# SEQUENTIAL PHOTOACTIVATION ON A1

1. Go to "Settings" in the A1 Compact GUI and select the appropriate laser & filter settings for your dye(s). In this example, we're looking at "Kaede" which is normally excited at 488nm and emits a green signal (like FITC). After photoactivation with 405nm, the excitation peak shifts to 561nm and a red signal is emitted (like TxRed).

2. Right-click on the desktop and select "A1 Stimulation" from the Acquisition Controls.
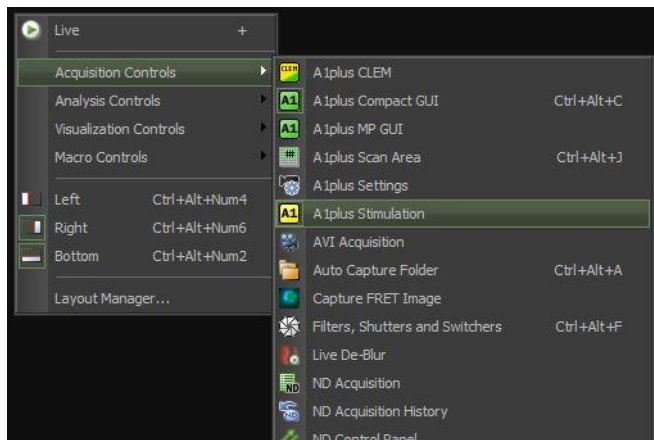
3. In the A1 Stimulation control box, press the 405 button to use that line for photoactivation. The optimal laser power for photoactivation will require some experimenting. Too little power will have no effect (red & green signals won't change) and too much power will cause bleaching rather than activation (red & green signals both decrease). In this example, I used 30% for a single 1fps pass.
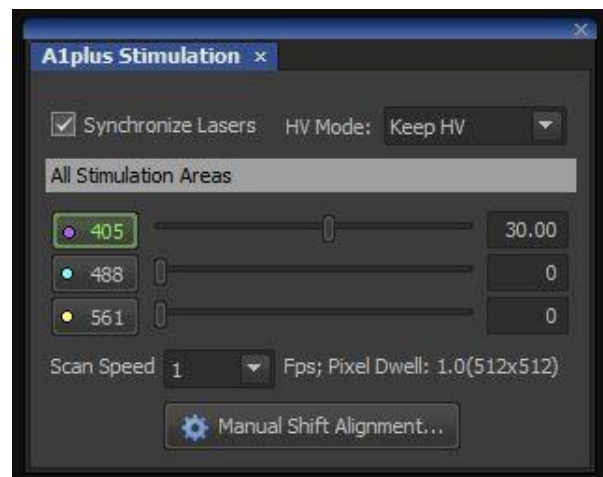
4. Right-click on the desktop and select "ND Stimulation" from the Acquisition Controls.

5. For a typical Photoactivation experiment, set up 3 phases:
   a) Pre-Activation Phase: select "Acquisition" and set interval, duration, etc.
   b) Activation Phase: select "Bleaching". For ROIs, select "S1"
   c) Post-Activation Phase: select "Acquisition" and set interval, duration, etc.
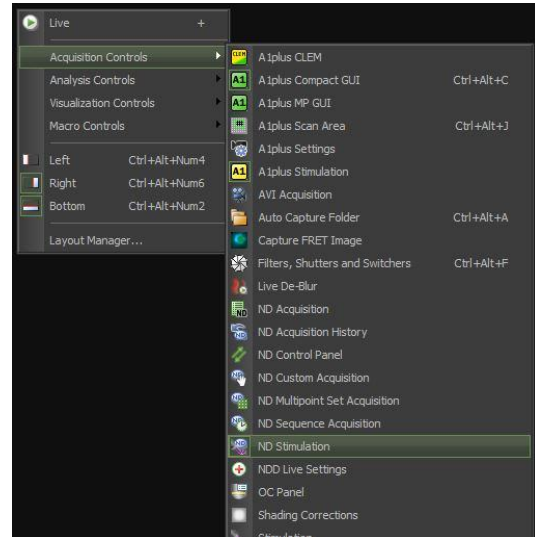
6. If desired, check the "Perform Time Measurement" box to display a plot of the fluorescence in the selected ROI(s) during the experiment.

7. Capture an image (by pressing "Live"), and draw an ROI for the activation area. Right click on the ROI and select "use as stimulation ROI" to designate this ROI for activation. If you do not designate the ROI as a stimulation ROI, then the software will not turn on the 405nm laser but instead just measure the fluorescence. In addition, you can draw one or more "standard" ROIs as controls. If desired, you can also designate multiple stimulation ROIs in a single experiment. Note though that, as you select multiple stimulation ROIs and/or increase the size of a stimulation ROI, you will need higher laser power and/or longer bleaching times. To keep things consistent, I usually recommend using a single stimulation ROI per image, and keeping the size of that ROI similar from experiment to experiment.
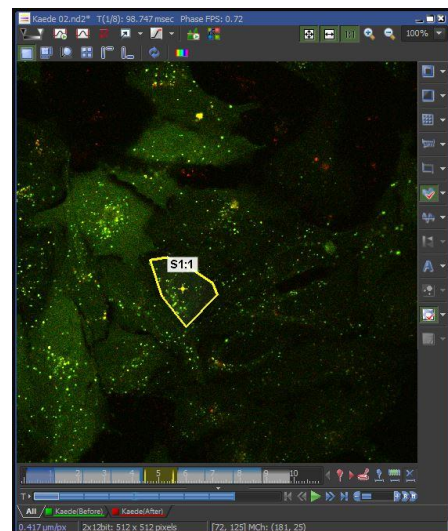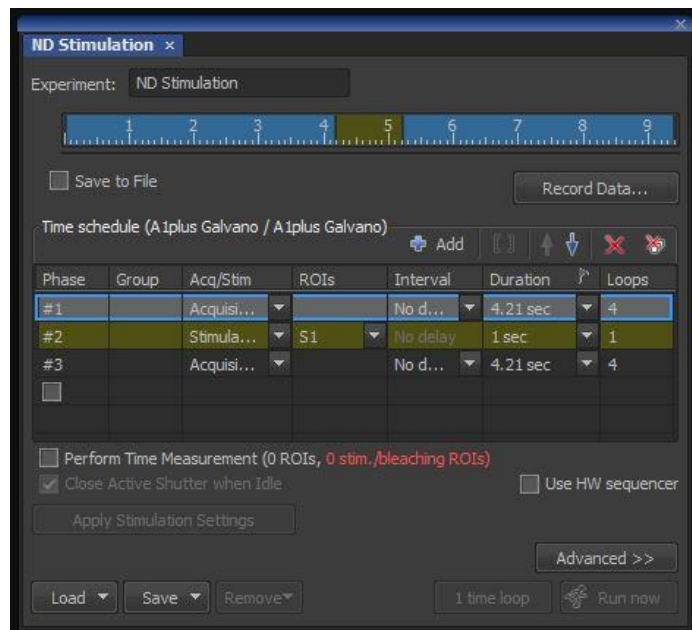
8. When done, click on "Apply Stimulation Settings" in the Sequential Stimulation/Bleaching control menu.

9. Click the "Run Now" button in the Sequential Stimulation/Bleaching control menu.

10. Data can be analyzed in a variety of ways, but the most common approach uses the Time Measurement module (Right-click on Elements desktop > Analysis Controls > Time Measurement). For the left and right axes, select "Kaede (Before)" and "Kaede (After)" respectively. The shaded area in the graph represents the activation pulse. After photoactivation, the green signal goes down while the red signal increases.