Chapter 2
Optical tweezers

2.1 Physics of optical tweezers

Is it possible to imagine one can pick up and move an object without actually touching it? It is indeed possible, but with the help of a laser as an optical tweezer. Optical tweezer is a scientific tool that can be used to trap and manipulate micron and sub micron sized particles in a non invasive manner [1]. First developed by Arthur Ashkin, researcher at Bell Labs (Murray Hills, NJ) in 1986 [2], it was initially used for trapping biological cells. Today its applications have grown to encompass the interest of scientists in areas ranging from study of molecular motors, protein folding, RNA folding, chromosomal movements and dynamics of biological cells to mechanical properties of polymers, measurement of picoNewton forces, microrheology of soft materials, study of micromachines, microfluidics and so on.

The basic principles of optical tweezing can be explained in terms of Newton’s laws of motion. Light carries momentum which is proportional to its energy and in the direction of propagation. Any change in the direction of light, by reflection or refraction, will result in a change of momentum of the light. If an object bends light thus changing its momentum, conservation of momentum requires that the object must undergo an equal and opposite momentum change. This gives rise to a force acting on the object.

![Diagram showing Mie and Rayleigh regimes](image)

Figure 2.1: Comparison of particle size and wavelength of light for understanding the two regimes.

This mechanical effect of light on matter can be largely understood in two separate regimes depending on the size of the particles: (i) Ray optics approach for Mie particles
(particle size, \(d > \lambda\)) and electric dipole approximation for Rayleigh particles (particle size, \(d < \lambda\)) [2] see in figure 1, where \(\lambda\) is the wavelength of light.

### 2.1.1 Mie Regime

A laser beam which is incident on the bead will be refracted twice, once as it enters the bead and once when it exits. Due to this refraction, the light beam will finally propagate in a direction different from its original direction of propagation. This change in direction causes a change in momentum of the light ray. From Newton's third law, we know that there should be an equal and opposite change in the momentum of the bead.

The linear momentum of a laser photon with wavelength \(\lambda\) can be described by the equation:

\[
p = \frac{E}{c}
\]  

(2.1)

where, \(p\) is the momentum of light, \(E\) is the energy of light photon and \(c\) is the velocity of light, which can be expressed in terms of wavelength \(\lambda\) and frequency \(f\) of light as

\[
c = \lambda f
\]  

(2.2)

while energy \(E\) of light photon is given by

\[
E = hf
\]  

(2.3)

\(h\) being the Planck's constant.

Hence, linear momentum \(p\) of the light photon in equation (2.1) becomes,

\[
p = \frac{E}{c} = \frac{hf}{\lambda} = \frac{h}{\lambda}
\]  

(2.4)

Force \(F\) due to change in momentum \(p\) is given by

\[
F = \frac{\sum \Delta p_i}{\Delta t}
\]  

(2.5)

where, summation is over all photons.

A simple two light ray diagram on a microsphere can be seen in figure 2.2, which qualitatively depicts the origin of the trapping forces in Mie regime. Let us assume that light rays \(p_1\) and \(p_2\) come from a Gaussian laser beam with different intensity. The changes in momentum of these rays after the exit from the bead are \(\Delta p_1\) and \(\Delta p_2\) respectively. The net force component due to this change in momentum is in the direction of highest intensity shown in white arrow, which is called the gradient force
that pulls the bead to the beam focus. In addition, green arrows show the scattering component of force due to reflection that tends to expel the object from the focus.

Figure 2.2: Qualitative picture of the origin of the trapping force. (Adapted from Ashkin [2,3]).

Roosen and Imbert [4] obtained expressions for gradient and scattering forces on a dispersive sphere due to a light beam of power \( P \), using the Fresnel reflection and transmission coefficients, \( R \) and \( T \).

For the gradient force they found that

\[
F_g = \frac{n_1 P}{c} \left( R \sin 2\theta - T^2 \frac{\sin(2\theta - 2\phi) + R \sin 2\theta}{1 + R^2 + 2R \cos 2\phi} \right) \hat{u}_\perp
\]

\[
F_s = \frac{n_1 P Q_g}{c} \hat{u}_\perp
\]

where, \( n_1 \) is the index of refraction of the medium of suspension, \( \theta \) and \( \phi \) the angles of incidence and refraction, \( c \) the speed of light and \( \hat{u}_\parallel \) and \( \hat{u}_\perp \) are the unit vectors respectively parallel and perpendicular to the incident ray. The term \( n_1 P/c \) is the
momentum per second of the light ray. The angle $\phi$ relates to $\theta$ via Snell's refraction law:

$$\frac{n_2}{n_1} = \frac{\sin \theta}{\sin \phi},$$

with $n_2$ the refractive index of the object.

Similarly for the scattering force they found that

$$F_s = \frac{n_1}{c} P \left\{1 + R \cos 2\theta - T^2 \frac{\cos (2\theta - 2\phi) + R \cos 2\theta}{1 + R^2 + 2R \cos 2\phi}\right\} \hat{u}_s$$

$$F_s = \frac{n_1}{c} P Q_s \hat{u}_s$$

(2.7)

Vectorial addition of these two trapping force components gives for the magnitude of the force due to a single laser beam of power $P$:

$$F_{total} = \frac{n_1}{c} P \sqrt{Q_s^2 + Q_g^2} = \frac{n_1}{c} P \left\{\theta, n_2, n_1, R, T\right\}$$

(2.8)

where, the variables determining $Q$, is a dimensionless factor called quality factor of the trap. It has two components, one for scattering and another for gradient. Therefore the total force on the object is found by summing over all rays passing through it. The magnitude of `$Q$' varies depending on the direction in which `$F$' is measured and is up to 0.30 in a direction perpendicular to the propagation of laser beam [3]. Ashkin calculated the forces on a dielectric sphere in the ray optic regime for both the TEM$_{00}$ and the TEM$_{01}$ (doughnut mode) intensity profiles. The quality factor depends strongly on the type of laser beam, the NA of the microscope objective and on the size of the trapped bead. When the bead is displaced from the beam center, the larger momentum change of the more intense rays cause a net force to be applied back toward the center of the trap. When the bead is laterally shifted from the center of the beam the net force points toward the beam waist.

If the particle is located at the center of the beam then individual rays of light refract through the particle symmetrically, resulting in no net lateral force. The net force in this case is along the axial direction of the trap, which cancels out the scattering force of the laser light. Not all of the light is refracted through the particle and some gets reflected. The force associated with these rays and the scattering force, pushes the particle away from the laser focus and causes the center of the optical trap to exist at a position displaced axially from the focus, slightly downstream of the beam waist as can
be seen in figure 2.4. It is also verified experimentally that the criteria for trap stability is satisfied from the Rayleigh regime into the full Mie regime in the range 0.2-5μm [3].

Figure 2.3: A qualitative view of the gradient forces on a dielectric bead due to refraction of light rays. (a) The net force, $F$, on the transparent bead is towards the focus (upward here) for the two rays of equal intensities, and (b) The net force, $F$, on the bead slightly right (towards the focus), for the light rays of unequal intensities (shown in rays of different thickness).

Figure 2.4: Schematic of scattering force, gradient force and resultant axial force in an optical trap. The axial equilibrium position of the trap is displaced slightly along the direction of the laser beam.
2.1.2 Rayleigh regime

In the Rayleigh regime, the trapped particles are treated as point dipoles, as the electromagnetic field is constant on the scale of the particle. Particles in the Rayleigh regime are subject to a scattering force due to radiation pressure and a gradient force due to the Lorentz force on a dipole [5,6]. Incident radiation can be absorbed and isotropically reemitted by the particle, but a net force results in the direction of incident photon flux. Hence the resulted scattering force in the direction of light propagation is given by

\[ F_s = n_i \frac{\langle S \rangle \sigma}{c} = \left( \frac{n_i}{c} \right) \sigma I(\vec{r}) \frac{\partial I(\vec{r})}{\partial r} \]

where, \( \langle S \rangle \) is the time-averaged Poynting vector of the electromagnetic wave and \( \sigma \) is the scattering cross section of a Rayleigh particle of radius a. For a spherical particle of radius a, the scattering cross section is:

\[ \sigma = \frac{8 \pi k^4 a^4}{3} \left( \frac{m^2 - 1}{m^2 + 2} \right)^2 \]

(2.10)

where, \( m = \frac{n_2}{n_1} \) is the relative refractive index of the particle and k is the wave number of the light used.

To have a strong and stable optical trap, the scattering force must be dominated over by the gradient force.

The induced dipole moment of a dipole with polarizability \( \alpha \) in the field \( \vec{E} \) of the laser is:

\[ \vec{p}(\vec{r}, t) = \alpha \vec{E}(\vec{r}, t) \]

(2.11)

The gradient force on this induced dipole is:

\[ \vec{F} = \left( \vec{p}(\vec{r}, t) \nabla \right) \vec{E}(\vec{r}, t) \]

(2.12)

Using the vector identity

\[ \nabla \vec{\nabla} = 2 \left( \nabla \nabla \right) \vec{V} + 2 \vec{V} \times (\nabla \times \vec{V}) \]

(2.13)

and the results of Maxwell's equations
\[ \nabla \times \overline{E} = 0 \]

The gradient force on a dipole reduces to

\[ \overline{F}(\vec{r}, t) = \frac{1}{2} \alpha \nabla \overline{F}(\vec{r}, t) \]

The gradient force which the particle experiences will be the time-average of the above force. We use the relation

\[ \left\langle \overline{E}^2(\vec{r}, t) \right\rangle = \frac{1}{2} \left\| \overline{E}(\vec{r}) \right\|^2 \]

(2.16)

to get

\[ \overline{F}_{\text{grad}}(\vec{r}) = \left\langle \overline{F}(\vec{r}, t) \right\rangle = \frac{1}{2} \alpha \nabla \left\langle \overline{E}^2(\vec{r}, t) \right\rangle = \frac{1}{4} \alpha \nabla \left\| \overline{E}(\vec{r}) \right\|^2 \]

(2.17)

The intensity \( I(\vec{r}) \) is defined by:

\[ I(\vec{r}) = \frac{n_2 \varepsilon_0 c}{2} \left\| \overline{E}(\vec{r}) \right\|^2 \]

(2.18)

where, \( n_2 \) is the refractive index of the exposed material, \( \varepsilon_0 \) is the permittivity of free space, and \( c \) is the speed of light. This force can be now linked to the gradient of the intensity by the relation

\[ \overline{F}_{\text{grad}}(\vec{r}) = \frac{1}{2 n_2 \varepsilon_0 c} \alpha \nabla I(\vec{r}) \]

(2.19)

From this it is clear that the force will be in the direction of the highest intensity. In the case of a focused Gaussian laser beam it will result in a force towards the focus, where the intensity is highest.

### 2.2 Design and Construction of dual optical tweezer

In the design of a dual optical tweezer, the selection of key components is an important aspect. The choices of some of these components are discussed below.

#### 2.2.1 Trapping laser

The most important component of an optical tweezer is the trapping laser. The important parameters to be considered for the trapping laser are, wavelength of laser, mode of laser beam, the power and power stability, pointing stability of the beam, polarization, and beam waist.
The wavelength of the laser selected is 1064 nm. This is because objects such as biological cells have low absorption at this wavelength. A single mode Gaussian beam (TEM00 mode) has been chosen so that a stable harmonic trap can be generated. The intensity of a Gaussian beam decreases exponentially away from the centre of the beam and hence gives sufficient transverse optical gradient to trap particles in three dimensions. A laser source with power varying option is used to create an optical trap of varying trap stiffness. A good quality laser beam with excellent pointing stability is essential to avoid unwanted optical trap displacements. A laser beam of beam quality: $M^2 < 1.1$ and Power stability $< 0.5\%$ are used in our setup. An un-polarized beam is chosen as it can be polarized according to the requirements of an experiment. Correct beam waist of 9 mm is made by using a beam expander to just overfill the back aperture of the microscope objective.

### 2.2.2 Microscope objective

![Numerical aperture of an objective](image)

**Figure 2.5:** Numerical aperture of an objective.

In order to create a sufficient gradient force to overcome the scattering force, it is necessary to have a high numerical aperture microscope objective. Numerical aperture (NA) being defined as $NA = n \sin(\theta)$, where, $n$ is the refractive index of the medium, and $\theta$ is the maximum angle subtended by light entering the objective. In addition, selection of high numerical aperture objective determines the trap stiffness of the optical trap. From the definition of numerical aperture, we see that NA above 1 is only possible when the index of refraction exceeds 1. As air has an index of refraction of 1, a high-NA optic is typically immersed in a fluid such as oil or water, which has a higher refractive index. Water immersion is convenient for biological specimens which tend to be prepared in
water, having a refractive index of 1.3. By using oil one can get a NA of more than 1.3. We have used an oil immersion objective having NA 1.4 in our optical tweezer setup. There is an inverse relation between trap depth and numerical aperture. Thus, when higher trap depths are required, it is necessary to use an objective of lower numerical aperture, sacrificing some of the trapping efficiency. In cases where low numerical aperture objective is used, there may not be a gradient force large enough to overcome the scattering force which will push the object out of the trap.

2.2.3 Tracking laser

A separate low power 980 nm diode laser (Thorlabs, USA) is used to track the position of a trapped bead rather than from the trapping beam itself. This allows flexible and independent handling of tracking and trapping laser beams. The power of this beam is so low that its influence on the trap is negligible. In addition, the detection laser can be adjusted to under fill the objective to maximize the collection of light, while overfilling with the trapping beam yields a high gradient force. Also, the tracking laser has to be carefully aligned onto the particle, to ensure detection in the linear regime. In the setup we have combined and separated the two trapping and tracking lasers using dichroic mirrors. Detection is then made using Quadrant Photo Detectors (QPD) using both back focal plane and back scattering techniques. Back focal plane detection is based on the interference between forward-scattered light from the bead and unscattered light, while back scattering detection relies on detection of the scattered laser beam using QPD by converting the signal to a normalized differential output in both lateral x and y directions.

2.2.4 $\theta_x, \theta_y$ tilting mirror

In order to steer a laser beam we have used a $\theta_x, \theta_y$ tilting mirror from Piezosystem Jena Germany. The tilting mirror is based on the concept of Piezoelectric effect. Tilting mirror platforms are equipped with two pairs of low-voltage piezoelectric linear drives (0 to 100 V) operating as a unit in push/pull mode. Tilt mirror top platforms are fast and compact tilt units, providing precise angular movements of the top platform in two orthogonal axes. The tilt range is equal to 2 mrad (each axis) with sub-$\mu$rad resolution. Closed-loop option is also available for highest accuracy and repeatability. Specifications of the mirror used in our setup are as follows.
<table>
<thead>
<tr>
<th>Model</th>
<th>PeizoJena, PSH 2z SG Top plate A 3-axis piezo tilting system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tilting angle</strong></td>
<td></td>
</tr>
<tr>
<td>Open loop</td>
<td>2 m rad</td>
</tr>
<tr>
<td>Closed loop</td>
<td>1.6 m rad</td>
</tr>
<tr>
<td><strong>Z-motion</strong></td>
<td></td>
</tr>
<tr>
<td>Open loop</td>
<td>16 μm</td>
</tr>
<tr>
<td>Closed loop</td>
<td>12.8 μm</td>
</tr>
<tr>
<td><strong>Dimensions (LxWxH)</strong></td>
<td>25x25x41 mm</td>
</tr>
<tr>
<td><strong>Voltage</strong></td>
<td>30……..130 V</td>
</tr>
</tbody>
</table>

### 2.2.5 Dichroic mirrors

Dichroic mirrors are needed to introduce the laser beam into the imaging path along with other laser beams. Dichroic mirrors have high reflectance for a certain wavelength range and high transmittance in another wavelength range. We have used a dichroic mirror of 30% reflectance and 70% transmittance for 1064 nm and maximum transmittance for visible light.

### 2.2.6 Vibration isolation system

To achieve maximum possible sensitivity, stability, and signal-to-noise ratio in optical trapping, mechanical vibrations should be isolated. Mechanical vibrations typically arise from compressors or pumps operating in the vicinity, or even from passing traffic on roads. Our experimental setup is built on an active mechanical isolation table (TMC) so that all vibrations (typically −20 dB at frequencies 2–10 Hz) are isolated.

### 2.2.7 XYZ Piezo stage

Piezoelectric stages permit three-dimensional control of the position of the trap relative to the trapping chamber. The ability to move precisely in the axial dimension permits characterization of the longitudinal properties of the optical trap. Position and force calibration routines employing the PZT stage are fast, highly reproducible, and very precise. We have used here an XYZ Piezo stage from PiezoJena Germany. This stage has strain gauge sensors, and gives excellent stability, positional resolution and repeatability. The unit is interfaced to a PC by analog means. The XYZ nanostage specifications are as follows.
### 2.2.8 Imaging system

The visualization of the sample can be done using a trans-illumination lamp. The trapping objective can also be used by the imaging system. Since the trapping and imaging light pass through the same objective, the trapped particle can be viewed either with a CCD camera or using one of the microscope eyepieces. In both cases we have used color filters to block the trapping beam, to prevent damage to the CCD camera or to the eye as the case may be. Here we have used a dichroic mirror as a color filter since it reflects most of the trapping light but transmits the imaging light. This permits the two beams to follow the same path through the objective, but allows only the imaging light to reach the camera or eyepiece. We have used two CCD cameras, one for fluorescence imaging and other for bright field imaging and fast scanning of the system. The camera from Princeton Instruments, USA may be used for fluorescence spectroscopy and can be seen in figure 2.7.
Figure 2.7: CCD camera CoolSNAP_{ee} for fluorescence imaging.

**Eyepieces:** Setup is also equipped with eyepiece for wider field of view visualization. Laser filters are used to block the trapping laser as a caution to avoid any accidents.

### 2.2.9 Quadrant photo detectors

Quadrant photo detectors work on the same principle as that of position sensitive light detectors. They consist of four photo detectors fixed on four quadrants of a circle as shown in the figure 2.12. Generally they work in the photovoltaic mode as it has slower frequency response and lower dark current. In this case four photodiodes share a common ground point (cathode) and separate anodes. The current output obtained from each photodiode is converted into a voltage and then it is processed electronically to obtain the x and y displacement signals. The potential well of the Gaussian laser beam can be approximated by a harmonic potential. In such a case, the force exerted on a particle scales linearly with the deviation of the particle from the trap center. As a consequence, the laser beam that falls on the QPD is a measure of the change in momentum flux, which is linearly related to the deviation of the particle, enabling position detection.

### 2.2.10 Data acquisition (DAQ) system

The goal of data acquisition is to measure a signal such as voltage, current, temperature, pressure, over time. It is usually through a combination of data acquisition hardware, application software, and a computer. DAQ card is what interfaces a signal with a computer with the help of appropriate software. It may be available in modules that can be connected to one of the I/O parts of the computer which may be parallel, serial, USB, PCI, PCM and so on. We have used an 8 channel, 16-bit, 250 kS/s/ch National Instruments DAQ card (PCI 6143, NI, USA) for acquisition.
2.3 Laser power calibration

![Graphs showing laser power calibration](image)

Figure 2.8: Variation of laser power between source end and at the sample plane.

Power of the trapping laser has been measured at the source as well as at the sample plane. The laser power variation between these positions is found to bear a linear relation.

2.4 Construction of dual optical tweezer

The schematic of a dual optical tweezer setup is shown in figure 2.9 [7]. It consists of a continuous wave Ytterbium fiber laser (1064 nm, 5 W, IPG, Germany) used for trapping micron sized particles and a diode laser (980nm, Thorlabs, USA) used for tracking the position of the trapped particle in two dimensions. The beams from these two lasers are merged into the same optical path using a dichroic mirror (DM1) and the combined beam is then split into two using a polarizing beam splitter (PBS1). The two split beams from PBS1 recombine at a second polarizing beam splitter PBS2, after undergoing reflections from a plane mirror (M1) and a $\theta_x\theta_y$ tilting mirror under dually.

A second dichroic mirror (DM2) reflects this beam towards a high numerical aperture oil-immersion microscope objective (1.4 NA, Olympus, Japan), which strongly focuses the beams to form two optical traps. The sample is placed in a sample holder made by a rubber ‘O’ ring on a clean cover-slip mounted on a nanometer precision three axis (XYZ) piezo-electric transducer stage (TRITOR 102 SG, Piezosystem Jena GmbH, Germany). The back scattered laser beam (980 nm) from the two independently trapped beads is reflected on a third polarizing beam splitter (PBS3). The resulting separated S
polarized and P polarized laser beams are then incident on two separate Quadrant Photo Detectors (EOS, USA), QPD1 and QPD2 fixed on XY stages. These detectors record the position information of the trapped beads. Data acquisition is through an 8 channel, 16-bit, 250 kS/s/ch DAQ card (PCI 6143, NI, USA). There is a provision for video imaging by a high speed CCD camera (200 frames/s, Voltrium, Singapore) and fluorescence imaging by yet another CCD camera (CoolSNAP.EZ, Princeton Instruments, USA) (not shown in figure). The entire set-up is built on a vibration isolation optical table (TMC, USA). All optics used here are from Thorlabs, USA or Casix Inc. China while the optomechanical components are from Holmarc, India.

Figure 2.9: Schematic of the optical dual trap built around an inverted microscope.
Figure 2.10: Dual optical tweezer setup.
Figure 2.11: A dual trap photo of 3 μm polystyrene beads in water.

Figure 2.11 shows the trapped polystyrene beads of size 3 μm in water 30 μm above the coverslip. Bright rings around the beads are due to the diffraction of light. A bead in the dark background can be also seen which is out of focus (not trapped).

2.4.1 Signal measurement through quadrant photo detector

Figure 2.12: Four photo detectors of the quadrant photo detector with current signals.

The detectors are placed in the setup in such a way that they can detect the position of the light backscattered from a trapped bead as well as the intensity shift in the back focal plane of the condenser lens due to displacement of the trapped bead from the trap center. We have used the IR laser (980nm) to get the position information of the trapped
bead by both backscattering and back focal plane detection techniques. Figure 2.12 shows the intensity in terms of the signals from the four segments of the quadrant photo detector. In a typical QPD, the four photo detectors share a common cathode and have separate anodes. When a laser beam falls on these photo diodes, they generate electric currents which can then be amplified to a desired level. The total current from all the detectors of the quadrant photo detector is

\[ I_{\text{sum}} = I_1 + I_2 + I_3 + I_4 \]  

(2.20)

The displacement of the beads are then obtained in terms of current signal as

\[ D_x \propto I_x = \frac{(I_2 + I_4) - (I_1 + I_3)}{I_{\text{sum}}} \]

\[ D_y \propto I_y = \frac{(I_1 + I_2) - (I_3 + I_4)}{I_{\text{sum}}} \]  

(2.21)

If a force of known magnitude and direction is applied to the trapped bead, then by observing the response of the quadrant photo detector signals, one can calibrate the detector to physically relevant units of force or displacement.

In order to make direct measurements of nanometer-scale motion, accurate calibration of the detector is necessary. Here we have used a method of calibration that involves moving a bead through a known displacement across the detector region and simultaneously recording the output signal. This operation can be performed either with a stuck bead moved by a calibrated displacement of the stage or with a trapped bead moved by a calibrated displacement of a steerable trap. We have chosen the former method of calibrating the detector. In this method, the position calibration is accomplished by moving a bead stuck to the surface of a coverslip through the detection region by a piezoelectric (PZT) stage and recording the detector output as a function of position. A 3\(\mu\)m bead is first stuck to a coverslip and translated through the detection area in small steps (200nm) using a PZT stage along both x and y directions, the signal is detected using a quadrant photo detector (QPD). For each relative position of the laser, 10,000 data points are acquired from the QPD using LabView through a DAQ card at a sampling rate of 10,000 per second, and the average value is stored on the hard disk. A plot of average voltage versus relative positions of the stuck bead is shown in figure 2.13, for the 'x' direction. The linear region of the detector has a width of nearly 0.32 \(\mu\)m and varies with a value -0.0682 V/\(\mu\)m. Almost the same values were obtained in the y direction.

39
Figure 2.13: Detectors responses for the signal from a 3 µm stuck bead when the stuck bead is moved in steps of 200 nm.

This method has certain drawbacks. It requires a precise knowledge of the stage movement and the position of the trap as position of the stuck bead may not coincide with the actual position of the trap. In addition, the bead used for the calibration may have a size that is different from that used for the experimental measurement. To overcome these drawbacks, other methods of calibrating the position detector are used. Calibration of the detector by power spectrum analysis is one such method, and is described in detail in the last section of this chapter.

2.5 Brownian motion and power spectra

There is an intensity shift in the signal in both back focal plane and back scattering due to displacements of the trapped bead from the trap center. QPD being sensitive to the intensity shift generates an output signal from which it is possible to measure the forces causing these displacements. The QPD response to light fluctuations from the trapped bead due to external forces is calibrated by making use of the diffusional Brownian motion of the bead due to the continuous and random bombardment by the solvent molecules. For a free bead, this bombardment gives rise to diffusion. After a time $t$, the mean square displacement of the spatial coordinate $x$ will be:

$$\text{Var}(x) = \langle x^2(t) \rangle - \langle x(t) \rangle^2 = \frac{2k_BT}{\gamma}t = 2Dt$$

(2.22)

where, $\frac{\gamma}{\pi \eta a}$, is Stoke’s drag coefficient on a sphere of radius ‘a’ and in a medium of viscosity ‘$\eta$’. $D$, the diffusion constant, $k_B$, the Boltzmann’s constant and $T$, the temperature.
Brownian force is also known as thermal force. A bead that is held in an optical trap instead of being free will feel the diffusion force as well as restoring optical force confining its motion within the laser focus. Assuming the confining force to be linear in the displacement, with a proportionality constant \( k \), the Langevin equation for the bead’s motion in the limit of low Reynolds number becomes

\[
\gamma \frac{dx}{dt} + k x = F(t)
\]  \hspace{1cm} (2.23)

\( F(t) \) being the random thermal force, which averages to zero over time. \( k = 2\pi\gamma f_c \) is the trap stiffness in terms of \( f_c \), the corner frequency of the optical trap.

Taking the Fourier transform of equation (2.23) [8], we get

\[
\gamma(2\pi i f)x(f) + k x(f) = F(f)
\]  \hspace{1cm} (2.24)

where, \( x(f) = \int_{-1/2}^{1/2} x(t') e^{-2\pi ift'} dt' \)

Product of the complex conjugate of equation (2.24) with the original, gives:

\[
x^2(f) = \frac{|F(f)|^2}{4\pi^2 \gamma^2 (f_c^2 + f^2)}
\]  \hspace{1cm} (2.25)

The quantity \( x^2(f) \) is the two-sided power spectrum of the random Brownian motion of the particle.

The one-sided power spectral density (PSD) can be written as,

\[
S_x(f) = x^2(f) + x^2(-f) = 2x^2(f) \text{ for } 0 < f < \infty
\]  \hspace{1cm} (2.26)

In equation (2.25) the time average of \( |F|^2 \) is given by

\[
|F|^2 = 2\gamma k_b T
\]

Hence, from equation (2.26), the power spectrum of the displacement fluctuations is found to be:

\[
S_x(f) = \frac{k_b T}{\gamma \pi^2 (f_c^2 + f^2)}
\]  \hspace{1cm} (2.27)

Here, \( f_c \) is a characteristic frequency of the trap as defined earlier \( f_c = k/2\pi\gamma \).

At frequencies \( f = f_c \), the power spectrum is roughly constant,
\[ S_x(f) = S_0 = \frac{4\gamma k_B T}{k^2}, \] which is called the plateau region.

At \( f \gg f_c \), \( S_x(f) \) falls off as \( 1/f^2 \). This is why \( f_c \) is known as the corner frequency. The high frequency behavior is characteristic of free diffusion, indicating that at short time scales the particle does not ‘feel’ the confinement of the trap.

Once the values for \( S_0 \) and \( f_c \) are measured by fitting the power spectrum of a trapped bead, these values can be used to find the trap stiffness \( \kappa \) \[ k = 2\pi \gamma f_c = \frac{2k_BT}{\pi S_0 f_c}, \] which has a units [pN/\mu m] \hspace{1cm} (2.28)

Also, it is important to note that the quadrant detector reads the displacement fluctuations \( x(t) \) as a voltage rather than a displacement in nanometers. Calibration factor \( R[m/V] \) of the detector can be obtained with known bead diameter and solvent viscosity[9]:

\[ R \left( \frac{m}{V} \right) = \sqrt{\frac{k_BT}{6\pi \eta S_0 f_c^3}} \] \hspace{1cm} (2.29)

In addition, to convert the data into forces, \( R \) should be multiplied by the trap stiffness \( \kappa \).

2.6 Power spectral density analysis of measured data

We have recorded position information data of a thermally fluctuating trapped 3 \( \mu \)m bead at a scan rate of \( 10^5 \) samples per second using a DAQ card and a program written in LabVIEW to interface with the computer. During the measurements care was taken to maintain a constant distance of 30 \( \mu \)m between trapped bead and cover slip to avoid surface effects. All measurements are carried out at a temperature of 22 \(^\circ\)C.

For each set of data, one sided PSD is calculated as described in the earlier section. A typical power spectrum averaged over 25 data sets is shown in figure 2.14.

By fitting the PSD curve using the equation (2.27), the corner frequency, \( f_c \), for a 3 \( \mu \)m trapped bead embedded in water is obtained. Figure 2.12, shows a corner frequency of 42.9 Hz, that corresponding to a laser at focal plane of 48 mW. The PSD data for frequencies higher than the corner frequency gives us the slope -2 as expected. Trap stiffness at a given laser power is calculated using equation (2.28) by considering the
The drag coefficient \( \gamma = 6 \pi \eta a \) for the trapped bead in water of viscosity \( \eta = 0.96E-3 \) Pa s.

The value of trap stiffness is found to be 7.389 pN/\( \mu \)m.

Figure 2.14: Typical power spectrum of a trapped bead, diameter, \( d = 3 \) \( \mu \)m (a) before calibration and (b) after calibration.

To obtain the PSD in real units (nm\(^2\)/Hz), voltage to distance conversion factor (calibration factor) \( R \) is calculated using the power spectral density value \( S_0 \) at the plateau region and corner frequency \( f_c \). Calibration factor is found to be \( 3.707 \times 10^{-8} \) m/V.

The plot of power spectral density after the calibrating to real units can be also seen in figure 2.14 (b).

2.7 References