

Possibility of Measuring of Erythrocyte Size Distribution Parameters by Laser Diffractometry of a Blood Smear

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Abstract. We consider the issues of obtaining information about the distribution of red blood cells in size from the scattering pattern of a laser beam on a wet blood smear. Within this article it is assumed to calculate the first three moments of the size distribution. The analytical model of light diffraction by blood smears was developed, as well as numerical experiments for verification suggested approach were carried out. The possibility of calculating average diameter of erythrocyte, width and asymmetry of size distribution by analyzing the angular distribution of light intensity in the diffraction pattern was shown. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: light scattering; laser diffractometry; data processing algorithms; red blood cells; size distribution; asymmetry.

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1 Introduction

In the human circulatory system, the microrheological properties of blood play an important role. The main microrheological parameters include the deformability and aggregation ability of erythrocytes [1]. The size distribution of red blood cells also affects the rheology of blood [2]. The importance of this distribution is evidenced, in particular, by the fact that the width of the erythrocyte size distribution is a reliable predictor of mortality in the general population of adults aged 45 years and older [3]. Typically, red blood cell size distribution is measured using a Coulter counter or flow cytometer [4–6]. Other measurement methods are also being developed, such as digital microscopy [7], hyperspectral holography [8], and laser diffractometry [9–14]. The advantage of the laser diffractometry method is that this method allows one to quickly process large ensembles of red blood cells without requiring complex equipment such as microscopes, microchannels, high-speed electronic circuits, etc.

The laser diffractometry method is based on the effect of scattering of a laser beam on a blood smear or a thin layer of red blood cell suspension. With such scattering, a pattern appears that resembles the pattern of light diffraction by a round aperture (the so-called Airy

pattern). The picture has axial symmetry. In its center there is a bright spot, the brightness of which decreases as the scattering angle increases. As a rule, this spot is surrounded by a dark ring, behind which a faint light ring is visible. The task of laser diffractometry is to determine the parameters of an ensemble of red blood cells based on measurements of the characteristics of the diffraction pattern. Note that the quality of the diffraction pattern strongly depends on the method of preparing the blood smear and its quality. For more details on this, see Ref. [15].

Some theoretical aspects of laser diffractometry of erythrocytes were considered in Refs. [16–20]. It is known that the angular size of the first dark ring in the diffraction pattern determines the average size of the red blood cells on the blood smear. The visibility of this ring determines the size distribution width of red blood cells. In this work, we identify the parameters of the diffraction pattern that determine the asymmetry of the size distribution of erythrocytes.

2 Characteristics of the Diffraction Pattern

Fig. 1 shows an example of a diffraction pattern that occurs when a laser beam is scattered on a blood smear, and the angular distribution of light intensity in this pattern $I = I(\theta)$. Normalizing this distribution to the

intensity of the central diffraction maximum $I(0)$, we obtain the function

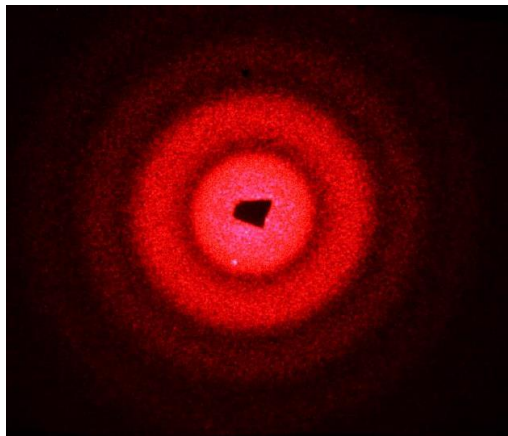
$$f(\theta) = \frac{I(\theta)}{I(0)}.$$

Let us consider a fragment of the diffraction pattern lying near the first minimum of light intensity (the first dark ring). In this region, the function $f(\theta)$ can be approximately represented as

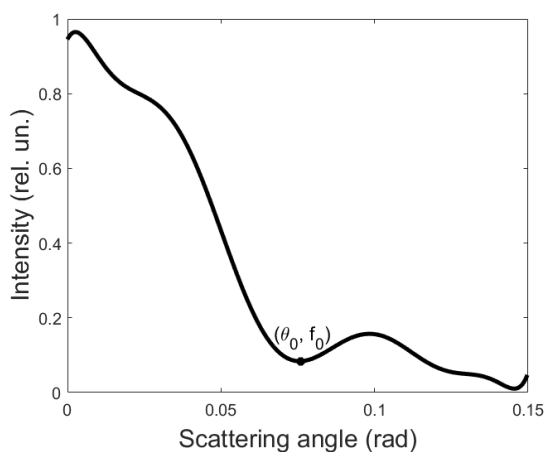
$$f(\theta) = f(\theta_0) + \frac{1}{2}f''(\theta_0) \cdot (\theta - \theta_0)^2. \quad (1)$$

Here θ_0 is the angular coordinate of the first minimum of light intensity in the diffraction pattern, determined by the condition

$$f'(\theta_0) = 0, \quad (2)$$



(a)



(b)

Fig. 1 (a) Diffraction pattern obtained from a wet blood smear and (b) the angular distribution of light intensity in this pattern. Laser wavelength $\lambda = 650$ nm.

where $f''(\theta_0)$ is the second derivative of the function $f(\theta)$ with respect to the scattering angle, taken at $\theta = \theta_0$. Let us introduce the dimensionless parameter

$$f_2 = f''(\theta_0) \cdot \theta_0^2 \quad (3)$$

and denote

$$f(\theta_0) = f_0. \quad (4)$$

The parameters θ_0 and f_0 are shown in the graph of the function $f(\theta)$ in Fig. 1b. These parameters, as well as the f_2 parameter, can be measured in an experiment on scattering of a laser beam on a blood smear.

3 Parameters of the Erythrocyte Ensemble

We will consider the red blood cells on a blood smear to be round discs and characterize them with radii R . The applicability of this model is justified by the fact that in the region of the diffraction pattern of interest to us, the indicatrix of light scattering by a biconcave disk and a flat disk differ negligibly little from each other [18]. Taking into account the scatter of red blood cells in size, we will consider the radius of the red blood cell to be a random variable and describe it with the following Eq.:

$$R = R_0 \cdot (1 + \varepsilon). \quad (5)$$

Here R_0 is the average radius of red blood cells on a blood smear, ε is a random parameter, the average value of which is considered equal to zero

$$\langle \varepsilon \rangle = 0. \quad (6)$$

The size distribution of red blood cells will be characterized by the parameters

$$\mu = \langle \varepsilon^2 \rangle \quad (7)$$

and

$$\nu = \langle \varepsilon^3 \rangle. \quad (8)$$

The μ value characterizes the width, and the ν value characterizes the asymmetry of the size distribution of erythrocytes. When calculating diffraction patterns, we will use the approximation of a weakly inhomogeneous ensemble of red blood cells. Mathematically, this approximation is expressed by the Eq.:

$$|\varepsilon| \ll 1. \quad (9)$$

For convenience, we also introduce the parameters

$$\mu' = \sqrt{\mu} \quad (10)$$

and

$$\nu' = \sqrt[3]{\nu}. \quad (11)$$

Our task is to express the parameters of erythrocytes R_0, μ, ν through the characteristics of the diffraction

pattern θ_0, f_0, f_2 and to construct an algorithm for measuring the average radius of an erythrocyte, as well as the width and asymmetry of the size distribution of erythrocytes using laser diffractometry of a blood smear.

4 Diffractometric Equations

Following the works [16, 17], we will model red blood cells as transparent flat disks. The angular distribution of light intensity in the diffraction pattern arising when a laser beam is scattered on a blood smear is approximately described by the following Eq.:

$$I(\theta) = \frac{1}{4} I_0 N |\alpha|^2 \left(\frac{k}{z}\right)^2 \langle R^4 G(kR\theta) \rangle. \quad (12)$$

Here I is the light intensity at a given point of the observation screen, R is the radius of a red blood cell, θ is the scattering angle, I_0 is the intensity of the incident laser beam, N is the number of red blood cells on the blood smear illuminated by the laser, z is the distance from the blood smear to the observation screen, $k = 2\pi/\lambda$ is the wave number, λ is the light wavelength, the parameter $|\alpha|^2$ characterizes the thickness and optical density of the red blood cell, angle brackets indicate averaging over the size of the red blood cells. The function G , called the Airy function, is defined by the Eq.:

$$G(x) = \left[\frac{2J_1(x)}{x} \right]^2, \quad (13)$$

where $J_1(x)$ is the first order Bessel function. The Airy function satisfies the condition $G(0) = 1$. Eq. (12) was obtained in the single scattering approximation and describes the distribution of light intensity in the far diffraction zone in that part of the observation screen where the radiation of the direct laser beam does not impinge. Let us represent the normalized distribution of light intensity in the diffraction pattern $f(\theta)$ in the form

$$\langle \rho^4 \rangle f(\theta) = \langle \rho^4 G(\rho\theta) \rangle. \quad (14)$$

Here

$$\rho = kR = 2\pi \frac{R}{\lambda} \quad (15)$$

is red blood cell size parameter.

Let us consider a limited part of the diffraction pattern located near the first minimum of light intensity. Mathematically, this region is determined by the condition $\rho\theta \approx x_0$, where the value x_0 is determined by the equation

$$J_1(x_0) = 0.$$

Here

$$x_0 = 3.8318. \quad (16)$$

Expanding the Bessel function into a Taylor series in the vicinity of the point x_0 , we obtain

$$J_1(x) = J_1'(x_0) \cdot (x - x_0) + \frac{1}{2} J_1''(x_0) \cdot (x - x_0)^2 + \frac{1}{6} J_1'''(x_0) \cdot (x - x_0)^3.$$

In the region $0.5 \leq x/x_0 \leq 1.3$ the error of this approximation does not exceed 3%. The derivatives of the Bessel function included in this expression can be calculated using the Eqs. [21]:

$$\begin{aligned} J_0'(x) &= -J_1(x), \\ J_1'(x) &= J_0(x) - \frac{1}{x} J_1(x). \end{aligned} \quad (17)$$

In this approximation, for the function $f(\theta)$ we obtain the expression

$$\langle \rho^4 \rangle f = \langle \rho^4 H(y) \rangle, \quad (18)$$

where

$$y = \frac{\rho\theta}{x_0} \quad (19)$$

is the normalized scattering angle. The function $H(y)$ is defined by the following Eq.:

$$H(y) = \frac{1}{x_0^2} \left[\frac{2\psi(y)}{y} \right]^2,$$

where

$$\begin{aligned} \psi(y) &= x_0 J_1'(x_0) \cdot (y - 1) + \frac{1}{2} x_0^2 J_1''(x_0) \cdot \\ &\cdot (y - 1)^2 + \frac{1}{6} x_0^3 J_1'''(x_0) \cdot (y - 1)^3. \end{aligned}$$

Using Eqs. (17), we obtain

$$\begin{aligned} J_1''(x) &= -\frac{1}{x} J_0(x) + \left(-1 + \frac{2}{x^2}\right) J_1(x), \\ J_1'''(x) &= \left(-1 + \frac{3}{x^2}\right) \left[J_0(x) - \frac{2}{x} J_1(x)\right]. \end{aligned}$$

Here $J_0(x)$ is the zeroth order Bessel function. In particular, for $x = x_0$

$$\begin{aligned} J_1'(x_0) &= J_0(x_0), \\ J_1''(x_0) &= -\frac{1}{x_0} J_0(x_0), \\ J_1'''(x_0) &= \left(-1 + \frac{3}{x_0^2}\right) J_0(x_0), \end{aligned}$$

Let us denote

$$J_0(x_0) = -\beta. \quad (20)$$

Here

$$\beta = 0.40276. \quad (21)$$

Then

$$J'_1(x_0) = -\beta,$$

$$J''_1(x_0) = \frac{1}{x_0}\beta,$$

$$J'''_1(x_0) = \left(1 - \frac{3}{x_0^2}\right)\beta.$$

The functions $\psi(y)$ and $H(y)$ take the form

$$\psi(y) = ay^3 - by^2 + cy - d$$

and

$$H(y) = c_1y^4 - c_2y^3 + c_3y^2 - c_4y + c_5 - \frac{c_6}{y} + \frac{c_7}{y^2}, \quad (22)$$

where

$$\begin{aligned} a &= \frac{\beta x_0}{6}(x_0^2 - 3), \\ b &= \frac{\beta x_0}{6}3(x_0^2 - 4), \\ c &= \frac{\beta x_0}{6}3(x_0^2 - 7), \\ d &= \frac{\beta x_0}{6}(x_0^2 - 12) \end{aligned} \quad (23)$$

and

$$\begin{aligned} a^2 \frac{4}{x_0^2} &= c_1, \\ ab \frac{8}{x_0^2} &= c_2, \\ (2ac + b^2) \frac{4}{x_0^2} &= c_3, \\ (ad + bc) \frac{8}{x_0^2} &= c_4, \\ (c^2 + 2bd) \frac{4}{x_0^2} &= c_5, \\ cd \frac{8}{x_0^2} &= c_6, \\ d^2 \frac{4}{x_0^2} &= c_7. \end{aligned} \quad (24)$$

Thus, the normalized angular distribution of light intensity in the diffraction pattern is represented as a polynomial relative to the normalized scattering angle. This distribution is expressed by Eqs. (18), (19), (22).

Derivatives of the function $f(\theta)$

$$f^{(n)}(\theta) = \frac{d^n}{d\theta^n} f(\theta)$$

are expressed by the following Eqs.:

$$\langle \rho^4 \rangle x_0 f^{(1)}(\theta) = \langle \rho^5 H^{(1)}(y) \rangle,$$

$$\langle \rho^4 \rangle x_0^2 f^{(2)}(\theta) = \langle \rho^6 H^{(2)}(y) \rangle$$

where

$$H^{(1)}(y) = \frac{dH}{dy} = 4c_1y^3 - 3c_2y^2 + 2c_3y - c_4 + \frac{c_6}{y^2} - 2\frac{c_7}{y^3},$$

$$H^{(2)}(y) = \frac{d^2H}{dy^2} = 12c_1y^2 - 6c_2y + 2c_3 - 2\frac{c_6}{y^3} + 6\frac{c_7}{y^4}.$$

The functions $f^{(n)}(\theta)$ are also polynomials with respect to the normalized scattering angle.

Let us find the angular coordinate θ_0 of the minimum light intensity in the diffraction pattern. Assuming

$$f^{(1)}(\theta_0) = 0$$

we get the Eq.:

$$\langle \rho^5 H^{(1)}(y_0) \rangle = 0,$$

where

$$y_0 = \rho \frac{\theta_0}{x_0}.$$

From here

$$\langle \rho^5 (4c_1y_0^3 - 3c_2y_0^2 + 2c_3y_0 - c_4 + \frac{c_6}{y_0^2} - 2\frac{c_7}{y_0^3}) \rangle = 0$$

or

$$4c_1 \langle \rho^5 y_0^3 \rangle - 3c_2 \langle \rho^5 y_0^2 \rangle + 2c_3 \langle \rho^5 y_0 \rangle - c_4 \langle \rho^5 \rangle + c_6 \langle \frac{\rho^5}{y_0^2} \rangle - 2c_7 \langle \frac{\rho^5}{y_0^3} \rangle = 0.$$

Here

$$\langle \rho^5 y_0^3 \rangle = \frac{\theta_0^3}{x_0^3} \langle \rho^8 \rangle,$$

$$\langle \rho^5 y_0^2 \rangle = \frac{\theta_0^2}{x_0^2} \langle \rho^7 \rangle,$$

$$\langle \rho^5 y_0 \rangle = \frac{\theta_0}{x_0} \langle \rho^6 \rangle,$$

$$\langle \frac{\rho^5}{y_0^2} \rangle = \frac{x_0^2}{\theta_0^2} \langle \rho^3 \rangle,$$

$$\langle \frac{\rho^5}{y_0^3} \rangle = \frac{x_0^3}{\theta_0^3} \langle \rho^2 \rangle.$$

Now

$$4c_1 \frac{\theta_0^3}{x_0^3} \langle \rho^8 \rangle - 3c_2 \frac{\theta_0^2}{x_0^2} \langle \rho^7 \rangle + 2c_3 \frac{\theta_0}{x_0} \langle \rho^6 \rangle - c_4 \langle \rho^5 \rangle + c_6 \frac{x_0^2}{\theta_0^2} \langle \rho^3 \rangle - 2c_7 \frac{x_0^3}{\theta_0^3} \langle \rho^2 \rangle = 0.$$

Let us consider a weakly inhomogeneous ensemble of erythrocytes. From Eqs. (9) and (15) it follows that

$$\rho = \rho_0 \cdot (1 + \varepsilon),$$

where

$$\langle \rho \rangle = \rho_0$$

is the average size of a red blood cell on a blood smear.
Let us enter the parameters

$$m_n = \frac{\langle \rho^n \rangle}{\rho_0^n} = \langle (1 + \varepsilon)^n \rangle.$$

Then

$$\langle \rho^n \rangle = m_n \rho_0^n$$

and the equation for the angle θ_0 takes the form

$$4c_1 \frac{\theta_0^3}{x_0^3} m_8 \rho_0^8 - 3c_2 \frac{\theta_0^2}{x_0^2} m_7 \rho_0^7 + 2c_3 \frac{\theta_0}{x_0} m_6 \rho_0^6 - \\ - c_4 m_5 \rho_0^5 + c_6 \frac{x_0^2}{\theta_0^2} m_3 \rho_0^3 - 2c_7 \frac{x_0^3}{\theta_0^3} m_2 \rho_0^2 = 0$$

or

$$4c_1 \frac{\theta_0^3}{x_0^3} m_8 \rho_0^3 - 3c_2 \frac{\theta_0^2}{x_0^2} m_7 \rho_0^2 + 2c_3 \frac{\theta_0}{x_0} m_6 \rho_0 - \\ - c_4 m_5 + c_6 \frac{x_0^2}{\theta_0^2 \rho_0^2} m_3 - 2c_7 \frac{x_0^3}{\theta_0^3 \rho_0^3} m_2 = 0.$$

Let us denote

$$z = \frac{\theta_0}{x_0} \rho_0.$$

Then

$$4c_1 m_8 z^3 - 3c_2 m_7 z^2 + 2c_3 m_6 z - c_4 m_5 + \\ + c_6 m_3 \frac{1}{z^2} - 2c_7 m_2 \frac{1}{z^3} = 0$$

or

$$4c_1 m_8 z^6 - 3c_2 m_7 z^5 + 2c_3 m_6 z^4 - \\ - c_4 m_5 z^3 + c_6 m_3 z - 2c_7 m_2 = 0. \quad (25)$$

Proceeding in the same way as when deriving Eq. (25), we obtain two more Eqs.:

$$m_4 z^2 f_0 = c_1 m_8 z^6 - c_2 m_7 z^5 + c_3 m_6 z^4 - \\ - c_4 m_5 z^3 + c_5 m_4 z^2 - c_6 m_3 z + c_7 m_2, \\ m_4 z^2 f_2 = 12c_1 m_8 z^6 - 6c_2 m_7 z^5 + \\ + 2c_3 m_6 z^4 - 2c_6 m_3 z + 6c_7 m_2. \quad (26)$$

Here the parameters f_0 and f_2 are determined by Eqs. (3), (4). For a weakly inhomogeneous ensemble of erythrocytes, the parameters m_n are related to the values of μ and ν by the relations

$$m_2 = 1 + \mu, \quad (27)$$

$$m_3 = 1 + 3\mu + \nu,$$

$$m_4 = 1 + 6\mu + 4\nu,$$

$$m_5 = 1 + 10\mu + 10\nu,$$

$$m_6 = 1 + 15\mu + 20\nu,$$

$$m_7 = 1 + 21\mu + 35\nu,$$

$$m_8 = 1 + 28\mu + 56\nu.$$

From Eqs. (23), (24) it follows that there are certain relationships between the coefficients of Eqs. (25), (26). These relations are expressed by the following Eqs.:

$$4c_1 - 3c_2 + 2c_3 - c_4 + c_6 - 2c_7 = 0,$$

$$c_1 - c_2 + c_3 - c_4 + c_5 - c_6 + c_7 = 0, \quad (28)$$

$$12c_1 - 6c_2 + 2c_3 - 2c_6 + 6c_7 = 8\beta^2.$$

For a weakly inhomogeneous ensemble of erythrocytes, we will look for a solution to Eqs. (25), (26) in the form

$$z = 1 + \alpha, \quad (29)$$

where

$$|\alpha| \ll 1$$

is a small parameter. Then, in an approximation linear in this parameter

$$z^n = 1 + n\alpha. \quad (30)$$

Let us introduce the values M_n , defining them by the Eqs.:

$$m_n = 1 + M_n. \quad (31)$$

From Eq. (27) it follows that

$$|M_n| \ll 1,$$

$$M_2 = \mu, \quad M_3 = 3\mu + \nu, \\ M_4 = 6\mu + 4\nu, \quad M_5 = 10\mu + 10\nu, \\ M_6 = 15\mu + 20\nu, \quad M_7 = 21\mu + 35\nu, \\ M_8 = 28\mu + 56\nu. \quad (32)$$

Substituting Eqs. (30), (31) into Eqs. (25), (26) and taking into account Eqs. (28), (32), we obtain the following Eqs.:

$$c_{1\alpha} \alpha + c_{1\mu} \mu + c_{1\nu} \nu = 0, \\ (1 + 6\mu + 4\nu + 2\alpha) f_0 = c_{2\alpha} \alpha + c_{2\mu} \mu + c_{2\nu} \nu, \\ (1 + 6\mu + 4\nu + 2\alpha) f_2 = 8\beta^2 + c_{3\alpha} \alpha + c_{3\mu} \mu + c_{3\nu} \nu. \quad (33)$$

Here

$$\alpha = \frac{\theta_0}{x_0} \rho_0 - 1. \quad (34)$$

The numbers x_0 and β are defined by Eqs. (16) and (21). The remaining coefficients are determined by the following Eqs.

$$\begin{aligned} c_{1\alpha} &= 24c_1 - 15c_2 + 8c_3 - 3c_4 + c_6, \\ c_{1\mu} &= 112c_1 - 63c_2 + 30c_3 - 10c_4 + 3c_6 - 2c_7, \\ c_{1\nu} &= 224c_1 - 105c_2 + 40c_3 - 10c_4 + c_6, \end{aligned} \quad (35)$$

$$\begin{aligned} c_{2\alpha} &= 6c_1 - 5c_2 + 4c_3 - 3c_4 + 2c_5 - c_6, \\ c_{2\mu} &= 28c_1 - 21c_2 + 15c_3 - 10c_4 + 6c_5 - 3c_6 + c_7, \\ c_{2\nu} &= 56c_1 - 35c_2 + 20c_3 - 10c_4 + 4c_5 - c_6, \end{aligned} \quad (36)$$

$$\begin{aligned} c_{3\alpha} &= 72c_1 - 30c_2 + 8c_3 - 2c_6, \\ c_{3\mu} &= 336c_1 - 126c_2 + 30c_3 - 6c_6 + 6c_7, \\ c_{3\nu} &= 672c_1 - 210c_2 + 40c_3 - 2c_6. \end{aligned} \quad (37)$$

From Eqs. (23), (24), (35) – (37) it follows that

$$\begin{aligned} c_{1\alpha} &= 8\beta^2, & c_{1\mu} &= 4\beta^2, \\ c_{1\nu} &= -\frac{16}{3}\beta^2 x_0^2, \\ c_{2\alpha} &= 0, & c_{2\mu} &= 4\beta^2, \\ c_{2\nu} &= 4\beta^2, \\ c_{3\alpha} &= -40\beta^2, & c_{3\mu} &= -4(4x_0^2 + 3)\beta^2, \\ c_{3\nu} &= -\frac{8}{3}(11x_0^2 - 30)\beta^2. \end{aligned} \quad (38)$$

Substituting Eq. (38) into Eq. (33), we obtain the following Eqs.:

$$8\beta^2\alpha + 4\beta^2\mu - \frac{16}{3}\beta^2 x_0^2\nu = 0,$$

$$(1 + 6\mu + 4\nu + 2\alpha)f_0 = 4\beta^2\mu + 4\beta^2\nu,$$

$$\begin{aligned} (1 + 6\mu + 4\nu + 2\alpha)f_2 &= \\ &= 8\beta^2 - 40\beta^2\alpha - 4(4x_0^2 + 3)\beta^2\mu - \frac{8}{3}(11x_0^2 - 30)\beta^2\nu \end{aligned}$$

or

$$2\alpha + \mu - \frac{4}{3}x_0^2\nu = 0, \quad (39)$$

$$\frac{1}{4\beta^2}(1 + 6\mu + 4\nu + 2\alpha)f_0 = \mu + \nu, \quad (40)$$

$$\begin{aligned} \frac{1}{8\beta^2}(1 + 6\mu + 4\nu + 2\alpha)f_2 &= \\ &= 1 - 5\alpha - \left(2x_0^2 + \frac{3}{2}\right)\mu - \frac{1}{3}(11x_0^2 - 30)\nu. \end{aligned} \quad (41)$$

From Eq. (39) it follows that

$$\alpha = -\frac{\mu}{2} + \frac{2}{3}x_0^2\nu. \quad (42)$$

Substituting Eq. (42) into Eqs. (40), (41), we obtain

$$\begin{aligned} \frac{1}{4\beta^2}\left[1 + 5\mu + 4\nu\left(1 + \frac{x_0^2}{3}\right)\right]f_0 &= \mu + \nu, \\ \frac{1}{8\beta^2}\left[1 + 5\mu + 4\nu\left(1 + \frac{x_0^2}{3}\right)\right]f_2 &= \\ &= 1 - (2x_0^2 - 1)\mu - (7x_0^2 - 10)\nu. \end{aligned} \quad (43)$$

Eqs. (42) and (43) solve the problem. They relate the characteristics of the diffraction pattern θ_0, f_0, f_2 with the parameters of the ensemble of erythrocytes ρ_0, μ, ν . The solution to these equations taking into account Eqs. (16), (21) has the form

$$\mu = \frac{-1.2977 + 233f_0 + f_2}{83.7 + 410f_0 + 18.6f_2}, \quad \nu = \frac{1.2977 - 66.7f_0 - f_2}{83.7 + 410f_0 + 18.6f_2}. \quad (44)$$

5 Measurement Algorithm

The algorithm for measuring parameters μ', ν', ρ_0 is expressed by the Eqs.:

$$\mu' = \sqrt{\frac{-1.2977 + 233f_0 + f_2}{83.7 + 410f_0 + 18.6f_2}}, \quad (45)$$

$$\nu' = \sqrt[3]{\frac{1.2977 - 66.7f_0 - f_2}{83.7 + 410f_0 + 18.6f_2}}, \quad (46)$$

$$\rho_0 = (1 - 0.5\mu + 9.79\nu) \frac{3.83}{\theta_0}. \quad (47)$$

The average diameter of a red blood cell on a blood smear can be found using the following Eq.:

$$D = 1.22 \frac{\lambda}{\theta_0} \cdot (1 - 0.5\mu + 9.79\nu). \quad (48)$$

In these Eqs., ρ_0, μ', ν' are the parameters of the size distribution of red blood cells, θ_0, f_0, f_2 are the characteristics of the diffraction pattern that occurs when the laser beam is scattered on a blood smear, λ is the laser wavelength. Namely, ρ_0 is the average size parameter of the erythrocyte, μ' is the relative width of the distribution of erythrocytes along the radii, ν' is the asymmetry coefficient of the distribution of erythrocytes along the radii, θ_0 is the scattering angle at which the first minimum light intensity in the diffraction pattern is achieved, f_0 – relative light intensity in the first minimum, f_2 – relative curvature of the angular distribution of light intensity at the first minimum intensity. The parameters $\lambda, \theta_0, f_0, f_2$ can be measured in an experiment by scattering a laser beam on a blood smear. The indicated values are mathematically determined by Eqs. (1) – (11).

On the stability of the algorithm. Estimates show that for the conditions of interest to us, the denominators of

Eqs. (44) weakly depend on the parameters f_0 and f_2 , which are the input data for the algorithm. If we neglect this dependence, then the parameters μ and ν become linear functions of the parameters f_0 and f_2 . This means that the relative error in measuring the parameters μ and ν is the same as the relative error in measuring the diffraction pattern parameters f_0 and f_2 . Further, by virtue of Eqs. (44) – (46), the relative error in measuring the parameter μ' is two times, and the parameter ν' is three times less than the relative error in measuring the parameters μ and ν . On this basis, we conclude that the measurement algorithm expressed by Eqs. (45), (46) is stable.

6 Verification of the Algorithm in a Numerical Experiment

Let us evaluate the accuracy of the algorithm using a model of a bimodal ensemble of red blood cells. This ensemble contains red blood cells of only two sizes. Let ρ_1 be the size parameter of the first component of the ensemble, ρ_2 be the size parameter of the second component of the ensemble, p be the fraction of particles of the first type. The parameters of the bimodal ensemble are found using the following Eqs.:

$$\begin{aligned}\rho_0 &= \langle \rho \rangle = p_1 \rho_1 + p_2 \rho_2, \\ \rho_1 &= \rho_0 (1 + \varepsilon_1), & \rho_2 &= \rho_0 (1 + \varepsilon_2), \\ \varepsilon_1 &= \frac{\rho_1}{\rho_0} - 1, & \varepsilon_2 &= \frac{\rho_2}{\rho_0} - 1, & (49) \\ \mu &= \langle \varepsilon^2 \rangle = p_1 \varepsilon_1^2 + p_2 \varepsilon_2^2, \\ \nu &= \langle \varepsilon^3 \rangle = p_1 \varepsilon_1^3 + p_2 \varepsilon_2^3.\end{aligned}$$

The angular distribution of light intensity in the diffraction pattern arising when a laser beam is scattered on a bimodal ensemble of erythrocytes is described by the Eq.:

$$f(\theta) = \frac{p_1 \rho_1^4 G(\rho_1 \theta) + p_2 \rho_2^4 G(\rho_2 \theta)}{p_1 \rho_1^4 + p_2 \rho_2^4},$$

where $p_1 = p$, $p_2 = 1 - p$, and the function $G(x)$ is determined by Eq. (13). Setting the parameters of the bimodal ensemble ρ_1 , ρ_2 and p , through numerical calculations we find the parameters of the diffraction pattern θ_0 , f_0 and f_2 , determined by Eqs. (2) – (4). The

characteristics of bimodal ensembles (BME) and the results of calculations of the parameters of diffraction patterns are presented in Table 1. The numerical values of the erythrocyte size parameters are selected for the laser radiation wavelength $\lambda = 0.63 \mu\text{m}$.

We use the found values of the parameters θ_0 , f_0 and f_2 as input data for the algorithm expressed by Eqs. (45) – (47). Using Eqs. (45) – (47), we calculate the parameters of the ensemble of erythrocytes ρ_0 , μ' , ν' and compare the obtained values with the exact values of the parameters found using Eqs. (10), (11), (49). The calculation results are presented in Table 2. In this table, ρ_{0c} , μ'_c , ν'_c are the exact values of the parameters of bimodal ensembles of erythrocytes, found using Eqs. (10), (11), (49). Approximate values of these parameters, found using algorithm (45) – (47), are designated ρ_0, μ', ν' . The errors in determining the parameters of an ensemble of erythrocytes using algorithm (45) – (47) are designated $\delta\rho_0, \delta\mu', \delta\nu'$. These errors are expressed as percentages.

Let us consider, as an example, the problem of reconstructing the parameters of a bimodal ensemble of erythrocytes from laser diffractometry data of a blood smear. The task is to determine the parameters of the bimodal ensemble p, ρ_1, ρ_2 from the measured parameters of the diffraction pattern θ_0, f_0, f_2 . Using the following Eqs.:

$$\begin{aligned}p\varepsilon_1 + (1-p)\varepsilon_2 &= 0, \\ \mu &= p\varepsilon_1^2 + (1-p)\varepsilon_2^2, \\ \nu &= p\varepsilon_1^3 + (1-p)\varepsilon_2^3\end{aligned}$$

we find [22]

$$\begin{aligned}p &= \frac{1}{2} \left(1 + \frac{\nu}{\sqrt{\nu^2 + 4\mu^3}} \right), \\ \varepsilon_1 &= \frac{\nu - \sqrt{\nu^2 + 4\mu^3}}{2\mu}, \\ \varepsilon_2 &= \frac{\nu + \sqrt{\nu^2 + 4\mu^3}}{2\mu}, \\ \rho_1 &= \rho_0 \cdot (1 + \varepsilon_1), \\ \rho_2 &= \rho_0 \cdot (1 + \varepsilon_2).\end{aligned} \quad (50)$$

Table 1 Parameters of diffraction patterns found by numerical experiment.

	BME 1	BME 2	BME 3	BME 4	BME 5
ρ_1	35	33	32	31	30
ρ_2	40	42	43	44	45
p	0.4	0.4	0.4	0.4	0.4
θ_0, rad	0.1005	0.0984	0.0968	0.0948	0.0923
f_0	0.002501	0.006984	0.009471	0.01181	0.01377
f_2	1.141	0.887	0.7777	0.7011	0.680

Table 2 Results on the evaluation of parameters of RBC size distribution, obtained in the numerical experiment.

	BME 1	BME 2	BME 3	BME 4	BME 5
ρ_{0c}	38	38.4	38.6	38.8	39
ρ_0	38	38.5	38.8	39.2	39.8
$\delta\rho_0, \%$	0	0.3	0.5	1	2
$\mu'_c, \%$	6.45	11.5	14.0	16.4	18.8
$\mu', \%$	6.34	10.9	12.9	14.6	15.9
$\delta\mu', \%$	1.7	5.2	7.9	11	15
ν'_c	-0.0478	-0.085	-0.104	-0.122	-0.140
ν'	-0.0458	-0.081	-0.103	-0.124	-0.144
$\delta\nu', \%$	4.2	4.7	1.0	1.6	2.9
p_c	0.4	0.4	0.4	0.4	0.4
p	0.41	0.40	0.37	0.35	0.33
$\delta p, \%$	2.5	0	7.5	12.5	17.5
ρ_{1c}	35	33	32	31	30
ρ_1	35.1	34.1	33.6	33.2	33.0
$\delta\rho_1, \%$	0.3	3.3	5.0	7.1	10
ρ_{2c}	40	42	43	44	45
ρ_2	40	42.6	43.9	45.1	46.5
$\delta\rho_2, \%$	0	1.4	2.1	2.5	3.3

The calculation results are presented in the same Table 2. In this table, $p_c, \rho_{1c}, \rho_{2c}$ are the exact values of the parameters of bimodal ensembles of erythrocytes. Approximate values of these parameters, found using Eqs. (45) – (47), (50) based on the analysis of diffraction patterns, are designated p, ρ_1, ρ_2 . The errors in determining parameters using algorithm (45) – (47), (50) are designated $\delta p, \delta\rho_1, \delta\rho_2$. These errors are expressed as percentages. Note that it is advisable to test the proposed algorithm on more complex models of red blood cell ensembles.

7 Discussion

In this paper we presented an algorithm to measure parameters of the size distribution of red blood cells on a blood smear. The algorithm is designed to measure the average size, as well as the width and asymmetry of the red blood cell size distribution. In the case of a bimodal ensemble of erythrocytes, these data make it possible to determine the individual size of the ensemble components under consideration, as well as the percentage ratio between them. The solution to the inverse scattering problem is presented in the form of explicit analytical expressions suitable for weakly homogeneous ensembles of erythrocytes. From a mathematical point of view, the possibility of analytically solving the inverse problem of scattering a laser beam on an inhomogeneous ensemble of red blood cells is associated with the use of two approximations. First, this

is a polynomial representation of the Bessel function and the Airy function, applicable to a limited part of the diffraction pattern that occurs when a laser beam is scattered on a blood smear. Second, the approximation of a weakly heterogeneous (in size) ensemble of erythrocytes. The first of these approximations makes it possible to write diffractometric equations in terms of elementary functions, the second makes it possible to linearize these equations with respect to small parameters and obtain their analytical solution. Both approximations introduce measurement errors, which we estimated using a numerical experiment. The results show that the error of the algorithm increases with increasing heterogeneity of the ensemble. The corresponding data are shown in Table 2. Thus, with an expansion of the size distribution of erythrocytes $\mu' = 14\%$, the measurement error of the ensemble parameters is not more than 8%, and with $\mu' = 18.8\%$ the measurement error reaches 17.5%. The results obtained can be used to create laser diffractometers of erythrocytes with expanded functionality.

For the practical implementation of the proposed measurement algorithm, several conditions must be met. Red blood cells on a blood smear should have their natural shapes, and their concentration should not be too high so that the condition of single light scattering is met. The laser beam must be sufficiently coherent. The width of the laser spectrum must be much less than its carrier frequency, and the coherence radius must significantly exceed the size of the red blood cell. Finally, the system

for recording diffraction patterns must have a sufficiently high resolution and a sufficiently wide dynamic range for measuring light intensity.

Let us also note the following circumstance. Eq. (48) shows that to accurately determine the average diameter of erythrocytes using laser diffractometry, it is necessary to know the width of the erythrocyte size distribution. This is due to the fact that the contribution of the red blood cell to the light intensity in the diffraction pattern is proportional to the fourth power of the radius of the red blood cell. In other words, the contribution of large erythrocytes dominates over the contribution of small erythrocytes. At a constant average radius of the erythrocyte, this leads to the fact that the scattering of erythrocytes in size shifts the minimum light intensity in the diffraction pattern to the region of small scattering angles. Therefore, the average size of a red blood cell on a blood smear depends not only on the angle θ_0 , but also on the width of the red blood cell size distribution.

Our theoretical model is based on the assumption of weak heterogeneity of the erythrocyte ensemble. We call an ensemble weakly heterogeneous for which the scatter of erythrocyte sizes is small compared to the average size of an erythrocyte. For blood samples close to normal, this condition is well met. Mathematically, the condition is expressed by the formula $\mu \ll 1$. In the numerical experiment we use a model of a bimodal ensemble of red

blood cells. This model is convenient because it is characterized by a minimum number of parameters. In the case of pathological blood samples (for example, with sickle cell anemia), the visibility of the dark ring in the diffraction pattern can be significantly reduced, which will be interpreted by our algorithm as an abnormally large dispersion of red blood cells in size.

Our algorithm is based on processing the diffraction pattern in the region of the first dark ring. As a rule, there is such a ring. But if it is not there, then this may indicate the presence of a large number of irregularly shaped cells, for example, echinocytes, in the blood sample.

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Disclosures

The authors declare that they have no conflict of interest.

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