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Features of Application of the Experimental Stand for Reception of the New Measuring Information Concerning Morphological Signs of An Erythrocyte

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Abstract— **During the research work to increase the resolution of three-dimensional image of erythrocytes to determine morphological parameters, the Laboratory of Biomedical Electronics of NTU KhPI developed and manufactured an experimental stand with better characteristics. The peculiarities of using an experimental stand for obtaining new measuring information on morphological features of erythrocytes, its design and methods of use, which allows obtaining new measuring information on geometric and morphological features of erythrocytes, with its further use to obtain new laboratory clinical features and improve diagnosing the relevant pathological processes of the patient's body.**

Keywords— medical laboratory diagnostics, erythrocyte, morphological parameters, three-dimensional image.

I. INTRODUCTION

The idea of using morphological features of erythrocytes has recently received universal recognition, and research in this direction has been conducted by a number of research organizations. The task was reduced to obtaining a mathematical model of real erythrocytes, allowing to calculate its geometric parameters.

Currently, mathematical modeling of the shape and deformation of the erythrocyte is carried out on the basis of continuous and discrete approaches [1]. Within the framework of the first approach, the membrane is considered as a two-dimensional continuum. In this case, the equilibrium configuration of the considered medium under isotropic loading is maintained due to internal tension, bending moments, and transverse forces.

In this case, it is calculated on the basis of various variants of the nonlinear theory of thin-walled shells [2].The second approach uses the representation of the membrane as a system of elastically bound mesoscopic particles, the movement of which is calculated on the basis of classical (Newtonian) mechanics, taking into account the forces of elastic deformations of stretching, bending, the dissipative force of viscous friction, and the conditions limiting the area of the membrane and the volume of the cell, simulating the weak extensibility of the membrane and incompressibility of cell contents [3].

In work [4], a posteriori features of the multiple implementation of a two-dimensional model of the shape of an erythrocyte by the particle method are considered. The two-dimensional model only illustrates the possibilities of a discrete approach to erythrocyte simulation by the particle method. For an adequate description, it is necessary to use a three-dimensional model.

In works [5 - 7], an optical system is proposed that allows obtaining a three-dimensional model of an erythrocyte. The principle of operation of the digital holographic interference microscope, considered in this work, is as follows. Interferograms of the investigated microscopic phase interference objects are formed by superimposing two coherent laser beams, one of which passes through the object, and the other goes parallel. After superimposing the rays, an interference picture is formed. The shift of the interference bands corresponds to the height of the microobject at the point with the corresponding coordinate.

By recognizing the bands on the interferogram, filtering, skeletonizing and calculating the shift of the band at individual points of the image, the original three-dimensional shape of the studied object is restored. The disadvantage of the proposed method is the large size of the optical device, as well as the instability of the interference image to various types of vibrations and, as a result, the complexity of its practical application.

The authors of this work conducted research on increasing the resolution of the three-dimensional image of blood erythrocytes to determine morphological parameters [8 - 11].

At the same time, an experimental stand with improved characteristics was developed and manufactured as part of the CTW carried out in the Laboratory of Biomedical Electronics of NTU KhPI.

The stand was designed to obtain new measurement information regarding geometric and morphological features of erythrocytes, with subsequent use of it to obtain new clinical features and improve the diagnosis of the corresponding pathological processes of the patient's body.

Taking into account the main goal of this work - improving the method of determining the morphological features of erythrocytes by visualizing a three-dimensional image, research was carried out on optical methods of monitoring the morphological features of erythrocytes of blood, and the directions of the search for optimal methods capable of obtaining geometric parameters of erythrocytes with the desired density for qualitative analysis were determined.

During the work, the authors considered various ways to improve the quality of the image obtained by optical devices, namely:

- improvement of the method of obtaining three-dimensional images from two-dimensional images for the synthesis of panoramic images of single erythrocytes;
- improvement of a method of the spectral analysis on illumination by coherent waves for definition of parameter of dynamics of absorption of light by phase objects;
- improvement of the method of spectral analysis to determine the informative parameters of the geometric structure of erythrocytes.

When developing an experimental stand to obtain new measurement information on the morphological features of erythrocytes, it should be noted:

- the ability to dynamically change the elements of the optical scheme,
- reduced weight;
- the possibility of determining the effective ratios of the structural parameters of optical elements and other elements of the stand;
- improved technological parameters of the stand;
- universality of research algorithms.

II. GENERAL FEATURES OF CONSTRUCTION OF EXPERIMENTAL STAND WITH IMPROVED CHARACTERISTICS

Based on the research, a number of technical solutions were proposed, which were the basis for the creation of an experimental stand to obtain new measuring information on the morphological features of blood erythrocytes.

The final version of the basic optical scheme of the device is shown in Fig. 1.

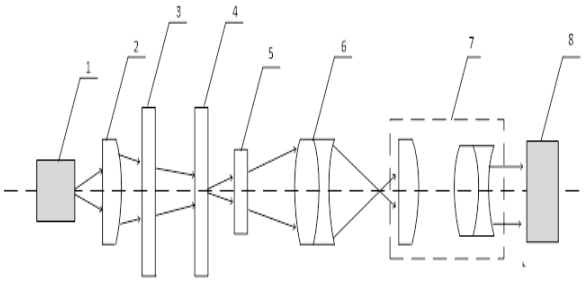


Fig. 1 - Schematic optical scheme of the experimental stand with improved characteristics: 1-coherent light source, 2 - condenser, 3 - polarizer, 4 - Goryaev camera with a sample, 5 - analyzer, 6 - microscope lens, 7 - digital camera eyepiece, 8 - photosensitive matrix.

In the basic optical scheme of the experimental stand with improved characteristics of Fig. 1, it is proposed to use two Galileo telescopic systems with different magnification, which will provide four options for the image size of the sample. The fifth option is achieved by completely removing the Galileo telescopic systems from the beam and inserting a tube with two diaphragms.

A semiconductor laser 1 with a radiation wavelength λ= 638 nm was used as the light source. The choice of this source was determined by its properties, the essence of which is to emit light in a narrow spectral range in the form of a directed, focused, highly coherent monochromatic beam of electromagnetic waves. Interference of light is obtained exclusively from the addition of coherent waves, ie waves that have the same frequency and time-constant phase difference.

The interference of light waves is obtained from a coherent source, in the proposed case of a laser, by dividing a monochromatic beam of light in two by means of a surface that partially reflects and partially refracts light. followed by superimposing them on each other. In order to determine the anisotropic areas in the sample, two polarization plates with mutually perpendicular direction of the optical axes were introduced into the beam. The first magnified image of the sample builds the microscope lens, and the final magnified image builds the eyepiece of the digital camera and builds it on a photosensitive matrix, which converts the optical signal into an electric one with the image output to a computer monitor. The experiment used a digital camera SIGETA M3CMOS 16000 16Mp USB3.0, which is an ocular digital camera with a high resolution of 16.0 Mpix.

The digital camera of this model can be used for stereoscopic, biological, metallographic microscopes. It is installed in an eyepiece tube or a separate optical port. The diameter of the chamber tube is designed for a standard tube width of 23.2 cm. For large tube diameters, two 30 and 30.5 mm adapters are included.

a)

b)

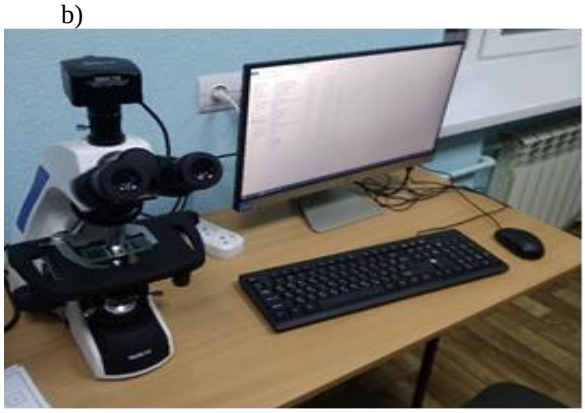


Fig. 2 - Appearance of the experimental stand with improved characteristics: a) front b) surface

The SIGETA M3CMOS 16000 eyepiece is powered via a USB cable that connects to the microscope via a USB3.0 interface.

ScopePhoto software (included on the CD), similar capture programs (ACD See, Amcap) and even photo editors such as PhotoShop are used to obtain images from the microscope. DShow & TWAIN driver, which is on the disk and is used to support third-party software.

Recommended software ScopePhoto is designed for translation and storage of images, functions for measuring linear and angular dimensions, editing, calculating the area of selected areas.

When capturing a video stream in maximum quality and setting a lower resolution, it is possible to watch video in real time at a specified speed. The appearance of the experimental stand with improved characteristics is shown in Fig. 2.

III. Assembly And Adjustment Of The Experimental Stand With Improved Characteristics.

The assembly of the experimental stand with improved characteristics was carried out in the Laboratory of Biomedical Electronics of the Department of Industrial and Biomedical Electronics of NTU KhPI. the object under study.

The basic basis for the sequential arrangement of optical and mechanical parts and components in general is the optical bench. Optical parts fixed in mechanical units were installed on the optical bench in accordance with the developed schematic diagram in a given order. It should be noted that the design of mechanical components must ensure compliance with all technical requirements for mounting optical parts in mechanical [12, 13].

Before attaching the optical parts to the mechanical, additional operations were performed to clean the surfaces of the optical elements. Mechanical parts that are in direct contact with the optical, such as: lens frames, glasses, intermediate and clamping rings of lenses, clamping bars of prisms, in the manufacturing process on machines are contaminated with cooling emulsions.

Therefore, for high-quality manufacturing of mechanisms and ensuring their operability during operation of the device, as well as in order to prevent contamination of optical parts in the process of assembling optical-mechanical components, washing of mechanical parts is provided. The process of washing mechanical parts is to remove various contaminants, degreasing all surfaces of the part. using aviation gasoline or petroleum ether, followed by drying with a stream of clean compressed air [14-16].

In accordance with the technical conditions for the manufacture and reception of optical-mechanical devices to the cleanliness of the surfaces of the optical parts of the device are high requirements. To meet these requirements in the assembly process it is necessary to clean the optical parts.

Optical details of the experimental stand should correspond to the I - III class of purity [17, 18]. Assembly is performed by ensuring the necessary mutual arrangement and interaction of circuit, especially optical, elements, among themselves or in relation to the design bases of assembly units and body parts.

Then the alignment is carried out, the essence of which is to bring the optical system of the device into working condition and to the compliance of the device with all the requirements of the technical conditions.

In the process of assembling optical-mechanical devices control their optical characteristics. The optical systems of all instruments must give high quality images, have specified characteristics (magnification, angular field), ensure high measurement accuracy and be reliable during operation.

The main condition that ensures high image quality is strict centering of optical elements. The essence of this operation is to combine the centers of curvature of all optical elements of the circuit with the optical axis of the device. And the optical axis must coincide with the geometric axis of the microscope tube.

To fulfill this condition, the design of mechanical parts provided for the possibility of longitudinal and transverse movements of optical and optical-mechanical components relative to each other. The designed mechanical units have three adjusting screws located at an angle of 120° relative to each other. The surface of mechanical parts was covered with black paint to eliminate light reflections.

After assembly and adjustment, control operations were performed to determine the quality of the image built by the optical system. The most common method of checking the quality of the image created by the optical system of the microscope is a visual assessment of the type of diffraction image of a glowing point.

The method is based on the study of light distribution in the diffraction image of an infinitely distant glowing point. lenses in frames, deviations in the centering of the lenses relative to the frames [21].

A diaphragm with a diameter of 0.02 mm is used to determine the image quality of a microobject constructed by an optical system. The microscope constructs an image of a point aperture, which creates a controlled lens (Fig. 3).

The following pictures of the field of view of the microscope are possible:

a) the image of the point is a bright unpainted spot surrounded by one or two concentric circles - the lens is assembled correctly (Fig. 3 a).

b) asymmetric image of the diffraction point means that there is a deviation in the centering of the lenses relative to the frames. The centering of the lens is considered satisfactory if the bright core of the diffraction image of the point when rotating the lens by 180 ° around the axis is slightly increased and the whole diffraction pattern does not change (Fig. 3b).

c) increased number of rings around the central spot - the presence of spherical aberration as a result of error in the length of air gaps or a large error in the manufacture of radii (Fig. 3 c).

d) the image of a point in the form of a cross, which passes when refocusing the microscope in a horizontal or vertical strip means the presence of astigmatism. The cause of astigmatism is the distortion of the lens surface in one direction, which is caused by deformation of the lens during its assembly or manufacture (Fig. 3 d).

e) the image of the point has a one-sided gap and when refocusing the microscope at the point of rupture of the enlarged halo of the point visible dark or light band crossing the halo means the heterogeneity of the refractive index in the glass (Fig.3e).

k) rupture of the first diffraction ring in the form of a tail means that one of the lenses on the edge there is a flooded surface - chamfer (Fig. 3k).

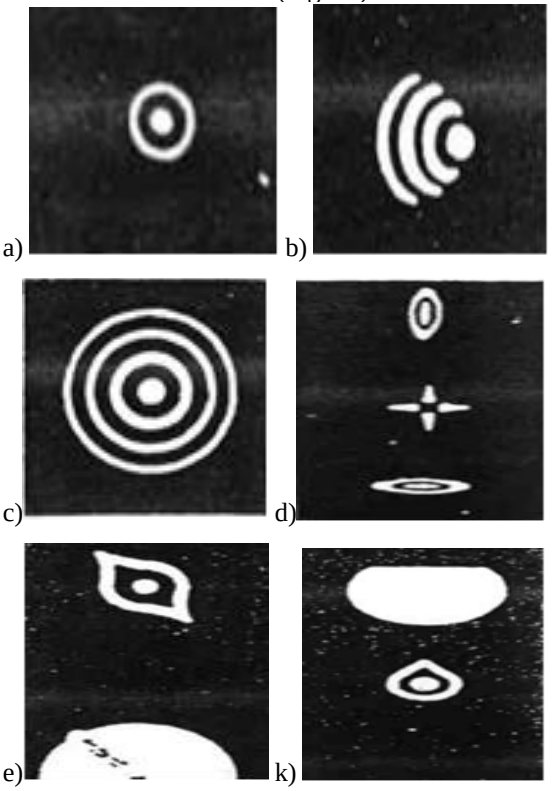


Figure 3 - Examples of diffraction point images: a) image of a point with a lens assembled correctly b) image of a point by a lens in which no centering is performed, c) image of a point by a lens with spherical aberration. d) image of a point by a lens having a non-constant radius of the surface in different meridians, e) image of a point by a lens having areas with different refractive indices, e) image of a point by a lens having a chamfer at the edge of the surface

The essence of control operations is that as a result of measurements and research to identify deviations of the actual characteristics of the optical system from the nominal values specified by the technical conditions. Errors and defects detected in the system are eliminated by adjusting, adjusting, replacing individual parts and components of the device [22].

IV. CALIBRATION OF THE EXPERIMENTAL STAND WITH IMPROVED CHARACTERICTICS

To test the magnification of the microscope, the Goryaev chamber grid was used, which is designed to count the formed elements of blood and cellular elements of cerebrospinal fluid (Fig. 4).

Technical data of Goryaev's camera:

- depth - 0.1 mm;
- area - 9 mm²;
- volume - 0.9 mm³ .

The chamber consists of a thick slide with transverse slots on it, forming three transversely located flat areas. The middle area is divided by a longitudinal slot into two sections, each of which has an engraved grid on it. On both sides of the middle platform in Goryaev's cell there are two others 0.1 mm above the average. The planes of these sites are used to grind the cover glass to the appearance of so-called Newtonian rings. After grinding the cover glass, a chamber is created, closed on two sides, and on the other two sides there are gaps (capillary space), through which the chamber is filled.

Fig. 4 - Microscope with Goryaev's camera located on the subject table

The microscopic grid of Goryaev's camera is drawn into large and small squares, grouped in different ways. Goryaev's grid contains 225 large squares (15 rows of 15 large squares in each), delineated vertically, horizontally, crosswise and undistributed. The sizes of small divisions of cells of a grid make 0.05 mm, and big - 0.2 mm. It is important that a small square with a side of 0.05 mm in all grids is a constant value. The area of the small square is 0.0025 mm2, and the area of the large square is 0.04 mm2. Then we obtain that the volume of liquid above the square formed by the large distributions of the Goryaev grid is 0.004 microliters.

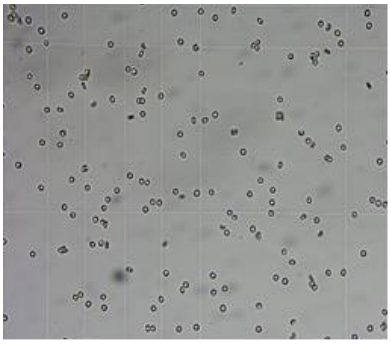
In addition to the intended use of Goryaev's camera to calculate the formed elements of the blood, this glass can be regarded as a kind of standard for determining the magnification of the microscope according to formula 1:

$$X = \frac{p_1 - p_2}{a \times N} \quad (1)$$

where: X - is the magnification of the microscope, lattice;
p_1 - is position of the left border of the Goryaev chamber cell;
p_2 - is the position of the right border of a cell or group of cells;
N - is the number of cells between the boundaries;
a - is the cell size of the Goryaev chamber (equal to 0.05 mm).

To calibrate the microscope magnification, a small square of the Goryaev camera grid was taken (Fig. 5).

a)



b)

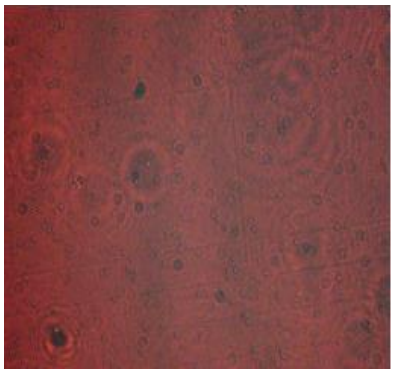


Fig. 5- Snapshot of the small square of the Goryaev camera grid: *a) in ordinary light, b) in coherent light*

The magnification of the microscope can be calculated by formula 2:

$$\Gamma_{мік} = \frac{l'}{l}. \quad (2)$$

where: l'– the value of the image of the Goryaev camera cell taken with the help of digital camera scales;

l – the size of the Goryaev chamber cell.

Fig. 6 - Snapshot of the plate inserted into the interference image

To test the program. allowing to construct a three-dimensional image of erythrocytes with superimposed vertical and horizontal scales, a picture of the interference pattern of the introduced plane-parallel plate of a given thickness was taken. 0.35 mm thick glass was used as a plate (Fig. 6).

V. METHODS OF USING THE EXPERIMENTAL STAND WITH IMPROVED CHARACTERISTICS TO OBTAIN A DIFRACTION IMAGE OF ERYTHRCYTES

Using the new qualities of laboratory equipment proposed in the experimental stand with improved characteristics, the authors have improved the method for obtaining images of erythrocytes.

For the study, the following algorithm was proposed for determining the morphological features of erythrocytes by visualizing a three-dimensional image:

1. Prepare a blood sample. Add 20 µl of blood to 4 ml of 0.9% NaCl solution and mix thoroughly.

2. Thoroughly wipe Goryaev's chamber and cover glass with a clean bandage slightly moistened with alcohol, then a gauze ball without alcohol. Grind the cover glass to the appearance of iridescent rings on both sides.

For better grinding, you can exhale a little air on the chamber and cover glass so that a small amount of moisture condenses on the glass surfaces to ensure better contact.

After grinding the cover glass, a chamber is created, closed on two sides, and on the other two there is a capillary space, through which the chamber is filled.

3. Place the prepared drug on the subject table.

4. Insert the digital camera connected to the computer via the USB cable into the eyepiece tube. ScopePhoto software (included on the CD) is used to obtain images from the microscope and must be pre-installed.

5. Connect the microscope illuminator to the mains by inserting a red laser into the light beam. The program for controlling the node responsible for the intensity of the laser radiation is given in Appendix D.

6. Gently move the microscope slide until a sharp image of erythrocytes appears on the computer monitor, using first coarse and then fine focusing screws.

7. Make panoramic views of the microscope.

8. Fine focusing screw to achieve diffraction image of erythrocytes formed by interfering

9. Take panoramic images of the field of view of the microscope.

10. To process the image by means of the offered algorithm of reception of a three-dimensional picture of an erythrocyte.

11. To detect anisotropic areas, enter the polarizer and analyzer in the course of the beam according to the location of the elements in the optical circuit. Rotating the analyzer around the optical axis of the device to achieve the most contrasting image of erythrocytes and take a screenshot.

13. When changing the magnification of the microscope, enter the lens smoothly into the course of the beam and focus with a fine guidance screw.

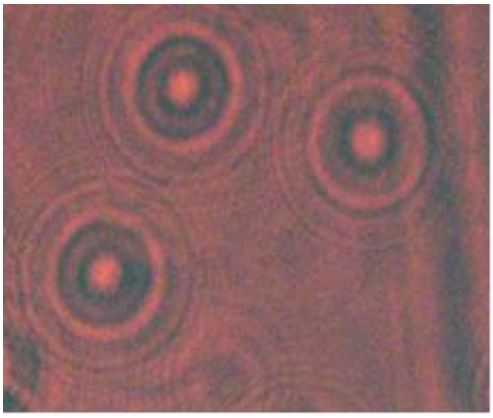
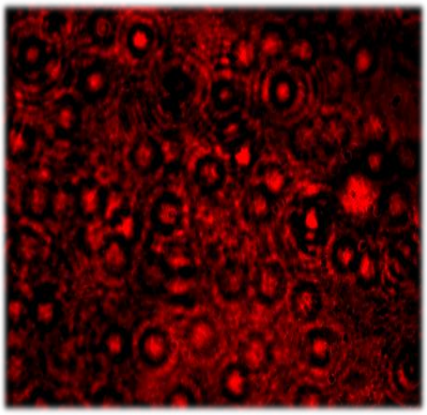
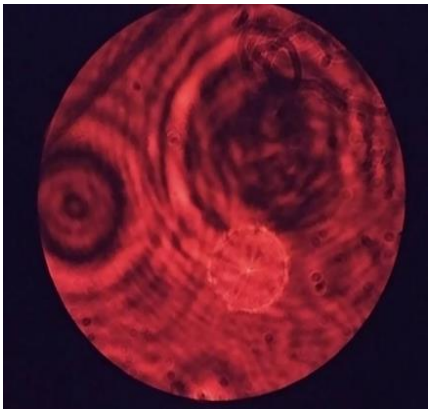


Fig. 7 - Diffraction image of erythrocytes formed by interfering rays



a)



b)

Fig. 8 - Interference pattern of erythrocytes in hypotonic solution with increasing: a) 400x and b) 900x.

For example, in Fig. 7 - 8 shows the photos obtained using the above method on the developed experimental stand with improved performance.

The obtained images are of sufficient quality for conducting research on the morphological features of human erythrocytes and further analysis.

VI. CONCLUSION

The peculiarities of application of the experimental stand for obtaining new measuring information on morphological features of erythrocytes of blood are given in the work.To improve the image quality of erythrocytes, the authors carried out targeted optimization of the optical elements of the device, which significantly reduced light loss with decreasing mass and dimensions of the device and allowed to obtain a clear image of microobjects with a standard size of 7.5-8.3 microns [23-24].

The optimal values of the numerical aperture of the lens were also selected, which provide sufficient resolution for this micro-object, namely in the range of 1-2 μm. The use of a multi-lens lens has reduced the aberrations of the optical system and improved the discreteness of the lens settings.

The introduction of polarizing light filters into the optical branch improves the quality and contrast of the interferogram, as well as the possibility of detecting anisotropic areas in the studied microobject. This significantly improves the optical and technical characteristics of the device (increasing the accuracy of the image relative to the object due to the optical magnification of x400 -x900 times) and simplifies the research process, thereby increasing the manufacturability of the process.

The experimental stand of the interference microscope developed as a result of work can be further used as a prototype of the medical device, for carrying out additional researches concerning morphology of erythrocytes and definition of superfluous diagnostic signs.

REFERENCES

[1] Ju M., Ye S.S., Namgung B. and et al. A review of numerical methods for red blood cell flow simulation // Computer Methods in Biomechanics and Biomedical Engineering. - 2015. - Vol. 18, No. 2. -P. 130 - 140.

[2] Pozrikidis C. Effect o f membrane bending stiffness on the deformation of capsules in simple shear flow // J Fluid Mech. - 2001. - Vol. 440. - P. 269 -291.

[3] Pivkin I.V, Karniadakis G.E. Accurate coarse-grained modeling of red blood cells // Phys. Rev. Lett. - 2008. - Vol. 101, No. 11: 118105.

[4] Golochalova, A.V. Features of calculating the two-dimensional shape of a human erythrocyte by the particle method / Natural science, engineering and economic research in technology, industry, medicine and agriculture: materials of the I Youth Scientific and Practical. conf. with international participation / Ministry of Education and Science of the Russian Federation, National Research University BelSU; under total ed. S.N. Devitsina. - Belgorod, 2017. - C. 409-412. http://dspace.bsu.edu.ru/handle/123456789/24967 (in Russia)

[5] Tishko T., Titar V., Tishko D., Nosov K. Digital holographic interference microscopy in the study of the 3D morphology and functionality of human blood erythrocytes .Laser Physics. 18. pp..486-490. 10.1134/s11490-008-4024-0.2.

[6] Safronov G.S., Tishko T.V., Forming difference holograms using orthogonal linear polarized waves, Ukrainsk. Fizich. Zh , 1989, 34, p.72-73.

[7] Tatyana Tishko; Tishko Dmitry; Titar Vladimir. Holographic microscopy of phase microscopic objects : theory and practice. London ; Singapore ; Beijing : World Scientific, cop. 2011.

[8] Y.I. Sokol, K.V. Kolesnik, S.G. Panibrattseva, T.V. Bernadskaya. “Methods of three-dimensional visualization of morphology of microobjects”. Materials of the II MNPK "Actual problems of automation and instrument making". Ukraine. Kharkiv: NTU KhPI. 2020. pp. 82-83. (in Russian).

[9] Y.I. Sokol , K.V. Kolisnyk, S.G. Panibrattseva, T.V. Bernadskaya, “The Use of Digital Interferometry Devices to Analyze the State of Red Blood Cell Membranes. 2020 IEEE KhPI Week on Advanced Technology. Kharkov. pp. 373-377. DOI:10.1109/KhPIWeek51551.2020.9250137

[10] Y.I. Sokol, K.V. Kolisnyk, R.S. Tomashevskyi, T.V. Bernadskaya, “Improving the Method of Interference Holography to Determine the State of Plasma Membranes”, IEEE 39ths international conference on electronics and nanotechnology / ELNANO-2019. Igor Sicorski Kyiv Polstechnic insninune . Kyiv. 2019. pp. 157-164. DOI: 10.1109/ELNANO.2019.8783675

[11] Y.I. Sokol, K.V. Kolisnyk, S.G. Panibrattseva, T.V. Bernadskaya, “Improving the accuracy of measuring the morphology of red blood cells”. 2020 IEEE 4thInternational Conference on Intelligent Energy and Power Systems. 2020. Istanbul. Turkey. pp.249-254. DOI: 10.1109/IEPS51250.2020.92631

[12] Panov V.A., Kruger M.Ya. Handbook of the designer of optical and mechanical devices. Improved ed. Panova VA. 3rd ed., Reworked. and ext. L .: Mashinostroenie, 1980. 742 P.

[13] 71. 71. Bardin A.N. Assembly and adjustment of optical devices. Moscow: Higher School, 1968. p. 115-250.

[14] Elnikov NT, Ditev AF, Yurusov IK Assembly and adjustment of optical-mechanical devices. Moscow: Mashinostroenie, 1974. pp. 156-272.

[15] Popov L.M. Handbook of the designer of optical and mechanical devices. Leningrad: Mashinostroenie, 1976. pp. 148-276.

[16] Fatykhov R.K., Fatykhov R.F., Kravtsov E.A. Control of optical and mechanical devices. Moscow: Mashinostroenie, 1988. 228 p.

[17] Semibratov M.N., Zubakov V.G. Technology of manufacturing optical parts. Moscow: Mashinostroenie, 1978. 248P.

[18] 76. Afasansev V.D. Optical measurements. M .: Higher School, 1981. 229 p.

[19] Creopalova G.V., Pureev D.T. Research and control of optical systems. M .: Mashinostroenie, 1978. pp. 78-179.

[20] Creopalova G.V., Lazoreva N.L., Pureev DT Optical measurements. M: Mashinostroenie, 1977. 264 s.

[21] Kuznetsov S.M., Okatov M.A. Handbook of optics technologists. L: Mashinostroenie, 1983. p.149-268.

[22] Bubis I.Ya., Weidenbach V.A., Dukhopel I.I. Handbook of optics technologists. Leningrad: Mashinostroenie, 1982. pp. 254-316.

[23] Bernadskaya TV, Kolesnik KV, Vodka AA, Tomashevsky RS Improvement of optical methods for studying the morphology of blood erythrocytes. Materials of MNPK "Modern information and electronic technologies". Ukraine. Odesa. 2020. P.106-109.

[24] Bernadskaya T.V., Kolesnik K.V., Tomashevsky R.S. Method of obtaining interference holographic image of microobjects. Proceedings of the First International Scientific and Practical Conference "Actual Problems of Automation and Instrument Making". Kharkiv: NTU KhPI. 2018. pp. 60–61.