The Correlation-Based Method for the Movement Compensation in the Analysis of the Results of FRAP Experiments

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Abstract: This paper presents a computational algorithm for the detection and compensation for intracellular movement in the FRAP experiments with focal adhesions in living cells. The developed approach is based on the calculation of correlation coefficient. It was validated on the series of the experimental datasets and shows the successful results in the comparison with other widely-established methods.

***Keywords*:** correlation, FRAP, cell movement, image processing, actin, focal adhesion.

1. INTRODUCTION

The cytoskeleton plays a major role in the cell motility. Actin forms part of the cytoskeleton in cells and is involved in the maintenance of cell shape as well as cell dynamics. The active form of actin is a helical polymer, called F-actin (F for filamentous), assembled from the monomeric subunits of G-actin (G for globular). The actin filaments are in the continuous process called treadmilling, when the G-actin molecules are added to the plus end of the filament and simultaneously they are removed from the minus end, without changing the filament length. Many proteins take part in the treadmilling, some of them are well-established, for example, Arp2/3 complex, which initiates new branches on the actin filaments, or capping proteins that block the growth of the branch of the actin filaments[1]. However, there are still a lot of proteins, such as testin, which functions in actin cytoskeleton are not well-studied [2].

The dynamic characteristac of the actin polimerization are studied by the Fluorescence Recovery After Photobleaching (FRAP) technique. Typically FRAP is used to examine the dynamics of fluorescently-labelled macromolecules in the living cells. In the FRAP experiment a small region of the fluorescent specimen is bleached rapidly (Fig 1A,B). As the photochemical bleaching of fluorophores is essentially irrevisible, the changes of fluorescent intensity in the bleached and unbleached regions are due to the exchange of bleached and fluorescent molecules between those regions(Fig 1C). Fluorescence recovery is quantified as average fluorescence intensity in the bleached region at each acquisition time point. The fitting of the recovery curve by the solution of the reaction-diffusion equation allows to estimate the parameters the intracellular processes, which change the mobility of fluorescently-labelled molecules (diffusion coefficients, association/dissociation rate constants, etc). However the accuracy of this analysis depends on the quality of quantification of recovery curves, which is very sensitive to the data artefacts due to the cell movement or a contamination [3].

A method based on modelling the received data with a parametric matrix model have been developed recently as an attempt to compensate for the image movement [4]. However this method is tackled by the definite assumption for the single particular experiment. On the other hand there are the specific approaches to consider the cell movement [5,6]. Nevertheless these methods are not well adjusted for to the inhomogeneous fluorescent distribution and the studies of the important cell structures, such as focal adhesions.

The aim of our work is the development of an automated procedure for the tracking of the movement of focal adhesions (the part of the cytoskeleton connecting to the extracellular matrix), suitable for the analysis of FRAP experiments. The proposed method is based on the alignment of the images by finding maximum of the correlation coefficient. The developed method was implemented as the plugin for ImageJ [7]. We tested our method on the collected experimental data and compared it with one of the popular tracking methods [5].

2. EXPERIMENTAL METHODS

The monkey kidney epithelial Vero cells were transfected with the actin-GFP by the electroporation method. FRAP experiments with these cells were performed with the laser scanning confocal microscope (LSM-510 Meta, Carl Zeiss, Jena, Germany) using a 63x/1.4NA oil-immersion objective.

In each experiment 20 prebleach images and 400 postbleach images were acquired with argon laser (488 nm laser line) and time interval of 1 s between scans. The size of every image was 512×128 pixels with 0.14 μm per pixel. The circular region for bleaching with the diameter (D_r) of 19 pixels was selected on focal adhesion before start of acquisition. The bleaching was performed in 0.11 s with 3 bleaching iterations using the same laser line at 100% power. The result of every experiment was stored as a stack of images in multi-tiff format.

3. COMPUTATIONAL ALGORITHM

The developed method is based on the calculation of the correlation coefficient (r), which characterises the linear dependence between two signals (Eq.1).

$$r = \frac{\sum_{i=1}^{N}(A_i - \overline{A})(B_i - \overline{B})}{\sqrt{\sum_{i=1}^{N}(A_i - \overline{A})^2}\sqrt{\sum_{i=1}^{N}(B_i - \overline{B})^2}}, \qquad (1)$$

where $A=\{A_1,...A_N\}, B=\{B_1,...B_N\}$ are studied signals, $\overline{A}, \overline{B}$ are their mean values.

Firstly the preparation of the reference image is described. Secondly the comparative procedure is presented.

Alternative for the previous paragraph: The algorithm consists of the several steps: i) preparation of the reference image for last prebleach time point and ii) alignment of images for other prime points to the prepared reference image. These steps are described in detail further.

Reference image preparation. Because different parts of the cell can move differently during the FRAP acquisition and the region of the interest (*ROI*) is smaller than 1 percent of the whole image area (*I*), therefore image comparison was not considered for entire slides in the stacks (Fig.1). The shifting was defined only for the small region (*RI*), which contains the *ROI* (Fig. 2A). The linear size of the region (D_r) is considered as the doubled size of the *ROI* (D_{roi}), which means that 25 percent of the processing pixels belongs to the *ROI*. The center of the *ROI* coincides with the center of the *RI*.
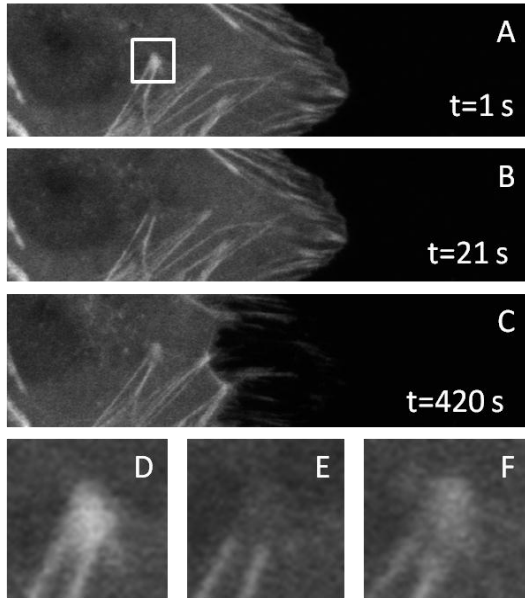

Fig. 1 –The FRAP experiment. A) The first image in the time series. B) The first image after bleaching (the 21st time point) C) Last acquired image in time series D-F) The enlargement of the region (*RI*) containing the *ROI* outlined in A with white square.

In the FRAP experiment the intensity recovery in the *ROI* with time occurs because of the diffusion labelled molecules, which leads to non-linear increase among the pixels in the *ROI*. In order to prevent the reduction of the correlation coefficient because of intensity changes at different time points, the intensities of the pixels belonging to the *ROI* are not taken into account by putting their intensities to zero.

The first image acquired after bleaching (S_{21}) was used to create the references image (*RI*). Hence the pixels belonging the *RI*, can be described by Eq.2

$$\text{If}(\,|\,(x - D_{roi})\,|\,>D_{roi}\,) \& (\,|\,(y - D_{roi})\,|\,>D_{roi}\,)$$

$$RI(x,y) = S_{21}(X_c - D_{roi} + x, Y_c - D_{roi} + y) \qquad (2)$$

$$else RI(x,y) = 0$$

where X_c, Y_c are the coordinates of the centre of the bleached region, $x,y=\{0,..,D_r\}$ – coordinates of the selected pixel. The prepared reference image (*RI*) is shown on the fig. 2B.

As the correlation coefficient can be calculated only for the one-dimensional arrays, the *RI* was transformed to the one-dimensional reference array (*RA*) using eq.3.

$$RA(x * D\_r + y) = RI(x,y)$$

$$x, y = \{0,...,D_r\} \qquad (3)$$

The profile for the *RA* is shown in the Fig. 2C.

Image alignment.

Let's assume that the processing slide (S_k) number is k, where $k=\{0,..., 419\}$ and the *ROI* shifting during an experiment does not exceed the half size of the *ROI*. Then the location of the *ROI* centre on the S_k can belong the area (*AREA*) as described by eq.4.

$$AREA_{kij} = \{X_{ki}, Y_{kj}\}$$

$$X_{ki} = X_c + i, Y_{kj} = Y_c + i \qquad (4)$$

$$i, j = \{-0.5 * D_{roi},...,0.5 * D_{roi}\}$$

For the each position of the centre calculated by the Eq.4 the pixel intensities for shifted image (PI_{kij}) were found by the Eq.5.

$$\text{If}(\,|\,(x - D_{roi})\,|\,>D_{roi}\,) \& (\,|\,(y - D_{roi})\,|\,>D_{roi}\,)$$

$$PI(x,y) = S_k (X_{ki} - D_{roi} + x, Y_{kj} - D_{roi} + y) \qquad (5)$$
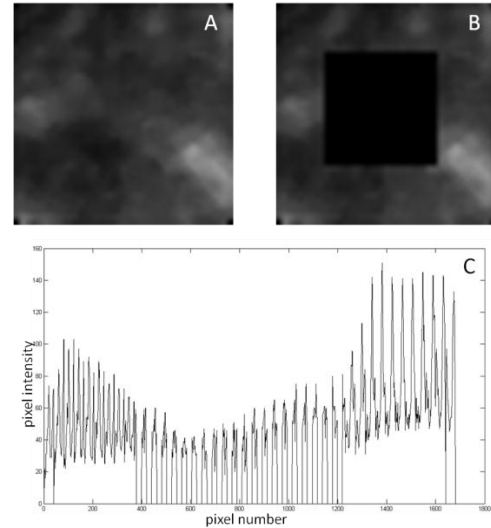
$$else\ PI(x,y) = 0,$$

Fig. 2- Reference image preparation. A) The *RI* B) The pixels belonging the *ROI* are nulled C) The linear interpretation of the *RI* (Eq,3).

Then the linear array PA_{kij} containing the PI_{kij} is constructed using the Eq. 3. The correlation coefficients ($C_k(i,j)$) are calculated for the each PA_{kij} with the *RA* by Eq.1. The desired shift values (I_k^{shift}, J_k^{shift}) along both axes are determined as the the values of i,j which lead to the maximum of the correlation coefficients ($C_k(i,j)$) (Eq.6).

$$(I_k^{shift}, J_k^{shift}) = \arg(\max_{(i,j)} C_k(i,j)) \qquad (6)$$

4. IMPLEMENTATION

The algorithm was implemented as the plugin for ImageJ [5] with the simple interface shown in the Fig. 3. The parameters are set before processing the image time series: the number of first postbleach image, the type of the bleaching region (square or circle), the size of the bleached region in pixels. As the OK button is pressed the image processing starts. After the image was analyzed the table containing the shift values and the mean intensities

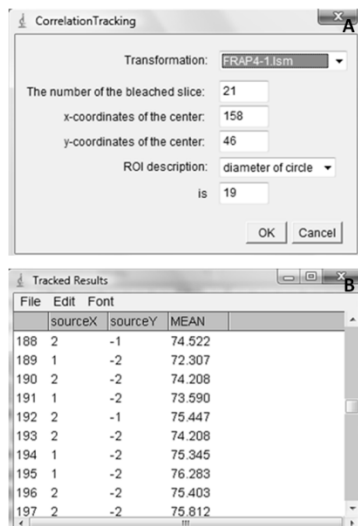in the ROI for each slice is automatically generated (Fig. 3B)

Fig. 3- The interface of plugin for ImageJ. A) Window for specification of initial parameters B) The result table

5. RESULTS

The developed method was compared with the well-established method for tracking on the experimental data of the actin dynamics at focal adhesions[3]. The set of tested images contains 28 undependable stacks, where there are four images with the strong contamination, two images has the gradual defocusing. The developed method showed tracked the movement of focal adhesions correctly for 86% of the images. The TurboReg plugin [5] has the successfully tracked 79% of images. Both methods do not process correctly only one image, for which cell parts moved a lot during the experiment.

All other uncorrected results for the proposed approach are received only for the contracting cells, which are not usually considered in the further analysis; in contrast TurboReg plugin was not resistant to the defocusing during time-series acquisition.

The use of the described tracking algorithm allows to improve the representativeness of the received data by diminishing effect of the movement of focal adhesions for the calculated recovery curves (Fig. 4).
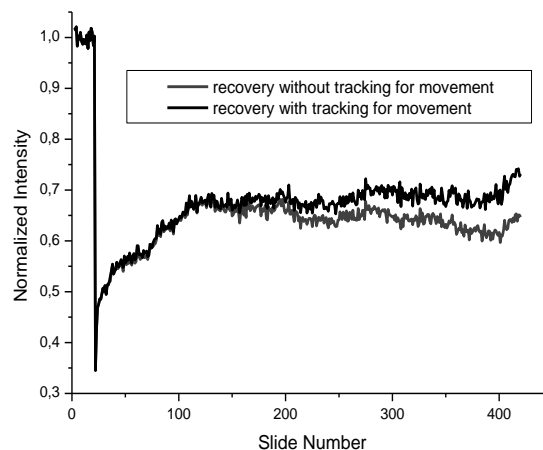

Fig. 4 – The recovery curves with and without compensation for the movement of focal adhesion.

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