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OPTICAL METHODS FOR INVESTIGATION OF ERYTHROCYTE DYNAMICS AND DEFORMATION IN FLOW CONDITIONS

ABSTRACT

Optical methods for investigation of erythrocyte dynamics and deformation are presented. In optical tweezers, tightly focused laser beam is used to trap and manipulate single microsized particles [1]. This allows observing deformation of single particles in various flow systems. In particular, this tool can be used for investigation of biological particles like red blood cells (RBC) behavior in different flow conditions. The second method is ektacytometry based on laser beam diffraction on dilute suspension of particles [2]. This method can determine the particle deformation by means of diffraction pattern change depending on the shear stress applied onto the particles or cells. The deformability of cells can be obtained implementing both methods using corresponding formulas. Here we present the two methods and the data that can be obtained using them. The objects under study are RBCs. These cells constitute the main part of blood mass and are responsible for oxygen supply to the tissues and removal of carbon dioxide. Their deformability is an important property determining the blood rheology.

RED BLOOD CELL, EKTACYTOMETRY, OPTICAL TWEEZERS, CELL DEFORMATION, FLOW, SHEAR STRESS

INRTODUCTION

Optical methods are important for non-invasive and non-contact investigation. For biological cells these properties are more crucial and the least interaction with cells is highly desirable. The optical trapping technique presented here is widely used for various applications since introduction of this method by Ashkin in 1984 [1]. The main feature of optical trapping is that it can used to measure the interaction forces between microparticles at piconewton range. On the other hand optical trapping can be used to hold a microparticle while observing its deformation in flow conditions. Also, the deformation properties of the particles can be studied using the ektacytometry method by analyzing the changes in the shape of the diffraction pattern depending on the extent of deformation of the cells. The main parameter that characterizes the deformation for these methods is the deformability index (*DI*) defined as the ratio of the difference of the cell longitudinal and transverse sizes to their sum. Because RBCs circulate throughout the body its deformability is very important, especially for passing through the terminal capillaries with diameters around 2~3μm,

which is much less than the RBC size. The RBC deformability was shown to reduce during various diseases like diabetes, sepsis, malaria and others. Lower deformability of RBCs induces the blood circulation problem and damaging the blood vessels [4]. These facts make the RBC deformability measurement an important and challenging task.

OPTICAL TRAPPING

Optical trapping method is based on the use of light pressure effect. The microsized particles can be trapped and manipulated by a tightly focused laser beam. The main requirement for trapping is that the beam should have a clearly Gaussian intensity profile and a high numerical aperture objective should be used for its focusing. The trapping can be described by considering the transmission and refraction of the light beam on the particle (Fig. 1). The resulting force holds the particle a bit lower than the focus plane of the objective. For convenience the forces acting on the particle are referred to as the "scattering" and "gradient" force. These forces are acting like "pushing" and "lifting" forces respectively. Generally the resulting trapping force can be calculated from the formula (1). However, for the sizes of the trapped particles that are about the wavelength of the trapping laser the mathematical definition of the trapping force is very complex. Therefore the experimental calibration is required.

$$F_{trap} = \frac{Qn_m P}{c}. \quad (1)$$

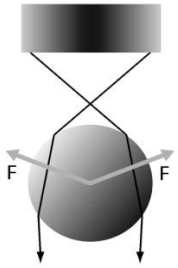

Fig. 1. Illustration of microparticle trapping.

Calibration of optical tweezers can be performed in various ways: by observing the Brownian motion of the trapped particle or by observing the response of the trapped particle using its periodical displacement. In our case, we used for calibration the viscous drag method. This method is the most simple and based on viscous flow made by movement of the suspending fluid with trapped particle. By increasing the flow speed until the viscous drag force

$$F_{vis} = 6\pi\eta v r \quad (2)$$

exceeds the trapping force and comparing the two forces the trapping force can be obtained using the effective radius of an RBC of 2.7μm.

Using a similar approach the shear stress can be applied onto the trapped cell. We have investigated the deformation of RBC at different flow rates ranging from 6 to 12 mm/s. The cuvette holding the diluted suspension of RBCs had the size of 15 mm in diameter and 0.1 mm in height, the RBCs were trapped at a distance of 20 μm from the bottom. Dilution was necessary to trap single cells. The schematic diagram of the experimental setup is shown in Fig.2. The laser wavelength (1064 nm) was chosen to correspond to the transparency window of the blood in order to minimize the heating effect while trapping. According to our calculations the heating effect was negligible[3]. The experimental procedure of an RBC deformation measurement is shown in the video fragment 1. The resulting RBC deformability index *DI* calculated according to formula 3 and was found to be equal to 0.44±0.4, which is similar to the value of *DI* measured by ektacytometry

method at high shear rates. The trapping force was 150 pN in all cases with the laser beam power of 72mW.

$$DI = \frac{a-b}{a+b} \tag{3}$$

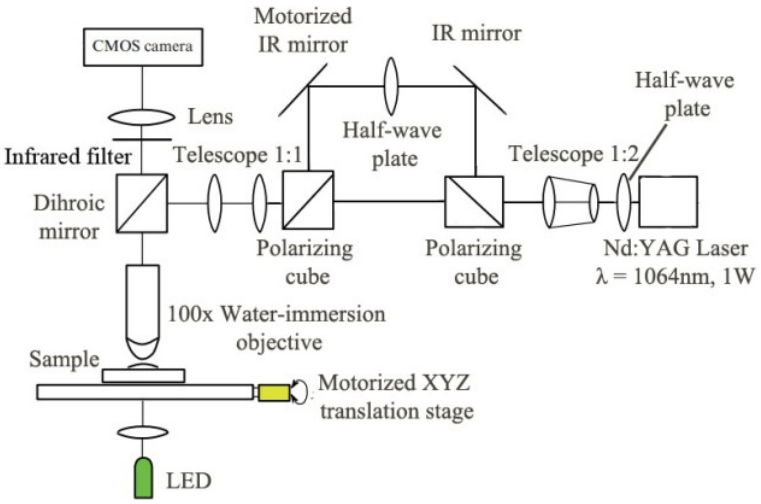

Fig. 2. Schematic diagram of the experimental setup for optical trapping.

ECTACYTOMETRY

In ektacytometry method, two coaxial cylinders are used to make a shear flow. Between the cylinders, there is a small gap about 1mm thick where a diluted suspension of RBCs is filled. The outer cylinder can be rotated relatively to the inner one producing the so called Couette flow with a linear distribution of flow velocities (Fig. 3) and, consequently, constant shear stress. The shear rate γ and shear stress τ can be calculated using formulas 4, 5. The measured parameter in this method is contained in the shape of the observable diffraction pattern. The pattern is formed by a laser beam passing through the diluted suspension of RBCs. RBCs are specially highly diluted (about 300 times) in a viscous suspension to form a single layer of cells in the shear flow. This allows to neglect the multiple scattering effect and consider only the diffraction on an ensemble of single cells. The schematic diagram of the ektacytometer used in our work is presented in Fig. 4.

$$\gamma = \frac{2\pi RN}{d}, \mathbf{R} = \frac{\mathbf{R_1} + \mathbf{R_2}}{\mathbf{2}}, \tag{4}$$

$$\tau = \gamma\eta . \tag{5}$$

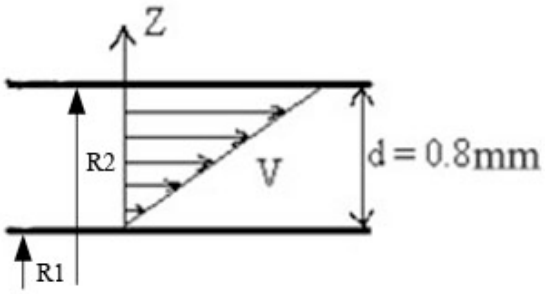

Fig. 3. Illustration of the shear flow in the ektacytometer.

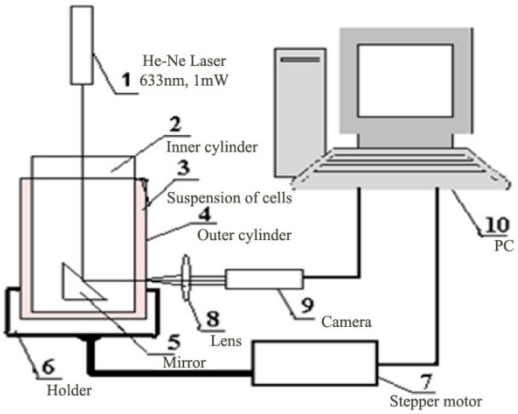

Fig. 4. Schematic diagram of the experimental setup of the ektacytometer.

The values of *DI* at different shear stresses were derived by observing the changes in the shape of the diffraction pattern depending on the cells deformation using the same formula as in optical trapping method (3) considering longitudinal and transverse sizes of the diffraction pattern. The change in the shape of the diffraction pattern is shown in Fig. 5. A typical dependence of *DI* on the shear stress is presented in Fig. 6.

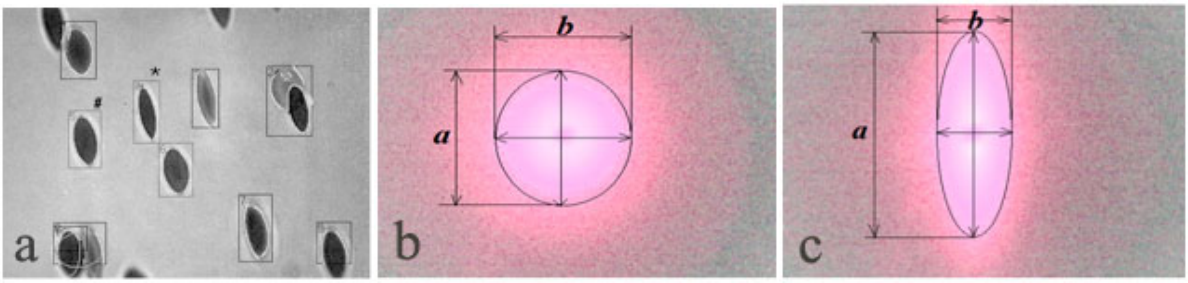

Fig. 5. (a) Illustration of RBCs deformed in a shear flow, (b) diffraction pattern from the non-deformed RBCs, (c) diffraction pattern from the shear deformed RBCs.

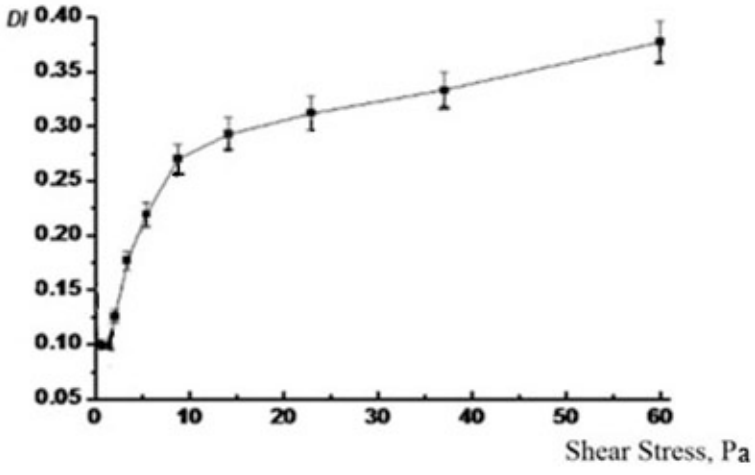

Fig. 6. Typical dependence of the deformability index on shear stress measured with an ektacytometer.

CONCLUSION

Measurement of RBC deformability is crucial, yet this property is highly dependent on the external conditions like temperature, pH, osmolality, etc. In a cell population, the deformability values may be characterized by a statistical distribution because individual RBCs have slightly

different sizes and mechanical properties. This makes the estimation of RBC deformation a difficult task. The presented methods of optical trapping and ektacytometry allow for measuring the deformability of cells with acceptable accuracy.

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LIST OF SYMBOLS

F_{trap} – trapping force, N
F_{vis} – viscous drag force, N
Q – trapping efficiency
n_m – relative refractive index
P – trapping laser power, W
c – speed of light, m/s
η – dynamic viscosity, $N \cdot s/m^2$
v – flow velocity, m/s
r – radius of a particle, m
DI – deformability index
a – longitudinal size, m
b – transverse size, m
τ – shear stress, Pa
γ – shear rate, s^{-1}
R – average radius of the cylinders, m
R_1 – radius of the inner cylinder, m
R_2 – radius of the outer cylinder, m
V – flow velocity vectors
d – gap between the cylinders, m

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