

FRAP GUIDE

This guide is written for FRAP on the Zeiss 510 Meta confocals but hopefully the principles can be adapted for FRAP experiments on other systems. All instructions within square brackets [] are specific to the Zeiss software.

Control experiments

Before starting the FRAP experiment, there are two control experiments that you should carry out to aid in the FRAP design.

1) Background bleaching

This involves a simple time-lapse to discover the extent of background bleaching during imaging. The idea is to establish how much your sample will bleach during the recovery phase of the FRAP experiment. You want to minimise background bleaching so that you do not mask your recovery. You are aiming for less than 10% background bleaching.

2) Bleach iterations

Again a simple time-lapse with multiple bleaching events to establish how many bleach iterations you need to bleach to the required intensity. You often do not need to bleach completely but might be satisfied with bleaching to 10-20% of the original intensity.

1) Background bleaching

- Choose a cell/object as similar as possible to the cell/object you wish to image during FRAP.
- Centre the cell/object in the field of view and adjust scan settings to achieve the fastest scan rate possible (for example, reduce the Y value of the box size [Scan Control Panel and Mode tab] to minimise the number of lines in a scan).
- Set up a time-series to scan continually for as many scans as you estimate will cover your recovery time in the final FRAP experiment (e.g., if you are scanning at 5 frames a second and expect your recovery to take at least 30 seconds then you should set a time-series to run at least $30 \times 5 = 150$ scans). [See Figure 1]
- Ensure you are imaging the entire cell/object to avoid any FRAP effects from non-imaged regions (so if you draw an ROI make sure it contains the entire cell/object - it doesn't matter what shape the ROI is at this stage).
- Collect the time series [Start T].
- [To analyse the time series data in the LSM software, go to Mean ROI in the image window. Draw an ROI **within** the cell/object and generate a graph over time. See Figure 2]
- Check that the final signal intensity is more than 90% of the initial intensity. If it is, then you can use these scan settings for the recovery phase of your FRAP experiment. If bleaching is occurring, then adjust the scan settings (for example lower the laser power) and repeat as above.

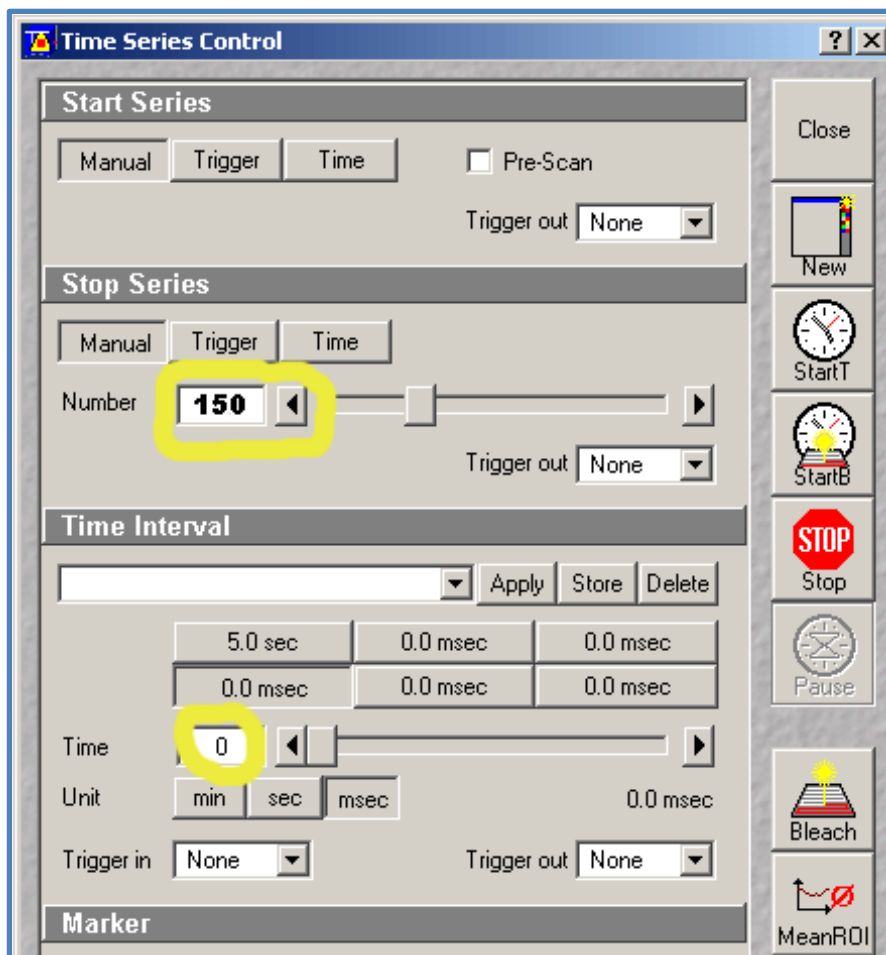


Figure 1.

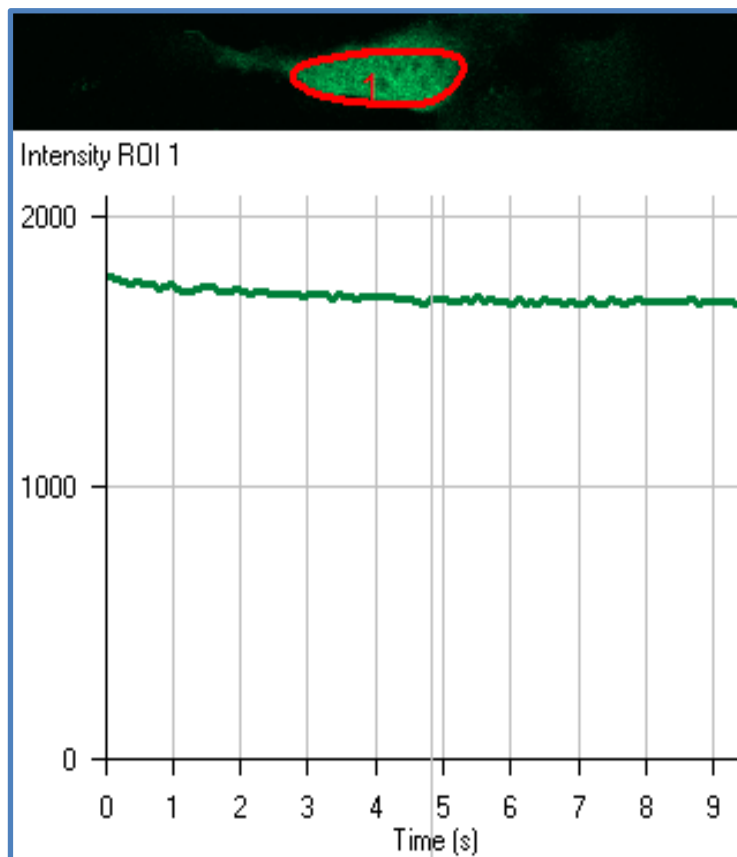


Figure 2.

2) Bleach iterations

- Choose a new cell/object as similar as possible to the cell/object you wish to image during FRAP.
- Centre the cell/object in the field of view and adjust scan settings to achieve the fastest scan rate possible (for example, reduce the Y value of the box size [Scan Control Panel and Mode tab] to minimise the number of lines in a scan).
- Set up the bleach parameters [Edit Bleach - Bleach control panel, as shown in [Figure 3](#)]. Set up the required number of pre-bleach scans [Bleach after number of scans (tick) Scan number 10]. Set to scan once after each bleach event [Bleach repeat after number of scans (tick) Scan number 1]. Set the number of bleach iterations for each bleach event [Bleach parameter - Iterations 3].
- Set the appropriate laser to 100% power for the bleaching events [Excitation of bleach track]. [NB If you are using the 488 laser you can use it together with the 477 line at 100% power.]
- Define a bleach area [in Define Region] to include the entire cell/object. Ensure you are bleaching the entire cell/object to avoid any FRAP effects from non-bleached regions. At this point a rectangle ROI is a good idea if possible - especially if you are scanning at rates of 10 frames per second or faster.
- Set up a time-series to include the pre bleach scans and then a reasonable number of bleach events (e.g. I estimate that 90 bleach iterations will be more than enough to bleach my sample. In the case of the example shown in [Figure 3](#) each bleach event is set to 3 iterations so 30 bleach events will give me 90 bleach iterations. There is one scan after each bleach event so 30 plus the 10 pre-bleach scans = 40 scans in total).
- Collect the bleaching time series [Start B].
- [To analyse the time series data in the Zeiss software, go to Mean ROI in the image window. Draw an ROI **within** the cell/object and generate a graph over time. See [Figure 4](#)]
- Count how many bleach events it takes to get down to the required percentage of the original intensity e.g. 20%. [The beginning and end of the bleach events are shown by the green bars]. Bleach events x bleach iterations in each event = number of iterations you use in your FRAP experiment.

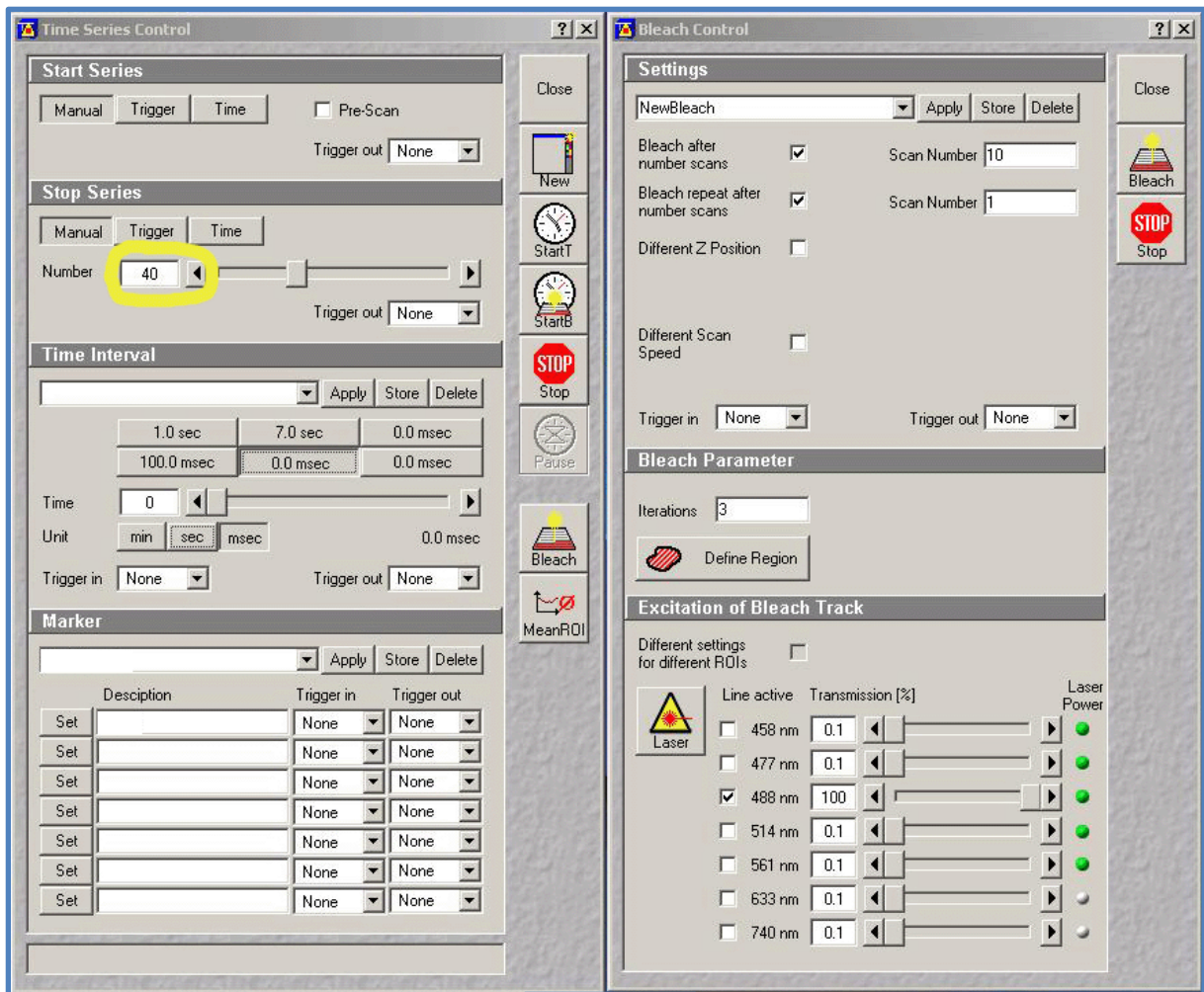


Figure 3.

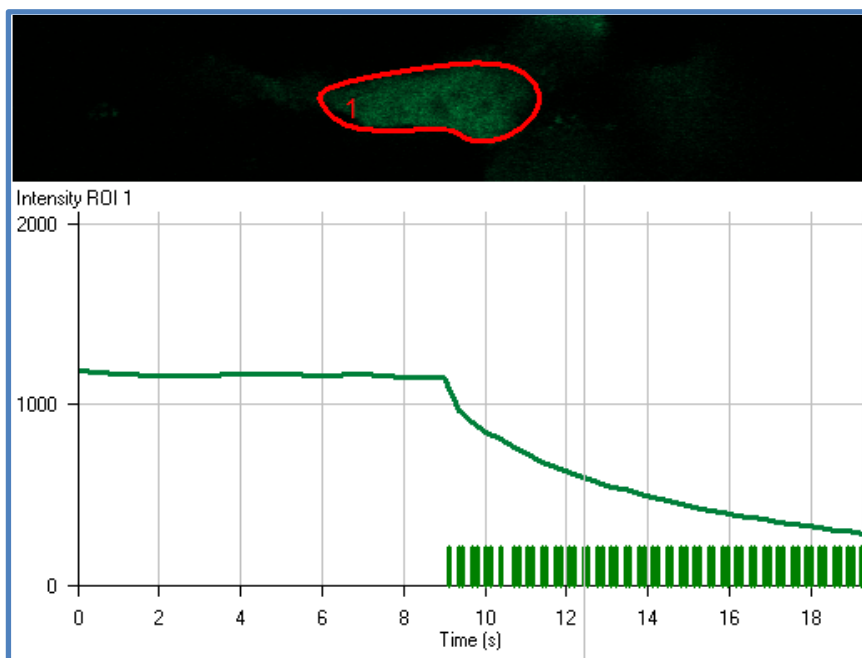


Figure 4.

The FRAP experiment

There are many different ways to set up FRAP. The one described below is based on a method for FRAP within transfected cells. In this method, you only image the bleached region which makes the data easier to analyse without dedicated software.

- Choose a cell, centre it in the field of view and adjust scan settings to achieve the fastest scan rate possible.
- Set an ROI for the FRAP experiment [use Edit ROI]. Choose a rectangle that preferably starts and ends outside the cell edges as you go from left to right (this is important if you are scanning at rates of more than 10 frames per second). The ROI should cover less than 10% of the total cell area so that there is enough fluorescence available for recovery. Save this ROI *.
- [In the Scan Control window, ensure that the 'Use ROI' button is activated.]
- Set up the bleach parameters [Edit Bleach - Bleach Control panel, [see Figure 5](#)]. Set up the required number of pre-bleach scans [Bleach after number of scans (tick) Scan number e.g. 50]. Set the number of bleach iterations for the bleach event [Bleach parameter - Iterations] using the value obtained from the Bleach iterations control experiment described above.
- Set the appropriate laser to 100% power for the bleaching events [Excitation of bleach track]. [NB If you are using the 488 laser you can use it together with the 477 line at 100% power.] (Use the same settings as for the Bleach iterations control experiment described above).
- Define Region - choose the ROI that you saved earlier *.
- Set up a time series to include the pre-bleach scans and then a reasonable number of scans for the recovery [see Figure 5].
- Collect the FRAP data [Start B].
- [To analyse the FRAP data in the Zeiss software, go to Mean ROI in the image window. Draw an ROI within the cell and generate a graph over time to see the bleach and recovery curve.]
- Export the intensity values [use the 'Show Table' button] to an appropriate software package and re-plot the graph. Draw a best fit curve to the recovery data to work out the half recovery time. Note that an exponential curve might not fit if there are two components to the recovery.

FRAP data can also be analysed using the Kinetic module in the LSM software.

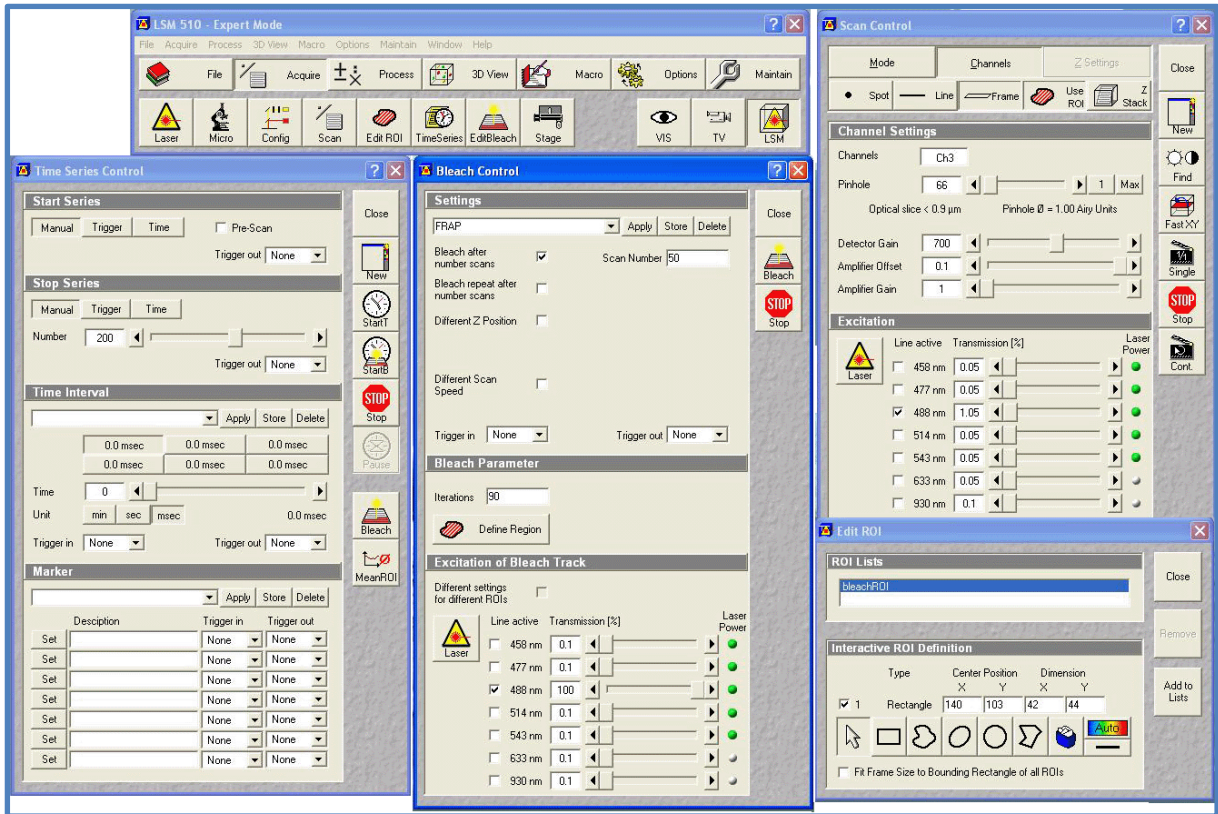


Figure 5.