**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.

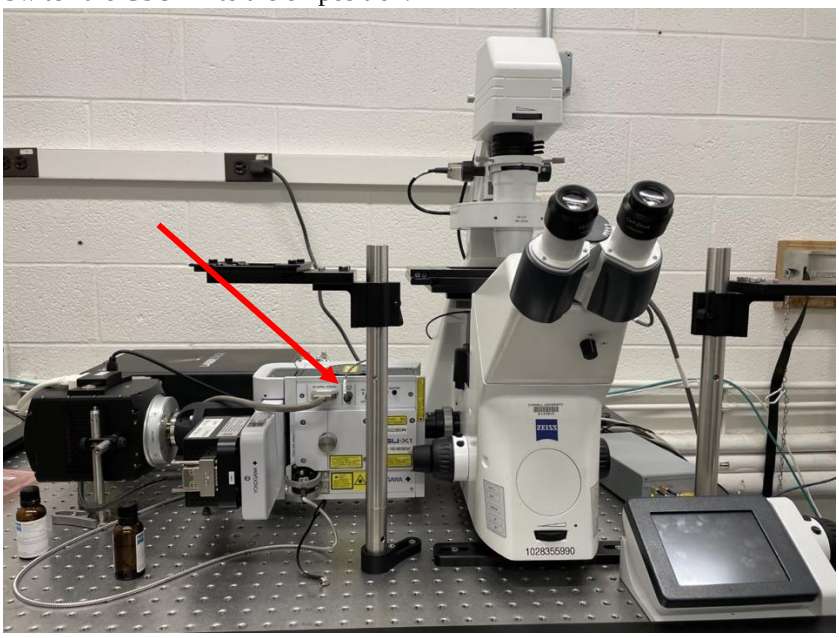
Please consult the Slidebook help (press F1 on the PC keyboard with Slidebook open) and/or the facility staff for help and troubleshooting information.

**1.1 – Logging on**

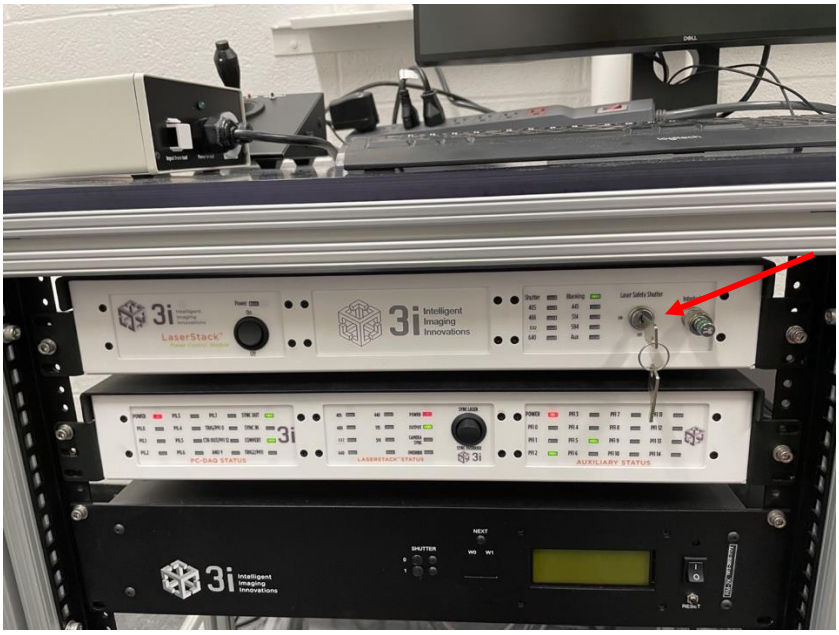
1. Log onto the instrument in FOM. The instrument will not turn on if you don't log onto FOM.

**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.

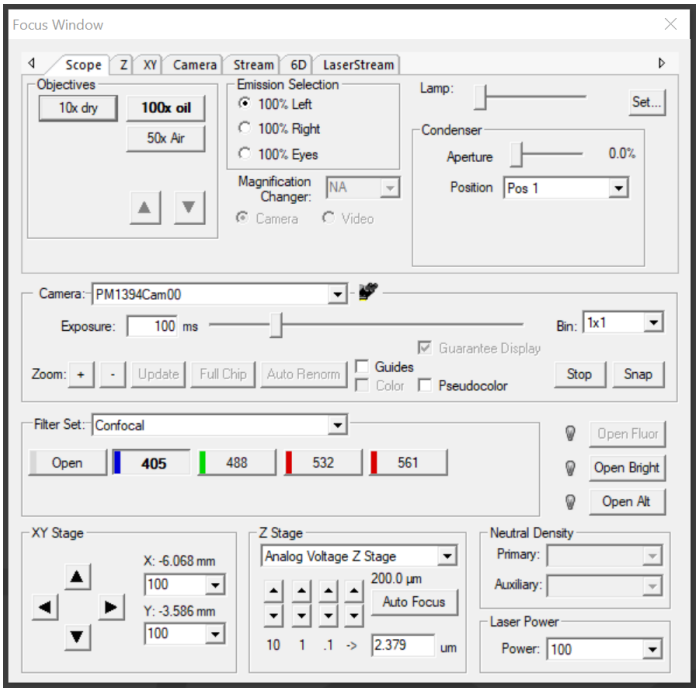



9. Switch the Laserstack on:




### 1.3 -View Brightfield

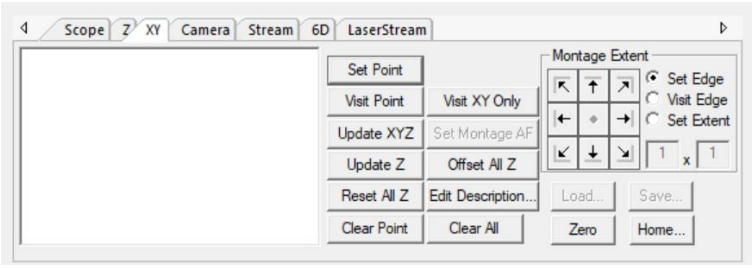
1. Open Slidebook on the computer
    a. If using the Z-stack piezo (100x lens), open the piezo version of Slidestack, otherwise open the no-piezo version.
2. Open the Focus window.




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

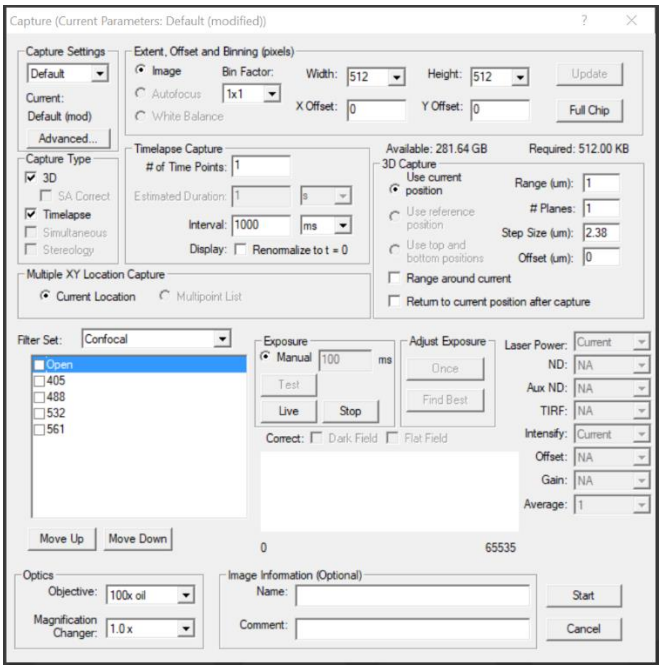
1. Select 100% Left in the focus window.
2. Select the Camera under the camera selector.
3. You should now see your sample live in the camera image on the screen.
4. Under Filter set, select confocal and select a laser.
5. Press Open Alt.
6. You may need to adjust the Laser power, Intensification or 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:




   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:




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.