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Characteristic point algorithm in laser ektacytometry of red blood cells

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Abstract. **We consider the problem of measuring red blood cell deformability by laser diffractometry in shear flow (ektacytometry). A new equation is derived that relates the parameters of the diffraction pattern to the width of the erythrocyte deformability distribution. The numerical simulation method shows that this equation provides a higher accuracy of measurements in comparison with the analogous equation obtained by us earlier.**

Keywords: *red blood cell deformability, diffractometry, laser ektacytometry, data processing algorithms.*

1. Introduction

An urgent task of medical diagnostics is to measure the deformability of red blood cells, defined as a measure of the cell ability to change shape under the action of external forces [1–3]. To assess the deformability, it is necessary to measure the deformation of red blood cells subjected to some known external force. The task is complicated by the fact that the erythrocyte ensemble is nonuniform in its properties, and measurements need to be carried out quickly and for a large number of cells. One way to solve this problem is the application of laser diffractometry of erythrocytes in shear flow (ektacytometry) [4–6].

In a laser ektacytometer, an erythrocyte suspension is placed between two glass plates, one of which is fixed and the other moves. Thus, erythrocytes are subjected to shear stress. Then, the suspension is illuminated by a laser beam. As a result, a diffraction pattern appears on the observation screen installed in the far zone. This pattern is recorded by a video camera and transmitted to a computer, where it is processed according to a certain algorithm. This method is described in more detail in our papers [7–9]. The algorithm of data processing is based on the idea of how the laser beam is scattered on an ensemble of blood cells. Thus, there arises a physical problem of finding a relation between the characteristics of the diffraction pattern observed in the ektacytometer and the parameters of the blood sample to be determined.

In [9–12], we proposed several data processing algorithms that allow one to measure the statistical characteristics of erythrocyte deformability, namely, the mean deformability, width and asymmetry of erythrocyte deformability distribution. Experiments with specially prepared blood samples [10, 11] show that these algorithms are quite efficient, but it is necessary to improve accuracy and reliability of measurements.

For the analysis of the diffraction pattern (DP), the concept of an isointensity curve (IC) is introduced. This is the curve on which the intensity I of the scattered light has a certain definite value. Uniformly deformable erythrocyte ensembles (blood of healthy donors) exhibit isointensity curves of ellipsoidal shape. At the same time, for nonuniform ensembles (blood of patients with sickle-cell anaemia or specially prepared blood samples), isointensity curves have a rhomboid shape. As was shown in [13], the features of the isointensity curve shape manifest themselves most vividly in the peripheral part of the DP, where the intensity of the scattered light is about an order of magnitude smaller than the intensity of the central diffraction maximum. In this paper, we propose an algorithm that makes it possible to work with that part of the diffraction pattern that is most sensitive to the parameters of the red blood cell ensemble. This region is located near the boundary of the central diffraction maximum.

2. Characteristic points of the diffraction pattern

A convenient characteristic of the isointensity curve is a dimensionless parameter

$$\tilde{I} = I/I(0), \tag{1}$$

where $I(0)$ is the light intensity at the DP centre. Let us assume that

$$\tilde{I} \ll 1, \tag{2}$$

i.e., the isointensity curve in question lies at the periphery of the central maximum of the DP.

An example of the isointensity curve is shown in Fig. 1. The same figure illustrates the concept of characteristic points. These are the intersection points of the isointensity curve with the diagonals of the rectangle that surrounds this line. The points located in the middle of the sides of the rectangle are called polar points.

We introduce a Cartesian coordinate system with the origin at the centre of the diffraction pattern (the centre of the rectangle in Fig. 1). We denote the coordinate of the right polar point and the upper polar point by x_p and y_p, respectively. The coordinates of the characteristic point lying in the region $x > 0$ and $y > 0$ will be denoted by x_c and y_c. We introduce the polar point parameter D and the parameter of the characteristic point Q, defining them by the formulas

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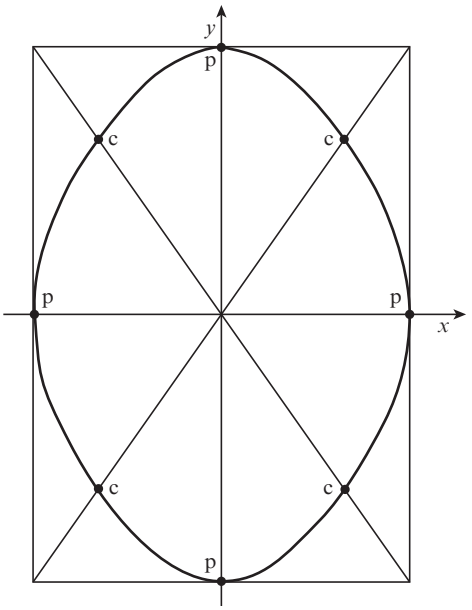

Figure 1. Isointensity curve, its polar (p) and characteristic (c) points.

$$D = \frac{y_p}{x_p}, \quad Q = \frac{1}{\sqrt{2}}\left(\frac{x_c}{x_p} + \frac{y_c}{y_p}\right). \tag{3}$$

We believe that the parameters D and Q, as well as the parameter $\tilde{I}$, can be measured using a laser ektacytometer.

3. Theoretical model

Following Refs [7–12], we model a red blood cell ensemble in shear flow of a laser ektacytometer by a set of flat elliptical disks with semi-axes $a = a_0(1 + \varepsilon)$ and $b = b_0(1 - \varepsilon)$, where a_0 and b_0 are the mean lengths of the semi-axes, and ε is a random parameter of the red blood cell shape. The validity of such a model is justified by the fact that in the region of small scattering angles, the erythrocyte model in the form of a flat disk provides sufficient accuracy [7]. We assume that the average value of the parameter ε is equal to zero ($\langle\varepsilon\rangle = 0$), and the probability density distribution $w(\varepsilon)$ is symmetric, i.e., $w(\varepsilon) = w(-\varepsilon)$.

The ensemble of red blood cells is characterised by the parameters

$$s = a_0/b_0, \quad \langle\varepsilon^2\rangle = \mu, \tag{4}$$

where s is the average deformability and μ is the width of the erythrocyte deformability distribution for the investigated blood sample; angular brackets denote averaging over the ensemble of particles. We assume that $\mu \ll 1$, i.e., the inhomogeneity of erythrocytes with respect to deformability is relatively weak. Our next task is to express the parameters of the blood sample (4) through the DP characteristics (3) and construct a measurement algorithm on this basis.

As was shown in [12], for the conditions in question, the isointensity curve is described by the formulas $x = Ar\cos\varphi$ and $y = Br\sin\varphi$. Here $A = q_1 z/(ka_0)$; $B = q_1 z/(kb_0)$; z is the distance from the measuring volume to the observation screen; $k = 2\pi/\lambda$ is the wavenumber; and λ is the wavelength of the light wave. The dependence $r(\varphi)$ is given by

$$r(\varphi) = \frac{1}{(1 + \sqrt{f_0})(1 - 2\mu)}\left[1 + \mu\left(\frac{H^2}{2\sqrt{f_0}} - \frac{5}{2} + 2H^2\right)\right],$$

where

$$f_0 = \tilde{I}/(4\beta^2); \tag{5}$$

and $H = \cos(2\varphi)$. The quantities $q_1 = 3.82$ and $\beta = -0.4$ are the parameters of the Bessel function of the first order. The first of them is equal to the argument of this function, at which the function vanishes, and the second one is equal to the derivative of the function at this point.

Using the above formulas, we can calculate the coordinates of the polar and characteristic points, namely, $x_p = Ar(0)$, $y_p = Br(\pi/2)$, $x_c = (1/\sqrt{2})Ar(\pi/4)$ and $y_c = (1/\sqrt{2})Br(\pi/4)$. Note that $r(\varphi = 0) = r(\varphi = \pi/2)$. It follows that $Q = r(\pi/4)/r(0)$,

$$x_c/x_p = y_c/y_p, \tag{6}$$

$$s = D, \tag{7}$$

and

$$Q = \frac{2 - 5\mu}{2 + \mu(1/\sqrt{f_0} - 1)}. \tag{8}$$

Relation (6) means that the coordinates of the polar and characteristic points of the isointensity curve are related to each other as shown in Fig. 1. Formulas (7) and (8) solve the above problem, relating DP parameters (3) and erythrocyte ensemble characteristics (4).

4. Algorithm of data processing

Solving equation (8) with respect to μ, we obtain

$$\mu = \frac{2(1 - Q)}{5 + (1/\sqrt{f_0} - 1)Q}. \tag{9}$$

Formulas (7) and (9) represent an algorithm for measuring the mean deformability s and the erythrocyte deformability dispersion μ in a blood sample under study. The algorithm consists in the following. First, one should choose the isointensity curve in accordance with condition (2) on the DP obtained with the help of an ektacytometer from this blood sample. Then it is necessary to determine the coordinates of the polar and characteristic points x_p, y_p, x_c and y_c (shown in Fig. 1) of the isointensity curve and also to measure the value of $\tilde{I}$, equal to the ratio of the light intensity at the given isointensity curve to the intensity of the central maximum of the DP. After that the parameters D, Q and f_0 are calculated from formulas (3) and (5) and, finally, the parameters s and μ are determined from formulas (7) and (9).

5. Testing the algorithm in a numerical experiment

Let us check the algorithm on the example of a bimodal ensemble of red blood cells. This ensemble consists of cells of only two types. In the model of elliptical disks, the cells of the first type have the dimensions a_1 and b_1 and the shape parameter ε_1, so that $a_1 = a_0(1 + \varepsilon_1)$ and $b_1 = b_0(1 - \varepsilon_1)$, and the cells of the second type have the dimensions a_2 and b_2 and the

shape parameter ε_2, so that $a_2 = a_0(1 + \varepsilon_2)$ and $b_2 = b_0(1 - \varepsilon_2)$. The thicknesses of all disks are assumed to be the same and equal to h. We denote the fraction of particles of the first type in the ensemble by p.

The shape of the particles of both components of the ensemble for the given shear stress will be characterised by the parameters

$$s_1 = a_1/b_1, \quad s_2 = a_2/b_2. \tag{10}$$

Three dimensionless parameters (s_1, s_2, p) completely determine the bimodal ensemble of red blood cells. These parameters are the initial data for a numerical experiment. The remaining parameters are expressed through them as follows [9]:

$$s = M + \sqrt{M^2 + s_1 s_2}, \quad M = (s_1 - s_2)(p - 1/2),$$

$$\varepsilon_1 = \frac{s_1 - s}{s_1 + s}, \quad \varepsilon_2 = \frac{s_2 - s}{s_2 + s}, \quad \mu = p\varepsilon_1^2 + (1 - p)\varepsilon_2^2.$$

Next we consider a symmetric bimodal ensemble of cells for which

$$p = 1/2, \quad s_1 = 1. \tag{11}$$

Physically, this ensemble corresponds to a mixture of deformable and undeformable blood cells. Such ensembles are interesting, in particular, from the point of view of testing algorithms for data processing [10, 11, 13, 14]. Under conditions (11), the only parameter of the ensemble of cells is the value of s_2, which characterises the deformation of the deformed component of the ensemble for the given shear stress. In this case,

$$s = \sqrt{s_2}, \quad \mu = \left(\frac{1 - \sqrt{s_2}}{1 + \sqrt{s_2}}\right)^2. \tag{12}$$

The light intensity distribution in the DP for such an ensemble has the form:

$$I(x,y) = \frac{1}{4} I_0 N |\gamma|^2 \left[\frac{1}{2}\left(a_1 b_1 \frac{k}{z}\right)^2 G\left(\frac{k}{z}\sqrt{a_1^2 x^2 + b_1^2 y^2}\right)\right.$$

$$\left. + \frac{1}{2}\left(a_2 b_2 \frac{k}{z}\right)^2 G\left(\frac{k}{z}\sqrt{a_2^2 x^2 + b_2^2 y^2}\right)\right], \tag{13}$$

where I_0 is the laser light intensity; N is the total number of red blood cells irradiated by the laser; $|\gamma|^2 = 4\sin^2(\Delta\varphi/2)$; $\Delta\varphi = kn_0h(n - 1)$; n is the absolute refractive index of the material from which the particles are composed; and n_0 is the absolute refractive index of the medium surrounding the particle.

In (13) we introduce the function

$$G(x) = [2J_1(x)/x]^2, \tag{14}$$

where $J_1(x)$ is the Bessel function of the first order. Function (14) satisfies the condition $G(0) = 1$. Note that formula (13) describes the distribution of the light intensity at those points of the observation screen where the direct laser beam does not fall.

The normalised light intensity distribution in the diffraction pattern has the form

$$\tilde{I}(x,y) = \frac{(a_1 b_1)^2 G\left(\frac{k}{z}\sqrt{a_1^2 x^2 + b_1^2 y^2}\right) + (a_2 b_2)^2 G\left(\frac{k}{z}\sqrt{a_2^2 x^2 + b_2^2 y^2}\right)}{(a_1 b_1)^2 + (a_2 b_2)^2}.$$

This function obeys the condition $\tilde{I}(0) = 1$.

We introduce the effective radius of the red blood cell, defining it by the formula

$$c_0 = \sqrt{a_0 b_0}. \tag{15}$$

This is the radius of a circle, the area of which is equal to the average area of the base of the elliptic disk that models the red blood cell. As the scale of the DP we choose

$$x_0 = z/(kc_0). \tag{16}$$

Then, we introduce dimensionless variables

$$\tilde{a} = a/c_0, \quad \tilde{b} = b/c_0, \quad \tilde{x} = x/x_0, \quad \tilde{y} = y/x_0. \tag{17}$$

In this case,

$$D = \frac{\tilde{y}_p}{\tilde{x}_p}, \quad Q = \frac{1}{\sqrt{2}}\left(\frac{\tilde{x}_c}{\tilde{x}_p} + \frac{\tilde{y}_c}{\tilde{y}_p}\right). \tag{18}$$

For the bimodal ensemble in question, $a_1 = \tilde{a}_1 c_0$, $b_1 = \tilde{b}_1 c_0$, and $a_2 = \tilde{a}_2 c_0$, $b_2 = \tilde{b}_2 c_0$, where

$$\tilde{a}_1 = \tilde{b}_1 = \frac{2\sqrt{s}}{1 + s}; \quad \tilde{a}_2 = \tilde{a}_1 s; \quad \tilde{b}_2 = \tilde{b}_1/s; \quad s = \sqrt{s_2}. \tag{19}$$

It follows that

$$\tilde{I}(\tilde{x},\tilde{y}) = \frac{1}{2} G\left(\sqrt{(\tilde{a}_1\tilde{x})^2 + (\tilde{b}_1\tilde{y})^2}\right)$$

$$+ \frac{1}{2} G\left(\sqrt{(\tilde{a}_2\tilde{x})^2 + (\tilde{b}_2\tilde{y})^2}\right). \tag{20}$$

This formula describes the light intensity distribution in the diffraction pattern for the considered bimodal ensemble of erythrocytes with characteristics (11). Here, the normalised light intensity $\tilde{I}$ is determined by the (1), the function $G(x)$ by formula (14), the dimensionless coordinates $\tilde{x}$ and $\tilde{y}$ are defined by formulas (15)–(17), the parameters $\tilde{a}_1$, $\tilde{b}_1$, $\tilde{a}_2$, $\tilde{b}_2$ by formulas (10), (19). An example of a DP constructed from formula (20) is shown in Fig. 2a. Figure 2b demonstrates one of the isointensity curves. Note that such diffraction patterns and isointensity curves give blood samples for certain diseases, in particular, for sickle-cell anaemia [1].

The procedure for verifying the algorithm is as follows. On the isointensity curve, we find polar and characteristic points (see Fig. 1), determine their coordinates $\tilde{x}_p$, $\tilde{y}_p$, $\tilde{x}_c$, $\tilde{y}_c$ and calculate the parameters D and Q using formulas (18). After this, using formulas (7) and (9), we find the parameters s and μ and compare the obtained values with the exact values of these parameters determined by formulas (12).

6. Results

Examples of the results of calculations are presented in Figs 3 and 4. Figure 3 shows the values of the parameter s, which characterises the mean deformability of red blood cells, and Fig. 4 – the values of the parameter μ, which characterises the

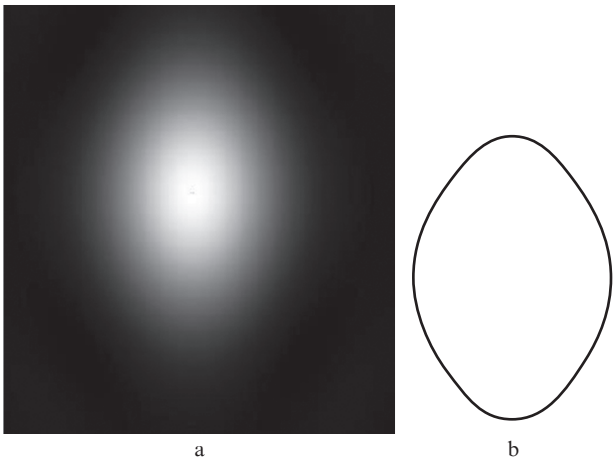

Figure 2. (a) Diffraction pattern constructed from formula (20) for $s_2 = 2$ and (b) isointensity curve corresponding to $\tilde{I} = 0.1$.

width of the erythrocyte deformability distribution. For comparison, the dashed line in Fig. 4 shows the results of measuring the erythrocyte deformability dispersion performed by the algorithm from [10].

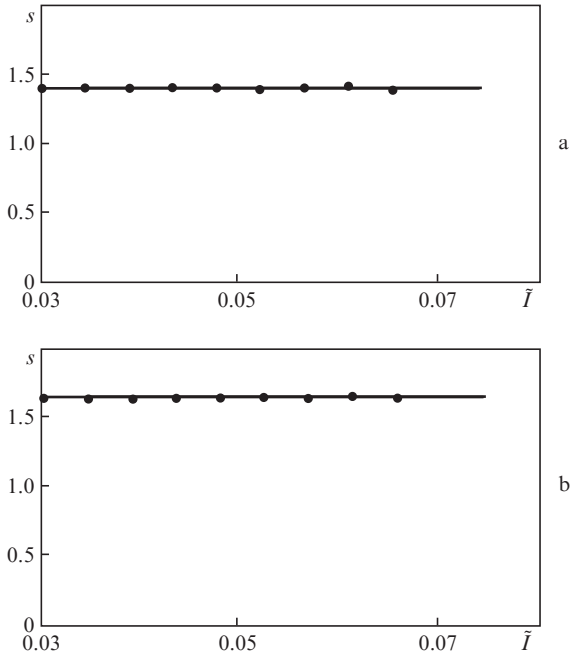

Figure 3. The results of measuring the mean deformability of erythrocytes at $s_2 = 2$ and (b) 3. The points are the values of the parameter s calculated by formula (7) on the basis of the analysis of the diffraction pattern constructed from formula (20). The solid lines are the exact values of the parameter s found from formula (12).

In the notation used here, this algorithm is described by formulas

$$\mu = \frac{1}{2}(\rho_3 + \sqrt{\rho_3^2 + \rho_4}), \quad \rho = Q - 1,$$

$$\rho_1 = \frac{f_0(Q^2-1) - \rho^2}{2\rho\sqrt{f_0}}, \quad \rho_2 = \frac{f_0(Q^2-1) + Q^2/2}{2\rho\sqrt{f_0}}, \qquad (21)$$

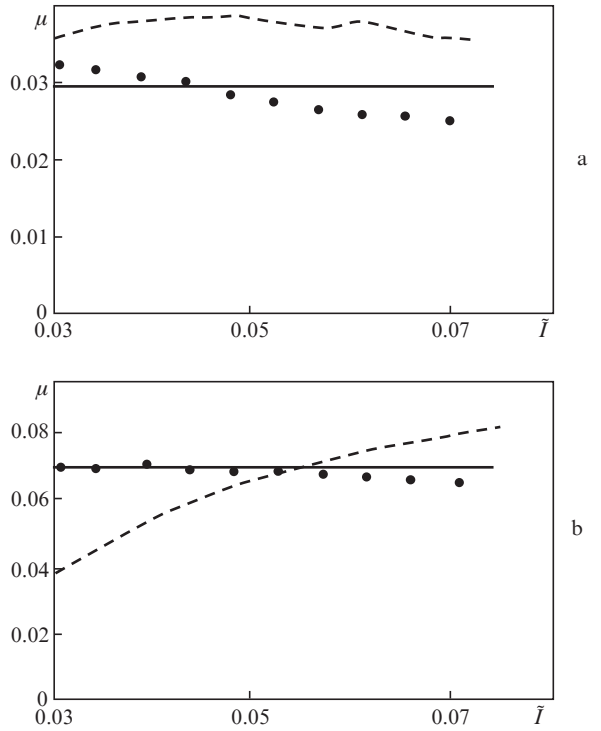

Figure 4. Results of measuring the dispersion of erythrocyte deformability at $s_2 = $ (a) 2 and (b) 3. The points are the values of the parameter μ calculated from formula (9) on the basis of the analysis of the diffraction pattern constructed from formula (20). The solid lines are the exact values of the parameter μ found from formula (12), the dashed lines are the results of measurements of the erythrocyte deformability dispersion performed by algorithm (21).

$$\rho_3 = \frac{2\rho_1\rho_2 - 1}{2\rho_2^2}, \quad \rho_4 = \frac{1 - \rho_1^2}{\rho_2^2}.$$

The data presented indicate that in the region of the diffraction pattern determined by the condition

$$0.03 \leqslant \tilde{I} \leqslant 0.075,$$

the algorithm presented in this paper provides a higher accuracy of measurements in comparison with the algorithm developed in [10]. Deviation of the erythrocyte deformability dispersion from the given value is explained by the approximations used in the derivation of formulas (9) and (21).

Let us pay attention to the following circumstance. In a real experiment, the scattered light intensity on the isointensity curve chosen for measurements can be determined with some uncertainty. The question arises about its effect on the accuracy of the measurement of red blood cell deformability. As can be seen from Figs 3 and 4, a small error in the measurement of the parameter $\tilde{I}$ does not lead to a significant error in the measurements of the mean deformability and the width of the erythrocyte deformability distribution if these measurements are carried out with the help of the proposed algorithm of a characteristic point.

7. Conclusions

We have considered the problem of measuring the erythrocyte deformability dispersion by laser diffractometry in shear flow (ektacytometry). The analysis has been carried out for

the region of the diffraction pattern lying on the periphery of the central diffraction maximum. This area is traditionally used in laser ektacytometry of red blood cells and is characterised by high sensitivity to the characteristics of the blood sample being studied. For an ensemble of cells with a symmetric deformability distribution function, we have developed an improved variant of the characteristic point algorithm. This algorithm allows one to measure the dispersion of erythrocyte deformability in the blood sample under study and is mathematically expressed by formula (9). A check carried out by the method of a numerical experiment has shown that the new algorithm provides a higher accuracy of measurements in comparison with the algorithm developed by us earlier in [10].

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