

PACS numbers: 42.25.Fx; 87.64.Cc
DOI: 10.1070/QE2010v040n12ABEH014504

On the problem of the diffraction pattern visibility in laser diffractometry of red blood cells

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***Abstract*. We consider the problem of the visibility of the diffraction pattern that is observed in scattering laser radiation on the erythrocyte suspension in ectacytometer. The theoretical estimates show that 10 % variation in the particle size reduces the diffraction pattern visibility by 1 % only.**

Keywords: *scattering of light, red blood cells, scatter in the particle size, visibility of the diffraction pattern.*

1. Introduction

Laser diffractometry of red blood cells (ectacytometry), proposed in 1975 [1 – 3], was used by many authors to measure the deformability of red blood cells under normal and pathological conditions (see, for example, [4 – 8]). Thus, the method of laser diffractometry showed that acute cerebral ischemia is accompanied by a significant decrease in erythrocyte deformability [9]. Some theoretical aspects of the method were considered in [10 – 13]. Streekstra et al. [10] suggested using an anomalous diffraction approximation to calculate the scattering of laser radiation by erythrocytes. The high accuracy of these calculations was confirmed in [11], where the red cells were simulated by three-dimensional ellipsoids. Wriedt et al. [12] compared different methods and approximations used in calculations of light scattering by individual red blood cells. Stoltz et al. [13] showed that the angular sizes of the zero diffraction maximum already contain information about the shape of red blood cells.

One of the drawbacks of this method in studying the blood cells is the low contrast of the interference pattern observed in the diffraction of the laser beam on a suspension of erythrocytes in the ectacytometer. According to our own experience and publications of other authors [7], the visibility of the observed pattern is usually much less than unity, and the number of distinct interference fringes does not exceed two or three. However, as follows from the experiments on the laser beam diffraction on transparent spherical particles [14], it is possible to observe interference patterns with a high contrast and visibility that is close to unity. In this connection, the question arises as to what factors determine the diffraction pattern visibility in the laser diffractometer of erythrocytes. One of such factors may be the spread of the particle size, which for a normal population of erythrocytes is approximately 10 % [15]. The purpose of this paper is to evaluate the impact of the size variation of red blood cells on the visibility of the diffraction pattern, observed in scattering laser radiation on the erythrocyte suspension in the ectacytometer.

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Received 28 October 2010
Kvantovaya Elektronika **40** (12) 1074 – 1076 (2010)
Translated by I.A. Ulitkin

2. Basic parameters

A normal erythrocyte is a biconcave disk of about 8 μm in diameter. The disk thickness h at the center is about 1 μm and along the edge is about 2 μm. The refractive index of the erythrocyte relative to the blood plasma is $n = 1.05$. At a wavelength of 0.633 μm, typically used in the diffractometer, the erythrocyte weakly absorbs light. Thus, in terms of optics, the erythrocyte can be viewed as a transparent, large, optically soft particle.

In the diffractometer, the laser beam diameter is $A \approx 1$ mm, the distance from the probed volume to the observation screen is $z = 10$ cm, the number of red blood cells in the laser beam is $N \approx 1000$. These data allow us to calculate the red blood cell size parameter $2\pi a/\lambda = 80$, the angle of diffraction divergence $\theta = \lambda/a \approx 0.08$ rad $\approx 4.6^\circ$, the diffraction length $z_a = ka^2/2 = 0.3$ mm, as well as the size of the diffraction pattern on the observation screen $x_0 = z\theta = 0.8$ cm and the diffraction length of the laser beam $z_A = kA^2/2 = 5$ m ($k = 2\pi/\lambda$).

Note that between the parameters of the system there exist strong inequalities: $z_a \ll z \ll z_A$. This means that the observation screen has an area in which the radiation diffracted on erythrocytes rather than radiation of a direct laser beam falls (Fig. 1).

Below, we calculated the distribution of the radiation intensity in this area.

3. Diffraction of a laser beam on a nonuniform-in-size ensemble of particles

As noted above, one of the factors affecting the diffraction pattern visibility is a size variation of red blood cells. To evaluate the influence of this factor, we consider the laser beam diffraction on a nonuniform-in-size ensemble of particles.

Using the anomalous diffraction approximation [16, 17], we will take into account the following in a laser diffractometry of red blood cells:

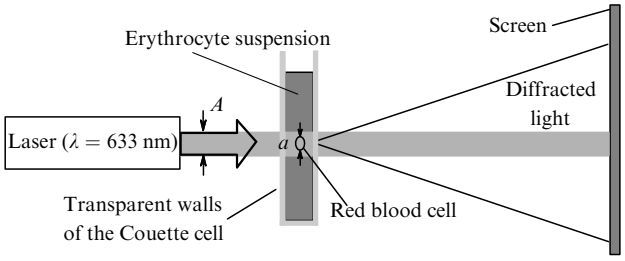

Figure 1. Experimental setup for the diffraction of the laser beam on a suspension of red blood cells.

(i) the diffraction light field, generated by a particle in the region of space lying outside the laser beam, is expressed by the diffraction integral, which taken over the particle surface;

(ii) in diffracting the laser beam on an ensemble of particles whose coordinates are independent random variables, the average light intensity at any point on the observation screen is equal to the sum of the light intensities produced at this point by the individual particles of the ensemble;

(iii) in diffracting the laser beam on a particle ensemble that is randomly nonuniform in size, the angular distribution of the light intensity $I(\theta)$ is proportional to the corresponding distribution for a single particle, averaged over the particle size:

$$I(\theta) = N\int_0^\infty I(\theta, R)w(R)\mathrm{d}R.$$

Here, θ is the light scattering angle; $I(\theta, R)$ is the angular light intenisty distribution in the diffraction pattern produced by a particle with a radius R; $w(R)$ is the probability density distribution for a particle radius.

We will simulate a separate particle by a transparent dielectric cylinder of radius R and height h from a material with a relative refractive index n. Let us assume that the bases of all the particles lie in the same plane, perpendicular to the laser beam. In this case [18]

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

Here, I_0 is the laser beam intensity; $x = \rho\theta$; $\rho = kR$ is the particle size parameter; $|\alpha|^2 = 4\sin^2(\Delta\varphi/2)$; $\Delta\varphi = kn_0h \times(n-1)$; n_0 is the absolute refractive index of the medium surrounding the particle. For the conditions interesting to us, we have $h = 1.5$ μm, $n_0 = 1.33$, $\Delta\varphi \approx 1$ rad, and $|\alpha|^2 \approx 1$.

We assume for simplicity that the values of the particle radius R are uniformly distributed in some interval, namely:

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

where $\bar{R}$ is the mean particle radius; ΔR is the maximal deviation of the particle radius from its mean value. The dispersion of the particle radii is found from the expression $\sigma^2 = (\Delta R)^2/3$. We also assume that $\Delta R \ll \bar{R}$, i.e., the nonuniformness of the ensemble in the particle size is relatively weak.

Consider the angular distribution of the light intensity near the first minimum of the diffraction pattern (the first dark fringe). In this region, the Bessel function can be approximated by a linear function

$$J_1(x) = \beta(x - x_1),$$

where x_1 is the argument of the Bessel function, for which this function vanishes; β is the derivative of the Bessel function at the point $x = x_1$. It is known that $x_1 = 3.82$ and $\beta = -0.4$. In the domain of arguments $3.0 \leqslant x \leqslant 4.2$, the error of the linear approximation of the Bessel function does not exceed 10 % [19]. It follows from these formulas that near the first minimum of the diffraction pattern, the angular distribution of the light intensity has the form

$$I(\theta, \rho) = I_0|\alpha|^2\left(\frac{\pi\rho^2}{k^2\lambda z}\right)^2\left[\frac{2\beta(x - x_1)}{x}\right]^2.$$

Let us introduce the quantities $\bar{\rho} = k\bar{R}$, $\Delta\rho = k\Delta R$, $\varepsilon = \Delta R/\bar{R}$, and $\theta_1 = x_1/\bar{\rho} = 0.61\lambda/\bar{R}$. Here, $\bar{\rho}$ is the average value of the particle size parameter, and θ_1 is the angle at which the first minimum of the diffraction pattern is seen in the absence of the spread of the particle size. Then, $x_1 = \bar{\rho}\theta_1$, and the expression for $I(\theta, \rho)$ can be rewritten in the form:

$$I(\theta, \rho) = I_0|\alpha|^2\left(\frac{\pi\rho^2}{k^2\lambda z}\right)^2\left[\frac{2\beta(\rho\theta - \bar{\rho}\theta_1)}{\rho\theta}\right]^2. \quad (1)$$

Now

$$I(\theta) = N\int_0^\infty I(\theta, \rho)w(\rho)\mathrm{d}\rho. \quad (2)$$

By substituting (1) into (2), we obtain

$$I(\theta) = I_0|\alpha|^2N\left(\frac{\pi}{k^2\lambda z}\right)^2 4\beta^2\frac{1}{2\Delta\rho}\int_{\bar{\rho}-\Delta\rho}^{\bar{\rho}+\Delta\rho}\rho^4\left(1 - \frac{\bar{\rho}\theta_1}{\rho\theta}\right)^2\mathrm{d}\rho.$$

Calculating the integral yields

$$f(\theta) \equiv I(\theta)/I(0) = \left[\frac{2\beta(\theta - \theta_1)}{\theta}\right]^2 + (2\beta\varepsilon)^2\left[2 - 2\frac{\theta_1}{\theta} + \frac{1}{3}\left(\frac{\theta_1}{\theta}\right)^2\right] + \frac{1}{5}(2\beta\varepsilon^2)^2,$$

where

$$I(0) = I_0|\alpha|^2N\left(\frac{\pi\bar{R}^2}{\lambda z}\right)^2$$

is the light intensity in the central maximum of the diffraction pattern in the absence of the spread of the particle size. For the conditions of interest, $\theta_1 = 0.096$ rad, $I(0)/I_0 = 0.64 \times 10^{-3}$.

Taking into account that the parameter ε is a small quantity, the final result can be presented in the form

$$\frac{f(\theta)}{(2\beta)^2} = \left(1 - \frac{\theta_1}{\theta}\right)^2 + \frac{\varepsilon^2}{3}\frac{\theta_1}{\theta}\left(\frac{\theta_1}{\theta} - 6\right) + 2\varepsilon^2.$$

Here, the first term in the right-hand side describes the angular distribution of the light intensity near the minimum of the diffraction pattern in the absence of the spread of the particle size. The remaining terms describe the corrections caused by this spread. Analysis of the derived expression

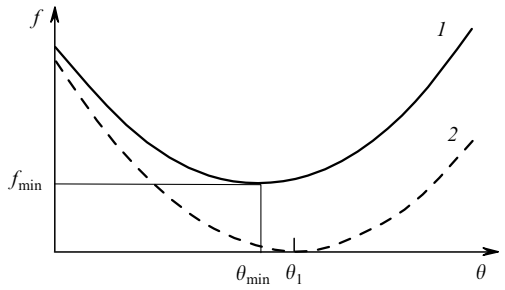

Figure 2. Angular distribution of the scattered light intensity near the first minimum of the diffraction pattern with allowance for (*1*) and without (*2*) the spread of the particle size ($\sigma/\bar{R} = 0.1$).

shows (Fig. 2) that the minimum light intensity in the diffraction pattern

$$I_{min} = I(0)\frac{(2\beta\varepsilon)^2}{3} \quad (3)$$

is observed at an angle

$$\theta_{min} \approx \left(1 - \frac{2}{3}\varepsilon^2\right)\theta_1.$$

Thus, due to the spread of the particle size, the radii of the interference fringes decrease, and the minimum light intensity in the diffraction pattern becomes different from zero. As seen from (1), the intensity of light scattered by a particle increases rapidly with increasing radius of the latter (as ρ^4). As a result, the contribution to the scattering pattern with respect to large particles becomes dominant, which leads to a decrease in the radii of the interference fringes.

4. Effect of the variations in the particle size on the diffraction pattern visibility

Let us estimate now the visibility of the interference pattern when the laser beam diffracts on a nonuniform ensemble of the particles. The visibility is defined as

$$v = \frac{I_{max} - I_{min}}{I_{max} + I_{min}},$$

where I_{max} and I_{min} is the light intensity in adjacent maximum and minimum of the diffraction pattern. Taking into account that $f_{min} = I_{min}/I(0) \ll 1$, $I_{max} = I(0)$, we obtain $v = 1 - 2f_{min}$. Then, using expression (3), we obtain

$$v = 1 - \frac{8}{3}\beta^2\varepsilon^2, \text{ or } v = 1 - 1.3\left(\frac{\sigma}{\bar{R}}\right)^2,$$

where σ^2 is the dispersion of the particle sizes. For example, putting $\sigma/\bar{R} = 0.1$, we obtain $v = 0.99$. This estimate shows that the natural size variation of red blood cells (about 10 %) has little effect on the visibility of the interference pattern, reducing it only by 1 %. Thus, we can conclude that the size variation of red blood cells does not prevent the attainment of a high visibility of the diffraction pattern in the case of the laser diffractometry of red blood cells.

5. Conclusions

Using the anomalous diffraction approximation, we have considered single scattering of the laser beam on an ensemble of transparent cylinders, simulating the red blood cells. We have taken into account the nonuniformity of the ensemble with respect to the particle size. We have derived an analytical expression for the angular distribution of the light intensity near the minimum of the interference pattern. We have found the dependence of the angular coordinates of the interference fringes and the visibility of the diffraction pattern on the spread of the particle size. More accurate estimates can be obtained by using numerical calculations of the diffraction patterns. Note, in this connection, the calculation methods based on the discrete-dipole [20] and ray-wave [21, 22] approximations.

Our calculations show that the natural variation in the particle size should not significantly reduce the visibility of the interference pattern in laser diffractometry of red blood cells. In practical terms, this means that under the experimental conditions it is important to make sure that the signal on the observation screen is absent in the absence of red blood cells in a solution. If so, the observed signal indeed characterises the red blood cells and is not a spurious signal scattered on any other particles such as dust in the air.

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