easyFRAP: an interactive, easy-to-use tool for qualitative and quantitative analysis of FRAP data

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ABSTRACT
Summary: We present easyFRAP, a versatile tool that assists quantitative and qualitative analysis of FRAP data. The user can handle simultaneously large datasets of raw data, visualize fluorescence recovery curves, exclude low quality data, perform data normalization, extract quantitative parameters, perform batch analysis and save the resulting data and figures for further use. Our tool is implemented as a single-screen Graphical User Interface and is highly interactive, as it permits parameterization and visual data quality assessment at various points during the analysis.
Availability: easyFRAP is free software, available under the General Public License (GPL). Executable and source files, supplementary material and sample datasets can be downloaded at: ccl.med.upatras.gr/easyfrap.html
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1 INTRODUCTION
Functional live cell imaging techniques such as Fluorescence Recovery after Photobleaching (FRAP) exploit the properties of fluorescent proteins coupled with modern microscopy systems and are increasingly used to visualize, track and quantify molecules in living cells. Like all photobleaching methods, FRAP involves the irreversible bleaching of emitted light from molecules tagged with a fluorescent protein. During a typical FRAP experiment, a defined Region of Interest (ROI) is bleached by a short laser pulse and the fluorescence recovery in the ROI is then monitored by time-lapse microscopy. Analysis of FRAP data provides information on the kinetic behavior of the studied molecules, such as diffusion and quantitative analysis of FRAP data. The user can easily exclude low quality data, extract quantitative information and save the resulting data and figures for further analysis. The FRAP analysis workflow is organized as follows (for a full description including quick start guide, manual and definitions see Supplementary Material):
(1) The user selects a dataset for uploading. Input data must contain intensity measurements from the bleached area (ROI1), the total fluorescence area (ROI2) and a background area (ROI3) and the corresponding time-points. EasyFRAP works with .csv, .txt and .xls file formats.
(2) Raw intensities in ROI1, ROI2 and ROI3 are plotted for visual examination and data quality assessment.
(3) Using the list box, a number of low quality samples can be excluded (and restored) from the analysis.
(4) The user is asked to insert the necessary parameters (number of prebleach, bleach and postbleach images).

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Optionally, a number of initial pre-bleach values can be deleted (and restored), as they exhibit loss of fluorescence due to non-intentional bleaching. The bleaching depth and the gap ratio values are then computed (see Supplementary material).

The normalized recovery curves are computed and visualized, according to the commonly used formulas of double (Phair, 2003) or full scale normalization (Ellenberg, 1997).

The user can select a sample of interest and perform curve fitting using a single or double term exponential equation. The t_{half} (half maximal recovery time) and mobile fraction values (individual and mean values) are computed. The data, fitted curve and the residuals are visualized in order to evaluate the fit and goodness-of-fit statistics (R-square) are also provided (see Supplementary material).

The top menu allows the user to save all resulting data (raw curves, normalized curves and curve fitting results) in separate files at any point in the analysis for further use. It also includes an extra feature for FRAP batch analysis (multiple experiment analysis). These features are incorporated in a simple and intuitive GUI, allowing the user to analyze a complete dataset in just a matter of minutes.

3 TEST CASE

EasyFRAP was tested using data from FRAP experiments on Cdt1GFP and on a nuclear localized construct of GFP (GFPnls), on a Leica SP5 confocal microscope (Fig.1B). The t_{half} and the mobile fraction values are computed for Cdt1GFP and GFPnls data (Table 1).

Based on the calculated t_{half} values we conclude that Cdt1GFP exhibits significantly slower mobility than GFPnls, but highly dynamic behavior, consistent with prior analyses (Xouri, 2007a) (Xouri, 2007b)(Roukos, 2011).

Table 1. Mean t_{half} and mean mobile fraction for Cdt1GFP and GFPnls.

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<thead>
<tr>
<th></th>
<th>t_{half} (s)</th>
<th>mobile fraction</th>
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<tbody>
<tr>
<td>Cdt1GFP</td>
<td>0.55 ± 0.09</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>GFPnls</td>
<td>0.27 ± 0.08</td>
<td>0.99 ± 0.01</td>
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REFERENCES


