# FRAP Acquisition and Analysis Guide in ZEN 2009

With the **LSM FRAP Module**, FRAP data generated using the LSM systems can be displayed and analyzed. This manual illustrates how to setup and acquire FRAP data on your Zeiss LSM using ZEN 2009. The FRAP module is not included in the base ZEN 2009 software and is available for purchase as an add-on module. Any questions or concerns can be directed to the ZEISS Product and Applications Support group at 1-800-509-3905 or Support@zeiss.com

Step 1: Selecting Appropriate Acquisition Parameters for FRAP

Upon starting ZEN 2009, click on the acquisition tab and check the bleaching option. After checking, both the time series and regions options will become selected as well.
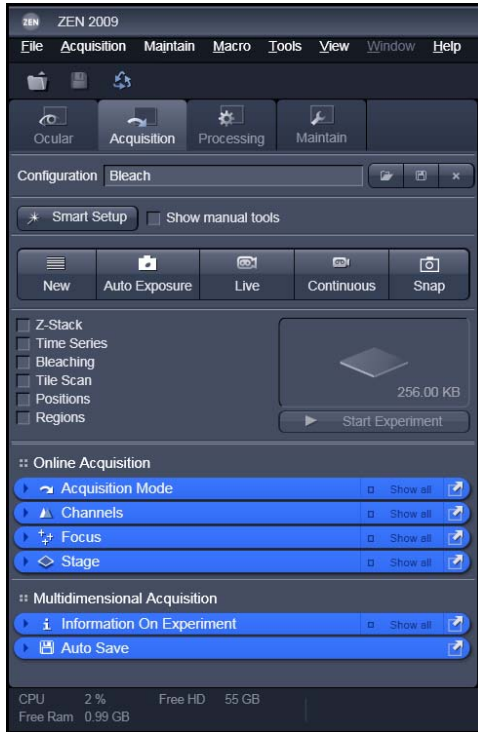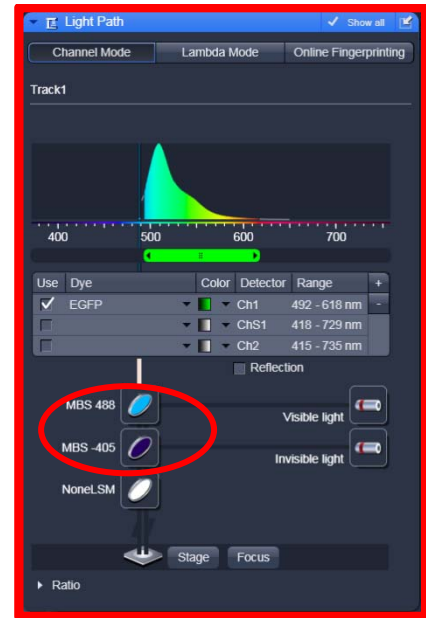

After selecting bleach option
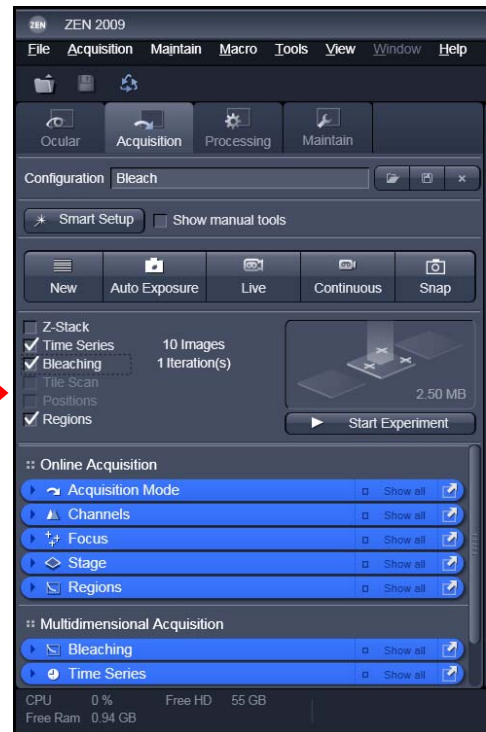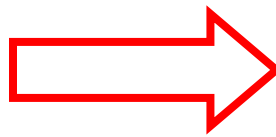
Step 2: Ensure Correct MBS is used

If the laser that will be used for imaging is different than the laser that will be used for bleaching/photomanipulation, then the correct dichroic must be chosen in the light path menu.


Imaging and bleaching with same laser

Imaging with 488nm laser and bleaching/ photomanupulation with 405 laser

Step 3: Selecting FRAP ROI from Regions Menu

Setup the LSM acquisition and channel parameters to collect desired image. Next, take a single image at desired position. After image acquisition, open regions menu and select bleach shape to use for FRAP experiment. After selecting, draw the shape in the desired location inside the open image. For FRAP experiments, make sure that the bleach and analysis options are checked in the regions menu and make sure that the acquisition option is NOT checked.

IMPORTANT: Make sure that the image used for selecting the ROI is taken with either the "Snap" function or "Continuous." Using the "Live" option will cause a spatial mismatch for the selected bleach ROI. Also, for fast kinetics, make sure to use a smaller ROI to keep the time interval between bleaching and imaging to a minimum.
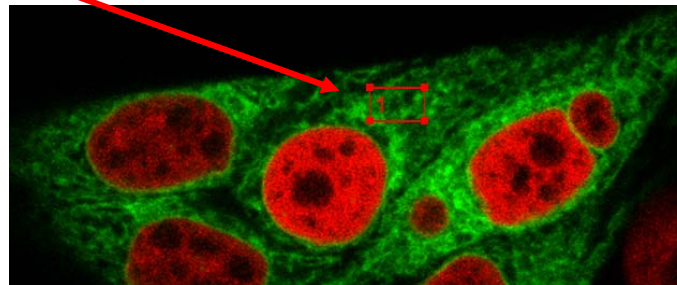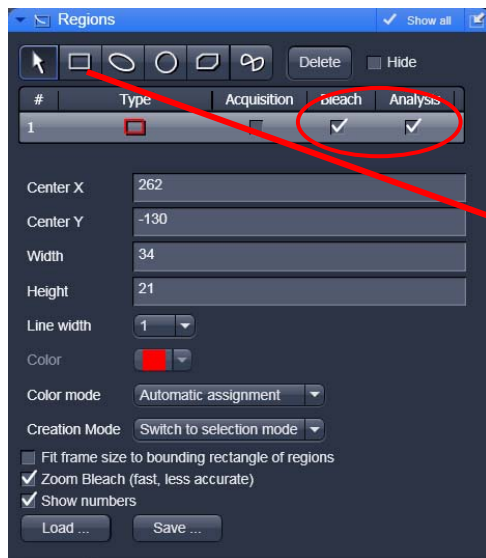
Step 4: Setup Bleaching Protocol

After designating a bleach region, the bleaching protocol needs to be specified under the bleaching menu.

1. Select Show all, if not checked
2. Select how many scans to make before the bleach (to get a baseline before bleach)
3. Specify how many iterations of the bleach laser over the bleach region
4. Decide if a different scan speed is needed over bleach region (default is the imaging scan speed)
5. Decide if Zoom Bleach is appropriate (Zoom bleach creates a bounding rectangle around the ROI for faster scanning, but less accurate. Default is to scan the entire range in x of the image and only bound the ROI in the y dimension)
6. Select which laser to use for bleaching the ROI. Again, make sure the appropriate dichroic is chose in the light path menu for the desired laser line. Multiple lasers can be used concurrently for bleaching.

Step 5: Define Time Interval and Number of Time Points

Once the bleaching protocol has been set, a time series needs to be specified. Open the Time Series menu and set the number of Cycles and duration of the Interval. The number of cylces will specify the total number of images in the final data set. The interval is measured as the time between the start of a scan to the start of the next scan (if the acquisition time is longer than the interval time, then the next scan will start as soon as the previous scan finishes). It is important to make sure that the time interval is appropriate for the dynamics to be measured to prevent any over sampling in time or temporal aliasing. Also, trial and error might be needed to make sure enough data points are collected such that the recovery curve plateaus at equilibrium to ensure the best fit of the data
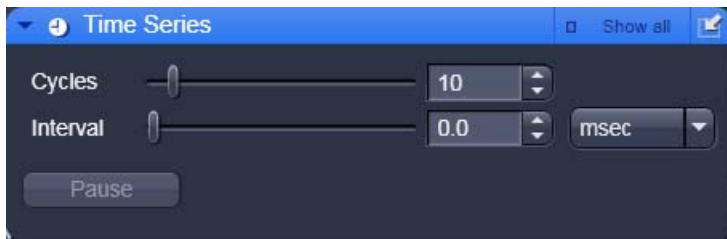
Step 6: Starting Experiment

To start the FRAP experiment, press the Start Experiment button under the Acquisition tab.
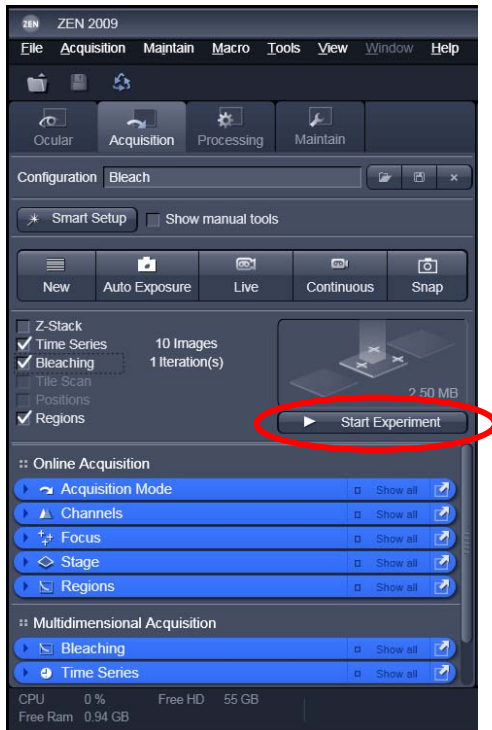
Step 7: Real-Time Monitoring of ROI's During Acquistition

The recovery of the fluorophore back into the bleach region can be monitored in real time by going to the Mean ROI tab to the left of the image window. Additional ROI's can be added in the Mean ROI menu located below the image window. (Note: Mean ROI is only available with the additional modules Physiology or FRAP. To check for these modules go to Help->About)
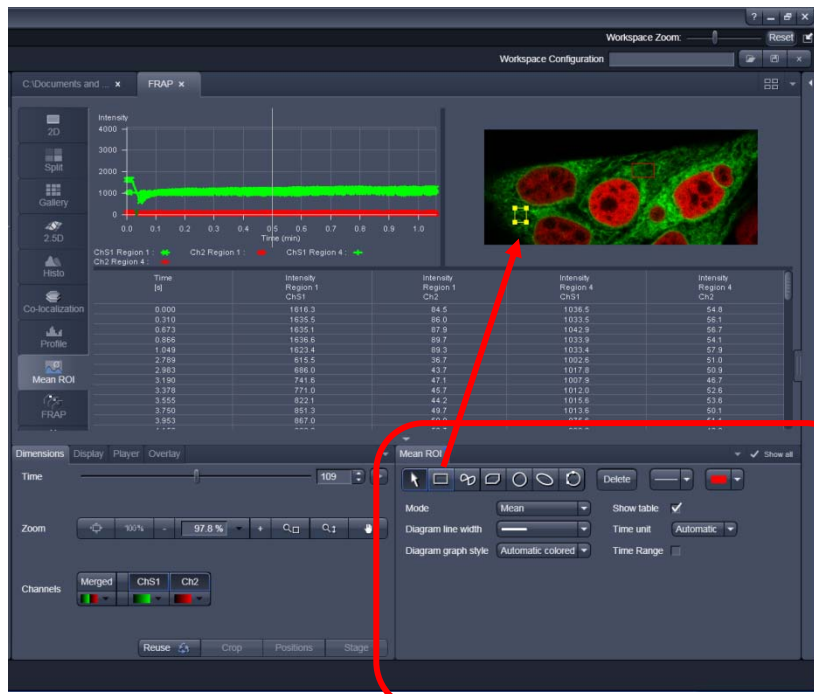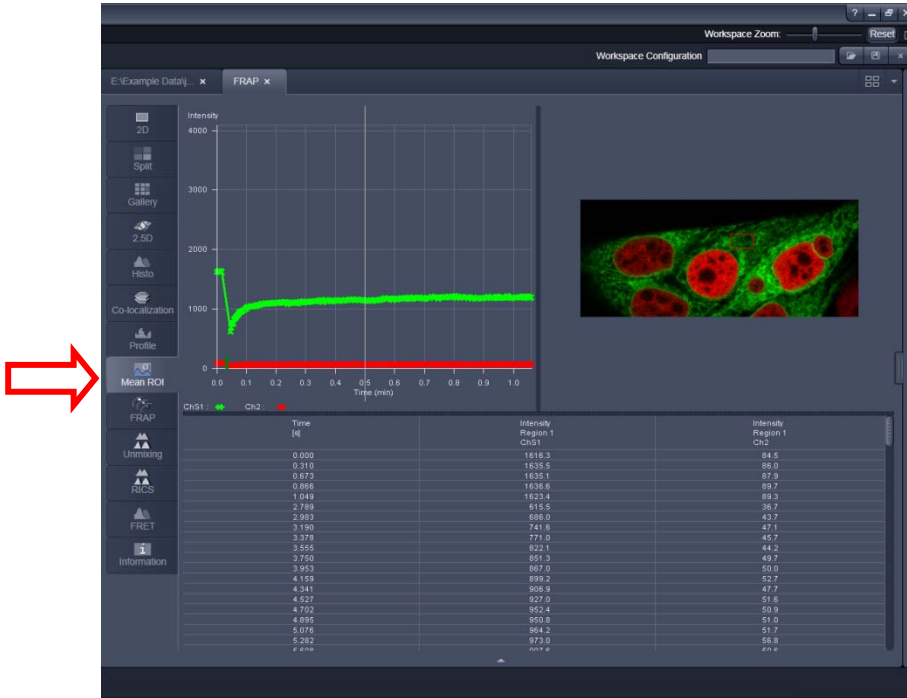

Add additional ROI's and edit ROI properties

Step 8: Analyzing FRAP Data

After the dataset is finished acquiring, the data can be analyzed in the FRAP module. Select the FRAP tab to the left of the image window to open the FRAP module. (Note: FRAP analysis is only available if FRAP module has been purchased. To check go to Help->About)

1. In the FRAP menu below the image select bleach ROI to group 1. If multiple ROI's are added to same group, then mean intensity trace of ROI's is averaged.
2. Select Normalize for easier viewing of data
3. To view data in a table form select Table
4. To correct data for background and incidental bleaching from imaging draw ROI's using FRAP menu. Define ROI's as Background or Reference.
5. Pick appropriate Fit formula. Fit results shown in table below graph.

To save data and fit results, right click on appropriate table to copy data to clip board or save as a text file.

5.
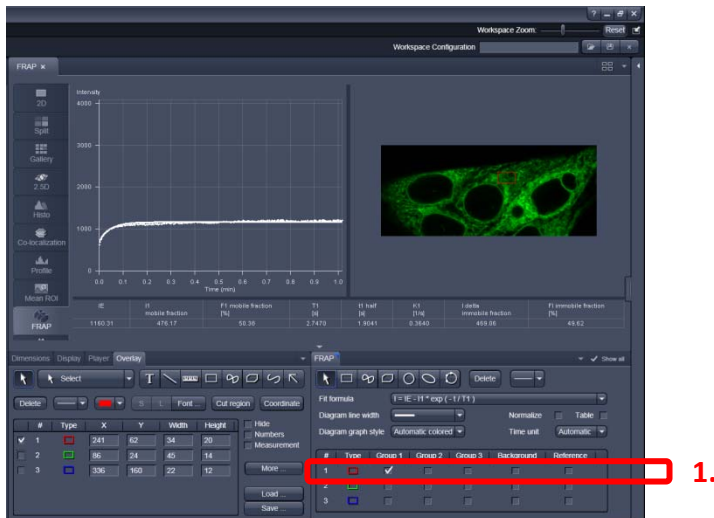
Note: The fit models available in ZEN 2009 are only for modeling free diffusion with 1 or 2 diffusing components. If other models are needed, then data must be analyzed in a separate software package of user choice.

Appendix

For additional information on FRAP and reference material see the Zeiss campus website.

http://zeiss-campus.magnet.fsu.edu/referencelibrary/frap.html