

3i Marianas Spinning Disk Confocal Microscope

Quick Start Guide

Turning the Instrument ON

1. In FOM, LogOn to the instrument in your reservation
 - a. The instrument will turn on, this may take a few minutes

Preparing and Mounting your sample

The 10X lens does not require a coverslip or immersion oil. The 100x lens requires a 0.17 mm (#1.5) coverslip and immersion oil (oil is supplied).

1. Lower the microscope to its lowest Z-position. The current Z-position can be read from the touchscreen main menu.
2. Remove the sample loading rack from the microscope stage.
3. Mount your sample face-down (sample side/coverslip side down) onto the loading rack and secure it in place, this is an inverted microscope.
4. Screw in the lens you are intending to use for your sample into the microscope.
 - a. If you are using the 100X lens, you will need to place a drop of the immersion oil on the lens after screwing it in. The drop should cover the lens and be thick enough to create a contact bridge with the coverslip of your sample.
 - b. There are two available slots for lenses. One is connected to a wired piezo attachment for z-stack images, the other (adjacent) is meant for normal use. If you would like to learn how the attachment works, please contact the facility manager.
 - c. You can select your lens slot via the Touchpad to the right of the microscope
5. Place the sample loading rack, with sample, back onto the stage
6. If using immersion oil, raise the Z-position until the lens immersion oil comes in contact and creates an oil bridge with the coverslip of your sample
 - a. Be very careful, **do not crash the lens into the sample.**
7. Tilt the head of the microscope forward, so that it is directly above your sample.
8. Switch the Laserstack Turnkey to ON

View Brightfield

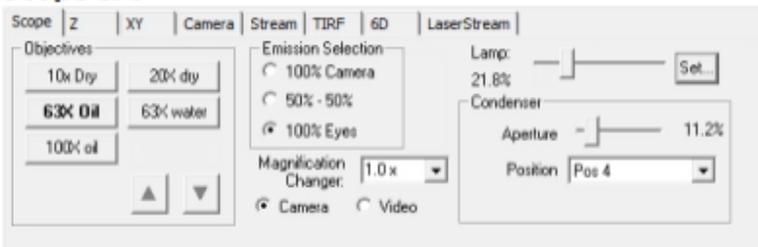
Press 'F1' at any time for a comprehensive Help resource.

Note that Slidebook is configured to switch the beamsplitter for either camera or eyepieces when any filter is chosen from the selected filter set

1. Open Slidebook
2. Open the Focus window (see images on next page)
3. View your sample with ambient light by clicking Open Bright on the right side of the focus window.
4. Select 100% Eyes in the Focus Window
5. Click Eyepieces icon next to the "Camera" selector
6. You can now review and focus your sample manually using the eyepieces on the microscope.
7. You can more finely adjust the Z-height using the Z Stage section of the Focus window



Scope tab

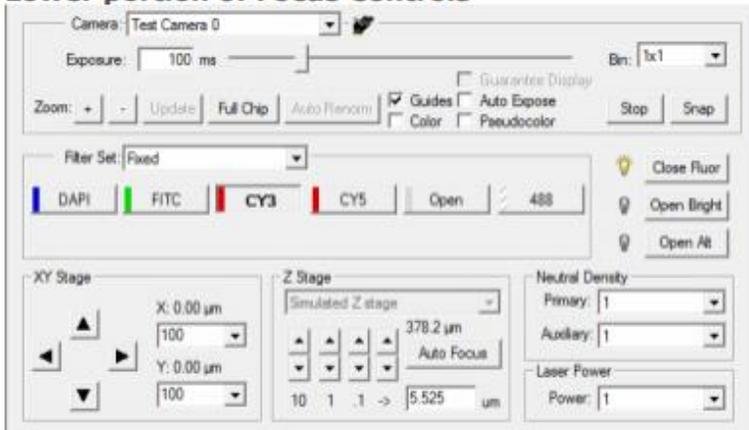


Select objective.

Direct light to eyes or camera.

Adjust transmitted light intensity and condenser.

Lower portion of Focus Controls



Adjust exposure time, binning, and appearance of live image window.
"Snap" a 2D single channel image.

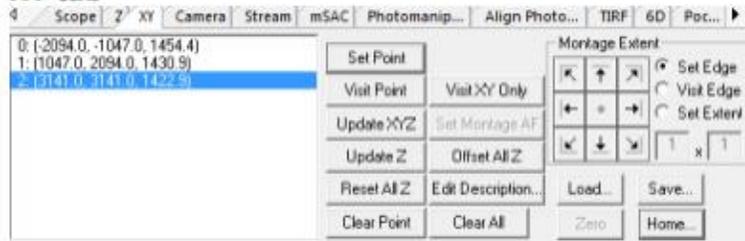
Select filter sets for viewing.
Open and close shutters.

Move the stage in X, Y, or Z.
Adjust movement size.
Test the "Auto Focus".
Neutral density settings.

View Fluorescent images

1. Select 100% Camera
2. Click Camera under the “Camera” selector
3. You should now see your sample live in the camera image on the screen.
4. Under Filter Set, select Confocal and select a laser. Press Open Alt.
5. You may need to adjust the Laser Power (Scope Tab) or the Intensification or Gain or speed (Camera tab).
6. You can move in the X/Y/Z direction across your sample using the X/Y/Z tab, or by clicking on the camera image.
 - a. You can save multiple points in the X/Y tab to perform multi-point image collection.

XY tab

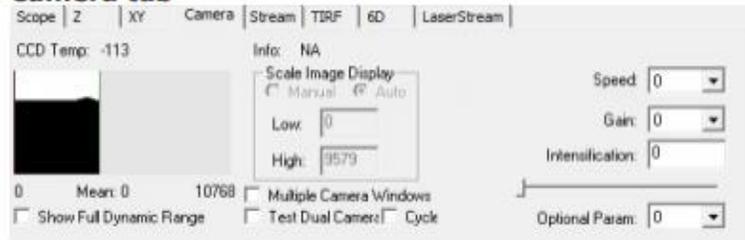


Set x,y,z positions for point visiting experiments.

Set extents for montage imaging.

Edit points.

Camera tab



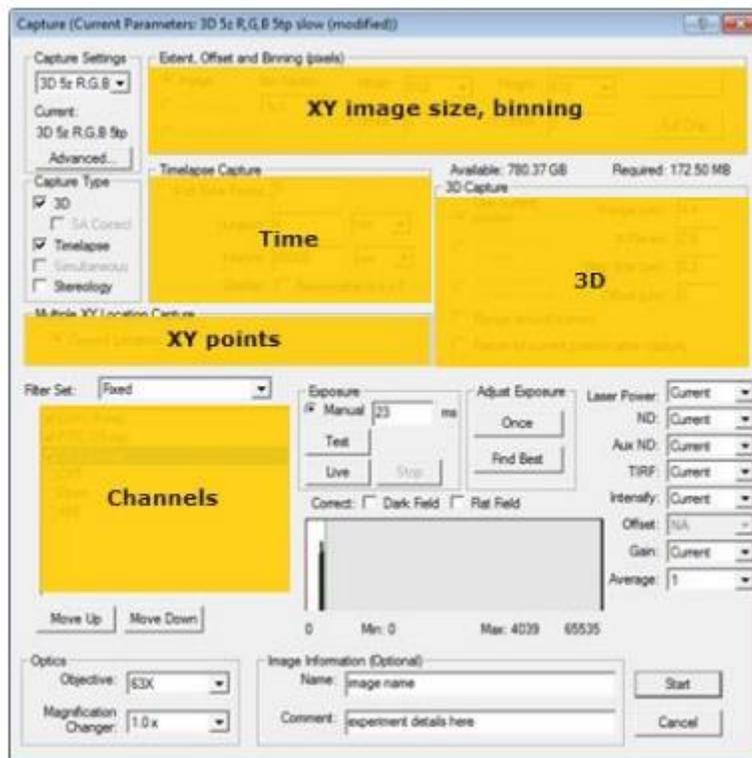
View intensity information based on sensitivity and exposure time.

Choose manual or auto-scaling display for live window.

Adjust intensification, speed, and gain for camera readout.

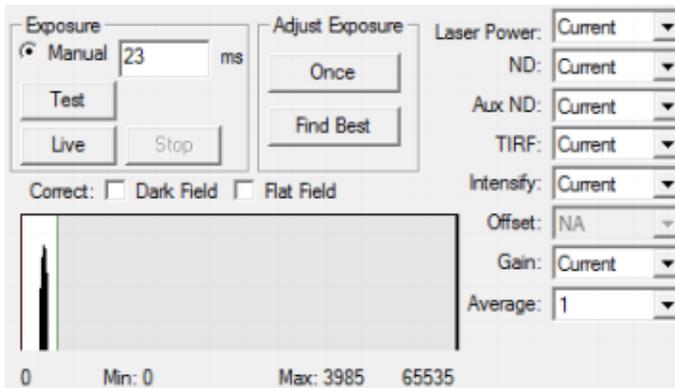
Collecting Fluorescent Images

1. Open the Capture window
2. Caution: pressing the Enter key at any time will start the imaging
3. Select capture type
 - a. This includes Z-stack, timelapse, multipoint, and others
 - b. More info in setting up different capture types can be found in the Slidebook 6 Help menu (F1) or in the Getting Started Slidebook 6 file on the computer desktop.
4. Check the boxes to select the channels you would like to capture.



5. Set the exposure

Exposure



Adjust the exposure time for each channel that will be captured. Use the **Live** button should you need to reposition your sample.

Refer to the histogram (see tips below) to determine optimal exposure times.

Try using the buttons in the adjust exposure dialog to allow SlideBook to optimize exposure time for your sample.

Adjust neutral density settings, gain, and intensification for the camera as desired. Use "Current" to use the settings from the focus controls. Different settings can be applied to each channel.

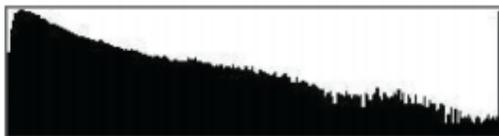
IDEAL



ACCEPTABLE FOR LIVE CELL



SATURATED



UNDEREXPOSED



TIPS

For fixed specimens, aim for filling the histogram 2/3 of the way. This gives you room for brighter planes.

Never saturate an image you would like to use for deconvolution or most other quantitative analysis techniques.

For live samples, experiment to determine how much light they can handle. You will often sacrifice intensity for cell viability. It is acceptable to have your signal at twice the background level, depending on the noise in the image.

When imaging live samples with an EM camera, you can increase the signal while keeping the exposure time low by increasing the intensification.

If possible, try to keep the intensification level well below the maximum available.

When using a non-EM, higher resolution camera, another way to increase signal without increasing exposure time is to utilize binning. It is important to note that binning will decrease the lateral resolution of an image.

Optimize the Captured Image

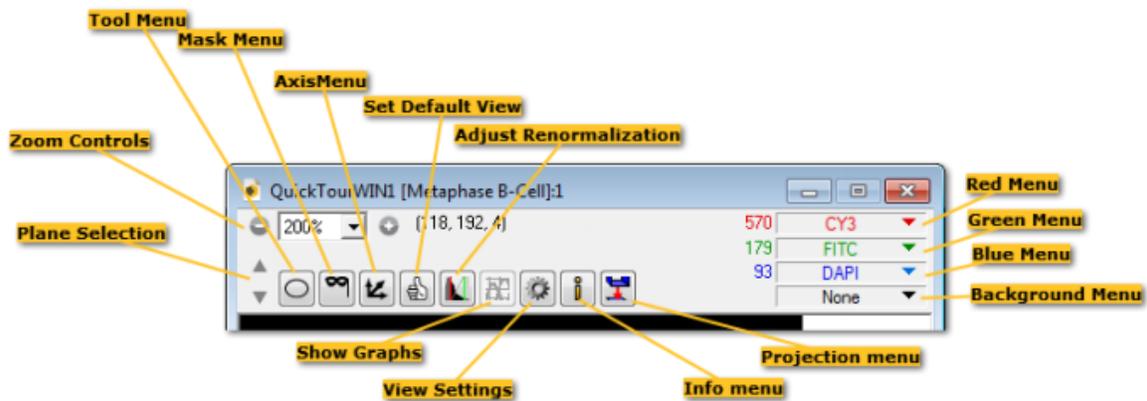
Observe the histogram in the Capture window as you test the exposure. Exposure times that display the brightest intensities at 1/3 to 1/2 of the maximum histogram are usually sufficient for fluorescent imaging. Attempting to fully fill the histogram usually causes high levels of photobleaching with little gain in signal to noise ratio.

Monitoring Image Appearance

1. Slidebook defaults to display the focus window with histogram stretch to make the dimmest images visible.
2. The best way to monitor image intensity is to observe the histogram while testing exposure time.
3. A grainy image indicates little light is reaching the camera and noise is very significant.
4. A red mask indicates saturated pixels. No information regarding spatial or intensity variation is possible from saturated pixels.
5. White regions lacking visible detail are over-exposed and close to saturation.
6. A solid black image indicates too much light is going to the camera and protective circuitry is blanking the chip to prevent damage.
7. Apply image corrections for dark field and flat field.
8. Make sure your coverslip is 170 μm thick (#1.5) and clean

Viewing Images

When you open an image by double-clicking on the thumbnail in a slide, a Main View will open. Alternatively, you may open a Main View or a Three View from the View Menu. All views contain many useful tools outlined below.



In any open view, the middle mouse scroll wheel will scroll through either z-planes or time.

Slidebook has many post-processing functions which you are welcome to explore. More information and tutorials on these functions can be found in the Slidebook Help menu (F1).

File Export

There are many functions for image analysis and options for image export within Slidebook 6.0, please consult the F1 Help menu for any guidance.

Press 'F1' in Slidebook for Help, search for "export" and select 'Topic: Export'.

1. Export as TIFF, Matlab or video
2. Bio-Formats plugin for Fiji opens Slidebook files (.slid)
3. Exporting quantitative images

Turning the System Off

1. Switch the Laserstack TurnKey to OFF
2. Close Slidebook
3. Lower the microscope stage.
4. Tilt the head of the microscope backwards, to its original angled starting position. It should no longer be directly above the sample.
5. Remove the loading rack from the stage and retrieve your sample. You can leave the loading rack off of the microscope stage.
6. Unscrew and remove the lens from the microscope.
 - a. If using the 100X, clean the immersion oil using isopropanol and the lens wipes, be very gentle, do not scratch or damage the lens.
7. Replace lens back into lens container
8. Log off of FOM, this will turn off the instrument components