AN ALGORITHM FOR PROCESSING THE FRAP IMAGES OF ACTIN FILAMENT TREADMILLING

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Automotive focal adhesion segmentation and its tracking is a critical part of actin filament treadmilling that is detected by the method of the fluorescent recovery after photobleaching(FRAP). However, the complex processes in live cells exhibit new challenging tasks before the cell image segmentation. In the developed tracking method we propose to use the correlation function to estimate the shift of cell compartments. We employ local thresholding for the fluorescence recovery intensity in order to segment the focal adhesions in the region of interest. We have applied this method for tracking the focal adhesions in HeLa cells.

Key words: actin polymerization, cell tracking, image processing.

INTRODUCTION

The cytoskeleton plays a major role in the cell motility [1]. 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)[1]. 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 [2,3,4]. However, there are still a lot of proteins, such as testin, which functions in actin cytoskeleton are not well-studied.

Testin is a protein that may play a role in cell adhesion, cell spreading and in reorganization of the actin cytoskeleton. According to its structure it might be in two conformation states. Being in the closed conformation state, testin is localized in the cytoplasm of the cell. When testin is in the opened conformation state, it is localized in the focal adhesion. As result, it might influence on the actin polymerization in the focal adhesions [5].

FRAP is the technology often used to determine the dynamic parameters of actin treadmilling. In a typical FRAP experiment, a small region of the fluorescent specimen is bleached once. Because the photochemical bleaching of fluorophores is essentially irrevisible, changes of fluorescent intensity in the bleached and unbleached regions are due to the exchange of bleached and fluorescent molecules between those regions. The obtained (or

acquired) images are analyzed to display the fluorescence recovery in the bleached region and to estimate the studied parameters (diffusion coefficients, association/dissociation rate constants, etc) [6].

Before evaluating and comparing any FRAP data [6], one should check that the cell or cell parts do not move. If there is the presence of the movement during time scanning series, the cells should be tracked by manually repositioning or using specific software [6]. For testin it is a more evident problem. Because testin is the protein that mainly localized in the focal adhesions, i.e. in the cell parts that are more dynamic in scene of movement.

In this paper we report a semi-automated algorithm based on using the thresholding method and correlation function [7]. We select the thresholding method because it allows receiving the edges of focal adhesions with high accuracy.

MATERIALS AND METHODS

Cell culturing. The human epithelial adenocarcinoma hela cells were studied. The cells were grown at 37 °C, under 5 % of CO_2.

DNA constructs. The testin variants were generated from pEGFP-N3 plasmid, expressing testin constructs fused to GFP [8].

Cell transfection. Cell transfection was done by the lipofectamine transfection according to the invitrogen protocol and by the calcium-phosphate mediated transfection according to the protocol reported in [9].

Transfected live and fixed Hela cells were analysed by confocal microscopy and the images received from the Zeiss laser scanning confocal microscope (LSM-510 Meta, Carl Zeiss, Jena, Germany).

IMAGE PROCESSING OF FRAP IMAGES

Algorithm

Processing of the FRAP images is done in five steps:

1. Focal adhesion tracking. It reduces influence of cell moviment.
2. Edge segmentation of focal adhesions. It focuses on analysis of the testin localization area.
3. Background subtraction. Takes into account the background fluorescence.
4. Correction for laser fluctuation and acquisition photobleaching. Considers that the pixel intensity is changed by the laser-intensity fluctuations, photobleaching reversibility.
5. Normalization. Allows to compare different experiments.

Cell Tracking

During the image acquisition the cell parts might shift. This shifting should be tracked.

The correlation function can be used to track the movement of the bleached cell parts.

$$Cor(X,Y) = E[XY], \quad (1)$$

where *X* and Y are two signals.

Eq. (1) is used to compare two signals in the time or in the space. According to the correlation function properties, if there are two signals, where the small signal should be

found in the large signal, the maximum of their correlation function shows the entry of the small signal into the long.

The main idea of the algorithm is shown in the fig.1. The intensity profile along the region of interest is taken from the reference image (fig. 1A,B). Then this profile is scanned in every image in time series (fig. 1C,D), where the position of the beginning of the small signal is defined by using the correlation function.

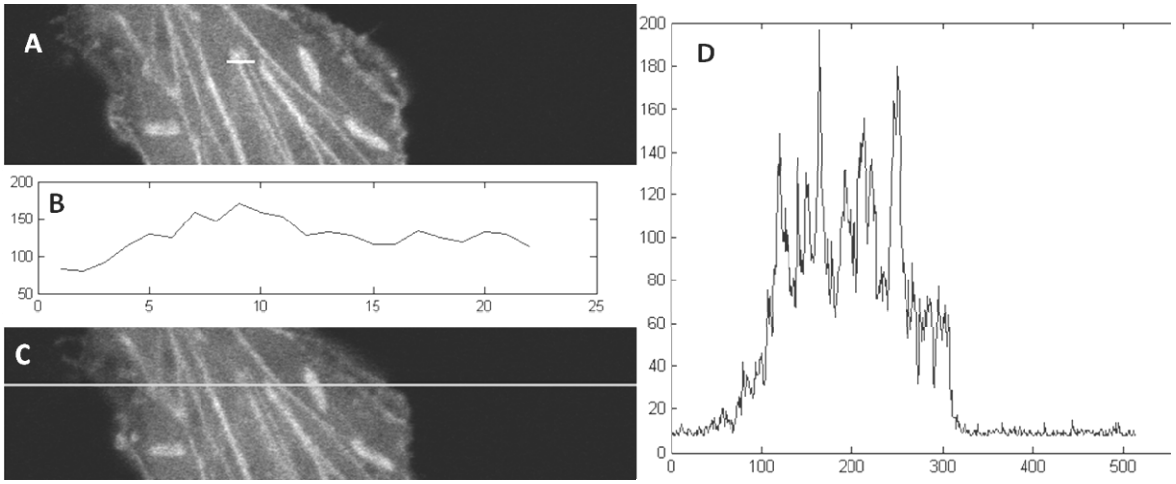

Fig.1. Cell tracking A) The first image of sequence. The yellow line corresponds to the resemblance B) The intensity values of the resemblance C,D) The intensity profile of the frame from time-series

FILAMENT SEGMENTATION

The algorithm for focal adhesion segmentation has been developed and is based on the threshold method.

The threshold method is chosen with aim to translate the greyscale image to the binary mask, where the pixels of the objects are marked as 1, and the background pixels as 0. Pixels above the threshold are marked as 1, pixels below threshold are marked as 0 [7].

Fig. 2 shows the main steps of the focal adhesion segmentation procedure. The rectangle, containing the region of the interest, is segmented (fig. 2B). Then the pixel intensity histogram is calculated and the threshold is calculated by the Otsu method (fig. 2C) [10]. The threshold is used to translate the greyscale image to the binary one (fig. 2D). Thus, only the cell parts, corresponding to filaments, are left in the binary mask, the small non-informative particles are removed (fig. 2E). If the mask contains holes then they are filled. The example of the resulting mask is shown in fig. 2E.

The developed algorithm is implemented in Matlab and ImageJ.

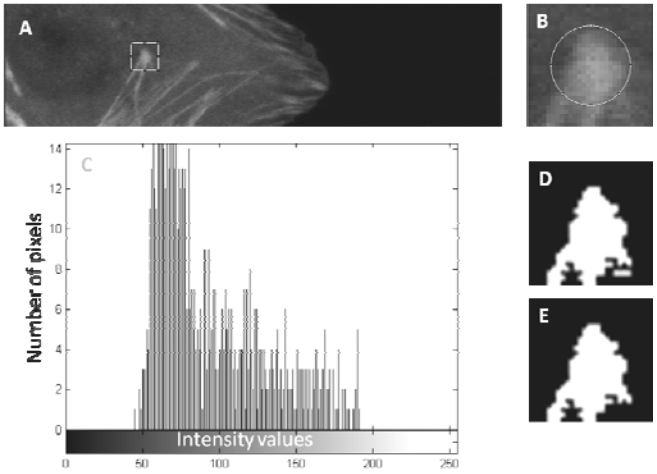

Fig.2. Focal adhesion segmentation A) The image of a cell part B) The rectangle around the region of interest (yellow circle) C) Histogram of the pixels intensities within the yellow circle. D) The binary image after thresholding E) The resulting binary image

RESULTS AND DISCUSSION

The algorithm of the focal adhesion segmentation in has been tested on series of images and it showed sufficient results.

The algorithm of the cell tracking has been tested on series of 18 image sequences and it showed wholly satisfactory results. According to the results of the cell tracking, it might detect movements only in the direction orthogonal to the filament orientation and it does not take into account the cell rotation.

Both algorithms have been combined in the single method allowing to detect cell movements and to measure the average fluorescence recovery only in the focal adhesions. The representative example of result of processing the FRAP image of actin filament treadmilling by our algorithm is shown in fig. 3.

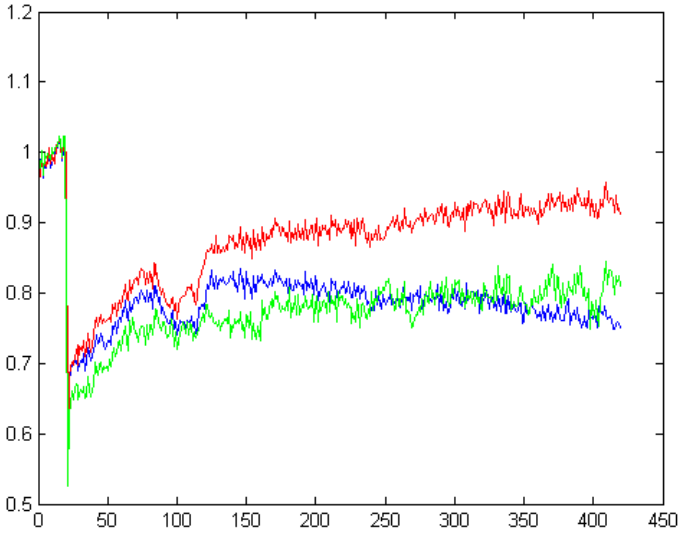

Fig.3. Image processing the results of the developed algorithm. Blue curve corresponds to the raw FRAP dataset. The red curve depicts the result after cell tracking. The green curve is the result of the developed algorithm

The cell tracking reduces an effect of cell movement (decreasing in the recovery curve). More accurate determination of the focal adhesions in the region of interest increases the robustness of the analysis to the fluctuations, takes into account different sizes of the focal adhesion areas. The application of our developed algorithm might significantly improve the FRAP analysis of actin filament treadmilling processes as well as enhance the estimation precision of system parameters obtained from the fluorescence recoveries.

LITERATURE

1. *Creighton, T. E.* The encyclopedia of molecular biology / T. E. Creighton, A Wiley-Interscience Publication. 1999. Vol. 1–4.
2. *Alberts, B*. Molecular Biology of the Cell / B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, J. D. Watson. Garland Science. 1994.
3. *Berk.* Molecular Cell Biology Fifth Edition / Berk. Matsudaira Lodish.
4. *Pollard, T. D.* Cellular motility driven by assembly and disassembly of actin filaments / T. D. Pollard, G. G. Borisy// Cell. 2003 May. Vol.16;113(4):549.
5. *Garvalov, B. K.* The conformational state of Tes regulates its zyxin-dependent recruitment to focal adhesion / B. K. Garvalov, T. E. Higgins, J. D. Sutherland et all // J Cell Biol. 2003. Vol.14;161(1):33–9.
6. *Goldman, R. D.* Live Cell Imaging: A Laboratory Manual / R. D. Goldman, D. L. Spector // Cold Spring Harbor Laboratory Press. 2004.
7. *Gonzalez, R. C.* Digital Image Processing Using MATLAB, 1st ed / R. C. Gonzalez, R. E. Woods, S. L. Eddins, Pearson Education. 2004.
8. *Tsien, R. Y.* The Green Fluorescent Protein / R. Y. Tsien // Annu. Rev. Biochem. 1998. Vol. 67:509–44
9. http://www.genomics.sbg.ac.at/meth/cacl2_transfection.pdf