

Microscopic flow measurements with optically trapped microprobes

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Received January 4, 2002

The use of optical tweezers to measure micrometer-resolution velocity fields in fluid flow is demonstrated as an extension of a scanning confocal viscosity microscope. This demonstration is achieved by detection of the motion of an optically trapped microsphere in an oscillating laser trap. The technique is validated by comparison with an independent video-based measurement and applied to obtain a two-dimensional map of the flow past a microscopic wedge. Since the velocity is measured simultaneously with the trap relaxation time, the technique requires no fluid-dependent calibration and is independent of the trap stiffness and the particle size. © 2002 Optical Society of America

OCIS codes: 180.5810, 180.1790, 170.3880, 020.7010.

Understanding and controlling the mechanics of fluids on the microscopic scale is important both from a basic research standpoint and for industrial applications. In recent years there has been a growing interest in a new branch of biotechnology—the field of microfluidics. The prospect of an integrated fluidic lab-on-a-chip that would eventually replace the manual handling of fluids in chemical and biological labs with an automated system that moves fluid volumes in the nanoliter range is very compelling and has the potential to revolutionize the biotech industry. Microfabricated devices such as microchannels, valves, and pumps will be used in basic biological research, from sample mixing to DNA sequencing and polymerase chain reaction (see, for example, Refs. 1 and 2 and references therein). As the technology of microfluidic systems matures, there will be a growing need for quantitative diagnostic techniques to measure the microscopic flow field and other fluid properties such as viscosity. The technique presented in this Letter answers some of these needs and demonstrates the potential for obtaining micrometer-resolution three-dimensional maps of fluid velocity with a simultaneous viscosity map.

In previous research, we developed a viscosity microprobe based on the motion of micrometer-sized particles in a viscous medium under the action of a rapidly oscillating optical trap.³ We showed that the viscosity of the fluid could be accurately measured by detection of the phase lag of the motion. In this Letter we extend this technique to measure local fluid flow.

A spherical micrometer-sized particle is trapped by a strongly focused beam from a near-infrared laser. An acousto-optic deflector is used to force the trapped particle periodically by movement of the laser tweezers back and forth across it at small amplitude (~ 100 nm) and frequencies in the kilohertz range. This forcing results in periodic motion of the particle with a phase lag that is due to hydrodynamic drag. Confocally detected backscattered light indicates the periodic motion of the particle relative to the laser trap. Since the particle lags behind the laser beam, the two coincide twice in each cycle, and as a result the confocal signal has a strong component at the second-harmonic

frequency. In the case of fluid flow, there is an additional drag force on the probe particle in the direction of the flow, which shifts the average position of the particle by a small amount from the center point of the oscillations. This offset is proportional to the fluid velocity and adds a component to the signal at the fundamental frequency. We measure the magnitude and phase of the fundamental and the second-harmonic components by use of two synchronous digital lock-in amplifiers (LIAs). The phase of the second-harmonic component is used to determine the relaxation time of the motion, τ , which is proportional to the fluid viscosity. The magnitudes of the fundamental and second-harmonic components, combined with the knowledge of τ , are used to determine the magnitude of the local velocity of the fluid in the axis of oscillations. The direction of the flow on this axis is determined by the phase of the fundamental component.

The one-dimensional equation of motion for a particle in a viscous Newtonian fluid in the presence of a periodically oscillating laser trap is

$$\gamma \frac{du}{dt} + \kappa u = -\gamma a \omega_0 \cos \omega_0 t + \gamma v, \quad (1)$$

where we assume that the focused laser beam forms a harmonic potential.⁴ The relative position between the particle and the laser trap is given by $u(t)$, $\gamma = 6\pi\eta r$ is the hydrodynamic drag coefficient given by the Stokes formula (for a sphere far from any surface⁵), r is the radius of the sphere, η is the dynamic viscosity, κ is the trap stiffness, which is proportional to the laser power, ω_0 is the spatial oscillation frequency, and v is the fluid velocity component in the axis of the oscillations. We neglect the inertial term (mass times acceleration) since it is small compared with the other forces—the motion of micrometer-sized particles in common fluids takes place at low Reynolds numbers.⁵

The solution of Eq. (1) is

$$u(t) = u_a(t) + v\tau, \quad (2)$$

where $u_a(t) = -u_0 \sin(\omega_0 t + \varphi_1)$ and $\varphi_1 = \cot^{-1} \omega_0 \tau$. $\tau = \gamma/\kappa$ is the relaxation time, and $u_0 = a\omega_0\tau/[1 + (\omega_0\tau)^2]^{1/2}$.

The effect of the flow is to add a small offset to the position of the bead relative to the trap center. Without specifying the actual form of the confocal detector response as a function of u , we assume that for sufficiently small u the confocal signal is quadratic in displacement:

$$I(t) \propto 1 - \alpha u(t)^2 \quad (3)$$

where α is an expansion parameter. Using Eq. (2), we find that

$$I(t) \propto 1 - \alpha(\nu\tau)^2 - \alpha u_a^2(t) - 2\alpha u_a(t)\nu\tau. \quad (4)$$

The first two terms add a dc component to the signal. The third term adds a dc component as well as a signal at the second-harmonic $2\omega_0$, with a phase given by $2\varphi_1$.

Measurement of this phase is the basis of the viscosity microscope.³ The last term adds a sinusoidal signal at the fundamental frequency, the magnitude of which is proportional to the fluid velocity. Denoting the second-harmonic rms magnitude as R_2 and the fundamental rms as R_1 , we see that the velocity is

$$\nu = \frac{R_1}{R_2} \frac{u_0}{4\tau}. \quad (5)$$

Assuming that we know the oscillation amplitude a in micrometers from a previous calibration, a simultaneous measurement of R_1 , R_2 , and φ_2 supplies all the information we need to obtain both the local velocity and viscosity. Since we use the ratio of the rms magnitudes, the conversion factor from intensity to signal voltage cancels out of the equation.

The optical tweezers setup and the experimental procedure for implementing the oscillating trap are identical to those described in detail previously.³ We use an inverted confocal microscope as the platform for the measurements and a Spectra-Physics 3900 Ti:sapphire laser for the laser tweezers. The innovation in this experiment is the use of two coupled digital LIAs, which are synchronously locked on the same frequency and receive the same analog signal from the confocal detector. LIA1 [Model SR830, Stanford Research Systems (SRS)] is used for the measurement of the fundamental magnitude and phase, and LIA2 (Model SR850 SRS) measures the second-harmonic magnitude and phase. LIA2 is also used to generate the driving voltage for the acousto-optic deflector and thus is internally locked on the reference signal at all times. The magnitude signal from LIA1 is fed to an auxiliary input on LIA2. The ratio of the two magnitudes is computed in real time by the digital signal processor of LIA2, and the results are sent to the computer by a serial port communication interface.

We measured nine different flow velocities, using a 1.9- μm -diameter silica microsphere (Bangs Laboratories) in water. We created the flow by use of the siphon effect in a flow cell placed on the microscope stage. The flow cell was made from a silicone rubber piece (molded from RTV 615, GE Silicones) the size of a microscope glass slide. The flow cell had a small rectangular indentation in the center (~ 1 cm long and 1 mm deep) for the fluid and two small holes for the inlet and outlet pipes. A glass coverslip sealed

the rubber and allowed high-magnification imaging. We adjusted the flow rate by varying the level of the water in the source and sink containers. The flow velocity was measured by the oscillating laser trap method and for comparison by video microscopy. The position of the optical tweezers was modulated by the acousto-optic deflector at 1000-Hz and 127-nm amplitude. The laser operating at 815 nm was kept at constant power (21 mW at the sample), which corresponds to a measured value of ~ 2 ms for τ . The duration of every flow measurement was 5 s, where we sampled (at 16 Hz) simultaneously the magnitudes and phases of the fundamental and the second-harmonic frequency components. The mean and the standard deviation of the velocity were calculated with Eq. (5). The time constant for both LIAs was 100 ms. To obtain an additional independent estimate of the flow, after every measurement with optical tweezers we took a short video clip of the microsphere moving in the fluid immediately after the laser beam was blocked and recorded the position of the microsphere in successive frames.

Figure 1 shows the velocity results from the two methods plotted against each other. Apart from a small intercept, which can be explained by noise induced by Brownian motion, one can see excellent agreement between the methods. Also shown is a fit to the data modeling the effects of noise in the velocity ν_{ex} measured by tweezers, $\nu_{\text{ex}} = [(A\nu)^2 + N^2]^{1/2}$, where A was found to be 1.05 ± 0.05 and N was found to be $3.3 \pm 1.5 \mu\text{m/s}$. The error bars for the video microscopy measurements were calculated from the uncertainty estimates for the time and position coordinates of the microsphere. The highest velocity point deviates from the fit because the trapping potential

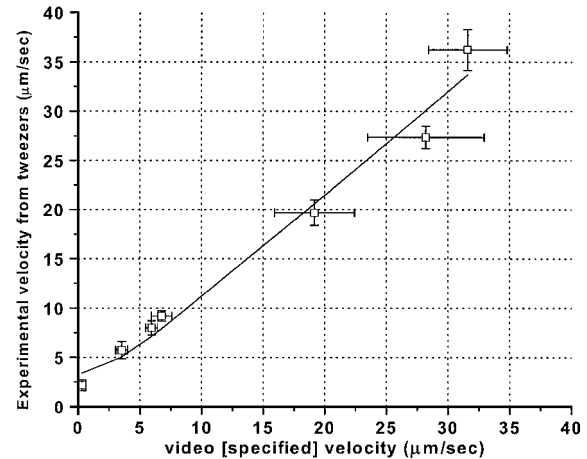


Fig. 1. Fluid velocity (in one dimension) measured by the oscillating laser tweezers versus velocity measured by tracking of the path of the bead by video microscopy once it is released from the trap. The solid curve is a fit to the data, including the effects of noise induced by Brownian motion. At velocities away from zero, where the effects of noise dominate, the proportionality constant is 1.05 ± 0.05 . Deviations from trap harmonicity when the bead is far away from the trap center explain the fact the highest-velocity point deviates from the fit. The tweezer oscillation amplitude was 127 nm, and the oscillation frequency was 1 kHz.

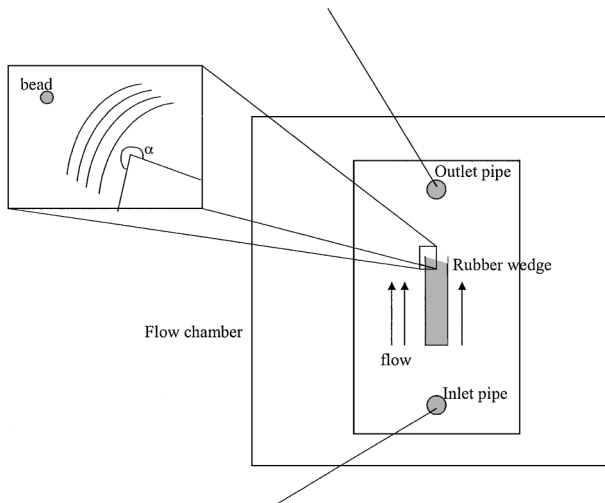


Fig. 2. Schematic diagram of the flow chamber in a top-down view. The length of the flow chamber is 1 cm, and the depth is 1 mm. The wedged rubber piece is placed roughly parallel to the general direction of the flow. The measurement field of view is also shown in the inset where the fluid curves past the edge of the rubber.

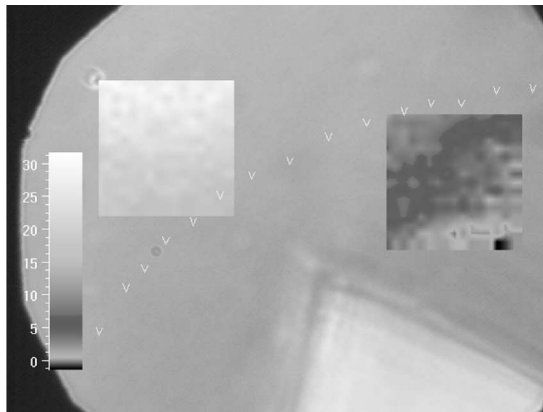


Fig. 3. Scalar velocity field v_y near a wedge. The gray scale in the inset boxes corresponds to the measured flow speed in the positive y direction (upward), and the values of the gray-scale bar are in micrometers per second. A trapped silica bead ($1.9\text{-}\mu\text{m}$ diameter) was moved by the galvanometer mirrors in steps of $1.06\text{ }\mu\text{m}$, and measurements of the flow in the y direction (v_y) were taken at each point to yield a matrix of 16×16 ($17\text{ }\mu\text{m} \times 17\text{ }\mu\text{m}$). The frequency of the trap oscillations was 1000 Hz, and the laser power was 30 mW at the sample. The LIA time constant was 100 ms. The V's show the trajectory of a particle moving freely in the flow and were obtained by recording of a series of images from the videotape and extraction of the coordinates of the particle at different times.

begins to deviate from harmonic when the bead displacement from the center of the trap is large. Our confocal scanning setup also allows us to obtain the spatial distribution of the flow. To demonstrate, we measured the two-dimensional distribution of the flow of water near the edge of a relatively long and narrow piece of rubber ($\sim 3\text{ mm} \times 0.5\text{ mm}$) that was placed approximately parallel to the flow created by the inlet and outlet pipes inside the flow chamber. A schematic of the flow chamber with the obstacle is shown in

Fig. 2. The flow cell is oriented in the y direction, with the general flow directed from the bottom to the top of the figure. For our measurements, we choose a region near the edge of the obstacle where we expect the flow to have a large spatial variation (Fig. 2, inset). The particle trajectories that we observed with the CCD camera were indeed found to curve around the wedge. In Fig. 3 we combine results from two velocity scans, one on either side of the wedge, with a conventional microscope image. One can see that, as expected, v_y varies considerably near the wedge.

The measurement gives absolute values for the velocity in the direction of oscillation without the need for any calibration other than a one-time calibration of α , the amplitude of the spatial oscillations of the laser trap. Once this amplitude is known, there is no need for further calibrations. The fact that just one calibration is needed, and that prior knowledge of the parameters of the probe bead is not necessary, makes our technique very practical and robust. In comparison, particle image velocimetry is a technique that uses small fluorescent particles ($\sim 300\text{-nm}$ diameter) to measure instantaneous and ensemble-averaged flow fields in micrometer-scale fluidic devices.⁶ In this technique, which employs an interline-transfer CCD camera, the motion of many seed particles is tracked with cross-correlation methods. This method has the advantage of being a wide-field technique, in contrast with our confocal scanning technique, which allows only one measurement at a given point in time and space. However, this method is computationally intensive and can provide only two-dimensional flow fields.

In conclusion, we have shown that our scanning confocal tweezers microscope can measure velocity and viscosity simultaneously and that absolute measurements in real time are obtained for the flow velocity with minimal calibration. We expect that the instrument will prove useful in the design and development of microfluidic devices.

We thank Nili Harnik for very useful discussions and comments on the manuscript. This research was partially supported by the National Science Foundation. M. Cronin-Golomb's e-mail address is mcroning@tufts.edu.

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