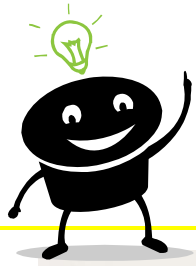




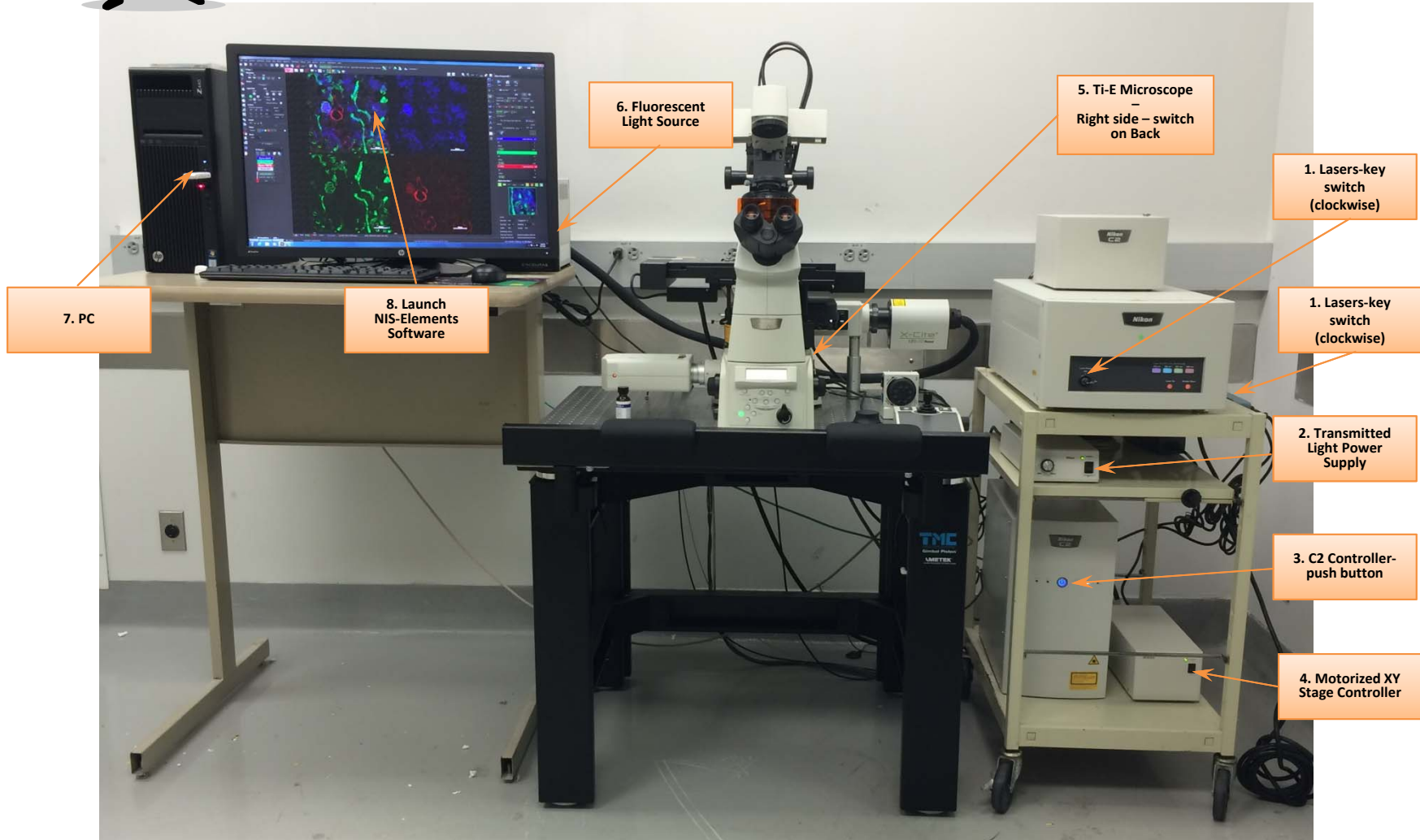
Nikon A1R Training Guide
Prepared for Florida International University



Hardware Guide



Turn On Sequence (off-reverse order)



Main System Components

Confocal PMTs
(DU3)

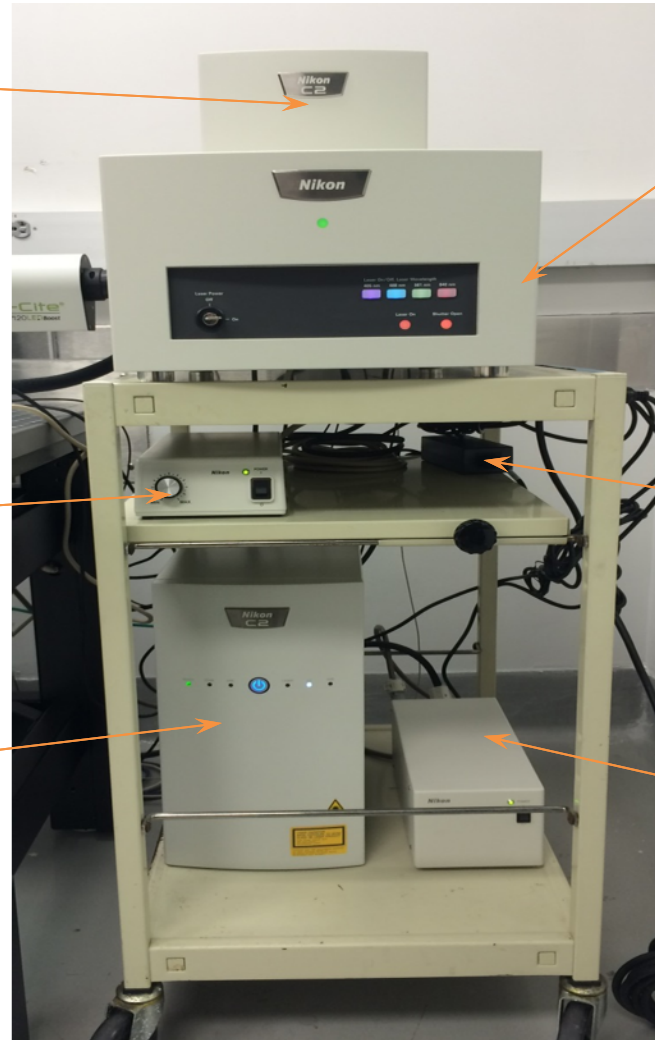
LU-N4
Laser Launch
(405nm, 488nm,
561nm, 640nm)

Transmitted Light
Power Supply

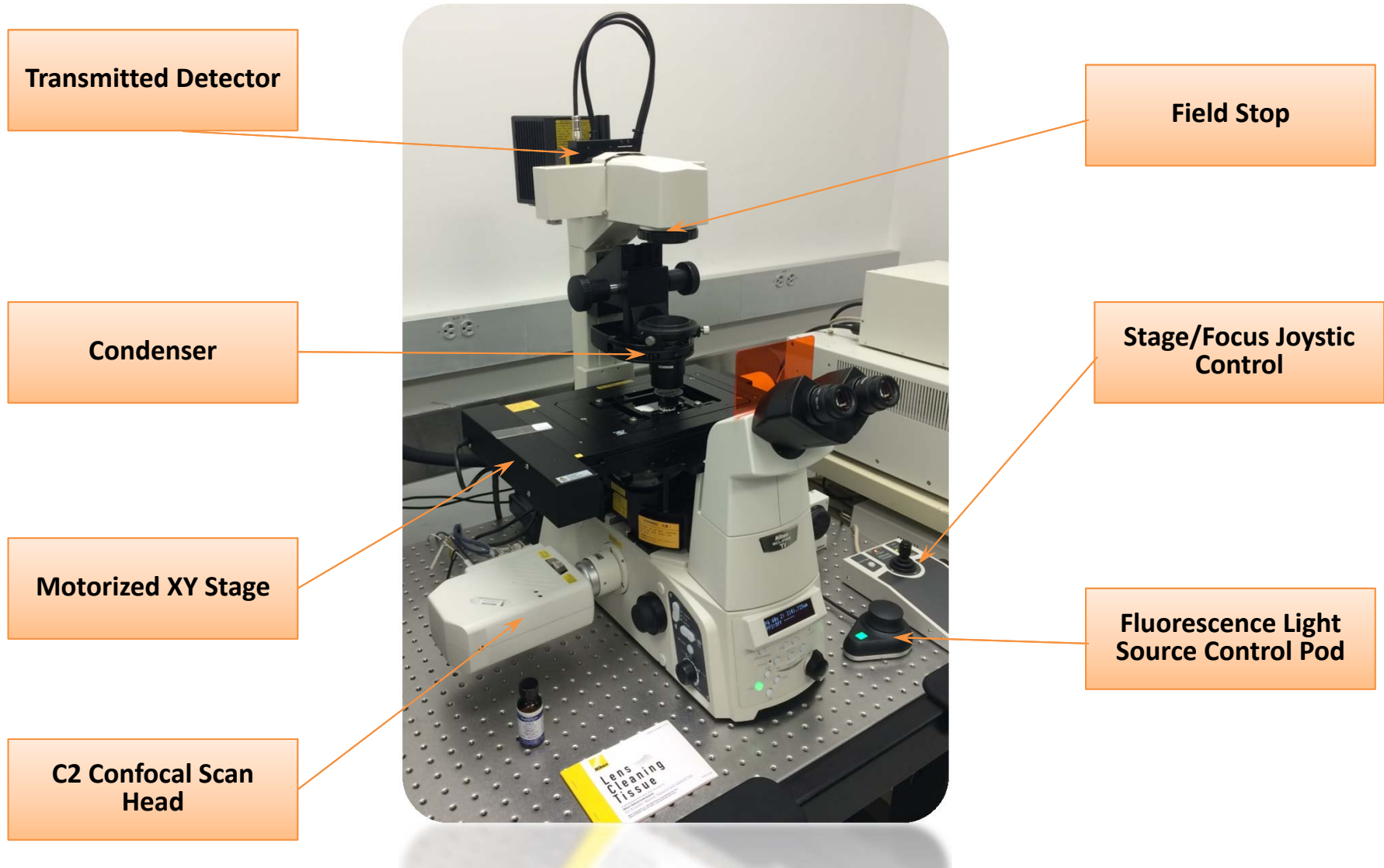
LUNV Laser
Controller

Confocal C2
Controller

Motorized XY Stage
Controller



Main System Components



Main System Components

Focus knob

Objectives Changer

Fluorescence Filter Cubes Changer

Escape & Refocus

Perfect Focus

Light Path Changer

1X – 1.5X Mag Changer



Quick Review on Kohler Alignment



**Use a 10x objective if available and fully open the condenser aperture diaphragm*

1. Focus on a sample



2. Close down your field stop



3. Focus your condenser (adjust condenser height)



4. Center your condenser



5. Open field aperture

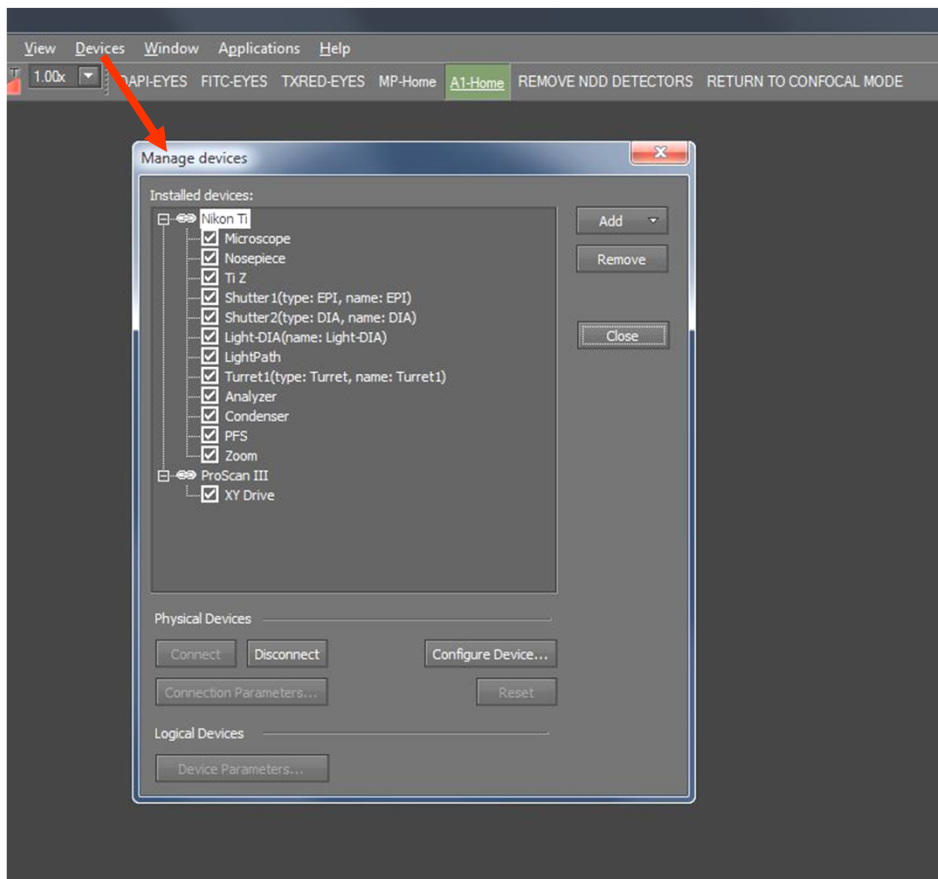





Software Guide

General Software Statements

As a general reference, the devices connected to the software can be reviewed in the “Device Manager” under “Devices”. This is a good place to confirm all portions of the system are connected and working properly.



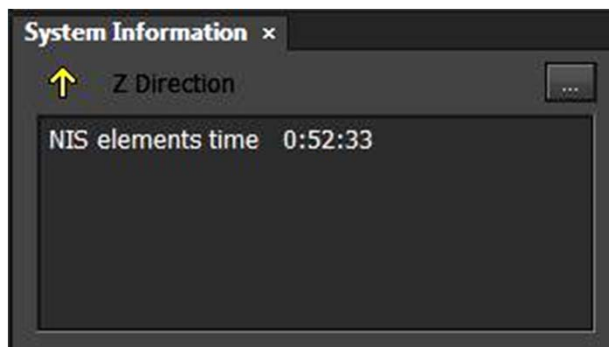
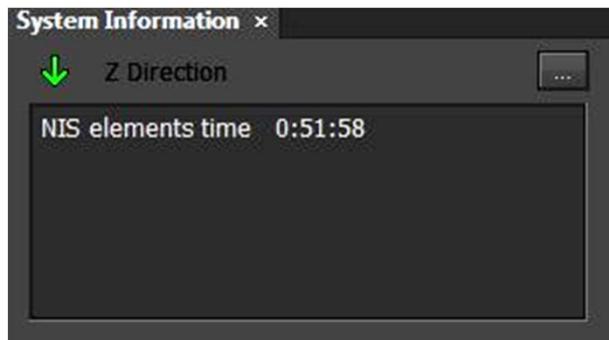
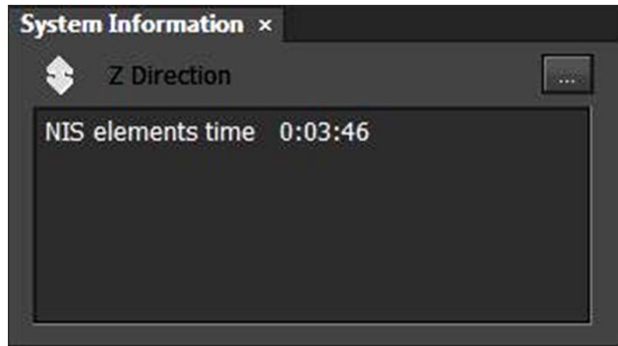
**On this system, you should see the Ti Microscope as being connected. If there is a break in the chain , make sure the device is physically turned on, then highlight the device and select “Connect” to reconnect.*

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 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 www.nis-elements.com. There is also a plugin for ImageJ.

System Information



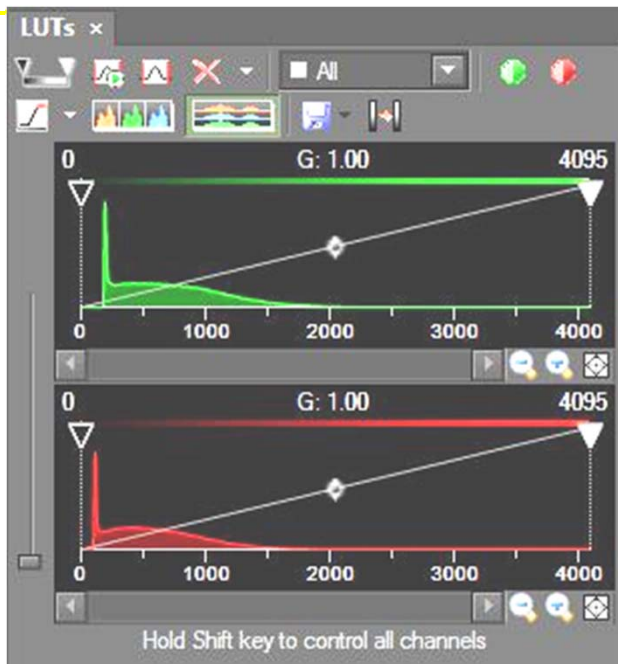
**The system information will tell you how long the software has been running for that session and will also tell you what direction you are focusing.*


Microscope Control

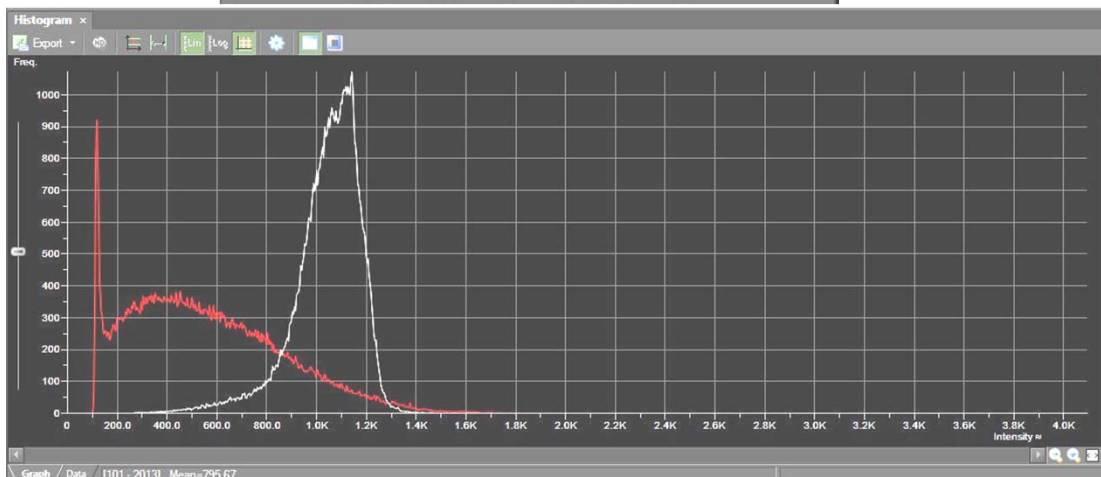


- The Ti Microscope Pad allows to:
 - Switch between objectives
 - Select light path
 - Use Perfect Focus
 - Change Filters

LUTs and Histogram

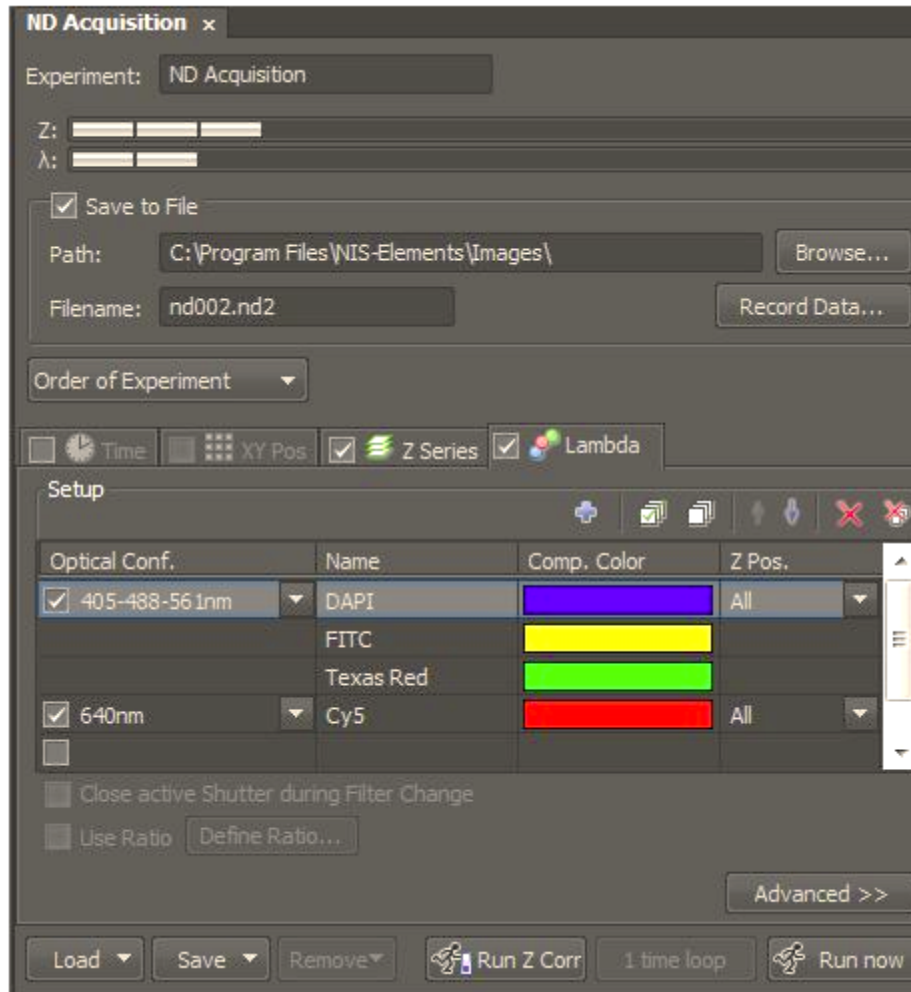


The LUTs menu can be used for brightness/contrast adjustment (using triangular sliders) as well as for showing a **saturation indicator**  which can be used to show what pixels are saturated. Use the drop down arrow to select “complementary color”



The traditional Histogram gives similar information as the LUTs but is very useful in determining a confocal “offset” adjustment

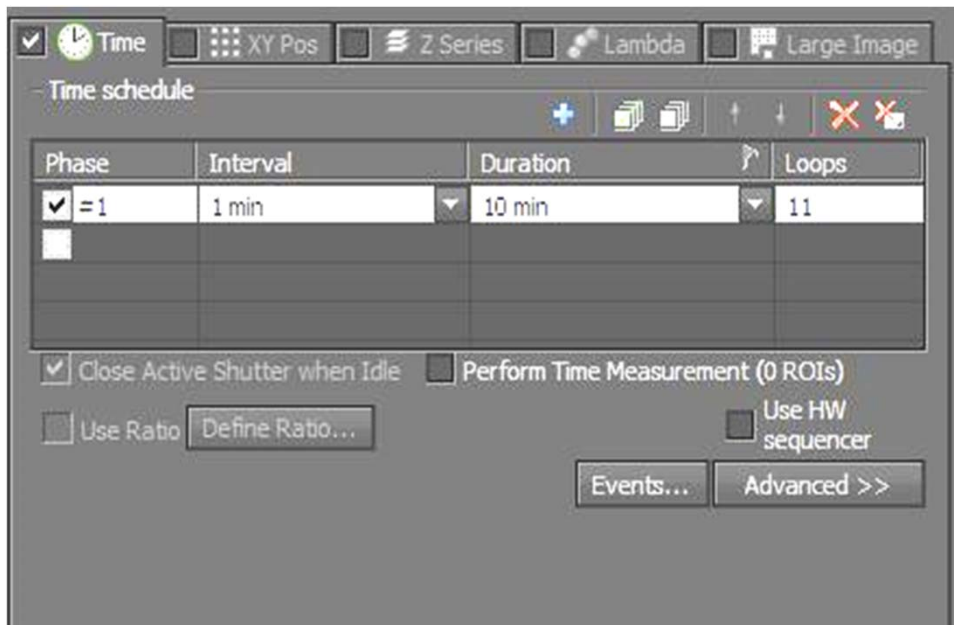
ND Experiments



**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.

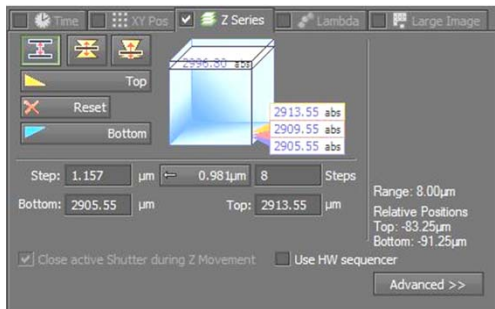
Time Lapse



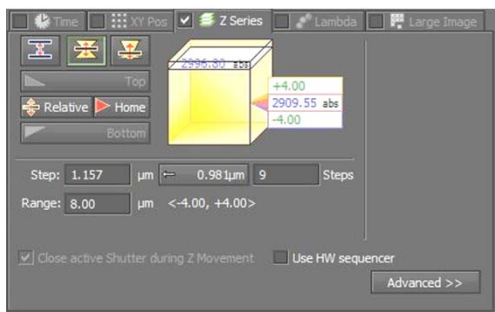
- To add a timelapse, check off the checkbox for “Time”.
- Add a time phase by checking one box; you can change the interval (how often to image) and the duration (total time). Alternatively you can input a number of loops (# of images required).
- You can add additional time phases to be run in sequence.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

Z-Stacking/Optical Sectioning

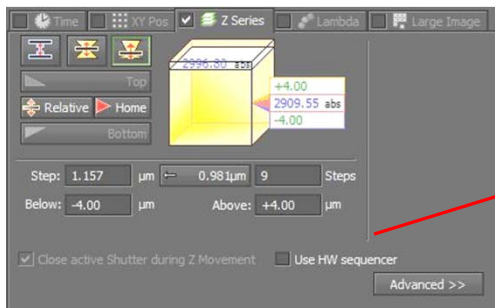
There are three methods for optical sectioning .select one



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)

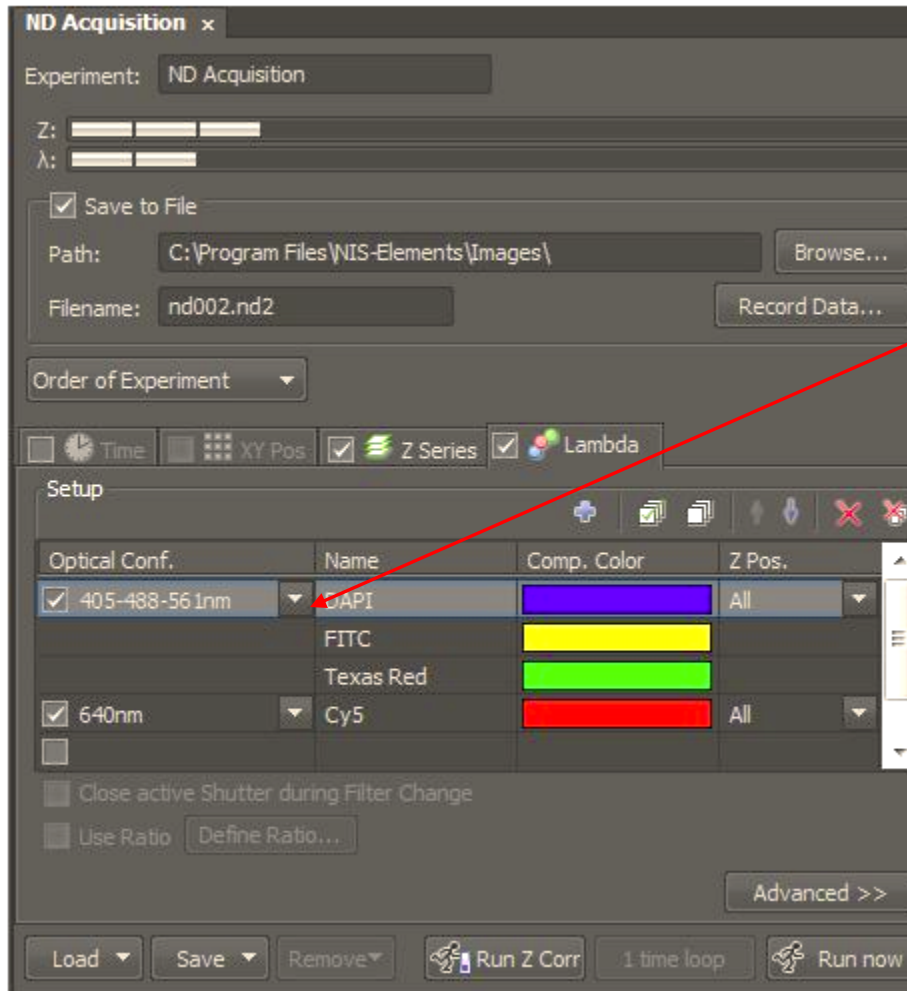


- *Set your step size (distance between each plane) and/or the # of steps you want (image total)
2. Choose a middle plane and click “home” then input a distance range (equal from top to bottom)



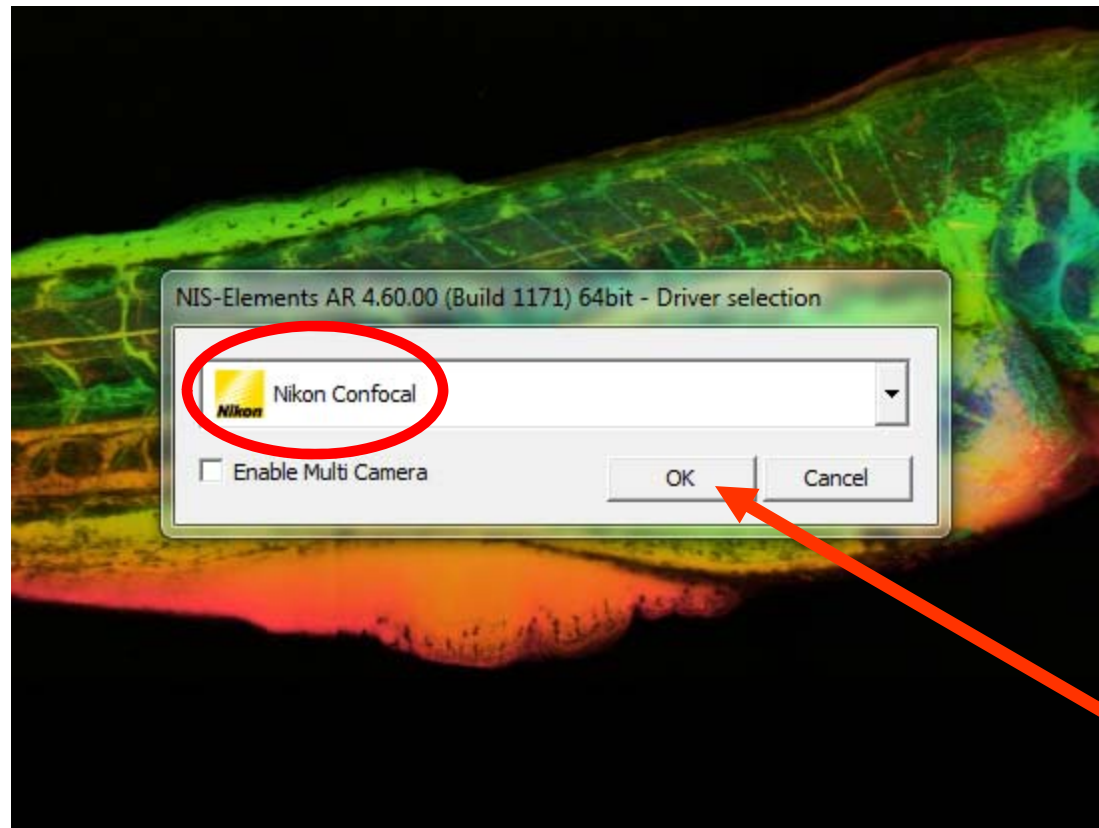
3. Choose a plane somewhere in the sample and click “home” then input a staggered range above and below

Lambda-wavelength

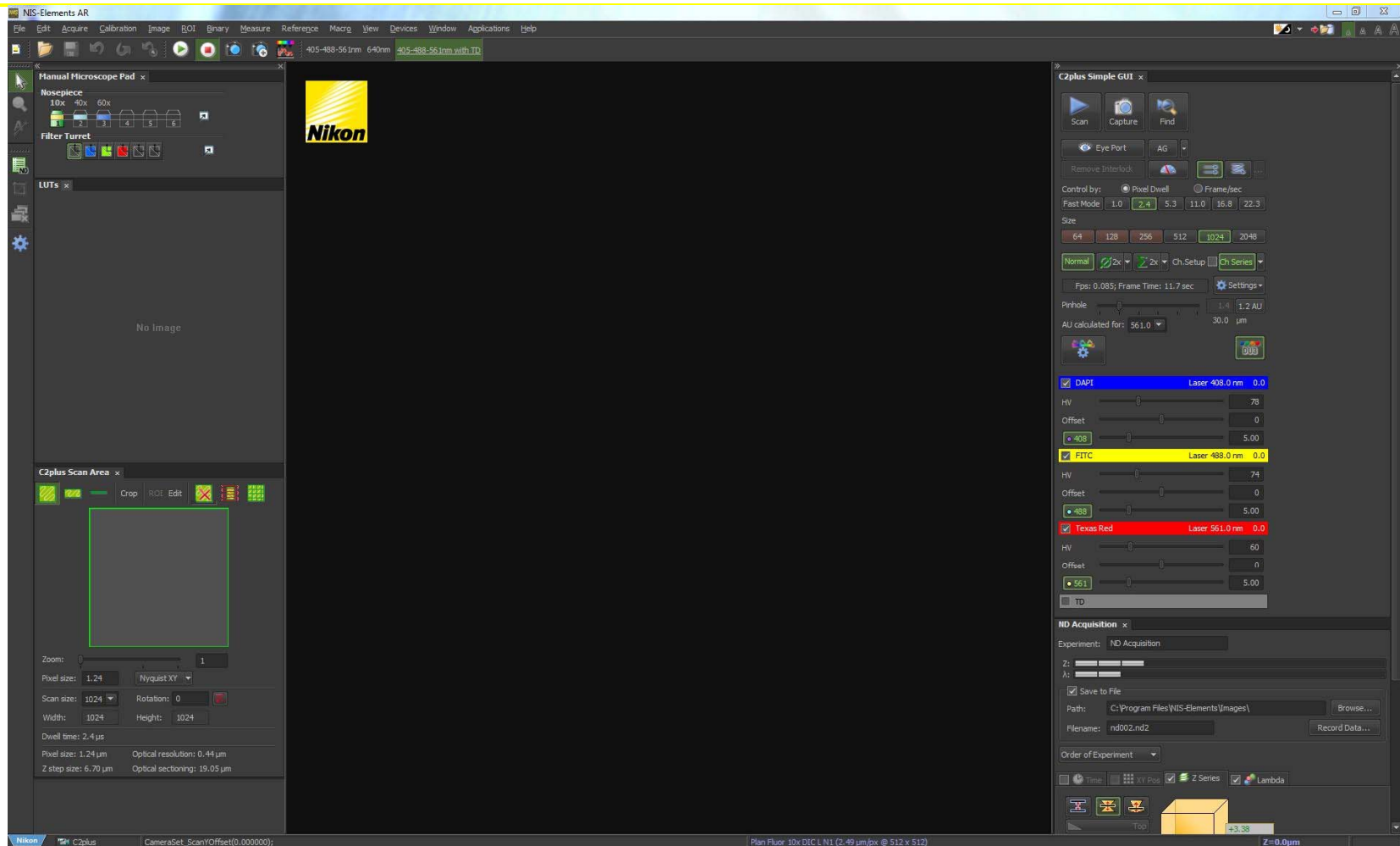


- You can add automated wavelength switching to an experiment by checking off the checkbox for “Lambda”.
 - You can add an optical configuration, if one was created, by clicking on the down arrows
- *Make sure to adjust your confocal scan settings before adding*

Confocal Imaging: C2+



Layout



Using Optical Configurations



405-488-561nm 640nm 405-488-561nm with TD

**You may have a series of “shortcut” keys on the top toolbar for confocal imaging and control of the microscope with a single button. These are referred to as “optical configurations”.*

Click on the configuration you want to use

On your system, click on:

405-488-561nm: this will use all three lasers/PMTs

640nm: this will use the 640 laser/PMT

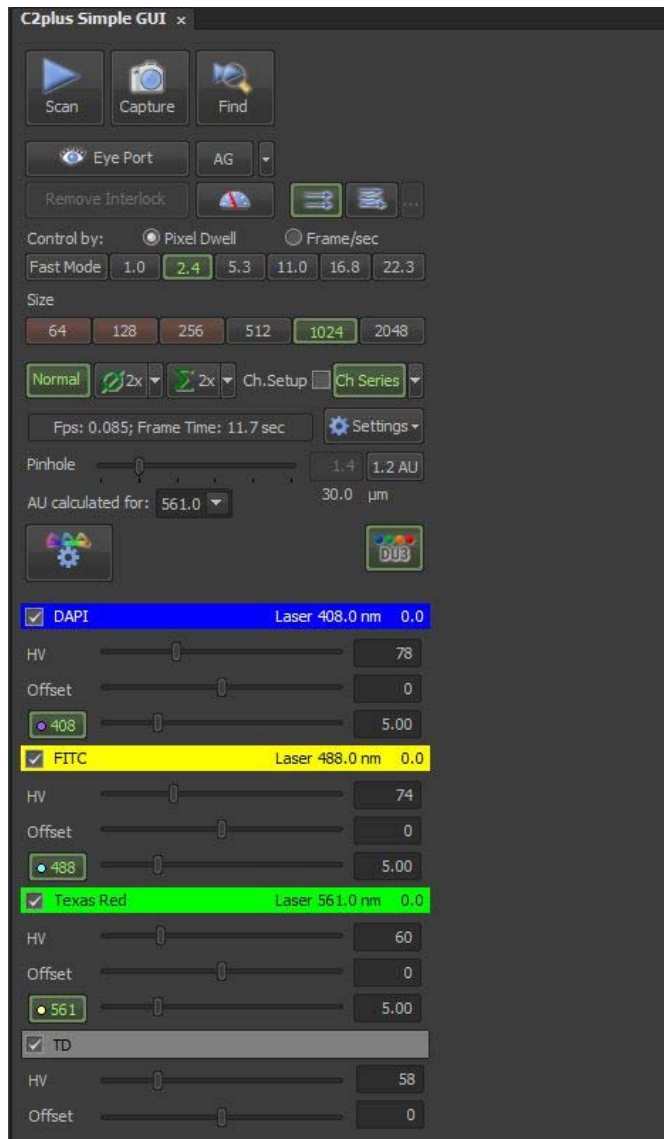
405-488-561nm with TD: this will use all three lasers/PMTs and also insert the TD (transmission detector)

Note: *Please remove the transmission detector if returning to the eyepieces to view a sample. The transmission detector is very sensitive to light and can be damaged if fluorescence or transmitted light is used instead of confocal!*

Confocal Acquisition: Quick Start



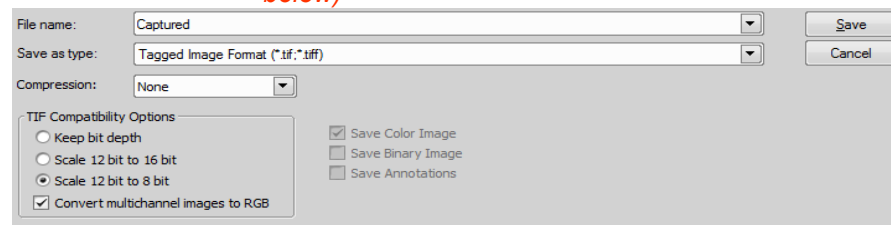
**The C2plus Simple GUI is a wizard that will walk you through acquiring a confocal image*




1. Make sure you have previewed your slide through the eyepieces and focused on an area of interest.
2. Select the objective that you're using in the manual microscope pad
3. Click on an optical configuration on the top toolbar to get started
4. Select what laser lines you want to acquire (check boxes for DAPI/FITC/TXRED/TD (transmission) or CY5)
 - **HV-Gain, start at 100**
 - **Offset-background adjustment, start at 0**
 - **Laser Power, start at 5%**
5. Start with a scan size of 512x512 and scan speed of 1.9usec.
6. Select "Normal" and "Ch Series"
7. Select 1.2 AU for Pinhole
8. Start "SCAN"
9. Adjust if needed
10. Click "CAPTURE"

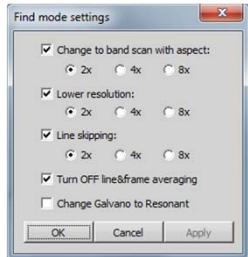
*To save, click on File→Save As and select either a **.tif** (keeping bit depth) or **.ip2** for raw data, **NOT** a JPG.



To convert into a viewable image, save an additional copy as a **converted tiff (shown below)*



The C2plus GUI-further explained (top to bottom)

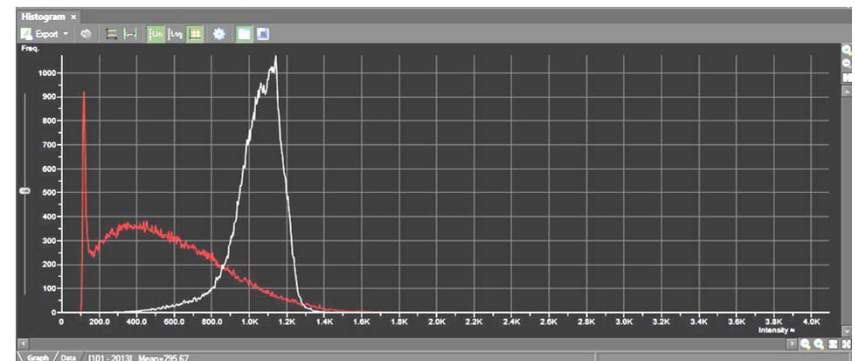
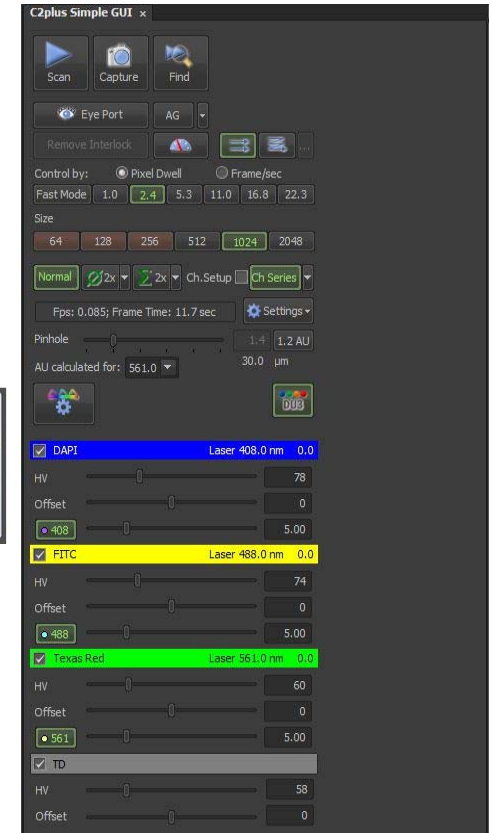
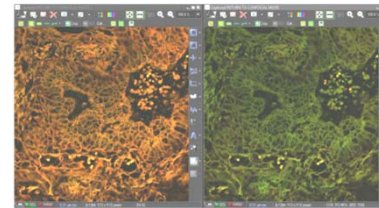
- 1) Stops/Starts Live Image Acquisition (as a default, please do not use any averaging or integrating in this mode, see #9)
- 2) Capture: captures an image (using user defined settings below)
- 3) "Find Mode": Implements a fast preview scan
a) Defined by Find Mode Settings in 



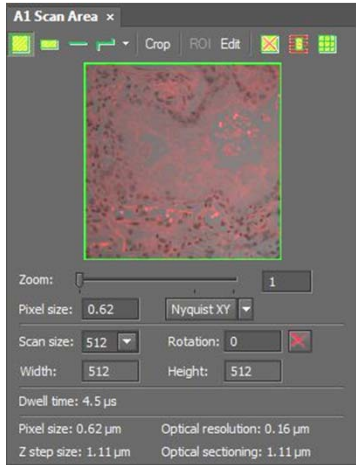
- 4) Eye Port-changes to last used non-confocal setting, click again to return to confocal mode (N/A on this microscope)
- 5) AG: Auto Gain feature; sets the HV (gain) for optimum based on user settings (for more info see Help Menu, 4.2.5 in software)
- 6) Unidirectional/Bidirectional: scan direction (Uni-one direction, traditional scanning; Bi-directional, used for live imaging for speed increase—does need to be aligned during scan). See Help, 8.1.3 for more info.
- 7) Pixel Dwell/Frame per Sec: scan speed; pixel dwell in micro seconds
- 8) Size: Scan Size in XY
- 9) Averaging  and Integrating  : average signal by line or integrate signal by line (see Help, 8.1.4). Normal mode does not use either. Typically averaging is used only for image capture, not for live preview.
- 10) Ch Series: Channel Series; setup will allow for each laser to be run sequentially rather than simultaneously ***important for closely excited fluorophores!** See Help, 4.1.4
- 11) Pinhole: adjusts size of pinhole, 1.2 AU can be set via button as standard, see Help 4.2.3 for more information.
- 12) Optical Path Settings: to set up lasers, dichroics, filters; for experienced users only!
- 13) Laser Selections: check off 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).

Note: Offset Adjustment

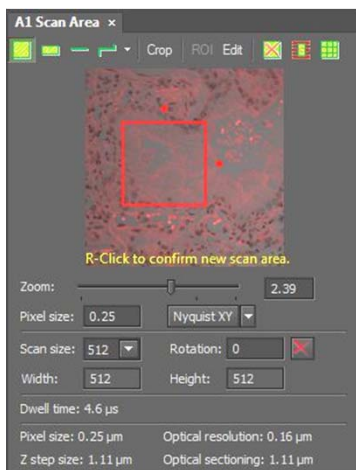
Offset values should be close to a number value of 0 (zero) but a histogram should be previewed to adjust this value accurately. The left side of each wavelength's peak should be close to 0,0 (bottom left of histogram). A more positive # will move the peak to the right, a more negative # will move it to the left. Please ask for assistance if you need help—this step is **VERY important!**



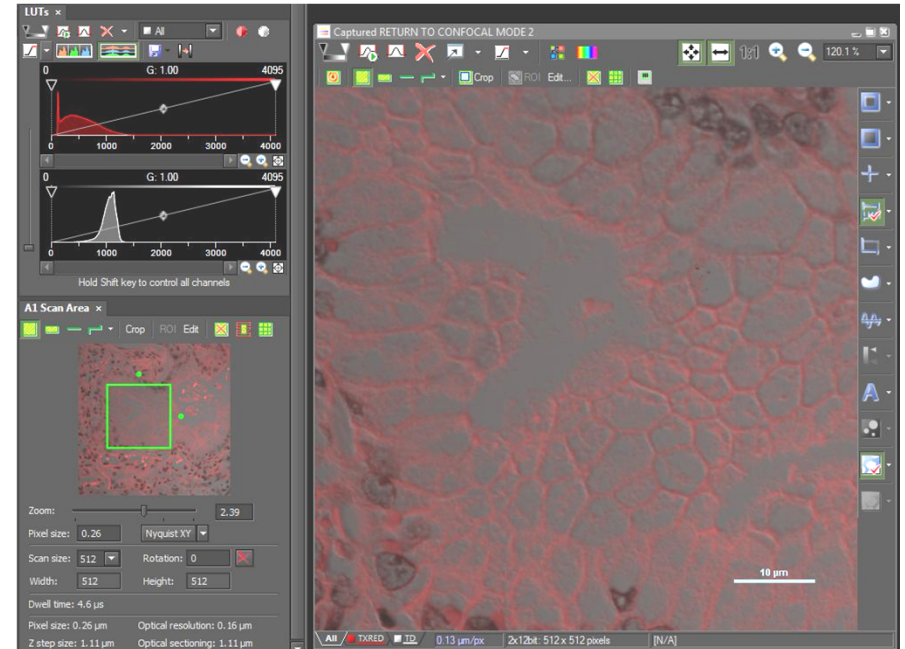
Scan Area/Zoom/Resolution Improvements



- Locate the “C2 Scan Area” dialog (View→Acquisition Controls→C2 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.



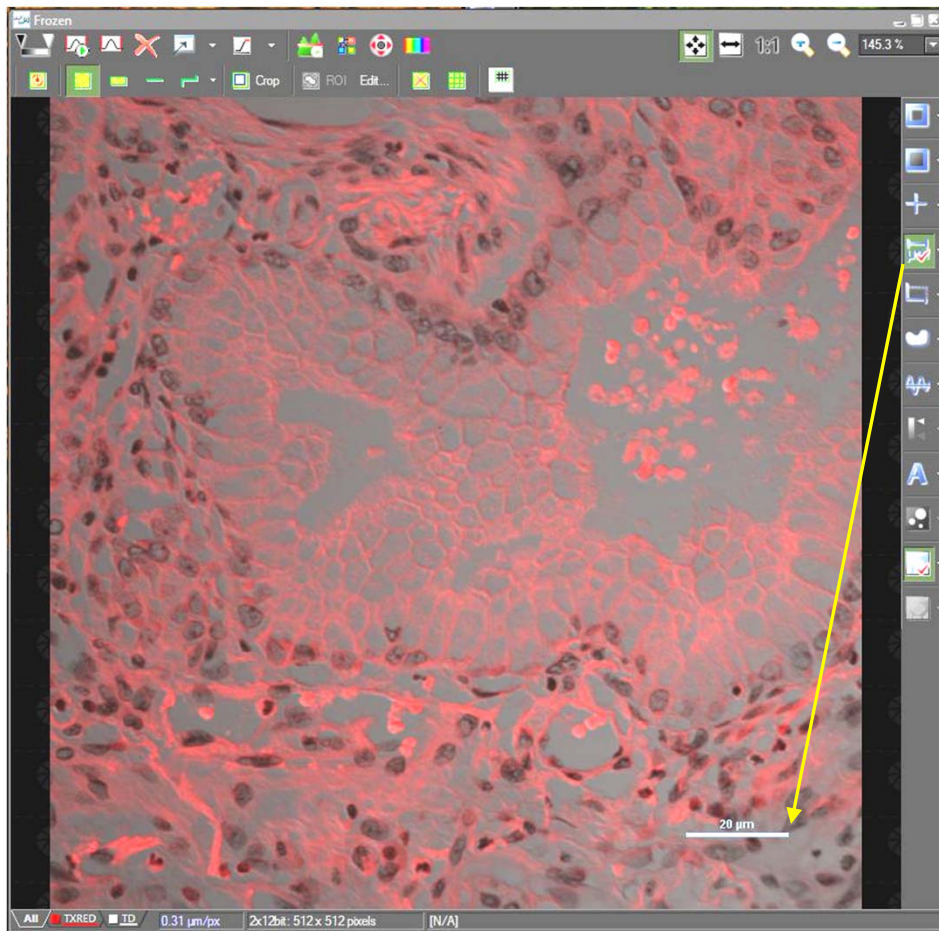
- *The “Nyquist XY” button will select the appropriate settings to set the selected area at Nyquist resolution (based on preferences set with drop down arrow).





Post Acquisition Image Adjustments

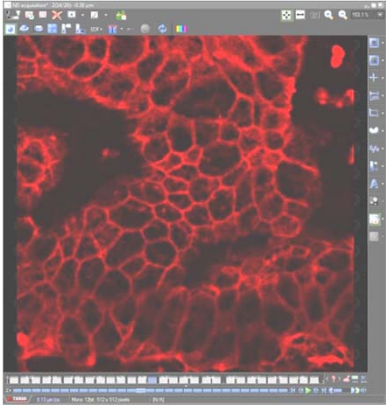
Scale Bars



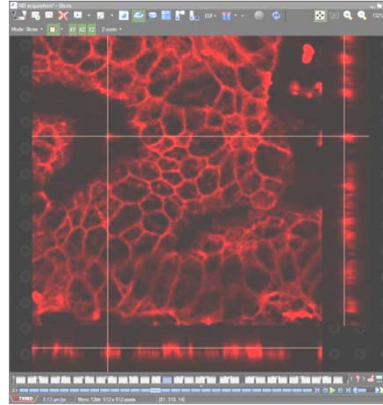
A Scale Bar can be added at any time from the right side image window's toolbar. Once positioned where you choose, you can right click on it to change its properties or to "burn it" into the image

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

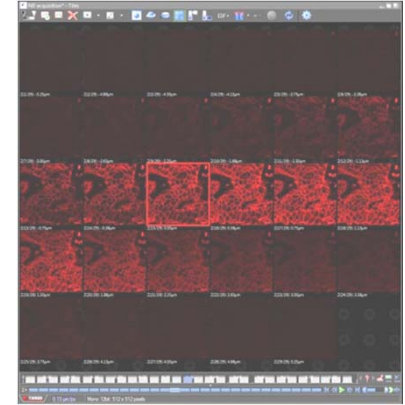
How you can visualize your data.....



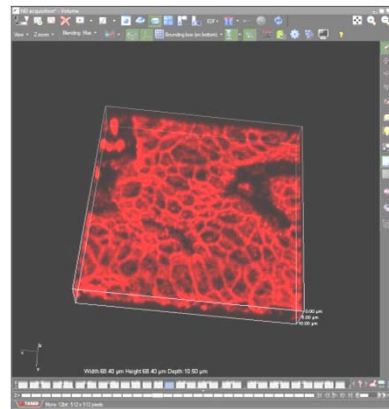
2D



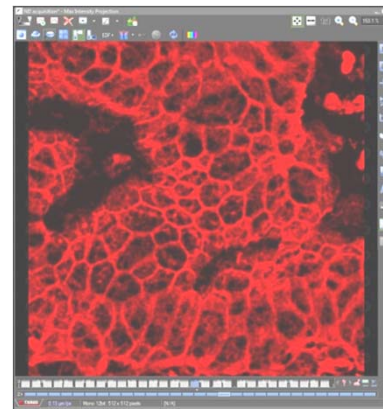
Sliced



Tiled



3D



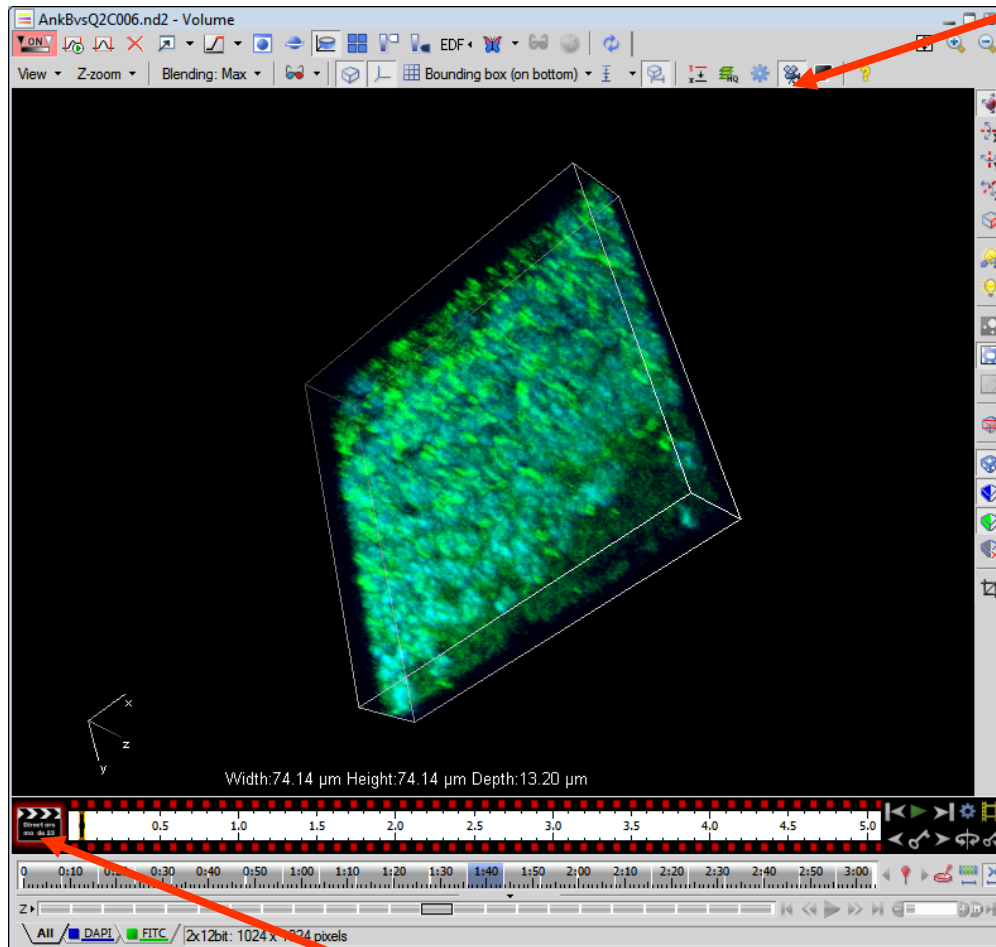
Projection

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

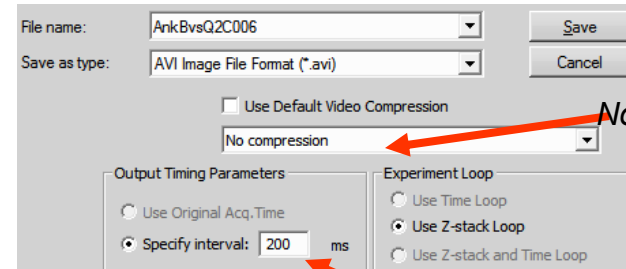
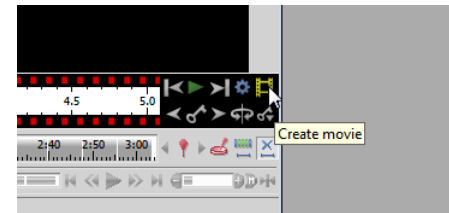
Movie Maker



Click the film icon to open movie maker



Make rotations/movements/adjustments and click where you want them to show up in the filmstrip. Once you play it back and it looks good, click on the film icon to create an .avi.

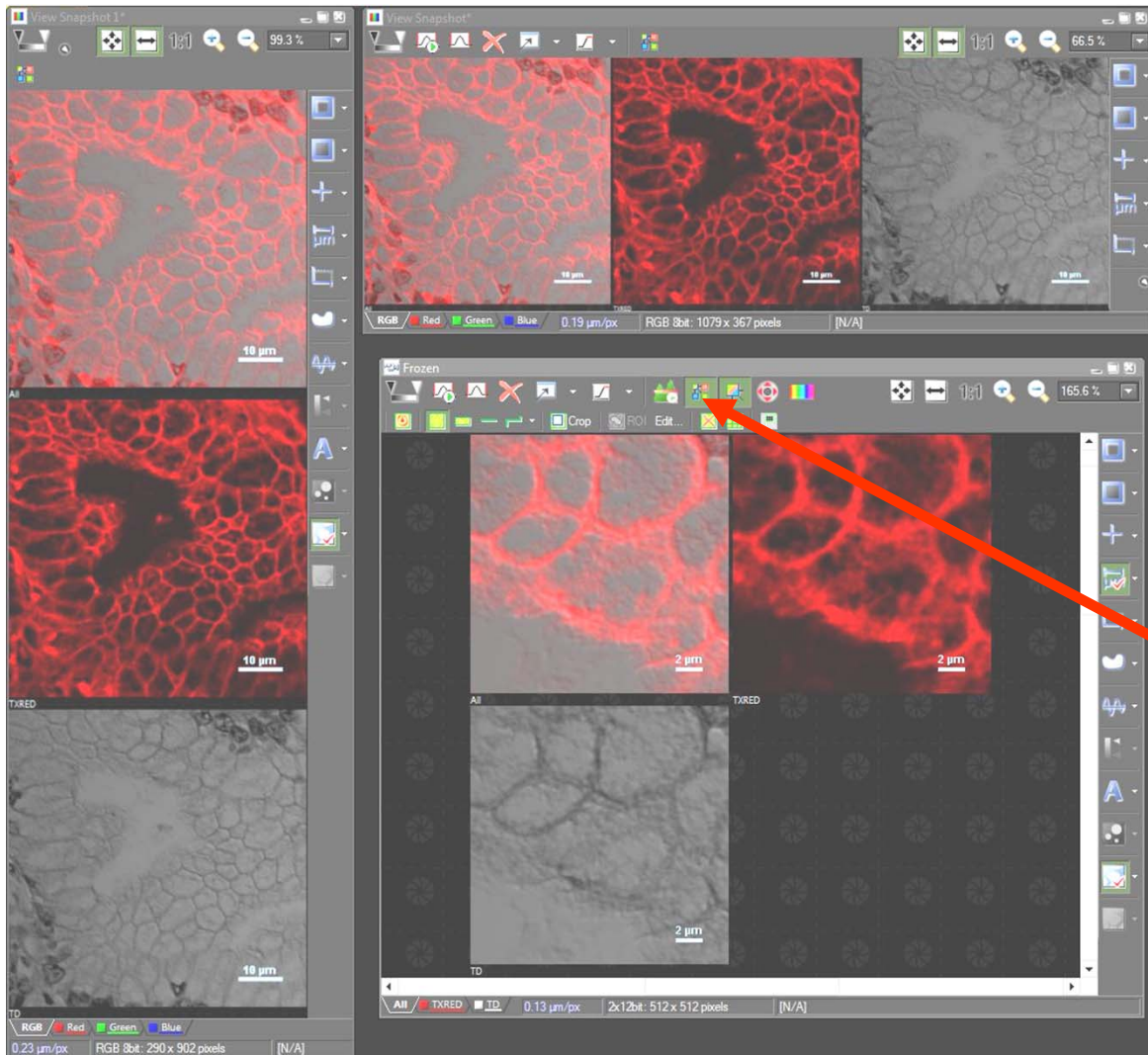


No compression

About 150-200ms is usually good

Click film strip (black to red) to go into editing mode

"X" Key Snapshots

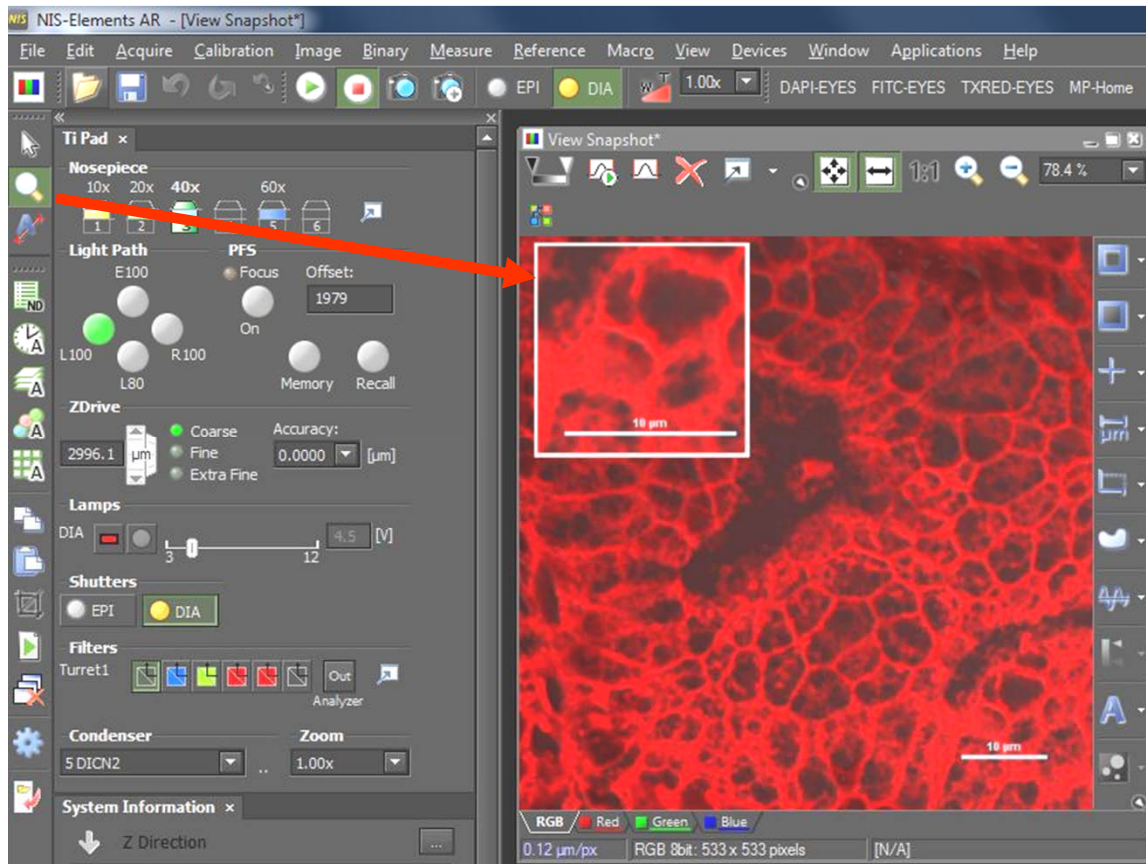


*Use the "x" key on your keyboard to take snapshots for publication/presentation

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

"Split Window"

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” to take a snapshot (as shown here)*