Orientation of Red Blood Cells and Rouleaux Disaggregation in Interference Laser Fields

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Abstract. The effect of interference laser fields on red blood cells (RBCs) was investigated both theoretically and experimentally. The optical trapping and orientation of individual RBC in interference fringes were observed. It was found that RBC rouleaux undergo disaggregation under the action of interference laser fields. To describe the effect of RBC orientation in interference fringes, we used the equation for torque exerted on a discoid dielectric particle in a gradient light field. The experimental results are in agreement with the predictions of the developed theoretical model.

Key words: interferometric trapping, red blood cell orientation, rouleau disaggregation

1. Introduction

It was first shown by Ashkin *et al.* in 1987, that a sharply focused laser beam can be used for noninvasive manipulation of bacteria, viruses and a variety of living cells [1, 2]. The mechanism of optical trapping and manipulation is based on the influence of radiation pressure and dipole gradient forces on a dielectric microparticle in a light field with spatial gradient of intensity [3]. Proper choice of wavelength and intensity of laser radiation allows to minimize optical damage of trapped biological objects. Based on these principles, these ‘optical tweezers’ are widely used both in investigation of individual biological object and in automated single-cell sorting systems for diagnostics [4, 5 and references therein]. To operate with a large array of cells simultaneously in processing it is necessary to develop the multiple-optical tweezer systems.

There are two main approaches towards multiple optical trapping: one based on strongly focused beams using the high numerical aperture objectives [6–10] and another based on interference methods when particles are trapped by interference field [11–17]. The first type systems were created by a number of means: by fast single beam scanning [6], by two counterpropagating Gaussian light beams [7], with methods of spatial light modulation, e.g., computer-generated holography [8] or generalized phase-contrast method [9], and with multiple light sources [10]. The

most powerful technique so far is that of holographic optical tweezers, where a large number of individually structured optical traps are created. These tweezers allow independent movement of trapped particles in three dimensions. However, such systems are quite complicated and require heavy calculations, which is a limiting factor for fast real-time manipulation of particles.

On the other hand, the large regular array of optical traps can be easily constructed interferometrically. The creation of two-dimensional array of microparticles by means of interference field was demonstrated for the first time in [11–13]. The simplest case of two-beam interference forms a pattern of linear interference fringes with easily adjustable width [14]. This approach does not require strong laser beam focusing, which makes it possible to create a wide interference field so that simultaneous operation with a large number of particles can be achieved. Moreover, the absence of focusing for each particle makes this kind of trapping less damaging. It should be noted that two-beam interference is a quite simple method of particle trapping, requiring a minimal number of optical elements. Furthermore, the light and dark fringe alternation allows for simultaneous manipulation of high- and low-index microobjects, as well as rod-like biological samples [15]. It is possible to employ more complicated interference schemes that form more sophisticated patterns by means of multiple beam interference, which allows creation of crystal-like particle structures [11]. It is also possible to form three-dimensional structures using different complicated wavefront configuration for the beams [16]. These advantages and possibilities make the interferometric technique a promising tool for the high speed operation with multicomponent suspensions and tissues. For instance, the selective capture and transportation of particles of different sizes by means of a moving interference field (the ‘optical sweep-net’ [17]) opens up interesting possibilities for biological applications. In particular, applications such as stimulation of blood transportation through microcapillaries, sorting and separation of lymphocytes, RBCs or other particles etc are expected.

Our previous paper [17] deals with the influence of interference laser fields on cells of human blood. We have demonstrated the trapping of lymphocytes and RBCs in interference fringes, and the influence of interference period on the lymphocyte apoptosis. To the best of our knowledge, in the present paper we provide the first theoretical assessment and experimental demonstration of the behavior of individual red blood cells in interference laser fields. We also consider the mechanism of disaggregation of linear RBC rouleaux in a suspension and estimate the shear stress acting on RBC during rouleau disaggregation under the influence of interference fields.

2. Orientation of RBC in the Fringes of Interference Laser Field (Theory)

The orientation of single RBC in two-beam traps has previously been described experimentally as well as theoretically by computer modeling based on individual beam effect [5]. Instead, we discuss RBC behavior under the influence of gradient

dipole forces that result from interaction with the interference laser field. In our approach, a red blood cell is considered as a discoid dielectric particle.

According to [3], the electromagnetic force density $\mathbf{f}(\mathbf{r})$ acting per unit volume of a dielectric placed in the light field with the spatial intensity gradient $\nabla I(\mathbf{r})$, and averaged over one period of oscillation is given by a formula:

$$\mathbf{f}(\mathbf{r}) = \mathbf{f}_{\text{grad}}(\mathbf{r}) + \mathbf{f}_{\text{sca}}(\mathbf{r}) = \frac{\alpha n}{2c} \nabla I(\mathbf{r}) + \alpha n \frac{\Delta \mathbf{m}(\mathbf{r})}{\Delta t}. \quad (1)$$

Here $I(\mathbf{r}) = \varepsilon_0 \cdot c \cdot |E(r)|^2 / 4\pi$ is the light intensity, ε_0 is the vacuum permittivity, c is the velocity of light in vacuum, α is the polarizability of the dielectric, n is the medium refractive index, $\Delta \mathbf{m} / \Delta t$ is the change of momentum density per unit time due to light scattering in the unit volume of the dielectric. The first term $\mathbf{f}_{\text{grad}}$ corresponds to the gradient force, resulting from the intensity gradient, and the second term $\mathbf{f}_{\text{sca}}$ is responsible for the light pressure force. In our case of the two-beam interference the light pressure acts on particles along a bisector to the angle of the two intersecting beams, which produce interference fringes. There is no component of the light pressure force acting across interference fringes [18]. Therefore, considering a particle moving across the interference fringes, one may disregard light pressure. Under the gradient force applied to the RBC center of gravity, it will move along the line of the intensity gradient towards the interference maximum.

Let us consider the rotational dynamics of RBC at the interference maximum. A discoid red blood cell is represented as a disc of a diameter D and a thickness h . Let us also introduce the system of axes attached to a cell in such a way that the plane YOZ lies in the disc plane and is perpendicular to the plane of Figure 1. The interference

Figure 1. Orientation of RBC under the influence of gradient forces in the interference light field.

fringes are parallel to the axis OY in Figure 1. Gradient forces act in the direction OZ', which is perpendicular to the interference fringes. Then the orientation of the discoid particle with respect to the direction of the intensity gradient will be determined by the angle ϕ between the particle axis OZ and the gradient direction OZ'. So when $\phi = 90^\circ$ the cell is oriented along the interference fringe.

The torque about the center of a particle with volume V is given by

$$\mathbf{T} = \int_V \mathbf{r} \times \mathbf{f}_{\text{grad}}(\mathbf{r}) dV, \quad (2)$$

where $\mathbf{r}$ is the radius-vector relative to the center of the cell, and $\mathbf{f}_{\text{grad}}(\mathbf{r})$ is a gradient force acting per unit volume in Cartesian coordinates:

$$\mathbf{r} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} \quad \mathbf{f}_{\text{grad}}(\mathbf{r}) = - \begin{pmatrix} \alpha \frac{8\pi^2}{c} I_0 \frac{1}{z_0} \cdot \sin\left(2\pi \frac{z'}{z_0}\right) \cdot \sin(\phi) \\ 0 \\ \alpha \frac{8\pi^2}{c} I_0 \frac{1}{z_0} \cdot \sin\left(2\pi \frac{z'}{z_0}\right) \cdot \cos(\phi) \end{pmatrix} \quad (3)$$

Here $z' = x \cdot \sin(\phi) + z \cdot \cos(\phi)$ is the projection of the radius-vector $\mathbf{r}$ of a volume element dV on the OZ'. Substituting Eq. (3) in (2) and taking into account the disc geometry, one can deduce the expression for the torque exerted on RBC in the interference fringe:

$$\mathbf{T} = -\alpha \frac{8\pi^2}{c} I_0 \frac{1}{z_0} \begin{pmatrix} 0 \\ \int_{-D/2}^{D/2} \int_{-\sqrt{D^2/4-z^2}}^{\sqrt{D^2/4-z^2}} \int_{-h/2}^{h/2} \left(x \cos(\phi) \sin\left(2\pi \frac{z'}{z_0}\right) - z \sin(\phi) \sin\left(2\pi \frac{z'}{z_0}\right) \right) dx dy dz \\ 0 \end{pmatrix}. \quad (4)$$

The calculated dependencies of the normalized torque on RBC orientation are presented in Figure 2(a) for different ratios $d = D/z_0$ of a cell diameter D and interference period z_0 . The value of the torque is presented in the normalized form T/T_0 , where $T_0 = 8(\alpha\pi I_0 D^4)/(cz_0)$. The points $\mathbf{T} = 0$ correspond to equilibrium RBC orientations. From these equilibrium orientations only those that correspond to the potential energy minima of RBC in the applied interference field are stable.

The energy of the dielectric particle in electric field is determined by the following integral over the particle volume:

$$U = - \int_V \alpha \mathbf{E}^2 dV. \quad (5)$$

Figure 2. Dependencies of the torque exerted on RBC (a), and the interaction energy of RBC with interference field (b) on the cell orientation ϕ for different ratios $d = D/z_0$ of the cell diameter D to the interference period z_0 .

The time-averaged distribution of electric field in the zone of interference along the direction of intensity modulation can be represented by a formula [18]:

$$\bar{\mathbf{E}}^2 = \mathbf{E}_1^2 \cdot \left(1 + \cos \left(\frac{2\pi z'}{z_0} \right) \right) \quad (6)$$

where z' is the coordinate along OZ' , z_0 is the period of interference fringes. From (5) and (6) we obtain:

$$U = -\alpha \frac{4\pi}{c} I_0 \int_V \left(1 + \cos \left(2\pi \frac{z'}{z_0} \right) \right) dV. \quad (7)$$

where z' is the OZ' projection of radius-vector $\mathbf{r}$, which indicates a point in the volume of the disc. Taking into account the disc geometry one can present the interaction energy as a function of the angle ϕ in the following form:

$$U = -\alpha \frac{4\pi}{c} I_0 \int_{-D/2}^{D/2} \int_{-\sqrt{D^2/4-z^2}}^{\sqrt{D^2/4-z^2}} \int_{-h/2}^{h/2} \left(1 + \cos \left(2\pi \frac{z'}{z_0} \right) \right) dx dy dz. \quad (8)$$

The potential energy U normalized to $U_0 = 4(\alpha\pi I_0 D^3)/(cz_0)$ was calculated as a function of the angle ϕ and the ratio $d = D/z_0$ (see Figure 2(b)).

Taking into account that RBC has a rotational symmetry and its center is positioned at the fringe maximum it is easy to see that the sum of all gradient forces acting on the cell is equal to zero. At the same time, as it is seen from Figure 2(a) for interference period z_0 larger than or equal to RBC diameter D ($d \leq 1$), the total torque is equal to zero only at three particular positions of the cell: $\phi = 0^\circ, 90^\circ, 180^\circ$. All three states are in equilibrium but the stable equilibrium corresponds only to orientation $\phi = 90^\circ$, which is characterized by the minimum of the potential energy (see Figure 2(b)).

If the interference period is less than the RBC diameter ($d > 1$) there might be more than one stable equilibrium state, as it follows from local minima on the corresponding potential energy curves (Figure 2(b)).

The effect of RBC rotation should be most pronounced in the case of $z_0 = D$, since the value of T_z/T_0 reaches maximum at $d = 1$ as shown in Figure 2(a).

3. Materials and Methods

3.1. PREPARATION OF RBC SUSPENSIONS

Reagents of analytical grade were from Merck (Germany). Water was triple distilled. Blood from adult donors was taken into 3% sodium citrate. Red blood cells were isolated by standard techniques: blood was centrifuged at 1500g for 10 minutes and supernatant plasma was collected for further use. Pelleted cells were resuspended in cold (4 °C) phosphate buffered saline (PBS: 0.15 M NaCl, 1.9 mM NaH_2PO_4 , 8.1 mM Na_2HPO_4 , pH 7.4) and centrifuged as above. A buffy coat was removed and the process was repeated three times to remove white blood cells and platelets. Finally, one part of washed RBC was resuspended in obtained plasma in ratio 1:200 and was vortex agitated to separate red cells in suspension. The other washed red cells were resuspended in PBS, refrigerated at 10 °C for a few hours to

Figure 3. Experimental set-up: 1: argon ion laser; 2, 3: Fresnel biprisms; 4: spherical lens; 5: folding mirror; 6: sample cell; 7: microscope objective; 8: CCD-camera.

aggregate. For experiments an aliquot of sedimentated RBC aggregates was diluted in 200 times with plasma without active stirring. RBC suspensions were placed in hemacytometer (Goriaev's chamber from Minimed, Russia) for observation.

3.2. EXPERIMENTAL SETUP

The two-beam experimental setup is shown in Figure 3. Argon ion laser (model 2017-04S Spectra-Physics) operating in TEM_{00} mode at 514.5 nm, with output power from 300 to 800 mW, was used as a light source. Its beam was passed through a system of two Fresnel biprisms to form two parallel beams of equal intensity. Interference pattern in the sample cell was formed by the $f = 30$ cm converging lens focusing two beams into the $100\ \mu\text{m}$ spots overlapping in space. The pattern period can be adjusted by varying the distance between the biprisms and, correspondingly, between the beams. The interference pattern was located on the RBC suspension in hemacytometer placed in the focal plane of the lens. After that the two beams were directed to a microscope objective and projection system for visual monitoring and photographing the sample by a CCD camera.

We have chosen Ar-laser as a light source because it provided both a clear interference pattern in the visible spectral range and the necessary intensity to obtain strong enough gradient forces. The RBC's hemoglobins are known to absorb at 514.5 nm [4, 5]. The intensity of the incident laser beam and the exposure time of cell suspensions were adjusted in such a way that the action of laser radiation by itself did not cause decomposition of RBC rouleaux and did not produce any pronounced photostress on RBCs.

4. Results and Discussions

The predicted phenomenon of optical orientation of RBC under the influence of gradient dipole force was detected experimentally. The RBC suspension was placed

Figure 4. Trapping and orientation of RBC by interference laser field. (a) The first moment after laser beams switching on. The cells are oriented by their planes across interference fringes. (b) The same RBC 40 seconds later. The cell in the right corner of the picture has been pulled into the interference maximum. Both levitated RBC have turned by 90° and aligned their flat sides along the interference fringes.

in the interference field of the argon laser radiation using the experimental setup described above. The period between fringes was chosen close to the RBC diameter. The power density of laser radiation at the maximum of the interference pattern was 2000 W/cm^2 . Under such conditions we have observed trapping and orientation of RBCs by gradient interference field. Figure 4(a) shows two red blood cells at the first moment after laser beams have been switched on. Both cells happened to be oriented with their planes perpendicular to planes of interference fringes and along the gradient direction ($\phi = 0^\circ$). Under the action of gradient forces the cell located on the right is pulled into the interference maximum. Both RBCs rotate by 90° and take the most stable position along the fringes, corresponding to $\phi = 90^\circ$, within forty seconds (Figure 4(b)).

We also investigated the effect of interference laser field on linear RBC aggregates, the rouleaux, which were formed under the static conditions *in vitro*. While trapped between two dark fringes of the interference field the regular rouleau remains unperturbed in time (Figure 5(a)). The irregular rouleau under the same conditions was aligned and slightly stretched along the fringe (Figure 5(b) and (c)).

Figure 6(a) shows a RBC rouleau oriented at 45° to the previous and exposed to a single laser beam. Under the action of one beam the rouleau remains stable, which shows that thermal effects produced by laser radiation do not cause the rouleau disaggregation. However, when the second laser beam creating the interference pattern was switched on, the rouleau disintegrated immediately (Figure 6(b)). The cells of the rouleau remained separated after the interference light field had been switched off.

It is known [19] that sliding cells side by side easily disrupts the contact between aggregated RBCs. In the case of trapping the regular linear rouleau into the interference maximum (as shown on Figure 5(a)), gradient forces act perpendicular

(a)

(b)

(c)

Figure 5. Position of the rouleau between two dark fringes of the interference field. (a) The regular rouleau remains unperturbed in time. (b) The irregular rouleau at the first moment after laser beams switching on. (c) Alignment and stretching of the irregular rouleau along the interference maximum fringe.

(a)

(b)

(c)

Figure 6. Splitting the rouleau into separate RBC under action of interference laser field. (a) The rouleau under action of a single laser beam. (b) The rouleau splitted by interference fringes oriented at an angle 45° to the rouleau axis. (c) The cells remain parted after the interference light field has been turned off.

Figure 7. The gradient forces acting on RBC in a rouleau placed in the interference light field.

to and towards the rouleau axis. Their resulting action on each cell of the rouleau is equal to zero, and decomposition does not happen.

In the case of irregular rouleau oriented along the interference fringe the gradient forces acting on those cells that are shifted away from the rouleau axis do not compensate each other. Their resulting action is directed towards the center of interference fringe maximum and cause sliding of the cells. As a result the rouleau becomes aligned along the fringe (Figure 5(b) and (c)).

The situation changes if a rouleau is oriented at some angle relative to fringes. In this case the gradient force $\mathbf{F}_{\text{grad}}$ has a component parallel to the plane of cells side-by-side sliding (components $\mathbf{F}_{\parallel}$ in Figure 7). At the right period of light field modulation the components of gradient forces $\mathbf{F}_{\parallel}$ acting on adjacent cells in the rouleau may pull them to the opposite directions as shown in Figure 7. It is these opposite forces that decompose the aggregate. The other component $\mathbf{F}_{\perp}$ of the gradient force is directed along the aggregate axis and acts as a stretching or compressive force dependent on the cell position in the rouleau and in the interference fringes. As is known [19], a much stronger force is needed to decompose the aggregated RBC in a stretching direction so the contribution of the force component $\mathbf{F}_{\perp}$ to disaggregation of the rouleau is hardly of any significance.

We have estimated the value of the gradient force acting on RBC in the rouleau oriented at 45° relative to fringes. The total force acting on RBC in the gradient field may be estimated by summation of all forces acting on the cell volume. Taking into account the geometry, orientation ϕ , and position Z' of RBC in the gradient field we derived a following expression for the total force:

$$\mathbf{F}_{\text{grad}}(\phi, Z') = \int_{-D/2}^{D/2} \int_{-\sqrt{D^2/4-z'^2}}^{\sqrt{D^2/4-z'^2}} \int_{-h/2}^{h/2} \mathbf{f}_{\text{grad}}(\mathbf{r}) \, dx \, dy \, dz. \quad (9)$$

Here $\mathbf{f}_{\text{grad}}$ is determined from (3) taking into account that $z' = x \sin(\phi) + z \cos(\phi) + Z'$, where Z' is the RBC displacement from the center of interference maximum. For numerical evaluation the following parameters of RBC were used: a diameter of $7 \mu\text{m}$, a thickness of $2.3 \mu\text{m}$, the polarizability $\alpha = 3(n^2 - 1)/(n^2 + 2)$ where $n = 1.0418$ is the refractive index of RBC relatively blood plasma at $\lambda = 500 \text{ nm}$ [20], the intensity of laser radiation $I_0 = 5000 \text{ W/cm}^2$, which corresponds to laser beam output power of 400 mW . By our calculations for the 45° orientation of RBC the maximal force of about 8 pN acts on the RBC when its shift from the center of interference maximum equals $Z' = 2 \mu\text{m}$. As a result the shear stress produced by such gradient forces can be as high as 0.9 N/m^2 . Such value of the shear stress is enough for rouleau disaggregation [21].

5. Conclusion

We have investigated the influence of the two-beam laser interference field on the behavior of individual red blood cells and their linear aggregates in suspension. The theoretical assessment of the RBC behavior in interference fringes based on the equation for torque exerted on a discoid dielectric particle in the gradient light field has been given. Experimentally we have observed optical trapping and orientation of RBC along the planes of interference fringes, which are in agreement with theoretical predictions. Our experiments have also demonstrated that the gradient forces created by interference laser fields can be sufficient for RBC rouleaux decomposition. The proposed method will hopefully be useful in implementation the optical based manipulation systems for single-cell diagnostics and therapeutics [22, 23].

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