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Review Report

X-ray Micro-Imaging of Flows in Opaque Conduits

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1. Introduction

Recently bio-fluid flows have received much attention in various research areas. However, most instruments used in these researches are unsatisfactory for the *in vivo* visualization of the biological flows. Therefore, most researches have been carried out *in vitro*, using transparent substitutes of the bio-fluids. Moreover, medical instruments such as MRI and ultrasonography also have limitations in directly measuring the flow information of bio-fluid flows, despite their permeability to living organisms.

Flow visualization has become an indispensable tool in the investigation of complex flow structures. Recent advances in digital image processing techniques have made it possible to extract quantitative flow information from visualized flow images of tracer particles (Adrian, 1991). As a quantitative flow visualization method, PIV/ PTV methods have been accepted as reliable and powerful velocity field measurement techniques. Optical visualizations or PIV systems commonly use lasers as a light source. Because they can be applied only to transparent fluids with a clear window, they are ill-suited in measuring fluid flows confined in opaque materials or non-transparent fluids such as blood. To resolve these limitations, a transmission-type light source such as an X-ray or ultrasonic wave is required (Jenneson et al., 2003).

For measuring opaque fluid flows or flows inside opaque conduits, we developed an X-ray micro-imaging technique in which an x-ray beam is used as a light source. To visualize tracers of flows inside an opaque tube, an X-ray beam from the synchrotron radiation source of PLS (Pohang Light Source) was used. In the X-ray micro-imaging, the refraction or Fresnel edge diffraction mechanism was adopted to improve the image quality, whereby the relative weights of the refraction and Fresnel diffraction depend on the given experimental conditions, the type of specimen, and the information to be extracted (Hwu et al., 1999). Westneat et al. (2003) visualized tracheal respiration in insects with synchrotron X-ray imaging method. Recently, we developed a new quantitative flow visualization technique using the synchrotron X-ray micro-imaging method and visualized several opaque flows, including: glycerin flow inside an opaque Teflon tube, sap flow inside the xylem vessels of a bamboo leaf, as well as blood flow and micro-bubbles moving in an opaque tube.

2. Flow in an Opaque Tube

Lee and Kim (2003) developed an X-ray PIV technique and applied it to a liquid flow in an opaque Teflon tube with an inner diameter of 750 μm. Figure 1 shows a schematic diagram of the X-ray PIV system. The X-ray propagating a test sample is converted into a visible wavelength by passing through a thin $CdWO_4$ scintillator crystal. X-ray flow images were recorded on a cooled CCD camera with a 1280×1024 pixel resolution. Since the synchrotron X-ray is a continuous beam, a mechanical shutter was installed to make a pulse type beam for PIV applications. A delay generator was used to synchronize the mechanical shutter and the CCD camera. Alumina flakes (Al_2O_3) of 3 μm, a strong absorber of x-ray, were used as tracer particles. To match the specific weight of the alumina particles, glycerin was used as the working fluid. The working fluid seeded with tracers was injected into the tube with a syringe pump. From preliminary tests, the optimum sample-to-detector distance (d) at which the refraction-based edge enhancement is effective was determined. The field of view was 1.5×1.5 mm^2, and the spatial resolution was 12.3×12.3 μm^2. A cross-correlation PIV algorithm was applied to each pair of consecutive X-ray particle images to get the instantaneous velocity field. The mean velocity field was obtained by ensemble averaging 100 instantaneous velocity fields.

Since the X-ray image captures all particles located in the pathway of the X-ray beam, it contains amassed flow information in the direction of the X-ray propagation. The amassed 3-D flow information gives the X-ray PIV remarkable ability to directly measure amassed volumetric flow rate. For two-dimensional or axi-symmetric flows, the velocity field information in any cross-section of the flow can be obtained using a simple mathematical formula. Figure 2 shows a typical streamwise mean velocity profile along a horizontal line extracted from the mean velocity field. In this figure, dots represent X-ray PIV results, the solid line denotes theoretical center-section velocity profile, and the dotted line shows theoretical amassed velocity profile. The mean velocity profile shows a parabolic velocity distribution, and its magnitude is about two-thirds of the theoretical values due to the amassed flow image. The X-ray PIV can be used to measure the velocity fields and volumetric flow rate of any liquid enclosed in an opaque conduit, for example, blood flow in a living organism.

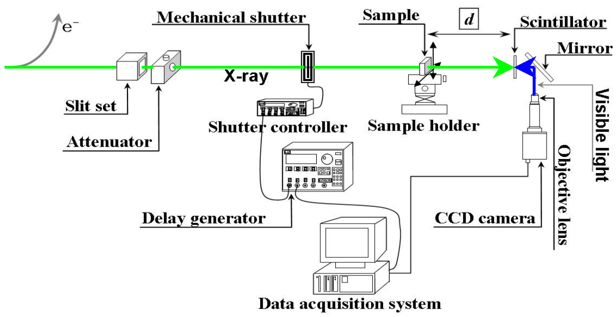

Fig. 1. Schematics of synchrotron X-ray PIV.

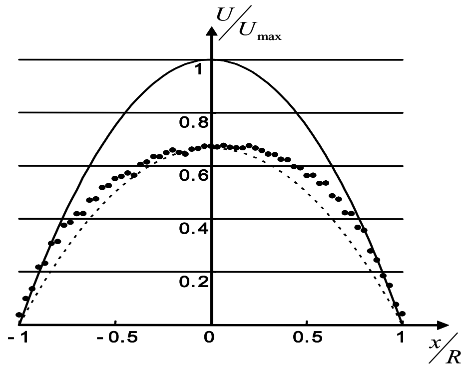

Fig. 2. Streamwise mean velocity profile.

3. Sap Flow inside Xylem Vessels

In most plants, long-distance transport of sap plays a crucial role in the exchange of nutrients and signal messengers between different plant organs. Plants have their own inherent xylem vessels to facilitate the water transport. However, most conventional measurement methods have limitations in the direct visualization of sap flow in intact plants. Kim and Lee (2003) employed the x-ray micro-imaging technique to monitor the refilling process inside the xylem vessels of a bamboo leaf *in situ*. After dehydrating the xylem vessels, water was supplied to the leaf placed vertically in a manipulation plate via the cut petiole, and x-ray micro-images were captured consecutively.

The water-rise kinetics was evaluated by tracking the position of water-front meniscus from the x-ray images. The traces of water-rise, vapor bubbles in the xylem vessels, and variations of contact angle were measured in real time. The consecutive phase-contrast X-ray images clearly show both plant anatomy and transport of water inside the vessels. The contact angle of sap with the xylem vessels was varied during the refilling process. Figure 3 shows a typical X-ray image of sap

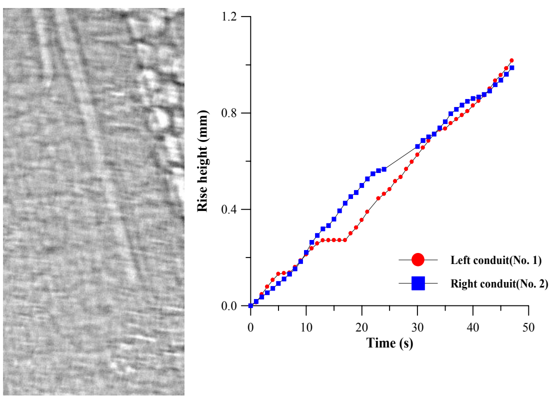

Fig. 3. X-ray image and water-rise kinetics of sap flow in xylem vessels of a bamboo leaf.

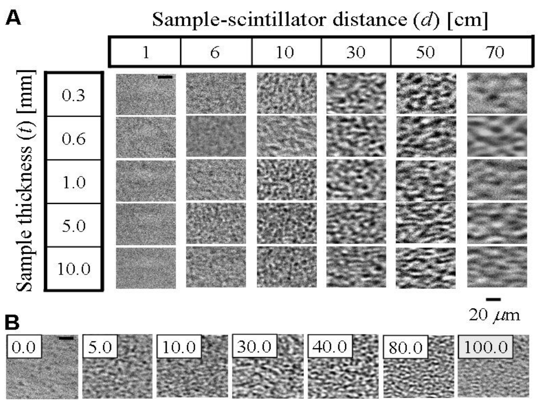

Fig. 4. Speckle patterns of blood samples.

flow in two parallel xylem vessels and their water-rise kinetics as a function of time. During the water refilling process, the rising water-front stopped at a vessel end of the left conduit for a while.

Thereafter it passed the vessel end with a higher velocity than the normal refilling speed. This indicates that the vessel end acts as a hydraulic valve in the water transport in vascular plants. Repeated cavitation was found to weaken the refilling ability of xylem vessels. From this study, the X-ray imaging technique was found to be a powerful tool in investigating the water refilling process in xylem vessels with a high resolution, compared with the MRI method (Holbrook et al., 2001).

4. Blood Flows in Opaque Tubes

Hemodynamic researches have mainly been carried out *in vitro* using transparent substitute of blood. It is not easy for MRI or ultrasonography to visualize blood flows in real time and acquire quantitative flow information due to poor spatial and temporal resolutions. On the other hand, a conventional PIV technique has a high spatial resolution, but is applicable only to transparent fluids inside a clear conduit. Therefore, it is nearly impossible to measure opaque blood flows *in vivo* or *in vitro*. For measuring blood flows, moreover, the seeding particles indispensable for PIV can affect the biological compatibility of blood.

Lee and Kim (2005a) applied the X-ray PIV technique to measure the velocity fields of real blood flow in an opaque microchannel using the speckle pattern enhancement method. Usually, the small density difference between blood cells and plasma makes it difficult to clearly visualize blood flows with the X-ray absorption or edge reflection methods. The speckle pattern of real blood was visualized with varying the sample-to-detector distance (d) and the sample thickness (t). As the sample-to-detector distance increases, as shown in Fig. 4(A), the speckle pattern of blood cells becomes detectable with induced Fresnel diffraction-based enhancement. The optimum distance was around 40 cm under the experimental condition tested. When the sample blood became thicker than 1 mm, the pattern images of blood cells became suitable in getting the velocity vectors with the X-ray PIV method. Hematocrit is another parameter for enhancing the speckle pattern of blood (Fig. 4B).

The instantaneous velocity fields were then obtained by applying a cross-correlation PIV algorithm to the acquired X-ray images without any contrast media or seeding particles. Figure 5 shows the mean velocity field obtained by ensemble averaging 200 instantaneous velocity fields. The measured velocity field data show good agreement with the typical velocity profile of flow in a rectangular channel (Lee and Kim, 2006). While the velocity profile, extracted from the mean velocity field along a horizontal line, shows some discrepancy with the theoretical profile of Newtonian flows, but agrees well with the hemorheological model suggested by Casson. From these results, we can see that the synchrotron X-ray PIV method can be used for investigating various hemodynamic phenomena experimentally.

5. Simultaneous Measurement of the Velocity and Size of Micro-Bubbles

Lee and Kim (2005b) developed an X-ray PTV technique to measure the size and velocity of micro-bubbles simultaneously. This advanced experimental technique is based on the combination of

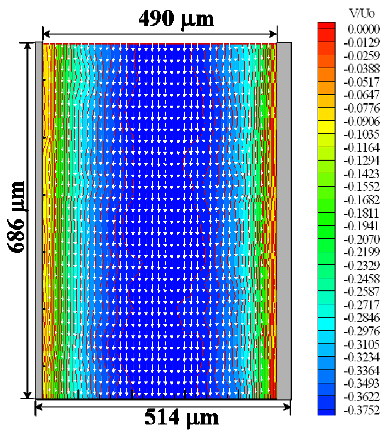

Fig. 5. Streamwise mean velocity field of blood flow.

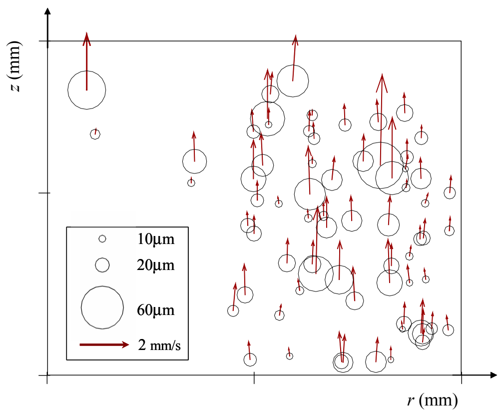

Fig. 6. Simultaneous measurement of size and velocity of micro bubbles.

the in-line X-ray holography and PTV method. Micro-bubbles (20-60 µm) were generated by heating a fine stainless wire and moved upward by buoyancy in an opaque straw (ϕ = 2.7 mm). Differently from the conventional PIV measurements of vapor bubbles (Cieslinski et al., 2005), phase contrast X-ray images clearly show the exact shape of the micro-bubbles without any optical aberrations. To measure bubble size, digital edge detection method was adopted, and the Fresnel diffraction pattern was used as a searching function. Velocity field data were obtained by tracking the centroids of individual micro-bubbles from two consecutive X-ray images using a 2-frame PTV algorithm. The field of view was 858 μm × 686 μm, and Fig. 6 shows a typical instantaneous distribution of the size and velocity of micro-bubbles. The overlapped micro-bubbles are also clearly distinguished. In addition, micro-bubbles show a spiral motion with a large pitch. For several working fluids of DI water and NaCl electrolyte solutions tested, the measured up-rising terminal velocity of the micro-bubbles is proportional to the square of the bubble diameter, agreeing with the theoretical formula (Clift, 1978).

6. Conclusion

We developed a novel X-ray micro-imaging technique in which a synchrotron X-ray beam was used as a light source. Using the quantitative X-ray imaging method combined with PIV/PTV velocity field measurement techniques, we visualized successfully several flows including: glycerin flow inside an opaque Teflon tube, sap flow inside the xylem vessels of a bamboo leaf, as well as blood and micro-bubbles moving in an opaque tube. This advanced x-ray imaging technique can be used to get useful information of various micro- and bio-fluid flows and will play an important role in visualizing veiled flow phenomena, for which conventional methods meet many difficulties to analyze.

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