Chapter 6

A pair of trapped beads in an optical tweezer - Analysis through simultaneous forward and back scattering techniques

6.1 Introduction

Optical manipulation of microscopic objects using optical tweezers have been used in a wide range of applications from microrheological studies of materials to probing dynamics of active suspensions [1-4]. When working with dense colloidal suspensions in an optical tweezer, one often ends up trapping more than one particle at a time. We have made an attempt to understand the interaction between a pair of beads through simultaneous position measurements carried out using forward and back scattering techniques on a single trapped bead as well as a pair of trapped beads. This was studied by Li and Arlt using a forward scattering detector and their results show that a trapped pair of beads could be mistaken to be a single bead in a trap with half the stiffness [5].

Understanding of forces between multiple trapped particles thus is a challenge. It is not clear whether optical binding is also, over and above the gradient force, responsible for trapping them. Additionally there can be hydrodynamics induced correlations between the beads, besides the effect of charging and roughness. Here, we attempt to extract information with regards to the inter bead interaction from monitoring their positions as a function of time.

6.2 Experimental techniques

Details of the optical tweezer setup used have been described in the second chapter. We have made a minor modification to the setup by configuring one of the beam paths for forward scattering detection and thus rendering the setup ready for simultaneous forward and back scattering based particle tracking, as can be seen in figure 6.1.
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Figure 6.1. Schematic diagram of an optical tweezer tracking system using both forward and back scattering techniques.

6.2.1 Simultaneous forward and back scattering detection

Position detection of the trapped particles through forward (back focal plane) and back scattering techniques has been explained under the heading 2.4.1 in chapter 2. Simultaneous measurements are carried out through both the detectors via 8 channels, 16-bit DAQ card. The DAQ card has an advantage that it acquires the data simultaneously without any time delay from all the eight photo detectors (four detectors from each QPD) at a sample rate of 250 kS/s/ch. The acquired data is then position converted as explained in 2.4.1 using a custom program written in LabVIEW, which is then used for the analysis.

In course of analysis, first we have determined the angle of tilt of one of the detector with respect to the other by manually displacing a bead stuck to the cover slip. We have plotted the graph of y displacement verses x displacement for both the detectors in a same graph within the sensitivity region of the detectors. The variation is found to be a straight line for both the detectors. Angle of tilt between the detectors has been measured
from the slope of the straight lines. The angle of tilt between the detectors is found to be 38 degree.

![Graph showing displacement](image)

Figure 6.2: Displacement of the stuck bead read by detectors 1 and 2.

Then the data collected from one of the detectors is corrected with respect to the other by following the rotational coordinate transformation which is given by

\[
\begin{pmatrix}
  x - x_0 \\
  y - y_0
\end{pmatrix} =
\begin{pmatrix}
  \cos \theta & \sin \theta \\
  -\sin \theta & \cos \theta
\end{pmatrix}
\begin{pmatrix}
  x - x_0 \\
  y - y_0
\end{pmatrix}
\]  

(6.1)

where \( x_0, y_0 \) and \( x'_0, y'_0 \) are the origins of the data sets of the first and second detectors respectively. When \( x_0 = y_0 = x'_0 = y'_0 = 0 \), our transformation equation becomes

\[
\begin{pmatrix}
  x' \\
  y
\end{pmatrix} =
\begin{pmatrix}
  \cos \theta & \sin \theta \\
  -\sin \theta & \cos \theta
\end{pmatrix}
\begin{pmatrix}
  x \\
  y
\end{pmatrix}
\]  

(6.2)

Data obtained from the detector 2 is run with rotational coordinate transformation before considering into analysis.

### 6.2.2 Power spectral density analysis

Power spectral density has been measured for both single and pair of trapped beads through both forward and back scattering techniques. The variation of the power spectral density (PSD) with frequency for a single trapped bead is shown in figure 6.3(a). We found the same estimate of the corner frequency from both detection techniques. However when a pair of beads are trapped, a large difference is observed in the corner frequencies measured by the two detectors (figure 6.3(b)). Forward scattering gives a
value of corner frequency which is about half that recorded for a single bead in the trap, in agreement with the work of Li and Arlt, while the back scattering gives a value which is an order of magnitude higher than the value obtained from forward scattering. Owing to the geometry of the situation, we conjecture that the back scattering is dominated strongly by the bead located closer to the focal point. Being deeper in the trap potential, the PSD for this bead shows a higher corner frequency. The forward scattering, on the other hand, bears information primarily from the bead away from the focal point and this being held by smaller trap stiffness yields a smaller value for the corner frequency in the PSD data. This conjecture, assumes that the scattered photons from the bead closer to the focus do not reach the detector facing the bead farther away from the focus when the beads are exactly aligned. We will discuss the effects of the “cross talk” due to photon signals from the bead closer to the focus reaching the detector monitoring forward scattering, later in the chapter.

Figure 6.3: (a) Power spectral density of single and (b) paired beads tracked through both forward and back scattering techniques at a laser power of 48 mW. Beads trapped here are of 3μm diameter.

The conjecture stated above is given weight via simulations carried out to generate histograms of normal projections of a pair of beads. In the first simulation, we assumed that the two beads were held with their corresponding trap stiffnesses measured in the experiment and were subjected to successive Brownian kicks for about 100,000 instances and the value of area of projection was computed for each instance by making use of a one dimensional Langevin equation which generated the initial values of co-ordinates for the centers of the two beads. Subsequently, a histogram of the projection area was plotted which was a half -Gaussian strongly peaked near the value of area of a single bead. In the other simulation, an ensemble average of the above situation was carried out
and a result identical with time average approach was obtained in agreement with the so-called Ergodic hypothesis of statistical physics.

### 6.3 Simulation of projected area of a pair of trapped beads

#### 6.3.1 Time average approach

We have considered two polystyrene beads of equal radius ‘r’ and they were placed one in front of the other in a Gaussian profile. Both the beads were subjected to random forces (100,000 in number) with fluctuation limits: assuming higher trap stiffness for the bead closer to the trap center and lower trap stiffness for the bead far from the trap center.

We have represented the two beads as circles in 2 dimension and have calculated the composite or total area of the two circles whose centers are at distance ‘d’ apart by using the formula 6.3.

Total area projected of two fluctuating spheres

\[ 2\pi r^2 - 2 \left( r^2 \cos^{-1} \left( \frac{rd}{2} \right) \frac{\pi}{180} \right) \left( \frac{1}{2} r^2 \sin \left( 2\cos^{-1} \left( \frac{rd}{2} \right) \right) \right) \] (6.3)

![Diagram of two overlapping circles](image)

Figure 6.4: a) Two dimensional representation of the two fluctuating spheres, (b) Histogram of the displacement of the fluctuating spherical beads from the centre limited by the trap stiffness. 

The calculated total area of projection of the fluctuating beads is shown in figure 6.5, which is always near \(1\pi r^2\) indicating that the displacement measured through forward scattering, is dominated by the displacement of the bead which is far from the focus or which is experiencing lower trap stiffness.
6.3.2 Ensemble average approach

Similarly, we have assumed Gaussian distribution data for a pair of two fluctuating beads by assuming one of the beads which is near to the focus experiences higher trap stiffness and the other which is away from the focus experiences lower trap stiffness. The "collective shadow" or overlapping area of the two beads for an ensemble average over 25 data sets was computed using equation 6.3. A histogram of total projection area was plotted and we got a half Gaussian with its peak at the value of a $\pi r^2$ and the entire histogram was confined between 1 and 1.002. The additional area obtained is due to the contribution from the bead which is nearer the focus but it is negligible compared to the total area. Therefore we can conclude that the signal from forward detection technique is dominated by the fluctuations of the front bead.

6.4 Estimation of correlation coefficient due to photon signal cross talk in the forward scattering detector

We have made an attempt to estimate the cross talk between the two beads based on the above simulation result and additionally by assuming that they show free Brownian motion in the absence of any interactive forces between them. Here, we have also considered the contribution from the bead which is near to the focus, and hence, the forward scattering data is the weighted average of both front (away from focus) and back bead (nearer the focus), while the back scattering data is just from the back bead itself.

Mathematically we modeled the position measured from forward scattering as

\[
\text{Forward scattering signal} = \alpha x_1(t) + \beta x_2(t)
\]
where, $\alpha$ and $\beta$ are the weight factors of the front and back bead respectively. $x_1(t)$ and $x_2(t)$ are the actual displacements of the front and back beads respectively.

Now by choosing the ratio $\alpha/\beta$ in the range 1 to 100, the correlation coefficients have been computed. Correlation coefficient can defined for the two time series $r_1(t)$ and $r_2(t)$ as

$$R_{r_1r_2} = \frac{Covar(r_1, r_2)}{\text{Var}(r_1) \cdot \text{Var}(r_2)} = \frac{n \sum r_1 r_2 - \sum r_1 \sum r_2}{\sqrt{n(\sum r_1^2 - (\sum r_1)^2)} \sqrt{n(\sum r_2^2 - (\sum r_2)^2)}}$$ (6.4)

The following table shows the variation of correlation coefficient as a function of the ratio $\alpha/\beta$.

As it can be seen in the table, the correlation coefficient increases with decrease in ratio $\alpha/\beta$. i.e., as the contribution to the forward detection from the bead at the back increases, we also see an increase in the correlation coefficient. In the worst case, assuming equal contribution to the forward scattering signal from both the trapped beads, i.e., when the ratio $\alpha/\beta = 1$, we get a correlation coefficient of 0.708. However, the experimentally determined correlation coefficient is centered at 0.9, which is well above the value obtained from simulation for the worst case of such cross talk. Therefore, we can say that correlation coefficient analysis does bear information about interaction between the two beads.

Table 6.1: Variation of measurement cross talk as a function of the ratio $\alpha/\beta$.

<table>
<thead>
<tr>
<th>Ratio $\alpha/\beta$</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.708</td>
</tr>
<tr>
<td>1.5</td>
<td>0.550</td>
</tr>
<tr>
<td>2</td>
<td>0.446</td>
</tr>
<tr>
<td>5</td>
<td>0.193</td>
</tr>
<tr>
<td>10</td>
<td>0.096</td>
</tr>
<tr>
<td>50</td>
<td>0.018</td>
</tr>
<tr>
<td>100</td>
<td>0.017</td>
</tr>
</tbody>
</table>
6.5 Analysis on a pair of trapped beads

6.5.1 Analysis through Correlation function

The amplitude variation of fluctuation tells one about the corner frequency or trap stiffness of an individual sample at various levels. But it is unable to say whether successive sample values are related to one another or not. In such a situation, autocorrelation measurement may be used to characterize the time domain structure of a sequence. The Auto Correlation Function (ACF), is defined as

$$ R_{xx}(\tau) = \int_{-\infty}^{\infty} f(t+\tau) f(t) \, dt $$ (6.5)

Cross-correlation is essentially the same process but instead of comparing a sequence with a time shifted version of itself, the comparison is between two different sequences. The Cross-Correlation Function (CCF) of two sequences x(t) and y(t) is defined as

$$ \rho_{xy}(\tau) = \int_{-\infty}^{\infty} x(t+\tau) y(t) \, dt $$ (6.6)

Figure 6.6(a) shows the auto correlation function of a single bead (black) and cross correlation of a pair of beads with time lag \( \tau \). The enlarged image can be seen in figure 6.6(b). It is inferred that there is no time correlation between the two data sequences in a paired bead case (red), but interestingly, the autocorrelation function of a single bead has a good interference pattern at very low lag times due to tracking lasers’ coherence properties. The laser temporal coherence has been measured using this autocorrelation, it is found to be equal to 12 ms can be seen in figure 6.6(b).

Figure 6.6: (a) Cross correlation function for single and pair of trapped beads as a function of lag time and (b) enlarged image centered at zero lag time.
6.5.2 Analysis through Correlation coefficient

We have tried to analyze the possible interaction between the two trapped beads by measuring the correlation coefficient of the two fluctuating beads tracked simultaneously through both forward and back scattering.

Figure 6.7 shows the histogram of the correlation coefficient of single and pair of beads in a trap for several sets of trapped beads. In case of a single bead, the correlation coefficient is centered on 0.96 indicating strong (expected) correlation while for a pair of beads the correlation coefficient is spread over 0.7 to 0.95. This indicates that the beads may be interacting with each other either through contact or through a hydrodynamic mediated interaction. The varying correlation coefficient may be an indication of the interaction depending on the surface morphology of the trapped beads.

![Histogram of correlation coefficient](image)

Figure 6.7: Histogram of the correlation coefficient for single and paired beads in a trap.

6.5.3 Analysis through corner frequency difference

Interestingly at a constant laser power, we have observed that corner frequency obtained through forward scattering for a pair of trapped beads is not always half that of the value at the single bead case. It is sometimes comparable with that of the value obtained through back scattering. As a result, the corner frequency difference spans over a certain range. This variation in corner frequency in a paired bead case can be seen in the figure 6.8. We speculate that this is due to the surface morphology and perhaps, the microscopic distribution of size of the trapped beads.

As discussed earlier, while, the back scattered signal bears information of the back bead’s fluctuation, this situation is no longer true in case of forward scattering, owing to the cross talk that results in information that is a (weighted) average of the fluctuation s of both front and back beads. Thus, although, the different values of the corner
frequencies that we measure corresponding to the front bead are always smaller, no doubt, than the ones corresponding to the back bead, the former being in a less intense region of the focused laser, the measured value of these frequencies need to be corrected after a measurement of the cross talk information from the beads. In future work we will measure the cross talk carefully and correct these values.

Figure 6.8: Power spectral density of paired beads tracked through both forward and back scattering techniques at a laser power of 24 mW showing different corner frequencies for different sets of trapped beads.

Figure 6.9: Histogram corner frequency difference measured for 3μm beads at a laser power of 24 mW in water.
We have also measured the corner frequency difference at a constant laser power. The spread of corner frequency difference can be seen in figure 6.9, it is a Gaussian centered on 60 Hz at a laser power of 24 mW at the sample plane.

A graph of correlation coefficient with corner frequency difference can be also seen in figure 6.10; we found that irrespective of the corner frequency difference, correlation coefficient is centered at 0.9.

![Graph showing correlation coefficient vs. frequency difference](image)

Figure 6.10: Plot showing the values of correlation coefficient for different corner frequency differences between pair of beads tracked by forward and back scattering technique. Values in the parenthesis are the corner frequencies measured through forward and back scattering respectively.

As an extension, of these measurements and to examine whether the viscosity of the liquid has a bearing on the distribution of correlation coefficients, we have measured the corner frequency difference between a pair of trapped beads tracked through simultaneous forward and back scattering techniques in a Glycerol-Water mixture which has a viscosity of 8.5 mPas.

![Histogram of corner frequency difference](image)

Figure 6.11: Histogram of corner frequency difference measured for 3μm at a laser power of 24 mW in Glycerol-Water mixture at a viscosity of 8.5 mPas.
At a laser power of 24 mW, again we have observed a spread in the corner frequency difference, which is Gaussian centered at 12 Hz, but correlation coefficient is found to be around 0.8 which is close to what we obtained for pure water. Hence it may not be possible to extract the viscosity dependent properties through this technique.

6.6 Simultaneous two side (multiple views) imaging

The schematic of the setup is shown in the figure 6.12. It consists of a conventional single-beam gradient force trap path which uses a $100 \times$ high NA objective (NA=1.4) in the inverted position and in addition a second $10 \times$ objective with moderate NA (0.65) is used to monitor the sample volume from a direction orthogonal to the trapping. The geometry followed here is similar to reference [6].

![Schematic of two-side imaging setup](image)

Figure 6.12: Schematic of the two-side imaging setup (not to scale) in an optical tweezer. The red path corresponds to the trapping laser while green indicates the imaging paths.

The conventional imaging path is composed of the fiber illumination lamp (I1), trapping objective, dichroic mirror and CCD camera (C1). The second imaging path, orthogonal to the first, is composed of illumination lamp (I2), lateral objective, converging lens (L2)
and CCD camera (C2). The trapping objective is mounted on a XYZ nano stage (PiezoJena GmbH) which enables axial displacement with 3 nm resolution. Due to space restriction to accommodate two microscopic objectives, the sample chamber built with microscope cover slips to form a cubic shape, so that sample is transparent in all three directions.

Figure 6.13: A picture of the multiple views imaging system.

Figure 6.14: Images obtained through (a) conventional and (b) side on view imaging system.
The images obtained by both conventional and side on view imaging are shown in the figure 6.14. From the figures and within the limitations of resolution it appears that the beads in the trap are touching each other.

6.7 Conclusions

The technique of simultaneous forward and back scattering detection in an optical tweezer has been demonstrated. Power spectral density analyses based on this technique clearly distinguish between the cases of single and a pair of trapped beads. A pair of trapped beads in a single beam tweezer is analyzed through differences in the corner frequencies and correlation coefficient measurement between two trapped beads. The results obtained from correlation coefficient analysis and side on view imaging system together conclude that the beads are in contact with each other.

Simulation studies on the cross talk between the two trapped beads and the influence of this on the resultant corner frequency difference can tell us about the true value of the trap stiffness seen by the front bead. In future we would like to extend our simulation studies to extract the true trap stiffness of both beads and from this, extend the work further, to investigate and quantify the nature of interaction between the trapped beads.

6.8 References