DETECTION PATTERNS OF LIVING CELLS FROM ITS AGGREGATION ON THE DIGITAL IMAGE

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One of the important problem of modern direction of cytological image analysis is cells segmentation. There are many algorithms in this field. But image properties and methods of cell investigation are changing every day. Today most perspective direction of cytological image analysis is lifing cells investigation. Such images lead to many troubles for cells analysis. In this paper we are proposed solution of one such problems: pattern extraction of cells from its aggregation.

Introduction

Investigation of the features of processes in cell cultures is essential for assessment and prediction of activity of a number of pathological processes. The ability to control these processes is connected to the development of appropriate technical equipment of monitoring of dynamical changes of cells and their biochemical properties in different phases of culture growth. At the same time, the quantitative interpretation of the dynamic changes of cell populations and clusters of cells may allow diagnosis of processes in different tissues of the body at an earlier stage of their development.

Quality monitoring of cell activity is performed in special boxes that significantly degrade the image. On the other hand cells are grouped together and stick together making it difficult to study them. This conditions leads to the fact that for the characterization of the cells during the process of their work requires the development of additional algorithms for their separation and image enhancement.

1. Properties of microscopic images acquisition

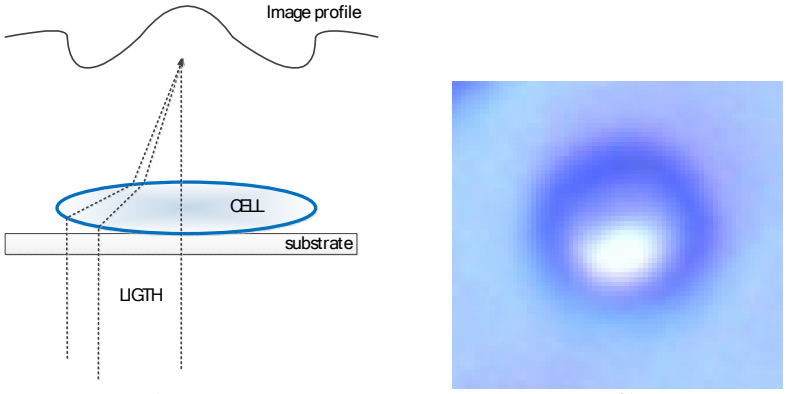
Microscope is an instrument used to produce magnified images of objects that are too small to be seen with the naked or unaided eye. Types of microscopes designed to study of microorganisms include the light microscope, dark field microscope, phase contrast microscope, confocal microscope, interference microscope, fluorescence microscope, electron microscope, and atomic force microscope.

Optical microscope (or compound microscope) consists of two lenses: an objective and an eyepiece (ocular). Light microscope uses a beam of light to create an enlarged image of the specimen.

Ray tracings in the optical microscope are the respective objective and eyepiece focal points. The object deform direction of rays. In result maximum intensity of light is concentrate near body center and borders of cells image are usually dark. The real image of the cell formed by the objective on camera matrix and every time support by this properties of cells borders (fig.1). Such image correspond to distortion of rays by cell and don't support information about density of matter in the cell.

The ability of an optical microscope to view an object depends on the size of the object relative to the wavelength of the light used to observe it. Sizes of small details that can be discerned through a microscope depend on the restrictions due to light diffraction. Diffraction is a deviation of light from the rectilinear propagation as it passes around the edge of an object that is physically the approximate size of, or even smaller than that light's wavelength.

Therefore the most important feature for a cell in the image is the shadow. Despite recent advances in modeling the Shape-from-Shading problem and its numerical solution, practical applications have been limited. This is primarily due to the lack of perspective Shape-from-Shading models without the assumption of a light source at the camera center and the non-metric spatial localization of the reconstructed shape. We try a modified formulation of the problem that was described in [1]. That allows the reconstruction of surfaces lit by a near point light source away opposite the camera center. The knowledge of the light source position can enable the recovery of depth information in a metric space by triangulating specular highlights. But such methods lead to many problems of border condition on the image and time required.



a) b)

Fig. 1. Properties of images acquisition by microscope: a) light ways for image forming; b) sample of microscopic image of cell

For solution of this problem we are developed simple algorithm for volume reconstruction of cells on the image.

2. Volume reconstruction of cells from image

Usually acquisition of images by optical microscope reflects all distortion optical way of equipment. Therefore light maximum on the image of cell is usually shifted. Cells are aggregate together and change image. In this case classical methods of geometrical reconstruction don't work. But we can use of properties of cell border. Every time the border region is corresponding to local minimum on profile line (fig. 2)

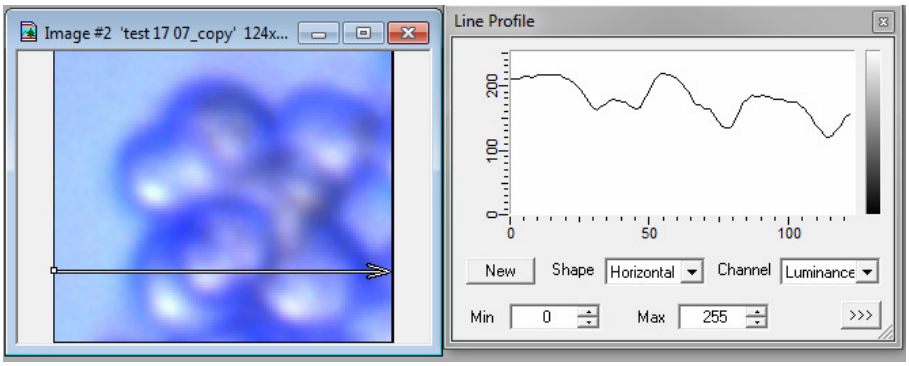


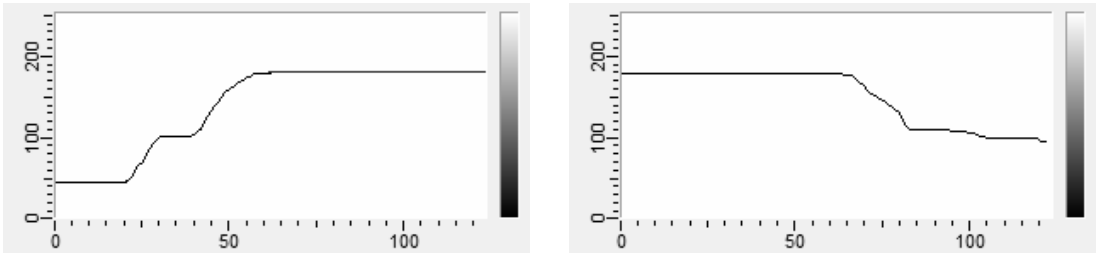
Fig. 2. Image of cells aggregation with profile line of intensity

For easy image understanding the algorithm invert intensity of image and for cells border intensity increase or decrease.

The algorithm is divided on tree stages. Those stages are corresponded to procedures of raster scans, but direction of scanning is changed for every iteration. The first iteration is completed from left to right and top to bottom direction. Every step of this iteration changed of intensity by low:

$$I_{x,y} = \max(I_{x,y}, I_{x-1,y}, I_{x,y-1}, I_{x-1,y-1}),$$

where $I_{x,y}$ is intensity of current pixel, $I_{x-1,y}$, $I_{x,y-1}$, $I_{x-1,y-1}$ are intensity of pixels from neighborhood. The new intensity include previous changes from neighborhood pixels. In result we take specific image with intensity integral properties (fig. 3).



a) b)

Fig. 3. Profiles of processing image of cells aggregation: a) from left to right and top to bottom direction; b) from right to left bottom and to top direction

The second stage is similar to the first but direction changed from right to left bottom and to top direction. In this case intensity is changed by low:

$$I_{x,y} = \max(I_{x,y}, I_{x+1,y}, I_{x,y+1}, I_{x+1,y+1}),$$

where $I_{x,y}$ is intensity of current pixel, $I_{x+1,y}$, $I_{x,y+1}$, $I_{x+1,y+1}$ are intensity of pixels from neighborhood.

On third stage the image are of formed as minimum from results of the first and second stages (fig. 4).

$$I_{x,y} = \min(I_{x,y}^{stage1}, I_{x,y}^{stage2}).$$

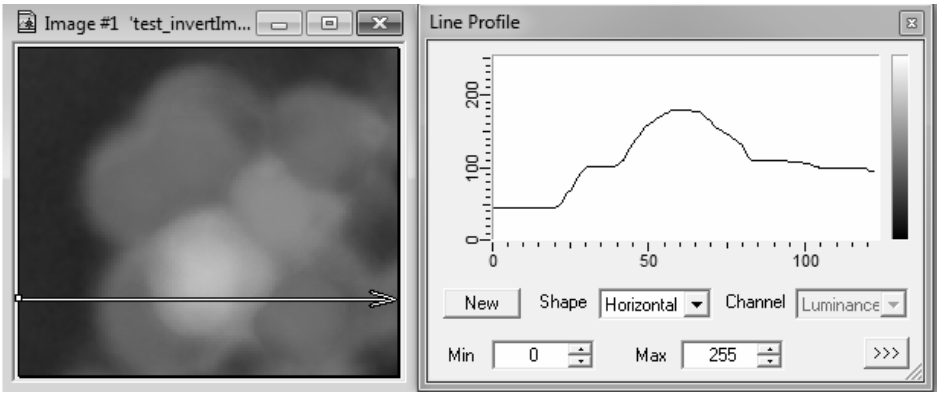


Fig. 4. Image of cells aggregation from third stage with profile line of intensity

In result we take image like as depth map that include volume properties (fig. 5).

3. Detection of cells patterns on the image

The depth map allows you to split image to layers of depth where cells are on different levels. It is realized it by multilayer binarization. We use multilayer Otsu algorithm of thresholding of image histogram (fig. 6) [2]. In result we take a few binary images with regions that are corresponding to cells of depth layers (fig. 7). The next trouble is separating such regions for cells.

The separating is very complex problem that connected with morphological properties of images [3]. We started investigate this problem from transformation of binary image to distance map [4] (fig. 8, a). Than skeleton is constructed from this map by gray thinning algorithm [5, 6] (fig. 8, a). The skeleton includes morphological features of cells aggregation regions. It consists from pixels where intensity index correspond to minimal distance from region border to this pixel. Changing of speed characterize existing circles that construct regions. Those circles correspond to cells. The special procedure spend analysis every branch of this skeleton from end points to the center and detect point of circles centers (fig. 9, a).

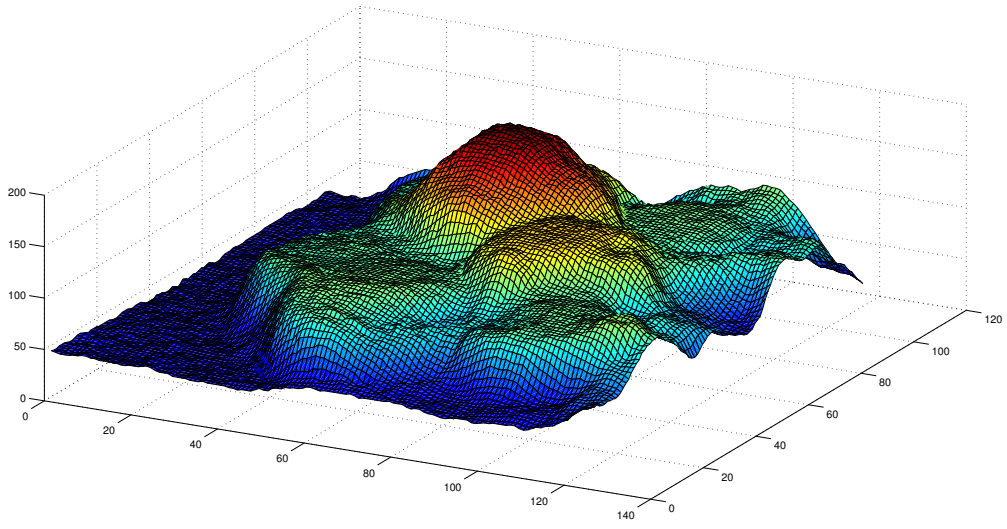


Fig. 5. Depth map of cells aggregation

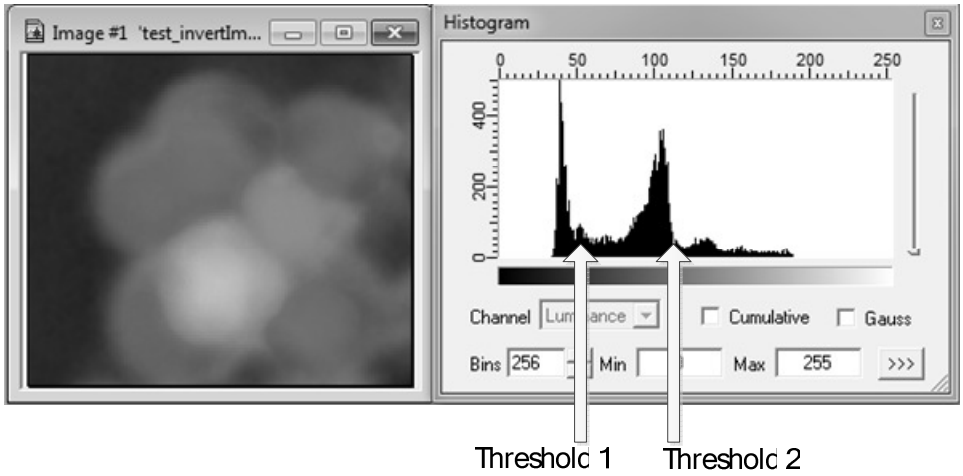
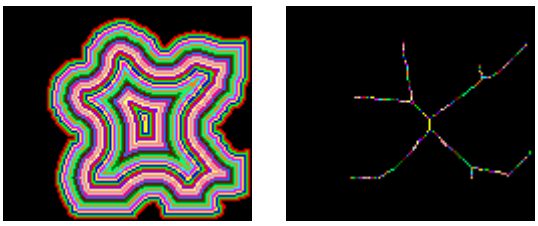


Fig. 6. Definition of binarization levels from depth map histogram

a) b)

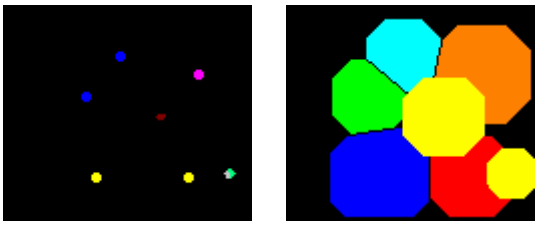
Fig. 7. Binary layers of cells aggregation: a) top; b) bottom

We detect specific pixels of center of cells where pixels index describe radius of cells (fig. 9, a). It is very easy reconstruct regions that are corresponding of separate cells by drawing circles (fig. 9, b).



a) b)

Fig. 8. Transformations of binary layers of cells aggregation: a) top; b) bottom



a) b)

Fig. 9. Regions of separate cells from aggregation: a) centers of cells; b) reconstructed regions

Conclusion

The method of detection regions of separate living cells from aggregation is proposed. It cosist of two algorithms for depth map reconstruction and cells separation. The first algorithm has complexity estimation mark which equals O(n). Such mark for the second algorithm equals $O(n^2)$. It is allow to decide the method is very fist. In other case this method leads to qualitative results. Of course shape of reconstructed cells is not ideal but this method can by improve in near feature.

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