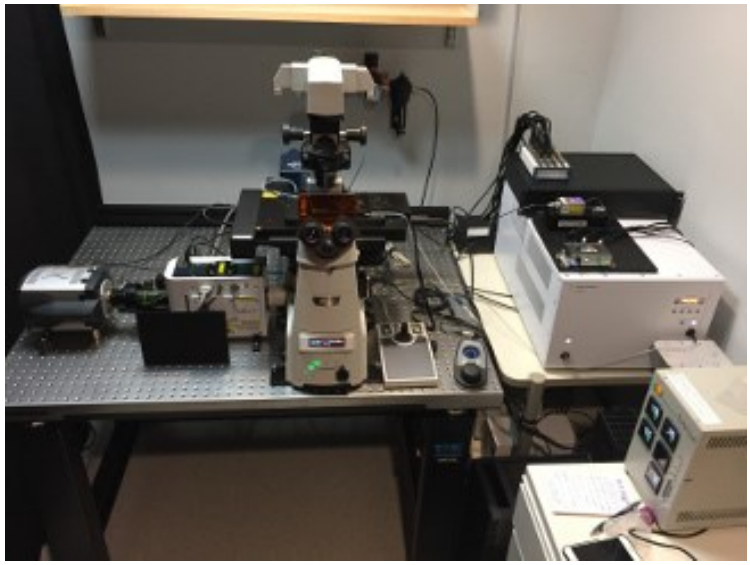
VANDERBILT School of Medicine | Cell Imaging Shared Resource

Nikon Spinning Disk Confocal (Opry)



Information

- Location: **MCN T-2216**
- Facility is open 24/7 to trained and badged users
- Book systems through iLab
  - Click on Schedule Equipment Tab
  - Click on "Confocal Microscopy"
  - Click on Nikon Spinning Disk

CENTER of EXCELLENCE
supported by Nikon

Acknowledgement for Cell Imaging Shared Resource

Our funding depends upon your acknowledgement of the Cell Imaging Shared Resource (CISR) when you publish or present data obtained with equipment or services from the CISR. This includes images from the microscopes, training in the use of software, use of image processing systems, experiment design, etc. Because we are fortunate to have so much Center support, the list of grant numbers is large.

Acknowledgment statement for using Nikon Spinning Disk Confocal:

"Experiments/Data analysis/presentation [include equipment] were performed in part through the use of the Vanderbilt Cell Imaging Shared Resource (supported by NIH grants **CA68485**, **DK20593**, **DK58404**, **DK59637** and **EY08126**)."

Please let us know when you publish, and if possible, send us a reprint of the paper.

You can also send us your images to broadcast on our TVs.

Technical Information

Microscope:

The Nikon Eclipse Ti inverted microscope incorporates a Yokogawa CSU-X1 spinning disk head, 1200x1200 pixel Photometrics Prime 95B sCMOS camera, high-speed piezo [Z] stage with 100µm range, live cell incubator, automated and encoded X/Y stage, a four-line high-power solid state laser launch, and a full complement of objectives.

Objectives:

Plan Fluor 4x (air) 0.13 NA WD 17.2mm

Plan Apo Lambda (air) 10x 0.45 NA WD 4.00mm

Plan Apo Lambda (air) 20x 0.75 NA WD 1.00mm

**Plan Fluor (oil) 40x 1.30 NA WD 0.24mm

**Plan Apo Lambda (oil) 60x 1.40 NA WD 0.13mm

**Apo TIRF (oil) 100x 1.49 NA WD 0.12mm

NOTE: Oil can be used only on the 40x, 60x, & 100x!

Nikon Laser Launch Diode Laser Lines:

405nm, 15mw

488nm, 15mW

561nm, 15mW

647nm, 15mW

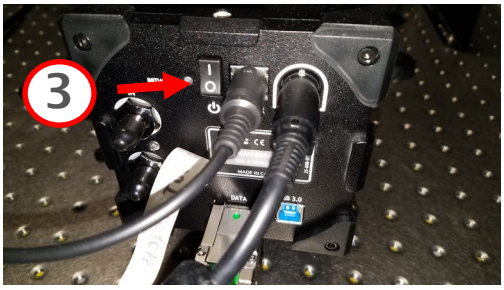
Obis Photo-Stimulation Laser:

405nm, 100mW

Start-Up

(1) Log into iLab to Start your time.
(2) Remove dust cover and place in lowest (3rd) drawer of cabinet. **Do not put on the shelf or floor.**
(3) Flip the switch on the left side of the Photometrics Prime 95B camera. Wait until the amber light stops flashing.


Camera



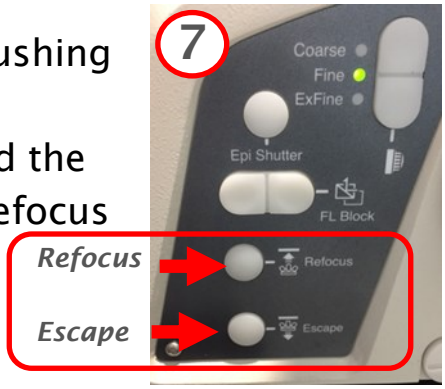
(4) Flip the red switch, marked "1" on tape, on the front of the Tripp-Lite (line conditioner) to the on position located on the shelf underneath the microscope.

(5) Turn the key, marked "2" on tape, on the laser launch from the off (vertical) to on (horizontal) position.

(6) Turn on the computer.

Start-Up (cont)

(7) Make sure that the objectives are lowered by pushing the Escape button on the right side of the inverted microscope to bring the objectives down, then hold the Escape button while simultaneously pushing the Refocus button. If you started up the system, the objective will be escaped by default.



If the system has been on before you, pressing the "Refocus" button to bring the objective up will cause the objective to return to the position it was in previous to your session, which may cause damage to your sample and the objective if this position is too high.

(8) If using an oil immersion objective (40X, 60X, 100X), apply a SINGLE drop of Type NF2 oil to the front lens of the objective. Allow gravity to drop the oil. **Never touch the glass rod to the objective!!**

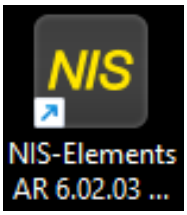
NOTE: It is not recommended to image a sample that has already been imaged using another type of oil, however, if this cannot be avoided, be sure to thoroughly remove all previous oil from the coverslip with Sparkle® (or other cleaner approved by CISR) to avoid mixing different types of oils (which causes emulsions and significantly degrades image quality).

Image Acquisition

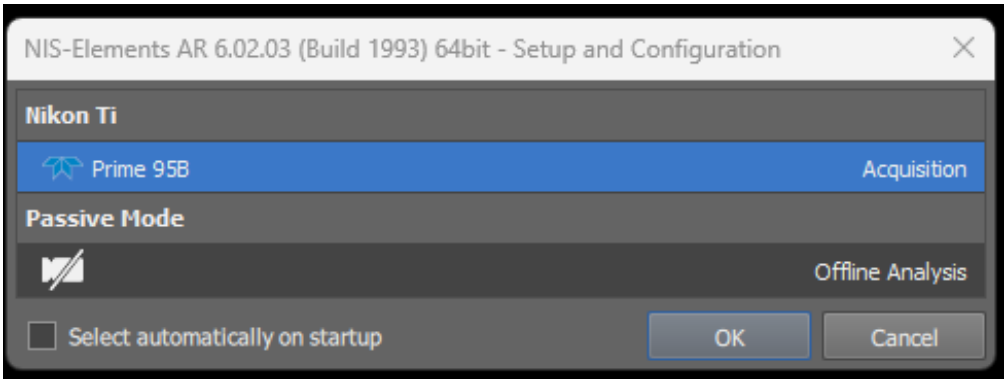
Place your slide on the stage, coverslip down towards the objective.

(1) Log into Windows with your VUNetID and password. Open NIS-Elements.

If VUMC Employee: use Dual Identity VUID acquired through *Pegasus* and password.



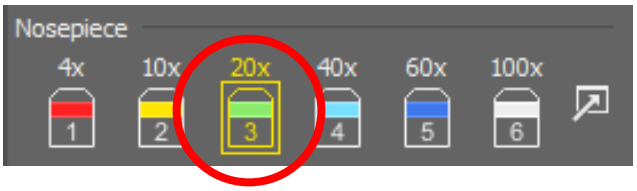
(2) Select Prime 95B Acquisition. Click OK.



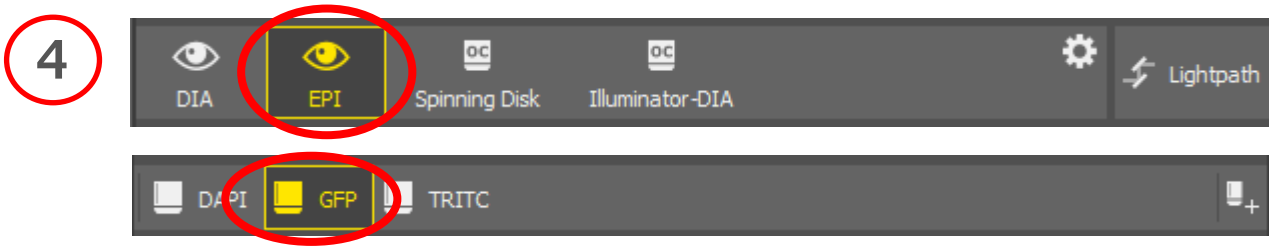
(3) Select your Objective by clicking on an objective button on the Ti Pad. Selected objective will be highlighted in yellow.

Remember:

Oil only for 40x, 60x and 100x

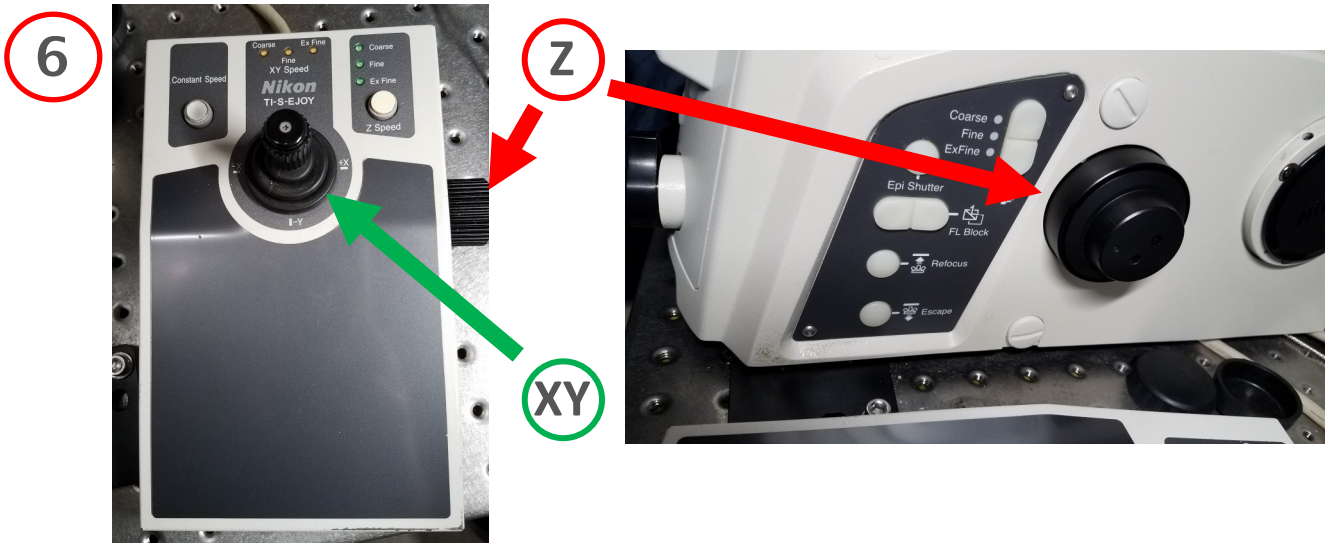


(4) On the Lightpath Panel, click the "EPI" tab. Select corresponding OC to one of your fluorophores. GFP selected in example.



(5) Click the mode button on the Lumencor device located next to the joystick on the floating microscope table, to unshutter the white light.

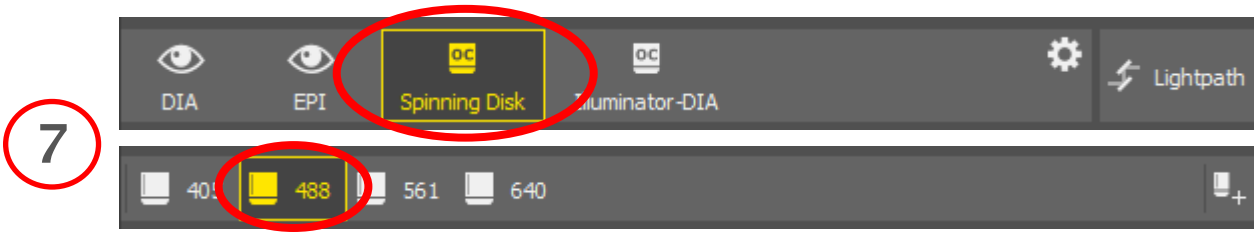
(6) Use the joystick (to the right of the microscope) and the focus knobs (on the microscope or on the joystick) to find and focus your sample.



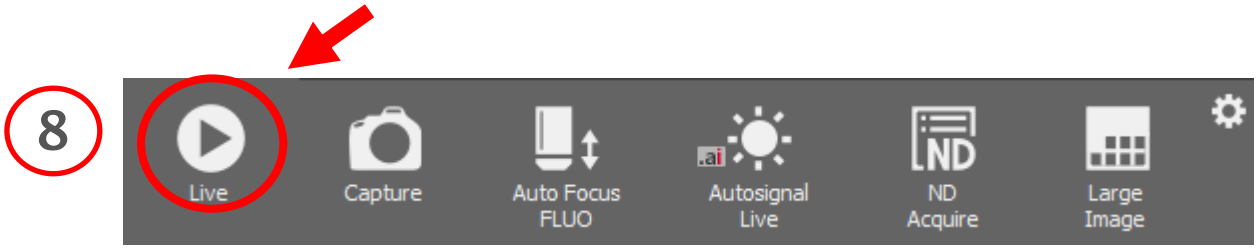
Z movement is controlled either by the knob on the right side of the joystick or by the large focus knobs on either side of the scope. Z-speed can be changed to course, fine, or extra-fine with buttons next to the focusing knobs, or on the joystick.

X and Y stage movement is controlled via the joystick to the right of the scope. Stage speed can be changed to course, fine, or extra-fine by twisting the knob at the top of the joystick.

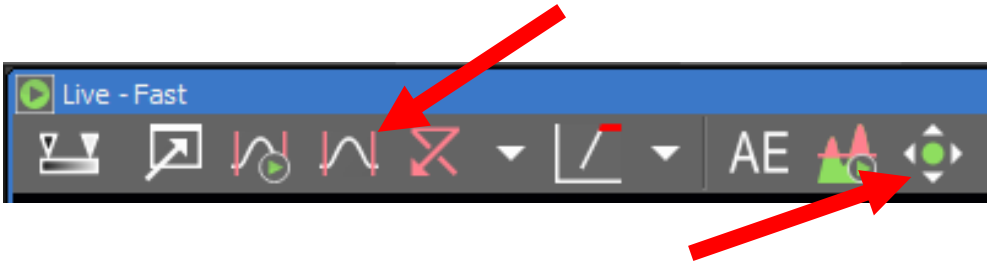
(7) Once you have found and focused on your sample, on the Lightpath Panel, click the “Spinning Disk” tab. Select corresponding OC to one of your fluorophores. 488 selected in example.



(8) Click “Live” from the Acquisition tab. A new window will pop up, and you will see your sample.



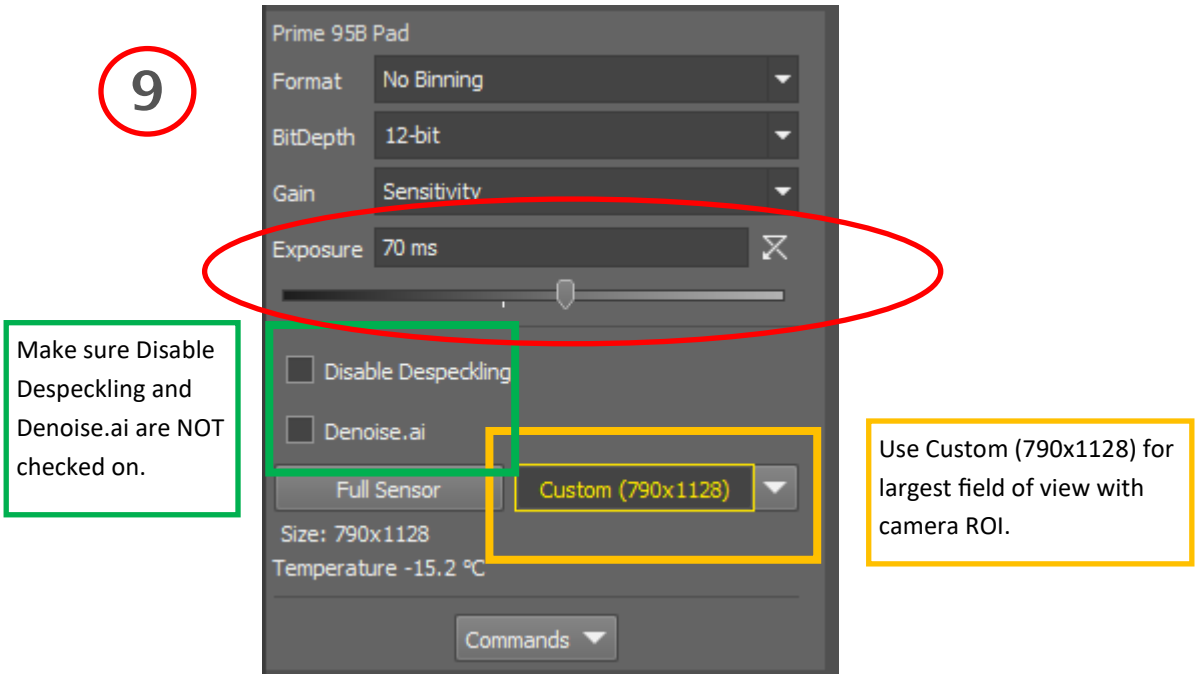
If the image looks blank, click Auto-scale from the Live window, then adjust focus with scroll wheel on the mouse to visualize the sample.



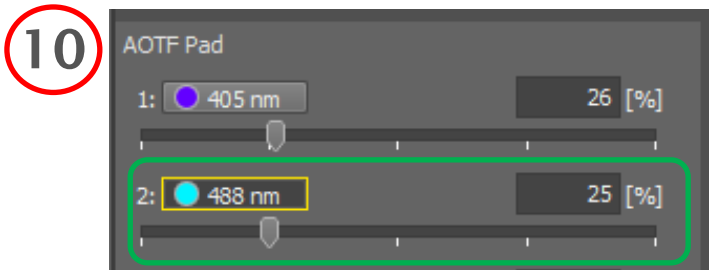
For Navigation: Click on the compass button on the Live window to enable the mouse pointer to click and drag the image to reposition.

****** To improve signal to noise ratio, you will need to adjust exposure time and laser power settings. ******

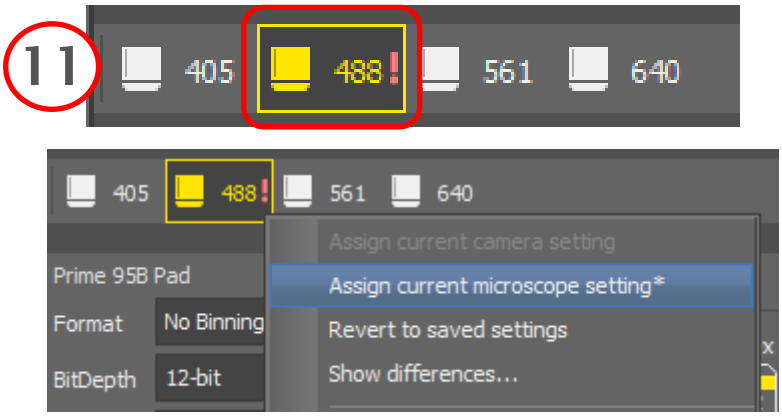
(9) Adjust **exposure time** on Prime 95B Pad by either typing in a time duration or clicking and dragging the slider.



(10) Adjust **Laser power** in AOTF Pad by clicking and dragging the slider associated with selected OC or typing in a number. 488 nm highlighted in **Green**, in example.

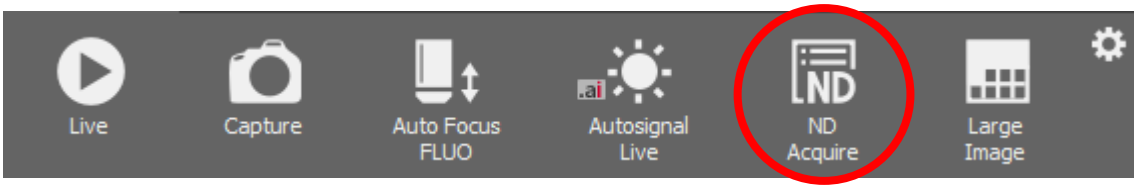


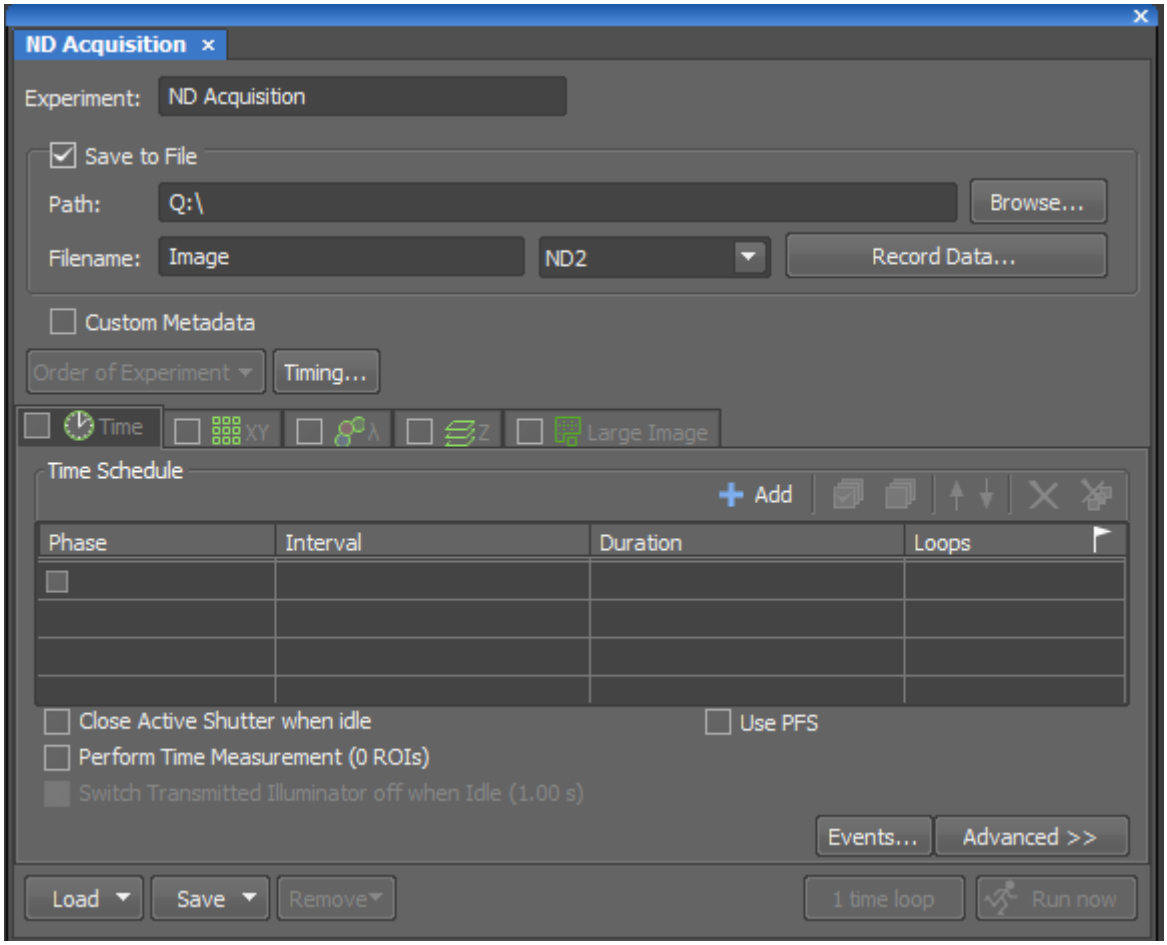
(11) After laser power adjustment, a red exclamation mark appears on OC. Right click on OC and click Assign current microscope setting to save



(12) Click Auto-scale again to adjust LUTs after each adjustment. Repeat steps 8 through 11 for each Spinning Disk OC that corresponds to your fluorophores.

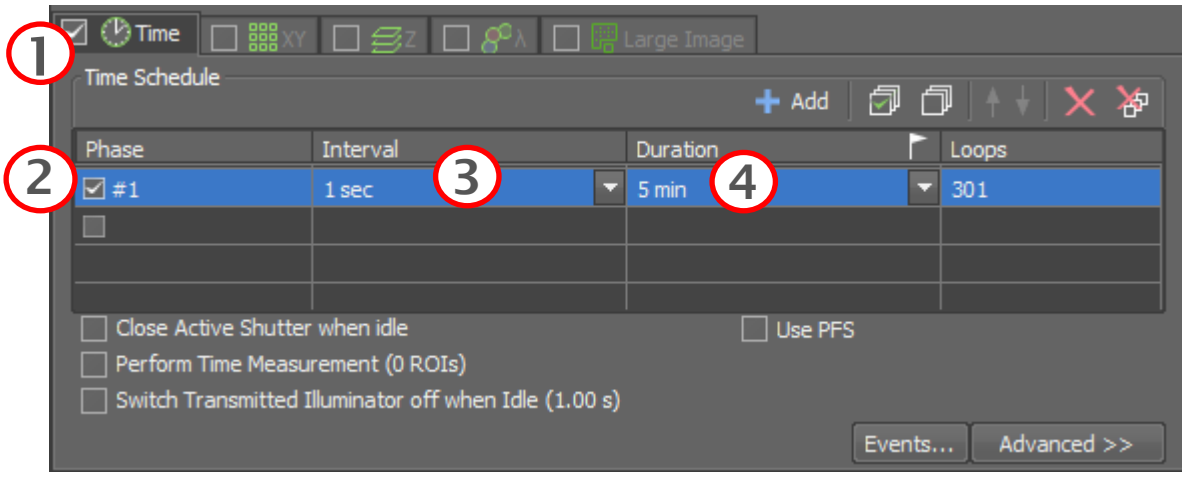
(13) Click ND Acquire button to open ND Acquisition window.



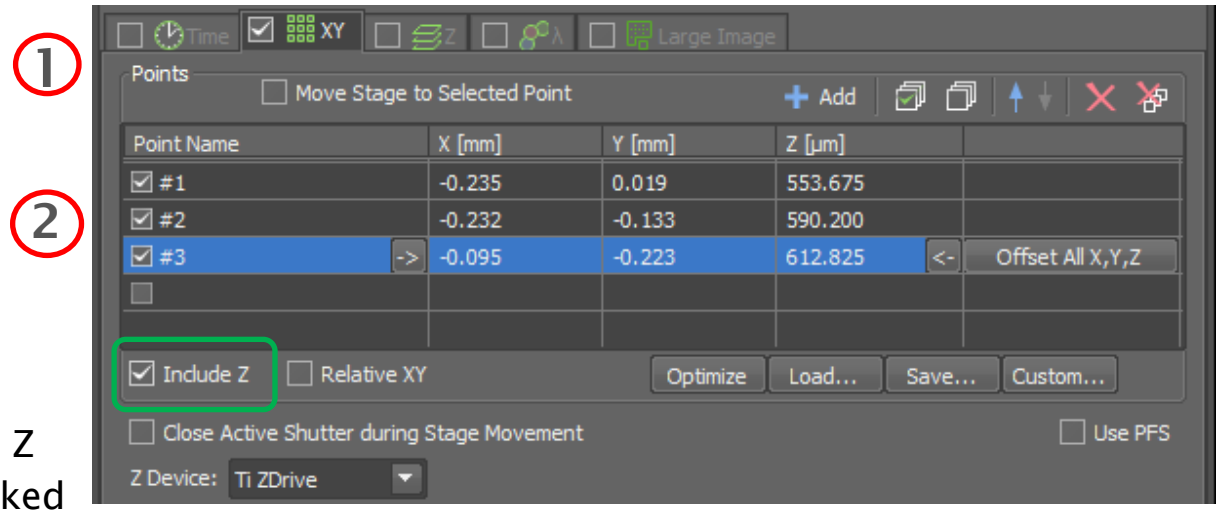


ND Acquisition - Let's Take Some Pictures!

<u>Time</u> allows for imaging over time, in essence making a movie. To use, (1) check the Time tab. (2) Click on the check box under Phase to include the timelapse you want to run. (3) Enter how often you want to acquire and image in Interval column. In the Duration column, (4) enter how long you want to image. In example below, an image will be taken every second for 5 minutes, giving 301 total images.



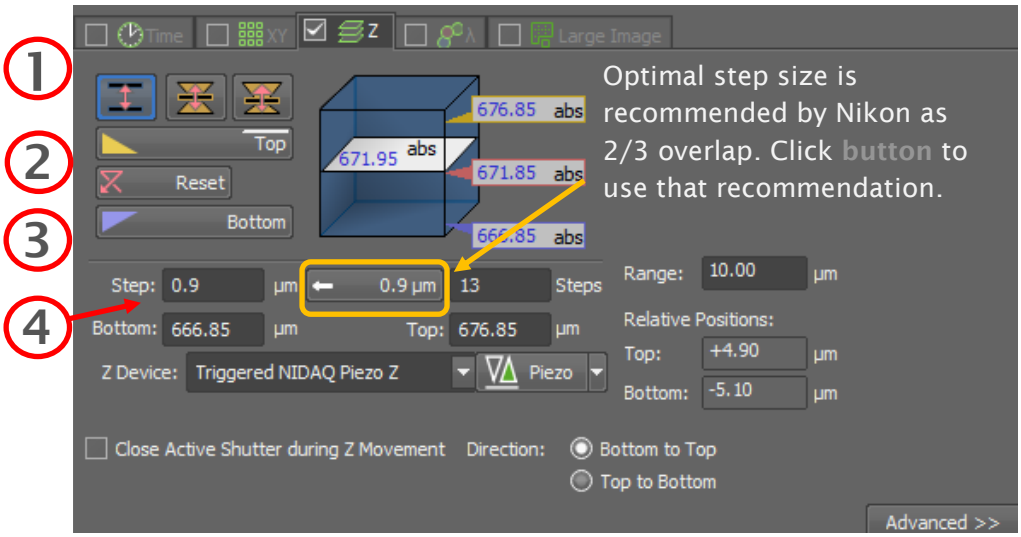
<u>XY</u> is the multipoint function. It stores multiple XY (and Z, if **Include Z** is checked) points. To add a point, (1) check the XY tab. (2) Click on the checkbox in the Point Name column. This adds your current location. Move to another location or Z to add more points. In example below there are 3 separate points of interest that will be captured.



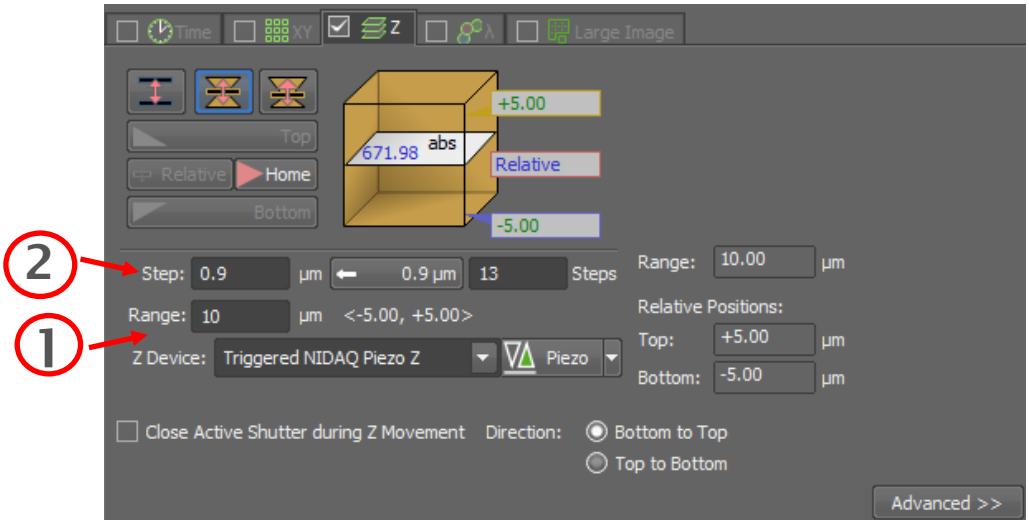
Include Z is Checked

ND Acquisition

Zstack allows you to take a volumetric image. There are 3 ways to do this. The simplest is setting the top and bottom limits of your sample. (1) Check the Z tab. (2) Navigate to the top of your stack using the mouse scroll wheel, and click the "Top" button. (3) Move to the bottom of your stack, and click the "Bottom" button. The Reset button will clear your Z points. It doesn't matter if you reverse the top and bottom, the software will fix it for you. (4) Set your step size.

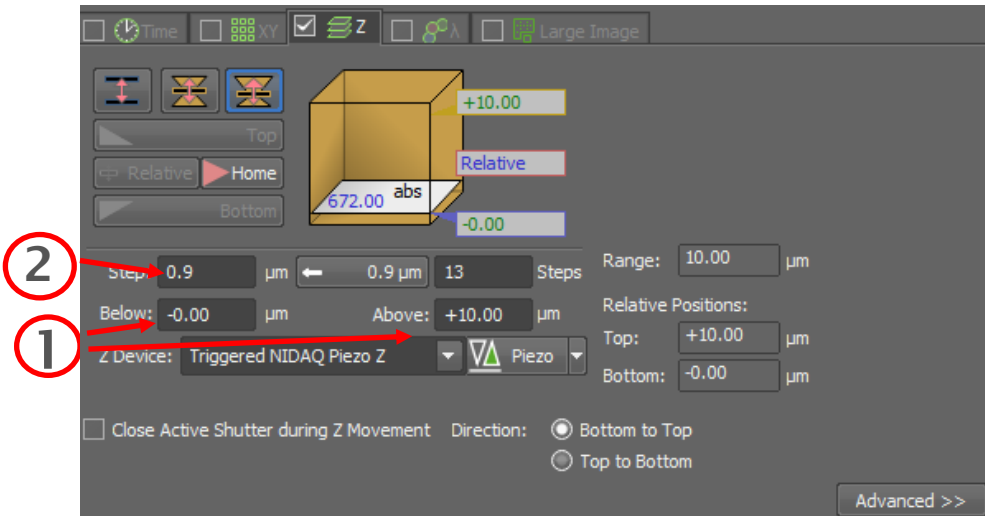


The second choice in Z-stacking is **symmetric** stacking. This will take a symmetric range above and below a relative point. This option is great when you have multi-points at different Z planes. You need to specify the (1) range in the range field, and the (2) step size, just like before. Pressing the "Relative" button will keep your range, and allow you to change your absolute point as you mouse up and down. Pressing "Home" will make your current Z position the absolute point.

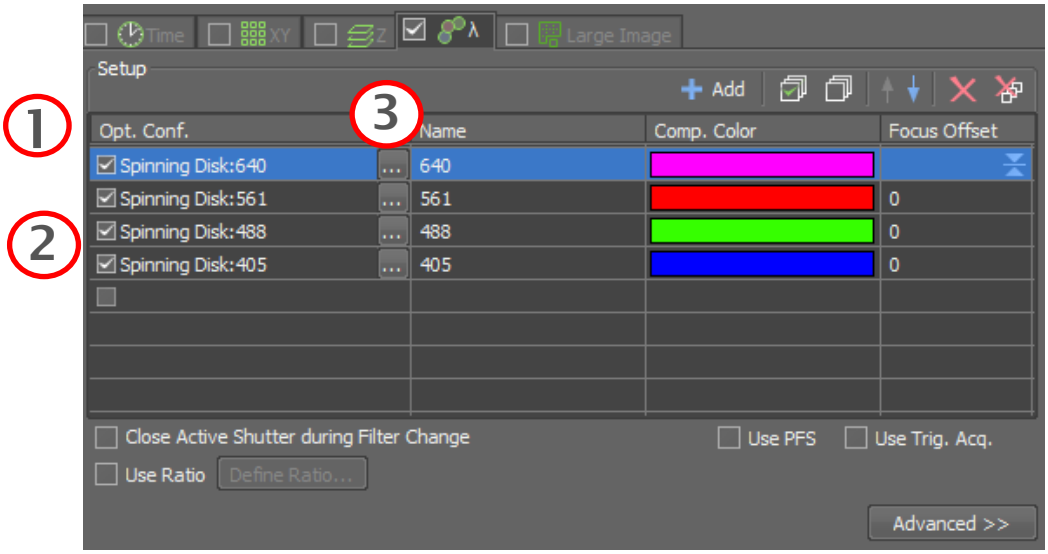


ND Acquisition

The third choice in Z-stacking is **asymmetric** stacking. This will take an asymmetric range above and below a relative point. This option is great when you have multi-points at different Z planes. You need to specify the (1) range above and below a relative Z position, and the (2) step size, just like before. Pressing the "Relative" button will keep your range, and allow you to change your absolute point as you mouse up and down. Pressing "Home" will make your current Z position the absolute point.

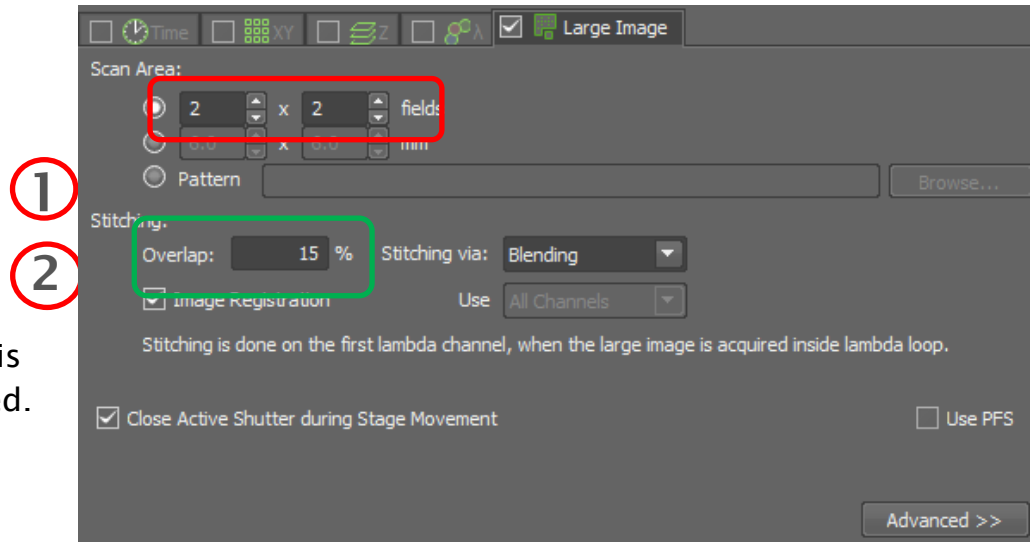


Lambda (λ) calls saved Optical Configurations. If you are imaging more than one channel, you will need to (1) check the λ tab. (2) Click on the check boxes to add OCs. (3) Click on the 3 dot button to select the proper OC for your fluorophores. **Note:** Set up as Highest to Lowest wavelength.



ND Acquisition

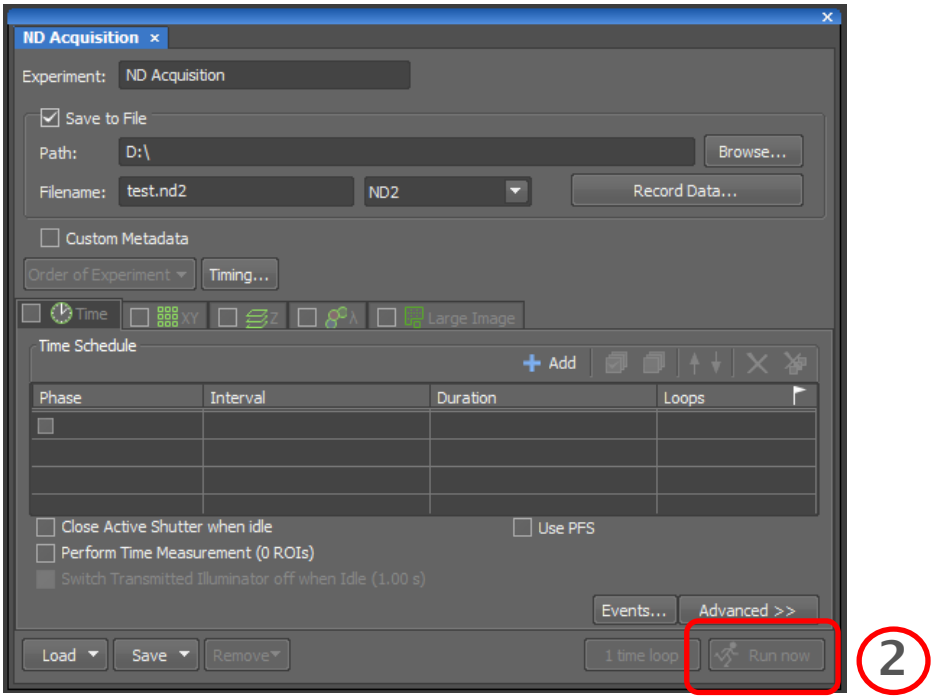
Large Image will stitch together multiple fields to make one image. To use, (1) check the Large Image tab. You must also have the point of interest selected in the XY tab. (2) Choose the number of fields in X and Y. It is recommended to use the default 15% overlap for stitching.



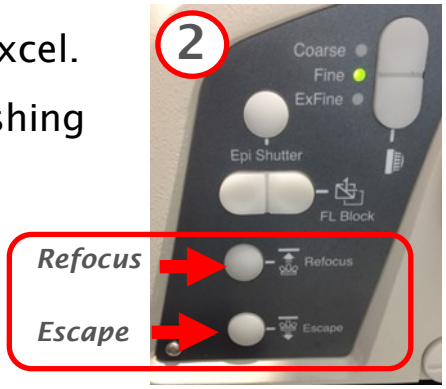
15% overlap is recommended.

Put it all together. You can select any or all of the tabs to perform an ND Acquisition. The software reads from right to left. Tabs can be moved around to image quicker or more accurately. Once you are ready to Run your experiment, (1) enter your file name, and (2) click Run Now.

NOTE: if you do not change the name of your file each time, the software will add consecutive numbers to the end of your file name.



Shutdown - Last User of the Day



(1) Close out of NIS-Elements. Record your time in Excel.

(2) Make sure that the objectives are lowered by pushing the Escape button on the right side of the inverted microscope to bring the objectives down. Reset the objective height by holding the Escape button while simultaneously pushing the Refocus button. Remove sample.

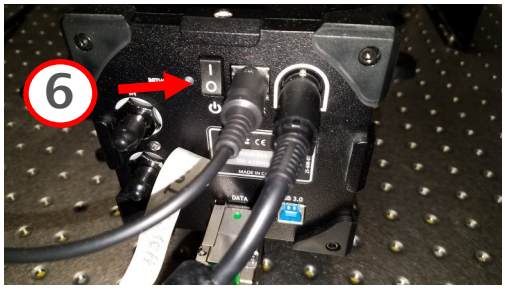
(3) If you used the 40X, 60X or 100X objective, clean off oil with lens paper and Sparkle solution.

(4) Turn the key on the Nikon laser launch from the on (horizontal) to off (vertical) position. You do not need to turn off individual lasers.

(5) Turn off Tripp-Lite by flipping the red switch to the off position located on the shelf underneath the microscope.

(6) Turn off Photometrics Prime95B Camera.

Camera



(7) Put dust cover, located in lowest (3rd) drawer of the cabinet, over top of the microscope.

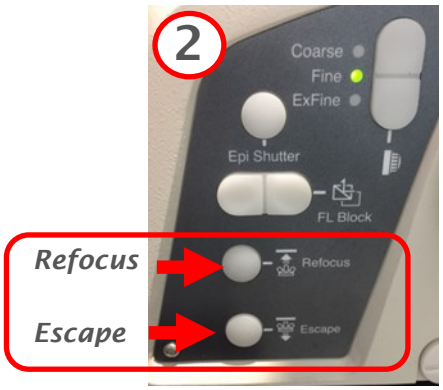
(8) Sign out of Windows. Turn off computer from the monitor. Finish your time on iLab.

Shutdown - Someone is Scheduled After You

(1) Close out of NIS-Elements. Record your time in Excel.

(2) Make sure that the objectives are lowered by pushing the Escape button on the right side of the inverted microscope to bring the objectives down. Reset the objective height by holding the Escape button while simultaneously pushing the Refocus button. Remove sample.

By resetting the objective height, you are preventing the next user from accidentally running an objective into their samples, potentially causing an objective to break.



(3) If you used the 40x, 60x or 100x objective, clean off oil with lens paper and Sparkle solution.

(4) Sign out of Windows. Finish your time on iLab.