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PIV measurements of a microchannel flow

C. D. Meinhart, S. T. Wereley, J. G. Santiago

Abstract A particle image velocimetry (PIV) system has been developed to measure velocity fields with order 1-μm spatial resolution. The technique uses 200 nm diameter flow-tracing particles, a pulsed Nd:YAG laser, an inverted epi-fluorescent microscope, and a cooled interline-transfer CCD camera to record high-resolution particle-image fields. The spatial resolution of the PIV technique is limited primarily by the diffraction-limited resolution of the recording optics. The accuracy of the PIV system was demonstrated by measuring the known flow field in a 30 μm × 300 μm (nominal dimension) microchannel. The resulting velocity fields have a spatial resolution, defined by the size of the first window of the interrogation spot and out of plane resolution of 13.6 μm × 0.9 μm × 1.8 μm, in the streamwise, wall-normal, and out of plane directions, respectively. By overlapping the interrogation spots by 50% to satisfy the Nyquist sampling criterion, a velocity-vector spacing of 450 nm in the wall-normal direction is achieved. These measurements are accurate to within 2% full-scale resolution, and are the highest spatially resolved PIV measurements published to date.

1 Introduction

Recently, there has been a growing interest to develop microscale devices that can manipulate and transport relatively small volumes of fluids. These devices have applications in many areas of engineering, including propulsion and power generation of micro-satellites, micro air vehicles, inkjet printer heads, and bioanalytical instruments. The recent surge of microfluidic devices has created a need for diagnostic tools with spatial resolutions on the order of several microns. These diagnostic techniques can be used as engineering tools to measure the flow performance of microfluidic devices, and as general scientific instruments to probe the physics of transport processes at the microscale.

Particle image velocimetry (PIV) is a well-established technique for measuring velocity fields in macroscopic fluid systems (Adrian 1991). In double-pulsed PIV, positions of flow-tracing particles are recorded at two known times by either illuminating the particles using a pulsed light source, or by illuminating the particles using a continuous light source and gating the light near the camera using a mechanical or electronic shutter. The displacement of the particle images is then estimated statistically by correlating the particle image pairs (Meinhart et al. 1993).

The first successful micro-PIV experiment was conducted by Santiago et al. (1998). In that experiment, we used an epi-fluorescent microscope with a continuous Hg-arc lamp, and a Princeton Instruments' intensified CCD camera to record the flow around a nominally 30 μm diacylinder in a Hele–Shaw flow cell. A bulk velocity of 50 $\mu m\, s^{-1}$ was measured with a spatial resolution of 6.9 μm × 6.9 μm × 1.5 μm, based upon the size of the first correlation window and the depth-of-field of the microscope. The flow-tracing particles were chosen to be 300 nm diameter polystyrene particles. These particles were large enough to emit sufficient light for recording and to reduce the effects of Brownian motion. The Hg-arc lamp continuously illuminated the particles, and the light signal was gated electronically by the CCD intensifier plate. In this experiment, the CCD array was exposed for $\delta t = 2$ ms, and the time delay between exposures was $\Delta t = 68.5$ ms.

The experimental setup of Santiago et al. (1998) is well suited for situations where high spatial resolution and low-light levels are required, such as investigating flows around living microorganisms. The minimum exposure time of the CCD camera necessary to record particle images is on the order of several milliseconds, and the time delay between image exposures is on the order of tens of milliseconds. This large time interval between image exposures limits the PIV system to relatively low velocities.

In this paper we describe an alternative approach where a 5 ns pulsed Nd:YAG laser is used to illuminate sub-micron fluorescent particles with ~1 mJ of light energy. The fluorescent images are recorded using a cooled interline transfer 1300 × 1030 × 12 bit CCD camera that is capable of taking back-to-back images within a time interval as short as 500 ns. The CCD camera's pixel spacing is 6.8 μm.

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2
Flow-tracing particles

Developing PIV to achieve microscale spatial resolution requires that the particles are chosen small enough to follow the flow faithfully without (1) disrupting the flow field, (2) clogging the microdevice, and (3) producing unnecessarily large images. At the same time, the particles must be chosen large enough so that they scatter sufficient light to be recorded and sufficiently dampen out Brownian motion.

Making accurate PIV measurements with spatial resolutions on the order of several microns requires that the diameter of flow-tracing particles, d_p, be on the order of 100–300 nm diameter. If visible light with a wavelength of $\lambda = 532$ nm is used to illuminate the particles, the particle diameter will be smaller than the wavelength of light, i.e. $d_p < \lambda$. In this regime, it is difficult to image particles using normal elastic scattering techniques. However, inelastic scattering techniques, such as epi-fluorescence, can be used to image sub-micron particles. Fluorescently labeled polystyrene particles with diameters of 100–300 nm and a specific gravity of $\rho_p = 1.055$ are suitable for many microfluidic liquid-flow applications. The particles can be doped with a fluorescent dye that is tuned to absorb light from a frequency-doubled Nd:YAG laser ($\lambda = 532$ nm), with a peak excitation wavelength of $\lambda_{abs} = 540$ nm and a peak emission wavelength of $\lambda_{emit} = 570$ nm.

When sub-micron particles are used to trace slow flows, one must consider errors due to particle diffusion resulting from Brownian motion. A first order estimate of this error relative to the displacement in the x-direction is given by Santiago et al. (1998)

$$\varepsilon_B = \frac{\langle s^2 \rangle^{1/2}}{\Delta x} = \frac{1}{u}\sqrt{\frac{2D}{\Delta t}} \tag{1}$$

where s^2 is the random mean square particle displacement associated with Brownian motion, and D is the Brownian diffusion coefficient, u is the characteristic velocity, and Δt is the time interval between pulses.

Since the errors due to Brownian motion are unbiased, they can be substantially reduced by averaging over several particle images in a single interrogation spot or by ensemble averaging over several realizations. In practice, errors due to Brownian motion place a lower limit on the size of particle that can be used to achieve the desired velocity measurement accuracy. This size is dependent on the characteristic velocity of the experiment.

The required density of flow-tracing particles in micro PIV experiments is commonly on the same order as that for macro PIV experiments. Consider a macro PIV experiment where one uses a 50 μm diameter particle to seed a water flow, and an interrogation spot size of 1 mm × 1 mm × 1 mm. Assuming an average of 10 particles per interrogation volume are required for adequate measurement signal (using standard autocorrelation techniques), the ratio of particle volume to interrogation spot volume is ~0.07%. In the current experiment, there was an average of 2.5 particles per a 13.6 μm × 0.9 μm × 1.8 μm interrogation volume. Given particle diameters of 200 nm, the ratio of particle volume to interrogation spot volume is 0.05%. In order to obtain high quality signal with only 2.5 particles per interrogation spot, we employed a specialized ensemble-averaged correlation technique. The algorithm is used to average over 20 instantaneous correlation functions, before searching for the signal peak. This algorithm increases the number of effective particles per measurement volume, and provides adequate signal for reliable measurements. A complete description of the ensemble-averaged correlation technique is discussed by Meinhart et al. (1999).

3
Imaging and recording

All optical diagnostic techniques are ultimately limited by the diffraction of light. For micro PIV, the spatial resolution is limited by the effective diameter of particle images when projected back into the flow field. For magnifications much larger than unity, the diameter of the diffraction-limited point spread function, in the image plane, is given by

$$d_s = 2.44M\frac{\lambda}{2NA} \tag{2}$$

where M is the total magnification of the microscope and NA is the numerical aperture of the lens (Born and Wolf 1997). Using an oil-immersion *Nikon CFI Plan Apochromat* 60x objective lens with a numerical aperture of $NA = 1.4$, and assuming the wavelength of the recording light is $\lambda \approx 560$ nm, the diameter of the point spread function in our experiment is $d_s \approx 29.3$ μm.

The actual image recorded on the CCD camera is the convolution of the diffraction-limited image with the geometric image (Adrian 1991). Approximating both the geometric and diffraction-limited images as Gaussian functions, the resulting convolution is a Gaussian function with an effective particle diameter d_e, where

$$d_e = [M^2 d_p^2 + d_s^2]^{1/2} \tag{3}$$

For a magnification of $M = 60$, a particle diameter of $d_p = 200$ nm, and numerical aperture of $NA = 1.4$, the effective particle image diameter projected onto the CCD camera is $d_e = 31.7$ μm. The effective particle diameter when projected back into the flow is 528 nm. According to Prasad et al. (1993) if a particle image diameter is resolved by 3–4 pixels, the location of a particle-image correlation peak can be determined to within 1/10th the particle-image diameter. This yields a measurement uncertainty of $\delta x \approx d_e/10M$. For the parameters considered here, the uncertainty reduces to $\delta x \approx 528$ nm/10 = 52.8 nm.

The fact that one can measure particle displacement to within 53 nm is somewhat surprising. In most microscopic applications, one is primarily interested in determining the shape of small objects. Obviously, the smallest resolvable shape is on the order of the resolution of the microscope. In micro PIV, one knows *a priori* the particle shape and is interested only in determining particle position. By over-sampling the image (i.e. resolving the image with 3–4 pixels across the image diameter), one can determine particle position to within an order of magnitude better resolution than the diffraction-limited resolution of the microscope.

In most PIV applications, the thickness of a laser light sheet, Δz, determines the out-of-plane measurement domain. This thickness is usually chosen to be smaller than the depth-of-field of the recording system, δz. Consequently, all particles illuminated by the light sheet produce in-focus images, reducing background noise in the image field (Adrian 1991).

In micro PIV, one is often interested in obtaining velocity measurements with out-of-plane resolutions on the order of 1–10 μm. Forming a light sheet in a microchannel that is 1–10 μm thick is extremely difficult. Aligning the light sheet so that it overlaps with the in-focus object plane of the image-recording system is nearly impossible. In principle, a light sheet could be formed in a microfluidic device by micro-machining optical wave guides and light sheet forming optics directly into the device (Sobek et al. 1994). Because of the added difficulty of integrating micromachined optics into our system, we have chosen an alternative method that uses broad field illumination.

Figure 1 shows a schematic of the micro PIV system. The illumination beam is produced by two Nd:YAG lasers manufactured by *New Wave, Inc*. The beam is adjusted by a set of lenses and delivered into an inverted epi-fluorescent microscope, where an optical filter assembly directs the beam to the objective lens. The objective lens relays the light onto the microfluidic device, where it illuminates the entire flow volume. Fluorescent particles in the flow field absorb the green illumination light, $\lambda = 532$ nm, and emit a distribution of red light (approximately $\lambda \sim 560$ nm). The emitted light is imaged through an oil-immersion lens and passed to the fluorescent filter cube, where green light from background reflections is filtered out and the red fluorescence from the sub-micron particles is recorded onto a *Princeton Instruments* cooled interline-transfer CCD camera. The camera is cooled to $T = -15^{\circ}C$, which lowers the readout noise so the weak fluorescent signal can be measured. The duration of the laser pulse is 5 ns. Within 500 ns after exposure, the image field is transferred to storage pixels on the CCD camera, so that a second image field can be recorded by the CCD camera. After a specified time delay, Δt, a second laser pulse is used to record a second set of particle images onto the CCD camera. Both of these images are then read out of the CCD camera and downloaded to a PC computer for processing.

The depth of field of a microscope system is somewhat arbitrary, because there are several definitions of when an image is considered to be unfocused. Inoué and Spring (1997) estimate the total depth of field as the sum of the depth of field due to diffraction and geometric affects

$$\delta z = \frac{n\lambda}{NA^2} + \frac{ne}{MNA} \tag{4}$$

where n is the index of refraction of the immersion medium between the microfluidic device and the objective lens ($n = 1.515$ for an oil immersion lens), λ is the wavelength of light in a vacuum ($\lambda \sim 560$ nm for our fluorescent dye), NA is the numerical aperture of the objective lens, M is the total magnification of the system, and e is the smallest resolvable distance of the image detector.

The first term in Eq. (4) is due to diffraction. The length represented by this term is chosen by convention to be one fourth of the total distance between the first intensity minima along the optical axis of the 3-D point spread function (Inoué and Spring 1997). The NA^{-2} dependence of this term suggests that the depth of field for a high NA imaging system decreases more rapidly than the lateral resolution of the image, as NA increases. For the present experiment, Eq. (4) yields $\delta z \approx 0.6$ μm.

Equation (4) should not be directly applied to determine out of plane resolution for micro PIV systems. A better criterion for out of plane resolution is to determine the distance along the optical axis when a particle becomes sufficiently unfocused so that it only contributes a fraction, say 1/10th, to the correlation function, compared to a similar particle that is located at the object plane. In our experiment, we estimated the out of plane resolution by focusing the objective lens on a set of particles fixed to a microscope slide, and recording a series of images with the objective lens placed at different axial positions. The out of plane resolution was then estimated by determining the distance the object plane moved to produce sufficiently out of focus particle images, which could not significantly contribute to particle-image correlation. We estimated our out of plane resolution to be $\Delta z \approx 1.8 \pm 0.5$ μm.

The process to determine out of plane resolution should be conducted in a timely fashion to minimize drift in the microscope (Crenshaw 1998). Since the human eye can accommodate from about 25 cm to infinity, out of plane resolution and depth of field cannot be determined accurately from direct visual observation (Inoué and Spring 1997).

Lastly, out of focus particle images produce background noise in the image field, which can decrease the signal of the in-focus particles. In order to obtain reliable measurements, the background noise must be minimized by using high dynamic range CCD cameras, applying special image processing algorithms during interrogation, and limiting the number density of particles in the flow field.

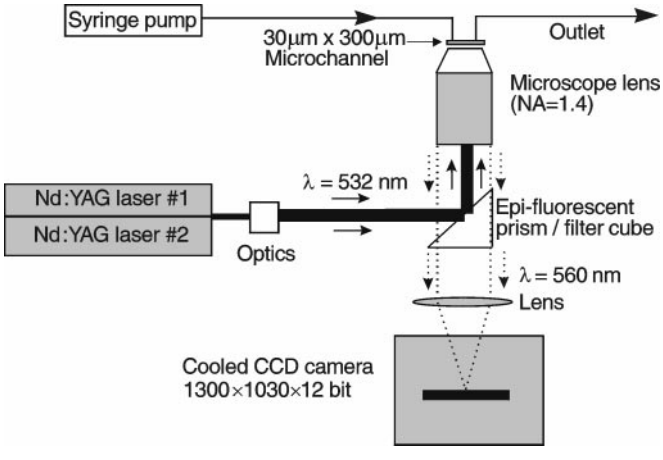

Fig. 1. Schematic of a micro PIV system. A pulsed Nd:YAG laser is used to illuminate 200 nm diameter fluorescent flow-tracing particles through an epi-fluorescent inverted microscope. A cooled 1300×1030 pixel $\times$ 12 bit interline-transfer CCD camera is used to record the particle images

4 Microchannel experiment

A 30 μm × 300 μm × 25 mm glass rectangular microchannel, fabricated by *Wilmad Industries*, was mounted to a 170 μm thick glass coverslip and a microscope slide (see Fig. 2). By carefully rotating the glass coverslip and the CCD camera, the channel was oriented to the optical plane of the microscope within 0.2°, in all three angles. The orientation was confirmed

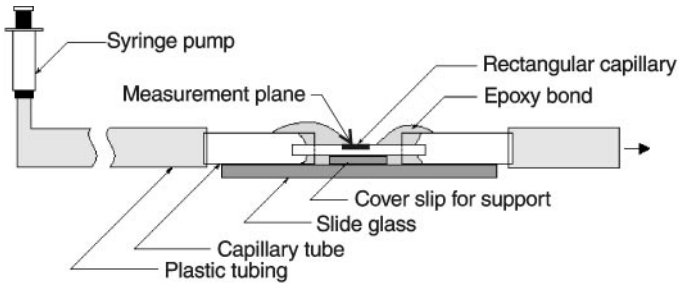

Fig. 2. Detail of the nominally 30 μm × 300 μm rectangular glass microchannel, which is glued to a circular capillary tube and a 170 μm glass coverslip for support. Plastic tubing connects the capillary tube to the syringe pump

optically by focusing the CCD camera on the microchannel walls. The microchannel was horizontally positioned using a high-precision *x*–*y* stage, and verified optically to within ∼400 nm using epi-fluorescent imaging and image enhancement.

The glass microchannel was imaged through an inverted epi-fluorescent microscope and a *Nikon Plan Apochromat* oil-immersion objective lens with a magnification $M = 60$ and a numerical aperture $NA = 1.4$. The object plane was placed at approximately 7.5 ± 1 μm from the bottom of the 30 μm thick microchannel. A Plan Apochromat lens was chosen for the experiment, because it is a high quality microscope objective designed with low curvature of field, low distortion, and corrected for spherical and chromatic aberrations (Inoué and Spring 1997).

Since deionized water was used as the working fluid, the effective numerical aperture of the objective lens was limited to $NA \approx 1.23$. A filtered continuous white light source was used to align the test section with the CCD camera and to test for proper particle concentration. During the experiment, the continuous light source was replaced by the pulsed Nd : YAG laser. A *Harvard Apparatus* syringe pump was used to produce a 200 $\mu l\,h^{-1}$ flow through the microchannel.

The particle-image fields were analyzed using a PIV interrogation program developed specifically for microfluidic applications. The program uses an ensemble-averaging correlation technique to estimate velocity vectors at each measurement point by (1) cross correlating particle-image fields from 20 instantaneous realizations, (2) ensemble averaging the cross correlation functions, and (3) determining the peak of the ensemble-averaged correlation function. This process is repeated for each velocity vector in the measurement domain. The signal-to-noise ratio is significantly increased by ensemble averaging the correlation function before peak detection, as opposed to either ensemble averaging the velocity vectors after peak detection, or ensemble averaging the particle-image field before correlation. The ensemble-averaging correlation technique is limited to steady or periodic flows. For the current experiment, twenty realizations were chosen because that was more than a sufficient number of realizations to give excellent signal, even with a first interrogation window of only 120 × 8 pixels.

The signal-to-noise ratio resulting from the ensemble-average correlation technique was high enough that there were no erroneous velocity measurements. Consequently, no vector validation was performed on the data after interrogation. The velocity field was smoothed using a 3 × 3 Gaussian kernel with a standard deviation of 1 grid spacing in both directions.

Figure 3 shows an ensemble-averaged velocity-vector field of the microchannel. The images were analyzed with low spatial resolution away from the wall, where the velocity gradient is low, and with a high spatial resolution near the wall, where the wall-normal velocity gradient is high. The interrogation spots were chosen to be longer in the streamwise direction than in the wall-normal direction. This allowed for a sufficient number of particle images to be captured in an interrogation spot, while providing the maximum possible spatial resolution in the wall-normal direction. The spatial resolution, defined by the size of the first interrogation window was 120 × 40 pixels in the region far from the wall, and 120 × 8 pixels near the wall. This corresponds to a spatial resolution of 13.6 μm × 4.4 μm and 13.6 μm × 0.9 μm, respectively. The interrogation spots were overlapped by 50% to satisfy the Nyquist sampling criterion. Consequently, the velocity-vector spacing in the wall-normal direction was 450 nm near the wall. The streamwise velocity profile was estimated by line-averaging the measured velocity data in the streamwise direction. Figure 4 compares the streamwise velocity profile estimated from the PIV measurements (shown as symbols) to the analytical solution for laminar Newtonian flow in a rectangular channel (shown as a solid line). The agreement is within 2% full-scale resolution. The bulk flow rate of the analytical curve was determined by matching the free-stream velocity data away from the wall. The wall position of the analytical curve was determined by extrapolating the velocity profile to zero near the wall.

Since the microchannel flow was fully developed, the wall-normal component of the velocity vectors should be close to zero. The average angle of inclination of the velocity field was found to be small, 0.0046 rad, suggesting that test section was slightly rotated relative to the CCD array. This was corrected mathematically by rotating the coordinate system of the velocity field by 0.0046 rad. The location of the wall was determined by applying the no-slip boundary condition and extrapolating the velocity profile to zero.

Most PIV experiments have difficulty measuring velocity vectors very close to the wall. In many situations, hydrodynamic interactions between the particles and the wall prevent the particles from traveling close to the wall, or background reflections from the wall overshadow particle images. By using 200 nm diameter particles and epi-fluorescence to remove background reflections, we have been able to make accurate velocity measurements to within about 450 nm of the wall (see Fig. 4). The position of the wall can be determined to within 400 nm by direct observation of the image.

5 Conclusions

A micro-PIV technique has been developed and demonstrated that can provide measurements of velocity fields with spatial resolutions approaching 0.9 μm. Using 50% overlap of the interrogation spot to satisfy the Nyquist sampling criterion,

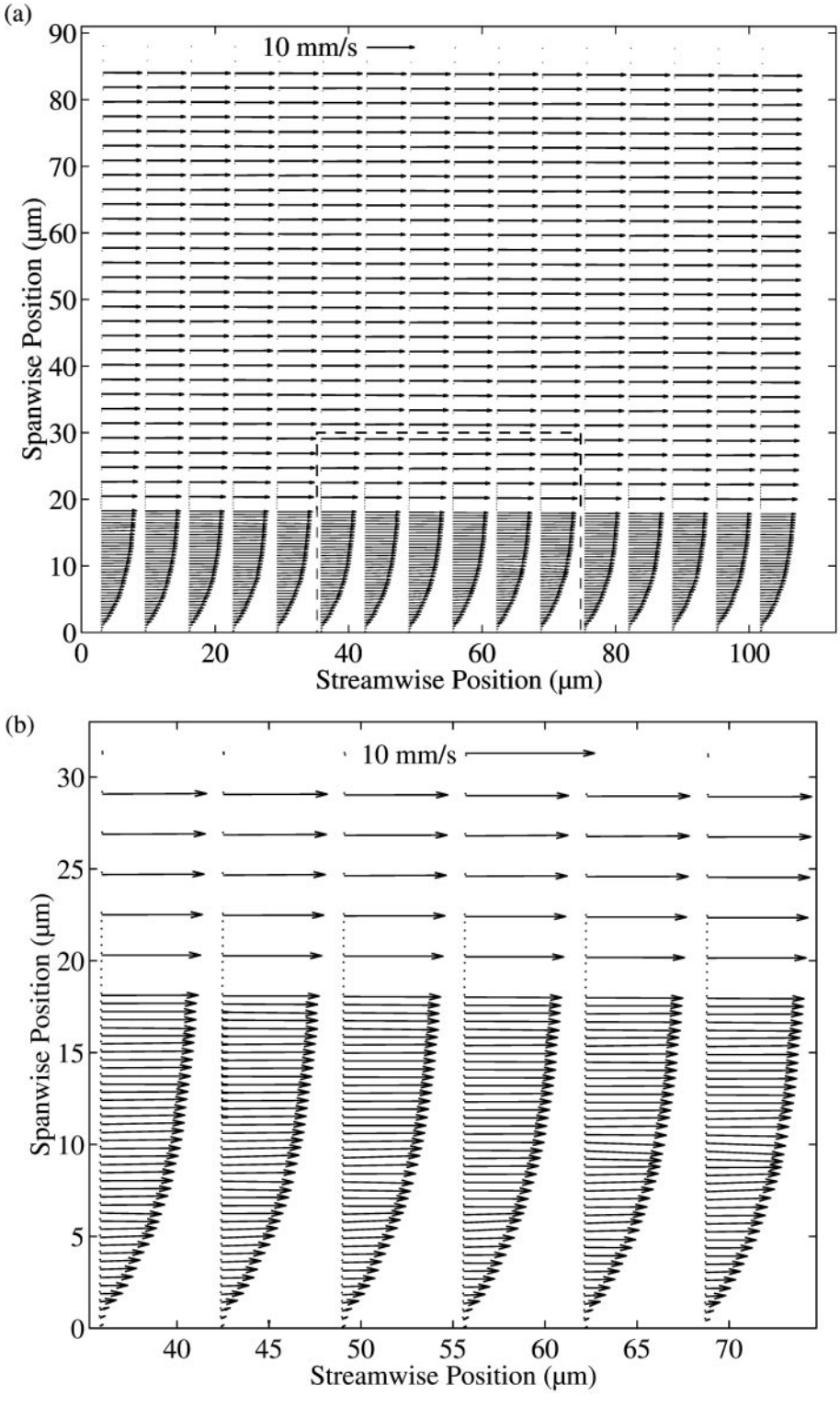

Fig. 3a, b. Ensemble-averaged velocity-vector field measured in a 30 μm deep × 300 μm wide × 25 mm channel. The spatial resolution, defined by the interrogation spot size of the first interrogation window, is 13.6 μm×4.4 μm away from the wall, and 13.6 μm × 0.9 μm near the wall. A 50% overlap between interrogation spots yields a velocity vector spacing of 450 nm in the wall-normal direction near the wall: **a** large-scale vector field showing a 100 μm × 85 μm cross section of the channel, **b** near-wall view of the lower 30 μm of vector field

a velocity-vector spacing of 450 nm is achieved. The technique uses epi-fluorescent microscopy, a CCD camera, and two Nd : YAG lasers to image 200 nm diameter fluorescent polystyrene particles. The spatial resolution and the accuracy of the measurements is limited primarily by the diffraction limit of the recording optics and the size of the sub-micron seed particles.

Ensemble-averaged velocity measurements in a 30 μm × 300 μm rectangular microchannel are presented. The measurements are obtained using a higher spatial resolution close to the wall, and a lower resolution away from the wall. The spatial resolution, defined by the size of the first data window in the interrogation spot, is 13.6 μm×0.9 μm near the wall, and 13.6 μm × 4.4 μm away from the wall. The out of

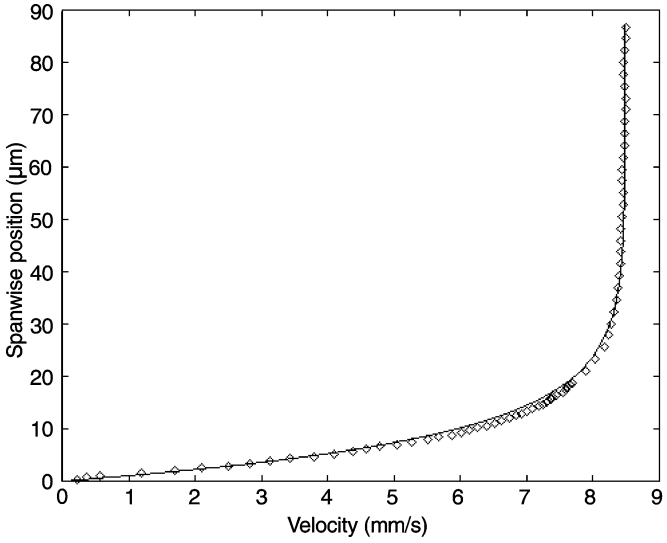

Fig. 4. Ensemble-averaged velocity profile measured in a nominally 30 μm × 300 μm channel. The symbols represent ensemble-averaged and streamwise-averaged PIV data. The solid line is the analytical solution for Newtonian flow through a rectangular channel

plane resolution of the measurements is estimated to be approximately 1.8 ± 0.5 μm. The resulting PIV measurements agree to within 2% of the analytical solution for flow in a rectangular channel.

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