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

Focus Controls	×
Scope Z XY Camera Stream XY Track 6D LaserStream Objectives Emission Selection C D0 NOT USE 31.0% 40x Water 100% clft C 100% Left 31.0% 63x Water 100x oil Magnification NA Pos Camera C Video Video Pos	Focus
Camera: C11440-22C S/N: 750988 💌 - 🚳 - 👗	
Exposure: 100 ms	Bin: 1x1 -
Zoom: + - Update Full Chip Auto Renorm Guides Auto R	ecolor Stop Snap
Filter Set: Visualization	Open Fluor
DAPI FITC Cy3	P Open Bright
	🔋 Open Alt
XY Stage X: 7.755 mm 100 ▼ Y: 13.257 mm 100 ▼ 100 ♥ 100 ♥	Neutral Density Primary: J Auxiliary: 10 Laser Power Power: 100

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)

Scope Z XY Camera Stream XY Track 60 LaserStream Focus CCD Temp:	Camera 1 (PM1394Cam00) 106.1% (Auto)	Focus Controls
▼ 100 ▼ 10 1 .1 -> 5.000 um (6) Power: 100 ▼ (6)		Scope Z XY Camera Stream XY Track 6D LaserStream Focus CCD Temp: -80 Info: NA Port: Multiplication Gain Image CD Temp: -80 Info: NA Port: Multiplication Gain Image CD Temp: -80 Info: NA Port: Multiplication Gain Image Camera: Max: 31430 65535 Multiple Camera O%: 93.5% Speed: Image 0 Max: 31430 65535 Multiple Camera Vindows Image Image

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)

🖸 Camera 2 (C11440-22C S/N: 750988) 26.5% (Auto)	Focus Controls
	Scope Z XY Camera Stream XY Track 6D LaserStream Focus CCD Temp: NA Info: NA Scale Image Display C Auto 0%-99.5% Speed: 2 - Unio: 0 High: 65535 Gain: 1 - 0 Max: 1854 65535 Multiple Camera Windows Image Test Dual Camera Cycle -
	Camera: C11440-22C S/N: 750988 • Image: Constraint of the sector of
	561_SC_Q 640_SC_Q Open_SC W Close Alt XY Stage Asia Asia Neutral Density Asia 100 Asia Asia Auto Focus Y: 13.266 mm 100 10 1.1 -> 5.000 um Image: Note that the state of t

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.

Focus Controls		
Scope Z XY Camera Stream X Top: 30.03 um Set Top (2 Center: Clear All	Track 6D LaserStream Focus Capture Information Number of Planes: 112 Total Travel (um): 70.00 μm	Z Stage: ASI (External Analog Voltage)
-4.97 <u>Go</u> Reference: (1) <u>Set</u> 0.03 um <u>Go</u> Bottom: -39.97 um <u>Set Bottom</u> (3	Step size (um): 0.63 @ 20x Optimal Motorized Spherical Aberation Correction	▼ ▼ ▼ ▼ Auto Pocus 10 1 .1 -> 5.000 um

- 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 state 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 state button.

Focus Controls				
Scope Z XY Camera Stream XY 0: (8619.6, 14121.4, 0.0) Montage (4 x 4)	Track 6D Set Point Visit Point Update XYZ Update Z Reset All Z Clear Point	LaserStream Foo Diagnose Visit XY Only Set Montage AF Offset All Z Edit Description Clear All	Montage Exten	₹ → × ave
1				

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.

ocus Controls Scope Z XY Camera	Stream XY Track 6D LaserStream Focus
	Number of frames to average: Elapsed Time: 00:00:00 - 0 Multi-channel stream 1 405_EM_S
0 Mean: 0 655	35 Multi (2) nel stream 2 405_EM_S (1) Capture 250 frames Start Record
Image Name: Stream to disk	Start recording when start button clicked

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.

Image Capture		
Capture Settings	Extent, Offset and Binning (pixels)	
Default 💌	Image Bin Factor: Width: 512 ▼ Height: 512 ▼ Update	
Current:	C Autofocus Ix1	
<none></none>	C White Balance X Offset: 0 Y Offset: 0 Full Chip	
Advanced	- Timelance Capture - Available: 821 32 CP - Beguired: 112 00 MP	-
Capture Type	# of Time Points: 1	_
I I SD Convert	Echicacido unchicacida (2) Use current Range (um): 70	
Timelance	Use reference # Planes: 112	
Simultaneous	Interval: 1000 ms position Step Size (um): 0.63	
Stereology	Display: Renormalize to t = 0	
Multiple XY Location	Capture	
(3) Current locat	ion O Montage	
(-) Currence been	Return to reference position after capture	
Filter Sets		_
Fliter Set: JEMCCD	Adjust Exposure Laser Power: Current	-
405_EM_S		-
561_EM_S	Test Aux ND: Current	-
640_EM_S	Live Stop TIRF: NA	-
405_EM_Q	Correct: Dark Field Flat Field Intensify: Current	.
✓ 488_EM_Q (20	ns) Offset: NA	Ţ
✓ 561_EM_Q (20	ns)	=
Open_EM	Gain. Jourrent	<u>-</u> -
	Average: 1	-
Move Up Mo	ve Down	
- Interep	0 Min: 498 Max: 4601 65535	
Optics	Image Information (Optional)	
Objective: 20	x Name: Start	
Magnification Changer: 1.0	Vx Comment: Cancel	

- 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)

- 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 multichannel 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.

Capture Controls	Capture Controls
Status Tracking Notes Live Stages MATLAB Tracking	Status Tracking Notes Live Stages MATLAB Tracking
488 EM Q 561 EM Q *Al*	Note - 1 test note
	Note - 2
	Note - 3
Show	Note - 4
	Note - 5
	Note - 6
Scale Display Low: 0 0 Mean: 1100 15608	Note - 7
Gamma: 1 High: 65535 Show Full Dynamic Range	Note - 8
Next Capture: - Elapsed Time: 00:04:18	Next Capture: - Elapsed Time: 00:05:12
Capturing channel 488_EM_Q, plane 88 of 112, position 12 of 25	Capturing channel 561_EM_Q, plane 31 of 112, position 15 of 25
- Graph Channels	Graph Channels
Show V	Show
XY Position: <u> Y</u> Cancel Stop Pause	XY Position: Cancel Stop Pause

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.

Slide2:1								×
Image	Comments	Capture Date	Capture Type	Channels	Dimensions	Size	Masks	
Capture 1		10/2/2013 10:38:26	3D Capture	1	512 x 512 x 112	56.0 MB	0	
Capture 2		10/2/2013 10:39:59	3D Capture	2	512 x 512 x 112	112.0 MB	0	
Capture 3		10/2/2013 10:40:35	3D Capture	2	512 x 512 x 112	112.0 MB	0	
Capture 4		10/2/2013 10:41:11	3D Capture	2	512 x 512 x 112	112.0 MB	0	
Capture 5 - Position 0 [25]		10/2/2013 10:57:29	2D Montage	2	512x512x112x1	2.7 GB	0	

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. <u>Gently wipe</u> any oil objectives you have used with <u>lens tissue</u> (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.

QUEENSLAND BRAIN INSTITUTE – STANDARD OPERATING PROCEDURE

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 <u>Eye Injuries</u> 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