

Probing the interaction between two microspheres in a single Gaussian beam Optical Trap

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ABSTRACT

Interactions between trapped microspheres have been studied in two geometries so far: (i) using line optical tweezers and (ii) in traps using two counter propagating laser beams. In both trap geometries, the stable inter bead separations have been attributed to optical binding. One could also trap two such beads in a single beam Gaussian laser trap. While there are reports that address this configuration through theoretical or simulation based treatments, there has so far been no detailed experimental work that measures the interactions.

In this work, we have recorded simultaneously the fluctuation spectra of two beads trapped along the laser propagation direction in a single Gaussian beam trap by measuring the back scattered signal from the trapping and a tracking laser beam that are counter propagating. The backscattering from the trapping laser monitors the bead encountered earlier in the propagation path. The counter propagating tracking laser, on the other hand, is used to monitor the fluctuations of the second bead. Detection is by using quadrant photo detectors placed at either end. The autocorrelation functions of both beads reveal marked departures from that obtained when there is only one bead in the trap. Moreover, the fall-off profiles of the autocorrelation indicates the presence of more than one relaxation time. This indicates a method of detecting the presence of a second bead in a trap without directly carrying out measurements on it. Further, a careful analysis of the relaxation times could also reveal the nature of interactions between the beads.

Keywords: Gaussian beam optical trap, back scattering, forward scattering, Quadrant Photo Detector, Colloidal interactions, Optical binding, Information crosstalk, Autocorrelation function

1. INTRODUCTION

Optical Tweezers [OT] enable non-contact trapping and manipulation of microscopic particles and can impart piconewton forces on trapped entities¹. This allows for the use of an OT to study colloidal systems without affecting the delicate balance between various forces such as the electrostatic forces, depletion forces, forces due to hydrodynamic interactions etc prevalent in such systems².

Apart from colloidal interactions, optical binding which is due to a rearrangement of trapping laser intensity between the trapped micro particles has also been seen³. However, optical binding has been studied pre-dominantly in two geometries: (i) a line tweezer arrangement formed using a cylindrical lens (ii) traps formed using two counter propagating laser beams⁴. One can trap two or more colloidal beads or micro spheres along the laser propagation direction in a Single Gaussian Beam OT [SGBOT] as in Fig.1 and a subsequent recording of the positional fluctuations can be used to study the interaction between them. However, previous attempts at recording position fluctuations of two micro spheres trapped axially in a SGBOT has resulted in an erroneous estimation of trap stiffness for a supposedly trapped single particle⁵. Therefore, it is pertinent that true fluctuation information of the trapped microspheres be recorded before attempting to understand inter bead interactions.

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In an earlier work⁶, we have shown that, in an axial two-bead trap, back scattering detection yields position fluctuation information of only the bead the laser encounters first whereas in forward scattering based detection schemes, the fluctuation information from both beads which we call the information crosstalk is present. This crosstalk ridden signal has a dominant contribution from the bead the laser encounters first on its propagation path⁶. Thus, a two laser wavelength simultaneous back scattering scheme allows for recording true fluctuation information of the two axially trapped beads in a SGBOT.

In this paper, we report auto correlation based analyses on the crosstalk free fluctuation data recorded using the two wavelength simultaneous back scattering detection scheme. We find that: (i) the nature of the autocorrelation plots for both front and back beads are significantly different from the plots obtained when a single particle is present in the OT (ii) the autocorrelation plots of the back bead show two relaxation times as against a single relaxation time expected for a particle executing Brownian motion. Thus, autocorrelation studies may be used to detect the presence of multiple microspheres in an OT where a direct measurement/detection of the same is not possible.

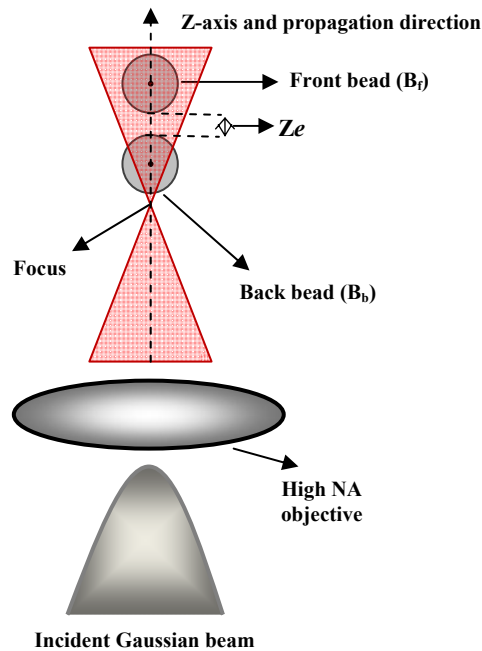


Figure 1: Schematic of two micro spheres trapped along the laser propagation direction in a Single Gaussian Beam Optical Trap. Z_e represents the edge to edge distance between the trapped beads.

2. EXPERIMENTAL DETAILS

2.1 Simultaneous Two Wavelength Back Scattering Detection Scheme

Our Optical Tweezer setup consists of a fiber coupled ytterbium laser whose free space wavelength is 1064nm. The fiber tip is fitted with a beam collimator which outputs an 8mm diameter circular beam which is unpolarized. This laser beam which is the trapping laser in all our experiments, is passed through a linear polarizer and then through a 1.4 Numerical Aperture oil immersion objective (O1 in Fig.2(a) and (b)). A second linearly polarized diode laser of wavelength 980nm is aligned to pass through another oil immersion objective of Numerical Aperture 1.25 (O2 in Fig.2(a) and (b)) aligned co-axial to O1. The 980nm laser power is attenuated so that it functions only as a tracking laser and does not affect the trap stiffness of the beads trapped using 1064nm laser. In order to align the two objectives, a bead was trapped with 980nm laser, the laser was moved till the trapped bead position coincided with predetermined position of the 1064nm laser before attenuating the power of the 980nm laser so as to use it only as a tracking laser. Also, in order to ensure co-linearity of the two objectives, the tilts of the objectives were varied till each objective formed in its back focal plane, a circular spot of the laser coming in from the other objective. Two Quadrant Photo Detectors (QPDs) on either sides of the sample stage were aligned to detect back scattering of a single wavelength by incorporating suitable line filters with

narrow pass bands centered around the two laser wavelengths. Thus, QPDb was used to detect 1064nm while QPDb was used for recording the fluctuation information carried by 980nm [Fig 2(a)]. Polystyrene beads of diameter 3 micrometer from Polyscience Inc., USA were suspended in double distilled water in a sample chamber formed by sticking an o-ring of diameter 1cm and thickness 1mm on a glass slip. The O-ring was slightly overfilled with the sample solution and another glass slip introduced from the top rendered this arrangement air tight.

2.2 Simultaneous Two Wavelength Forward Scattering Detection Scheme

By simply swapping the line filters from the previous arrangement, forward scattering detection from the two trapped micro beads was carried out. Thus in this arrangement, QPDb recorded the 1064nm and QPDb recorded the 980nm laser wavelength.

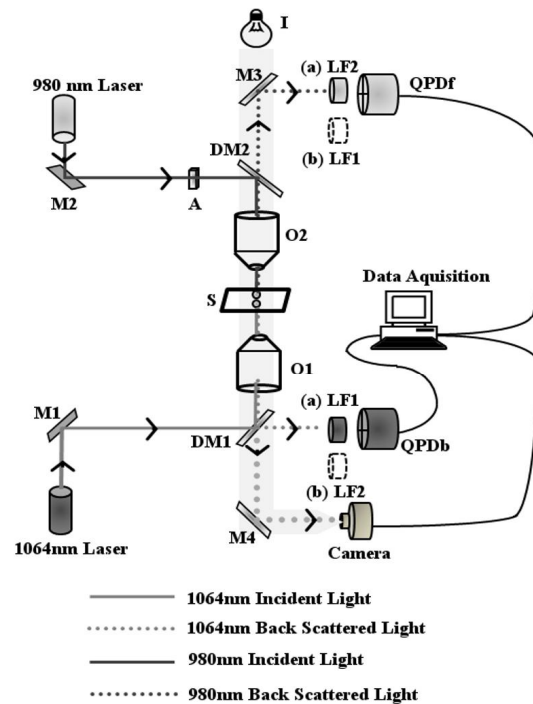


Figure 2: (a) Schematic of Two wavelength simultaneous back scattering detection setup with 1064nm line pass filter and 980nm line pass filter fixed in front of QPDb and QPDb respectively. M1, M2, M3 and M4 are mirrors, A- attenuators, S – sample Plane, I – illumination lamp, DM1 to DM3 are dichroic mirrors, O1(NA=1.4) and O2(NA=1.25) are tapping and tracking objectives respectively. LF1 and LF2 are 1064nm and 980nm line filters respectively. (b): For two wavelengths simultaneous forward scattering detection setup with the positions of the two line pass filters exchanged as shown by dotted LF1 and LF2.

3. EXPERIMENTAL RESULTS AND DISCUSSION

The entire process of trapping was visualized using a high frame rate camera from Point Grey Research USA [Fig.2 (a) and (b)]. The number of beads in the trap was monitored using the camera with its speed set at 15 frames per second. Before recording data from a double bead trap, a single bead was trapped and its fluctuations were recorded using both simultaneous back scattering detection scheme [Fig. 3(a) and (b)] and simultaneous forward scattering detection scheme [Fig. 3(c) and (d)] and we obtained almost identical corner frequencies on both detectors. This calibrated our setup.

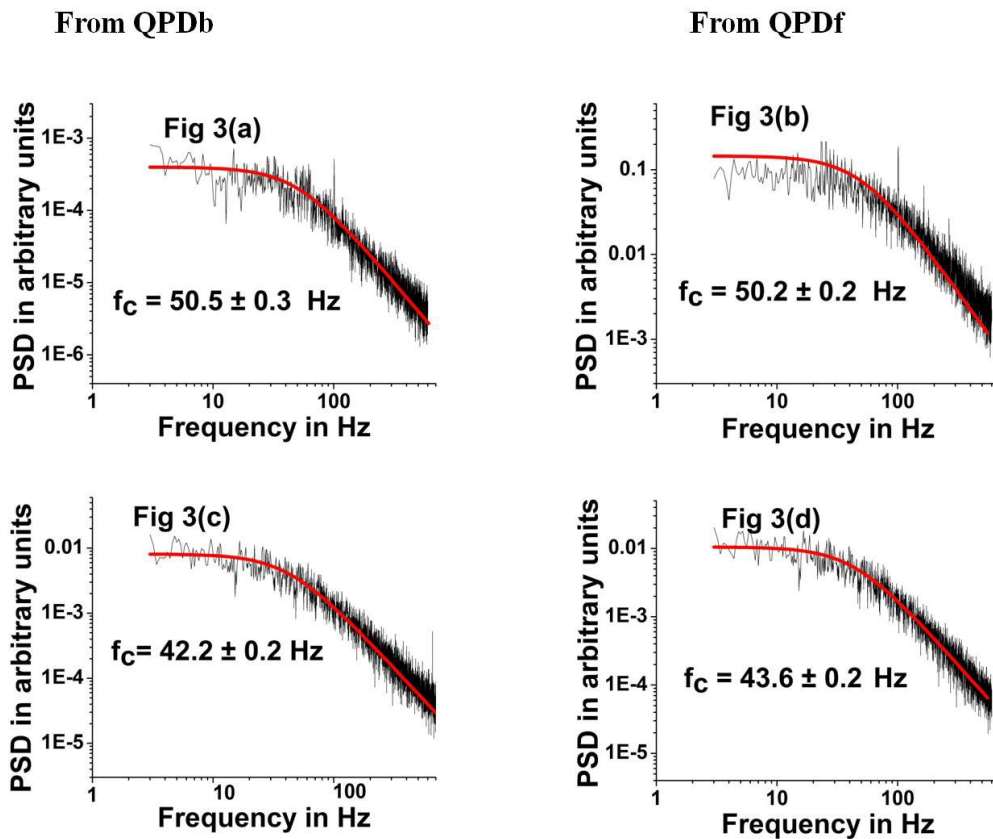


Figure 3(a) and (b): Power Spectra recorded using the setup in Figure 2(a). Figure 3(c) and (d): Power Spectra recorded using the setup in Figure 2(b). Trapping laser power is set to 32mW in cases (a) and (b) and to 25mW in cases (c) and (d)

3.1 Trapping two microspheres along the laser propagation direction

In Fig. 4, we show a real time Power Spectral Density (PSD) based monitoring of the process of trapping a second bead in a trap that already has a microsphere. Data is recorded at the rate of 100,000 data points each second. PSDs are computed for data recorded for 1 second duration. In this experiment, the laser power at the sample was about 30mW and we show data as recorded on QPDb aligned to record back scattering of 1064nm laser.

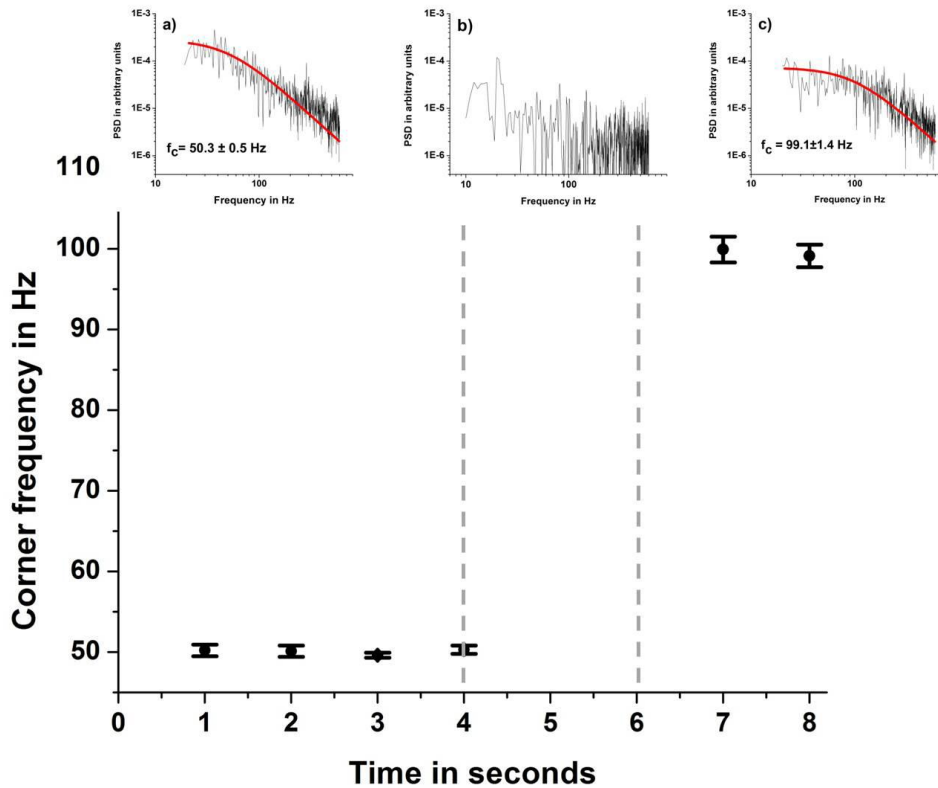


Fig 4: Variation in the corner frequency of data recorded on QPDb with time as a second bead is trapped. Data logged at the end of each second is subjected to power spectral density analysis and a corner frequency with standard deviation error is reported. Insets (a) and (c) show PSDs with fits for single and double beads respectively where, a corner frequency of $50.3 \pm 0.5 \text{ Hz}$ is obtained for the case of a single bead while, the value for the same parameter after the second bead is stably trapped is $99.1 \pm 1.4 \text{ Hz}$. Inset (b) shows data recorded in the time period between the dashed lines where a fit to a modified Lorentzian was not possible. This is when the second bead is close enough to affect the signal detected by QPDb but is yet to be stably trapped. Throughout this experiment, the trapping laser power in the sample plane was set to 32mW.

It may be seen in inset (a) in Fig. 4 that a single trapped bead is held with a corner frequency of about 50Hz. In inset (b) of Fig. 4, a second bead is approaching the trap and is yet to be trapped stably. Therefore, a fit to a Lorentzian is not possible during this event. While it is not possible from this method to comment upon where the second bead will be trapped with respect to the bead already in the trap, it can be shown that the back scattering data recorded on QPDb reveals a corner frequency much higher (about 99Hz) than that obtained in the case of a single bead trap as can be seen in inset (c) of Fig.4. Therefore it is reasonable to conjecture that the back bead is positioned closer to the laser focus compared to a single bead and also that the front bead will be stably trapped farther from the laser focus. Thus, the corner frequency of the front bead might be expected to be lower than that of the back bead.

3.2 Two wavelength simultaneous back scattering detection

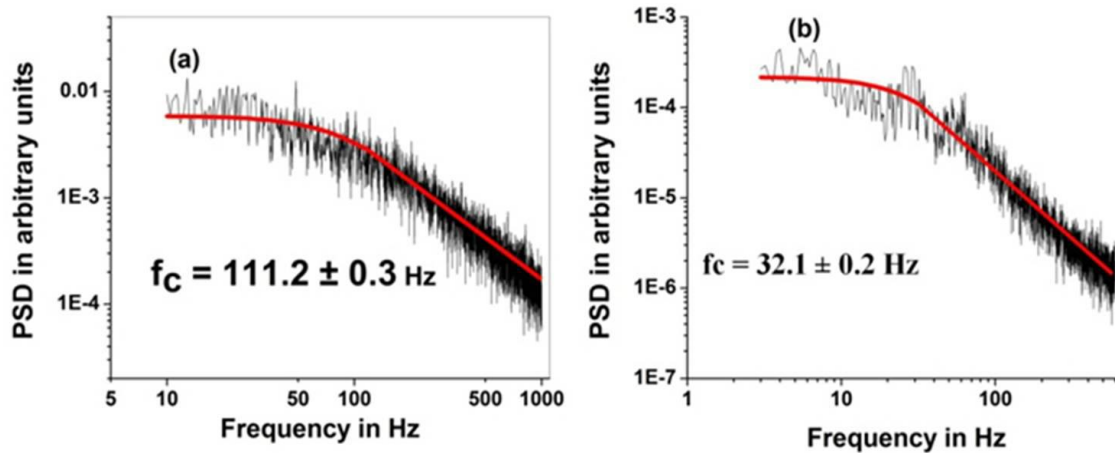


Figure 5(a) and (b): Power Spectra of the data recorded using the simultaneous back scattering detection scheme. Trapping laser power is set to 32mW

The experiment was carried out at 32mW of laser power at the sample plane. Before trapping a pair of beads, a single bead was trapped and its corner frequency was recorded on both detectors. A representative data from this experiment is shown in Fig. 3(a) and 3(b) where it can be seen that the two corner frequencies lie within range of each other considering signal-to-noise ratio in each case. Then, a pair of beads was trapped. Power spectra computed on recorded data are shown in Figures 5(a) and 5(b) where it can be seen that the back bead is held more strongly compared to the front bead and also a single bead.

3.3 Two wavelength simultaneous forward scattering detection

A representative data of a single bead trap detected using two wavelength simultaneous forward scattering setup is shown in Fig. 3(c) and (d) where it can be seen that the two corner frequencies lie within a range allowed upon considerations of signal-to-noise ratio in this detection scheme. Then, for a double-bead trap, it is seen that the corner frequency obtained on QPDb is greater than that obtained on QPDb. It is worth mentioning again here that, since the back bead is held closer to the laser focus than the front bead, a higher corner frequency for the back bead and a lower corner frequency for the front bead is expected and indeed, seen in the previous detection scheme. Therefore, it can be concluded that in the case of simultaneous forward scattering detection scheme, both detectors record data that has substantial position information crosstalk. Furthermore, since data recorded on QPDb shows a higher corner frequency than that recorded on QPDb, it is reasonable to conclude that the data recorded on QPDb is dominated by fluctuation information from the back bead and that the data recorded on QPDb is dominated by fluctuation information from back bead reversing the observations made in the previous detection scheme. Hence, a more general conclusion that in the forward scattering detection scheme, the recorded data is not free from crosstalk and is dominated by the fluctuation information from the bead the laser encounters first on its propagation path can be drawn.

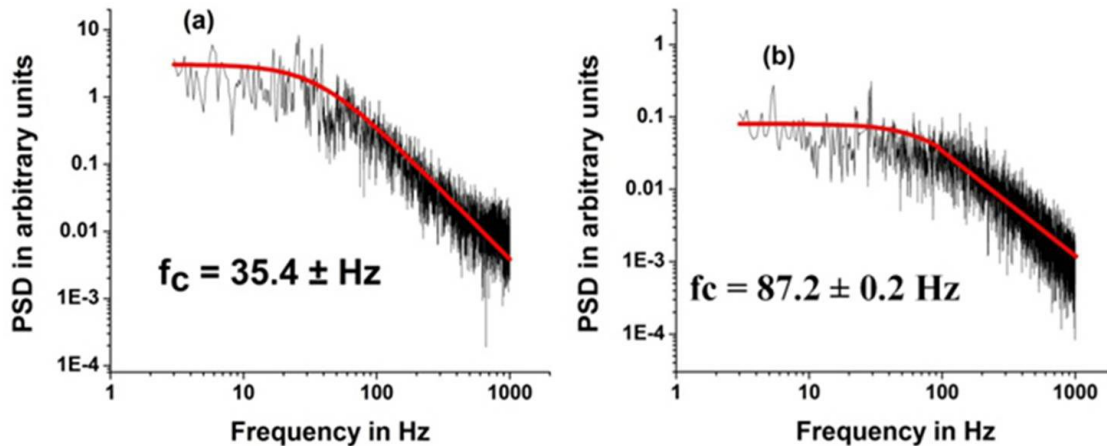


Figure 6(a) and (b): Power Spectra of the data recorded using the simultaneous forward scattering detection scheme. Trapping laser power is set to 32mW.

In Ref.[6], we have also used Mie Scattering based Optical Tweezer Toolbox and Ray Optics codes that we have developed, to further garner support to the fact that back scattering detection from a pair of beads trapped along the laser propagation direction in a SGBOT allows for crosstalk free detection of the fluctuations of the individual beads. On the other hand, the fluctuation information obtained from forward scattering detection carries an admixture of information from both beads.

4. AUTOCORRELATION ANALYSES

Data recorded using two wavelength simultaneous back scattering scheme has been shown to be free from crosstalk. This is used to compute autocorrelations. The threshold sample plane power for stably trapping a pair of 3 micrometer polystyrene beads was found to be 12-13mW. We show in Figures 7 (a) and 7(b), data recorded from a single trapped bead on QPDb and QPDb respectively using the simultaneous two wavelength back scattering method with the trapping power set to 32mW at the sample plane. Autocorrelation values with lag time ranging from zero to 20 milli seconds were computed. The autocorrelation data was subjected to a two step box averaging with the first box averaging done with a window size of 100 data points which was then box averaged again with a window size of 2 so that the overall box average window was over 200 autocorrelation data points which correspond to a time of 20 milli seconds.

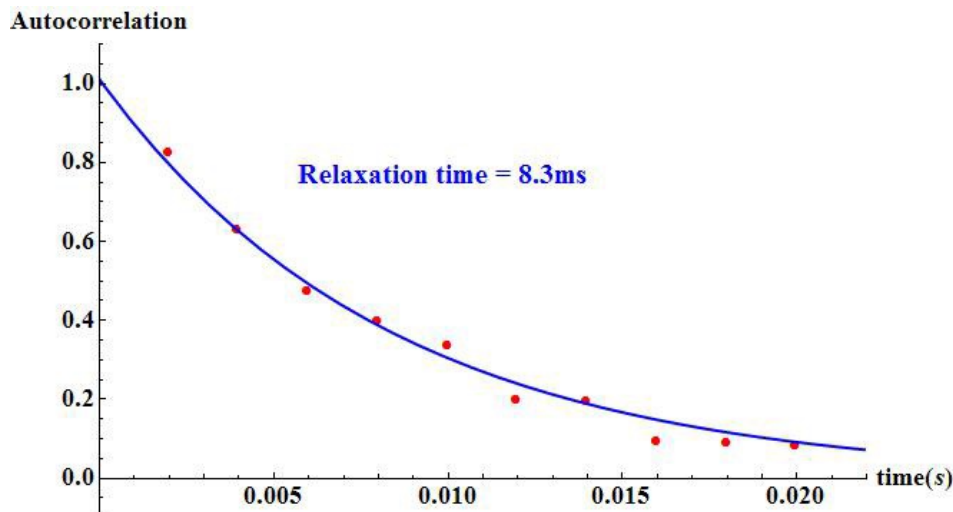


Figure 7(a): Single bead autocorrelation of the data recorded using the simultaneous backscattering detection scheme on QPDb. Trapping laser power in the sample plane is set to 32mW.

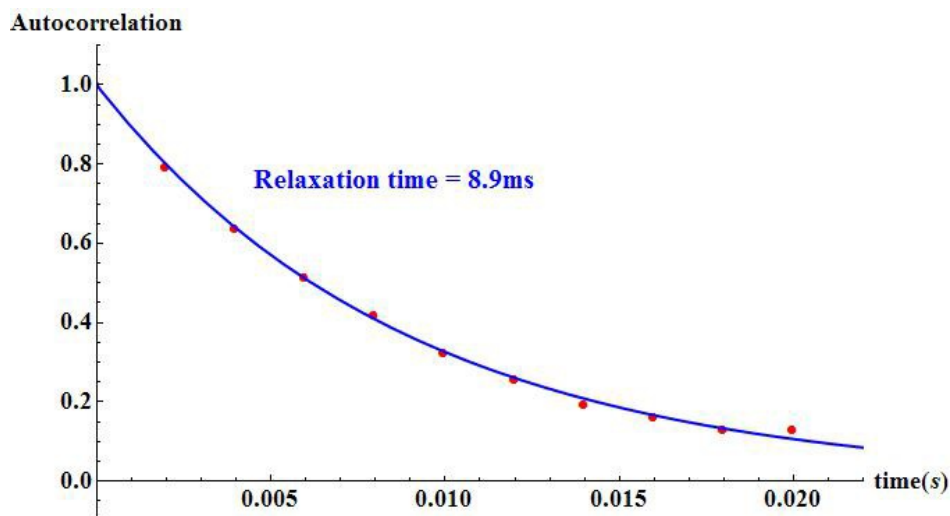


Figure 7(b): Single bead autocorrelation of the data recorded using the simultaneous backscattering detection scheme on QPDef. Trapping laser power in the sample plane is set to 32mW.

A representative autocorrelation plot of the front bead, when two beads are trapped is shown in Fig. 8 where a fit to a single exponential is carried out and the relaxation time is found to be 10ms. It may be noted that the relaxation time obtained in this case is larger than those obtained for a single bead, indicating that the front bead is held more loosely. This is in accordance with the lower corner frequency measured for the front bead, compared to a single trapped bead

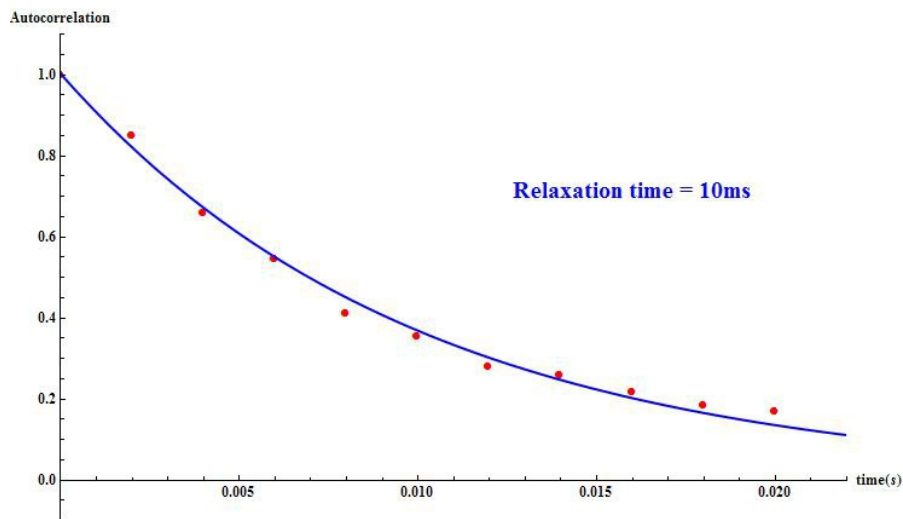


Figure 8: Front bead autocorrelation of the data recorded using the simultaneous backscattering detection scheme. Trapping laser power in the sample plane is set to 32mW.

In Fig. 9(a) a representative autocorrelation plot of the back bead data is shown along with a fit obtained to a single exponential after 100 iterations. It is seen clearly that a single exponential function cannot represent the data over the full range. However, in Fig. 9(b), it is seen that two different exponential functions, one for the lag time 0 to 8 ms and the other for data points corresponding to lag times 10 to 20 ms represent the data reasonably well.

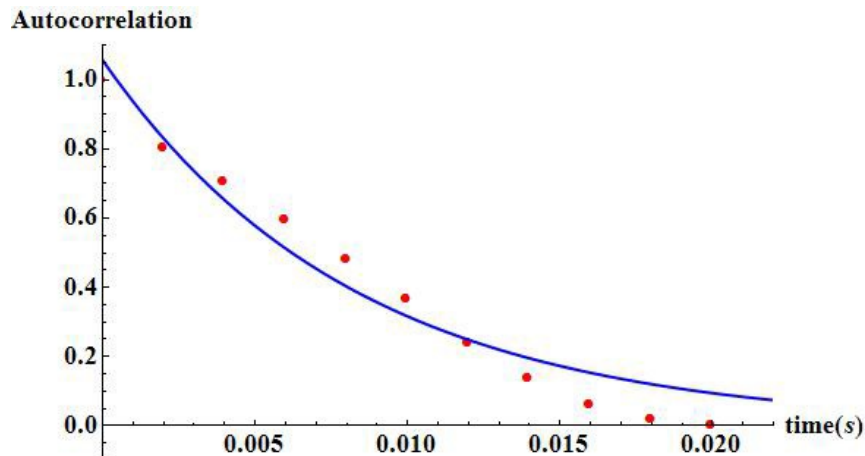


Figure 9(a): Back bead autocorrelation of the data recorded using the simultaneous backscattering detection scheme along with a fit to an exponential function over the full range of data.

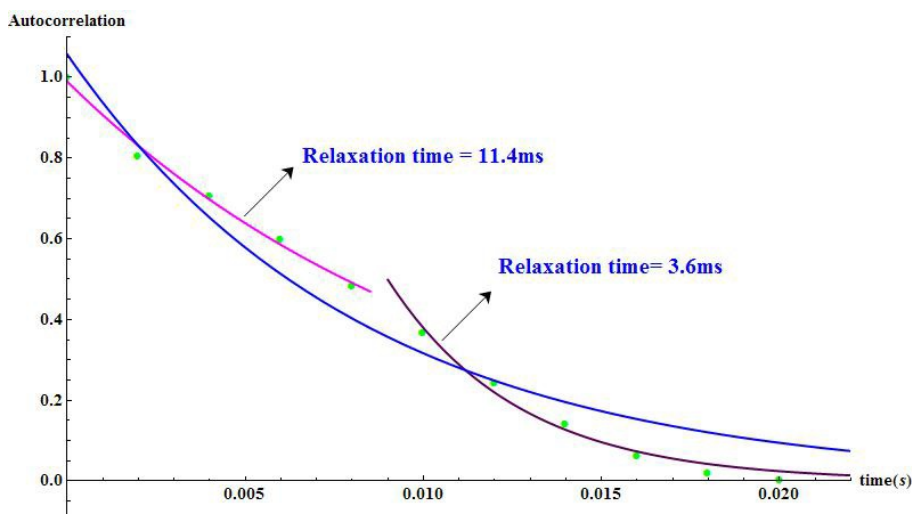


Figure 9(b): Data shown in Fig.8(a) is fit to an exponential function over the full range of data (blue), an exponential fit from 0 to 8ms (purple) 10 to 20ms (black)

We speculate that this behavior is indicative of an interaction between the trapped pair of beads that is limited to distances within the thermal fluctuation amplitudes of the beads and is therefore seen on the back bead which undergoes smaller displacements from the trap center due to a higher trap stiffness. The front bead autocorrelation plot does not show signatures of the interaction as it undergoes larger positional fluctuations due to a lower trap stiffness. Furthermore, we attribute the smaller relaxation time of 3.6ms to the Optical Trap as it may be noted that a trap stiffness of about 111Hz (Refer to Fig.5(a)) corresponds to a relaxation time of about 1.4ms. Thus, the larger relaxation time of 11.4ms may be thought of as leading to an interaction induced Corner frequency of 13.9Hz.

4.1 Autocorrelations of data recorded at trapping power equal to the threshold for trapping double beads

The trapping laser power at the sample plane was set at 13mW. This corresponds to the lowest power at which two 3 micron polystyrene beads can be stably trapped in our setup. We show in Fig.10 the autocorrelation plots of the back bead fluctuation data. It can be seen clearly that, the signatures of interaction as seen in Fig.9(b) (where the trapping was carried out at 32mW) are not present. Therefore, we speculate that, an interplay between the relative strengths of the trap potential, inter bead interaction potential and the random thermal forces determine if the signatures of the interaction are seen. In fact, it is reasonable to expect that as the trap strength is increased, the trap potential might overwhelm the signatures of inter bead interaction on the back bead, whereas, for the case of the front bead, it could be comparable with

the strength of inter bead interaction potential and thereby, these features might be visible on the front bead. This may help us set limits on the strength of the inter bead interaction.

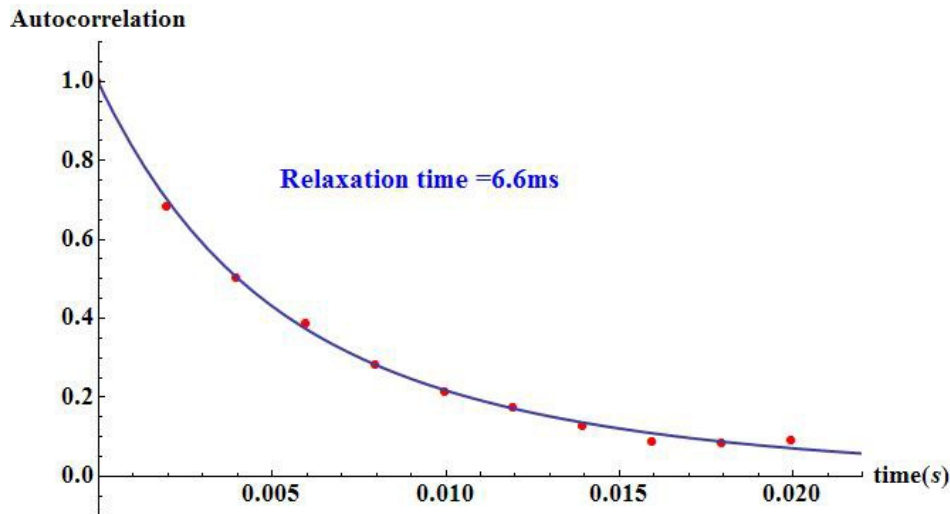


Figure 10: Autocorrelation plot of fluctuation data of the back bead recorded using back scattering detection technique. The trapping power of the laser at the sample plane is set to 13mW.

5. CONCLUSIONS AND OUTLOOK

We have shown that when two micro beads are trapped along the propagation direction in a single Gaussian beam optical trap, detection of back scattered light yields cross talk free position fluctuation information of the individual beads whereas forward scattering based detection carries an admixture of fluctuation information from the two trapped beads. Autocorrelation based analyses of the data recorded using simultaneous two wavelength back scattering for the two trapped beads reveal that signatures of inter bead interaction can be seen on the autocorrelation plots of the back bead which is held more tightly compared to the front bead and therefore suffers smaller displacements. Furthermore, we could also setup a limit on the strength of the interaction as the signatures of interaction seen on back bead fluctuation autocorrelation plots were seen to disappear upon lowering the power of the trapping laser.

We are currently at work to develop an analytical method with an interaction term in the Lagrangian whose strength and form are being varied to obtain best fits to the experimentally observed data points. In another approach, we are developing codes that numerically solve coupled Langevin equations with an interaction force setup to represent the equations of motion of the two trapped beads. The strength and form of the interaction force will be varied till convergence with the experimental data is obtained.

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