## Optical Trapping: Laser Tweezers

In this lab you will learn a fairly modern technique, trapping a small sphere using the field gradient of a focused laser beam. The spheres you will use are made of plastic and are 1.2 μm in diameter. After setting up the optics and aligning the trap, you will make some "movies" of the ball diffusing within the trap to measure the strength of the trap as a function of the laser power.

SAFETY WARNING: the lasers used in this lab are more than 30 times more powerful than our usual red lasers, so please use extra caution in avoiding eye contact.

The experimental setup is shown below.
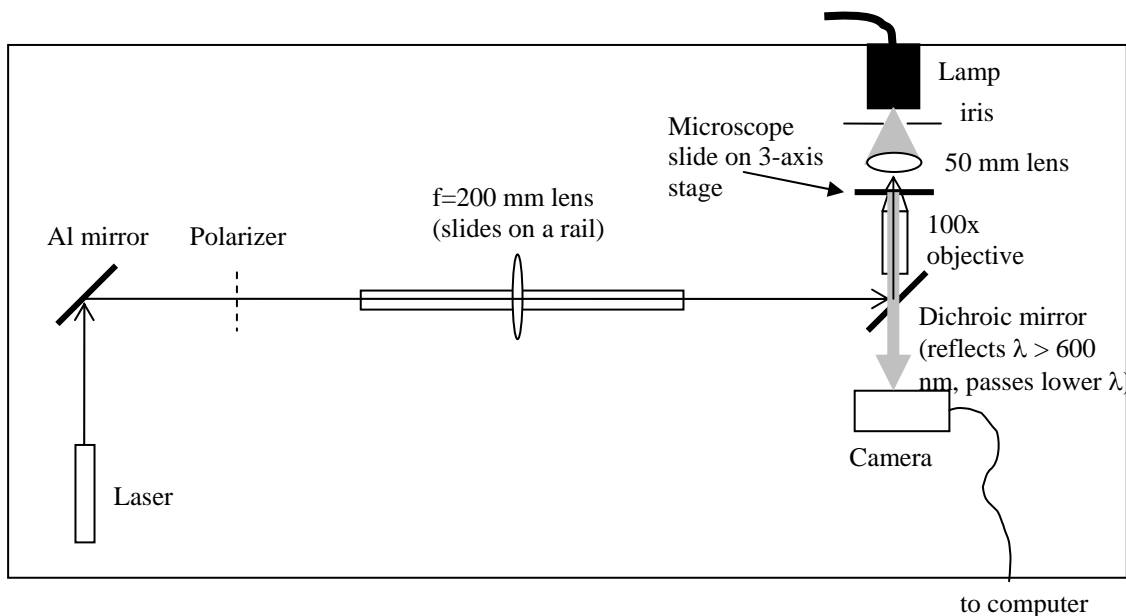


One setup has this arrangement reversed. When you get started, all of the components will be on the table except the Al mirror, 200 mm lens, dichroic mirror and rotating polarizer. These you will need to put in place and align to make the tweezers. But first you should check your microscope:

A. To make a sample for the microscope, take a slide, put one drop of the bead solution on the right end of the slide (left end for the reversed setup) and cover with a coverslip. Place the slide in the holder with the <u>coverslip facing the objective</u> and advance the 3-axis stage towards the objective (this axis is the "focus"). When the slide is very close, add a little immersion oil between the coverslip and objective. **Q1.** What is the purpose of the oil?

B. Turn on the white light and start the camera in "Preview" mode on the computer. You should see beads moving on the screen. If not, move the stage forward and

backward until they come into focus. **Q2.** Which way are the beads moving and why?

C. Now place the two mirrors in their holders and align the beam to the back of the objective. The two adjustment screws behind each mirror adjust horizontal (lower screw) and vertical (upper screw). The beam should run parallel to the table and along the grid of holes in the table. You can check this by measuring the beam position on a ruler at various points in the path. Finally add the lens to the rail. If the alignment by the mirrors is perfect, the beam should go straight through the lens. Slide the lens along the rail until the beam at the back of the objective is slightly smaller than the hole.

D. The final adjustment is to make sure the beam goes straight through the objective. This can be checked with a card behind the microscope slide (you might need to turn off the white light). The image should be bright fringes in an hourglass shape. Adjust the two mirrors until you see this image. Now look at the image on the camera. You should see a circular diffraction pattern that enlarges symmetrically as you change the focus. Adjust the mirrors until you see this. (If you see a red speckle pattern, it means the beam is not going straight through the objective).

E. Turn on the white light again and watch the beads. Adjust the focus until you see beads get trapped in the laser beam. With full power, you will probably see several beads get trapped. Put in the rotating polarizer and turn it to lower the power until you can trap just one bead (note the angle you set). Try carefully moving the slide vertically and horizontally to see if you can keep the bead trapped as you drag it around. (This is how researchers generally use this technique to apply small forces to molecules).

F. Now try to collect some data. Stop the camera in preview mode and click on "frame capture." Make the time between frames 50 ms and set the number of frames to 100. Also chose a directory to collect data (make a new folder for each data set) and click "OK." Now start the camera with both "Preview" and "Frame Capture" selected. Take data for a few seconds and stop. Look at the folder where the data was collected. You will probably need to delete the first frame because it won't have a good image.

G. Open the Scion Image program and follow these steps:

   a. Load Macros
   b. Click Open, find your data and click on the first file, click on "Open All" and click OK
   c. In the Stacks menu, click "Make Stack"
   d. If you want to see a movie of the measurements, click "Animate" in the Stacks menu
   e. Draw a small box around the trapped bead; in the Special menu, click on "Crop and Scale" (scale should be 1).

      f.   Click on "Invert Stack."

      g.   Click on "Correct Background."

      h.   Move your cursor around the image and look at the values on the info window, figure out what values will specify the bead and leave out the background. Click on "Find Displacement" in the Special menu and pick a threshold larger than the background but smaller than the bead.

      i.   In the Edit menu, click on "Copy Measurement" and paste the data into Kgraph.

H.  Make a histogram of the x or y values of the displacement. If the histogram does not look like a Gaussian distribution, try adjusting the threshold in Scion and make a new displacement measurement. You can also try making the crop of the image smaller (the bead should take up most of the image). Find the variance in this histogram.

I.  Repeat steps F and G for at least two other power settings. The exact power in the trap can't be measured accurately but you can get a scale of the power by using the angle of the polarizer. Find the angle of minimum power, then turn 90° to get the maximum. Assume this power is 30 mW. The relative power of your other measurements are $30\cos^2(\theta)$, where $\theta$ is the angle between maximum power and your measured power (remember $P \sim <E^2>$).

J.  Plot the variance vs. 1/P. **Q3.** What does the slope of this curve tell you?

Appendix

For a particle with a radius much larger than the wavelength of light, the trapping force can be treated with geometric optics. In the figure below, the rays from the focused laser beam enter the beam at some large angle relative to the normal. If the index of refraction of the bead is higher than the surrounding medium (water), then some light will be refracted, producing a downward force, and some light will be reflected, producing an upward force. These forces are equal if the bead is centered in the focus of the beam; however, a restoring force is exerted if the beam or bead are displaced in any direction. Therefore the trap can be treated as a spring with a spring constant $k$ proportional to the angles of the rays entering the bead (how tight the focus is) and the total power of the beam.
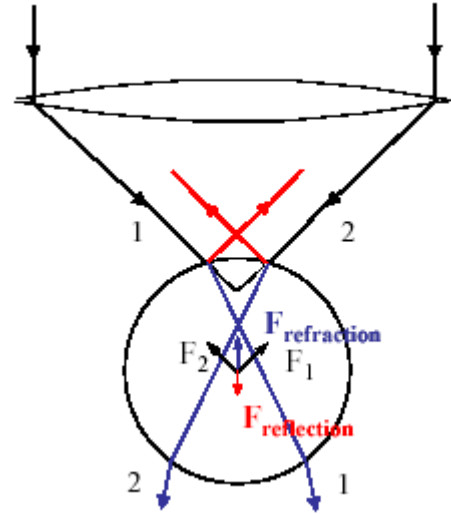
For a particle with a radius less than or equal to the wavelength, you can treat the system as a collection of dipoles where the energy of the particle is proportional to the energy density (or light intensity) of the trap. A large gradient in the energy density gives a gradient in the energy which also produces a spring-like restoring force. The particle can be visualized as trapped in a nearly harmonic potential well. The viscosity of the water acts to damp large excursions of the particle.

Since the bead is in water, even when it is trapped, it will receive random kicks from water molecules, making it jump about in the trap. How much it jumps, or diffuses, depends on the energy of the kicks and the strength of the trap. Since the system is in equilibrium we can equate two energies, the mean potential energy of the particle in the trap and the thermal energy:

$$\frac{1}{2}k_x\left\langle x^2\right\rangle = \frac{1}{2}k_BT$$

where $k_B$ is Boltzmann's constant and $T$ the temperature and $\left\langle x^2\right\rangle$ is the variance of the displacement in the trap in one direction. This model assumes that we can treat the particle as a 1-dimensional, damped harmonic oscillator driven by a random force.