

# LabVIEW Lightsheet Guide

## Getting Started

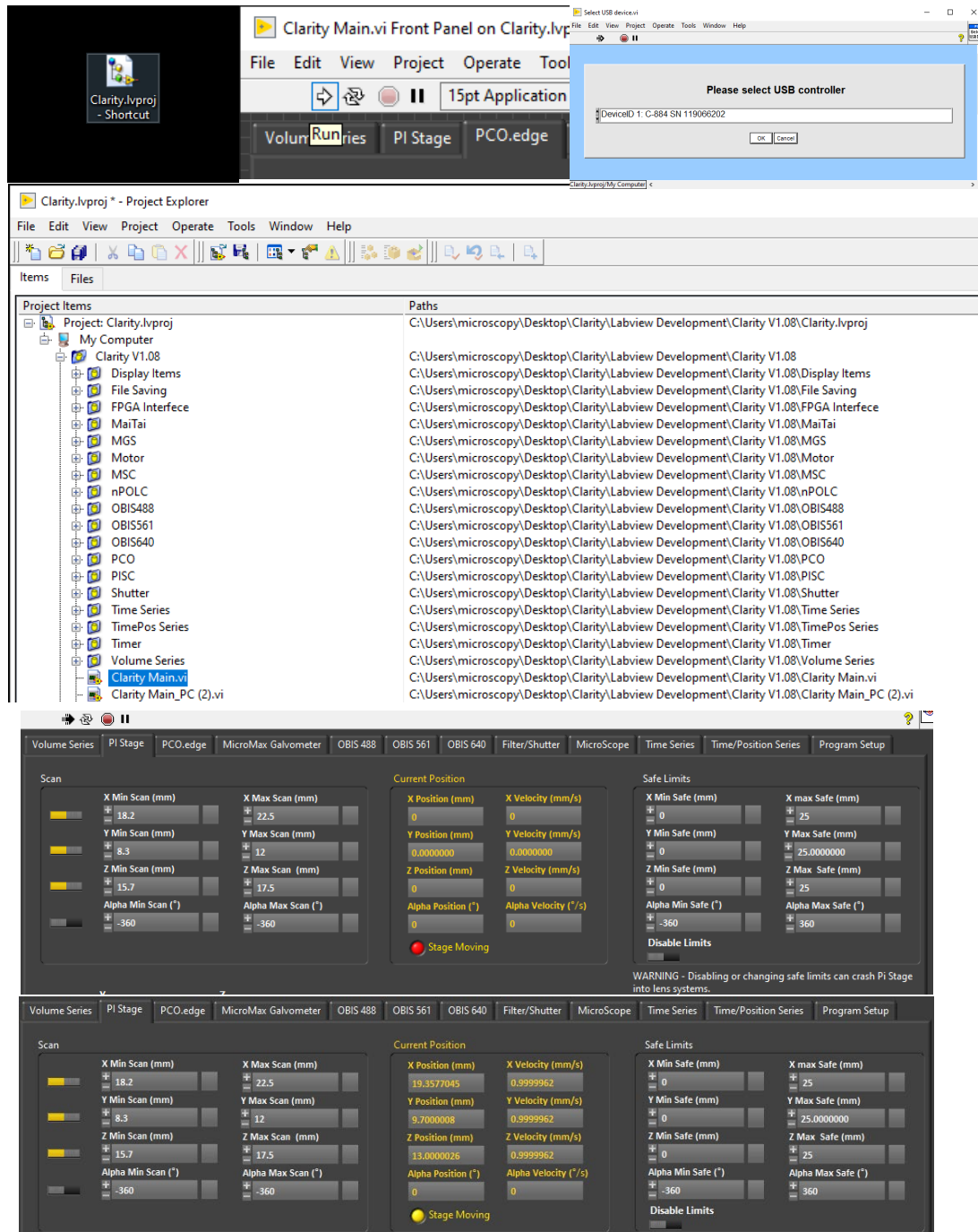
All peripherals must be turned on prior to the microscope PC

1. Switch on main power switches on the rear wall
2. Turn the camera, National Instruments PXIe power supply and Galvanometer power supply
3. To open the front panel of the microscope, pull outwards to disengage the top magnets, then lift upwards to remove cover



Queensland Brain Institute Microscopy

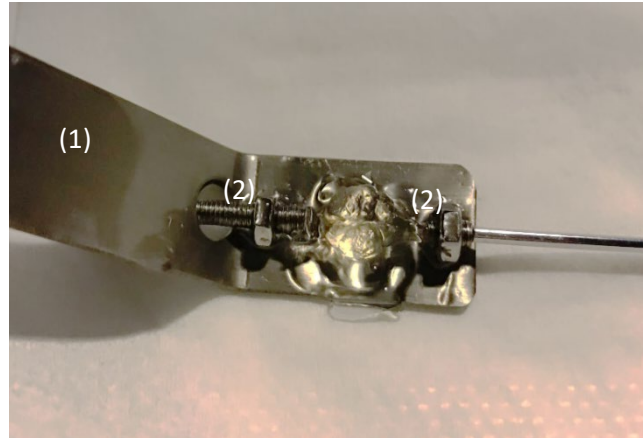
4. Turn on the microscope PC
5. Log into QBI Microscopy account
6. Run "Clarity.vlproj" on desktop with LabVIEW
7. Navigate to "Clarity Main.vi" and double-click to launch the program
8. Run the program with the small arrow in the top left corner
9. Press 'OK' to connect to the PI stage (C-884SN 119066202) when asked to select USB controller
10. Navigate to **PI Stage** tab. If stage readouts read zeros, stop the program, close and re-run the .vi file from the .lvproj in the Project Explorer



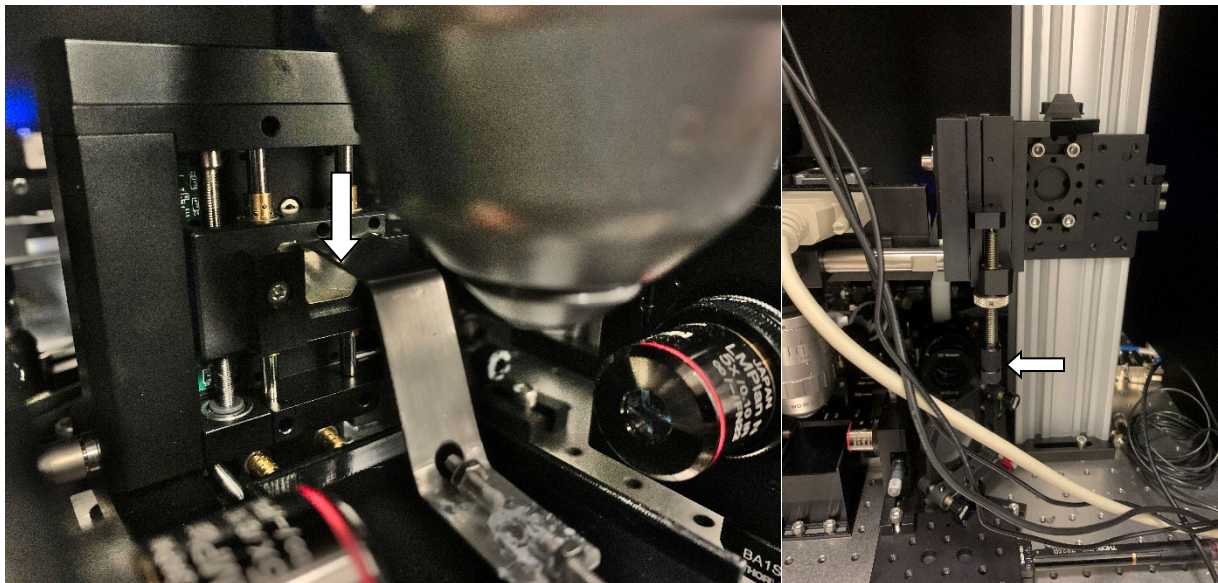
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## Mount the Sample

The sample is mounted on a metal platform (1), and can be held in place by two metal grub screws on either side (2). Samples are additionally secured by a UV-cured adhesive (Bondic) which can be applied both underneath the sample and to the two grub screws. Be sure not to apply adhesive anywhere that will obstruct the light sheet penetrating the sample where you wish to image.



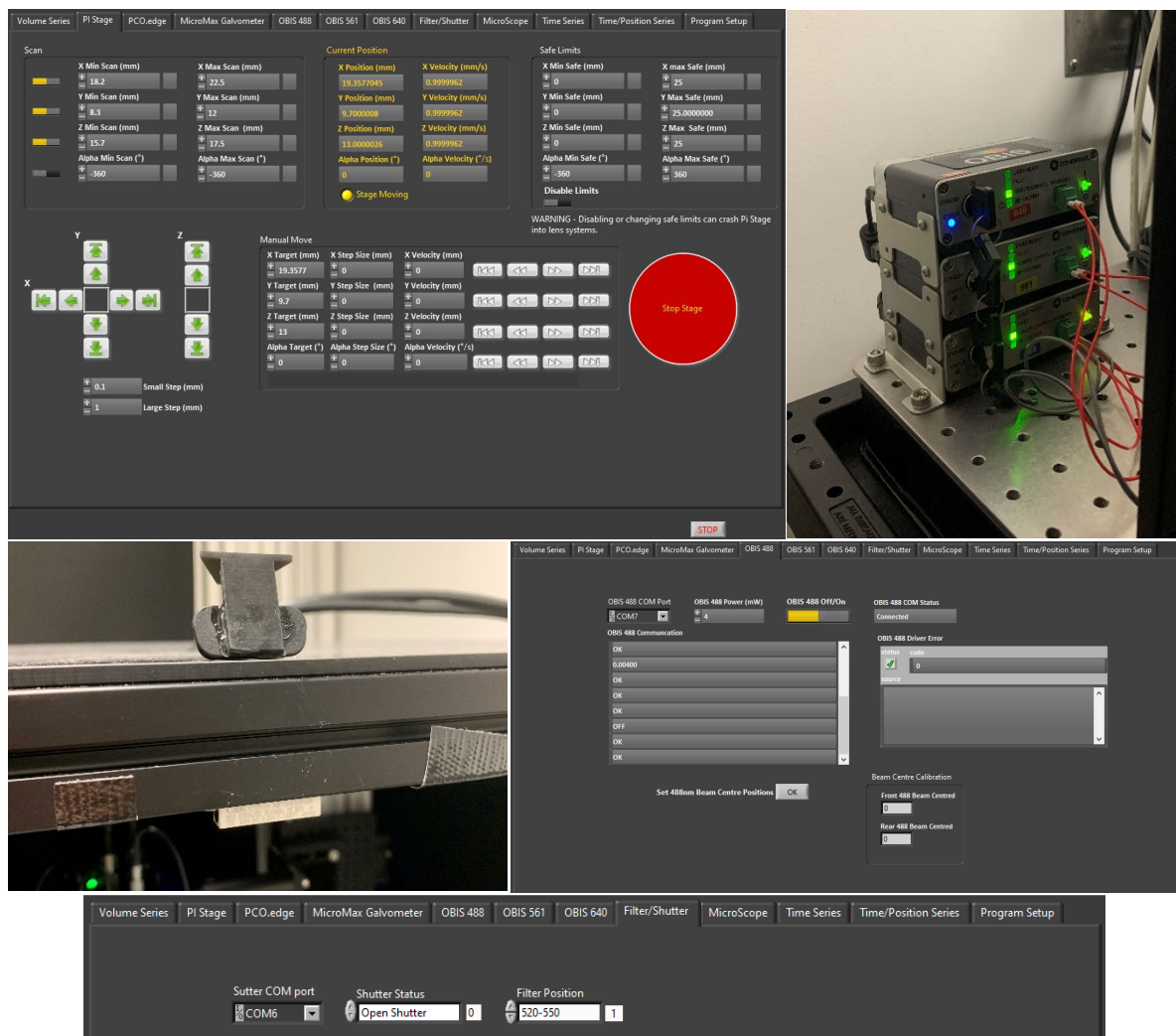
The metal platform is then submerged in the imaging chamber and then both are slid into position under the imaging objective. The metal platform can then be mounted to the X-Y-Z stage by hooking it into position as shown below (talk to QBI Microscopy about how to do this as it can be tricky). Once this is in place, lower the imaging objective into the imaging media with the screw closest to the large post.





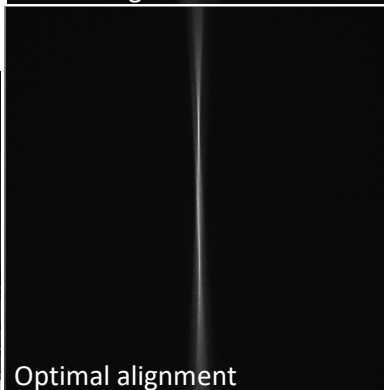
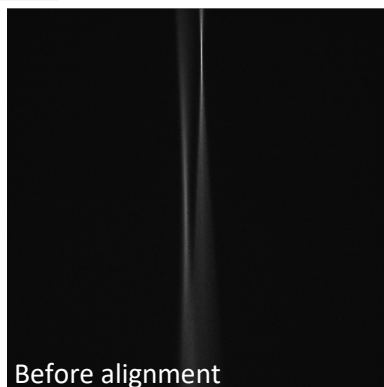
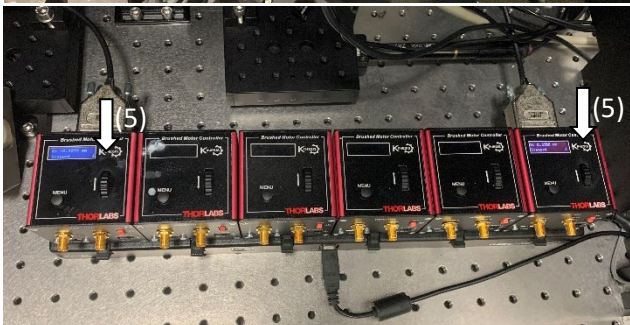
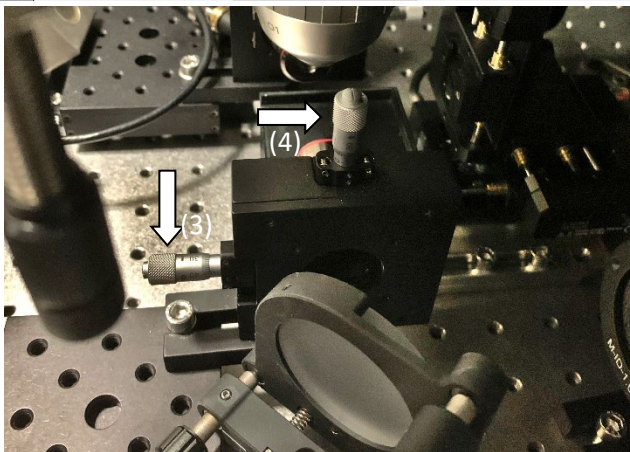
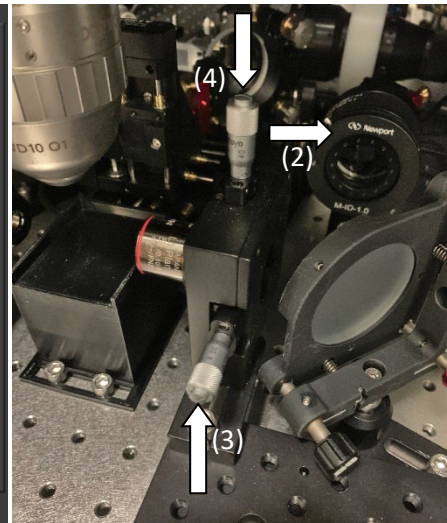
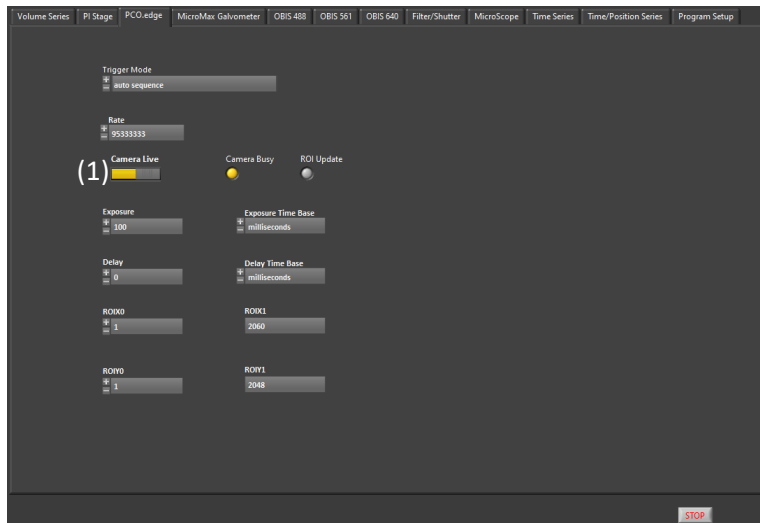
# Set up

1. Ensure the sample is lowered below the laser lightsheet path before turning on the lasers. This should be roughly ~12mm on the Z axis in the **PI Stage** tab (1). Press the UP arrows to move the sample down (UP arrows move the sample down through the lightsheet thus appearing as more superficial layers of the sample on the camera).
2. Engage the interlock to allow the lasers to be turned ON. Do this by placing the magnet against the top interlock (2). This should only be done after you have been trained how to safely align the beams and are authorized to do so. Ensure you have signed the corresponding QBI SOP122 and Risk Assessment.#18295 in the UQSafe database.
3. Toggle the laser interlock keys for each of the OBIS controllers on the back left of the airtable (3)
4. Turn each of the lasers **ON** then **OFF** again in the software under their respective **OBIS tabs** (4). This is important because once the interlock keys are turned ON, the lasers will turn on at their previously set power.
5. Turn **ON** one laser in the software to a low power and set the **Filter/Shutter** to the appropriate corresponding position.

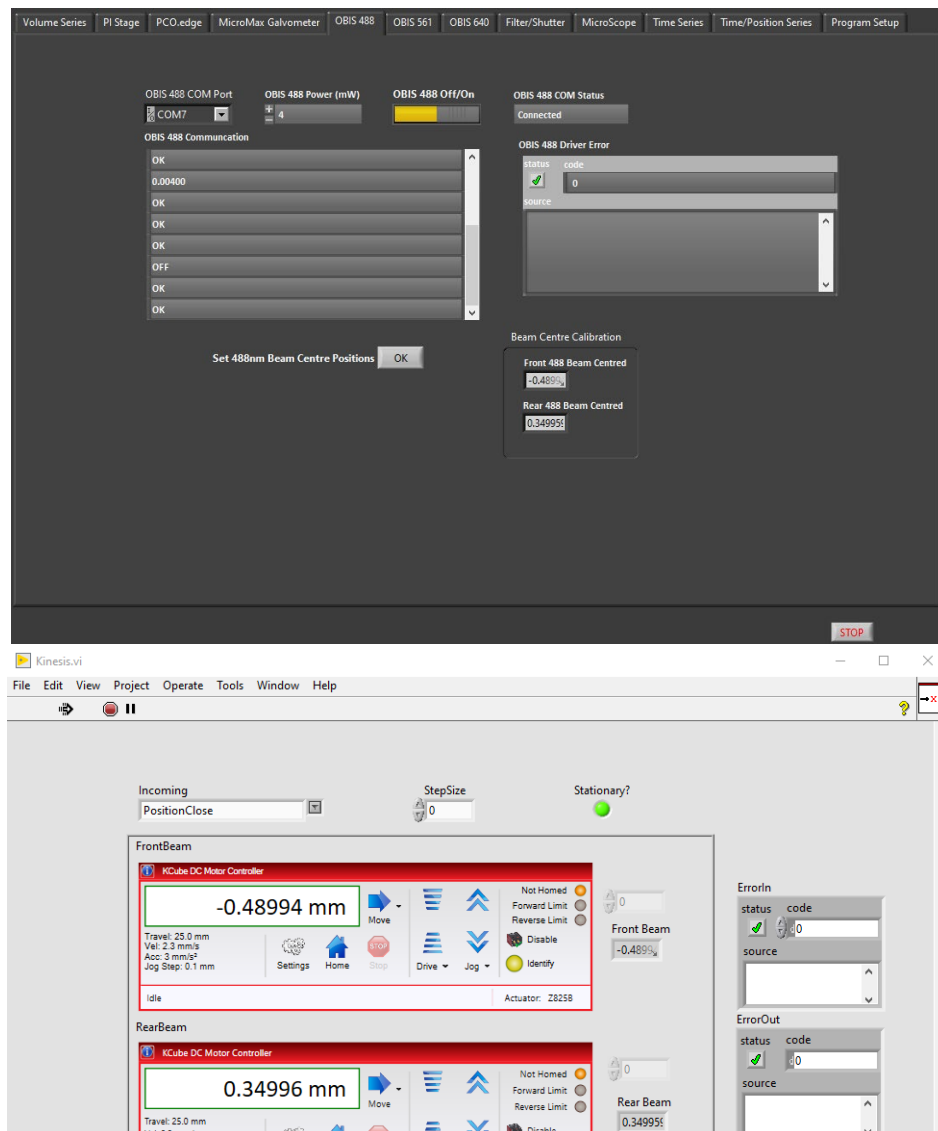


# Centering the beam

1. In the **PCO.edge** camera tab, **press start** to see a live image displayed (1)
2. Focus the imaging objective by turning the screw until the beam comes into sharp focus
3. Close one of the two beams with the iris (2) on the lightpath
4. Adjust the position of the beam to be centered in the X axis (3) and in sharp focus in the Z axis (4) using the screws shown below
5. Position the beam in the Y axis with the corresponding Kinesis Thorlabs Controller (5) so that the beam waist is centered about 1/3<sup>rd</sup> of the way along the Y-axis
6. Open the closed iris and repeat steps 3 and 4 for the second beam, positioning this beam 1/3<sup>rd</sup> of the way along the Y-axis from the other direction



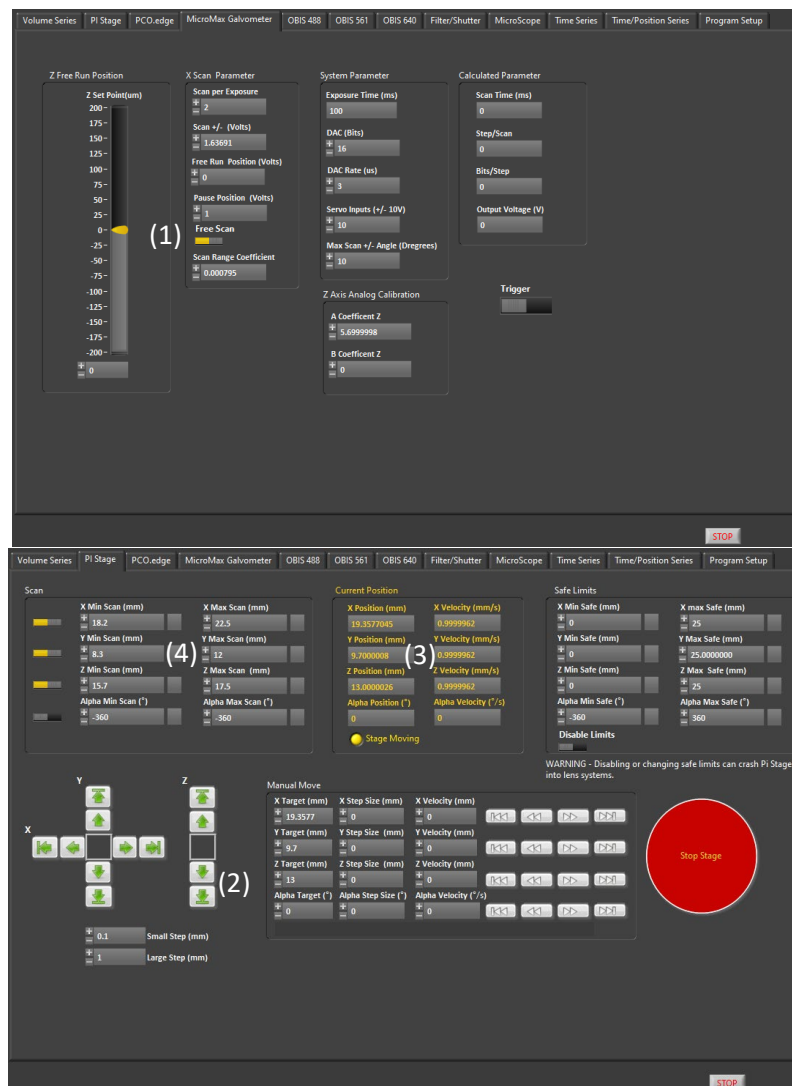
7. Once both beams are centered, press the “Set ???nm Beam Centre Position - OK” button in the **OBIS** tab and the Kinesis plugin will open and set the beam centers for that wavelength.
8. Each wavelength will focus through the illumination objective to a different point, so turn of the first laser and repeat steps 5-7 for the remaining wavelengths required



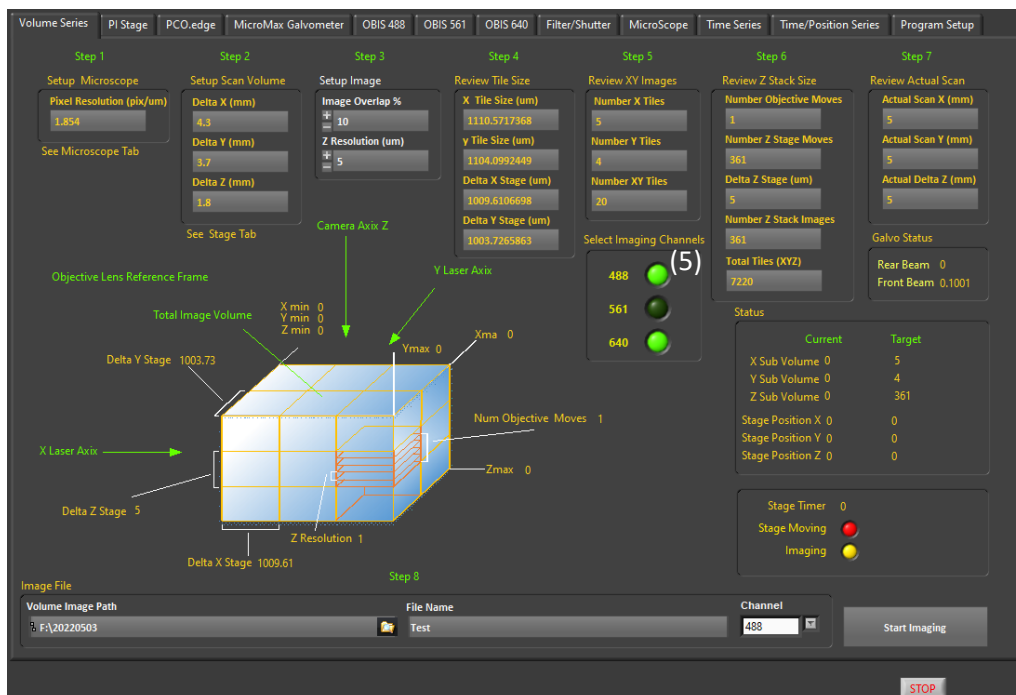
9. Remove the magnet from the interlock and replace the microscope Cover
10. Toggle the laser interlock keys for each of the OBIS controllers on the back left of the airtable again to reset these after the interlock was disengaged. Turn each of the lasers **ON** then **OFF** again in the software under their respective **OBIS tabs** as they will now be **ON**, despite what the software reads.

# Set up the acquisition parameters

1. Once the beams are centered, you can initiate the galvo mirrors to scan the beam and create one seamless 'sheet' of light. To do this, open the **MicroMax Galvometer** tab and toggle **ON** 'Free Scan' (1).
2. Turn on one of the lasers and filters in which you expect to see signal
3. Raise the sample into the lightsheet path using the Down arrows (2) and begin navigating to determine the extents of your sample in X, Y and Z. You can adjust the size of the small and large steps where indicated.
4. Find the Minimum and Maximum positions of the sample on the stage coordinates (3) and add these values to the Scan limits dialogue box (4).



5. Find part of your sample where you expect strong fluorescence and check the maximum signal in the live camera window. If needed, increase or decrease the laser power as required.
6. Check the signal intensity in each of the remaining channels required by turning on the correct laser and filter settings. Adjust the laser power as required.
7. If you cannot adjust the laser power to reach the desired signal strength, you can Stop the **Camera Live** and adjust the exposure time higher or lower as required (**DO NOT ADJUST THIS WHILE THE CAMERA IS LIVE**). This is a global parameter and will be applied to all channels so you may need to re-adjust the laser powers for any previously set channels.
8. Once the appropriate laser powers and exposure time is set, lower the sample below the lightsheet, turn **OFF** the **Camera Live** and any lasers that are currently **ON**.
9. Open the **Volume Series** tab, set your Tile Overlap (10% is default), Z Resolution (Z step size – 5um is default) and toggle ON the desired Imaging Channels (5).
10. Give your Images a File Path and File Name and press **Start Imaging**
11. After a few seconds the stage will move to the start of X, Y and Z Sub Volume 1 and begin imaging. No live images are displayed during acquisition but can be checked within your imaging File Path after each Sub Volume has been saved using ImageJ or similar.



12. Talk to QBI Microscopy about post-processing your data (shading correction, deconvolution, volume stitching)



## Shutting Down

1. Lower the sample to ~12mm
2. Open the microscope cover and raise the imaging objective with the focusing screw until it is higher than the top of the imaging chamber
3. Unhook the metal sample mount from the XYZ stage
4. Slide the sample chamber and sample out from below the imaging objective
5. Gently wipe any immersion media from the imaging objective with lens tissue (**do not use kim wipes to clean objectives**)
6. Press **STOP** in the bottom right of the Clarity Main.vi to stop the imaging program. You can then close this and the Clarity.lvproj

## WORKING WITH LIGHTSHEET MICROSCOPES



**Ergonomics: Use of mouse and keyboard / viewing computer screen** – Prolonged use of the microscope and microscope computer without breaks can increase the risk of muscular strain.



**Eye strain and fatigue** – Viewing samples through microscope eye piece or computer monitor over lengthy periods of time can result in eyestrain and headaches.



**Exposure to sharps** – Exposure to razor blades, scalpels, forceps, cover slips, glass slides could result in cuts or puncture wounds to hands or other areas of the body. Any microscope slide shards or glass debris must be disposed of in the appropriate sharps disposable bin in accordance with PC2 regulations.



**Exposure to intense fluorescent and laser light** – Lasers and a xenon light source are attached to this microscope and are the source of intense and potentially dangerous light. Under no circumstances should any optical elements be removed from the microscope light path or fail-safe switches be circumvented. Do not attempt to adjust the lasers, laser light path, or laser modules in any way. Avoid direct exposure to the light.

## Scope

This procedure details the method for using the microscopes equipped with laser light sources.

## Safety Considerations

### Personal Protective Equipment (PPE):

Laboratory coat, latex gloves and closed in shoes should be worn to prevent injury.

### Ergonomics and Risk Exposure:

Appropriate ergonomics, including adjustment of the seat, computer screen and microscope oculars should be undertaken to reduce risk of strain injuries.

### Emergency Procedures:

First aid may be required for:

**Exposure to sharps** – Contact the nearest first aid officer from the list that is beside all first aid kits and on safety notice board.

**Exposure to intense fluorescent and laser light** – Seek immediate medical assistance if you have been exposed to intense direct light or laser light.

In the event of a laser accident, do the following:

1. Shut down the laser system.
2. Provide for the safety of personnel (first aid, evacuation, etc). If needed, provide further medical assistance for Eye Injuries by:  
Proceed directly to: **Royal Brisbane and Women's Hospital at  
Cnr Butterfield St and Bowen Bridge Rd  
HERSTON, QUEENSLAND AUSTRALIA 4029  
(07) 3636 8222**

**Note:** If a laser eye injury is suspected, have the injured person keep still and looking straight up to restrict bleeding in the eye. Laser eye injuries should be evaluated by a physician as soon as possible.

3. Contact UQ Security Emergency on 336 5333.
4. Inform QBI's Laser Safety Officer, Rumelo Amor on 04 4907 8485, of the accident as soon as possible.
5. A UQ online incident report must be completed as soon as possible after the incident.

All incidents must be reported to the OH&S Manager and on UQs online incident reporting system.

**Contacts:** Security x53333 or OH&S Manager Ross Dixon 0401 673 654