To do a FRAP experiment with the A1, run through the following steps:

1. First acquire an image; this will allow you to draw a region of interest (ROI) to designate the area to photobleach.

2. To draw an ROI, chose the kidney shaped tool bar on the right hand side of the image window and then right click on the image window to bring up options.

   **Define the ROI for the stimulation.**

   1. Press and make ROI on the image.

   2. Right click on the ROI and select ‘Use as Stimulation ROI’ from the menu window.
3. Make sure you’ve opened all the dialog boxes you need for FRAP acquisition, which include **SEQUENTIAL STIMULATION/BLEACHING, A1 STIMULATION, AND STIMULATION** (more on this one later).
4. Your docking panels should look somewhat like this:

![Docking panel image]

5. Choose your laser stimulation wavelength and power, as well as scan speed (this is the duration time for single stimulation ie 1 fps would mean a 1 second stimulation time).

![Laser stimulation panel image]
6. Make sure your stimulation device is set to be the A1. We’ve seen some recent instances where it was changed to either a camera or some other device, causing the A1 to not properly do the stimulation. Please check the STIMULATION dialog box and make sure the active device is set to the “A1 Stimulation Device.”

![Stimulation Dialog Box](image)

You will know you did this correctly when you look at the sequential stim dialog box and see “A1 Galvano/A1 Galvano”.

![Sequential Stimulation/Bleaching Dialog Box](image)

Make sure this is correct, otherwise will not work.
7. So far, you have set your Stimulation ROI, set the Stimulation laser wavelength and power. You know just need to set your acquisition parameters in the above dialog box. You will have 3 phases- 1) acquisition for a few seconds to get a baseline intensity measurement 2) a period of stimulation 3) acquisition of the recovery of intensity. Once you have set these phases, please make sure to click on the “Apply Stimulation Settings” at the bottom of the dialog box and then hit “RUN NOW”.

Set the time phases here for acquisition

Make sure to apply STIM settings, otherwise experiment will not work
8. To analyze the data, right click on the desktop, then Analysis ---> Time Measurement.

Click on “Measure” and you will see the graph of intensity versus time (make sure your ROI is selected)