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Trapping multiple particles in single optical tweezers

Martin Li [1], Jochen Arlt [*,1]

Department of Physics, University of Reading, Whiteknights, Reading RG6 6AF, UK

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Abstract

Optical trapping of multiple particles in three dimensions in a single inverted optical tweezers is investigated. The effect of trapping pairs of beads on the voltage signal recorded by a quadrant photo diode detector is examined, with particular emphasise on its power spectral density. It is found that trapped pairs of beads could be mistaken for single beads in a trap of half the strength. Stokes drag measurements reveal that both beads of the pair are confined less than a single particle in the same trap and that the quadrant signal provides some information of their mean displacements.

1. Introduction

Over the last three decades optical forces have found increasingly widespread use within a large variety of scientific disciplines. Especially the first implementation of the single beam optical gradient trap [1], so called optical tweezers, has led to a surge of activity both for contactless micromanipulation and precision measurements [2]. Early pioneering work was mostly done in the context of biological and biophysical investigations but during the last decade applications have spread to diverse areas in physical sciences, chemistry and engineering [3].

Typically, optical tweezers are used in very dilute samples: there are very few 'trappable' particles within the immediate vicinity of the trap to avoid attracting more than one particle into the trap. But more recently, optical tweezers have also started to be employed for microrheological measurements of materials such as polymer solutions, dense colloidal suspensions and even living cells [4–6], where such dilute conditions cannot always be ensured. In some cases it is still possible to have only a very small amount of tracer particles, which act as handles for the optical tweezers, in a material of interest that does not interact with the tweezers. For example, in dense colloidal suspensions the host colloids can be matched to the refractive index of the solvent, making them invisible for the trapping beam [5]. However, for other experimentally interesting systems this is not always feasible. For example, in dense suspensions of motile bacteria [7], the trap does not only interact with the tracer beads but also the bacteria themselves [8].

Unfortunately, in dense samples it happens fairly frequently that two or more objects are attracted by a single optical tweezers, especially if a strong trap is used. Depending on the sample used, this might not be immediately obvious from either direct visual observation of the trapped particle or the recorded time trace of the position. Especially, in microrheology experiments it could therefore be quite easy to accidentally mistake measurements with two trapped particles for 'real' results, and attribute the effects to the visco-elastic properties of the host medium. In this paper, we investigate how 3D-trapping of two or more beads within a single optical tweezers affects the recorded power spectrum and stiffness of the trap.

2. Background

The position fluctuations of single spherical particles trapped in optical tweezers within a purely viscous fluid

* Corresponding author. Tel./fax: +44 131 651 7066.
E-mail address: j.arlt@ed.ac.uk (J. Arlt).
[1] Present address: COSMIC, SUPA, School of Physics, The University of Edinburgh, King's Buildings, Edinburgh EH9 3JZ, Scotland, UK.

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are well understood, and several experimental methods actually exploit the Brownian motion of the trapped particle to characterise the trap completely [9], often in conjunction with drag measurements [10].

The motion of the trapped particle is described by the Langevin equation

$$\gamma \frac{dx}{dt} + kx = F(t), \tag{1}$$

where γ is the particle's drag coefficient, k the spring constant of the optical trap and $F(t)$ are random thermal fluctuations which average to zero and are completely uncorrelated in time. In the above equation the inertial term is omitted as it is only relevant at much higher frequencies than considered here. For an isolated spherical particle of radius r far away from any other surface the drag coefficient is given by $\gamma = 6\pi\eta r$ (Stokes law).

As this system is in thermal equilibrium, equipartition of energy holds and can be used to immediately calculate the variance of the position fluctuations $\langle \Delta x^2 \rangle = k_B T/k$, where k_B is the Boltzmann constant and T the absolute temperature of the sample.

One common method of analysing the motion in some more detail is to look at its power spectrum. It can be easily derived from Eq. (1) that the power spectrum for a single trapped object is a Lorentzian

$$S_x(f) = \frac{k_B T}{\gamma \pi^2 (f_c^2 + f^2)} \tag{2}$$

with the corner frequency given by $f_c = k/(2\pi\gamma)$.

Experimentally, the position fluctuations are often not measured directly but deduced from the fluctuations of trapping laser light transmitted or reflected from the trapped particle. It can be shown that for a single scattering particle trapped within the focus the normalised voltage signals of a quadrant photo diode detector are directly proportional to the particle's position over a limited range [11]. Experimentally, the proportionality factor β is often found by simply fitting the Lorentzian Equation (2) to the power spectral density (PSD) of the normalised voltage fluctuations $S_V(f) = \beta^{-2} S_x(f)$ assuming the value for γ is known [9,10].

When trapping two particles within the same optical trap, one would as a first approximation expect that each of the particles moves independently within the harmonic trap according to Eq. (1). But as each of the particles scatters light it modifies the optical forces experienced by the other particle, which can give rise to an additional attractive force in between the two particles. Such optical binding [12] has been observed experimentally using different experimental geometries [13,14]. The particles are also coupled by hydrodynamic interaction, giving rise to anti-correlated motion of the particles [15]. Furthermore, if the particle size is comparable to the volume of the focus, spatial exclusion implies that the two particles sit at different positions relative to the focus and thus experience different trap stiffness k_1 and k_2.

3. Experimental methods and results

Our experimental setup uses a standard photonic force microscopy design [11] on an open-bench inverted microscope. A near-infrared laser ($\lambda = 1064$ nm; Ventus HP 1064, Laserquantum, UK) is used for trapping through an oil-immersion objective (Nikon, CFI E Plan Achromat 100× NA1.25). The objective is also used to image the trapped particles onto a digital CCD camera (TM-6740CL, Pulnix, CA) with a 200× overall magnification. The transmitted laser light was collected by an oil-immersion condenser and imaged onto a quadrant photo diode (QPD) detector. The particle's movement was monitored using the voltage signal of the QPD, typically sampled at 20 kHz, and/or the camera image, which was recorded digitally at up to 200 fps. Most of the experiments were performed trapping polystyrene beads of 1 μm diameter suspended in distilled water but some measurements were also done using 0.7 and 2.0 μm diameter polystyrene spheres (Duke Scientific Corp., Palo Alto, CA and Fluka, Sigma–Aldrich, Germany).

We first trapped a single bead and recorded its fluctuations as a reference. Then we moved the sample stage to capture a second bead within the same optical trap. This bead enters the trap from below and shunts the originally trapped particle deeper into the sample. The two particles thus end up stacked on top of each other with the initial bead being displaced further away from the laser focus and experiencing a somewhat weaker confinement due to the optical forces, whereas the second bead seems to sit closer to the optimal position. When the image of two trapped spheres is directly compared to that of a single bead in a trap they are easily distinguished (see Fig. 1), but without reference it could also be mistaken for a single bead in a

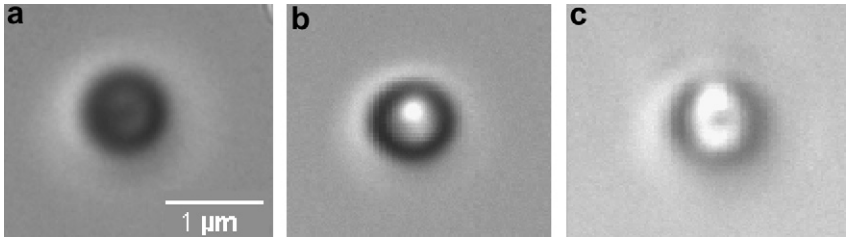

Fig. 1. CCD image of (a) a single 1 μm bead; (b) two 1 μm beads; (c) three 1 μm (at higher laser power to give a stable configuration) trapped in a single optical tweezers.

slightly different focal plane. With our coaxial view of the bead configuration (which is the most widely used configuration) it is thus difficult to identify the actual number of beads without careful considerations. Obviously, the beads could be easily distinguished using a side view geometry [1] or by using confocal imaging [16].

When a second bead was trapped the voltage fluctuations recorded by the QPD detector increase noticeably; in case of two 1 μm beads, the variance almost exactly doubled. However, somewhat surprisingly, the power spectrum of the normalised voltage signal retained a Lorentzian shape (see Fig. 2). Comparison with the power spectrum for a single bead showed that the high frequency components overlapped almost perfectly. The additional variance of the signal was due to more low frequency noise and a corresponding lowering of the corner frequency f_c. Fitting the data to a Lorentzian, and assuming Eq. (2) for the power spectrum as derived for a single bead in the trap was still applicable, yielded a good fit with a corner frequency reduced by a factor of two. If the drag coefficient γ for a single bead was used this would correspond to a harmonic trap with half the trap stiffness k. Thus the voltage fluctuations recorded by the QPD for two 1 μm beads confined in the same optical trap, were the same as for a single 1 μm bead confined in a trap of approximately half the trap stiffness k.

To verify this behaviour we repeated the experiments for several different power settings, each about half of the previous laser power. The corresponding power spectra are shown in Fig. 3. For the single beads it can be clearly seen that the corner frequency reduced proportional to the laser power as one would expect. The data for a pair of beads trapped overlaps almost perfectly the single bead data at half the laser power and thus half the trap stiffness, reconfirming the previous observation.

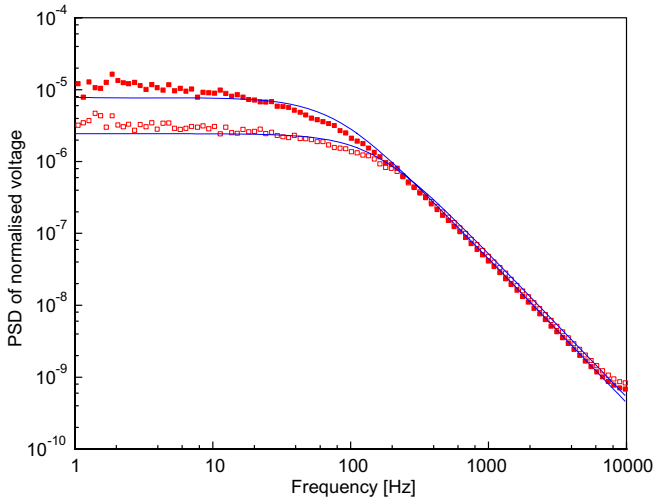

Fig. 2. Power spectra of the normalised voltage signal $(V_+ - V_-)/(V_+ + V_-)$ for single and two 1 μm beads trapped, together with their Lorentzian fit. The corner frequency for the pair has almost exactly halved (148 Hz vs. 76 Hz) while the sensitivity has hardly changed (1.11×10^3 nm/V).

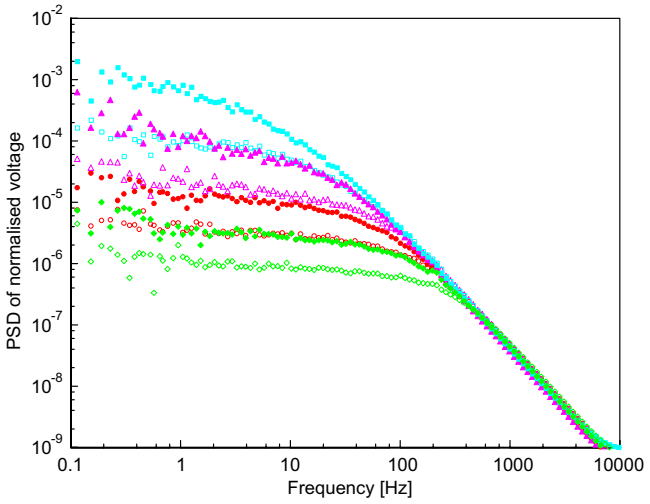

Fig. 3. Power spectra for single 1 μm beads (open symbols) and pairs of 1 μm beads (filled symbols) trapped for various laser powers. Notice overlapping pairs of power spectra for pair and single bead data at half the laser power. The laser power in the back focal plane of the objective were 5.4, 14, 23 and 37 mW, respectively, from top to bottom.

Repeating the experiment with 0.7 and 2 μm beads to generalise our findings gave similar results, although in both cases the variance of voltage fluctuations for two trapped beads increased by more than a factor of two. The power spectra of the normalised voltage fluctuations for two beads were once again Lorentzian in shape but the high frequency components did not directly coincide with those of the single bead data. However, if the data was scaled such that the variance for two beads is double the variance for a single bead data, one finds for both bead sizes comparable behaviour as the one observed for 1 μm beads (see Fig. 4a). That is, we find that independent of the bead radius the voltage fluctuations on the QPD for two trapped particles look the same as the ones recorded for a single bead at half the trap stiffness/laser power. The same result was obtained if, instead of scaling the variances, the power spectra were fitted by Eq. (2) to determine corner frequency and sensitivity assuming γ of a single sphere. Note that for the smallest beads the power spectra are not perfectly Lorentzian any more, but this only becomes obvious for weak traps (filled squares in Fig. 4a).

Our data for the 2 μm beads is very limited as trapping a pair of these beads proved to be more difficult than for the smaller spheres. Even though it was possible to trap pairs of 2 μm beads, the trap became unstable and trapping was only sustained for a limited period (typically on the order of minutes, depending on the trap strength).

Interestingly, the power spectrum of pairs of beads even stays Lorentzian if different size spheres are trapped together. As an example, Fig. 4b shows the PSD of a 2.0 and a 0.7 μm bead trapped together, where the larger bead was trapped first before capturing a 0.7 μm bead underneath in the same optical trap. Note that the sensor is

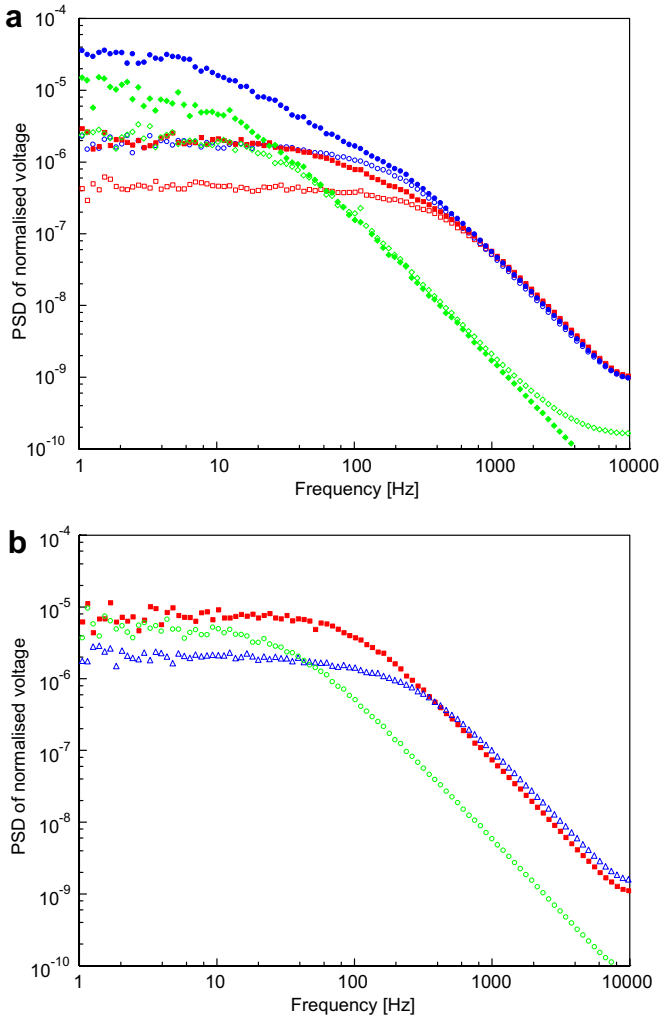

Fig. 4. (a) Scaled power spectra for trapping single (open symbols) and pairs (filled symbols) of same sized beads: 0.7 μm beads (squares for $P = 23$ mW and circles for $P = 37$ mW) and 2.0 μm beads (diamonds for $P = 37$ mW); (b) power spectrum of a 2.0 μm and a 0.7 μm trapped together (squares) compared to the PSD for a single 2.0 μm (circles) and 0.7 μm (triangles) bead at the same power.

about four times more sensitive to position fluctuations of the 0.7 μm beads than the 2.0 μm beads, which makes the PSD of the two spheres trapped simultaneously look more similar to the 0.7 μm single bead spectrum.

There is no immediately obvious relationship between the voltage fluctuations on the QPD and the position fluctuations if two beads are trapped simultaneously but the resemblance of the power spectra suggests that the QPD signal might provide an estimate of the mean displacement of the two beads relative to the equilibrium position.

To find further evidence for this suggestion we displaced the trapped 1 μm particles from their equilibrium position by performing drag experiments. The sample stage was translated at a constant speed v using a computer controlled stepper motor (Thorlabs, ZST25 & TST001, UK), applying a Stokes drag force of $F = 6\pi\eta r v = \gamma v$ in case of a single particle of radius r. For bead pairs the drag on each bead is actually reduced due to hydrodynamic interactions; for a centre-to-centre separation of 1–2 diameters the drag force is only between 70% and 80% of the Stokes drag [17]. The resulting displacement from equilibrium was recorded with both QPD and CCD camera.

For a single bead the displacement calculated from the QPD data corresponded very nicely with the displacements predicted from the measured trap stiffness (Fig. 5). The bead position was also traced directly from recorded videos using a particle tracking program written in house based on a pattern matching algorithm (LabView 7.1, National Instruments, Austin, Texas). For a single bead the camera data was consistent with the QPD data although the displacements at slow speeds were close to the resolution limit of the tracking method (≈10 nm).

For two beads in a trap we found much larger displacements from the equilibrium position, as one might expect from their static power spectra. The recorded videos furthermore revealed very clearly that the two beads were bound with different strengths (see Fig. 6). The lower particle (the one trapped second) was trapped more tightly, but both particles got displaced much further than a single trapped particle. The image of the two beads thus changed shape as the drag speed was increased which made automatic tracking of the mean particle position by pattern matching impossible. However, an estimate of the beads displacement was obtained by analysing intensity shifts along line profiles of the recorded images (Fig. 6c). The shift of the upper and lower edge can be determined independently, giving an estimate for the displacement of the top and bottom bead. Three mean displacements estimated from CCD images are included in Fig. 5 clearly showing the large displacement of the pair.

Interestingly, displacements calculated from the QPD readings, assuming the sensitivity determined by a fit of Eq. (2) to the static power spectrum of the pair of beads,

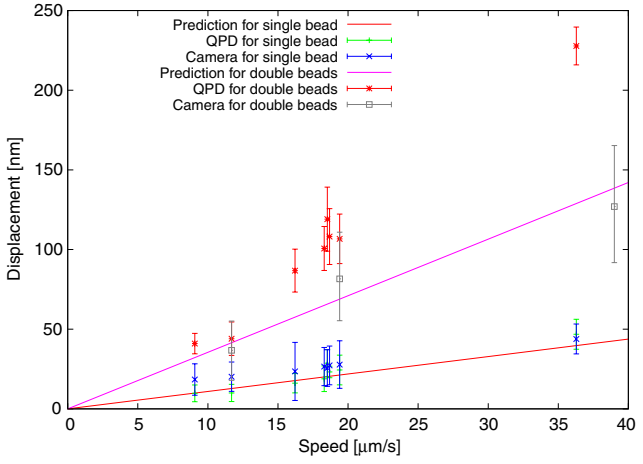

Fig. 5. Displacements from equilibrium as a function of drag speed for single 1 μm bead and a pair of beads in a weak optical trap with $k = 8.6 \times 10^{-3}$ pN/nm (slightly weaker than for open triangles shown in Fig. 3). Note that the predicted displacement for the double bead does not depend on the actual value of the drag coefficient γ.

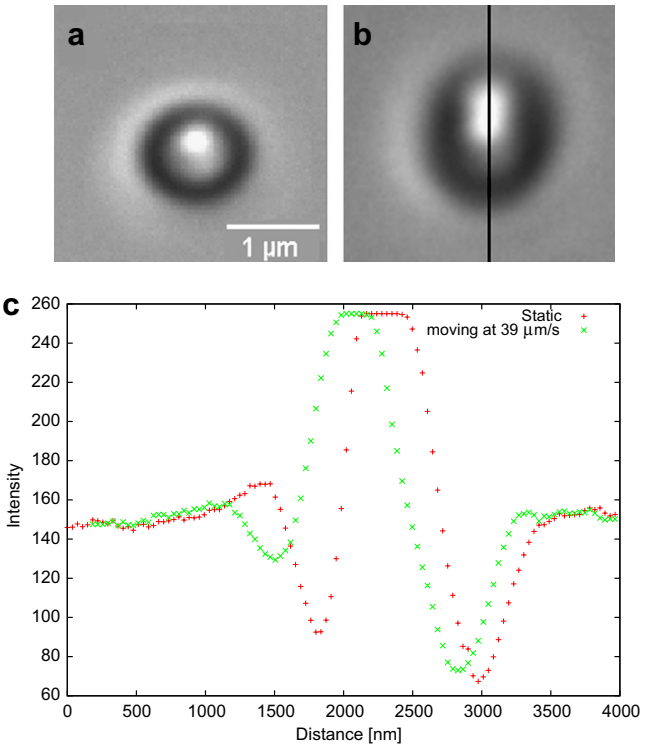

Fig. 6. Image of a trapped pair of beads: (a) in a stationary sample; (b) at the fastest recorded drag speed ($v = 39$ μm/s); (c) the displacement of the spheres was estimated by comparing averaged line profiles of 10 images, indicating the shift of the upper and lower edges.

agreed reasonably well with the displacements determined from the CCD images. Thus the QPD data did provide a reasonable estimate of the mean displacement even for a pair of particles. For small displacements, these experimental results also agreed with predictions based on the corner frequency obtained from the same fits (Fig. 5). For fast drag speeds the displacements deduced from the QPD data are larger than the predictions, but this is also observed for single beads due to non-linearity of the position detection [18]. Overall, the parameters extracted from a 'naive' fit of the QPD data were surprisingly consistent with the displacements observed in the drag measurements.

If the drag speed was increased beyond 40 μm/s, both beads were either lost together or in close succession (<1 s). Single trapped beads where only lost at a much higher drag force.

4. Discussion

Computer simulations by Gauthier and Ashman [19] of trapping a pair of beads simultaneously suggest that the two beads should experience different trap stiffness. These simulations assume the ray optics regime and consider large (5 μm) and heavy glass spheres, which means they can neglect Brownian motion. Therefore these results are not directly applicable to our experiments, but one would expect the stable equilibrium position (see Fig. 2 of Ref. [19]) to be qualitatively similar: both spheres are displaced from the equilibrium position of a single particle, with one bead experiencing a substantially weaker trap as the waist size at its z equilibrium position has about doubled.

There have been some previous experiments stacking multiple particles using a single beam, such as 'non-diffracting' Bessel beams [20] or interference patterns of fairly low divergence beams [21]. In our current experiment, a high numerical aperture microscope objective is used to tightly focus the laser beam to a diffraction limited spot of radius $w_0 \approx 0.4$ μm. If, for simplicity, the beam is assumed to be Gaussian its Rayleigh range is slightly less than $z_R = 0.7$ μm. So even for the smallest beads used in our experiments the second bead will be displaced from the optimum trapping position by more than a Rayleigh range. It will thus feel a much weaker confinement both in terms of trap stiffness and depth of the trapping potential. This is reflected in our experimental results, in particular the drag measurements, where the bead deeper inside the sample displaces further and escapes the trap more easily. When considering the bead size relative to the focal volume it is also not surprising that we found that 3D trapping of pairs of 2 μm beads was fairly unstable.

Measuring the intensity shift of the transmitted trapping laser light using a quadrant photo diode provides very precise high-bandwidth measurements for single trapped beads [9]. Here we found that when a second bead entered the trap the variance of the QPD voltage signals increased noticeably but their fluctuations still had a Gaussian distribution and even their power spectral density (PSD) retained a Lorentzian shape. However, the PSD did not just simply show a proportional increase for all different frequencies but the additional variance was mostly due to additional low frequency noise (Fig. 2).

The Gaussian distribution of the signal histogram is to be expected from the Central Limit Theorem but the Lorentzian shape of the PSD is quite surprising: As mentioned earlier, scattering of laser light by either of the particles modifies the optical forces experienced by the other and the presence of a solvent gives rise to hydrodynamic coupling of the particles motion. Both of these effects lead to correlations of the particles position fluctuations, and thus one might expect an effect on the QPD signal, leading to deviations from Lorentzian shape. However, as similar constellations of the two spheres can result in the same overall signal on the QPD, at least the short-time correlations due to hydrodynamic coupling do not show up in the overall power spectrum.

However, if the QPD signal is assumed to be insensitive to correlations in the beads movement, then the Lorentzian shape of the PSD has a simple explanation. Without correlations, the overall power spectrum is just a linear combination of the individual bead's power spectral densities. The individual beads PSDs are both Lorentzian, but with different corner frequencies due to the different trap stiffness k_1 and k_2, and the PSDs contribute with different weighting due to the change of sensitivity with distance from the focus [22]. However, linear combinations of two

Lorentzians with similar corner frequencies are almost of Lorentzian shape themselves. Deviations from the Lorentzian shape would only be appreciable close to the corner frequency and might not be noticeable within our experimental noise.

Note that for the smallest beads used in our experiments, the PSD does not look perfectly Lorentzian any more (Fig. 4a), suggesting that in this case, correlations in the two beads motion start to become relevant. The 0.7 μm beads scatter the laser light most effectively, thus modifying the light forces experienced by the other sphere more noticeably, which leads to more long-time correlation in the signal.

We found no evidence that optical binding effects play any role in our experiments, but they might have just been masked by the much more dominant effects of volume exclusion and limited trap volume.

5. Conclusions

We have shown that it is possible to trap multiple beads in three dimensions using a single beam optical trap in an inverted microscope setup. The trapped spheres are stacked on top of each other and thus can be easily mistaken for a single trapped sphere when using the typical coaxial observation geometry. Fluctuations recorded by a quadrant photo diode detector increase markedly, but both their histogram and power spectral density retain the same shape expected for trapping a single bead, i.e. Gaussian and Lorentzian, respectively. If we used the common procedure of fitting the power spectrum to extract both sensitivity and trap stiffness simultaneously, we found that within the range of bead sizes examined here (0.7–2.0 μm) the data could easily be mistaken for the signal of a single trapped bead.

This could easily lead to erroneous results when performing microrheology experiments in dense samples. As the visco-elastic response of the suspending medium is unknown, it will be less obvious if a second particle is accidentally trapped together with the tracer than in the simple experimental system investigated in this manuscript. This is particularly true if the second particle has a lower refractive index contrast and/or is much smaller than the tracer, as might happen within living cells [6] or bacterial suspensions [8]. Extra care must be taken to ensure that only the tracer is confined by the optical trap. It is not enough to rely on the 'right' shape of the measured power spectrum, but by monitoring the signal's variance carefully irregular results can be clearly identified.

Acknowledgements

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References

[1] A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm, S. Chu, Opt. Lett. 11 (1986) 288.
[2] J.E. Molloy, M.J. Padgett, Contemp. Phys. 43 (2002) 241.
[3] D.G. Grier, Nature 424 (2003) 810.
[4] K.M. Addas, C.F. Schmidt, J.X. Tang, Phys. Rev. E 70 (2004).
[5] L. Wilson, R. Besseling, J. Arlt, W.C.K. Poon, in: K. Dholakia, G.C. Spalding (Eds.), Optical Trapping and Optical Micromanipulation II, Proceedings of SPIE, vol. 5930, 2005, p. 593016.
[6] I.M. Tolic-Norrelykke, E.L. Munteanu, G. Thon, L. Oddershede, K. Berg-Sorensen, Phys. Rev. Lett. 93 (2004).
[7] G.V. Soni, B.M.J. Ali, Y. Hatwalne, G.V. Shivashankar, Biophys. J. 84 (2003) 2634.
[8] J. Arlt, W.J. Duncan, W.C.K. Poon, in: K. Dholakia, G.C. Spalding (Eds.), Optical Trapping and Optical Micromanipulation II, Proceedings of SPIE, vol. 5930, 2005, p. 59300L.
[9] F. Gittes, C.F. SchmidtMethods in Cell Biology, vol. 55, Academic Press, SanDiego, 1998, p. 129.
[10] A. Buosciolo, G. Pesce, A. Sasso, Opt. Commun. 230 (2004) 357.
[11] F. Gittes, C.F. Schmidt, Opt. Lett. 23 (1998) 7.
[12] M.M. Burns, J.M. Fournier, J.A. Golovchenko, Phys. Rev. Lett. 63 (1989) 1233.
[13] W. Singer, M. Frick, S. Bernet, M. Ritsch-Marte, J. Opt. Soc. Am. B 20 (2003) 1568.
[14] N. Metzger, K. Dholakia, E. Wright, Phys. Rev. Lett. 96 (2006) 068102.
[15] J.-C. Meiners, S.R. Quake, Phys. Rev. Lett. 82 (1999) 2211.
[16] D.L.J. Vossen, A. van der Horst, M. Dogterom, A. van Blaaderen, Rev. Sci. Instrum. 75 (2004) 2960.
[17] J. Happel, H. Brenner, Low Reynolds Number Hydrodynamics With Special Applications to Particulate Media, Prentice-Hall Inc., Englewood Cliffs, NJ, 1965.
[18] J.H.G. Huisstede, K.O. van der Werf, M.L. Bennink, V. Subramaniam, Opt. Exp. 13 (2005) 1113.
[19] R.C. Gauthier, M. Ashman, Appl. Opt. 37 (1998) 6421.
[20] J. Arlt, V. Garces-Chavez, W. Sibbett, K. Dholakia, Opt. Commun. 197 (2001) 239.
[21] M.P. MacDonald, L. Paterson, K. Volke-Sepulveda, J. Arlt, W. Sibbett, K. Dholakia, Science 296 (2002) 1101.
[22] A. Rohrbach, E.H.K. Stelzer, J. Appl. Phys. 91 (2002) 5474.