OFFPRINT

LASCA with a small number of scatterers: Application for monitoring of microflow

S. Ulyanov, Y. Ganilova, Dan Zhu, Jianjun Qiu, Pengcheng Li, O. Ulianova and Qingming Luo

EPL, 82 (2008) 18005

Please visit the new website
www.epljournal.org
TAKE A LOOK AT THE NEW EPL

Europhysics Letters (EPL) has a new online home at www.epljournal.org

Take a look for the latest journal news and information on:

- reading the latest articles, free!
- receiving free e-mail alerts
- submitting your work to EPL

www.epljournal.org
LASCA with a small number of scatterers: Application for monitoring of microflow

S. Ulyanov\textsuperscript{1(a)}, Y. Ganilova\textsuperscript{2}, Dan Zhu\textsuperscript{3}, Jianjun Qiu\textsuperscript{3}, Pengcheng Li\textsuperscript{3}, O. Ulianova\textsuperscript{4,1} and Qingming Luo\textsuperscript{3}

\textsuperscript{1} Saratov State University - Russia
\textsuperscript{2} Saratov State Medical University - Russia
\textsuperscript{3} Huazhong University of Science and Technology - Wuhan, China
\textsuperscript{4} Saratov State Agrarian University - Russia

received 3 December 2007; accepted in final form 13 February 2008
published online 17 March 2008

PACS 87.64.-t – Spectroscopic and microscopic techniques in biophysics and medical physics
PACS 82.70.-y – Disperse systems; complex fluids

Abstract – LASCA, combined with cross-correlation technique, has been applied for high-resolution measurements of the velocity of microflow and its structure in small blood capillaries in vivo. Such measurements have been carried out for the first time behind the resolution limit of the optical system.

Alteration of blood flow characteristics and the structure of blood flow in a single microvessel must be considered to contain important pathogenic and diagnostic information. Essential change of the character of blood motion in capillaries may arise at the application of drugs. Investigation of such disorders, for instance in the vessels of the rat mesentery, may form the basis of the screening of medical preparations and study of the influence of toxins [1].

It is important to note that the character of microflows in small blood vessels is very complicated. The plasma fluid in such vessels cannot be considered as a Poiseuille flow in the gap “erythrocyte membrane”-“vessel wall”. The velocity of erythrocytes is not equal to the average velocity of blood plasma. The mentioned facts impede the development of an adequate optical model of the smallest microvessels. So, the analysis of blood microfluids in vessels with diameter of about the erythrocyte size is very difficult from the optical viewpoint.

Video capillaroscopy (videomicroscopy) is very often used for the characterization of blood microflow, see, for example, ref. [2]. This technique allows observing single red blood cells (RBC), tracking them and measuring their velocity. However, video capillaroscopy cannot be used for the characterization of blood plasma flow.

The method of laser speckle contrast analysis (so-called LASCA) has been recently developed [3–7]. Another name for LASCA, which is also often used in the literature, is LSCI (laser speckle contrast image). Unlike the classical videomicroscopy, the method of LASCA/LSCI allows to visualize hidden microvessels, for example, cerebral vessels.

Unfortunately, LASCA [3,8] is characterized by relatively low spatial resolution (typically about 100\(\mu\)m [9]) and does not allow to measure the velocity of blood flow, but only the contour of a single vessel. So, in practice, LASCA does not have any advantages over video capillaroscopy. The quality of the images of the vessel, obtained using conventional videomicroscopy, is higher than the quality of the laser speckle contrast image, compare fig. 1a and fig. 1b. Moreover, the application of specific algorithms allows to essentially enhance the resolution of reconstructed images [10].

Doppler microscopy [11] and speckle microscopy [1,12] also may be effectively applied for diagnostics of blood microvessels. The spatial resolution of these methods is very high. Nevertheless, efforts to apply Doppler microscopy, speckle microscopy and diffusing-wave spectroscopy (DWS with a small number of scattering events [13]) for the investigation of blood flow in the smallest vessels with diameter of about RBC size did not give positive results [14,15]. So, the mentioned methods permit only
to roughly estimate the average velocity of blood flow in microvessels.

The main purpose of this brief report is to demonstrate the possibility of high-resolution LASCA (implying speckles with a small number of scatterers), combined with the cross-correlation technique (CCT) [16], for blood flow measurements in smallest capillaries in vivo.

As is commonly known, LASCA and classical CCT operate with fully developed speckles [17]. In this case, the optical system should not resolve single inhomogeneities of the investigated scattering object. If the imaging system comes to resolve single inhomogeneities, then speckles with a small number of scatterers [18–21] or small-N speckles [22] are formed. This is a very specific class of speckles which is not investigated in detail at the present time. The dynamics of these speckles in the image plane does not have character of pure translation. The average speckle size in the image plane is essentially larger than the size of the image of the observed microvessels. The scattering from a single moving red blood cell is very weak; that is why the temporal contrast of dynamic image speckles is very low. At the same time, the statistics of these speckles is a non-Gaussian one, as a result, the spatial contrast of small-N static speckles [20] is higher than unity. Low values of the temporal contrast of dynamic speckles and the high contrast of static speckles make extremely difficult the direct observation of images of the smallest microvessels using a high-resolution laser microscope.

The typical coherent image of a small blood vessel of the white-rat mesentery is presented on fig. 2. Evidently, the contour of the blood vessel cannot be clearly observed with microscope with a laser source because of the influence of small-N image speckles. So, the situation in the area of coherent microscopy is the following: on the one hand, the magnification and the numerical aperture of the microscope should be increased to resolve smallest microvessels. On the other hand, with the increase of the resolution of the imaging system, fully developed speckles are destroyed because of the formation of speckles with a small number of scatterers, so the traditional CCT breaks down.

Nevertheless, as will be demonstrated below, high-resolution CCT can provide further development of coherent imaging of the smallest microvessels.

The experimental set-up is mounted on the base of a standard transmittance microscope with immersion micro-objective (magnification $M = 90\times$, numerical aperture $NA = 1.35$). A laser source with wavelength of 650 nm is used for the illumination of the white-rat mesentery. A phoenix PC-1280 CMOS camera (MuTech Co, USA)
with resolution 1280 × 1024 pixels and pixel size of 5.2 µm is placed in the image plane. Sequences of one hundred speckle images (grabbed at a rate of 15 frames per second) are stored in a computer. The averaged speckle size (∆S) in the image plane, evaluated as

$$\Delta S = 3\lambda \frac{(M + 1)}{2NA}, \quad (1)$$

is about 60 µm (which is more than ten times larger than the pixel size), thus the speckle structure is completely resolved by the CMOS camera.

In the described set-up, the size of the field of vision is about 90 µm. The size of the resolution cell (RS), estimated as the width of the central lobe of the Airy disc according to

$$RS = 1.22 \frac{\lambda}{NA}, \quad (2)$$

equals 0.57 µm, i.e. a little less than the wavelength.

Clearly, when the position of the object plane of the micro-objective (in the z-direction) is matched with the center of the vessel, speckles of the largest size are observed by the CMOS camera. This effect allows to adjust the optical scheme very easily.

The depth of focusing (DOF) can be estimated as

$$DOF = \frac{8\lambda}{\pi} \left( \frac{1}{2NA} \right)^2. \quad (3)$$

In the considered scheme, the DOF is about 0.2 µm. Such small depth of imaging permits to precisely select the extremely thin central layer of microflow.

So, since RS and DOF are smaller than the wavelength, the described optical scheme provides a sub-wavelength resolution of the spatial structure of microflow in the smallest microvessels.

It is important to note that such small values of RS and DOF make the optical system very sensitive to the shift of the mesentery, caused by the breath of the animal and involuntary muscle contractions. The motion of the mesentery should be carefully suppressed during the registration of speckle patterns.

Microvessels with diameters a little smaller than 5 µm are taken for the analysis. In such vessels red blood cells move like “train-like” particles [1,23]. They are slightly deformed, but keep their forms, while moving along the vessel.

The procedure of speckle processing consists of two steps. At the first step the laser speckle contrast image (LSCI) is calculated in accord with a standard procedure [8,9]. To obtain LSCI, local contrast is calculated over a small neighbourhood of 7 × 7 pixels. Evidently, the LSCI size is 7 times smaller than the initial size of raw frames (if contrast is calculated over a neighbourhood of 7 × 7 pixels). So, the original white-light image (fig. 1a) is also reduced by 7 times using local averaging, to be compared with LSCI (fig. 1b).

Clearly, the quality of the LSCI of a blood microvessel, presented in fig. 1b, is very low. As has been already mentioned, the application of methods of classical white-light video microscopy has provided so far better results, see fig. 1a. Nevertheless, incoherent microscopy has a serious disadvantage. It does not allow identifying the vessels, which are definitely containing moving red blood cells. Sometimes, in the smallest vessels, even in norm, blood flow can be stopped. In spite of the application of classical white-light microscopy, smallest vessels with moving blood can be found using the LASCA method.

At the second step of speckle processing, the fragment of LSCI, containing the image of a microvessel with moving RBC, is selected, see fig. 3. The parts of LSCI which relates to moving particles are characterized by lower values of the local contrast, and this acts as a criterion for selection. Then displacement of speckles in a selected area of LSCI has been analyzed using the standard CCT algorithm [16,24].

The typical curve of a cross-correlation coefficient (CCC) of LSCI, formed with a small number of scatterers is shown in fig. 4. Usually, CCC contains several maxima. As experimental investigations show, the amplitudes of these maxima exceed at least by 3 times false peaks which are conditioned by sample errors.
As can be seen from fig. 4, the positions of the maxima of CCC correspond to the displacement of LSCI speckles to 80 and 160 $\mu$m, respectively. Taking into account that the magnification of the imaging system is 90× and the frame rate is 15 FPS, we can conclude that microflow in the considered blood microvessel has multivelocity character (with main velocities approximately 13 and 26 $\mu$m/s).

At the present moment, despite the great interest in monitoring the motion of scattering objects using CCT, this method has never been applied for the study of blood microcirculation in the vessels with diameter of about or less than the erythrocyte size, i.e. over the resolution limit of the LASCA imaging system. In this brief report it has been done for the first time in the case of the formation of small-$N$ image speckles. It is shown that the combination of LASCA and CCT opens the perspectives of analysis of complicated multivelocity microflows.

***

This work has been funded by the Russian Foundation of Basic Researchers (Grant No. 06-04-39016) and the National Science Foundation of China (Grants No. 3071120171, No. 90508003, and No. 30770552).

REFERENCES
