CONSTRUCTION OF A THREE-DIMENSIONAL IMAGE OF ERYTHROCYTES USING AN INTERFERENCE MICROSCOPE

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Abstract. *This paper examines the possibility of creating a biometric device for controlling the morphology of erythrocytes, the principle of which is based on the use of an improved method of spectral analysis using illumination with coherent waves to determine the dynamics of light absorption by phase objects, followed by mathematical processing of the obtained images.*

Keywords: *medical equipment, interference microscopy, spectral analysis, red blood cells, laboratory diagnostics.*

Introduction

In practical medicine, changes in blood composition caused by hematological diseases often cause a change in the morphology of its main component - erythrocytes. The morphological study of these organoids requires appropriate medical equipment that combines advances in nanoelectronics, biophysics, and microbiology. To improve the quality of determining the morphological parameters of erythrocytes, it is necessary to improve existing methods and laboratory equipment, which will open up new prospects for development in the field of laboratory diagnostics.

Existing methods and means of measuring the morphological parameters of erythrocytes have a number of significant shortcomings. They either measure planar coordinates in the projection of an erythrocyte onto a plane, like traditional methods of light microscopy, or have a rather labor-intensive technology at a high cost (like an electron microscope), which does not allow them to be effectively used in practical laboratory medicine.[1]

This paper examines the dwells into an idea of creating means of morphology analysis, devoid of these shortcomings. The authors propose to use an improved method of monitoring the morphology of phase micro-objects, based on the spectral analysis of their parameters when illuminated by coherent waves, followed by determining their light absorption parameters.

1. Features of erythrocyte and its morphology control

The morphology of erythrocytes is an important parameter to consider when analyzing blood. Normal erythrocyte has a form of biconcave disc. The main function of red blood cell is to transport oxygen through the bloodstream and its form helps maintain small volume but at the same time provides large enough surface area for diffusion. Protein layers overlapped by lipid sheets with total thickness of 20nm form erythrocytes outer membrane. Dimensions of normal erythrocyte are following: diameter – 7-8 µm, height depends on the distance from center of the cell ranging from 1,5 to 2,5 µm, 140 μm^2 of surface area and total volume of the cell lies in the range of 80 to 100 μm^3 [2–3].

In order to pass through the capillaries that are smaller than its dimensions erythrocyte change their shape, by twisting or folding. After passing through a narrow area, the erythrocyte returns to its original shape.

Deviation from the above parameters indicates certain diseases [4], which greatly simplifies their diagnosis.

For the most accurate analysis of the erythrocyte morphology, it is important to obtain its three-dimensional image. For this type of analysis microscopy methods which can only obtain a two-dimensional image of the samples are not suitable. A three-dimensional image, in turn, can be obtained using an electron microscope [5], but the economic aspect of this method outweighs its effectiveness.

Based on this, as an alternative, the authors propose to use an improved method of spectral analysis. The point of the improved method of spectral analysis consists in illumination by coherent waves of a phase object, defining its optical parameters such as the amount of light, and subsequent mathematical processing of the resulting image.

2. Improvement of the spectral analysis method

To practical use the proposed method of spectral analysis, a necessary device is required - a digital interference microscope, the main components of which are a source of coherent light, a microscope providing the necessary magnification, a digital camera as a photoreceiver of digital signals, and a computer running software that processes the received signal and result. The authors improved the device for the spectral analysis of erythrocytes, using for the study of erythrocyte blood samples and obtaining input digital images obtained by a coherent light source with wavelengths of 405, 560 and 650 nm [6-9].

The use of a coherent source with three wavelengths (fig. 1) allows you to see features of morphology that may not be visible in some spectrum, remove aberrations that could happen when light of one of the wavelengths passes through the sample and as a result increases the accuracy of the obtained three-dimensional image of erythrocytes.

a) b) c)

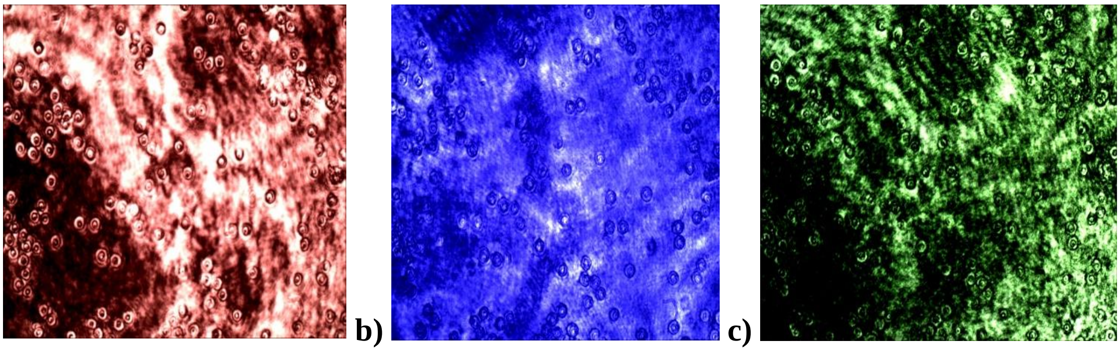


Figure 1. Image using red-a, blue-b and green-c light source

In this case, based on the laws of optical scattering of a light flux in a semitransparent medium, the brightness of the image at each point of its cross section will be proportional to the length of the path of the optical flux, and therefore will determine its geometric dimensions in the direction of its propagation.

Thus, the differential dependence of the change in brightness in two-dimensional space allows us to determine the third coordinate of the object's morphology, and build its 3-D model.

The result of the spectral analysis of the image may have distortions due to the aberrations of the optical system, however, these shortcomings can be eliminated by applying the method of simulating double annealing of the image, in relation to its 2D image (fig. 2 a,b).

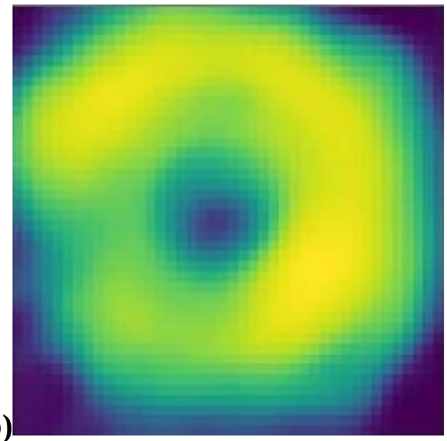
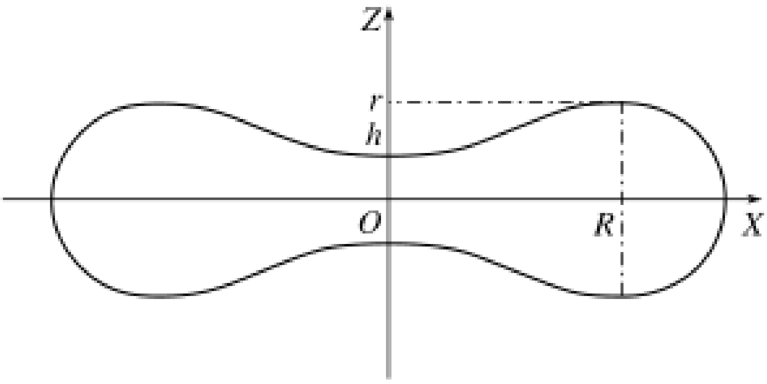
a)  **b)** 

Figure 2. Single erythrocyte image in red light(a) and after dual annealing (b)

The next stage of the analysis is the evaluation of the geometric parameters h, r, R of the erythrocyte surface in accordance with the proposed model of fig. 3. In order to obtain these parameters simulation of dual annealing is used but on three-dimensional scale.



a)

$$F(x) = \begin{cases} h - 3\dfrac{h-r}{R^2}x^2 + 2\dfrac{h-r}{R^3}x^3, & t \in [0, R], \\ \sqrt{r^2 - (x-R)^2}, & t \in [R, R+r]. \end{cases}$$

b)

Figure 3. Geometric model of normal erythrocyte section -
a and the system of equations describing it – b

Subsequent processing of the obtained experimental data is carried out using digital signal processing methods [10, 11].

Visualization of the resulting image of the erythrocyte can be obtained in the form of a height map, which allows you to make a qualitative assessment of it (Fig. 4).

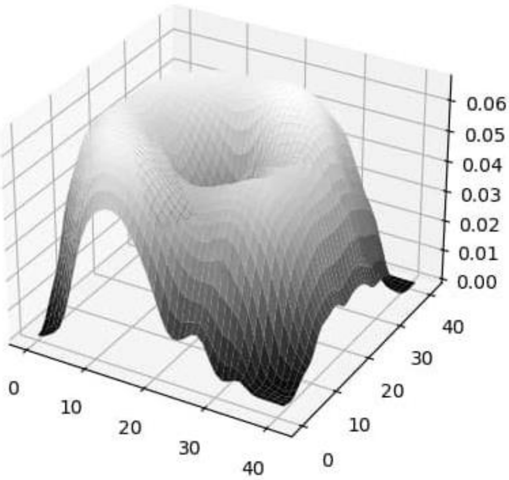


Figure 4. Resulting 3d image

Results show that the method is valid for analyzing micro-objects, however the restored three-dimensional surfaces contain restoration artifacts associated with the specifics of the shooting process, noise of the camera matrix, which can be removed at the next stages by applying classical image processing methods in the spatial domain, for example, Gaussian smoothing [12].

The use of the Fourier transform in the implementation of the digital signal processing method made it possible to obtain an algorithm for estimating the morphological parameters of translucent micro-objects with high accuracy in measuring geometric parameters. In turn, this made it possible to improve the quality of monitoring the state of erythrocyte plasma membranes and form the prerequisites for improving diagnostic methods for laboratory diagnosis of hematological diseases.

Conclusions

This research confirmed that the use of an improved method of spectral analysis with digital processing of images of erythrocytes obtained in three frequency ranges of coherent light, after further comparison of the results obtained, can significantly improve the accuracy of measuring the geometric parameters of erythrocytes.

The improved method of interference holography considered by the authors with the use of mathematical processing of its results makes it possible to improve the quality of the assessment of the state of the plasma membranes of erythrocytes in order to assess possible pathologies. The application of this method in practice will significantly reduce the cost of laboratory research, with a sufficiently high quality of results.

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