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Micro-ultrasound biofluid imaging and multi-component velocity measurement with Micro Echo Particle Image Velocimetry technique

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***Abstract*—This paper presents a high-resolution microscale ultrasonic particle image velocimetry technique (termed as Micro-EPIV) for measuring multi-component velocity vectors in microscale opaque flows such as blood and biofluid flow in microvessel. The method was tested by in vitro flow imaging and in vivo small animal blood flow imaging studies. The bioflow and blood flow were seeded with ultrasound contrast microbubbles, and were "illuminated" acoustically by 50 MHz and 30 MHz ultrasound, respectively. B-mode images obtained at imaging frame rate of 10 frames per second (fps) and 110 fps were constructed from back-scattered RF signals from bubbles. Then, consecutive images were processed with optimized PIV algorithm, to acquire multi-component velocity vectors. The results were in good agreement with analytical solutions and the velocities measured by ultrasound Doppler technique.**

I. Introduction

Recently, small animal models are generally adopted in areas of medical and biological research, in order to further investigate the mechanisms of blood flow triggering the development, the regulation, and also the pathology of the circulatory system. Typically, fluid shear stress, which is related to the differentiation and regulation of tissue cells, is considered an important mediator in the development of many cardiovascular problems such as athermoma, intimal hyperplasia, thrombus and hemolysis. For example, recent molecular and cellular level studies found that hemodynamic shear gradient stimulates the endothelial cell proliferation and favors atherosclerosis development [1]-[3]. Also, many *in vitro* and *in vivo* studies found that the magnitude and rate of change of shear stress nearby vessel wall are linked to the pathogenesis of atherosclerosis [4]-[7].

Normally, wall shear stress, τ, can hardly be measured *in vivo* directly, and should be extracted from a velocity field by the calculation of the wall-normal velocity gradients, *du/dz*,

$$\tau = \eta \, du/dz \qquad (1)$$

where η is the fluid dynamic viscosity, a function of the shear rate too. The derivation of the wall shear stress from equation (1) is strongly determined by the resolution of the velocity measurement. However, Current fluid measuring methods including magnetic resonance imaging (MRI), ultrasound Doppler velocimetry techniques (UDV), and contrast-based ultrasonic particle imaging velocimetry technique (Echo PIV) [8] can only be applied to large dimension flow imaging, due to the limitation in spatial resolution. By applying high-frequency ultrasound, the imaging resolution can be greatly enhanced [9]-[14].

This paper aims to establish a contrast-based microscale ultrasonic particle image velocimetry technique (termed as Micro-EPIV) for measuring multi-component velocity vectors in microscale opaque flows. Micro-EPIV system was established, and both in vitro and in vivo experiments were conducted. Ultrasound contrast microbubbles were added as fluid tracers, and high-resolution B-mode images of bubbles were acquired. Then more accurate velocity vectors were obtained by processing consecutive images with optimized PIV algorithms.

II. Experimental Methods and Materials

A. Design of Micro-EPIV System

The technique is based on the fusion of three existing techniques, particle image velocimetry (PIV), high-frequency ultrasound imaging, and ultrasound contrast imaging, and it is termed Micro-EPIV. The system was composed of computer, ultrasound system, high-frequency ultrasound transducer, B-mode image construction, and PIV analysis algorithm (as shown in Fig.1). Ultrasound contrast microbubbles were used as flow tracers. The ultrasound transducer driven by the PC-controlled ultrasound system scanned over the field of view. Based on back-scattered RF signals from bubbles, B-mode images could be constructed. Two sequential images were then subjected to PIV analysis: the images were divided into interrogation windows and cross-correlation between the two images was applied to obtain particle displacement, allowing a velocity vector field to be determined based on the time interval between the two images.

The in vitro biofluid imaging was performed on a self-configured high-frequency ultrasound system with a 50MHz transducer, and the maximum imaging frame rate is 10 fps. The in vivo small animal imaging was performed on a Vevo 770 system (VisualSonics Inc, Toronto, Ontario, Canada) using a 30-MHz transducer mounted to a 3D motor on a rail system. The ultrasound system was especially coupled with an anesthetization system for animal experiments. The frame rate can reach 110 fps.

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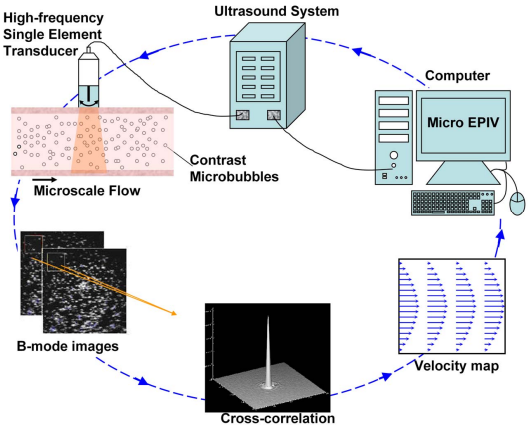

Fig. 1. Self-configured Micro-EPIV system is composed of PC, ultrasound system, high-frequency ultrasound transducer, B-mode image construction, and PIV analysis algorithm.

B. *Ultrasound Contrast Microbubbles*

Ultrasound contrast microbubbles were used as flow tracers. The bubble layer was composed of serum albumin, and sulfur hexafluoride (SF_6) was filled inside. The size distribution of microbubbles was measured by Optical Particle Sizer (Accusizer 780, PSS, Santa Barbara California), and the mean diameter is in the range of 2.0~4.5 μm. Micro-EPIV technique required higher concentration of microbubbles than Echo PIV, because of greater magnification ratio and smaller field of view. The bubble concentration of the solution used in our experiments was about $5.0\sim8.0\times10^9$ bubbles/ml. To obtain robust data, we determine the optimum concentration of bubbles for Micro-EPIV by using cross-correlation index (CCI) method. The quality of the velocity vector obtained can be quantitatively evaluated by the CCI, which is produced by cross-correlation function that indicates the effectiveness of the pattern-matching between two sub-windows.

C. *Optimized PIV Processing of B-mode Images*

Here, PIV image processing method was optimized to enhance correlation accuracy. First, sub-pixel calculation based on Gaussian peak fitting formula [15], [16] was adopted, the formula is

$$\begin{cases} \Delta x = i + \dfrac{\ln\left(r_{pq}(i-1,j)\right) - \ln\left(r_{pq}(i+1,j)\right)}{2\ln\left(r_{pq}(i-1,j)\right) - 4\ln\left(r_{pq}(i,j)\right) + 2\ln\left(r_{pq}(i+1,j)\right)} \\ \Delta y = j + \dfrac{\ln\left(r_{pq}(i,j-1)\right) - \ln\left(r_{pq}(i,j+1)\right)}{2\ln\left(r_{pq}(i,j-1)\right) - 4\ln\left(r_{pq}(i,j)\right) + 2\ln\left(r_{pq}(i,j+1)\right)} \end{cases} \quad (2)$$

where $r_{pq}(i,j)$ is the maximum cross-correlation function. i and j are the corresponding coordinates when the cross-correlation function obtains the maximum value. $r_{pq}(i-1,j)$, $r_{pq}(i+1,j)$, $r_{pq}(i,j-1)$ and $r_{pq}(i,j+1)$ are cross-correlation functions around four nearest grid points. This sub-pixel calculation can accurate the PIV process to decimal precision pixel level.

Second, the iterative scheme was processed by performing cross-correlation twice or more times. The second correlation is performed with a slightly smaller window size. The multiplication of two correlations can result in an amplification of the true velocity correlation peak, while peaks due to noise are not reinforced. This increases the accuracy of the resulting velocity vector. The iterative scheme can result in a final spatial resolution that is four times that of the first pass in each direction [17].

Third, the algorithm of filtering in frequency domain was adopted. Suppose the velocity vectors in two directions were $u(x,y)$ and $v(x,y)$ respectively, two-dimensional complex function $W(x,y) = u(x,y) + iv(x,y)$ was structured. By using Fourier transform, the frequency domain function $\tilde{W}(\omega_x,\omega_y)$ was obtained. Then low-pass filter in frequency domain was applied to get $\tilde{W}(\omega_x,\omega_y)$. Thereafter, two-dimensional inverse Fourier transform was processed and revised two-dimensional complex function can be obtained. The real part of the function corresponds to transverse component of $u(x,y)$ and the imaginary part corresponds to $v(x,y)$.

Besides, the correction based on continuity equation was utilized to correct improper vectors. The following formula is applied for the error vector analysis

$$val = \frac{\sum_{i,j}\left|u_{i,j} - u_{0,0}\right| + \sum_{i,j}\left|v_{i,j} - v_{0,0}\right|}{\sum_{i,j}\left|u_{i,j}\right| + \sum_{i,j}\left|v_{i,j}\right|} \quad (3)$$

According to the initial vector distribution, the allow value is set up. If the *val* value is greater than allow value, the vector should be corrected. If the *val* value is smaller than allow value, the vector unchanged. Wrong vectors were corrected by the formula as follow

$$u_{0,0} = \frac{\sum_{i,j} u_{i,j}}{8},\ v_{0,0} = \frac{\sum_{i,j} v_{i,j}}{8} \quad (4)$$

III. Experiments and Results

A. *In vitro artery model experiments*

The *in vitro* experimental system was established as Fig. 2. A pipe flow circulation system was constructed to simulate blood flows in microscale blood vessels. The pipe was 110 cm long with a 0.8 mm inner diameter. The replaceable *in vitro* artery model (straight model, stenosed model, or curved model) was immersed in a water tank, with water as the medium for ultrasound transmission. A micro electric pump was employed to generate steady flow with adjustable flow rate. Ultrasound contrast microbubbles were seeded into the flow as tracers. Bubbles were injected into the stirring bottle and stirred to be evenly distributed by the rotating bar. The 50MHz high-frequency ultrasound transducer dipped into water and scanned along the micro-vessel. Due to the huge acoustic impedance mismatch on the interface of bubble and fluid, the bubbles scatter strongly and "shine" acoustically in ultrasound field, resulting in a clear brightness mode (B-mode) image of the particle positions with excellent signal to noise ratio. Then, B-mode images were processed with optimized PIV algorithm to acquire 2-D velocity distribution.

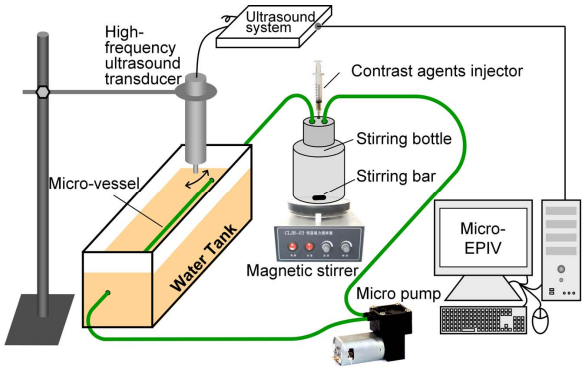

Fig. 2. The experimental system: the ultrasound transducer was driven by the PC-controlled ultrasound system, and scanned over the field of view. RF-derived B-mode images were processed with optimized PIV algorithm to obtain velocity distribution of flow in micro-vessel.

Both the laminar flow and the stenosed flow were investigated. First, a straight plastic pipe model was used, with inner diameter of 0.8 μm. The fluid was driven by a micro electric pump, and the flow rate was maintained at 1ml/h (average flow speed 0.35 mm/s) to guarantee that the flow was always stable and laminar. The flow was measured with Micro-EPIV method and the results were shown in Fig. 3. The velocity vectors along axial distance are in good agreement with analytical profile. The maximum velocity at the axis is 0.7 mm/s, twice as the average flow speed, showing good agreement between Micro-EPIV measuring results and the value acquired from the micro pump. Also, stenosed flow was measured with Micro-EPIV technique, and the results are shown in Fig. 4. The flow rate of stenosed flow is 1 ml/h, and the measured maximum velocity is 0.7 mm/s. The B-mode images and the velocity map together show the flowing feature in vicinity of the curve point.

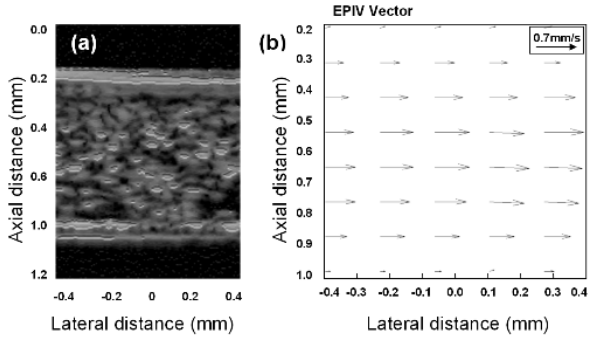

Fig. 3. Laminar pipe flow measured by Micro-EPIV: (a), B-mode image of laminar flow, the pipe inner diameter is 0.8 mm; (b) velocity map obtained, the max velocity at the axis is 0.7 mm/s.

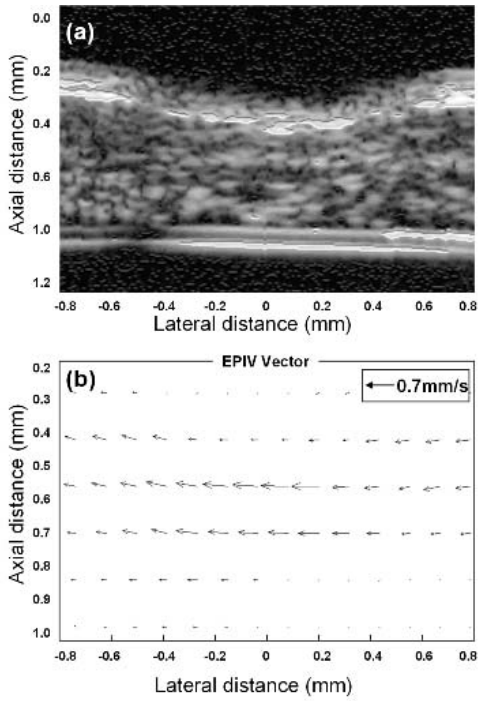

Fig. 4. Stenosed flow measured by Micro-EPIV. (a), B-mode image of stenosed flow, the pipe diameter is 0.8 mm, and 0.6 mm for the narrowest point; (b) velocity map obtained, the max value is 0.7 mm/s.

B. *In vivo animal experiments*

For the *in vivo* experiments, all procedures adhered to our institution's Animal Care and Use Committee guidelines. Rats were anaesthetized with isoflurane gas at a dose of 1% in pure oxygen at a flow rate of 1 L/min, and the body temperature was monitored and maintained at 37°C by a warming plate. The heart rate was also monitored and remained approximately 350 to 400 beats per minute. The fur on the rat abdomen around the scanning site was removed with a depilatory cream. Microbubble solution was injected into rats via tail vein, with concentration of $5\text{-}7\times10^{9}$ microbubbles per milliliter. A medical ultrasound acoustic gel was applied to the appropriate areas of the abdominal skin before performing ultrasound scans. The rats were imaged on a Vevo 770 system (VisualSonics Inc, Toronto, Ontario, Canada) using a 30-MHz transducer mounted to a 3D motor on a rail system, and the frame rate is 110 frames per second. In contrast mode, a sequence of B-mode images (as shown in Fig. 5) was recorded for latter PIV analysis, to obtain velocity vectors. Fig. 6(a) depicts the vector map obtained by processing the red square area of two consecutive images. The maximum velocity at the axis is 54cm/s. According to Fig. 6(b), the velocity obtained by ultrasound Doppler technique was about 50cm/s.

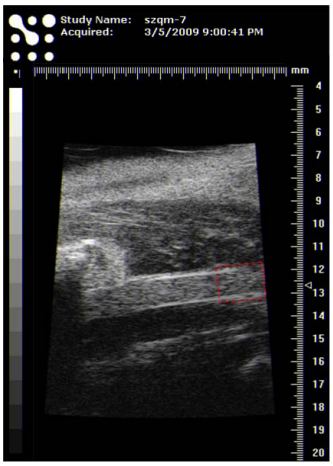

Fig. 5. B-mode image of a rat artery recorded by Visualsonics Vevo 770 system, when ultrasound contrast bubbles are inject via the rat's tail vein. The area noted with the red square was chosen to conduct PIV processing.

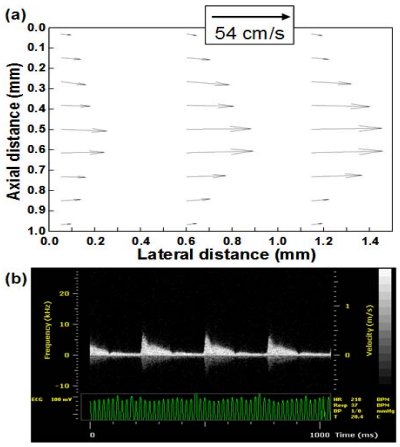

Fig. 6. (a) velocity vectors obtained by processing the area noted with the red square in Fig. 7, the max velocity is 54cm/s; (b) measured velocity by ultrasound Doppler technique, the velocity is 50cm/s.

IV. Discussion and Conclusion

This paper presents a high-frequency ultrasound-based velocimetry technique termed as Micro-EPIV and its application in imaging microscale opaque flow such as biofluids and blood flow, and measuring multi-component flow velocity distribution in microscale in vitro and in vivo. Two measuring systems were constructed for test. The in vitro system was based on a self-configured high-frequency system, and the frame rate is limited to 10 frames per second. So, the maximum velocity could be measured is relatively low. The in vivo Micro-EPIV system was based on Vevo 770 ultrasound system, coupled with an anesthetization system necessary for animal study. The Vevo 770 ultrasound system provides imaging frame rate up to 240 fps. Therefore, the maximum velocity could be measured is much bigger than the former system.

Ultrasound contrast microbubbles were used as fluid tracers, and high-frequency ultrasound beam was used to "illuminate" acoustically the bubbles in the flow, to receive and to record the scattered RF signals from bubbles. B-mode images can be constructed with good signal-to-noise and high spatial resolution. Thereafter, multi-component velocity map would be acquired by processing B-mode images with optimized PIV algorithm. Due to the utilization of high-frequency ultrasound to image contrast microbubbles, Micro-EPIV technique is a promising candidate to visualize and non-invasively characterize the complex hydrodynamics in micro blood flow and other microscale opaque flow.

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