

ANALYSIS OF LASER BEAM SCATTERING BY AN ENSEMBLE OF PARTICLES MODELING RED BLOOD CELLS IN EKTACYTOMETER

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ABSTRACT. Using a simple theoretical model, we have obtained approximate relations between the characteristics of particles, modeling red blood cells, and the parameters of the diffraction pattern, produced by a laser beam diffracted in the ektacytometer. We have estimated, in particular, the effect of the particles size dispersion on the diffraction pattern visibility. The estimate shows, that relation of light intensities in the first minimum and the first maximum in the diffraction pattern is a parameter, which is rather sensitive to the particles size dispersion.

1. Introduction

Laser ektacytometry is used for determination the sizes and ability of red blood cells (RBCs) to deform and change their shape due to shear stress in the flow, e.g., while passing through capillaries with small diameters [2]. In in-vitro measurements with this method, the diffraction of laser beam on a dilute suspension of RBCs is observed. Usually the suspension is placed into a thin gap between a stationary cup and a bob that can be rotated with variable rate (so called Couette chamber). Rotation of the bob causes a suspension flow, which orients the RBCs so that their axes of symmetry become parallel to the illuminating laser beam. The concentrations of the particles in the suspension usually are chosen so that single light scattering processes dominate.

If the shear rate in the flow is low, the laser beam diffraction pattern has an axial symmetry and represents a system of concentric dark and light rings. The rings radii and brightness depend on the particles sizes and shapes as well as on the hemoglobin concentration inside the particles. An increase in shear rate results in elongation of deformable RBCs and consequent deformation of the diffraction pattern in the direction perpendicular to the direction of the suspension flow. This testifies to the particles deformation by shear stress. The deformation dependence on the shear stress is a measure of the RBCs deformability.

In principle, this method enables one to obtain more information, for example to estimate the relative number of undeformable RBCs in a specially prepared sample of blood [1]. However, this requires further development of the experimental technique and data processing algorithms. In particular, this concerns the theoretical models of light scattering by inhomogeneous ensembles of particles and a more accurate analysis of factors that

affect the size and visibility of the diffraction pattern. Note that in this connection the calculation techniques based on the anomalous diffraction approximation [3-4], discrete-dipole [5] and ray-wave [6] approximations may be used.

2. Red blood cells size dispersion and visibility of the diffraction pattern

2.1. Model of RBC. We model a single RBC as a circular transparent disk. The average disk radius $R = 4 \mu\text{m}$, thickness $h = 1.5 \mu\text{m}$, relative refractive index depends on the hemoglobin concentration inside the cell. In our calculations we assume $n = 1.05$.

2.2. Light scattering by an ensemble of equal sized particles. Let us consider scattering of a laser beam by a thin layer of RBC suspension in an ektacytometer. We assume that all particles are located in one plane so that their symmetry axes are parallel to the laser beam. The method of calculation of the angular distribution of scattered light intensity is described in our paper [4]. In particular, the angular distribution of the intensity of light scattered by a size-uniform ensemble of particles is described by the formula:

$$I(\theta, \rho) = I_0 \cdot N \cdot |\alpha|^2 \left(\frac{\rho^2}{2kz} \right)^2 \cdot \left[\frac{2J_1(\rho\theta)}{\rho\theta} \right]^2. \quad (1)$$

Here I_0 - laser beam intensity, θ - scattering angle, $\rho = kR$ - size parameter of a particle, R - particle radius, $k = 2\pi/\lambda$ - wave number, λ - light wavelength, N - number of particles illuminated by the laser beam, z - distance from the measurement volume to the observation screen, $J_1(x)$ - first order Bessel function, $|\alpha|^2 = 4 \cdot \sin^2(\Delta\varphi/2)$. $\Delta\varphi = k \cdot n_0 \cdot h \cdot (n - 1)$, where n - relative refractive index of the particle, n_0 - absolute refractive index of the medium surrounding the particle. Assume $\lambda = 0.633\mu\text{m}$ and $n_0 = 1.33$. Then $\Delta\varphi \approx 1\text{rad}$, and $|\alpha|^2 \approx 1$.

2.3. Light scattering by a nonuniform ensemble particles. Let us consider the RBC radius R a random value. We assume for simplicity that R is uniformly distributed in the interval from $\bar{R} - \Delta R$ till $\bar{R} + \Delta R$. The particle radii dispersion is defined as $\sigma^2 = (\Delta R)^2 / 3$. We shall assume also that $\Delta R \ll \bar{R}$, i.e. the nonuniformity of the ensemble in particle sizes is relatively weak.

We account for the particle size dispersion by averaging of the relation (1) over the particle size parameter. Possibility of such procedure is substantiated in paper [4]. Thus we obtain the following relation for the angular distribution of scattered light intensity: $I(\theta) = \langle I(\theta, \rho) \rangle_\rho$. We shall further consider the regions of the diffraction pattern close to the diffraction minimum and diffraction maximum. In these regions, the Bessel function allows for a simple (linear or quadratic) approximation.

2.4. First diffraction minimum. In the region of the first minimum of the diffraction pattern (first dark ring on the observation screen), the Bessel function can be approximated with linear function $J_1(x) = \beta \cdot (x - x_1)$. Here x_1 - the value of the argument of the Bessel function at which it becomes zero, β - the value of the derivative of the Bessel function in point $x = x_1$. It is known that $x_1 = 3.82$ and $\beta = -0.4$. The angle θ_1 at which the first diffraction minimum is seen is defined by the formula $\theta_1 = x_1/\bar{\rho}$. In the case interesting for us $\bar{\rho} = 39.7$ and $\theta_1 = 0.09$ rad. The calculations result in the following relation for

the light intensity in the first minimum of the diffraction pattern $I_{\min} = I(0) \cdot (2\beta\varepsilon)^2/3$, where $I(0) = I_0 \cdot N \cdot |\alpha|^2 (\bar{\rho}^2/2kz)^2$ - light intensity in the zero (central) maximum of the diffraction pattern. The dimensionless value $\varepsilon = \Delta R/\bar{R}$ is a measure of size dispersion of the particles.

2.5. First diffraction maximum. In the region of the first maximum of the diffraction pattern (first light ring on the observation screen), the Bessel function can be approximated with quadratic function $J_1(x) = a + (b/2) \cdot (x - x_2)^2$ where x_2 - the value of the argument at which the Bessel function reaches minimum $a = J_1(x_2)$, b - value of the second derivative of the Bessel function in point $x = x_2$. It is known that $x_2 = 5.32$, $a = -0.346$ and $b = 0.4$. The angle θ_2 , at which the first diffraction maximum is seen, is defined by the formula $\theta_2 = x_2/\bar{\rho}$. In the case interesting for us $\theta_2 = 0.13\text{rad}$.

Calculation according to the above shown formulae yields the following relation for light intensity in the first maximum of the diffraction pattern

$$I_{\max} = I(0) \cdot (2a/x_2)^2 \{1 + (\varepsilon^2/3) \cdot [1 + (bx_2^2/a)]\}. \quad (2)$$

2.6. Estimate of the diffraction pattern visibility. Visibility of the diffraction pattern is defined as $v = (I_{\max} - I_{\min}) / (I_{\max} + I_{\min})$, where I_{\max} and I_{\min} - light intensities in the neighbouring maximum and minimum of the diffraction pattern. In the case interesting for us an approximate estimate of the visibility of the diffraction pattern is given by the formula $v = 1 - \gamma \cdot \delta_R^2$. Here we have introduced the value $\delta_R = \sigma/\bar{R}$ which has the meaning of relative dispersion of the particles in sizes. The parameter γ is defined as $\gamma = 2 \cdot (\beta x_2/a)^2 \approx 76$. For example, by assuming $v = 1/2$ we obtain $\delta_R = 0.08$. Thus a two fold reduction in the visibility of the diffraction pattern in the region of the first minimum and first maximum of the intensity takes place already at the standard deviation of the particle sizes from the average value equal to 8%. For reliable measurements with RBC suspension, a calibration of the ektacytometer with mono- or polydisperse suspensions of spherical particles should be made similarly to [7].

3. Results

We have considered single scattering of a laser beam on a nonuniform ensemble of particles mimicking red blood cells in the anomalous diffraction approximation. We concluded that it is possible to estimate the particle size dispersion by measuring the light intensities in the first minimum and first maximum of the diffraction pattern.

4. Conclusions

Laser ektacytometry allows, in principle, to estimate such characteristics of red blood cells population as particle size dispersion. Accounting for the effect of other parameters like the dispersion of shear elongated particles orientations in space is necessary for the practical implementation of such possibility.

Acknowledgments

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References

- [1] G.J. Streekstra, J.G.G. Dobbe, A.G. Hoekstra, “Quantification of the poorly deformable red blood cells using ektacytometry”, *Optics Express*, **18(13)**, 4173 (2010).
- [2] M. Bessis, placeN. Mohandas, “A diffractometric method for the measurement of cellular deformability”, *Blood Cells*, **1**, 307 (1975).
- [3] G.J. Streekstra, A.G. Hoekstra, E.-J. Nijhof, R.M. Heethaar, “Light scattering by red blood cells in ektacytometry: Fraunhofer versus anomalous diffraction”, *Applied Optics*, **32(13)**, 2266 (1993).
- [4] S.Yu. Nikitin, A.E. Lugovtsov, A.V. Priezzhev, “On the problem of the diffraction pattern visibility in laser diffractometry of red blood cells”, *Quantum Electronics*, **40(12)**, 1074 (2010).
- [5] M.A. Yurkin, V.P. Maltsev, A.G. Hoekstra, “The discrete dipole approximation for simulation of light scattering by particles much larger than the wavelength”, *Journal of Quantitative Spectroscopy and Radiative Transfer*, **106**, 546 (2007).
- [6] A.V. Priezzhev, S.Yu. Nikitin, A.E. Lugovtsov, “Ray-wave approximation for the calculation of laser light scattering by transparent dielectric particles, mimicking red blood cells or their aggregates”, *Journal of Quantitative Spectroscopy and Radiative Transfer*, **110**, 1535 (2009).
- [7] M.J. Berg, S.C. Hill, G. Videen, K.P. Gurton, “Spatial filtering technique to image and measure two-dimensional near-forward scattering from single particles”, *Optics Express*, **18(9)**, 9486 (2010).

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