Optical Tweezers

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This experiment investigated the motion of micron-sized silica beads in an optical trap. Two different methods of calculating the optical trap stiffness as a function of laser power were then compared. The first method used the Boltzmann distribution to predict the probability of a particles position. The second method used the mean squared displacement of the particles position. It was concluded that the Boltzmann distribution method was more accurate as it produced a better linear fit.

In this experiment, a diluted sample of 2.06 $\pm$ 0.13 μm silica micro-spheres was used in conjunction with a 658 nm laser diode to investigate optical traps. The concept of optical traps was pioneered by Ashkin in 1970 when he first moved particles using a laser [1]. In 1986 he developed these ideas into optical traps similar to ones used today by focusing a laser to create an intensity gradient along the beam [2]. Optical tweezers are of paramount importance in biophysics as the magnitude of the force they exert are comparable to those used in biological motors[3]. They have also been used in experiments to determine properties of small molecules such as DNA[4]. The force that the laser exerts on a particle can be split into two components, the scattering and the gradient force. The scattering force acts along the direction of propagation of the laser whereas the gradient force acts likewise as well as towards the focus of the laser in the plane perpendicular to propagation. The combination of these forces confines the particle in a potential well. However, the particle will still move via Brownian motion within the trap. By analysing this motion using the equipartition theorem, the stiffness of the optical trap, κ, was calculated. The mean squared displacement of the particle in a trap was also used to calculate the optical trap stiffness. These two methods were compared based on how well the data fits a linear model.

The apparatus is shown in Figure 1. The sample of silica micro-spheres was diluted in water and prepared on a slide. This was able to be moved precisely due to a motorised positioning stage. The laser beam was initially collimated but then expanded by a beam expander. It was then reflected by a series of right angled mirrors and focused by the 63x microscope lens. A CMOS camera was used to record the motion of particles on the slide. The radiation pressure caused by incident photons exerted a scattering force acting in the direction of propagation. As a fraction of the light was reflected, there was a momentum transfer and therefore a force exerted on the particle. The magnitude of this force is given by[5:p24]

$$|\boldsymbol{F}| = \frac{2\Gamma P}{c} \tag{1}$$

where Γ is the reflection coefficient, P is the laser power and c is the speed of light. Due to a Gaussian profile of the laser, there was an intensity gradient across the beam. This caused a particle with a higher refractive index than the solution to move towards the center of the beam. If the particle was off center, more photons were incident at the center of the beam which reflected inside the particle and pushed it back towards the center. Due to the focusing of the laser through the lens, there was also an intensity gradient along the beam. The magnitude of this gradient was increased by the beam expander as the beam was more spread before it was focused. Therefore, the particle also moved towards the focus of the beam which, if strong enough, counteracted the scattering force and gravity.

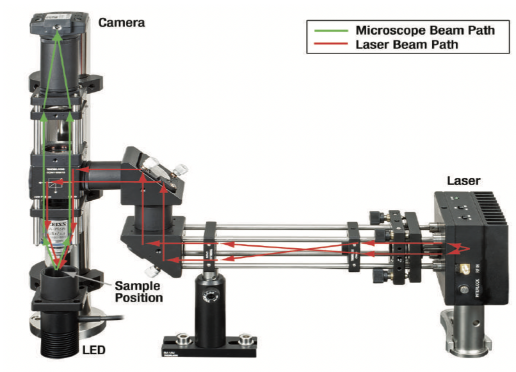

FIG. 1. A diagram of the equipment used to optically trap micro-spheres. The path of the laser is shown in red and the path of light from the microscope is shown in green. The LED, CMOS camera and sample position are also labelled [6].

For this section it was assumed that the potential, $U(x)$, created by the optical trap is harmonic

$$U(x) = \frac{1}{2}\kappa x^2 \tag{2}$$

where κ is the optical trap stiffness and x is the displacement from equilibrium. Therefore, using the Boltzmann distribution, the probability of a particle being displaced by x from equilibrium is given by

$$P(x) = Aexp(\frac{-\kappa x^2}{2k_BT}) \tag{3}$$

which is the form of a Gaussian Distribution. This probability is represented experimentally through a histogram of the particles position from equilibrium in an optical trap. Therefore, by fitting a Gaussian distribution to this, the optical trap stiffness can be calculated by

$$\kappa = \frac{k_B T}{\sigma^2} \tag{4}$$

where σ is the standard deviation of the Gaussian.

Another method of calculating the optical trap stiffness is by solving

$$m\ddot{x} + \gamma\dot{x} + \kappa x = \sqrt{2k_B T}W(t) \tag{5}$$

which is the Langevin equation of motion and m is the mass of the particle, γ is the friction coefficient and the right hand side represents stochastic Brownian motion [7]. In a solution with a low Reynold's number, the inertial forces are much less than the drag forces so the first term can be dropped. The result of this is the over-damped Langevin equation and the solution to this is the autocorrelation function (ACF)[5:p301]

$$\langle x(t+\tau)x(t)\rangle = \frac{k_B T}{\kappa} exp(\frac{-\kappa\tau}{\gamma}) \tag{6}$$

where τ is the autocorrelation time. The ACF is related to the mean squared displacement (MSD) of the particle, $\langle \Delta x(\tau)^2 \rangle$ by

$$\langle \Delta x(\tau)^2 \rangle = 2\langle x^2 \rangle - 2\langle x(t+\tau)x(t)\rangle. \tag{7}$$

Therefore, combining equations (6) and (7)

$$\langle \Delta x(\tau)^2 \rangle) = \frac{2k_B T}{\kappa}[1 - exp(\frac{-\kappa\tau}{\gamma})]. \tag{8}$$

By taking the natural log of this equation and plotting $\ln(msd)$ against $\ln(\tau)$, the optical trap stiffness is given by

$$\kappa = \frac{2k_B T}{exp(y)} \tag{9}$$

where y is the y intercept.

Before taking measurements, the equipment had to be calibrated. First a 10μm graticule was used under the microscope to relate pixels to meters. This introduced a random error in all displacements; however, this was negligible in comparison to other errors. In order to account for any background thermal noise the path of a dried micro-sphere was tracked. As it was not in a solution, the only motion it experienced was due to thermal vibrations. Therefore, a quantitative value of how this affects the system was found by plotting the mean squared displacement and taking the average. This was a systematic error for the experiment and was taken off of all subsequent measurements.

For the Boltzmann distribution method, the particle was confined and tracked in an optical trap. The particles

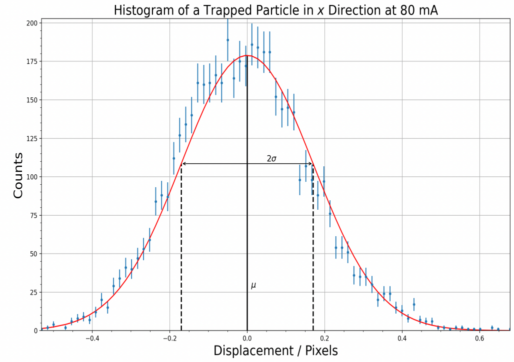

FIG. 2. An example of a histogram of particle displacement from the equilibrium position of an optical trap at 75.04±0.01 mA. Fitted Gaussian distribution is shown in red with A=178.9±3.2 counts , σ=0.170±0.002 $\chi^2_{red} = 1.12$

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displacement from the equilibrium position was plotted as a histogram with 80 bins as shown in Figure 2. The error on the counts was equal to the square root of counts as per Poisson statistics. Therefore, the counts were maximised to reduce the random error on each point. A Gaussian distribution was fitted to the data using chi-square minimisation. The coefficients of the Gaussian were varied and the corresponding chi-square was calculated. These were then plotted on a contour plot and the $\chi^2 + 1$ contour was used to calculate the error on the width of the Gaussian with a 68% confidence level. The width of the Gaussian was used in Equation 4 to calculate the optical trap stiffness. The error on the temperature was negligible in comparison to the error on the width, therefore the dominant error for this method was from the counts. This was repeated for a range of laser powers and optical trap stiffness was plotted as a function of power as shown in Figure 3. Repeats were also taken at each laser power to reduce random errors. As can be seen from the graph, the optical trap stiffness increases linearly as theory suggests. A straight line was fitted to this with $\chi^2_{red} = 10.94$, this indicates that the fit is good; however, the errors were underestimated. The coefficients of this fit are dependent on the parameters of the laser so this cannot be compared to literature. However, the optical trap stiffness values are of the same order as expected values.

The path of a particle in an optical trap was was also used to calculate the mean squared displacement. This data was noisy due to the small displacements measured so it was split into bins. Outliers more than 2 standard deviations from the mean in each bin were ignored and the remaining data points were averaged. The error on each point was equal to the standard deviation of the mean in each bin. This resulted in a data set which resembled theory. The natural log was taken of the data and plotted, these are both shown in Figure 4. The y-intercept of this graph was used to calculate the optical

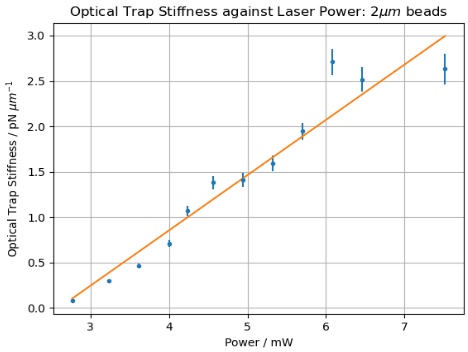

FIG. 3. A plot of optical trap stiffness against laser power from the Boltzmann distribution method. The linear fit shown in orange is given by $y = (0.608 \pm 0.003)x - (1.579 \pm 0.069)$ and has $\chi^2_{red} = 10.94$

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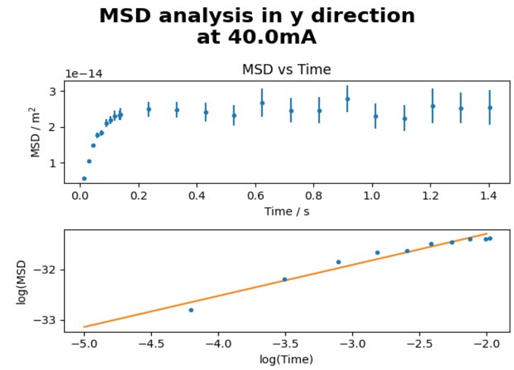

FIG. 4. (Top) An example of an MSD of a trapped particle in an optical trap at 40.00±0.01mA. (Bottom) An example of the log(MSD) of the above data before the cornering frequency. The errors bars are too small to be seen. The fit is shown in orange and is given be $y = (0.598 \pm 0.005)x - (30.71 \pm 0.02)$ and has $\chi^2_{red} = 68.4$

trap stiffness using Equation (9). Again, the error on temperature was negligible compared to the error on the y intercept. Therefore, the dominant error came from the spread of data points in each bin. As before, this was repeated for a range of laser powers and optical trap stiffness was plotted as a function of power as shown in Figure 5. Repeats were taken at each power to reduce random errors. After removing anomalous data points, this fit had $\chi^2_{red} = 49.6$ which indicated that errors were underestimated. Again, optical trap stiffness increased linearly with laser power as theory suggests. The errors from the tracking process were not accounted for, this would increase the errors and reduce the χ^2_{red}. The tracker used for this experiment had a large variation in results; therefore, for a more accurate method, a different tracker should be used. The χ^2_{red} offered comparison between the two methods. The Boltzmann method had a smaller χ^2_{red} which indicated that the data fits a linear relationship better. It also gave smaller percentage errors on optical trap stiffness. These errors are also easier to minimise than the MSD method. Therefore, the Boltzmann method is better for calculating optical trap stiffness.

In conclusion, using a Boltzmann distribution to investigate the relationship between optical trap stiffness and laser power is more reliable than using the mean squared displacement. This is reinforced by the Boltzmann method producing a linear fit with $\chi^2_{red} = 10.94$ whereas using mean squared displacements gives $\chi^2_{red} = 49.60$. Therefore, the Boltzmann method produces data which fits a linear relationship more than using mean squared displacements. Also the Boltzmann method gives smaller percentage errors on the values of optical trap stiffness, but this could be decreased by increasing the counts. However, this is limited by the time it takes the particle to sediment. The MSD method can be improved by using a more accurate tracker and including errors from the tracking.

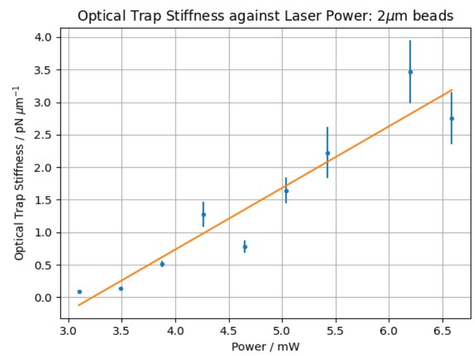

FIG. 5. A plot of optical trap stiffness against laser power from the log(MSD) method. The linear fit shown in orange is given by $y = (0.951 \pm 0.124)x - (3.062 \pm 0.581)$ and has $\chi^2_{red} = 49.6$

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