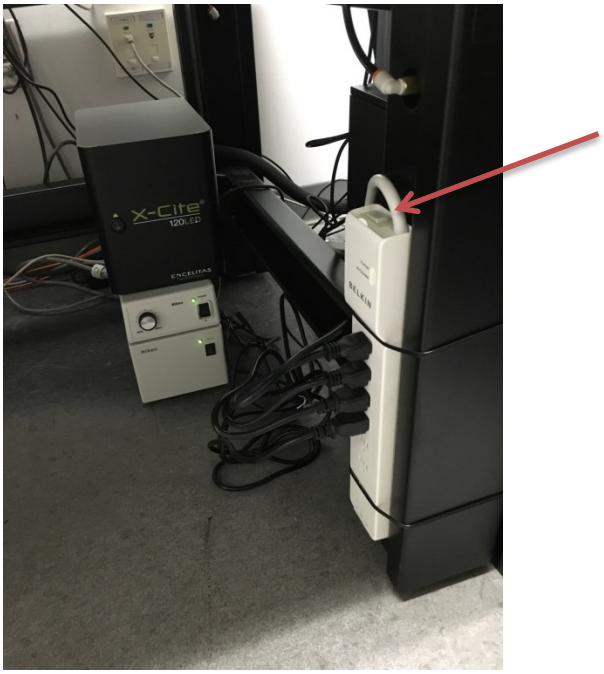
Nikon A1Rsi Confocal Start-Up Sequence

1. **Turn the key on the Nikon LUN-V Laser Launch.**

2. **Press the button the left side of the A1Rsi Controller unit.**

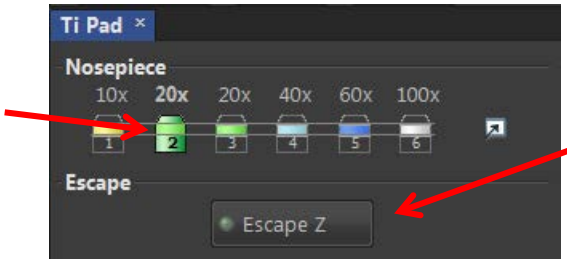
3. **Turn on the power strip underneath the microscope.**



4. **Turn on the H.P. workstation (P.C. can be turned on first).**
5. **Open NIS-Elements.**

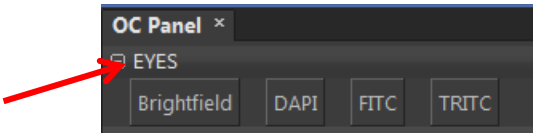
Nikon A1RSi Confocal Workflow

1. **Start by selecting your desired objective and then place your sample in the sample holder on the stage.**



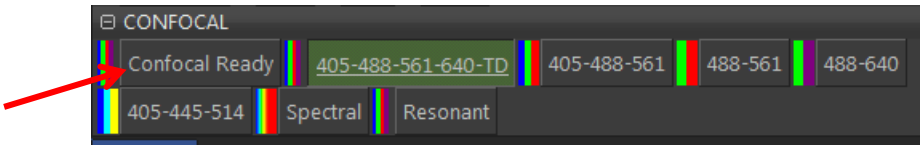
Remember to use the Escape Z function when loading and unloading your sample.

2. **In the EYES optical configuration (OC) panel click brightfield/fluorescence color of interest.** This will allow you to focus on your sample prior to using confocal.



3. **Press the X-Cite controller to turn on the fluorescence light.**

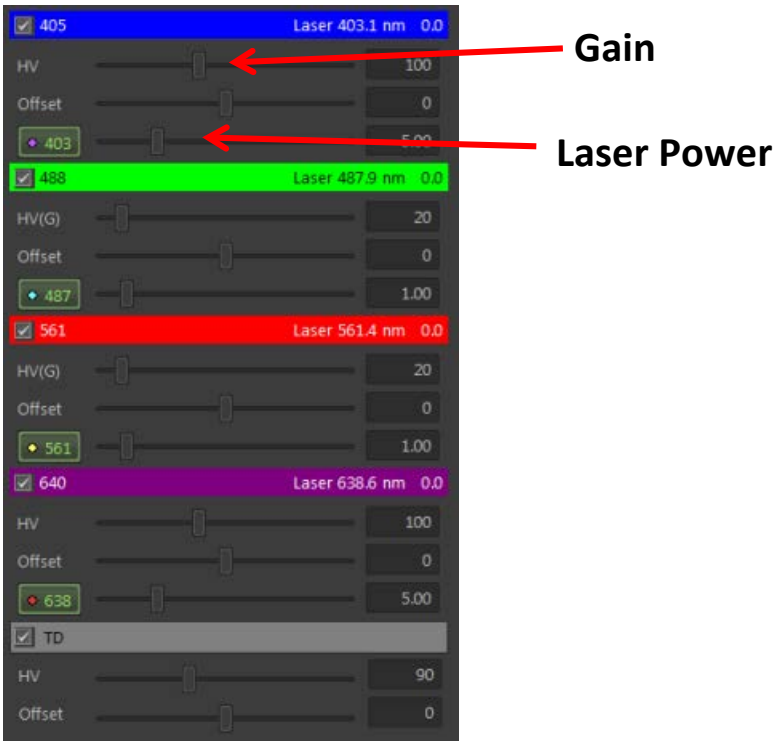
4. **Once you have your sample in focus turn off the fluorescence light by pressing the controller again.**
5. **Next you will click on the Confocal Ready button in the CONFOCAL optical configuration menu.**

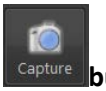


6. **The Confocal Ready button will allow the scanning of samples for all four channels plus transmitted light (TD). If you don't need all the laser lines you can select one of the other OC buttons for your fluorescent probe of interest.**
7. **Press the** 

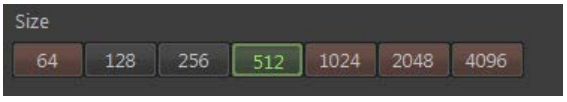
button and the live image window will appear with your sample. Press the scan button again to stop scanning.

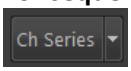
8. **Adjust the laser power and gain (HV) settings to your desired level for visualizing your sample.**



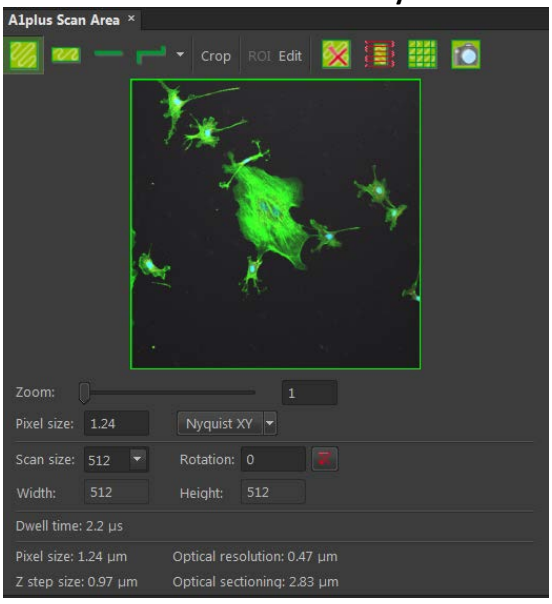
9. **Once you have set your laser power and gain, press the** 

button to obtain a 2D image.
10. **To improve the resolution you can change the Pixel dimensions. Default is 512x512.**



11. **For sequential scanning to prevent bleed-through or crosstalk between fluorescence channels, press the** 

button.
12. **Be aware of photobleaching when scanning for long periods of time on one particular region of your sample.**
13. **Utilize the Scan Zoom feature if your structure of interest is much smaller than the scanning window.**



14. **Once you have captured an image we recommend you save the image as an ND2 file which is the Elements preferred file format. Once you have saved as an ND2 file then you can export to a Tiff file.**
15. **Following your imaging session please clean any objectives used if you used oil or water immersion.**
16. **Leave the system on and the Imaging Center Director will turn off the equipment at the end of the day.**

    a. **Please Note – If it is afterhours please turn off the system following usage.**
        i. **Close Elements**
        ii. **Turn off powerstrip**
        iii. **Turn off controller**
        iv. **Turn off laser unit**

Nikon Confocal Training Document

Ti Pad

- Nosepiece – use to change objective
- Zoom – should be at 1.00x unless using the manual 1.50x mag changer on the TiE

OCs – Optical Configurations

- Eyepiece OCs for finding sample through eyepieces
- Confocal OCs for imaging with the A1 confocal

Confocal GUI

- Scan (starts and stops live image); Capture (take picture); Fast (for faster preview live scan)

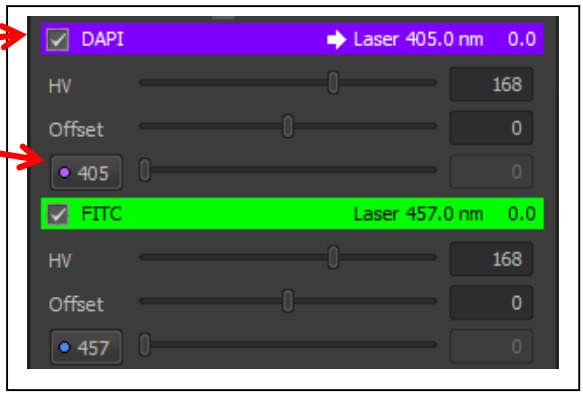
NOTE: If you are LIVE but do not see an image, make sure the red Interlock is turned off!

- Galvono – slower scan mirrors
- Resonant – faster scan mirrors (used mainly for live cell imaging)
- Eyeport – changes to last used eyepiece setting; click again to return to confocal mode
- Autogain – cycles through some gain settings and finds a good starting point for HV
- Single vs. Bidirectional Scan
  - Single – need more resolution, don't need speed
  - Bi – ONLY for fast speed, live-cell - lose flexibility

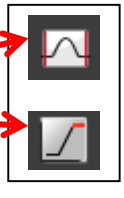
- Pixel Dwell/Frame per Second – scan speed (how long laser scanning over each pixel)
  Start at 1 Frame/sec
- Size – Scan size in XY; pixel size (resolution = how many pixels we put in the field)
  Start at 512; if want pretty picture with no z in a fixed sample, use 1024
- Average – cleans up image by decreasing noise – Mainly used when Imaging with Resonant Scanner

  - Use 2x to start and can go to 16x – keep in mind the equivalent decrease in scan speed
- Ch Series = Channel Series – excites and collects in sequentially rather than simultaneously
- Settings – you don't need to use
- Laser Settings – do not open

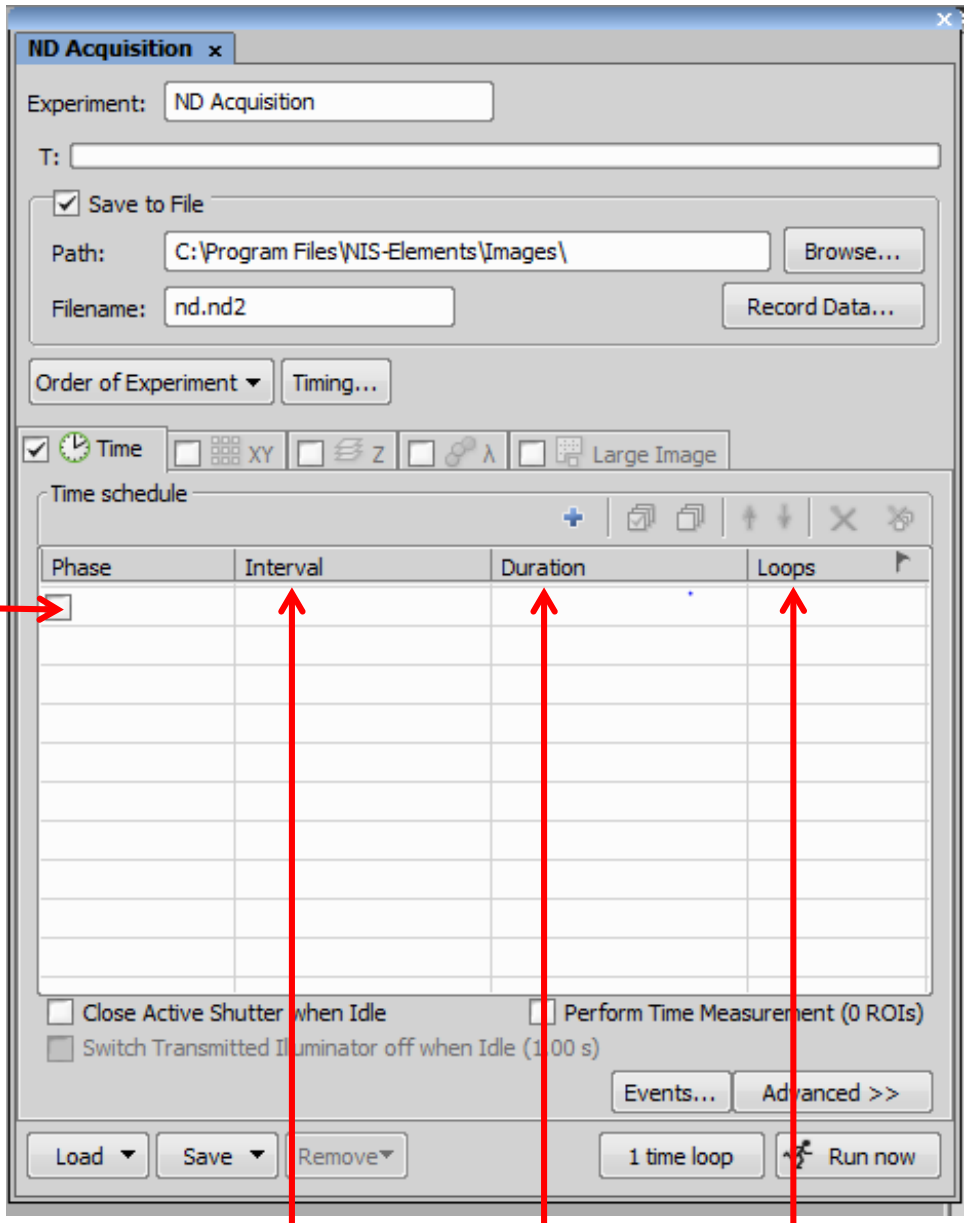
- Pinhole – click square 1.2 AU button to use recommended pinhole size
- Laser Lines
  - Checked = collecting that channel
  - Depressed = laser on
  - HV (Gain)
    Start at 80-110 for 405 and 647 lasers
    Start at 20% for 488 and 561 lasers
  - Offset
    keep at 0
  - Laser Power
    Start at 5% for 405 and 647 lasers
    Start at 1% for 488 and 561 lasers



- Increasing Laser Power Increases signal w/o increasing noise, but photobleachs sample faster
- Increasing HV (Gain) doesn't bleach more, but increases noise
- LUTs – Look Up Tables
  - Autoscale doesn't change data – just scales visual image
  - Oversaturation Indicator



Timelapse



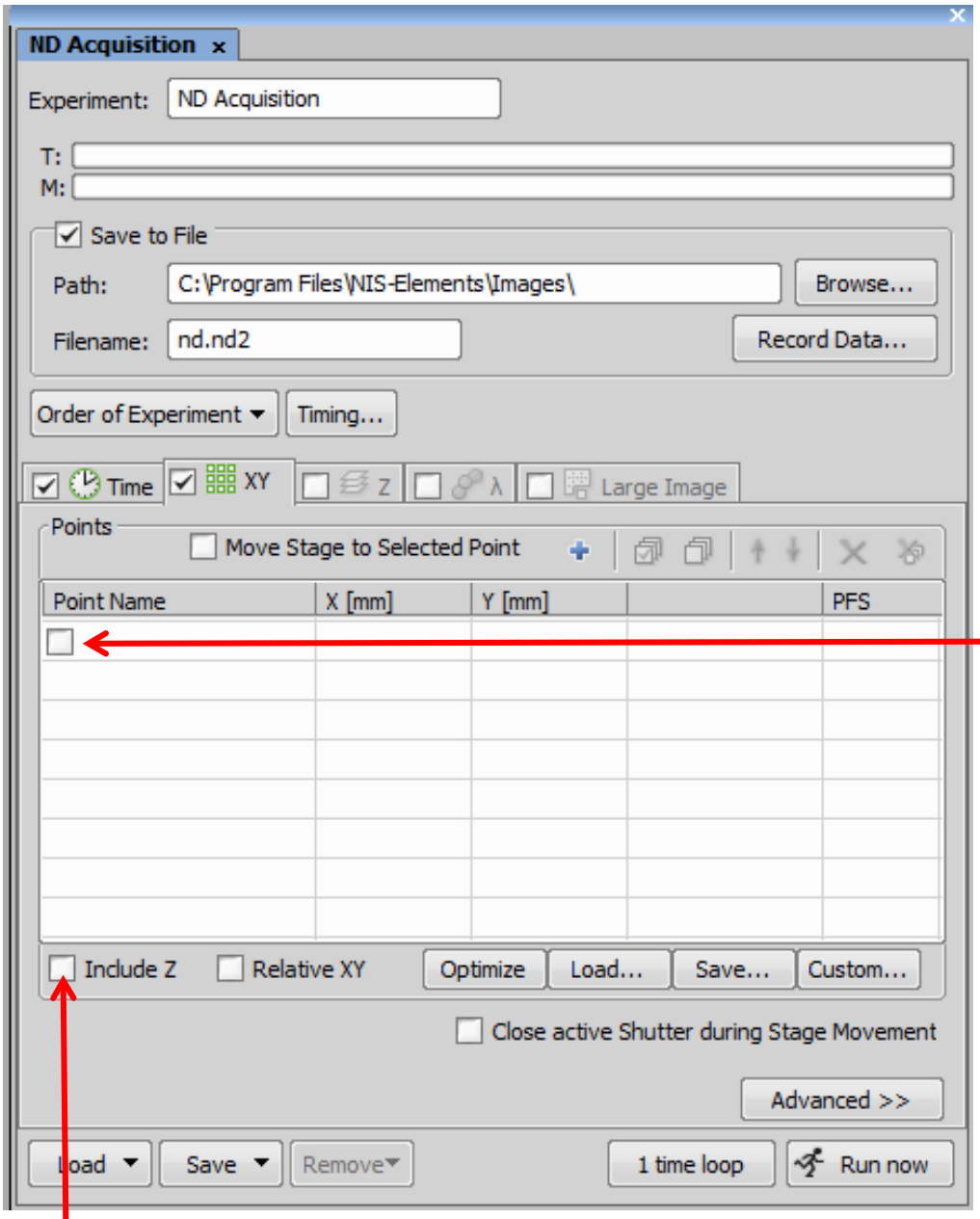
Click to add a new Time Phase

Interval – How often you want to image (ie. every 3 sec, every 10 min, no delay = image) continuously)

Interval – How long you want the timelapse experiment to run

Loops – How many images will be acquired during this duration

Multipoint



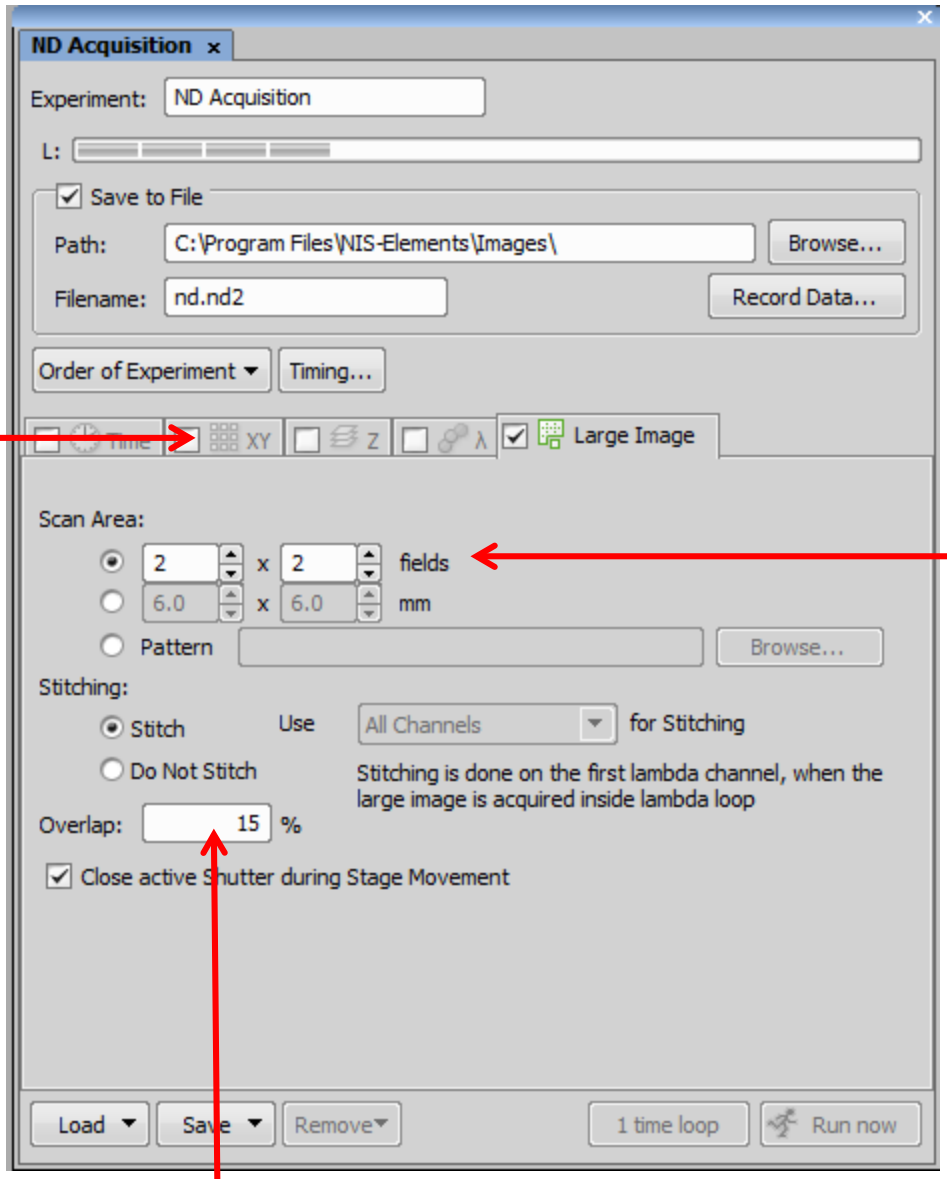
Click this box to add a point.

Pressing the spacebar will also add points.

Once a point is added, XYZ can be updated by clicking the arrow next to those values.

Check this box to have Z remembered, too. Your set focal plane will be remembered when this point is visited.

Large Image Stitching – Tile Scanning

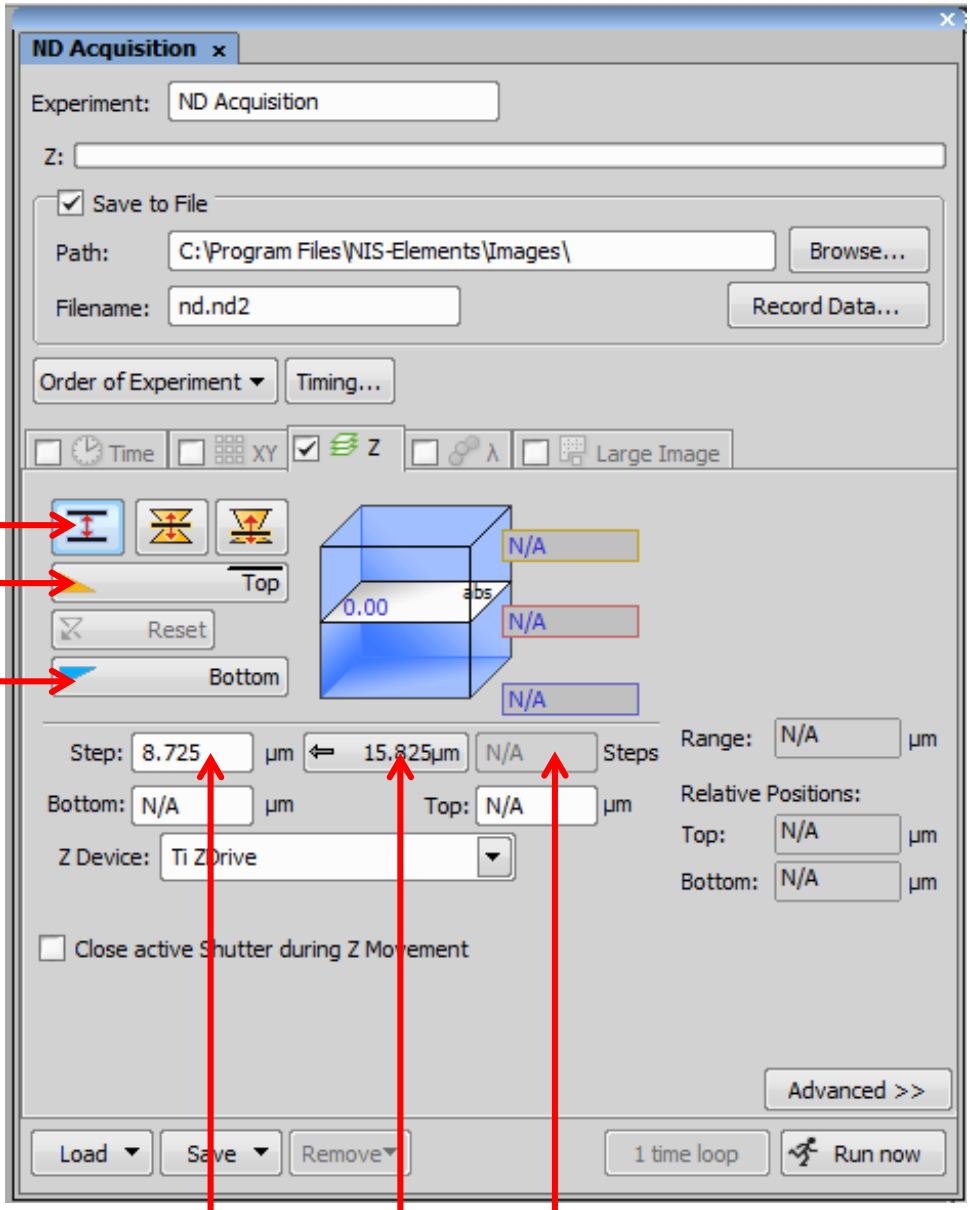


You must add an XY point! This will serve as your center point to stitch around.

Choose size of stitch

Use 10-15% Overlap

Z Stack



Set Top and Bottom of Stack

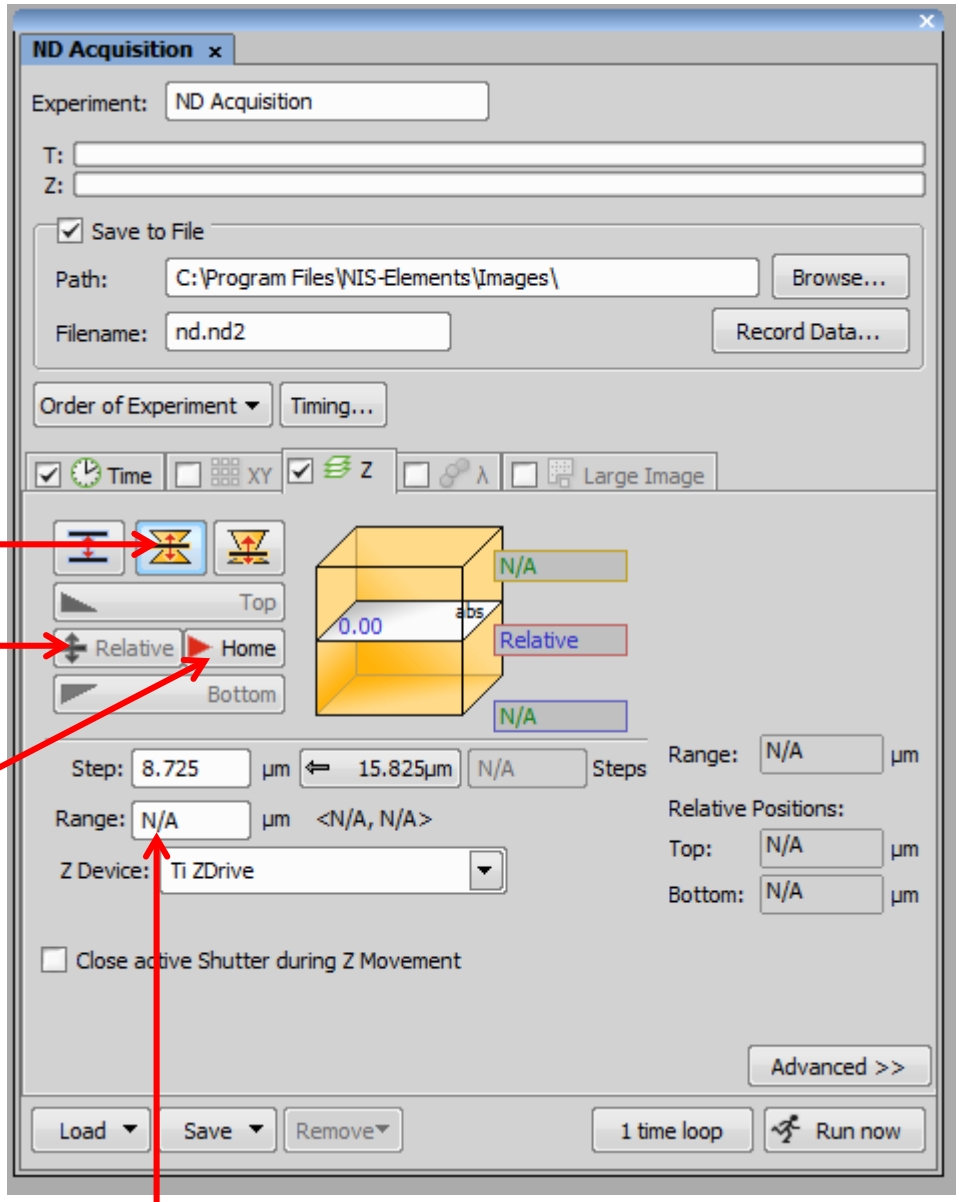
Scroll to top and click to set

Scroll to bottom and click to set

Set step size here

Use recommended step size

Set number of steps here



Set Z Stack by Defining a Range

Choose Relative if combining z stack with multipoint XY

Choose Home to set the focal plane you would like to take a stack around

Set the range of the stack (ie.range of 10 = 5 above and 5 below)

Don't forget you can click here to automatically save your ND experiments as they are acquired!

Set Z Stack by Defining an Asymmetrical Range