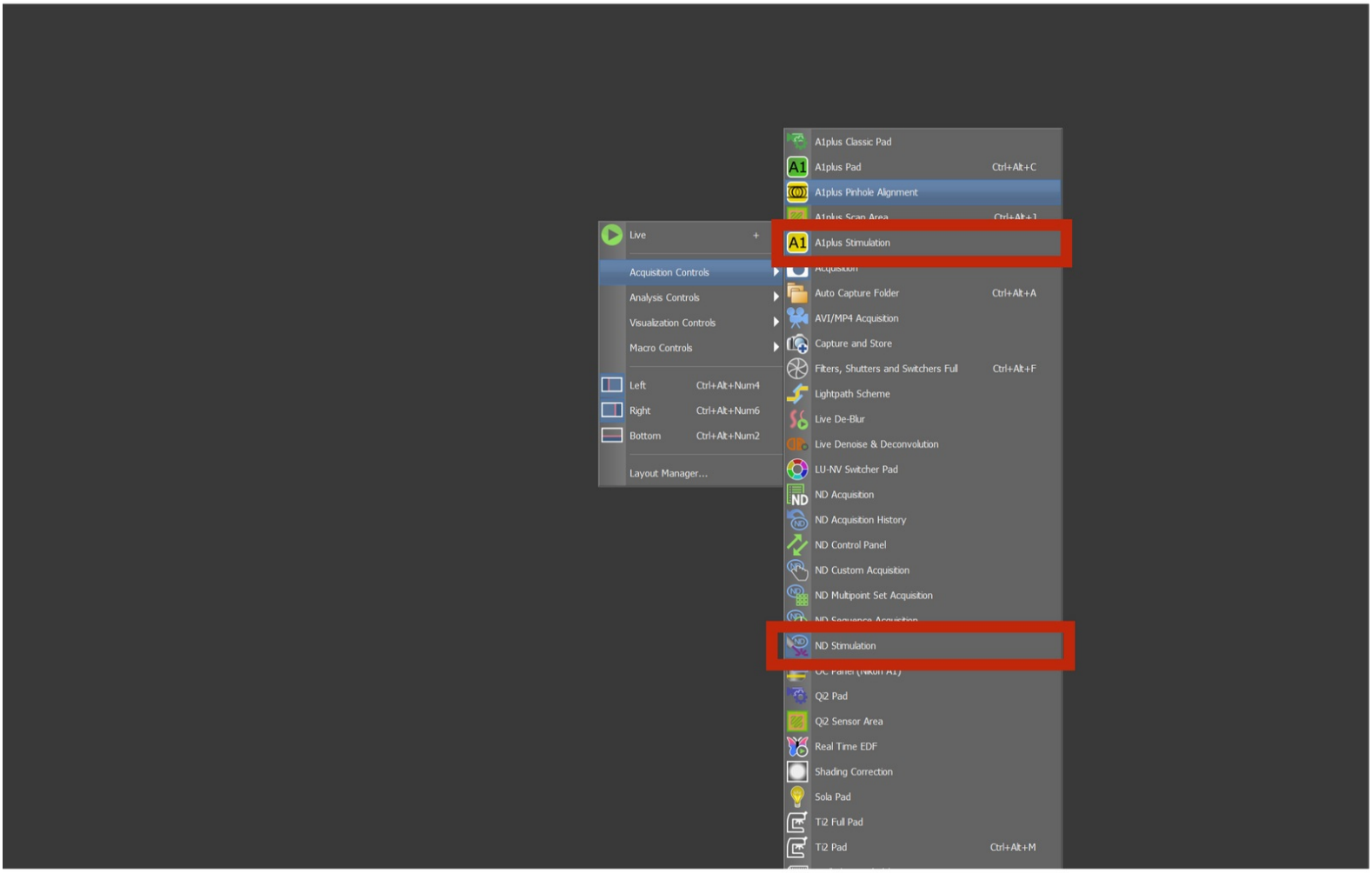
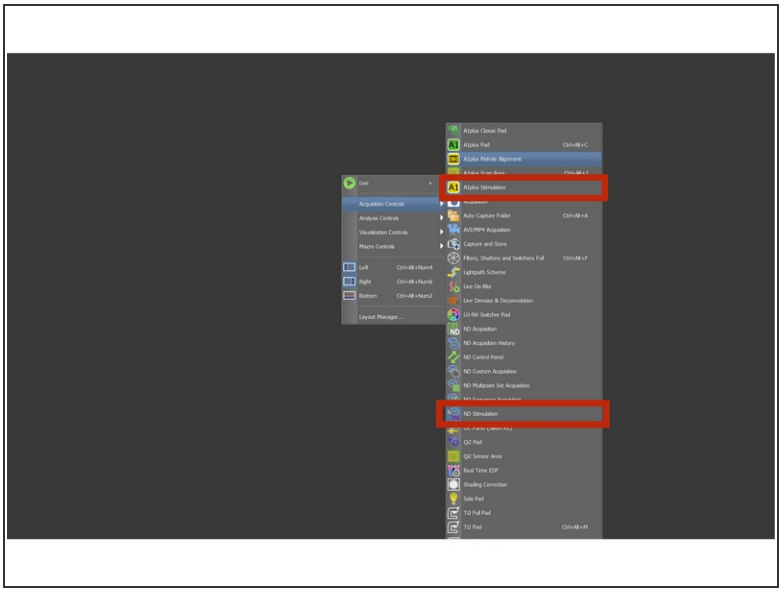
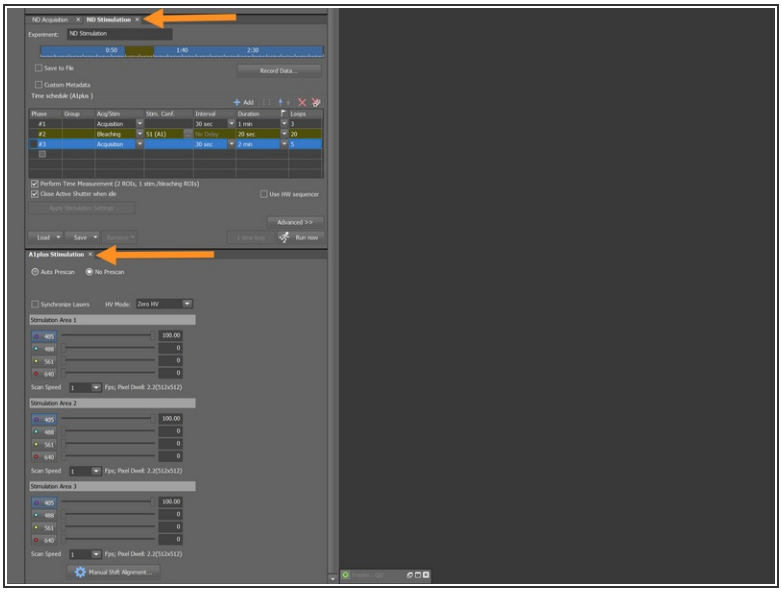
Nikon A1r+ (Balgrist) - FRAP

Written By: Eliska Macickova



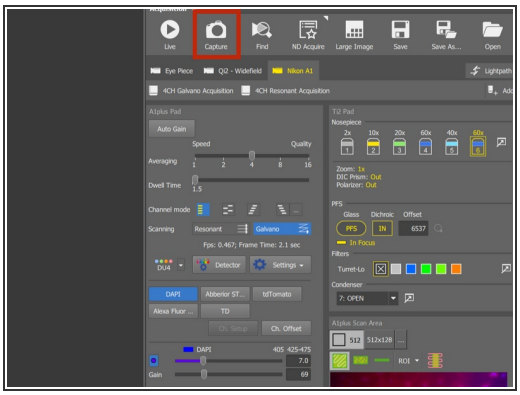
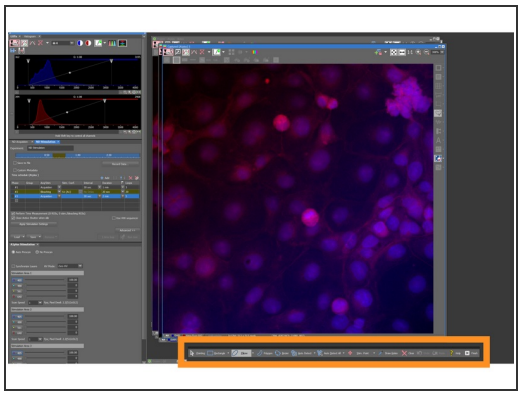
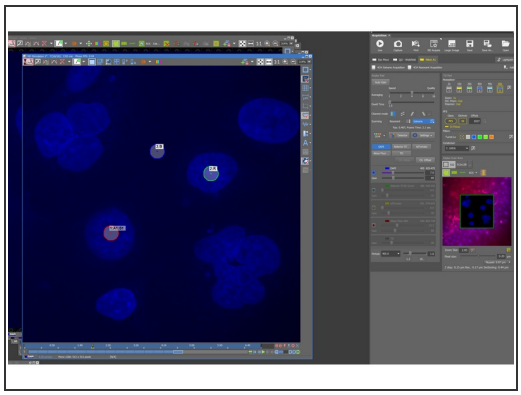
Step 1 — Open FRAP acquistion panels





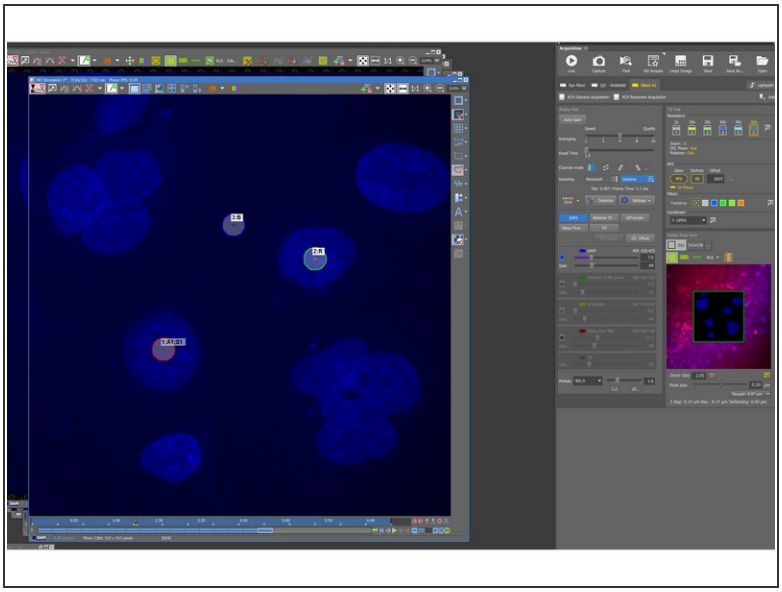
- Rightclick in the SW and in the acquisition control open **A1plus Stimulation** and **ND Stimulation** panel
- Dock it somewhere in the SW

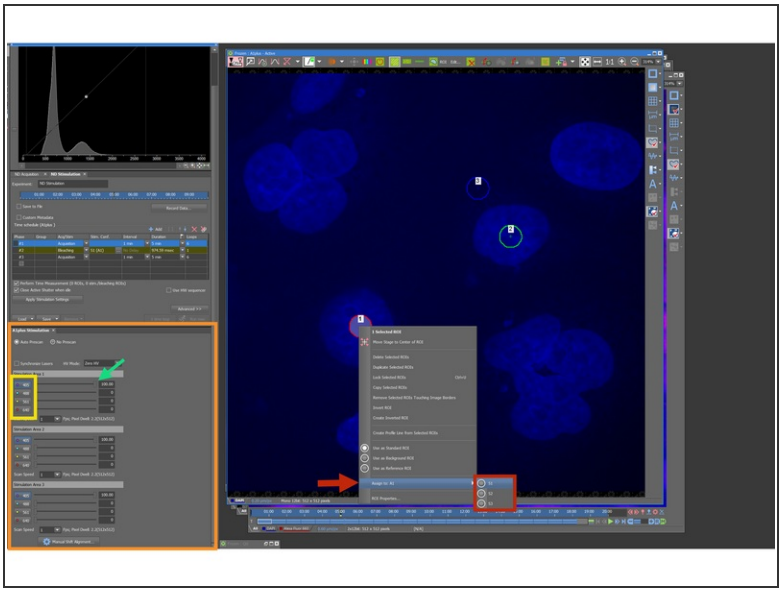
Step 2 — Optimize your acquisition parameters as normal

- Optimize the laser pw and scanning parameters as usual.
- Capture image with those settings
- press ***Ctrl+Shift+R*** to display ROI Editor to draw 3 ROI
  - Draw a ROI of 1-3 um in diameter and then right-click and duplicate twice, so you have in the end three ROI

Step 3 — Bleaching ROI

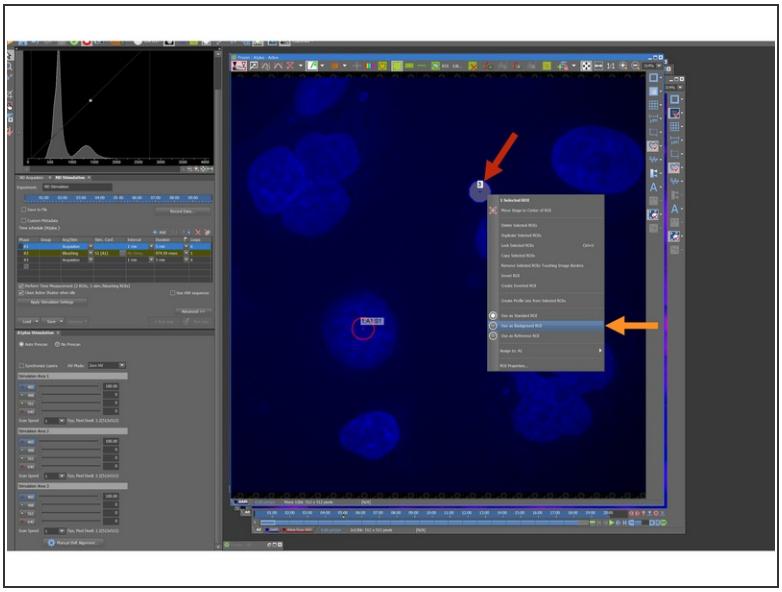


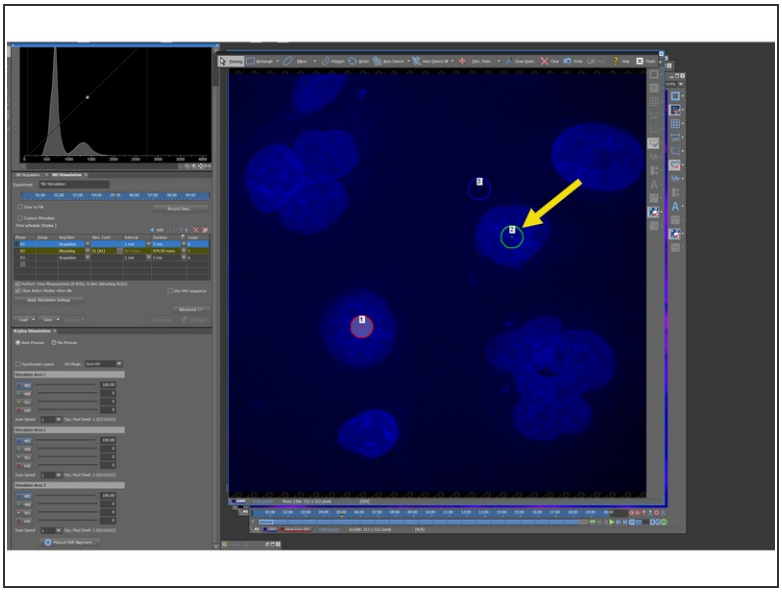


- Should be small enough ~1-3 um. If too large, the diffusion won't be correct because your molecule will diffuse into the ROI, while bleaching will be still running.
- Position in the area, where you want to bleach signal.
- Rightclick on the ROI > Assign to: A1 > S1/S2/S3
  - Parameters of the Stimulation/Bleaching settings you can find in this window
  - Choose the wavelength for bleaching
  - Change the laser power - Aim for ~50–70% intensity loss. This typically requires several test runs and intensity measurements.

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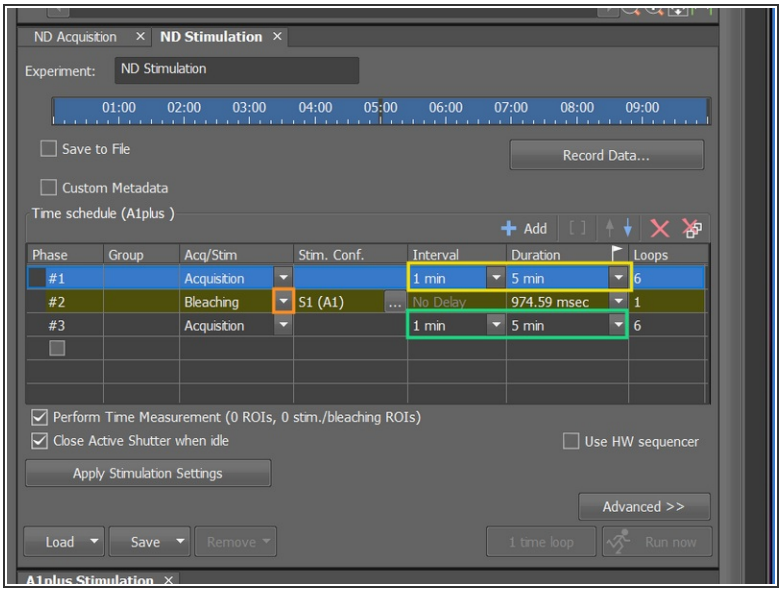
Step 4 — Reference ROI, Background ROI

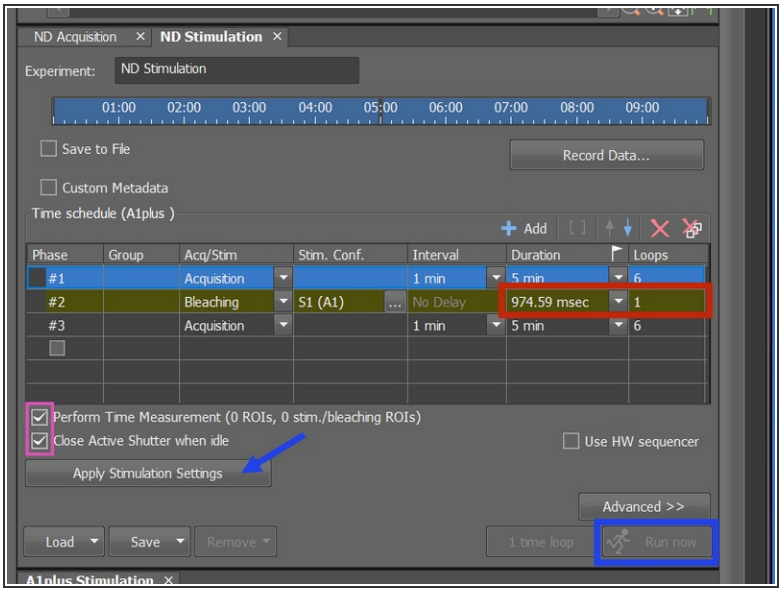




- One of the ROI place where is only background signal
  - Right-click → Assign to Background.
- Last ROI will be placed in the region, where is your specific signal, but no bleaching will happen
  - Right-click → Assign to Reference.
  - The reference ROI should show minimal intensity drop during the experiment. If the reference curve shows bleaching, adjust imaging parameters

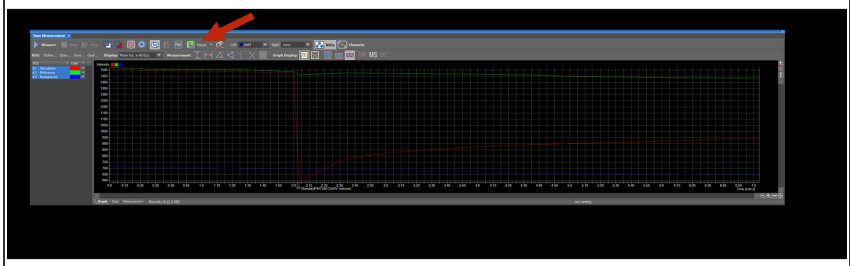
Step 5 — ND Stimulation window





- The acquisition consist of three phases: Pre-bleaching, Bleaching, Post-Bleaching (Recovery)
  - You can rename phase depending on whether you perform stimulation or bleaching.
- Define the timelapse duration before bleaching and the interval between frames.
  - ⓘ 5-10 loops to measure the intensity before bleaching
- Define the timelapse duration after bleaching, where signal recovery will be captured. Use the same interval as in Phase #1.
- In the bleaching phase, you can use a very short duration (~100ms). SW redefines the duration, so the bleaching occurs only once
- Check the relevant boxes to enable Time Measurement.
- Click Apply Stimulation Settings, then proceed with Run.

Step 6 — Time Measurement



- After the experiment, the Time Measurement window opens automatically, showing intensity curves for all ROIs.
- You can export the data in the excel sheet here.
- Reference ROI: Should have minimal drop of the intensity, otherwise you bleach already with your acquisition settings: If yes, you have to adjust them to avoid the bleaching or perform Reference correction during Data analysis
- Background ROI: could be used to subtract all non-specific fluorescence and camera/detector noise and backgroud signal coming from the sample, so that only the true signal from your sample is analyzed.