PIV Measurement of Red Blood Cell Velocity Field in Microvessels

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ABSTRACT
As endothelial cells are subject to flow shear stress, it is important to determine the detailed velocity distribution in microvessels in the study of mechanical interactions between blood and endothelium. Recently, particle image velocimetry (PIV) has been proposed as a quantitative method of measuring velocity fields instantaneously in experimental fluid mechanics. The authors have developed a highly accurate PIV technique with improved dynamic range, spatial resolution and measurement accuracy. In this paper, the proposed method was applied to images of the arteriole in the rat mesentery using an intravital microscope and high-speed digital video system. Taking the mesentery motion into account, the PIV technique was improved to measure red blood cell (RBC) velocity. Velocity distributions with spatial resolutions of 0.8 x 0.8 μm were obtained even near the wall in the center plane of the arteriole. Ensemble averaged time-series of velocity profiles in cross sections were compared. The arteriole velocity profile was blunt in the center region of the vessel cross-section and sharp in the near-wall region.

Keywords: Blood flow, RBC velocity, Microcirculation, Highly accurate PIV technique

1. Introduction
Microcirculation in arterioles, capillaries and venules, which have diameters of 5 to 50 μm, is essential in the process of maintaining healthy tissues and organs. In particular, the measurement of the circulation velocity with high spatial resolution and high measurement accuracy is crucial for scientific and clinical study in evaluating supply to the tissues and organs and the shear stress of blood cells and endothelial cells. Various experimental techniques, such as the electromagnetic blood flowmeter, the ultrasonic Doppler flowmeter, laser Doppler velocimetry, the dual slit method, microscopic video images techniques and so on, have been developed to measure blood velocity in microcirculation\(^1\)\(^-\)\(^3\). However, a spatial resolution and measurement accuracy were also not suitable for microcirculation analysis.

Particle image velocimetry (PIV) is a quantitative method for measuring velocity fields instantaneously in experimental fluid mechanics systems\(^6\). The authors have applied a highly accurate PIV technique\(^5\) to blood flow images in 30-μm diameter bifurcation arteriole using an intravital microscope and high-speed digital video system\(^6\), and have also improved the accuracy of RBC (Red Blood Cell) velocity by taking the mesentery motion into account\(^7\). The results indicated that the dynamics of blood flow were complex due to multi-phase flow, the non-Newtonian fluid, the cardiac cycle and so on.

This paper described in vivo blood velocity measurement technique and time-series analysis about ensemble averaged velocity profiles in a rat mesentery arteriole.

2. Experimental Setup
Figure 1 shows schematic view of experimental set up. A male Wister rat (8 weeks, 310 g body weight) was anesthetized with thiobutabarbital sodium intraperitoneously and allowed to respire spontaneously. An intestinal loop was mounted on the stage of an intravital microscope with water-immersion objective lens with a magnification M=60 and a numerical aperture NA=1.0. The mesentery was placed on an observation window and perfused with Krebs-Ringer solution maintained at 37 degrees shown in Fig.2. Blood flow images were recorded into a computer for two seconds using a high-speed CCD camera at a rate of 1000 frames/sec. The images consisted of 512 x 512 pixels with 8 bit gray levels. The measurement region was illuminated
from the bottom using back light illumination by metal halide.

![Diagram](image)

**Fig. 1 Schematic view of experimental set up**

**Fig. 2 Observation of the rat mesentery under intravital microscope**

**Fig. 3 Image of blood flow in an arteriole**

Figure 3 shows the visualized image on blood flow in an arteriole with internal diameter of about 24-26 μm at the center of the image. The observed region is 136 x 136 μm in size. One pixel is equivalent to 0.27 μm. The vessel curved slightly to the right at around x = 50 μm. Generally, the diameter of endothelial cells is approximately 5 μm. A plasma layer sometime called as cell free layer, where erythrocytes hardly pass, is clearly observed near the wall.

Initially, in order to improve measurement accuracy by taking a mesentery motion into account the highly accurate PIV technique was applied to the first blood flow image at t = 0 as a reference, and then sequentially to subsequent images at t = k (k = 1, 2, ..., 2047). The displacement and temporal derivation value of it were determined as the relative distance from the reference image and the velocity of the mesentery, respectively. The spatial gradients and variation in displacement in the upper and lower areas of the arteriole were small. It is considered that the motion involved only parallel translation, without higher-order displacements such as deformation or rotation. The displacements varied from -1.9 to 1.6 μm in the x direction from the reference position and the instantaneous velocity of the mesentery also varied from -0.26 to 0.17 mm/s in the x direction. These amplitudes correspond to about 14% of the vessel diameter and 13% of the time-averaged RBC (Red Blood Cell) velocity at the center of the vessel, respectively. Without taking the motion into account, the time-averaged and spatially averaged velocity exhibited a broad profile, and bias error of 13% was introduced.

In order to reduce the effect of mesentery motion, both the blood image and RBC (Red Blood Cell) velocity were modified using the obtained mesentery motion. The PIV technique was applied to two successive shifted images in order to obtain the RBC velocity distributions. The measurement accuracy of RBC velocity improved after the relative positions of the arteriole in all images were arranged consistently and the effect of the mesentery motions eliminated.

### 3. Results

Figure 4 shows time averaged velocity distribution of 2008 images for about 2 sec. calculated using the highly accurate PIV technique. An interrogation window of 7 x 7 pixels was taken, with a 50% overlap rate. This corresponded to a spatial resolution of 0.8 x 0.8 μm. The velocities in the horizontal direction were thinned out in order to be displayed clearly. A velocity distribution with high spatial resolution and
highly measurement accuracy was obtained. The velocity vectors very close to the wall were measured and it was found that the wall-normal component of the velocity vectors was close to zero. The maximum velocity is about 11.0 pixel/frame or 3.0 mm/sec at the center of the arteriole. The flow around inner side of the bent corner of arteriole was lower. The lower velocity areas near the wall were in the plasma layer. Averaged velocity profiles show the blood flow volume to be constant at the vessel cross-sections.

Analyzing the time series of axial blood velocity in several cross sections on the lower stream side at the bend in the vessel, the velocities showed periodical because of a heartbeat. The amplitudes and phases in all sections were relatively consistent. The pulsatile flow was similar to that of regular circulatory flow. The peak frequency was about 6.4 Hz obtained by spectrum analysis. The result shows that the velocity is synchronized with the cardiac cycle of 6-7 Hz even in microvessels.

Figure 5 shows the ensemble-averaged maximum axial velocity in three cross sections. These profiles were obtained using 13 cardiac cycles. The velocities in every section repeatedly increased sharply from end diastole at time $t = 30$ msec toward a peak systole at time $t = 70$ msec and then decreased gradually toward a late diastole. High cross correlations for these series were recognized. The diameters of vessels at $x = 85, 101$ and 125 $\mu$m were 24.7, 25.7 and 23.6 $\mu$m, respectively. Since the most downstream section at $x = 125$ $\mu$m had the smallest diameter the velocity profile was largest varied from 3.0 mm/sec to 4.2 mm/sec. However, in other two profiles with different diameters showed almost same values varied from 2.6 mm/sec to 3.7 mm/sec.

Because a very low velocity region was observed near the downer wall due to a marginal cell-free plasma layer at $x = 101$ $\mu$m.

Figure 6 shows the ensemble-averaged axial blood velocity profiles in three sections shown in Fig. 5. Thirty velocity values were obtained along the capillary diameter at a spacing of 0.8 $\mu$m. The time averaged velocity profiles in every section display as dot lines. The velocity of all profiles became maximal around the center of the vessel, and decreased to zero near the wall. The arteriole velocity profiles were broad at the center of the vessel, and sharp near the wall compared with a parabolic flow profile. This suggests that the shear stress on the vessel wall was higher than expected. The profiles shows symmetric at $x = 85$ $\mu$m in Fig. 6 (a). The profile at $x = 101$ $\mu$m in Fig. 6 (b) in particular shows the typical flow features for a non-Newtonian fluid: a more broad axial velocity distribution and a steep velocity gradient near the wall. A very low velocity region was identified near the downside wall due to a marginal cell-free plasma layer. This exhibited the broadest profile. At $x = 125$ $\mu$m in Fig. 6 (c). for the smallest diameter section. the dip was not observed and the cell-free plasma layer was less predominant.

**4. Conclusion**

A highly accurate PIV technique was applied to in vivo blood images of the arteriole in the rat mesentery. The images were recorded using the intravital microscope with water-immersion objective lens and illumination by metal halide, and the high-speed digital video system. The velocity distributions with spatial resolutions of 0.8 $\times$ 0.8 $\mu$m were measured even near the wall in the center plane.
of arteriole. The arteriole velocity profiles were blunt at the center region of the vessel cross-section and sharp near the wall. The results show that the proposed method can be used to measure the blood flow velocity profiles with highly accurate temporal and spatial resolution even in the micro-vessels.

References

Fig. 6 Cross-sectional ensemble-averaged axial velocity profile