BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

Nikon SoRa Spinning Disk Confocal

Twin-Camera with Optogenetic Capabilities

Austin Edwards John Eichorst Mohammad Naser Kyle Marchuk

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Contents

1 Introduction

The Nikon CSU-W1 SoRa Spinning Disk Microscope is a cutting-edge platform designed for high-resolution fast-acquisition live-sample imaging. The system also supports a DMD for optogenetic experiments.

Objectives Include:
4x Air for Large Overview
20x Air N.A. 0.75, W.D. 1mm
40x Water N.A. 1.15, W.D. 0.59-0.61mm, Correction Collar 0.15-0.19mm
60x Oil N.A. 1.49, W.D. 0.12mm

The microscope also includes components and features such as:

CSU-W1 SoRa Spinning Disk Large field-of-view with Super-Resolution option.

4-Line Laser Launch 405nm, 488nm, 561nm, 647 nm. Each 100 mW.

Dual Camera Option Hamamatsu ORCA-FusionBT for simultaneous dual-wavelength imaging.

Environmental Chamber Full enclosure for temperature and CO_2 control with quick-release front panel access.

Polygon 100 DMD For Optogenetics with 395nm, 440nm, 475nm, 555nm, 575nm, 640nm, and 730nm LEDs.

Phase Contrast Brightfield sample alignment.

Nikon Elements Software Includes 3 offline licenses for image analysis.

2 Initial Setup

Sign up for microscope time using the iLab calendar.

2.1 Startup

1. Turn on the computer.
2. Turn **On** the two red switches within the box and close the door (Figure 1).
3. Turn on the CO_2 flow for live samples.
   (a) Wake the display using the **Power Button** (Figure 2).
   (b) Go to Settings (Gear icon) $\rightarrow$Gas $\rightarrow$Flow Rate = 0.4
4. Launch NIS Elements from the software and choose your configuration.

   Fusion 1 Main camera for single-camera imaging.
   Dual Fusion Twin-camera for simultaneous imaging.
   Fusion 2 Second camera for single-camera imaging (Unlikely needed).
5. If you will use the 40x Water or 60x Oil objective load it now.
   (a) In the **Ti2 Full Pad** (software) in *Nosepiece* select the objective to load.
   (b) The objective is located in its case within the environmental chamber.
   (c) *Carefully* unscrew from the case and screw the objective into the vertical objective threading.

2.2 Startup Figures

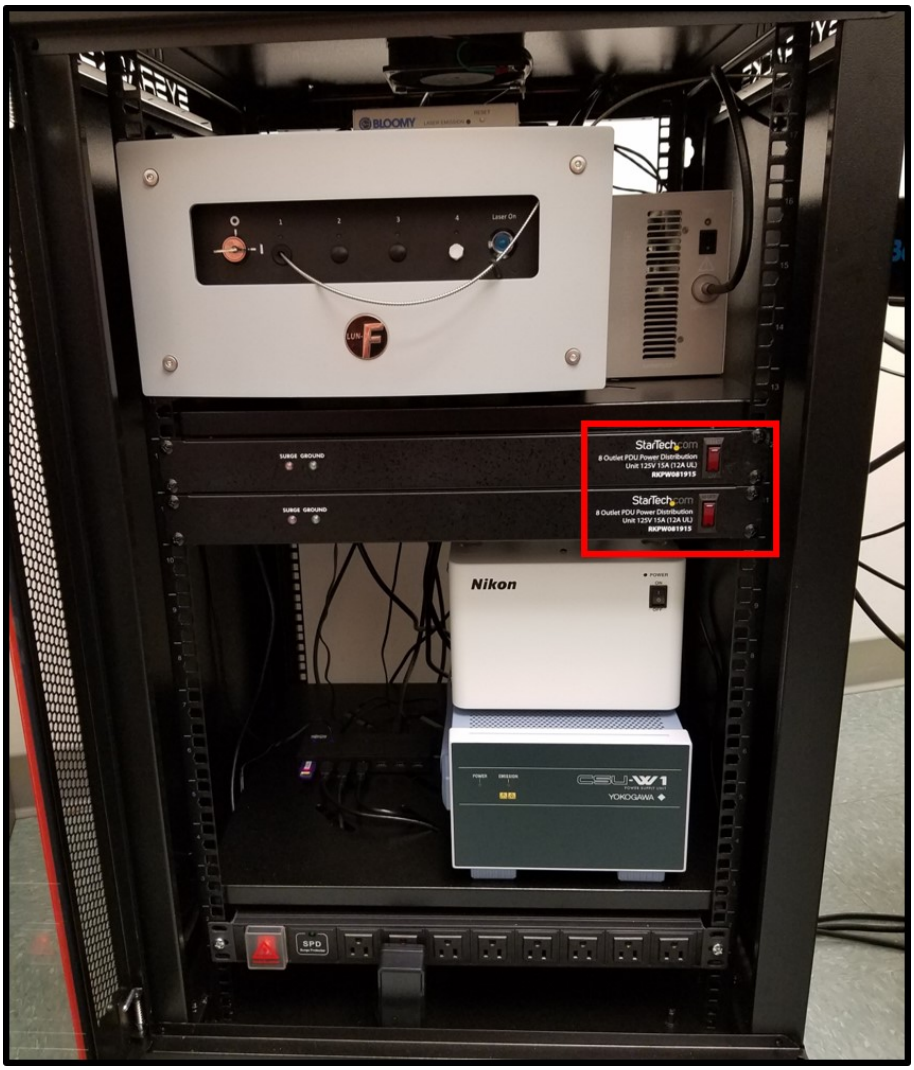

Figure 1: Server Rack with Power Boxes

Figure 2: Okolab Control Touchpad

2.3 Finding Sample by Eye

1. In the **Ti2 Full Pad** (software) in *Nosepiece* select your objective.
2. Add the appropriate immersion liquid if needed.
3. Load your sample onto the appropriate sample holder.
4. Place the sample holder into the stage and tighten the screw with the provided hex wrench.
5. Using the joystick move your sample over the objective.
6. Raise the objective to the sample using the focus knob.
7. Using the **footpad** (Figure 3) turn off the light in the environmental chamber.
8. In the **OC Panel** select *Eyepiece - EPI* and the channel you would like to illuminate with.
9. Open the shutter by pressing "'Mode"' on the Lamp controller (Figure 4). You should see the light at the slide.
10. Using the focus knob, bring the sample into view.
11. Close the shutter using the same "'Mode"' button.

2.4 Finding Sample Figures

Figure 3: Footpad for Lights in Environmental Chamber

Figure 4: Mode Button for Epifluorescence

3 Control Layout

3.1 OC Panel (Eyes)

Various options for viewing the sample through the eyepiece.

Eyepiece - EPI Selects the optical configuration to image with eyepiece.

Spinning Disk - LEFT Selects the optical configuation to image through spinning disk.

DAPI Selects filter cube to image DAPI by eyepiece.

GFP Selects filter cube to image GFP by eyepiece.

RFP Selects filter cube to image RFP by eyepiece.

DIA Selects configuration for brightfield imaging through the eyepiece.

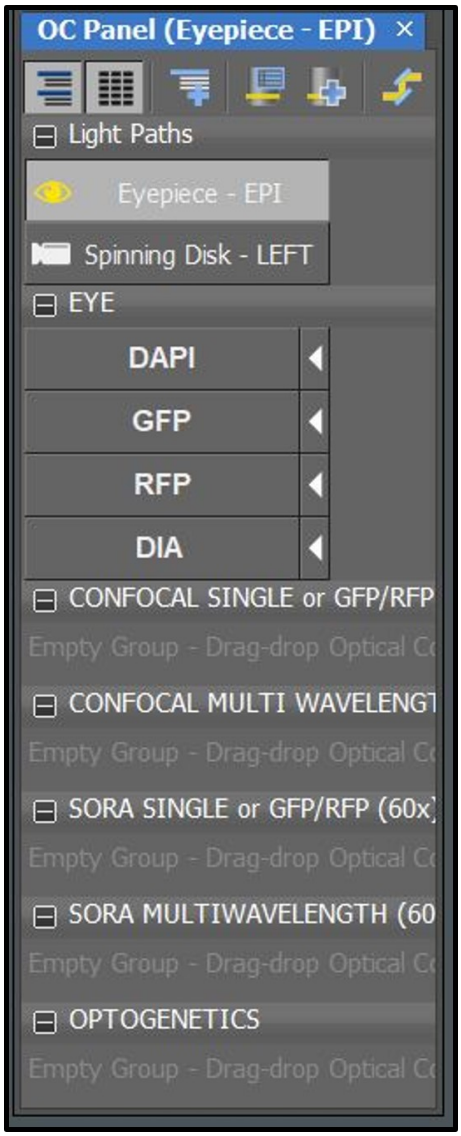

Figure 5: OC Panel (Eyes)

3.2 OC Panel (Spinning Disk)

Various options for imaging with the spinning disk unit.

Eyepiece - EPI Selects the optical configuration to image with eyepiece.

Spinning Disk - LEFT Selects the optical configuation to image through spinning disk.

CONFOCAL SINGLE or GFP/RFP Extended filter windows for GFP and/or RFP imaging.

CONFOCAL MULTI WAVELENGTH Bandpass filters for DAPI, GFP, RFP, and Cy5.

SORA SINGLE or GFP/RFP Extended filter windows for GFP and/or RFP imaging with SoRa.

SORA MULTI WAVELENGTH Bandpass filters for DAPI, GFP, RFP, and Cy5 with SoRa.

OPTOGENETICS For use with the DMD.

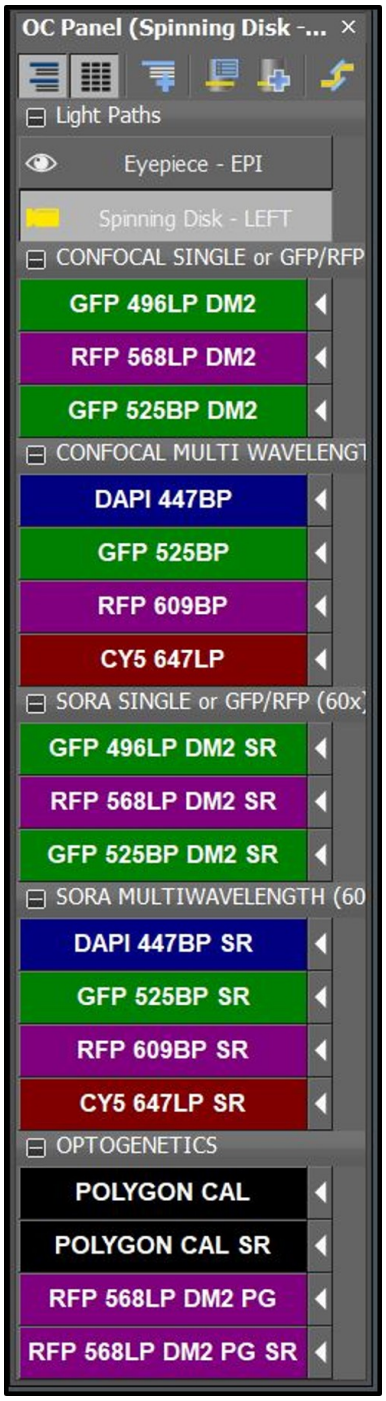

Figure 6: OC Panel (Spinning Disk)

3.3 Fusion 1 Pad

Camera setup for live and capture imaging.

Format For Live Bit depth and binning options for live (continuous) imaging.

Format for Capture Bit depth and binning options for Capture (record) imaging.

Auto Exposure The computer attempts to optimize the exposure based on signal.

Exposure Time How long the camera collects light per image.

Set Exact FPS Takes camera readout time into account.

Scan Mode

ROI Image only a Region of Interest on the camera.

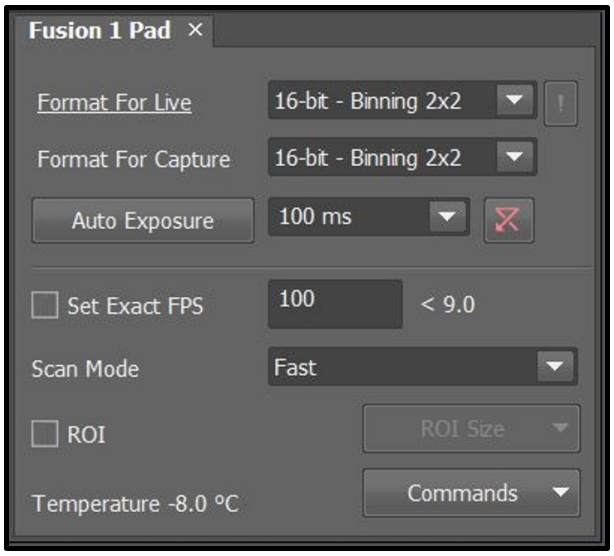

Figure 7: Fusion 1 Pad

3.4 LUN-F pad

The laser power settings for each channel.

Check Boxes Displayed channel selections. Keep **checked** at all times.

Laser Buttons Whether the laser is on or off. Keep **unchecked** at all times.

Laser Power For maximum speed, these percentages should be matched for each channel being imaged.

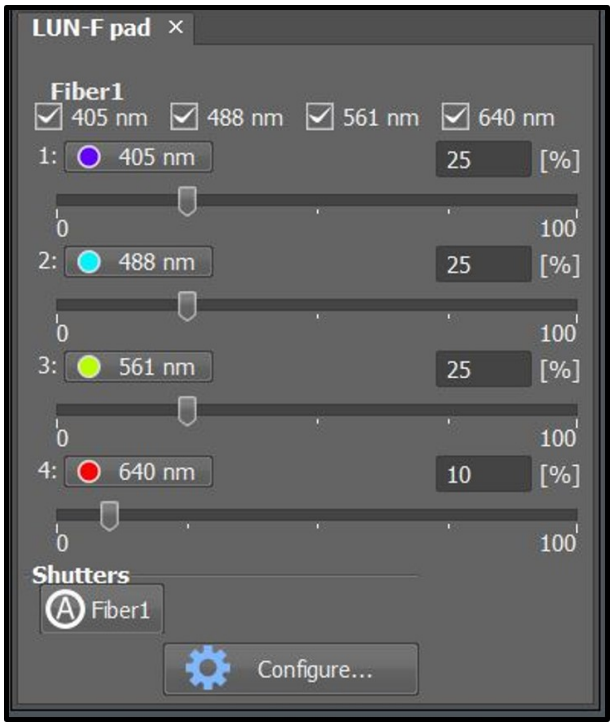

Figure 8: LUN-F pad

3.5 XYZ Navigation

Software control for moving the XY and Z stages by discrete steps. This is not commonly needed as the system can be controlled through the Mouse XY (drag and drop) and the Mouse Scroll Wheel for Z.

XY The direction to move by the amount in the XY Step box.

Coarse, Fine, FOV Determines the XY Step size.

Z1, Z2 Options for moving the Z drives by discrete steps.

XY - Ti2 XYDrive Enter values to move the XY stage to absolute positions.

Z1 - Ti2 ZDrive Enter the value to move the stepper motor to an absolute position.

Z2 - NIDAQ Piezo Enter the value to move the z-piezo to an absolute position.

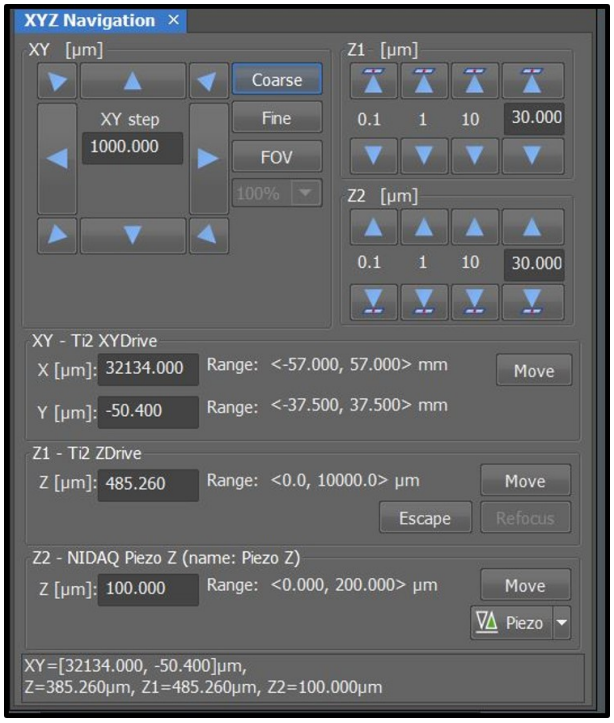

Figure 9: XYZ Navigation

3.6 LUTs

Look up Tables change the display of your image on the screen by assigning a pixel intensity to black and a higher pixel intensity to white (or the color equivalent). This is to increase contrast and does not affect how data is collected.

Autoscale Continously (red square) Each image that is acquired is automatically scaled by the dimmest and brightest pixel.

Austoscale Once (blue square) The image is scaled by the dimmest and brightest pixel once.

Oversaturation Indicator (green square) Highlights pixels that exceed the pixel depth of the camera.

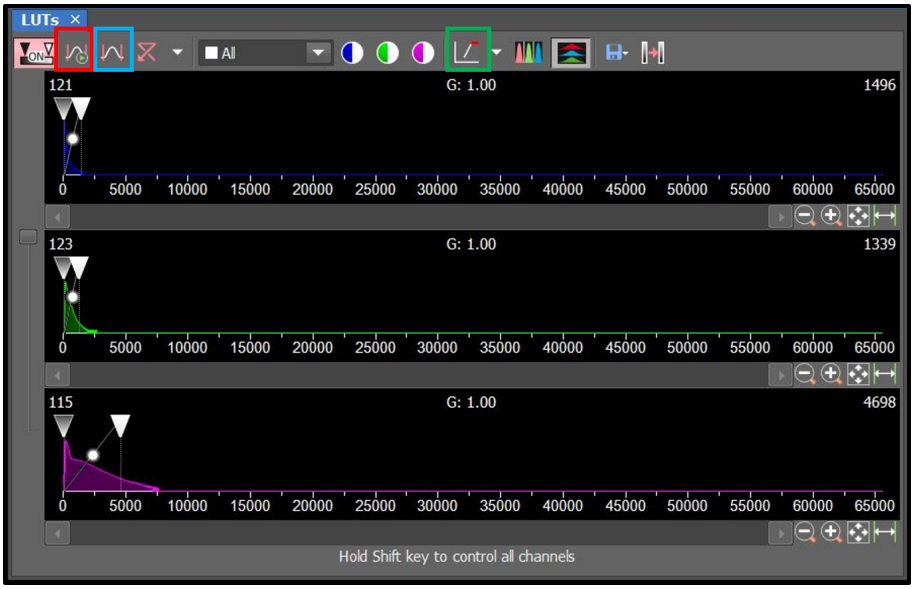

Figure 10: LUTs

3.7 NDAcquisition

Setup for multi-dimensional experiments.

Experiment ND Acquisition

Save to File Automatically saves the file to disk at the conclusion of the experiment.

Path Directory to save in.

Filename Name of the file to be saved.

Order of Experiment How the microscope goes about acquiring all the dimensions of the experiment.

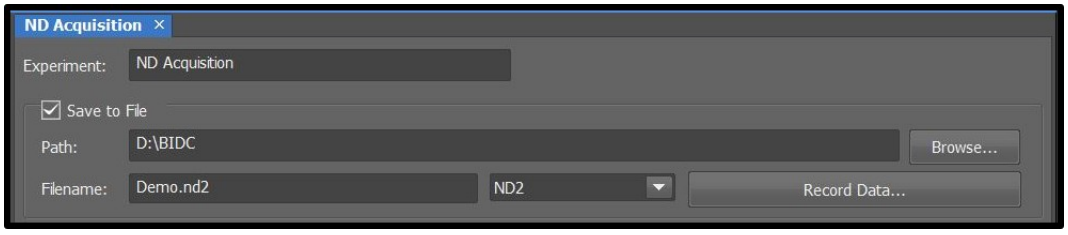

Figure 11: NDAcquisition Saving

3.7.1 Time

Setup for timelapse experiments for live-imaging.

Time Schedule Customization for different components of the timelapse experiment.

Phase Number Components.

Interval Time between images in that phase.

Duration How long the phase lasts for.

Loops How many acquisition loops (images, z-stacks, channels, etc) will complete in the phase.

Use PFS Engage or disengage the perfect focus system.

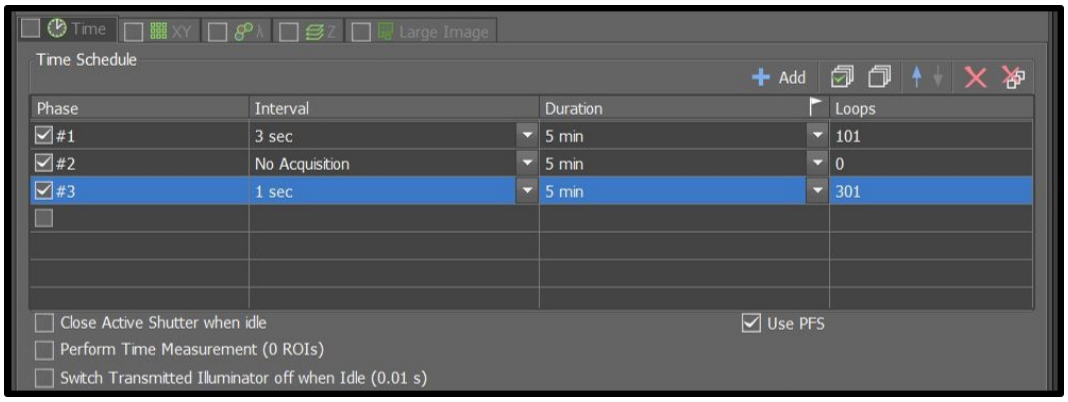

Figure 12: NDAcquisition Time

3.7.2 XY

Setup for multiple positions on the sample.

Move Stage to Selected Point Will move the stage when you highlight a position.

Point Name Description of point.

X, Y, Z Location of the point on the slide.

PFS Perfect focus system offset from the Z stage position.

Include Z Use is positions of interest are at different z positions.

Use PFS Engage or disengage the perfect focus system.

Z Device The drive that the z position is recorded from.

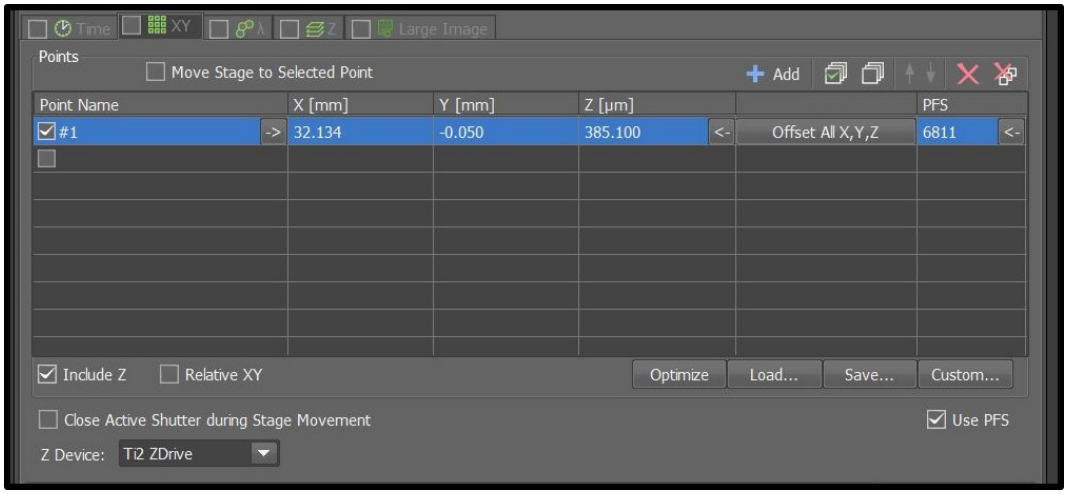

Figure 13: NDAcquisition XY

3.7.3 λ

Choose the optical configurations (channels) for imaging.

Opt. Conf. Selected optical configurations for the acquisition.

Name Recorded name in the file.

Comp. Color The LUT used for display.

Focus Offset A correction for chromatic aberrations if needed.

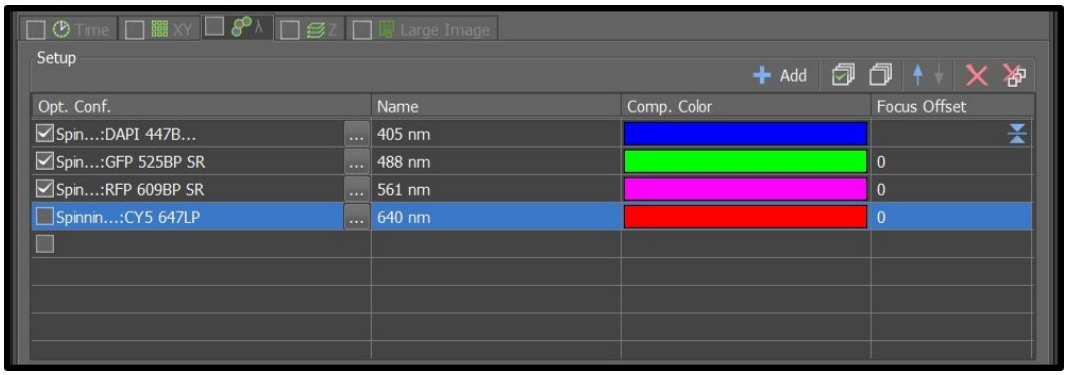

Figure 14: NDAcquisition λ

3.7.4 Z

Assigning the volume for z-stacks.

Absolute (red), Symetrical (blue), Asymetrical (green) Method for assigning Top and Bottom.

Top Assigns the current z-position as the "'Top"' of the volume (larger number).

Bottom Assigns the current z-position as the "'Bottom"' of the volume (smaller number).

Reset Clears the assigned positions.

Step The step distance between z-steps.

Range Distance between Top and Bottom of the volume.

Steps The numbers of steps calculated based on the Range and Step size.

Z Device The z-stage which performs the scan.

Direction The Start and Stop of the z-scan.

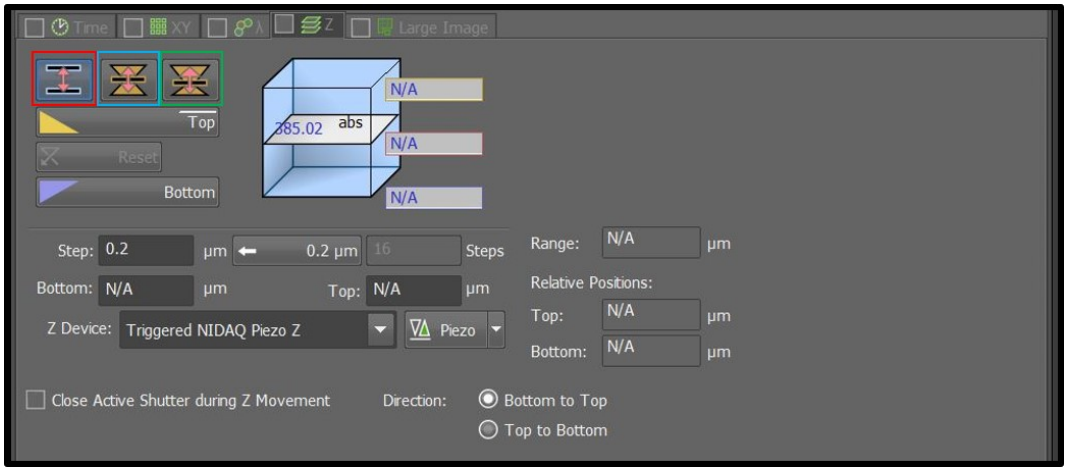

Figure 15: NDAcquisition Z

3.7.5 Large Image

The "'Tiling"' or "'Stitching"' setup of the acquisition.

Scan Area The size of the stitched area in fields [of view], millimeters, or a saved pattern.

Overlap Amount at which the tiled images are overlapped.

Stitching via: Algorithm in which the tiles are combined.

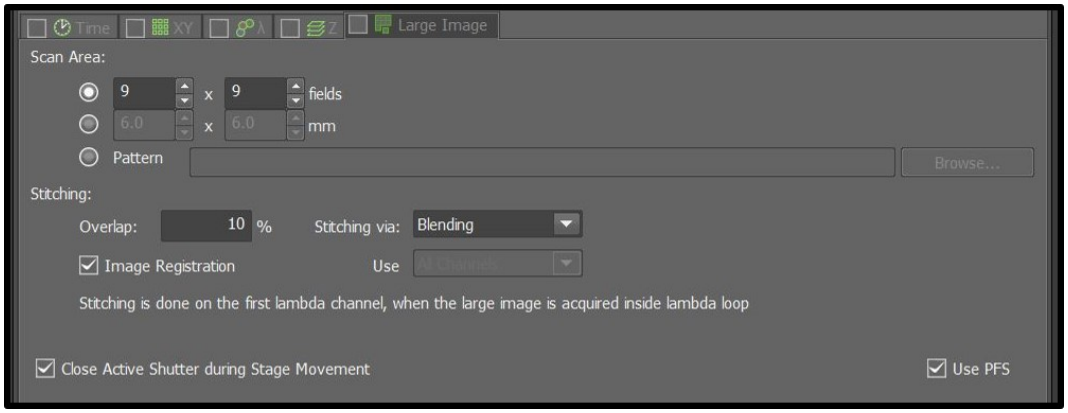

Figure 16: NDAcquisition Large Image

3.8 Ti2 Full Pad

This panel is useful for referencing the state of some microscope components.

Nosepiece Select the objective you wish to image with.

Escape Lowers the objective to the escape position. Can be returned by pressing **Escape Z** a second time.

Light Path Shows the current light path and whether perfect focus is engaged.

Z Drive Another option for moving the **Z Drive** by a specific amount.

DIA The light intensity for brightfield.

Shutters Displays the status of the **EPI** and **Polygon** shutters.

Filters Displays which filters are engaged in the two turrets.

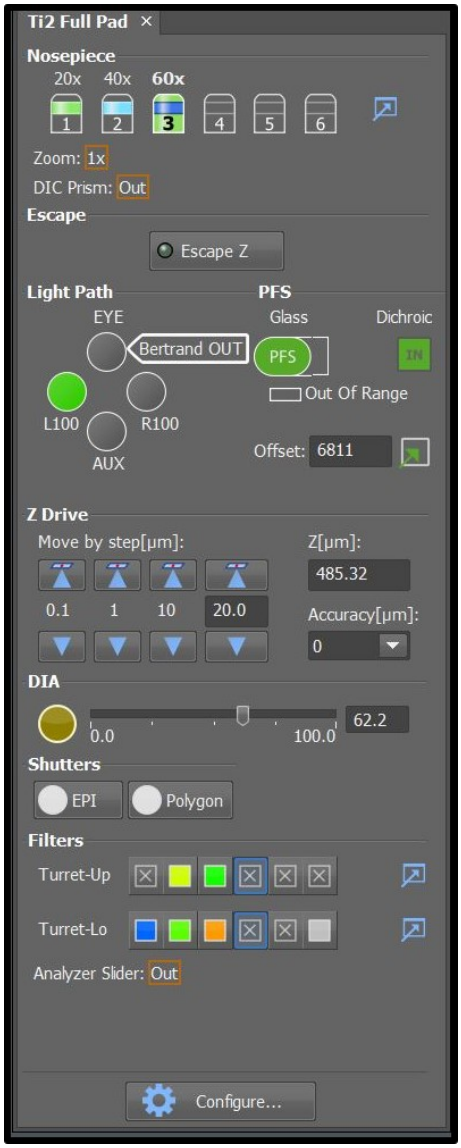

Figure 17: Ti2 Full Pad

3.9 CSU-W1 Pad

This panel shows the settings and configuration of the spinning disk unit.

Observation Mode Displays whether the system is in confocal or widefield mode.

Pinhole Displays whether the pinhole for spinning disk confocal (50 µm) or SoRa is engaged.

Disk Speed Sets the speed at which the pinhole disk rotates. **Sync** can be used to remove streaking artifacts that arise as fast frame rates.

Aperture Size of the aperture.

Filter Wheel Display of what filters are currently engaged for confocal.

SoRa Magnifier Displays which magnifier is being used.

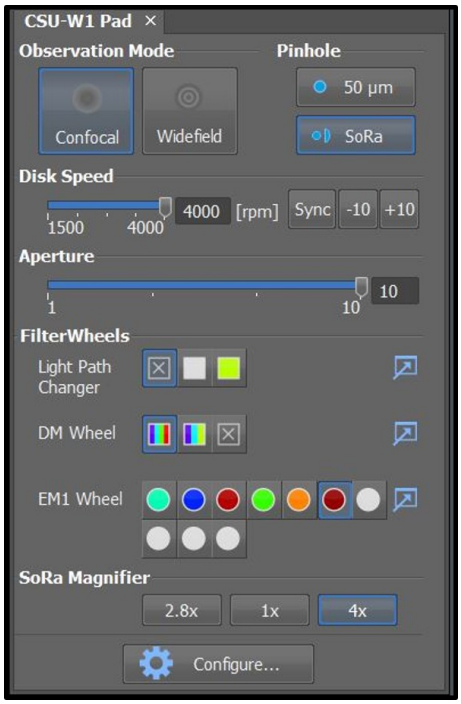

Figure 18: CSU-W1 Pad

3.10 Mightex Polygon1000 (DMD)

This panel is for the DMD for optogenetics experiments.

Diode Powers Power in percentage.

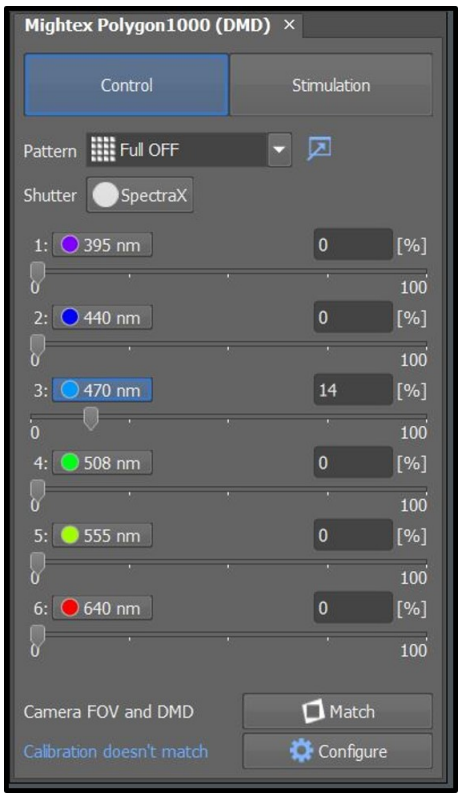

Figure 19: Mightex Polygon1000 (DMD)

4 Data Analysis

The microscope comes with 3 offline analysis seats that can be accessed:

1. Remotely on your laptop or desktop computer.
2. Remotely on the BIDC's analysis stations (useful if you need a powerful machine).

There are two Nikon specific analysis modules that can be useful.

Deconvolution To take full advantage of SoRa imaging, the data needs to be deconvolved. Use the 3D deconvolution option. Enter the excitation and emission value in the bottom of the screen. There are various deconvolution algorithms. It can take time to find the best algorithm for your dataset.

Denoise.ai This module uses AI to find and remove shot noise from images. It can be quite powerful on low signal images.

5 Shut Down Procedure

It is important to leave the microscope clean and in a consistent state for the next user.

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1. Make sure your images are saved and start transferring them to your hard drive.
2. Exit the software.
3. Return the gas flow rate to 0.0.
4. Lower the objective and take off the sample holder and sample.

5. Carefully remove the objective and screw it into the case cap.
6. Clean the objective of oil or water using the provided solvent and lens paper.
7. Store the objective "'upside down"' in the environmental chamber.
8. Turn off the two switched in the server cabinet.
9. Turn off the computer through the Start menu.
10. Ensure the area is clean and ready for the next user.

6 Trouble Shooting

When finding the sample by eye, the image has a very bright background Make sure the light inside the environmental chamber is off.

The lamp light is not illuminating the sample Be sure you are opening the correct shutter. Click the filter in the **OC Panel - Eyepiece** to reset the configuration.

7 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/emergency assistance and no one is available in the office, or it is after business hours, please call the **BIDC Hotline** at 415-745-2432.