StripFRAP ON LEICA SP8 MANUAL

Fluorescence recovery after photobleaching

This protocol describes the FRAP Wizard set-up and is an extension on the [SP8 Quick start Manual](#) found on the website. FRAP is only possible on the SP8-775a.
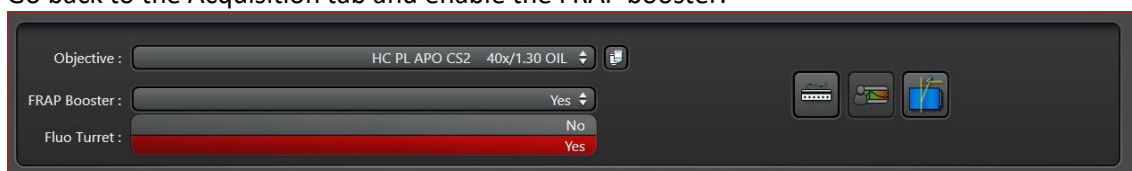
General Image set-up (also in SP8 Quick start manual)

Turn on the temperature, CO_2 and humidity.

Start LAS-AF

1. Use as configuration: Machine without AFC.
   a. Initialize stage
2. Start the lasers in the Configure tab and turn the Argon laser to 50%
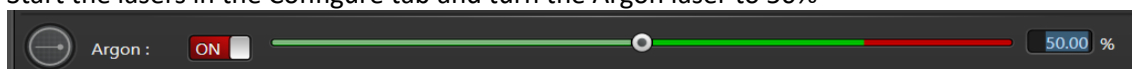
3. Go back to the Acquisition tab and enable the FRAP booster.

4. Place your samples on the microscope and focus.
5. Setup the desired channel(s) in sequential mode, like you would do for live cell imaging experiments e.g. low laser powers and high gain.
   Note: PMT's are preferred above Hyd detectors. Hyd detectors can turn off during the high bleaching laser powers and need to reset in between. This takes time and there for not optimal for the short time intervals we want to measure during recovery.
6. Take a first picture to store your settings.

Experiment setup

Imaging setup:

- Pixels: 512*512
- Average: line, 2 times
- Laser: 1-2%
- Speed: 400Hz
- Gain: 800-1000V (no saturation)
- Pinhole: 1AU

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FRAP WIZARD

1. Go to the left top of the screen and click the small arrow directing down. A menu will appear, select FRAP Wizard.

Setup tab

2. Press live and search for a cell of interest. By using a target (press Ctrl+t) move the cell to the middle of the screen. Press Ctrl+t again to hide the target.
3. Rotate the view until the longest axis of the cell is vertical
4. Zoom in to the cell in such a way that it is completely filling the screen (e.g. for nucleus about 8x zoom)
5. Stop live imaging.
6. Press "Capture Image" to have the basic initial image of your cell.
7. Change you field of view to perform strip-FRAP
   a. Go to the XY menu and click the double arrow (4a).
   b. In the drop down menu, that appears there will be the option to choose "More".
   c. A second window will appear where you can change the Y to 16 pixels and click OK.
8. Change the speed to 1400Hz (5)
9. Enable the Pinhole in the XY menu and set to 1-2 AU
10. Press "Live" again to see the result of changing the settings.

Longest axes vertical
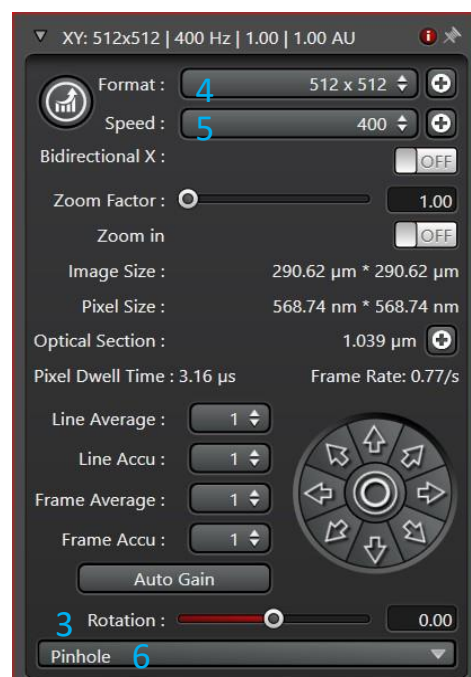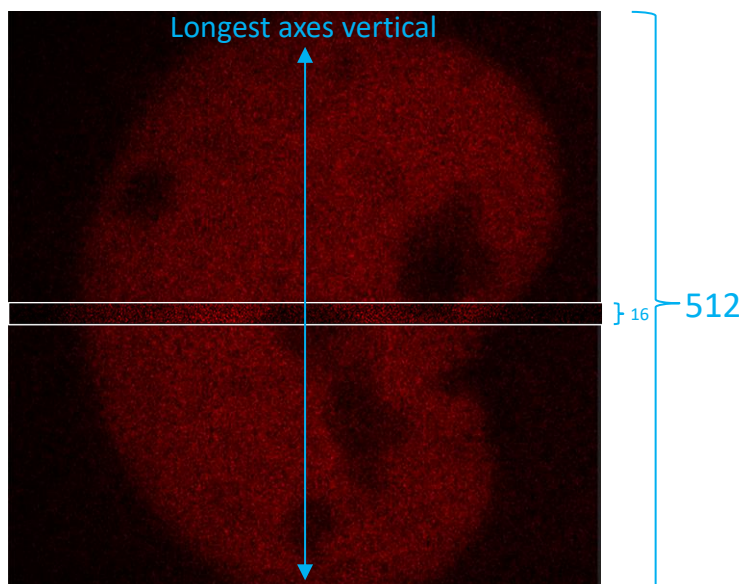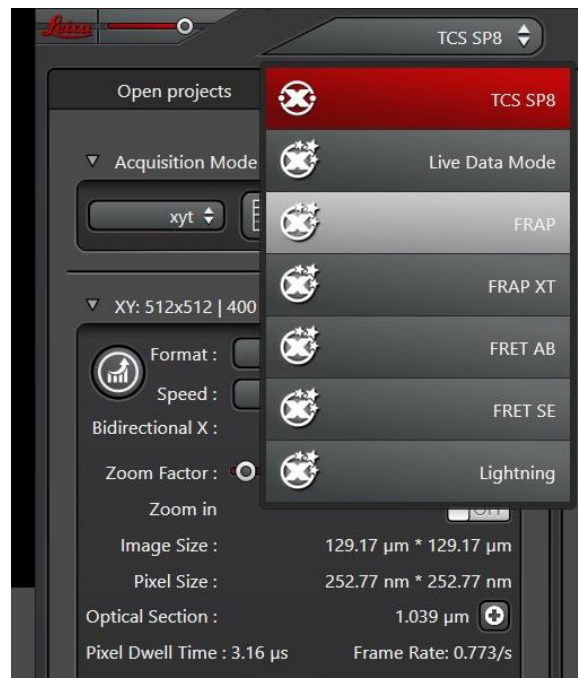
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Bleach tab

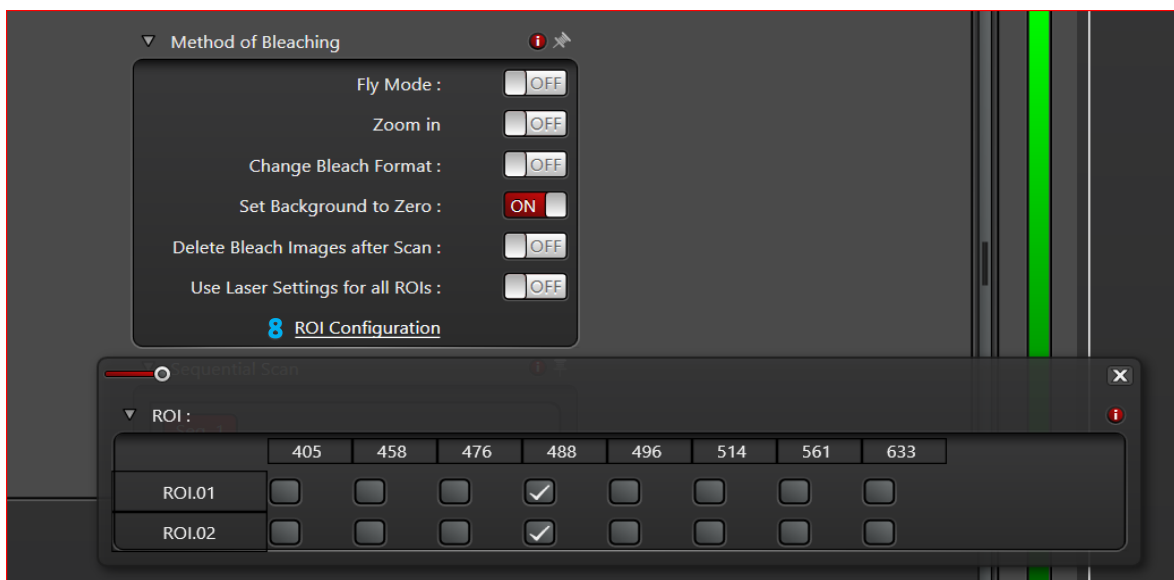11. Draw a square ROI in the cell (ROI 1; covering the whole nucleus)

      a.   Place lines of ROI half inside image, half outside

12. Draw a second ROI in the background (ROI 2)

13. Click in ROI Configuration and make sure the correct lasers are selected for the ROI's (8)
14. Make sure the shutter of the bleaching laser is open and that the laser has a power of 100%.

Method of bleaching

- Fly mode: OFF
- Zoom in: OFF
- Change Bleach format: OFF
- Set background to zero: OFF
- Delete Bleach Images after Scan: ON
- Use laser settings for all ROI's: Depending on your experiment

Time Course Tab

Interval settings

      a.   Pre-bleach   100 frames, 22ms interval (uncheck minimize)
      b.   Bleach   9 frames or ~100ms,
      c.   Post-Bleach 1   2000 frames, 22ms interval (uncheck minimize)
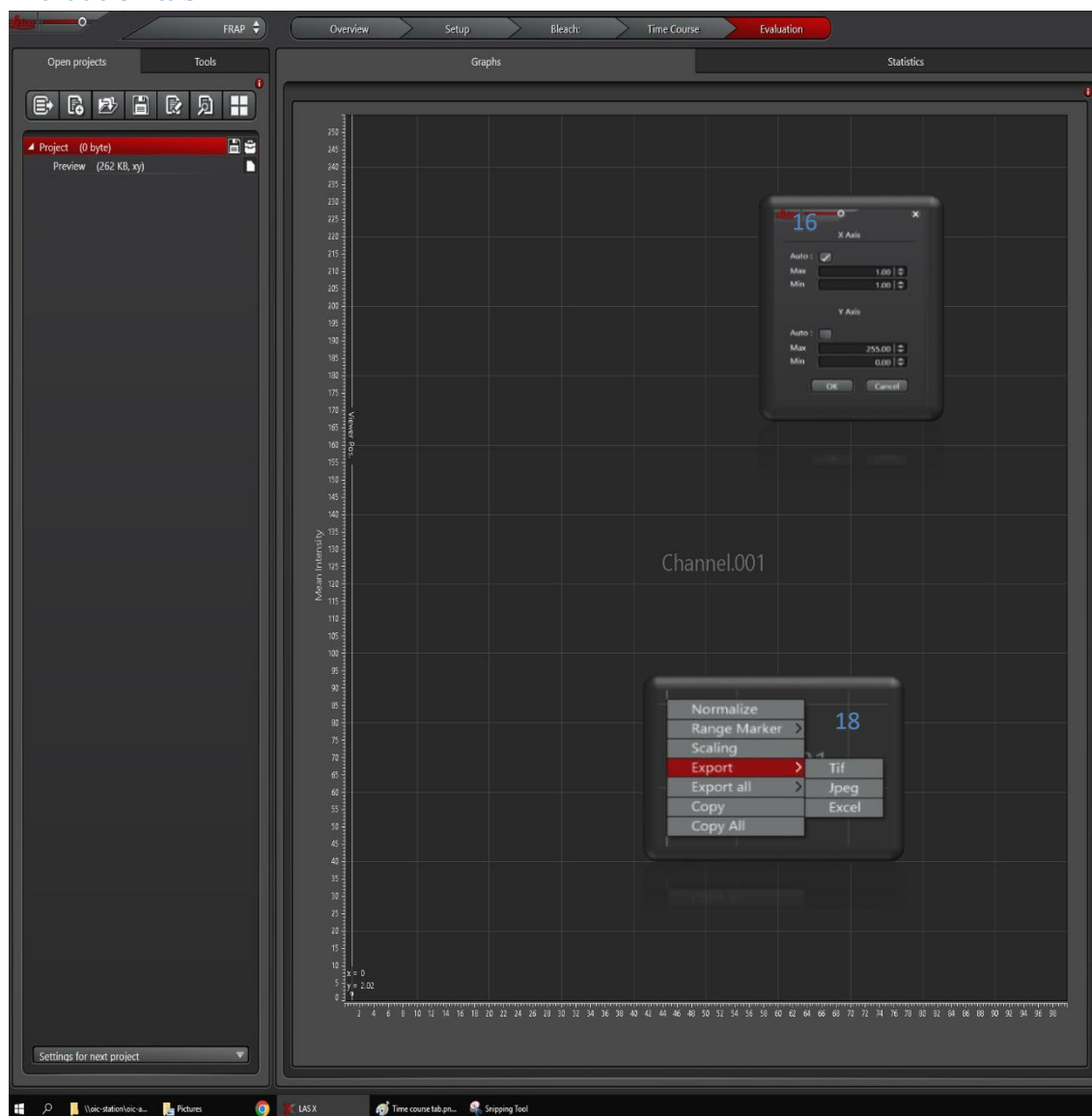
15. Check settings and press start experiment

Evaluation tab

16. Enable a graph in the left window and scale accordingly by clicking right mouse button and go to scaling. A new window will open and you can change the scaling.
17. Check the curves created by the bleaching.
    a. Is the pre-bleach signal stable or is it declining; if declining this means that the Imaging laser is to strong and bleaching your fluorophore. Change the settings for the general image set-up.
    b. Is the Bleach "drop" almost as low as the background? No, then more Iterations are needed to bleach the fluorophore in the Time-Course settings.
    c. Is the signal coming back in the post-bleach? No, use more Iterations to see the signal coming back.

18. When finished, export the data by clicking the right mouse button and export the data to a csv file.