Optical Microrheology of Biopolymers

Simon Parkin, Gregor Knöner, Timo A. Nieminen, Norman R. Heckenberg and Halina
Rubinsztein-Dunlop

Centre for Biophotonics and Laser Science, School of Physical Sciences, The University of
Queensland, QLD 4072, Australia.

ABSTRACT

We use passive and active techniques to study microrheology of a biopolymer solution. The passive technique is video tracking of tracer particles in the biopolymer, a technique which is well established. The active technique is based on rotating optical tweezers, which is used to study viscosity. A method to actively measure viscoelascity using time varying rotation of a particle trapped in optical tweezers is also presented.

Keywords: Microrheology, Optical Tweezers, Particle Tracking

1. INTRODUCTION

The field of microrheology has existed since the 1950's. Small magnetic particles were manipulated using magnetic fields to probe the physical properties of cytoplasm in chick fibroplasts.[1] Optical methods for microrheology have since become more popular, most likely due to the convenience of integration with optical microscopes. Particle tracking is a technique that allows this integration, the simplest implementation being video tracking. Video tracking uses high speed cameras and motion detection software to track the motion of tracer particles embedded in the medium of interest. The technique's simplicity has led to its suggestion as a replacement for conventional rheology for high-throughput screening of polymer solutions.[2] Multiple particles can be tracked simultaneously to improve statistics, however the camera frame rate limits the measured frequency spectrum of the Brownian motion. Laser deflection particle tracking improves the frequency range by measuring the deflection of a laser beam by a tracer particle using a photodetector with a much faster response than a CCD camera.[3, 4] The hydrodynamic interaction between the tracer particle and the medium affects the motion of the particle in the medium. This effect can be removed by measuring the cross correlated Brownian motion of two particles.[5, 6]

Optical tweezers offer convenient microrheological techniques due to their ability to manipulate tracer particles which enables the study of localised rheological properties of the medium. Optical tweezers are an optical trap based on the gradient force generated by a tightly focused laser beam on transparent micron sized particles. Quantitative studies using optical tweezers were demonstrated in 1989 by measuring the force exerted by bacteria flagella.[7] Force measurements are made possible by calibrating the trap stiffness against the Stokes drag on the trapped particle. The motion of the particle for calibration can be applied externally by movement of the microscope's translation stage, or the thermal energy of the particle can provide the motion. So we see the similarity to a particle tracking microrheological technique. However, optical tweezers suffer from a complication due to the difficulty of distinguishing between the optical trap stiffness and the viscoelastic properties of the medium. One solution to this problem is to consider motion of a trapped particle that is independent of the trap stiffness, which is rotational motion.

Rotating optical tweezers apply torque to optically trapped particles. A light beam can carry angular momentum in its polarisation ('spin') or in its phase structure ('orbital'). While it is possible to transfer orbital angular momentum,[8] spin is the most convenient to apply[9] and measure.[10, 11] The most efficient transfer of spin angular momentum requires the use of birefringent objects, while simple rotational drag torque calculations require a spherical objects. Fortunately such a particle exists as vaterite, a calcium carbonate crystal, and has previously been used for viscosity measurements which were determined from the particle's rotational motion.[12]

In this paper we use a passive and an active microrheological technique to study the properties of a biopolymer solution (hyaluronic acid). One and two point video tracking techniques are used to passively study viscoelasticity

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and rotating optical tweezers are used to actively apply stresses to the fluid and measure viscosity. A technique to use oscillating rotation motion to apply a shear force to the fluid using rotating optical tweezers is also demonstrated.

2. THEORY

In order to determine the viscoelastic properties of a medium the mean square displacement of tracer particles embedded in the medium can be determined by tracking their motion. The mean square displacement is related to the diffusion coefficient(D) by

$$\langle \Delta \vec{x}^2(\tau) \rangle = 2dD\tau \tag{1}$$

where d is the number of dimensions and τ is the time. For spherical particles the Stokes–Einstein relation can be used to determine viscosity (η) from the diffusion coefficient:

$$D = \frac{k_B T}{6\pi \eta a} \tag{2}$$

where k_B is the Boltzmann constant, T is the temperature and a is the radius of the particle. However for viscoelastic media we use a generalised Stokes–Einstein relation to find a complex viscoelastic modulus[13]:

$$\tilde{G}(s) = \frac{k_B T}{\pi a s \langle \Delta \tilde{r}^2(s) \rangle} \tag{3}$$

where $k_B T$ is the thermal energy, a is the particle radius, s is the Laplace frequency. This equation is transformed to give the loss ($G'(\omega)$) and ($G''(\omega)$) storage moduli:

$$\tilde{G}(\omega) = G'(\omega) + iG''(\omega) \tag{4}$$

where ω is frequency. These two moduli characterise the viscous and elastic behaviours of the medium.

To determine the viscosity of a fluid using rotating optical tweezers we calculate the spin angular momentum transferred to a birefringent crystal. The trapping beam can be represented as a sum of two circularly polarised components and defining a 'degree of circular polarisation'

$$\sigma = \frac{P_L - P_R}{P_L + P_R} \tag{5}$$

where P_L and P_R are the powers associated with the left and right circularly polarised components respectively. A change in either of these two components as the beam passes through a birefringent particle results in a change in the total angular momentum flux of the beam and the transfer of angular momentum from the beam to the particle. The resulting reaction torque on the particle is given by[10]

$$\tau_R = \frac{\Delta \sigma P}{\omega} \tag{6}$$

where $\Delta \sigma$ is the change in the degree of circular polarisation as the beam passes through the particle, P is the laser power and ω is the optical angular frequency. The viscosity can then be determined, if the particle is spherical, by calculating the rotational viscous drag on a sphere and measuring the applied torque and rotational rate.[12] The viscous drag is given by[14]

$$\tau_D = 8\pi \eta a^3 \Omega \tag{7}$$

where η is the viscosity of the surrounding liquid, a is the radius of the sphere and Ω is its angular frequency.
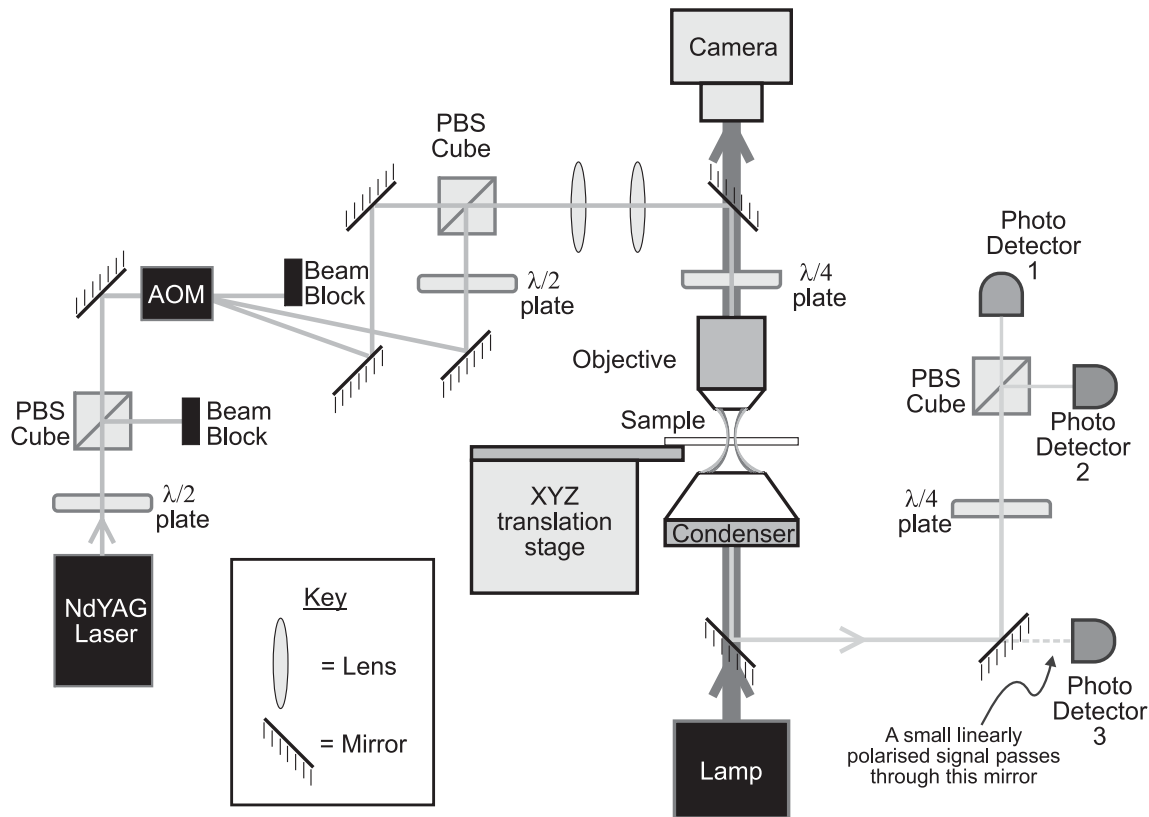
Figure 1. The rotating optical tweezers setup used for viscosity measurements. The light transmitted through the microscope is sent to a detection system which measures the torque applied to a probe particle and its rotation rate. The acousto-optic modulator (AOM) gives more flexibility to the system, allowing the direction and rate of rotation to be rapidly controlled.

3. EXPERIMENT

The setup used for particle tracking experiments consisted of a inverted microscope fitted with a high speed Phantom V5 camera, capable of capturing 512×512 pixel images at 4000 frames per second. The samples were placed in a sealed sample chamber containing approximately 20μL of fluid. This helped to avoid drift and currents during the experiments. The tracer particles used were 1.5μm in diameter and made from polystyrene. The volume fraction of tracer particles was 0.04%. A particle tracking algorithm was used to track the motion of at least 100 particles for 8000 video frames, and determine their mean square displacement. Two particle tracking was also used, whereby the cross-correlated motion of two particles is measured. Storage and loss moduli were calculated for the one and two particle motions.

The experimental setup used to make viscosity measurements using a rotating optically trapped particle consisted of a typical optical tweezers setup combined with a detection system to analyse the transmitted laser light (figure 1). Viscosity measurements were made without the acousto-optic modulator in the setup. Instead the single beam from the laser was used for trapping. The power from the NdYAG laser (Crystal Laser) was controlled with a half wave plate and a polarising beam splitting (PBS) cube. The maximum power available was 800 mW at a wavelength of 1064 nm. The beam is expanded before a Olympus 100X oil immersion objective (NA 1.3). The quarter wave plate before the objective was used to create either left or right handed circularly polarised light or linearly polarised light. A piezo driven XYZ translation stage held the sample and an oil immersion condenser (NA 1.4) collected the transmitted beam. The transmitted light is analysed by three photodetectors.

Photo detector 3 measures a very small fraction of the beam that passes through a mirror that acts as a linear polariser. The two photo detectors (photo detectors 1 and 2) behind the quarter wave plate and the PBS cube measured the two orthogonal circularly polarised components of the laser light transmitted from the optical tweezers. These two detectors were able to measure whether the polarisation was changed by the trapped and rotating particle. The degree of this change was used to determine the torque applied to the particle by the laser beam. The third photo detector was used to measure the rotation rate of the linearly polarised component of the transmitted laser light caused by the rotating particle. Hence this detector measures the particle's rotation rate.

A second micro-viscometer setup involves replacing the quarter wave plate in front of the objective with a half wave plate that can be rotated via a stepper motor, in order that a constant rotation rate of the plane of polarisation can be achieved. This allows for direct control of the rotation rate of the particle rather than controlling the applied torque, as is the case when using a quarter wave plate to generate circularly polarised light. These two configurations are similar to constant stress and constant strain rheometers used in conventional rheometry.

An acousto-optic modulator (AOM) was introduced into the setup to allow advanced control over the polarisation of the laser beam going into the objective. The AOM is an Intraaction DTD-274HAG Deflector with two crystals allowing deflection of the laser beam in two dimensions. For this application only one axis deflection was used. Two frequencies (25 MHz and 30 MHz) are used to drive the crystal which gives two beams, and the AOM is aligned so that there is equal power in each beam. The AOM can switch between each deflection angle or time share between the two angles so that effectively both beams are on at the same time. The power in each beam can also be individually controlled. The two beams are directed by different mirrors to different entrances of a PBS cube and are recombined. A half wave plate in one of the paths ensures the beam has the correct polarisation to leave from the same exit of the PBS cube as the other beam. This system allows for versatile control of the rotation of the trapped particle. The AOM can rapidly switch between the two paths which means a particles direction of rotation can be changed 'instantaneously'. If the two paths are time shared then the applied torque can be varied from zero to a maximum value (in either direction) while maintaining a constant trapping power. The AOM shifts the laser's frequency so that the beams in the two paths are no longer coherent. Instead of the beams interfering to give linear polarisation when the beams are of equal power, instead they apply torques to the trapped particle which are equal in magnitude and in the opposite direction. Therefore the resultant torque on the particle is zero. By varying the magnitude of each beam a well defined time varying torque can be applied to the particle.

4. RESULTS

The results for one and two particle video tracking in hyaluronic acid are shown in figure 2. The loss modulus clearly dominates in this plot across the entire frequency range strongly suggesting that the biopolymer behaves as a viscous fluid. The two particle loss modulus is on average 25% larger than the single particle result. This suggests that a depletion layer may form around the tracer particles in the hyaluronic acid solution, which would give rise to a locally reduced viscosity and thus a higher mean squared displacement. Consequently any microrheological technique based on a single probe particle must consider the coupling of the particle with the surrounding medium.

Viscosity measurements were made using the rotating optical tweezers without the AOM in the setup. Such a measurement for a methanol sample is shown in figure 3(a) and shows the viscosity varying with laser power. Varying the laser power varies the torque on the particle and the shear stress on the surrounding medium. As methanol is a Newtonian fluid we can be confident that the variation in viscosity is not caused by varying the shear in the methanol, but instead is due to laser induced heating of the fluid as a temperature increase is known to decrease the viscosity of methanol. The data is fitted to a temperature dependent curve which has been determined by the well characterised relationship between viscosity and temperature for methanol. The fit gives values for the room temperature viscosity and the temperature increase due to laser induced heating. Different sized particles can be used for this measurement and figure 3(b) shows the results for some different sized particles. A mean viscosity of 0.54 cP (centiPoise) was measured which agrees well with 0.56 cP, the accepted viscosity of methanol for room temperature.[15] Laser induced heating was typically 60 K per Watt of laser power.
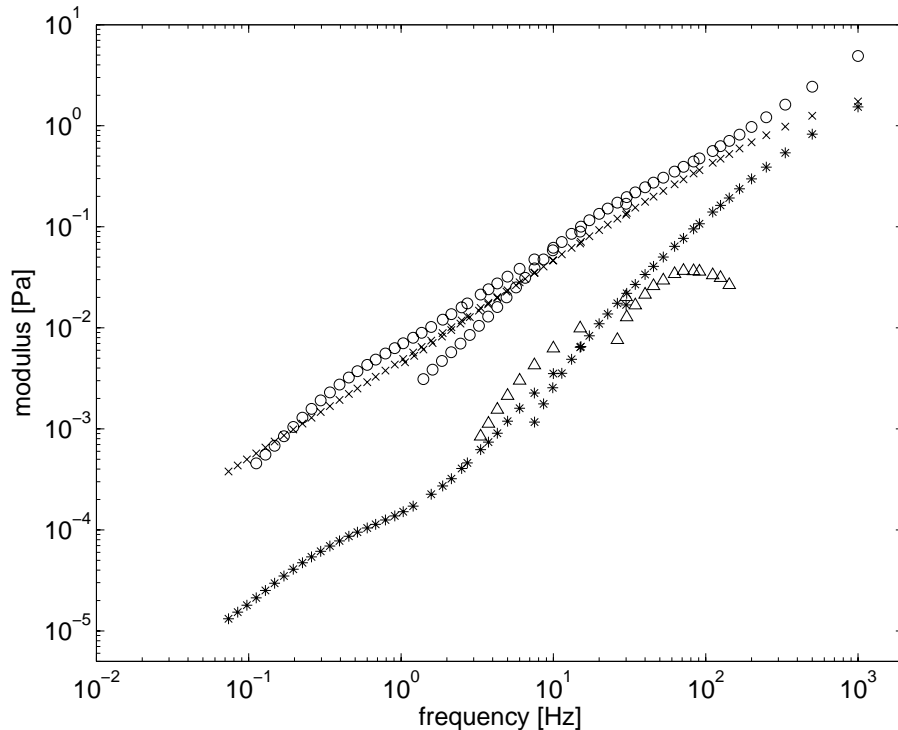
Figure 2. The plot shows the storage ($G'(\omega)$) and loss ($G''(\omega)$) moduli for one and two particle video tracking in hyaluronic acid. Across the whole frequency range the loss modulus ($\circ$, $\times$) dominates the storage modulus ($\triangle$, $*$), which means the biopolymer solution acts as a viscous fluid in this frequency range. The single particle result ($\times$) slightly underestimates the loss modulus compared to the two particle result ($\circ$). The single particle storage modulus ($*$) almost crosses the loss modulus at higher frequencies which suggests measurements at higher frequencies would be interesting for this fluid. The two particle result for the storage modulus ($\triangle$) does not provide a lot of information due to its small magnitude compared to the loss modulus and the smaller amount of statistics for the two particle technique.

The viscosity for hyaluronic acid was also measured with the same setup. Figure 4(a) shows this result. A temperature dependent curve has been fitted to this data. Hyaluronic acid has exhibited non-Newtonian effects, such as shear thinning, in macrorheology experiments.[16] However the accuracy of the fit suggests that shear thinning does not occur within the range of shear rates available for this micro-viscometer. Results for the controlled rotation rate micro-viscometer setup are shown in figure 4(b). In this experiment the rotation rate is fixed and the laser power is varied. Shear effects that might effect the viscosity's laser power dependence are removed in this setup. This result seems the confirm the idea that the shape of the experimental curves shown in figures 3 and 4 are due to laser induced heating and not due to varying shear rate.

5. CONCLUSION

In this paper we have used both active and passive techniques to study the biopolymer hyaluronic acid. The passive technique allows us to characterise the frequency response of the viscoelasticity of a fluid. Also by using one and two particle video tracking, we were able to investigate the coupling between the tracer particle and the fluid. The active technique, based on rotating optical tweezers, uses a single probe particle. Therefore the results from two particle tracking could assist in correcting for a depletion layer that seems to form around the probe particle.

The active microrheological technique allows the shear rate dependence of viscosity of the fluid to be measured. This is because actively applying shear to the fluid increases the range of shear rates available, and offers control over the shear rate. The development of the technique that utilises an AOM to switch polarisations of the
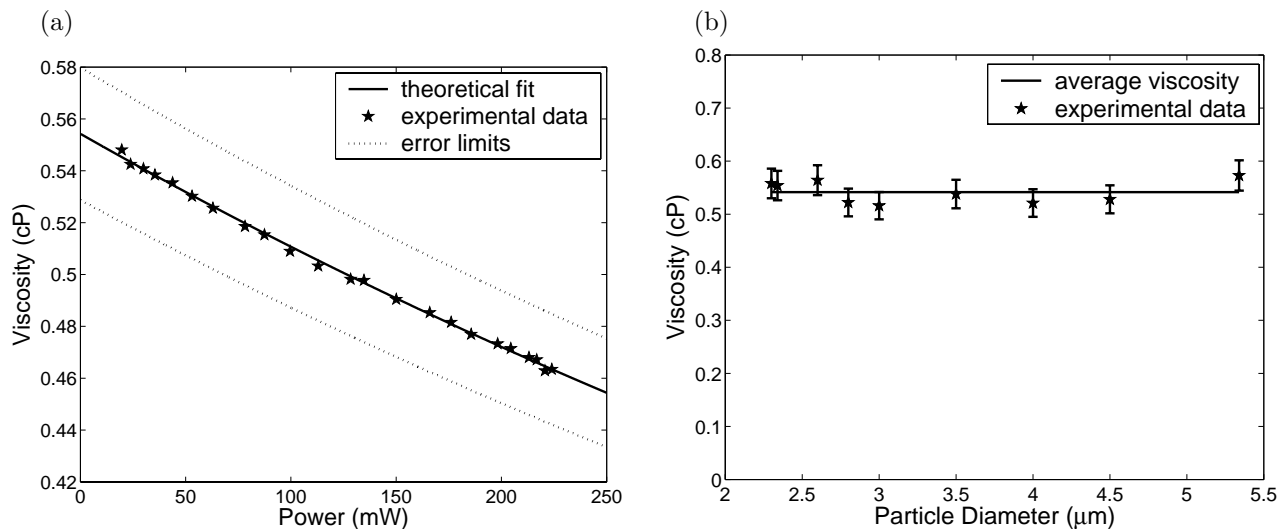
Figure 3. Plot (a) shows measured viscosities at different laser powers for a methanol sample. A temperature dependent curve is fitted to the data to determine the room temperature viscosity and the temperature rise due to laser heating. Plot (b) shows the variation in measured viscosity when particles of different diameters were used. The results suggest particles with diameters of 2–5 μm are suitable for measuring viscosity.
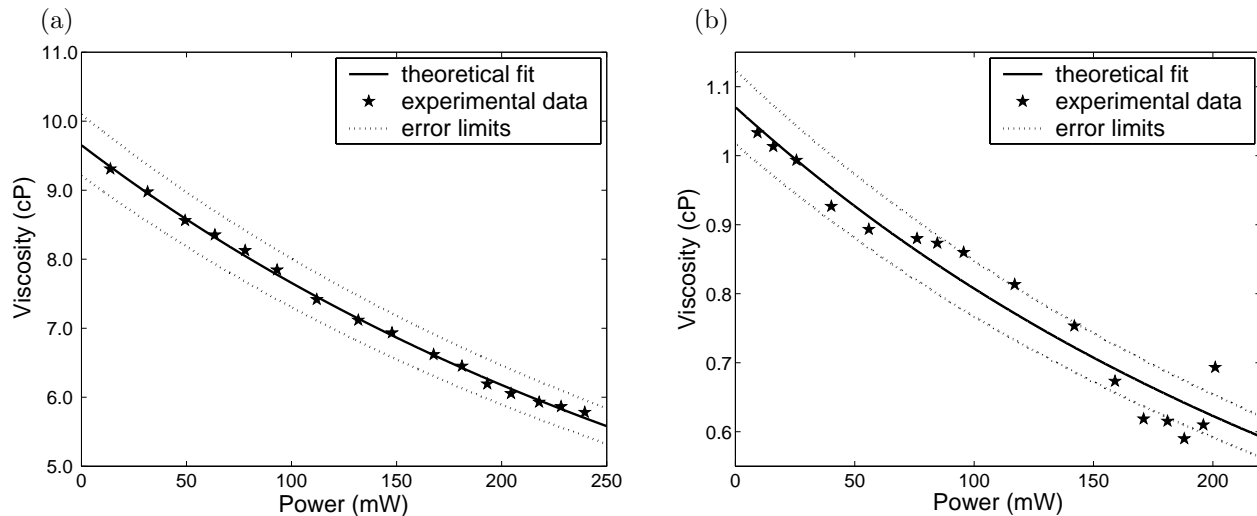

Figure 4. The plot (a) shows the viscosity's dependence on laser power for the non-Newtonian fluid hyaluronic acid (HA). A temperature dependent curve is fitted to the data, the accuracy of the fir suggests that no non-Newtonian behaviour can be observed in this polymer solution with the available shear rates of the micro-viscometer. Plot (b) shows the variation of waters viscosity with laser power only, not shear rate, as the rotation rate in this experiment is fixed for all the different laser powers. A temperature dependent curve is again fitted to the data.

trapping laser beam, will allow measurement of the shear rate dependence of the loss and storage moduli. This can be achieved by applying a time varying stress and measuring the in and out of phase response of the fluid. Combining the techniques described in this paper, more complete microrheological studies of biopolymers will be possible.

6. ACKNOWLEDGEMENTS

REFERENCES

1. F. H. C. Crick and A. F. W. Hughes, "The physical properties of cytoplasm," *Experimental Cell Research* **1**, pp. 37–80, 1950.
2. V. Breedvald and D. J. Pine, "Microrheology as a tool for high-throughput screening," *Journal of Material Science* **38**, pp. 4461–4470, 2003.
3. T. G. Mason, K. Ganesan, J. H. van Zanten, D. Wirtz, and S. C. Kuo, "Particle tracking microrheology of complex fluids," *Physical Review Letters* **79**, pp. 3282–3285, 1997.
4. F. Gittes, B. Schnurr, P. D. Olmsted, F. C. MacKintosh, and C. F. Schmidt, "Microscopic viscoelasicity: Shear moduli of soft materials determined from thermal fluctuations," *Physical Review Letters* **79**, pp. 3286–3289, 1997.
5. A. J. Levine and T. C. Lubensky, "One- and two- particle microrheology," *Physical Review Letters* **85**, p. 1774, 2000.
6. J. C. Crocker, M. T. Valentine, E. R. Weeks, T. Gisler, P. D. Kaplan, A. G. Yodh, and D. A. Weitz, "Two-point microrheology of inhomogeneous soft materials," *Physical Review Letters* **85**, pp. 888–891, 2000.
7. S. M. Block, D. F. Blair, and H. C. Berg, "Compliance of bacterial flagella measured with optical tweezers," *Nature* **338**, pp. 514–518, 1989.
8. H. He, M. E. J. Friese, N. R. Heckenberg, and H. Rubinsztein-Dunlop, "Direct observation of transfer of angular momentum to absorptive particles from a laser beam with a phase singularity," *Physical Review Letters* **75**, p. 826, 1995.
9. M. E. J. Friese, J. Enger, H. Rubinsztein-Dunlop, and N. R. Heckenberg, "Optical angular-momentum transfer to trapped absorbing particles," *Physical Review A* **54**, p. 1593, 1996.
10. T. A. Nieminen, N. R. Heckenberg, and H. Rubinsztein-Dunlop, "Optical measurement of microscopic torques," *Journal of Modern Optics* **48**, pp. 405–413, 2001.
11. A. I. Bishop, T. A. Nieminen, N. R. Heckenberg, and H. Rubinsztein-Dunlop, "Optical application and measurement of torque on microparticles of isotropic nonabsorbing material," *Physical Review A* **68**, p. 033802, 2003.
12. A. I. Bishop, T. A. Nieminen, N. R. Heckenberg, and H. Rubinsztein-Dunlop, "Optical microrheology using rotating laser-trapped particles," *Physical Review Letters* **92**, p. 198104, 2004.
13. T. G. Mason and D. A. Weitz, "Optical measurements of frequency dependent linear viscoelastic moduli of complex fluids," *Physical Review Letters* **74**, pp. 1250–1253, 1995.
14. L. D. Landau and E. M. Lifshitz, *Fluid Mechanics*, Pergamon Press, 1987.
15. D. R. Lide, ed., *CRC Handbook of Chemsitry and Physics*, Taylor & Francis CRC Press, New York, 85th ed., 2004.
16. E. Fouissac, M. Milas, and M. Rinaudo, "Shear-rate, concentration, molecular weight, and temperature viscosity dependences of hyaluronate, a wormlike polyelectrolyte," *Macromolecules* **26**, pp. 6945–6951, 1993.