Blood flow velocity measurements in chicken embryo vascular network via PIV approach

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Abstract

A method for measuring of blood velocity in the native vasculature of a chick embryo by the method of micro anemometry from particle images (μPIV) is improved. A method for interrogation regions sorting by the mask of the vasculature is proposed. A method for sorting of the velocity field of capillary blood flow is implemented. The *in vitro* method was evaluated for accuracy in a glass phantom of a blood vessel with a diameter of 50 μm and *in vivo* on the bloodstream of a chicken embryo, by comparing the transverse profile of the blood velocity obtained by the μPIV method with the theoretical Poiseuille laminar flow profile.

Keywords*:* digital image processing, μPIV, blood flow velocity measurements, vascular network, chicken embryo, chorioallantoic membrane, vascular morphology, in vivo.

1. Introduction

Microcirculatory bed is a transport system that is responsible for the timely delivery of nutrients and oxygen to the tissues through the blood, followed by the removal of by-products of metabolism.[1,2] The functional state of endothelial cells is one of the factors for the formation of adequate blood supply to the tissue.[3,4] Endothelial mechanisms of synthesis of biological regulators affect the tone of smooth muscles, blood coagulability and permeability of the vascular wall.[5-8]

Movement of blood cells causes shear deformation of endothelial cells, which determines the physiological response of arteries and arterioles to an increase in blood flow velocity.[9] Abnormal blood flow and modification of hemorheological blood characteristics in accordance with the configuration of blood vessels are of critical importance in the diagnosis of vascular diseases. To date, it is known that the shear stress on the vessel wall plays a decisive role in the development of the pathology of atherosclerosis and cardiogenesis.[10,11] Thus, it is the quantitative data on the rate of blood flow in vivo that can provide important information for early detection of the pathogenesis of diseases of the circulatory system.[12]

For a more detailed understanding of the mechanisms of the occurrence of vascular pathological conditions, it critical not only to measure the volume flow of blood in a separate segment of the vasculature, but also to assess its spatial distribution along a branching network section.[13]

Speckle-imaging methods (LASCA),[14] Doppler optical coherence tomography (DOCT),[15] and particle image anemometry (μPIV) are widely used for *in vivo* measurement of blood velocity.[16] Methods based on the analysis of the dynamics of laser speckles do not allow one to obtain absolute values of the velocity and direction of the blood flow.[14]

Saratov Fall Meeting 2017: Optical Technologies in Biophysics and Medicine XIX, edited by Elina A. Genina, Irina Yu. Goryacheva. Valery V. Tuchin, Proc. of SPIE Vol. 10716, 107160H

The resolving power of DOCT does not allow for measuring of blood velocity in the smallest capillaries, and it is also extremely sensitive to involuntary movement of the object under study,[15] which makes it impossible to analyze the processes of local regulation of blood supply by endothelial cells.

The μPIV method is used for measurements of the instantaneous flow rate by analyzing two or more consecutively recorded images of tracer particles moving in the flow. Normally, tracers are not distinguishable among themselves; therefore the averaged displacement of a group of tracers is determined over a certain interrogation regions of finite size to estimate the local flow velocity.[16]

The most common method in this case utilizes an estimate of 2D cross correlation of a pair of consequently captured images of the same interrogation region (IR). The shift of the maximum of the correlation function corresponds to the average displacement of particles in the image plane. When multiplied by frame rate it produces the averaged flow velocity over the IR.

Since one IR corresponds to a single velocity vector estimate a set of IRs uniformly distributed over a field of view makes it possible to get a 2D vector map of tracer velocity. Spatial resolution of the map is determined by the size of the IR that has to be small in comparison with the spatial scale of the flow velocity gradient. However it should be large enough with respect to the maximum displacement of tracer particles over the time separating of two successive images.[17]

Although the successful use of μPIV method for measurements of blood flow velocity has been reported[18] the application of the technique for blood circulation studies is still challenging and it requires further modification. The principal difference between μPIV applications for experimental fluid dynamics and intravital blood microcirculation studies relates to 1) involuntary movement of an object relative to the imaging system and 2) accurate matching of velocity map to the actual vascular network structure.

To solve the first issue we proposed to use the correlation based digital method of image stabilization prior to PIV analysis.[19] For this purpose, the rectangular image area, occupying 80% of the total frame size, was placed in the center of each frame of the image series. The offset of the maximum of the cross-correlation function calculated for the first and subsequent frames in the series displays the direction and magnitude of the displacement of the image elements relative to the first frame.[19]

The second important problem is the location of IRs of the PIV analysis with respect to the boundaries of the blood vessel. Partial overlap between IR and the blood vessel may result in an underestimation of the measured blood velocity values. A method for centering the IRs by the mask of the capillary network is proposed in this paper. We determined the mask of the capillary network manually and then we only took into account those IR whose centers are located within the vascular mask. The performance of the PIV assay method on a model of a blood vessel of 50μm in diameter was checked. Velocity map of the velocities of the capillary blood flow of the chicken embrio measured with the PIV technique is presented.

2. Materials and methods

2.1 Experimental setup

The experimental setup is an optical imaging system consisting of a digital microscope, a three-dimensional slide, a fixed base, a stationary stage and a lighting fixture. Ring illuminator composed of green light-emitting diodes with a total irradiation power of 1.56 W at a wavelength of 565 nm was used as a light source. Green LEDs were chosen to match the hemoglobin absorption peak in this spectral region.

The image of the capillaries was recorded with a digital microscope consisting of a 10x/0.27 lens, a cylindrical tube and a digital monochrome camera Basler acA2040-180km, having a resolution of 2040x2048 pixels, which corresponded to 1275x1280 μm in the object space. The camera was connected to a personal computer used for image processing. Focusing of the microscope on the object under investigation was performed by moving the imaging system along the optical axis using a screw alignment device mounted on a stationary base. Original software was developed in the LabVIEW programming environment (National Instruments, USA) for registering images of the capillary network of a chicken embryo. Recording parameters: 8-bit color depth, frame size 2040x2048, frames per second 95, exposure time 10.5 ms.

2.2 Chicken embryo

The capillary network of the chorioallantoic membrane of the 12-14 day-old chick embryo of the Cross breed "Super Nick" was used as a research object. The eggshell was removed from the side of the air chamber. Next, the outer and inner shells were removed to open access to the capillary network of the chorioallantoic membrane. A square 15x15 mm cover slip with a thickness of 170 μm was placed over a membrane prevent drying of the surface of the capillary network as well as for leveling the surface of the investigated area of the tissue.

2.3 Measurement of blood velocity

The concentration of erythrocytes in different parts of the capillary is not uniform, and therefore the movement of blood in the capillary is clearly visible in the form of displacement of sites of different brightness along the axial line of the capillary. This phenomenon allows us to estimate the rate of blood flow in the capillary using correlation algorithms.[16-18] To estimate the displacement of erythrocytes along the capillaries, the cross-correlation functions for the corresponding zones were calculated. The correlation function of the unit calculation zone has the form:[16-18]

$$\Phi_{\mathrm{k}}(m,n)=\sum_{j=1}^{q}\sum_{i=1}^{p} f_{\mathrm{k}}(i,j)g_{\mathrm{k}}(i+m,j+n), \tag{1}$$

where $f_{\mathrm{k}}(i,j)$ and $g_{\mathrm{k}}(i,j)$ are distribution of the intensity values of the corresponding calculated areas with sizes $p\times q$ pixels of two consecutive frames. Correlation algorithm implies splitting each frame of a series of images into elementary rectangular or square IR. Since blood movement takes place only within the capillary, the velocity of this movement should be measured within the areas completely overlapping the capillary. Thus, it is necessary to determine the boundaries of a branched network of capillaries.

The boundaries of the capillary network were determined by means of threshold brightness conversion. For this purpose, the image of the capillary network was binarized in such a way that the image areas corresponding to the background (brightness value from 125 to 255) assumed zero values. The remaining non-zero elements of the image were replaced by the maximum brightness value of the 8-bit image. The obtained mask of the capillary network was used to place the calculated PIV interrogation region. For the IR of 60x60 pixels, the minimum displacement of the image elements corresponds to a displacement of 1 pixel, or the erythrocyte movement speed is 59 μm/s. The maximum possible displacement of the image elements will be a displacement of 59 pixels that corresponds to the erythrocyte speed of 3.5 mm/s.

Since the image of a capillary captured *in vivo* is characterized by low contrast and significant noise, pre-processing of the image of the series was carried out before the μPIV processed; it includes subtraction of the average over the entire series of images from each image of the series. This procedure completely excludes all fixed objects from the subsequent image analysis and significantly reduces the effect of image noise on the estimation of the mutual correlation function of the pixel intensity.

Compensation for involuntary movements of the capillary network in the field of view of the microscope was carried out by the method of digital stabilization, based on the calculation of the mutual correlation function of the first and subsequent frames of a series of images.[19]

In this work, the μPIV method was also used to determine the blood velocity in the smallest vessels of the chorioellantoic membrane of a chick embryo. The proposed algorithms were tested first by using a blood vessel model. The model was made of a glass capillary tube with an internal diameter of 50 μm, glued to the slide. To balance the capillary forces, glass tubes with an internal diameter of 1 mm were glued on either side of the capillary. The capillary was filled in such a way that the water menisci were located in the central parts of the millimeter tubes, completely filling the 50-micron capillary without air bubbles.

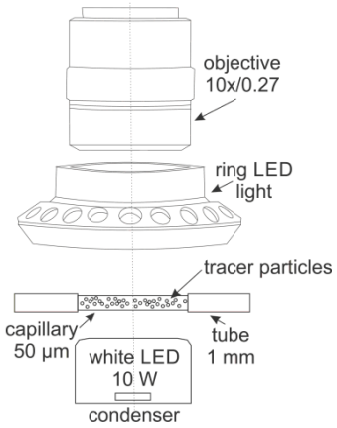

Figure 1. A glass phantom of a blood vessel.

To reduce the effects of scattering and light reflection on the outer cylindrical surfaces of the glass capillary, the entire capillary was immersed in immersion oil and covered with a cover slip. A 10% suspension of polystyrene latex with 1.5 μm diameter (Diaem, Russian Federation) diluted in deionized water (dilution 1: 100) was used as tracer particles. The particle size was 1.5 μm.

The pressure difference at the ends of the glass capillary was measured using a water manometer. An insulin syringe was used to pressurize the system. The pressure in the system varied in steps of 10 mm, water gauge.

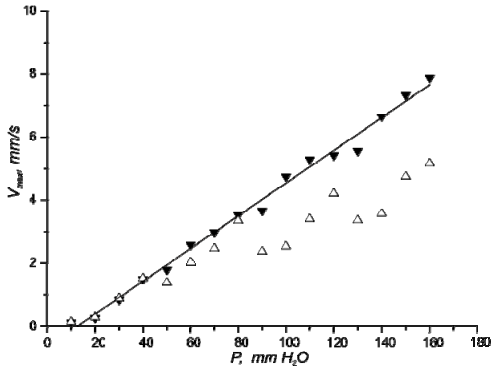

Figure 2. Maximal flow velocity vs pressure difference. Filled triangles with filling correspond to the measured values of the speed of the water meniscus. Triangles without filling correspond to the calculated values of the speed using the PIV analysis. Direct - linear regression of measured values of speed.

During each measurement, a series of images of tracer particles in a capillary with a diameter of 50 μm was registered, as well as a movement of the water meniscus in a 1 mm diameter end tube. The speed of movement of the water meniscus corresponds to the average flow velocity in the tube, and the velocity of tracer particles in the central part of the capillary is the maximum flow velocity in it. Thus the volume of fluid flowing through the cross section of the flow per unit time:

$$Q=V_{av}\,S, \tag{2}$$

where V_{av} is the average flow velocity, S is the cross-sectional area of the flow. According to Eq. 2, the average velocity in the capillary will be 400 times greater than the average flow rate in the millimeter tube glued into the capillary. For laminar flow in a cylindrical tube, the following equality holds:[20]

$$V_{av} = 0.5V_{max}, \tag{3}$$

where $V_{\max}$ is the maximum value of the flow velocity.

The maximum value of the velocity was measured by the PIV method and, according to Eq. 3, the mean value of the flow rate of the microsphere suspension was calculated. The data for velocity of the tracer particles were normalized and compared with the average values of the water meniscus velocity. The results are shown in Fig. 4. Observation of the motion of tracer particles was carried out in the transmitted white light (10 W LED), the Basler acA2040-180km camera, the x10/0.27 lens (Fig. 2). The CAM imaging parameters: 8-bit color depth, frame size 2040x2048, frames per second 187, exposure time 5 ms, number of frames in each series 100. The size of the calculated area was 200x10 pixels.

The graph clearly shows that for velocities less than 1.5 mm/s, the speed values obtained by μPIV coincide with the measured velocities. For the speed range 1.5 mm/s - 3.5 mm/s, the experimental and measured values differ by 20%. At a flow rate of more than 3.5 mm/s, the absolute values of the velocity of particle tracers in the deionized water stream cannot be calculated by the μPIV method for the IR sizes. The effects of decreasing of calculated velocity of tracer for high velocity fluid streams measured by μPIV, to some extent can be compensated by increase of the frame rate and reduction of the exposure time.

3. Results and discussion

In this paper, we proposed to center the interrogation regions according to the mask of the vascular network. For this, we put a grid with a constant step of 10 pixels on the mask of the vascular network. The point of intersection of the vertical and horizontal grid lines that hit the mask became the centers of the interrogation regions. Next we measure blood flow velocity in these IR.

a) b)

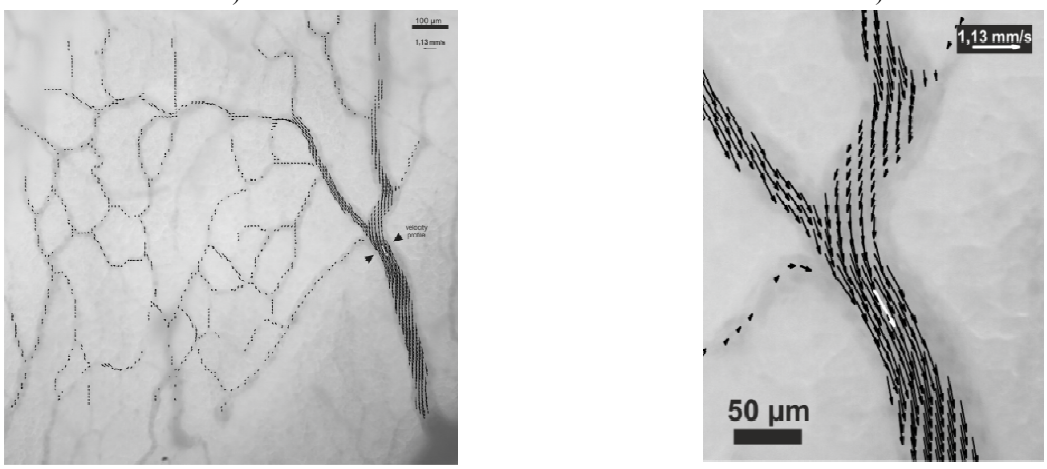

Figure 3.The instantaneous velocity field of the capillary blood flow of the chorioallantoic membrane of a 12-14 day old chick embryo (a) and an enlarged portion of the image (b).

Figure 3 shows the instantaneous velocity field of the capillary blood flow of the chorioallantoic membrane of a 12-14 day old chicken embryo (a) and an enlarged portion of the image (b). The solid line denotes the transverse blood velocity profile measured by the adaptive μPIV method. The results of the μPIV analysis were compared with the parabolic velocity profile because in small venules blood flows are laminar. Of course, the blood can be considered as a two-phase medium consisting of plasma and red blood cells, but in this paper we used a simplified model of the parabolic Poiseuille flow profile, specifically for laminar fluid flows.

$$v(r)=\frac{p_1-p_2}{4\eta\eta}(R^2-r^2), \tag{4}$$

or

$$v(r)=v_{max}[1-\frac{r^2}{R^2}], \tag{5}$$

where v is the blood velocity, r is the distance from the capillary axis, R is the radius of the capillary, $p_1 - p_2$ is the pressure difference at the ends of the capillary, L is the length of the capillary and η is the viscosity of the blood.

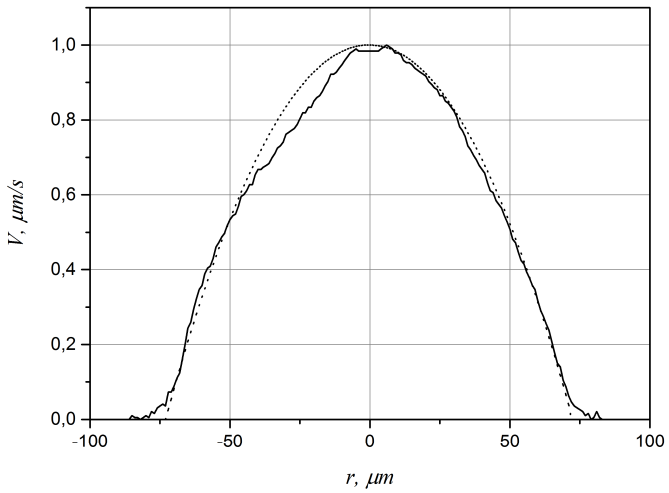

Figure 4. The solid line corresponds to the normalized measured cross-sectional blood velocity profile by the μPIV method. The dotted line corresponds to the normalized parabolic profile of the velocity of the laminar flow of a liquid according to Poiseuille distrubution. The profile was calculated by the line mask of the blood vessel cross-section in the zone after y-bifurcation with a step of 1 pixel using μPIV method.

The resulting velocity profile, indicated by the dashed line in Fig. 4, almost completely coincides with the Poiseuille flow profile. It can be explained by the fact that in a real blood vessel the concentration of erythrocytes, and therefore the relative viscosity of the blood, assumes the maximum value on the axis of the vessel and the minimum in its near-wall region.[18] The difference in shape, as well as the lack of symmetry of the measured μPIV velocity profile by the method, is determined by the character of blood flows near the bifurcation zones.[20] On the left side of the measured velocity profile, a small dip is observed due to the presence of a vessel flowing into the bifurcation above the measurement zone on the same side and with a lower blood speed relative to the remaining vessels flowing into the y-bifurcation.

Conclusion

In this paper, we present an adaptation the classical anemometry method for particle imaging (μPIV) for working with living objects and calculating the instantaneous velocity field of a visualized capillary network. The method of sorting of

the IR by the mask of capillary blood flow is implemented. An instantaneous velocity field for a branched network of vessels is calculated. The original software for recording images and digital analysis of capillary blood flow is developed.

The use of the μPIV method for calculating the rate of capillary blood flow in a chicken embryo *in vitro* and *in vivo* was evaluated. It was found that the *in vitro* method gives a 5% variance in the measured and calculated values of the flow velocity of tracer particles for the latex suspension rate up to 1.5 mm/s, which corresponds to the range of capillary flow rates in small vessels. An estimate of the accuracy *in vivo* showed a very good coincidence of the measured transverse blood velocity profile with the theoretical Poiseuille velocity profile.

In this paper, it was shown that for latex spheres, the liquid flow rate up to 1.5 mm/s can be qualitatively measured by the μPIV analysis method. For flows with a velocity of 1.5 mm/s to 4.5 mm/s, a variation of approx. 20% was found. However, in a real blood vessel it should be somewhat higher, as the vessel wall thickness and hematocrit also increase with increasing vessel diameter, making the particles tracers (erythrocytes) indistinguishable.

It can be concluded that for blood vessels with a blood velocity above 1.5 mm/s, the μPIV analysis method is able to determine the direction of the blood flow, as well as its relative change to a greater or lesser extent. However for the measurement of absolute velocities this method becomes unsuitable. However, the blood velocity in the smallest capillaries does not exceed 1.5 mm/s.

In this paper, we demonstrated measurements of the RBCs velocity profile in a chick embryo and calculate the blood velocity for a branched network of capillaries. This means that further modification of the method will allow for calculating blood volume flow maps over a branched network of blood vessels in tissues, which in a good characterization of tissue functioning.

Acknowledgments

This work has been performed in the framework of research supported by the Russian Ministry of Education and Science, project No. 3.1586.2017/4.6. MAK acknowledges the support from the grant No. 8959ГУ/2015 of Fund for Assistance to Small Innovative Enterprises in Science and Technology UMNIK-2015, and VVT to RFBR grant No. 17-02-00358.

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