

PACS numbers: 42.62.Be; 87.54.Fj
DOI: 10.1070/QE2002v032n10ABEH002317

Diagnostics of a laser-induced response of capillary vessels in tissues

A.A. Stratonnikov, N.V. Ermishova, V.B. Loshchenov

Abstract. **The effect of 675-nm semiconductor laser radiation of various power densities on the degree of oxygenation and the relative concentration of hemoglobin in blood in the microcirculatory vessels in the skin of a hand is studied *in vivo* by the spectroscopy of backward diffuse reflection in the visible range. No noticeable variations in these quantities were observed at relatively low power densities (up to 0.5 W cm^{-2}) of the radiation power density. At higher power densities, an increase in the degree of oxygenation and the relative concentration of hemoglobin was observed, which is apparently caused exclusively by heating.**

Keywords: *optical diagnostics, tissue spectroscopy, laser therapy, photodynamic therapy, diffuse reflection.*

1. Introduction

The response of the capillary vessels of tissues to laser irradiation is manifested quite clearly in photodynamic therapy (PDT). The PDT technique involves the introduction of a photosensitiser (PS) into the patient's organism, which is accumulated selectively in tumour tissues. Then, the tumour tissue is exposed to radiation in a certain spectral range where the PS absorbs light, which leads to a destruction of the tissue due to photochemical reactions. During irradiation, the response of a capillary vessel is clearly observed and can be perceived by the naked eye from a change in the colour of the irradiated tissue caused by a change in the degree of oxygenation and the relative concentration of hemoglobin. The reaction of the capillary vessel can be observed especially clearly if laser irradiation is carried out soon (a few hours) after intravenous injection of a PS, when its concentration in the blood is still quite high. The irradiation leads to a contraction of capillaries (spasm) and a stoppage of blood circulation (stasis).

In this work, the capillary vessel diagnostic technique based on monitoring of the degree of blood oxygenation by the method of backward diffuse reflection [1–3] is used for the first time to study the effect of laser irradiation on nonsensitised tissues, in particular, on the human skin.

A.A. Stratonnikov, N.V. Ermishova, V.B. Loshchenov Natural Science Research Center, A.M. Prokhorov General Physics Institute, Russian Academy of Sciences, ul. Vavilova 38, 119991 Moscow, Russia; e-mail: biospec_sys@rol.ru

Received 27 May 2002
Kvantovaya Elektronika **32** (10) 917–922 (2002)
Translated by Ram Wadhwa

2. Noncontact technique for measuring the degree of hemoglobin oxygenation upon laser irradiation

To monitor the degree of hemoglobin oxygenation upon laser irradiation, we measured the backward diffuse reflection spectra in the centre of the irradiated region. Fig. 1 shows the scheme of the experimental setup. Radiation from a 675-nm, 2.5-W LFT-01-Biospec semiconductor laser (*1*) was coupled to the tissue through a quartz fibre (*4*) of diameter 600 μm at an angle 15° to the normal to the tissue surface. The distance between the end of the fibre and the tissue surface was chosen in such a way that the diameter of the light spot on the surface was 6 mm. Such a small size of the spot was dictated by the need to ensure a sufficiently high power density in the exposure region without damaging large areas of the skin.

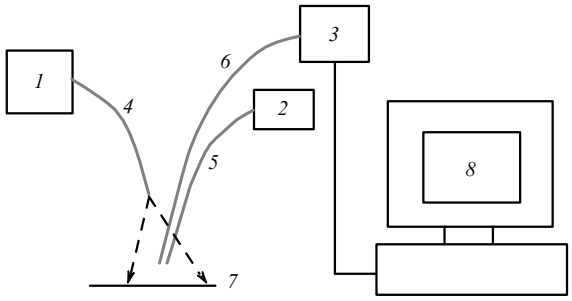

Figure 1. Experimental setup for monitoring the degree of oxygenation and the relative concentration of hemoglobin during laser irradiation of tissues: (*1*) LFT-01-Biospec semiconducting laser; (*2*) halogen lamp; (*3*) LESA-5 (Biospeck) fibreoptic spectrometer; (*4,5*) transmitting fibres; (*6*) receiving fibre; (*7*) irradiated tissue surface; (*8*) computer.

White light from a halogen lamp (*2*) was coupled to the tissue through a quartz fibre (*5*) of diameter 600 μm. After passing through the tissue in the irradiated region, the diffusively reflected light was collected by a quartz fibre (*6*) of diameter 200 μm and coupled to the entrance of a LESA-5 (Biospec) fibreoptic spectrometer (*3*). The distance d between the receiving and transmitting fibres was 2 mm. The distal parts of the transmitting fibre (*5*) and of the receiving fibre (*6*) were fastened parallel to each other, forming an angle of 15° with the normal to the tissue surface (see Fig. 1), while the ends of the fibres were fixed at a distance of 1 mm (or less) from the tissue surface. In such a measuring scheme, the receiving fibre collected only diffu-

sively reflected photons, and the contact of tissues with the ends of the fibres was excluded entirely. At the entrance slit of the spectrometer a filter was placed, which rejected light at wavelengths above 630 nm, thereby rejecting intense scattered laser radiation. By using this setup, we measured the diffuse reflection spectra of tissues in the range 400–630 nm at the centre of the irradiated region. The spectrometer was computer-controlled with the help of a special program.

It is well known that blood in arteries and veins has different colours. This is due to the fact that hemoglobin, which is the main pigment of the blood and is responsible for the transport of oxygen, has different absorption spectra in the free state and when it is bound with oxygen. This property can be used for a quantitative measurement of the degree of oxygenation and the relative concentration of hemoglobin from the spectra of backward diffuse reflection [4]. However, because biological tissues and, in particular, human skin are strongly scattering media, the photon trajectories in them are zigzag lines with different path lengths. The probing depth and mean free path of photons in the tissue depends on the absorbing and scattering properties of the tissue and the geometry of measurements (on the distance between the receiving and transmitting fibres).

Fig. 2 shows the geometry of the probed region for the measuring scheme used by us. The probed region, i.e., the region containing a larger part of the trajectories of phonons contributing to the signal being measured, has the shape of a banana whose ends are close to the ends of the receiving and transmitting fibres. We determined the probing depth Z from simple analytic expressions [5]:

$$Z \approx \frac{\sqrt{2}d}{4}, \quad d \ll \delta \text{ (weak absorption)},$$
$$Z \approx \left(\frac{d\delta}{2}\right)^{1/2}, \quad d \gg \delta \text{ (strong absorption)}, \tag{1}$$

where $\delta = [3\mu_a(\mu_a + \mu_s')]^{-1/2}$ is the depth of light penetration into the tissue (diffusion length); μ_s' and μ_a are reduced (transport) scattering coefficient and the absorption coefficient, respectively. In the visible spectral region (530–590 nm), which we use below for determining the degree of hemoglobin oxygenation, the penetration depth δ at the wavelength $\lambda = 585$ nm in the epidermis ($\mu_a = 36$ cm^{-1}, $\mu_s' = 99$ cm^{-1}) and derma ($\mu_a = 2.2$ cm^{-1}, $\mu_s' = 41$ cm^{-1}) [6] is 0.1 and 0.6 mm, respectively. In the strong absorption limit ($d \gg \delta$), the probing depth Z (see Fig. 2) at this wavelength for the fibreoptic probe we used is 0.2 and 0.7 mm at the epidermis and derma, respectively. Since measurements were made on the skin of the hand, the contribution to the signal being measured comes from the epidermis and derma, and we obtain some average value of probing depth. In fact, the main contribution to the measured hemoglobin absorption signal comes from the capillary loops of the microcirculatory vessels, extending to the border between epidermis and derma.

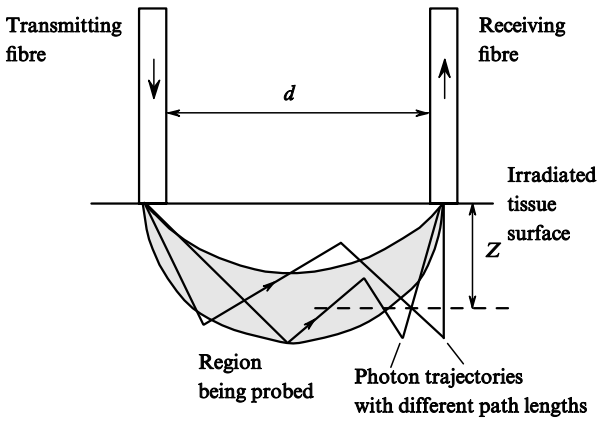

Figure 2. Geometry of the probed region for measuring the backward diffuse reflection spectra of tissues.

Due to scattering, the mean path length $\langle L \rangle$ of photons in the tissue between the receiving and transmitting fibres is larger than the separation between them, and is defined in terms of the dimensionless coefficient β of the differential path length:

$$\langle L \rangle = \beta d. \tag{2}$$

In the diffusion approximation, when scattering prevails over absorption, and the separation between fibres is larger than the scattering length $1/\mu_s'$, the coefficient of the differential mean path length is determined by the expression [4, 7]:

$$\beta = \frac{\sqrt{3}}{2}\frac{d}{d+\delta}\left(\frac{\mu_s'}{\mu_a}\right)^{1/2}. \tag{3}$$

Substituting the above values of μ_s', μ_a and δ at $\lambda = 585$ nm into (3), we find that mean path length of photons through the tissue exceeds the distance between the receiving and transmitting fibres ($d = 2$ mm) by a factor of 1.4 and 2.9 for epidermis and derma, respectively.

For a quantitative measurement of the degree of oxygenation and the relative concentration of hemoglobin, we used the logarithm of the backward reflection

$$A_{exp}(\lambda) = \ln\left(\frac{I_{ref} - I_{dark}}{I - I_{dark}}\right), \tag{4}$$

where I_{ref} is the reflection signal from the standard $BaSO_4$ sample, whose reflection coefficient is close to unity in the spectral range under study; I_{dark} is the signal in the absence of light (dark current of the detector); and I is the signal of diffuse reflection from the tissue. Equation (4) takes into account the spectral inhomogeneity of the light source and of the transmission of fibres, as well as the sensitivity of the detector. Note that $A_{exp}(\lambda)$ in this equation is determined to within a certain constant. However, the value of this constant is not significant in our algorithm for calculating the degree of oxygenation, and all we need to know is the wavelength dependence of $A_{exp}(\lambda)$.

The experimentally obtained dependence $A_{exp}(\lambda)$ can be described by the following model function:

$$A_{model}(\lambda) = c_0 + c_1\lambda + \langle L \rangle [c_{Hb}\varepsilon_{Hb}(\lambda) + c_{HbO_2}\varepsilon_{HbO_2}(\lambda)] \ln 10, \tag{5}$$

where c_0 and c_1 are the coefficients that take into account the contribution of scattering and absorption of the tissue not related to hemoglobin; ε_{Hb,HbO_2} and c_{Hb,HbO_2} are the extinction coefficients and concentrations of the oxygenated

and deoxygenated hemoglobin. The coefficient c_0 also takes into account the contribution from a certain indefinite constant component [see expression (4) and comments]. The values of these coefficients and the quantities $\langle L\rangle c_{\mathrm{Hb}}$ and $\langle L\rangle c_{\mathrm{HbO_2}}$ are obtained by minimising the difference between the experimental and model spectra in the interval 510–590 nm by the method of least squares. This spectral range was chosen because the absorption spectra of the oxygenated and deoxygenated hemoglobin differ most strongly in this interval [7].

The degree of haemoglobin oxygenation $S_{\mathrm{O_2}}$ and the product of the total hemoglobin concentration $c_{\mathrm{Hb+HbO_2}}$ and the mean path length $\langle L\rangle$ of photons were determined from the calculated coefficients as:

$$S_{\mathrm{O_2}} = \frac{\langle L\rangle c_{\mathrm{HbO_2}}}{\langle L\rangle c_{\mathrm{HbO_2}} + \langle L\rangle c_{\mathrm{Hb}}} = \frac{c_{\mathrm{HbO_2}}}{c_{\mathrm{HbO_2}} + c_{\mathrm{Hb}}},$$
$$\langle L\rangle c_{\mathrm{Hb+HbO_2}} = \langle L\rangle c_{\mathrm{Hb}} + \langle L\rangle c_{\mathrm{HbO_2}}. \qquad (6)$$

The product $\langle L\rangle c_{\mathrm{Hb+HbO_2}}$ depends on the geometry of the probed zone through the quantity $\langle L\rangle$, hence only dynamic variations in the relative concentration $C = c_{\mathrm{Hb+HbO_2}}(t)/c_{\mathrm{Hb+HbO_2}}(0)$ of hemoglobin are of any significance. Note also that in noncontact *in vivo* measurements, the involuntary movements of the hand cause a slight variation in the distance between the ends of the fibres and the tissue, leading to random variations in $\langle L\rangle$ and hence to errors in the monitoring of C. However, the degree of hemoglobin oxygenation is less sensitive to errors caused by the variations in the geometry during measurements because it is independent of the photon mean path length in the tissue [see expression (6)].

3. Discussion of results

To determine the extent to which the chosen technique reflects the state of microcirculation, we measured the degree of oxygenation and the relative hemoglobin concentration in the microcirculatory vessels of skin of the upper phalanx of a finger in the case of arterial occlusion caused by tightening the arm with a rubber tourniquet. Fig. 3 shows the diffuse reflection spectra $A_{\mathrm{exp}}(\lambda)$ measured at different instants of time after the beginning of occlusion. For clarity, the spectra are displaced along the y axis. One can see that the shape of the spectral curves depends on the oxygenation degree. The figure also shows the model spectra calculated in the range 510–590 nm using Eqn (5) for different oxygenation degrees obtained from expression (6). Good agreement between the experimental and model curves confirms the validity of the algorithm used.

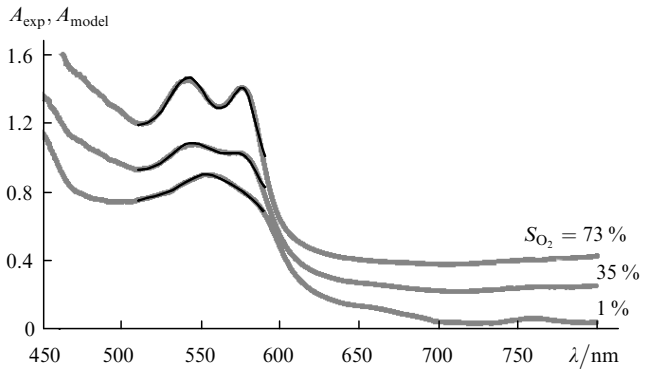

Figure 3. Backward diffuse reflection spectra of the skin of the hand, measured at different instants of time after arterial occlusion (light curves), and model spectra (dark curves) in the range 510–590 nm, calculated from Eqn (5) for different values oxygenation degrees obtained from Eqn (6).

Fig. 4 shows the time monitoring of the oxygenation degree $S_{\mathrm{O_2}}$ and the relative concentration C of hemoglobin in the skin of the hand before, during, and after the removal of arterial occlusion at the normal ambient temperature (20–22 °C). The time $t = 0$ corresponds to the beginning of occlusion, and $t = 700$ s corresponds to its termination. One can see that irregular oscillations of the oxygenation degree are observed in the range 50 %–70 % under normal conditions (before the beginning of occlusion). These are not random errors of measurement, but actual variations caused by the space–time oscillations of microcirculation. Unfortunately, these oscillations mask to a certain extent the effect of the laser radiation, especially for low power densities (see below). Note also that, at a lower ambient temperature and, hence, of the skin, the oxygenation degree in the microcirculatory vessel dropped to 10 %–30 %, and the oscillation range increased. Hemoglobin completely deoxygenated in 10 min after the beginning of occlusion. After the removal of occlusion, the oxygenation degree increases sharply, and the relative concentration of hemoglobin increases slightly due to intense blood circulation (reactive hyperemia). After some time, the hemoglobin oxygenation and concentration returned to their initial values. Similar results were also obtained by other authors investigating arterial occlusion [8, 9].

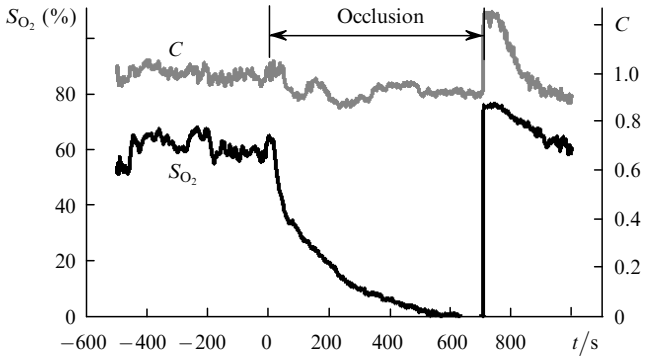

Figure 4. Dynamics of the degree of oxygenation $S_{\mathrm{O_2}}$ (dark curve) and relative concentration $C = c_{\mathrm{Hb+HbO_2}}(t)/c_{\mathrm{Hb+HbO_2}}(0)$ of hemoglobin (light curve) in microcirculatory vessels of the skin of the hand during arterial occlusion.

The response of capillary vessels to laser radiation is clearly manifested in the blood containing PS. This effect was studied earlier to explore the possibility of monitoring microcirculation during PDT using this technique [1–3]. Fig. 5 shows the results of monitoring the oxygenation degree in the tumour of a mouse upon laser irradiation during PDT by using sulphonated aluminium phthalocyanine (Photosens, produced by the NIOPIK State Research Center) as a PS. The tumour was exposed to laser radiation

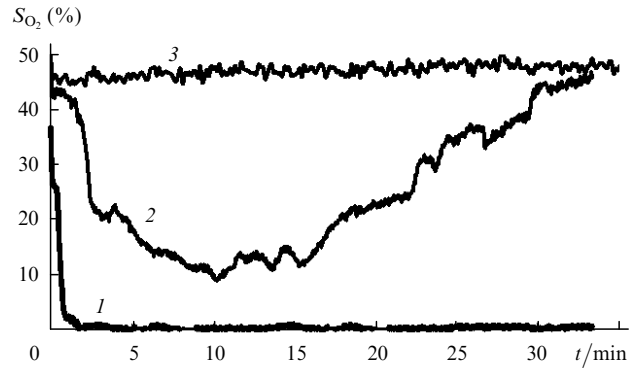

Figure 5. Dynamics of the degree of hemoglobin oxygenation in the tumour of a mouse during laser irradiation for 30 min with a power density 50 mW cm^{-2} in the course of PDT. The PS Photosens was introduced intravenously in amounts of 4 mg kg^{-1}. The time elapsed between the introduction of PS and laser irradiation was 1 (curve *1*) and 24 h (curve *2*). The reference curve (*3*) was obtained for laser irradiation without introducing a PS.

1 and 24 h after the intravenous injection of the PS. One can see that, in the absence of the PS, no variations were observed in the tissues for power densities typical of PDT (50 – 300 mW cm^{-2}). If the time interval between the PS injection and laser irradiation is small (the major portion of the PS is still circulating in the blood during this period), a sharp decrease in the oxygenation degree was observed, apparently, due to contraction of capillaries.

If the blood does not contain the PS, the response of the capillary vessel to the laser radiation is observed only for quite high power densities. In this case, the oxygenation degree increases upon laser irradiation instead of decreasing (as in the case when the blood contains the PS) (see Fig. 5). Fig. 6 shows the dynamics of oxygenation and the relative concentration of hemoglobin in the region of the lower phalanx of a finger exposed to the 7.5-W cm^{-2} laser radiation. The oxygenation degree increased sharply 15 – 20 s after the beginning of irradiation, while the relative concentration of hemoglobin remained almost unchanged (a slight increase was observed in some cases). The curves in Fig. 6 were obtained at a lower ambient temperature

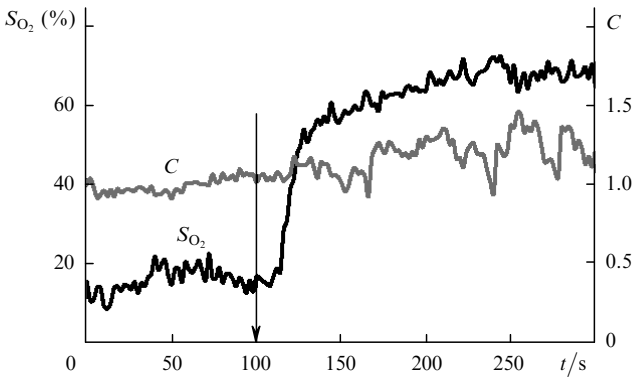

Figure 6. Dynamics of the degree of oxygenation S_{O_2} (dark curve) and relative concentration $C = c_{Hb+HbO_2}(t)/c_{Hb+HbO_2}(0)$ of hemoglobin (light curve) during laser irradiation of the skin of the human hand (lower phalanx of a finger) at a power density of 7.5 W cm^{-2}. The arrow shows the beginning of irradiation.

(14 °C). In this case, the initial oxygenation degree is low, and the jump caused by laser radiation is manifested most clearly. Note also that the laser power density was quite high, and microscopic burns appeared in the region exposed to radiation in some cases.

Fig. 7 shows the oxygenation dynamics for different power densities of irradiation of the skin of the hand. One can see that high power densities of radiation lead to a greater jump in the oxygenation degree and to a decrease in the time elapsing between the instants of exposure of the skin to radiation and the jump in the oxygenation degree. No noticeable variations of the degree of hemoglobin oxygenation were observed for a power density of 0.3 W cm^{-2}, which is the upper limit of the power density normally used in PDT. No noticeable changes in the oxygenation degree were observed at lower power densities (50 and 100 mW cm^{-2}), which are used in low-intensity laser therapy.

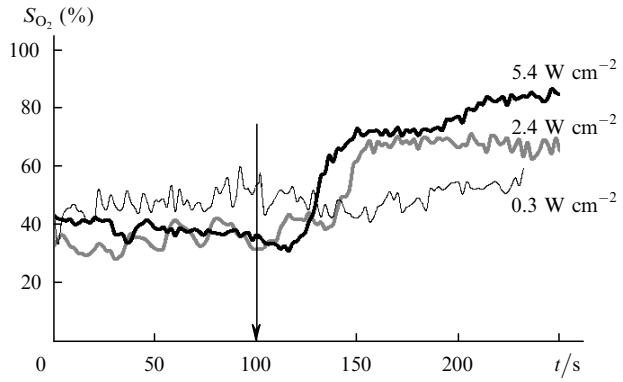

Figure 7. Dynamics of the degree of hemoglobin oxygenation during laser irradiation of the skin of the human hand at various power densities. The arrow shows the beginning of irradiation.

Thus, we can make the following conclusions from a comparison of the response of the capillary vessel during PDT and upon laser irradiation without using a PS. First, PDT reduces the oxygenation degree, while laser irradiation without using a PS increases this quantity. Second, the response of the capillary vessel during PDT can be detected even for quite low power densities (a few tens of milliwatt), especially if active PSs like Photosens are used, while a power density more than 0.5 W cm^{-2} is required in the case when laser irradiation alone is used.

Fig. 8 shows the results of monitoring of the oxygenation degree with and without laser irradiation. The oxygenation degree was restored to its initial value 150 – 200 s after the termination of irradiation. The oxygenation degree increased sharply again upon repeated irradiation, and its initial value was restored more smoothly after the termination of irradiation. The results obtained in different regions of the hand correlate well with one another. An analysis of such a behaviour of the oxygenation degree upon laser irradiation leads to the conclusion that the observed phenomenon is related exclusively to the thermal effect. Laser radiation absorbed primarily by blood leads to an increase in the tissue temperature. In turn, the response of the organism to an increase in temperature is manifested as an enhancement of microcirculation, leading to an increase in the degree of hemoglobin oxygenation.

This was proved by comparing the oxygenation dynam-

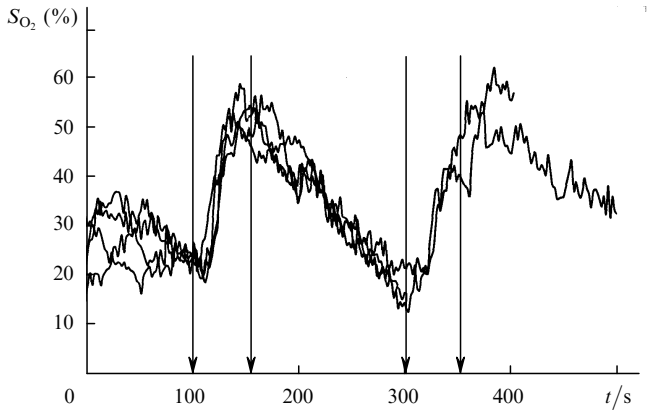

Figure 8. Dynamics of restoration of the degree of hemoglobin oxygenation after termination of laser irradiation at a power density of 3.7 W cm^{-2}. The laser was switched on at time intervals 100–150 s and 300–350 s. The arrows show the beginning and the end of irradiation. The curves were obtained at different points on the skin of the same human hand.

ics upon laser irradiation of the hand and its heating in hot water at a temperature $T = 50\,^{\circ}$C (Fig. 9). We found that the oxygenation dynamics was similar in both cases. During laser irradiation, the temperature of the skin in the exposed region was monitored simultaneously with a thermocouple. A sharp increase in the temperature was observed immediately after the exposure, while a sharp increase in the oxygenation degree followed the irradiation with a certain delay. The actual shape of the curve describing the temperature dynamics corresponds to heating of a body with a constant heat capacity, having a heat sink when a constant heat source was used (exponential approach to the equilibrium value). The response of the capillary vessel apparently appears after the attainment of a certain temperature, followed by a rapid triggering of the regulatory mechanisms. The results of our observations made during the laser irradiation of the hand immersed in water at room temperature (water ensures an effective heat removal from the irradiated region) also confirm that the observed effect is related to the temperature regulation. The jump in the

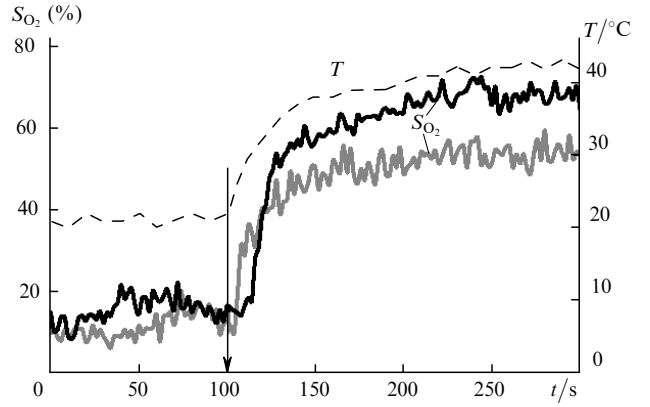

Figure 9. Comparison of the oxygenation dynamics of hemoglobin during laser irradiation at a power density of 7.5 W cm^{-2} (dark curve) and heating of skin of the human hand in hot water at a temperature 50 °C (light curve). The arrow shows the beginning of irradiation and the moment of immersion of hand in water. The dashed curve shows the monitoring of the skin temperature in the irradiated region.

oxygenation degree in this case was much smaller than upon laser irradiation in air.

Apart from the oxygenation dynamics at the centre of the irradiated region, we also measured the spatial distribution of the oxygenation degree outside the irradiated region immediately after the termination of laser irradiation. We found that the oxygenation degree was enhanced at a distance up to 2–3 cm from the irradiated region. Probably, this is caused, on the one hand, by the heat conduction in the tissue during heating and by dissipation of laser radiation in it, and, on the other hand, by purely physiological reasons. For example, the response of the organism to any stimulus is often spread over a much larger area than the stimulated region.

In our experiments, we studied only the normal skin of the hands of healthy people. It is quite possible that the oxygenation dynamics of hemoglobin (jump of the oxygenation degree from its normal value at the beginning and restoration to the initial value at end of the irradiation) in the region of irregular microcirculation may differ from that in normal tissues. We believe that this technique of studying the response of capillary vessels to laser irradiation can be used in the diagnostics of vascular diseases.

As mentioned above, the technique we proposed could be used successfully for monitoring and dosimetry in PDT. For example, the destruction of capillary vessels in a tumour can be monitored directly during the therapy (see Fig. 5), and appropriate measures (e.g., increasing the power density of laser radiation or additional injection of PS) can be taken, if necessary.

One of the aims of this research was to ascertain whether this method could be used for determining the response of the capillary vessels (if such a response exists) in low-intensity laser therapy. As a matter of fact, diverse opinions have been expressed on the mechanisms of laser action in the case of low-intensity laser therapy. The photodynamic effect in which the chromophores of the tissues themselves (in particular, endogenous porphyrines [10]) serve as a PS could be one of such mechanisms. In small doses, the photodynamic effect could stimulate various physiological processes rather than causing the destruction of tissues as in the case of PDT. Since we have established beyond doubt the response of the capillary vessels in PDT, it can be expected that a response will also be observed upon laser irradiation of nonsensitised tissues. If the response of capillary vessels to the low-intensity laser radiation could be detected, a quantitative approach could be developed to the dosimetry of radiation in this method. Some researchers have observed a change in the colour of blood upon laser irradiation and have even coined the term 'scarlet blood' for this effect. From the physical point of view, this could indeed be due to an increase in the degree of hemoglobin oxygenation, which could be reliably measured by using the technique described in this work. Note that the change in the tissue colour is visible to the naked eye only if the oxygenation degree changes significantly, while the diffuse reflection spectroscopy used by us makes it possible to determine quantitatively even small changes that are not perceived by the eye. Our investigations show that laser irradiation at power densities used in low-intensity laser therapy (normally less than 100 mW cm^{-2}) at wavelengths 630 and 675 nm does not noticeably change the oxygenation degree and the relative concentration of hemoglobin in microcirculatory blood vessels of human beings. It is

possible that the response of tissues to low-intensity laser irradiation can be determined by other methods, e.g., Doppler scattering or coherent optical tomography. In particular, the photobleaching of the intrinsic fluorescence of skin upon laser irradiation was discussed in Ref. [10].

4. Conclusions

In this work, we have described in detail the technique for studying the response of capillary vessels of tissues to laser irradiation. We found that, in contrast to PDT, where laser irradiation leads to a sharp decrease in the degree of hemoglobin oxygenation down to zero, the oxygenation degree increases during irradiation of nonsensitised tissues, including the skin of the hand. However, this effect can be observed only for quite high power densities of laser radiation. This is apparently explained by heating caused by radiation absorbed by the tissue. Although, we have not studied in this work the response of capillary vessels in pathological tissues, the method proposed here can be used in the future for diagnostics of microcirculation disorders.

References

1. Stratonnikov A.A., Douplik A.Y., Loschenov V.B., in *Oxygen Transport to Tissues XXI*. Ed. by Eke A., Delpy D.T. (New York: Kluwer Acad. Publ., 1999) pp 515–524.
2. Stratonnikov A.A., Meerovich G.A., Loschenov V.B. *Proc. SPIE Int. Soc. Opt. Eng.,* **3909**, 81 (2000).
3. Stratonnikov A.A., Loshchenov V.B., Duplik A.Yu. *Ros. Khim. Zh.*, **52** (5), 63 (1998).
4. Stratonnikov A.A., Loschenov V.B. *J. Biomed. Opt.*, **6** 457 (2001). doi>
5. Feng S., Zeng F., Chance B. *Proc. SPIE Int. Soc. Opt. Eng.,* **1888**, 78 (1993).
6. Tuchin V.V. *Lazery i volokonnaya optika v biomeditsinskikh issledovaniyakh* (Lasers and Fibre Optics in Biomedical Research) (Saratov: Saratov University Press, 1998).
7. Stratonnikov A.A., Edinak N.E., Klimov D.V., Linkov K.G., Loschenov V.B., Luckjanets E.A., Meerovich G.A., Vakulovskaya E.G. *Proc. SPIE Int. Soc. Opt. Eng.,* **2924**, 49 (1996).
8. Harrison D.K., Evans S.D., Abbot N.C., Swanson J.B., McCollumn P.T. *Clin. Phys. Physiol. Meas.*, **13**, 349 (1992) doi>
9. Wallace D.J., Michener B., Choudhary D., Levi M., Fennelly P., Hueber D.M., Barbieri B. *Proc. SPIE Int. Soc. Opt. Eng.,* **3597**, 300 (1999).
10. Stratonnikov A.A., Polikarpov V.S., Loschenov V.B. *Proc. SPIE Int. Soc. Opt. Eng.,* **4241**, 13 (2001).