

FRAPAnalyser
USER MANUAL

University of Luxembourg, 2009.

Installation

The latest version of *FRAPAnalyser* program can be downloaded from the internet site <http://actinsim.uni.lu/>. Program does not require any specific installation. Unpack the archive on your PC and run the file *FRAPAnalyser.exe*.

System Requirements

Software is developed for the Windows operating system (Windows NT or further versions). Program was tested successfully on PC with the following parameters: processor Intel Pentium (CPU 1400 MHz), RAM 512 MB. Executable file requires just 2 MB of the hard drive space. User also needs some free space on hard drive for storing experimental data (input files) and the results of the analysis (output files).

Description

FRAPAnalyser is independent software package for modelling and fitting FRAP experiments.

FRAPAnalyser provides:

- Data import/export;
- Graphical support;
- Normalization;
- Modelling and data fitting by: one- and two-binding state, diffusion for circular and rectangular bleached spots, reaction-diffusion, and the polymerization models;
- Error analysis.

Main window

The main work window of the *FRAPAnalyser* (see in Figure 1) consists of four blocks marked with red frames: **Command Panel**, **Data Table**, **Datasets**, and **Report**.

Command Panel manages the program activities and is organized as a *Windows* bar combining the items into the menus: **Data**, **Plots**, **Normalization**, **Analysis**, **Help**.

Menu **Data** provides access to data manipulation operations:

- Item **[Load files]** allows loading data from the text file(s). Pressing this item launches the window for selecting **Input file(s)**. For the format of the loaded file(s) see *Appendix A*.
- Item **[Set ROIs]** opens the submenu for proper associating the table columns with measured curves (FRAP, reference/whole cell and background intensities). If the column for background is not determined then the background intensity is set automatically to 0.

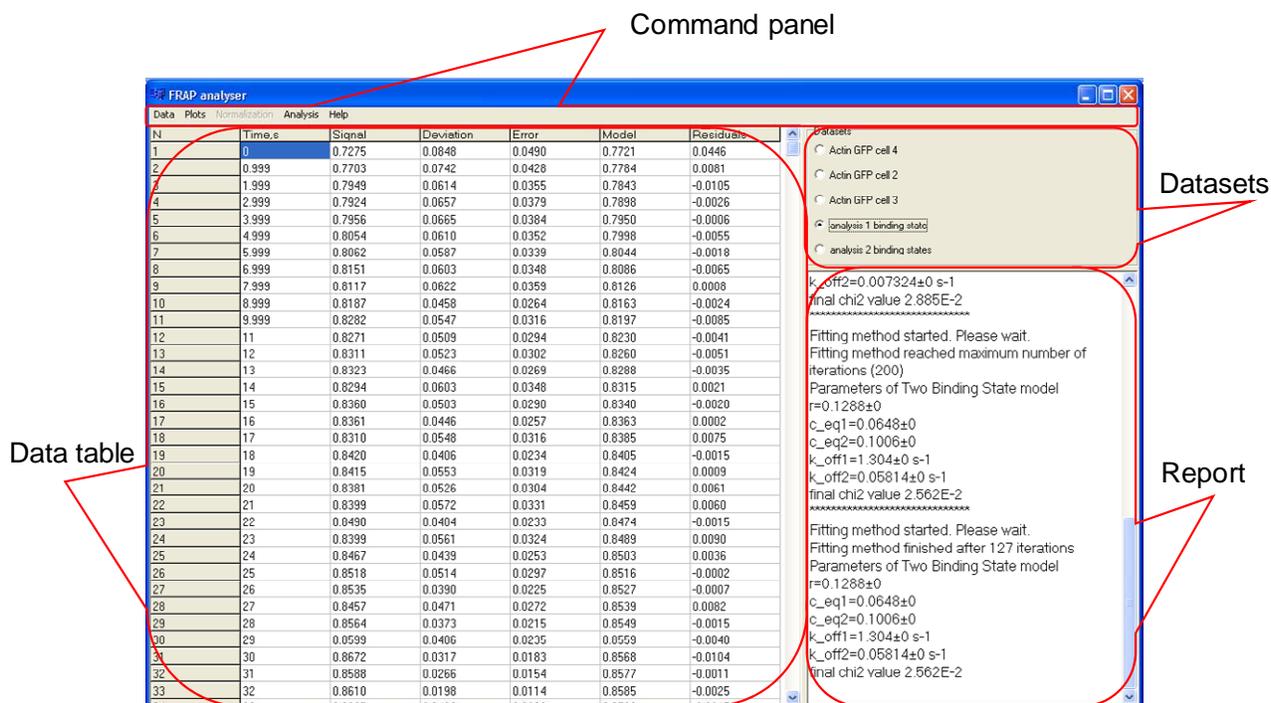


Figure 1. Main window of the FRAPAnalyser.

- Item **[Set bleach channel]** opens the submenu to input the row index of the first time point after the bleach.
- Item **[Delete Data]** erases the workspace including the data table and the results of the analysis.
- Pressing the item **[Save data]** stores current dataset to a text (*.txt) file. Standard *Windows* output dialog **Save current dataset** helps to specify name and location of the output file.

Item **[Show Plots]** of the menu **Plots** loads the subwindow depicting the FRAP data.

Menu **Normalization** runs the normalization procedures according different algorithms [1]:

- **[Double normalization]** – the procedure accounts for the background fluorescence and acquisition photobleaching.
 - **[Single normalization]** – the procedure accounts only for the background fluorescence.
 - **[Double normalization + Full Scale]** – the double normalization procedure with scaling the FRAP data between 0 to 1.
 - **[Single normalization + Full Scale]** – the single normalization procedure with scaling the FRAP data between 0 to 1.
 - **[Data are already normalized]** – is used in case the loaded data are already normalized.
- See *Appendix C* for technical description the normalization procedures.

Menu **Analysis** activates the submenus of data modeling, fitting and error analysis.

- Item [**New Analysis**] opens an accessory window for creating of the new analysis dataset.

See *Appendix B* to find out the structure of a dataset for the current version of the program.

- Pressing the item [**Delete Analysis**] deletes the current analysis dataset.
- Item **Models** launches a submenu for modeling the FRAP data by:
 - [**One binding state**] the one-binding state model [2];
 - [**Two binding states**] the two-binding state model [2];
 - [**Diffusion circular spot**] – the diffusion model for a circular bleached spot [3];
 - [**Diffusion rectangular spot**] the diffusion model for a rectangular bleached spot [4];
 - [**Binding + diffusion**] – the full reaction-diffusion model [2].
 - [**Polymerization**] – the model of actin filaments turnover {the paper is submitted}.

After a corresponding subitem is chosen the subwindow **Model parameters** is loaded to input parameters of the selected model. For mathematical description of the FRAP models see *Appendix D*.

- Item **Analysis** launches a multi-step dialog for data fitting. Analysis Step1: input (1) the fitting model [from the abovementioned list of the FRAP models], (2) the optimization algorithm (the Levenberg-Marquardt method or the Nelder and Mead method), (3) number of iterations for a fitting method, (4) the first time channel and (5) the last time channel. Analysis Step2: input the initial values for fitting model parameter. Check-out the fitting parameter(s) if it is fixed.

Item [**About**] of the menu **Help** displays information about current program version and contact details of the developers. This information could be used for any citing the software and for announcement for the updates.

Data table visualizes the current dataset in the tabular form. Upper row contains the column names. The first column counts the indexes of time channels. The second column represents the scanned time moments. Other columns are defined for intensity arrays.

Datasets let make a quick overview of the loaded or processed datasets via the **Data table**.

Report times the performed operations as “successful” or “failed”, the attained value of the correspondence criterion χ^2 , and the estimated parameters.

Plots window

Plots (Fig. 2) is used to plot the FRAP recoveries and the weighted residuals (for judging the fit quality). It contains **Command Panel**, **Plot of intensities**, **Plot of residuals** and the menu **Available plots**.

The button **[Save]** of the **Command Panel** stores the current **Plot of intensities** into the image file. The standard output Windows dialog helps to specify name, type and location of the output file. The Plot can be stored as the bitmap file (*.bmp) or the Windows MetaFile (*.wmf).

Plot of intensities displays the experimental and/or fitted intensity curves as chosen in the menu **Available plots**:

- Non-normalized **FRAP** curves for the loaded experimental datasets;
- **Reference** curves for the loaded experimental datasets;
- **Background** curves for the loaded experimental datasets;
- **Normalized** curves for the loaded experimental datasets;
- Analysis datasets: averaged normalized intensity (red boxes), upper and lower limits for experimental data as calculated from estimated standard errors (red and blue lines), calculated (modeled or fitted) FRAP curve (green line). The additional subplot **Plot of residuals** appears depicting the graph of weighted residuals. The random distribution of the weighted residuals along the zero line indicates the good fit.

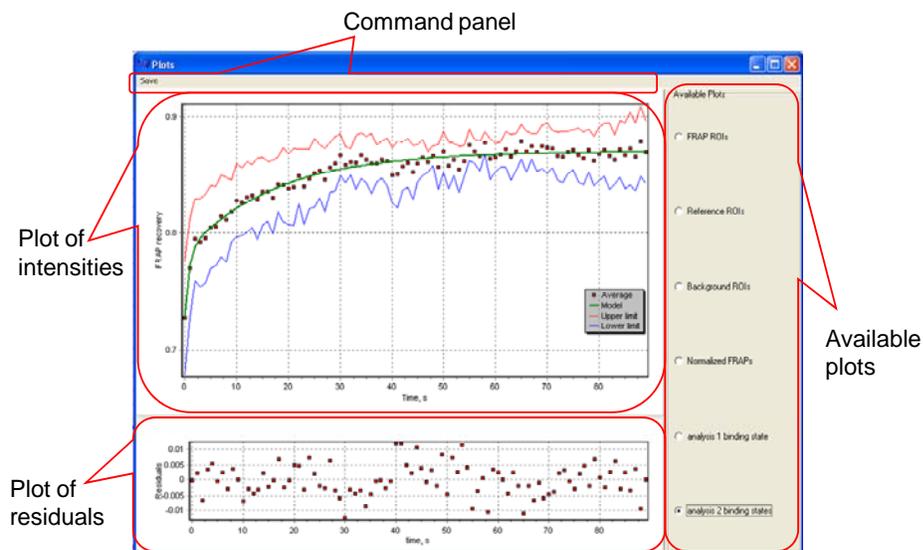


Figure 2. **Plots** window.

Parameters window

Parameters window (Figure 3) leads to the determination of different parameters and options. Its appearance is determined by performed activity and/or chosen model. Parameters could be determined as numerical values or as options from provided lists.

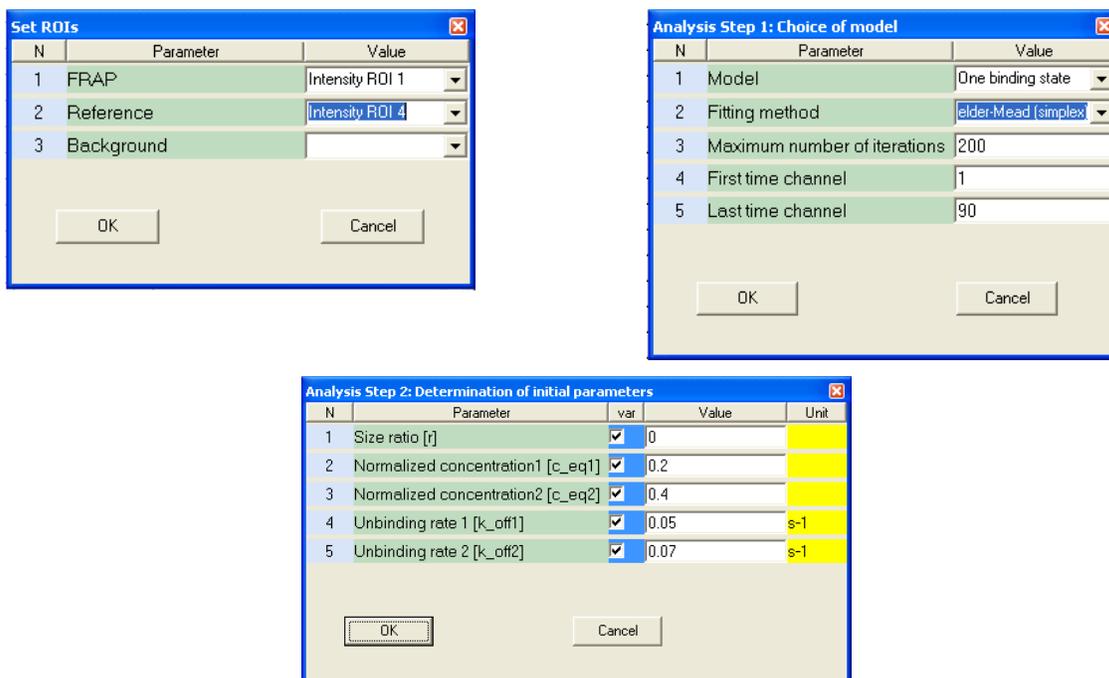


Figure 3. **Parameters** window (different occurrences).

Example

Here we give an example of analysis of FRAP data with the *FRAPAnalyser*. This example includes five text files obtained from the FRAP experiment on actin-GFP in vero cells. The corresponding achieve is attached to the software. The first column in each file is the time. Other two columns collect intensities for the FRAP and the reference.

1. Open the files with **the Windows Notepad** and study the file structure.
 2. Launch the *FRAPAnalyser*. The main window will appear.
 3. Press **Data->Load Files**. **Input Files** dialog will appear.
 4. Find and select five test files. Press **OK** to upload the data. **Data table** and **Datasets** regions will appear in the main window. The accessory window **SetROIs** will be loaded.
 5. Choose the following options:
 - a. **FRAP** =Intensity ROI 1;
 - b. **Reference** = Intensity ROI 4;
 - c. Leave **Background** field empty;
- And press **OK**. The window **Set bleach channel** will be loaded.
6. Set **Bleach time channel** =11 and press OK.
 7. Control the datasets of loaded files. Be sure that the columns contain the proper data.
 8. Press **Normalization->Double normalization** to apply double normalization method. Pay attention that numbers in column **Normalized** of each dataset have been updated.

9. Press **Analysis->New Analysis**. In the opened window leave the name of new dataset as proposed and press **OK**. Notify that new dataset was added and study its contents.
10. Press **Analysis->Fitting Panel** to start the data fitting. Select the following options:
 - a. **Model** = One binding state
 - b. **Fitting method** = Levenberg-Marquardt (gradient);
 - c. **Maximum number of iterations** = 200;
 - d. **First time channel** = 1;
 - e. **Last time channel** = 90;
11. Press **OK** and go to the second step. Control the list of fitted parameters corresponds to one binding state model. Put the mouse arrow in the editing zone of a parameter – then the parameter range is highlighted. Keep the default initial values for the model parameters. Press **OK** to run fitting procedure.
12. The final values of the correspondence criterion and model parameters will appear in the **Report**. Contents of two columns Model and Residuals in the Analysis 1 dataset will be updated.
13. Press **Plots->Show plots**. In the appeared **Plots** window check all available options one after another to see experimental and best fitted FRAP curves. Analyze the **Plot of residuals**.
14. Perform the analysis using two binding state model.
 - a. Create new Analysis dataset;
 - b. Perform the same steps data fitting however with two binding states model;
 - c. Compare the results of data fittings by one- and two-binding state model.
 - d. Visualize the results of data analysis by the menu **Plots**.

Appendixes

Appendix A. Formats of input data files

Experimental data are stored in format of the tab- delimited text files (*.txt). The first line may contain the column names, the rest of the file are filled in by numerical values. The first column represents the time points, other columns are the intensities (FRAP, reference/whole cell, background). Organization of the columns in the loaded files must be absolutely similar. Number of uploaded files is not limited.

Appendix B. Datasets

Dataset is a structure for storage of simulated and/or experimental time series for intensities. Each dataset is stored as a table and visualized via the **Data table**. When the input files are loaded, new dataset is created for each file. Name of the dataset is the same as the name of the file from that it was generated. Number of columns in a dataset is equal to the number of columns in the original file with one additional column reserved for the normalized intensity.

For reasons of modelling or fitting a new analysis dataset has to be created ([**New analysis**] item of the menu **Analysis**). The analysis dataset contains the following columns:

- **Time** – calculated as an average of the column **Time** from the experimental datasets.
- Averaged **Signal** intensity – calculated automatically as average of normalized intensities from all experimental datasets.
- Standard **Deviations** of the FRAP intensity – estimated from the experimental datasets when number of loaded files >1.
- Standard **Errors** of the FRAP intensity – estimated from the experimental datasets when number of loaded files >1.
- Simulated FRAP intensity (**Model**) - recalculated each time when modelling or fitting is performed.
- **Residuals** – inconsistencies between experimental and simulated data, recalculated simultaneously with **Model** column.

FRAPAnalyser allows creating a number of the analysis datasets for the same experimental data. This feature can be used to test and to compare the fitting quality by several analysis procedures (made by varying different normalization, modelling, fitting algorithms).

Appendix C. Normalization methods [1]

Double normalization

$$I_{norm}(t) = \frac{I_{ref_pre}}{I_{ref}(t) - I_{back}(t)} \cdot \frac{I_{frap}(t) - I_{back}(t)}{I_{frap_pre}}$$

where

$I_{norm}(t)$ - normalized intensity;

$I_{frap}(t)$ - measured average intensity inside the bleached spot;

$I_{ref}(t)$ - measured average reference or whole studied compartment (cell, nucleus, etc.) intensity;

$I_{back}(t)$ - measured average background intensity outside the cell;

Subscript *_pre* means the averaging of intensity in the corresponding ROI before bleach moment after subtraction of background intensity;

Single normalization

$$I_{norm}(t) = \frac{I_{frap}(t) - I_{back}(t)}{I_{frap_pre}}$$

The notations are the same as above.

Full scale calibration

$$I_{norm1}(t) = \frac{I_{norm}(t) - I_{norm}(t_{bleach})}{I_{norm_pre} - I_{norm}(t_{bleach})}$$

Here t_{bleach} is the bleach time.

Appendix D. FRAP models – intensity functions and parameters description

One binding state model [2]

$$FRAP(t) = (1 - r) \left(1 - c_{eq} e^{-k_{off}(t-t_{bleach})} \right)$$

Parameters are: unbinding rate constant (k_{off}), fraction of bound molecules (c_{eq}). An additional parameter (r) is added to account for the effect of non-complete recovery.

Two binding states model [2]

$$FRAP(t) = (1 - r) \left(1 - c_{eq1} e^{-k_{off1}(t-t_{bleach})} - c_{eq2} e^{-k_{off2}(t-t_{bleach})} \right)$$

Parameters are: the unbinding rate constants for two binding states (k_{off1}, k_{off2}), fractions of bound molecules (c_{eq1}, c_{eq2}). An additional parameter (r) is added to account for the effect of non-complete recovery.

Diffusion model for circular spot [3]

$$FRAP(t) = a_0 + a_1 \cdot e^{-\frac{\tau}{2(t-t_{bleach})}} \cdot \left(I_0 \left(\frac{\tau}{2(t-t_{bleach})} \right) + I_1 \left(\frac{\tau}{2(t-t_{bleach})} \right) \right) \quad \tau = \frac{w^2}{D}$$

Here $I_0(x)$, $I_1(x)$ – modified Bessel functions.

Parameters are: the radius of bleach spot (w) and the diffusion coefficient (D). Two normalizing coefficients (a_0, a_1) are introduced to account for the non-zero intensity at bleach moment and incomplete recovery.

Diffusion model rectangular spot [4]

$$FRAP(t) = a_0 + a_1 \left(1 - \sqrt{\frac{w^2}{w^2 + 4\pi D(t-t_{bleach})}} \right)$$

Parameters are: the width of bleach spot (w) and the diffusion coefficient (D). Two normalizing coefficients (a_0, a_1) are introduced to account for the non-zero intensity at bleach moment and incomplete recovery.

Full reaction-diffusion model [2]

$$FRAP(t) = (1 - r) \text{invlap}(\overline{FRAP}(p))$$

Here $\text{invlap}()$ – function representing inverse Laplace transform. Function $\tilde{I}(p)$ is defined by Eq. (6) in [2].

Parameters are: the pseudo binding rate constant (k_{ons}), the unbinding rate constant (k_{off}), the diffusion coefficient (D), the radius of a bleached spot (w). An additional parameter (r) is added to account for the effect of non-complete recovery.

Polymerization model

Equations are introduced in: Halavatyi et.al. “A Mathematical Model of Actin Filament Turnover for Fitting FRAP Data” [submitted to the Biophysical Chemistry].

Parameters are: the association and dissociation rate constants for barbed and pointed ends ($k_{(+)b}$, $k_{(-)b}$, $k_{(-)p}$), the average length of filaments in actin subunits (L), F-actin concentration (c_f).

References

- [1] K. Miura, Analysis of FRAP Curves, <http://www.embl.org/cmci/downloads/FRAPmanual.htm> (2007).
- [2] B.L. Sprague, R.L. Pego, D.A. Stavreva, J.G. McNally, Analysis of binding reactions by fluorescence recovery after photobleaching, *Biophys J* 86 (2004) 3473-3495.
- [3] D.M. Soumpasis, Theoretical analysis of fluorescence photobleaching recovery experiments, *Biophys J* 41 (1983) 95-97.
- [4] J. Ellenberg, E.D. Siggia, J.E. Moreira, C.L. Smith, J.F. Presley, H.J. Worman, J. Lippincott-Schwartz, Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis, *J Cell Biol* 138 (1997) 1193-1206.