Quick Start: Structured Illumination Microscopy with Nikon Elements

Starting the Instrument

There are 3 separate pieces of equipment to turn on. Each piece of equipment has a numbered orange label, and is listed below.

1. Lasers – To the left of the table.
(2. For confocal only – NOT NEEDED)
3. Hamamatsu Camera – Attached to the bottom right side of the microscope.
4. LEDs – On shelf above microscope to the far right.

Stage Preparation and Sample Placement

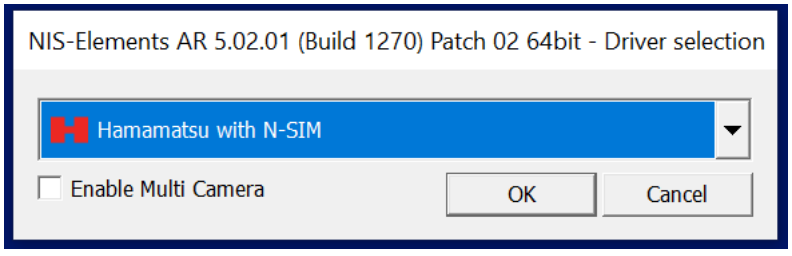
The SIM has multiple stage inserts available for both live and fixed samples. Prior to use, users should select the stage insert that best suits their experimental needs. Stage inserts click into place in the main stage, allowing users the ability to quickly swap out stages.

With a suitable stage insert in place, users should select the objective that best fits their imaging application. Switching objectives can be done in Nikon Elements on the Ti2 Pad (see The Ti2 Pad section). Always check to make sure the objective is clean regardless of if the objective is an immersion objective or not. With a clean objective, users can now add immersion media to the objective, or proceed to placing the sample into the stage insert.

Nikon Elements

Start the software by clicking the Nikon Elements icon on the desktop labeled NIS-Elements AR Acquisition.

As Elements begins to load the user will be presented with window allowing the user to select the mode to be used. If “Hamamatsu with N-SIM” is not selected, select it from the drop down menu. The “Enable Multi Camera” option should be left unchecked.



Once Elements opens the user will be presented with an interface that consists of a main window and a series of tabbed sub-windows that the user can arrange and move around as needed. These tabbed windows can be connected and disconnected from one another by dragging and dropping the tabs.

The Main Window

Once Nikon Elements has fully loaded, check the bottom left corner of the main page to see if the SIM tab is selected as shown below. If it is not selected, select it now. This will assure the proper interface for SIM is loaded.

While the main window provides the user with a multitude of options, users will only regularly use the buttons shown below. The menu bar at the top of the main window (shown below) allows users to quickly load or save acquisitions.

This menu bar also allows the user to initiate a live capture, a single capture, or stop the current capture; however, controlling capture from this menu is only recommended if using a Widefield OC. If the system is in SIM mode, it is best to control capture via the N-SIM pad (see N-SIM Pad section).

Another useful piece of the main window is the Close All button on the left side of the main window. This button will close all of the current open data windows. If there are windows containing unsaved data, the user will be prompted to save the data prior to Elements closing the window. The OC Restore button also resides on the left side of the main window. This button will restore all of the OCs to their current stock settings. This is best used if an OC appears to quit working or break during use.

The Sub-Windows

The OC Panel

The OC Panel consists of a series of preset system parameters that categorized by use. When an OC is selected, Elements loads and sets all of the associated system parameters. The OCs allow users to quickly switch between different system functions in a single click. Once an OC is loaded only minor tweaks, such as laser power or camera integration times, are needed prior to collecting images.

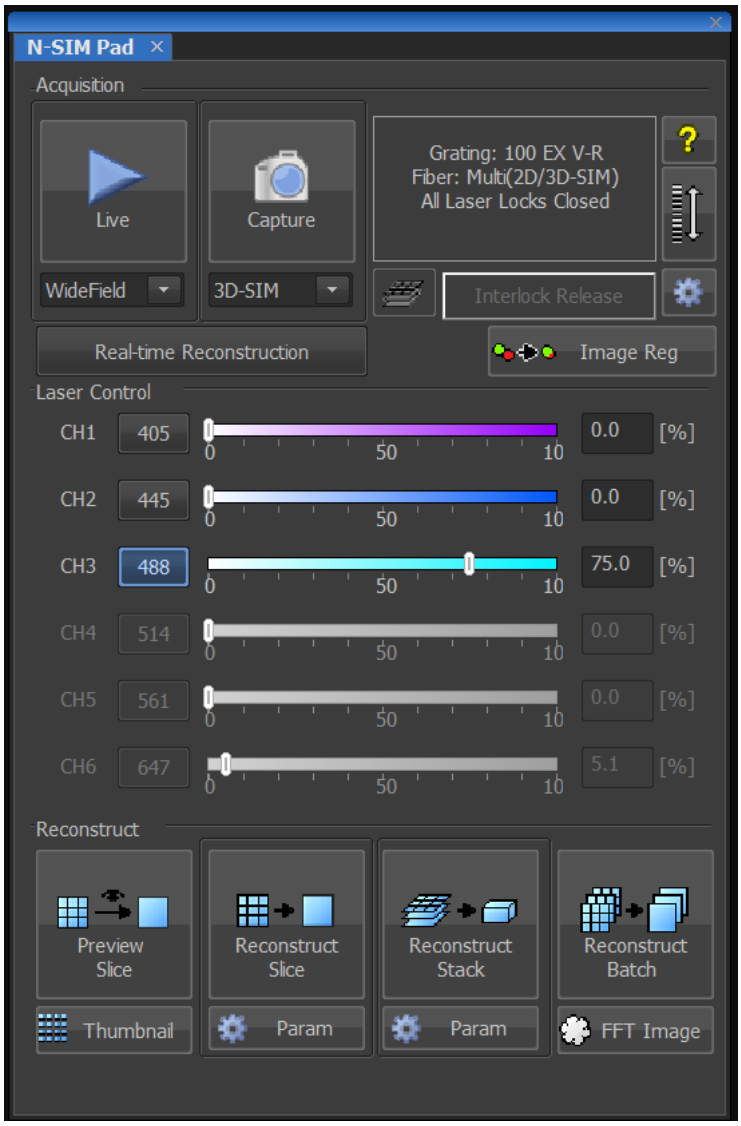
As the user makes adjustments to the OC, a red exclamation point will show up next to the arrow box in the active OC. To keep the changes made to the OC, click the arrow box next to the exclamation point. This will update the OC with the current user settings. If the user does not want to save the settings, reloading the OC (double clicking the current OC) or switching to a different OC will discard the changes.

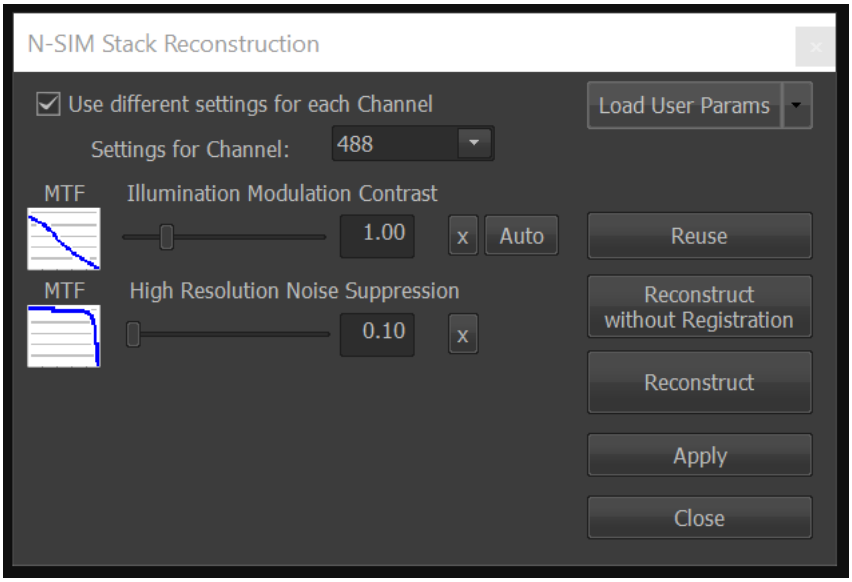
The N-SIM Pad

The N-SIM pad is used to set SIM acquisition parameters, initiate image capture, and reconstructed collected SIM images. The Live button initiates a live capture utilizing the imaging modality selected from the drop down list below the button. In Live mode it is common to utilize WideField acquisition to bring the sample into focus and to find areas of interest.

The Capture button initiates data capture using the imaging modality from the drop down list below the button. For z-stacks users should select 3D-SIM, while 2D-SIM can be used for single plane imaging. The active laser and laser parameters used are set by clicking and highlighting the wavelength of interest. Laser power is then set by adjusting the slider next to the wavelength.

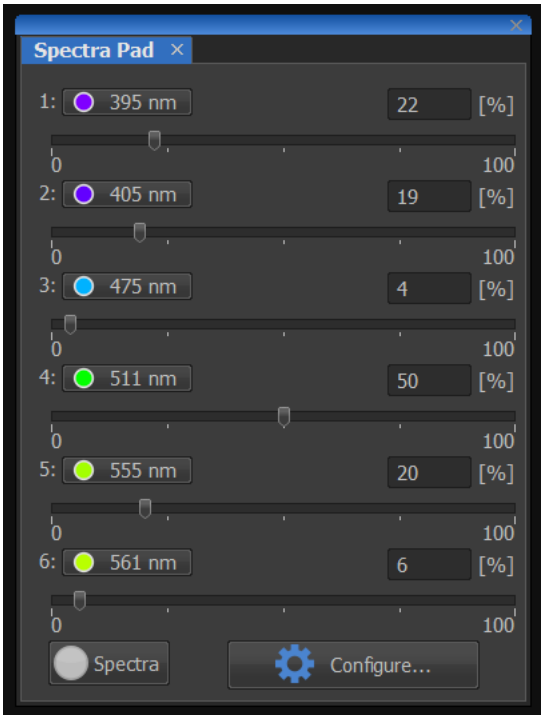
The collected SIM images must be reconstructed in order to achieve the desired resolution increase. Users can reconstruct a single plane by selecting the Param button below the Reconstruct Slice button, or an entire stack of images by selecting the Param button below the Reconstruct Stack button. An example of the N-SIM Stack Reconstruction can be seen below. Reconstruction parameters can be tuned by the user, and the stack can be reconstructed by clicking the Reconstruct button.





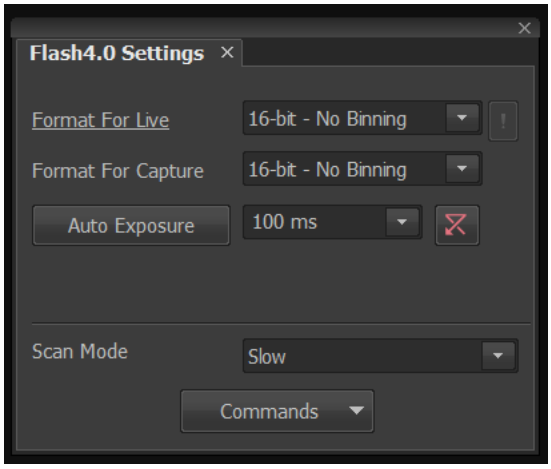
The Spectra Pad

The Spectra pad is used to select the active LED, set LED power, and open/shutter the LED source. LED power is adjusted via the slider, while the active LED is selected by highlighting the LED wavelength. The LED will auto shutter when not in use, however the shutter can be opened manually by clicking the “Spectra” button.



Flash4.0 Settings (The Camera Window)

The Flash4.0 Settings window allows the user to adjust the camera settings used for acquisition. Users will normally only adjust exposure time for optimal acquisition, however the window does allow users to change the scan speed and initiate binning.

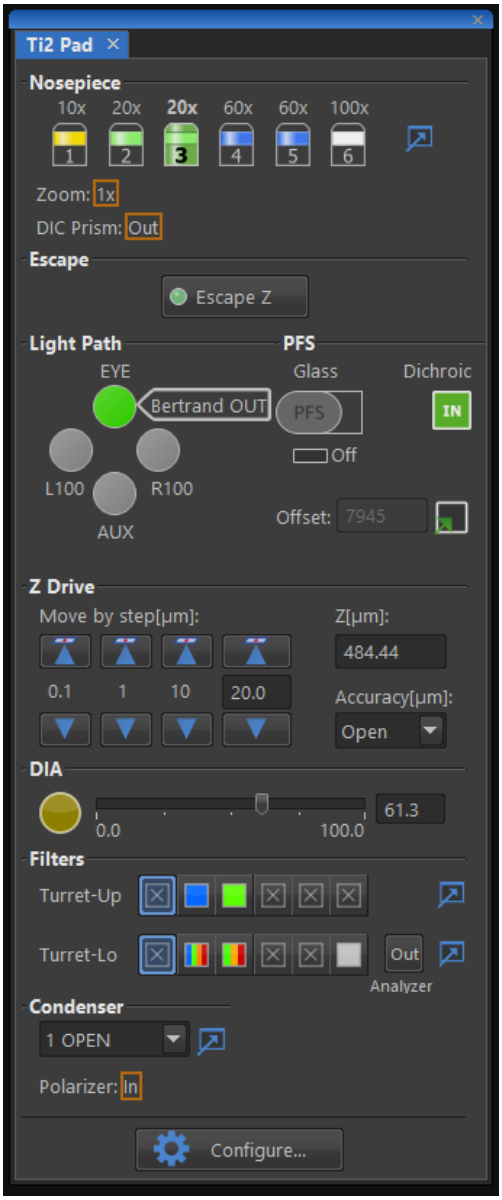


The Ti2 Pad

The Ti2 Pad controls all of the basic functions of the microscope. Selection of the objective can be done here. By hovering over any of the available objectives, the properties of each objective are displayed. Elements will also display if the current objective has a DIC prism in place. DIC PRISM MUST BE REMOVED TO ACQUIRE A SIM IMAGE.

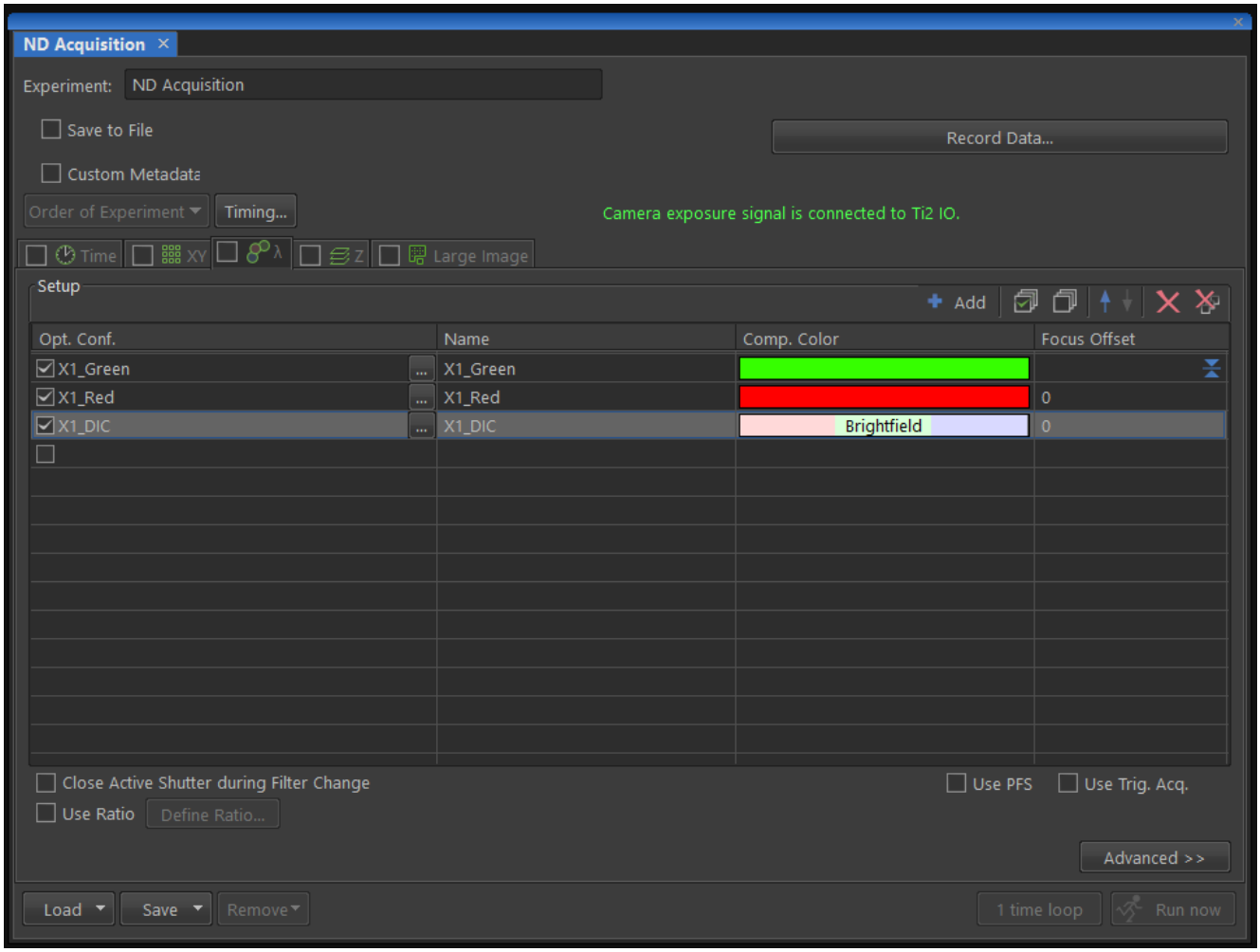
The Perfect Focus System (PFS) is controlled via the Ti2 Pad. Perfect focus maintains a set distance between the objective and the coverslip, to prevent any focus drift during long acquisition periods. To set the perfect focus, bring the sample into focus and press the box with the green arrow. This sets the offset for the Perfect Focus and initializes the system. PFS DICHROIC MUST BE IN TO ACQUIRE A SIM IMAGE.

The Ti2 Pad has multiple additional functions; however, it is not recommended that users alter these settings. Many of these settings, such as the light path and filters, are saved in the OC's and do not require user adjustment.



ND Acquisition

The ND Acquisition window is used to set acquisition parameters for complex image captures.



Time – The Time tab is used when acquisitions will occur over a large time period, but do not require continuous acquisition. Phases with a defined duration and set intervals can be set.

XY – If there are multiple areas of interest within a sample, the XY tab can be used to set the X,Y,Z coordinates of a series of areas to be image.

λ - The λ tab is used for multi-color acquisitions. The colors of interest are set by selecting OC's that capture the colors of interest.

Z – The Z tab is used to collect image stacks that scan through the Z axis of the sample based on the user entered parameters.

Large Image – The Large Image tab is used to create images larger than the field of view. This is done by taking multiple fields and stitching them together.

The LUTs Window

The LUTS window allows the user to adjust the look up table (brightness/contrast) for each channel in the images displayed. LUTs only adjust how the data is displayed on the screen. The tables can be scaled manually or automatically using the buttons in the top left corner of the window. For highest image quality, the pixel intensity histograms should extend to roughly half the x axis of the histogram (roughly 30,000-40,000 counts in this example).