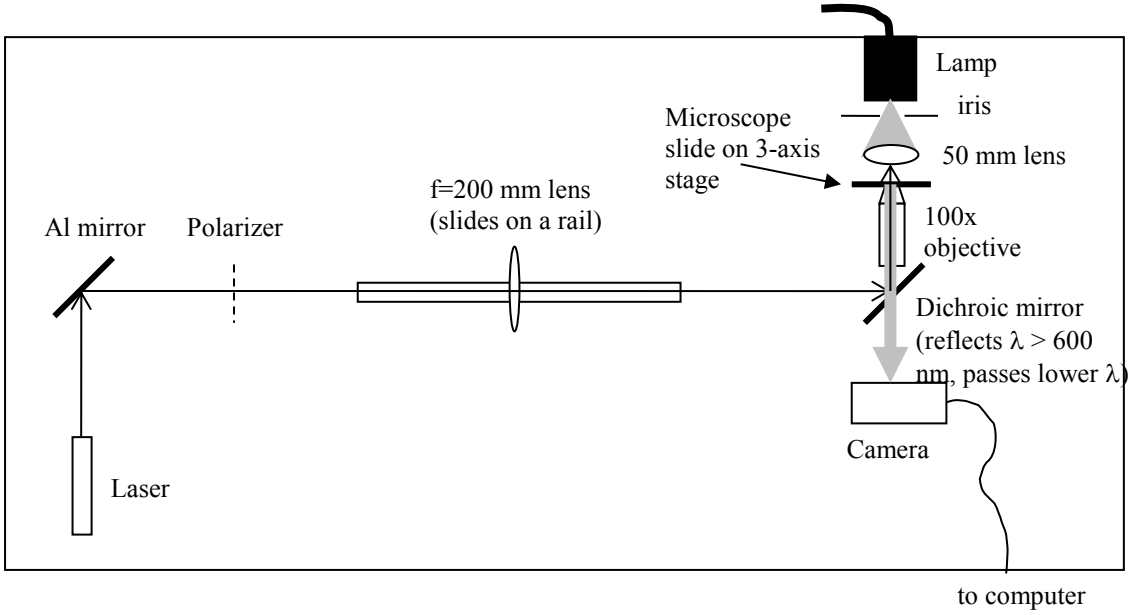
Experiment 10: Laser Tweezers Phys 431

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 looks like this:



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 coverslip facing the objective 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** Why do you need 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. Rotate the polarizer 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. You will need to insert a filter in front of the camera to remove the rest of the laser light. *There is only one filter for the whole class that must be shared for data capture*. 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 ImageJ and follow the steps in the appendix

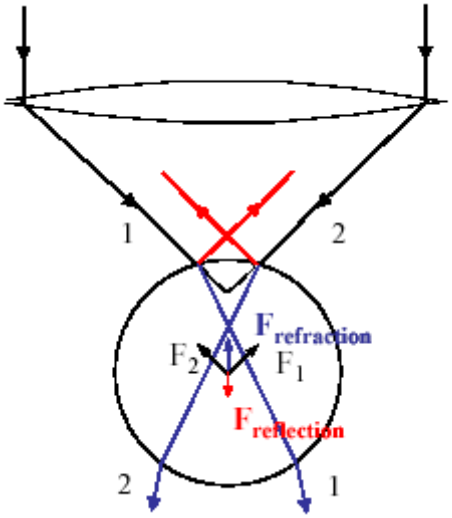
H. Make a histogram of the x or y values of the displacement. It should look roughly Gaussian. If not try adjusting the various parameters in ImageJ or take a new data set. Find the variance in this histogram.

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 measuring the power with the power meter before the dichroic mirror.

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 acute 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 qre equal if the bead is centered in the focus of the beam and a restoring force is exerted if the beam is displaced in any direction. Therefore the trap can be treated like a spring with a spring constant *k* proportional to both 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.

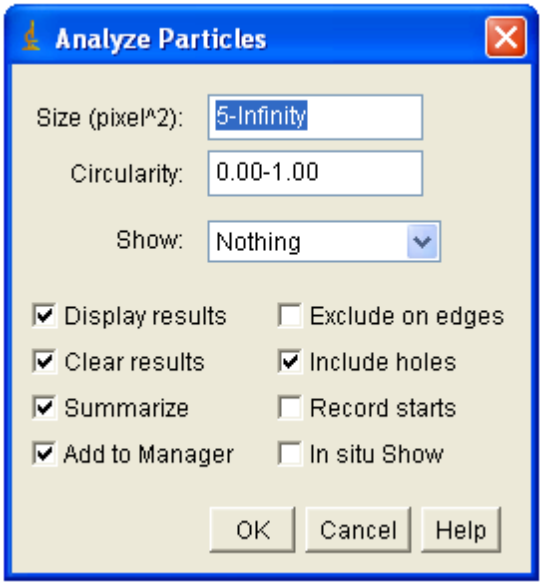
Since the bead is in water, even as it is trapped, it will receive random kicks from water molecules, making it diffuse slightly in the trap. How much it diffuses depends on the energy of the kicks and the strength of the trap. Since the system is in equilibrium we can relate these two forces

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

Using ImageJ

1. Open your images as a "stack" by going to **File -> Inport -> Image Sequence** and selection one of the images in your series. (It doesn't matter which one).
2. If the initial slide looks like garbage, remove it by going to **Image -> Stacks -> Remove Slice**. Check the end of your images as well.
3. Now convert the images into an 8bit grayscale by clicking **Image -> Type -> 8Bit**.
4. Next convert them to black and white binary images **Process -> Binary -> Make Binary**.
5. Now set up the measurements you're going to use by going to **Analyze -> Set Measurements** and selecting Area and Center of Mass. Anything else you want to try is optional, but these two you'll need.

6. Now we want to crop out the part we don't care about. Using the select tool (the button with a square below File Edit etc.) make a selection around the particle you're interested in, and slide through the slices to assure it never leaves your box. You can zoom in and out with Ctrl+ and Ctrl- or through **Image -> Zoom** which will make this easier. Once you have your selection, go to **Image -> Crop**
7. Now we can start using the analyze particle function. However, we want to remove as much background noise as we can, so try a few different tools.
    a. Note, you can't undo these feature, so you may want to save before filtering via **File -> Save As… -> Image Sequence**. Be sure to save these in a different folder so you don't accidentally overwrite your starting data.
    b. Manually Crop more: You can select areas and use Edit -> Clear to remove the stuff in there, this can be good for remove all the other beads that go by.
    c. Remove Noise: **Process -> Noise -> Remove Outliers**. Use a radius of 1 pixel to start, but play around and see what works best. Also, its important to select Dark or Bright outliers correctly. Use preview to make sure it looks good.
8. Once you've removed most of the background and outliers, go to **Analyze -> Analyze Particles**. This will attempt to label particles and count them, as well as do some measurements. Set your options as follows, with the exception of Size, which you can adjust to see what works.



You're going to get a bunch of windows, one of which has a list of data. The goal is to only have around 100 entries in the list, which would correspond to your one particle, on 100 slides.

Now you've got the X and Y coordinants, Select them all and copy them into Excell or Kaleidograph and make a histogram of the positions of X and Y. I rounded my numbers to the nearest decimal to make it easier to group into bins.