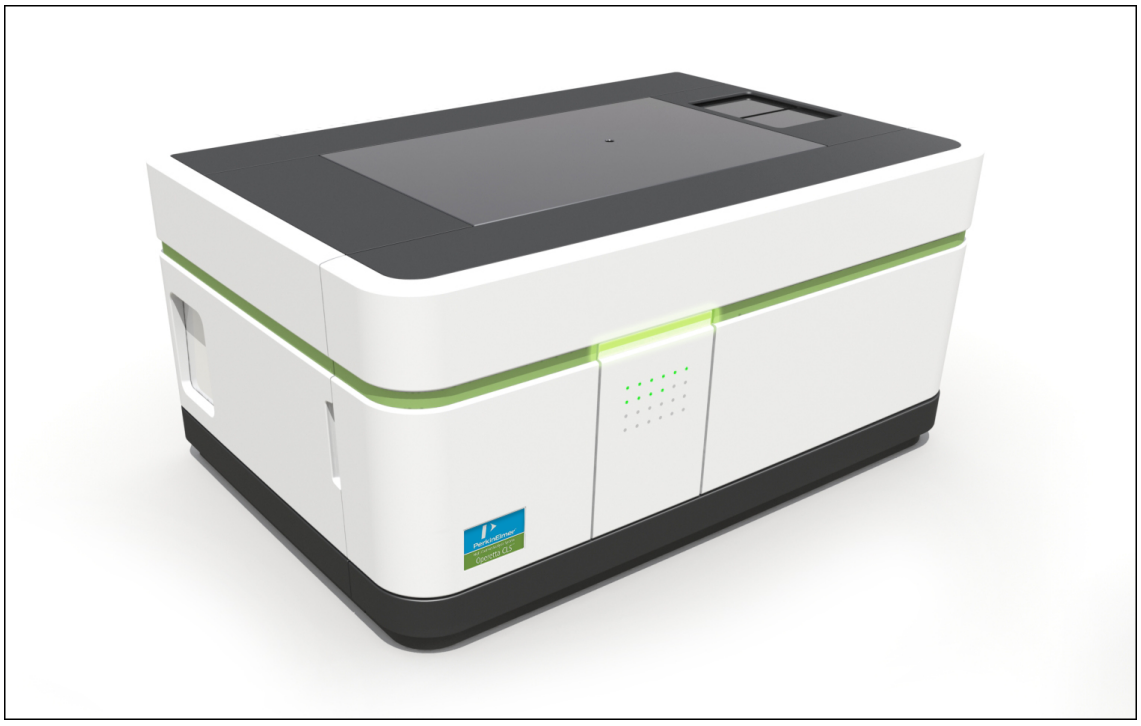
CELLULAR IMAGING AND ANALYSIS

OPERETTA CLS™



User Manual

PerkinElmer®
For the Better

Release History

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Service Contact
Information: http://www.cellularimaging.com
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Image was recorded using an Opera Phenix™ and human cardiomyocytes labeled with the hypertrophy marker proBNP/488, Rhodamine-Phalloidin, Hoechst and CellMaskBlue, 20x water objective. Cor.4U cardiomyocytes provided by Axiogenesis AG.

Contents

1 Safety Symbols

The following signal words are used in this manual:

Danger!

Indicates a hazardous situation which, if not avoided, can result in death or irreversible injury.

Warning!

Indicates a hazardous situation which, if not avoided, can result in severe but normally reversible injury.

Caution!

Indicates a hazardous situation which, if not avoided, can result in pain or minor injury.

Notice

Failure to observe may result in invalid measurement results or damage of the instrument.

Specific symbols are used which show you the type of hazard:

Biohazard!

Risk of crushing!

Failure to observe may result in injury or damage to the system.

Concentrated laser beam!

Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Electrical shock!

Direct and indirect electrical contact.

2 Safety Instructions

2.1 Usage of the Operetta CLS system

Caution!

Use only as intended.

The Operetta CLS system, including the original accessories may only be used in conjunction with the methods described in this manual. PerkinElmer does not assume any liability for any other applications or procedures, including use of individual subassemblies or components for other purposes.

The manufacturer does not assume any liability for any other kind of application, including individual subassemblies and the addition of individual components. This exclusion of liability also applies to all service or repair work which was not carried out by authorized PerkinElmer service personnel.

For research use only – not for use in diagnostic procedures

The Operetta CLS is an automated fluorescence imaging reader suitable for use in research applications such as academic, pre-clinical, and for forensic applications only. Due to its technical design the Operetta CLS is particularly suitable for cellular applications.

Exclusion of Liability for Measurement Results

PerkinElmer does not assume any liability for the correctness of measurement results obtained with the PerkinElmer Operetta CLS system nor for conclusions based on these measurement results.

2.2 Power Requirements

- Mains voltage: 100-240 V AC, 50/60 Hz
- Power consumption: 1000 VA max.

2.3 Network Connection (PC/LAN)

Network connection between Operetta CLS and Harmony PC:

- Gigabit Ethernet according to IEEE 802.3
- LAN cable max. length: 3 m (118 in)

2.4 Operating and Maintenance Staff

- The Operetta CLS may only be operated by qualified staff who have been specially trained and are familiar with the contents of this manual and the operating instructions of the device.

- The system must only be operated when it is in a fully assembled and installed condition. There are no user-serviceable parts inside the instrument.
- Unauthorized intervention or tampering inside the instrument is prohibited.
- Corrective maintenance work and service may only be performed by the PerkinElmer Service. Operational and maintenance procedures described in the provided manuals are excepted and may be carried out by the user.
- Additional safety instructions and warnings must be observed by service personnel during maintenance or repair. Such information is *not* part of this manual, except for mandatory laser safety information according to IEC 60825-1 (servicing information).
- The manufacturer can accept no liability for this product if the provisions of this documentation are not complied with.
- Wear your personal safety equipment, i.e. safety goggles and laboratory coat.

2.5 Environmental Conditions

The instrument has been designed to be safe under the following conditions:

- Operating conditions:
  +20 °C to +25 °C (66 °F to 77 °F), relative humidity < 70%, non-condensing
- Indoor use only
- A climatized, clean and dust-free laboratory is recommended
- Atmospheric pressure up to 2000 m altitude
- Installation category (overvoltage category): II, according to IEC 60664-1 (see note)
- Pollution degree: 2 according to IEC 60664-1 (see note)

Notice

- ***Installation category*** *(overvoltage category) defines the level of transient overvoltage which the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its overvoltage protection means. CAT II is the category used for instruments in installations supplied from supply comparable to public mains such as hospitals and research laboratories and most industrial laboratories.*
- ***Pollution degree*** *describes the amount of conductive pollution present in the operating environment. Pollution degree 2 assumes that normally only non-conductive pollution such as dust occurs with the exception of occasional conductivity caused by condensation.*
- *Both of these affect the dimensioning of the electrical insulation within the instrument.*

2.6 Indication of Hazards and Danger Zones

Before operating the Operetta CLS system for the first time please read this manual carefully to avoid incorrect operation of the Operetta CLS system.

2.6.1 Mechanical Hazard

Caution!

The lid on top of the instrument is not entirely stopped by the pneumatic springs if you let it fall down, so that you could hurt your fingers (especially at the lock in the middle).

- *Close the lid slowly and carefully.*

Caution!

You could jam your fingers at the front doors (access to emission filter wheel and immersion water bottles).

- *Open and close the front doors carefully and keep your hands away from the hinges.*

Caution!

You could jam your fingers at the emission filter wheel (behind left front door). The wheel can suddenly start rotating without notice (with low force but at high speed).

- *Open the left front door only when requested by the Harmony software and follow exactly the instructions of the **Change Filter** wizard.*
- *Keep your hands away from the filter wheel if it is rotating.*

Warning!

You could jam your fingers at the plate lift (transfer position).

- *Do not reach into the compartment of the plate lift.*
- *Keep your hands away from the plate lift when it is moving.*

Caution!

Glass elements are used for the upper surface of the instrument. Broken glass can lead to cuts and injuries.

- *Do not let heavy objects drop onto the glass plates.*
- *In case of a damaged glass plate, do not touch the glass fragments. Let PerkinElmer replace the broken part immediately. Do not operate a damaged instrument.*

2.6.2 Laser Radiation

Danger!

Use of this instrument other than specified in the user instructions may result in exposure to hazardous laser radiation.

Classified according to standard
IEC 60825-1:2014 (wavelength: 785 ± 10 nm;
accessible radiation: 3.4 µW)

Warning!

Class 3B invisible laser radiation – Eye injury

Invisible Class 3B laser radiation may be present inside the Operetta's protective housing during operation. The safety interlock will switch off the autofocus laser as soon as the instrument's lid is is opened so that no harmful radiation can become accessible.

- *Do not defeat the safety interlocks!*
- *Do not remove any housing components!*
- *AVOID DIRECT AND INDIRECT EXPOSURE TO BEAM!*
- *Service tasks that require access to the instrument while the interlocks are defeated may only be carried out by qualified service personnel.*

2.6.3 Optical Radiation

The Operetta CLS is equipped with up to 8 LED light sources for sample excitation.

Optical Radiation – Risk Group 3

WARNING: UV emitted from this product.

- *Avoid eye and skin exposure to unshielded products.*

WARNING: Possibly hazardous optical radiation emitted from this product.

- *Do not look at operating lamp. Eye injury may result.*

Since the instrument's lid is protected by laser safety interlocks. No harmful radiation will become accessible when it is opened for setup and maintenance.

- *Do not defeat the laser safety interlock.*

2.6.4 Electric Shock

2.6.4.1 General

The Operetta CLS operates with up to 240 V AC. Highly dangerous electric voltages occur in a number of places throughout the system.

Danger!

Direct electrical contact – Electrical shock.

- *Use only the provided power cable which has a mains plug with earthing contact (Europe: King-Cord K5A031H6250BB, USA: King-Cord K01031C5200BB-L).*
- *The mains plug must only be inserted in a socket outlet with earthing contact.*
- *No user serviceable parts inside the housing. Service has to be carried out by qualified PerkinElmer service personnel only!*
- *Unplug from mains for a complete disconnection.*
- *Disconnect the power cable by grasping the plug, not the cord. Otherwise the cable can be damaged.*
- *Check the instrument and power cable for damages before switching on the Operetta CLS. Do not use the instrument with damaged power cable or obviously defective or opened enclosure.*
- *In case of a damaged power cable contact PerkinElmer to request a new cable.*

2.6.4.2 Disconnecting from Mains Supply

The on/off switch does not switch off the Operetta CLS immediately. There will be a controlled shutdown of the electronics with a certain delay before the instrument is really switched off.

Danger!

Direct electrical contact – Electrical shock.

- *In case of emergency unplug the power cable to disconnect the Operetta CLS from mains supply immediately.*
- *Ensure easy access to the mains connection at any time.*

2.6.4.3 Replacing a Fuse

If one of the two fuses of the Operetta CLS has blown, it can be exchanged by the user.

Danger!

Direct electrical contact – Electrical shock.

- *Unplug from mains before replacing fuses.*
- *For continued protection against risk of fire, replace only with certified fuse of same type and rating:*
  - *100 V – 127 V: 2x T12A / 250 V (T = time-delay)*
  - *220 V – 240 V: 2x T6.3A / 250 V*

Please see section 7.2.3 “Replacing a Fuse”, page 537 for detailed instructions.

2.6.5 Spillage or Leakage of Liquids

Danger!

Direct electrical contact - Electrical shock.

- *Do not handle large amounts of liquids near or above the Operetta CLS and observe the cleaning instructions.*
- *If liquids should be spilled into the instrument accidentally, switch off the Operetta CLS immediately, i.e. unplug the power cable (see also section “Disconnecting from Mains Supply”, page 1).*
  - *Remove the liquid from areas which may be cleaned by the user (see cleaning instructions).*
  - *If liquid was spilled in other areas and may have entered the inside of the instrument, please contact PerkinElmer Service to let them check the instrument. Do not operate the instrument.*
  - *If potentially infective substances have been used, the instrument has to be decontaminated together with PerkinElmer Service.*
- *Service and repair may be carried out by qualified PerkinElmer service personnel only!*

Danger!

Direct electrical contact - Electrical shock.

- *Check the filling levels of the immersion water bottles regularly, especially to avoid an overflow of the "WASTE" bottle and thereby immersion water getting into the instrument.*
- *When refilling or emptying a bottle, remove the respective bottle from its position. Refill or empty the bottle at a certain distance from the instrument only.*
- *Do not refill the "SUPPLY" bottle if the "WASTE" bottle does not fill up even though a water objective is in use. This could be an indication of a defective suction or a leakage resulting in immersion water being spilled into the instrument. In this case switch off the instrument immediately, i.e. unplug the power cable (see also section "Disconnecting from Mains Supply"). Contact PerkinElmer Service and do not operate the instrument.*
- *Do not obstruct the outlet at the bottom of the instrument. If clear water drops out under the instrument (without having spilled liquids), this can indicate a leakage of the immersion water system. In this case switch off the instrument immediately, i.e. unplug the power cable (see also section "Disconnecting from Mains Supply"). Contact PerkinElmer Service and do not operate the instrument.*
- *Service and repair may be carried out by qualified PerkinElmer service personnel only!*

2.6.6 Cleaning

Warning!

Ethanol or other cleaning solvents may cause fire or injury if used in great amounts.

- *Switch off the instrument before cleaning.*
- *Observe the cleaning instructions.*
- *Do not handle large amounts of liquids near or above the instrument.*
- *Keep the concentration of dangerous solvents as low as possible.*
- *Ensure the area is well ventilated.*

Please see section 7.2.1 "Cleaning", page 531 for detailed cleaning instructions.

2.6.7 Process Liquids or Substances

Danger! Biohazard!

This device is designed for analyzing biological substances at biosafety level 1 and 2 (BSL-1 or BSL-2).

- *Do not use more hazardous substances due to limited possibilities of decontamination.*
- *Observe the safety instructions for the substances used.*
- *Observe the instructions for decontamination and cleaning.*
- *Always make sure to comply with national and international laboratory safety instructions.*
- *Always wear your personnel safety equipment.*
- *Attach a biohazard label (included in delivery) as soon as potentially infective substances are used.*

The processing of samples on the Operetta CLS covers only probes at biosafety level 1 and 2 (BSL-1 or BSL-2, CDC http://www.cdc.gov/biosafety/publications/bmbl5/BMBL5_sect_IV.pdf).
BSL-1 includes “well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment”. BSL-2 is “suitable for work involving agents of moderate potential hazard to personnel and the environment”.

All procedures performed on the Operetta CLS should be done carefully to minimize the creation of splashes. Biological substances higher than BSL-2 may not be used. As though developed for easy cleaning and low internal contamination, a sufficient sterilization and decontamination especially of internal mechanics and electronics cannot be guaranteed!

It is fully within the responsibility of the operator to ensure that all appropriate measures are taken to ensure that no substance processed by Operetta CLS exposes any hazard to the system, personnel or the environment.

As supplied, Operetta CLS does not provide any particular protection against the hazards evolving from aggressive chemicals, biological active substances or radioactive substances.

2.6.8 Carbon Dioxide

If you are using the TCO unit (Temperature and CO_2 Control) to control the carbon dioxide level inside the instrument, you have to observe the following safety instructions regarding carbon dioxide handling.

Danger!

Carbon Dioxide Gas

In high concentration, carbon dioxide is harmful to health. Depending on the concentration it can lead to headache, tiredness, and dizziness, in higher concentrations even to unconciousness, spasms, and death. Carbon dioxide is hardly perceptible.

- *Do not install the Operetta CLS in very small rooms or without sufficient room ventilation.*
- *The customer has to install a pressure controller which provides the required carbon dioxide working pressure of 3 bar. Lower pressures can lead to malfunctions, higher pressures could destroy the instrument or lead to harmful concentrations in case of an instrument malfunction. See the Operetta CLS Site Readiness Instructions for a connection diagram.*
- *Use only a robust high quality CO_2 tube (see next section). Check tube and tube connection regularly for visible damages and do not operate the instrument with an obviously damaged tube or tube connection.*
- *If carbon dioxide control has been activated and you open the lid, wait 10 seconds before bending over the instrument to avoid unnecessary inhalation of the CO_2 gas.*
- *CO_2 supply must be switched off during downtime of the instrument.*
- *Do not try to set a CO_2 concentration higher than 10 % in the Harmony software, even if the software should allow that in case of malfunction.*

2.6.8.1 Supply Tubing

Danger!

Carbon Dioxide Gas - Supply Tubing

- *Do not expose the tubing to any mechanical or thermal stress.*
- *Comply with the maximum bending radius of the tubing (see below).*
- *If the tubing has to be laid on the floor, provide a corresponding protection to prevent people from stumbling or stepping on the tubing.*
- *Do not lay the tubing close to radiators or other heated objects.*
- *Do not expose the tubing to direct sunlight.*
- *Check the tubing for damages each time before using carbon dioxide control.*
- *Do not operate the instrument with a damaged carbon dioxide supply tubing. Contact PerkinElmer to request a new tubing.*
- *Do not manipulate the carbon dioxide tubing or fitting.*
- *The tubing may only be shortened using an approved hose cutter.*

Specification

- Outer diameter: 6 mm
- Min. bending radius: 21 mm
- Shore hardness: D 62 ±3
- Material: TPE-A (polyamide)

2.6.8.2 Recommended Protective Measures

The TCO is equipped with a flow limiter for carbon dioxide. Thereby, only 15 l carbon dioxide per hour can flow into the instrument. This corresponds approximately to the amount of carbon dioxide which one exhales during one hour and can be considered as relatively safe.

However, the flow limiter does not protect you if the carbon dioxide supply itself (or other components outside the Operetta CLS like tubing, pressure controller, fitting etc.) is damaged or not installed correctly. To reduce the residual risk, at least one of the following measures should be carried out by the customer.

Notice

It is the customer's responsibility to carry out the recommended measures correctly and in a professional manner.

- Ensure sufficient air exchange in the installation room to avoid dangerous carbon dioxide concentrations in case of a malfunction. For operating the instrument, a flow rate of 15 l/h is sufficient. It is recommended to limit also the carbon dioxide supply itself to this flow rate.

- Use only a small carbon dioxide cylinder with a maximum filling of 1 kg CO_2 per 40 m³ volume of the installation room. In case of a leakage, no dangerous carbon dioxide concentration can occur, even if the whole volume of the cylinder should escape.
- Monitor the carbon dioxide concentration in the installation room and install an automatic mechanism which shuts off the carbon dioxide supply if dangerous concentrations of carbon dioxide are detected.

2.6.9 Ventilation

Caution!

Covered ventilation openings may cause malfunctions or damage to the equipment.

- *Make sure that none of the ventilation openings of the instrument (in the back and bottom) are obstructed.*
- *Keep a distance of at least 15 cm (5.9 in) to walls and other objects.*

2.6.10 Transport

Warning!

The instrument is very heavy (up to 180 kg / 397 lb). If it falls down or tips over it can cause severe injuries.

- *The four handles of the Operetta CLS are not designed to carry the device for relocation!*
- *It is only permitted to move the Operetta CLS on the bench or table by four persons using the provided handles. Do not try to carry the instrument.*
- *All four transport handles have to be screwed in completely. Do not use inadequate force or tools for this manual process, otherwise the handle threads may be damaged.*
- *The instrument may only be transported by PerkinElmer Service (requires a special transport frame and fork-lift).*

Please see section 7.2.4 “Transport”, page 538 for detailed transport instructions.

2.6.11 Disposal

Caution!

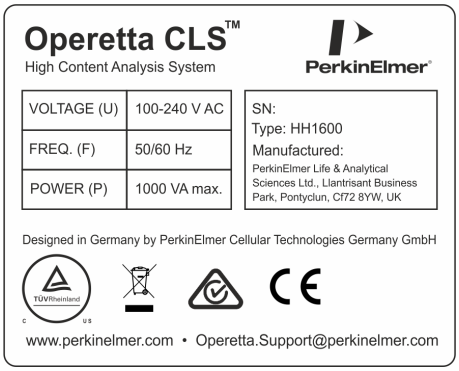
Device may be contaminated by hazardous substances.

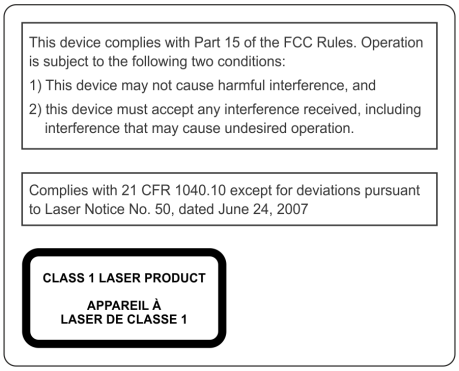
- *Before disposal, ensure that the device is decontaminated.*

The user is responsible for protecting the environment against any hazardous substances used in the process, in particular the environmentally appropriate disposal of process residues. Relevant local regulations must be observed. See also section 10.3 “WEEE Instructions”, page 567.

2.7 Location of Warning Signs and Labels

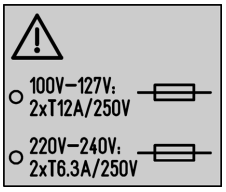
On the back of the instrument:





Classified as Class 1 Laser Product according to standard IEC 60825-1:2014 (wavelength: 785 ± 10 nm; accessible radiation: 3.4 µW).

On the side of the instrument:



Bottom side of instrument lid and plate lift compartment (transfer position):

3 Servicing Information

This chapter contains laser warnings and protective procedures which have to be observed by service personnel when opening the housing in case of service or repair (according to IEC 60825-1 {Ed3.0}, 8.2 b). This information is not intended for the user and it is not part of any user maintenance!

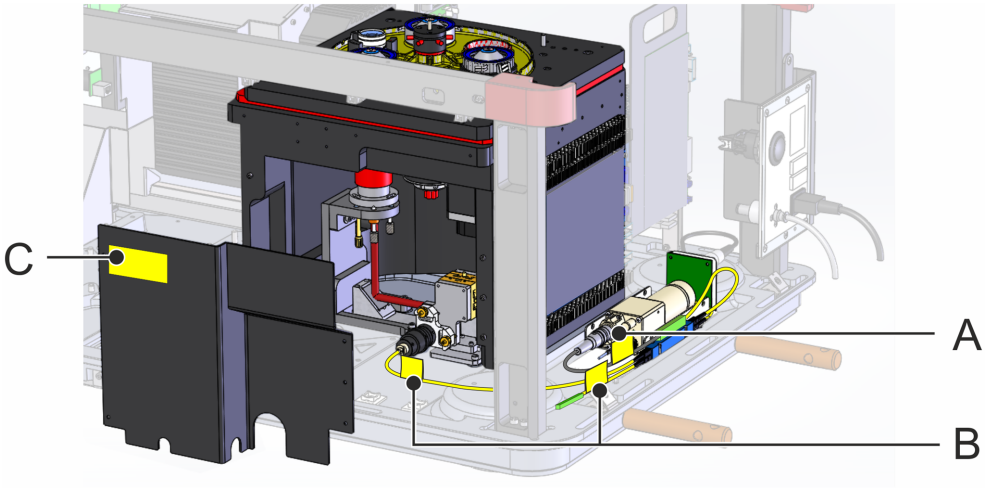
Caution!

There are no user-serviceable parts inside the instrument.

- *Repair and maintenance (excluding the user maintenance tasks described in the User Manual) may only be performed by specially trained and authorized persons (e.g. PerkinElmer Service).*

3.1 Autofocus Laser

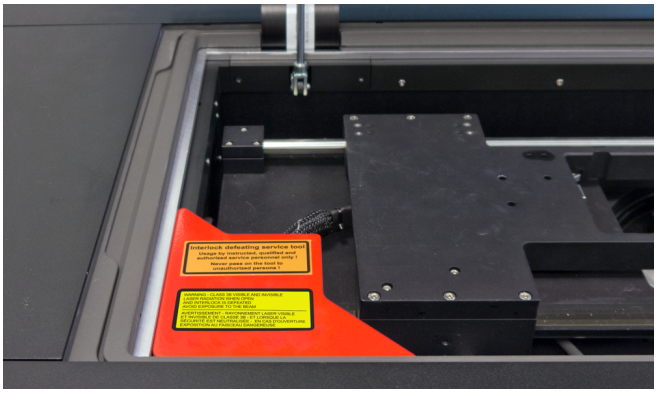
The autofocus laser belongs to Laser Class 3B. The laser is automatically disabled by the interlock system when the lid is opened.



A: Autofocus laser with fiber exit
B: Fiber coupler exits
C: Metal sheet of microscope body

The light from the autofocus laser is guided into the microscope body by optical fibers. The laser light is encased by the microscope body from three sides. The front side is sealed off by a black anodized aluminum metal sheet. The fibers and the metal sheet (see positions in the figure above) are labeled with warning labels for Class 3B invisible laser radiation (see next chapter, label 1).

Before using the interlock defeating tool (in red in the image below), service personnel must take measures to protect themselves and others from harmful laser radiation which may be present above the objective. If the tool is used, the interlock will also be defeated for the LED light sources. The tool has a warning label for Class 3B visible and invisible laser radiation (see next chapter, label 2).

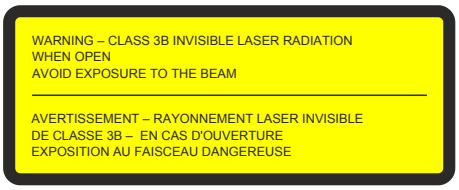


Interlock defeating service tool

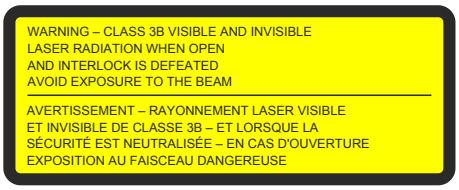
3.2 Internal Warning Labels

The following laser warning labels are placed inside the instrument. If the component or housing part bearing such a label is opened or removed, there will be access to laser radiation above Class 1. The exact nature of the hazard and corresponding instructions are stated on the warning label and in the table below.

Label 1



Label 2



Instrument Position	Component	Warning	Safety Instructions
Fiber exit laser	Autofocus laser	See label 1	• Switch off the autofocus laser before disconnecting the optical fiber.
Fiber coupler exits	Autofocus laser – fiber connection	See label 1	• Switch off the autofocus laser before disconnecting the optical fiber. • Fix fiber outputs if radiation is required for service purposes. • Ensure that emission is directed to a non scattering beam block.
Microscope body	z-Drive area seal off sheets	See label 1	• Switch off the autofocus laser before removing the metal sheets.
Above objective	Interlock defeating service tool	See label 2	• Interlock defeating tool may be used by instructed, authorized and qualified service personnel only! Never pass on the tool to unauthorized persons! • Do not look to the objective from above. • Use beam blocks on objective or remove interlock defeating tool if you leave the instrument.

4 Software Installation

4.1 Prerequesites

4.1.1 USB Dongle and SMA License

Handling of the USB license dongle depends on the installation scenario:

- **Update existing Harmony installation:** Before Harmony is installed, your SMA license (Software Maintenance Agreement) stored on the USB dongle will be checked by the setup. Make sure that the USB dongle has been connected before starting the setup.
- **Initial Harmony installation:** Do not connect the USB dongle until Harmony and the dongle driver have been installed. Connect the dongle before starting Harmony for the first time.

For further information and instructions how to obtain a new license please see section “License Management”, page 320.

4.1.2 Office PC Requirements

For installing Harmony 4.6 on an office PC, the following requirements must be met:

Basic:

- Windows® 7, SP1, 64 bit, English or Windows® 10, 64 bit, English (Windows® 8 is not supported)
- Harmony Office License (incl. USB dongle)
- One free USB port
- 2 GB free harddisk
- DVD drive (for installation)
- Gigabit Ethernet connection to Harmony PC (to connect to database)
- Administrator rights (only for the installation)
- Internet Explorer 8 or later, JavaScript® enabled, cookies allowed
- Display Language: English
- Region and Language Setting: English
- DPI setting: normal size (96 DPI)

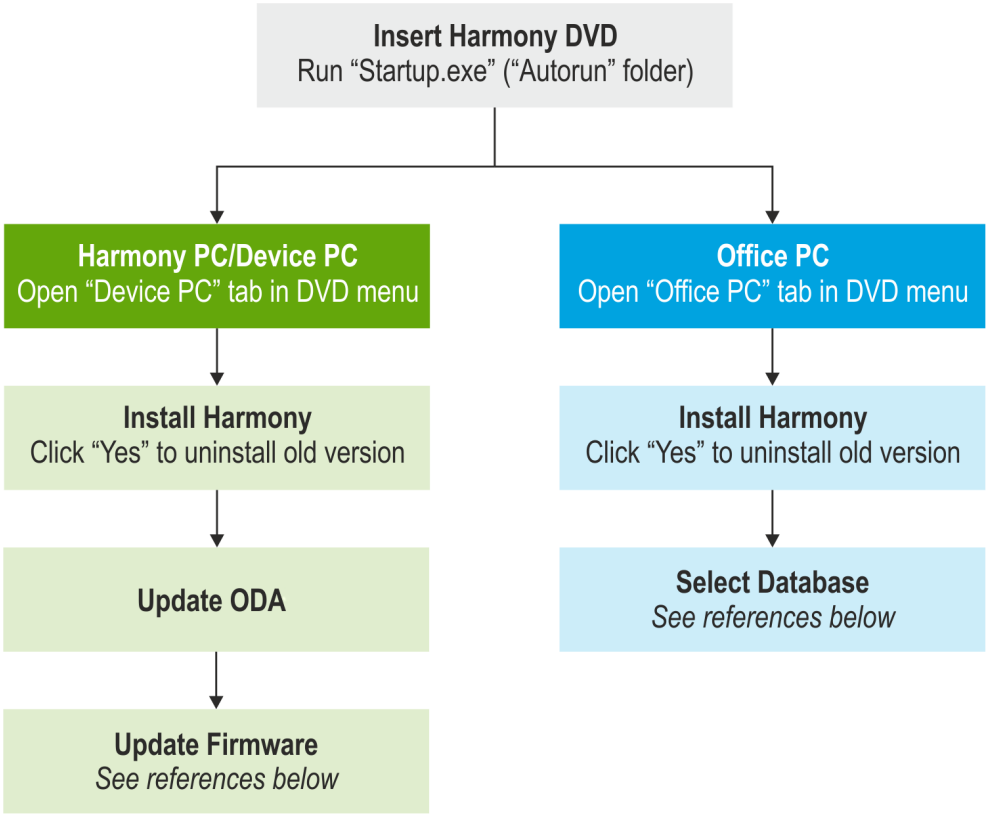
Minimum:

- Processor with 4 cores (Intel® Xeon®, Core™ i5, Core™ i7)
- 8 GB RAM
- Screen resolution of 1280 x 1024 (19")

Optimum:

- Intel® Xeon® processor with 12 cores
- 32 GB RAM
- Screen resolution of 1920 x 1200 (24") or 2560 x 1600 (30”)

4.2 Install or Update Harmony



For detailed instructions see the following sections:

- “Update Device Firmware”, page 244
- “Change Database”, page 292

5 Harmony Software

5.1 User Interface

5.1.1 Start Up

Notice
Wait at least 30 sec. before you restart the Operetta CLS after a shutdown.

1. Switch on the Operetta CLS.

   The instrument is initialized (status light blinking green). As soon as the status light stops blinking (constantly green), the Operetta CLS is ready and can be used. See also section 6.5 “Instrument Status”, page 510.
2. Switch on the Harmony PC and log into Windows®.
3. Double-click the Harmony icon on the desktop.

   Harmony is started and a login dialog appears.
4. Log into Harmony (see section 5.1.3 “Login”, page 33).

Notice
It is recommended to let the instrument warm up for 1 hour before starting measurements.

5.1.2 Shut Down

It is recommended to switch off the Operetta CLS (instrument) to extend the life time of the components if the instrument is not required for several hours or even longer. Harmony and the Harmony PC can also be shut down if not required anymore (see exceptions below).

Notice
You should not close Harmony or shut down the Harmony PC in the following cases:

- Background jobs are still running (see section “Job Status”, page 300).
- Scheduled tasks have been defined for the near future (see section “Schedule Tasks”, page 280).
- Database needs to be accessible for office installations of Harmony (Harmony can be closed, but the Harmony PC must be running).

How to shut down the whole system

1. Close the Harmony software by closing the window in the usual way (X button).
2. Shut down the Harmony PC.
3. Close the main valve of the carbon dioxide supply (if applicable).
4. Switch off Operetta CLS using the power switch.

The on/off switch does not switch off the Operetta CLS immediately. There will be a controlled shutdown of the electronics with a certain delay before the instrument is really switched off.

Danger!

Direct electrical contact – Electrical shock.

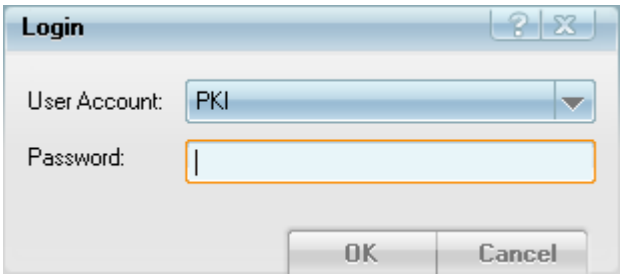
- *In case of emergency unplug the power cable to disconnect the Operetta CLS from mains supply immediately.*
- *Ensure easy access to the mains connection at any time.*

Notice

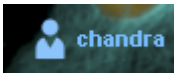
Wait at least 30 sec. before you restart the Operetta CLS after a shutdown.

5.1.3 Login

After starting Harmony, the **Login** dialog is displayed where you can select a user account and enter the corresponding password.



- The initial user account is **PKI** with password **PKI**. This account has administrative rights and should only be used for the first login. You can (and should) create new user accounts for each Operetta CLS user. For details see section 5.1.17.8 “User Accounts (Harmony + Columbus)”, page 304.
- The user account **PKI Service** is required by the PerkinElmer Service. It cannot be deleted.
- If you are logged in and want to switch to a different user account, click the user icon in the **Navigation bar** (see section 5.1.5 “Screen Arrangement”, page 36). The **Login** dialog is opened.



5.1.4 Instrument and Data Type

Harmony can be used with different instruments and you can load data from other instruments (Operetta, Opera Phenix or Operetta CLS). The appearance of the graphical user interface (GUI) is influenced by a few properties which are visualized in the **Navigation Bar**:

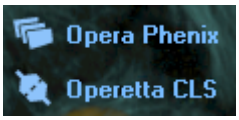
Legend

Property	Description
Connection status	**Connected:** Harmony is directly connected to an instrument. **Disconnected:** Harmony automatically tries to connect to an instrument. If no instrument can be detected or the instrument is switched off, Harmony will run as an office version (disconnected). If the instrument is switched on or connected in the meantime, you have to restart Harmony to try again.
Data type	Type of the currently loaded measurement or evaluation (generated by Operetta, Opera Phenix or Operetta CLS).
Instrument type	Type of the current instrument (Opera Phenix or Operetta CLS). • If Harmony is in *connected* state, the instrument type is determined by the connected device. • If Harmony is in *disconnected* state, the instrument type is retrieved from the currently selected database. The displayed edition of Harmony Help depends on the current instrument type.

5.1.4.1 *Harmony GUI States*

The combination of these properties leads to three basic states of the Harmony GUI:

Harmony GUI	Description
Connected Version 	Harmony is directly connected to an instrument. Data type and instrument type match. • All functions enabled • Harmony Help describes the current GUI
Office Version 	Harmony is not directly connected to an instrument (office installation) or the instrument is switched off. Data type and instrument type match. • Hardware-related functions are disabled or hidden • Harmony Help describes the current GUI

Harmony GUI	Description
Mismatch 	Data type and instrument type do **not** match. • Restricted mode, parts of the GUI are disabled or read-only • Harmony Help does not describe the displayed GUI • Further restrictions apply if Harmony is disconnected (office version) See the following section 5.1.4.2 “Mismatch”, page 35 for details.

5.1.4.2 Mismatch

In **mismatch** state, the Harmony GUI will look different than usual and might be confusing. The restrictions are similar to those in the office version (Harmony disconnected).

What can cause a mismatch?

You have loaded a measurement or evaluation of a different data type.

Example
Harmony is connected to an Operetta CLS and you load a measurement from the Application Guide example datasets. These datasets have been generated using an Operetta. Therefore, the data type changes to “Operetta”.

What are the effects of a mismatch?

- Various functions are disabled
  - All measurement functions
  - Save functions
  - Experiment is displayed on **Setup** screen, but cannot be edited.
- Harmony Help does not describe the displayed GUI.
  There are multiple editions of the online help (Opera Phenix and Operetta CLS). The displayed version depends on the instrument type, not on the current GUI or data type.

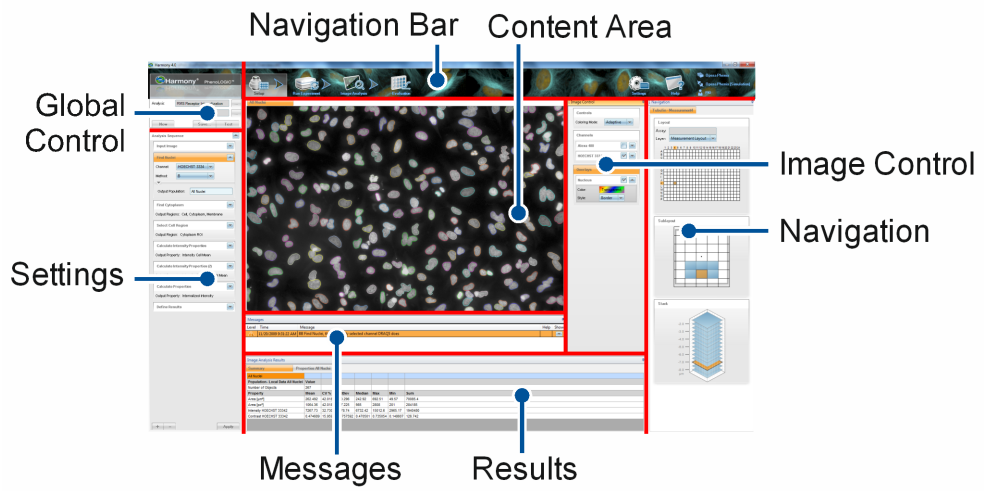
How can I get rid of a mismatch?

You can return to a normal mode by one of the following actions:

- Click the **New** button on the **Setup** screen.
- Load an experiment on the **Setup** screen.
  You can only select experiments which are compatible with your instrument type.
- Load a measurement (or an evaluation including a measurement) which has a data type compatible with your instrument type.

In all cases, the previously loaded measurement which caused the mismatch is removed from the memory. Data type and instrument type match again.

5.1.5 Screen Arrangement



While certain screen areas in Harmony are fix (e.g. the upper **Navigation Bar** and the left part of the screen), the following panels can be arranged and resized in multiple ways:

- **Navigation**
- **Image Control**
- **Results** (per default only visible on the **Image Analysis** and **Evaluation** tab)
- **Messages**

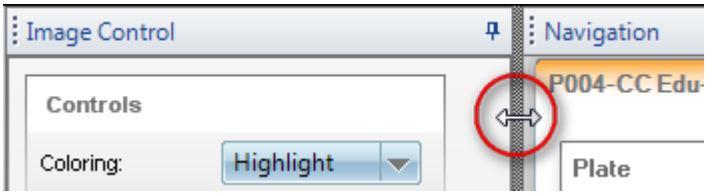
The remaining space in the center of the screen is automatically occupied by the **Content Area**, e.g. for image display.

Notice

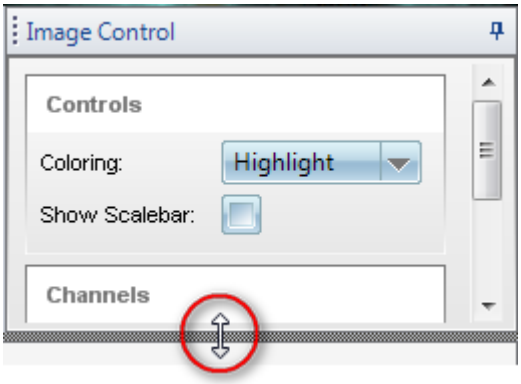
The default window configuration can be restored using the function **Reset Windows and Panels** (see section 5.1.17.13 “Display Settings”, page 322).

How to resize panels

- In docked state (not detached), a panel can be resized by dragging the outer border or the border between two panels.

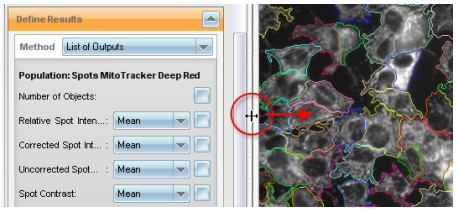


- In detached state, you can drag the borders or corners of the window to resize it.

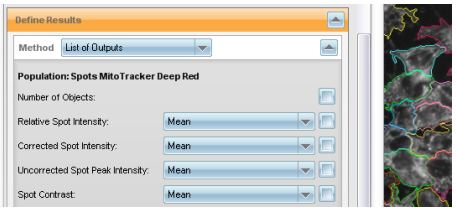


How to resize the Analysis Sequence pane

The **Analysis Sequence** pane on the **Image Analysis** tab can be resized to wide view (e.g. for formulas or long property names) by dragging the border to the right. The size can only be adjusted in two steps: regular view or wide view.



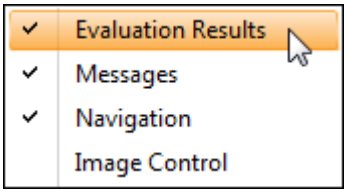
Regular view



Wide view

How to show/hide panels

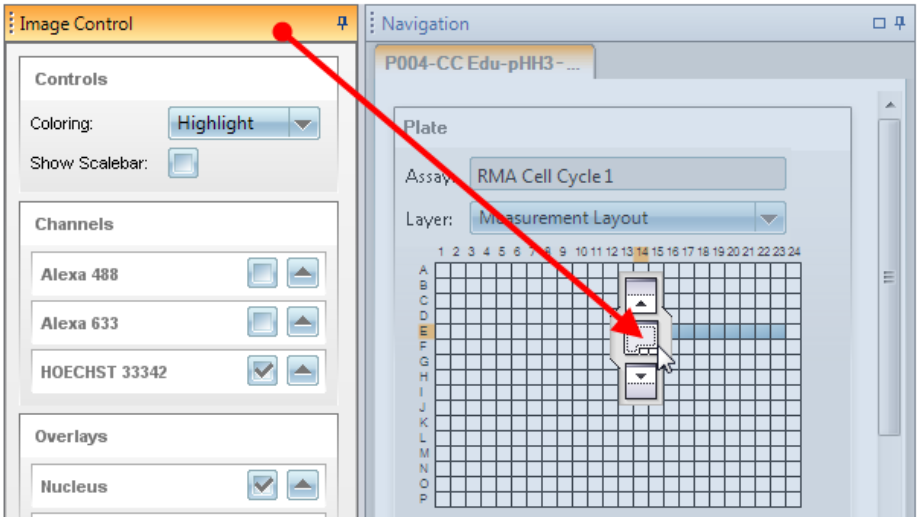
1. Right-click on a pane's headline.
   A context menu is opened. All panels are listed with their current status (visible/hidden).



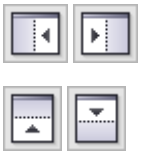
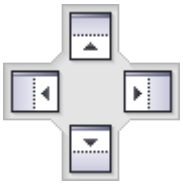
2. Click on a panel name to show/hide the corresponding panel.
   At least one panel has to remain visible.

How to arrange and dock panels

1. Click on a panel's headline and hold the mouse button.
2. Drag the panel and drop it on the desired docking position.



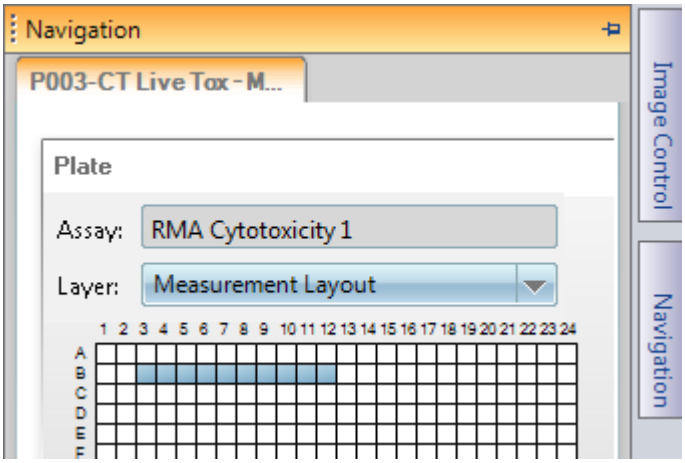
The docking symbols indicate the future position of the panel (in relation to the screen section beneath):

Docking Position	Description
	**Docking positions of the whole working area** Drop the panel on these symbols to arrange them in the outmost positions.
	**Docking positions of a panel** Allows to arrange the panel above or under another panel. The center position allows to arrange the panels in tabs.
	**Docking position of the Content Area** Allows to select a docking position around the **Content Area**.

How to pin panels to the sidebar

- Click in a panel's headline to pin the panel to the sidebar.

The panel slides open if you move the mouse over the tab. Only one panel is displayed at a time.



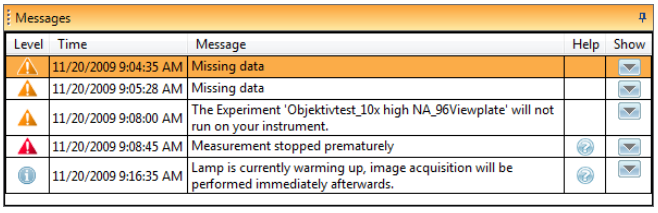
- To unpin the panel, click again.

How to detach panels

- Double-click a pane's headline to detach the panel. It is put into a separate window and can be resized and moved anywhere on the screen.
- Double-click the headline again to dock the panel.

5.1.6 Messages

5.1.6.1 Messages Pane



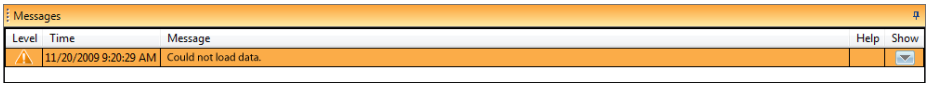
This screen section is used to display system messages. There are three levels of messages:

- Error
- Warning
- Information

You can expand a message using the button to read the full message text. Some messages also provide a direct link to the corresponding help topic, indicated by the button.

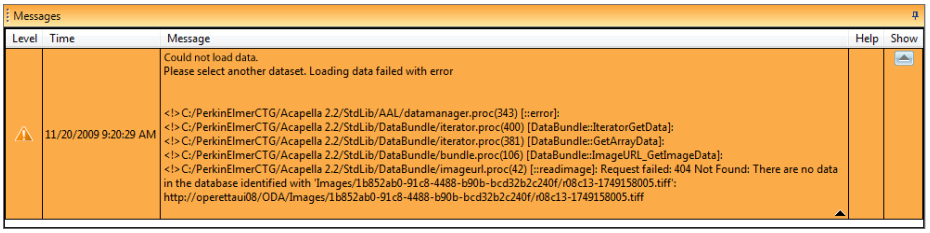
How to read the full message text

1. Per default only a short version of each system message is displayed.



2. Double-click the desired message to expand it or click the button. A more detailed description is displayed or instructions are given how to avoid the error.

3. Some messages have a further level which can be expanded: Click the button in the lower right corner of the message. This level can contain technical information for troubleshooting.



4. To collapse the message, double-click it again or click the button.

How to copy the message text to the clipboard

The message text can be copied to the clipboard, e.g. for troubleshooting:

1. Select the desired message.
2. Right-click the message and select **Copy** or **Copy All** (recommended when reporting a technical issue to PerkinElmer Support) from the context menu.

   The selected message is copied to the clipboard.

Buttons and Elements

The table can be sorted by **Time** or **Message** if you click the corresponding column title.

Element	Description
Level	Error level of the message, indicated by an icon: • Error • Warning • Information
Time	Time stamp of the message.

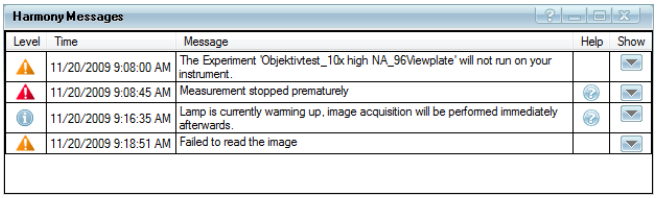
Element	Description
Event	Messages can have up to three levels. You can expand/collapse a message using the corresponding buttons or by a double-click. • In collapsed state, only the first level (title) is visible. • If you expand the message, a longer description is displayed. • Some messages also have a third level with technical information for troubleshooting.
Help	Opens the context-sensitive help for this screen section.
Show	The button can be used to expand or collapse a message. The third level (if existing) can be opened using the button in the lower right corner of the message. If the button is disabled (gray), then there is no third level.

Context Menu

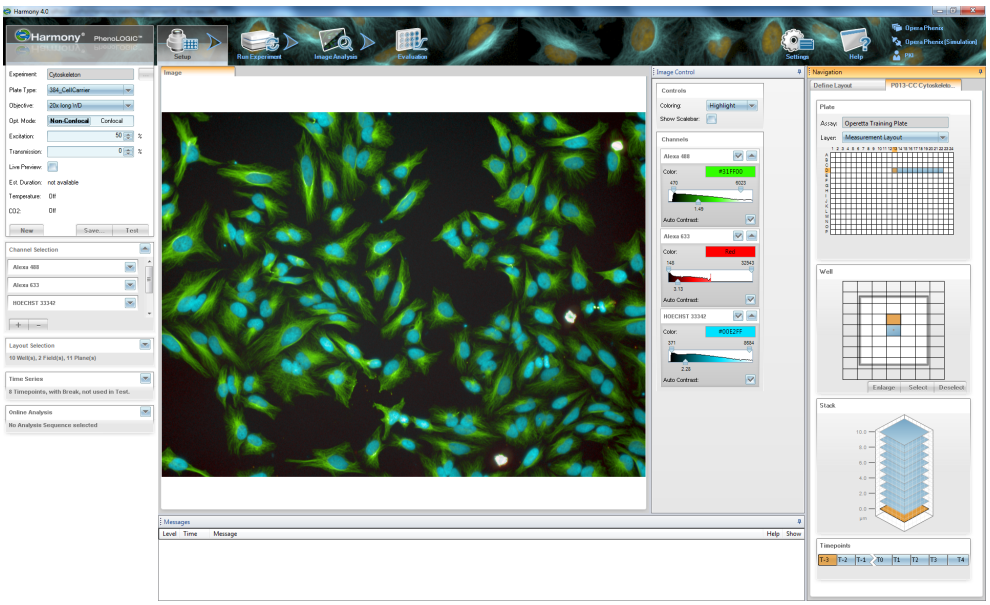
Element	Description
Copy	Copies the selected message to the Windows® clipboard.
Copy All	Copies all messages to the Windows® clipboard.
Delete All	Deletes all messages from the list.
Delete	Deletes the selected message.
Help	Opens the context-sensitive help for this screen section.

5.1.6.2 *Messages Window*

If the **Messages** pane is covered by another window (e.g. **Settings** or one of its sub-dialogs), you can click the button in the title bar of these dialogs. **Messages** will then be opened in a separate window so that you have full access to the system messages. The button becomes red to indicate that a new message has just been announced.



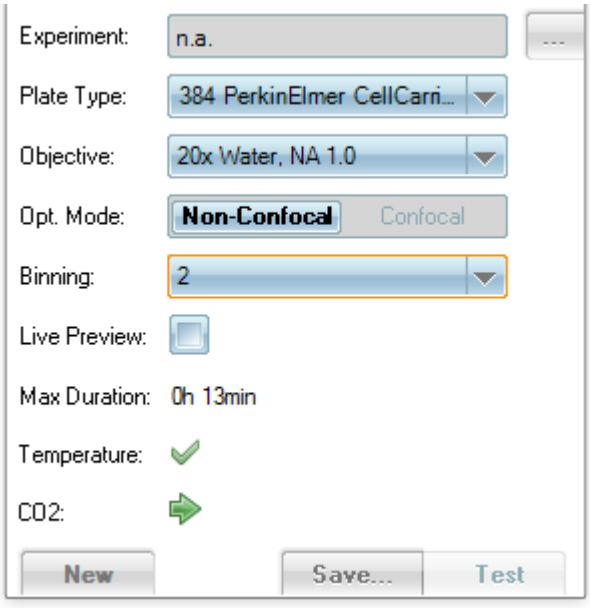
5.1.7 Setup



The **Setup** tab is used for the following purposes:

- Load or define an experiment including global settings, channel settings, layout, time series and online jobs.
- Take snapshots or make test measurements to find the best settings for your experiment.

5.1.7.1 Global Control



In section **Global Control** you define global experiment settings. These settings are identical for all channels. If you save your experiment, it will also include the settings you have entered in sections **Channel Selection**, **Layout Selection**, **Time Series** and **Online Analysis**.

An experiment file includes:

- Global experiment settings such as optical mode (confocal/non-confocal, only available with confocal upgrade), binning, plate type and objective (section **Global Control**).
- Channels and exposure settings (section **Channel Selection**).
- Plate layout, well layout, stack (if configured, section **Layout Selection**).
- Time series (if configured, section **Time Series**).
- Analysis sequence for online analysis or screen name for Columbus transfer (if configured, section **Online Jobs**).

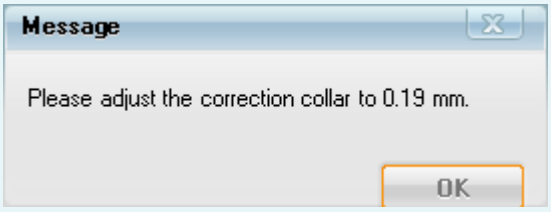
Notice

- Please refer to the **Operetta CLS Application Guide**, chapter 2 “Experimental Setup” for detailed information and strategies for the experiment setup and to chapter 1.5 “Optical Modes” for an explanation of the confocal principle and its advantages.
- To quickly identify areas of interest on the slide we recommend to use the **PreciScan** function (requires optional license, see section 5.1.8.3 “PreciScan™”, page 119) or the **Manual PreScan/ReScan** procedure (see section 5.1.11.4 “Manual PreScan/ReScan”, page 191).

A **test measurement** can be used to test the current experiment settings including all channels in one single well of the plate. If selected, well layout, stack, time series and online analysis are also taken into account. The test results are only temporary and will be overwritten by the next test measurement. The images can be selected and viewed in **Navigation – Test Images**.

Notice

Do not forget to adjust the correction collar of the objective to the corrected plate bottom thickness of the selected plate type. After selecting a different objective or plate type, a message box will display the required setting of the correction collar (if applicable):



How to make a test measurement

1. Open the **Setup** tab.
2. Configure your experiment using sections **Global Control**, **Channel Selection**, **Time Series** and **Layout Selection**.

3. If you have configured a well layout, stack, time series or an online analysis, and you want them to be used in the test measurement, activate the **Use in Test** check boxes on the **Well**, **Stack** , **Time Series** or **Online Jobs – Analysis** pane.
4. In **Navigation** open **Define Layout** to see the **Plate** pane.
5. Select one single well which is to be used for the test measurement.
6. In the **Global Control** section, click Test to start the test measurement. The selected well is measured using the current experiment settings.

> **Notice**
> During a snapshot or test measurement the selected combination of plate type and objective is checked for compatibility. Restrictions regarding the usable plate area (indicated by crossed wells and red well borders) and the focus height are displayed in **Navigation – Define Layout – Plate**. For details see section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89.

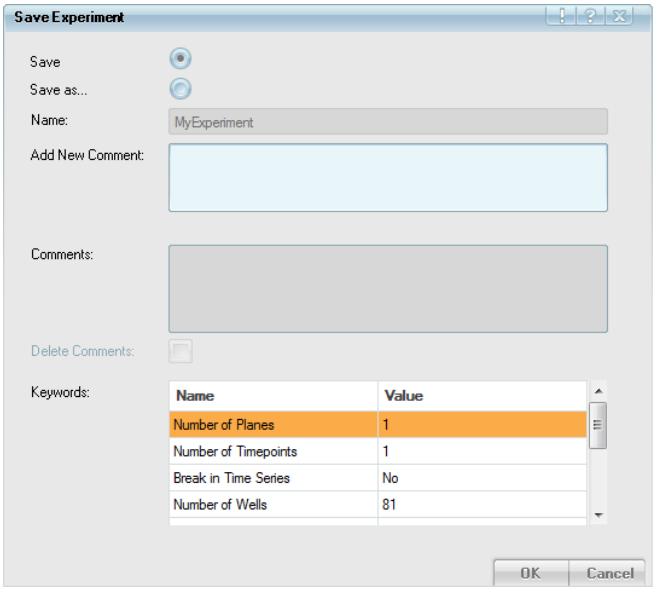
7. In **Navigation – Test Images** click the desired well layout field and/or stack plane. The image will be displayed in the **Content Area**. Selection and coloring of the channels can be configured in the **Image Control** pane.

How to save an experiment

Notice

It is not necessary to save an experiment. If it has not been saved, it will be "contained" in the measurement. If you save the experiment, it will be available as a separate data object in the database. If you do not save it and start a measurement, the keyword for *Experiment Name* will be set to *n.a.* (not available).

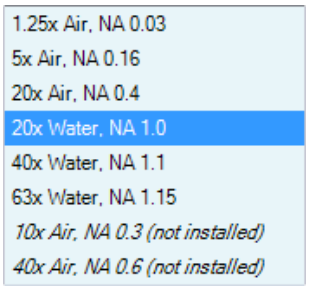
1. Click Save... . The **Save Experiment** dialog is opened.



2. Select **Save** or **Save as**:
   - **Save:** Overwrites the existing experiment with a new version. This is useful when saving interim versions. The existing experiment name is displayed and cannot be edited. This option is only available if the experiment has been saved before and if you are the owner of the experiment. The previous version of the experiment will not be accessible in the **Database Explorer** anymore.
   - **Save as:** Saves the experiment as a new object (new signature) in the database. You can enter a new **Name** or keep/modify the existing one (if the experiment has been saved before). The previously saved experiment will also remain accessible in the **Database Explorer**.
3. Enter a **Comment**, if desired.
   Keywords describing the experiment configuration are created automatically.
4. Click OK to save the experiment.

Buttons and Elements

Element	Description
Experiment	Displays the name of the current experiment. An asterisk * in front of the experiment name indicates that the experiment has unsaved changes. Click ... to open the **Database Browser** and load an experiment.
Plate Type	Allows you to select the plate type for the experiment. Depending on the selected plate type and objective, there may be restrictions regarding the maximum focus height and the measurable plate area. Especially plate types with a high plate bottom can lead to restrictions. For details see section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89. **Notice** Do not forget to adjust the correction collar of the objective (if applicable) to the corrected plate bottom thickness of the selected plate. A message box will display the required value.

Element	Description
Objective 	Allows you to select the objective for the experiment. In the drop-down menu, all available objectives are listed. The currently installed objectives are displayed in regular text, not installed objectives are written in *italics*. Depending on the selected plate type and objective, there may be restrictions regarding the maximum focus height and the measurable plate area. Especially objectives with a short working distance can lead to restrictions. When planning your experiment, you can also select an objective which is not installed, e.g. to check for eventual plate restrictions. For details see section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89. **Notice** • Do not forget to adjust the correction collar of the objective (if applicable) to the corrected plate bottom thickness of the selected plate. A message box will display the required value. • After switching from a water immersion objective to an air objective while using the same plate, you should clean the plate bottom to remove any water droplets and prevent focusing errors.
Opt. Mode 	Select between confocal and non-confocal optical setup. **Notice** For details on the confocal mode see also: • **Operetta CLS Application Guide**, section “Introduction to Confocal Imaging”

Element	Description
Binning	With **binning 1** the full resolution of the camera is used (2160 x 2160 px): 1 camera pixel corresponds to 1 pixel in the final image. With **binning 2** the signal of 2 x 2 camera pixels is integrated into 1 pixel in the final image. This leads to a doubling of the signal to noise ratio and to smaller image files. However, the effective resolution of the cameras is reduced accordingly (1080 x 1080 px). In order to avoid non-square pixels only symmetrical binning (identical in horizontal and vertical direction) is supported.
Live Preview	If activated, the last processed image is displayed automatically during a test measurement. Live preview will be disabled if you have added a Digital Phase Contrast (DPC) channel. **Notice** Due to delays during image processing, the images may be displayed in a different order and not necessarily in chronological order.
Max Duration	Maximum duration of the current experiment (only measurement without online analysis). The maximum duration is estimated very conservatively, actual measurement times may be up to 50 % faster. Format: *hours:minutes* **Notice** The calculation of the maximum duration is not possible with office versions of Harmony (connection to the instrument is required).

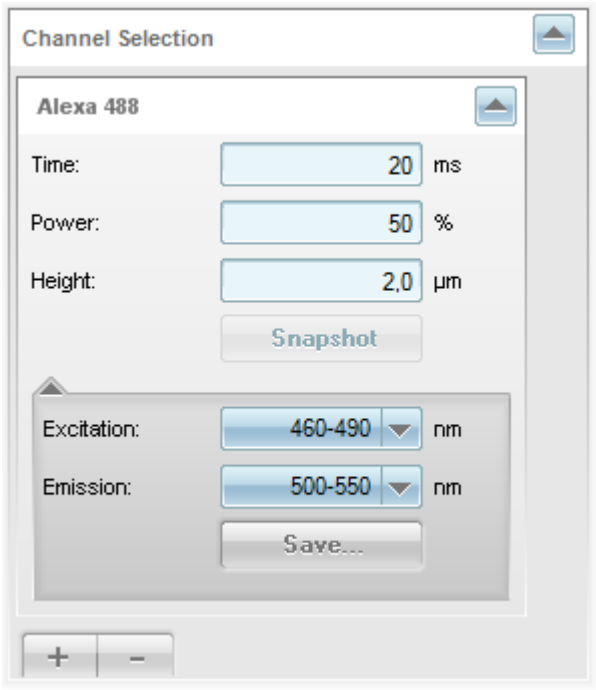
Element	Description
Temperature / CO_2	Only displayed with TCO upgrade, see also section 5.1.17.5 “TCO Settings”, page 246.  : **Start-up**, current value is still significantly lower than target value. : **Regulating**, target value nearly reached, but not stabilized yet. In this phase temperature is at maximum 4 °C below target value and CO_2 is at maximum one percentage point below target value. Transiently slightly higher values for temperature and CO_2 may also occur in this phase, please check the **Messages** window. : **Ready**, target value reached (target temperature ±1 °C; target concentration ±0.5 percentage points). **Off**: Not activated in **TCO Settings** or switched off by shutdown. **Standby:** Deactivated temporarily between two measurements (only for **Remote** mode, see also section 5.1.17.6 “Standby/Shutdown Settings”, page 249) : **Operating Error** (e.g. lid open), see also section 5.3.18 “TCO Issues”, page 478. The status of the temperature control is also visualized by the Operetta's progress LEDs. A blinking “T” indicates that the TCO unit is warming up or reheating. If the target temperature has been reached “T” will shine continuously. See also section 6.5 “Instrument Status”, page 510. **Notice** During the start-up sequence the status of temperature and carbon dioxide concentration will alternate several times between **Regulating** and **Ready**. The target values only have been stabilized if status **Ready** is displayed continuously.

Element	Description
New	Discards all changes in **Channel Selection**, **Layout Selection**, **Time Series** and **Online Jobs**. The global settings are reset to default values, only the selected plate type is maintained. The currently loaded measurement is removed from memory, i.e. it is not displayed anymore on **Setup** or any other screen. Any analysis sequence or evaluation will also be removed on the corresponding screens. The current assay layout is preserved if it was loaded separately and not attached to the measurement. If there had been a "Harmony mismatch", the GUI will be reset to be compatible with the current instrument description again. See also section 5.1.4 "Instrument and Data Type", page 33.
Test Stop	Starts a test measurement of all channels in the selected well. Well layout and stack are also taken into account, if selected. If you click Test it turns into a Stop button which allows you to cancel the test measurement. • In **Navigation – Test Images** you can select one of the resulting images. It will be displayed in the **Content Area**. • In **Image Control**, you can control which channels of the selected image are displayed by activating or deactivating their check boxes (see 5.1.12 "Image Control", page 195).
Save...	Allows to save the experiment (global settings, channels, layout, time series, and online jobs). As a default, this includes one field in the center of the well, stack is optional.

Context Menu

Element	Description
Plate Type Details	Opens the currently selected plate type in the **Plate Wizard** where you can check and/or modify the plate type definition. See also section 5.1.17.10 "Define Plate Type", page 309.
Help	Opens the help topic for this section.

5.1.7.2 Channel Selection



Using a sequence of channels you can define multiple independent measurements at the same position using different optical settings, e.g. color (wavelength), exposure time or focus height. The channels are processed sequentially in top-down order. An experiment has to include at least one channel.

You can load default channels for typical dyes and wavelengths from the database. Each default channel includes an excitation filter and an emission filter suitable for the respective dye/wavelength (visible in the nested pane of the channel). Exposure time, power and focus height have to be adapted for your application. The default channels cannot be overwritten, but you can save your modified channel with a different name.

Notice

- The default color of a channel is automatically derived from the filter wavelengths. If you change the excitation or emission wavelength, you have to save the channel before the new color is applied.
- The keyword **Channel Type** is automatically created for each channel. It divides the channels into the following categories: *Fluorescence*, *Brightfield*, or *Phase*.
- The keyword **Instrument Type** is automatically created for each channel. It can have the values *Operetta*, *Opera Phenix* or *Operetta CLS*. If you load a channel, only channels for the currently used instrument type will be displayed. See also section 5.1.4 “Instrument and Data Type”, page 33.
- Experiments, measurements and evaluations using a channel inherit these keywords so that you can use them when searching objects in the **Database Browser**.

How to add and configure a channel

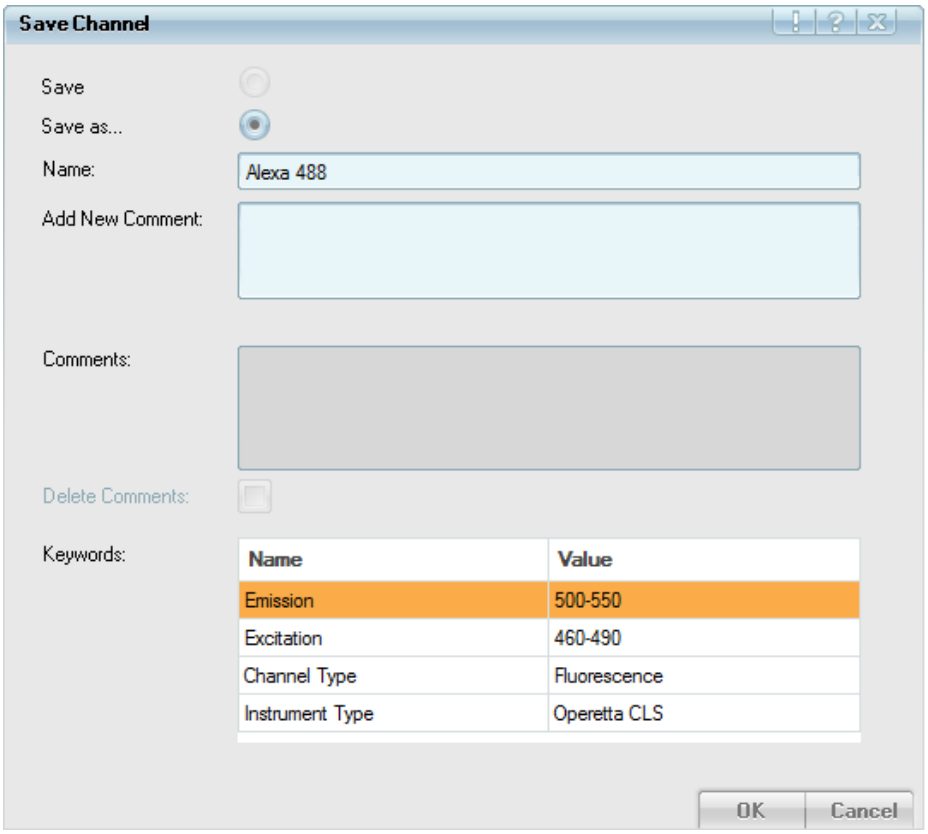
1. Click + . The **Database Browser** is opened.
   For details see section 5.1.16 “Database Browser”, page 220.
2. Select one of the provided default channels under the owner name “PKI Service” or one of your previously saved channels which corresponds to the desired dye/wavelength. If there is no channel yet matching your requirements, just select any channel and modify it in the next steps. Only channels suitable for your current instrument type and configuration will be displayed.
3. Enter the desired **Time**, **Power** and **Height**.

   Notice
   If the **Optical Mode** is **Confocal** (see section 5.1.7.1 “Global Control”, page 42), you can only enter exposure times which are a multiple of 20 ms (e.g. 20, 40, 60 ms etc.) because the pinhole disk needs 20 ms for one rotation. If you enter other values for **Time**, the value will automatically be corrected.
4. If you need to select a different excitation or emission filter, open the nested pane with the filter settings. Try different settings for **Excitation** and **Emission**.
5. Take snapshots to check the result:
   - In **Navigation – Define Layout** select a well (and a well layout field, if other than the central one is desired).
   - Click Snapshot to take an image with the current channel settings. The image is displayed in the **Content Area**.

   Notice
   During a snapshot or test measurement the selected combination of plate type and objective is checked for compatibility. Restrictions regarding the usable plate area (indicated by crossed wells and red well borders) and the focus height are displayed in **Navigation – Define Layout – Plate**. For details see section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89.
6. If you want to reuse the modified channel in future experiments, click Save... and save it to the database.

How to save a channel

1. Click Save... . The **Save Channel** dialog is opened.

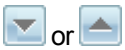


2. Select **Save** or **Save as**:
   - **Save:** Overwrites the existing channel with a new version. This is useful when saving interim versions. The existing channel name is displayed and cannot be edited. This option is only available if the channel has been saved before and if you are the owner of the channel. The previous version of the channel will not be accessible in the **Database Explorer** anymore.

     Notice
     The provided default channels cannot be overwritten, you can only choose **Save as**. Your channel may have the same name, but the keyword **Owner** will be different.

   - **Save as:** Saves the channel as a new object (new signature) in the database. You can enter a new **Name** or keep/modify the existing one (if the channel has been saved before). The previously saved channel will also remain accessible in the **Database Explorer**.
3. Enter a **Comment**, if desired.
   Keywords describing the channel configuration are created automatically.
4. Click OK to save the experiment.

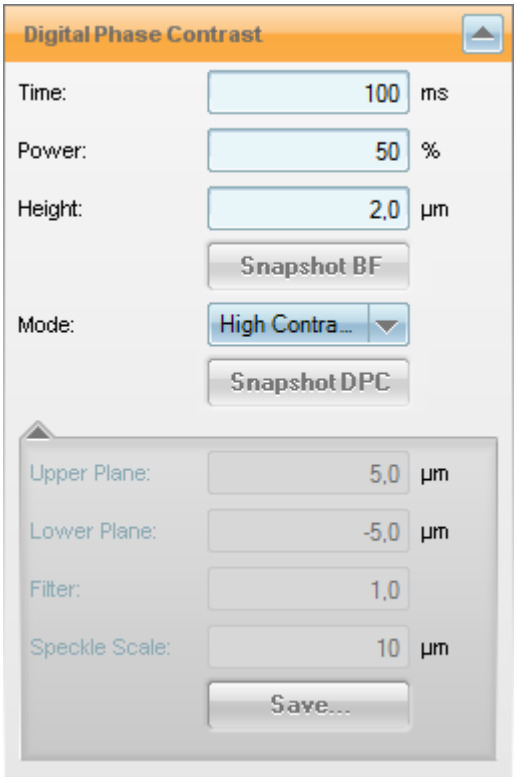
Buttons and Elements

Element	Description
	Click the corresponding disclosure button to open or close a channel or the channel sequence.
Time [ms]	Exposure time, determines image brightness (in combination with excitation/transmission power). **Notice** If the **Optical Mode** is **Confocal** (see section 5.1.7.1 “Global Control”, page 42), you can only enter exposure times which are a multiple of 20 ms (e.g. 20, 40, 60 ms etc.) because the pinhole disk needs 20 ms for one rotation. If you enter other values for **Time**, the value will automatically be corrected. For details on the confocal mode see also: • **Operetta CLS Application Guide**, section “Introduction to Confocal Imaging”
Power [%]	Individual power setting of the excitation LED or transmission LED used for this channel.
Height [µm]	Focus height above the plate bottom. **Notice** If you define a stack, the focus height selected in the channel will be ignored.
	Takes an image in the selected well using the global experiment settings and the settings of this channel. • Mark a single well in **Navigation – Define Layout – Plate**. • In **Navigation – Define Layout – Well** you can mark the desired field. Otherwise the field in the center of the well is measured. • Stacks are ignored. The snapshot is taken at the focus height defined in the channel.
	Click the arrow to open or close the nested pane with further settings.
	Select the desired excitation light source (excitation wavelength/filter or **Transmission** for using the transmission LED).
Emission	Selects the emission filter. The emission filter wheel holds eight filters which are exchangeable by the user (see section 5.1.17.3 “Change Filter”, page 242). The selected emission filter is automatically moved into position before the channel is measured.

Element	Description
Save...	Saves the channel to the database. The provided default channels cannot be overwritten. If you modify such a channel, your channel may have the same name, but it will have a different owner.
+	Opens the **Database Browser** to load an existing channel from the database and adds it to the **Channel Selection**. This can be one of the provided default channels or a channel that you have previously saved to the database. Only channels suitable for your current instrument type and configuration will be displayed.
–	Opens a popup menu where you can select the channel to be removed.

Digital Phase Contrast

Digital phase image reconstruction describes a computational approach for the generation of phase images based on brightfield images. By combining two defocused brightfield images at a certain distance below and above the focal plane, a digital phase image can be generated showing an excellent signal to noise ratio allowing the detection of cells without any additional fluorescent staining.



A Digital Phase Contrast (DPC) channel has fixed filter settings (which are identical to a brightfield channel). You can use the **Snapshot BF** button to take brightfield snapshots and optimize the exposure parameters.

The channel offers three modes: **High Contrast**, **High Detail** and a **Manual** mode where you can manually define the z-heights of the two brightfield images. In the automatic modes (High Contrast and High Detail) the planes are determined automatically. When clicking **Snapshot DPC** two main steps are executed:

- A brightfield stack is recorded automatically around the focal plane. This is done only once in the background (or after the channel's parameters have been modified).
- Subsequently, an algorithm picks the appropriate two planes (upper plane and lower plane) from this stack in order to compute the digital phase image.

For troubleshooting please see section "Digital Phase Contrast", page 490.

Notice

- Two brightfield images have to be recorded to compute one DPC image. Therefore, measure times for a DPC channel will be longer.
- You cannot acquire a **stack** of DPC images but you can combine the DPC channel with a stack measurement of a fluorescent channel. See also section "Stack", page 181.
- A **flatfield correction** method cannot be selected for DPC images. They are already flatness optimized. If further (fluorescent) channels are included in the measurement, a correction method can be selected. However, only the fluorescent channel(s) will be corrected. See also section 5.1.12.5 "Flatfield Correction", page 205.
- If restrictions of the measurable plate area (see section 5.1.7.7 "Restrictions of Measurable Plate Area", page 89) are displayed, it is not taken into account that the DPC channel needs to acquire a stack if the automatic modes are used (after clicking **Snapshot DPC**). Therefore you might get an error message even though the selected well is not in a restricted area. In this case, select a well closer to the center of the plate before using **Snapshot DPC** or reduce the focal height.
- The quality (contrast) of the DPC images generated with the 5x objective, depends on the sample carrier (due to optical phenomena). In general we have observed that the contrast is better in 384 well plates, less good in 96 well plates and worst with cells on slides.
- For the 1.25x objective, DPC is generally disabled for all modes. This lens is causing a strong field curvature which is not compatible with the DPC algorithm.

How to add and configure a Digital Phase Contrast channel

1. Click + . The **Database Browser** is opened.
   For details see section 5.1.16 "Database Browser", page 220.
2. Select the channel **Digital Phase Contrast** and click **OK**.
3. Insert a plate (using **Load/Eject Plate**).
4. In the **Navigation – Define Layout** pane, click on a well with a suitable sample.
5. In the DPC channel, enter the desired **Time** and **Height**.

6. Click **Snapshot BF** to take brightfield snapshots and find the height of the sharpest plane (usually where hardly any objects are visible).
7. Select a **Mode**. First try **High Contrast** or **High Detail**, because in these modes, all parameters are determined automatically. In **Manual** mode, you can define all parameters like upper and lower plane manually.
8. Click **Snapshot DPC**.
   - **Step 1** (only once or after modifying parameters, only performed if using an automatic mode): A stack of brightfield images is acquired in the background (see progress LEDs at the instrument), and z-heights of upper and lower plane are determined.
   - **Step 2:** Two brightfield images are recorded (upper and lower plane) and the computed DPC image is displayed.

> **Notice**
> During a snapshot or test measurement the selected combination of plate type and objective is checked for compatibility. Restrictions regarding the usable plate area (indicated by crossed wells and red well borders) and the focus height are displayed in **Navigation – Define Layout – Plate**. For details see section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89.

6. If you want to reuse the modified channel in future experiments, click Save... and save it to the database.

Buttons and Elements

Element	Description
 or 	Click the corresponding disclosure button to open or close a channel or the channel sequence.
Time [ms]	Exposure time, determines image brightness (in combination with transmission power). Adjust to approx. 10,000 counts.
Height [µm]	Focus height above the plate bottom. Enter the height of the sharpest plane (hardly any objects visible).
Power [%]	Individual power setting of the transmission light source used for this channel.
Snapshot BF	Takes a brightfield image in the selected well/field using the global experiment settings and the settings of this channel. Use this function to optimize **Time** and **Height** before using **Snapshot DPC**.

Element	Description
Mode	Algorithm selecting the two z-planes for computing the phase image: • **High Contrast:** Automatic selection of upper and lower plane, optimized for cell segmentation. • **High Detail:** Automatic selection of upper and lower plane, optimized for analyzing structural details. • **Manual:** Allows the user to adjust the upper and lower plane as well as two more parameters (**Filter** and **Speckle Scale**) manually. It is used in these situations: ◦ The automatically generated DPC image quality is insufficient e.g. because artifacts disturbed the algorithm. ◦ When the automatic digital phase reconstruction fails due to a certain sample characteristic.

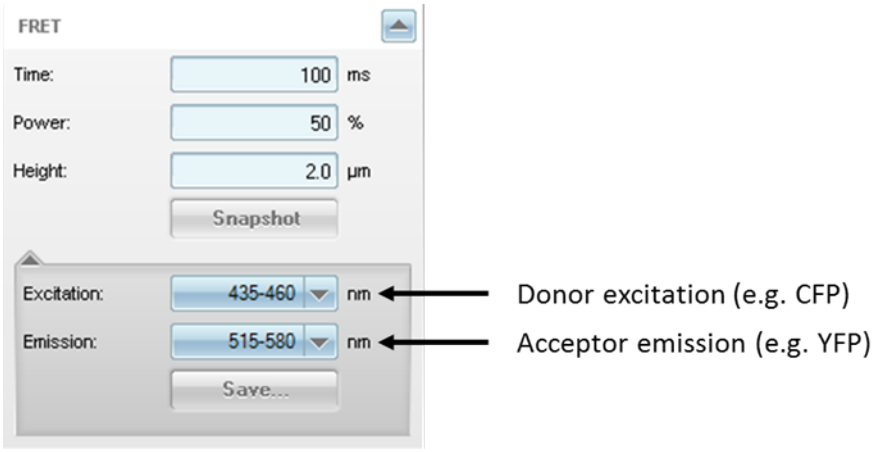
Element	Description
Snapshot DPC	The **Snapshot DPC** button combines two functions: • **Step 1** (only performed if using an automatic mode): Automatic acquisition of a brightfield stack (in the background) and applying an algorithm for selecting two mode-dependent z-heights (upper/lower plane). This procedure can take a while. The progress is indicated by the progress LEDs on the instrument. • **Step 2:** Recording the two brightfield images for upper and lower plane and computing the phase image. **Step 1** is only performed once in the automatic modes unless you modify one of the following variables: • Height • Mode • Objective The next time you click **Snapshot DPC** (e.g. after changing the well or field), only **step 2** will be executed which is much faster. The computed phase image is displayed in the **Content Area**. If you want to reset the values for the now computed upper and lower planes, simply change the **Mode** or **Height** back and forth. This way, pressing **Snapshot DPC** initiates a complete re-calculation of the DCP image. **Notice** • Before you can start a (test) measurement, **Snapshot DPC** must have been executed at least once (for each DPC channel in the channel sequence). In addition, you have to repeat this procedure after saving a DPC channel under a different name. • Please carefully select the best possible well/field for the first **Snapshot DPC** in order to avoid imprecise or faulty plane calculations.
	Click the arrow to open or close the nested pane with further settings.
Upper Plane	Upper and lower plane specify the z-height of the two brightfield images which are used to compute the DPC image. • In the automatic modes (**High Contrast**, **High Detail**), these fields are disabled and the automatically selected z-heights will be displayed. • In the **Manual** mode, you can define upper and lower plane manually.
Lower Plane	

Element	Description
Filter	This parameter is used to remove large scale background noise. The default value 1.0 corresponds to a typical background threshold that we typically see at the given magnification. • If there is too much residual background, try higher values (1.5, 2, 3, 4) • If the cells look dark inside, too much has been cut: Lower the threshold (0.75, 0.5, 0.25).
Speckle Scale	Speckle scale is the typical size of dust particles (in µm). Typical values in lab environments have been observed to be 15-20 µm. Dust particles are best visible in the brightfield channel as dark objects and the size can be directly measured on the image. In phase images the unremoved dust looks like a bright burst. Well compensated areas look like the signal has been slightly suppressed. Making the parameter too small or too high both will fail to remove speckles. There is a range of values that works well enough and small values usually work better than higher ones. Start from smaller values and increase until dust gets cleaned. Speckle Size = 0 is a special value that switches the correction off.
	Saves the channel to the database. The provided default channels cannot be overwritten. If you modify such a channel, your channel may have the same name, but it will have a different owner. **Notice** • If you save a DPC channel and load it again when setting up a new experiment, you will be asked to run **Snapshot DPC** again before the measurement can be started. This happens because the saved DPC channel does not include the required experiment information (e.g. used objective). You can avoid this by saving the entire experiment, not only the channel. If you then load the experiment, it can be started directly.
	Opens the **Database Browser** to load an existing channel from the database and adds it to the **Channel Selection**. This can be one of the provided default channels or a channel that you have previously saved to the database. If you add a DPC channel, it will always be inserted at the top of the channel sequence so that it is processed first during the measurement.
	Opens a popup menu where you can select the channel to be removed.

FRET

FRET (Förster Resonance Energy Transfer) is a mechanism describing the energy transfer between two chromophores (**donor** and **acceptor**). It can be used to determine if two fluorophores are within a certain distance of each other.

All images have to be acquired in sequential exposures. Depending on the information to be acquired, up to three channels have to be defined (for donor, acceptor and FRET). For most fluorophores predefined channels can be selected from the Harmony database to acquire the donor and the acceptor image. To acquire the FRET image, a custom channel has to be defined that uses the excitation filter for the donor and the emission filter for the acceptor fluorophore (see below).



5.1.7.3 Layout Selection

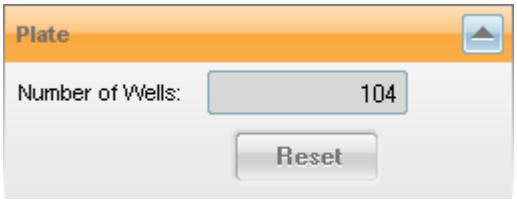
In the **Layout Selection** pane you can define physical points of the plate which are to be measured during the experiment, oriented in X-Y-axis and Z-axis.

- **Plate:** Defines which **wells** are included.
- **Well:** Defines which **area of one well** (also called “field") is included.
- **Stack:** Defines which **planes** of one well are included (Z-stack).

Notice
The measuring path (i.e. the order in which the selected wells and fields are measured) is optimized by the system and cannot be modified.

Layout Selection is closely connected to the **Navigation – Define Layout** pane. When creating plate layouts, well layouts and stacks you will have to switch between these two panes frequently. For details see section 5.1.7.6 “Navigation (Setup)”, page 81.

Plate

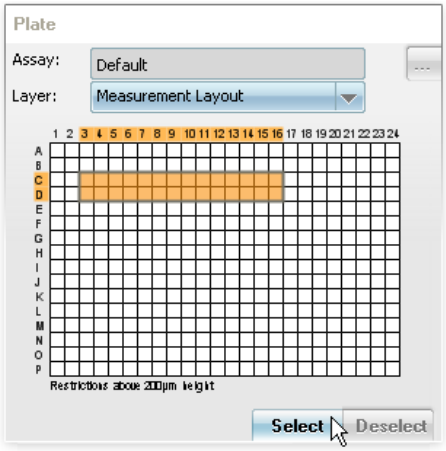


The layout determines which wells on the plate are measured during the experiment. If you define no well layout or stack, the instrument will take one image field per channel. The image field will be in the center of the well with the height defined in the channel.

The wells to be measured can be selected in the **Navigation – Define Layout – Plate** pane. For details see section 5.1.7.6 “Navigation (Setup)”, page 81.

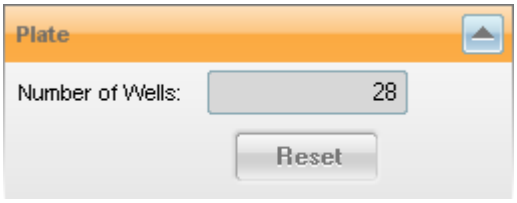
How to create a layout

1. On the **Setup** tab open the **Layout Selection** pane.
2. Open the **Plate** pane.
3. In **Navigation – Define Layout – Plate** mark the desired well(s). If you press and hold the **Ctrl** key, you can mark multiple wells or areas in one step.



Optionally: If you have created and loaded an assay layout, you can also select the desired **Layer** as background. This helps to select the desired wells.

4. Click **Select**.
   The selected orange wells become gray and the number of selected wells is displayed in the **Plate** pane.



5. To deselect wells, mark the desired wells and click **Deselect**. The marked wells are deselected and become white.
6. If necessary, **Select**/**Deselect** further wells to achieve the desired layout.

Buttons and Elements

Element	Description
 or	Click the disclosure button to open or close the **Plate** pane.
Number of Wells	Displays the number of wells which have been selected in **Navigation – Define Layout – Plate**. **Note:** It is not sufficient to just mark the wells in **Navigation – Define Layout – Plate**. You also need to click **Select** to accept the selection. The selected wells become gray afterwards.
	Deselects all wells.

Well

The well layout determines number and position (X/Y) of image fields inside the well. There are two ways of defining a well layout:

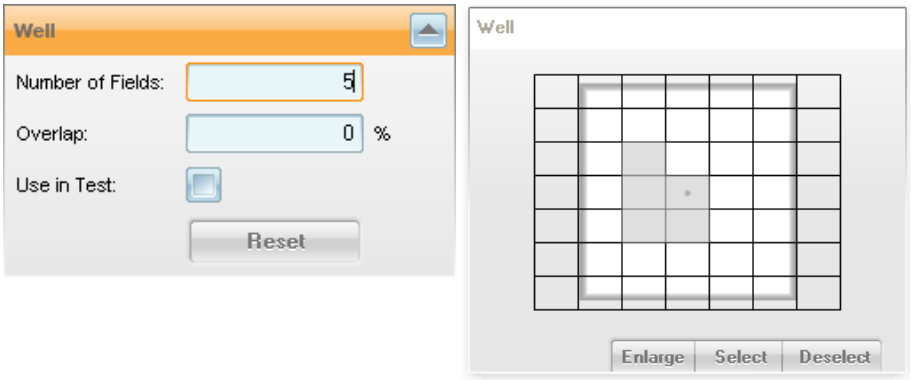
- **Select number of fields:** The number of fields entered is automatically selected in or around the center of the well. It is possible to modify this selection.
- **Select/deselect individual fields manually:** You can also directly mark and select/deselect the desired fields in **Navigation – Define Layout – Well**.

Notice

If you want to use overlapping fields, please define the overlap **before** you define your well layout. The well layout is reset as soon as you enter an overlap.

How to create a well layout by entering the number of fields

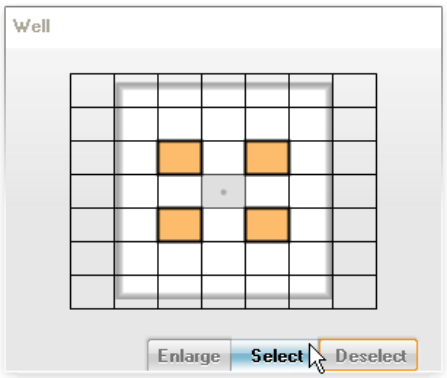
1. On the **Setup** tab open the **Layout Selection** pane.
2. Open the **Well** pane.
3. In text box **Number of fields** enter the desired number of fields and press **Enter**. In **Navigation – Define Layout – Well** the entered number of wells has been selected automatically. The field positions are in or around the center of the well.



How to create a well layout by manually selecting fields

1. On the **Setup** tab open the **Layout Selection** pane.
2. Open the **Well** pane.

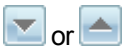
3. In **Navigation – Define Layout – Well** mark the desired field. Press and hold the **Ctrl** key to mark multiple fields (orange selection).



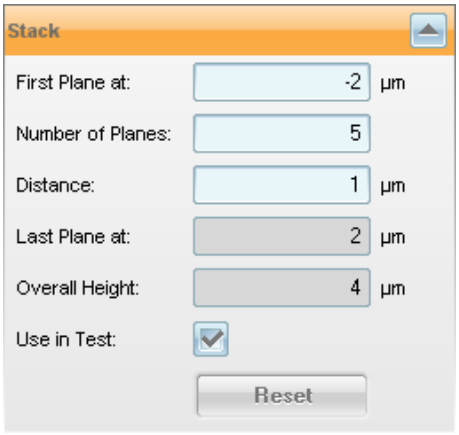
4. Optional: Click Enlarge to view an enlarged version of the well layout in the **Content Area**. This can be helpful if there are many small fields (due to large well dimension and/or small field of view).
5. Click **Select** to accept the selected fields. The orange fields become gray, and the number of selected fields is displayed in **Layout Selection – Well**.

6. To deselect fields, mark the desired fields and click **Deselect**. The marked wells are deselected and become white.
7. If necessary, **Select**/**Deselect** further fields to achieve the desired layout.

Buttons and Elements

Element	Description
	Click the disclosure button to open or close the **Well** pane.
Number of Fields	Displays the number of wells which have been selected in **Navigation – Define Layout – Plate**. **Note:** It is not sufficient to just mark the wells in **Navigation – Define Layout – Plate**. You also need to click **Select** to accept the selection. The selected wells become gray afterwards.
Overlap [%]	Allows you to define an overlap of the image fields (0-45 %). The size of each field remains the same, but they will be displayed as smaller fields because they overlap each other. **Notice** Please define the overlap **before** you define your well layout. The well layout is reset as soon as you enter an overlap.
Use in Test	If activated, the well layout will also be used during a test measurement (see section 5.1.7.1 “Global Control”, page 42). Otherwise the test measurement will be done with just one field in the center of the well.
	Deselects all fields except the one which is in the center of the well.

Stack

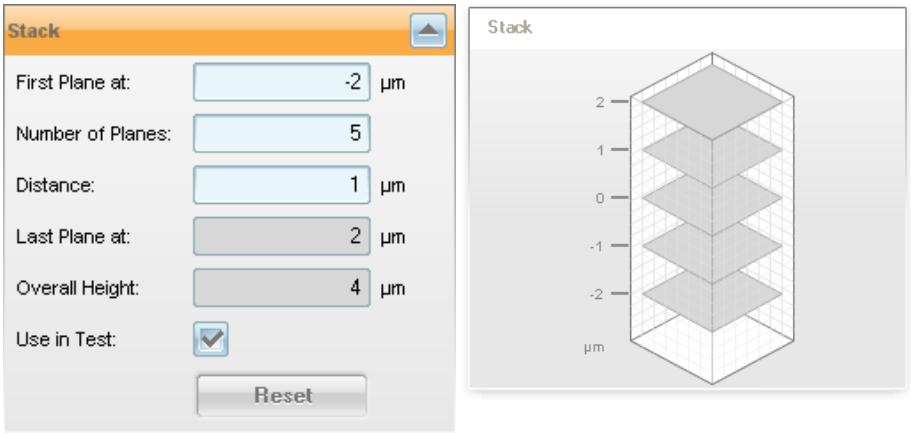


The stack defines which planes of the well are measured (Z-stack). It is defined (and measured) 'bottom-up', i.e. you define the bottom plane and enter distance and number of planes. The resulting stack is visualized in **Navigation – Define Layout – Stack**.

Notice
If you define a stack, the height defined in each individual channel will be ignored.

How to define a stack

1. On the **Setup** tab open the **Layout Selection** pane.
2. Open the **Stack** pane.
3. In **First Plane at** enter the height of the bottom plane of your stack. Press **Enter**.



4. In **Number of Planes** enter the total number of planes in your stack. Press **Enter**.
5. In **Distance** enter the vertical distance between the stack planes. Press **Enter**.
6. The resulting stack is displayed in **Navigation – Define Layout – Stack**.

Buttons and Elements

Element	Description
 or	Click the disclosure button to open or close the **Stack** pane.
First Plane at [µm]	Position (Z-axis) of the bottom plane of your stack.
Number of Planes	Total number of stack planes.
Distance [µm]	Vertical distance between the stack planes.
Last Plane at [µm]	Displays the position (Z-axis) of the top plane of your stack.
Overall Height [µm]	Displays the distance from bottom plane to top plane.
Use in Test	If activated, the stack will also be used during a test measurement (see section 5.1.7.1 “Global Control”, page 42). Otherwise the test measurement will be done with the focus height defined in the channel(s).
	Deletes the stack.

5.1.7.4 Time Series

Working Principle

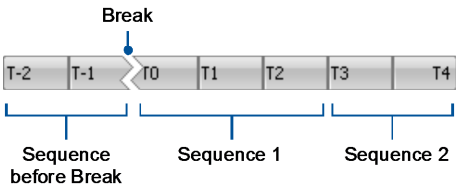
Time series measurements in Harmony are multiple scheduled measurements of the same plate at different time points. At each time point, the whole plate is measured as defined in sections **Channel Selection** and **Layout Selection**. Therefore, the shortest interval between two time points is the time which is required to measure one time point. The interval can be “fixed”, or the time points can be processed “as fast as possible”.

Notice

- Before defining a time series you have to define all other elements of your experiment (channels, wells, fields, planes), because the time required to measure one time point has to be determined.
- The **Shortest Interval** and the resulting **Max. Duration** for the whole experiment can only be calculated and displayed directly on the Harmony PC (connected to the instrument). Therefore, it is not recommended to plan and create a time series experiment using an office installation of Harmony.
- If the experiment is intended for automated measurements controlled by an external scheduler (**Remote** mode), the time series may not include a break.

A time series measurement can consist of one or two sequences (see example below). Each sequence can have various time points and a different interval between the time points (fixed or as fast as possible). You can pre-define one **break** before sequence 1 for manual user interaction (e.g. adding liquids). In this case, an additional **Sequence before Break** will be inserted at the beginning of the time series. It can be configured in the same way as the other sequence(s).

The first time point of sequence 1 is defined as **T0** (0:00:00). All other time points are defined in relation to **T0**. If the time series includes a break, the time points before the break (and thereby before **T0**) get negative time stamps.



- A visualization of the **scheduled** time series is displayed in the **Navigation – Define Layout** pane. See also section “Timepoints”, page 88.
- The time stamps of the **actually measured** time points are displayed in the **Navigation – Measurement** pane during and after the measurement (tooltip). See also section “Timepoints”, page 182.

If you run a time series experiment, you will get one large measurement including the various time points. It is also possible to add separate measurements to an existing time series or to combine multiple separate measurements subsequently, so that a regular time series measurement is created. For details and requirements please refer to section “Combine Measurements”, page 274.

Example

Sequence	Timepoint	Scheduled Time
Sequence before Break Interval = As fast as possible (shortest interval e.g. 0:01:30)	T-2	-0:03:00
	T-1	-0:01:30
Sequence 1 Interval = Fixed interval: 6 min.	**T0**	**0:00:00**
	T1	0:06:00
	T2	0:12:00
Sequence 2 Interval = Fixed interval: 30 min.	T3	0:42:00
	T4	1:12:00

How to define a time series measurement

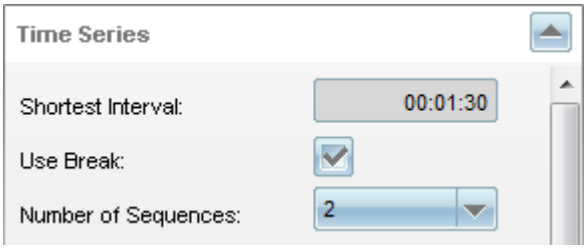
This step-by-step instruction demonstrates how to define the time series shown in the previous example.

Define measurement of one time point

1. Open the **Setup** tab.
2. Define the global experiment settings (plate type, objective, etc.).
3. Add and configure the desired channels in **Channel Selection**.
4. Select the wells, fields, planes to be measured in **Layout Selection**.

The experiment definition for a single time point is now complete. The maximum duration is calculated and displayed in the **Global Control** section (**Max. Duration**) and in the **Time Series** pane (**Shortest Interval**).

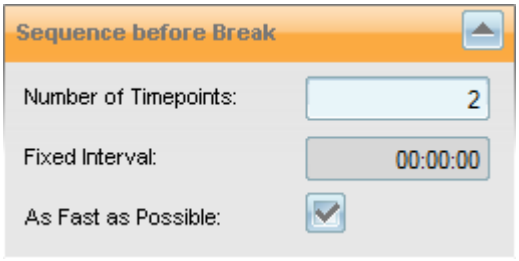
Define structure of time series



1. Open the **Time Series** pane.
   Per default, there is only one sequence without break.
2. Optionally: Add one break (before sequence 1).
   - Enable the **Use Break** check box.
     A **Sequence before Break** is inserted at the beginning of the time series. The break will be before sequence 1.

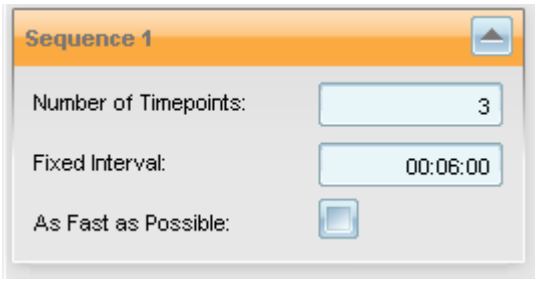
3. Select the number of sequences.
   If you select “2”, a second sequence is added at the end of the time series.

Define “Sequence before Break” (only if you activated “Use Break")



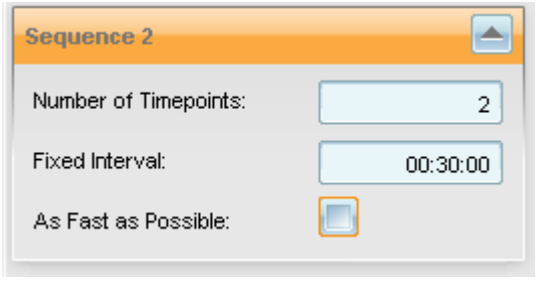
1. Open the **Sequence before Break** pane.
2. Enter “2” for **Number of Timepoints**.
3. Enable the check box **As Fast as Possible**.
   The field **Fixed Interval** is disabled. The next time point will be measured as soon as the previous time point has been finished.

Define “Sequence 1"



1. Enter “3” for **Number of Timepoints**.
2. Disable the check box **As Fast as Possible**.
   The field **Fixed Interval** is enabled.
3. Enter a **Fixed Interval** of 6 min. (0:06:00).

Define “Sequence 2"



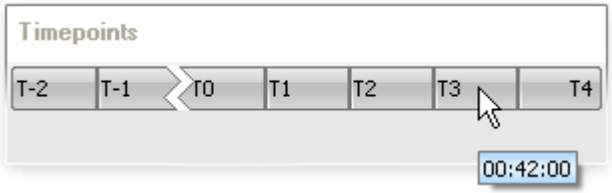
Only if you defined a sequence 2:

1. Enter “2” for **Number of Timepoints**.
2. Disable the check box **As Fast as Possible**.

The field **Fixed Interval** is enabled.

3. Enter a **Fixed Interval** of 30 min. (0:30:00).

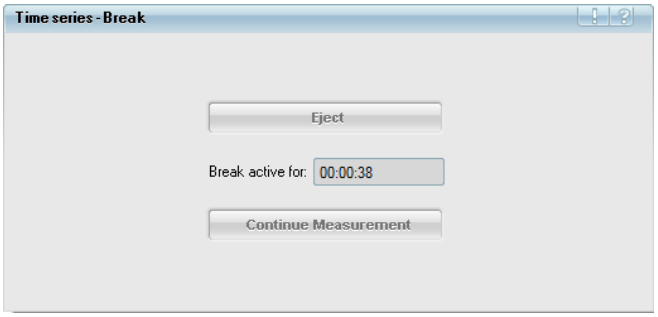
Check Timepoints



The time series is visualized in the **Navigation – Define Layout** pane (see also section "Timepoints", page 88). If you move the mouse cursor over the time points, the scheduled measurement time (in relation to **T0**) is displayed by a tooltip.

How to run a time series measurement including a break

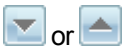
1. Define an experiment including time series and break on the **Setup** tab.
2. Open the **Run Experiment** tab and start the experiment.
   The time point(s) of the **Sequence before Break** are processed.
3. As soon as the **Sequence before Break** has been completed, the following **Break** dialog is displayed:



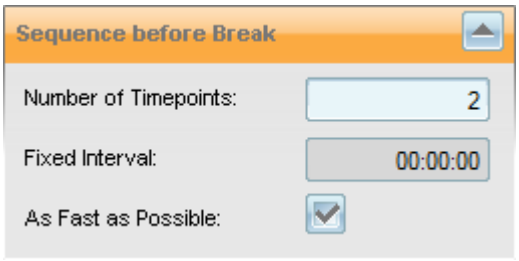
   The Operetta's status light is blinking green and red to indicate that the user's interaction is required.

4. Click **Eject**.
   The plate is ejected at the transfer position. The **Eject** button turns into a **Load** button.
5. Execute the desired assay steps (e.g. adding liquids).
6. Click **Load**.
   The plate is loaded into the instrument.
7. Click **Continue Measurement**.
   The dialog is closed, and the measurement of the remaining time points is started immediately.

Buttons and Elements

Element	Description
	Click the corresponding disclosure button to open or close the **Time Series** pane.
Shortest Interval	Displays the shortest possible interval between two time points, i.e. it is the estimated duration for measuring one time point (including all channels, wells, fields, planes). **Notice** The calculation of **Shortest Interval** is just an estimate. It is possible to enter a fixed interval which is shorter than the calculated Shortest Interval. In this case a blue outline is displayed around the text box. However, it may happen, that the selected interval cannot be realized. To achieve a shorter interval time please reduce the measurement time of one time point by reducing channel number and/or exposure time and/or well number and/or field number and/or plane number.
Use Break	Allows you to insert a break for manual user interaction (e.g. adding liquids). Only one break is possible, and it is automatically inserted before **Sequence 1**. If the check box is enabled, the block **Sequence before Break** appears where you can configure an additional sequence before the break, e.g. to measure a baseline.
Number of Sequences	Determines the number of sequences (sequence before break not included). Each sequence can have a different interval and a different number of time points. You can choose between **1** or **2** sequences. If you select **2**, the **Sequence 2** block appears.
Sequence panes	Please refer to the following sections.
Use in Test	If selected, the time series will also be processed if you start a test measurement in one well (including break and the defined intervals). **Notice** • If you have selected the option **As Fast As Possible** as interval for a sequence, the actual interval during a test measurement may be much shorter than the displayed **Shortest Interval**. During a test measurement, only one selected well of the defined layout is processed. Therefore, an internally calculated value is used instead of the **Shortest Interval** (calculated for the whole layout). • If you have selected a **Fixed Interval** for a sequence, this interval will also be used during a test measurement.
Reset	Resets all parameters to default (one sequence, no break).

Sequence before Break



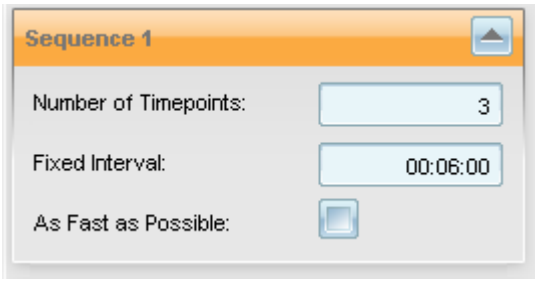
This section is only displayed if you activated **Use Break** in the **Time Series** pane. It can be used to define time points before the break, e.g. for measuring a baseline.

Buttons and Elements

Element	Description
or	Click the corresponding disclosure button to open or close the **Sequence before Break** pane.
Number of Timepoints	Determines the number of time points in the sequence. Possible range: 0-99. If you select 0, there will be no time point before the break and the experiment will start with a break. This can be useful if no baseline is required but you want to document the break in the measurement data.

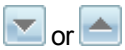
Element	Description
Fixed Interval and **As Fast as Possible**	Allows you to select the interval between the time points of a sequence: • **As fast as possible:** Starts the measurement of the next time point as soon as the previous time point has been finished completely. The interval will approximately correspond to the calculated **Shortest Interval**, but variations are possible (e.g. due to focus errors etc.). The actual time stamp of the measured image can be viewed after the measurement as a tooltip in **Navigation – Measurement – Timepoints**. To measure the time points of a sequence as fast as possible: ◦ Enable the check box **As Fast as Possible**. **Fixed Interval** will be disabled. • **Fixed Interval:** Allows you to define a fixed interval between the time points within a sequence. The **Fixed Interval** should be at least as long as the calculated **Shortest Interval**. You can enter shorter times, but probably the scheduled time will not be kept (a warning message will be displayed if you start the measurement). The next time point will then be measured as fast as possible. In the case of a delay (e.g. due to focus errors), the software will try to make up for it, so that the next time point can be started at the scheduled time. However, such a delay may also affect other subsequently measured wells and time points. The actual time stamp of the measured image can be viewed after the measurement as a tooltip in **Navigation – Measurement – Timepoints**. If a fixed interval could not be met, this will also be logged as a comment in the measurement (see also section 5.1.16 “Database Browser”, page 220). To measure the time points of a sequence with a fixed interval: ◦ Disable the check box **As Fast as Possible**. **Fixed Interval** will be enabled. ◦ Enter the desired **Fixed Interval** (hh:mm:ss).

Sequence 1 and 2

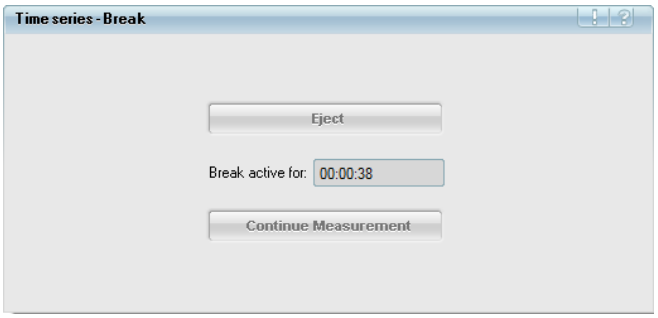


This section allows you to define the number of time points within the sequence and the interval between the time points. The parameters of section **Sequence 1** and **Sequence 2** are the same.

Buttons and Elements

Element	Description
	Click the corresponding disclosure button to open or close the **Sequence 1** or **2** pane.
Number of Timepoints	Determines the number of time points in the sequence. Possible range: 1-500.
Fixed Interval and **As Fast as Possible**	Allows you to select the interval between the time points of a sequence: • **As fast as possible:** Starts the measurement of the next time point as soon as the previous time point has been finished completely. The interval will approximately correspond to the calculated **Shortest Interval**, but variations are possible (e.g. due to focus errors etc.). The actual time stamp of the measured image can be viewed after the measurement as a tooltip in **Navigation – Measurement – Timepoints**. To measure the time points of a sequence as fast as possible: ◦ Enable the check box **As Fast as Possible**. **Fixed Interval** will be disabled. • **Fixed Interval:** Allows you to define a fixed interval between the time points within a sequence. The **Fixed Interval** should be at least as long as the calculated **Shortest Interval**. You can enter shorter times, but probably the scheduled time will not be kept (a warning message will be displayed if you start the measurement). The next time point will then be measured as fast as possible. In the case of a delay (e.g. due to focus errors), the software will try to make up for it, so that the next time point can be started at the scheduled time. However, such a delay may also affect other subsequently measured wells and time points. The actual time stamp of the measured image can be viewed after the measurement as a tooltip in **Navigation – Measurement – Timepoints**. If a fixed interval could not be met, this will also be logged as a comment in the measurement (see also section 5.1.16 “Database Browser”, page 220). To measure the time points of a sequence with a fixed interval: ◦ Disable the check box **As Fast as Possible**. **Fixed Interval** will be enabled. ◦ Enter the desired **Fixed Interval** (hh:mm:ss).

Break Dialog



If you run a time series experiment which includes a break, this dialog will pop up at the beginning of the break. It will be displayed until you continue the measurement.

Buttons and Elements

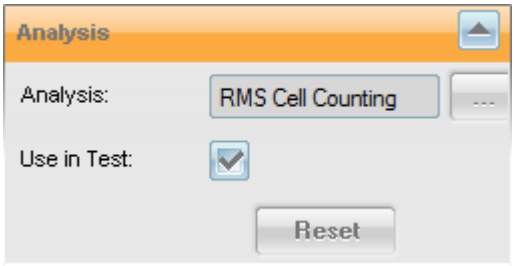
Element	Description
Eject/Load	Allows you to eject the plate, execute the desired assay steps (e.g. adding liquids) and load it back into the instrument. The displayed button depends on the instrument's status (plate detected or not).
Break active for	Displays the time elapsed since the beginning of the break.
Continue Measurement	Click this button to continue the measurement. The first time point of sequence 1 will be processed immediately.

5.1.7.5 Online Jobs

Notice

Online **Analysis** and online **Columbus Transfer** cannot be combined in one experiment.

Analysis



In this section you can select an existing analysis sequence to be used for an online analysis of the current measurement (or test measurement). Each measured well will automatically be analyzed using the specified analysis sequence.

If you do not define an online analysis, you can manually create or apply an analysis sequence on the **Image Analysis** or **Evaluation** tab.

Notice

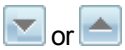
- **Cell tracking** cannot be combined with online analysis. Please make sure that the selected analysis sequence does not include the **Track Objects** building block.
- Flatfield correction cannot be combined with online analysis. Only analysis sequences with **Flatfield Correction: None** (**Input Image** building block) are accepted. See also section 5.1.12.5 “Flatfield Correction”, page 205.
- The online analysis of a measurement with only **one single field** will be slower than a subsequent evaluation of the same measurement on the **Evaluation** tab. For technical reasons the calculation cannot be done in parallel if there is only one field. In such a case it is better to run the measurement without online analysis and start a manual evaluation after the measurement has been finished.

How to perform a measurement with online analysis

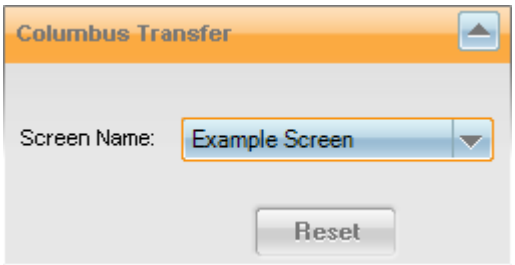
1. Define your experiment on the **Setup** tab.
2. Open the **Online Jobs** pane.
3. Make sure that no online transfer to Columbus has been defined in the **Columbus Transfer** pane. If necessary, click Reset to disable the Columbus Transfer.
4. Open the **Analysis** pane.

5. Click ... (next to **Analysis** text box) to open the **Database Browser** and select an analysis sequence. Click **OK**.
   The name of the selected analysis sequence is displayed in the **Analysis** box.
6. Optionally: In the **Global Control** section, click Save... to save the experiment.
7. Start the measurement on the **Run Experiment** tab.
   Each measured well will automatically be analyzed using the specified analysis sequence.

Buttons and Elements

Element	Description
	Click the disclosure button to open or close the **Online Jobs** pane or the **Analysis** sub-pane.
Analysis	Displays the name of the specified analysis sequence for online analysis. Click  to open the **Database Browser** and load an existing analysis sequence. • If you load an analysis sequence, your plate will automatically be analyzed using this analysis sequence as soon as you start the measurement on the **Run Experiment** tab (online analysis). The analysis sequence will be part of the experiment when you save it. • If you leave this field empty, the experiment will not be analyzed automatically, and you have to manually create or apply an analysis sequence on the **Image Analysis** or **Evaluation** tab after the plate has been measured. No analysis will be saved with the experiment.
Use in Test	If activated, the online analysis will also be performed after a test measurement (see section 5.1.7.1 “Global Control”, page 42). Otherwise the test measurement will not be analyzed automatically.
	Removes a selected analysis sequence; online analysis is disabled.

Columbus Transfer



If you define an online Columbus Transfer, the measurement will be transferred automatically to a Columbus system within the network as soon as the measurement has been completed.

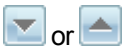
The URL of the Columbus server and a Columbus user account must have been configured before in the **User Accounts** dialog. See also sections “Manage Columbus Account”, page 305 and 5.3.20 “Columbus Network Integration”, page 480.

Instead of defining an online transfer to Columbus, measurements can also be transferred later on using the **Columbus Transfer** function (see section “Columbus Transfer”, page 271). If the Columbus server is not accessible via network, you have to use the **Export Data** function (see section “Export Data”, page 262).

How to perform a measurement with online Columbus Transfer

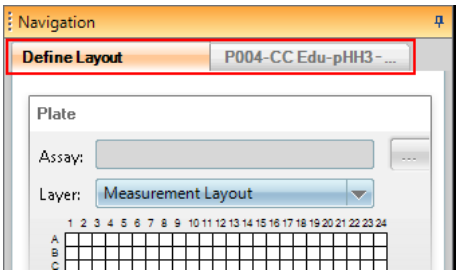
1. Define your experiment on the **Setup** tab.
2. Open the **Online Jobs** pane.
3. Make sure that no online analysis has been defined in the **Analysis** pane. If necessary, click Reset to disable the online analysis.
4. Open the **Columbus Transfer** pane.
5. Select a **Screen Name**. The measurement will be added to this screen in Columbus.
6. Optionally: In the **Global Control** section, click Save... to save the experiment.
7. Start the measurement on the **Run Experiment** tab.
   The measurement data will automatically be transferred to Columbus using a background job.
8. If you want check the status of the transfer, open **Settings – Data Management – Job Status**. See also section "Job Status", page 300.

Buttons and Elements

Element	Description
	Click the disclosure button to open or close the **Online Jobs** pane or the **Columbus Transfer** sub-pane.
Screen Name	The measurement will be added to the selected screen in Columbus. **Notice** The list of available **Screen Names** depends on the selected Columbus account (configured in **Settings – User Accounts**), and these settings can be different for each Harmony user. See also section "Manage Columbus Account", page 305. If you load an existing experiment, the **Screen Name** defined in the experiment must also be available for the currently set Columbus user account. Otherwise you have to select a different screen name or configure the same Columbus account which was originally used by the user who created the experiment.
Reset	Removes a selected online transfer to Columbus; Columbus Transfer is disabled.

5.1.7.6 Navigation (Setup)

This chapter describes the **Navigation** pane on the **Setup** tab, especially the **Define Layout** sub-pane. For a general introduction to the **Navigation** pane and its function on other tabs see section 5.1.11 “Navigation”, page 169.



Tabs to switch between Define Layout and Measurement pane

The **Navigation** pane on the **Setup** tab includes the following sub-panes which can be opened by clicking on the corresponding tab:

- **Define Layout**: This pane only appears on the **Setup** tab. It is required for selecting a well for snapshot or test measurement and to create plate layouts, well layouts, time series and stacks. Via the context menu of the **Plate** pane you can open the **Assay Layout Editor** (see also section 5.1.7.8 “Assay Layout Editor”, page 94).
- **Test Image(s)** or **Plate Measurement**: This pane is used to browse measurement results (i.e. images).The button for selecting this pane is named according to the origin of the measurement:
  - Test images resulting from a temporary test measurement or snapshot in one single well are labeled with **Test Images**.
  - Images of a regular plate measurement (started on **Run Experiment**) are labeled according to the following pattern: **[Plate Name] – Measurement[#]**.

  For a detailed description of this pane please see section 5.1.11 “Navigation”, page 169.

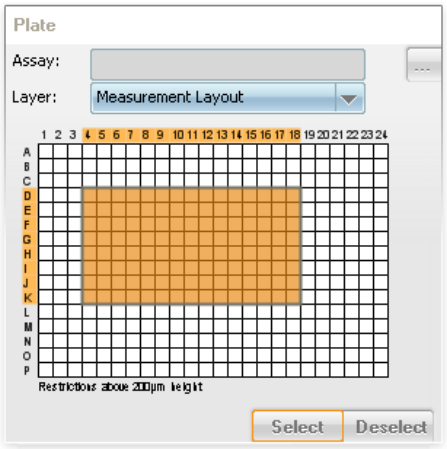
Define Layout

The **Navigation – Define Layout** pane is closely connected to the **Layout Selection** and the **Time Series** pane.

Layout Selection (Function)	Navigation – Define Layout (Function)
Plate: • View number of selected wells	**Plate:** • Select single well for snapshot or test measurement • Select one or multiple wells for measurement layout
Well: • View number of selected fields • Enter number of fields to be measured	**Well:** • Select single field for snapshot • Select one or multiple fields for well layout
Stack: • Define stack	**Stack:** • View stack

Time Series (Function)	Navigation – Define Layout (Function)
Time Series: • Define time series	**Timepoints:** • View time series and scheduled time points

Plate



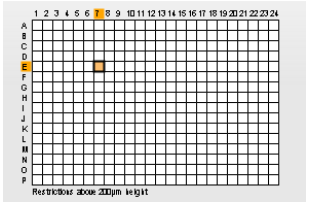
The **Plate** pane is either used to select a single well for a snapshot/test measurement or you can select wells on the plate for a layout to be used during an automated measurement. For step-by-step instructions see section “Plate”, page 62 (Layout Selection).

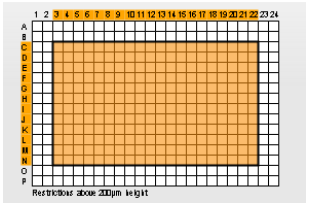
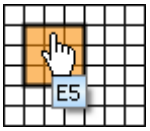
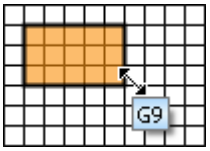
Certain combinations of plate type and objective can lead to non-measurable areas on the plate which will be indicated on the plate layout. For detailed information please see section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89.

Notice

Wells of a plate format with more than 384 wells are always displayed as rectangular wells, regardless of the real shape of the well.

Buttons and Elements

Element	Description
Name	Displays the name of the assay layout. An asterisk * in front of the name indicates that the assay layout has unsaved changes. For details see section 5.1.7.8 “Assay Layout Editor”, page 94.
Layer	Allows to select a layer of the assay layout which is displayed on the plate layout. An assay layout facilitates orientation and navigation on the plate. Select **Measurement Layout** to return to the regular view. You can also select wells for a measurement layout while an assay layout layer is displayed. Selected wells will become gray, but when switching to a different layer, the original assay layout will be displayed again. The **Measurement Layout** layer always displays the wells to be measured.
	Opens the **Database Browser** to load an existing assay layout.
	Allows to select a single well, e.g. for a snapshot or a test measurement. The well is marked in orange.

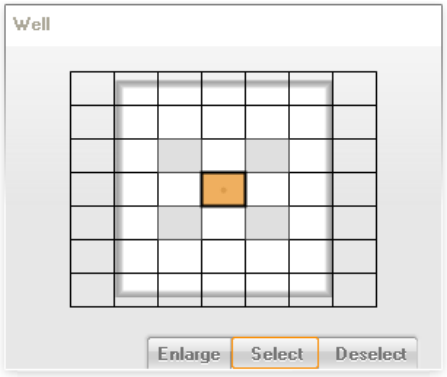
Element	Description
 **Move:**  **Resize:** 	Allows to mark a single well or a region of wells for a measurement layout. • Click and hold the mouse button to mark multiple wells in a rectangular area. • Press and hold the **Ctrl** key to mark multiple separate wells or well areas in one step. • **Note:** The marked wells are not selected for the layout until you click **Select**. Selected wells are gray. • The orange mark can be moved and resized. The mouse cursor changes accordingly, if you move the mouse on the mark or over the edges and corners of the mark (see on the left). A tooltip indicates the well coordinates of the current cursor position.
 **Restrictions** (due to selected combination of plate type, objective and focus height)	Certain combinations of plate type and objective can lead to restrictions, i.e. not all wells of a plate can be measured, because otherwise the objective would collide with the plate or table. The influence of the focus height is explained directly below the plate illustration. For details see section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89.
Select	Selects the marked wells (orange) for the layout. These wells become gray. They will be measured during the automated experiment.
Deselect	Removes the marked wells (orange) from the layout. These wells become white. They will not be measured during the automated experiment.

Context Menu

Element	Description
Assay Layout Editor	Opens the **Assay Layout Editor** to edit the current assay layout, load an assay layout, or create a new one. See also section 5.1.7.8 “Assay Layout Editor”, page 94.
Remove current Assay Layout	Removes the currently loaded assay layout.

Element	Description
Help	Opens the corresponding topic in the Harmony Help.

Well



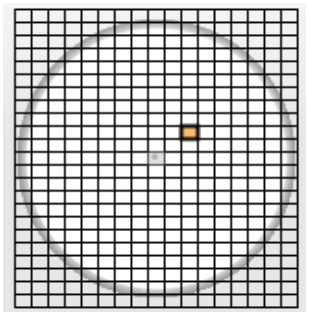
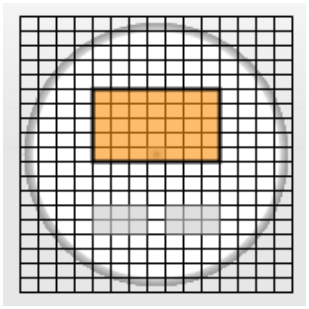
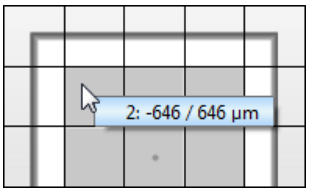
The **Well** pane is either used to select a single field in the well for a snapshot or you can select fields in the well for a well layout to be used during an automated measurement.

If you scroll the mouse wheel you can **zoom** in the well layout. This can be helpful if you have a plate type with large wells (e.g. a slide) and an objective with high magnification, which will result in many small image fields. You can also use the Enlarge button to display the well layout much larger in the **Content Area**.

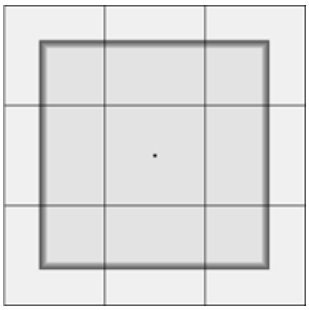
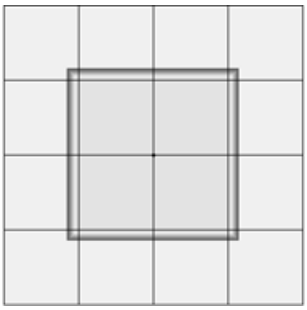
Using the context menu option **Field Centered** you can select how the grid of fields is arranged within the well (see context menu). If the option is activated, the default field is positioned in the center of the well. If you deactivate the option, the whole grid is shifted by half a field, so that a corner of the field is aligned in the center of the well. This can help you to reduce the number of required fields or to avoid border fields.

For step-by-step instructions see also section “Well”, page 64 (**Layout Selection** pane).

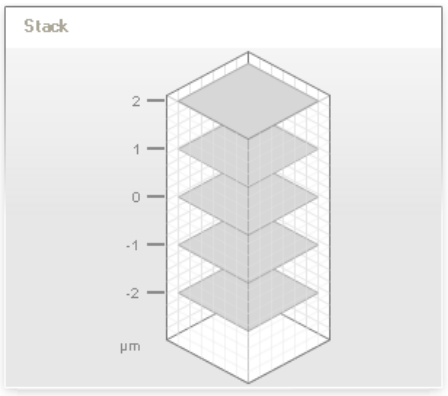
Buttons and Elements

Element	Description
Enlarge or Reduce	**Enlarge** displays the well layout as a larger version in the **Content Area** (useful for large wells with small image fields). **Reduce** switches back to the normal size in the **Well** pane.
	Allows to select a single field in the well, e.g. for a snapshot or a test measurement. The well is marked in orange.
	Allows you to mark a single field or multiple fields for a well layout. • Click and hold the mouse button to mark multiple fields in a rectangular area. • Press and hold the **Ctrl** key to mark multiple separate fields or field areas in one step. • **Note:** The marked fields are not selected for the well layout until you click **Select**. Selected fields are gray.
	For selected fields (gray) a tooltip indicates the distance of the current field to the center of the well in microns.
Select	Selects the marked fields (orange) for the layout. These fields become gray. They will be measured during the experiment.
Deselect	Removes the marked fields (orange) from the layout. These wells become white. They will not be measured during the experiment.

Context Menu

Element	Description
Field Centered	**Activated:** A grid is displayed, the central field has its center on the middle of the well (default). **Not activated:** The grid can optionally have its corner on the middle of the well. This can reduce the number of fields required to cover a well completely (especially when using low magnification objectives).  *Field centered*  *Field not centered*
Panning	If **Panning** is activated, you can click and move well layout (useful if the view is zoomed in). Selection of fields is not possible. To use panning temporarily without activating/deactivating the selection mode, hold down the **Shift** key before you click and move the view.
Help	Displays the context-sensitive help for this section.

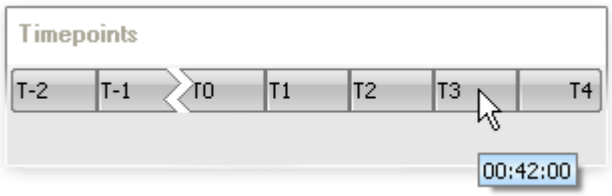
Stack



The **Stack** pane displays the current stack configuration. It is only visible if you configure a stack in **Layout Selection – Stack**.

For step-by-step instructions see also section “Stack”, page 66 (Layout Selection).

Timepoints



The **Timepoints** pane is used to visualize the current time series configuration. It is only visible if you configure a time series in the **Time Series** pane. For detailed information and step-by-step instructions see also section 5.1.7.4 “Time Series”, page 68.

A tooltip indicates the **scheduled** measurement time for each time point in relation to time point **T0** (beginning of the kinetics).

Notice

The tooltip displays only the **scheduled** measurement time of the time point. The **actual** measurement time may be different due to a pre-defined break (duration cannot be calculated) or delays during the measurement (e.g. focus issues).

The real time stamps can be viewed after the measurement in the **Navigation – Plate Measurement – Timepoints**. See also section “Timepoints”, page 182.

Test Images/Plate Measurement

For a detailed description of this pane please see section 5.1.11 “Navigation”, page 169.

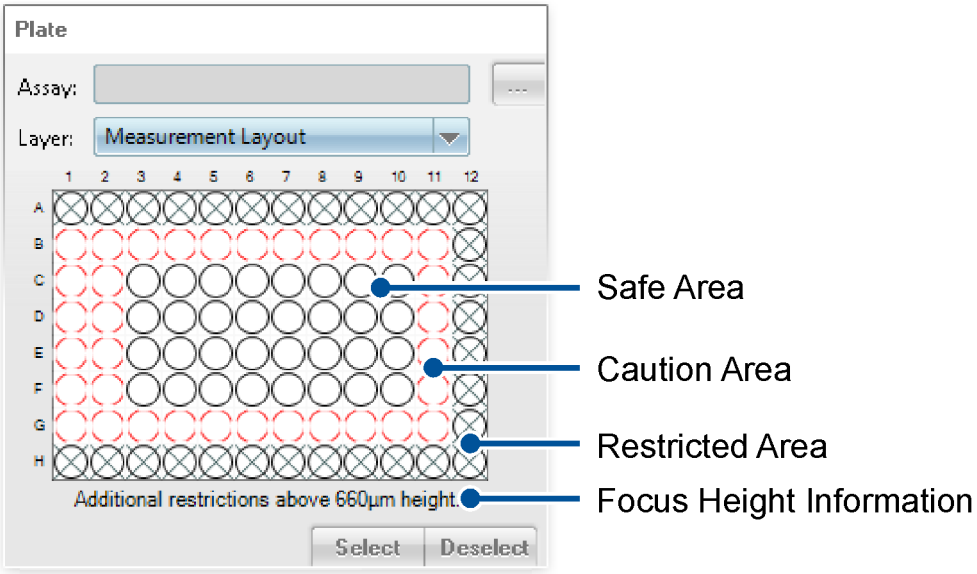
5.1.7.7 Restrictions of Measurable Plate Area

Certain combinations of plate type and objective can lead to non-measurable areas on the plate, because the objective would collide with the plate or with the table. The probability of restrictions is increased by the following factors:

- Objective with short working distance (e.g. high NA objectives)
- Plate type with high plate bottom or very thick plate bottom
- High focus height

Area Types

During the definition of an experiment, the **Plate** pane indicates which wells on the plate cannot be measured (when using the currently selected plate type and objective) and how the focus height affects the measurable plate area. The measurability of a plate is visualized by three different areas:



- **Safe Area:** No collision of objective with scan table or plate. All wells can be measured safely.
- **Caution Area (wells with red dotted border line):** Collision of objective with plate rim or ribs is possible but not fatal. All wells can be measured. However, it is the responsibility of the user to check for potential issues (see below).
- **Restricted Area (crossed wells):** Collision of objective with scan table could be possible and would be fatal. These wells cannot be selected or measured.

The influence of the **focus height** is explained by a short info below the plate layout (see section "Focus Height", page 92). The displayed plate restrictions are only valid for the currently selected fields of the **well layout** (see section "Well Layout", page 93).

Notice

- Please note that these regions are calculated based on the technical data provided by the plate manufacturer or during plate definition by the user. During focusing the instrument measures the actual height of the plate bottom for each imaging position. If the actual plate bottom of an outer well is located so high that imaging at that position would cause a collision between objective lens and table, the software will prevent the collision and report an autofocus failure.
- If you are using an office version of Harmony (not connected to the instrument) to prepare your experiment, the focus heights are probably not known yet. Please note that the measurable plate area may change again when setting the final focus heights at the instrument.
- For a **DPC** channel, the restrictions are displayed based on the values for **Upper Plane** and **Lower Plane** which will be used for the plate measurement. The **Height** parameter is only required for taking snapshots during setup. It has no influence on the displayed restrictions and you may get error messages if the entered height cannot be measured. For details see also section "Digital Phase Contrast", page 55.

How to view restrictions of the measurable plate area

1. Open the **Setup** tab.
2. Select the **Plate Type** used.
3. Select an **Objective** suitable for your readout.

   Notice
   The plate restrictions can also be checked if the objective is currently not installed in the instrument or if you are using an office version of Harmony.

4. Add a new channel (only one channel should be present).
5. Enter the focus **Height** required for your sample.
   - If the exact focus height is still unknown, you should test a range of focus heights to be on the safe side, i.e. the expected min. and max. focus height, e.g. -20 µm and 40 µm.
   - If you want to measure a stack, define the stack as desired. The restrictions will be calculated and displayed based on the upper stack plane (focus height in the channel will be ignored).
6. Select the desired fields in the **Well** pane.

   Notice
   Plate restrictions are only valid for the selected fields, not for the entire well. To be sure that the entire well can be measured without restrictions, you should select multiple fields close to the well borders (in all directions).

7. Check the **Plate** pane for restrictions of the measurable plate area:
   - If a **Restricted Area** (crossed wells) is displayed, these wells are automatically excluded from the measurement layout.
   - If a **Caution Area** (wells with red dotted border line) is displayed, these wells should be checked for potential autofocus issues (see next section).
8. If the plate is not measurable at all or if there are too many restrictions, repeat the procedure and try the following:
   - Try different field positions in the well layout.
   - Reduce the focus height (if possible).
   - Use an objective with longer working distance.
   - Use a plate type with lower or thinner bottom or different plate ribs.

How to check measurability of wells in the Caution Area

If some wells of your layout are classified as **Caution Area** (wells with red dotted border line), you should perform a test measurement to verify measurability of these wells. Ideally, prepare a sample plate reflecting the later assay conditions as close as possible. Otherwise, take a sample plate filled with water.

1. Select the wells of the **Caution Area** in the **Plate** pane (only the outer rows and columns directly at the border).
2. Select fields in the **Well** pane as required for your experiment. To save time you can include only extreme positions (corner fields, fields close to the border etc.).

   Notice
   Plate restrictions are only valid for the selected fields, not for the entire well.

3. Enter the focus **Height** required for your sample.
4. Open the **Run Experiment** tab and click 

to start an automatic measurement.
5. Check all wells and fields for focus failures and check the sharpness of the images (if possible).
6. If focus failures occurred (or if the measurement was even canceled due to focus errors), exclude the corresponding wells/fields from your plate/well layout, i.e.:
   - Try a different selection of fields in the well layout.
   - Remove wells in the outer plate regions, i.e. deselect corner and border wells.
7. Repeat the measurement until all wells and fields can be measured without errors.

How to document the measurable area for later use

- The measurable area of a plate can be documented in an **assay layout**. Create an assay layout, add a custom layer e.g. for "Focusability" and enter the corresponding data. Further information such as focus height, plate type and objective should be inserted in the name of the assay layout or attached as a

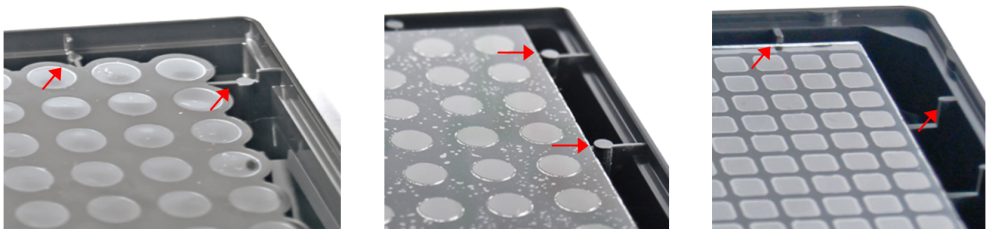
comment. See also sections 5.1.7.8 “Assay Layout Editor”, page 94 and “Comments ”, page 229.

- The selected field positions of the well layout are essential for determining the measurable plate area, but they cannot be saved in an assay layout. You have to document the well layout separately, e.g. using screenshots.

Influences on Measurable Plate Area

Plate Rim and Ribs

Many microplates have ribs on the plate rim, protruding towards the wells. The shapes of these ribs are not standardized i.e. not part of the SBS standard description. As no reliable information is available about the rib geometry it is not possible to exactly calculate which areas of the plate can be measured successfully. That is why the **Caution Area** has been introduced in the Harmony plate layout. Wells within this area are measurable but if ribs are present potential collisions of objective and plate rib can occur (based on the worst case scenario). The collision would lift the plate and the visible result might be either focus failures or unfocused sample images. The Caution Area should be tested before the actual measurement to confirm which part can be measured reliably.



Examples for ribs on the plate rim

Focus Height

The influence of the focus height on the measurable plate area is explained directly below the plate illustration:

- **Restrictions above ###µm height:** With the selected combination of objective, plate type and focus height, all wells can currently be measured (no wells are crossed), but if you exceed the stated focus height, there will be non-measurable areas.
- **Additional restrictions above ###µm height:** With the selected combination of objective, plate type and focus height, there are already non-measurable areas on the plate (crossed wells). If you exceed the stated focus height, there will be additional non-measurable areas.
- **No info text:** Indicates that the focus height has no influence on the plate restrictions (with current combination of plate type, objective and well layout). If restrictions are displayed, they are valid for the whole range of allowed focus heights.

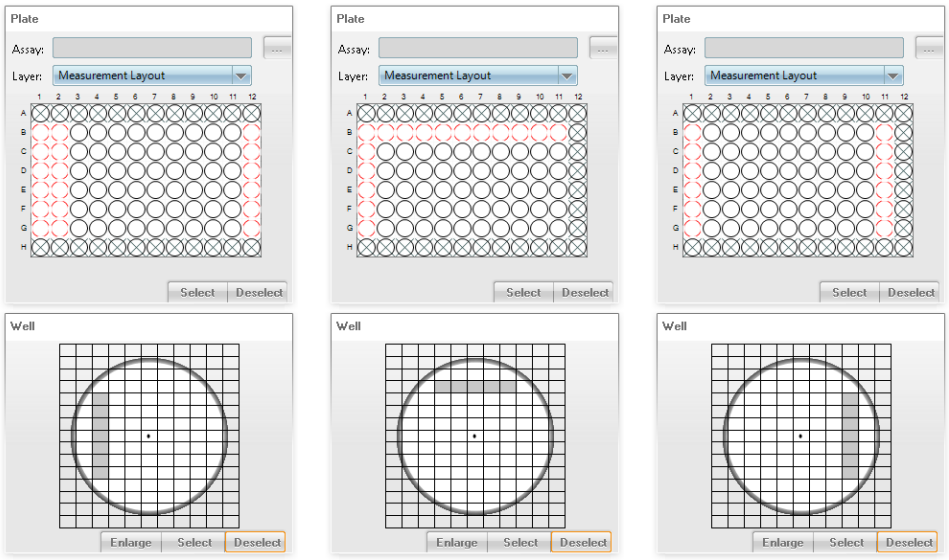
Non-measurable areas (crossed wells) cannot be selected for a layout. If you have created a layout and switch e.g. to an objective which leads to restrictions, the non-measurable wells will be removed from the layout.

Well Layout

The measurable plate area is furthermore influenced by the position of the selected fields in the well layout (see examples below). The restrictions displayed in the **Plate** pane are only valid for the selected fields in the **Well** pane. To be sure that the entire well can be measured without restrictions, you should select multiple fields close to the well borders (in all directions).

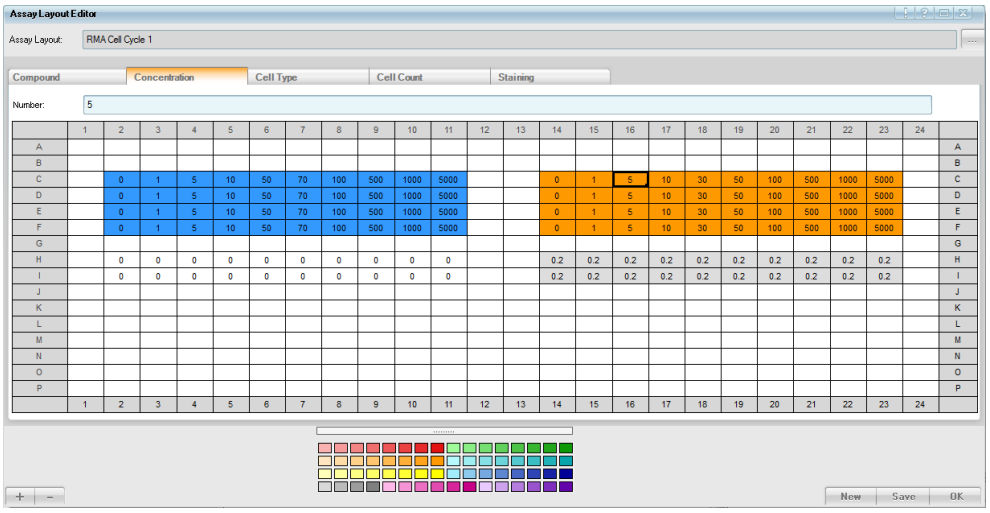
Notice

Since Harmony version 3.1 the displayed plate restrictions are only valid for the selected fields. In earlier versions, the restrictions were valid for the whole well.



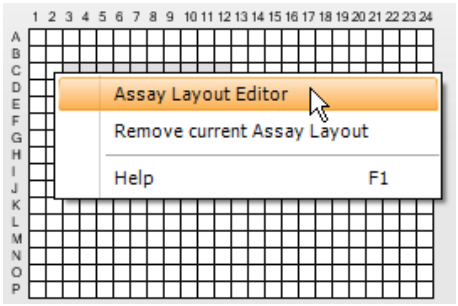
The position of the selected fields in the well layout can influence the plate restrictions.

5.1.7.8 Assay Layout Editor



The **Assay Layout Editor** can be opened in two different ways:

- Open the **Settings** window and double-click the **Assay Layout Editor** icon.
- Right-click on the plate control (**Navigation – Define Layout – Plate (Setup tab)**) and select **Assay Layout Editor** from the context menu.



An assay layout can be created (optionally) to describe the well contents of a microplate, e.g. concentration of compounds, location of control wells, etc. A separate layer is added for each attribute. These layers can be selected as colored overlay for the plate control (**Navigation** pane).

Notice

An assay layout provides meta information by describing the content of a plate. It does not specify where or how to measure a plate. This is done by the measurement layout. For details see section “Plate”, page 62.

Benefits of creating an assay layout

- Makes it easier to select the desired well (e.g. when defining an experiment or during image analysis).
- Allows you to retrace the content of a measured well.
- The assay layout information is also included when exporting evaluation results

for use with third-party applications. See section "Export Data", page 262.

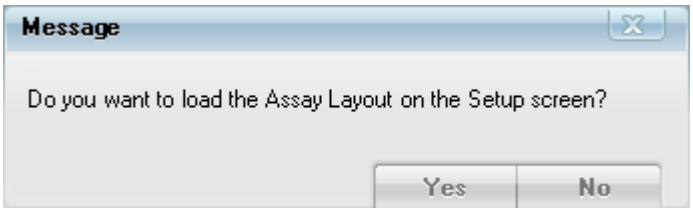
- Allows you to create EC50 calculations (compound concentrations required).

The assay layout is a separate database object, but it is saved with the measurement if you assign it before starting the measurement. If no assay layout had been selected before starting the measurement, you can also assign or change it subsequently in the **Database Browser**. See section 5.1.16.5 "Attach Assay Layout", page 235.

Evaluations inherit the current assay layout of the measurement. If you change the assay layout of the measurement (i.e. detach and attach a new one) the assay layout will also be updated for existing evaluations. For detailed information see also section 5.1.16.3 "About Keywords", page 232.

Buttons and Elements

Element	Description
Layer Title	Displays the name of the layer. You can rename a layer if you right-click the title and select **Rename** from the context menu.
Number or **Text or Number**	The data format of the layer is indicated by this label. It can be selected when adding a layer. In numeric layers you can only enter numbers. In alphanumeric layers you can enter any text (also numbers are treated as text). This may be important if you copy data to or paste from other applications. The data format of a layer cannot be changed. The input field displays the content of the selected well and allows you to edit the entry; especially useful for longer descriptions.
Plate	Displays the wells on the plate as a grid of table cells where you can enter descriptions for each well. • Click on a well to select it (black frame) and to enter a description. • There are several ways to select multiple wells: ◦ Click a well, hold down the mouse button and drag the mouse to expand the frame. ◦ Click a well, hold the **Shift** key and click another well. A rectangular selection is spanned between these wells. ◦ Hold the **Ctrl** key and click multiple wells. ◦ Click the row or column label to select the whole row or column. Can also be combined with **Shift** and **Ctrl** key (see above). • The context menu offers functions to copy, paste and reset wells (see below). • The value and color of a well can be copied to multiple wells if you click the lower right corner of the frame and expand the frame.
	Opens the **Database Browser** to load an assay layout. The **Assay Layout Format** (keyword of assay layout) has to be compatible with the selected plate type, otherwise you will get an error message.

Element	Description
+	Allows to add a layer. A new dialog is opened where you can enter a layer name and select the data format (**Type**).
–	Removes the selected layer.
New	Deletes the current assay layout and opens the **Select Plate Format** dialog where you can choose the plate format for the new assay layout.
Save...	Opens a new dialog to save the assay layout.
OK	Closes the **Assay Layout Editor** and loads the assay layout. • If the currently selected plate type is compatible with the assay layout format, it will be loaded automatically in section **Navigation – Define Layout – Plate** of the **Setup** screen. • If you opened the editor from the **Settings** window, you can decide whether the assay layout is loaded or not. Afterwards you will get back to the **Settings** window.  • If you want to close the editor and discard all changes or avoid loading the assay layout, click the X button of the editor's window.
Color Palette	If you select one or multiple wells and click on a color field, the wells change their background color. The color palette can be detached as a movable color picker if you click on the thin bar above it.

Context Menu

Context Menu	Description
Cut (Ctrl + X)	Copies the selected well(s) to the clipboard and deletes them on the layer (color and value).

Context Menu	Description
Copy (Ctrl + C)	Copies the selected well(s) to the clipboard (color and value). **Notice** If you copy wells to the clipboard, these data can only be pasted in Microsoft Excel® due to the used data format. If you need to copy wells to a different application (e.g. Microsoft Word®, GraphPad Prism® etc.), you first have to paste them in Excel® and copy them again. Afterwards you can paste them in the desired program.
Paste (Ctrl + V)	Inserts the content of the clipboard (color and value).
Paste Color	Inserts only the background color of the copied well(s).
Select All	Selects all wells.
Reset	Deletes the selected well(s) (color and value).
Reset color	Removes the background color of the selected well(s). The values are not modified.
Help	Opens the corresponding topic in the Harmony help.

Creating a New Assay Layout

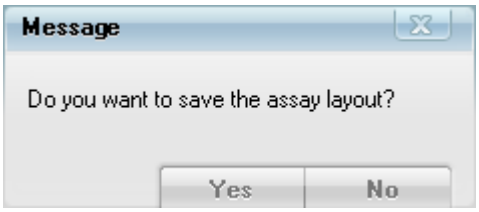
If you open the **Assay Layout Editor**, there are four default layers predefined: *Compound, Concentration, Cell Type* and *Number of Cells*. You can use them directly and enter data, add new layers or start from scratch.

- Right-click on a layer title and select **Rename** from the context menu to rename it.
- You can **add** or **remove** layers using the + and – buttons.
- You can **load** an existing assay layout using the ... button and use it as a template. The assay layout format has to be compatible with the selected plate type. To start from scratch (and to have the option to select a different plate format) use the New button.
- There are two types of layers: **numeric** and **alphanumeric** layers. The data format is indicated in the upper left corner of the plate (**Number** or **Text or Number**) and can be selected when adding a layer. In numeric layers you can only enter numbers. In alphanumeric layers you can enter any text (numbers are also treated as text). This may be important if you copy data to or paste from other applications.
- The values which you enter on the four default layers are automatically saved as keywords, so that you can search e.g. for a compound name in the **Database Browser**.

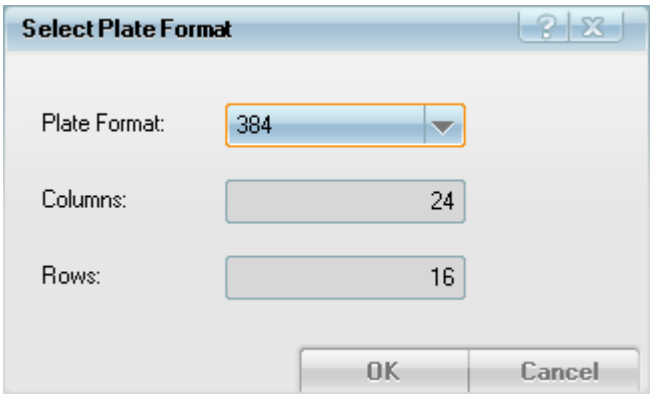
How to create a new assay layout

1. Click New.

A message box is displayed:



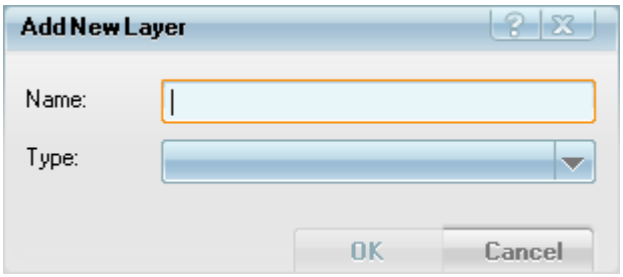
2. Click Yes if you first want to save the current layout or click No to discard any unsaved changes and start with an empty layout.
   The **Select Plate Format** dialog is opened.



3. Select the desired plate format.
4. Click OK.
   An empty assay layout is displayed in the **Assay Layout Editor**. See the following sections for editing options.

How to add a layer and enter data

1. Click +.
   The **New Layer** dialog is opened.

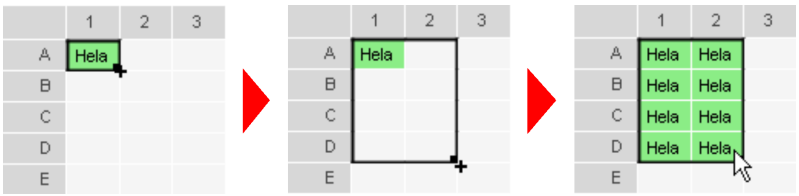


2. Enter a **Name** for the new layer.
3. Select a **Type** from the combo box (numeric or alphanumeric data format).
4. Click OK.
   The new layer is added.
5. Click one well on the layer (black frame is displayed).
6. Enter the desired number or text.

See also the following sections for further options.

How to copy a value to multiple wells

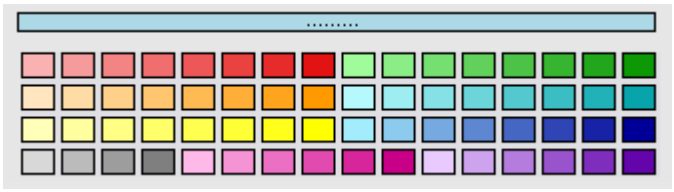
1. Click on the desired well so that it is selected (black frame).



2. Click on the lower right corner of the frame and drag it down and/or to the right to expand the frame.
3. Release the mouse button.
   The original value (and its background color) is copied to all selected wells.

How to select background colors

1. Select one or multiple wells.
2. Click on a color in the color palette.



The selected wells are colored according to your selection. These background colors of the wells are also visible if you select this layer as overlay for the plate control (**Navigation** pane).

How to paste data from other applications

1. Copy the desired data to the clipboard.
   This can be a single value, a text string or a group of table cells, e.g. from Microsoft Excel®.

> **Notice**
> Text can only be pasted on layers of type **alphanumeric** (**Text or Number** is displayed as data format).

2. In the **Assay Layout Editor** click on the desired well where the data is to be inserted. Right-click the well and select **Paste** from the context menu or press **Ctrl + V**.
   The data is attached to the well. If multiple cells from the Excel® spread sheet are copied, the data is attached to multiple wells.

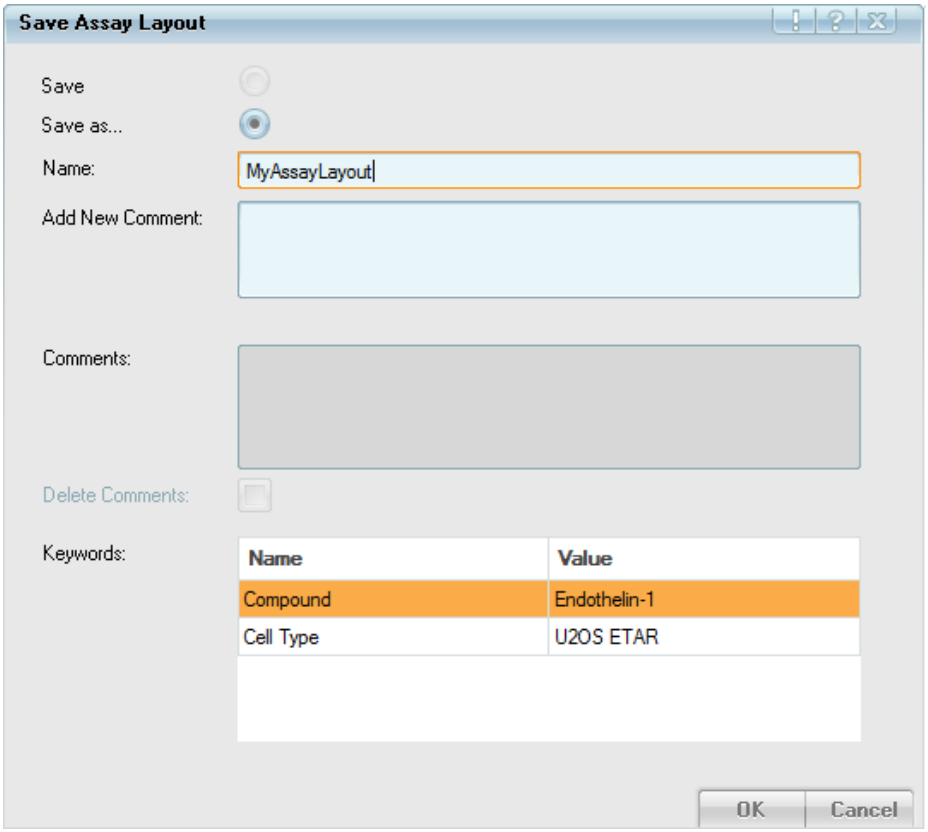
How to copy data to other applications

Copied data from the **Assay Layout Editor** can only be pasted in Microsoft Excel®. If you want to copy the data to other applications (e.g. Microsoft Word® or GraphPad Prism®), you have to take the detour via Excel®:

1. Select the desired wells.
2. Right-click the selection and choose **Copy** from the context menu or press **Ctrl + C**.
3. Paste the data in Microsoft Excel® (**Ctrl + V**).
4. Still being in Excel®, select the pasted cells and copy them to the clipboard again (**Ctrl + C**).
5. Open the desired application and paste the data.

How to save an assay layout

1. Click Save... . The **Save Assay Layout** dialog is opened.



2. Select **Save** or **Save as**:
   - **Save:** Overwrites the existing assay layout with a new version. This is useful when saving interim versions. The existing assay layout name is displayed and cannot be edited. This option is only available if the assay layout has been saved before and if you are the owner of the assay layout . The previous version of the assay layout will not be accessible in the **Database Explorer** anymore.

- **Save as:** Saves the assay layout as a new object (new signature) in the database. You can enter a new **Name** or keep/modify the existing one (if the assay layout has been saved before). The previously saved assay layout will also remain accessible in the **Database Explorer**.

3. Enter a **Comment**, if desired.
   Keywords describing the assay layout are created automatically.
4. Click 

to save the experiment.

5. Enter a **Comment**, if desired.
   Keywords describing the assay layout are created automatically (only for data inserted in the default layers **Compound**, **Cell Type** or **Number of Cells**).
6. Click OK to save the assay layout.

Assigning an Assay Layout

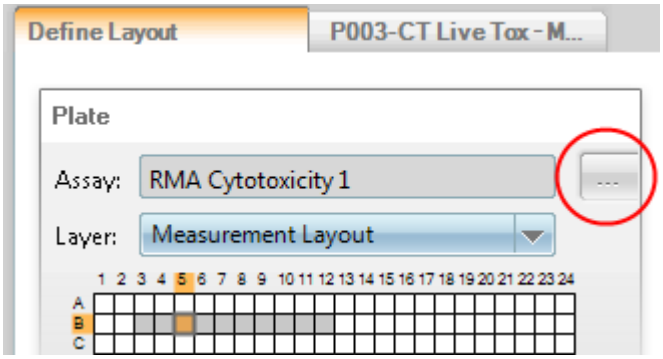
You can create and/or select an assay layout before starting a measurement or an evaluation. If an assay layout has been attached to both measurement and evaluation, only one assay layout is visible at a time (depending on whether you load the measurement or the evaluation). For background information see also section 5.1.16.3 “About Keywords”, page 232.

How to select an assay layout before measurement

There are two ways to load an assay layout (for selecting it *before* starting a measurement):

Option 1: During experiment definition (**Setup** tab)

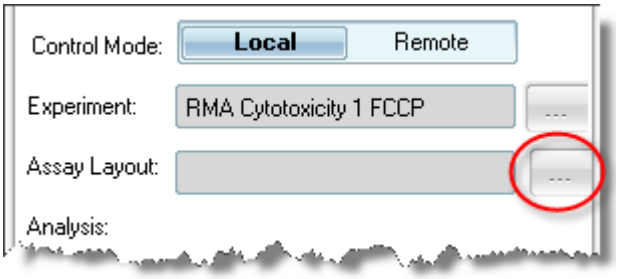
1. On the **Setup** tab click ... in section **Navigation – Define Layout – Plate**.
   The **Database Browser** is opened.



2. Select the desired assay layout and click OK.
   The name of the loaded assay layout is displayed.
3. Optionally: Select the layer to be displayed from the **Layer** box.

Option 2: Before starting a measurement (**Run Experiment** tab)

1. On the **Run Experiment** tab click ... in **Global Control** section (next to **Assay Layout** box).



   The **Database Browser** is opened.

2. Select the desired assay layout and click OK.
   The name of the assay layout is displayed in the **Assay Layout** box.

The selected assay layout will be saved with the measurement when you start it.

How to select/change an assay layout of a measurement subsequently

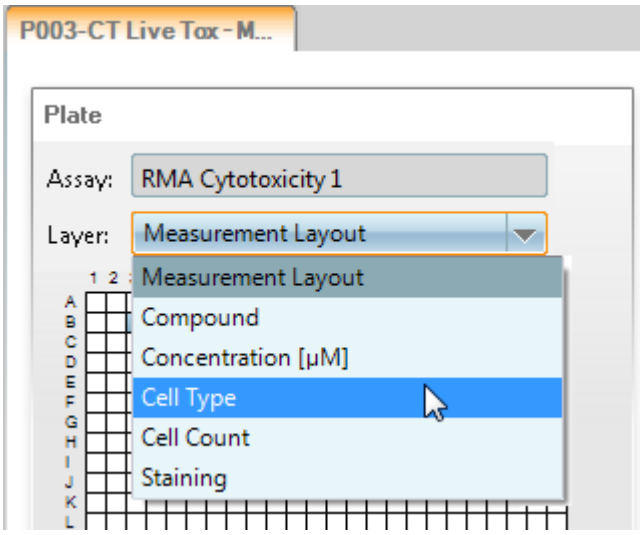
You can use the **Database Browser** to assign an assay layout to a measurement subsequently or to change the assigned assay layout. For details see section 5.1.16.5 “Attach Assay Layout”, page 235.

Displaying an Assay Layout Layer

How to view the assay layout as plate overlay (Navigation pane)

Precondition: Assay layout (or measurement including an assay layout) has been loaded

1. In **Navigation – Measurement/Define Layout – Plate** select the desired layer from the **Layer** combo box.

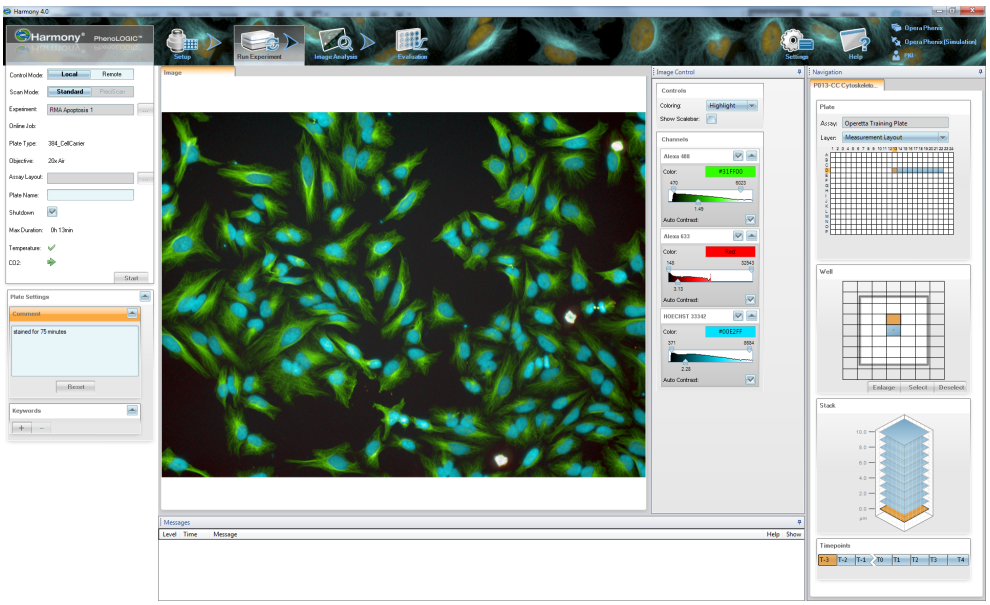


   The selected assay layout layer is displayed on the plate (only by well colors, values are not displayed).

2. Select **Measurement Layout** if you want to go back to the regular view (colors indicate well status and progress).

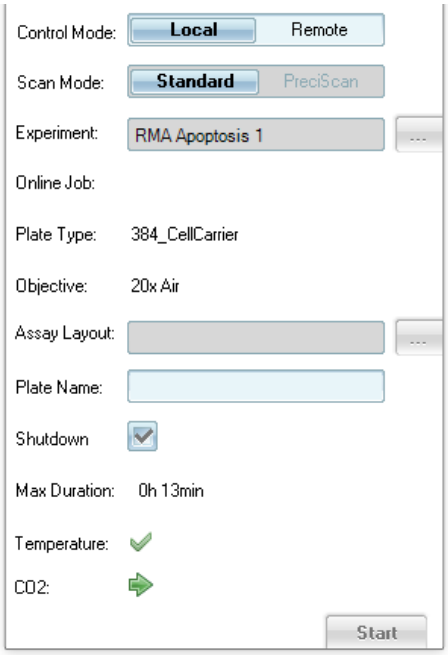
See also section 5.1.11.1 “Plate Measurement”, page 171.

5.1.8 Run Experiment



The **Run Experiment** tab is used for running a plate measurement with the current experiment settings.

5.1.8.1 Global Control



There are two different **Control Modes**:

- **Local:** Normal measurement mode, local control and manual plate loading.
- **Remote:** Automated measurement controlled by external scheduler (remote control), automatic plate loading via robot. Most Harmony functions are disabled while the instrument is under remote control.

Local Mode

In section **Global Control** you can start a plate measurement with the current experiment settings. If you have defined an experiment on the **Setup** tab, this experiment is loaded automatically. You can also load an experiment from the database.

Notice
It is not necessary to save an experiment. If it has not been saved, it will be "contained" in the measurement. If you save the experiment, it will be available as a separate object in the database.

The **PreciScan** scan mode allows you to define two different experiments (PreScan and ReScan) to scan a sample at a low magnification and to automatically rescan only the desired objects within a second measurement at a higher magnification. This feature is only available with the optional PreciScan license. For details please see the following sections:

- 5.1.8.3 "PreciScan™", page 119
- "License Management", page 320

How to start a plate measurement

1. Open the **Run Experiment** tab.
2. Click ... to load an experiment from the database or use the current experiment defined on the **Setup** tab (default).
3. In the **Plate Name** text box enter the desired plate name, e.g. a short description or a barcode.
4. Optional: Use the **Plate Settings** pane to enter keywords and a comment for describing the plate.
   For details see section 5.1.8.2 "Plate Settings", page 117.
5. Click Start to start the plate measurement.
   The progress is indicated by the progress bar and by the progress LEDs on the instrument.

How to view measurement progress and images

In **Navigation – Plate Measurement** the measurement progress is indicated by the color of the wells, fields and stack planes. For details and examples see section 5.1.11.3 “Color Coding”, page 189. Furthermore, indicate the measurement progress on a percentage basis.

Viewing measured images:

- Select a well, field and stack plane which have already been measured. The corresponding image will be displayed in the **Content Area** (see section 5.1.13 “Content Area”, page 208).
- Selection and coloring of the channels can be configured in the **Image Control** pane (see section 5.1.12 “Image Control”, page 195).

Buttons and Elements (for Scan Mode: Standard)

Element	Description
Control Mode 	• **Local Control:** Default mode for regular plate measurements, manual plate handling. • **Remote Control:** Allows an external scheduling software to control the instrument (for measuring multiple plates automatically) **Note:** This requires an instrument with plate::handler™ II upgrade (optional).
Scan Mode 	Only available with optional PreciScan license (see section "License Management", page 320): • **Standard:** Default mode for regular plate measurements (one experiment). • **PreciScan:** Fully automated and sequential measurement of two different experiments (PreScan and ReScan). If this option is selected, additional parameters will be available in the Global Control section. For details please see section 5.1.8.3 "PreciScan™", page 119.
Experiment	Displays the name of the current experiment. Click ... to open the **Database Browser** and load an experiment.
Online Job	Displays the name of the current online job (online analysis or Columbus transfer). An online job can be selected when defining an experiment on the **Setup** tab. See also section 5.1.7.5 "Online Jobs", page 77. If no online job had been selected, this field is empty and the plate is measured without automatic evaluation or Columbus transfer.
Plate Type	Displays the selected plate type for the experiment.
Objective	Displays the selected objective for the experiment.
Assay Layout	Displays the name of the current assay layout. Click ... to open the **Database Browser** and load an assay layout. For more information on assay layouts see also section 5.1.7.8 "Assay Layout Editor", page 94.

Element	Description
Plate Name	Enter a name for the plate, e.g. a barcode or a description (64 characters maximum). **Notice** It is recommended to keep the plate name short (64 characters maximum), because the plate name will be part of the storage path if you archive, relocate or export objects from the database. The Windows® operating system is limited to 256 characters for the total length of a file path (including all subfolders and file names), i.e. there will be errors if this limit is exceeded.
Shutdown	If you activate this option, the instrument components selected for **Shutdown** in the **Standby/Shutdown Settings** dialog (e.g. temperature and carbon dioxide control) will be switched off after measurement. • In **Local** mode, the selected components will be switched off after the experiment. • In **Remote** mode, this checkbox will be ignored. The shutdown has to be triggered by your external scheduler sending a shutdown command. **Notice** In **Local** mode, a component is only switched off ... • if the **Shutdown** option on **Run Experiment** has been activated ***AND*** • if the component has been selected for shutdown in the **Standby/Shutdown Settings** dialog. In **Remote** mode, a component is only switched off ... • if the component has been selected for shutdown in the **Standby/Shutdown Settings** dialog ***AND*** • if the scheduler sends a shutdown command. The shutdown and standby settings only apply to TCO components which had been activated in the **TCO Settings** dialog before the measurement. For further details and the difference between **shutdown** and **standby** please see section 5.1.17.6 “Standby/Shutdown Settings”, page 249.

Element	Description
Max Duration	Maximum duration of the current experiment (only measurement without online analysis). The maximum duration is estimated very conservatively, actual measurement times may be up to 50 % faster. Format: *hours:minutes* **Notice** The calculation of the maximum duration is not possible with office versions of Harmony (connection to instrument is required).

Element	Description
Temperature / CO_2	Only displayed with optional TCO upgrade, see also section 5.1.17.5 “TCO Settings”, page 246.  : **Start-up**, current value is still significantly lower than target value. : **Regulating**, target value nearly reached, but not stabilized yet. In this phase temperature is at maximum 4 °C below target value and CO_2 is at maximum one percentage point below target value. Transiently slightly higher values for temperature and CO_2 may also occur in this phase, please check the **Messages** window. : **Ready**, target value reached (target temperature ±1 °C; target concentration ±0.5 percentage points). **Off**: Not activated in **TCO Settings** or switched off by shutdown. **Standby:** Deactivated temporarily between two measurements (only for **Remote** mode, see also section 5.1.17.6 “Standby/Shutdown Settings”, page 249) : **Operating Error** (e.g. lid open), see also section 5.3.18 “TCO Issues”, page 478. The status of the temperature control is also visualized by the Operetta's progress LEDs. A blinking “T” indicates that the TCO unit is warming up or reheating. If the target temperature has been reached “T” will shine continuously. See also section 6.5 “Instrument Status”, page 510. **Notice** During the start-up sequence the status of temperature and carbon dioxide concentration will alternate several times between **Regulating** and **Ready**. The target values only have been stabilized if status **Ready** is displayed continuously.

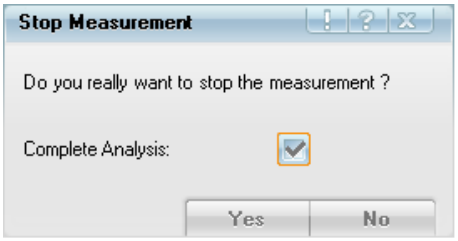
Element	Description
Start Stop	Starts a plate measurement using the current experiment. The button will turn into a Stop button which allows you to cancel the measurement (after confirming the warning message). For details see section "Stop Measurement", page 112. The measurement progress is indicated by the progress bar above the button. • In **Navigation – Plate Measurement** you can select one of the resulting images. It will be displayed in the **Content Area**. • In **Image Control**, you can control which channels of the selected image are displayed by activating or deactivating their check boxes (see 5.1.12 "Image Control", page 195).

Context Menu

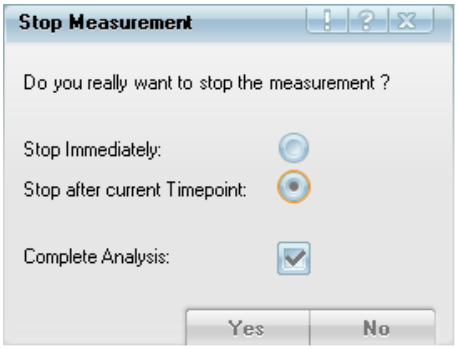
Element	Description
Remove current Assay Layout	Removes the currently loaded assay layout.
Help	Opens the help topic for this section.

Stop Measurement

If you click Stop to stop a running plate measurement, one of the following dialogs is opened.



Without time series



With time series

- **Complete Analysis:** If your experiment includes an online analysis, you can decide whether the analysis will be completed for the already measured wells.
- **Stop Immediately/Stop after current Timepoint:** If your experiment includes a time series, a different dialog is displayed. You can then decide to stop the measurement immediately or after the current time point has been measured completely (including all wells, field, and planes).

Remote Mode (Automation)

Activate the **Remote** mode to allow an external scheduling software to control the instrument (for using Operetta CLS in an automated environment). As long as the remote mode is active, most functions in Harmony are disabled.

Harmony functions behaving differently in Remote mode

- **Time Series with Break**
  Not allowed in remote mode (experiment will be rejected). Time series without break can be used.
- **Online analysis using "Track Objects"**
  Not supported in remote mode. The measurement will be performed, but the online analysis will fail (yellow wells).
- **Notifications + popup dialogs**
  Any Harmony prompts or blocking message windows will be suppressed. Please check the **Messages** pane for error messages and notifications.
- **Missing hardware**
  In case of missing hardware (objective, filter etc.) the experiment will be rejected.
- **Shutdown/Standby**
  Compared to local mode: Different shutdown behavior and additional standby option. See also section 5.1.17.6 “Standby/Shutdown Settings”, page 249.

How to prepare automated measurements (remote mode)

Notice

- Eject and remove any sample plate before starting an automated run. Such a plate could lead to a crash of the robot and damage the instrument.
- Check prior to your run if the Harmony experiment can be executed and no error message regarding the plate type is shown.
  - If you load an experiment, the plate type details will be read directly from the experiment, not from the plate type object in the database (which may have been changed in the meantime).
  - If the experiment is rejected, please switch Harmony to local mode and go to the **Setup** tab. Change the plate type (even if the name is the same) and save the experiment again. Vice versa, to prevent any crashes, please check prior to your run if the plate type of the Harmony experiment can *really* be handled by the robot. We recommend checking this under supervision.

Checklist for automated runs
Plate type selected which can be handled by the robot and which is reliably detected by the instrument's plate sensor?
Experiment saved in the database?

Checklist for automated runs
Test measurement of one plate successful (to determine experiment duration for plate::works scheduler)?
Evaluation not slower than measurement (if online analysis selected)?
Free disk space on the database PC for all plates available? See also section 5.3.6 “Calculating Required Disk Space”, page 464.
All used objectives are installed?
All correction collars are adjusted to the plate bottom thickness of the used plate type?
All required emission filters are installed?
Operetta CLS status light is green?
No plate loaded? Transfer position empty?
Harmony set to “Remote” mode?

Manuals for plate::handler II + plate::works

If you are using the PerkinElmer automation upgrade (plate::handler™ II robot + plate::works scheduler) please see also the following manuals which are delivered with the upgrade.

Manual	Content
plate::handler II with Opera Phenix or Operetta CLS	Upgrade description and Quick Guide
plate::handler II User Guide	Hardware and software documentation of the robot
plate::works Software Manual	Description of the scheduling software

How to specify the experiment to be measured in the external scheduler

In your external scheduling software you need to define the exact Harmony experiment which is to be measured. One of the following options can be used to uniquely identify a single experiment:

- *Signature*
- *User$ExperimentName* (latest experiment version is used)
- *User$ExperimentName$Date*

If using the **PreciScan** feature in Harmony, 2 experiments must be specified for PreScan and ReScan:

- *Signature1$$Signature2*
- *User1$ExperimentName1$$User2$ExperimentName2* (latest experiment

versions are used)

- *User1$ExperimentName1$Date1$$User2$ExperimentName2$Date2*

Notice

- The exact way how to enter the required parameters and which of the above options are supported will depend on the used scheduling software. Please refer to the manufacturer's documentation. For using the **plate::works** scheduler see the example below.
- It is recommended to copy and paste the required parameters directly from Harmony to avoid typos. The **Database Browser** allows you to copy keyword values via context menu.

Legend:

- **Signature:** Database signature of the protocol (displayed in the **Database Browser**)
- **User:** Owner of the protocol
- **ExperimentName:** Name of the experiment
- **Date:** Timestamp of the experiment in the database, if not present the latest version will be used.

  Supported date formats:

  - YYYY-MM-DD HH:MM:SS
  - MM/DD/YYYY HH:MM:SS a
  - YYYY-MM-DD HH:MM:SS a

Example: plate::works scheduler

In plate::works you have to enter the parameters separately into the *Experiment name* and *Experiment owner* fields of the *READ_HCS* method. Plate::works will assemble both entries according to the following pattern: *[Experiment owner]$[Experiment name]*.

- **Experiment owner:** Enter only the name of the experiment owner (in case of PreciScan: owner of first experiment).
- **Experiment name:** Enter the rest of the parameter string (see options above), but without "$" separator at the beginning.
- In case of using the experiment **signature**, enter it into the *Experiment name* field and leave *Experiment owner* empty.

Examples for valid configurations:

Properties	
Name	READ_HCS
DisplayName	Name: $Experiment_name, Owner: $Experi
Experiment name	MyExperiment
Experiment owner	MyUser
Simulation duration	20 min
Timeout error tolerance	130 %
Timeout warning tolerance	110 %

User name + experiment name

Properties	
Name	READ_HCS
DisplayName	Name: $Experiment_name, Owner: $Experi
Experiment name	MyExperiment$11/20/2016 10:33:26 AM
Experiment owner	MyUser
Simulation duration	20 min
Timeout error tolerance	130 %
Timeout warning tolerance	110 %

User name + experiment name + date

Properties	
Name	READ_HCS
DisplayName	Name: $Experiment_name, Owner: $Experi
Experiment name	b87a790e-e0e1-4eba-828a-dcd5f36f5d2a
Experiment owner	
Simulation duration	20 min
Timeout error tolerance	130 %
Timeout warning tolerance	110 %

Signature only

Properties	
Name	READ_HCS
DisplayName	Name: $Experiment_name, Owner: $Experi
Experiment name	MyExperiment1$$MyUser2$MyExperiment2
Experiment owner	MyUser1
Simulation duration	20 min
Timeout error tolerance	130 %
Timeout warning tolerance	110 %

PreciScan (two experiments for PreScan + ReScan)

How to stop an automated run

Ideally, an automated run should be stopped via the external scheduler. If it does not offer a corresponding function, you can stop the run locally:

1. Switch to **Local** mode.
2. Click **Stop**.

   The **Stop Measurement** dialog is opened. For details see section “Stop Measurement”, page 112.
3. Select the desired options and click **Yes** to stop the measurement.

5.1.8.2 Plate Settings

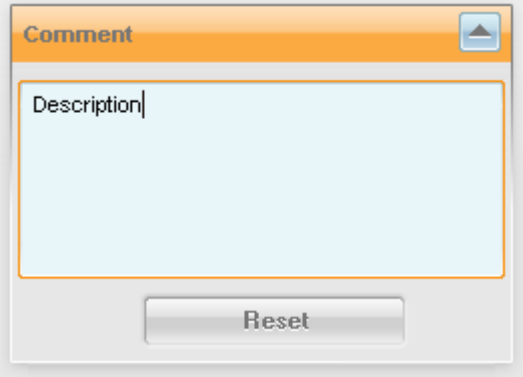
The **Plate Settings** can be used optionally to add information to the measurement to facilitate the search for the measurement in the database.

The measurement itself is named automatically (*Measurement#*). If you measure the same plate again, the measurement number is incremented.

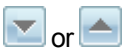
> **Notice**
> The information in **Plate Settings** has to be entered *before* starting a measurement. Otherwise it is not saved with the measurement.

Comments

This section allows you to enter free text describing the experiment. This can be useful for adding specific information which is not included in the automatically generated keywords. You can search in these comments using the **Database Browser** (**Search in Comments ...**). See also section 5.1.16 “Database Browser”, page 220.

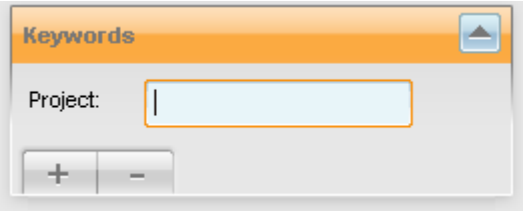


Buttons and Elements

Element	Description
 or	Click the disclosure button to open or close the **Comments** pane.
Comment	Field for entering free text.
	Removes the entered comment.

Keywords

This section allows you to add user-defined keywords which will be saved with the measurement. For more information on the concept of keywords see sections 5.1.16.3 “About Keywords”, page 232 and “Define Keywords”, page 278.



Buttons and Elements

Element	Description
 or	Click the disclosure button to open or close the **Keywords** pane.
Keyword	Field for entering the desired keyword value.
	Opens a context menu where you can select a user-defined keyword which you want to add. **Notice** New keyword categories can only be created by users with administrative rights. See sections 5.1.17.8 “User Accounts (Harmony + Columbus)”, page 304 and “Define Keywords”, page 278.
	Opens a context menu where you can select the keyword which you want to remove.

5.1.8.3 PreciScan™

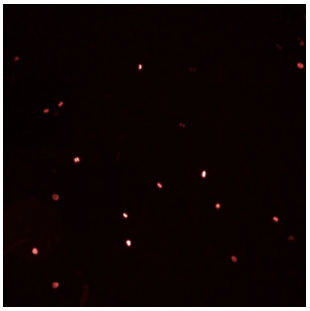
The PreciScan™ feature allows the user to scan a sample at a low magnification and to automatically rescan only the desired objects within a second measurement at a higher magnification. Therefore, the user has to prepare two experiments called **PreScan** and **ReScan**.

Working Principle

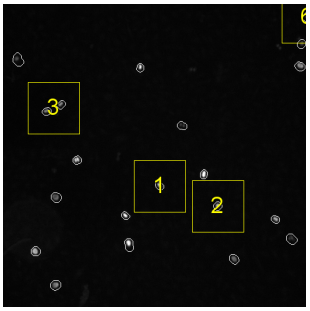
The user measures a sample at first with a lower magnification (first experiment: **PreScan**). This measurement is automatically analyzed using an online analysis. The result of the analysis are the image fields capturing the desired objects for the second experiment with a higher magnification (second experiment: **ReScan**).

In the analysis of the PreScan the user defines a population of objects that shall be measured (e.g. all transfected cells or a microtissue). The analysis (i.e. the *Determine Well Layout* building block, see section 5.2.23 “Determine Well Layout”, page 436) yields as a result the well layout for the ReScan. This second experiment is also pre-defined by the user, i.e. all measurement parameters are fix except the plate and well layout which result from the analysis of the PreScan. Both experiments are executed without any user intervention directly one after the other.

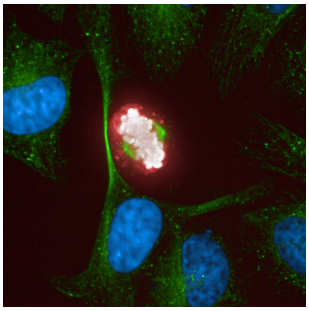
The result is a measurement where in each well only the desired objects are measured in the second experiment, thus speeding up the second measurement (which is inherently slow as it either uses a higher magnification or time-consuming z-stacks/time series) and avoiding the recording of unnecessary image fields which do not contain desired objects.



PreScan with low magnification



Field positions for ReScan calculated by Determine Well Layout building block



ReScan with high magnification

Notice

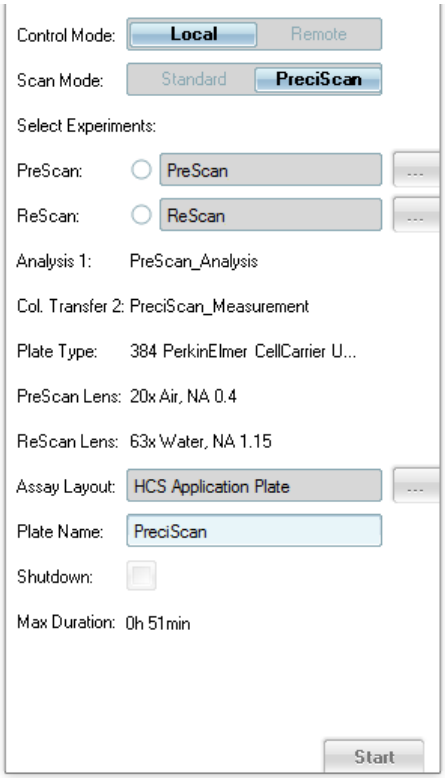
- The PreciScan feature is only available with an optional license. For details see section "License Management", page 320.
- The similar **Manual PreScan/ReScan** function in Harmony can also be used for sequential measurements at different magnifications (no license required). However, this is a much simpler manual procedure without automatic selection of image fields. For details please see section 5.1.11.4 "Manual PreScan/ReScan", page 191.
- In addition this manual function is helpful during the experiment definition for the PreScan and ReScan experiments of the PreciScan. See also section "How to create a ReScan experiment", page 125.

Notice

- PreciScan measurements (in contrast to normal measurements) will be named with a number and an additional letter: "Measurement **#a**" for PreScan, "Measurement **#b**" for ReScan. This helps you to recognize the type of measurement.
- PreciScan measurements will get the keyword **PreciScan** (value "PreScan" or "ReScan") which can be used when searching for a measurement in the **Database Explorer**.
  The ReScan measurement will also get the keyword **PreScan Evaluation Signature** so that the measurement can be traced back to the corresponding PreScan evaluation.
- If a PreScan or ReScan measurement is loaded on the **Image Analysis** screen, you can use the **Load PreScan/ReScan** button in the Global Control section to switch between the PreScan evaluation and the ReScan measurement (including their contained objects like measurement, experiment and analysis). For details see section "Load PreScan/ReScan", page 137.
- For testing the entire PreciScan sequence you could e.g. define a "one well" version of the PreScan experiment and then run such a short version of your PreciScan on the **Run Experiment** screen and inspect the results.
- The well layout determined by the PreScan analysis cannot take into account any **plate restrictions** that may apply for the selected combination of plate type, objective and focus height in the ReScan experiment. It is recommended to check the border wells (all fields selected) of your ReScan experiment for possible restrictions before using it in a PreciScan. See also section 5.1.7.7 "Restrictions of Measurable Plate Area", page 89.
- If the image alignment procedure fails, repositioning accuracy of PreciScan might be impaired. For troubleshooting see section 5.3.12 "Image Alignment Procedure Fails Repeatedly", page 470.

Additional Parameters

If the **Scan Mode** is set to **PreciScan**, additional parameters will be available on the **Run Experiment** screen (Gobal Control section).



Additional Buttons & Elements in PreciScan mode

Element	Description
Scan Mode	• **Standard:** Default mode for running normal experiments. For a description of the default GUI please see section 5.1.8.1 “Global Control”, page 105. • **PreciScan:** The Global Control section is changed and additional parameters and buttons are shown. The user can select two different experiments (PreScan and ReScan) which will be measured automatically one after the other.
PreScan Exp.	Click the ... button to select the experiments for **PreScan** and **ReScan**. See also section “Requirements for PreScan and ReScan experiments”, page 122. The dogma of Harmony is that only one experiment can be loaded/active in the GUI (the one which is also displayed on the **Setup** screen). By selecting an experiment in one of these fields, no experiment is loaded yet. You just select the experiments to be loaded and measured sequentially when starting the PreciScan. The status of the experiments is indicated by the following icons: ○ : Experiment **not running** ↻ : Experiment loaded and **running**
ReScan Exp.	

Element	Description
Online Job 1 (PreScan) **Online Job 2** (ReScan)	Displays the online jobs defined in the selected experiments. If an online job has been defined, the label is changed to indicate the job type (e.g. **Analysis 1** or **Col. Transfer 2**). • It is mandatory to define an **online analysis** (including the building block *Determine Well Layout*) for the **PreScan** experiment. See also section 5.2.23 “Determine Well Layout”, page 436. A Columbus transfer cannot be defined for the PreScan, because there can be only one online job. • Optional: You can define an online analysis *or* an online Columbus transfer for the **ReScan** experiment.
PreScan Lens	Displays the objective selected in the PreScan experiment.
ReScan Lens	Displays the objective selected in the ReScan experiment.
Max Duration	Estimated max. duration of the currently loaded experiment (displayed on **Setup** screen). • **Note:** Before starting the PreciScan this parameter does not necessarily display the max. duration of one of the *selected* PreciScan experiments! It will display the duration of the currently *loaded* experiment which is always displayed on the **Setup** screen. • As soon as the PreciScan is started, it will display the max. duration for the running experiment (PreScan or ReScan), indicated by the icon. **Notice** The displayed value is the pure measurement time of the current experiment (PreScan *or* ReScan). It does not include the mandatory online analysis of the PreScan, any online jobs defined for the ReScan or other delays.
Start	If you click Start the two selected experiments will be processed sequentially without user intervention.

Using PreciScan

Requirements for PreScan and ReScan experiments

Requirement	PreScan	ReScan
Plate Type	Must be identical	
Objective	Must not be a water objective if the ReScan uses an air objective	Must not be a lower magnification than the objective of the PreScan

Requirement	PreScan	ReScan
Optical Mode	Can be different	
Binning		
Channel Selection		
Plate Layout	Normal plate layout	Plate layout must contain at least one well to be valid. Will be replaced by plate layout and well layout resulting from PreScan.
Well Layout	Normal well layout	
Stack allowed	Yes	Yes
Time Series allowed	No	Yes
Online Job	Mandatory (online analysis) • Must contain building block "Determine Well Layout" • Magnification selected in "Determine well layout" BB and magnification of ReScan experiment have to match • If using a stack, "Maximum Projection" must be chosen in the "Input Image" building block • If using multiple fields, it is recommended to activate "Create Global Image" in the "Input Image" building block • Online Columbus transfer not possible	Optional • May contain online analysis *or* Columbus transfer

How to create a PreScan experiment

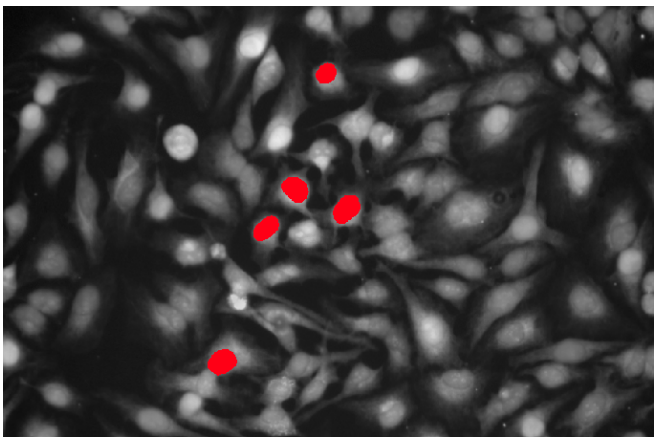
1. Insert the plate to be measured using **Load/Eject**.
2. Open the **Setup** screen and click **New** to create new experiment.
3. Set up a normal experiment as required to capture all objects of interests using a low magnification objective. For detailed requirements see table before).

Notice

In case the PreScan cannot be done with sufficiently low magnification to see the full region of interest in a single field of view, it is needed to use multiple fields in the PreScan. For analysis it is needed to:

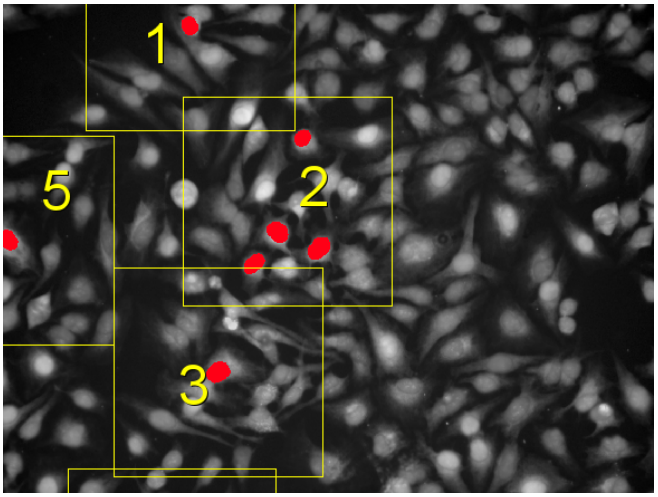
1. Create a **global image** of the PreScan.
   See also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.
2. Detect objects of interest on the global image.
3. Use the *Determine Well Layout* building block on these objects (global population).

4. Make test measurements in different wells to optimize exposure parameters and focus heights for all channels.
5. Define plate layout and well layout so that all objects of interest are captured.
6. Open the **Image Analysis** screen and click **New** to create a new analysis sequence.
7. Select one well and add buildings blocks as desired to create a population of objects which you want to rescan at a higher resolution during the ReScan experiment. For example using:
   - Find Cells
   - Calculate Intensities
   - Select Population



Overlay of objects which have been selected for ReScan

8. Add building block **Determine Well Layout** (mandatory).
   - Select the **Population** of desired objects for the ReScan and a **Region**.
   - Select the desired **Rescan Magnification**.
     The objective lens of the ReScan experiment has to be selected accordingly.
   - The calculated well layout will be displayed.
   - For further options see section 5.2.23 “Determine Well Layout”, page 436.



Calculated field positions (yellow frames) for ReScan

The calculated field positions (yellow frames) are not restricted by a grid anymore. The positions are optimized by the analysis to cover all selected objects using as few fields as possible. The object(s) will be located in the center of the field.

9. **Save** the analysis sequence.
10. Return to the **Setup** screen, select a different well and make a test measurement.
11. Open the **Image Analysis** screen again (*Determine Well Layout*) and check the fields of the calculated well layout.
12. Optimize the analysis sequence until it is robust enough to produce satisfying results in each well.
13. **Save** the final analysis sequence.
14. Switch to the **Setup** screen and select this analysis sequence as online analysis for the PreScan experiment (**Online Jobs – Analysis** pane).
15. **Save** the PreScan experiment.

How to create a ReScan experiment

It is assumed that you have just completed and saved the PreScan experiment so that it is still loaded on the **Setup** screen.

You first need to locate one or multiple typical objects which are selected by the analysis of the PreScan. Then you can optimize the experiment settings for capturing these objects.

Locate objects

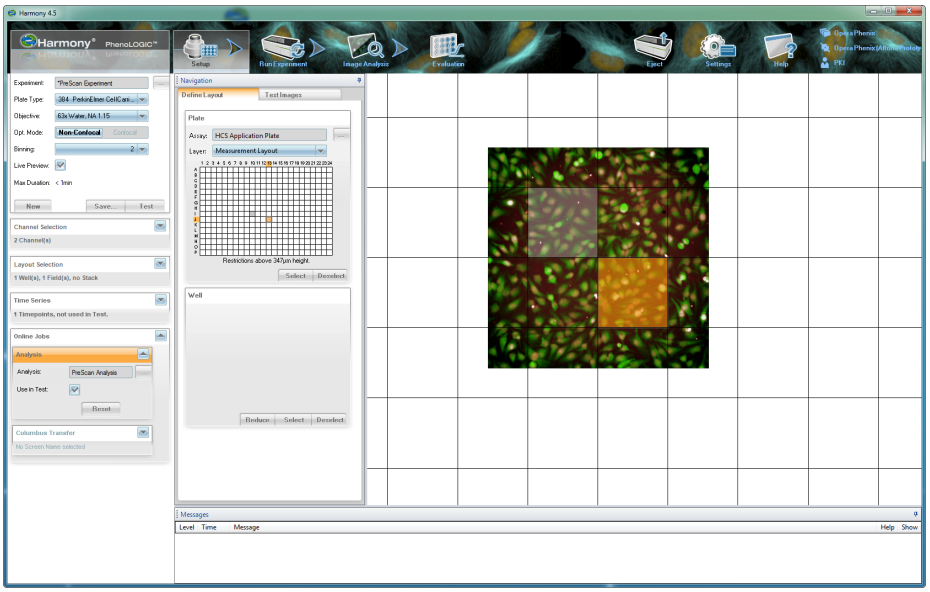
The test measurement of the PreScan should still be present. Otherwise select one well and make a new test measurement (still using the low magnification objective).

1. On the **Image Analysis** screen, open the *Determine Well Layout* building block. Locate a selected object (yellow frame) and memorize its position within the

well/field.

2. Open **Setup – Navigation – Test Images**.
3. Use the **Background for Well** function:
   - Right-click on the well layout in the **Well** pane and select **Background for Well**.

     The current image is used as background image for the well layout.
   - Click **Enlarge** to view the well layout in the **Content Area**.
4. Select the high magnification **Objective** for the ReScan. It must have the same magnification as specified in the online analysis of the PreScan experiment (*Determine Well Layout*, parameter **ReScan Magnification**).



   The fields of the displayed grid are smaller now (due to the higher magnification).
5. Find the memorized object (see step 2) on the background image and select the corresponding field.

Setup experiment parameters for ReScan

You can now start to find the best experiment settings for the ReScan of these objects. This may include changes of channel selection, optical setup, exposure settings etc. For a list of requirements which have to be fulfilled, please see section “Requirements for PreScan and ReScan experiments”, page 122.

1. Make test measurements and optimize the settings by testing different wells.

> **Notice**
> Check the border wells (all fields selected) of your ReScan experiment for possible restrictions of the measurable plate area and modify your selection of objective and focus heights, if necessary. See also section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89.

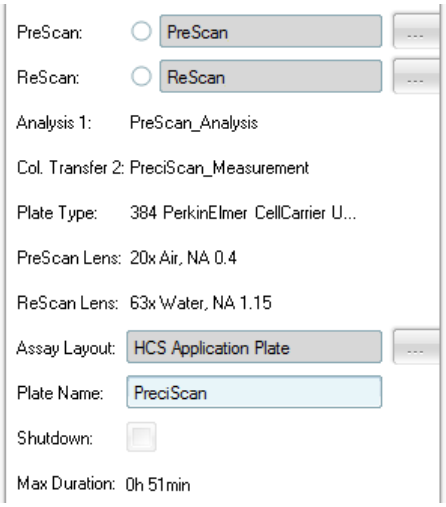
2. Plate layout and well layout are not relevant, because they will be replaced by the well and field positions resulting from the PreScan analysis. You just have to select at least one well, otherwise the plate layout is not valid.
3. Optional: Define a new online job (online analysis or online Columbus transfer). Otherwise remove the existing online analysis (remains of the PreScan experiment).
4. **Save** the ReScan experiment.

How to run a PreciScan measurement

It is assumed that the experiments for PreScan and ReScan have been set up correctly and saved in the database.

1. On the **Run Experiment** screen, set **Scan Mode** to **PreciScan**.

   The **Global Control** section is changed and additional parameters are displayed.
2. Click ... and select the desired **PreScan** experiment (new dialog is opened).
3. Click ... select the desired **ReScan** experiment (new dialog is opened).



   Some properties of the experiments are displayed:

   - Online jobs defined for PreScan/ReScan
   - Objective for PreScan/ReScan
   - Max. duration (currently pure measurement time of PreScan)
4. Check the experiment properties.
   - If you need to modify one of the experiments, see section "How to modify/update one of the experiments selected for a PreciScan", page 129.
   - If you need to modify the PreScan analysis (**Analysis 1**), see section "How to modify/update the online analysis of a PreScan experiment", page 129.
5. If necessary: Click **Eject**, remove the plate and clean the plate bottom. **Load** the plate again.

   This can be necessary if you have been using a water immersion objective before (e.g. during setup of the ReScan experiment) and if you want to use an air

objective for the PreScan. Otherwise the remaining water droplets can lead to focusing errors or distorted images.

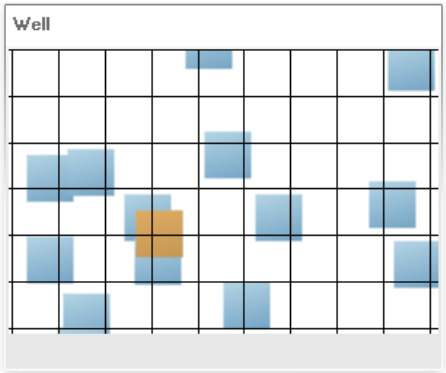
6. Optional: Select an **Assay Layout**. The selected assay layout will be attached to both measurements (PreScan and ReScan).
7. Enter a **Plate Name**.
8. Click Start.

   The PreScan and ReScan experiments are loaded and processed automatically one after the other.

How to view results of a PreciScan measurement

After running a PreciScan, the ReScan measurement will be the active measurement in Harmony.

1. Open the **Image Analysis** screen and click on a measured well.
2. The **Well** pane displays the well layout which was automatically calculated for this well by the online analysis of the PreScan measurement. Every well has a different well layout.



3. Select one field to view the corresponding image.
4. Use the **Load PreScan/ReScan** button to switch between the PreScan/ReScan measurements and to view the corresponding experiments on the **Setup** screen. If you load the PreScan, also the corresponding evaluation will be displayed on the **Evaluation** screen.

Notice

The ReScan experiment that is contained in the ReScan measurement cannot be saved or tested.

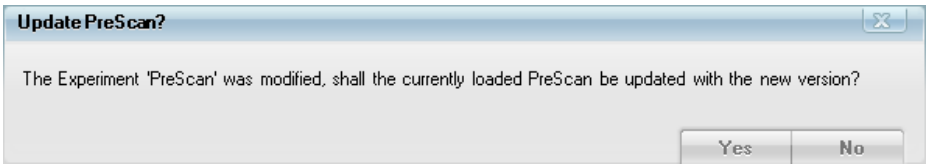
- The only available button for the user on the **Setup** screen is **New**.
- The resulting well layouts cannot be saved or reused in any form.
- You can start the same experiment again on the **Run Experiment** screen if you switch to scan mode **Standard**.

How to modify/update one of the experiments selected for a PreciScan

It is assumed that the experiment to be modified is currently selected for a PreciScan on the **Run Experiment** screen.

1. Open the **Setup** screen and load the PreScan/ReScan experiment.
2. Modify the experiment as required.
3. Click **Save** and overwrite the experiment (option **Save**). Click **OK**.

   Harmony recognizes that an experiment with the same name is currently selected for a PreciScan on the **Run Experiment** screen and displays the following request:



4. Click **Yes** to automatically update the experiment selected for the PreciScan with this new version.

   If you now start a new PreciScan, the updated experiment will be used.

How to modify/update the online analysis of a PreScan experiment

If you modify the online analysis of a PreScan experiment, this change will not immediately be effective in your current PreciScan setup. Please follow one of the following procedures, depending on your starting situation.

It is assumed that the analysis to be modified is used as online analysis in the currently selected PreScan experiment (**Run Experiment** screen).

Situation 1: PreciScan not measured yet

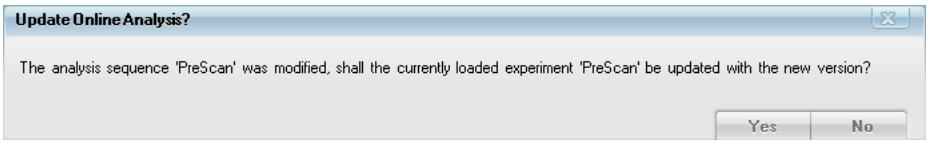
Typical example: You try to start a PreciScan measurement, but validation of the experiments fails (e.g. “Rescan Magnification” in the PreScan analysis does not match selected objective in the ReScan experiment). You have to correct the PreScan analysis.

1. Open the **Setup** screen and load the PreScan experiment.
2. Make sure that the option **Use in Test** is checked for the online analysis (**Online Jobs – Analysis**).
3. Select a well and make a **Test** measurement.

   The measured image is displayed and the analysis sequence of the online analysis is loaded on the **Image Analysis** screen.
4. Open the **Image Analysis** screen and modify the analysis sequence as required.
5. Before saving, open the *Define Results* building block so that the whole analysis sequence is executed.
6. Click **Save**. In the save dialog select option **Save** to overwrite the analysis.

Confirm with **OK**.

Harmony recognizes that an analysis with the same name is currently used as online analysis by the selected PreScan experiment on the **Run Experiment** screen and displays the following request:

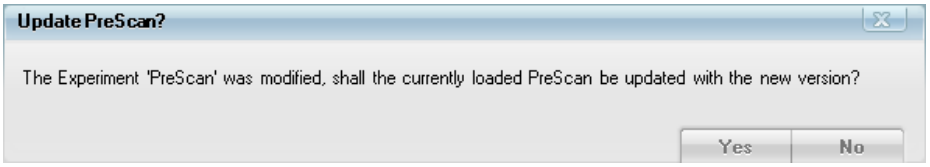


7. Click **Yes** to automatically update the online analysis in the PreScan experiment.

   The PreScan experiment on the **Setup** screen is modified so that it now uses the updated version of the online analysis. The experiment name has an asterisk * in front of its name to indicate that the changes have not been saved yet.

8. On the **Setup** screen, click **Save**. In the save dialog select option **Save** to overwrite the experiment. Confirm with **OK**.

   Harmony recognizes that an experiment with the same name is currently selected for a PreciScan on the **Run Experiment** screen and displays the following request:



9. Click **Yes** to update the selected PreScan experiment.

   If you now start a PreciScan, the updated analysis will be used.

Situation 2: PreciScan measurement existing

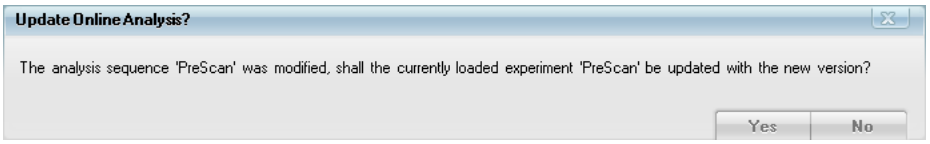
Typical example: You have measured a PreciScan successfully, but you are not happy with the selection of fields and need to improve the PreScan analysis.

1. It is assumed that the **ReScan measurement** is still loaded. Otherwise load it on the **Image Analysis** screen.
2. On the **Image Analysis** screen, click **Load PreScan**.

   The PreScan *evaluation* will be loaded (and thereby the contained measurement, experiment and analysis sequence).
3. Modify the analysis sequence as desired.
4. Before saving, open the *Define Results* building block so that the whole analysis sequence is executed.
5. Click **Save**. The only available option in the save dialog is **Save as**. Confirm with **OK**.

Notice
Do not change the suggested name for the analysis. Otherwise the PreScan online analysis cannot be updated automatically.

Harmony recognizes that an analysis with the same name is currently used as online analysis by the selected PreScan experiment on the **Run Experiment** screen and displays the following request:



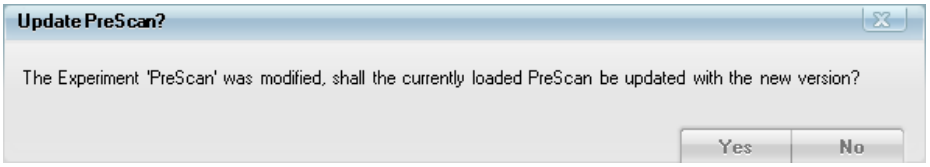
6. Click **Yes** to automatically update the online analysis in the PreScan experiment.

   The PreScan experiment on the **Setup** screen is modified so that it now uses the updated version of the online analysis. The experiment name has an asterisk * in front of its name to indicate that the changes have not been saved yet.

7. On the **Setup** screen, click **Save**. The only available option in the save dialog is **Save as**. Confirm with **OK**.

Notice
Do not change the suggested name for the experiment. Otherwise the PreScan experiment cannot be updated automatically in the PreciScan configuration.

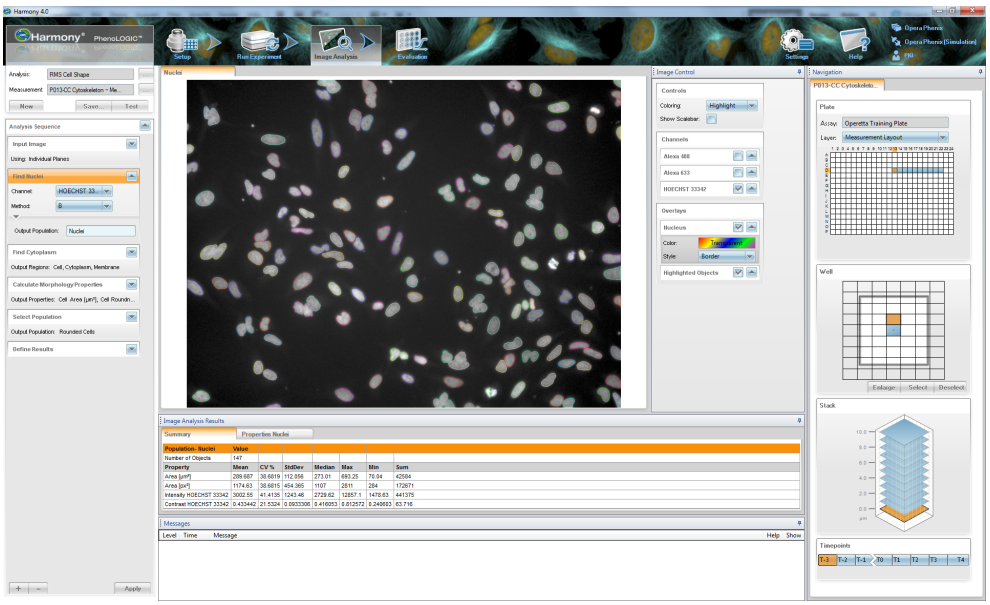
Harmony recognizes that an experiment with the same name is currently selected for a PreciScan on the **Run Experiment** screen and displays the following request:



8. Click **Yes** to update the selected PreScan experiment.

   If you now start a new PreciScan, the updated analysis will be used.

5.1.9 Image Analysis



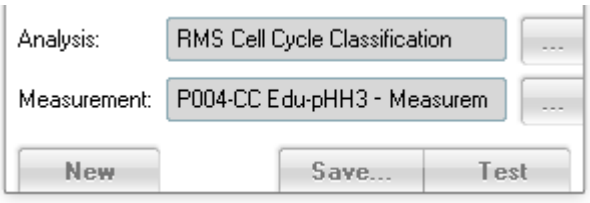
The **Image Analysis** tab is used to define an automated image analysis (analysis sequence) to detect and quantify effects in your images. There are multiple options how to create an analysis sequence:

- **Option 1** - Loading a pre-configured Ready-Made Solution from the database.
- **Option 2** - Using a Ready-Made Solution as a starting point but modifying it as necessary to adapt it to your application.
- **Option 3** - Creating a new analysis sequence from scratch by combining building blocks.

Notice

Please refer to the **Image Analysis Guide** for detailed information on image analysis strategies and a description of the Ready-Made Solutions.

5.1.9.1 Global Control



Common tasks in the **Global Control** section are:

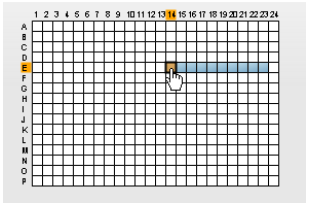
- **Load measurement:**
  Load the measurement to be analyzed. If you have performed a measurement on **Run Experiment** (or a test measurement on **Setup**) and switch to **Image Analysis**, the measurement is already loaded.
- **Load analysis:**
  Load an analysis from the database (e.g. a Ready-Made Solution or a previously created analysis) and adapt it, if necessary. Of course, you can also create a new analysis sequence from scratch.
- **Test analysis sequence:**
  Test the complete analysis sequence in one selected well. The results can be inspected in the **Results** pane. They are not saved in the database.
- **Save analysis sequence**

Notice
If the currently loaded measurement was part of a **PreciScan** (only available with optional license, see section 5.1.8.3 “PreciScan™”, page 119), you can use the **Load PreScan/ReScan** button to easily switch between the corresponding measurements.

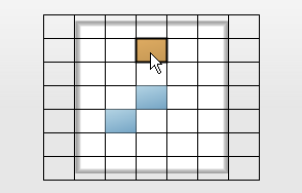
How to test an analysis sequence

Precondition: Measurement performed or loaded, analysis sequence created or loaded

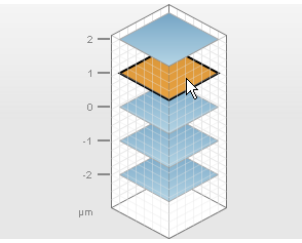
1. In **Navigation – Measurement – Plate** select a single measured well.



2. In **Navigation – Measurement – Well** select a single field.
   A global analysis will always use all fields in the well.

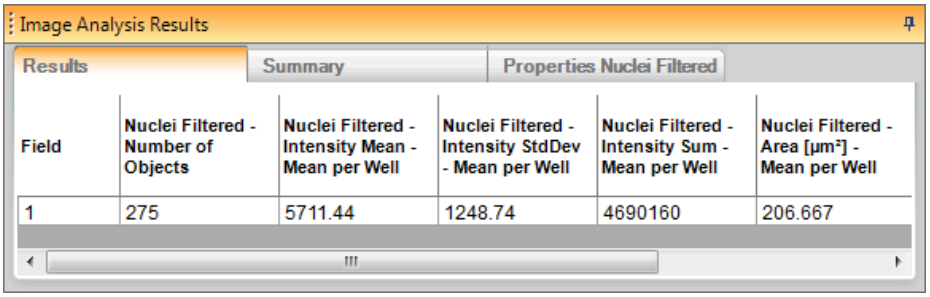


3. In **Navigation – Measurement – Stack** select a single plane (if available).



4. Click Test.
   The selected image is analyzed using the analysis sequence.

5. Results can be inspected in the **Results** pane.

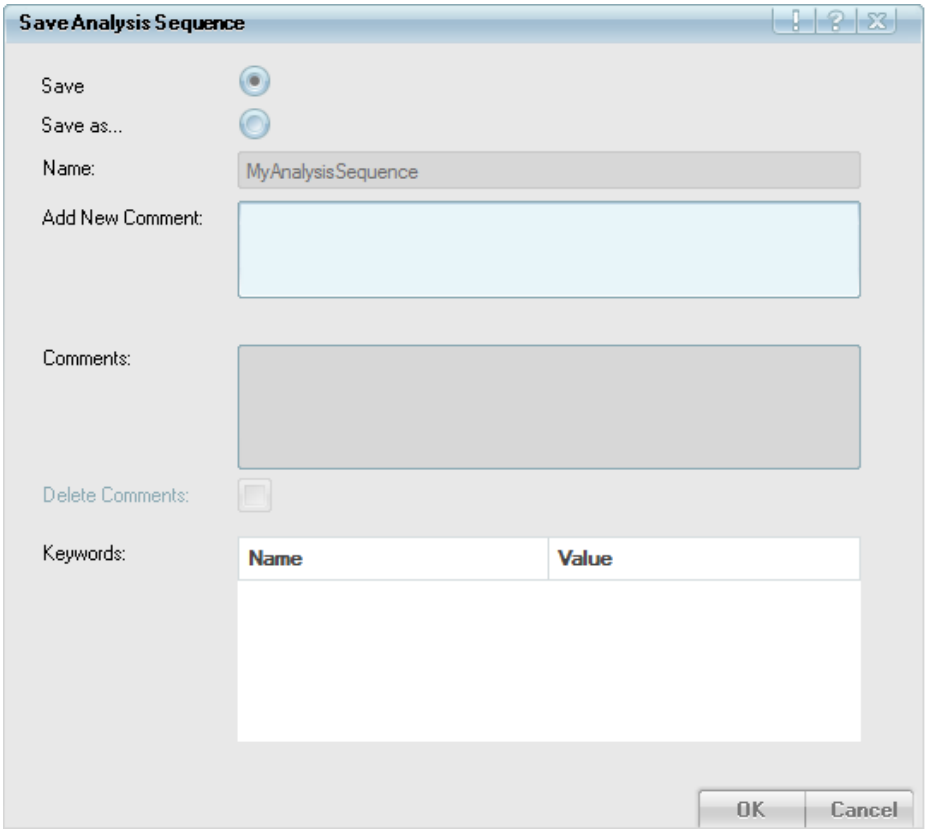


Field	Nuclei Filtered - Number of Objects	Nuclei Filtered - Intensity Mean - Mean per Well	Nuclei Filtered - Intensity StdDev - Mean per Well	Nuclei Filtered - Intensity Sum - Mean per Well	Nuclei Filtered - Area [µm²] - Mean per Well
1	275	5711.44	1248.74	4690160	206.667

How to save an analysis sequence

Notice
It is not necessary to save an analysis sequence. If it has not been saved, it will be "contained" in the evaluation. If you save the analysis sequence, it will be available as a separate data object in the database. If you do not save it and start an evaluation, the keyword for *Analysis Sequence* will be set to *n.a.* (not available).

1. Click Save... . The **Save Analysis Sequence** dialog is opened.



2. Select **Save** or **Save as**:
   - **Save:** Overwrites the existing analysis sequence with a new version. This is useful when saving interim versions. The existing analysis name is displayed and cannot be edited. This option is only available if the analysis sequence has been saved before and if you are the owner of the analysis sequence . The previous version of the analysis sequence will not be accessible in the **Database Explorer** anymore.
   - **Save as:** Saves the analysis sequence as a new object (new signature) in the database. You can enter a new **Name** or keep/modify the existing one (if the analysis sequence has been saved before). The previously saved analysis sequence will also remain accessible in the **Database Explorer**.
3. Enter a **Comment**, if desired.
   Keywords describing the configuration are created automatically, if applicable.
4. Click OK to save the analysis sequence .

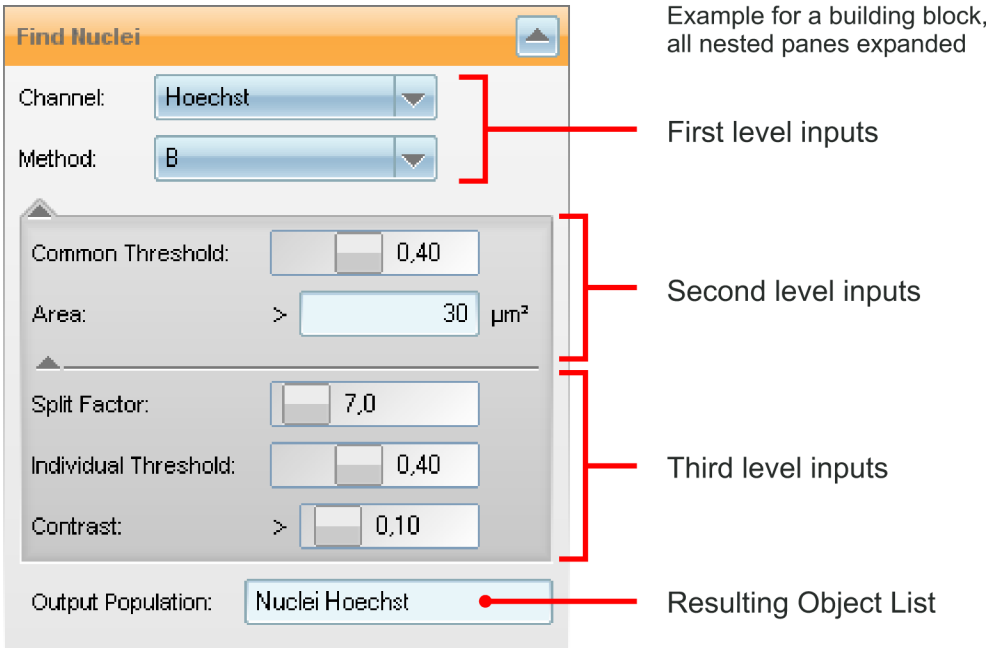
Buttons and Elements

Element	Description
Analysis	Displays the name of the current analysis. An asterisk * in front of the name indicates that the analysis sequence has unsaved changes. The asterisk for unsaved changes is only set after you click Apply or Test or change a property which is applied automatically (via check box or combo box). Click ... to open the **Database Browser** and load an analysis.
Measurement	Displays the name of the currently loaded measurement. Only one measurement can be opened at a time. If the measurement is named with an additional letter (e.g. Measurement 1a), this indicates that it was part of a PreciScan measurement (see section 5.1.8.3 “PreciScan™”, page 119). • Measurement #a: PreScan • Measurement #b: ReScan Click ... to open the **Database Browser** and load a measurement.
Load PreScan/ReScan	If you load a measurement which was part of a PreciScan (see also section 5.1.8.3 “PreciScan™”, page 119), you can use this button to easily load the corresponding PreScan evaluation or ReScan measurement. **Load PreScan:** • Actually, the PreScan *evaluation* is loaded (visible on the **Evaluation** screen). • PreScan measurement, experiment and analysis are contained in the evaluation and are also displayed on the corresponding screens. **Load ReScan:** • The ReScan measurement and the contained experiment will be loaded and displayed on the corresponding screens. • If the ReScan experiment contains an online analysis, it will also be loaded and displayed. Otherwise the current analysis sequence will be kept. • One or multiple evaluations may exist, but will **not** be loaded automatically.
New	Removes/empties all building blocks (except **Input Image** and **Define Results**) to start a new analysis sequence.
Test	Applies the whole analysis sequence to the selected image. Only a single well, field and plane may be selected.
Save...	Allows to save the current analysis sequence to the database.

5.1.9.2 *Analysis Sequence*

To allow you to easily create customized analysis sequences, the Harmony software contains a set of building blocks for each step, which can be combined into a full analysis sequence. The building blocks are processed in top-down order. Intermediate results of the selected (and previous) building blocks are immediately displayed, e.g. as overlays and/or results tables.

Each building block offers multiple parameters to adapt it to your specific application. The first level inputs determine e.g. the data source (channel) or the mathematical approach for the analysis (method). Fine tuning can be done using the second and third level inputs.



Input Image is the first building block of each analysis sequence. If you select this block, the original image (selected in the **Navigation** pane) is displayed in the **Content Area**.

The last building block **Define Results** is used for performing the statistical analysis of arbitrary populations. It also defines the assay readout values for each well of a microplate (results per well). All properties calculated by the preceding building blocks can be included in this readout, as well as any combination (by formula).

All other illustrations and intermediate results of the building blocks are only temporary. Only the readout values that you select in **Define Results** are stored in the database.

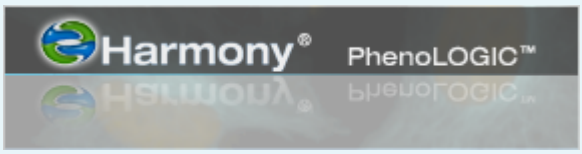
Further information:

- For a complete description of all building blocks, including all their adjustable parameters, please see section 5.2 “Building Block Reference”, page 325.
- For detailed information on image analysis strategies and many examples of how the building blocks can be used for a wide range of different applications see the **Image Analysis Guide**.

Notice

Building blocks (or single methods within a building block) which use the PhenoLOGIC™ technology are only available with an optional license. For details see section "License Management", page 320.

If the required license is activated on your license dongle, the PhenoLOGIC™ logo will be displayed next to the Harmony logo.

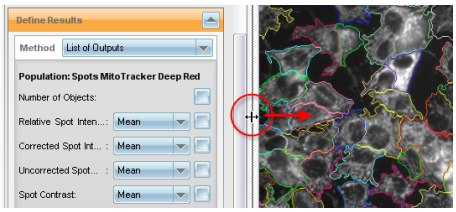


Notice

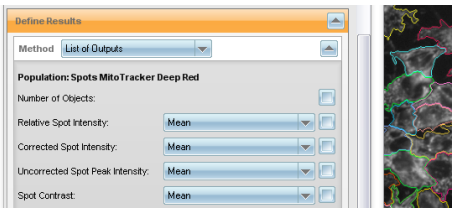
- You can generate a print version of the analysis sequence with all its settings if you right-click the headline **Analysis Sequence** and select **Show Print Version...** A formatted HTML page will be opened in your web browser. See also the description of the context menu below for details.
- You can add comments to the analysis sequence or to a building block using the context menu option **Edit comment ...** These comments will also appear in the print version.

Screen Adjustment

The **Analysis Sequence** pane can be resized to wide view (e.g. for formulas or long property names) if you drag the border to the right using the mouse. The size can only be adjusted in two steps: regular view or wide view.



Regular view

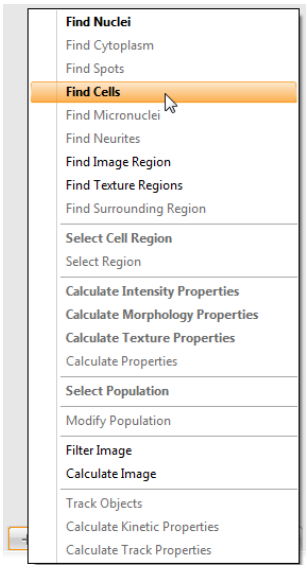


Wide view

How to add and configure a building block

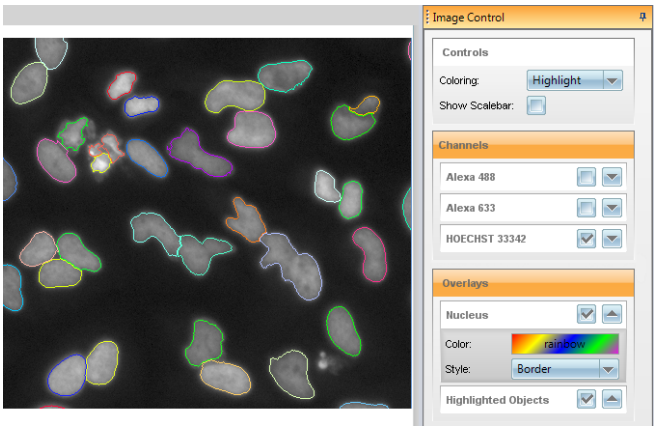
Precondition: Measurement to be analyzed is loaded or has been performed before.

1. Click + and select the desired building block from the popup list. Only building blocks suitable for the selected position in the sequence can be selected.

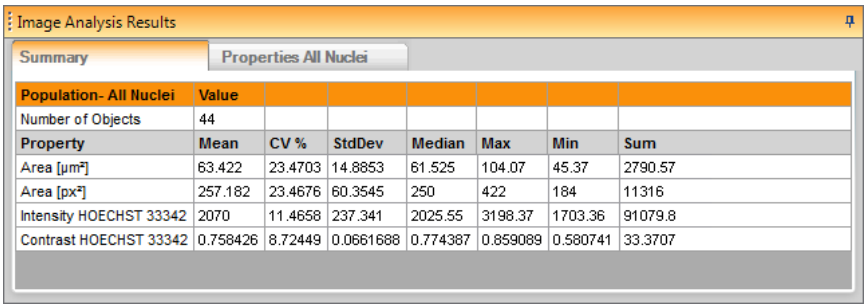


The new building block is inserted after the current position in the analysis sequence. It is executed immediately using default settings.

- If the building block generates (temporary) visual outputs (e.g. overlay mask with detected nuclei), they are displayed in the **Content Area**. The appearance of image and overlays can be controlled in the **Image Control** pane.

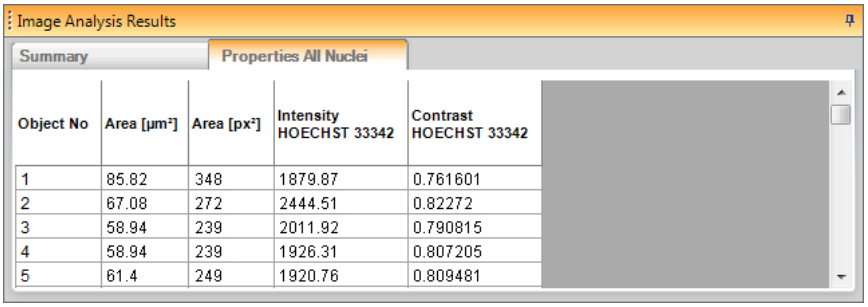


- In **Results – Summary** you get intermediate results (averaged results for the whole well) of the building block.



Population- All Nuclei	Value						
Number of Objects	44						
Property	**Mean**	**CV %**	**StdDev**	**Median**	**Max**	**Min**	**Sum**
Area [µm²]	63.422	23.4703	14.8853	61.525	104.07	45.37	2790.57
Area [px²]	257.182	23.4676	60.3545	250	422	184	11316
Intensity HOECHST 33342	2070	11.4658	237.341	2025.55	3198.37	1703.36	91079.8
Contrast HOECHST 33342	0.758426	8.72449	0.0661688	0.774387	0.859089	0.580741	33.3707

- In **Results – Properties** you can see the detailed list of detected objects and their properties (on single object basis).



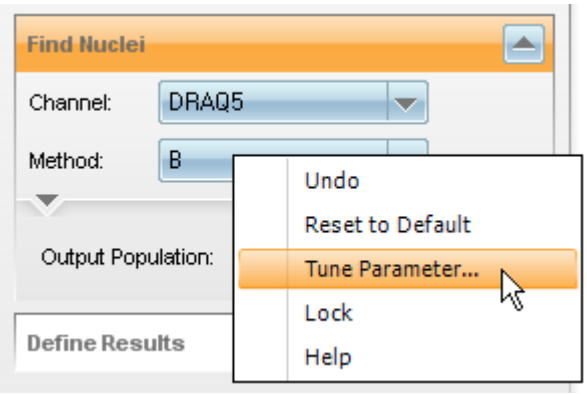
2. Select the first level inputs of the building block.
   This will typically be the **channel** to be analyzed and a **method**, i.e. a different algorithm. Further inputs can be **object lists** or other outputs generated by previous building blocks.
   After each modification the resulting overlay image in the **Content Area** and the **Results** pane are updated.
3. Adjust the second and/or third level parameters, if necessary.
   See also next section “How to tune a parameter”.
4. Optionally: Rename the **Output** of the building block.
5. Add further building blocks as required.

Further steps:

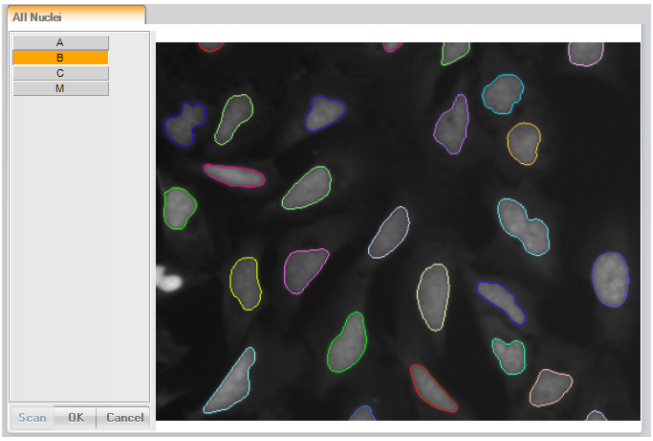
- Configure the **Define Results** block to select the final outputs of the analysis sequence.
- **Test** the analysis sequence and modify, if necessary.
- **Save** the analysis sequence.

How to tune a parameter (combo box entry)

1. Right-click on the respective parameter and select **Tune Parameter** from the context menu.



In the **Content Area**, the **Tune Parameter** control is displayed on the left. Each value of the combo box is represented by a button.

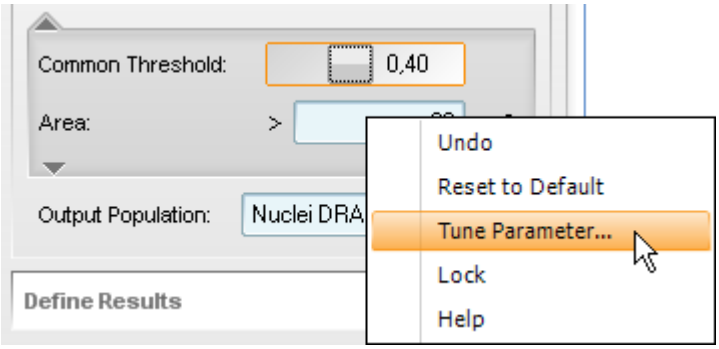


Notice
It can take a few seconds until all overlays have been calculated.

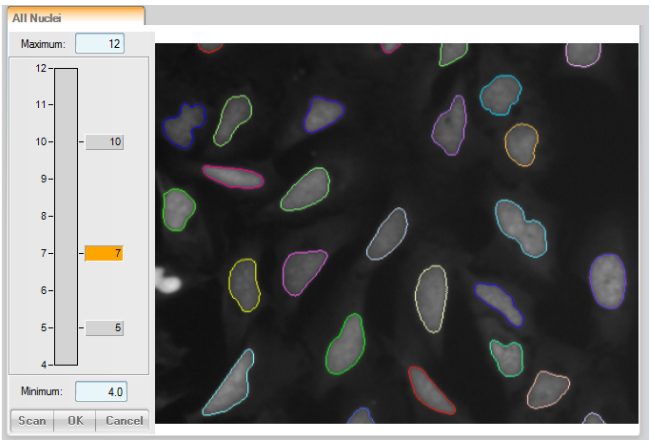
2. Move the mouse cursor over the buttons to see the corresponding overlay immediately.
3. Click the desired button of the value which produces the best visual result. The button becomes orange.
4. Click **OK** to accept the value and return to the building block.

How to tune a parameter (numerical parameter)

1. Right-click on the input box or slider of a numerical parameter and select **Tune Parameter** from the context menu.



In the **Content Area**, the **Tune Parameter** control is displayed on the left as a scale of values. The current value is indicated by a marker (orange). Additionally, there are markers for one higher and one lower value. For building blocks *Find Nuclei* and *Find Cells* certain parameters have a “Best” marker indicating the recommended value (only within the selected range, the really best value may be outside the current range).



Notice
It can take a few seconds until all overlays have been calculated.

2. Move the mouse cursor over the markers to see the corresponding overlay immediately.
3. If none of the values produces the desired result:
   - Insert markers to test additional values: **Left-click** on the scale at the desired position. You can also **right-click** the scale to enter a numerical value (a small window with an input field is opened).
   - Extend the range of the scale: Enter a higher value for **Max** and/or a lower value for **Min** (accept by pressing **Enter**) or move the scale with the mouse (see notice below) to see a different value range. Insert markers to test further values.

- If you want to test a wide variety of different values, click **Scan**. The range of the scale is extended and multiple new markers are added automatically.

Notice

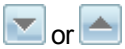
- **Zoom scale:** After a scan, the new markers may overlap each other which makes it difficult to select them. If you place the mouse cursor on the scale and scroll the mouse wheel, you can zoom in and out of the scale. The **Min** and **Max** values are adapted automatically.
- **Move scale:** If you click next to the scale, keep the button pressed and drag the mouse, the visible section of the scale can be moved.

4. Click the marker of the value which produces the best result. The button becomes orange.
5. Click **OK** to accept the value and return to the building block.

How to define results

For detailed information please see section 5.2.27 “Define Results”, page 450.

Analysis Sequence: Buttons and Elements

Element	Description
	Click the corresponding disclosure button to open or close the analysis sequence.
	Adds a building block after the currently selected position (orange block title) in the analysis sequence. The list of available building blocks will depend on the selected position because of dependencies between the building blocks.
	Removes the selected building block from the analysis sequence. Select **Remove All** from the menu to reset the analysis sequence.
	Applies the changes made to the analysis sequence, i.e. the output of the building block or the whole analysis sequence is generated again using the new configuration. Parameter values which you select from a combo box (e.g. **Method**) are applied immediately. For all numerical parameters (with slider or input box) you have to click **Apply** before the new value is applied.

Analysis Sequence: Context Menu

Context Menu	Description
Show Print Version...	Opens a formatted HTML page in your web browser showing the current analysis sequence including all building blocks, inputs, outputs and parameter settings. The list has a printable format and can be used for documentation and review of the sequence at a glance. Changed parameters (other than default value) are marked. The print version is self-contained. No other information is needed to restore the sequence.
Edit Comment...	Opens a new window where you can enter a comment for the whole analysis sequence. This comment will be displayed in the "Print Version" of the analysis (see above). Comments can be formatted in multiple lines and may contain any character. Formatting is preserved in the print version. The comment can be saved using the **Save** button or discarded by just closing the edit window.
Help	Opens the help function for the Analysis Sequence in the Harmony Help.

Buidling Block – Headline: Context Menu

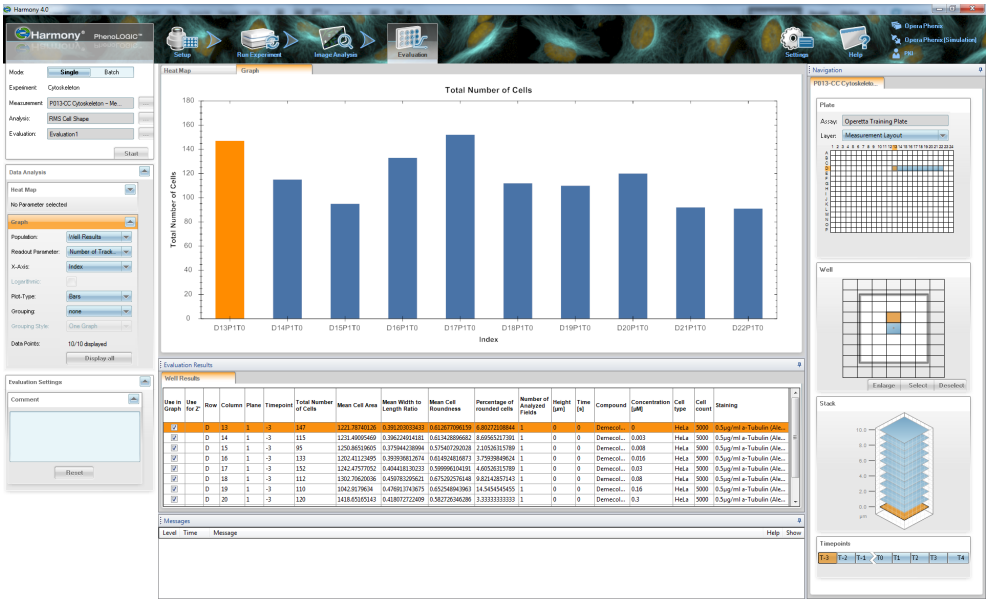
Context Menu	Description
Help	Opens the help function for this building block in the Harmony Help.
Edit Comment...	Opens a new window where you can enter a comment for this building block. The comment will be displayed in the "Print Version" of the analysis. Comments can be formatted in multiple lines and may contain any character. Formatting is preserved in the print version. The comment can be saved using the **Save** button or discarded by just closing the edit window.

Building Block – Parameters: Context Menu

Context Menu	Description
Undo	Undo the previous modification of the parameter value.
Reset to Default	Resets the parameter value to the default value.

Context Menu	Description
Tune Parameter ...	Allows you to find the best value for a parameter by testing and previewing different settings. A new control element is displayed in the **Content Area**. Depending on the type of the parameter there are two versions of this control: • **Value selectable from a combo box (e.g. Method):** In this case, the results for all selectable options are calculated. Then you can easily and quickly switch between the resulting overlays (by mouse-over) of the different parameter values to compare them and find the best setting. • **Numerical value:** In this case, the control element in the **Content Area** is shown as a scale of values. The current value is indicated by a marker, and a lower and a higher setting is pre-defined. If you move the mouse over the respective marker, you can see the resulting overlay. You have the option to insert further markers for desired values or modify the scale range.
Lock	Locks this parameter to avoid modification. The locked state is indicated by a little lock icon next to the parameter name. Click the lock icon to unlock the parameter. **Example application** An experienced user creates the analysis sequence for a new user. The locks could be used to indicate those parameters which have already been optimized and should not be modified anymore.
Help	Opens the help function for this building block in the Harmony Help.

5.1.10 Evaluation

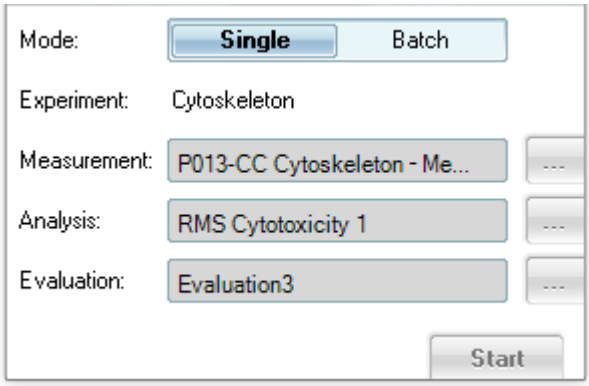


The **Evaluation** tab offers the following functions:

- Evaluate measurements:
  - **Single mode:** Evaluate or re-evaluate selected wells (or the whole plate) of a measurement with an analysis sequence.
    See section “Single Mode”, page 147.
  - **Batch mode:** Evaluate multiple measurements with the same analysis sequence.
    See section “Batch Mode ”, page 151).
  - **Evaluation Settings:** Add meta information (e.g. enter a description or comment) before starting an evaluation.
    See section 5.1.10.3 “Evaluation Settings”, page 169.
- Visualize evaluation results:
  - **Results table:** The resulting readout values can be inspected in the **Results** pane.
  - **Heat map:** Select one readout parameter and visualize the readout values by a heat map.
    See section “Heat Map”, page 154.
  - **Graph:** Select one readout parameter and visualize the readout values by a graph. Create IC50 and Z' statistics.
    See section “Graph”, page 157.

5.1.10.1 Global Control

Single Mode



If a measurement or analysis has not yet been loaded or created on a previous tab, you need to load a measurement and an analysis sequence for the evaluation. You can also load existing results for a re-evaluation. In this case, the corresponding experiment, measurement and analysis sequence are loaded automatically.

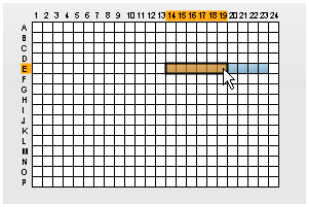
Notice
If you have selected a flatfield correction method in the analysis sequence, this correction must be available for the measurement(s) to be analyzed. Otherwise the best available correction method will be used. For further details please see section 5.1.12.5 “Flatfield Correction”, page 205.

How to evaluate a single measurement

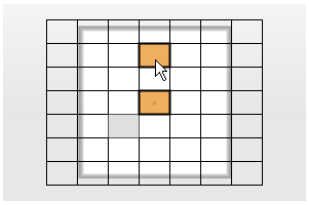
Notice

- Please note that the evaluation may take very long time if you select many images (e.g. multiple fields, stack planes or time points).
- If you select multiple wells and if the measurement is the ReScan of a PreciScan, then all fields are selected automatically. Every well has a different well layout. See also section 5.1.8.3 “PreciScan™”, page 119.

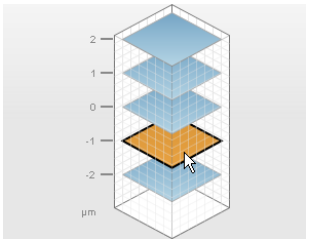
1. Make sure that **Single** mode is active.
2. Click ... next to the **Measurement** box and load a measurement (if necessary).
3. Click ... next to the **Analysis** box and load an analysis sequence.
4. In the **Navigation – Measurement – Plate** pane, mark the desired well(s) to be evaluated.



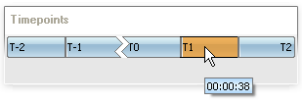
5. In the **Navigation – Measurement – Well** pane, mark one or multiple fields to be evaluated.



6. In the **Navigation – Measurement – Stack** pane, mark one or multiple planes (if available).



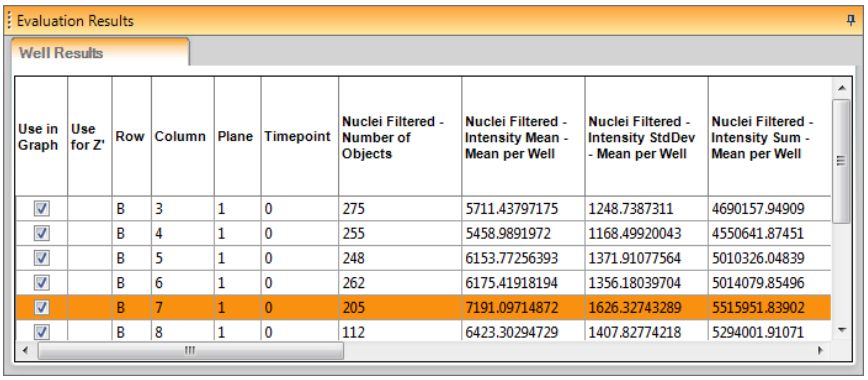
7. In the **Navigation – Measurement – Timepoints** pane, mark one or multiple time points (if available).



8. Click Start to start the evaluation.

Notice
The selected wells are not necessarily processed in the same order as during the measurement. The evaluation order cannot be influenced.

- The readout values (well results) can be inspected in the **Results** pane.



Use in Graph	Use for Z'	Row	Column	Plane	Timepoint	Nuclei Filtered - Number of Objects	Nuclei Filtered - Intensity Mean - Mean per Well	Nuclei Filtered - Intensity StdDev - Mean per Well	Nuclei Filtered - Intensity Sum - Mean per Well
☑		B	3	1	0	275	5711.43797175	1248.7387311	4690157.94909
☑		B	4	1	0	255	5458.9891972	1168.49920043	4550641.87451
☑		B	5	1	0	248	6153.77256393	1371.91077564	5010326.04839
☑		B	6	1	0	262	6175.41918194	1356.18039704	5014079.85496
☑		B	7	1	0	205	7191.09714872	1626.32743289	5515951.83902
☑		B	8	1	0	112	6423.30294729	1407.82774218	5294001.91071

- The results of the evaluation are saved in the database (named *Evaluation#*). The evaluation number is increased if you re-evaluate the measurement.

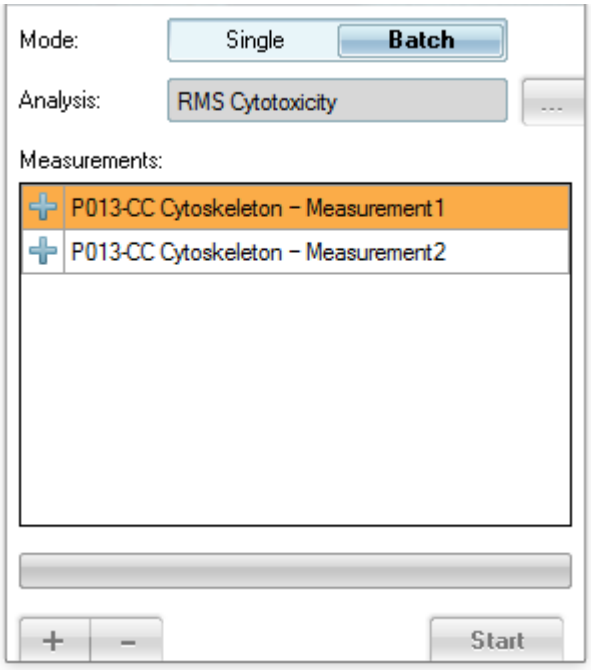
How to inspect an existing evaluation

1. Click ... next to the **Evaluation** box and load the evaluation of the desired measurement from the database (if necessary). The corresponding experiment, measurement and analysis sequence is loaded automatically.
2. The readout values (well results) can be inspected on the **Evaluation** tab:
   - The **Results** pane displays the averaged readout values for each well.
   - In **Data Analysis – Heat Map** you can select one readout parameter to be visualized as a heat map in the **Content Area** or as a layer in **Navigation – Measurement – Plate**.
   - In **Data Analysis – Graph** you can select one readout parameter to be visualized as a graph of selected data points in the **Content Area**.
3. Results on single object basis (e.g. object properties, images, overlays etc.) can be inspected on the **Image Analysis** tab.

Buttons and Elements

Element	Description
Mode 	• **Single Mode:** Allows to evaluate a single measurement. You have the option to evaluate only selected wells or the whole plate. • **Batch Mode:** Allows to evaluate a batch of multiple measurements. Always the whole plate is evaluated.
Experiment	Displays the current experiment.
Measurement	Displays the current measurement. Click ... to load a different measurement to be evaluated.
Analysis	Displays the current analysis sequence. Click ... to load a different analysis sequence for the evaluation.
Evaluation	Displays the current results file. Click ... to load a result file (e.g. for re-evaluation). The corresponding experiment, measurement and analysis is loaded automatically.
	Starts the evaluation of the selected well(s). The processing order of the wells cannot be influenced. During the evaluation the button turns into a  button which can be used to cancel the evaluation. • If you stop an evaluation, all non-evaluated wells become yellow. These wells will not be saved with the evaluation, but you can still view the corresponding image e.g. on the **Image Analysis** tab. • If you stop an evaluation before at least one well has been evaluated successfully (blue), the evaluation will not be saved.

Batch Mode



In **Batch** mode you can evaluate multiple existing measurements using the same analysis sequence. All measured wells (including all fields) are processed, you cannot select the wells or fields to be evaluated.

Notice
Please note that the evaluation may take very long time if the measurement includes many images (e.g. due to multiple fields, stack planes or time points).

You can add multiple measurements to the list which are processed one after another. While a measurement is evaluated, you can use all functions such as heat map or graph as you would do during a manual evaluation.

However, you have only access to the active evaluation (indicated by the icon). As soon as the evaluation of one measurement is finished, the evaluation is saved and the evaluation of the next measurement is started. To view an earlier evaluation you have to switch to **Single** mode and load the evaluation manually (after the batch evaluation has been completed).

Notice
If you have selected a flatfield correction method in the analysis sequence, this correction must be available for the measurement(s) to be analyzed. Otherwise the evaluation will fail. For further details please see section 5.1.12.5 “Flatfield Correction”, page 205.

How to evaluate multiple measurements

1. Activate the **Batch** mode.
   The **Global Control** section for the **Batch** mode is displayed.
2. Click [...] next to the **Analysis** box and load an analysis sequence.
3. Click [+] to load a measurement and add it to the list.
4. Add further measurements as desired.
5. Click [Start] to start the evaluation.

All measurements in the list will be evaluated one after another using the selected analysis sequence.

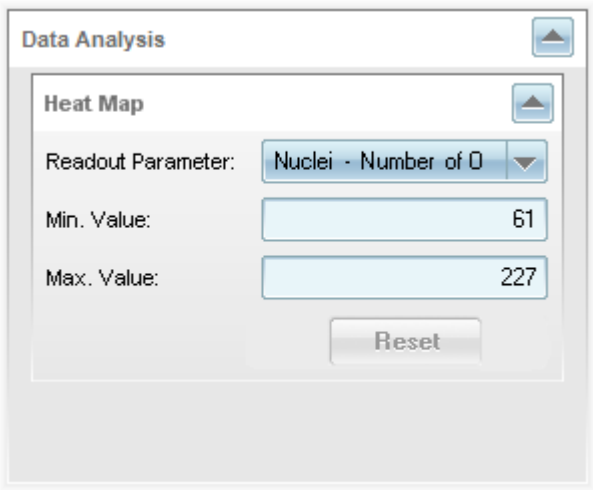
Buttons and Elements

Element	Description
Mode Single Batch	• **Single Mode:** Allows to evaluate a single measurement. You have the option to evaluate only selected wells or the whole plate. • **Batch Mode:** Allows to evaluate a batch of multiple measurements. Always the whole plate is evaluated.
Analysis	Displays the analysis sequence which is applied to the whole batch of measurements. Click [...] to load a different analysis sequence for the evaluation.
Measurements	Displays the list of measurements to be evaluated. You can add/remove measurements using the [+] and [–] buttons. **Status icons:** To do Active Evaluation successful Evaluation with warnings (see **Messages** window for details) Evaluation failed
Progress bar	Displays the total progress of the batch evaluation.
[+]	Opens the **Database Browser** to load a measurement and adds it to the list.

Element	Description
	Removes the selected measurement from the list.
 	Starts the evaluation of the selected measurements. The processing order of the wells cannot be influenced. During the evaluation the button turns into a  button which can be used to cancel the evaluation. • If you stop an evaluation, all non-evaluated wells become yellow. These wells will not be saved with the evaluation, but you can still view the corresponding image e.g. on the **Image Analysis** tab. • If you stop an evaluation before at least one well has been evaluated successfully (blue), the evaluation will not be saved.

5.1.10.2 Data Analysis

Heat Map

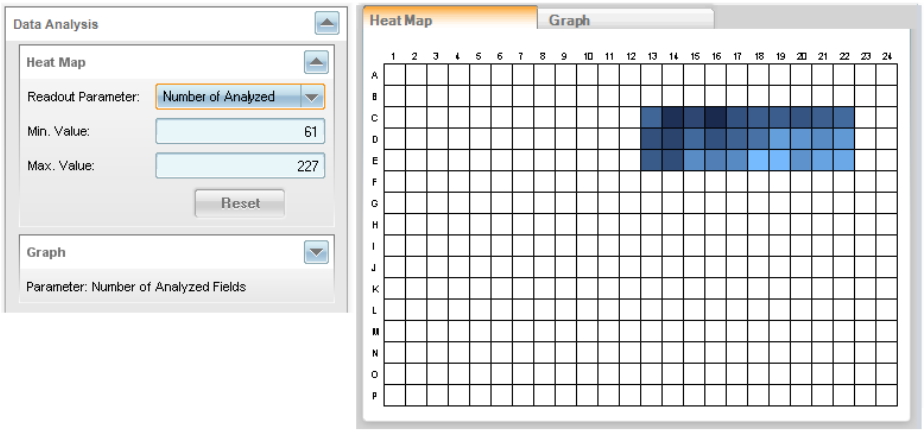


A heat map is used to visualize the values of one selected readout parameter (well result) by the color of each well of the plate. Light blue indicates low values, dark blue indicates high values. The heat map is displayed in the **Content Area**. It can also be selected as a layer in the **Navigation – Measurement – Plate** pane.

How to view a heat map

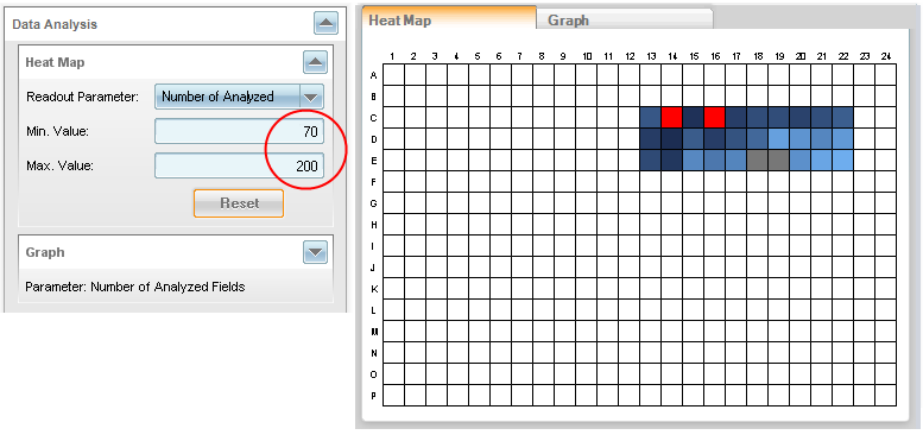
Precondition: Evaluation has been performed

1. Open the **Data Analysis – Heat Map** pane.
2. Select the desired readout parameter from the **Readout Parameter** box.



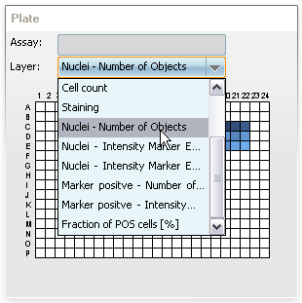
The heat map is displayed in the **Content Area**. **Min. Value** is set to the smallest, **Max. Value** to the highest readout value on the plate, i.e. all evaluated wells are displayed.

3. To exclude extremely low or high values, modify **Min. Value** and **Max. Value** and press **Enter**.

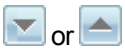


Wells exceeding the new parameter range become gray (lower than **Min. Value**) or red (higher than **Max. Value**). The color coding of the other wells is adapted to reflect the new parameter range.

4. The heat map can also be selected as a layer in **Navigation – Measurement – Plate**. If you switch e.g. to the **Image Analysis** tab, you could activate this layer and click on a well to see the corresponding image or an overlay of detected objects (e.g. to examine outliers).



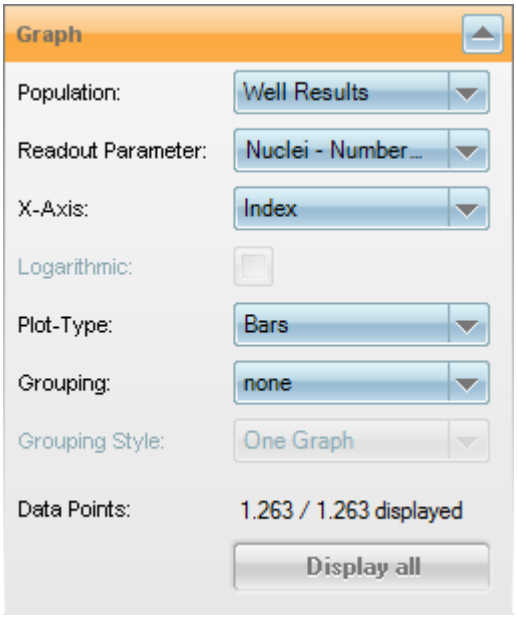
Buttons and Elements

Element	Description
	Click the disclosure button to open or close the **Data Analysis** pane and/or the **Heat Map** pane. The **Heat Map**/**Graph** tab of the **Content Area** is switched accordingly.
Readout Parameter	Allows you to select the readout parameter which will be visualized in the heat map.
Min. Value	• These options determine the lower and upper limit of the visualized parameter range. They can be used to exclude extremely small or high values. • Color coding: ◦ **Min. Value** defines the value represented by light blue. ◦ **Max. Value** defines the value represented by dark blue. ◦ Outliers become gray (lower than **Min. Value**) or red (higher than **Max. Value**). ◦ Wells with evaluation errors remain white.
Max. Value	
	Restores the default settings for **Min. Value** and **Max. Value**.

Context Menu (Heat Map)

Element	Description
Save Image ...	Allows you to save the current heat map as an image file.
Help	Opens the context-sensitive help for this screen section.

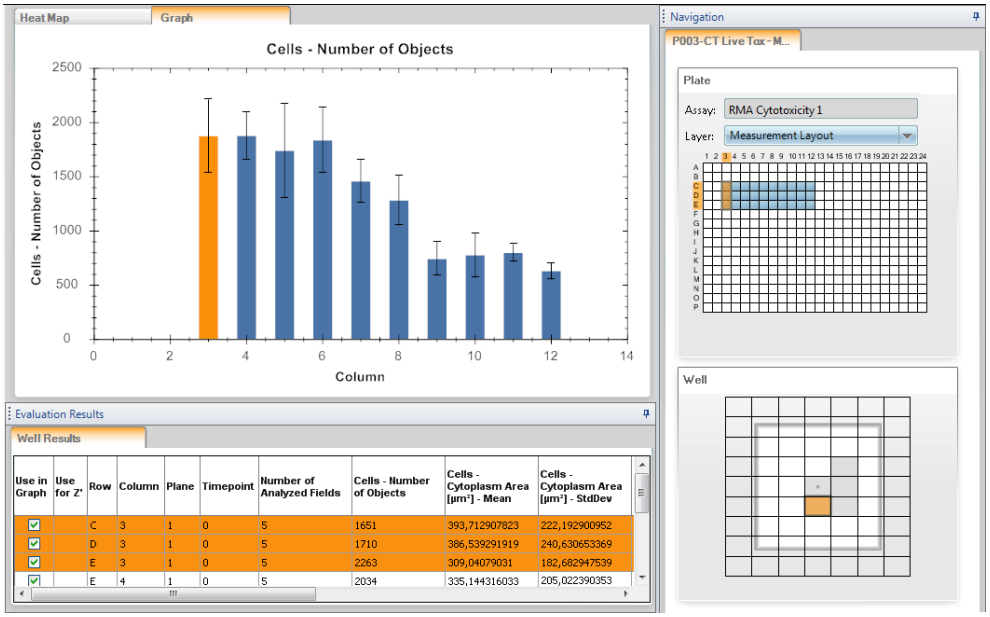
Graph



This section is used to visualize the values of one selected readout parameter (well result) for selected wells as a two-dimensional graph. The readout values are displayed on the y-axis, the definition of the x-axis is arbitrary. If you select a population (local or global) instead of well results, you can also visualize object results.

There is a close interaction between the graph, the **Navigation – Measurement** pane and the **Evaluation Results** table. If you select one data point in the graph, the corresponding well, field, plane and time point and the corresponding data row in the table are selected and vice versa.

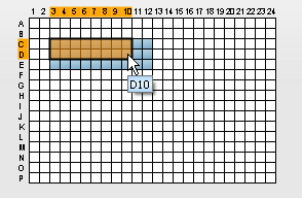
It is also possible to display an EC50 curve (dose-response) or calculate a Z' value. For detailed instructions please refer to the following sections.



How to create a graph (well results)

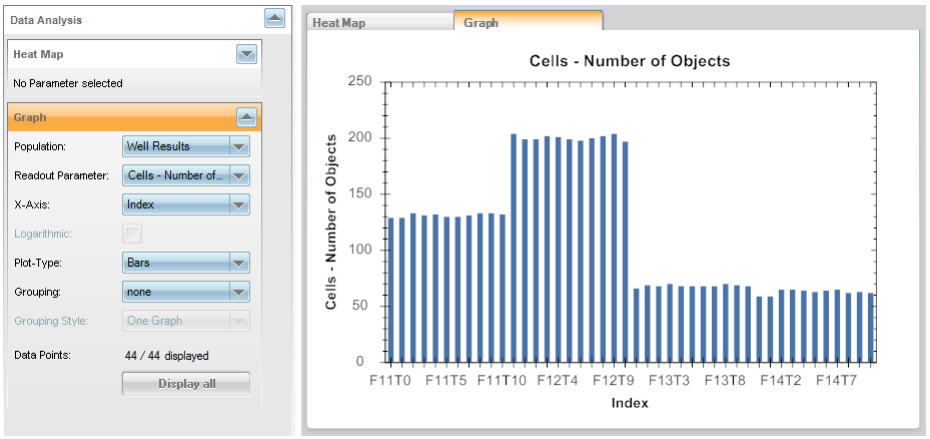
Precondition: Evaluation has been performed

1. Open the **Data Analysis – Graph** pane and make sure that **Population: Well Results** has been selected.
2. Select the desired wells for the graph in **Navigation – Measurement – Plate**.



3. Optionally: Select the desired planes and time points in **Stack** and **Timepoints** (if applicable). Fields cannot be selected because the evaluation results are well results (one readout value for the whole well).
4. Right-click the plate view in the **Plate** pane and select **Graph – Display Selection**. A default graph is displayed which includes the selected data points.
5. In the **Graph** pane, select the desired readout parameter from the **Readout Parameter** box.
6. Define the **X-Axis**.

   Default setting: **Index** (data points are displayed in the same order as the results in the **Evaluation Results** table; click a column title to sort the table by the desired parameter).
7. Optionally: You can select a **Logarithmic** scale for the x-axis (if applicable) or select a different **Grouping** or **Plot-Type** (see section “Buttons and Elements").
8. The graph is displayed in the **Content Area**.



   If you click one of the data points, the corresponding well (and plane/time point if available) is highlighted in the **Navigation** pane and the corresponding data row is highlighted in the **Evaluation Results** table.
9. To include or exclude data points:

- Select or deselect rows in the **Evaluation Results** table and select **Graph – Display Selection** from the context menu.
- Select different wells (and planes or time points if applicable) in the **Navigation** pane and select **Graph – Display Selection** from the context menu to apply the new selection.
- Click Display all if you want to include all data points of the evaluation.

How to create a graph (object results)

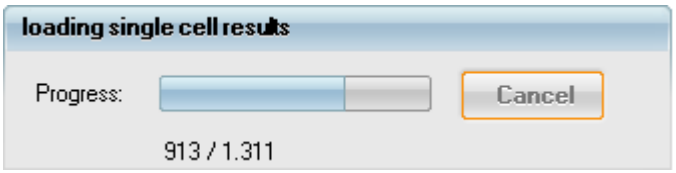
Precondition: Evaluation has been performed and includes object results

Notice

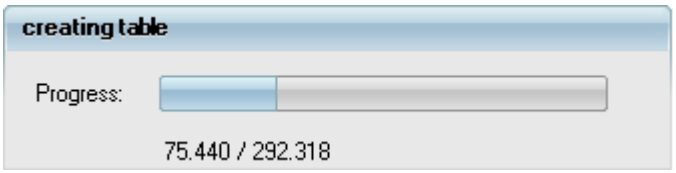
When using object results, the number of data points will increase significantly. The result table is limited to **500,000** rows. If this limit is reached, you have to reduce the number of selected wells, fields, planes, and time points.

It is recommended to select only one well initially and to extend the selection afterwards as far as required.

1. Identify wells of interest by analyzing the well results (see description before).
2. Select the desired wells, planes and time points in the **Navigation** pane.
3. Reduce the data points displayed in the graph by using **Graph – Display Selection** from the context menu of the **Navigation** area or the results table.
4. In the **Data Analysis – Graph** pane select a **Population** which has associated object results.
   - The object results of the selected images are loaded.



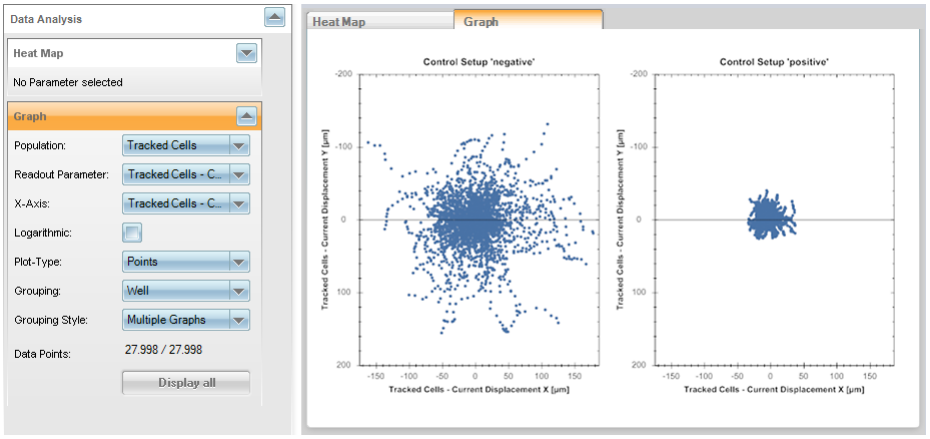
   - The result table is created.



     All loaded data points will be listed in the table and displayed in the graph.
5. Select the desired readout parameter from the **Readout Parameter** box.
6. Define the **X-Axis**.
   Default setting: **Index** (data points are displayed in the same order as the results in the **Evaluation Results** table; click a column title to sort the table by the desired parameter).
7. Optionally: You can select a **Logarithmic** scale for the x-axis (if applicable) or select a different **Grouping**, **Grouping Style** or **Plot-Type** (see section “Buttons

and Elements").

8. The graph is displayed in the **Content Area**.



If you click one of the data points, the corresponding well (and plane/time point if available) is highlighted in the **Navigation** pane and the corresponding data row is highlighted in the **Evaluation Results** table.

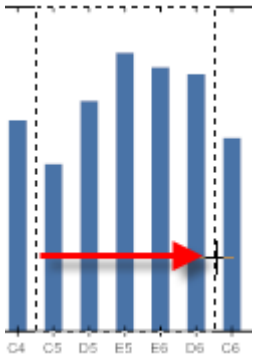
9. To include or exclude data points:
   - Select or deselect rows in the **Evaluation Results** table and select **Graph – Add Selection** or **Graph – Remove Selection** from the context menu.
   - Activate/deactivate the **Display** check box of the corresponding row(s) in the **Evaluation Results** table.
   - Select different wells (and planes or time points if applicable) in the **Navigation** pane and select **Results table – Display Selection** from the context menu to apply the new selection.
   - Click Display all if you want to display all data points which have been loaded (i.e. all data points listed in the **Evaluation Results** table).

How to zoom the graph

You can zoom in/out using the **mouse wheel**. If you press and hold the **Shift** key, you can temporarily switch to panning mode and move the graph.

To zoom in a desired area:

1. Click on the graph and hold the mouse button.
2. Drag the mouse to the left or right.



3. Release the mouse button.
   The selected part of the graph is magnified.

To zoom out:

- Select **Set Scale to Default** to view the whole graph.

How to create an EC50 curve

Precondition: Assay layout with compound concentrations is included or has been attached to the measurement. Make sure that at least one layer is named "Concentration" at the beginning, the remaining characters do not matter. The layer type must be of type "numeric". The first layer matching these conditions will be used.

1. Select the desired wells in the **Plate** pane, right-click the wells and select **Graph – Display Selection** from the context menu.
2. Select the desired **Readout Parameter**.
3. Select **EC50** as **Plot Type**. The **X-Axis** is automatically set to **Concentration** and the EC50 curve is displayed.

How to calculate a Z' value

Precondition: Measurement includes positive and negative control wells.

1. Select neg. control wells in the **Plate** pane, right-click the wells and select **Use for Z' negative Control** from the context menu.
2. Select pos. control wells in the **Plate** pane, right-click the wells and select **Use for Z' positive Control** from the context menu.
3. Select **Z'** as **Plot Type**.
4. Select the desired **Readout Parameter**. The corresponding Z' value is displayed.
5. Try different readout parameters to determine the most appropriate one for your assay.

Definition: EC50

The concentration of a compound that is needed to provoke a response of the assay readout value halfway between the baseline and maximum response (half maximal effective concentration).

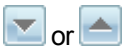
$$Y = (\text{Bottom}) + \frac{(\text{Top}) - (\text{Bottom})}{1 + \left(\frac{X}{EC_{50}}\right)^{-(\text{Hill coefficient})}}$$

Definition: Z' Value (Z prime)

A number, which measures the accuracy and sensitivity of an assay (including the image analysis algorithm). High Z' values indicate low noise and a large signal window (SNR = Signal to Noise Ratio). Z' values above ~0.4 are considered sufficient for screening cellular assays, Z' values above ~0.6 are considered a good value. The theoretical maximum Z' value is 1.0 (= no noise).

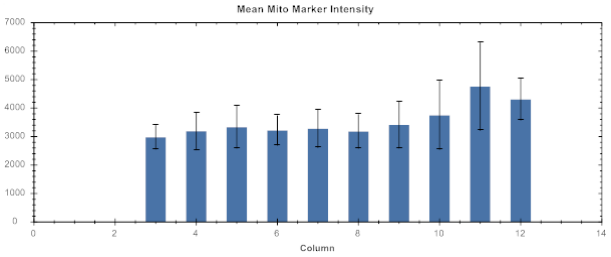
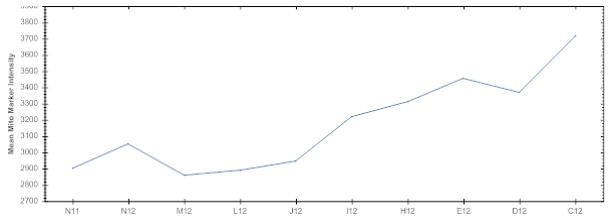
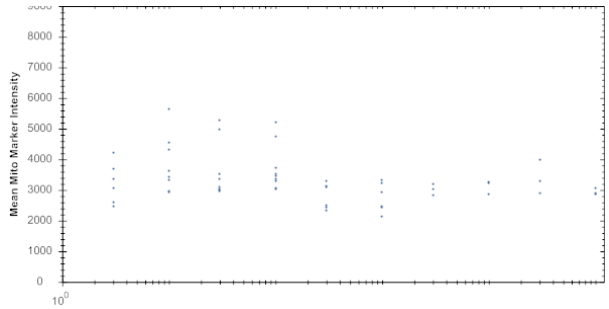
$$Z' = 1 - \frac{3*(stddev(\text{Negative Control}) + stddev(\text{Positive Control}))}{|mean(\text{Negative Control}) - mean(\text{Positive Control})|} = 1 - \frac{3}{SNR}$$

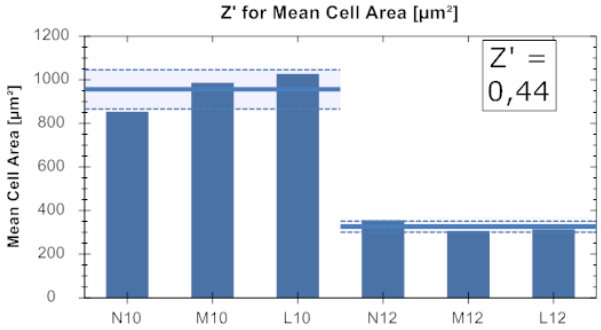
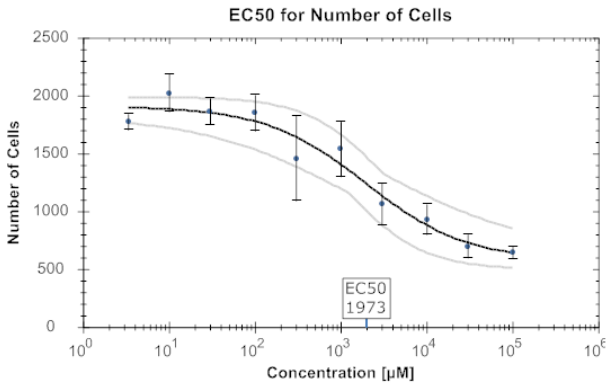
Buttons and Elements

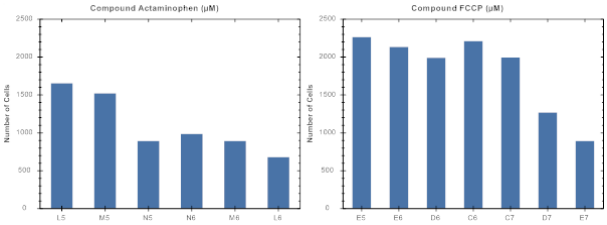
Element	Description
 or	Click the disclosure button to open or close the **Data Analysis** pane and/or the **Graph** pane. The **Heat Map**/**Graph** tab of the **Content Area** is switched accordingly.

Element	Description
Population	Allows you to select **well results** or **object results** of a population for the graph display. If there are multiple populations with object results, the population to display can be chosen from the drop-down list (e.g. Spots, Cells, Nuclei etc.). **If you select Well Results:** • The **Evaluation Results** table will contain all well results. • The data points to be displayed can be selected and changed via the context menus of **Results** table and **Navigation** pane. **If you select any of the listed populations:** • All object results associated with the currently selected well results are loaded and displayed in the table and graph. The selection can be modified using the context menu of the **Navigation** pane (**Results table - Add/Remove Selection**). • The data points to be displayed in the graph can be selected and changed using the context menu of the **Results** table (**Graph – Add/Remove Selection**). **Notice** When using object results, the number of data points will increase significantly. The result table is limited to **500,000** rows. If this limit is reached, the loading process will stop and you have to reduce the number of data points: 1. Select a reduced number of wells, fields, planes, and time points. It is recommended to select only one well initially and to extend the selection afterwards as far as required. 2. Select **Results table – Display Selection** from the context menu of the **Navigation** area to apply the selection. Only the selected data points will be loaded and displayed in table and graph.
Readout Parameter	Allows you to select the readout parameter which will be visualized in the graph.

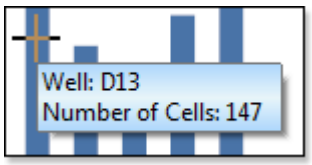
Element	Description
X-Axis	Allows to define the plotting mode of the x-axis, for example: • **Index:** Data points are displayed in the same order as the results in the **Evaluation Results** table. This mode can be used for a plate overview, to check for fluctuations/homogeneity, to find outliers or to check for drift effects. ◦ Click a column title to sort the table by the desired parameter. • **Row:** Averages the results for each row. This mode can be used to view dose response (row-wise pipetting) or to view high-low areas without assay layout. • **Column:** Same as **Row**, but for column-wise pipetting. • **Numeric assay layout layer:** If an assay layout has been attached to the measurement, you can also plot the readout values against a numeric layer of the assay layout, e.g. concentration or cell count. • **Readout parameter:** Can be used for plotting readout parameters against each other, e.g. to find correlated parameters. Once you analyze a **time series** there are three options to display the time on the x-axis: • **Timepoint:** Number of the time point. The number can be negative (time point before break), zero (T0, first time point of sequence 1) or positive (time point of sequence 1 or 2). See also section 5.1.7.4 “Time Series”, page 68. • **Time:** Time in seconds of each time point in relation to *T0* (*T0* is defined as time 0:00:00 in every well). This is done separately for every well. If you look at the same timepoint over different wells, you will see that they differ slightly within in a few hundred ms. • **Time (avg.):** Average of your y-axis variable and the time of each well and time point. Hence if you zoom in you will also see an error bar for the x-axis.
Logarithmic	Allows you to select a logarithmic scale for the x-axis.

Element	Description
Plot-Type	• **Bars:** Displays one bar per data point or the mean value with error bar (standard deviation) if there are multiple values per x-value (e.g. if **X-Axis** is **Column**).  • **Lines:** Displays the data points as a curve of connected points.  • **Points:** Displays each data point, multiple points per x-value are possible. 

Element	Description
Plot Type (continued)	• **Z':** Displays a number measuring the accuracy and sensitivity of an assay (including the image analysis algorithm); useful to determine the most appropriate readout parameter. To calculate a Z' value, you need to define positive and negative control data points.  • **EC50:** Displays a dose-response curve to determine the half maximal effective concentration.  **Notice** An EC50 curve can only be displayed if there is an assay layout with compound concentrations attached to the measurement. For details see section 5.1.7.8 “Assay Layout Editor”, page 94.

Element	Description
Grouping	The data points will be grouped by the values of the selected property. Assay layout layers of type “Text” (if existing).can also be selected. There will be a separate graph for each unique value. This can be used to group the results e.g. by compound, by area (high/low), or by object. **Grouping** will only have an effect if there are at least two different values for the selected property. 
Grouping Style	Only available if **Grouping** has been selected: • **One Graph:** The curves of the grouped data points will be plotted to one single diagram, i.e. with overlaid curves. • **Multiple Graphs:** Multiple diagrams are displayed, one for each group item.
Data Points	Depending on the selected **Population** parameter: • **Well Results:** ◦ *First value:* Number of well results currently displayed in the graph. ◦ *Second value:* Total number of well results in the evaluation. • **Population with object results:** ◦ *First value:* Number of object results currently displayed in the graph. ◦ *Second value:* Number of object results which have been loaded. This number is identical to the number of data points in the result table. The number of object results which can be loaded is limited to 500,000.
Display all	Depending on the selected **Population** parameter: • **Well Results:** All data points of the evaluation will be displayed in the graph. • **Population with object results:** Displays the set of object data in the graph that was loaded initially. To load further object results, select additional wells, fields, time points or planes and choose **Results table – Add Selection** from the context menu of the **Navigation** pane. Note that the limit of 500,000 data points still applies.

Context Menu (Graph)

Element	Description
Panning	Allows you to permanently activate the panning mode, i.e. you can move the graph instead of zooming.
Zoom	Allows you to permanently activate the zoom mode, i.e. you can select a rectangular area which is zoomed in.
Extended Selection	Allows you to select a rectangular area on the graph. All data points within this area are selected.
Set Scale To Default	Displays an overall view of the graph.
Show Value Tooltip	If this option is activated, there will be a tooltip if you move the mouse over a data point stating the well coordinate and the readout value. 
Copy	Copies an image of the graph to the clipboard.
Save Image As...	Allows you to save the current graph as an image file.
Page Setup...	Opens a dialog where you can define the page setup for printing the graph.
Print...	Opens a dialog to print the graph.
Help	Opens the context-sensitive help for this screen section.

5.1.10.3 Evaluation Settings

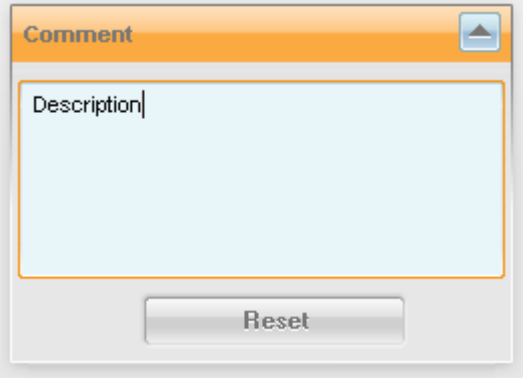
The **Evaluation Settings** can be used optionally to add information to the evaluation. The evaluation itself is named automatically (*Evaluation#*). If you evaluate the same plate again, the evaluation number is incremented.

Notice

- The information in **Evaluation Settings** has to be entered *before* starting an evaluation. Otherwise it is not saved with the evaluation.
- Evaluations inherit the current **assay layout** of the measurement. If you change the assay layout of the measurement (i.e. detach and attach a new one) the assay layout will also be updated for existing evaluations. For detailed information see also section 5.1.16.3 “About Keywords”, page 232.

Comments

This section allows you to enter free text describing the evaluation. This can be useful for adding specific information which is not included in the automatically generated keywords. You can search in these comments using the **Database Browser** (**Search in Comments ...**). See also section 5.1.16 “Database Browser”, page 220.



Buttons and Elements

Element	Description
or	Click the disclosure button to open or close the **Comments** pane.
Comment	Field for entering free text.
Reset	Removes the entered comment.

5.1.11 Navigation

The **Navigation** pane displays physical positions on the plate and time points of a time series:

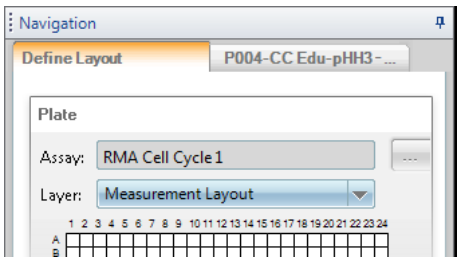
- **Plate** for displaying/selecting wells on the plate.
- **Well** for displaying/selecting image fields within the well.

- **Stack** for displaying/selecting planes (z-axis).
- **Timepoints** for displaying/selecting time points of a time series.

Navigation is available on each of the four main tabs. However, its function depends on the tab and on the context.

There are two different types of navigation controls: **Define Layout** and **Measurement** (with slightly different titles). They look identical, but they differ in their function.

- **Define Layout** only appears on the **Setup** tab and is used for defining a measurement layout. For a detailed description see section 5.1.7.6 “Navigation (Setup)”, page 81.



Define Layout (only on Setup)

- **Measurement** appears on all tabs and exists in two different types. Its name depends on the kind of the current measurement (plate measurement or temporary test measurement or snapshot).

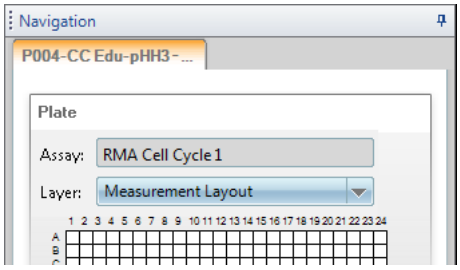
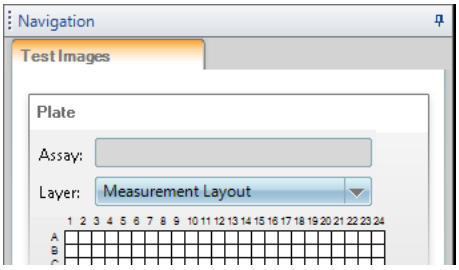


Plate measurement

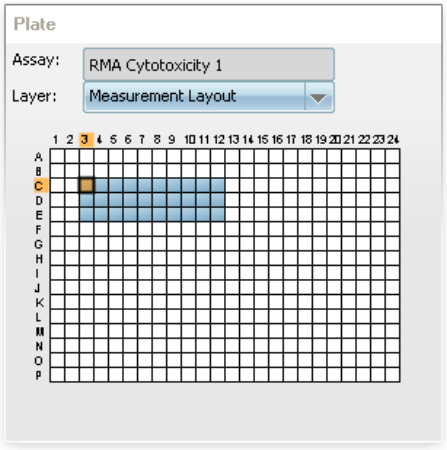


Snapshot or test measurement

1. **Plate Measurement** (the pane is named like *[Plate Name] – Measurement [#]*) is used to view measured images of a regular plate measurement and for progress display.
   On the **Image Analysis** tab it is also used to select images for the analysis.
   On the **Evaluation** tab it is used to select images for the evaluation.
   For a details see section 5.1.11.1 “Plate Measurement”, page 171.
2. **Test Images** is very similar to **Plate Measurement**. The difference is that a test measurement always consists of one single well (including well layout/stack, if selected). A snapshot only consists of one single image (for selected channel and current position). For a detailed description see section 5.1.11.2 “Test Images”, page 187.
   For more information on test measurements see section 5.1.7.1 “Global Control”, page 42 (**Setup** tab).

5.1.11.1 Plate Measurement

Plate



The **Plate** pane is used to:

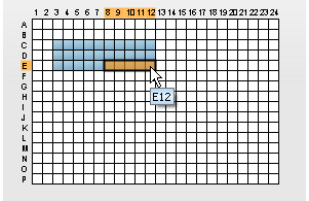
- Select a single well of which you want to view the measured image (field and plane can be selected in **Well** and **Stack**).
- View progress of a measurement, analysis or evaluation (for an explanation of the color coding of the wells see section 5.1.11.3 “Color Coding”, page 189).
- View thumbnail images of the selected wells, fields, planes and time points (**Overview**) to get an overview of the plate quickly.
- Select images for a **Time Window** (**Image Analysis** tab only).
  A time window is a subset of a time series measurement, used to develop an analysis sequence which makes use of object tracking. For details see below and section 5.2.24 “Track Objects”, page 438.

Notice
Wells of a plate format with more than 384 wells are always displayed as rectangular wells, regardless of the real shape of the well.

How to open an Overview

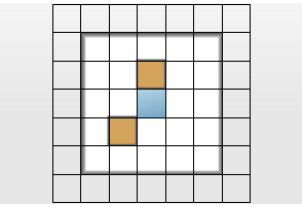
Precondition: Measurement performed or loaded

1. In the **Navigation – Measurement – Plate** pane, select one or multiple wells which have already been measured (blue).



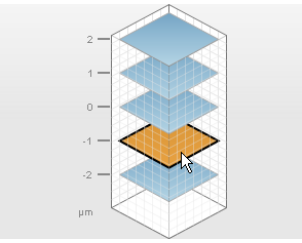
2. In the **Navigation – Measurement – Well** pane, select one or multiple measured fields (blue).

> **Notice**
> If multiple wells have been selected and if the measurement is the ReScan of a PreciScan, then all fields are selected automatically. Every well has a different well layout. See also section 5.1.8.3 “PreciScan™”, page 119.

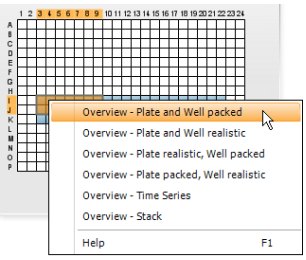


3. In the **Navigation – Measurement – Stack** pane, select one single plane (if available).

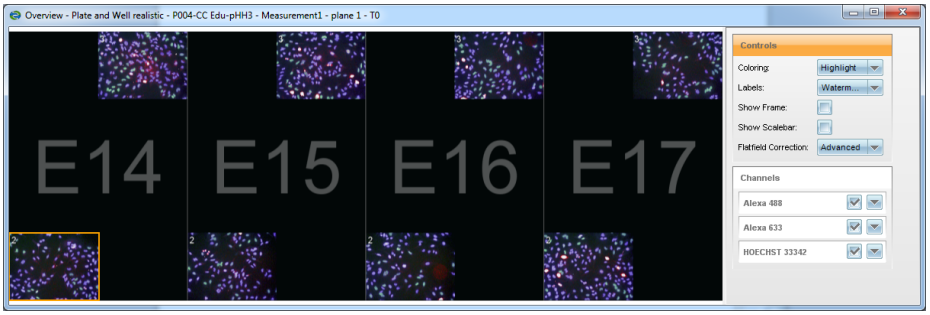
> **Notice**
> If multiple stack planes are selected, the maximum projection is shown in the overview.



4. Right-click on the selected well(s) (**Plate** pane) to open the context menu and select **Overview**. Depending on the selected images you can select between different visualizations (combinations of realistic or packed view of the wells and fields).
Stacks and **Time Series** can also be visualized, but to select one of these two options you only may select one single well. For a detailed description of the options see section “Context Menu”, page 175.



The **Overview** window is opened. Each selected well is represented by a miniaturized image. If multiple fields have been selected, the fields are grouped well by well.



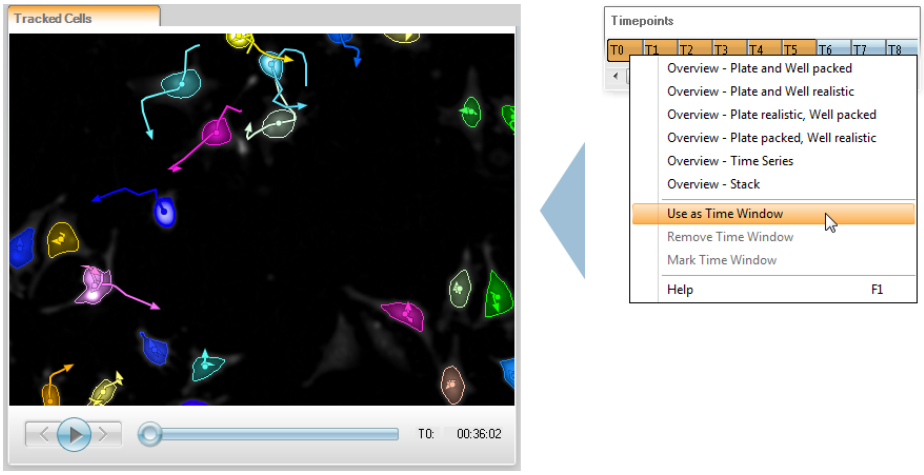
For a description of the **Overview** window see also section "Overview", page 184.

How to define a Time Window

1. Select one single well.
2. Select one single field.
3. If there is a stack, select one or multiple planes.
4. Select multiple time points.

> **Notice**
> It is recommended to select only a few (e.g. five) time points to reduce calculation times during object tracking.

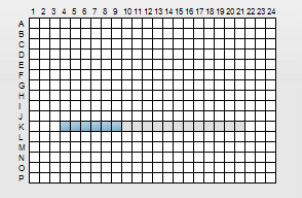
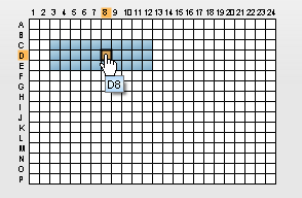
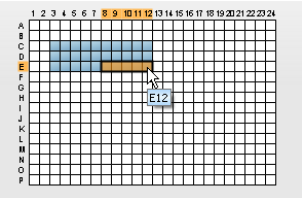
5. Right-click on the selection and select **Use as Time Window** from the context menu.



The time points are displayed as a movie in the **Content Area** (if supported by current building block). See also section 5.1.13.2 "Movie Control", page 213.

The time window can be removed and re-defined as required using the context menu (see below).

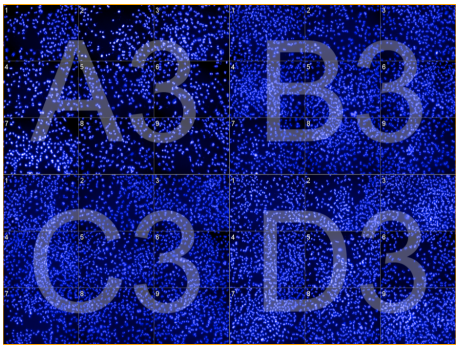
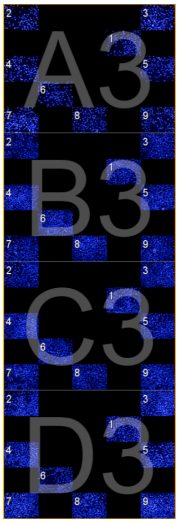
Buttons and Elements

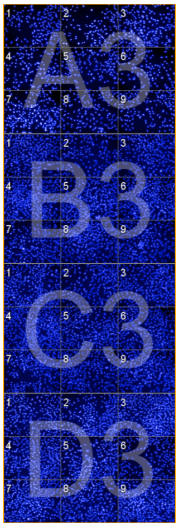
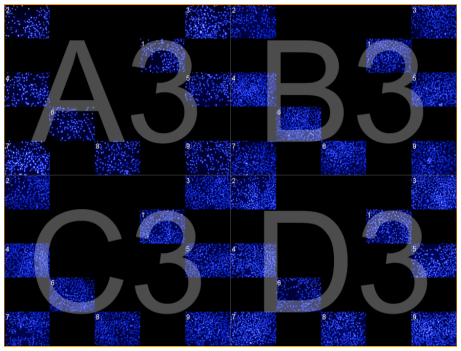
Element	Description
Name	Displays the name of the current assay layout.
Layer	Allows you to select a layer of the assay layout to be displayed on the plate. If you have defined a heat map on the **Evaluation** tab, it can be selected here as a layer. Select **Measurement Layout** to return to the regular view.
	**View progress/status** During a measurement, analysis or evaluation the progress is displayed by the color of the wells. For details see section 5.1.11.3 “Color Coding”, page 189.
	**View image** Click on a measured well (blue). The first image in this well is displayed in the **Content Area**. Different fields or planes can be selected in **Well** and **Stack**. Using the cursor keys you can move the selection to view the images of neighboring wells.
	**Select multiple wells** e.g. for overview or evaluation • Click and hold the mouse button to select multiple wells in a rectangular area. • Press and hold the **Ctrl** key to select multiple single wells successively.

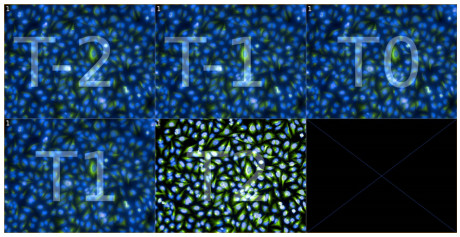
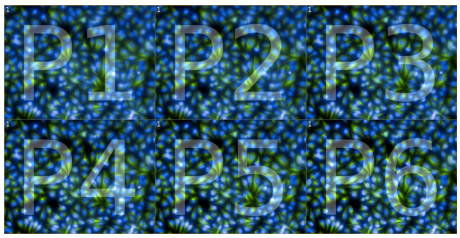
Context Menu

Notice

Regardless of whether you select **packed** or **realistic** style for an **overview**, unmeasured fields in the outer region of the selection and unselected or unmeasured columns/rows of wells will not be displayed.

Element	Description
Overview – Plate and Well packed (not available during an evaluation)	Opens the **Overview** window for the selected wells and fields. The thumbnails of the selected wells and fields are arranged in a space-saving way. The arrangement does not correspond to their real position in the well anymore. Unselected or unmeasured wells and fields are not displayed. 
Overview – Plate and Well realistic (not available during an evaluation)	 Opens the **Overview** window for the selected wells and fields. The arrangement of the thumbnail images corresponds to the real position of the wells and fields on the plate. Unselected or unmeasured wells/fields are displayed black.

Element	Description
Overview – Plate realistic, Well packed (not available during an evaluation)	 Opens the **Overview** window for the selected wells and fields. The arrangement of the thumbnail images corresponds to the real position of the wells on the plate. Unselected/unmeasured wells are displayed black with a cross. The thumbnails of the selected fields are arranged in a space-saving way. The arrangement does not correspond to their real position in the well anymore. Unselected fields are not displayed.
Overview – Plate packed, Well realistic (not available during an evaluation)	Opens the **Overview** window for the selected wells and fields. The thumbnails of the selected wells are arranged in a space-saving way. The arrangement does not correspond to their real position on the plate anymore. Unselected wells are not displayed. The thumbnail images of the fields are arranged according to their real position in the wells. Unselected/unmeasured fields are displayed black. 

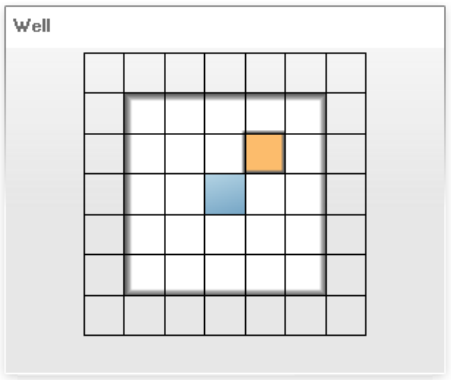
Element	Description
Overview – Time Series (not available during an evaluation)	Only possible if one single well and one plane have been selected. Opens the **Overview** window for the selected time points. The thumbnails of the selected time points are arranged in a space-saving way labeled with the time point number. Unselected time points are not displayed. 
Overview – Stack (not available during an evaluation)	Only possible if one single well and one time point have been selected. Opens the **Overview** window for the selected stack planes. The thumbnails of the selected planes are arranged in a space-saving way labeled with the plane number. Unselected planes are not displayed. 
Use as Time Window (only on Image Analysis tab)	**Requirement:** One well, one field, and multiple time points selected. A time window is created based on the selected elements of the measurement. Only the images included in the time window (orange or blue) can be used, all other elements become gray. The **Content Area** will switch to movie mode, i.e. you can view the time points of the time window as a movie clip, useful for watching object movements. This is only supported by certain “time-aware” building blocks, e.g. *Input Image* and the building blocks involved in object tracking.
Remove Time Window (only on Image Analysis tab)	The time window is removed. The entire measurement can be accessed again, not only elements selected for the time window.

<table>
<tr><th>Element</th><th>Description</th></tr>
<tr><td>Mark Time Window
(only on Image Analysis tab)</td><td>The elements belonging to the current time window are selected again (orange mark). Useful for modifying the time window:
1. Select Mark Time Window.
2. Modify mark, e.g. select a different well.
3. Select Use as time window.</td></tr>
<tr><td>Graph/Results table – Display Selection
(only on Evaluation tab)</td><td rowspan="3">Depending on the selected Population in the Graph pane (see section “Graph”, page 157), different menu items are displayed:
• If population Well Results is selected, the three Graph – ... menu items can be used to select data points which will be displayed directly in the graph. The Results table will contain all well results.
• If a population with object results is selected, the three Results table – ... menu items can be used to select the data points to be included in the Results table of object results. Only the selected results will be listed in the table. The same data points will be displayed directly in the graph.

Notice
When using object results the number of data points will increase exponentially. The Results table is limited to 500,000 rows. If this limit is reached, you have to reduce the number of selected wells, fields, planes, and time points.
It is recommended to select only one well initially and to extend the selection afterwards as far as required.</td></tr>
<tr><td>Graph/Results table – Add Selection
(only on Evaluation tab)</td></tr>
<tr><td>Graph/Results table – Remove Selection
(only on Evaluation tab)</td><td>Functions:
• ... – Display Selection: The results of the selected images will be displayed in the results table and in the graph. All other data points will be excluded.
• ... – Add Selection: The results of the selected images will be added to the data points which are currently displayed in the graph and in the results table.
• ... – Remove Selection: The results of the selected images will be removed from the graph and the results table.</td></tr>
<tr><td>Use for Z' negative Control
(only on Evaluation tab)</td><td rowspan="2">The results of the selected wells, planes and time points will be used as negative or positive control for calculating a Z' value. For details see section “Graph”, page 157.</td></tr>
<tr><td>Use for Z' positive Control
(only on Evaluation tab)</td></tr>
</table>

Element	Description
Help	Opens the corresponding topic in the Harmony Help.

Well



The **Well** pane is used to:

- Select a single field of which you want to view the measured image.
- View progress of a measurement, analysis or evaluation (for an explanation of the color coding of the fields see section 5.1.11.3 “Color Coding”, page 189).
- Select multiple fields for an overview image (Overview). The Overview function is triggered by a context menu command in the **Plate** or **Well** pane (for detailed instructions see section “How to open an Overview”, page 171).

If you scroll the mouse wheel you can **zoom in** the well layout. This can be helpful if you have a plate type with large wells (e.g. a slide) and an objective with high magnification, which will result in many small image fields.

Background for Well

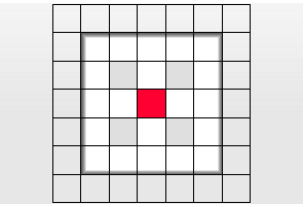
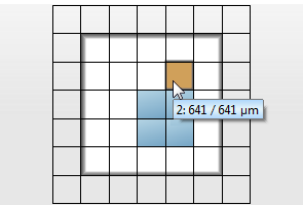
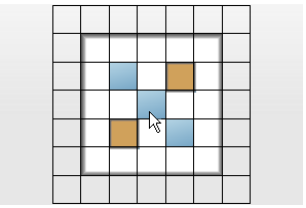
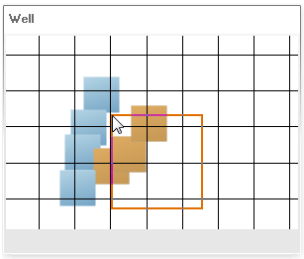
Via the context menu you have the option to select one or multiple fields as **Background for Well** (only available on **Setup** tab), useful for a pre-scan/re-scan procedure e.g. when looking for interesting areas on a slide. For detailed instructions please refer to section 5.1.11.4 “Manual PreScan/ReScan”, page 191.

The background image will be displayed on the well layout until you ...

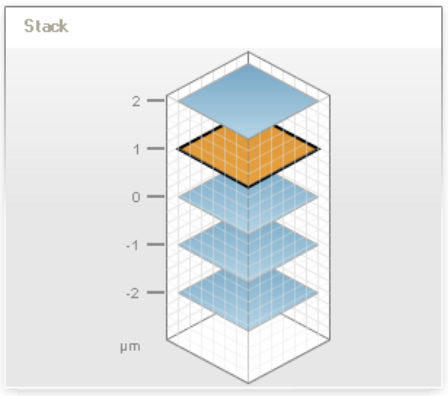
- select a different plate type.
- load an experiment which has a different plate type.
- load a measurement.
- start a measurement (**Run Experiment** tab).
- select a different well and run **Background for Well** again (replacing the existing image).

For further context menu options please refer to section “Context Menu”, page 175 (**Plate** pane).

Buttons and Elements

Element	Description
	**View progress/status** First, select a well in the **Plate** pane. During a measurement or evaluation the progress/status is displayed by the color of the field(s). For details see section 5.1.11.3 “Color Coding”, page 189.
	**View image** First, select a measured well in the **Plate** plane. Click on a measured field (blue). The corresponding image is displayed in the **Content Area**. Different planes (if configured) can be selected in the **Stack** plane. Using the cursor keys you can move the selection to view the images of neighboring fields. If no well layout was defined, only the default field is displayed in the center of the well (blue). A tooltip displays the field number and the distance of the field to the center of the well in microns.
	**Select multiple fields** e.g. for viewing an overview or for data analysis on the **Evaluation** tab • Click and hold the mouse button to select multiple fields in a rectangular area. • Press and hold the **Ctrl** key to select multiple separate fields successively.
	**PreciScan measurement** The ReScan of a PreciScan measurement (see section 5.1.8.3 “PreciScan™”, page 119) has individual well layouts for each well. The field positions may overlap and will differ from the usual grid structure. • Select single or multiple fields as described above. • When selecting multiple fields in a rectangular area (red frame), a field will be selected if its center is within this area.

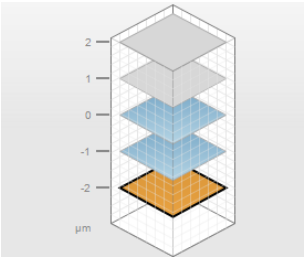
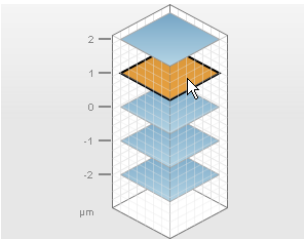
Stack

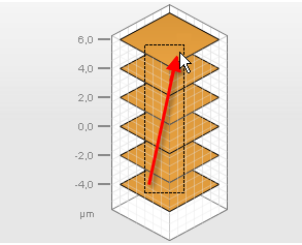
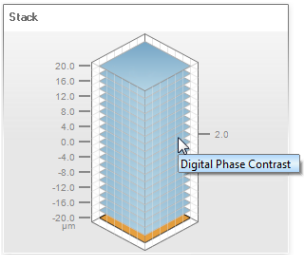


The **Stack** pane is used to select a plane in the selected well and field and to view the progress of a measurement, analysis or evaluation. If no stack was defined, this section is not displayed.

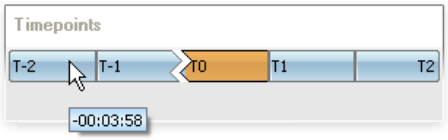
For context menu options please refer to section “Context Menu”, page 175 (**Plate** pane).

Buttons and Elements

Element	Description
	**View progress/status** First, select a well in the **Plate** pane and select a field in the **Well** pane (if configured). During a measurement, analysis or evaluation the progress/status is displayed by the color of the field(s). For details see section 5.1.11.3 “Color Coding”, page 189.
	**View image** First, select a measured well in the **Plate** plane and a field in the **Well** pane. Click on a measured plane (blue). The corresponding image is displayed in the **Content Area**. Using the cursor keys you can browse through the stack.

Element	Description
	**Select multiple planes** e.g. for viewing a stack overview or for data analysis on the **Evaluation** tab • Click and hold the mouse button to select multiple planes in a rectangular area (see screenshot). • Press and hold the **Ctrl** key to select multiple separate fields successively. • Press **Ctrl + A** to select all planes. You may first have to click on any plane to set the focus to the **Stack** control.
	**Digital Phase Contrast (DPC)** Stacks cannot be acquired for the DPC channel. If a DPC channel is combined e.g. with a fluorescent channel, the stack of the fluorescent channel will be recorded and displayed as usual. But there will be only one DPC image, so that the same DPC channel is displayed for all planes of the stack. On the right side of the stack view, a height is displayed at the Z plane which is closest to the calculated height of the DPC image. This plane is highlighted by a tooltip named “Digital Phase Contrast”. **Notice** The displayed height might not be the real height of the DPC image. This situation occurs if the calculated height of the DPC image is not within the range of the regular stack. See also section “Digital Phase Contrast”, page 55.

Timepoints



The **Timepoints** pane is used to select one or multiple time points of a time series measurement and to view the progress of a measurement, analysis or evaluation. A tooltip displays the actual measurement time of the time point in relation to time point **T0** (beginning of the kinetics). If no time series was defined, this section will not be displayed.

Notice

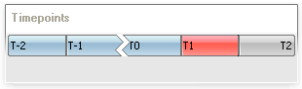
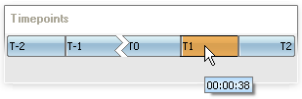
The time stamps (in relation to **T0**) displayed by the tooltip may change until the measurement has been completed. For example, if the time series includes a break, the correct time stamps of the time points before the break can only be displayed after the break when its duration is known.

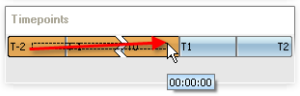
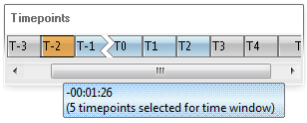
Deviations from the scheduled time points can also be caused by delays during the measurement (e.g. focus issues). This can also affect subsequently measured wells and time points and can lead to slightly different time stamps for wells of the same time point.

If a fixed interval could not be measured as scheduled, there will be a message and a comment will be attached to the measurement (see also section 5.1.16 “Database Browser”, page 220).

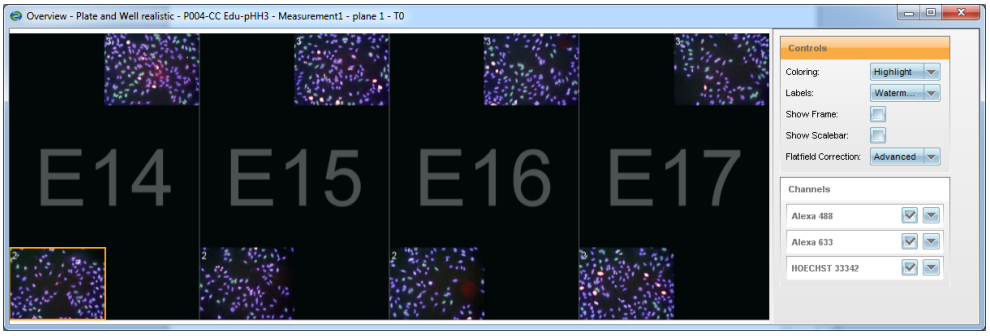
- For detailed information on time series please refer to section 5.1.7.4 “Time Series”, page 68.
- For context menu options please refer to section “Context Menu”, page 175 (**Plate** pane).

Buttons and Elements

Element	Description
	**View progress/status** During a measurement or evaluation the progress/status is displayed by the color of the time points. For details see section 5.1.11.3 “Color Coding”, page 189.
	**View image** First, select the desired well, field and plane (if available). Then click on a measured time point (blue) to view the corresponding image in the **Content Area**. A tooltip displays the actual measurement time of the time point (hh:mm:ss). If no time series was defined, this section will not be displayed. **Move time point selection via keyboard** • Previous/next time point: **Arrow left/right** key • First/last timepoint: **Pos 1**/**End** key

Element	Description
	**Select multiple time points** e.g. for viewing an overview of the time series or for data analysis on the **Evaluation** tab • Click and hold the mouse button to select multiple time points in a rectangular area. • Press and hold the **Ctrl** key to select multiple separate time points successively. • To select a group of neighboring time points, select the first time point, press and hold the **Shift** key and the select the last time point (may be using the scrollbar first, if not all time points are visible). • Press **Ctrl + A** to select all time points. You may first have to click on any time point to set the focus to the **Timepoints** control.
	**Time window** (Image Analysis tab) If a time window has been defined, the total number of time points in the time window is displayed as a tool tip. Time points belonging to the time window are colored blue (or orange if currently selected). All other time points are gray and cannot be displayed as long as the time window exists.

Overview



This window displays thumbnail images of selected wells and fields and arranges them according to their position on the plate (option “realistic") or in a more space-saving way (option “packed"). It can be used to get an overview of the plate quickly. You can open this function from section **Navigation – Measurement – Plate** (see also section 5.1.11.1 “Plate Measurement”, page 171).

In the **Controls** section on the right you can select display options, e.g. for the style of the image labels. In the **Channels** section you can select/deselect the desired channels and adjust brightness and color for each channel. The functions are identical with the **Image Control** panel (see also section 5.1.12 “Image Control”, page 195).

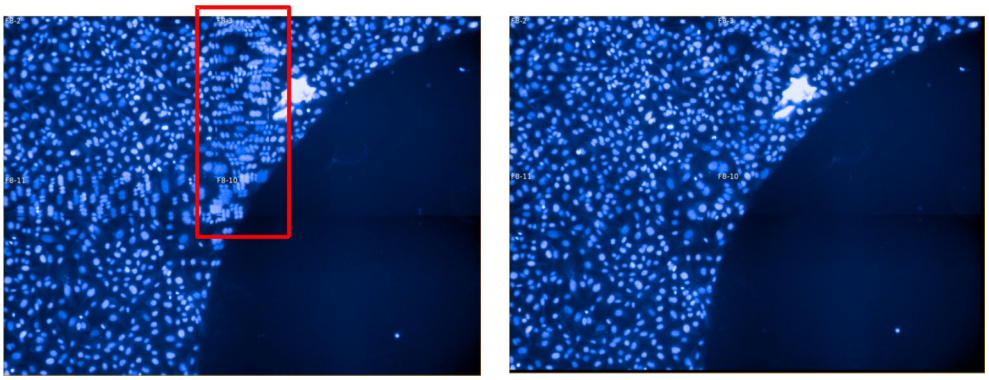
The **Stitching** section allows you to correct slightly incorrect matching at the borders of adjacent fields to create one large seamless image. This function is only available if you have selected an **Overview** with a **realistic** view of the well.

Right-click on the image area to open the context menu where you can e.g. define how to calculate the **autocontrast** for the whole overview image.

Notice

- The overview can be zoomed at any time using the **mouse wheel**.
- You can also press and hold the **Shift** key to toggle temporarily between **Zoom** mode and **Panning** mode.

About Stitching

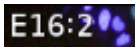
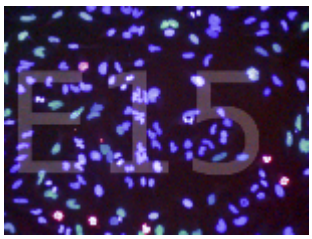


Stitching of an overlapping image. Four image fields with 20% overlap. Left: without stitching – the nuclei in the overlapping area do not match perfectly (see e.g. red frame). Right: Stitching applied, magnification correction was adjusted to 0.9802 by pressing the "Autoadjust" button.

Due to slight differences in the magnification from the nominal magnification of the objective (e.g. 20x), a tiled image usually shows slightly incorrect matching at the junctions between the tiles. This inaccuracy can be corrected by setting a **magnification correction factor**:

- For **overlapping** images the correction factor can be automatically adjusted using the **Autoadjust** function. If **Autoadjust** does not find the optimum value, enter a different start value (e.g. 1.0 or a value close to the expected optimum) and try again (press **Enter**).
- For **non-overlapping** images the factor can be determined manually (range: 0.5–2.0):
  - Start at 0.98 which is good for most situations. Press **Enter** to apply the factor.
  - Turn stitching on and off (**Stitching** check box) and see if the correction is fine.
  - If the magnification is over- or undercorrected, in- or decrease the correction factor in small steps (e.g. ±0.01).
  - The magnification correction only has to be determined once for an optical setup (instrument, objective, plate) and can be used for all fields/wells.

Buttons and Elements

Element	Description
Coloring Mode	Please refer to section 5.1.12 “Image Control”, page 195.
Labels	• **None:** No labels for wells, fields, planes or time points. • **Small:** Well coordinate, plane or time point number and the field number are displayed in the upper left corner of each thumbnail image. In this example, this means: well E16, field 2.  1536-well plates are labeled numerically, i.e. “22/13:1” means row 22, column 13, field 1. • **Watermark:** The label (well coordinate, plane or time point number) is displayed across the whole well like a watermark. The field number is displayed in the upper left corner of each field . Stack planes are labeled with P#, time points with T# (where # stands for number). 
Show Frame	The thumbnail(s) used for calculating the autocontrast of the whole overview image (see context menu) will be indicated by an orange frame. This option allows to show/hide the frame.
Show Scalebar	Displays a scale bar in the left bottom corner of the overview which allows you to estimate the dimensions of the objects.
Flatfield Correction	If a flatfield correction profile could be calculated for the current measurement, you can select a correction method: • **None:** Uncorrected image is displayed (default). This is the only available option if no correction profile could be calculated. • **Basic:** Image with corrected background is displayed. Foreground objects have not been corrected. • **Advanced:** Corrected image (background and foreground) is displayed. For more information on see section 5.1.12.5 “Flatfield Correction”, page 205.
Stitching	Please refer to section “About Stitching”, page 185.
Channels	Please refer to section 5.1.12 “Image Control”, page 195.

Context Menu

Element	Description
Set Zoom 1:1	Displays the thumbnail images in original size, i.e. one pixel in the thumbnail image corresponds to one pixel on the screen.
Zoom to Fit	Displays the plate overview so that it fits into the **Content Area**.
Panning	In **Panning** mode you can move the image within the **Content Area** to see a different section (if zoomed in). The mouse cursor turns into a hand.
Zoom	In **Zoom** mode you can span a rectangle with the mouse. The marked region of the image is enlarged to the full size of the **Content Area**.
Copy	Copies the plate overview (full size, no zoom) to the Windows® clipboard to paste it in a different program.
Save Image ...	Allows to save the plate overview as a BMP, JPG or PNG file.
Select image for autocontrast	Right-click on the desired thumbnail image and select this option to use only the selected image for calculating the autocontrast for the whole plate overview image. This thumbnail image will be highlighted by an orange frame (if **Show Frame** has been selected).
Select all images for autocontrast (default)	By default, the automatic contrast adjustment for the whole plate overview image is based on all images. If **Show Frame** has been activated, the whole overview image will be displayed with an orange frame. The selected autocontrast method of the overview is also applied to the autocontrast function in the **Channels** section.
Help	Opens the context-sensitive help for this screen section.

Keyboard Commands

Key	Description
Shift	Toggles temporarily between **Zoom** mode and **Panning** mode.
PageUp	Zoom in.
PageDown	Zoom out.
Pos1	Corresponds to **Set zoom 1:1** (see context menu).

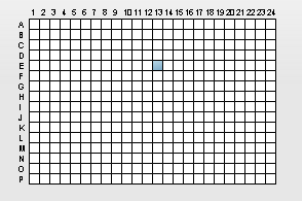
5.1.11.2 Test Images

The **Test Images** pane is used to select and view images of a test measurement. It offers the same functionality as the **Plate Measurement** pane. Only the **Plate** sub-pane is different, because a test measurement always consists of one single well.

- For a description of the other panes see section 5.1.11.1 “Plate Measurement”, page 171.

- For detailed information on test measurements see section 5.1.7.1 “Global Control”, page 42 (**Setup** tab).

Plate

Element	Description
	Displays only the single well of the test measurement. No other well can be selected.

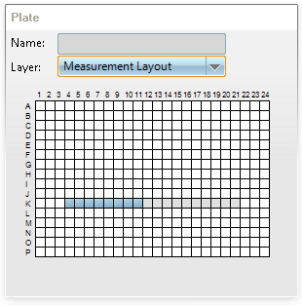
5.1.11.3 Color Coding

Navigation

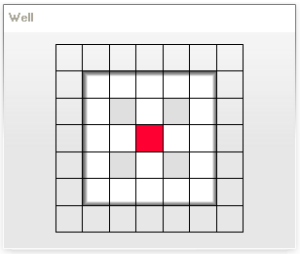
In the **Navigation – Measurement** pane, the progress of a measurement or evaluation is indicated by the color of the respective well, field, stack plane or time point. In the **Navigation – Define Layout** pane (**Setup** tab), the colors indicate if a position will be measured or not.

Color	Basic meaning
Blue	• Measurement successful • Evaluation successful
Orange	Selected
Yellow	Evaluation failed
Red	Measurement failed, no image available
Gray	• Selected for measurement • Selected for evaluation
White	• Well or field not selected for measurement • Not measured

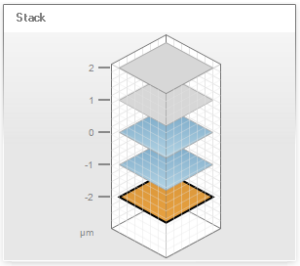
Examples



Measured wells (blue) and unmeasured wells (gray)



Unmeasured fields (gray) and field with error (red)



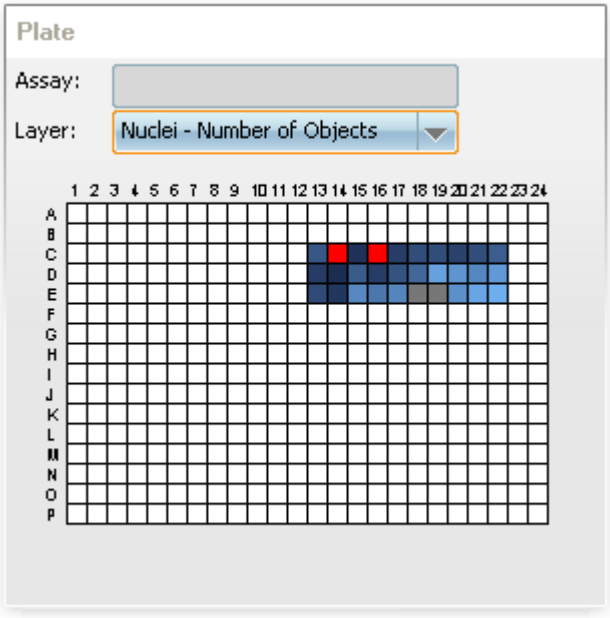
Measured planes (blue), unmeasured planes (gray) and a selected plane (orange)

Heat Map

If you have created a heat map for an evaluation (see section "Heat Map", page 154), you can select this heat map as layer in **Navigation – Measurement – Plate**. The heat map is used to visualize the values of one selected readout parameter by the color of each well of the plate. Light blue indicates low values, dark blue indicates high values. Outliers have separate colors.

Color	Meaning
Light blue	Min. Value
Dark blue	Max. Value
Gray	Lower than Min. Value
Red	Higher than Max. Value

Example



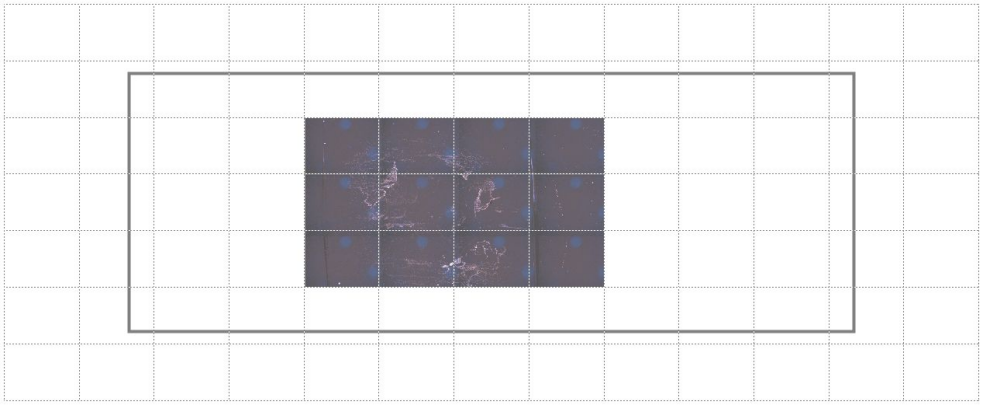
5.1.11.4 Manual PreScan/ReScan

Notice

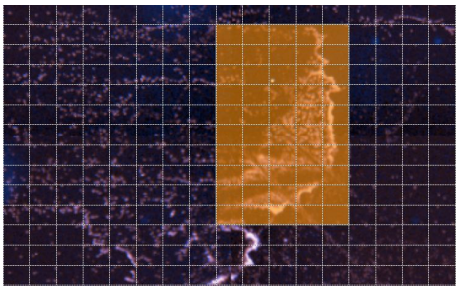
The **Manual PreScan/ReScan** function described in this chapter is a manual procedure for sequential measurements at different magnifications. The **PreciScan** feature (optional license required) offers a fully automated workflow and allows to analyze the sample and rescan only a population of desired objects. For details please see section 5.1.8.3 “PreciScan™”, page 119.

Especially when working with slides or large wells, you may want to **prescan** the whole well using a low-magnification objective (e.g. 1.25x) to get an overview and identify areas of interest. In a second step you can then use a high-magnification objective (e.g. 40x) to **rescan** the desired fields.

Harmony supports this workflow by allowing you to select an overview image of a low-magnification measurement as background of the well layout (**Navigation – Measurement – Well** pane). If you then switch to a high-magnification objective, the background image of the low-magnification measurement is still visible and you can easily select the fields of interest and define the well layout for the final high-resolution measurement.



Prescan of a slide using a 2x objective (Operetta). The resulting images are used as background for the well layout.



After switching to a high-magnification objective you can easily select the fields of interest for rescan.

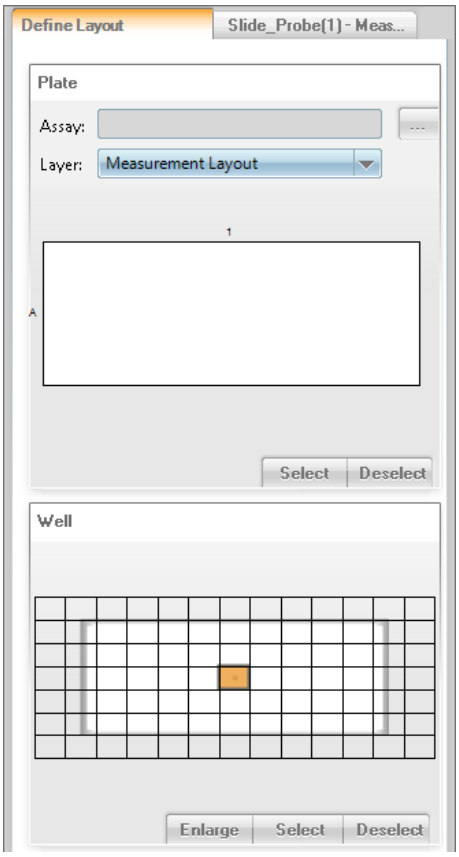
Notice
After switching from a water immersion objective to an air objective you should eject the plate and clean the plate bottom to remove any water droplets and prevent focusing errors.

How to perform a manual Prescan/Rescan

In this example it is assumed that you use a slide in a slideholder.

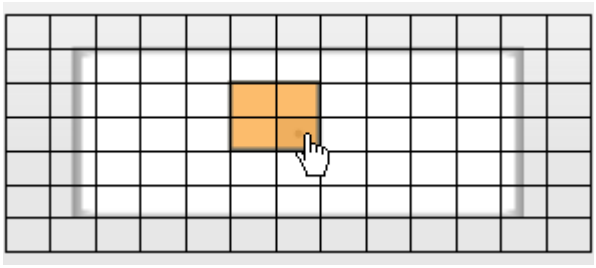
Prescan

1. Open the **Setup** tab.
2. Select the corresponding **Plate Type**, in this case the used slideholder.
3. Select a low-magnification **Objective**.
4. Open the **Navigation - Define Layout** pane.
   The slide is displayed in the **Plate** and **Well** panes. Per definition, one slide corresponds to one well.



5. In the **Plate** pane select the slide (i.e. the well).

6. Click Select.
   The well becomes gray and is added to the plate layout.
7. In the **Well** pane select a few fields where you expect areas of interest.



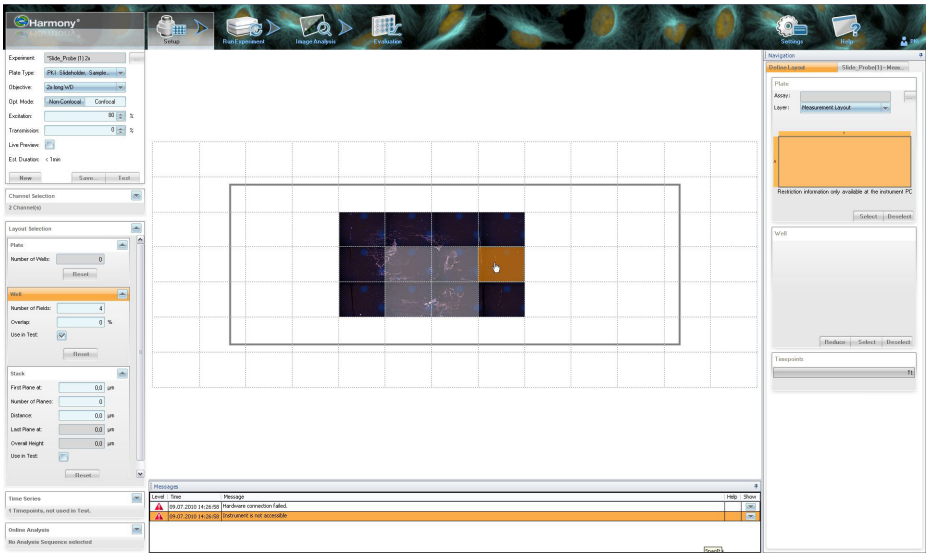
8. Click Select.
   The fields become gray and are added to the well layout.
9. In **Layout Selection – Well** activate the option **Use in Test** so that the well layout is also used for the test measurement.

10. Select a single well (i.e. the slide) in the **Navigation – Define Layout – Plate** pane and click Test.
    The selected fields on the slide are measured as a test measurement.

Create Background Image

1. Open the **Navigation – Test Images** pane.
2. Select the desired well in the **Plate** pane.
   In this example, there is only one well (i.e. one slide), but if you use a slideholder with more than one slide or a microplate, you first have to select a well.
3. Select the measured fields (blue) in the **Well** pane.
4. Right-click on the well layout and choose **Background for Well** from the context menu.

The selected fields are displayed as background images on the well layout. The well layout is automatically enlarged and displayed in the **Content Area**. You can **zoom** the view using the mouse wheel.



Notice

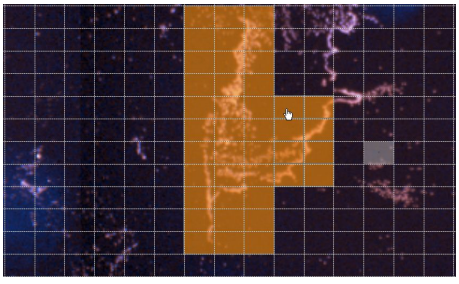
The background image created by the **Background for Well** function is only valid for the selected well. If there is more than one well and you select a different well to check areas of interest for rescan, the background image displayed on the well layout will still be the same. **It will not be updated automatically.** In this case, select the new well and run **Background for Well** again to create a new background image.

Rescan

1. Select a high-magnification **Objective**.

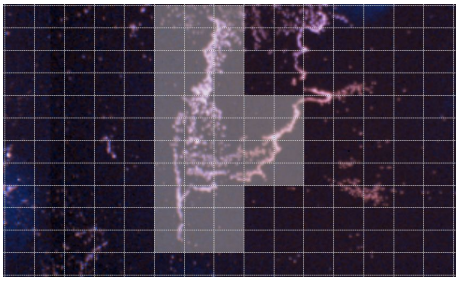
   The grid over the well layout is now much tighter, because the image fields are smaller. Probably, the field borders are not visible at all. Use the mouse wheel to zoom in the well layout.

2. Select the fields of interest (orange mark) for the high-resolution measurement.



3. In the **Navigation – Define Layout – Well** pane click Select.

   The selected fields are added to the well layout (indicated by a gray overlay over the fields).

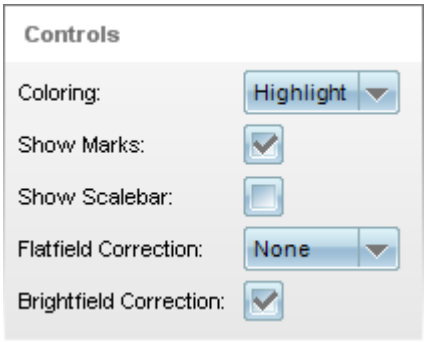


4. Start the final measurement on the **Run Experiment** tab.

5.1.12 Image Control

The **Image Control** pane allows you to modify the visualization of the selected image in the **Content Area**. The desired image (well, field, plane, time point) has to be selected before in **Navigation – Measurement**.

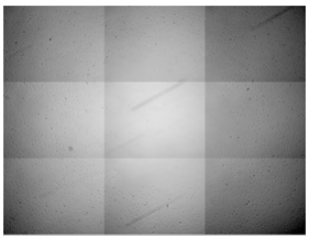
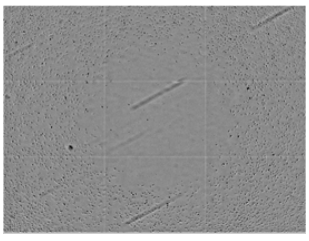
5.1.12.1 Controls



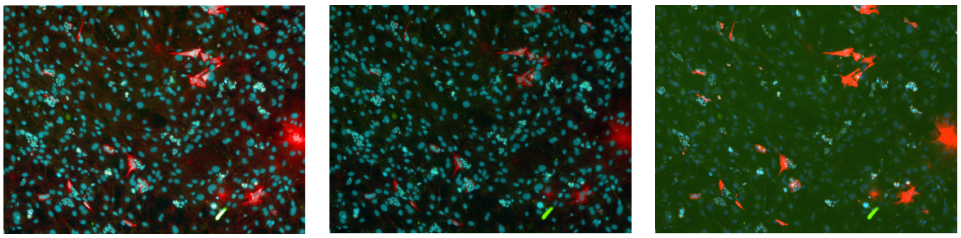
This section allows you to select a display mode which is suitable for your application.

Buttons and Elements

Element	Description
Coloring	The images recorded by the Operetta CLS have a high dynamic range (16 bit cameras). There is no way to display all information in a single image on a computer screen (RGB, low dynamic range, 8 bit) without loss of information. In Harmony, the user can select one of three display modes to view the image as required (see also the examples after this table). • In the **Highlight** mode (default), the color channels are intelligently adapted to show as many details as possible within the dynamic range of the display. Colors are more pure and saturated. *Drawback:* Very bright objects are all white, independent from which color they have been assigned to. This is a good tradeoff to get a better representation of "middle intensity" regions in the image. In many applications it is clear from the location to which channel the "white" areas belong. • The **Enhanced** mode is mostly identical to the **Highlight** mode, except for the fact that very bright regions are colored in the assigned channel color (or a mixture of colors for co-localized regions). *Drawback:* Gives a less clear view for the middle intensity range (which mostly is the largest part of the image). • In the **Standard** mode, image intensities are not modified to enhance image details. Intensities can be directly judged on the displayed image. **Notice** The **Coloring** mode has no influence on the intensity values which are displayed by the **Show intensity** tool in the **Content Area** (see section 5.1.13.1 "Image Display", page 209).
Show Marks (only Image Analysis tab)	If **object result** data points have been selected on the **Evaluation** tab, the corresponding cells in the image will be marked. You can disable this function by removing the check mark.
Show Scalebar	Displays a scale bar on the image [µm].

Element	Description
Track Coloring	Only for building block *Track Objects*: Allows you to configure how the tracks of moving objects are displayed: • **None:** Tracks are not displayed. • **Root ID:** The tracks of objects which descend from the same root object (i.e. have the same **Root ID**) have the same color. As long there are no split objects, there will be no difference to the **Track No.** style. • **Track No.:** Each track has an individual color (same color as the corresponding object). • **Generation:** The color of the track (or the track section) indicates the generation of the object (first generation: green, second generation: red, etc.).
Flatfield Correction	If a flatfield correction profile could be calculated for the current measurement, you can select a correction method: • **None:** Uncorrected image is displayed (default). This is the only available option if no correction profile could be calculated. • **Basic:** Image with corrected background is displayed. Foreground objects have not been corrected. • **Advanced:** Corrected image (background and foreground) is displayed. For more information on see section 5.1.12.5 “Flatfield Correction”, page 205. This parameter is not available when viewing global images. The corresponding option can only be activated/deactivated during global image generation in *Input Image* (see section 5.2.3 “Input Image”, page 327).
Brightfield Correction	Removes the background profile of brightfield channels, affects display only. Allows better visualization and smooth montage.   *Uncorrected* *Corrected* This parameter is not available when viewing global images. The corresponding option can only be activated/deactivated during global image generation in *Input Image* (see section 5.2.3 “Input Image”, page 327).
Log X, Log Y	Only available if a scatter plot is displayed in the **Content Area**. Activates logarithmic sale for the X- or Y-axis of the plot.

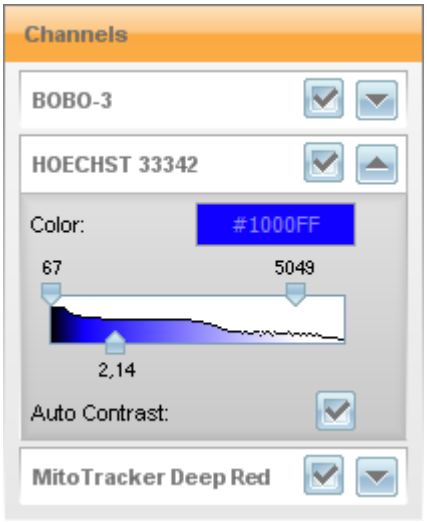
Coloring Mode: Example



Highlight *Enhanced* *Standard*

- **Highlight** is the most colorful mode.
- **Enhanced** shows the bright green dye agglomerate in the bottom right of the image in the correct green color instead of white.
- **Standard** reveals that there is a high background, that the red stain has a high contrast, and that the small blue objects (nuclei of stem cell colonies) are much brighter than the larger ones (nuclei of feeder cells).

5.1.12.2 Channels

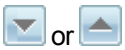
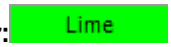
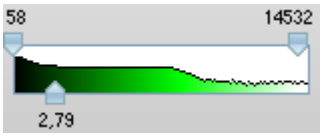


- If the selected image includes multiple channels, you can activate or deactivate each channel to achieve the desired multi color overlay.
- Each channel can be modified in terms of brightness, contrast, coloring etc. These changes are only temporary (for visualization), the images are not modified.
- Instead of a single color you can also select a pre-defined color palette to create pseudocolor images and highlight specific intensity changes.

Notice

For detailed background information on image visualization and correction and how to avoid common pitfalls see chapter 1.2.1 “Image Display Hints” in the **Image Analysis Guide**.

Buttons and Elements

Element	Description
	Click the disclosure button to open or close this channel.
	• **Activate** the check box to display this channel in the image. If multiple channels are activated, they will be displayed as an overlay. • **Deactivate** the check box to ignore this channel for image display. This does not modify or delete the channel, it just controls the appearance of the image.
Color: 	Each channel has a default color corresponding to the wavelength of its emission filter. The **Brightfield** channel is black and white by default. **Notice** If a channel name is unknown to Harmony, the respective channel will be displayed in gray. See also section 5.3.17 “Channel Displayed in Gray”, page 478. You can click on the color field to open the **Select Color** dialog and select a different color for this channel. The color field displays the name of the color or the precise RGB values (hexadecimal). For details see also section 5.1.12.4 “Select Color”, page 202. The new color will only be used for the current session.
	The sliders of the histogram can be used to adjust brightness and contrast of the channel (temporarily). • Upper left slider: Adjust the **minimum intensity** value of the image. • Upper right slider: Adjust the **maximum intensity** value of the image. • Lower slider: Adjust the **Gamma** value. Gamma correction is a nonlinear intensity enhancement of dark image areas in order to make the structure inside these areas visible.

Element	Description
Auto Contrast	Activates or deactivates automatic contrast adjustment. This does not modify the image, it just controls the appearance of the image. **Notice** The **Auto Contrast**feature makes browsing a set of images very easy without having to adapt any settings. But be cautious when comparing images. For comparing absolute image intensities the auto contrast must be deactivated, so that fixed settings are used. If a time window has been created, the same contrast adjustment will be applied to all images of the time window, based on the time point where **Auto Contrast** was activated.

5.1.12.3 Regions and Overlays

These sections are only available on the **Image Analysis** tab. They can be used to select and configure overlay masks visualizing detected objects and regions of the current building block or of the whole analysis sequence.

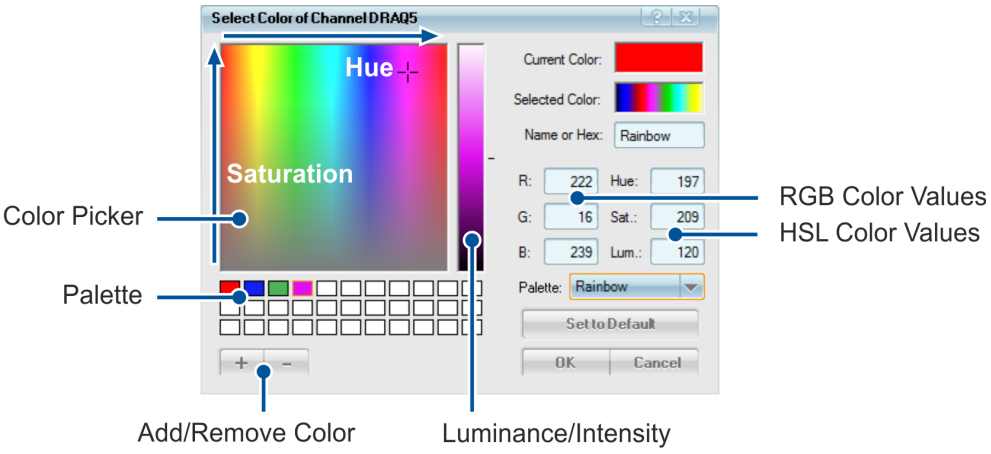
The list of available masks depends on the context: If you have opened a building block, you can select masks visualizing all detected objects up to this position in the analysis sequence. Masks for detected objects of subsequent building blocks are not available.

Buttons and Elements

Element	Description
or	Click the disclosure button to open or close the overlay settings.
or	• **Activate** the check box to display this overlay mask in the image. • **Deactivate** the check box to ignore this overlay for image display.
Color Lime	Click the field to open the **Select Color** dialog and modify the color of the region/overlay. See also section 5.1.12.4 “Select Color”, page 202. For certain regions the color may be **fixed**. In this case the color is pre-defined by the building block and cannot be modified (e.g. color of trained objects in *Select Population*). **Notice** If you select the **Rainbow** palette in the **Select Color** dialog, multiple colors are used, so that adjacent objects never get the same color and can easily be recognized as detected and correctly separated objects.

Element	Description
Style	Determines which object information is displayed in the overlay mask. • **Border:** Displays a border around the detected object. • **Body:** Displays the whole area of the object (semi-transparent). • **Solid:** Displays the whole area of the object (no transparency). • **Center:** Indicates only the center of the object with a small circle. • **Numbers:** Displays only the object number. • **Circle:** Draws a circle around the detected object.

5.1.12.4 Select Color



This dialog is used to select a color for a channel or for an overlay. It is opened if you click on the **Color** field in section **Channels** , **Regions** or **Overlays**.

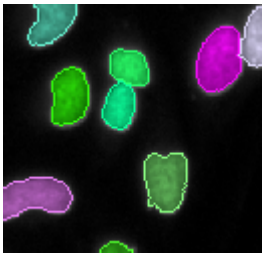
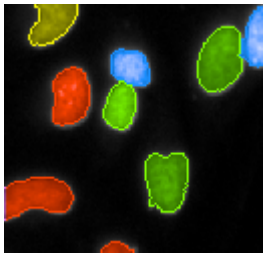
How to select a color

1. There are multiple ways to select a color:
   - **Pick color:** Pick a color from the **Palette** of user-defined colors or from the **Color Picker** (hue/saturation). Optionally: Adjust **Luminance** as desired.
   - **Enter name/hex code:** Enter the name of a basic color (e.g. “Red”) or enter an RGB value (hexadecimal) in the **Name or Hex** box.
   - **Enter RGB/HSL values:** Enter the desired RGB or HSL values directly in the corresponding fields.
   - **Palette (for channels):** Select a pre-defined coloring palette to create pseudocolor images.
     Coloring (for regions and overlays): Select a coloring style for the region/overlay (see table below for details).

   After selecting a color, it is applied to the image/overlay in the **Content Area** so that you can preview the result.
2. Click OK to confirm the color selection and close the dialog.

Buttons and Elements

Element	Description
Color Picker (Hue/Saturation)	Allows to pick a color. The different **hues** are aligned horizontally. **Saturation** increases towards the top of the color picker. The current color is indicated by crosshairs.
Luminance	Allows to modify the **luminance/intensity** of the selected color. The current color is indicated by a marker on the right.
Current Color	Original color before opening and using this dialog.
Selected Color	Preview of the selected color (after picking a color or entering color values).
Name or Hex	• Displays the name (e.g. “Red” or “Lime”) of basic colors or the RGB values in hexadecimal notation. • You can select a color by entering a name or a RGB color definition (hexadecimal).
RGB Color Values	Allows to define the desired color by entering RGB values (Red, Green, Blue with range 0-255 each).
HSL Color Values	Allows to define the desired color by using the HSL color space. This color model is more intuitive than RGB and corresponds to the color picker elements on the left. • **Hue:** Specifies the basic color (0-240). • **Sat:** Saturation of the color (0: neutral gray; 240: fully saturated). • **Lum:** Luminance or intensity of the color (120: pure color; < 120: adds black; > 120: adds white).

Element	Description
Palette or **Coloring**	**Palette** (only for channels): Pre-defined color palettes for creating pseudocolor image from grayscale images. For a detailed description and examples see also Image Analysis Guide, section 1.2.2 “Pseudocolor Images”. **Coloring** styles (only for regions and overlays): • **Monochrome:** One single color for the entire overlay. • **Rainbow:** In "Rainbow" mode slight changes in object shape and position do usually not drastically change the object colors (useful e.g. for parameter tuning or time series). Draw back is that very similar colors of touching objects cannot be avoided in some cases. • **Shuffle:** In contrast to this, "Shuffle" mode focuses on best visual discrimination, but has less good color persistence. Switch to "Shuffle" in case your main interest is object splitting.   *Rainbow* *Shuffle*
Set to Default	Resets the color to the building block's default color for the selected channel or overlay. This only applies to the selected image tab in the currently active building block.
Color Palette	Palette of user-defined colors. • Click a color to select it. • To create a new color: ◦ Click a color field (an existing or a white one). ◦ Select the color by using the color picker or entering values. ◦ Click + to save the color in the palette.
+	Saves the current color at the selected position in the palette of user-defined colors.
–	Removes the selected color field from the palette.
Cancel	Discards all changes and closes the dialog.
OK	Applies the changes and closes the dialog.

5.1.12.5 Flatfield Correction

What's the Use of Flatfield Correction?

The illumination of the Operetta CLS is not perfectly homogenous which leads to an intensity drop at the image borders/corners (vignetting). This affects viewing the images, segmentation, and analysis of absolute intensities. The fully automated flatfield correction eliminates this effect by calculating a correction profile from the sample images which can be applied to all images of the measurement. Thereby you get almost flat (i.e. homogenous) images, both for visualization and analysis.

See also:

- **Image Analysis Guide**, section 1.6 “Flatfield Correction”
- Harmony Help, section 5.3.13 “Flatfield Correction”, page 472 (troubleshooting)

Calculation of Correction Profile

A flatfield correction profile is calculated automatically in the following situations:

- Measurement (Setup, Run Experiment)
- Loading a measurement which does not have a profile yet (profile "n.a.").

Flatfield correction is **NOT** available for:

- Snapshot
- Brightfield channel
- Phase Contrast channel
- Online analysis (profile will be calculated, but cannot be used directly for online analysis)
- Measurement with only one stack (no further wells, fields, time points)

As soon as you start a measurement (or load a measurement without profile) and at least seven images are available, Harmony begins to calculate a flatfield correction profile for each channel. This is indicated by a message in the **Messages** window:

- *“Flatfield correction profile estimation started for […]”*
  Another message is posted when the calculation is finished:
- *“Flatfield correction profile estimation completed (available method: Basic) for […]”*
  The best correction profile type which could be achieved is stated in brackets (“Advanced”, “Basic”, or “None”).

A new keyword **Available Correction** will be attached to the measurement to indicate the best available correction method. You can sort or search measurements by this criterion in the **Database Browser**. The keyword has the value "n.a." until the calculation of the correction profile is completed. In case the calculation was aborted (no final profile determined yet) the correction profile type will be stated as "n.a.". The next time you load such a measurement, the calculation of the correction profile will be started again.

The correction profile can now be applied to view or analyze images. The corrected images themselves are not saved. They are generated on-the-fly by applying the correction profile to the uncorrected images. With default settings uncorrected images will be used as before. For detailed instructions see section "Using Corrected Images", page 206.

Correction Methods

There are three different correction methods:

- **None:** No correction.
- **Basic:** Background is corrected, foreground objects are not corrected.
- **Advanced:** Background and foreground objects are corrected.

The available correction methods depend on the number of available images and on the content of the images. The more images, the better is usually the available correction.

Using Corrected Images

If a correction profile could be calculated for a measurement, you can apply the profile and use it both to visualize and analyze the corrected images.

How to view corrected images

1. Load a measurement, start a test measurement, or use a running plate measurement.
2. If no flatfield correction profile exists for the measurement, profile calculation will be started automatically. Wait until the calculation has been completed (see **Messages**).
3. Select an image in the **Navigation** pane. The uncorrected image is displayed.
4. Select a method for **Flatfield Correction** (**Basic** or **Advanced**) in section **Image Control – Controls**. The corrected image is displayed.

> **Notice**
> A flatfield correction method can also be selected as described above when viewing a plate overview. See also section "Overview", page 184.

Corrected images can only be displayed if the best available correction method is **Basic** or **Advanced**. If only **None** is available, please refer to the troubleshooting section 5.3.13 "Flatfield Correction", page 472.

How to use corrected images for analysis

1. On the **Image Analysis** tab, load a measurement and select an image.
2. Load an analysis sequence or create a new one.
3. Open the **Input Image** building block.
4. Select a method for **Flatfield Correction** (**Basic** or **Advanced**). The images will be corrected accordingly before the analysis.

Notice

- As soon as you modify the method for **Flatfield Correction** in the **Input Image** building block, the same correction will be applied to the currently displayed image (**Image Control – Controls – Flatfield Correction**). There is no such dependency in the opposite direction, i.e. you can change the displayed correction without modifying your analysis sequence.
- The correction method selected in the analysis sequence must be available for the measurement(s) to be analyzed.
  Example: If you set **Flatfield Correction** to *Advanced*, then the measurement can only be analyzed if an *Advanced* correction profile could be calculated.

Interaction with other Harmony Functions

Function/Measurement Mode	Important Notes
Image Control **Overview**	The combobox **Flatfield Correction** allows you to select a correction method which is applied to the currently displayed image or overview. See also sections 5.1.12 “Image Control”, page 195, and “Overview”, page 184.
Input Image	The input parameter **Flatfield Correction** of this building block allows you to select a correction method which is applied to the images during analysis. See also section 5.2.3 “Input Image”, page 327.
Evaluation	The selected correction method in the analysis sequence must be available for all measurement(s) to be analyzed. • **Single evaluation:** If the specified correction method is not available, the analysis sequence will automatically be corrected. The best available method will be used. • **Batch evaluation:** The evaluation will fail if the required method set in the analysis sequence is not available. See also section 5.1.10 “Evaluation”, page 146.
Online Analysis	A flatfield correction profile will be calculated during the measurement, but it cannot be used directly for online analysis. Therefore, only analysis sequences with **Flatfield Correction** set to **None** will be accepted for online analysis. Otherwise there will be an error message. Nevertheless, to use flatfield correction you can re-evaluate the measurement on the **Evaluation** tab. See also section 5.1.7.5 “Online Jobs”, page 77.

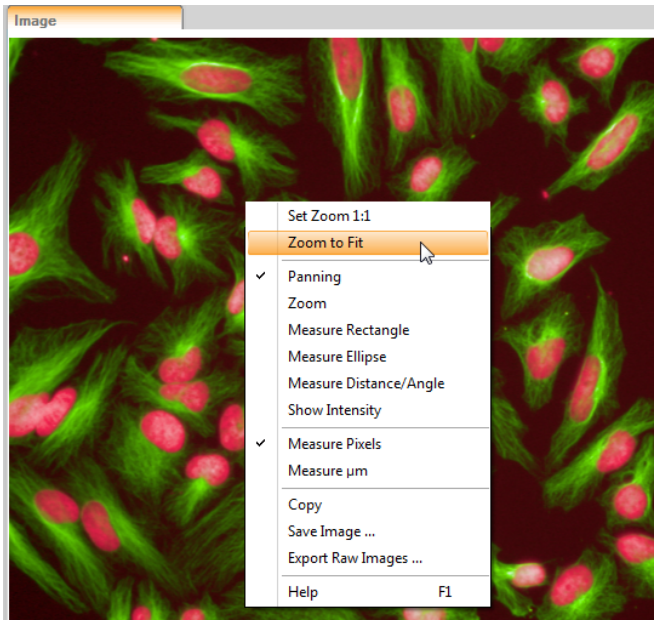
Function/Measurement Mode	Important Notes
Combine Measurement	The best correction available in one of the measurements to be combined will also be kept for the resulting measurement (indicated by the keyword **Available Correction**). See also section "Combine Measurements", page 274.
Columbus Transfer	Corrected Operetta CLS images are also available in Columbus after transfer. See also section "Columbus Transfer", page 271.
Stack	Only the mid plane of a stack is used for calculating the correction profile. This can be useful to know because the flatfield correction needs a certain minimum amount of images.For example, if you have defined a large stack, but only a few wells/fields you still might not have enough images to calculate a better correction profile. However, the resulting correction profile can be applied to all images of the measurement including stack images.
Brightfield / Digital Phase Contrast	Flatfield correction is not supported for the Brightfield or Digital Phase Contrast channel. Phase contrast images are inherently flat. Other channels of the same measurement can be corrected.

5.1.13 Content Area

The **Content Area** has various functions:

- **Display images:** These images can result from a test or plate measurement which you select in **Navigation – Measurement**, but also from intermediate results of an image analysis with overlay of recognized objects (**Image Analysis** tab). The desired channel overlay can be configured in the **Image Control** pane.
- **Movie Control:** On the **Image Analysis** tab, images selected for a time window (subset of a time series measurement with multiple time points) can be displayed as a movie clip (if supported by the selected building block). For details see section 5.1.13.2 "Movie Control", page 213.
- **Display heat map or graph:** Heat map and graph on the **Evaluation** tab are also displayed in the **Content Area**. For details see section 5.1.10 "Evaluation", page 146.
- **Tune Parameter Dialog:** This dialog is displayed on the left of the **Content Area** if you tune a parameter of a building block on the **Image Analysis** tab. For details see section "Analysis Sequence", page 142.
- **Display enlarged screen elements:** Some screen elements like the well layout (on **Setup – Navigation – Define Layout**) have an **Enlarge** button and can be displayed larger and more comfortably in the **Content Area**. For details see section "Define Layout", page 82.

5.1.13.1 Image Display



Notice

- The currently displayed image can be zoomed at any time using the **mouse wheel**.
- You can also press and hold the **Shift** key to toggle temporarily between **Zoom** mode and **Panning** mode.

At zoom levels lower than 1:1 the image display is interpolated to scale the image appropriately. For zoom levels higher than 1:1 smoothing can be activated or deactivated using the **Display Settings** (see section 5.1.17.13 “Display Settings”, page 322).

Any interpolation is only applied to the image display within Harmony. The original images which are used for image analysis or any image which is manually saved/exported (using the menu functions **Save Image** or **Export Raw Images**) will never be interpolated. These functions are recommended if you need the unscaled images in full resolution (e.g. for scientific publications).

The context menu function **Copy** will copy the image exactly as displayed at current zoom level (possibly interpolated and with reduced resolution). Right-click on the image to open the context menu.

The zoom levels between different tabs of the same scale (local/global image) are synchronized.

If you open the building block **Input Image** on the **Image Analysis** tab, there will be up to three different tabs in the **Content Area**:

- **Input Image:** Displays the original image without any overlays.

- **Global Image:** Displays a stitched global image of all fields in the current well. Only available if **Create Global Image** has been selected. See also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.
- **Histograms:** Displays a histogram for each channel of the image.
  If a time window is selected, the histogram is shown for multiple time points to allow seeing changes over time. The first and last time point's curve is marked bold (first continuous, last dotted line).
- **XYZ View:** Displays the images of a stack in a special viewer which allows to view sectional planes in x-, y- and z-direction. Only for stacks and if *Maximum Projection* has been selected. Zooming and panning is not possible (reduced context menu).

For details see section 5.2.3 “Input Image”, page 327.

Context Menu

The measuring tools work correctly on global images as well. [px] always refers to the current image pixels (local/global), not camera pixels.

Element	Description
Set Zoom 1:1	Displays the image with its original size, i.e. one pixel in the image corresponds to one pixel on the screen.
Zoom to Fit	Displays the image so that it fits into the **Content Area**.
Select Objects	Available in **Training** mode with building block *Select Population*, method **Linear Classifier**: • By clicking on a single object, the object is added to the currently selected training class (radio buttons Class A, Class B, ... on the left side of the image). If the object was already in that class, it is removed from the class and set to unselected ("toggling"). • Dragging a box on the image has the same effect as manually clicking on all objects inside the box. This allows quick selection of many cells, e.g. all cells on the image.
Highlight Objects	In **Highlight objects** mode you can select recognized objects on the **Image Analysis** tab to view object data in the **Results** table and vice versa. Available in all building blocks that show illustrations of a population. • Clicking on a single object highlights the object (default style: orange, solid) and marks the corresponding row in the object table. Any existing current highlight is removed. • When the CTRL-key is pressed when clicking on an object, the object is added to the current selection if it was not part of it, or removed if it was already selected ("toggling"). • Dragging a box on the image has the same effect as manually clicking on all objects inside the box. This allows quick selection of many cells, e.g. all cells on the image.

Element	Description
Panning	In **Panning** mode you can move the image within the **Content Area** to see a different section (if zoomed in). The mouse cursor turns into a hand.
Zoom	In **Zoom** mode you can select a rectangle with the mouse. The marked region of the image is enlarged to the full size of the **Content Area**.
Measure Rectangle	In **Measure rectangle** mode you can select a rectangle with the mouse. Width, height and area of the selected region are displayed (in px or µm, see below). Furthermore, the mean intensities for each channel are displayed.
Measure Ellipse	In **Measure ellipse** mode you can select an ellipse with the mouse. Width, height and area of the selected region are displayed (in px or µm, see below).
Measure Distance/Angle	In **Measure distance/angle** mode you can draw a straight line with the mouse. The distance from start point to end point and the angle of the line are displayed (in px or µm and °).
Show Intensity	In **Show Intensity** mode you can click the point of interest in the image to display the intensity value at this position (brightness of one single pixel) for all channels.
Measure Pixels	Sets the unit for the measuring tools to **pixels** (px). Refers always to the current image pixels (local/global image), not camera pixels.
Measure µm	Sets the unit for the measuring tools to **µm**.
Copy	Copies the visible image section (exactly as displayed at current zoom level, possibly interpolated and with reduced resolution) to the Windows® clipboard to paste it in a different program.
Save Image ...	Allows you to save the current image (full size and resolution) as configured with colored channels and overlays. This will reduce the color depth from 16 bit grayscale per channel to 8 bit color (no separate channels anymore). The file format can be selected (one BMP, JPG or PNG file). If a flatfield correction profile has been selected, the corrected image will be saved. See also section 5.1.12.5 “Flatfield Correction”, page 205.
Save Movie (full resolution) ...	Only available for movies: Allows you to save the current movie with full resolution (WMV format).
Save Movie (current view) ...	Only available for movies: Allows you to save the current movie as displayed with reduced resolution (WMV format).

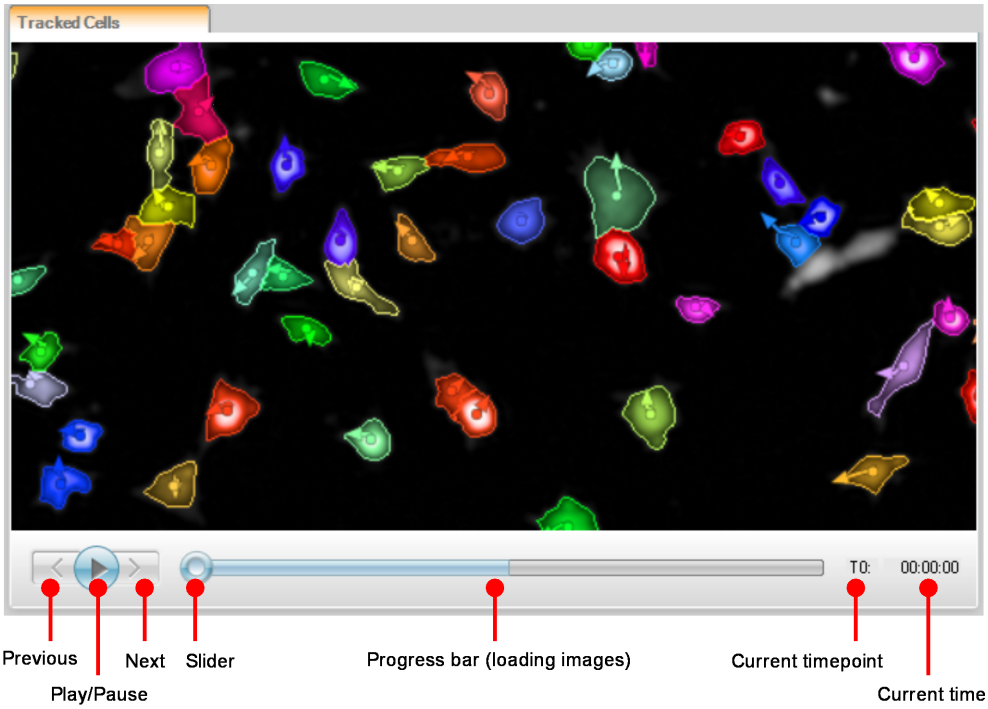
Element	Description
Export Raw Images ...	Only available on the **Image Analysis** screen: Allows you to save the unscaled RAW images with with full resolution and original color depth (one TIFF file per channel, 16 bit grayscale). To view these images outside of Harmony you need a third-party image viewer which is capable of displaying 16 bit TIFF images (e.g. IrfanView).
Help	Opens the context-sensitive help for this screen section.

Keyboard Commands

Key	Description
Shift	Toggles temporarily between **Zoom** mode and **Panning** mode.
PageUp	Zoom in.
PageDown	Zoom out.
Pos1	Corresponds to **Set Zoom 1:1**. Image rotation is undone.

5.1.13.2 Movie Control

On the **Image Analysis** tab, the movie control is displayed if you have defined a time window (see section 5.1.11.1 “Plate Measurement”, page 171). This is useful to browse through the images of the time window and to watch object movements.



Buttons and Elements

Key	Description
Previous	Go to previous time point.
Play/Pause	Play movie or pause movie.
Next	Go to next time point.
Slider	Indicates the position of the current frame in the movie clip. You can drag the slider back and forth to browse the images.
Progress bar	The blue bar indicates the progress of loading the images after a time window has been defined. As soon as the whole bar is blue, all images are available. It does *not* indicate the current position within the movie.
Current timepoint	Number of the current time point.
Current time	Time stamp of the current time point.

5.1.14 Results

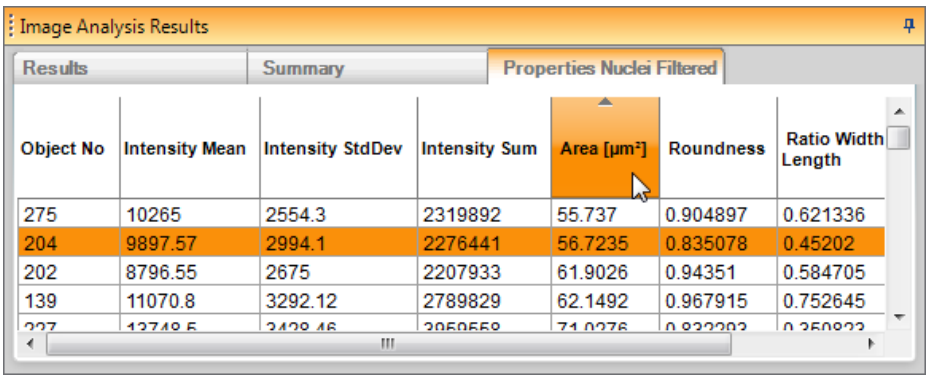
The **Results** pane is displayed on the **Image Analysis** tab and on the **Evaluation** tab and is named according to the selected tab.

- **Image Analysis Results:** Allows you to examine intermediate results of a building block on the tabs **Properties** and **Summary**. These tables display properties on the object level and give a summary for the whole well using multiple statistical methods. If the **Define Results** block is selected, you can examine the final readout values (well results) for the selected field on the **Results** tab.
- **Evaluation Results:** Allows you to inspect final readout values (well results) of an analysis sequence. The table displays the defined results for each well. If object results have been included, there will be separate tables for results per object.

How to sort a table

Notice
The table on **Image Analysis Results – Summary** cannot be sorted.

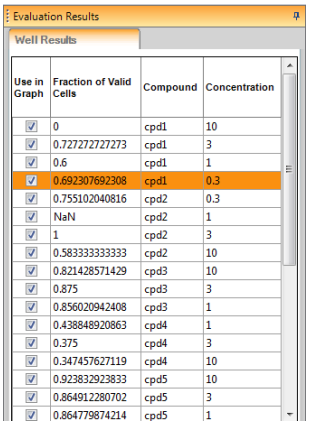
1. Click the column title to sort the object list by the corresponding property. The column title is highlighted and the sorting order is indicated by a small arrow.



2. Click the title again to reverse the sorting order.

How to sort a table by two properties

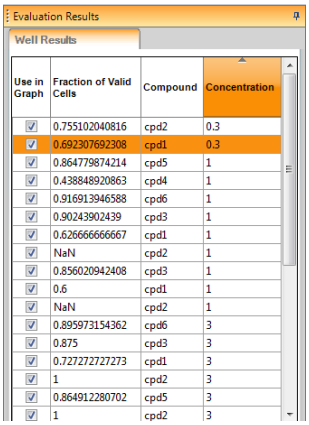
1. Click one column title to sort the object list by the corresponding property (e.g. “Concentration”).
2. Click the title of another column (e.g. “Compound”). The table is now sorted by compound, but each group of lines which has the same compound will be sorted by concentration. The primary sorting criterion is always determined by the column which you clicked last.


Evaluation Results

Well Results

Use in Graph	Fraction of Valid Cells	Compound	Concentration
☑	0	cpd1	10
☑	0.727272727273	cpd1	3
☑	0.6	cpd1	1
☑	0.692307692308	cpd1	0.3
☑	0.755102040816	cpd2	0.3
☑	NaN	cpd2	1
☑	1	cpd2	3
☑	0.583333333333	cpd2	10
☑	0.821428571429	cpd3	10
☑	0.875	cpd3	3
☑	0.856020942408	cpd3	1
☑	0.438848920863	cpd4	1
☑	0.375	cpd4	3
☑	0.347457627119	cpd4	10
☑	0.923832923833	cpd5	10
☑	0.864912280702	cpd5	3
☑	0.864779874214	cpd5	1

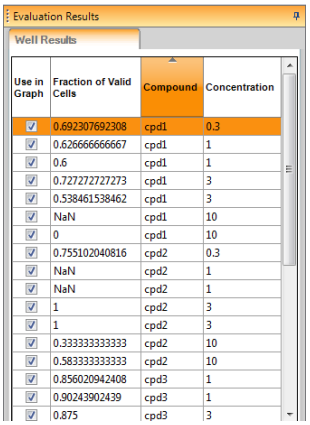
Unsorted table


Evaluation Results

Well Results

Use in Graph	Fraction of Valid Cells	Compound	Concentration
☑	0.755102040816	cpd2	0.3
☑	0.692307692308	cpd1	0.3
☑	0.864779874214	cpd5	1
☑	0.438848920863	cpd4	1
☑	0.916913946588	cpd6	1
☑	0.90243902439	cpd3	1
☑	0.626666666667	cpd1	1
☑	NaN	cpd2	1
☑	0.856020942408	cpd3	1
☑	0.6	cpd1	1
☑	NaN	cpd2	1
☑	0.895973154362	cpd6	3
☑	0.875	cpd3	3
☑	0.727272727273	cpd1	3
☑	1	cpd2	3
☑	0.864912280702	cpd5	3
☑	1	cpd2	3

Sorted by concentration


Evaluation Results

Well Results

Use in Graph	Fraction of Valid Cells	Compound	Concentration
☑	0.692307692308	cpd1	0.3
☑	0.626666666667	cpd1	1
☑	0.6	cpd1	1
☑	0.727272727273	cpd1	3
☑	0.538461538462	cpd1	3
☑	NaN	cpd1	10
☑	0	cpd1	10
☑	0.755102040816	cpd2	0.3
☑	NaN	cpd2	1
☑	NaN	cpd2	1
☑	1	cpd2	3
☑	1	cpd2	3
☑	0.333333333333	cpd2	10
☑	0.583333333333	cpd2	10
☑	0.856020942408	cpd3	1
☑	0.90243902439	cpd3	1
☑	0.875	cpd3	3

Sorted by compound, then by concentration

How to change the order of the table columns

Notice

The order of the table columns on **Image Analysis Results – Summary** cannot be changed.

- Click a column title and drag it to the desired position.

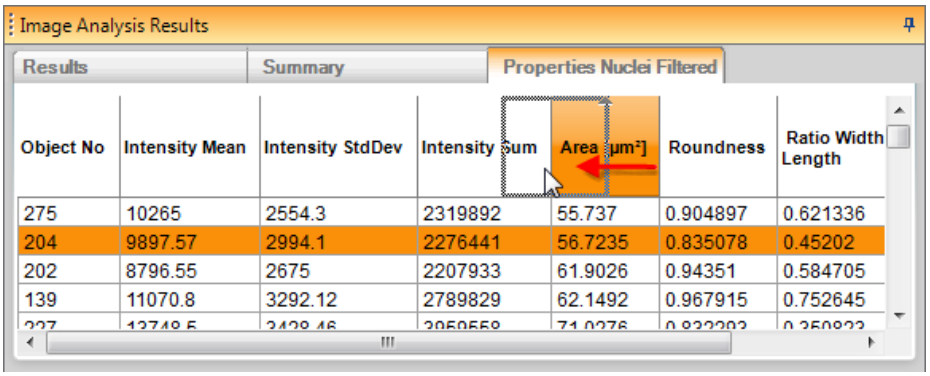


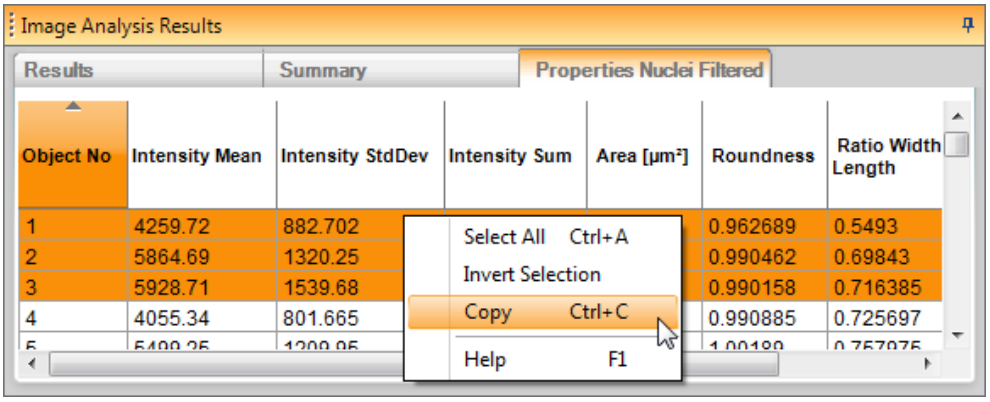
Image Analysis Results

Results | Summary | Properties Nuclei Filtered

Object No	Intensity Mean	Intensity StdDev	Intensity Sum	Area [µm²]	Roundness	Ratio Width Length
275	10265	2554.3	2319892	55.737	0.904897	0.621336
204	9897.57	2994.1	2276441	56.7235	0.835078	0.45202
202	8796.55	2675	2207933	61.9026	0.94351	0.584705
139	11070.8	3292.12	2789829	62.1492	0.967915	0.752645

How to copy results to the clipboard

1. Select the desired table rows which you want to copy.
2. Right-click on the selection and choose **Copy** from the context menu.



The selected data rows are copied to the Windows® clipboard from where you can paste them e.g. in Microsoft® Excel or other applications.

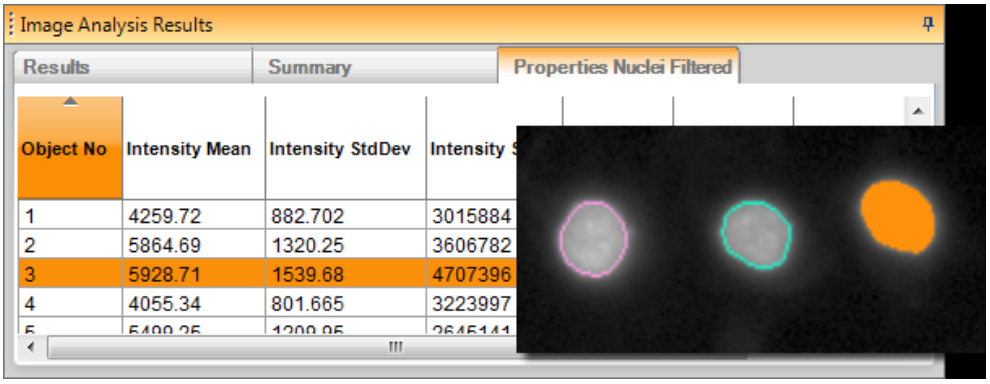
Context Menu

Element	Description
Select All	Selects all data rows.
Invert Selection	Selected rows become unselected and vice versa.
Copy	Copies the selected data row to the clipboard.
Graph – Display Selection (only on Evaluation tab)	The three **Graph – ...** menu items can be used to select the data points to be displayed in the graph: • **... – Display Selection:** The selected data points will be displayed in the graph. All other data points will be excluded. • **... – Add Selection:** The selected data points will be added to the data points which are currently displayed in the graph. • **... – Remove Selection:** The selected data points will be removed from the graph. **Notice** Be careful when using object results, since the number of data points can become extremely high. The table is limited to **500,000** rows. For further information please see section “Graph”, page 157,
Graph – Add Selection (only on Evaluation tab)	
Graph – Remove Selection (only on Evaluation tab)	
Use for Z' negative Control (Evaluation Results only)	The data points will be used as negative control for calculating a Z' value. For details see section “Graph”, page 157.

Element	Description
Use for Z' positive Control (Evaluation Results only)	The data points will be used as positive control for calculating a Z' value. For details see section “Graph”, page 157.
Help	Opens the context-sensitive help for this screen section.

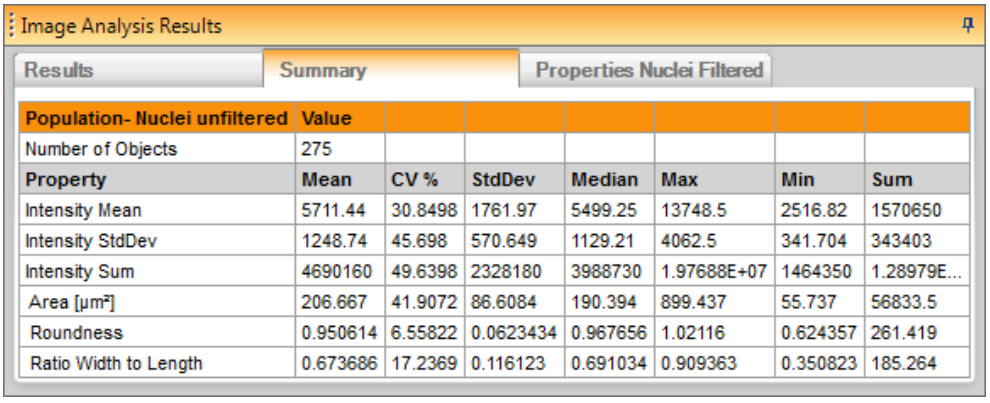
5.1.14.1 Properties

This section is only available on **Image Analysis Results**. It displays a list of detected objects with properties of each object (e.g. cell area, intensity, contrast). It depends on the selected building block which properties are displayed. The object list can be sorted by the desired property.



If you select an object in the table, the corresponding object in the image will be highlighted (orange).

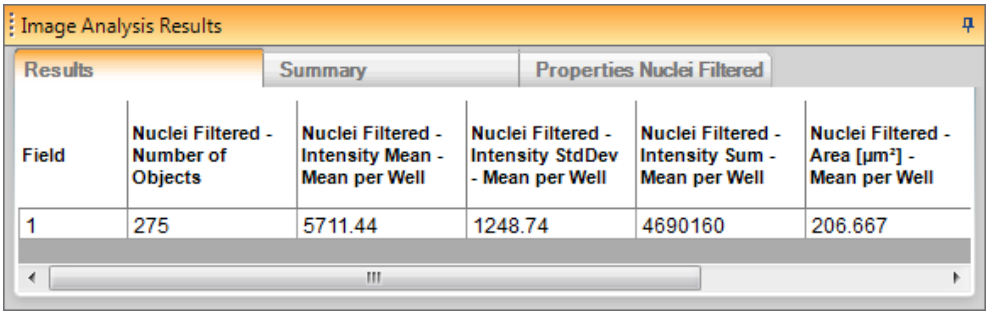
5.1.14.2 Summary



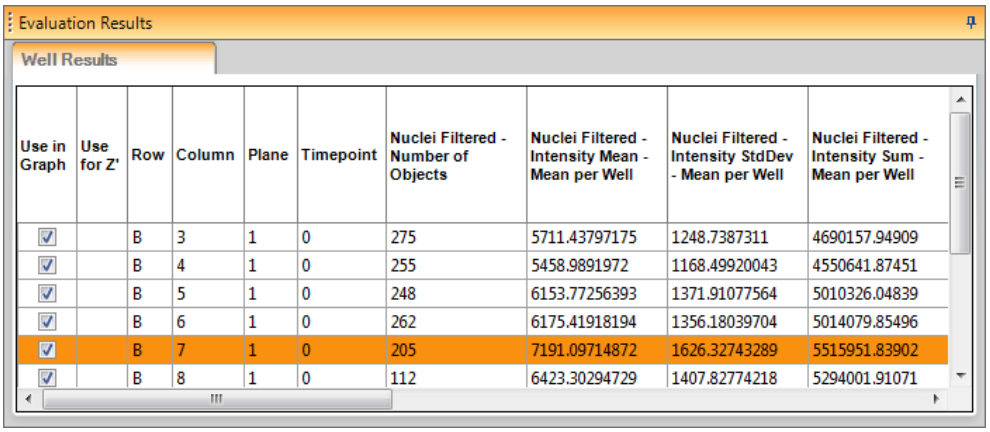
This section is only available on **Image Analysis Results**. It summarizes the properties of all detected objects in the selected field and calculates a number of statistical values. There will be separate sections for each population. The list of the properties depends on the selected building block.

5.1.14.3 Results

On the **Image Analysis** tab (**Image Analysis Results** pane), this section displays the final readout values of the analysis sequence (for the selected field) as defined in the **Define Results** block (e.g. if you test an analysis sequence).



On the **Evaluation** tab (**Evaluation Results**), this section is called **Well Results**. It displays the readout values for all evaluated wells. If multiple fields have been selected for the evaluation, the field results are calculated to one value for the whole well (mean value or sum, depending on the parameter). The result table can be sorted.



If you select a row, the corresponding well is highlighted in **Navigation – Measurement – Plate** and (if a graph is currently displayed in the **Content Area**) the corresponding data point of the graph is highlighted (and vice versa).

Default columns in Evaluation Results

These are the default columns of the **Evaluation Results** table. Additional readout parameters can be defined using the **Define Results** building block. If the analyzed measurement included an assay layout, the values of each layer will also be displayed.

Element	Description
Display	Indicates whether the data point is displayed in the graph. Activate/deactivate the check box to include/exclude a data point. To change this property for multiple data points, it is more convenient to use the context menus of the **Results** table (**Graph - Display Selection**).
Use for Z' (only for well results)	Displays “neg” or “pos” if the data point will be taken into account as negative or positive control for calculating a Z' value. For details see section “Graph”, page 157.
Row	Row letter of the analyzed well.
Column	Column number of the analyzed well.
Plane	Number of the stack plane. If no stack has been defined, the value is '1'.
Timepoint	Number of the time point. If no time series has been defined, the value is always '0'. For measurements including a time series, the number can be negative (time point before break), zero (T0, first time point of sequence 1) or positive (time point of sequence 1 or 2). See also section 5.1.7.4 “Time Series”, page 68.
Number of Analyzed Fields	Number of fields used for evaluating this well. If multiple fields had been selected, the readout values for each field are calculated to one readout value for the whole well.
Global Image Binning	Displays the binning value of the global image for the current well. This helps analyzing data with varying number of fields per well, e.g. from PreciScan.
Height [µm]	Height of the plane in relation to the focus height. If no stack has been defined, the value is '0'.
Time [s]	Time stamp of the time point in relation to T0. If no time series has been defined, the value is '0'.
Temperature	Only with TCO upgrade and if temperature control had been activated; status during measurement: • **-1:** Temperature was below target value. • **0:** Temperature was within the range of tolerance (target value ±1 °C). • **+1:** Temperature was above target value. The corresponding TCO settings (target values) are also logged in the measurement as a comment. See also section 5.1.16 “Database Browser”, page 220.

Element	Description
Target Temperature	Only with TCO upgrade and if temperature control had been activated: Target temperature during measurement. The corresponding TCO settings (target values) are also logged in the measurement as a comment. See also section 5.1.16 “Database Browser”, page 220.
CO2	Only with TCO upgrade and if control of carbon dioxide control had been activated; status during measurement: • **-1:** Carbon dioxide concentration was below target value. • **0:** Carbon dioxide concentration was within the range of tolerance (target value ±0.5 percentage points). • **+1:** Carbon dioxide concentration was above target value. The corresponding TCO settings (target values) are also logged in the measurement as a comment. See also section 5.1.16 “Database Browser”, page 220.

5.1.15 Load/Eject Plate

This function is used to insert a sample carrier into the system or to remove it from the instrument. Depending on the instrument’s status (plate detected or not) the button will change its function. The button is only available with directly connected instrument.

Notice

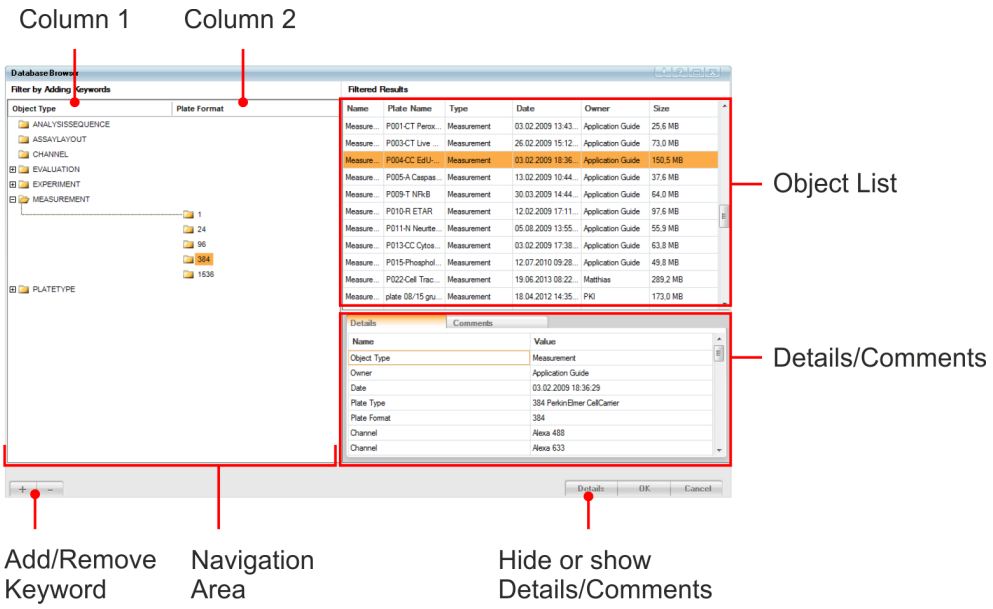
Do not try to insert or remove a plate directly via the opened lid. Please use the **Load/Eject** function (via transfer position).

See also section 6.8 “Transfer Position”, page 516.

5.1.16 Database Browser

The **Database Browser** is the interface for all database-related tasks. Keywords (see also section 5.1.16.3 “About Keywords”, page 232) help you to filter the database objects and find the desired objects quickly. You can also use the search function to search in the manually entered comments (click the + button and select **Search...**).

5.1.16.1 Overview

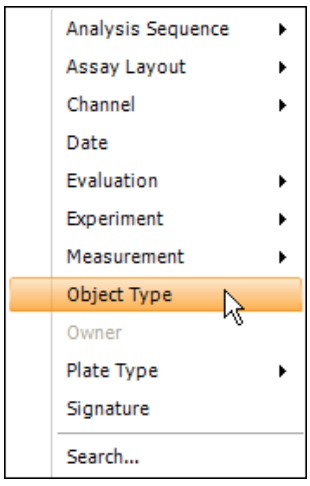


How to find an object in the database

Example: You are looking for a **measurement** and remember the following properties:

- The user **Dennis** performed the measurement.
- One of the channels was **DRAQ5**.

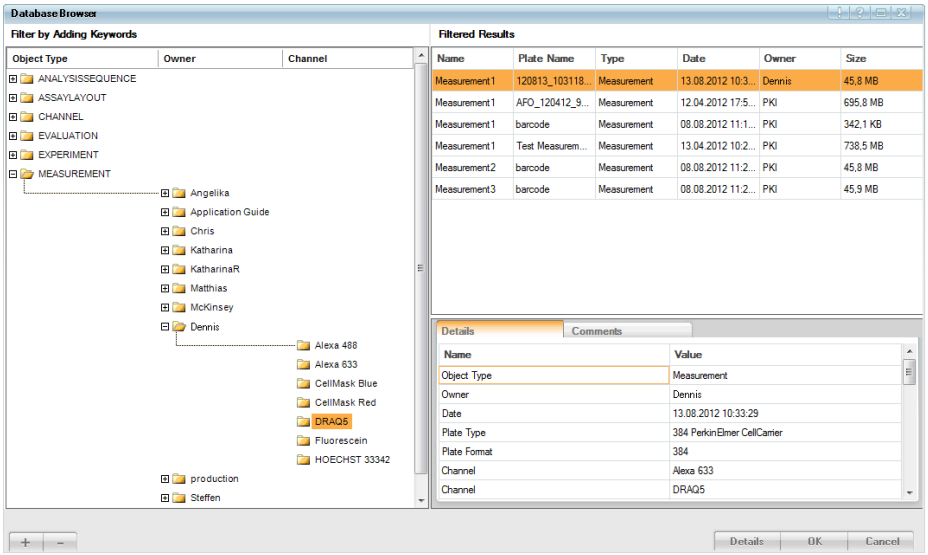
1. Add keywords (i.e. search terms) to narrow down the search. The order in which you add the keywords is arbitrary and can be changed subsequently by drag & drop (drag the column title to the desired position). The **Object List** displays all objects matching the selected keywords.



- Click + to add a keyword and select **Object Type** from the menu, so that you can limit the search to a certain object type, in this case **Measurement**.
  The keyword **Object Type** is inserted as first column in the **Navigation** area.
- Click + and select **Owner** (user who created the object).
  The keyword **Owner** is inserted as second column.
- Click + and select **Channel > Channel**.
  The keyword **Channel** is inserted as third column.

2. In the first column, double-click on **Measurement** or click the ⊞ symbol to expand this item.
   In column 2 (*Owner*), all users are listed who have performed a measurement already.

3. Double-click on owner **Dennis** or click the ⊞ symbol to expand this item.
   In column 3 (*Channel*), all channels are listed which were used in measurements performed by **Dennis**.
4. In column 3 (*Channel*) click on **DRAQ5**.
   The **Object List** displays all measurements done by **Dennis** using the **DRAQ5** channel.

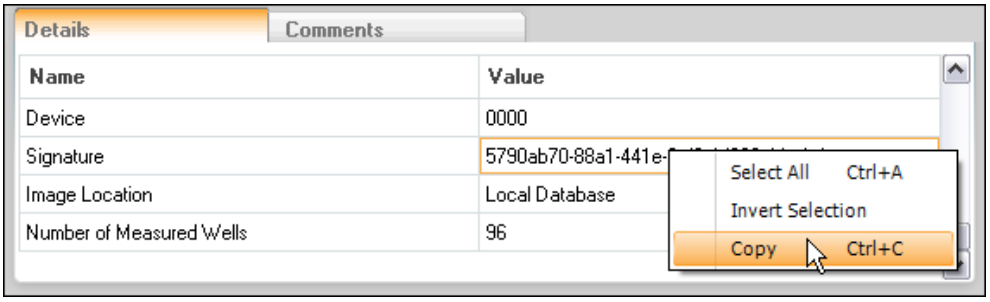


> **Notice**
> In the right-most column you can also select multiple keywords if you hold down the **CTRL** or **Shift** key and click on the keywords. The elements from all selected folders will be displayed in the **Object List**.

5. Select the desired measurement in the **Object List**.
   - If the search produces still too many results, add further keywords, e.g. **Date**.
   - If the desired object is not listed, extend the search by removing keywords (– button).

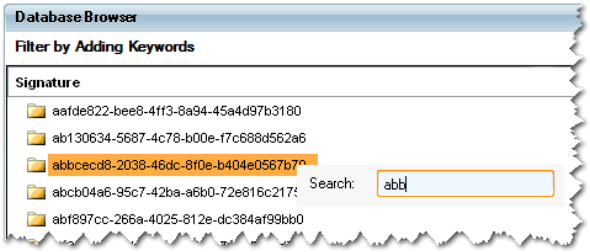
How to find an object by using its signature

Another fast and easy way to find the desired object is to search for its unique signature. The signature is visible as a property on the **Details** tab if you select an object. If you write down or copy the signature, you can use it as a “shortcut” to find that object again at a later time.



To find the object again:

1. If you want to search the whole database, use the – button to remove any other keywords which narrow down the search.
2. Click + and select **Signature** from the menu.
   The signatures of all database objects will be listed.
3. Select any item in the **Signature** column to set the focus to that column and then just start typing the first characters of the desired signature. A small search box is opened while you type. Usually the first 4-5 characters are already sufficient to identify the object. You do not have to enter the whole signature.
   Alternatively, you can also press **CTRL + F** to open an empty search box and e.g. paste a signature from the clipboard.
4. If you press **Enter**, the first signature in the column matching your query is selected.

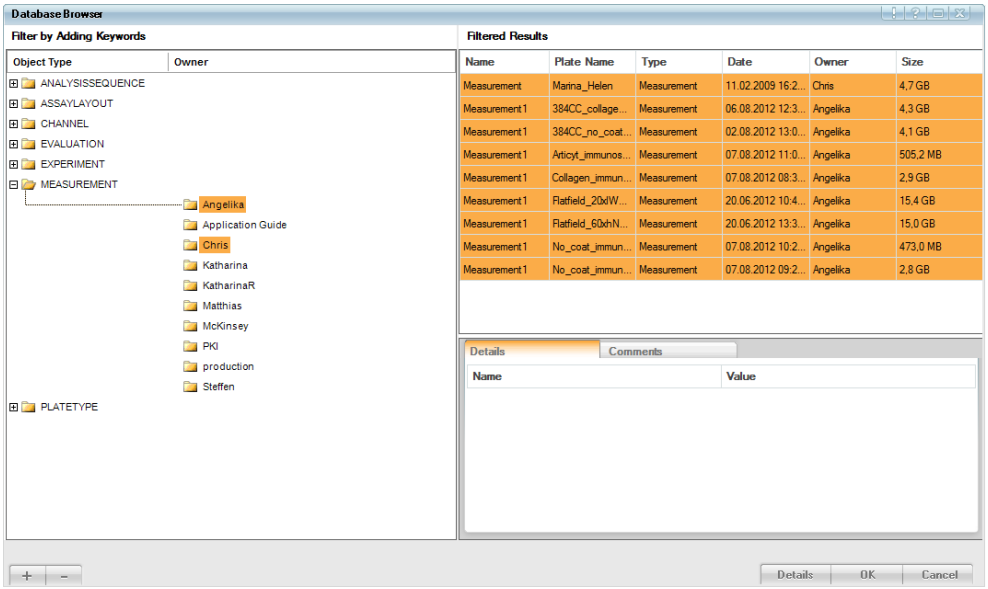


Notice
This “intra-column search” also works for other keywords, not only for **Signature**.

How to select objects from multiple keywords

In the right-most column of the **Navigation** area you can also select multiple keywords if you hold down the **CTRL** or **Shift** key and click on the keywords. The objects from all selected keywords will be displayed in the **Object List**. This can be useful if you want to select many objects, e.g. for **Write Archive**, **Delete Data**, or **Relocate Images**.

Example: You want to select all measurements from two different owners.



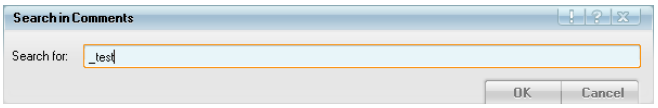
1. Add keywords **Object Type** and **Owner**. Remove all other keywords.
2. In column **Object Type**, double-click **MEASUREMENT**.
   The measurement owners appear in the second column.
3. Hold down the **CTRL** key and click on the desired owners.
   All measurements performed by these owners will be displayed in the Object List.
4. Select the desired objects in the **Object List** (in this example: all objects).

How to use the search function (search in comments)

In addition to employing the different keywords to generate your sorting path, the **Search…** function can be used. The **Database Browser** will search for the occurrence of the search term within all manually entered **comments**.

Comments are useful to add any relevant information which is not included in the automatically created keywords. They can be entered before starting a measurement or an evaluation. You can also add them subsequently to any object in the **Database Browser** (**Comments** tab).

1. If keywords have already been added which you do not want to combine with the search, use the **–** button to remove these keywords.
2. Click **+** and select **Search...** from the menu.
3. You will be prompted to enter a search term:

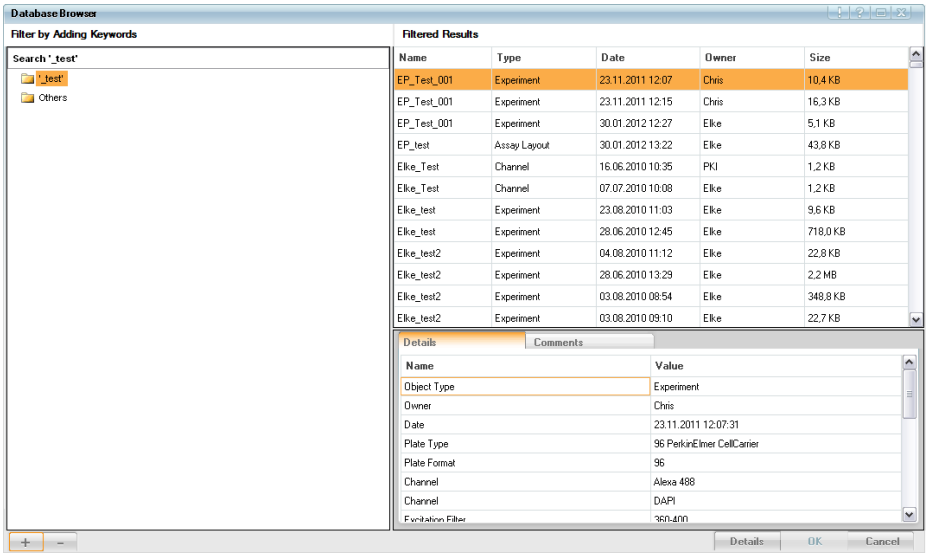


Notice

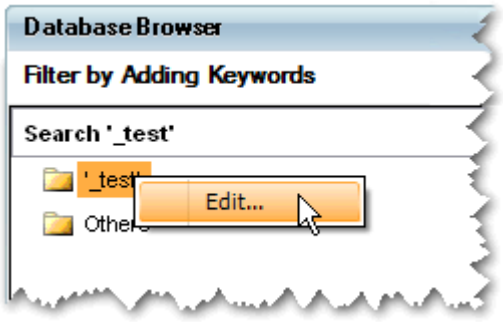
The search term must be included entirely in the comment to be found. Numbers are treated like text. Example:

- "2" will be found in the comment "I have used version 2.0".
- "3.0" will **not** be found in the comment "I have used version 3".

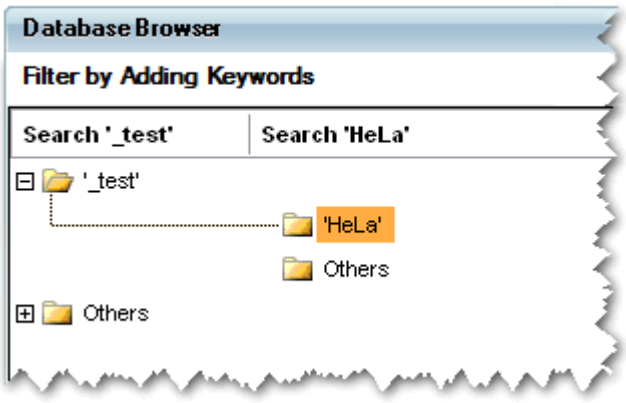
4. Click OK.
   The **Database Browser** will identify all comments in the database that contain the search term. A new **Search** column will be inserted in the **Navigation** area:



5. Two folders are displayed in the new column:
   - **'SearchTerm':** Click this folder to view all objects containing your search term.
   - **Others:** Click this folder to view all objects that do **not** contain the term.
6. Optional steps:
   - If the provided search results are unsatisfactory, the search term can be redefined: Right-click the folder with the old search term and select **Edit...** from the context menu. You will be prompted to enter a new search term.



   - You can add further separate search columns which will be combined by a logical **AND** operation (results should contain term 1 AND term 2). Of course, you can also combine search columns and regular keywords.



Notice

The search is not case-sensitive, i.e. the search term “ab” will provide the same results as “Ab” or “AB”.

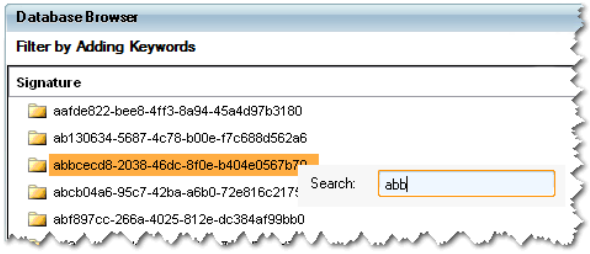
Wildcards can be used to create a more complex search term:

- The **asterisk** can replace an *arbitrary number* of characters and/or numbers: “A*B” will e.g. provide an object named “A_12345_B” as a result. You do not have to enter an asterisk at the beginning or at the end of the search term to find expressions that contain the term. This is done automatically.
- The **question mark** can replace exactly *one* character or number: "A?B" will e.g. provide an object named "A2B" as a result.
- Please note that it is not possible to search for the occurrence of the characters '*' and '?' as they will always be interpreted as wildcards in your search term.

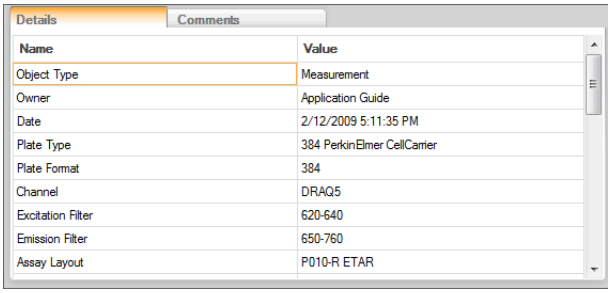
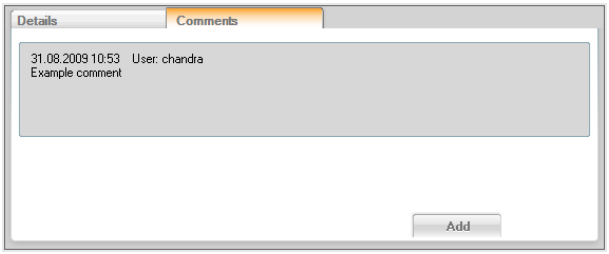
Special characters and German umlauts:

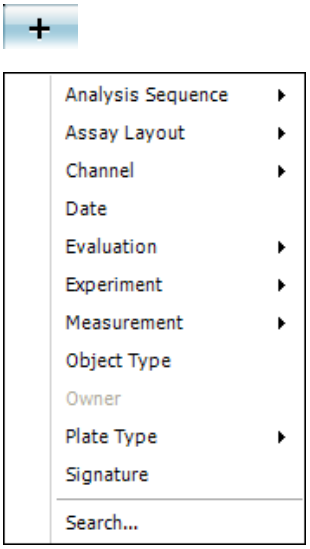
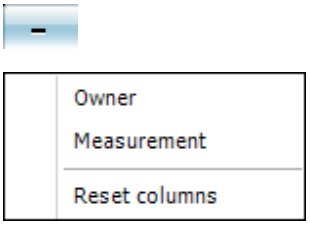
- Special characters may be used in the search term (e.g. %"$/\-' etc.).
- The German letter “ß” is treated like “ss” and vice versa: “paß” will e.g. also find an object named “pass”.
- German umlauts (ö, ä, ü) may not be replaced by normal letters (e.g. oe, ae, ue) in the search term and vice versa: “Geraet” will e.g. not find an object named “Gerät”.

Buttons and Elements

Area/Element	Description
Navigation	In the **Navigation** area you can add and select keywords to narrow down the search. For each keyword a new column is created. The keywords are displayed in a “tree structure”, similar to the file tree of the Windows® Explorer. Click on the ⊞ symbol or double-click a keyword to expand it and view the keywords of the next column. The order of the keywords can be changed (drag the column title to the desired position). The configuration of keywords/columns is saved and restored if you open the same dialog the next time. You can search a column for a keyword if you select one item in the respective column and just start typing the first characters of the desired keyword (or press **CTRL + F** to open an empty search box, e.g. if you intend to paste a search term from the clipboard). If you press **Enter**, the first keyword in the column matching your query is selected. This can be especially useful if you have added the keyword **Signature** to find e.g. a measurement by using its unique signature.  **Notice** Depending on the context in which you open the **Database Browser**, certain keywords may be preset. For example, if you load an experiment, the Object Type **Experiment** is set for the first column, and **Owner** is set for the second column. For details see section 5.1.16.2 “Variations of the Database Browser”, page 230. **Notice** In the right-most column of the **Navigation** area you can also select multiple keywords if you hold down the **CTRL** or **Shift** key and click on the keywords. The objects from all selected keywords will be displayed in the **Object List**.

Area/Element	Description
Object List	The **Object List** displays all objects matching the selected keywords. • The list can be sorted if you click on a column title. • If you double-click an object, it is automatically chosen (as if you had clicked **OK**). • To select multiple objects: (available only in certain variants of the dialog) ◦ Click on an object, keep the mouse button pressed and drag the mouse. ◦ Hold the **Ctrl** key and click on multiple objects. ◦ Group of subsequent objects: Select the first object, hold the **SHIFT** key and select the last object. • Selected objects can be copied to the clipboard: ◦ Press **Ctrl + C** or ◦ Right-click on the selection and select **Copy** from the context menu. **Context menu** (only in certain variants of the dialog): • **Select All:** All objects in the list are selected. • **Invert Selection:** Unselected objects become selected, selected objects become unselected. • **Copy:** Selected objects are copied to the clipboard. • **Attach Assay Layout:** Opens a new dialog where you can select or change the assay layout of the selected measurement (only available if a measurement has been selected). For details see section 5.1.16.5 “Attach Assay Layout”, page 235. • **Detach Assay Layout:** Allows you to remove an assay layout from a measurement (only available if a measurement with assay layout has been selected). For details see section 5.1.16.6 “Detach Assay Layout”, page 237. • **Edit Keywords:** Opens a new dialog where you can edit the keywords of an object (only availble for measurements). For details see section 5.1.16.4 “Edit Keywords”, page 234.

Area/Element	Description
Details/Comments	This section displays the **Details** tab or the **Comments** tab. You can switch between them by clicking on the tab title. The active tab is indicated by an orange line. **Details**  This tab displays the keywords of the selected object. • Selected table cells can be copied to the clipboard: ○ Press **Ctrl + C** or right-click on the selection and select **Copy Selected** from the context menu. **Comments**  This tab displays comments which have been entered for the selected object. • Click **Add** to add a comment. A new dialog is opened where you can enter the comment. Click **Save** to add the new comment at the end of the list with a time stamp. Comments cannot be deleted.

Area/Element	Description
	Opens a context menu to add keywords/columns. • **Keywords:** If you select a keyword, a new column is added in the **Navigation** area. Disabled keywords (gray) have already been added or are preset. If you add the keyword **Date**, the dialog **Select Period** is opened where you can specify the desired period of time. • **Search...:** Allows you to search for an arbitrary expression in all manually entered comments.
	Opens a context menu to remove a keyword/column from the **Navigation** area. Certain keywords/columns may not be removable, because they are preset for the respective variant of the **Database Browser**. • **Reset columns:** Restores the original default configuration of the columns. See section 5.1.16.2 “Variations of the Database Browser”, page 230.
	Allows to hide or show the **Details/Comments** area.
	The function of this button depends on the variant of the **Database Browser**. In general, you thereby confirm the selected object(s) and trigger an action, e.g. loading the selected object.
	Closes the dialog without changes or actions.

5.1.16.2 *Variations of the Database Browser*

The **Database Browser** can be opened in many different contexts. Depending on the context, it will be opened with slightly different presetting and functionality:

- **Load Object:** If you click the ... button (e.g. in one of the **Global Control** sections, to load an experiment, a measurement, etc.), the **Database Browser** is opened in *Load Object Mode*. The corresponding object type (e.g. *Experiment*) is already preset.
- **Settings – Data Management – Database Browser:** In this version the **Database Browser** is opened without any presets. You can add any combination of keywords to search for the desired object(s). If you select a measurement, you can use the context menu functions **Attach Assay Layout** and **Edit Keywords**.

Notice
Using the **Database Browser** in the **Settings** dialog you cannot load objects or use data management functions like archiving or exporting.

For details please see section "Database Browser (Settings)", page 299.

- **Data Management:** Various dialogs in **Settings – Data Management** open the **Database Browser** to let the user select objects in the database (e.g. for exporting data). The presets depend on the respective dialog (see table below).

Overview of Database Browser Presets:

Property	Load Object	Settings – DM – Database Browser	Data Management	
			Write/Read Archive, Delete Data	Relocate Images, Export Data
Column 1	*Object Type*	Owner	Owner	*Object Type: Measurement*
Column 2	Owner			Owner
Multiselect	No	Yes	Yes	Yes
Context Menu				
Select All/Invert Selection	No	Yes	Yes	Yes
Attach/Detach Assay Layout	No (except Load Measurement)	Yes	No	No
Edit Keywords	No (except Load Measurement)	Yes	No	No

Expressions printed in *italic* cannot be modified by the user.

5.1.16.3 About Keywords

In Harmony, keywords are created automatically by the system to describe the properties of a database object. In the **Database Browser** you can combine multiple keywords and use them as search terms to find the desired object.

Each object has the same four default keywords (Signature, Owner, Date, Object Type) and – depending on the object type – a number of individual keywords describing the object.

Assay Layouts

- **Measurements** can have an assay layout attached (see section 5.1.7.8 “Assay Layout Editor”, page 94) or additional user-defined keywords (see section “Define Keywords”, page 278).
- **Evaluations** inherit the current assay layout of the measurement and display the data from the assay layout in the **Evaluation Results** table. If you change the assay layout of the measurement (i.e. detach and attach a new one) the assay layout of existing evaluations will also be updated. See also sections 5.1.16.6 “Detach Assay Layout”, page 237and 5.1.16.5 “Attach Assay Layout”, page 235.

Example: Keywords for object type CHANNEL

Default keywords:

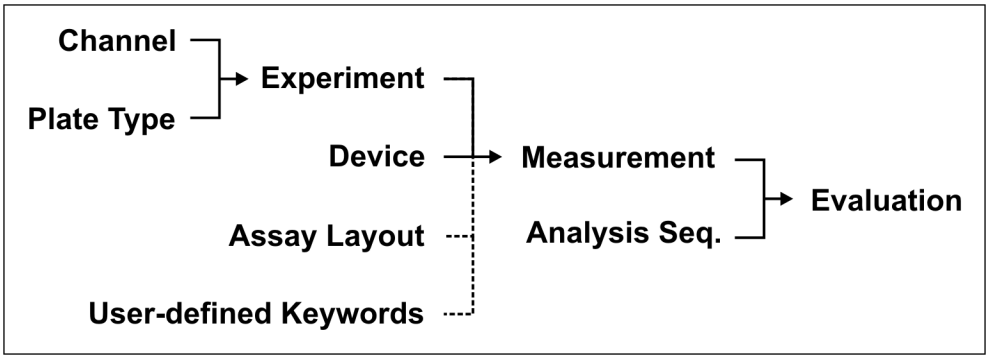
- SIGNATURE (unique identifier)
- OWNER (user who created the channel)
- DATE (creation date)
- OBJECTTYPE

Individual keywords:

- INSTRUMENT TYPE (suitable instrument type: Opera Phenix, Operetta or Operetta CLS)
- CHANNEL (name of the channel)
- CHANNEL TYPE (Fluorescence, Brightfield or Phase)
- EMFILTER (emission filter name)
- EXFILTER (excitation filter name)

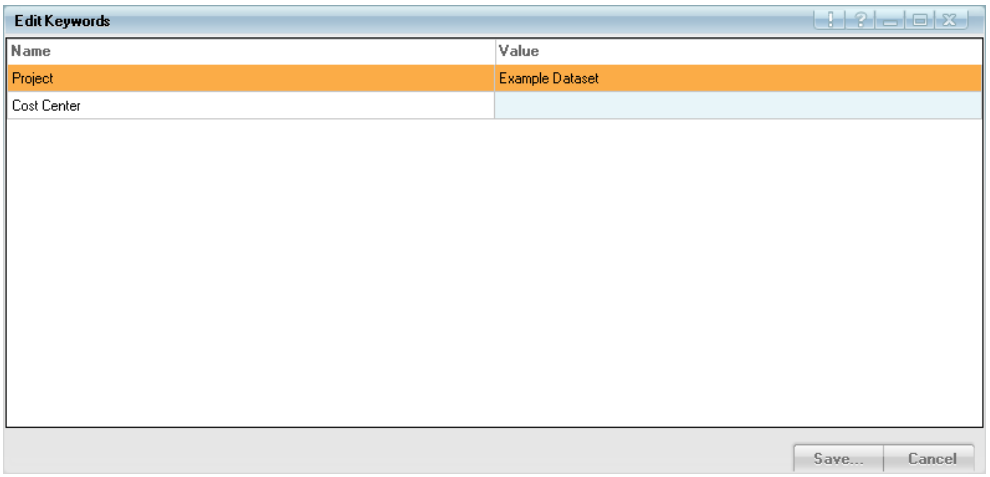
If you use an object in an experiment, measurement or evaluation, its individual keywords are passed on to the next higher object.

Example
If you use a channel in an experiment, the individual channel keywords are copied and added to the keywords of the experiment. This new connection allows you to do a combined search in the **Database Browser**, e.g. for all experiments including a certain channel.



Inheritance of keywords: *Objects pass on their keywords when used in an experiment, measurement or evaluation*

5.1.16.4 Edit Keywords



This dialog can be opened from the **Database Browser** (**Settings** dialog) if you select one or multiple measurements in the **Object List** and choose **Edit Keywords** from the context menu. It can be used to enter or modify the values of predefined keywords to describe the measurement. This function is only available if you are the owner of the corresponding measurement or if you have administrative rights.

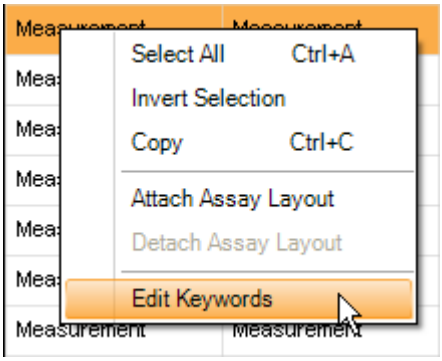
Notice

It is recommended to enter the keyword values for a measurement in **Run Experiment – Plate Settings** directly before starting a measurement. In this case, **Edit Keywords** is only required if you want to change the value of a keyword subsequently. For details see section 5.1.8.2 “Plate Settings”, page 117.

The dialog only displays user-defined keywords. They can be defined in **Settings – Data Management – Define Keywords** (requires administrative rights). See also section “Define Keywords”, page 278.

How to edit keywords

1. In **Settings – Database Browser** select one or multiple objects and choose **Edit Keywords** from the context menu.



2. Double-click the **Value** of a keyword which you want to edit and enter the new value. Values which can be edited have a light blue table cell background.

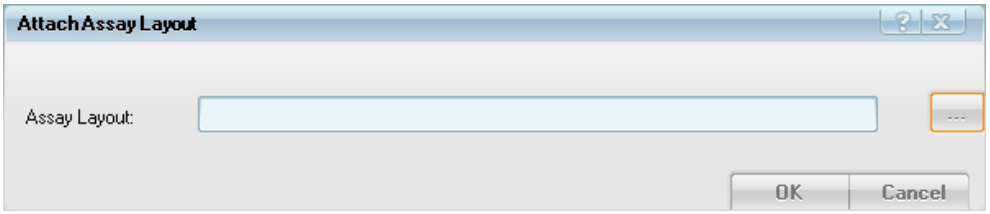
Notice
If multiple objects were selected in the **Database Browser** and the objects have different values for this keyword, this is indicated by a dot pattern of the value's cell background and no value is displayed. If you enter a new value, it will be applied to all selected objects.

3. Click Save... to save the modified keywords.

Buttons and Elements

Element	Description
Name	Name of the keyword.
Value	Value of the keyword. Double-click the field to enter a new value. **Color coding** of the table cell background: • **Light blue (with black text):** Value can be edited. • **White (with gray text):** Keyword has been removed, value cannot be edited anymore. See also section "Define Keywords", page 278. • **Dotted background:** Multiple objects were selected in the **Database Browser** and the objects have different values for this keyword, no value is displayed. If you enter a new value, it will be applied to all selected objects.
Save...	Saves the modified keywords.
Cancel	Closes the dialog discarding any changes.

5.1.16.5 *Attach Assay Layout*



This dialog can be opened from the **Database Browser** (**Settings** dialog) if you select one or multiple measurements in the **Object List** and choose **Attach Assay Layout** from the context menu. It can be used to attach an assay layout to a measurement.

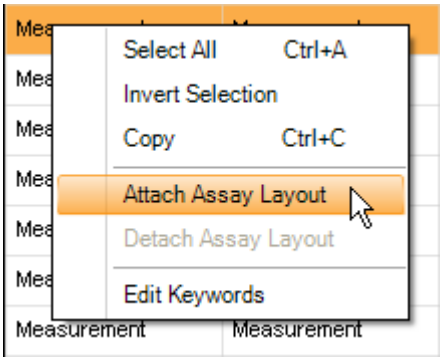
Notice
This function is only available if there is no assay layout attached to the selected measurement(s) yet.

- For detailed information on assay layouts see section 5.1.7.8 "Assay Layout Editor", page 94.

- If there is already an assay layout attached to the measurement, you will first have to remove it before you can attach a new assay layout (see section 5.1.16.6 “Detach Assay Layout”, page 237).
- Evaluations inherit the current assay layout of the measurement. If you change the assay layout of the measurement (i.e. detach and attach a new one) the assay layout will also be updated for existing evaluations. For detailed information see also section 5.1.16.3 “About Keywords”, page 232.
- Each attach or detach action (including the user who triggered it) is tracked and listed in the comments of a measurement (see section 5.1.16 “Database Browser”, page 220).

How to attach an assay layout to a measurement

1. In **Settings – Database Browser** select one or multiple measurements which have no assay layout yet and choose **Attach Assay Layout** from the context menu.



   The **Attach Assay Layout** dialog is opened. If there is already an assay layout attached, the name will be displayed in the input box.

2. Click ... to add or change the assay layout.
   A new **Database Browser** is opened where you can select an assay layout.
3. Click OK to save the changes and attach the assay layout to all selected measurements.

5.1.16.6 Detach Assay Layout

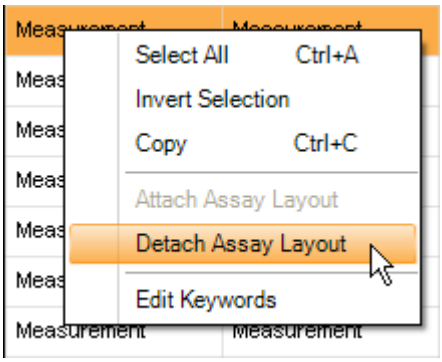
This command can be selected in the **Database Browser** (**Settings** dialog) if you select one single measurement in the **Object List** and choose **Detach Assay Layout** from the context menu. It can be used to remove an assay layout from a measurement.

Notice
This function is only available if there is already an assay layout attached to the selected measurement.

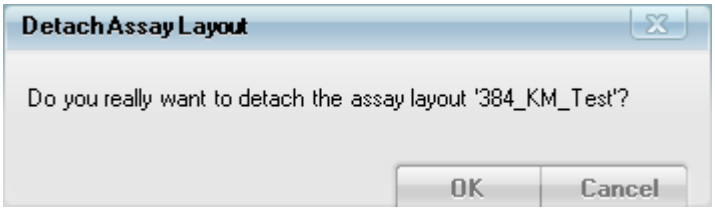
- For detailed information on assay layouts see section 5.1.7.8 “Assay Layout Editor”, page 94.
- To attach an assay layout to a measurement see section 5.1.16.5 “Attach Assay Layout”, page 235.
- Evaluations inherit the current assay layout of the measurement. If you change the assay layout of the measurement (i.e. detach and attach a new one) the assay layout will also be updated for existing evaluations. For detailed information see also section 5.1.16.3 “About Keywords”, page 232.
- Each attach or detach action (including the user who triggered it) is tracked and listed in the comments of a measurement (see section 5.1.16 “Database Browser”, page 220).

How to detach an assay layout from a measurement

1. In **Settings – Database Browser** select one single measurement and choose **Detach Assay Layout** from the context menu.

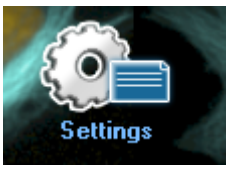


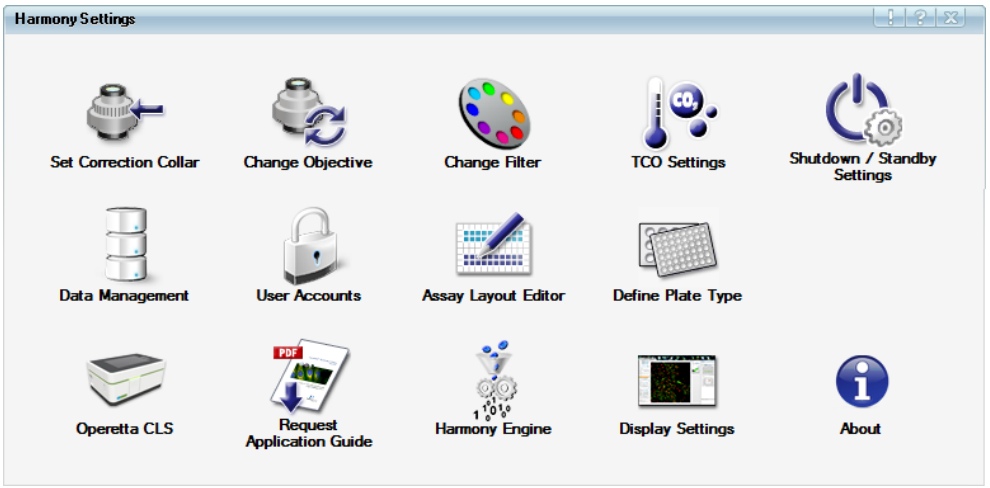
A confirmation prompt appears:



2. Click OK to detach the assay layout from the selected measurement.

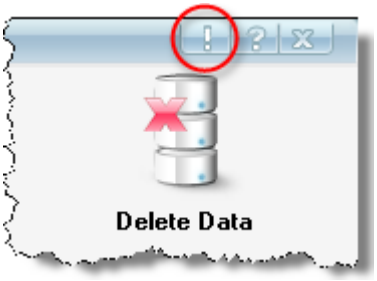
5.1.17 Settings

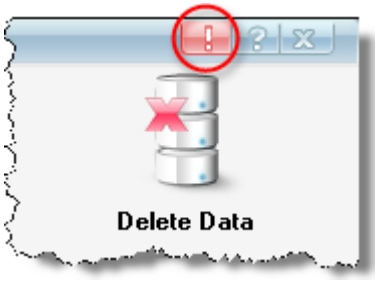




The **Settings** dialog provides access to a couple of options and administrative functions. Most functions can be used by any user, some require administrative rights. Please see the following sections for details.

While **Settings** or one of its sub-dialogs is opened, these dialogs may cover the **Messages** pane. Most dialogs under **Settings** have a  button in the title bar. This button can be used to open the **Messages** area in a separate window so that you can access the system messages. The button becomes red  to indicate that a new message has just been announced. See also section 5.1.6 “Messages”, page 39.





5.1.17.1 Set Correction Collar

Some objective lenses for the Operetta CLS have an integrated correction collar. It can be rotated and allows the correction of spherical aberrations mainly caused by differences in plate bottom thickness. A wrong setting of the correction collar can compromise image quality significantly.

The Harmony software will remind you to adjust the correction collar if you select a different plate type or objective during experiment definition. The required correction collar setting for the current plate type will also be stated.

Notice

The correction collar must be set to the **optical thickness** of the plate bottom multiplied by the standard refractive index of 1.52 for glass, not to the real thickness. In case of glass bottom plates this value is of cause identical to the physical thickness. Harmony gives this corrected thickness value automatically.

If you want to adjust or check the correction collar position at a different occasion, you can use the **Set Correction Collar** function to move the table to a position where you can access the objective.

See also section 7.1.2 “Adjust Correction Collar”, page 528 for detailed instructions.

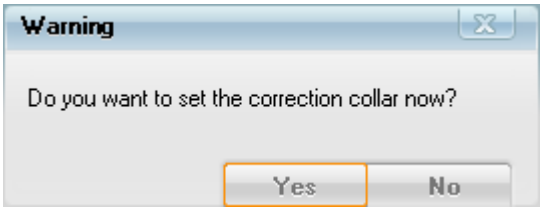
Notice

Set Correction Collar is not available if the Operetta CLS is under remote control (automation upgrade only). Switch to local control first (see section 5.1.8.1 “Global Control”, page 105).

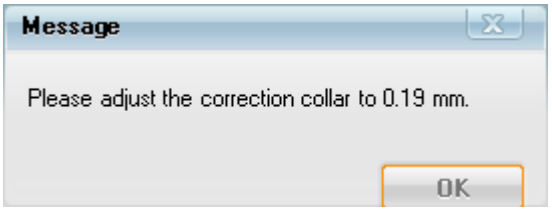
If the bottom of the used plate type is too thick to be compensated by the correction collar (i.e.the values on the correction collar are smaller than the corrected plate bottom thickness), set the correction collar to its maximum position and try again. However, this may lead to bad images and/or focus failures. In this case it is recommended to use a different combination of plate type and objective.

How to set the correction collar

1. Click the **Set Correction Collar** icon in the **Settings** dialog.
   A warning is displayed:

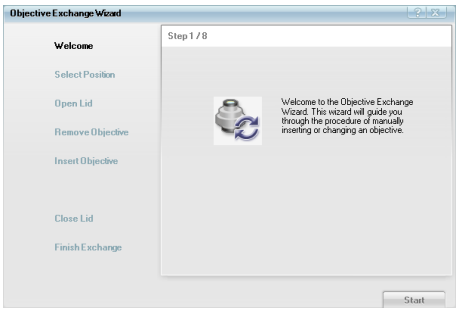


2. Click Yes to continue.
   The currently selected objective lens is moved into service position (12 o'clock position in the objective turret).
3. The required setting for the correction collar is displayed (corresponding to the selected objective and plate type):



   The lid is unlocked.
4. Open the lid of the Operetta CLS.
5. Check or adjust the correction collar. For detailed instructions see section 7.1.2 "Adjust Correction Collar", page 528.
6. Close the lid so that is snaps into place.
7. Click OK.
   The adjustment is finished.

5.1.17.2 Change Objective



The **Objective Exchange Wizard** allows you to replace one of the currently installed air objectives by a new one. The objective turret can hold up to three air objectives. If all three positions are occupied, you will be prompted to remove one objective before the new one can be installed. The wizard will guide you through the procedure step-by-step.

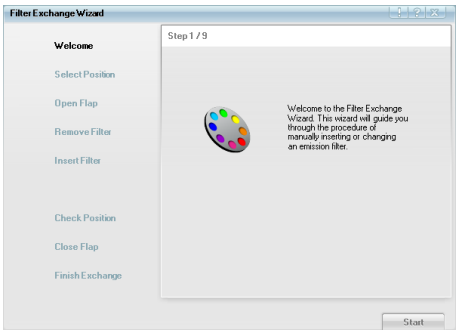
The objectives are labeled with a barcode which can be read by the Operetta CLS. Harmony will therefore always know which objective is inserted at which position.

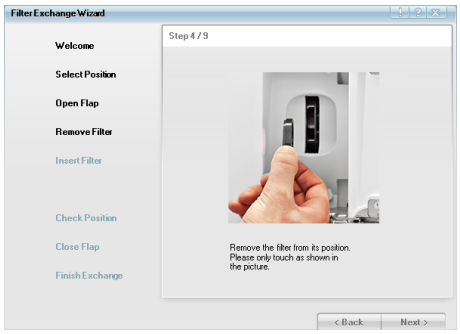
Notice

- Do not forget to adjust the correction collar of the objective to the corrected plate bottom thickness of the current plate type.
- You can only exchange the selected objective which has been moved to the service position (12 o'clock position in the objective turret). Do not try to remove a water immersion objective or an objective at a different position.

Please see section 7.1.1 "Change Air Objective", page 527 for detailed instructions.

5.1.17.3 Change Filter





The **Filter Exchange Wizard** allows you to add an emission filter or to replace one of the existing filters in the emission filter wheel with a different filter. If all eight positions of the filter wheel are occupied, one of the existing filters has to be removed. The wizard will guide you through the procedure step-by-step.

Notice

We recommend to expand the second level of all channels used in your experiment **(Setup – Channel Selection)** before using the **Filter Exchange Wizard**. In this screen configuration you can see which filter is currently not required and can be taken out of the filter wheel to have an empty position for the new filter.

The emission filters are labeled with a barcode which can be read by Operetta CLS. Harmony will therefore always know which filter is inserted at which position. The list of currently installed filters (steps 2 and 9 of the wizard) is sorted by the position of the filters in the wheel (scan order).

Notice

If you insert the filters used in an experiment in neighboring positions, this can slightly speed up measurement time.

You can also use this dialog to remove a filter, e.g. for using it in a different instrument. In this case, skip the step where the new filter is inserted. The filter list will be updated automatically.

Caution!

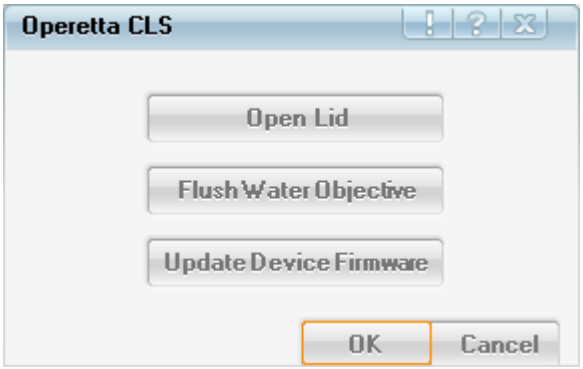
You could jam your fingers at the emission filter wheel (behind left front door). The wheel can suddenly start rotating without notice (with low force but at high speed).

- *Open the left front door only when requested by the Harmony software and follow exactly the instructions of the **Change Filter** wizard.*
- *Keep your hands away from the filter wheel if it is rotating.*

See also section 7.1.3 “Change Emission Filter”, page 530 for details and safety instructions.

5.1.17.4 Operetta CLS

This dialog is only available if Harmony is directly connected to the instrument.



Open Lid

Using the **Open Lid** button you can open the lid without prior movement of the scanning stage or the objective turret. This can be useful e.g. if a plate is tilted inside the instrument. Please note that this function is intended for troubleshooting and error recovery only.

Notice

Do not use this function to insert or remove a plate or change an objective. Please use the following software functions instead:

- 5.1.15 “Load/Eject Plate”, page 220
- 5.1.17.2 “Change Objective”, page 241

Flush Water Objective

If you click **Flush Water Objective**, the currently selected water objective is flushed for a few seconds. You can use this function to remove air bubbles from the tubing (after refilling the immersion water supply bottle) or to check whether the immersion water droplet can be formed on the lens.

During start-up of the Operetta CLS, one of the water objectives is rinsed in order to fill the tubes with water. With the first snapshot or test measurement using a water objective, this objective is rinsed again so usually there is an adequate water droplet between objective and plate bottom.

However, it can happen that, if your Operetta CLS has been in operation without use of any water objective, the very first focus scan of a well could not build a sufficient water droplet, or that there are tiny air bubbles in the droplet. The well cannot be focused then, or the image does not look as expected (it is e.g. blurry or even shows a bubble). In that case, please check the filling level of the immersion water supply. If there is sufficient water in the bottle so that the tube will not take air instead of water, click **Flush Water Objective**. Then perform another snapshot of your assay plate.

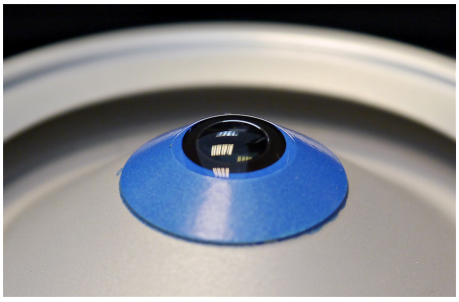
If you are sure that you already found the correct exposure settings (time, power and especially focus height), and you are still not satisfied with the image quality, or if the plate cannot be focused at all, check the water droplet on the objective.

How to check the water droplet on the objective

It is assumed that you have selected a water objective and taken snapshots already, so that the objective is in measuring position.

1. Click **Eject** and remove the sample plate.
2. Click the **Load** button again.
3. Go to **Settings** and open the **Operetta CLS** dialog.
4. Click **Open Lid** and open the lid completely.
5. Click the **Flush Water Objective** button.

   The selected objective will be flushed for a few seconds.
6. Inspect the objective:

   The just flushed water objective is located beneath the plate carrier and holds a water droplet.
7. If the water droplet is not stable or contains bubbles, repeat steps 5-6.
8. Close the lid and close the **Settings** windows.
9. Load the plate again via **Eject/Load** and click **Snapshot** to verify the image quality.

If the immersion water droplet cannot be formed on the lens, you may have to clean the blue hydrophobic ring of the water objective as described in the cleaning instructions. See also the following sections:

- 7.2.1 “Cleaning”, page 531
- 7.2.2 “Checking the Water Bottles”, page 534

Update Device Firmware

After installing a new version of the Harmony software, you also have to update the device firmware using the **Update Device Firmware** function. The required firmware file is located on the Harmony DVD.

Notice
We recommend updating the Operetta CLS firmware only after a complete restart of the whole system. So please restart your Operetta CLS and the Harmony PC before you update the Operetta CLS firmware using this dialog.

How to update the firmware of Operetta CLS

1. Insert the Harmony DVD into the DVD drive of the Harmony PC. The setup menu will be started automatically.
2. Click on **Device PC (Operetta CLS)** in the DVD menu.
3. Select **Operetta CLS Firmware** (i.e. click **DOWNLOAD**) to save the firmware file on the local hard disk.
4. Switch to Harmony and open **Settings – Operetta CLS**.
5. Click **Update Device Firmware**.
   A new dialog is opened where you can select the path to the update file.
6. Click ... .
7. Locate the previously downloaded firmware file **OperettaCLSFirmware4.6.xxxx.xxx.bin** which you have saved on your local disk and click OK.

   Notice
   Make sure that the version number of the firmware file does exactly match the version of your Harmony installation (check **Settings – About** dialog). Otherwise the update will fail.

8. Click Start.
   You will be asked to confirm your selection.
9. Click **Yes** to confirm and start the update.

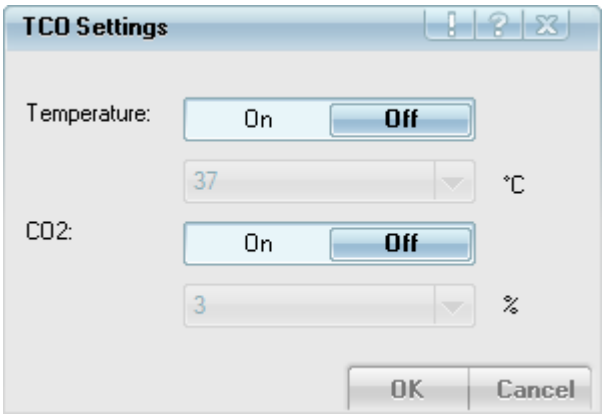
   Notice
   Do not cancel the update process or switch off the Operetta CLS while an update is running. The Operetta CLS may remain inoperative.

10. The firmware is updated. The status light is blinking red.
11. After the update, the status light is blinking green because the Operetta CLS is restarted and initialized.

    Notice
    You will get the following message: “Communication to Operetta CLS lost”. This is a normal behavior caused by restarting the Operetta CLS.

12. Wait until the status light indicates “ready” (constantly green) before you continue working with the Operetta CLS.

5.1.17.5 TCO Settings



If your Operetta CLS is equipped with the TCO upgrade (Temperature and CO_2 Option), this dialog allows you to select target values for temperature and carbon dioxide concentration in the sample chamber. Temperature and carbon dioxide control can be activated or deactivated independently of each other.

Warning!

Carbon dioxide

- *Refer to section 6.12.2 "CO2 Supply", page 521 for detailed safety instructions.*

- The flow of carbon dioxide is only active ...
  - if CO_2 control has been activated in the **TCO Settings** *and*
  - if the lid is closed so that the sample chamber is sealed.
- Check your CO_2 supply before activating carbon dioxide control (working pressure: 3 bar).
- The selected TCO settings will be logged during the measurement and added as a comment. See also section 5.1.16 "Database Browser", page 220.
- The TCO status of each well will be documented in the **Evaluation Results** table (parameters **Temperature** and **CO2**, see also section 5.1.14 "Results", page 214).
- For troubleshooting see also section 5.3.18 "TCO Issues", page 478.

How to activate TCO and start a measurement

1. Open **Settings – TCO Settings**.
2. Turn temperature control **On** or **Off**.
3. If activated: Select the desired target temperature from the combo box (possible values: 37 to 42 °C, 0.5 °C steps).

4. Turn carbon dioxide control **On** or **Off**.
5. If activated: Select the desired concentration from the combo box (possible values: 1 to 10%, 0.5% steps).
6. Click OK.
   A message box with further instructions is displayed (depending on which controls have been activated/deactivated):
7. Follow the instructions of the message, i.e.
   - for carbon dioxide control: Turn on the carbon dioxide valve and check the working pressure of your CO_2 supply (3 bar).
9. Click OK. The dialog is closed and the TCO unit starts to regulate the climate (start-up sequence).

Notice

Please note that the system needs time to establish and stabilize the target values for temperature and CO_2 concentration.

- Temperature has typically been stabilized after 1 h. If the room temperature is low and you select a temperature higher than 37 °C, the warm-up procedure may take much longer.
- CO_2 concentration has typically been stabilized after 5 min. (i.e. carbon dioxide control can be activated shortly before starting the measurement if you want to save carbon dioxide).
- It is possible to start a measurement before reaching the target temperature and CO_2 values.
- The heating mechanism is carefully optimized to avoid any transient overheating of the sample even during the warm-up cycle. As a result, it is safe to load the plate as soon as the green checkmark appears for the very first time. Stabilizing the plate and instrument together will be faster.

The current status is displayed in the **Global Control** sections of **Setup** tab and **Run Experiment** tab:

: **Start-up**, current value is still significantly lower than target value.

: **Regulating**, target value nearly reached, but not stabilized yet. In this phase temperature is at maximum 4 °C below target value and CO_2 is at maximum one percentage point below target value. Transiently slightly higher values for temperature and CO_2 may also occur in this phase, please check the **Messages** window.

: **Ready**, target value reached (target temperature ±1 °C; target concentration ±0.5 percentage points).

Off: Not activated in **TCO Settings** or switched off by shutdown.

Standby: Deactivated temporarily between two measurements (only for **Remote** mode, see also section 5.1.17.6 “Standby/Shutdown Settings”, page 249)

: **Operating Error** (e.g. lid open), see also section 5.3.18 “TCO Issues”, page 478.

The status of the temperature control is also visualized by the Operetta's progress LEDs. A blinking “T” indicates that the TCO unit is warming up or reheating. If the target temperature has been reached “T” will shine continuously. See also section 6.5 “Instrument Status”, page 510.

Notice

During the start-up sequence the status of temperature and carbon dioxide concentration will alternate several times between **Regulating** and **Ready**. The target values only have been stabilized if status **Ready** is displayed continuously.

10. When the target values have been reached: Click **Eject Plate**, insert your sample plate at the transfer position and click **Load Plate**.
11. Open the **Run Experiment** tab.
12. Optionally: Activate the **Shutdown**. Using this function you can automatically switch off temperature control and/or carbon dioxide control after the experiment. For details see section 5.1.17.6 “Standby/Shutdown Settings”, page 249.
13. Click Start to start the measurement.

How to deactivate TCO

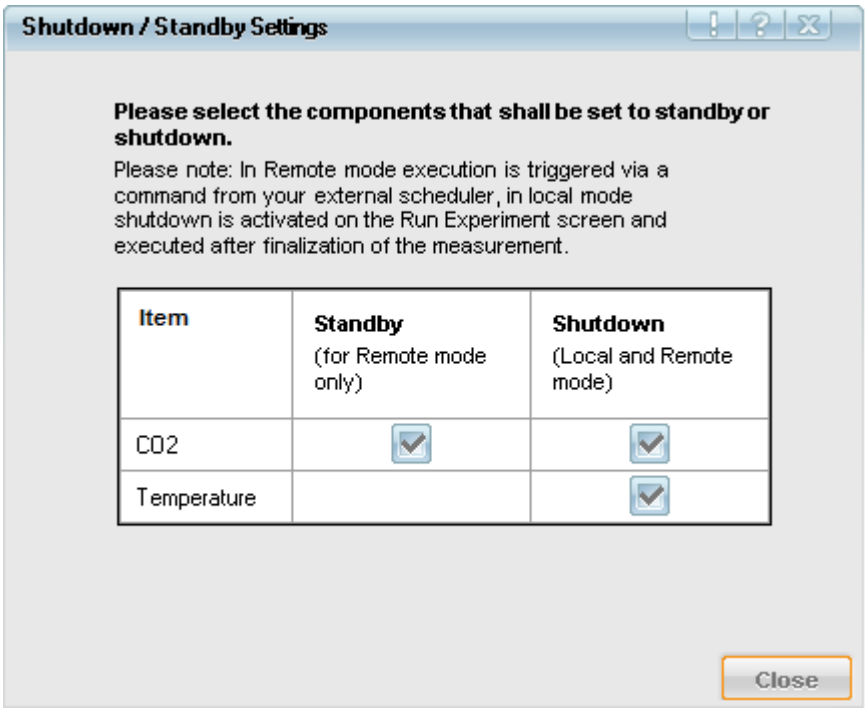
1. Open **Settings – TCO Settings**.
2. Turn temperature control **Off**.
3. Turn carbon dioxide control **Off**.
4. Click OK.
   A message box with further instructions is displayed (depending on which controls have been activated/deactivated):
5. Follow the instructions of the message, i.e. turn off the carbon dioxide valve (if carbon dioxide control was deactivated).
6. Click OK. The dialog is closed and the TCO unit is deactivated.
   The status of temperature and carbon dioxide control changes to **Off**:

Buttons and Elements

Element	Description
Temperature	**On/Off:** Allows you to activate or deactivate temperature control. If it is activated, the target temperature can be selected in the combo box. Range: 37 to 42 °C (0.5 °C steps).
CO2	**On/Off:** Allows you to activate or deactivate carbon dioxide control. If it is activated, the target value for the carbon dioxide concentration can be selected in the combo box. Range: 1 to 10 % (0.5 % steps).
Cancel	Closes the dialog and discards all changes.
OK	Closes the dialog and applies the changes.

5.1.17.6 Standby/Shutdown Settings

Using this dialog you can select the instrument components which shall be switched off during standby or shutdown. This can be useful e.g. to save carbon dioxide when using the TCO. The list of components which can be selected depends on the instrument configuration. The shutdown and standby settings only apply to TCO components which had been activated in the **TCO Settings** dialog before the measurement.



- **Shutdown** means:
  - In **Local** mode: Switching off components automatically after the experiment has been finished.

- In **Remote** mode: Switching off components at the end of a screening run, triggered by your external scheduler sending a shutdown command.

- **Standby** means:
  - Only for **Remote** mode: Deactivating components temporarily between two experiments, triggered by your external scheduler sending a standby command. The components will be “waked up” automatically when the next measurement is started.

How to switch off components after a measurement (Local mode)

1. Open the **Standby/Shutdown Settings** dialog and check the **Shutdown** option for the desired components.
2. On the **Run Experiment** screen, activate the **Shutdown** option.

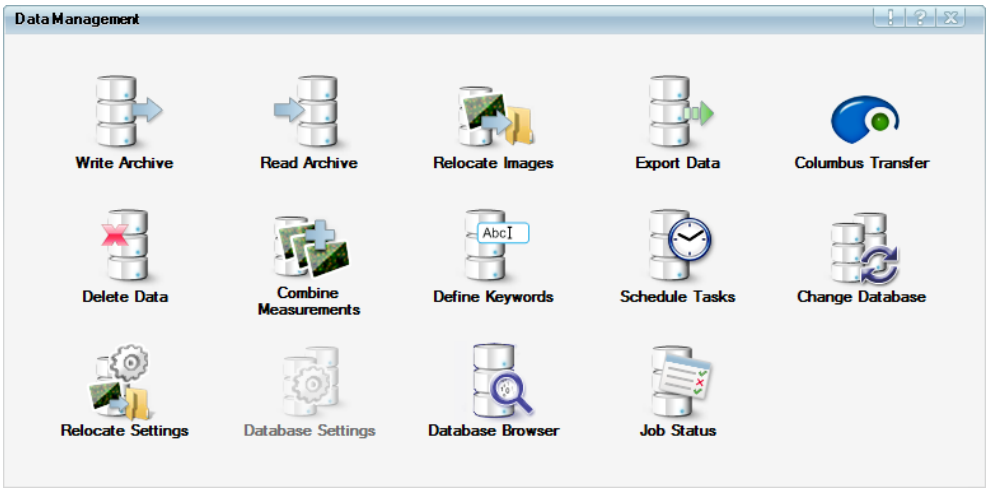
How to switch off components after a screening run (Remote mode)

1. Open the **Standby/Shutdown Settings** dialog and check the **Shutdown** option for the desired components.
2. On the **Run Experiment** screen, switch to **Remote** mode.
3. Make sure that your scheduling software will send a Shutdown command at the end of the screening run.

How to set components to standby during a screening run (Remote mode only)

1. Open the **Standby/Shutdown Settings** dialog and check the **Standby** option for the desired components. Please note that standby is not supported by all components.
2. On the **Run Experiment** screen, switch to **Remote** mode.
3. Make sure that your scheduling software will send a **Standby** command after the experiment.

5.1.17.7 Data Management

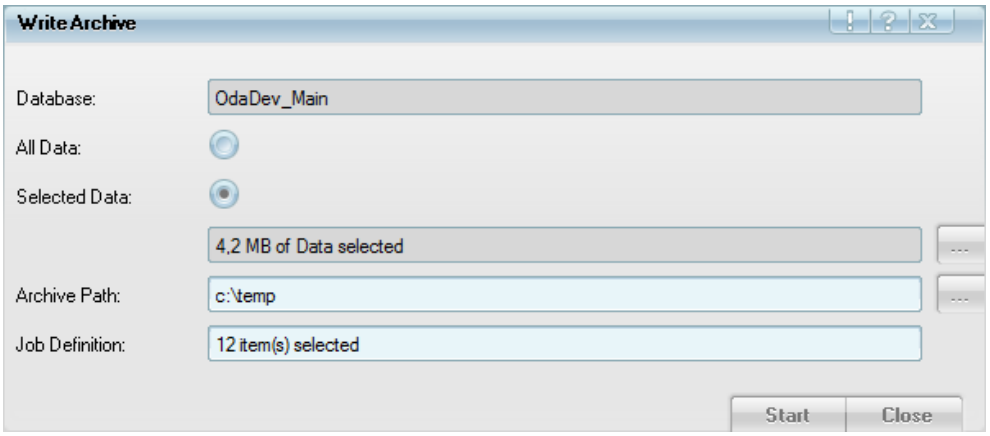


The **Data Management** dialog offers a number of functions for data handling and database administration:

- **Write Archive:** Copy data to another location.
  See section "Write Archive", page 253.
- **Read Archive:** Import archived data.
  See section "Read Archive", page 256.
- **Relocate Images:** Move images to another location.
  See section "Relocate Images", page 259.
- **Export Data:** Export data for use with other applications.
  See section "Export Data", page 262.
- **Columbus Transfer:** Transfer measurements (and associated images, assay layouts and evaluations) to the Columbus image database. See section "Columbus Transfer", page 271.
- **Delete Data:** Delete data from database.
  See section "Delete Data", page 272.
- **Combine Measurements:** Combine single measurements to a time series or add single measurements to an existing time series.
  See section "Combine Measurements", page 274.
- **Define Keywords:** Add user-defined keywords (requires administrative rights).
  See section "Define Keywords", page 278.
- **Schedule Tasks:** Create automatic and periodic tasks for relocating images or archiving data (disabled for office installation).
  See section "Schedule Tasks", page 280.
- **Change Database:** Select database and view database properties.
  See section "Change Database", page 292.
- **Relocate Settings:** Define paths for relocation (requires administrative rights).
  See section "Relocate Settings", page 294.
- **Database Settings:** Add or remove databases (paths), only available for office installation (or if no instrument connected). See section "Database Settings", page 298.

- **Database Browser:** Search for objects in the database and view their keywords and comments. It is not possible to load objects using this dialog. See section “Database Browser (Settings)”, page 299.
- **Job Status:** View status and log files of background jobs. Cancel jobs, if necessary.
  See section “Job Status”, page 300.

Write Archive



The **Write Archive** function can be used to copy selected data objects to a different location, e.g. to archive measurements on a DVD or server or copy them into an archive which can be transferred to a different Operetta CLS database. The original objects remain in the database, they are not deleted. Associated images are included automatically in the archive if you select a measurement or evaluation.

Notice

- The counterpart of **Write Archive** is **Read Archive**. Archived data remains compatible with Harmony and can be re-imported into the database using the **Read Archive** function. For details see section “Read Archive”, page 256.
- If you want to export and convert data for use in other applications (e.g. Columbus® or Microsoft® Excel), use the function **Export Data** (see section “Export Data”, page 262). Please note that data exported by this function **cannot be re-imported into the database**.

Hint

When selecting the data to be archived, it is sufficient to select only evaluations to archive all important data that was necessary to perform this experiment. Due to the inheritance structure in Harmony (see section 5.1.16.3 “About Keywords”, page 232) the evaluations will contain all the additional object information and keywords (measurement, experiment, channels, plate types, analysis sequence etc.), assay layouts and user-defined keywords, if applicable.

- Measurements that have **not** been evaluated have to be selected and archived separately!
- To archive a complete **PreciScan** you have to select the PreScan and ReScan **evaluations**! Otherwise the link between the measurements cannot be restored and the analysis sequences will be missing. If no evaluation exists for the ReScan, you have to select the ReScan measurement instead (e.g. Measurement 1b).

Hint

The archived data can be written to a new archive or added to an existing one. On the one hand the latter is useful, because it is much faster and easier to search through a larger archive for the desired object (using the sorting mechanism of the **Database Browser**) than having to open multiple smaller archives successively. On the other hand, an archive should not become too large (e.g. multiple terabytes/more than 10.000 data objects), otherwise it will be difficult to handle.

Notice: Before adding data to an existing archive, please make sure that no other user is currently adding data to the same archive (e.g. from a Harmony office installation). In rare cases, this could lead to the loss of data.

Hint

If you want to delete the archived data from the database, open the **Delete Data** dialog directly after archiving has been finished. The archived objects will be selected as a preset and you can delete them very easily without making a new selection.

How to archive data

1. Click the **Write Archive** icon in **Settings – Data Management**.
   The **Write Archive** dialog is opened.
2. To archive all objects in the database:
   - Activate **All Data**. The database is analyzed and the required disk space is displayed in the text box under **Selected Data**. The hourglass cursor will be displayed until the analysis has been finished.

   To archive only selected objects:
   - Activate **Selected Data**.
   - Click the ... button next to the **Selected Data** field.
     The **Database Browser** is opened.
   - Select the desired object(s) and click **OK**.
     For detailed instructions see section 5.1.16 “Database Browser”, page 220.
     In the **Selected Data** field, the required disk space for all selected objects is displayed.
4. Click the ... button next to the **Archive** field.
   A new dialog is opened where you can select (or create) a folder for the archive. You can also select a folder with an existing archive if you want to add the selected data to this archive. This can be useful, because one large archive can be re-imported more easily than multiple smaller ones.
   Confirm with **OK**.
5. Optional: Enter a description of the task in the **Job Definition** field (default text: number of selected items). This text will be displayed in the **Job Status** window.
6. Click Start.

   A background job is created for the archiving task and the dialog is closed.

Notice

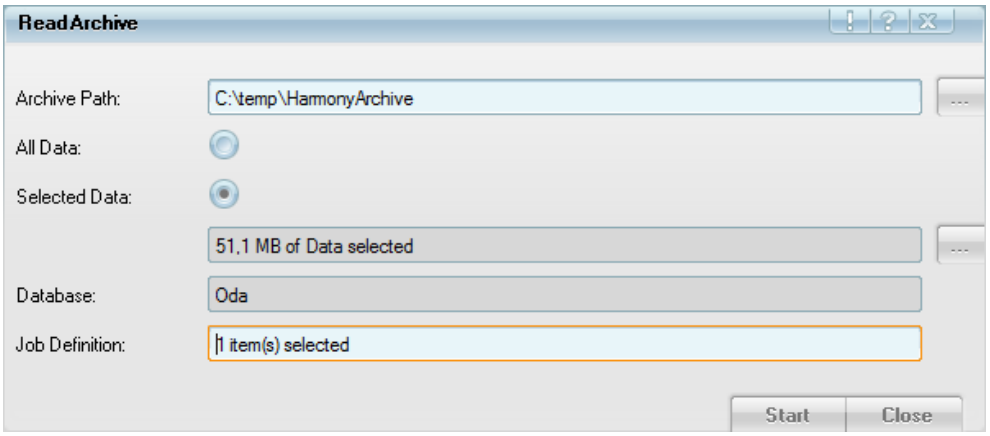
If the target folder contains already an archive which includes also objects that are identical with some of the selected objects, the **Replace Object?** dialog is opened which allows you to decide how to handle these objects.

7. If you want check the status of the archiving process, open **Settings – Data Management – Job Status**. See also section "Job Status", page 300.

Buttons and Elements

Element	Description
Database	Displays the name of the connected database. If you want to archive data from a different database, you must connect to that database first. See section "Change Database", page 292.
All Data	Selects all objects in the database. If you click this option, the database is analyzed and the required space for all objects is displayed in the text box under **Selected Data**. The hourglass cursor will be displayed until the analysis has been finished.
Selected Data	Click the ... button to open the **Database Browser** and select the objects to be archived. If the selection has been made, this field displays the required disk space for all selected objects.
Archive Path	Displays the archive path. Click the ... button to select a target folder. If the folder is empty, a new archive will be created. If there is already an archive, the data will be added to this archive. **Notice** It is recommended to keep the archive path short and to avoid many subfolders (total length: 140 characters maximum). The Windows® operating system is limited to 256 characters for the total length of a file path. The remaining characters are required by Harmony for creating subfolders and file names within the archive folder.
Job Definition	Here the user can optionally give a description of the task. Default value is number of data items selected.
Start	Creates a background job for the archiving task and closes the dialog. The progress can be monitored in the **Job Status** window, see section "Job Status", page 300.
Close	Closes the dialog without starting an archiving job.

Read Archive



The **Read Archive** function can be used to import data into the database which has been archived from the current or from a different database. You can also import archives generated by other Operetta or Opera Phenix users (i.e. from other instruments running the same or an older Harmony version).

Depending on your selection of objects, you can import an archive completely or only the desired objects. Images associated with the selected objects are imported automatically from the archive.

Notice

- The counterpart of **Read Archive** is **Write Archive**. Only data generated using the **Write Archive** function can be imported into a database. For details see section "Write Archive", page 253.
- Data generated by the **Export Data** function **cannot be re-imported into the database**.

Notice

If you import objects which already exist in the database, the **Replace Object?** dialog is opened which allows you to decide how to handle these objects (overwrite, skip, etc.).

Overwriting is only possible if you are a Harmony admin or owner of the objects. See the corresponding info message in the **Messages** window for a list of files (and their owners) which could not be replaced.

How to import data

1. Click the **Read Archive** icon in **Settings – Data Management**.
   The **Read Archive** dialog is opened.
2. Click the ... button next to the **Archive** field.
3. Select the folder where the desired archive is stored and click **OK**.
4. To import all objects from the archive:
   - Activate **All Data**. The archive is analyzed and the required disk space is displayed in the text box under **Selected Data**. The hourglass cursor will be displayed until the analysis has been finished.

   To import only selected objects from the archive:
   - Activate **Selected Data**.
   - Click the ... button next to the **Selected Data** field.
     The **Database Browser** is opened.
   - Select the desired object(s) and click **OK**.
     For detailed instructions see section 5.1.16 "Database Browser", page 220.
     In the **Selected Data**field, the required disk space for all selected objects is displayed.
4. Optional: Enter a description of the task in the **Job Definition**field (default text: number of selected items). This text will be displayed in the **Job Status** window.

5. Click Start.

   A background job is created for the read archive task and the dialog is closed.

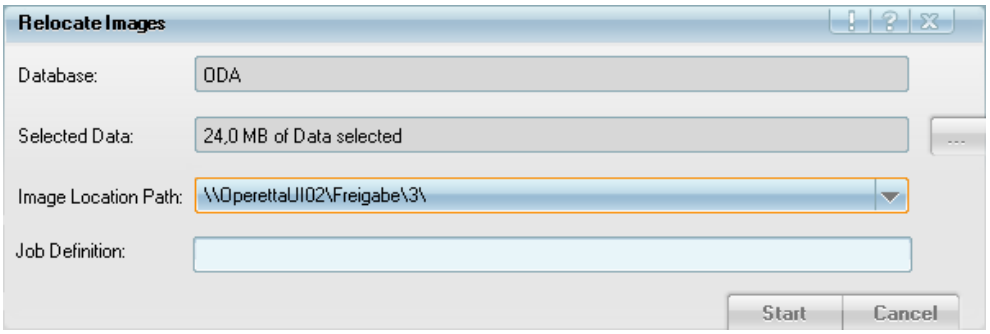
   Notice
   If you import objects which already exist in the database, the **Replace Object?** dialog is opened which allows you to decide how to handle these objects.

6. If you want check the status of the read archive process, open **Settings – Data Management – Job Status**. See also section "Job Status", page 300.
7. Once the background job has been completed, restart Harmony to refresh the view of all list elements.

Buttons and Elements

Element	Description
Archive	Displays the archive path. Click the ... button to select the folder where the archive is stored.
All Data	Selects all objects in the archive. If you click this option, the archive is analyzed (progress window appears) and the required space for all objects is displayed in the text box under **Selected Data**. The hourglass cursor will be displayed until the analysis has been finished.
Selected Data	Click the ... button to open the **Database Browser** and select the objects within the archive to be imported. If the selection has been made, this field displays the required disk space for all selected objects.
Database	Displays the name of the connected database. If you want to import the data into a different database, you have to connect to that database first. See section "Change Database", page 292.
Job Definition	Here the user can optionally give a description of the task. Default value is number of data items selected.
Start	Creates a background job for the read archive task and closes the dialog. The progress can be monitored in the **Job Status** window, see section "Job Status", page 300.
Close	Closes the dialog without starting a read archive job.

Relocate Images



The **Relocate Images** function can be used to move selected images to a different computer or hard disk. This can be useful e.g. to **free up disk space** on the local hard disk. The relocated images remain accessible in Harmony. References in the database are updated to the new image location. The meta data (keywords, numerical data) remain in the database, only images are moved.

Notice

The destination path for the relocated data has to be accessible at any time (e.g. on a network server). It is not recommended to use USB sticks or other removable media. Otherwise the relocated images cannot be opened if you select them in Harmony. Furthermore, you must have write access to the destination folder and there must be sufficient disk space.

Image location paths (and the required network account settings) have to be defined in **Settings – Database Management – Relocate Settings**, see section “Relocate Settings”, page 294.

The relocation process can also be automated and triggered periodically using a scheduled task (see section “Schedule Tasks”, page 280).

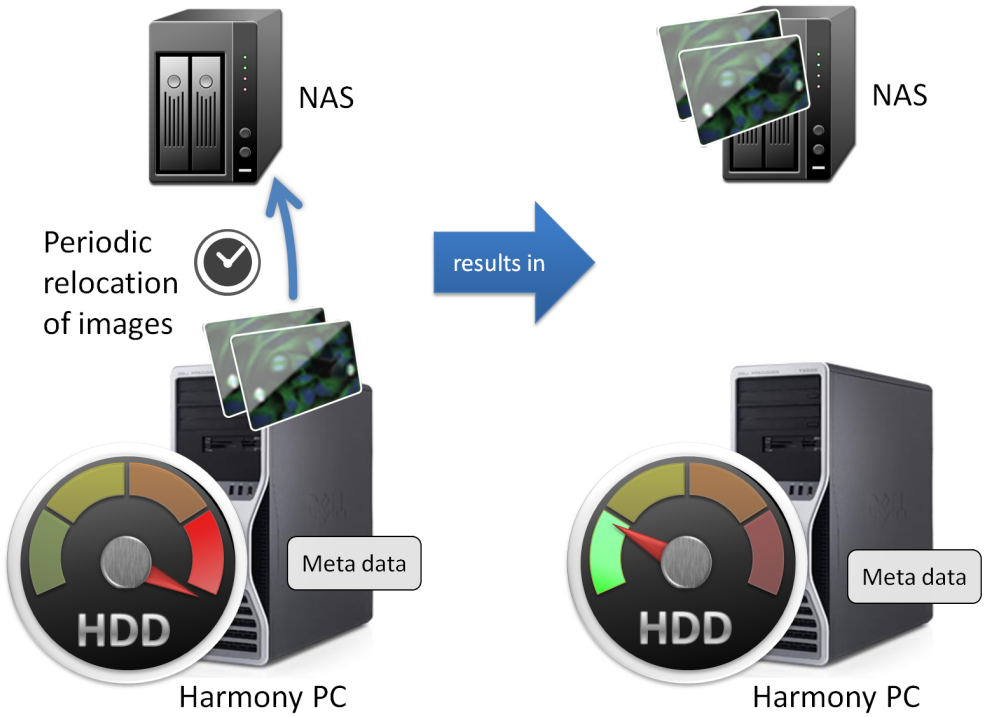
Notice

- Normal users can only relocate **their own** images. To relocate images, the user has to be the owner of the corresponding measurement.
- Administrators can relocate **all** images in the database. Take care not to relocate data of other users accidentally.

Notice

- **Relocate Images** is **not** a suitable tool to backup your data. Please refer to the **Operetta CLS Application Guide** (chapter 4) for a description of recommended backup strategies.
- Harmony database performance depends on the number of database objects. To prevent the database of getting slow and unresponsive, please archive finished projects using the **Write Archive** function and then delete them from the database using the **Delete Data** function. Please note that the **Relocate Images** function will not reduce the number of objects in the database, but only free up disc space on the Harmony PC.

Typical application of "Relocate Images"



Relocate images to free up hard drive space on the instrument PC. Only images are moved to a secondary storage device (NAS), metadata will remain on the Harmony PC. Moved data (images) are still fully accessible in Harmony, even if not stored on the primary device. The secondary device is just an extension to the primary storage. Relocate Images was designed for one purpose: free up disk space.

How to relocate images

1. Click the **Relocate** icon in **Settings – Data Management**.

   The **Relocate Images** dialog is opened.

2. Click the ... button next to the **Selected Data** text box.

   The **Database Browser** is opened.

3. The images to be relocated cannot be selected directly. Instead, select the corresponding measurements and click **OK**.

   If you do not have administrative rights, you may only select your own measurements. Otherwise you will get an error message as soon as you click Start. For detailed selection instructions see section 5.1.16 "Database Browser", page 220.

   In the **Selected Data** text box, the required disk space for all images belonging to the selected measurements is displayed.

4. Select a path from the **Image Location Path** combo box.

   Destination paths for relocation have to be defined in **Settings – Database Management – Relocate Settings**, see section "Relocate Settings", page 294.

5. Optional: Enter a description of the task in the **Job Definition** text box (default

text: number of selected items). This text will be displayed in the **Job Status** window.

6. Click Start.

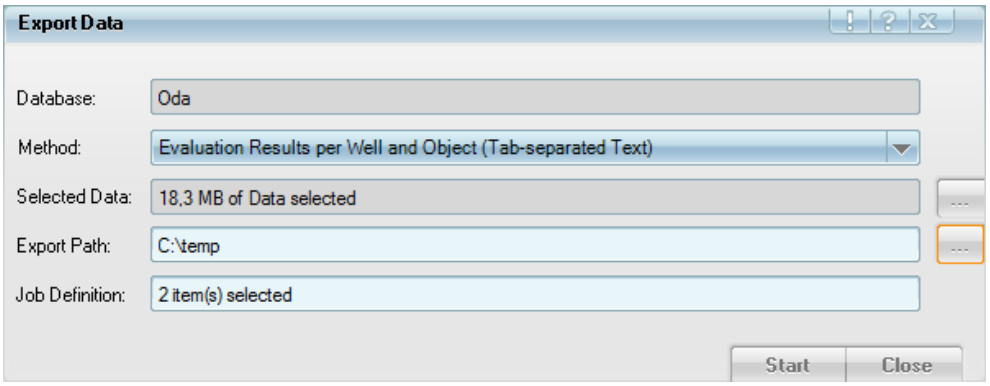
   A background job is created for the relocating task and the dialog is closed.

7. If you want check the status of the relocating process, open **Settings – Data Management – Job Status**. See also section "Job Status", page 300.

Buttons and Elements

Element	Description
Database	Displays the name of the connected database. If you want to relocate images from a different database, you have to connect to that database first. See section "Change Database", page 292.
Selected Data	Click the ... button to open the **Database Browser** and select the objects to be archived. If the selection has been made, this text box displays the required disk space for all selected objects.
Image Location Path	Allows you to select a predefined destination path from the combo box. Image location paths have to be defined in **Settings – Database Management – Relocate Settings**, see section "Relocate Settings", page 294. If you select **Local Database**, you can move selected images back into the local database which had been relocated to a different computer/hard disk. Images located on a server can directly be relocated to another server, if desired. It is not necessary to first move them back in the local database. **Notice** The current storage location of an image is displayed in the **Database Browser** if you select the corresponding measurement (see section **Details**, keyword *Image Location*).
Job Definition	Here the user can optionally give a description of the task. Default value is number of data items selected.
Start	Creates a background job for the relocating task and closes the dialog. The progress can be monitored in the **Job Status** window, see section "Job Status", page 300.
Close	Closes the dialog without starting an relocating job.

Export Data



The **Export Data** function can be used to export selected measurements and results for use with third-party applications (e.g. for post-processing of results in Microsoft® Excel or Spotfire® or for importing images into the Columbus image database). During the export, the objects are converted to the required format. The original objects are not modified and remain in the database.

Several export **methods** for different purposes are available. For details and examples see section "Methods", page 264.

Notice

Exported data is modified and converted to another format which **cannot be re-imported into the database**. To export and import unmodified Harmony datasets, use the functions **Write Archive** and **Read Archive**.

- **Write Archive:** See section "Write Archive", page 253.
- **Read Archive:** See section "Read Archive", page 256.

Notice

Please use the faster and easier **Columbus Transfer** instead of **Export Data** if you have Columbus available in your network and want to transfer data directly. For details please refer to section "Columbus Transfer", page 271.

If you do **not** have direct access to Columbus please use the **Export Data** option with the method **Measurement – incl. associated files** to store the data and transport them to the Columbus location for import.

How to export data

1. Click the **Export Data** icon in **Settings – Data Management**.
   The **Export Data** dialog is opened.
2. Select an export method from the **Method** combo box.
3. Click the ... button next to the **Selected Data** field.
   The **Database Browser** is opened.
4. Select the desired object(s) and click **OK**.

Depending on the selected method, the **Object Type** is preset to **Measurement** or **Evaluation**. For detailed instructions see section 5.1.16 “Database Browser”, page 220.

In the **Selected Data** field, the required disk space for all selected objects is displayed.

5. Click the [...] button next to the **Export Path** field.

   A new dialog is opened where you can select (or create) a folder for the exported data.
   Confirm with **OK**.

6. Optional: Enter a description of the task in the **Job Definition**field (default text: number of selected items). This text will be displayed in the **Job Status** window.

7. Click [Start].

   A background job is created for the export task and the dialog is closed.

8. If you want check the status of the export process, open **Settings – Data Management – Job Status**. See also section “Job Status”, page 300.

Buttons and Elements

Element	Description
Database	Displays the name of the connected database. If you want to export data from a different database, you must connect to that database first. See section “Change Database”, page 292.
Method	Allows to select an export method. See section “Methods”, page 264 for details.
Selected Data	Click the [...] button to open the **Database Browser** and select the objects to be exported. If the selection has been made, this field displays the required disk space for all selected objects.
Export Path	Displays the destination path. Click the [...] button to create or select a folder. **Notice** It is recommended to keep the export path short and to avoid many subfolders (total length: 140 characters maximum). The Windows® operating system is limited to 256 characters for the total length of a file path. The remaining characters are required by Harmony for creating subfolders and file names within the destination folder.
Job Definition	Here the user can optionally give a description of the task. Default value is number of data items selected.
[Start]	Creates a background job for the export task and closes the dialog. The progress can be monitored in the **Job Status** window, see section “Job Status”, page 300.
[Close]	Closes the dialog without starting a export job.

Methods

Here all available export methods and their applications are described.

For more technical details on the exported data objects see next section "Export Summary and Description of Exported Data Formats", page 266.

Method: Analysis Sequence

Purpose: Import analysis sequence to Columbus

This method exports the selected analysis sequence as an AAS file (Assay Analysis Sequence). This will allow you to use the analysis sequence in Columbus. Only one analysis sequence can be exported at a time.

Method: Measurements

Purpose: Chose this format if you wish to **transfer measurement data to a remote location without network connection**. One application example is transfer to a **remote Volocity installation**, another one is data sharing with colleagues who wish to analyze the data in 3rd party software.

The export is self-contained, a copy of all image files in TIFF format as well as any assay layout associated with the measurements are included, so for large measurements data volume and export time are high.

Method: Measurements – incl. associated files

Purpose: Choose this format if you wish to take a complete measurement to a **remote Columbus system** (not connected via network) for import (using import type **Harmony IDX/TIF**).

The export is self-contained, a copy of all image files in TIFF format is included, as well as all files associated with the selected measurements like assay layouts, flatfield correction parameters, analysis sequences and evaluations. So for large measurements data volume and export time are high.

Notice

Please use this method only if you do **not** have direct access to your Columbus server in the network and need to store the data intermediately. Otherwise please use the faster and easier **Columbus Transfer** (see section "Columbus Transfer", page 271).

Method: Measurements – referenced images

Purpose: Choose this format if you wish to **transfer data to a Volocity installation that is connected to Harmony via network** (but is not on the same PC as Harmony, in that case just use drag & drop).

Content is the same as in method **Measurement**, but only a reference to the images is contained, which can then be imported by Volocity directly from the Harmony database. This has been described in detail here: http://cellularimaging.perkinelmer.com/pdfs/technotes/Data%20Transfer%20from%20Operetta%20to%20Volocity.pdf

The export is NOT self-contained, only a reference to all image files is included, so even for large measurements data volume and export time are low.

Method: Measurement – referenced images, incl. associated files

Purpose: This format contains everything except the TIFF images. It can e.g. be used as **source for the evaluation data** for **FCS Express** and **CellProfiler**.

Notice
Previously this export format was named *Columbus Export –Referenced images*, however, the respective functionality was replaced by the direct Columbus Transfer, which is far easier to use.

Method: Evaluation Results per Well

Purpose: Export of numerical results for usage with e.g. **Excel** and **Spotfire**.

This method exports the well results of the selected evaluation as a TXT file which can be read by all programs with ASCII file import.

In addition the included new “indexfile.txt” contains information that allows reconstruction of object location (e.g. for slides) in **Spotfire**.

Notice
The content of the assay layout is included in the TXT files (not as separate data object).

Method: Evaluation Results per Well and Object

Purpose: Export of numerical results for usage with e.g. **Excel** and **Spotfire**.

This method exports the selected evaluation as ...

- one TXT file for the well results (same format as method **Evaluation Results per Well**)
  and
- separate TXT files for each population with object results. These population files have an additional population name in the header, and the **Data** section lists the readout values for each object (one data row per object). The data columns are separated by tabs.

In addition the included new “indexfile.txt” contains information that allows to reconstruct the object location (e.g. for slides) in **Spotfire**.

Notice
The content of the assay layout is included in the TXT files (not as separate data object).

Export Summary and Description of Exported Data Formats

In the following table the data objects of each export method are listed and below you can find further explanations to the data formats.

Method	Index file (.idx.xml)	Index file (.ref.xml)	Images	Assay layouts	Flatfield correction profile (new)	Analysis sequences	Evaluations	(Pre-) selected object type for export
Analysis Sequence	–	–	–	–	–	AAS	–	ANALYSISSEQUENCE
Measurement	XML	–	TIFF	XML	–	–	–	MEASUREMENT
Measurement – incl. associated files	XML	–	TIFF	XML	XML	AAS	XML	MEASUREMENT
Measurement – referenced images	–	XML	–	XML	–	–	–	MEASUREMENT
Measurement – referenced images, incl. associated files	–	XML	–	XML	–	AAS	XML	MEASUREMENT
Evaluation Results per Well (Tab-separated Text)	TXT	–	–	within TXT files	–	–	TXT	EVALUATION
Evaluation Results per Well and Object (Tab-separated Text)	TXT	–	–	within TXT files	–	–	TXT	EVALUATION

Green background = included in method, exported file type is stated

TIFF Files

The images are 16 bit TIFF files, for viewing outside of Harmony you need a third-party image viewer which is capable of displaying 16 bit TIFF images (e.g. IrfanView).

AAS File

Notice

AAS files exported by Harmony 4.1 are fully supported by Columbus 2.6 (or higher). For AAS files created with Harmony 4.5 or 4.6 please refer to the Columbus product information to identify a compatible Columbus version.

If in doubt if an exported analysis sequence works on a specific Columbus version, you should test the sequence with one well and check the results for plausibility. There may be error messages if the analysis sequence makes use of new features which are not supported by the old Columbus version yet (e.g. new building blocks or modified methods or parameters). Try to replace missing features by using the building blocks and functions that your Columbus version offers. To get rid of warning messages saying that the analysis sequence was created on a newer Harmony version than the one you are currently using, load and save the analysis sequence on your target system. It is recommended to update your Columbus system to the latest version.

XML Files

Double-click XML files to open them in the Internet Explorer.

Notice

The content of any XML-file may change without further notice. Please check after a software upgrade if import filters of 3rd party software have to be adapted.

Index files (XML)

The index files include global measurement information (plate type and plate name) and information which is specific for each image.

- **Indexfile.idx.xml**
  In case of export methods which include the TIFF images (“Measurements” and “Measurements incl. associated files”) the indexfile.idx.xml gives the image location in the URL relative to the indexfile location.
  The description of an image can be found in section Images, where the file name of the image is stated followed by a list of properties (see figure below).

The image file name is composed as follows:

r02c07f01p01rc1-ch1sk1fk1fl1.tiff

r = row, c = column, f = field, p = plane, rc = record, ch = channel, sk = time point (the remaining parts of the file name are currently not in use)

Notice

Do not rely on image properties derived from image file names. The naming convention is subject to change without notice. Please see the meta information in the XML files for reliable data.

- **Indexfile.ref.xml**

  This type of index file is created if the TIFF images are NOT included in the export (“Measurement – references images” and “Measurements – referenced images, incl. associated files”). In this case only the storage location of each image is included in the XML file.

  The description of an image can be found in section Images, where the full storage path and the file name of the image is stated (URL), followed by a list of properties (see figure below).

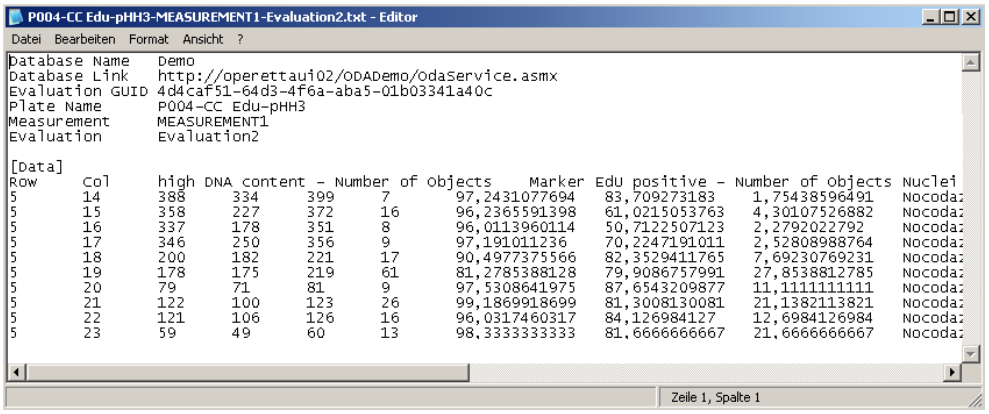
The image file name is composed as follows:

r02c07-1822390628.tiff

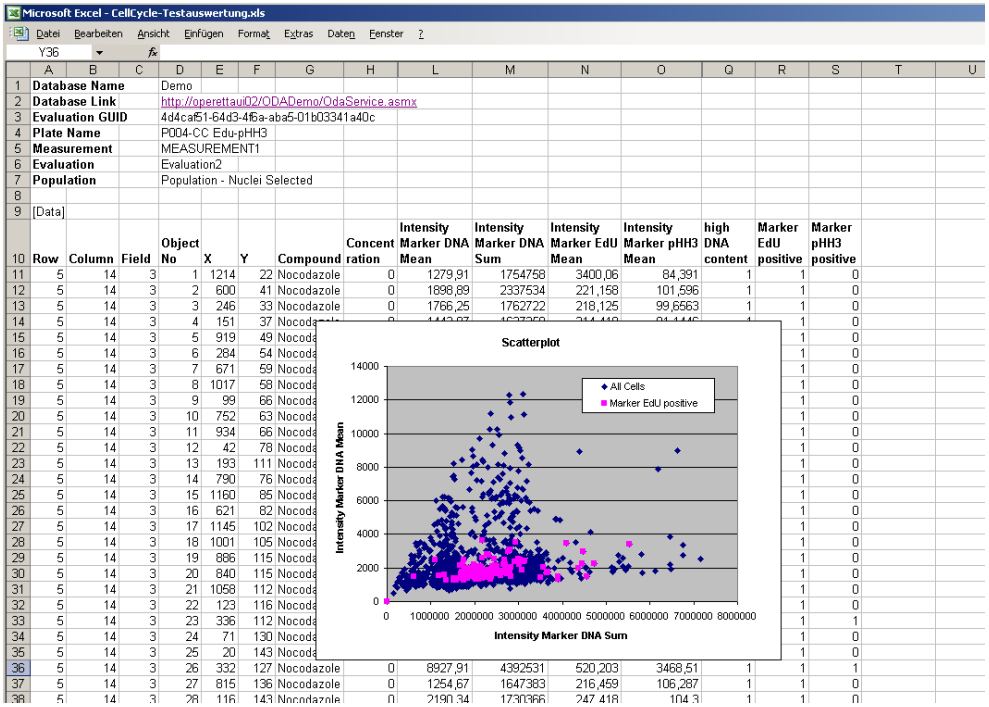
r = row, c = column, random number

TXT Files

- The file **header** includes meta information describing the origin of the **evaluation**.
- The **Data** section lists the readout values (one data row per well) as defined in the **Define Results** building block. It also includes the assay layout information entered during assay setup, e.g. compound concentrations and location of control areas. The data columns are separated by tabs.



Evaluation results per well exported as TXT file, opened in a text editor

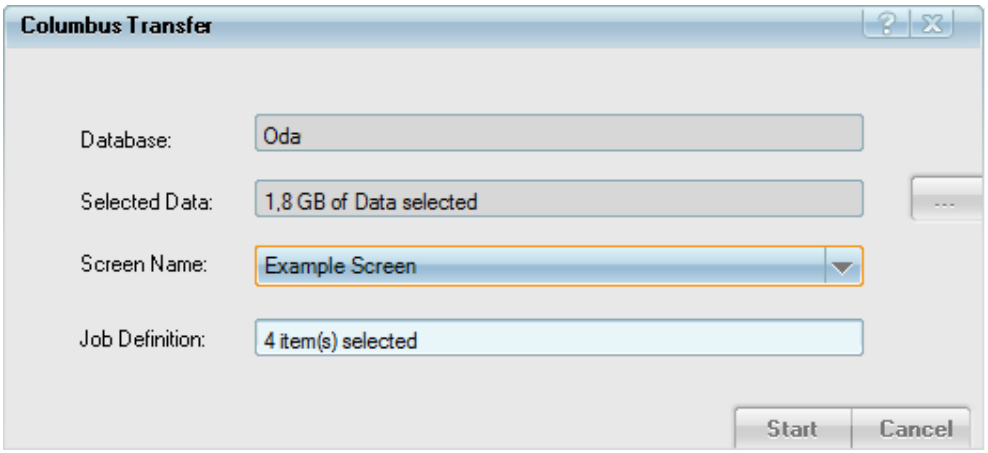


"Evaluation Results per Well and Object" exported as TXT files, one population file imported into Microsoft® Excel, column widths adjusted, scatter plot created to visualize the results

Notice

- Single cell results can only be exported if they have been saved in the database. You can activate this option in the **Define Results** building block (see section 5.2.27.4 "Object Results", page 455).
- Due to long parameter names, the column titles may not be displayed properly aligned above the respective column if you open the file in a text editor. You can avoid this problem by increasing the tab size of the editor (if possible) or by importing the file into Microsoft® Excel.
- If you want to open TXT files in Microsoft® Excel, make sure to select format **"Unicode (UTF-8)"** in Excel's **Text Import Wizard**. Otherwise certain characters may not be displayed correctly.

Columbus Transfer



This dialog can be used to transfer **measurements** directly to a Columbus system (version 2.4 or later) within the network. A copy of all image files in TIFF format, as well as all files associated with the selected measurements like assay layouts, flatfield correction parameters, analysis sequences and evaluations will be transferred. The transfer will be processed as a background job, so that you can continue using Harmony. The status of the transfer can be checked in the **Job Status** dialog. See also section "Job Status", page 300.

To use this function, the URL of the Columbus server and a Columbus user account must have been configured before in the **User Accounts** dialog. See also sections "Manage Columbus Account", page 305 and 5.3.20 "Columbus Network Integration", page 480.

Notice

- Please use the **Columbus Transfer** if you have Columbus available in your network. It will work faster and easier than the **Export Data** function, because the data is transferred directly.
- If you do **not** have access to Columbus please use the **Export Data** function with the method **Measurement – incl. associated files** to store the data intermediately before importing them manually into Columbus. For details see also section "Export Data", page 262.

When setting up an experiment you can also define an **Online Job** for the **Columbus Transfer**. In this case, the data will be transferred as soon as the measurement has been completed. See also section "Columbus Transfer", page 79 (Online Jobs).

How to transfer data to Columbus

1. Click the **Columbus Transfer** icon in **Settings – Data Management**.
   The **Columbus Transfer** dialog is opened.
2. Click the ... button next to the **Selected Data** field.
   The **Database Browser** is opened.
3. Select the desired measurement(s) and click **OK**.

Associated images, assay layouts and evaluations will be included automatically.

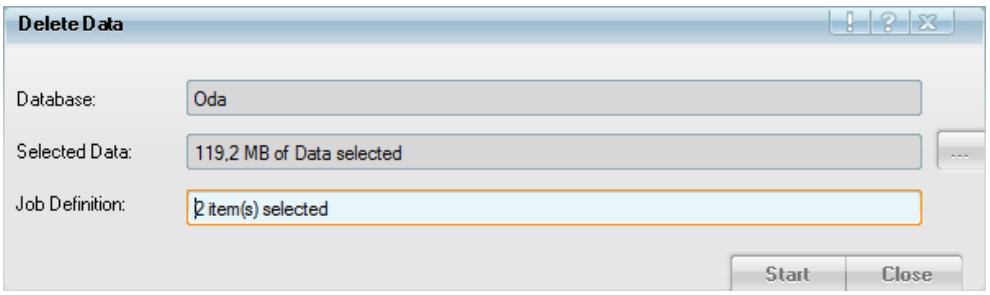
In the **Selected Data** field, the required disk space for all selected objects is displayed.

4. Select a **Screen Name**. The measurement(s) will be added to this screen in Columbus.
5. Optional: Enter a description of the task in the **Job Definition**field (default text: number of selected items). This text will be displayed in the **Job Status** window.
6. Click Start.
   A background job is started and the dialog is closed.
7. If you want check the status of the transfer, open **Settings – Data Management – Job Status**. See also section "Job Status", page 300.

Buttons and Elements

Element	Description
Database	Displays the name of the connected database. If you want to export data from a different database, you must connect to that database first. See section "Change Database", page 292.
Selected Data	Click the ... button to open the **Database Browser** and select the measurements to be transferred. If the selection has been made, this field displays the required disk space for all selected objects.
Screen Name	Select one Columbus screen from the drop-down list. The measurement(s) will be added to this screen in Columbus. Each Columbus user account has its own list of screens in Columbus. The list of available **Screen Names** in Harmony depends on the currently configured Columbus account.
Job Definition	Here the user can optionally give a description of the task. Default value is number of data items selected.
Start	Closes the dialog and starts a background job for the transfer to Columbus. The status of the transfer can be checked in **Settings – Data Management – Job Status** dialog. See also section "Job Status", page 300.
Cancel	Closes the dialog without starting the transfer.

Delete Data



The **Delete Data** function can be used to delete the following objects in the database:

- Selected measurements including images and evaluations (if existing)
- Any other objects

Notice

- Users can only delete **their own** objects. To delete images, the user has to be the owner of the corresponding measurement.
- Administrators can delete **all** objects in the database. Take care not to delete data of other users accidentally.
- If you delete a measurement, the evaluations based on this measurement (if existing) are also deleted.

Notice

After deleting objects via **Delete Data** you should restart Harmony. Otherwise deleted objects might still be displayed in the corresponding combo boxes of the user interface (e.g. plate types). See also section 5.3.16 “Display Errors”, page 476.

How to delete objects

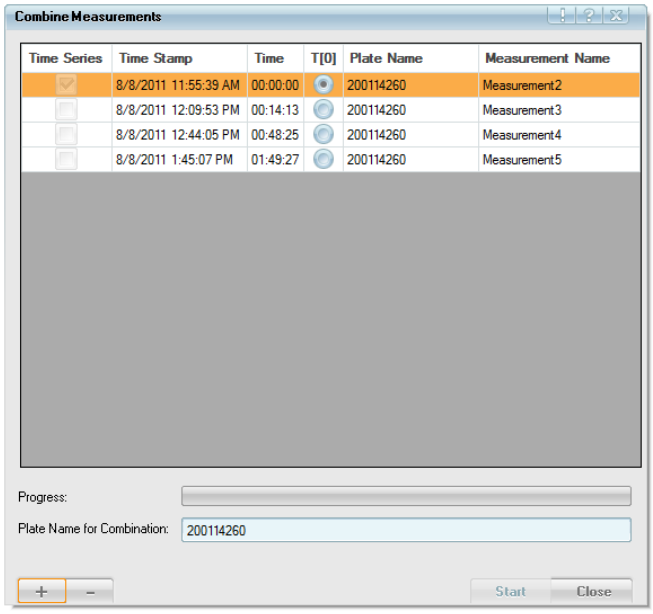
1. Click the **Delete Data** icon in **Settings – Data Management**.
   The **Delete Data** dialog is opened.
2. Click the ... button next to the **Selected Data** field.
   The **Database Browser** is opened.
3. Select the desired objects. Images to be deleted cannot be selected directly. Instead, select the corresponding measurements and click **OK**.
   If you do not have administrative rights, you may only select your own objects. Otherwise you will get an error message as soon as you click Start. For detailed selection instructions see section 5.1.16 “Database Browser”, page 220.
   In the **Selected Data** field, the selected amount of data is displayed.
4. Optional: Enter a description of the task in the **Job Definition**field (default text: number of selected items). This text will be displayed in the **Job Status** window.
5. Click Start.
   A background job is created for the delete task and the dialog is closed.
6. If you want check the status of the delete process, open **Settings – Data Management – Job Status**. See also section “Job Status”, page 300.
7. Once the job has been completed, restart Harmony to refresh the view of all list elements.

Buttons and Elements

Element	Description
Database	Displays the name of the connected database. If you want to delete objects from a different database, you must connect to that database first. See section “Change Database”, page 292.

Element	Description
Selected Data	Click the ... button to open the **Database Browser** and select the objects to be deleted. If the selection has been made, this field displays the number of selected objects.
Job Definition	Here the user can optionally give a description of the task. Default value is number of data items selected.
Start	Creates a background job for the delete task and closes the dialog. The progress can be monitored in the **Job Status** window, see section "Job Status", page 300.
Close	Closes the dialog without starting a delete job.

Combine Measurements



This dialog can be used to combine multiple measurements so that one regular time series measurement is created which can be evaluated as a whole. The following scenarios are possible:

- **Combine single measurements** (one time point each)
  These measurements can e.g. result from manually started measurements of the same plate or from automated measurements triggered e.g. by the plate::works scheduler (automation upgrade).
- **Add single measurements** (one time point each) **to an existing time series** (multiple time points)
  This can be useful if the kinetics you want to analyze takes longer than expected and you want to add subsequently measured endpoint(s) to the time series. Measurements can only be inserted before or after the time series, not between two time points of a time series.

For details on time series measurements see also section 5.1.7.4 "Time Series", page 68.

Notice

- It is not possible to combine two time series (measurements with multiple time points). A time series may only be combined with single measurements.
- We recommend to combine all measurements in one step and not to add measurements one by one using this function multiple times. This could lead to technical issues.
- The measurements must have been performed using the same version of the Harmony software.

The experiment settings of the measurements to be combined have to be identical. Furthermore, the measurements must have been performed on the same instrument. Only the following parameters may be different:

- Exposure time
- Focus height

If you combine single measurements, the time point **T0** (beginning of the kinetics) can be chosen freely. If you add measurements to a time series, the time point **T0** of the time series is kept and cannot be modified. The time points will be sorted chronologically, and the time in relation to **T0** is displayed.

The combined measurement is saved as one new time series measurement in the database.

- The **keyword values** of the new measurement are determined by the first measurement in the time series.
  The only exception is the keyword **Available Correction** which indicates the best available flatfield correction method of a measurement. The best correction found in any of the measurements will be kept for the combined measurement.
- The user who combined the measurement will be the **owner** of the new measurement.

How to combine measurements to one time series

1. Open **Settings – Data Management – Combine Measurements**.
2. Click  .

   The **Database Browser** is opened.
3. Select the desired measurements (not more than one time series, an arbitrary number of single time point measurements).

   Multiple measurements can be selected if you hold the **Ctrl** key.
4. Click  .

   The selected measurements are added to the list sorted chronologically. If you have added a time series, this will be indicated in the column **Time Series**.
5. Define **T0**.
   - If you have only selected single measurements, you can freely define time point **T0** (beginning of the kinetics). Check the **T[0]** box of the corresponding measurement. The times of the other measurements in relation to **T0** will be

adapted.

- If the list includes a time series measurement, **T0** of the time series is kept and cannot be modified.

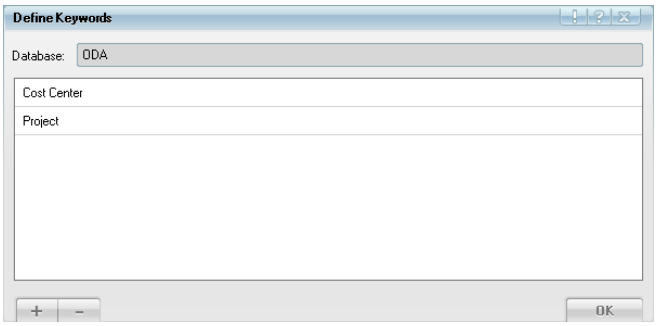
6. Optionally: Enter a new **Plate Name** if desired.
7. Click Start.

   The measurements are combined and saved in the database as a new measurement (measurement number is incremented if the default plate name is kept).

Buttons and Elements

Element	Description
Time Series	Indicates whether a measurement is a time series (multiple time points) or a single time point measurement; non-editable check box.
Time Stamp	Displays the time stamp (real measurement time) of the measurement (for time series the first time point of the measurement is displayed).
Time	Displays the measurement time in relation to time point **T0**.
T[0]	Allows to define time point **T0** (beginning of the kinetics). If there is a time series in the list, time point **T0** of the time series is kept and cannot be modified.
Plate Name	Displays the plate name of the measurement.
Measurement Name	Displays the name of the measurement.
Progress	The bar indicates the progress of the combination process. It does not tell you whether the process has been successful or not. Errors will be reported in the **Messages** window as required.
Plate Name for Combination	Displays the plate name of the measurement. Default: plate name of the first measurement in the list. You can also enter a new plate named for the combined measurement.
	Combines the measurements and saves the new measurement to the database (measurement number is incremented if the default plate name is kept).
	Stops the combining process. You will be asked if you really want to cancel. • Click **Yes** to cancel (resulting in an incomplete combination). • Click **No** to continue the process.

Define Keywords

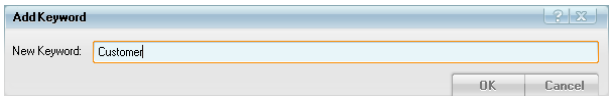


Administrative rights are required to open this dialog. If you are not an administrator, a login dialog is displayed so that you can switch to a user account which has administrative rights.

Keywords are used to describe objects in the database. You can search for keywords when selecting objects in the **Database Browser**. Most keywords are added automatically by the system when the objects are created. This dialog allows you to create **user-defined keywords** to enter additional measurement descriptions.

- Values for these keywords can be entered before starting a measurement in **Run Experiment – Plate Settings**. See section 5.1.8.2 “Plate Settings”, page 117.
- You can also edit keywords subsequently if you select a measurement in **Settings – Database Browser** and select **Edit Keywords** from the context menu. See also section 5.1.16.4 “Edit Keywords”, page 234.

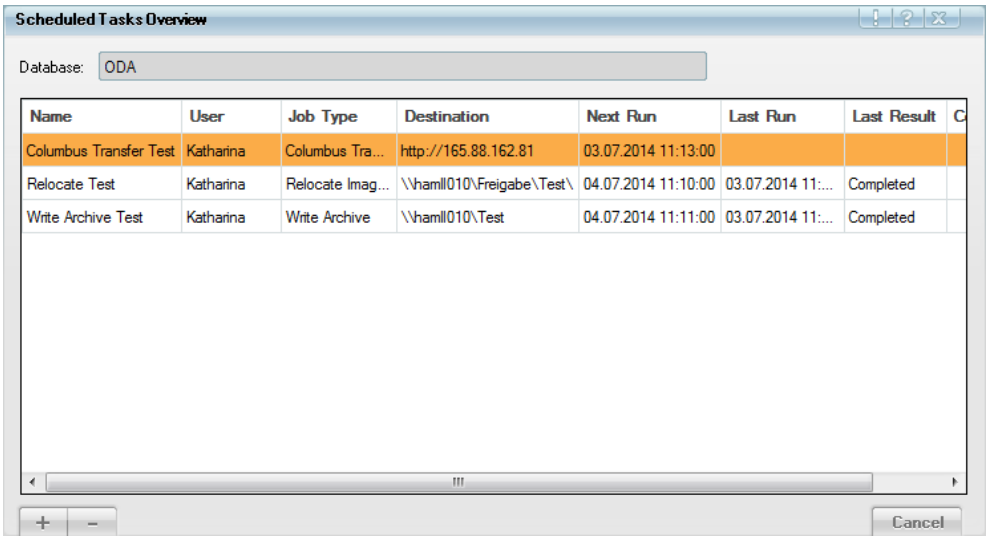
Buttons and Elements

Element	Description
+	Opens the **Add Keyword** dialog where you can enter the name for the new keyword. Confirm with **OK** and restart Harmony. 
-	Allows you to select one keyword which is removed from the list (after confirmation). • If no user has entered values for this keyword yet, it is deleted completely. • If a user ever entered a value for this keyword, the keyword is only disabled, i.e. it is not shown in the keyword list and you cannot enter values for the keyword anymore. The existing values for this keyword can still be viewed if you select the corresponding measurement in the **Database Browser** and browse the **Details**. They are also displayed in **Edit Keywords**, but they cannot be edited anymore. • To reactivate a disabled keyword, add the same keyword again as new keyword.

Element	Description
	Closes the dialog and applies the changes.

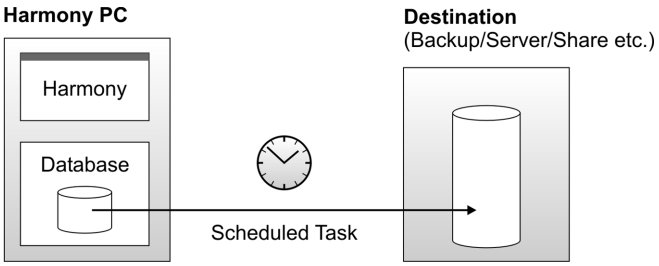
Schedule Tasks

Overview



The **Schedule Tasks** dialog allows you to define automatic and periodic tasks for:

- **Relocating images** of selected measurements, e.g. to free up disk space on the Harmony PC.
- **Writing an archive** of selected data, e.g. to copy the data to a backup server.
- **Transferring** selected measurements to **Columbus**.



Example

Using a couple of selection criteria you could e.g. create a scheduled task running each Monday which relocates all images to a server which are older than 7 days and which were measured by a certain user.

The **Schedule Tasks** dialog can only be used on the Harmony PC, not with an office installation of Harmony. When you open the dialog, you see the list of existing tasks. To define or edit scheduled tasks you need to be logged in as Harmony administrator. For detailed information on the user accounts involved in creating and running scheduled tasks, please see section 5.3.14 “User Accounts”, page 473.

Scheduled tasks are processed as background jobs on the Harmony PC. A task can only be executed if the Harmony PC is switched on and the Harmony software is running at the scheduled time (standby or hibernate mode is not sufficient). The **Job Status** dialog can be used to view status information and log files of the corresponding jobs (see section "Job Status", page 300).

Notice

For an explanation of the data management functions which are used in the scheduled tasks, see the following sections:

- "Relocate Images", page 259
- "Write Archive", page 253
- "Columbus Transfer", page 271

Notice

All scheduled tasks are linked to the name of the Harmony PC. If you rename this computer, all scheduled tasks will disappear from the list and will not be executed anymore. For details see section 5.3.9 "Scheduled Tasks and Jobs Disappeared", page 467.

How to delete a scheduled task

Notice

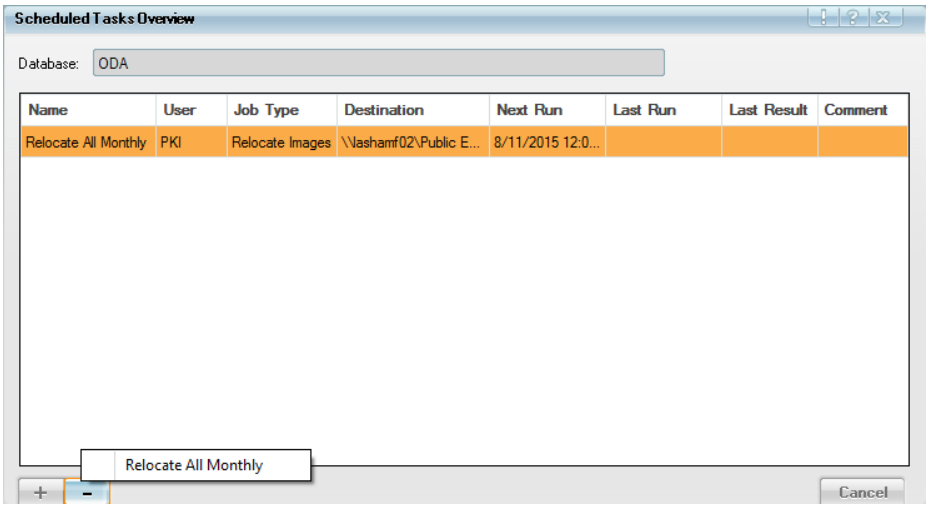
Instead of deleting a scheduled task you can also deactivate it. This allows you to reactivate the task at a later time, and the history of completed jobs for this task (incl. log files) will still be available in the **Job Status** dialog. See also section "How to enable/disable a scheduled task", page 283.

Precondition:

Logged in as Harmony administrator

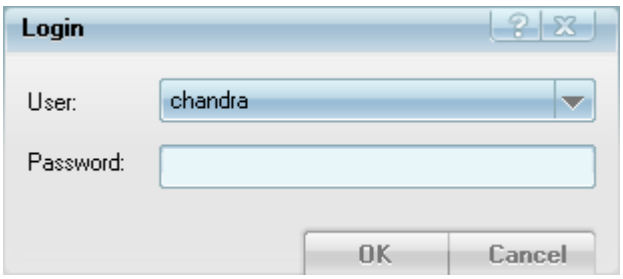
1. Click – .

2. Select the desired task from the pop-up menu.



A login dialog appears.

3. Enter your Harmony user password and click **OK**.



Notice

You cannot select a different user account at this point. You have to be logged in as administrator already and just confirm your password.

If you need to change the user account first, close all dialogs and switch to a user account with administrative rights. See also section 5.1.3 “Login”, page 33.

The task is removed from the list.

How to edit a scheduled task

- Right-click the desired scheduled task and select **Edit** from the context menu to edit the task in the **Scheduled Task Definition Wizard**.

How to check whether a scheduled task was processed

- In the table of scheduled tasks in Harmony, check the columns **Last Run** and **Last Result**. If the task passed successfully, **Last Run** should display the scheduled date and **Last Result** should state **“Passed”**.
- Scheduled tasks are processed as background jobs on the Harmony PC. Therefore you can also use the **Job Status** dialog to view status information and log files (see section “Job Status”, page 300).

- Optional steps:
  - For a **Relocate Images** task, open the target directory and check if the images have been moved to this folder.
  - For a **Write Archive** task, use the **Read Archive** function (see section "Read Archive", page 256) to open the archive and check whether it includes the selected data.
  - For a **Columbus Transfer** task, log into Columbus and check whether the selected screen includes the transferred data.

How to enable/disable a scheduled task

- Right-click the desired scheduled task and select **Disable** from the context menu.

  The task will not be executed anymore. All "waiting" jobs for this task will be removed from the **Job Status** dialog (see section "Job Status", page 300), but the history of completed jobs (incl. log files) will be kept.
- To re-activate the task, select **Enable** from the context menu.

  The next scheduled run of this task is displayed under **Next Run**.

Buttons and Elements

Element	Description
	Opens the **Scheduled Task Definition Wizard** to create a new scheduled task. You need to be logged in as administrator before opening the wizard.
	Allows you to delete a task. You need to be logged in as administrator before you can use this function.
	Closes the dialog without saving changes.

Context Menu

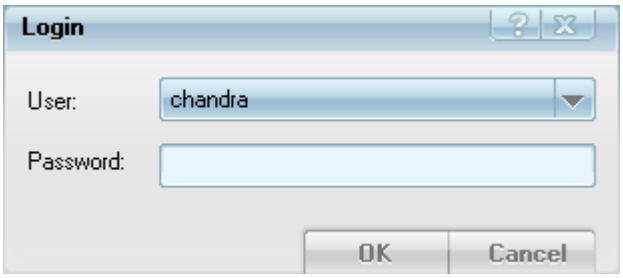
Menu Item	Description
Copy	Copies the selected task to the clipboard.
Edit	Allows you to edit the selected task. You need to be logged in as administrator before you can use this function.
Enable/Disable	Activates/deactivates a task. The status can be seen in column **Next Run** ("Disabled" or next run time). Disabled tasks will not appear in the **Job Status** dialog anymore (see section "Job Status", page 300).
Help	Opens this topic in the Harmony Help.

Scheduled Task Definition Wizard

How to create a new scheduled task

Precondition: You have to be logged in as Harmony administrator.

1. Click + .
   A login dialog appears.

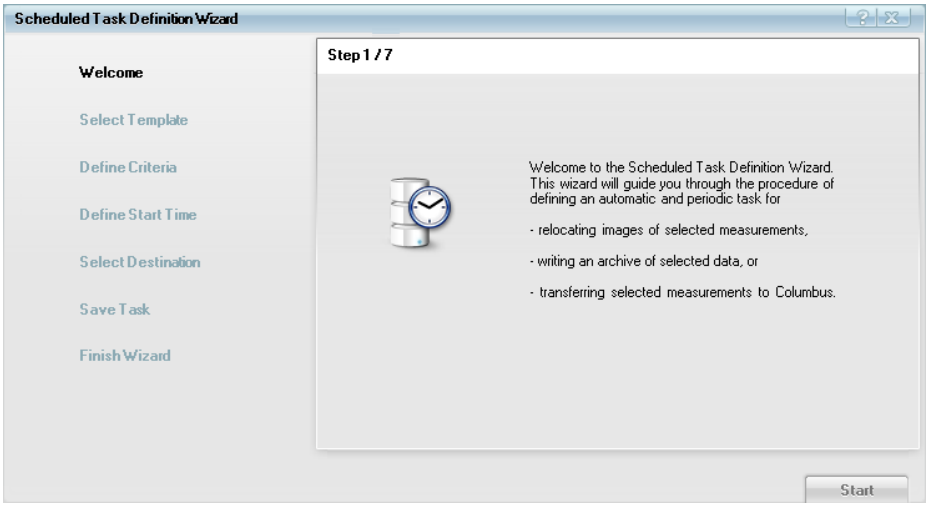


2. Enter your Harmony user password and click OK .

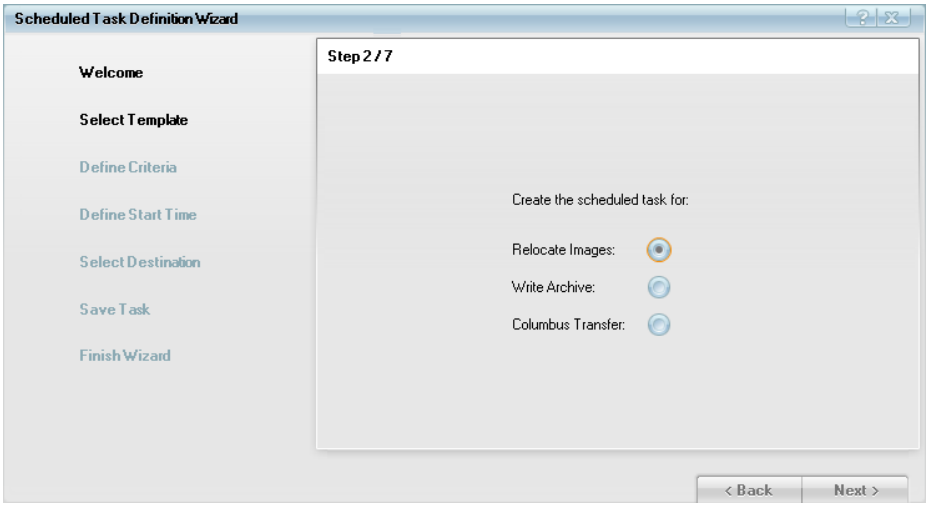
> **Notice**
> You cannot select a different user account a this point. You have to be logged in as administrator already and just confirm your password.
> If you need to change the user account first, close all dialogs and switch to a user account with administrative rights. See also section 5.1.3 “Login”, page 33.

   The **Scheduled Task Definition Wizard** is opened.

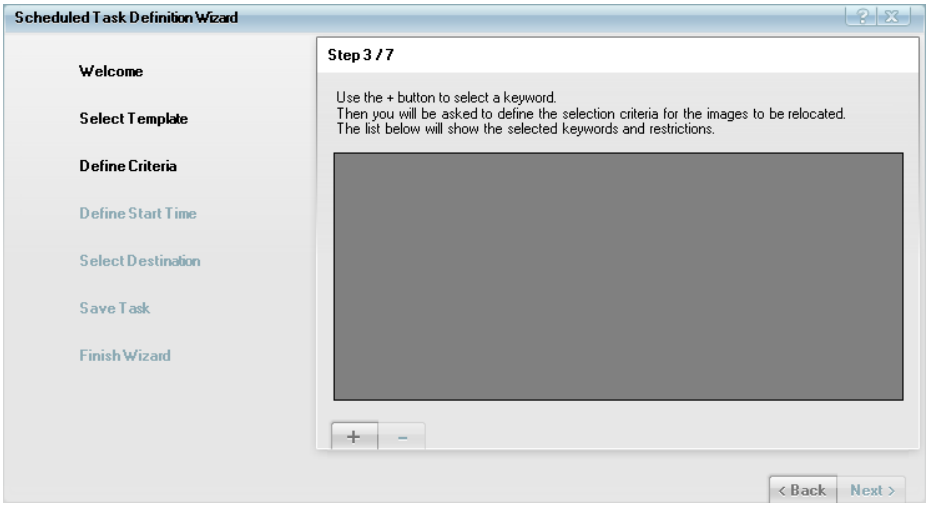
3. Click Start .



4. Select the desired purpose of the task and click Next > .

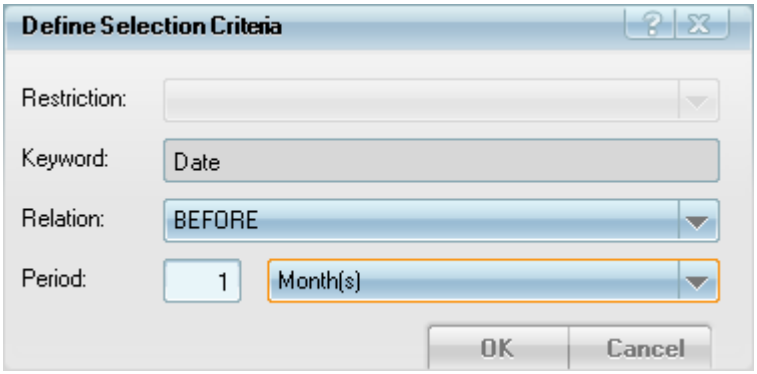


5. Click + to and select a keyword from the pop-up menu.
   - If the task is of type **Relocate Images** or **Columbus Transfer**, you can only filter the objects by date, owner or user-defined keyword (group “Measurement").
   - If the task is of type **Write Archive**, you can use all keywords in the database to filter the objects. For a clear overview, the keywords are grouped (as in the **Database Browser**).



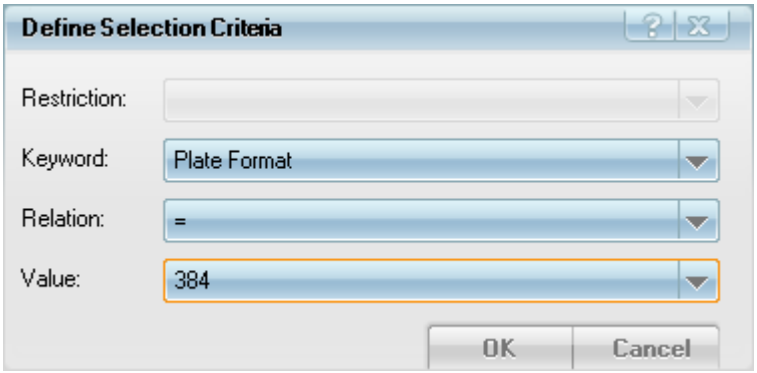
   The dialog **Define Selection Criteria** is opened.

6. If you have selected the keyword **Date**, the dialog allows you to filter the objects by their date of creation.



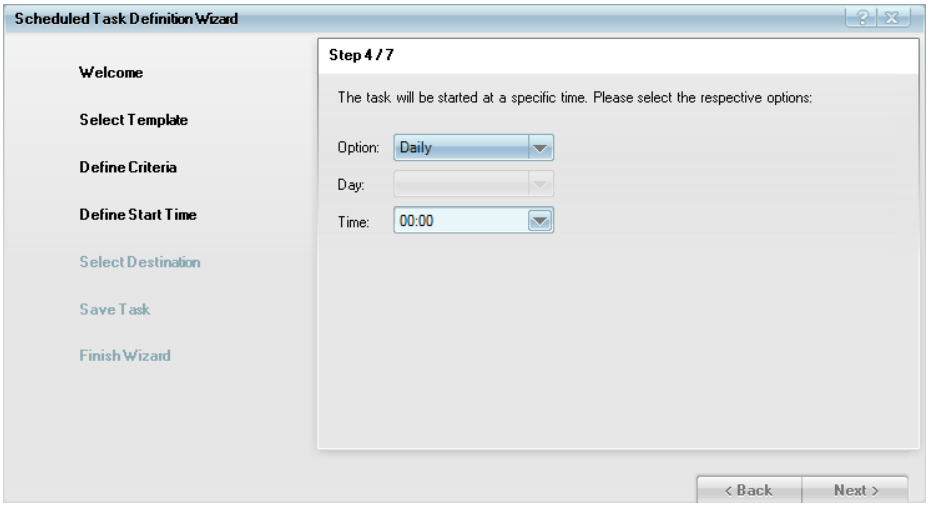
Example
The settings shown above would select all objects which were created 1 month ago or before.

If you have selected any other keyword, you can filter the objects by the desired keyword value.

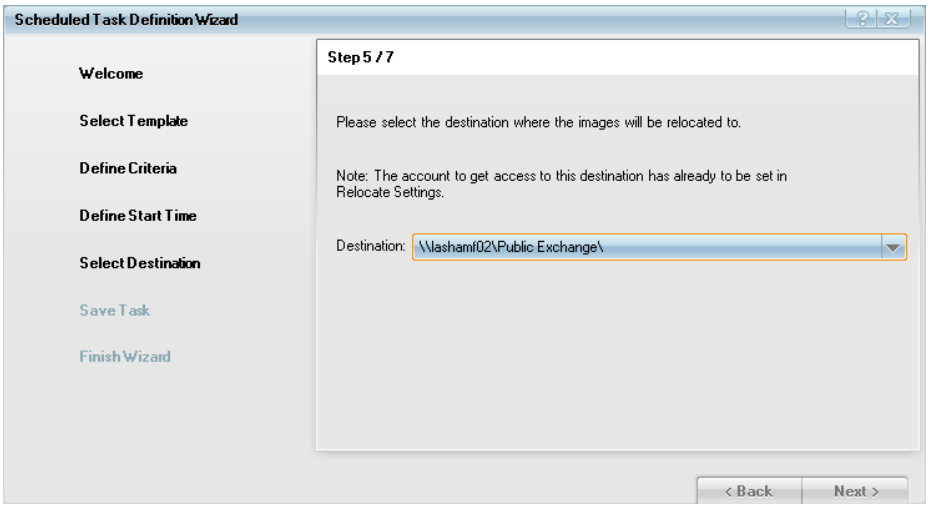


A detailed description of this dialog can be found in section "Buttons and Elements: Define Selection Criteria", page 290.

7. Click OK.
   The selection criterion is added to the filter list.
8. If necessary, add further selection criteria using the + button to narrow down the selection.
   There will be a logical AND relation between the criteria, i.e. an object has to fulfill each criterion to be selected.
9. Click Next >.
10. Select the start time of the task:



11. Select a destination path.
    If you have selected **Relocate Images** in step 2 of the wizard:

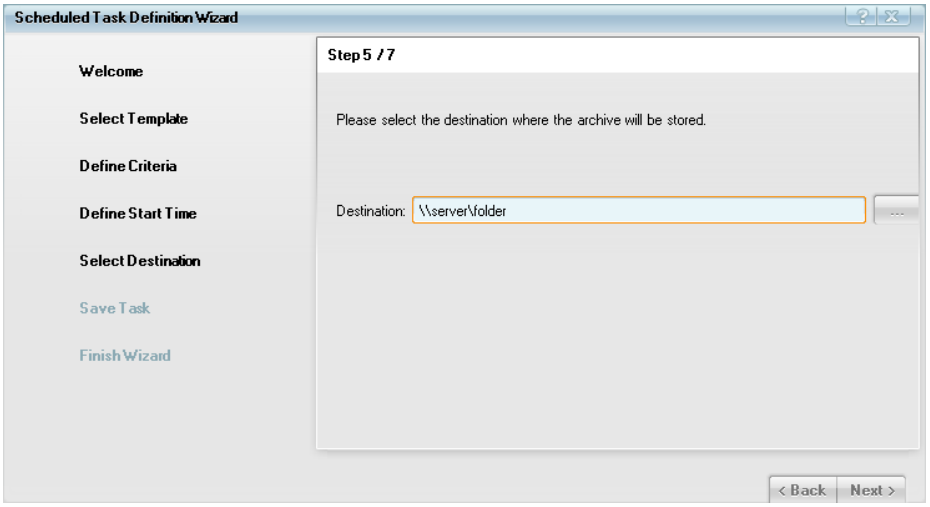


    - Select a predefined **Destination** for the images.

Notice

- Destinations for **Relocate Images** can be defined in the **Relocate Settings** dialog. For details see section “Relocate Settings”, page 294.
- Please notice that automatic relocation of images to the same computer (i.e. Harmony PC) is not possible.

If you have selected **Write Archive** in step 2 of the wizard:

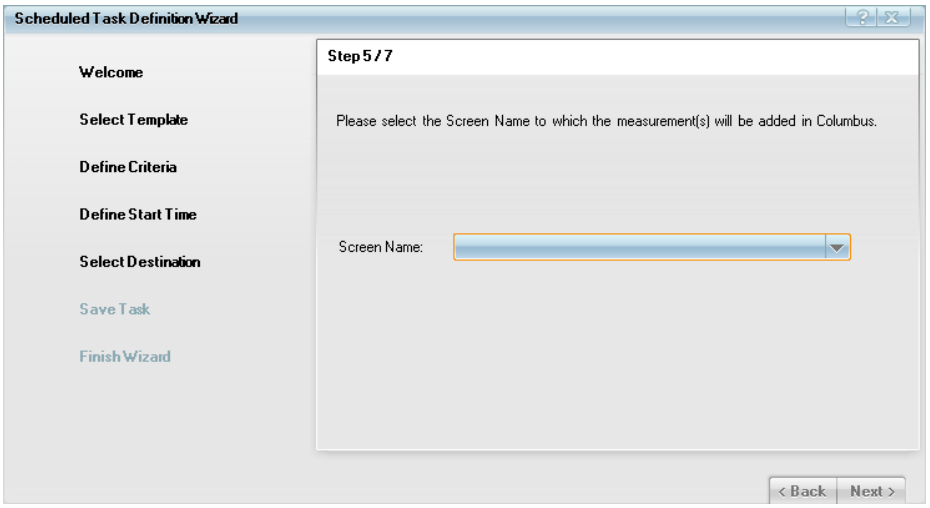


- Enter the desired **Destination** path (UNC path) or click ... to open a **Browse for folder** dialog.

Notice

- If a login is required to access the selected destination, please make sure that the default user account of the Harmony PC (default “Harmony") has read/write access is allowed to create subfolders. For details see section 5.3.14 “User Accounts”, page 473.
- It is recommended to keep the destination path short and not to avoid many subfolders (total length: 140 characters maximum). The Windows® operating system is limited to 256 characters for the total length of a file path. The remaining characters are required by Harmony for creating subfolders and file names within the destination folder.

If you have selected **Columbus Transfer** in step 2 of the wizard:

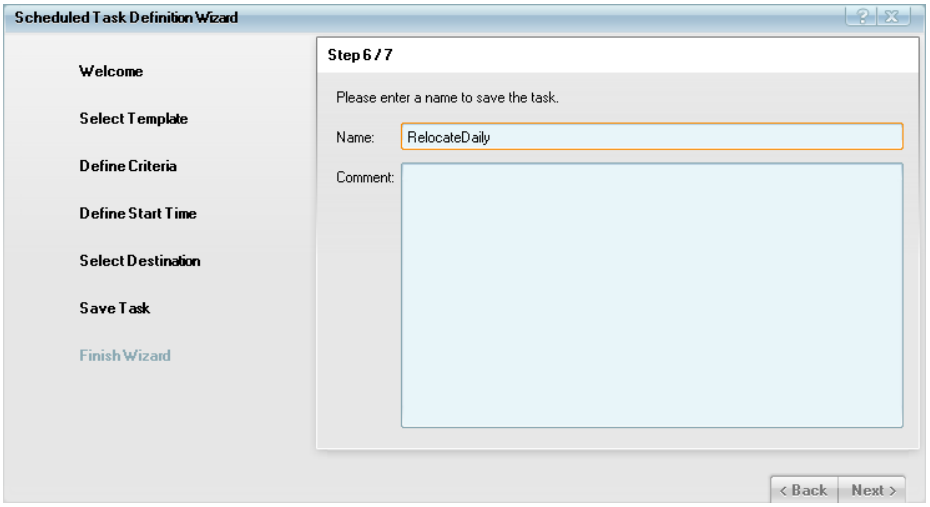


- Select a Columbus **Screen Name** to which the measurements will be added.

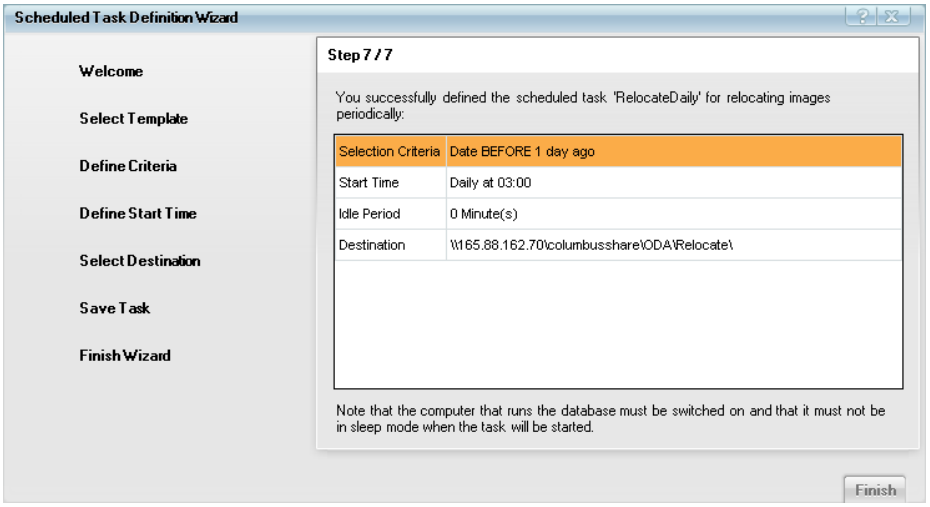
Notice

- To use this function, the URL of the Columbus server and a Columbus user account must have been configured before in the **User Accounts** dialog. See also sections "Manage Columbus Account", page 305
- Each Columbus user account has its own list of screens in Columbus. The list of available **Screen Names** in Harmony depends on the currently configured Columbus account.

12. Click Next >.
13. Enter a **Name** for the task and, if desired, a description.



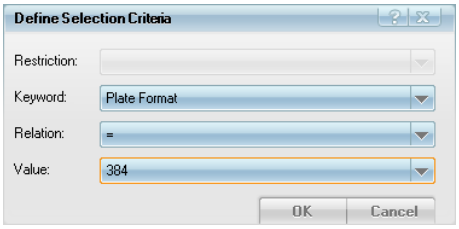
14. Click Next >.
A summary of the task properties is displayed.



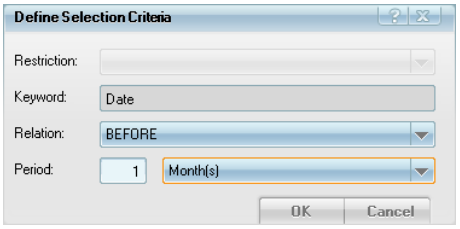
15. Click Finish.
The new scheduled task is added to the task list. It will also appear in the **Job Status** dialog with status **Waiting**. See also section "Job Status", page 300.

Buttons and Elements: Define Selection Criteria

This dialog belongs to the **Scheduled Task Definition Wizard**, step 3.



Parameters for normal keyword and value

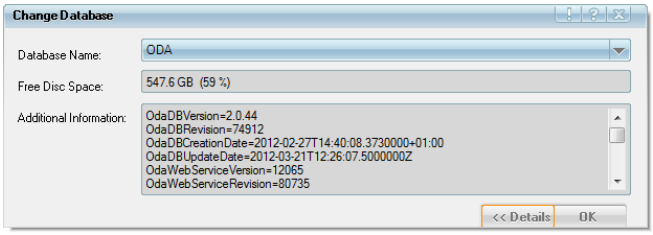


*Parameters for keyword **Date***

Element	Description
Restriction	Logical relation between the selection criteria. Currently the only option is **AND**, i.e. a database object has to fulfill all criteria to be selected.
Keyword	Allows to select a keyword (system-defined or user-defined) which is used to filter the database objects. The keyword **Date** allows you to filter the objects by their creation date. If you select it, the dialog offers slightly different parameters (see below).
Relation (normal keyword)	Relation between selected **Value** and value of a database object: • **=** : The object's value must be *identical* with the selected **Value** to fulfill the criterion. • **LIKE**: You can enter the **Value** directly and use wildcard characters: **?** replaces one single character. ***** replaces an arbitrary number of characters. The wildcards can be inserted at the beginning, in the middle or at the end of the value. **Example** The selected keyword is **Channel**, the relation is **LIKE**: • **Alexa*** would select all channels beginning with “Alexa”, e.g. “Alexa 488”, “Alexa_532_modified” etc. • **Alexa 5??** would select all channels beginning with “Alexa 5” followed by two arbitrary characters, e.g. “Alexa 532”, “Alexa 568”, but *not* “Alexa 532_modified”.

Element	Description
Relation (keyword is **Date**)	Defines the relation between the selected **Period** and the object's creation date: • **BEFORE**: The object's creation date must be **older** than the selected **Period**. • **AFTER**: The object's creation date must be **newer** than the selected **Period**. **Example** The selected keyword is **Date**, the relation is **BEFORE**, the period is **7 days**: • All objects which are at least 7 days old (or older) will be selected.
Value (normal keyword)	Allows to select the keyword value which is used for filtering the database objects. Depending on the selected **Relation**, you can select a value from the combo box **(Relation** is **=**) or enter a value including wildcard characters (**Relation** is **LIKE**). **Notice** If you use wildcards (e.g. '*' or '?') for selecting the desired objects, you have to make sure that the names of the objects do not contain such characters. In this case you should not use special characters if you save objects or enter keywords or plate names. Otherwise the selection of these objects will fail.
Period (keyword is **Date**)	Defines the point in time (always in the past, calculated from runtime of the scheduled task) which is used to filter the objects. The selected **Relation** defines whether the object's creation date has to be **BEFORE** or **AFTER** this point in time.
OK	Closes the dialog and adds the new selection criterion to the list.
Cancel	Closes the dialog without saving the changes.

Change Database



In the **Change Database** dialog you can select a database to work with. Properties like remaining disk space are displayed for the current database. Per default the database is installed locally on the Harmony PC. If you use an office installation of Harmony, you will want to connect to the Operetta's database (see instructions below).

Although there seems to be only “one database”, it consists in fact of two different systems:

- Images, experiments, analysis sequences and results are stored in a folder structure on the local hard disk (TIFF and XML files).
- Meta information (e.g. references to images, well co-ordinates etc.) is kept in an SQL database (also stored on the local hard disk).

You have to make sure that a sufficient amount of **Free Disk Space** is available before starting new experiments. If you are running out of disk space (especially due to a huge amount of image files) you have the following options:

- **Delete** not required measurements and the associated images. See section “Delete Data”, page 272.
- **Relocate** (i.e. move) selected images out of the database, e.g. to a network server. The images can still be accessed in Harmony. See section “Relocate Images”, page 259.
- **Archive** data (i.e. copy out of the database) and delete the original objects in the database. See section “Write Archive”, page 253.

How to connect to the instrument’s database (for office installation)

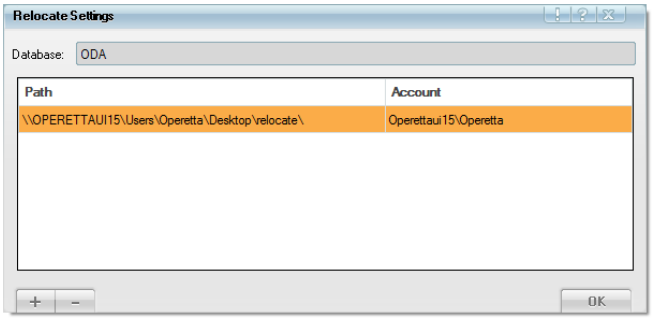
If you run Harmony as an office installation (without instrument), you can also work with the instrument's database:

1. First you have to add the name of the computer where the database is located (Harmony PC) to the list of available databases (see section “Database Settings”, page 298).
2. Afterwards you can select this database in the **Change Database** dialog.

Buttons and Elements

Element	Description
Database Name	Allows you to select the database to work with. *ODA* is the name of the standard database upon delivery (**O Da**ta Manager). After the selection you have to login again (with a valid account of the selected database).
Free Disk Space	Displays the remaining disk space which is available for new measurements, images and results. If there is not sufficient disk space left, you might not be able to run experiments or complete a running measurement or evaluation. Free up disk space using the data management functions (see above).
Additional Information	Version of the database and its services.
Details >>	Show/hide the additional information field.
OK	Closes the dialog and confirms the database selection.

Relocate Settings



Administrative rights are required to open this dialog. If you are not an administrator, a login dialog is displayed so that you can switch to a user account which has administrative rights.

In the **Relocate Settings** dialog you can define a number of image location paths (UNC format, no local paths). Only shared folders can be used as destination, and only these paths can be selected when relocating images in **Settings – Data Management – Relocate Images** (see section "Relocate Images", page 259).

Using the context menu you can **Edit** a relocate path, e.g. to update the account details.

For each image location path you have to specify a valid network account which is used to access the destination folder.

Notice

- The destination folder must be available permanently so that the relocated images can be accessed any time.
- Only shared folders can be used as destination (even if the destination folder is on a local drive, e.g. external USB drive connected to the Harmony PC). You have to share the destination folder and grant access for the account specified for that destination. The account must have read and write access and the right to create subfolders (see also section 5.3.22.2 "Cannot access data", page 483).
- If you get an "unknown error" (Windows system message), please also make sure that the access rights to the destination are correct and updated (see above).
- The account information is also used to access the image location path if it is used in scheduled tasks (**Relocate Images** tasks). For details see sections "Schedule Tasks", page 280 and 5.3.14 "User Accounts", page 473.
- Do not create multiple relocate destinations on the same computer, because this may lead to login errors.

How to specify a relocate path – Example: External USB drive

Example: Defining an external USB drive as destination; instructions for creating shared folders etc. based on Windows® 7.

Share USB drive

1. Connect the USB drive to a free USB port of the Harmony PC.

   The USB drive is detected and a drive letter is assigned (e.g. 'D').
2. Open the Windows® Explorer.
3. Right-click the D drive and select **Properties** from the context menu.

   The Properties window is opened.
4. Open the **Sharing** tab and click **Advanced Sharing ...**.
5. Enter the password for the current Windows® login (i.e. on the Harmony PC enter 'Harmony').
6. Activate the checkbox **Share this folder**.

   The **Settings** section is enabled.

   Click **Permissions**.

   A new window is opened.
7. Select **Everyone** in the list of group/user names and activate the checkbox **Allow Full Control**.
8. Click **OK** to close the dialog.
   - Everyone now has the right to write and read files on this drive.
   - If you want, you can also add the user 'Harmony' to the list and provide him full control as well.

   The **Properties** window (**Sharing** tab) should now display that D:\ is shared and uses the network path **\\ComputerName\d**.
9. Close the **Properties** window.

Add Relocate Path in Harmony

1. Open **Settings – Data Management – Relocate Settings**.
2. Click + to add a new relocate path.

   The window **Specify Relocate Path** is opened.
3. Enter the **UNC Path** of the shared USB drive:
   - **\\localhost\d** (if USB drive is directly connected to the Harmony PC)
   - **\\ComputerName\d** (if USB drive is connected to a different computer within the network)
4. Enter the network **Account** which is used to access the destination: **ComputerOrDomain\Harmony**
5. Enter the **Password** of the network account: Harmony

6. Click OK.

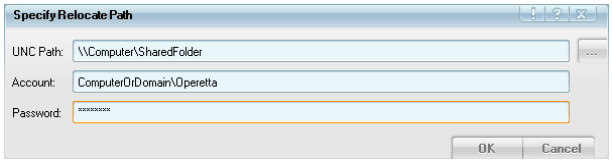
   The new relocate path is listed in the table.

7. Click OK to save the changes and close the **Relocate Settings** window.

Context Menu

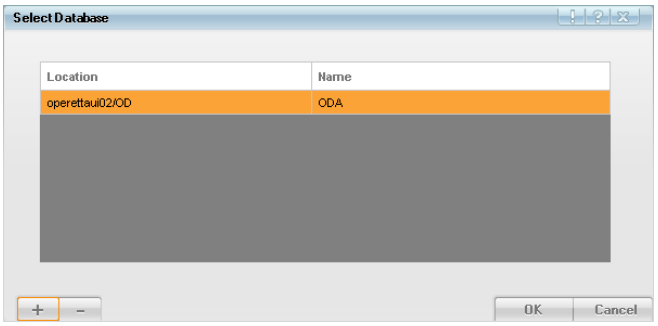
Element	Description
Copy	Copies the selected path to the clipboard.
Edit	Opens a dialog where you can edit the relocate path and the corresponding account details.
Help	Opens this topic in Harmony Help.

Buttons and Elements

Element	Description
+	Opens a dialog where you can enter a new relocate path.  See below for further details. **Notice** Do not create multiple relocate destinations on the same computer, because this may lead to login errors.
–	Allows you to select one destination which will be removed from the list. **Notice** Do not delete image location paths which are still being used, i.e. which still contain relocated images. Otherwise these images cannot be accessed anymore.

Element	Description
UNC Path	UNC path of the destination folder. Format: **\\ComputerName\share** Using the ... button you can also open the **Browse for Folder** dialog and browse for the shared folder. Please note that you have to navigate to that folder via the **Network** branch of the folder tree. If you select the folder in the **Computer** branch, it will be inserted as a local path and not as a UNC path. In case of a USB drive connected to the local computer (Harmony PC), please use this format: **\\localhost\share** **Browse for Folder** will not work in this case. See the example above for detailed instructions. **Notice** It is recommended to keep the relocate path short and to avoid many subfolders (total length: 140 characters maximum). The Windows® operating system is limited to 256 characters for the total length of a file path. The remaining characters are required by Harmony for creating subfolders and file names within the destination folder.
Account and Password	Network account and password which is used to access the destination folder. We recommend to use the default user account of the Harmony PC (Harmony/Harmony). To enable access to the destination folder, this account has to be created locally on the destination computer or in the corresponding domain (with full access to the folder). Depending on the account type (local/domain) the account has to be entered with a prefix: • **Local** account on the destination computer: **Computer\Account** *(Computer = computer name of the destination computer)* • **Domain** account: **Domain\Account** *(Domain = domain name of the destination computer)*
OK	Closes the dialog and applies the changes.
Cancel	Closes the dialog without changes.

Database Settings

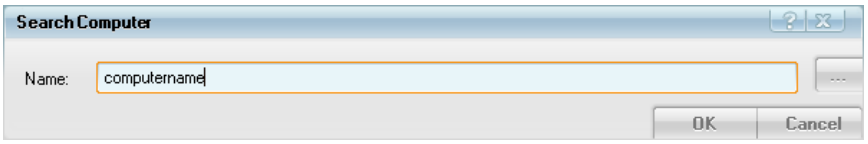


This dialog displays a list of available databases. You can add or remove further databases as desired. If working with an office installation of Harmony, you will typically use this dialog to add the database of your instrument, so that you can connect to it from your office PC.

To connect to one of these databases, you have to use the **Change Database** dialog (see section "Change Database", page 292).

How to add a database

1. Click + .
   The **Search Computer** dialog is opened.
2. Specify the location of the database. There are two ways:
   - Recommended: Enter the name of the computer where the database is installed directly. If you want to add the database of your Operetta CLS, you have to enter the name of the Harmony PC.



   - Alternative: Click ... to open a new dialog which allows you to browse the network for the required computer. This dialog only accepts folders, i.e. you have to select any folder on the corresponding PC. This can only work if the PC has at least one shared folder which can be selected. If you click OK the dialog is closed and the name of the computer (without folder path) is transferred to the **Search Computer** dialog.

2. Click **OK** to close the **Search Computer** dialog.
   The computer name is added to the list.
3. Enter a name for this database in the **Name** column. This name will be displayed in the **Change Database** dialog.
4. Click **OK** to close the dialog.

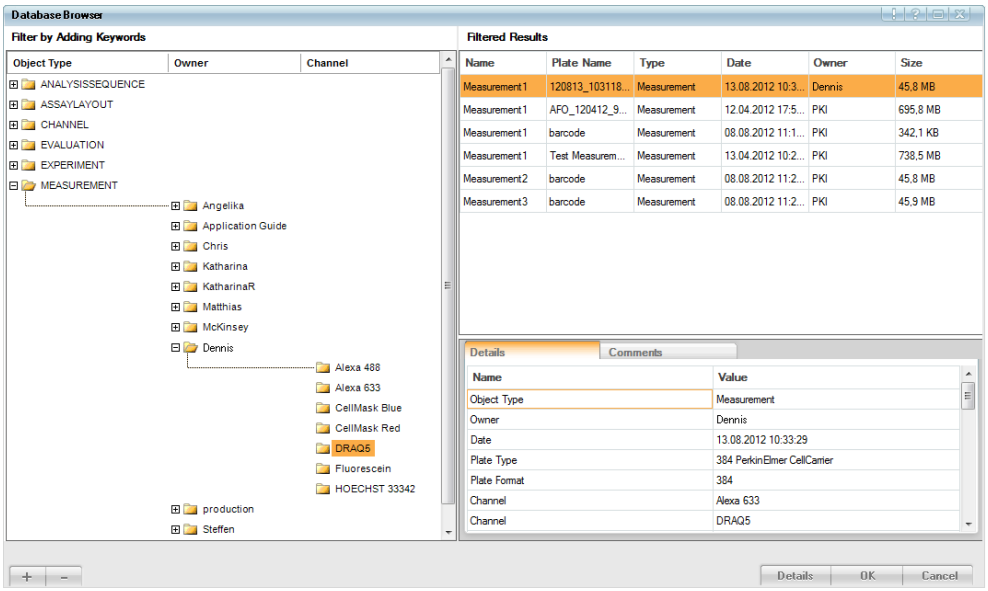
Notice

The connection to the new database is not verified automatically. To test the connection open the **Change Database** dialog and select the new database.

Buttons and Elements

Element	Description
Computer Name	Displays the name of the computer where the database is located.
Name	Name of the database connection (alias). This name is displayed in the **Change Database** dialog. You can edit the name as desired if you click on the name.
+	Allows you to add a database (see instructions above).
–	Removes the selected database from the list.
OK	Closes the dialog and saves the database settings.
Cancel	Closes the dialog without saving the changes.

Database Browser (Settings)



The **Database Browser** in the **Settings – Data Management** dialog can be used to:

- Search for objects and view their keywords and comments.
- Add comments to an object.
- Add/change the assay layout for a measurement.
- Edit a measurement's keywords (user-defined keywords only).

For a detailed description of the **Database Browser** and its variants please see section 5.1.16 “Database Browser”, page 220.

Notice

The **Database Browser** (opened via **Settings – Data Management** dialog) cannot be used to load objects or to perform data management tasks like archiving or exporting.

- **Loading objects:** Click [...] in the **Global Control** section of one of the main tabs to load the corresponding object.
- **Data management:** See section 5.1.17.7 “Data Management”, page 251 and sub-chapters.

Job Status

This dialog displays a list of background jobs which are currently running or which have been processed within the last 30 days. Such jobs are started if you ...

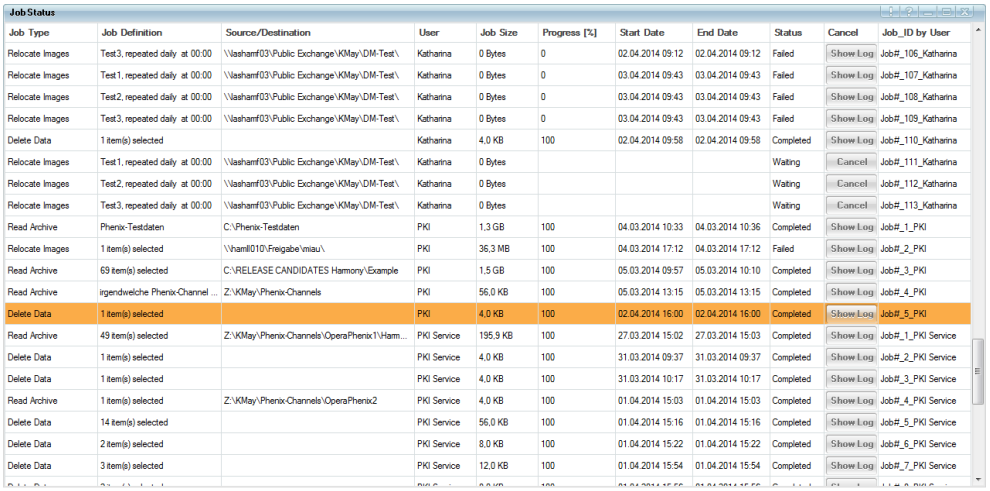
- use any of the data management functions in the **Settings** dialog (except for **Combine Measurement**).
- start a measurement including an **online Columbus Transfer**.

In the table you can see properties and status of each job. The view is refreshed every 30 seconds (or by pressing the **F5** key). You have the option to cancel a job and to view log files. Jobs older than 30 days will be removed from the list.

If you open the dialog, the jobs are sorted by user and the last job of the currently logged in Harmony user is highlighted. The job list can be sorted if you click on the column title of the respective property.

The **Job Status** dialog resides in a separate window which can be minimized and restored when needed. Please note that you cannot use other functions on the user interface as long as the **Settings** and **Data Management** dialogs are opened.

To view the **Job Status** table during a measurement, you have to open and minimize the dialog *before* starting the measurement.


Job Status

Job Type	Job Definition	Source/Destination	User	Job Size	Progress [%]	Start Date	End Date	Status	Cancel	Job_ID by User
Relocate Images	Test3, repeated daily at 00:00	\\ashamf03\Public Exchange\KMay\DM-Test\	Katharina	0 Bytes	0	02.04.2014 09:12	02.04.2014 09:12	Failed	Show Log	Job#_106_Katharina
Relocate Images	Test1, repeated daily at 00:00	\\ashamf03\Public Exchange\KMay\DM-Test\	Katharina	0 Bytes	0	03.04.2014 09:43	03.04.2014 09:43	Failed	Show Log	Job#_107_Katharina
Relocate Images	Test2, repeated daily at 00:00	\\ashamf03\Public Exchange\KMay\DM-Test\	Katharina	0 Bytes	0	03.04.2014 09:43	03.04.2014 09:43	Failed	Show Log	Job#_108_Katharina
Relocate Images	Test3, repeated daily at 00:00	\\ashamf03\Public Exchange\KMay\DM-Test\	Katharina	0 Bytes	0	03.04.2014 09:43	03.04.2014 09:43	Failed	Show Log	Job#_109_Katharina
Delete Data	1 item(s) selected		Katharina	4,0 KB	100	02.04.2014 09:58	02.04.2014 09:58	Completed	Show Log	Job#_110_Katharina
Relocate Images	Test1, repeated daily at 00:00	\\ashamf03\Public Exchange\KMay\DM-Test\	Katharina	0 Bytes				Waiting	Cancel	Job#_111_Katharina
Relocate Images	Test2, repeated daily at 00:00	\\ashamf03\Public Exchange\KMay\DM-Test\	Katharina	0 Bytes				Waiting	Cancel	Job#_112_Katharina
Relocate Images	Test3, repeated daily at 00:00	\\ashamf03\Public Exchange\KMay\DM-Test\	Katharina	0 Bytes				Waiting	Cancel	Job#_113_Katharina
Read Archive	Phenix-Testdaten	C:\Phenix-Testdaten	PKI	1,3 GB	100	04.03.2014 10:33	04.03.2014 10:36	Completed	Show Log	Job#_1_PKI
Relocate Images	1 item(s) selected	\\hamll010\Freigabe\miau\	PKI	36,3 MB	100	04.03.2014 17:12	04.03.2014 17:12	Failed	Show Log	Job#_2_PKI
Read Archive	69 item(s) selected	C:\RELEASE CANDIDATES Harmony\Example	PKI	1,5 GB	100	05.03.2014 09:57	05.03.2014 10:10	Completed	Show Log	Job#_3_PKI
Read Archive	irgendwelche Phenix-Channel ...	Z:\KMay\Phenix-Channels	PKI	56,0 KB	100	05.03.2014 13:15	05.03.2014 13:15	Completed	Show Log	Job#_4_PKI
Delete Data	1 item(s) selected		PKI	4,0 KB	100	02.04.2014 16:00	02.04.2014 16:00	Completed	Show Log	Job#_5_PKI
Read Archive	49 item(s) selected	Z:\KMay\Phenix-Channels\OperaPhenix1\Harm...	PKI Service	195,9 KB	100	27.03.2014 15:02	27.03.2014 15:03	Completed	Show Log	Job#_1_PKI Service
Delete Data	1 item(s) selected		PKI Service	4,0 KB	100	31.03.2014 09:37	31.03.2014 09:37	Completed	Show Log	Job#_2_PKI Service
Delete Data	1 item(s) selected		PKI Service	4,0 KB	100	31.03.2014 10:17	31.03.2014 10:17	Completed	Show Log	Job#_3_PKI Service
Read Archive	1 item(s) selected	Z:\KMay\Phenix-Channels\OperaPhenix2	PKI Service	4,0 KB	100	01.04.2014 15:03	01.04.2014 15:03	Completed	Show Log	Job#_4_PKI Service
Delete Data	14 item(s) selected		PKI Service	56,0 KB	100	01.04.2014 15:16	01.04.2014 15:16	Completed	Show Log	Job#_5_PKI Service
Delete Data	2 item(s) selected		PKI Service	8,0 KB	100	01.04.2014 15:22	01.04.2014 15:22	Completed	Show Log	Job#_6_PKI Service
Delete Data	3 item(s) selected		PKI Service	12,0 KB	100	01.04.2014 15:54	01.04.2014 15:54	Completed	Show Log	Job#_7_PKI Service

Notice

- The table shows only jobs which have been started on this computer. For example, if you open this dialog on the Harmony PC, you won't be able to see jobs which have been started using an office version of Harmony although the same database is involved.
- All jobs and scheduled tasks are linked to the name of the computer which was used to create them. If you rename this computer, all jobs will disappear from this list and scheduled tasks will not be executed anymore. For details see section 5.3.9 “Scheduled Tasks and Jobs Disappeared”, page 467.

If you create a **scheduled task** (see section “Schedule Tasks”, page 280), it will appear as a new job in the list with status *Waiting*. The start interval of the task is displayed in the **Job Definition** column. At the start time of the task, the job status changes to *Running* and a new job is added for the next run time of this task, again with status *Waiting*.

If a job has status *Completed*, *Failed*, or *Canceled*, you can click **Show Log** to view the log file. There may be listed more objects and signatures in the log file than you had previously selected for the job. The reason is that also files attached to or linked from the selected objects will be included, even though they may not be visible in the database. This can lead to additional signatures listed in the log file.

Examples

- If you select an evaluation, the corresponding measurement will also be included.
- If you select a measurement, the attached flatfield correction files will also be included.

After a job has got status *Completed* it may take a while until the changes are displayed by all Harmony functions and dialogs. It is recommended to wait 1 minute before proceeding.

If the job of a scheduled task with status *Completed* shows objects and signatures with the keyword “no value” in the log file, no data could be processed. The job was run successfully, but e.g. no objects were found matching the search pattern.

Buttons and Elements

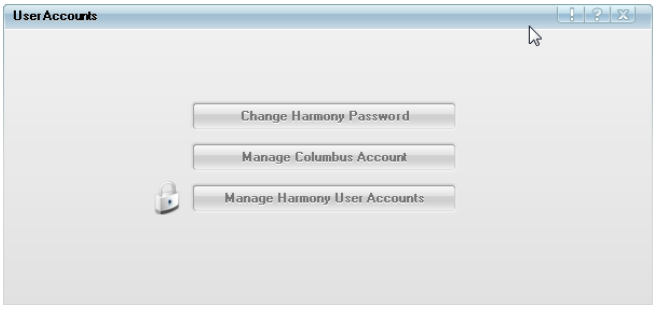
Element	Description
Job Type	Currently there are the following job types: • Columbus Transfer • Columbus – Online Transfer • Write Archive • Read Archive • Relocate Images • Export Data • Delete Data
Job Definition	Displays a user-defined name or comment for the job, or a default text is displayed. If the job was created by a scheduled task, the name and the start interval of the task are displayed.
Source/Destination	Depending on the job type: • **Columbus Transfer:** URL of the Columbus server (see also section "Manage Columbus Account", page 305). • **Delete Data:** Empty. • **Read Archive:** Source path of the archive. • **Relocate Images:** New image location (old image location can be seen in the log file) • **All other job types:** UNC path of the destination folder or name of the destination database (ODA).
User	Harmony user who started the background job. By default, the job list is sorted alphabetically by user.
Job Size	Total size of the objects to be processed.
Progress [%]	Job progress [%].
Start Date	Start date and time of the background job (status changed from *Waiting* to *Running*).
End Date	End date and time of the background job (status changed from *Running* to *Completed*, *Failed*, or *Canceled*).
Status	Current status of the job. Possible states: *Waiting*, *Running*, *Completed*, *Failed*, *Canceled*
Cancel / Show Log	• Job status *Waiting* or *Running*: **Cancel** button allows to cancel the job. • Job status *Completed*, *Failed*, *Canceled*: **Show Log** button allows to view the log file.

Element	Description
Job_ID by User	Consecutive job number and user who started the job, unique only for jobs started on this PC. If you click the column title to sort the list by this property, the jobs will be sorted (1) alphabetically by user name and (2) numerically by job ID (within the same user).

Context Menu

Element	Description
Copy	Copies the selected data to the clipboard.
Save Table...	Allows you to save the job list (*.csv) and all available log files (*.txt) of the listed jobs.
Help	Opens the corresponding topic in the Harmony Help.

5.1.17.8 User Accounts (Harmony + Columbus)

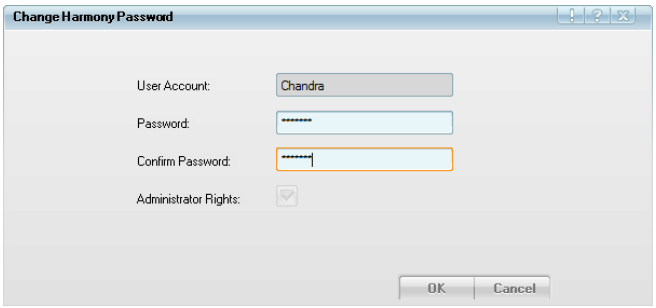


This dialog allows you to administrate the Harmony user accounts, i.e. modify accounts, change passwords and add or delete accounts.
For details on the Windows® user accounts involved in the Operetta CLS system, please refer to section 5.3.14 “User Accounts”, page 473.

Furthermore, you can configure connection details required for transferring measurements directly to Columbus. For details please refer to section “Columbus Transfer”, page 271.

If you open the **User Accounts** dialog, a **Login** dialog is displayed first. To be able to use the functions under **Manage Harmony User Accounts**, you have to login as an administrator. The other functions can also be used without administrative rights.

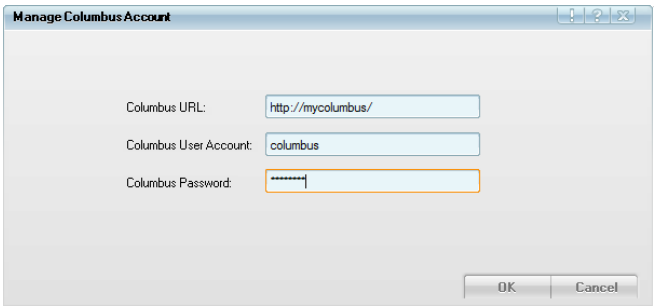
Change Harmony Password



Each user can change his/her own password:

1. Enter the new password in the **Password** box.
2. Enter the same password in the **Confirm Password** box.
3. Click OK.
   The password is changed.

Manage Columbus Account



This dialog allows you to configure connection details required for transferring data directly to a Columbus server within the network.

Notice

These settings are only valid for the currently logged in Harmony user. Other Harmony users can enter their own Columbus user account (if applicable), which has the following consequences:

- Each Columbus user account has its own list of screens in Columbus. The list of available **Screen Names** in Harmony (to be selected before transfer) depends on the selected Columbus account – and therefore on the currently logged in Harmony user if different Columbus accounts are used.
- This becomes important if you want to re-use an experiment of a different user where an **Online Job** for the **Columbus Transfer** has been defined. The **Screen Name** defined in the experiment must also be available for the currently set Columbus user account. Otherwise you have to select a different screen name or configure the same Columbus account which was originally used by the user who created the experiment.

For details see also sections “Columbus Transfer”, page 271 and 5.1.7.5 “Online Jobs”, page 77.

Notice

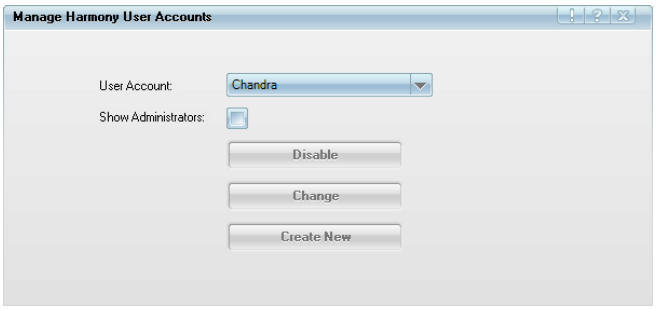
After changing the Columbus connection details you will have to delete and re-define any existing **scheduled task** which includes a Columbus Transfer. See also section “Schedule Tasks”, page 280.

Buttons and Elements

Element	Description
Columbus URL	URL of the Columbus server within the network, e.g. “http://ColumbusServerName/”.
Columbus User Account	Enter the name of a Columbus user account. The transferred data will be associated with this account in Columbus.

Element	Description
Columbus Password	Enter the password for the selected Columbus user account.
OK	The entered connection details will be verified, and the dialog will be closed if the check was successful. Otherwise errors will be reported in the **Messages** pane. If the connection cannot be established, you should recheck the URL and your network connection (see also section 5.3.20 “Columbus Network Integration”, page 480).
Cancel	Closes the dialog without saving the changes.

Manage Harmony User Accounts



This dialog allows to modify Harmony user accounts. Administrative rights are required.

Buttons and Elements

Element	Description
User Account	Allows to select the user account to be modified.
Show Administrators	If activated, only users with administrative rights are listed when you open the combo box above.
Disable	Disables a user account, i.e. this user account is not offered in the **Login** dialog anymore. You cannot delete a user account completely, because database objects created by this user may exist.
Change	Opens a dialog where you can change a user's password. Furthermore, you can set or remove the administrator status.
Create New	Allows you to define a new user account.

Administrator Privileges

Harmony user accounts with administrative rights have the following privileges:

- **Attach (or detach) an assay layout** to a measurement owned by another user. See sections 5.1.16.5 “Attach Assay Layout”, page 235 and 5.1.16.6 “Detach

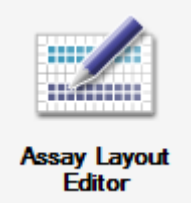
Assay Layout", page 237.

- **Edit keywords** of a measurement owned by another user.
  See section 5.1.16.4 "Edit Keywords", page 234.
- **Create user-defined keywords.**
  See section "Define Keywords", page 278.
- **Delete objects** owned by another user. Objects with owner **PKI Service** cannot be deleted.
  See section "Delete Data", page 272.
- **Relocate images** of a measurement owned by another user.
  See section "Relocate Images", page 259.
- **Define destinations for relocating images.**
  See section "Relocate Settings", page 294.
- **Manage Harmony user accounts.**
  See section "Manage Harmony User Accounts", page 306.
- **Create or delete scheduled tasks** (automated relocating or archiving).
  See section "Schedule Tasks", page 280.

Default User Accounts/Owners

- **PKI:** Initial user account (password **PKI**). This account has administrative rights and should only be used for the first login. It is recommended to create new user accounts for each Operetta CLS user.
- **PKI Service:** This user account is required by the PerkinElmer Service. It cannot be deleted.
- **Application Guide:** All objects in the database that belong to the example data of the Application Guide (e.g. the Ready-Made Solutions) are listed under this owner by default. The user "Application Guide" cannot be selected for login, and the user account cannot be deleted.

5.1.17.9 Assay Layout Editor

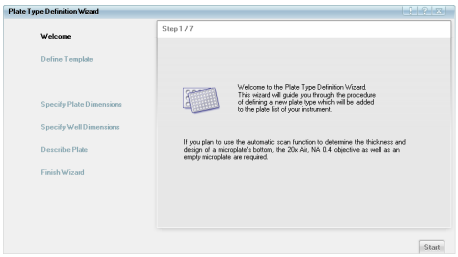


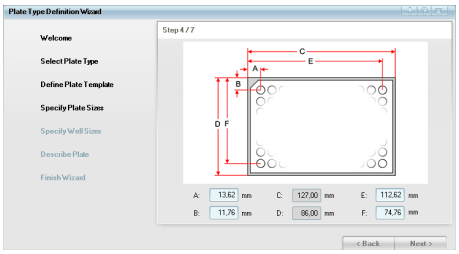
This function is used to open the **Assay Layout Editor**. For more information please refer to section 5.1.7.8 “Assay Layout Editor”, page 94.

Notice

Another way to open this editor is to right-click on the plate control on the **Setup** screen (**Navigation – Define Layout – Plate**) and select **Assay Layout Editor** from the context menu.

5.1.17.10 Define Plate Type





The **Plate Type Definition Wizard** allows you to define new plate types which will be added to the plate list of the Operetta CLS. There is a template **Plate** for regular microplates and a template **Slide** for slideholders with one or multiple slides. The wizard will guide you through the procedure step-by-step.

Notice

The dimensions of each new plate type have to be measured exactly using a vernier caliper. Incorrect plate dimensions can e.g. lead to focus failures.

Do not rely entirely on the plate manufacturer's specification. In case of focus failures please use **Scan H & I** to determine the height and thickness of the plate bottom automatically (not available for slides, see section "Scan Plate Bottom", page 310).

After the new plate type has been added, it can be selected for an experiment in **Setup – Global Control**.

Microplates

Plate Wizard

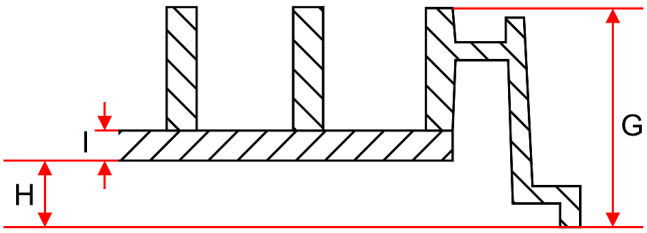
New plate types for microplates can be created if you start the wizard and select the template **Plate** in step 3. In the following steps you have to enter various plate dimensions and properties. This is not explained here in detail, because the wizard will provide you with all necessary information.

Notice

- The plate height (parameter **G** in the plate wizard) may not exceed 22 mm. When using plates with lids, it is the user's responsibility to make sure that the maximum plate height (plate *and* lid) is not exceeded. Otherwise the plate may crash inside the instrument.
- *Only for optional Automation Upgrade:* Before starting an automated run you have to verify whether the used plate type can really be handled by the robot and is properly detected by the Operetta's plate sensor.

Scan Plate Bottom

A common source of focusing errors are incorrect or imprecise values in the plate type definition. This is especially true for parameters **H** and **I** (step 5 of the plate wizard) which define the height and the thickness of the microplate's plate bottom:



H: Height of the plate bottom above the outer rim
I: Thickness of the plate bottom

The **Scan H & I** button allows you to determine these two parameters automatically and with high precision. This can be helpful when defining new plate types or verifying existing plate definitions. This function is only available for microplates, not for slides. The following requirements must be met before you can use the **Scan H & I** function:

- Objective **20x Air** installed (will be selected automatically if installed)
- Well dimensions entered correctly (parameters **J** and **N** for round wells or parameters **L**, **M**, **O**, and **P** for rectangular wells)

During the scan, the center fields of six different wells in the center region of the plate will be scanned:

- 96-well plate: Col 6 Row 4 ... Col 8 Row 5
- 384-well plate: Col 12 Row 8 ... Col 14 Row 9
- 1536-well plate: Col 24 Row 16 ... Col 26 Row 17

H and **I** will be determined by calculating the median of the resulting values.

Notice
It is not recommended to use plates with a plate bottom thickness higher than 1 mm as the automatic image alignment procedure requires the correction collar to be set to ≤ 1 mm. A wrong correction collar setting might impact the image quality.

For further reasons for focusing issues see also troubleshooting section 5.3.22.10 “Focus failure”, page 492.

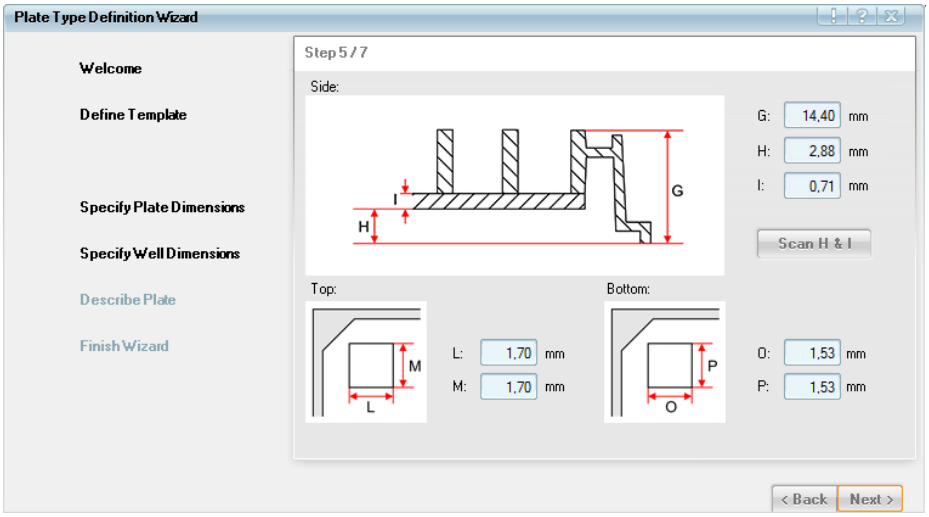
How to use the "Scan H & I" function

Preparations

1. Make sure that the objective **20x Air** is currently installed. You do not have to select it, it just has to be present in the instrument.

Start Plate Wizard

1. Open the **Plate Definition Wizard** (**Settings – Define Plate Type**) and click **Start**.
2. In step 2 you can define a new plate type or modify an existing plate type:
   - Select **New** to create a new plate type from scratch.
   - Select **New Based On** to select an existing plate type and check its settings or use it to create a similar plate type.
3. Enter or verify the values in step 3 and 4 of the wizard and proceed to step 5 (Specify Well Dimensions).
4. Enter the well dimensions correctly (parameters **J** and **N** for round wells or parameters **L**, **M**, **O**, and **P** for rectangular wells).
   The **Scan H & I** button will remain disabled until you have entered values for these parameters.



Scan Plate Bottom

1. Click **Scan H & I**.

   The scanning stage is moved to the transfer position and the transfer gate is opened. A message box with instructions will be displayed.
2. Insert the microplate of which you want to scan **H** and **I**. The plate must be empty.
3. Click **OK**.

   The plate is loaded and the 20x Air objective is moved into service position. Then the lid is unlocked to allow access to the objective. A new message box with instructions is displayed.
4. Open the lid, take out the 20x Air objective and set the correction collar to the expected thickness of the microplate's plate bottom. For detailed instructions see section 7.1.2 “Adjust Correction Collar”, page 528.
   - If this value is unknown, set the correction collar to “0.5”.
5. Insert the objective again and close the lid.

   The objective is moved into measuring position and the **OK** button is enabled.

6. Click **OK**.
7. Click **Start**.

   The plate scan is started. After the scan the detected values for parameter **H** and **I** will be displayed.
8. Click **OK**.

   The determined values are changed automatically in the plate definition. The previously used objective will be selected again, if applicable.

Optional: Second Plate Scan

A second plate scan is only necessary if the plate bottom thickness (**I**) was unknown before the first scan (see step 4 in the previous section).

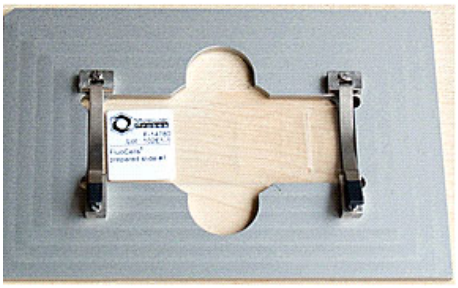
1. Click **Start H & I** again to start a second plate scan.
2. Repeat the procedure as described above, but with the following modification:
   - Set the correction collar to the value of **I** which resulted from the first scan.
3. **H** and **I** will be finally determined and changed in the plate definition.

Finish Plate Definition Wizard

Proceed with step 6 and 7 of the wizard and finally click **Finish** to add the new/modified plate type to the database.

Slides

As a slide does not have the standard dimensions of a microplate, it needs to be inserted into a slideholder. This slideholder must have the outer dimensions of a microplate and therefore corresponds to a new plate type. Accordingly, the slide corresponds to a large rectangular well.



Slideholder for one horizontal slide

Slideholder for up to four vertical slides

- For background information and instructions how to prepare the sample on the slide see also **Operetta CLS Application Guide**, chapter 1.3 “Slides”.
- To quickly identify areas of interest on the slide we recommend to use the **PreciScan** function (see section 5.1.8.3 “PreciScan™”, page 119) or the **Manual PreScan/ReScan** procedure (see section 5.1.11.4 “Manual PreScan/ReScan”, page 191).

Notice

- If you define a new plate type for a slideholder, it is important to select the **Slide** template in the wizard. Otherwise the objective will collide with the rims surrounding the slide positions and the autofocus may not work reliably.
- Please note that the area on the slideholder which can be defined as measuring area may be restricted to avoid collisions of scanning stage and objective. See also section 5.1.7.7 “Restrictions of Measurable Plate Area”, page 89.

How to create a new plate type for slides

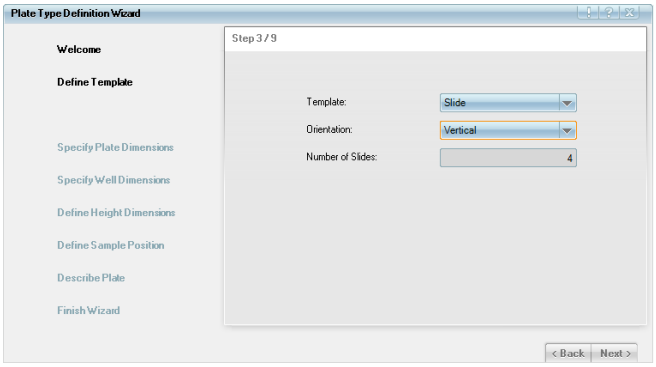
Step 1: Welcome

- Click **Start**.

Step 2: Define Template

- Select **New** to define a new plate type and click **Next**.

Step 3: Define Template

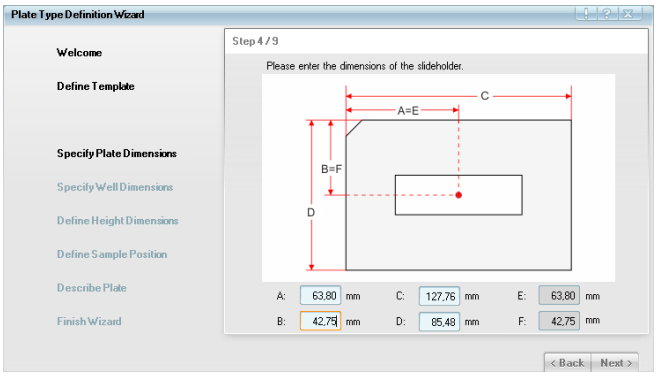


1. Select **Slide** as **Template**. The options of the **Slide** template are displayed.
2. Select the **Orientation** of the slide(s): **Horizontal** or **Vertical**.
3. The **Number Of Slides** in the slideholder is displayed (vertical: 4, horizontal: 1). Not all positions in the slideholder must be used. You can select the desired slides (i.e. wells) in the plate layout when you define the experiment.
4. Click **Next**.

Step 4: Specify Plate Dimensions

Depending on the selected orientation and the number of slides, one of these screens will be displayed in step 4:

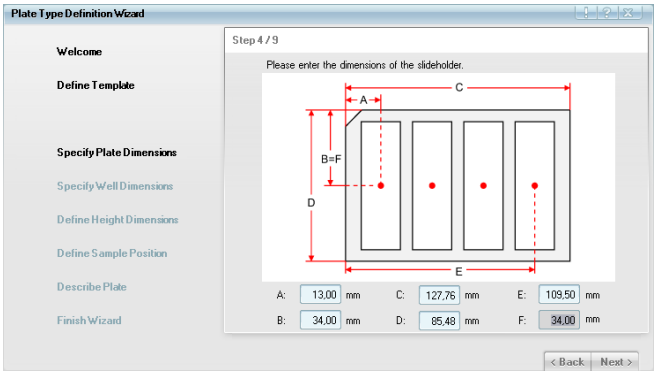
One horizontal slide:



1. Enter the dimensions of the slideholder (**C** and **D**). These parameters can also be reset to default values via the context menu.
2. Specify the center of the slide position (**A** and **B**). The red dot represents the center of the slide position (and thereby the center of the well).
3. Click **Next**.

Four vertical slides:

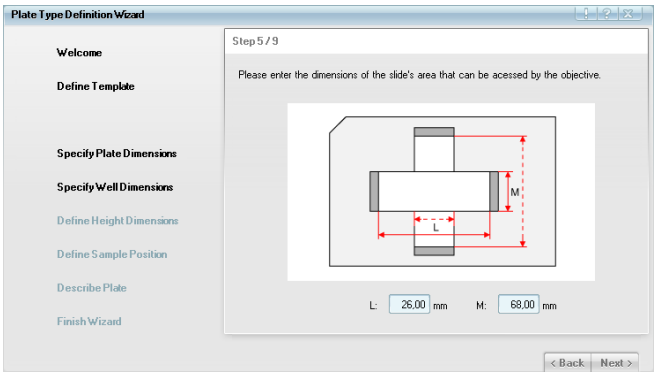
If you have selected vertical slides, the following slideholder with four slide positions will be displayed. Only the center of the first and the last slide (**A**, **B** and **E**) have to be specified. The positions in between will be calculated automatically.



1. Enter the dimensions of the slideholder (**C** and **D**). These parameters can also be reset to default values via the context menu.
2. Specify the center of the first and the last slide (**A**, **B** and **E**). The red dots represent the center of each slide position (and thereby the center of each well).
3. Click **Next**.

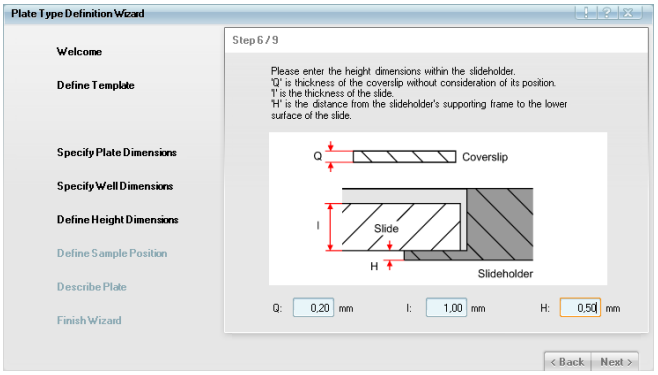
Step 5: Specify Well Dimensions

The following screen with one slide will be displayed independently of the selected orientation and number of slides.



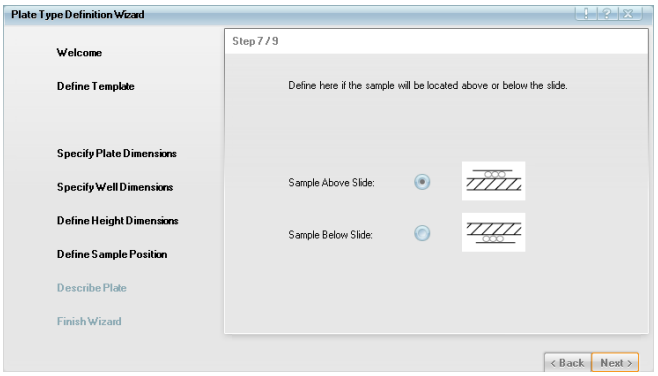
1. Enter the dimensions of the well, i.e. only the visible and usable area of each slide which is not covered by the slideholder (**L** and **M**). Do not just enter the outer slide dimensions. At least at the supporting points in the slideholder a certain area of the slide will be covered.
2. Click **Next**.

Step 6: Define Height Dimensions



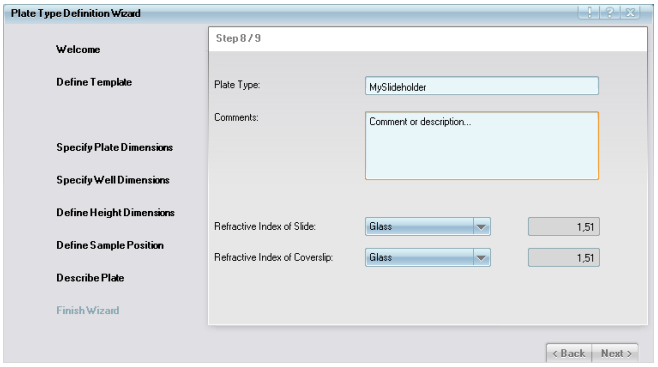
1. Enter the thickness of the coverslip (**Q**). The position of the coverslip will be defined in the next step. If you do not want to use a coverslip, enter '0'.
2. Enter the thickness of the slide (**I**).
3. Enter the distance from the slideholder's bottom to the lower surface of the slide (**H**).
4. Click **Next**.

Step 7: Define Sample Position



1. Define the position of the sample (above or below the slide).
2. Click **Next**.

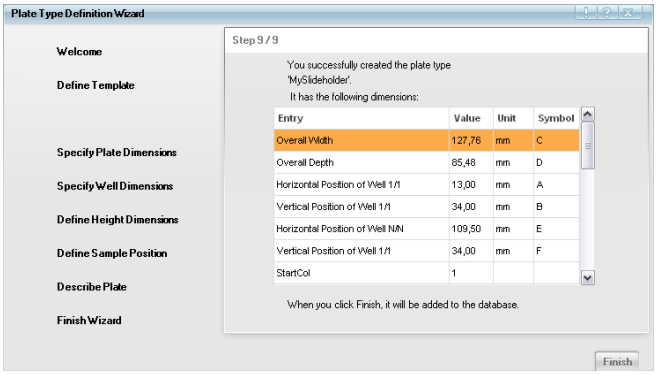
Step 8: Describe Plate



1. Enter a name for the new **Plate Type**.
2. Enter a comment, if desired.
3. Specify the **Refractive Index** of slide and coverslip.
4. Click **Next**.

Step 9: Finish Wizard

A summary of the plate type properties is displayed.



- Click **Finish**. The new plate type is saved to the database.

5.1.17.11 Request Application Guide

The **Application Guide package Operetta CLS** consists of two documents:

- Operetta CLS Application Guide
- Image Analysis Guide

You can use this dialog to request personalized electronic copies of these guides (see below for detailed instructions). PerkinElmer will send you an email with a web link from where you can download the PDF files. The guides are not available as printed manuals.

Copyright Information
The documents may not be copied or published in any form. The PDF files are stamped with your personal information and the serial number of your instrument.

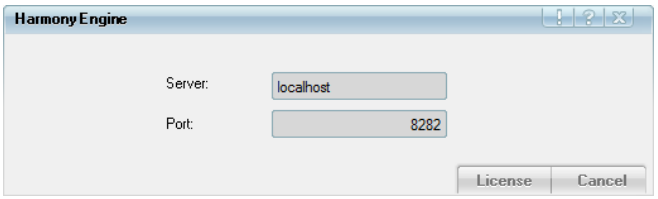
For troubleshooting please see section 5.3.4 “Application Guide Request Not Successful”, page 463.

How to request your Application Guide package

1. Open **Settings – Request Application Guide**.
2. Enter your **Name**.
3. Enter the name of your **Organization**.
4. Enter your **Mail Address**.
   The download link will be sent to this email address.
5. The requested documents are displayed in the lower section of the dialog. Make sure that the package is intended for your instrument. If a different instrument name is displayed, please see 5.3.4 “Application Guide Request Not Successful”, page 463 for instructions.
6. Click **Save...**
7. Select a destination folder for saving the request file (**RequestApplicationGuide.rag**), e.g. on the desktop. This file includes the entered data and further information on your instrument. Click **Save**. The file has to be sent to PerkinElmer by email.
8. Open your email program and open a new email:
   - **Subject:** Application Guide
   - **Recipient:** request.applicationguide@perkinelmer.com
   - **Attach** the previously saved request file (**RequestApplicationGuide.rag**).
   - The text of the email will be ignored.
9. Send the email.

   PerkinElmer will send you the download link to the email address which you entered in the request dialog. You can expect an answer within 24 hours.

5.1.17.12 Harmony Engine



The Harmony Engine is responsible for the image analysis of the measured images. This dialog displays the server name and the port number of the **Acapella Server** which manages the flow of data between Harmony and the Harmony Engine (Acapella). Furthermore, you can view or update your Acapella license (see section “License Management”, page 320).

Buttons and Elements

Element	Description
Server	Server name of the **Acapella Server** (usually installed locally on the Harmony PC)
Port	Port number of the **Acapella Server**
License	Opens a new dialog for viewing and updating license information, see also section “License Management”, page 320).
Cancel	Closes the dialog.

Acapella Server

The **Acapella Server** is an application that manages the flow of data between Harmony and the Harmony Engine (Acapella) where the image analysis occurs. It is started automatically during Harmony start-up.

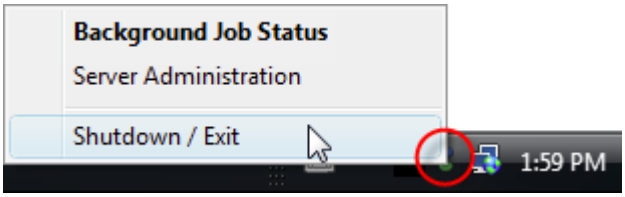
If you right-click on the **Acapella Server** icon in the Taskbar Notification Area, you can open a web interface in your browser to watch the current status and use a number of administrative functions.

How to shutdown the Acapella Server

If you shutdown Harmony, the **Acapella Server** keeps running. It will be automatically restarted by Harmony during start-up. To shutdown the **Acapella Server** manually:

Notice
Do not shutdown the **Acapella Server** while Harmony is running or batch analyses are still being processed. This could lead to the loss of data.

1. Right-click the **Acapella Server** icon in the Taskbar Notification Area.



2. Select **Shutdown/Exit** from the context menu.
   The **Acapella Server** is shutdown.

Acapella Server – Context Menu

Right-click the **Acapella Server** icon in the Taskbar Notification Area to open the context menu.

Element	Description
Background Job Status	Opens the web interface of the **Acapella Server** and displays a list of image analysis tasks which are being processed.
Server Administration	Opens the web interface of the **Acapella Server** and offers administrative functions.
Shutdown / Exit	Closes the **Acapella Server**.

License Management

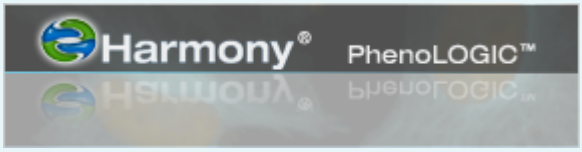
To use the image analysis functionality of Harmony, you need a valid license for the Harmony Engine, i.e. for Acapella. Upon delivery, your Operetta CLS is equipped with a **USB license dongle** which grants usage of the provided Harmony Engine. This license does not expire.

The USB dongle also stores an **SMA expiration date** (SMA: Software Maintenance Agreement). The SMA allows you to obtain and install free software updates (Harmony and/or Acapella) within one year after purchasing the Operetta CLS. If your SMA has expired, you can still use your existing installation (for unlimited time).

- To be able to install and run new software updates, the SMA has to be renewed. In this case please contact the License Admin to request a new license key.
- If you want to extend the image analysis functionality of Acapella by further detection libraries or plug-ins (e.g. Neurite Detection, the PreciScan feature or building blocks using the PhenoLOGIC™ technology), you also need a new license key. Please contact the PerkinElmer Service.

Notice

- Building blocks (or single methods within a building block) which use the **PhenoLOGIC™** technology are only available with an optional license. If the required license is activated on your license dongle, the PhenoLOGIC™ logo will be displayed next to the Harmony logo.



- An optional license is also required to use the **PreciScan** feature including the building block “Determine Well Layout”. See also section 5.1.8.3 “PreciScan™”, page 119.

Technical Support

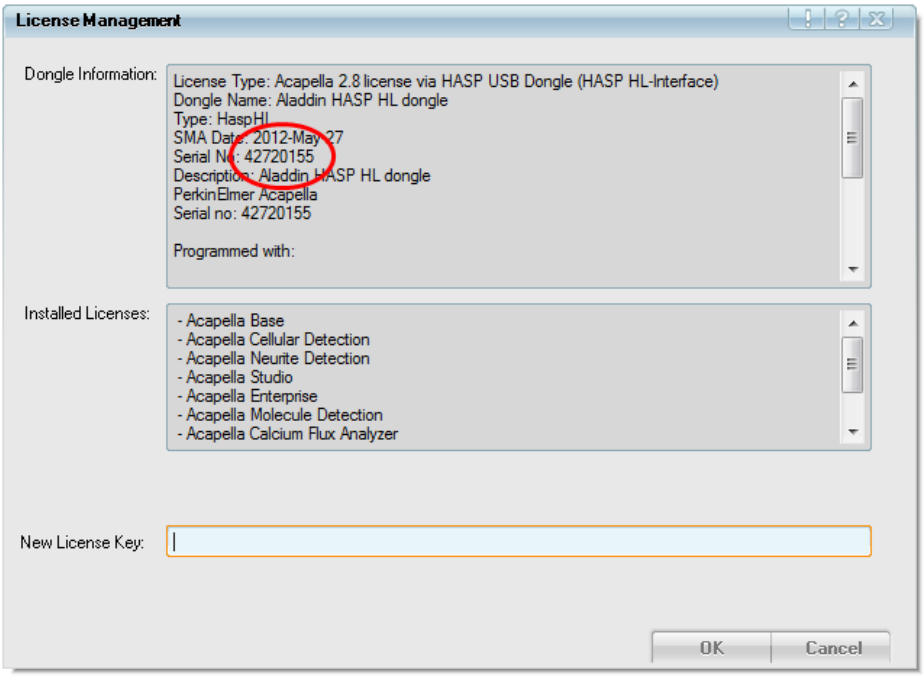
For requesting a new license key please contact:

- operetta.support@perkinelmer.com

How to update an Acapella license

1. Open **Settings – Harmony Engine**.
2. Click License.

   A new dialog is opened.

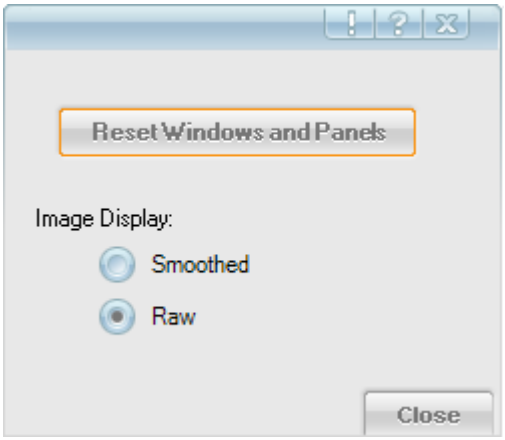


3. Mark the serial number of your USB license dongle and copy it to the clipboard (Ctrl + C).

4. Send the serial number to the following email address: **operetta.support@perkinelmer.com**
5. PerkinElmer will send you a new license key.
6. Copy the new key from the email and paste it into the field **New License Key**.
7. Click OK to apply the license key and close the dialog.

   The new license is activated and written to your USB dongle.

5.1.17.13 Display Settings



Reset Windows and Panels

If you click the **Reset Windows and Panels** button, the arrangement of windows and panels is reset to condition of delivery. This can be useful if you have disarranged or hidden the movable panes **Navigation**, **Image Control**, **Messages** and **Results**.

For detailed information on the arrangement of screen elements please see section 5.1.5 “Screen Arrangement”, page 36.

Image Display

Using this option you can enable or disable image smoothing at zoom levels higher than 1:1 for all images displayed in Harmony. This is a global setting, valid for all Harmony users.

Notice

The display mode influences only the image display within Harmony. The original images which are used for image analysis or any image which is manually saved/exported (using the context menu functions **Save Image** or **Export Raw Images**) will never be interpolated.

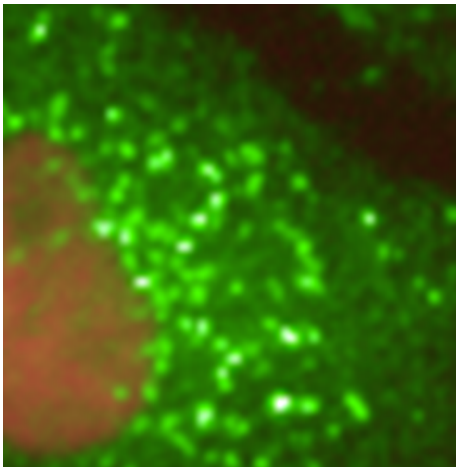
Only the **Copy** function will copy the image exactly as displayed at current zoom level (possibly interpolated and with reduced resolution). See also section 5.1.13.1 “Image Display”, page 209.

- **Smoothed:** Displays an interpolated version of the image at zoom levels higher than 1:1.
  This option produces nicely smoothed images, but it makes it more difficult to

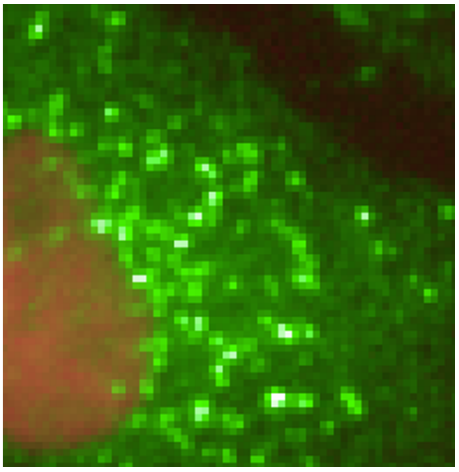
judge the real image quality and the meaning of image analysis results.

- **Raw:** Displays the original pixel structure at zoom levels higher than 1:1. For scientifically accurate representation it is recommended to use this option.

The visible pixel structure at zoom levels higher than 1:1 is often confused with “bad image quality” while smoothing of the image display may seem to produce “better image quality”. To the contrary, smoothing makes it more difficult to judge the real image quality and the meaning of image analysis results. The resolution of the image is not improved. The image is blurred and contrast and details are reduced.



Smoothed: *Image display with smoothing at high zoom level*



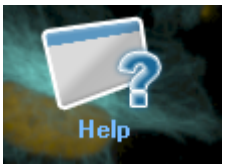
Raw: *Image display without smoothing at high zoom level*

5.1.17.14 About

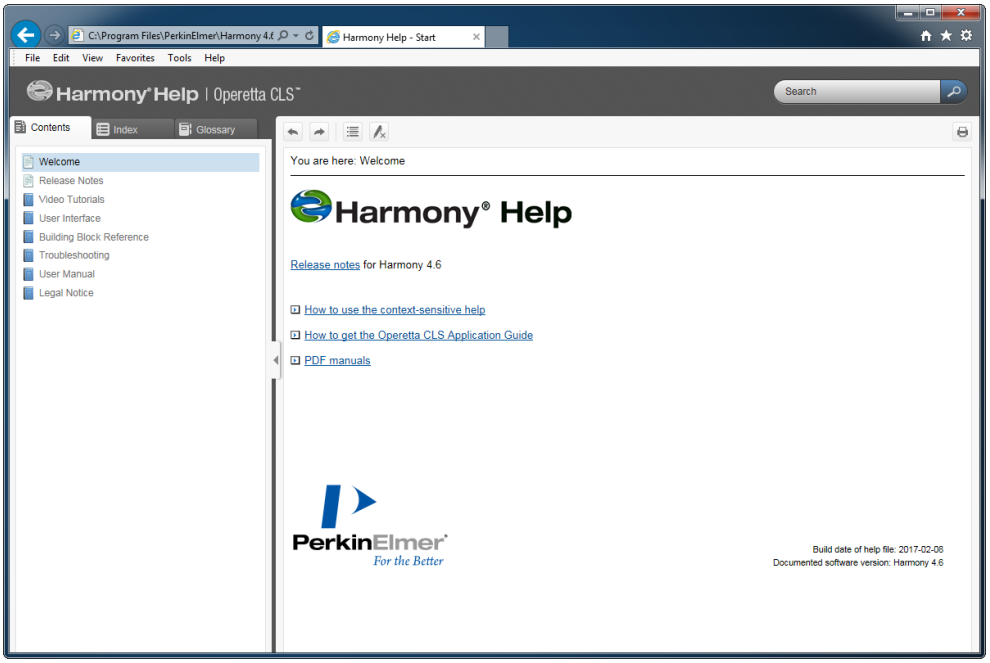
In this dialog you can find various information on your Operetta CLS system:

- PerkinElmer contact details
- Instrument type and serial number
- Instrumentation of the connected device
- Software version details (Harmony, Acapella, firmware, database)

5.1.18 Help



Click the help icon in the **Navigation Bar** to open the Harmony Help (welcome page) in your default web browser.



Notice

Many dialogs offer a **context-sensitive help**, i.e. the help topic for the corresponding screen element can be opened directly:

- Right-click the corresponding element to open the context menu and select **Help**.
- Alternatively, you can use the **F1** key:
  Set the focus to the corresponding screen section, i.e. set the mouse cursor into a text box within the section or click on a pane title. Press the **F1** key.

The help topic for this section of the user interface will be displayed. If there is no specific topic available, the welcome page will be opened.

5.2 Building Block Reference

5.2.1 Overview

The building blocks can be divided into the following categories. Frequently used building blocks are written in bold text.

Define Input

- ***Input Image*** (mandatory)
  See section 5.2.3 “Input Image”, page 327

Find Objects

- ***Find Nuclei***
  See section 5.2.4 “Find Nuclei”, page 336
- ***Find Cytoplasm*** (around previously detected nuclei)
  See section 5.2.5 “Find Cytoplasm”, page 341
- ***Find Spots*** (in previously defined regions)
  See section 5.2.6 “Find Spots”, page 345
- ***Find Cells***
  See section 5.2.7 “Find Cells”, page 355
- *Find Neurites*
  See section 5.2.9 “Find Neurites”, page 366
- *Find Micronuclei*
  See section 5.2.8 “Find Micronuclei”, page 361
- *Find Image Region*
  See section 5.2.10 “Find Image Region”, page 371
- *Find Texture Regions* (uses PhenoLOGIC™)
  See section 5.2.11 “Find Texture Regions”, page 374
- *Find Surrounding Region*
  See section 5.2.12 “Find Surrounding Region”, page 382

Select Region in Objects

- ***Select Cell Region*** (for a complete “map” of the cell)
  See section 5.2.13 “Select Cell Region”, page 386
- *Select Region* (can be used with arbitrary objects)
  See section 5.2.14 “Select Region”, page 390

Calculate Object Properties

- ***Calculate Intensity Properties***
  See section 5.2.15 “Calculate Intensity Properties”, page 394
- ***Calculate Morphology Properties***
  See section 5.2.16 “Calculate Morphology Properties”, page 397
- ***Calculate Texture Properties***
  See section 5.2.17 “Calculate Texture Properties”, page 406

- *Calculate Properties*
  See section 5.2.18 “Calculate Properties”, page 411

Select a Population of Objects

- ***Select Population*** (uses PhenoLOGIC™)
  See section 5.2.19 “Select Population”, page 414

Modify a Population

- *Modify Population*
  See section 5.2.20 “Modify Population”, page 425

Process Images

- *Filter Image*
  See section 5.2.21 “Filter Image”, page 426
- *Calculate Image*
  See section 5.2.22 “Calculate Image”, page 432

PreciScan

- ***Determine Well Layout*** (requires optional PreciScan license)
  See section 5.2.23 “Determine Well Layout”, page 436

Track Objects

- ***Track Objects***
  See section 5.2.24 “Track Objects”, page 438
- *Calculate Kinetic Properties*
  See section 5.2.25 “Calculate Kinetic Properties”, page 445
- *Calculate Track Properties*
  See section 5.2.26 “Calculate Track Properties”, page 447

Calculate Collated Readout Values

- ***Define Results*** (mandatory)
  See section 5.2.27 “Define Results”, page 450

Notice
See also the **Image Analysis Guide** (section “Setting up an Analysis Sequence") for an introduction to the building blocks.

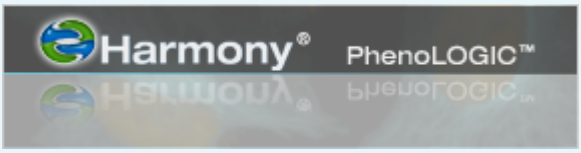
5.2.2 PhenoLOGIC™

Some of the building blocks implement PerkinElmer's PhenoLOGIC™ technology which includes an interactive training mode to identify cellular phenotypes. The user can train the software by selecting positive/negative examples in the image. Once the training has been finished, the search pattern can automatically be applied to similar images.

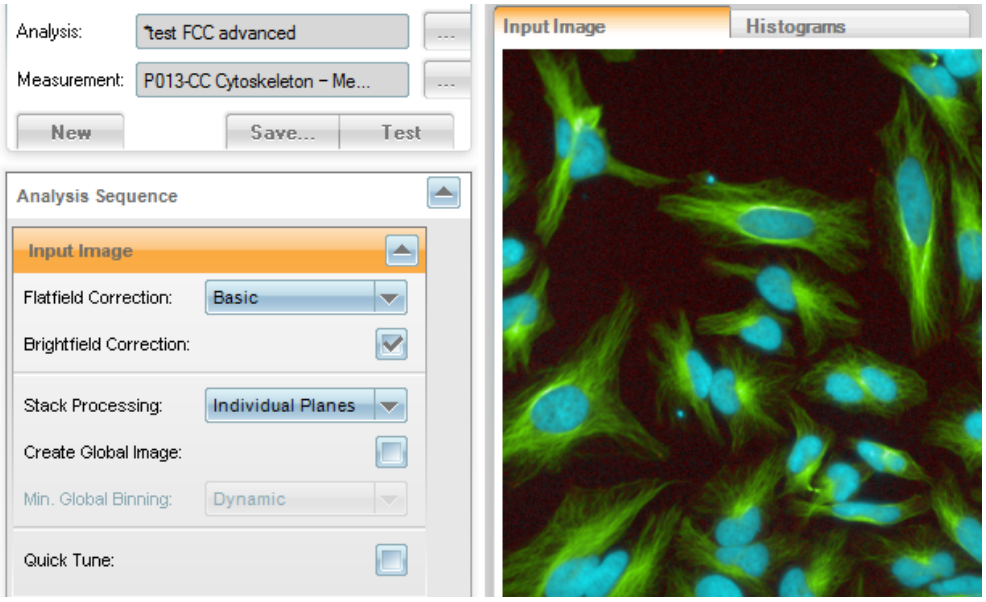
Notice

Building blocks (or single methods within a building block) which use the PhenoLOGIC™ technology are only available with an optional license. For details see section "License Management", page 320.

If the required license is activated on your license dongle, the PhenoLOGIC™ logo will be displayed next to the Harmony logo.



5.2.3 Input Image



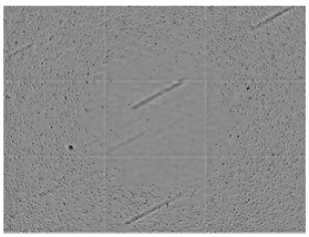
Input Image is the first building block of each analysis sequence. If you select this block, there will be up to three different tabs displayed in the **Content Area**:

- **Input Image:** Displays the original image without any overlays. The image can be selected in the **Navigation** pane.
- **Global Image:** If **Create Global Image** is checked, all measured fields are stitched to create a "global image" of the well. This global image can be used in the analysis sequence like a normal channel. For details see section 5.2.3.1 "Global Image / MultiScale Analysis", page 329.
- **Histograms:** Displays a histogram for each channel of the image.
  If a time window is selected, the histogram is shown for multiple time points to allow seeing changes over time. The first and last time point's curve is marked bold (first continuous, last dotted line).
- **XYZ View:** Only for stacks and if *Maximum Projection* has been selected. Displays the images of a stack in a special viewer which allows to view sectional planes in x-, y- and z-direction. See also section 5.2.3.4 "XYZ View", page 334.

Input Parameters

Input parameters	

Flatfield Correction	Flatfield correction method which is used to correct the image before analysis. The same correction method (or a better one) must be available for the measurement to be analyzed:
	• **None:** Uncorrected image is used (default). This option must be used …
	  ◦ if no correction profile could be calculated for the measurement to be analyzed.
	  ◦ if the analysis sequence is to be used for online analysis.
	• **Basic:** Image with background correction is used, no foreground correction.
	• **Advanced:** Corrected image (background and foreground) is used.
	The selected method will automatically be used as display method for the current image (**Image Control – Controls – Flatfield Correction**), but not vice versa.
	For detailed information see section 5.1.12.5 “Flatfield Correction”, page 205.

Brightfield Correction	Removes the background profile of brightfield channels to allow creating a smooth montage for analysis and visualization. Affects the images which are analyzed (in contrast to the identically named option in the **Image Control** section which affects display only).   *Uncorrected* *Corrected*
Stack Processing	Determines how multiple planes of a stack measurement are handled during an evaluation: • **Maximum Projection:** All planes are reduced to one single image. Each pixel gets the maximum value across the stack planes. • **Individual Planes:** Each plane is processed and reported individually.
Create Global Image	Creates a montage of all image fields in a well, called **Global Image**. An additional tab **Global Image** will be added to the existing tabs in the **Content Area**. For details see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.

Input parameters	
Min. Global Binning	*If "Create Global Image" was selected:* Allows you to control the binning (resolution reduction) of the global image. The stitched global image can become very large (depending on the number of image fields). The resolution must be reduced so that it fits into the computer's memory and can be processed efficiently. • **Dynamic (default):** Binning is automatically adapted to the size of the global image while preserving as many details as possible, fits most applications. • **Minimal value:** Defines a minimal binning for the global images of all wells (to support special applications, e.g. in combination with PreciScan where the number of fields per well can vary). For detailed instructions see also section 5.2.3.3 “Binning Adjustment of Global Image”, page 332. The actually used **Binning** value is displayed in the **Image Info** tab (**Image Analysis Results** pane) and will also be reported together with the well results.
Quick Tune	If activated, the central area of the image is cropped out to allow faster parameter tuning. Once the analysis sequence has been assembled, it is recommended to test and run it with uncropped images. The option has no effect on the single/batch evaluations which you run on the **Evaluation** tab. **Notice** **Quick Tune** should not be used in combination with building blocks which offer a training mode (PhenoLOGIC™). This would falsify the results.

Context Menu (Content Area)

- For a description of the context menu options of the image display please refer to section 5.1.13.1 “Image Display”, page 209.
- For a description of the context menu options of the histogram please refer to section “Graph”, page 157.

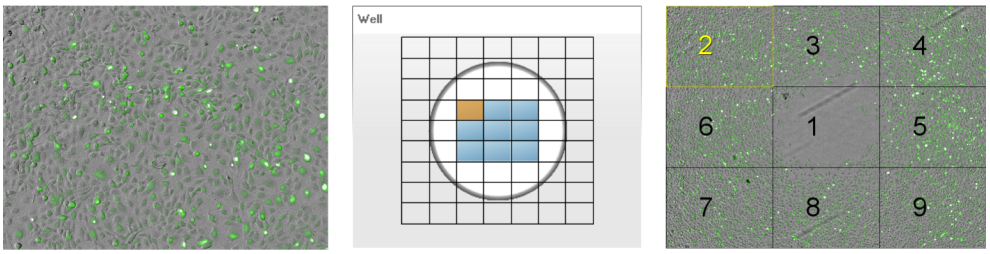
5.2.3.1 Global Image / MultiScale Analysis

MultiScale Analysis allows you to analyze samples (e.g. cell colonies, tissue slices or cell spots/grids, on slides and plates) that span multiple fields by generating a montage of all fields in a well ("global image" with reduced resolution). This allows you to choose the region of interest and then analyze the original high resolution images ("local" images) of this region.

Notice

MultiScale Analysis is an expert feature only. Good understanding of image analysis and the concept of related populations and ROIs (Regions of Interest) is needed.

Example



Left: *Local image (high resolution)* ***Middle:*** *Well layout with selected field* ***Right:*** *Global image with "Field Map" overlay (reduced resolution)*

Working Principle

Before Harmony 4.5, image analysis was limited to a scope of one image field at a time. Large objects, like e.g. cell colonies or small animals, could not extend over multiple image fields. Therefore the objective magnification had to be sufficiently low (= large field of view) to fit the full object of interest into one image field (e.g. cell colony). On the other hand the objective magnification had to be sufficiently high to capture all details of interest (e.g. cells, nuclei, spots). To overcome this limitation, the analysis is split in two parts:

1. Detect large objects (e.g. colonies) on a binned, low detail, montage image of all image fields in the current well (**global image**). Detection of large objects does not need memory consuming full resolution images.
2. The detected large objects can be used as a region of interest (ROI) for the detailed analysis on the **original full resolution images**, e.g. nuclei detection within the colonies. The ROI can spread over multiple image fields.

The global image is composed of **all** image fields in the current well (i.e. all fields displayed in the well control in the right pane). For each measured channel (e.g. “Alexa 488”) a corresponding new channel with the global image is created, e.g. “Alexa 488 (global)”. To reduce the image size of the global image, the imaging fields may be re-arranged and the resolution will be reduced (see the following sections for details).

Notice

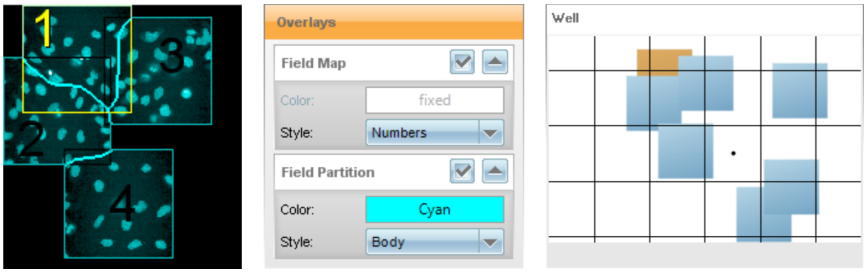
The creation of the global image can be time consuming if many fields, planes, channels etc. have been measured. The calculation is now done as a background task and you can cancel the process if required:

- Uncheck **Create Global Image** (if it was accidentially activated) or select a different well (if a wrong well was selected).

  The global current image creation is cancelled in this case (takes some seconds). As long as the global image creation is in progress, it is updated from time to time on the **Global Image** tab and a progress bar is shown.

How to create a global image

1. Open the **Image Analysis** building block and load a measurement with multiple fields.
2. In the *Input Image* building block, check the option **Create Global Image**.
   - A global image (montage) is generated using all image fields of the selected well. It is displayed on the **Global Image** tab in the **Content Area**.
   - In addition to the regular "local" channel(s), *Input Image* now generates "global" channels (e.g. "Alexa 488 (global)") which can be used in the following building blocks.
   - The overlay **Field Map** is created which marks the locations of all image fields and highlights the currently selected image field . By default the overlay style “Numbers” is selected. The displayed numbers correspond to the field numbers used in the well control and reported image analysis result tables.
   - A second overlay **Field Partition** is available which uniquely assigns the full imaged region to exactly one field in case there is an overlap of fields. In case of no field overlap, field partition and field map are identical.



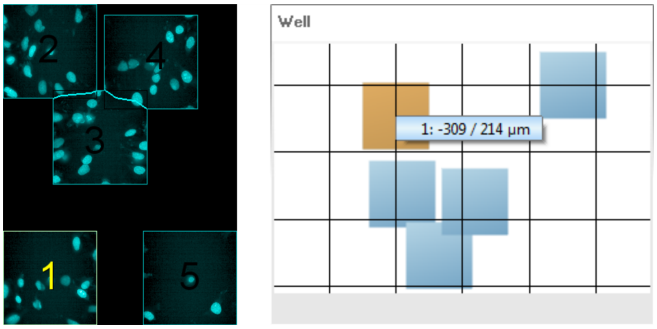
Field Map (yellow frame and number) and Field Partition (cyan borders)

   - The actual binning and other properties of the global image are displayed on the **Image Info** tab (**Image Analysis Results**).
3. If you are analyzing the ReScan of a PreciScan, you might need to define a **Min. Binning Value**. See also section 5.2.3.3 “Binning Adjustment of Global Image”, page 332.

5.2.3.2 *Compression / Field Packing of the Global Image*

In case of sparse coverage of the well with image fields (e.g. random rare events), a realistic montage image may contain a lot of empty regions. This would result in high binning and loss of information in the image fields of interest. Therefore, the fields of a global image are packed automatically. To find the best balance between the contradicting needs of keeping the topology (e.g. in a gridded sample) and highest possible resolution (dense packing) one of the following packing strategies is selected automatically:

1. **Unpacked:** A realistic montage image is generated (using original placement of fields).
2. **Squeezed:** All full horizontal and vertical empty ribbons are reduced to half an image field width. This very efficiently collapses grid structures while preserving rows and columns of fields.
   Squeezing is used in case it gives 10% reduction of image size compared to the realistic image.
3. **Packed:** Best arrangement of field in terms of resulting image size. Blocks of touching fields are preserved, including diagonally touching fields. This ensures that objects extending multiple fields are not separated in the montage image.
   Packing is used in case it gives 10% reduction compared to both other methods.



Packed global image with re-arranged fields (left) and original well layout (right)

To find out the former/realistic position of an image field:

- Look at the field numbers of the **Field Partition** overlay and locate the corresponding field in the well layout (see tooltip).
- In the other direction you can also click on a field in the well layout to highlight the corresponding field in the global image.

The absolute coordinates of detected objects in the result tables ("Position X [µm]" and "Position Y [µm]") are not affected by squeezing and packing.

5.2.3.3 *Binning Adjustment of Global Image*

The default mode for global image binning (“Dynamic”) selects an optimal binning value for the currently loaded well. A binning is chosen which still fits the image into the computer memory while preserving the highest possible level of details. The used binning value is displayed in the “Image Info” table of the global image.

- In case all wells are imaged identically (same well layout), **Dynamic** is optimal.
- In case PreciScan is used (e.g. to pick rare events on a plate), different wells of the ReScan might have a very different number of image fields and different well layouts. In this case the dynamic binning might produce binning values that vary significantly from well to well. This may introduce unwanted bias effects in the analysis. The best binning cannot be determined fully automatically any more. The scientist who designs the experiment needs to set up the binning characteristics according to the properties of the biological samples (e.g. minimal, typical and maximal sizes), features of interest and needed accuracy. In order to do this, a **Minimal Global Binning** value can be selected from a list of choices in a drop down list.

Suggested basic workflow for determination of Minimal Global Binning for a ReScan analysis

1. Pick a "worst case" well with very many fields, e.g. by visual inspection of the PreScan.
2. Set up the analysis sequence with **Dynamic** binning until it works fine.
3. Get **Binning** value from the **Image Info** table of the global image and choose a **Minimal Global Binning** value of at least as high as this value. A bit higher minimal global binning can be used to increase robustness.
4. If you now check the analysis on a well with only a few image fields, the global image binning is bound to the **Minimal Global Binning** value, the analysis is identical to the analysis of the well with more fields.
5. If the analysis encounters a well with more fields than your "worst case" well, a higher binning value than "Minimum Binning" is used to fit the image into the memory. The actually used binning values are reported together with the well results, so these wells can be easily identified in the follow-up data analysis.
6. Check if the higher binning has a systematic effect on the results. In case of large objects a small change of the binning, e.g. from 11 to 15, very likely has virtually no effect on the results.
7. In case there are bias effects seen or expected, the outlier wells with higher binning can be excluded during follow up analysis (discard wells with "Global Binning > x").

Analysis of Global Image with Building Blocks

The new global image channels created by the *Input Image* building block can be used like any other image channel in the building blocks.

Additions in case of global analysis

- If a global channel is selected as input in a building block, the image view shows the set of global channels in the **Channels** part of the image view controls. In the **Overlays** part an overlay **Field Partition** and **Current Field** are available to illustrate how the individual original images are related to this (global) image.
- In case a new population is created based on a global image (e.g. by an object detection building block), it is a "global" type population. Objects in the global population (e.g. cell colonies) can cross field borders.

Restrictions to the use of global images

- Global images and populations cannot be mixed with standard images and populations in one building block (except for ROI definition, see below). If a global image is selected, only global images and populations are available for other selections in this building block (and vice versa).

Using the Result of a Global Analysis as a ROI

Detailed analysis of objects in a global population (e.g. cell colonies) can be done by choosing one of the original measured (local) channels as input in a building block and the global population as a ROI. The result of the analysis is a standard population (in case of object or region detection).

Restrictions

Note that objects that cross field borders inside a ROI may need special attention. The situation is analogous to handling objects at the image border in a standard analysis with multiple fields.

General options for handling objects that cross field borders

- Discard those objects (equivalent to using "Select Population – Remove Border Objects" in standard analysis). Object count is lower than true count.
- No special treatment: Object parts in different fields are treated as separate objects (leads to double counting of those objects, corresponds to standard analysis)
- Use overlap of image fields and analyze an object only in the field that best covers it using *Select Population* with method **Select by Mask** (to avoid double counting). This allows you to use the **Field Partition** mask to select only objects which have their center within the selection mask or have more than 50 % overlap with this mask. For details see section 5.2.19.6 "Method: Select by Mask", page 424.

Reporting Results per ROI

- Results (e.g. a list of colonies or list of nuclei detected inside the colonies), need to be exported as **Object Results** in the *Define Results* building block.
- All information which is needed to allow further "per ROI" aggregation is included in the results.

The actual calculation (e.g. to determine the number of nuclei in a colony) has to be done in the follow up analysis, e.g. in Excel or Spotfire. Results cannot be aggregated within the analysis sequence.

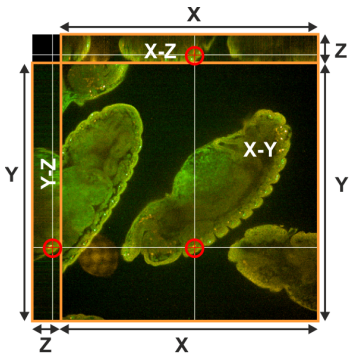
5.2.3.4 XYZ View

The **XYZ View** displays the (local) images of a stack measurement in a special viewer which allows to view sectional planes in x-, y- and z-direction. The XYZ view is calculated as soon as you select multiple stack planes and activate *Maximum Projection*.

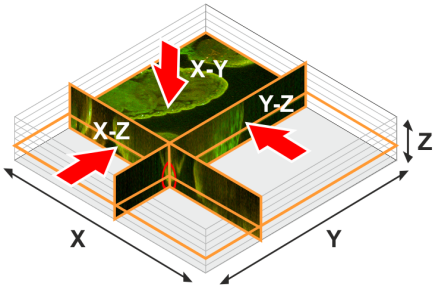
The view consists of three sections, one for each viewing direction:

- the large **X-Y** view in the middle (top view),
- a **X-Z** view at the top (front view) and
- a **Y-Z** view at the left (right side view).

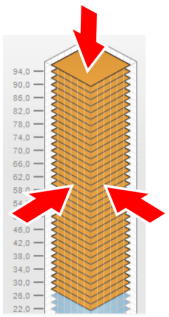
Crosshairs indicate the current position of the sectional planes in the sample. If you click on a position in one of the three sections, the other sectional planes will be updated accordingly.



XYZ View



Viewing directions in the sample


Stack Control

Notice

- Zooming and panning is not possible in the **XYZ View**.
- The **XYZ View** is not available for DPC channels.

How to create an XYZ view

1. Load a suitable stack measurement.
2. Select a single well and field in the **Navigation** pane.
3. Select multiple planes in the **Stack** pane.
4. If necessary, switch to the **Image Analysis** screen and expand the **Input Image** building block.
5. For **Stack Processing** select the option **Maximum Projection**.
   The XYZ view is calculated. It may take up to several minutes until the **XYZ View** tab appears.
6. Open the **XYZ View** tab in the **Content Area**.
   The crosshairs indicate the position of the sectional planes.
7. If you click on a position in one of the three sections of the view, the other sectional planes are updated accordingly.

5.2.4 Find Nuclei

This is a building block for detecting nuclei in a nuclei stained image.

5.2.4.1 Working Principle

- The task is to detect regions on the image belonging to cell nuclei.
- Each nucleus is detected as a region on the image having a higher intensity than its surrounding. The name of the output population of *Find Nuclei* can be selected in the box **Output** population.
- The first level inputs of *Find Nuclei* are **Channel**, **ROI** (Region of Interest), and **Method**.
- If a **global** channel is selected:
  - You have to use a ROI to restrict the analysis to measured parts of the image.
  - New ROI **Imaged Area (global)** is available (default).
  - New overlays **Field Partition** and **Current Field** are available for information.
  - For details see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.
- If Regions of Interest (**ROIs**) are selected, the detection is done inside each of the specified ROIs.
  - Results are reported per ROI, e.g. cell colony.
  - Parts of the image which are outside any ROI are ignored.
  - To select a set of ROIs specify any previously created population as **ROI Population**. Each object in the ROI population defines a separate ROI.
  - The ROI population is usually created by a preceding sequence of object detection and region selection building blocks.
  - If the ROI population objects contain multiple regions, use **ROI Region** to pick one of these regions as ROI.
  - If a local channel is used, the ROI input allows choosing local and global populations.
  - If using a local channel and a global ROI, the result is a local population. The “ROI No” column in the property table indicates to which global ROI the local object belongs.
- The set of second level inputs is specific to each method.

How to improve performance of Find Nuclei

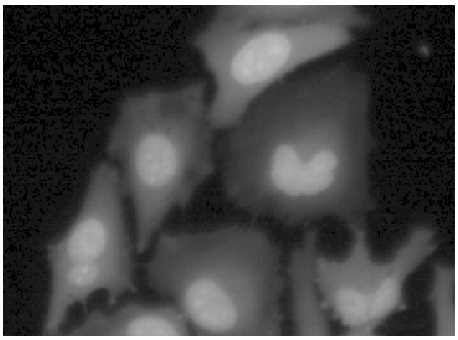
If errors of nuclei detection are observed then you should attempt to reduce them in the following steps:

1. Select **Channel**, i.e. check whether the most appropriate image channel for nuclei detection is selected.

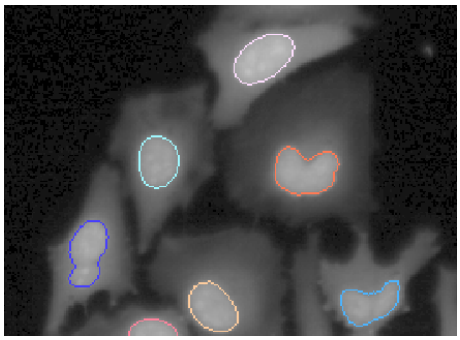
2. Test how different methods work at default values of the second and third level parameters.
3. Select the most promising method and fine-tune the second and third level parameters.
4. If errors of nuclei detection are still observed then select another method and fine-tune the second and third level parameters.

> **Notice**
> The set of input parameters is different for different methods. Even if names of parameters are the same, their optimal values may be different.

5.2.4.2 Example



Visual input: *A nuclei stained image*



Visual result: *Detected nuclei as overlay on the image*

5.2.4.3 Methods

Overview of methods of *Find Nuclei*:

Method	General robustness	High background	Low background	Intensity variations	Stuck nuclei	Calculation speed
A		+			+	+
B	++					+
C			+	+	+	+
M	++			+	++	+

Method A

+ More active in separating clump nuclei compared to method **B.**
+ Supports high background images.
– Higher rate of excessive (false-positive) borders within a single nucleus.

Second level parameters (similar to methods B and C)	
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to nuclei. • If dark objects that you think are not nuclei are still detected then **increase Common Threshold** to reduce that chance. • If some relatively dark nuclei are not detected then try to **decrease Common Threshold**.
Area	Parameter determining the **lower limit of the area** of a detected nucleus. If a detected nucleus has a lower area than this then the algorithm first attempts to connect it with a neighbor; if this is impossible then the small object will be removed from the population.

Third level parameters (similar to methods B and C)	
Split Factor	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. If splitting remains your basic concern then select method **M**.
Individual Threshold	Parameter determining the **intensity threshold** for each object individually. Thus, a brighter object has a higher absolute **intensity threshold** determining its border line, compared to a darker object.
Contrast	Parameter setting a **lower threshold to the contrast of detected nuclei**. If the contrast of a particular object is smaller than the threshold, such objects will be removed from the population. The **Contrast** of a particular object is defined as the difference of intensities inside and outside the object divided by the sum of the intensities.

Method B

+ Provides good results for typical nuclei stained images.
+ Supports high background images, e.g. when the cytoplasm is also stained.
+ Short calculation time.
– Weaker splitting of stuck nuclei compared to methods **A** or **M**.

Second level parameters (similar to methods A and C)	
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to nuclei. • If dark objects that you think are not nuclei are still detected then **increase Common Threshold** to reduce that chance. • If some relatively dark nuclei are not detected then try to **decrease Common Threshold**.
Area	Parameter determining the lower limit of the **area** of a detected nucleus. If a detected nucleus has a lower **area** than this then the algorithm first attempts to connect it with a neighbor; if this is impossible then the small object will be removed from the population.

Third level parameters (similar to methods A and C)	
Split Factor	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. If splitting remains your basic concern then select method **M**.
Individual Threshold	Parameter determining the **intensity threshold** for each object individually. Thus, a brighter object has a higher absolute **intensity threshold** determining its border line, compared to a darker object.
Contrast	Parameter setting a lower threshold to the contrast of detected nuclei. If the contrast of a particular object is smaller than the threshold, such objects will be removed from the population. The **Contrast** of a particular object is defined as the difference of intensities inside and outside the object divided by the sum of the intensities.

Method C

+ Provides good results for images with low background or with size variations of nuclei.
+ Supports images with large variations in intensity or contrast of nuclei.
– Bad splitting of stuck nuclei.
– Fails with high background images.

Second level parameters (similar to methods A and B)	
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to nuclei. • If dark objects that you think are not nuclei are still detected then **increase Common Threshold** to reduce that chance. • If some relatively dark nuclei are not detected then try to **decrease Common Threshold**.
Area	Parameter determining the lower limit of the **area** of a detected nucleus. If a detected nucleus has a lower **area** than this then the algorithm first attempts to connect it with a neighbor; if this is impossible then the small object will be removed from the population.

Third level parameters (similar to methods A and B)	
Split Factor	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. If splitting remains your basic concern then select method **M**.
Individual Threshold	Parameter determining the **intensity threshold** for each object individually. Thus, a brighter object has a higher absolute **intensity threshold** determining its border line, compared to a darker object.
Contrast	Parameter setting a lower threshold to the contrast of detected nuclei. If the contrast of a particular object is smaller than the threshold, such objects will be removed from the population. The **Contrast** of a particular object is defined as the difference of intensities inside and outside the object divided by the sum of the intensities.

Method M

+ Provides good results for typical nuclei stained images.
+ Better splitting of stuck nuclei compared to method **A**, **B** or **C.**
+ This is a rather universal method: by tuning a few input parameters, it can be applied to very different type of images.
– Not working well for various sized nuclei.

Second level parameters	
Diameter	Parameter determining the lower limit of the area of a detected nucleus. Furthermore, splitting algorithm works best if **Diameter** is in correspondence with reality.
Splitting Coefficient	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. **Splitting Coefficient** within the method **M** allows one to tune splitting reliably in a wide range. The value 0.0 corresponds to no splitting and the value 1.0 forces splitting into the smallest objects allowed by **Diameter**.

Second level parameters	
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to nuclei. If dark objects that you think are not nuclei are still detected then increase **Common Threshold** to reduce that chance. From the other side, if some relatively dark nuclei are not detected then try to decrease **Common Threshold**.

5.2.5 *Find Cytoplasm*

This is a building block for detecting cytoplasm around nuclei.

5.2.5.1 *Working Principle*

- The task is to detect regions around nuclei belonging to the cell cytoplasm.
- Nuclei must have been detected beforehand.
- Either nucleus- or cytoplasm-stained images can be used to detect cytoplasm.
- Each cytoplasm is detected as a region on the image having a higher intensity than the background.
- The first level inputs of *Find Cytoplasm* are **Channel**, **Nuclei** and **Method**. The input channel must be of the same scope (local/global) as selected nuclei population.
- For **global** populations:
  - Use of a **Restrictive Region** is mandatory (cannot be unchecked), default: “Imaged Area”.
  - Overlays **Field Partition** and **Current Field** are available.
- The set of second level inputs is specific to each method, but **Individual Threshold** is always among them.
- In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.
- **Outputs** of *Find Cytoplasm* are the regions **Cell**, **Membrane**, and **Cytoplasm**.
- If you select method **E**, a new population is created which is different from the input population (Nuclei), because more than one nucleus is allowed for a single cell. The desired name can be entered in the box **Output Population**.

How to improve performance of Find Cytoplasm

If errors of nuclei detection are observed, then attempt to reduce them in the following steps:

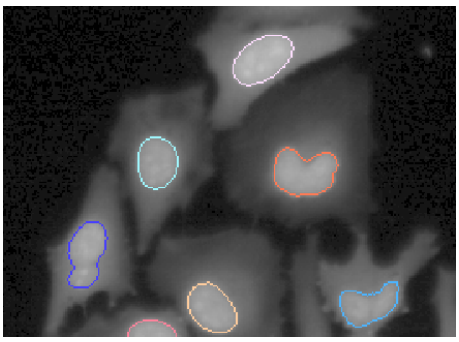
1. Select **Channel**, i.e. check whether the most appropriate image channel for cytoplasm detection is selected.
2. Select an appropriate method:

- If a cell may contain more than a single nucleus then try method **E** first.
- If an additional channel with membrane stain is available then try method **F** first.
- Otherwise, test how different methods work at default values of the second level parameters and select the most promising method.

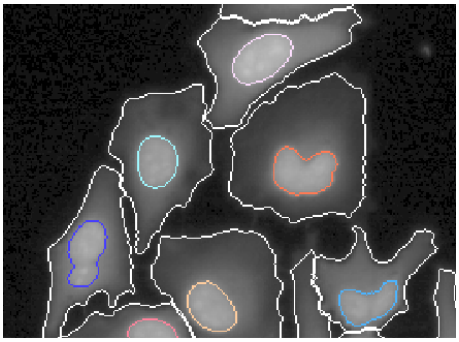
3. Fine-tune second level parameters.
   If errors of cytoplasm detection are still observed then select another method and fine-tune second level parameters.

> **Notice**
> The set of input parameters varies for different methods. Even if names of parameters are the same, their optimal values may be different

5.2.5.2 Example



Visual input: *A nuclei/cytoplasm stained image with nucleus overlay*



Visual result: *Detected cytoplasm as additional overlay on the image*

5.2.5.3 Methods

Overview of methods of *Find Cytoplasm*:

Method	Number of tuning parameters	Nuclei-stained image	Cytoplasm-stained image	Additional membrane channel	Multiple nuclei
A	1	+	–	–	–
B	2	+	–	–	–
C	2	–	+	–	–
D	1	+	–	–	–
E	2	–	+	–	+
F	1	(+)	(+)	+	–

Further details about individual methods:

Method A

\+ It is the most robust method of *Find Cytoplasm*

+ There is only one tuning parameter
+ It is most applicable in cases when the intensity decreases with the distance from nucleus
– Errors may occur with the detected cytoplasm spreading out to the background area

Tuning parameter (similar to methods D and F)	
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.

Method B

+ It is the recommended method in the case when method **A** yields errors with cytoplasm spreading out to the background area.
– There may appear other type of errors compared to method **A**, e.g. only a part of cytoplasm is detected.

Tuning parameters (similar to methods C and E)	
Common Threshold	Parameter determining the first guess borders of cytoplasm.
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.

Method C

+ This method is best suited in the case of cytoplasm-stained images when borders of neighbor cells are visible as intensity steps rather than intensity valleys.
– A typical error is detecting only a part of cytoplasm near nucleus.
– This method fails in cases of nucleus-stained images.

Tuning parameters (similar to methods B and E)	
Common Threshold	Parameter determining the best guess borders of cytoplasm.
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.

Method D

+ There is only one tuning parameter.
+ It is best applicable in cases when intensity decreases with the distance from nucleus.
– Errors may occur with the detected cytoplasm spreading out to the background area.

Tuning parameter	
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.

Method E

This method is similar to method **C**, but more than one nuclei per cell is allowed.

+ Applicable in cases with more than one nucleus per cell.
– This method fails in cases of nucleus-stained images.

Tuning parameters (similar to methods B and C)	
Common Threshold	Parameter determining the first guess borders of cytoplasm.
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.

Method F

Cytoplasm/nuclei stained image is the basic means for cytoplasm detection. The membrane stained image is used mainly in the step when the whole image is divided between cells. The method is otherwise similar to method **A**.

+ Applicable in the case when a membrane stained image is available, in addition to a cytoplasm/nuclei stained image.
+ There is only one tuning parameter.
– The membrane-stained image has only a minor influence on the detection results.

Tuning parameter (similar to method A)	
Membrane Channel	Allows you to select the membrane stained channel.
Individual Threshold	Parameter determining the intensity threshold for each object individually.

Tuning parameter (similar to method A)	
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.

5.2.5.4 Limitations

For speed reasons the cytoplasm region detection is limited to a distance of about 200 px from the nucleus. This should be no limitation for typical cells.

5.2.6 Find Spots

This is a building block for detecting spots and quantifying their properties.

5.2.6.1 Working Principle

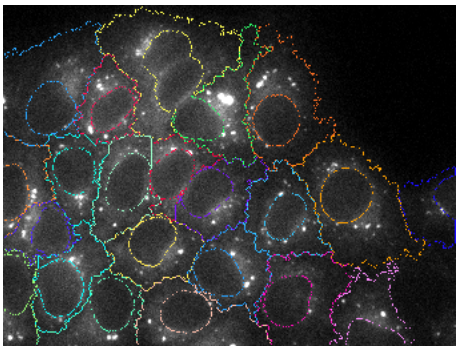
- The task is to detect spots and quantify their properties.
- Each spot is detected as a small region on the image having a higher intensity than its surrounding.
- Spots are searched in a specified regions of interest (**ROIs**).
  - The regions of interest are defined by the first level inputs **ROI Population** and **ROI Region**, e.g. the cytoplasm region of a cell population.
  - If no ROI is defined ("None"), spots are searched on the whole image.
- Additional first level input of *Find Spots* are **Channel** and **Method**. There are four different methods available.
  The set of second and third level inputs is specific to each method.
- Allowed spot population scope (local/global) depends on selected input channel
  - For global channels:
    - Use of a ROI is mandatory (default: “Imaged Area (global)”).
    - Only global populations can be used as ROI.
    - A “global” suffix is added to the default output name.
    - Overlays **Field Partition** and **Current Field** are available
  - For local channels:
    - ROI can be any existing local or global populations
    - “None” as a ROI to find spots in the full image area.
- The output is an **Output Population** and (optionally) properties of the detected spots.

How to achieve a wanted performance of Find Spots

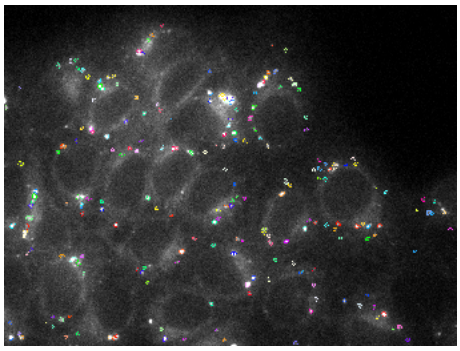
1. Select **Channel**, i.e. check whether the most appropriate image channel for spot detection is selected.
2. If your interest is concentrated at a certain region then select the appropriate region of interest (**ROI**), otherwise set ROI to **None**.
3. Select a **Method**. There are four different methods available (**A**, **B**, **C** and **D**).

4. Fine-tune the second and third level parameters using visual feedback from an image with visible spots of your interest.
5. Compare the results of a high control image and a low control image. They must be significantly different.
6. Repeat tuning with each method until you have found a satisfactory method and set of parameters.

5.2.6.2 Example



Input: *An image with borders of the specified Regions of Interest (ROI)*



Visual result: *Detected spots as overlay on the image*

5.2.6.3 Methods

Notice

- Do not expect that default values of input parameters are good enough when comparing different methods of spot detection. The situation here is different from that of detection of nuclei or cytoplasm, for which default parameters are usually good enough. The set of optimal parameters of spot detection depends on image data and user's preferences about what is wanted and what is not wanted to be detected.

Overview of methods of *Find Spots*:

Method	First basic step of the algorithm	Adaption to spot size	Number of tuning parameters	Number of calculated properties
A	Detection of local intensity maxima	+	2	8
B	Estimation of background image	+	2	8
C	Detection of local intensity maxima	–	5	8
D	Estimation of background image	+	3	8

Deprecated spot detection methods

In Harmony 4.6 the calculation of some automatically generated numerical spot properties has been updated. No special user attention is needed.

Background information

Results of old analysis sequences created with earlier Harmony versions do not

change. The spot detection method names in these sequences are displayed as "A (deprecated)" to "D (deprecated)" to indicate that a newer version is available.

In new analysis sequences only the updated methods "A" to "D" can be used, deprecated methods are not visible to the user. Compared to the deprecated versions the following values have changed (calculated according to the documentation now):

- Method A: Spot population property "Spot to Region Intensity"
- Method C: Spot population property "Corrected Spot Intensity"
- Method A, B, D: ROI population property "Relative Spot Intensity"

Note that the spot detection itself did not change.

Method A

Second level input parameters	
Relative Spot Intensity	Determines how intense a spot must be to be detected. **Relative Spot Intensity** is defined as the ratio of the "Uncorrected Spot Peak Intensity" to the "Region Intensity" (mean intensity of the cell region where the spot is searched, see sketch below for definition of intensity terms). Note that this value is different from the output property "Relative Spot Intensity".
Splitting Coefficient	Responsible for split-or-merge decisions of adjacent spots. • At the **lowest** value (0.0), detected regions if connected are considered a single spot. • At the **highest** value (1.0), there is a maximum number of split lines.

Third level input parameter	
Calculate Spot Properties	Determines if a set of pre-defined numerical spot properties is calculated (default: on).

Method B

Second level input parameters	
Detection Sensitivity	Determines how intense a spot must be to be detected. • At the **lowest** value (0.0), only extremely intense spots are detected. • At the **highest** value (1.0), extremely weak spots are also detected.
Splitting Coefficient	Responsible for split-or-merge decisions of adjacent spots. • At the **lowest** value (0.0), detected regions if connected are considered a single spot. • At the **highest** value (1.0), there is a maximum number of split lines.

Third level input parameter	
Calculate Spot Properties	Determines if a set of pre-defined numerical spot properties is calculated (default: on).

Method C

Second level input parameters	
Radius	Sets an upper threshold for spot radius; spots are not allowed to expand more than this.
Contrast	Sets a lower threshold for contrast. **Contrast** is defined as the difference to sum ratio of (i) the spot peak intensity and (ii) the intensity in its neighbourhood.
Uncorrected Spot To Region Intensity	Sets a lower threshold for spot to region intensity. **Uncorrected Spot to Region Intensity** is defined as the ratio of the spot peak intensity to the mean intensity of the cell region where the spot is searched.

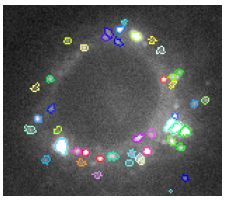
Third level input parameter	
Distance	Sets a lower threshold for the distance between maxima of two adjacent spots.
Spot Peak Radius	Radius of the disk over which the spot peak intensity is integrated. Zero returns the intensity of the brightest spot pixel as peak intensity.
Calculate Spot Properties	Determines if a set of pre-defined numerical spot properties is calculated (default: on).

Method D

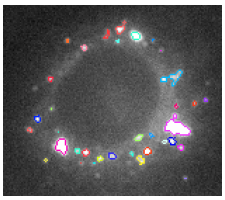
Second level input parameters	
Detection Sensitivity	Determines how intense a spot must be to be detected. • At the **lowest** value (0.0), only extremely intense spots are detected. • At the **highest** value (1.0), extremely weak spots are also detected.
Splitting Coefficient	Responsible for split-or-merge decisions of adjacent spots. • At the **lowest** value (0.0), detected regions if connected are considered a single spot. • At the **highest** value (1.0), there is a maximum number of split lines.

Third level input parameter	
Background Correction	Responsible for an adequate estimation and subtraction of the background. • At the **lowest** value (0.0), only very slow changes in background intensity are allowed. There is a danger that some of the natural intensity changes on images will be detected as spots. See example image with background correction set to “0.1”. • At the **highest** value (1.0), the background intensity is allowed to change very fast. There is a danger that some weak or big spots are considered as background and therefore missed. See example image with background correction set to “0.9”. • When tuning the **Background Correction** parameter, the background-corrected image can be monitored. The background-corrected image must be black everywhere except spots of our interest. At overcorrection, the weakest spots disappear and the biggest ones contract in size and lose a significant part of intensity. See example images.
Calculate Spot Properties	Determines if a set of pre-defined numerical spot properties is calculated (default: on).

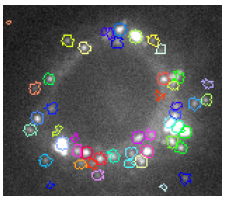
Illustrations of Methods and Tuning Parameters



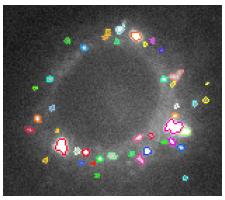
Method A
Default parameters



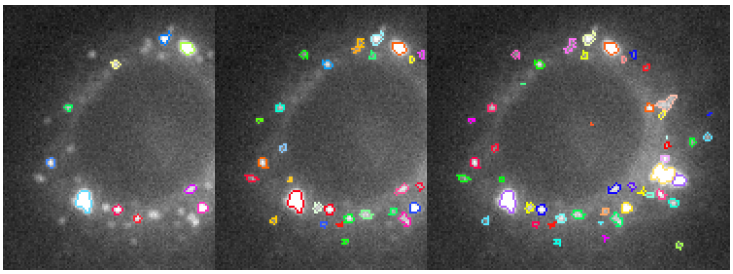
Method B
Default parameters



Method C
Default parameters



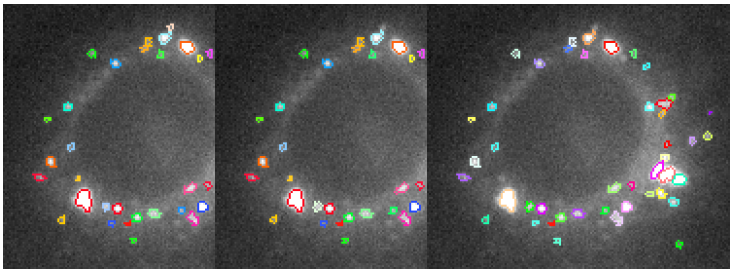
Method D
Default parameters



Method D
Detection Sensitivity = 0.2

Method D
Detection Sensitivity = 0.5 (default)

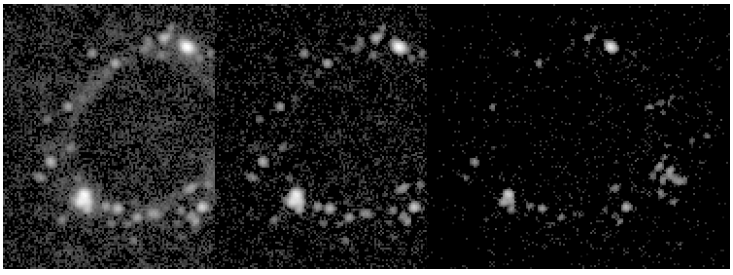
Method D
Detection Sensitivity = 1.0



Method D
Splitting Coefficient = 0.0

Method D
Splitting Coefficient = 0.5 (default)

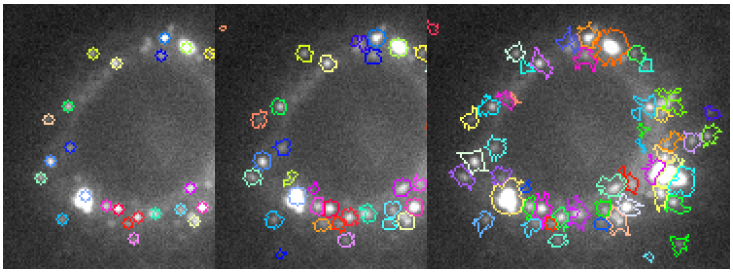
Method D
Splitting Coefficient = 1.0



Method D
Background-corrected image Background Correction = 0.1 (undercorrection)

Method D
Background-corrected image Background Correction = 0.5 (default)

Method D
Background-corrected image Background Correction = 0.9 (overcorrection)



Method C
Radius = 3

Method C
Radius = 5
(default)

Method C
Radius = 10

5.2.6.4 Outputs

Always

A new population of spots is created in addition to the input population.

- The name of the output population can be selected in the box **Output Population** (default name: "Spots").
- The population includes a region "Spot" marking the outlines of each individual spot.
- For Methods A and C an additional region "Spot Maximum" is included, marking the initial local intensity maximum around that the spot was detected.

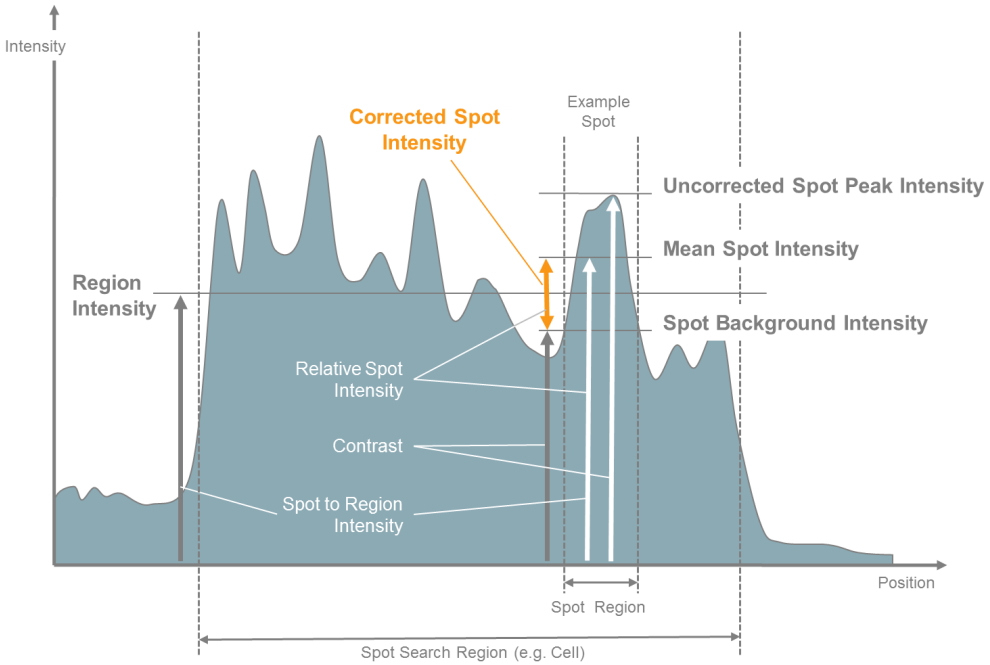
If a ROI is used:

- Each individual spot in the spots population gets a property **Object No in ...** (ROI population) referencing to the ROI region (e.g. cell) in which the spot was found.
- A new region "Spots" is added to the ROI population. In this region all spots in one ROI (e.g. cell) are combined into one region, i.e. all spots in one ROI are addressed as a single object.
- The spot population is related to the ROI population. *Calculate Properties* – Method "By Related Population" can be used to access properties of individual spots in ROI objects or vice versa.

When calculation of spot properties is activated – Properties added to the Spot Population

Each object (spot) in the spots population is characterized by the following properties (see sketch below for definition of intensity terms):

- **Relative Spot Intensity** which is the ratio of (i) the "Corrected Spot Intensity" and (ii) the "Mean Spot Intensity".
- **Corrected Spot Intensity** which is the "Mean Spot Intensity" minus "Spot Background Intensity".
- **Uncorrected Spot Peak Intensity** which is intensity of the most intense spot pixel. For Method C: intensity of the "Spot Maximum" pixel or if Spot Peak Radius ≥1 the average intensity in a disk around "Spot Maximum".
- **Spot Contrast** which is defined as (A-B)/(A+B) where A is the "Uncorrected Spot Peak Intensity" and B is the "Spot Background Intensity".
- **Spot Background Intensity** which is the mean intensity of the spot border. For Methods B and D: an estimate of the spot background intensity determined by a sliding parabola filter (see building block *Filter Image*, method "Sliding Parabola", described in section “Sliding Parabola”, page 431).
- **Spot Area** is the area of the spot region in pixel units.
- **Region Intensity** which is the mean intensity of the spot search region where the particular spot is located.
- **Spot To Region Intensity** which is defined as the "Mean Spot Intensity" divided by the "Region Intensity".



Sketch of pre-calculated spot properties:
Four basic intensities are calculated – two of them characterizing the spot intensity, one characterizing the local spot background and one characterizing the spot search region - denoted by white and gray vertical arrows. The "Corrected Spot Intensity" (orange font) is most relevant for many applications. In addition, three intensity relations are calculated, which are independent of the absolute intensity of the image (small white font).

When calculation of spot properties is activated – Properties added to the ROI Population

Each object of the ROI population (e.g. cell) is additionally characterized by the following properties:

- **Total Spot Area** which is the total area of all spots detected in the object (e.g. cell) in pixel units.
- **Relative Spot Intensity** which is defined as the ratio of (i) integrated signal from all pixels of all spots, the signal being intensity within the spot minus the local "Spot Background Intensity"; (ii) intensity integrated over the "Spot Search Region". This quantity corresponds to the fraction of dye aggregated in spots compared to total amount of dye in the cell. Note that this property is different from the "Relative Spot Intensity" property in the spot output population (see above).
- **Number of Spots in each ROI object** (e.g. cell).
- **Number of Spots per Area of ...** (ROI Region name) which is the number of detected spots divided by the area of the ROI object (e.g. cell).

5.2.6.5 References

For further information on *Find Spots* please refer to the following sections of the Image Analysis Guide (6th Edition):

- Section 1.17: "Various sized Spots – Spot Co-localization"
- Section 2.8: Ready Made Solution "Spot Analysis"
- Section 2.18: Ready Made Solution "Nuclear Spots"
- Section 2.24: Ready Made Solution "Lipid Droplet Analysis – Various sized Spots"

5.2.7 Find Cells

This is a building block for detecting cells, either in a whole cell stained image or in a digital phase image. Technically, methods **A**, **B**, **C**, and **M** are identical to those in the *Find Nuclei* building block except for default values of some tuning parameters.

5.2.7.1 Working Principle

- The task is to detect regions on the image that correspond to cells.
- Each cell is detected as a region on the image that has a higher intensity than its surrounding.
- The first level inputs of *Find Cells* are **Channel** , **ROI**, and **Method**.
  The set of second level inputs is specific to each method.
- The name of the output population of *Find Cells* can be selected in the box **Output population**.
- If a **global** channel is selected:
  - You have to use a ROI to restrict the analysis to measured parts of the image.
  - New ROI **Imaged Area (global)** is available (default).
  - New overlays **Field Partition** and **Current Field** are available for information.
  - For details see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.
- If Regions of Interest (**ROIs**) are selected, the detection is done inside each of the specified ROIs.
  - Results are reported per ROI, e.g. cell colony.
  - Parts of the image which are outside any ROI are ignored.
  - To select a set of ROIs specify any previously created population as **ROI Population**. Each object in the ROI population defines a separate ROI.
  - The ROI population is usually created by a preceding sequence of object detection and region selection building blocks.
  - If the ROI population objects contain multiple regions, use **ROI Region** to pick one of these regions as ROI.
  - If a local channel is used, the ROI input allows choosing local and global populations.
  - If using a local channel and a global ROI, the result is a local population. The “ROI No” column in the property table indicates to which global ROI the local object belongs.

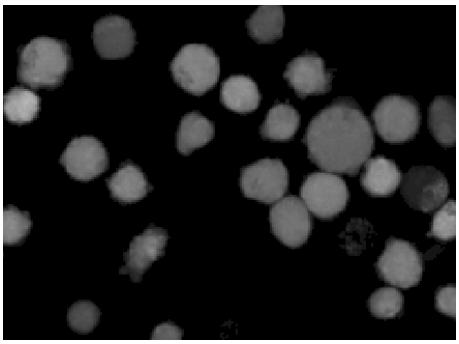
How to improve performance of Find Cells

If errors of cell detection are observed you should attempt to reduce them in the following steps:

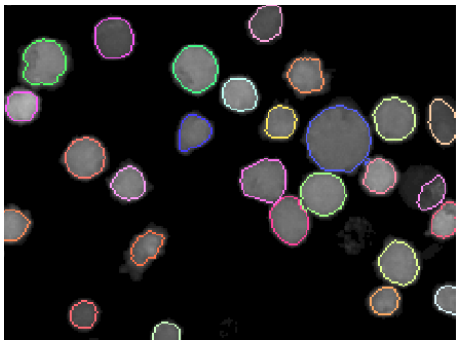
1. Select **Channel**, i.e. check whether the most appropriate image channel for cell detection is selected.
2. Test how different methods work at default values of the second and third level parameters.
3. Select the most promising method and fine-tune the second and third level parameters.
4. If errors of cell detection are still observed then select another method and fine-tune the second and third level parameters.

> **Notice**
> The set of input parameters is different for different methods. Even if names of parameters are the same, their optimal values may be different.

5.2.7.2 Example



Visual input: *A whole cell stained image*



Visual result: *Detected cells as overlay on the image*

5.2.7.3 Methods

Overview of methods of *Find Cells*:

	Designed for channel	General robustness	High backgr.	Low backgr.	Intensity variations	Stuck cells	Calc. speed
A	Fluorescence		+			+	+
B	Fluorescence	++					+
C	Fluorescence			+	+	+	+
M	Fluorescence	++			+	++	
P	DPC				+	++	

Method A

+ More active in separating clump cells compared to method **B**
+ Supports high background images
– There is a danger that excessive (false-positive) borders are created within a single cell.

Second level parameters (similar to methods B and C)	
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to cells. • If dark objects that you think are not cells are still detected then **increase Common Threshold** to reduce that chance. • If some relatively dark cells are not detected then try to **decrease Common Threshold**.
Area	Parameter determining the **lower limit of the area** of a detected cell. If a detected cell exceeds this lower limit, then the algorithm first attempts to connect it with a neighbor; if this is impossible then the small object will be removed from the population.

Third level parameters (similar to methods B and C)	
Split Factor	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. **Split Factor** can be selected in the range 1..100. The lowest value corresponds to maximum splitting, the highest value corresponds to minimum splitting.If splitting remains your basic concern then try method **M**.
Individual Threshold	Parameter determining the **intensity threshold** for each object individually. Thus, a brighter object has a higher absolute intensity threshold determining its border line, compared to a darker object.
Contrast	Parameter setting a lower threshold to the contrast of detected cell. If contrast of a particular object is smaller than the threshold, such object will be removed from the population. The **Contrast** of a particular object is defined as the difference of intensities inside and outside the object divided by the sum of the intensities.

Method B

+ Provides good results for typical cell stained images
+ Supports high background images
+ Short calculation time
– Weaker splitting of stuck cells compared to methods **A** or **M**.

Second level parameters (similar to methods A and C)	
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to cell. • If dark objects that you think are not cells are still detected then **increase Common Threshold** to reduce that chance. • If some relatively dark cells are not detected then try to **decrease Common Threshold**.
Area	Parameter determining the lower limit of the **Area** of a detected cell. If a detected cell has a lower **Area** than this then the algorithm first attempts to connect it with a neighbor; if this is impossible then the small object will be removed from the population.

Third level parameters (similar to methods A and C)	
Split Factor	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. **Split Factor** can be selected in the range 1..100. The lowest value corresponds to maximum splitting, the highest value corresponds to minimum splitting.If splitting remains your basic concern then try method **M**.
Individual Threshold	Parameter determining the **intensity threshold** for each object individually. Thus, a brighter object has a higher absolute **intensity threshold** determining its border line, compared to a darker object.

Third level parameters (similar to methods A and C)	
Contrast	Parameter setting a lower threshold to the contrast of detected cells. If the contrast of a particular object is smaller than the threshold, such object will be removed from the population. The **Contrast** of a particular object is defined as the difference of intensities inside and outside the object divided by the sum of the intensities.

Method C

+ Provides good results for images with low background or with size variations of cells
+ Supports images with large variations in intensity or contrast of cells
– Bad splitting of stuck cells
– Fails with high background images

Second level parameters (similar to methods A and B)	
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to cells. • If dark objects that you think are not cells are still detected then **increase Common Threshold** to reduce that chance. • If some relatively dark cells are not detected then try to **decrease Common Threshold**.
Area	Parameter determining the lower limit of the **Area** of a detected cell. If a detected cell has a lower **area** than this then the algorithm first attempts to connect it with a neighbor; if this is impossible then the small object will be removed from the population.

Third level parameters (similar to methods A and B)	
Split Factor	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. **Split Factor** can be selected in the range 1..100. The lowest value corresponds to maximum splitting, the highest value corresponds to minimum splitting.If splitting remains your basic concern then try method **M**.
Individual Threshold	Parameter determining the **intensity threshold** for each object individually. Thus, a brighter object has a higher absolute **intensity threshold** determining its border line, compared to a darker object.
Contrast	Parameter setting a lower threshold to the contrast of detected cells. If the contrast of a particular object is smaller than the threshold, such object will be removed from the population. The **Contrast** of a particular object is defined as the difference of intensities inside and outside the object divided by the sum of the intensities.

Method M

+ Provides good results for typical cell stained images
+ Better splitting of stuck cells compared to method **A**, **B** or **C**

+ This is a rather universal method: by tuning a few input parameters, it can be applied to very different type of images.

– Relatively long calculation time

Second level parameters	
Diameter	Parameter determining the lower limit of the area of a detected cell. Furthermore, splitting algorithm works best if **Diameter** is in correspondence with reality.
Splitting Coefficient	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. **Splitting Coefficient** within the method **M** allows to tune splitting reliably in a wide range. The value 0.0 corresponds to no splitting and the value 1.0 forces splitting into the smallest objects allowed by **Diameter**.
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to cells. • If dark objects that you think are not cells are still detected then increase **Common Threshold** to reduce that chance. • From the other side, if some relatively dark cells are not detected then try to decrease **Common Threshold**.

Method P

+ Optimized for Digital Phase Contrast (DPC) images

+ Good splitting of stuck cells

Second level parameters	
Area	Parameter determining the lower limit of the **Area** (in pixels) of a detected cell. If a detected cell has a lower **area** than this, the algorithm first attempts to connect it with a neighbor; if this is impossible then the small object will be removed from the population. The splitting algorithm works best if **Area** is close to the real size of the objects.
Splitting Coefficient	Parameter influencing the decision of the computer whether a large object is split into two or more smaller objects or not. **Splitting Coefficient** within the method **P** allows to tune splitting reliably in a wide range. The value 0.0 corresponds to no splitting and the value 1.0 forces splitting into the smallest objects allowed by **Area**.
Common Threshold	Parameter determining the lower level of pixel intensity for the whole image that may belong to cells. • If dark objects that you think are not cells are still detected then increase **Common Threshold** to reduce that chance. • From the other side, if some relatively dark cells are not detected then try to decrease **Common Threshold**.

5.2.8 Find Micronuclei

This is a building block for detecting micronuclei.

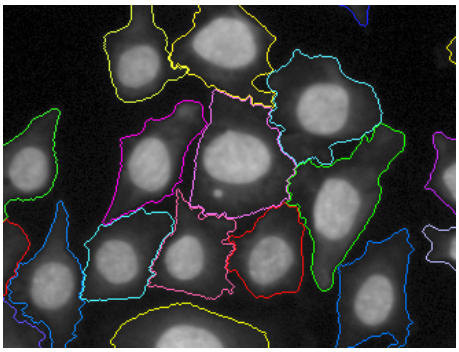
5.2.8.1 Working Principle

- The task is to detect micronuclei and quantify their properties.
- Each micronucleus is detected as a small region on the image having a higher intensity than its surrounding.
- Micronuclei can be searched in a specified region.
- The first level inputs of *Find Micronuclei* are **Channel**, **Population**, **Cell Region**, and **Method**. Only local channels can be selected as input.
- There are two methods available (**A** and **B**). The set of second level input parameters is specific to each method.

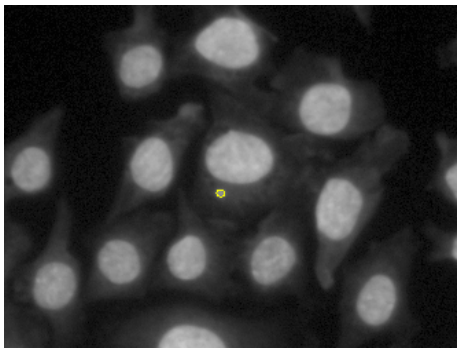
How to configure *Find Micronuclei*

1. First of all, make sure that the previous building blocks of the sequence (in particular, *Find Nuclei* and *Find Cytoplasm*) are well tuned.
   Errors in detecting nuclei or cytoplasm affect the accuracy of detecting micronuclei.
   Especially make sure that micronuclei that are very close to the nucleus are not detected as part of the nucleus, e.g.
   - In the *Find Nuclei* building block prefer method **A** in contrast to B (which tends to join nuclei and very close objects to one object).
   - Prefer an **Individual Threshold** value in *Find Nuclei* that is higher than the default value of 0.4, to “tighten” the detected nucleus border. E.g. use a value of 0.6.
2. Select a **Channel**, i.e. check whether the most appropriate image channel for micronuclei detection is selected.
3. Select a **Population** - if there is more than a single population then check that the most appropriate one is selected.
4. If your interest is concentrated at a certain cell region then select the appropriate **Cell Region**.
5. Select a **Method** (**A** or **B**).
6. Fine-tune second and third level parameters using visual feedback from an image with visible spots of your interest.
7. Select the other **Method** and fine-tune input parameters. Compare the results of the two methods and select the one with best results.

5.2.8.2 Example



Input: *An image with borders of the search region*



Visual result: *Detected micronuclei as overlay on the image*

5.2.8.3 Methods

Method A

Second level input parameters	
Micronucleus to Cytoplasm Intensity >	Determines how intense a spot must be to be detected. The default is 0.2.

Third level input parameters	
Calculate Micronuclei Properties	• If unchecked, micronuclei are only detected and counted. • If checked, micronuclei properties are also calculated.
Distance unit	Allows you to define the measuring unit of the resulting distances.

Method B

Second level input parameters	
Detection Sensitivity	Determines how intense a spot must be to be detected. • At the lowest value (0.0), only extremely intense micronuclei are detected. • At the highest value (1.0), extremely weak objects are detected as well.
Splitting Coefficient	Responsible for split-or-merge decisions. This parameters matters in rare cases when micronuclei and nuclei are very close to each other.

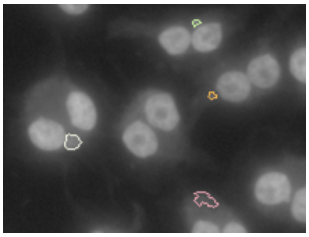
Third level input parameters	
Background Correction	Responsible for determining the background image which will be subtracted from the original image before object detection.
Contrast >	Threshold parameter determining how clear the border of a potential micronucleus must be in order to be accepted.
Typical Micronucleus Diameter	Measure of a typical micronucleus diameter. It can be presented either in µm or px units. We recommend to measure this value from an image, rather than to tune this parameter by performance.
Micronucleus Diameter <	Measure of the upper limit of micronucleus diameter. Detected objects larger than this will be considered as nuclei. The value can be input either in µm or px units.
Typical Nucleus Diameter	Measure of a typical nucleus diameter. It can be input either in µm or px units. We recommend to measure this value from an image, rather than to tune this parameter by performance.
Calculate Micronuclei Properties	• If unchecked, micronuclei are only detected and counted. • If checked, micronuclei properties are also calculated.
Distance unit	Allows you to define the measuring unit of the resulting distances.

5.2.8.4 Illustration of Tuning Parameters

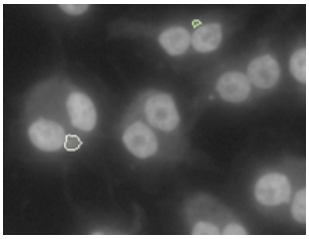
The important point at this step of the analysis is that **no micronuclei are missed**. Artifacts can be filtered out in the following steps, but missed micronuclei cannot be restored. Filtering is usually done by a following *Select Population* building block. That means for tuning that e.g. the sensitivity has to be set high enough to detect all micronuclei. On the other hand, the sensitivity should be as low as possible to limit the number of detected artifacts.

Method A

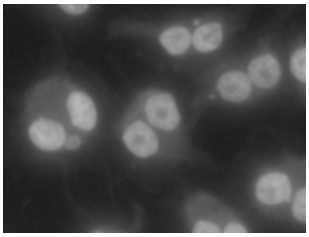
Tuning of parameter Micronucleus to Cytoplasm Intensity



Micronucleus to Cytoplasm Intensity = 0.05



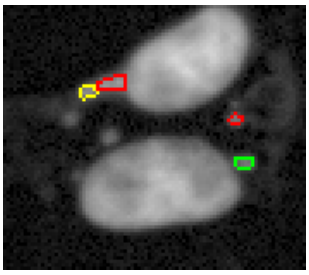
Micronucleus to Cytoplasm Intensity = 0.1



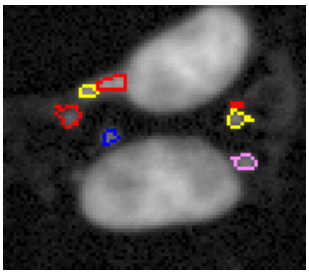
Micronucleus to Cytoplasm Intensity = 0.2

Method B

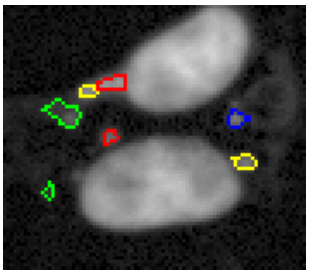
Tuning of parameter Detection Sensitivity



Detection Sensitivity = 0.2
Obvious micronuclei are not detected

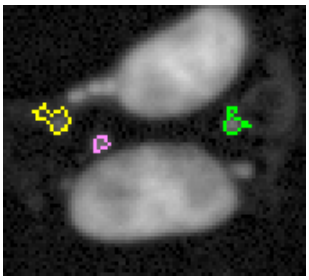


Detection Sensitivity = 0.5
Nearly wanted performance

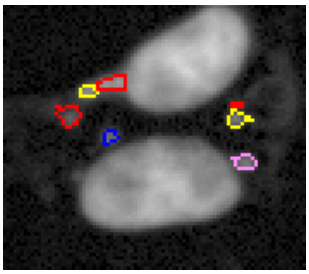


Detection Sensitivity = 0.8
Too weak spots are detected

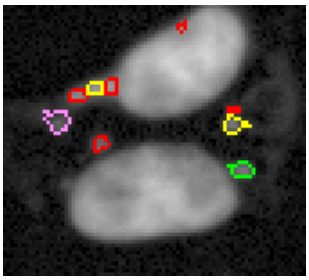
Tuning of parameter Splitting Coefficient



Splitting Coefficient = 0.2
Undersplitting: objects touching each other tend to be connected



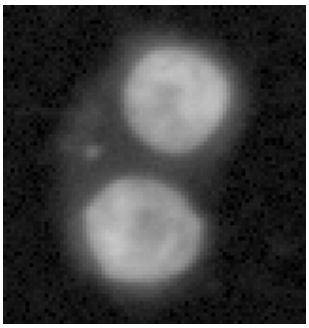
Splitting Coefficient = 0.5
Nearly wanted performance



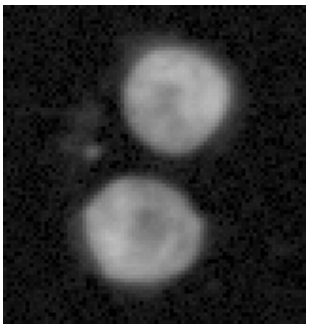
Splitting Coefficient = 0.8
Oversplitting: even a part of a nucleus is considered as micronucleus

Tuning of parameter Background Correction

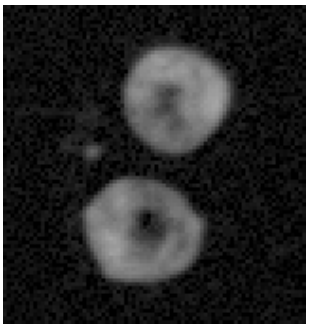
In order to see background-corrected image, enter tuning mode and select the illustration tab **Background Correction**.



Background Correction = 0.2
Undercorrection: Cytoplasm is visible

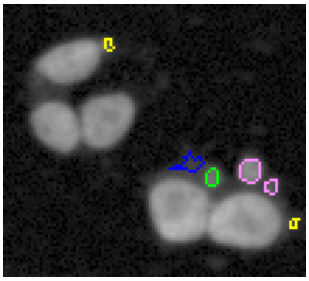


Background Correction = 0.5
Nearly wanted performance

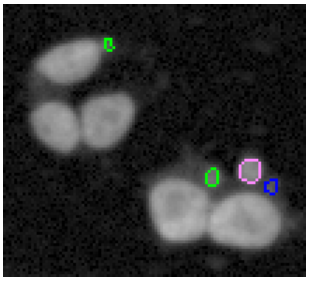


Background Correction = 0.8
Overcorrection: Holes are created in the middle of nuclei

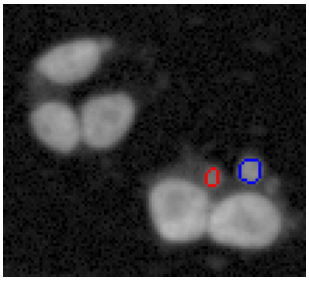
Tuning of parameter Contrast >



Contrast > 0.2
Some ghost (hazy) objects are detected



Contrast > 0.5
Nearly wanted performance



Contrast > 0.8
Some micronuclei are missed

5.2.8.5 Outputs

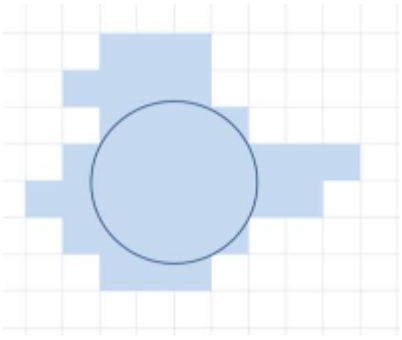
Output Population

- A new population including all detected micronuclei is created. The name can be selected in the box **Output Population**.
- An additional property is added to the input population, the **Number of Micronuclei** detected in each object (cell) in the population.
- An additional region **Nucleus modified** is added to the input population.

Micronuclei Properties (optional)

The following properties are only calculated if you have activated third-level parameter **Calculate Micronuclei Properties**. Each micronucleus is characterized by the following properties:

- **Fraction of Nucleus Intensity** which is the ratio of the micronucleus to nucleus mean intensities.
- *With Method A:* **Relative Intensity to Border** which is the mean intensity above the mean border intensity divided by mean border intensity.
- *With Method B:* **Relative Intensity to Background** which is the mean intensity above the mean background divided by the mean background intensity.
- **Distance from Nucleus** [px or µm] which is distance from the micronucleus center to the nucleus border.
- **Gap to Nucleus** [px or µm] which is minimal distance between border pixels of the micronucleus and the nucleus.
- **Area of Micronucleus** [px² or µm²]
- **Fraction of Nucleus Area** which is the ratio of the micronucleus area to the nucleus area.
- **Compactness** which is area of the maximum size disk fitting into the micronucleus divided by the area of the whole micronucleus. See sketch below.



Please note, that the compactness (as well as roundness) calculation of very small objects (only a few pixels in diameter) usually shows high noise due to pixel quantization effects.

- **Object No in input population** identifies the object (cell) in which the micronucleus is located.

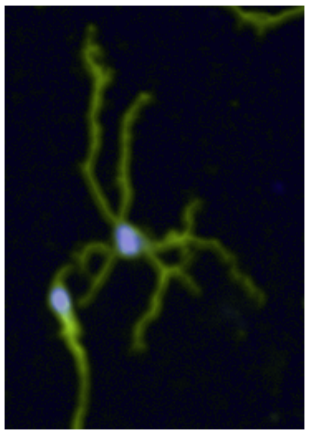
5.2.9 Find Neurites

This building block identifies neurites growing from a cell body. It can also be used to detect any kind of dendritic structures growing from a region of interest.

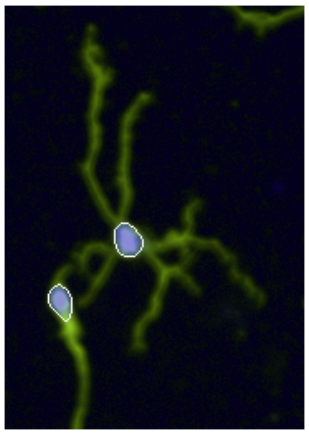
Notice

An additional software license is needed to use this building block. The license needs to be activated on your Harmony dongle. See also section "License Management", page 320.

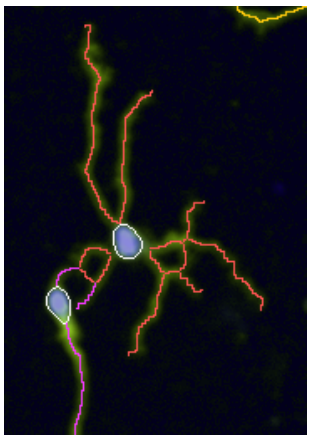
5.2.9.1 Example



Input: *Color overlay of a nuclear stain image (blue) and neurite stain image (green)*



Preparation for neurite detection: *Detected outlines of the cell body (white line) based on the nuclear stain channel. Starting point for neurite detection.*



Visual result: *Detected Neurites based on the neurite stain image (color overlay on input images)*

5.2.9.2 *Working Principle*

- Neurites are detected on the neurite stain image by its higher intensity than the surrounding.
- The first level inputs of *Find Neurites* are **Channel**, **Population**, **Region**, and **Method**.
  - **Channel** specifies the neurite stain image.
  - **Population** specifies the population of individual cell bodies to be used a starting point for neurites.
  - **Region** specifies the cell body region of each object of the population.
- If using **global** images:
  - If a global channel is selected only a global population can be selected.
  - If a local channel is selected only a local population can be selected
  - A “global” suffix is added to the default output name
- Only one method is available: **CSIRO Neurite Analysis 2**. The outdated method **CSIRO Neurite Analysis** is not supported anymore. This method was available on 32-bit operating systems for backward compatibility.
- The name of the resulting population can be entered in the **Output Population** box.
- The neurite analysis consists of two steps:
  - Neurite detection: The neurites are detected on the neurite channel image.
  - Neurite tree analysis: The neurites are split into segments, assigned to individual cells and quantified. The neurite tree analysis part of both methods is identical.

How to set up a neurite analysis

1. Select the **Channel**, i.e. check whether the most appropriate image channel for neurite detection is selected.
2. Select a **Population** and a **Region** as a starting point for finding neurites. Region and population must have been identified by other building blocks before.
3. Run the *Find Neurites* analysis on a typical image you want to analyze and check if the neurites of interest are correctly identified.
4. The default parameters provide sufficient results in most cases. In case you need to adjust the analysis to your images:
   - Identify the most obvious deviation of the analysis with your desired results, e.g. dim neurites are not detected.
   - Select an appropriate tuning parameter by studying the descriptions in the tuning parameter section below.
   - Use the interactive tuning dialog for the corresponding tuning parameter to correct the analysis. See also section “How to tune a parameter (numerical parameter)”, page 143.
5. Try the analysis on different images for validation.

Notice

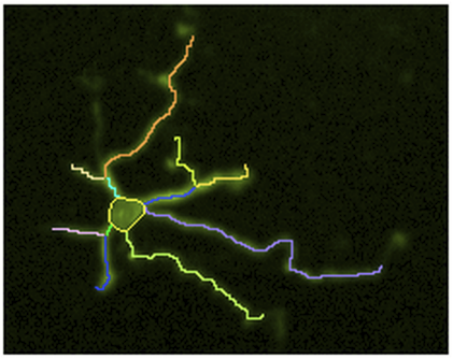
Do not adjust parameters too perfectly for one selected image; then most likely other images will fail. In case you do not see a significant benefit of adjusting a specific tuning parameter keep the default value.

6. If you have positive and negative control images, validate that the analysis works well on both types of images.
7. Identify the properties best describing your desired effect to quantify. You may either use one of the pre calculated properties (see method description) or use the *Calculate Intensity* or *Calculate Morphology* building blocks to calculate your own properties using the “Neurites” region, e.g. total length of all neurites emerging from a cell.

5.2.9.3 *Description of Results*

A default set of output values is calculated to quantify the neurite tree for each individual cell:

- **Maximum Neurite Length** - length of the longest neurite attached to the cell body
- **Number of Extremities** - see figure below
- **Number of Roots** - see figure below
- **Number of Segments** - see figure below
- **Number of Nodes type 1** - see figure below
- **Number of Nodes type 2** - corresponds to “Number of Segments” divided by “Number of Roots"
- **Total Neurite Length** - sum of the length of all neurites attached to the cell



*Break down of the detected neurite tree to individual segments (marked by a different colors) defining the property **Number of Segments**.*

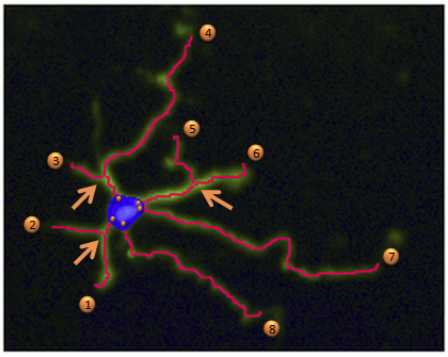


*Illustration of **Number of Extremities** corresponding to the number of ends of individual neurites (marked by numbered orange circles); Illustration of **Number of Roots** (marked by small orange circles inside the cell body) and **Number of Nodes type 1** marked by arrows.*

A new **Region** “Neurite Tree” is added to the population of cell bodies. It can be used …

- as an overlay on images to visualize the detected neurites.
- as a region of interest for further building blocks, e.g. to search spot-like features inside the neurite region.

A new **Population** “Neurite Segments” is created, which can be further processed in the same way like e.g. spots found in a region (available for method “CSIRO Neurite Analysis 2” only). The population of neurite segments can be …

- filtered by their properties
- associated intensity properties can be calculated
- associated morphology properties can be calculated
- associated texture properties can be calculated

5.2.9.4 Methods

Method: CSIRO Neurite Analysis 2

This is the default method. The analysis is done by an algorithm developed by the Australian CSIRO research institute (http://www.csiro.au).

Step by Step Parameter adjustment

For parameter adjustment, please walk step by step (top down) through the list of parameters and correct if needed.

Neurite Detection:

1. **Smoothing (Gaussian Filtering):** Select the level of Gaussian Blur by tuning parameter “Smoothing Width”. This filter helps to spread intensity.
2. **Linear Feature Detection:** The neurites are detected in this step. Tuning parameters are “Linear Window” and “Contrast”.
3. **Remove Small Objects:** Small objects that are not neurites can be removed in this step. Tuning parameter is “Diameter”.
4. **Gap Closing:** Gaps between detected neurites can be closed in this step. Tuning parameters are “Gap Closure Distance” and “Gap Closure Quality”.

Neurite Tree Analysis:

1. **Debarb Small Neurite Branches:** Parameter “Debarb Length”.
2. **Thicken Neuron Bodies:** Parameter “Body Thickening”.
3. **Remove Small Trees:** Parameter “Tree Length”.

Tuning Parameters

Second level parameters	
Smoothing Width	Smoothing is useful to suppress noise and obtain a single intensity maximum across neurites. Filtering should not modify the global appearance of cells significantly. Make sure that neurites are not suppressed with higher values.
Linear Window	The linear window size specifies the dimension in pixels of the window used to find local intensity maxima. The larger the window, the fewer the number of features and the slower the computations. Larger windows help to suppress spurious features. The contrast parameter specifies how prominent a pixel has to be in relation to its local neighborhood. Make sure all neurites are detected. Choose the linear window size that produces optimum results.
Contrast	Adjust the contrast to increase or decrease sensitivity. Reduce contrast if not all neurites are detected, but avoid producing excessive background noise.

Second level parameters	
Diameter	Objects with small diameter can be removed here, as they mostly correspond to spurious features. Make sure that actual neurites are not removed.
Gap Closure Distance/Quality	The extremities of linear features can be connected to other linear features lying within a range to be chosen here. False connections may be made if the distance is too high. • The “Quality” parameter defines the minimum average intensity along a gap in order to fill it (measured in percent of average intensity of the connected endpoints). • If set to zero, endpoints are connected by a straight line. • Increasing the Quality will reduce the likelihood of false connections appearing.
Debarb Length	Debarbing is the process of removing small lateral branches of the neurite trees to clean them. Small branches that may be due to noise. • Use the neurites overlay on the neurite stain image to check how much debarbing is required. • Lower the Length value if actual neurite branches are removed in this step.
Body Thickening	Thickening the neurite bodies ensures that the neurites are connected to their mother cell. Be aware that cell bodies should not be so thick as to cover smaller neurites.
Tree Length	Neurites smaller than a few pixels in length are most likely the result of noise and can be removed here. If noise is not present, set Length to '0' to bypass this step.

5.2.10 Find Image Region

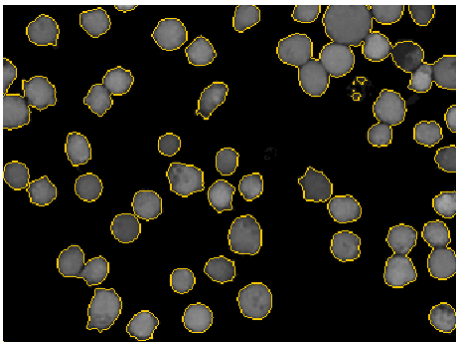
Find Image Region allows you to define regions on the image comprising parts of the image or the whole image.

5.2.10.1 Working Principle

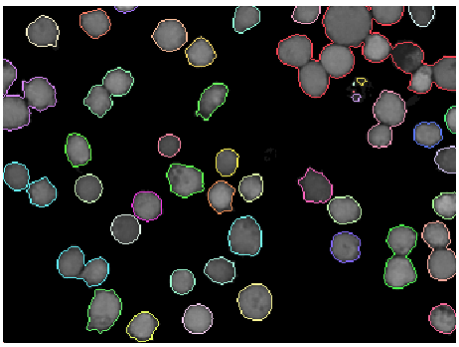
- The *Find Image Region* building block creates a single region or a set of regions of the input image, according to one of the following three options:
  - Option 1: Region includes the set of all pixels with intensity higher than a threshold
    (select method **Common Threshold**).
  - Option 2: Regions includes all pixels with intensity higher than a threshold, but spatially distinct regions are separate objects
    (select method **Common Threshold** and enable switch **Split into Objects**).
  - Option 3: Region includes the whole image
    (select method **Whole Image Region**).
- The first level inputs of *Find Image Region* are **Channel** , **ROI** (Region of Interest), and **Method**. The input channel can be either local or global.

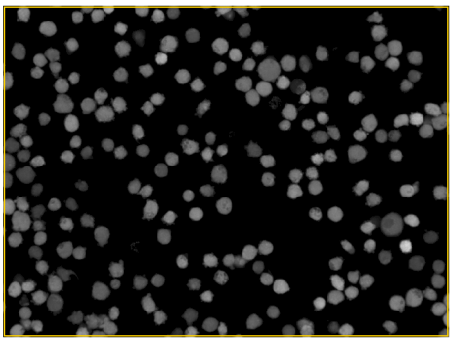
- In case of a global input channel:
  - The use of a ROI is mandatory (default: “Imaged Area (global)”)
  - New overlays **Field Partition** and **Current Field** are available to see relations.
- If Regions of Interest (**ROIs**) are selected, the detection is done inside each of the specified ROIs.
  - Results are reported per ROI, e.g. cell colony.
  - Parts of the image which are outside any ROI are ignored.
  - To select a set of ROIs specify any previously created population as **ROI Population**. Each object in the ROI population defines a separate ROI.
  - The ROI population is usually created by a preceding sequence of object detection and region selection building blocks.
  - If the ROI population objects contain multiple regions, use **ROI Region** to pick one of these regions as ROI.
- The name of the output population can be selected in the box **Output Population**.
- The name of the output region can be selected in the box **Output Region**.

5.2.10.2 Example



Option 1: *The region includes all pixels with intensity higher than a threshold. The orange line is the border of the created region.*





Option 2: *The regions consist of all pixels with intensity higher than a threshold. Spatially distinct regions are separate objects, indicated by different colors.*

Option 3: *The region includes the whole image. The orange line around the whole image is the border of the created region.*

5.2.10.3 Methods

There are two methods available:

Common Threshold

Creates a region or a set of regions covering all pixels of the image with intensity higher than a threshold.

Exemplary applications for Common Threshold

- Preparatory step when e.g. morphology properties of the image are requested.
- Preparatory step for a fast and robust estimation of cell confluency.
- Can be used to find a bright well region, excluding dark well borders.

Second level parameters	
Threshold	Arbitrary correction factor ranging from 0…1. It allows to influence the automatically determined threshold to take more or less background into account for the region of interest. The default value is 0.5, lower values enlarge the detected region, higher values reduce the size of it. The automatic determination of the threshold is based on detection of bright areas in the image in relation to the surrounding background. In contrast to the *Find Nuclei*, *Find Cells* and *Find Spots* building blocks a single threshold is used for the whole image (no per-object thresholds).
Split into Objects	**Selected:** Spatially distinct regions will be separate objects. **Unselected:** One single region will be created.

Third level parameters	
Area	Sets a lower threshold to the area of objects; objects which are smaller than this are discarded.
Fill Holes	If **Fill Holes** is selected then each region not belonging to any object but surrounded by a single object will be a part of that object.

Whole Image Region

Creates a region covering all pixels of the image. There are no tuning parameters.

Exemplary applications:

- Preparatory step when e.g. intensity properties of the whole image are requested.
- Preparatory step for detecting spots in the whole image.

5.2.11 Find Texture Regions

Find Texture Regions allows to separate regions of an image with different textures using an interactive training mode (PhenoLOGIC™).

Notice
This building blocks uses the PhenoLOGIC™ technology and is only available with an optional license. For details see the following sections:

- 5.2.2 “PhenoLOGIC™”, page 326
- “License Management”, page 320

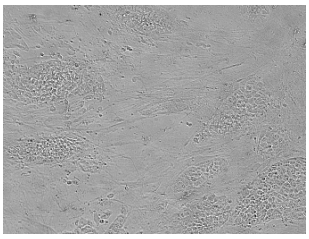
5.2.11.1 Working Principle

- This building block performs a task known as *supervised texture segmentation*.
- In the training phase, the user (supervisor) selects some examples of a single or few texture classes.
  - The computer divides each image into regions, each being similar to a particular example class and different from the other example classes.
  - Usually, training is performed on a single or a few images while segmentation can then be applied to a great number of images.
- The first level input parameters of *Find Texture Regions* are **Channel**, **ROI** (Region of Interest) and **Method**. The input can be a local or global channel.
- For global images:
  - Use of a ROI is mandatory (default: “Imaged Area (global)”).
  - Overlays **Field Partition** and **Current Field** are available
  - Switching between local and global channels during the training needs reset of the training. A warning and instructions are given.
  - Using global images of different sizes but same binning is allowed. This enables using *Find Texture Regions* in combination with **PreciScan**. See also section 5.1.8.3 “PreciScan™”, page 119.
  - The user has to take care that the classifier is trained on images with the appropriate binning.
  - For detailed information see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.
- If Regions of Interest (**ROIs**) are selected, the detection is done inside each of the specified ROIs.
  - Results are reported per ROI, e.g. cell colony.
  - Parts of the image which are outside any ROI are ignored.
  - To select a set of ROIs specify any previously created population as **ROI Population**. Each object in the ROI population defines a separate ROI.
  - The ROI population is usually created by a preceding sequence of object detection and region selection building blocks.

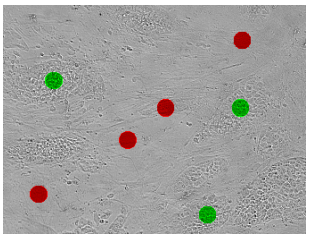
- If the ROI population objects contain multiple regions, use **ROI Region** to pick one of these regions as ROI.

- Methods:
  - **Find a Class** detects regions of a single texture class.
  - **Split into Classes** splits the whole image into texture regions of multiple different classes using a similarity criterion.
- The name of the resulting output population(s) can be selected in the box **Output Population**. The output is a local or global population depending on the input channel. For global populations the suffix "(global)" is automatically added to the output name.

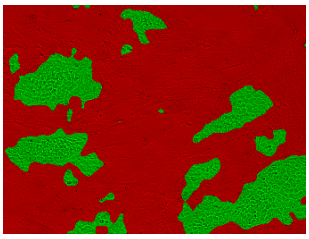
5.2.11.2 Example



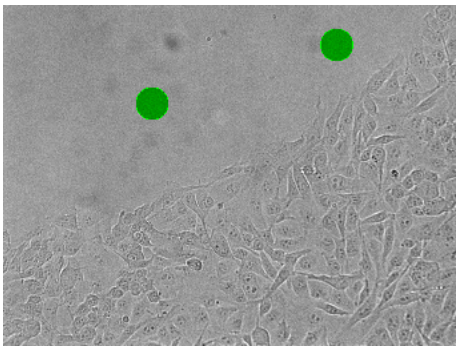
Input: *A brightfield image*



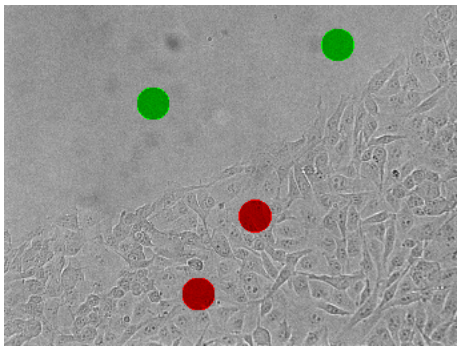
Training: *Selected examples of texture classes*



Visual result: *Detected regions*

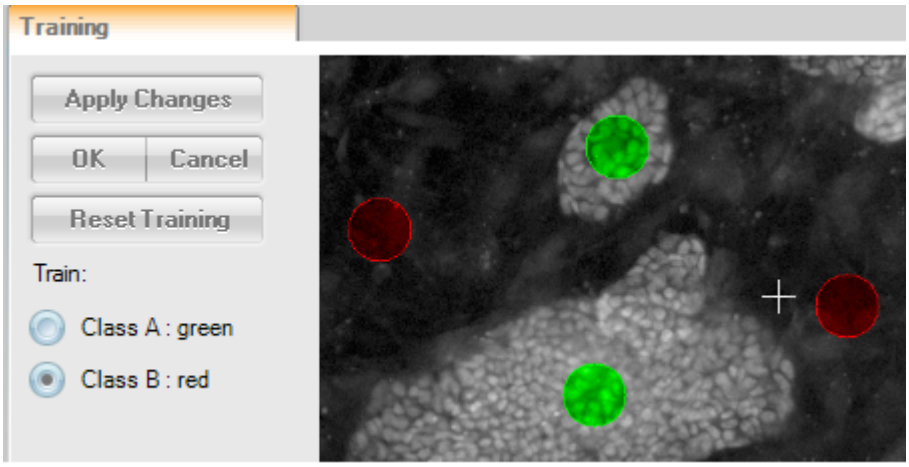


Training for ***Find a Class***



Training for ***Split into Classes***

5.2.11.3 Training Mode



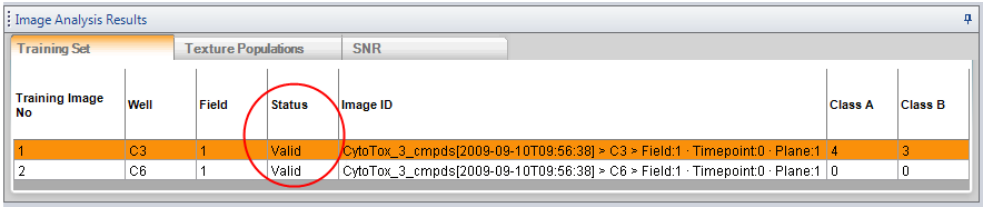
After adding the *Find Texture Regions* building block you are automatically in training mode. Otherwise use the **Train...** button to enter the training mode. Only in this mode you can modify the input parameters and select examples for each class on the image.

- Select a class and add (or remove) training examples for each class by clicking on the image.
- Use **Apply Changes** to update the preview of the segmentation after you have changed the input parameters or added/removed training examples.
- Click **OK** to exit the training mode and view the resulting texture classes.

Notice

You have to leave the training mode before you can open or add another building block.

Training Set



In the **Image Analysis Results** pane (**Training Set** tab) you can see the list of training images and their **Status**:

- **Valid:** The training image is in valid state and is taken into account for classifier calculation.
- **Invalid:** Not all classes are trained on the image. The training image is in invalid state and is not taken into account for classifier calculation.

Each class must have at least one training point on each of the training images. If there are invalid training images, you need to select or load that image again (see **Image ID**) and enter the training mode. Add training examples for the missing classes and click **OK**. The training image will become valid again.

To remove a training image from the training set, open the corresponding image, enter training mode and remove all training examples.

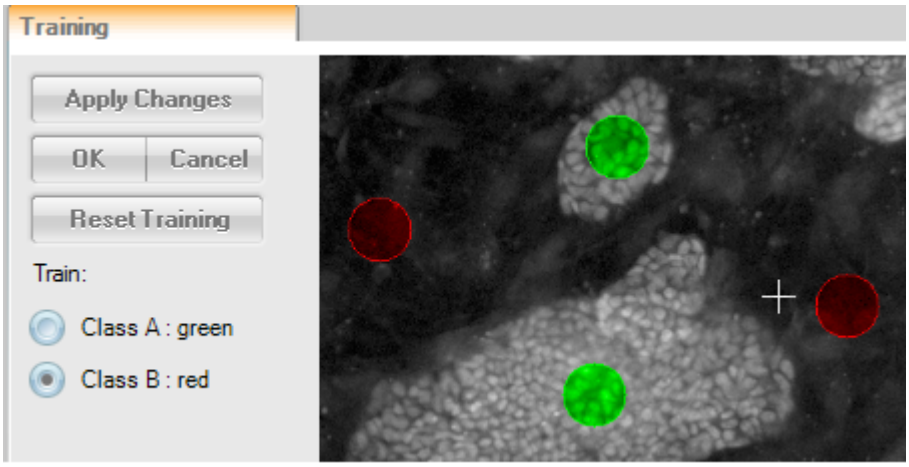
When training on a **global image**:

- The training points are assigned to the global image instead of the currently selected image field.
- The field number of the global image is set to "*" indicating that all fields are used instead of a specific field. The field number is visible in the **Image ID** and the **Field** columns of the training set table.
- If an old analysis sequence with a classifier trained with Harmony version < 4.6 is loaded, the trained classifier still works, but field numbers are reported as trained in Harmony 4.5. The training of these classifiers cannot be modified, it must be reset and trained again.

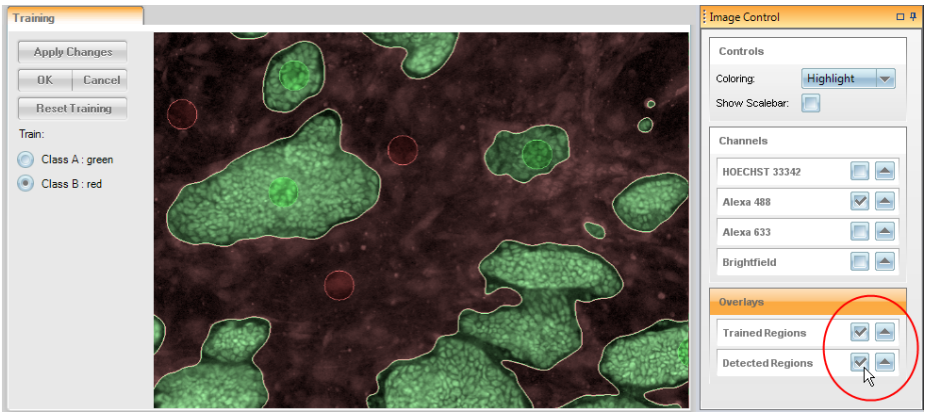
How to configure *Find Texture Regions*

1. Select the appropriate **Channel**.
2. Select the desired **Method**.
3. If you have selected method **Split into Classes**: Select the **Number of Classes** which shall be created.
4. Click on the image and select a few examples of **Class A**. The training spots are indicated by a green circle. To remove a training spot, click the circle again. It is not recommended to add more than 10 examples per class.

5. If you have selected method **Split into Classes**: Select the next class and select a few examples of this class. The training spots will be indicated by a different color. Continue until you have selected examples for each class.

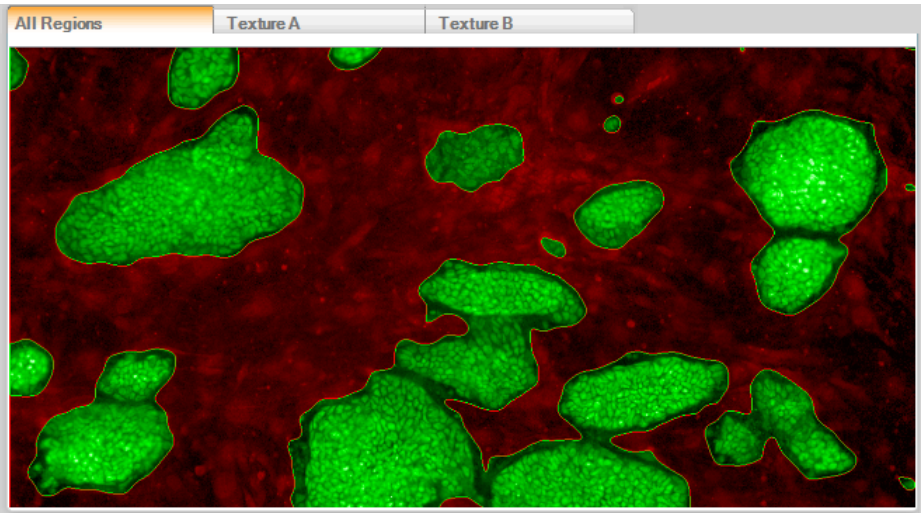


6. Click **Apply Changes** in the training section.
   The image will be segmented into one or multiple classes, you are still in training mode.



7. Check the quality of the segmentation:
   - To check the results, switch on and off the overlays **Trained Regions** and **Detected Regions** (**Image Control** pane).
   - Check the **Image Analysis Results** pane for statistical information on the segmentation, e.g. the signal-to-noise ratio on the **SNR** tab.
8. Possible steps to improve the result:
   - Check whether the most appropriate image channel for segmentation is selected.
   - Add or remove training examples for a class, in particular at places of segmentation errors. You can remove a training spot by clicking on it.
   - Fine-tune the second and third level parameters.
   - Try a different selection of training examples.
   - If errors of segmentation are still significant then select the other method, repeat the training and fine-tune the second and third level parameters.

9. Select a different well or field and check if the segmentation is also working with other images.
10. Click **OK** in the **Training** section.
    The image will be segmented into one or multiple classes, the training mode is finished. The detected texture regions are visualized by illustrations.



11. If you need to modify the training set, click **Train...** to enter training mode again.
12. Further optional steps:
    - Use *Modify Population* to split objects which could not be separated during segmentation.

Buttons and Elements

Element	Description
Apply Changes	Applies the changes (e.g. modified input parameter or new training examples). Training mode is not terminated.
OK	Terminates the training mode and applies the changes.
Cancel	Terminates the training mode and discards all changes.
Reset Training	Deletes all training examples from all training images, i.e. the whole **Training Set** (see **Image Analysis Results** pane).
Train section	Displays the available classes. Select the class for which you want to add/remove training examples.

5.2.11.4 Methods

Notice
The parameters can only be edited in **Training Mode**.

Find a Class

The method **Find a Class** is adequate in either of the following cases:

- if there is a single homogeneous texture class (e.g. a cell-free region) while the other class is heterogeneous
- if the user does not care much about the border lines between the other classes
- if performance of the other method **Split into Classes** is poor

Notice

The **Find a Class** method is replacing the old method **Find a Class** (legacy) which is still supported in existing analysis sequences, but cannot be added to new sequences any more. The new method gives very similar results but with much less calculation time (up to 10x faster).

Second level input parameters	
Tolerance	Specifies how much the texture properties are allowed to differ from those of the examples (range: 1-10).
Split into Objects	If activated, the detected texture regions are split into separate objects.

Third level input parameters	
Texture Scale	Specifies the distance at which the texture pattern is characterized (range: 0-20).
Region Scale	Determines the smoothness of border lines (range: 2-20).
Training Region Radius	Radius of training region which is used to select examples in the image. The default value of 25 px should only be modified by experienced users.
Include Intensity Information	Option to include the intensity of the original image as a feature used for classification. Default: Don't use intensity information. If regions can be reliably identified by their intensity in addition to their texture, detection results will improve. Drawback: The classification becomes dependent on absolute image intensities, e.g. plate-to-plate or batch-to-batch variations. Avoid using this option if the analysis should be robust against absolute intensity changes.

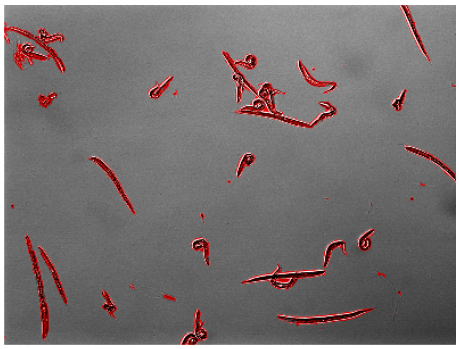
Split into Classes

The method **Split into Classes** is the adequate choice if there are two or more homogeneous texture classes.

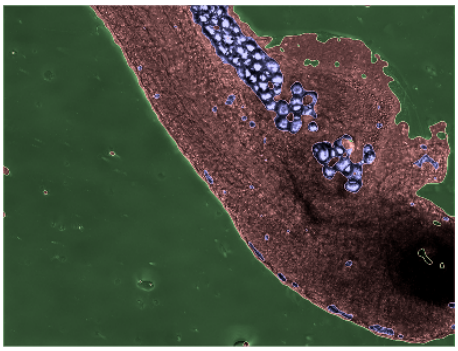
Second level input parameters	
Number of Classes	Desired number of resulting texture classes.
Split into Objects	If activated, the detected texture regions are split into separate objects.

Third level input parameters	
Texture Scale	Specifies the distance at which the texture pattern is characterized (range: 0-20).
Region Scale	Determines the smoothness of border lines (range: 2-20).
Training Region Radius	Radius of training region which is used to select examples in the image. The default value of 25 px should only be modified by experienced users.
Include Intensity Information	Option to include the intensity of the original image as a feature used for classification. Default: Don't use intensity information. If regions can be reliably identified by their intensity in addition to their texture, detection results will improve. Drawback: The classification becomes dependent on absolute image intensities, e.g. plate-to-plate or batch-to-batch variations. Avoid using this option if the analysis should be robust against absolute intensity changes.

5.2.11.5 More Examples



C. elegans segmentation



Zebrafish segmentation

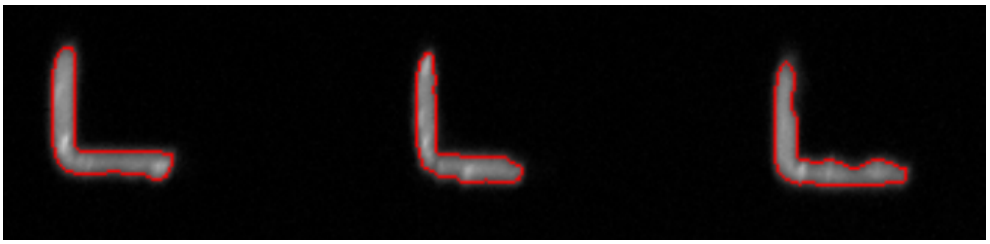
5.2.12 Find Surrounding Region

This is a building block for detecting a surrounding region around arbitrary objects.

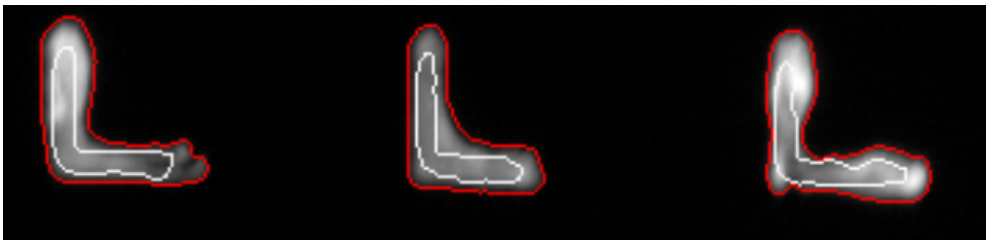
5.2.12.1 Working Principle

- The task is to detect regions around arbitrary objects.
- The objects must have been detected beforehand.
- The surrounding region is detected as a region on the image having a higher intensity than the background.
- The name of the detected region can be selected in the box **Output Region**.
- The first level inputs of *Find Surrounding Region* are **Channel**, **Population**, **Region** and **Method**. The input channel must be of the same scope (local/global) as selected nuclei population.
- For **global** populations:
  - Use of a **Restrictive Region** is mandatory (cannot be unchecked), default: “Imaged Area”.
  - Overlays **Field Partition** and **Current Field** are available.
- The set of second level inputs is specific to each method, but **Individual Threshold** and **Include Input Region** is always among them.
- In case the surrounded objects were detected in a user defined region of interest (ROI), the surrounding region detection can be restricted to the same or a modified region by the **Restrictive Region** input.
- Technically the methods **A** to **D** correspond to the methods **A** to **D** used in *Find Cytoplasm*.

5.2.12.2 Example



Visual input: *Fluorescently stained microstuctures detected by Find Image Region (outlines marked red).*



Visual result: *Detected surrounding regions (outlines marked red) around the previously detected microstructures (outlines marked white). Detection was done on a tubulin stained image (shown as background image).*

How to improve performance of Find Surrounding Region

If errors of the surrounding region detection are observed then attempt to reduce them in the following steps:

1. Select **Channel**, i.e. check whether the most appropriate image channel for detection is selected.
2. Test how different methods work at default values of the second level parameters and select the most promising method.
3. Fine-tune second level parameters:
4. If errors of detection are still observed select another method and fine-tune second level parameters.

Notice
The set of input parameters is different for different methods. Even if names of parameters are the same, their optimal values may be different.

5.2.12.3 Methods

Overview of methods of *Find Surrounding Region*:

Method	Number of tuning parameters	Surrounded object is visible very bright on surrounding region channel	Surrounded object is not visible on surrounding region channel
A	1	+	–
B	2	+	–
C	2	–	+
D	1	+	–

Method A

+ It is the most robust method.
+ There is only one tuning parameter.
+ It is best applicable in cases when intensity decreases with the distance from surrounded object.
– Errors may occur with the detected surrounding region spreading out to the background area.

Second level parameter (similar to method D)	
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.
Include Input Region	See section 5.2.12.4 “Include Input Region”, page 386.

Method B

+ It is the recommended method in the case when method **A** yields errors with surrounding region spreading out to the background area.
– There may appear other type of errors compared to method **A**, e.g. only a part of surrounding region is detected.

Second level parameters (similar to method C)	
Common Threshold	Parameter determining the first guess borders of surrounding region.
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.

Second level parameters (similar to method C)	
Include Input Region	See section 5.2.12.4 “Include Input Region”, page 386.

Method C

+ This method is best suited when borders of neighbor cells are visible as intensity steps rather than intensity valleys.
– A typical error is detecting only a part of the surrounding region near the surrounded object.
– This method fails in cases when the surrounded objects are very brightly visible in the channel used to detect the surrounding region.

There are two tuning parameters (similar to method **B**):

Second level parameters (similar to method B)	
Common Threshold	Parameter determining the first guess borders of surrounding region.
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.
Include Input Region	See section 5.2.12.4 “Include Input Region”, page 386.

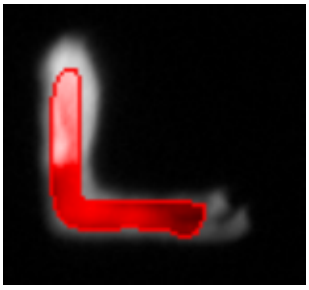
Method D

+ There is only one tuning parameter.
+ It is best applicable in cases when intensity decreases with the distance from the surrounded object.
– Errors may occur with the detected surrounding region spreading out to the background area.

Second level parameter (similar to method A)	
Individual Threshold	Parameter determining the intensity threshold for each object individually.
Restrictive Region	In case nuclei were detected in a user-defined region of interest (ROI), the cytoplasm detection can be restricted to the same or a modified region by the **Restrictive Region** input.
Include Input Region	See section 5.2.12.4 “Include Input Region”, page 386.

5.2.12.4 Include Input Region

Using the **Include Input Region** switch you can determine whether the original (surrounded) region is included in the output region of the building block.



Input Region

Reported surrounding region

*Reported surrounding region; **Include Input Region** activated*

5.2.12.5 Limitations

For speed reasons the surrounding region is limited to a distance of about 200 px around the initial region. If larger surrounding regions are needed, you may detect a second surrounding region around the first incomplete region.

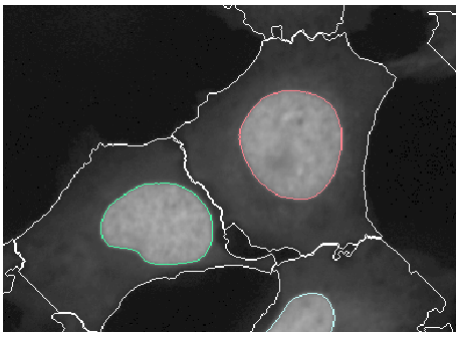
5.2.13 Select Cell Region

This is a building block for creating a cell region.

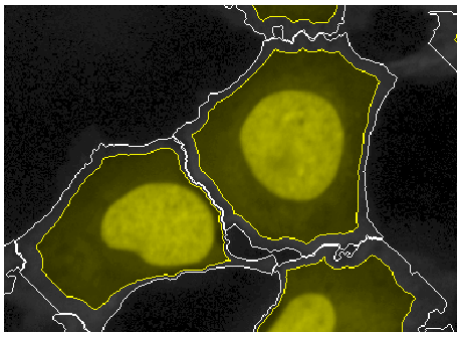
5.2.13.1 Working Principle

- The task is to create a region of interest for each cell.
- The basic first level input of *Select Cell Region* is **Region Type**.
  - **Population** is an additional input parameter whenever there is more than one single population.
- The created region is located in respect to detected nuclei and cytoplasm.
- Borders of the created region can be shifted inwards or outwards using second level parameters **Outer Border** and **Inner Border** (relative to cell dimensions (%) or as a constant value (µm/px)).
- The name of the output population of *Select Cell Region* can be selected in the box **Output population**.

5.2.13.2 Example



Visual input: *A nuclei stained image with overlays of detected nuclei (in rainbow colors) and cytoplasm (in white)*



Visual result: *Overlay of the created cell region (in yellow)*

5.2.13.3 Region Type

There are five default **Region Types** to start from:

- Cell Region
- Nucleus Region
- Cytoplasm Region
- Membrane Region
- Ring Region

The final shape and expansion of the region is determined by the tuning parameters **Inner Border** and **Outer Border**.

Notice

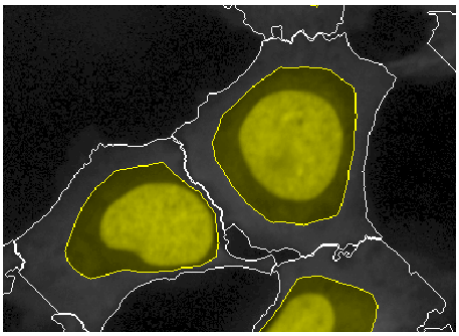
The **Region Type** determines only the initial values of the tuning parameters. After changing the tuning parameters, the result possibly does not correspond to the selected **Region Type** any more.

5.2.13.4 *Tuning Parameters*

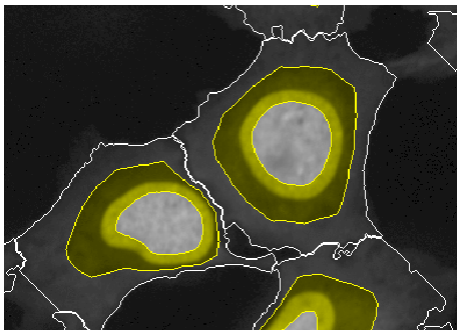
How to interpret values for Inner Border and Outer Border

Resize Region Method	Inner Border	Outer Border
µm/px: Interpretation of parameters **Outer Border** and **Inner Border** depends on **Region Type**.	For region types **Cell Region** and **Membrane Region**, the value 0 corresponds to cytoplasm outer border.	For region types **Cell Region**, **Cytoplasm Region** and **Membrane Region**, the value 0 corresponds to cytoplasm outer border.
	For region types **Nucleus Region**, **Cytoplasm Region** and **Ring Region**, the value 0 corresponds to nucleus border.	For region types **Nucleus Region** and **Ring Region**, the value 0 corresponds to nucleus border.
%: Location of created region depends only on parameters **Inner Border** and **Outer Border** and not on **Region Type**.	• 0 % corresponds to cytoplasm outer border • 50 % corresponds to nucleus border • 100 % corresponds to nucleus center	

Example: Closed and ring-like shape of the created region

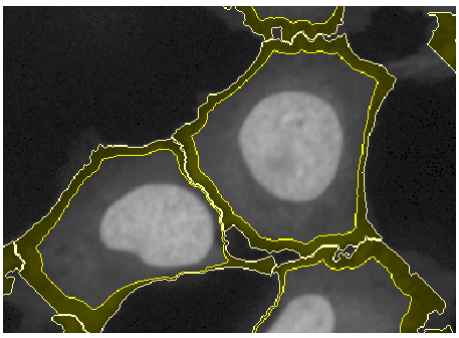


A closed region:
Outer Border = 25 %, Inner Border = 100 %

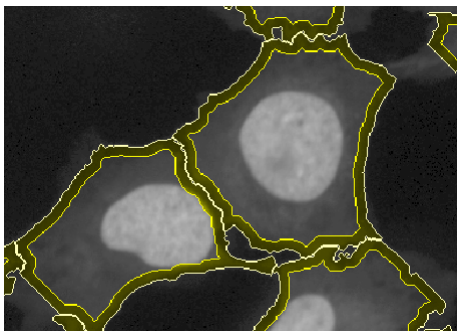


A ring region:
Outer Border = 25 %, Inner Border = 60 %

Example: Relative and constant region width



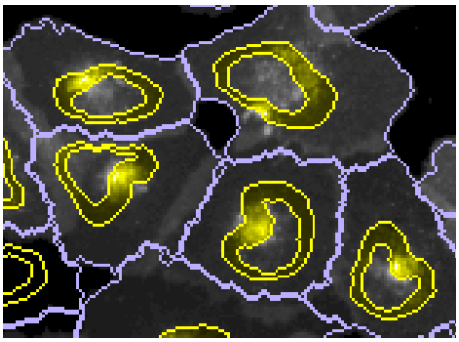
A membrane region with relative width:
Outer Border = 0 %, Inner Border = 10 %



A membrane region with constant width:

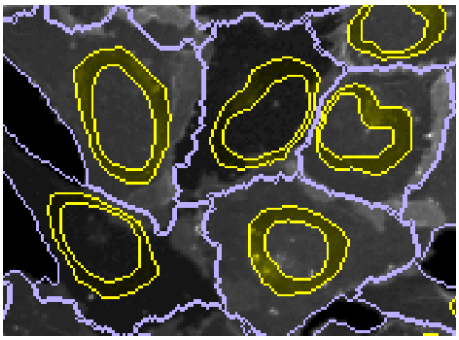
Outer Border = 0 px, Inner Border = 9 px

Example: Compare positive and negative control wells



Signal image from a positive control well

Achieve that the created region (in yellow) covers bright spots.



Signal image from a negative control well
As the ultimate goal of creating the region, signal intensities from the region will be significantly different in positive and negative control wells.

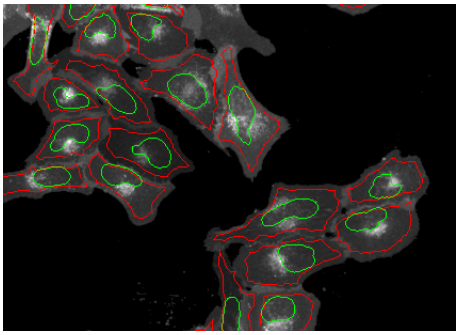
5.2.14 Select Region

This is a building block for creating a region of interest for an arbitrary region in input.

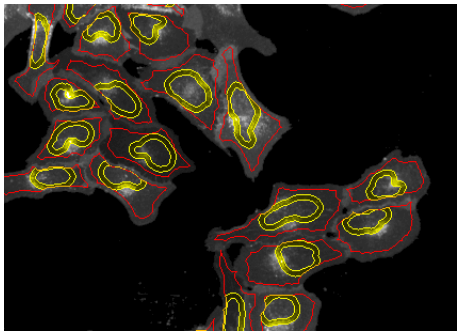
5.2.14.1 Working Principle

- The task is to create a region of interest starting from an arbitrary region (not necessarily nucleus, cytoplasm or cell).
- The first level inputs of *Select Region* are **Population**, **Region** and **Method**. The input population can be either local or global.
- The created region is located in respect to the input **Region**.
- Output region name/prefix:
  - For the **Standard** method you can enter a prefix for the name of the output region in the box **Region Prefix**. It will be added to the automatically generated region name.
  - For all other methods you can enter the name of the output region in the box **Output Region**.

5.2.14.2 Example



Visual input: *A signal channel image with overlays of an input region (nucleus in green) and an outer region used for relative resizing (cell subregion in red).*



Visual result: *Overlay of the created region of interest (in yellow), together with the outer region (in red).*

5.2.14.3 Methods

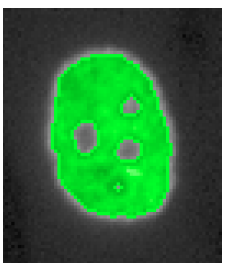
Overview of methods of *Select Region*:

Standard

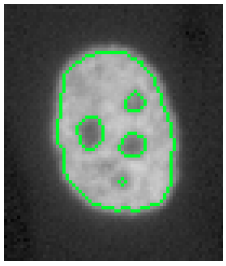
- More than a single region can be created in parallel.
- There are no tuning parameters.
- Each created region is of a predefined type.
  - The predefined types of the created regions are *Border, Fill holes up to* (with an additional input to specify the size limit in µm² or px²), *Geometrical Center, Skeleton, Inner Center, Weighted Center* (with an additional input selecting image channel for weighting), *Outer Border, Inner Border*, and *Holes up to* (with an additional input to specify the size limit in µm² or px²).

- For the *Filled* and the *Holes* regions a maximum area can be specified. Holes which are bigger than the specified area are ignored. The default is *INF*, i.e. all holes are filled / part of the region.

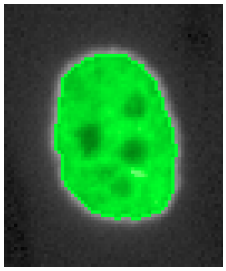
Examples of regions of some predefined types are presented below:



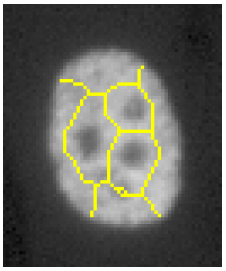
Input



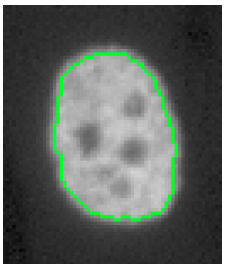
Border



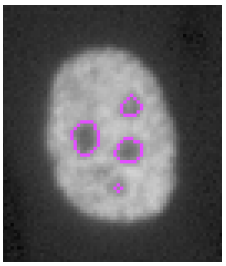
Filled



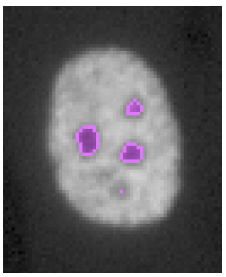
Skeleton



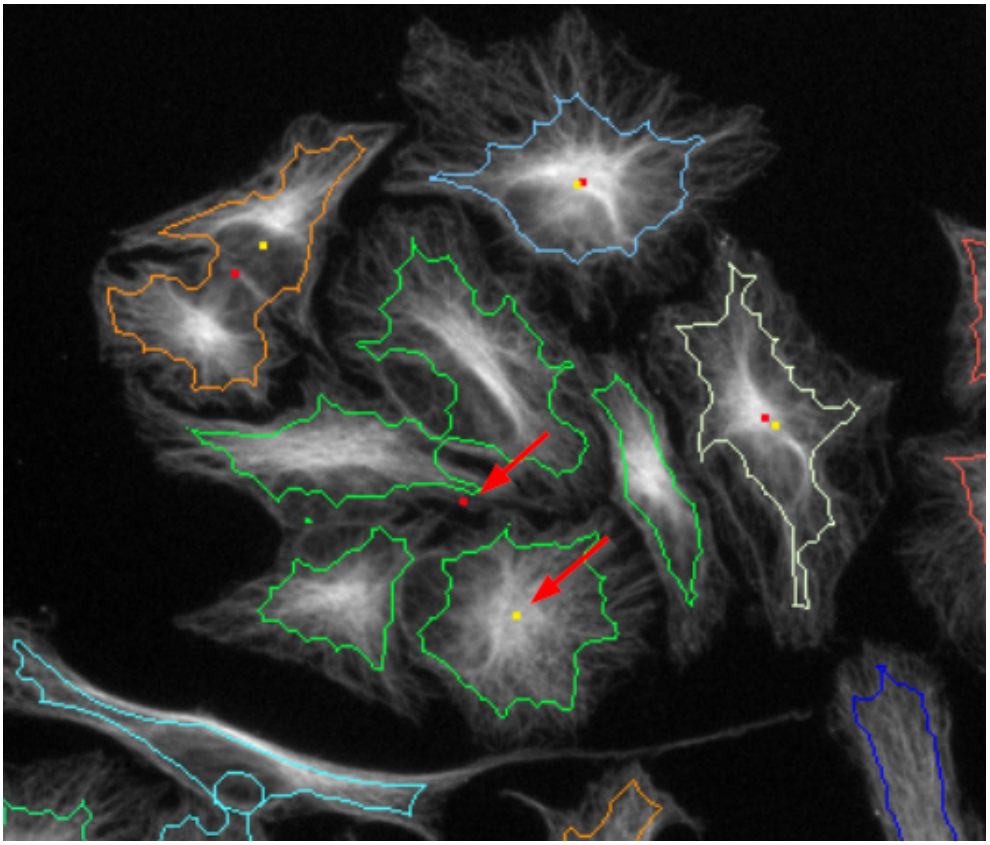
Outer Border



Inner Border



Holes



Example for *Inner Center* (yellow dots) and *Mass Center* (red dots) of some objects. The object outlines are marked by colored lines. Note that the green object in the middle consists of multiple parts. For this object the *Inner Center* and *Mass Center* are marked by red arrows. The *Inner Center* (yellow dot) is the point inside the object which has the largest distance from any object border, so it is located in the middle of the biggest part of the green object.

Resize Region [µm, px]

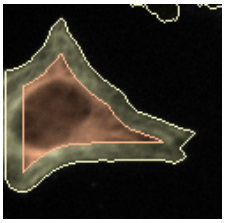
- A single region is created at a time.
- Border of the created region can be tuned by second level parameters **Outer Border** and **Inner Border**.
  - If the value of the parameter **Inner Border** is infinity then the parameter **Outer Border** alone determines the border of the created region.
- **Increasing** a tuning parameter causes an **inward** shift of the border that is adjusted (i.e. the shift direction is from external towards internal of the input region).
- The unit of tuning parameters is an absolute distance, either micrometer (µm) or pixel (px).
- The value 0 of each tuning parameter corresponds to the original border (i.e. border of the input region).

  Notice

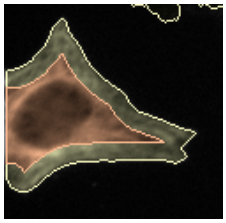
  - If the original region has two or more borders (e.g. it is cytoplasm) then all borders are treated in the same way. For example, the value of a tuning parameter -2 px corresponds to new borders at 2 pixel distance from the original borders outside the original region.
  - If the original region has two or more borders then it is recommended that **Inner Border** = infinity, otherwise the logic may be difficult to understand.

- The created region can never be extended outside the **Restrictive Region**.
  - If using a global population, it is mandatory to select any **Restrictive Region** (**Population** and **Region**), the default is “Imaged Area (global)”.
  - Note that this is also very useful for analyses in local context. The ROI does not need to be from the same population, e.g. the result of the *Modify Population* building block can be used for restriction.
- The **Keep Image Border** option defines the behavior when the original region touches the image border.
  - If the option is activated pixels at the image border are not modified. That corresponds to the assumption the object continues outside of the image border.
  - If the option is deactivated pixels at the image border are not specially treated. That corresponds to the assumption that the object border coincides with the image border.

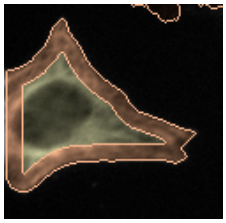
Here is an example of the resulting inner and outer borders of a cell region resized with different **Keep Image Border** settings. The original region is the full cell, the resized region is overlaid red.



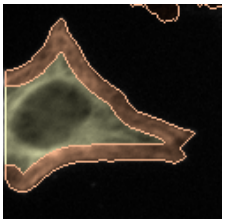
Outer Border = 10 px
Keep Image Border
OFF



Outer Border = 10 px
Keep Image Border
ON



Inner Border = 10 px
Keep Image Border
OFF



Inner Border = 10 px
Keep Image Border
ON

Resize Region [%]

- A single region is created at a time.
- Borders of the created region can be tuned by second level parameters **Outer Border** and **Inner Border**.
  - The value 0 % of a tuning parameter corresponds to the original border.
  - The value 100 % of a tuning parameter corresponds to the inner center of the original region.
- The algorithm of creating a new region is the following. For each pixel two distance characteristics are calculated. One of them is the distance to the closest border pixel ("border distance"); it is given a negative sign for pixels outside the original region. The second characteristic is the distance to the inner center of the input region ("center distance").Thereafter, a relative distance is calculated defined as the ratio of "border distance" to "border distance + center distance". A given pixel belongs to the output region if the calculated relative distance is larger than **Inner Border** and smaller than **Outer Border**.
  - The inner center of a given region is defined as the point inside the region having the largest distance to the closest border point.
- It is recommended that the original region is of filled type, i.e. not a ring area with a hole. Otherwise, the logic may be difficult to understand.
- If an optional **Outer Region** is specified the created region is defined relative to the inner and outer region:
  - A value of 0 % corresponds to the outer region border.
  - A value of 50 % corresponds to the original region border (including border, use 49 % to start outside the region).
  - A value of 100 % corresponds to the inner center.

Restrict by Mask

- The existing regions are cropped by another region serving as a “Mask”.
- The new regions are created by a pixelwise AND operation of the existing regions and the mask region, i.e. all pixels of the original region are preserved, which overlap with the mask region.
- The mask region can be specified by the **Population** and **Mask Region** second level input parameter.
- The **Use Inverted Mask** check box inverts the mask region, i.e. all pixels of the original region are preserved, which do NOT overlap with the mask region.

Examples

Original region

Mask region (from a different population)

Original region restricted by mask region

Original region restricted by inverted mask region

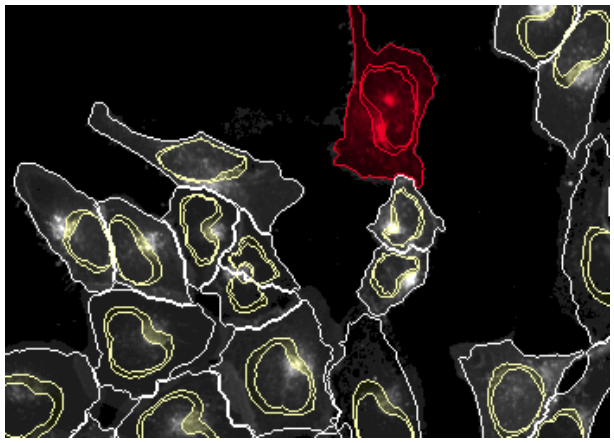
5.2.15 Calculate Intensity Properties

This is a building block for calculating one or more intensity properties in a cell region.

5.2.15.1 Working Principle

- The task is to calculate intensity properties such as mean intensity for a region of interest.
- The basic first level inputs of *Calculate Intensity Properties* are **Channel**, **Region** and **Method**.
- If a **global** channel is selected:
  - Mixing between local and global context is not possible
    - If a local channel is selected: only local populations can be selected
    - If a global channel is selected: only global populations can be selected
  - New overlays **Field Partition** and **Current Field** are available for information.
  - For details see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.
- The prefix of names for output properties can be selected in the box **Property Prefix**.
- There is a single method available, **Standard**, with a number of intensity properties that can be calculated in parallel upon request.

5.2.15.2 Example



Summary | **Properties [0]**

Object No	Region Signal Mean
1	765.915
2	702.979
3	859.744
4	618.995
5	389.532
6	1071.15
7	1195.96
8	665.108
9	890.566
10	793.08
11	690.635
12	1281.03
13	494.743
14	696.382
15	951.772
16	936.151
17	840.248
18	311.827
19	308.125
20	326.042
21	814.089
22	247.711
23	414.085
24	508.085

Visual input: *A signal channel image with overlays of detected cells (white) and a region of interest (yellow) and selected object (red).*

Result: *Calculated intensities in the selected region of interest. For example, the average pixel intensity in the selected region for cell number 6 is 1071.15. The particular value is highlighted in blue in the table and the particular cell is highlighted in red on the image.*

5.2.15.3 Method

There is only a single method available:

Standard

A series of properties characterizing an intensity histogram can be calculated by *Calculate Intensity Properties*:

- Mean value
- Standard deviation
- Coefficient of variation, i.e. standard deviation divided by mean
- Median value
- Quantile value (at any specified quantile fraction)

- Minimal value
- Maximal value

Furthermore, in addition to histogram properties, contrast can be calculated by *Calculate Intensity Properties:*

- Contrast *c* is the following function of the mean intensity in the region (excluding a part at the border), *a*, and the mean intensity in the neighborhood of the region, *b: c=(a-b)/(a+b)*.

5.2.16 Calculate Morphology Properties

This is a building block for calculating one or more morphology properties of a cell region.

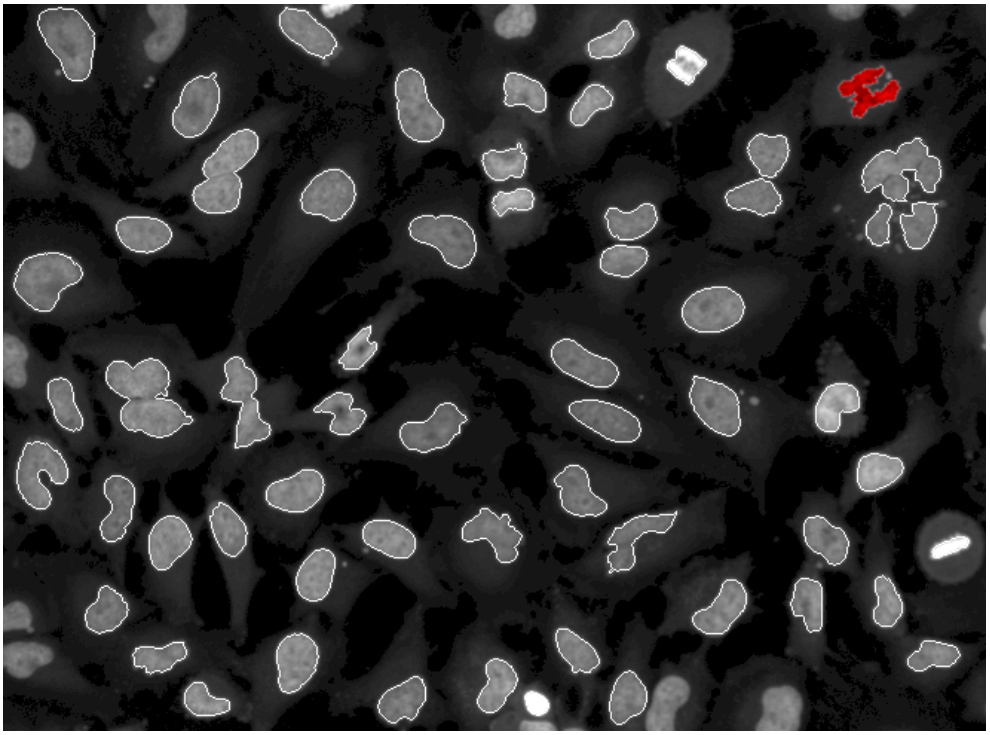
5.2.16.1 Working Principle

- The task is to calculate morphology properties such as area and roundness for a region of interest.
- The basic first level inputs of *Calculate Morphology Properties* are **Population**, **Region** and **Method**.
- If a **global** population is selected:
  - New overlays **Field Partition** and **Current Field** are available for information.
  - For details see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.
- The prefix for names of output properties can be selected in the box **Property Prefix**.

Notice

It is recommended to remove objects crossing the image border beforehand. This can be done using the **Select Population** building block. Otherwise, morphology properties of the objects crossing the image border are distorted.

5.2.16.2 Example



Visual input: *Nuclei stained image with overlays of detected nuclei. (Nuclei crossing the image border have been filtered out.)*

Object No	Index in Nuclei	Nucleus Area [px²]	Nucleus Roundness
1	5	1323	0.906596
2	6	786	0.892209
3	7	465	0.850523
4	8	421	0.925275
5	9	1099	0.868564
6	10	609	0.427125
7	11	982	0.899641
8	12	572	0.847814
9	13	473	0.872579
10	15	1456	0.790197
11	16	618	0.938168
12	17	1390	0.605835
13	18	539	0.817775
14	19	949	0.973641
15	20	660	0.865413
16	21	361	0.814123
17	22	578	0.788509
18	23	632	0.854048
19	24	355	0.805671
20	25	1083	0.878132
21	26	728	0.929109

Result: *Calculated morphology properties of nuclei. Here, the selected row in the table corresponds to the cell of the lowest roundness value. The particular cell is highlighted red on the image above.*

5.2.16.3 Methods

There are two methods available:

Standard

A single or more morphology properties from the following list can be calculated:

- Area
- Roundness
- Width
- Length
- Ratio width to length

STAR

The STAR method includes a large and diverse set of morphology properties for phenotype classification and quantification of morphology changes. Here, “morphology” includes the outer shape of objects but also the distribution of intensity inside the objects, e.g. symmetry.

Second Level Input Parameters

- **Channel** selects a gray-scale image on which basis properties of objects are calculated
- There are five switches determining if a given subfamily of STAR morphology properties will be calculated or not.
  - **Symmetry**
  - **Threshold compactness**

- **Axial**
- **Radial**
- **Profile**

- For a detailed description of the properties see the following sections.

Third Level Input Parameters

In addition to calculating the properties on the original image, several filters can be selected to preprocess the images before calculating the properties. For each selected filter, the whole set of properties is calculated on the filtered image as well. There are two different types of filters, **Sliding Parabola** and **Texture SER**, each with several parameters to adjust.

- **Profile Inner Region** effects the Profile properties only. It determines if an inner region should be used inside the selected region of interest, e.g. the nucleus region inside the cell region. A list of choices of suitable inner regions is offered (default: on, first choice selected).
- **Profile Width** adjusts the width of the regions (default: 4px)
- **Sliding Parabola**
  - A checkbox can be set to enable calculation of sliding parabola filtered features (default: off). The filter is a background subtraction algorithm similar to the “rolling ball” algorithm.
  - **Curvature** adjusts the filtering, higher values lead to suppression of smaller structures. The filtered image can be visually inspected by selecting image tab **Sliding Parabola**.
  - **Use for Center** determines if the original or sliding parabola-filtered image is used when calculating the mass center of an object. The reference radius is also calculated from the image selected here.
    Using the filtered image for center determination influences the numerical values of **Symmetry**, **Axial** and **Radial** properties.
- **Texture SER**
  The texture filters are the same as used in the *Calculate Texture Properties* building block's “SER” method. For detailed help see *Calculate Texture Properties*, section “SER Features”, page 407.
  - **Scale** adjusts the characteristic length of structures of interest (default: 1px)
  - **Normalization by** determines if intensity information is removed by normalization (default: Kernel)
  - Illustrations of the texture filtered images can be displayed for visual inspection by inserting a Calculate Texture Properties building block.
  - A set of 8 checkboxes can be set select the filters to use (default: all off)
    - **SER-Spot** (recommended choice)
    - **SER-Hole**
    - **SER-Edge**
    - **SER-Ridge** (recommended choice)
    - **SER-Valley**

- **SER-Saddle**
- **SER-Bright**
- **SER-Dark**

Names of STAR properties

Names of morphology properties will be automatically generated according to the following rules:

- The first part of the name is a user-selectable string specified by **Property Prefix**, e.g. **Cell**.
- The second part of the name is the name of the subfamily of properties, e.g. **Symmetry**.
- The third part of the name is the subfamily-specific name of the individual property, e.g. **02**.
- The fourth part of the name is the filter if the property characterizes a filtered image, e.g. **SER-Bright**.

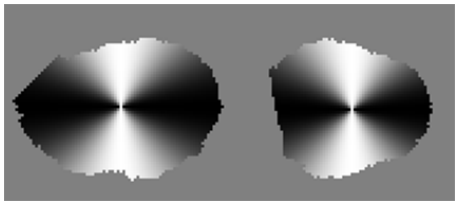
The full name of the property in the example above is **Cell Symmetry 02 SER-Bright**.

Symmetry

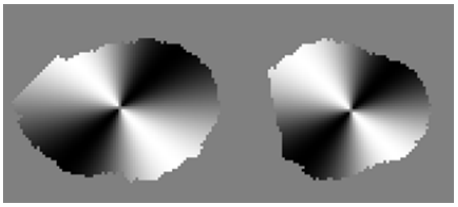
Symmetry properties are calculated via a selected set of polynomial functions,

$$R_{nm}(\rho,\varphi) = \rho^n e^{-im\varphi}$$

On the following pair of images, real and imaginary parts of a polynomial with n=0, m=2, calculated for two objects are illustrated.

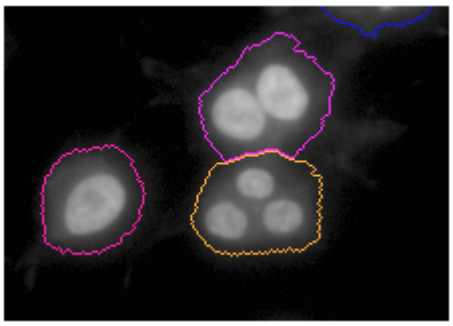


Real part of the polynomial



Imaginary part of the polynomial

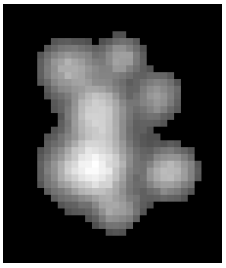
Symmetry properties are normalized moments of the polynomial functions above. Here is a typical example of cells that can be very well characterized by symmetry features:



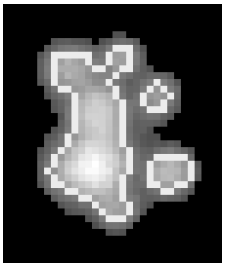
Multinucleated cells with one, two and three nuclei

Threshold Compactness

Threshold compactness is a numeric property describing an object but being calculated via applying a threshold to the image in location of the original object. The following pair of images illustrates how a threshold creates a set of provisional objects on location of an originally detected object.



Input image on location of a detected nucleus

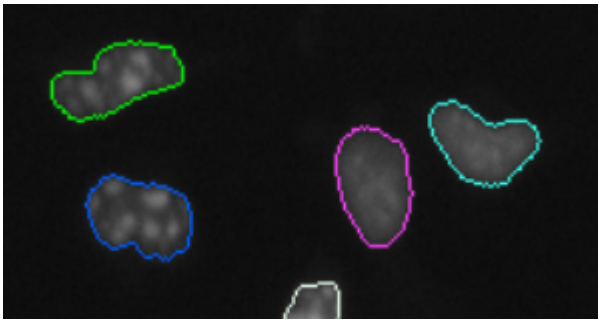


The same image with borders of provisional objects created by a threshold which is set to 40% of the maximal intensity

Compactness of objects created by the threshold is calculated by the following function of area of the body of the provisional objects and area of their border:

$$C = \frac{2\sqrt{\pi S_{body}}}{S_{border}}$$

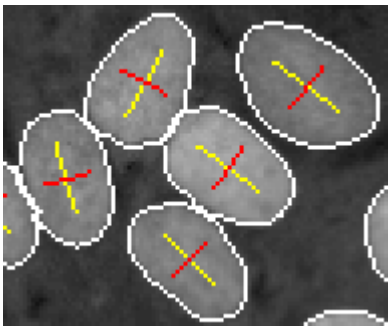
Here is a typical example of nuclei that can be very well characterized by threshold compactness features:



Healthy and fragmented nuclei of HeLa cells

Axial

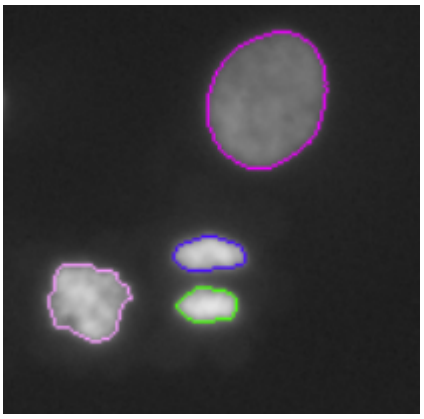
From the second order central moments of the object image, lengths of the two principal axes of each object are calculated. The following image illustrates longer and shorter principal axes calculated for each object.



Longer and shorter principal axes as an overlay

The length of the shorter axis (in pixel units) is a straightforward morphology property (**Axial Small Length**) while the other axial property is the ratio of the shorter to the longer axis lengths (**Axial Length Ratio**).

Here is an example of nuclei that can be very well characterized by axial features:



Stained DNA at different stages of the cell cycle

Radial

Radial properties are calculated via radial moments. Radial moments are special cases of the polynomial moments used in calculation of symmetry properties, namely those with angular index m=0. Thus, the polynomials are

$$R_n(\rho) = \rho^n$$

The n-th order radial moment is

$$\Phi_n = \iint P(\rho, \varphi)\rho^n d^2 S$$

In this application, radial properties are derived from radial moments up to the order of two (n=0,1,2). The ratio of the first to the zeroth moment is reported by the **Radial Mean** property. It is the intensity-weighted mean radial coordinate in pixel units.

The other radial property is the relative deviation of the radial coordinate. The corresponding output property is named **Radial Relative Deviation**. In terms of radial moments, it is expressed as

$$RelDev = \frac{\sqrt{\phi_0\phi_2 - \phi_1{}^2}}{\phi_1}$$

The third radial property is **Radial Mean Ratio** which is the mean radius on a filtered image divided by a reference radius. The reference radius is the mean radius calculated on the original image. This property is provided for filtered images only.

Profile

Profile properties are calculated with the help of profile images. Intensity of a profile image is a function of the closest distance from a given pixel to a cell border. For example, the following pair of images illustrate two complementary profiles. The first one is bright near the cell border and dark at long distances from the border. The other one is dark near the border and bright elsewhere.

Profile image 1/2

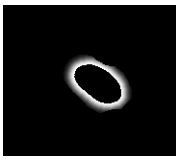
Profile image 2/2

Each profile image is used to characterize the intensity of an input image (original or filtered) but only in those locations where the profile image is bright. The profile image serves as a weight function when the mean intensity of an input image is calculated. Profile properties are unitless; this is achieved by dividing the profile-weighted mean intensity by a reference intensity (except cases when the image in input is already normalized, e.g. when characterizing a normalized texture-filtered image).

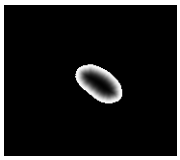
If a single region is defined where morphology is wanted to be characterized (e.g. whole cell) then two profile images are generated, as described by the pair of profile images above. If, however, in addition to the region of interest (e.g., cytoplasm or whole cell) an inner region is defined (usually, cell nucleus) then five profile images are generated.

Profile image 1/5 e.g. “inner side of plasma membrane"

Profile image 2/5 e.g. “inside the cytoplasm"



Profile image 3/5 e.g. “outside of the nuclear membrane"



Profile image 4/5 e.g. “inner side of the nuclear membrane"

Profile image 5/5 e.g. “inside the nucleus"

How fast the profile changes with the distance from border depends on the input parameter **Profile Width**.

Profile Width = 2

Profile Width = 8

The profile images are displayed on image view tabs, e.g. Profile 1/5, Profile 2/5 etc. In particular, this is useful when selecting an appropriate value for the **Profile Width** parameter.

5.2.17 Calculate Texture Properties

This is a building block for calculating texture properties (or features) in a cell region.

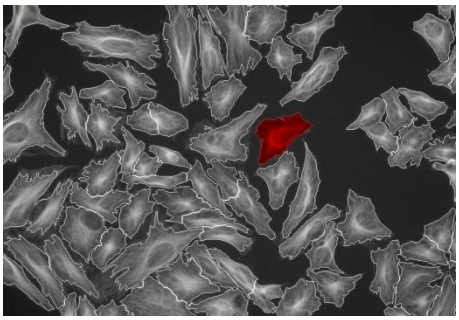
5.2.17.1 Working Principle

- The task is to calculate texture features for a region of interest.
- The basic first level inputs of *Calculate Texture Properties* are **Channel**, **Region** and **Method**.
- If a **global** channel is selected:
  - Mixing between local and global context is not possible
    - If a local channel is selected: only local populations can be selected
    - If a global channel is selected: only global populations can be selected
  - New overlays **Field Partition** and **Current Field** are available for information.
  - For details see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.
- The prefix of names for output properties can be selected in the box **Property Prefix**.
- There are three methods available:
  - **SER Features**
  - **Haralick Features**
  - **Gabor Features**

How to improve performance of *Calculate Texture Properties*

1. Select an image or a pair of images with two (or more) different classes of cells which you want to distinguish by texture.
2. Select a **Channel**, i.e. check whether the most appropriate image channel for texture classification is selected.
3. Select a **Method** and test how well the different features distinguish the selected classes of cells.
4. Test different methods using default values of second level parameters.
5. Select the most promising method and fine-tune the second level parameters.
6. (Advanced:) Search for the best combination of two or more features.

5.2.17.2 Example



Visual input: *A signal channel image with cell borders. One cell is highlighted.*

Object No	Cell Alexa 488 SER Spot 0 px	Cell Alexa 488 SER Hole 0 px	Cell Alexa 488 SER Edge 0 px	Cell Alexa 488 SER Ridge 0 px
41	0.0356053	0.035791	0.100209	0.0458127
42	0.0360488	0.0342373	0.0991811	0.0456148
43	0.0317592	0.0298008	0.0939852	0.0410497
44	0.0382544	0.0379041	0.11651	0.0543482
45	0.0435537	0.0421868	0.119758	0.0596666
46	0.038456	0.0361017	0.0986678	0.0481999
47	0.034028	0.0290055	0.117933	0.0485956
48	0.0431858	0.0388855	0.103176	0.0552805
49	0.0301866	0.0275876	0.129818	0.0435697
50	0.0354486	0.0357711	0.120501	0.0531318
51	0.0309399	0.0288291	0.103296	0.0393964
52	0.0373413	0.0347324	0.127387	0.0601364
53	0.0357531	0.0322895	0.111447	0.0501022
54	0.0434293	0.0399989	0.110651	0.0618549
55	0.0408188	0.0405767	0.0963689	0.052634
56	0.0411069	0.0397193	0.113968	0.0577486

Result: *Excerpt from a table of calculated texture features. For example, for cell number 44, the value of Spot feature is 0.0382544. The particular row is highlighted in the table (corresponds to the cell that is highlighted on the image).*

5.2.17.3 Methods

Overview of methods of *Calculate Texture Properties*:

Method	Mathematical basis for texture features	Keywords
SER	Gaussian derivative images	Gaussian filter, Gaussian derivative filter, scale-space, edge detection, ridge detection, Laws texture features
Haralick	Co-occurrence matrix	Co-occurrence, Haralick texture features, Robert Haralick
Gabor	Gabor-filtered images	Gabor filter, Gabor texture features, Dennis Gabor

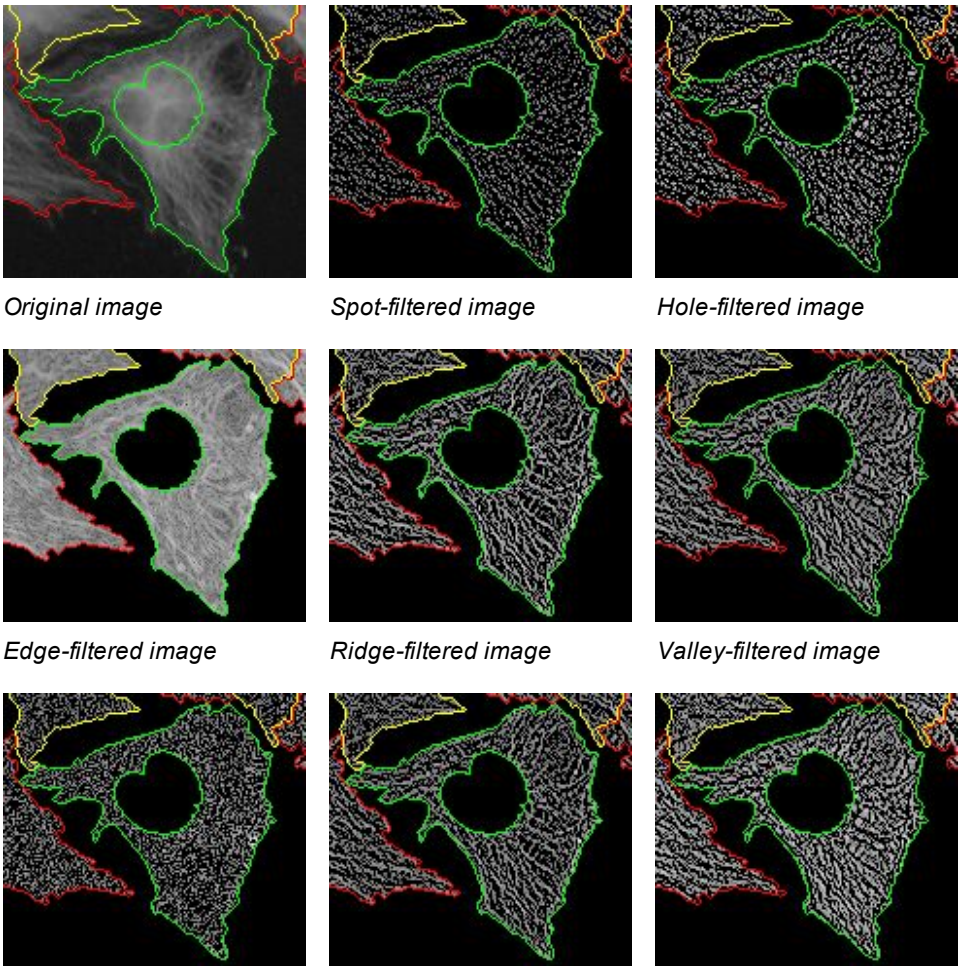
SER Features

+ It is frequently the best method for classification of cells.
+ For each feature, an illustrative filtered image is provided.
– This method is not widely known.

- There are eight SER features: Spot, Hole, Edge, Ridge, Valley, Saddle, Bright and Dark. SER is an acronym for Spots, Edges and Ridges.
- Each SER feature is calculated as intensity of a corresponding filtered image averaged over the corresponding object. See illustration 1 below.
- SER features have two second order input parameters: **Scale** and **Normalization**.
- **Scale** is the characteristic length of a smoothing operation when calculating filtered images. See illustration 2 below.

- There are three options for normalization of texture features.
  - Normalization by **Kernel** means that the filtered images are pixelwise divided by the smoothed original image.
  - Normalization by **Region Intensity** means that the filtered images are pixelwise divided by the average intensity of the original image in the corresponding object.
  - The third option is **Unnormalized** – objectwise intensity fluctuations are kept uncompensated (not recommended).

Illustration 1: Original image and eight SER-filtered images

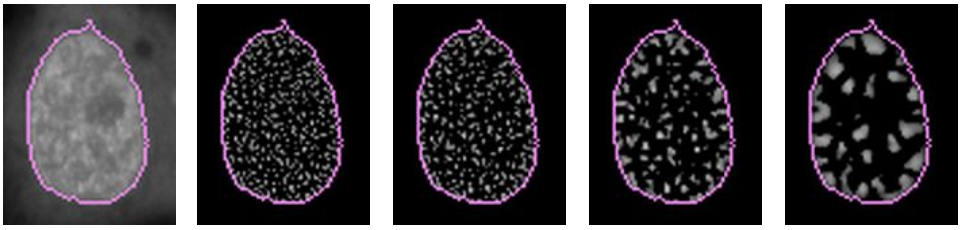


Original image | *Spot-filtered image* | *Hole-filtered image*

Edge-filtered image | *Ridge-filtered image* | *Valley-filtered image*

Saddle-filtered image | *Bright-filtered image* | *Dark-filtered image*

Illustration 2: Influence of scale on spot-filtered images



Original image | *Spot-filtered image at scale=0* | *Spot-filtered image at scale=0.5 px* | *Spot-filtered image at scale=1.0 px* | *Spot-filtered image at scale=2.0 px*

Haralick Features

+ Haralick features are a “standard” texture approach of 1970's.
– It is not based on image filtering. Therefore, illustrative filtered images are not provided.
– This building block calculates not all but a subset of four Haralick features.

- The four features calculated by this building block are:
  - Correlation
  - Contrast
  - Homogeneity (also known as Inverse Difference Moment)
  - Sum Variance
- Haralick features have a second level input parameter: **Distance**. Distance is a scalar input parameter characterizing the (orientation-independent) distance between the two pixels that are studied at a time.
- The calculated features are normalized in a way that the outcome does not significantly depend neither on intensity unit nor object-to-object intensity fluctuations.

Gabor Features

+ Gabor features seem to be most widely used texture features in literature.
+ The approach has been introduced by a Nobel Prize winner in physics, Dennis Gabor.
+ For each feature, an illustrative filtered image is provided.
– Gabor filter has many parameters. Therefore, this method has more second level parameters than the other two.
– Spatial aspect ratio is fixed to unity in this realization.

- There are two Gabor features calculated at a time: one corresponding to the minimum and the other to the maximum projection of Gabor energy images.
- Each numerical Gabor feature is intensity of a projection image averaged over the corresponding object.
- Gabor features have four second level input parameters: **Scale, Wavelength, Number of Angles and Normalization**.
  - **Scale** is the characteristic half-width of the Gaussian envelope function when calculating Gabor images.
  - **Wavelength** is the characteristic length of sinusoidal modulation.
  - **Number of Angles** is a technical parameter specifying in how many different orientations Gabor filter is applied for determining minimum and maximum projections. The recommended value is eight.
- There are three options for normalization of texture features:
  - Normalization by **Kernel** means that the filtered images are pixelwise divided by a Gaussian-filtered original image.

- Normalization by **Region Intensity** means that the filtered images are pixelwise divided by the average intensity of the original image in the corresponding object.
- The third option is **Unnormalized** -- objectwise intensity fluctuations are kept uncompensated (not recommended).

- Spatial axial ratio which is generally an additional argument of Gabor filters is fixed to unity.

5.2.18 Calculate Properties

This is a building block for calculating an additional property of objects.

5.2.18.1 Working Principle

- The task is to calculate an additional property of objects.
- The first level input of *Calculate Properties* is **Method**.
- Output property name/suffix:
  - For the method **By Formula** you can enter the name of the output property in the box **Output Property**.
  - For the method **By Related Region** a suffix can be entered in the box **Property Suffix**. It will be added to the automatically generated property name.

5.2.18.2 Example

Object No	Region Intensity Mean	Cell Intensity Mean
1	765.915	570.31
2	702.979	645.269
3	859.744	520.846
4	618.995	486.519
5	389.532	573.404
6	1071.15	689.356
7	1195.96	850.899
8	665.108	462.458
9	890.566	859.279
10	793.08	494.834
11	690.635	659.669
12	1281.03	786.891
13	494.743	374.244
14	696.382	475.435
15	951.772	725.697
16	936.151	550.105
17	840.248	566.47
18	311.827	246.035
19	308.125	294.762
20	326.042	313.075
21	814.089	679.678
22	247.711	315.871
23	414.085	364.76
24	508.085	302.234
25	642.665	453.086

Input: *Pre-calculated intensities in a region of interest and in the whole cell.*

Relative Intensity in Region
1.34298
1.08943
1.65067
1.27229
0.679332
1.55383
1.40553
1.4382
1.03641
1.60272
1.04694
1.62797
1.32198
1.46473
1.31153
1.70177
1.4833
1.26741
1.04534
1.04142
1.19776
0.784216
1.13522
1.6811
1.41842

Result: *Calculated relative intensity in the region of interest.*

In the example above, noise of the output property of the region of interest (CV = 20 %) is significantly lower than that of the input property (CV = 44 %). This is the reason why relative intensities are often preferred over absolute ones.

5.2.18.3 Methods

There are two methods available:

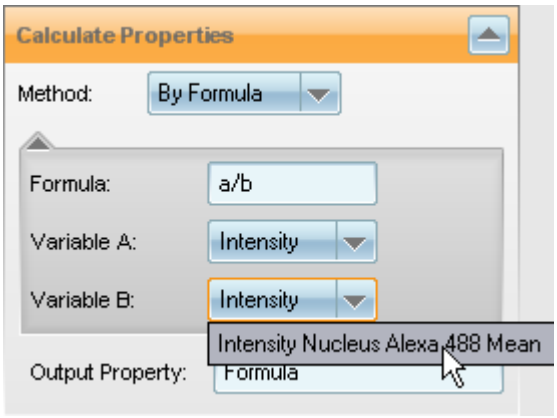
By Formula

A great number of functions of input properties can be calculated, according to a formula freely defined by the user, e.g.

- a/b
- (a-b)/(a+b)
- a/(a+b+c)

Characters in the formula such as *a*, *b* and *c*, are considered to denote existing properties, except standard functions, such as *cos* or *exp*.

- The user will be offered to select the meaning of the denotations (*a*, *b*...) among existing properties (e.g. **Region Intensity Mean**, **Cell Intensity Mean**).



Example: *Selecting the meaning of variable B.*

By Related Population

This method can be used to access properties of a related population. Populations are related if one population was created by finding objects inside the objects of another population. For example: A population of spots was created by finding spots in a population of cells. The properties of the spot population (e.g. spot roundness) can then be accessed in the cell population by the **By Related Population** method.

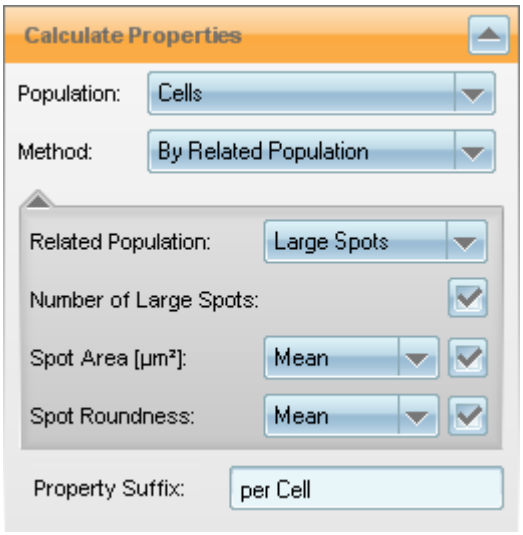
Relations are inherited when selecting a part of the population by *Select Population* building block. For example if you create a population “Large Spots” from a population “Spots”, all relations of “Spots” will be preserved.

Example:

Count the number of spots in each cell, that match a specific criterion, e.g. that are large and irregular.

1. **Find Nuclei** (output population named “Cells")
2. **Find Cytoplasm**

3. **Find Spots** inside the “Cell” region
4. **Calculate Morphology Properties** of the spots in the “Spots” population
5. Use **Select Population** to create a population “Large Spots” by filtering the “Spots” population
6. **Calculate Properties** in the “Cells” population using related population “Large Spots"



Adding properties from the population 'Large Spots' to the 'Cells' population

Summary | Properties Cells

Population- Cells	Value						
Number of Objects	9						
Property	**Mean**	**CV %**	**StdDev**	**Median**	**Max**	**Min**	**Sum**
Number of Large Spots- per Cell	131.444	17.3905	22.8589	132	158	97	1183
Spot Area [µm²]- Mean per Cell	0.797493	6.13781	0.0489486	0.77737	0.886994	0.741339	7.17743
Spot Roundness- Mean per Cell	0.896763	0.500073	0.00448447	0.896805	0.902736	0.887897	8.07087

Resulting properties of the 'Cells' population

Parameters

- **Related Population:** List of all populations that are related to the selected population. One can be selected.
- **List of related properties:** All properties of the selected related population are listed and each property can be selected or deselected. By activating the checkbox the property is added to the properties of the current population. A statistical operation (Mean, StdDev, CV %, Sum, Max, Min) can be selected to define how to calculate the value for multiple related objects (e.g. spots) in one object (e.g. cell).
- **Property Suffix:** Suffix which will be added to the name of the output property.

Hints and Tips

Relations work in both directions. In the above example also the properties of the “Cells” can be accessed in the “Spot” population as well. For example, for each spot could be determined if it was found in a binulceated cell.

Other building blocks that create related populations are

- **Find Neurites** - detects neurites attached to cell bodies. The detected neurites are related objects to the corresponding cell bodies.
- **Find Cytoplasm** - Method “E” finds polynucleated cells around previously detected nuclei. The nuclei are related to corresponding cells. The method “By Related Population” can be used to quantify the number and properties of the nuclei to the cells.
- **Modify Population** – the method “Cluster by Distance” creates a new population of objects from an existing one. For example you can create a population “Colonies” from a population “Cells”. Each colony consists of a set of cells. The method “By Related Population” in the *Calculate Properties* building block can then, for example, be used
  - To count the number of cells in the colony.
  - To count the number of cells of a certain phenotype in the colony.
  - To calculate minimum, maximum, average values, etc. of properties of the cells in the colony.

5.2.19 Select Population

This is a building block for selecting (creating) a subpopulation out of an existing one.

Notice

The method **Linear Classifier** uses the PhenoLOGIC™ technology and is only available with an optional license. For details see the following sections:

- “License Management”, page 320
- 5.2.2 “PhenoLOGIC™”, page 326

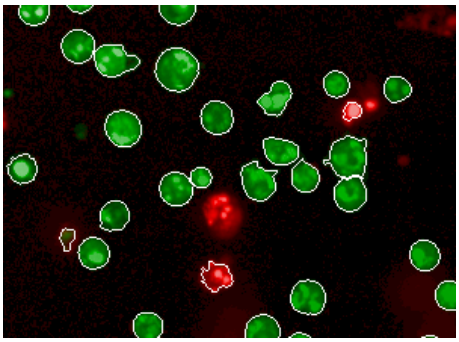
5.2.19.1 Working Principle

- The task is to select a subpopulation of the input population by applying a condition or multiple conditions.
- The first level inputs of *Select Population* are **Population** and **Method**. Local or global populations can be used.
- There are four methods available: **Filter by property**, **Common filters**, **Linear Classifier** and **Select by Mask**.
- The name of the output population of *Select Population* can be selected in the box **Output Population/Output Flag**.

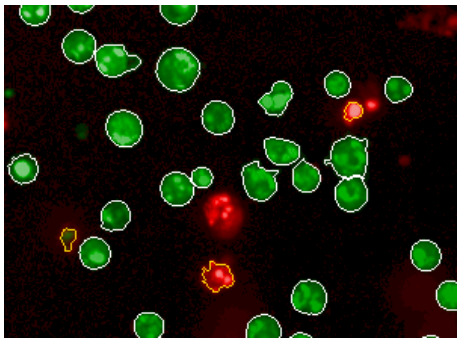
Notice

The building block *Select Population* cannot create sub-populations of **kinetic populations** (see section 5.2.24 “Track Objects”, page 438). However, using this building block it is possible to create an object flag, which indicates whether the object meets a user-defined criterion or not.

5.2.19.2 Example



Input population: *In this example, the input population of cells has been created by detecting cells from the green channel image. The detected cells are labeled with white borders. Not only cells that are green, but also some cells that are dominantly red have been detected.*



Output population: *We have created a subpopulation (labeled with white borders) applying the condition that fractional intensity in the green channel must be more than 90 %. The discarded cells are labeled with orange borders.*

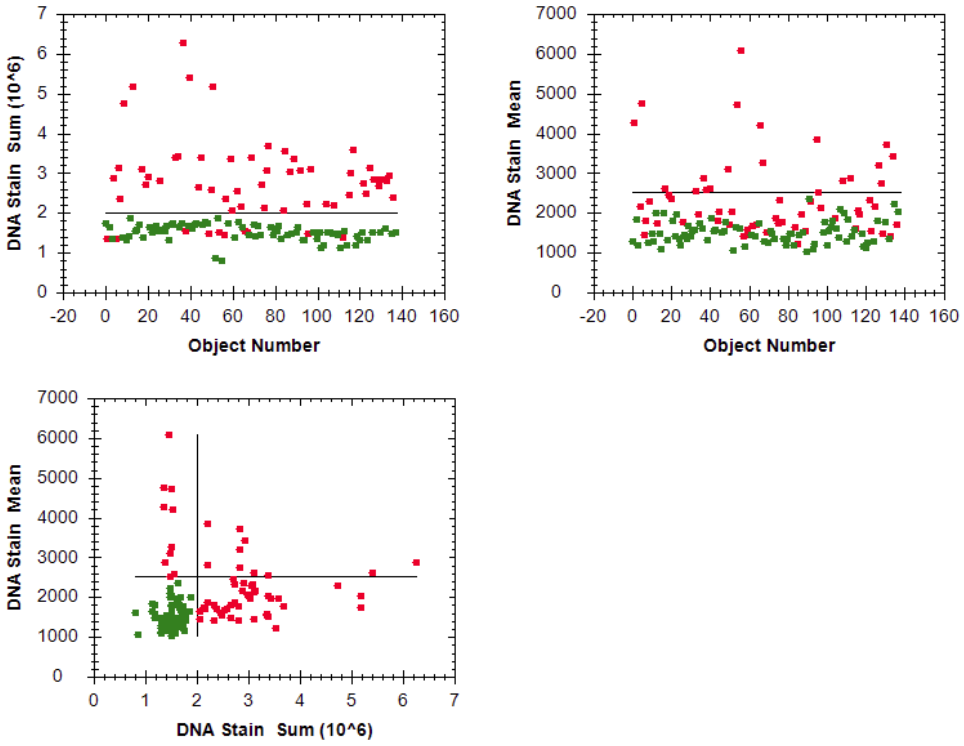
5.2.19.3 Method: Filter by property

- You may select one or more property filters.
- For each filter, you may select any available numeric property, a qualifier and a constant (e.g. **Green Fraction > 0.9**).
- If more than one filter is defined, then boolean operations with the results of individual filters are needed. The filters are referred to as **F1**, **F2**, etc.The default boolean operation with individual filters is **AND**, but the user may modify this.

> **Example:** The default boolean operation **F1 AND F2** may be modified to **F1 OR F2**.

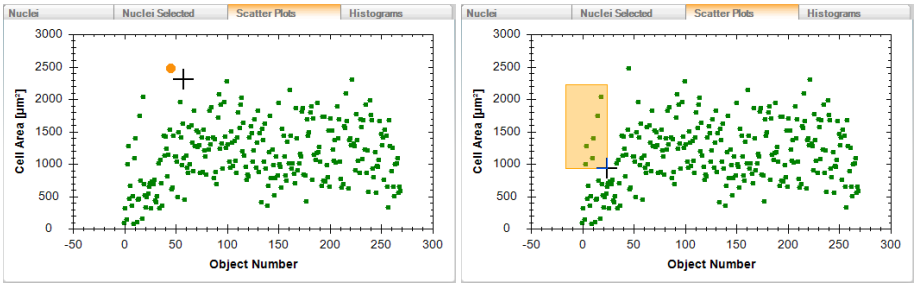
Scatter Plots

Each data point in the scatter plot corresponds to one individual cell. A green color indicates the selected cells which pass all criteria of the filter and the red color indicates the discarded cells. The black lines show the specified thresholds. A separate scatter plot is provided for each filter criterion (two properties selected in the example below). In addition, one scatter plot for each pair of properties is shown.



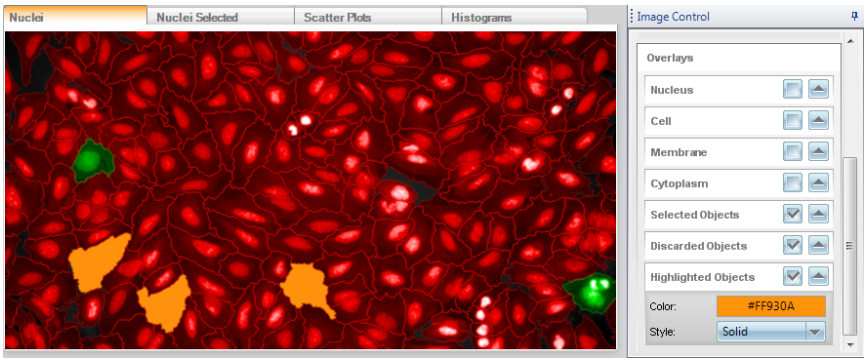
How to select data points and highlight cells

1. Define one or multiple filter criteria using the **Filter by Property** method.
2. Open the **Scatter Plots** tab.
3. Select the desired data points in the plot. Selected data points will be displayed as big orange dots. There are multiple ways to make the selection:



   - Click on a single data point.
   - Hold down the mouse button and drag the mouse to draw a rectangle on the plot. All data points covered by the rectangle will be selected.
   - To add data points to the selection, hold down the **CTRL** key and click on the data points or draw a new rectangle.
4. The selection has the following effects:
   - If there are multiple scatter plots, the corresponding data points will be selected in all scatter plots.

- Switch to the **Nuclei** or **Nuclei Selected** tab. The corresponding cells are highlighted (orange). Style and color of the overlay can be configured in the **Image Control** pane (**Highlighted Objects**).



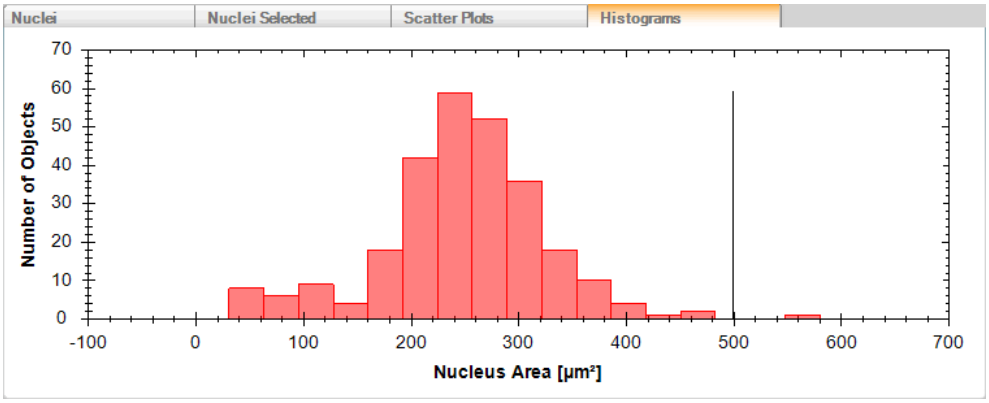
- The rows corresponding to the selected objects are highlighted in the **Image Analysis Results** table.

Hint

The selection works the other way round as well: If you click on a cell or select a dataset in the results table, the corresponding data point will be highlighted in the scatter plot.

Histograms

A histogram displays the distribution of values for the selected property. This makes it easier to define a threshold (black vertical line) which separates the populations correctly. If multiple filter criteria have been selected, one histogram per selected property is displayed.



5.2.19.4 *Method: Common filters*

The only option is **Remove Border Objects**.

- If the option **Remove Border Objects** is selected then the user is asked to specify the **Region** (e.g. **Nucleus** or **Cell**) which is not allowed to cross image borders.

5.2.19.5 Method: Linear Classifier

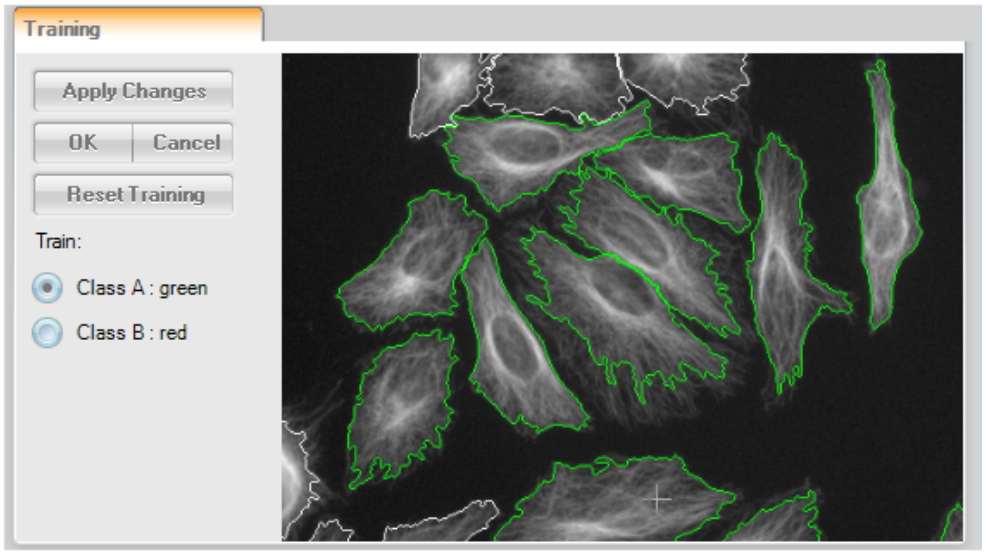
Working Principle

This method realizes a supervised classification task.

- It requires several properties (e.g., a set of texture or morphology features) precalculated for each object of the input population. The user may restrict the list of precalculated properties by manual switches.
- Supervised classification requires a training phase. As the result of training, a classifier will be created.

See also the Image Analysis Guide (section 1.13 “PhenoLOGIC™ – Texture Based Segmentation”) for background information and analysis strategies.

Training Mode

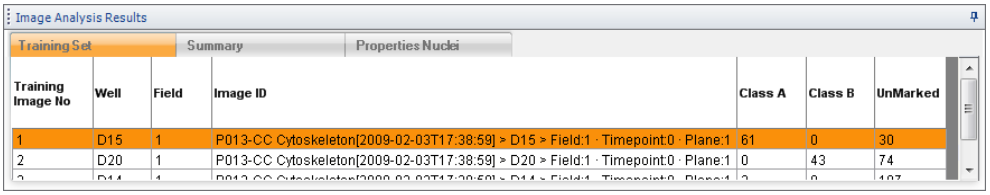


After selecting the method *Linear Classifier* you are automatically in training mode. Otherwise use the **Train...** button to enter the training mode. Only in this mode you can modify the input parameters of the method and select examples for each class on the image.

- Select a class and add (or remove) training examples for each class by clicking on the image. You can also drag a box on the image to select/deselect all objects inside the box.
- Use **Apply Changes** to update the preview of the classification after you have changed the input parameters or added/removed training examples.
- Click **OK** to exit the training mode and view the resulting populations.

Notice
You have to leave the training mode before you can open or add another building block.

Training Set



In the **Image Analysis Results** pane (**Training Set** tab) you can see the list of training images used so far.

- To add a training image, select the desired image in the **Navigation** pane, enter training mode and select training examples for each class.
- To remove a training image from the training set, open the corresponding image (see **Image ID**), enter training mode and remove all training examples.

When training on a **global image**:

- The training points are assigned to the global image instead of the currently selected image field.
- The field number of the global image is set to "*" indicating that all fields are used instead of a specific field. The field number is visible in the **Image ID** and the **Field** columns of the training set table.
- If an old analysis sequence with a classifier trained with Harmony version < 4.6 is loaded, the trained classifier still works, but field numbers are reported as trained in Harmony 4.5. The training of these classifiers cannot be modified, it must be reset and trained again.

How to use the method Linear Classifier

Precondition: Properties have been calculated which can be used to classify the objects (e.g. by *Calculate Morphology Properties*, *Calculate Texture Properties* or *Calculate Intensity Properties*).

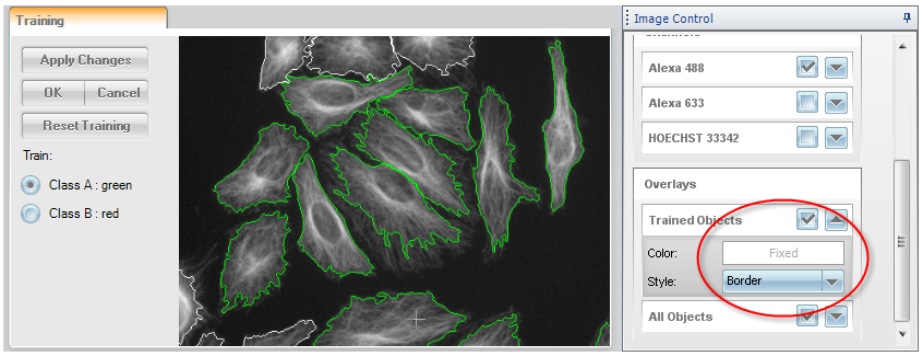
Initial Training

1. Insert building block *Select Population*.
2. Select method **Linear Classifier**.
3. Select the appropriate input **Population**.
4. Select the number of classes which shall be created (in this example: 2). If you select more than two classes, a linear classifier will be calculated for each combination of classes (A versus B, A versus C etc.).
5. Enter a name for each **Output Population**.
6. Select an image in the **Navigation** pane showing typical objects of **Class A**.
7. Click on the image and select example objects of **Class A**:
    - By clicking on a single object the object is added to the currently selected training class (radio buttons Class A, Class B, ... on the left side of the image). If the object was already in that class, it is removed from the class

and set to unselected ("toggling").

- Dragging a box on the image has the same effect as manually clicking on all objects inside the box. This allows quick selection of many cells, e.g. all cells on the image.

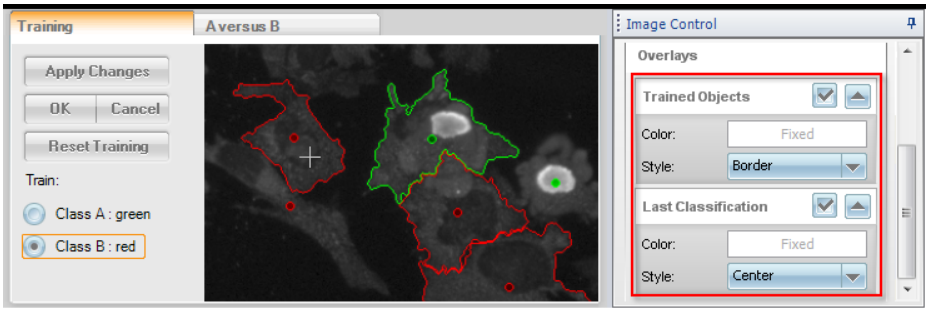
The training examples are indicated by a green circle. Depending on the objects, it can be helpful to select a different overlay style in the **Image Control** pane, e.g. “Borders”. To remove a training example, click the object again. It is recommended to add 50-100 examples per class (usually from multiple images).



8. Select an image in the **Navigation** pane that shows typical objects of **Class B** (only if needed).
9. Select **Class B** and select 50-100 examples of this class. The training examples will be indicated by a different color. Continue until you have selected a sufficient amount of examples for each class.
10. Click **Apply Changes** in the training section.
    All objects in the image will be classified, you are still in training mode.

Check Quality of Classification

1. To check the results, switch on and off the overlays **Trained Objects** and **Last Classification** (**Image Control** pane). Depending on the objects it may be helpful to select a different overlay style, e.g. “Center”.

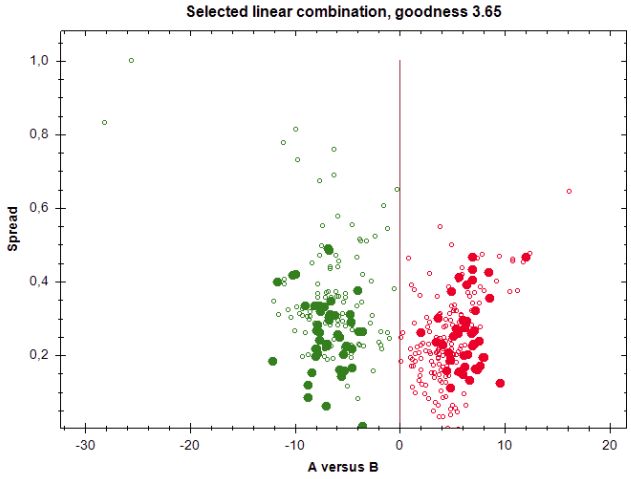


Meaning of object colors:

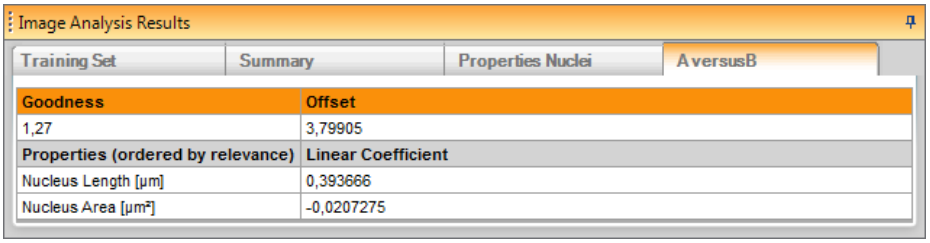
- **Green:** Class A
- **Red:** Class B
- **Blue:** Class C etc. for the other up to 6 different classes
- **Gray:** The object either does not belong to one of the displayed classes (e.g. in the illustration tab **A versus B** it is in the class C) OR it could not be

classified because a property selected in the linear classifier does not exist for this object (the value is “NaN").

2. Open the **A versus B** tab. In the scatter plot each training sample is represented by a solid dot; the color indicates the training class. The value of the classifier's linear combination is shown on the x-axis, a value greater than zero indicates class B (red), less than zero class A (green). Other objects in the training images are represented by a small circle, the color of the circle indicates the classified class for that object. The vertical red line divides the two classes.



3. Check the **Image Analysis Results** pane to see which properties have been used for the classification. On the **A versus B** tab the used properties are listed and sorted by relevance. If properties have been selected which seem to be artifacts, you can exclude them manually (see below).



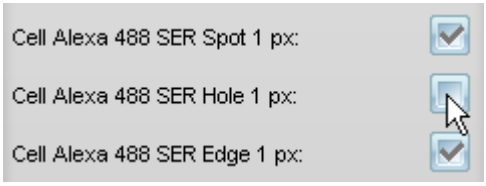
4. If the separation of the classes is not satisfying, try the following steps to optimize the results:

Improve Results

- Select more objects of each class.

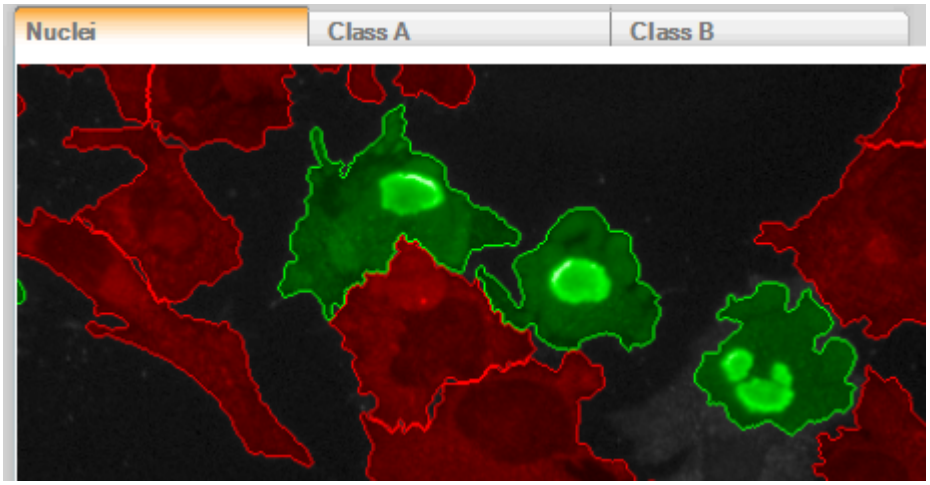
Manual Property Selection

- Exclude properties which are not relevant or which lead to artifacts. Disable the corresponding check boxes and click **Apply Changes**.



Finish Training

1. Click **OK** in the **Training** section.
   The objects will be classified, the training mode is finished. The detected populations are visualized by illustrations.



2. If you need to modify the training set, click **Train...** to enter training mode again.

How "good" is the classification?

The "goodness" of the separation is reported in the table and the title of the scatter plot. This is the signal to noise ratio based on the distance of the training points from the classifier line.

This value expresses, to some extent, the quality of the separation. It does however, provide information about the distribution of classification results – outliers, separation and shape of the populations (focused, splattered, multiple focus regions). The scatter plot is essential to fully understand the quality of the classification.

For details please see Image Analysis Guide, chapter 1.14 "PhenoLOGIC – Classification".

Buttons and Elements

Element	Description
Apply Changes	Applies the changes (e.g. modified input parameter or new training examples). Training mode is not terminated.
OK	Terminates the training mode and applies the changes.
Cancel	Terminates the training mode and discards all changes.
Reset Training	Deletes all training examples from all training images, i.e. the whole **Training Set** (see **Image Analysis Results** pane).
Train section	Displays the available classes. Select the class for which you want to add/remove training examples.

Parameters

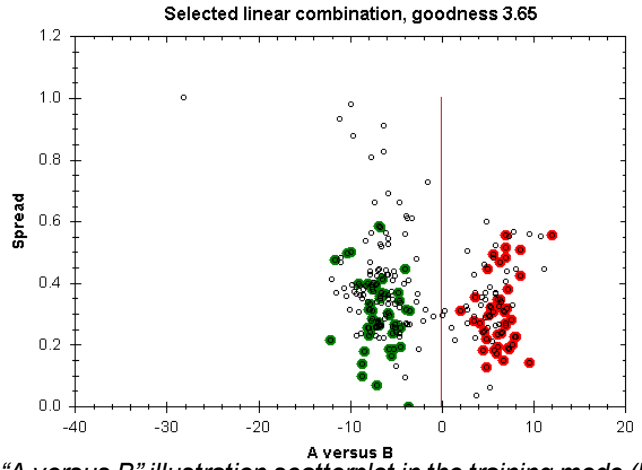
Second Level Parameter	
Number of Classes	Determines the number of classes which will be created (up to six).

Third Level Parameters	
List of calculated properties	Full list of properties of the input population calculated by previous building blocks. To exclude certain properties from being used for the classification (e.g. properties known to cause artifacts), disable the corresponding check boxes.

Output Properties

The following properties are added to the **input population**:

- **Class:** Class of the object (A, B, C …).
- **Regression A-B:** Value of the linear classifier as displayed on the x-axis of the "A versus B" illustration scatterplot in the training mode (see figures below).
- One **Flag** for each output population: Indicates if the object is contained in the population ("Phenotype A" and "Phenotype B" populations in the figure below).



"A versus B" illustration scatterplot in the training mode (Method: Linear Classifier)

Image Analysis Results

Summary | Properties All Cells

xture 2 px	Marker Texture SER Saddle 2 px	Marker Texture SER Bright 2 px	Marker Texture SER Dark 2 px	Class	Regression A-B	Phenotype A	Phenotype B
32	0.00803306	0.0124164	0.00697399	B	1.36703	0	1
31	0.0100336	0.0130504	0.0079402	B	0.750386	0	1
45	0.00501324	0.00678559	0.00852232	A	-48.5354	1	0
37	0.00802524	0.0113402	0.00790932	A	-1.88752	1	0
12	0.00955743	0.0141046	0.00637607	A	-3.21415	1	0
3	0.00764477	0.010007	0.00849745	B	3.24848	0	1
01	0.00749029	0.00884696	0.00803474	B	2.14866	0	1
22	0.00911446	0.0110431	0.00679175	A	-0.979277	1	0

Properties added to the input population (Method: Linear Classifier)

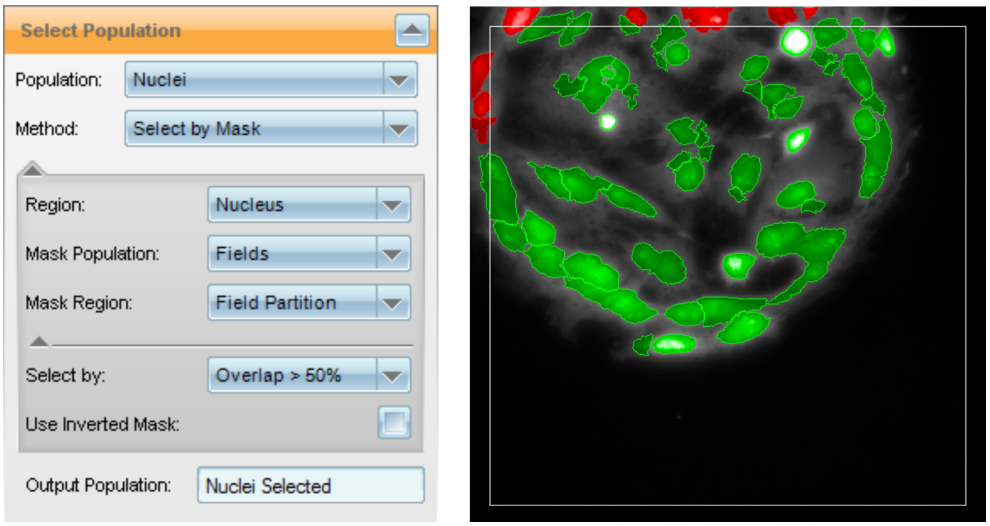
For each class a new **output population** is created and the following default properties are added:

- **Object Number in parent population**
- **Regression A-B** (see above)

Please note that for simplicity we always output only one property “Regression A-B”, also in case of 3 or more classes. In case other regression values are needed, they can be calculated by the *Calculate Properties* building block (see **Image Analysis Guide** for an example).

5.2.19.6 *Method: Select by Mask*

Allows to select objects by checking if their mass center, inner center or more than 50% overlap is inside the specified mask region. This is very useful for local populations to remove double counting of objects in overlapping fields.



Example: Only objects are selected (green) which have more than 50 % overlap with the Field Partition mask (white border)

- The mask region can be specified by a **Mask Population** and **Mask Region** input.
  - For global populations only global mask populations can be selected.
  - For local populations both local and global populations can be selected.
- The ROI “Fields - Field Partition” is available for local populations to remove double counting of objects in overlapping fields.
- Use the parameter **Select by** to define the selection criterion:
  - **Geometrical Center:** Object is selected if its geometrical center (center of mass) is inside the mask region.
  - **Inner Center:** Object is selected if its inner center is inside the mask region (needed for discontinuous objects).
  - **Overlap > 50 %:** Object is selected if more than 50 % of its area overlap with the mask region.
- The option **Use Inverted Mask** can be used to invert the selected mask and

thereby the selection of objects.

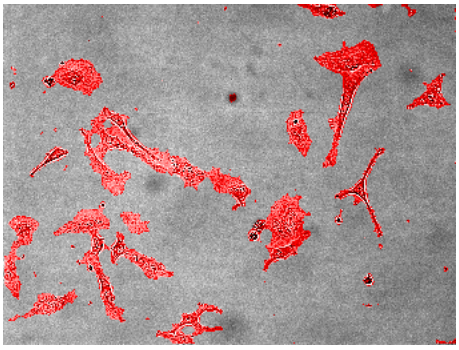
5.2.20 Modify Population

This is a building block for modification, e.g. splitting or merging objects, of an existing population, creating a new population.

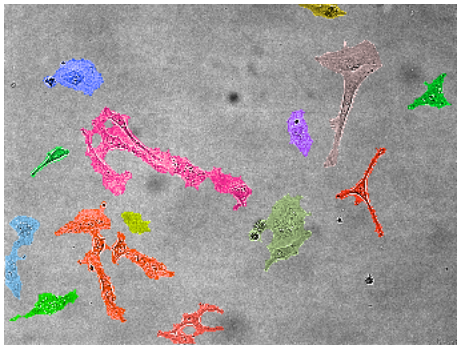
5.2.20.1 Working Principle

- The task is to create a new population, with objects consisting of fragments and/or several pieces of objects of the input population.
- The first level input parameters are **Population**, **Region** and **Method**.
- There is a single method available: **Cluster by Distance**.
- The name of the output population can be entered in the box **Output Population**.

5.2.20.2 Example



Visual input: *Image with overlay of the input population; here: a texture region*



Visual result: *Overlay of the created population*

How to configure *Modify Population*

1. Select a **Population**. If you already have more than a single population then check whether you have selected the most useful one.
2. Select a **Region**. If your interest is concentrated at a certain region of the input population then select the appropriate one.
3. Fine-tune the second level parameters using visual feedback of the created objects.

5.2.20.3 Method

Cluster By Distance

Second level input parameters	
Distance	• **Distance = 0:** Considers that distinct parts of the input region are different objects of the output population. • **Distance > 0:** Considers that only parts of the input region sufficiently far from each other are different objects. • **Distance < 0:** Considers that parts of the input region even if connected by a relatively narrow bridge belong to different objects.
Area	**>** sets lower threshold for the area of an object. Objects which are smaller than will be discarded.

Third level input parameter	
Fill Holes	If **Fill Holes** is selected then each region not belonging to any object but surrounded by a single object will be a part of that object.

5.2.21 Filter Image

Apply a filter to an image. The filtered image is available in a new channel, which can be used by subsequent building blocks.

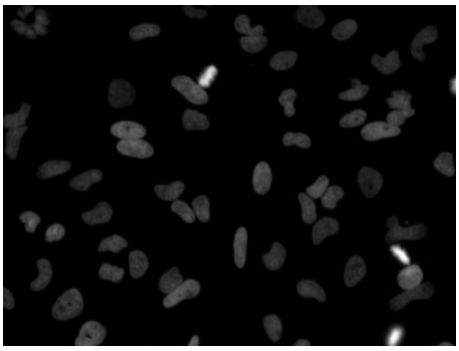
Typical applications are

- Image inversion to allow detecting dark objects instead of bright objects
- Image smoothing for noise reduction
- Background removal
- Object detection on texture filtered images

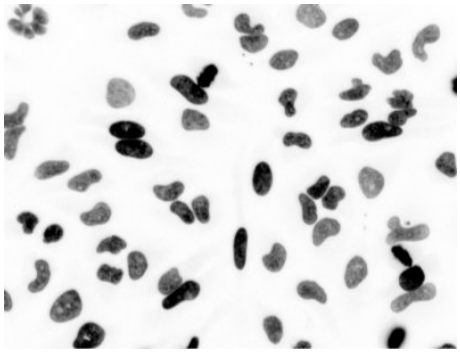
Notice

For the ready made object detection building blocks like *Find Nuclei, Find Cytoplasm, Find Neurites*, etc. no pre-processing is needed. These building blocks work best on the raw images.

5.2.21.1 Example



Original image



Inverted image

5.2.21.2 General Inputs

- **Channel:** Select an input image (local or global) to be filtered.
  - In case of global images, the overlays **Field Partition** and **Current Field** are available.
- **Method:** Select a filtering method.

5.2.21.3 Methods

Invert

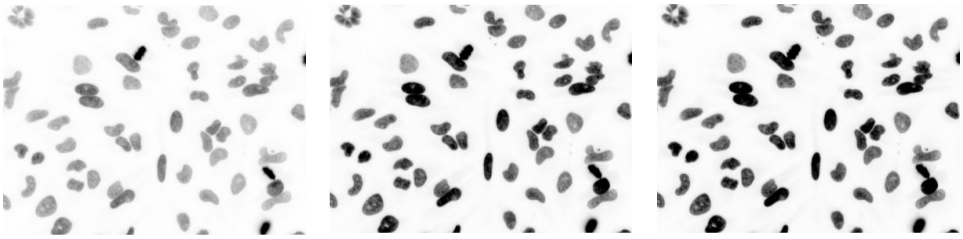
Invert the image. The highest intensity is mapped to the lowest and vice versa. There is one input parameter:

Cut-off Quantile

Can be used to ignore a fraction of very high intensity pixels when calculating the mapping. Useful to prevent overexposed pixels or bright artifacts from hiding image content in the inverted image. Valid range is between 0...100. Default is 100 (i.e. no cut-off). Typical values are 98..100%.

> **Notice**
> Intensity details are lost in the cut-off part of the image. All values above the cut-off threshold are mapped to minimum intensity.

Example of different cut-off quantiles:



Cut-off Quantile: 100. *Three very bright mitotic nuclei cause all other nuclei to be mapped to very high intensity values.*

Cut-off Quantile: 99. *Ignoring the 1% highest intensity pixels in the mapping causes more uniform mapping of nuclei.*

Cut-off Quantile: 98. *Same for ignoring the 2% highest intensity pixels.*

Notice

Make sure to be aware of the effect of the **Auto Contrast** and **Coloring** image view control settings when comparing images. **Auto Contrast** in coloring modes **Highlight** or **Extended** nonlinearly modifies the displayed (visual) image intensities. See also section 5.1.12 “Image Control”, page 195.

Texture SER

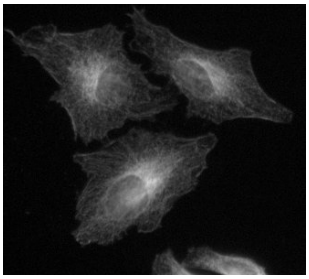
Calculate texture filtered images for use in other building blocks, e.g. for object detection or property calculation on the filtered image.

The algorithm and parameters are identical to the building blocks *Calculate Texture Properties* and *Calculate Morphology Properties*. You may refer to section5.2.17 “Calculate Texture Properties”, page 406 for additional information.

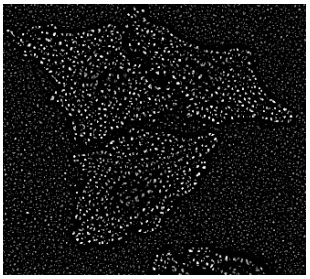
Filter

Available filters are: SER Spot, SER Hole, SER Ridge, SER Valley, SER Edge, SER Saddle, SER Bright, SER Dark

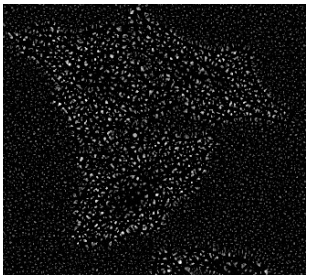
Examples of filtered images. A detail of a full image is shown. **Scale** is 1 px:



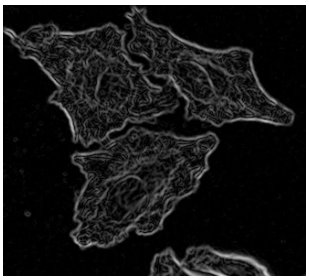
Original image (tubulin stained image)



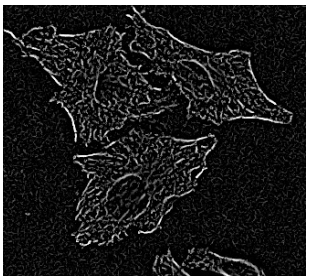
Filtered image SER Spot



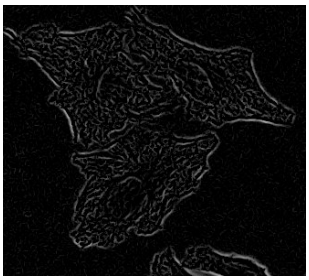
Filtered image SER Hole



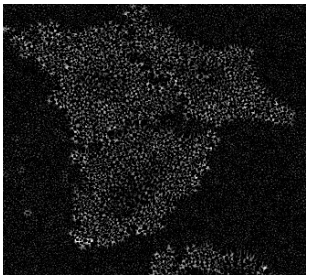
Filtered image SER Edge



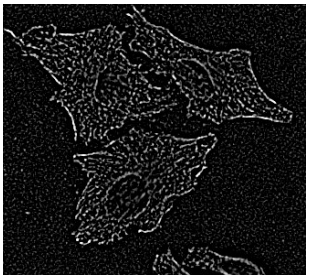
Filtered image SER Ridge



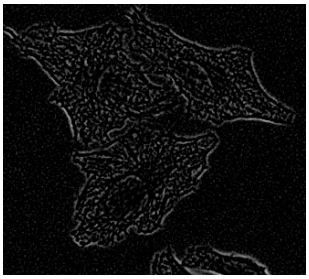
Filtered image SER Valley



Filtered image SER Saddle



Filtered image SER Bright



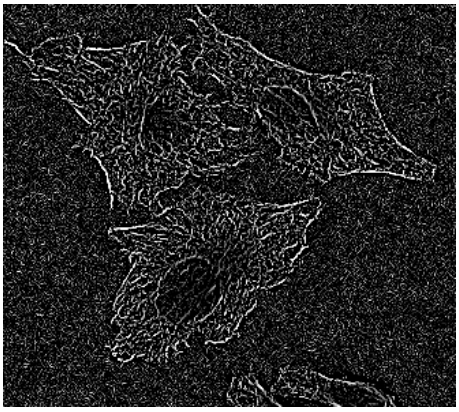
Filtered image SER Dark

Scale

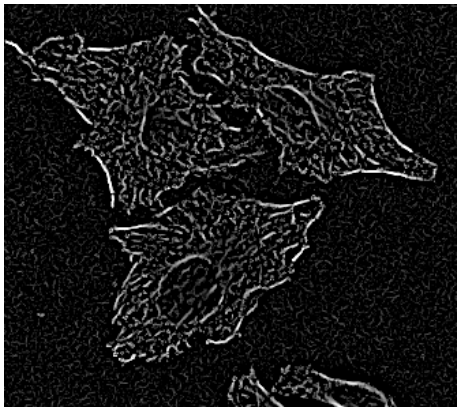
Characteristic size of the texture structures the filter is most sensitive for. The value corresponds to the standard deviation of a Gauss filter applied to the image during SER filtering. Valid range is 0...20px. Default is 1px.

Also fractions of pixels are a valid input. A value of zero means no Gauss filtering, in this case the texture detection is done on the original image, i.e. the smallest possible characteristic length is used.

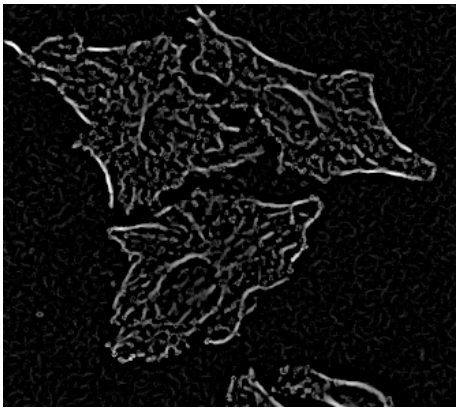
Here is an example of a **SER Ridge** filtered image with different scales (detail of the full image shown). Smaller scales allow smaller structures to pass the filter, bigger scales allow only bigger structures to pass the filter:



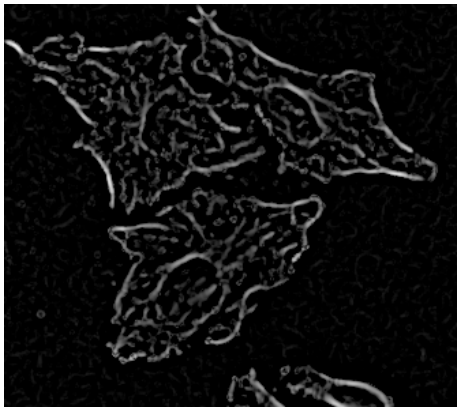
Scale 0.5 px



Scale 1 px



Scale 1.5 px



Scale 2 px

Normalization By

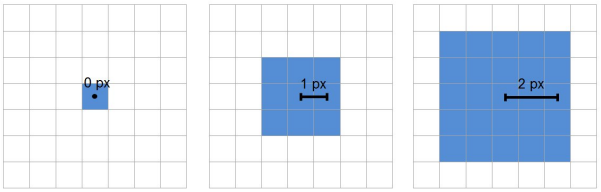
There are three options for normalization of texture features.

- **Kernel**: The filtered images are pixelwise divided by the smoothed original image.
- **Region Intensity**: The filtered images are pixelwise divided by the average intensity of the original image.
- **Unnormalized:** Image-to-image intensity fluctuations are kept uncompensated (not recommended).

Smoothing

Three common smoothing filter methods are available.

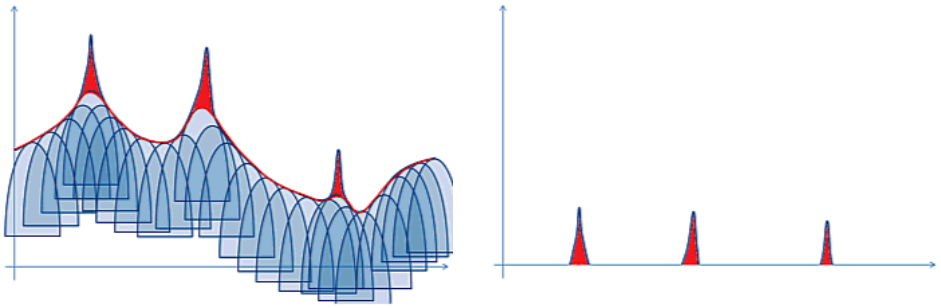
Second level parameters	
Filter	Choose the filter: • Gaussian • Mean • Median

Second level parameters	
Width	Characteristic size of the kernel for the **Gauss** filter. It corresponds to the standard deviation of the Gauss filter. Valid range is 0...1000 px, default 3 px. Click **px/µm** to switch the unit of the parameter. µm input values are translated to different pixel values for local and global images.
Scale	Characteristic size of the kernel for the **Mean** and **Median** filter. It corresponds to the half width of the kernel, see sketch below.  • Valid range for **Mean** is 0...1000 px, default 3 px. • Valid range for **Median** is 0...20 px, default 3 px.

Sliding Parabola

Used to remove smooth and continuous background intensity from the image. The image intensity is split into a background and a foreground part. The foreground part is returned as filtered image.

Here is a sketch of the principle. The image intensity on a line through the original image is shown on the y-axis. It shows how some high intensity spots (red area) are separated from smooth background (blue parabola area). The result of the filter is the spot intensity area (marked red) on a flat background.



Intensity of original image *Result of the filter*

Curvature

Smaller values mean more details are preserved in the filtered image (the parabola becomes "wider"), bigger values mean more details are removed (the parabola becomes "narrower"). A suitable curvature depends both on intensity and size of the objects which we want that they remain on the scene. The curvature must be

significantly less than two times peak intensity divided by radius square of a typical object. For example, if intensity is 1000 and radius is 10 pixels then an appropriate curvature is 1 or 10 but not 200.

Curvature has a logarithmic scale, useful values may cover a very wide range, e.g. 0.01, 0.1, 1, 10, 100. Allowed value are between 0...INF. Default is 10.

Notice
Use the **Tune Parameter...** function (right mouse button context menu) to quickly pick the best value from a typical range of **Curvature** values. See also section “How to tune a parameter (numerical parameter)”, page 143.

5.2.21.4 Output

- The resulting filtered image is returned as a new channel (local or global, depending on input channel).
- The channel name can be defined by the parameter **Output Name**. Default name is dependent on the method, e.g. “Inverted Image”. In case of a global channel the suffix "(global)" is automatically attached to the output name.
- For quick comparison of the filtered and original image, the original image is shown on a second illustration tab in the image view. Switch back and forth between the two image to compare details.
- No numerical output is generated.

5.2.22 Calculate Image

This building block calculates a new image from existing images. A formula can be specified including any number of images. The resulting image can be used as input for subsequent building blocks.

Potential applications are

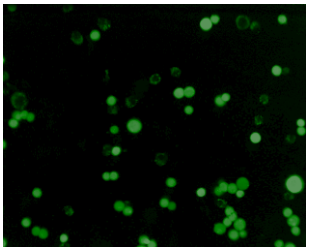
- Add fluorescent marker channels to perform object detection on the combined signal
- Calculate FRET ratios
- Normalize image intensity

Notes for using global images:

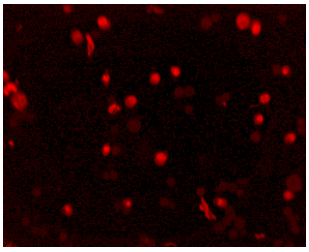
- Only images of the same scope can be used in a calculation:
  - The first image can be arbitrarily selected (global or local).
  - The following images can only be of the same type as the first.
- The overlays **Field Partition** and **Current Field** are available.
- The resulting image is local or global, depending on the input images.
- For detailed information see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329.

5.2.22.1 Example

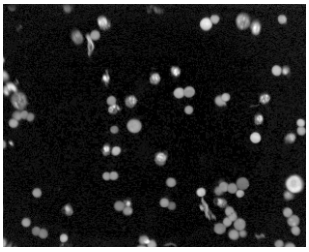
Adding two channels to create a combined image of two channels for object detection.



Live cells stained with CMFDA



Dead cells stained with ethidium homodimer-1



Combination of both channels shows all cells. Formula: "A/A.mean + B/B.mean" with A=CMFDA and B=ethidium homodimer-1

5.2.22.2 Methods

Currently there is only one method available: **By Formula**

Method "By Formula"

Each image pixel of the calculated image is determined by a formula from the values of the corresponding pixels in the input images.

Second level parameters	
Formula	An arbitrary formula can be entered as a text, e.g. *A+B* or *(A+B)/C*. Single characters in the formula such as **A**, **B** and **C**, are considered to denote existing images. For each character used in the formula, a drop down list appears to select one of the existing channels. • The formula may contain the following operators, brackets can be used to define operator precedence: *+, - , *, /, >, <, ==, >=, <=, &&, ||, not, (,)* • All common functions like *sin, cos, log10*, etc. can be used, e.g. *log10 (A)*. • For conditinos use *iif(Condition, ExpressionTrue, ExpressionFalse)*, e.g. *iif(A>B, A, B)* to take the higher intensity value of two images. • Some image properties can be denoted using the syntax *A.mean*: *mean, min, max, sum, median, fmedian, stddev, finitemin, finitemax*. • The syntax *quantile(A, 0.75).quantile* can be used to access the 75% intensity quantile value for use in the formula. Examples of advanced formulas: • *A/A.mean* – normalize the image intensity, a value of 1 represents the average intensity. • *A>A.mean* – all pixels above average intensity in image A are set to 1, all other pixels are zero. • *A>B* – results in an image which is 1 if the intensity in image A is bigger than in image B, all other pixels are zero. Use the Find Image Region building block to segment this image. • *(A>B) && A>500* – find pixels with intensity in image A bigger than in image B AND intensity in image B is bigger than 500. • *iif(A>2500, A, 0)* – keep only pixels with intensity values bigger than 2500, set the rest to zero.
Channel A, B, ...	For each character used in the formula, a drop down list appears to select one of the existing channels.

Third level parameters	
Negative Values	Defines if negative pixel values are allowed in the output image. If the result may contains negative values, the image cannot be handled by building blocks which are not prepared for handling negative intensity values. This is true for most of the object detection building blocks, since they are tailored for measured images from a camera and assume that the image don't contain negative values. Currently only the following building blocks support negative values: • *Calculate Image* • *Calculate Intensity Properties* • *Filter Image* Options are: • **Set to Zero:** Set all negative values to zero. This is the default setting. • **Shift Intensities:** Add a constant value to all pixels of the image so that the lowest intensity pixel is zero. This is equivaluent to using the formula *iif(A.min<0, A+A.min, A)*. • **Keep:** Keeps negative values. If this option is used, the resulting image is blocked to be used in building blocks which don't support negative values handling.
Undefined Values	Defines how undefind values (*NaN*, "Not a Number") will be represented in result images. These values may e.g. occur by divisions by zero or sqare root of negative values. Options are: • **Set to Zero:** Set pixels with undefined value to zero. • **Set to Local Average:** Use average value of the surrounding pixels. This fills gaps more smoothly than setting the pixel to zero. E.g. average calculation or texture feature calculation is less affected. This is the default setting.

5.2.22.3 Output

- A new channel, available for use in the subsequent building blocks. The new channel can be used in the same way as the measured channels. The name for the newly created channel can be selected in the **Output Image** box (default is “Calculated Image”). For global images, the suffix "(global" is attached to the output name.
- A table with statistical properties (mean, min, max, etc.) of the calculated image is displayed. Information about the number of undefied and negative pixels before and after correction is included.
- An overlay indicating any negative pixel in the image (before potential correction by the **Set to Zero** or **Shift Intensity** option). This allows to be warned if pixel values occur which might need special care when used by following building blocks or which have been automatically modified in the output image. The default color is red, default style is **Center** to avoid missing single pixels with negative values.
- An overlay indicating undefined pixels in the image (before potential correction by **Set to Zero** or **Use Local Average**). This allows to be warned in case undefined values have occured. Default color is yellow, default style is **Center**.

In case there are red or yellow overlays indicating negative and/or undefined pixels:

- Double check if the formula is correct and the result is expected.
- Double check if the handling options for negative or undefined values are configured appropriate for your application. Adjust **Undefined Values** and **Negative Values** parameters. Alternatively you may add explicit handling in the formula to avoid undefined values, e.g. *iif(A!=1000, A/(A-1000), 1)* to avoid the undefined value at intensity value 1000.

5.2.23 Determine Well Layout

This building block is a key element for setting up a **PreciScan** measurement. It must be included in the online analysis of the PreScan experiment.

Notice
This building block is part of the **PreciScan** feature. It is only available with an optional license. For details see the following sections:

- “License Management”, page 320
- 5.1.8.3 “PreciScan™”, page 119

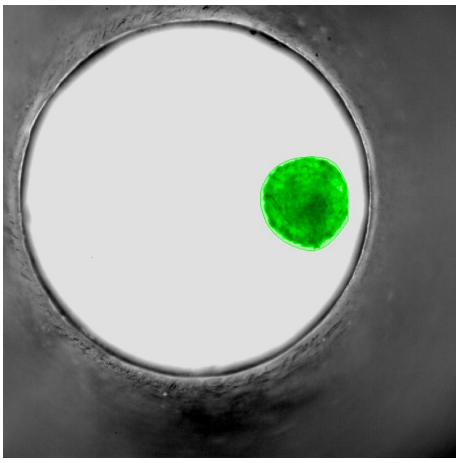
5.2.23.1 Working Principle

The user sets up an analysis sequence for the online analysis of the PreScan experiment in which he identifies the desired objects that shall be measured in the ReScan. All currently available building blocks can be used to detect and filter these objects with the exception of the kinetic building blocks. At least one population of objects must be present before *Determine Well Results* can be added.

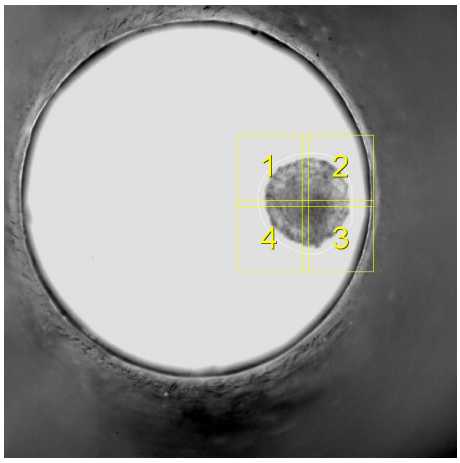
The output of this building block is a well layout with optimized field positions for every well capturing all objects of the selected population. This is visualized by an illustration on the **Well Layout** tab. This well layout will be used by the ReScan experiment.

- The first level inputs of *Determine Well Results* are **Population**, **Region** and **Method**. The input population can be local or global.
- There is one method **Standard** with several input parameters.
- The name of the output population of *Determine Well Results* can be selected in the box **Output Population**. Depending on the selected input population the output is a local or global population. In case of a global population, the suffix " (global)" is automatically attached to the output name.

5.2.23.2 Example



Input: *Population of objects for the ReScan*



Output: *Well layout with optimized field positions (yellow frames)*

5.2.23.3 Method: Standard

Second Level Parameters	
Rescan Magnification (mandatory)	Magnification of the objective lens which will be used for the subsequent ReScan experiment.
Max. No Fields	Allows you to limit the number of fields that are measured in the ReScan (default: empty). If you enter a limit, quasi-random positions are selected from the number of all fields determined by the analysis until the limit is reached, starting in the center. Non-overlapping fields will be selected as long as possible, then fields with increasing overlap. If you want to remove an existing limitation, please delete the entered value.
Object Margin [µm]	Allows you to define a margin around each object as a buffer to compensate for offsets. The buffer "inflates" the selected objects (also shown in the "Covered Objects" overlay), thus forcing the field placement to keep them further away from the field rims. • Default: 10 µm • Range: 0-100 µm
Field Overlap [%]	A certain minimum overlap of the fields of the ReScan is required to be able to match them exactly. • Default value: 2% • Can be increased if required, but should not be reduced to avoid gaps in the stitched images.

5.2.24 Track Objects

Track Objects allows to follow the position of moving objects (e.g. cells) over multiple time points.

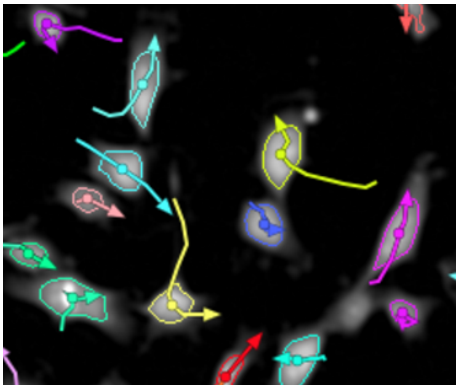
> **Notice**
>
> Analysis sequences including the **Track Objects** building block cannot be used for online analysis.

See also the **Image Analysis Guide** (section 1.15 “Object Tracking”) for background information and analysis strategies.

5.2.24.1 Working Principle

- The first level inputs of *Track Objects* are:
  - **Population:** Population of objects to be tracked.
    Tracking local population objects in the presence of a global image is supported. Tracking global population objects is not supported.
  - **Region:** Region to be used for tracking, e.g. “Nucleus” or “Whole Cell”
  - **Method:** Only one method “Standard” is available
- The input population is transformed into a new **kinetic population**. The name of the new population can be defined by the **Output Population** parameter. By default the prefix „Tracked“ is added to the input population name.
- See also below section 5.2.24.6 “Outputs”, page 442 for details on the output properties.

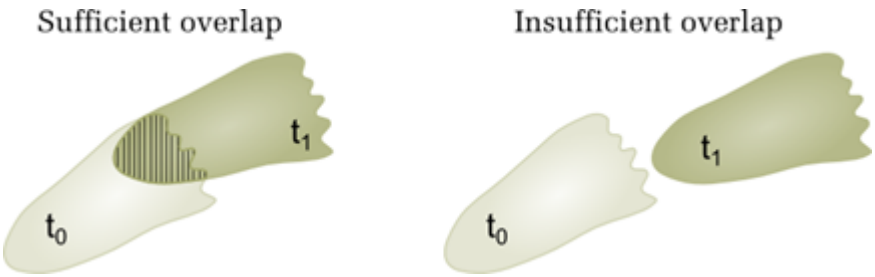
5.2.24.2 Example



Tracks of moving cells

5.2.24.3 Prerequisites

1. **Time series measurement:**
   The same sample must have been measured at a series of consecutive time points. The object positions in two consecutive images must have an overlap to allow robust tracking (see figure below).



Cell positions in two consecutive time points must have an overlap for object tracking

2. **Object detection:**
   The objects to be tracked must have been identified by a previous building block (e.g. by *Find Nuclei* or *Find Cells*). Correct splitting of the objects is essential for subsequent tracking.

5.2.24.4 Tracking Objects

Time Window

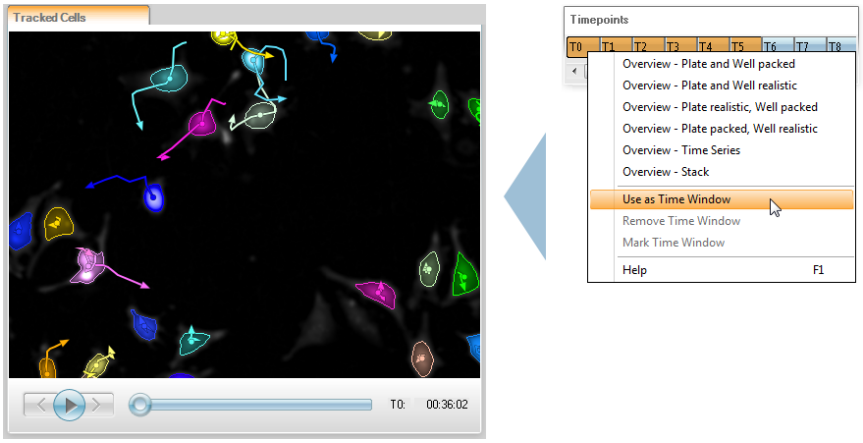
A time window is a subset of a time series measurement, used to develop an analysis sequence which makes use of object tracking. It consists of 1 well, 1 field and multiple time points. As long as the time window exists, you can only select and work with the images included in the time window. Other wells, fields, time points, etc. cannot be selected. This is done to reduce calculation times when developing an analysis sequence with object tracking. When using this analysis sequence on the **Evaluation** tab, the whole measurement will be analyzed of course. See also section 5.1.11.1 “Plate Measurement”, page 171.

How to use Track Objects

1. Click + and select *Find Nuclei* or *Find Cells*.
   The building block is added to the analysis sequence.
2. Click + and select *Track Objects*.
   The building block is added to the analysis sequence.
3. Select an input **Population** of objects to be tracked.
4. Define a time window in the **Navigation** pane:
   - Select one single well and one single field. If there is a stack, select one or multiple planes.
   - Select multiple time points.

     Notice
     It is recommended to select only a few time points (e.g. five) to reduce calculation times during object tracking and allow interactivity.

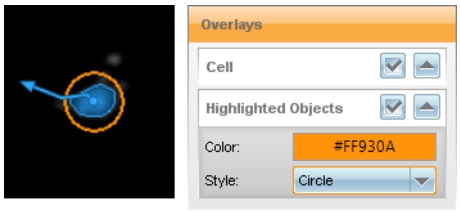
   - Right-click on the selection and select **Use as Time Window** from the context menu.



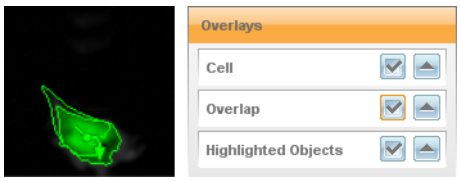
The object tracks will be calculated immediately by analyzing the overlap between adjacent time points of the time window. The objects and the determined object tracks are shown as a movie clip in the **Content Area** area. See also section 5.1.13.2 “Movie Control”, page 213.

Numerical properties of the tracks are shown in the **Track Properties** table. For details see section 5.2.24.6 “Outputs”, page 442.

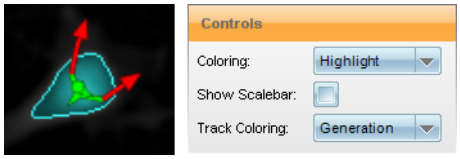
5. Useful visualization options (**Image Control** pane):



Change the style of overlay **Highlighted Objects** to **Circle**. This can help to find and watch selected objects. In contrast to the default style **Solid** the object itself remains visible.



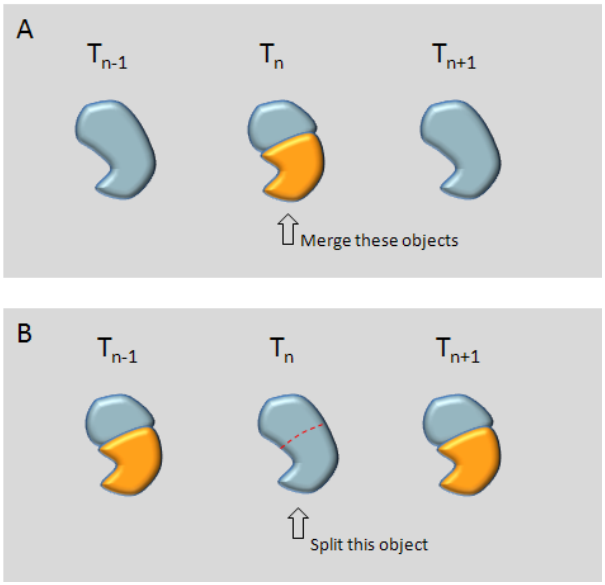
Activate the overlay **Overlap** to view the overlap between two adjacent time points.



Select the desired **Track Coloring**, see section 5.1.12.1 “Controls”, page 195. E.g. coloring by generation allows you to easily find split cells (red).

6. You can add building blocks to calculate further properties of the tracked objects. These can be conventional properties like object morphology, intensity or texture, and **time-dependent properties** like current speed or object displacement, using the following building blocks:
   - 5.2.25 “Calculate Kinetic Properties”, page 445: Object properties calculated per object and time point.
   - 5.2.26 “Calculate Track Properties”, page 447: Properties for the entire object track.

5.2.24.5 *Method: Standard*

Second Level Parameters	
Track Object Division	If switched off splitting and merging of objects is not tracked. The biggest object is followed after splits. No cell relations are recorded.
Correct Detection Errors	By default objects which exist for only a single time point are considered as detection errors and corrected automatically (split or merged), see figure below. This can be switched off by deactivating the check box. 
Discard Single Timepoint Tracks	Tracks of objects which are visible for only one time point are removed by default (as long as they are not referenced as a parent object of other objects). This behavior can be switched off by deactivating this check box.

Third Level Parameters	
Overlap [%]	Minimum object overlap between two time points needed to consider the objects are the same. Default is 1 %. In case multiple objects have an overlap, the one with the biggest overlap wins.
Unit for Properties	Object properties can be reported in pixels [px] or micrometers [µm]. Default is [µm].

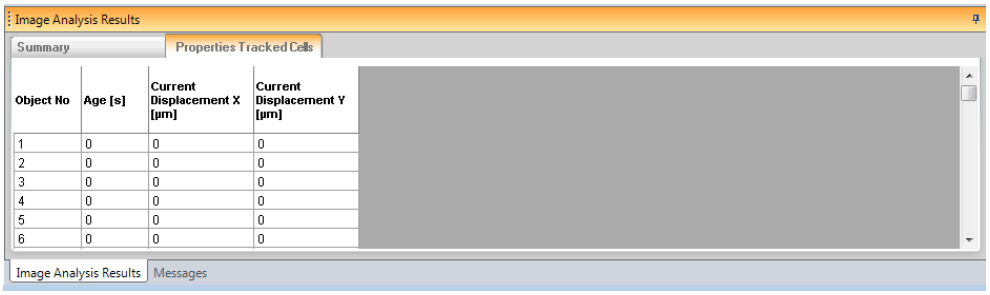
5.2.24.6 Outputs

Notice

The building block *Select Population* (see section 5.2.19 “Select Population”, page 414) cannot create sub-populations of **kinetic populations**. However, using this building block it is possible to create an object flag, which indicates whether the object meets a user-defined criterion or not.

Properties

In the **kinetic population**, object numbers are globally aligned across all time points, i.e. object number 6 is always the same object in all time points. If it is not visible in an image, the object number is not shown in the corresponding property table. That means: The **Properties** tables of kinetic populations do not need to start with object one and usually do not have a continuous numbering.



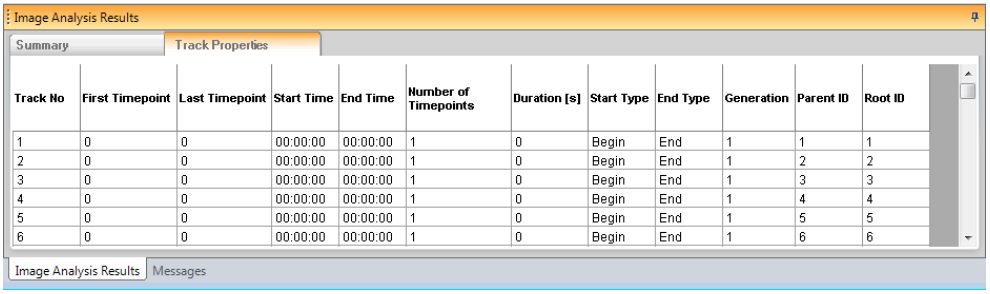
Default properties added to a kinetic population

These properties are calculated for each individual time point:

- **Age [s]:** Time passed after the object was detected first. By using “Age” as x-axis in a graph cells can be easily displayed synchronized in cell cycle stage.
- **Current displacement X [µm/px]:** Displacement of the cell from the position it was detected first.
- **Current displacement Y [µm/px]:** Same for y coordinate.

Track Properties

A kinetic population has a second associated table, the **Track Properties**. This table collects all properties that belong to the whole track, e.g. the **Track Length**. These properties cannot be associated with a single image or time point. The track properties are time-aggregated information, they do not have a time point information. Track numbers and object numbers are the same, i.e. track 6 refers to the track generated by object no. 6.

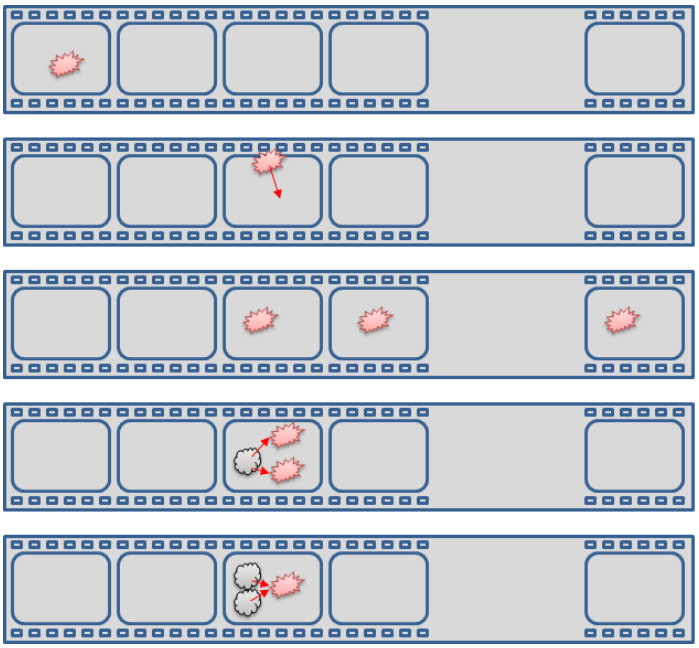


Default track properties

These properties are calculated for the entire track:

- **First Timepoint:** Timepoint number at which the object appeared.
- **Last Timepoint:** Timepoint number at which the object disappeared.
- **Start Time:** Time the object appeared (relative to the begin of the measurement).
- **End Time:** Time the object disappeared (relative to the begin of the measurement).
- **Number of Timepoints:** Track length in number of time points.
- **Duration:** Track length in number of seconds.
- **Start Type:** The way the object appeared, see section 5.2.24.7 “Start Types”, page 444 for an explanation of the classes.
- **End Type:** The way the object disappeared, see section 5.2.24.8 “End Types”, page 444 for an explanation of the classes.
- **Generation:** Counts how many splits resulted in the current object, see section 5.2.24.9 “Assignment of Object IDs”, page 445.
- **Partent ID:** If start type is „Split“ this is a reference to the parent of this object, see section 5.2.24.9 “Assignment of Object IDs”, page 445.
- **Root ID:** Object ID of the very first parent object generating this object by a series of splits. All objects with the same Root ID form a complete family tree of objects resulting from the root object.

5.2.24.7 Start Types



Begin: Object is there from the first timepoint

Border: Object enters at the border

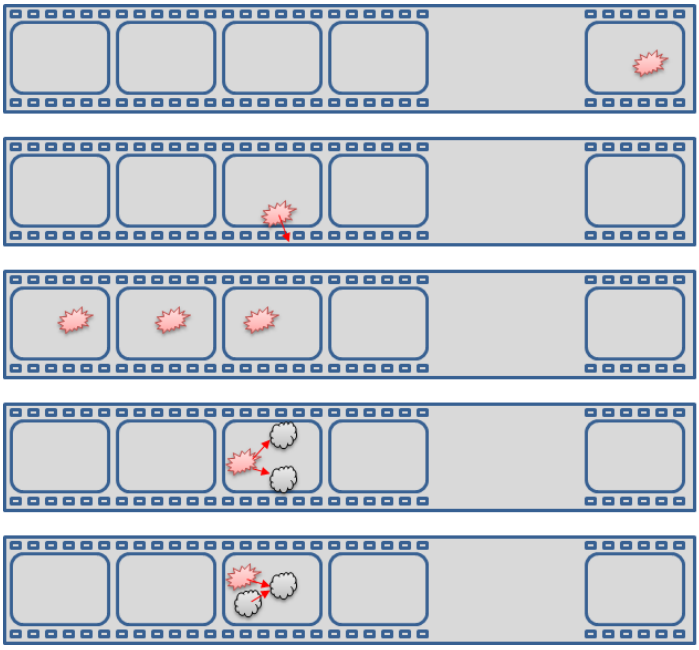
Cosmos: The object just appears

Split: From a split (e.g. mitosis)

Merged: From a merge (e.g. cluster formation)

Start types classifying how an object appears. This information is very helpful to select or discard wanted or unwanted tracks.

5.2.24.8 End Types



End: Object is still visible on last frame

Border: Object leaves at border

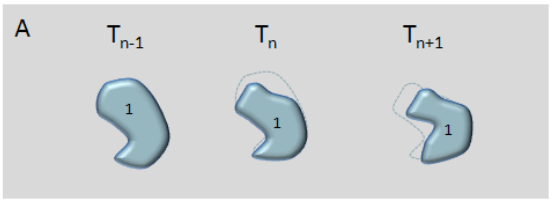
Cosmos: Object just disappears (off to cosmos)

Split: Object splits into two new objects (e.g. mitosis)

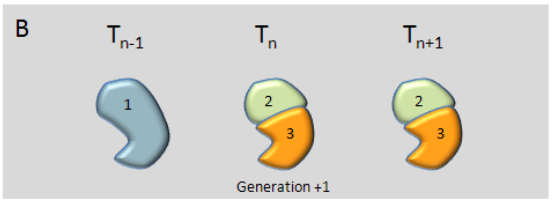
Merged: Merged with another object (e.g. cluster formation)

End types classifying how an object disappears. This information is very helpful to select or discard wanted or unwanted tracks.

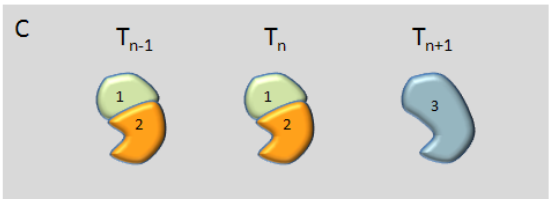
5.2.24.9 Assignment of Object IDs



Movement of objects



Splitting of objects* (e.g. mitosis)



Merging of objects
(e.g. cluster formation)

***A**: Moving object*
***B**: Splitting of objects. The two new objects get new object IDs (here: 2 and 3) and the parent object ID (here: 1) is recorded in their properties. Their "Generation" property is incremented by one (parent's generation +1).*
** When split into more than two parts, the parts have start type "Cosmos" and generation zero.*
***C**: Merging of objects.*

5.2.25 Calculate Kinetic Properties

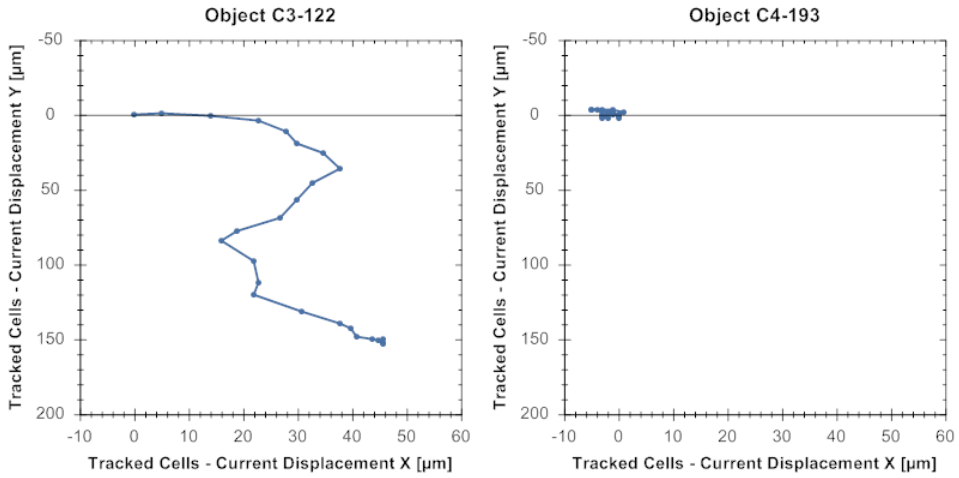
Calculate Kinetic Properties allows to characterize the current movement and speed of objects by comparing the current object position with the positions in the neighboring time points. A kinetic population created by a *Track Objects* building block is required.

5.2.25.1 Working Principle

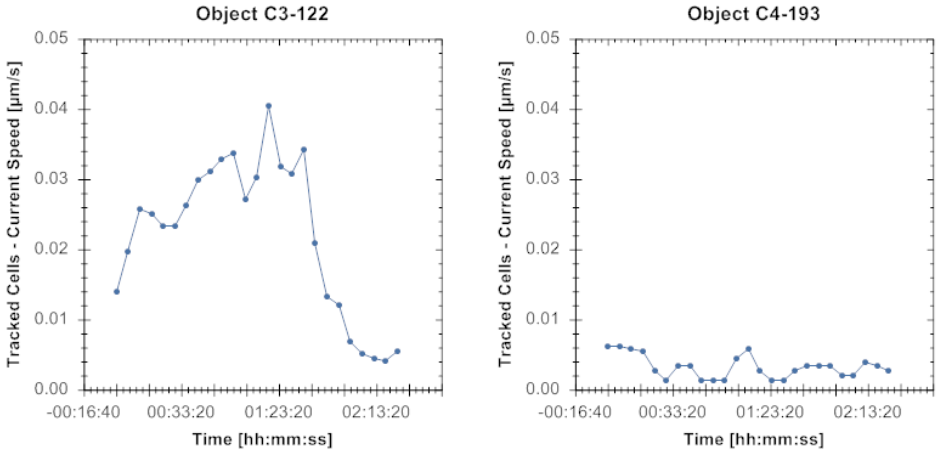
- The first level inputs of *Calculate Kinetic Properties* are:
  - **Population:** Kinetic population of tracked objects
  - **Method:** Only one method "Standard" is available
- The selected output properties are added to the kinetic population. See also below section 5.2.25.4 "Outputs", page 447 for details on the output properties.

5.2.25.2 Example

Here is an example for two cells, one moving fast and slowing down after a while (left panels). The other cell (right panels) is moving slowly:



Position of two cells monitored over 2.5 hours



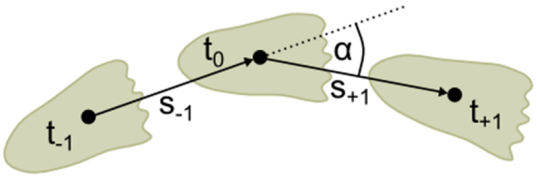
Measured speed of the objects plotted as a function of time

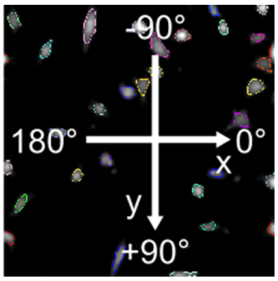
5.2.25.3 Method: Standard

Second Level Parameters	
Current Step Size	Tick check box to output this property, definition see figure below. Default: off.
Current Speed	Tick check box to output this property, definition see figure below. Default: on.
Current Direction	Tick check box to output this property, definition see figure below. Default: off.
Current Turning Angle	Tick check box to output this property, definition see figure below. Default: off.

5.2.25.4 Outputs

To calculate properties at least two time points are needed. In case only one time point is available for an object “NaN” is returned as a result.





$$\text{Current Step Size} = (s_{-1} + s_{+1})/2$$
$$\text{Current Speed} = (s_{-1} + s_{+1})/(t_{+1} - t_{-1})$$
$$\text{Current Turning Angle} = \alpha$$

Definition of output properties. Right: Coordinate system for “Current Direction” property

5.2.26 Calculate Track Properties

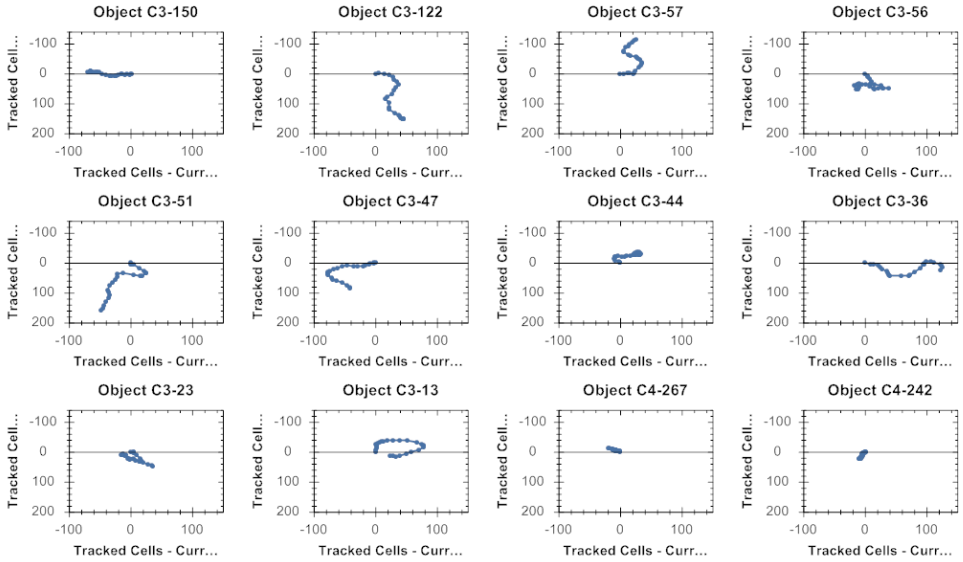
Calculate Track Properties allows to characterize the tracks of moving objects (e.g. cells). A kinetic population created by a *Track Objects* building block is required.

5.2.26.1 Working Principle

- The first level inputs of *Calculate Track Properties* are:
  - **Population:** Kinetic population of tracked objects
  - **Method:**
    - **Standard:** Set of common measures of whole track properties.
    - **Time Aggregation:** Calculate statistics of cell properties over a full track, e.g. average cell intensity while moving on the track.
- The new properties are added to the **Track Properties** table of the kinetic population.

5.2.26.2 Example

Here is a selection of different tracks. *Calculate Track Properties* can generate parameters like displacement or straightness to classify them or quantify differences.



5.2.26.3 *Method: Standard*

Activate the corresponding check box to calculate a property and add it to the **Track Properties** table of the kinetic population.

Second Level Parameters	
Accumulated Distance [µm/px]	Total length of the track from start point to end point (sum of all individual sections).
Displacement [µm]	Length of a straight line from track start to track end (see figure below).
Speed [µm/s]	**Accumulated Distance** divided by **Duration** (definition see building block *Track Objects*, section "Track Properties", page 442)
Straightness	**Displacement** divided by **Accumulated Distance**. It is equal to one for a straight line. "Meandering" of the track decreases the value.
Displacement X [µm]	X-direction component of the **Displacement** vector, see figure below.
Displacement Y [µm]	Y-direction component of the **Displacement** vector, see figure below.

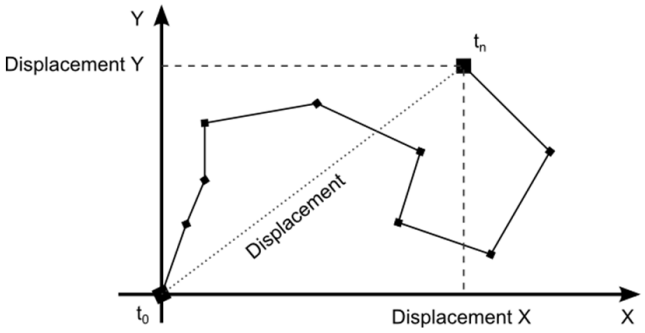
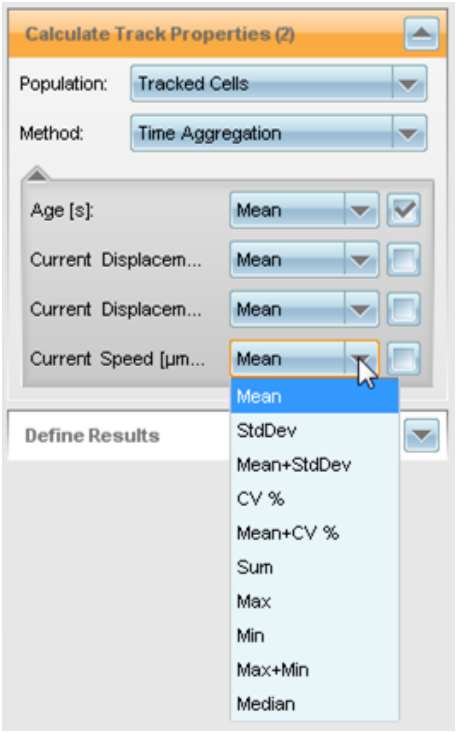


Illustration of the “Displacement” properties

5.2.26.4 Method: Time Aggregation

A list of all available properties of the tracked objects is shown. By activating a checkbox and specifying a statistical operation, the *Mean, StdDev, CV%, Sum, Min, Max, Median* or combinations of it can be calculated over the tracks. For example, the minimum speed on the track or the maximum intensity of a marker can be calculated as a track property.



5.2.27 Define Results

This is a building block for performing the statistical analysis of arbitrary populations, including kinetic populations. It also defines the **assay readout values** for each well of a microplate (results per well). If desired, you can specify to save also **Object Results** to the database.

5.2.27.1 Working Principle

- All properties calculated by the preceding building blocks (for local and global populations) can be included in this readout, as well as any combination (by formula).
- For global populations there are two new overlays **Field Partition** and **Current Field**.
- For each readout value to be reported, the building block contains one method block which can be added in the building block to specify what method to use to generate this value.
- First level input of *Define Results* is **Method**. You can add an arbitrary number of methods, i.e. readout values.
- The set of second level inputs is specific to each method.

- In addition to the standard properties, well results can also be defined based on **track properties** of a **kinetic population**.
- Results per object are also saved to the database if you select *All* or *Use Selected Well Results* for a population in section **Object Results**.

How to add a readout value

Precondition: Analysis sequence includes building block(s) calculating properties (e.g. *Calculate Properties*, *Calculate Intensity Properties*, *Calculate Morphology Properties*).

1. Open the **Define Results** building block.
2. Select a **Method** for selecting the readout value.
   The corresponding parameters are expanded and a new empty method is added to insert further readout values.
3. Configure the second level parameters to select the desired property and statistical analysis to report.
   Please refer to section 5.2.27.3 “Methods”, page 452for detailed information.
4. If applicable, define an **Output Name** for the readout value.
5. Click Apply to update the view in the **Results** pane. The defined readout values are listed on the **Results** tab.

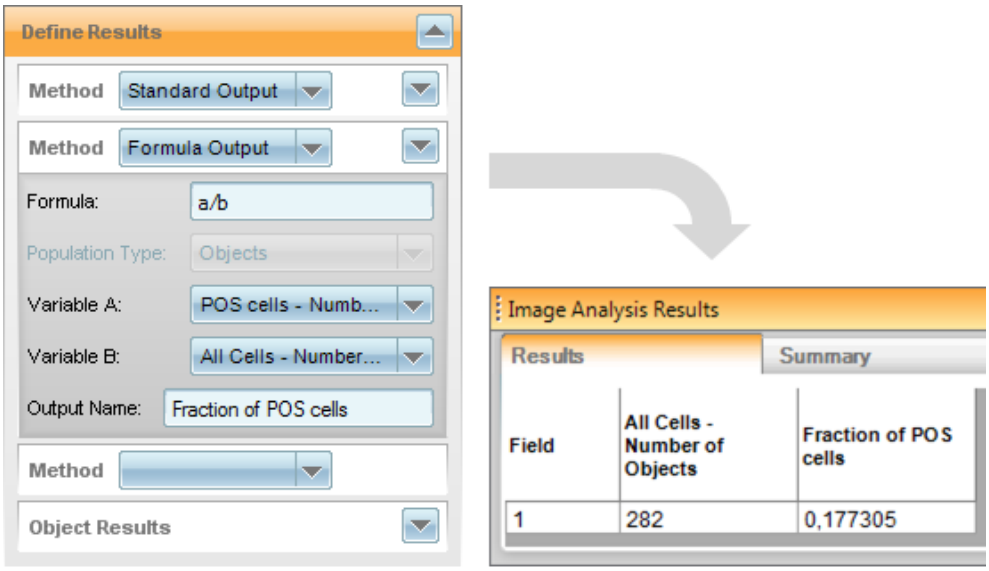
How to remove a readout value

1. Open the **Define Results** building block.
2. For the readout value which you want to remove, select the “*empty position*” from the **Method** combo box.
   The whole method block is removed.

Illustrations

Depending on the building blocks used before, illustrations will be displayed in the **Content Area**. As a default, the first channel used in the analysis sequence will be shown. You can configure the appearance of each illustration using the elements in the **Image Control** pane (channels, overlay styles, colors etc.). If you click Apply and Save..., these settings will also be stored with the analysis sequence in the database.

5.2.27.2 Example



Define Results building block generating two readout values: "All Cells – Number of Objects" and "Fraction of POS cells"

5.2.27.3 Methods

List of Outputs

Offers a list of all previously calculated properties (for example the calculations determined by building blocks such as *Calculate Properties*). The properties are grouped by population. The prefix of the group name is determined by the population type:

- **Objects:** Contains object properties of a standard or kinetic population.
- **Tracks:** Contains track properties of a kinetic population (properties which are valid for the entire track).

Select a statistical method for all values to be reported (individually or one method for all).

Second level parameters	
Number of Objects	Default property of any population. Activate the check box to report this value.
Apply to All	If you select a statistical method from this combo box, it will be applied to all properties of that population (individual selection is disabled). If you want to select individual methods for each property, choose **Individual Selection**.

Second level parameters	
Property / Statistical Method	Select one or multiple previously calculated properties to report by choosing a statistical method. If you select the *"empty"* position, the value will not be reported. The statistical method defines how to process the object results into a single value for the whole population: • Mean, Standard deviation, Mean + Standard deviation, CV %, Mean + CV %, Sum, Max, Min, Max + Min, Median, ALL • Empty: Value is not reported

Standard Output

This method is similar to **List of Outputs**. The differences are that you can only select one property to report per method block, and you have the option to rename this readout value.

Second level parameters	
Property	Choose one previously calculated property to report.
Statistical Method	Select the desired statistical method to define how to process the object results into a single value for the whole population: • Object Count, Mean, Standard deviation, CV %, Sum, Min, Max, Median
Output Name	Enter a name for the readout value.

Formula Output

This method allows you to create a formula, e.g. for calculating a ratio of two properties.

Second level parameters	
Formula	Enter a formula using an arbitrary number of properties. The arithmetic operations "+", "-", "*", "/" and "()" can be used. After adding this method block, there are two variables, **A** and **B**, displayed. You can add further variables to the formula. Any character from **A** to **Z** is allowed. **Example** You want to use a third variable **X**:Enter your formula, e.g. "*a*+*b*+*x*" into the **Formula** box and press **Enter**. A new combo box is added automatically, where you can define the meaning of the variable **X**.

Second level parameters	
Population Type	Track properties and object properties *cannot* be mixed in one formula. To avoid this, you have to select a **Population Type**: • **Objects:** Only object properties of a standard or kinetic populations are displayed and can be used in the formula. • **Tracks:** Only track properties of a kinetic population are displayed and can be used in the formula. If there is no kinetic population available, population type **Objects** will be selected automatically.
Variable A, B, ...	Defines the meaning of the variables: Select one of the previously calculated properties. Select the desired statistical method to define how to process the object results into a single value for the whole population.
Output name	Enter a name for the readout value.

5.2.27.4 *Object Results*

The **Object Results** section allows you to save also results per object to the database. This can be configured individually for each population (local or global). After the evaluation they can be exported for use in statistical programs via **Settings – Export Data**. See also section "Export Data", page 262.

First level parameter	
Population	• **None:** Object results are not saved in the database to save disk space (default setting). • **ALL:** All object properties which are explicitly calculated by building blocks (e.g. *Calculate Intensity Properties*, *Calculate Morphology Properties*) are saved in the database. I.e. all columns of the object tables displayed by the *Define Results* building block are saved. **Notice for "ALL"** This option generates large and space-consuming tables in the database. Only choose this option if you really need it. • **Use Selected Well Results:** Only those cell properties which have corresponding well results defined are saved in the database. E.g. if the "mean nuclear area" is defined as a well result, the "nuclear area" is also saved for each individual cell. **Notice for "Use Selected Well Results":** • Properties referenced in formula outputs are not saved in the database. • The object table displayed by the *Define Results* building block shows all properties (not only the selected ones).

5.3 Troubleshooting

5.3.1 General Tips

In this section you can find tips and tricks which help you to avoid common pitfalls when working with Harmony.

5.3.1.1 Installation

- **Verify access to Application Guide's data before installing ODA**
  Only relevant when updating a Harmony installation on the device PC.
  - The ODA setup will also update provided data objects like plate types and analysis sequences. If you had relocated the images of “Application Guide"’s measurements and the image location path is not accessible any more, the setup cannot update the complete data set – it will be available, but out of date. This will be shown at the end of the setup.
- If the error message “Communication to <instrument name> lost – Please check power and cables” occurs while updating the device firmware, please ignore this error message and also do not check power and cables as suggested. The update process will not be affected by this incident.
- When you switch off the Operetta CLS, the time for the shutdown procedure may vary. It can take up to 8 minutes until the Operetta CLS is completely off. Do not remove the power cable .
- It is possible that UV excitation light causes auto fluorescence of the transmission light source’s diffusion disk. This fluorescence can be seen with the 1.25x Air objective due to its large detection aperture. Workaround: Cover the assay plate with a lightproof lid when using the 1.25x Air objective.

5.3.1.2 General

- Please make sure that your instrument operates in an environment as specified in the corresponding **Site Readiness Document** (Site-, Electrical-, Environmental-, Safety-, IT and Software requirements).
- We strictly recommend saving backups of the Harmony PC on a regular basis. In case of loss of the database it will not be possible to restore any results.
- Please make sure that your Harmony PC always provides enough disk space (> 1 GB) to avoid errors. Use **Relocate Images**, **Write Archive** and/or **Delete Data** to free up disk space.
- **Power settings:** Please make sure that the sleep mode is disabled on every PC running Harmony. Awaking from the sleep mode may result in a not-responding Harmony software and requires a reboot of the computer.
- In case of an unexpected error message, please click the **Continue** button. Usually the software will work again afterwards.
- If the Acapella Server has been closed during a measurement, it is restarted automatically. Please note that the image display and online evaluation may not work anymore until you restarted Harmony. However, the data transfer to the database will not be affected.
- Please keep in mind that accessing shared folders in the network via Harmony's data management functions may lead to Harmony “not responding”. Please select “Wait for the program to respond” and wait patiently until Harmony is accessible again.
- In some cases, the tooltip indicating an error says “Input string was not in correct format.” This means that you entered e.g. decimal numbers, negative numbers, special or alphabetic characters, but the text box requires a positive integer. Please correct your input.
- Please do not change the monitor settings in the **Display Properties** of Windows for the Harmony PC. The **DPI Settings** must be set to “normal size” (96 dots/pixels per inch).
- If Harmony was closed in an unexpected manner during a measurement, or the instrument restarted during a measurement, the measurement will be continued and completed and stored in the database. So just be patient and wait for the measurement to be completed.
- **Image view:** If you zoom in on an image, there may be different patterns on the image depending on the zoom level. This is just a digital image display artifact. The image itself (1:1 view) does not have this pattern.
- **Lifted plates:** Due to reduced plate restrictions (introduced in Harmony 3.1) it might happen that a plate is lifted by the objective, but the focus is working anyway. If your images look blurred even if the software captured the image, please check if the plate sits correctly in the plate holder. Try again in the middle of the plate, away from any potential restrictions.
- The size of evaluations displayed in the **Database Browser** might be wrong if the evaluation was created with Harmony software <3.1.
- Depending on the performance of the (office) PC, **flatfield-corrected** images may be displayed with a certain delay.

- If the connection between Harmony and the instrument was lost during a measurement, Harmony may not react as usual any more. Please restart Harmony after ending its process in the Windows Task Manager and load the measurement again.
- If you use Harmony as an office installation, please always make sure that the network connection to the database PC (i.e. the PC directly attached to your instrument) is working and that this PC is running. In case of error messages saying “no endpoint listening”, also a restart of the database PC may help.
- Keep in mind that only one Windows account can run Harmony when using several accounts on the same (office) PC.
- In order to avoid the acquisition of slightly defocussed images, please omit the fields very next to the well border from the **well layout**. Measuring in the well border might not lead to a focus failure as the residual results of the focus routine might still be so good that no error is identified and the image is taken anyway (but is then slightly defocussed).

5.3.1.3 Setup

- **Background for Well** feature:
  - Background images cannot be removed. Please load a different measurement to reset the view.
  - Please do not include any red fields.
  - It may happen that the background images are not loaded completely or not at all. In this case, please restart Harmony and try again.
  - Background images cannot be removed. Please load a different measurement or click the **New** button to reset the view.
- **Live preview** during a test measurement will show the images correctly but with a little delay and in different order.
- **Field selection in Well pane:** When using objectives with a large magnification it may be possible to select fields outside of the well. Please do not include these fields in your measurement layout.
- When using the **manual PreScan/ReScan** function with a slide plate type and a high magnification, it may happen that the well view in **Navigation – Define Layout – Well** cannot be moved any more via the scroll bars. Please zoom out using the mouse wheel and try again.

5.3.1.4 Assay Layout Editor

- Please avoid using blanks as first or last character in the entered data.
- Pasting entries from Microsoft Excel® to the **Assay Layout Editor** may cause unexpected errors. In such cases please restart Harmony and try again. We recommend to save the changes regularly while editing the assay layout.

5.3.1.5 Run Experiment

- Clicking the **Start** button triggers initializing processes of your Operetta CLS. If you already started a measurement and stop it immediately, it may happen that the measurement cannot be stopped at all. We recommend waiting until the first well has been processed before stopping a measurement.

- The plate name will automatically be shortened to 64 characters. Please keep in mind when typing in more than 65 characters, that the input will be deleted and you need to re-enter a name with less than 65 characters.
- Please note that any entries (comments/keywords) in the **Plate Settings** section are not updated if another experiment is loaded. The **Plate Settings** have to be adapted manually within a Harmony session.
- Please do not enter blanks or more than 256 characters for a keyword value.
- When browsing already measured images of a z-stack during an ongoing measurement, it may happen that the images are displayed with a long delay. Please be patient and select the planes one after another and wait for every image to be displayed.
- During a **Time Series Break**, the button **Continue Measurement** will remain inactive if the assay plate is missing. Please click the **Eject** button, insert your assay plate and try again.

5.3.1.6 Image Analysis

- After installing a new version of the Harmony software you may experience errors if you load and run analysis sequences created with an older version of Harmony. This is due to modifications of the Harmony Engine. You may have to **check and update existing analysis sequences** before you can use them for evaluations in the new Harmony software.
- Most interactions with the user interface on the **Image Analysis** screen trigger a re-evaluation of the current image. This calculation may take a few seconds. During this period of time it is not recommended to perform any other actions on the user interface. Please be patient and wait until the last changes are processed.
- **Image Control:** Any changes of the histogram for the visualization of an image done on the **Setup** or **Run Experiment** screen will not be adapted automatically on the **Image Analysis** screen and vice versa. Please select the respective well again to see your changes on every screen.
- Please mind any automatic changes that are announced in **Harmony Messages**. This will occur if you test an analysis sequence that uses different channels in the building blocks than you used in your measurement. Please check the sequence using the **Test** button *and* check each building block for correct assignment of the channel.
- The building block **Input Image** offers a maximum projection of z-stack images. Note that if you select several planes on the **Evaluation** screen and go back to the **Image Analysis** screen, the maximum projection can only be displayed if it was selected. Choosing the individual plane option will display the topmost plane only.
- Selecting rows in the **Properties** results table will highlight the corresponding objects in the image overlay. However, after selecting several rows it can take up to one minute until all objects are highlighted in the image overlay.
- It is currently not possible to select more than 200 properties per population in the building block **Define Results**. If your desired property is not displayed in the list, try to reduce the number of calculated properties in the previous building blocks by disabling the calculation of unneeded properties.

- **Changes of image color in building block:** When changing the image channel color in a building block and at the same time changing also a parameter, e.g. the "Method" in *Find Nuclei*, then this new channel color is also applied to already existing building blocks in the sequence following the current one.
- **Special characters:** Please do not use the special characters **\ + "** in the output text boxes of a building block.
- The building block **Input Image** offers a maximum projection of z-stack images. Note that if you select several planes on the **Evaluation** screen and go back to the **Image Analysis** screen, the maximum projection can only be displayed if it was selected. Choosing the individual plane option will display the topmost plane only.
- **Building block "Find Texture Regions":** Please make sure that at least one training point is displayed in the image view when leaving the training with "OK". Otherwise the building block will not generate results.
- **Building block "Select Population" – Method "Linear Classifier":**
  - Training points added in Harmony cannot be viewed or modified in Columbus. In Columbus the classifier can only be used as it is and training points can be added (but not removed).
  - It is not possible to modify a training which was done in an older software version (only option: reset training).
- **Building block "Define Results" – Method "Formula Output":** Track Properties and Population Properties cannot be mixed in one formula.

5.3.1.7 Evaluation

- If you load an analysis sequence on the **Image Analysis** screen, it is automatically loaded as well on the **Evaluation** screen. If you now run a measurement that uses an online analysis, the evaluation results table shows the results of the online analysis.
- During a measurement, you may adapt and save the analysis sequence that has been loaded before you started the measurement.
- The evaluation results are always generated using the online analysis sequence.
- Please keep in mind that any changes in the analysis sequence done on the **Image Analysis** screen are used directly on the **Evaluation** screen as well. Both on the **Image Analysis** and the **Evaluation** screen there is an asterisk "*" added to the name of the analysis sequence if it has been modified.
- Depending on the complexity of the analysis sequence, an online evaluation may take longer than the measurement. Please keep this in mind if you plan to measure experiments using an online analysis in an automated run.
- The evaluation results always include the default assay layout layers (Compound, Concentration, Cell Type and Cell Count), even if no assay layout is attached to the measurement. This will not have any impact on the data but a selection for the graph display will not be possible.

5.3.1.8 Settings

- **Data Management:** Please make sure that the Operetta's Harmony PC is always running. If you run Harmony as an office installation and you restarted the

Operetta's Harmony PC, it may occur that the ODA connection fails. This happens when you restarted the Operetta's Harmony PC in the meantime. Please refresh the Harmony login as well at your office PC using the account icon.

- **Define Plate Type:** If you defined a new plate type, please check the selection of the plate type on the **Setup** screen. It will not be loaded automatically.
- **Define Plate Type:** We pre-defined a list of plate types to the best of our knowledge. Please keep in mind that it can happen that the plate's manufacturer has changed the dimensions in the meantime so this can result in focus failures or effects on the images' field of view. Please also consider that each plate has factory tolerances which can especially have an impact on the focus-ability. If you are sure that the plate type definition is correct and the plate still cannot be focused, check the values for 'H' and 'I' (bottom thickness and the distance between bottom and the rim of the plate) in the Plate Wizard using the **Scan H&I** function.
- **Scheduled Task Wizard:** Any "unknown error" is a Windows system message. If you get such an error, please make sure that e.g. the access rights to the destination are correct and updated.
- **Change Objective:** For an error-free operation, only use the Operetta CLS with at least one objective and do not install objectives with identical barcodes.
- **Change Filter:** For an error-free operation, only use the Operetta CLS with at least one emission filter and do not install filters with identical barcodes.
- **Export Data:** Exported evaluation results may show an incorrect display of special characters if opened in Microsoft® Excel. This does not have any impact on the results. It can easily be corrected by either removing these characters via Find & Replace or by pasting the data from a text editor directly into Excel.
- **Relocate Settings:** If you relocated images in the past using an incomplete account (no UNC path or no qualified account), please remove this incorrect relocate path and specify it again. This will guarantee that images can be relocated and relocated images can be accessed. For further information please see Harmony help.
- **Relocate Settings:** We recommend using as few relocate paths as possible. Please also make sure that **Relocate Settings** only contains existing relocate paths which are accessible. Both will speed up accessing relocated images.
- **Database Settings:** For an error-free operation, please do not use any special characters in the computer name.
- **Schedule Tasks:** Please keep in mind that if any object name contains a "*" or a "?", the wild cards in the selection criteria might not work. In this case, the task will not process any data.
- **Combine Measurements:** There will be no notification if you selected the same measurement twice. Please note that each measurement can only be used once for the current combination.
- **Define Plate Type:** The comments included in the provided plate definitions of microplates are valid for the Harmony release date. Over the time these comments may become outdated because plate manufactures change their specifications or part numbers.

- **Missing plates:** If you miss a plate type after updating to a new Harmony version, you can easily restore your plates by loading an old measurement, switching to the **Setup** screen, opening the context menu in the global control section and choosing **Plate Type Details**. Now you can save the plate after finishing the **Plate Wizard**.
- Under certain conditions, it is possible to attach an assay layout that has a different plate format than the measurement. This would result in an apparently wrong display. Please detach the wrong assay layout from the measurement and attach the correct one.
- The **Scan H&I** function can only be used for plate types with 6 to 1536 wells.

5.3.2 Reporting Technical Issues

If you experience technical issues while using Harmony, please contact PerkinElmer Support and provide the following information:

1. Copy **all** error messages from the **Messages** window:
   - Right-click the list of messages.
   - Select **Copy All** from the context menu.
2. Paste the copied messages into a new email or a new request in the support portal and add a description of the issue.
   - **Support Portal:** https://evoportal.perkinelmer.com/
   - **Email:** operetta.support@perkinelmer.com

Notice

- Do not send screenshots of the **Messages** window.
- Please copy and send **all** messages as described above, even though this may be a large amount of text.

Collect Log Files

PerkinElmer Support may ask you to send instrument log files for deeper analysis of an issue. In this case please perform the following steps directly at the Harmony PC which is connected to the instrument:

1. Run the program **Collect Log Files** (Windows® Start Menu – All Programs – PerkinElmer).

   The log files are automatically copied from the instrument to the Harmony PC's desktop (password-protected *.zip file).
2. Use the **Support Portal** (see above) to upload the *.zip file. It will typically be too large for sending via email.

5.3.3 Starting Harmony Takes a Long Time

If you start Harmony, the most recently used measurement will be loaded again automatically. If this was an extremely large measurement with many images, this will delay the start of Harmony significantly and meanwhile Harmony may be not responding. Please be patient and wait until Harmony's user interface is displayed.

5.3.4 Application Guide Request Not Successful

No Response

If you request an Application Guide package for your instrument, you should get a response with a download link within 24 hours.

Possible reason	Possible solution
The response was blocked by your spam filter.	Check your spam folder for an email from request.applicationguide@perkinelmer.com.
Your email address was not entered correctly in the **Request Application Guide** dialog.	Repeat the whole process, enter the information again and carefully check your email address for typing errors. Save a new request file (RequestApplicationGuide.rag) and send it via email to request.applicationguide@perkinelmer.com. If you still get no response, please contact PerkinElmer via the support portal (http://evoportal.perkinelmer.com).

Notice
The response will be sent to the email address which you entered in the **Request Application Guide** dialog, not to the address which you used to send the request file to PerkinElmer.

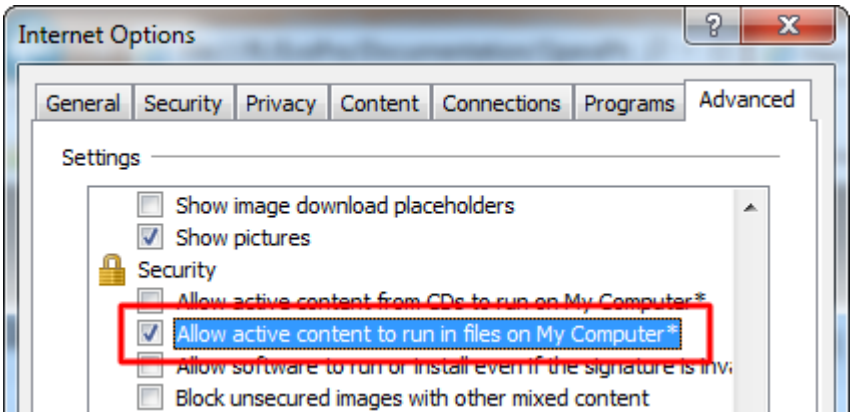
Application Guide Package for Wrong Instrument Displayed/Received

Possible reason	Possible solution
Harmony can be used with different instruments. If you request an Application Guide, your current **instrument type** is detected and automatically included in the request. If Harmony is connected to a database of a different instrument (e.g. Opera Phenix), this will also change the instrument type to “Opera Phenix”.	Use **Change Database** and select the database of the instrument for which you want to request the Application Guide package. Repeat the whole process and make sure that the correct **instrument type** is displayed in the lower section of the dialog (Requested documents: ...). Save a new request file (RequestApplicationGuide.rag) and send it via email to request.applicationguide@perkinelmer.com.

5.3.5 Video Tutorial Does Not Play

If video files embedded in the Harmony Help cannot be played, please check the following setting in your Internet Explorer:

1. Open the Internet Explorer.
2. Open menu **Tools – Internet Options – Advanced**.
3. Make sure that **Allow active content to run in files on My Computer** is checked.



4. Click **OK**.
5. Restart the Internet Explorer to apply the change.

5.3.6 *Calculating Required Disk Space*

Before starting a measurement you should check the Harmony PC (where the database is located) for sufficient disk space.

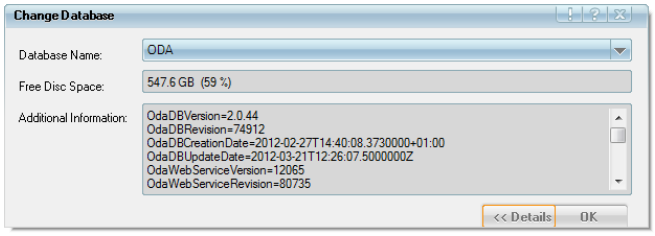
How to calculate the required disk space of a measurement

Disk space = channels × fields × planes × wells × time points × plates × average image file size

The average size of an image file can fluctuate and depends on the visual content. 2.2 MB (binning 2) and 6.5 MB (binning 1) are typical maximum values. Modify this value according to your specific images.

How to check the free disk space

1. Open **Settings – Data Management – Change Database**.

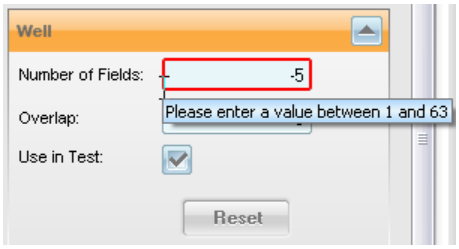


2. The **Free Disk Space** on the database PC is displayed.

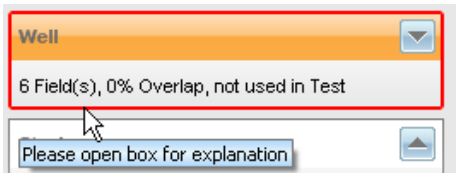
5.3.7 *Feedback via Tooltips*

Data entered into the input boxes of the user interface is checked for plausibility (if possible) and errors or instructions are indicated by a colored outline:

- **Red outline:** The entered data is incorrect (e.g. parameter value out of range or logical error detected). A tooltip explains how to enter the data correctly.

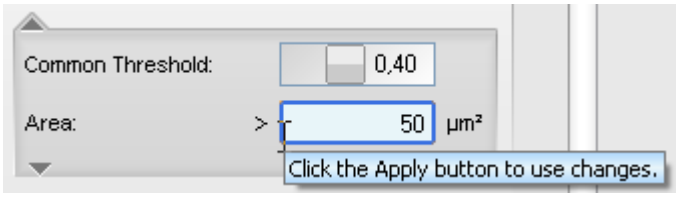


Parameter range exceeded



Input error inside the closed group box

- **Blue outline:** A tooltip is available explaining the behavior of the user interface and giving helpful instructions.



Furthermore, tooltips are displayed in the following situations:

- Display of well and field coordinates in the **Navigation** pane
- Explanation of abbreviated text (e.g. in input boxes or tab labels)

All other errors will be reported in the **Harmony Messages** window. For details see section 5.1.6 “Messages”, page 39.

5.3.8 Archive vs. Relocate

This following illustrations explain the differences between the data management functions **Write Archive** and **Relocate Images**.

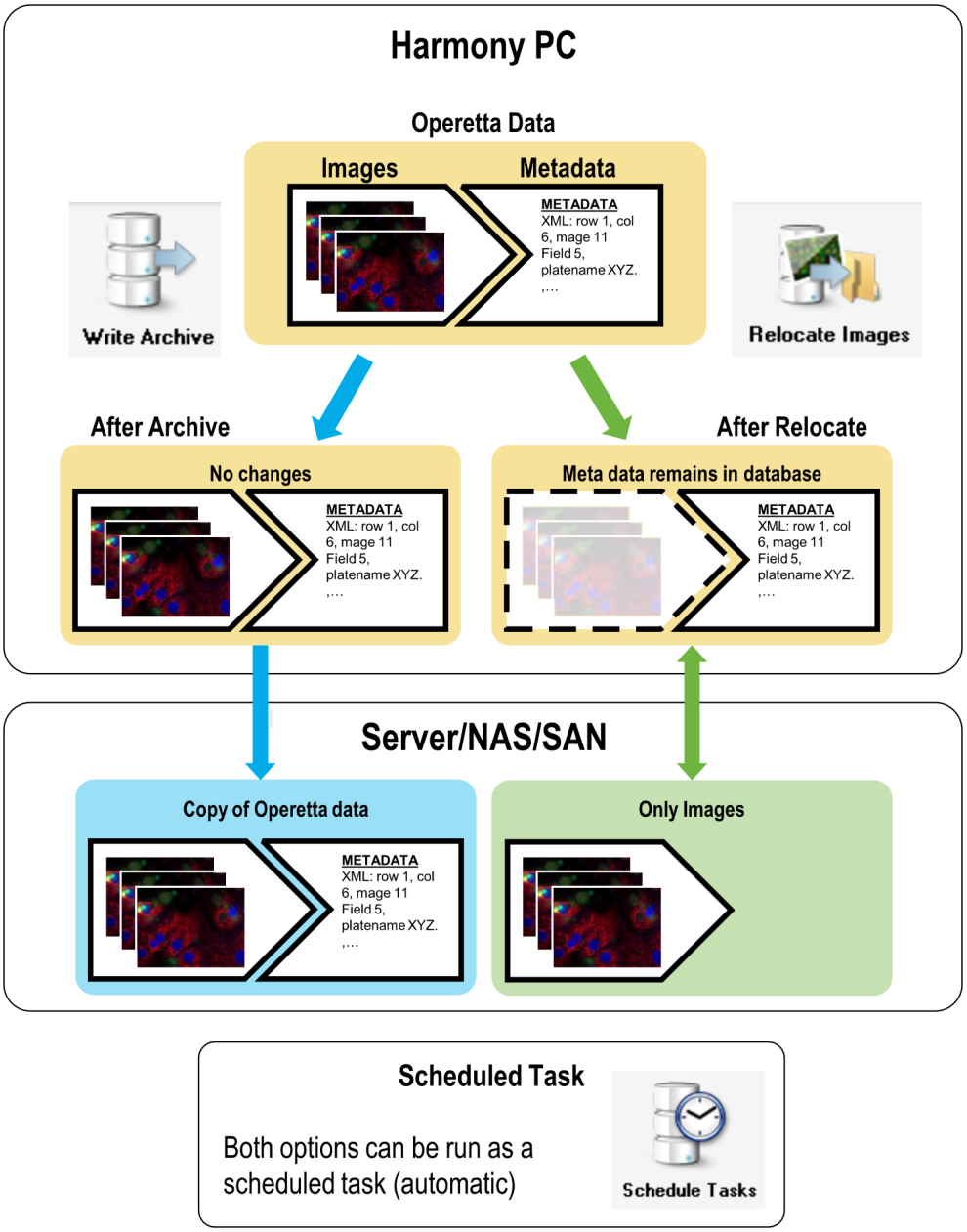
For details please refer to:

- section “Write Archive”, page 253
- section “Relocate Images”, page 259
- section “Schedule Tasks”, page 280

Notice

Harmony database performance depends on the number of database objects. To prevent the database of getting slow and unresponsive, please archive finished projects using the **Write Archive** function and then delete them from the database using the **Delete Data** function. Please note that the **Relocate Images** function will not reduce the number of objects in the database, but only free up disc space on the Harmony PC.

WRITE ARCHIVE Backup/Archive your data	RELOCATE IMAGES Free up disk space on Harmony PC
Why? • To Archive all Data which belongs to a project. • To create a backup of your Data **Disadvantage:** Only accessible after re-import	**Why?** To free up disk space on Harmony PC with the ability to still view the images in the Harmony software. **Advantage:** Data/Images still accessible
When? • At the end of each project (Manual task) • Daily or weekly (Scheduled Task)	**When?** Whenever disk space is low on Harmony PC
What? The Archive contains all Metadata and all images	**What?** Only Images
Restore? An Archive (or parts from an archive) can be loaded into any ODA system. After that the Data can be used in Harmony software.	**Restore?** Images can be located back to the Harmony PC



5.3.9 *Scheduled Tasks and Jobs Disappeared*

Possible reason: Computer name was changed

Whenever you create a scheduled task in Harmony (only possible on Harmony PC at the instrument) or start a data management task running as a background job (on Harmony PC or Office PC), this task will be linked to the name of the currently used computer. If you change the name of this computer, the links cannot be resolved anymore.

- All scheduled tasks will disappear in the **Scheduled Tasks Overview** and will not be executed anymore.
- All background jobs listed in the **Job Status** dialog will also disappear (incl. log files of completed jobs).

How to avoid this issue

- Avoid renaming the Harmony PC or Office PC.
- If you have to rename the Harmony PC (e.g. due to internal IT requirements), it should be renamed before creating any scheduled tasks. Existing scheduled tasks will disappear and have to be created again.

Hint for restoring scheduled tasks

If you restore the initial computer name and restart the PC, the jobs and scheduled tasks will re-appear and work again. You can then make notes of your existing scheduled tasks or save log files of the desired jobs before you finally rename the computer and re-create the scheduled tasks.

See also the following sections:

- "Schedule Tasks", page 280
- "Job Status", page 300

5.3.10 Backup all Data on Harmony PC

We highly recommend to backup all Harmony data on the Harmony PC daily using third-party backup software so that it can be restored in case of a crash. To restore such a backup you will need to contact PerkinElmer Service.

Warning

If no backup procedure is established, a crash of the system will not only mean the **loss of all data on the Harmony PC**, but also the **loss of all relocated images**. Without the meta data in the Operetta CLS database (ODA) these images cannot be accessed anymore and they cannot be re-imported into the system.

What needs to be backed up?

The following paths must be included to backup all Harmony data:

- C:\ProgramData\PerkinElmer\ODA_DATA\IMAGES
- C:\ProgramData\PerkinElmer\ODA_DATA\RESULTS
- C:\ProgramData\PerkinElmer\ODA_DATA\XML
- C:\ProgramData\PerkinElmer\ODA_DATA\BAK (daily database dump)

Notice

If images have been moved to a different location using the function **Relocate Images** (see section "Relocate Images", page 259), these relocated images must be subjected to a separate backup procedure and are not included when backing up the data on the Harmony PC.

Database Dump and Scheduled Task

The important meta data contained in the Operetta CLS database (ODA) cannot be backed up directly by third-party backup software. Therefore, the Harmony software will automatically create a daily dump of the database and write it to the "...\BAK" folder (see above). Thereby, the meta data contained in the database becomes accessible for the backup software and can also be saved.

The creation of the daily database dump is realized by a scheduled task running at 10 p.m. each night (default). The scheduled task can be found in the Windows Task Scheduler:

- Task Scheduler Library\PerkinElmer\ODA Backup Dump File

Notice
You have to make sure that the Harmony PC is switched on at the start time of the scheduled task.

How can I restore a backup in case of a crash?

Only if a backup created with above mentioned procedure is available, the Harmony PC can be restored by the PerkinElmer Service.

Troubleshooting

Warning: "The daily dump of the database was not used by any backup software so far."

It was detected that no external backup has been executed since the last creation of the database dump (archive flag of the file was not set).

- Make sure that the external backup is executed regularly and that all paths listed above are included.

Error: "No SQL Server backup executed"

The database dump could not be written. Possible causes of failure:

- Hard disk is full.
  Please check the free disk space on the Harmony PC where the database is installed (see section "How to check the free disk space", page 464).
- Backup was started while new ODA version was being installed.
- Harmony PC was switched off while backup was running.
- Target folder for daily database dump does not exist (C:\ProgramData\PerkinElmer\ODA_DATA\BAK).
- SQL Server process does not have permission to write into the target folder.
- Database is corrupt.

5.3.11 Poor Image Quality

Blurred or faulty images can e.g. result from dirt or fingerprints on optical components or from autofocus issues. See the following table for further causes.

Possible causes	How to fix it
Plate bottom is not clean.	Inspect the plate bottom and clean it, if necessary.
Objective lens is not clean.	**Air objective:** Clean the objective lens (see section 7.2.1 “Cleaning”, page 531). **Water objective:** Clean the objective lens and/or the hydrophobic ring (follow the instructions in section 7.2.1 “Cleaning”, page 531). Further cleaning may only be done by PerkinElmer Service. Do not touch the lens, the water collar or the blue hydrophobic ring with your fingers. This could affect the position and stability of the water droplet and thereby cause focus errors.
Air bubbles in the well.	Remove the air bubbles.
Autofocus issue.	See section 5.3.22.10 “Focus failure”, page 492.

If the error persists, please contact the PerkinElmer Service.

5.3.12 Image Alignment Procedure Fails Repeatedly

If the image alignment procedure fails repeatedly, the reason is usually a soiled objective or (if using water immersion objectives) residual water droplets at the plate bottom or the alignment sample inside the instrument.

For general information on image alignment see also section 6.10 “Image Alignment”, page 518.

5.3.12.1 Using Air Objective (exclusively)

Possible causes	How to fix it
Objective is soiled.	Clean the air objective (see section 7.2.1 “Cleaning”, page 531).
Plate bottom is soiled.	Inspect the plate bottom and clean/dry it, if necessary.
Alignment sample is soiled.	Carefully clean the alignment sample (see section 7.2.1 “Cleaning”, page 531).

5.3.12.2 *Using Water Objective (exclusively)*

Possible causes	How to fix it
Objective or blue hydrophobic ring is soiled.	Clean the objective lens and/or the blue hydrophobic ring of the water objective (see section 7.2.1 “Cleaning”, page 531). The water collar may only be cleaned by PerkinElmer Service.
Air bubbles got trapped in the residual water drop at the alignment sample.	Carefully dry the alignment sample (see section 7.2.1 “Cleaning”, page 531).

5.3.12.3 *Using Air and Water Objectives Alternately*

If you are using water and air objectives alternately (e.g. using the PreciScan function), you have to be very careful not to carry over immersion water droplets from the water objective to the plate bottom and to parts inside the instrument. If the plate bottom, the air objective or the alignment sample are soiled with residual water droplets, this can lead to failure of image alignment, autofocus routine or the entire measurement when using an air objective afterwards.

Notice

- Before switching from a **water** objective to an **air** objective (measuring the same plate), you first have to eject the plate and clean/dry the plate bottom.
- High NA air objectives with short working distance (20x high NA, 40x high NA) are so close to the plate bottom (if using low bottom plates) that they come in contact with residual water droplets if the plate bottom is not clean. Once the objective is soiled, the water droplets can be carried over to parts inside the instrument, especially to the image alignment sample. If this happens, image alignment may fail with any air objective until the alignment sample is cleaned.
- PreciScan: For the PreScan experiment you cannot select a water objective if the ReScan uses an air objective (to avoid residual water at the plate bottom). See also section 5.1.8.3 “PreciScan™”, page 119.

Possible causes (when using air objective AFTER water objective)	How to fix it
Water objective used before. There is residual water on the plate bottom.	Inspect the plate bottom and clean it before using an air objective.
Water objective used before. Residual water droplet carried over from plate bottom to air objective (e.g. high NA objective with short working distance and low bottom plate). Objective is not clean.	Inspect the plate bottom and clean it. Clean/dry the air objective (see section 7.2.1 “Cleaning”, page 531).

Possible causes (when using air objective AFTER water objective)	How to fix it
Water objective used before. Immersion water droplet carried over from plate bottom and objective to alignment sample. Alignment sample is soiled.	Check plate bottom and air objective and clean/dry, if necessary. Clean/dry the alignment sample (see section 7.2.1 “Cleaning”, page 531).

If none of the above helps, please contact the PerkinElmer Service.

5.3.12.4 Consequences of Image Alignment Failure

The measurement(s) will be executed anyway, however the repositioning accuracy of PreciScan and the stitching accuracy for Multiscale Analysis and overviews might be impaired.

Tips for using the instrument in the meantime:

- For PreciScan experiments: Increase the **Object Margin** in the *Determine Well Layout* building block to account for the decreased repositioning accuracy between the experiments. See also section 5.2.23 “Determine Well Layout”, page 436.
- For MultiScale Analysis and overviews: Stitching artifacts might occur, in general increased overlap is recommended.

5.3.13 Flatfield Correction

Please note that flatfield correction is not supported for:

- Snapshot
- Brightfield channel
- Digital Phase Contrast channel
- Online analysis

For detailed information on this feature please see section 5.1.12.5 “Flatfield Correction”, page 205 and the **Image Analysis Guide**, chapter 1.6 “Flatfield Correction”.

For troubleshooting of flatfield correction issues related to an evaluation see also section “Flatfield Correction”, page 488 (evaluation error).

Error message

“Flatfield correction profile estimation completed (available method: 'None'/'Basic') for […]“

Possible reason	Possible solution
Not enough images available for calculation of a (better) correction profile	Add more images to the measurement layout (wells, fields, time points). At least seven images are required.

Possible reason	Possible solution
Image content is difficult to analyze	Add images from different plate regions, with more/less objects etc.

Error message

“Flatfield correction profile estimation incomplete for [...]“

Possible reason	Possible solution
User canceled the measurement, not enough images to calculate correction profile	Select a sufficient amount of images (at least seven) and restart the measurement.
Network error	Check the network connection between Harmony PC and Operetta CLS.

Error message

“Calculation of flatfield profile not completed. [...]“

Possible reason	Possible solution
Calculation of correction profile was aborted (e.g. by loading a different measurement or experiment immediately after the measurement has been finished). This can also happen with the PreScan measurement of a PreciScan, because the ReScan experiment will be loaded automatically directly afterwards. Keyword "Available Correction" for such a measurement remains "n.a.".	Load the measurement manually in Harmony again. Flatfield correction will then be calculated automatically.

5.3.14 User Accounts

The expression “user account” is used in different contexts:

- **User accounts for Harmony software**
  Harmony comes with two pre-defined user accounts:
  - **PKI** (password “PKI")
    This is the default user account to login at the Harmony software. It has administrative rights. We recommend to use this account only for the first login and to create new accounts for each user of the Operetta CLS. For details see section 5.1.17.8 “User Accounts (Harmony + Columbus)”, page 304.
  - **PKI Service**
    This user account is required by the PerkinElmer Service.
- **Windows® user account for Harmony PC**
  The Harmony PC comes with two pre-defined Windows® user accounts:
  - **Harmony** (password “Harmony”)
    This the Windows® user account of the Harmony PC.

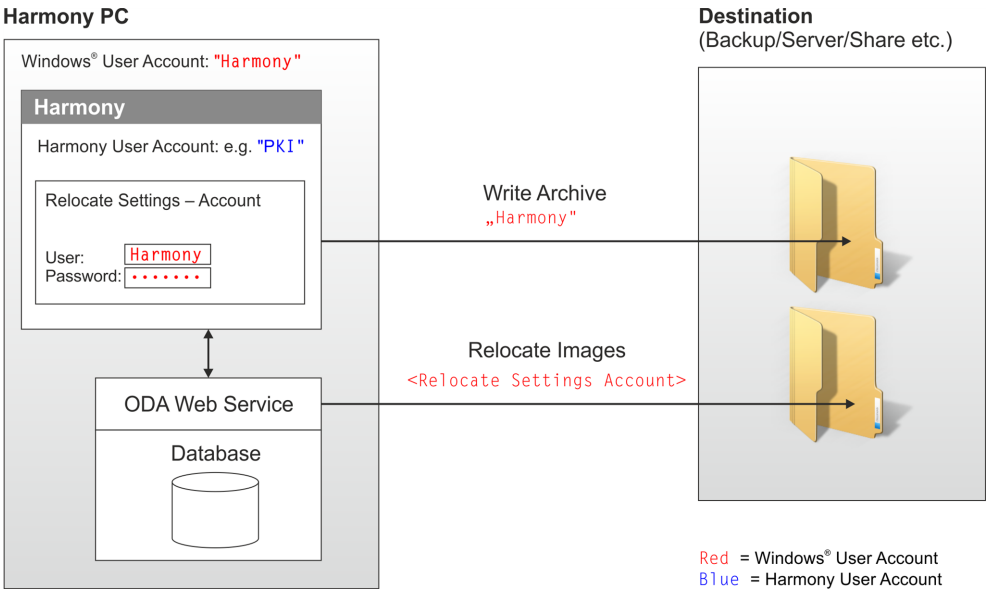
- **Administrator** (password “Harmony”)
  This account is not required for operating the Operetta CLS system, only e.g. for installing additional software or updates.

- **Columbus user account**
  This account (and the URL of the Columbus server) is required for transferring data directly to a Columbus system within the network. For details see section “Manage Columbus Account”, page 305.

This topic focuses on the Windows® user accounts involved in the data management functions of the Operetta CLS system.

5.3.14.1 *Data Management*

When using Harmony's data management functions **Write Archive** and **Relocate Images** manually, the following accounts are involved:



Write Archive

Writing the archive is done using the current Windows® user account of the Harmony PC (default: “Harmony”). You have to make sure that this account can access the selected destination folder (read/write access and the right to create subfolders). See also section “Write Archive”, page 253.

Relocate Images

Relocating images is not performed by Harmony directly, but by the ODA web service. As this service runs under a different account, you have to specify an account for each destination in **Relocate Settings – Account** which is then used to access the destination folder. You have to make sure that this account can access the selected destination folder (shared folder with read/write access and the right to create subfolders). See also section “Relocate Images”, page 259.

Notice

We recommend to enter the default account “Harmony” when adding relocate paths in **Relocate Settings**, so that the same account is used for accessing the destination folder, both for archiving and relocating. The destination folder has to be entered as a UNC path. See also section “Relocate Settings”, page 294.

5.3.14.2 Requirements

The default user account of the Harmony PC (default: “Harmony”) must have the following property:

- Password does not expire.
  Otherwise the user account details for all relocate paths in the **Relocate Settings** have to be updated each time after changing the password.

Required steps after modifying the default Windows® user account “Harmony”

We recommend **not** to modify the default user account ("Harmony”). However, if renaming or modifying the default account cannot be avoided (e.g. due to local IT policies), the following parts of the Operetta CLS system have to be adapted.

1. Edit all relocate paths in **Relocate Settings** and enter the new account details for each destination in **Specify Relocate Path**.
2. Check all destinations used for manual or scheduled archiving/relocating to make sure that the new default user account can access the corresponding destination folders (read/write access and the right to create subfolders is required).

5.3.15 Measurement Contains No Images

If a measurement contains no images (gray wells), this can have one of the following reasons:

- You started the measurement and stopped it before Operetta CLS could take any images.
- You started the measurement and Operetta CLS canceled it, because 5 focus errors occurred successively right at the beginning.

Hint

The object size of a measurement or evaluation can indicate whether it includes images. Operetta CLS images usually have a file size of 2 MB (binning 2) or 6 MB (binning 1).

- If the object includes **no images** but only the measurement/evaluation data, the object size is usually **< 1 MB**.
- If the object includes **images**, the object size is usually much larger.

The size of an object is displayed in the object list of the **Database Browser** (see section 5.1.16 “Database Browser”, page 220).

To be perfectly clear whether images are included or not, load the measurement.

5.3.16 Display Errors

5.3.16.1 Regional Settings

Harmony accepts both dot and comma as decimal separators. If necessary, the separator will be converted automatically according to your Windows® regional settings.

Notice

In the regional settings of Windows® (Control Panel – Region and Language) you may select the corresponding format and location for your region (i.e. your country). The correct formats for numbers, dates and times will then be used automatically. Do not edit the “Additional settings ...” manually, e.g. to modify only the decimal separator. This would lead to wrong calculations in Harmony.

After you have changed the regional settings of Windows®, you have to restart Harmony to apply the changes and to avoid errors. For example, if Harmony is not restarted and the decimal separator (dot or comma) has changed, this can lead to wrong calculations when you enter decimal numbers in Harmony.

5.3.16.2 Display Properties

If you change the appearance of windows and buttons in the **Display Properties** dialog of Windows® (e.g. by selecting a new theme or changing the style for buttons and windows), you have to restart Harmony to apply the changes and to avoid display errors.

5.3.17 Channel Displayed in Gray

Normally, each channel (displayed in **Image Control – Channels**) has a default color matching the wavelength of the channel.

- If a channel is unknown to Harmony, the correct color cannot be assigned automatically, and the channel color will be gray. In this case, you have to select the desired color manually.
- If you modify the wavelength of a channel, the new default color is only applied after you have saved the channel.

5.3.18 TCO Issues

Notice

Please note that the system needs time to establish and stabilize the target values for temperature and CO_2 concentration.

- Temperature has typically been stabilized after 1 h. If the room temperature is low and you select a temperature higher than 37 °C, the warm-up procedure may take much longer.
- CO_2 concentration has typically been stabilized after 5 min. (i.e. carbon dioxide control can be activated shortly before starting the measurement if you want to save carbon dioxide).
- It is possible to start a measurement before reaching the target temperature and CO_2 values.
- The heating mechanism is carefully optimized to avoid any transient overheating of the sample even during the warm-up cycle. As a result, it is safe to load the plate as soon as the green checkmark appears for the very first time. Stabilizing the plate and instrument together will be faster.

5.3.18.1 CO_2 Concentration

Danger!

Carbon Dioxide Gas

If the carbon dioxide concentration displayed in the software is permanently higher than the selected target concentration, this may indicate a defect of the instrument.

- *Shut off the carbon dioxide main valve.*
- *Contact PerkinElmer Service and report the issue.*

Issue	Possible causes	How to fix it
CO_2 concentration too low	CO_2 pressure is not correct.	Check the pressure regulator and make sure that the pressure is 3 bar.
	CO_2 cylinder is empty.	Check the filling level of the CO_2 cylinder.
	CO_2 connection is leaking.	Check the connection valve of the CO_2 cylinder for leakages.
CO_2 concentration too high	After setting a new CO_2 target value, the system needs several minutes to stabilize the concentration. During this process it is normal that slightly higher concentrations occur.	Wait a few minutes until the desired CO_2 level has been stabilized. If the concentration is too high permanently, switch off CO_2 supply and contact PerkinElmer Service.

5.3.18.2 Temperature

Issue	Possible causes	How to fix it
Temperature too high	Room temperature is too high.	Make sure that the room temperature is < 25 °C. Otherwise the target temperature may be exceeded.
Temperature too low	TCO defect.	Please contact PerkinElmer Service.

5.3.19 Update Analysis Sequences after Harmony Update

After installing a new version of the Harmony software you may experience errors if you load and run analysis sequences created with an older version of Harmony. This is due to modifications of the Harmony Engine. You may have to check and update your analysis sequence before you can use it for evaluations in the new Harmony software.

How to update an analysis sequence

1. Open the **Image Analysis** tab.
2. Load a measurement which you want to analyze.
3. Load the analysis sequence which was created using an older version of Harmony.
4. Select a measured well in the **Navigation** pane.
5. Click Test to run the analysis sequence.
   The building blocks are processed one after the other. If incompatibilities are detected, the analysis is aborted and you will be notified by an error message in the **Messages** window. It will typically include detailed instructions how to fix the problem. Sometimes it is already sufficient to click Test again and save the analysis sequence. Harmony will then automatically update the corresponding building block, if possible. If further modifications are necessary, this will be stated in the error messages.
6. Repeat the previous step until the analysis sequence is completed without errors.
7. Please check carefully if the results of the building blocks and the final results of the analysis sequence are still as expected.
8. **Save** the analysis sequence.

5.3.20 Columbus Network Integration

Error Message

"Cannot establish connection to Columbus"

Possible causes	How to fix it
Columbus version is not compatible.	For using the direct Columbus Transfer, the Columbus Server version must be 2.4 or higher.
Columbus user account name or Columbus server URL not entered correctly.	Check the Columbus user account name and the Columbus URL for typos. See section "Manage Columbus Account", page 305.
Password of the Columbus user account has been changed in Columbus.	Update the password in Harmony, too. See section "Manage Columbus Account", page 305.

Log Files

If you encounter further problems please first have a look at the log files created by Harmony and Columbus:

- **Harmony:** Open the **Job Status** dialog and click the **Show Log** button of the corresponding job. See also section "Job Status", page 300.
- **Columbus:** Log in, select **Job Status** from the menu bar and click the **Show** button of the corresponding job. The Columbus log file is more detailed and will be required if you contact Columbus Support.

Network Configuration

The usage of the Columbus integration (see "Columbus Transfer", page 271) requires that the Columbus server is reachable from the machine Harmony is running on. This means that the URL that provides the Columbus user interface can be resolved from the Harmony machine.

- You can check that manually by starting a browser on the Harmony machine and putting the Columbus URL into the address field.
- A more basic connectivity check can be performed by using the ping command from the Windows® command line.

Furthermore you should check that

- no firewall is blocking the communication (URL or port 80 and 8081)
- the IP addresses of both Harmony and Columbus installation share the same subnet
- your DNS server can resolve the URL of the Columbus installation
- the Columbus installation accepts connections at all
- the Columbus installation is running

Since the communication is bidirectional you should also have a look at the settings of the Columbus installation, if necessary.

Columbus Support Contact

If the issue could not be solved using the information listed above, please contact Columbus Support and provide the Columbus log file:

- **Support:** http://www.perkinelmer.com/cellularimagingsupport
- **Email:** imaging.support@perkinelmer.com

5.3.21 Firewall Settings

If the ODA setup shows an error message “Firewall configured causes problems” after the installation, please make sure that the firewall is configured correctly, especially when not using the Windows® firewall.

On the Harmony PC including the ODA database, the following firewall rules are required:

- **Harmony.exe** requires:
  - listen (in) ports 8257, 8258 and 8213
  - additional calling (out) ports 80, 8255, 8256, 8282, and 8300
- **ODA database web service** requires the listen (in) port 80 (Internet Information Services (IIS) based web service).
  - **OdaCmd.exe** requires the calling (out) port 80.
  - **OdaWJobDispatch.exe** requires the calling (out) port 80.
- **AcapellaServer.exe** needs the listen (in) port 8282.
  If the firewall blocks the Acapella Server, you have to make sure that the Acapella Server is listed in the “Allowed Programs” and that all check boxes are enabled.

Depending on the firewall software additional rules may be necessary. All communication is based on TCP and is configured for any profile (local, group, domain).

5.3.22 Error Messages

5.3.22.1 Acapella Server terminated

Error message

"Acapella Server terminated” or “New Acapella Server started”

Possible causes	How to fix it
Acapella Server crashed.	Restart Harmony. • You do NOT have to cancel ongoing measurements. The instrument will continue to write measured data to the database. An online analysis will also be continued (eventually after longer period of time). • If the Acapella Server is restarted during an online analysis, this evaluation may be incomplete. The well which was analyzed at that moment will be missing. This might also be the well which caused the crash in the first place. • The **Image Analysis** screen may not be fully functional until Harmony has been restarted.
Acapella Server was terminated manually.	

5.3.22.2 Cannot access data

Error message

"Cannot access data"

This error can occur during relocation of images or if you try to access images which have been relocated.

Possible causes	How to fix it
Image source file cannot be read or does not exist.	Check if the image file has accidentally been moved, renamed or deleted.
Path to source folder or destination folder cannot be found.	• Check if the corresponding computer is switched on. • Check the network connection. • Check the relocate path entered in **Relocate Settings** for typing errors. See section "Relocate Settings", page 294. • Check if the folder stated in the error message has accidentally been moved, renamed or deleted.
Source/destination folder is not a shared folder, access is denied.	Check if the folder is shared so that it can be accessed by the user account specified in **Relocate Settings**.

Possible causes	How to fix it
User account specified in **Relocate Settings** has been modified, for example: • Password has been changed • Password has expired • User account has been renamed	• Update the user account specified in **Relocate Settings**. See section “Relocate Settings”, page 294. • Update the access rights of the source/destination folder.
The corresponding destination (relocate path) in **Relocate Settings** has been deleted.	• Do not delete relocate paths in **Relocate Settings** which are still being used, i.e. where relocated images have been stored. • Restore the relocate path in **Relocate Settings**, i.e. add a new relocate path and enter the same path and account information which have been used for the relocation. See also section “Relocate Settings”, page 294.

For further information see also sections “Relocate Settings”, page 294and 5.3.14 “User Accounts”, page 473. If the error persists, please contact the PerkinElmer Service.

5.3.22.3 Could not connect to database

Error message

“Could not connect to the selected database" or “Connection to database lost”

This message is displayed if the connection to a database fails or gets lost which is located on a different PC in the network, e.g. if you use an office installation of Harmony and try to connect to the Operetta's database on the Harmony PC. To localize the source of error you can try to connect to a different database (if available), see section “Change Database”, page 292.

Possible causes	How to fix it
Database computer is switched off.	Switch on the database computer.
Database computer is not connected to the network.	Check the network connection of the database computer.
The computer trying to connect to the database is not connected to the network.	• Check the network connection of the computer running Harmony. • Restart Harmony.
Database settings are wrong (e.g. typo in computer name).	Check the database settings in Harmony. See also section “Database Settings”, page 298.
Harmony installation and connected database/web service are not compatible (different version number).	Please refer to section 5.3.22.11 “Incompatible Web Service”, page 494.

If the error persists, please contact the PerkinElmer Service.

5.3.22.4 Could not detect assay plate

Error message

"Could not detect assay plate."

Possible causes	How to fix it
No plate inserted.	Use the **Load/Eject** function to insert a sample plate. See section 5.1.15 “Load/Eject Plate”, page 220.
Plate could not be detected.	Eject the plate and check it: Correct plate type? Plate bottom bent?

If the error persists, please contact the PerkinElmer Service.

5.3.22.5 Could not read barcode

Error message

"Could not read barcode"

Possible causes	How to fix it
Barcode of a filter is damaged, soiled or missing.	• Open the **Filter Exchange Wizard** and select the corresponding filter position (will be displayed as “empty"). Check the barcode label on the corresponding filter. See also section 5.1.17.3 “Change Filter”, page 242. • If the label cannot be cleaned or fixed, please contact PerkinElmer Service to request a new barcode label.
Barcode of an objective is damaged, soiled or missing.	• Open the **Objective Exchange Wizard** and select the corresponding objective position (will be displayed as “empty"). Check the barcode label on the corresponding objective. See also section 5.1.17.2 “Change Objective”, page 241. • If the label cannot be cleaned or fixed, please contact PerkinElmer Service to request a new barcode label.

5.3.22.6 Could not store image

Error message

"Could not store image C:\ProgramData\PerkinElmer\Harmony 4.6\Buffer\D2_ 2775672895.tmp in ODA, will retry later"

Possible causes	How to fix it
Operetta CLS tries to send an image to the database which already exists (e.g. after a crash of the Acapella Server). This duplicate image now blocks the measurement process.	Open the Windows® Explorer, locate the *.tmp file stated in the error message and delete the file manually. The measurement will then be continued.

5.3.22.7 Device reported error

Error message

"Device reported error"

Possible causes	How to fix it
Hardware component of the Operetta CLS could not be initialized (e.g. a camera).	Shutdown and restart the Operetta CLS system (including instrument).
Barcode of a filter or objective could not be read.	• Check if the barcode label on the corresponding filter or objective is damaged or missing. • Shutdown and restart the Operetta CLS system (including instrument).
Hardware defect.	Please contact the PerkinElmer Service.

5.3.22.8 Duplicate barcodes detected

Error message

"Duplicate barcodes detected"

Possible causes	How to fix it
Two identical objectives or emission filters have been installed.	Remove one of the identical objectives/filters using the corresponding wizard in the **Settings** window: • 5.1.17.2 “Change Objective”, page 241 • 5.1.17.3 “Change Filter”, page 242

5.3.22.9 *Evaluation or Harmony Engine error*

There are many different variations of errors where the evaluation process or the Harmony Engine (Acapella) are involved. Please check the following list for the corresponding error message.

Error message

"Evaluation error" or
"Evaluation aborted"

Possible causes	How to fix it
Out of memory: One or multiple images cannot be evaluated.	• Check the corresponding images to find a reason for the failed analysis (e.g. no objects or too many objects in the image, well contaminated etc.). • Adapt the analysis sequence or exclude these images from evaluation.
Evaluation aborted: Wrong or non-existing channel selected in analysis sequence.	Select the correct channel in the analysis sequence. Expand the error message to find detailed information.
There are further variations of this error. If such an error occurs, perform the steps listed in the right column.	• Check the analysis sequence. • Load the analysis sequence again and restart the evaluation. • If the issue is not resolved, try to restart Harmony and the Acapella Server. See section "Acapella Server", page 319.
Analysis sequence is incompatible with current Harmony version.	Click the **Test** button on **Image Analysis**, save the analysis sequence and start your evaluation again.

Error message

"Acapella could not obtain licensing information"
or message including "HASP dongle error" or "USB license dongle is required"

Possible causes	How to fix it
USB license dongle is not connected.	• Plug in the USB license dongle and restart Harmony.

Possible causes	How to fix it
The SMA (Software Maintenance Agreement) of your license has expired and you have tried to install new software which was built after the SMA expiration date.	• Contact PerkinElmer to extend the SMA and request a license update key which allows you to install and run new software updates. • Install an older software version which was built before the SMA expiration date. For detailed information see section “License Management”, page 320.

If the error persists, please contact the PerkinElmer Service.

Error message

“<Name of building block> can not be used because of a missing license”

Possible causes	How to fix it
Required license (e.g. for using PhenoLOGIC™ building blocks) is missing.	Contact PerkinElmer to request a license update key which unlocks further image analysis capabilities. For detailed information see section “License Management”, page 320.

Warning message

“Missing data” (during batch evaluation)
“Missing data” (not enough disk space)

Possible causes	How to fix it
The missing image(s) have been relocated and cannot be accessed anymore (e.g. access rights or password have been changed).	Check if the relocate path can still be accessed and the relocate settings are correct. For further tips see also section 5.3.22.2 “Cannot access data”, page 483.
The missing image(s) have *not* been relocated and are saved in the database (on the Harmony PC), but the file is missing or defective.	In this case, it is not possible to recover the data. However, if only a few images of a larger measurement are concerned, the evaluation might still be usable.
Not enough disk space available.	Free up disk space on the Harmony PC, e.g. by deleting measurements. To check free disk space see also section “Change Database”, page 292.

Flatfield Correction

For general troubleshooting of flatfield correction issues see also section 5.3.13 “Flatfield Correction”, page 472.

Warning message

“Flatfield correction setting was automatically changed”

Possible causes	How to fix it
The user has defined an image analysis sequence requiring a flatfield correction method (**Basic** or **Advanced**) and then loads a measurement for which this method is not available.	The flatfield correction method in the image analysis sequence is automatically changed to **None**. Please always make sure that the correction method specified in the analysis is available for the measurement to be analyzed.

Error message

“Evaluation aborted at Building Block 'Input Image'”

Possible causes	How to fix it
The analysis sequence selected for an **online analysis** requires a flatfield correction method (**Basic** or **Advanced**).	Flatfield correction cannot be combined with online analysis. Please change the flatfield correction method of the analysis to **None** (**Image Analysis** screen, building block **Input Image**).
The analysis sequence selected for an **evaluation** requires a flatfield correction method which is not available for the selected measurement.	Please change the flatfield correction method of the analysis to a method which is available for your measurement or set it to **None** (**Image Analysis** screen, building block **Input Image**).

Error message

“Flatfield correction 'Advanced'/'Basic' is not possible for all measurements”

Possible causes	How to fix it
The analysis sequence selected for a **batch evaluation** requires a flatfield correction method which is not available for **all** measurements to be analyzed.	Please change the flatfield correction method of the analysis to a method which is available for **all** measurements or set it to **None** (**Image Analysis** screen, building block **Input Image**).

Error message

"'Calculation of flatfield correction profile for measurement '...' has not been completed yet"

Possible causes	How to fix it
The analysis sequence selected for a **batch evaluation** requires a flatfield correction method and one of the measurements to be analyzed does not have a correction profile yet, e.g. because it had been measured using an older version of Harmony (before version 3.5).	Load the corresponding measurement manually. A flatfield correction profile will be calculated automatically.

Digital Phase Contrast

For detailed information on the DPC channel see also section "Digital Phase Contrast", page 55.

Error message

"Automatic plane selection failed"

Possible causes	How to fix it
Sample is not suitable.	Try a different well or field. • Make sure that the sample is not too thick. • Avoid objects in different heights. • Make sure that no well borders are visible in the images. See also Operetta CLS Application Guide for further hints.
Exposure settings are not suitable.	Optimize **Height** and **Time**. Use the **Snapshot BF** button to take brightfield images: • Check the exact height of the sharpest plane. • Check the exposure time and avoid overexposure. See also Operetta CLS Application Guide for a recommended workflow.
Snapshot DPC was stopped.	Please click **Snapshot DPC** again.

If automatic plane selection still fails, please try the **Manual** mode and select upper and lower planes manually.

Error message

"For the stack, the range of possible focus heights is exceeded ..."

Possible causes	How to fix it
For the automatic modes (high contrast and high detail) an internal stack of brightfield images is acquired around the given focal height when clicking **Snapshot DPC**. This internal stack is not considered for the visualized plate restrictions.	There should be a red frame around the value for focal height. Please have a look at the corresponding tooltip and follow the instructions.

Error message

"Constant image detected"

Possible causes	How to fix it
Completely overexposed images. Digital Phase Contrast cannot be applied to such images.	Please reduce exposure time and/or LED power.

5.3.22.10 Focus failure

Error message

"Focus failure", "Autofocus failure", "Could not detect the plate bottom." or "Discrepancy in plate bottom height H detected"

Possible causes	How to fix it
Wrong plate type selected (inserted plate has a different plate type).	Select the correct plate type.
Plate type definition is not correct or too inaccurate.	Check the dimensions of this plate type in the **Plate Type Definition Wizard**. • Use the **Scan H & I** button to determine height and thickness of the plate bottom automatically (see section "Scan Plate Bottom", page 310). • Measure the other plate dimensions using a vernier caliper. Do not rely on the plate manufacturer's specification.
Correction collar of the objective has not been set correctly.	Adjust the correction collar (see section 5.1.17.1 "Set Correction Collar", page 239). If the bottom of the used plate type is too thick to be compensated by the correction collar (i.e. the values on the correction collar are smaller than the corrected plate bottom thickness), set the correction collar to its maximum position and try again. However, this may lead to bad images and/or focus failures. In this case it is recommended to use a different combination of plate type and objective.
Selected field is too close to the edge of the well.	Select a field which is closer to the center of the well.
Plate bottom is not clean.	Inspect the plate bottom and clean it, if necessary (e.g. to remove water droplets from previously used water objectives).
A water immersion objective is used, and the immersion water supply bottle is empty.	Refill the supply bottle (see section 7.2.2 "Checking the Water Bottles", page 534) and then flush the objective for 1 min. (see section "Flush Water Objective", page 243).

Possible causes	How to fix it
A water immersion objective is used, and there are air bubbles in the tubing.	Flush the objective for 1 min. to remove the air bubbles (see section "Flush Water Objective", page 243).
A water immersion objective has been used before and there are still water droplets at the plate bottom.	Inspect the plate bottom and clean it, if necessary.
Using a water objective: The instrument is switched on and no water objective has been used for a long time (e.g. not switched off over night). The immersion water tubing has dried out so that the automatic flushing routine before the snapshot/measurement is not sufficient.	• Select a water objective and flush it for 1 min. (see section "Flush Water Objective", page 243). • Perform multiple snapshots or test measurements using a water objective until focusing is successful. **Notice** When restarting the instrument, a proper flushing routine is carried out automatically.
Objective lens is not clean.	**Air objective:** Clean the objective lens (see section 7.2.1 "Cleaning", page 531). **Water objective:** Clean the objective lens and/or the blue hydrophobic ring (see section 7.2.1 "Cleaning", page 531). Further cleaning may only be done by PerkinElmer Service. Do not touch the lens, the water collar or the blue hydrophobic ring with your fingers. This could affect the position and stability of the water droplet and thereby cause focus errors.
Not enough liquid in the well.	Check the liquid level of the well. Fill in enough buffer and do not let the well dry up.
If the plate had been stored at low temperatures: • Plate is bent, thermal expansion is not finished yet. • Plate bottom is not clean (condensed water)	Wait at least 30 minutes to let the plate warm up. Inspect the plate bottom and clean it, if necessary.

Possible causes	How to fix it
Plate is too bent to be measured.	Verify that the plate is still according to specifications, e.g. centrifugation can alter the plate characteristics like the height of the bottom above the outer rim (value “H” as depicted in the **Plate Type Definition Wizard**) considerably and thus lead to focus failures. Use a different plate.

If the error persists, please contact the PerkinElmer Service.

5.3.22.11 Incompatible Web Service

Error message

"Incompatible version of the ODA web service detected.”

Harmony and the ODA web service are only compatible to each other if the internal version numbers match. These versions are stated in the error message:

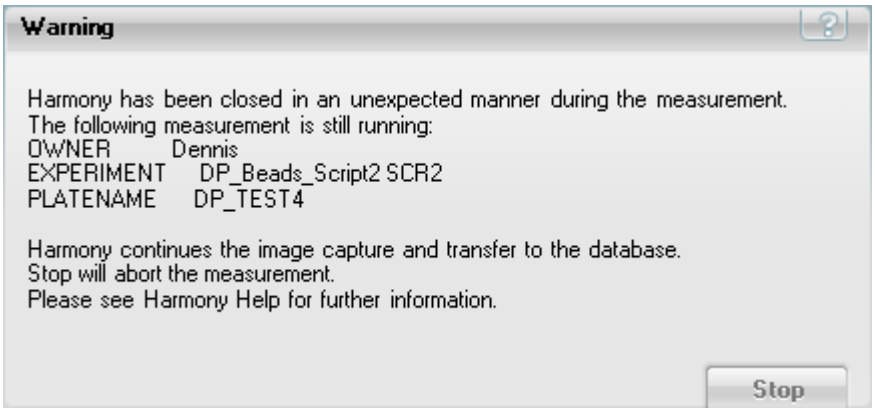
- **Detected version:** Version of **ODA web service** (instrument or database which is to be connected)
- **Expected version:** Version number of ODA web service which is expected/supported by your installation of **Harmony**

Make sure that Harmony (on device PC and all office PCs) and the ODA web service (device PC) are always updated together. Use setups from the same Harmony Setup DVD only to ensure compatibility.

Possible causes	How to fix it
Detected version < expected version: • ODA web service is outdated (e.g. ODA setup not executed after installing a new Harmony version) *or* • Your current Harmony version is not compatible with the instrument/database which is to be connected	• Make sure that the latest ODA setup compatible with your instrument is installed on the Harmony PC (device PC). If necessary, run the ODA setup from your Harmony Setup DVD to update the ODA web service. **Notice** Do not try to install the ODA setup on an office PC. ODA may only be installed on the Harmony PC (device PC). • Make sure that your version of Harmony is compatible with the selected instrument/database. For example, Harmony 4.5 can only be used with Operetta CLS and Opera Phenix, not with Operetta. • If multiple databases/instruments are available, you can try to select a different database to work with via **Settings – Change Database**.
Detected version > expected version: Harmony installation is outdated (e.g. office PC not updated yet)	Update your Harmony installation. Make sure that the same version of Harmony is installed on the Harmony PC (device PC) and on all office PCs.

If the error persists, contact the PerkinElmer Service to let them check your installation and provide you with a compatible Harmony or ODA setup.

5.3.22.12 Measurement still Running at Start



This dialog is displayed if you open Harmony (e.g. after it had crashed) and there is still a measurement running. You can stop the measurement or wait until it has been finished.

Notice

- If you decide to wait until the measurement is finished, the user interface will be blocked until the measurement is done. If you have Harmony installed on a second PC (e.g. office installation with access to the Operetta's database), you can load the measurement from there to view the images and check the progress. Reload the measurement to update the progress information.
- If your experiment included an online evaluation, the plate was only evaluated until Harmony was terminated. In contrast to a measurement, an online evaluation is not resumed when you restart Harmony. A re-evaluation of the whole plate or an evaluation of the non-evaluated wells can be done on the **Evaluation** tab.

If Harmony is started three times with the same measurement still running, there will be no more attempts to resume the measurement. In this case restart the measurement manually.

How to continue a measurement after restarting Harmony

1. Restart Harmony and login.
   If there is still a measurement running, this will be indicated by a warning message.
2. **Wait** until the measurement is finished (e.g. indicated by Operetta's progress LEDs).
   In the meantime: To view images and check measurement progress you can use a Harmony installation on a different PC and load the relevant measurement.
3. The dialog closes automatically as soon as the measurement is complete.
4. To view the measurement and the measured images, load the measurement from the database (**Image Analysis** tab).
5. Optional: If your experiment included an online evaluation, open the **Evaluation** tab to re-evaluate the whole plate or only the wells which have not been evaluated yet.

Buttons and Elements

Element	Description
Stop	Stops the measurement (after further confirmation). The measurement (as measured so far) remains in the database.

5.3.22.13 No valid signal found

Error message

"No valid signal found" or "Error while scanning plate"

This message can occur when using the **Scan H & I** function in the **Plate Type Definition Wizard** (see also section "Scan Plate Bottom", page 310).

Possible causes	How to fix it
Plate is not properly mounted on the table.	Remove the plate and mount it correctly on the table.
Correction collar of the objective has not been set correctly.	Adjust the correction collar (see section 5.1.17.1 “Set Correction Collar”, page 239). If the bottom of the used plate type is too thick to be compensated by the correction collar (i.e. the values on the correction collar are smaller than the corrected plate bottom thickness), set the correction collar to its maximum position and try again. However, this may lead to bad images and/or focus failures. In this case it is recommended to use a different combination of plate type and objective.
Plate bottom is not clean.	Inspect the plate bottom and clean it, if necessary.
Objective lens is not clean.	**Air objective:** Clean the objective lens (see section 7.2.1 “Cleaning”, page 531). **Water objective:** Clean the objective lens and/or the blue hydrophobic ring (see section 7.2.1 “Cleaning”, page 531). Further cleaning may only be done by PerkinElmer Service. Do not touch the lens, the water collar or the blue hydrophobic ring with your fingers. This could affect the position and stability of the water droplet and thereby cause focus errors.
Well is not empty.	Make sure to use an empty plate when using the **Scan H & I** function.
Plate type definition is not correct or too inaccurate.	Measure the other plate dimensions using a vernier caliper. Do not rely on the plate manufacturer's specification.

If the error persists, please contact the PerkinElmer Service.

5.3.22.14 Online Evaluation Timeout

Error message

"Evaluation of well x/y timed out."

Possible causes	How to fix it
One well could not be evaluated which lead to an evaluation timeout and an abortion of the online analysis.	• Check the corresponding image to find a reason for the failed analysis (e.g. no objects or too many objects in the image, well contaminated etc.). • Adapt the analysis sequence or exclude these images from evaluation. • Re-evaluate the measurement when it is finished.

If the error persists, please contact the PerkinElmer Service.

5.3.22.15 Operetta CLS is in Service Mode

Error message

"Operetta CLS is in Service Mode"

This message can occur during service and maintenance by the PerkinElmer Service.

Possible causes	How to fix it
The Operetta CLS cannot be controlled by Harmony because the instrument is currently serviced.	Shutdown and restart the Operetta CLS system (including instrument).

5.3.22.16 SMA License will expire soon

Error message

"SMA License will expire soon." or "SMA License expired."

A message reminds you that your **SMA (Software Maintenance Agreement)** will expire within the next 60 days. The SMA allows you to obtain and install free software updates (Harmony and/or Acapella) within one year after purchasing the Operetta CLS. If your SMA has expired, you can still use your then current installation (for unlimited time). However, to install new software updates which were built after the SMA expiration date, you have to renew the SMA and request a license update key from PerkinElmer.

For detailed information see section "License Management", page 320.

6 Instrument Description

6.1 Specifications

6.1.1 Hardware

Detection

- Widefield fluorescence
- Confocal fluorescence
- Innovative, fast switching LED light source with up to eight wavelengths and high efficiency coupling
- Optional true point confocal, wide view Nipkow spinning disk
- 16 bit sCMOS camera, 4.7 Megapixel (2160 x 2160), 6.5 µm pixel size
- Transmitted light capabilities (brightfield, digital phase contrast)
- High speed, high resolution linear drive scanning stage, 50 nm resolution, 1 µm repeatability, z-stage resolution 50 nm
- Measurement time: less than 8 min/384-well plate for a two color experiment and one field, depending on instrument setting and assay

Excitation

- Configurations with either four or eight LEDs
  - UV: 365 nm
  - Violet: 405 nm (optional)
  - Violet: 440 nm (optional)
  - Blue: 475 nm
  - Cyan: 510 nm (optional)
  - Green/Yellow: 550 nm
  - Red: 630 nm
  - Far Red: 660 nm (optional)
- LED light source for transmission mode
- Fully automated, user accessible eight position emission filter wheel
- Choice of emission filters available, barcoded and user exchangeable

See sections 9.5 “Light Sources”, page 558 and 9.6 “Optical Configuration”, page 559 for detailed specifications.

Objective lenses

- Six position objective turret for automated change
- Proprietary, fully automated high NA water immersion objectives*
- Default objective: 20x air long WD, NA 0.4
- 20x water immersion lens*, NA 1.0
- 40x water immersion lens*, NA 1.1

- 63x water immersion lens*, NA 1.15
- 1.25x air long WD, NA 0.03
- 5x air long WD, NA 0.16
- 10x air long WD, NA 0.3
- 20x air high NA, NA 0.8
- 40x air long WD, NA 0.6
- 40x air high NA, NA 0.75

** Requires water immersion kit*

See section 9.7 “Objective Lenses”, page 560 for detailed specifications.

Environmental control unit

- Temperature control: 37 °-42 °C +/- 1 °C
- CO_2 control: 1-10 % +/- 0.5 %

Physical dimensions

- Width: 98 cm / 39 in
- Depth: 66 cm / 26.0 in
- Height: 47 cm / 18.5 in

See section 9.1 “Physical Dimensions”, page 556 for detailed specifications.

Robotic automation

- Cell::explorer™ robotic automation platform for fully automated cellular and high content screening
- Integrates with plate::handler™ robotic workstation and plate::works™ scheduling software
- Compatible with third party automation systems
- Add stackers, incubators and liquid handling

Computer (Harmony PC)

Computer specifications subject to change without notice

Standard PC	PC with expandable HDD
≥ 2x Intel® Six Core™	
≥ 32 GB RAM (8x4 GB)	
Operating system: Microsoft® Windows® 7 or 10, 64 bit	
Network interface: Gigabit Ethernet	
Hard drive: ~ 2 TB usable storage space, RAID 1 (no further hard drives available)	Hard drive: ~ 2 TB usable storage space, RAID 5 (additional hard drives available)

Standard PC	PC with expandable HDD
≥ 24“ LCD Monitor	≥ 30“ LCD Monitor

6.1.2 Harmony® High Content Imaging and Analysis Software

Image Acquisition

- Computer controlled excitation power
- Laser-based, high speed autofocus in every field
- Full exposure control and channel editor
- Digital phase contrast imaging
- Acquire and analyze simultaneously, and visualize data during acquisition
- Variable plate formats following SBS standard (6, 24, 48, 96, 384, 1536-well), user-defined formats and slides (in slide holder)
- Compatible with a wide range of microtiter and nanotiter plates from various manufacturers including CellCarrier™ Ultra
- Full choice of number and locations of fields, focus height, volume imaging, plate format and type
- Multi-field imaging acquisition enabling full coverage of each well
- Z-sectioning for identifying the z-plane of interest for screening in various planes
- Automated flat field correction
- Time kinetics capabilities for time course experiments

Image analysis

- Ready-made turnkey applications
- Fully automated cell segmentation with automatic or manual parameter tuning
- Easy and intuitive creation and modification of analysis sequences
- PhenoLOGIC™ machine learning plug-in available, enabling users to teach the software to create tailored algorithms
- Texture analysis capabilities

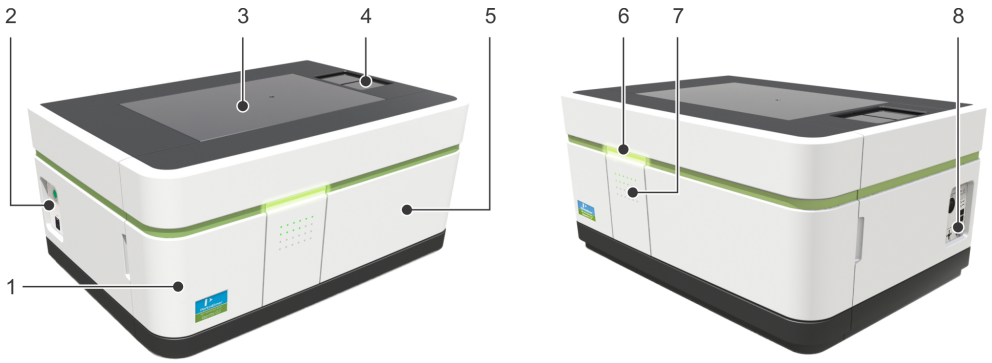
Data Management

- Scalable database
- Search via sortable tree
- Stores all metadata automatically (assay layout, instrument setting, analysis results)
- Stores additional experiment information (user defined keywords, annotations)
- Allows for image storage on a remote server
- Export results automatically or in batches into Columbus™ image data storage and analysis system so that you can access, re-analyze, store, and share your image data

- Export results for further analysis, for example into High Content Profiler™ platform powered by TIBCO Spotfire®
- Accessible from office installations of the Harmony software for data inspection, analysis and result export

6.2 Overview

6.2.1 Instrument



1. Left front door
   Access to emission filter wheel
2. Controls and connections (left)
   Network connection, power switch
3. Lid
   Access to objectives and sample chamber
4. Transfer position
   Load/eject sample plate
5. Right front door
   Access to bottles of water immersion system
6. Status light
7. Progress LEDs
8. Controls and connections (right)
   Manual release for lid, CO_2 connection, mains connection, fuses

6.2.2 Harmony PC

The Harmony PC is directly connected to Operetta CLS via network cable. The Harmony software running on that computer is used to control the instrument and analyze the measured data. The Operetta CLS Database (ODA) is also installed on this computer.

6.2.3 Monitor/Keyboard Cart (optional)

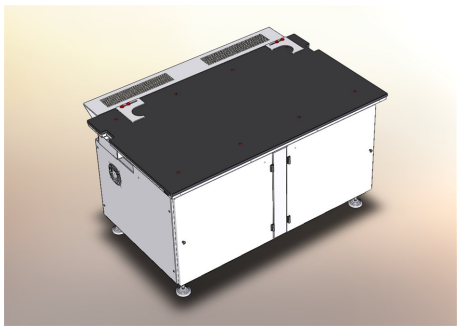
The optionally available mobile computer cart can hold the LCD monitor, keyboard and mouse. The cart is easily adjustable so that you can move from a sitting to a standing position whenever you want. An integrated brake secures the table in place.

Notice

The Harmony PC cannot to be mounted on the cart. It should be placed inside the Heavy Duty Lab Cart (optional) or next to the cart on the floor.

Please refer to the manufacturer's assembly instructions for details on safety, assembly and adjustment of the cart.

6.2.4 Heavy Duty Lab Cart (optional)



We recommend to install the Operetta CLS on the optionally available Heavy Duty Lab Cart. It is a stable basis for the instrument and especially designed to carry the Operetta CLS. Since the table is rollable you can easily move the whole system, if necessary. Optimal ventilation is ensured by perforated metal plates in the back and a perforated rear shelf which ensures sufficient wall stand-off. Behind the table doors there is plenty of space for the Harmony PC and other accessories. Positioning brackets allow secure and accurate connection to PerkinElmer automation upgrades.

6.3 Setup Requirements

Notice

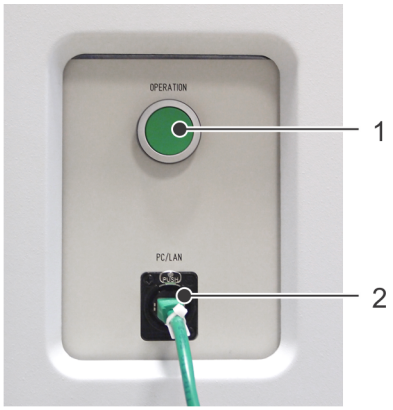
The Operetta CLS is sensitive to vibrations. Blurred images can be the consequence.

- *Put the Operetta CLS on a very stable bench or table. We recommend the optional Heavy Duty Lab Cart.*
- *Do not put other devices on the same table which cause vibrations (e.g. a centrifuge).*
- *Avoid any shocks or vibrations during measurements.*
- *If the Operetta CLS is integrated into an automated screening system, you have to reduce the speed or frequency and amplitude of components which cause vibrations (e.g. robots, centrifuges, shakers etc.).*

For a detailed list of requirements please refer to the **Operetta CLS Site Readiness Instructions**.

6.4 Controls and Connections

6.4.1 Left Panel



1. Power switch
2. Network connection

6.4.1.1 Power Switch

The on/off switch does not switch off the Operetta CLS immediately. There will be a controlled shutdown of the electronics with a certain delay before the instrument is really switched off.

Danger!

Direct electrical contact – Electrical shock.

- *In case of emergency unplug the power cable to disconnect the Operetta CLS from mains supply immediately.*
- *Ensure easy access to the mains connection at any time.*

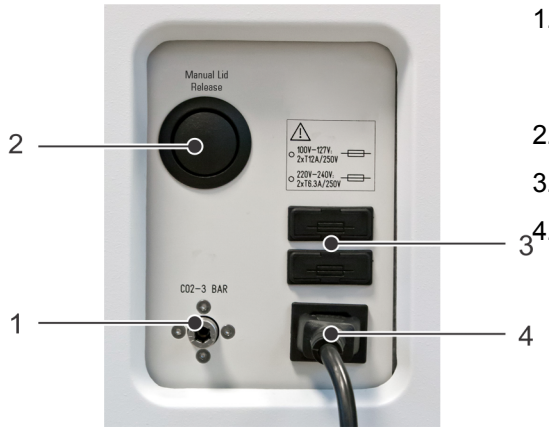
Notice

Wait at least 30 sec. before you restart the Operetta CLS after a shutdown.

6.4.1.2 Network Connection (LAN/PC)

LAN connection (Gigabit Ethernet according to IEEE 802.3) to the Harmony PC. Do not connect network cables longer than 3 m (118 in).

6.4.2 Right Panel



1. Carbon dioxide connection (3 bar) See also section 6.12 “Temperature and CO2 Control (TCO)”, page 521.
2. Manual release for lid
3. Fuse holders
4. Mains connection

6.4.2.1 Manual Lid Release

This mechanical function allows you to unlock the lid if the corresponding software functions for opening the lid cannot be used (e.g. instrument is switched off or defective). Do not press the button during operation. This can lead to the loss of data. See also section 6.6 “Lid and Sample Chamber”, page 512.

6.4.2.2 Fuse Holders

If one of the two fuses of the Operetta CLS has blown, it can be exchanged by the user.

Danger!

Direct electrical contact – Electrical shock.

- *Unplug from mains before replacing fuses.*
- *For continued protection against risk of fire, replace only with certified fuse of same type and rating:*
  - *100 V – 127 V: 2x T12A / 250 V (T = time-delay)*
  - *220 V – 240 V: 2x T6.3A / 250 V*

The Operetta CLS has two fuse holders:

- One electrical line is secured by the upper fuse holder.
- The other line is secured by the lower fuse holder.

Each fuse holder has two fuses. The left one is the active fuse, the right one is a spare fuse. See also section 7.2.3 “Replacing a Fuse”, page 537 for detailed instructions.

6.4.2.3 Mains Connection

Danger!

Direct electrical contact – Electrical shock.

- *Use only the provided power cable which has a mains plug with earthing contact (Europe: King-Cord K5A031H6250BB, USA: King-Cord K01031C5200BB-L).*
- *The mains plug must only be inserted in a socket outlet with earthing contact.*
- *No user serviceable parts inside the housing. Service has to be carried out by qualified PerkinElmer service personnel only!*
- *Unplug from mains for a complete disconnection.*
- *Disconnect the power cable by grasping the plug, not the cord. Otherwise the cable can be damaged.*
- *Check the instrument and power cable for damages before switching on the Operetta CLS. Do not use the instrument with damaged power cable or obviously defective or opened enclosure.*
- *In case of a damaged power cable contact PerkinElmer to request a new cable.*

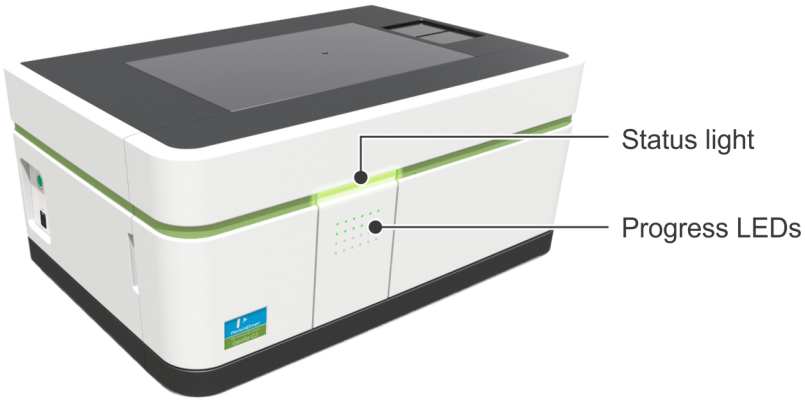
Danger!

Direct electrical contact – Electrical shock.

- *In case of emergency unplug the power cable to disconnect the Operetta CLS from mains supply immediately.*
- *Ensure easy access to the mains connection at any time.*

- Mains voltage: 100-240 V AC, 50/60 Hz
- Power consumption: 1000 VA max.

6.5 Instrument Status



6.5.1 Status Light

The status light of Operetta CLS indicates the instrument status.

General color coding:

- Green color: Information
- Red color: Error
- Green/red alternating: Waiting for user

Signal	Description
No Light	**Instrument off**
Blinking Green 	**Initializing / Load/eject plate / Shutdown**
Green Light 	**Ready / Measuring** • Operetta CLS is ready. • If the progress LEDs are visible, the instrument is in operation (measuring). The LEDs indicate the progress of the measurement on a percentage basis.
Blinking Green/Red 	**Waiting for user / Time series break** The instrument is waiting for user interaction. This does not indicate an error. The user's feedback is required to continue.
Red Light 	**Error** An error occured (hardware or software). The measurement has been stopped. A restart of the system may be necessary.

Signal	Description
Blinking Red  	**Fatal error (call Service) / Firmware Update** The instrument has a defect. Please call the PerkinElmer Service. The light is also blinking red during firmware update (no error). Do not cancel the procedure and do not switch off the instrument.

6.5.2 Progress LEDs

The 24 LEDs arranged in a 6×4 pattern are also used for indicating the instrument's status and the progress of a measurement:

Display	Description
	**Measurement Progress:** Progress of the measurement (on a percentage basis).
	**Close Lid:** The lid is currently open/unlocked. Close the lid (e.g. after changing an objective) to continue. See also section 6.6 “Lid and Sample Chamber”, page 512.
	**Temperature:** (only with TCO upgrade and temperature control activated). See also section 5.1.17.5 “TCO Settings”, page 246. • **'T' blinking:** Regulating the temperature inside the sample chamber, target value not stabilized yet. The 'T' starts blinking again if the TCO unit is reheating to maintain the temperature. • **'T' steady:** Target temperature has been reached.

6.6 Lid and Sample Chamber

Caution!

The lid on top of the instrument is not entirely stopped by the pneumatic springs if you let it fall down, so that you could hurt your fingers (especially at the lock in the middle).

- *Close the lid slowly and carefully.*

Danger!

Direct electrical contact - Electrical shock.

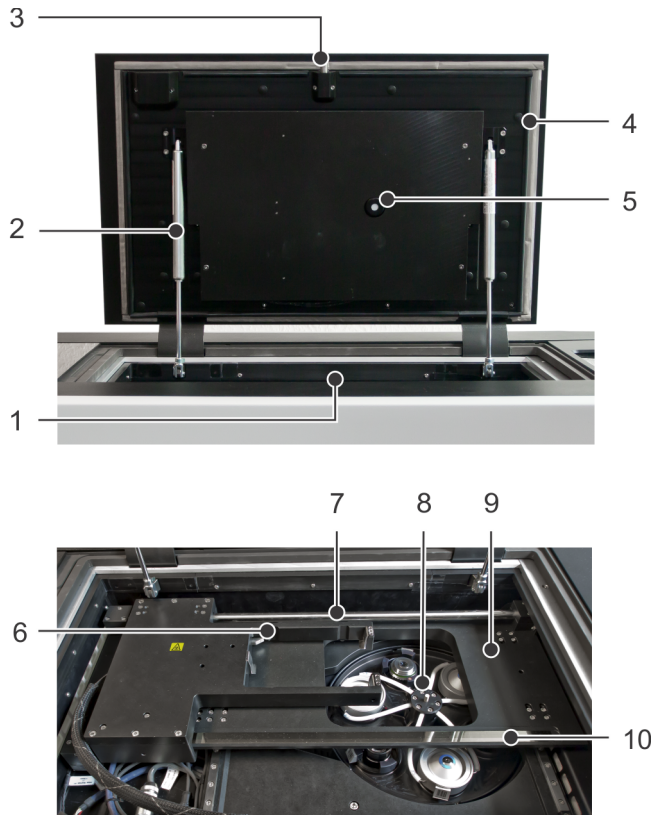
- *Do not handle large amounts of liquids near or above the Operetta CLS and observe the cleaning instructions.*
- *If liquids should be spilled into the instrument accidentally, switch off the Operetta CLS immediately, i.e. unplug the power cable (see also section "Disconnecting from Mains Supply", page 1).*
  - *Remove the liquid from areas which may be cleaned by the user (see cleaning instructions).*
  - *If liquid was spilled in other areas and may have entered the inside of the instrument, please contact PerkinElmer Service to let them check the instrument. Do not operate the instrument.*
  - *If potentially infective substances have been used, the instrument has to be decontaminated together with PerkinElmer Service.*
- *Service and repair may be carried out by qualified PerkinElmer service personnel only!*

Caution!

Glass elements are used for the upper surface of the instrument. Broken glass can lead to cuts and injuries.

- *Do not let heavy objects drop onto the glass plates.*
- *In case of a damaged glass plate, do not touch the glass fragments. Let PerkinElmer replace the broken part immediately. Do not operate a damaged instrument.*

For detailed cleaning instructions see section 7.2.1 "Cleaning", page 531.



Legend:

1. Sample chamber
2. Pneumatic springs
3. Locking bolt
4. Sealing
5. Transmission light source
6. Plate carrier
7. Linear motor (strong magnetic field, keep away metal tools and watches)
8. Objective turret
9. Scanning stage
10. Magnetic encoder scale of scanning stage (do not touch)

The lid on top of the instrument allows access to the sample chamber and the objective turret. A transmission light source (required for brightfield or digital phase imaging) is located on the bottom side of the lid. The lid is protected by safety interlocks. All lasers will be switched off as soon as the lid is opened.

The lid is locked by default. It can only be unlocked by the software using one of the following functions when access to the objectives is required:

- 5.1.17.2 “Change Objective”, page 241
- 5.1.17.1 “Set Correction Collar”, page 239
- 5.1.17.10 “Define Plate Type”, page 309 (when adjusting the correction collar)

Before the lid is unlocked, the scanning stage and the objective turret will be moved to their service positions.

Notice

Do not try to insert or remove a plate directly via the opened lid. Please use the **Load/Eject** function (via transfer position) instead. See also section 5.1.15 “Load/Eject Plate”, page 220.

If the lid is unlocked by the software, it pops up a bit so that you can open it manually. Close the lid carefully, then push it down until it clicks into place. The lid will be locked again.

Open lid

Close lid

Error Recovery

If you need to open the lid without prior movement of the scanning stage (e.g. plate tilted inside instrument) there are two further ways:

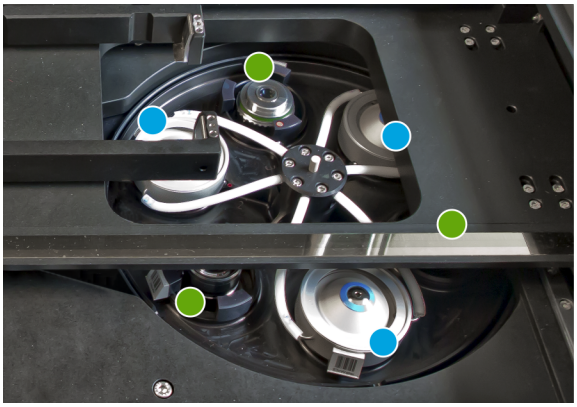
- Use the **Open Lid** button in the **Settings – Operetta CLS** dialog (see section “Open Lid”, page 1).
- Press the **Manual Lid Release** button (see section 6.4.2.1 “Manual Lid Release”, page 508).

Notice
Please note that these functions are intended for error recovery only.

6.7 Objective Turret

The objective turret can hold up to six objective lenses. Three positions are exclusively designed for air objectives, the other three positions for water immersion objectives (optional upgrade).

- **Air objectives** can be exchanged easily be the user.
  See also section 7.1.1 “Change Air Objective”, page 527.
- **Water immersion objectives** can only be installed or exchanged by PerkinElmer Service.
  See section6.11 “Water Immersion Objectives”, page 519 for further information.



Green: Air objective – Blue: Water immersion objective

The objective lens selected in the software is automatically turned into measuring position. During the autofocus procedure, the objective is then lifted out of the turret by the z-drive and moved to the correct focus height.

The objectives are labeled with barcodes. After exchanging an air objective, the list of available objectives in the software is automatically updated.

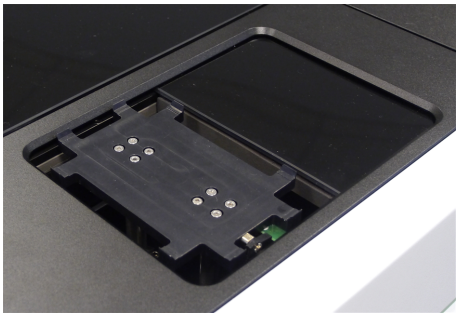
6.8 Transfer Position

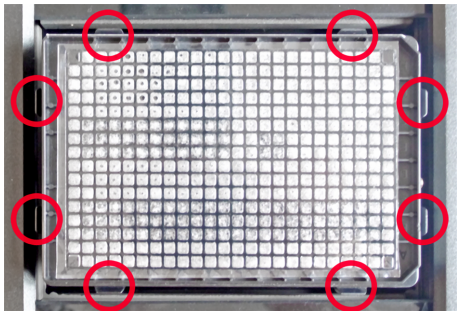
Warning!

You could jam your fingers at the plate lift (transfer position).

- *Do not reach into the compartment of the plate lift.*
- *Keep your hands away from the plate lift when it is moving.*

The transfer position is used for loading or ejecting a sample plate via the **Load/Eject** function in Harmony. Below the transfer gate there is a plate lift. The inserted plate will be moved down and placed into the plate carrier of the scanning stage. Then it will be moved into the sample chamber.





Make sure that the plate is correctly centered after putting it onto the plate lift (see red markers in the photo above).

Notice

Do not try to insert or remove a plate directly via the opened lid. Please use the **Load/Eject** function (via transfer position).

See also section 5.1.15 “Load/Eject Plate”, page 220.

6.9 Emission Filter Wheel

Access to emission filter wheel behind left front door

The Operetta CLS is equipped with an eight position emission filter wheel (behind left front door). Four standard emission filters are always included in the instrument. Further emission filters are available as an option and can be added anytime to further optimize the detection. Emission filters are barcoded and can be exchanged by the user. For details see section 9.6 “Optical Configuration”, page 559.

To change the configuration of the filter wheel please follow the instructions of the **Change Filter** wizard in the Harmony software
(see section 7.1.3 “Change Emission Filter”, page 530

Caution!

You could jam your fingers at the emission filter wheel (behind left front door). The wheel can suddenly start rotating without notice (with low force but at high speed).

- *Open the left front door only when requested by the Harmony software and follow exactly the instructions of the **Change Filter** wizard.*
- *Keep your hands away from the filter wheel if it is rotating.*

6.10 Image Alignment

Operetta CLS features a fully automatic image alignment procedure using a proprietary alignment sample attached to the scan table. Images from this durable sample are acquired each time a (test) measurement is started. The image alignment is skipped for snapshots to speed up experiment setup.

The structures detected in the acquired images of the alignment sample are then mapped to the ideal image of the sample. A set of correction parameters is determined and saved with the uncorrected raw image. If you view single (unstitched) images in Harmony, the original images will be displayed. The correction parameters will only be applied transiently to the images in the following situations:

- **Correction of stitched images**
  - Creating a global image (see also section 5.2.3.1 “Global Image / MultiScale Analysis”, page 329)
  - Creating a plate or well overview (see also section “How to open an Overview”, page 171)
- **Offset correction for PreciScan**
  See also section 5.1.8.3 “PreciScan™”, page 119.

There is a build-in quality control in the alignment procedure. If the alignment does not pass the control the procedure is repeated. If it fails repeatedly the measurement(s) will be executed anyway, however the repositioning accuracy of PreciScan and the stitching accuracy for MultiScale Analysis and overviews will be impaired. Please see section 5.3.12.4 “Consequences of Image Alignment Failure”, page 472 for further information.

If the image alignment procedure fails repeatedly, please first clean the objective. If this does not lead to an improvement please see section 5.3.12 “Image Alignment Procedure Fails Repeatedly”, page 470 for troubleshooting.

Please be aware that while the instrument is heating up (esp. if TCO is used) the optical properties of the system might change so that the previously calculated alignment parameters do not fit optimally anymore. Therefore, it is recommended to trigger an image alignment right before you do your measurement.

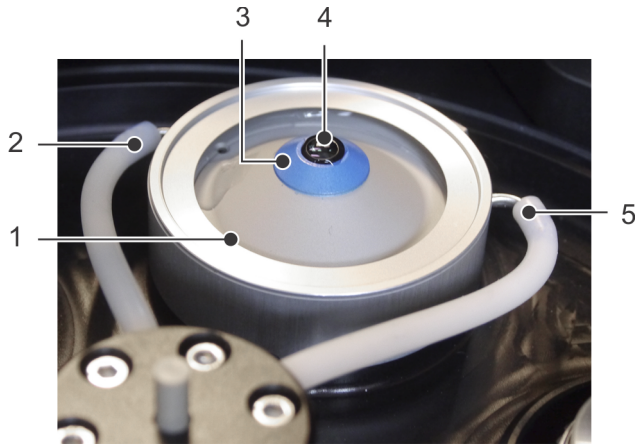
An easy way to replace the existing parameters is to select a different objective and click **Test**. Then switch back to the previous objective, and once you start the measurement the image alignment will be repeated automatically.

Please note that the alignment procedure uses the 475 nm LED. The corresponding emission filter **500-500 nm** must always be inserted in the emission filter wheel.

6.11 Water Immersion Objectives

Operetta CLS can be equipped with optional water immersion objectives. Immersion water supply via peristaltic pumps is fully automated and controlled by the software.

6.11.1 Objectives



Legend:

1. Water collar
2. Drain
3. Hydrophobic ring
4. Lens with water droplet
5. Supply

The water immersion objectives have fixed positions and cannot be exchanged by the user (see also section 6.7 “Objective Turret”, page 515). Each objective has a water collar with immersion water supply and drain. The blue hydrophobic ring keeps the water droplet in place.

Notice

Do not touch the hydrophobic ring with your fingers. A soiled ring will affect the water immersion and may lead to focus errors.

For cleaning instructions see also section 7.2.1 “Cleaning”, page 531.

The currently selected water objective can be flushed via Harmony, e.g. to remove air bubbles from the tubing or for troubleshooting. Please see section “Flush Water Objective”.

6.11.2 Water Bottles

The bottles for immersion water supply and waste collection are located behind the Operetta's front door. The bottles have to be emptied/refilled with laboratory water regularly (see also section 7.2.2 “Checking the Water Bottles”, page 534). Each bottle has a volume of 1 liter.

Caution!

You could jam your fingers at the front doors (access to emission filter wheel and immersion water bottles).

- *Open and close the front doors carefully and keep your hands away from the hinges.*

Danger!

Direct electrical contact - Electrical shock.

- *Check the filling levels of the immersion water bottles regularly, especially to avoid an overflow of the "WASTE" bottle and thereby immersion water getting into the instrument.*
- *When refilling or emptying a bottle, remove the respective bottle from its position. Refill or empty the bottle at a certain distance from the instrument only.*
- *Do not refill the "SUPPLY" bottle if the "WASTE" bottle does not fill up even though a water objective is in use. This could be an indication of a defective suction or a leakage resulting in immersion water being spilled into the instrument. In this case switch off the instrument immediately, i.e. unplug the power cable (see also section "Disconnecting from Mains Supply"). Contact PerkinElmer Service and do not operate the instrument.*
- *Do not obstruct the outlet at the bottom of the instrument. If clear water drops out under the instrument (without having spilled liquids), this can indicate a leakage of the immersion water system. In this case switch off the instrument immediately, i.e. unplug the power cable (see also section "Disconnecting from Mains Supply"). Contact PerkinElmer Service and do not operate the instrument.*
- *Service and repair may be carried out by qualified PerkinElmer service personnel only!*

6.12 Temperature and CO_2 Control (TCO)

6.12.1 Overview

With the **Temperature and CO_2 Control (TCO)**, the climate inside the sample chamber can be controlled via the Harmony software:

- **Temperature:** 37-42 °C (0.5 °C steps) or unregulated
- **Carbon dioxide (CO_2) concentration:** 1 to 10 % (0.5 % steps) or unregulated

Notice

The TCO unit needs approximately one hour to reach and stabilize the target temperature and 5 min. to stabilize the selected.CO_2 concentration.

Inside the Operetta CLS there is an air-circulating unit which regulates the CO_2 level in the sample chamber. Temperature is controlled by heating elements inside the sample chamber; the scanning stage and the bottom side of the lid can be warmed up. A temperature sensor is located directly below the plate. The sample chamber is sealed so that almost no air or CO_2 gas can escape.

The flow of carbon dioxide is only active if the lid is closed and CO_2 has been activated in the **TCO Settings** of the Harmony software (see section 5.1.17.5 “TCO Settings”, page 246). If the lid is opened, the climate in the sample chamber is lost and you have to wait until the target values have been reached and stabilized again.

6.12.2 CO_2 Supply

Notice

The CO_2 tubing is provided by PerkinElmer. CO_2 supply, pressure controller, stop valve and pneumatic air fitting are not part of the delivery and have to be provided by the customer. For details and a connection diagram please refer to the **Operetta CLS Site Readiness Instructions**.

Danger!

Carbon Dioxide Gas

In high concentration, carbon dioxide is harmful to health. Depending on the concentration it can lead to headache, tiredness, and dizziness, in higher concentrations even to unconciousness, spasms, and death. Carbon dioxide is hardly perceptible.

- *Do not install the Operetta CLS in very small rooms or without sufficient room ventilation.*
- *The customer has to install a pressure controller which provides the required carbon dioxide working pressure of 3 bar. Lower pressures can lead to malfunctions, higher pressures could destroy the instrument or lead to harmful concentrations in case of an instrument malfunction. See the Operetta CLS Site Readiness Instructions for a connection diagram.*
- *Use only a robust high quality CO_2 tube (see next section). Check tube and tube connection regularly for visible damages and do not operate the instrument with an obviously damaged tube or tube connection.*
- *If carbon dioxide control has been activated and you open the lid, wait 10 seconds before bending over the instrument to avoid unnecessary inhalation of the CO_2 gas.*
- *CO_2 supply must be switched off during downtime of the instrument.*
- *Do not try to set a CO_2 concentration higher than 10 % in the Harmony software, even if the software should allow that in case of malfunction.*

For additional protective measures please see section “Recommended Protective Measures”.

Danger!

Carbon Dioxide Gas - Supply Tubing

- *Do not expose the tubing to any mechanical or thermal stress.*
- *Comply with the maximum bending radius of the tubing (see below).*
- *If the tubing has to be laid on the floor, provide a corresponding protection to prevent people from stumbling or stepping on the tubing.*
- *Do not lay the tubing close to radiators or other heated objects.*
- *Do not expose the tubing to direct sunlight.*
- *Check the tubing for damages each time before using carbon dioxide control.*
- *Do not operate the instrument with a damaged carbon dioxide supply tubing. Contact PerkinElmer to request a new tubing.*
- *Do not manipulate the carbon dioxide tubing or fitting.*
- *The tubing may only be shortened using an approved hose cutter.*

Specifications and Requirements

- CO_2 tubing:
  - Outer diameter: 6 mm
  - Min. bending radius: 21 mm
  - Shore hardness: D 62 ±3
  - Material: TPE-A (polyamide)
- Working pressure: 3 bar
- Required CO_2 flow: > 15 l/h
- Required CO_2 purity: ≥ 99.5 %, particle-free

Notice

If you connect or disconnect the Operetta CLS from the CO_2 supply, dust particles may be introduced into the tubing. This can lead to malfunctions and blockages.

- *Blow through the tubing using CO_2 before reconnecting it to the instrument.*

6.12.3 CO_2 Control

The flow of carbon dioxide is only active ...

- if CO_2 control has been activated in the **TCO Settings** (see section 5.1.17.5 “TCO Settings”, page 246)
  and
- if the lid is closed so that the sample chamber is sealed
  and
- if the internal door to the transfer position is closed.

7 Retooling and Maintenance

This chapter describes retooling tasks for changing the Operetta's optical setup and maintenance procedures, which need to be carried out periodically to keep the instrument in good operating condition.

Danger!

Do not attempt to perform any kind of maintenance or retooling on the Operetta CLS beyond those listed and described here! All other repair or technical service tasks may only be performed by the PerkinElmer service personnel.

The retooling and maintenance steps outlined in the following may be carried out by the user. Please be sure to follow the instructions closely and observe both the general safety instructions (see section 2 “Safety Instructions”, page 9), and any specific safety instructions which may be given at the beginning of each section.

7.1 Retooling

7.1.1 Change Air Objective

Up to three air objectives can be installed in the objective turret at the same time. Air objectives can be exchanged by the user. Water immersion objectives can only be exchanged by PerkinElmer Service. See section 9.7 “Objective Lenses”, page 560 for a list of objectives available for Operetta CLS.

The 20x Air objective is the standard objective and should always be installed in the instrument. It is mandatory for certain software functions, e.g. in the **Plate Definition Wizard** (see section “Scan Plate Bottom”, page 310).

How to Change an Air Objective

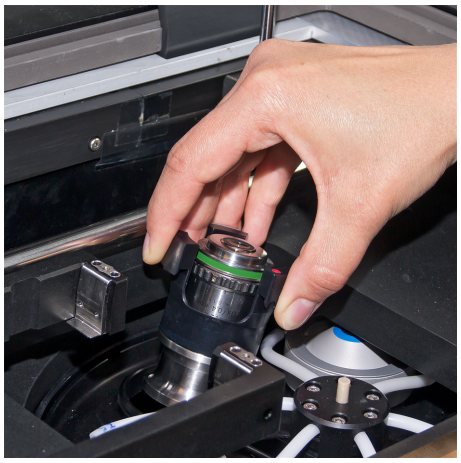
1. Open the **Settings** dialog in Harmony and select **Change Objective**.

   Follow the instruction of the **Objective Exchange Wizard** (see also section 5.1.17.2 “Change Objective”, page 241).

2. If all three positions for air objectives in the objective turret are occupied: Select the objective which is to be exchanged.

   The selected objective is moved into service position and the lid is unlocked.

3. If applicable: Remove the objective from its position (12 o’clock position in the objective turret) by just lifting it up.



4. Take the new objective and adjust the correction collar to the given value [mm]. Please only touch the objective as shown in the picture, do not touch the lens.

5. Insert the new objective into the objective turret. There is only one possible orientation where the notch can snap into place: Align the red marks with each other and take care that the objective is correctly installed.

6. Close the lid so that is snaps into place.

   The objective turret starts homing and the objective information will be read by the internal barcode reader.

7. A summary table shows all installed objectives. Close the wizard.

7.1.2 Adjust Correction Collar

Some air objectives for the Operetta CLS have an integrated correction collar (see section 9.7 “Objective Lenses”, page 560). It can be rotated and allows the correction of spherical aberrations mainly caused by differences in plate bottom thickness. The correction collar has to be adjusted to the value calculated by Harmony.

Notice

The correction collar must be set to the **optical thickness** of the plate bottom multiplied by the standard refractive index of 1.52 for glass, not to the real thickness. In case of glass bottom plates this value is of cause identical to the physical thickness. Harmony gives this corrected thickness value automatically.

The Harmony software will remind you to adjust the correction collar if you select a different plate type or objective during experiment definition. The required correction collar setting for the current plate type will also be stated. You can also use the **Set Correction Collar** function in the **Settings** dialog (see section 5.1.17.1 “Set Correction Collar”, page 239).

The objectives have a scale engraved. The unit of values of the scale is typically [mm]. There are different scales used depending on the range of compatible plate bottom thicknesses. On the ring above the scale you can find a thin black mark which indicates the current setting of the correction collar (see photo below).

Notice

It is not recommended to use plates with a plate bottom thickness higher than 1 mm as the automatic image alignment procedure requires the correction collar to be set to ≤ 1 mm. A wrong correction collar setting might impact the image quality.

How to Adjust the Correction Collar

1. If the message *"Please adjust the correction collar to ... mm"* appears in Harmony, the selected objective is moved into service position and the lid is unlocked.
2. Open the lid.
3. Remove the objective from its position (12 o'clock position in the objective turret) by just lifting it up.

4. If necessary, adjust the correction collar to the given value [mm].

> **Notice**
>
> - Only touch the objective as shown in the picture, do not touch the lens.
> - Make sure not to screw the objective out of its mount when adjusting the correction collar. The objective must remain screwed in firmly.

5. Insert the objective back to its position in the objective turret. There is only one possible orientation where the notch can snap into place: Align the red marks with each other and take care that the objective is correctly installed.

6. Close the lid so that is snaps into place.

7.1.3 Change Emission Filter

To change the configuration of the filter wheel please follow the instructions of the **Change Filter** wizard in the Harmony software
(see section 5.1.17.3 “Change Filter”, page 242).

Caution!

You could jam your fingers at the emission filter wheel (behind left front door). The wheel can suddenly start rotating without notice (with low force but at high speed).

- *Open the left front door only when requested by the Harmony software and follow exactly the instructions of the **Change Filter** wizard.*
- *Keep your hands away from the filter wheel if it is rotating.*

How to change an emission filter

1. Open the **Settings** dialog in Harmony and select **Change Filter**.

   Follow the instructions of the **Filter Exchange Wizard**. Use the **Next** button to proceed from step to step.
2. Select a position for the new filter. If all positions are occupied, you have to select and remove an existing filter.

   The filter wheel is moved so that you can access the selected position.
3. Open the left front door.
4. If necessary, remove the existing filter.
5. Insert the new filter (barcode facing to the left).
6. Close the left front door.

   The filters in the wheel will be read by the internal barcode reader.
7. A summary table shows all installed filters.
8. Click **OK** to close the wizard.

7.2 Maintenance

7.2.1 Cleaning

Warning!

Ethanol or other cleaning solvents may cause fire or injury if used in great amounts.

- *Switch off the instrument before cleaning.*
- *Observe the cleaning instructions.*
- *Do not handle large amounts of liquids near or above the instrument.*
- *Keep the concentration of dangerous solvents as low as possible.*
- *Ensure the area is well ventilated.*

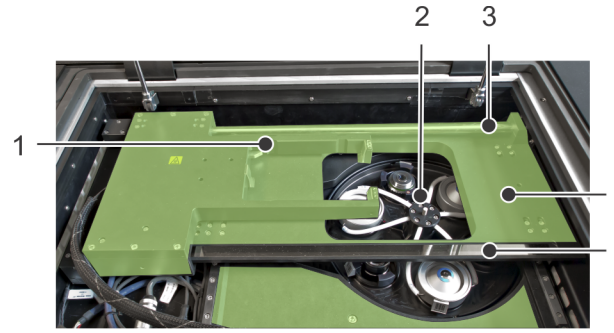
Cleaning the Instrument (Outside)

- For cleaning the outer surface of the Operetta CLS use only a soft cloth slightly moistened with water or a mild soap solution. Do not use any aggressive detergents or alcohol.
- Keep the area below the instrument free from dust to reduce the need for maintenance of the air filters at the bottom of the instrument. The air filters may only be cleaned or exchanged by PerkinElmer Service.
- Glass elements can be cleaned using a soft cloth moistened with glass cleaner.

Cleaning the Instrument (Inside)

The following places inside the housing may be cleaned by the user (only if necessary). All other areas and components can only be cleaned by PerkinElmer Service.

- Plate carrier of the plate lift (transfer position except area around and below the plate lift)
- Compartment for water bottles (behind front door)
- Certain areas inside the sample chamber (to open the lid while the system is switched off press the **Manual Lid Release** button on the right side of the instrument):
  - Scanning stage and plate carrier (except magnetic encoder scale)
  - Free surfaces of the sample chamber (except area with cables on the left)
  - Do **not** try to clean the objective turret. For cleaning the objectives see next sections.



Legend:

1. Plate carrier
2. Objective turret
3. Linear motor (strong magnetic field, keep away metal tools and watches)
4. Scanning stage
5. Magnetic encoder scale of scanning stage (do not touch)

Cleaning instructions:

- Soiled parts in the green areas described above can be cleaned using a soft, lint-free cloth moistened with a mild detergent or 70 % ethanol.
- If the magnetic encoder scale is soiled, you can clean it with a soft, lint-free cloth moistened with pure isopropanol without additives (residue-free). Do not use abrasive cleaners or cleaning agents which can corrode steal, chromium or gold.

Cleaning an Air Objective Lens

Air objectives should be removed for easier and safer cleaning.

1. Use the **Change Objective** wizard to take the desired objective out of the instrument. See section 5.1.17.2 “Change Objective”, page 241 for detailed instructions.
2. Clean the objective:
   - Please wear gloves during this procedure. Do not touch the lens (and the water collar or the blue hydrophobic ring of a water immersion objective) with your fingers.
   - Wet a lint-free lens cleaning tissue (e.g. Whatman® lens cleaning tissue, Grade 105) with 70% ethanol.
   - Wipe the lens a few times very carefully without force. Do not wipe back and forth but carefully in one direction.
   - Take a dry tissue and wipe the lens carefully in one direction until it is dry.
3. Put the objective back to its former position and check for correct positioning (see photos and instructions in the wizard).
4. Close the lid and finish the wizard.

Cleaning a Water Immersion Objective Lens

Water immersion objectives cannot be removed from the turret. To get access to the desired objective, you first you have to move the objective into measuring position.

1. Select the desired **Objective** on the **Setup** screen.
2. Select a well and click **Snapshot** to move this objective into measuring position.

3. Open **Settings – Operetta CLS** and click **Open Lid** to unlock the lid.
4. Clean the objective lens:
   - Please wear gloves during this procedure. Do not touch the lens, the water collar or the blue hydrophobic ring with your fingers.
   - Wet a lint-free lens cleaning tissue (e.g. Whatman® lens cleaning tissue, Grade 105) with 70% ethanol.
   - Wipe the lens a few times very carefully without force. Do not wipe back and forth but carefully in one direction.
   - Take a dry tissue and wipe the lens carefully in one direction until it is dry.
5. Close the lid.

Cleaning The Blue Collar of a Water Immersion Objective

Check each water objective unit once a week for correct water supply (see also section "Flush Water Objective", page 243). If the immersion water droplet cannot be formed on the lens, you may clean the blue hydrophobic ring as described below.

- Clean the blue coated area of the objective water collar with **warm water** if necessary:
  - Please wear gloves during this procedure. Do not touch the lens, the water collar or the blue hydrophobic ring with your fingers.
  - Wet a lint-free lens cleaning tissue (e.g. Whatman® lens cleaning tissue, Grade 105) with warm water.
  - Clean the blue coating gently and only for a few seconds.
  - Avoid rubbing and intense cleaning as this might harm the blue coating.
  - Make sure to avoid any scratches.
- If cleaning with water is not sufficient, you can clean the blue coated area of the objective water collar with **70 % ethanol** instead of warm water.

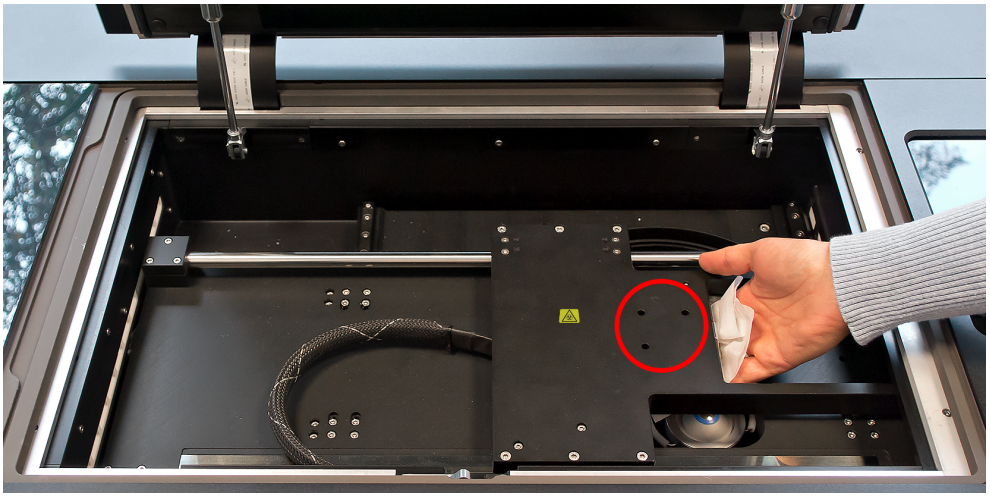
> **Notice**
> Cleaning with 70 % ethanol should not be performed more than once a month!

If you still experience issues with the water supply of a water objective unit after cleaning (with warm water and 70 % ethanol) please contact PerkinElmer Service.

> **Notice**
> Further cleaning of the water objectives can only be done by PerkinElmer Service.
> - Do not touch the lens, the water collar or the blue hydrophobic ring with your fingers.

Cleaning the Alignment Sample

Notice
Please only clean the alignment sample on your own if the image alignment procedure fails repeatedly and you cannot wait until the next service visit.



The alignment sample is located beneath the three screw holes in the scanning stage to the left of the plate carrier (see photo).

1. Click **Eject**.
2. Open the **Settings – Operetta CLS** dialog.
3. Click **Open Lid**.

   The lid is unlocked.
4. Open the lid.

   The table is now in a position where the alignment sample is accessible.
5. Clean the alignment sample:
   - Wet a lint-free lens cleaning tissue (e.g. Whatman® lens cleaning tissue, Grade 105) with 99% ethanol.
   - Gently wipe the alignment sample. Please note that the alignment sample is made of glass and might break if too much pressure is applied. Do not touch the alignment sample with bare fingers.
6. Close the lid.

7.2.2 Checking the Water Bottles

The fill levels of the fresh and waste water bottles should be checked daily before starting a screening run. The immersion water has to be exchanged at least every two weeks. Use only ultrapure laboratory water as specified below.

Specifications for immersion water (typical for ultrapure Millipore water)

- De-ionized
- Filtered through 0.22 µm sterile filter
- Conductivity of 0.056 µS at 25 °C
- Resistivity of 18.2 MΩ.cm at 25 °C
- Total organic content (TOC) ≤ 5 ppb
- Particulates (size > 0.22 µm) < 1 particulate/mL
- Bacteria < 0.1 CFU/mL

Caution!

You could jam your fingers at the front doors (access to emission filter wheel and immersion water bottles).

- *Open and close the front doors carefully and keep your hands away from the hinges.*

Danger!

Direct electrical contact - Electrical shock.

- *Check the filling levels of the immersion water bottles regularly, especially to avoid an overflow of the "WASTE" bottle and thereby immersion water getting into the instrument.*
- *When refilling or emptying a bottle, remove the respective bottle from its position. Refill or empty the bottle at a certain distance from the instrument only.*
- *Do not refill the "SUPPLY" bottle if the "WASTE" bottle does not fill up even though a water objective is in use. This could be an indication of a defective suction or a leakage resulting in immersion water being spilled into the instrument. In this case switch off the instrument immediately, i.e. unplug the power cable (see also section "Disconnecting from Mains Supply"). Contact PerkinElmer Service and do not operate the instrument.*
- *Do not obstruct the outlet at the bottom of the instrument. If clear water drops out under the instrument (without having spilled liquids), this can indicate a leakage of the immersion water system. In this case switch off the instrument immediately, i.e. unplug the power cable (see also section "Disconnecting from Mains Supply"). Contact PerkinElmer Service and do not operate the instrument.*
- *Service and repair may be carried out by qualified PerkinElmer service personnel only!*

1. Open the front door.
2. Check the fill levels of the bottles.

   Perform the following steps if a bottle needs to be refilled or emptied:
3. Screw off the blue cap of each bottle while holding the bottle's white tubing connector in place (to prevent it from rotating).
4. Remove the bottles and perform the following steps at a certain distance from the instrument.
5. Empty the **WASTE** bottle.
6. Refill the **SUPPLY** bottle with ultrapure laboratory water (see specifications before).
7. Put the bottles back to their former positions (left: waste; right: supply).
8. Screw on the blue caps while holding the white tubing connectors in place.
9. Close the front door.

After refilling the immersion water bottle you should flush the objective for 1 min. to remove air bubbles from the tubing. For details see section "Flush Water Objective", page 243 (**Settings – Operetta CLS** dialog).

7.2.3 Replacing a Fuse

If one of the two fuses of the Operetta CLS has blown, it can be exchanged by the user.

Danger!

Direct electrical contact – Electrical shock.

- *Unplug from mains before replacing fuses.*
- *For continued protection against risk of fire, replace only with certified fuse of same type and rating:*
  - *100 V – 127 V: 2x T12A / 250 V (T = time-delay)*
  - *220 V – 240 V: 2x T6.3A / 250 V*

1. Switch off the Operetta CLS and unplug the power cable.
2. Pull out both the upper and the lower fuse holder.
   - One electrical line is protected by the fuse in the upper holder, the other line is protected by the fuse in the lower holder.
   - Each fuse holder has up to two fuses: The fuse in the rear position is the active fuse, the one in the front position is a spare fuse.

3. Replace the blown fuse(s) in both fuse holders with the same type and rating of fuse suitable for your mains voltage.
4. Close the fuse holders and push them into their former position.
5. Reconnect the power cable.

7.2.4 Transport

Warning!

The instrument is very heavy (up to 180 kg / 397 lb). If it falls down or tips over it can cause severe injuries.

- *The four handles of the Operetta CLS are not designed to carry the device for relocation!*
- *It is only permitted to move the Operetta CLS on the bench or table by four persons using the provided handles. Do not try to carry the instrument.*
- *All four transport handles have to be screwed in completely. Do not use inadequate force or tools for this manual process, otherwise the handle threads may be damaged.*
- *The instrument may only be transported by PerkinElmer Service (requires a special transport frame and fork-lift).*

1. Pull off the magnetic covers on both sides of the instrument's base plate.
2. Screw the four provided handles completely into the threaded holes (see photo above).
3. Move the instrument carefully using the four handles (4 persons required).
4. After moving the instrument, unscrew the handles and mount the magnetic covers.

8 IT Policy

This chapter contains policies and general information about the IT infrastructure and rules for integration of the Operetta CLS System into any network environment.

Notice

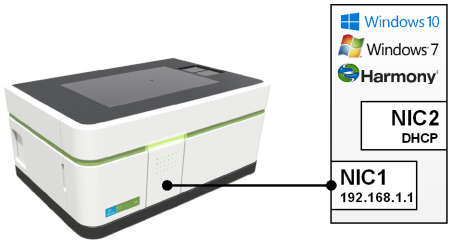
The purpose of the Operetta CLS IT Policy is to ensure the effective protection and proper usage of the computer systems belonging to the “Operetta CLS System”. The IT Policy will assist in maintaining systems at operational level. Contraventions of the IT Policy could seriously disrupt the operation of the “Operetta CLS System” and could involve PerkinElmer support billable at the current Service rate.

Notice

PerkinElmer is not responsible for problems caused by violating the following policies. Any effort required to verify this type of problem is billable at the current service rate and is not covered by guarantee and/or service contract.

8.1 Network Integration

8.1.1 Overview



The “Operetta CLS System” is composed of:

- Operetta CLS device (cell imaging reader)
- Harmony PC (to control the Operetta CLS using the Harmony software)

Optional components (provided by the customer) may be:

- SAN / Image Server to store/archive images
- Office PCs (for an office installation of the Harmony software)
- Columbus Server

Upgrade components (automation upgrade):

- plate::handler II robot (for automated plate handling)
- plate::works PC (running the scheduling software plate::works)
- Network Switch, 1Gbit speed, provided by PerkinElmer

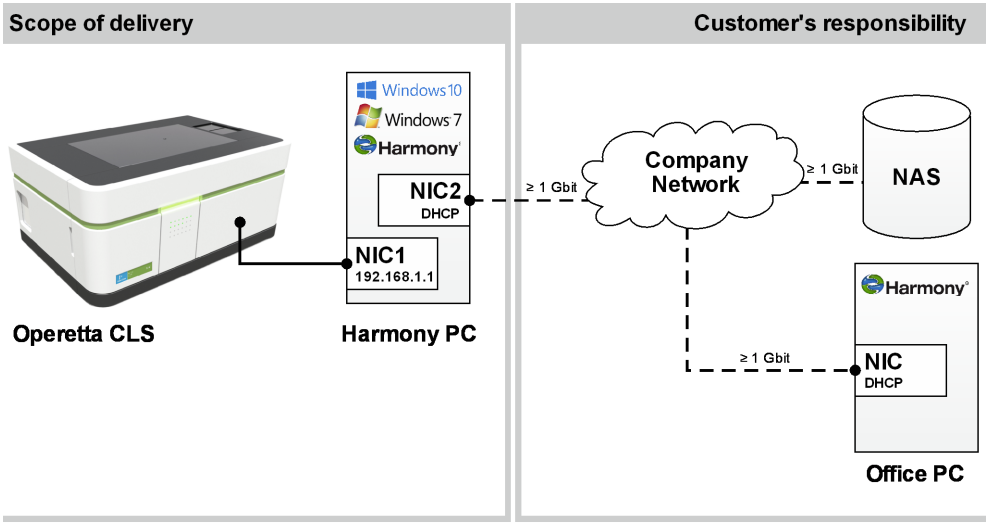
Notice

- The Harmony PC is a dedicated computer to control the Operetta CLS. This PC runs with Windows® 7 (64 bit) or Windows® 10 (64 bit), includes the database and cannot be substituted by any customer-supplied computer.
- The hardware of the Harmony PC must not be modified, replaced or extended (e.g. by additional network interface cards).
- The optional Columbus software (especially the Acapella Server) may not be installed on the Harmony PC or on a PC with Harmony office installation.

General Network Requirements

- The subnet mask for all network adapters has to be 255.255.255.0.
- All network connections must be Gigabit Ethernet connections using ≥ CAT-5e cable. All devices shown in the following configuration examples must support Gigabit Ethernet.

8.1.2 Simple Integration

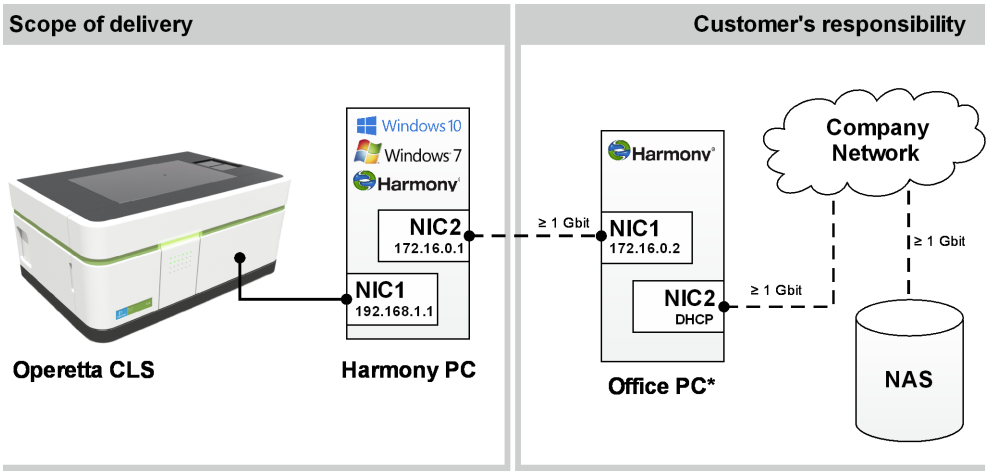


NIC: Network Interface Card – NAS: Network Attached Storage

8.1.3 Alternative Solutions

In the case where Windows® 7 or 10 is not allowed in the customer's company network, use one of the following solutions to isolate the “Operetta CLS network” from the company network.

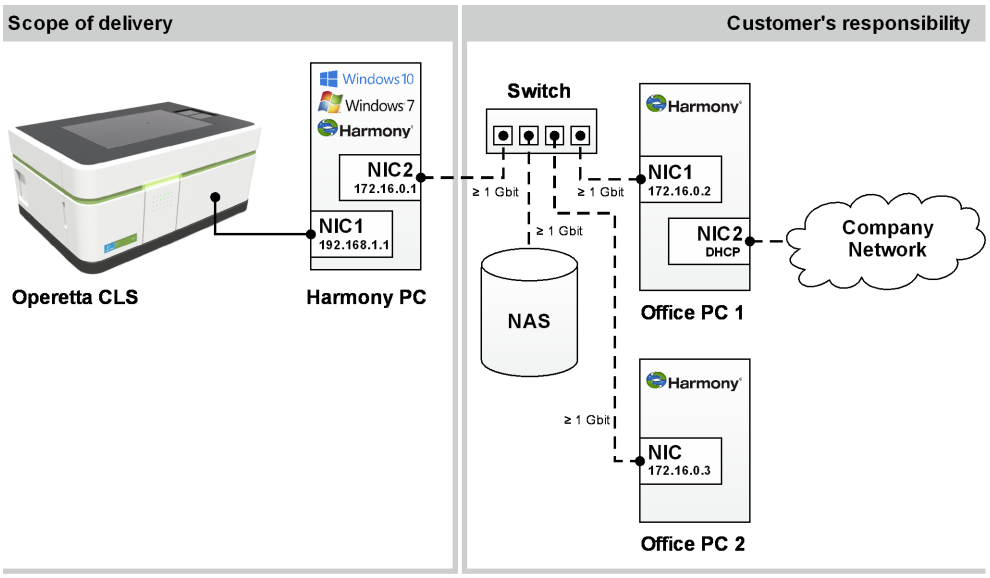
8.1.3.1 Office PC as interface (small solution)



NIC: Network Interface Card – NAS: Network Attached Storage

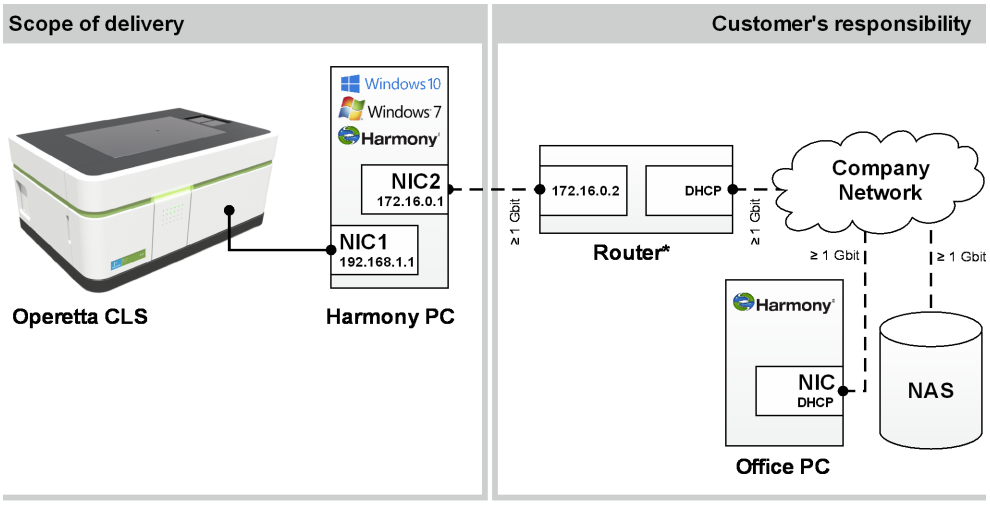
* Special NIC configuration required

8.1.3.2 Office PC as interface (big solution)



NIC: Network Interface Card – NAS: Network Attached Storage

8.1.3.3 Router



NIC: Network Interface Card – NAS: Network Attached Storage

* Special configuration required

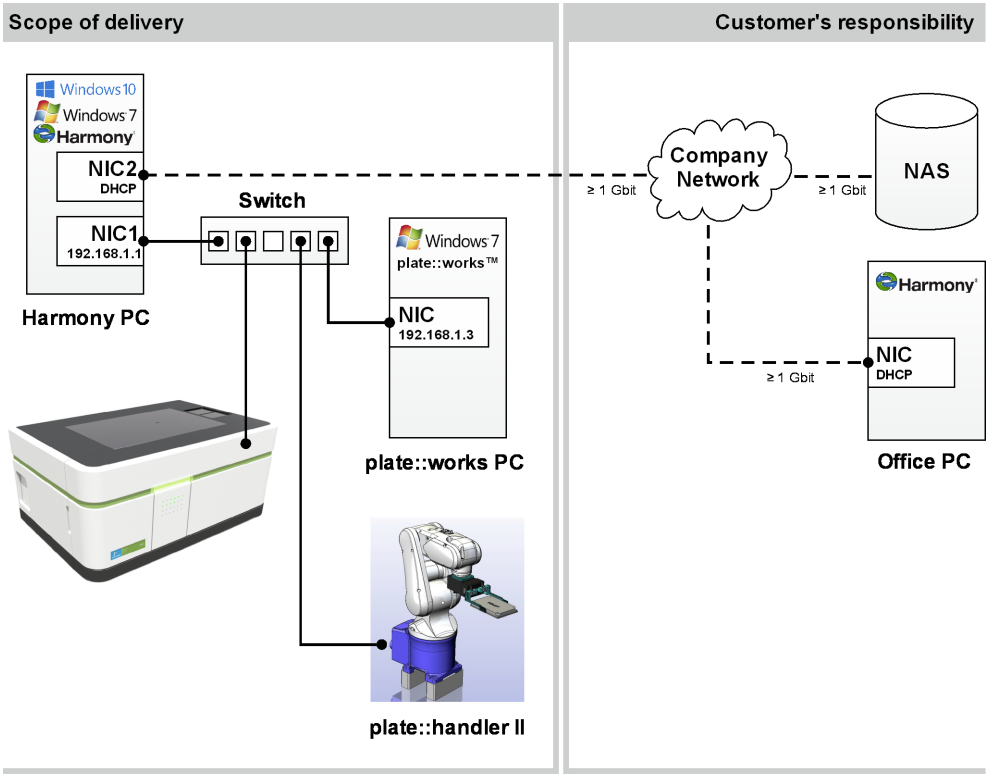
8.1.4 Automation Upgrade (plate::handler II)

Notice

The plate::works PC is a dedicated computer to control the automated plate handling (via plate::works scheduling software). It runs with Windows® 7 and cannot be substituted by any customer-supplied computer.

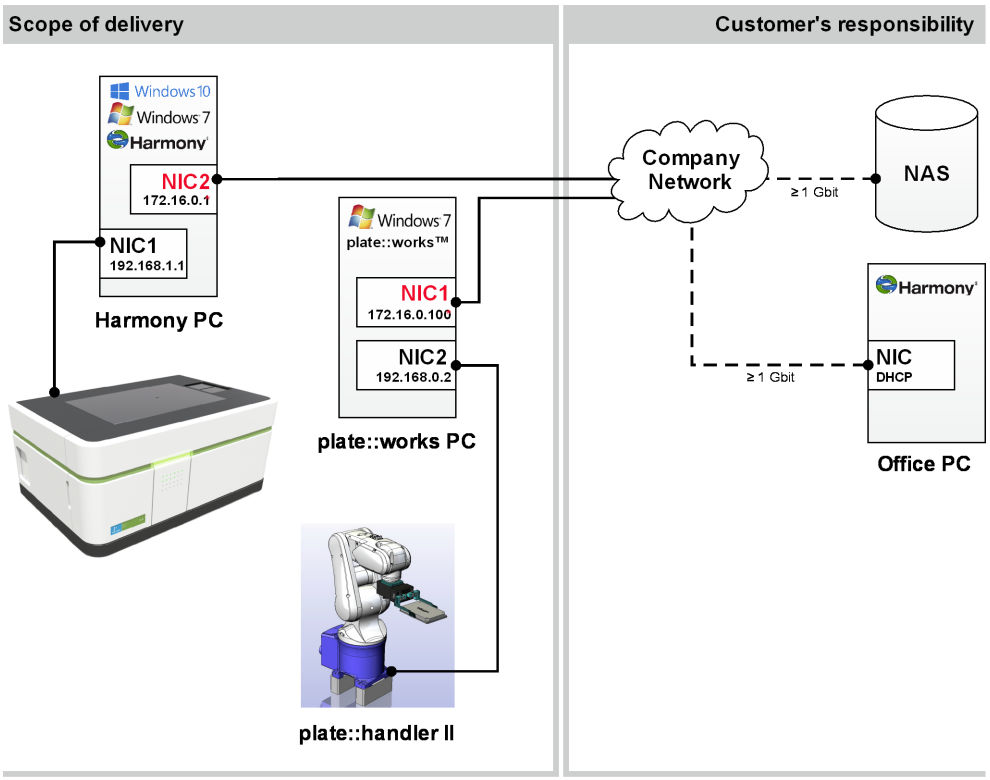
- The computer may not be integrated into a domain.
- Anti-virus or malware protection software may not be installed on this computer.

8.1.4.1 Standard Configuration with PKI Switch



NIC: Network Interface Card – NAS: Network Attached Storage

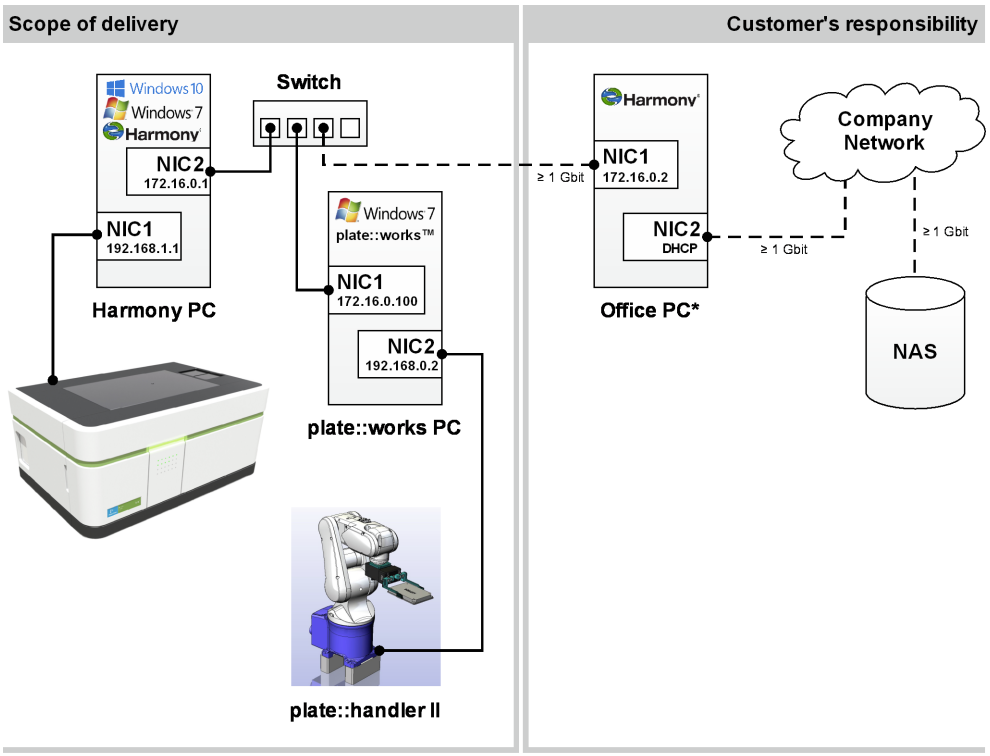
8.1.4.2 Standard Configuration w/o PKI Switch



* Network configuration must be adapted to company network, see below
NIC: Network Interface Card – NAS: Network Attached Storage

This scenario is only possible if NIC2 of the Harmony PC and NIC1 of the plate::works PC (both in red, see figure above) are configured with static IP addresses by your local IT department. The network configuration (IP, Gateway, Subnet, Wins, DNS) has to be adapted according to the configuration of the company network.

8.1.4.3 Office PC as interface



NIC: Network Interface Card – NAS: Network Attached Storage

* Special NIC configuration required

8.1.5 Columbus

The Columbus image storage and analysis system is installed as a client-server system. The Columbus Server is a dedicated computer which has to be integrated into the customer's network. Columbus can be used via web interface on any PC within the network.

8.1.5.1 Network Configuration

Columbus Scope Server

- The Columbus Server requires a free IP address in a local area network:
  - Either a **static IP address** with a **host name** provided by local network admin,
  - or an address provided by **DHCP**.
- The network settings of the Columbus Server must be configured for your network:
  - Enter DNS server IP address(es)
  - Enter optional gateway IP address
- Add an entry for the Columbus Server on the DNS server of your network, if access via host name is required.

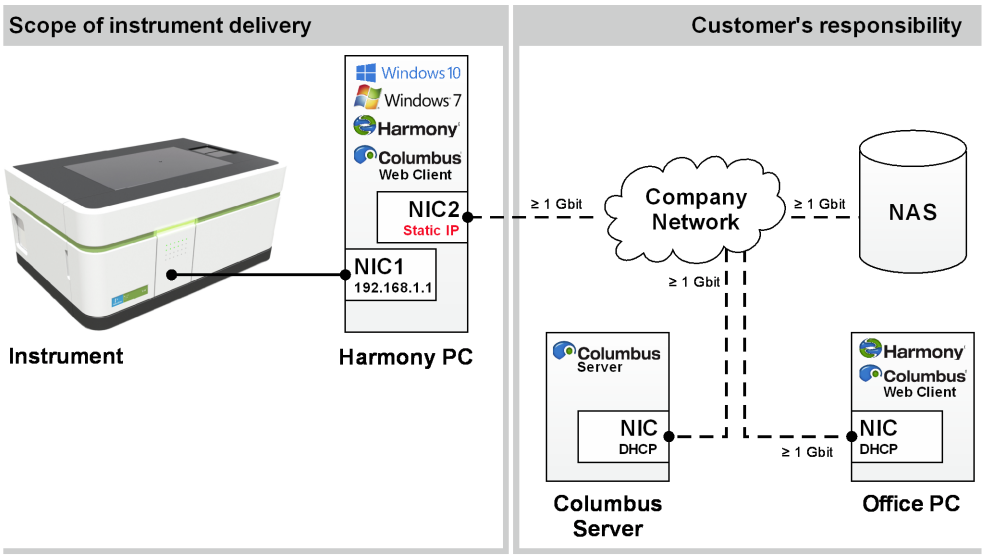
- If there is a firewall between the server and the clients, the following ports must be opened. On the Columbus Server, the local ports have been set accordingly during setup.
  - TCP/22 – SSH – Remote Access
  - TCP/80 – http – Columbus webapp
  - TCP/443 – https – Columbus webapp
  - TCP/8081 – http – Columbus SOAP webservice interface
  - TCP/4063-4064 – Columbus Java clients

Communication between Columbus and Harmony

> **Notice**
>
> To ensure proper communication between the Harmony software and Columbus via the **Columbus Transfer** function of Harmony, it is essential to read the following instructions.

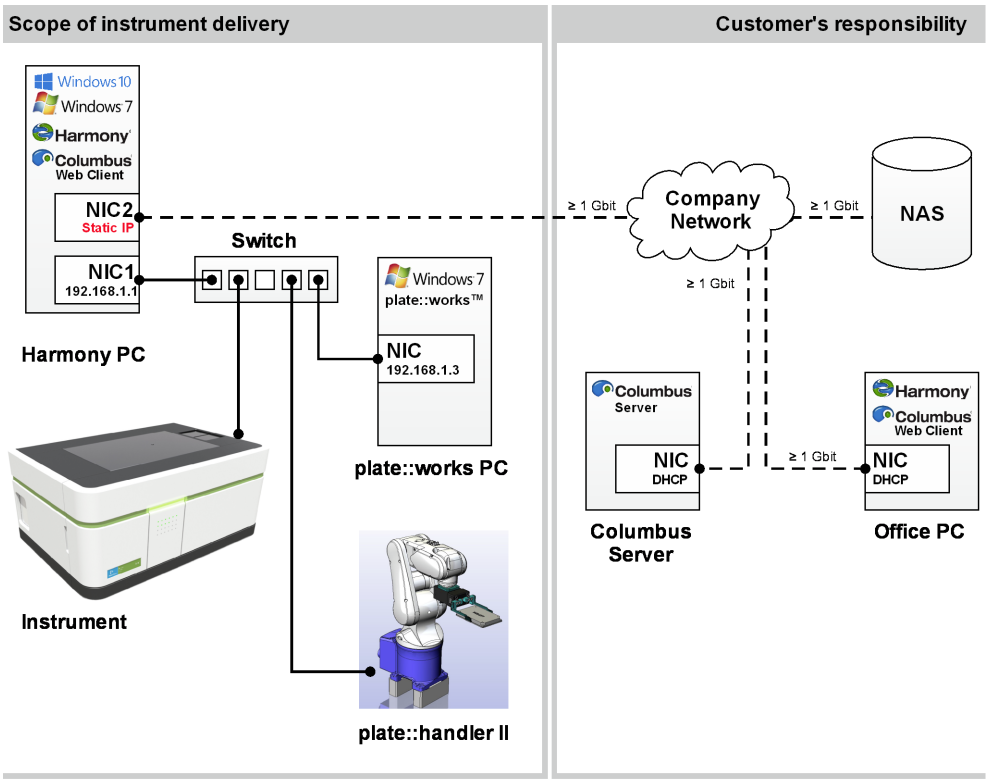
- For the Harmony workstation (instrument PC) a **static IP address** with **host name** must be configured (see also diagrams in the following chapter).
- The IP address and host name of the Harmony workstation must be known to the Columbus instance. It must be added as a new host to the **host configuration** of the Columbus Server (Network Services – Hostnames, /etc/hosts configuration file).

8.1.5.2 *Standard Configuration with Columbus*



NIC: Network Interface Card – NAS: Network Attached Storage

8.1.5.3 Automation Upgrade with Columbus



NIC: Network Interface Card – NAS: Network Attached Storage

8.2 Security Updates / Service Packs

8.2.1 Microsoft Windows

The Harmony software was tested and released with:

- Microsoft Windows® 7, Service Pack 1, 64 bit
- Microsoft Windows® 10, 64 bit

This includes all security patches until the Harmony release date (see Release Notes).

Notice

PerkinElmer cannot guarantee that future Windows updates provided by Microsoft will not compromise the stability of the “Operetta CLS System”. We assume that the impact is low but to ensure to always have a stable “Operetta CLS System” it is recommended to disable Windows updates.

8.2.2 Microsoft SQL Server

- Harmony 4.6 has been tested with MSSQL 2012 SP3.

8.3 Requirements for Harmony Installation on an Office PC

Notice

- Due to numerous differences in PC hardware, PerkinElmer cannot guarantee that our software will run on a computer supplied by the customer, even if the system meets the minimum specifications described on the next pages. PerkinElmer installation of a computer supplied by the customer is available for an additional fee. PerkinElmer is not responsible for problems caused by unspecified system components, software, and/or accessories.
  Any effort required to verify this type of problem is billable at the current service rate. PerkinElmer may not provide maintenance service on the computers supplied by the customer.
- It is the customer's responsibility to update office installations of the Harmony software (same version as on Harmony PC is required).

Basic:

- Windows® 7, SP1, 64 bit, English or Windows® 10, 64 bit, English (Windows® 8 is not supported)
- Harmony Office License (incl. USB dongle)
- One free USB port
- 2 GB free harddisk
- DVD drive (for installation)
- Gigabit Ethernet connection to Harmony PC (to connect to database)
- Administrator rights (only for the installation)
- Internet Explorer 8 or later, JavaScript® enabled, cookies allowed
- Display Language: English
- Region and Language Setting: English
- DPI setting: normal size (96 DPI)

Minimum:

- Processor with 4 cores (Intel® Xeon®, Core™ i5, Core™ i7)
- 8 GB RAM
- Screen resolution of 1280 x 1024 (19")

Optimum:

- Intel® Xeon® processor with 12 cores
- 32 GB RAM
- Screen resolution of 1920 x 1200 (24") or 2560 x 1600 (30”)

8.4 Computer Systems

8.4.1 Network

The Harmony PC can receive the network address from a DHCP server. The general configuration is to obtain the IP address automatically from a DHCP.

1. The usage of any additional network adapter of any computer of the “Operetta CLS System” is not allowed.
2. Changing the configuration of the Harmony PC's Network Interface Card 1 is not allowed.
3. Bridging of the two network cards of the Harmony PC is not allowed (no Internet Connection Sharing).

Notice

A stable Gigabit network is essential for the correct function of the Operetta CLS System, because permanent network connection is required between the different components (particularly Operetta CLS device, Harmony PC, Office PCs, image servers, Columbus Server). In addition, your DNS server must be able to resolve each computer's name.

8.4.2 Hardware

Requirements for new PC hardware should be discussed in advance with PerkinElmer Service to assess the detailed specification. Problems with hardware should also be reported to PerkinElmer Service.

1. The relocation of the Harmony PC is not allowed. This PC must have a direct connection to the Operetta CLS device. The relocation of any other hardware within the “Operetta CLS System” should be discussed with the PerkinElmer Service in advance to ensure good reason for relocation, determine the most appropriate means of relocation and to ensure a fully operational “Operetta CLS System”.
2. Modification of any hardware of the Harmony PC is not allowed.
3. In order to get most performance in image evaluation from your office computer we highly recommend to check your PC’s memory module (DIMM) configuration. If your motherboard/CPU supports multichannel bandwidth operations then make sure that each channel has its own DIMM. Please refer to your local IT and/or to the PC’s manual.

8.4.3 Operating System, Software & Software Applications

Problems with software should be reported to the PerkinElmer Service.

1. Supported operating systems:
   Windows® 7, SP1, 64 bit, English
   or Windows® 10, 64 bit, English
   - 32 bit operating systems are generally not supported.
   - The 64 bit versions of Windows® 8, Windows Vista® and Windows® XP are not supported.
   - Using any virtual PC (e.g. VMWare, Virtual-PC) is not supported.
2. Set “Automatic Microsoft Updates” to “Turn Off Automatic Updates” or “Notify me but don´t automatically download and install them.” because they may disturb the “Operetta CLS System”.
3. Deactivating the virtual memory for Microsoft Windows® is not allowed.
4. Making any changes in the [HKLM\Software\PerkinElmerCTG] registry is not allowed.
5. Make sure that the Local Security Policy of Windows® is configured correctly:
   - “Network access: Do not allow storage of credentials or .NET Passports for network authentication” must be set to “Disabled”. Otherwise you cannot create a new scheduled task via the Harmony software (if using the option “Run whether user is logged on or not”).
   - You can find this policy here: “Control Panel\All Control Panel Items\ Administrative Tools\Local Security Policy\Local Policies”.

8.4.4 Data

1. PerkinElmer is not liable for any data loss due to data management processes like backups, etc.
2. When harddisk space on the Harmony PC (2 TB) is exhausted we recommend to a) archive data that is (currently) not actively required or b) relocate images to free disk space. Relocated images remain accessible via the Harmony software.
3. Images should only be relocated to reliable servers which are integrated into the customer's backup system.
4. PerkinElmer recommends a Gigabit connection and to include this server in the internal backup system.

8.4.5 Back Up

Warning
If no backup procedure is established, a crash of the system will not only mean the **loss of all data on the Harmony PC**, but also the **loss of all relocated images**. Without the meta data in the Operetta CLS database (ODA) these images cannot be accessed anymore and they cannot be re-imported into the system.

1. PerkinElmer is not responsible for the implementation of an effective backup strategy.
2. PerkinElmer is not responsible for the backup of any files from the “Operetta CLS System”.
3. PerkinElmer recommends to backup all data on the Harmony PC once a day using third-party backup software. so that it can be restored in case of a crash. To restore such a backup you will need to contact PerkinElmer Service.
4. The following paths must be included to backup all Harmony data:
   - C:\ProgramData\PerkinElmer\ODA_DATA\IMAGES
   - C:\ProgramData\PerkinElmer\ODA_DATA\RESULTS
   - C:\ProgramData\PerkinElmer\ODA_DATA\XML
   - C:\ProgramData\PerkinElmer\ODA_DATA\BAK (daily database dump)
5. If images have been moved to a different location using the function “Relocate Images”, page 259, these relocated images must be subjected to a separate backup procedure and are not included when backing up the data on the Harmony PC.

For further information see section 5.3.10 “Backup all Data on Harmony PC”, page 468.

8.4.6 Security Settings & Anti-Virus Protection

The Harmony software is tested with Microsoft Windows Defender.

1. PerkinElmer is not responsible for the implementation of an effective virus security strategy.
2. It is suggested to exclude the following file types and protocols from any security scan as this may slow down some Harmony processes tremendously:
   - HTTP protocols
   - TIFF (images)
   - XML (database files)
3. The “Operetta CLS System” needs the following ports for communication (TCP/UDP). Please ensure that these ports are unblocked:
   - 80, 8213, 8255-8258, 8282, 8300

   For more details please see section 5.3.21 “Firewall Settings”, page 482.
4. In the unexpected case of returning a PC back to PerkinElmer any security software like virus scanner or firewall has to be removed before shipping.

8.5 Computer Users

1. Training: Ensure an appropriate Harmony training for any user of the “Operetta CLS System”.
2. User Accounts: The Harmony PC comes with two pre-defined Windows® user accounts:
   - **Harmony** (password “Harmony")
     This is the default Windows® user account for operating the Operetta CLS system.
   - **Administrator** (password “Harmony")
     This account is only required for installing additional software or updates.

   Modifying or deleting the existing user accounts is not recommended. If you change the default user account “Harmony", this will require additional modifications in Harmony and in the network (see section 5.3.14 “User Accounts”, page 473). The default user account ("Harmony") must have the following property:
   - Password does not expire.
     Otherwise the user account details for all relocate paths in the **Relocate Settings** have to be updated each time after changing the password.
3. Network share: Computers in the network which shall be used as destinations for the functions **Relocate Images** or **Write Archive** (as scheduled task or triggered manually) must be accessible for the default user account “Harmony” (read/write access including the right to create subfolders).
4. Passwords: “Operetta CLS System” users should not change any passwords. Any changes are only allowed upon consultation with PerkinElmer Service.

8.6 Remote Support

PerkinElmer uses the service of LogMeIn (https://secure.logmeinrescue.com/) to solve issues of “Operetta CLS Systems” by remote support. If LogMeIn support is not allowed please contact PerkinElmer Service and ask for other options. Please note: Other options may increase costs for the service contract.

Minimum system requirement for LogMeIn remote support:

- Windows® 10, 8.1, 8, 7, Vista, XP, Server 2003 & 2008 (all including 64-bit)
- Broadband connectivity to the internet (i.e. T1, cable modem, ISDN, or DSL)

9 Technical Data

9.1 Physical Dimensions

Physical Dimensions	
Width	98 cm / 38.6 in
Depth	66 cm / 26.0 in
Height	• Lid closed: 47 cm / 18.5 in • Lid opened: 85 cm / 33.5 in
Weight	150-180 kg / 331-397 lb

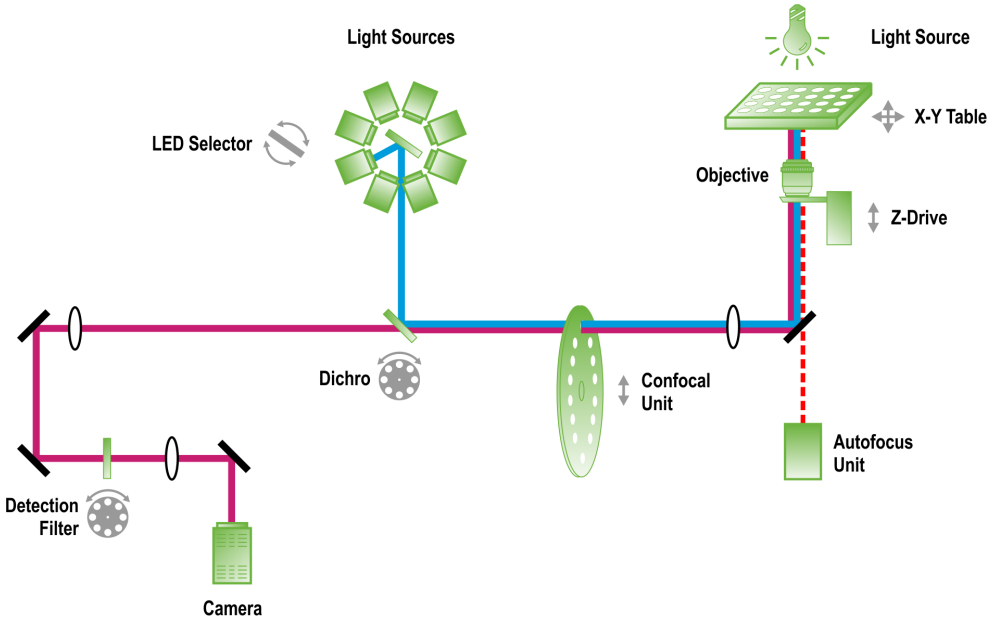
9.2 Power Requirements

- Mains voltage: 100-240 V AC, 50/60 Hz
- Power consumption: 1000 VA max.

9.3 Connections

Connection	Description
LAN/PC	• Gigabit Ethernet according to IEEE 802.3 • LAN cable max. length: 3 m (118 in)
CO_2 (carbon dioxide)	• Working pressure: 3 bar
Fuses	• 100 V – 127 V: 2x T12A / 250 V (T = time-delay) • 220 V – 240 V: 2x T6.3A / 250 V
Mains connection	• Mains voltage: 100-240 V AC, 50/60 Hz • Power consumption: 1000 VA max. • Power cord Europe: King-Cord K5A031H6250BB USA: King-Cord K01031C5200BB-L

9.4 Optical Path



9.5 Light Sources

Operetta CLS is a Class 1 Laser Product (classified according to standard IEC 60825-1:2014).

- Wavelength: 785 ± 10 nm
- Accessible radiation: 3.4 µW

9.5.1 LED Light Sources

Depending on the selected LED configuration, the instrument is equipped with 4 or 8 LEDs for excitation of the sample. In the table below, the 4 standard LEDs are highlighted with gray background.

Wavelength	Power	Excitation Filter	Classification of Optical Radiation
365 nm (UV)	130 mW	355-385 nm	Risk Group 3
405 nm	230 mW	390-420 nm	Risk Group 3
440 nm	230 mW	435-460 nm	Risk Group 3
475 nm	110 mW	460-490 nm	Risk Group 2
510 nm	100 mW	490-515 nm	Risk Group 2
550 nm	170 mW	530-560 nm	Risk Group 2
630 nm	120 mW	615-645 nm	Risk Group 2
660 nm	250 mW	650-675 nm	Risk Group 3

9.5.2 Autofocus Laser

Wavelength	Laser Power	Description
785 ±10 nm	12 mW	• Solid state laser • Laser class 3B

9.5.3 Transmission Light Source

Wavelength	Power	Description
740 nm	80 mW	• LED for Brightfield and Digital Phase Contrast measurements • Integrated in sample lid

9.6 Optical Configuration

9.6.1 Emission Bandpass Filters

The emission filter is used to additionally block the excitation wavelength and transmit light emitted by the sample to the camera. Additionally the wavelength of the autofocus laser is blocked.

Up to 8 emission filters can be installed simultaneously in the instrument. These filters can be exchanged by the user (see section 7.1.3 “Change Emission Filter”, page 530).

Standard Configuration	Optional Configuration
430-500 nm	460-515 nm
500-550 nm*	470-515 nm
570-650 nm	470-540 nm
655-760 nm	515-580 nm
	525-580 nm
	570-620 nm
	600-640 nm
	655-705 nm
	685-760 nm

*required for image alignment, should always be inserted

For an overview of excitation filters see section 9.5 “Light Sources”, page 558.

9.6.2 Dichroic Mirrors

The dichroic mirror is used to couple in the excitation wavelength band and transmit the emission wavelength bands to the camera.

Configuration
Longpass 425 nm
Longpass 465 nm
Longpass 495 nm
Longpass 520 nm
Longpass 565 nm
Longpass 650 nm
Longpass 680 nm

9.7 Objective Lenses

9.7.1 Configuration

Component	Description
Standard Objective	20x Air, NA 0.4
Optional Air Objectives	1.25x Air, NA 0.03 5x Air, NA 0.16 10x Air, NA 0.3 20x HNA Air, NA 0.8 40x Air, NA 0.6 40x HNA Air, NA 0.75
Optional Water Immersion Objectives	20x Water, NA 1.0 40x Water, NA 1.1 63x Water, NA 1.15

9.7.2 Objective Specifications

Part No.	Objective	NA	Working distance [mm]	Correction collar	Corrected for plate thickness [mm]	Field of view [mm]	Depth of focus** [µm]	Effective xy resolution*** [µm]		Recommended minimum distance between planes**** [µm]
								Binning 1	Binning 2	
HH14000401	1.25x Air	0.03	4	No	0.17	10.33 × 10.33	1501.0	10.6	21.2	682.3
HH14000402	5x Air	0.16	12.1*	No	0.17	2.58 × 2.58	58.2	2.6	5.3	26.5
HH14000403	10x Air	0.3	5.2	No	0.17	1.29 × 1.29	16.2	1.3	2.6	7.4
HH14000404	20x Air	0.4	8.28*	Yes	0 – 1.5	0.65 × 0.65	8.0	0.79	1.32	3.6
HH14000407	20x hNA Air	0.8	0.55	No	0.17	0.65 × 0.65	2.6	0.66	1.32	1.2
HH14000421	20x Water	1.0	1.7	No	0.17	0.65 × 0.65	1.8	0.66	1.32	0.8
HH14000405	40x Air	0.6	3.28	Yes	0 – 1.5	0.32 × 0.32	3.3	0.53	0.66	1.5
HH14000408	40x hNA Air	0.75	0.71	No	0.17	0.32 × 0.32	2.2	0.42	0.66	1.0
HH14000422	40x Water	1.1	0.62	No	0.17	0.32 × 0.32	1.2	0.33	0.66	0.5
HH14000423	63x Water	1.15	0.6	No	0.17	0.21 × 0.21	1.0	0.28	0.42	0.5

The default objective which is always included in the instrument is highlighted in green.

* The working distance of the objective is so large that it will not be completely used due to the given limitation of the z -drive.

** Depth of focus = 1.4 λ n / NA^2 with λ = 500 nm, n = 1.33, NA = numerical aperture. Eventually with a reduction factor due to undersampling on the camera.

*** Calculated at 520 nm. Optical resolution = 0.61 λ / NA. Eventually with a reduction factor due to undersampling on the camera.

**** Calculated as "Depth of focus / 2.2" (according to Nyquist's theorem). Please note that using smaller distances will not improve the results.

The working distance given here is the total distance from lens to focal point. Please note that manufacturers of objectives often use the maximum working distance for historical reasons. The maximum working distance is defined by objective manufacturers as the distance from lens to focal point minus 0.11 mm (0.11 mm equals the thickness of a typical cover slip reduced by its refractive index).

The 10x Air objective is best suited for plates with a bottom thickness of 170 µm. As it has no correction collar, a much thicker plate bottom will affect the optical image quality.

9.8 Camera

Component	Description
Detection Camera	sCMOS sensor 16 bit resolution 4.7 Megapixel (2160 x 2160 px) 6.5 µm pixel size

9.9 Microplates

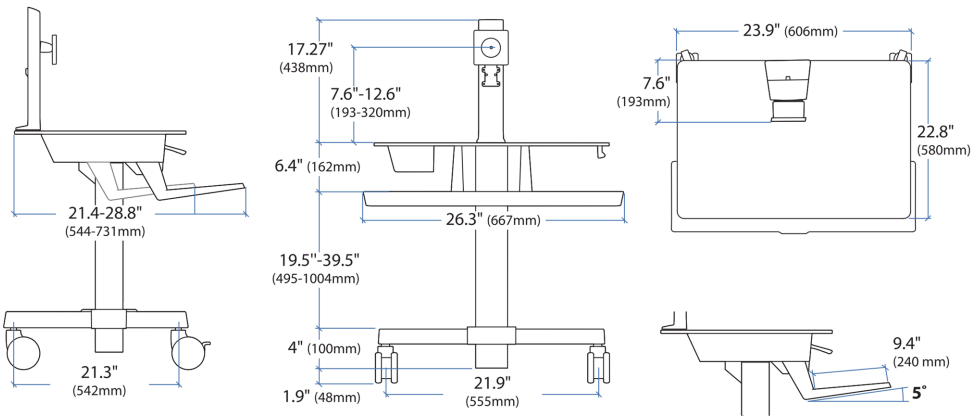
The Operetta CLS is compatible with SBS compliant microplates. Please see the **Operetta CLS Application Guide** (appendix) for a list of recommended plate types which are suitable for cell imaging.

Notice

- The plate height (parameter **G** in the plate wizard) may not exceed 22 mm. When using plates with lids, it is the user's responsibility to make sure that the maximum plate height (plate *and* lid) is not exceeded. Otherwise the plate may crash inside the instrument.
- *Only for optional Automation Upgrade:* Before starting an automated run you have to verify whether the used plate type can really be handled by the robot and is properly detected by the Operetta's plate sensor.

9.10 Monitor/Keyboard Cart (optional)

Dimensions and Range of Motion

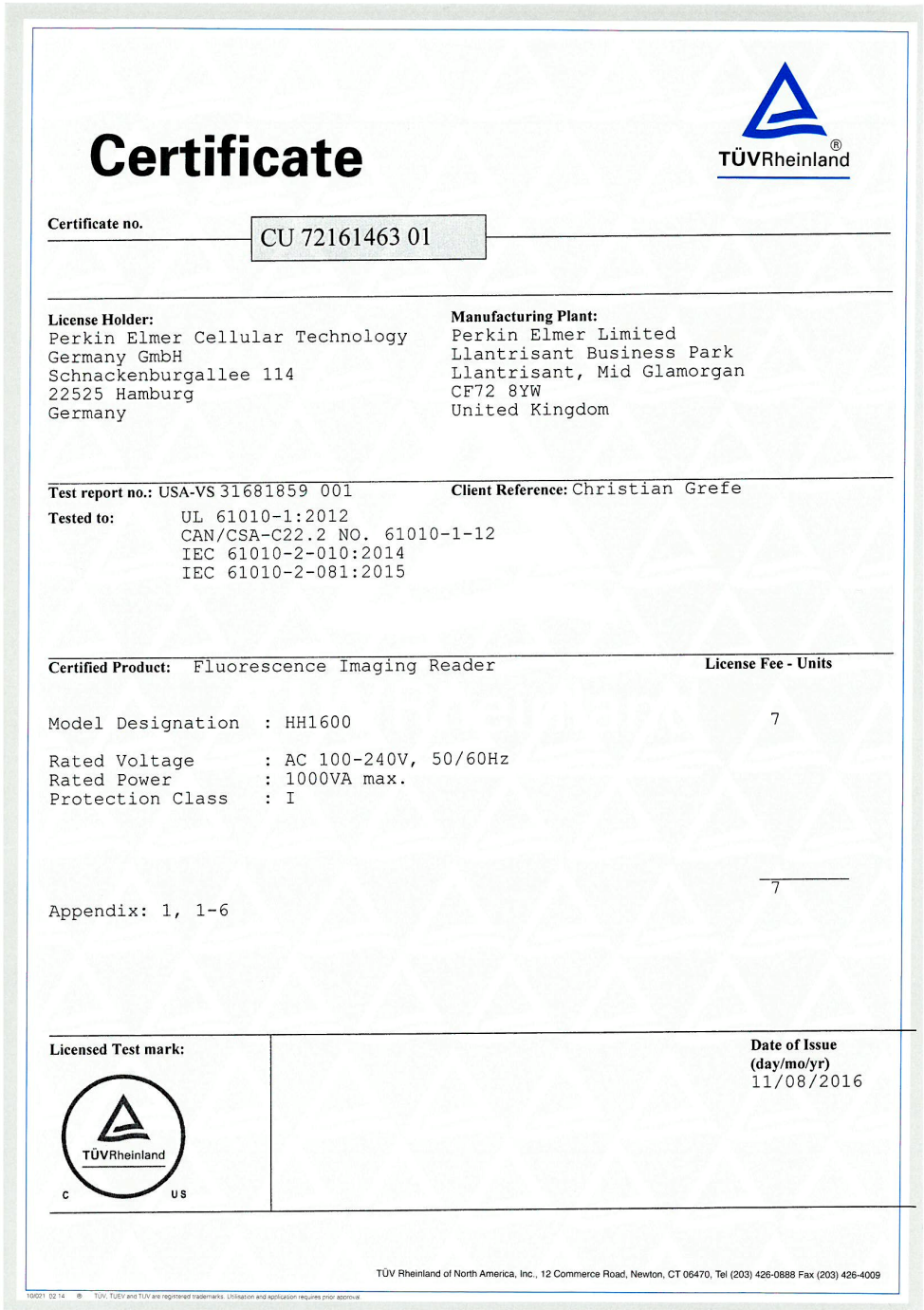


9.11 Heavy Duty Lab Cart (optional)

Property	Value
Width	1400 mm / 55.1 in
Depth	950 mm / 37.4 in 800 mm / 31.5 in (rear shelf removed for transport)
Height (w/o positioning brackets)	780 mm / 30.7 in
Weight	~ 100 kg / 221 lb
Max. Load	~ 400 kg / 882 lb

10 Compliance

10.1 Certificate



Certificate

TÜVRheinland®

Certificate no. CU 72161463 01

License Holder:
Perkin Elmer Cellular Technology
Germany GmbH
Schnackenburgallee 114
22525 Hamburg
Germany

Manufacturing Plant:
Perkin Elmer Limited
Llantrisant Business Park
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United Kingdom

Test report no.: USA-VS 31681859 001 **Client Reference:** Christian Grefe

Tested to:
UL 61010-1:2012
CAN/CSA-C22.2 NO. 61010-1-12
IEC 61010-2-010:2014
IEC 61010-2-081:2015

Certified Product: Fluorescence Imaging Reader **License Fee - Units**

Model Designation : HH1600 7

Rated Voltage : AC 100-240V, 50/60Hz
Rated Power : 1000VA max.
Protection Class : I

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Appendix: 1, 1-6

Licensed Test mark:

TÜVRheinland C US

Date of Issue (day/mo/yr)
11/08/2016

TÜV Rheinland of North America, Inc., 12 Commerce Road, Newton, CT 06470, Tel (203) 426-0888 Fax (203) 426-4009

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10.2 Protection Against Harmful Interference

This equipment has been tested and found to comply with the limits for a **Class A** digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate frequency energy and, if not installed and used in accordance with the provided manuals, may cause harmful interference to radio communications. Operation of this equipment in a residual area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

10.3 WEEE Instructions

A label with a crossed-out wheeled bin symbol and a rectangular bar indicates that the product is covered by the Waste Electrical and Electronic Equipment (WEEE) Directive and is not to be disposed of as unsorted municipal waste. Any products marked with this symbol must be collected separately, according to the regulatory guidelines in your area.

The objectives of this program are to preserve, protect and improve the quality of the environment, protect human health, and utilize natural resources prudently and rationally. Specific treatment of WEEE is indispensable in order to avoid the dispersion of pollutants into the recycled material or waste stream. Such treatment is the most effective means of protecting the customer's environment.

Requirements for waste collection, reuse, recycling, and recovery programs vary by regulatory authority at your location. Instructions to both PerkinElmer customers and recyclers/treatment facilities wishing to obtain disassembly information are provided on the PerkinElmer website:

http://www.perkinelmer.com/lab-products-and-services/environmental-health-and-safety/environmental-directives-compliance.html

Products from other manufacturers may also form a part of your PerkinElmer system. These other producers are directly responsible for the collection and processing of their own waste products under the terms of the WEEE Directive. Please contact these producers directly before discarding any of their products.

11 Glossary

A

analysis algorithm
The description of a step-by-step procedure to calculate the assay readout from an image set. In the Harmony software the algorithm is defined by an analysis sequence.

analysis parameter
Adjustable input parameter of an analysis algorithm to adapt the analysis to specific experimental conditions without modifying the algorithm. Do not mix up with readout values!

analysis sequence
An image analysis algorithm consisting of a sequence of building blocks. Each subsequent building block takes the output of the proceeding blocks as an input and refines or generates new information. The last building block returns the readout values of the analysis.

assay
A biological test system. Only cellular assays are considered here.

assay layout
Information about the well contents of a microtiter plate, e.g. which compounds are added at which concentrations, location of control wells.

assay readout
The set of different readout values generated by a specific assay. “High Content” means multiple readout values.

B

background
Area of the image where no objects of interest are located, e.g. the area not covered by cells.

batch analysis
Fully automated analysis of a group of wells or batch of plates. The assay readout is calculated for each well. Private results are discarded.

binning
Procedure of combining a cluster of pixels into a single pixel. For example, in 2x2 binning, an array of 4 pixels becomes a single larger pixel, reducing the overall number of pixels.

building block
Ready to use processing module performing a specific image analysis task, e.g. nuclei detection or measurement of object properties. A building block takes images and/or populations as an input and outputs populations and illustrations. The behavior of the building block can be adjusted by analysis parameters. Building blocks can be combined to create an analysis sequence and/or ready-made solution.

C

Canned Solution
A “black box” analysis algorithm for a specific task which cannot be modified by the user. Only analysis parameters can be adjusted.

channel
Independent optical measurement performed at the same position but using different optical settings, e.g. color (wavelength), imaging mode (brightfield/fluorescence), exposure time. Also called “color” when referring to different wavelengths.

channel sequence
Defines a sequence of channels, i.e independent measurements at the same position using different filter and exposure settings.

classification
Putting each object into one of a set of groups, e.g. “strong responder”, “weak responder”, “non responder”.

Columbus
PerkinElmer's universal image data storage and analysis system.

compound
Chemical substance that is tested by the assay.

CSV
Comma-Separated Values: File format often used for moving tabular data between two different computer programs.

D

data analysis
Further processing of the well results or single cell results in order to come to a statistically significant conclusion about the tested compounds. Extract the key information out of the huge amount of generated numbers.

data point
One value in a graph or table representing a result derived from one well or one cell.

domain
Windows Server domain: Logical group of computers running versions of the Microsoft Windows operating system that share a central directory database which contains the user accounts and security information for the resources in that domain.

DPC
Digital Phase Contrast; a computational approach for the generation of phase images based on brightfield images.

E

EC50

The concentration of a compound that is needed to provoke a response of the assay readout value halfway between the baseline and maximum response (half maximal effective concentration).

F

field

Image field, position in a well at which images are taken. The size of the image field depends on the objective magnification.

field of view

The area (width and length) which is imaged in the sample in every exposure. Depending on the optical set up of the microscope and the objective magnification.

flagging

Assigning a new binary property “Belongs to the population X” (Yes/No) to an object.

G

Gamma

Gamma correction is a nonlinear intensity enhancement of dark image areas in order to make the structure inside these areas visible.

GUI

Graphical User Interface of a software.

GUID

Globally Unique Identifier; unique reference number for objects in the database.

H

Harmony

PerkinElmer’s fully integrated image acquisition, image analysis and data management software. For image analysis either ready-made solutions can be used or custom algorithms can be created by interactively composing ready to use building blocks in a visual editor.

I

illustration

Graphical representation of multi channel images, overlays and populations at the user interface for visual inspection. A building block or analysis sequence can create predefined illustrations for guided data inspection or parameter tuning.

image

1) Matrix of intensity values (pixels) generated by a digital camera. 2) General term meaning any kind of picture.

image analysis
Calculating the assay readout for one well by applying an analysis algorithm to an image set.

image set
All images taken in one well in order to generate the assay readout for that well. The image set can include multiple channels, multiple locations in the well (image fields), multiple time points (time series) and multiple z-planes (stack).

interactive analysis
Manual selection of images and start of an analysis sequence. All private results can be interactively studied. The impact of analysis parameter changes are immediately visible.

L

layout
Plate layout: defines the wells on a plate which are to be measured; Well layout: defines the fields inside a well which are to be measured

M

measurement
All images and meta information collected from one plate during a specific image acquisition run. Multiple measurements may exist for the same plate.

meta information
For images/measurements: additional information describing the image contents, e.g. date, time, instrument used to acquire the image, instrument settings (e.g. magnification of a microscope), the assay type, the barcode of a microtiter plate, the well, the imaged positions in the well, user annotations.

method
Special analysis parameter of a building block to select different mathematical approaches how to solve the building blocks task, e.g. to choose an optimal nuclei detection algorithm in the “Find Nuclei” building block.

microtiter plate
Collection of 96 or 384 wells, also just called plate.

multi channel image
Set of images captured at the same position but each on a different channel.

O

object
1) Any kind of well defined spatially defined similar items visible in an image, e.g. cells, nuclei, spots, the cytosol, beads, clusters of cells, bright areas, etc. 2) Data object in the database (e.g. experiment, measurement, analysis sequence etc.)

object detection
Finding objects of interest an image, e.g. whole cells, nuclei, spots, beads. Each detected object is represented by an overlay defining the position of the object in the image and its properties (numbers associated with the object), e.g. the object number, size, mean intensity. All detected objects are summarized in a population.

object list
A table listing the individual properties of all objects in a population (one line per object, one column per property).

object result
Individual readout values for each cell or object in a well (in contrast to well results).

ODA
ODA: Operetta Data Manager; name of the standard database upon delivery

online analysis
Automatic analysis of a plate directly after the measurement. This is triggered if you select an analysis sequence for your experiment (Setup tab, Online Analysis).

overlay
Defines the positions of individual objects in an image. Overlays are used to refer to object positions in further analysis steps and for visualization of detected objects.

P

parameter tuning
Adjustment of the analysis parameters in order to optimize image analysis results.

PhenoLOGIC
Optional software plug-in for identifying cellular phenotypes using an interactive training mode; requires an optional license.

pixel
“Picture element”. Smallest piece of information in an image generated by a digital camera.

plane
The x-y image at a specific height in a stack (z-position).

plate layout
Defines the wells on a plate which are to be measured.

population
A set of objects, e.g. found by an object detection step. A population is represented by an object list and an associated overlay which specifies the object locations.

PreciScan
The PreciScan feature allows the user to automatically scan a sample at a low magnification and to rescan only the desired objects resulting from the first experiment within a second measurement at a higher magnification. This feature is only available for Opera Phenix and Operetta CLS and requires an optional license.

private result

Intermediate images and overlays (generated by a building block) which are not returned as a readout value. The private results can be displayed for control and parameter adjustment purposes in an interactive analysis.

property

Numerical values associated with an object, e.g. its size, fluorescence intensity, number of spots inside the object.

R

readout value

A number quantifying a biological feature of interest, e.g. the cell number, the intensity of a marker or a fraction of cells showing an effect.

Ready-Made Solution

An analysis algorithm which can be modified by the user, e.g. to add additional readout values or to combine multiple analysis algorithms for a multiplexing assay.

region

Region definition inside objects which is used to refer to this region in subsequent image analysis steps, e.g. to measure the intensity of a fluorescent marker inside the region. Also called region of interest (ROI).

S

screening

Use of an assay to test a (large) set of compounds.

segmentation

Breaking an image down into discrete objects of interest, e.g. whole cells, nuclei, spots and background. Segmentation is frequently done by object detection(s).

snapshot

Test image in the selected well using the global experiment settings and the settings of the selected channel.

stack

Also called z-stack. A set of images taken at different heights (z-positions) but at the same x-y-position (field) in a well. Also see plane.

Synchrony™ Optics

Proprietary principle developed by PerkinElmer to separate excitation of adjacent fluorescent channels in time and space to reduce spectral crosstalk during simultaneous acquisition in confocal mode

T

TCO

Temperature and Carbon Dioxide Option: Optional upgrade for controlling temperature and carbon dioxide concentration in the sample chamber.

test measurement

Measurement of one single well on the plate to test the current experiment settings including all channels. If selected, sublayout, stack and online analysis are also taken into account. The test results are only temporary and not saved in the database.

time series

A set of images typically taken at 5-30 different timepoints to monitor changes over time (short time lapse movie). Typical time steps are 1 minute to 1 hour.

time window

Subset of a time series measurement which can be defined on the Image Analysis screen; required to develop an analysis sequence which makes use of object tracking.

U

UNC

Universal Naming Convention: Specifies a common syntax to describe the location of a network resource, such as a shared file, directory, or printer. The UNC syntax for Windows systems has the generic form: \\ComputerName\SharedFolder\Resource

URL

Web address of a ressource in the network or on the internet.

W

well

Sample chamber of a microtiter plate. Each well contains an independent instance of the same assay with a different compound.

well layout

Defines the positions (x/y-direction) of image fields inside a well.

well result

The assay readout values for a single well.

Z

Z'

Z' Value (Z prime): A number measuring the accuracy and sensitivity of an assay (including the image analysis algorithm). High Z' values indicate low noise and a large signal window. Z' values above ~0.4 are considered sufficient for screening cellular assays, Z' values above ~0.6 are considered a good value. The theoretical maximum Z' value is 1.0 (= no noise).

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M

X

Z

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