Nikon A1R Appendix

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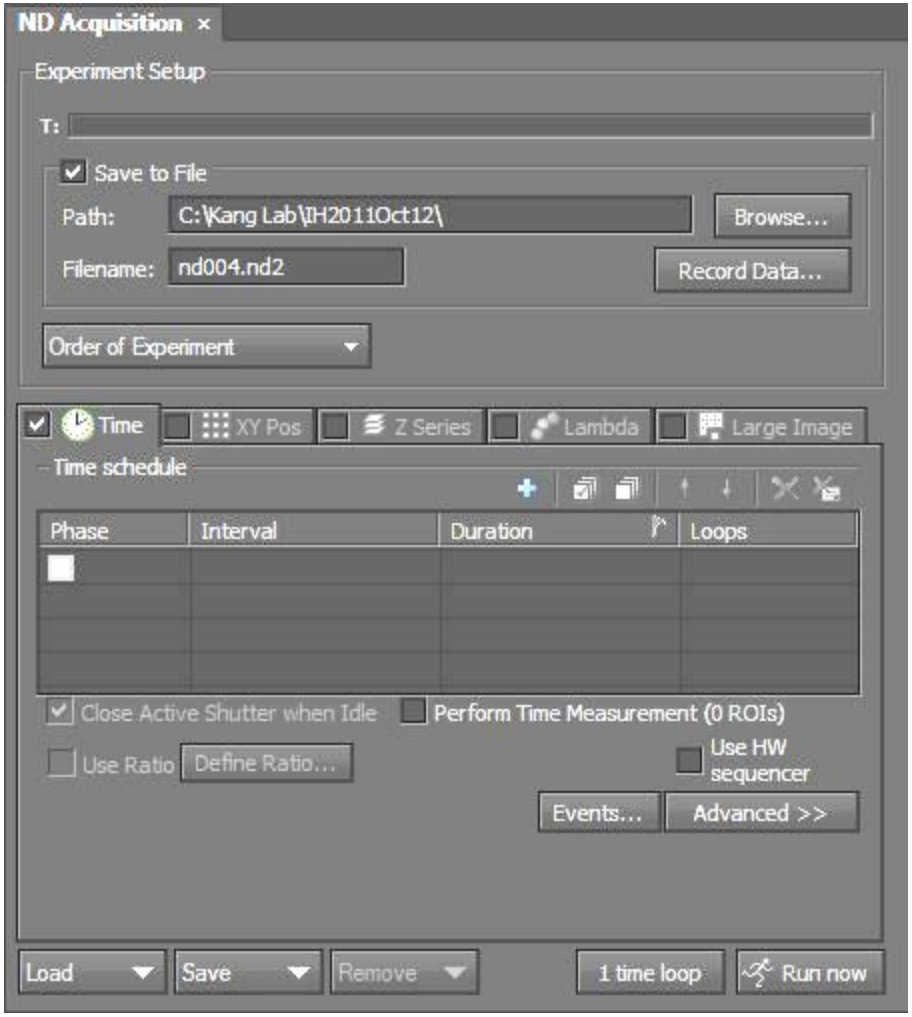
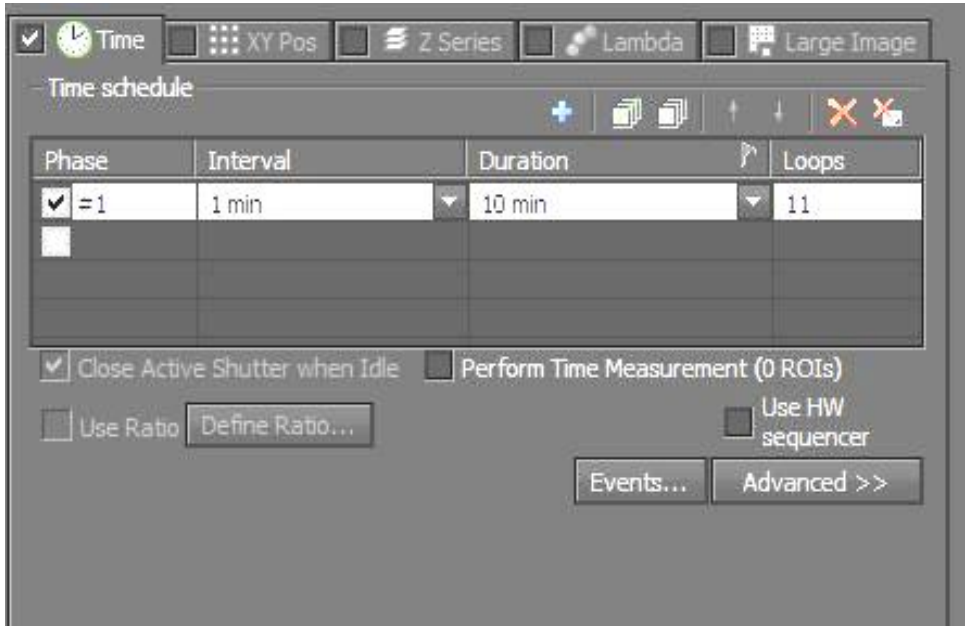
ND Experiments



**Experiments can be run on time, stage positions, Z-stacks, wavelength switching and/or image stitching.*

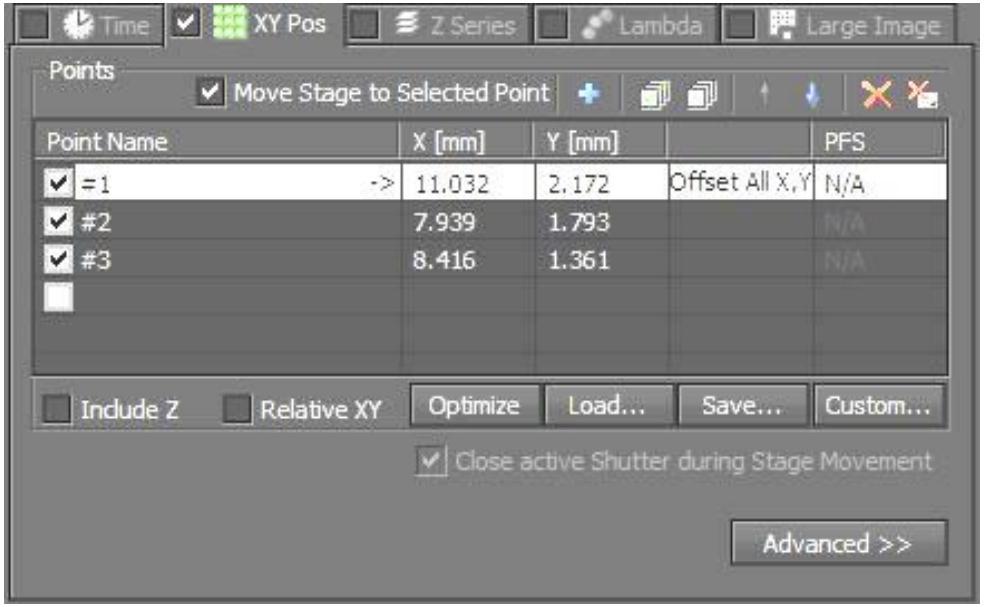
-The next few slides will explain each dimension.

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.

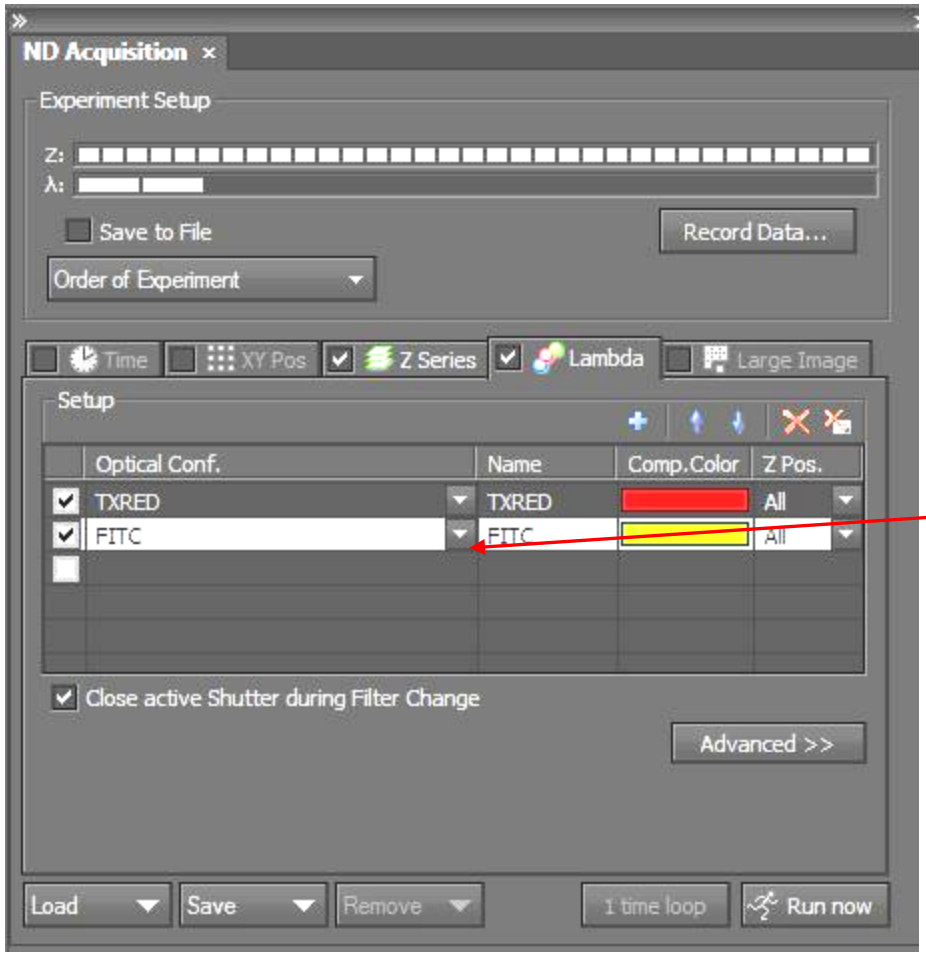
XY-Stage Positions



- 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).

*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.

Lambda-wavelength



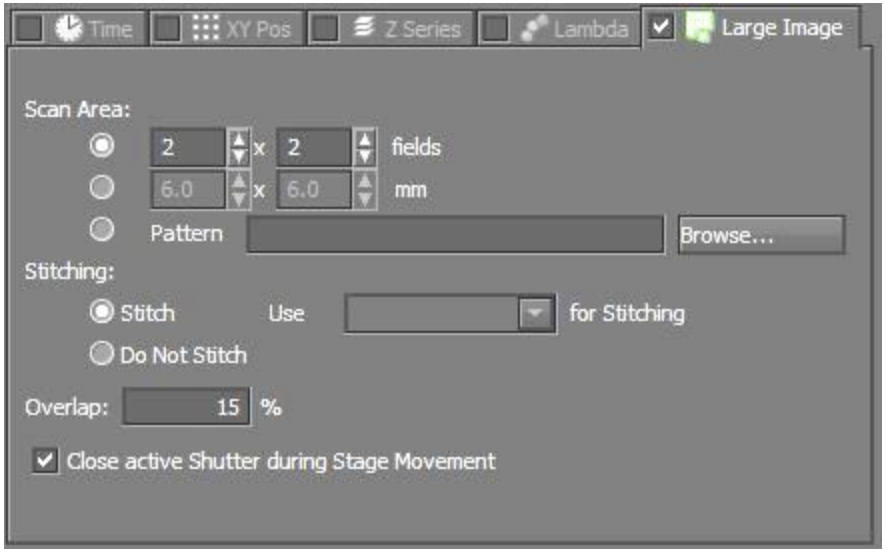
- You can add automated wavelength switching to an experiment by checking off the checkbox for “Lambda”.

**This is most used for widefield imaging as this is already automated for confocal as default*

- You can add an optical configuration (DAPI/FITC/CY3/DSRED) by clicking on the down arrows

**Make sure to adjust your camera settings before adding*

Image Stitching

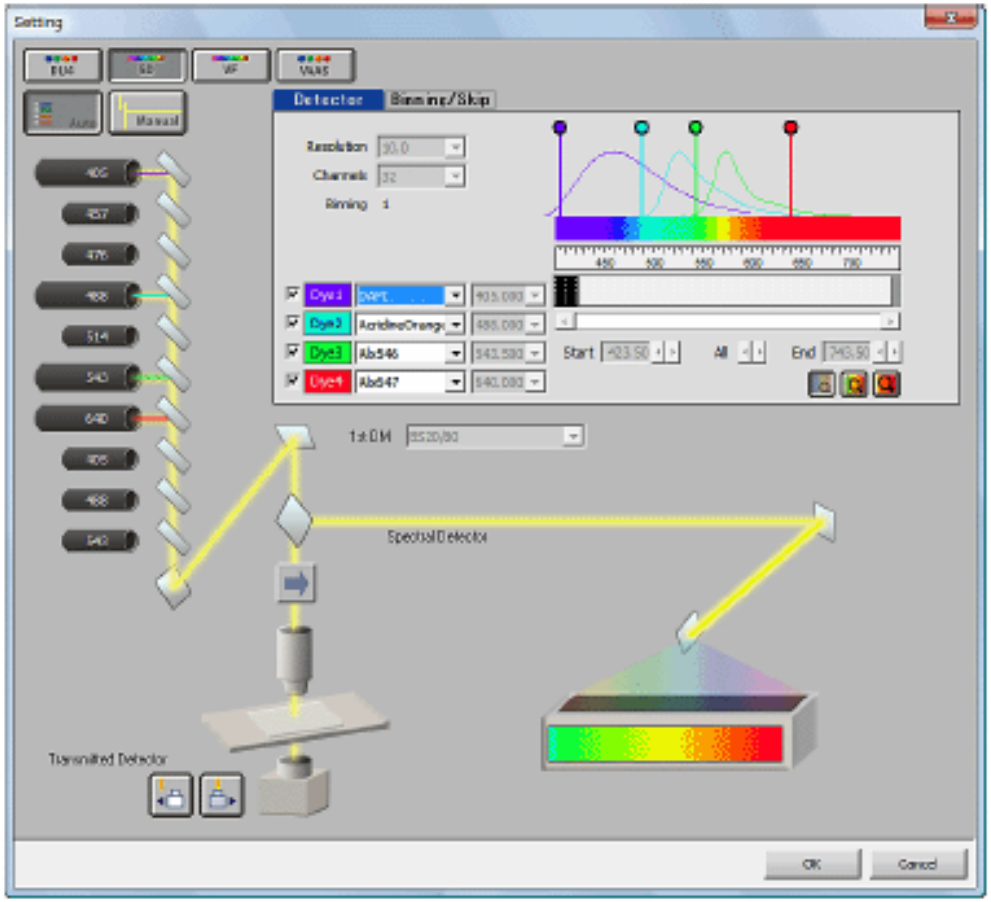


- You can add image stitching to an experiment by checking off the checkbox for “Large Image”.

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

1. Place a high contrast slide on the stage (like an H&E)
2. Locate Calibration→Recalibrate “x” objective
3. Select “Auto” for calibration

Spectral Acquisition



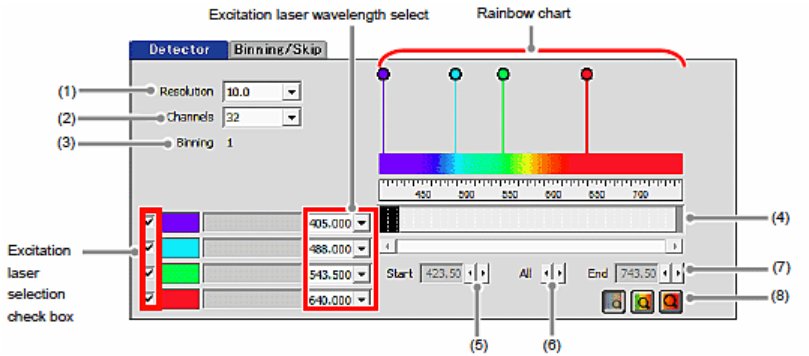
- To acquire a spectral image, choose the 

from the A1 Simple Gui.

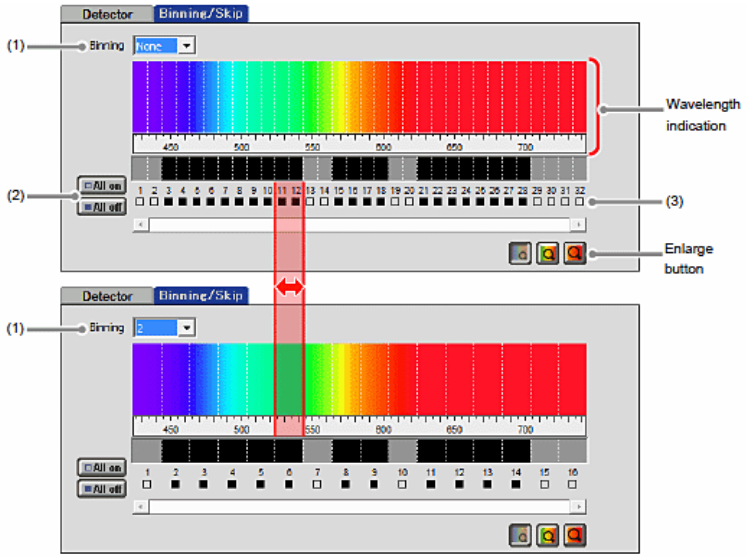
- This will allow you to adjust the resolution and range for spectral detection.

**Spectral detection can be great for increasing the # of fluorophores you can use in a single experiment; it can also be used to subtract autofluorescence.*

Spectral Settings



With the inter-channel binning, the dark image can be brightened. (Enabled in the manual mode only.)
Further, channels within the set wavelength range can be arbitrarily skipped. Since masked channel data is not acquired, the data volume can be reduced.
Set this tab after the setting of the [Detector] tab is determined.



	Name	Function
(1)	Resolution	Selects a wavelength resolution. (Enabled in the manual mode only.) Selectable from 2.5, 6, or 10nm.
(2)	Channels	Selects the number of channels (number of PMTs). (Enabled in the manual mode only.) Up to 32 channels can be selected in the wavelength range of 400nm to 750nm.
(3)	Binning	Displays the number of channel binning currently set.
(4)	Wavelength range setting bar	Sets a wavelength range in a wavelength range from 400nm to 75nm. (Enabled in the manual mode only.) Sets a range by shifting the wavelength range setting bar to the right or left or by enlarging or reducing it. (Linked with the above setting of the number of channels.) * A part of the wavelength range may be displayed in black depending on the setting conditions. In the wavelength range displayed in black, no wavelength range can be set.
(5)	Start	Displays the start wavelength of the wavelength range currently selected. Enabled to enlarge or reduce the range in units of wavelength resolution with the right or left button in the manual mode.
(6)	All	Enabled to shift to the right or left within the currently selected wavelength range without the wavelength interval being changed. (Enabled in the manual mode only.)
(7)	End	Displays the end wavelength of the wavelength range currently selected. In the manual mode, the range in units of wavelength resolution can be enlarged or reduced using the right and left buttons.
(8)	Enlarge button	Enlarges the rainbow chart. The display is switched in three levels.

	Name	Function
(1)	Binning	Sets the number of channels to be combined into one channel. Two to four channels can be set. When Binning is set, the number of channels set with the [Detector] tab is automatically re-set to the closest number of channels that can be divided by the binning value.
(2)	PMT All on/off button	**All on** - Resets all PMT skips that have been set.
		All off - Leaves one channel and skips all of other PMTs.
(3)	PMT skip selection check box	Sets skip in each channel. If this box is clicked, i (black) is displayed and skip is set. Channel data with skip set is not acquired during scan.

** If the setting of the [Detector] tab is changed, the setting with the [Binning/Skip] tab is cancelled.*

Spectral Unmixing

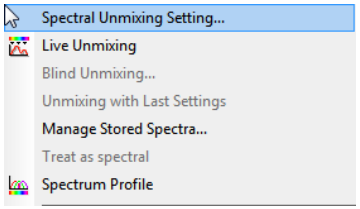
Spectral Images can be unmixed a few different ways.

It is best to decide which method based on the following questions:

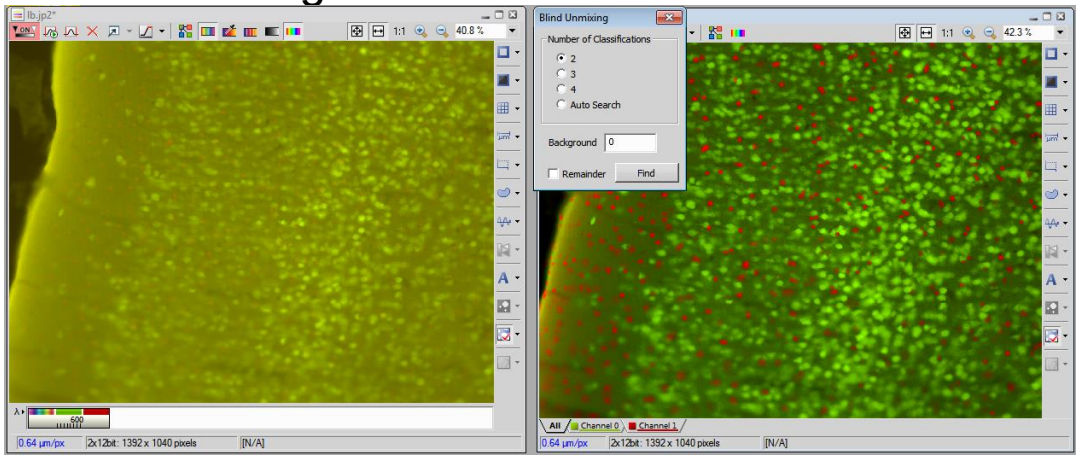
1. Do you have all of your controls, i.e. separate slides per wavelength as well as a slide will all wavelengths?
   - Yes-we will need to acquire spectrums from each of your control slides in order to use them when unmixing your combined wavelength's slide
   - No-see next question

2. Do you only have a slide will all wavelengths?
   - Yes-ok, instead of preparing controls, we will use either a "blind" unmixing algorithm, pick from regions in your sample, or choose from stored known spectra.

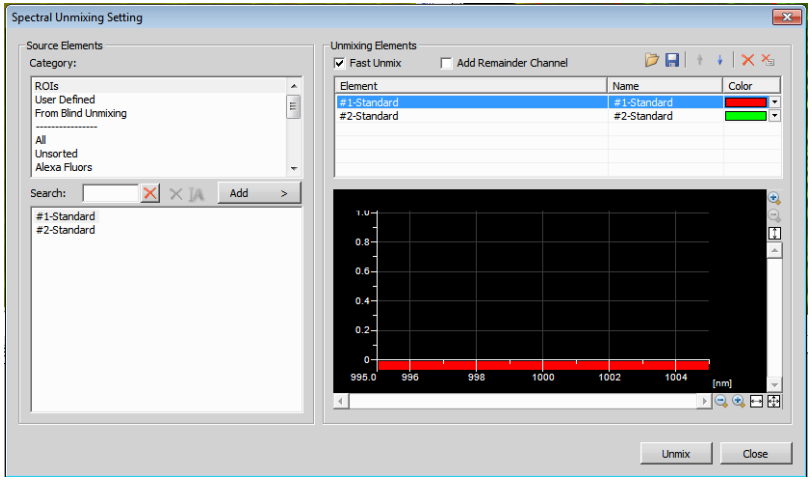
Under Image→ you can find all of the spectral unmixing adjustments

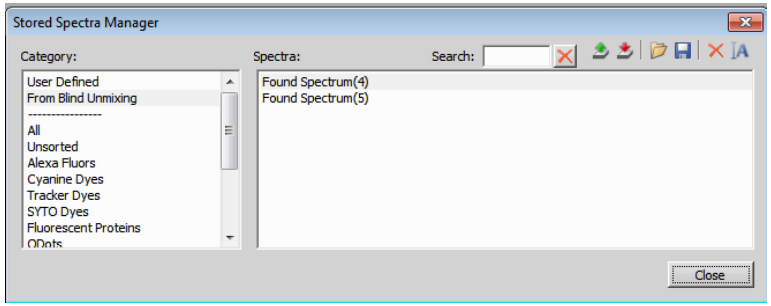


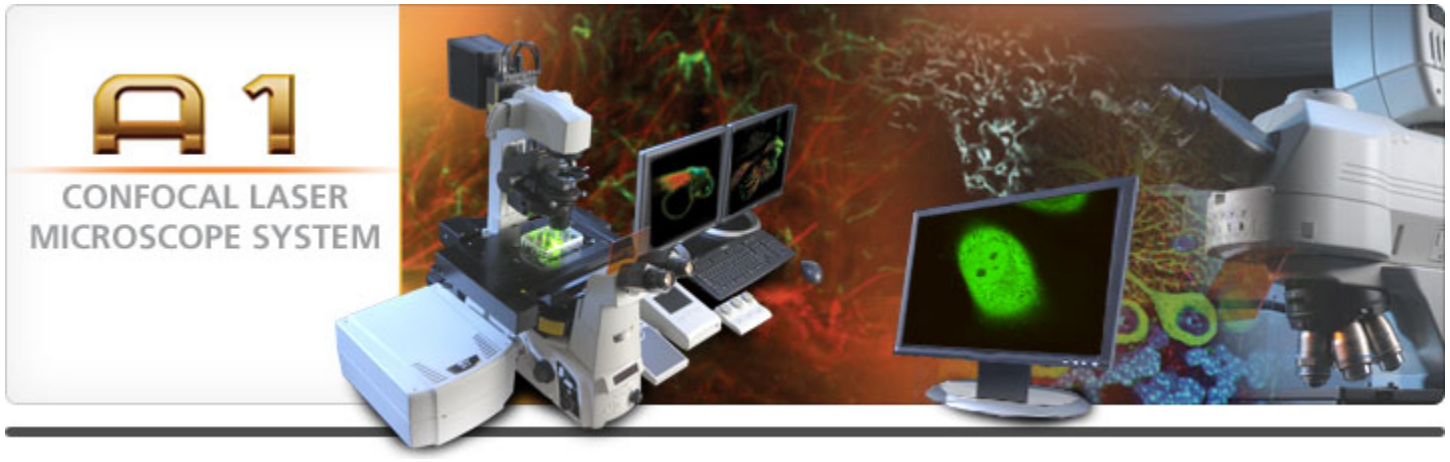
Blind Unmixing:



Unmixing from ROI/Library:

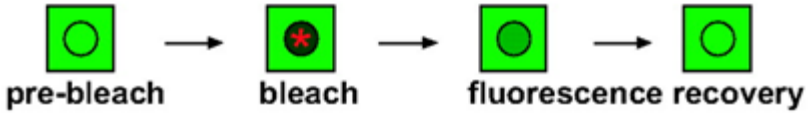






FRAP Experiments on Nikon A1Rsi

July 2012



Galvano or Resonant?

Before you start a FRAP or PA experiment, you will need to decide which scanner to use.

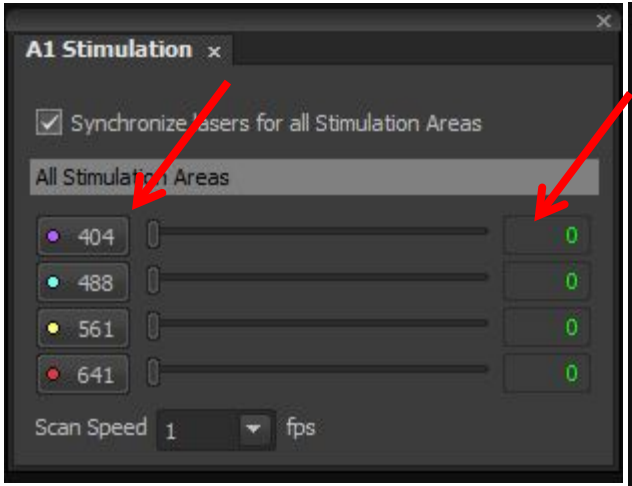
Galvano: fairly standard, average speeds, can select any laser for bleaching/imaging

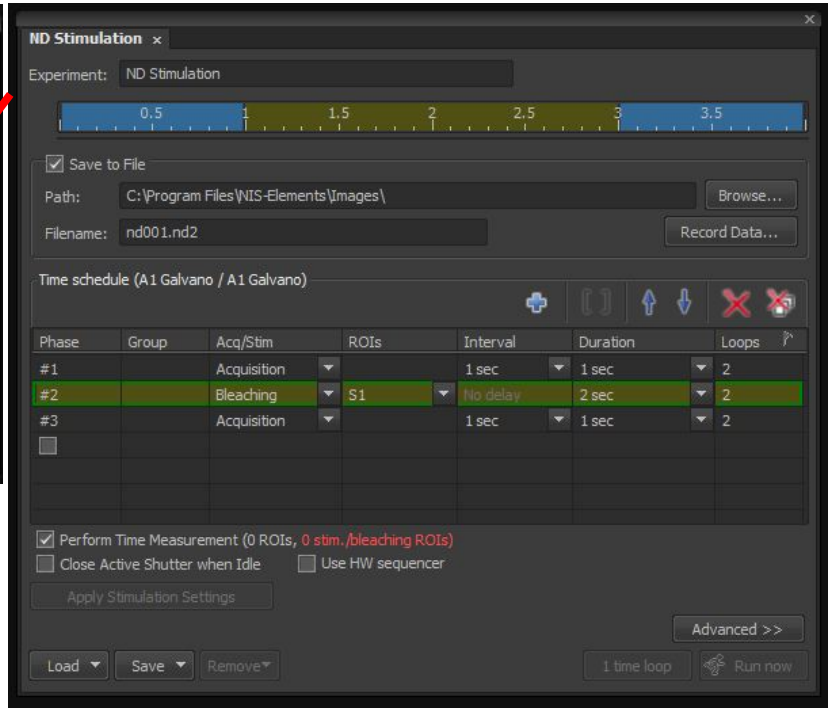
Resonant: limited to using the 405nm laser to bleach (can use any to image), but can image simultaneously while bleaching for an increase in dynamic-recording

*The following pages will walk you through how to set up the experiment in either method

Galvano-FRAP

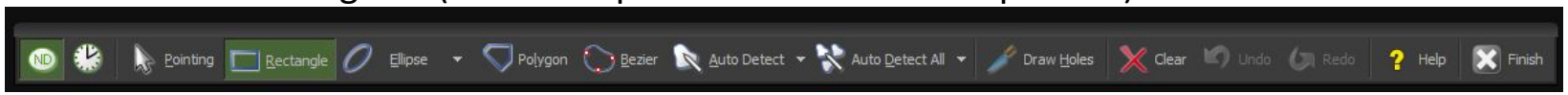
1. First, set your confocal settings as needed for imaging.
2. Open the “A1 Stimulation” and “ND Stimulation” dialog boxes under View→Acquistion Controls on the top toolbar.
3. Set up as shown below. Select your bleaching laser(s) and adjust power (usually 100%)
4. The ND experiment should be saved to file (choose your location) and should have at least three time phases (Acquisition/Bleach/Acquisition). You can choose the interval (time between image cycles) and the duration (total time).

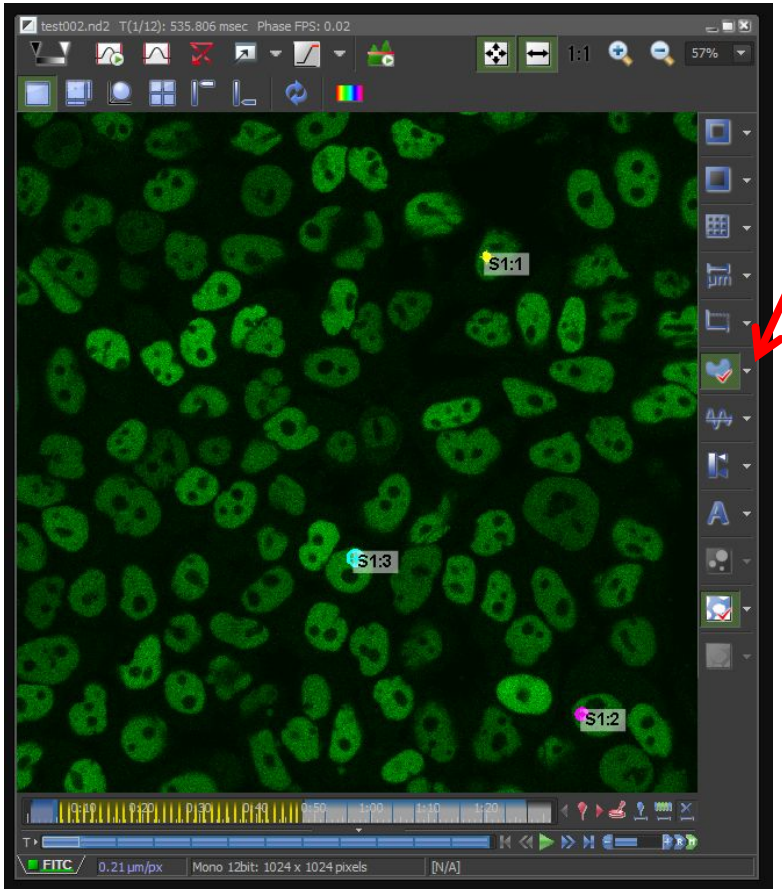




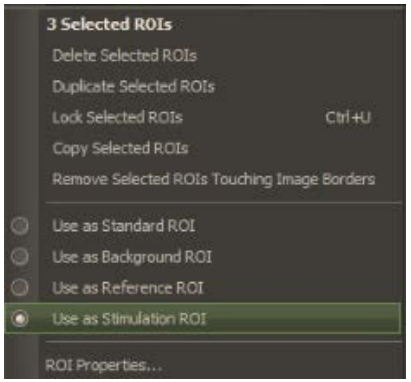
Cont.

5. On an open image window, select the kidney bean icon on the right toolbar to draw your bleach regions (select Simple ROI Editor from drop down)



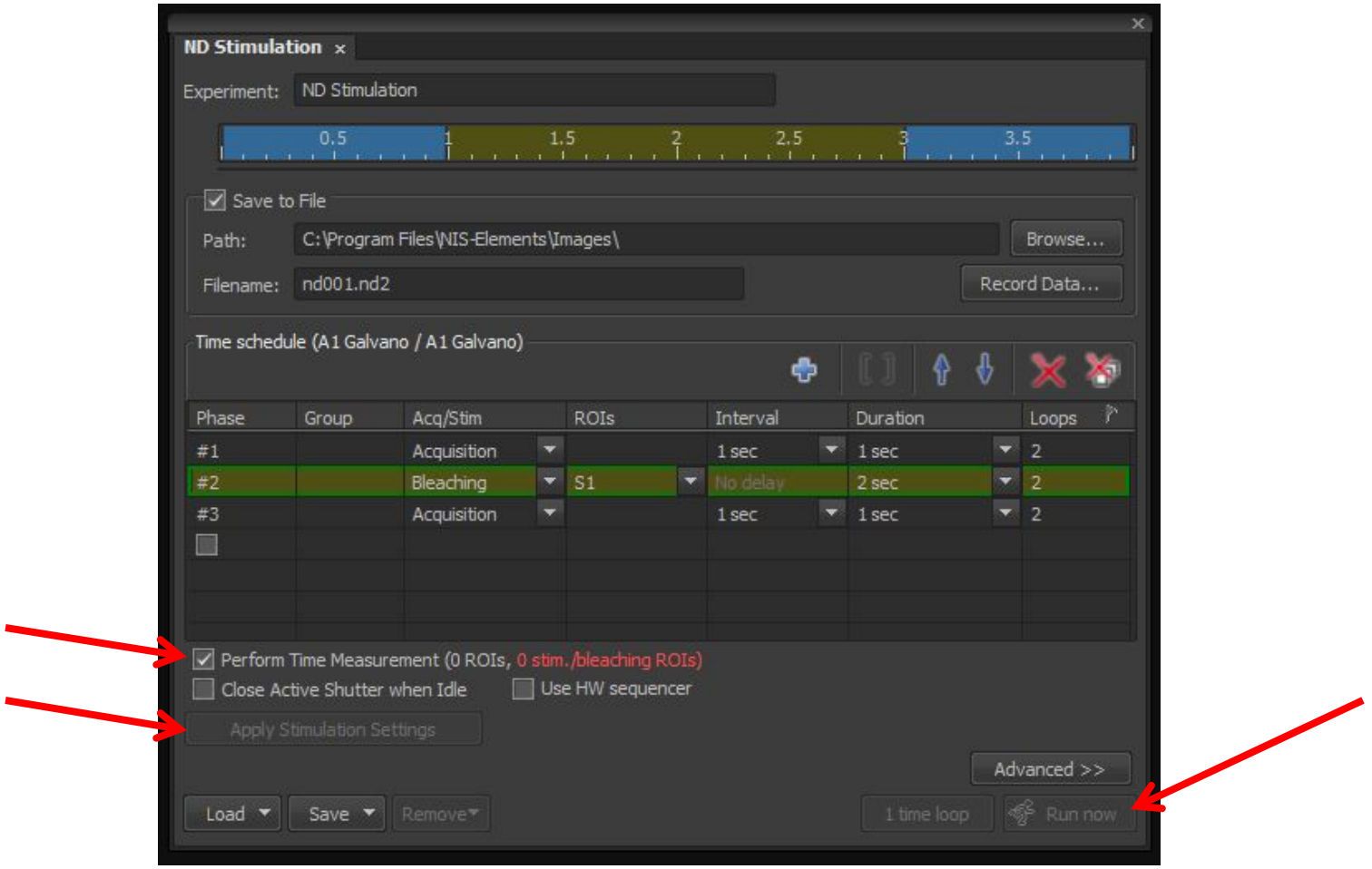


6. When you are finished drawing the ROI's, click "Finish"
7. You will need to right click on each of your drawn ROI's to select it to be a "Stimulation ROI"
8. You can have up to three groups of ROI's (S1/S2/S3) and each can have different bleach lasers/laser powers associated with them if you choose to



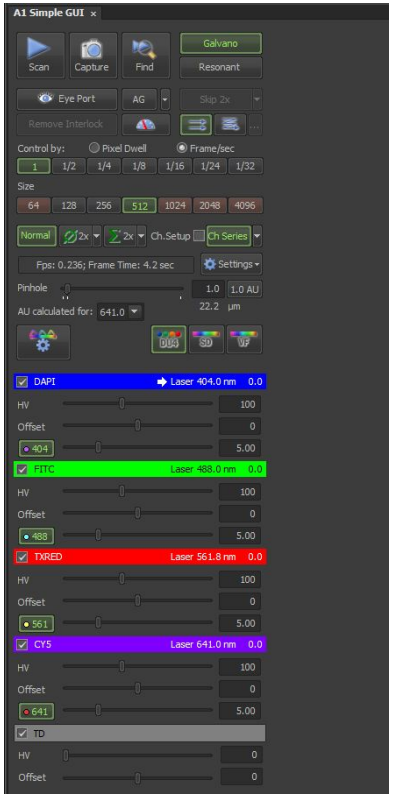
Cont.

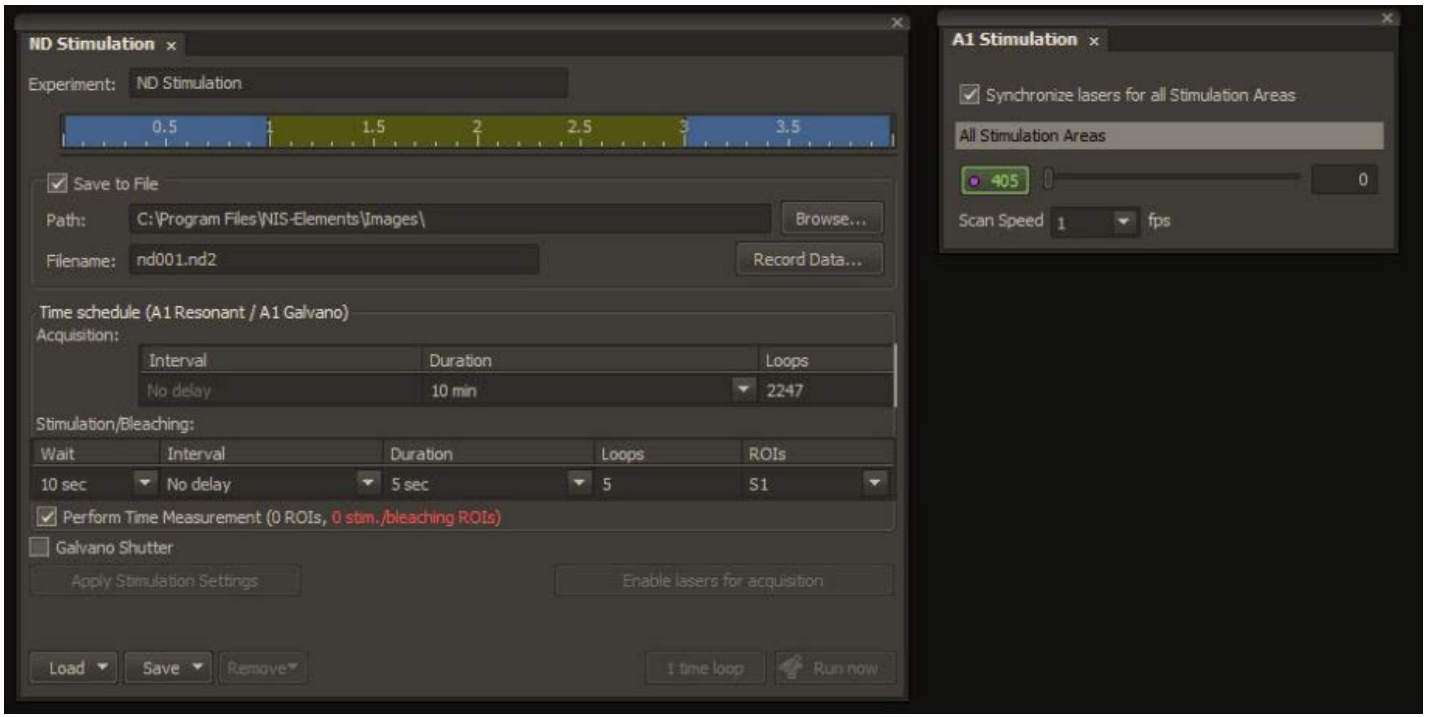
9. When you are ready to run the experiment, make sure to click on “Perform Time Measurement” if you would like real time graph/data results.
10. Click “Apply Stimulation Settings”
11. Click “Run now” when you are ready to start the experiment.



Resonant-FRAP

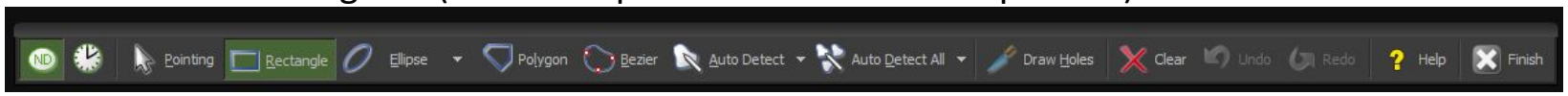
1. First, set your confocal settings as needed for imaging.
2. Open the “A1 Stimulation” and “ND Stimulation” dialog boxes under View→Acquistion Controls on the top toolbar.
3. Set up as shown below. Select your bleaching laser (405) and adjust power (usually 100%)
4. The ND experiment should be saved to file (choose your location). You can choose the interval (time between bleach cycles) and the duration (total time) of the bleaching phase (this will be done with the Galvano scanner), while the acquisition interval is fixed (as this will be done with the resonance scanner); you can choose the total imaging duration however.

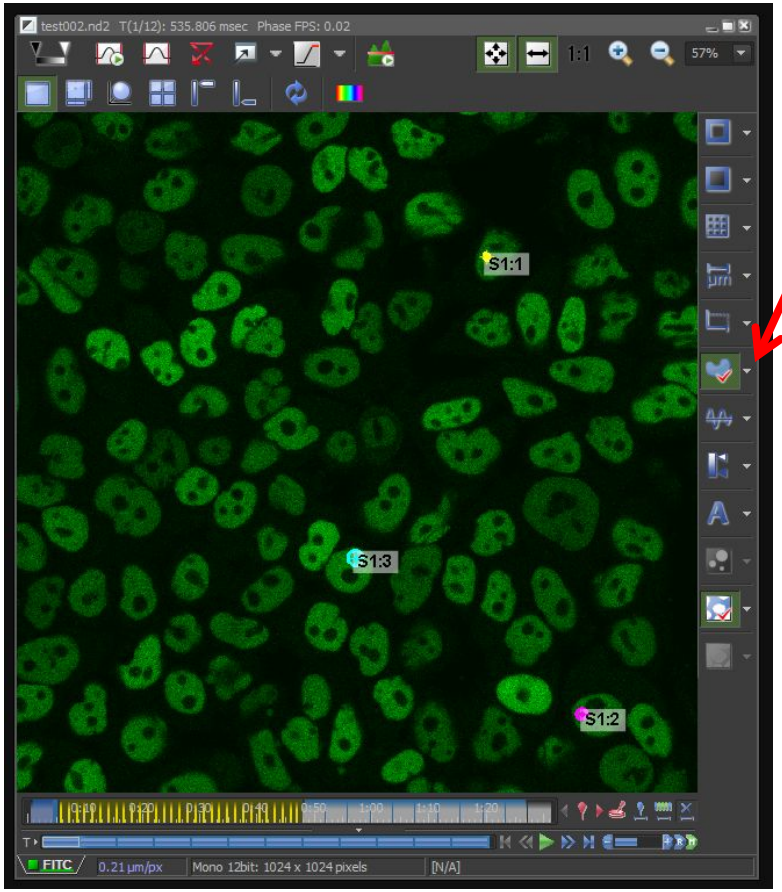




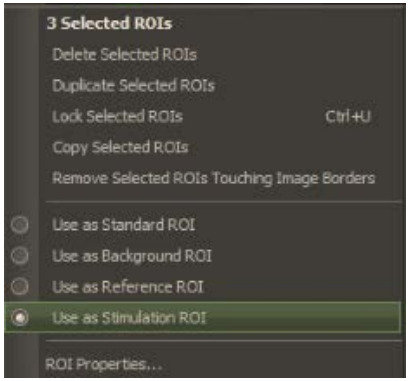
Cont.

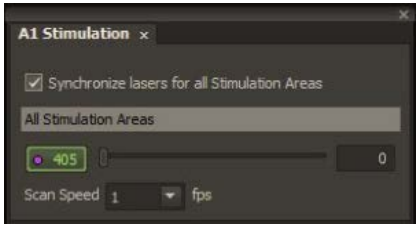
5. On an open image window, select the kidney bean icon on the right toolbar to draw your bleach regions (select Simple ROI Editor from drop down)





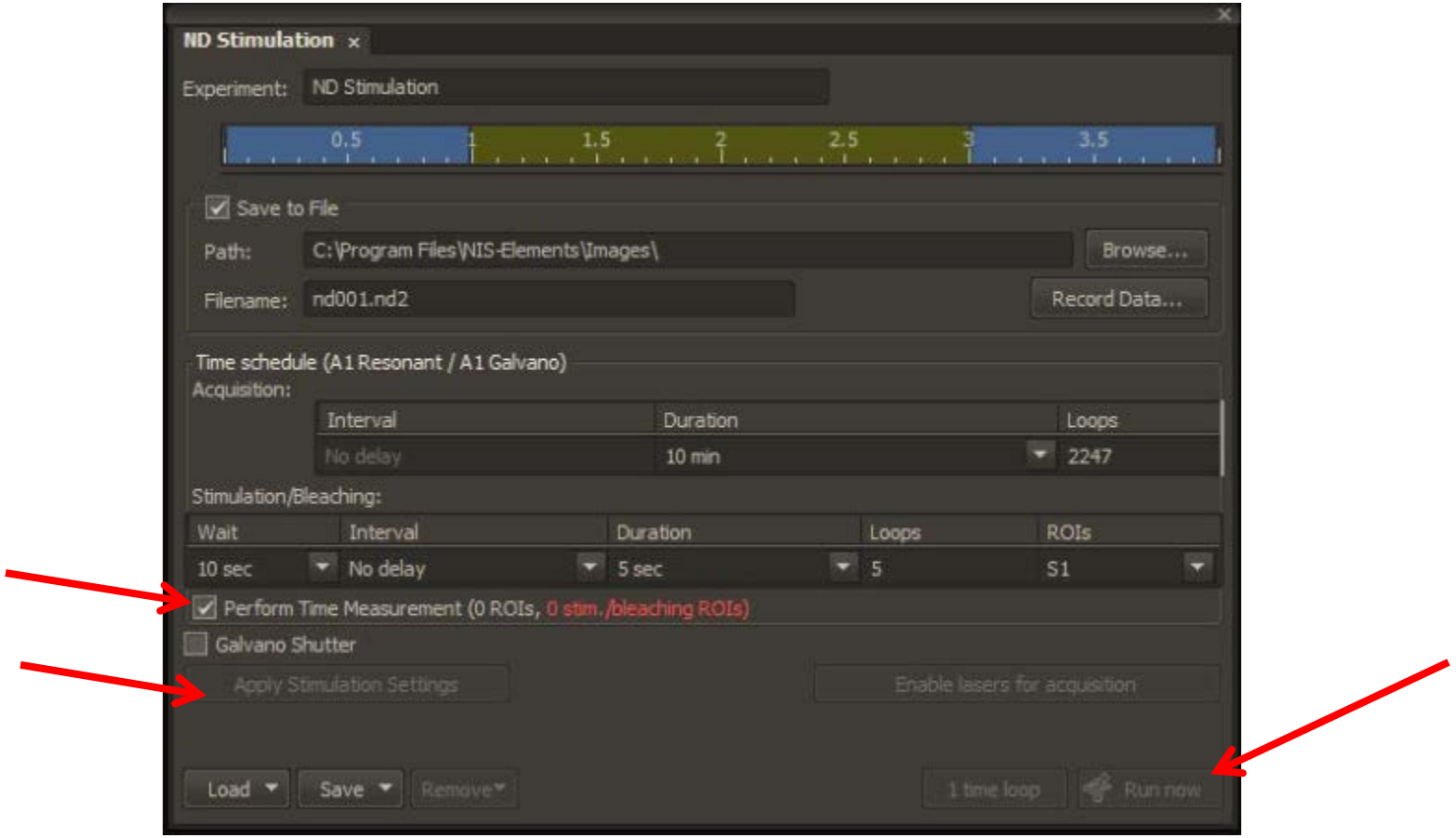
6. When you are finished drawing the ROI's, click "Finish"
7. You will need to right click on each of your drawn ROI's to select it to be a "Stimulation ROI"
8. You can have up to three groups of ROI's (S1/S2/S3) and each can have different bleach-laser powers associated with them if you choose to. (uncheck synchonize if you want different powers per ROI)





Cont.

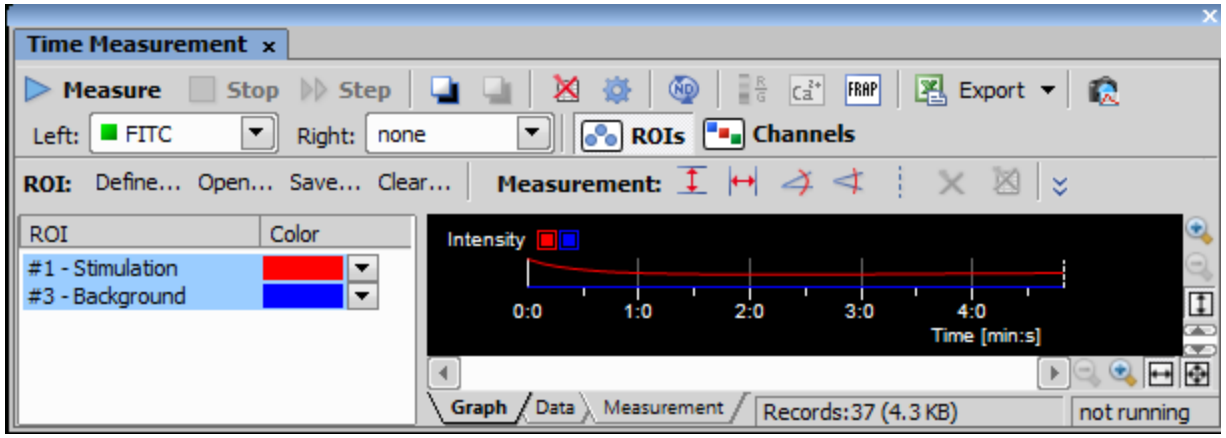
9. When you are ready to run the experiment, make sure to click on “Perform Time Measurement” if you would like real time graph/data results.
10. Click “Apply Stimulation Settings”
11. Click “Run now” when you are ready to start the experiment.

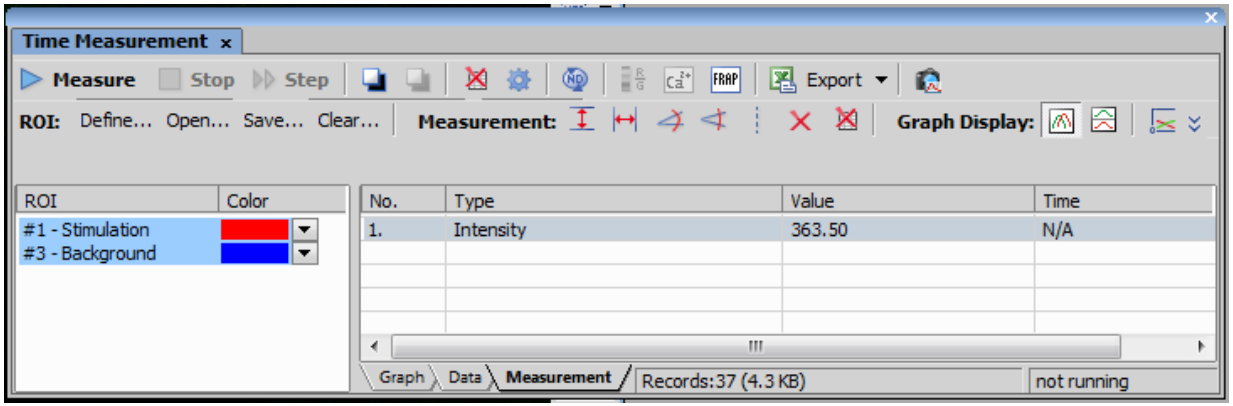


Analysis

Measurements can be run either during or post acquisition via the “Time Measurement” dialog (under View->Analysis Controls) on the top toolbar.

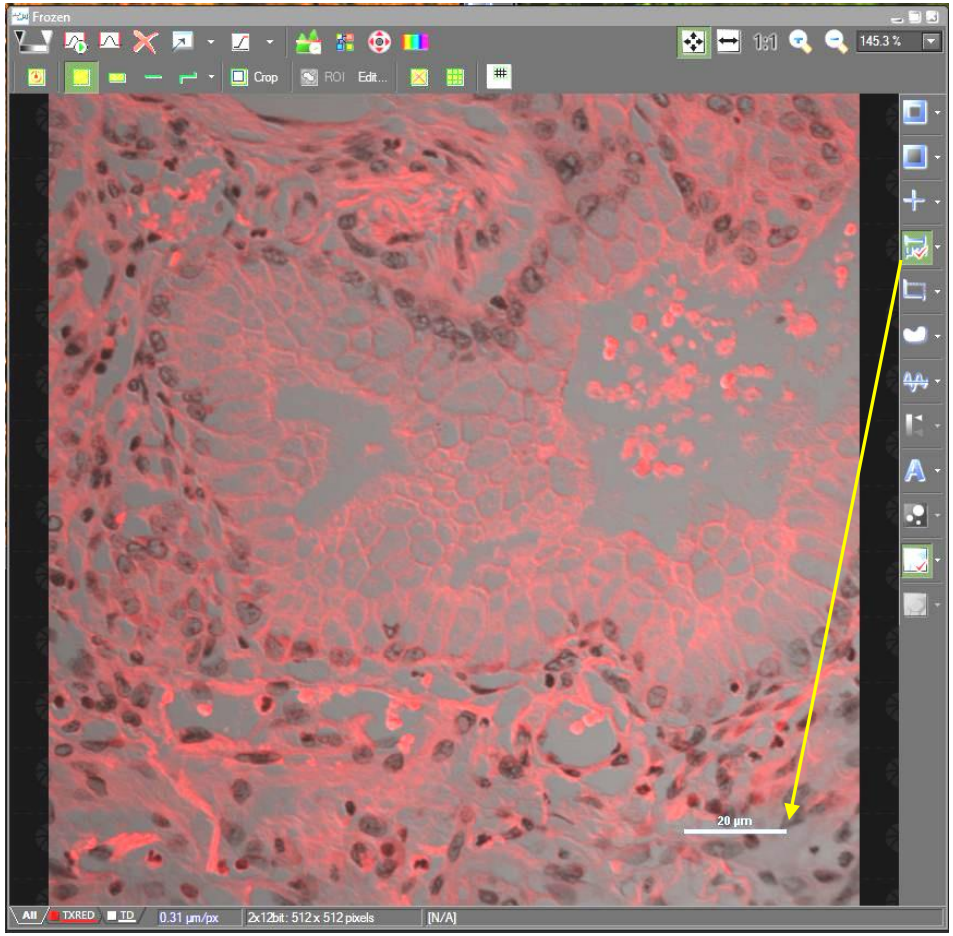
You can calculate intensity changes, etc and export to excel.





Post Acquisition Image Adjustments

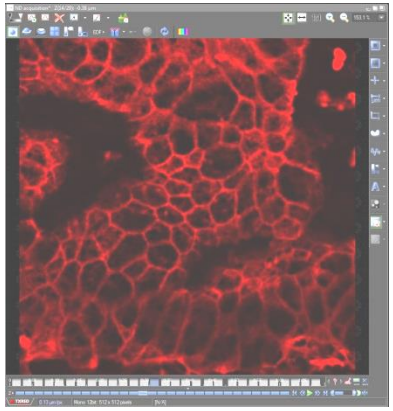
Scale Bars



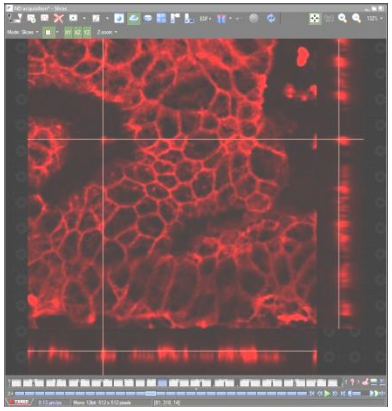
A Scale Bar can be added at any time from the right side image window's toolbar. Once positioned where you choose, you can right click on it to change its properties or to "burn it" into the image

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

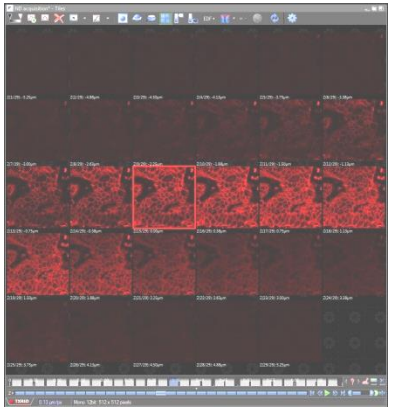
How you can visualize your data…….



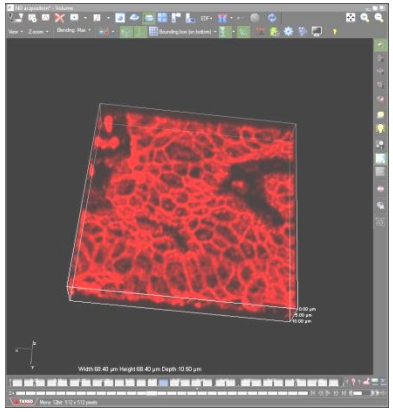
2D



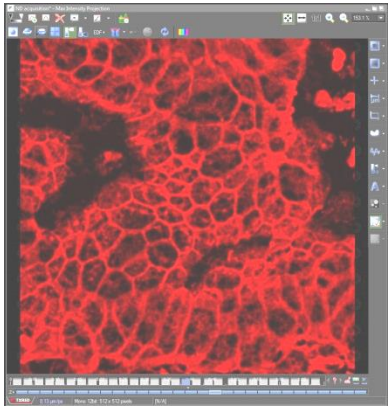
Sliced



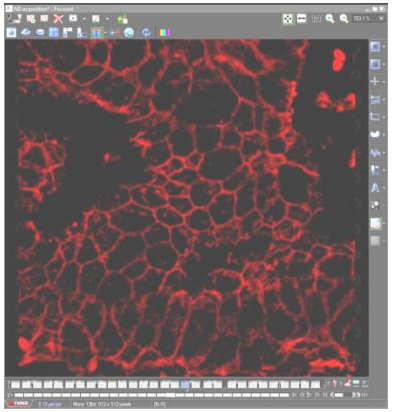
Tiled



3D



Projection

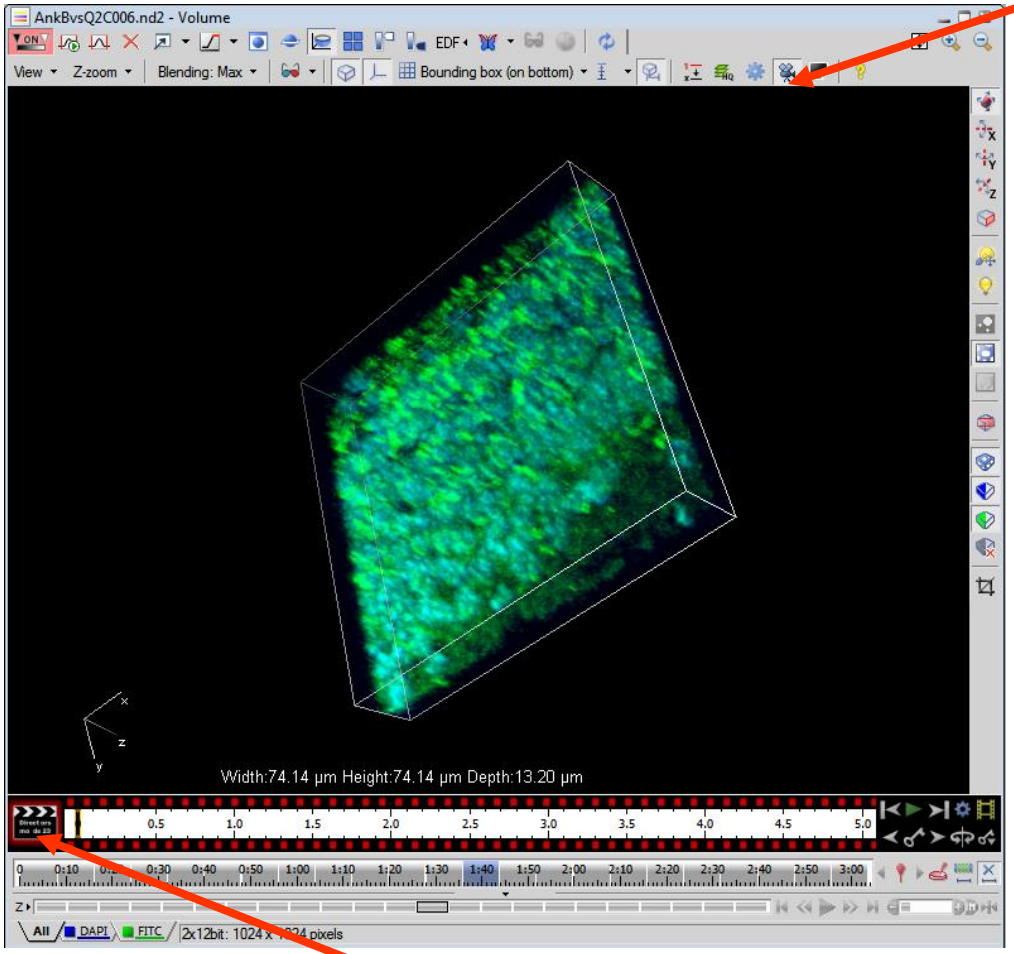


EDF

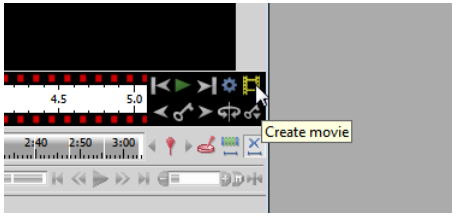
*Can also make really nice 3D rotational movies as .avi's!

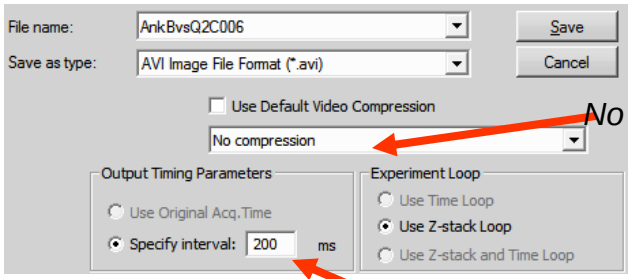
Movie Maker

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.



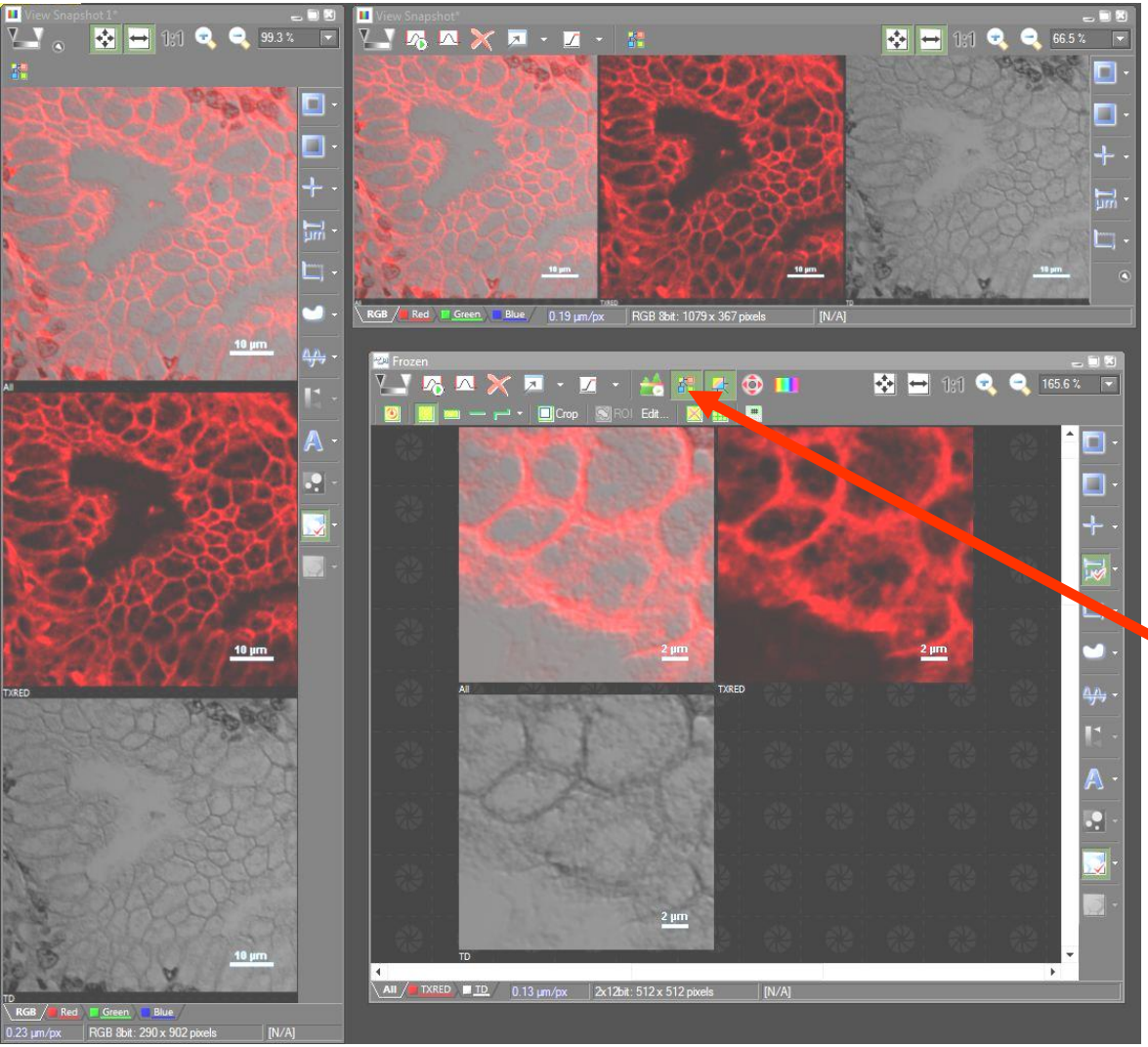


No compression

About 150-200ms is usually good

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

“X” Key Snapshots

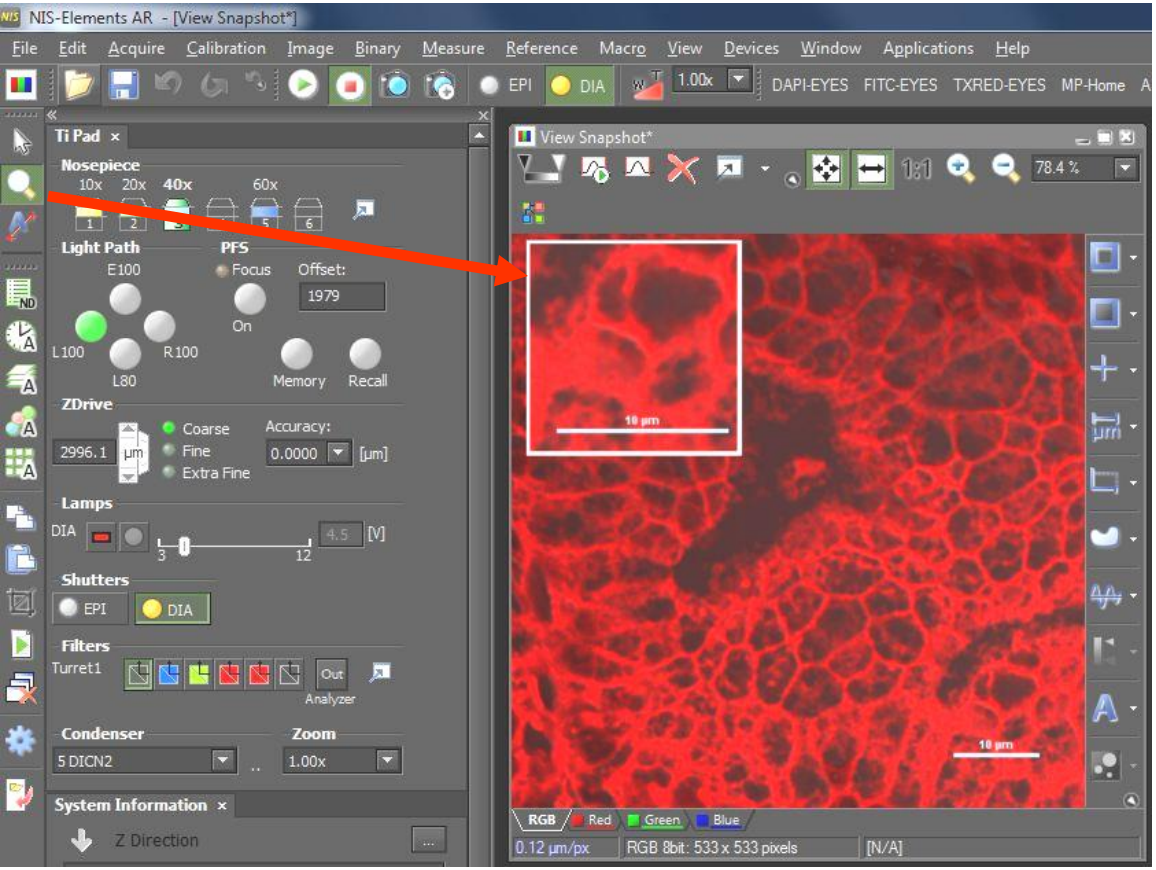


*Use the “x” key on your keyboard to take snapshots for publication/presentation

-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)*

Image Saving Helper….