Calibration of Optical Tweezers

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In this experiment, we calibrate a pair of optical tweezers. The goal of this experiment is to measure the spring constant k of each of the optical tweezers. This work is part of a series of experiments to study DNA unwinding. DNA unwinding is important for gene expression and therefore of great interest to cancer researchers.

I. INTRODUCTION

Optical tweezers use a laser beam to trap and manipulate microscopic particles, such as small beads. The laser beam creates an optical gradient force, which pushes the particle toward the center of the beam. By adjusting the laser's position, we can move the trapped particle in three dimensions and measure mechanical properties. Optical tweezers have a wide range of applications in biophysics, cell biology, materials science, and nanotechnology, and have enabled important discoveries, such as the mechanical properties of DNA or the behavior of motor proteins. The basic design of our optical tweezer setup is shown in figure 1.

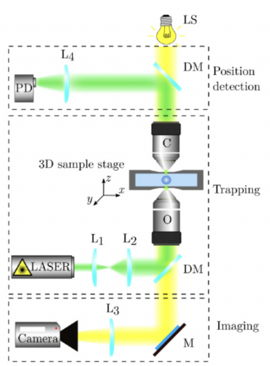

Fig 1: Optical Tweezers Apparatus.

II. CALIBRATION GOALS

The optical tweezers reports signal in voltage, so we need to calibrate a conversion factor, β, to go from voltage to displacement:

$$x = \beta V. \tag{1}$$

For small displacements, the restoring force on the optical tweezer is linear with stiffness κ,

$$F = -\kappa x. \tag{2}$$

Our objective is to calculate β and κ. We will use two methods: fitting the power spectral density and signal auto-correlation. We perform this analysis in each direction, for each trap, and for beads of sizes 2.14 μm, 4.5 μm.

III. DATA COLLECTION/FITTING

The experiment consists of several steps. Two beads of the same radius are caught in an optical tweezer. We collect displacement data for approximately 20 seconds at three different power levels. We fit a Lorentzian to the power spectral density (PSD) of the displacement data. Using the fitted curve we extracted the spring constants and volts to distance calibration factor. We repeat the entire process of catching the beads, collecting displacement data, and calibrating the trap stiffness with two different bead sizes. A fitted PSD is shown in figure 2.

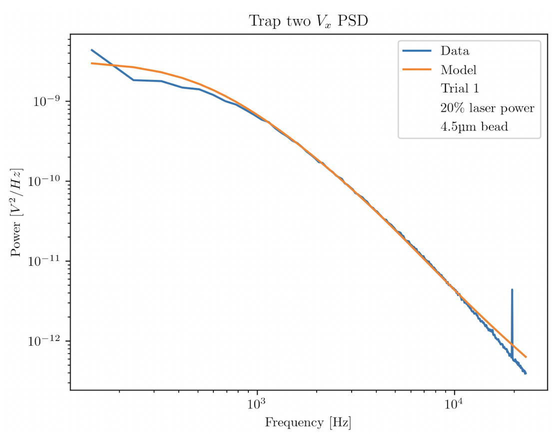

Fig 2: Fitted Power Spectral Density

IV. LANGEVIN MOTION

A common method for trap stiffness calibration is based on the analysis of the Brownian motion of a trapped particle in a viscous fluid: Langevin motion. In Langevin motion, the movement of a particle is determined by three forces: an external force, a frictional force, and a random force. The differential equation describing the motion of the particle is given by

$$m\ddot{x} = -\gamma\dot{x} + \sqrt{2\gamma k_B T m}\ \eta(t) - \frac{d}{dx}U(x) \tag{3}$$

where γ is the damping constant and $\eta(t)$ is a white noise term with a mean of 0 and standard deviation of 1. In our experiment, the potential can be approximated as harmonic, which gives

$$m\ddot{x} = -\gamma\dot{x} + \sqrt{2\gamma k_B T m}\,\eta(t) - kx \tag{4}$$

We use equation 2 to perform numerical simulations generating particle trajectories. A simulated trajectory is shown in figure 3.

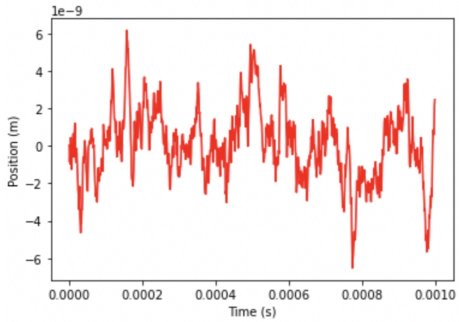

Fig 3: A Numerically simulated trajectory.

By tuning the simulation parameters to match measured parameters we reproduce particle trajectories with statistical behavior similar to the measured behavior.

V. DATA ANALYSIS

We use the autocorrelation method to find calibration constants. From the Volpe paper [1], we have

$$C_x(t) = \langle x(t'+t)x(t')\rangle_{t'} = \frac{k_B T}{\kappa} e^{-t/\tau}, \tag{5}$$

where $\tau = \gamma/\kappa$. Because $V(t) = x(t)/\beta$, the autocorrelation function for $V(t)$ should follow:

$$C_V(t) = \langle V(t'+t)V(t')\rangle_{t'} = \frac{C_x(t)}{\beta^2} \tag{6}$$

Therefore, if we fit $\ln(C_v(t)/(1\,\mathrm{V}^2))$ to the model $a + bt$, then,

$$\kappa = -\gamma b, \qquad \beta = \sqrt{\frac{k_B T}{-\gamma b \cdot (1\,\mathrm{V})^2} e^{-a}} \tag{7}$$

Fitting the autocorrelation function and extracting the calibration constants. We find that the parameters are all within 10 percent of the automated values.

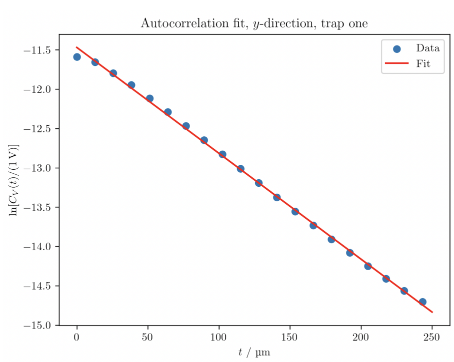

Fig 4: Fitting Autocorrelation Function

We plot the stiffness for each laser power for each bead size. We note a clear linear relationship between the trap stiffness and the laser power. This makes sense because as we increase the laser power there are more photons pushing the bead toward the center of the trap.

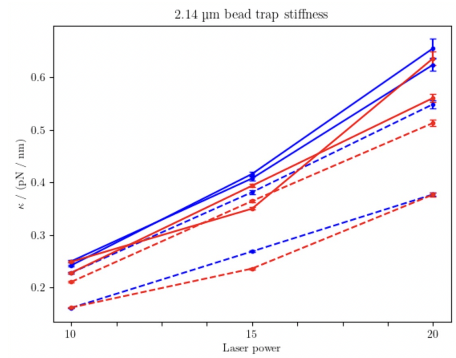

Fig 5: Trap Stiffness vs Laser Power

The motion of a bead in the optical trap is described by Langevin motion. We use the parameters extracted from fitting the PSD and the Langevin equation to simulate particle trajectories. A plot of a simulated trajectory is shown in figure 6. We note that the simulated and measured trajectories fluctuate on the same time scale.

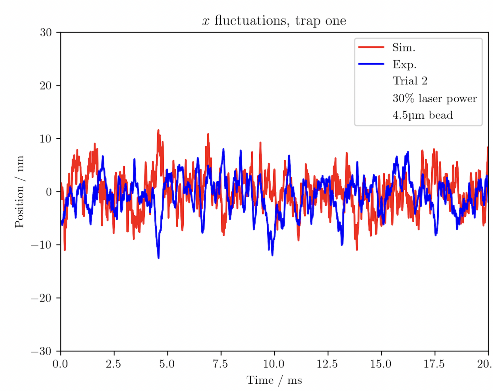

Fig 6: Comparison of Simulated and Measured Trajectories with the same parameters

We are interested in the statistical behavior of beads. We compare the stationary probability distribution of the simulated and measured trajectories. In figure 5, we see that the stationary probability distributions are essentially the same. We note a similar relation for all data sets.

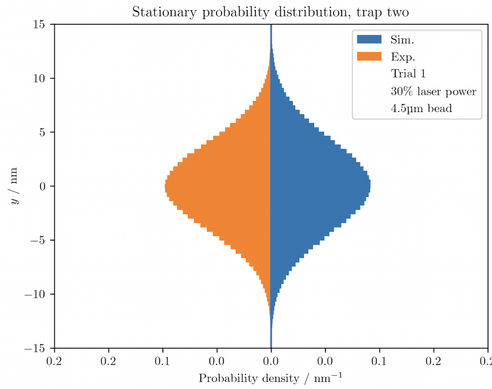

Fig 7: Comparison of Simulated and Measured statistical behavior

The variance of the stationary probability distribution match quite well. What is the variance of the displacement? Does this agree with the value expected from thermodynamics?

We used the Langevin code to model the diffusion behavior at different viscosities. We observed that viscosity does not have a qualitative effect on the motion. This makes sense because probability distribution is described by Boltzmann statistics:

$$\rho(x) = \alpha e^{-\frac{U(x)}{K_B T}}, \tag{8}$$

where α is the normalization. Thus, the statistical behavior of the trajectories is not affected by viscosity.

VI. CONCLUSION

In this work, we used two separate methods to calculate the calibration constants for the optical traps. We used autocorrelation functions and fitting spectral densities. We were able to reproduce the results that the software automatically calculates to within 10 percent.

VII. ACKNOWLEDGEMENTS

We would like to thank Professor Masha Kamenetska and Brian Dawes for directing the wet lab section of this experiment.

[1] J. Gieseler, *Optical tweezers: from calibration to applications: a tutoria*, Optical Publishing Group (2021).