

Chapter 2

Theory and methods

This chapter covers the theory behind light and particle interactions, and how the exact theory varies for differently sized particles. After that we show how this can be applied to existing diffusion models to accurately simulate the behaviour of a particle within the vicinity of an optical trap. Lastly, we highlight the techniques utilised in experiments to both calibrate and characterise an optical trap based on our understanding of the trap.

2.1 Electromagnetism and optical tweezing

Proper understanding of optical tweezing requires a description of how the trapped particle interacts with the laser. From an electromagnetism perspective the laser creates a spatially and temporally coherent electric field that scatters light when passing through a medium. The laws governing electric and magnetic fields are summarised most succinctly via the Maxwell equations. These four equations describe how the electric and magnetic fields behave and relate to one another. Any discussion of optical trapping is underpinned by the fact that in all cases the Maxwell equations must be satisfied.

Macroscopic forms of the Maxwell equations are given below [1]:

$$\nabla \cdot \mathbf{D} = \rho_f \quad (2.1)$$

$$\nabla \cdot \mathbf{B} = 0 \quad (2.2)$$

$$\nabla \times \mathbf{E} = \frac{\partial \mathbf{B}}{\partial t} \quad (2.3)$$

$$\nabla \times \mathbf{H} = \mathbf{J}_f + \frac{\partial \mathbf{D}}{\partial t} \quad (2.4)$$

where ρ_f and \mathbf{J}_f are the charge density and current caused by the presence of free charges in the medium, and \mathbf{D} and \mathbf{H} are the displacement and magnetising fields respectively. The latter two are defined by:

$$\mathbf{D} = \epsilon \mathbf{E} + \mathbf{P} \quad (2.5)$$

$$\mathbf{H} = \frac{1}{\mu} \mathbf{B} - \mathbf{M} \quad (2.6)$$

where μ is the vacuum permeability,, ϵ is the permittivity of free space, \mathbf{P} is the polarization field and \mathbf{M} is the magnetisation field, these fields arise due to the bound charges throughout the medium interacting with the EM field.

The force exerted by an optical tweezer can be subdivided into the gradient and scattering components, for most modelling research this is how the force fields are reported. The gradient force is a conservative force brought about by the polarisation of dielectric materials which is directed towards the point of maximum intensity (for a simple Gaussian beam this would be at it's focal point) [2]. Typically, optical tweezers will utilise a higher numerical aperture lens in order to increase the intensity, thus allowing for stronger gradient forces at the trade off of decreased trapping depth.

The scattering force arises from the motion of bound charges generating secondary scattering as they move through the electric field [2]. Even if the particle experiences no external forces the scattering force is still significant as the electric field oscillates, though for most practical applications the time dependence is ignored. While some trapping arrangements can induce transverse scattering forces for simple spheres the scattering force is only significant in the direction of propagation [3]. In order to

determine where a particle will remain at rest in an optical trap requires determining where the potential well formed by the gradient force is equal to the thermal energy of the system.

2.2 Lorenz-Mie Theory

The Lorenz-Mie theory provides an exact solution to the Maxwell equations for the scattering caused by an isotropic sphere. This theory describes the scattered wave given off by a dielectric sphere when incident by a plane wave as a summation of partial spherical waves. For any wave equation the vector fields must solve the Helmholtz equation given by:

$$\nabla^2 \mathbf{E} + k^2 \mathbf{E} = 0 \quad (2.7)$$

where k is the wave number of the electromagnetic radiation ($k = \omega^2 \mu \epsilon / c^2$). This combined with the constraints of Maxwell's equations leaves very few exact solutions apart from spherical or planar waves. For a laser of arbitrary shape the electric field can be described as a summation of partial spherical waves multiplied by an expansion coefficient. Depending on the beam in question the expansion coefficients can be tailored to match the desired beam shape. For an approximate Gaussian beam interacting with a sphere of radius a , the incident, internal, and scattered fields are given as [4]:

$$\mathbf{E}_{\text{inc}}(\mathbf{r}) = E_0 \sum_n \sum_{m=-n}^n \left[a_{nm} \mathbf{M}_{nm}^{(1)}(\mathbf{r}) + b_{nm} \mathbf{N}_{nm}^{(1)}(\mathbf{r}) \right] \quad (2.8)$$

$$\mathbf{E}_{\text{scat}}(\mathbf{r}) = E_0 \sum_n \sum_{m=-n}^n \left[p_{nm} \mathbf{N}_{nm}^{(3)}(\mathbf{r}) + q_{nm} \mathbf{M}_{nm}^{(3)}(\mathbf{r}) \right] \quad (2.9)$$

$$\mathbf{E}_{\text{int}}(\mathbf{r}) = \mathbf{E}_{\text{inc}}(\mathbf{r}) + \mathbf{E}_{\text{scat}}(\mathbf{r}) \quad (2.10)$$

where a_{nm} , b_{nm} , p_{nm} , and q_{nm} are the expansion coefficients of each of the fields, and \mathbf{M}_{nm} and \mathbf{N}_{nm} are the magnetic and electric vector spherical harmonics respectively. The superscript denote whether the field is inward [(1)] or outward propagating [(3)]. By linearity of Maxwell's equations, its possible to relate the expansion coefficients

of the scattered field directly to those of the incident field. As such we can write the scattering coefficients in terms of their incident components and the target's 'T-matrix'.

$$\begin{pmatrix} q_{mn} \\ p_{mn} \end{pmatrix} = \mathbf{T} \begin{pmatrix} a_{mn} \\ b_{mn} \end{pmatrix} = \begin{pmatrix} T_{11} & T_{12} \\ T_{21} & T_{22} \end{pmatrix} \begin{pmatrix} a_{mn} \\ b_{mn} \end{pmatrix} \quad (2.11)$$

Where the matrix \mathbf{T} accounts for the targets shape, and refractive index. The T -matrix method can be easily applied to any solution of Maxwell's equations. The T -matrix method allows us to relate directly the momentum carried by the incoming wave 2.8 to the momentum retained by the scattered wave 2.9, where the difference between these two quantities is the momentum transferred to the scattering object. The time averaged change in this momentum transfer provides an approximation of the force imparted to the target scatterer.

TO BE MOVED And the time averaged linear momentum carried by the scattered beam is given by:

$$\begin{aligned} \langle P_{z, out} \rangle = & -\frac{1}{4\pi k^2} \sum_{n,m} \left(\frac{m}{n(n+1)} \Im[p_{nm} q_{nm}^*] \right. \\ & + \frac{1}{n+1} \left[\frac{n(n+2)(n-m+1)(n+m+1)}{(2n+1)(2n+3)} \right]^{1/2} \\ & \times \Im[q_{nm} q_{nm}^* + p_{nm} p_{n+1m}^*] \Big) \end{aligned} \quad (2.12)$$

Therefore the total force imparted to the trapped particle is thus:

$$\mathbf{F}_z = \frac{dP}{dt} = \langle P_{z, in} \rangle - \langle P_{z, out} \rangle \quad (2.13)$$

Where $a_{nm}, b_{nm}, p_{nm}, \& q_{nm}$ are the beam expansion coefficients used in (2.8) & (2.9). Similar expressions are shown in Sec. 2.6 for calculating the optical torque. Lorenz-Mie theory can be applied to describe the scattering from any particle regardless of size. Though as the size of the target particle changes - relative to the wavelength of light - the theory describing the electromagnetic fields are easier to approximate by alternative theories. When the particle radius is $\gg \lambda$ the optical force is best described by the Ray-Optics model. In the opposite case the target particle is better approximated as a

single electric dipole as described by the Rayleigh approximation.

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2.2.1 T-matrix Method

The T -matrix method was first developed by Peter Waterman with his research into acoustic wave scattering [9], this would later be extended to electromagnetic waves. Sometimes referred to as the extended boundary condition method (EBCM), the method replaces the scatterer with a series of surface currents over the targets surface. These currents are chosen so that the electromagnetic field outside is identical to the original problem [10]. By expanding the harmonic functions one can derive the individual elements of \mathbf{T} until a desired accuracy is achieved. One can either use the EBCM to compute the scattering for a fixed particle orientation and position. Or they can evaluate the scattering for any particle orientation by computing its T -matrix. Because the T -matrix is based only on the properties of the scatterer it can be reused in calculations regardless of the incident field in question.

This is in truth merely an extension of the Lorenz-Mie theory as the T -matrix of a single sphere can be found from the electric field solutions. The method developed by Waterman allows for any arbitrary shaped particle to produce similar solutions. One of the biggest advantages to the method is the fact that it can leverage symmetries in particle geometries to speed up calculations.

For this project we utilised an extension of the EBCM known as multi-sphere T -matrix method (*mstm*) developed by Mackowski [11]. Where Lorenz-Mie Theory centres the spherical harmonics expansion about the sphere's origin it often struggles to adjust the expansion to any arbitrary origin. *mstm* utilises an addition theorem to translate the individual spherical expansions from the centres of each individual sphere to a preset target origin. *mstm*'s primary function is to compute the averaged scattering from large spherical aggregates, but for our purposes it can be also utilised to compute T matrices for arbitrary shaped spherical aggregates.

The T -matrix method is exceptionally useful for computing the scattering from any arbitrary spherical aggregate. However, the T -matrix method by itself can be

computationally taxing as the number of spheres increases [11]. While it is possible to solve for the full electromagnetic field for the entire cluster this is only applicable for a single orientation and can be even slower for large aggregates [12], [13]. The benefit of *mstm* is that the major scattering properties (scattering and extinction cross sections, scattering matrices, etc) can all be computed both for single orientations, or averaged over multiple orientations to determine the average scattering from the target particle.

2.2.2 Discrete Dipole Approximation

The discrete dipole approximation (DDA) is a general method that can be applied to the scattering from particles of arbitrary composition and geometry. Developed by Purcell and Pennypacker [14], the DDA method approximates the particle as being constructed of discrete dipoles. This is essentially an extension of the Rayleigh approximation, though in order to compute the optical force one now requires a full description of the electric field. Each dipole interacts with both the incident field and the scattered fields from every other dipole surrounding it. The resulting scattered field is identical to the scattered field produced by direct integration of Eq. (2.10) throughout the full particle volume [15]. The integral form for the total electric field inside a scatterer is given as [10]:

$$\mathbf{E}(\mathbf{r}) = \mathbf{E}_{inc}(r) + \sum_i^{N_d} \mathbf{E}_{scat,i} = \mathbf{E}_{inc}(r) + \int_{V/V_0} d^3r \bar{\mathbf{G}}(\mathbf{r}, \mathbf{r}') \chi(\mathbf{r}') \mathbf{E}(\mathbf{r}') \quad (2.14)$$

Where $\bar{\mathbf{G}}$ is the Greens dyadic function of free space, which defines the impulse response between two separate dipoles; and χ is the susceptibility of the medium, which describes the degree of polarisation of the medium in the presence of an electric field. One of the primary advantages of DDA over the T-matrix method is that the composition of the target can be changed freely. When comparing different computational scattering methods, the EBCM was found to be better suited for simulating the scattering of symmetric targets as the EBCM can use the target's symmetry to speed up calculations [10]. But when dealing with inhomogeneous media DDA is more efficient compared to EBCM.

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The DDA method can be used to derive the target's T -matrix. After generating the far field expansion of the electric field (Eq. (2.14)), the vector spherical expansion far field (Eq. (2.9)) can be matched to it. Solving for the expansion coefficients p_{nm} , and q_{nm} allows one to construct the T-matrix of the target.

2.3 Ray-Optics Regime

The Ray-Optics model is the simplest to understand, this theory models the electromagnetic field as a collection of individual 'rays' that propagate and are refracted according to Snell's Law. The Ray optics model is generally only used in cases where the particle size is far greater than the wavelength of light. Based on the change in direction momentum is transferred to the target particle; with rays closest to the centre of the beam having greater intensity than those rays at the very edge of the beam. Consider a particle struck by two rays in a Gaussian beam, one coming close to the centre, and the other ray coming from the edge. As each ray is refracted by the target sphere a force is imparted onto it, the total force imparted is given by:

$$F_i = Q_i \frac{\Delta n P_i}{c} \quad (2.15)$$

where Q_i is the trapping efficiency, Δn is the difference in refractive indices between the solution and the target particle, and P_i is the power of the individual ray. The net force can be subdivided into its gradient and scattering components, where the latter directs the particle to the centre of the beam, and the former acts on the particle in the direction of beam propagation. For a beam with a Gaussian intensity distribution P_i will fall off as you move from the centre of the beam. While the ray optics model can be useful in predicting the forces experienced by said particle's it does not fully capture the behaviour of light when considering interference between different rays. And as such no information is learned about the scattered field.

2.4 Rayleigh Scattering

The Rayleigh approximation is for describing a particle when the particle radius is $\ll \lambda$, as the particle shape decreases we find the effective scattered area approaches zero as the incident light diffracts around the target particle. As such we can approximate a dielectric sphere as a dipole while in the presence of the electromagnetic field. In which case the scattering force is given simply by the scattering of the induced dipole,

and the gradient force is due to the Lorentz force [7]. The gradient forces in the principle Cartesian axis are described by Harada et al [2] in MKS units as a restorative rectangular force field:

$$F_{grad,x} = -\hat{x} \frac{2\pi n_2 a^3}{c} \left(\frac{m^2 - 1}{m^2 + 2} \right) \frac{4\tilde{x}/w_0}{1 + (2\tilde{z})^2} \times I(r) \quad (2.16)$$

$$F_{grad,y} = -\hat{y} \frac{2\pi n_2 a^3}{c} \left(\frac{m^2 - 1}{m^2 + 2} \right) \frac{4\tilde{y}/w_0}{1 + (2\tilde{z})^2} \times I(r) \quad (2.17)$$

$$F_{grad,z} = -\hat{z} \frac{2\pi n_2 a^3}{c} \left(\frac{m^2 - 1}{m^2 + 2} \right) \frac{4\tilde{z}/w_0}{1 + (2\tilde{z})^2} \times I(r) \times \left[1 - \frac{2(\tilde{x}^2 + \tilde{y}^2)}{1 + (2\tilde{z})^2} \right] \quad (2.18)$$

$$(2.19)$$

where, for a approximate Gaussian beam the intensity $I(r)$ close to the beam focus can be calculated as such.

$$I(r) = \left(\frac{2P}{\pi w_0^2} \right) \frac{1}{1 + (2\tilde{z}^2)} \exp \left[-\frac{2(\tilde{x}^2 + \tilde{y}^2)}{1 + (2\tilde{z})^2} \right]$$

Where m is the relative refractive index (n_1/n_2), n_2 is the particle's refractive index, ω_0 is the beam waist, a is the radius of the particle, and $\tilde{x}, \tilde{y}, \tilde{z}$ are simply the x, y, z coordinates but scaled by the beam radius. The scattering force however, is dependent on the effective scattering cross-sectional area.

$$F_{scat} = \hat{z} \left(\frac{n_2}{2} \right) C_{pr} I(r) \quad (2.20)$$

where:

$$C_{pr} = \frac{8}{3} \pi (ka)^4 a^2 \left(\frac{m^2 - 1}{m^2 + 2} \right)^2 \quad (2.21)$$

There is a clear difference between Eq. (2.16)- (2.19) and Eq. (??)- (2.13), namely that the Rayleigh regime is not concerned with the state of the scattered field. This is because if the particle is approximated by a point dipole we can ignore the momentum transfer calculation and simply compute the Lorenz force for a dipole in an electric

field. While not important for optical force calculation higher complexity scattering problems can be simplified by subdividing the particle into discrete dipoles (see Sec ??), in which case the scattered field is now significant. As the target particle gets larger this approximation fails to accurately describe the trapping force [8]. For larger particles the optical force and scattering is better described via the Lorenz-Mie theory.

2.5 Describing the trajectory of a trapped particle

While describing the optical forces is sufficient for theoretical characterisation of an optical trap, in an experimental setting the observed motion is not as simple. As such it is often far simpler to characterise the optical trap by recording the particle's trajectory (see ?? for specifics regarding the equipment), and then fitting it to our presumed description of the beam. The trajectory is easily understood if we understand the particle's diffusive behaviour.

2.5.1 Diffusion Models

For the case of optical tweezers the most complete model of diffusion is the Langevin equation. Models such as the Fickian, and Einstein derivations are sufficient for macroscopic behaviours but the Langevin model better describes the microscopic characteristics of any diffusive behaviour.

The Fickian model describes the net flux of solute molecules into a finite volume of fluid being proportional to the density gradient $\delta\rho(u,t)/\delta u$ [16]. It assumes that the solute molecules do not collide with one another or with molecules in the solution; overall, the Fickian model is used to describe how solute molecules disperse over long periods of time. It does not provide any insight into the forces acting on individual molecules, nor does it prove particularly useful over smaller time scales. Whereas the Langevin model captures the physical interactions between an individual molecule and the surrounding fluid over a wide range of time scales.

The Einstein model of diffusion expands upon the Fickian model by considering the collisions between individual molecules. If we consider a single particle suspended in

a solution it will experience multiple collisions with other molecules [17] over a given time interval δt . Applying statistical analysis to an observed trajectory allows one to make measurements of a particle's diffusive behaviour within a fluid. Where it begins to falter is when we consider bringing δt to 0; in this scenario the molecule experiences not many but only individual collisions. The Einstein model does not consider the inertia of the molecule in question and so for very short time frames the model implies that the molecule's velocity changes instantly after each collision [17], [18]. Furthermore, the kinetic energy of each collision is not limited by the thermal energy of the system, meaning that using the Einstein model to predict a particle's trajectory to an finite degree of accuracy implies that the particle has infinite kinetic energy [18]. This failure to describe motion over smaller time frames is addressed in the Langevin model by accounting for the fluid drag of the system, where any sudden change in velocity must result in a proportionally opposed drag force [19].

The Langevin model of diffusion assumes that the net force on a particular particle is described fully by these individual collisions [19]. Unlike the Fickian model it provides a full description of the interactions between the target molecule and the surrounding fluid; while also being able to describe its motion over any time scale, unlike the Einstein model. Unlike the Einstein model that views collisions as discrete but finite events, the Langevin model instead considers the collisions as one random. The standard form of the Langevin model for a particle undergoing Brownian motion is given as:

$$m \frac{d\vec{v}}{dt} = -\gamma_0 \vec{v} + F(t) + W(t) \quad (2.22)$$

Where the first term accounts for inertial forces, the second term accounts for friction forces which counteract the particles current motion (γ_0 is the friction coefficient), and the final term accounts for the random Brownian force. The $F(t)$ is there for convention which accounts for any external forces acting on the particle. These three terms are equal to the random Brownian motion of the surrounding fluid. We can say that the noise term $W(t)$ has a normal distribution, being scaled by the thermal energy

of the system, with a correlation function of:

$$W(t) = \sqrt{2k_B T \gamma_0} \eta(t) \quad (2.23)$$

$$\langle W_i(t) W_j(t') \rangle = 2k_B T \gamma_0 \delta(t - t') \quad (2.24)$$

The Langevin model can be extrapolated to describe the diffusive behaviour of an overall system, but for this project we can instead consider the behaviour of a singular particle with a diffusion tensor D suspended in a viscous fluid and spacially localised by an optical potential with trap strength κ . Assuming the only external force acting on our particle is the laser, the net force should be exactly equal to force of the stochastic collisions due to the fluids thermal energy. If we focus our analysis when the particle is stably trapped and assume that the trap is harmonic, we can model the trapping force as a Hookean spring ($F(t) \approx \kappa x(t)$). The full Langevin equation for an optically trapped particle is therefore given as:

$$m \frac{\delta^2 x(t)}{\delta t^2} + \gamma_0 \frac{\delta x(t)}{\delta t} + \kappa x(t) = \sqrt{2k_B T} \eta(t) \quad (2.25)$$

Eq. (2.25) provides an accurate description of strongly trapped particles. Despite this the analytical solution of the Langevin equation requires integration of the noise term making it difficult to simulate the trajectory of a given particle [20]. Instead, it is often far easier to solve the equation numerically and use the analytical solution to calibrate and extract information about the particle and fluid, and how the two interact with the optical trap. Solving the Langevin equation numerically is far easier when considering small time steps.

2.5.2 Finite Difference

The Finite Difference approach involves discretizing the time and spatial elements in order to approximate the higher order terms. If we assume that $x(t)$ is differentiable

to n (we can find its n^{th} derivative) then we can use the Taylor series expansion to get:

$$x(t + \Delta t) = x(t) + \frac{x'(t)}{1!}\Delta t + \frac{x''(t)}{2!}\Delta t^2 + \dots + \frac{x^{(n)}(t)}{n!}\Delta t^n + R_n(x(t)) \quad (2.26)$$

Where $R_n(x(t))$ is the remainder term between the Taylor expansion to term n and the actual expression. If we limit our approach to the first derivative only, we find that for sufficiently small values of R_1 the velocity and acceleration can be approximated as:

$$x'(t) \approx \frac{x(t + \Delta t) - x(t)}{\Delta t} \quad (2.27)$$

$$x''(t) \approx \frac{x'(t + \Delta t) - x'(t)}{\Delta t} = \frac{x(t) - 2x(t + \Delta t) + x(t + 2\Delta t)}{\Delta t^2} \quad (2.28)$$

By reversing the time step (i.e. use $-\Delta t$) to approximate the velocity and acceleration based on the previous time steps, we can discretise the position by taking finitely small time steps (i.e. $x(t) = x_i$, $x(t - \Delta t) = x_{i-1}$). The same cannot be done for noise, as no information is known about $W(t)$ at any time. We can instead say that the velocity of a Brownian particle should approximate our noise as a random walk, where at each new time step, the position changes randomly within a given range. Constricting the variance to $\sqrt{2D}/\Delta t$ allows us to represent the noise using the finite-difference approach as:

$$m \frac{x_i - 2x_{i-1} + x_{i-2}}{\Delta t^2} = -\gamma \frac{x_i - x_{i-1}}{\Delta t} + \sqrt{2k_B T \gamma} \frac{w_i}{\sqrt{\Delta t}} \quad (2.29)$$

Where w_i is a Gaussian random real number between -1 and 1, we can say that it is normally distributed around 0 for simulation purposes. We can rearrange this for x_i to approximate the Brownian motion of a particle (setting $x_0 = 0$), where the characteristic time is $\tau = m/\gamma$. Now in the case of an optical trap, the restoration time scale is given by $\tau_{OT} = \kappa_x/\gamma$ which for strongly trapped particles is far greater than the characteristic time. As such we can neglect the particle's inertia which allows the

motion of an optically trapped particle to be written as:

$$x_i = x_{i-1} - \tau_{OT} \dot{x}_{i-1} \Delta t + \sqrt{2D\Delta t} w_i \quad (2.30)$$

This result can be generalised for a 3-dimensional description of an optically trapped particle, where each Cartesian direction has its own unique characteristic restoration time. We see from the result that trajectory is dependent on only a handful of factors, the trap stiffness κ_x , the fluid viscosity γ , and the thermal energy of the system $k_B T$ (with the latter two being related by Einstein's formulation of the diffusion coefficient $D = k_B T / \gamma$). Therefore, by calculating these parameters to a high degree of precision allows one to get a precise description of the forces experienced by a target particle, which in the past has been used for highly accurate force transduction [21], [22].

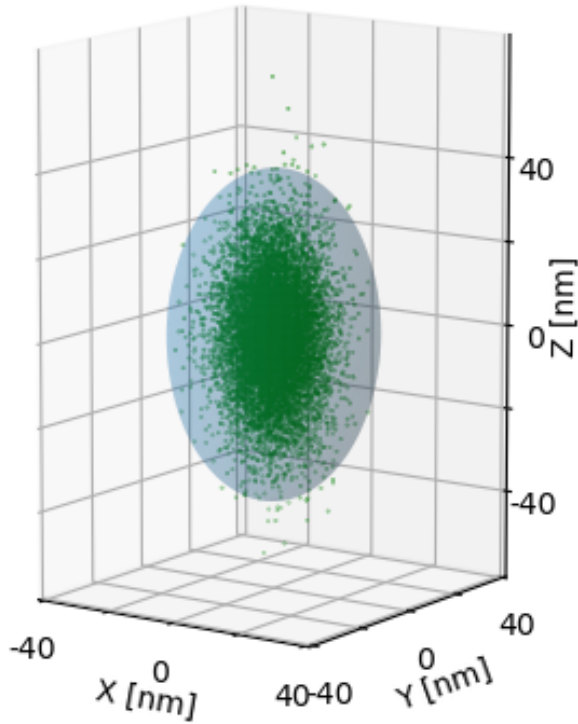


Figure 2.1: Example trajectory created using Finite Differences method for a $2\mu m$ diameter sphere. Trap stiffness's were estimated using *ott* at $\kappa_x = \kappa_y = -100 \text{ pN}/\mu m$ and $\kappa_z = -25 \text{ pN}/\mu m$. The particle's motion can be localised around the shaded ellipsoid.

2.6 Simulation of spherical aggregates

Later chapters cover the dynamics of spherical aggregates and anisotropic scatterers, these subjects are particularly difficult to characterise using conventional calibration techniques [29], [30]. As an example consider a symmetric dimer as a paradigmatic aggregate; if we consider the Langevin equation for such a aggregate within an optical trap we have:

$$\frac{d\vec{r}(t)}{dt} = \frac{\vec{\kappa}_x}{\gamma} \vec{r}(t) + \sqrt{2\vec{D}_x\eta(t)} \quad (2.31)$$

Where $x(t)$ is replaced with $\vec{r}(t)$ to signify that the translational motion is generalised to a 3 dimensional case. Here we omit the inertia term as the restoration time of the optical trap is far greater than the characteristic time of the particle ($\kappa/\gamma \gg m/\gamma$). Now, the dimer is undergoing random rotational motion in addition to its Brownian translational motion the first term on the right hand side is no longer purely a function of the dimer's position but also of its orientation. The rotational form of the Langevin equation for a dipole within an external potential is given as [31]:

$$\frac{d\vec{u}(t)}{dt} = \frac{\mu}{\gamma_R} [\vec{u}(t) \times E(t)] \times \vec{u}(t) + \sqrt{2k_B T \vec{D}_R \lambda(t)} \times \vec{u}(t) \quad (2.32)$$

Where $\vec{u}(t)$ is the unit vector aligned along the centres of the two spheres, μ is its dipole moment, and γ_R is the rotational friction coefficient which is given as $\gamma_R = 8\pi\eta r^3$ for a sphere. If we assume that over the course of a trajectory the dimer has a 'equilibrium' orientation in which the optical torque is minimised we can say that the first right hand term can be replaced with $\vec{\kappa}_u(\vec{r}(t))$ to represent a harmonic potential due to the torque. $\lambda(t)$ is the Brownian rotations from the surrounding fluid, like in the translational case the Brownian rotations are normally distributed and are also uncorrelated so that:

$$\langle \lambda(t) \lambda(t') \rangle = \delta_{ij} \delta(t - t') \quad (2.33)$$

For an asymmetric scatterer whose radius is comparable to that of the electric field's

wavelength we now have a system of simultaneous equations:

$$\frac{d\vec{r}(t)}{dt} = \frac{\vec{\kappa}_x(\vec{u}(t))}{\gamma} \vec{r}(t) + \sqrt{2k_B T \vec{D}_\eta(t)} \quad (2.34)$$

$$\frac{d\vec{u}(t)}{dt} = \frac{\vec{\kappa}_u(\vec{r}(t))}{\gamma_R} \times \vec{u}(t) + \sqrt{2k_B T \vec{D}_R \lambda(t)} \times \vec{u}(t) \quad (2.35)$$

Fortunately, we do not need to solve these directly as the latter two random variables can be easily approximated if the thermal energy of the system is known, and the rate of change can be assumed as linear if we take a sufficiently small time step that $\Delta t \ll \kappa_x/\gamma$ & $\Delta t \ll \kappa_u/\gamma_R$. In doing so we now only need to compute the optical force and torque applied to the dimer.

2.6.0.1 Harmonic Traps

When a sphere is located at the centre of a laser the gradient force will try to recentre the sphere if it is displaced. If the restoring force is proportional to the sphere's displacement this is referred to as a harmonic trap. The ratio of the restoring force to the displacement is called the trap stiffness (denoted by κ), and is an important characteristic of the trap. Fig. 2.2 demonstrates an example case of the force-displacement curve for a simple sphere in an optical trap. Laser power and numerical aperture are omitted as the exact shape is not of importance, only the linear region about the origin.

The strength of the optical trap is directly proportional to both the laser power and the contrast in refractive indices between the medium and the particle. Fully describing the optical forces is dependent on the ratio between the particle size (a) and the trapping wavelength (λ). The scattering theory that describes the optical force is dependent on this ratio.

2.7 Calibration Techniques

There are several approaches for calibrating and characterizing the optical trap, each approach has its drawbacks and benefits so each option should be chosen based on what elements want to be characterized. The basis for each of these methods stems from the

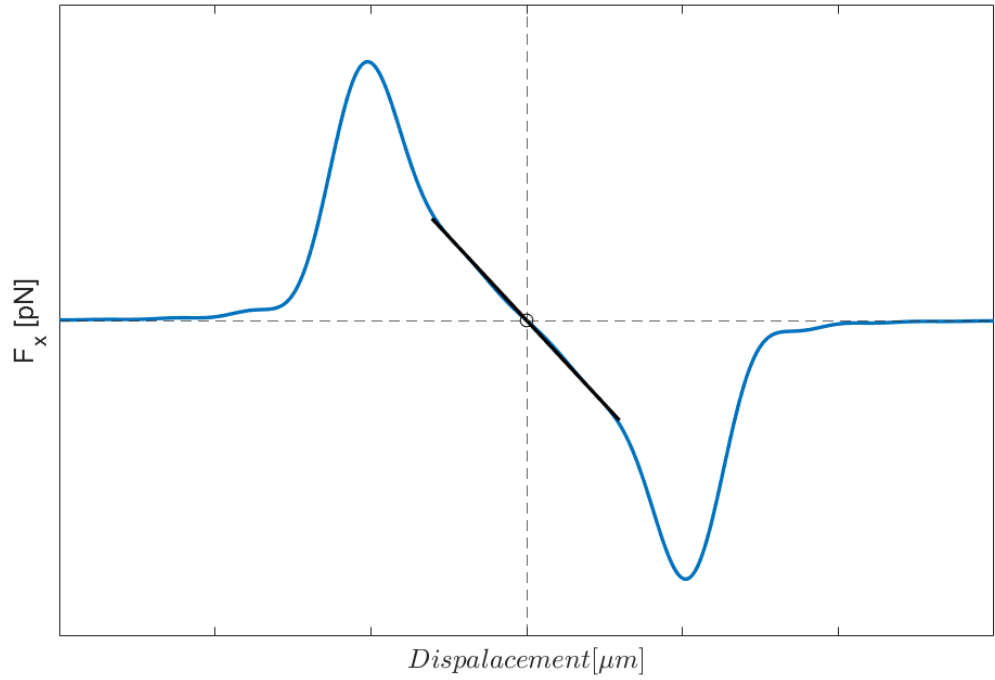


Figure 2.2: Example of a sphere's ($r = 1 \mu m$) force-displacement curve while moving along the x-axis, where $x = 0$ is the centre of the beam. The harmonic regime is highlight by the linear fit given in black.

analytical solution of the Langevin equation:

$$x(t) = x(0)e^{-t/\tau_{OT}} + \sqrt{2D} \int_0^t ds W_x(s) e^{-(t-s)/\tau_{OT}} \quad (2.36)$$

Positional data is often acquired in an experimental setting either using image analysis or photodiodes to infer the particles position relative to the beam focus. The former is often used in cases when precision is not a key concern, as often a standard CCD camera will have limited spacial and temporal resolution. The latter method often requires the use of a quadrant photo diode (QPD), the data provided by the QPD will often need to be converted to physical units to compute the force directly - see Eq. (2.51).

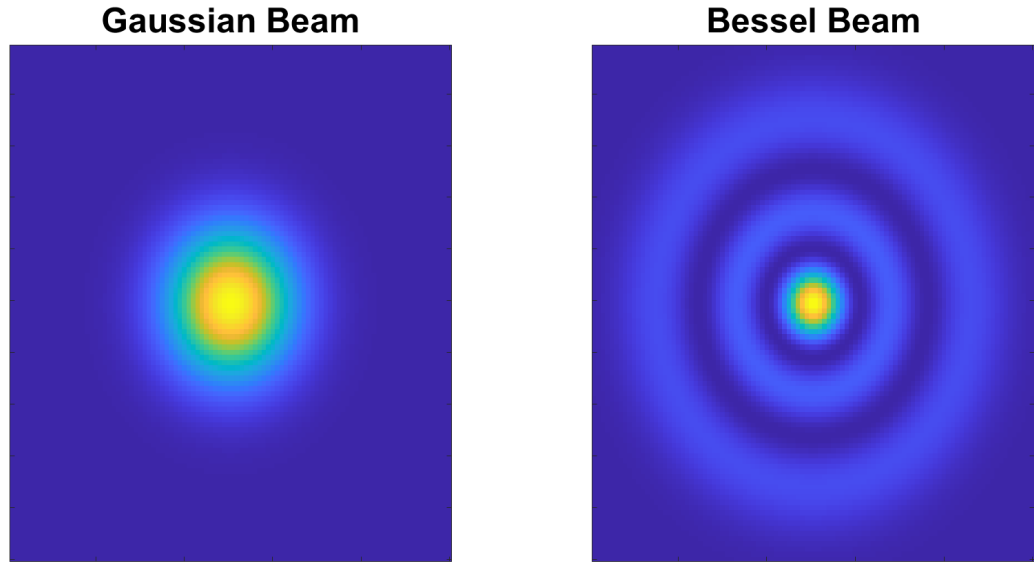


Figure 2.3: Intensity distribution from a Gaussian and Bessel beam.

2.7.1 Beam shapes

A quick aside is necessary prior to the discussion of calibration techniques, namely on the assumption of the beams shape. It has been previously mentioned that the focused beam can be approximated as a Gaussian beam, what this implies is that the beam has a circularly symmetrical intensity profile that can be described by the Gaussian function. There are alternative beam formations that also preserve the circularly symmetric profile, such as Bessel beams that have an intensity profile that is described by the Bessel function.

An idealised form of a Gaussian and Bessel beam would be unbounded, meaning the intensity of the electric field never goes to 0 - approaching it asymptotically instead. This however breaks Maxwell's Equations (see Eq. (??) - (??)), what this means is that in reality is that a true Gaussian or Bessel beam cannot exist, only a close approximation [23]. Due to the converging fields, a focused beam will experience fluctuations in its electric field that distort the intensity profile [24]. This effect is insignificant for slowly converging beams but for trapping objectives used in optical tweezing there is a notable effect.

When a Gaussian beam is linearly polarised and focused to a point the focal point will not be perfectly symmetrical but instead show some degree of ellipticity even if the initial beam itself is kept parallel. In practical terms this means that regardless of the calibration technique used the trap stiffness along the x and y axis will not be equal to one another. Proper alignment can bring them close together and longer data acquisition times can reduce the uncertainty of the measured values, but there will always be a slight variance in the two values. We can measure the ellipticity of the beam using the acquired values from our calibration.

$$e = \left(1 - \frac{\kappa_x}{\kappa_y}\right)^{1/2} \quad (2.37)$$

Generally the concern is more over the uncertainty in the trap stiffness values and not the overall shape. Even laser manufacturers will often state a maximum ellipticity, assuming perfect alignment and focusing, in most cases a ellipticity of 10-20% is still considered acceptable for most calibration results [21].

2.7.2 Potential Well Analysis

The Langevin equation for an optically trapped particle assumes that the trap acts similar to a Hookean spring that creates a potential well about its centre. Therefore a simple analysis method is to understand the height and width of said potential well.

Potential analysis is a useful technique for estimating the strength of an optical trap; this method assumes that the force acting on the particle is purely conservative, an accurate presupposition if we ignore the motion of the particle as it enters the trap. This is because the scattering force is far more significant far away from the potential well and is negligible if the trap strength is much greater than the thermal fluctuations. With this in mind we can write the probability of finding the particle at position x as:

$$\frac{\rho(x)}{\rho_0} = e^{-\frac{U(x)}{k_B T}} \quad (2.38)$$

which therefore means we can write the potential well as:

$$U(x) = -k_B T \ln \left(\frac{\rho(x)}{\rho_0} \right) \quad (2.39)$$

Now assuming our laser approximates a Gaussian beam we should be able to describe the probability distribution $\rho(x)$ centred at some equilibrium position x_0 :

$$\rho(x) = \sqrt{\frac{\kappa_x}{2\pi k_B T}} \exp \left(-\frac{\kappa_x}{2k_B T} x^2 \right) \quad (2.40)$$

After observing the particles position over a long enough period of time we can construct an experimental estimation of $\rho(x)$. By inserting (2.40) into (2.39) we can fit the potential well in order to determine the trap strength κ_x . This has some limitations in that the large fluctuations can throw off the fit meaning a longer acquisition time is necessary to properly fit the potential well, making it difficult to characterise weakly trapped particles who may not remain trapped for long. It also provides no information on the particle itself (i.e. the friction coefficient γ and diffusion tensor D).

2.7.3 Equipartition method

The Equipartition method is by far the fastest and simplest means for estimating the trap strength but unlike Potential Analysis is limited strictly to harmonic potentials. This can be often not the case for highly focused beams, as the trap strength can vary due to polarisation differences. Simply put we can use the equipartition theorem to relate the potential well to the particle's thermal energy using (2.40):

$$\langle U(x) \rangle = \frac{1}{2} \kappa_x \langle (x - x_{eq})^2 \rangle = \int_{-\infty}^{\infty} \rho(x) (x - x_{eq})^2 = \frac{1}{2} k_B T \quad (2.41)$$

$$\implies \kappa_x = \frac{k_B T}{\langle (x - x_{eq})^2 \rangle} \quad (2.42)$$

By taking a time average over multiple trajectories to get $\langle x - x_{eq} \rangle$ we can get a fairly accurate estimation of the trap strength. Because this requires a time average of the particle's displacement any large errors in the position measurement can have knock-on effects. Likewise with the potential analysis route, no information is gleaned

about the particle itself.

2.7.4 Mean Square Displacement

Mean square displacement (MSD) is a common means of describing the random motion of a given particle (or group of particles). This is useful information if say for example we want to understand reaction kinetics on the surface of a catalyst, if we know how far its likely to move from the surface we can tell if its likely to react when a catalytic site becomes available. As it pertains to colloids, consider a suspension of silica spheres immersed in a fluid undergoing Brownian motion (as described by the Langevin Equation) so that:

$$m \frac{\delta^2 x}{\delta t^2} + \gamma \frac{\delta x}{\delta t} = \eta(t) \quad (2.43)$$

Where γ is the objects friction coefficient which for spheres is given as $\gamma = 6\pi\eta r$, and $\eta(t)$ is a random white noise variable that is directly related to the thermal energy of the surrounding fluid. If the motion is truly random then we should see the average displacement increase over longer observation periods. This can be useful for measuring characteristic times of a system, or in the case of optical trapping, characterise the optical field.

For each sphere we can record its position in the $x - y$ plane and measure its displacement from a set reference point; for example with an optical tweezer this could be the beam focus. We can measure the MSD by forming a 'window' between two points in time of the trajectory (i.e. t & $t + \tau$) and sliding this window along the entire trajectory length and then take the average of this series. Repeating over a range of time lags (τ) allows us to describe the MSD as a function of τ :

$$MSD(\tau) = \langle |x(t + \tau) - x(t)|^2 \rangle \quad (2.44)$$

If we sub in (2.36) for $x(t)$ we can expand out the squared term to get an analytical

expression for the MSD as a function of time lags:

$$MSD(\tau) = \langle |x(t+\tau)^2 - 2x(t+\tau)x(t) + x(t)^2| \rangle \quad (2.45)$$

$$\rightarrow MSD(\tau) = \frac{2k_B T}{\kappa_x} \left[1 - e^{-\frac{\tau}{\tau_{OT}}} \right] \quad (2.46)$$

From this expression its evident that the mean squared displacement increases with larger values of τ until it reaches a maximum value as shown in fig. 2.4 by the dotted line.

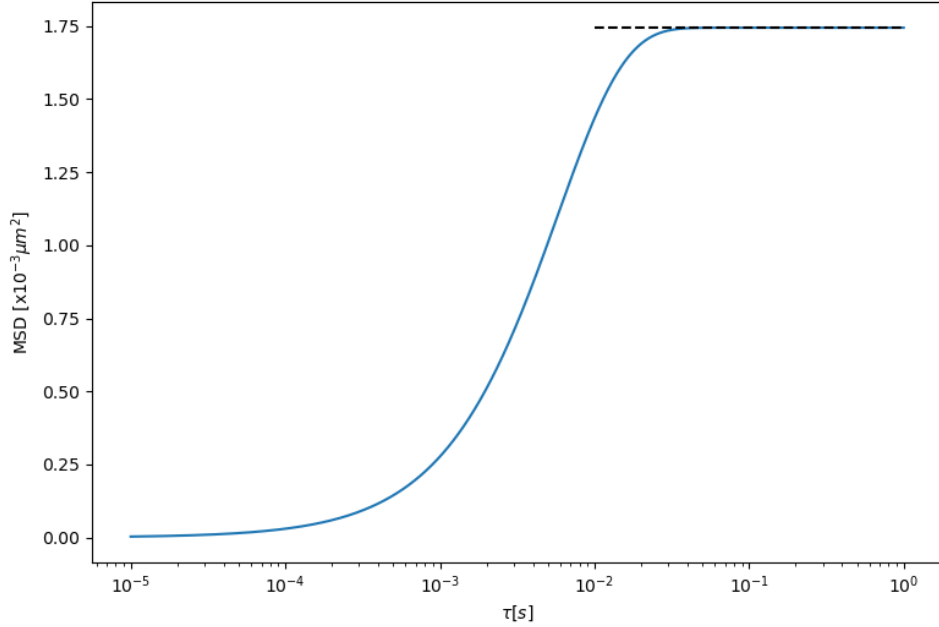


Figure 2.4: Example mean squared displacement using (2.45), for a $1\mu m$ sphere trapped by an optical potential well. The dotted line represents the upper limit of the sphere's displacement due to the optical trap.

The MSD plot can be subdivided into two regimes, when $\tau \gg \tau_{OT}$ the particle is experiencing the harmonic potential described by the equipartition theorem, and when $\tau \ll \tau_{OT}$ the particle is said to be freely diffusing within the trap focus. Of course for a freely diffusing object the MSD will never reach a plateau value, comparing MSD's for different particles provides a simple visual indicator of the difference in trapping

strength. The MSD method is an already very versatile analytical tool for diffusive motion, however it is rather slow in computing time meaning it is only really beneficial when a high degree of accuracy is required and shorter time resolutions are unavailable - such as using a quadrant photo diode instead or a high speed CCD.

2.7.4.1 Mean Square Angular Displacement (MSAD)

It is also possible to plot the angular MSD (MSAD) using simulative or experimental data, but there is no complementary expression for Eq. (2.45). This is because while the particle is close to the focus the optical force is easily modelled as a Hookean spring, even if the particle undergoes some rotation. Conversely, the optical torque applied to a particle is far more sensitive to changes in position meaning a simple linear approximation is not satisfactory. Vigilante *et al* [25] derived the upper limit of a dimer's MSAD along its long axis by assuming it was strongly trapped and so had limited angular motion, there expression gives:

$$\lim_{\tau \rightarrow \infty} \langle (\Delta u_z)^2 \rangle = 2 \left[1 - \frac{1}{4\beta\kappa_r} \left(\frac{\exp(\beta\kappa_r) - 1}{\exp(\beta\kappa_r)F(\sqrt{\beta\kappa_r})} \right)^2 \right] \quad (2.47)$$

Where, u_z is the unit vector connecting the two spheres of the dimer, $\beta = 1/k_B T$, κ_r is the rotational stiffness of the trap, and F is Dawson's integral [26]. Vigilante's paper expressed that they couldn't compute $MSAD(\tau)$ because they couldn't solve the Einstein-Smoluchowski equation which describes the diffusion constant for dielectric particles. This would require a full description of a particle's electrical mobility and charge distribution - the latter could be achieved via a discrete dipole approximation, the former would be dependent on both the particle's position and relative orientation to the electric field.

2.7.5 Power Spectrum Density (PSD)

The power spectral density (PSD) method is by far the most versatile method for observing the dynamics of any object within an optical trap, allowing for fast calibration times while also quickly filtering out typical noise sources. After recording a trajectory

over some time t_{msr} , taking the Fourier transform of a particle's trajectory yields:

$$\hat{x}(f) = \frac{(2D)^{1/2}\hat{\eta}}{2\pi(f_c - if)} \quad (2.48)$$

where x can either be the physical position (relative to the trap focus) or a recorded signal from a position-detector, and $\hat{\eta}$ is the Fourier transform of the white noise (see Eq. (2.34)). Where the values are exponentially distributed as opposed to being normally distributed in the time domain [21]. We can therefore ignore the white noise from our analysis by looking at the spectral density of $\hat{x}(f)$ that produces a Lorentzian curve:

$$S_x = \frac{\hat{x}^2}{t_{msr}} = \frac{D}{2\pi(f_c^2 + f^2)} \quad (2.49)$$

Eq. (2.49) can be fitted via a simplified geometric series $S_x = 1/(A + Bf_k^2)$ which allows us to compute both the diffusion coefficient (in arbitrary units) and the corner frequency f_c which is directly related to the trap strength via $f_c = \kappa_x/(2\pi\gamma)$. The Lorentzian shape implies that the trap is harmonic - but not symmetric - which assumes that the particle itself is an isotropic scatterer. Anisotropic scatterers can produce an Lorentzian curve if the angular component of the power spectra is insignificant. The magnitude of rotational motion (whether it is stochastic [27] or periodic [28]) will effect how drastically it departs from a typical Lorentzian curve.

Like with the analytical expression of the mean squared displacement we see two distinct regions, when $f \ll f_c$ the PSD reaches a plateau value that when converted to length units represents the maximum displacement the particle can move beyond the focus. When $f \gg f_c$ the PSD falls off exponentially which denotes the particle is freely diffusing within the beam focus.

The Lorentzian relationship is only valid for frequency terms up to the Nyquist frequency (half of our sampling rate), this is because we are only taking a finite sampling of the particle's trajectory meaning the signal is aliased. Berg and Sorensen provide a

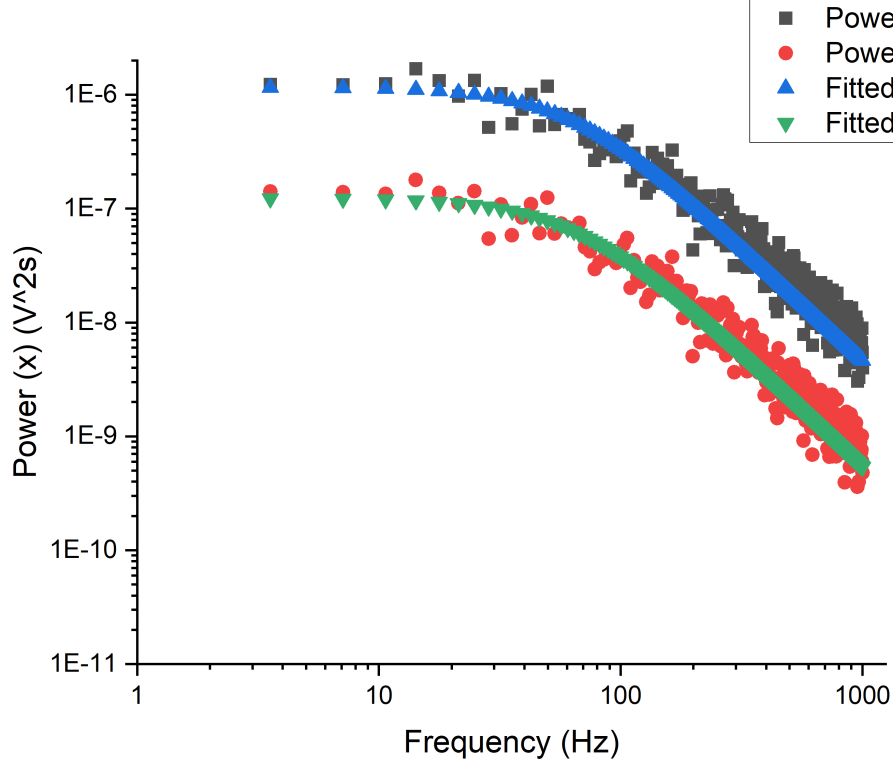


Figure 2.5: Example PSD fitted using (2.49), power spectra is collected from an optically trapped silica sphere suspended in water. The difference in magnitude is due to the asymmetry of the quadrant photo diode having a stronger signal response in the direction of the polarisation vector. Using a conversion factor (see (2.51)) will adjust the power spectra to better describe the trap shape.

suitable modified Lorentzian to account for the aliasing effects [21]:

$$S_x = \frac{(\Delta x)^2 \Delta t}{1 + c^2 - 2c \cos 2\pi f_k \Delta t / N} \quad (2.50)$$

Where N is the total number of samples taken, Δx & c have no direct physical interpretation and are defined in [21]. Further modifications can be made to the power spectrum model but this is only useful when a high degree of accuracy is necessary. Typically power spectra are recorded using a Quadrant Photo Diode (QPD), which records motion in voltage units, not in units of length. There are two main methods for converting to physical units: If multiple photodetectors are available then a differential

interference contrast (DIC) system can be used to compute the linear relationship between the beads displacement and the photodiodes signal [3]. While this is useful when high force precision is necessary it also allows you to collect information about the particle's motion along a single direction, making it ideal for less focused beams. Alternatively, if the size distribution is very wide but each particle can be accurately sized, then a conversion factor can be approximated by comparing the fitted value of the diffusion coefficient, and the reported value given by the Stokes-Einstein relation.

$$D_{SE} = \frac{k_B T}{\gamma_0} \Rightarrow \text{Conversion Factor } [m/V] = \sqrt{\frac{D_{SE}}{D_{fit}}} \quad (2.51)$$

With the latter method, the local fluid viscosity must be known to a high degree of accuracy, depending on the local heating effect this may be a trivial increase or it may be significant enough to drastically alter the characterisation. This also assumes that the particle shape is known, and by implication its diffusion tensor. PSD analysis is often seen as the gold standard for calibration as it can be fine tuned to the point that optical forces can be computed on the order of $10^{-15}N$ [21], it captures all of the information acquired by other calibration techniques while filtering out noise and requiring a relatively small amount of data collection.

2.7.5.1 Power spectral analysis for rotating objects

The scattering from rotating objects can be partially characterised via the power spectrum density method. If the particle is birefringent then as it rotates the detected signal will oscillate periodically. When a Fourier transform of the time series is taken the power spectra will have a peak signal at that periodic frequency. Typically papers reporting on the rotation rate of a trapped object will collect a power spectrum and look at the maximum frequency term to determine its rotation rate. However this often neglects any information on the trapping forces acting on particle and only looks at the torque applying around one of the particle's primary axis. There has only been one notable effort to fully characterise the full trapping dynamics on a rotating body, [28] developed a theoretical model that relates the power spectrum to both the rota-

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tional and translational motion simultaneously. This is applicable to particles with a polarizable long axis but is insufficient for birefringent particles.

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