

BIOLOGICAL IMAGING DEVELOPMENT COLAB



Nikon AZ100 Laser Scanning Macro Confocal Microscope

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Contents

1	Introduction	2
2	Hardware - Startup	2
3	Software/Operation	4
3.1	Multidimensional Acquisition	10
4	OKO Lab Incubator Chamber	11
5	Shut Down Procedure	13
6	Troubleshooting	13
7	Considerations	14

1 Introduction

The Nikon AZ100M, also known as the “Macro Confocal,” is the newest addition to the BIDC family. The Macro Confocal is a laser scanning confocal with four low-mag objectives, 0.5X, 1X, 4X, and 5X, all with long working distances. Not only does this microscope utilize digital zooming like other Nikon instruments, it also boasts a 1-8x optical zoom, making the Macro a one platform solution for whole specimen to single cell imaging.

It uses four laser lines for excitation including 405, 488, 561, and 540 nm. The Macro has three standard PMTs (Nikon C2-DU3) with a detection range from 400 - 700 nm, as well as a spectral detection (Nikon C2-DUS) with a detection range from 400 - 750 nm. The instrument can achieve up to 100 fps in “fast” mode, and the objectives can cover a field of view over 1 cm.

This microscope can produce confocal as well as diascopic DIC images and uses Nikon Elements software for both standard and spectral imaging. Spectral unmixing is available with this Elements pack, with preset or custom references.

2 Hardware - Startup

1. Sign up for time on the BIDC MyCores page. This can be done up to 2 weeks in advance.
2. Under the desk, turn on the PC.



Figure 1: Computer.

3. Turn ON the power strip using the white remote provided. This will supply power to the components, but you will still need to turn some of them on yourself. Please return the remote to its place on the shelf above the computer monitor next to the OKO Lab controller.



Figure 2: Find the remote above the computer by the OKO controller. Place back on shelf when done.

4. Turn on the lasers by turning the key clockwise a quarter turn. This will begin the laser warm-up.



Figure 3: Key for Lasers.

5. Turn on the C2si control box located underneath the air table.



Figure 4: Control Box.

6. On the main screen on the desktop, double-click the NIS Elements Software and log in.

3 Software/Operation

1. Continue with NIS Elements open.
2. Choose your objective. This is a manual selection.
3. Place the proper sample holder on the stage, and put your sample in place. Using the coarse adjustment knob, carefully raise the stage slightly from its lower limit. This will allow you more room to adjust for focus later.
4. Using the joystick located to the right of the microscope, center your sample under the objective lens.
5. (a) In Elements, Look for the OC Panel in the top right of the window, below Macro Panel. Select how you would like to visualize your sample through the eyepieces by selecting an option in the Wide Field panel (DAPI, FITC, CFP, etc).

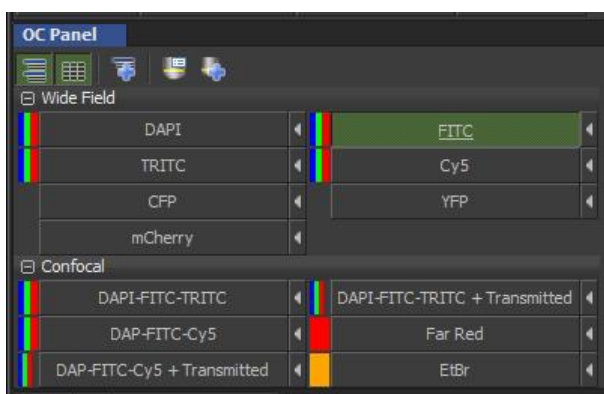


Figure 5: OC Panel.

- (b) Make sure the filter cube is set to the proper position. The filter cube knob is located on the right side of the microscope, above the objective turret.

Position 1 - Confocal (empty)
Position 2 - DAPI, FITC, TRITC, Cy5
Position 3 - CFP, YFP, mCherry
Position 4 - Confocal (empty)



Figure 6: Filter Cube Knob.

6. On the top right side of the body tube of the microscope, above the filter cube knob, push the lever in to allow the light through the eyepieces.



Figure 7: Light Pass Lever.

7. In the AZ Pad window, just below the OC Panel, check that your objective you chose manually matches the objective that the software is highlighting in green. Use the arrows in the Zoom tool to zoom in at least

2x (this minimizes falloff due to the zoom optics).

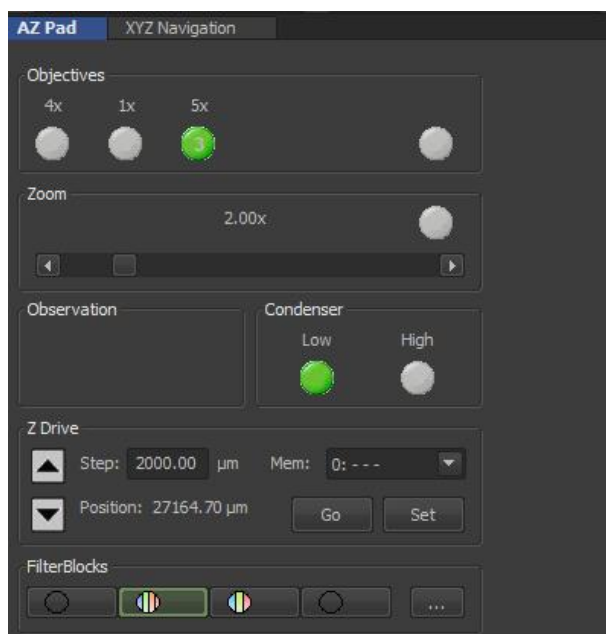


Figure 8: AZ Pad.

8. In the XYZ Navigation window, located next to the AZ Pad controls, use the Z controls to adjust your focus in short increments. This adjustment moves the objective closer to and farther from your sample, instead of moving the stage. Be mindful of the objective's distance from your sample.
 - (a) Keep in mind that samples in well plates require objective lenses that have a longer working distance, so as to not hit the objective with the top of the plate.

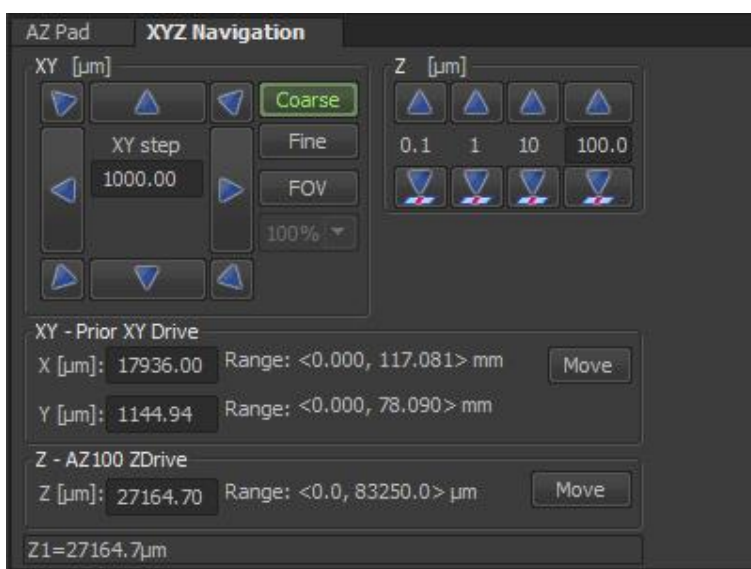


Figure 9: XYZ Navigation.

9. Once you have found your sample and focused it, click the Spectra button on the top menu bar. This will close a shutter and keep light from bleaching your sample.

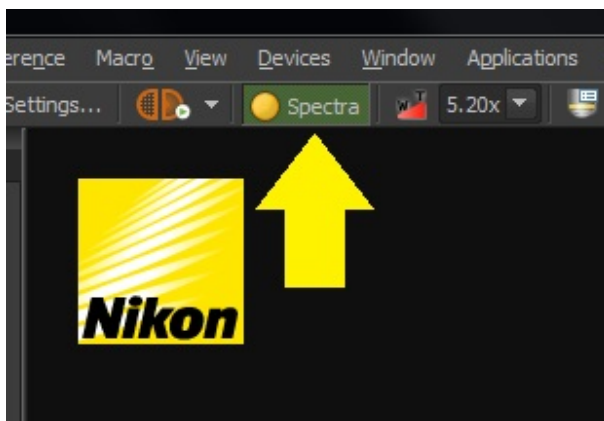


Figure 10: Spectra.

10. Pull the top lever out to allow light to go to the detectors. You will no longer be able to see through the eyepieces.
11. In the OC Panel, choose the Confocal mode you would like to image in (ex. DAPI, TRITC, FITC; or Far Red; etc.). Change the filter cube using the knob on the right to Position 1, which is empty.

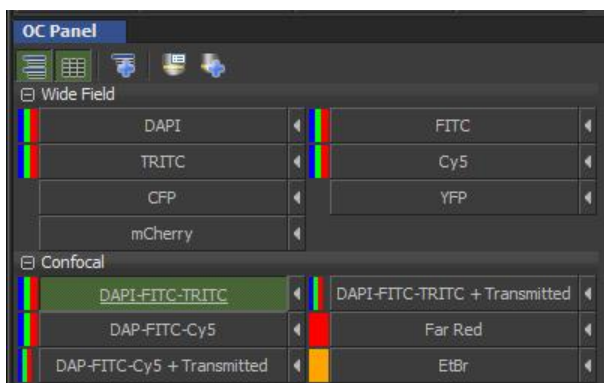


Figure 11: OC Panel - Confocal.

12. Click the "Remove Interlock" button on the top left-hand side of the window. This button will only disengage the interlock if the beam path lever is set to send light to the detector. This is a fail-safe to protect your eyes.



Figure 12: Remove Interlock.

13. Find the C2 Plus Compact GUI on the left-hand side of the window. This is where you will set your imaging parameters. Some of your choices are as follows:
 - (a) Unidirectional (slower scan) or bidirectional (faster scan).
 - (b) Frame/sec or Pixel Dwell (how much time will be spent acquiring the frame/ acquiring per pixel).
 - (c) Frame Size in Pixels (ex. 512 x 512, 1024 x 1024, etc.)
 - (d) Frame Average or Frame Integrate (image quality settings).
 - (e) Channel Series and Setup.
 - (f) Pinhole Setting (6 options).
 - (g) Beam path Settings.

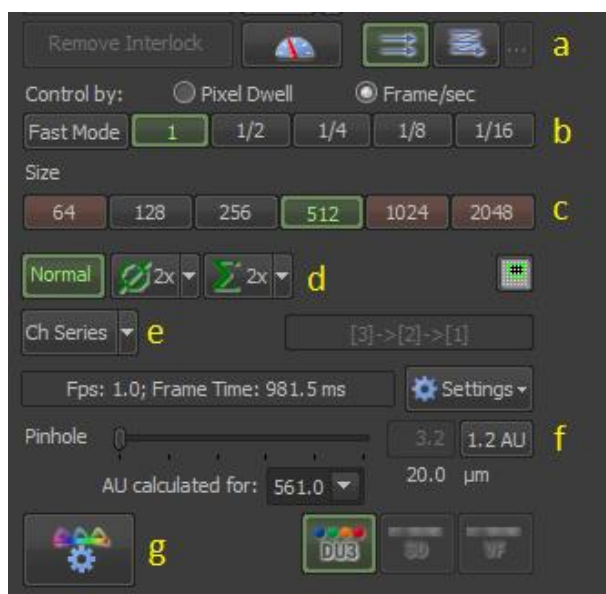


Figure 13: C2 Plus Compact GUI.

14. After setting your acquisition settings, look at your laser and detector settings. Start with a low laser power, to avoid unnecessary photobleaching. When you're satisfied with your settings to start, click the Scan button at the top of the C2 Plus Compact GUI.

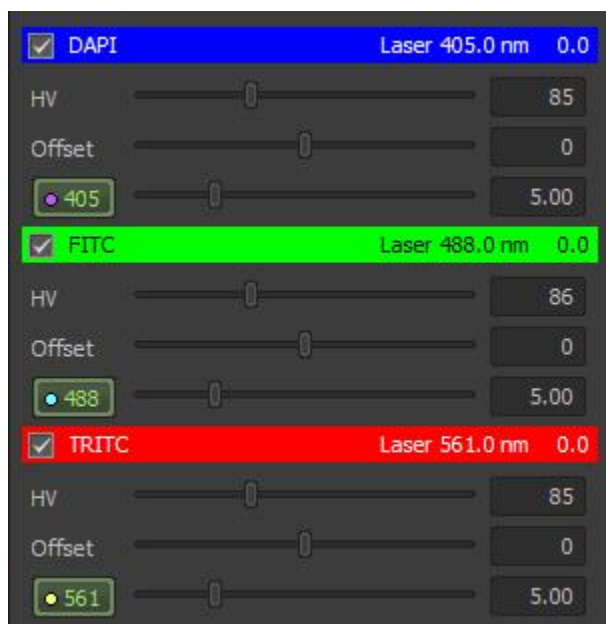


Figure 14: Laser Settings.

15. Adjust your laser power and detector gain (HV) appropriately for your sample. Make any necessary adjustments to your acquisition settings, such as selecting Averaging or changing the frame size. Remember that changing your frame size may influence the relative brightness of your image. Keep your Offset at zero.
16. If you would like to change the scan area, i.e. zoom in on your sample, you have two options. One would be to change the optical zoom in the AZ Pad window (will change focus), and the other is to change the digital zoom using the Scan Area window located at the bottom left of the Elements window. It's best to keep both of these zoom controls within their limits, not at the outer limits. For example, the optical zoom should be somewhere between 2-7, and the digital zoom should be greater than 1.

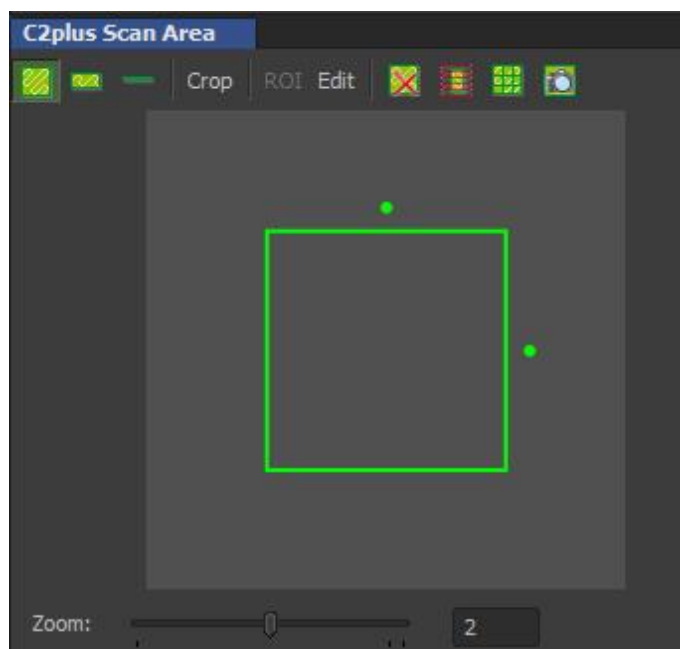


Figure 15: C2Plus Scan Area.

17. The LookUp Table (LUT) Panel is located on the bottom panel in the Elements window. This controls the scaling of the image you have selected.
- (a) Auto scale will scale the contrast of your image based on its histogram.
 - (b) Saturation indicator will color code oversaturated pixels.

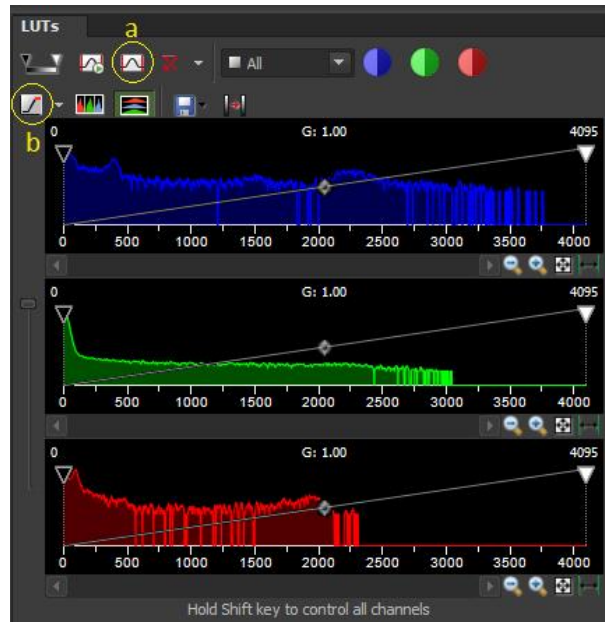


Figure 16: Look Up Tables.

3.1 Multidimensional Acquisition

Allows you to set up time lapse, multi-position, large image, and Z-stack experiments.

1. Z-Stack

- (a) Select "Reset" before setting any values.
- (b) You may select absolute, relative, or asymmetrical.
 - i. For absolute scroll through Z while scanning and define the top and bottom of your sample.
 - ii. For relative set the center of your sample to "Home" and enter the distance you want to acquire above and below this point
 - iii. For asymmetrical select a point and enter the distance you would like to capture above and the distance below

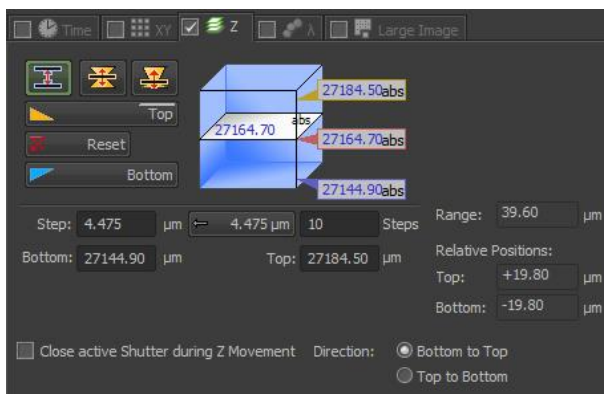


Figure 17: Z-Stack.

2. Time Lapse clock tab (specify 2 of the following three) .

- (a) Interval is how often time points are acquired.
- (b) Duration is how long the experiment should run.
- (c) Loops is the total number of time points.

3. Multi-point XY tab

- (a) As you navigate around your sample, mark areas of interest to be acquired.

NOTE: if Z is included in your coordinates, you will be required to use relative for your Z stacks.

4. Large Image

- (a) Allows you to acquire a grid of images of any size with the current field as the center.
- (b) Stitch will attempt to identify common features between tiles and use them to stitch the final image.
- (c) Do Not Stitch simply places tiles next to each other without processing them.

NOTE: If you have multi-point and Large image checked, it will do a grid of images around each XY point.

5. Channels - Lambda

- (a) This allows you to switch between optical configurations that require a change in dichroic, or light source.

6. Order of Experiment

- (a) The drop down menu is written multiplicatively, the experiment will be executed in the order of options RIGHT to LEFT

4 OKO Lab Incubator Chamber

NOTE: Everything for the OKO Lab Incubator is controlled through the touch screen. Incubator must be on before starting the software in order to collect incubator metadata with images. It is highly recommended to turn on the incubator **at least two hours prior** to your imaging session to equilibrate all components. Please reserve time accordingly.

1. Press and hold button on the touch screen panel to turn on the incubator. Pressing the button quickly will turn the screen on and off, but not the system.
2. Check T valves for the gasses on the wall behind the instrument. Open the valves if they are not open. The handle should be parallel to the tubing when open.

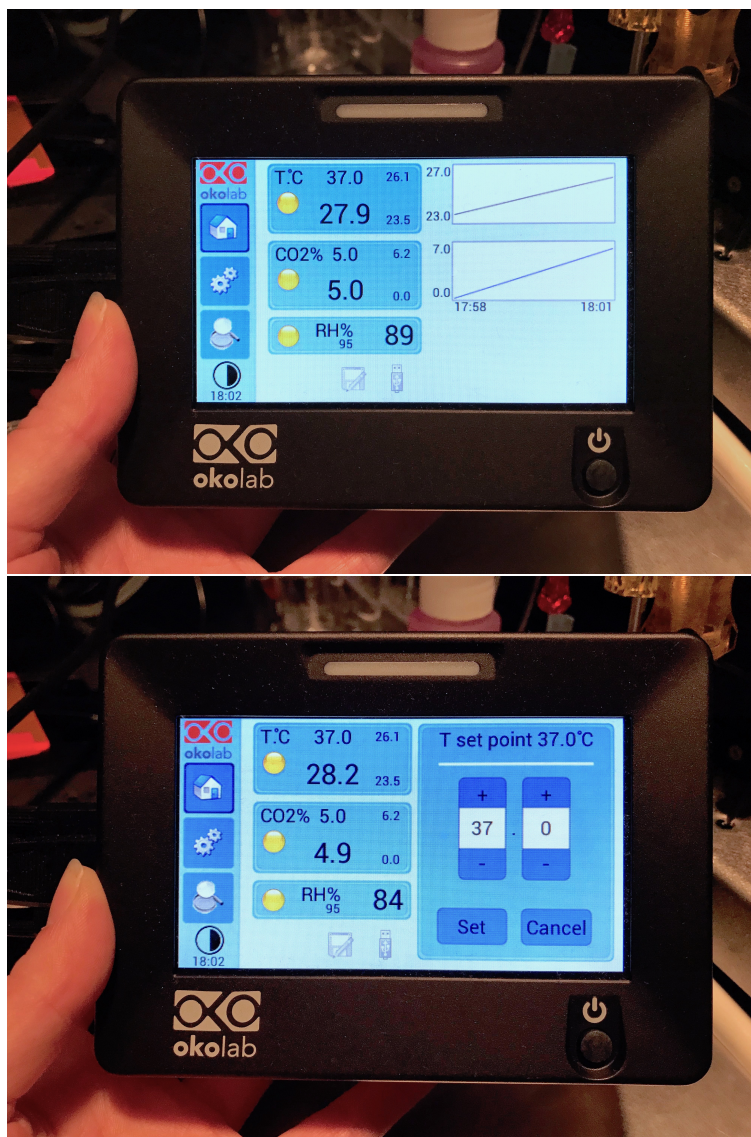


Figure 18: OKO Lab Touch Screen

3. Set the temperature and humidity using the Home screen on the touch panel. (Figure 18)
4. Check the humidity bottle for an appropriate level of distilled water. ONLY distilled water may be used. Please ask the BIDC for more, do not refill. **NOTE:** *It is recommended to keep the Relative Humidity (RH) between 80-95%.*
5. Choose appropriate chamber insert/riser combination for your sample. All components are magnetic and align via red dots, but chamber risers must be screwed in using a 1.5mm hex key (found on table).
6. If your sample does not fill all available open slots in chamber insert, please add additional slides/dishes to fill empty spaces, in order to maintain humidity and gas exchange within chamber.
7. Connect heated tubing to the brass fitting on the stage chamber. Be sure when moving the stage that this tube does not get caught. (Figure 19)
8. Users may change the flow rate of the gasses using the settings panel on the touch pad (the gear set button). The recommended setting is approximately 0.3 l/min, which will replace the gas in the stage chamber every

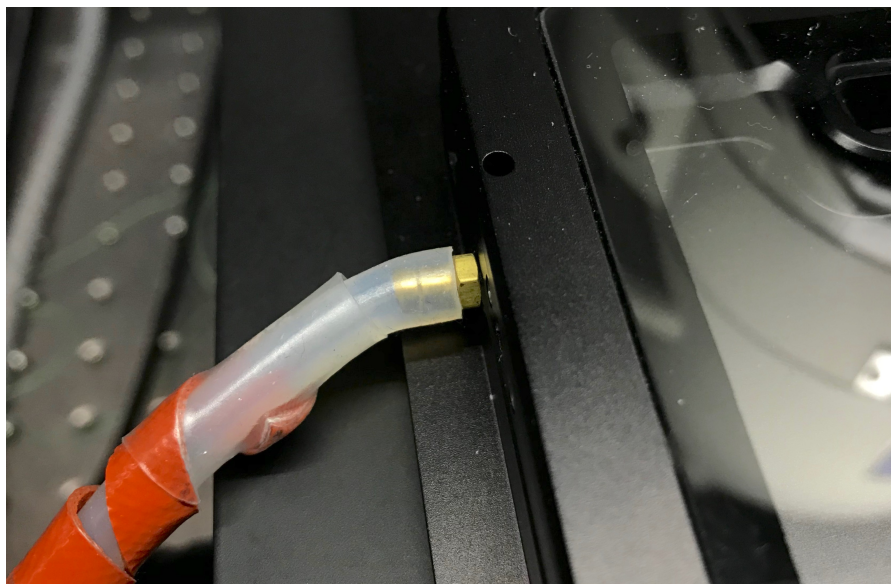


Figure 19: Brass fitting with heated tubing attached

minute or so.

9. When your imaging is complete, press and hold button on the touch screen panel to turn off the incubator.
NOTE: *Lights will blink even when the system is off, because power is connected.*

5 Shut Down Procedure

1. Once you have saved your data, exit the software first.
2. Shut down the computer by going to the Start menu in the bottom left corner and clicking Shut Down.
3. Turn the key on the laser launch to “Off” to shut down the lasers. They do not need time to cool.
4. Turn off the C2si control box.
5. Turn off the switch on the power strip that is located underneath the computer table next to the PC.

6 Troubleshooting

1. No light reaching the sample
 - (a) Filter block settings are manual. If you can't see any light hitting your sample, check to make sure your filter block is set to the proper position. An exclamation point (!) will also appear by your configuration type in the OC Panel if your filter block is not set to the correct position.
 - (b) Check the manual shutter on the right-hand side of the body tube. It's situated in a position that isn't easily moved, but if it is closed, no light will reach your sample.
2. If you're having trouble getting your sample into focus
 - (a) First check that your sample holder is firmly placed in the stage. If your sample holder wobbles, remove your sample, and try placing the sample holder in the correct position. It should be flat, and feel continuous with the stage.
3. Sample appears dim or fuzzy

- (a) Double check the aperture located on the body tube of the microscope- for fluorescence work, it should always be set to be 100% open to allow the most light through the system.



Figure 20: Aperture

7 Considerations

1. This microscope has a large working distance for most lenses, but it is also set so that the objectives will not run into the stage. For this reason, you may be at the upper limit of the stage in Z, but the objectives themselves have more room to go. For ease of use, try setting the stage at about half of its Z limit, focus with the piezo (the objectives via the software) and then you'll have room for focus adjustments via the stage and the piezo.
2. These objective lenses are meant for low magnification imaging. Planning your experiment to work with this microscope will pay off later. Try to plan your experiment so that things you know may be dimmer (ex. Those features with variable receptor sites, features that may or may not be present, etc.) are paired with bright fluorophores well in the visible spectrum range, like blue-greens, greens, oranges, and orange-reds. Those things that you know to be bright (ex. Those features with many receptor sites, that bind well, generally appear bright regardless of fluorophore, etc.) are better paired with fluorophores that are harder to excite, like reds and far-reds, than those features that you may have trouble finding. While red and far red fluorophores have many benefits (less energy, less damaging to the sample, less cross talk), they can be hard to detect with lower magnification objectives (like 0.5X and 1.0X), and should be used thoughtfully. It's good to remember that there are many aspects of successful experimental design- ask a BIDC member for help if you would like to talk through choosing fluorophores for your experiment.
3. Large samples have a lot of information ready to be captured and analyzed! Be sure to design your imaging in a way that allows you to get the most data in the most efficient way possible. If you are working with a large, tiled Z stack and your research requires it (ex. for 3D representations), keep in mind the file size will be large and may require special considerations. If you don't need a very large image stack, perhaps limiting the number of slices in your Z stack will be beneficial, and you will still gather the data you need. These are choices you, as the researcher, must make, however the BIDC is always available to help you walk through your choices.

Contact the BIDC

The BIDC office is located in Medical Sciences Building Room S1109.

The BIDC office phone number is 415-476-4550.

If you need immediate assistance and no one is available in the office, or it is after business hours, please call the **BIDC Hotline** at 415-745-2432.