Quick Guide to the 3i Confocal Microscope

The following is a quick guide for using the 3i. This assumes the user has already been trained on the 3i and only needs a reminder of the steps for using it.

Please consult the Slidebook help (press F1 on the PC keyboard with Slidebook open) and/or the facility staff for help and troubleshooting information.

1.1 – Logging on

1. Log onto the instrument in FOM. The instrument will not turn on if you don't log onto FOM.

1.2 – Preparing and mounting your sample:

- 1. Use Zeiss touchpad for switching between objectives.
 - a) To change objectives: touchpad -> microscope -> control -> select the objective you want to use.
 - b) Use "load position" button on the touchpad before mounting or removing your sample.
- 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. Select the lens slot via the Touchpad.
 - a) Screw in the lens you are intending to use for your sample into the microscope.
 - b) If you are using the 10x lens, you need to use the lens extender in order for it to be at the correct height in the microscope
 - c) 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.
- 5. Place the sample loading rack, with the sample, back on the stage.
- 6. If using the immersion oil, raise the Z-position of the lens until the lens immersion oil comes in contact and creates an oil bridge with the coverslip of your sample.
 - a) Be very careful not to crash the lens into the sample.
- 7. Tilt the head of the microscope forwards, so that it is directly above your sample.
- 8. Switch the CSU-XI to the on position.



9. Switch the Laserstack on:



1.3 -View Brightfield

- 1. Open Slidebook on the computer
 - a. If using the Z-stack piezo (100x lens), open the piezo version of Slidestack, otherwise open the nopiezo version.
- 2. Open the Focus window.



Focus Window Scope Z XY Camera Stream 6D LaserStream Objectives 10x dy 50x Air Magnification Changer: C Camera C Video Common Common	Lamp:Set Condenser Aperture0.0% Position Pos 1
Camera: PM1394Cam00 Exposure: 100 ms Zoom: + - Update Full Chip Auto Renorm Color	Bin: 1x1 Image: Guarantee Display Pseudocolor Stop Snap
Filter Set: Confocal Open 405 488 532 567	1 Open Fluor Open Right Open At
XY Stage X:-6.068 mm 100 V Y:-3.586 mm 100 V 101 1 .1 > 2.379	Image: Second

- 3. View your sample with ambient light by clicking Open Bright on the right side of the focus window and select 'Make Visible' button on the Zeiss touchpad.
- 4. Select 100% Eyes in the Focus Window.
- 5. You can now view and focus your sample manually using the eye pieces on the microscope.
- 6. You can more finely adjust the Z-height using the Z Stage section of the focus window.

1.4 Viewing Fluorescent images

- 1. Select 100% Left in the focus window.
- 2. Select the 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.
- 5. Press Open Alt.
- 6. You may need to adjust the Laser power, Intensification or Exposure time to find the best settings for your sample.
- 7. You can move in the X/Y direction across your sample using the joystick, the X/Y tab, or by double clicking in the camera image.
- 8. In the X/Y tab:

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Set Point					Get Edge
Visit Point	Visit XY Only	-	Т		C Visit Edge
Update XYZ	Set Montage AF	+		+	C Set Extent
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Clear Point	Clear All	Z	ero	11	Home

- a. You can save multiple points in the X/Y tab to perform multi-point image collection.
- b. You can create a montage in the X/Y tab as well.

1.5 Collecting Fluorescent images

1. Open the Capture window:

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- 2. Caution: pressing the enter key at any time will start the imaging.
- 3. Select capture type:
 - a. This includes Z-stack, timeless, multipoint, and others.
 - b. Mover info in setting up different capture types can be found in the Slidebook help menu (F1) or in the Getting Started Slidebook file on the desktop.
- 4. Check the boxes to select the channels you would like to capture.
- 5. Set the exposure.
- 6. To create a 3D image:
 - a. Focus on the sample and then either use the current position or reference position or set the top and bottom of the sample.
 - b. If using the Z-piezo 'Use current position' and then check the 'Range around current' box.
 - c. Make sure the step size is set to the value you want.
 - i. To use the Nyquist resolution of the camera, click on the optimal step size button in the Z tab in the focus window.

1.6 Turning the System Off

- 1. Switch the Laserstack Turnkey to OFF.
- 2. Switch the CSU-XI to the off position.
- 3. Close Slidebook.
- 4. Lower the microscope stage.
- 5. Tilt the head of the microscope backwards to its original angled starting position.
- 6. Remove the loading rack from the stage and retrieve your sample. You can leave the loading rack off of the microscope stage.
- 7. Unscrew and remove the lens from the microscope.
 - a. If using the 100x, clean the immersion oil off of the lens using IPA and the lens wipes. Be very gentle, do not scratch or damage the lens.
 - b. Replace the lens back into the container.
 - c. Place the cap back onto the lens carousel.
 - d. Log off of FOM, this will turn the instrument and computer off.