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Optical shield: measuring viscosity of turbid fluids using optical tweezers

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Abstract: The viscosity of a fluid can be measured by tracking the motion of a suspended micron-sized particle trapped by optical tweezers. However, when the particle density is high, additional particles entering the trap compromise the tracking procedure and degrade the accuracy of the measurement. In this work we introduce an additional Laguerre–Gaussian, i.e. annular, beam surrounding the trap, acting as an optical *shield* to exclude contaminating particles.

OCIS codes: (140.7010) Laser trapping; (230.6120) Spatial light modulators.

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1. Introduction

In a typical optical tweezers (OT) set up, the gradient forces created by tightly focused laser beams trap particles [1] in a colloidal solution. OT have been used in a host of experiments across many disciplines as varied as biology [2], micro fabrication [3], and colloidal science [4]. In each of these areas there is, to varying degrees, a requirement to control freely diffusing particles of the colloid. For example, Reference [3] spatially separated particles in a reservoir, from which they were transported to a substrate using OT, thus demonstrating a technique for micro fabrication. Another application of OT is microrheology [5], where it is possible to measure the viscoelasticity of the surrounding fluid by monitoring the Brownian motion of trapped particles [6, 7]. This technique is relevant to microfluidics [8], and biology, where changes in viscosity arise from biological processes [9].

In microrheology, it is necessary to limit the number of freely diffusing particles in the sample where the measurement is taking place; otherwise, these particles can diffuse into the trap, compromising the tracking procedure. Local heating of the sample can also create convection currents which draws additional particles to the optical trap [10]. Controlling the number of particles or contaminants is not always easy, especially when measurements are made in turbid samples.

In this work, we present a method of optically shielding the region of the sample where the measurement is taking place. Holographic OT [11] use spatial light modulators (SLM’s) to manipulate many trapped objects independently. In addition they can be used to create unusual beams, e.g. those with azimuthal phase structure. We use a Laguerre-Gaussian (LG) beam [12] to create an annular ring around the trap. With an azimuthal index of $l = 40$ and high numerical aperture (NA), the scattering forces are stronger than the gradient force, and particles are propelled in the beam direction, away from the trap. Previous work has been done using LG beams, arrays of Airy beams and inverted axicon beams to clear paths through turbid samples [13]. Here, we choose the simplicity of encoding the trap and an LG shield beam on the same SLM and find a considerable increase in the length of time we were able to successfully measure viscosity.

2. Measuring viscosity with optical tweezers

In a viscous fluid, the thermal fluctuations cause displacement of a trapped particle away from the centre of the potential well created by OT. The motion of the particle can be modelled as a thermally driven, over damped oscillator in a potential well given in one dimension by,

$$E(\vec{x}) = \frac{1}{2}\kappa\langle(\vec{x})^2\rangle, \tag{1}$$

where κ is the trap stiffness, $\vec{x}$ is the particle position from the center of the trap. The trap stiffness depends on factors such as laser power and the refractive index difference between

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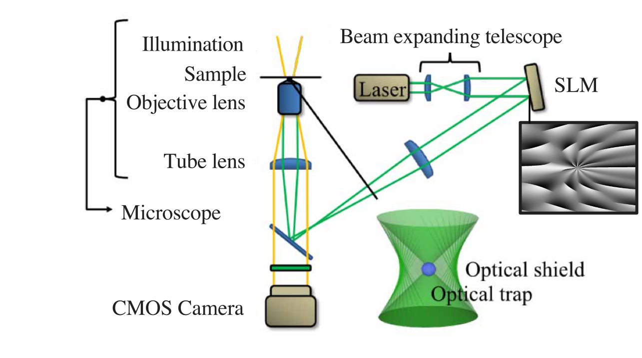

Fig. 1. Holographic optical tweezers. Laser beam is diffracted by the SLM which is imaged onto the back aperture of the microscope objective. A diffraction pattern (represented on the right) is displayed on the SLM to create a trapping beam surrounded by a LG beam (inset shows a not to scale illustration of this arrangement, with a particle placed in the trapping beam). The trapping plane is imaged onto a high speed camera.

fluid and particle. The restoring force exerted by the OT on the particle is expressed as $\vec{F} = -\nabla E(\vec{x}) = -\kappa\vec{x}$, which is linearly proportional to the bead position for displacements smaller than ~ 0.8 times the bead radius, a [14]. The trap stiffness, κ, can be calculated from the equipartition principle, i.e. $\frac{1}{2}k_B T = \frac{1}{2}\kappa\langle x^2\rangle$, where k_B is the Boltzmann constant, T is the temperature and $\langle x^2\rangle$ is the time–independent variance of the bead position from the trap centre. For a Newtonian fluid (i.e. a fluid with time–independent viscosity), the particle's motion can be described by means of a Langevin equation [5, 15], with the inertia term typically being neglected for frequencies up to MHz. At thermal equilibrium, the Langevin equation can be solved in terms of the normalised position autocorrelation function [16], giving,

$$A(\tau) \rightarrow \exp(-\Gamma\tau)\,, \tag{2}$$

where $\Gamma = \kappa/(6\pi\eta a)$ is the characteristic relaxation rate of the system. Once κ and a are known, this can be used to determine viscosity, $\eta = \kappa/(6\pi a\Gamma)$. Note that Eq. (1) and Eq. (2) are applicable for each Cartesian axis provided that the potential is quadratic. Other methods using optical tweezers to measure viscosity exist, such as rotating the particle [7, 17] and observing phase lag whilst periodic forcing [9]. However, as we are testing a new procedure using a Newtonian fluid, Eq. (2) is the most appropriate for our purposes.

3. Experiment

Our OT system (Fig. 1) is built around a Zeiss inverted microscope using a $100\times$, 1.3 NA, objective lens. The optical fields are created using a 532 nm laser (Laser Quantum Ltd., excel). The beam is expanded through a telescope, then incident on a SLM (Hamamatsu X8267). Using software written within the National Instruments LabVIEW environment [18], a phase modulation is calculated by summing the effect of a grating (for diffraction angle) and lens (for defocus). It is also possible to create LG beams simply by adding a phase modulation that is proportional to the azimuthal angle around the centre of the beam. The optical shield used here consists of such a beam with $l = 40$, which focuses to a ring around 4 μm in diameter, and Rayleigh range ~ 3 μm. The diffracted beams then pass through $4f$ imaging system, such that the SLM is imaged onto the back focal plane of the objective lens. The objective lens tightly

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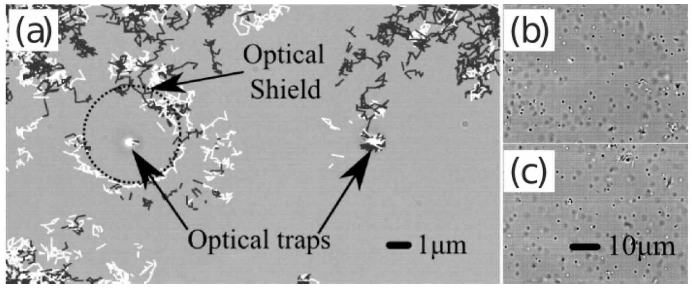

Fig. 2. (a) Tracked particle trajectories superimposed on an image of the experiment. White lines indicate particles in or just above the focal plane, which appear brighter in the image compared to the background. Dark lines indicate particles below the focal plane, which appear darker. The tracks appear and disappear as particles go in and out of focus. On the left is an optical trap surrounded by a optical shield, on the right is an unshielded trap. The tracks of the freely diffusing particles can be seen to enter the right hand, unshielded trap, whereas the optical shield around the left hand trap prevents such an event. (b) and (c) are images of the sample at the time of the measurements with and without a shield respectively.

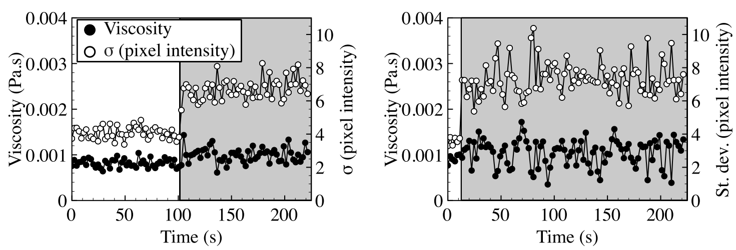

Fig. 3. Two examples of viscosity measurements (• points, on the left y-axis) against time, together with the standard deviation, σ, of pixel intensity (○ points, on the right y-axis). The left plot shows results taken with the shield on. Here, after around 100 seconds, the standard deviation of pixel intensity jumps to a higher value, indicating the arrival of another particle in the optical trap. The measurement of viscosity can also be seen to be altered, though not as considerably. The right plot shows results where no shield is used. The viscosity is expected to be 0.89 mPa.s.

focuses the beams creating optical fields inside the sample, Fig. 2. Our sample is a microscope slide with a concave recess, prepared with a solution of spherical silica particles ($a = 0.4\ \mu$m, Bangs Laboratories) in de-ionised, distilled water and sealed with a coverslip and secured with UV curing glue. These particles tend to sediment on the coverslip, however, we observed that thermal energy is sufficient for these particles to diffuse up to $\sim 20\ \mu$m above coverslip. The trapping plane is then imaged on to a high speed CMOS camera (Prosilica GC650). Using a centre of mass algorithm and reduced region of interest, we can track particle position at 1.34 kHz, sufficient to measure the autocorrelation time of the trapped particle [19].

The experiment is automated to take 250 measurements with a shield, then the system is reconfigured to take 250 measurements without a shield. Each measurement involves tracking a trapped particle for approximately 3 minutes 40 seconds, i.e. 3×10^5 data points taken at 1.34 kHz. In the configuration with a shield, ~ 50 mW of laser radiation is used with the optical

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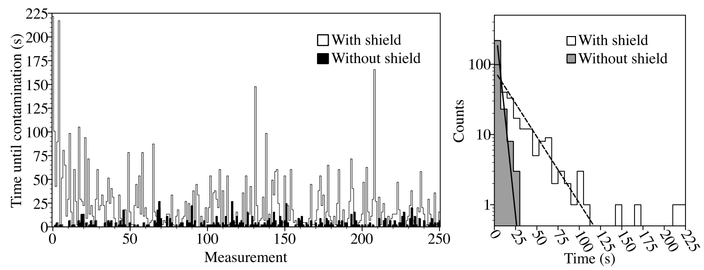

Fig. 4. Left: the length of time of successfully measuring viscosity in 500 attempts. 250 white bars indicate the use of an optical shield, whereas the 250 black bars indicated no shield was used. Right: A histogram showing the number of times the arrival of a particle in the optical trap occurred in time bins of length 7.5 seconds. The exponential fits are calculated from the mean time until contamination.

power of the shield weighted approximately twice that of the power in the trap. Without a shield, the laser power is reduced to give a similar trap strength.

In both configurations, an optical trap is created 5 μm above the coverslip in a sample of highly concentrated particles (Figs. 2(b) and 2(c)). The software then waits for a particle to diffuse into the trap. This event can be identified computationally by analysing images recorded by the camera. Background subtraction on these images means the average intensity of the brightest pixel in the image is near zero, with only camera noise contributing. The standard deviation of the brightest pixel value is then small. When a particle diffuses into the empty trap, this standard deviation increases (Fig. 3). Therefore, we use this criterion to identify the presence of a particle. If the standard deviation increases further (typically because two or more particles have diffused into the trap) the trap is switched off, and the software waits 30 seconds before restarting. If the particle meets the criterion, then, in the case using a shield, the shield is switched on. In the case without a shield, no change is made to the hologram. 3×10^5 position data and pixel intensities are saved to file for later analysis. The trap then switches off for 30 seconds and the experiment repeats, allowing many measurements to be taken with different particles. Figure 2(a) shows tracks of diffusing particles, two of which are trapped in optical traps. The left hand of these traps is surrounded by an optical shield which is seen to deflect material from the trap.

4. Results

In order to obtain time-resolved viscosity information, the 3×10^5 position data are divided into 100 blocks, each 2.2 seconds (Fig. 3). Assuming a temperature of 25°C, we calculate the trap stiffness to be around 1.3 μNm^{-1}. For each block, an autocorrelation is calculated, yielding the characteristic decay rate of around 200 s^{-1}. In our case, 2.2 seconds of data gives a measured fluctuation in the decay rate of 12% causing our calculation of viscosity to also fluctuate. The uncertainty in particle size (in our case 10%) gives a systematic error in the viscosity measurement. We found an average viscosity of (0.88 ± 0.10) mPa.s with shield, and (0.88 ± 0.24) mPa.s without shield, where standard deviation is larger in the case without a shield as more of the data is excluded due to the arrival of contaminating particles. These results are in good agreement with each other, and the expected value of 0.89 mPa.s. This result assures that the presence of the shield does not effect the measurement of viscosity.

We determine the arrival time of a contaminating particle as the time when the standard deviation of the maximum pixel intensity exceeds a predetermined level. Figure 4 (left) is a

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bar chart detailing the arrival time for every measurement. Figure 4 (right) is a histogram of these arrival times, showing an improvement in the length of time the trap stayed clear of other particles when the shield is used. The mean arrival times with and without a shield are 23 seconds and 3.8 seconds respectively. The effect of a contaminating particle typically increases the viscosity measurement by $\sim$ 25% and standard deviation by $\sim$ 90%.

If the particles are more difficult to trap than standard dielectric spheres, the scattering force provided by the optical shield is greater, and the shield should prove more effective. If the scattering force on a particle is less than the particle's weight, the particle will not be cleared from the region. This can be overcome with increased laser power or, if possible, reducing the size of particles used. Focusing the shield and trap 5 μm above the coverslip helped prevent particles entering the trap from below, and is far enough from the coverslip that hydrodynamic influences are small. Using 532 nm laser light helps reduce the effect of laser heating on the sample [10]. Reference [13] outlines some difficulties of using LG beams to clear paths through turbid media. The experimental requirements differ in this work as only a small volume around the trap is required to be kept clear, rather than a continuous path. We also have no requirement to clear the area inside the annulus, which is arranged to be clear at the start of the experiment.

In conclusion, we have shown that introducing an LG beam centred around an optical trap reduces the probability that freely diffusing material will enter that trap. We have devised a procedure for detecting such an event and find nearly an order of magnitude increase in arrival time of contaminates compared to standard procedures. We use the approach to measure viscosity in a turbid sample and obtain the expected value. With the simplicity of generating the LG beam with holographic OT, we foresee that this approach may assist measurements in samples which involve a plethora of diffusing material.

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