PHYS-E0411 - Advanced Physics Laboratory

Optical Tweezers

A. Väkeväinen, M. Hautakorpi, K.Lindfors, A. Priimägi, P. Järvinen,
H. Rekola ja M. Kaivola
translated by Rebecca Heilmann

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Contents

1 Introduction

"What is light?" is a question, that played an important role in guiding physics development over several centuries. Our current understanding of physics based on quantum mechanics provides an answer to this, while the dual nature might be confusing: light is electromagnetic radiation which has the properties of both particles and waves at the same time. These seemingly contradictory descriptions of the particle and wave model are combined in the equations $E = \hbar\omega$ and $p = \hbar k$, where properties of the light wave, the frequency ω and the wave vector k, are coupled to the energy E and momentum p that are properties of the light particle (the photon) by the Planck constant $\hbar$. According to Newtonian mechanics, in the process of interaction between light and matter, both energy and momentum must be preserved. In traditional optics, where the direction of light is changed, for example by a lens, the lens is thus subjected to an opposing force, which, however, is negligibly small in the case of macroscopic specimens. The situation changes crucially if the interacting object is sufficiently small. At its extreme, a laser beam of high intensity can be aimed at an individual atom resulting in an atomic force equivalent to an acceleration of up to 10^5g!

The concept of light pressure was already clear to J. C. Maxwell when he constructed his famous equations, but he had to wait until a laser of sufficiently intense light sources was available to exploit this phenomenon. In this experiment, mechanical forces caused by light are studied, and in particular the ability of a focused laser beam to capture a microscopic dielectric particle, which is in thermal motion in a liquid environment. This concept of the *optical tweezers* as a diverse tool was developed quickly after Arthur Ashkins pioneering work during the 1970s-1980s [1], which, for example, offers a completely new type of method for the study of biomolecules, cells and molecular motors [2]. At the same time, the same force effects have been used in basic physics research, when a gas cloud of millions of atoms has been cooled to the temperature of nanokelvins by laser beams, and as a result, the atoms form a Bose-Einstein condensate. This research has led to significant new discoveries in modern physics and was already awarded with two Nobel Prizes in physics in 1997 and 2001.

In this experiment, the momentum and angular momentum of an electromagnetic field are studied using optical tweezers. The structure of these instructions is as follows: Section 2 describes the forces exerted by light on a dielectric particle in the ray and and wave optical picture. Section 3 discusses the manipulation of the rotational motion of birefringent particles in a liquid environment by light polarization. The particle interacts with both light and the surrounding liquid molecules. This interaction is briefly described in section 4. Section 5 introduces the setup of the optical tweezers used in this experiment and the measurement process is explained in section 6. Section 7 contains instructions regarding laser safety. The preliminary tasks done by the student are described in section 8. The necessarily tasks to be done for the report are found in Section 9.

2 Operating principle of the optical tweezers

2.1 Ray optical picture

In this section, the working principle of the optical tweezers are discussed from a ray optical picture point of view. The other possible picture, the wave optical picture, and its theory are briefly explained in section 2.2, where the polarization of a captured particle in a focused (spatially inhomogeneous) electromagnetic field is considered.
According to ray optics, the light moves straight through a homogeneous medium and is refracted at a surface between two media according to Snell's law

$$n_1 \sin\theta_1 = n_2 \sin\theta_2 \tag{1}$$

where the angles are defined with respect to the normal (see Fig. 1). A part of the light coming from the surface reflects symmetrically with respect to the normal. In optical tweezers, a collimated beam (advancing in the direction of the optical axis) is focused by a lens toward the back focal point of the lens, as shown in Fig. 2.

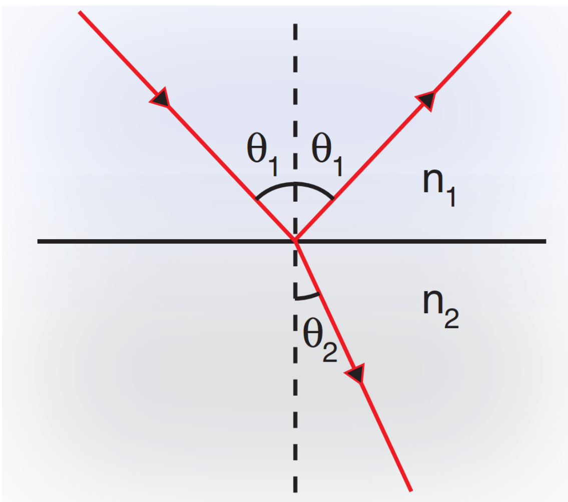

Figure 1: Light refraction and reflection at the interface between two media. The parameters n_1 and n_2 are the refractive indices of the media and in this case $n_2 > n_1$.

The rays refract in the lens according to Snell's law and a small point of light is formed from the focused light at the back focal point. This point of light is smaller in size (i.e. the focus is tighter), if the light rays can be focused coming from a larger angle θ. The ability of a lens to capture and focus light is described by the *numerical aperture*

$$\mathrm{NA} = n \sin\theta, \tag{2}$$

where n is the refractive index of the medium, where the focal point is located. The largest numerical apertures are generated with microscope objectives, where *immersion oil* ($n \approx 1.5$) is added between the sample and the lens of the objective.

Let us now consider the refraction of the outermost rays of a converging (focused) bundle of rays from a small dielectric sphere immersed in a homogeneous liquid (see

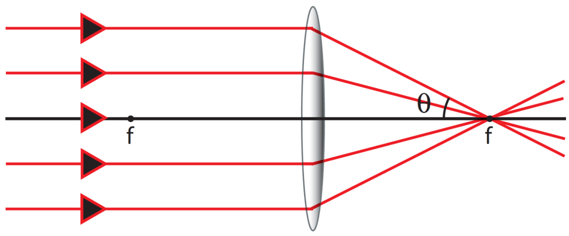

Figure 2: Focusing a collimated beam in air with a positive lens. The focal length is f. The angle between the outermost rays and the optical axis is θ

Fig. 3), where the refractive index of the sphere is larger than of the surrounding liquid. The ability of the optical tweezers to capture a particle in three dimensions can be explained by looking at the change in the momentum of light as the beam of light passes through the sphere and refracts at the interfaces according to Snell's law. The momentum of light is proportional to its intensity (unit $\mathrm{W/m^2}$), and the momentum vector $\mathbf{p}$ points into the direction of propagation of the light beam. The propagation direction of the light and thus the momentum will change upon refraction. According to Newton's third law, if the change in momentum of the light is $\Delta\mathbf{p}$, the change in momentum of the particle in which the light refracts has to be $-\Delta\mathbf{p}$. The change in momentum results in a force F per unit time, the direction of which depends on the position of the center of the particle o with respect to focal point f. Fig. 3 shows the forces applied from the light to the particle depending on the relative positions of the points o and f. In Fig. 3 (a), ray A refracts at the interface such, that its momentum increases in the z-direction and decreases in the x-direction. Correspondingly, the particle center o is subjected to a force $\mathbf{F}_A$ that has components in the $-z$- and $+x$-directions. For ray B one can argue correspondingly, leading to a force $\mathbf{F}_B$. The vector sum of forces $\mathbf{F}_A$ and $\mathbf{F}_B$ leads to the resultant force $\mathbf{F}$, which is directed towards the focal point f. A particle located behind the focal point f as viewed from the direction of indicidence of the light, is therefore subjected to a force pulling the particle towards focus. In Fig. 3 (b), the particle is located in in front of the focal point f, and the force F tends to move the particle again towards f. Also in Fig. 3 (c), where the particle is next to the focal point, the force acts such, that the particles is moved toward f. The operation of the optical tweezers can thus be explained based on simple ray optical argument [3].

In the ray optical analysis presented above, only the outermost rays of the bundle were considered and the rays reflected from the interface were not taken into account. If the rays that are propagating closer to the to the optical axis as well as the reflected rays are considered, an additional force component into the positive z-direction acts on the trapped particle in Fig. 3 [3]. This *scattering force* can be thought of as radiant pressure of the light acting on the particle. Due to this scattering force the position of the trapped particle at equilibrium is slightly behind the focal point f, viewed from the propagation direction of light. In order to prevent the scattering force from pushing the particle out of focus, the light should be focused tightly enough (sufficiently large NA). In this case, the share of the outermost rays in the beam is emphasized and a stable trapping of the particle is possible.

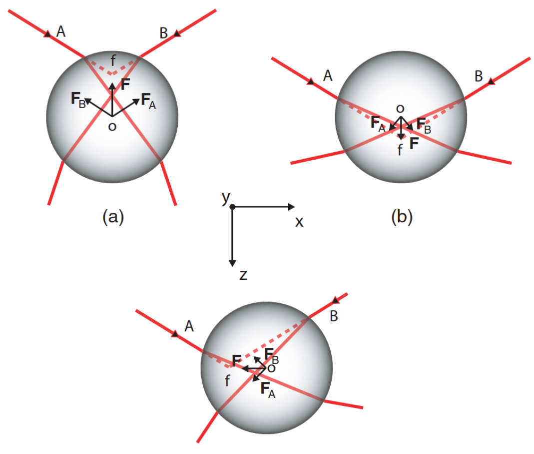

Figure 3: Forces F that arise from the refraction of the outermost rays of a focused bundle of rays, when the focal point f is (a) behind, (b) in front, and (c) next to the center of the particle. The total force F points in all cases toward the focal point f.

2.2 Wave optical picture

The ray optical picture can only be applied to the optical tweezers if the particle is much larger than the wavelength of light. If the size of the particle is of the same order of magnitude or much smaller than the wavelength of the light, the phenomenon has to be analyzed with the wave optical picture. If the particle is smaller than the wavelength, the interaction between light and particle can be approximated by an electric dipole (polarizability α), where the dipole moment in an external electric field is of the form [4, 5]

$$\mu(\mathbf{r}) = \alpha \mathbf{E}(\mathbf{r}). \tag{3}$$

The potential energy U of the dipole can be expressed as

$$U(\mathbf{r}) = -\mu(\mathbf{r}) \cdot \mathbf{E}(\mathbf{r}) \equiv -\alpha \mathbf{E}(\mathbf{r}) \cdot \mathbf{E}(\mathbf{r}) \tag{4}$$

where the quantity $\mathbf{E} \cdot \mathbf{E}$ is proportional to the intensity of light $I(\mathbf{r})$. From Eq. 4 one can see that the minimum of the potential energy is reached in the local maxima of the intensity, if α is positive (normal case). The *gradient force* transferred from the optical field onto the particle can be expressed by

$$\mathbf{F}(\mathbf{r}) = -\nabla U(\mathbf{r}), \tag{5}$$

from where the dependence $\mathbf{F}(\mathbf{r}) \propto \nabla I(\mathbf{r})$ can be derived.
Thus, in a inhomogeneous field, a polarizable particle is subjected to a force whose magnitude is proportional to the intensity gradient. In a tightly focused field, a particle ($\alpha > 0$) tends to shift toward the focus in all three dimensions as the focused light has an intensity gradient not only in the x- and y-directions, but also in the z-direction. The intensity gradient in x- and y-direction is directly proportional to the numerical aperture of the objective $\left[(\nabla I)_{x,y} \propto \mathrm{NA}\right]$, and opposed to that the dependence in z-direction is quadratic $\left[(\nabla I)_z \propto \mathrm{NA}^2\right]$. In addition to the gradient force, also in this case a scattering force acts onto the particle in z-direction, the

effect of which is however small, when the numerical aperture is large.
Near the focal point, the light intensity distribution can be approximated as a parabolic and the electric dipole (a polarizable particle) as a harmonic oscillator which is subjected to a spring force

$$F(x) = -k_x x, \tag{6}$$

where k_x is the spring constant in x-direction (correspondingly k_y, k_z in y- and z-direction). The potential energy of the harmonic oscillator is of the form

$$U = \frac{1}{2} k_x x^2. \tag{7}$$

Although the particle was assumed to be much smaller than the wavelength, this formalism can also be used to describe fairly accurately particles of the order of magnitude of the wavelength and the process of trapping even larger particles.

3 Capturing birefringent particles

3.1 Light polarization and angular momentum

In the ray optical picture the working principle of the optical tweezers was described by the state of momentum of the light before and after refraction. The properties of light polarization were not taken into account, as in case of a homogeneous and isotropic spherical particle the polarization of light does not have any effect onto the capturing process. However, when manipulating an anisotropic, birefringent particle, the polarization of light and the associated spin angular momentum plays a key role [6]. The angular momentum transmitted by polarized light to a particle can be determined by looking at the change in the angular momentum of the light as it passes through the particle.
An elliptically polarized plane wave, which oscillates with an angular frequency ω and propagates into positive z-direction, can be expressed by

$$\mathbf{E} = E_0 e^{i(\omega t - kz)} \left(\cos \Phi \hat{x} + i \sin \Phi \hat{y} \right), \tag{8}$$

where E_0 is the ampilutde of the electric field, t is the time, $k = \omega/c$ the wave number and c the speed of light. The parameter Φ is the phase, which determines the degree of elliptical polarization, and the vectors $\hat{x}$ and $\hat{y}$ are the unity vectors in x- and y-direction. A genuine measurable electric field is always complex and has a real part of the electric field vector, for which we have

$$Re\{\mathbf{E}\} = E_0 \left(\cos(\omega t - kz) \cos \Phi \hat{x} - \sin(\omega t - kz) \sin \Phi \hat{y} \right). \tag{9}$$

In the special case of $\Phi = \pi/4$, the light is circular polarized, i.e. the components of the electric field in x- and y-direction have equal amplitudes. From Eq. 9 one can see, that the phase difference of x and y components is $\pi/2$ and the degree of elliptical polarization determined by the relationship between the amplitudes, which depends on phase angle Φ.

The momentum density (in unit volume) of the electromagnetic field can be expressed based on Maxwell's equations in the form of

$$\mathbf{p} = \epsilon \mathbf{E} \times \mathbf{B}, \tag{10}$$

where $\mathbf{B}$ is the magnetic flux density and ϵ is the permittivity of the medium. The total angular momentum density of the field on the other hand can be calculated by the expression known from classical mechanics

$$\mathbf{J} = \mathbf{r} \times \mathbf{p}. \tag{11}$$

In the general case, the expression for $\mathbf{J}$ can be divided by the spin angular momentum associated with the polarization state of light and the orbital angular momentum associated with the spatial distribution of light. For the plane wave in Eq. 8, the orbital angular momentum is zero, and an expression for the spin angular momentum density can be derived [7].

$$\mathbf{J}_{\mathrm{spin}} = \frac{\epsilon}{2i\omega} \mathbf{E}^* \times \mathbf{E} \tag{12}$$

3.2 Interaction between polarized light and a birefringent particle

The refractive index in birefringent materials depends on the oscillation direction of the electric field $\mathbf{E}$. Let's assume that a disk-like birefringent particle in Fig. 4 is located at the origin such, that $z = 0$ is the side of the disk onto which the light is impinging. The thickness of the particle into z-direction is assumed to be d. When the electric field vector oscillates in x'-direction, the refractive index of the material is n_e and correspondingly, when the electric field vector oscillates in y'-direction, the refractive index of the material is n_o. When Eq. 8 is rewritten in the rest coordinate system of the particle (x',y'), the electric field at $z = 0$ is of the form

$$\mathbf{E}_1 = E_0 e^{i\omega t} \left[(\cos\Phi \cos\theta + i \sin\Phi \sin\theta)\, \hat{x}' + (-\cos\Phi \sin\theta + i \sin\Phi \cos\theta)\, \hat{y}' \right], \tag{13}$$

where θ is the angle between the coordinate systems (for instance between x- and x'-axis; rotation clockwise from the (x, y)-coordinate system from the point of view into positive z-direction). In dielectric materials the value of the wave number k depends on the refractive index via the speed of light c/n. Generally $k = n\omega/c \equiv k_0 n$. After the light has travelled the distance d to the the other side of the particle, we get for the outgoing light the expression

$$\begin{aligned} \mathbf{E}_2 = E_0 e^{i\omega t} [& e^{-ik_0 n_e d} (\cos\Phi \cos\theta + i \sin\Phi \sin\theta)\, \hat{x}' + \\ & e^{-ik_0 n_o d} (-\cos\Phi \sin\theta + i \sin\Phi \cos\theta)\, \hat{y}'], \end{aligned} \tag{14}$$

the exponential phase factors take into account the wave number dependence on the refractive indices n_e and n_0. The spin-angular momentum at the planes $z = 0$ and $z = d$ can be calculated by integrating the angular momentum density of Eq. 12 over the particles surface. The torque applied to the particle per unit time can be calculated as the difference between the angular velocities of the equation

$$\tau = \frac{\sqrt{\epsilon/\mu_0}}{2i\omega} \int_\sigma (\mathbf{E}_1^* \times \mathbf{E}_1 - \mathbf{E}_2^* \times \mathbf{E}_2)\, \mathrm{d}x \mathrm{d}y, \tag{15}$$

where μ_0 is the vacuum permeability and σ the cross-sectional area of a disk-like particle. Inserting the expressions for the electric field of Eqs. 13 and 14 into Eq. 15 gives the expression of the torque in z-direction [6, 8]

$$\begin{aligned}\tau = &-\frac{\sigma}{2\omega}\sqrt{\frac{\epsilon}{\mu_0}} \mid E_0 \mid^2 \sin[k_0 d(n_o - n_e)] \cos 2\Phi \sin 2\theta \\ &+\frac{\sigma}{2\omega}\sqrt{\frac{\epsilon}{\mu_0}} \mid E_0 \mid^2 \{1 - \cos[k_0 d(n_o - n_e)]\} \sin 2\Phi.\end{aligned} \tag{16}$$

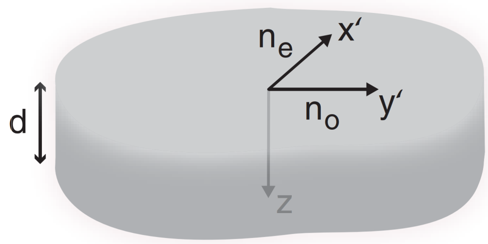

Figure 4: Birefringent particle which has a rest coordinate system (x',y'). The refractive index for light oscillating in the x'-direction is n_e and for light oscillating in the y'-direction n_o.

When the light is linearly polarized ($\Phi = 0$ or $\Phi = \pi/2$), only the first part of Eq. 16 is left. Then, because of the $\sin 2\theta$-dependence, the particle is subjected to a torque as long as $\theta \neq m\pi/2$ (m = 0, 1, 2,...) and the x'- or y'-axis of the particle tends to rotate in the direction of the polarization of the light. In the case of circularly polarized light only the second term of Eq. 16 is left. Then the torque does not depend at all on the orientation of the particle, and the particle can rotate in a continuous motion, where the angular velocity Ω depends on the intensity of the light and the flow resistance of the surrounding medium, which in this experiment is a liquid. The from the flow resistance resulting rotation leads to a corresponding torque of the form

$$\tau_D = D\Omega, \tag{17}$$

where the parameter D is a proportionality factor depending on the shape of the particle and the viscosity of the liquid.

3.3 Rotation of a birefringent particle

In case of elliptically polarized light the particle is subjected to a continuous rotational motion if the latter term in Eq. (16) is greater in absolute value than the first term for all angles θ. In the boundary case the torque is zero, from which the threshold conditions for the phase angle leading to rotation are obtained

$$\Phi_0^{\pm} = [\pi \pm k_0 d(n_o - n_e)]/4. \tag{18}$$

When the particle is in a continuous rotational movement, the work done by the torque in Eq. 16 during one rotation corresponds to the work done by the torque τ_D which acts contrary to the rotation. By integrating Eq. 16 over one rotation one notes, that the work done by the linearly polarized component is zero. The work done by the circularly polarized component (over one rotation) can be described by

$$W_\Phi = 2\pi AP \sin 2\Phi, \tag{19}$$

where the proportionality factor A contains a set of constants. In addition P is the power of the light impinging onto the particle $P = I\sigma$, with the expression for the intensity

$$I = \frac{1}{2}\sqrt{\frac{\epsilon}{\mu_0}} \mid E_0 \mid^2 . \tag{20}$$

The work done by the friction torque can be calculated using Eq. 17 as follows:

$$\begin{aligned} W_D &= D \int_0^{2\pi} \Omega(\theta)\mathrm{d}\theta \\ &= D \int_0^{T} \frac{\mathrm{d}\theta}{\mathrm{d}t}\frac{\mathrm{d}\theta}{\mathrm{d}t}\mathrm{d}t \\ &= D \int_0^{T} [\Omega(t)]^2 \,\mathrm{d}t \\ &= DT\langle[\Omega(t)]^2\rangle, \end{aligned} \tag{21}$$

where the angle dependency was change to a time dependency and T is the time needed for one rotation. The time average of the angular velocity during one rotation can be calculated from the equation

$$\langle\Omega(t)\rangle = \frac{1}{T}\int_0^T \Omega(t)\mathrm{d}t \equiv \frac{2\pi}{T}, \tag{22}$$

where the last form follows directly from the definition of the time average. By placing this into Eq. 21 and assuming that the rotational movement is almost completely smooth ($\langle\Omega^2\rangle \approx \langle\Omega\rangle^2$) , we get under the assumption that $W_\Phi = W_D$ following expression for the average angular velocity:

$$\langle\Omega(t)\rangle = \frac{AP}{D}\sin 2\Phi \tag{23}$$

4 Brownian motion and spring constant of the tweezers

The Brownian movement was first discovered by botanist Robert Brown while observing pollen particles dispersed in water. Particles in a liquid environment are in constant motion due to the thermal movement of molecules of the liquid [see Fig. 5 (a)]. In 1905 Albert Einstein justified with this phenomenon the structure of matter from molecules. Later, J. B. Perrin was able to confirm the accuracy of Einstein's predictions by microscopy, and the atomic nature of matter began to be considered factual rather than hypothetical. When the particle is not subjected to external forces, its motion in one dimension can be described by the Langevin equation

$$m\frac{\mathrm{d}^2x}{\mathrm{d}t^2} + \mu\frac{\mathrm{d}x}{\mathrm{d}t} - F(t) = 0, \tag{24}$$

where $F(t)$ describes the random force on a particle and μ is the viscosity of the surrounding medium as well as a factor that describes the geometry of the particle. Let's assume, that $F(t)$ does not depend on the velocity v of the particle and that $F(t)$ changes considerably faster than v. In this case, the work done by $F(t)$ over a long period of time is zero, and only this part of the equation needs to be solved:

$$m\frac{\mathrm{d}^2x}{\mathrm{d}t^2} + \mu\frac{\mathrm{d}x}{\mathrm{d}t} = 0 \tag{25}$$

From Eq. 25 the expectation value of the squared displacement $\langle x^2\rangle$ can be can be solved by multiplying the equation with x and using following identities:

$$x\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{1}{2}\frac{\mathrm{d}x^2}{\mathrm{d}t}, \tag{26}$$

$$x\frac{\mathrm{d}^2x}{\mathrm{d}t^2} = \frac{1}{2}\frac{\mathrm{d}^2x^2}{\mathrm{d}t^2} - \left(\frac{\mathrm{d}x}{\mathrm{d}t}\right)^2 \tag{27}$$

By placing these into Eq. 25, one gets

$$m\left(\frac{1}{2}\frac{\mathrm{d}^2\langle x^2\rangle}{\mathrm{d}t^2} - \langle\frac{\mathrm{d}x}{\mathrm{d}t}\rangle^2\right) + \frac{1}{2}\mu\frac{\mathrm{d}\langle x^2\rangle}{\mathrm{d}t} = 0 \tag{28}$$

By using the equipartition theorem $\frac{1}{2}m\langle v\rangle^2 = \frac{1}{2}k_BT$, which connects the total energy of the system with the temperature of the system, one can write the equation as follows:

$$\frac{1}{2}m\frac{\mathrm{d}^2\langle x^2\rangle}{\mathrm{d}t^2} - k_BT + \frac{1}{2}\mu\frac{\mathrm{d}\langle x^2\rangle}{\mathrm{d}t} = 0 \tag{29}$$

which leads to the solution

$$\frac{\mathrm{d}\langle x^2\rangle}{\mathrm{d}t} = \frac{2k_BT}{\mu} + Ae^{-\mu t/m}, \tag{30}$$

where A is the integration constant. The exponential term vanishes in equilibrium ($t \to \infty$) and for the square displacement one gets

$$\langle x^2\rangle = \frac{2k_BT}{\mu}t. \tag{31}$$

In case of a rotating particle we have

$$\mu = 6\pi\eta a, \tag{32}$$

where η is the viscosity of the surrounding medium and a the radius of the particle. In this case, the squared displacement increases linearly with time t as a function according to

$$\langle x^2\rangle = \frac{k_BT}{3\pi\eta a}t \tag{33}$$

A quantitative analysis of Brownian motion has been used in order to determine the Boltzmann constant [9].
The thermal motion of a particle captured with optical tweezers differs significantly

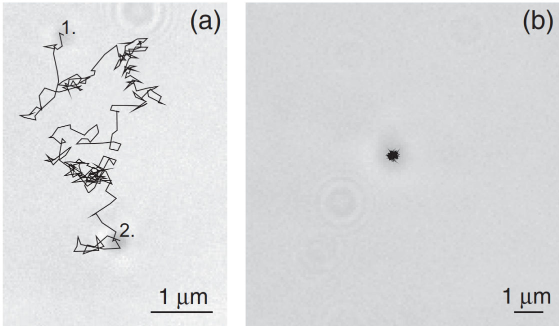

Figure 5: (a) Brownian motion of a small glass sphere (diameter 1 μm in water. The start and end points of the trajectory of the particle are marked with the numbers 1. and 2. (b) In the potential well of optical tweezers, the thermal motion of the particle is significantly more confined. This small movement can be used to determine the spring constant of optical tweezers using the equipartition theorem. The trajectories of the particle are combined from a series of 275 images.

to Brownian motion [see Fig.5 (b)]. In this case the motion of the particle is described by

$$m\frac{\mathrm{d}^2x}{\mathrm{d}t^2} + \mu\frac{\mathrm{d}x}{\mathrm{d}t} - F(t) - U(t) = 0, \tag{34}$$

where $U(t)$ is the parabolic potential caused by the tweezers. In this case the squared displacement is

$$\langle x^2 \rangle = \frac{k_B T}{k_x}, \tag{35}$$

which is obtained by comparing the average potential energy of the particle in the potential caused by the tweezers with the average kinetic energy of the particle at a specific temperature

$$U = \frac{1}{2}k_x\langle x^2\rangle = \frac{1}{2}k_B T. \tag{36}$$

Based on this Eq. (36), the spring constant of the tweezers k_x can be determined by recording the position coordinates of the captured particle over a sufficiently long period of time. The typical order of magnitude of the spring constant is $\approx$ fN / nm and is linearly proportional to power of the laser beam.

5 Setup

The experimental implementation of the optical tweezers requires only a tightly focused laser beam to trap the particles and imaging optics that is used both to monitor the trapping process and to collect measurement results. A schematic diagram of the equipment is shown in Fig. 6. The main components of the equipment are a laser, a microscope objective and imaging optics. In the following is a brief summary of the components.

The most important properties of the laser for operating as optical tweezers are beam spatial quality, power and wavelength. The quality and diameter of the beam determine how well the beam is focused at the sample plane and how strong the generated intensity gradient is at the focal point. The applied power of the laser

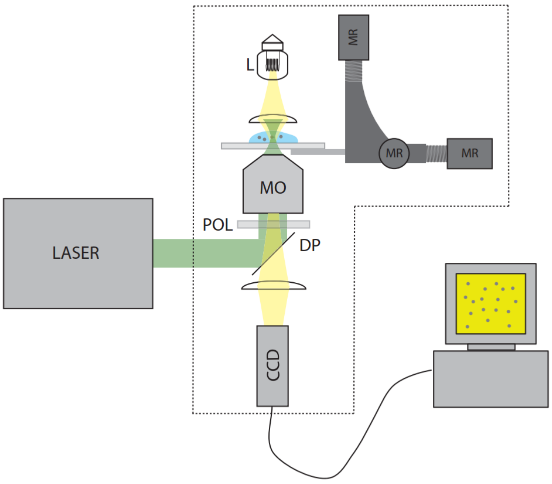

Figure 6: Schematic of the setup. A collimated laser beam is guided by a dichromatic mirror (DP) through polarization optics (POL) to a microscope objective (MO) that focuses the beam on the sample. The sample can be moved in three dimensions by a motorized xyz-translator which can also be adjusted manually with micrometer screws (MR). The sample is illuminated by a lamp (L), and the trapped particle is monitored with a CCD camera. The analysis of the results of the measurements is performed on the basis of a series of images stored on a computer

beam has a strong effect on the ability to trap a particle and in addition the spring constant of the tweezers is directly proportional to the power of the laser. The choice of wavelength, in turn, depends on the intended use of the tweezers. A laser of light in the visible regime is the best option for capturing small glass spheres due to its easy positioning and safety. Most often, however, optical tweezers are used to study biological samples that are sensitive to even small changes in temperature due to light absorption. In this case, near-infrared lasers (for example at a wavelength of 1064 nm) are typically used in optical tweezers, where the absorption is small.
The microscope objective is the most important single component of optical tweezers, as it determines how large the intensity gradient is at the focal plane. In order for the gradient to be large enough to trap the particles in three dimensions, it is most often necessary to use an oil or water immersion lens with a large numerical aperture (NA = 1.2 1.4). The focusing ability of the objective is also affected by the diameter of the input beam. To obtain the smallest possible focal point, the beam diameter must be at least as large as the lens inlet. Because the laser beam has typically a diameter of only 2-3 mm, and for example, the inlet of the objective used in this experiment is about 7 mm, the diameter of the beam has to be expanded.
Imaging of the sample is typically accomplished by using the same objective that is used to focus the laser beam; the dichromatic mirror reflects the laser beam into the sample and transmits enough other wavelengths to a CCD-camera, where an image of the sample plane is formed, which is illuminated by a lamp above the sample plane.

6 Measurements

The work is divided into two parts. First, the operation of optical tweezers is introduced and data is collected to determine the spring constant of tweezers based on Eq. 36. The goal is to get an idea of the magnitude of the optical forces. Glass (silica) spheres with a diameter of 1 μm are used as samples.
In the second part the utilization of the angular momentum of light in the manipulation of birefringent particles is investigated. Birefringent and irregularly shaped calcite particles in the size range of about 5μm are used as samples. In the experiment half- and quarter-wave plates are used to change the polarization state of the light. The polarization direction of the incoming linearly polarized light can be rotated with the half-wave plate. The quarter-wave plate, in turn, converts the incoming linearly polarized light to elliptically polarized light. The ellipticity is determined by the orientation of the wave plate. Rotating the wave plate corresponds directly to changing the angle Φ in Eq. 8.
The following is a detailed description of the sequence of the work.

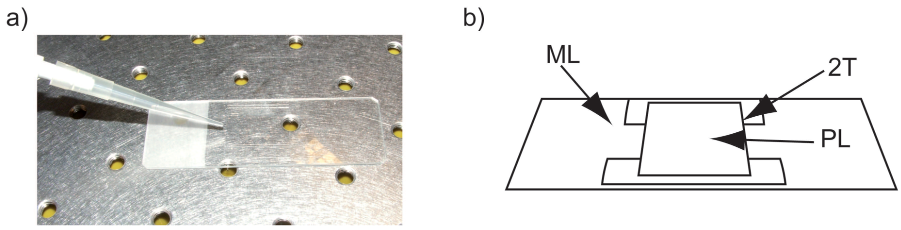

Figure 7: (a) A sample is prepared by pipetting a liquid containing particles between a microscope slide and a coverslip. (b) Schematic diagram of the sample chamber. The microscope slide (ML) and the coverslip (PL) are attached to each other with double-sided tape (2T).

1. Familiarize yourself with the measurement equipment with the help of the assistant and calibrate the power measurement. Compare the light output of the laser beam at the input of the microscope lens and at the measuring point where the power meter is held during the measurements. This will give you the ratio needed to calculate the power. Take a measurement with at least three different power values to get a reliable result.

2. Prepare a sample with a solution of 1μm glass spheres: take a small amount of the solution with a pipette and spread it into the sample chamber as shown in Fig. 7.

3. Drop a drop of immersion oil onto of the microscope lens and place the sample on the microscope (see Fig. 8). Be sure to place the sample in the correct orientation (cover glass down). Also check that the sample holder is high enough so that the sample does not collide with the objective.

4. Carefully lower the sample until you see the glass spheres on the computer screen. The sample can be moved by turning the micrometer screws on the xyz-translator or with a joystick.

5. Move the sample and trap a particle with the tweezers.
6. Record a series of images of the motion of a particle in the tweezers with different light intensities to determine the spring constant of the tweezers. Each series of images should consist of about 300 images. Use a shooting speed of about 15 fps. The assistant guides you through taking and saving images. Remember to take a sufficiently large enough set of measurements.
7. Remove the sample from the microscope and prepare a new sample of calcite particles in the same way as the glass spheres.
8. Adjust the distance of the sample from the objective lens so that you can see the calcite particles on the computer screen.
9. Capture the calcite particle and study the effect of the direction of linear polarization on the orientation of the particle by rotating the half-wave in 10° steps. Save an image of each measurement point. The assistant guides you through saving the pictures.
10. Replace the half-wave plate with a quarter-wave plate and study the effect of the degree of ellipticity of the polarization on the rotational speed of the particle. Measure the rotation speed as a function of the angle of the wave plate as follows: First, find the orientation of the quarter-wave plate at which the particle clearly rotates. Next, rotate the quarter-wave plate every 2°-5° until the particle stops. Then turn the wave plate back to the first measurement point and repeat the measurement every 2°-5° into the other direction until you reach a position where the particle stops again. Determine the rotational speed of the particle at each measurement point by recording a series of images for at least 20 seconds (15 fps). The assistant guides you through saving images.
11. Compare the effect of right- and left-handed circularly polarized light on the motion state of a particle: Capture a calcite particle and make it rotate by rotating the quarter-wave plate. Save a short series of pictures of the rotation. Rotate the wave plate by 90° and save another set of images of the rotation.

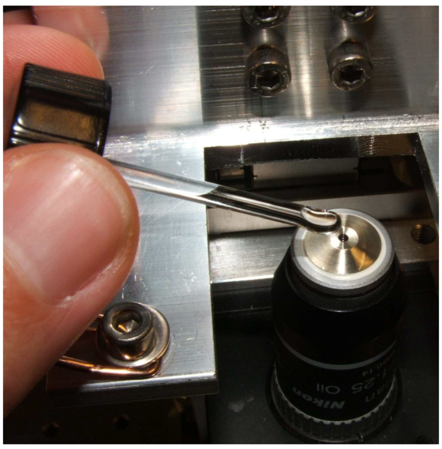

Figure 8: Adding immersion oil onto the objective.

Remember the following things:

- Always mark down the used intensity of the beam in a specific measurement. You will need it.
- In experiments involving the orientation of particles, it is easiest to examine particles that are sufficiently irregular to make their orientation easy to determine.
- In your report, always remember to reflect upon and interpret your results. Try to understand the results you get!

7 Laser safety

The laser used in this experiment is a class IV laser, and the power of the laser used for the optical tweezers is at its maximum several tens of milliwatts. The wavelength of the laser is 1064 nm, which cannot be observed with bare eye. As a comparison, the pen-like laser pointers typically belong to class I (power about 1 mW). The laser light used only poses a danger if it hits the eye and does not damage the skin of the hands, for example. The following rules of thumb should be kept in mind when working with lasers:

- Never look directly into the laser beam or reflected radiation.
- Do not position yourself so that your eyes are level with the laser beam (e.g. when sitting)
- Block the beam always when it is not needed.
- The safest way to protect yourself from laser light is to wear goggles. **Always wear safety goggles during the experiment!**
- **Follow the instructions given by the assistant!**

8 Preliminary tasks

- How does a particle with a refractive index smaller than the one of the surrounding liquid behave in a tightly focused field according to the ray optical picture?
- Find an expression for the proportionality factor A in Eq. 19.

9 Report

1. Briefly describe the principle of operation of the optical tweezers and the concept of light polarization. It is not advisable to explain all the details of the work instructions again. Think about what are the most important things in terms of measurements. Explain both the ray and wave optical pictures. Direct copying (copy/paste) from the instruction is not allowed.

2. Go through the equations needed to process the measurement results. How to determine the spring constant of tweezers? Why does a birefringent particle begin to rotate under the influence of elliptically polarized light? How are the angular velocity and the shape parameter D determined?

3. Describe the measurement setup and procedure so that the measurements could be repeated based on that description.

4. Explain how the measurement results have been analyzed and briefly describe the image processing. Explain how the error limits are determined.

5. Determine the dependence of the spring constant of the tweezers on the laser power in both the x- and y-direction using the image series you collected. Plot graphs and remember the error limits. Reflect, what the possible reasons for differences in the spring constants could be. For this task, you need to determine the location of the particle in the optical tweezers.

6. Discuss the magnitude of the force exerted by optical tweezers and the magnitude of the forces that can be measured with optical tweezers.

7. Describe the effect of the polarization state of light (linear, left- and right-handed circular polarization, elliptical) on the orientation and motion of birefringent particles. Explain your findings.

8. Analyze the dependence of the orientation of the calcite particle on the direction of linear polarization. What is the relationship between the orientation of the particle and the position of the half-wave plate? Use images and graphs to support the analysis. Remember the error limits.

9. Determine the angular velocity of the particle rotation dependent on the orientation of the quarter-wave plate by analyzing the time series you measured. Compare the dependence of the angular velocity you get on the position of the wave plate with the theory presented in the instruction. Determine the value of parameter D from the graph using equation (23). Remember the error limits.

10. How is the rotation of a calcite particle affected by left- and right-handed circular polarization?

11. Repeat the results with the numerical values and error limits in the summary. Reflect on the reliability of the results and how the measurement could be improved.

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