# Optical Tweezers

Phys 2010 – Brown University – November, 2010

## Purpose

Learn about the principles behind the optical tweezers or optical trapping experiment. Become familiar with the use of standard optics including optical alignment, and collection of data through the use of a camera-computer system. Examine the forces on a bead trapped by a laser.

## Safety

The laser used in this experiment is the strongest laser in the instructional labs. Do not attempt to perform this lab unless you have received laser training from EH&S and training from the dept specific to this lab. In general, you must wear laser safety goggles designed for red HeNe laser light at all times unless the beam intensity is set below 1 mW.

## Introduction

Optical tweezers is one of the most effective methods of manipulating micron and sub-micron sized particles. Their use covers a number of research areas in biology, chemistry and physics; and has found its greatest use in the growing field of biophysics.

Those working in biophysics often need to manipulate small biological samples, strands of DNA, or the constituents of a cell. Researchers have used optical tweezers to stretch a single strand of DNA in order to study its elasticity and observe numerous other properties. Optical traps have been used to study the motility of sperm and more successful methods of in vitro fertilization. Organelles of a cell or even single proteins moving within a cell have been manipulated with no observable damage to the cell. To biologists, optical traps are an invaluable tool.

Atomic physicists have also found a use for optical tweezers. Using these setups, they have managed to trap individual molecules and atoms. Trapped atoms can be cooled to temperatures of a fraction of a microkelvin. Atomic fountains have been created, which can be used as the most accurate of atomic clocks (1). Superfluid helium has been optically levitated using these traps; microscopic "pinwheels" have been created and observed with torques and rotations generated by the traps. New scanning-force microscopes are being developed with much lower "spring constants" than the traditional cantilever method.

Chemists are also finding uses for this versatile tool. They have trapped particles of gold in suspension; demonstrating metallic materials can be trapped. Colloids and suspensions have been manipulated using optical tweezers. Even gas bubbles in various solutions have been controlled with traps.

A more complete list of recent accomplishments with optical traps, through 1997, is referenced in Arthur Ashkin's article (1).

## Theory

Optical tweezers can be used with atoms, molecules and particles ranging in size from nanometers to hundreds of microns. For this reason, it is difficult to devise a simple theory explaining how optical tweezers work. Each theory is used to explain the phenomenon at a different length scale relative to the wavelength of the light used. Because any type of laser, infrared, visible, or ultraviolet, may be used, depending on the application of the trap, the divisions between each of these theories are fluid.

For particles much larger than the wavelength of the laser, the Rayleigh regime, conservation of momentum and geometrical optics are used to explain optical tweezers. For particles much smaller than the wavelength of the laser, the Mie regime, the theory of electromagnetism must be employed. Finally, between the two regions a combination of theories can be employed to understand the phenomenon.

In this experiment, one micron beads and a laser with a wavelength of 632nm will be used. The experiment takes place in the region between the Rayleigh and Mie regimes. For this reason simple ray optics do not accurately describe the physics of the system; however, they do provide a simple approach to the underlying effect. For a more complete explanation of optical tweezers in this and the Mie regime consult the references (2,3,4).

## The Ray Optics Approach

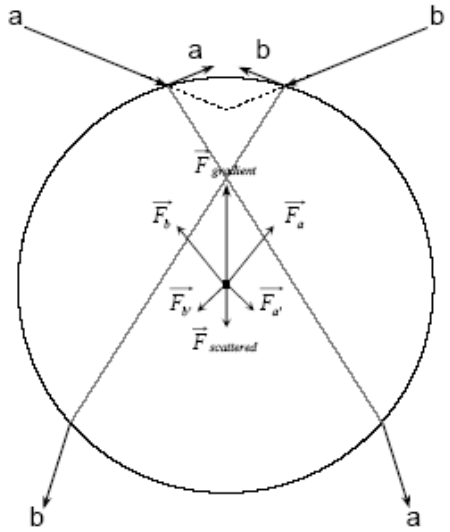


**Figure 1.** Ray Optics of a trapped bead. A gradient force is created by the refracted rays, while a scattered force is created by the reflected rays.

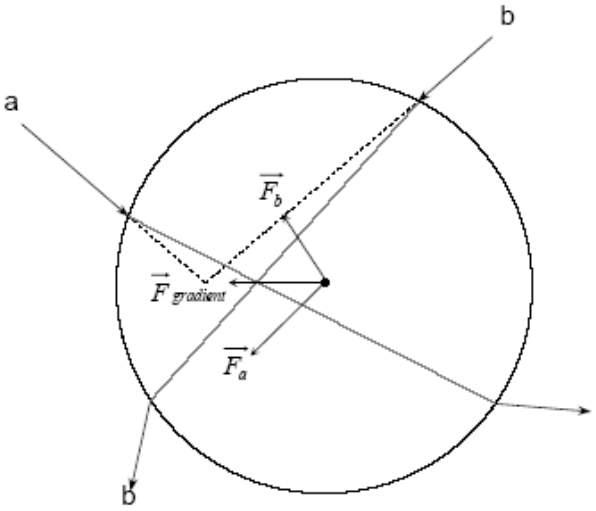


**Figure 2.** Forces on a trapped bead being pulled to the left.

When a ray enters a dielectric sphere, part of it is refracted according to Snell's law into the sphere, while the remainder is reflected off the sphere, as seen in Figure 1. As the photons reflect

and refract off the surface, there exists a transfer of momentum between the incident photons and the sphere. Because each photon has a change in momentum of $\Delta p$, the sphere must undergo a change in momentum of $-\Delta p$. The refracted photons will create a restoring force towards the focus of the beam. This force, the gradient force, must outweigh the force created by the reflected photons, the scattering force. If the scattering force is stronger than the gradient force, a particle will be pushed through the trap rather than held at its focus. The created trap becomes a three dimensional potential well. Figure 2 illustrates the restoring force in a direction other than that of the beam's propagation.

**Oil Immersion Objectives:**

Three key principles exist in understanding the objective used in the optical tweezers setup: the back focal length of the objective, numerical aperture and the use of immersion oil.

First, the back focal length: Contrary to simple lenses, common microscope objectives are not designed to work properly with parallel light, instead they are designed to receive light with a particular divergence. This divergence defines the back focal length of the objective; the distance between the back of the microscope objective and the light source. This distance is generally a number etched into the objective and is usually a value of either 160 or 170 mm. As a result of this feature, an object at the front focus of the objective will generate an image at the back focus; hence, placing the camera's CCD at the back focus results in an in focus image. Note, it is possible to purchase microscope objectives designed to work with parallel light, these are referred to as infinity corrected.

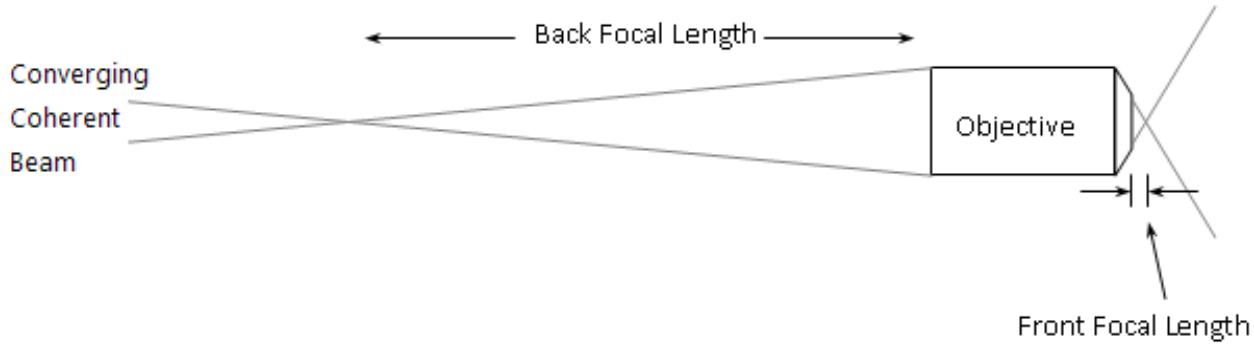


Figure 3. Focal lengths of a microscope objective

The concept of numerical aperture (NA) is relevant to all microscope objectives. The NA is a good measure of the resolution of an objective. Low magnification objectives have very low NA's, around 0.1, while high powered objectives can have NA's as high as 1.6. The NA is related to the angular aperture of the objective and the index of refraction of the medium following the objective, usually either air or oil. As the numerical aperture is increased, the convergence angle of light entering the objective is increased. The NA is given by the simple formula:

$$NA = n \cdot \sin(\theta)$$

where θ is the angular aperture of the objective, a value fixed by its internal optics.
If the imaging medium following the objective is air, Figure 4a, the numerical aperture can never be greater than the angular aperture and a significant amount of light will be lost. For the purpose of optical tweezers, a NA comparable to that of the glass cover slip is desirable. To meet this requirement, immersion oil, with an index of refraction of approximately 1.51, is used. In using materials of comparable indices of refraction, the amount of refraction occurring at the surface between these two materials is greatly limited, Figure 4b. Using this immersion oil, the NA of the objective is increased to the value etched on the oil immersion objective.

Figure 5 details the ray optics of a simplified oil immersion objective. The sample is located at point P between the slide and cover slip. The light rays arrive at the Meniscus lens appear to originate from P(1). Upon exiting this lens they appear to diverge from P(2). Another series of lenses collects these rays and is used to refract them towards the viewer's eye or a camera attached to the microscope.

Adjusting the numerical aperture of the system has a significant effect on trapping in an optical tweezers setup. A low NA will lead to a deep trapping depth, but a low trapping efficiency and a high power loss. A high NA will lead to a high trapping efficiency, a low power loss, but a shallow trapping depth. The NA must be carefully selected for an optical tweezers experiment given its consequences on the efficiency, depth and power loss of the trap.

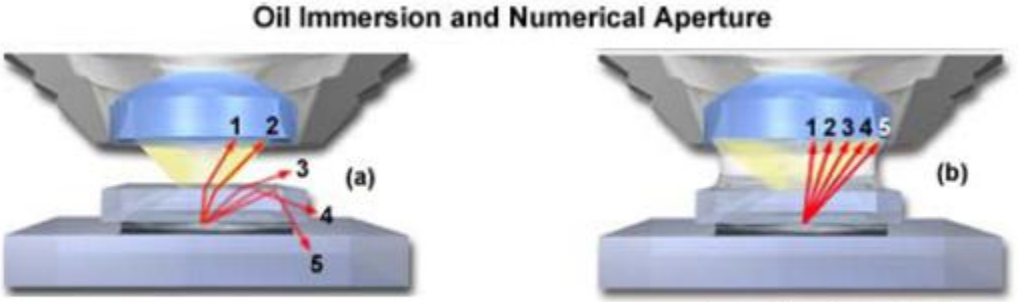


**Figure 4.** a) an objective with no immersion medium.

**Figure 4.** b) an objective with oil as an immersion medium.

From MicroscopyU.com

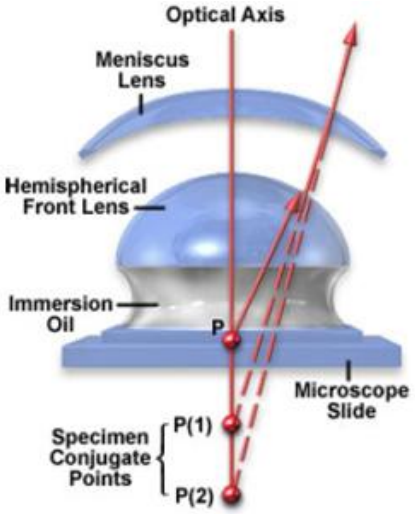


**Figure 5.** Ray optics for an oil immersion microscope objective. From MicroscopyU.com

Note: These figures show an objective as used in a standard microscope, not as used in optical tweezers. The figures show light from a sample entering the front of the objective. In optical tweezers, laser light enters the back of the objective and exits the front. Despite this change in direction, the concepts presented here are still very pertinent and important.

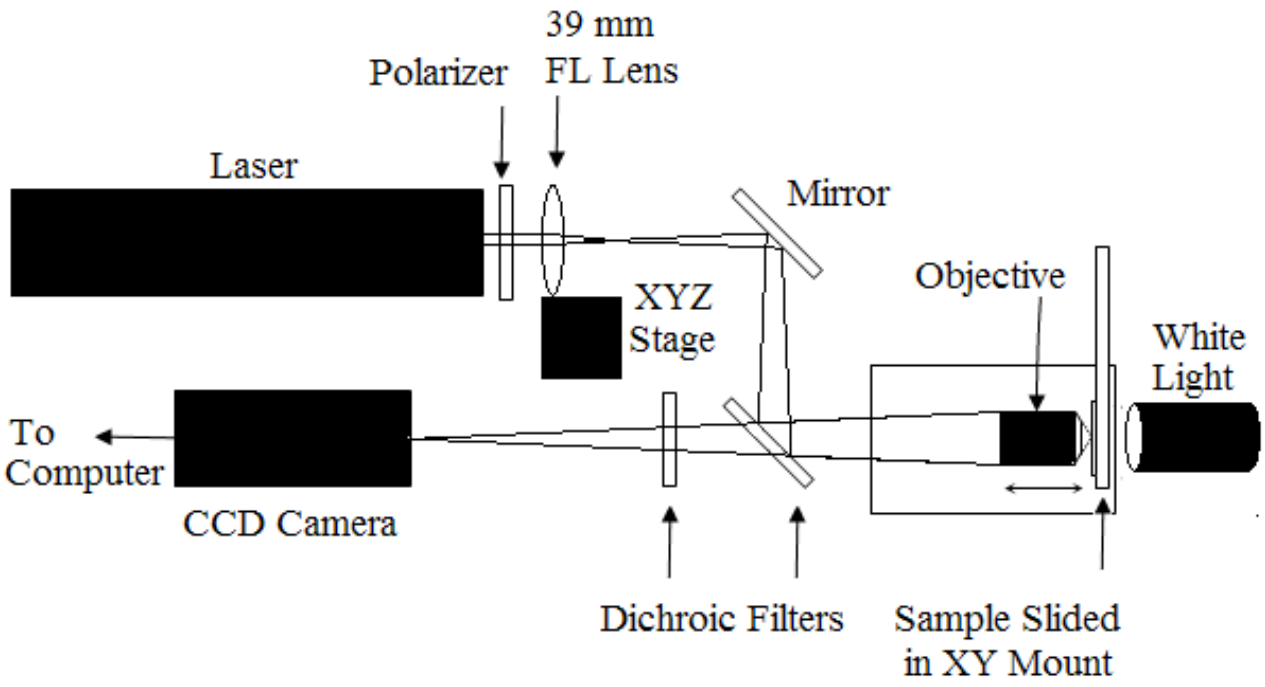


Figure 6. Optics configuration for optical tweezers

**Equipment**

Laser ~17 mW
1 Silver Mirror in mount
2 Dichroic Filters in mounts
Polarizer in mount
Lens on XYZ stage w/ focal length of 39 mm
100x Oil Immersion Objective w/ 160 mm back focus
White light source
Slides, cover slips and parafilm
Solution of water and one-micron polystyrene beads
Firewire CCD Camera, Computer and Fire-I and Scion Image software
2 Apertures

**Experimental Procedure**

Setting up the optics:
One of the most difficult elements of an optics experiment is setting up and aligning the optical components. In this experiment the beam must follow the path illustrated in Figure 6.

The laser beam first passes through a focusing lens serving two purposes, to expand the beam filling the back lens of the microscope objective and to focus the beam to a point at the back focal length of the objective. **Why are these two purposes critical to an optical tweezers setup?**

From the lens, the beam reflects off a dichroic mirror also serving a dual purpose. This dichroic mirror is used to reflect the beam into the objective and, due to its transparency, it allows us to use the objective not only to create our trap, but also view it with our camera. The dichroic mirror only reflects specific wavelengths. By placing the camera at the proper distance behind the objective, the trapping optics act as a microscope for the camera.

Once the beam passes through the objective, it reaches the sample, where it focuses the beam to a point. By moving the objective toward or away from the sample slide, the position of the focal point can be placed anywhere within the sample solution. A backlight is used to illuminate the slide for viewing on the camera.

The camera is positioned behind the dichroic mirror. By adjusting the position of the camera different parts of the sample are viewed through the objective. The goal is to be able to focus on trapped beads. When collecting images of a trapped bead, a second dichroic mirror will filter the reflection of the laser beam off the trapped bead.

In setting up the optics begin with the simplest pieces first, the mirrors. Once the mirrors have been aligned, add the objective and the lens. A polarizer will be used to vary the intensity of the beam while trapping, but should also be used to minimize the intensity of the beam while aligning up the optics. Use the apertures and the principle of back reflection to align the beam along the proper optical paths.

Making a slide:

1. Cut out a square of parafilm larger than the size of a cover slip and apply it to the slide by pressing it down on the slide. Place Parafilm and slide in front of the heat source for about a minute and press it more securely onto the slide. The parafilm adhesive is activated by both pressure and heat.
2. Cut out a square from the parafilm, large enough to hold a single drop of solution such that the drop should not touch the parafilm, if possible, and small enough to be covered by a cover slip.
3. Put a drop of solution at the center of the cutout. The drop should be small enough so that it will not touch the parafilm.
4. Place a cover slip on top of the solution and parafilm, pressing down on it hard enough to create a bond between it and the parafilm. Pressing too hard may cause the cover slip to break.
5. Place a drop of immersion oil over the sample on the cover slip.
6. Insert the slide into the slide mount and bring the objective up to the slide so the immersion oil adheres to both the cover slip and the objective.

Using Fire-I:

Fire-I is a simple program used to view the camera image and record frames from it. The program should only be used with resolutions of only “1024x768” at 30fps or “User Defined (MAX 1024x768)”. The exposure for the camera can be adjusted through the Camera Properties window. It is often useful to look at the “negative image” of the camera to view the beads more

clearly. Also when saving frames, avoid saving the full "1024x768" image, but rather use a user defined resolution, 320x240 or smaller is sufficient. Enough frames should be captured to obtain accurate particle statistics.

Using Scion Image:

Scion image is used to obtain the particle statistics from the images captured using Fire-I. The macros that allow measurement of particle displacement must first be loaded; the captured frames are then placed into a stack, or sequence, of images; finally particle statistics are obtained.

Loading Macro's:

1. From the "Special" menu, select "Load Macros…"
2. Navigate to the desktop and select the text file "bead_macros"

Creating a stack:

1. From the "File" menu, select "Open…"
2. In the dialog box that appears check the box that says "Open All" and navigate to the folder where the captured frames are saved. Select the first image in the list and click "Open." All of the images will now open in separate windows; this may take a minute depending on how many images are saved.
3. From the "Stacks" menu, select "Windows to Stack," placing the open windows in a single stack. Save this file.

Obtaining particle position statistics:

1. Crop the image, using the "Crop and Scale" macro to contain only the particle of interest.
2. Run the "Correct Background" macro to eliminate much of the background from the image. Some noise may exist in the rest of your image, producing data that may need to be discarded.
3. Run the "Find Displacement" macro. The threshold and minimum particle size values should be adjusted depending on the captured images. This macro may need to be run several times with different input values to obtain only the relevant statistics.
4. Once complete, the macro displays the particle statistics of anything it interpreted to be a particle in the "Results" window. All of the data that appears in this window is stored in the clipboard to be pasted into any other file. If the program detects particles other than the bead, then the value used for the minimum particle size is too small and the find displacement macro should be run again.

A preferable alternative to using Scion image is a MATLAB algorithm (OpticalTweezersAnalysis.m) written by some previous students, which is available on the lab wiki under resources.

**Helpful hints**

- The ultimate goal in alignment is to obtain a trap that appears as perfectly symmetrical concentric rings.
- It is important to make very sure that the axis of the microscope objective is perfectly aligned with the beam.

- A good test of alignment is ensuring that the reflected beam returns along the incident beam, i.e. back into the laser.
- A focus should occur at each interface, i.e. between the oil and the cover slip, the cover slip and the water, and the water and the slide. Think carefully about where you want the focus to be.
- The two knobs on the slide holder are the fine adjustments for the X-Y position of the slide, be aware that they may also move the slide relative to the focus.
- Be a perfectionist about everything related to alignment.
- Ask Dean for a thermometer!

## Data analysis

The trap strength can be approximated using the Equipartition Theorem:

$$\langle U(x) \rangle = \frac{1}{2} k_x \langle x^2 \rangle = \frac{1}{2} k_b T$$

where $k_x$ is the "spring constant" or our trap, $k_b$ is Boltzmann's constant, T is temperature in Kelvin and $<x^2>$ is the average of the squared deviation from the mean particle position. Calculate the force per unit displacement in the x and y directions on a particle using sensible force and length units. Measure this restoring force for a number of different beam intensities and plot the force as a function of intensity. **In making this calculation what assumptions are made about the trap?**

In using the Equipartition Theorem, the assumption of a parabolic potential was made. To check the validity of this assumption a probability density for particle position can be created. From this Boltzmann distribution, the potential U(x) can be solved for. Create a frequency distribution of the particle statistics for each of beam intensity and obtain a Gaussian fit for each distribution. Using this Gaussian fit as the probability density:

$$\rho(x) = \frac{e^{-U(x)/k_b T}}{Z}$$

where Z is the partition function normalizing $\rho$, approximate the potential.

## References


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