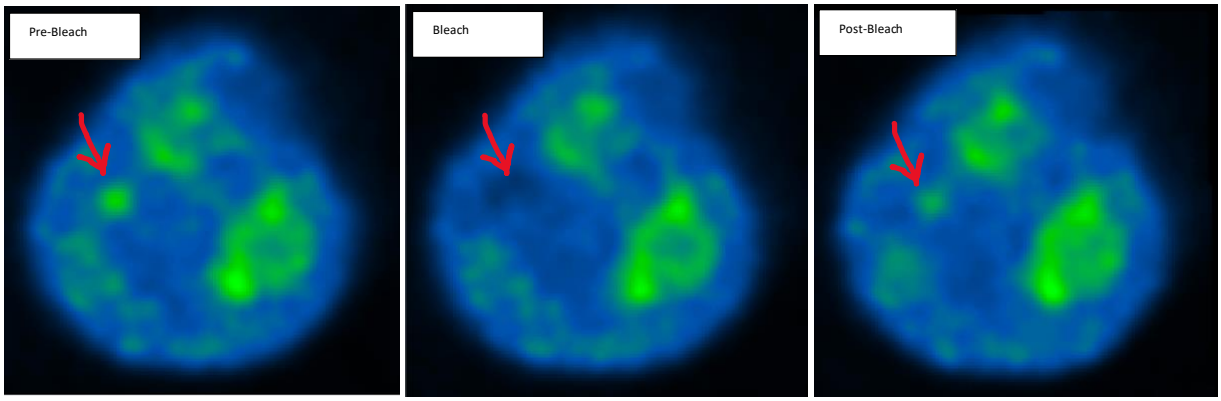
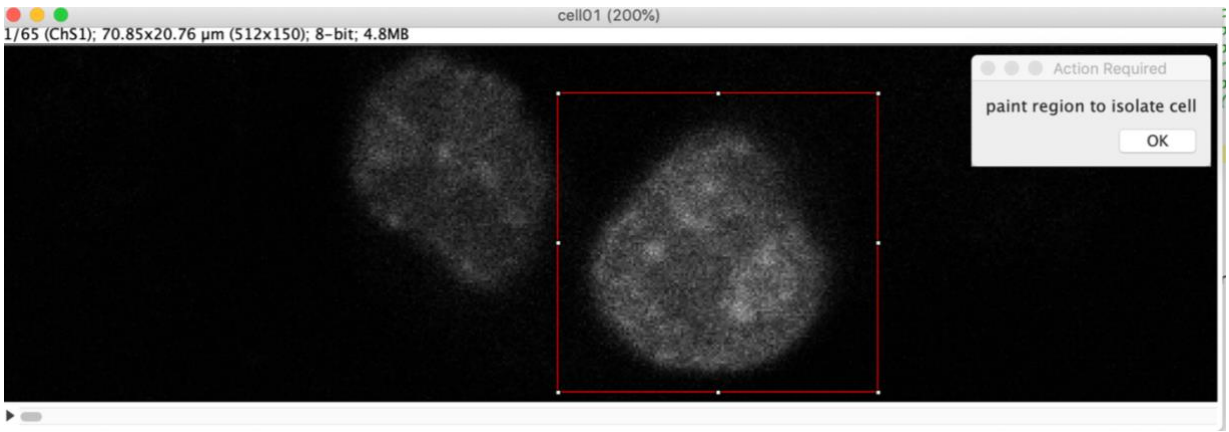
measureFRAP.ijm macro instructions (E. Rebollo)

The macro ***measureFRAP.ijm*** is an interactive routine to obtain FRAP measurements from fluorescence time lapses where a small dot-like fluorescent structure has been bleached. The macro helps select the regions of interest (ROI Bleach, ROI Background and ROI Cell) in a format compatible with the on-line FRAP analysis application "easy FRAP" (https://easyfrap.vmnet.upatras.gr/), and automatically measures the intensity fluorescence of all the selected ROIs along the time stack, delivering the corresponding result´s table.

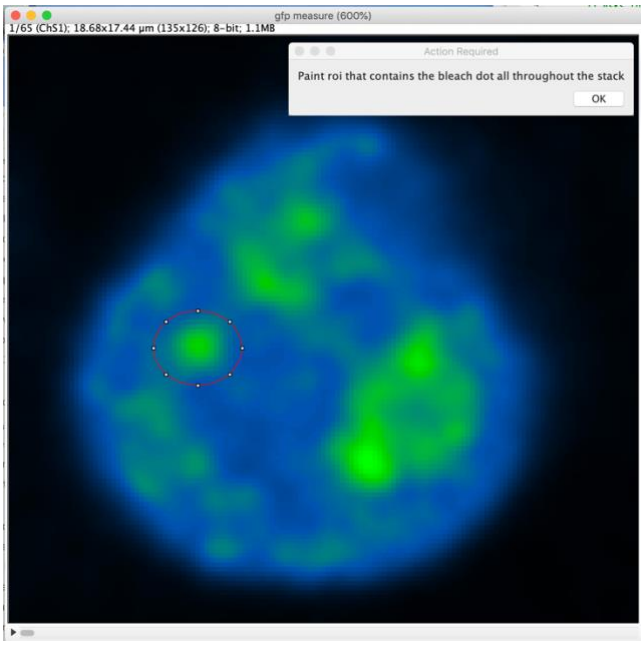


Macro description and *how to use*:

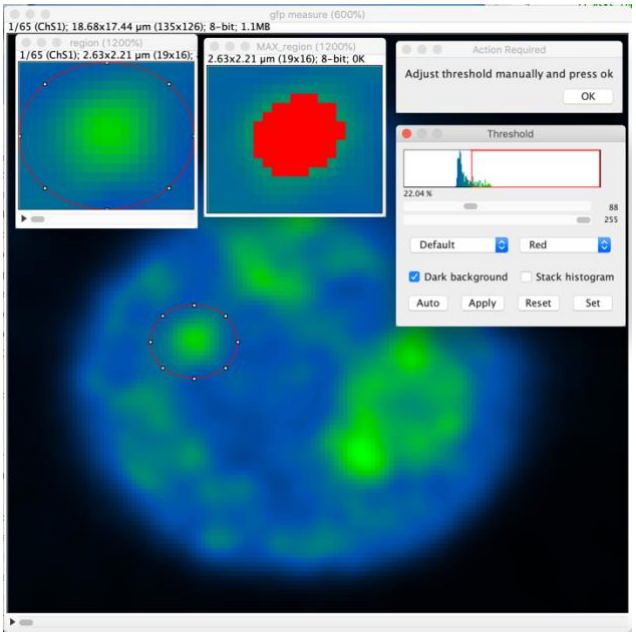
1. *Open* the image file (one channel fluorescence time lapse where the bleaching has been performed). You can use the example image provided (cell_frap.lsm).
2. *Drag and drop* the macro to the Fiji menu bar and hit *Run*.
3. The macro will prompt the user to paint a ROI to select the cell of interest. Select the region using the "rectangular selection" at the Fiji tools bar and *Hit OK* on the message "*paint region to isolate cell"*. Upon hitting *OK*, the ROI will be automatically cropped and registered to minimized cell movements and drifts.



4. Next, the macro will ask the user to paint a ROI (red circle on the image) around the bleaching target (signal spot). Paint the region using the "oval selection" at the Fiji tools bar and *hit OK* on the message *"Paint roi that contains the bleach dot all throughout the stack"*.



5. Upon hitting OK, the bleach ROI will be automatically duplicated in a new stack containing the original signal (left inset) and all the time-frames; a time projection will be performed on this stack and the "Default" threshold method will be applied on it, so as to select the region that better represents the signal along the stack (middle inset, red pixels conform the ROI that will be measured). The user is asked to fine tune the thresholding on the show up menu to make sure no irrelevant signal will be measured along the time stack. Once adjusted, *hit OK* on the message *"Adjust threshold and press OK"*; the macro will automatically measure the "bleach ROI" intensity along the time stack containing the original signal (left inset).



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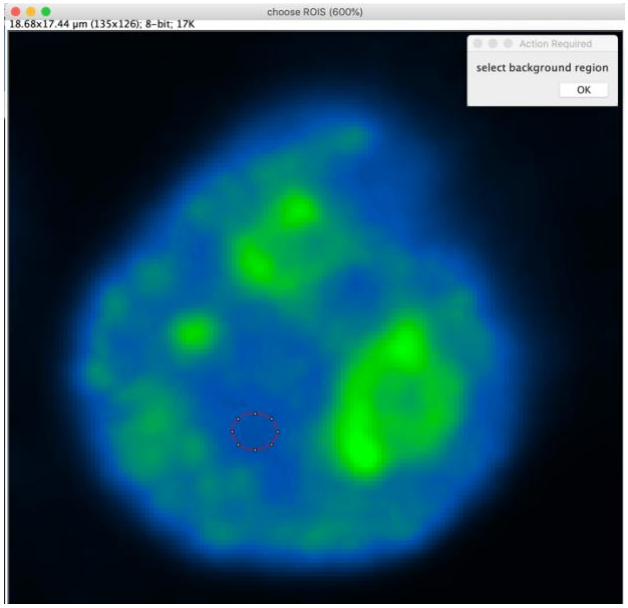
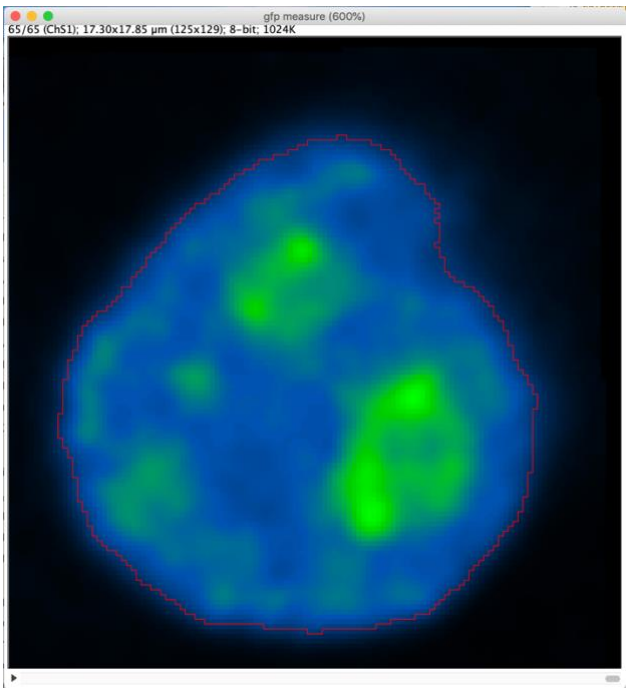
6. The same strategy will be used to select the background region, but now applied to the whole image. The macro will create a time projection of the whole image and will ask the user to select the background ROI (red circle) using the "oval selection" at the Fiji tolls bar. Upon hitting *OK* the macro will automatically measure the background ROI along the original time stack.



7. Last the macro will automatically segment the whole cell area using a local contrast strategy that may serve for a wide range of labellings. This step may need further programming for each particular application. If automatic segmentation is not viable, a manual step can be used as in the previous steps (not included in the present macro). Once segmented, the macro will measure the intensity of the "cell ROI" (red selection) along the time stack.



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8. All measurements are stored in arrays that are finally organized in a results table shown at the end of the process. This table is given the name of the original image. The data is organized in three columns as required for analysis in the on-line application easyFRAP. Save the results preferably as an excel file.

spot_frap

ROIbleach	ROIcell	ROIbackground
95.6267	69.903	35.6993
96.72	69.5661	35.5933
93.6667	69.1369	35.3637
50.0267	66.8137	34.3247
59.9333	66.9208	34.3629
66.1867	67.6612	34.7188
69.5067	67.787	34.7731
71.0267	68.2794	34.9317
73.7333	68.1916	34.928
78.6	68.5212	35.0679
76.0267	68.4513	35.0415
78.4	68.6072	35.0702
76.2133	68.6953	35.1262
77.1733	68.2538	34.952
80.16	69.0358	35.3004
80.0933	68.6327	35.1108
83.6933	68.771	35.1702
79.3467	69.2515	35.4216
80.4667	68.9226	35.2113
79.6667	69.3726	35.4151
79.1867	69.0067	35.2998
78.28	69.2228	35.3173