OPERETTA®

Technical Manual

PerkinElmer®

For the Better

Release History

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DE 103 33326, EP 1 646 902, US 7 424 401, EP 1 449 081, DE 101 23027, EP 1 386 189 ,
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US 6 902 883, US 6 573 039, US 5 989 835, US 6 671 624, US 6 759 206, CA 2 328 194,
EP 1 095 277, JP 3 466 568, US 6 416 959, EP 1155304, CA 2,362,117, JP 3576491,
US 6 727 071, US 6 875 578, US 6 716 588, EP 1 348 124, US 6 917 884, US 6 365 367,
EP 1 314 980, EP 1 203 214, CA 2 381 344, EP 1 368 653, EP 1 368 689

Contents

1   System Safety

1.1 *Purpose-Oriented Application*

⚠️ **Caution!**

Use only as intended.

The Operetta 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.

Intended Use and Biosafety

The Operetta is an automated fluorescence imaging reader for usage in biological-medical and pre-clinical research (biosafety levels 1 and 2, see also section 1.3.3 "Process Liquids or Substances", page 9). Due to its technical design the Operetta is particularly suitable for cellular applications. The instrument is not designed to be used in the clinical or medical-diagnostical area.

Exclusion of Medical Applications

The Operetta is not approved for use in human or veterinary medical applications, including but not limited to *in vivo* or *in vitro* diagnostics. PerkinElmer explicitly excludes such applications of the Operetta, and will not assume liability for any unauthorized use of the instrument in these areas.

Exclusion of Liability for Measurement Results

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

1.2 *Signal Words and 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.

Automatic start!
Parts can suddenly start moving.

Hot Surface!

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.

UV/Vis Light!

1.3 Indication of Hazards and Danger Zones

Before operating the Operetta system for the first time please read this manual carefully to avoid incorrect operation of the Operetta system. Also read the safety instructions given in the separate manual of the Xenon lamp.

1.3.1 Operating and Maintenance Staff

- The Operetta 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.

- Unauthorized intervention or tampering inside the machine is prohibited.

- Corrective maintenance work and service may only be performed by the PerkinElmer service. Operational and maintenance procedures described in this manual are excepted and may be carried out by the user.

- The manufacturer can accept no liability for this product if the provisions of this documentation are not complied with.

- Always wear your personal safety equipment, i.e. safety goggles and laboratory coat.

1.3.2 Mechanical Hazard

Warning!
Moving scanning table – Risk of crushing
The Operetta scanning table operates automatically. It can suddenly start moving.

- *Do not manipulate the interlocks of the lid or the protective doors and shields (automation upgrade).*
- *Do not reach into the danger area of the scanning table.*
- *Do not reach inside the Operetta through the scanning table flap.*

The Operetta has a moving scanning table. This table is accessible if driven out of the device for loading of new sample carriers (only for automation upgrade). An interlock switch stops driving of the table motors immediately as soon as the Operetta's lid is opened.

1.3.3   *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.*

The processing of samples on the Operetta covers only probes at biosafety level 1 and 2 (BSL-1 or BSL-2, CDC http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm). 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 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 exposes any hazard to the system, personnel or the environment. It is also within the responsibility of the user to attach a biohazard label as soon as potential infective substances are used.

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

1.3.4   Light Sources

The Operetta is equipped with a class 3B autofocus laser and a high voltage Xenon lamp.

Danger!
Laser radiation - Eye injury

Class 3B invisible laser radiation may be present inside the Operetta`s protective housing during operation. Since the housing's access doors are protected by laser safety interlocks, no harmful radiation will become accessible when the doors are opened for setup and maintenance.

- *Do not defeat the laser safety interlock!*

- *Do not remove any housing components!*

- *AVOID DIRECT EXPOSURE TO BEAM!*

- *Switch off the Operetta system before starting any permitted retooling and maintenance tasks.*

- *Service tasks that require access to the instrument while the interlocks are defeated may only be carried out by qualified service personnel. Service personnel must observe the instructions of the separate Service Manual.*

Danger!
Scattered laser radiation (only automated version)!

Scattered laser radiation may occur if the automation sample carrier access door on the right for sample carrier input / output is opened during operation.

- *Do not open the sample carrier access door!*

Danger!
Ultraviolet light - Eye injury

Ultraviolet light (visible and invisible) from the Xenon lamp is present inside the Operetta. Since the housing's access doors are protected by laser safety interlocks, no harmful radiation will become accessible when the doors are opened for setup and maintenance.

- *Do not defeat the laser safety interlock.*

1.3.5 *Carbon Dioxide (only with TCO Upgrade)*

If your instrument is equipped with the **Temperature and CO$_2$ Option** (TCO), 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 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. See the Operetta Site Readiness Instructions for a connection diagram.*
> - *If carbon dioxide control has been activated and you open the Live Cell Chamber, wait 10 seconds before bending over the instrument to avoid unnecessary inhalation of the CO$_2$ gas.*

1.3.5.1 *Recommended Protective Measures*

The TCO unit 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 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 customers'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.

1.3.6 **Electric Shock**

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

⚠	**Danger!** **Direct electrical contact - Electrical shock.** ● *The system may only be connected with properly installed protective earthing conductor.* ● *Service has to be carried out by qualified PerkinElmer service personnel only!* ● *Turn main power off and unplug from mains for a complete disconnection.* ● *Unplug from mains before replacing the fuses.* ● *Use only the provided power cable.* ● *Check the power cable for damages before switching on the Operetta. Do not use the instrument with damaged power cable.* ● *In case of a damaged power cable contact PerkinElmer to request a new cable.*

1.3.7 **Disconnecting from Mains Supply**

The on/off switch does not switch off the Operetta 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 from mains supply immediately.* ● *The mains connection must be accessible at any time.*

1.3.8   Spilling of Liquids

	Danger! **Direct electrical contact - Electrical shock.** ● *Do not handle large amounts of liquids near or above the Operetta.* ● *If liquids should be spilled into the instrument accidentally, switch off the Operetta immediately, i.e. unplug the power cable.* ● *Contact PerkinElmer Service to let them check the instrument and repair the damages, if necessary.* ● *Service and repair has may be carried out by qualified PerkinElmer service personnel only!*

1.3.9   Cleaning

	Warning! Ethanol or other cleaning solvents may cause fire or injury if used in great amounts. ● *Observe the cleaning instructions.* ● *Keep the concentration of dangerous solvents as low as possible.* ● *Ensure the area is well ventilated.*

For cleaning the outer surface of the Operetta use a slightly moistened, damp cloth. For cleaning the workstation inside, the operator must specify suitable precautions, especially with regard to biological contaminated and infectious materials.

1.3.10   Ventilation

Take care to ensure that none of the ventilation openings of the workstations and the various devices are obstructed. Keep a distance of at least 20 cm to walls and other objects. Covered venting slots, louvers etc. may cause malfunctions or damage to the equipment.

1.3.11   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.

1.4   *Warning Signs*

Attention must always be paid to the black and yellow warning signs. If these signs are not attached to the system, contact the PerkinElmer customer support. Replacement labels will be supplied free of charge.

**Class 1
Laser Product**

1.5   *Protection against Harmful Interference*

This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications.

However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.

- Increase the separation between the equipment and receiver.

- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.

- Consult the dealer or an experienced radio/TV technician for help.

1.6   *Warranty Information*

The manufacturer of the equipment described in this manual warrants that the equipment is delivered free from defects of material and workmanship. The purchaser shall, without delay, inform the manufacturer of any defects that may be found after delivery and do everything within his power to limit the possible damage. If a defect is reported within the warranty period, the manufacturer will correct the defect by his choice of either repair or replacement.

This warranty does not cover defects due to natural wear (especially with regard to wearing parts) or improper handling, neither are any consumables subject to warranty services.

The manufacturer cannot be held liable for any damage caused by faulty operation, negligence or unauthorized interference with the equipment, including but not limited to the removal or exchange of parts, and the use of accessories of other origin. Any such action or negligence will invalidate any claim under warranty.

Maintenance work or repairs beyond the operations described in this manual may only be carried out by PerkinElmer service staff or by persons expressly authorized by PerkinElmer to do so. In case of a malfunction, contact customer support.

1.7   Conformity

document no. :	**DOC/PE/1007**

PerkinElmer®

DOC
Declaration Of Conformity

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We declare in accordance with the guidelines
Wir erklären in Übereinstimmung mit den Richtlinien,

EMC-Directive 89/336/EWG *(EMV-Richtlinie)*
Low Voltage Directive 73/23/EWG *(Niederspannungsrichtlinie)*
Machinery Directive 98/37/EC *(Maschinenrichtlinie)*

that the product of Perkin Elmer with the marking :
dass das Produkt von Perkin Elmer mit der Bezeichnung :

Operetta™   (basic instrument with/without confocal option)

fulfils the requirements of these directives, using the following standards :
die Vorgaben dieser Richtlinien, unter Anwendung der folgenden Normen :

$\mathsf{C}\mathsf{E}$

EN60825-1 : 11/2001	EN1050:19997
DIN EN61010-1 : 08/2002	EN954-1:1997
EN 61326-1 : 01/1998	EN61010-2-081:12/2002
EN 61326/A1 : 05/1999	EN55011
DIN EN 61000-4-2 : 03/1996	FCC part 15
DIN EN 61000-4-2/A1 . 10/1998	EN 61000-3-3 : 03/1996
DIN EN 61000-4-3 : 03/2003	
DIN EN 61000-4-4 : 03/1996	
DIN EN 61000-4-5 : 09/1996	
DIN EN 61000-4-6 : 12/2001	
DIN EN 61000-4-11 : 12/2001	
EN 61000-3-2 : 10/1998	
DIN EN 61000-4-8	

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Hamburg, 30th of january 2009

Geschäftsführer / CEO

DOC	date / Datum	30.01.2009	document no.	DOC/ET/1007	page 1/1
Perkin Elmer	reviewed by / sachl. geprüft	C.Grefe	approved / Freigabe	Dr.Henning Menke	

1.8   Service Schedule

In order to guarantee a perfect and reliable function, the Operetta system should be subjected to a regular inspection by the PerkinElmer service staff.

Certain maintenance tasks have to be performed regularly by the user. Please see chapter 3 "Retooling and Maintenance" for further information.

1.9   Setup Requirements

Notice

The Operetta is sensitive to vibrations. Blurred images can be the consequence.

- *Put the Operetta on a very stable bench or table.*
- *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 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 Site Readiness Instructions**.

2 Hardware Description

2.1   Introduction

This chapter describes the most relevant hardware components of the Operetta®.
Please note that your specific instrument may not be equipped with all features or optional upgrades described in this manual (dependent on your selected instrumentation)!
For a description of the Operetta control software (Harmony®) please refer to the Harmony Help or to the separate Operation Manual.

The Operetta is an entry level to medium throughput microplate reader for high content screening (HCS). It can acquire, analyze and manage fluorescence and brightfield images. A set of ready-made image analysis solutions is included consisting of building blocks which can also be assembled to new analysis solutions.

The Operetta Application Guide helps you getting started with HCS. It describes the essentials of setting up an HCS assay and how to use the Operetta and image analysis software for the most common applications.

2.1.1   Instrument Features

- High content imaging reader

- Switchable between non-confocal and confocal

- High power Xenon lamp (300W) providing entire visible spectrum

- Peltier cooled high resolution 14 bit CCD camera

- Brightfield excitation

- 4 or 8 excitation filters, up to 8 emission filters choosable from 17

- Up to 4 automatically exchangeable objective lenses for different fields of view / resolutions (2x to 100x objectives, high NA or long WD)

- Fast laser based auto focus

- Focal plane thickness 1.5 – 145 µm

- Simple experiment set-up

- Calibration free

- Harmony software for fully automated image acquisition, online evaluation and data management

- Supports various sample carrier formats, e.g. 96 and 386 well plates

- Plug and play applications with ready-made image analysis solutions as a starting point

2.2   *Operetta Device*

2.2.1   *Right Side*

1. Power line

2. Fuses

3. Flap for scanning table (only for automation upgrade): Only with the automation upgrade the scanning table can move out of the housing to a handover position for sample carrier exchange by the plate::handler II robot. Due to safety reasons this flap is locked for instruments without this upgrade.

Warning!

Moving scanning table – Risk of crushing

If the table can move out of the housing (automation upgrade), the table can cause injuries if the provided protective shields are not installed and if you reach into the danger area of table or robot.

- *Do not use an Operetta without protective shields installed if the scanning table flap is unlocked.*
- *The table in/out functionality may not be used for manual exchange of sample plates.*
- *Do not manipulate the interlocks of the lid or the protective doors and shields (automation upgrade).*
- *Do not reach into the danger area of the scanning table.*
- *Do not reach inside the Operetta through the scanning table flap.*
- *If the Operetta is integrated into a third-party automation system, the manufacturer has to install suitable protective shields so that the moving scanning table is no source of danger anymore.*

2.2.2   *Left Side*

1. Louver: Airflow intake / exhaust. Do not cover!

2. Main switch (on/off)

3. Network (1Gbit): Connection to external control PC (must be connected to the separate network socket labeled with "OPERETTA"), not intended to connect TNV

4. Fiber port: Port for Liquid Light Guide (Xenon lamp fiber)

5. RS232 interface for communication with Xenon light source

6. USB (Barcode Reader): Port for connecting a PerkinElmer barcode reader

> **Notice**
> Please use a PerkinElmer barcode reader only, otherwise a faultless operation cannot be guaranteed.

2.2.3   *Front Side*

1. Filter access door
   This small door allows access to the emission filter wheel and to a storage possibility for unused filters.

   ○ 8 position filter wheel equipped with user changeable bar coded filters

   ○ 4 filters in standard instrument, up to 4 further selectable from a catalogue with 13 main and side band filters

> **Notice**
>
> Do not open the filter access door unless requested by the **Change Filter** wizard (see chapter 3 "Retooling and Maintenance", page 33) when changing the emission filter.
>
> The filter access door is not interlock-protected. If the filter wheel is rotating and you reach into the wheel with your hand, this can damage the instrument.

2. Balcony with status light
   The LEDs inside the balcony indicate the Operetta status. See also next chapter.

3. Sample lid with progress LEDs
   The 24 LEDs arranged in a 4×6 pattern on the lid indicate the progress of a measurement on a percentage basis. During normal operation, the sample lid of the Operetta is closed. The lid may only be opened in the following situations upon prior permission from Harmony:

   o **Exchange sample carrier:**
     Use the **Open Lid** button in Harmony. For details please see **Operetta Operation Manual**, section 3.15 "Open Lid".

   o **Exchange or clean objective:**
     Use the **Change Objective** function in Harmony (**Settings** dialog). For details please see **Operetta Operation Manual**, section 3.16.2 "Change Objective".

   o **Adjust correction collar:**
     Use the **Set Correction Collar** function in Harmony (**Settings** dialog). For details please see **Operetta Operation Manual**, section 3.16.1 "Set Correction Collar".

> **Notice**
>
> - *Do not open the sample lid without prior permission from Harmony. If you open the lid during a measurement or during initialization, this can lead to errors and to the loss of data.*
> - *If you open the lid, lift it at least by 10 cm before you close it again. Otherwise the interlock cannot detect that the lid has been closed.*

2.2.4   Instrument Status
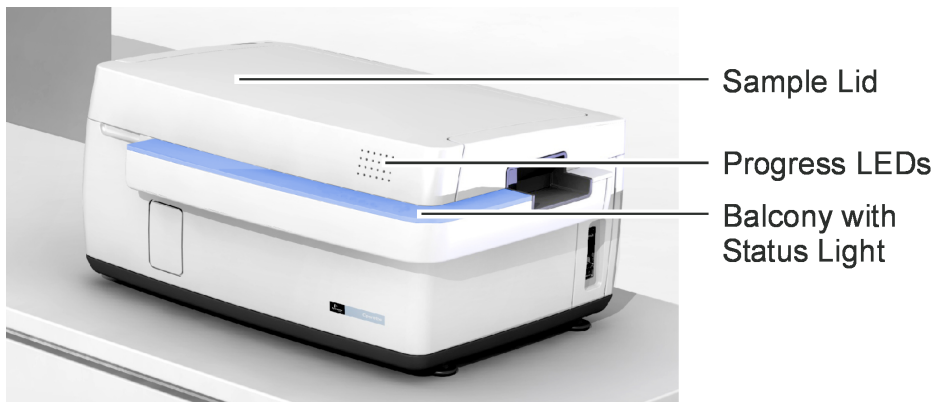
The status light in the balcony of the Operetta indicates the instrument status:
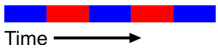
Signal	Description
No Light	**Off/Dimmed** The Operetta is switched off or the status light has been dimmed.
Blinking Blue Time ➝	**Pre/Post Operation** Indicates … • power up/down of the instrument • initialization of the instrument
Blue Light Time ➝	**Ready/Measuring** • Operetta is ready. • If the progress LEDs are visible, the instrument is in operation (measuring). The LEDs indicate the progress of the measurement on a percentage basis.
Blinking Blue/Red Time ➝	**Waiting for User** The instrument is waiting for user interaction. This does not indicate an error. The user's feedback is required to continue.
Red Light Time ➝	**Sample Lid Open / Error** This signal is used on two occasions: • The sample lid is open. • An error occured (hardware or software). The measurement is stopped. A restart of the system may be necessary.
Red Flash Time ➝	**Serious Error (Call Service)** The instrument has a defect. Please call the PerkinElmer Service.

2.2.5   *Sample Chamber*

1. Generously proportioned working space which can easily be cleaned. The chamber is illuminated (blue) as soon as you open the sample lid. In contrast to the status light this is no status indication (see also previous chapter).

2. Scanning stage with plate holder
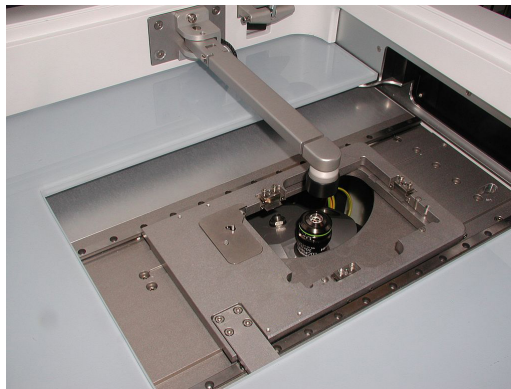
   - Mechanism to hold or release the sample carrier
   - Sample carrier bedding
   - Above the objective lens: Swivel arm with transmission light source

> ⚠ **Caution!**
>
> Sharp-edged sample carrier holder!
>
> - *Be careful when performing retooling or maintenance tasks on the scanning table and the objective lens changer.*
> - *Do not touch the sample carrier holders.*
> - *Switch off the Operetta before working on the scanning table, so that the table is in the service position.*

3. Objective lens changer
   The objective lens changer automatically changes between up to 4 installed lenses via software control. The selected lens is moved to measuring position. Available objectives: 2x, 10x, 20x, 40x, 60x, 100x (long WD and high NA)

4. Transmission light source for brightfield excitation

   ○ Detect cells independent of dye, label-free techniques

   ○ 740nm Infrared to minimize cell damaging

   ○ Extension arm with integrated transmission light source (has to be moved into measuring position above the objective before starting brightfield measurements)

   ○ Maintenance-free LED

   ○ For brightfield measurments in combination with climatization see chapter 2.3.2 "Live Cell Chamber", page 28.

2.2.6 CCD Camera

The Operetta uses one high resolution 14 bit CCD camera for cell imaging.
The camera is cooled down (20 °C offset) by a Peltier element.

2.3 Temperature and CO$_2$ Option (TCO)

2.3.1 Overview

If the Operetta is equipped with the TCO upgrade, the climate in the sample chamber can be controlled via the Harmony software:

- **Temperature:** 37 °C or unregulated
- **CO$_2$ concentration:** 3 to 8.5 % (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 there is an air-circulating unit which regulates the CO$_2$ level in the sample chamber. Temperature is controlled by heating elements in the **Live Cell Chamber** and inside the sample chamber. The sample chamber is sealed so that almost no air or CO$_2$ gas can escape.
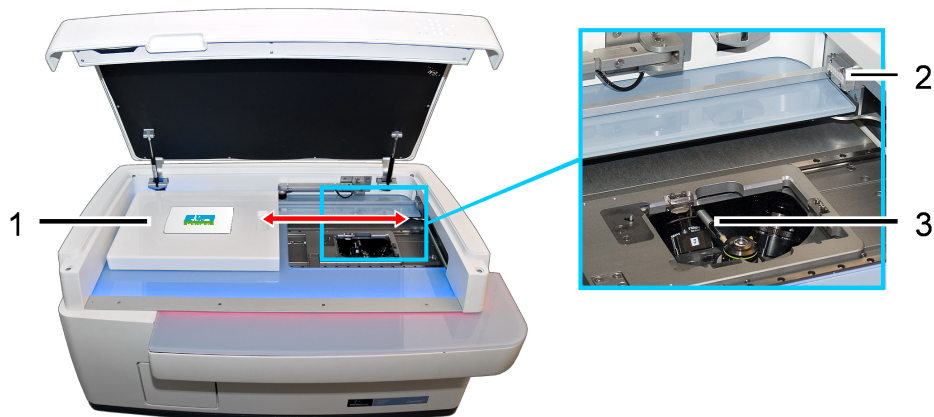
1. Live Cell Chamber
2. Electrical connector
3. Temperature sensor

2.3.2   Live Cell Chamber

The **Live Cell Chamber** can be moved on a guide rail. If you move it to the left you can access the sample chamber, the table, the objectives etc. If you want to use climatization, you have to move it all the way to the right, so that it seals the sample chamber and the electrical connector is plugged to its counterpart. The flow of carbon dioxide is only active if the **Live Cell Chamber** is closed (moved to the right) and CO_2 has been activated in the **TCO Settings** of the Harmony software. If you move the **Live Cell Chamber** to the left, the climate in the sample chamber is lost and you have to wait until the target values have been reached and stabilized again.

Inside the **Live Cell Chamber** there are heating elements for controlling the temperature. On the bottom of the **Live Cell Chamber** there is an additional transmission light source, because the default transmission light source inside the swivel arm cannot be used in combination with climatization. Although both LEDs have the same specification, slightly different exposure times or transmission power values may be necessary to achieve the same results (due to adjustment and tolerances).

The **Live Cell Chamber** can be removed completely, e.g. for cleaning.
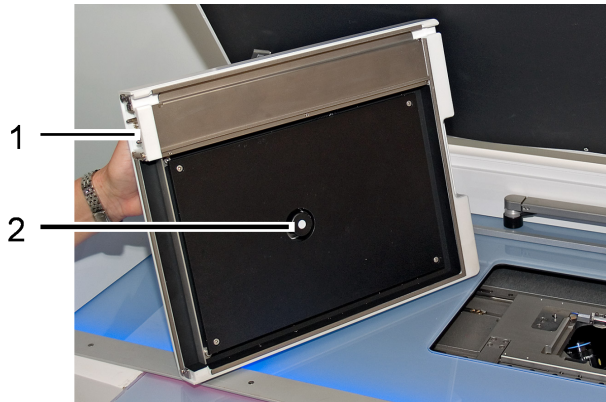
1. Electrical connector
2. Transmission light source

2.3.3 CO_2 Supply

With the TCO upgrade the Operetta's side panel is extended by a CO_2 connection. The tubing has to be connected to your CO_2 supply.

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.

- *If carbon dioxide control has been activated and you open the Live Cell Chamber, wait 10 seconds before bending over the instrument to avoid unnecessary inhalation of the CO_2 gas.*
- *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. See the Operetta Site Readiness Instructions for a connection diagram.*

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 next page).*
- *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.*
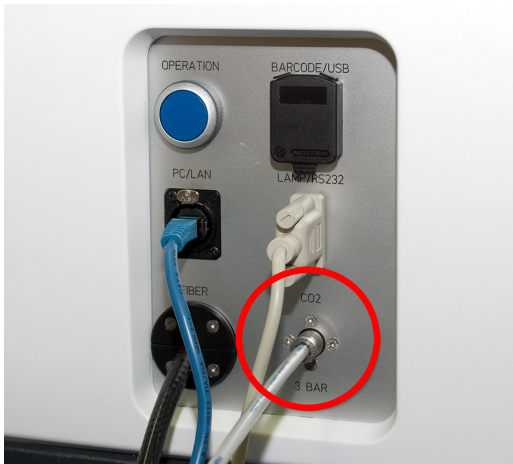

Notice

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 Site Readiness Instructions**.

The flow of carbon dioxide is only active ...

- if CO_2 control has been activated in the **TCO Settings** (Harmony software) *and*

- if the Live Cell Chamber is connected so that the sample chamber is sealed *and*

- if the flap for the scanning table is closed (only with automation upgrade).

- CO_2 tubing:
    - Outer diameter: 6 mm
    - Max. 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: ≥ 99.998 %, particle-free

Notice

If you connect or disconnect the Operetta 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 Operetta.*

2.4 Control PC (Harmony PC)

The Operetta system is equipped with a control PC, TFT monitor, mouse and keyboard. The user can control the Operetta using the Harmony software installed on the control PC.
The Harmony software allows for fully automated image acquisition, online image evaluation and data management.

> **Notice**
>
> In order to guarantee a successful operation of the Operetta system it is very important not to change any settings on the Harmony PC. Please see chapter 4 "IT Policy", page 44.

2.5 Xenon Light Source

The Operetta is equipped with an external Xenon light source.

> **Notice**
>
> This chapter is an overview for the most important information. For a complete description please see the Xenon Light source manual!

- Xenon lamp high power 300 W

- More continuous spectrum than HBO or Metal Halide

- Adjustable output power

- Maximum 2000 lm white light

- Easily exchangeable bulb

- Color selection by filter wheel

> **Danger!**
> High Voltage!
> For more information please see Xenon Light Source manual.

1. Standby Switch
   When the Standby switch is depressed the system will become energized. With pressing the Standby Switch again, the system will become deenergized. Energizing the system will not automatically turn the lamp on.

2. Lamp On / Off
   Switch to control lamp once system has been energized. The Blue LED indicator will flash until lamp ignition occurs. Then, the LED will stay on until the lamp is turned off.

3. Lamp Hours
   Button to display the operating hours of the lamp module. For detailed instructions please refer to section 3.2.1.1 "Displaying Lamp Hours", page 40.

4. Four-Port Turret
   Accepts ACMI, Olympus, Storz or Wolf fiber-optic light cables. The names of the ports are clearly visible on the perimeter of the adapter outboard from the port inputs.
   Operetta: ACMI.

Danger!

Danger of white, blue and UV light exposure!

- *Do not remove liquid light guide from Xenon light source.*

2.6 Additional Upgrades

- Plate handling robotics (plate::handler™ II)
  Housing and scanning stage prepared for the most articulate robot systems like plate::handler II.

- Temperature and CO_2 Option (TCO)
  See section 2.3 "Temperature and CO2 Option (TCO)", page 27.

- Field upgrades
  The confocal upgrade, more excitation filters, objectives, automation components (plate::handler II, plate hotel, incubator) and server extensions can be provided as field upgrades.

3 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.

The individual steps for each task are described and a schedule for the maintenance tasks is given.
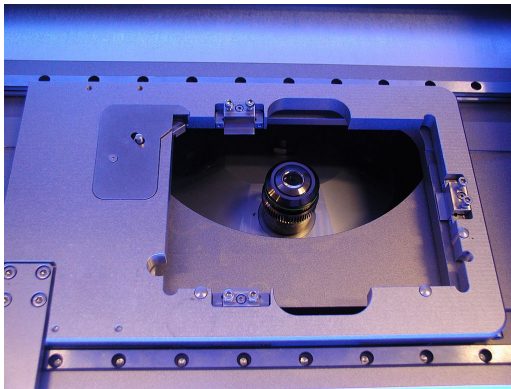
> ⚠ **Danger!**
> Do not attempt to perform any kind of maintenance or retooling on the Operetta beyond those listed and described here! All other technical service tasks – including but not limited to those described in the Service Manual – may only be performed by the PerkinElmer service personnel.

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 chapter 1 "System Safety", page 5), and any specific safety instructions which may be given at the beginning of each section.

3.1 Retooling

3.1.1 Objective Lens

The motorized four position changer can automatically change objectives. The user has the choice of 10x, 20x, 40x, 60x and 100x objectives. There is a wide range of objectives available, optimized either for high numerical aperture / resolution or high working distance for thick bottom plates or thick sample carriers. The 20x long WD objective is always included in the instrument.

All of the other selections can be chosen as an option when purchasing the instrument, or at any other time for customer installation. Objectives are barcoded and can be exchanged by the user. Up to four objectives can be fitted onto the objective turret for automated change.

> **Caution!**
> Sharp-edged sample carrier holders!
>
> - *Be careful when performing retooling or maintenance tasks on the scanning table.*
> - *Do not touch the sample carrier holders.*

> **Notice**
> If you screw in an objective which is colder than the interior of the instrument, it may be difficult to screw out the objective at a later time. You can avoid this if you let the objective warm up before you screw it in, e.g. by putting it on the glass plate inside the Operetta for 30-60 minutes.

Follow the instruction of the **Objective Exchange Wizard** (**Harmony – Settings – Change Objective**):

1. If all four positions of the turret are occupied: Select the objective which is to be exchanged. The selected objective is moved into position.

2. Open the sample lid.

3. Unscrew the old objective.

4. Screw in the new objective.

5. Close lid. The objective turret starts homing and the objective information will be read by the internal barcode reader.

6. A summary table shows all installed objectives. Close the wizard.

3.1.2   *Correction Collar*

Some objective lenses for the Operetta (see table on the next page) have an integrated correction collar. It can be rotated and allows the correction of spherical aberrations mainly caused by differences in plate bottom thickness. Underneath the colored ring of the objective you can find a small white line which should be set directly on the value of the plate bottom thickness.

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 plate bottom thickness of the current plate type will also be stated.

Also please note, that while the message box "Please adjust the correction collar" is displayed the table is positioned so as to give easy access to the objective.

The objectives have a scale engraved, which you can see on the photograph below. The unit of values of the scale is typically [mm]. There are different scales used depending on the range of compatible plate bottom thicknesses. E.g. the scale of the 20x WD lens ranges from 0 to 2 mm, often the value of 0.17 is also depicted, as this is a very common thickness of cover slips used in microscopy.
Next to the scale you can find a small singular mark, which indicates the current setting of the correction collar.

Below you find some example settings:

- 0.17 mm
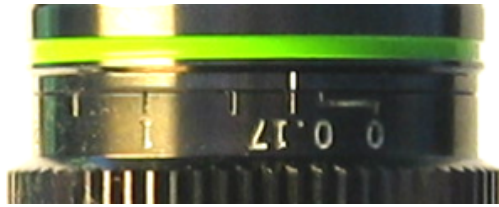
- 0.50 mm
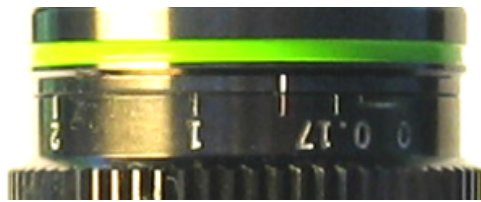
- 0.75 mm

Some objectives also feature a secondary scale, which does not contain the numerical values. In the case of the "20x WD" lens this secondary scale consists of a single mark at the 1 mm value. Below is the secondary scale for a 0.75 mm adjustment is shown.
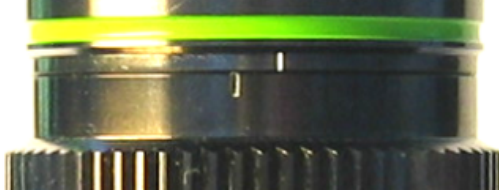
How to Adjust the Correction Collar

Objective with correction collar

1. If the message *"Please adjust the correction collar to ... mm"* appears, open the sample lid.

2. Turn the correction collar and adjust it to the given value [mm].

3. If the mark of the correction collar is not visible from your position, use the provided mirror to adjust for the correct value.

4. Close the sample lid.

5. Confirm the message box and click **OK**.

If the plate type – objective lens combination is not working together, the software will warn you beforehand.

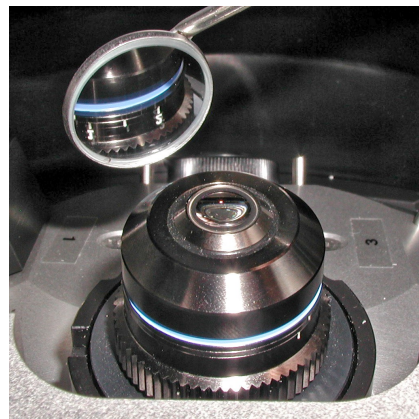| Objective Lens	Correction Collar	Possible Values [mm]
2x long WD	No	–
10x long WD	No	–
10x high NA	No	–
20x long WD	Yes	0-2
20x high NA	No	–
40x long WD	Yes	0-2
40x high NA	Yes	0.11-0.23
60x long WD	Yes	0.1-1.3
60x high NA	Yes	0.11-0.23
100x long WD	Yes	0-0.7

3.1.3   Emission Filter

The Operetta is equipped with an eight position emission filter wheel. The four emission filters 410 - 480 nm, 500 – 550 nm, 560 – 630 nm and 650 – 760 nm are always included in the instrument. All of the other selections can be chosen as an option at any time for customer installation. Emission filters are barcoded and can be exchanged by the user. Unused filters can be stored in the filter hotel behind the door.

Follow the instructions of the **Change Filter** wizard in the Harmony software (**Settings – Change Filter**).

> **Notice**
>
> Do not open the filter access door unless requested by the **Change Filter** wizard.
>
> The filter access door is not interlock-protected. If the filter wheel is rotating and you reach into the wheel with your hand, this can damage the instrument.

1. Open the filter access door to reach the filter wheel.

2. If necessary remove the old filter.

3. Insert the new filter to the intended position.

4. Close the door. The filter wheel starts homing and the filter information will be read by the internal barcode reader.

5. A summary table shows all installed filters.Click OK to close the wizard.

3.2   *User Maintenance*

3.2.1   *Cermax® Xenon Lamp*

3.2.1.1   *Displaying Lamp Hours*

Perform the following steps to display the operating hours of the lamp module:

1. Switch off the Operetta.

2. Switch off the Xenon light source and switch it on again.

3. Press the **Lamp Hours** button at the front panel of the light source.
    The operating hours are displayed.

4. If you want to reset the counter, hold down the **Lamp Hours** button for 3 sec.

3.2.1.2   *Exchanging the Lamp*

The Cermax® Xenon Lamp will typically provide more than 500 hours of service. The symptoms indicating the need for replacement are:

*   Repeated ignition attempts without lamp going into operation.

*   Low light output evident with dark picture at high light level adjustment.

The delivery of the Operetta is carried out including a replacement lamp. Alternatively the whole lamp module can be easily exchanged, which has to be ordered separately. You can find both ways of replacing either the lamp or the lamp module in the Xenon Light Source manual.

	Caution! Xenon lamp could still be hot in case of replacement. • *Wait a suitable time (5 min) before replacing the lamp.* • *For more information and detailed instructions please refer to Xenon Light Source manual.*

3.2.2 Cleaning

Cleaning Schedule

Daily: Clean the housing surfaces (outside and sample room).
Only if required: Clean the microscope objective lens(es) carefully using optical wiper and 70% ethanol.

Cleaning the Housing Surfaces

> **Notice**
> The Operetta is not protected against spraying or even splashing water! The water could get into the housing and damage optical and electrical components of the instrument.
>
> - *Do not drop or pour water over the instrument.*

- Switch off the Operetta.
- Clean the surfaces of the housing and the sample room with a slightly damp cloth. Soiled areas may also be cleaned using 70% ethanol.
- The glass panels should be cleaned with a suitable glass cleaning agent.
- Do not handle with greater amounts of fluids near the device.

Cleaning the Objective Lens

> **Caution!**
> Sharp-edged sample carrier holders! Be careful when performing retooling or maintenance tasks on the scanning table.
>
> - *Do not touch the sample carrier holders.*
> - *The objective lens(es) should only be cleaned if they are obviously soiled or dusty.*

- Shut down the Operetta to move the scanning table to the service position.
- Wet the objective lens with 70% ethanol.
- Take a dust-free optical tissue, wipe the lens a few times very carefully without force and let it dry. Do not wipe back and forth but carefully in one direction.

3.2.3   *Replacing a Blown Fuse*

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

⚡	**Danger!** Electric Shock! ● *Switch off the Operetta and disconnect the power cable before changing the fuse(s).*

⚡	**Caution!** A defective fuse may only be replaced by exactly the same part from the following manufacturer: LITTLEFUSE 218000 Series 5 × 20 mm Time Lag Fuse (Slo-Blo) 15 A / 250 V (US), 8 A / 250 V (EU).

1. Switch off the Operetta and disconnect the power cable.

2. Pull out the fuse holder.

3. Replace the blown fuse(s).

4. Close the fuse holder and push it into its former position.

5. Reconnect the power cable.

3.2.4   Transportation

It is only permitted to move the Operetta on the bench by four persons.

⚠	**Warning!** The four handles of the Operetta are not designed to carry the device for relocation!

- *The instrument is very heavy (120 kg). If it falls down or tips over it can cause severe injuries.*
- *In the case of relocation please contact the PerkinElmer Service. A subsequent optical adjustment is recommended.*

3.3   Maintenance by PerkinElmer Service

The following maintenance jobs have to be carried out as specified to guarantee the correct and reliable operation of the Operetta.

⚠	**Warning!** The jobs listed here may only be performed by the **PerkinElmer Service**.

- *If you do not have a maintenance contract which includes these tasks, you have to order on-site service by the PerkinElmer Service at your own expense.*
- *These maintenance jobs may not be carried out by the customer or by third-party service providers.*

Maintenance Job	Interval
Replace both air filters of housing fan.	≤ 1 year
Only with TCO upgrade: Replace air filter in the TCO's air recirculation unit.	1 year
Only with TCO upgrade: Replace CO_2 sensor.	3 years

4 IT Policy

This chapter contains policies and general information about the IT infrastructure and rules for integration of the Operetta System into any network environment.

|	**Notice**
	The purpose of the Operetta IT Policy is to ensure the effective protection and proper usage of the computer systems belonging to the "Operetta 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 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.

4.1 Network Integration

4.1.1 Overview
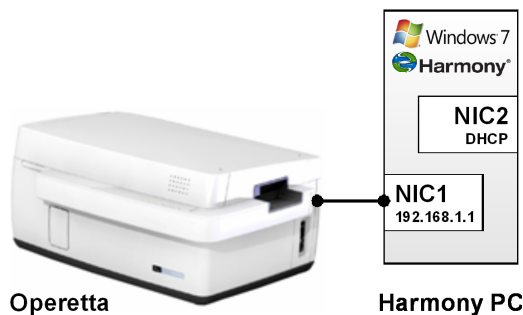
The Operetta System is composed of:

- Operetta device (cell imaging reader)
- Harmony PC (to control the Operetta 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)

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. This PC runs with Windows$^{®}$ 7 (64 bit), includes the database and cannot be substituted by any customer-supplied computer.
> - The optional Columbus software (especially the Acapella Server) may not be installed on the Harmony PC or on a PC with Harmony office installation. This does not apply to the Acapella Client or Acapella Proxy which are part of the Columbus 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.

- On Office PCs with a Harmony installation, the network card which is used for the communication with the database (Harmony PC) has to rank first regarding the binding order. This has to be verified in the "Advanced Settings" dialog of the Network Configuration window, "Adapters and Bindings" tab (Windows$^{®}$ XP).

4.1.2   Simple Integration
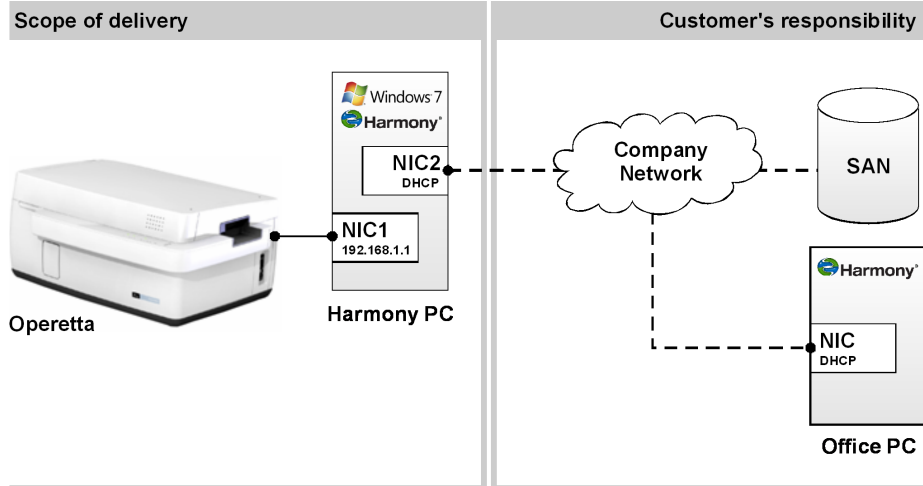
NIC: Network Interface Card – SAN: Storage Area Network

4.1.3 Alternative Solutions

In the case where Windows® 7 is not allowed in the customer's company network, use one of the following solutions to isolate the "Operetta network" from the company network.

4.1.3.1 Office PC as interface (small solution)
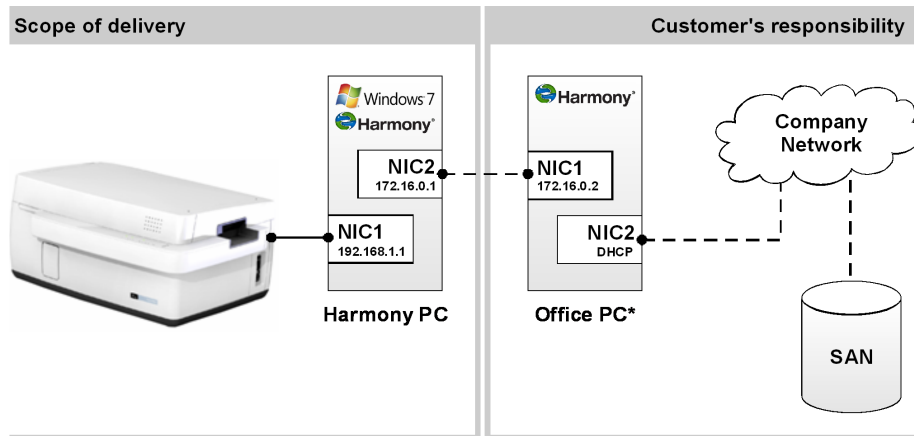
NIC: Network Interface Card – SAN: Storage Area Network   * Special NIC configuration required

4.1.3.2 Office PC as interface (big solution)
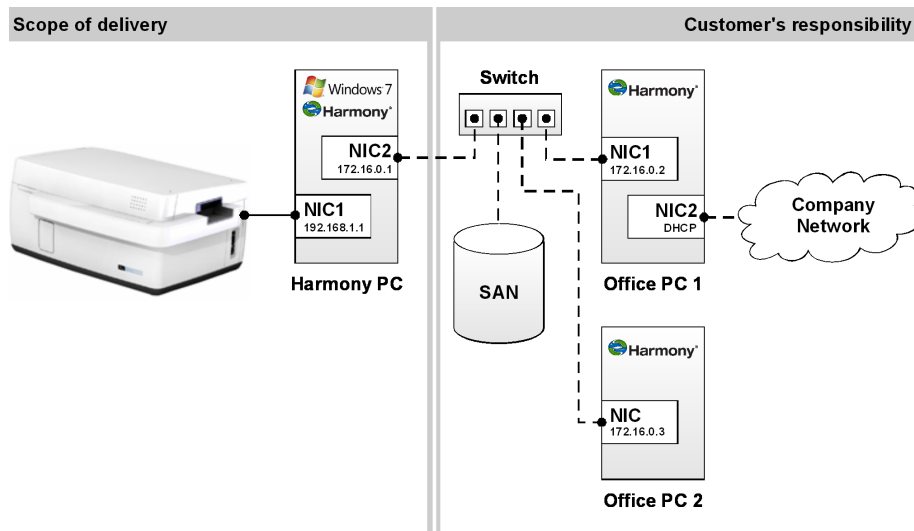
NIC: Network Interface Card – SAN: Storage Area Network

4.1.3.3 Router

| Scope of delivery | Customer's responsibility |

Windows 7
Harmony
NIC2
172.16.0.1
NIC1
192.168.1.1
Harmony PC

172.16.0.2   DHCP
Router*

Company Network

Harmony
NIC DHCP   **SAN**
Office PC
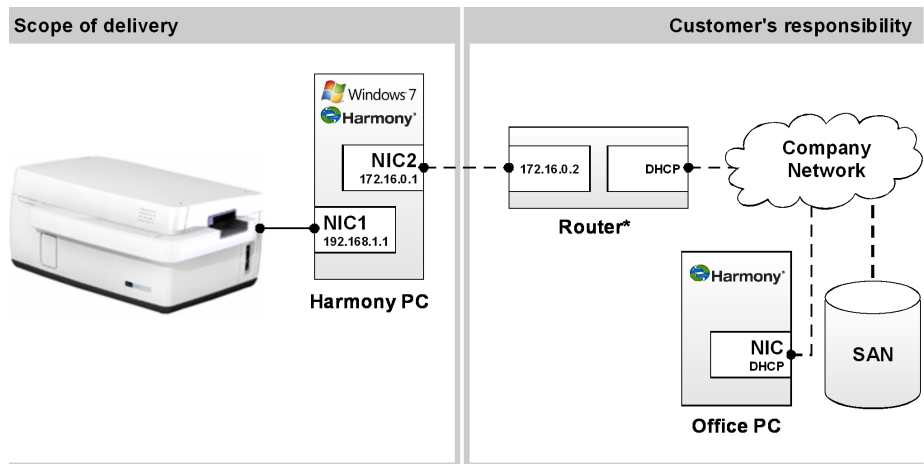
NIC: Network Interface Card – SAN: Storage Area Network   * Special configuration required

4.1.4   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® XP 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.

4.1.4.1   Standard Configuration
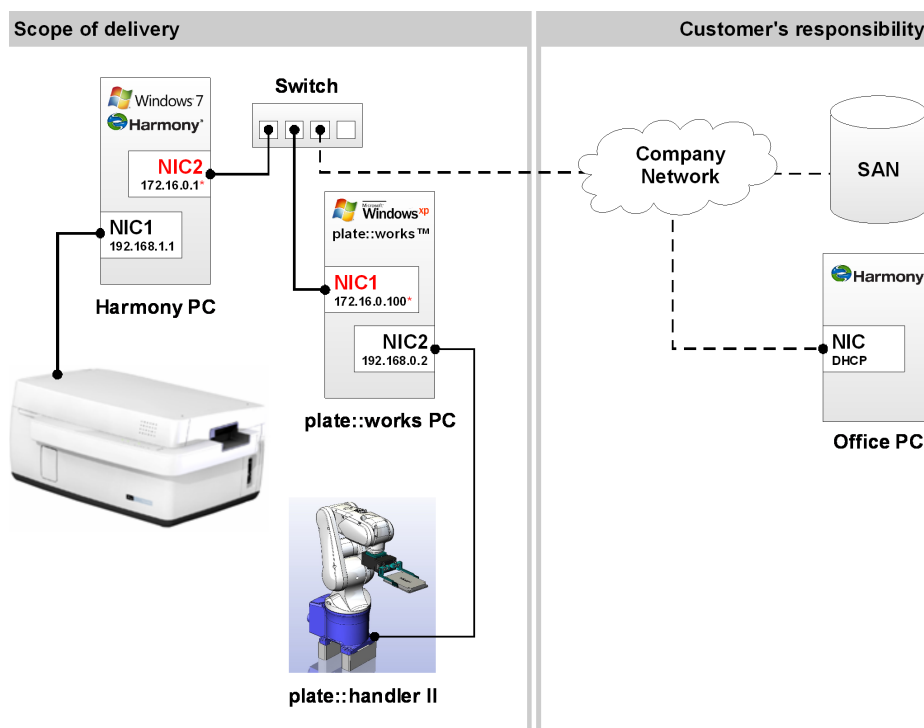
* Network configuration must be adapted to company network, see below
NIC: Network Interface Card – SAN: Storage Area Network

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.

4.1.4.2   *Office PC as interface*
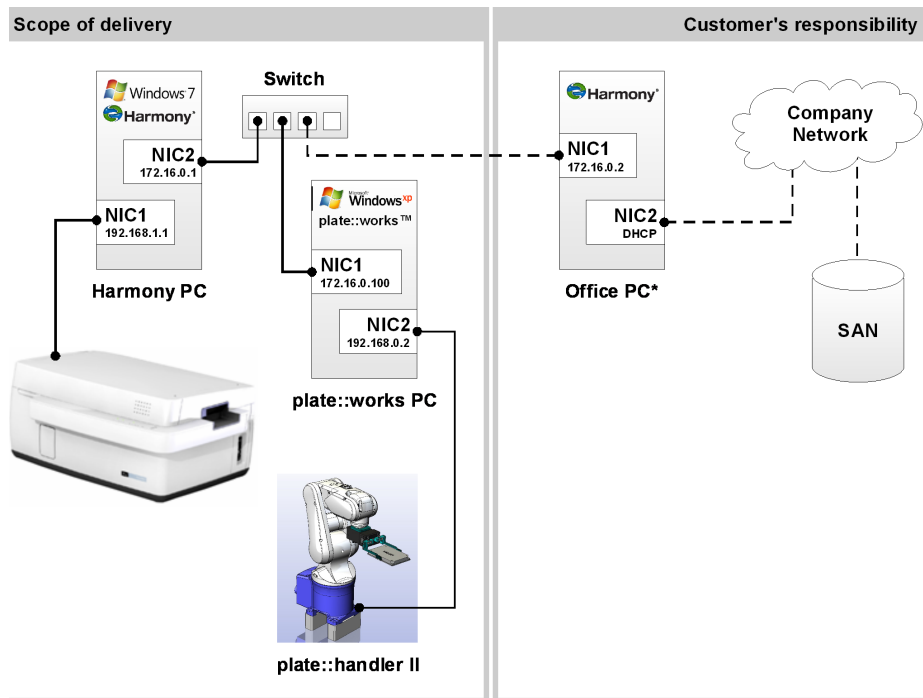
| Scope of delivery | Customer's responsibility |

NIC: Network Interface Card – SAN: Storage Area Network   * Special NIC configuration required

4.2   Microsoft Windows Security Updates / Service Packs

The Harmony software was tested and released with:

1. Microsoft Windows$^{®}$ 7

2. Microsoft Windows$^{®}$ Vista Service Pack 2

3. Microsoft Windows$^{®}$ XP Service Pack 3 (only for office PC installations)

This includes all security patches until October 1st, 2010).

> **Notice**
>
> PerkinElmer cannot guarantee that future Windows updates provided by Microsoft will not compromise the stability of the "Operetta System". We assume that the impact is low but to ensure to always have a stable "Operetta System" it is recommended to disable Windows updates.

4.3 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.

Basic:

- Harmony Office License (incl. USB dongle) with valid Software Maintenance Agreement (SMA)
- 2 GB free harddisk
- One free USB port
- DVD drive (for installation)
- Network to Harmony PC (to connect to database)
- Administrator rights (only for the installation)
- Internet Explorer 7 or later

Minimum:

- Windows® XP SP3
- Intel® Pentium 4 with 3.2 GHz
- 1 GB RAM
- Screen resolution of 1280 x 1024 (19"),
  DPI setting: normal size (96 DPI)

Optimum:

- Windows® 7 (64 bit)
- Intel® Xeon® Quad Core with 2.6 GHz
- ≥ 8 GB RAM
- Screen resolution of 1920 x 1200 (24"),
  DPI setting: normal size (96 DPI)
- Gigabit Ethernet connection to Harmony PC

> **Notice**
> Harmony will not run on any virtual PC (e.g. VMWare).

4.4 Computer Systems

4.4.1 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 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 network is essential for the correct function of the Operetta System, because permanent network connection is required between the different components (particularly Operetta device, Harmony PC, Office PCs, image servers).

4.4.2 Hardware

Requirements for new 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 device. The relocation of any other hardware within the "Operetta 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 System".

2. Modification of any hardware of the Harmony PC is not allowed.

4.4.3 Operating System, Software & Software Applications

Problems with software should be reported to the PerkinElmer service.

1. 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 System".

2. Deactivating the virtual memory for microsoft windows is not allowed.

3. Making any changes in the [HKLM\Software\PerkinElmerCTG] registry is not allowed.

4.4.4   Data

1. PerkinElmer is not liable for any data loss due to Data management processes like backups, etc.

2. Due to limited harddisk space on the Harmony PC (500GB), Harmony provides a functionality to relocate the data to an external data medium. This could be a USB-hard drive as well as a server (SAN). The relocated data remain reachable via the Harmony software.

3. PerkinElmer recommends a Gigabit connection and to include this server in the internal backup system.

4.4.5   Back Up

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 System".

4.4.6   Security Settings & Anti-Virus Protection

The Harmony software is tested with Microsoft Forefront Client Security.

1. PerkinElmer is not responsible for the implementation of an effective virus security strategy.It is suggested to exclude the following file types from any scan:
   - TIFF (images)
   - XML (database files)

2. The "Operetta System" needs the following ports for communication (TCP/UDP). Please ensure that these ports are unblocked:
   - 80
   - 8250 - 8300

3. 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.

4.5   Computer Users

1. Training: Ensure an appropriate Harmony training for any user of the "Operetta System".

2. User Accounts: The Harmony PC comes with two pre-defined Windows$^®$ user accounts:
   - **Operetta** (password "Operetta")
     This is the default Windows$^®$ user account for operating the Operetta system.
   - **Administrator** (password "Operetta")
     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 "Operetta", this will require additional modifications in Harmony and in the network (see **Operetta Operation Manual**, section "User Accounts"). The default user account ("Operetta") must have the following properties:

- ○ Can create and run scheduled tasks.

- ○ Password does not expire.
  Otherwise all scheduled tasks have to be updated regularly.

- ○ The account must have the right to "Log on as a batch job". This right must be granted *before* creating scheduled tasks.

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 "Operetta" (read/write access including the right to create subfolders).

4. Passwords: "Operetta System" users should not change any passwords. Any changes are only allowed upon consultation with PerkinElmer service.

4.6   *Remote Support*

PerkinElmer uses the service of WebEx (http://evoportal.webex.com) to solve remote support issues in real time for "Operetta Systems". If WebEx 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 WebEx remote support:

- Windows® XP, Windows® Vista, or Windows® 7

- Intel® x86 (Pentium 400 MHz +) or compatible processor with 128 MB RAM

- Microsoft® Internet Explorer® 6

- JavaScript® and cookies enabled in the browser

- ActiveX enabled

- 56 K or faster internet connection

5   *Technical Data*

5.1 Optical Configuration

The Operetta is available in a non-confocal and a confocal version. It can sequentially acquire an arbitrary number of fluorescence and brightfield images.

The confocal version can switch between non-confocal and confocal mode by guiding excitation and emission light either in a non-confocal path or through a pinhole disc. It can acquire either a series of confocal or a series of non-confocal images in one experiment.

The Operetta has a high speed high precision auto focus unit based on an infrared laser for detection of the sample carrier surface and measurements in a defined imaging plane above the sample carrier surface.
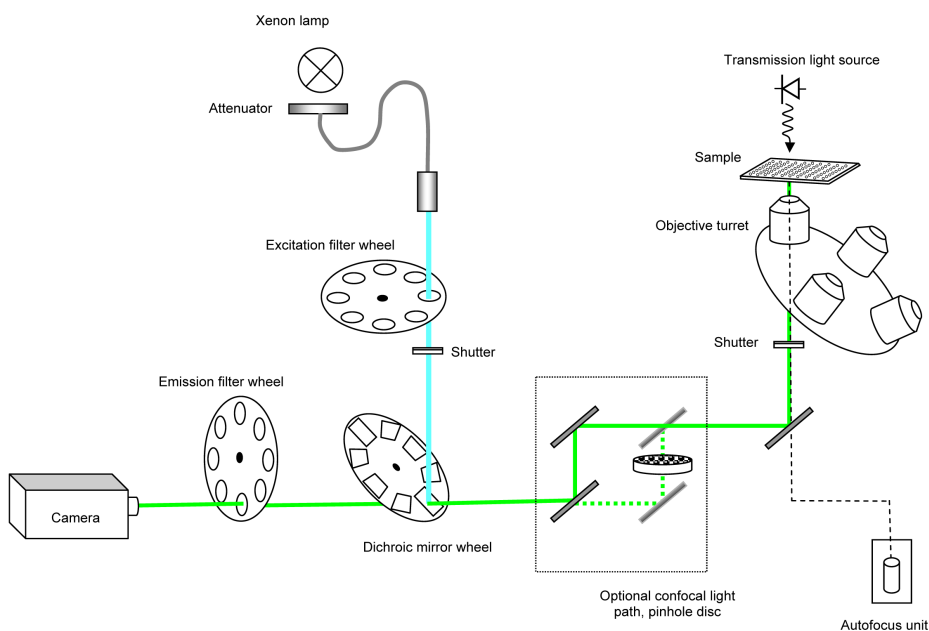
The light source for fluorescence excitation is a 300 W Xenon lamp with continuous spectrum in the visible wavelength range. The illumination intensity in the sample can be tuned in the software using a mechanical attenuator. The fluorescence excitation wavelength is selected by choosing an excitation filter on an eight position excitation filter wheel.

The light is guided into the sample via two mirrors which are mounted on a slider. In the confocal version the slider can move and guide the light through a spinning pinhole disc. This allows simultaneous scanning of an array of diffraction limited points across the sample. This leads to high speed confocal imaging with greatly reduced background noise. Please note that non-confocal and confocal imaging cannot be used in combination in a single measurement or screen.

Up to four objectives can be mounted on the objective turret for automated change. A transmission light source is mounted above the sample for automated brightfield imaging, either separately or in combination with fluorescence imaging.

Fluorescence emission and transmitted light from the sample is collected through bandpass filters on a emission filter wheel and detected by a high sensitivity Peltier cooled CCD camera. Special optics enable viewing of an enlarged field of view / number of cells in an image view without compromising optical resolution.

The Operetta unit contains all necessary hardware and software for four color measurements (UV, green, orange and far red) using a 20x long WD objective – see below for the accessories included. Briefly they comprise the instrument with four excitation filters, four dichroics, four emission filters, 20x objectives, camera, Xenon lamp, laser based auto focus and one set of Harmony Software included in the instrument.

Please see the following table for an overview of standard and optional components of the optical setup such as lenses, filters, dichroic mirrors etc.

Component	Standard Configuration	Optional Configurations
Objective lens	20x long WD	2x long WD 10x long WD 10x high NA 20x high NA 40x long WD 40x high NA 60x long WD 60x high NA 100x long WD
Excitation filter	380/40 (UV) 475/30 (Green) 535/30 (Yellow) 630/20 (Far Red)	420/20 500/20 570/20 615/30
Dichroic mirror	405 435 495 515 555 585 635 645	
Emission bandpass filter	445/70 525/50 595/70 705/110	

5.2 Basic Components

Components	
Operetta unit	Optical imaging unit Confocal scanning module (optional) Laser based high precision auto focus Automated objective lens changer High resolution table Xenon light source Control electronics Control PC
Scanning table	High precision X- and Y-axis Plate holder
Automation	plate::handler II (optional)

5.2.1 Detection

Components	
Detector	CCD camera with 1.3 mega pixel Peltier-cooled 14 bit resolution Emission band pass changer

5.2.2 Light Sources

Components	
Xenon light source	External Xenon lamp, fiber-coupled Spectral width: 14 nm General wavelength range: 350-680 nm Input Voltage: 100 – 240 V AC, 50/60 Hz, 6.0A Lamp module: • 300 W • Lifetime: 500 hours • Easy exchange
Brightfield LED	

5.3 Specifications

5.3.1 Objective Lenses

Objective	Numerical aperture	Working distance [mm]	Field of view [µm^2]	Depth of focus [µm]	Optical resolution xy [µm]
2x long WD	0.08	6.2	6754 × 5086	145.5	3.75
10x long WD	0.3	10	1351 × 1017	10.3	1
10x high NA	0.4	3.1	1351 × 1017	5.8	0.75
20x long WD	0.45	6.6-7.8	675 × 509	4.6	0.67
20x high NA	0.75	0.6	675 × 509	1.7	0.4
40x long WD	0.6	2.7-4.0	338 × 254	2.6	0.5
40x high NA	0.95	0.18	338 × 254	1.0	0.32
60x long WD	0.7	1.5-2.2	225 × 170	1.9	0.43
60x high NA	0.9	0.2	225 × 170	1.1	0.33
100x long WD	0.85	1.2-1.9	135 × 102	1.3	0.35

5.3.2 Sample Carrier

Recommended sample carriers for the Operetta:

Plate type	Bottom material supports adherence of cells	Plate bottom allows use of high NA objectives	Suitable for the following objectives
ViewPlate-96 F TC (black, with lid, TC, sterile, individually pouched) bottom thickness of 0.8 mm	yes	no	2x long WD 10x long WD 10x high NA* 20x long WD 40x long WD*
ViewPlate-384 F TC (black, with lid, TC, sterile)	yes	no	2x long WD 10x long WD 10x high NA* 20x long WD 40x long WD*

Plate type	Bottom material supports adherence of cells	Plate bottom allows use of high NA objectives	Suitable for the following objectives
CellCarrier-384, TC, black, sterile	yes	yes	2x long WD 10x high NA 10x long WD 20x high NA 20x long WD 40x high NA* 40x long WD 60x high NA* 60x long WD 100x long WD*

* Restrictions - some edge wells cannot be measured

5.3.3 Power Supply

The Operetta is connected to mains by a separate power cable which is specified as follows:

Specifications	Europe	US
Cordage Rating	16A/250V~AC	15A/125V~ACI
Plug	16A/250V~AC, IEC 60884-1	15A/125V~AC, NEMA 5-15P UL817
Connector	10A/250V~AC, EN 60320/C13	15A/125V~AC, IEC 320/C13
Approvals	VDE	UL

5.4 Consumables

Part Number	Item
HH12040041	Operetta Xenon Exchange Bulb
HH12040039	Operetta Mod A Xenon Bulb Unit

Please contact your local sales representative to order consumables.

PerkinElmer

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