How to optimize your FRAP experiments

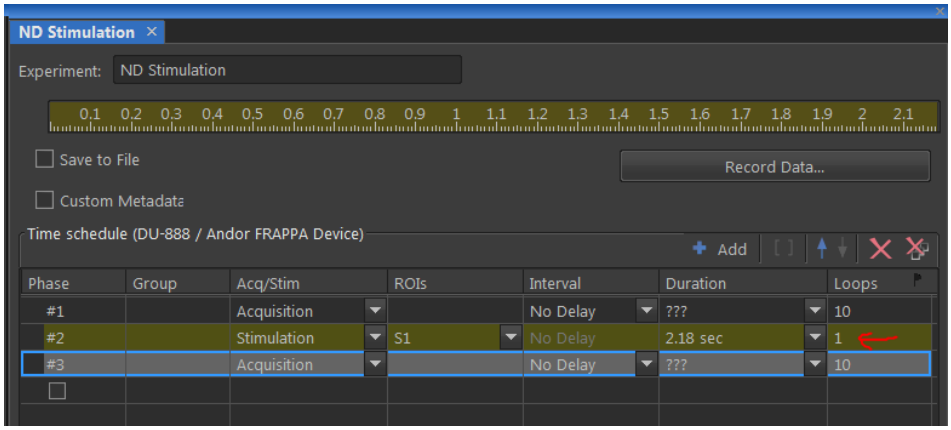
Before setting up a FRAP experiment you need to consider the following:

1. **To prevent unnecessary sample damage you want to bleach with as little power as necessary!**
2. **To obtain reproducible FRAP data between experiments and while using different size ROIs you need to keep the FRAP power density constant.**

Therefore, you should understand the difference between the following FRAP (Stimulation) parameters:

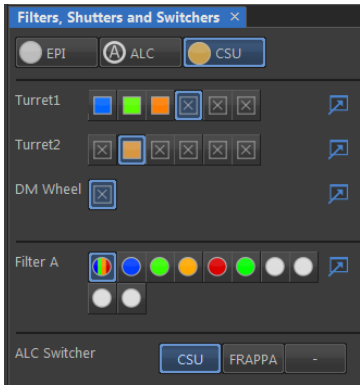
- FRAP laser power = laser power during the point scan through the stimulation ROI
- **Duration** = Total time for the FRAP laser to scan through the total ROI area (will be calculated by the system automatically, no need to be set!)
- **Interval** = normally on 'No Delay' to FRAP as fast as possible
- **Loops** = number of scans through the total ROI area in a defined raster (Start with 1 loop!)

Thus, to **keep the power density constant** between each FRAP experiment you need to keep both the **FRAP laser power** and the **number of loops** constant. The **ROI size and shape are irrelevant** to keep the power density constant. Changing the ROI size only adds time to the time 'Interval' that is needed to scan the FRAP beam through a given ROI. Changing the loops, however, changes the times the FRAP laser scans through the ROIs.

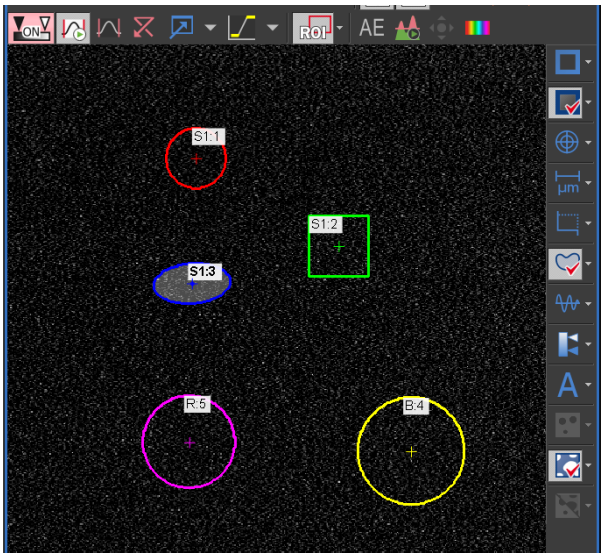


Recommendations

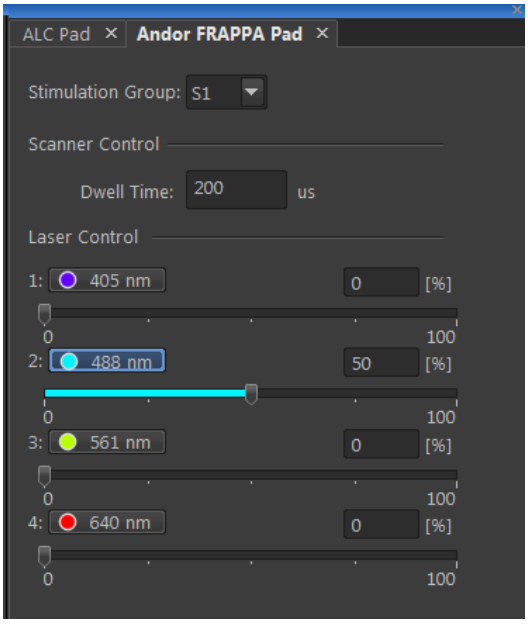
1. Make sure the FRAP dichroic is in place (Turret2) and the Turret1 is in an empty position.



2. Choose your FRAP Stimulation ROIs (S) that can be different sizes and shapes.
3. Choose the Background ROI (B) to subtract background and the Reference ROI (R) to check for the bleaching rate in a time-lapse experiment.



4. Start FRAPing with 1 loop at 50% FRAP laser power.



5. If bleaching is complete, reduce the FRAP laser power until you just have enough power to bleach.
6. If bleaching is not complete, elevate the FRAP laser power.
7. If 100% FRAP laser power is not enough, elevate the number of loops that scan through the ROI. Keep this number of loops constant for each comparable experiment.