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A simplified algorithm for measuring erythrocyte deformability dispersion by laser ektacytometry

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Abstract. **The possibility of measuring the dispersion of red blood cell deformability by laser diffractometry in shear flow (ektacytometry) is analysed theoretically. A diffraction pattern parameter is found, which is sensitive to the dispersion of erythrocyte deformability and to a lesser extent – to such parameters as the level of the scattered light intensity, the shape of red blood cells, the concentration of red blood cells in the suspension, the geometric dimensions of the experimental setup, etc. A new algorithm is proposed for measuring erythrocyte deformability dispersion by using data of laser ektacytometry.**

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

1. Introduction

One of the main rheological parameters of blood is the deformability of red blood cells, defined as the ability of these cells to change shape under applied external forces. Authors of many papers note the importance of this parameter for the diagnosis and treatment of various diseases. The objective in this case is to measure not only the average deformability, but also the distribution function of the deformability of red blood cells. Thus, the aim of our paper is to measure the characteristics of erythrocyte deformability by using the data of laser ektacytometry.

Laser diffractometry of red blood cells in shear flow (ektacytometry) [1] is based on the observation of diffraction patterns (DPs). A diffraction pattern is formed when a laser beam passes through a highly diluted erythrocyte suspension, in which red blood cells are deformed by the viscous friction. Examples of such pattern are presented in [2–6]. The measurement accuracy of this method depends on the accuracy of determining the shapes of iso-intensity lines (ILs) of the diffraction pattern. The accuracy of measuring the IL shape, in turn, depends on the part of the pattern where this line is located. In other words, it depends on the scattered light intensity. Thus, the highest measurement accuracy is achieved in that region of the observation screen, where the intensity of scattered light changes most rapidly with the coordinate, i.e., in the region of a maximum intensity gradient. The previously developed models [2–5] are not applicable in this field and therefore there is an urgent need to develop new theoretical models that would cover the specified part of the diffraction pattern. One such model is presented in this paper.

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2. Basic idea

The basic idea of the model consists in the fact that in the region of a maximum gradient the dependence of the scattered light intensity on the coordinates of a point on the observation screen is described by a linear function. For example, the Airy function [7] (Fig. 1)

$$\psi(q) = \left[\frac{2J_1(q)}{q}\right]^2 \qquad (1)$$

is well approximated by a linear function in the region $1 \leqslant q \leqslant 2$, where $0.3 \leqslant \psi \leqslant 0.7$ [8]. In this region, the Airy function can be written as

$$\psi(q) = \alpha - \beta q \quad (\alpha = 1.24, \beta = 0.46), \qquad (2)$$

and the linear approximation error does not exceed 10%.

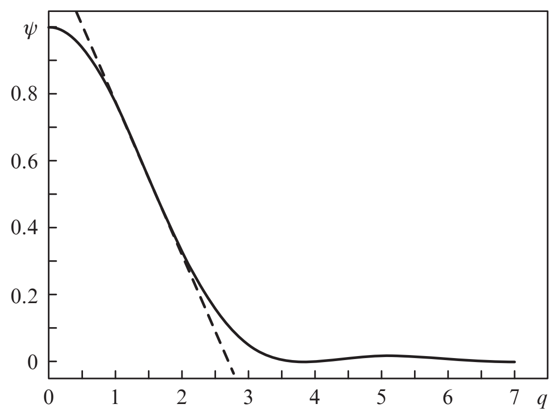

Figure 1. Airy function (solid curve) and its linear approximation (dashed line) in the region of a maximum gradient of the function.

The function $\psi(q)$, given by formula (1), describes diffraction by a circular aperture [7, 9], as well as by round or elliptic discs [10]. However, an approximation of type (2) is applicable to a wider class of objects, such as spherical or spheroidal particles, biconcave discs [11, 12]. In the region of a maximum gradient, the functions for such particles, describing the spatial distribution of the scattered light intensity, are also linear, although they may differ from (2) by the values of constant coefficients α and β.

3. Model of elliptical discs

As shown in [13], erythrocytes in shear flow stretch along the flow and acquire a shape similar to an ellipsoid. We assume that in these conditions a red blood cell can be modelled by a flat elliptical disc [10]. Calculations [2–6] show that this model provides sufficient accuracy. At the same time, it allows one to analytically calculate the scattering pattern of a laser beam by an ensemble of red blood cells in an ektacytometer and establish functional relationships between the main parameters of the problem, which is important for the development of new data processing algorithms.

When a laser beam is scattered by an ensemble of identical equally oriented elliptical disc with semiaxes a and b, the light intensity distribution on the observation screen located in the far field of diffraction is described by the formula [10]

$$I(x,y) = I_0 N |\gamma|^2 \left(\frac{S}{\lambda z}\right)^2 \psi(q), \tag{3}$$

where the function $\psi(q)$ is defined by formula (1) and

$$q = \frac{k}{z}\sqrt{a^2x^2 + b^2y^2}. \tag{4}$$

Formula (3) corresponds to the distribution of light in a DP, averaged over the coordinates of erythrocytes. In a real laser ektacytometry experiment, this averaging occurs automatically as a result of a continuous movement of red blood cells through the laser beam and a finite exposure in DP photographing.

In formulas (3) and (4), x and y are the Cartesian coordinates of the point on the observation screen in a coordinate system whose origin is chosen at the point of incidence of a laser beam on the screen, and z is the distance from the measuring volume to the observation screen. The axis X is directed horizontally, and the axis Y – vertically. Physically, the directions of these axes are chosen such that one of them is parallel and the other is perpendicular to the shear flow in an ektacytometer. Other values denote the following: I_0 is the intensity of an incident laser beam; N is the number of particles illuminated by a laser beam; $k = 2\pi/\lambda$ is the wave number; λ is wavelength of laser light; $S = \pi ab$ is the area of the disc base; and γ is a parameter, which is proportional to the thickness of a red blood cell. Under conditions of laser diffractometry, $|\gamma|^2 \approx 1$. We note that formula (3) describes the light intensity distribution at the points of the observation screen where a direct (unscattered) laser beam does not fall.

4. Inhomogeneous ensemble of red blood cells

Human red blood cells or erythrocytes have the property of extensive deformation (deformability) [13–16]. Taking into account this fact, we will consider the sizes a and b of the semiaxes of an elliptical disc to be random quantities and will define them by the formulas [2–6]

$$a = a_0(1 + \varepsilon), \quad b = b_0(1 - \varepsilon). \tag{5}$$

Here, a_0 and b_0 are the average sizes of the semiaxes; and ε is a random parameter. The average value of this parameter is assumed equal to zero:

$$\langle \varepsilon \rangle = 0. \tag{6}$$

The characteristics of an ensemble of particles are

$$s = a_0/b_0, \tag{7}$$

and the following statistical moments of the quantity ε:

$$\langle \varepsilon^2 \rangle = \mu, \quad \langle \varepsilon^3 \rangle = \nu. \tag{8}$$

Here, s, μ, and ν are the average erythrocyte deformability, the width and the asymmetry of the deformability distribution, respectively. We believe that the inhomogeneity of the ensemble with respect to the shape of the particles is relatively weak, that is, $|\varepsilon| \ll 1$. In this case, $\mu \ll 1$, $\nu \ll 1$. These relations are generally well met for real ensembles of red blood cells.

5. Scattered light intensity distribution in the central part of the diffraction pattern

In this section we calculate the scattered light intensity distribution in the part of the DP, where approximation (2) is valid. This part of the pattern lies outside the direct laser beam and represents an elliptical annular zone defined by the formula

$$0.4 \leqslant I(x,y)/I(0) \leqslant 0.6. \tag{9}$$

Here, $I(x,y)$ is the light intensity at a given point of the DP; and $I(0)$ is the intensity of the central diffraction peak.

Substituting expressions (5) into formulas (3) and (4) leads to the fact that the light intensity distribution on the observation screen becomes a random function. In this case, the observed distribution can be calculated by averaging the intensity in the parameter ε:

$$I(x,y) = \left\langle I_0 N |\gamma|^2 \left(\frac{S}{\lambda z}\right)^2 \psi(q) \right\rangle_\varepsilon, \text{ or}$$

$$I(x,y) = I_0 N |\gamma|^2 \left(\frac{S_0}{\lambda z}\right)^2 \langle (1-\varepsilon^2)^2 \psi(q) \rangle_\varepsilon,$$

where $S_0 = \pi a_0 b_0$;

$$q = (k/z)\sqrt{a_0^2(1+\varepsilon)^2x^2 + b_0^2(1-\varepsilon)^2y^2}. \tag{10}$$

In particular, the light intensity in the DP centre is

$$I(0) = I_0 N |\gamma|^2 \left(\frac{S_0}{\lambda z}\right)^2 \langle (1-\varepsilon^2)^2 \rangle_\varepsilon,$$

or, approximately,

$$I(0) = I_0 N |\gamma|^2 \left(\frac{S_0}{\lambda z}\right)^2 (1 - 2\mu).$$

Note that these equations describe the light intensity distribution in those points of the observation screen, where no direct laser beam radiation is incident. We call (for brevity) the DP centre the vicinity of the direct laser beam.

The intensity $I(0)$ of the central diffraction maximum can be conveniently used to normalise the scattered light intensity. By introducing the function

$$f(x,y) = I(x,y)/I(0), \quad (11)$$

we obtain

$$(1-2\mu)f(x,y) = \langle(1-\varepsilon^2)^2\psi(q)\rangle_\varepsilon. \quad (12)$$

For the normalisation of the coordinates on the observation screen we introduce the quantities

$$A = z/(ka_0), \quad B = z/(kb_0), \quad (13)$$

which determine the horizontal and vertical sizes of the DP. Then, expression (10) can be represented in the form $q = \sqrt{(1+\varepsilon)^2u^2 + (1-\varepsilon)^2v^2}$ with the normalised coordinates

$$u = x/A, \quad v = y/B. \quad (14)$$

By using the explicit dependence of q on the parameter ε, we obtain $q = \sqrt{(1+\varepsilon^2)g + 2h\varepsilon}$ with the functions of the coordinates $g = u^2 + v^2$, $h = u^2 - v^2$.

Using approximation (2) and formula (12), the intensity distribution in the central part of the DP can be expressed as

$$(1-2\mu)f = \langle\Phi(\varepsilon)\rangle_\varepsilon. \quad (15)$$

Here, the function $\Phi(\varepsilon) = (1-\varepsilon^2)^2[\alpha - \beta q(\varepsilon)]$. Expanding Φ in powers of the small parameter ε and retaining the terms up to third order, we obtain

$$\Phi(\varepsilon) = \alpha - \beta\sqrt{g} - \frac{\beta h}{\sqrt{g}}\varepsilon + \left(-4\alpha + 3\beta\sqrt{g} + \frac{\beta h^2}{g\sqrt{g}}\right)\frac{\varepsilon^2}{2} + \frac{\beta h}{2\sqrt{g}}\left(5 - \frac{h^2}{g^2}\right)\varepsilon^3. \quad (16)$$

Let us substitute (16) into (15) and average the resultant expression for the random parameters ε, by taking into account (6) and (8). Next, we introduce the polar coordinates r and φ, by defining them by formulas

$$u = r\cos\varphi, \; v = r\sin\varphi. \quad (17)$$

Then, $g = r^2$ and $h = r^2\cos2\varphi$, and the expression for the normalised light intensity takes the form

$$(1-2\mu)f = \alpha - \beta r + \frac{\mu}{2}(-4\alpha + 3\beta r + \beta r\cos^2 2\varphi) + \frac{\nu}{2}\beta r\cos2\varphi(5 - \cos^2 2\varphi). \quad (18)$$

This formula describes the desired distribution of the normalised scattered light intensity in the DP centre.

6. Iso-intensity line, its polar and characteristic points

In laser ektacytometry, the DP is analysed by using the concept of an iso-intensity line, which is a line on the observation screen, on which the scattered light intensity has a constant value. Mathematically, such a line is defined by the equation

$$f = \text{const.} \quad (19)$$

From (18) and (19) it follows that

$$\frac{\beta}{(\alpha - f)(1-2\mu)}r(\varphi) = [1 - \frac{\mu}{2}(3 + \cos^2 2\varphi) - \frac{\nu}{2}(5 - \cos^2 2\varphi)\cos2\varphi]^{-1}. \quad (20)$$

The function $r(\varphi)$, determined by this formula, describes the IL shape in polar coordinates.

We assume that the random parameter ε is described by an even function of the probability density distribution $w(\varepsilon)$, i.e.,

$$w(\varepsilon) = w(-\varepsilon). \quad (21)$$

According to (8) for such an ensemble $\nu = 0$, and formula (20) takes the form

$$\frac{\beta}{(\alpha - f)(1-2\mu)}r(\varphi) = [1 - \frac{\mu}{2}(3 + \cos^2 2\varphi)]^{-1}. \quad (22)$$

An inhomogeneous ensemble of particles satisfying condition (21) is called a symmetrical ensemble. Below, we confine ourselves to a symmetric inhomogeneous ensemble of red blood cells.

We call the polar points of an iso-intensity line the points of its intersection with the horizontal and vertical axes. We calculate the coordinates of the polar points. The Cartesian coordinates of an arbitrary IL point are defined by formulas (14), (17) and can be represented as $x = Ar\cos\varphi$, $y = Br\sin\varphi$.

The coordinate x_p of the polar point lying on the axis X is defined by the condition $y = 0$ or $\varphi = 0$. Therefore,

$$x_p = Ar(0). \quad (23)$$

The coordinate y_p of the polar point lying on the axis Y is defined by the condition $x = 0$ or $\varphi = \pi/2$. Therefore,

$$y_p = Br(\pi/2) = Br(0). \quad (24)$$

Let us denote

$$D = y_p/x_p. \quad (25)$$

Then, it follows from (7), (13), (23)–(25) that $D = s$. Thus, in the case of a symmetric ensemble of particles the coordinates of the polar IL points characterise the average the deformability of red blood cells in shear flow of a laser ektacytometer.

We call the characteristic IL points the points whose coordinates are defined by formulas $x_c = Ar(\pi/4)\cos(\pi/4)$, $y_c = Br(\pi/4)\sin(\pi/4)$, or

$$x_c = (A/\sqrt{2})r_c, \; y_c = (B/\sqrt{2})r_c, \quad (26)$$

where

$$r_c = r(\pi/4), \quad (27)$$

and the function $r(\varphi)$ is defined by formula (22). It follows from (23), (24) and (26) that $y_c/x_c = B/A = y_p/x_p$. This relation allows one to find the characteristic points on the iso-intensity line using the following procedure. Let us construct a rectangle with vertical and horizontal sides passing through the polar points. This rectangle surrounds the IL, by touching it at the polar points. After drawing the diagonals of this rectangle we find the point of intersection of the diagonals with the IL. They are the characteristic points. The polar and characteristic points of the IL are shown in Fig. 2.

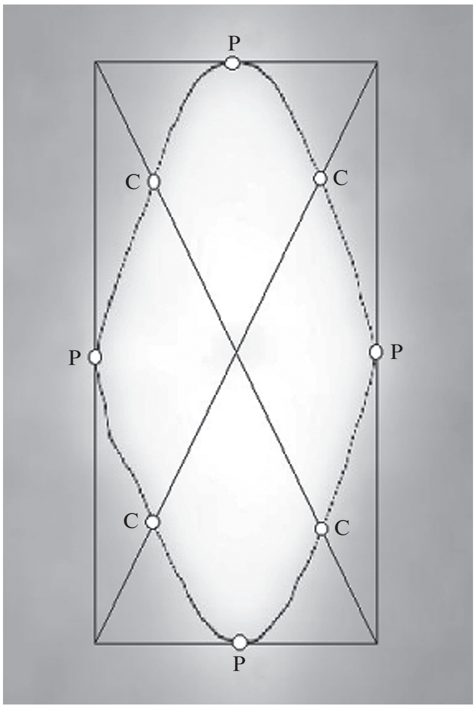

Figure 2. Iso-intensity line, its polar (P) and the characteristic (C) points.

In our model, the dispersion of the erythrocyte deformability is characterised by the parameter μ defined by formula (8). The above-derived formulas allow one to construct an algorithm for determining this parameter, consisting in the following. Let us introduce the parameter

$$P = \frac{1}{2}\left(\frac{x_c}{x_p} + \frac{y_c}{y_p}\right), \tag{28}$$

which we call the characteristic point parameter. Substituting (23), (24) and (26) into (28), we obtain $P = r_c/[\sqrt{2}\,r(0)]$, or by virtue of (22) and (27), $P = \sqrt{2}(1 - 2\mu)/(2 - 3\mu)$. Then,

$$\mu = \frac{\sqrt{2} - 2P}{2\sqrt{2} - 3P}. \tag{29}$$

This equation expresses the main result of the calculation. It relates the DP parameter P defined by (28) with the erythrocyte ensemble characteristic μ determined by formula (8). Equation (29) is applicable, provided that the IL selected for the measurement is located in the central part of the DP, defined by condition (9). Note that equation (29) contains neither the parameter f defined by formula (11), nor parameters α and β given by formula (2). This means that the IL in this part of the diffraction pattern is not very sensitive to the level of the scattered light intensity, as well as to the peculiarities of the shape of red blood cells. However, these lines remain sensitive to such a population characteristic as dispersion of the deformability of erythrocytes in a blood sample under study.

7. Algorithm for measuring erythrocyte deformability dispersion

Formula (29) makes it possible to determine the erythrocyte deformability dispersion in a blood sample by laser ektacytometry. To do this, it is sufficient to select the IL from that part of the diffraction pattern, which satisfies condition (9). Then, it is needed to find polar and characteristic IL points (see Fig. 2), to measure their coordinates and calculate the parameter P defined by formula (28). Thereafter, the parameter μ can be calculated from formula (29).

We have tested this algorithm, using the experimental data given in [16]. Streekstra et al. [16] investigated specially prepared blood samples with known erythrocyte deformability distributions. We have selected the IL obtained for a symmetrical ensemble of red blood cells and corresponding to the level of the scattered light intensity $f = 0.5$. This line is shown in Fig. 3. Using the algorithm described above, we obtained for this line $P = 0.67$, $\mu = 0.09$ and $\sqrt{\mu} = 0.30$. As shown in [3], the assessment of the parameter P, based on the known composition of the blood sample, yields $\sqrt{\mu} = 0.27$. Thus, the proposed algorithm for measuring the dispersion of red blood cell deformability allows one to obtain reliable data.

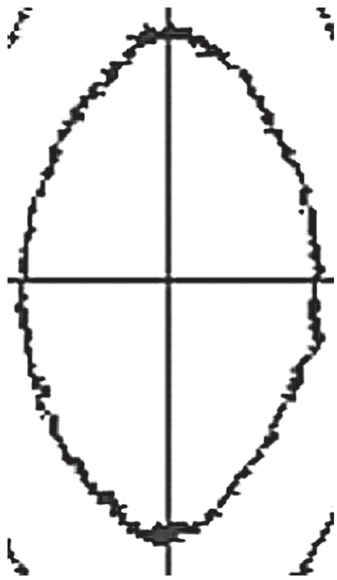

Figure 3. Iso-intensity line of the diffraction pattern obtained for a symmetrical ensemble of red blood cells and scattered light intensity $I/I_0 = 0.5$ [16].

8. Discussion of the results

We have analysed the IL lying in the central part of the diffraction pattern. The boundaries of this region are defined by condition (9). The data obtained in [16, 4, 5] show that the central part of the DP is less sensitive to the parameters of an erythrocyte ensemble than its peripheral part. Probably, for this reason, in laser ektacytometry of erythrocytes the IL lying near the first DP minimum is chosen for measurements. However, in other respects, the central part of the DP has certain advantages. In this part of the pattern the level of the scattered light intensity is higher and therefore the noises are less influential. In this part the IL shape can be measured more accurately. In the centre of the diffraction pattern the IL shape is less sensitive to the peculiarities of the shape of red blood cells, as well as to the level of the scattered light intensity. At the same time, the IL retains its sensitivity to such a parameter as the spread of erythrocyte deformability. Thus, the use of the IL lying in the central part of the diffraction pattern is promising for measuring the characteristics of red blood cell deformability.

9. Conclusions

Thus, we have analysed theoretically the possibility of measuring the erythrocyte deformability dispersion by laser diffractometry of red blood cells in shear flow (ektacytometry). We have found the parameter of the diffraction pattern which is sensitive to the dispersion of erythrocyte deformability and less sensitive to such parameters as the level of the scattered light intensity, the shape of red blood cells, the concentration of red blood cells in the suspension, the geometric dimensions of the experimental setup, etc. We have proposed a new algorithm for measuring red blood cell deformability dispersion by using the data of laser ektacytometry.

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References

1. Bessis M., Mohandas N. *Blood Cells*, **1**, 307 (1975).
2. Nikitin S.Yu., Kormacheva M.A., Priezzhev A.V., Lugovtsov A.E. *Kvantovaya Elektron.*, **43** (1), 90 (2013) [*Quantum Electron.*, **43** (1), 90 (2013)].
3. Nikitin S.Yu., Priezzhev A.V., Lugovtsov A.E. *J. Quantitat. Spectrosc. Rad. Transfer*, **121**, 1 (2013).
4. Nikitin S.Yu., Priezzhev A.V., Lugovtsov A.E., Ustinov V.D. *Kvantovaya Elektron.*, **44** (8), 774 (2014) [*Quantum Electron.*, **44** (8), 774 (2014)].
5. Nikitin S.Yu., Priezzhev A.V., Lugovtsov A.E., Ustinov V.D., Razgulin A.V. *J. Quantitat. Spectrosc. Rad. Transfer*, **146**, 365 (2014).
6. Nikitin S.Yu., Lugovtsov A.E., Ustinov V.D., Lin M.D., Priezzhev A.V. *J. Innovat. Opt. Health Sci.*, **8** (4), 1550031 (2015).
7. Born M., Wolf E. *Principles of Optics* (Oxford: Pergamon Press, 1984; Moscow: Nauka, 1973).
8. Jahnke E., Emde F., Lösch F. *Tables of Higher Functions* (New York: McGraw-Hill, 1960; Moscow: Nauka, 1977).
9. Akhmanov S.A., Nikitin S.Yu. *Physical Optics* (Oxford: Clarendon Press, 1997; Moscow: Nauka, 2004).
10. Nikitin S.Yu., Priezzhev A.V., Lugovtsov A.E. In: *Advanced Optical Flow Cytometry: Methods and Disease Diagnoses*. Ed. by V.V. Tuchin (Weinheim: Wiley-VCH, 2011) p. 133.
11. Lugovtsov A.E., Nikitin S.Yu., Priezzhev A.V. *Kvantovaya Elektron.*, **38** (6), 606 (2008) [*Quantum Electron.*, **38** (6), 606 (2008)].
12. Priezzhev A.V., Nikitin S.Yu., Lugovtsov A.E. *J. Quantitat. Spectrosc. Rad. Transfer*, **110**, 1535 (2009).
13. Dobbe J.G.G., Streekstra G.J., Hardeman M.R., Ince C., Grimbergen C.A. *Cytometry*, **50**, 313 (2002).
14. Plasek J., Marik T. *Appl. Opt.*, **21** (23), 4335 (1982).
15. Rasia R.J., Schutz G. *Clin. Hemorheol.*, **13**, 641 (1993).
16. Streekstra G.J., Dobbe J.G.G., Hoekstra A.G. *Opt. Express*, **18** (13), 14173 (2010).