FREDIS – FRAP Reaction Diffusion Solver

FREDIS is a Java program written for the analysis of Fluorescence Recovery After Photobleaching experiments. FREDIS features the calculation of the corrected recovery curve as well as the fitting with three different models.

Installation notes

1. In order to use all the implemented fit models, the program GNU Octave has to be installed. If this is not the case, you can download GNU Octave from the following website: http://octave.sourceforge.net

   GNU Octave is freely available under the terms of the GNU General Public License (GPL).

   If you use Mac, please install Octave in the "/Applications" folder; if you use windows, please use "C:\Program files\Octave".
2. Copy the folder "fredis" into the following subfolder (of the Octave installation directory): "Contents/Resources/share/octave/<version>/m". <version> denotes the Octave version you are using, e.g. "3.2.3". If you are using Mac, you have to control-click (i.e. right-click) on "/Applications/Octave.app" and choose "Show Package Contents" in order to access the respective subfolders.
3. Copy the file "FRAP_Analyzer.jar" into an arbitrary folder.
4. Copy the folder "lib" in the same folder.
5. FREDIS can now be launched from the command line with the following syntax:

   java –Xms64m –Xmx512m –jar FRAP_Analyzer.jar

   The values 64m and 512m denote the initial and maximum memory to be allocated by FREDIS. You can adjust the values according to the memory available in your computer and the size of the image stacks you are working with.

It is noted that it is convenient to use a script to start-up FREDIS, in the case of Mac this can be a ".command" file, for Windows a ".bat" file. For further information about such files look their structure up in the Internet or ask your system administrator.

Quick guide to FREDIS

1. Open a file

   To open your FRAP data, choose File -> Open LIF series, Open TIF stack or Open FREDIS data. Choose LIF series (LIF = Leica Image File) if you generated you data using a Leica SP5 microscope, choose TIF stack otherwise (FREDIS reads 8-bit layered TIF files, if you acquired your data differently you have to convert them using for example ImageJ). Choose FREDIS data if you want to re-analyze FREDIS data.
2. Choose Series -> FRAP Analysis in order to start the procedure for calculating the recovery curve. If no metadata could be read-in (this is only the case for LIF series), you have to insert the voxel size and the time-step size manually in the appearing dialog and to confirm with "OK". Then, click Series -> FRAP Analysis again.
3. In Step 1, select the image in your time series for defining where the cell and the background is located. You should take an image where this is clearly visible, e.g. the first image (where nothing has been bleached and most details should be appreciable. If you have acquired multi-color data, you can choose the corresponding color channel.
4. In Step 2, select the area occupied by the cell. This can be done by manually drawing a polygon (one click generates one corner) or by choosing pixels above a certain offset (Auto).
5. In Step 3, select the area occupied by the background as in Step 2. Assure that background pixels are chosen, because the average background value is used for the calculation of the corrected recovery

curve. If no background is chosen, this will result in improper termination of FREDIS.

6. In Step 4, select where the bleach frames are. The buttons (> and >>) allow for skipping 1 or 25 images in your image series, respectively. Images before the first bleach frames are used to calculate the 100% fluorescence intensity value, images after the last bleach frames are used to calculate the recovery curve.
7. In Step 5, choose the bleach region. Initially, there is the possibility to determine potentially bleached pixels by defining a maximum intensity (1st slider) and a minimum intensity difference between last pre- and first post-bleach frame (2nd slider). By clicking on “Manual”, one can insert a ROI (region of interest) radius and select a corresponding circle by clicking in the image. If one clicks on “Auto” again, a ROI with the inserted radius and with a considerable intensity difference is proposed. This ROI does not have to be the correct one, so always check manually before confirming.
8. After having completed all these steps, the recovery curve is calculated and displayed. Upon clicking on “Fit”, one can fit one of the three models “Reaction-dominant”, “Diffusion-dominant” or “Reaction-diffusion” (Sprague et al., Analysis of Binding Reactions by Fluorescence Recovery after Photobleaching. Biophys J. 86:3473-3495 (2004)). With “Save”, the recovery curve and the fit can be saved (three files are saved, a layered “tif” with an image of the cell and the positions of the selected ROIs, one “dat” file containing the values for recovery and fit curve, and one “fit” file with the fit parameters). With the different buttons “FRAP”, “Cell”, “Bleach ROI” and “Background” the corresponding normalized intensity traces can be displayed or not. With “Set tmax”, all values above a certain time are not considered for the fit; this is useful if the cell has suddenly moved after some time, there was a microscope drift or some other disturbance. The “Profile” button opens a window for more sophisticated bleach profile analyses.