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New diffractometric equations and data processing algorithm for laser ektacytometry of red blood cells

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ABSTRACT

The problem of measuring the deformability of red blood cells in shear flow by laser diffractometry (ektacytometry) is considered. New diffractometric equations are obtained, which relate the parameters of the diffraction pattern to the characteristics of the erythrocyte ensemble, such as mean deformability as well as width and asymmetry of the erythrocytes distribution in deformability. A feature of these equations is that they include only geometric parameters of the diffraction pattern but do not contain its energy parameters. Basing on these equations, we propose a new data processing algorithm for the laser ektacytometry of red blood cells.

1. Introduction

An actual problem in the contemporary medical diagnostics is the problem of measurement the deformability of red blood cells (RBC) [1,2]. It is known that the deformability of RBCs is reduced in such diseases as sickle cell disease [3–5], hereditary spherocytosis [3], thalassemia [6], cerebral ischemia [7], and diabetes [8,9]. The measurement of erythrocyte deformability is also important in connection with the problem of storage of blood for the transfusion [10].

One of the most efficient methods for measuring the deformability of RBCs is laser diffractometry in a shear flow (ektacytometry) [11]. This method is based on the observation and analysis of the diffraction patterns (DP), which arise when a laser beam is scattered by a suspension of erythrocytes deformed in a shear flow by forces of viscous friction. There are commercial devices for such measurements [12–14]. Namely, LORCA (Laser-assisted Optical Rotational Cell Analyzer, RR Mechatronics, Hoorn, The Netherlands), Rheodyn SSD (Myrenne GmbH, Roetgen, Germany), RheoScan-D (RheoMeditech, Seoul, Korea), LADE (Laser Aggregometer – Deformometer of Erythrocytes, RheoMedLab, Moscow, Russia). However, until now these devices are not widely used in clinical practice. A pressing problem is the standardization of measurements with these devices, as well as raising their reliability and functionality [5,14–17].

Another important issue is that in human blood different erythrocytes have different ability to deform. From this point of view the deformability should be considered as a statistical characteristic of the erythrocyte ensemble that can be described by such notions as the mean value, variance and distribution function. In principle, all these parameters can be measured by means of direct observation of the erythrocytes in a shear flow [3,18]. However this approach is difficult to implement. Several studies

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were focused on the problem of measuring the statistical characteristics of the deformability of RBCs by means of laser ektacytometry. In paper [19], it was suggested to determine the proportion of poorly deformable erythrocytes in a blood sample by measuring the light intensity at a certain point of DP as a function of shear stress. In ref. [20], the authors discuss a possibility of measuring the average value and variance of the deformability by assuming that the erythrocytes have the Gaussian distribution in the deformability. In ref. [21], it was shown that for an ensemble of RBCs bimodal in deformability, it is possible to evaluate the fraction of poorly deformable cells, if the DPs are known at a given shear stress for both components of the ensemble. In ref. [22], a multi-parametric model was proposed, which allows to simulate the laser beam scattering by an ensemble of RBCs bimodal in the deformability for an arbitrary shear stress. Using this model, the authors were able to measure the cell deformability of the two components of the RBC ensemble separately as a function of shear stress, and to assess the proportion of weakly deformable cells in specially prepared blood samples.

Our papers [23,24] are devoted to the theory of the laser ektacytometry of erythrocytes. Theoretical modeling helps to understand better the laser light scattering by RBCs and to develop new data processing algorithms. In refs [23,24] we proposed two such algorithms: the characteristic point algorithm, and the line curvature algorithm. These algorithms are experimentally verified with specially prepared samples of rat blood [25]. These results relate to the peripheral part of the DP, which is located near the first minimum of the scattered light intensity. In this area, the intensity of the scattered light I is approximately an order of magnitude lower than that of the main diffraction peak $I(0)$. In ref. [26], we considered the central part of the diffraction pattern, which is determined by the condition $0.4 \le I/I(0) \le 0.6$. For this area an equation was obtained, which relates the RBCs variance in deformability and geometric parameters of the DP. The advantage of this equation is that it does not contain the energy parameters of the DP. This simplifies essentially the measurement procedure. However, the analysis carried out in [26], is limited by the case of an RBC ensemble with a symmetrical distribution in the deformability. In the present paper, we generalize the theory described in ref. [26] for the case of a weakly inhomogeneous ensemble of RBCs with an arbitrary distribution function in the deformability.

We propose a new technique for measuring the mean deformability, width and asymmetry of erythrocytes distribution in deformability. This technique is based on analyzing the curvatures of the isointensity lines curvature at the polar points. To test the new algorithm we use numerically simulated diffraction patterns as well as the experimental diffraction patterns obtained with the laser ektacytometer and real samples of blood cells.

2. Model of erythrocyte ensemble

As in refs [23,24,26], we model the RBCs in shear flow of the ektacytometer by an inhomogeneous ensemble of flat elliptical discs. The advantage of this model is that for a limited area of DP it allows to obtain approximate analytical relations between the characteristics of the DP and the parameters of RBCs that need to be determined ("diffractometric equations"). In principle flat ellipses cannot model the diffraction by real erythrocytes in all regions of the diffraction pattern. However, as we have shown in [23], within the central diffraction maximum the diffraction pattern is essentially the same in cases of the flat disk and biconcave disk modeling the erythrocyte. Thus, we state that our model can be applied in this limited region of the diffraction pattern. Besides, as we have shown in [26], the new algorithm is generally low sensitive to the shape of the particles but remains still sensitive to the parameters of the particle distribution in shapes.

Let the dimensions of semi-axes of the elliptical discs modeling erythrocytes be

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

where a_0, b_0 are the mean values of a and b, and ε is a random parameter. This model allows one to describe analytically the scattering of a laser beam by erythrocytes, taking into account the fact that different RBCs have different ability to deform (deformability). We assume that

$$|\varepsilon| < < 1 \tag{2}$$

and $\langle \varepsilon \rangle = 0$. Condition (2) corresponds to a weak inhomogeneity of the RBCs ensemble in the deformability. As a rule, this condition is satisfied for real blood samples. This condition was used in our paper [26] in derivation of Eq. (16), which describes the shape of the isointensity line. As one can see from Eqs. (46), (47) the maximum value of the parameter ε in our calculations is 0.3 . Thus, Eq. (2) approximately holds. The population characteristics of RBCs are the average deformability and the width and asymmetry of the RBCs distribution in the deformability. These quantities are defined by the formulas

$$s = a_0/b_0, \mu = \langle \varepsilon^2 \rangle, \nu = \langle \varepsilon^3 \rangle. \tag{3}$$

Parameters μ and ν characterize the width and asymmetry of the erythrocytes distribution in deformability. As one can see from Eqs. (1) and (3), these parameters have the meaning of the second and third moments of the deviation of the maximum (and minimum) sizes of the erythrocytes in a shear flow of the ektacytometer from the mean values. They need to be measured with the laser ektacytometer. The parameters μ and ν satisfy the conditions

$$\mu < < 1, |\nu| < < 1. \tag{4}$$

A more detailed model is described in our papers [23,24].

3. Coordinate system

We introduce the Cartesian coordinates in the plane of the observation screen. Axis x is directed horizontally, axis y – vertically. Physically, the directions of these axes are distinguished so that one of them is parallel to the fluid flow in the ektacytometer, and the other one is perpendicular to the flow. The origin of coordinates is chosen in