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Measurement of Red Blood Cell Geometry Using Holographic Interferometry

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Problems associated with changes in the shape and size of red blood cells in patients with atherosclerosis are considered. The applicability of physical optics techniques (in particular, holography) for the examination of red blood cells is discussed. An interferographic technique for analyzing the shape of red blood cells and estimating quantitatively their cross section and size is suggested. Experimental data on the comparative characteristics of red blood cells in normal and pathological states are presented.

Introduction

According to WHO, in industrially developed countries the cardiovascular mortality constitutes 46% of the total mortality. Atherosclerosis is the most frequent cardiovascular disease. It often affects coronary arteries, leading to the development of cardiac ischemia and myocardial infarction. Changes in red blood cell geometry are among the symptoms of atherosclerosis. Red blood cells are shaped as biconcave discs 6-7 μm in diameter [1-3]. Their main constituent is hemoglobin. Red blood cells have antigenic properties and participate in hemostasis. However, their main purpose is to release oxygen into tissues and carry some of the carbon dioxide back from the tissues. Red blood cells respond to biochemical and physicochemical effects of atherosclerosis by changing their shape.

All existing techniques for measuring the red blood cell size [2-4], concentration, and shape can be divided into two groups: visual and automated methods. The visual methods can be subdivided into optical, electron, and laser scanning microscopy; the automated methods, into those based on measurement of the scattering pattern and on measurement of the permittivity. However, all these methods are not free from disadvantages. Their common disadvantage is that measurements are performed in red blood cells whose intra vitam properties are not conserved. This disadvantage can be avoided using a physical optics technique (holography) [4-6]. This method has a number of advantages: it is contactless and provides high precision and high information value of measurement results. Up to this date, holography has not been applied to examination of blood cells. Techniques developed for holographic examination of other types of microscopic objects cannot be used without modification for studying red blood cells. The goal of this work was to assess the applicability of holographic interferometry for the examination of red blood cell geometry.

To attain this goal, the following problems should be solved:

– hologram recording geometry and wavefront reconstruction process should be selected;

– experimental technique should be developed;

– red blood cell size should be measured using the developed technique.

Experimental Interferography of Red Blood Cells

At this stage of the study, the problem of obtaining experimental interferograms of red blood cells for quanti-

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tative evaluation of their size and shape had to be solved. For this purpose, the optical circuit of the experimental set-up was optimized and properly aligned. Experimental interferograms of red blood cells were obtained in two stages. First, the hologram was recorded. Then, holographic interferograms of red blood cells were observed and recorded. Let us consider both stages in more detail.

First stage. The optical circuit for recording holographic interferograms of red blood cells is shown in Fig. 1. He-Ne laser 1 radiation is received by a telescopic system of two lenses 2 and 4. The aperture 3 installed in the focal plane of the system isolates the single-mode component of the radiation beam, which is then split by the beam splitter 5 into the object and reference beams. The object beam is reflected by the mirror 6, focused by the condenser 7 on the surface of the optically homogeneous object glass 8, and transmitted through the objective 9 to the hologram 10. The reference beam is reflected from the mirror 12 and passes through optical system 13 and 14 to the hologram 10.

In the experiment, in addition to PFG-01 photographic plates, PFG-03 red-sensitized (0.6328 μm) photographic plates were used for recording holograms. The modes of exposure and photochemical processing of holograms had been optimized. The exposure time was 2 s. The non-emulsion side of the holographic plate faced the object.

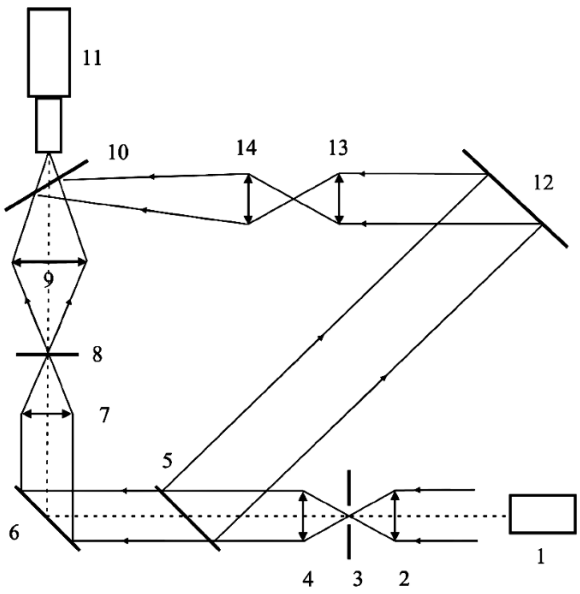

Fig. 1. Optical circuit of the experimental set-up: 1) laser; 2-4, 13-14) telescopic systems; 3) aperture; 5) beam splitter; 6, 12) mirrors; 7, 9) objectives; 8) object glass; 10) hologram; 11) microscope.

Before recording, photographic plates were treated for 5 min with 10% triethanolamine solution and dried. Hologram processing with 20 mL Petrov developer in 400 mL H_2O took 7 min.

After photochemical processing, the plate was installed in the same position, tilted by a small angle of several arc minutes, and exposed to the reference beam. Considering that a hologram of a phase object (object glass) was recorded and the plate had been removed from the exposure site for development, installation and reconstruction of the hologram gave rise to a system of straight interference fringes observed with the microscope. The fringe period depended on the plate tilt with respect to its original position. Interferograms of this type are called interferograms with fringes of finite width [7-10].

Second stage. At the stage of observation, objects under study (red blood cells) were smeared on the object glass. The object glass was then placed in the observation position. At this stage, the radiation passes through the object (red blood cell), which leads to deformation of its wavefront according to the phase structure (thickness) of the object.

Then, the radiation passes through the objective and the hologram. Superposition of the object-scattered wave and the wave reconstructed from the hologram using the reference beam leads to an interference pattern being formed behind the hologram. The interference pattern formed by alternating straight dark and light fringes can be visually observed with the microscope [8-11]. These fringes become curved as a red blood cell is brought into the objective field. The deviation of the interference fringe at given point bears information about the red blood cell thickness at this point. Calculating the cell thickness values along its diameter makes it possible to construct its cross section. This, in turn, allows (considering red blood cells as axially symmetric) the cell volume and surface area to be calculated.

Experimental interferograms of red blood cells were recorded on a photographic film with sensitivity of 64 units. Microscope eyepiece with 25× magnification was used. The aperture was 1:4; the exposure time, 1/125, 1/250.

The resulting fringe pattern is shown in Fig. 2. It allows the red blood cell thickness to be determined with an accuracy of up to 0.06 μm.

Calculation of Red Blood Cell Cross-Section Size from Interferograms

Figure 3 illustrates the procedure for calculation of red blood cell cross-section size from interferograms.

The deviation of the interference fringe from the straight line caused by the red blood cell is determined by the following equation [12]:

$$2\pi\frac{\Delta h(x, y)}{S} = \frac{2\pi}{\lambda}T(x, y)\Delta n(x, y),$$

where $\Delta h(x, y)$ is the deviation of the interference fringe from the straight line, S is the spacing of interference fringes, $T(x, y)$ is the red blood cell thickness at given point, $\Delta n(x, y)$ is the difference of refraction indices inside and outside the cell, and λ is the wavelength of He-Ne laser (0.6328 μm).

It follows from Eq. (12) that

$$T(x, y) = \frac{\lambda\Delta h(x, y)}{S\Delta n(x, y)}.$$

Thus, the red blood cell cross-section size $T(x, y)$ can be determined by measuring the spacing of interference fringes S and the fringe deviation $\Delta h(x, y)$, provided that the difference $\Delta n(x, y)$ of refraction indices inside and outside the cell is known. For a red blood cell, $n = 1.4$. Therefore, $\Delta n(x, y) = 0.4$.

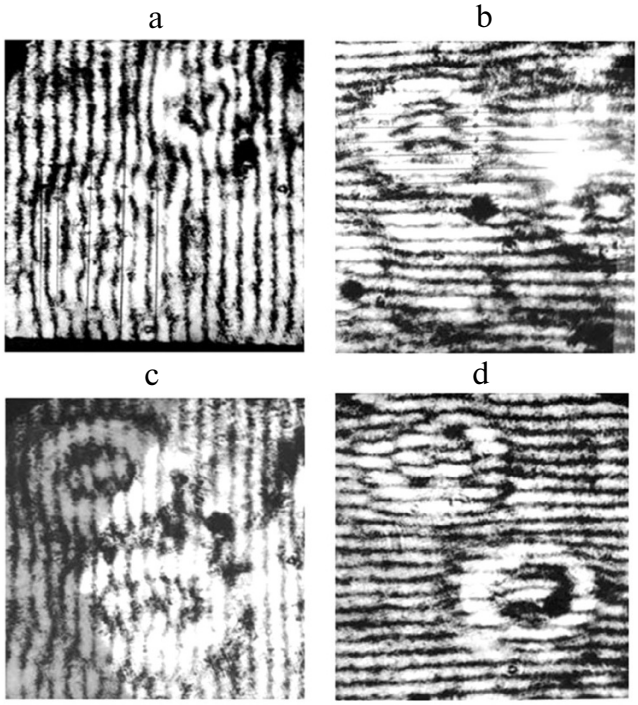

Fig. 2. Red blood cell interferograms.

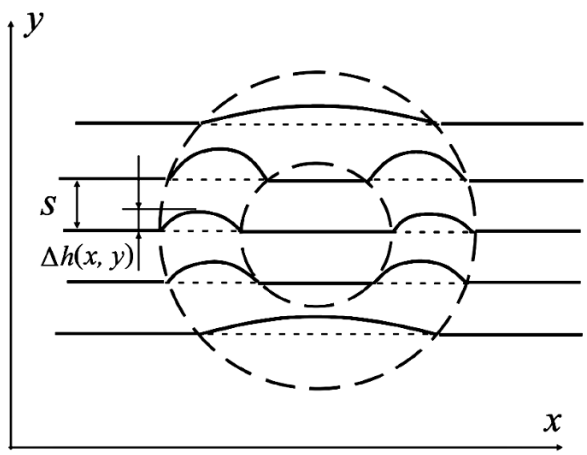

Fig. 3. Calculation of red blood cell cross-section size from interferograms: explanatory scheme.

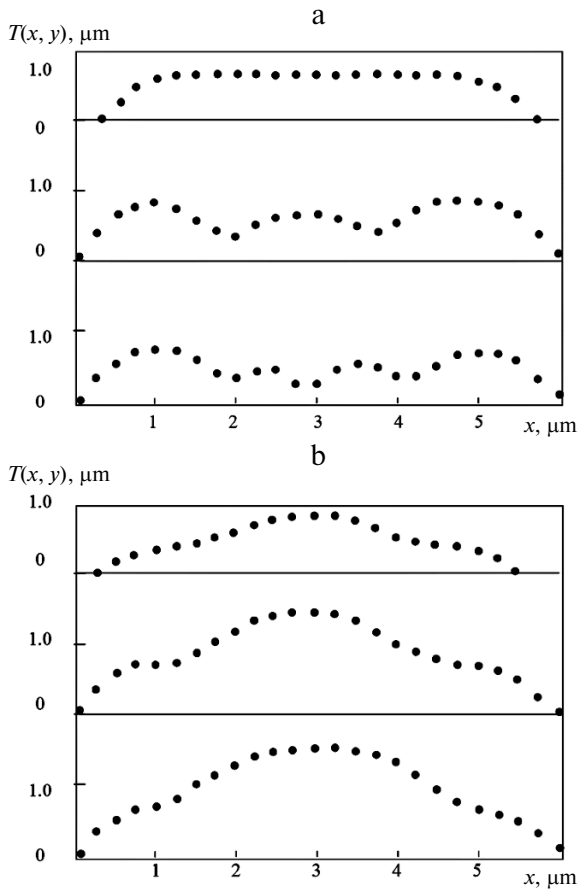

Fig. 4. Red blood cell cross section (as determined from interferograms); a) norm; b) pathology.

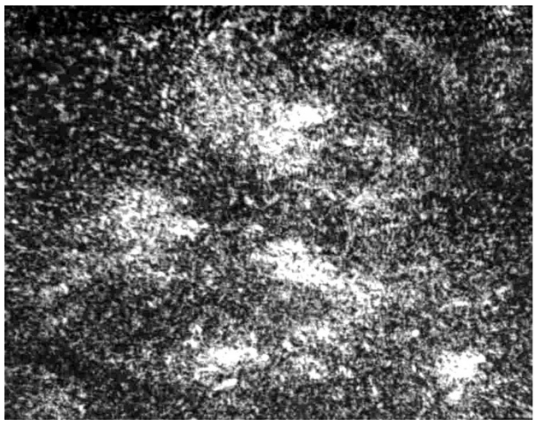

Fig. 5. Interferogram of cuvette with red blood cell solution.

The results of calculation of the red blood cell cross section from the obtained interferograms are shown in Fig. 4. An attempt was also made to obtain interferograms for red blood cells in a solution. However, because of strong scattering, these interferograms contained only coherent noise [4, 5, 10, 11] (Fig. 5).

Conclusions

Thus, the experimental set-up for interferographic examination of red blood cells has been presented. The procedure for photographic recording of experimental interferograms of red blood cells has been considered in detail. The technique for obtaining and analyzing red blood cell interferograms with subsequent calculation of the cell cross-section size has been described and the results of calculation have been presented. The suggested technique has been shown to provide a novel opportunity for studying the shape of normal and pathological cells.

Technoeconomic analysis of the suggested technique shows it to hold much promise for practical applications [13-15]. Its accuracy matches that of the methods described in [1-4], while its information value is comparable to that of the devices described in [6-11]. As for its economic features, the cost of the basic set-up and the operating cost remain the same, while the cost of expendable materials at the stage of applicability testing is negligible.

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