Optical Deformability of Soft Biological Dielectrics

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Two counterpropagating laser beams were used to significantly stretch soft dielectrics such as cells. The deforming forces act on the surface between the object and the surrounding medium and are considerably higher than the trapping forces on the object. Radiation damage is avoided since a double-beam trap does not require focusing for stable trapping. Ray optics was used to describe the stress profile on the surface of the trapped object. Measuring the total forces and deformations of well-defined elastic objects validated this approach.

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Because of the small momentum of photons, radiation pressure can be neglected in our immediate environment. In the microscopic world, however, the effects of the interaction of light with matter can be significant. Lasers are used to manipulate objects ranging from atoms to micron-sized beads or biological cells [1–3]. The total momentum transfer from a laser beam to a transparent object results in a propulsive force in the direction of the light propagation (scattering force) and an attractive force along the intensity gradient perpendicular to the laser axis (gradient force) [4]. One-beam gradient traps, called optical tweezers, have been used for a variety of biological experiments in which cells, organelles, or beads, attached to biological objects as tiny handles, have been trapped and moved [5,6]. However, attempts to deform whole cells by pulling on two handles have been limited by the small holding forces on the handles. Even soft red blood cells could be deformed only by 15% of the original cell size with this method [7].

In spite of this, laser beams can be used for the deformation of cells. While scattering and gradient forces are due to the total momentum transferred to the particle's center of gravity, the transfer actually occurs on the particle's surface. Our study shows that the resulting local surface forces are much larger than the total forces. This effect can be observed in a two-beam trap [1,8,9], where two slightly divergent, counterpropagating laser beams are used to trap single cells. Intuitively, one might expect that the scattering forces from the two beams compress the cell. In contrast, exactly the opposite occurs: The cell is stretched out along the beam axis. This optical deformability of soft dielectrics can be motivated by a simple *gedankenexperiment.*

The momentum p_1 of a ray of light with energy E traveling in water is $p_1 = n_1 E/c$ (n_1: refractive index of water, ≈ 1.33; c: speed of light in vacuum) [10,11]. Let such a ray hit the surface of a dielectric transparent cube with length $l = 10\ \mu$m and a refractive index $n_2 = 1.45$, which is typical for biological materials. At normal incidence (incident angle $\alpha = 0°$) only a fraction $R = 0.2\%$ of the light is reflected. Almost all the light enters the cube and gains momentum due to the higher index of refraction. Upon exiting the cube, the same fraction of light, R, is reflected and the exiting light loses momentum. The conserving momenta transferred to the two surfaces per second, i.e., the forces experienced by the two surfaces, are

$$F_{\text{front}} = [n_1 - (1 - R)n_2 + Rn_1]P/c \qquad (1a)$$

and

$$F_{\text{back}} = [n_2 - (1 - R)n_1 + Rn_2](1 - R)P/c\,, \qquad (1b)$$

where P is the total light power. The forces F_{front} ($\approx$190 pN for $P = 500$ mW) and F_{back} ($\approx$210 pN) point in opposite directions—away from the cube. The total force acting on the cube is the difference between those two surface forces $F_{\text{total}} = F_{\text{back}} - F_{\text{front}} \approx 20$ pN. This total force is in essence the scattering force. In addition, the surface forces stretch the cube with $(F_{\text{back}} + F_{\text{front}})/2 \approx 200$ pN, which is 10 times greater than the scattering force. If an identical ray hits the cube from the opposite side, there is no total force acting on the center of the cube. However, the forces stretching the cube are now twice as large as before ($\approx$400 pN). The cube experiences a deforming stress $\sigma = 400\ \text{pN}/(10\ \mu\text{m})^2 \approx 4\ \text{N m}^{-2}$ that results in a deformation of $\Delta l = l\sigma/E \approx 400$ nm for a Young's modulus $E = 100\ \text{N m}^{-2}$. Any soft dielectric material can be stretched in this fashion as long as its refractive index is larger than the refractive index of the surrounding medium. Thus, we termed this two-beam setup the *optical stretcher.*

An essential benefit of using a two-beam trap for cells is the possibility to work with higher laser powers than in a one-beam trap. Radiation damage is avoided because there is no focusing required for the trap's stability. In fact, the laser beams must be divergent. The trap is stable as long as the radii w of the divergent laser beams at the position of the cell are larger than the cell size ($w \approx 10\ \mu$m) [8]. In optical tweezers there are also surface forces present that distort the cell shape. These deformations are very small because the light power is limited to $P < 20$–250 mW

depending on the cell type and the wavelength used [3,4]. The reason is that the laser beam has to be highly focused for the trap to be stable, which can lead to opticution of living biological cells. Typical beam sizes at the focal point of optical tweezers are on the order of $w \approx \lambda/2 \approx 500$ nm. The light power, i.e., the deforming stresses, in the optical stretcher can be 400 times greater than in optical tweezers before similar light intensities in the cell are reached. Thus, the stresses accessible for cell elasticity measurements with an optical stretcher range between the highest stresses possible with optical tweezers and the lowest stresses exerted by an atomic force microscope.

In our experiments, even sensitive eukaryotic cells, such as PC12 cells, were trapped in the optical stretcher without any sign of radiation damage with up to 700 mW of light power in each beam [12]. Red blood cells (RBCs) were used to test the concept of optical deformability because they are well-defined mechanical objects, intensively studied, and easy to handle. The use of oil drops was disregarded. Their shape is determined by surface tension that is sensitive to miniscule temperature changes due to light absorption. Giant vesicles did not seem to be a good choice because laser beams induce instabilities in their often multilamellar membranes [13].

The experimental setup consists of a cw-Ti:sapphire laser emitting at $\lambda = 785$ nm, an acousto-optic modulator as light power control, and an inverted microscope equipped with a CCD camera. Images of the trapping and stretching of cells are captured and analyzed with a computer. Similar to the method described in [9], two single mode optical fibers are used to deliver the light to the microscope for ease of use and as spatial filters for the Gaussian beam profile. The maximum light power output from each optical fiber is $P \approx 700$ mW.

Ray optics (RO) was used to calculate the deforming stress acting on the surface of a cell trapped in the optical stretcher. The cells studied can be well approximated by nonabsorbing spheres with an isotropic index of refraction. This is justified because the eukaryotic cells assumed a spherical shape in suspension and the RBCs were osmotically swollen into a sphere. Cells are almost transparent in the near infrared, so absorption can be neglected. The relative refractive index is $n = n_2/n_1 = 1.05-1.15$ for biological objects, where n_1 and n_2 are the refractive indices of the medium and the object, respectively. The value of n can be determined by index matching in phase contrast microscopy [14]. The size of cells is on the order of tens of microns (typical radius of an eukaryotic cell, $\rho = 8-15$ μm; radius of a spherical RBC, $\rho = 3.0-3.4$ μm). Thus, RO can be used to describe their interaction with $\lambda = 785$ nm light [15]. This approach is commonly used for laser traps [1,4–6,8]. The deforming stress calculation proceeds similar to the simple estimate above, with the rays intersecting the cell surface in general at angles $\alpha \neq 0$. The direction of the transmitted ray is given by Snell's law. The fraction of reflected light, R, varies depending on α and the state of polarization and can be calculated from the Fresnel formulas. To simplify the calculation and to maintain symmetry with respect to the laser axis, R is taken to be the average of the coefficients for perpendicular and parallel polarization relative to the plane of incidence. This is a negligible deviation from the true situation [16].

With this simple RO model, the forces acting on any surface element, i.e., the stress on the cell surface, was calculated. One result is that all surface forces act normal to the surface. Figure 1 shows the stress profiles for one laser beam shining on the cell. The profile has rotational symmetry around the beam axis (z axis). The cell acts as a lens and focuses the beam towards the axis. The resulting asymmetry between front and back profile leads to a total propulsive force in the positive z direction. This is the origin of the scattering force. The exact shape of the stress distribution and the magnitude of the total force F_{total} depend on the ratio between the cell radius ρ and the beam radius w, which in turn depends on the distance d from the tip of the optical fiber.

In order to test the RO calculations, we measured the total force acting on different objects. After trapping silica beads, polystyrene beads, or cells in the optical stretcher, one of the beams was blocked. The total force from the other beam then accelerated the object in the direction of the light propagation. The resulting velocities were measured and the total force on the object was estimated to equal the Stokes drag force. Figure 2 shows the agreement between the measured and calculated total force. The fact

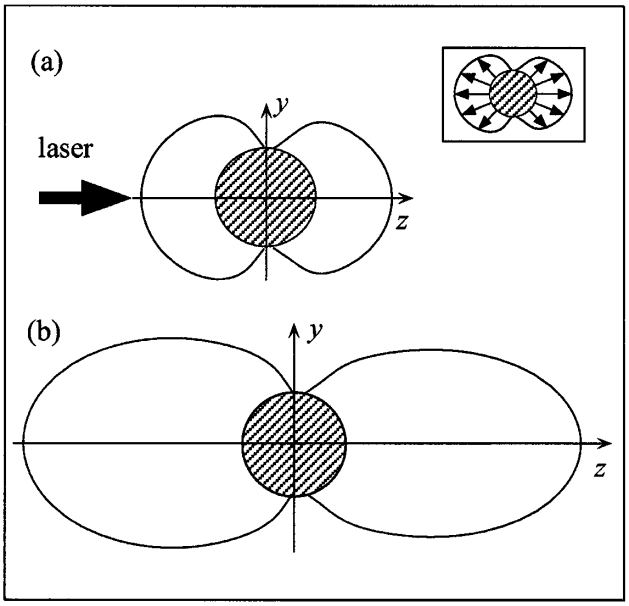

FIG. 1. Stress profiles on the surface of a sphere ($n_1 = 1.33$, $n_2 = 1.45$) due to one laser beam with Gaussian profile and total power $P = 500$ mW; (a) for a ratio of $w/\rho = 2.0$ the stresses along the z axis are $\sigma_{\text{front}} = 2.8\ \text{N m}^{-2}$ and $\sigma_{\text{back}} = 3.1\ \text{N m}^{-2}$ resulting in a total force $F_{\text{total}} = 25$ pN and (b) for $w/\rho = 1.1$, $\sigma_{\text{front}} = 9.0\ \text{N m}^{-2}$ and $\sigma_{\text{back}} = 9.8\ \text{N m}^{-2}$ resulting in $F_{\text{total}} = 38$ pN. The inset illustrates the direction of the surface forces.

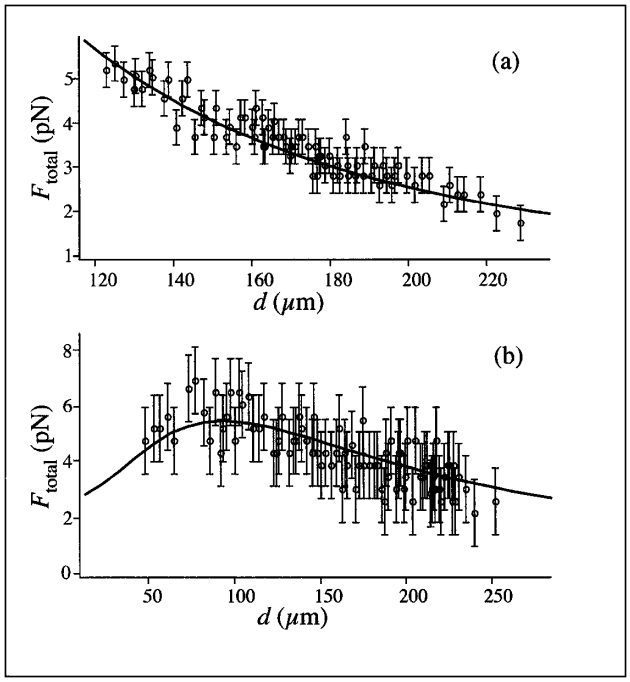

FIG. 2. Calculated and measured total force F_{total} as a function of the distance d between fiber tip and particle for (a) silica beads ($\rho = 2.50 \pm 0.02$ μm, $n_2 = 1.43 \pm 0.01$, $P = 350$ mW) and (b) PC12 cells ($\rho = 7.7 \pm 0.2$ μm, $n_2 = 1.38 \pm 0.01$, $P = 300$ mW) in aqueous solution.

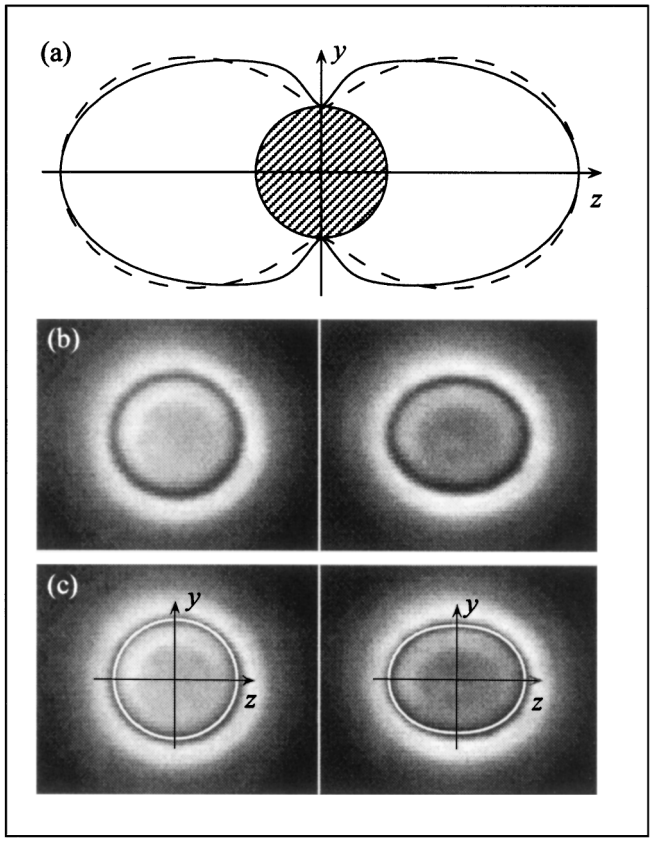

FIG. 3. (a) Stress profile for a spherical RBC with radius $\rho = 3.32 \pm 0.02$ μm ($n_1 = 1.335$, $n_2 = 1.380$) in between two 86 mW laser beams ($w/\rho \approx 1.1$). The peak stress along the z axis is $\sigma_0 = 1.02\ \mathrm{N\,m^{-2}}$. The solid line is for the calculated stress; the dashed line is for the $\sigma(\alpha) = \sigma_0 \cos^2(\alpha)$ approximation. (b) Phase-contrast images of a RBC in the optical stretcher at 5 mW and at 86 mW light power in each laser beam. (c) The white line shows shapes expected from linear membrane theory due to the stress shown in (a).

that the RO model works as well for spherical beads with homogeneous index of refraction (silica beads and polystyrene beads) as for cells justifies the assumptions about the physical properties of biological cells. The magnitude and the d dependence of the total force also agree well with previous results [8].

In the optical stretcher the cell was trapped between two identical, counterpropagating laser beams. Thus, the total force on the cell was zero, and the cell was stably trapped if $w/\rho > 1$. However, when the stress on the cell surface was large enough, the cell was stretched out along the beam axis. To verify this optical deformability, osmotically swollen spherical RBCs were investigated because their elastic properties are very well characterized [17] and, due to their softness, deformations are easily quantified. Figure 3(a) shows a typical stress profile calculated for a RBC. It can be approximated by $\sigma(\alpha) = \sigma_0 \cos^2(\alpha)$, σ_0 being the peak stress along the z axis.

RBCs consist mainly of a thin elastic shell with a ratio of radius ρ to thickness h, $\rho/h \approx 100$. They are filled with hemoglobin, which leads to a homogeneous index of refraction of $n_2 = 1.380$ (for spherical shape) [18]. In contrast to eukaryotic cells, RBCs do not have a three-dimensional polymer scaffold throughout the cytoplasm, which makes them much softer. The buffer for the RBCs with osmolarity of 270 mOsm was adapted from [19]. Under physiological conditions, they have a biconcave, disk-like shape. However, the osmolarity of the buffer was adjusted to $\approx$130 mOsm ($n_1 = 1.335$) and the RBCs assumed a spherical shape prior to the trapping and stretching. Prepared this way, they perfectly resembled the model cell: They were spherical, had an isotropic index of refraction, and had virtually no absorption at the wavelength used ($\lambda = 785$ nm).

Single RBCs were trapped in the optical stretcher at low light powers ($\approx$5 mW). The distance d between cell and fibers was adjusted so that $w/\rho \approx 1.1$–1.2. The light power was then increased in steps up to 350 mW and the resulting deformation of the cell was recorded [see Fig. 3(b)]. In the linear regime the maximum expansions in the z direction were $\approx$800 nm at 350 mW, while in the y direction the cells contracted ≈ -600 nm. At light powers higher than 350 mW the elastic response of the RBCs became nonlinear, deformations reached values up to 160% of the original cell size at $\approx$600 mW, and finally the cells ruptured. For each step, the stress distribution on the cell was calculated using the power P, the radius ρ, and the distance d measured. Figure 4 shows the relative deformations in the linear regime along the beam axis (positive values) and perpendicular to it (negative values) as a function of the peak stress σ_0 along the z axis.

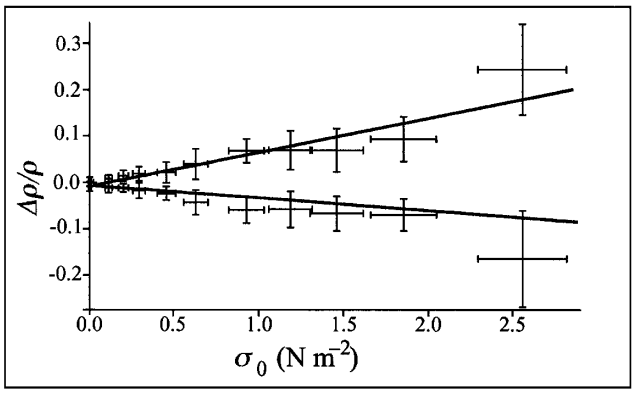

FIG. 4. Relative deformation of RBCs measured along (positive) and perpendicular to the laser axis (negative) as a function of the peak stress σ_0 on each cell. Solid lines show the prediction from linear membrane theory.

To verify our stress profile calculations we related the observed deformations of the RBCs to the known material constants of their membranes by using linear membrane theory [20]. The stress on a thin shell with isotropic Young's modulus E, thickness h, and radius ρ leads to tensions in the shell that result in displacements of surface elements. The rotational symmetry makes spherical coordinates a natural choice, which are oriented so that the incident angle α is identical to the polar angle. For a thin shell the bending energy is negligible compared to the membrane (stretching) energy [21]. Because of the rotational symmetry of the stress profile, the radial displacements $\Delta\rho(\alpha)$ of the surface elements depend only on the polar angle,

$$\Delta\rho(\alpha) = \frac{\rho^2\sigma_0}{4Eh}[(5 + \nu)\cos^2(\alpha) - \nu - 1], \quad (2)$$

where the Poisson ratio $\nu \approx 0.5$ for biological membranes. These displacements result in an ellipsoid with major axis $a = \rho + \Delta\rho(0)$ and minor axes $b = \rho + \Delta\rho(\pi/2)$. Figure 3(c) shows the agreement between the theoretical and the observed shape of a RBC in the optical stretcher. The relative deformations along the z axis $[\Delta\rho(0)/\rho]$ and the y axis $[\Delta\rho(\pi/2)/\rho]$ are both linearly proportional to the peak stress and the material properties ρ/Eh. Plotting the relative deformation for $Eh \approx 3.9 \times 10^{-5}\ \mathrm{N\,m^{-1}}$ (see Fig. 4) shows the consistency between experiment and theory. This value for Eh is in agreement with literature values for RBC membranes [22].

In conclusion, we have demonstrated the possibility of stretching soft biological dielectrics in a two-beam laser trap. A RO approach is sufficient to explain this stretching. The momentum transferred from the light to the surface of the trapped object results in forces on the object's surface. The surface forces lead to stretching of an elastic object [23]. The deforming forces can exceed the total trapping forces. As illustrated with the *gedankenexperiment*, the stretching forces of two counterpropagating 500 mW laser beams on an object with a relative refractive index of $n \approx 1.1$ are $F \approx 400$ pN. They could be even greater for higher indices of refraction and higher light powers. The optical stretcher can be used to measure the elasticity of biological cells. The advantages are that optical deformation does not require any kind of mechanical contact and covers a stress range previously inaccessible to cell elasticity measurements.

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