Segmentation and tracking of cells in the image sequences

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Abstract

In the paper the method of automated cells tracking in the sequence of images is presented. The method starts with a sequence of images processing by the use of an adaptive threshold operator. Then the cells are detected, labeled and parameterized by their size, position, orientation, bounding box, etc. Each pair of two consecutive images in the sequence is analyzed next in order to find corresponding (i.e. matching) cells. In that way all observed cells might be recognized and traced. The tracking, associated with cells parameters measurements, provides data in a form of time series. This data can be used for a particular cell activity observation, complex interaction modeling of multicellular organisms, etc. Such analysis can give detailed information about cells time-space dynamics. The method proposed is demonstrated on a set of biological data.

1 Introduction

In the biological research in vivo live confocal microscopy has enabled scientists to perform observation and store their results for further analysis, [1]. Usually the data is available in the form of sequences of images with time stamps assigned. Especially the GFP (*Green Fluorescent Protein*) images became the sources of valuable data. It comes from the fact that GPF intensity may reflect protein concentration and thus GFP intensity observation within a cell can serve as a method of its inspection. In the consequence the data provided can be used for cells activity observation, complex interaction modeling of multicellular organisms, cell growth and proliferation examination, gene expression modeling, etc. But to draw valid conclusions or build models with a great accuracy the amount and quality of collected data should be significant enough. Because the amount of data to be analyzed is huge usually, there is a need for automated data processing. The problems of GFP fluorescence intensities measurements and cells tracking were concern in [2, 3].

In this paper we present the method of automated cells tracking in the sequence of images, which is based on image segmentation. The method proposed assumes that sampling rate of image taking is fast enough to assure that a new cell positions and old cell positions on two consecutive images are relatively closed. The methods consist of some computation steps, which are repeated for each image in the sequence analyzed. Thus, the computation starts with an image processing by the use of an adaptive threshold operator. Then the cell detection is done. The cells detected are labeled and parameterized by their size, position, orientation, centroid, bounding box, etc. (recognition of cell splits and merges events is not addressed here, for the details on this problem please refer to [4]). Two consecutive images in the sequence are analyzed then in order to find corresponding cells.The result of tracking can be used for many purposes. It can serve, for example, as a base for the changes in the cells color intensities detection. The averaged intensity within a cell can be interpreted as a relative protein concentration, while the number of a particular image corresponds to the time of observation. Other cell attributes (such as: position, size) can be used to observe the cell movement and growing dynamics. The method is demonstrated on a set of biological data which is a sequence of images with living cells colonies.

2 Cell tracking algorithm

The cell tracking problem can be formulated as follows: Given a sequence of images with species, de-

termine the positions of each cell at each time point (track cells through time). In the method proposed we assumed that the images are captured from a video stream with a preset sampling rate, and stored in a sequence for the further processing. Thus the idea of the algorithm proposed can be expressed as follows:

- segment each image in the sequence in order to find the areas covered by the cells (create a segmented image for each original image in the sequence);
- analyze the original images within areas corresponding to the areas found (analyze the interiors of the cells detected, and extract individual cell attributes such as: cell placement, shape, average of lightness, etc.);
- find the matching cells for every two consecutive images in the sequence and evaluate the dynamics of parameters changes and cells movements (identify the same cells on two consecutive images using cells attributes just extracted, and estimate dynamics of changes knowing the time slice's length between two image captures).

In a confocal microscopy the observations can be captured by different video capturing devices. It may happen, that a lighting conditions in the experimental setup are varying due to some reasons (voltage instabilities for example may produce different lighting intensities). More over, the distribution of the lighting can be non uniform (most often the distribution is radial as a result of the light source's characteristic). Thus before performing any observation some preliminary steps are needed. These steps can include a lighting distribution measuring in an experimental setup without species. The result of such steps can be used later to adjust the observed light intensities by removing a non uniform background. The influence of the varying lighting intensity can be canceled by the intensity tuning during image processing. The following subsection provides some clues on the way it can be done.

2.1 Adaptive threshold operator

The cell detection method discussed in the next section requires that the images provided are monochromatic with different colors for the background and the areas of interest. The simplest way to create such images is to apply the threshold operator on the re-quantized original images (images with a reduced number of colors). But the threshold value can not be kept on the same level for all images. If it would be so, the varying lighting intensities would cause filtering out underexposed cells, or accepting overexposed background as an area of interests. The solution to this problem can be an application of an adaptive threshold operator.

The adaptive threshold operator uses the threshold level which is calculated on the base of the histogram of the re-quantized original image. It appeared (see Figure 2) that all histograms of the re-quantized images in the sequence analyzed were of the similar shape. The maximums of histograms were observed for the values which were standing in correspondence to the lighting intensities. Thus the threshold value in the adaptive threshold operator can be calculated as follows:

$$v_t = x_{MAX} + d \tag{1}$$

where v_t is the threshold value, x_{MAX} is the most often observed pixel intensity in the re-quantized original image, d is a parameter which assure the proper background elimination (in the experiments performed this value was set to 4).

2.2 Cell detection

The images obtained after adaptive threshold operator application contains areas of interest. These areas are groups of pixels but not objects yet. To find the objects the following algorithm is proposed:

let A, B, T, O be the colors of pixels belonging to, respectively, the area of interest, the background, the temporary area, and the area of an object.

1. trace an image starting from the upper left pixel in order to find a pixel whose color is A
2. beginning with the pixel found fill the area with a color T
3. extract parameters of the area filled in 2 (size, position, orientation, centroid, bounding box, etc.)
4. if the area filled in 2 is interesting (is a cell), fill it with a color O and memorize the parameters of it in a list, otherwise fill this area with a color B
5. repeat 2, 3, 4 until the lower right pixel is not reached

The fill procedure (step 2) tries to mark all connected points having the same color A with a color T, starting from a given pixel. This procedure returns the bounding box of the area filled. There are, of course, several more general algorithms for region filling, which are

working with non monochromatic images. In these algorithms some criterions are defined, which are used to classify the neighboring pixels as belonging to the region being filled or not. In our case we have an image obtained after adaptive threshold operator use, so the areas of interest are already filled. Now we want to known, what kind of the object is represented by each area, and what are the objects parameters. Thus our fill method is used mainly to find the bounding box of the area filled. Having bounding box we can simply extract objects parameters by looking at pixels in this box and at corresponding pixels in the original image (step 3).

There might be several objects parameters. One of the most important parameters of the object is the position of its geometric center. This parameter is used in the corresponding objects finding (see section 2.3). The coordinates of the object center expressed in the coordinate frame associated with a bounding box can be calculated from the formula:

$$x_c = \frac{M_{10}}{M_{00}} \quad , \quad y_c = \frac{M_{01}}{M_{00}} \tag{2}$$

where: $M_{ji} = \sum_{x,y} \sigma(x_j, y_i) x_j \cdot y_i$ is a spacial moment; $\sigma(x_j, y_i)$ is an indicator function which returns 1 if a pixel at position (x_j, y_i) has color T, and 0 otherwise. The average intensity of the cell, avg, can be calculated from the formula:

$$avg = \sum_{x,y} \frac{I(x + dx, y + dy)}{M_{00}} \tag{3}$$

where dx, dy are the coordinates of the upper left corner of the bounding box expressed in the image coordinate frame. Several other parameters can be obtained in similar manner.

It is not known, weather objects detected are cells or uninteresting parts of the observed field. Once the objects parameters are extracted, the task of objects identification becomes a problem of classification with extracted parameters as features. Finding the correspondence between cells at different time points simplifies then to finding the correspondence between cells detected on the consecutive images.

2.3 Cell tracking

The problem of finding correspondences between image objects is well known in the computer image analysis field. It can be NP-complete, if an object in one image potentially match every object in another image. To limit the number of possible matches we propose to consider the distance between matching candidates. The assumption we have made about the sampling rate allows as to define matching candidates as objects from two consecutive images which are most closely, and lying within a distance which does not exceed an assumed limit. Lets assume that the detection of cells ended successfully and the cell parameters have been collected. Thus to find the matching candidates for an object from one image it is enough to search for them in the second image within a circular area centered at the object center. The matching object should be the closest one (and most similar one, if we take into account extracted earlier objects parameters). If there is no matching objects it means, that cell "disappeared". The correspondence analysis can be summarized with some information about cells movements, appearance, etc.

3 Experiments

The experiments were performed on the sequence of captured images, which was processed off-line. Each captured image was, in fact, a frame of 672×512 pixel size, stored in 48 bit grayscale image format. The images were converted into 8 bit grayscale and then processed by the method described. In the Figure 1 the characteristics of the lighting conditions we met can be observed. It is clear, that the radial background intensity should be considered. The use of an

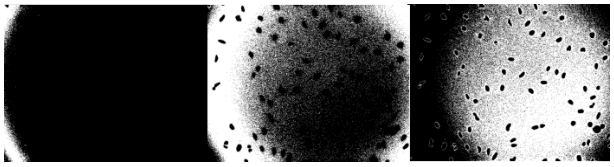

Figure 1: Three pictures representing the same image after filtering with gradual increased threshold.

adaptive threshold operator was connected with histogram's analysis. In the Figure 2 two histograms (in a logarithmic scale) are shown. In most cases the histograms were similar. Thus on the base of observations the value of d parameter (equation (2) was set to 3. After thresholding the objects detection was done. For each object detected different parameters were extracted (this included object's area and position of its center). The objects detected with the are of 20 pixels were filtered out. The rest of the objects was classified as cells. The cells centers calculated are shown in the Figure 3b) as black dots within cells interior. The part a) of this figure represent the first, original image in the sequence analyzed. The part b) represents final

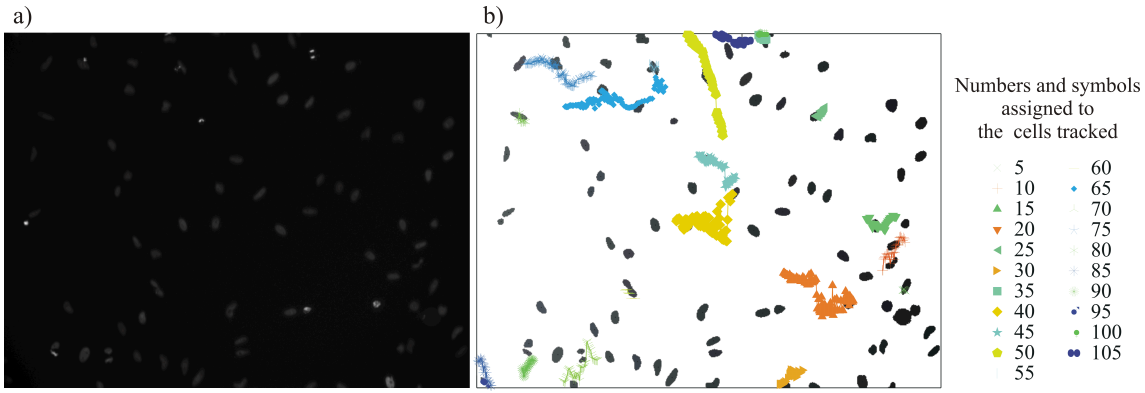

Figure 3: The first image in the sequence of 238 original images analyzed, a); and results of 21 cells tracking with cells detected for the first image in the background, b).

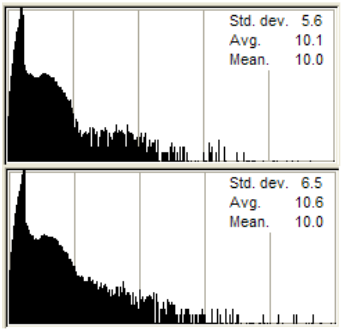

Figure 2: Histogram examples of images analyzed, drown in the logarithmic scale.

results of cell tracking. For better visualization the tracks of 21 chosen cells are shown only.

4 Conclusions

In the paper a method of automated cells tracking was presented. The method was successfully applied to the sequence of GFP images of biological species. For the good method's performance method parameters tuning might be necessary.

The data used in the experiment performed were processed off-line, but processing on-line is also possible. The experiments showed, that adaptive thresholding shortens the time necessary for cells detection and their parametrization coming next (the thresholding depends on the image histogram shape). The cell tracking was implemented as a problem of cell matching on two consecutive images, while the sampling time provided the clue about time line. The cells parameters found were extensively used there (cells parameters are calculated on the base of cells detected in a thresholded image and image intensities inside corresponding areas of the original image).

The data collected during processing can be used for different purposes, for example for a particular cell activity observation, intercellular processes modeling, complex interaction modeling of multicellular organisms, etc.

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References

[1] V. Gor, T. Bacarian, M. Elowitz, and E. Mjolsness: Tracking Cell Signals in Fluorescent Images. In *Proceedings of the 2005 IEEE Computer Society Conference on Computer Vision and Pattern Recognition (CVPR05)*, (2005).

[2] L. Vincent and P. Soille: Watersheds in digital spaces: an efficient algorithm based on immersion simulations. *Trans. Patt. Anal. Mach. Intell.*, 13(6), (1991).

[3] H. Shen, G. Nelson, S. Kennedy, D. Nelson, J. Johnson, D. Spiller, M. R. White, and D. B. Kell: Automatic tracking of biological cells and compartments using particle filters and active contours. *Chemometrics and Intelligent Laboratory Systems*, 82(1-2):276–282, (2006).

[4] J. Withers and K. A. Robbins: Tracking cell splits and merges. In *Proceedings of the IEEE Southwest Symposium on Image Analysis and Interpretation*, (1996).