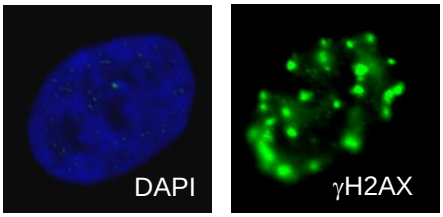
# Immunofluorescence

## Reagents required

- Complete media
- Trypsin
- 3% paraformaldehyde, 2% sucrose, in PBS
- 0.2% TritonX-100 in PBS
- PBS
- PBS supplemented with 2% bovine serum fraction V albumin (BSA)
- Primary and secondary antibodies
- DAPI
- Vectorsheild
- Nail varnish




Foci analysis 15' post 3Gy IR

(100x magnification)

## Slide Preparation

Place coverslips into 3cm dishes and add $2x10^5$ cells. For G0/G1 analysis grow at 37°C until confluent or serum starve using 0.5% FCS for 3-4 days. For G2 analysis culture until cells reach 60-80% confluency

## Treatment

To monitor repair kinetics after IR exposure, each dish is irradiated with 3Gy (9 sec/Gy) at 0.25, 2 ,6 ,24, 48, and 72h intervals and processed together.

## Fixation

Cells are fixed for 10min at room temperature using 3% paraformaldehyde, 2% sucrose, in PBS.

## Permeabilization

Cells are permeabilized using 0.2% TritonX-100 in PBS for 2.5 min at RT, followed by 3x PBS washes.

## Primary and Secondary Antibody Incubations

Primary and secondary antibodies are diluted in PBS supplemented with 2% bovine serum fraction V albumin (BSA). Primary antibody incubations are performed for 30 min at 37°C and secondary for 20 min at 37°C, followed by 3x PBS washes.

## Dapi and Mounting

Nuclei are counterstained by DAPI (0.000025% in PBS) for 10min at RT, followed by 3x PBS washes. Coverslips are mounted onto microscope slides in Vectashield, and sealed with nail varnish before analysis using your favourite microscope and imaging software.

For advice please see Lisa/Elena