

# Laser Particle Trapping and Brownian Motion

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## I. INTRODUCTION

An optical trap or "optical tweezers" is a device that can trap and move micrometer sized dielectric objects. These traps use highly focused light beam to apply forces on these beads. Optical trapping serves as a major tool in biophysics, such as unzipping DNA or manipulating cellular components, NASA Science, such as pulling dust out of a volcano, or pulling high density residue off of comet tails not accessible by astronauts, and other equally interesting applications. Additionally, since our trap 'strength' is a function of our laser intensity, we are able to change trap strength therefore measuring the Brownian motion of a particle in a viscous fluid.

This method of trapping was first discovered accidentally by a physicist names Arthur Ashkin in 1970. Ashkin thought that radiation pressure from a high power beam of light focused onto a single bead would propel the bead forward. While this was true he also realized that other beads would be attracted to the beam and follow suit.

In this lab, we not only show this property but also seek to find Boltzmann's constant using several different methodologies.

## II. THEORY

### A. Optical Traps

Although seemingly counter-intuitive, light can impart a force on things in its path due to the principle that photons carry momentum. We can recall this by remembering that photons are massless but still follow the equation  $E^2 = c^2p^2 + m^2c^4$  which leads us to  $E = pc$  which relates energy momentum and the speed of light. Although these forces can be small they can be very large relative to forces that typically act on single small particles at micro and nanometer scale. Using a focused laser beam, incident photons can impart force or to trap these particles.

We must first however understand the change of momentum of light that is scattered and refracted in the dielectric material or in this experiment a glass silica bead. For objects larger than the wavelength of the laser, light will refract through and scatter off of the particle causing an equal and opposite force to be imparted on the bead. Drawing a ray-tracing diagram, we can argue that the scattering force caused by this momentum is in the direction of light propagation while the refracted light creates an opposing force due to conservation of momentum. We may also notice that because of this refraction and scattering, the beams final angle if the bead is not centered will be slightly at an angle when recorded by our software. This is important because it allows

us to measure position of the bead relative to the initial trap placement based on the lasers deflection. When the bead is centered in the trap all forces cancel leading the bead to reach an equilibrium state in the 'waist' of the laser<sup>[1]</sup>. Essentially, the glass beads experience a restoring force towards the center of the trap. (See figure 1). We also realize that the laser is more intense towards the center, following a Gaussian intensity profile, therefore always creating a stable equilibrium potential well at the center of the beam.

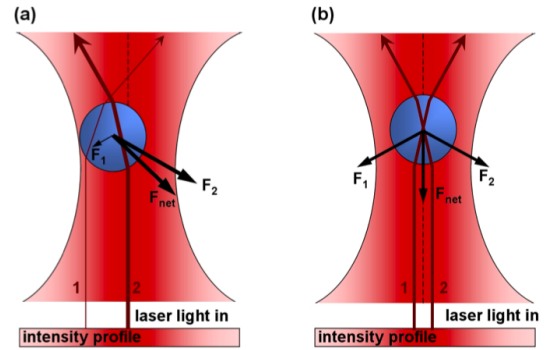


Fig. 1. A Schematic of the Imaging and Trapping Apparatus.

### B. Measuring Trap Strength and Boltzmann's Constant

The trap strength is a function of laser intensity, numerical aperture, bead diameter, bead composition, and optical properties of the fluid containing the particles. Luckily for us, we can model the system as a linear driven damped spring oscillating about the center of the waist of our laser with spring constant  $\alpha$ . In this way we are able to determine the trap strength only by measuring particle perturbations. These perturbations are caused by Brownian motion in the fluid which causes particles to collide with the bead in the trap therefore knocking it away from the trap center. A restoring force (the laser) acts to recenter the bead while a damping viscous force in the fluid acts to dampen the system.

To understand the interactions of our 'spring' and our viscous fluid, we must first revisit the thermodynamics which govern the movement of the particle trapped in the photon stream. We recall the ideal gas law

$$PV = nk_bT$$

<sup>1</sup>This only really holds for particles considerably larger than the wavelength of the light, which in our case isn't true. Our 1 $\mu$ m bead is only slightly larger than our 975 nm laser. Therefore we must treat the bead as a point dipole and Rayleigh scattering theory must be used which details the absorption and reradiation of light by the dipole interacting with an inhomogeneous electromagnetic field. In our case, we will ignore this and simply use ray optics because it works experimentally.

where  $P$  is the pressure in the volume,  $V$  is the volume,  $n$  is the number of particles present, and  $T$  is room temperature at which we keep our sample. We can immediately understand using our trap to find Boltzmann's constant based on the ideal gas law would be ridiculous. We could never innumerate the particles in the liquid or really find the pressure of the contained liquid. We are able to use instead the intrinsic connection between temperature and kinetic energy revealed through the perturbations of the gas due to fluctuation forces imparted by the gas. The equipartition theorem holds that each degree of freedom in a physical system at thermal equilibrium will have energy equal to  $\frac{1}{2}k_bT$ . Additionally, we know the energy of a harmonic oscillator  $\frac{1}{2}\alpha x^2$  where  $x$  is the displacement from the center of the trap. In three dimensions with three degrees of freedom we have that the constant  $\frac{1}{2} \rightarrow \frac{3}{2}$  and that

$$\alpha \langle x^2 \rangle = k_bT$$

. Here, instead of using the displacement of the particle we use the statistical variance in the position  $x$  due to random Brownian motion. So, by measuring temperature, variance, and trap strength, we are able to measure Boltzmann's constant.

In addition to finding the variance of the silica sphere, we also are able to find the power spectrum of these variations from the theory of Brownian Motion. This is given by

$$P_x(f) = \frac{P_0 f_0^2}{f^2 + f_0^2}$$

. The power spectrum is found by taking the Fourier Transform of the variance in position measurement. Additionally Perceval's Theorem tells us that the area under the power spectral density is equal to the expectation value of  $x^2$  or the variance. By solving the transform and using dimensional analysis we reach

$$\langle x^2 \rangle = \int_0^\infty \frac{P_0 f_0^2}{f^2 + f_0^2} df = \frac{\pi}{2} P_0 f_0 = \frac{k_bT}{\alpha}$$

where  $P_0 = \frac{2k_bT}{\pi\alpha f_0}$ ,  $\beta = 3\pi\eta d$ , and  $f_0 = \frac{\alpha}{2\pi\beta}$  where  $d$  is the diameter of the bead, 3 micrometers, and  $\eta$  is the viscosity of the medium, water. We find the power spectrum of the variations and fit them to a curve as expected to determine values for  $P_0$  and  $f_0$ . ..

### C. Measuring Forces in Microbiology and Intracellular Transport.

In the cell, organelles are transported over large distances by tiny walking creatures called myosin motors. These motors walk along small microfiber filaments, called microtubules and often look adorable if watched<sup>2</sup>. These adorable crawlers are attached to cargo vesicles in order to transport them from one place in the cell to another. We are able to determine the strength of this complex as a constant

<sup>2</sup>After showing these crawlers to my non-STEM friends I have been told this assumption that they look adorable to all may be exaggerated. In fact, the general consensus was that they actually look like White Walkers from Game Of Thrones.

force along the microtubules. The vesicle sacs being carried have optical refractive and geometric properties for trapping and we are able to trap these vesicles using our techniques, probably sending the cell into utter panic because some plant-god like force has just taken an important(probably) thing right out of its hands(or myosin motors). We may adjust the force applied by the laser to watch for when we are able to trap these sacs to determine the strength of the motors and in that sense, the entire transport mechanism.

### III. APPARATUS

For optical trapping we desire a set up that provides an intense laser beam that is focused at a desired point within a sample cell. The setup also allows visual imaging of the organisms, and quantitative measurement of the position of the particles based on the deflection angle of laser light as described below.

#### A. Light Sources and Optical Traps

In this experiment we use two light sources, a 975 nm coherent laser beam and a white LED used for observation and illumination of the sample. We choose a wavelength of 975 nm because it is far from typical absorption lines in most biological specimens, it also isn't too expensive. The output of the laser is controlled and monitored by an output current specified by the user. It ranges between 100mA and 400mA. The trapping force is clearly related to the intensity of the laser beam because more photons are inducing a change in momentum on particles.

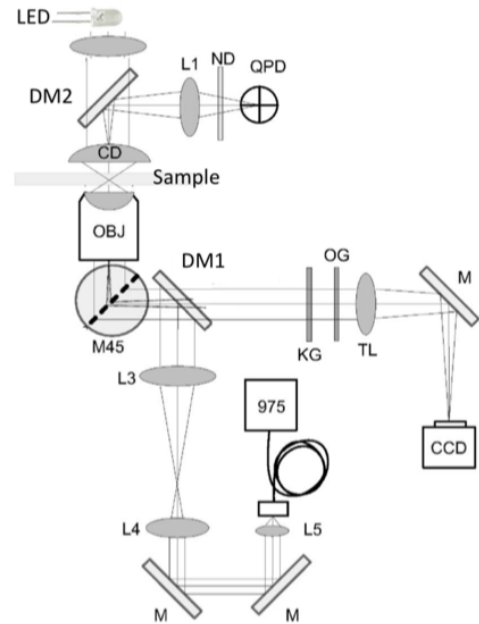


Fig. 2. A Schematic of the Imaging and Trapping Apparatus

Fig 2. describes the optical set up. The beam from the 975nm laser beam is first collimated, or made straight and calibrated, by a lens (1). The beam is then reflected into two mirrors by a set of expanding lenses (2). This light is then

reflected by a "hot mirror" which only reflects infrared wavelengths, allowing visible light to transfer through the lens thereby 'leaving' out setup(3). A turning mirror then focuses the light on our inverted oil immersion Nikon 100X oil objective which focuses the beam to 1.1 micrometer focus to a position between the cover slip and glass slide where the particles are (4). The reflected and scattered light that governs the optical trap is then re-collimated and focused by a condenser(5). This beam is then reflected by another 'hot mirror', and attenuated by a neutral density filter which refocuses light onto the QPD, the quadrant photo detector to be described which we use to measure position(6). Additionally, White light is emitted from an LED to visualize the sample by being filtered and focused onto a CCD camera(7).

This experiment is dependent on knowing the position of the scattered beam and the sample. The position of the sample is determined by the stage while the laser beam is determined by the QPD (Fig 3). The QPD is a semiconductor photodiode which is segmented into four quadrants. An electric circuit embedded with the QPD, with difference amplifiers, computes differences between the quadrants, for example, to find the X position we find the difference between the sum of the intensity on the left and right of the QPD and then normalize based on total intensity. This information provides information about the position of the light beam relative to the center of the QPD. If the beam is centered after calibration, the total voltages when examined as above we should arrive at zero output voltage. If we know the beam displacements for a certain voltage the computer mechanism is able to translate any voltage measurement to any distance. The QPD also acts at an extremely fast frame rate allowing us to monitor Brownian Motion.

### B. Microscopic Stage

The microscopic stage has three degrees of freedom and can be controlled manually and electrically, finely and coarsely. The overall range of these adjustments is 4mm. The stage can be controlled using little knobs on the sides of the stage as well as by piezo controllers. These are driven by high voltage controllers based on feedback from strain gauges, which convert voltage output to displacement.

### C. Samples

The sample used is created by first mixing the proper solution then using a small flow channel to suck up the solution using hydrodynamic forces. This is then glued down to a slide and set to dry. In our experiment we use both a stuck sample, a free sample, and an onion sample. 3.21 micrometer Silica is obtained from the chem lab and is diluted by a factor of  $\frac{1}{100}$ . The fixed silica sample is made by mixing a bead sample containing 3.21 micrometer silica glass beads ( $SiO_2$ ) with NaCL buffer which shields the intrinsic repelling surface charge of silica so that electric forces can bind the silica to the glass channel. These sample is used to calibrate the QPD and general apparatus. The free sample contains the same silica beads this time mixed only with

$H_2O$  because surface charges repel the particles from the surface of the tube, particles can float around freely, and therefore can be trapped optically! For both samples we attempt to make a dilute enough sample so that we only have one particle on the screen at a time. The biological sample finally, is a monolayer cell of onion with drops of saline solution held under a cover slip. All samples are held with glue and dried for several minutes by a UV light.

## IV. METHODS

### A. Setup

1) *Samples*: We begin by preparing the samples which we will examine as discussed in our apparatus section. We then check if all samples allow us to view only one bead in the frame at a time so that we can gather data appropriately.

2) *Software*: We power on the OTKB-CAL and the APT-User from which we control the experiment. We also assure the camera and piezo controllers are on.

3) *Laser*: The laser is powered on whenever any measurements are being done. To power the laser on, we turn the key at the back of the laser controller and make sure that the temperature control is on. We choose the intensity of the Laser in mA. We then switch the laser on. At all times when the laser is on we will be wearing safety goggles so we don't sizzle out eyes.

4) *Piezo Calibration*: In the APT user tab we select setting and for each piezo controller window choose the appropriate settings as defined in the manual provided by ThorLabs. Sometimes in our experiment, the piezo controllers get stuck which leads our calibration to be faulty, so, we have to watch these controls often.

5) *Position Calibration*: We acquire data from the QPD detector in volts, which is not particularly useful to us. We therefore need to transform it to data on particle position and force on the particle by the trap. We must determine the detector 'responsivity' factor. To do this we place our free sample on the viewing area making sure that the laser light is aligned with the sample. We very slowly lower our sample onto the stage until the beads are in focus and their holograms are visible. We then activate the laser on, turning the trap on making sure to trap only a single bead. We draw a circle in the APT user signifying where the trap is. We then place a stuck sample on the stage and with the trap off and after moving our trap circle over a single bead run the calibration. This records the detector voltage while steps of known size are used which then allow plotting of the position signal in volts versus the position signal in microns. When the bead moves across the trap position a graph is formed as shown in Fig 4.

We then choose a linear range signified by the blue bars to choose the conversion factor of voltage to position. Unfortunately, our calibration was extremely noisy, and may have influenced our data. The number of steps was increased however this only made the data recording more noisy. This issue was not able to be solved. This calibration must happen every time the apparatus has been turned off.

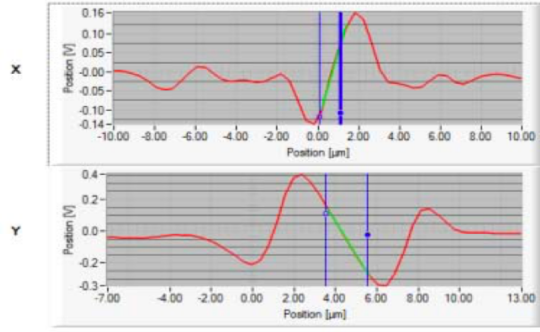


Fig. 3. Calibration Fit

6) *Measuring Boltzmann's Constant Using Trap Strength.*: After calibrating the trap position we are able to begin the actual experiment. We begin by measuring trap strength in relation to laser intensity. To do this we look for the variance in particle position as a function of laser intensity and temperature, which we assume to be room temperature. We measure the particles position as a function of time using the Data Recording tab of the OTKB-CAL software which saves QPD data in X and Y from which we are able to determine variance. We use laser intensities ranging from 50mA to 150mA. This intensity is determined to be best experimentally because all frequencies above 150mA are too strong, which allows no decipherable variance, while trap strengths too low are not strong enough. We suspect that with higher laser intensity, trap strength should increase and therefore, variance from the trap decrease!

We must also find the power spectrum of the bead to a value of frequency which allows us to determine a value for trap strength for which we can find a value for  $k_B$ . To do so, we trap free floating beads under laser intensities from 90mA to 110mA and use the Force Calibration tool to gather our data.

### B. Biophysical Application

We examine an onion layer under the microscope to look for tiny vesicles which we can trap within the cytoskeleton. We observe the effects of our trap on these particles for different trap strengths ranging from 40mA to about 250mA. Sophie and I then tried to play a game where we saw how far we could move vesicles inside the cell without them escaping our traps. Poor onion.

## V. ANALYSIS

### A. Determining Boltzmann's Constant and Trap Strength as a Function of Laser Intensity

As we increase laser intensity, we expect that trapped particles should move freely less and less. In other words as we increase the force we exert with our optical trapping beam the variance of the trapped particles should decrease. We should be able to check this by extracting an x-y position measurement from the QPD and finding the standard

deviation from our zero point for different laser intensities. These laser intensities are chosen to run from 50mA-250mA, the full range of practical laser intensities. These values are found below. For this part of the experiment, we may not ignore uncertainty, but not pay much attention to it, since when calculating the standard deviation, the error that comes from x-y data does not really effect our final result since we are looking for a standard deviation from the norm and not a general movement of the particle.

TABLE I  
PARTICLE VARIANCE AS A FUNCTION OF LASER INTENSITY

Laser Intensity (mA)	Particle Variance ( $\mu m^2$ )
50	0.02008
60	0.03351
70	0.03871
80	0.04547
90	0.04975
100	0.06454
110	0.08238
120	0.08956
130	0.0992
140	0.11668
150	0.12190
160	0.12729
170	0.13560
180	0.14743
190	0.15186
200	0.16052
210	0.16668
220	0.17878
230	0.19135
240	0.18272
250	0.19272

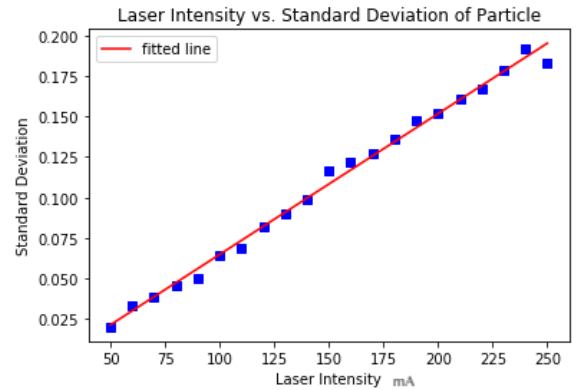


Fig. 4. Laser Intensity vs. Particle Variance

Graphically, we may realize this relationship shows that particle variance increases with laser intensity! We recall that trap strength is related to particle variance by

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

. We realize here that this is antithetical to our data since trap strength is inversely proportional to variance,  $\alpha = \frac{1}{\langle x^2 \rangle}$ . This is confusing because not only does our data seem exactly opposite of what it should be, but also is extremely linear. Lets consider what the problems may be.

## B. Why isn't laser intensity experimentally correlated with Trap Strength

Let's return to how optical traps were discovered. Ashkin, a scientist in 1970 who attempted to understand the optical properties of radiation pressure accidentally discovered that he not only could push small particles with beams of light, but also realized he could trap these beads. Ironically enough, radiation pressure may be the reason our bead is failing to be systematically trapped. Proposed by Ashkin, and later confirmed by Chaumet and Nieto-Vesperinas in 2000 was a theory to compute the force due to light upon a particle on a dielectric plane by the coupled dipole method. This theory said that light imparts a force on dielectric material spheres like silica, as modeled by

$$\vec{F} = \frac{1}{2} \text{Im} \alpha E \nabla E^*$$

where  $\alpha$  is the dipole polarizability of the silica sphere or dielectric material through which light propagates modeled by  $\vec{d} = \alpha \vec{E}$  which has both a real and an uncompromisable imaginary part proved by Draine. We also know, from groups like the David Grier group at NYU that a light wave can be modeled in three dimensions as

$$\vec{E} = \sum u_j e^{i\phi_j} \hat{e}_j$$

. Using these two equations together we calculate a formula for the radiation pressure imparted on a bead by a beam of light.

$$\vec{F} = \frac{1}{2} \alpha' \nabla U^2 + \frac{1}{4} \alpha'' U^2 \nabla \phi$$

We also know that  $\vec{F} = mg\hat{z}$  due to the force of gravity giving us

$$\frac{1}{2} \alpha' \nabla U^2 + \frac{1}{4} \alpha'' U^2 \nabla \phi = mg\hat{z}$$

. Wait but what does all this mean? It means, that for a heavy particle like silica, which weighs approximately 160fN, large for a small particle, the bead may not be properly trapped in the center of the beam. If for example, the gradient second order term of the dipole force is larger smaller than the force of gravity, which it most likely is for such a particle, we may see that the bead is actually lower than the true center of the trap. Additionally, as the laser intensity increasing, the bead may slowly be moving up the trap, which may lead to the particle physically going through different available ranges of motion which are not only correlated to laser intensity! This is a complicated issue because for one, a lighter bead might just should up out of the trap and just lead to worse measurements. Additionally, to fix this problem we would need to calculate an optical bead weight and locate a bead with the proper dielectric constant and weight.

We can actually experimentally confirm this and I would recommend it be added to future labs. If I knew this information before the lab, I would have chosen to take image data of the particle in a trap at 50mA and 250mA and compare both the images to see if the holographic rings had changed position indicating a change in the z of the particle, this is easy to do using Lorenz-Mie software

available through professor Grier's lab at NYU for particular beads. The theory is extremely complex but the experimental procedures are relative to other labs, simple, if the code is given and explained. I think this would be a great way to introduce holograms and how they work to undergraduates who have never learned this before. Over heating may also be a cause of fluctuations that are not inherently properly related to Brownian motion.

In addition, although I shudder to say it, there may be yet another problem with the calibration conducted to receive experimental data, which is a problem that is not specified in any of the lab manuals but I think should be investigated further and may be why its so hard for all groups to get good data here. The problem with the calibration here might be that when the sample is switched between stuck and free bead, the position of the stage may be moving slightly, aka skewing the QPD measurement. Even if the QPD has been properly calibrated, any slight movement in the stage, via the manual knob or the piezo might lead to the QPD converted values actually sitting on and around a saturated value of the linear fit, this may lead to the QPD reading large particle fluctuations as not just fluctuating around its own zero, but also the saturated peaks zero leading to lower values associated with the QPD, aka, one side of the position measurement in either x-y is skewing data. We would expect if this is true, when particle variance actually increases the QPD reads off values that are decreasing because of this saturated peak, where before, the variance didn't even reach this peak. This problem may be solved by enforcing a strict no adjustment to the manual knobs after the system is calibrated rule. I'd be happy to discuss all of this further if it means the experiment can run smoother in coming year. I'll be here for the Summer! Additionally, this would mean a cleaner data taking process over all since if the manual knobs can't be touched, there is no room for the apparatus to break once the actual data taking process begins.

### 1) Particle Strength derived from the Power Spectrum:

Luckily, we recall that we may use a different data collection method to not only find trap strength but also find Boltzmann's constant and that we will not be penalized for cherry picking the data we want to use because people have done this before us.

We may extract  $\alpha$  from the power spectrum of the variations of the particle. We collect power spectrum data for laser intensity values of 50mA-250mA in increments of 20mA to enhance the trend. We may fit out data extracted for each intensity to a fitted logarithmic curve for  $\frac{P_0 f_0^2}{f^2 + f_0^2}$  and from this observe values for  $P_0$ , the y axis intercept of the magnitude of the power spectrum and  $f_0$ , which is the cutoff frequency for which the spectrum begins to slope downwards. We from this can find alpha using  $P_0 = \frac{2k_B T}{\pi \alpha f_0}$ ,  $\beta = 3\pi \nu d$ , and  $f_0 = \frac{\alpha}{2\pi \beta}$  where d is the diameter of the bead,  $d = 3.21 \pm 10^{-4} \mu m$ , and  $\nu = 9.783 \cdot 10^{-7} \pm 10^{-8} \frac{m}{s}$  is the viscosity of the medium, water .

Using the graphs

We can graphically see, aside from a few outliers, a direct

TABLE II  
INFORMATION OBTAINED FROM POWER SPECTRUM

Intensity in mA	$P_0 \cdot 10^{-12}(ms^2) \pm 5 \cdot 10^{-6}$	$f_0 \pm 5 \cdot 10^{-2}(Hz)$	$\alpha \pm 10^{-11}(J)$
50	$2 \cdot 10^{-17}$	4.0	$7.44 \cdot 10^{-10}$
70	$6 \cdot 10^{-17}$	4.2	$7.81 \cdot 10^{-10}$
90	$7 \cdot 10^{-17}$	5.2	$9.67 \cdot 10^{-10}$
110	$7.5 \cdot 10^{-17}$	6.0	$1.12 \cdot 10^{-9}$
130	$2 \cdot 10^{-16}$	6.5	$1.2 \cdot 10^{-9}$
150	$2.1 \cdot 10^{-16}$	6.7	$1.25 \cdot 10^{-9}$
170	$2.2 \cdot 10^{-16}$	6.7	$1.25 \cdot 10^{-9}$
190	$3 \cdot 10^{-16}$	5.0	$9.3 \cdot 10^{-10}$
210	$3.1 \cdot 10^{-16}$	5.0	$9.3 \cdot 10^{-10}$
230	$3.4 \cdot 10^{-16}$	7.0	$1.3 \cdot 10^{-9}$
250	$3.5 \cdot 10^{-16}$	7.5	$1.4 \cdot 10^{-9}$

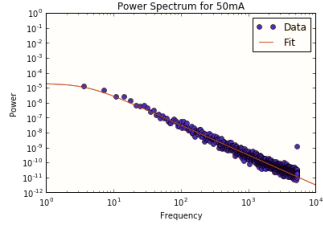


Fig. 5.

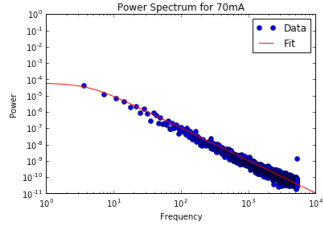


Fig. 6.

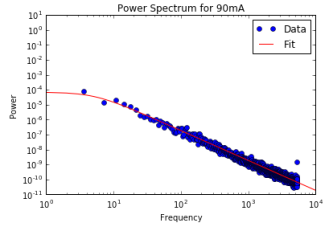


Fig. 7.

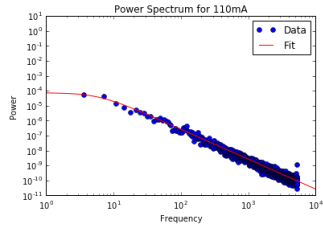


Fig. 8.

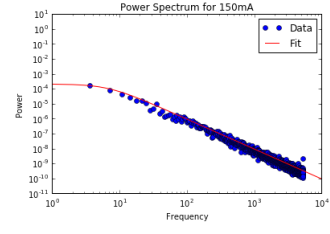


Fig. 9.

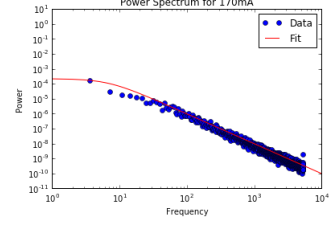


Fig. 10.

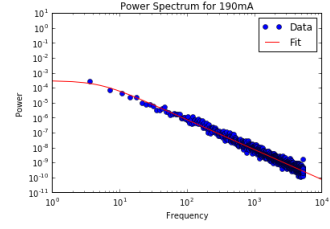


Fig. 11.

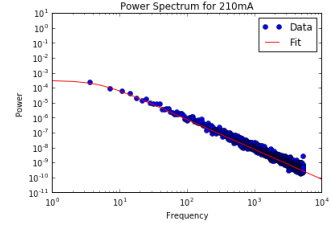


Fig. 12.

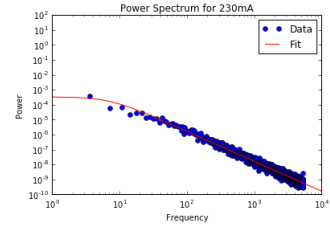


Fig. 13.

After finding these values, we may find a value for Boltzmann's constant again using the derived

$$P_0 = \frac{2k_B T}{\pi \alpha f_0}$$

to find a value of  $k_B$  for each value assuming room temperature.

correlation between trap strength and laser intensity, as is expected. Science is not broken! (At least not that we know of.)



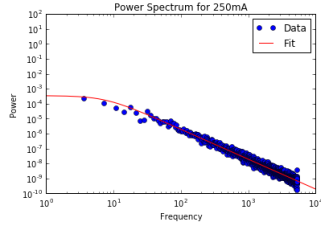


Fig. 14.

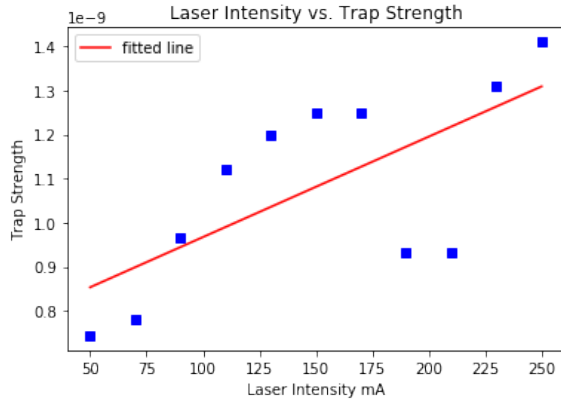


Fig. 15. Laser Intensity vs. Trap Strength collected from Power Spectrum

From this we can find an average value of Boltzmann's Constant of  $k_B = 1.21 \cdot 10^{-25} \pm 10^{-26}$ . This is off from the actual  $k_B = 1.38 \cdot 10^{-23}$  by two orders of magnitude which in relative terms to the rest of our data is pretty adequate, our uncertainty measurement does! If we choose to only average values of 50mA-150mA we are even closer to the actual constant, with a measured value of  $k_B = 6.06 \cdot 10^{-24} \pm 10^{-25}$ , which is within our margin of error!

Seven orders of magnitude is pretty terrible, even with error analysis, but recall that we have, although seemingly skewed, appropriately ranged values for the variance of particles as a function of laser intensity, and have now gathered alpha by using the power spectrum. We may return

TABLE III  
BOLTZMANN'S CONSTANT FROM THE POWER SPECTRUM TRAP STRENGTHS

Laser Intensity (mA)	Boltzmann's Constant ( $\frac{J}{K}$ ) $\pm 10^{-26}$
50	$3.14 \cdot 10^{-25}$
70	$1.96 \cdot 10^{-24}$
90	$1.75 \cdot 10^{-23}$
110	$9.47 \cdot 10^{-24}$
130	$8.26 \cdot 10^{-24}$
150	$9.37 \cdot 10^{-24}$
170	$9.76 \cdot 10^{-24}$
190	$7.39 \cdot 10^{-24}$
210	$7.63 \cdot 10^{-24}$
230	$1.63 \cdot 10^{-25}$
250	$1.94 \cdot 10^{-25}$

to

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

and calculate  $k_B$ . Values are shown below.

TABLE IV  
BOLTZMANN'S CONSTANT FROM PARTICLE VARIANCE

Laser Intensity (mA)	Boltzmann's Constant ( $\frac{J}{K}$ ) $\pm 10^{-15}$
50	$5.25 \cdot 10^{-14}$
70	$1.0 \cdot 10^{-14}$
90	$1.6 \cdot 10^{-14}$
110	$2.6 \cdot 10^{-13}$
130	$3.7 \cdot 10^{-13}$
150	$4.9 \cdot 10^{-13}$
170	$5.7 \cdot 10^{-13}$
190	$4.7 \cdot 10^{-13}$
210	$5.2 \cdot 10^{-13}$
230	$8.4 \cdot 10^{-13}$
250	$9.1 \cdot 10^{-13}$

From this we can calculate an average value of  $k_B = 1.0 \cdot 10^{-14}$ , a whopping 9 orders of magnitude off of the theoretical constant value  $k_B = 1.38 \cdot 10^{-23}$ . We will just ignore this data. And analyze a time series later. We hope this discrepancy is related to inherit problems in our

### C. Error Analysis

As one can tell from our data, the data gathered from this experiment has no physical meaning whatsoever. For one, the extremely sensitive optical table is not properly set up on an air table so it is able to be deterred by subways, elevators, or any walking movements. We realized when taking data that if anything in the lab moved, a huge spike in the data was observed momentarily. With an entire lab course going on during our data taking period, it is impossible to monitor all of these random fluctuations. Additionally, our value for  $k_B$  is simply put, trash. Finally, in observing particle strength as a function of laser intensity for several sets of data, we realize something must be wrong with the way the data is being collected or the experiment is being set up. We were not able to find anyone in the last year and a half who successfully correlated these two things correctly through their data analysis. Although our margin of error helps us to explain the values we received a little bit, we also inherently can not tell what is problematic about the experiment because we have not programmed the experiment or physically built the apparatus. Additionally, we do not have the ability to understand the calibration of the QPD exactly because we do not have access to the circuitry of the QPD and therefore do not know where the exact data is being stored in order to check it. Overall, standard error analysis is used in this experiment.

### D. Optically Trapping Onion Vesicles

Once we have determined trap strength, we are able to determine how much force it takes to trap vesicles inside an onion cell. We are able to, for varying trap strengths, qualitatively describe the effectiveness of our laser traps.

- +250mA:  $\alpha = 1.4 \cdot 10^{-9}+$ : Particles are trapped well only escaping if trap is pushed through a wall.
- 230mA - 210mA:  $\alpha = 1.3 \cdot 10^{-9} - 9.3 \cdot 10^{-10}$  : Larger particles are no longer trapped reliably though small particles are still trapped well.
- 210mA - 190mA:  $\alpha = 9.3 \cdot 10^{-10} - 9.3 \cdot 10^{-10}$  : All particles are trapped however do not remain trapped for long.
- 190mA - 170mA:  $\alpha = 9.3 \cdot 10^{-10} - 1.25 \cdot 10^{-9}$  : Some particles are trapped but are hard to move around.
- 170mA - 130mA:  $\alpha = 1.25 \cdot 10^{-9} - 1.2 \cdot 10^{-9}$  : Particle trapping becomes exponentially worse. Only small particles are able to be trapped reliably and are not able to be trapped for long.
- 130mA - 110mA:  $\alpha = 1.2 \cdot 10^{-9} - 1.12 \cdot 10^{-9}$  : We are able to see very small particles trapped infrequently.
- 110mA - 60mA:  $\alpha = 1.12 \cdot 10^{-9} - 7.44 \cdot 10^{-10}$  : We are only able to push small particles around slightly with optical forces.
- 50mA and under:  $\alpha = 7.44 \cdot 10^{-10} -$  : No effect of the optical trap is noticed.

## VI. CONCLUSIONS

Overall, this lab served as a learning experience. I have a particular interest in optical setups as well as the ruling principles of non-equilibrium thermodynamics so I was excited to perform this lab. The theory of this lab is easy to grasp and conceptually interesting and fun. However, after approximately 9-10 different attempts at a working calibrations and attempting to collect data, only 3 times was a successful calibration received, and of this, only once was data able to be collected. Additionally, part of our experiment, measuring particle variance as a function of laser intensity resulted in counter intuitive results which leads us to question either the data we had collected or the software from which data is downloaded. Luckily, alternative methods derived from Fourier analysis allow us to find a relationship between trap strength and laser intensity, and as predicted these to be proportional. Finally, we measure Boltzmann's constant, in two ways, one fruitful, one not.

Although I preferred Optical Pumping to this lab, because of its in depth theory section, and demand for a deep understanding of all controls of the system, as someone who wants to be an experimental physicist, the frustration I came across in this lab is probably most likely one that I will feel again, so it was nice to experience it when the stakes are a bit lower. Realistically, even though one can understand the apparatus fully and follow the guidance of those who came before there will always be systematic problems in experimental procedures that one must learn to work around and fix. This lab allowed me to become more comfortable with just doing what I had to do until things worked (or in the case of variance, didn't work) and when need be, find other ways of achieving the results one is seeking.

Overall, I would recommend this lab to anyone who wants to learn how to deal with power spectrum data and experimental derailment.

## REFERENCES

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