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Microfluidic Velocity Measurements using Three-Dimensional Confocal Micro Particle Tracking Velocimetry (CM-PTV)

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MICROFLUIDIC VELOCITY MEASUREMENTS USING THREE-DIMENSIONAL CONFOCAL MICRO PARTICLE TRACKING VELOCIMETRY (CM-PTV)

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ABSTRACT

In the past, confocal microscopy was limited by its slow temporal resolution and was not a practical tool for imaging dynamic systems. Recent improvements in spinning disk confocal microscopy and high-speed camera technology have made it a viable technique for imaging of microfluidic systems. Here, a confocal micro particle tracking velocimetry (CM-PTV) system is demonstrated with the ability to obtain 3D velocity profiles at high flow rates.

INTRODUCTION

Confocal microscopy is a well-developed technique that is widely used in biomedical research due to its advantages over conventional widefield microscopy, which is used in conventional micro-PIV systems. High-speed spinning disk confocal microscopy was first used to obtain micro-scale flow field measurements in 2004 and was able to show an increase in resolution and accuracy by reducing the signal to noise ratio for PIV measurements (1). This so called confocal laser scanning microscopy (CLSM) micro-PIV system was developed by Park *et al* (1) and was able to achieve high spatial accuracy, but was limited to a low temporal resolution of up to 120 frames per second, which limits the systems usefulness for microfluidic velocity measurements. Additionally, they did not utilize the optical sectioning capabilities of confocal microscopy to generate 3D velocity data. Since then, advances in camera and spinning disk technology lead to the development of confocal systems capable of up to 1,000 frames per second. This type of system then was utilized to generate 3D velocity profiles of fluid flow through square microchannels (2). However, the system only generated results at a temporal resolution of 200 frames per second due to sensitivity issues at higher frame rates. The ability of the confocal system to track multiphase fluid flow was also explored in the same paper using suspended blood cells. However, they were only able to investigate the effect of adding blood cells on the fluids velocity profile, while the ability to track the actual movement of the cells was not demonstrated.

A method for recording high speed, high sensitivity, and high-resolution images of fluid flowing through microchannels is critical to enable control and design of novel microfluidic devices. Additionally, if multiphase systems such as solid

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particles suspended in a liquid can be studied this could provide essential information to assist in the design of microfluidic processes involving liquid-particulate interactions such as micro reactions, particulate sampling, and blood flow. This study demonstrates the use of a high speed confocal imaging system to generate 3D velocity profiles of fluid flowing in trapezoidal microchannels at speeds of 1000 frames per second, which is five times faster than any results previously reported in literature. Additionally, the ability to track a separate solid particle phase is demonstrated.

METHODS AND MATERIALS

1 Confocal Microscopy

Confocal microscopy, developed in 1957 (3), improves axial and lateral resolution along with delivering extremely thin optical slices of data enabling 3D reconstruction of images. High-speed spinning disk confocal microscopy is an emerging technology in the field of microfluidics. It offers several advantages over widefield micro-PIV techniques, such as the ability to obtain 3-D results of multiphase flow systems with increased signal to noise ratio and spatial resolution. In order to achieve high frame rates spinning disk confocal microscopy replaces the single pinhole used in traditional confocal microscopy with a rotating array of pinholes. This allows full-field images to be captured at much higher frame rates and allows the use of a camera instead of a PMT detector. The key feature to the spinning disk system is the use of dual disks in which the upper disk contains 20,000 micro-lenses in an identical pattern to the 20,000 pinholes on the bottom disk. The illumination source is pumped through the microlenses, which focus the light through the 50-micron pinholes; this greatly increases the amount of light passing through to the sample making it possible to illuminate fluorescent objects. The emission signal from the fluorescent material then passes back through the pinholes and is reflected off a dichroic to the detector.

2 Microchannel

The trapezoid microchannel was fabricated in PMMA by use of a newly developed mechanical micro-machining process. The process has the advantages of (1) higher efficiency, (2) more flexible 3D shape generation, and (3) easier tool preparation over the currently well-used micro-milling technique. In this novel mechanical micro-machining process, a non-rotating diamond tool is employed to move against the work piece with the assistance of multi-axis numerically controlled ultra precision motions, and to write or generate directly the required patterns on the surface of the work piece.

As the first demonstration of the process, a commercially available ultra precision machine, Nanotech 350FG, was used in our experiments to provide the 3-axis ultra precision motions. The machine has three linear axes that are equipped with linear laser scales capable of resolving 8.6 nm at a maximum speed of 1800 mm/min. The motion straightness on all slides is less than 250 nm. The diamond tool used in these experiments has the same trapezoid shape as the profile of the microchannel. Its tip width is 5 μm, and its inclination angle of each side is 15 degrees. To generate the microchannel, the diamond tool is moving at the rate of 200mm/min against the work piece, and at a depth of cut of 1 micrometer, and a rake angle of 0 degree.

The microchannel has dimensions of 52.9 micron across the top of the channel, 47.0 micron deep, and 23.8 micron across the bottom, see figure 1. The channel was 10 mm long with 1/16-inch inlet and outlet holes drilled at each end. Inlet and outlet ports were attached using a UV curable adhesive and the channel was sealed using optically clear tape, which could be removed after each experiment to clean out the channel. The dimensions of the channel were obtained using a WYKO NT-3300 optical profilometer with the results shown in figure 1.

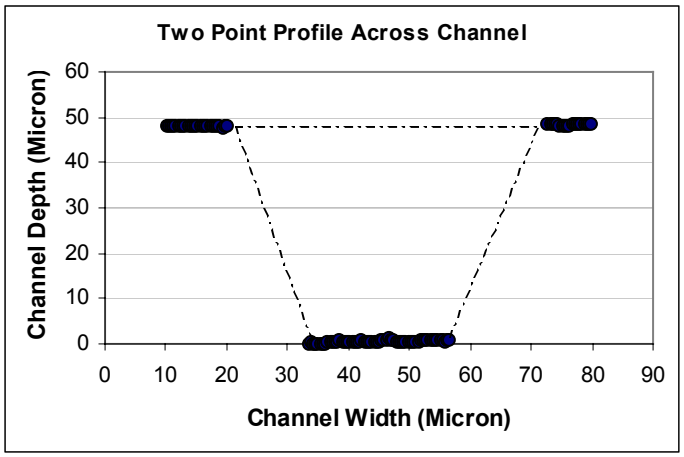

Figure 1. Optical profilometry measurements of channel dimensions. Two point profile across channel, solid line is actual data, dashed line is theoretical channel cross section.

3 Confocal Micro-Particle Tracking Velocimetry (PTV) System

The system used in this study is similar to the CLSM micro-PIV system used by Park *et al* (1) in 2004, with the exception of several key upgrades. Instead of the Yokogawa CSU-10 spinning disk this CM-PTV system utilizes the Yokogawa CSU-22, which increases the maximum temporal resolution from 120 frames per second up to 1000 fps. In order to realize these higher frame rates the traditional CCD camera was replaced with a high-speed CMOS camera from Photron that is capable of 2000 frames per second at full frame resolution (1024x1024 pixels). While there is always a trade off between speed and sensitivity when dealing with detectors, it was found that the very bright 1-micron fluorescent tracer particles used could easily be seen at 1000 frames per second. This represents a significant increase in temporal resolution for a confocal microfluidic tracking system, while still maintaining adequate sensitivity. Two solid state laser lines were employed with the first being 50 mW at 491 nm and the second supplying 25 mW at 561 nm wavelength. The lasers were controlled using an AOTF. An Olympus IX-81 inverted microscope was utilized as the base microscope with a 60x 1.42 NA oil objective that was equipped with a Jena piezo objective-focusing collar for submicron control in the z direction. The camera and spinning disk were synchronized and the entire system was controlled using Vox Cell software from Visitech International.

To track fluid motion, distilled water was seeded with 1 micron Fluosphere® tracer particles obtained from Molecular Probes. These fluorescent particles are made from polystyrene with a density of 1.055-g/cm^3 and peak illumination/excitation of 505/515 nm. The water was seeded with 0.07% by volume of the tracer particles.

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Details of the flow system and the optical set up are summarized in table 1. The sealed microchannel was taped to the microscope stage and clear silicon tubing was used to connect the inlet of the channel to a 10 mL syringe that had previously been loaded with the water/tracer solution. A Harvard Apparatus syringe pump was used to deliver 5 micro liters per hour of the fluid to the channel. The fluid was allowed to fill the microchannel and outlet port to avoid wetting effects and measurements were taken half way between the two ends, 5 mm from the inlet, to eliminate entrance effects. The top and bottom of the channel were found by scanning though the z direction of the channel and image slices were captured every 4 microns at a rate of 1000 fps.

Table 1: Experimental Parameters

Flow system		**Optical Set Up**	
Trapezoid channel		Confocal System	
Top width	52.9 μm	Pinhole diameter	50 μm
Bottom width	23.8 μm	Magnification (M)	60x
Height	47.0 μm	Refractive index (n)	1.52
Flow rate	5 μL/hr	Numerical Aperture (NA)	1.42
Particle diameter	1.1 μm	Excitation wavelength	491 nm
Particle concentration	0.07% by vol.	Emission wavelength	515 nm

4 Particle Tracking

Particle tracking was accomplished using commercially available Simple PCI software from Compix Inc. At velocities above 1500 microns/sec particles were easily tracked from one picture to the next due to the high frame rate of the camera and the low seeding of tracer particles, which ensures that particles are generally spread apart. Three sets of 10 consecutive images were analyzed at each z slice to increase the number of data points. Experimental results were then plotted and compared to simulation results. Data obtained from this system can also be analyzed using micro-PIV algorithms. However, this system readily lends itself to PTV because consecutive images can be taken with no lag time between image pairs as in micro-PIV. The use of PTV increases accuracy over PIV by allowing lower particle seeding and producing velocity results for each individual particle (4).

THEORETICAL

1 Spatial Resolution and Optical Slice Thickness

The spatial filtering of the pinhole apertures gives confocal increased lateral and axial resolution as well as enabling optical sectioning. The diameter of the pinholes plays an important role in determining the optical properties of a confocal microscopy system. By comparing the modified pinhole diameter (MPD) (see table 2) to the Airy unit (AU) value you can determine if a geometric-optical analysis or a wave-optical analysis should be used (5). Following the equations developed by Wilhelm *et al* and using the experimental parameters given in table 1 we calculated our lateral and axial resolutions, see table 2. The optical slice thickness (OST) for this system was calculated to be 1.34 microns, which allows us to capture thin slices of data no mater how thick the sample is. These slices can then be reconstructed to give 3D results. The optical slice thickness should be less that the step size in the z

direction to avoid overlap between optically sectioned images (6). For the theoretical OST of 1.34 microns a step size of 4.0 microns was used. Previous studies have conducted similar optical analysis to evaluate their spinning disk confocal micro-PIV systems based on the equations presented by Wilhelm *et al* (5) and a more detailed comparison between conventional and confocal microscopy is presented by Park *et al* (1).

Table 2. Optical parameters

Modified pinhole diameter (μm)	0.83
Airy unit	0.42
Lateral resolution (μm)	0.18
Axial resolution (μm)	0.44
Optical Slice Thickness (μm)	1.34

2 Simulation

Flow in the micro channel was simulated with the commercial CFD software FLUENT. The length of the simulation domain was 600 micron, which was long enough for the flow to reach fully developed state near the outlet of the channel. Hexagonal cells were used to generate the computation mesh, and the grid number was 60, 60, 300 in x, y, and z directions, respectively. No-slip boundary condition was imposed on the solid walls, while the mass flow rate was given at the inlet of the channel, and fully developed flow was assumed at the outlet. The flow was assumed to be steady, incompressible, and laminar. The continuity equation and the momentum equations were discretized with 2^{nd} order accuracy, and the SIMPLE method was used for the coupling of the velocity and the pressure terms. Convergence was assumed when the residuals fell below 10^{-7}. The velocities at different locations along the flow direction were compared to make sure that the flow was fully developed near the outlet.

RESULTS AND DISCUSSION

1 Experimental vs. Simulation Results

Experimental results are compared to the simulation results for 3D velocity profiles. The results are compared in figure 2 at three different heights within the channel. The value at 24 μm is approximately half way up the 47-micron tall channel; the other 2 heights represent half way to the top and bottom surfaces from the middle. Good agreement between simulation and experimental results is observed. Experimental values near the center height of the channel had less variation and followed the simulation curve more closely than values near the top or bottom surface. This is most likely due to wall effects.

Cross sectional surface plots were constructed to compare all experimental data from a height of 4 μm up to 44 μm with a 4 μm step size. Raw data is plotted in figure 3.A and smoothed data is plotted in figure 3.B by constructing a second order polynomial fit for each optical slice. The systems ability to accurately track flows in 3 dimensions is seen when compared to simulation results in figure 3.C. The experimental values appear to be shifted up slightly. This may be due to bulging in the tape at the top surface making the height of the channel somewhat higher than 47 μm and leading to a shift up in the 3D velocity profile. The z-axis velocity profile

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was constructed using velocities from the center of the channel at each optical slice and this data was then compiled and compared to simulation in figure 3.D. 3D surface plots were constructed using the smoothed experimental values and simulation with results shown in figure 4.

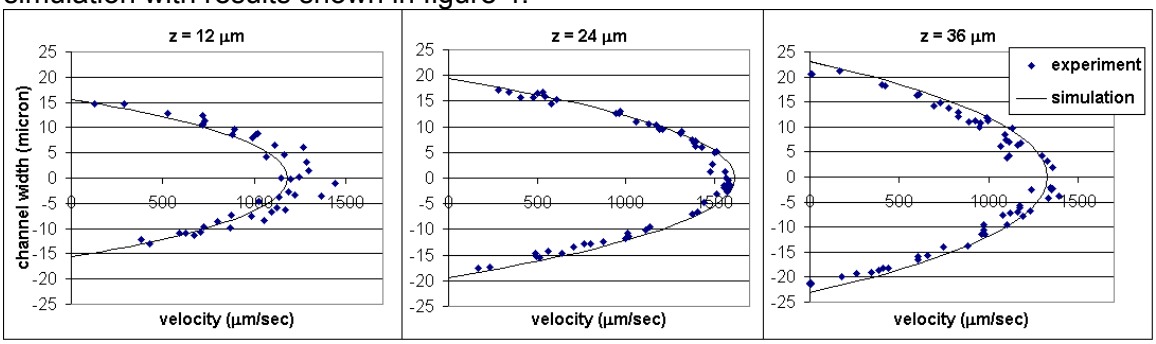

Figure 2. Experimental vs. simulation at 12, 24, and 36 micron heights in the channel at a volumetric flow rate of 5 micro liters per hour.

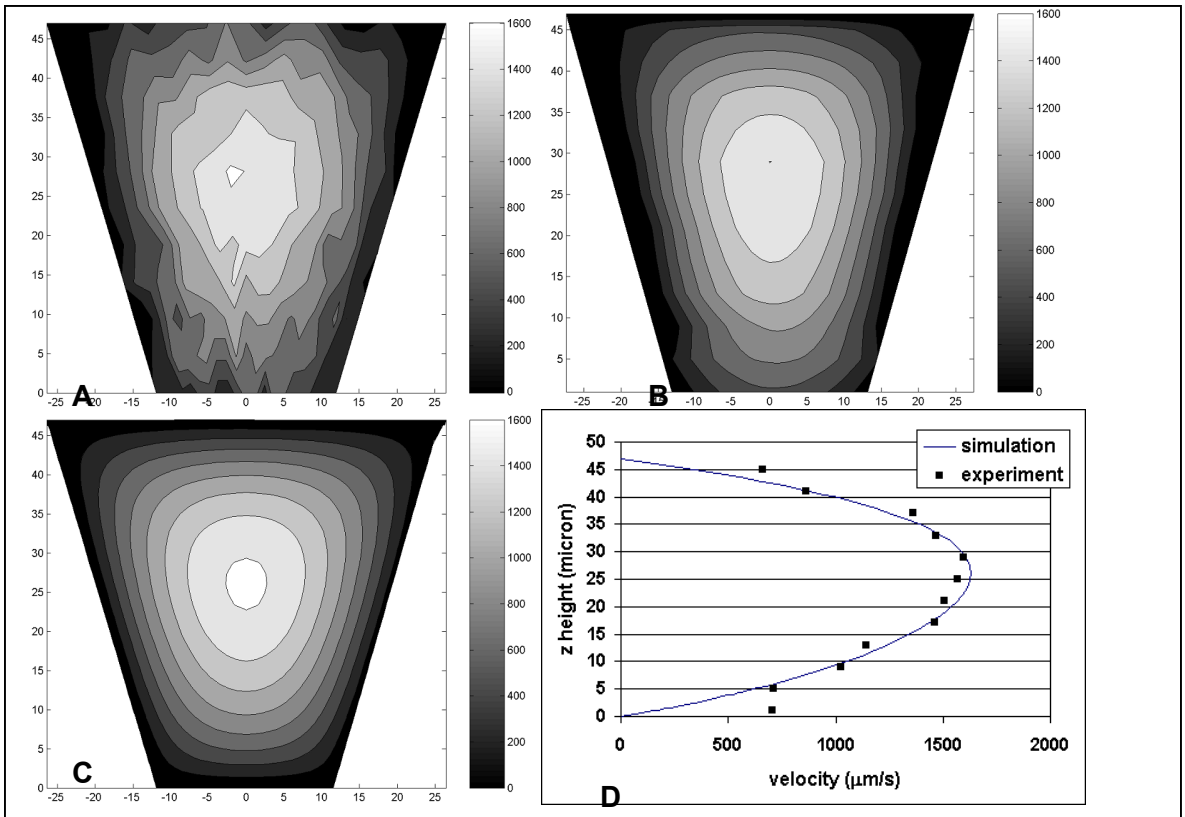

Figure 3: Cross sectional surface contour plot of A: Experimental data, B: Smoothed experimental data, C: Simulation. D: Experimental vs. simulation plot of z-axis velocity profile from the top to bottom of the channel.

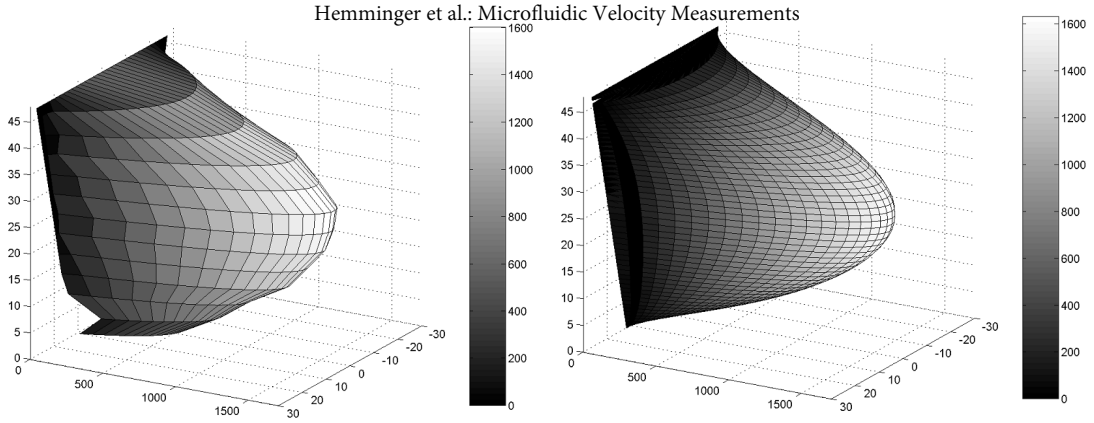

Figure 4. 3D surface plots. A: Smoothed experimental. B: Simulation.

2 Multiphase Particle Tracking

The ability to track not only the liquid phase tracers but also a separate solid particle phase is a valuable tool for developing novel microfluidic devices. Tracking dynamic solid phase systems such as DNA stretching in an extensional flow or measuring the movement and deformation of blood cells or other cells as they flow through specific features of a microchannel would provide valuable information for design of new biomedical microdevices. This capability is demonstrated here by seeding the water/tracer solution with a small amount of 4-micron fluorescent particles (Spherotech, 570/600). Low seeding had to be used to avoid clogging of the channel. Both the tracer particles and the 4-micron particles were tracked simultaneously using the 491 and 561 lasers at the same time and then differentiating the particles based on a size threshold. Figure 5 shows velocity results from tracking 20 consecutive images taken at 1000 fps. Experimental results are presented here to demonstrate the multiphase capability of the developed confocal micro-PTV system.

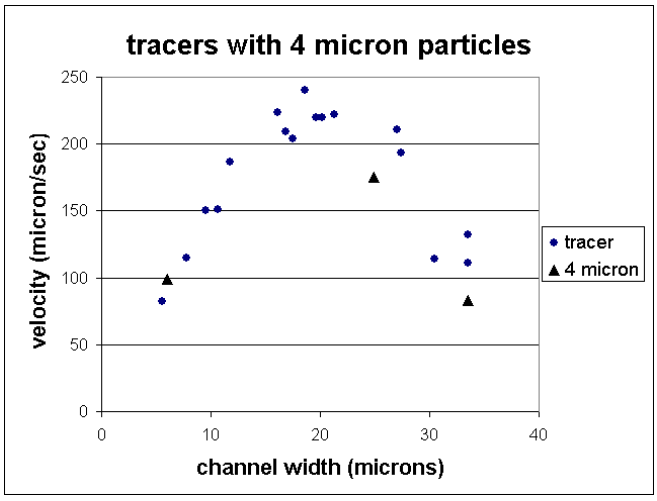

Figure 5. Velocity of tracer particles and 4 micron solid particles.

3 Discussion

The system developed here is capable of measuring 3D velocity profiles, which is critical to understanding microfluidics, but are not obtainable using traditional micro-PIV techniques. Compared to previous confocal micro-PIV studies the spatial resolution was increased and using a 60x oil objective with a NA of 1.42 decreased optical slice thickness. While this required the use of a smaller microchannel due to the decreased field of view, it enabled the full benefits of confocal microscopy to be realized. The pinholes used in the spinning disk confocal system are optimized for use with high NA objectives, which results in thinner slices and higher resolution. Additionally, results were obtained at a rate of 1,000 frames per second, which is five times faster than any confocal microfluidic results previously reported. This increase in temporal resolution is critical for establishing high-speed confocal microscopy techniques in the field of microfluidics. The ability to track two separate phased was demonstrated and will be explored further in future work. The combination of high-speed, high-resolution, 3D, and multiphase capabilities make this a valuable instrument in the field of microfluidics.

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