Setting up an Optical Trap

Using light to trap and manipulate particles? That sounded like science fiction, when Arthur Ashkin first published that idea in 1970. Today, this method for studying and manipulating objects on the microscopic scale is well-established in biology and medicine. Until the mid-80s, interactions on single-molecule level could not be studied and knowledge about biomolecular processes was limited to studies of macroscopic samples. The invention of optical tweezers (optical trap, laser trap) opened up new vistas. Nowadays, optical tweezers setups are commercially available and can be found in numerous biological and medical laboratories. This experiment is supposed to demonstrate how optical tweezers can be set up from standard optical elements. With this optical trap, particles can be trapped in three dimensions. Furthermore, the optical trap will be calibrated to enable measuring forces in the pN range.

Theory

The Principle of Optical Trapping

An optical trap uses the radiation pressure of light to trap, move, or even rotate microscopic objects. Johannes Kepler postulated the radiation pressure of light more than 400 years ago after he had observed that a comet's tail always points away from the sun. Kepler's idea of moving particles with light can be used in optical trapping experiments. A force $\vec{F}$ is exerted on a particle when it is placed in a light beam. This force can be explained by the transfer of momentum $\vec{P}$ from photons that are scattered or refracted at the surface of the particle. The particle's momentum is changed by $\vec{F}=\frac{d\vec{P}}{dt}$. The force which acts upon the particle can be separated into two components: The scattering force $\vec{F_{scat}}$ and the gradient force $\vec{F_{grad}}$:

$$\vec{F}=\vec{F_{scat}}+\vec{F_{grad}} \quad (1)$$

Force generation by laser traps can be theoretically understood in two regimes, namely the Mie regime ($\lambda \gg r$) using geometric optics, and in the Rayleigh regime ($\lambda \ll r$) where the trapped object can be considered as a point dipole induced by the external electric field. λ is the wavelength of the laser light and r the radius of the trapped bead. For biological samples, radiation damage is minimal with infrared light and therefore $\lambda \approx r$. Nevertheless, as it is instructive to consider the principle of force generation in the limiting cases, both will be sketched below.

Geometric limit

Scattering force:

Photons cause a force in direction of incidence ($\vec{z}$ direction), when they are scattered or absorbed by the particle. The so-called scattering force pushes the particle in $\vec{z}$ direction until the force is fully compensated by other forces or a mechanic barrier (such as a microscope slide).

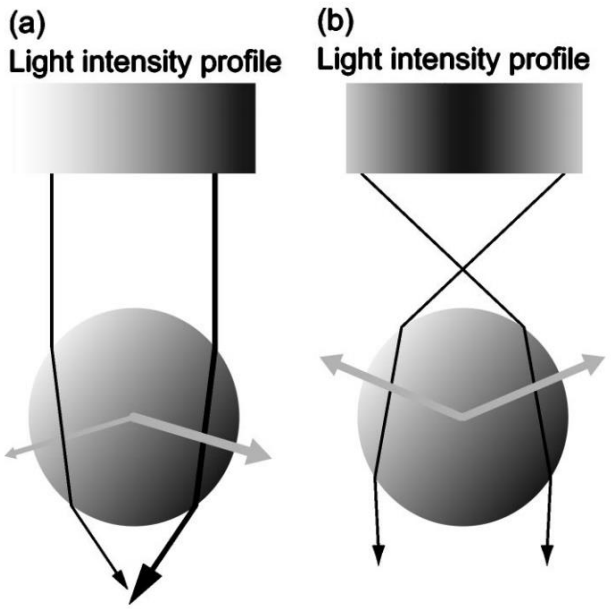

Figure 1.1: Ray optics description of the gradient force. Black lines represent light rays, gray lines represent forces, thick lines represent higher intensities/forces. (a) A bead is drawn towards high light intensities. (b) A trapped bead is in equilibrium in the focus. [Neuman, Block 2004]

Gradient force:

The gradient force depends on the geometry of the light beam and the position of the particle, i.e. its distance to the optical axis. Ideally, the cross section of the beam can be described as an intensity distribution that is decreasing towards the edges. If a particle is placed in the light beam, but not on the optical axis, the intensity is not symmetrically distributed with respect to the center of the particle. As a consequence, the intensity of light that is refracted away from the optical axis is higher than the intensity of light that is refracted towards the optical axis. Thus for the majority of refracted photons, the momentum change (force) points away from the optical axis. As an equal force in the opposite direction is exerted on the particle (Newton's 3rd law, actio = reactio), the particle moves towards the center of the beam. The gradient force is always directed towards the intensity maximum (Fig. 1.1). It allows trapping a particle laterally, that means in a plane perpendicular to the direction of the light beam. Once a particle is trapped in the center of the light beam, it will follow the beam if it is displaced. In that way, a particle can be trapped in the observation plane and moved around there.

2D-Trapping:

The first optical traps used the gradient force to trap a particle on the optical axis. The trapped particle cannot freely diffuse in the observation plane and is trapped in two dimensions. The scattering force in the direction of light propagation pushes the particle against the microscope slide.

3D-Trapping:

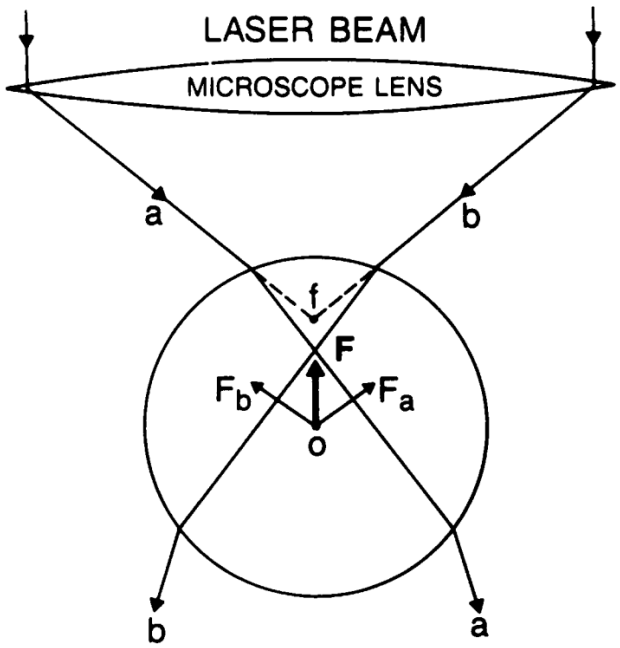

Figure 1.2: Trapping a bead in 3D requires a focused laser beam to create a 3D intensity gradient. The particle is pulled towards the focus. Two representative beams a and b, the forces $\vec{F}_a$ *and* $\vec{F}_b$ *they exert on the bead while being scattered, and the resulting gradient force* $\vec{F}$ *towards the focus f are shown. [Ashkin 1994]*

To trap a particle in three dimensions and move it along the optical axis, a force component in the direction of the propagation of light $\vec{z}$ is needed. It should create a balance of forces with the scattering force $\vec{F}_{scat}$ which pushes the particle in $\vec{z}$ direction, therefore the entity of all rays under all angles must fulfill the condition $\vec{F}_{grad} > \vec{F}_{scat}$. To create this force component, the beam must be focused such that it is strongly divergent out of the focal plane. Practically this is achieved by the objective lens of the microscope. That way an intensity profile is created along the optical axis with its maximum in the focus of the objective. In analogy to the lateral intensity profile, gradient forces along the optical axis are created that will accelerate the particle towards the focus (where the light intensity reaches its maximum). The absolute value of the axial gradient force must at least equal the absolute value of the scattering force.

Optical Trapping in the Rayleigh regime

The geometric description of optical trapping is true for the case $d \gg \lambda$. For the limit $d \ll \lambda$, optical trapping cannot be explained by geometric optics. In this case, the particle can be considered as an induced

dipole in an external electromagnetic field. It can be shown that the scattering force is $\vec{F_{scat}} \propto \langle \vec{S} \rangle$ where $\vec{S}$ is the Poynting vector. In case of $d \ll \lambda$, the gradient force can be derived by calculating the Lorentz-force on a dipole in an electromagnetic field. The Lorentz-force on a point charge q reads

$$F = q\left(\vec{E} + \frac{1}{c}\frac{d\vec{x}}{dt} \times \vec{B} \right)$$

which leads to the force that acts on a point like dipole

$$F = q\left(\vec{E}_1 - \vec{E}_2 + \frac{1}{c}\frac{d\vec{x}}{dt} \times \vec{B} \right)$$

$$F = q\left(\vec{E}_1 + \left(d\vec{x}\vec{\nabla}\right)\vec{E} - \vec{E}_1 + \frac{1}{c}\frac{d\vec{x}}{dt} \times \vec{B} \right)$$

The electric dipole moment is defined as $\vec{p} = qd\vec{x}$, yielding

$$\vec{F} = \left(\vec{p}\vec{\nabla}\right)\vec{E} + \frac{1}{c}\frac{\partial \vec{p}}{\partial t} \times \vec{B}$$

For a dielectric particle the polarizability α, the dipole moment is $\vec{p} = \alpha\vec{E}$, leading to

$$\Rightarrow \vec{F} = \alpha\left(\left(\vec{E}\vec{\nabla}\right)\vec{E} + \frac{1}{c}\frac{\partial \vec{E}}{\partial t} \times \vec{B} \right)$$

Using $\left(\vec{E}\vec{\nabla}\right)\vec{E} = \vec{\nabla}\left(\frac{1}{2}E^2\right) - \vec{E} \times \left(\vec{\nabla} \times \vec{E}\right)$ and $\vec{\nabla} \times \vec{E} = -\frac{1}{c}\frac{\partial \vec{B}}{\partial t}$ (Maxwell equation) one can derive that

$$F = \alpha\left(\frac{1}{2}\vec{\nabla}E^2 - \frac{1}{c}\frac{\partial}{\partial t}\left(\vec{E} \times \vec{B}\right) \right)$$

For a continuous power (cw) laser we know that $\frac{\partial}{\partial t}\left(\vec{E} \times \vec{B}\right) = 0$. Hence, the gradient force is proportional to the squared electrical field and thus to the intensity:

$$\Rightarrow \vec{F} = \frac{1}{2}\alpha\nabla\vec{E}^2$$

Experimental setup

The experimental setup of an optical trap has two major components: A microscope and a laser trap.

Microscope

A microscope uses several lenses to magnify small objects. The illumination of the microscope is very important for proper imaging of the object. The beam diameter should be adjusted to the numerical aperture of the objective to optimize the objective's resolution. Furthermore, the illuminated area in the object plane

should be adjusted to the size of the observed area. Köhler illumination allows adjusting both parameters using two iris apertures.

Figure 1.3 shows a schematic of the microscope setup that will be used in this experiment. A red LED lamp is used to illuminate the sample. The light is focused into the object plane by the condenser lens. The microscope objective (MO) creates a virtual image of the sample which can be imaged on the CCD camera with another lens (L3). The distance between condenser lens and object plane and the distance between object plane and MO are determined by the respective focal lengths. The distance between MO and L3 can be varied. The magnification of the microscope $M_{microscope}$ can be determined from the magnification of the microscope objective M_{MO} and the focal length f_{L3} of L3:

$$M_{microscope} = \frac{f_{L3}}{200} M_{MO} \quad (2)$$

The spatial resolution of a microscope is the minimum distance d_{min} between two distinguishable objects in an image. It can be calculated from the wavelength λ and the numerical aperture NA:

$$d_{min} = \frac{1.22 \cdot \lambda}{2 \cdot NA} \quad (3)$$

Laser trap

The most important components of an optical trap are a laser and an objective which focuses the laser beam in the object plane.

Light Source

For building an optical trap, a light source with a high power density is required. A laser fulfills this requirement. Laser stands for *Light Amplification by Stimulated Emission of Radiation*. A laser is basically made up of two mirrors (resonator) that reflect the beam from one side to the other, and an active laser medium. In the active laser medium, electrons are pumped into an excited state by an external power supply. Electrons can spontaneously return to the ground state, emitting a photon. They can also be stimulated to return to the ground state by a photon with an energy that equals the energy difference between the excited and the ground state, emitting a photon of the same wavelength during the process. As the photons are reflected between the two mirrors, a cascade of coherent photons of identical wavelength is created.

Lasers have some characteristics that make them different from other light sources:

- The waves produced by a laser have a well-defined phase relationship that is conserved over long distances (coherent radiation).
- Lasers usually emit linearly polarized light.
- Lasers usually emit monochromatic light (depending on the active laser medium).
- The intensity distribution of the beam cross-section ideally depicts a two-dimensional Gaussian distribution (TEM_{00}).

In this experiment, an infrared laser with a wavelength of 1064 nm and an output power of 500 mW is used.

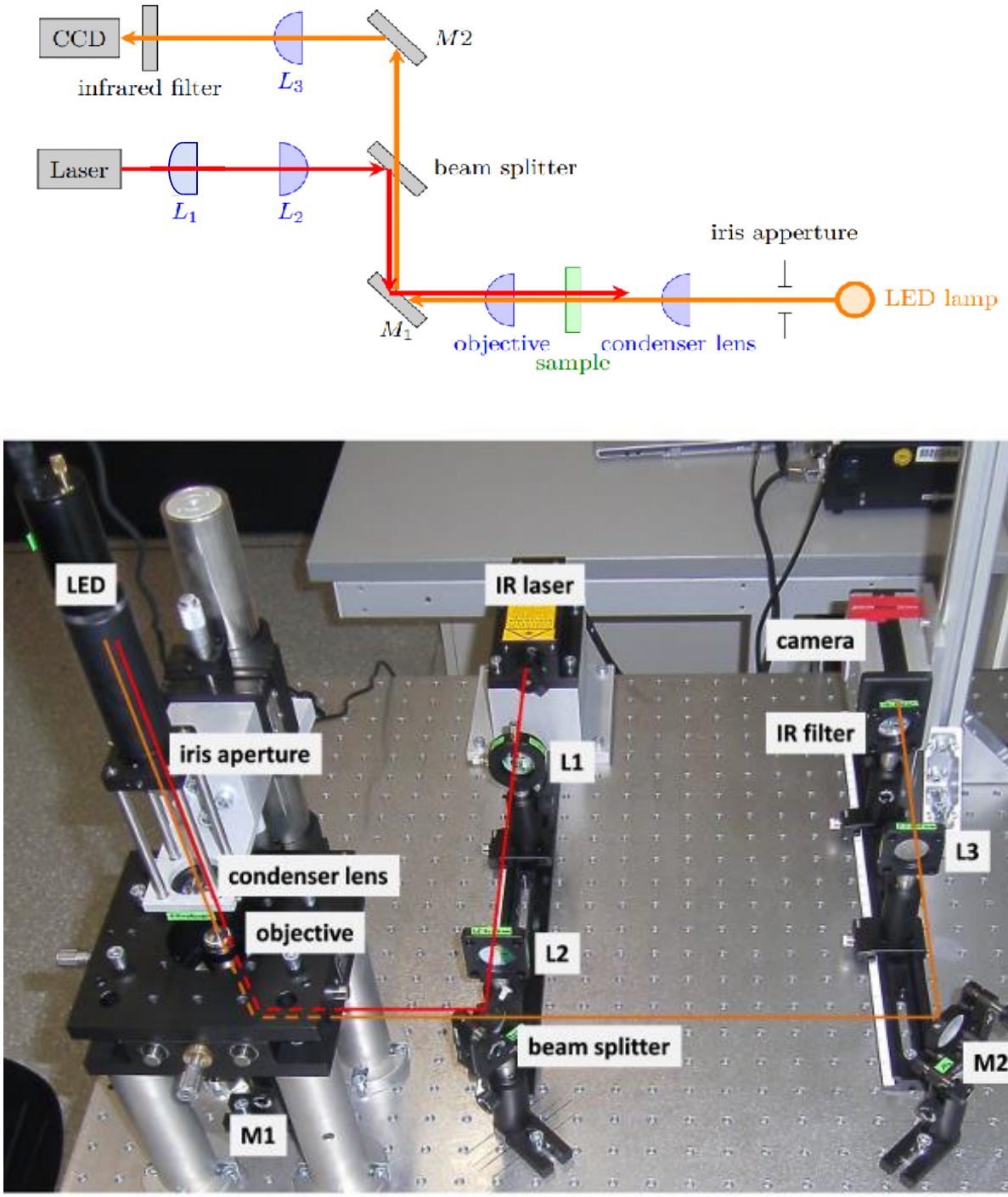

Figure 1.3: Upper figure: Schematic of the microscope and laser trap setup in this experiment. Lower figure: photograph of the setup with the light paths of microscope and laser trap.

Microscope objective

In order to trap particles, a high gradient force in $-\hat{z}$ direction is required. That means the beam must be focused sharply to create a narrow beam waist. Microscope objectives with a short focal length f and high numerical aperture NA are used to focus the trapping laser. It is important that the beam overfills the aperture of the objective and the NA is maximized, since $NA = \frac{r}{f}$ (r is the radius of the incident beam).

Optical Tweezers

Optical tweezers can be created by combining a microscope and an optical trap. Small particles can be trapped and observed simultaneously. The microscope objective can be used both for observing the sample and focusing the trapping laser, so trapped particles will always be in focus. Dichroic mirrors are used to separate the different light sources (microscope illumination and trapping laser) outside of the focal plane. A dichroic mirror separates incident the light with respect to wavelength by reflecting only a certain part of the light spectrum and transmitting the other part.

Applications of optical tweezers

There are numerous applications of optical tweezers. The simplest application is the combination of a microscope with optical tweezers to trap and observe small particles simultaneously. Optical tweezers are very useful for biological applications, as they are non-invasive and there is no direct contact to the sample which also minimizes the risk of contamination. Furthermore, forces in the range of nano- or pico-Newton can be measured with optical tweezers, allowing to investigate molecular motors on the single-molecule level.

Calibrating the trap potential

While trapped in an optical trap, a particle resides at the point of maximal light intensity. This is equivalent to residing at the minimum of the trap potential. The trap potential can be described as a harmonic potential for small deflections from the center. The trapped particle will move to the minimum of the potential and can only escape the potential well, if the equilibrium is distorted by external influences, such as thermal fluctuations. As long as the particle is not deflected beyond the maximum of the potential through external perturbation, it will return to its equilibrium position. Due to Brownian motion, a trapped bead fluctuates around its equilibrium position. By recording a video of the trapped particle's motion, the position can be determined as a function of time and the deflections from the equilibrium position can be calculated.

There are various methods for calibrating the trap potential. One method uses the **equipartition theorem** which links the energy in thermal equilibrium to the number of degrees of freedom. After determining the mean position x_{mean}, the distance $d = x - x_{mean}$ can be calculated. From the distance, the variance $< d^2 >$ can be calculated. The whole system's thermal energy can be obtained from the equipartition theorem, as each degree of freedom in a harmonic potential has the same energy $W = \frac{1}{2} k_B T$, where T is the temperature and $k_B = 1{,}38 \cdot 10^{-23} J/K$ the Boltzmann constant.

The trap stiffness (which corresponds to the spring constant k of the trap) can be obtained from the following relation:

$$\frac{1}{2} k_B T = \frac{1}{2} k < d^2 > \quad (4)$$

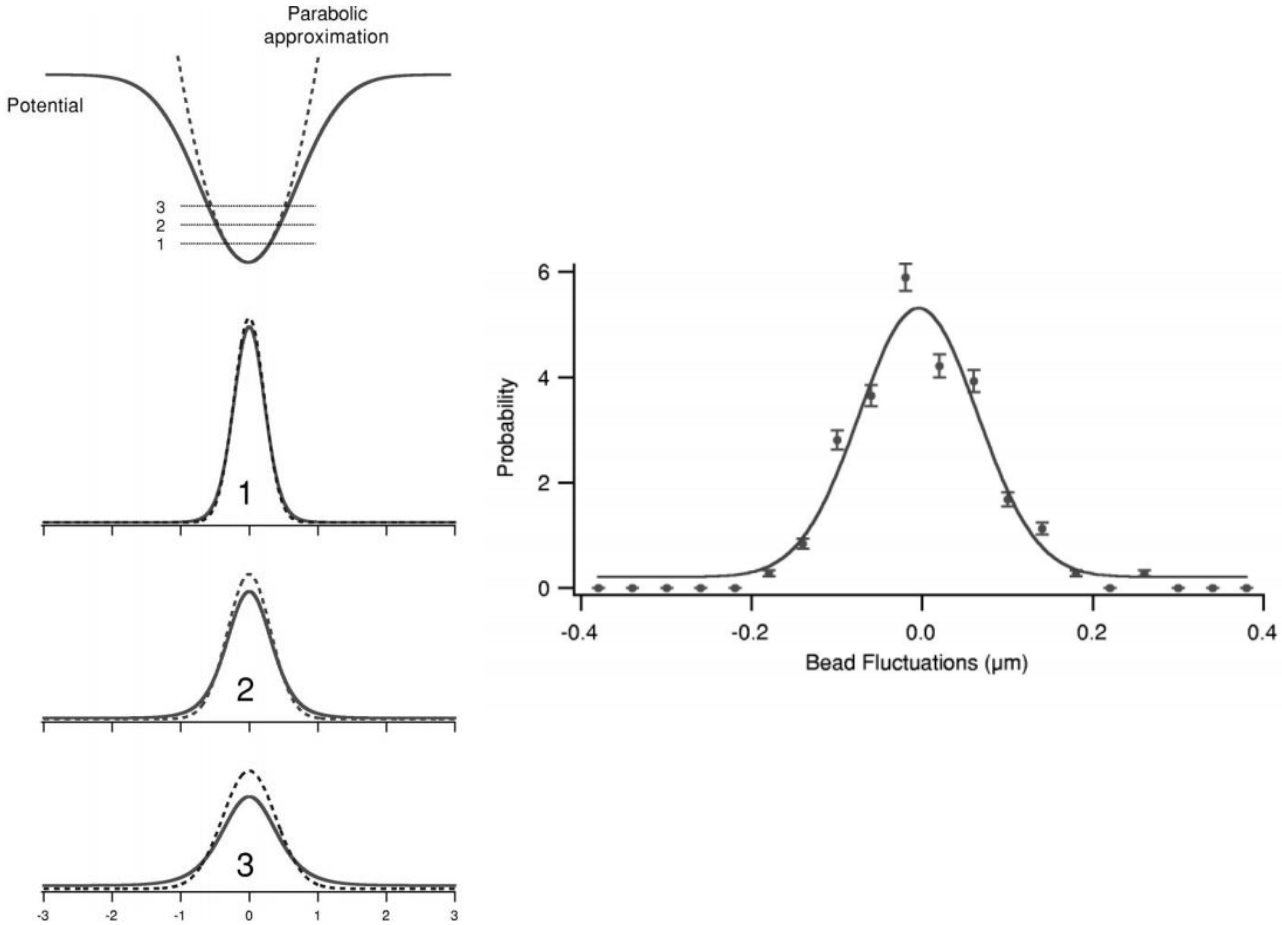

Figure 1.4: Upper left side: Schematic of a potential well seen by a trapped particle, solid line: actual potential, dashed line: parabolic approximation. Below: Position distributions for three different

temperatures. Right side: Observed distribution of particle positions for one-dimensional displacements in the trap. [Bechhoefer, Wilson, 2001]

The advantage of calibrating the trap potential as described is that only the temperature and the particle position must be measured. Other methods for calibrating the trap potential additionally require information about the medium, the local viscosity, the size of the particle and the shape of the particle. As those can only be determined with a certain error, further uncertainties in trap stiffness may arise.

Experimental procedure

As this experiment includes working with laser radiation, it is very important to familiarize with the necessary safety precautions.

Laser safety

Lasers are more dangerous than other light sources, especially for the eyes. Laser radiation can irreversibly damage the cornea and/or the retina (**risk of blindness!**). The laser used in this experiment emits at 1064 nm and is **invisible!**

Risks can be minimized by following some basic rules:

- Never directly look into the laser beam!
- Take off all reflecting objects such as watches or jewelry before starting the experiment!
- Always wear laser goggles as long as the laser is turned on!
- The laser beam must never leave the optic table!
- Never bring your eyes in beam height!
- Warn other persons in the lab before turning on the laser!
- Turn on the laser warning lamp!
- Do not work alone if it can be avoided!

Equipment

- Infrared laser (λ 1064 nm, 500 mW, CNI-1064-TTL-AC/LED, Laser2000)
- Red LED (λ 630 nm)
- condenser
- sample stage
- microscope objective (100x, CFI E Planachromat, N.A. 1.25, working distance 0.23 mm, Nikon)

- two mirrors (M_1 , M_2) for controlling the light path
- dichroic mirror (transmits visible light, reflects infrared light)
- two lenses for expanding the laser beam (L_1 , L_2 ; focal lengths: 40 mm, 150 mm)
- a lens for imaging the sample on the camera (L_3)
- CCD camera
- infrared filter for protecting the camera
- tools that help aligning the setup: indicator card with graticule, black metal tubes with screw thread that fits into the objective holder

Tasks and advises

1. Planning the experimental setup:

- How much space is available on the optic table?
- Where do you want to place the components?
- Which distances can be chosen freely? Which distances have to be kept at a fixed value?

2. Preparing the sample:

- mix 1 µl beads (polystyrene beads, 2 µm, Kisker) with 99 µl Milli-Q H_2O, mix thoroughly and dilute the beads solution 1:100 by mixing 1 µl of it with 99 µl Milli-Q H_2O (final dilution of beads is 1:1000)
- pipet 50 µl in the middle of a 24 mm x 24 mm coverslip
- put a drop of silicon grease in each corner of the cover slip (as spacers)
- attach a microscope slide to the cover slip (it should stick to the spacers), gently press on the spacers (you can use a pencil for that) until the solution has filled the whole area under the cover slip

3. Setting up the microscope:

The microscope is already set up for this experiment. Do not move mirror M_1 (the one under the objective), as it affects both the microscope and the laser trap. If you see a black rim in the field of view, you can adjust the height of the camera or turn the screws of mirror M_2 to center the image on the camera chip.

4. Inserting the laser trap:

Always remember that the laser light is invisible and can damage your eyes! It can also damage the CCD camera, so make sure that you have placed the infrared filter in front of the camera! Take off all reflective elements from your hands and arms, such as watches or jewelry! Ask your supervisor for laser goggles! Turn on the laser warning lamp and warn other persons in the lab! There is a laser indicator card which can make the laser visible if it is held into the light path of the laser. It quickly bleaches once it is hit by the laser and can be recharged by illuminating it with visible light (e.g. a torch). Also make sure that you block the light path after the beam splitter, as it does not reflect 100 % of the laser light and some will be transmitted and may leave the optical table.

- Turn on the laser and align it, so that the beam propagates parallel to the optic rails (not necessary for this experiment as this is already done). Use the indicator card to make the laser visible. Estimate the beam diameter.
- Add the lenses L_1 and L_2 to the light path. They form a Keplerian telescope to expand the laser beam. Where should they be placed and at which distance?
- Add lens L_1 to the light path. The flat side of a lens should always face towards the collimated beam to minimize aberrations. You can fix the IR indicator card with the graticule in front of the lens. Set the laser to very low power if you do so as the indicator card bleaches quickly. Adjust the height of the lens in a way that it is hit by the beam exactly in the center. Remove the indicator card and use the screws of L_1 to adjust it in a way that the (divergent) laser beam is still parallel to the optical rail.
- Add lens L_2 to the light path. The only degrees of freedom are the height of the lens and the position on the rail. Adjust L_2 such that the laser beam is collimated after L_2 and still propagating parallel to the table.
- Insert the beam splitter into the light path. It is important to adjust it properly because the field of view in the microscope can easily be cut if the light path is not centered on the beam splitter and on mirror M_2. You can use the indicator card to check the position of the laser on the beam splitter. Remove the objective and place a black tube with the indicator card with the graticule in its place. Align the beam splitter such that the laser is centered when it leaves the objective. Check both position and angle by using black tubes of different length. You have aligned everything correctly if the laser is always centered, no matter if you use a short or a long black tube.

- Once the laser trap is properly aligned, try to find out its position. You can do so by preparing a sample with a high density of beads and try to trap a bead. How will you recognize a trapped bead? If this does not work and you have verified that the laser beam is properly aligned, you can also remove the infrared filter and image the scattered laser light on the camera to find the trap position. Laser light that has been scattered by the sample will not destroy the camera. Anyway, make sure that you cannot detect laser light after the beam splitter by using the indicator card before you remove the infrared filter.
- If you can see the laser, but still cannot trap a bead, the trapping plane might be different from the focus plane. This can happen if the laser beam is not completely parallel before it enters the microscope objective. You can carefully adjust the second lens of the Keplerian telescope (L_2) to move the trapping plane into the focal plane.

5. Trapping a particle

- If you have inserted the laser trap successfully, you can observe how small particles are drawn into the trap. You can move the trap relatively to the sample by moving the sample stage.
- To test if a particle is really trapped in 2D, move the stage in x- and y-direction. The particle should stay at the same position of the screen.
- To test if the particle is also trapped in 3D, move the stage in z-direction. The trapped particle should stay focused, whereas all other particles should get blurred as they are moved out of focus. Move the stage downwards (to move the bead upwards within the sample), as beads often stick to the surface of the cover slip. If you move the bead downwards in the sample and hit the cover slip, the bead might get stuck there and you have to trap another bead.
- Make snapshots to show that the bead is trapped. Figure 2.1 shows a possibility to prove 3D trapping with snapshots.
- If you cannot trap a bead, it might be stuck to the cover slide. Beads will move by Brownian motion if they are not stuck to the cover slide.

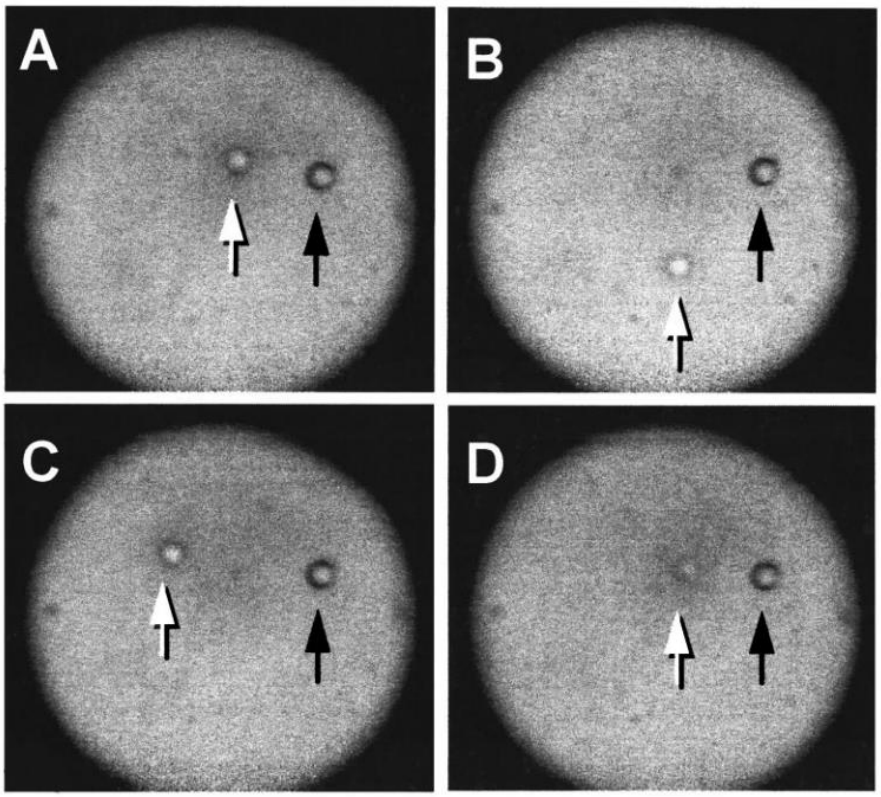

Figure 2.1: A bead is trapped in the laser trap (solid arrow) and a second bead is stuck to the microscope slide (hollow arrow). A shows the starting position, B and C show translations in the x and y plane and D shows translation in the z plane [Smith et al, 1998].

6. Calibrating the trap potential

- To calibrate the trap potential, you need a fairly dilute bead solution to make sure that there is only one bead in the trap.
- Trap a particle and make sure that there are no other particles close by, as they might fall into the trap if they move closer. You can test how many beads you have trapped by blocking the laser beam. Then you will see how many beads diffuse away.
- Record a video of the bead's movements in the trap (at least 300 frames).
- Analyze the recorded video to extract the bead positions (program is found on the computer, ask your supervisor). Calculate the mean square displacement.
- You need the pixel/µm ratio to evaluate your data. Take pictures of a calibration slide with a µm scale. Measure the pixel/µm ratio in both x and y direction.

7. Trapping swimming *Bacillus subtilis*

Swimming is a common strategy for motility, driven by rotating flagella, which extend from the cell body. Each flagellum consists of a long (~10µm), thin (~20 nm), helical filament, turned like a screw by a rotary motor at its base.

We use wild type *Bacillus subtilis* bacteria that use flagella-driven motility to demonstrate that also actively moving particles can be tracked by laser traps and to see how they behave while being trapped.

Motile *B. subtilis* will be prepared for you in an appropiate media.

Evaluation

Trapping in 2D and 3D

Document that you can trap a particle in 2D (x,y plane) and in 3D (z plane). You can make snapshots of a particle that is moved within the sample to demonstrate the trapping.

Trap stiffness

Further, calculate the trap stiffness using the two methods explained before:

The equipartition theorem:

Calculate the variance from the distances that have been determined in the analysis of the video:

$$< d^2 > = \frac{\Sigma d^2}{N} \quad (9)$$

N is the number of values. The spring constant can then be determined from the equipartition theorem (formula 4).

Further calculations:

Calculate the restoring force on a particle in a distance of $d_1 = 100\,nm$ ($d_2 = 200\,nm$) from the trap center. What happens, if *d* is very large?

Literatur

Keir C. Neuman, Steven M. Block: Optical Trapping. Review of Scientific instruments, 2787-2809, 2004

A. Ashkin: Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime. Biopys. J., 569-582, 1992

John Bechhoefer, Scott Wilson: Faster, cheaper, safer optical tweezers for the undergraduate laboratory. Am. J. Phys., 393-400, 2001

Stephen P. Smith, Sameer R. Bhalotra, Anne L. Brody, Benjamin L. Brown, Edward K. Boyda, Mara Prentiss: Inexpensive optical tweezers for undergraduate laboratories. , 26-35, 1998

The following publication will be relevant for the oral exam:
Min TL, Mears PJ, Chubiz LM, Rao CV, Golding I, Chemla YR. High-resolution, long-term characterization of bacterial motility using optical tweezers. Nature methods. 2009; 6(11):831-835. doi:10.1038/nmeth.1380