Universität Leipzig

Biophysics Lab Course

Forces on Small Spheres in a One-Beam Gradient Trap (BONG)

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1. Introduction

In 1970 Ashkin demonstrated that dielectric particles can be accelerated by the radiation pressure of a laser beam and trapped by two counter-propagating beams [1]. Sixteen years later he succeeded in trapping particles using a single, highly focused laser beam [2], a setup that was called "optical tweezers". Optical tweezers have become a powerful tool, in physics as well as in biology, for manipulating objects as large as 100 μm and as small as a single atom without mechanical contact. For example, inside biological objects such as cells or cell organelles, small probes can be held, moved and rotated by exertion of forces as small as several piconewtons with optical tweezers.

In the case of biological samples, light in the near infrared (λ = 700–1100 nm) is used in order to prevent radiation damage, which occur with shorter or longer wavelengths. The cause for this "opticution" could be absorptive heating or photochemical processes, but this is still poorly understood and subject to controversy.

The list of experiments for which optical tweezers have been used includes the trapping of cells and bacteria [3,4], the measuring of the forces exerted by molecular motors such as myosin or kinesin [5,6] and the study of the mechanical properties of single macromolecule strands (DNA, RNA, polypeptides) fully expanded by the means of beads attached to their ends [7]. A good overview of biological applications can be found in reference [8].

The historically older two-beam trap has played a major role in the fields of atom trapping and cooling and has been a prerequisite for the first experimental proof of Bose-Einstein condensation. Both achievements have been honored with a Nobel Prize in recent years. However, in biology, this kind of trap has only recently acquired some importance through its use in deforming cells by optically induced forces. In this context, this setup is also called “optical stretcher”.

Theoretical Background

In general, scattering, i.e. the interaction of light with an object, can be divided into two components [9]. The first is the reflection and refraction at the surface of the particle, the second is the diffraction from the rearrangement of the wavefront after it interacts with the particle. While the radiation pattern due to reflection and refraction emanates from the particle in all directions and depends on the refractive index of the particle, the diffraction pattern is primarily in the forward direction and depends only on the particle geometry.

Two different regimes of theoretical approach can be distinguished. They are determined by the ratio of the incident light's wavelength λ to the diameter D of the irradiated particle. In the ray optics regime regime, the particle is very large compared to the wavelength ($D \gg \lambda$), whereas in the Rayleigh regime the opposite is true ($D \ll \lambda$). The calculation of optical forces for arbitrary particle sizes $D \approx \lambda$ is nontrivial. For a full arbitrary theory, the solution of Maxwell's equations with the appropriate boundary condition is required [9]. The Lorenz-Mie Theory [10] was the first step in that direction and describes scattering of a plane wave by a spherical particle for arbitrary particle size, refractive index, and wavelength. However, the Lorenz-Mie Theory cannot describe a Gaussian beam, such as that produced by a TEM_{00} laser, which is a particular point of interest to accurately describe laser-induced forces. The calculation of optical forces of Gaussian beams and arbitrarily shaped objects can be achieved by the Generalized Lorenz-Mie Theory (GLMT) [11, 12].

Rayleigh Regime (D « λ)

In the Rayleigh regime, the particle is very small compared to the wavelength (D « λ). The distinction between the components of reflection, refraction, and diffraction can be ignored. Since the perturbation of the incident wavefront is minimal, the particle can be viewed as an induced dipole behaving according to simple electromagnetic laws.

Scattering Force:

One of the two arising forces is the *scattering force* due to the radiation pressure on the particle. Incident radiation can be absorbed and isotropically reemitted by atoms or molecules. With this, two momenta are received by the molecule: one along the beam propagation of the incident light and one opposite to the direction of the emitted photon. Since the photon emission has no preferred direction, a net force results in the direction of incident photon flux. This force is directed along the propagation of light and is given by

$$F_{scat} = n_m \frac{\sigma \langle S \rangle}{c}, \qquad (1)$$

where n_{m} is the refractive index of the surrounding medium, <S> is the time-averaged Poynting vector, c is the speed of light, and σ is the particle's scattering cross section, which in case of a spherical particle is given by

$$\sigma = \frac{8}{3}\pi (kr)^4 r^2 \left(\frac{n^2-1}{n^2+2}\right)^2, \qquad (2)$$

with r, particle radius, n, refractive index of the particle and k, wave vector of the used light.

Gradient Force

The second force arising is the *gradient force*. This force is due to the Lorenz force acting on the dipole, induced by the electromagnetic field. In the field of the laser, the gradient force on an induced dipole

$$\vec{p}(\vec{r},t) = \alpha \cdot \vec{E}(\vec{r},t) \qquad (3)$$

of polarizability α is given by

$$\vec{F}(\vec{r},t) = \left[\vec{p}(\vec{r},t)\nabla\right] \cdot \vec{E}(\vec{r},t), \qquad (4)$$

where $\vec{E}(\vec{r},t)$ is the electric field vector of the laser light [13]. With the identity

$$\nabla \vec{E}^2 = 2\left(\vec{E}\nabla\right)\vec{E} + 2\vec{E} \times \left(\nabla \times \vec{E}\right) \qquad (5)$$

and the result from Maxwell's equations

$$\nabla \times \vec{E} = 0 \qquad (6)$$

eq. (4) becomes

$$\vec{F}(\vec{r},t) = \frac{1}{2}\alpha \nabla \vec{E}^2(\vec{r},t). \qquad (7)$$

The gradient force that the particle experiences is the time-averaged version. The relation

$$\left\langle \vec{E}^2(\vec{r},t)\right\rangle_T = \frac{1}{2}\left|\vec{E}(\vec{r})\right|^2 \qquad (8)$$

yields

$$\vec{F}_{grad}(\vec{r}) = \left\langle \vec{F}(\vec{r},t)\right\rangle_T = \frac{1}{2}\alpha\nabla\left\langle \vec{E}^2(\vec{r},t)\right\rangle_T = \frac{1}{4}\alpha\left|\vec{E}(\vec{r})\right|^2. \qquad (9)$$

The light intensity I associated with the field is

$$I(\vec{r}) = \frac{n_m \varepsilon_o c}{2}\left|\vec{E}(\vec{r})\right|^2, \qquad (10)$$

where n_{m} is the refractive index of the exposed material, ε_o is the permittivity of free space, and c is the speed of light [13]. This leads to the force relation

$$\vec{F}_{grad}(\vec{r}) = \frac{1}{2n_m \varepsilon_o c} \alpha \nabla I(\vec{r}). \quad (11)$$

The gradient force's direction is along the gradient (hence the name) toward the area of highest light intensity, i.e., toward the beam axis in case of a Gaussian beam profile, and toward the focus of the laser if the beam is focused.

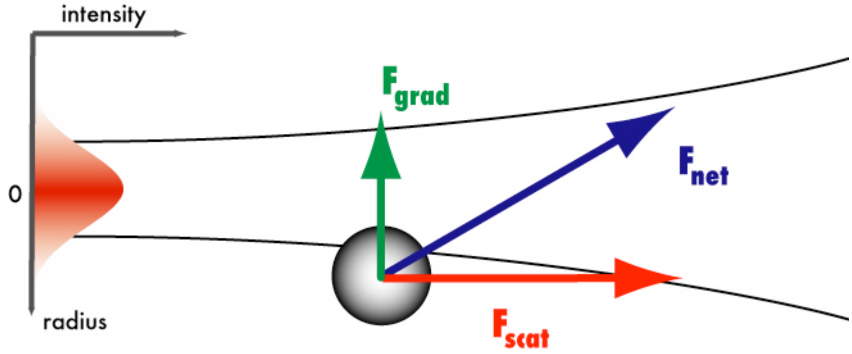

Fig. 1*. The forces arising for a slightly diverging laser beam.*

A particle displaced from the beam axis experiences the gradient force as a restoring force toward the beam axis. Due to the low curvature of the beam, a component of the gradient force parallel to the direction of light propagation can be neglected. The scattering force due to radiation pressure pushes the particle in the direction of light propagation, i.e., away from the light source. For the trapping of particles, either two counter-propagating beams are required in order for the scattering forces to cancel out (geometry of the two-beam trap or optical stretcher), or a single laser beam has to be focused very tightly.

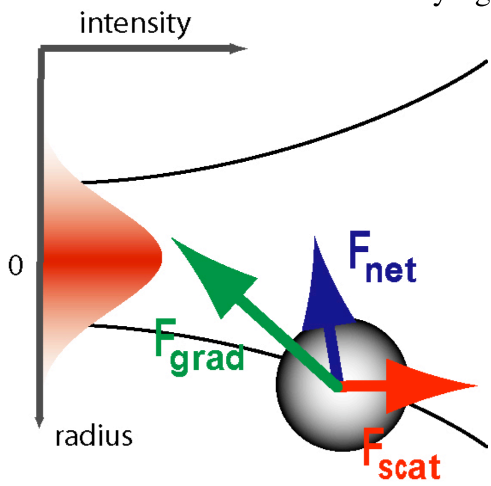

Fig. 2*. The forces arising for a tightly focused laser beam (optical tweezers).*

In a highly focused beam (see Fig. 2), the gradient force possesses a component against the Poynting vector in addition to its component perpendicular to it. This component prevents the particle from being pushed in the direction of light propagation by the scattering force. The net force acts as a restoring force toward the focus of the beam with respect to all three dimensions.

***Ray Optics Regime* (D » λ)**

In the ray optics regime, the size of the object is much larger than the wavelength λ of the light, and a single ray can be tracked throughout the particle. If the ratio of the refractive index of the particle to that of the surrounding medium is not close to one but sufficiently large, diffraction effects can be neglected, which is thoroughly explained in [9] and assumed for further discussion. This situation is for example given when whole cells, which are microns or tens of microns in size, are trapped using infrared light while suspended in a dilute aqueous solution. The incident laser beam can be decomposed into individual rays with appropriate intensity, momentum, and direction. These rays propagate in a straight line in uniform, nondispersive media and can be described by geometrical optics.

Consider a Gaussian laser beam hitting a spherical particle of refractive index n_1, which is surrounded by a medium of refractive index n_2 (see Fig. 3a).

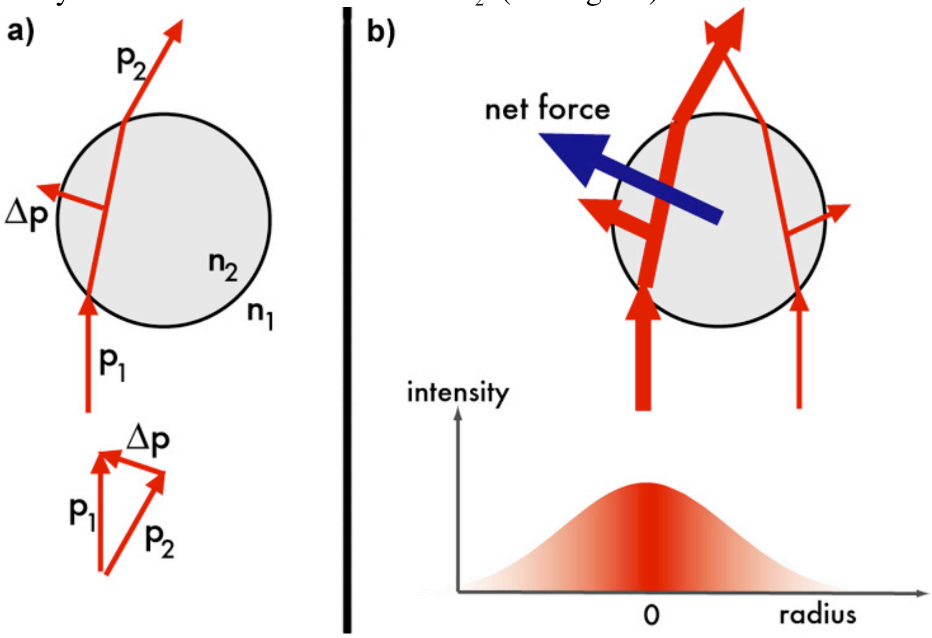

Fig. 3. *The momentum (red arrows) of (a) one ray and (b) two rays with different intensities propagating through a sphere. The blue arrow indicates the restoring net force.*

Each incoming ray carries a certain amount of momentum p proportional to its energy E and to the refractive index n_i of the medium it travels in,

$$p_i = \frac{En_i}{c} \tag{12}$$

After a light ray traveled through the particle, its momentum has changed in direction and magnitude. The momentum difference is picked up by the particle. The force due to the directional change of a ray's momentum has components in the forward direction as well as to the side. However, there are many rays incident on the particle. The net force has only a forward component due to the rotational symmetry of the problem. This symmetry is broken if the particle is not centered exactly on the optical axis of the Gaussian beam. In this case the particle feels a restoring net force (see Fig. 3b).

The net force's component perpendicular to the beam propagation is called the *gradient force,* its component along the direction of beam propagation the *scattering force*, in reference to Rayleigh scattering (Fig. 1). In the ray optics regime, however, these two force-components stem from just one single physical effect as discussed.

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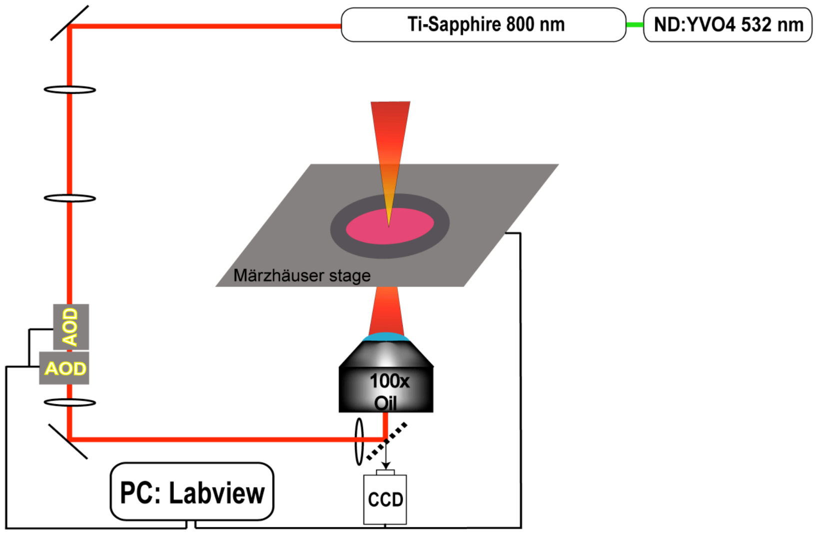
Experimental set-up

Considering the laser part of the setup a tunable Ti:Sapphire CW laser is used which is pumped by a 10 Watt diode driven Nd:YVO4 ring laser. The pump beam (λ=532nm) is directly coupled into a Coherent 890 Ti:Sapphire Laser (λ=800nm).



After the light is emitted by the laser, the beam diameter is increased and collimated by a lens system to a final diameter of 6mm.
An AOD (acousto-optical deflector) is aligned in a way that a Bragg configuration gives a single first order output beam, its intensity is directly linked to the power of the RF control signal, and its angle is directly linked to the RF frequency. By varying the frequency, the output laser beam angle is modified and by varying the RF power, the intensity of the diffracted light is controlled. Thus an AOD works as a scanner and a modulator. To gain XY control, two perpendicular AODs are used. Frequency and amplitude of each channel are controllable by an analog input signal controlled by a PC.
The laser beam is coupled into an inverted microscope by an adjustable mirror beneath the objective revolver. A 100x oil objective with high numerical aperture (NA=1.35) is used in the experiment.
In order to move the sample a movable stage (Märzhäuser stage) is used which can either be controlled by a joystick or the PC.
Images are taken with an analog CCD camera.

2. Tasks and Experimental Procedures

- Determination of the holding force of a laser beam on a polystyrene bead in water solution via the Stokes relation
- Determination of the curve behaviour of holding force depending on power and holding force depending on bead radius
- Evaluation of a correction factor
- Discussion of limitations and possible sources of error

Experimental procedure and evaluation

1. Märzhäuser stage calibration: Use the program stagecalibration.vi to calibrate the movable stage. Obey instructions in the program.

2. Field of view: Put grating slide with scale bar on microscope. Make a snapshot with the CCD-camera and determine the field of view (use Photoshop). Draw scale bar five times and calculate error.

3. Bead size: Make snapshots of five beads and evaluate the radius ρ of the beads by using the previously found scale. Include error calculation.

4. Center laser beam and calibrate the power: Center the laser beam via the adjustable mirror beneath the objective revolver. Set laser power to a certain value before the objective via the modulation channel of the AOD and measure the power after the objective. Do so for at least 5 different power values varying between 5mW and 50mW. Evaluate the absorption in the objective in %.

5. Drag experiment: Trap a bead suspended in water (check temperature before every experiment to find appropriate viscosity) with the laser beam (power P~10mW after objective). Move the bead over a certain distance with linearly increasing velocity. Find the maximal velocity for the Stokes relation and calculate the holding force by using Stokes drag force

$$F_{Stokes} = 6\pi\rho v\eta \overset{!}{=} F_{hold} .$$

   Use the program beaddrag.vi which steers automatically the Märzhäuser stage. Input parameters are start velocity and step size. The stage will then move back and forth and the stage´s velocity will increase every consecutive cycle. The trapped bead will be displaced from the trap center of the laser beam by viscous drag. With increasing velocity this displacement will rise until the laser loses the bead. A bead detection routine incorporated in the program determines and records the bead displacement and the according stage velocity *v*. Via the Stokes relation the holding force can be determined.

6. Plot Stokes drag force versus bead displacement and calculate via linear regression the stiffness of the laser trap in units of Newton/meter, according to

$$F_{Stokes} = \kappa x ,$$

   where κ is the trap stiffness and x the bead displacement.

   Plot κ versus power P. Verify a linear relationship and find a power independent trap stiffness γ, so that κ=γP.

7. Do the experiment with another two different bead sizes and use the same laser power you used in one of the previous experiments. Plot trap stiffness versus bead radius. What behaviour do you find? Can you explain it (theory section might be helpful)?

8. The viscous drag on a spherical object can depend strongly on the proximity of boundaries. A correction factor *k* can be found in terms of the ratio between the radius of a sphere *ρ* and the distance to the closest boundary *d* (Svoboda and Block, 1994),

$$k = \frac{1}{1 - \frac{9}{16}\frac{\rho}{d} + \frac{1}{8}\left(\frac{\rho}{d}\right)^3 - \frac{45}{256}\left(\frac{\rho}{d}\right)^4 - \ldots} .$$

Make another drag experiment with a laser power you used before and lift the bead high enough over the substrate. Try to estimate the correction factor and the distance to the substrate by comparison to a drag experiment where the bead was close to the substrate.

9. Where does this correction term come from? What other sources of error can you imagine?

10. Do an error calculation or alternatively an error estimation on all your calculated values.

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