BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

Nikon A1R

Multi-Photon & Laser Scanning Confocal Microscope

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Contents

1 Introduction

The Nikon A1R microscope consists of two illumination options; four confocal laser lines (408 nm, 488 nm, 561 nm, 638 nm) and a Mai Tai DeepSee IR laser (740 - 1040 nm). For the confocal laser lines there are 4 PMT detectors (blue, green, red, far red) that can reach video rate and faster. For multi-photon imaging there is a high sensitivity non-descanned detector (NDD) for deep tissue imaging.

The system utilizes an Nikon base in the upright geometry, an automated Prior XY stage, and a PIFOC objective piezo for scanning. Additionally, there is a perfusion system with an in-line and stage heater and an anesthesia setup making the microscope suitable for slide, slice, explant, and intravital imaging.

Currently, there are 3 long working distance objectives consisting of a 16x/0.8 NA Plan Apo water immersion, 20x/1.0 NA Plan Apo water immersion, and 25x/1.1 NA Plan Apo water immersion. Also, there are higher magnification objectives consisting of a 40x/1.3 NA Plan Fluor Oil immersion, 60x/1.2 NA Plan Apo water immersion, and a 100x/1.4 NA Plan Apo oil immersion.

Please sign up for time using iLab.

2 Start-Up

1. Check the Mai Tai laser (Figure 1).
   (a) The power supply should **always** be on. **DO NOT** turn it off.
   (b) The key should always be turned to "ON".
   (c) If the screen on the lower box indicates coolant needs to be added, add a small volume. If you have not done this before, please contact a BIDC employee and we will show you how.
   Note: *Even if you are only using the diode lasers to image, the Mai Tai needs to be on and talking to the computer for the software to run.*

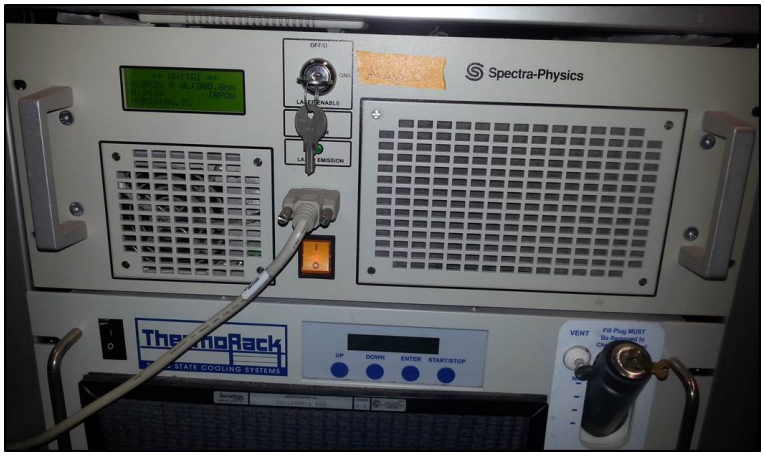

Figure 1: Power and Key always ON.

2. Make sure the large stage is completely down (Figure 2).
3. Install objective.
   (a) Screw the objective into the z-axis piezo.

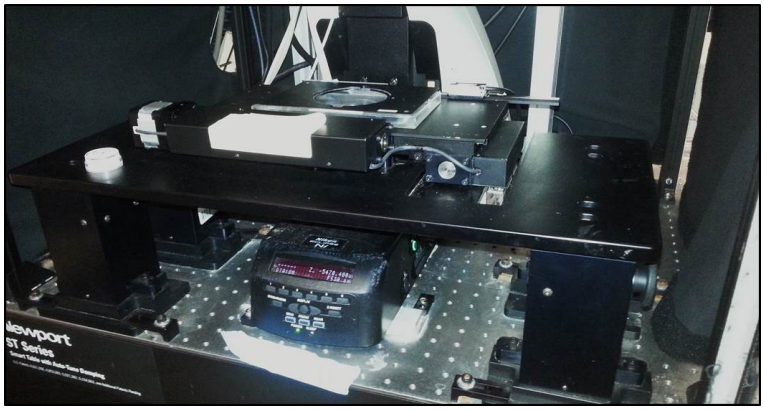

Figure 2: Stage Down on Startup.

4. Turn on the microscope peripherals (Figure 3).
   (a) Turn on the Prior XY stage (1).
   (b) Turn on the z-axis piezo (2).
   (c) Turn on the microscope power supply (3) and wait until initialization is complete.
   (d) Turn on the microscope control box (4).
5. Turn on the arc lamp for visualization (Figure 3).
   Note: *The lamp must be on for* **at least** *10 minutes before shutting it off.*
   (a) Turn on the power switch located on the back of the box.
   (b) Press "local" for lamp control.
   (c) Press "1" to start the lamp.
   Note: *If your acquisition will run for hours, please turn off the lamp to conserve bulb lifetime.*

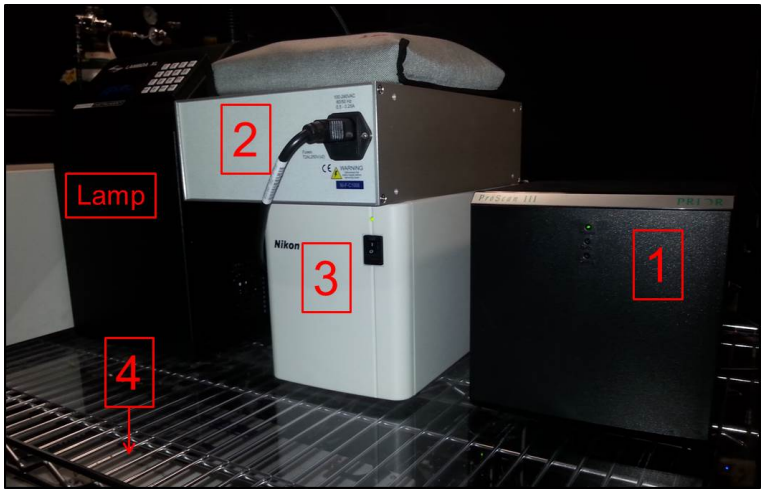

Figure 3: Peripherals and Lamp.

6. Turn the keys on confocal lasers from "Standby" to "ON" (Figure 4).
   Note: *Only turn on the lasers you will be using.*
7. Launch the NIS-Elements software from the desktop.

8. Clear previous user's presets:
   (a) Clear the "Z Intensity Correction" tab in the lower left of the GUI.
   (b) Reset the Z range under the "ND Acquistion Z" tab.
   (c) Clear all XY stage positions under the "ND Acqusition XY" tab.

Figure 4: Turn keys from Standby to On.

3 Imaging

3.1 Sample Alignment

1. Place your sample on stage.
   (a) For "open solution" samples, first put down the plastic drop tray.
   Note: *Cut-out must be in the upper-right corner to avoid collision with the micro-encoder.*
   (b) Add the black metal piece with the circular hole for slides.
   (c) Place your slide and add the correct immersion fluid for the objective.
   (d) If you are using the heated stage and/or the perfusion pump, have a BIDC employee show you the proper setup.
2. Move sample under the objective.
   (a) Carefully raise the large stage by hand.
   Pro Tip: *Work your hands under the stage by the adjustable legs instead of pulling up. This will reduce the risk a hitting the objective with your sample.*
   (b) In combination with the XY stage, move your sample so that the objective is touching your immersion liquid.
   (c) Tighten the knobs so the stage does not drift downwards.
   (d) Using the coarse adjustment, lower the objective until it is just above your sample.

3. Align sample by eye.

   (a) Change the microscope's light path using the knob on the right side of the microscope stage to "BINO".

   (b) Select how you would like to visualize your sample through the eyepiece by selecting "GFP Eyes", "DAPI Eyes", etc in the **OCPanel**.
   Pro Tip: *Use your brightest or most concentrated dye for initial alignment*

   (c) Looking through the eyepiece, turn the microscope focus towards you (thumbs up) until your sample comes into focus.

4. Switch to imaging with software.

   (a) Once you have found your sample with the eyepieces, change the light path to "F" with the knob at the side of the microscope.

   (b) In the **OC Panel** turn off the lamp illumination by selecting "Close Lamp Shutter".

   (c) In the software select the proper objective under the **OC Panel** (at the top right of the screen). This affects the metadata for your acquisition (stitching, pinhole size, etc.)

   (d) On the left side of the screen, click the red "Remove Interlock" button to open the light path.

There are 4 main components of the software's Graphical User Interface. Many of the settings and selections will work in cooperation with others. The following sections of this user guide will attempt to explain how to use the most popular buttons and settings.

3.2 OC Panel

This panel is mostly only used during the start of your imaging session (Figure 5).

Visualization: Selects the filter used for finding your sample by eye. Press "Close Lamp Shutter" to turn off lamp illumination.

Confocal: Saved settings for different imaging techniques. "Default Confocal Settings" is a good place to start for a new sample. "Shut Down" is pressed before closing the software.

Objectives: Select the objective used for imaging. This is important for stage stitching, calculated parameters, and metadata.

Special Apps: Saved settings for particular imaging conditions. Not useful to the average user.

3.3 A1plus Compact GUI

Within this tab many of the options for laser and PMT control are found (Figure 6).

Scan: Runs a live preview.

Capture: Takes a single image.

Galvano vs Resonant: This switches between scan settings within the microscope. Resonant scans at a continuous frequency, while Galvano will dwell at each pixel position. Resonant is fast and is useful for alignment. Galvano is slower, but will produce "nicer" images.

uni- vs bi-drectional scanning Sets the PMTs to collect light while the "y" mirror is scanned in only in one direction or both. Bi-directional is faster, while uni- is more precise.

Control by: Determines how the user sets the frame rate for acquistion, whether by the pixel dwell time, or by setting a "frame per second".

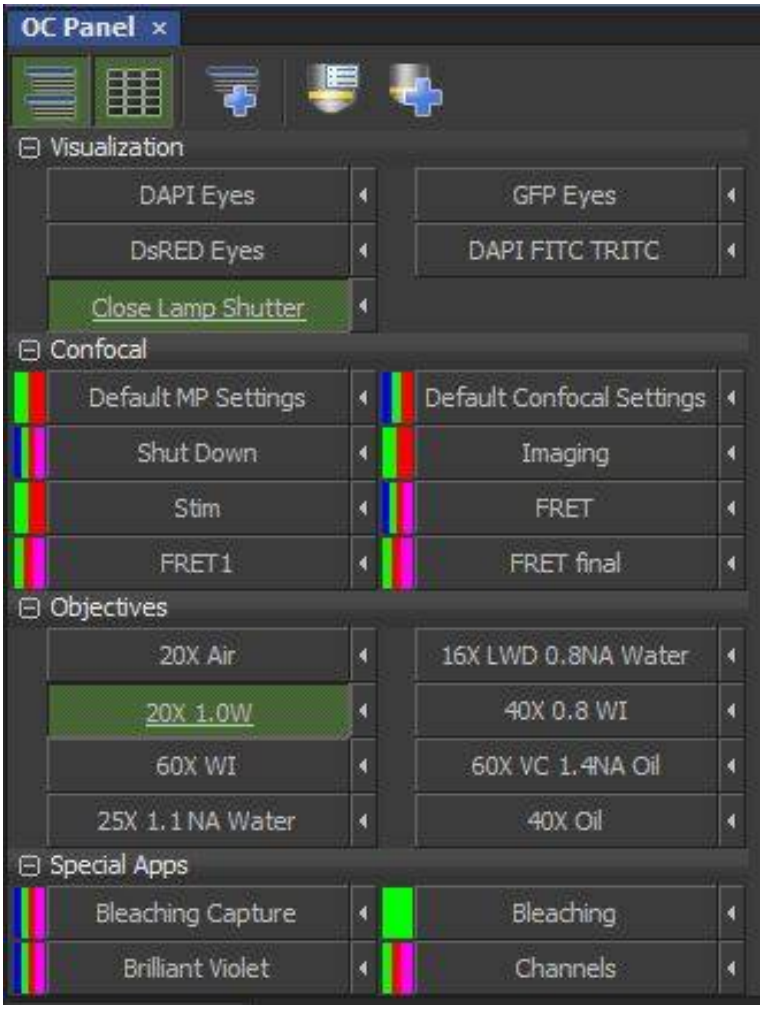

Figure 5: OC Panel

Size: The size, in pixels, of your image. Some sizes are not accessible due to the imaging speed set above. If you want to access larger image areas, change the frame rate to a longer acquisition setting.

Normal vs line averaging vs line integration: These settings allow the user to scan lines multiple times and either average or integrate the signal. Sample acquisition will take longer depending on the amount of averaging or integration chosen. The "Normal" button clears those settings.

Ch Series: The dropdown allows the user to choose the channels and the order in which they are fired. This is a useful feature to limit emission bleedthrough. The more "passes" selected, the slower the acquisition. Unselecting the "Ch Series" button fires all channels at once.

Pinhole: Changes the physical size of the pinhole for light collection. Pressing the "1.2 AU" sets the size to 1.2 Airy Units based on your selected objective. This is a good starting point for most experiments. Opening the pinhole increases light throughput, but descreases resolution. The dropdown menu chooses which wavelength (laser illumination) the pinhole is calculated for.

DU4 vs IR NDD: This selects which detectors are used. **DO NOT** select IR NDD if the lights are on or you're using the visible diode lasers. This can cause permanent harm to the detectors.

Use IR Laser: Select this option when doing 2P-imaging (see below).

Detectors and Lasers: The checkboxes select which PMTs to use in imaging. HV is the gain setting (typical 80-110). Offset is a current bias on the PMTs (keep at 0). The pushbuttons turn on or off the emission of the laser. The slider and input box is the power of the laser in percentage (typical 1-20). We do not recommend going below 1-2% as the laser intensities will become unstable.

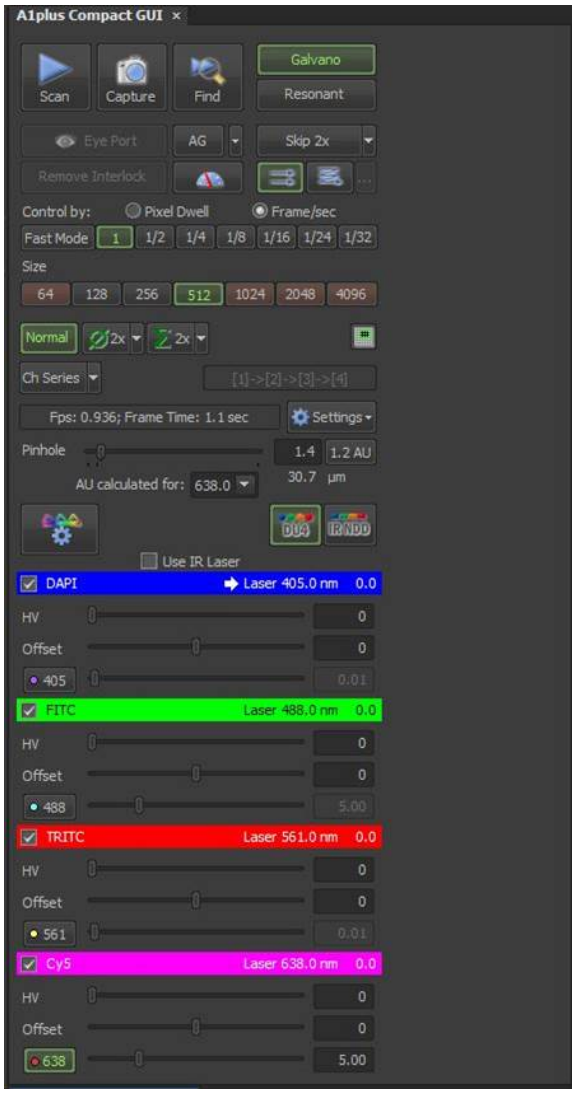

Figure 6: A1plusCompactGUI

3.4 LUTs

Look up tables (LUTs) control how the intensity is displayed on the screen. They always control the active image (whether during or post acquisition). They do not change how the data is collected (Figure 7).

Autoscale (All): The computer gives a best guess on the best way to view the data. This is a good button to press, as you can then adjust the min and max sliders to dial in your display.

Dropdown: Selects which LUTs are displayed.

Saturation Display: Highlights which pixels are being saturated with the current illumination settings. It's best to have this on while setting up acquisition parameters.

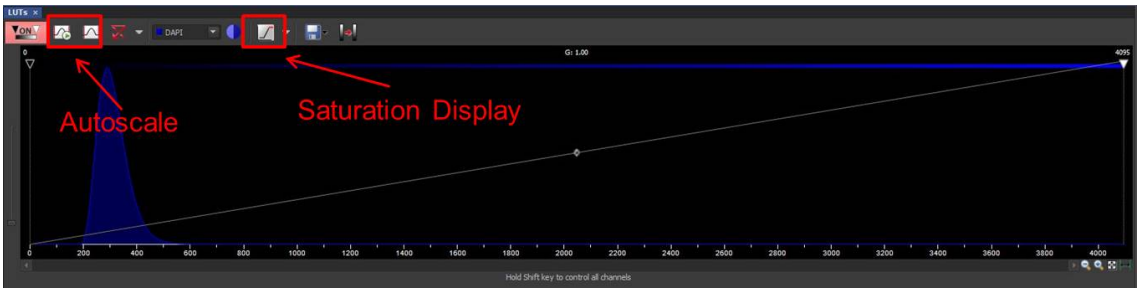

Figure 7: LUTs

3.5 ND Acquistion

The ND Acquisition tab is where most of your experimental setup will take place (Figure 8).

Save to File: Check this box to automatically save your file after completion to the directory with the chosen filename. The filename will continuously update itself so no data is overwritten. It is good practice to always have this checked.

Order of Experiment: Choose the order in which the data is acquired. The rule of thumb is to minimize physical movements. Channels are faster than z stacks are faster than stage movements.

Time: Set up a time lapse experiment.

XY: Choose different positions across your sample.

Z: Used to set up scanning through your sample in the z direction.

1. Top: Assign the top of the z stack from the current objective position.
2. Bottom: Assign the bottom of the z stack from the current objective position.
3. Step: The size of the steps through the z stack.
4. Steps: The number of the steps through the z stack.
5. Step push button: Assigns the steps and step size based upon the Nyquist sampling limit calculated for that objective.

Lambda: Used to set up non traditional illumination schemes. See a BIDC employee if help is needed.

Large Image: Used to set up a large tiled image.

Run Now: When everything has been selected. Run Now will start the acquisition using the laser and PMT settings from the A1plus Compact GUI.

Run Z Corr: Runs the experiment like Run Now with the exception that it using the interpolated power and PMT settings from the Z Intensity Correction tab (See Below).

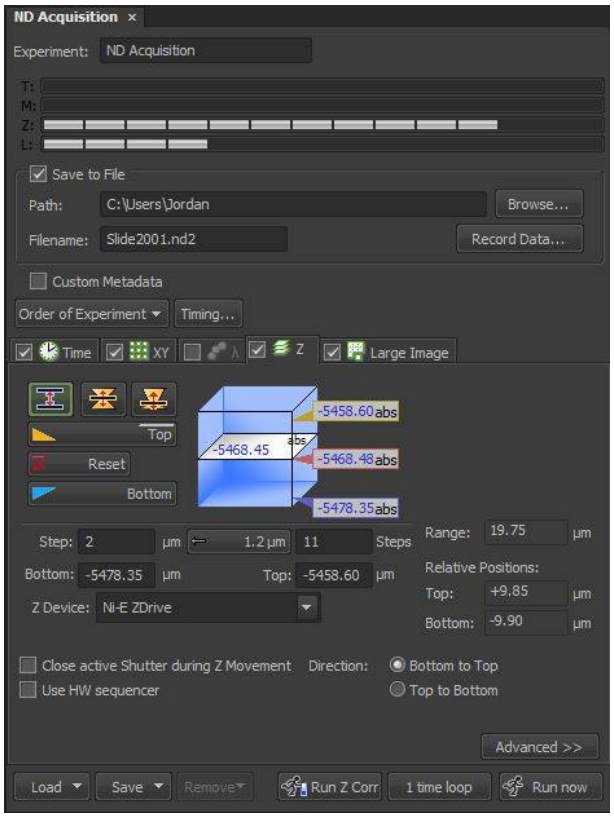

Figure 8: ND Acquisition

3.6 Other Useful Features

Some other quality of life features can be found around the software (Figures 9,10,11).

XY Mouse At the top of the imaging window press the target looking button. This will allow you to control the stage with the mouse.

Z Intensity Correction Located in the lower left of the GUI. This allows you to set laser intensity and PMT gain specific to the z position. Intensity at z steps is linearly interpolated.

Split Channel View Collected images can be split into their channels.

Volume View A 3D view of collected data.

Tile View Data is broken into z positions.

Max Projection View the max intensity at all pixels through a dimension.

Sliders The blue tile buttons at the bottom of your image allow you to slide through z positions and time.

Scan Large Image Under the "Aquire" menu there is the Scan Large Image option. This is a useful menu that quickly creates a tiled view at the current z position. Once the image is collected, the user can right-click on the image and interact with it, such as setting a feature to the image center. Users must select the proper objectives.

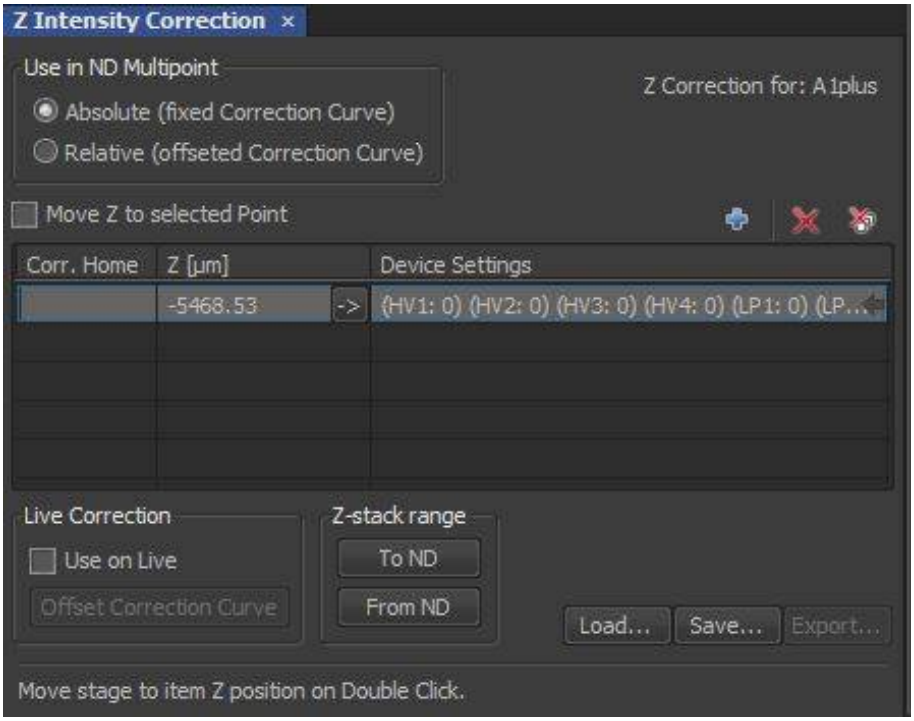

Figure 9: Z Intensity Correction

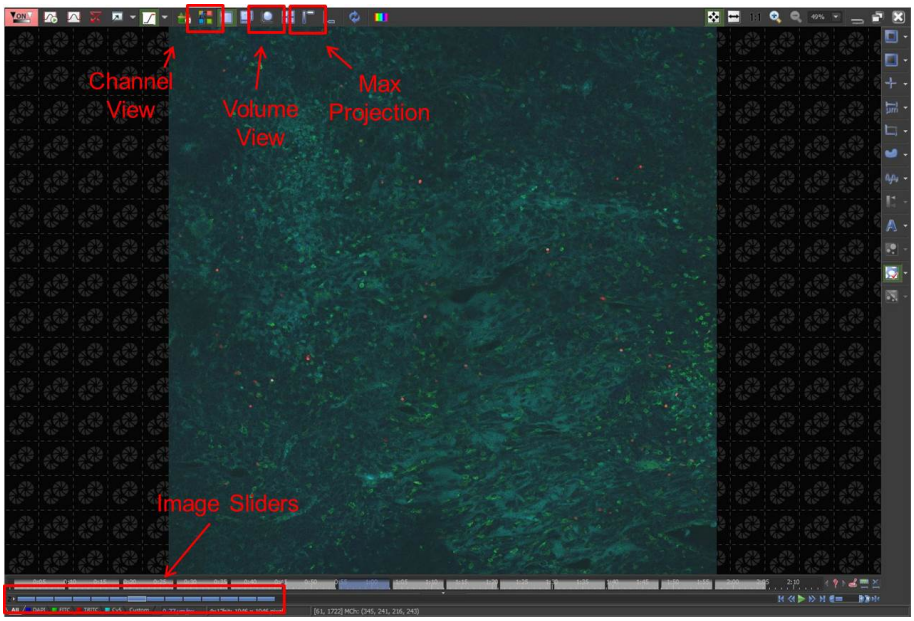

Figure 10: Image Interaction

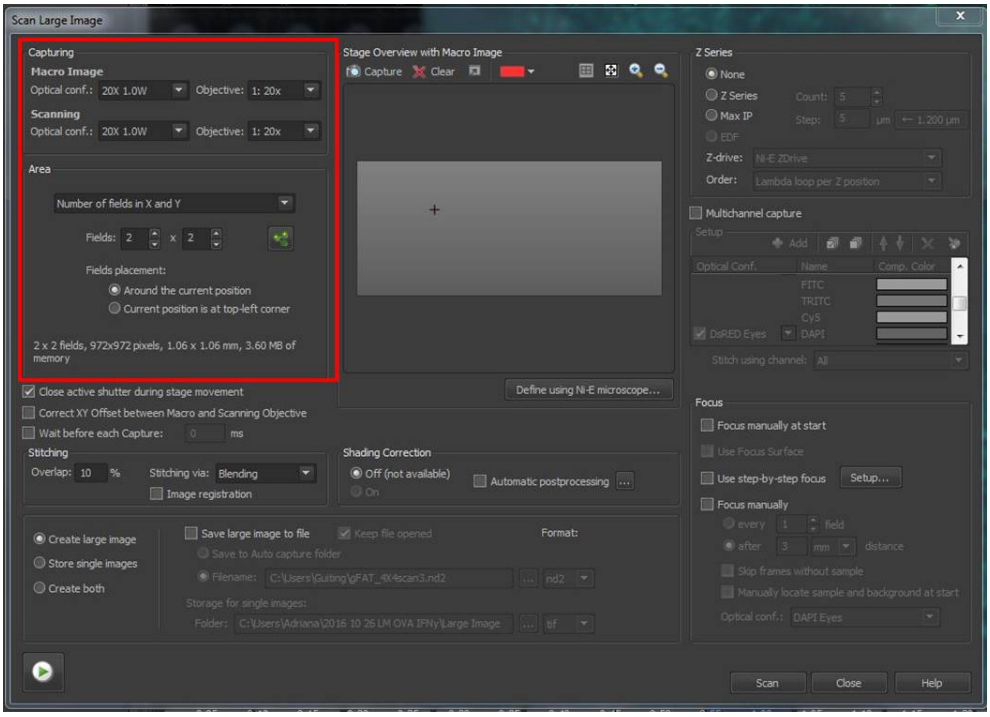

Figure 11: Scan Large Area

4 Two-Photon Imaging

Two-Photon Imaging parameters and acquisition setup works in much the same way as the visible diode lasers.

1. In the A1plus Compact GUI, check the Use IR Laser Box. This will enable the A1plus MP GUI (upper left).
2. Press the "On" button to enable the Mai Tai Deep See Laser. This will take a few minutes as indicated by the flashing yellow square.
3. Clicking on the yellow circle option opens the shutter.
4. Choose the wavelength to set the laser. Switching wavelengths takes a few seconds as indicated by the flashing yellow square.
5. Change the power within any of the activated PMT windows. Since there is only one laser line, relative channel intensities can only be balanced by wavelength selection and PMT gain.
6. **WARNING:** Only press the IR NDD option once the lights are off and the microscope cover is closed. This button selects the sensitive IR NDD detectors for two-photon imaging. Each time you need to turn on the lights, select the DU4 detectors.

5 Shutdown

Check the scheduler. If there is no one scheduled within two hours of your experiment's completion, follow the complete shutdown procedure, otherwise only shutdown the software.

1. Software
   (a) In the OC panel select "Shut Down."
   (b) Close the software.
2. Microscope
   (a) Turn off the microscope control box (4).
3. Devices
   (a) Turn off the microscope power supply (3).
   (b) Turn off the z-axis piezo (2).
   (c) Turn off the Prior XY stage (1).
4. Lamp
   (a) Under the "local" setting, press "2" to shut down the lamp.
   (b) Turn off power.
5. Lasers
   (a) Turn the lasers you used from "ON" to "Standby."
6. Objective
   (a) Gently unscrew the objective from the piezo.
   (b) Clean the objective with water and lens paper.
   Note: *If you have not cleaned an objective before, please have a BIDC employee show you a proper technique.*

6 Trouble Shooting

Please let us know when you run into a software or hardware problem, so we can add it to the list.

1. There is no light getting to the eyepiece.
   (a) Check that the arc lamp is powered and emitting.
   (b) Check that the light path knob is set to "BINO."
   (c) Open the "filters and shutters" menu in the software.
      i. Check if the shutter is open.
      ii. Check that the correct filter cube is selected.
   (d) Check whether the neutral density filters on the left side of the microscope are engaged.
2. No light is getting to the detector.
   (a) Make sure the light path knob is set to "F" (not "R" or "BINO").
   (b) Click the red interlock button in the software.
3. During software startup an error pops up saying it cannot connect to a device.
   (a) Press the "Shut Down" button in the OC Panel.
   (b) Close the software.
   (c) Lower the microscope stage.
   (d) Check the piece of hardware and cycle the power, if needed.
   (e) Restart the software.
4. During acquisition your sample seems to be zoomed in.
   (a) Check the viewing area zoom factor.
   (b) Check your pixel array size (it sometime becomes bigger than the screen).
   (c) Check the Scan Area tab to make sure you are scanning the whole image area.