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Measuring skewness of red blood cell deformability distribution by laser ektacytometry

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Abstract. **An algorithm is proposed for measuring the parameters of red blood cell deformability distribution based on laser diffractometry of red blood cells in shear flow (ektacytometry). The algorithm is tested on specially prepared samples of rat blood. In these experiments we succeeded in measuring the mean deformability, deformability variance and skewness of red blood cell deformability distribution with errors of 10%, 15% and 35%, respectively.**

Keywords: *red blood cells, the deformability, laser ektacytometry.*

1. Introduction

Diagnosis and treatment of many diseases is highly dependent on the ability to constantly monitor such a characteristic of human blood as red blood cell deformability distribution [1]. In principle, this can be done by photographing red blood cells in shear flow [2]. However, this method proposed in 2002 and also known as rheoscopy has not found a wide practical application so far; therefore, the search for alternative solutions is still urgent. We believe that the distribution of red blood cell deformability can be measured by using laser diffractometry of red blood cells in shear flow (ektacytometry).

Laser ektacytometry is a method for measuring the deformability of red blood cells, based on recording and analysis of diffraction patterns. A diffraction pattern is observed when a laser beam is scattered on a suspension of red blood cells deformed in shear flow by viscous friction forces. In a rotational ektacytometre this flow is produced in a Couette cell. The diffraction pattern is analysed using iso-intensity curves, i.e., curves on the observation screen, on which the intensity of the scattered light has a constant value. Usually the iso-intensity curve is approximated by an ellipse. Then the ratio of its semiaxes, which is a measure of red blood cell deformation in shear flow, is found. Deformation as a function of shear stress characterises the deformability of red blood cells, which is determined in these experiments. Studies show that red blood cell deformability, greatly affecting the blood microcirculation, on the one hand, depends on the state of the human body, and on the other, largely determines this state. For example, in patients with acute cerebral ischemia deformability of red blood cells is markedly lower than in healthy people of the same age [3]. In this and in other cases, laser ektacytometry helps identify the rheological disturbance, assess its extent and choose the appropriate treatment. In more detail the method of laser ektacytometry of red blood cells is described elsewhere [3–7].

Laser ektacytometry was proposed in 1975 [4] and has since been used without substantial changes, allowing one to estimate only the mean deformability of red blood cells in a blood sample under study. The question arises whether it is possible to measure by this method the red blood cell deformability distribution, or at least to assess the basic parameters of this distribution? To answer this question, we have investigated theoretically the scattering of a laser beam on an ensemble of elliptical discs simulating blood cells [3, 6, 7]. We have shown [7] that the method of laser ektacytometry allows one to evaluate the spread of red blood cell deformability. In this evaluation, it is necessary to know the shape of the iso-intensity curve and the scattered light intensity on this curve relative to the intensity of the central diffraction maximum. These results are directly applicable in the case of a symmetric distribution of red blood cell deformability. Verification of the algorithm [7] with the help of the experimental data [8] obtained from specially prepared blood samples showed that it can produce reliable data. The next step is to develop an algorithm for measuring the third momentum of the red blood deformability distribution, i.e., the distribution skewness. In this paper we consider the problem of measuring this parameter on the basis of the data of red blood cell ektacytometry.

2. Model of an ensemble of red blood cells

As in [3, 6, 7], we will represent red blood cells in the form of transparent elliptical discs. The basis for such a model are the images of red blood cells in shear flow, obtained using a microscope in [2]. In addition, in the region of small-angle scattering, the phase functions of flat and biconcave discs hardly differ from each other [7]. We assume that the discs have approximately the same surface areas and volumes but different eccentricity (elongation). The shape of the red blood cell is treated as a random quantity and is characterised by such concepts as the mean value, variance and shape distribution skewness. This model allows us to describe the population of red blood cells, taking into account the fact that different red blood cells differ in their ability to deform. In more detail our model is described elsewhere [6, 7].

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Semiaxes of the bases of elliptical discs simulating red blood cells are considered random variables and described by the formulas

$$a = a_0(1 + \varepsilon),\ b = b_0(1 - \varepsilon). \tag{1}$$

Here, a_0 and b_0 are the average lengths of the semiaxes, and ε is a random parameter of the particle shape. We assume that the inhomogeneity of the ensemble with respect to the shape of the particles is relatively weak, i.e., $|\varepsilon| \ll 1$. We also assume that the average value of the parameter is $\langle\varepsilon\rangle = 0$. The characteristics of the distribution of red blood cells with respect to the shapes are the statistical moments of the quantity ε, namely the particle shape distribution variance and skewness

$$\mu \equiv \langle\varepsilon^2\rangle \text{ and } \nu \equiv \langle\varepsilon^3\rangle \tag{2}$$

respectively. The condition $|\varepsilon| \ll 1$ leads to restrictions on these parameters

$$\mu, \nu \ll 1. \tag{3}$$

Below, we discuss the question of how to determine the parameters $s = a_0/b_0$, μ and ν, using the experimental data of laser ektacytometry. To this end, we generalise the theory developed in our papers [6, 7] to the case of an inhomogeneous ensemble with a skewed distribution of particle shapes.

3. Shape of the iso-intensity curve

For the shape of the iso-intensity curve to be calculated, we have assumed that red blood cells in shear flow of the ektacytomer represent an inhomogeneous ensemble of transparent elliptical discs. Calculations have shown that in the region of the observation screen (the boundary of the central diffraction maximum or the first minimum of the diffraction pattern), the shape of the iso-intensity curve is approximately described by the expression

$$r = 1 - \sqrt{f} + \mu\frac{\cos^2 2\varphi}{2\sqrt{f}} + \nu\cos 2\varphi\left(1 - \frac{2 - 3\sin^2 2\varphi}{2\sqrt{f}}\right), \tag{4}$$

where

$$f = \frac{1}{4\beta^2}\frac{I}{I(0)}(1 - 2\mu) \tag{5}$$

is the normalised intensity of scattered light; I is the intensity of scattered light on a given iso-intensity curve; $I(0)$ is the intensity of the central diffraction maximum; and $\beta = -0.4$ is a parameter of the Bessel function. The quantities r and φ are the generalised polar coordinates of a point on the observation screen and are defined by the expressions

$$x = Ar\cos\varphi,\ y = Br\sin\varphi. \tag{6}$$

Here, x and y are the Cartesian coordinates of a point on the observation screen in a coordinate system whose origin is located in the centre of the diffraction pattern (point of incidence of the laser beam). The x axis is directed horizontally (parallel to the shear flow in the ektacytometre), and the y axis – vertically (perpendicular to it). The parameters

$$A = m_1 z/(ka_0) \text{ and } B = m_1 z/(kb_0) \tag{7}$$

determine the size of the diffraction pattern (i.e., distances from the centre of the pattern to the first diffraction minimum along the horizontal and vertical directions, respectively); z is the distance from the measuring volume to the observation screen; $k = 2\pi/\lambda$ is the wavenumber; λ is the wavelength; and $m_1 = 3.82$ is the root of the Bessel function J_1. It will be convenient to represent expression (4) in the form

$$r = r_0 + \mu f_2(\varphi) + \nu f_3(\varphi), \tag{8}$$

where

$$r_0 = 1 - \sqrt{f_0};\ f_2(\varphi) = \sqrt{f_0} + \frac{\cos^2 2\varphi}{2\sqrt{f_0}};$$
$$f_3(\varphi) = \left(1 - \frac{2 - 3\sin^2 2\varphi}{2\sqrt{f_0}}\right)\cos 2\varphi; \tag{9}$$

$$f_0 = \frac{1}{4\beta^2}\frac{I}{I(0)}. \tag{10}$$

The reason for introducing the parameter f_0 is that it is the characteristic of the diffraction pattern only. At the same time, the parameter f (5) depends both on the appearance of the diffraction pattern and the properties of the red blood cell ensemble under study. Between the parameters f and f_0, there is an obvious relation: $f = f_0(1 - 2\mu)$, which consequently leads to $\sqrt{f} \approx \sqrt{f_0} - \mu\sqrt{f_0}$ because of the smallness of μ. This equality is used in deriving (8) and (9). Thus, formulas (8)–(10) approximately describe the shape of the iso-intensity curve in polar coordinates with account for the red blood cell shape distribution skewness.

4. Polar points of the iso-intensity curve

Let us call polar such points on the iso-intensity curves, in which these curves intersect the Cartesian coordinate axes x and y. Let x_p and y_p be polar coordinates of points on the horizontal and vertical coordinate axes, respectively. Both of these quantities can be measured experimentally. We calculate the ratio $D = y_p/x_p$. Using formulas (6), (8), (9) and taking into account the smallness of ν, we obtain

$$D = \frac{B}{A}\left(1 + 2\frac{\nu}{\sqrt{f_0}}\right). \tag{11}$$

From (11) it follows that due to the skewness of the particle shape distribution, the iso-intensity curve either elongates or flattens, depending on the sign of ν. It also shows that in the case of a skewed distribution of the ensemble of particles in shapes, the ratio of the semiaxes of the iso-intensity curve is not equal to the average ratio of the semiaxes of the particles, as is usually assumed in the laser ektacytometry of red blood cells.

The final result is represented in the form

$$D/s = 1 + \nu q_0, \tag{12}$$

where $s = a_0/b_0 = B/A$ and

$$q_0 = 2/\sqrt{f_0}. \tag{13}$$

5. Curvature of the iso-intensity curve in polar points

Other characteristics of the iso-intensity curves, which can be measured experimentally, are the radii of curvature of the curve in polar points $R(x_p)$ and $R(y_p)$. For convenience we introduce dimensionless parameters characterising the curvature of the iso-intensity curve in polar points:

$$C_1 = \sqrt{x_p/R(x_p)},\ C_2 = \sqrt{y_p/R(y_p)}. \tag{14}$$

The radii of curvature can be calculated by the formulas

$$\frac{1}{R(x_p)} = \left|\frac{d^2x_p}{dy^2}\right|,\ \frac{1}{R(y_p)} = \left|\frac{d^2y_p}{dx^2}\right|, \tag{15}$$

where d^2x_p/dy^2 and d^2y_p/dx^2 are the derivatives taken in the polar points x_p and y_p. It follows that

$$C_1 = \sqrt{x_p\left|\frac{d^2x_p}{dy^2}\right|},\ C_2 = \sqrt{y_p\left|\frac{d^2y_p}{dx^2}\right|}.$$

Using formulas (6), (8) and (9), we obtain

$$C_1 s = 1 + \mu q_1 - \nu q_2, \tag{16}$$

$$C_2/s = 1 + \mu q_1 + \nu q_2. \tag{17}$$

Parameters q_1 and q_2 are expressed by

$$q_1 = \frac{q_0}{1-\sqrt{f_0}},\ q_2 = (4-\sqrt{f_0})q_1. \tag{18}$$

Equations (12), (16) and (17) relate the population characteristics of the ensemble of red blood cells (mean deformability, deformability variance and skewness of the red blood cell deformability distribution in the blood sample under study) and the parameters of the diffraction pattern, which can be measured experimentally [the coordinates of the polar points of the iso-intensity curve, the radii of curvature of the iso-intensity curve in the polar points and the quantity $I/I(0)$]. Using these equations, we construct an algorithm for measuring the statistical characteristics of the distribution of red blood cells in deformability.

6. Algorithm of curvature of the iso-intensity curve

The proposed algorithm, which we refer to as the algorithm of curvature of the iso-intensity curve, is as follows. In a laser diffractometry experiment, we should measure six quantities: $I(0)$, I, x_p, y_p, $R(x_p)$ and $R(y_p)$. Using the experimental data obtained, it is necessary to calculate four dimensionless parameters: f_0, D, C_1 and C_2, which are related with the quantities s, μ and ν that have been introduced above and are to be determined by relations (12), (16), (17) and (13), (18). The solution to equations (12), (16) and (17) has the form:

$$s = \frac{Q}{1+\sqrt{1-2QqC_1}},\ \mu = \frac{1}{2q_1}(C_2/s + C_1 s) - \frac{1}{q_1},$$
$$\nu = \frac{1}{2q_2}(C_2/s - C_1 s), \tag{19}$$

where $Q = 2(D - qC_2)$ and $q = q_0/2q_2$.

Formulas (19) express the measurement algorithm: they yield explicit expressions of the desired characteristics of an ensemble of red blood cells through the parameters of the diffraction pattern available for measurement.

7. Experimental studies. Verification of the algorithm

To test the efficiency of the algorithm of curvature of the curve we performed experiments with red blood cells of rats by using the LADE 6 device (RheoMedLab, Russia). Diffraction patterns were obtained for the bimodal ensembles of red blood cells, i.e., ensembles consisting of particles of only two types (shapes). Experimentally, such ensembles are created by processing the cells with a glutaraldehyde solution reducing their deformability.

The data obtained are presented in Fig. 1, which shows the iso-intensity curves of four different blood samples with different concentrations of cells treated with a glutaraldehyde solution (0, 20%, 50% and 100%). All the curves refer to the same shear rate and to the same level of the scattered light intensity: $I/I(0) = 0.06$.

For the analysis, we chose the curve shown in Fig. 1b. Figure 2 demonstrates the diffraction pattern, the iso-intensity curve and the processing procedure of this curve.

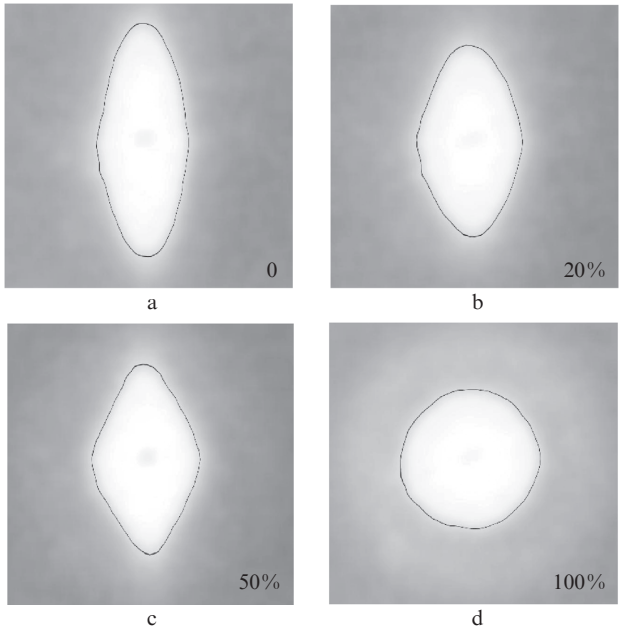
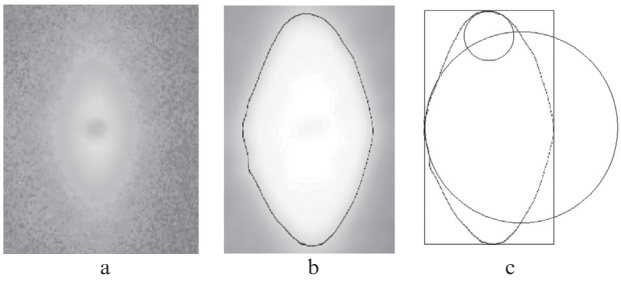


Figure 1. Iso-intensity curves obtained for bimodal ensembles of red blood cells of a rat at various concentrations (0–100%) of cells treated with a glutaraldehyde solution.

In our model, the bimodal ensemble is characterised by three quantities. Two of them, $s_1 = a_1/b_1$ and $s_2 = a_2/b_2$, describe the shape of the cells of both components of the ensemble, and p describes the fraction of the particles of the first type. The above-introduced parameters s, μ and ν in the particular case of the bimodal ensemble of cells can be defined in two ways. In the calculation by the first method, the shapes of the particles and the ratio of their concentrations are assumed known. In the calculation by the second method, the light intensity distribution in the diffraction pattern produced due



a b c

Figure 2. (a) Diffraction pattern, (b) iso-intensity curve and (c) curve processing procedure.

to the laser beam scattering by the considered ensemble of particles is assumed known. In particular, the shape of the iso-intensity curve located near the boundary of the central diffraction maximum is considered known and corresponding to some certain scattered light intensity.

In our model of elliptical discs that is described by formulas (1), the bimodal ensemble is given by the equations

$$a_1 = a_0(1+\varepsilon_1),\ b_1 = b_0(1-\varepsilon_1),$$
$$a_2 = a_0(1+\varepsilon_2),\ b_2 = b_0(1-\varepsilon_2), \qquad (20)$$
$$p\varepsilon_1 + (1-p)\varepsilon_2 = 0.$$

Note that the last equation follows from the condition $\langle\varepsilon\rangle = 0$ in the case of the bimodal ensemble. Solving equations (20), we obtain

$$s = M + \sqrt{M^2 + s_1 s_2},\ \varepsilon_1 = \frac{s_1 - s}{s_1 + s},\ \varepsilon_2 = \frac{s_2 - s}{s_2 + s}, \qquad (21)$$

$$\langle\varepsilon^2\rangle = p\varepsilon_1^2 + (1-p)\varepsilon_2^2,\ \langle\varepsilon^3\rangle = p\varepsilon_1^3 + (1-p)\varepsilon_2^3, \qquad (22)$$

where

$$M = (s_1 - s_2)(p - 1/2). \qquad (23)$$

In particular, for the conditions of our experiments ($s_1 = 1$, $s_2 = 2.54$, $p = 0.2$) from formulas (21)–(23) we obtain

$$s = 2.1, \mu = 0.032, \nu = -0.0087. \qquad (24)$$

Let us now estimate the same quantities using the algorithm of curvature of the iso-intensity curve. Using the iso-intensity curve shown in Fig. 2, we have calculated the parameters

$$D = y_p/x_p = 1.82,$$
$$C_1 = \sqrt{x_p/R(x_p)} = 0.82,\ C_2 = \sqrt{y_p/R(y_p)} = 2.18. \qquad (25)$$

Since we have defined the level of the scattered light intensity on the curve as $I/I(0) = 0.06$, according to formula (10) ($\beta = -0.4$), we obtain $f_0 = 0.094$. Using the values of the parameters D, C_1, C_2 and f_0, from the formulas of our model (19) we obtain

$$s = 1.9,\ \mu = 0.037 \text{ и } \nu = -0.0056. \qquad (26)$$

It follows from the comparison of the results (24) and (26) that the errors in determining the parameters s, μ and ν using the algorithm of curvature of the iso-intensity curve are 10%, 15% and 35%, respectively.

8. Results and discussion

One can see from Fig. 1 that with increasing fraction of undeformed red blood cells, p, in the blood sample, the iso-intensity curve continuously changes its shape from an ellipse to a rhomb and then to a circle. This character of the change in the shape of the iso-intensity curve predicts the model of an inhomogeneous ensemble of elliptical discs set forth above. Thus, our theoretical model is confirmed experimentally.

According to formula (11), the skewness of the distribution of red blood cells in deformability leads to the fact that the ratio of the semiaxes of the iso-intensity curve, $D = y_p/x_p$, is not equal to the average ratio of the semiaxes of the particles, $s = a_0/b_0$, as is usually assumed in the laser ektacytometry of red blood cells. As our analysis shows, the relationship between these parameters depends on the magnitude and sign of the skewness of the red blood cell deformability distribution. In particular, in the example considered in Section 7 we have $D/s = 0.94$. This means that under these conditions the parameters s and D differ from each other by about 6%. Thus, our model not only allows one to measure the new characteristics of the red blood cell deformability distribution, but also to make more precise the evaluation of the parameter measured by laser ektacytometry, namely mean deformability of red blood cells in a blood sample under study.

Speaking about the error in measuring the parameters s, μ and ν with the help of the algorithm of curvature of the iso-intensity curve, we note the following. The error in determining these parameters can be introduced by the approximation employed and the inaccuracy of measurement of the scattered light intensity on the selected iso-intensity curve. In addition, the factors that are not taken into account in our model can make a contribution to the error. They are the scatter of red blood cells in size and orientation in space, finite size of the measurement volume, finite time of averaging of the diffraction patterns, fluctuations of the various parameters, etc. Nevertheless, it is clear that in general, the new data processing algorithm is efficient. We hope that in its further development, the accuracy of measuring the red blood cell deformability parameters will be increased.

9. Conclusions

We have proposed a new algorithm for measuring the population characteristics of red blood cells, based on the analysis of data of laser diffractometry of red blood cells in shear flow (ektacytometry). These are such characteristics as mean deformability of red blood cells, deformability variance and skewness of the red blood cell deformability distribution.

For these parameters to be measured using the algorithm of curvature of the iso-intensity curve, we need to find the scattered light intensity relative to the intensity of the central diffraction maximum on some iso-intensity curve located near the boundary of the central maximum of the diffraction pattern. In addition, it is necessary to measure the coordinates of the polar point of this iso-intensity curve (they are located at the intersections of the iso-intensity curve with hor-

izontal and vertical coordinate axes) and the radii of curvature of the iso-intensity curve in the polar points.

To test the algorithm, we have conducted experiments with specially prepared samples of rat blood. We have succeeded in measuring the mean deformability, deformability variance and skewness of the red blood sample deformability distribution with an accuracy of 10%, 15% and 35%, respectively.

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