Measurements of Boltzmann's constant through the motion of beads in an optical trap

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Optical trapping is a method of manipulating and making measurements of microscopic particles using a focused laser beam. In this paper, we calibrate an optical trap to determine its sensitivity to position changes, then use Stokes' drag and position time-series data to make measurements of Boltzmann's constant. We obtain $k_B = 6.47 \pm 2.04(stat.) \pm 4.85(sys.) \times 10^{-24} \frac{J}{K}$, which is in good agreement with the known value.

I. RELEVANT THEORY

I.1. Brownian motion

Brownian motion is the random motion of particles suspended in a fluid, such as dust in the air or pollen in water. Due to the relatively large size of the particle (compared to the fluid molecules), collisions occur very frequently and each impart a very small impulse. This system is too complex to model deterministically, so we study it using statistical mechanics instead.

The dynamics of a particle undergoing Brownian motion are described by the Langevin equation:

$$m\frac{dv}{dt} = -\lambda v + F(t) \tag{1}$$

where m is the mass of the particle, v is its velocity, and λ is the damping coefficient. F is a random forcing term with Gaussian probability distribution and the correlation function

$$\langle F(t)F(t')\rangle = 2\lambda k_B T\delta(t-t') \tag{2}$$

Note that the correlation time is zero due to the delta function. In reality the correlation function will have a small but nonzero correlation time, but this is a good approximation.

Adding a harmonic oscillator potential gradient term αx to (1) and considering the overdamped system with a spherical particle, we get

$$\beta v + \alpha x = F(t) \tag{3}$$

where $\beta = 3\pi\eta d$ is the hydrodynamic drag coefficient for a sphere of diameter d in a fluid of dynamic viscosity η.

Additionally, for the system described in (3), the equipartition theorem gives us

$$\alpha\langle x^2\rangle = k_B T \tag{4}$$

which, knowing α, allows us to measure k_B. [1]

I.2. Optical trapping

Optical trapping is a non-invasive method of manipulating and making precise measurements of small particles. It is often used in fields like biology, where it is useful for studying the behavior of cells.

Optical trapping involves focusing a high power laser onto the region of interest. Due to radiation pressure, objects near the focal point of the laser (also called the "waist") are attracted to the center. This is caused by the scattering of laser light off of the object. When a particle is perturbed from the waist, it scatters light unevenly so that radiation pressure provides a restorative force. (See diagram in Fig. 1).

There may also be additional forces due to effects such as Rayleigh scattering that occur when the size of the particle is comparable to the laser wavelength. Therefore, rather than predicting the force from theory, the trap must be calibrated through direct measurements to determine the "trap stiffness" α for a particle of a given size. [1]

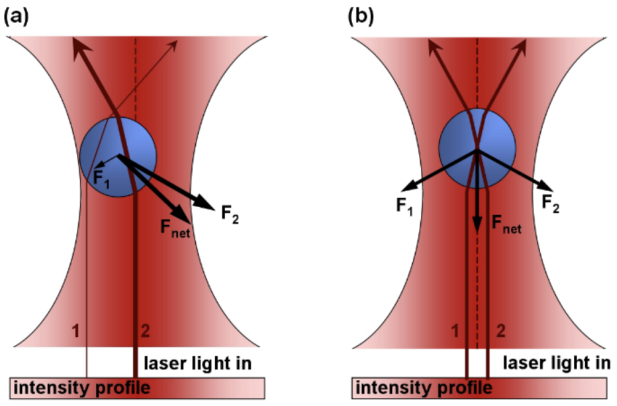

FIG. 1. Particles that are perturbed from the waist of the trap experience a restorative force due to radiation pressure. Image from [1].

I.3. Stokes' Drag

Recall the overdamped Langevin equation with a harmonic oscillator term, where we had the drag force term βv. This is known as Stokes' drag, which is the resisting force experienced by a spherical particle traveling through a fluid with relative velocity v. When the velocity is large enough compared to the Langevin random force $F(t)$, the Brownian motion of the particle becomes negligible compared to the drag force and we obtain

$$\alpha x = -\beta v \tag{5}$$

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which, given v, allows us to measure the trap stiffness α.

II. EXPERIMENTAL SETUP

The experimental setup is shown in Fig 2. A high power laser is focused onto a point within the sample, creating an optical trap as described in section I.2. To make measurements we use a quadrant photodetector (QPD), which measures the voltage due to light hitting each of four quadrants. The laser light is incident on the center of the QPD when no object is trapped, providing a uniform voltage across the quadrants. However, a trapped object will deflect some of the light, making the voltage non-uniform. The QPD translates and records this data as QPD X and Y voltage, which will later be translated to X and Y position data through calibration.

Additionally, to allow us to find interesting objects within a sample, the setup also contains an optical microscope. A LED light illuminates the sample and a CCD camera captures real time video that is sent to the monitor.

Finally, the sample sits on a stage that moves in the X, Y, and Z directions. The stage is primarily moved by hand turning the knobs to find an interesting region. It can then be finely tuned using piezoelectric motors to make very small adjustments. These motors can also be used to precisely oscillate the position of the stage, which is useful when calibrating QPD voltage to position and for making Stokes' drag measurements.

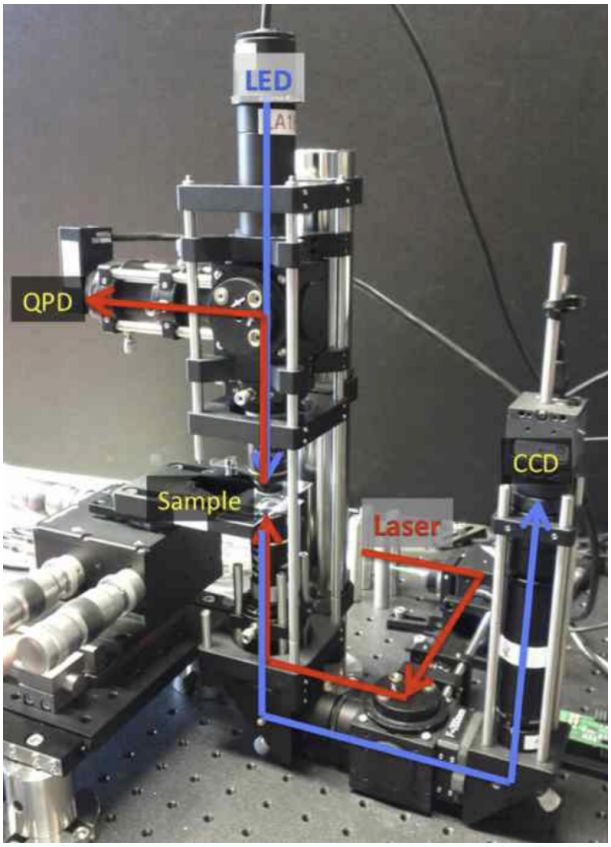

FIG. 2. Picture of the Optical Tweezers setup showing paths of the laser and LED light. Image from [1].

III. EXPERIMENTAL PROCEDURE

III.1. QPD time series data for floating beads

We began by studying the Brownian motion of 1μm and 2μm diameter silicon beads. We made dilutions of bead stock solutions until we could isolate one bead at a time under the optical microscope. Deionized water was used since any ions present in the water can cause the beads to become charged and stick to the plates, where they will no longer undergo Brownian motion.

We trapped one bead at a time under several different laser powers (throughout the experiment, we use laser currents 150mA to 450mA in increments of 50mA). We saved QPD time series data for 10 seconds, sampling at a rate of 20kHz.

III.2. Stokes' Drag measurements

Using the same bead solutions as in III.1, we now turned on stage oscillation using the piezoelectric motors. We oscillated the stage in both the X and Y directions. We found that an amplitude of 4μm and a frequency of 0.5Hz provided enough flow for the discussion in I.3 to be valid, while also not being enough to overcome our weakest trap. Therefore, we decided to keep these same settings across all laser powers and bead diameters.

III.3. Calibration

In the previous two sections, we obtained time series data for the QPD voltage. However, most of our measurements require knowing the position time series data, which means we must calibrate the trap to know how a given QPD voltage value maps to a position.

For each bead diameter, we prepared a 1M NaCl solution with the same bead dilution as before. The salt ions in the solution cause the beads to become charged and stick to the plates. We then found a lone bead in each solution using the optical microscope, and centered it on the known position of the trap which was marked from our floating bead measurements.

We then performed an XY scan using the piezoelectric motors, where the stage oscillates quickly along the "fast axis" and moves in slow increments along a slow axis. We saved the data when the center of the trap crossed the center of the bead. The slope of the line through this point gives the conversion factor between the QPD voltage and position (see Fig. 3). The calibration was done for both the X and Y axes due to the possibility of an asymmetric trap (which we found to be the case in our analysis).

Note that the line in the Y calibration shown does not cross the origin. This is due to the trap crossing the bead slightly off-center. It was caused due to the increments along the slow axis being too large, which made it difficult to get a crossing through the center. This was especially

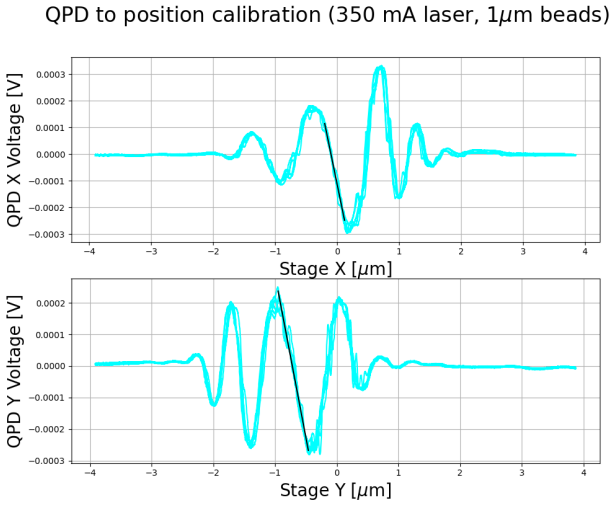

FIG. 3. Linear fit to central crossing regions of the bead over the optical trap for 1μm beads under a 350mA laser.

a problem for the 1μm beads as they were the smallest, so a potential improvement of our measurements could be made in the future by changing the XY scan settings to use a smaller slow axis increment.

IV. DATA ANALYSIS

IV.1. Calibration: Fitting to the linear region

As explained in III.3 and shown in Fig. 3, we performed linear fits to the region where the bead center aligns with the waist of the trap. The fit intervals were determined manually by visually estimating the linear region for each plot. The results of these fits are summarized for 2μm beads in Fig. 4, where each data point is the slope of the line fit to the corresponding laser power data.

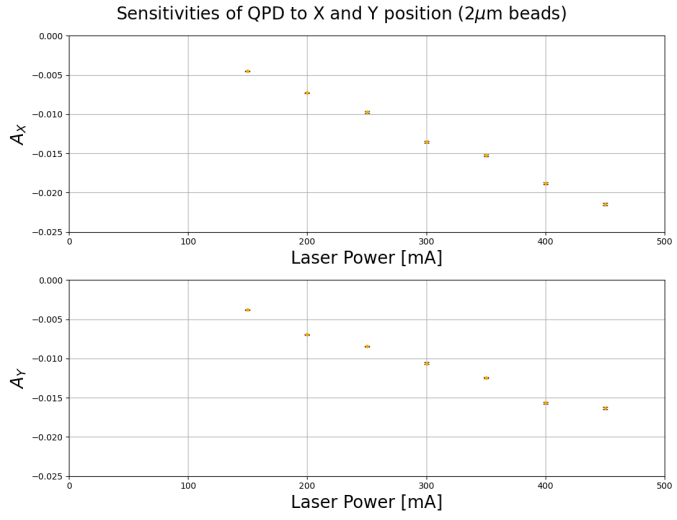

FIG. 4. Results of calibration fits for each laser power; 2μm beads shown.

The errors on these fits, which are statistical, were very small since our calibration data was very linear. However, these results do not account for the human error in determining the fit intervals. To estimate this, we reselected the fit regions to be slightly larger (to contain regions that do not look linear). This changed our slopes by about 5% on average, which we considered to be systematic error.

IV.2. Stokes' drag

Using our calibration results, we converted our Stokes' drag QPD data to position data. Eq.(5) predicts that the bead displacement is proportional to its velocity through the fluid. Since the bead remains trapped, this is just the stage velocity. Therefore, since the stage velocity is sinusoidal, we fit the function $A\sin(\omega x+\phi)+B$ to the position time-series data (shown in Fig. 5). As expected, all values of B were 0 within uncertainty, which makes sense since our data is centered. Additionally, all the frequencies ω were πHz within uncertainty, matching our stage oscillation frequency. Using the fact that our stage oscillated

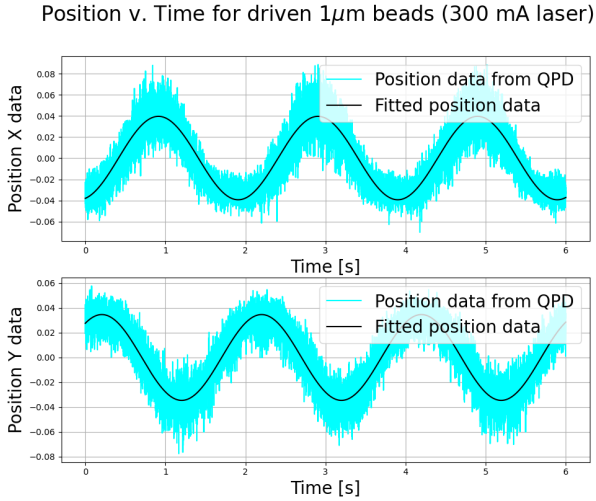

FIG. 5. Fit of $A\sin(\omega x+\phi)+B$ to Stokes' drag position time series data.

with amplitude 4μm and frequency 0.5Hz, we computed $v_{max}=4\pi\ \frac{\mu m}{s}$. We then extracted the amplitude A from each of our fits, which we used along with eq. (5) to obtain the trap stiffness as

$$\alpha=\frac{\beta v_{max}}{A}. \tag{6}$$

The resulting values are summarized below in Fig. 6. The Y data appears to follow a roughly linear trend as expected, but interestingly, the X data was almost constant. This was most likely caused by us not centering the bead in the trap properly or having the bead stick slightly to the plate during measurements. It is also interesting to note that on average, the trap stiffness was significantly higher for the Y axis than for the X.

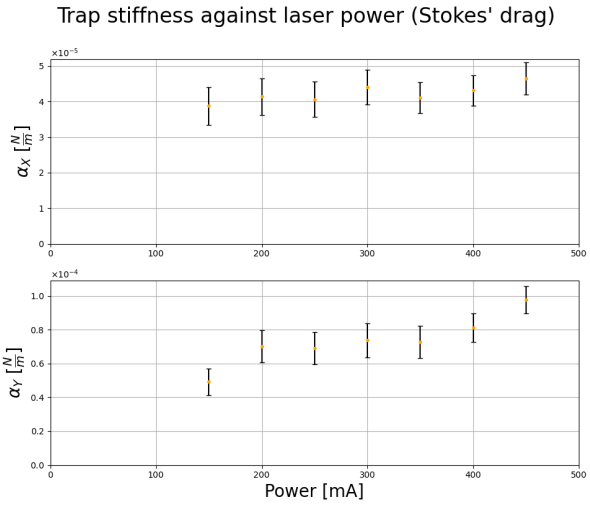

FIG. 6. Trap stiffness at each laser power for 2μm beads.

IV.3. Position time-series

Lastly, we used the calibration results to convert the floating QPD voltage time series data from III.1 into position time series data for X and Y. As explained in (4), the time-averaged variance of the position time-series data gives us the ratio between k_BT and the trap stiffness. We have already computed the trap stiffness in IV.2, so we can now compute k_BT.

We begin by computing the time-averaged variance $\langle x^2 \rangle$ for each bead diameter and laser power, which was straightforward. However, estimating the uncertainty on this was more difficult. We derived the formula $\Delta\langle x^2 \rangle \sim \sqrt{\frac{2\langle x^2 \rangle^2}{N-1}}$, where N is the number of samples. However, since the autocorrelation time τ_c of the time series data is nonzero, we estimate the number of independent samples $N_{ind.}$ as $\frac{NT}{\tau_c}$ where $T = 10$s is the total duration of the time series data. We then use $\tau_c = \frac{1}{f_c}$, where f_c is the corner frequency obtained from fitting to the power spectral density (PSD) done by Maryna [2]. Using these uncertainties in our fit, which turned out to be quite small, we obtain the plot shown Fig. 7. Combining these results with our previous methods of computing the trap stiffness α gives us a measurement of k_B. We summarize our results in the table below, where our two methods are the Stokes' drag measurement and the power spectral density.

d	Stokes X	Stokes Y	PSD X	PSD Y
1	1.581 ± 0.455	1.042 ± 0.276	0.455 ± 0.017	0.133 ± 0.009
2	0.581 ± 0.085	1.022 ± 0.157	0.193 ± 0.010	0.194 ± 0.010

TABLE I. Summary of k_B values for each bead diameter d [μm]. All k_B values are given in units of $10^{-23}\frac{J}{K}$.

We then take our measurement of k_B to be the average of the above values. Our statistical error is then computed from the uncertainties in the table. Additionally, since different methods give us different values of k_B, our

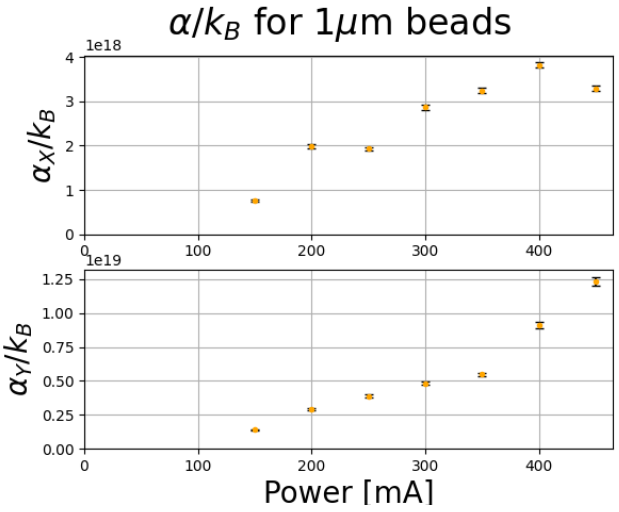

FIG. 7. Resulting $\frac{k_B}{\alpha}$ ratios for 1μm beads.

systematic error is estimated using the standard deviation of the values in the table from the mean. We finally obtain

$$k_B = 6.47 \pm 2.04(stat.) \pm 4.85(sys.) \times 10^{-24}\frac{J}{K}.$$

Within our uncertainty, this is in agreement with the true value of $k_B = 1.380 \times 10^{-23}\frac{J}{K}$.

V. CONCLUSIONS

Our measurement of k_B is in good agreement with the known value, indicating that our optical tweezers setup can successfully manipulate microscopic particles and make measurements of the minuscule forces that act on them. With the calibration we have done in this paper, the tweezers can be used to make measurements of biological systems, allowing us to study and understand more complicated dynamics such as active matter transport in biological cells.

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[1] M. D. of Physics, *8.13 Optical Trapping Lab Guide*, MIT (2023).

[2] A. Gennerich, *Optical Tweezers: Methods and Protocols* (Springer, 2022).