

Experiment 6: Optical Tweezers

1. Introduction

NOTE: Before attempting this experiment, read this entire lab description.

Optical tweezers instruments use the forces of laser radiation pressure to trap small particles. Using various techniques, these trapped particles can then be manipulated and forces on the objects in the trap can be measured. The forces that such an instrument is capable of measuring are of the order of 0.01 to 300 piconewtons (pN). While this technique has been used for over 20 years to manipulate and study the properties of micron-sized dielectric particles, it is only recently that this precise force measurement instrument has been applied to the study of biological systems.

There are two major types of optical tweezers instruments. In the Williams lab here at Northeastern University, dual-beam optical tweezers instruments are used to measure single molecule DNA-protein interactions. In a dual-beam instrument, two microscope objectives are used to focus two counterpropagating beams to the same spot. The point to which the two beams are focused forms the optical trap. Dual-beam instruments have the advantage that, using a relatively low laser power of 400 mW, they can generate trapping forces of up to 300 pN. The disadvantage of these instruments is that they must be built on an optical table, they involve many optical components, and they require careful alignment.

A single high power laser beam that is focused to a small spot using a high numerical aperture microscope objective will cause small dielectric particles (which in these experiments are usually floating in water) to be attracted to the focus of the beam. This attractive force is due to the refraction of the laser light by the surface of the particle, as shown in Figure 1. For this to work, the index of refraction n of the particle must be greater than that of the surrounding medium. In our experiments we will use polystyrene spheres ($n=1.5$) in water ($n=1.33$).

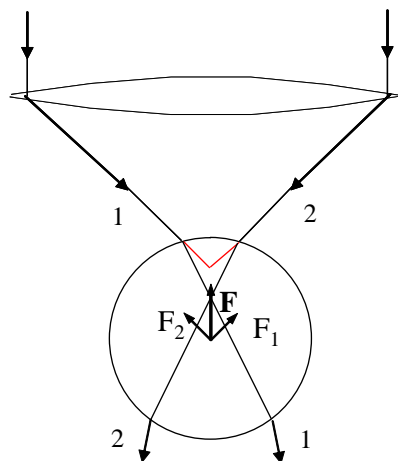


Figure 1. Schematic diagram showing the force on a dielectric sphere due to refraction of two rays of light, 1 and 2. The resultant force on the bead due to refraction is towards the focus.

2. Instrument Design

For our experiments, we will use a single beam optical tweezers design. However, the instrument design is not standard. In fact, almost all single beam optical tweezers instruments are designed as part of a standard commercial microscope. You will see that a very good microscope can easily be constructed on an optical table with very few

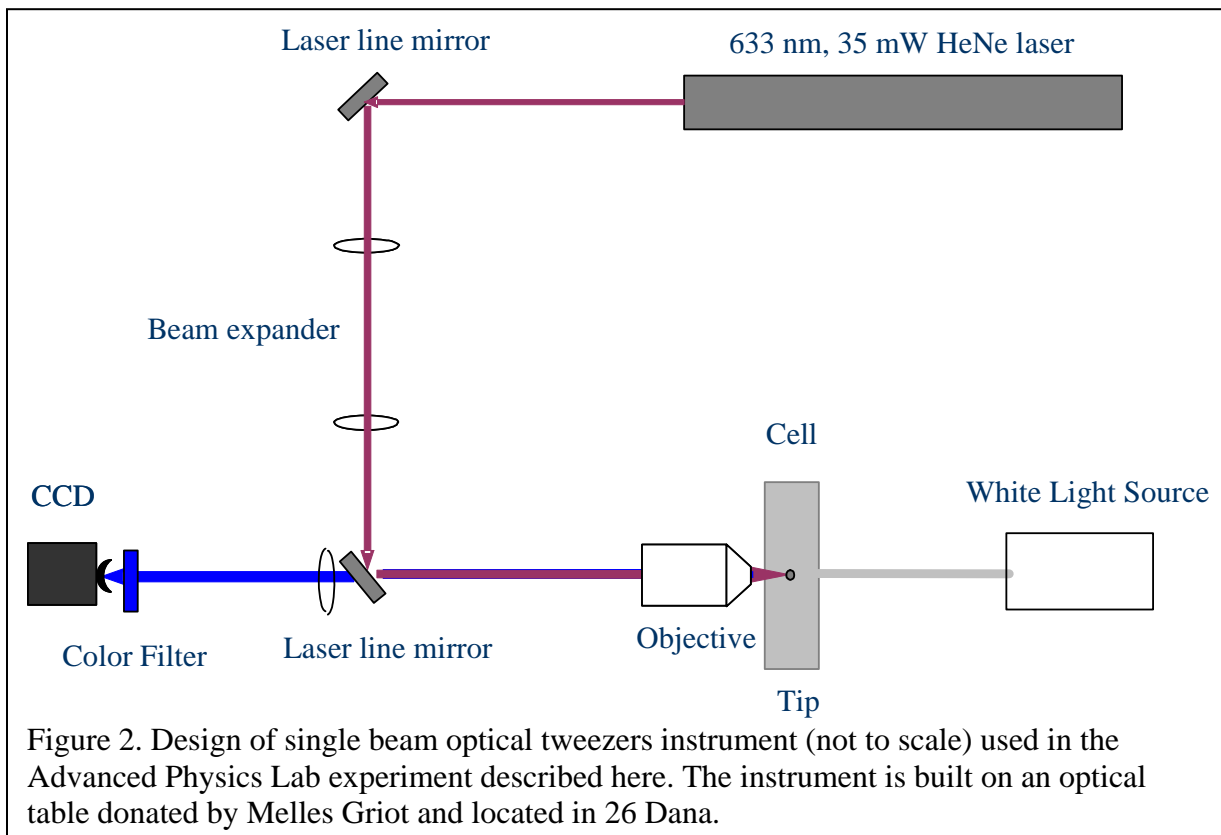
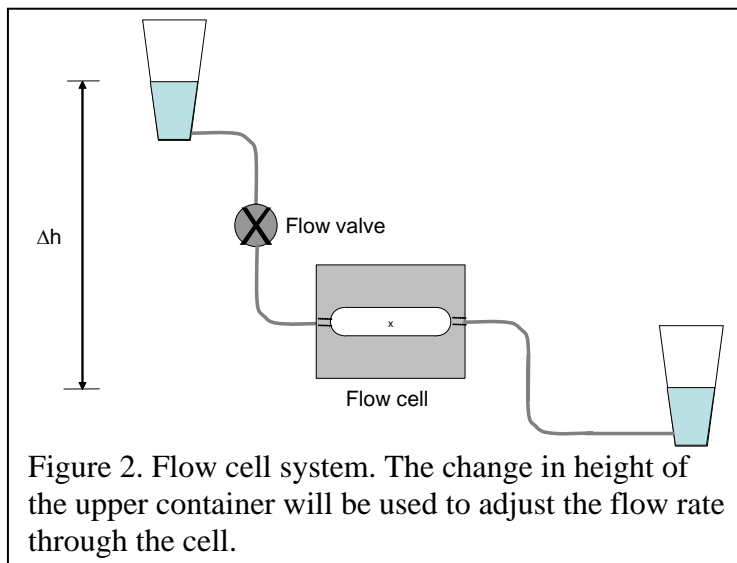


Figure 2. Design of single beam optical tweezers instrument (not to scale) used in the Advanced Physics Lab experiment described here. The instrument is built on an optical table donated by Melles Griot and located in 26 Dana.

optical elements. A diagram of the instrument is shown in Figure 2. Before operating the instrument, be sure to wear the supplied safety glasses, as the 35 mW helium neon laser can be dangerous if it is deflected into unprotected eyes. The protective eyewear will reduce the laser intensity by at least two orders of magnitude, rendering even the full 35 mW harmless at 0.35 mW.

Most of the instrument optics will be already set up when you start. However, you will have to set up and mount the flow cell so that you can take data. The flow cell is designed to allow water to flow from into and out of the cell at a fixed rate, such that the liquid flow rate and direction is uniform at the optical trap. Before mounting the flow cell in the instrument, you must fill it with water. The cell consists of a Plexiglas spacer with two glass cover slips glued to each side to form a sealed container with an input and output tube. At the ends of each tube, there is a container with lines on it every 0.5 ml of liquid volume, as shown in Figure 3. Make sure the flow valve is set to off. Fill the input container, which has a much longer tube, up to 5 ml with water and mount it on the ring stand. You will use this to fill the cell. Also, fill a syringe with bead solution and connect it to the flow valve. Before mounting the cell in the instrument, make sure all of the bubbles are out of the tubing and valve system.

The laser beam is reflected off two laser line mirrors. These mirrors are designed to reflect only light in a narrow band around 633 nm. Between the two mirrors, the beam is expanded using two lenses. Using a card and the information on the lenses, investigate how the beam expander works. Measure the beam diameters before and after the lenses.



Put this information in your lab report, explaining how the beam expander works in a diagram. The purpose of the beam expander is to increase the beam diameter so that it is the same size as the input aperture of the microscope objective. As you can see from Figure 1, the more light you have coming from the edges of the input aperture of the objective, the higher the trapping force will be.

After being reflected off the second mirror, the light is focused by the objective. If the laser light is collimated (not converging or diverging) before the objective, the light will be focused to a spot directly after the objective, about 0.2 mm from the its front face. (The front is usually defined as the side after the objective in this geometry.) The laser light must be focused into the cell, which we have designed for you. This flow cell must be filled with water and mounted properly very close to the objective. This objective is an “oil immersion” objective, which means you must also have a drop of oil in between the objective and the cell for it to work properly. After being focused into the cell, the laser beam diverges rapidly.

In order to see what is happening in the cell, we must use white light to create an image. The image comes from looking into the objective, so a camera is placed on the back side of the objective, before the mirror. A white light source is focused into a large fiber-optic bundle, and the output of the bundle is placed directly after the flow cell. This light enters from the front of the objective and most of it goes through the laser line mirror. It is then focused with another lens into the CCD camera. The CCD camera image is displayed on the monitor directly above the table. If a bead is held in the trap, you will see an image on the monitor if the optics are placed properly. Study the optical elements and compare them with what you would expect in a standard microscope. Which parts correspond to the parts of a microscope? **Discuss this in your report.**

Because of the geometry in this design, you will not see the laser beam on your camera image. How will you know the beam is focused into the cell? If you use the translation stage to move the cell along the axis of the beam (away from and towards the objective), you should be able to find a place where the laser beam is reflected back towards you and you see an image of the focused laser spot. Then move then cell towards the objective using the translation stage for 4 or 5 turns of the micrometer. Thus, you will know the trap is inside the cell and you can trap beads. At this point, you can use the syringe to inject a small amount of beads into the cell. As they float by, they should be attracted to

the focus of the laser beam and they will suddenly come into focus and stay in the trap. Now that you have trapped a bead, you need to figure out how to use it to measure forces.

3. Calibration of the optical tweezers.

The easiest way to measure the force on the bead in the optical trap is to measure its position. The distance of the bead from the center of the trap is directly proportional to the force exerted upon it:

$$\vec{F} = -k\vec{x}, \quad (1)$$

where \vec{x} is the position vector relative to the center of the trap. The parameter k is referred to as the trap stiffness. Once the trap stiffness has been measured, a measurement of the bead position is equivalent to a measurement of the force exerted on the bead. How can we measure this? If we apply a known force and measure the position, we can use linear least-squares fitting to determine k and the error in k .

One common method for applying a known force is to use the viscous drag force on a bead in the trap. The force due to viscous drag on a sphere of known radius can be calculated. If a liquid with viscosity η flows past a sphere of radius r with velocity v , the force due to viscous drag F_{vis} is given by:

$$F_{vis} = \gamma v = 6\pi\eta r v, \quad (2)$$

where γ is the viscous drag coefficient. Since the viscosity of the liquid is known and polystyrene spheres of known radius can be obtained, we can apply a known force if we can measure the velocity of the liquid.

The method we will use for inducing liquid flow is shown in Figure 3. In a previous year, students would determine the force on the bead by measuring the volume flow rate of the liquid from the top container to the bottom container. Thus, one can measure the volume flow rate as a function of height for several values of Δh and calculate the linear flow rate and its error for each height. However, this was very tedious and it is not clear that there is a direct relationship between the volume flow rate and the linear flow rate at the bead (due to turbulence). This year, we will measure the flow rate directly by video imaging.

To do this, turn on the laser and trap a polystyrene bead. Set up the computer to save images of the bead using Pinnacle Studio 8.0. Please only save a few seconds at a time, since we do not have unlimited hard drive space for video capture. After capturing an image without flow, turn on the liquid flow and set your first Δh . Capture a video for three values of Δh , three times each. Then measure the flow rate at which the bead leaves the trap for each height. After analyzing these images and determining the flow rate, you will have a measurement of the trap stiffness.

To analyze your images and find the change in position for each velocity, you will use National Instruments Vision 7.0. This program allows you to measure the position of the center of the bead in pixels. To find the distance per pixel, measure the size of the bead in pixels, and use the fact that these polystyrene beads are $6.02 \pm 0.37 \mu\text{m}$ in diameter.

From this data obtained in this experiment, calculate the trap stiffness, maximum trapping force, and the error in each of these parameters. In your lab write-up, present a few pictures of trapped beads as well as graphs showing velocity and force calibrations, including error bars. You have now learned to use a powerful modern instrument that is capable of measuring very small forces on single molecules.

For general information about optical tweezers, see:

Mark C. Williams. 2002. [Optical Tweezers: Measuring Piconewton Forces](#), in [Single Molecule Techniques](#), Petra Schwille, ed., a volume of the *Biophysics Textbook Online*, L. DeFelice, editor-in-chief, Biophysical Society, Bethesda, MD. Now available at <http://www.biophysics.org/education/techniques.htm>.