

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 counter-propagating 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 ($n=1.33$).

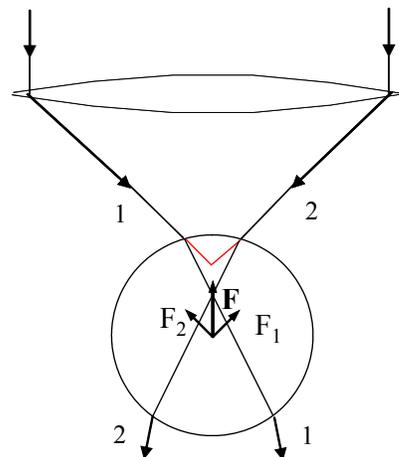
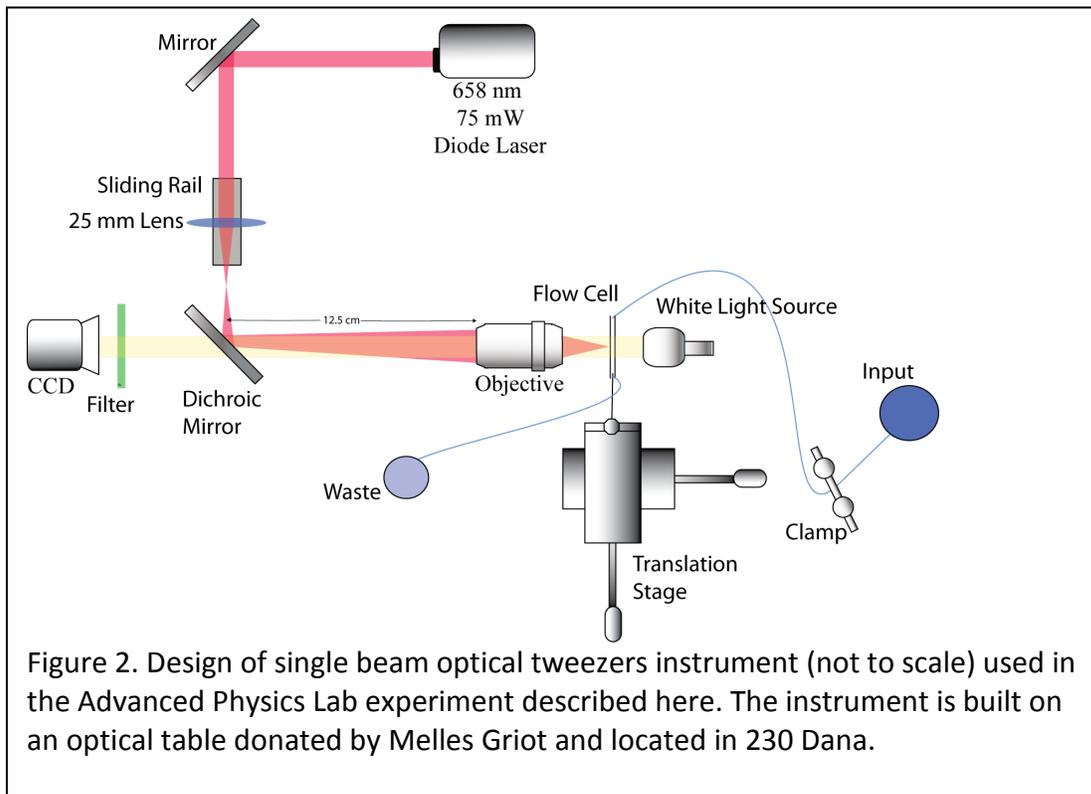


Figure 1. Schematic diagram showing the force on a dielectric sphere due to refraction of two rays of light, 1 and 2. The resultant force on the bead due to refraction is towards the focus.

2. Instrument Design

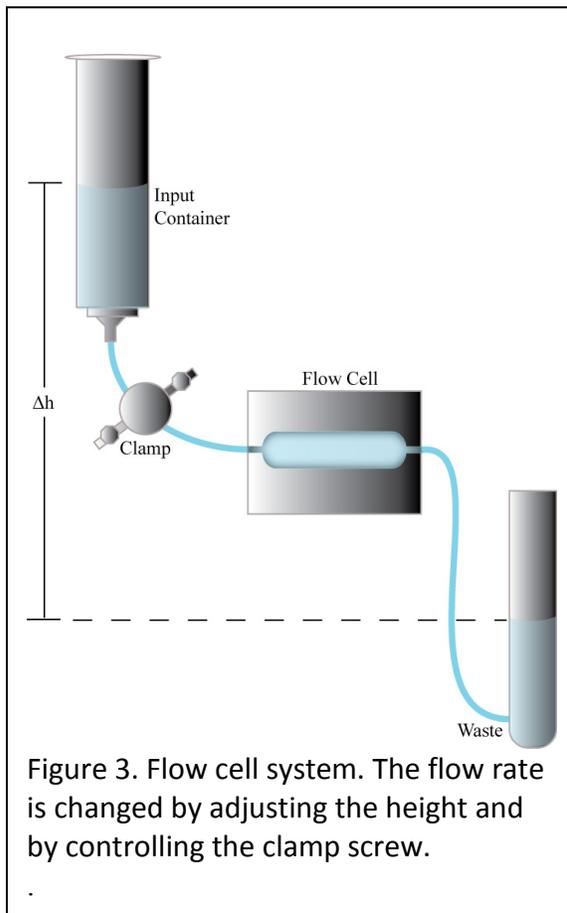


For our experiments, we will use a single beam optical tweezers design. However, the instrument design is not standard. In fact, most single beam optical tweezers instruments are designed as part of a standard commercial microscope. You will see that a very good microscope can easily be constructed on an optical table with very few 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 75 mW 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 75 mW harmless at 0.75 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 clamp screw is tightened so there is no flow. 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. 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 relatively narrow band around 633 nm, which includes 675 nm. Between the two mirrors, the beam is expanded by a single convex lens mounted on a sliding rail. The purpose of the lens is to increase the beam diameter so that it fills the



back focal plane of the microscope objective. As you can see from Figure 1, the more illumination you get through the objective, the higher the trapping force will be.

After being reflected off the second mirror, the light is focused by the objective. Then 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 the 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 flow 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. Instead of adjusting the flow rate only via height, we use a variable clamp screw as well in order to control/change the flow rate. Therefore now, we can get

different flow rates for a particular height by dialing the clamp screw. This excludes using the direct relationship of height vs flow rate, so we will use direct measurements of the flow rate to determine the force for each measurement.

To do this, turn on the laser and trap a polystyrene bead and close the clamp so there is no flow at the moment. 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, pick a particular height, and turn on the liquid flow by slowly opening the clamp screw. You'll see the displacement of the trapped bead at this point. Now you have the displacement data but you do not know the force. In order to find the force, we measure the velocity of a non-trapped bead. That force is proportional to the flow rate at that moment. Record the trapped beads for different flow rates by adjusting the height and dialing the clamp screw. Make sure to record a non-trapped bead flowing for each trapped instance in order to measure the force and make sure you carefully document the files you are saving under different conditions. Obtain measurements of bead displacement with uncertainties at six different flow rates, and make sure these points cover a large range of approximately equally spaced flow rates. In order to determine the uncertainty, you will need at least three measurements at each flow rate (of both the flow rate and the displacement). After analyzing these images and determining the flow rate, you will have a measurement of the trap stiffness. Record the data at the maximum flow rate at which a bead can be trapped in order to estimate the maximum trapping force with uncertainty.

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 $3.18 \pm 0.16 \mu\text{m}$ in diameter. Check with the TA to make sure the beads you are using are described by these numbers. ***From the data obtained in this experiment, calculate the trap stiffness, maximum trapping force, and the uncertainty in each of these parameters.*** In your lab write-up, present a few pictures of trapped beads as well as a graph showing force or flow rate vs. displacement, including error bars. Describe how the data is analyzed very clearly, including examples of the measurement from the image. You have now learned to use a powerful modern instrument that is capable of measuring very small forces on single molecules.

NOTE: Please save the bead solution by reusing the waste as input and please do not leave the flow system open to air when you leave. Undergraduate students are not permitted to use the lasers if a TA is not present.

For general information about optical tweezers, see:

Mark C. Williams. 2002. [Optical Tweezers: Measuring Piconewton Forces](http://www.biophysics.org/Portals/1/PDFs/Education/williams.pdf), in *Single Molecule Techniques*, Petra Schille, ed., a volume of the *Biophysics Textbook Online*, L. DeFelice, editor-in-chief, Biophysical Society, Bethesda, MD. Now available at <http://www.biophysics.org/Portals/1/PDFs/Education/williams.pdf>