

## VELOCITY FIELDS OF BLOOD FLOW IN MICROCHANNELS USING A CONFOCAL MICRO-PIV SYSTEM

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The *in vitro* experimental investigations provide an excellent approach to understand complex blood flow phenomena involved at a microscopic level. This paper emphasizes an emerging experimental technique capable to quantify the flow patterns inside microchannels with high spatial and temporal resolution. This technique, known as confocal micro-PIV, consists of a spinning disk confocal microscope, high speed camera and a diode-pumped solid state (DPSS) laser. Velocity profiles of pure water (PW), physiological saline (PS) and *in vitro* blood were measured in a 100 $\mu$ m glass square and rectangular polydimethylsiloxane (PDMS) microchannel. The good agreement obtained between measured and estimated results suggests that this system is a very promising technique to obtain detail information about micro-scale effects in microchannels by using both homogeneous and non-homogeneous fluids such as blood flow.

### 1. Introduction

Detailed knowledge on flow velocity profiles of blood flow in microchannels is essential to provide a better understanding on the blood rheological properties and disorders in microvessels. Despite the high amount of research in microcirculation, there is not yet any detailed experimental information about flow velocity profiles, RBCs deformability and aggregation in microvessels. This lack of knowledge is mainly due to the absence of adequate techniques to measure and quantitatively evaluate fluid mechanical effects at a microscopic level [1, 2].

During the years the most research work in this area has focused in experimental studies using techniques such as laser Doppler anemometry (LDA)

or conventional particle image velocimetry (PIV). However, due to limitations of those techniques to study effects at a micro-scale level, Meinhart and his colleagues [3] have proposed a measurement technique that combines the PIV system with an inverted epi-fluorescent microscope, which increases the resolution of the conventional PIV systems [3, 4]. More recently, considerable progress in the development of confocal microscopy and consequent advantages of this microscope over the conventional microscopes have led to a new technique known as confocal micro-PIV [5-9]. This technique combines the conventional PIV system with a spinning disk confocal microscope (SDCM). Due to its outstanding spatial filtering technique together with the multiple point light illumination system, this kind of microscope has the ability to obtain in-focus images with optical thickness less than 1  $\mu\text{m}$ , task extremely difficult to be achieved by using a conventional microscope. As a result, by combining SDCM with the conventional PIV system it is possible to achieve a PIV system with not only extremely high spatial resolution but also with capability to generate 3D velocity profiles.

The main purpose of this paper is to investigate the ability of the confocal micro-PIV system to measure velocity fields of both homogeneous and non-homogeneous fluids in two different kinds of microchannels, i. e., 100 $\mu\text{m}$  glass square and rectangular (300 $\mu\text{m}$  wide, 45 $\mu\text{m}$  deep) PDMS microchannel.

## **2. Materials and methods**

### **2.1. Working fluids and microchannels**

Four working fluids were used in this study: pure water (PW), physiological saline (PS) and PS containing about 17%(17Hct) and 20% (20Hct) of human red blood cells (RBCs). All fluids were seeded with 0.1 to 0.15% (by volume) 1- $\mu\text{m}$ -diameter red fluorescent solid polymer microspheres (R0100; Duke Scientific, USA). The blood was collected from a healthy adult volunteer, where ethylenediaminetetraacetic acid (EDTA) was added to prevent coagulation. The RBCs were separated from the bulk blood by centrifugation and aspiration of the plasma and buffy coat and then washed twice with PS. The washed RBCs were diluted with PS to make up the required RBCs concentration by volume. All blood samples were stored hermetical at 4°C until the experiment was performed at room temperature (25 to 27°C).

Two microchannels were used in this study. The first was 100  $\mu\text{m} \times 100 \mu\text{m}$  borosilicate glass square microchannel fabricated by *Vitrocom*. The square microchannel was mounted on a slide glass with thickness of approximately 120  $\mu\text{m}$ , which was immersed in glycerine in order to minimize some possible refraction from the walls [9]. A second microchannel was a PDMS rectangular

microchannel (300 $\mu\text{m}$  wide, 45 $\mu\text{m}$  deep) fabricated by a soft lithographic technique at the Esashi, Ono and Tanaka Laboratory, Department of Nanomechanics, Tohoku University [10].

## 2.2. Confocal micro-PIV experimental set-up

The confocal micro-PIV system used in our experiment consists of an inverted microscope (IX71, Olympus, Japan) combined with a confocal scanning unit (CSU22, Yokogawa, Japan) and a diode-pumped solid state (DPSS) laser (Laser Quantum Ltd, England) with an excitation wavelength of 532 nm. Moreover, a high-speed camera (Phantom v7.1, U.S.A.) was connected into the outlet port of the CSU22. The microchannels were placed on the stage of the inverted microscope where the flow rate of the working fluids was kept constant by means of a syringe pump (KD Scientific Inc., U.S.A.). For the square microchannel the flow rate was 0.15  $\mu\text{l}/\text{min}$  ( $\text{Re} = 0.014$ ), whereas for the PDMS rectangular microchannel the flow rate was 0.22  $\mu\text{l}/\text{min}$  ( $\text{Re} = 0.021$ ).

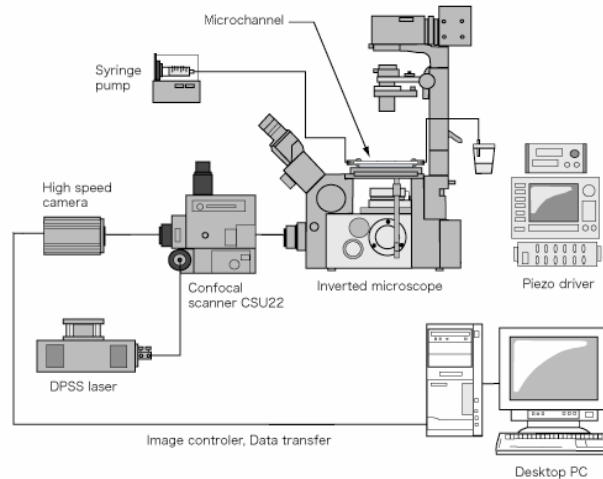


Fig. 1. Confocal micro-PIV experimental set-up.

The microscopic observations and the capturing of the confocal PIV images were performed in the middle of the microchannels. By using a RT3D software it was possible to collect a series of xy confocal images at different z positions. The images were captured with a resolution of 640 $\times$ 480 pixels, 12-bit grayscale, at a rate of 200 frames/s with an exposure time of 4995 ms. The recorded images were transferred to the computer and then evaluated by the PivView version 2.3 (PivTec) using a cross-correlation method [11]. As a result it was possible to obtain the velocity vector fields at the interrogation area of interest. Detailed

information about the experimental set-up, used in the present study, has already been described previously [9].

### 3. Results and discussion

#### 3.1. Comparison between Experimental and Analytical Results

By using the optical sectioning ability of the confocal system it was possible to obtain series of optical sectioned images along z axis. Figures 2 and 3 shows a comparison between analytical solutions [12] and average fluid velocities of 100 PIV image pairs at several optical sectioned images for both microchannels. Generally, the experimental results from Figures 2 and 3 show good agreement with the analytical solution, especially the results from the middle plane where we found errors less than 5%. As one moves out of the middle plane the deviations start to increase and at locations close to the wall the errors can be slightly larger than 10%. We believe that these latter errors are mainly attributable to "second-order effects" such as the surface roughness of the wall and background noise generated from particles adhered into the wall.

The results from Figure 3 also show that the velocity patterns were approximately parabolic which is characteristic of laminar flow in a square microchannel. In contrast, the velocity profiles from the PDMS microchannel (see Figure 4) were markedly flat in the central region, which is characteristic of laminar flow in a rectangular microchannel with low aspect ratio ( $h/w = 0.15$ ).

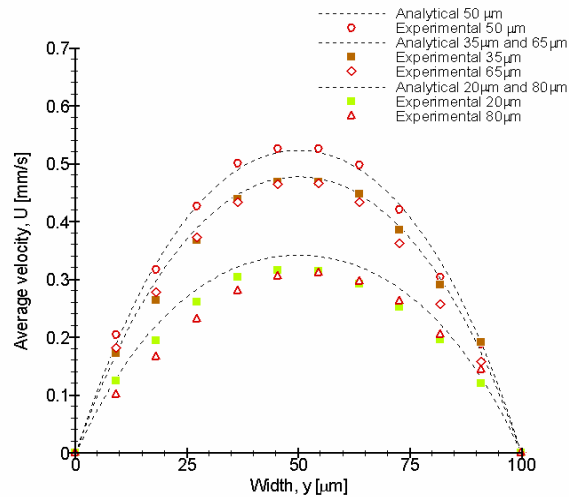


Fig. 2. Comparison between experimental data and analytical solutions at several optical sectioned images in 100 $\mu$ m square microchannel. The working fluid is PW.

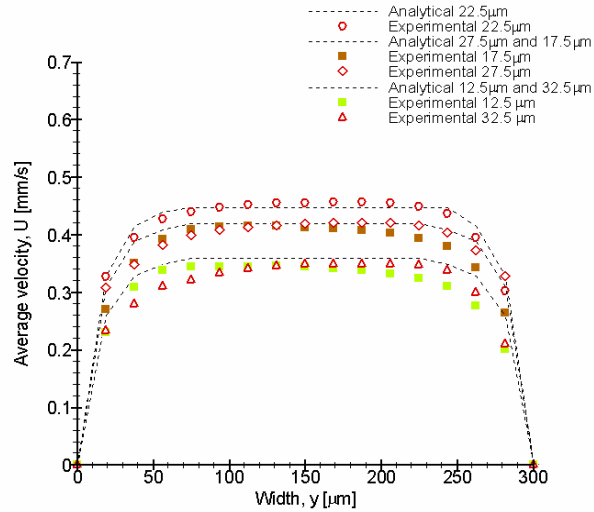


Fig. 3. Comparison between experimental data and analytical solutions at several optical sectioned images in a rectangular PDMS microchannel. The working fluid is PS.

### 3.2. Velocity fields of *in vitro* blood flow

Besides the employment of PW and PS, in this study PS containing around 17% and 20% of suspended blood cells was also used in order to evaluate the potentialities of the confocal micro-PIV system to investigate the flow behaviour of *in vitro* blood in both microchannels.

Figure 4 and 5 show the ability of the system to obtain velocity vector fields of *in vitro* blood at different xy planes. However, for the case of the square microchannel it was not possible to obtain reliable results along the full depth of the microchannel, especially in the upper part. One of the reasons for this limitation is the thickness of the wall and slide glass which imply a much high working distance to obtain the velocity fields. Another reason is the high concentration of RBCs in the middle region of the square microchannel, which decreases the intensity of the emitted light from the flowing fluorescent particles within the plasma flow, especially from the upper part of the microchannel. Conversely, for the case of the PDMS microchannel it was possible to record images along the full depth, in order to be processed by the PIV software. In this latter microchannel, due mainly to its geometry, we did not visualize any plasma layer, but rather a homogenous distribution of the RBCs which could contribute to the improvement on the images quality recorded by the high speed camera.

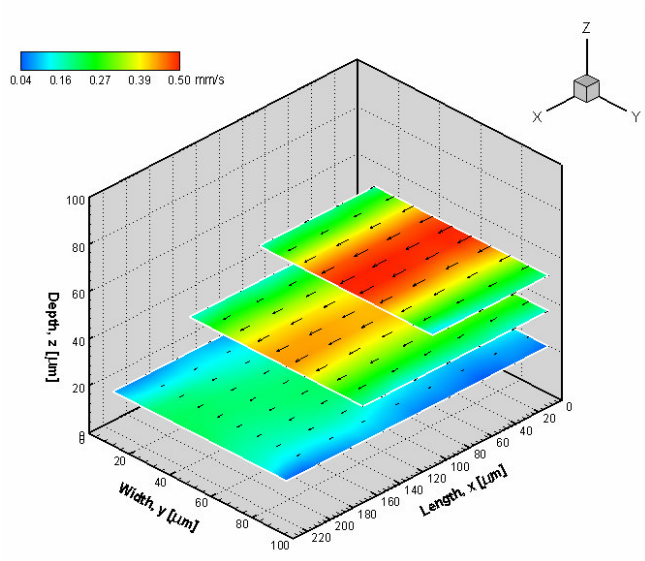


Fig. 4. Velocity vector fields from several optical sectioned images along z axis, in a 100  $\mu\text{m}$  glass square microchannel. The working fluid is *in vitro* blood with 17% Hct.

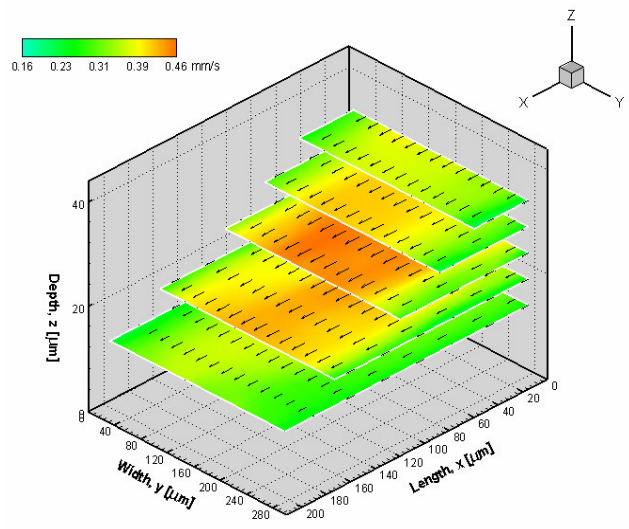


Fig. 5. Velocity vector fields from several optical sectioned images along z axis, in a rectangular PDMS microchannel. The working fluid is *in vitro* blood with 20% Hct.

### **3.3. Comparison between glass and PDMS microchannels**

Although it is possible to manufacture glass microchannels with circular or rectangular cross sections this kind of microchannels are limited to only straight geometries. Thus, it is crucial to use a microfabrication technique able to produce microfluidic devices with complex geometries similar to human blood arterioles and capillaries networks. The PDMS microchannel used in this study have shown a number of very useful properties to study *in vitro* blood flow, such as good optical transparency, easy reversible sealing to glass, good mechanical rigidity, chemical inertness, low toxicity, low cost and ability to have very complex geometries. Furthermore, due to the small working distance (less 100  $\mu\text{m}$ ) required to obtain confocal images, the PDMS microchannel have shown to be extremely suitable to study several phenomena in microcirculation by combining it with our confocal micro-PIV system.

### **4. Conclusions**

We applied a confocal micro-PIV system to measure PW, PS and *in vitro* blood flowing in two kinds of micorchannels, i. e., glass square and rectangular PDMS microchannel. The measured velocity profiles of PW and PS agree well with established analytical solution. Moreover, the measurements of *in vitro* blood (17% and 20%Hct) have demonstrated the ability of this system to obtain confocal images with just the fluorescent particles within the plasma flow. As a result, the confocal micro-PIV system has demonstrated potential to obtain accurate detail information about micro-scale effects in both microchannels.

This study also demonstrated that the combination of confocal micro-PIV systems with PDMS microchannels would play an important role in the assessment of several phenomena in microcirculation.

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