

LASER METHODS IN MEDICINE

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Relation between the diffraction pattern visibility and dispersion of particle sizes in an ektacytometer

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Abstract. **We have calculated the angular distribution of the light intensity in the diffraction pattern arising upon scattering of a laser beam on a suspension of red blood cells in an ektacytometer. We have estimated the diffraction pattern visibility in the region of the first diffraction minimum and the first diffraction maximum as a function of particle size variation. It is shown that in this fragment of the diffraction pattern its visibility decreases already twofold in the case of a standard deviation of the particle size from the average value, equal to 8 %.**

Keywords: *diffraction, particle, red blood cell, ektacytometer, pattern visibility, scatter in the particle size.*

1. Introduction

Laser diffractometry of erythrocytes (ektacytometry) [1–3] is a method for studying red blood cells (erythrocytes), based on the analysis of diffraction patterns. The diffraction pattern is observed when a laser beam is scattered on a thin layer of the erythrocyte suspension confined between two transparent walls of the cups, one of which is fixed and the other is rotating (so-called Couette cell). Rotation of the cup causes the fluid flow and the appearance of shear stress in it. The orientation of red blood cells in the fluid flow is determined by the direction of this flow, namely, the red blood cells are aligned in the flow so that their resistance to the fluid movement is minimal. The laser beam in the ektacytometer is perpendicular to the flow, and so the symmetry axes of the particles are parallel or nearly parallel to the laser beam. At low rotation speeds of the cup red blood cells retain their natural shape (biconcave disk). In this scattering regime, the observed diffraction pattern is similar to the pattern of light diffraction on a round hole (so-called Airy pattern).

Measurements are performed as follows. A gradual increase in the rotation speed of the cup of the Couette cell results in an increase of the shear stress in the suspension of the erythrocytes. This leads to deformation of the particles that begin to extend in the direction of the flow. Deformation of the particles leads to corresponding changes in the shape of the interference lines, which acquire an ellipsoidal shape, on the observation screen. By measuring the axes of these ellipses a and b, the deformability index of red blood cells, defined as $D = (a - b)/(a + b)$, is found. The ratio of the deformability index to the shear stress characterises the deformability of red blood cells, which is measured in such experiments (see details in [4]).

In our paper [5], we established a relation between the dispersion of the erythrocyte sizes and the diffraction pattern visibility, observed upon scattering of the laser beam on a suspension of red blood cells in an ektacytometer. The question arises: Is it possible to use data of the laser diffractometry of red blood cells to estimate the dispersion of these particles by their sizes? The central part of the diffraction pattern (the central bright spot and the first dark ring on the observation screen) is inconvenient for solving this problem, because the visibility of a fragment of the diffraction pattern depends only weakly on the particle size dispersion [5]. At the same time, the visibility of other parts of the diffraction pattern may depend on the particle size dispersion much stronger. In this paper, we give an analytical assessment of the diffraction pattern visibility in the vicinity of the first diffraction maximum, taking into account the scatter in the particle sizes.

2. Model of an ensemble of erythrocytes

Let us represent a single red blood cell in the form of a circular transparent disk. The average radius of the disk is $\bar{R} = 4$ μm, the disk thickness is $h = 1.5$ μm, and the relative refractive index of the particle is $\tilde{n} = 1.05$. The erythrocyte radius R is considered to be a random quantity. We assume for simplicity that R is uniformly distributed within certain limits, namely

$$w(R) = \frac{1}{2\Delta R}\begin{Bmatrix} 1, |R - \bar{R}| \leqslant \Delta R \\ 0, |R - \bar{R}| > \Delta R \end{Bmatrix},$$

where $\bar{R}$ is the average radius of the particle; ΔR is the maximum deviation of the particle radius from its average value. The dispersion of the particle radii is $\sigma^2 = (\Delta R)^2/3$. We also assume that $\Delta R \ll \bar{R}$, i.e., the nonuniformity of an ensemble of particles in their sizes is relatively weak.

A similar distribution is true for the parameter of the particle size $\rho = kR$, where $k = 2\pi/\lambda$ is the wave number; λ is the radiation wavelength. Statistical moments of the size parameter can be calculated by the formula

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

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Here n is any positive number; $\varepsilon = \Delta\rho/\bar{\rho}$ is the measure of scatter in the particle sizes; $\bar{\rho} = k\bar{R}$ is the average value of the particle size parameter; $\Delta\rho = k\Delta R$ is the maximum deviation of the size parameter from its average value. We assume that the laser radiation wavelength is $\lambda = 0.633$ µm. In this case, $\bar{\rho} = 39.7$. The function

$$f_n(\varepsilon) = \frac{(1+\varepsilon)^n - (1-\varepsilon)^n}{2\varepsilon}.$$

In particular, when $n = 2, 3, 4$

$$\langle\rho^2\rangle = \bar{\rho}^2(1+\varepsilon^2/3), \quad \langle\rho^3\rangle = \bar{\rho}^3(1+\varepsilon^2),$$
$$\langle\rho^4\rangle = \bar{\rho}^4(1+2\varepsilon^2). \tag{1}$$

These expressions are written with accuracy up to terms of the order ε^2 inclusive. The aim of the paper is to establish the relation between the quantity ε and the diffraction pattern visibility, observed with the help of the method of laser diffractometry of red blood cells.

3. Light scattering from an ensemble of particles

Consider the scattering of the laser beam on a thin layer of the erythrocyte suspension in an ektacytometer. We assume that all particles are located in the same plane so that the symmetry axes of the particles are parallel to the laser beam. The method of calculating the angular distribution of the scattered light intensity is presented in our paper [5]. In particular, in the case of the laser beam scattering on a uniform-in-size ensemble of particles, the angular distribution of the light intensity is described by the formula

$$I(R,\theta) = I_0 N|\alpha|^2\left(\frac{\pi R^2}{\lambda z}\right)^2\left[\frac{J_1(kR\theta)}{0.5kR\theta}\right]^2. \tag{2}$$

Here I_0 is the laser beam intensity; θ is the scattering angle; R is the particle radius; N is the number of particles illuminated by the laser beam; z is the distance from the measuring volume to the observation screen; $J_1(x)$ is the first-order Bessel function; $|\alpha|^2 = 4\sin^2(\Delta\varphi/2)$; $\Delta\varphi = kn_0h(\tilde{n}-1)$; $\tilde{n}$ is the relative refractive index of the particle; n_0 is the absolute refractive index of the medium surrounding the particle. For the conditions we are interested in, $h = 1.5$ µm, $n_0 = 1.33$, $\Delta\varphi \approx 1$ rad, and $|\alpha|^2 \approx 1$. Distribution (2) can be written as

$$I(\theta,\rho) = I_0 N|\alpha|^2\left(\frac{\rho^2}{2kz}\right)^2\left[\frac{2J_1(\rho\theta)}{\rho\theta}\right]^2. \tag{3}$$

We will take into account the scatter in the particle sizes by averaging expression (3) with respect to the particle size parameter ρ. The possibility of such a procedure is justified in [5]. Thus, we obtain an expression for the angular distribution of the light intensity:

$$I(\theta) = \langle I(\theta,\rho)\rangle_\rho.$$

This formula describes the diffraction pattern arising upon scattering of the laser beam by a nonuniform-in-size ensemble of the particles. Below, we will consider the regions of the diffraction pattern that are close to the first diffraction minimum and the first diffraction maximum. In these regions, the Bessel function has a simple (linear or quadratic) approximation.

4. First diffraction minimum

In the region of the first diffraction minimum (the first dark ring on the observation screen), the Bessel function can be approximated by a linear function

$$J_1(x) = \beta(x - x_1). \tag{4}$$

Here x_1 is the argument of the Bessel function at which it vanishes; β is the derivative of the Bessel function at the point $x = x_1$. It is known [6] that $x_1 = 3.82$ and $\beta = -0.4$. The angle θ_1 at which the first diffraction minimum is visible is found from the expression $\theta_1 = x_1/\bar{\rho}$. In our case, θ_1=0.09 rad. Substituting (4) into (3), we obtain

$$I_1(\theta,\rho) = I_0 N|\alpha|^2\left(\frac{1}{2kz}\right)^2(2\beta)^2\left(\rho^4 - 2\rho^3\frac{x_1}{\theta} + \rho^2\frac{x_1^2}{\theta^2}\right).$$

Averaging this function over ρ and taking into account formula (1), we find

$$I_1(\theta) = I_0 N|\alpha|^2\left(\frac{1}{2kz}\right)^2(2\beta)^2\bar{\rho}^4$$
$$\times\left[(1+2\varepsilon^2) - 2(1+\varepsilon^2)\frac{x_1}{\bar{\rho}\theta} + \left(1+\frac{\varepsilon^2}{3}\right)\frac{x_1^2}{\bar{\rho}^2\theta^2}\right].$$

This function describes the angular distribution of the light intensity near the first diffraction minimum. To estimate this minimum we set $\theta = \theta_1$. Then

$$I(\theta_1) = I(0)(2\beta)^2\frac{\varepsilon^2}{3}, \tag{5}$$

where

$$I(0) = I_0 N|\alpha|^2\left(\frac{\bar{\rho}^2}{2kz}\right)^2$$

is the light intensity in the zero (central) maximum of the diffraction pattern.

5. First diffraction maximum

In the region of the first diffraction maximum (the first bright ring on the observation screen) the Bessel function can be approximated by a quadratic function

$$J_1(x) = a + \frac{1}{2}b(x - x_2)^2,$$

where x_2 is the value of the argument at which the Bessel function becomes minimal; $a = J_1(x_2)$; b is the value of the second derivative of the Bessel function at the point $x = x_2$. It is known [6] that $x_2 = 5.32$, $a = -0.346$ and $b = 0.4$. The angle θ_2 at which the first diffraction maximum is visible is found from the expression $\theta_2 = x_2/\bar{\rho}$. In our case, $\theta_2 = 0.13$ rad. The square of the Bessel function can be approximately represented in the form

$$J_1^2(x) = a^2 + ab(x - x_2)^2. \qquad (6)$$

Substituting (6) into (3), we obtain

$$I_2(\theta,\rho) = I_0 N|\alpha|^2\left(\frac{\bar\rho^2}{2kz}\right)^2\left(\frac{2a}{\bar\rho\theta}\right)^2\left(\frac{1}{\bar\rho^2}\right)$$

$$\times\left(\rho^2 + \frac{b}{a}\rho^4\theta^2 - 2\frac{b}{a}x_2\rho^3\theta + \frac{b}{a}x_2^2\rho^2\right).$$

Averaging this function over ρ and taking into account formula (1), we find

$$I_2(\theta) = I_0 N|\alpha|^2\left(\frac{\bar\rho^2}{2kz}\right)^2\left(\frac{2a}{\bar\rho\theta}\right)^2$$

$$\times\left[1 + \frac{\varepsilon^2}{3} + (1+2\varepsilon^2)\frac{b}{a}\bar\rho^2\theta^2 - 2(1+\varepsilon^2)\frac{b}{a}x_2\bar\rho\theta + \left(1+\frac{\varepsilon^2}{3}\right)\frac{b}{a}x_2^2\right].$$

This function describes the angular distribution of the light intensity near the first diffraction maximum. To estimate this maximum we set $\theta = \theta_2$. Then

$$I(\theta_2) = I(0)\left(\frac{2a}{x_2}\right)^2\left[1 + \frac{\varepsilon^2}{3}\left(1 + \frac{b}{a}x_2^2\right)\right]. \qquad (7)$$

6. Estimate of the diffraction pattern visibility

According to the standard definition [7], evaluation of the diffraction pattern visibility in the region of the first diffraction minimum and the first diffraction maximum of the light intensity is given by the expression

$$v = \frac{I(\theta_2) - I(\theta_1)}{I(\theta_2) + I(\theta_1)}. \qquad (8)$$

Substituting (5) and (7) into (8), we obtain

$$v = \left\{1 + \frac{\varepsilon^2}{3}\left[1 + \frac{b}{a}x_2^2 - \left(\beta\frac{x_2}{a}\right)^2\right]\right\}$$

$$\times\left\{1 + \frac{\varepsilon^2}{3}\left[1 + \frac{b}{a}x_2^2 + \left(\beta\frac{x_2}{a}\right)^2\right]\right\}^{-1}$$

or, approximately,

$$v = 1 - 2\frac{\varepsilon^2}{3}\left(\beta\frac{x_2}{a}\right)^2.$$

This formula determines the diffraction pattern visibility in the region of the first diffraction minimum and the first diffraction maximum, calculated with account for the scatter in the particle sizes. The obtained result can be represented in the form

$$v = 1 - \gamma\delta_R^2, \qquad (9)$$

where $\delta_R = \sigma_R/\bar R$ is the relative scatter in the particle size. The parameter γ is found from the formula

$$\gamma = 2\left(\beta\frac{x_2}{a}\right)^2 \approx 76. \qquad (10)$$

For example, setting $v = 1/2$, we obtain $\delta_R = 0.08$. Thus, the twofold decrease in the visibility of the diffraction pattern near the first minimum and first maximum of the light intensity occurs already in the case of a standard deviation of the particle sizes from the average value, equal to 8%.

Note that according to formulas (9) and (10), the visibility of the diffraction pattern does not depend on such parameters as the mean particle radius $\bar R$, the laser wavelength λ and the distance z from the measuring volume to the observation screen. In our model, the visibility of the diffraction pattern depends only on the scatter in the particle sizes. Analytic evaluation of the visibility (9) is obtained under the assumption of a rectangular particle size distribution.

7. Numerical simulation of laser radiation scattering on a nonuniform-in-size ensemble of particles

To take into account the possible scatter in the particle sizes, it is necessary to average expression (2) over the particle radii R. In the computer calculations, we assume the particle radius to be a discrete random quantity that can take the value of R_j with the probability p_j. Then the angular intensity distribution of the light scattered by a nonuniform ensemble of particles can be represented as

$$I(\theta) = \sum_{j=-m}^{m} I(R_j,\theta)p_j, \qquad (11)$$

where

$$I(R_j,\theta) = I_0 N|\alpha|^2\left(\frac{\pi R_j^2}{\lambda z}\right)^2\left[\frac{J_1(kR_j\theta)}{0.5kR_j\theta}\right]^2. \qquad (12)$$

Probabilities p_j obey the normalisation condition $\sum_{j=-m}^{m} p_j = 1$. We will characterise an ensemble of the particles by an average particle radius $\bar R$, the dispersion of particle sizes σ_R^2 and the total number of particle types M. The quantity $\delta_R = \sigma_R/\bar R$ means the relative scatter in the particle sizes. The possible values of the particle radii are defined by the expression $R_j = \bar R + jr$, where $j = 0, \pm1, \pm2, ..., \pm m$. In the examples presented below, we assume $\bar R$ = 4 μm, λ = 0.63 μm, z = 10 cm.

(i) An ensemble of particles contains only two types of particles (bimodal ensemble). In this case, we have $M = 2$, $R_{-1} = \bar R - \sigma_R$, $R_1 = \bar R + \sigma_R$ and $p_{-1} = p_1 = 1/2$. For the given values of $\bar R$ and σ_R, these formulas allow us to calculate the angular intensity distribution of the light scattered by this ensemble of particles and find the dependence of the diffraction pattern visibility (of one or another of its fragments) on the scatter in the particle sizes. The corresponding dependence $v_{12}(\delta_R)$ is shown in Fig. 1 [curve (*1*)].

(ii) The particles are uniformly distributed in sizes in a certain range. To simulate this distribution, we set $M = 100$ and $p_j = 1/M$. The half-width of the ΔR distribution is obtained from the formula $\Delta R = \sigma_R\sqrt{3}$, the quantity r is found from the ratio $\Delta R = \frac{1}{2}Mr$. Thus, $r = 2\sqrt{3}\,\sigma_R/M$. The dependence $v_{12}(\delta_R)$ for this case is shown in Fig. 1 [curve (*2*)].

(iii) The Gaussian particle size distribution. For its simulation, we set

$$p_j = \frac{r}{\sigma_R\sqrt{2\pi}}\exp\left(-\frac{r^2 j^2}{2\sigma_R^2}\right)$$

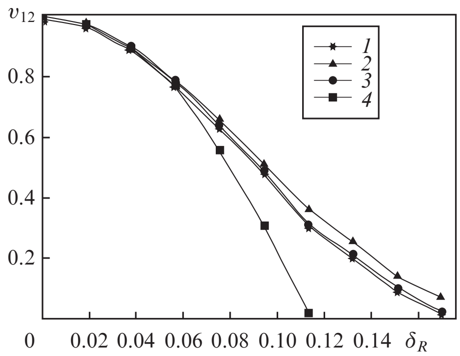

Figure 1. Dependences of the diffraction pattern visibility of the scatter in the particle sizes. Curves (*1–3*) are obtained numerically from formulas (11), (12) and correspond to different types of particle size distributions – bimodal (*1*), uniform (*2*), Gaussian (*3*); curve (*4*) is plotted using formulas (9) and (10).

and $M = 100$. The choice of the proportionality factor between σ_R and $\Delta R = \frac{1}{2}Mr$ is largely arbitrary. For our calculations we have chosen it to be equal to 3. Then, $r = 6\sigma_R/M$. The dependence $v_{12}(\delta_R)$ for this case is shown by curve (*3*). Here, for comparison, we present curve (*4*) plotted by using formulas (9), (10). One can see that in the region of small scatter in the particle sizes all the shown dependences are in good agreement with each other.

We have performed similar calculations for different values of such parameters as the mean particle radius $\bar{R}$, the laser wavelength λ and the distance z from the measuring volume to the observation screen. Analysis of the obtained data suggests that these parameters have virtually no effect on the form of the function $v = v(\delta_R)$, which expresses the dependence of the diffraction pattern visibility on the scatter in the particle sizes. The numerical results presented in Fig. 1 show that the particle size distribution also has little effect on the visibility of the diffraction pattern, at least for the investigated cases.

8. Conclusions

We have analytically assessed the visibility of the diffraction pattern, observed in the laser ektacytometer in the vicinity of the first diffraction maximum. We have shown that the diffraction pattern visibility is very sensitive to the level of the scatter in the particle sizes. Such parameters as the mean particle radius, the laser wavelength, the distance from the measuring volume to the observation screen, and the type of the particle size distribution have little effect on the form of the function, expressing the dependence of the diffraction pattern visibility on the scatter in the particle sizes. This opens up a possibility of measuring the size dispersion of red blood cells by the method of laser diffractometry.

The implementation of this method would mean expanding the functionality of laser diffractometry as a diagnostic method of red blood cells. We believe that in addition to the existing functions the laser diffractometer should be capable of measuring the visibility (contrast) of the diffraction pattern. To improve the reliability of the obtained data, it is reasonable to calibrate the device by observing the pattern of light scattering on suspensions of reference monodisperse or polydisperse particles.

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