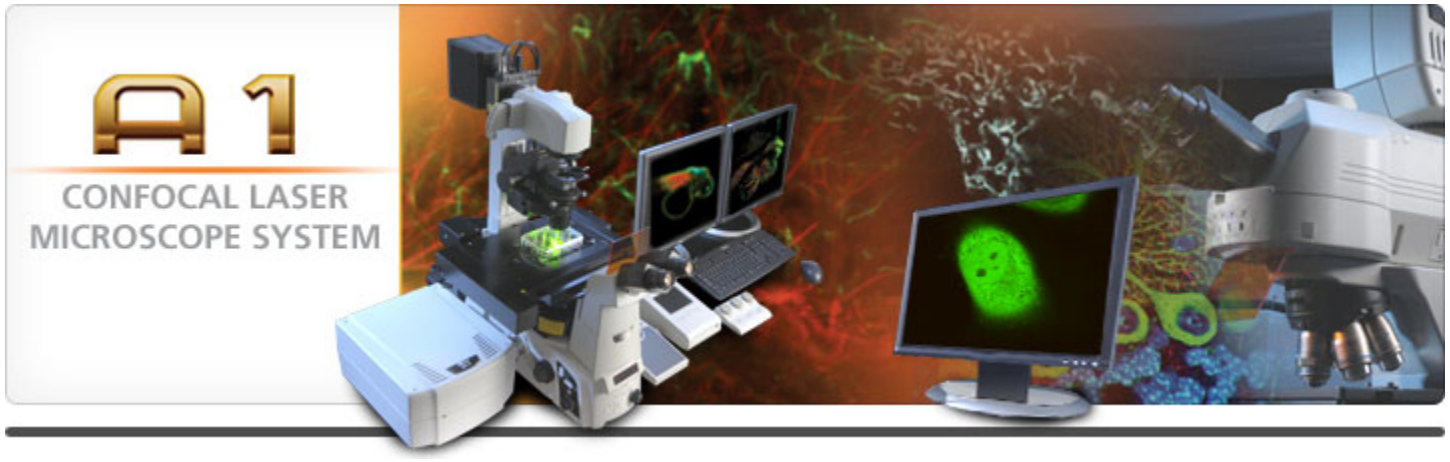
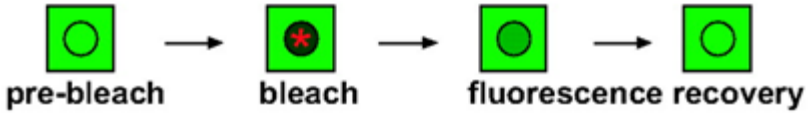


FRAP Experiments on Nikon A1Rsi

July 2012



Galvano or Resonant?

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

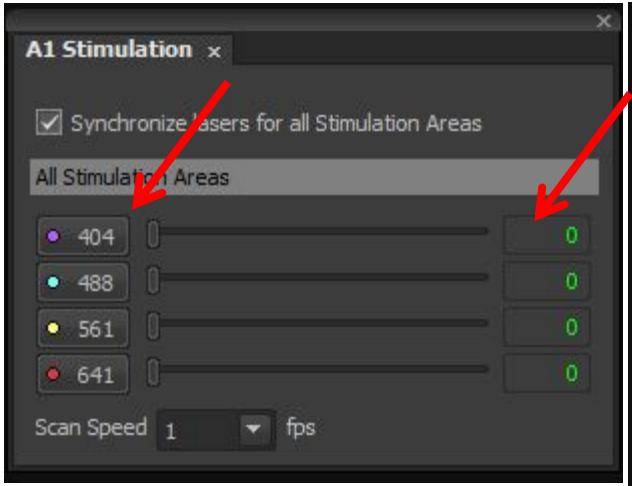
Galvano: fairly standard, average speeds, can select any laser for bleaching/imaging

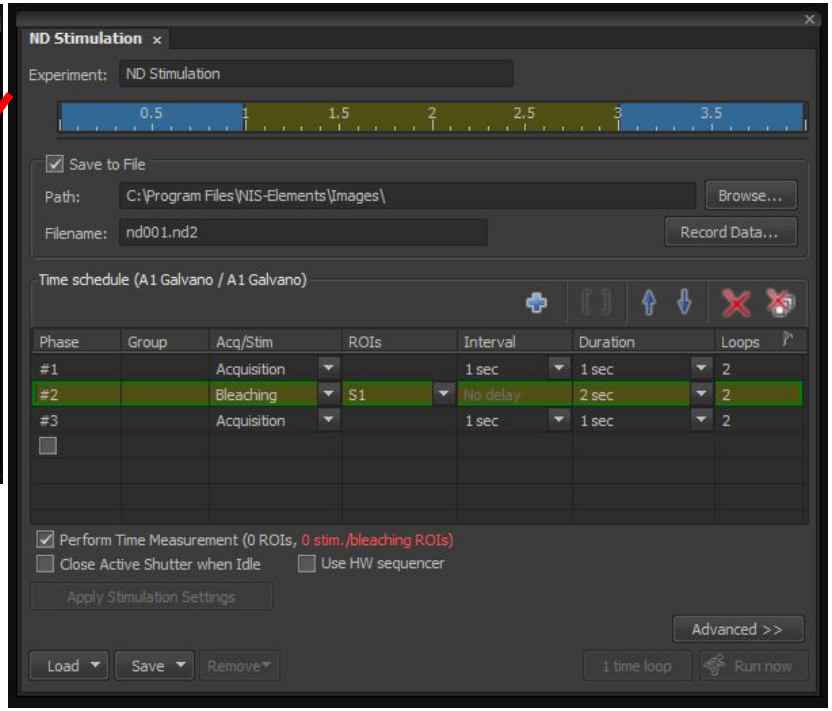
Resonant: limited to using the 405nm laser to bleach (can use any to image), but can image simultaneously while bleaching for an increase in dynamic-recording

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

Galvano-FRAP

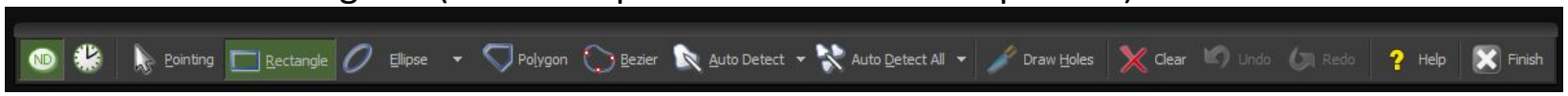
1. First, set your confocal settings as needed for imaging.
2. Open the “A1 Stimulation” and “ND Stimulation” dialog boxes under View→Acquistion Controls on the top toolbar.
3. Set up as shown below. Select your bleaching laser(s) and adjust power (usually 100%)
4. The ND experiment should be saved to 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).

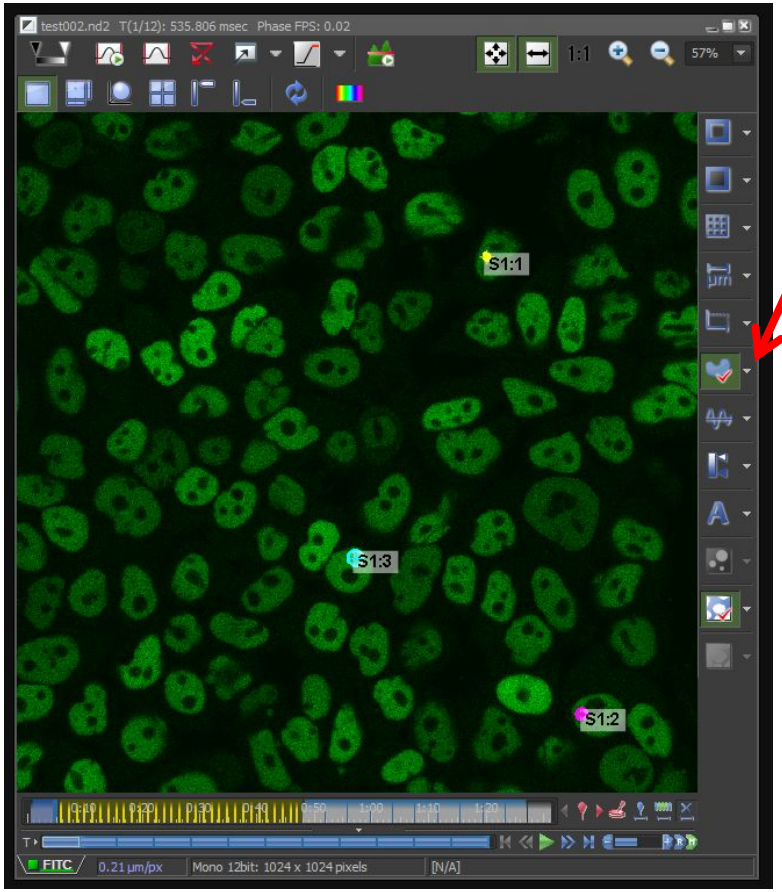




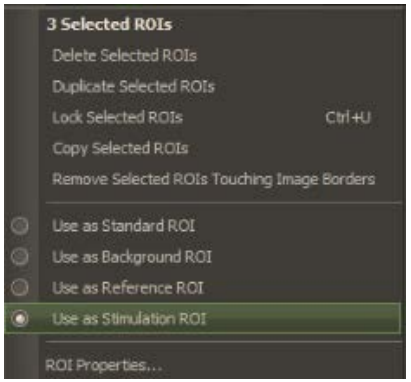
Cont.

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)



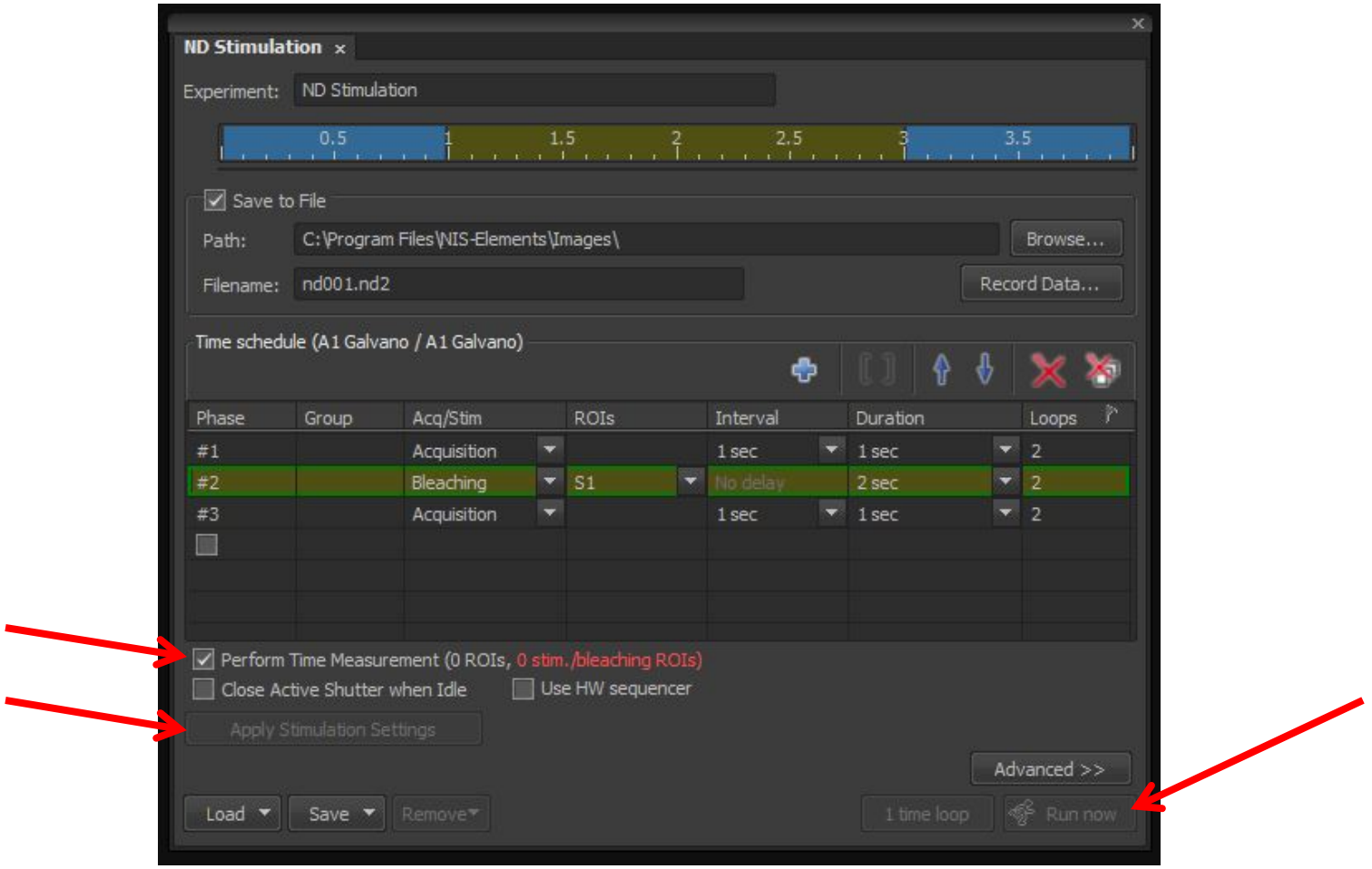


6. When you are finished drawing the ROI's, click "Finish"
7. You will need to right click on each of your drawn ROI's to select it to be a "Stimulation ROI"
8. You can have up to three groups of ROI's (S1/S2/S3) and each can have different bleach lasers/laser powers associated with them if you choose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



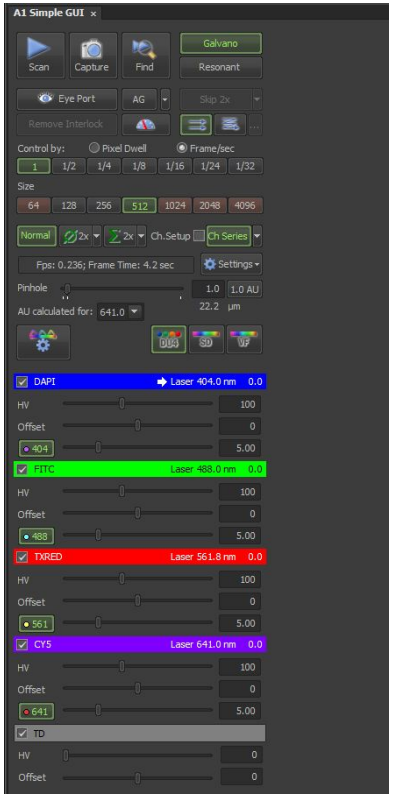
Cont.

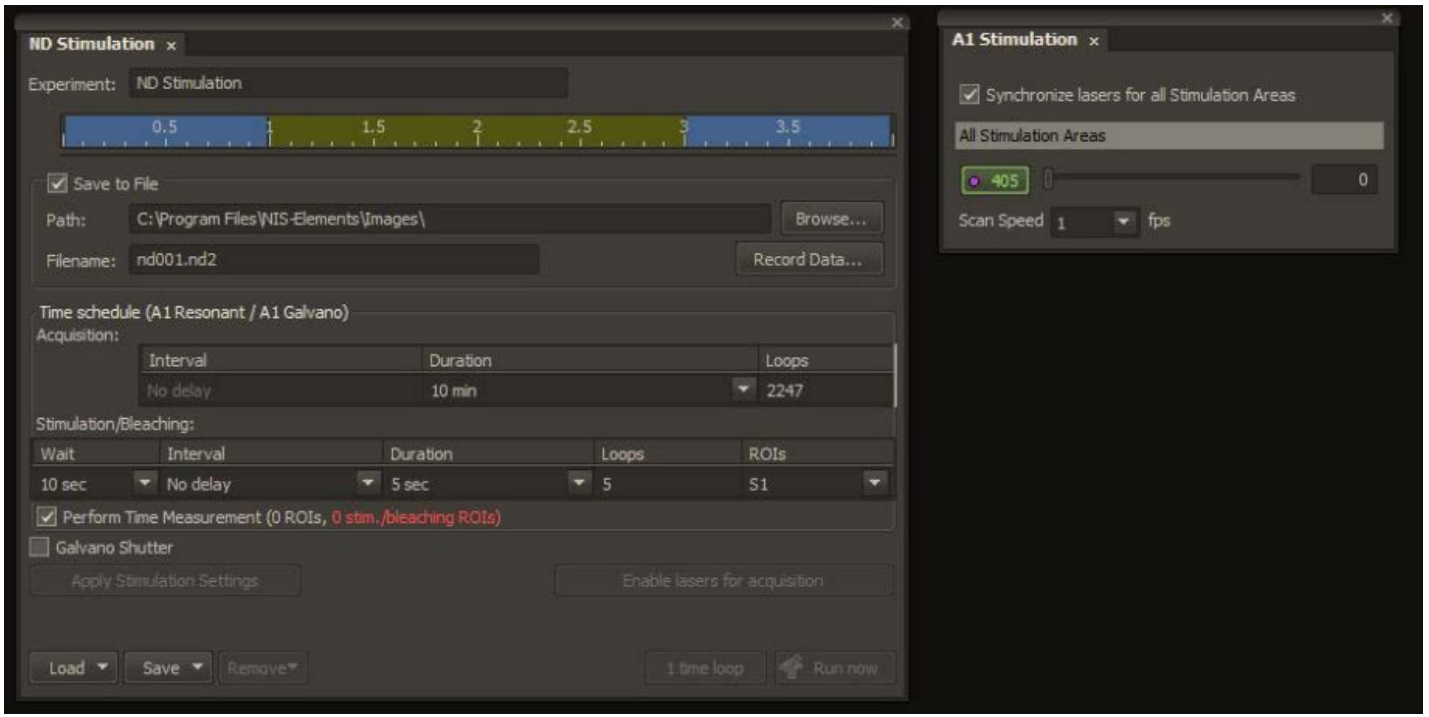
9. When you are ready to run the experiment, make sure to click on “Perform Time Measurement” if you would like real time graph/data results.
10. Click “Apply Stimulation Settings”
11. Click “Run now” when you are ready to start the experiment.



Resonant-FRAP

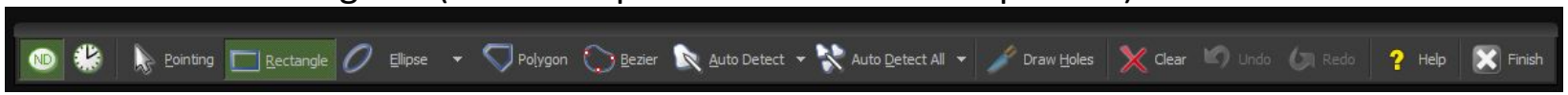
1. First, set your confocal settings as needed for imaging.
2. Open the “A1 Stimulation” and “ND Stimulation” dialog boxes under View→Acquistion Controls on the top toolbar.
3. Set up as shown below. Select your bleaching laser (405) and adjust power (usually 100%)
4. The ND experiment should be saved 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.

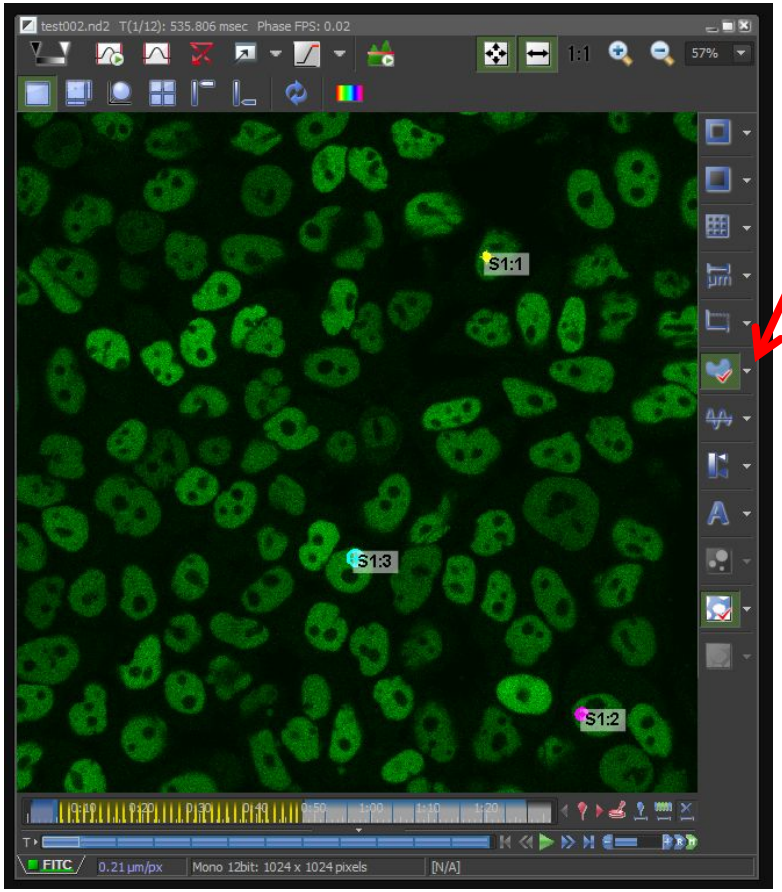




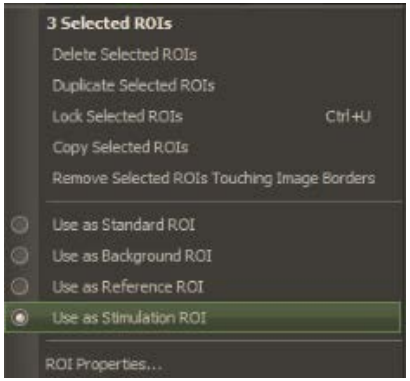
Cont.

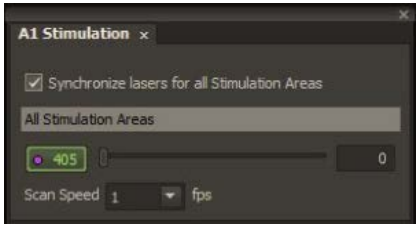
5. On an open image window, select the kidney bean icon on the right toolbar to draw your bleach regions (select Simple ROI Editor from drop down)





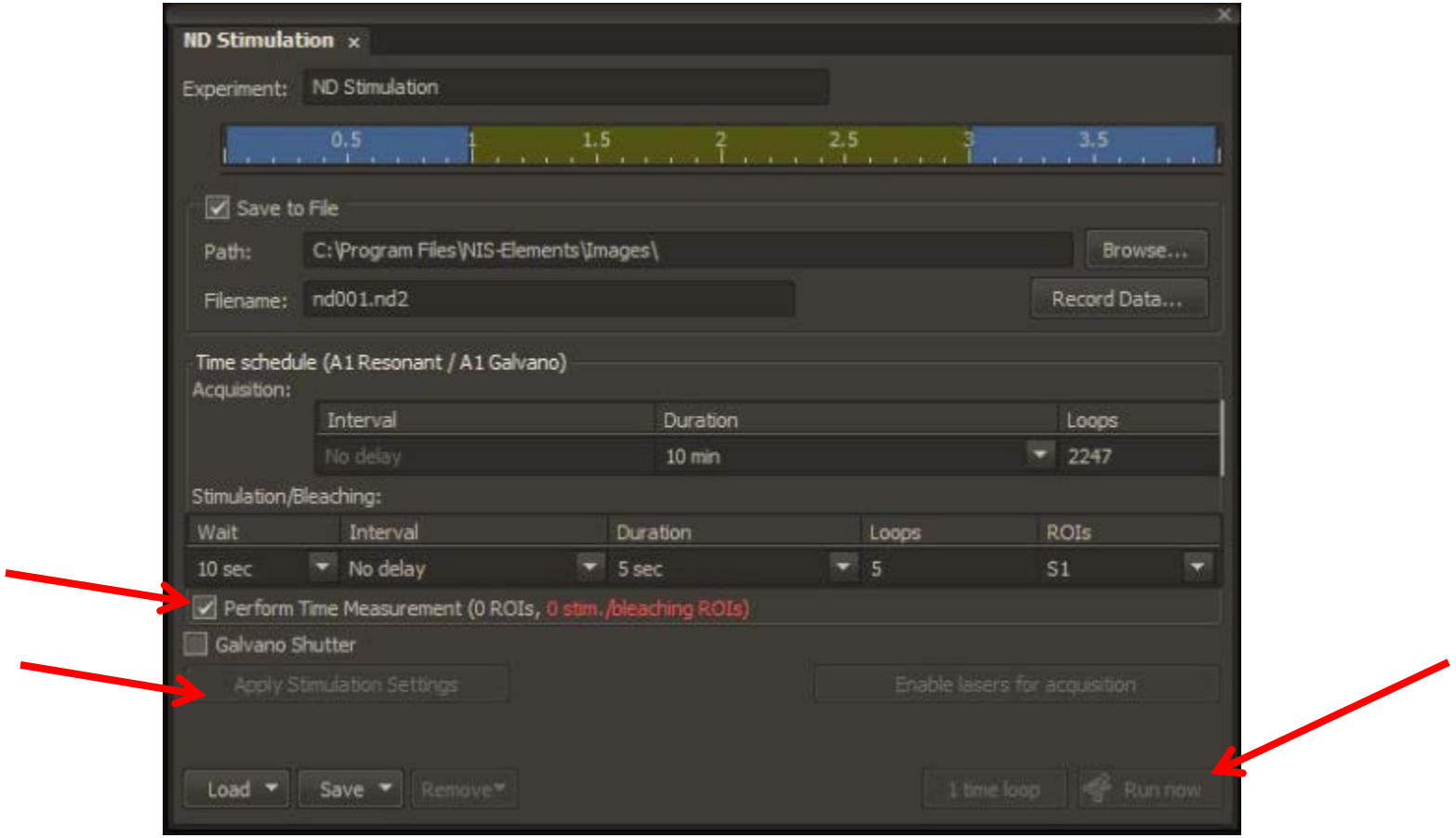
6. When you are finished drawing the ROI's, click "Finish"
7. You will need to right click on each of your drawn ROI's to select it to be a "Stimulation ROI"
8. You can have up to three groups of ROI's (S1/S2/S3) and each can have different bleach-laser powers associated with them if you choose to. (uncheck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