

Optical Tweezers Experiment

Optical Trapping

Advanced Physics Laboratory

Developed By: Jimmy Shen (August 9, 2010)

Revised By: Hasmita Singh, Shannon O'Keefe, Laura Poloni and Maryam Badakhshi (December 2010)

1. Introduction

Optical trapping began as a proposed method for atomic trapping and laser-cooling A. Ashkin. "Acceleration and Trapping of Particles by Radiation Pressure". Phys. Rev. Lett, vol. 24, no. 4, pp. 156-159, 1970. A. Ashkin et al. "Observation of a single-beam gradient force optical trap for dielectric particles". Optics Letters, vol.11, no.5, pp. 288-290, 1986., but evolved into a tremendously powerful tool in biological physics. Applications for optical traps range from manipulating single bacterial cells, to measuring the forces generated by singular motor proteins A. Ashkin et al. "Observation of a single-beam gradient force optical trap for dielectric particles". Optics Letters, vol.11, no.5, pp. 288-290, 1986.. Most experiments involving optical traps will require the determination of trap stiffness, which is the effective spring constant

experienced by a trapped bead displaced from equilibrium. Since the factors involved in determining the stiffness of optical traps are often complicated and theories for calculation are not entirely accurate, the stiffness should be measured empirically each time a parameter is altered. The goal of this lab is to measure the stiffness of the optical trap through multiple methods.

2. Theory

2.1 – Background on Optical Tweezers

The optical tweezers apparatus is used to hold microscopic particles stable during examination. This is done through the use of a highly focused laser beam. The photons comprising the beam each carry momentum and therefore exert force. By aligning the laser beam, the photons are focused and the resulting electric field gradient is strong enough to hold a dielectric particle or bead in position.

Optical traps, originally designed to trap atoms and study laser cooling, now have ample use in the biological sciences. Any biological particle to be studied can be attached to a small dielectric bead [3]. This bead will become attracted to the photon generated electric field and remain in this position for observation. This process is used to investigate, examine, and compare the various intrinsic aspects of small particles, such as cell motility and optical intensity. The most common use of optical trapping involves the study of cell stiffness. Once the light force exerted upon the trapped bead and the bead displacement is measured, this information can be used to determine the force exerted by the trapped cell.

2.2 – Optical Trapping Theory

2.2.1 – Forces Affecting Trapped Particles

Each photon in a laser beam carries momentum and can exert force. When the beam of the laser is focused, through a microscopic objective, each photon will exert force in a single direction. This combined focus, which provides enough force to trap and move small particles, is manipulated and applied using the optical tweezers apparatus. There are two types of forces, the gradient and scattering force, which exert themselves on a particle and trap it in the path of the laser beam [3].

The gradient force is a result of the electric field gradient present when a laser beam is focused and photons are aligned. This electric field is strongest at the narrowest part of a focused beam, the beam waist. The dielectric particles to be studied in the optical trap become attracted to the electric field gradient of the beam waist.

The second force affecting dielectric particle movement, the scattering force, is due to the change in momentum experienced by photons traveling in the direction of beam propagation. This force slightly displaces the trapped particle downstream from its original position at the center of the beam waist.

As a result of the combination of both the scattering and gradient force, a particle will be trapped in the optical tweezers apparatus slightly downstream of the laser beam waist [3]. The lateral displacement from the center of the beam is dependent on the strength of the scattering force and the stiffness of the optical trap. The optical trap stiffness can be thought of as the

effective spring constant, k , of Hooke's Law [4]. Trapping only occurs when the gradient force is stronger than the scattering force.

2.2.2 – Modeling Optical Trapping Forces

Predictions on the affect optical forces have on trapped beads are directly dependent on the diameter of the bead relative to the wavelength of the incident laser. The classic ray optics model is sufficient to explain how forces trap and displace bead particles only when the radius of the particle is a great deal larger than the laser wavelength. Once the diameter of a bead to be trapped is much smaller than the incident light wavelength, then the particle can be treated as an electric dipole and the electric dipole approximation can then be used to predict force interactions.

The Ray Optics Model

When the diameter of the trapped particle is far greater than the wavelength of the incident laser, the classic ray optics model of ray refraction can be used to describe the affect scattering forces have on trapped particles and the resulting gradient forces which withstand the scattering effects and keep the particle trapped just downstream of the beam center.

As emitted photons travel the path of the laser, they carry momentum. The momentum associated with each photon changes as it interacts with the trapped dielectric bead. This is because light particles colliding with the glass bead are refracted as they rebound off of the particle. This refraction results in a change of photon direction and therefore an alteration in the photon momentum. This change in momentum will cause a force to be exerted on the photon and, as a result of Newton's Third Law, a force of equal magnitude will also be exerted upon the trapped bead.

The laser beam used to operate the optical tweezers apparatus maintains a Gaussian profile. This means that the affect each photon has on the trapped bead is dependent upon its distance from the bead. As a result, the photons near the beam waist and trapped bead, which exert an attracting force and pull the bead towards the center of the beam, will have a more intense affect than those photons further from the trapped bead attempting to displace the particle from the beam waist [5]. When these distant scattering forces attempt to move the trapped particle to the left or right of the beam center, the high intensity attractive forces overcome this displacement and maintain bead position, as seen in Figure 1 [4]. These high intensity forces restoring the radial position of trapped particles are referred to as the gradient force [4].

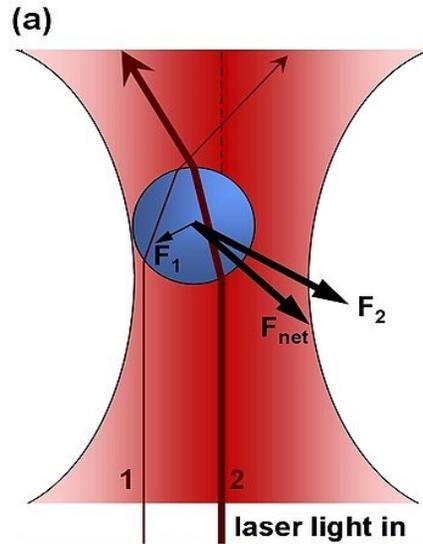


Figure 1: Any attempts to radially displace the trapped bead from the beam waist are overcome by the higher intensity attractive forces closer to the bead. [4]

Now, once the gradient forces have returned the bead position to the center of the trap, then all refracted photon rays will be symmetric about the bead, leading to a net momentum of zero. Without a change in momentum, there will be no forces pulling the bead from the center of the trap, and so it will remain in this lateral position. Thus, without a lateral net force, only the scattering forces in the axial direction of the beam path will be able to influence bead movement. It is these scattering forces which cause the radially centered bead to undergo lateral displacement and be trapped slightly downstream of the beam waist, as seen in figure 2 [5].

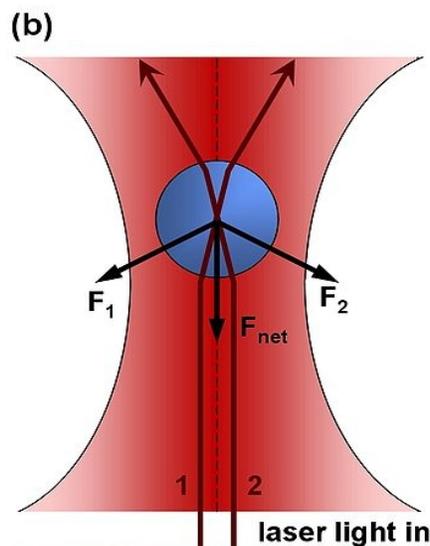


Figure 2: Once the trapped particle is centered radially, scattering forces in the lateral direction cause the bead to be displaced slightly downstream of the beam waist. [4]

The Electric Dipole Model

Once the radius of the particle to be trapped is sufficiently less than the wavelength of the incident laser beams, then the electrical dipole model can be used to approximate the photon and particle interactions. Because the trapped bead is so much smaller than the laser wavelength, it can be thought of as a point dipole in the photon electromagnetic field.

The force acting on a single point charge placed in a magnetic field is called a Lorentz force [6] and can be mathematically described through the equation:

$$\mathbf{F} = (\mathbf{p} \cdot \nabla) \mathbf{E} + \frac{d\mathbf{p}}{dt} \times \mathbf{B} \quad (1)$$

where:

\mathbf{F} is the force [N]

\mathbf{E} is the electric field [V/m]

\mathbf{B} is the magnetic field [T]

\mathbf{p} is the polarization of the dipole, $\mathbf{p} = q\mathbf{d}$

q is the particle electric charge [C]

and

\mathbf{d} is the distance between charges [m]

Now, because we assume that the trapped point charge is linear, we can eliminate the dipole polarization from equation (1) through the use of the polarizability, α , where $\mathbf{p} = \alpha \mathbf{E}$. Equation (1) can be then be rearranged into the form of:

$$\mathbf{F} = \alpha \left[(\mathbf{E} \cdot \nabla) \mathbf{E} + \frac{d\mathbf{E}}{dt} \times \mathbf{B} \right] \quad (2)$$

which is simplified to:

$$\mathbf{F} = \alpha \left[\frac{1}{2} \nabla E^2 + \frac{d}{dt} (\mathbf{E} \times \mathbf{B}) \right] \quad (3)$$

The last term on the right hand side of equation (3) is the time derivative of the Poynting vector, which represents the power flux through an electromagnetic field. During the optical tweezers experiment, the sampling frequencies are much shorter than the frequency of the laser beam, $\sim 10^{14}$ Hz, and so the power of the laser will be constant [7]. Constant power will lead to a zero value of the time derivative of the Poynting vector and so this term can be removed from the equation. The force acting on the electric dipole can then be represented with the equation:

$$\mathbf{F} = \frac{1}{2} \alpha \nabla E^2 \quad (4)$$

Because the E^2 term in equation (4) represents the electromagnetic intensity of the photons, the strongest light forces acting on the particle will be those with the highest intensity. As the peak photon intensity occurs at the center of the beam waist, the forces acting on the bead to be studied will draw it to this position. These forces are then of gradient type, as they attract particles to the center of the beam.

As in the ray optics approximation, once the gradient forces have radially centered the beam, only axial displacement due to scattering forces can now occur. These scattering forces, represented by the Rayleigh approximation:

$$\mathbf{F}_{\text{scat}}(\mathbf{r}) = \frac{k^4 \alpha^2}{6\pi c n^3 \epsilon_0^2} I(\mathbf{r}) \hat{z} \quad (5)$$

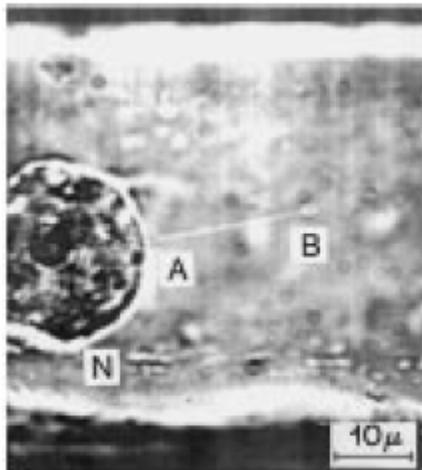
once again displace the trapped bead slightly downstream of the beam waist [6].

2.3 – Applications of Optical Tweezers

Although the optical tweezers trap was initially designed as an atom trap, it has found a wide range of applications in physics, chemistry and biology. The first application of optical trapping in biology was on the trapping of viruses and bacteria [L1]. With manipulation of the laser wavelength being used, it was eventually possible to trap bacteria cells without damaging them. From here, researchers proceeded to trap a variety of cells, including pigmented red blood cells, green algae, diatoms, amoebas, and other protozoans [L2]. Further work used scallion cells to create an “artificial cytoplasmic filament” by using the trap to pull a filament from the surface of the nucleus of the cell into the central vacuole of the cell, as shown in Fig. 3 [L3]. Optical tweezers have more recently been used to study the viscoelastic behaviour of single strands of polymeric molecules, such as DNA [L4].

Optical trapping has also been widely used in the fields of physics and chemistry. Optically induced torques and rotations of micromachined $\mu\text{-m}$ sized anisotropic particles held in a tweezers trap have been observed [L5]. It has also been shown that small metallic Rayleigh particles have polarizabilities larger than dielectric particles and can be trapped in tweezers [L6]. In studies pertaining to colloidal science, direct measurements using tweezers showed that an attractive force can exist between like-charged particles in a colloidal suspension near a surface, contrary to theory. Based on this work, metastable colloidal crystals were created [L7].

Further references regarding applications of optical trapping can be found in the *Papers* section of Appendix I. In particular, Ashkin provides a thorough history of optical trapping from 1970-2000 in *History of Optical Trapping and Manipulation of Small-Neutral Particle, Atoms, and Molecules*. Another useful resource is the guide to the literature on optical tweezers provided in *Resource Letter: LBOT-1: Laser-based optical tweezers*.



a scallion cell.
the nucleus
AB [10]

3.1 – Important Safety Rules

- 1) The optical fibres used to transport the output of the laser source to the rest of the apparatus are extremely delicate. Do not remove the protective plastic enclosure surrounding these fibres.

- 2) After exiting the FibrePort Collimator, the laser beam is concentrated and extremely dangerous. To prevent retinal damage, the laser is encased in an opaque enclosure. **If access into the enclosure is required, contact the lab TA's for assistance. Under no circumstances should students remove this safety enclosure with express permission and assistance from a lab coordinator.**

3.2 General Description of the Optical Trapping Apparatus

The optical trapping apparatus and a schematic of the components of the apparatus are shown in Fig. 4a and 4b, respectively.

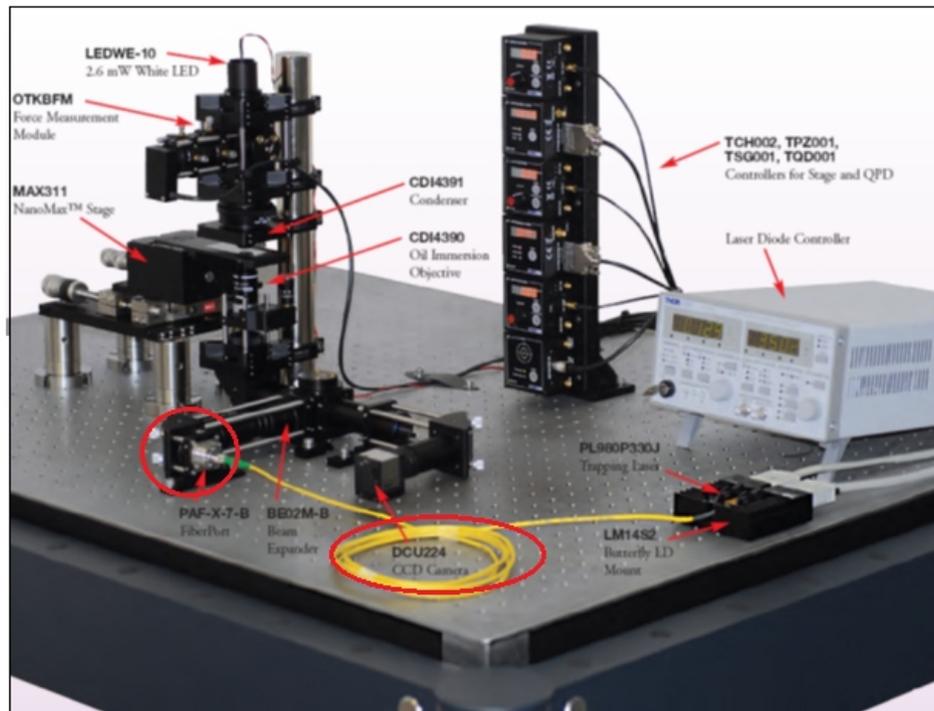


Figure 4a: Optical Tweezer Apparatus [8]

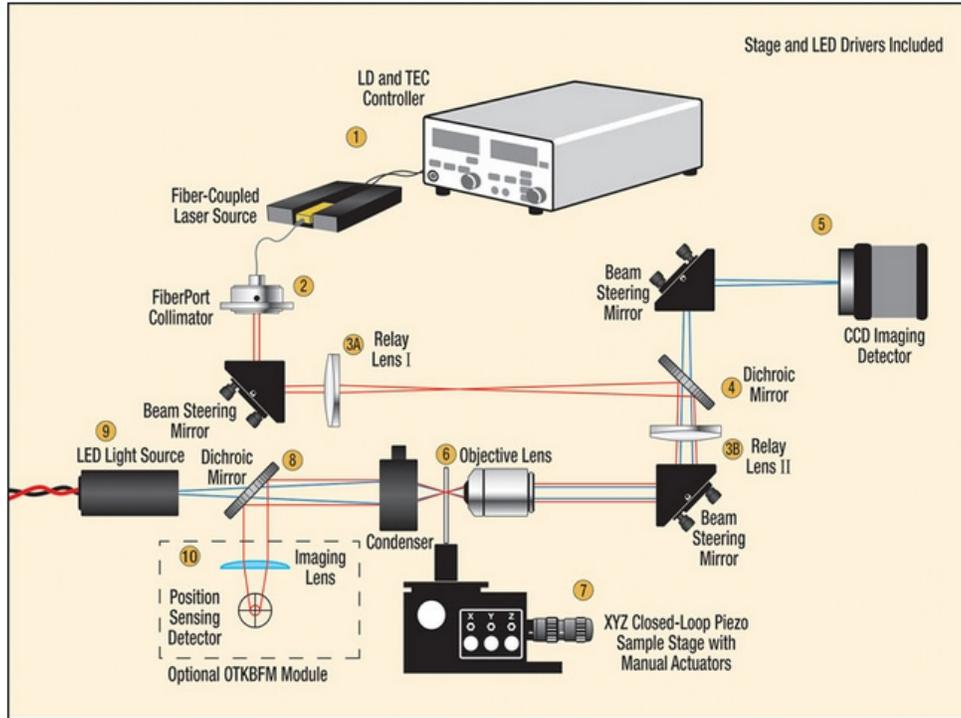


Figure 4b: Schematic of Optical Tweezer Apparatus [8]

Table 1 provides a brief description of each major component comprising the optical trapping system. Detailed instructions regarding operation of these components can be found in Section 6, the experimental procedure section of this laboratory report. Further information can be found in the Thorlabs manuals for the Optical Trap Apparatus and the Laser Diode Controller, links to which are provided in Appendix I.

Table 1: General Description of Optical Trapping Apparatus

Component Name	Object Number	Description
Laser Diode Controller	1	-the 330mW, 980nm laser is generated from this source - the temperature of the diode is monitored and controlled through an integrated TEC (thermoelectric cooling unit) element
FiberPort Collimator	2	- aligns photon rays in a single unified direction. After exiting the collimator, the laser beam is focused and extremely dangerous. <i>Do not remove covering without supervision.</i>
Relay Lenses and Beam Steering Mirrors	3A,3B,4,8	-relay lenses and dichroic mirrors are required to direct the collimated laser beam into the back aperture of the microscope focusing objective and imaging detector of the CCD camera
CCD Imaging Detector	5	-the charge coupled device camera is used to image the sample -images can be viewed through the <i>uc480viewer</i> software

Piezo Sample Stage	7	-consists of a microscope slide holder mounted to a 3-axis translation stage -stage is capable of movement in the x,y, and z directions and contains both coarse and fine knobs to ensure accurate sample positioning
Position Sensing Detector	10	-the signal generated by the Quadrant Position Detector is sensitive to the relative displacement of a trapped particle from the laser beam axis. As a result, the QPD is used to measure the position, stiffness, and force of the optical trap.

4. Pre-lab

1. Using the schematic provided, describe the path of the laser leading up to the sample.

5. Laser Safety

5.1 – Laser Characteristics



Figure 5

The laser diode used by the optical tweezers system has a maximum power output of 330mW emits light at a wavelength of 980nm, which is in the infrared range. It employs a collimated beam and is classified as a Class 3B laser. A Class 3B laser is considered hazardous under direct and specular reflections, and direct exposure to the eye is considered a hazard. Through the inclusion of safety interlocks to enclose the open beam region, the system has been re-classified as a Class 1 working environment.

5.2 – Hazards

1. Stray beams: Diffuse reflections can occur if objects are placed in the path of the beam, which is especially dangerous since the position of the invisible light is unknown. Do not place your hands or any object in the path of the laser at any time to avoid such reflections.
2. Beam Alignment: This is an extremely dangerous procedure since it involves working with an open beam. Please contact the TA if beam alignment must be performed. Please do not tamper with the safety cover enclosing the open beam region [18].
3. Biological Hazards: Eye injuries could occur if direct exposure to the focused laser beam occurs without the laser safety glasses. The laser used in this set-up falls within the Retinal Hazard Region, and can result in retinal burns, scars, overheating, blind spots or even permanent loss of central or peripheral vision. Due to the invisibility of the light, you may not be aware of the damage until after it occurs.

The open-beam region has been enclosed for your safety. Please follow the safety procedures outlined below.

[Note for the TA: The student may be inclined to take their glasses off when viewing the sample through the camera or looking at the sample on the holder. Please reinforce that they **MUST** have their glasses on AT ALL TIMES.]

5.3 – Hazards Control

Before beginning the experiment:

1. Place the “Laser Work in Progress” warning sign on the door.
2. Obtain the interlock key.

3. See that all unauthorized people leave the room.
4. Close the room door. **If someone unexpectedly enters the room, shut off the laser immediately.**
5. Remove any wristwatches or reflective jewellery.
6. Wear laser safety glasses AT ALL TIMES. Check the wavelength and the optical density to ensure these are appropriate.
7. Remove any unnecessary items from the apparatus table.
8. Remember to turn off the laser when changing samples.
9. In case of emergency, contact your TA/Instructor or UofT Campus Police: **416-978-2222**.
If an eye injury occurs, see an ophthalmologist.

6. Experimental Procedure

Optical traps operate best when the trapping laser wavelength and the diameter of the trapped bead are comparable. A. Ashkin et al. "Observation of a single-beam gradient force optical trap for dielectric particles". Optics Letters, vol.11, no.5, pp. 288-290, 1986.. In this regime, neither of the two approximations are valid and theories regarding trapping in this regime are very complex [16]. Thus, the stiffness of traps within this regime cannot be accurately predicted. Given the fact that different beads and power of the laser will often be required during experiments, empirical methods of determining the stiffness of an optical trap is crucial. The calibration experiments outlined in this lab will take you through a variety of methods to determine the trap stiffness. The numerous sub-experiments outlined will demonstrate a variety of methods to determine the trap stiffness.

6.1 – Equipartition through CCD Camera

The Equipartition theorem will be employed for a trapped bead undergoing thermal fluctuations in order to determine trap stiffness.

For each degree of freedom in the thermal motion of the particle, there will be $\frac{1}{2}k_B T$ of thermal energy, where k_B is the Boltzmann constant and T is the temperature in Kelvin. When the bead is not trapped, the thermal energy is converted into kinetic energy and the bead will undergo random walk in the medium. If the bead is in a harmonic trap, the thermal energies become potential energies manifested in a small displacement. Over large sample sizes the fluctuations will give the result:

$$(6)$$

where $\langle x^2 \rangle$ is the variance of the x displacements. In this experiment, you will be recording *.avi* videos from the CCD camera (Instructions on recording *.avi* videos from CCD camera which will be converted to position data.

It is important to note that precision of the positional data is critical for this method. Since the fluctuations in position are squared, it is highly sensitive to noise.

6.2 – Experimental Operating Procedure

Put the Immersion oil on the Nikon oil Immersion objective lens.

[Note for the TA: Sufficient oil must be present on the objective. If the sample slide is loaded and unloaded, put additional oil.]

6.2.1 – Sample Preparation

[Note for the TA: to prepare the dilution, use a stock bead solution with polystyrene beads that are 1-2 μ m in diameter and distilled water. Stock solutions of polystyrene beads with varying diameters are available for use.]

Figure 6: a) Components of the sample slide: microscope slide, two strips of double-sided tape, and a cover slip. [18]. B) Student trimming off the excess tape from the sample slide with a razor blade.

1. The following materials will be required: microscope slide, cover slip, double-sided tape, P200 micro-pipette, pipette tips, kim wipes, razor blade, diluted bead solution (beads – 1:100 dilution), and vacuum grease.
2. Clean the microscope slide with ethanol and ensure that it is kept clean. Dirt or oil can affect the measurements as it may cause scattering of the beam [18].
3. Place two pieces of double-sided tape separated by about 3-4mm across the centre of the slide. Place another layer of double-sided tape on the first layer. This creates a channel with sufficient height that the beads can move through. Press on the tape to eliminate air bubbles near the channel to ensure that the liquid will not escape the channel.
4. Place the cover slip on top of the channel and the double-sided tape strips which will hold it in place. Press on the tape contact to ensure that the liquid cannot escape from the channel, and carefully trim off the excess tape from the ends using a razor blade.
5. Adding the sample:
 - a. Shake the sample solution thoroughly to evenly distribute the beads.
 - b. Using the P200 micro-pipette and the appropriate plastic tip, obtain 15-20 μ L of the bead solution and insert it into the channel from one end just outside of the cover slip. The bead solution will fill the channel through capillary action and gravity.
6. Seal the open ends of the channel using vacuum grease and carefully recap the syringe.
7. Discard the plastic tip.
8. To load the sample, use the translating breadboard to position the sample holder between the objective and condenser. Place the slide onto the sample slide holder with the cover slip facing down. Carefully press down the glass slide firmly into the holder.

Figure 7: Sample stage onto which the microscope slide containing the sample is placed.

Note:

- The beads in the channel should be somewhat scarce, so as to allow isolation and trapping.
- Once the sample is created and sealed appropriately, it may be used for upto 24 hours.

- It is recommended that you use two bead concentrations during the course of the experiment – a higher concentration (eg. $10^{-5}\%$) to practice trapping and a lower concentration (eg. $10^{-8}\%$) to perform actual measurements.
- The entire stage can be translated along the direction perpendicular to the beam path to facilitate loading and unloading of the sample.

WARNING: Please turn OFF the laser when changing samples.

6.2.2 – Powering the Trapping Laser

Please contact your TA/Instructor before turning on the laser to ensure that all the safety precautions have been accounted for.

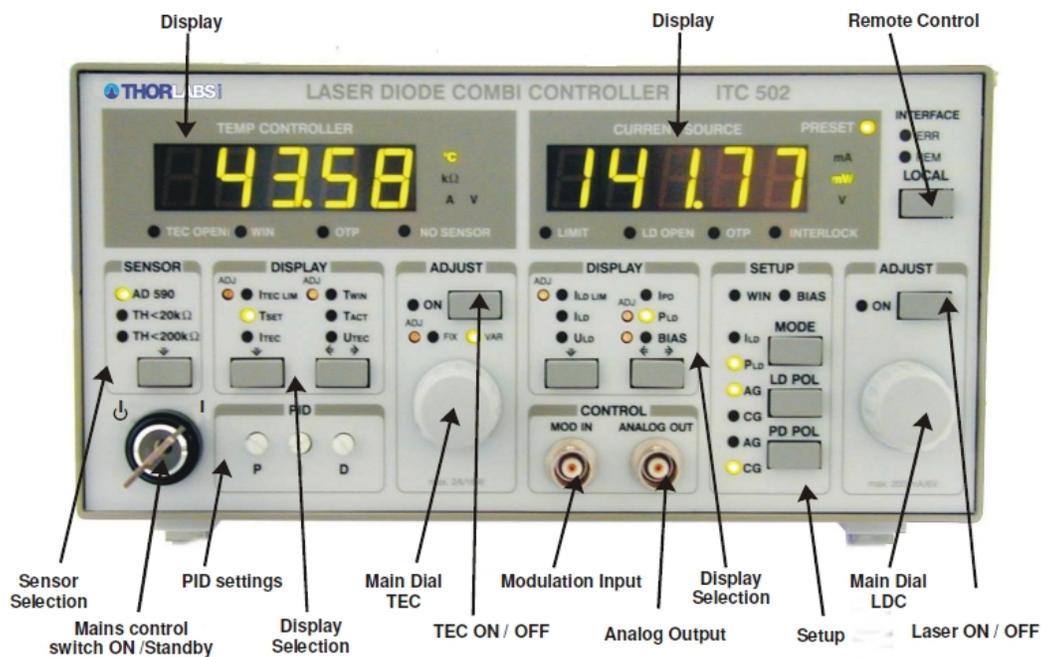


Figure 8: ITC 510 Laser Diode Combi Controller. Thorlabs. [17].

Insert the laser key into the Mains Control Switch on the ITC 510 Laser Diode Combi Controller and turn it to the ON position.

Temperature Controller

Using the display selection keys:

1. Select $I_{TEC\ LIM}$ and change the limit value of the TEC current to 1.4 A by using the screwdriver-potentiometer (ADJ) besides the LED $I_{TEC\ LIM}$. [This part should be done by the TA].
2. Ensure that $TH < 20k\Omega$ is selected under the Sensor Selection keys.
3. Check that T_{ACT} is around $10.5k\Omega$. This should already be set.
4. Select T_{SET} and set the value to $10k\Omega$ using the Main Dial TEC. The TEC, when set properly and 'enabled', will control the temperature of the laser diode, which prevents temperature-related power fluctuations. It should not need to be adjusted [18].

5. Power on the TEC.

Current Source

The laser diode is powered through the Laser Diode Controller (LDC) upon setting and enabling the TEC.

1. Select $I_{LD\ LIM}$ and set it to 350mA by using the screwdriver-potentiometer (ADJ) beside the $I_{LD\ LIM}$.
2. Select I_{LD} and set it to 0mA using the Main Dial LDC.
3. Power on the Laser.
4. Turn the Main Dial LDC to adjust the power of the trapping laser (this adjusts the I_{LD}).

Note for TA: The maximum current limit ($I_{LD\ LIM}$) for the laser is 720 mA [19]. If I_{LD} goes beyond the maximum set current ($I_{LD\ LIM}$), there will be a ‘beep’ and the current will not increase beyond the $I_{LD\ LIM}$. The LIMIT light will light up if the current limit is reached.

[Pg. 40 of the ITC manual.]

6.2.3 – Camera Settings

The CCD camera is controlled through the uc480viewer software. Before beginning, turn on the LED light to illuminate the sample by powering the LED (connect wire from the LED on top of the apparatus to the wire labelled “LED Power”).

1. Start → All Programs → Thorlabs DCx Camera → uc480viewer
2. Press the *Open Camera* button to display the live image.
3. Press the *Camera properties* button to configure the camera:
 - a. Format tab: debayering method *Direct raw bayer (Y8)* and the Hardware configuration 3x3 should be selected
 - b. Once the bead is trapped, you should crop the image by accessing the *Size* tab and changing both *Width* and *Height* to 120 pixels and adjusting *Left* and *Top* accordingly to capture the trapped bead in the field of view.
 - c. You can control the Exposure time through the *Camera* tab should the need arise. Exposure time should be set at approximately 15ms.
 - d. To record a video using the CCD camera, go to *File* → *Record video sequence* and press the *Create* button. Set the file path, change the *JPEG Quality* to 100 and *Max Frames* to 500 then press *Record*. The data will be saved in .avi format.

Note: no calibration is necessary.

6.2.4 – Viewing the Beads

1. Set I_{LD} to 100mA (laser diode current).
2. The cover glass should be in contact with the immersion oil. Use small adjustments to move the Nikon objective upwards until you see the beads.
3. Use the x and y micrometers to view the channel and adjust the brightness of the camera as necessary.

Note for TA:

- Turning the z-position knob will raise the objective closer to the sample, which is necessary for you to be able to view the beads.
- Once the z-knob has been positioned, use the x and y knobs to position the channel within the viewing frame.
- Stationary defects are an indication that you are at the edge of the cover slip. Use the micrometers to move the bead away from tape/dust/other beads.
- The beads are the same size, so if spherical particles of varying shapes sizes can be observed, then you are looking at the immersion oil [17].
- If the students are having difficulty viewing the beads, adjust the positioning or add more oil onto the objective lens.

6.2.5 – Trapping a Bead

1. For initial trapping of a bead, a current of about 350mA is required. A 100mA current is sufficient to maintain a trapped bead in a weak trap.
2. To find the trapping position, scan the beam in the X and Y directions using the coarse and differential knobs. When a bead is near the trap axis, it will either be captured by the trap or be shot out of the field of view due to the scattering force.
3. If you are having trouble trapping, gradually move the sample higher such that the focus is closer to the coverslip.
4. Once you have trapped a bead, record a video sequence through the uc480viewer software as outlined above.

Note:

- When a bead is close to the laser, the gradient force is strong enough to hold it in place. Trapping must be done from the bottom of the channel, such that the beads will be pushed through the channel past the focal point.
- Currents greater than 350mA are generally not required. Greater currents provide too strong a force and tend to attract more beads into the trap, which can be problematic when collecting data.
- A sample video of a trapped bead can be found in the Optical Trapping folder.
- Once a bead is trapped, it will maintain a relatively fixed position and “glow”. It will appear slightly unfocused, since the equilibrium position of the bead is above the optical focus.
- While you are collecting the data, you should ensure that other beads do not get stuck in the trap. This can be an issue, especially at a high bead concentration.

If the sample slide looks this like the following, the bead solution may have leaked from the channel. Discard the glass slide and prepare a new sample.

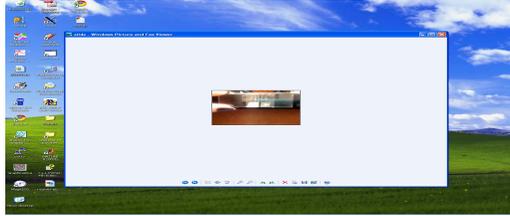


Figure 9: Sample slide with solution that may have leaked from the channel.

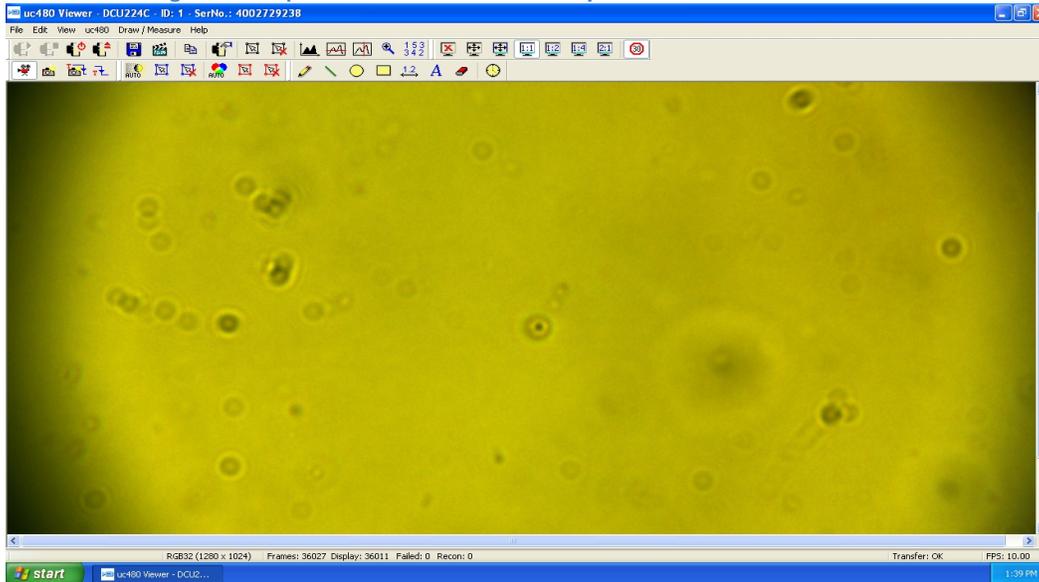


Figure 10: Image of a trapped bead using the CCD camera.

Perform this experiment for a bead with a different diameter.

6.3 – After Completing the Experiment

1. Ensure that the laser is OFF before removing your laser safety glasses.
2. Remove the key from the laser controller and return it to Rob.
3. Turn off the LED light.
4. Dispose the slides in a glass disposal box. The pipette tips and kimwipes can be put into the regular trash.

7. Additional Experiments for Trap Stiffness

7.1 – Stokes' Drag

The deflection of the trapped bead under external forces produced by Stokes' Drag will be used to determine trap stiffness.

A more direct way of measuring the stiffness is simply to apply an external force and measure the deflection. At low Reynolds numbers, external force may be applied to the trapped bead by oscillating the sample stage. The liquid inside will move in unison with the sample stage and for liquid velocity v the drag force applied to the bead will be:

$$F_d = \beta v \quad (7)$$

Since the strength of the optical trap decreases as you move further into the medium, the optimal working distance for the optical trap is a few microns above the coverslip. Due to the proximity of the trapped bead to the boundary, Stokes' drag will not be sufficient in calculating the drag coefficient β . For a better approximation of the drag coefficient you will have to correct for wall effects with the formula:

$$\beta = \frac{6\pi\eta r}{1 - \frac{9}{16}\left(\frac{r}{h}\right) + \frac{1}{8}\left(\frac{r}{h}\right)^3 - \frac{45}{256}\left(\frac{r}{h}\right)^4 + \frac{1}{16}\left(\frac{r}{h}\right)^5} \quad (8)$$

Where r is the radius of the bead and h is the height of the center of the bead with respect to the coverslip surface. Once the bead is trapped, you can begin recording the positional data from the CCD camera while oscillating the sample stage. If the bead is assumed to be in equilibrium, then the drag force F_d will be balanced by the trapping force $F_t = kx$ from which the stiffness k may be calculated.

The maximal deflections of the bead will correspond to the maximal external force from the surrounding liquid. Thus you will need to measure the maximal deflections at several different frequencies and find the displacement to force relationship to calculate the stiffness. The maximal deflection x_{\max} should be half the width of the data in the x-direction and the maximal force F_{\max} should correspond to the maximal velocity $F_{\max} = \beta v_{\max}$.

Finding the height of a trapped bead:

When a bead is properly trapped, it will stay at the same z-position as you translate the stage up and down, only going slightly out of focus due to changing amount of refraction from the medium. If a bead is held by a weak trap (100mW), there should be noticeable thermal fluctuations which can be seen in the CCD image. If the stage is translated very slowly, the fluctuations will stop when the bead touches the coverslip, but the bead will not be dislodged from the trap. Following this, the bead may simply be moved back to the original z-position for data acquisition.

A less sensitive method for measuring the trapping height is to move the stage up until the trapped bead is dislodged and then moving back to the original height to trap another bead. You could also perform all the experiments and then check the height. However, this is not recommended at low power since the bead may be dislodged accidentally.

7.2 - Power Spectrum

The power spectrum of a trapped bead will be produced by taking advantage of the high data acquisition rates offered by the quadrant detector to infer the trap stiffness from theoretically predicted features.

In the low Reynolds number regime, the equation of motion for the bead will be that of a massless damped oscillator:

$$\beta \dot{x}(t) + kx(t) = F(t) \quad (9)$$

Where x is the displacement of the bead and β is the drag coefficient.

The external Brownian force $F(t)$ is random, and is essentially white noise with an amplitude of :
 (10)

$$|\tilde{F}(f)|^2 = 4\beta k_B T$$

The Fourier transform of equation (9) is:
(11)

$$2\pi\beta\left(\frac{k}{2\pi\beta} - if\right)\tilde{x}(f) = \tilde{F}(f)$$

Thus, the power spectrum of the position is given by:
(12)

$$|\tilde{x}(f)|^2 = \frac{k_B T}{\pi^2 \beta \left[\left(\frac{k}{2\pi\beta}\right)^2 + f^2 \right]}$$

Equation (12) is a Lorentzian with a cornering frequency of:

$$f_c = k/2\pi\beta$$

which will give the stiffness k .

7.3 – Equipartition and Stokes’ Drag using Quadrant Photo-Diode (QPD)

The Equipartition and Stokes’ Drag experiments can be performed using the quadrant detector. The *Force Measurement Module* (OTKBFM) uses a *quadrant detector* and a high frequency *Data Acquisition (DAQ) Device* to obtain force, stiffness and positional data from the base optical trap system. THE DAQ device transfers the analog signal as a digital signal to the computer and is capable of recording the data at over 10, 000 MHz.

Piezo-Stage Control:

The *NanoMax MAX311* piezo transition stage allows nanometric movements of the sample in all three dimensions using piezo actuators. The stage is controlled through a *T-Cube* controller on the *THORLABS T-Cube USB Controller Hub*. Two axes (X and Y) of the stage’s piezo actuators operate in *closed loop* configuration with strain gauge readers to give high precision position control. The system may be controlled through the APT software and LabView ActiveX VI’s. For the purpose of this lab, only the LabView VI OTcontroller.vi will be used. The OTcontroller.vi file is used to move the stage in nanometer steps. The power for the controllers is switched on through the power box located underneath the optical breadboard table.

Calibrating and using the quadrant detector:

The quadrant detector consists of four photo-diodes that divide the detector surface into four quadrants. The output *XDiff* and *YDiff* report the combined voltage difference between the X and Y quadrants respectively. The detector also measures the total voltage for all four sectors, *Sum*, which will be divided into the *XDiff* and *YDiff* value to normalize them.

The data is sent to the computer through both the *T-Cube USB Controller Hub* and the *DAQ device*. The lower speed USB data is displayed inside the *OTControls.vi* software while the high speed data will be captured by the DAQ device when you press the *Record* button in the software.

To calibrate the Voltage vs. Position relationship of the quadrant detector, you will need to use a bead that is stuck to the coverslip (using a solution with PBS).

- Use the position controls to centre the bead in the trap. If the bead is centered, you will notice linear deflections in the *X* voltage as you tune the *X* positions and only slight changes in the *Y* voltage.
- Upon centering the bead, change the controller to *Sine* mode and oscillate the bead at a very low frequency and high amplitude.
- Record the position and voltage data. You may linearize the relationship around the centre to obtain the normalized voltage to position conversion.
- Use the *Record* button to gather data

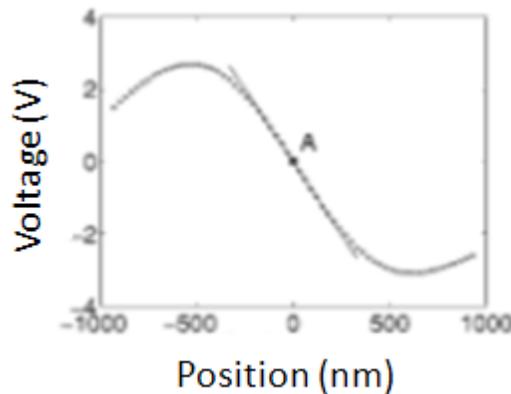


Figure 11: Calibrating the quadrant detector. (Appleyard, 2007).

For optimum results, it is advisable to keep the power on the quadrant detector between 250 μW and 1 mW.

8. Post-lab Questions

8.1 – Questions

1. Derive the expression for the gradient force for \mathbf{F}_g in detail.
2. Show that the forces acting on the bead are in the low Reynold's number regime, and that the assumptions of Stokes' Drag are appropriate.
3. Derive equation (8.1) and include an argument for the validity of the approximation $\mathbf{F}_g \approx -\kappa \mathbf{r}$.
4. Explain the disadvantage of the second method for determining trapping height.

5. Comment on the differences for varying bead diameters and its effect on the respective trap stiffness.

6. Discuss the advantages of using the Quadrant Detector for data collection versus the CCD method.

8.2 – Data Analysis

Using the recorded video of the trapped bead (.avi file) from the CCD Camera:

- Use the Matlab program “*positions.m*” to convert the recorded .avi video of the trapped bead from the camera into x and y position data in Excel.
- Each program requires the user to specify the file paths according to the instructions in the comments. When running the program, a graph of the positions of the center of the bead should appear.
- If the plot shows many outliers to the large grouping of points, it means the image has too many bright spots that do not represent the bead. In this case, you will need to go to *Camera Properties* → *Image* and adjust the *Master gain* to a lower level.

Using data gathered inside OTControls.vi from the quadrant detector:

- The data will be saved in a .lvm file
- Access the data through Excel by right-clicking on the icon and pressing *Open With* → *Microsoft Excel*.
- The Excel data will be formatted in two columns, *XDiff* and *Sum*, which will be used to generate the power spectrum of the normalized voltage.

8.3 – Student Assessment

The following is the basis upon which the student is to be assessed:

Category	Percent Composition
Pre-lab	3%
Notebook	17%
Calculation of Trap Stiffness (Excel/Matlab)	40%
Post-lab discussion with TA/ Professor	40%

100% on Laser Safety Quiz	2%
---------------------------	----

Table 2: Mark break down

Notebook

A notebook should be kept throughout the labs to keep a record of all activities undertaken.

This notebook is to be brought in with the students on the day of their oral examination. The post-lab questions must be must be answered in the notebook with any additional Excel/Matlab based calculations printed and attached. As well, the write-up should include a brief discussion of a research paper that utilizes Optical Tweezers.

Oral Lab Exam

In accordance with the existing Advanced Physics Lab structure, an oral examination an oral examination will take place following the final laboratory session. The oral exam will be 25 minutes long. There will be three examiners, all of whom are either professors or demonstrators in the course [20], and at least one of the three examiners will be a professor. The student must bring their experiment notebook to the examination, which will follow the Advanced Physics Lab Oral Exam guide lines. It will take place after two weeks following the completion of the last session of the lab to provide adequate time for the students to complete the lab write-up.

9. References

- [1] A. Ashkin. "Acceleration and Trapping of Particles by Radiation Pressure". *Phys. Rev. Lett*, vol. 24, no. 4, pp. 156-159, 1970.
- [2] A. Ashkin et al. "Observation of a single-beam gradient force optical trap for dielectric particles". *Optics Letters*, vol.11, no.5, pp. 288-290, 1986.
- [3] Ethier C. Ross, Simmons Craig A. (2007). "Introductory Biomechanics: From Cells to Organisms." Cambridge, New York. 41-42.
- [4] Neuman KC, Block SM (2004). "[Optical trapping](#)". *Review of Scientific Instruments* **75** (9): 2787–2809.
- [5] [4] Gordon JP (1973). "Radiation Forces and Momenta in Dielectric Media". *Physical Review A* **8** (1): 14–21.
- [6] [5] Harada Y, Asakura T (1996). "Radiation Forces on a dielectric sphere in the Rayleigh Scattering Regime". *Optics Communications* **124**: 529–541.
- [7] [6] Ashkin A, Dziedzic JM, Bjorkholm JE, Chu S (1986). "Observation of a single-beam gradient force optical trap for dielectric particles". *Opt. Lett.* **11**: 288–290.
- [8] [7] ThorLabs Inc. (2010). "Optical Trap Application Setup". <http://www.thorlabs.com/Thorcat/19500/19590-M01.pdf>

- [9] Ashkin, A., and J.M. Dziedzic. "Optical Trapping and Manipulation of Viruses and Bacteria". *Science*. Vol. 235, p. 1517 (1987)
- [10] Ashkin, A. "*History of Optical Trapping and Manipulation of Small-Neutral Particle, Atoms, and Molecules*". *IEEE Journal on Selected Topics in Quantum Electronics*. Vol. 6 (6), p. 841, 2000
- [11] A. Ashkin and J.M. Dziedzic, "Internal Cell Manipulation Using Infrared Laser Traps". *Proceedings of the National Academy of Science, USA*. vol. 86, p. 7914 (1989)
- [12] S.B. Smith, Y. Cui, and C. Bustamante. "Overstretching of B-DNA: The Elastic Response of Individual Double-Stranded and Single-Stranded DNA Molecules". *Science*, vol. 271, p. 795, 1996
- [13] E. Higurashi, H. Ukita, H. Tanaka, and O. Ohguchi. "Optically induced rotation of anisotropic micro-objects fabricated by surface micromachining". *Applied Physics Letters*. Vol. 64, p. 2209, 1994
- [14] K. Svoboda and S.M. Block. "Optically trapping of metallic Rayleigh particles". *Optical Letters*. Vol. 19, p. 930, 1994
- [15] C.A. Murray. "When like charges attract". *Nature*. Vol. 15, p. 1088, 1962.
- [16] A. Rohrbach. "Stiffness of optical traps: quantitative agreement between experiment and electromagnetic theory." *Phys Rev Lett*, vol.95, no.16, pp. 168102-2 – 168102-4, 2005.
- [17] <http://www.thorlabs.com/thorcat/7100/7111-D02.pdf>
- [18] http://www.advancedlab.org/mediawiki/index.php/Optical_Trapping
- [19] http://www.thorlabs.com/NewGroupPage9.cfm?ObjectGroup_ID=4190&Guide=127&Category_ID=94
- [20] <http://www.physics.utoronto.ca/~phy326/oralguidelines.htm>

Appendix I – Useful Links

Thorlabs' Manuals:

ITC510 Laser Diode Combi Controller:

<http://www.thorlabs.com/thorcat/7100/7111-D02.pdf>

Optical Trap Manual with Components:

<http://www.thorlabs.com/Thorcat/19500/19590-M01.pdf>

Papers:

A practical guide to optical tweezers:

http://www.genomics.princeton.edu/shaevitzlab/OT_Practicle_Guide.pdf

Optical trapping for undergraduates:

[http://web.mit.edu/~langlab/Publications/Appleyard-etal\(2007\).pdf](http://web.mit.edu/~langlab/Publications/Appleyard-etal(2007).pdf)

A list of relevant websites and papers that will aid the student in understanding the laboratory concepts or equipment should be included.

Review Papers for Optical Tweezers:

Ashkin, A. *History of optical trapping and manipulation of small-neutral particle, atoms, and molecules*. IEEE Journal on Selected Topics in Quantum Electronics. 6 (6), pp. 841-856 (2000)

Ashkin, A. *Optical trapping and manipulation of neutral particles using lasers*. Proceedings of the National Academy of Sciences of the United States of America. 94 (10), pp. 4853-4860 (1997)

Neuman, K.C., and S.M. Block. *Optical Trapping*. Review of Scientific Instruments. 75 (9): 2787-2809 (2004)

Lang, M.J. and S.M. Block. *Resource Letter: LBOT-1; Laser-based optical tweezers*. American Journal of Physics. 71 (3), pp. 201-215 (2003)

Optical Tweezers Blog:

<http://opticaltweezers.blogspot.com/>