Wellcome Trust Centre for Human Genetics
Cellular-Imaging Microscopy Core

The Zeiss Spinning Disk Confocal

Abridged INSTRUCTIONS

Introduction

The Zeiss Cell Observer spinning disk confocal microscope is located in the category three viral suite in the Oxford Particle Imaging Centre (STRUBI). The spinning disk's principle advantage over our Leica SP8 and Zeiss 510 LSM microscopes is speed of acquisition rather than image quality, and so the spinning disk is particularly suited to live cell imaging, although it can image specimens on slides as well.

The new **Zeiss Cell Observer spinning disk confocal** microscope has:

- Zeiss Observer Z1 Inverted Microscope
- NewPort Vision IsoStation anti-vibration isolation table
- Yokogawa CSU-X1 Filter Wheel and Spinning Disk Unit
- Photometrics Evolve 512 Delta EM-CCD camera (512 x 512 pixels)
- Applied Scientific MS2000 Piezo motorised XY stage
- Applied Scientific fast galvo Z focus (150um range)
- Definite Focus with NIR LED Hardware Focus to eliminate thermal drift
- PeCon Temp Module S1 and XL Multi S1 live cell stage incubator (rated 3^{o}C to 45^{o}C)
- 10x EC Plan Neofluar NA 0.3 air objective
- 40x C-Apochromat NA 1.2 water-immersion objective (with coverslip correction collar)
- 63x C-Apochromat NA 1.2 UV-VIS-IR water-immersion objective (with coverslip correction collar)
- 100x Plan Apochromat NA 1.4 oil-immersion objective
- Zeiss HXP-120V short-arc fluorescence Hg lamp and controller (2,000h)
- DAPI, GFP and DSRed diochroic filters for eyepiece viewing
- 405nm line DAPI laser
- 480nm line GFP and 468nm line DsRed laser
- Zeiss ZEN Blue image aquisition software

General information

The system **can only image** green GFP/FITC, red DsRed/RFP/TexsRed and blue DAPI dyes. As well as single dichroics, we have a double GFP/DS-Red for faster imaging of these two dyes. We don't have the optional Cy5 far red laser.

The Photometrics Evolve 512 Delta 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).

The Evolve camera's fastest frame rate is 15ms (67 frames per second). Images are stored in the Zeiss *.czi format. To view these *.czi files on another Windows 7 PC download Zen Lite via the Core's Spinning Disk microscope webpages.

All scans by Zeiss's Zen Blue image acquisition software are automatically saved in 'My Pictures/Temp' on the PC, but will be deleted when you leave Zen (so save them first). In event of Zen crashing or PC mains failure the files will remain in Temp.

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'.

Don't go above 300 in EM Gain with the camera (there's not safety warning). 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 cameras EMCCD sensor. The Evolve camera needs to be triggered if you want extra speed.

Click Live for faster 'preview' imaging with more noise.

Smart setup for selecting fluorochromes (Channels) starts the laser power at 50% **which will be too high** for most samples. Fixed samples seem to bleach very rapidly at these higher laser powers.

You can save configurations and the menu screen setup. You can reuse microscope and camera settings from a stored image.

Using the Zeiss Observer Z1 microscope

The Cell Observer microscope mixes **air** (10x), **oil** (100x), and **water** objectives (40x and 63x). Make sure you use the **appropriate immersion fluid** with the objective. The immersion oil for the 100x oil is left on the air table and the Immersol W 2010 for water immersion is left under the PC monitor. The two immersion fluids don't mix. The Immersol water immersion fluid is particularly prone to drips from the applicator so take care when using it not to overload the applicator (if oil or water Immersol drips from its applicator you are using too much).

It is far easier to control the DAPI, GFP and DsRed mercury lamp fluorescence (as viewed down the eyepieces) from the PC's 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 oil and Immserol water fluid from the objectives.

We don't have the Tile module for the confocal system, so we can only raster scan and stitch images around one single field. We can time-lapse at multiple locations though (but it must be with the same dye filter configuration at each location).

Using the Zeiss Observer XL stage incubator

The Zeiss 37oC incubator controller **must be switched on before any other equipment** otherwise the control link to the microscope stand won't be established and 'Incubation' will not appear as a tab in the Observer Z1's LCD controller.

To switch on the Temp Controller press its rear rocker switch **to the left** (the lowest LED lights up green). Then switch on the upper Heating Unit XLS by pressing its rear rocker switch **to the right** - the middle yellow 'heating on' LED will light up. To set the microscope stage incubator temperature press Home, Microscope, and Incubation on the microscope's LCD panel.

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

The spinning disk system has the Zeiss 'Definite Focus' NIR LED hardware autofocus system that fires an LED at the coverslip and keeps that distance constant. This only works well if your cells are growing are on top of the coverslip.

Using the PeCon CO2 system

The full manual is located on our core web pages and as a printed copy with the spinning disk system manuals. The CO2 controller should be switched on **15 minutes before use** and allowed to stabilise before the CO2 feed is activated.

Operation

- Switch on the CO2-Controller. Power on/off on the back of the device. The CO2-Controller should have a lead time of approx. 15 minutes before the CO2 is fed in.
- After approx. 5 min. the CO2 value in the display shows 0.0. This zero value can be corrected with the potentiometer "sensor calibration" on the back of the device (see section maintenance).
- Press the "display" button to view the CO2 nominal value. The LED should mark "nom". Press the “+" and "–" buttons to set the nominal value. To view the CO2 real value press the button "display" again.
- Regulate the CO2 influx with the CO2 reducing valve. The valve should be adjusted carefully to avoid a permanent overshoot of the CO2 value (see guideline table 1).
- The carrying capacity of the pump can be chosen by pressing the "pump" button. The power is increased or decreased by repeated pushing. For 5% CO2, a pump power of 2 is recommended.
- Activate the CO2 control by pressing the "valve" button. This button has a two-color LED, Red → no gas influx and Green → CO2 flow into the mixing chamber.
- Press the button "valve" again to deactivate the CO2 control. The LED in the button is off.

The PeCon CO2 controller is **on loan** from the Zeiss 510 confocal system and may have be used on other microscope systems occasionally (principally with the Leica SP8 confocal).

Switch on Procedure

1) Switch on the 13A mains socket labelled HXP-120V. Then switch on the epi-fluorescence HXP-120V short-arc mercury lamp power module
2) Switch on all the other 13A mains socket switches. All the microscope control boxes should come on (always leave the Zeiss modules in the switched on position). The Yokogawa spinning head will also come on (the key is left in the on position). The Evolve camera should be left off
3) Switch on all the lasers using the remote control box (4 black switches)
4) Switch the HP workstation PC on, and the monitor
5) Log into the PC as Zeiss user (left option, no password)
6) When you get into the Windows 7 desktop, switch on the large Evolve Delta 512x512 EMCCD camera
7) Start Zen Blue
8) Select Zen System (Start System)
9) Place the specimen onto the microscope
10) Use the DAPI, GFP, RFP and BF (bright field transmission) and All-Off to select dichroics for viewing down the microscope eyepieces (under 'Locate' in Zen Blue).
11) Go to Zen Blue's Acquisition 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)
12) Use the GFP/DsRed dual emission filter for faster red/green scanning
13) For complex operations like scanning multiple positions with the motorised stage or time-lapse, press the 'Start Experiment' button under Acquisition
14) The microscope will produce better images with less photo-bleaching if the room light is left off

Switch off procedure

1) Remove the specimen from the microscope stage
2) Wipe off excess immersion fluid from the objective lens/top using the Whatman 105 lens cleaning tissues provided
   The 10x air objective doesn'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
4) Drop the 10x objective 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 the large Evolve Delta 512x512 EMCCD camera
8) Switch off all the lasers using the remote control box (4 black switches)
9) When the PC is off switch off the epi-fluorescence HXP-120V short-arc mercury lamp power box via the illuminated button on the front
10) Switch off all 13A mains sockets around the microscope, leaving the module boxes themselves switched on
11) Tidy up the microscope area and leave. Switch the room light back on as required when you leave the room.

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 28th November 2014.