Calculation of spherical red blood cell deformation in a dual-beam optical stretcher

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Abstract: We present a numerical method based on the linear elastic membrane theory to compute the morphological deformation of a spherical cell from the photonics stress distribution over the cellular membrane. The method is applied to fit the experimental data for deformation of a spherical human red blood cell trapped and stretched in a fiber-optical dual-beam trap with a single fitting parameter $Eh$ where $E$ is the Young’s modulus of elasticity and $h$ is the thickness of the cell membrane. We obtained $Eh = (20 ± 2) \text{μN m}^{-1}$ which is comparable to results reported earlier. This numerical method can be applied in general experimental conditions.

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1. Introduction

Dual-beam optical stretchers consisting of two counter-propagating and slightly diverging laser beams from two well-aligned single-mode optical fibers have been used for noninvasive and non-contact trapping and stretching of biological cells to measure their viscoelastic properties [1-3]. In these applications, optical forces on the cell are smoother and less localized, compared with those forces obtained by the atomic force microscope (AFM) [4], the micropipette [5] or the dual optical tweezers [6]. In contrary to the cases where the cell under investigation is disturbed by the contact of mechanical probes or beads; in the dual-beam optical stretcher the elasticity of the cell as a whole is measured with minimum extraneous loading.

To measure the elasticity of a cell with the dual-beam optical stretcher, the local stress distribution on the cell surface must be first calculated for a given experimental condition [1, 2]. Then, the morphological deformation of the cell is calculated from the stress distribution.
The parameters specifying the cellular elasticity are finally determined by comparing the experimentally measured cell’s deformation in optical stretcher with the theoretical cell deformation result.

The stress distribution in a dual-beam optical stretcher depends on the ratio of the laser beam-spot radius \( W \) to the cell’s radius \( R \). Guck et al. have shown that when \( W/R \approx 1.1 \) the stress profile on a spherical cell can be approximated by \( \sigma_r = \sigma_0 \cos^2(\phi) \) where \( \sigma_0 \) is the peak stress along the beam axis and \( \phi \) is the polar angle with respect to beam axis [1]. A more general calculation taking into consideration the laser beam divergence and its Gaussian intensity profile has been reported recently [2], by which the optical stress distribution over the cellular surface can be calculated for different distances between the cell and the fiber end-faces and different numerical apertures of the fibers.

Deformation of the cell’s membrane by a given stress distribution is usually computed with the principle of minimum energy and the Euler-Lagrange equations [1], where the energy functional describing the membrane energy and the work done by the stress is solved for a given analytic expression of the stress distribution, such as \( \sigma_r = \sigma_0 \cos^2(\phi) \) [1]. However, in general experimental conditions when the condition \( W/R \approx 1.1 \) is not fulfilled, or when more precise calculation as presented in Ref. 2 is desirable, the stress distribution may no longer be expressed as \( \sigma_r = \sigma_0 \cos^2(\phi) \) so that the equations for the cell deformation used in Ref. 1 are no longer appropriate. One approach to circumvent this problem might be to fit the calculated numerical stress distribution with a polynomial and from each term in the polynomial find differential equations for the cell deformation. However, the differential equations derived from the Euler-Lagrange equations can be too complex, and the fitting of the stress profile to the polynomial would unavoidably introduce additional errors.

In this paper, we present a numerical method to compute the cell’s deformation from the stress profile. The method is more general in the sense that it is applicable to various value of \( W/R \) in the dual-beam optical stretcher and also to any other stress distribution, including the cases where the stress distribution can not be represented by analytical expressions. We apply this model to fit the experimental data for measurement of the deformation of a spherical human red blood cell (RBC) trapped and stretched in a fiber-optical dual-beam stretcher with a single fitting parameter \( Eh \) where \( E \) is the Young’s modulus of elasticity and \( h \) is the thickness of the cell membrane. Although a red blood cell, in its natural physiological environment, resembles a compliant biconcave disk, it can be swollen to the shape of a microsphere via osmotic pressure [1]. The experimental data taken with the spherical RBCs allowed us to check the validity of our linear elastic membrane model in simple geometry and to modify the mechanical model for future applications with the compliant biconcave geometry.

2. Theory

We consider a deformable spherical cell trapped in a fiber-optical dual-beam stretcher, in which two counter-propagating and slightly diverging Gaussian laser beams are reflected and refracted at the cell’s surface. The photon’s momentum change at the cell’s surface results in a photonic radiation pressure. As the index of refraction inside the cell is usually greater than that of the surrounding medium, the changes in the photon momentum due to Fresnel reflection and refraction of the beams at the interface generate a pair of net optical forces which not only trap but also stretch the cell [1-3]. The cell is confined along the common optical axis of the two beams by transverse gradient forces, and is stabilized at a point on the optical axis where the axial scattering forces of the two beams balance each other. Besides, photonic stress on the cell surface results in the cell’s deformation. The stress distribution in the dual-beam optical stretcher can be calculated by ray optics model as the cell size is usually much larger than the laser wavelength. In the case of the spherical cell it has been demonstrated analytically that the local photonic forces are perpendicular to the refracting surface, and independent of the incidence angle and the position of the incident beam on the sphere as well as the reflectance and the transmittance of the beam at the cell surface [2].
As in Ref. 1, a swollen RBC is modeled as a spherical elastic shell filled with homogeneous and isotropic fluid. The only elastic component in the RBC is then the thin shell made of plasma membrane. The ratio of the membrane thickness \( h \) to the cell radius \( R \), \( h/R \), is on the order of 0.01. It is well known that the error introduced using the membrane theory instead of the thin shell theory with finite shell thickness is on the order of \( h/R \) [7].

We consider only the cases where the cell’s deformation is small compared with the initial shape, so that the cell is not severely and irrecoverably deformed. In this case the deformation can be described by the linear elastic theory of membrane. In general, a thin elastic shell supports an external loading by means of internal forces, bending and twisting moments. We assume that RBC membrane cannot sustain bending or twisting moments because of its small flexural stiffness. It only supports tensile and compressive forces. We also assume that the applied stress does not generate bending forces or torques so that the deformed surface remains relatively smooth; i.e., the changes in curvature and twisting of the membrane are assumed to be very small. This model is valid for the RBCs in the dual-beam optical stretcher, as visual examination under microscope of deformed cells clearly shows smooth cell surfaces.

We propose a numerical approach to the problem using the classical mechanical theory of linear elastic membrane [7]. In the dual-beam optical stretcher the spherical RBC is a shell of revolution around the optical axis \( z \). In the spherical coordinate system the photonic stress is expressed by its radial, zenithal and azimuthal components \( (\sigma_r, \sigma_\theta, \sigma_\phi) \). As the stress is normal to the surface we have \( \sigma_\theta = \sigma_\phi = 0 \), and the radial stress \( \sigma_r \) normal to the membrane corresponds to the external surface load to the RBC. We consider a differential membrane element isolated from the shell by means of four cuts with two meridians with \( \theta, \theta+d\theta \) and two azimuths with \( \phi, \phi+d\phi \), respectively as shown in Fig. 1. The element is in an orthogonal curvilinear system \( \alpha = \theta, \beta = \phi \) with the origin on the element. The element is in a static equilibrium state under external and internal forces. The internal forces to the differential element are in-plane normal forces \( N_\phi, N_\theta \), which are in the plane where the element is laid and normal to the coordinates \( \alpha \) and \( \beta \) respectively, and the in-plane shear force \( S \) in the case of the shells of revolution. The \( N_\phi, N_\theta \) and \( S \) are resultants of the integration of stress distribution through the membrane thickness, so that their dimensions are the force per unit length of the element. The \( S \) is related to the shear strain \( \gamma_{\phi\theta} \) according to the relation \( S = hG\gamma_{\phi\theta} \) where \( G \) is the shear modulus. In the membrane theory for the shells of revolution and for the spherical membrane, the Laplace equations are expressed as [7]

\[
\begin{align*}
R \frac{\partial S}{\partial \theta} + \frac{\partial}{\partial \phi} (N_\phi R \sin \phi) - N_\theta R \cos \phi + R^2 \sin(\phi)\sigma_\phi &= 0 \\
\frac{R \partial N_\theta}{\partial \theta} + \frac{1}{R \sin \phi} \frac{\partial}{\partial \phi} (R^2 \sin^2(\phi)S) + R^2 \sin(\phi)\sigma_\theta &= 0 \\
N_\phi + N_\theta + \sigma_r &= 0
\end{align*}
\]

which allow to calculate the internal forces \( N_\phi, N_\theta \) and \( S \) from the external load \( \sigma_r \) where \( \sigma_\theta = \sigma_\phi = 0 \) and \( R \) is the sphere radius. As the system is rotationally symmetric around the \( z \)-axis, all derivatives with respect to the zenithal angle \( \theta \) vanish in Eqs. (1) and (2). As \( \sigma_\phi = 0 \) the shear force is independent of the external load according to Eq. (2), and we have \( S = 0 \). We rewrite Eq. (3) as

\[
N_\phi = -\sigma_r(\phi)R - N_\phi
\]

where the local photonic stress profile \( \sigma_r(\phi) \) normal to the membrane can be computed according to experimental conditions, such as the size of the RBC, the refractive indices of the
RBC and of the surrounding medium, the distance from the RBC to the fibers, and the laser power [2]. Substituting Eq. (4) into Eq. (1) yields

$$N_\phi(\phi) = -\frac{R}{\sin^2(\phi)} \int_{0}^{\phi} \sin(\phi') \cos(\phi') \sigma_r(\phi') d\phi'$$  \hspace{1cm} (5)

where the upper limit of integration designates the position of the membrane element in the azimuth coordinate. Equation (5) can be computed by numerical integration using the Lobatto Quadrature method.

![Coordinate system for a spherical cell. Two laser beams propagate in the ±Z direction.](image)

Once the internal forces $N_\phi$ and $N_\theta$ are computed from the given photonics stress profile $\sigma_r(\phi)$ by Eqs. (5) and (4), one may now calculate the strains of the differential membrane element, $\varepsilon_\phi$ and $\varepsilon_\theta$, which represent the total deformation per unit length of a segment on the membrane element in the azimuthal and zenithal directions, respectively. The strains are related to $N_\phi$ and $N_\theta$ via Hooke’s law under the linear elastic membrane approximation for small deformation of cells as:

$$\varepsilon_\phi = \frac{1}{Eh} (N_\phi - \nu N_\theta) \quad \text{and} \quad \varepsilon_\theta = \frac{1}{Eh} (N_\theta - \nu N_\phi)$$  \hspace{1cm} (6)

where $E$ is the modulus of elasticity and $\nu$ is the Poisson coefficient representing the membrane change in volume due to the deformation. For small deformation, the volume of the membrane is approximately constant and $\nu=0.5$.

The local deformation of the membrane is described by the displacement of a material point on the surface due to the strains in the differential element. Denoting the projections of the displacement vector on the directions of $U$, $V$ and $W$ axes, where $U$, $V$ are tangent to the curvilinear coordinate axes $\alpha$, $\beta$ on the element and $W$ is normal to the surface element, by $u$, $v$ and $w$ respectively, and using the kinematic equation for the strain-displacement relation we have [7]:

$$\varepsilon_\phi = \frac{1}{R} \frac{\partial u}{\partial \phi} - \frac{w}{R} \quad \text{and} \quad \varepsilon_\theta = \frac{1}{R} (u \cot \phi - w)$$  \hspace{1cm} (7)

Equation (7) can be obtained by considering a line segment along $\alpha$ and a line segment along $\beta$ in the unstrained membrane element, and computing their displacements due to the strains of the membrane element in the small deformation approximation with the high-order infinitesimal terms neglected. Eliminating $w$ in Eq. (7), one obtains

$$\frac{du}{d\phi} = u \cos \phi + R(1+\nu)(N_\theta - N_\phi)$$  \hspace{1cm} (8)
The solution of this differential equation is

\[ u(\phi) = \sin \phi \int_{0}^{\phi} \frac{R(1+v) (N_{\phi} - N_{\phi})}{Eh \sin \psi} d\phi + C \sin \phi \]  

(9)

where \( C \) is a constant determined from the boundary condition. It is easy to see that \( u(0) = 0 \).

The displacement of the membrane in the radial direction \( w \) is simply the change in the radius of the cell and can be measured experimentally. We computed \( w \) from Eqs. (6) and (7), as

\[ w = u \cot(\phi) - \frac{R}{Eh} (N_{\theta} - vN_{\phi}) \]  

(10)

Thus, the deformations \( u \) and \( w \) can be calculated numerically from Eqs. (9) and (10) for any given stress profile. In our case, we used Runge-Kutta of order 4 with error control. This approach can be applied in general experimental conditions. Moreover, in the special case when \( \phi = 90^\circ \), and when \( \phi = 0^\circ \), Eq. (10) gives the RBC deformation normal to optical axis \( (\phi = 90^\circ) \) and along the optical axis \( (\phi = 0^\circ) \) as

\[ w_{90} = \frac{R}{Eh} \left( vN_{\phi}(90^\circ) - N_{\phi}(90^\circ) \right) \]  

(11a)

\[ w_{0} = \frac{R}{Eh} \left( vN_{\phi}(0^\circ) - N_{\phi}(0^\circ) \right) \]  

(11b)

To obtain Eq. (11b) we notice that \( \lim_{\phi \to 0} (\cos \phi) = 0 \) which approximately equals zero according to Eq. (9). In Eq. (11), the membrane stresses \( N_{\phi} \) and \( N_{\phi} \) are obtained from Eqs. (5) and (4). We verified that in the special case when the stress distribution is given by \( \sigma_r = \sigma_r \cos^2(\phi) \), the deformation obtained by our numerical method is identical to that obtained from the semi-analytical solution in Ref. 1. Moreover, we compared the theoretical results, obtained from Eq. (11), with the experimental data.

3. Comparison with experimental results

The theoretical RBC deformations calculated using Eqs. (4), (5) and (11) can be compared with experimental results. Within the linear elastic membrane approximation for small deformations of the RBC, Eq. (11) shows that the deformations \( w_{90} \) and \( w_{0} \) are linear functions of the laser beam power because both the internal stresses \( N_{\phi} \) and \( N_{\phi} \) depend linearly on the photonic stress \( \sigma_r \) according to Eqs. (4) and (5), and \( \sigma_r \) is proportional to the laser power. To determine the modulus of elasticity of the cell’s membrane, we plotted the experimental data \( w/R \) (representing the relative cell deformation along the optical axis) and \( w_{90}/R \) (representing the relative cell deformation normal to the optical axis) of spherical RBCs in an optical stretcher as a function of the laser power. From the best fit of Eq. (11) to the experimental data, we obtained the slopes \( Eh \) of the two deformation curves \( w/R \) and \( w_{90}/R \), as illustrated in Fig. 2(b).

The experiments were performed in a fiber-optical counter-propagating dual-beam stretcher with laser wavelength \( \lambda = 1064 \text{ nm} \), fiber numerical aperture \( NA = 0.14 \) and the distance between two fiber end-faces \( d = 150 \mu\text{m} \). Human RBC samples were osmotically swollen into spherical shape with the radius \( R = 3.3 \mu\text{m} \). The refractive index of RBC \( n_2 = 1.378 \) was taken from Ref. 8, and that of the buffer was \( n_1 = 1.335 \). Micrographs of spherical human RBC trapped and stretched at various optical powers are depicted in Fig. 2(a), for laser power varying from 19mW to 230mW. The corresponding relative deformations in the length of the elongated sample along the major axis and the minor axis as a function of the optical power.
are plotted in Fig. 2(b). Each data point represents a mean of the values measured for 10 RBC samples. The experimental details were reported in Ref. 3.

In Fig. 2(b) the solid lines are the theoretical fits obtained from the computational method described previously with a single fitting parameter $E_h$. Deviation of the experimental data from the straight lines in the high power regime is expected since our calculation is applicable only for small deformation associated with small stretching power. In this specific example, the deviations are pronounced when the laser power is higher than 150-175 mW and the relative deformation exceeds 10%. Those data points were neglected. The best fit, as shown in Fig. 2(b), was recalculated by taking into account only the data points with the relative deformation within the ±10% range. We obtain $E_h=(20±2)\mu$Nm$^{-1}$. The corresponding shear modulus $G_h=E_h/(1+\nu)\approx(6.67±0.07)\mu$Nm$^{-1}$ which is comparable to the results reported earlier: $8.5\mu$Nm$^{-1}$ by Dao et al. [8], $13\mu$Nm$^{-1}$ by Guck et al. [1] and $2.5\mu$Nm$^{-1}$ by Hénon et al. [6].

4. Conclusion

We report a new numerical method for calculating the deformation of a spherical red blood cell from an arbitrary stress distribution over the cell membrane. In the particular case when the stress distribution can be approximated analytically by $\sigma_r = \sigma_0 \cos^2(\phi)$, our results reduce to those reported earlier for this special case [1]. We applied the method to fit the experimental data for the deformation of a red blood cell obtained in general experimental conditions in a fiber-optical dual-beam stretcher, for a general stress distribution. From the best fit, the modulus of elasticity and the shear modulus of the red blood cell were determined to be comparable with the results reported earlier.

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