The Algorithm for Monitoring of Evolution of Stem Cells

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Abstract: *In this paper algorithm for monitoring of stem cell populations in vitro is present. The algorithm allows to spend analysis of evolution of tissue. It includes five stages: acquisition and preprocessing, segmentation, measurement, constructing of tissue description, cell classification. The basic tasks are oriented to detection of mitosis, apoptosis and intermediate stage of cells. The result of this this detection allows construct graph of cells evolution in tissue. Due to the combination of simple solutions this algorithm can be easy realized on base of many computer systems of image analysis.*

***Keywords*:** monitoring, stem cells, image analysis, object characteristics.

1. INTRODUCTION

An importance of analysis and automatization of human stem cells video images is due to their potential application for cell-based regenerative therapy. Using of stem cells are based on their two main properties that are important mechanisms for normal tissue regeneration and renewal: differentiation into specialized cells and proliferation through mitosis to produce more stem cells. Using of phase-contrast time-lapse microscopy provides opportunity for monitoring intact stem cells. This technique makes the analysis of a cell population completely label-free and gives possibility to study population of thousands of cells in vitro.

The modern level of stem cellular technology has a strong relationship with the development of software and hardware tools and methods that obtain information about the structure of cells and their associates. Its important part is automation technology of live cells monitoring by video images. Automated cell tracking system shall consider that adult stem cell populations has varying and inhomogeneous character. Most interesting questions for investigation include dynamical properties of cells and cell conglomerates and cellular interactions.

An important aim of the automated systems is detection of spatio-temporal localization of mitosis events. Every mitosis event is the division of cell into two daughter cells, which is always accompanied by a change in size, shape and brightness of the area around cells.

Currently used mitosis detection methods on base of phase-contrast time-lapse microscopy images can be divided into two groups: tracking-based methods, where cells trajectories are examined to detect mitosis, and tracking-free methods, where mitosis visual features are examined [1]. These methods based on morphology and brightness changes of mitotic cells. Different mitosis detection methods unclude: mean shift algorithms; multiple-object matching methods based on the frame-by-frame segmentation; tracking algorithms based on determination of blob region's characteristics; methods of detection of mitosis based on brightness change [2, 3].

Another classification of approaches to detection of mitosis divides all methods on temporal and spatio-temporal [1, 4]. According to this classification, temporal methods detect moment of time, when one cell divides into two cells. Spatio-temporal methods detect as size, shape, velocity and brightness of cells change.

All described algorithms are based on monitoring of separate cells. There are two problems with the work of these algorithms: the quality of cell detection and redundancy of information.

Investigated cells are alive and there are many processes inside them. Sometimes a border of cell has low contrast and cell is not detected. Such cell can be omitted from tracking. After some time this cell can occur on image again. It is conflict situation, because system hasn't information about state of this cell and translates such situation as creation of new cell.

Many information about every cell is accumulated during tissue investigation. This information is excessive because most of cells have similar evolution. There are many papers concentrated on mitosis stage. However, for high quality of tissue construction it is necessary to consider other state of cell evolution in tissue like differentiation and apoptosis. Monitoring of these events allows to decrease information flow for the tissue evolution analysis.

In this paper, we present a cell monitoring algorithm that includes registration of basic states of cells in tissue: normal state, mitosis, apoptosis and intermediate state. The information about cell state is significantly important for the monitoring of tissue evolution, it results in more qualitative cell lineage construction.

2. CAPTURING AND PREPROCESSING OF CELL'S IMAGES

The basic factor for description of cells state is shape changing. Shapes of stem cells are changed incessantly. The task of monitoring of tissue evolution from stem cells can be solved by control of shape characteristics. The algorithm of such monitoring consist of five stage (fig. 1):

- image acquisition and preprocessing;
- cell's segmentation and detection;
- characteristics measurement;
- determination of common characteristics of description;
- cells classification.

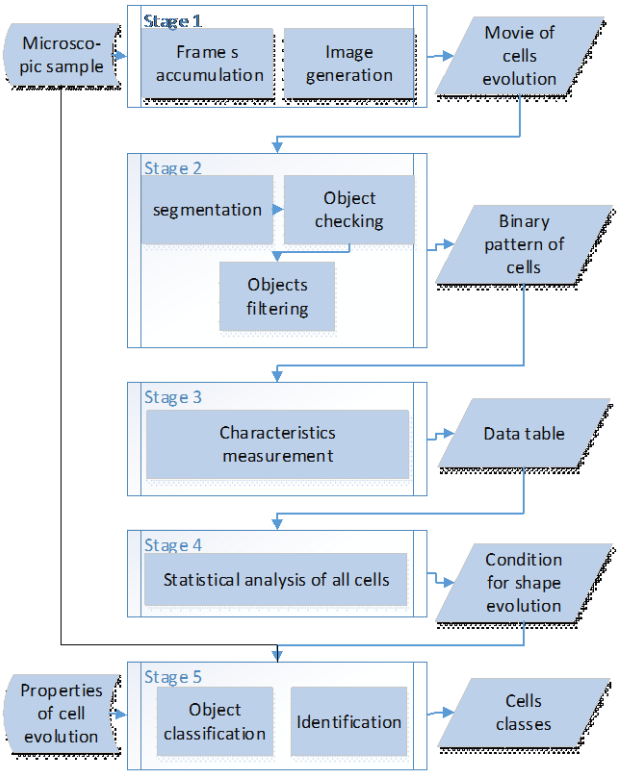

Fig.1 – Algorithm of monitoring of stem cell growing and evolution

3. CAPTURING AND PREPROCESSING OF CELL IMAGES

The first stage of algorithm of monitoring includes image acquisition and preprocessing for quality improving. Usually image acquisition process is going with very slow speed, about one frame per a few hours. Therefore, image of tissue has a little changing in short time period. This property can be used for image improving. It is possible to use averaging of frames through short period for image quality improving. As result, the procedure of acquisition is irregular and nonlinear in time. The quality image is constructed by averaging of frames captured in short range of time. In this way a pixel on image of every iteration of capture operation is calculated as

$$I''(x,y) = \frac{I'(x,y)\cdot(n-1) + I(x,y)}{n},$$

where *(x,y)* – coordinate of pocessed pixel, *I''(x,y)* – new value of intensity of pixel, *I'(x,y)* – previous value of pixel intensity, *I(x,y)* – value of pixel intensity in current captured frame, *n* – count of capture iteration in short time.

This acquisition process is stopping by checking of distortion error that calculated for every new frame as

$$\sigma''(x,y) = \frac{\sigma'(x,y)\cdot(n-1) + I''(x,y) - I(x,y)}{n} < \varepsilon,$$

where *(x,y)* – coordinate of processed pixel, *σ''(x,y)* – new error of intensity of pixel, *σ'(x,y)* – error of pixel intensity for previous iteration, *I''(x,y)* – new value of intensity of pixel, *I(x,y)* – value of pixel intensity in current captured frame, *n* – count of capture iteration in short time, *ε* – accuracy for error detection.

This procedure of quality image construction is repeating through long time. Such images form video sequence for cells evolution (fig. 2).

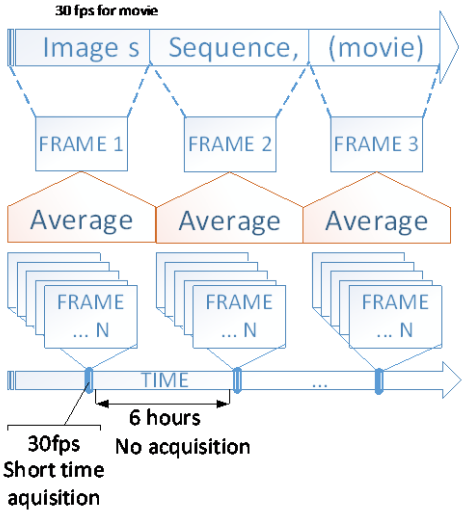

Fig.2 – Process of video capture with preprocessing

4. SEGMENTATION OF CELL

Second stage is segmentation of cells on image. After procedure of background correction, we have images with high quality and can use gray thresholding for cells border detection.

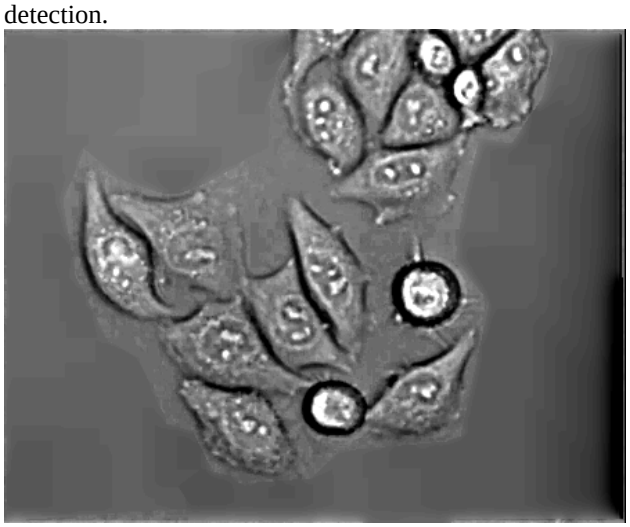

Fig.3 – Image or frame of video about steam cells evolution

Operations of morphological filtration (close and open) and small object removing allow to correct this borders. The thinning operation by Zong-Suen algorithm allows taking closed border of cells. Regions inside extracted closed border correspond to cells pattern on the image. After operation of filling of hole, binary patterns are constructed on the image. Unfortunately, these binary patterns include regions that do not correspond to the cells (fig. 4).

Fig.4 – Result of image segmentation with morphological correction.

Such regions have flat gray level on full area. We can check dispersion or bandwidth of gray levels in every region and remove regions with large values of these characteristics (fig. 5) by object filtering. In result binary image consist only cell's pattern.

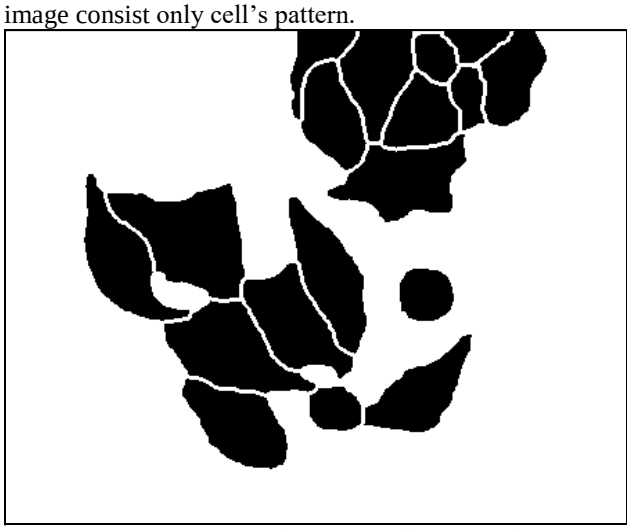

Fig.5 – Binary pattern of stem cell after object filtering

5. CALCULATION OF CELLS CHARACTERISTICS

Third stage is characteristics calculation. Unfortunately, there is no common shape for cells of any tissue. However, changing of shape of stem cells during their differentiation occurs according specific laws for every type of tissue. Therefore, we can define common tendency of cells shape evolution for every tissue. In this stage, next characteristics are calculated: compactness, sphericity, convexity, compactness, solidity, geometrical center of mass [5, 6].

The compactness correspond to relation area to perimeter:

$$\text{compactness} = \frac{4\pi \cdot \text{area}}{\text{perimeter}^2}.$$

The sphericity is defined as:

$$\text{sphericity} = \frac{R_{\text{in}}}{R_{\text{out}}},$$

where R_{in} – interior (minimal) radius; R_{out} – external (maximal) radius.

The convexity allow to describe of cell's smoothness:

$$\text{convexity} = \frac{\text{convexperimeter}}{\text{perimeter}}.$$

The solidity is defined through area:

$$\text{solidity} = \frac{\text{area}}{\text{convex area}}.$$

Coordinate of center of mass calculate as

$$\bar{x} = \frac{1}{\text{area}} \sum_{x \in Obj} x,$$

$$\bar{y} = \frac{1}{\text{area}} \sum_{y \in Obj} y.$$

6. DETERMINATION OF COMMON CHARACTERISTICS FOR DESCRIPTION OF CELLS

Fourth stage is cell differentiation. Stem cells colony usually has the same growth factor to stimulate differentiation in tissue. This leads to a uniform evolution of cells in sample. Their shapes become similar. Some cells have similar dimensions and orientation (fig. 6).

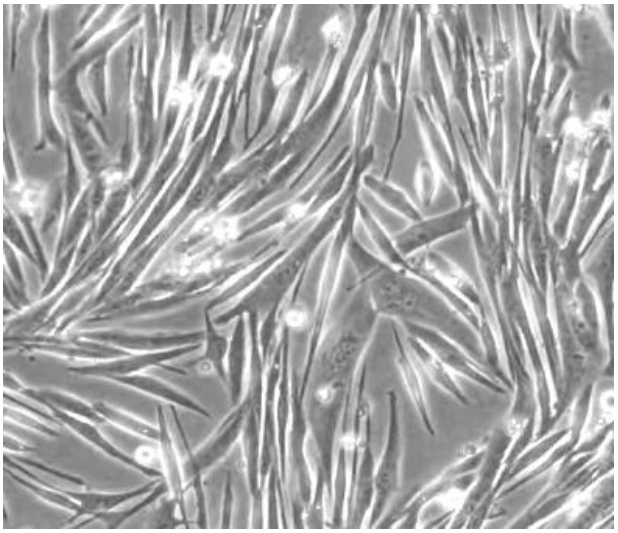

Fig.6 – Differentiation of stem cells to skeletal muscle cells

First of all general laws for shape for all cells are defined by statistical analysis methods. Mean value for every measurement characteristics describes common properties cells in tissue.

7. CLASSIFICATION OF CELLS

The five stage of algorithm is classification of cell states. There are four basic stages: normal state (fig. 7)), mitosis (division of cell to two ones (fig. 8)), apoptosis (death of cell (fig. 9)) and intermediate (between other states (fig. 10)).

Fig. 7 – Stem cell in normal state

Fig. 8 – Stem cell in mitosis state

Fig. 9 – Stem cell in apoptosis state

Fig. 10 – Stem cell in intermediate state

Differences of mean characteristics for previous and current states allows to classify state of cells. Nevertheless, we can determine only abnormal cells. It is necessary to classify these cells by state. Before mitosis (fig. 10), a shape of cell tends to circle. Therefore, we can use compactness as a sign of changing of state. For circular cells the value of compactness ranges around one.

After beginning of the state of mitosis the cell takes the shape like as eight (fig. 8). This state of the cell is determined only after previous circular state. For these cells value of sphericity is decreased. This process leads to cell division, after which new cells take the normal shape.

Fig. 11 – Stem cell division to new two cells

We can no use circular shape of cell for definition of mitosis stage because some time after circular cell state apoptosis state occurs(fig. 9). A cell is destroyed and loses its shape. This state is described by characteristics of convexity and solidity with their values going to zero.

8. CONCLUSION

Monitoring of the evolution of stem cells is a complex procedure. For its implementation it necessary to take into account the shape of the cells of the tissue. Changing of cells is performed by acquiring of circular shape. After this change, the cell either divided or destroyed. Based on these features an efficient algorithm has been developed for automatization of the monitoring and description of culture of stem cells. Using this algorithm has improved the quality control of cultivated tissue.

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