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VISUALIZATION AND MEASUREMENT OF FLOW-INDUCED DYNAMIC MOTION OF RED BLOOD CELLS USING TRACKING CONFOCAL MICRO-PIV SYSTEM

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INTRODUCTION

RBC(Red Blood Cell)s have a biconcave shape with diameters of about 8 μm and thicknesses of about 2 μm like a capsule structure with highly deformable membrane. In arterioles having diameters of less than 100μm, the effect of RBCs becomes pronounced because the scales of the flow and the RBCs become similar. RBCs exhibit the axial migration [1] toward the center of blood vessel. The axial migration leads to non-Newtonian flow behavior such as decrease in flow resistance. The tank-tread motion [2] makes an important role for the axial migration and it is dependent on the shear rate of the surrounding flow, which ranges up to 500 s^{-1} in arteriole.

In order to understand these distinctive phenomena of RBCs suspended flow, the measurement of interaction between the RBC and the surrounding fluid with high spatial resolution and continuous measurements in an physiological environment is required. Although, a confocal micro-PIV [3] is a powerful tool that can non-invasively measure the velocity distribution of a microflow with high-resolution, the measurable velocity is limited to approximately 0.70 mm/s using a 100x objective lens because of the limitation of its scanning speed. Moreover, the narrow field of view of a high-magnification observation causes the target to be lost from the field of view.

In this study, a novel "target-tacking confocal micro-PIV system" has been constructed in order to overcome the limitations of the conventional system. Then, we try to measure the interaction between RBCs and the surrounding fluid continuously with high resolution in a realistic physiological environment using present system.

METHODOLOGY

Figure 1 shows the basic concept of the present system. The targeted microchannel is moved in the direction opposite to the flow in the channel by a motorized stage to keep the measurement target in the field of view. The proposed technique can measure not only faster flows but can also measure flows for a longer period of time than was possible using the previous confocal micro-PIV system. The absolute flow velocity is calculated by subtracting the velocity of the tracer particles from that of the marker particles, which imbedded in a sub-channel adjacent to the main channel.

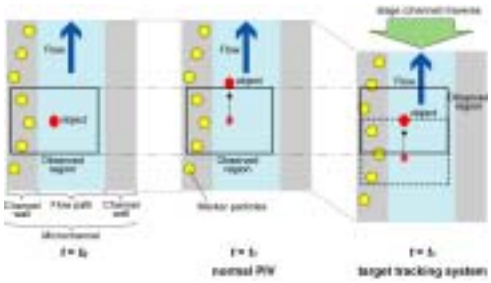

Fig.1: Schematic illustration of tracking system

The target tracking system is installed on the confocal Micro-PIV system developed by the authors [4]. A motorized stage moves at constant velocity with high precision and little vibration. A stepping motorized stage with an air bearing (VMDA-100, PMT Corp., Japan) was chosen for this purpose. The 100x oil-immersion objective lens (PL APO CS 100x/1.4-1.25 HCX, Leica Microsystems, Germany) achieve the sufficient spatial resolution of 0.116 μm/pixel in plane and the confocal depth of 0.58 μm with ϕ0.2 μm tracer particle.

Figure 2 shows the geometry of target microchannel. The field of view of the measurement slice is 92.8 x 69.6 μm at channel center height. The flow shear stress is adjusted to 0 – 2.9 Pa by controlling

flow rate at 2.5 μl/h with physiologically-controlled surrounding fluid. Under the conditions above, the shear rate becomes 0 ~ 152.4 s^{-1}, and this value satisfies physiological condition with the possibility of tank tread motion. The translational velocity of the moving stage is set to 0.7 mm/s to cover all velocity range from zero to the maximum flow velocity of 1.25 mm/s. The carboxylate-modified yellow-green fluorescent tracer particle 0.2 μm in diameter (F8813, Invitrogen Molecular Probes, USA) is attached electrically to the surface of RBC to visualize the deformation and movement of the membrane of RBC.

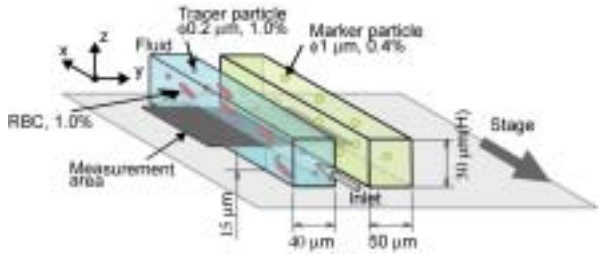

Fig. 2: Measurement target microchannel

RESULTS AND DISCUSSION

Figure 3 shows PIV images of RBCs and the surrounding flow separated by different wavelengths. The channels run in the vertical direction of the images, and the RBC suspended fluid flows downward. We succeeded in recording continuous images of RBC A for 0.1 s. It means the present system enables 2.56 times longer continuous measurement compared with the conventional system.

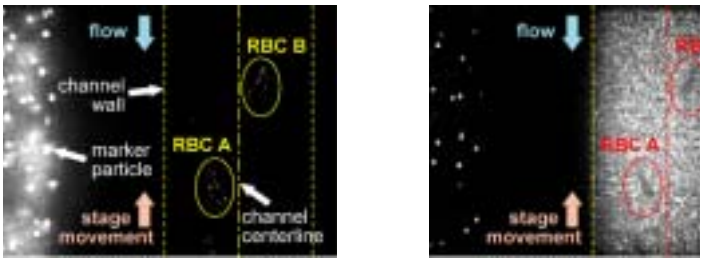

Fig.3 : PIV images separated by wavelength at t = 0.06 (left) Shorter-wavelength image for RBCs (right) Longer-wavelength image for surrounding flow

Figure 4 shows the relative velocity map of the RBC membrane and the surrounding fluid overlapped at t = 0.04 sec. The relative velocities of these two phases are calculated by subtracting the translational velocity of the center of gravity of the RBC in order to investigate the interaction. Since the absolute velocity of RBC A was 1.15 mm/s, the present system succeeded to measure the faster flow than the limit of conventional system. The map is cut out only region around RBC A, and the channel centerline is indicated as dashed line on the right side of RBC. The velocities of tracer particle on the RBC membrane are indicated by red circles.

The flow around RBC is distorted in the same direction as the rotational direction of the RBC membrane. The velocities on RBC membrane also show the same magnitudes of the adjacent flow velocities. It indicates there is strong interaction between RBC motion and surrounding flow. Moreover, particle velocity on the RBC membrane varies depending on their position. The membrane velocity of channel centerline side of RBCs is larger than that of channel sidewall side. It suggests that RBC membrane may have stretch and show hyper elastic material depending on the shear stress induced by the surrounding flow. The membrane curvature of channel centerline side of RBCs is larger than that of channel sidewall side. We consider that this difference of membrane curvature enhances the difference of lateral velocity component, and it result in the one reason of axial migration.

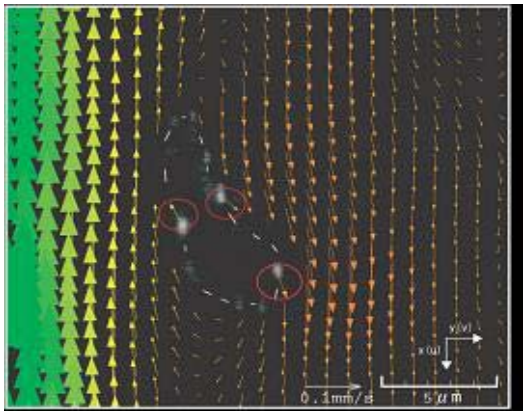

Fig. 4: Distribution of streamline

CONCLUSIONS

We have developed and evaluated a target-tracking confocal micro-PIV system that can measure faster flows than the conventional system with a motorized stage. The movement of the RBCs and the velocity distribution of the surrounding flow are successfully measured simultaneously using present system. The proposed system enables continuous observation of moving RBCs over a distance 2.5 times longer than that of the conventional system. It can also measure higher absolute velocities than the conventional system does.

As a result, the tank-tread motion of RBCs and the corresponding movement of the surrounding flow structure are measured simultaneously and quantitatively. The rotational direction of the tank-tread motion corresponds to the shear stress gradient of the surrounding flow. The tank-tread motion also affects the surrounding flow structure. The relationship between the tank-tread frequency and the shear rate is investigated quantitatively through comparison with previous studies.

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