1 Prelab

- Draw the path of a single photon when it passes through a circle of glass in vacuum, not along the center axis of the circle (this doesn’t need to be a precise, just a sketch). At each interface, the light should refract. At each of these interfaces, draw the change in momentum of the photon. From this, draw the change of momentum of the circle.

- Now that you’ve done this, draw the circle in a Gaussian laser gradient, such that the circle is not in the center of the Gaussian. Then, repeat the previous exercise with two paths that are symmetric across the center axis of the circle. You should note that paths that originate closer to the center of the Gaussian have a higher intensity, and therefore a greater momentum. From here, you should be able to draw the change in momentum of the circle as pointing to the center of the Gaussian. If you have trouble doing this, check the figures in the theory section.

1.1 Precautions

- The laser used in this lab is extremely powerful, and can cause significant retinal damage and/or blindness. Whenever the keylock is engaged, make sure that the door is shut and that you’re wearing safety glasses.

- **Do not exceed 75V on the Piezo Controllers**, doing so can permanently damage the servos in the stage.

2 Overview

This experiment is an exploration of an optical tweezers kit, and a brief introduction to the use of optical trapping, including measurements of optical trap strength as a function of current, and measurements of the power spectra and Brownian motion experienced by particles whilst in the trap. The particles used for the calibration process are 3 µm glass beads, either free floating (for Brownian motion measurements) or ’stuck’ to the slide (for position calibration). These measurements are then used in a biophysical application: using a trap to capture and manipulate cellular components and vesicles, and measuring the strength of intracellular transport mechanisms.

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1 While you’ll be drawing a 2D Gaussian, keep in mind that the actual laser is a 3D cylindrical symmetric Gaussian, but due to azimuthal symmetry, the forces are the same as they are in the 2D case.
3 Introduction

Optical trapping utilizes the reflection and refraction of photons between different media and their momentum shift as a result of their nonlinear paths as a method of creating a potential gradient well that can hold a particle stationary in an x-y plane, applying a force on the order of piconewtons. The gradient is generated by a focused laser such that the center of the laser has a greater intensity than the periphery. The geometry of a glass sphere is such that the light rays passing through it on the right side will cause it to refract to the left, and the inverse for the left side, creating an equilibrium point at the center of the trap. The applications of this method are primarily in biology and biophysics, where a piconewton-strength is useful for surgically manipulating cellular organisms.

The apparatus allows for variable laser intensity, so the primary work done is calibrating the laser, taking measurements of known particles to determine the strength of the trap in relation to the laser intensity, and applying this data to understand both thermodynamic systems (making measurements of Brownian motion to determine Boltzmann’s constant, $k_B$), and biological systems, in our case, an onion cell.

Vesicles, small sacs containing organic material within a cell, are transported throughout the cell using an intracellular transport system that utilizes the cytoskeleton in addition to several "walking" transport proteins. By using the laser trap to capture the vesicles, with a known strength, we can estimate the force generated by these walking proteins.

4 Theory

4.1 Optical Trapping

Laser beams are highly coherent light sources that can impart force onto particles through transfer of photon momentum. Laser light can be thought of as a beam of particles that will change in momentum when they refract through, or are reflected by, a surface. Stronger laser intensity corresponds to an increase in the number of incoming photons, and thus a larger force exerted on the surface. In this case, spherical glass beads reflect and refract the light from a focused laser in such a way that the beads experience a restoring force towards the center of the trap location.

More precisely, a high numerical aperture lens brings the laser beam to a sharp focus at the location of the trap. The ray tracing diagram in Figure (1) is a two dimensional simplification of the trajectories of the light rays incident on the bead. Some portion of the incoming light will be reflected perpendicular to the surface of the bead. The light that passes through the glass-medium interface is then refracted at a slightly different angle, and refracted again as it leaves the bead. Each time the light changes direction, conser-
vation of momentum dictates that the resulting change in momentum of the glass bead is equal and opposite. For a bead located in the center of the trap, these forces exactly cancel, and the bead feels no net force.

Once the bead becomes displaced from the center of the trap, the angles of reflection and refraction are slightly changed, such that there is a resultant net force towards the center of the trap. Figure (2) illustrates this net force. Since the incident angle of the beam is determined by the numerical aperture of the lens, it is easy to see how a larger numerical aperture corresponds to a stronger trap. Notice also, that the laser beam’s final angle is changed as a result of the placement of the bead. This becomes important later, as it allows us to measure the position of the bead, based off how much the laser is deflected from its resting position.

Figures (1) and (2) are both simplifications of the trapping mechanism. An important factor that is not visible in these reductions is that the intensity of the beam varies across the location of the trap. In general, a circular laser spot fits a Gaussian intensity profile. This means that when the bead is moved away from the center of the trap, as in Figure (3), the ray that passes through the right side of the bead has a stronger intensity than the ray passing through the left. This implies that the force contribution of this ray is stronger, and even with a parallel incoming beam, there is a net restoring force on the bead.

Figure (3) is yet another simplification as it shows parallel incoming beams rather than a focused point. In reality, the trap operates with a combination of these two effects. It can be seen in Figure (3) that given parallel incident beams, the bead will experience a slight forward force. The high numerical aperture and large angle of incidence compensate for this effect, such that in the end, the bead feels a net restoring force in both the lateral and axial directions. A more realistic view of the trap location can be seen in Figure (4), which shows both effects at play.
The properties of the trap are a function of the laser power, numerical aperture, bead diameter, bead composition, as well as the optical properties of the surrounding medium. Since the force on the bead is very nearly proportional to distance from the center of the trap, the system can be modeled as a linear spring, with spring constant $\alpha$. This allows us to determine the strength of the trap without knowing the precise nature of each of these factors. If the position of the bead is $x$, we can model the movement of the bead in the fluid as a driven, damped harmonic oscillator. Brownian motion in the fluid causes particles to collide with the bead in the trap, driving its displacement from the center. The trap forces act to restore the bead to the center, and the viscosity of the medium resists this motion, and thus dampens the system. Overall, the average kinetic and potential energy of the bead is given as follows in Equations (1) and (2),

$$\langle E_k \rangle = \frac{3}{2}k_BT$$
$$\langle E_p \rangle = \frac{3}{2}\alpha \langle x^2 \rangle$$

where $\langle E_k \rangle$ is the average kinetic energy, $\langle E_p \rangle$ is the average potential, $T$ is the temperature, $\langle x^2 \rangle$ is the variance of the particle’s distance from the center of the trap, and $k_B$ is the Boltzmann constant. For a harmonic oscillator, we know that $\langle E_k \rangle = \langle E_p \rangle$, and we can solve for the trap strength $\alpha$.

$$\alpha = \frac{k_BT}{\langle x^2 \rangle}$$

Thus, by measuring the time averaged variance of a bead in the trap, we can determine the strength of the trap $\alpha$. In addition to predicting the variance of the bead, the theory of Brownian motion also tells us what we should expect the power spectrum of these variations to be [1]:

$$P_x(f) = \frac{P_0f_0^2}{f^2 + f_0^2}$$

The power spectrum is found by taking the Fourier Transform of the variations in position measurements. By Parceval’s Theorem, the area under the power spectrum is equal to the expectation value of $x^2$. Combine this with Equation (3), and do some dimensional analysis to derive the values of $P_0$ and $f_0$ in terms of $k_B$, $T$, and $\alpha$.

$$\langle x^2 \rangle = \int_0^\infty \frac{P_0f_0^2}{f^2 + f_0^2} = \frac{\pi}{2} P_0f_0 = \frac{k_BT}{\alpha}$$

$$P_0 = \frac{2k_BT}{\pi \alpha f_0} \beta = 3\pi vd \quad f_0 = \alpha/2\pi \beta$$

Figure 4: A more accurate representation of the trap location. Gradient forces and ray angles both play a role in keeping the bead in the center of the trap [1].
where $d$ is the diameter of the bead (3 $\mu$m in our case) and $\nu$ is the viscosity of the medium (which is probably water). Thus, by finding the power spectrum of the variations for a trapped bead, a fit of the curve to the expected function in Equation (4) can be made, and used to determine values for $P_0$ and $f_0$.

Figure 5: This figure shows a log-log plot of the expected power spectrum for various trap strengths $\alpha$. $P_0$ corresponds to the $y$ axis intercept, and $f_0$ corresponds to the frequency where the spectrum begins to slope downwards.

With fit parameters for variance of position measurements, it’s straightforward to calculate trap strength from Eq. (6) and then use the variance measurements to estimate a value for Boltzmann’s constant $k_B$.

### 4.2 Intracellular Transport

In this experiment, the apparatus takes advantage of the refractory properties of vesicles, a cellular organelle used for transport, and their interactions with the optical trap. These vesicles, which are essentially sacs of some material, are transported using a protein complex, which attaches and detaches from the cytoskeletal microtubules. The strength of this complex can be approximated to a constant force along the microtubule. The vesicles are approximately spherical, and thus can be captured by the optical trap. By adjusting the force applied by the laser (modulating the laser current), trapping the vesicles with a known trap strength can be used to determine the strength of the transport mechanism.
5 Apparatus

Figure 7: A simplified beam diagram of the optical tweezers kit

The apparatus of this device is relatively simple, with most of the apparatus being a basic microscope, with an ultrabright blue LED shining light through a condenser and through the sample, passing through an objective and into the CCD camera. The laser is passed through several beam modifying lenses, and bounces off of a dichroic mirror through the microscope, after which it’s reflected off of another dichroic mirror, and focused onto the quadrant position detector (QPD). Any refraction of the laser will result in the laser moving across the QPD. The QPD is simply 4 separate photo detectors in a quadrant orientation.

Using this, the refraction of the laser can be calculated in terms of x and y coordinates. Knowing that the sample will refract the laser by some significant amount, these readings can be used to measure the samples location in real time as a function of the readings from this QPD.
6 Procedure

Setup

1. Ensure that everything you need to begin is present. Make sure the following has been completed:
   - You have the two samples (free and stuck bead samples) prepared by the TA or instructor
   - The computer, controller hub, and laser are all powered on
   - The camera, OTKB-CAL, and APT-User software all open
   - The Piezo Controllers are configured in the APT-User software as specified in the user manual for the OTKB-CAL

2. Carefully insert the free bead sample capillary-side down into the stage. Place a drop of oil onto the objective, and use the z-axis knob to carefully lower the slide down onto the objective. Watching the camera feed on the monitor, lower the slide slowly until you are in focus with the beads. **Do not** lower the sample too much, or the objective will break the slide.

3. Close the door, put on safety glasses, turn the keylock, and enable the laser. You should be able to capture a free-floating bead. Once you’ve identified the location of the bead, use the drawing tools in the camera software to mark the location of the trap on the screen.

Calibrations

![Calibration Graph](image)

Figure 9: The calibration graph reading raw QPD data into x-y data for the bead

4. Now that you’ve located your trap, you need to complete a position calibration so you can associate data from the QPD with the position of the bead in relation to the trap.
To do this, replace the free-floating sample with the stuck bead sample, and repeat the previous procedure to focus the objective on the beads.

5. Once you’ve focused your sample, center the trap on one of the beads. Close the door, equip eye protection, and enable the laser again. In the OTKB-CAL software (shown in figure 9), click on the “Run Calibration” button. This has the stage scan over a certain range in the x, then y direction, centered on the bead, and records the data read by the QPD which is displayed below.

6. Due to the diffractive nature of the silica beads, there should be a linear relationship between the recorded voltage, and the x-y positioning in the beads. Using the blue sliders, isolate the linear section of the data. Now, the QPD will translate the read data into x-y data for the beads. Note that this calibration is for the assumption that the bead remains relatively close to the center of the trap, as the linear approximation does not hold for large distances.

6.1 Measurements

![Figure 10: A photo of the bead, trapped, next to two untrapped beads](image)

**Note:** You will want to run the following experiment several times

7. Now that you’ve calibrated the bead position, you can start taking data. For measuring the trap strength, you need to measure the variance of the particle’s distance from the center of the trap, as well as the temperature, but we can assume that it’s relatively close to room temperature. To measure the particle’s position as a function of time, use the Data Recording tab of the OTKB-CAL software, which will stream QPD data as a function of time to a file, which you can later use to determine $\langle x^2 \rangle$ with Python, or some similar data analysis tool.

8. To calculate the power spectrum of the bead in the trap, use the “Force Calibration” section of the software. Collect the power spectrum of a free-floating bead undergoing Brownian motion. In python, you can fit the function to the expected values using
your results from the previous section for trap strength $\alpha$ using equation 4. From this fit, you should be able to pull out a value of $k_B$.

9. Take multiple measurements at different laser intensities to measure a linear relationship between $\alpha$ and laser intensity.

### 6.2 Biophysical Application

10. To examine a known system, first create a biological sample to be used in the observation. The suggested sample is a fresh onion, sliced open, with a peeled layer, and placed onto a slide with a slim slide cover with a biological saline solution provided.

![Figure 11: An image of the onion cell, with the round vesicles traveling along the cytoskeleton](image)

11. Make several attempts to manipulate and trap the traveling vesicles, and make note of laser intensity, and therefore the strength required to hold them in place.

### 7 Data/Analysis

Calculating the power spectrum of the variations in position measurements at various laser intensities to compare values of $f_0$, and determine $\alpha$ as a function of laser intensities. Using these values of $\alpha$ and calculated values of the variance $\langle x^2 \rangle$, estimate values of $k_B$.

### References

[1] MIT Department of Physics, *Optical Trapping*, Massachusetts Institute of Technology

[2] MIT Department of Physics, *An Introduction to Optical Trapping*