

Requirement Analysis - "FRAP Tool" - project MRI Cell Image Analyzer	Montpellier RIO Imaging	
Contact: Volker Bäcker / 04 34 35 95 19 / volker.baecker@mri.cnrs.fr	Version 0.1 / 09/06/11	

Requirement Analysis - "FRAP Tool" - project MRI Cell Image Analyzer

Montpellier RIO Imaging

09/06/11

Responsible: Volker Bäcker

Contact: 04 34 35 95 19 / volker.baecker@mri.cnrs.fr

Requirement Analysis - "FRAP Tool" - project MRI Cell Image Analyzer	Montpellier RIO Imaging	
Contact: Volker Bäcker / 04 34 35 95 19 / volker.baecker@mri.cnrs.fr	Version 0.1 / 09/06/11	


Table of Contents

1 Measure the brightest cube in a region in a series of stacks	5
1.1 Adding cells.....	5
1.2 Removing cells.....	6
1.3 Creating projections.....	6
1.4 Display of stacks or projections.....	6
1.5 Creating and editing selections.....	6
1.6 Running the batch processing and results.....	7
1.7 Delimitations.....	7

Requirement Analysis - "FRAP Tool" - project MRI Cell Image Analyzer	Montpellier RIO Imaging	
Contact: Volker Bäcker / 04 34 35 95 19 / volker.baecker@mri.cnrs.fr	Version 0.1 / 09/06/11	

Illustration Index

figure 1: The user interface of the FRAP tool.....	5
figure 2: The list editor.....	6

Requirement Analysis - "FRAP Tool" - project MRI Cell Image Analyzer	Montpellier RIO Imaging	
Contact: Volker Bäcker / 04 34 35 95 19 / volker.baecker@mri.cnrs.fr	Version 0.1 / 09/06/11	

Changes

Version 0.1	09/06/11	first version
-------------	----------	---------------

1 Measure the brightest cube in a region in a series of stacks

Reference user: Edouard Bertrand, Stephanie Boireau

Issue: none, 102

In FRAP experiments a spot of a fluorescently stained cell is hit by a laser in a way that the fluorescence is destroyed. The fluorescent molecule will then come back and this is the interesting information that has to be measured. The data the user wants to extract is the intensity in the spot in a time series of 3d images, as the fluorescence comes back into the spot.

In order to measure the brightest spot the user indicates a region in which the spot is searched. This minimizes the risk of errors. The region is defined on a stack build from the z-projections of the stacks of each timepoint. As a new requirement multiple selections per cell must be possible. This can be useful when the cell moves because there is a risk that other bright spots might enter the fixed selection while the FRAP-spot might leave it.

The user can add multiple cells, define the selections for all cells and run the processing in batch mode. The results are a spreadsheet file with the coordinates of the brightest cube in the region, the integrated intensity and the mean intensity. Furthermore a control images (stacks) with the brightest cubes that have been used are saved in a folder.

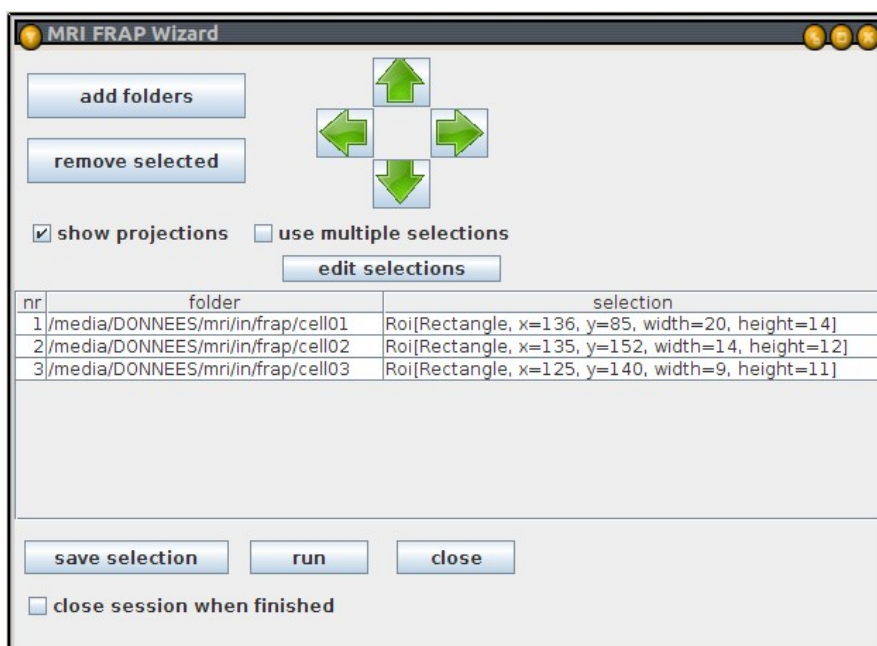



figure 1: The user interface of the FRAP tool.

1.1 Adding cells

The user can add cells by using the *add folders* button. This will open the list editor. The add button of the list editor allows to add one or multiple folders in a time. When the list is setup, it will be transferred to the FRAP-wizard when the list editor is closed.

Requirement Analysis - "FRAP Tool" - project MRI Cell Image Analyzer	Montpellier RIO Imaging	
Contact: Volker Bäcker / 04 34 35 95 19 / volker.baecker@mri.cnrs.fr	Version 0.1 / 09/06/11	

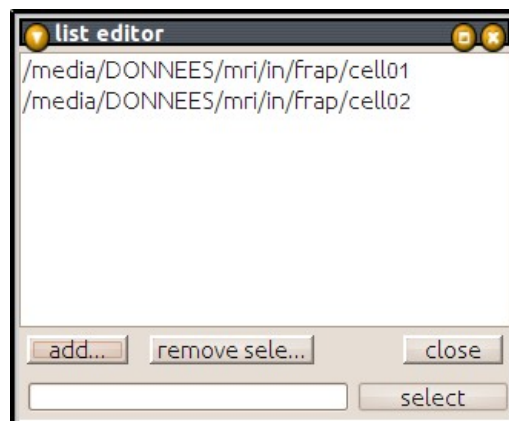


figure 2: The list editor.

Each folder representing a FRAP experiment must contain a series of image-stacks in tif or stk format. The image names must end with the number of the image in the time series. The numerical order is taken into account.

1.2 Removing cells

By clicking on the *remove selected* button, users can remove folders from the list of folder to be process.

1.3 Creating projections

Clicking on an entry of the list opens by default a stack created from projections of the different time-points. If the folder contains a sub-folder named projections, these projection are used otherwise the projections are created (time consuming).

The projections in the folder projections must have the same names as the stacks from which they have been created.

1.4 Display of stacks or projections

By clicking on a list entry, the corresponding image is shown. It is possible to navigate through the time dimension using the image scroll bar.


Vertical arrows allows the navigate between different cells (or folders).

If the checkbox *show projection* is not selected, the separate stasks of the cell are shown instead of the series of projections. In the case, horizontal arrows allow to navigate between the time-points.

1.5 Creating and editing selections

A selection can be created on the image and can be saved by clicking on the *save selection* button.

A checkbox *allow multiple selections*, changes the behaviour of the interface by using the ROI manager to handle multiple selection in time. A button *edit selections* opens the selections in the roi

Requirement Analysis - "FRAP Tool" - project MRI Cell Image Analyzer	Montpellier RIO Imaging	
Contact: Volker Bäcker / 04 34 35 95 19 / volker.baecker@mri.cnrs.fr	Version 0.1 / 09/06/11	

manager. The *save selections* button will save the selections currently in the roi manager and update the display of the selections in the list. The different selections act as key images. A selection is valid until another is encountered. A selection is needed on the first image.

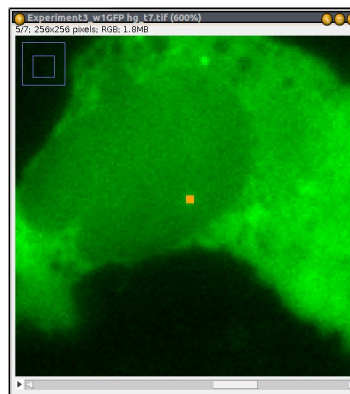
1.6 Running the batch processing and results

When the run button is pressed the cells are processed one after the other. The selection or the list of selections associated with the cell is taken into account. The application executed for each cell is the one that can be opened under *_applications/stacks/measure best cube batch auto_3pix.cia*. The size of the cube that is actually used for measurements is configured in this application.

The result is a spreadsheet file of the following form:

Image	number of objects	Integrated Intensity	average Intensity	x	y	z	radius	folder
Experiment3_w1GFP_hg_t1.TIF	1	357196	2857.5681152344	142	92	3		2 D:\Utilisateurs\Ute Schmidt\101122 50.11 GFP-MS2\cell-3\
Experiment3_w1GFP_hg_t2.TIF	1	352079	2816.6320800781	142	91	4		2 D:\Utilisateurs\Ute Schmidt\101122 50.11 GFP-MS2\cell-3\

A control stack-image for each time-point stack, containing the cube used in red, is saved into the folder cubes:



1.7 Delimitations

- The tool only exports the intensity data. It does not fit a curve or extract parameters from it