Analysis of the behaviour of erythrocytes in an optical trapping system

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Abstract: We present a theoretical analysis of the behaviour of erythrocytes in an optical trapping system. We modeled erythrocyte behaviour in an optical trap by an algorithm which divided the cell surface into a large number of elements and recursively summed the force and torque on each element. We present a relationship between the torque and angle of orientation of the cell, showing that stable equilibrium orientations are at angles of 0^o, 180^o and 360^o and unstable equilibrium orientations are at 90^o and 270^o relative to the axis of beam propagation. This is consistent with our experimental observations and with results described in the literature. We also model behaviour of the erythrocyte during micromanipulation by calculating the net force on it. Such theoretical analysis is practical as it allows for the optimization of the optical parameters of a trapping system prior to performing a specific optical micromanipulation application, such as cell sorting or construction of a cell pattern for lab-on-a-chip applications.

OCIS Codes: (170.0170) Medical optics; (170.3660) Light propagation in tissues.

References and Links

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The need for the manipulation of single cells has been demonstrated in applications ranging from the development of cell biosensors [1,2] to single cell molecular biology applications [3,4] to laser-assisted in vitro fertilization [5]. The use of optical trapping has proven to be ideal for this purpose, due to the ease of manipulation of single cells to specific locations, and the ability to perform this manipulation in a closed, sterile environment without danger of contamination. Many commercial devices based on optical trapping have been developed to allow for the incorporation of these micromanipulation techniques to medical and molecular biology laboratories [3].

Optical trapping takes advantage of the radiation pressure exerted by one or more focused laser beams onto a micron-sized biological particle [6]. A focused laser beam exerts two categories of forces on objects in its path. The scattering force results from the transfer of momentum from photons striking the surface of the particle [6]. Two counterpropagating laser beams can thus trap a particle by applying equal scattering forces to two sides of the particle. The gradient force is derived from fluctuating electrical dipoles induced when light passes through a transparent or near transparent object, and acts proportional to and in the same direction as the spatial gradient in light intensity created by focusing the laser beam. The gradient force tends to draw objects toward regions of greater light intensity, allowing for a single focused laser beam to trap a particle in its focus, where the light intensity is greatest [6]. The single beam gradient force optical trap, or optical tweezers, was first achieved in 1986 by Ashkin and colleagues [7].

While many experimental studies have demonstrated the feasibility of optical tweezers in the trapping and manipulation of cells, none of these have applied optical theory to determine the behaviour of cells in optical traps. Such optical modelling is of practical importance as it allows the experimenter to investigate the optical parameters of the trapping system for optimization of trapping and manipulation of the cell prior to performing a specific application. Different types of biological cells manipulated in various types of media will be subject to a multifactorial array of forces. These forces, however, can be easily approximated and calculated using computer modelling based on optical theory.

Theoretical models exist which have been used to calculate the behaviour of simple physical shapes, including spheres and cylinders in optical traps [8,9]. These models have subsequently been verified by experimental results. The difficulty in modelling biological cells in a similar fashion is due to the heterogeneity of cells in various optical parameters including shape, optical density, and absorptive properties.

In this paper, we present for the first time, to the best of our knowledge, a theoretical determination of the behaviour of a biological particle in an optical trapping system. We have achieved this by developing an algorithm for modelling the behaviour of cells of arbitrary shape and characteristics in an optical trapping system. We present an analysis of the forces exerted by a dual-beam optical trap on an erythrocyte (red blood cell) and determine the maximal velocity for manipulation of the cell. Previous experimental studies have shown that erythrocytes orient themselves with their maximum diameter in the direction of the laser beam in a single beam optical trap [10].

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Modelling of the behaviour of objects with a high degree of symmetry, such as spheres, can be achieved with exact analytical calculations that allow one to trace rays through the structure [9]. However, for irregularly shaped objects such calculations cannot be performed with ease. One cannot use simple analytical calculations to trace rays through the structure of an erythrocyte, despite its radial symmetry. In order to model the behaviour of an erythrocyte, we developed a computerized algorithm for ray tracing and calculation of scattering and gradient force elements. We developed our program to represent the erythrocyte's shape and to divide its surface into 1024 triangular elements. Up to 6000 rays were propagated through the cell and resulting scattering and gradient force components were calculated for each triangular element. The resultant net force and torque were then summed and the cell was rotated and translated prior to the next iteration. The iterations were performed recursively until the cell reached an equilibrium position, specifically the angle at which torque was at a minimum.

The shape of the erythrocyte was approximated to be a biconcave disk [11], with a maximum diameter of 7.2 μm, a maximum thickness of 2.1 μm, and a minimum thickness of 0.9 μm [12,13], optical density of 1.407 [14] and specific gravity of 1.057 g/ml [14]. Simulations were performed for the dual beam trapping configuration with a laser power of 15 mW for the top beam and 30 mW for the bottom beam. The beam diameters were taken to be 5 μm. Experimental confirmation of the results of the theoretical modelling was performed using a He-Ne laser dual beam setup [15] with similar parameters.

The forces exerted on the cell can be calculated by summing all the force elements, dF, produced by the interaction of photons with the cell surface area element, dA. Each force element is comprised of scattering (SC) and gradient (GR) components such that $dF=dF_{SC} + dF_{GR}$ [9,15]. Each reflection or refraction from the erythrocyte surface can be interpreted as scattering from a spherical surface provided that the angle of refraction or reflection is coordinate dependent. We have derived the forces acting on an erythrocyte by adopting an approach similar to that used in calculating forces acting on spheres [9,15]. These forces can be expressed as follows:

$$dF_{SC} = \frac{n_M I}{c} dA\left\{1 + R\cos(2\vartheta(r_i)) + (1+m\sum_{n=0}^{\infty} T^{2n})T^2 \sum_{n=0}^{\infty} R^n(\cos(\alpha(r_i) + n\beta(r_i))\right\} \quad (1)$$

$$dF_{GR} = \frac{n_M I}{c} dA\left\{R\sin(2\vartheta(r_i)) + (1+m\sum_{n=0}^{\infty} T^{2n})T^2 \sum_{n=0}^{\infty} R^n(\sin(\alpha(r_i) + n\beta(r_i))\right\} \quad (2)$$

where n_M is the refractive index of the medium, I is the total intensity per unit area, c is velocity of light, θ is the angle of incidence, r_i is the spatial coordinate (x_i, y_i, z_i), and R and T are reflection and transmission coefficients, respectively. R and T depend on angle of incidence and refractive index of the erythrocyte and surrounding medium and were calculated for each surface area element. Angles α and β are defined in Fig. 1. The term containing the factor m accounts for the possibility of photons re-entering the cell due to its biconcave shape. Internal reflections account for approximately 3 % of the force induced on a particle and the literature suggests that these can be neglected in calculations [8].

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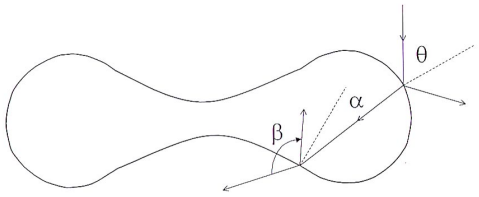

Fig. 1. Schematic of erythrocyte showing angle of incidence (θ) and angles α and β, as defined for modeling studies. This schematic shows minimal cross section of erythrocyte.

The torque about the centre of the cell induces it to rotate and align in the optical trap [8]. The torque about the centre of the cell was calculated by summing over all of the elements:

$$T = \sum_{\substack{surface \\ elements}} r_i \times dF \tag{3}$$

Fig. 2 shows the y-component of the torque, T_Y, as a function of the angle of orientation of the cell (ϕ) in Fig. 2. An equilibrium is considered to be stable if the system always returns to its original state after a small disturbance and unstable if it moves away from its equilibrium after the disturbance. Unstable equilibrium orientations for the erythrocyte are found to be at ϕ=90° and ϕ =270°, and stable equilibrium orientations are at ϕ =0°, ϕ =180°, and ϕ =360°. Subjected to the total radiation force, the erythrocyte is centred in the beam.

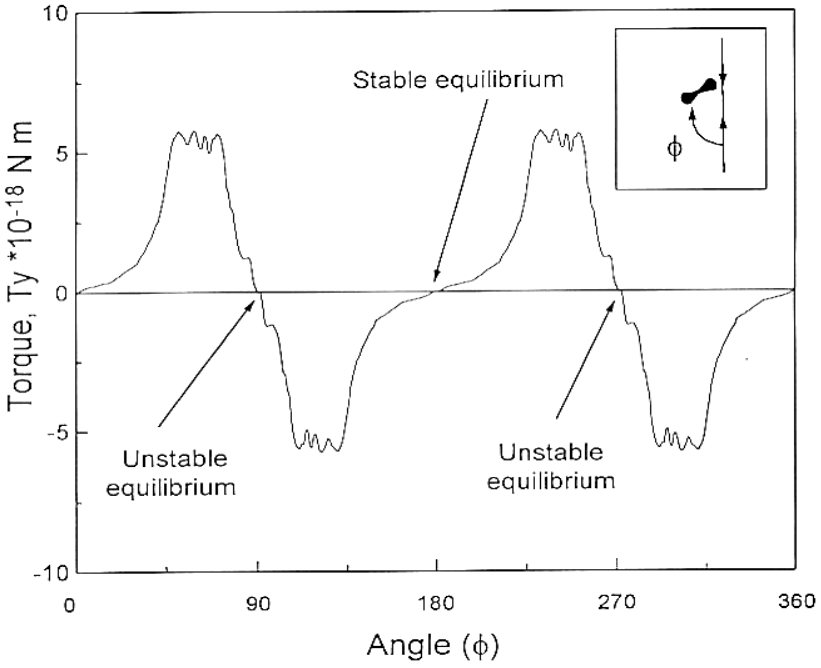

Fig. 2 . Torque exerted on an erythrocyte versus the angle of the cell in a dual beam trapping system. Unstable and stable equilibrium positions as shown. The inset defines the angle, ϕ with respect to the bottom beam.

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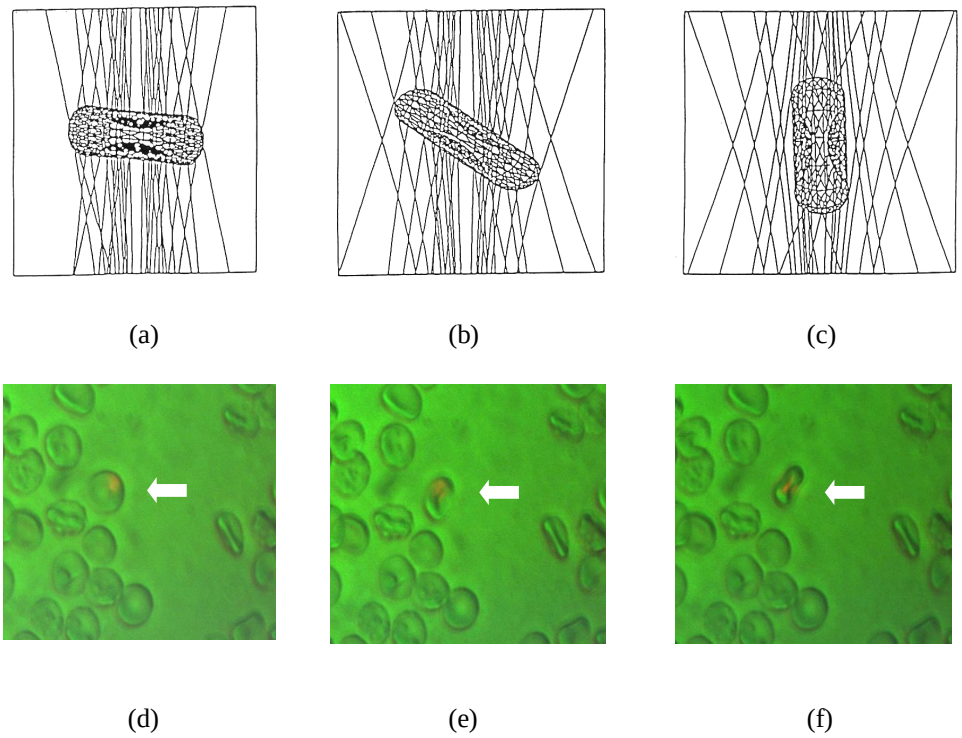

Fig. 3. Results of theoretical modelling (top row) and experimental results (bottom row) showing an erythrocyte before trapping (a,d), during reorientation in a dual beam optical trap (b,e), and after the stable trapping is achieved (c,f). Figures in the top row demonstrate triangular elements used in the algorithm for theoretical determination of behaviour.

Fig. 3 shows pictorial results of theoretical modelling (top row) of the erythrocyte orientation in a dual beam optical trapping system by propagation of rays through the region containing the cell. Fig. 3(a) shows an erythrocyte originally oriented at $\phi = 90°$ relative to the line along the propagation direction of the two laser beams. The erythrocyte turns, as seen in Fig. 3(b), to until it is oriented at $\phi = 180°$ where it is stably trapped as shown in Fig. 3(c). This is in accordance with the torque calculation presented in Fig. 2.

Fig. 4 is a movie clip that demonstrates experimental results showing the rotation of an erythrocyte in an optical trapping system. It is clear from this that the erythrocyte rotates from an orientation of $\phi = 90^{o}$ to a stable equilibrium orientation at $\phi = 180^{o}$. These findings are concordant with the theoretical calculation of torque and equilibrium orientations of the erythrocyte shown in Fig. 2. Still images of the erythrocyte rotation are shown with their corresponding theoretical pictorial images in Fig. 3 (bottom row).

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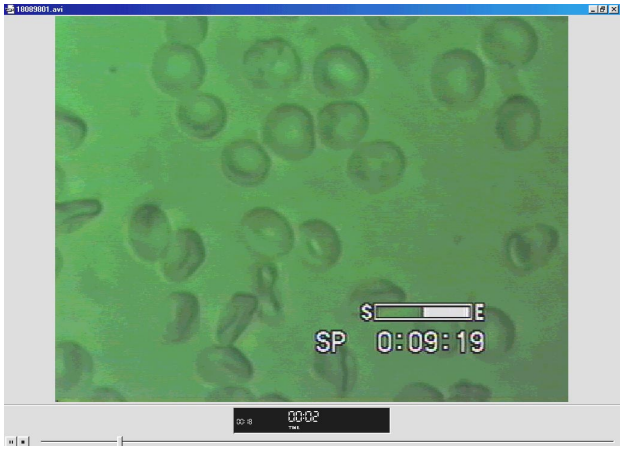

Fig. 4. Movie clip showing rotation of erythrocyte in our experimental optical trapping system (2.7 MB version).

Optical micromanipulation is achieved by displacing the laser beams relative to the medium when a cell is trapped and oriented; as a result, the trapped cell will follow the beam. We have additionally calculated the forces acting on an erythrocyte under these dynamic conditions. Stable manipulation of the trapped erythrocyte at a constant velocity can be achieved if the drag force is in equilibrium with the radiation pressure force. The equation of motion of the trapped cell can be expressed as follows:

$$m\,\mathbf{a} = \mathbf{F} - \gamma\,\mathbf{v} \tag{3}$$

where **v** is the maximum velocity of the cell which is displaced by a given force **F** and γ is the so-called drag coefficient, or damping factor.

At low Reynolds numbers, the damping factor or drag coefficient can be estimated by approximating the disk shape of erythrocyte by a sphere of the same cross-sectional area [16, 17]:

$$\gamma = 3\,\pi\,\eta\,D \tag{4}$$

Here η is the viscosity of the solution (water based 1.02 x 10^{-3} N s/m^2) and D is the diameter of the corresponding cross sectional area of the cell.

Stable manipulation of the trapped cell at a constant velocity can be achieved if the drag force is in the equilibrium with the radiation pressure force, the velocity is equal to the force divided by the damping factor $v = F/\gamma$. We examined two possible translations of the blood cell – one with the maximum cross section, and the other with the minimum cross section in the direction of the manipulation. The force was computed versus the offset of the cell centre and beam axis, and is shown in Fig. 5. From this graph the maximum velocity at which the cell can be translated was estimated to be 29 μm/s. This data is comparable with a previous calculation on polystyrene spheres [18] and serves as an approximation for establishing the displacement properties of erythrocytes during micromanipulation. An experimental demonstration of the manipulation of an erythrocyte with its minimum cross section in the direction of translation is shown in the movie clip (Fig. 6).

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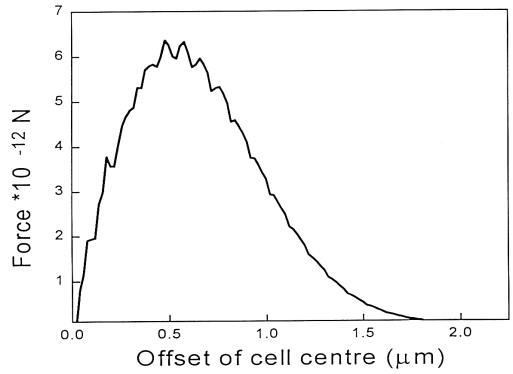

Fig. 5. The force of the optical trapping system versus the offset of the cell center in the Z-direction, for an erythrocyte in a dual beam trapping system. A maximum in the displacement defines the equilibrium location of the cell.

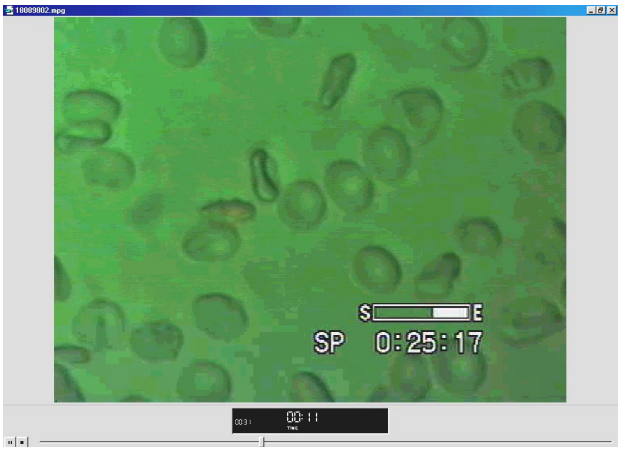

Fig. 6. Movie clip showing micromanipulation of a single erythrocyte with its smallest cross section in the direction of translation (4.7 MB version).

In conclusion, we have demonstrated an algorithm that enables one to model the behaviour of cells of arbitrary shape in an optical trapping system. We have applied this algorithm to determine the behaviour of the erythrocyte in an optical trap, and have shown that our theoretical calculations are concordant both with experimental results and with results found previously in the literature. As optical manipulation becomes increasing important for cell sorting [2], for single cell molecular biology [3,4], and for the construction of cell patterns for lab-on-a-chip type [1,2] applications, the need is growing for optimization of optical trapping systems for specific applications. Investigation into the behaviour of erythrocytes specifically in optical trapping systems can potentially be important in the single cell testing of erythrocytes linked to pharmacophores for use in drug therapy [19]. As such, theoretical analysis of the behaviour of biological particles in optical trapping systems is both practical and important in tailoring the optical parameters of a trapping system for specific cells and other biological particles.

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