

Picoliter viscometry using optically rotated particles

Simon J. 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,
 Brisbane, Queensland 4072, Australia*

(Received 26 April 2007; revised manuscript received 23 August 2007; published 26 October 2007)

Important aspects in the field of microrheology are studies of the viscosity of fluids within structures with micrometer dimensions and fluid samples where only microliter volumes are available. We have quantitatively investigated the performance and accuracy of a microviscometer based on rotating optical tweezers, which requires as little as one microliter of sample. We have characterized our microviscometer, including effects due to heating, and demonstrated its ability to perform measurements over a large dynamic range of viscosities (at least two orders of magnitude). We have also inserted a probe particle through the membrane of a cell and measured the viscosity of the intramembranous contents. Viscosity measurements of tears have also been made with our microviscometer, which demonstrate its potential use to study unstimulated eye fluid.

DOI: [10.1103/PhysRevE.76.041507](https://doi.org/10.1103/PhysRevE.76.041507)

PACS number(s): 83.85.-c

Recently, there has been increasing interest in microrheology, the study of flows and deformations of a material or medium using probes of microscopic size. In this paper, we will concentrate on microrheological methods that probe viscosity on micrometer length scales. Suitable existing techniques are magnetic tweezers [1,2], particle tracking [3], and optical tweezers based techniques [4,5]. Magnetic tweezers allow comparatively large forces to be applied to probe particles and thus the effects of high rates of shear can be studied. Particle tracking elegantly extracts the viscoelasticity of a medium over a large frequency range and allows fluid-probe coupling effects to be removed [6]. Optical tweezers allow the viscoelasticity of very localized regions to be probed, which enables the investigation of picoliter volumes and micrometer structures, such as the interior of cells. The region can be further localized by studying rotational motion of the trapped particle [5], which is also true for passive techniques [7–9]. These techniques have been used to study the viscoelasticity of cells [10,11] and also polymer solutions, where small volumes and high throughput are advantageous [12]. Another potential application is small-volume medical samples, such as eye fluid [13].

Rotating optical tweezers have been discussed in detail by Parkin *et al.* [14]. A spherical birefringent microparticle, combined with an optical measurement of the torque applied to it, can be used to probe fluid properties [5,15]. Using the light transmitted through the probe particle trapped in optical tweezers, the rotation rate of the probe particle and the change in the polarization of the light are measured. We use vaterite, which is a calcium carbonate crystal that forms spherical structures under certain growth conditions [5], as our probe particle. This particle has also been used to create and study microfluidic flows [16–18]. We present the characterization of our microviscometer, based on this rotating sphere, and the application of this device to measure intramembranous liquid and tear fluid. This technique allows a flow to be generated in a very localized region, of picoliter volume, and the viscosity of the fluid in this region can be measured. The use of rotational motion means that a smaller volume is probed compared to methods based on translational motion, due to the tighter confinement of the flow. This demonstrates the potential of this method as a high-

resolution active-probe method for microviscometry.

The optical torque applied to a birefringent sphere by the trapping laser is [5]

$$\tau_{\text{optical}} = \frac{\Delta\sigma P}{\omega}, \quad (1)$$

where $\Delta\sigma$ is the change in the degree of circular polarization as the beam passes through the particle, P is the laser power, and ω is the optical angular frequency. Viscosity is found by equating the applied torque and the viscous drag torque on a rotating sphere. The drag torque is complicated by the fact that experimentally we show that the viscosity varies with the trapping laser power, which is explained by heating of the fluid due to slight absorption of the trapping laser by the probe sphere. This leads to a nonuniform temperature distribution within the liquid. The steady state temperature of the fluid around a sphere that has a fixed uniform surface temperature as a function of the distance from the center of the sphere, r , is given by

$$T(r) = \frac{\gamma}{r} + T_0, \quad (2)$$

where γ is a constant and T_0 is room temperature. The viscosity of a fluid varies with temperature, which means there will be a nonuniform distribution of fluid viscosity around the sphere. For certain liquids, experimental data exist which can be used to determine viscosity from temperature [19]. Theoretical models for viscosity as a function of temperature tend to be inaccurate over significant temperature ranges, so we use interpolated data to determine viscosity as a function of temperature.

For steady state creeping flow, in an infinite viscous medium, driven by a rotating sphere, the fluid flow at any radius can be characterized by an angular velocity. As a torque must be applied to the rotating sphere to maintain the flow against viscous drag, there is a uniform outward flux of angular momentum equal to [20]

$$\tau = 8\pi\eta(r)r^6 \frac{d\Omega}{d(r^3)}, \quad (3)$$

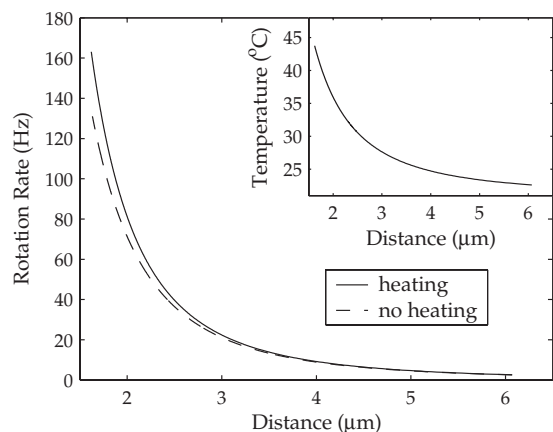


FIG. 1. Rotation rate of the fluid in the equatorial plane as a function of the distance from the center of the particle. The rotation rates correspond to a particular optical torque from an experiment with a vaterite particle, $3.2 \mu\text{m}$ in diameter. The solid line represents the case where the fluid has a temperature variation as shown in the inset. The temperature is constant throughout the fluid for the dashed line.

where η is the viscosity of the surrounding liquid and Ω is the angular frequency of rotation of the sphere. This is the case even when the viscosity is nonuniform as long as its distribution within the fluid is spherically symmetric. The rotation rate of the fluid is equal to the rotation rate of the particle, at the particle's surface, which we experimentally measure, and is given by

$$\Omega = \frac{\tau}{8\pi} \int_{r=\infty}^{r=a} \frac{1}{\eta(r)} d(1/r^3). \quad (4)$$

We do not have an analytical expression for $\eta(r)$; however, in this form the integral can easily be calculated numerically. The torque τ in this equation is equal to the optically applied torque, given by Eq. (1) and is measured experimentally. The surface temperature of the particle is unknown and is required to determine $\eta(r)$. The surface temperature, according to Eq. (2), depends on the parameter γ . However, from the empirical relation of viscosity as a function of temperature, which is derived from tabulated experimental data, and the relationship between temperature and distance r from Eq. (2), it is possible to rewrite Eq. (4) as

$$F(\gamma) = 0, \quad (5)$$

which can be numerically solved using the Newton-Raphson method to find γ . The rotation rate of the fluid shells in this model, as a function of distance from the center of the particle, is shown in Fig. 1. It can be seen that, although the heating effect is quite localized, there can be a significant effect on the rotation rate and hence the estimated viscosity.

The experimental setup used for this experiment is described in [5], and in more detail in [21]. To characterize the viscometer, the power dependence of the rotation rate and polarization were determined (Fig. 2). A linear fit, through the origin, of the rotation rate as a function of power is shown [Fig. 2(a)]. Contrary to expectation, the dependence is

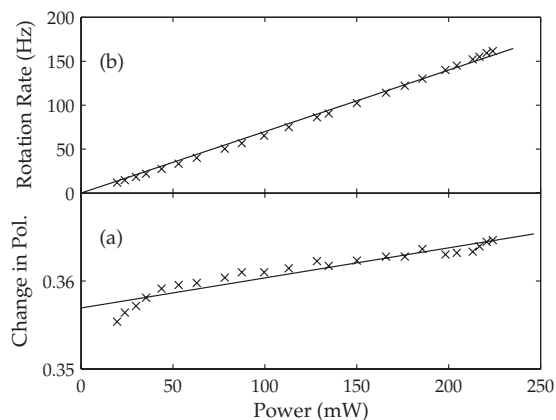


FIG. 2. Variation of rotation rate (a) and change in polarization (b) with trapping laser power. The data are for a vaterite particle with a diameter $3.2 \mu\text{m}$ in methanol. The errors in these plots are small enough that the trends are significant. The absolute value of the power is not known precisely, but the relative values of the powers in these plots are, and are limited only by the detector's precision. The rotation rate is precise, provided enough revolutions of the particle are recorded, which means the deviation from linearity is significant. The error in the polarization measurement is 0.005, yet the scatter is less than this, which means the linear decrease with power is a real effect.

not perfectly linear, which suggests that another parameter varies with the laser power. There is also unexpected behavior of the change in polarization as a function of power [Fig. 2(b)], which varies by several percent over the range of powers measured. In this case the dependence seems to be linear, and a linear fit of the data is shown. It is possible that this trend could be due to convection caused by heating of the fluid surrounding the sphere, or maybe to an increase in trap strength due to increasing the trapping laser power. However, the effect is minimal and would be difficult to confirm experimentally. Therefore we have not investigated the trend in this paper.

Typically, a viscometer measures viscosity by applying a known or controlled stress to the medium of interest, and then measures the resulting strain, which manifests as a shear rate in a liquid. In our microviscometer, the applied stress is represented by the optically applied torque to the particle and the shear rate is related to the rotation rate. Therefore the rotation rate as a function of optical torque is the relationship of interest, and is depicted in Fig. 3. The form of the fit is

$$\Omega(\tau) = \alpha\tau + \beta\tau^2, \quad (6)$$

where α and β are constants and τ is the optically applied torque. The nonlinear response could either be due to non-Newtonian behavior of the fluid or a temperature effect. In this case, as the fluid (methanol) is Newtonian, there must be some heating occurring as the trapping laser power is increased. Absorption of the laser light by the liquid itself turns out to be of insufficient magnitude to explain the decrease in viscosity [22]. However, if the particle is itself slightly absorbing, then the heating could be of sufficient magnitude to explain the observed behavior. If this is the case, then the

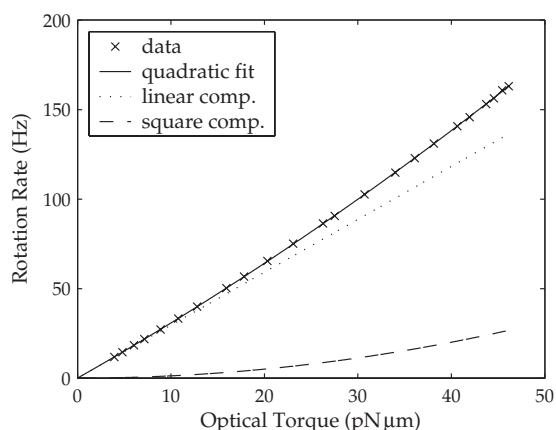


FIG. 3. Variation of rotation rate with the optically applied torque. The components of the quadratic fit are shown. The linear component is the expected rotation rate without heating or non-Newtonian effects. The square component represents the nonlinear effects on viscosity, which in this case are due to heating, which increases with laser power.

temperature of the fluid surrounding the particle is described by Eq. (2). Solving Eq. (5) for γ gave a value for the rotation rate that matched the experimentally observed rotation rate of the particle. The surface temperature of the vaterite particle as a function of optical torque was found and is plotted in Fig. 4. A linear fit of the surface temperature data yields a temperature increase of $66\text{ }^\circ\text{C/W}$ of laser power. Commonly used laser powers, in our experiments and other optical tweezers experiments, are of the order of 100 mW, which corresponds to only a $7\text{ }^\circ\text{C}$ temperature increase. The observed temperature increase corresponds to 0.08% of the laser power being absorbed. Now that the power dependence of the measurements is characterized and understood, accurate measurement of viscosity at room temperature is easily derived by making measurements at several power levels, which is easily done, and extrapolating to zero power. All the measurements discussed in this paper were made in this way.

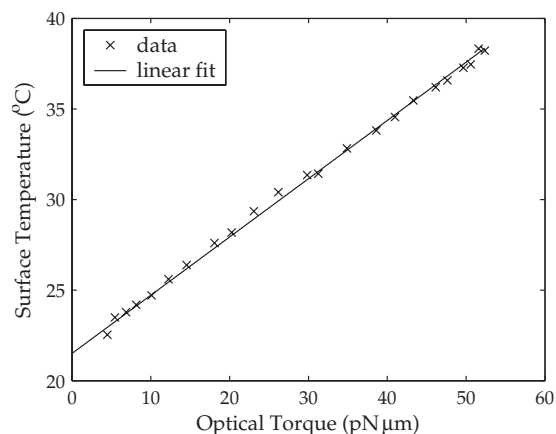


FIG. 4. Surface temperature of the vaterite particle in methanol due to heating from absorption of the trapping laser. This is the maximum temperature in the surrounding liquid; the temperature falls off as r^{-1} , which is depicted in Fig. 1.

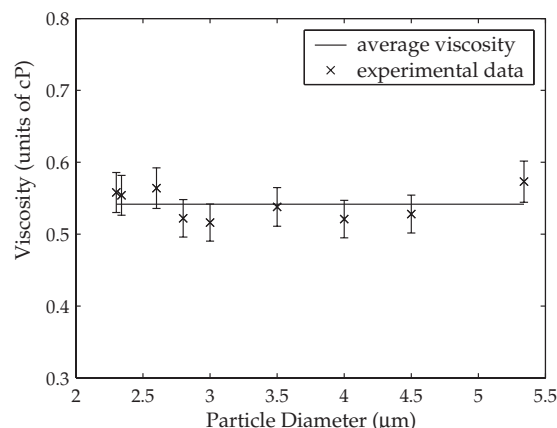


FIG. 5. Viscosity of methanol measured using vaterite particles with different diameters. This does not represent the limit of the size range of vaterite particles, as particles as small as $1\text{ }\mu\text{m}$ and as large as $10\text{ }\mu\text{m}$ have been observed. However, viscosity measurements were not carried out with these spheres as the measurement of diameter become less accurate for small spheres and the larger particles tend to be less spherical.

A good test of reproducibility is the microviscometer's performance over a range of particle sizes. The result of this test is plotted in Fig. 5. For probe particles 2–5 μm in diameter, the viscosity measurements are consistent with the expected independence of viscosity on particle size.

We also investigated the performance of our microviscometer over a range of viscosities. A series of solutions with different concentrations of glycerol were chosen because the relationship between viscosity and glycerol concentration has been well characterized [23]. In addition the glycerol polymers are short, which means the fluid exhibits Newtonian behavior. The viscosities of different concentrations of glycerol, as measured by our microviscometer, are shown in Fig. 6 compared to accepted values, and show good agreement. The dynamic range tested here was two orders of magnitude; however, that does not represent the limits of our technique. Measurements of both lower and higher viscosities are possible, with the upper limit being determined by any user-imposed time restrictions on acquiring an accurate rotation signal. It is important to note that the volume of the sample used for these measurements was 10–15 μl , approximately one drop of fluid. Dried vaterite particles were added to the sample using the tip of a brass wire while the sample was on the microscope slide. This *in situ* addition of vaterite is advantageous as it allows volumes as small as $1\text{ }\mu\text{l}$ to be handled.

Now that the microviscometer is fully characterized, it can be used for practical applications. An example of a medical sample where only microliter volumes are available is eye fluid. The viscosity of eye fluid has previously been measured to be 3–4 cP [13]. Without stimulating a tear response, only about 1–5 μl of eye fluid can be collected [13]. In a proof of principle experiment, we measured the viscosity of a similar volume of stimulated tears using our microviscometer. We found the viscosity to be $1.1 \pm 0.1\text{ cP}$. In our experiment the collection procedure was safe but crude, so we expected the measured tear fluid viscosity to be close to that

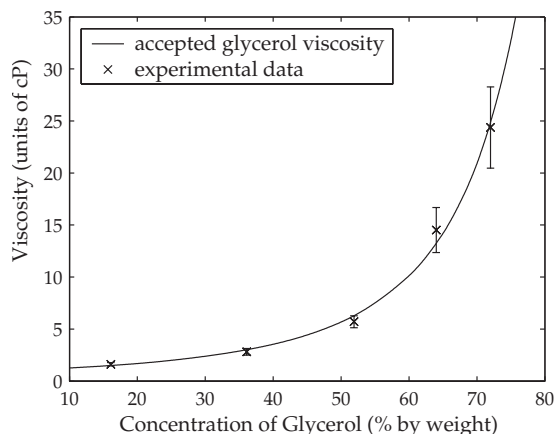


FIG. 6. Viscosity measurements for different concentrations of glycerol. Each data point is the average of five measurements using five different particles. The error bars represent the standard deviation, and the error in measurement of particle diameter is primarily responsible for the data spread. The relative error for each data point is about 10–15 %.

of water (0.97 cP at 21.5 °C). In the future, more quantitative studies could be carried out by employing controlled and reproducible eye fluid extraction procedures developed by ophthalmologists [24].

Probing very localized regions of fluid is an application that we have previously demonstrated by measuring the viscosity of a fluid inside a micelle [5]. In that experiment, the vaterite particles were added during the formation of micelles so that, on occasion, a vaterite particle was engulfed by a micelle. A more interesting case is the viscosity inside a cell. In a proof of principle experiment, we trapped and rotated a vaterite particle within a “bleb” on a macrophage cell (Fig. 7). The bleb, an extended region of the cell membrane, was formed by the cell in response to exposure to a focused femtosecond laser. The vaterite was then inserted into the cell by simultaneously cutting a hole in the cell membrane with a femtosecond laser and pushing the vaterite through the hole in the membrane using an optical trapping laser

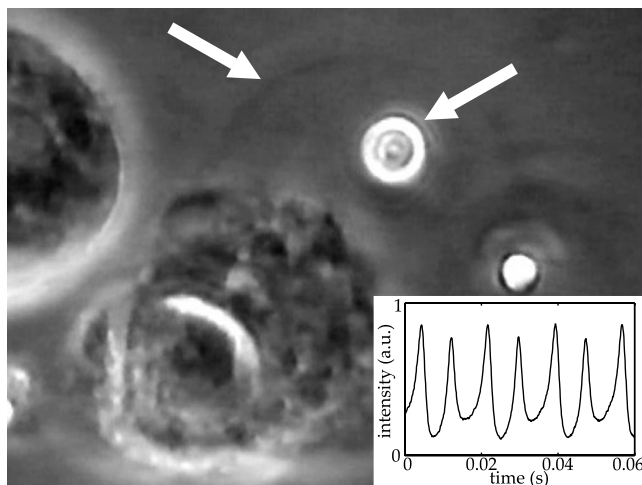


FIG. 7. A phase contrast image of a vaterite particle inside a cell. The left arrow points to the cell membrane, or the “bleb.” The right arrow points to the vaterite within the cell membrane. The inset shows the normalized intensity, in arbitrary units, measured by the photodetector used to determine the rotation rate of the vaterite particle.

beam. The viscosity was measured to be 3.3 ± 0.5 cP, which suggests that the fluid was drawn into the cell through the membrane during bleb formation, as the surrounding fluid has a viscosity close to that of water, while intercellular viscosity has been reported to be orders of magnitude higher [10,11].

We have demonstrated a microviscometer that measures viscosity by optically applying torque to a spherical probe particle and optically measuring both the torque and the particle’s rotation rate. The viscosities of glycerol solutions varying by two orders of magnitude have been measured, which demonstrates the lower limit of the dynamic range. Effects due to absorption of the trapping laser, which causes local heating, have been accounted for and quantified. Experiments within cells and with eye fluid have demonstrated the practical applications of this technique.

-
- [1] F. H. C. Crick and A. F. W. Hughes, *Exp. Cell Res.* **1**, 37 (1950).
- [2] A. R. Bausch, W. Moller, and E. Sackmann, *Biophys. J.* **76**, 573 (1999).
- [3] T. G. Mason, K. Ganesan, J. H. van Zanten, D. Wirtz, and S. C. Kuo, *Phys. Rev. Lett.* **79**, 3282 (1997).
- [4] L. Starrs and P. Bartlett, *Faraday Discuss.* **123**, 323 (2003).
- [5] A. I. Bishop, T. A. Nieminen, N. R. Heckenberg, and H. Rubinsztein-Dunlop, *Phys. Rev. Lett.* **92**, 198104 (2004).
- [6] J. C. Crocker, M. T. Valentine, E. R. Weeks, T. Gisler, P. D. Kaplan, A. G. Yodh, and D. A. Weitz, *Phys. Rev. Lett.* **85**, 888 (2000).
- [7] Z. Cheng and T. G. Mason, *Phys. Rev. Lett.* **90**, 018304 (2003).
- [8] M. Schmiedeberg and H. Stark, *Europhys. Lett.* **69**, 629 (2005).
- [9] E. Andablo-Reyes, P. Díaz-Leyva, and J. L. Arauz-Lara, *Phys. Rev. Lett.* **94**, 106001 (2005).
- [10] P. A. Valberg and D. F. Albertini, *J. Cell Biol.* **101**, 130 (1985).
- [11] B. R. Daniels, B. C. Masi, and D. Wirtz, *Biophys. J.* **90**, 4712 (2006).
- [12] V. Breedveld and D. J. Pine, *J. Mater. Sci.* **38**, 4461 (2003).
- [13] J. M. Tiffany, *Int. Ophthalmol.* **15**, 371 (1991).
- [14] S. Parkin, G. Knöner, W. Singer, T. A. Nieminen, N. R. Heckenberg, and H. Rubinsztein-Dunlop, in *Laser Manipulation of Cells and Tissues*, edited by M. W. Berns and K. O. Greulich, *Methods in Cell Biology* Vol. 82 (Academic Press, New York, 2007), Chap. 19, pp. 525–561.

- [15] T. A. Nieminen, N. R. Heckenberg, and H. Rubinsztein-Dunlop, *J. Mod. Opt.* **48**, 405 (2001).
- [16] G. Knöner, S. Parkin, N. R. Heckenberg, and H. Rubinsztein-Dunlop, *Phys. Rev. E* **72**, 031507 (2005).
- [17] J. Leach, H. Mushfique, R. di Leonardo, M. Padgett, and J. Cooper, *Lab Chip* **6**, 735 (2006).
- [18] R. Di Leonardo, J. Leach, H. Mushfique, J. M. Cooper, G. Ruocco, and M. J. Padgett, *Phys. Rev. Lett.* **96**, 134502 (2006).
- [19] H. A. Barnes, J. F. Hutton, and K. Walters, *An Introduction to Rheology* (Elsevier, Amsterdam, 1989).
- [20] L. D. Landau and E. M. Lifshitz, *Fluid Mechanics*, 2nd ed. (Butterworth-Heinemann, Oxford, 1987).
- [21] S. J. W. Parkin, G. Knöner, T. A. Nieminen, N. R. Heckenberg, and H. Rubinsztein-Dunlop, *Proc. SPIE* **5736**, 59 (2005).
- [22] E. J. Peterman, F. Gittes, and C. F. Schmidt, *Biophys. J.* **84**, 1308 (2003).
- [23] J. B. Segur and H. E. Oberstak, *Ind. Eng. Chem.* **43**, 2117 (1951).
- [24] J. C. Pandit, B. Nagyova, A. J. Bron, and J. M. Tiffany, *Exp. Eye Res.* **68**, 247 (1999).