Velocity Measurements of Physiological Flows 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 both pure water and physiological fluid were measured within a square 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 physiological fluids

Key words: Microcirculation, Confocal micro-PIV, Nipkow disk, Blood cell suspension, Microchannel.

1. INTRODUCTION

The detail measurements of velocity profiles of in vitro blood flow in micorchannels are fundamental for a better understanding on the biomechanics of the microcirculation. 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 (diameter in the order of 100μm or less). These 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]. More recently, considerable progress in the development of confocal microscopy and consequent advantages of this microscope over the conventional microscopes [4, 5] have led to a new technique known as confocal micro-PIV. 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 μm, 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 the present study is to evaluate the performance of our confocal micro-PIV system in order to investigate its ability to study the behaviour of non-homogenous fluids such as physiological fluids.

2. MATERIALS AND METHODS

2.1. Working fluids and microchannel

Two working fluids were used in this study. The first was pure water (PW) seeded with 1% (by volume) of 1μm diameter red fluorescent solid polymer microspheres (R0100, Duke Scientific). A second fluid was Hanks solution (HS) seeded with 10% of human blood and 1% of 1μm diameter red fluorescent solid polymer microspheres (R0100, Duke Scientific).

In this study a 100 μm × 100 μm borosilicate glass square microchannel fabricated by *Vitrocom* was used to evaluate the performance of a our confocal micro-PIV system. The square microchannel was mounted on a slide glass with thickness of approximately 120 μm which was immersed in pure water in order to minimize some possible refraction from the walls of the microchannel.

2.2. 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 microchannel was placed on the stage of the inverted microscope where the flow rate of the working fluid was kept constant at 0.15 μl/min (Re = 0.014) by means of a syringe pump (KD Scientific Inc. U.S.A.).

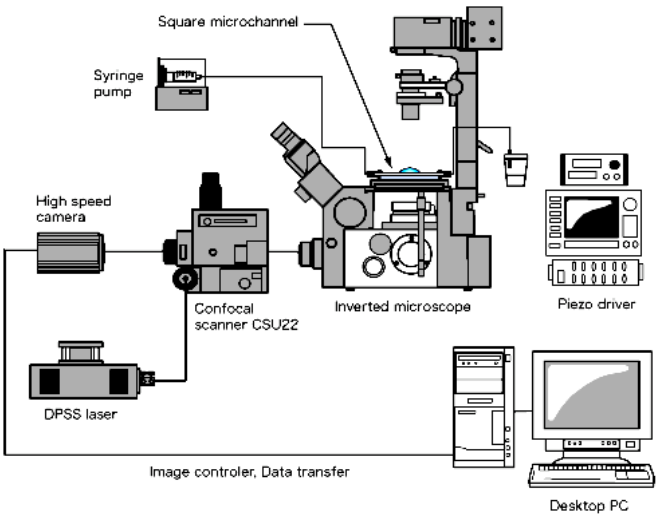

Fig. 1 Main components of the experimental set-up.

The microscopic observations and the capturing of the confocal PIV images were performed in middle of the microchannel. By using a RT3D software it was possible to collect a series of xy images at different z positions. The recorded PIV images were digitized directly in the camera and then transferred to the computer to be processed by the PIV data analysis. Due mainly to the complex physiological fluid (PW with 10% of blood) used in

our study we have decided to capture images with a resolution of 640×480 pixels, 12-bit grayscale, at a rate of 200 frames/s with an exposure time of 4995 ms. By using the PivView version 2.3 (PivTec) the images were evaluated by using a cross-correlation method and as a result it was possible to obtain the velocity vector fields at the interrogation area of interest. Deatailed information about the experimental set-up, used in the present study, has already been described previously [5].

3. RESULTS AND DISCUSSION

By using the optical sectioning ability of our system it was possible to obtain series of optical sectioned images along z axis. Figure 2 shows a comparison between analytical solutions [5] and average fluid velocities of 20 PIV image pairs at several optical sectioned images. According to the results shown in Figure 2, the averaged velocity data and analytical solutions at the centre plane and 15 μm away from the centre plane show very close agreement with errors less than 3% and 6% respectively. However, at locations closer to the wall and far away from the focal plane (xy planes located at 30 μm from the centre plane) the deviations were more pronounced with errors from 10% to 13%. We believe that the latter errors are mainly due to the increase of the degree of defocusing as one moves out of the ideal focus plane and to "second-order-effects" such as surface roughness of the wall.

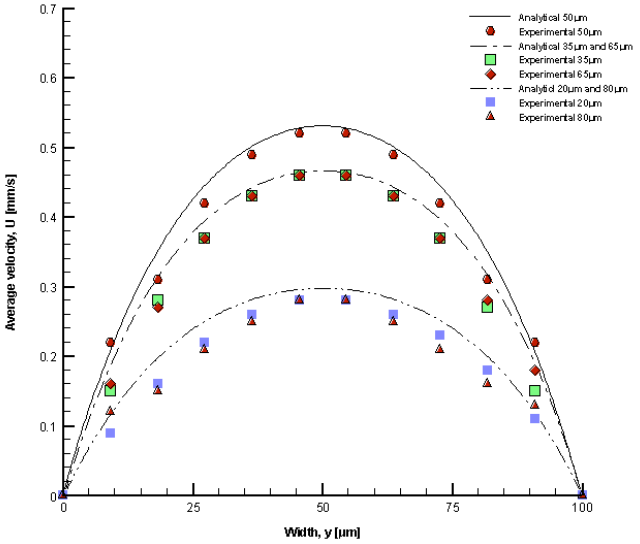

Fig. 2 Comparison between experimental data and analytical solutions at several optical sectioned images.

Besides the employment of pure water, in this study a physiological fluid containing of about 10% of suspended blood cells was also used in order to evaluate the potentialities of our confocal micro-PIV system to investigate the flow behaviour of complex fluids such as *in vitro* blood flow. Figure 3 shows the ability of our system to obtain confocal images with just the fluorescent particles within the plasma where as the blood cells around the particles were not captured as they had different emission wave lengths. As a result, we believe that this system is the best technique to study blood flow phenomena at microscopic level, such as interactions between blood cells and plasma. In fact, from the measurements shown in Figure 4 it is possible to observe some very small deviations when compared physiological fluid with pure water. These results suggest that around 10% of suspended blood cells have almost a negligible effect in the plasma flow and that this physiological fluid behaves as a poiseuille flow. We believe that the reason for this behaviour is mainly due to the small hematocrit used in this experiment. An attempt to implement our confocal micro-PIV system to investigate the behaviour of *in vitro* blood with different hematocrits is in its initial stage and now facing some difficulties mainly due to the complex task to control the hematocrit through a microchannel.

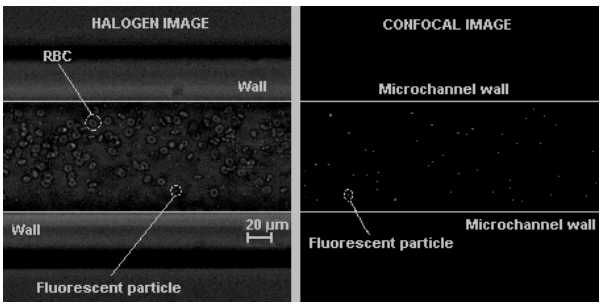

Fig. 3 Halogen image Vs confocal image of the physiological fluid used in this experiment.

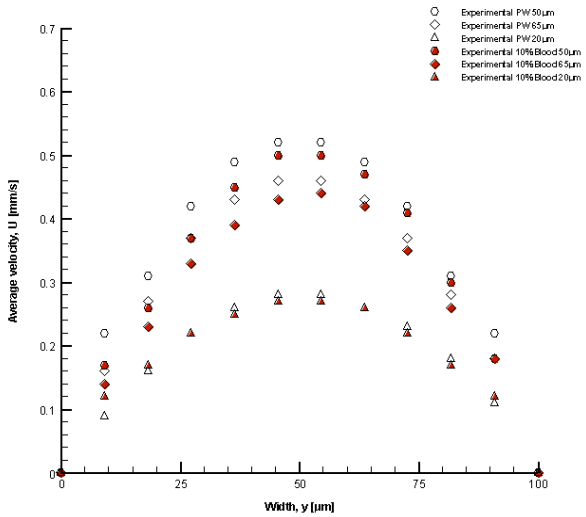

Fig. 4 Average velocity profiles at several xy planes of pure water and Hanks solution with 10% human blood

4. CONCLUSIONS

The present study corresponds to ongoing work in order to evaluate the performance of a new technique, known as confocal micro-PIV, to investigate phenomena of blood flow at a microscopic level. The measured velocity profiles of pure water agree well with predicted Poiseuille profiles. Moreover, the measurements of a physiological fluid containing 10% of suspended blood cells have demonstrated the ability of this system to obtain confocal images with just the fluorescent particles within the plasma. As result, our confocal micro-PIV system have demonstrated the ability to obtain accurate detail information about micro-scale effects in microchannels.

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