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Recording of lymph flow dynamics in microvessels using correlation properties of scattered coherent radiation

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***Abstract*. The direction-sensitive method of microflow velocity measurements based on the space – time correlation properties of the dynamic speckle field is described and used for *in vivo* monitoring of lymph flow in the vessels of rat mesentery. The results of measurements are compared with the data obtained from functional video microscopy of the microvessel region.**

Keywords: *speckles, radiation scattering, laser medicine.*

1. Introduction

The field of laser radiation scattered by a diffusely scattering object has a characteristic speckle structure. This structure results from the interference of independent contributions from a large number of scattering centres, is of random nature, and is called speckle field [1, 2]. For a moving scattering object, the intensity of the speckle field varies in time and space. The intensity fluctuations are statistical and depend on the structure of the scattering object, parameters of the laser beam illuminating the object, and the position of the observation plane [1 – 4]. The interrelation between the dynamics of the object scattering the laser radiation and the statistical properties of the speckle field formed in the process has been studied for the last several decades and is used widely in laser measuring techniques [1 – 5].

Biological and medical research is one of the rapidly growing areas in which the laser measuring technique is applied [6, 7]. In particular, various methods employing the properties of the speckle field formed as a result of scattering of laser radiation by blood vessels are used for measuring the velocity of blood flowing in individual vessels and for evaluating the intensity of microcirculation in various organs and biological tissues [6 – 12].

The speckle field of a laser beam scattered by a blood vessel is the result of interference of light waves scattered by blood cells moving at different velocities in the flow, and those scattered by immobile tissues surrounding a vessel. The fluctuations of the speckle field intensity have a quite complicated form in this case. The properties of such a speckle field differ considerably from the properties of speckle fields that are formed, for example, upon a single scattering of laser radiation on a moving screen with a rough surface, and have been studied extensively [10]. A special term 'biospeckles' was introduced to emphasise the peculiar nature of this speckle field. Because of the extreme complexity of biospeckles, a theoretical explanation of this effect has not been obtained so far. Even an exact interrelation between speckle field intensity fluctuations and the velocity of blood flow has not been established [9]. However, numerous experiments using blood vessel models indicate that the width of the autocorrelation function or of the power spectrum of the speckle field intensity fluctuations is linearly connected with the mean velocity of flow of the blood cells [10 – 12].

In the blood or lymph flow studies, the speckle field intensity fluctuations are detected at one point, and the flow velocity is estimated from the width of power spectrum of these fluctuations or from the width of their autocorrelation function [6 – 13]. Measurements of this type cannot be used to determine the direction of flow in blood or lymph vessels because the speckle field fluctuations recorded at a point do not depend on the direction of motion of the scattering object.

Another method of measuring the velocity of motion of an object is based on the recording of intensity fluctuations of the speckle field at two points separated in space and on an analysis of their mutual correlation [3, 5]. This method makes it possible to determine the velocity as well as direction of motion of an object and is used in various technical applications [5]. However, the possibility of using this method for measuring the blood and lymph flow velocity has not been investigated so far.

Earlier, it was shown in experiments that the space – time correlation properties of intensity fluctuations of the speckle field formed upon a single scattering of a focused beam of coherent radiation from a small number of particles moving in the flow allow us to determine the velocity of the flow and its direction by analysing the mutual correlation of speckle field intensity fluctuations recorded at two points separated in space [14 – 16].

In this work, we describe the experimental setup for *in vivo* measurements of the blood and lymph microcirculation by the method of cross-correlation analysis of speckle field intensity fluctuations recorded at two points separated in space. This setup can be used to detect the changes in the velocity and direction of flow. The results of experiments

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based on the lymphatic microvessel model and the *in vivo* measurements of the lymph flow velocities in the lymphatic vessel of the mesentery in the small intestine of a rat are presented. The results of *in vivo* measurements are compared with the flow velocity data obtained by using the functional video microscopy technique.

2. Space – time correlation properties of dynamic speckle fields

Consider the basic principles of the method. It is known that the space and time fluctuations of the intensity of the speckle field formed upon scattering of coherent radiation by a moving object are not statistically independent [1 – 3]. In particular, for the scattering of a Gaussian beam of coherent radiation by a moving random phase screen (RPS), the space – time correlation function of the speckle field intensities recorded at two points has the form [3]

$$g_I(\boldsymbol{r},\tau)-1=\exp\left(-\frac{|\boldsymbol{v}|^2}{w^2}\tau^2\right)\exp\left[-\frac{1}{r_s^2}\left|\boldsymbol{r}-\left(1+\frac{l}{\rho}\right)\boldsymbol{v}\tau\right|^2\right], \quad (1)$$

where $\boldsymbol{r}$ is the difference in the coordinates of the points where measurements are made in the observation plane; τ is the time delay; $\boldsymbol{v}$ is the velocity of motion of the RPS; w is the radius of the illuminated RPS region; ρ is the radius of curvature of the wave front in the RPS plane; l is the distance between the RPS plane and the observation plane; $r_s = 2l/(k_0 w)$ is the mean speckle size; and k_0 is the wave number of the incident radiation. One can seen from (1) that the translation velocity of the speckle field in the observation plane is [3]

$$\boldsymbol{v}_s=\frac{\boldsymbol{r}}{\tau_d}=\left(1+\frac{l}{\rho}\right)\boldsymbol{v}, \quad (2)$$

where τ_d is the position of the correlation function maximum. The speckle field will continuously change its structure, and the correlation decreases by a factor of e as the speckle field traverses a distance

$$r_{tr}=\left(1+\frac{l}{\rho}\right)w. \quad (3)$$

If $\boldsymbol{r}$ is parallel to $\boldsymbol{v}$, $|\boldsymbol{r}| \ll r_{tr}$ и $\rho \ll 1$, we obtain the following expression for the flow velocity by using (2) [14]:

$$v \approx \frac{\rho}{l}\frac{r}{\tau_d}. \quad (4)$$

Thus, for a fixed geometry of scattering, the position τ_d of the maximum of the cross-correlation function of the developed dynamic speckle field will be inversely proportional to the velocity of RPS, the sign of τ_d corresponding to the sign of the projection of $\boldsymbol{v}$ on $\boldsymbol{r}$. Therefore, by recording the speckle field intensity fluctuations and estimating their cross-correlation function, we can obtain information not only on the velocity of the scattering object, but also on the direction of its motion.

This principle forms the basis of the method used for measuring the velocity of various scattering objects. Expression (1) is valid only for describing the scattering of light by a single moving RPS and only under the condition that the correlation length of the inhomogeneities of this RPS is much smaller than the size of the illuminated region, i.e., the illuminated part covers a large number of inhomogeneities [2, 3]. Statistical properties of speckle fields formed upon scattering of coherent radiation by the blood or lymph flow in a vessel of small diameter, when the average number of scatterers in the illuminated region is small, have not been studied in detail, and it is quite difficult to obtain the explicit form of space – time correlation functions for the intensities of such speckle fields. However, the experimental studies of the spacetime correlation for the intensities of speckle fields carried out by using lymphatic and blood vessel models confirmed the validity of expression (4) for scattering of light by the capillary flow of a liquid containing the scattering particles [14 – 16].

3. Experimental

The experimental setup was based on a microscope for studying the lymph flow dynamics in the vessels of rat mesentery using the transmission functional microscopy technique. The setup not only made it possible to perform experiments with blood and lymph vessel models but also to study real microvessels *in vivo*. The setup is shown schematically in Fig. 1. Radiation from a LG-207 633-nm He – Ne laser was delivered through the illuminator channel and focused by the objective of the microscope (*2*) to a spot of diameter about 2 μm in a plane situated at a distance $z \approx 100$ μm above the axis of the microvessel (*13*) under study. The radius of curvature of the wavefront of the beam illuminating the microvessel is quite small to ensure the acceptable translation length of the speckle field defined in the solitary RPS approximation by formula (3). The measuring volume is formed by the intersection of the diverging laser beam with the microvessel being inves-

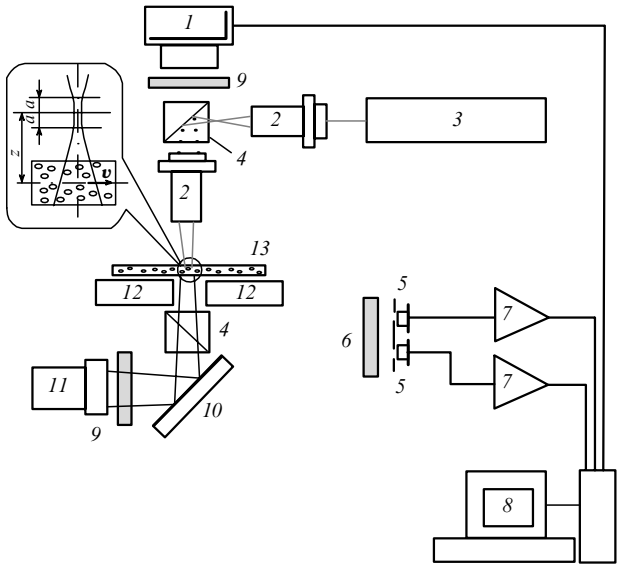

Figure 1. Scheme of the experimental setup: (*1*) digital video camera; (*2*) micro-objective; (*3*) He – Ne laser; (*4*) beamsplitter; (*5*) photodiodes; (*6*) red light filter; (*7*) photocurrent converters; (*8*) PC; (*9*) green light filters; (*10*) mirror; (*11*) illuminator; (*12*) thermally stabilised table; (*13*) lymph microvessel of mesentery. The inset shows illumination of a lymphatic vessel by a focused Gaussian laser beam (*a* is the length of the laser beam waist and *z* is the separation between the flow axis and the waist plane of the laser beam).

tigated, and has the shape of a truncated cone (whose elements have a slope 10° and a mean diameter of the order of 30 μm) placed across the microvessel under investigation.

The laser radiation scattered by the lymph flow is directed with the help of the beamsplitter (*4*) to the photodetector (*5*) situated at a distance of 300 mm from the objective plane of the microscope. The diameter of the photosensitive region of each photodetector is 3 mm, which corresponds to the mean speckle diameter in the observation plane. The centres of the photodetectors are situated 7 mm apart on a straight line parallel to the direction of translation of the speckle field. Signals from the photodetectors are amplified with the help of photocurrent transducers (*7*) and digitised with the help of a two-channel 16-digit analogue-to-digital converter with a quantisation frequency 44.1 kHz. A PC is used to determine the cross-correlation function of the photodetector signals as well as the position of its peak, from which the flow velocity is calculated with the help of expression (4). The time dependence of the flow velocity is determined automatically. Depending on the time resolution, the processing of photodetector signals of duration 60 s takes between 90 and 300 s. The setup makes it possible to detect changes in the velocity and direction of motion of cells in the region of intersection of the laser beam and a lymph vessel with a time resolution up to 50 ms in the velocity range from 10 μm s^{-1} to 10 mm s^{-1}.

A digital video camera (*1*) installed on the microscope is used for videography of the mesentery region under study, and also for determining the mean flow velocity and its direction by functional videomicroscopy, as well as for measuring the diameter of the microvessels. In order to prevent the illumination of the video camera by laser radiation and exposure of the photodetectors to the microscopic illuminator (*11*), green light filters (*9*) are installed on the objective of the chamber and on the illuminator and a red light filter (*6*) is mounted on the photodetectors. The linear field of the video camera in the objective plane of the microscope was 250–350 μm. Digital video images were processed with a specially developed software. In order to determine the velocity of blood flow, the movement of cells from frame to frame was traced in a square field of side 150 μm in the objective plane of the microscope. The cell velocity was determined as the ratio of the difference in cell coordinates in two consecutive frames to the time interval between two frames. The mean flow velocity was calculated by averaging the velocities of four to six cells.

This method allows us to record the lymph flow velocity in the range from 25 μm s^{-1} to 4–5 mm s^{-1} with a time resolution of 40 ms, determined by the frame frequency during video recording (25 Hz). The complicated structure of lymph vessels requires the participation of the operator in the processing of video recordings, which makes the method quite cumbersome. The processing of a video recording of duration 15 s (375 frames) takes about 10 h, and involves the measurement of the motion of about 2000 cells.

4. Experiments based on the lymph vessel model

The efficiency of the system was verified in a series of experiments using the lymph vessel model. A thin-walled plastic capillary tube of diameter 200 μm, through which water containing suspended particles of red pigment with an average diameter 3 μm flowed due to a pressure difference created at the ends of the tube, served as such a model. The concentration of particles was about 1%. Fig. 2 shows the time dependence of the mean flow velocity in the lymph vessel model measured by a velocimeter upon a stepwise variation of the pressure difference at the ends of the capillary during recording between 100 and −100 mm of water column with a step of 10 mm water column, and upon a subsequent equalisation of the pressures at the tube ends. The sensitivity of the speckle-correlation method to the flow direction is illustrated because the mean flow velocity in the experiments was directly proportional to the difference in pressures at the ends of the capillary, and the reversal of the sign of the difference corresponds to a reversal of the direction of flow, as shown in Fig. 2. The velocity measured by the velocimeter is expressed in relative units because the velocimeter described above does not allow measurements of velocity without a preliminary calibration. This is due to the fact that the radius of curvature ρ of the wave front of the laser beam in the plane of the microvessel, determined by the distance z between the axis of the vessel and the plane of the beam waist, cannot be determined quite accurately during *in vivo* measurements.

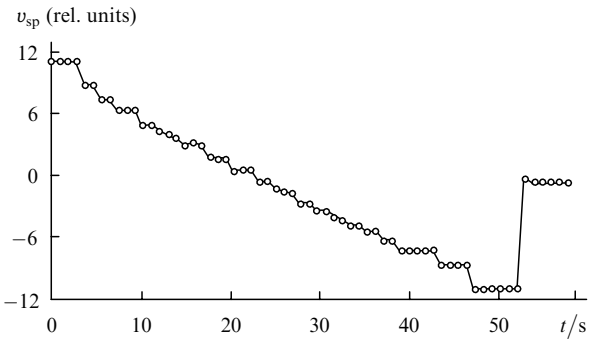

Figure 2. Time dependence of the mean flow velocity in the lymph microvessel model.

Fig. 3 shows the estimates of cross-correlation functions of speckle field intensity fluctuations, corresponding to the 7th and 24th seconds of recording shown in Fig. 2. Test measurements confirmed the linear dependence of the measured flow velocity on the difference in pressures at the ends of the capillary. The dependence of the relative flow velocity on the difference in pressures at the ends of the

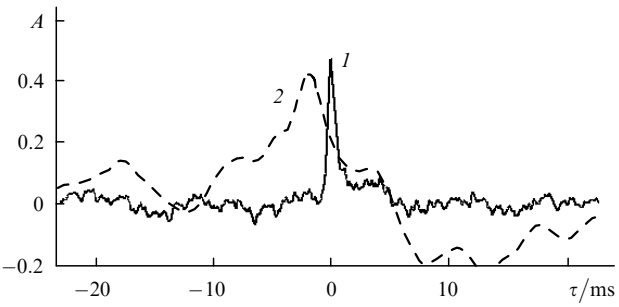

Figure 3. Cross-correlation functions of signals corresponding to 7th (curve *1*) and 24th (curve *2*) seconds of the recording shown in Fig. 2, for delays 0.15 ms (curve *1*) and 1.9 ms (curve *2*) corresponding to the maxima of correlation functions.

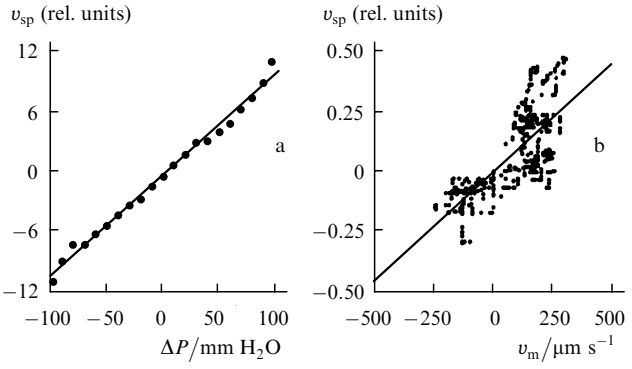

Figure 4. Dependences of the flow velocity determined by a laser velocimeter on the difference in pressures at the ends of a capillary tube (a), and on the lymph flow velocity in the lymphatic of rat mesentery, measured by the method of functional videomicroscopy (b). The solid straight line correspond to linear regression, the correlation coefficients of linear regression being 0.996 (a) and 0.723 (b) respectively.

capillary is shown in Fig. 4a. The solid curve shows the linear regression straight line with a correlation coefficient equal to 0.996. This result confirms the validity of expression (4).

5. Measurement of the lymph flow velocity in the lymphatic of the rat mesentery

The setup for such measurements was also tested *in vivo* on the lymph vessel. Experiments were performed with white rats of no breed. The anaesthised animal was placed on the thermally stabilised table (*12*) of the microscope (see Fig. 1). Lymph microvessels on the mesentery of the small intestine were studied *in vivo* in transmitted light. The mesentery is a transparent weakly scattering thin (20 – 100 μm) film of the connective tissue, which contains the blood and lymph vessels and is a very convenient object for studying the blood and lymph microcirculation in the transmitted light. The lymph flowing through the mesentery vessels is a transparent weakly scattering liquid, with 10 % of its volume being occupied by protein and lipid molecules. The lymph also contains cells, predominantly lymphocytes having a spheroid shape. The mean diameter of lymphocytes is 7 – 10 μm. The lymph composition and the concentration of cells in it depend to a considerable extent on the specific physiological conditions, especially on the time elapsed after the food intake [17 – 19]. The mean refractive index of the lymph varies in the interval 1.35 – 1.36, that of the lymphocyte and cytoplasm membranes is 1.43 – 1.46 and 1.36 – 1.37 respectively. The volume concentration of the lymphocytes varies between 0.1 % and 20 %. A characteristic feature of the lymph flow is a periodic reversal of the flow direction, called the back throw [19 – 21]. The dynamics of the lymph flow, especially the back throw frequency, depends on various processes occurring in the organism, and is of considerable interest from physiological point of view [18 – 21].

Fig. 5 shows the time dependence of the lymph flow velocity in the lymphatic of the mesentery of a white rat. This dependence was obtained from recordings with the help of a velocimeter and processing of the video recording. The mean diameter of the investigated region of the vessel was 170 ± 5 μm, and the mean velocity of the lymph flow was 169 ± 4.6 μm s^{-1}. It was mentioned above that laser velocimeter allows lymphocyte velocity measurements only in relative units. The proportionality factor between the readings v_{sp} on the laser velocimeter and the mean flow velocity v_m measured by the functional video microscopy was determined from the slope of the regression straight line (Fig. 4b). The coefficient of linear regression correlation for the dependence of v_{sp} on v_m was 0.72.

6. Discussion

Experiments on scattering of focused beams of coherent radiation from the blood and lymph microvessel models as well as lymph microvessels *in vivo* revealed that a correlation exists between the speckle field intensity fluctuations recorded at two spatially separated points. This correlation indicates the manifestation of translational dynamics of the speckle field, analogous to the translational dynamics of such fields appearing upon scattering of a laser beam by a moving solitary RPS. Moreover, a linear dependence is established between the flow velocity, radius of curvature of the laser beam wave front, and the speckle field translational velocity [14 – 16]. The linear dependence between the mean flow velocity and the peak of the cross-correlation function of the intensities is confirmed, in particular, by the experimental results based on the lymphatic model presented in this work (see Fig. 4a).

However, in contrast to an RPS moving as a single entity, the cells in a lymphatic move at different velocities depending on their position relative to the flow axis. Therefore, further investigations must be carried out in order to find the relation between the flow velocity calculated in the moving RPS approximation and the mean flow velocity. Moreover, while the radius of curvature of the wave front can be treated as constant over the illuminated region during an analysis of scattering of the focussed beam by an RPS, the radius of curvature of the wave front at the centre of a vessel is comparable with the radius of this vessel (see the inset to Fig. 1) in the case of scattering by the flow in a vessel of diameter 170 μm. Therefore, the proportionality factor between τ_d and the velocity of a cell crossing the beam will depend on the position of this cell relative to the plane of the beam waist . This effect will be manifested especially clearly in the case when the number of cells in the scattering volume does not exceed one or two.

The microscopy of the model of the lymph vessel and of the real lymphatic of the mesentery shows that the measuring volume contained about ten particles on the average in experiments on the model of the lymph vessel, while the corresponding number in the case of the real vessel was 23

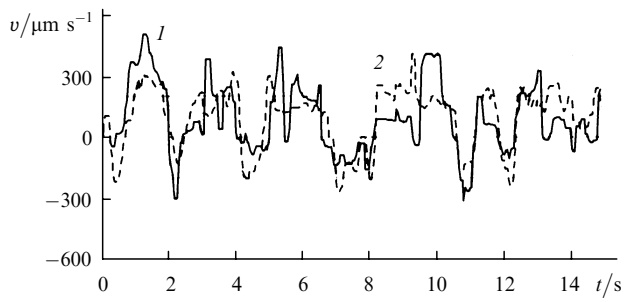

Figure 5. Time dependence of the lymph flow velocity in the lymphatic vessel of diameter 150 μm of a white rat mesentery, recorded with a velocimeter (curve *1*) and by processing of video recording (curve *2*).

cells, and not a single cell was observed in the measuring volume at certain instants of time. These observations explain to a certain extent the discrepancy in the data on flow velocity in the lymphatic of the mesentery obtained by the laser technique and from the video image processing since the mean flow velocity was defined as the average velocity of the particles in a 150-μm 'square' field in the objective plane of the microscope, while the laser velocimeter recorded the velocities of particles passing through the measuring volume of diameter 30 μm. The velocity of the cell crossing the measuring volume may differ significantly from the mean velocity of the cells moving within a field of characteristic size 150 μm. However, the results of measurements made by two entirely independent methods are quite convincing since the correlation coefficient of linear regression of the dependence of v_{sp} on v_m is quite large. The method proposed here is also supported by the fact that the time dependences of the lymph flow velocity shown in Fig. 5 required an operator time of 10 h (video recording of duration 15 s was processed), while the processing of the signals registered by photodetectors over the same period of time was completed in just 30 s.

7. Conclusions

Thus, we have studied experimentally the space–time correlation properties of the dynamic speckle fields formed upon a single scattering of a focused beam of coherent radiation by liquid flows containing scattering particles, and considered the possibility of their application for measuring flow velocity.

Optical measurements of the blood or lymph flow velocity taking into account the direction of flow for a single scattering of light can be performed at present only by functional microscopy [8, 21, 22] or Doppler laser microscopy [6, 23, 24] technique. It was mentioned above that functional microscopy requires a prolonged and cumbersome processing of images, while Doppler laser microscopy requires quite complex and expensive equipment. Of course, the method described here for measuring the lymph and blood flow velocities in biological and medical experiments requires a more detailed analysis of the properties of speckle fields formed as a result of scattering of coherent radiation beams from blood and lymph vessels of various diameters, and also a modification of the existing experimental equipment. However, even the experimental results presented in this work indicate that the changes in the velocity as well as the direction of lymph microflow can be recorded quite expediently by using relatively simple equipment.

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References

1. Francon M. *Laser Speckle and Applications In Optics* (New York: Academic Press, 1979; Moscow: Mir, 1980).
2. Goodman J. *Statistical Optics* [Russian translation] (Moscow: Mir, 1985).
3. Yoshimura T. *J. Opt. Soc. Am. A.*, **3** (7), 1032 (1986).
4. Jakeman E. *Opt. Eng.*, **23** (3), 453 (1984).
5. Jones R., Wykes C. *Holographic and Speckle Interferometry* (Cambridge: Cambridge Univ. Press, 1983 Moscow: Mir, 1986).
6. Priezzhev A.V., Tuchin V.V., Shubochkin L.P. *Lazernaya diagnostika v biologii i meditsine* (Laser Diagnostics in Biology and Medicine) (Moscow: Nauka, 1989).
7. Tuchin V.V. *Lazery i volokonnaya optika v biomeditsinskikh issledovaniyakh* (Lasers and Fibre Optics in Biomedical Research) (Saratov: Izd. Saratov Gos. Univ., 1998).
8. Galanzha E.I., Brill G.E., Aizu Y., Ulyanov S.S., Tuchin V.V., in *Handbook of Optical Biomedical Diagnostics*. Ed. by V.V. Tuchin (Bellingham: SPIE Press, 2002).
9. Aizu Y., Asakura T. *J. Biomed. Opt.*, **4** (1), 61 (1999).
10. Aizu Y., Asakura T. *Opt. Laser Technol.*, **23** (4), 205 (1991).
11. Aizu Y., Ambar H., Yamamoto T., Asakura T. *Opt. Commun.*, **72** (5), 269 (1989).
12. Aizu Y., Asakura T., Ogino K., Sugita T., Suzuki Y., Masuda K. *Proc. SPIE Int. Soc. Opt. Eng.*, **2678**, 360 (1996).
13. Ulyanov S.S., Tuchin V.V., Bednov A.A., Brill G.E., Zakharova E.I. *Lasers in Medical Science*, **12** (1), 31 (1997).
14. Fedosov I.V., Tuchin V.V. *Proc. SPIE Int. Soc. Opt. Eng.*, **4241**, 384 (2001).
15. Fedosov I.V., Tuchin V.V. *Proc. SPIE Int. Soc. Opt. Eng.*, **4434**, 192 (2001).
16. Fedosov I.V., Tuchin V.V. *Optika Spektr.,* **93** (3), 473 (2002).
17. Levtov V.A., Regirer S.A., Shadrina N.Kh. *Reologiya krovi* (Rheology of Blood) (Moscow: Meditsina, 1990).
18. Tkachenko B.I. (Ed.) *Fiziologiya krovoobrashcheniya: Fiziologiya sosudistoi sistemy* (Physiology of Blood Circulation: Physiology of Blood Vessel System) (Leningrad: Nauka, 1984).
19. Buyanov V.M., Alekseev A.A. *Limfologiya endotoksikoza* (Lymphology of Endogenous Toxicosis) (Moscow: Meditsina, 1990).
20. Gashev A.A. *Fiziol. Zh.,* **77** (7), 63 (1991).
21. Berk D.A., Swartz M.A., Leu A.J., Jain R.K. *Am. J. Physiol.*, **270**, 330 (1996).
22. Gurfinkel Yu.I., Lyubimov V.V., Oraevskii V.N., et al. *Biofizika,* **40**, 793 (1995).
23. Levenko B.A., Priezzhev A.V., Proskurin S.G., Savchenko N.B. *Izv. Ross. Akad. Nauk. Ser. Fiz.,* **59** (6), 162 (1995).
24. Eiju T., Nagai M., Matsuda K., Ohtsubo J., Homma K., Shimizu K. *Opt. Eng.*, **32** (1), 15 (1993).