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

Maria Anna Rapsomaniki[1,*], Panagiotis Kotsantis[1], Ioanna-Eleni Symeonidou[1], Nickolaos-Nikiforos Giakoumakis[1], Stavros Taraviras[2] and Zoi Lygerou[1,*]

[1]Laboratory of Biology and [2]Laboratory of Physiology, School of Medicine, University of Patras, 26505 Rio, Patras, Greece

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ABSTRACT

Summary: We present easyFRAP, a versatile tool that assists quantitative and qualitative analysis of fluorescence recovery after photobleaching (FRAP) data. The user can handle simultaneously large data sets 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 (GUI) 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 data sets can be downloaded at: ccl.med.upatras.gr/easyfrap.html.

Contact: lygerou@med.upatras.gr; rapsoman@upatras.gr

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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 macromolecular interactions (Sprague and McNally, 2005).

A typical FRAP data set consists of a number of noisy images acquired before, during and after photobleaching. These raw experimental data must be carefully processed to permit biologically significant conclusions to be drawn. FRAP data analysis consists of multiple steps. Following quantification of fluorescence intensity in the various ROIs, intensity values are processed to remove noise, systematic bias and artifacts (referred to as normalization), raw and normalized single-cell curves are visually inspected for quality assessment and mean values from multiple cells are plotted for qualitative analysis. Curve fitting and parameter extraction permits quantitative analysis (Reits and Neefjes, 2001). This process is usually performed manually using common spreadsheet software packages and is time-consuming and prone to errors. The need for automated analysis has led to the development of new software, such as FrapCalc (http://cmci.embl.de/downloads/frap_analysis), VirtualFRAP (Cowan, 2009) and FRAPAnalyser (http://actinsim.uni.lu/eng/Downloads/FRAPAnalyser). However, as functional live cell imaging becomes wide-spread in biology laboratories, there is a lack of fast, interactive and easy-to-use tools that do not require access to commercially licensed software and are not computationally intensive.

2 PROGRAM OVERVIEW

In this note, we present easyFRAP, a stand-alone application that facilitates qualitative and quantitative analysis of FRAP data. EasyFRAP allows the user to handle large data sets of raw data under various experimental conditions and thus facilitates differential analysis and comparison. The main functions of easyFRAP include data visualization, normalization of the raw recovery curves and curve fitting and are accessible through a single screen GUI (Fig. 1A). 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 pre-bleach, bleach and post-bleach images).
Optionally, a number of initial pre-bleach values can be deleted (and restored), as they exhibit loss of fluorescence

*To whom correspondence should be addressed.

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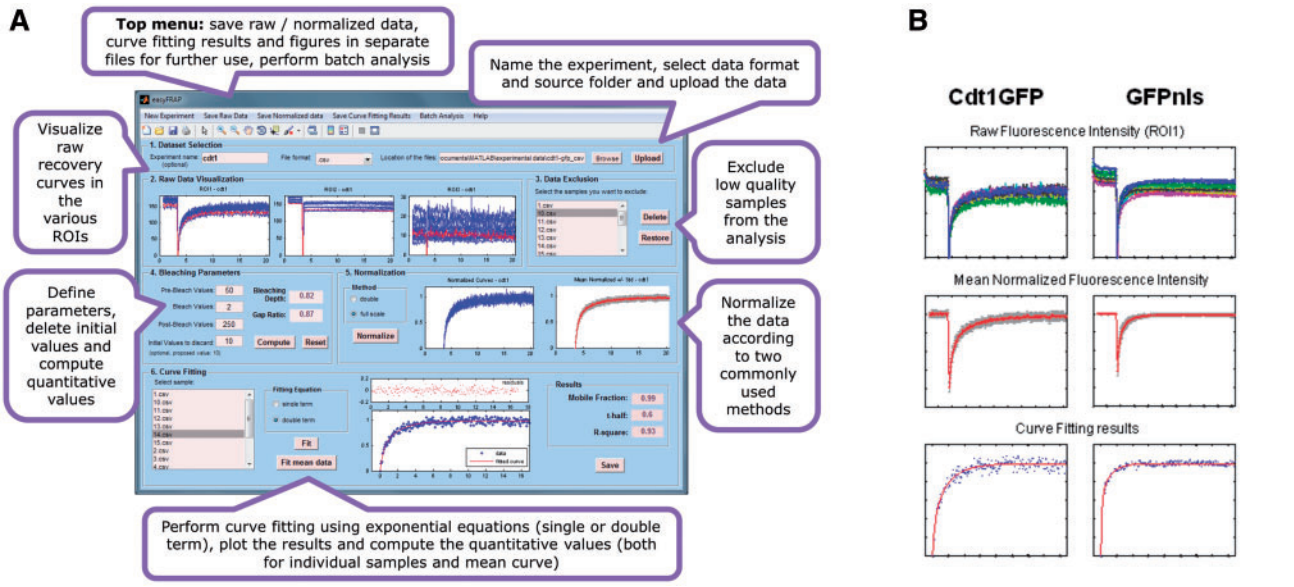

Fig. 1. (**A**) easyFRAP's main Graphical User Interface. (**B**) Exported figures for Cdt1GFP (*left*) and GFPnls (*right*) data

due to non-intentional bleaching. The bleaching depth and the gap ratio values are then computed (see Supplementary Material).

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

(6) 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^2) 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 data set 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 (Roukos, 2011; Xouri, 2007a, b).

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

Protein	t_{half} (s)	Mobile fraction
Cdt1GFP	0.55 ± 0.09	0.98 ± 0.02
GFPnls	0.27 ± 0.08	0.99 ± 0.01

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Conflict of Interest: none declared.

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