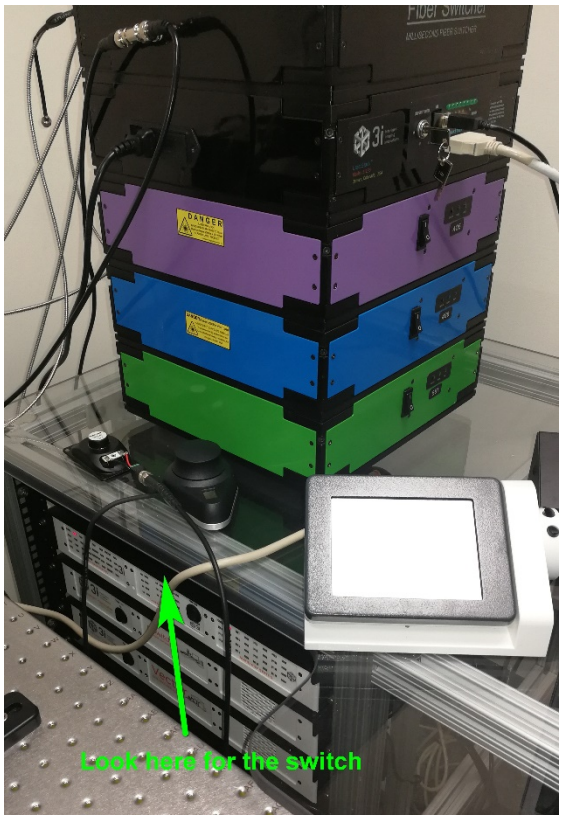
3i Marianas Spinning Disk Confocal Microscope – Quick Start

Please note: There is a context sensitive and very extensive help information available for the software. Press F1 key on the PC keyboard to get information about section that is currently open.

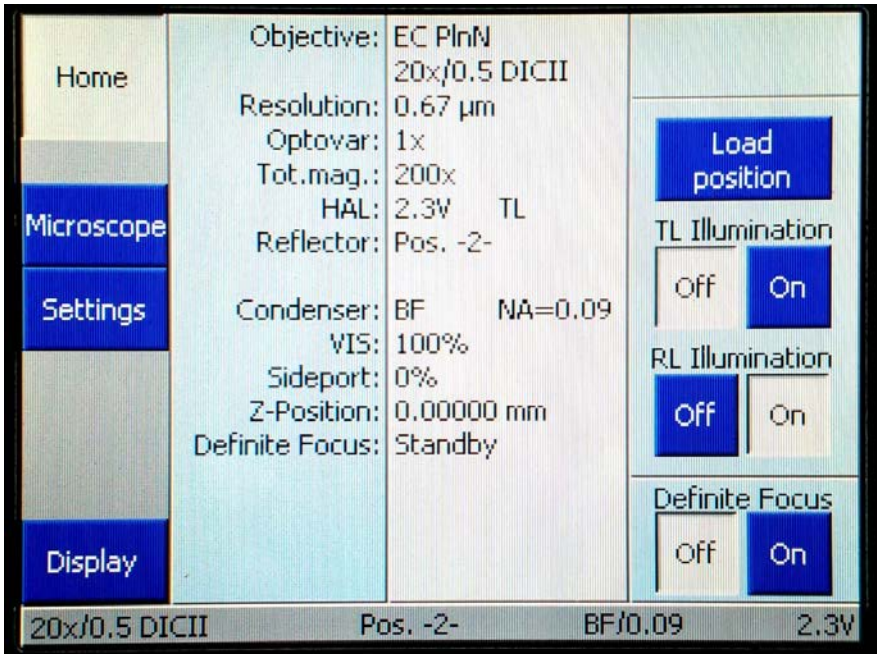
Turning the instrument ON

Important! There is only one ON/OFF switch for the whole system plus a key on laser stack and ON/OFF button on the PC. Please **DO NOT** touch any other switches or keys!

1. Locate the module called “G5 Rack Power Filter”. It is a flat, black box located directly under the surface on which the laser stack stands. There is an ON/OFF button in the middle of it. Press it for about one second – the little blue screen will lighten and the microscope components and the touchpad will initialize.



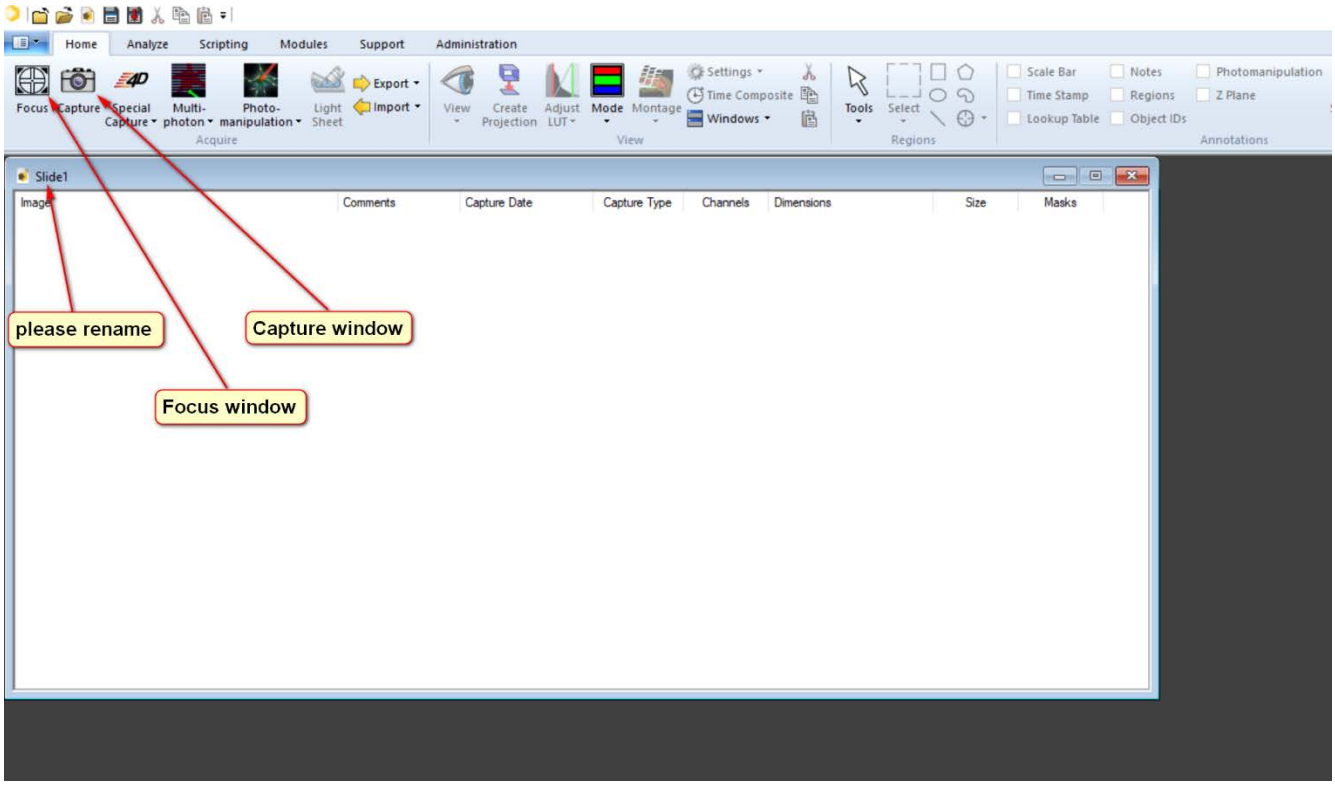
2. Watch the touchpad – there will be a progress bar for several activities. The last two are called “waiting for definite focus” and “starting application”. After the activities are complete (it may take about 30-40 seconds). The touchpad should look like this:



3. If the PC is off, turn it on and start SlideBook software (a yellow hexagon icon on a desktop). Wait until software loads completely and a window called “Slide 1” is displayed. This is a container into which all imaging data will be saved. Rename it (using “save as” command) according to your needs.

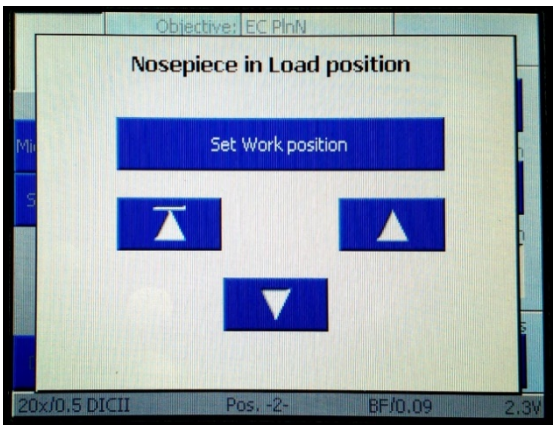
4. Switch the key in laser stack to ON position. You are ready to start imaging.

SlideBook window after starting the software.



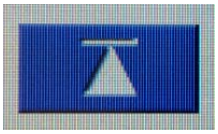
Loading the sample

Important! Use Zeiss touchpad for switching between objectives. Use “load position” button on the touchpad before mounting or removing your sample. Touchpad will look like this:



Important!

Press this button:

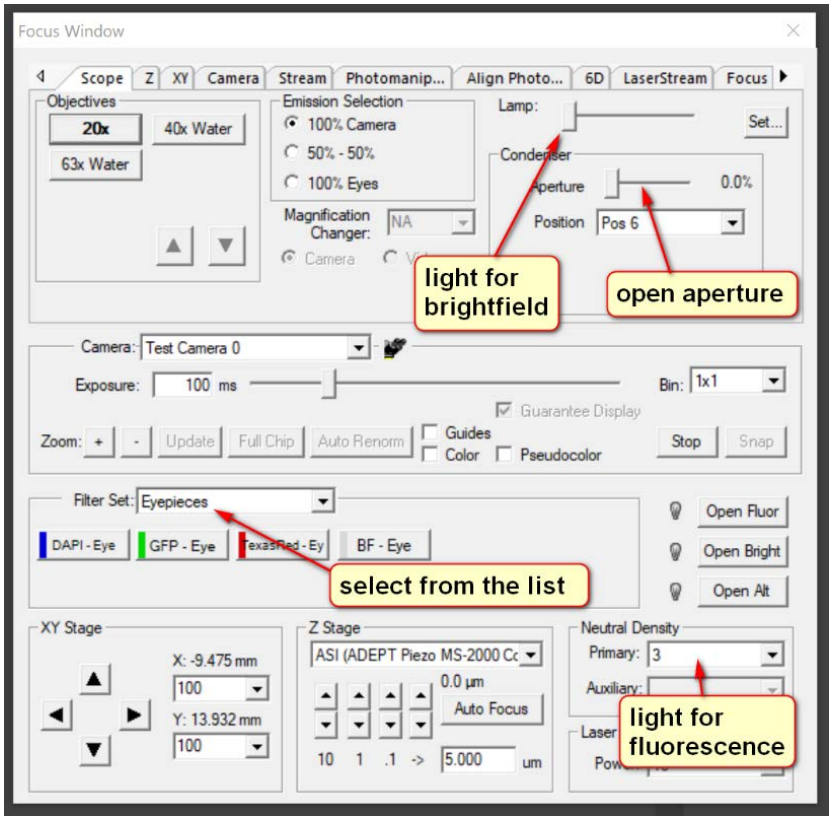


To get back to focus position

Before going confocal

Checking the specimen using the eyepieces

- In Focus window, select "Eyepieces" from the "Filter Set" list
- For **fluorescence imaging** – select an appropriate filter (DAPI, GFP or TxRed) and press "open fluor" button. Adjust Neutral Density if necessary (higher number = higher intensity)
- For **brightfield imaging** – select "BF-Eye" filter, press "open bright", open condenser aperture and increase light intensity with the slider (or with the regulator in the front part of the microscope body)

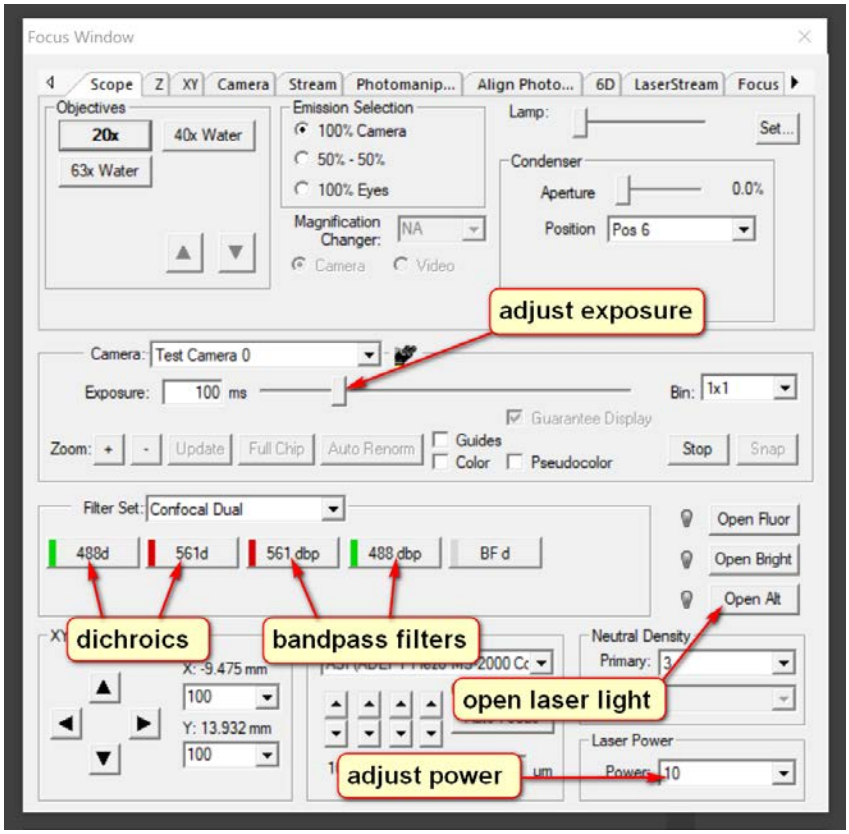


Using camera to view wide-field fluorescence or bright-field images.

- In Focus window, select "Epi" from the "Filter Set" list
- For **fluorescence imaging** – select an appropriate filter (DAPI, GFP or TxRed) and press "open fluor" button. Adjust Neutral Density if necessary (higher number = higher intensity)
- For **brightfield imaging** – select "BF-Eye" filter, press "open bright", open condenser aperture and increase light intensity with the slider (or with the regulator in the front part of the microscope body)
- Adjust Exposure

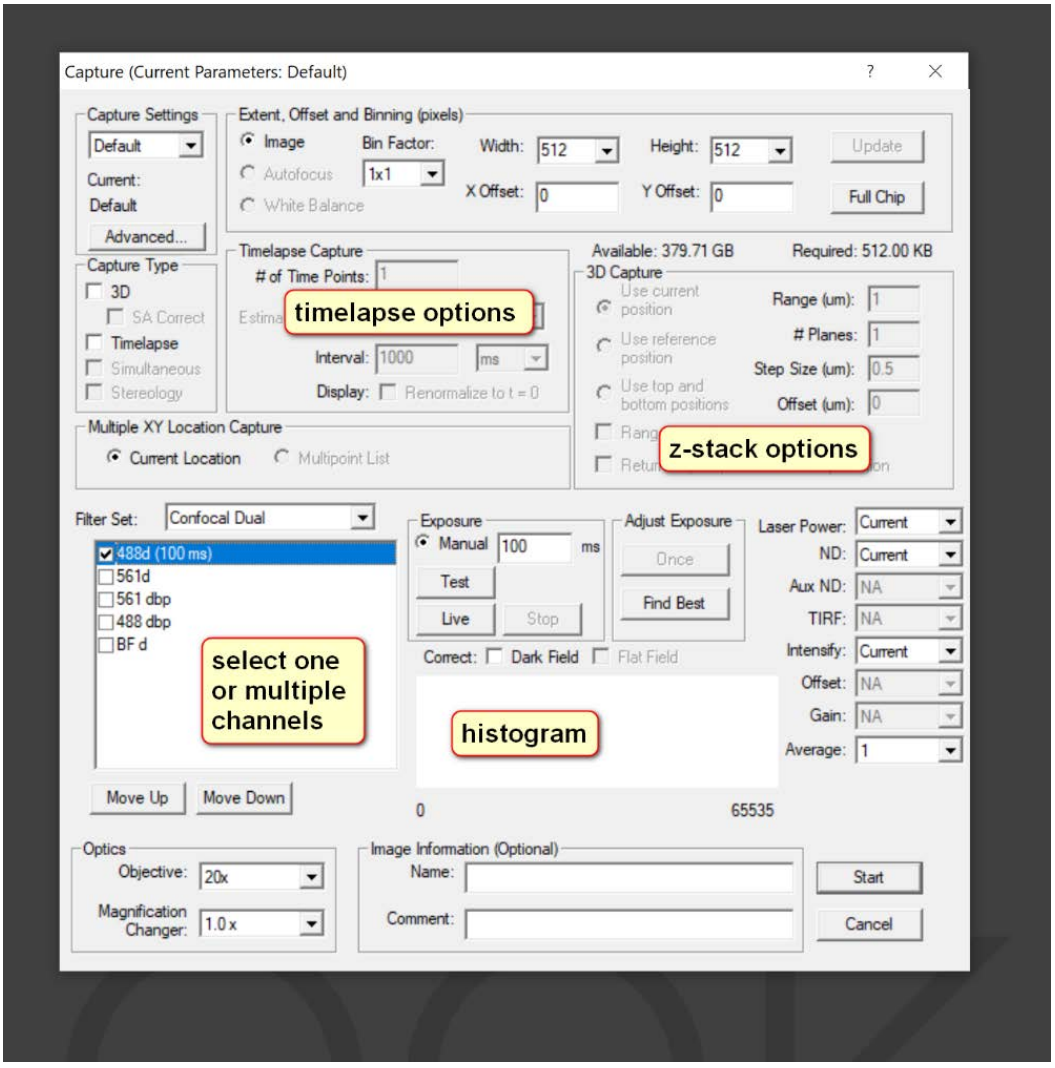
Setup for confocal imaging.

- Make sure that the key in the laser stack unit is switched to “ON”
- In Focus window select Camera tab
- Set Intensification to 100 (you can change it later)
- Select desired camera speed (1, 2 or 3; 3 is the fastest mode but the noisiest)
- From “Filter Set” list, select “Confocal Duo” (488nm and 561nm lasers available) or “Confocal Quad” (405nm, 488nm, and 561nm)
- Click on a button in order to selest a desired excitation wavelength – buttons labelled with “d” or “q” are assigned to dichroics, “dbp” and “qbp” are assigned to bandpass filters
- Click on “Open Alt” to enable laser illumination
- Adjust laser power



Capturing images

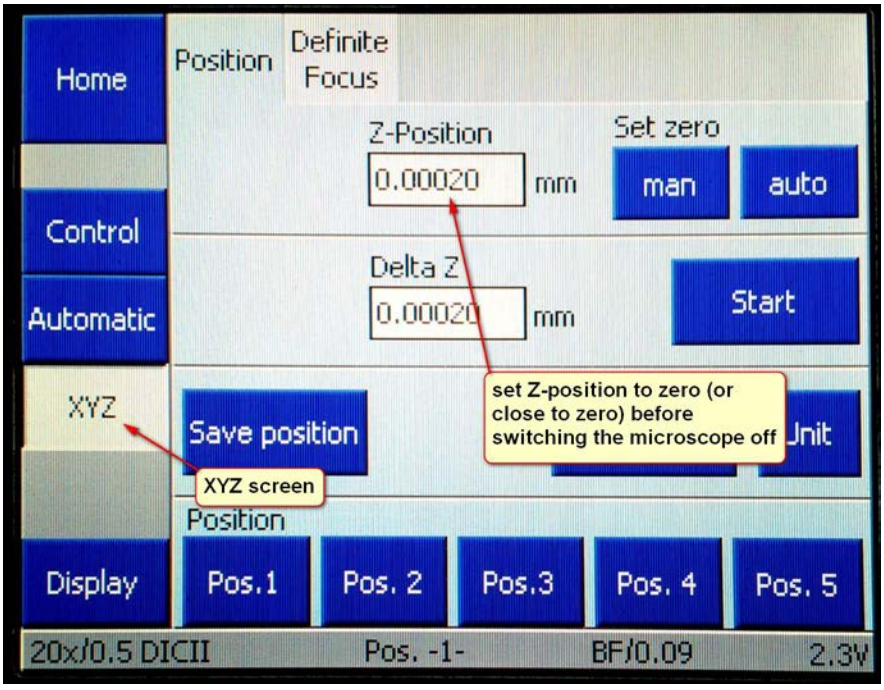
- Open “Capture” window
- Caution! Pressing “Enter” key at any time will start imaging
- Select Capture Type – 3D (pre-define the range in “Focus” window), Timelapse or Multiple XY Location (for positions or area pre-defined in “Focus” window
- Select apropriate Filter Set from the list
- Check box for one or more channels that will be included in the capture
- Select a channel to display or edit its settings - values can be left as “current” (pre-set in “Focus” window) or modified for each channel; press “Test” or “Live” to visualise the changes
- Enter name of the collection and comment. Each capture will be saved in the container that was named at the beginning.



Turning the system OFF

Important! Before swithing the microscope off, please make sure that:

1. The objectives are clean
2. Low magnification (20x) objective is selected
3. Focus is set to ZERO (or close to zero)



To turn OFF

1: Exit the software

2. Turn the key in laser stack to OFF position

3. Briefly press the ON/OFF button located in the middle of module called “G5 Rack Power Filter” (a flat, black box located directly under the surface on which the laser stack stands)