

# The Zeiss Elyra TIRF Confocal

Abridged INSTRUCTIONS

# Introduction

The Zeiss Elyra TIRF PALM/STORM super-resolution microscope is located in room 00/071, Lab 1 in the WTCHG building.

The new Zeiss Elyra TIRF microscope has:

- Zeiss Observer wide-field inverted TIRF fluorescence microscope
- X-Cite 120Q fluorescence excitation illuminator
- Triple dichroic filter for FITC, TRITC and Cy5 far red
- Newport Vision IsoStation anti-vibration isolation table for Elyra system
- PALM super-resolution illumination module
- Piezzo fast Z stage with 100µm range
- Motorised XY stage
- Black box XL stage incubator for super-resolution with temperature control
- Plan Apochromat 10x NA 0.3 air objective
- Plan Apochromat 20x objective NA 0.8 air objective
- Alpha Plan Apochromat 100x NA 1.46 oil objective
- Andor iXon DU 897 EM-CCD (512x512 pixel) camera
- DAPI 405nm line 50mW diode laser
- GFP 488nm line 100mW laser
- DsRed 561nm line 100mW laser
- Imaging PC workstation for Elyra systems S.1, P.1 and PS.1
- 30" LCD TFT PC monitor
- Zeiss ZEN Black image acquisition software with super-resolution PALM/STORM module
- Two hardware USB2 licence keys for Zen Black imaging software, one on the microscope PC and the other on the Core's Imaging Workstation

To view the Zeiss image files on your office PC you will need to download and install Zeiss's Zen Lite program (Windows PCs only).

# General information

The system can only image green GFP/FITC, red DsRed/RFP/TexasRed and blue DAPI dyes.

The Andor iXon DU 897 EM-CCD camera has a maximum resolution of 512 x 512 pixels. It captures black and white images with a 16-bit depth of 65,536 grey levels (with 0 being pure black- and 65,535 being pure white).

Zen Black doesn't automatically save images – you must save them as you go along.

Use 'snap' in Zen Blue to clear noise from the camera detectors before imaging. Setting 'snap' for every image will slow down acquisition a lot, but you can just 'snap' clear at the beginning of an image sequence. Adjust exposure time, laser power and EM Gain for brightness in 'Acquisition'.

Unlike the Zeiss Spinning Disk microscope in OPIC, you can't go above 300 in EM Gain with the camera (it's locked in software). The EM Gain going from 1 to 200 goes from the equivalent of ½ second to a 20ms to frame rate. Avoid putting too much light onto the camera's EMCCD sensor. The EM-CCD camera is 16-bit (64,000 shades of grey).

Leave the Elrya system on for 5 minutes before scanning as the Andor EM-CCD camera needs time for its electronics to cool down.

The system has three laser lines at 405nm DAPI, 488nm GFP and 561nm TRITC/DsRed. There are four different emission filter sets for DAPI, GFP, DsRed and Cy5 far red, selectable via Zen Black.

In Zen Black click Live for a faster 'preview' imaging with more noise.

You can save configurations and the menu screen setup in Zen Black. You can reuse microscope settings from a stored image. The images are generally saved in Zeiss \*.czi format although you can use \*.ism. To prevent PC crashes with Zen Black, it's a good idea to limit the number of open images.

Log-in to the PC via the account '**cyto\ism user**'.

# The Zeiss Axio Observer Microscope

The Zeiss Elyra TIRF PALM/STORM super-resolution microscope mixes air (10x, 20x) and oil (100x) objectives. Make sure you use the appropriate immersion fluid with the objective. The Zeiss immersion oil for the 100x oil objective is left on the air table. The oil immersion fluid is prone to drips from the applicator so take care when using it not to overload the applicator.

It is far easier to control the DAPI, GFP and DsRed mercury lamp fluorescence (as viewed down the eyepieces) from Zen Blue software.

Alcohol 70% is provided along with gloves and green-box lab tissues. These are supplied for cleaning the samples and spillages and are not to be used on the objectives. Be careful where you stand the 70% alcohol bottle as the alcohol can drip out from the spout into electronics.

Only Whatman 105 Lens Cleaning tissues are to be used to wipe immersion oil from the objectives. The air objectives shouldn't need cleaning after use.

If you are using the heated incubator at 37°C you should switch on the incubator at least 2 hours before imaging. For the most stable time-lapse imaging at 37°C leave the incubator on overnight.

To view fluorescence down the microscope you have to press the X-Cite Start (Stop) button or use the footswitch, so that the X-Cites 'Open' LED comes on. You set the microscope to view down the eyepieces via Zen Black software, under 'Locate'. Set the emission filters to those required for the eyepieces (FSet77HE, GFP/DsRed) and the lasers (other filters).

**WARNING:** *To lift out the slide/Petri dish insert from the Piezzo Z motor stage always pick up the insert by the two middle finger-grip indent holes – NEVER use the levelling adjuster screws at the top left and bottom right.*

The motorised Piezo Z stage is only controlled by the Zen Black software. The microscope stand and the Zeiss microscope remote LCD station only move the main microscope Z motor. The Piezo stage has a total range of 100µm.

The two buttons on the right of the microscope on the focus knobs raise the objectives to the work position (far button) and drop the objective to the load position (near button).

The two buttons on the left side by the focus knob control the reflector turret and light path (this is far easier selected by Zen Black software on the PC).

You can switch microscope objectives via the Zeiss microscope remote LCD station or within Zen Black software.

There is no 'definite focus' autofocus hardware on the Elyra microscope.

The tube lens can be 1x or 1.6x – always use the 1.6x with super-resolution. If the 1x tube lens is selected you can see the shadow of the 1.6x down the microscope (vanishes when the 1.6x is selected).

You can do a multi-location time lapse with an identical z stack, and say a 3x3 raster scan at each location in time-lapse.

The Elyra isn't a confocal and the Core's Zeiss Spinning Disk confocal system should be more 'gentle' with live cells. Start with lower laser power as the Elyra has high powered lasers (around 100mW).

# The X-cite Series 120PC Q

The X-Cite takes a minute or so warm up

Use the Mode button to select three modes:

1. Press mode and when OOOO. appears on the display this is the number of hours the arc lamp has been on
2. Press mode and when OOO.O appears on the display this is the timer mode. The display is in seconds and 1/10 seconds. It times how long the lamp will be on in seconds. Set the time and press Start (Stop) and lamp will come on and the timer will count down to switch-off.
3. Press mode and when OOO appears on the display this is the lamp intensity. Press Up/Down to select 0, 12, 25, 50 and 100% lamp intensity. Warning things can very bright down the eyepieces even with the standard FITC/TRITC/Far red triple filter selected.

Press Start/Stop to switch open/close the X-Cite shutter and illuminate the specimen. The footswitch under the microscope also activates the Start/Stop sequence.

Note that the Excite 120PC Q only works with the triple filter block FSet77HE (for GFP/DsRed/Cy5 only and so no DAPI) when viewing down the eyepieces. The triple filter FSet77HE excites at 483 + 564 + 642 nm (HE) and shows all three fluorescence colours at the same time, although our eyes can't see the far red Cy5 emission.

# Zeiss Elyra Operating Manual

For details on the operation of the Elyra TIRF super-resolution microscope see the Zeiss Elyra manual, and in particular the 'Elyra Quick Guide' (after chapter 7 of the manual) – a pdf download of the Quick Guide is available via the Core's Elyra web-pages.



# Switch on Procedure

- 1) Switch on all the labelled 13A mains sockets with the Microscope + X-Cite socket on first.
- 2) All the microscope control boxes including the Andor EM-CCD camera should come on with the exception of the Tokai Hit incubator heater unit that is only switched on as required.
- 3) Switch on the system PC and 32" monitor and login to the WTCHG network.
- 4) Start Zen Black (don't select the Zen Blue option as that is for off-line image analysis only). Start up the Elyra hardware via the software prompt.
- 5) Place the specimen onto the microscope
- 6) Use the triple filter block FSet77HE (GFP/DsRed) or BF (bright field transmission) to view the specimen down the microscope via the eyepieces (filter selection and open/close shutters options are under 'Locate' in Zen Black).
- 7) Use the X-Cite footswitch or Start-Stop button to illuminate the specimen with excitation light, in combination with open/close filter options in Zen Black.
- 8) Ensure that the doors and viewing panels are closed on the black stage incubator or the lasers will switch off. Wait ~5 minutes until the Andor EM-CCD camera electronics have cooled down before acquiring images.
- 9) Go to Acquisition in Zen Black to capture images  
*The microscope stand must not be left pushed backwards on the microscope or the lasers will not fire (there is a safety cut-out).*
- 10) Create and save filter and EM-CCD camera settings for the fluorochromes in your sample.
- 11) Leave the Elyra microscope system on for at least 5 minutes before scanning as the Andor EM-CCD camera needs time for its electronics to cool down.
- 12) The system has three laser lines at 405nm DAPI, 488nm GFP and 561nm TRITC/DsRed. There are four different emission filter sets for DAPI, GFP, DsRed and Cy5 far red, selectable via Zen Black.  
*Warning: The Elyra lasers are shuttered off by magnetic micro-switches if one of the access doors is left open on the blackened live cell incubator chamber.*



# Switch off procedure

- 1) Remove the specimen from the microscope stage
- 2) Wipe off excess immersion fluid from the 100x oil objective lens/top using the Whatman 105 lens cleaning tissues provided  
*The 10x and 20x air objectives don't need cleaning after use.*
- 3) Leave the 10x objective selected on the microscope nose-piece to minimise any possibility of damage during start-up when the XY motorised stage initialises  
*Note: The top of the 100x objective rotates, so avoid accidentally turning it.*
- 4) Drop the 10x objective nearer to the Load (lowest) position using the microscope coarse focus control
- 5) Transfer image files to the network or data key
- 6) Shut Down the PC workstation
- 7) Switch off all 13A mains sockets around the microscope, leaving the modules switched on
- 8) Tidy up the microscope area and leave.
- 9) Switch the room light, desk lamp, and red lamps off as you leave the room.

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If you have any microscope/imaging problems or queries, just ask a member of the Microscopy Core staff for help.

There is additional help and advice on our Core web-pages:

<http://www.well.ox.ac.uk/microscopy>

*WTCHG Zeiss Cell Observer SD microscope user guide. This version updated 26<sup>th</sup> June 2014.*