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In vitro micro PIV measurements of velocity profiles near a wall

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ABSTRACT

A scanning Micro Particle Image Velocimetry (μPIV) technique is used to measure the near wall velocity profile. The flow characteristics around a fixed thrombus in a rectangular glass micro channel in multiple parallel planes are examined in order to understand the role of flow mechanics in cell adhesion, platelet aggregation and thrombus formation mechanism.

1. INTRODUCTION

μPIV is a non-invasive flow field measurement technique, which is becoming the method of choice for many experimental micro fluid mechanics investigations. This technique, introduced by Santiago et al. [11] in 1998 is now being used to aid our understanding of blood flow within the human body. Significant physiological and pathological phenomena occur in the circulation and consequently flow behaviour inside micro channels has been intensively analyzed both *in vivo* and *in vitro* [1, 2, 4, 5, 6, 7, 8]. Despite the large number of studies dedicated to micro channel flows there is a lack of detailed experimental information on the velocity profile near the wall. Knowledge of the near wall flow is important for a number of reasons; firstly the flow induced shear stress is maximized at the wall. Additionally, the hemodynamic forces in the near wall region are believed to affect platelet activation and adhesion resulting in thrombus formation. Thus the near wall flow conditions play a significant role in arterial diseases leading to heart attacks or strokes. Thrombus formation initially develops through the excessive aggregation of discoid platelets. A recent study has demonstrated that platelets can form stable aggregation without a detectable increase in soluble agonists in vessels [9]. It has been shown that thrombus development depends on rapid changes in blood velocity gradients with the effects of soluble agonists considered as a secondary factor in stabilised discoid platelet aggregation. A new mechano-sensory platelet activation mechanism was introduced which suggested a fundamental view of platelet activation and thrombi formation and was able to predict the thrombus growth rate [9].

The objective of this work is to investigate the ability to measure velocity fields around a fixed thrombus next to the micro channel's wall. A robust two-dimensional scanning μPIV technique was used to conduct experiments in different parallel planes with small spatial resolution. Scanning μPIV is a reliable alternative technique for more complicated three-dimensional μPIV techniques and can be coupled with the concept of continuity to calculate velocity gradients.

2. EXPERIMENTAL METHOD

As shown in Figure 1(a) the experimental setup consisted of an epi-fluorescent inverted microscope (DM IL, Leica Microsystems, Germany) equipped with a submicron resolution vertical stage, a high-speed camera (MotionPro X3, Redlake Inc., USA), a diode-pumped solid-state continuous laser and a syringe pump (Harvard Apparatus, USA). The high-speed camera was connected to the outlet port of the microscope with a 0.7x adapter to improve image intensity and maximise the signal-to-noise ratio.

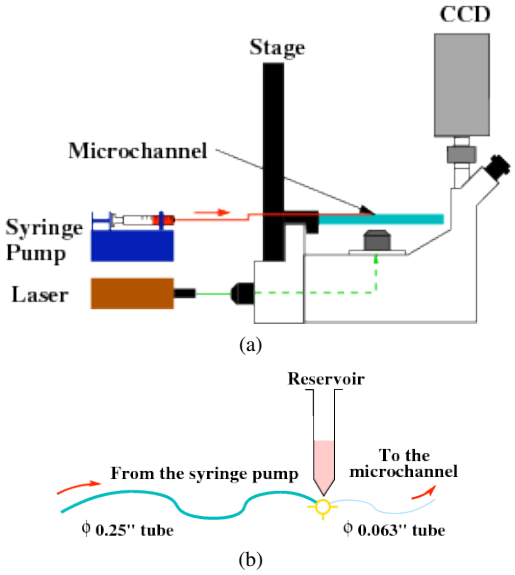

Figure 1: (a) Schematic showing experimental setup, (b) tubing setup

The Harvard syringe pump is widely used for biomedical research; however the screw mechanism within the pump appears to introduce oscillations into the flow. To reduce these oscillations an experimental setup has been chosen with an additional long tygon tube (Cole-Parmer instrument co., USA, ID .25 in) and a reservoir was placed between the syringe pump and micro channel as shown in Figure 1(b). Thus the pump infuses fluid through a 69cm long, 6.35mm diameter tygon tube through the reservoir damper, then a 7cm section of 1.6 mm diameter section of silicon tubing (Cole-Parmer instrument co., USA, ID .063 in) before entering the micro channel. This setup was found to generate steady flow in the micro channel.

The experiment was carried out in a rectangular glass micro channel, cross-section 2 mm × 0.2 mm and 100 mm long (Vitrotubes, VITRO COM, USA). As shown in Figure 2, the reference axes are indicated by x, y and z, with corresponding velocity components u, v and w, where the direction of the upstream flow is parallel to the unit vector x. The glass micro channel was placed horizontally on the vertical stage and illuminated by a 200 mJ 532 nm laser, with the laser beam passing through the microscope objective. The flow was imaged using a 63x dry objective (HCX PL FLUOTAR, NA 0.7 and WD 1.8-2.6 mm). The flow rate of working fluid inside the

micro channel was kept constant at 0.32 ml.min^{-1} ($\gamma_{z,th}$=400 s^{-1}, V_{max}=20 mm.s^{-1}).

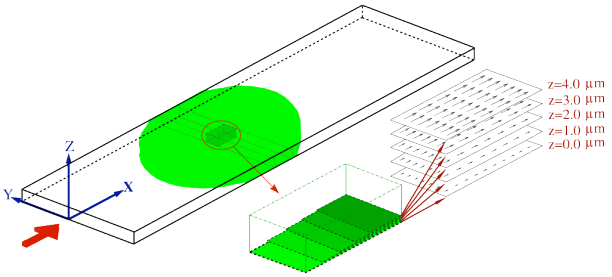

Figure 2: Schematic diagram illustrating the scanning method. The figure shows the coordinate system, the volume illumination and the PIV measurement planes

The working fluid was distilled water seeded with 0.3% (by volume) 1 µm diameter red fluorescent microspheres (R0100; Duke Scientific, USA).

Image pairs were captured using the high speed camera with a maximum resolution of 1280×1024 pixels and 12-bit grayscale; at frame rates up to 1000 frame/s. Each image pair was processed using inhouse PIV software [3]. This software uses a double-frame, cross-correlation multi-window algorithm, with temporal correlation averaging, to extract a grid of velocity vectors from the PIV images. PIV was performed using a cross-correlation analysis, with the dynamic range enhanced using an iterative approach to select the sampling window size starting at 128×128 pixels with a final window size of 16×16 pixels (corresponding to a spatial resolution of 4.32×4.32 µm) with an overlap of 50%. Erroneous vectors were defined as those which deviated by more than 2 pixels from a local fit of the data. Less than 1% of vectors failed this criterion and where subsequently replaced by the local fit.

To confirm the accuracy of the system the near wall out-of-plane velocity profile was measured in a plane micro channel using scanning µPIV. Subsequent experiments were performed to measure the velocity vector fields around a fixed thrombus at different planes covering the thrombus' height. The thrombus was fixed prior to µPIV experiment. The fixed thrombus was prepared by coating the micro channel with collagen (10 μg/ml) to promote platelet adhesion on glass. The micro channel was then washed out with Tyrode's buffer. Whole blood (anticoagulated with Hirudin) obtained from healthy human donors was then perfused through the coated micro channel at the constant flow rate of 1.44 ml.min^{-1} to form the thrombus. Once the thrombus was fully formed and had stopped growing the blood flow was stopped and the thrombus was fixed [12].

3. RESULTS AND DISCUSSION

Figure 3 shows the average velocity in a z-planes between z = 0 µm and 37 µm. Each measurement was obtained using 150 image pairs where the vertical error bars represent a standard deviation (95% confidence interval) of the 5120 spatial locations within each z-plane. Two set of data are presented and compared with the theoretical Poiseuille flow profile for steady flow through a long, straight rigid rectangular channel. The experimental results are in good agreement with the theoretical flow profile. In the very near wall region ($z \leq 2$ µm) the seeding density is low and the PIV images are contaminated by higher velocity out-off-focus particles. In this region the measured velocity is typically over-estimated by µPIV techniques [10].

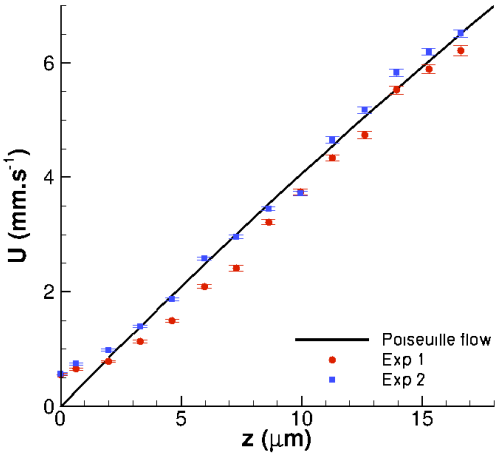

Figure 3: Out-of-plane velocity profile obtained with experimental data (dots) compared with the theoretical profile (solid line) at a bulk shear rate of $\gamma_{z,th}$=400 s^{-1}

The in-plane velocity vector fields around a fixed thrombus in selected z-planes are shown in Figure 4. µPIV was conducted in parallel planes (0.0≤z≤37µm) covering the whole height of the thrombus. The data were acquired at a theoretical mean wall shear rate of $\gamma_{z,th}$=400s^{-1}. Due to out-of-focus particles the measured velocity field is not zero at bottom wall (Figure 4a). The local flow pattern is considerably altered by the thrombus with zero velocity adjacent to the thrombus increasing gradually to the uniform theoretical value far from the thrombus.

As the thrombus grows the velocity over the top of the thrombus and hence the local shear rate, will increase. High shear rates are not favourable for stable platelets adhesion, however after passing over the top of the thrombus the platelets are pushed downward into the suitable low shear region behind the thrombus. It has been observed that initial platelet recruitment to the surface occurs at high shear region. These initial interactions are always unstable resulting in rapid platelet translocation to the trailing edge of the thrombi. As a consequence, more than 75% of stable discoid platelet aggregation occurs within the low shear region behind the thrombi [9].

4. CONCLUSION

This study provides an insight into how advanced experimental fluid dynamics may be utilised to analyse flows for biological applications. Scanning µPIV has been used to examine near wall flow conditions. Conventional two-dimensional µPIV data has been obtained at a number of parallel planes in an empty rectangular micro channel. The measured velocity profile agreed well with the theoretical value. For z less than 2 µm the low density of seeding particles causes the correlation averaging method to over-estimate the velocity.

Investigation of flow fields around the fixed thrombus showed detailed velocity information around the thrombus. It is confirmed that the thrombus is pyramid in shape. Detailed measurements of the flow field combined with knowledge of the biological processes occurring during platelet adhesion can allow us to determine the effect of the local flow on thrombus growth as well as the final geometry of the thrombus.

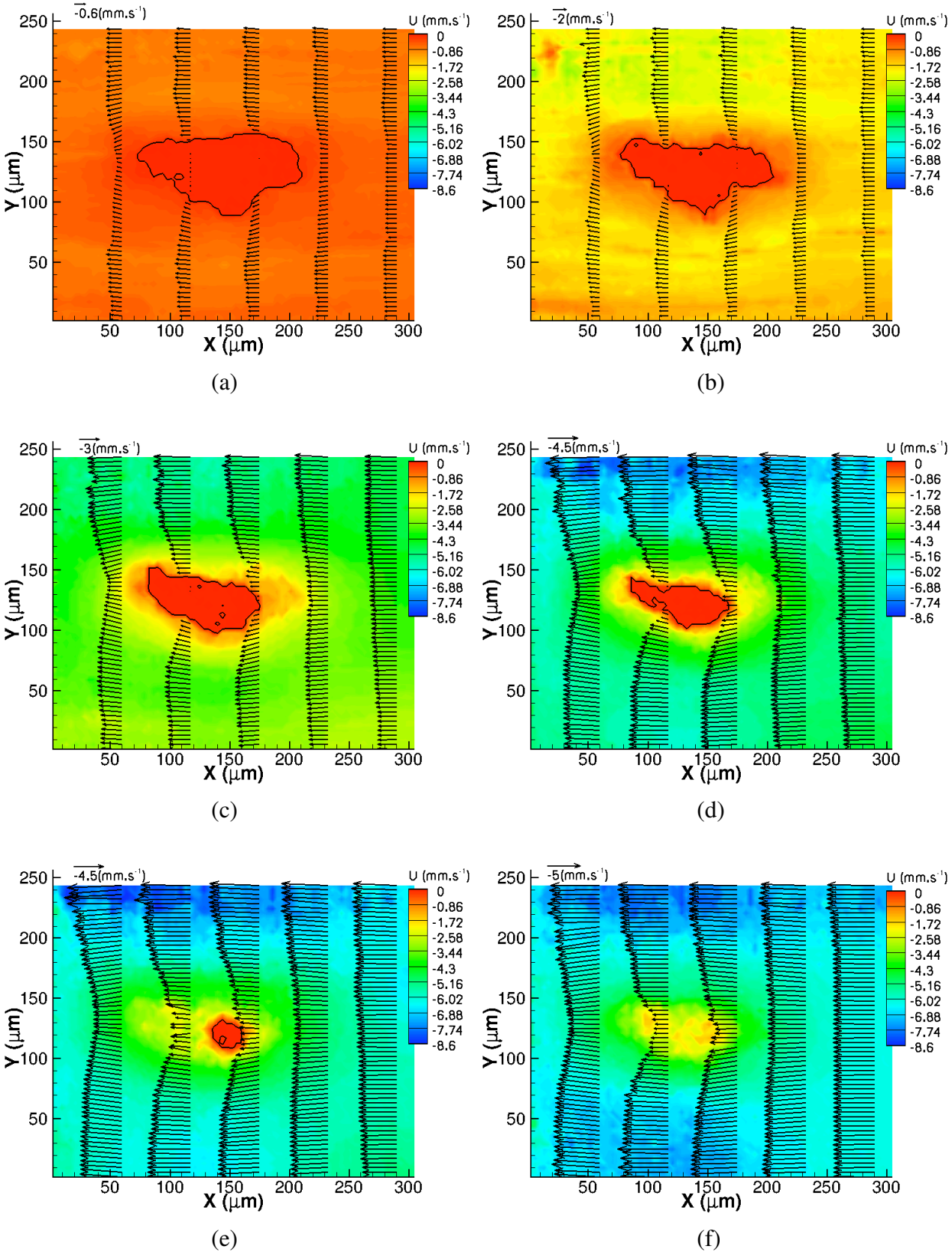

Figure 4: Velocity vector fields in different z planes (a) z=0.0μm, (b) z=8μm, (c) z=16μm,(d) z=24μm, (e) z=29μm, (f) z=37μm

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