3D Mapping of Microfluidic Flow in Laboratory-on-a-Chip Structures Using Optical Tweezers

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3D Mapping of Microfluidic Flow in Laboratory-on-a-Chip Structures Using Optical Tweezers

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Accurate measurement of flow in microfluidic systems is both challenging and important, providing information that can be used to better understand flow fields within laboratory-on-a-chip devices and validate computational simulations. Here, we use optical tweezers within a microfluidic system to measure the velocity vectors of flow fields in two and three dimensions around a microstructures including both molded features within channels and cells. The experimental results are compared to a complex fluid dynamics model showing an agreement between the two of better than 3 μm/s. This measurement is highly reproducible and minimally invasive, which in the future could be used to provided more in-depth studies of the rheological properties of biological cells and microstructures in laboratory-on-a-chip devices.

It is already established that the miniaturization of the components associated with fluidic flow can lead to significant advantages when compared with conventional macroscale analytical systems. Faster reaction times, the local management of heat, and the reduction of sample and reagent volumes (often leading to reduced costs) are considered as important in deciding to miniaturize standard analytical techniques.[1,2] As a consequence, microfluidics has already shown significant potential for widespread applications in a number of fields including clinical diagnostics, analytical biotechnology, and microchemical synthesis.[3]

The flow of fluids in such microfluidic or laboratory-on-a-chip formats can be characterized by their Reynolds number, expressed as the ratio of the inertial and viscous forces. In most circumstances where there is fast flow in small channels, microflow is strongly influenced by viscous forces and consequently the fluid behaves very differently from that observed at the macroscale. Given the potential for the widespread application of laboratory-on-a-chip technologies, there is now a need to develop noninvasive methods that can be used both to experimentally show how fluids behave in such structures and to validate computational fluid dynamic, CFD, finite-element models used to numerically simulate flow.

Currently, there are a number of existing techniques that have been used to explore the complex internal geometries of microfluidic devices, including scalar image velocimetry, laser doppler velocimetry, and particle image velocimetry (PIV),[4–7] all of which have merits and limitations. For example, although combining PIV with confocal imaging may provide accurate three-dimensional (3D) maps in microfluidic channels there are practical issues associated with the effect that the many microspheres, used to track the flow fields, may have on the functioning of the device being studied.

Optical tweezers use tightly focused beams of light to trap micron-sized particles.[8–10] They have previously been used within microfluidics systems to manipulate microscopic objects, such as cells, or to create micropumps, valves, and sensors.[11–15] Previously, optical tweezers have been used to measure fluid flow,[16] relying on the fact that the displacement of the particle from the center of the optical trap is proportional to the Stokes drag force exerted by the moving fluid. Recently, we have demonstrated a multipoint technique for measuring flow, where the intensity of trapping beams generated with holographic optical tweezers is modulated so that the particle is repeatedly released into the fluid

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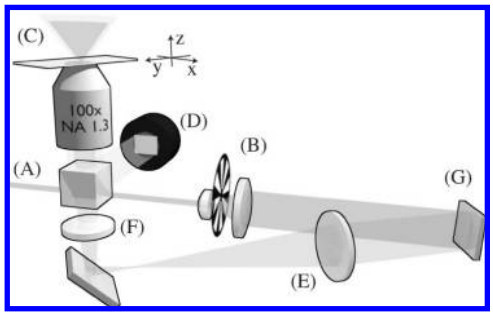

Figure 1. Experimental setup of the optical microflow sensor. The laser (A) is passed through a beam-chopper (B) such that the particle (C) is repeatedly trapped and released into the flow. The particle is imaged with video microscopy (D). The relay lenses (E and F) image the beam steering mirror (G) onto the back aperture of the objective lens.

flow then retrapped.[17] The technique has the advantage over PIV in that the test particles are captive and do not block the microchannels.

In this work we use optical trapping as a quantitative analytical technique to measure the velocity components of fluid flow parallel to the image plane, i.e., v_x and v_y, at any 3D position. We use this technique in microchannels containing complex microstructures and subsequently compare these measurements to those predicted from finite-element modeling of flow.

MATERIALS AND METHODS

Figure 1 shows the schematic of the tweezers-based, flow sensor system. The optical trap was generated from a frequency-doubled, continuous-wave laser (Laser Quantum, $\lambda = 532$nm). The beam was coupled via a dichroic mirror into an inverted microscope (Zeiss Axiovert 200) and focused using a 100× high numerical aperture objective (NA = 1.3, working distance 200 μm). The illumination light was focused into the sample with a standard 0.4 NA condensing lens, and the resulting images were recorded using an interfaced camera (QImaging Retiga EXi). The velocity field of a particular fluid flow was measured using video microscopy of trapped silica microspheres[18] (1.1 μm diameter, Bangs Laboratories). Initially, a probe particle was trapped at the beam focus, and it was then released into the surrounding fluid using a mechanical chopper to block the beam at the focus of one of the relay lenses. In our experiments the probe particles were moved to the desired location in the $x-y$ plane by adjusting a beam steering mirror and moving to different heights (z) using the microscope focus control.

As the particle is in a fluid with a velocity v, it experiences a Stokes force which is given by $F_{\text{Stokes}} = 6\pi\eta r v$, where η is the viscosity of the fluid and r is the radius of the particle. In a low Reynolds number system, this force rapidly (<1 ms) accelerates the particle so that its velocity matches that of the surrounding fluid and it moves downstream, and its new location, d_1, is measured by triggering the camera at a time t_1, after the chopper blocks the beam. The particle was then retrapped as the light next passed through the chopper. The chopper then blocked the beam, and the probe was once again released; the camera was then time-triggered at a time t_2, and the location, d_2, of the particle was measured.

The velocity of the fluid is simply given by $v = \Delta d/\Delta t = (d_2 - d_1)/(t_2 - t_1)$. The rate of the chopper is set such that on each release of the particle, the distance traveled is no more than one particle diameter, meaning that it was readily retrapped in the next chopper cycle. Note that neither the acceleration phase nor precise dynamics of the retrapping was important with repect to these measurements. For a 1.1 μm particle in a fluid flow of 50 μm/s, the rate of chopper was typically set to 50 Hz and $\Delta t = (t_2 - t_1)$ was set to 10 ms.

In addition to the downstream displacement of the particle due to Stokes force, the inherent Brownian fluctuations of the particle introduce random displacements into the measurement of position. In a given time interval Δt, one can predict the average distance a particle will travel due to Brownian motion. As the direction of this displacement is random, the time average of these fluctuations is zero and for n measurements, the standard deviation of the mean value can be estimated by[4,17]

$$\sigma(\Delta t) = \frac{1}{\sqrt{n}}\sqrt{\frac{k_B T}{\pi\eta r}\Delta t} \tag{1}$$

where k_B is Boltzmann's constant and T is temperature.

We therefore repeated the measurement outlined above many times at a rate of 50 Hz to reduce the random error in our velocity measurement. For a typical measurement on a 1.1 μm diameter particle with $n \approx 200$ and $\Delta t = 10$ ms the acquisition time for a single point is on the order of 4 s. This relates to a standard deviation in the position measurements of 0.01 μm and a corresponding error in the velocity measurement of ≈ 1 μm/s. It should be noted that this error depends only on the number of measurements and the time interval; the relative error in the velocity measurement therefore decreases as one measures faster flowing fluids. For example, one has to measure for 600 s to have a 10% error if the flow is on the order of 1 μm/s but only for 0.5 s to have the same confidence if the flow is 40 μm/s. The error can be further reduced by approximately 25% if a 2 μm diameter bead is used (due to the reduced displacements from Brownian motion). However, as probe particles will perturb the velocity profile of the fluid in accordance with their size, we choose to use the 1.1 μm particles for all subsequent measurements. Rather than increasing the size of the probe particle to reduce the error, a better approach is to increase the number of samples thereby averaging over more displacements.

The microfluidic structures were made using soft lithographic techniques involving photolithographic pattern transfer, mask deposition, and deep dry etch to create a smooth, high-aspect-ratio replica mold against which the elastomeric polymer, (polydimethylsiloxane), PDMS, Sylgard 184, was cast. Coverglass slides (150 μm thick) were coated with a positive photoresist, and a photolithographic mask was used to produce a cuboid microstructure (as a fluidic obstacle). The PDMS channels were aligned against the microstructured obstacles before the structure was sealed. The silica microspheres used as the probes were diluted in water before being introduced

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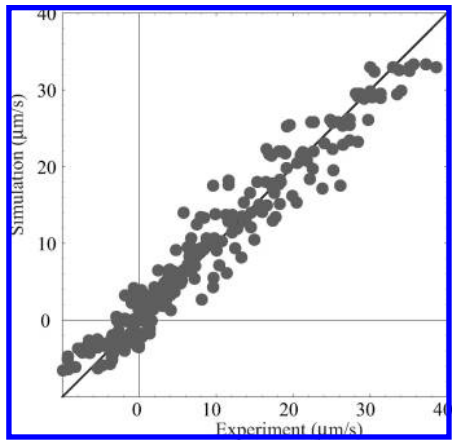

Figure 2. Comparison between modeled and experimentally measured fluid velocities. The test structure was a PMDS cuboid.

into the channel using a pressure-driven syringe pump (KD Scientific, U.S.A.) at rates up to 40 μm/s.

The cell culture media and serum were supplied by the Invitrogen Corporation. Chinese hamster ovary (CHO) cells were cultured in 25 cm^2 tissue culture flasks at 37 °C in a humidified atmosphere with 5% CO_2, 95% air using a DMEM/F12 medium supplemented with 10% fetal calf serum and 4 mM L-glutamine. The cells were suspended with 0.05% trypsin-EDTA solution, loaded into a microfluidic channel, and left undisturbed for 30 min to allow attachment to the channel surface. Subsequently, unattached cells were washed away at a flow rate of 1 μL/min. The cell was finally positioned close to the optical trap using the translation stage.

RESULTS AND DISCUSSION

We investigated the correlation between experimentally measured velocities and those predicted using a computational fluid dynamics (CFD) simulation (Comsol, Femlab). The test system comprised a PDMS cuboid structure within a much larger channel and a surrounding fluid flow, driven by a syringe pump. The finite-element model assumed no-slip boundary conditions applied along the channel floor, smooth channel walls, and constant fluid viscosity. The fluid flow velocities and physical dimensions of the microfluidic device were such that the Reynolds number was <0.001 and, hence, we always operated in a regime of laminar flow. Figure 2 compares the experimentally obtained velocities at various positions in the channel with those of the CFD simulation. The standard deviation between the measured and modeled values is <3 μm/s. It was calculated that the Brownian motion of the probe (eq 1) accounts for a disprepancy of only 1 μm/s, suggesting other factors were contributing to the overall measured error. These might include inaccuracies in the position of the comparison points or incorrect assumptions being made when establishing the boundary conditions within the simulation. For example, the error in the position measurement of the particle in the $x-y$ plane using video microscopy is on the order of ±10 nm whereas the error in the height of the particle above the coverslip is significantly higher and on the order of ±100 nm.

To map the compete flow around the test structure, a total of 20 different positions in 7 planes at different heights were measured. The distance between each measurement point in the $x-y$ plane was 10 μm, and the height between measurement planes

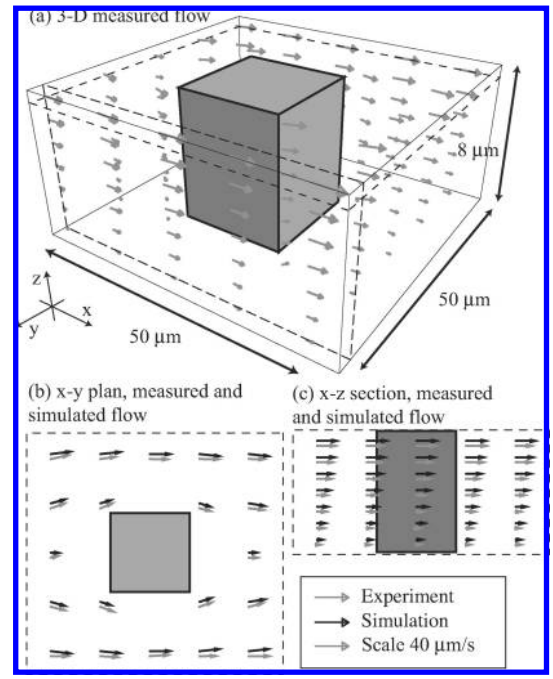

Figure 3. (a) Measured 3D flow map around a PDMS cuboid structure. The arrows show the experimentally measured and simulated flow map surrounding the block. The dotted lines indicate the cross sections of b and c. (b) $x-y$ plan. (c) $x-z$ section. Note that in a the z scale has been extended to separate the measurement points and that in b and c the experimental and simulated points have been separated for clarity.

was 1 μm. Figure 3a shows the experimentally measured velocity vectors around the PDMS cuboid. The arrows represent the flow vectors, and as expected illustrate a laminar flow profile due to the low Reynolds number. Cross-sections of the experimental and simulated flows are shown in parts b and c of Figure 3 in the $x-y$ plan and $x-z$ section, respectively.

Provided that the viscosity of the surrounding fluid is low enough such that trapping of a probe particle is possible, the vectors of fluid flow can be measured in any experimental system. This is particularly useful in situations where the flow is difficult to analytically solve or numerically simulate. Such flows may occur in chemical or biological systems where the slip length of a surface or visco-elastic properties of the fluid are not known. As an illustration of this, Figure 4 shows the measured fluid flow around a CHO cell fixed to the surface of a microfluidic channel.

One of the key benefits of the flow measurement technique is that it neither relies on the trap characteristics of the optical tweezer nor on the structure of the particle.[16] As all the displacements were measured while the optical trap was off, we were not subject to fluctuations in laser power or optical aberrations. Also, the technique is equally robust for asymmetric particles, provided that the center of mass of the probe can be located accurately. One area where this may be of concern is imaging close to structures that have large height profiles. Accurately determining the center of mass of the probe particle is made difficult when the nearby structure casts a significant shadow on the image.

The technique is also limited when the structure of interest influences the light cone of the laser such that aberrations are introduced and trapping is not possible. This will occur under a number of circumstances, for example, when measuring flow at

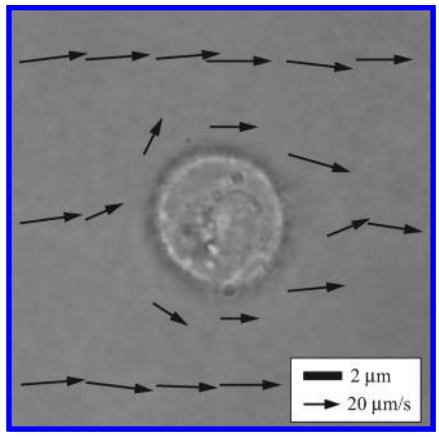

Figure 4. The measured 2D flow around a CHO cell, positioned within a PDMS molded microfluidic channel.

large depths next to structures with high aspect ratios, next to absorbing structures, or when attempting to measure flow over the top of samples. Ultimately, the range over which this technique can be applied is limited to the trapping range within the volume defined by the working distance and field of view of the microscope objective lens.[19]

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We also note that as the acquisition time for a single site (comprising the average of many individual measurements) is on the order of a few seconds, the total time to acquire multiple points in three dimensions can be several minutes. This has an obvious disadvantage when attempting to measure flow rates that change on a shorter time scale than this. In our experiment, we choose to measure steady flow rates; however, to combat this problem, multiple particles can be used in several positions[17] or the acquisition time for a single particle can be reduced at the expense of accuracy of measurement.

CONCLUSIONS

We have demonstrated a minimally invasive technique of measuring the velocity vectors v_x and v_y of flow fields in three dimensions. The technique can be applied to better understand flow fields in microfluidic structures. In the future, it will be possible to extend the technique to measure flow in the axial direction by measuring probe displacements normal to the imaging plane using an advanced particle tracking algorithm.

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