Measuring Microscopic Forces with Optical Trapping

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We investigate the use of an optical trap to measure Boltzmann's constant. We investigate a variety of analyses that are robust to different errors. Using the equipartition theorem proves to work poorly when a quadrant photodetector is used, but shows promising results with a computer vision technique. Using a power spectral density method determines Boltzmann's constant to be $1.5 \pm 0.6 \times 10^{-23}\,\mathrm{J\,K^{-1}}$, which is in good agreement with the established value of $1.3 \times 10^{-23}\ \mathrm{J\,K^{-1}}$. Finally, we measure the Stokes drag to get a measurement of trap strength independent of Boltzmann's constant.

I. INTRODUCTION

Humans have been fascinated by the power of light since Archimedes focused the rays of the sun to burn ships attacking his hometown of Syracuse[6]. In some ways, the optical trap used here is even more exciting than that. In 1970, Arthur Ashkin discovered that a beam of light being used to push glass microspheres would sometimes trap the microspheres[5]. Soon afterwards, people began using this trap to investigate biological samples, and it has proved to be an incredibly useful tool.

II. THEORY

II.1. Trapping Forces

When light shines on an object, it refracts and reflects the light, changing the momentum of the incoming photons, and causing a corresponding change in momentum on the object. This is the principle behind optical trapping. An object is placed at the focus of a laser beam, which can be roughly approximated as two intersecting rays[1], as shown in Figure 1. The reflections off the particle push it laterally into the trap and forward, while the refractions push it laterally out of the trap and backwards. Increasing the numerical aperture, which means focusing the trapping laser from a closer distance, increases the backwards force from the refraction and the lateral trapping force from the reflection, so a large numerical aperture is required for a successful trap.

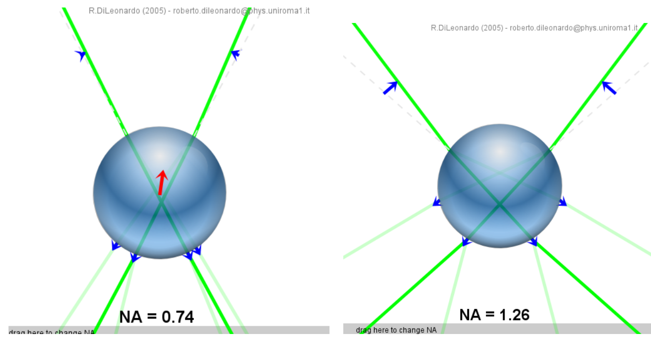

FIG. 1. A large numerical aperture is required for a stable trap.

II.2. Equipartition Theorem

A d-dimensional physical system with temperature T has energy $\frac{d}{2}k_B T$. A harmonic oscillator with restoring force α has energy $\frac{1}{2}\alpha\langle x^2\rangle$. Thus, if we measure the variance of the position of a particle at a known temperature, we can find the ratio $\frac{\alpha}{k_B}$.

III. APPARATUS

A 975 nm laser passes through a fiber optic cable and is reflected off a hot mirror, which passes visible light but reflects infrared light. It is focused onto the sample, then passes through another hot mirror onto a quadrant photodiode. This device allows for a very precise determination of position by measuring the difference between the left and right halves and top and bottom halves of a circular photodiode. At the same time, the light from a white LED passes through each of the hot mirrors into an oil-immersion microscope that feeds into a CCD camera, which allows the sample to be viewed on an attached computer.

Two samples were examined. For calibration of the QPD, a 3.2 µm bead was moved through the beam on a motorized stage. To measure Boltzmann's constant, a floating 3.2 µm bead was held in the trap and the brownian motion was measured. Then, to measure the Stokes drag coefficient for the bead in the medium, the floating

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[1] This is only accurate for particles much larger than the wavelength of the light, which is not really the case for this experiment. Nevertheless, this is a good illustration of the principles used.

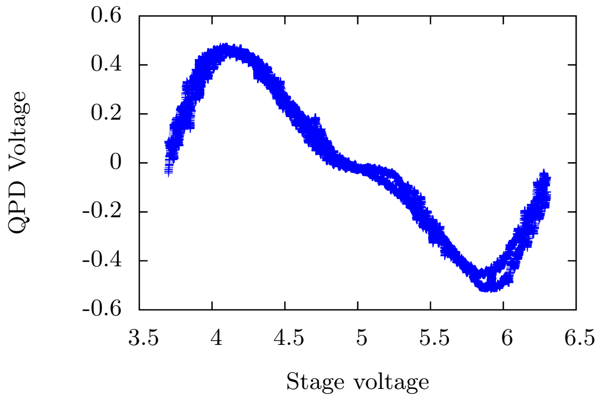

FIG. 2. The photodiode response from moving a fixed bead through a 120mA trapping beam. Only the central linear portion is used for calibration.

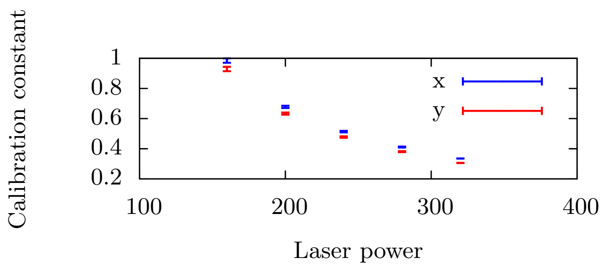

FIG. 3. The calibration constant decreases with laser power

bead was moved through the medium on the stage.

IV. RESULTS

IV.1. Calibration

The QPD was calibrated by measuring the response to moving the bead to a known position with the PZTs on the stage. The response to moving the beam over the entire bead, as shown in Figure 2, is linear when the beam is near the center of the bead, but becomes non-linear outside this region. However, in the actual experiments, the bead is confined to a very small region near the center of the trap, so a linear fit to the central region is sufficient to convert the QPD voltage to stage voltage, which determines the position to within 0.02 µm. As expected, the calibration constant, which is the derivative of actual position with respect to QPD position, decreases with increasing laser power, as shown in Figure 3.

Since the anisotropy of the laser beam is not along the same axes as the QPD, the points were rotated to the least covariant axes, as found by a singular value decomposition of the points.

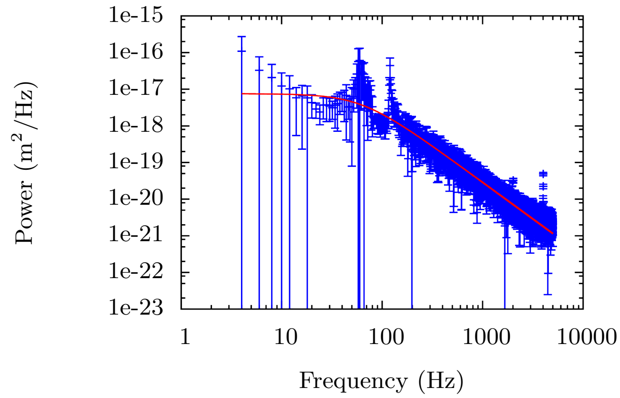

FIG. 4. The power spectral density of a floating bead in a 280 mA trap showing the decreased motion at higher frequencies

IV.2. PSD Method

To measure α and k_B, a floating bead sample was trapped by a variety of laser powers. The Wiener-Khinchin theorem gives the result that the power spectral distribution given by the Fourier transform of the auto-correlation function is given by

$$S_{xx}(f) = \frac{k_B T}{\pi^2 \beta (f^2 + f_0^2)}$$

The data for the 280 mA trap is given in Figure 4. The best-fit line is calculated ignoring the 60 Hz peak and 120 Hz harmonic due to electrical noise and the very low frequencies that result from mechanical vibration in the environment. The χ^2 values for these fits range between 1.5 and 5. As seen in Figure 5, the measured value of k_B rises as the laser power increases; this may be due to the sample heating at higher powers. The average value of k_B is measured to be $1.5 \pm 0.6 \times 10^{-23}\,\mathrm{J\,K^{-1}}$, which is in good agreement with the accepted value of 1.3×10^{-23} $\mathrm{J\,K^{-1}}$.

The trap strength, shown in Figure 6, appears to increase linearly with laser power. As with the k_B measurement, laser powers above 280 mA appear to violate this trend. We conjecture that this is due to sample heating.

IV.3. Equipartition Method

The equipartition method provides for a much simpler analysis than the PSD method above. However, it only allows for a measurement of $\frac{\alpha}{k_B}$, not the individual values, and it is much more susceptible to noise.

IV.3.1. QPD Measurement

First, the same data set used for the PSD measurements was used for the equipartition measurement. We

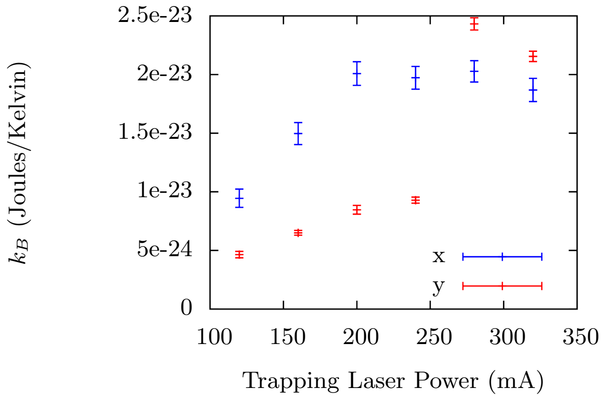

FIG. 5. The value of Boltzmann's constant measured with the PSD method increases with higher laser powers, possibly due to heating of the sample

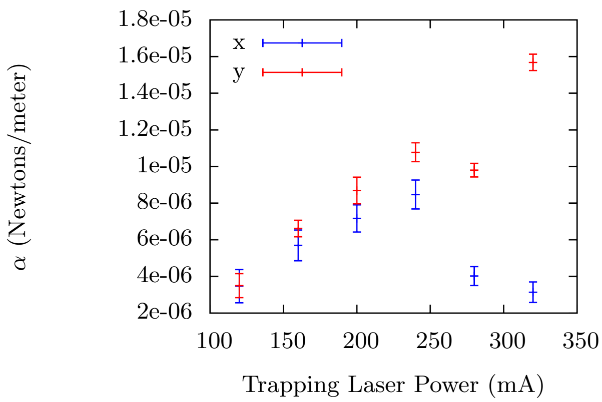

FIG. 6. The trap strength as measured with the PSD method tends to increase with laser power

sampled the data set at a 20 Hz sampling rate to get a set of uncorrelated measurements. As shown in Figure 7, these measurements did not show the expected trend of a decreasing $\frac{\alpha}{k_B}$, though they did echo the ratios measured by the PSD method. We analyze some reasons why this did not work as well as expected below.

IV.3.2. Camera Measurement

Due to the low quality of the equipartition data measured with the PSD, a novel computer vision approach was tested to measure the variance in the position. A SIFT feature detector[3] is applied to a region of video where the sample is known to be. Since the bead is the only object in this area, the keypoints are evenly spaced around the rim of the bead, as shown in Figure 8.

Due to the limitations of the camera used, the sampling rate was only about 6 Hz, so only the equipartition method could be used here. Furthermore, because the camera has a limited resolution, only weak traps allowed

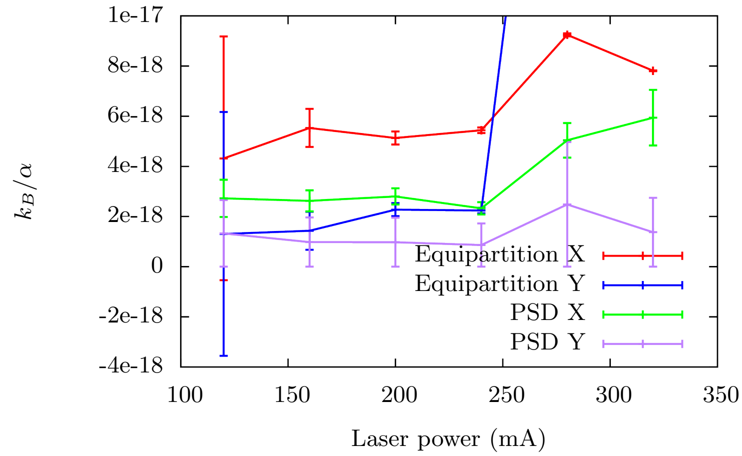

FIG. 7. Neither the equipartition method nor the PSD method shows the expected downward trend for $\frac{k_B}{\alpha}$

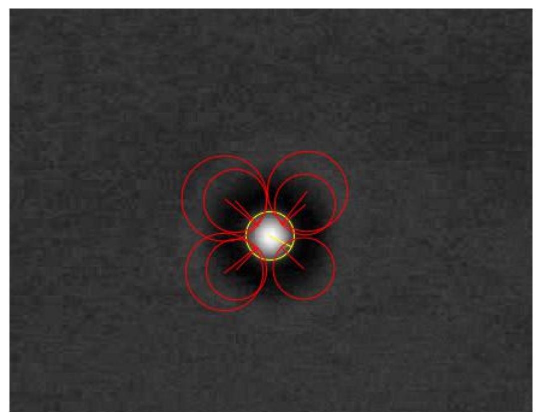

FIG. 8. The SIFT keypoints detected on one frame of video

the bead to move enough to be seen by the camera. Nevertheless, this method showed a much clearer relationship between trap strength and $\frac{k_B}{\alpha}$, as shown in Figure 9.

IV.4. Stokes Drag

The Stokes drag was measured by dragging a floating bead while trapped. We should measure $\alpha = \frac{\beta v}{x - x_0}$. To minimize the effect of transients, we ignore the data taken at the "turning points" of the stage and only take data while the stage is moving at a steady rate. We derive α as shown in Figure 10.

V. ERROR ANALYSIS

This experiment had significant error in each of the measurements. The diversity of techniques provides unique ways to compensate for different sources of error, but some sources of error were present in all the methods

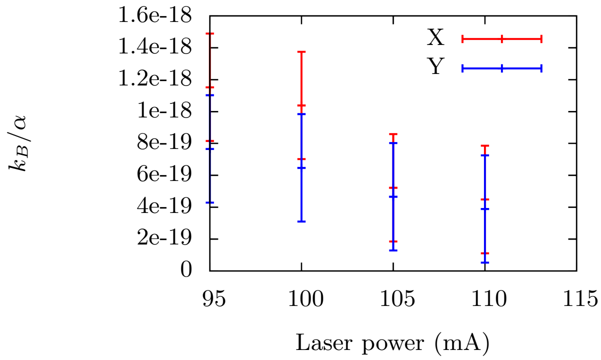

FIG. 9. The computer vision method shows a clear decreasing trend of $\frac{k_B}{\alpha}$ with increasing trap power

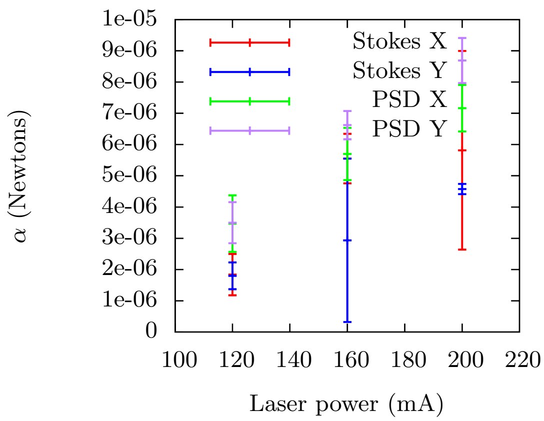

FIG. 10. The Stokes drag method gives a value of α close to that from the PSD method

used.

V.1. Sample Heating

While the laser had a wavelength with a low absorption coefficient in the materials used, some of the light was inevitably absorbed by the sample. Given that at the highest power used, a 320 mA laser was focused to a point only a few µm in diameter, the heating could be significant, although figuring out exactly how significant would require significant knowledge of the thermodynamic properties of the samples.

V.2. Discretization

The resolution of the equipment used in most of these experiments was not a limiting factor. The stage was accurate to 20 nm, and the linearity of the QPD allowed us to measure essentially arbitrary differences in position near the center of the trap. On the other hand, the resolution of the camera was a highly limiting factor in the vision analysis. A single pixel had a size of 0.03 µm, so movements of the bead were not visible for stronger traps. However, it was possible to measure position differences more finely than a single pixel's width by combining measurements from several pixels, which is actually done automatically by the SIFT transform.

V.3. External Vibrations

The biggest source of error, especially in the QPD equipartition measurements, was electrical and vibration noise. Analysis of the bead position over several seconds showed a slow drift of the bead that was much bigger than the brownian motion. This had the effect of significantly decreasing the measured trap strength. This is visible in the PSD plots as the high values for low frequencies. The PSD plots also show a large spike due to 60Hz line noise. This also had a significant effect on the equipartition measurements.

VI. SUMMARY

We have examined several techniques for measuring Boltzmann's constant and the strength of an optical trap. The PSD method, which finds the rolloff frequency of the trap, is the most robust to external vibrations and AC line noise. The equipartition method proves to be unwieldy when used with the QPD, as these errors overwhelm the signal, but computer vision approaches appear promising, especially if cameras and frame grabbers with higher frame rates and resolution can be obtained. However, the equipartition method is fundamentally limited in utility, as it can only measure the ratio of the trap strength to Boltzmann's constant, as opposed to the Stokes drag method and the PSD method, which can measure α or both α and k_B directly. We find a k_B of $1.5 \pm 0.6 \times 10^{-23}\,\mathrm{J\,K^{-1}}$, which agrees with the known value.

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