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Determination of Volume Characteristics of Cells from Dynamical Microscopic Image

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Abstract. *The algorithm for the determination of 3D-characteristics of a dynamic biological object based on the recovery of a stereo pair was proposed. Images for stereo pair construction were obtained via a single camera before and after the displacement of the object. The algorithm is based on the example of living one-celled organisms.*
Keywords: *Image analysis, dynamical image, live cell, cytology, optical flow.*

1. Introduction

The study of the mobility of cells allows us to understand phenomena such as tissue repair, chemotaxis of leukocytes, the proliferation of drugs, and the motion

of antibodies. The process of cell growth is accompanied by the shifting of cellular organelles and dimensional changes which can also be considered as motion. Construction of three-dimensional models of moving microscopic objects is one of the auxiliary tool for such studies.

The problem of three-dimensional reconstruction of a biological moving object has several features that require specific methods of solution [1]. It is primarily due to the diffuse, low-contrast nature of the borders of object, as well as difficulties associated with the object detection. Furthermore, this type of dynamic scene does not provide a standard stereo pair at an exact moment [2].

In this paper, we consider the possibility of stereo reconstruction of dynamic microscopic objects, provided that the displaced image can be used as an additional image in the construction of a stereo pair.

2. Properties of dynamic objects in microscopic images

Most light microscopes produce two-dimensional images of physical objects. Stereomicroscope allows us to obtain three-dimensional image and more realistic representation of the form of an object. But the limitation of the depth of field for such type of microscopes hinders us to receive distinct images. Only one direction can be used for photography of an object, and two-dimensional digital image is received in this case. Therefore, additional processing is required to receive a 3D image.

In modern medicine and biology, receiving and processing three-dimensional images of cell cultures is one of the problems faced. However, most of the equipment that is used in medical imaging is based on one sensor which means that only one image is the source of information for the analysis.

There are several methods of sensing of an object in an optical microscope to obtain volume characteristics (figure 1). It is believed that the lighting system provides a uniform illumination of the field of the microscope. In real life, the optical system of the microscope always has defects on the optical axis. Optical axis of the lightning system and imaging system are displaced relative to each other (figure 2). This causes an uneven illumination of the field of view and formation of the maximum lighting. As a result of this, different positions of moving objects forms shadows with different shapes. This feature allows us to obtain the projections of microscopic objects from different points of view. This method of determination of

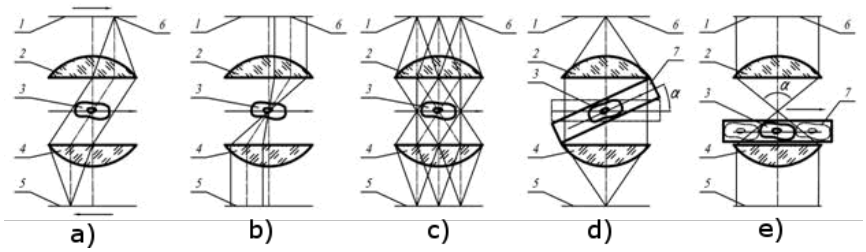


Figure 1. The scheme of sensing of an object in optical microscope: a-c) With scanning probe light beam relative to a stationary object; d, e) With the motion of the object relative to a fixed beam of probing light way. 1, 5 - Front and rear focal planes of the microscope objectives, 2, 4 – Micro-objectives, 3 - Object, 6 - Source 7 - Holder

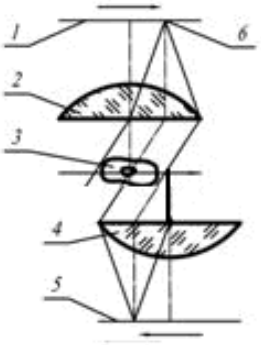


Figure 2. Scheme sensing an object in a microscope with a displaced optical axis

the correspondence between the fragments of two images appropriate to the same elements of the scene is fundamental of any system stereo reconstruction.

3. Construction of the anisotropy map

There are two main approaches for stereo reconstruction: local methods and global ones. Local methods are based on the computation of a function of two frames. There are functions such as AD (absolute differences), NCC (normalized

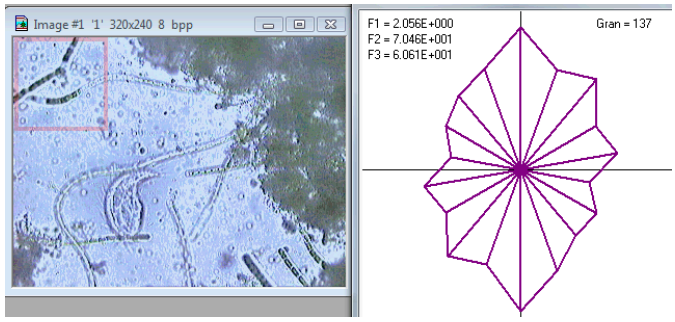


Figure 3. The distribution of the anisotropy for local region

cross correlation) and others. The accuracy of work of local methods is worse but they are faster. Smoothing of contour and incorrect detection of displacement on poorly textured regions due to lack of data is typical of them.

Global methods allows us to create a decision based on all the pixels of the image. They work better on poorly textured regions but the time for them is larger on one order of magnitude. There are several approaches to optimize the global function: simultaneous annealing, dynamic programming, belief propagation, graph cuts. Working time of simultaneous annealing method is very large. Dynamic programming is the fastest of global methods but this method does not provide a satisfactory image quality. Graph cuts method is one of the most modern methods. Its running time is sufficiently large but less than the time for the simultaneous annealing.

In this paper, we proposed a method for the stereo reconstruction based on determining the position of the object relative to the maximum lighting. To find the location of the maximum lighting, a specialized algorithm based on the anisotropy of the brightness of the image was developed. The anisotropy of the brightness gradient is calculated for each local region. It represents the general direction of the shadows for each image point (figure 3). As a result, the anisotropy map is created for the whole image based on the absolute values of the anisotropy. This map has strong smoothing and contrasting for best results. The minimum value of the anisotropy is at the point corresponding to the maximum lighting (figure 4). After determination of the direction of the maximum lighting shadows in the image can be found in (figure 5). After that, the stereo pair was obtain on a base of two images of the same object captured before and after displacement (figure 6).

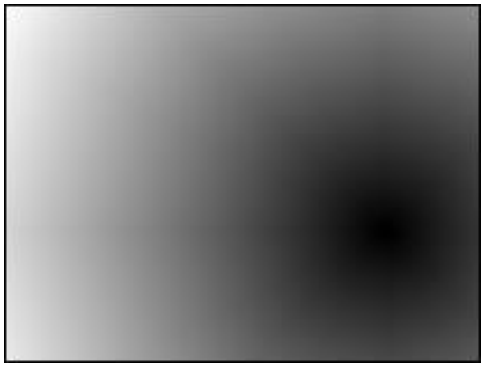


Figure 4. Map of anisotropy distribution

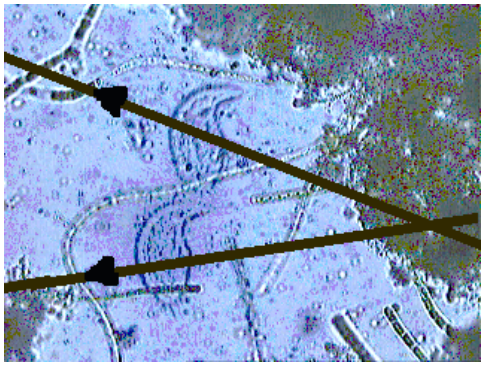


Figure 5. Determination of the direction of shadows in the image

However, solving this problem requires determination of the position of objects and their selection.

4. Segmentation of dynamic objects

There are many algorithms for dynamic objects extraction in images. Most of them are based on the determination of the background and its difference with the current image. The easiest way to determine the background is a creation of the histogram for each image pixel (figure 7).

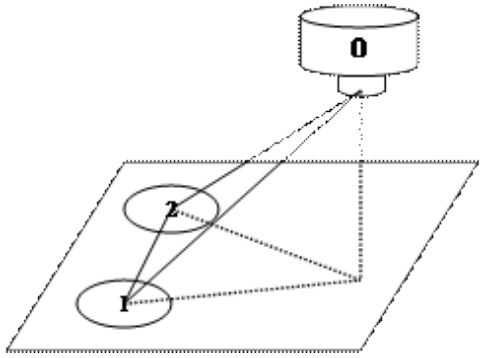


Figure 6. The optical scheme for obtaining a stereo pair: camera, the initial position of the object and the final one

The sequence of images taken at certain time intervals was represented as a cube. A profile of brightness for each pixel along a line across all the image sequence was obtained. Then the histogram of brightness was constructed. The median for each point of the sequence was determined by the histogram; its value has been assigned to the corresponding pixel of the background (figure 7).

As a result, a background image was created (figure 8). The resulting image of background has a slight error that occurs when noise of the camera superimposes on dynamic changes in the image. These noises are not big and they were removed by median filtering [3].

When the background image is known, it is easy to identify the position of the dynamic objects (figure 9) by calculating the modulus difference between the images (figure 10).

To determine the position of the object, binarization of the difference is necessary. Binarization is performed by the Otsu threshold segmentation [4] (figure 11). This method allows us to determine a threshold that minimizes the ratio of the combined variance to the variance between classes and separate the object pixels from background pixels. Binary image with multitype objects was obtained as a result (figure 11).

Determination of geometrical properties was performed for classification of objects. Objects that fall out of a certain interval of geometric features were removed (figure 12). Shape defects of objects are corrected by morphological operations and filling (figure 13).

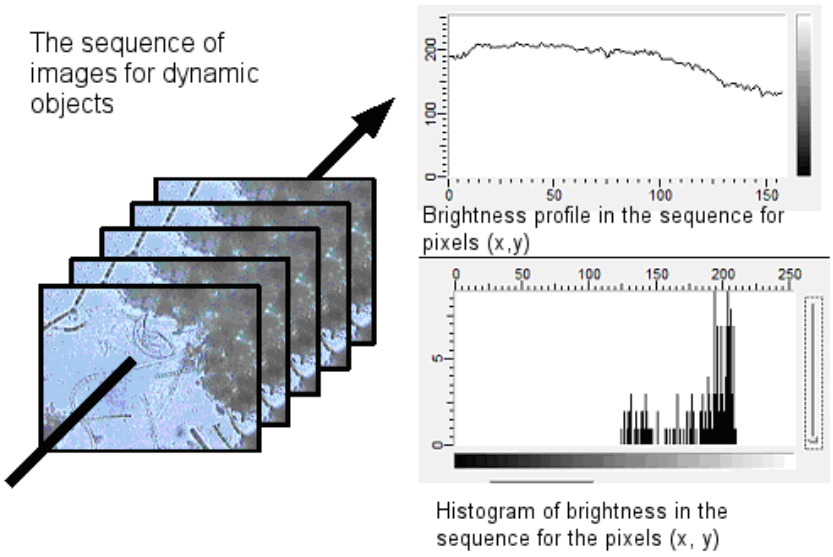


Figure 7. Determination of the background pixel values

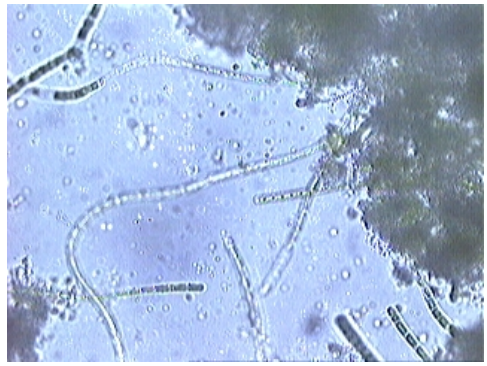


Figure 8. The synthesised background image

5. A construction of disparity map

A binary image of an object allows us to determine the center of mass, the minimum and maximum coordinates of the contour of the object, and the surface

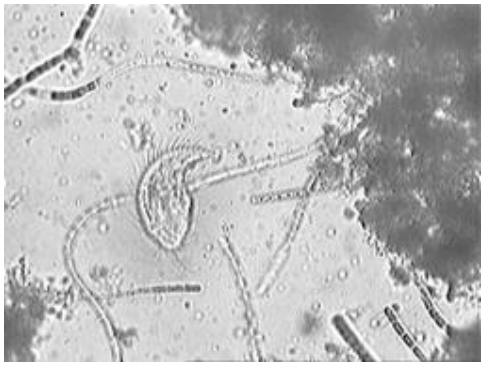


Figure 9. The original image of living cell

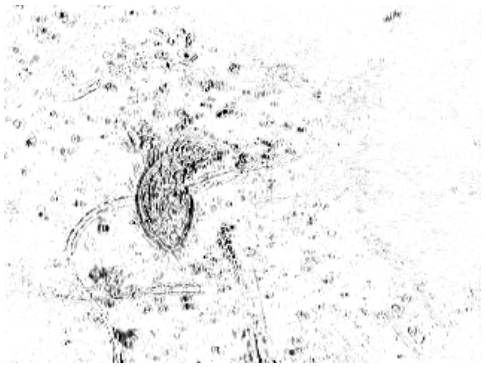


Figure 10. The modulus of difference of original image with the background

area. These characteristics are used to determine the parameters of the stereo pair for three-dimensional reconstruction of the object [5, 6, 7].

To determine the stereo-pair characteristics, it's necessary to construct a disparity map. In computer vision binocular, disparity refers to the difference in coordinates of the similar features between the right and left images [5, 6, 7]. To find correspondences in the images, the images must be converted to the form to be easy to compare. Otherwise, the comparison of the images will take a very long time as it is necessary to go through all the possible image rotations and scales. This is a very time-consuming process that can take several hours.

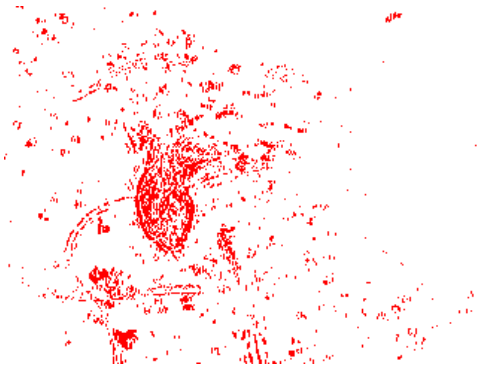


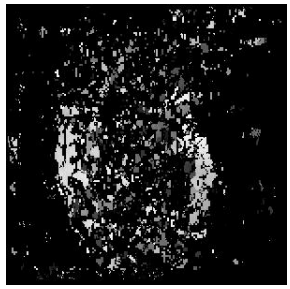
Figure 11. The binary image after thresholding

Figure 12. The binary image after removing small objects

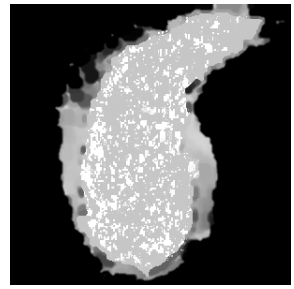
To significantly accelerate the comparison of images, their approximate comparison is done. The easiest way is to use reduced-size images. A comparison with different rotations and scales is then performed for each pixel of images. After finding the best solution, comparison is performed on full scale images [8].

The construction of the disparity map for stereo pair is quite a complicated process [9]. Uncertainty of the optical system of microscope leads to the appearance of shadows and distortions. This problem was solved by removing the shadows. The draft version of disparity map was constructed as result. The disparity map enhancement was carried out by means of mathematical morphology and watershed operations (figure 14).

Figure 13. Shape correction by morphological processing



a)



b)

Figure 14. Disparity map: a) draft version constructed by fragments, b) draft disparity map constructed by fragments

6. 3D measurements of the characteristics of dynamical cells images

A further description of the dynamic behavior of the microscopic object requires the formalization of the problem. The characteristics describing the behavior of the cells must be defined for this purpose. The most important characteristics are the change of volume, the change of shape, and the change of the position of the object.

Volume characteristics allow us to obtain important information about the cell structure for optical microscopy. Disparity map provides the estimation of such 3D

characteristics as volume, external surface, and shape on the base of a 2D image. These characteristics are determined on the base of the surface area of an object.

The external surface corresponds to the sum of the perimeters of all cross-section areas accordingly to z-coordinate. The perimeter characterizes the length of contour of an object. It is defined as the sum of distances between the boundary pixels. There are two types of perimeters. The first type consists of 4-connected boundary points (N_4) and second one consists of 8-connected (N_8) boundary points.

The number of diagonal connections is ($N_4 - N_8$) and the number of remained pixels in 8- neighborhood is ($N_8 - (N_4 - N_8)$). As a result, the general perimeter is determined as:

$$perimiter = (\sqrt{2} - 1) \cdot N_4 + (2 - \sqrt{2}) \cdot N_8. \quad (1)$$

The convexity is used for description of the shape complexity:

$$convexity = \frac{convexsurface}{surface}, \quad (2)$$

where convex surface is the entire boundary of a convex object calculated from new generated shape by convex hull algorithm. The convexity allows us to describe smoothness of a contour.

The brightness characteristics are also important for the description of the dynamical changes in cell. The brightness (average brightness) characterizes the mean optical density of object. The maximum brightness and the minimum one characterize the brightness range of an object. The dispersion of brightness characterizes the optical heterogeneity of an object. All these characteristics form a clear description of the changes in the cell and cell movement. Resulting stereo pair for volume shape reconstruction is presented in (figure 15).

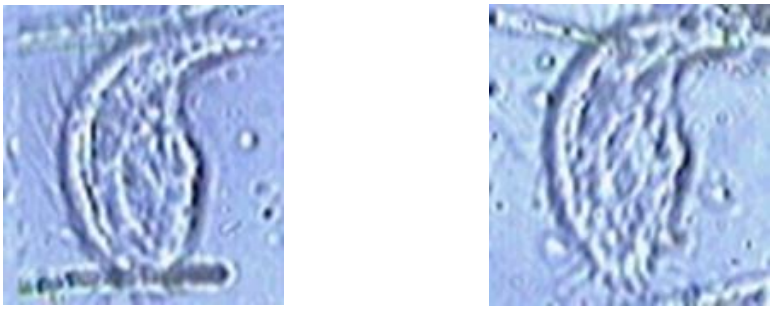


Figure 15. Resulting stereo pair for volume shape reconstruction

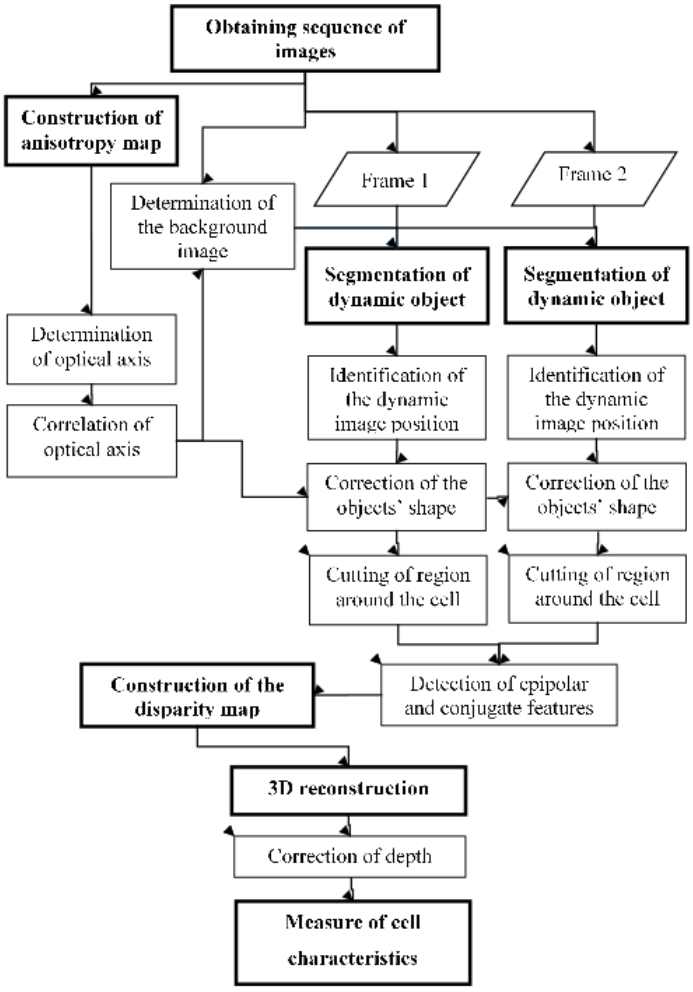


Figure 16. Scheme of algorithm to obtain a stereo-pair

7. General description of the algorithm

The algorithm described above is shown in (figure 16).

These tasks can be supplemented by calibration and pretreatment of images. However, pre-calibration is performed both with the settings of the optical system and pre-processing task strongly depends on the parameters of the original image. So these tasks were not included in the specified scheme. As a result, the algorithm identifies areas and the parameters of the projective transformation [10] to obtain a stereo-pair (figure 17).

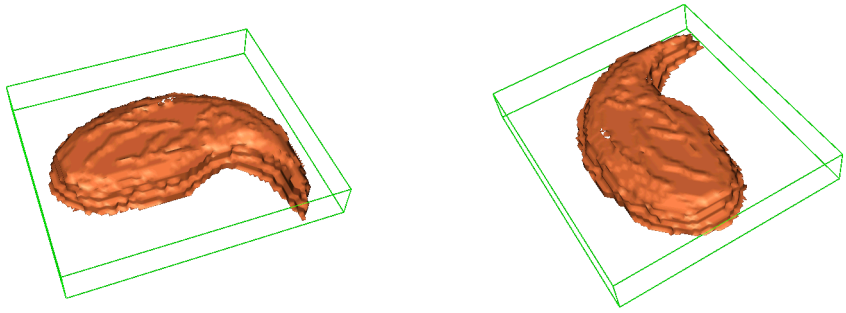


Figure 17. 3D cell reconstruction

8. Conclusions

The developed algorithm allows us to obtain the 3D object pattern of dynamic biological image based on the stereo-pair principle. Usage of additional stereo methods such as the segmentation of dynamic objects, determination of the optical axis, and correction of the disparity map allows us to use this algorithm for work with such complex objects as objects for a dynamic microscopic. Using combinations of these principles can quickly and efficiently determine the actual dimensions of the cell contour. Therefore, this algorithm is most effective for solving problems associated with the analysis of dynamical microscopic objects.

These results will contribute to the development of analytical software for cytological studies.

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