Quick Start: Spinning Disk Confocal with Nikon Elements

Starting the Instrument

There are 4 separate pieces of equipment to turn on. Each piece of equipment has a numbered orange label, and is listed below.

1. Microscope
2. Spinning Disk Unit

3A. EM-CCD (3B only used for dual camera acquisitions)

4A. Lasers

Optional Equipment – The equipment listed is not required for conventional use, but may be required for specific experiments (such as live cell imaging).

- Piezo Stage – The switch on the front of control box with the MCL logo. Box sits below the table.
- Live Cell Incubator – The control box is labeled Tokai and is located on the floor below the table. If CO_2 is needed, the tank is located in the far corner of the room behind the SIM system.
- Second EM-CCD – Switch 3B. Only needed for dual camera acquisition.
- Dichroic Cube – Used only for dual camera acquisition. Cubes are located in a box next to the laser source. To insert cubes into the splitter, remove the cover and gently slide the cube into the splitter. Cubes are locked in place magnetically. Replace cover prior to use.
- TIRF Lasers – Switch 4B on the laser source.

Stage Preparation and Sample Placement

The SDC 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 based on their needs.

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.

Once Elements opens you will be presented with a user interface consisting of a main window and a series of sub-windows that can be connected and disconnected from one another by dragging and dropping the tabs on each window.

The Main Window

While the main window provides the user with a multitude of options, it can be simplified down to the buttons shown below.

The play button initiates Live mode, where the instrument will continuously collect images with the current instrument configuration. This is best used to find the regions of interest and to determine the optimal acquisitions settings. The camera button will also initiate a collection with the current instrument settings, however it will only perform one interval.

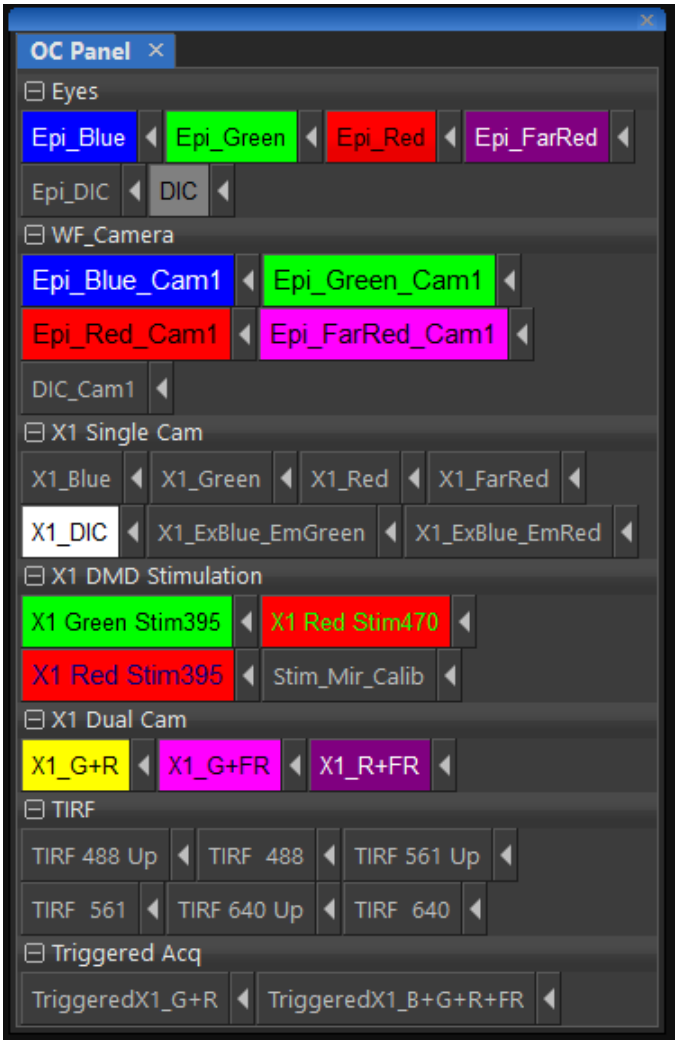
Other useful buttons in the main window are the close all button and the restore OC button. The close all button (pictured top right) will close all of the current open data windows. You will be prompted to save any unsaved data. The restore OC button (pictured bottom right) will restore the OC's to their original settings. This is best used if an OC appears to quit working or break.

The Sub-Windows

The OC Panel

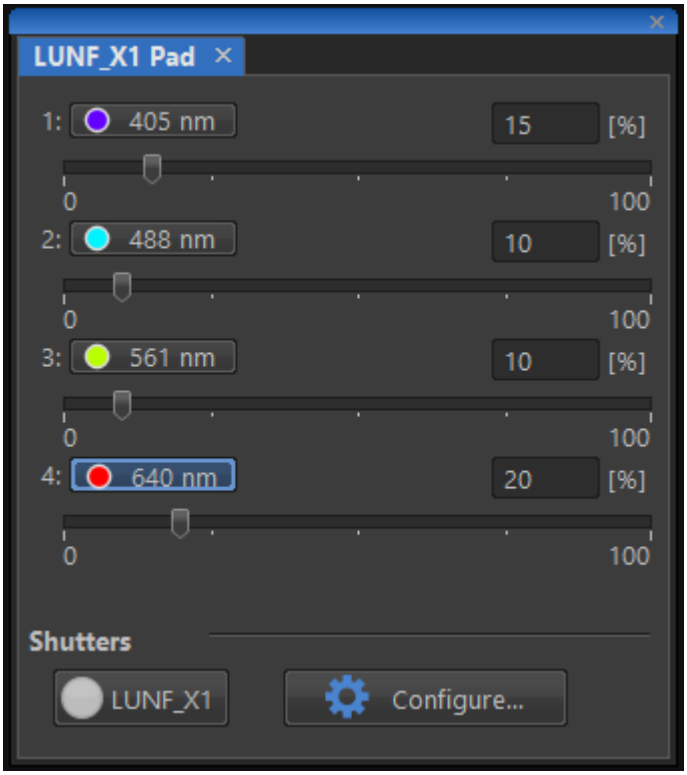
The OC Panel (pictured below) consists of a series of preset system parameters. The OC's are categorized by use, and can be modified as needed. When an OC is selected, Elements loads the system parameters that are saved to the OC. Once loaded only minor tweaks, such as laser power or camera integration 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.



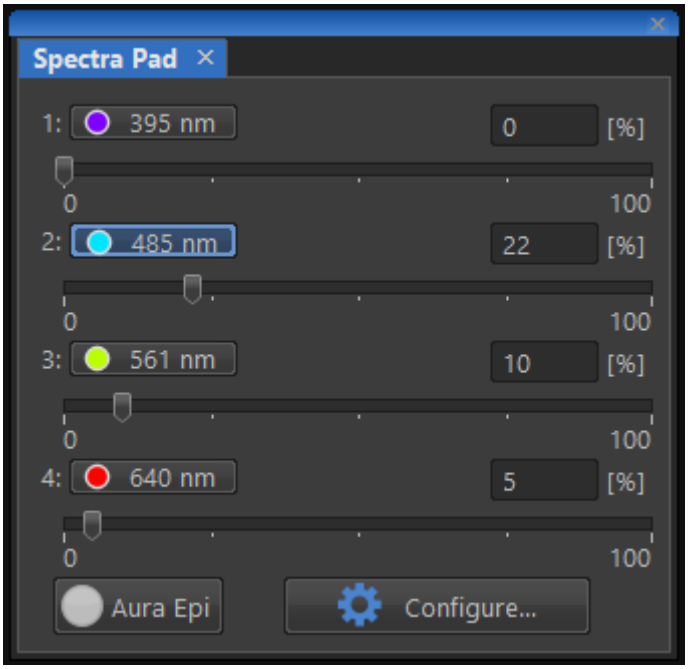
The X1 Pad

The X1 Pad is used to select the active laser, set laser power, and open/shutter the laser source. Laser power is adjusted via the slider, while the active laser is selected by highlighting the laser wavelength. The laser will auto shutter when not in use, however the shutter can be opened manually by clicking the “LUNF_X1” 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 “Aura Epi” button.

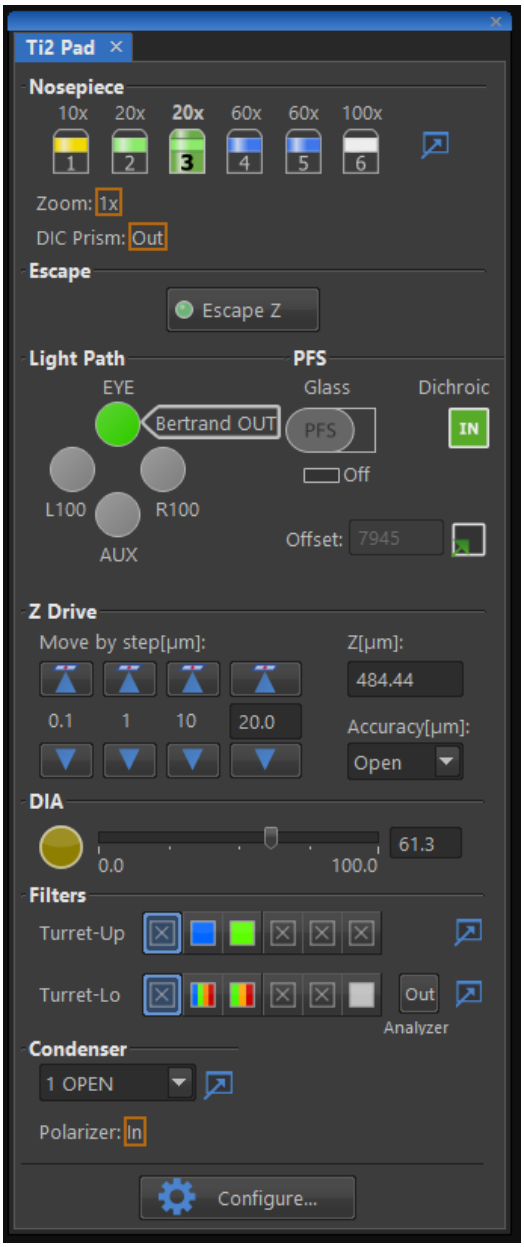


The Ti2 Pad

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.

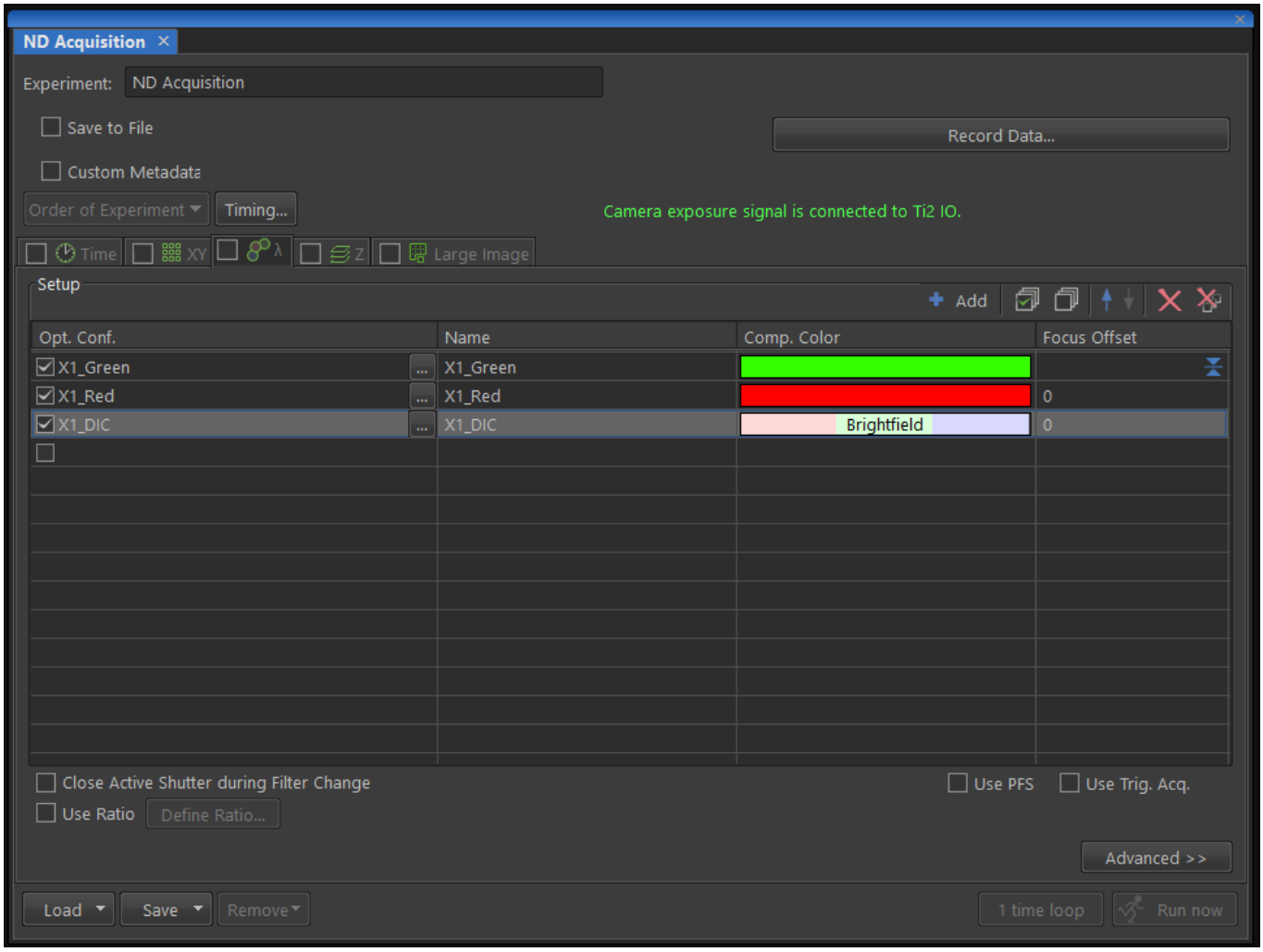
The Perfect Focus System (PFS) is controlled via the Ti2 Pad. Perfect focus maintains a set distance between the objective and the coverslip, to prevent any focus drift during long acquisition periods. To set the perfect focus, bring the sample into focus and press the box with the green arrow. This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

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.



The ND Acquisition Window

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.

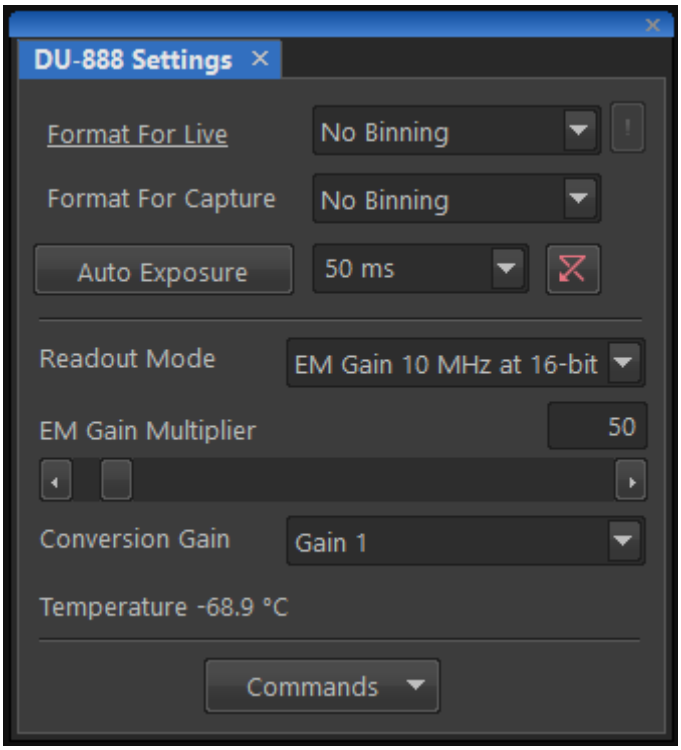
λ - The λ tab is used for multi-color acquisitions. The colors of interest are set by selecting OC's that capture the colors of interest.

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 capture images larger than the field of view. This is done by taking multiple fields and stitching them together.

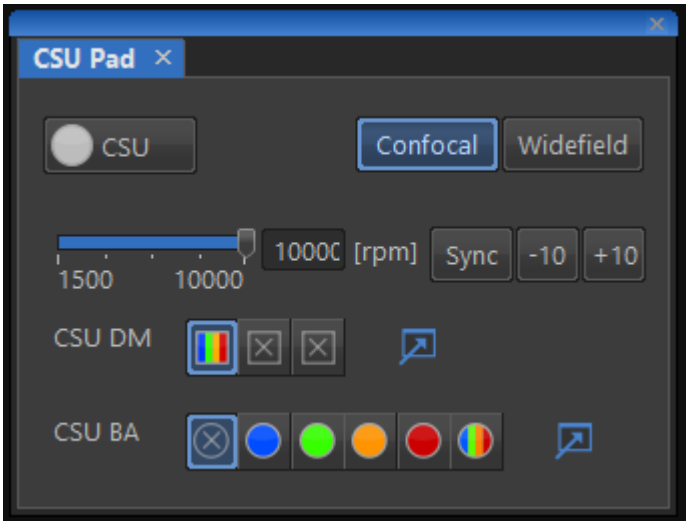
The DU-888 Settings Tab

The DU-888 Settings tab controls the camera settings. The camera gain, readout mode, and exposure time are all set here. Exposure times and gain are sample dependent and are saved for each individual OC, allowing the system to use optimal camera settings for each color imaged. **Settings shown here are for example only and are unlikely to be optimal for your experiment.**



The CSU Pad Tab

The CSU Pad tab controls the speed at which the spinning disk rotates. Additionally, users can select the light path within the spinning disk unit and the emission filter used. The light path and filters are pre-set in the OC and do not require adjustment by the user.



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).