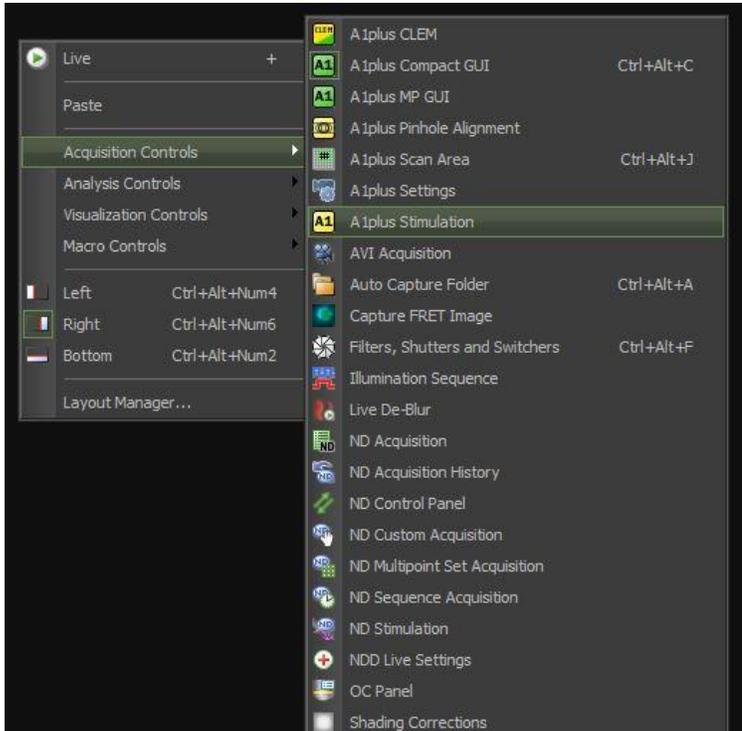
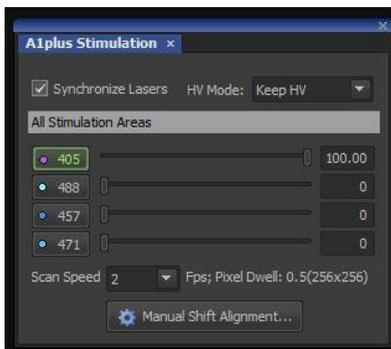


## SEQUENTIAL FRAP ON A1

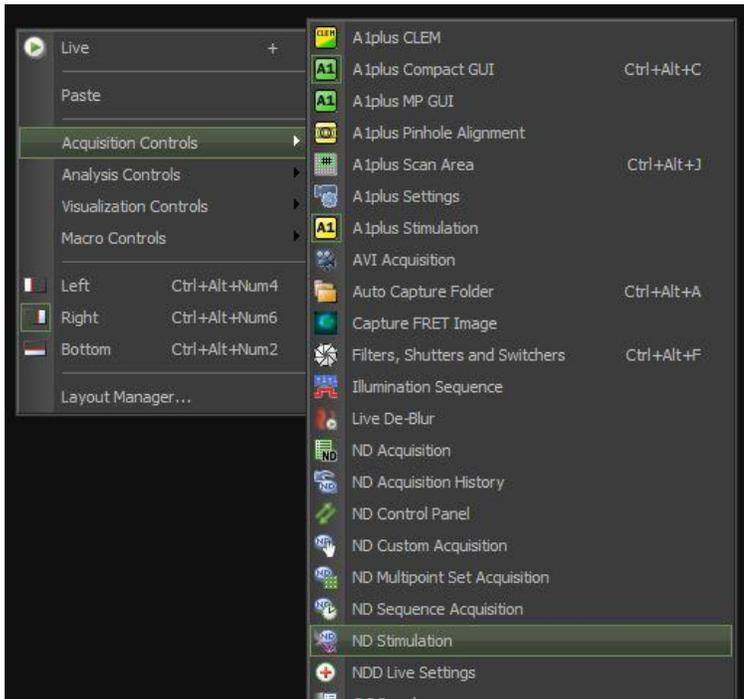
1. Go to "Settings" in the A1 menu and select the appropriate filter settings for your dyes (for example, select "FITC" in Channel 2 for CFDA) and turn on the transmitted detector if needed.
2. Right-click on the desktop and select "A1 Stimulation" from the Acquisition Controls.



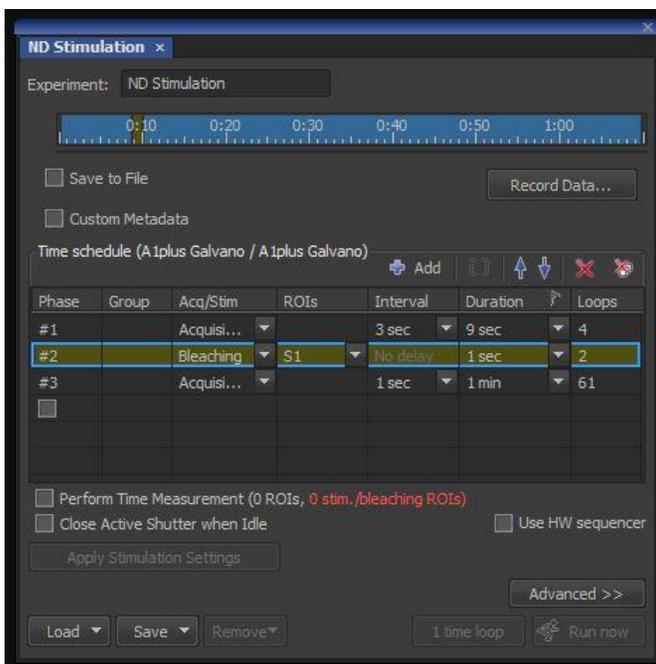
3. In the A1 Stimulation control box, select the desired laser line for bleaching. Typically, this is either the same line used for acquisition or the 405nm line. I often set the power (for bleaching) to 100%, but you can try lower power if desired. For starters, I would leave the scan speed at the default setting (probably 1 sec/frame).



4. Right-click on the desktop and select "ND Stimulation" from the Acquisition Controls.



5. For a typical FRAP experiment, set up 3 phases:
  - a) Prebleach Phase: select "Acquisition" and set interval, duration, etc.
  - b) Bleach Phase: select "Bleaching". For ROIs, set "S1"
  - c) Postbleach Phase: select "Acquisition" and set interval, duration, etc.



6. If desired, check the "Perform Time Measurement" box to display a plot of the fluorescence in the selected ROIs during the experiment.
7. Capture an image (by pressing "Live"), and draw an ROI for the bleach area. Right click on the ROI and select "use as stimulation ROI" to designate this ROI for bleaching. If you do not designate the ROI as a stimulation ROI, then the software will not bleach it but instead just measure the fluorescence without bleaching. You can draw one or more "standard" ROIs as controls for the bleach area. You can also designate multiple bleach ROIs. Note though that, as you select multiple Bleach ROIs and/or increase the size of a Bleach ROI, you will need higher laser power and/or longer bleaching times. To keep things consistent, I usually recommend using a single bleach area per image.
8. When done, click on "Apply Stimulation Settings" in the Sequential Stimulation/Bleaching control menu.
9. Click the "Run Now" button in the Sequential Stimulation/Bleaching control menu.