Laser Tweezers: An Investigation into the Brownian Motion and Laser Trapping of Micrometre Spheres in Solution

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An optical tweezers setup was used to trap spherical microbeads in solution, and the forces trapping these particles were measured. The relationship between the laser power, P [mW], and the optical trap stiffness, κ [pNμm^{-1}], was found to be $\kappa = (0.35 \pm 0.02)P$ in water, and $\kappa = (0.38 \pm 0.02)P$ in 25% aqueous glycerol (by weight). By measuring the Brownian motion of microbeads without laser trapping applied, the viscosity of water at $22 \pm 1°C$ was calculated to be 0.986 ± 0.081 mPas. The relationship of the viscosity of aqueous glycerol with concentration was also investigated.

I. INTRODUCTION

Pioneered by Arthur Ashkin in the 1970s, earning him the 2018 Nobel Prize for Physics, optical tweezers allow the trapping of micrometre-sized particles using lasers [1]. Useful in the biological sciences for manipulating DNA strands and in nanoengineering for building up molecules, optical tweezers use differences in the respective refractive indexes of particles and a medium to exert small forces on the particle [2]. A single focused laser can be used to trap individual particles, and the trapping forces applied to the particle measured. The motion of untrapped particles can also be measured, and the viscosity calculated from their diffusion.

II. THEORETICAL BACKGROUND

A. BROWNIAN MOTION

Particles freely suspended in a fluid undergo Brownian motion - small, random fluctuations over time. Measuring the mean square displacement (MSD) of the position of a Brownian particle over time produces a relationship that is linear with time [3],

$$\langle (x(t+\tau) - x_0 \rangle = 2nDt \tag{1}$$

where $x(t)$ is the position of a particle at time t, $x(t+\tau)$ is the position of a particle at time t plus a small time step τ and n is the number of dimensions the MSD is being taken over ($n = 1$ as x and y displacements are considered separately). D is the diffusion constant, which quantifies the spread of the particle over time and depends on the radius of the particle used. It can be related to the viscosity of the suspending fluid, a more comparable value, by [3]

$$\eta = \frac{k_B T}{6\pi D r} \tag{2}$$

where η is the viscosity, T is the temperature of the fluid and r is the radius of the suspended particle.

B. LASER TRAPPING

A particle in the focused beam of a laser experiences a force along the gradient of the laser's intensity, provided by one of two mechanisms. In the Mie regime, for particle radius $r \gg \lambda/2\pi$, rays from the laser beam will reflect through the particle, exerting a force due to radiation pressure. [1]. The refractive index of the particle must be higher than that of the suspending fluid for these forces to act to trap the particle into the centre of the beam. In the Rayleigh regime, for radii $r \leq \lambda/2\pi$, the particle is treated as a dipole, with electromagnetic forces [1].

At 2μm radii and with a 658 nm wavelength laser, the particles used in this experiment lie between these two regimes, and therefore the forces are a result of both factors and difficult to calculate accurately. Instead, for a Gaussian laser beam [1], the laser trap can be considered to be a harmonic potential [4] with form

$$U(x) = \frac{1}{2}\kappa(x - x_0)^2 \tag{3}$$

where if a trapped particle is displaced from position x_0 by x, a proportional restoring force will act to move the particle back to the centre of the trap. The magnitude of this force is determined by the optical trap stiffness, κ pNμm^{-1} . Assuming the system is in thermal equilibrium, the probability of a particle having energy corresponding to a particular displacement is given by a Boltzmann distribution [5] of form

$$P(x) \propto exp\left(\frac{-U(x)}{k_B T}\right) = exp\left(\frac{-\kappa}{2k_B T}(x - x_0)^2\right) \tag{4}$$

This is the equation of a Gaussian function, and therefore a histogram of particle displacements over a time interval should follow a Gaussian curve with variance inversely proportional to κ. The optical trap stiffness is proportional to the force applied to the particle [4], so κ is expected to be proportional to the laser power.

III. EXPERIMENTAL SETUP

A Thorlabs Optical Tweezers kit was used, with a 658 nm laser diode. Samples were recorded using a CMOS camera, with two precision motors used to adjust the position of the sample slide. Figure 1 shows the path of the laser through the apparatus, starting from a wide beam emitted from the diode before passing through a collimating lens to maximise the gradient of the beam, which in turn maximises the gradient forces trapping the particle. The beam is then reflected into the microscope and is focused at the focal point of the microscope, a Zeiss Optics 63x air objective lens.

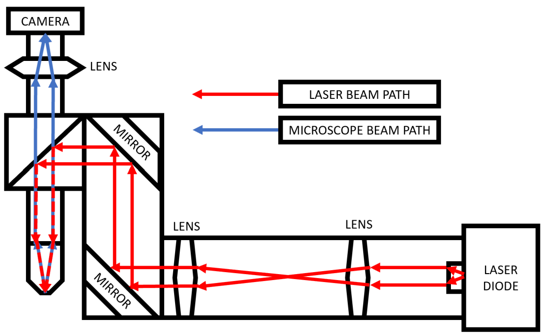

FIG. 1. The paths taken by the laser beam (red) from the laser diode to the objective lens, and from the sample to the camera (blue) are shown.

IV. EXPERIMENTAL METHOD

Samples were prepared by adding a drop of a concentrated solution of Polysciences 19814 2μm beads [6] to de-ionised water, and then transferring this solution onto a sample slide using a pipette. The slide cover slip was sealed with nail varnish to prevent any leakage.

A. Measuring Viscosity from Brownian Motion

With the microscope focused on a single free particle, a short video ($\sim$1000 frames) was taken, with the recording area cropped to improve frame rate. The position of the particle over time was then tracked using Blender. A ruled calibration slide was used to convert displacement in video pixels into micrometres. From this position-time data, the MSD could be calculated in the x and y directions. Equation 1 was fitted to the MSD to recover D, and from this the viscosity using Equation 2.

B. Measuring forces on a trapped particle

With the laser at its highest power (100mA of supplied current), a sample slide was moved so a free particle was within a few micrometres of the laser beam. The particle was observed to be pulled into the beam and held still. The position over time of the particle was measured as before, and a histogram of the particle displacements plotted. Equation 4 was fitted to recover the optical trap stiffness. The current supplied to the laser can then be reduced in steps, and κ measured for each step of current. A laser power meter was used to find a linear relationship between current supplied and power emitted so these results could be stated in terms of laser power.

V. EXPERIMENTAL RESULTS & DISCUSSION

A. Measuring Viscosity from Brownian Motion

Figure 2 shows the MSD plot for a particle undergoing Brownian motion in water. The diffusion constant was found by a linear fit with Equation 1. From Equation 2, the viscosity was found to be 0.902 ± 0.075 mPas and 1.069 ± 0.086 mPas using the x and y MSD data respectively. This gives a mean value of 0.986 ± 0.081 mPas. Water has a viscosity of 0.890 mPas at 25°C and 1.016 mPas at 20°C, so this value is within the expected range. [7].

The uncertainty in each value of the MSD was taken as the standard deviation of the values used to calculate each point of the MSD, and this was then used to weight a least squares first order polynomial fit, giving a fitted value for D and its uncertainty. The solution temperature was measured using a thermometer to the nearest degree, and the radii of the particles was accurate to within $\pm 0.20\mu$m. [6]. These uncertainties were combined in quadrature when calculating viscosity. To investigate uncertainties resulting from either vibrations of the equipment or the tracking software, a stationary particle was tracked for 1500 frames. The mean displacement found significantly smaller than other uncertainties and can therefore assumed to be negligible.

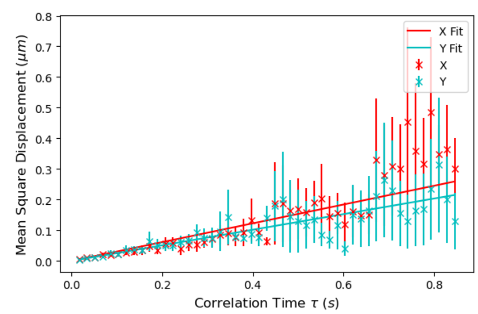

FIG. 2. The MSD in x and y against time interval for a polystyrene microsphere undergoing Brownian motion in water. Linear fits of $x(t) = (0.239 \pm 0.010)t$ and $y(t) = (0.202 \pm 0.008)t$ fitted to the x and y data, with χ^2_{red} values of 0.630 and 0.540 respectively.

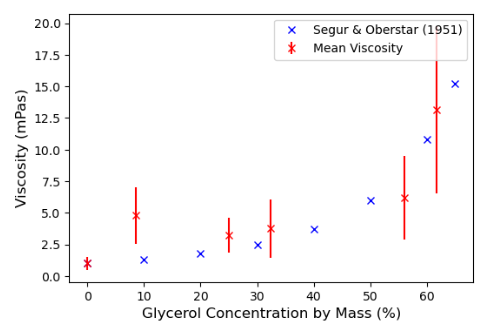

FIG. 3. This figure shows the viscosity values of aqueous glycerol for different concentrations of glycerol by mass. Data from Segar & Oberstar [8], measured using a viscometer, for discrete values of concentration has also been plotted.

Figure 3 shows the mean viscosity of solutions of aqueous glycerol against their concentration by mass. Five measurements of particles were taken for each concentration from 0% to 60%, in approximate steps of 10%. The standard deviation for each of these sets of measurements was used as the uncertainty, as the individual errors for each data point were smaller than the range of values. The data follows that found in Segar & Oberstar [8]. At high viscosities ($\gtrsim$60%), particles appeared fixed and Brownian motion measurements were not possible.

B. Measuring forces on a trapped particle

Figure 4 shows the power-trap stiffness relationship for a particle in water trapped in the focus of the laser. A linear relationship is expected, but this appeared to only be the case for the lowest five powers. At higher powers, the trapping force appears to taper off, with a larger discrepancy between the measured stiffness in x and y. The relationship $\kappa = (0.35 \pm 0.03)P$ for κ in pNμm^{-1} and power in milliwatts was found for water. This was repeated for particles in 25% glycerol, with the results shown in Figure 5 and the relationship $\kappa = (0.38 \pm 0.02)P$ was found. This is similar to that of water, as expected - the trap stiffness should not depend on viscosity [4].

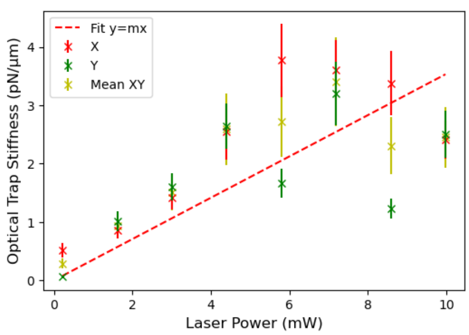

FIG. 4. Optical trap stiffness plotted against laser power in water. A straight line through the origin was fitted with equation $\kappa = (0.35 \pm 0.02)P$, and $\chi^2_{red} = 63$.

When creating histograms of the particle displacements, as large numbers of frames were taken ($\gtrsim$1000) the error in each bin was taken as a Poisson error, the square root of the number of counts for each bin. Non-linear least squares fitting was then used to fit Equation 4, taking into account the weighting of the error of each bin. The uncertainty in the fit was then used as the final uncertainty in the optical trap stiffness. There are several reasons why non-linear behaviour may be seen when measuring the optical trap stiffness with power. If the laser is not focused completely correctly, the particle may appear trapped in the x and y directions, but be pushed up or down the z axis, which was not measured. If the particle contacts the cover slip or base of the slide, it can become sedimented and will no longer be free to move in the solution, decreasing the observed displacement. Even with the cover slip sealed, some convection may still occur, increasing the observed displacement. Similarly, the focus of the laser was observed to drift slightly over the course of the experiment, requiring weekly recalibration, and this may also have increased the observed displacement.

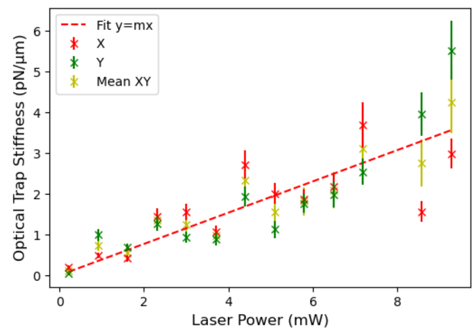

FIG. 5. Optical trap stiffness plotted against laser power in 25% aqueous glycerol. A straight line passing through the origin was fitted with equation $\kappa = (0.38 \pm 0.02)P$, and χ^2_{red} 64. An outlier at 10mW was removed as this was greater than 2 standard deviations from the fit.

VI. CONCLUSION

By measuring the Brownian motions of particles in solution, the viscosity of water was measured to be 0.986 $\pm$ 0.081 at $22 \pm 1°C$, in accordance with the values in the HRC Handbook of Chemistry and Physics [7]. A relationship for the viscosity of aqueous glycerol with solution concentration was found to be in agreement with Segur & Oberstar [8]. When investigating laser trapping forces microbeads suspended in both water and glycerol, the optical trap stiffness and laser power had respective linear relations $\kappa = (0.35 \pm 0.03)P$ and $\kappa = (0.38 \pm 0.02)P$.

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