

Quantification of absolute blood velocity using LDA

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ABSTRACT

We developed novel schematics of a Laser Doppler anemometer where measuring volume is comparable with the red blood cell (RBC) size and a small period of interference fringes improves device resolution. The technique was used to estimate Doppler frequency shift at flow velocity measurements. It has been shown that technique is applicable for measurements in whole blood.

Keywords: laser Doppler anemometer, blood microcirculation, light scattering, digital signal processing

INTRODUCTION

Laser Doppler anemometry (LDA) is widely used for blood flow velocity measurements and is well established as a method for analyzing particle movement at a single point in a fluid based on the Doppler effect.^{1,2} Quantitative assessment of blood velocity is very demanded in different fields of physiology and medicine.³⁻⁵ Currently available devices for evaluating and validating blood flow used in medical diagnosis and physiological studies include Doppler optical coherence tomography (DOCT),⁶⁻¹¹ laser speckle contrast analysis (LASCA),^{12,13} ultrasonic Doppler,¹⁴⁻¹⁵ particle image velocimetry,^{16,17} scanning laser Doppler flowmetry.¹⁸ Ultrasonic measurement alone cannot result in microvascular imaging, since the spatial resolution is poor. Spatial resolution of microvascular imaging is dramatically improved when the laser Doppler velocimeter (LDV) is used.^{13,14} However, measurements done by LASCA is qualitative, and the absolute velocity cannot be measured. In large vessels such as arterioles and venules due to the specific movement of RBC the image of the cells is blurred and make the application of the particle image velocimetry method impossible.^{16,17} DOCT has been proposed to determine blood vessel spatial position by scanning two adjacent sections¹⁹ or by eliminating of uncertainty in blood flow velocity at integration velocity components over a vessel cross-section area.²⁰

In our previous works,^{21,22} we proposed the novel technique to suppress low frequency components of LDA signal. Our approach is based on elimination of spectral components caused by movement of the scattering particles outside the measuring volume. This method does not require any devices for pre-shifting frequency of laser light. Also we have illustrated the application of the developed modification of LDA device to the *in vivo* measurement under the conditions of pronounced modulation of the blood flow. Our results show that

normal living pulsations of blood flow can considerably complicate the operation of LDA. Specifically, since the measurements are based on determination of peak position in power spectra, its broadening and splitting makes the use of laser Doppler anemometer *in vivo* rather challenging.²³ In the framework of current research we apply our method using a new schematics where the intersection angle of the two beams is 90° that will improve device resolution.

2. MATERIALS AND METHODS

2.1 Laser Doppler Anemometer (LDA)

An LDA scheme is presented in Fig. 1. As a light source we used the diode laser module ML-09 (Skat-R, Russia) with *cw* radiation output of 15 mW at wavelength of 650 nm. Collimated laser beam passes through a lens used as a beam expander. Expanded laser beam was divided into two perpendicular beams with beam splitter cube. One of the beams is directed by the mirror 1 and the other by the mirror 2, and falls on the entrance pupil of the micro-lens 1 and the micro-lens 2, respectively. Beams focused in fluid flow by microobjectives with the equal focal lengths of 27 mm and clear aperture diameter of 6 mm. LDA probe volume is an intersection of coherent laser beams, which forms an interference fringes system transversely to flow direction. The intersection angle of the two beams is close to 90° .

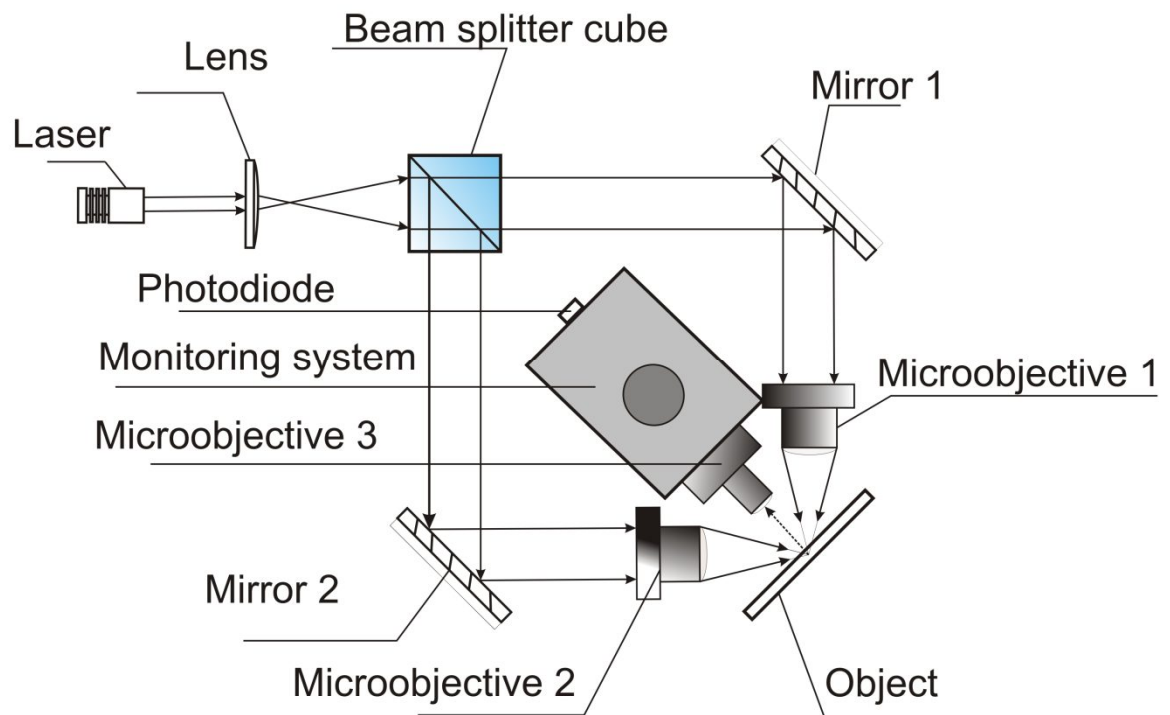


Fig. 1. The experimental setup

When a particle in the flow intersects interference fringes within probe volume it scatters light toward detector (photodiode PD-256) which is located in the system for monitoring/detection.

2.2 Monitoring/detection system

Monitoring system is presented in Fig. 2. Thus each time a particle passes through the measurement volume, backscattered light passes through the microobjective 3 with focal length of 8 mm and 6 mm clear aperture diameter. The triangular prism directs the beam onto the dichroic mirror and then an image of the measuring volume projected simultaneously onto camera (Thorlabs) and onto a 300 μm pinhole, placed in front of a photodetector that registers pulsed modulation, which frequency is proportional to particle velocity. Electric signal from the photodetector is recorded by a personal computer sound card.

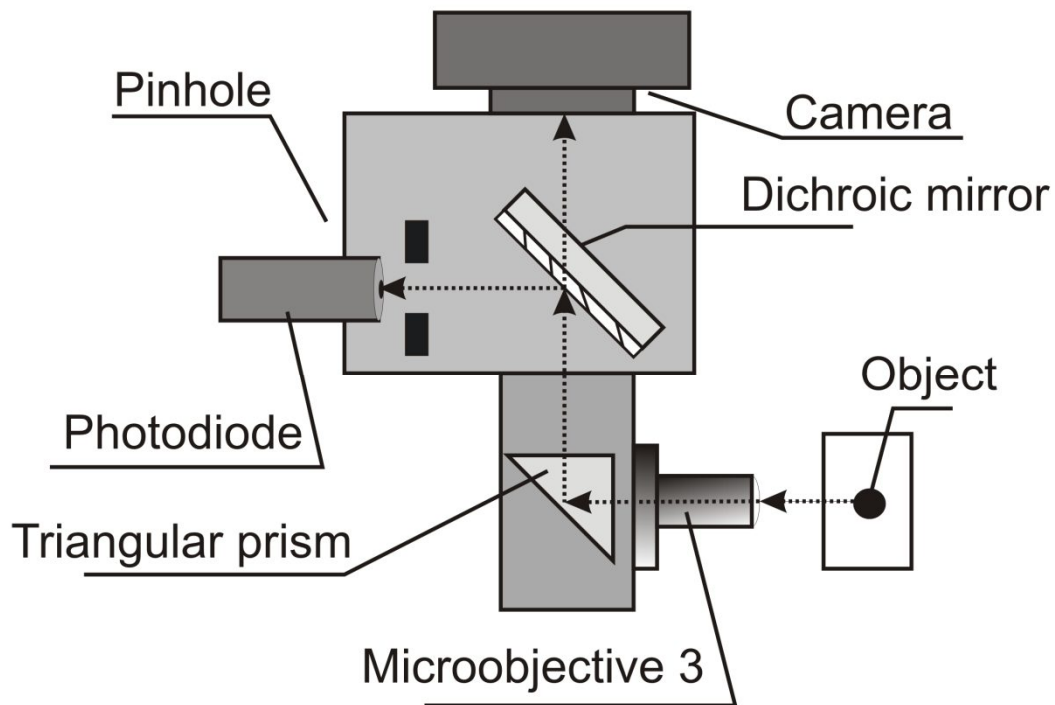


Fig. 2 Monitoring/detection system

Such a system is very important for tuning the intersection of beams in the area of interest, where we are going to measure the velocity

2.3 Object of study

To demonstrate the capability of the proposed technique to measure flow quantitatively, we performed measurements for a human blood pumped through the glass tube with diameter 100 μm . The tube has been placed between 2 glasses with thickness 15 μm . As an immersion medium between glasses and tube external wall we used silicon glue. At the ends of the tube there are plastic tubes with a suitable diameter. LDA measurement volume was located within the center of the tube

2.4 LDA signal analysis

Photodetector's signal was passed to the audio adapter of a personal computer with a frequency 44100 kHz and 16-bit resolution. In order to determine the Doppler frequency shift we use the Fast Fourier Transform (FFT) of recorded time series. Specifically, both FFT and other stages of signal processing were performed with the LabVIEW (National Instruments, USA). The typical observation time was 10 s, it was splitted into periodograms with length of 512 points each. This delivered us about 861 periodograms per measurement. Since a high-frequency component of the power spectrum which corresponds to the modulation of the scattered light is hidden by the low-frequency component (which is often has higher intensity and broader frequency band), we performed the averaging of non-overlapping modified periodograms using the Hanning data window²⁴

3. RESULTS AND DISCUSSION

3.1 The dependence of the signal on probe volume

Velocity measurement is directly related to intersection angle between two laser beams directed into the flow. At increase of angle between the beams, the fringe period within measuring volume decreases. This makes possible to make the size of the probe volume small and at the same time to preserve a large number of interference fringes.

In this study, the length of the measuring volume was 10 μm that is close to the diameter of human erythrocyte 6-8 μm , intersection angle between two laser beams is approximately 90° , and fringe period is 0.46 μm . In Fig. 3 (a), the spectrum obtained by measuring the flow velocity on the axis of glass tube with diameter 100 μm , through which whole blood was passed, flow velocity is 203 $\mu\text{m/s}$ and blood diluted with saline 1:10 flow velocity is 649 $\mu\text{m/s}$ (b).

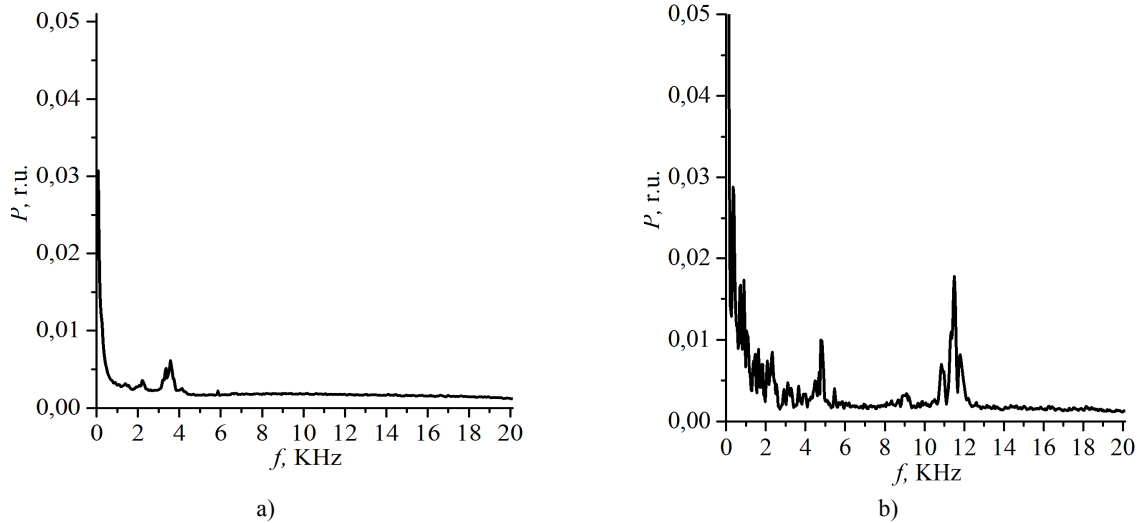


Fig. 3 a) The normalized spectra of the positive values of obtained by measuring the flow rate of whole and diluted blood used the setup where the angle between the beams was close to 90° and fringe period was $0.46 \mu\text{m}$ spectrum obtained by measuring the flow velocity on the axis of glass tube with diameter $100 \mu\text{m}$, through which whole blood was passed; flow velocity is $203 \mu\text{m/s}$ (a) and blood diluted with saline 1:10 flow velocity is $649 \mu\text{m/s}$ (b). The maximum corresponding to the frequency shift on these spectra is well distinguishable in whole blood as well as when the blood dilution is 10 times.

The maximum corresponding to the frequency shift on these spectra is well distinguishable in whole blood as well as when the blood dilution is 10 times. It is connected with the fact that at the size of the measuring volume is comparable with the particle size and period of interference fringes is small. We can measure the velocities of the individual particles and a large number of particles moving at lower velocities in the stream do not fall into the measuring volume and cannot lead to broadening of the peak.

3.2 Measuring depth and particle concentration

Although the size of the measuring volume is comparable with the RBC size, due to high concentration of RBC in a whole blood, it is still not small enough to obtain velocity information from the individual particle. Figure 4 illustrates how several particles with different velocities in the range from 137 to $220 \mu\text{m/s}$ moving across the measuring volume give several DFS (Doppler Frequency Shift) peaks.

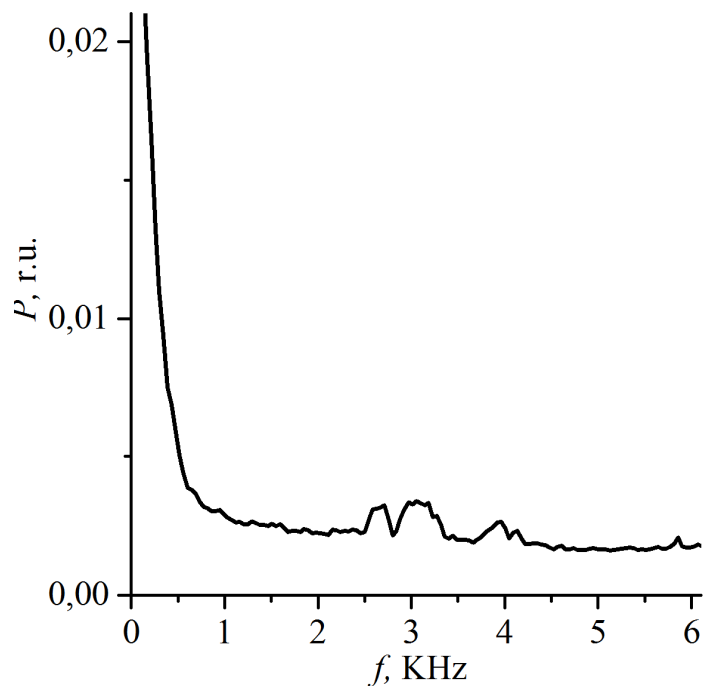


Fig. 4. The normalized spectra of intensity fluctuations: several DFS peaks, obtained from the particles, simultaneously

When velocity is measured on the surface, a distinct peak is visible because of a small size of the measuring volume. When the measuring volume is displaced in depth more than $15\ \mu\text{m}$, the peak broadens. That happens due to scattering of laser light outside the measuring volume and thus makes the largest contribution to the low-frequency component of the LDA spectrum. In Fig. 5 LDA spectrum with the low-frequency component (curve 1) is shown. As curve 2 shows spectrum with subtracted signals obtained for the measuring volume illuminated by each beam separately at blood flow velocity of $20\ \mu\text{m/s}$

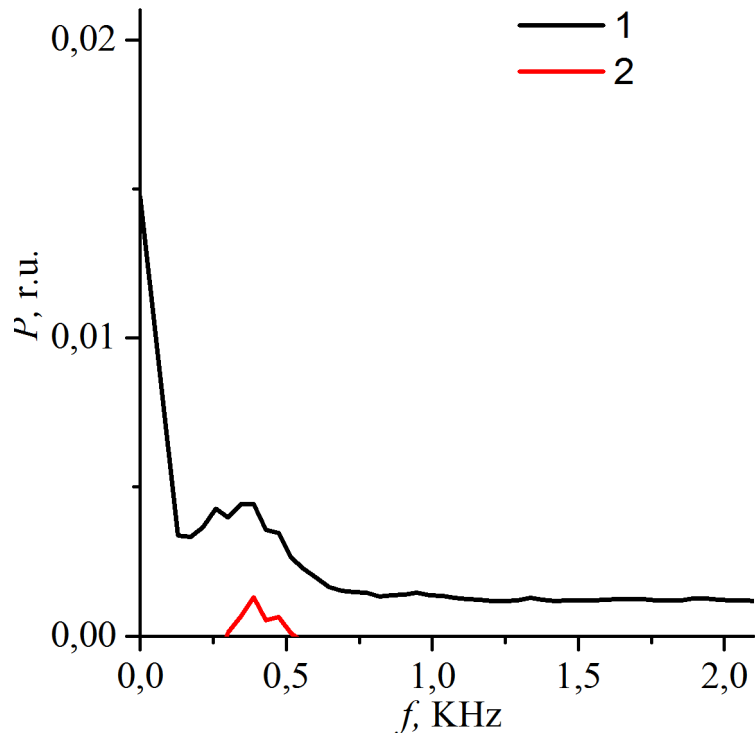


Fig. 5 The normalized spectra of intensity fluctuations: 1- raw data, 2- differential spectrum

We considered and illustrated with examples flow velocity estimates in assumption of a constant velocity of a flow. Applied method based on elimination of spectral components caused by movement of the RBC outside the measuring volume,^{21,22} using a new schematics where the intersection angle of the two beams is around 90° that is improved device resolution.

CONCLUSION

We proposed method of elimination of spectral components caused by movement of the scattering particles outside the measuring volume. New schematics are based on the use of measuring volume comparable with the particle size. In the same time, a small period of interference fringes will improve device resolution. During measurements on a surface of the tube the resolution does not depend on concentration of particles. For in depth measurements, peak is broadening due to scattering by RBC travelling outside the measuring volume makes the largest contribution to the low-frequency component. Proposed LDA differential scheme provides possibility of measuring the flow rate of whole blood in a 100 μ m vessel.

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