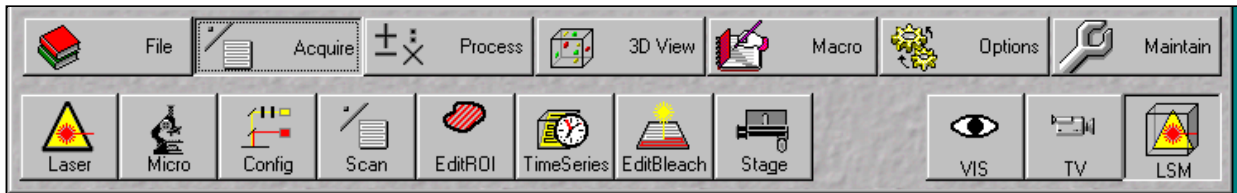


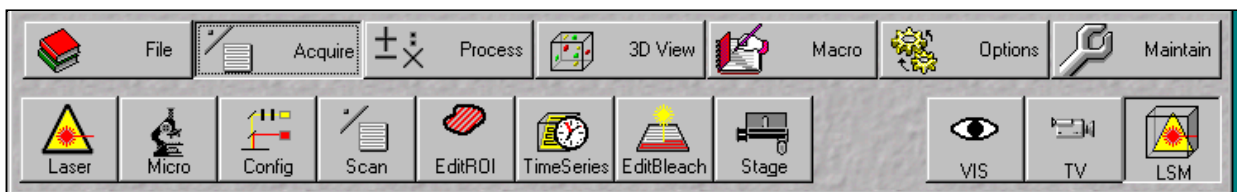
Zeiss LSM510 META

Fluorescence Recovery After Photobleaching (FRAP)

- 1) After final adjustments to obtain best image quality, click [EditBleach](#).



- 2) In "Bleach Control" dialogue box click on "Define Region" (under **Bleach Parameter**).
- 3) In "Bleach Regions" dialogue box click on type of ROI you want to use (*i.e.*, rectangle, circle, *etc.*).
- 4) On the image apply the ROI, adjust position and size, then in "Bleach Regions" dialogue box click on "Add to lists". Give ROI a name and click OK. NOTE: LEAVE "Bleach Regions" dialogue box OPEN.
- 5) In "Bleach Control" dialogue box tick "Bleach after number of scans" and set Scan Number (*e.g.*, 10).
- 6) Under **Bleach Parameter** set number of iterations (more iterations = more bleaching; should probably be >25).
- 7) Under **Excitation Bleach Track**, select laser (same as for fluorophore) and set strength (probably 100%, to get most effective bleaching).
- 8) Click on [TimeSeries](#).



- 9) Under **Stop Series** set total number of scans for experiment (should be greater than number set in step 5).
- 10) Under **Time Interval** set the interval between scans (should be longer than the time needed to complete a single scan).
- 11) To begin experiment click on **StartB**.
- 12) After experiment is complete, save images to database.
- 13) To view results, click on the **Mean ROI** button in the image window (to the right of the image).
- 14) Select your ROI from the drop down menu at the top righthand corner of the dialogue box.
- 15) To extract data, click on **Show Table**, then **Save Table** (table will be stored as a text file that can be opened in Excel/Word, *etc.*).