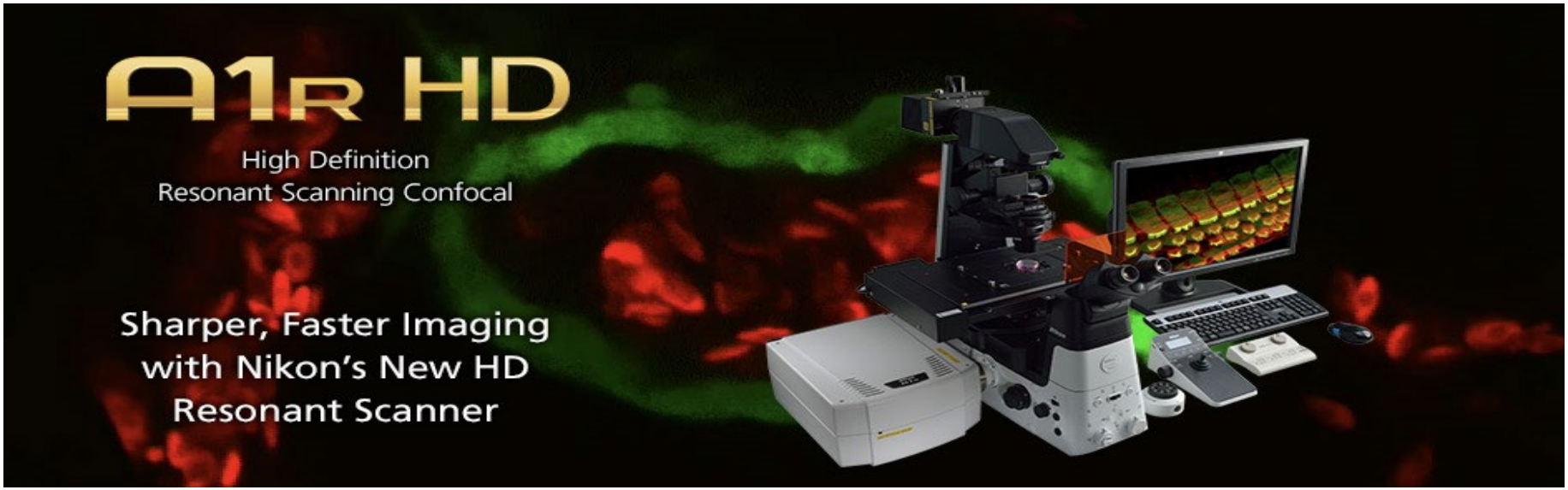


Nikon A1R/Ti2E Training Guide

Prepared for Missouri University of Science and Technology (MST)

2018

(For internal use only)

Hardware Guide

Table of Contents

Turn On Sequence

- TURNING ON:
- 1.) Turn computer on-->Let it initialize and load to the main screen
- 2.) Turn on Ti2 controller
- 3.) Turn on Ti2 microscope (wait for it to finish initializing)
- 4.) Turn on LUN4 laser launch-->Turn Key to ON position
- 5.) Turn on A1R Controller-->Press white switch on left side of controller tower.
- 6.) Turn on SOLA
- 7.) Wait 30 seconds before starting up Elements so that the hardware can initialize properly.

Turn Off Sequence

- TURNING OFF:
- 7.) Close Elements
- 1.) Turn computer off-->Give it about 30 seconds to completely turn off before proceeding to next step.
- 4.) Turn LUN4 laser launch off--> Turn key to OFF position
- 5.) Turn A1R controller off--> Press white switch on controller tower
- 3.) Turn Ti2 microscope off--> Turn switch to off position on the Ti2 microscope, wait for green lights to turn off.
- 2.)Turn Ti2 Controller off
- 6.) Turn SOLA off

- Keep power strip on

Main System Components

Light Path:

Eye-Eyepiece Viewing

L (left)-Confocal

R (right)-NOT USED

L (bottom)-NOT USED

Objective changer switch: Rotating the switch changes the objectives

Escape button: Moves the objective to escape position. The z will not move until the escape is released

Magnification Changer: 1x or 1.5x

**Please default to 1x*

PFS indicator:

On (locked on)

Blinking: Interface detected but PFS is off

Off: PFS is off

DIC indicator

On: all components in the light path

DIA indicator: status brightfield light

Open=Lit,Close=unlit

DIA illumination indicator: Lights when DIA is on

Z course mode indicator: indicates when coarse Z mode is activated

EPI 1 indicator: indicates when EPI1 turret is open

PFS button: Turns the PFS on and off

Z focus knob: Focus the objective up and down

Z course mode button: Changes the speed of z movement

DIA on/off switch: Toggles the diascopic lamp on and off

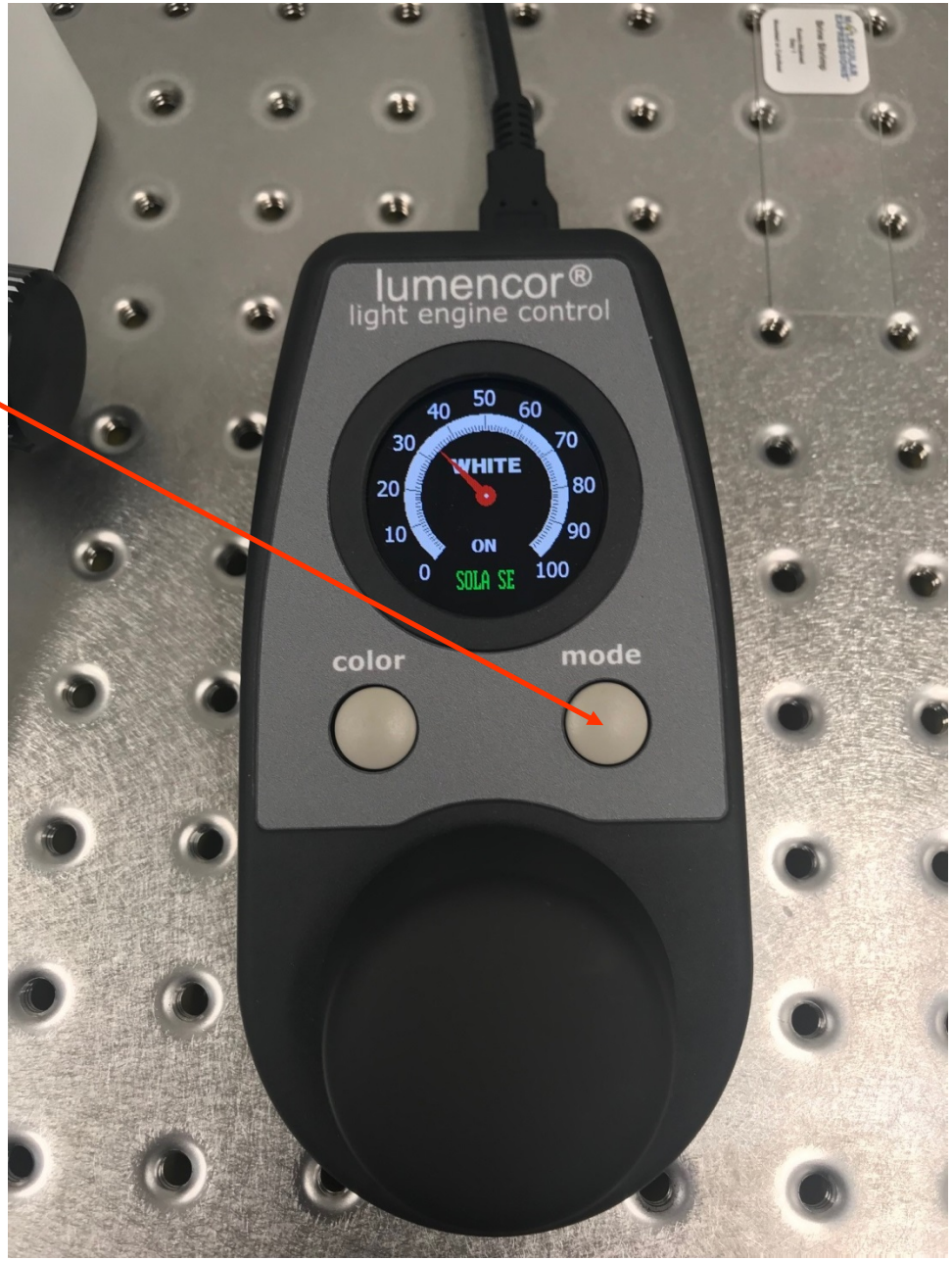
DIA brightness adjuster: adjusts the brightness of the diascopic lamp

PFS button:
Turns the PFS on and off

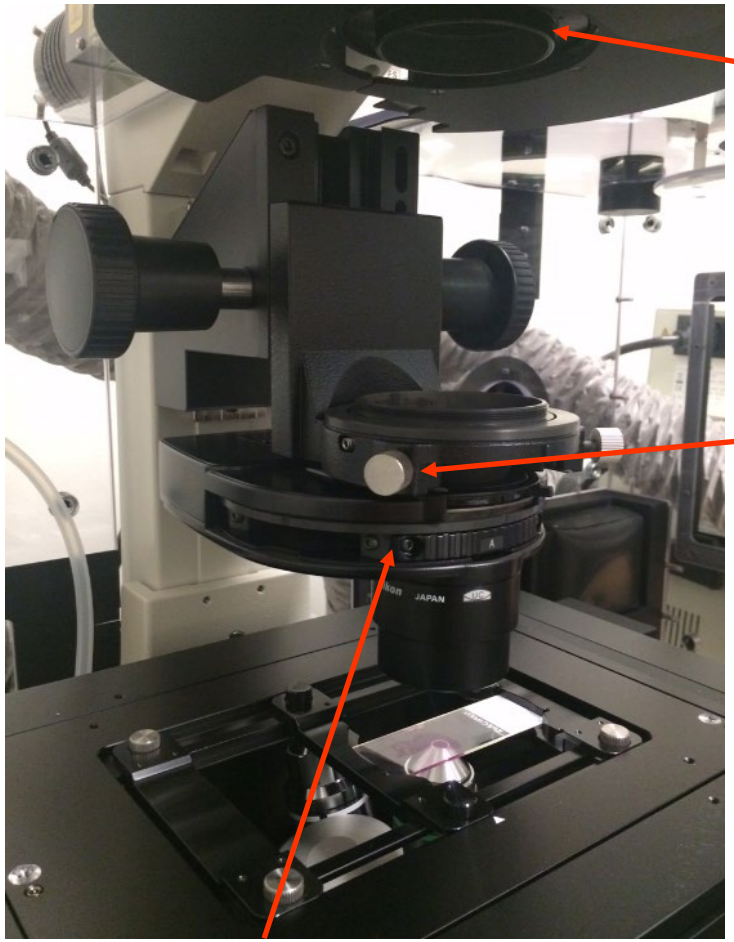
EPI 1: Changes the position of the filters (DAPI,FITC, TRITC etc)

Z focus knob: Focus the objective up and down

Mode : turns the fluorescence on and off



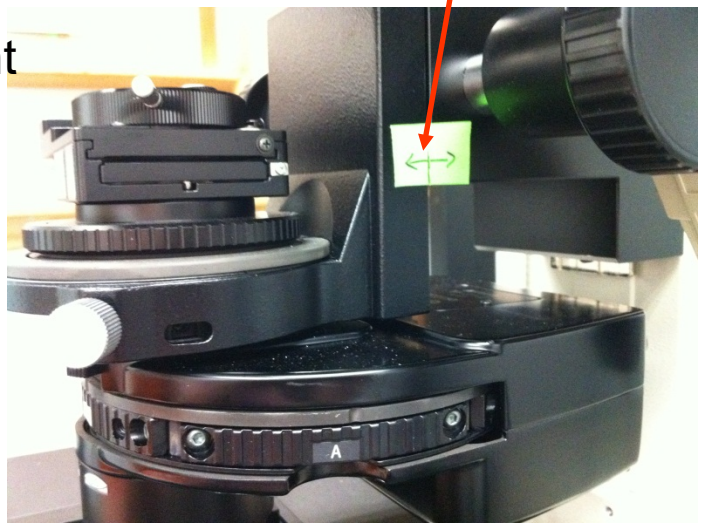
Transmitted Light Adjustments



Field Diaphragm
for adjusting
Kohler Alignment

Centering
Screws for
adjusting
Kohler
Alignment

Condenser
Height-set at
arrows



Condenser Prisms-
manually controlled by hand

Quick Review on Kohler Alignment

**Use a 10x objective (if available) and put the condenser in the A position*

1. Focus on a sample 

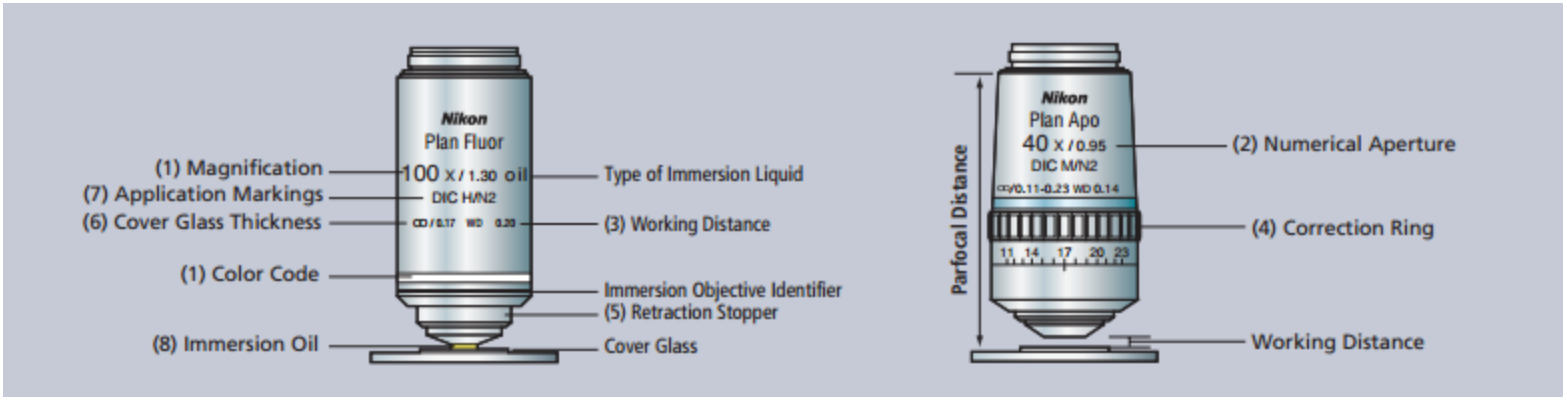
2. Close down your field diaphragm 

3. Focus your condenser (adjust condenser height) 

4. Center your condenser 

5. Open field diaphragm 

Your Objective Lenses



Plan Apo 10x
.45 NA, 4.0mm WD
Good for coverslip #1.5

Plan Apo VC 20x
.75 NA, 1.0mm WD
Good for coverslip #1.5

Plan Fluor 40x OIL
1.30 NA, 0.20mm WD
Good for coverslip #1.5

Plan Apo Lambda 60x OIL
1.40 NA, 0.13mm WD
Good for coverslip #1.5

Basic Operation

NIS Elements
Confocal
Microscope Imaging Software

Software Guide

Good to know….

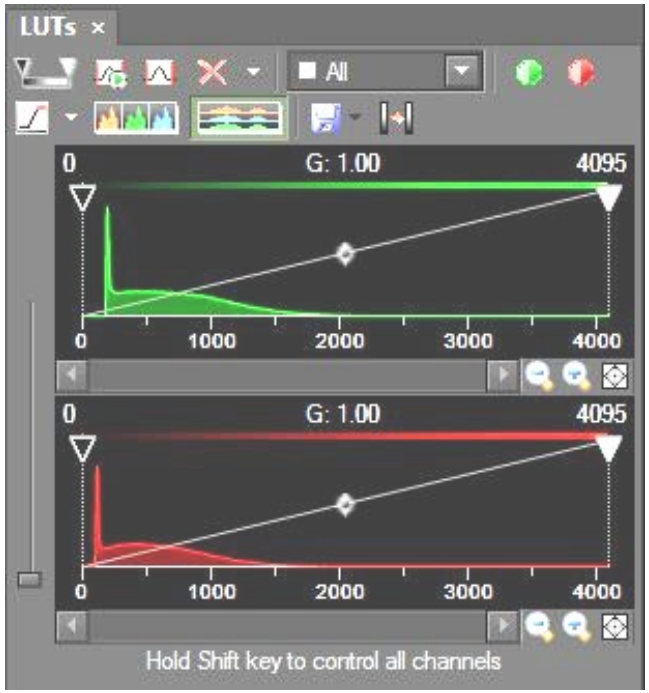
- Most dialogs can be found under the **View** tab (Acquisition/Analysis/Visualization Controls) in Elements

  *-As a shortcut these can also be found by **right clicking** in the back open space of Elements*
- Elements obeys most Windows-based functions, shift/control and Alt are used often; **right-clicks** are used to display options/properties
- Elements also uses “drag and drop” for overlaying windows/datasets
- ND2 Files can be opened/viewed outside of Elements with a free download on: http://www.nikoninstruments.com/Products/Software/NIS-Elements-Advanced-Research/NIS-Elements-Viewer. There is also a plugin for ImageJ from: http://rsb.info.nih.gov/ij/plugins/nd2-reader.html

Microscope Control

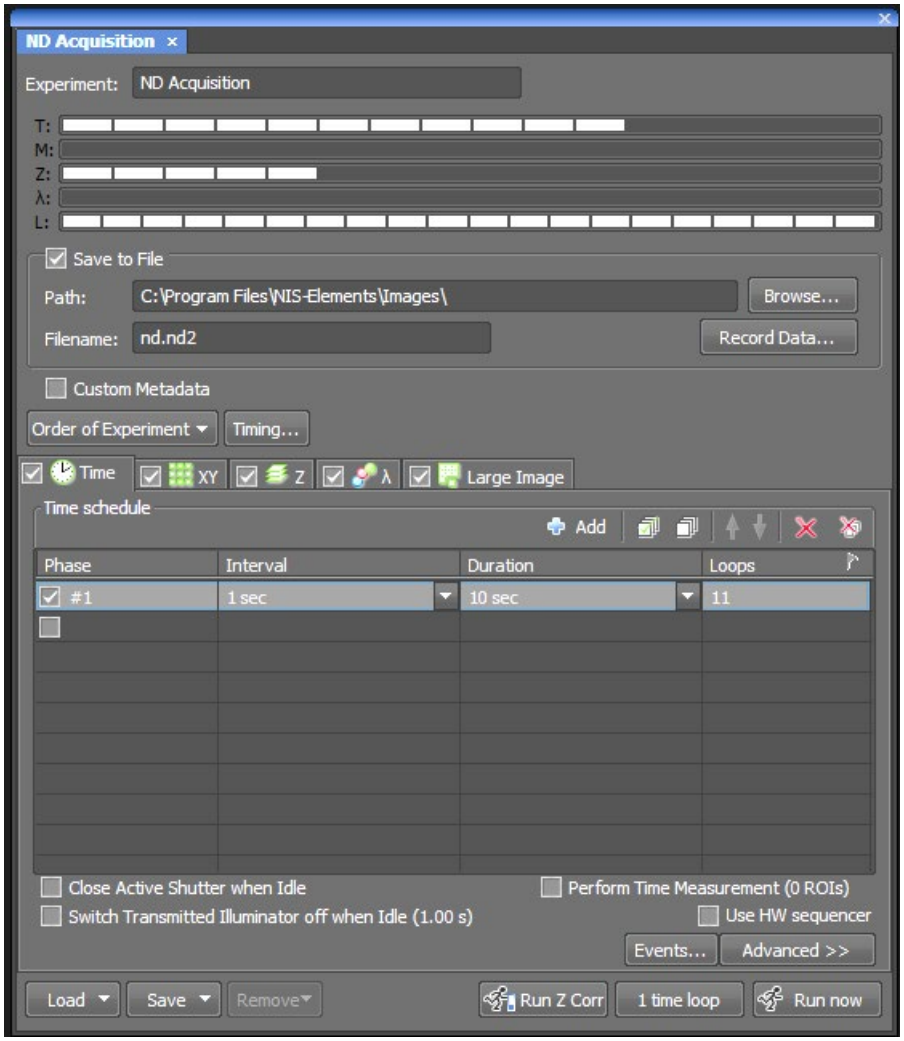
- The Ti2Microscope Pad (found under View→Acquisition Controls) shows all motorized components on the microscope that can be adjusted
- Note-your condenser and mag changer (zoom) is intelligent; the mag changer must match what is set on the microscope base to insure proper calibration of images

LUTS



The LUTs menu can be used for brightness/contrast adjustment (using triangular sliders) as well as for showing a saturation indicator which can be used to show what pixels are saturated. Use the drop down arrow to select "complementary color"

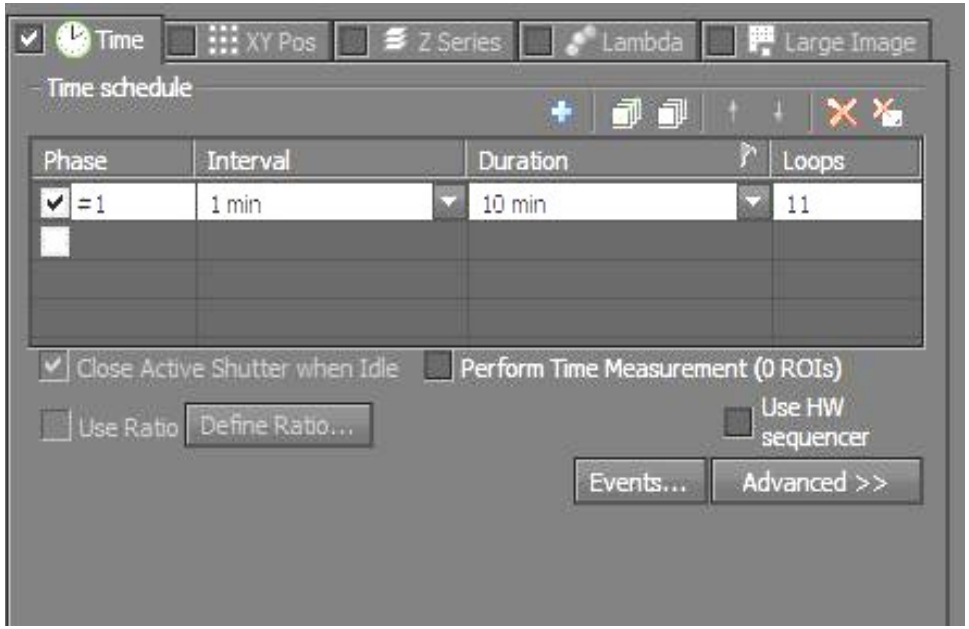
ND Experiments



**Experiments can be run on time, Z-stacks, wavelength switching or stage positions and image stitching (if a motorized stage is supplied).*

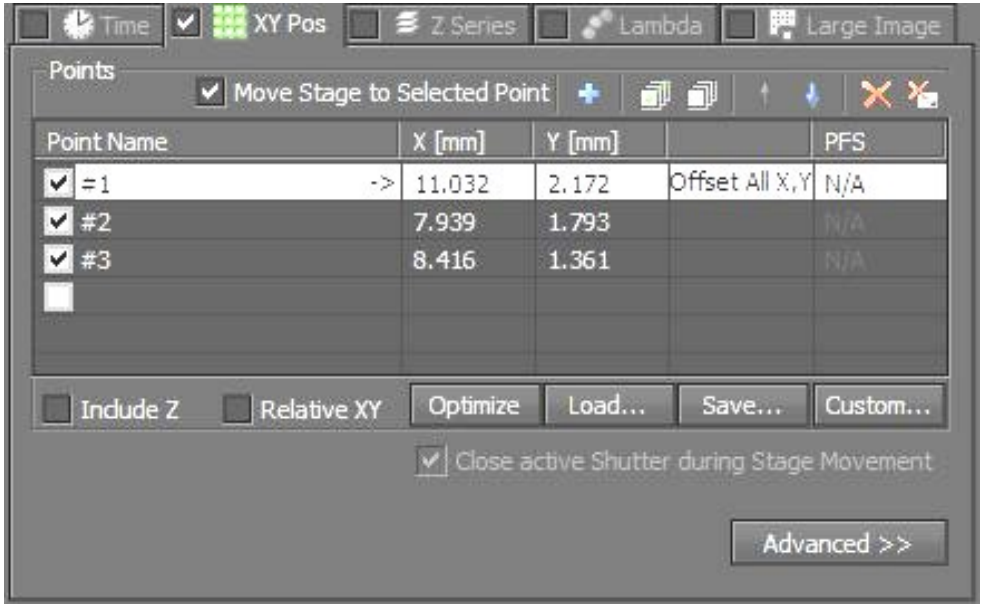
-The next few slides will explain each dimension.

Time Lapse



- To add a timelapse, check off the checkbox for “Time”.
- Add a time phase by checking one box; you can change the interval (how often to image) and the duration (total time). Alternatively you can input a number of loops (# of images required).
- You can add additional time phases to be run in sequence.
- The “X” will reset the time phases.

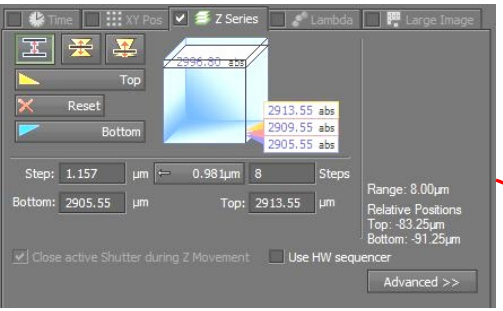
XY-Stage Positions



- To add XY positions, check off the checkbox for “XY Pos”.
- Anytime you would like a position to be remembered, check off a box. This will add the XY coordinate as well as Z (if checkbox for Include Z is checked) or the **PFS offset** (if PFS is set). You do not need Z if PFS offset it used.

*There is also a checkbox for “move stage to selected point”, which will allow you to freely move between points by clicking on them in the XY Pos box.

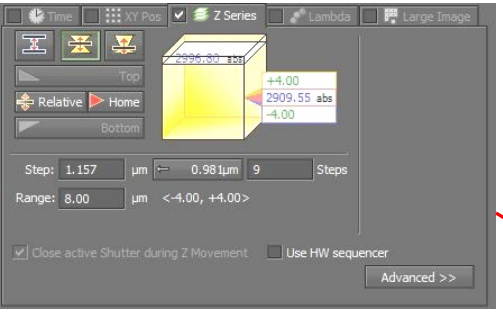
Z-Stacking/Optical Sectioning



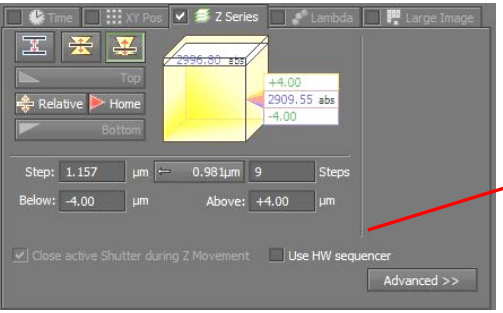
There are three methods for optical sectioning….select one

1. Choose a middle plane and click “reset”, go to a live view and focus towards the bottom (click bottom) then focus to top (click top)

   *Set your step size (distance between each plane) and/or the # of steps you want (image total)

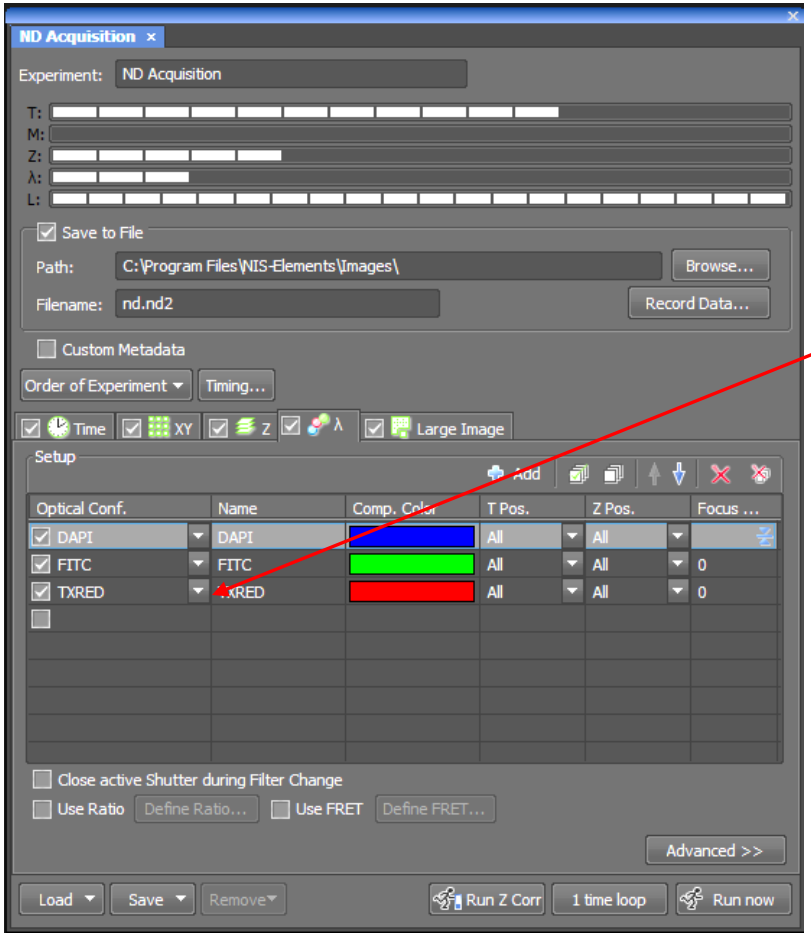


2. Choose a middle plane and click “home” then input a distance range (equal from top to bottom)



3. Choose a plane somewhere in the sample and click “home” then input a staggered range above and below

Lambda-wavelength

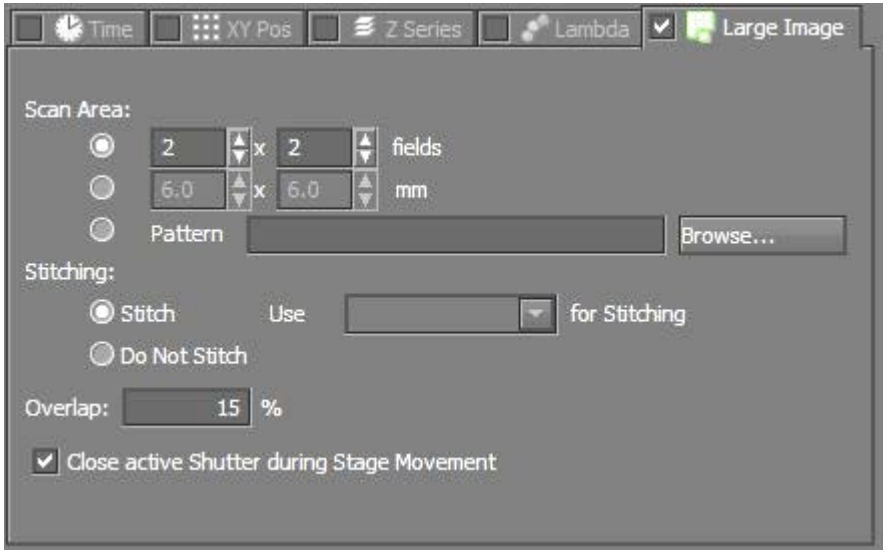


- You can add automated wavelength switching to an experiment by checking off the checkbox for “Lambda”.

- You can add an optical configuration, if one was created, by clicking on the down arrows

Not needed for the confocal, will be useful later if a secondary camera is added

Image Stitching



- You can add image stitching to an experiment by checking off the checkbox for “Large Image”.

You will need to make sure to run a calibration before starting

✓ *Place a high contrast slide on the stage (like an H&E) and focus it*

✓ *Locate Calibration→Recalibrate “x” objective*

✓ *Select “Auto” for calibration*

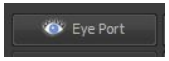
Imaging: Nikon A1 Confocal

Confocal Acquisition: Quick Start

*<u>*The A1 Simple GUI is a wizard that will walk you through acquiring a confocal image*</u>

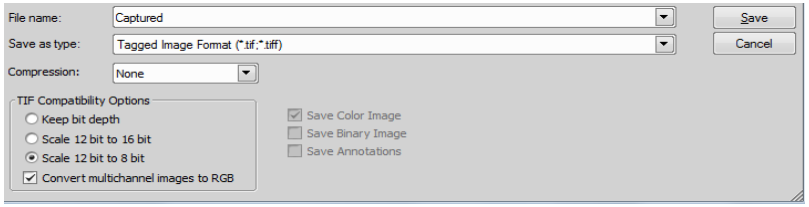
1. Make sure you have previewed your slide through the eyepieces and focused on an area of interest.
2. Click on the “Confocal” optical configuration on the left panel to load default settings
3. Remove interlock if showing in RED by clicking on it
4. Choose *Galvano* for standard imaging, *Resonant* for fast imaging
5. Select what laser lines you want to acquire (check boxes for DAPI/FITC/TXRED/CY5/TD (TD is for DIC)
   - ***HV-Gain, start at 100 for 405/640 and 25 for 488/561 PMTs***
   - ***Offset-background adjustment, start at 0***
   - ***Laser Power, start at 5% for DAPI and cy5, 2.00% for FITC and TRITC***
6. Start with a scan size of 512x512 and scan speed (pixel dwell) of 2.2usec.
7. Select “Normal” and “Ch Series” if using more than one laser
8. Select 1.0 AU for Pinhole
9. Start “SCAN”
10. Adjust if needed
11. Click “CAPTURE”

<u>If you need to go back to the eyepieces to check your slides, click EYE PORT, click it again to return to the confocal!**</u>



*To save a quantitative image, click on File→Save As and select either a <u>.tif</u> (check “keep bit depth ONLY”) or <u>.jp2</u> for raw data

**To convert into a viewable “COLOR” image, save as a <u>converted</u> tiff (shown below)*

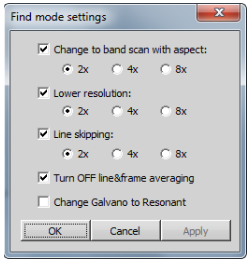


The A1 GUI-further explained (top to bottom)

1) SCAN Stops/Starts Live Image Acquisition (as a default, please do not use any averaging or integrating in this mode, see #10)

2) Capture: captures an image (using user defined settings below)

3) "Find Mode": Implements a fast preview scan
a) Defined by Find Mode Settings in 



4) Eye Port-changes to last used non-confocal setting, **click again** to return to confocal mode

5) AG: Auto Gain feature; sets the HV (gain) for optimum based on user settings

6) Skip: skips scan lines, increases speed

7) Unidirectional/Bidirectional: scan direction (Uni-one direction, traditional scanning; Bi-directional, used for live imaging for speed increase—does need to be aligned during scan).

8) Pixel Dwell/Frame per Sec: scan speed; pixel dwell in micro seconds

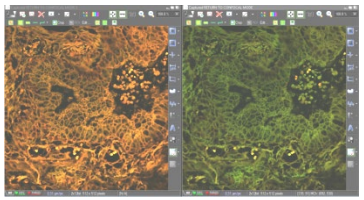
9) Size: Scan Size in XY

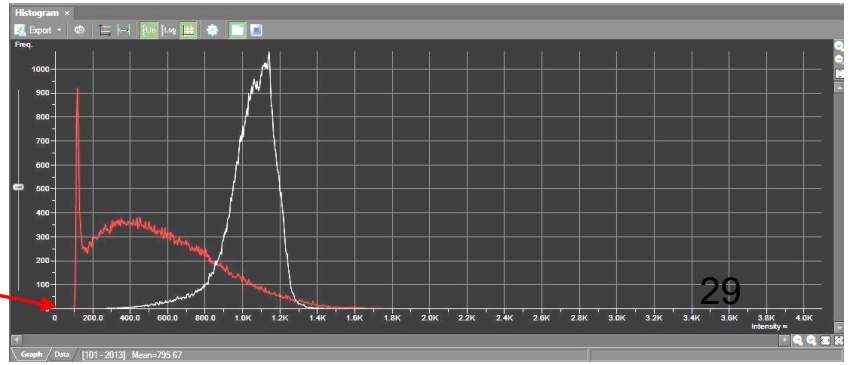
10) Averaging and Integrating : average signal by line or integrate signal by line (see Help, 8 4). Normal mode es not use either. Typically averaging is used only for image capture, not for live preview.

11) Ch Series: Channel Series; setup will allow for each laser to be run sequentially rather than simultaneously ***important for closely excited fluorophores!**

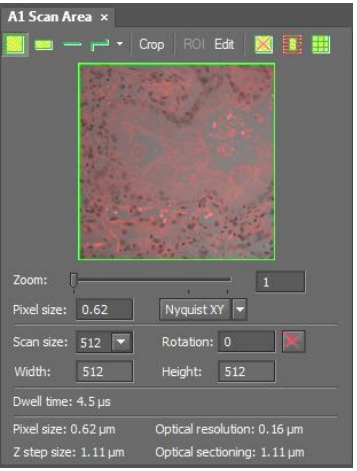
12) Pinhole: adjusts size of pinhole, 1.0 AU can be set via button as standard,

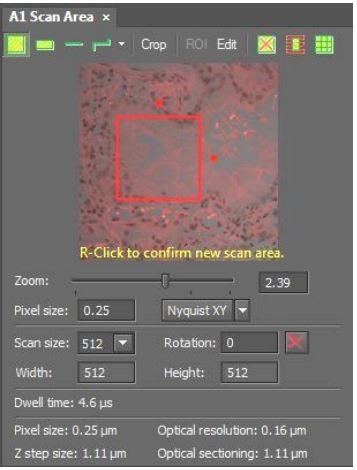
13) Laser Selections: check laser lines to be used. HV is your amplification gain (100 is a good starting point), offset will black values (0 is a good starting point) and the wavelength shown last is your laser power in % (5 is a good starting point).





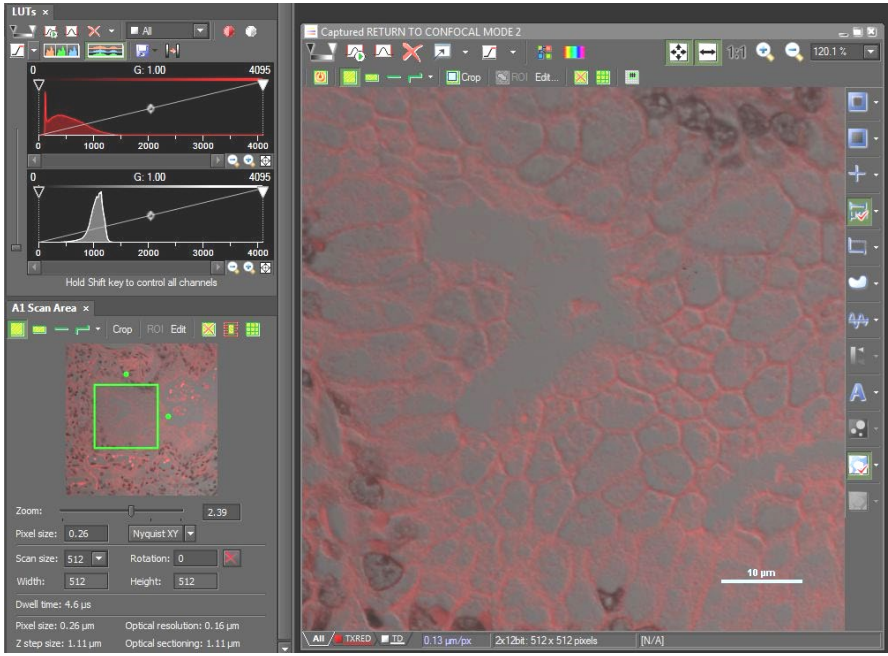
Scan Area/Zoom/ Resolution Improvements





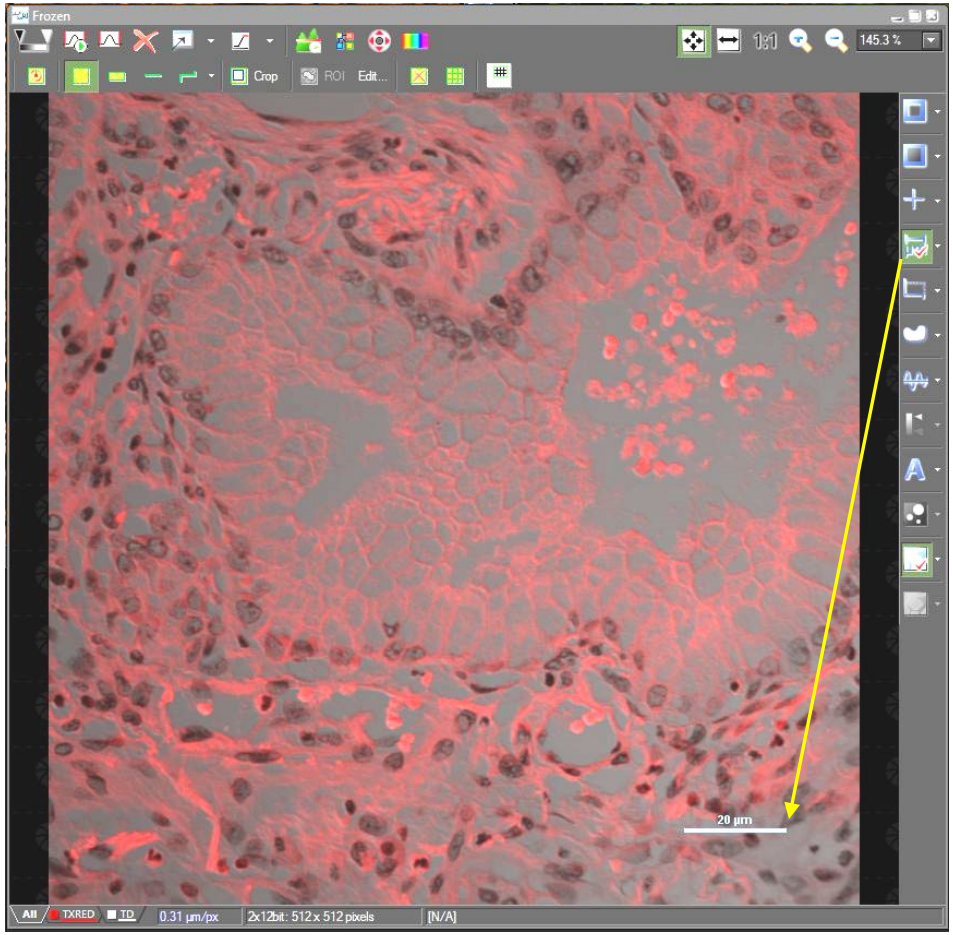
- If not already open, locate the "A1 Scan Area" dialog (View→Acquisition Controls→A1 Scan Area)
- You can click and drag the green borders of the image to zoom into an area of interest. While zooming in normally decreases resolution, it can increase resolution when using a confocal. The pixel size will decrease and keep the image size the same.

*The "Nyquist XY" button will select the appropriate settings to set the selected area at Nyquist resolution (based on preferences set with drop down arrow).



Post Acquisition Image Adjustments

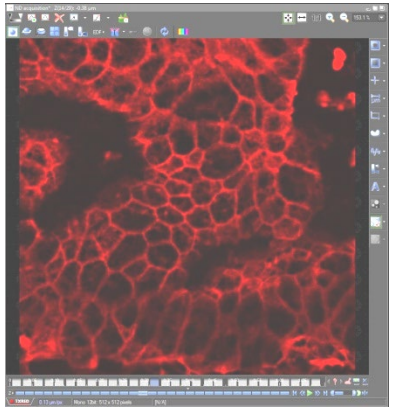
Scale Bars



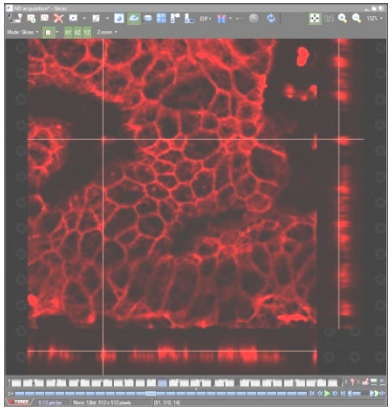
A Scale Bar can be added at any time from the right side image window's toolbar. Once positioned where you choose, you can *right click on it to change its properties* or to "burn it" into the image

**it is recommended to save an original image before burning a scalebar into an image, save the second as a copy*

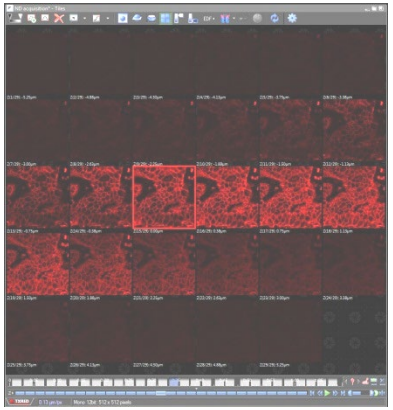
How you can visualize your data…….



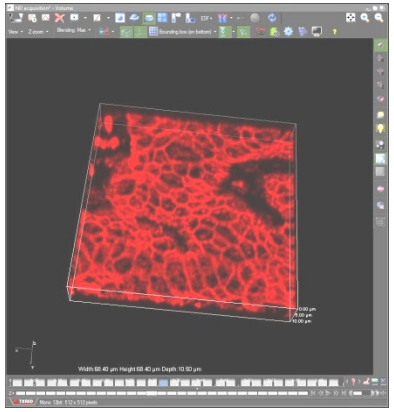
2D



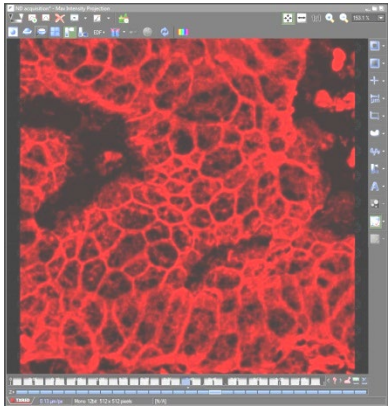
Sliced



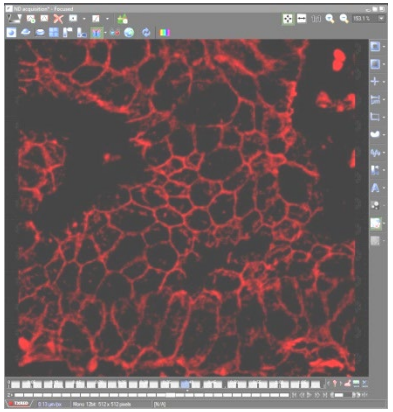
Tiled



3D



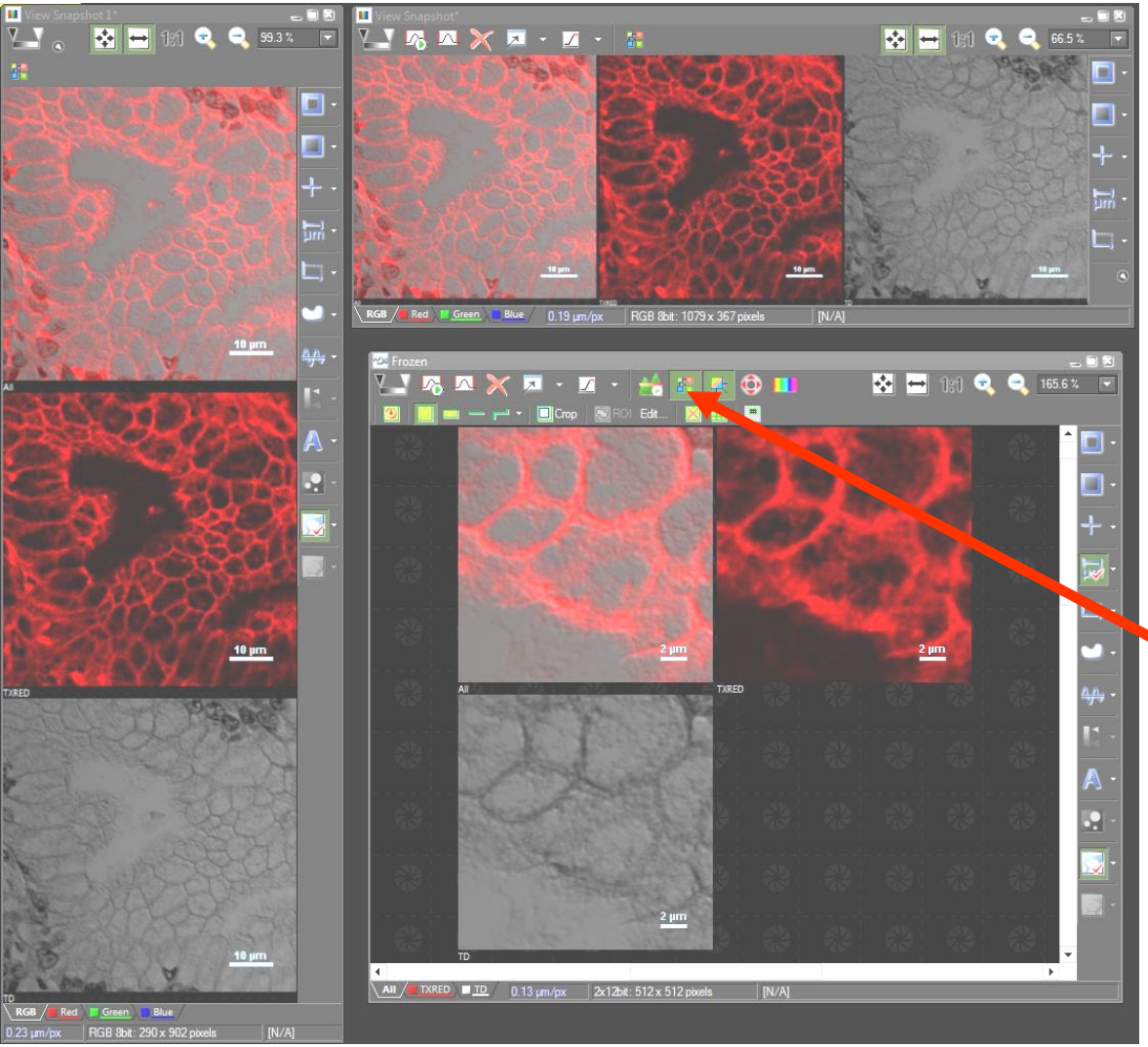
Projection



EDF

*Can also make really nice 3D rotational movies as .avi's!

“X” Key Snapshots

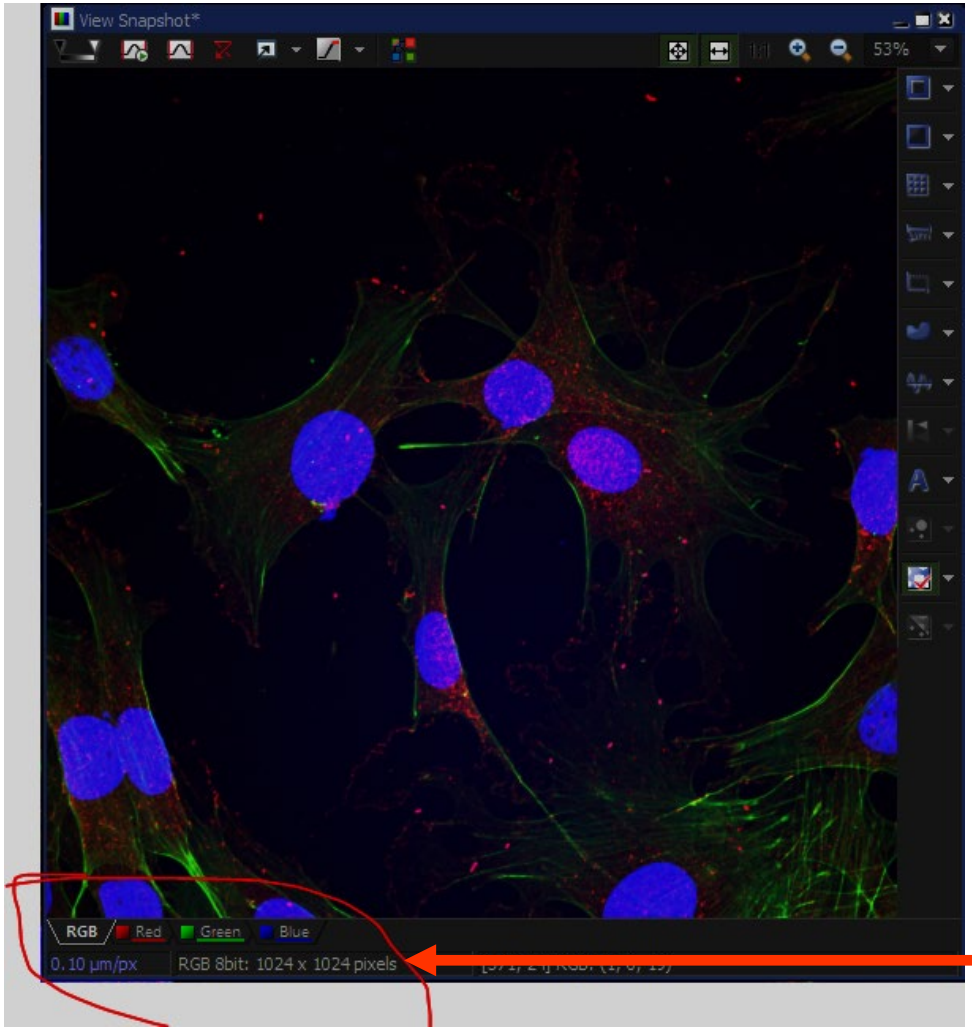


*Use the “x” key on your keyboard to take snapshots for publication/presentation

-can be used on any open image window, in any orientation

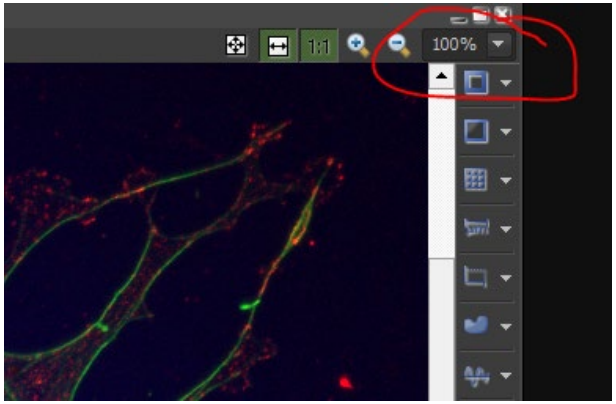
“Split Window”

“Shift then Z” Full Resolution Snapshots



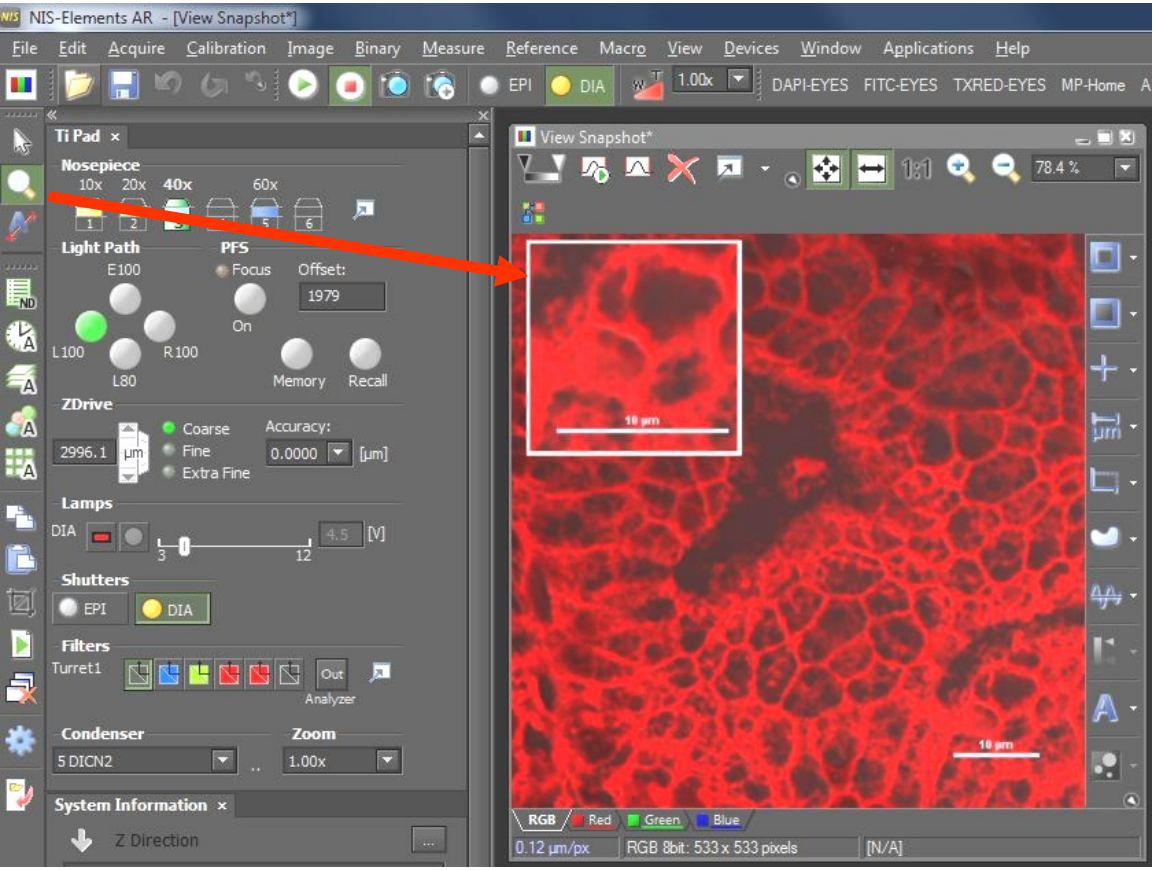
*Use the “shift then Z” keys on your keyboard to take full resolution snapshots for publication/presentation

-zoom must be set to 100% before setting snapshot



Keeps original resolution or higher!

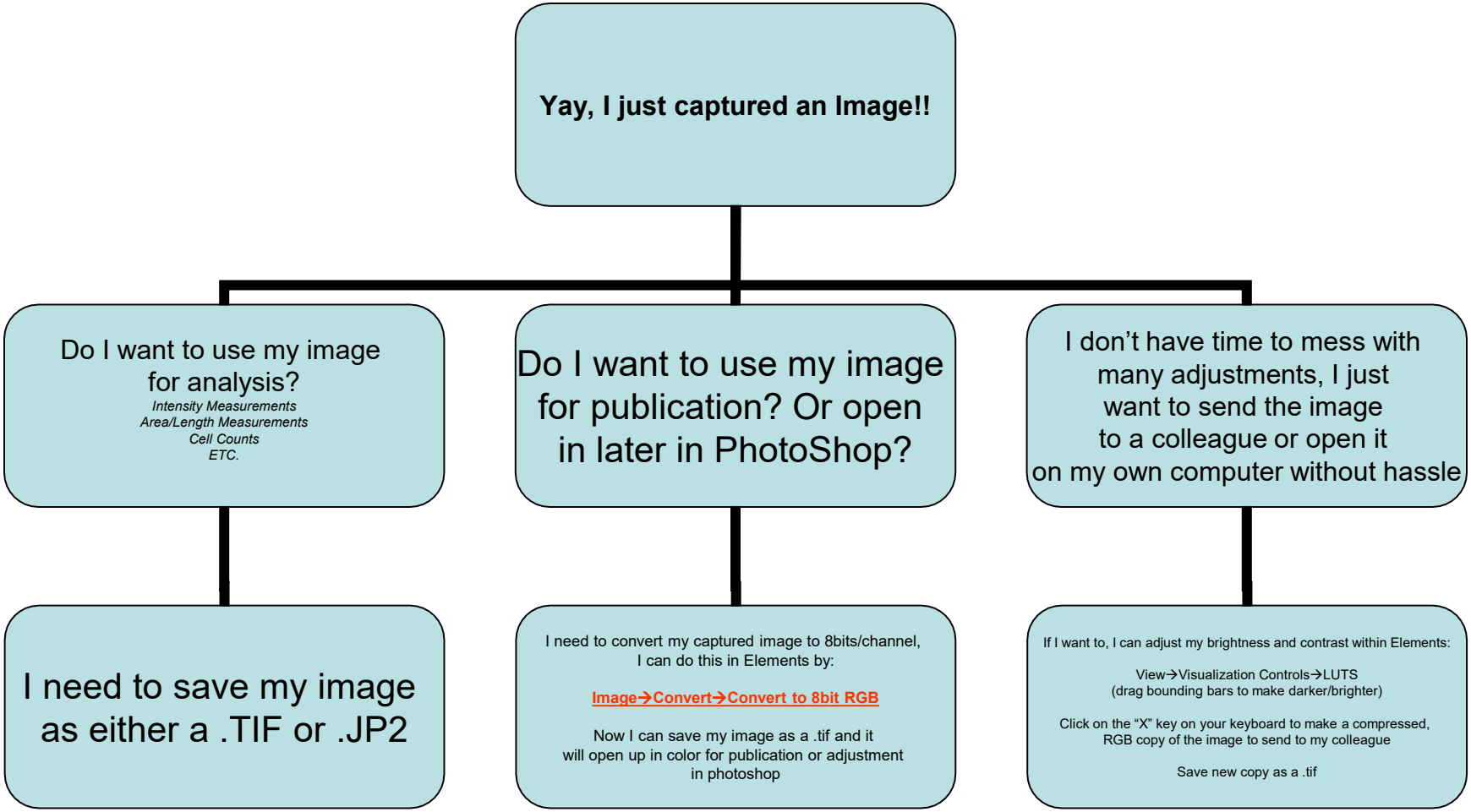
Magnifying Glass



- The magnifying glass can be used to show zoomed portions of an image
- Under View→Magnifying glass options, you can change the zoom and shape/border

**Holding down "shift" after choosing an area can allow it to be moved to another spot on the image; click on "X" while still holding "shift" to take a snapshot (as shown here)*

Image Saving Helper….



*For saving timelapse movies, simply go to file→save as→AVI and select no compression and 150-200ms for time interval