SlideBook Spinning Disk Guide

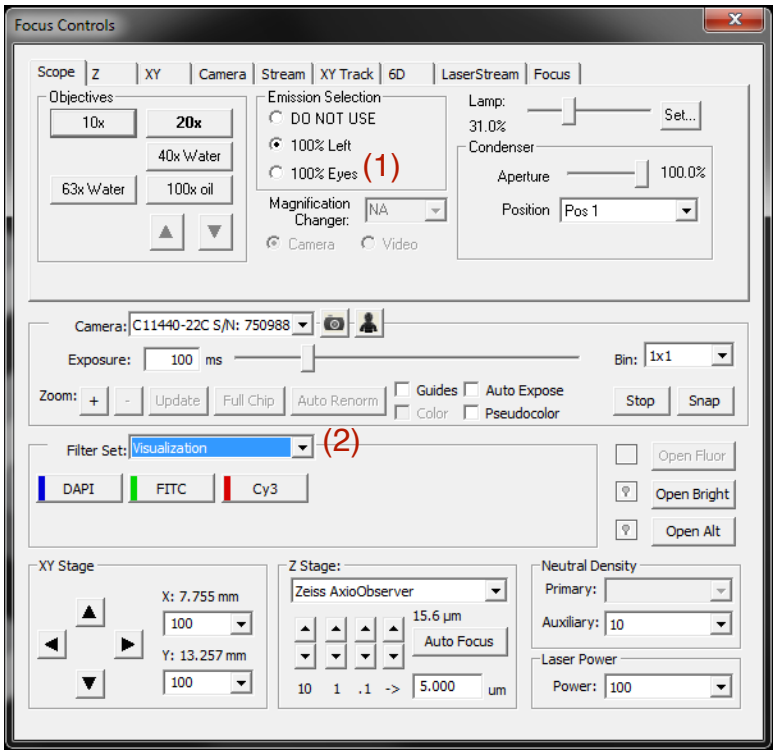
Getting Started

The microscope stand and all peripherals are powered through the main 3i power supply box (with the exception of the HBO lamp and environmental controls).

1. Switch on main power supply to start system (large white switch).
2. Switch on the lasers by turning the key in the laser stack off then on again.
3. If required switch on HBO lamp power supply. If switched on, leave on for at least 20mins.
4. Start the computer and log in to PPMS when prompted
5. Start SlideBook once the microscope has finished switching on.

Visualising a sample through the oculars

1. On the touch screen attached to the microscope press "**load position**" and position the slide on stage. Pressing the button will return you to the working position.
2. Press **microscope** on the far left of the touch screen to gain access to the microscope controls.
3. In the main SlideBook window press the **F** button on the main toolbar which will present the **Focus**



Controls window.

4. Under the **Scope** tab of SlideBook select **100% eyes** and select the **Visualization** from **Filter Set**. (1) (2)

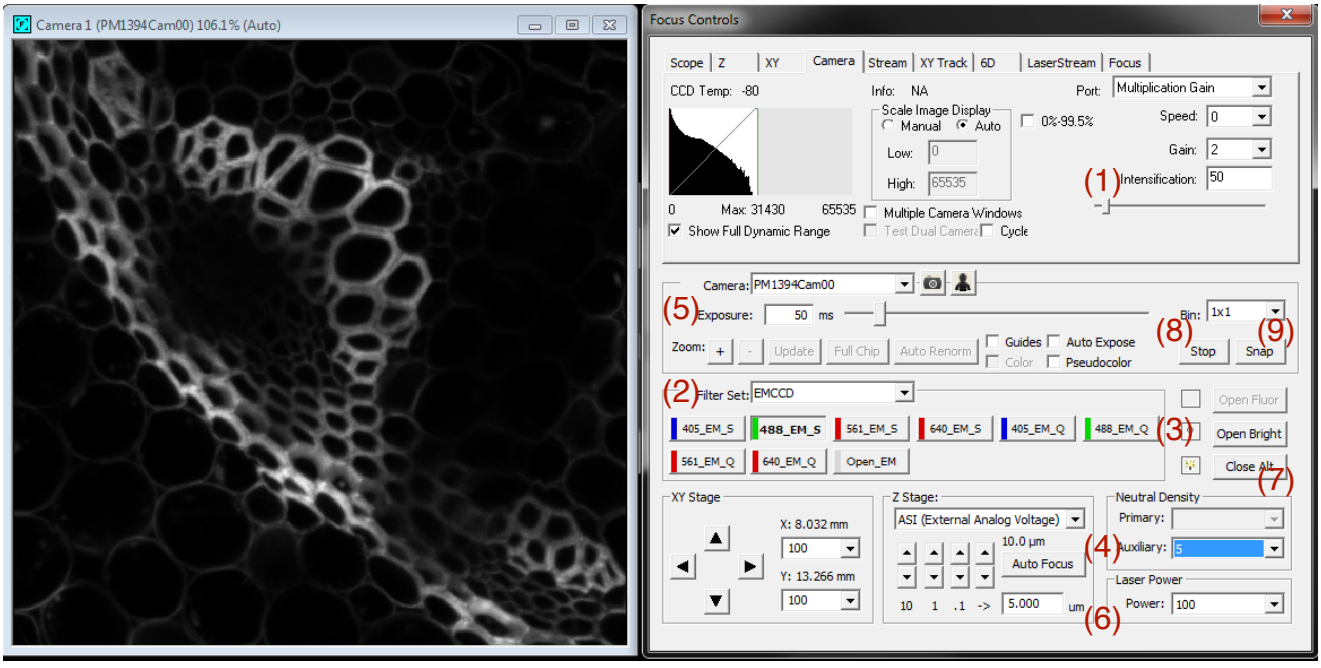
Capturing a confocal image

The microscope is equipped with two cameras: a Photometrics Evolve electron multiplying CCD (emCCD) and a Hamamatsu Flash4 scientific complementary metal-oxide semiconductor (sCMOS) camera. The emCCD is a high sensitivity, high-speed camera (with 512x512 format chip) which is useful for recording highly dynamic events. The 4Mpixel sCMOS (2048x2048, 6.5um pixels) camera provides a much larger field-of-view making it ideal for capturing large montage images at high-speed.

Capturing a confocal image - emCCD

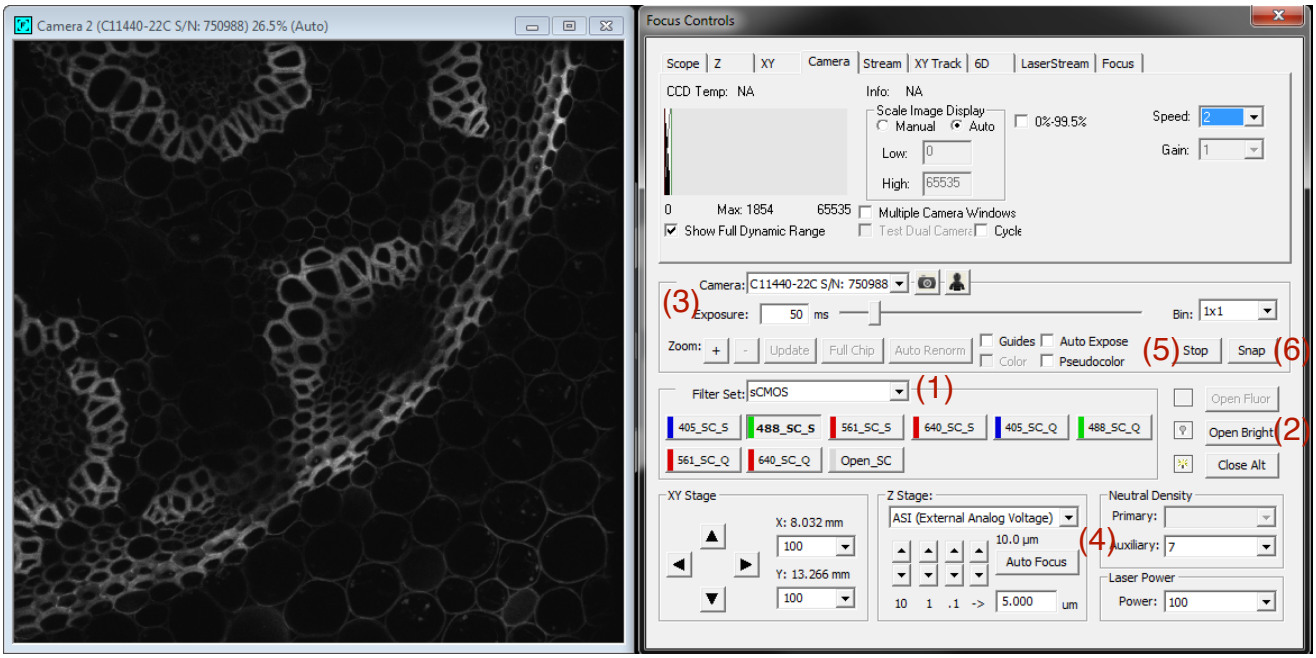
*The Evolve emCCD requires 10 minutes to cool down before being ready to image at best performance

1. In the **Focus Controls** window change the **Filter Set** to **EMCCD**.
2. Switch to the **Camera** tab to see the controls specific for the camera. Here you can set an appropriate value for **Intensification** (can only be set with the camera running live) and view the image histogram to aide parameter selection. Note that the **Port** should be set to **Multiplication Gain**. (1)
3. Choose an a configuration under **Filter Set** appropriate for the fluorophore on your sample. Each configuration is labelled according to which laser line is used for excitation and whether the single channel or quad-channel reflector cube is used in the microscope. For example **488_EM_S** uses the 488nm laser line in combination with the emCCD and the single pass reflector cube. (2)
4. You can use a transmitted light image to locate your sample using the **Open_EM** configuration and pushing **Open Bright**. (3)
5. Adjust **Auxillary** under **Neutral Density** to a value of **5** (see point 6 below for explanation). (4)
6. Set the **Exposure** time for the camera, set the **Laser Power** (represented as a percentage) and **Open Alt** to illuminate the sample. (5) (6) (7)
7. Start a live acquisition by pressing **Start**. (8)
8. The spinning disk always operates in confocal mode and there is no pin-hole to adjust. To acquire an optically sectioned image simple press **Snap**. (9)



Capturing a confocal image - sCMOS

1. In the **Focus Controls** window change the **Filter Set** to **sCMOS**.
2. Switch to the **Camera** tab to see the controls specific for the camera. Note that this camera does not have an intensification or gain control.
3. Choose an a configuration under **Filter Set** appropriate for the fluorophore on your sample. As with the EMCCD filter set, each configuration is labelled according to the laser line is used for excitation and whether the single channel or quad-channel reflector cube is used in the microscope. (1)
4. You can use a transmitted light image to locate your sample using the **Open_EM** configuration and pushing **Open Bright**. (2)
5. Set the **Exposure** time for the camera, set the **Laser Power** (represented as a percentage) and **Open Alt** to illuminate the sample. (3)
6. Usually, in conventional microscopes, the field-of-view of the camera is much smaller than the region being illuminated by the excitation light source. This is also that case with the spinning disk microscope except that an aperture is included in the light path to avoid bleaching regions not being imaged. Since the field-of-view is different for the two cameras this aperture needs to be adjusted according to which camera is used. For the sCMOS camera adjust **Auxillary** under **Neutral Density** to a value of **7**. (4)
7. Start a live acquisition by pressing **Start**. Note the increase in the field-of-view and the horizontal flip in camera orientation. (5)
8. To acquire an optically sectioned image simple press **Snap**. (6)

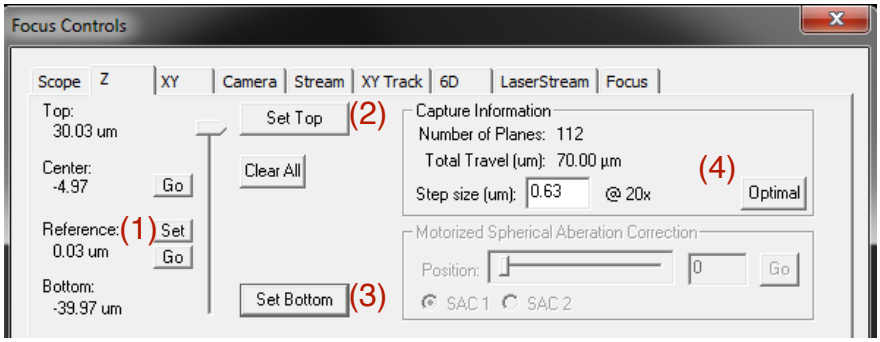


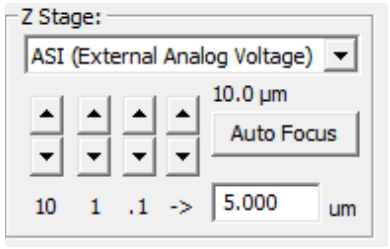
Configuring multi-dimensional acquisitions

In general the **Focus Controls** window is not used to acquire multi-dimensional data (the exception is streaming) but it is used to configure z-stacks, montage images and for making position lists.

Z-stacks

The microscope is equipped with two z-position control devices: the Zeiss AxioObserver Z-drive and an ASI piezo-device. Here we make use of the latter (the ASI device) to set the range over which our Z-stack will be recorded. This device has a limited range of 150um and can only be controlled through the software interface.





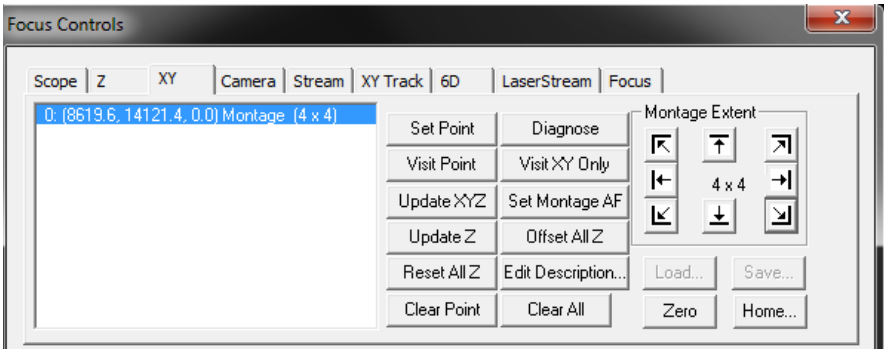
1. In the **Focus Controls** window select the **Z** tab.
2. Make sure that **ASI (External Analog Voltage)** is selected under the **Z Stage** panel.
3. Adjust the manual focus control of the microscope to roughly the centre of the Z-range being recorded. This becomes the zero point for the ASI device. Save this **Reference** by pushing the **Set** button. (1)
4. Use the buttons on the **Z Stage** panel to adjust the z-position to the extremes of the z-range being recorded and press the **Set Bottom** and **Set Top** buttons. (2) (3)
5. The range of z-positions is now saved ready to be used in an image capture.
6. The number of planes is assigned by set the **Step size**. Use the **Optimal** to achieve Nyquist sampling for your chosen objective. (4)

Montage images (or mosiacs) and points lists

In SlideBook mosiac images are known as montage images. Again the **Focus Controls** window does not allow a montage to be acquired but allows the parameters to be set ready for an image capture.

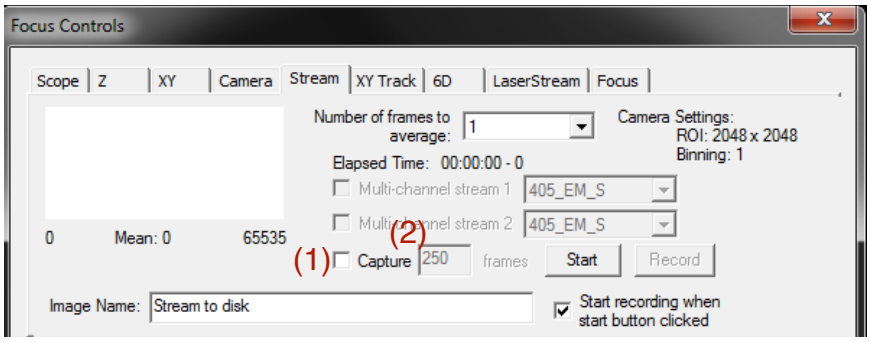
1. In the **Focus Controls** window select the **XY** tab.
2. Adjust the XY stage to a region of interest.
3. Add this position to the list using the **Set Point** button. When multiple points exist in the list, they can be revisited by highlighting the desired point and pressing the **Visit Point** button.
4. Any point can be updated by highlighting the point in the list and using the **Update XYZ** or **Update Z** button.
5. A description of the region of interest can be added by pressing **Edit Description** and individual points or all points can be removed using the **Clear Point** and **Clear All** buttons respectively.

6. To create a montage for any of the points in the list adjust the XY stage position and use the buttons in the **Montage Extent** panel to define the region of interest. For example, adjust the XY stage position and use the ⇱ button to mark the top left-hand corner of the region-of-interest. To mark the bottom right hand corner of the region use the ⇲ button.



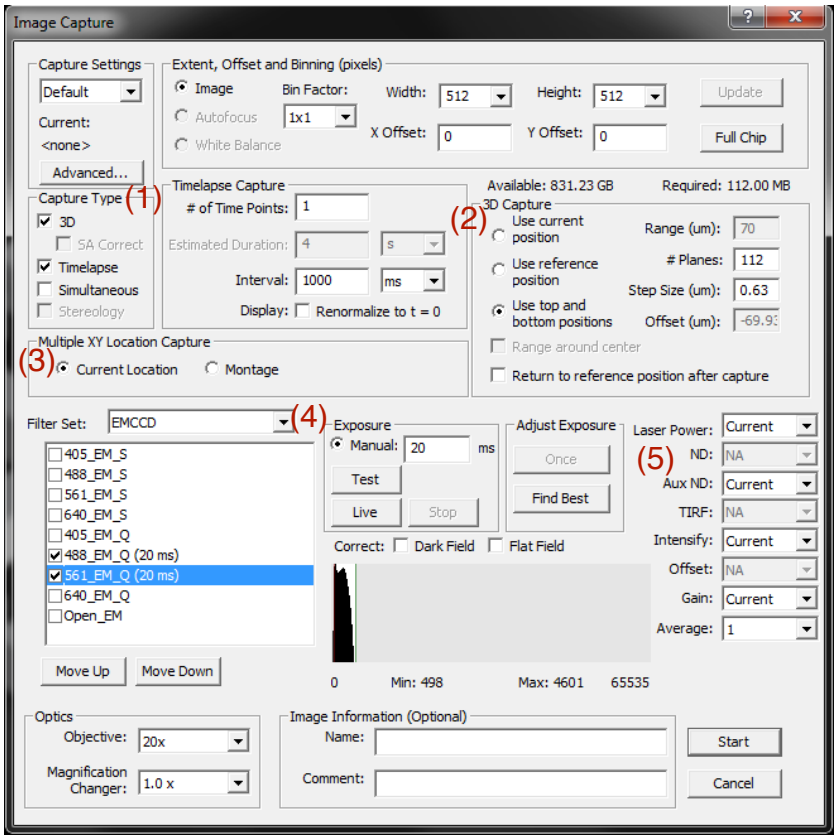
Streaming images to disk

To obtain the highest acquisition speeds, images can be streamed from the cameras directly to the hard disk drive. This is done using the **Stream** tab of the **Focus Controls** window. Simply check **Capture (1)**, set the number of frames to be acquired **(2)** and press the **Start** button.



Multi-dimensional acquisition using Image Capture

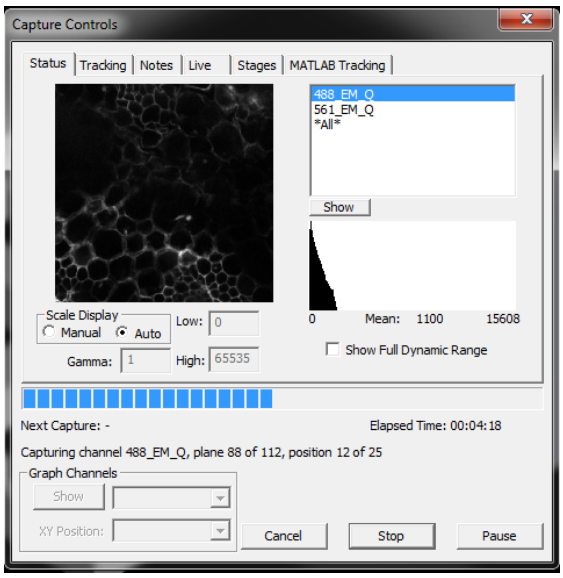
All multi-dimensional acquisitions are recorded using the **Image Capture** window. Push the **C** button to make the window active after setting up any required **XYZ** parameters in the **Focus Controls** window.

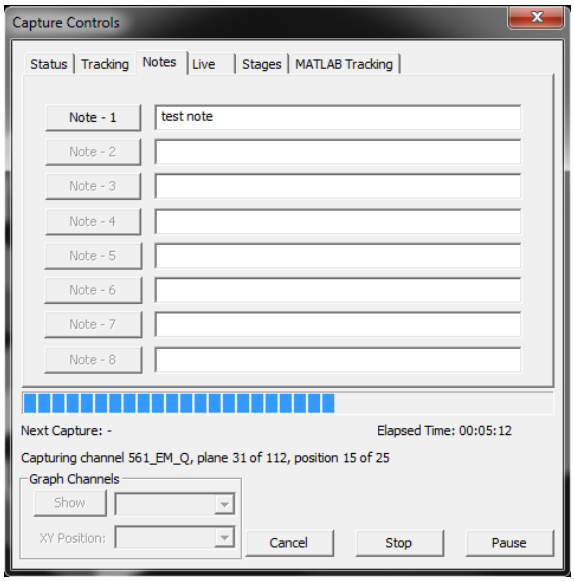


1. First set the **Capture Type**. Choose from **3D**, **Timelapse** or **Simultaneous**. (1)
2. When using **3D**, the z-range for the stack can be configured under the **3D Capture** panel. If using parameters that were set in the Focus Controls select the **Use top and bottom positions** radio button. Alternatively, select the **Use current position** radio button to use the current z-position as the center of

the stack and set any two out of **Range**, **# Planes** and **Step Size (um)** the parameter not set will be calculated from the other two. Similarly it is also possible to **Use reference position** that was set in the Z tab of the Focus Controls. (2)

3. Select either **Current Location** or **Montage** in the **Multiple XY Location Capture** panel to acquire data at the current XY position or at positions saved the list under the XY tab of the Focus Controls window. (3)
4. Select an appropriate **Filter Set** for the acquisition and select one or more configurations for a multi-channel acquisition. Highlight each channel in turn and set an exposure time. The exposure time can be checked using **Test** to capture a single frame or **Live** to start a live capture for the selected camera. (4)
5. The laser and camera parameters can either be left as **Current** to use the parameters set in the Focus Controls window or values can be individually set. (5)
6. Begin the acquisition using the **Start** button.

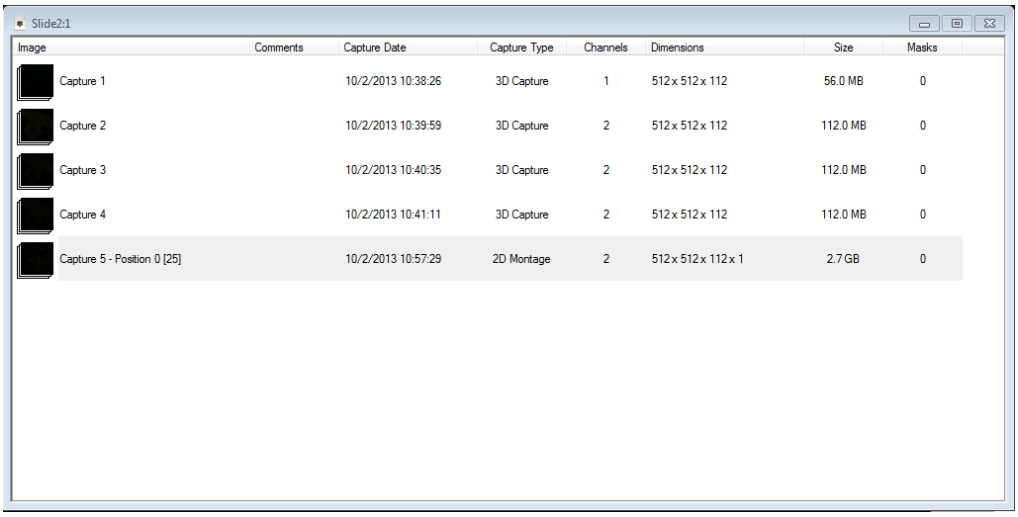




7. The **Status** tab of the **Capture Controls** window shows the progress of the multi-dimensional acquisition. The **Notes** tab can be used to mark events during the acquisition.

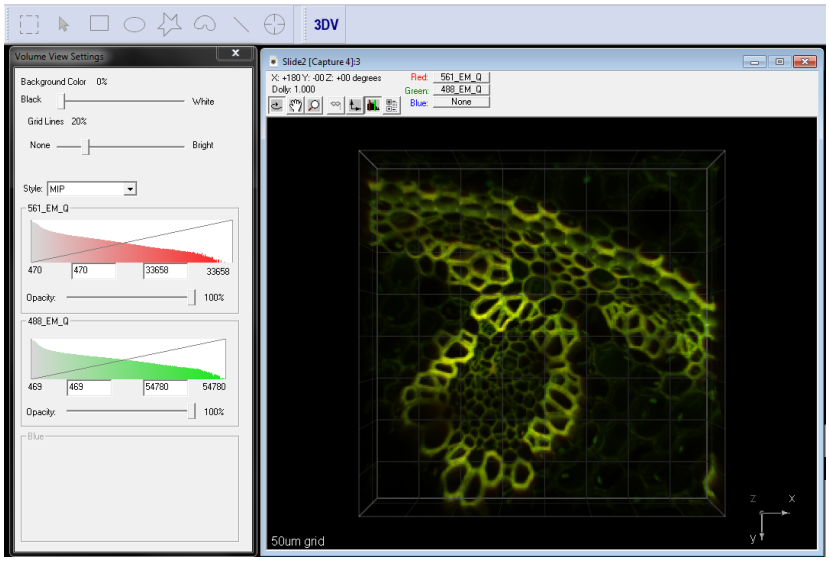
Exploring acquired data

SlideBook stores data in the *.sld file format. As data is acquired a container called a Slide is populated. Before starting an Image Capture you will be prompted to save your slide. When saved, all data contained within the slide is saved to the same file. When the data in a slide reaches 2Gb the user will be prompted to start a new slide.



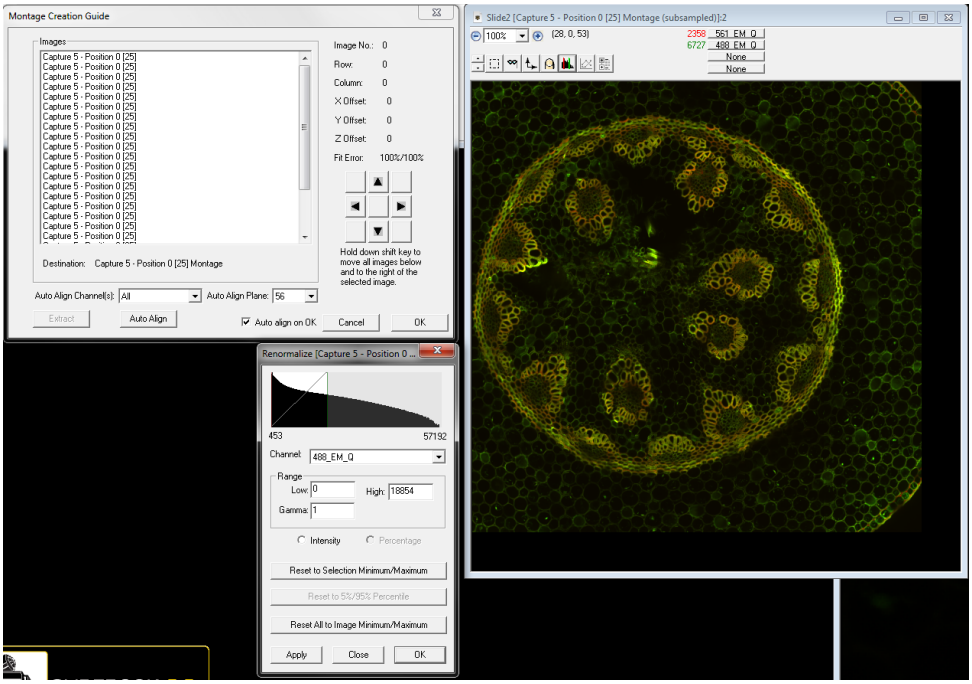
Viewing 3D data

Z-stacks can be visualised by pressing the **3DV** button on the main toolbar. This launches a two windows: the **Volume View Settings** window and a rendering of the 3D volume. The **Volume View Settings** window allows the display contrast to be adjusted for each channel and the **Style** of the rendering can be changed between maximum intensity projection (**MIP**) and several others...



Stitching montage data

To stitch montage data with one or more z-planes choose *Image* --> *Generate Montage* ...



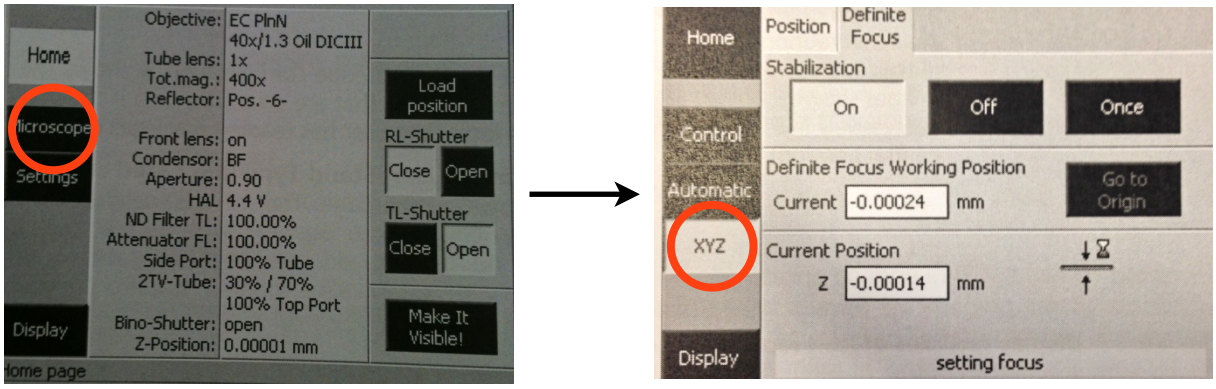
Use the **Montage Creation Guide** to generate a stitched, merged montage. Select the z-plane to be used to calculate the alignment and push the **Auto Align** button at the bottom of the window. Use the data viewing window to inspect the result but note that the displayed image is subsampled to improve the responsiveness of the display. **Auto Align** can be used repeatedly to attempt to improve the alignment. If the calculation fails the XY position of the individual tiles can be manually adjusted using the arrow buttons in the **Montage Creation Guide**. Press **OK** to accept the alignment. The new, full resolution, montage image generated in the slide data container can be displayed and inspected to check the quality of the stitching.

Using Definite Focus

The definite focus module allows for consistent focus regardless of changes in environmental conditions. The module operates by detecting the coverslip and keeping the focus constant relative to the position of the coverslip.

To use definite focus:

1. Activate definite focus on the microscope touch screen - via the XYZ tab.
2. Once activated definite focus works in the background - you do not need to set any additional autofocusing settings.
3. Once active, definite focus can be controlled in SlideBook via the focus tab.



Definite focus allows different offsets to be used in time-lapse experiments using multi-positions. To enable this feature use **advanced features** in the **Image Capture** window. If using different offsets for multi-positions click “compute offsets”.

6D Experiments

SlideBook allows highly complex image acquisition experiments - these include parallel experiments on the same sample/ mulit-well dish using different lasers or different acquisition settings.

Please contact the facility staff if you are interested in setting up a 6D experiment.

Exporting data

Currently LOCI Bioformats does support the SlideBook *.sld format. To view data in ImageJ or Imaris acquired data will need to be exported to tiff.

Shutting Down

1. Lower the stage and remove your sample.
2. *Gently wipe* any oil objectives you have used with *lens tissue* (**do not use kim wipes to clean objectives**).
3. Exit the software and copy your files to your *home or group network/USB drive.*
4. Turn off the microscope power supply box.

WORKING WITH CONFOCAL AND TIRF 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 shapes 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