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Numerical study of the influence of oxyhemoglobin, deoxyhemoglobin, and methemoglobin content on the reflection, absorption, and transmission spectra of human blood

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The Monte Carlo method was used to numerically investigate the effect of human blood oxygenation and methemoglobin content on the reflection, absorption, and transmission spectra in the wavelength range of 400–1100 nm. It was established that replacing oxyhemoglobin with deoxyhemoglobin causes major spectral changes in the reflection, absorption, and transmission within the wavelength ranges of 450–520, 590–780, and 780–1100 nm, while replacing deoxyhemoglobin with methemoglobin leads to significant changes in the ranges of 520–590, 590–780, and 780–1100 nm. It was found that within the 520–590 nm range, with a peak at 580 ± 5 nm, replacing oxyhemoglobin with deoxyhemoglobin does not significantly alter reflection, absorption, or transmission, whereas changes in methemoglobin content result in an increase in reflection and transmission in this spectral range while decreasing absorption. Numerical analysis demonstrated that decreasing blood oxygen saturation leads to increased transmission (optical clearing) in the 450–520 and 780–1100 nm wavelength ranges, while the presence of methemoglobin causes optical clearing in the 520–590 nm range.

Keywords: Spectra, reflection, absorption, transmission, blood, methemoglobin.

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Introduction

Blood performs vital functions, including transporting oxygen and nutrients, maintaining body temperature, and participating in immune responses [1]. Plasma and cellular elements (erythrocytes, leukocytes, platelets) are its main components. Erythrocytes make up about 99% of all cellular elements of the blood, hemoglobin accounts for 25% of the volume of erythrocytes, leukocytes and platelets are present in much smaller quantities. Plasma makes up approximately 55% of blood volume, while cellular elements account for about 45% of blood volume [2]. The hematocrit, which reflects the volume fraction of red blood cells in the blood, averages 40% for women and 45% for men. The plasma composition is dominated by water (about 92%), proteins, nutrients and dissolved gases, which plays an important role in maintaining the body's homeostasis [3].

Red blood cells containing hemoglobin are key participants in the transport of oxygen and carbon dioxide. Their size, shape, and concentration have a significant effect on the optical properties of blood. It is known that the refractive index of red blood cells is higher than that of blood plasma, and the difference in their values is the main source of light scattering [4]. The size and shape of red blood cells determine their scattering ability: when red blood cells aggregate, the number of diffusion surfaces decreases, which leads to a decrease in the backscattered signal. The concentration of red blood cells also directly affects the absorption and scattering of light by the blood, as it determines the intensity of light exposure to it [5].

The possibilities of modern optical, including laser, medical technologies are limited by the small depth of light penetration into biological tissues [6]. In this regard, the task of increasing the depth of light penetration, or, in other words, the task of biological tissues and body fluids optical clearing, including blood, is very relevant.

Blood optical clearing methods based on immersion techniques can reduce scattering and increase blood transparency by reducing the difference in refractive indices between plasma and red blood cells. Osmotically active solutions such as saline, glucose, glycerin, propylene glycol, dextran and radiopaque substances are used for this purpose. These substances change the properties of the plasma, which helps to harmonize the refractive indices and reduce scattering [5]. The matching of the refractive indices of erythrocytes and plasma can also be achieved by local hemolysis by introducing hypoosmotic solutions, which leads to the destruction of erythrocytes and the release of hemoglobin into plasma, in addition, injections of a hemoglobin solution into biological tissues can also change their optical properties [7–9]. Blood optical clearing makes it possible to expand the areas for research and improve the quality of diagnostics using optical methods. However, immersion techniques are limited by the diffusion time of immersion liquids, which means that a sufficiently long period of time is required to clear the tissue to a significant depth [10].

At the same time, the clearing effect can be achieved not only through the use of immersion, but also by changing the composition of the components of the biological tissue itself. It is known that blood can change its composition when heated due to the thermal transformation of hemoglobin. Thus, at temperatures of 46–48°C, oxyhemoglobin is converted to deoxyhemoglobin, and at 65–70°C, deoxyhemoglobin is oxidized to methemoglobin [11]. Methemoglobin, being a dysfunctional form of hemoglobin, has unique optical characteristics. The absorption spectrum of methemoglobin differs significantly from the absorption spectrum of deoxyhemoglobin in the visible region in the range of 415–630 nm and in the infrared region in the range of 800–1200 nm, which can significantly affect the absorption spectrum of whole blood, in which deoxyhemoglobin is replaced with methemoglobin [12]. The influence of laser heating of blood on its optical properties is also discussed in Ref. [13, 14]. Unfortunately, there is currently no comprehensive, including numerical, analysis in the literature of the relationship between the reflection, absorption, and transmittance spectra of blood in the visible and near-infrared regions of the spectrum with the dynamics of its content of deoxyhemoglobin, oxyhemoglobin, and methemoglobin, which makes the study of this relationship relevant, especially in the context of laser multiwave technologies for the treatment of vascular diseases the skin.

Multi-wavelength laser technologies for the treatment of vascular skin diseases involve the use of laser radiation with one wavelength to create optimal conditions for exposure to biological tissue radiation with a different wavelength. Thus, in laser sclerosis of telangiectasias, the most effective treatment is achieved when exposed first to a wavelength of 585 nm, and then to a wavelength of 1064 nm [15]. Obviously, this may be due to laser-induced changes in blood composition, which significantly alter the optical properties of blood, and this should be taken into account to achieve optimal results of laser treatment. The optimal result in this case can be achieved by monitoring the optical properties of the biological tissue during laser exposure with one wavelength and controlling the parameters of laser radiation with another wavelength when the optical characteristic of the biological tissue reaches the optimal value for laser exposure at this wavelength, i.e. when used feedback system in a laser.

Feedback systems play an important role in improving the safety and accuracy of energy dosing in case of the laser exposure [16]. In the absence of accurate dosing of laser exposure, undesirable effects may occur, such as perforation of biological tissues, burns, hypo- or hyperpigmentation, necrosis. Feedback systems can reduce the risk of these complications by changing the power, wavelength, exposure time, and more. The feedback mechanisms most common in laser medical systems today control the temperature of the working end of a fiber-optic instrument [17,18]. However, temperature cannot fully characterize the changes in biological tissues that occur under the influence of laser radiation, including due to the inertia of the thermal field and the technical difficulties associated with measuring temperature in the area of laser exposure or inside the biological tissue [19]. Feedback based on optical methods makes it possible to evaluate the result of laser exposure to tissues in real time. For example, monitoring the intensity of reflected light at the wavelengths most sensitive to changes in the state of chromophores in biological tissue can make it possible, by assessing the content of chromophores in biological tissue, to adapt the parameters of laser exposure in real time to achieve the desired treatment result. Thus, monitoring changes in reflected light in the content of deoxyhemoglobin and methemoglobin in the blood of a tissue when it is heated can determine the moment in time when, in a certain spectral region, the tissue becomes most transparent (clear) due to the thermostimulated substitution of these blood chromophores with each other. It is obvious that at present, the development of an adequate algorithm for operation and the construction of any feedback in a laser medical system, including one based on measuring the optical characteristics of biological tissue, is impossible without performing careful numerical simulation.

Using numerical simulation, it is possible to determine the wavelengths that are most effective for a particular laser treatment. For example, the wavelengths in the visible range of the spectrum absorbed by deoxyhemoglobin (420–450 and 590–800 nm) are well suited for effective photothermolysis, but do not allow laser energy to be delivered to deep tissue layers without damaging surface structures [20]. In this regard, in the context of creating an effective feedback system in medical laser systems, a comprehensive analysis of reflected, absorbed and transmitted light both at the wavelength of laser radiation and in a wide spectral range is very relevant. In some cases, diffuse reflectance spectroscopy and RGB imaging methods are effectively used to detect the state of biological tissue, which make it possible to noninvasively determine chromophore concentrations from diffuse reflectance spectra of the biological tissue under study or its image [21,22]. This allows, for example, to separate groups of patients with different levels of hemoglobin in the blood, as was done in Ref. [21]. This approach makes it possible to evaluate laser-induced changes in the optical properties of biological tissue in real time, which is critically important for optimizing the parameters of laser exposure.

Blood, as the main chromophore of blood-filled soft tissues, plays a key role in the formation of their optical properties. Reflection, absorption, and transmittance of light by blood are very well studied [5,23]. Numerical optical modeling of blood makes it possible to identify key wavelengths that are most sensitive to changes in methemoglobin saturation and concentration, which opens up wide opportunities for optimizing laser medical technologies and developing feedback systems. Such systems can adapt laser exposure in real time, minimizing tissue damage and increasing the effectiveness of treatment. It is important that numerical simulation allows taking into account the peculiarities of the composition of biological tissues and blood, which is critically important for the development of modern medical technologies. Changes in the concentrations of oxyhemoglobin, deoxyhemoglobin, and methemoglobin in the blood can characterize the state of biological tissues during laser exposure. The identification of spectral ranges that are most sensitive to changes in the concentration of various forms of hemoglobin makes it possible to improve the accuracy of diagnostic and therapeutic procedures, including feedback technologies. However, the lack of information on the effect of changes in the concentration of methemoglobin in the blood during laser heating on its optical parameters, including the reflection, absorption and transmittance spectra, limits the capabilities of modern laser technologies, including the treatment of deep-lying tissues. As a part of this work, it is proposed to fill this gap by numerically evaluating the behavior of light reflected, absorbed and transmitted through the human blood layer at various wavelengths with changes in the concentration of deoxyhemoglobin, oxyhemoglobin and methemoglobin.

Thus, in the context of optimizing the laser effect on blood and its containing tissues and searching for an optimal algorithm for the operation of a feedback laser system, the purpose and objectives of this study were to develop a computer model of an experimental setup to evaluate the powers of reflected, absorbed and transmitted light fluxes through a human blood layer and determine, as a result of numerical simulation, the wavelengths at which these powers vary as much as possible with changes in blood oxygen saturation and the concentration of methemoglobin in the blood, as well as the wavelengths at which the powers of these light streams do not change with changes in the same parameters.

Materials and methods

A computer simulation of the experimental setup for estimating the power of reflected, absorbed and transmitted light streams through the human blood layer is shown in Fig. 1. The thickness of the blood layer was chosen to be 150, 300, and 1000 μm, which is comparable to the size of small, medium, and large telangiectasias in human skin [24]. A numerical optical model of human blood presented in Ref. [25] was used to describe the processes accompanying the interaction of light with blood.

Optical numerical 3D simulation was performed in the „TracePro 7.0.1“ program (Lambda Research Corporation, USA) using the Monte Carlo method. The light source emitted in the wavelength range of 400–1100 nm. It was a completely transparent in the wavelength range of 400–1100 nm hollow infinitely thin cylinder with a diameter of 10 mm, which was located at a distance of 20 mm from the surface of the blood layer facing it (Fig. 1). The light from the source normally fell to the surface of the blood layer with a diameter of 50 mm and was a parallel beam of rays with a diameter of 10 mm. The beam diameter was selected in accordance with the data on radiation parameters for laser telangiectasia sclerosis presented in Ref. [26]. The spectral and spatial distribution of radiation in the source beam was uniform. 10,000 rays were used in the simulation, the total power of which was 1 W. The reflected and transmitted light power after interaction with the blood layer was intercepted by corresponding detectors with a diameter of 100 mm and evaluated using the built-in software tools of „TracePro 7.0.1“ as a result of integration over the area of the spatial distribution of the luminous flux power, which reached and was completely absorbed by the detector. The light power absorbed by the blood layer was calculated by subtracting the reflected and transmitted light fluxes from the power of the light source.

Combinations of concentrations of oxyhemoglobin ($C_{\mathrm{HbO_2}}$), deoxyhemoglobin (C_{Hb}) and methemoglobin (C_{MetHb}) in blood used in numerical simulation

№	$C_{\mathrm{HbO_2}}$, %	C_{Hb}, %	C_{MetHb}, %
1	100	0	0
2	80	20	0
3	60	40	0
4	40	60	0
5	20	80	0
6	0	100	0
7	0	80	20
8	0	60	40
9	0	40	60
10	0	20	80
11	0	0	100

The numerical simulation used eleven combinations of concentrations of various forms of hemoglobin, shown in the table. The selected combinations cover a wide range of physiological and pathological conditions, which makes it possible to approximate the results of numerical analysis of changes in the optical characteristics of blood to the results that may occur in a clinic setting. The combinations № 1–6 describe changes in blood saturation without the formation of methemoglobin, which corresponds to both normal physiological processes, such as blood oxygen saturation or the development of hypoxia, and pathological ones, when blood oxygen saturation decreases as a result of temperature exposure [11,21,27]. Combinations of No 7–11 correspond to the substitution of deoxyhemoglobin with methemoglobin, which is observed when exposed to thermal, including laser-induced or chemical factors [28,29].

As a result of numerical simulation, the power spectra of reflected ($R(\lambda)$), absorbed ($A(\lambda)$) and transmitted ($T(\lambda)$) through the blood layer of light in the wavelength range of 400–1100 nm were calculated with various combinations of concentrations of oxyhemoglobin, deoxyhemoglobin and methemoglobin according to the table. Further, the following equations were used to determine the parameters $dR(\lambda)$,

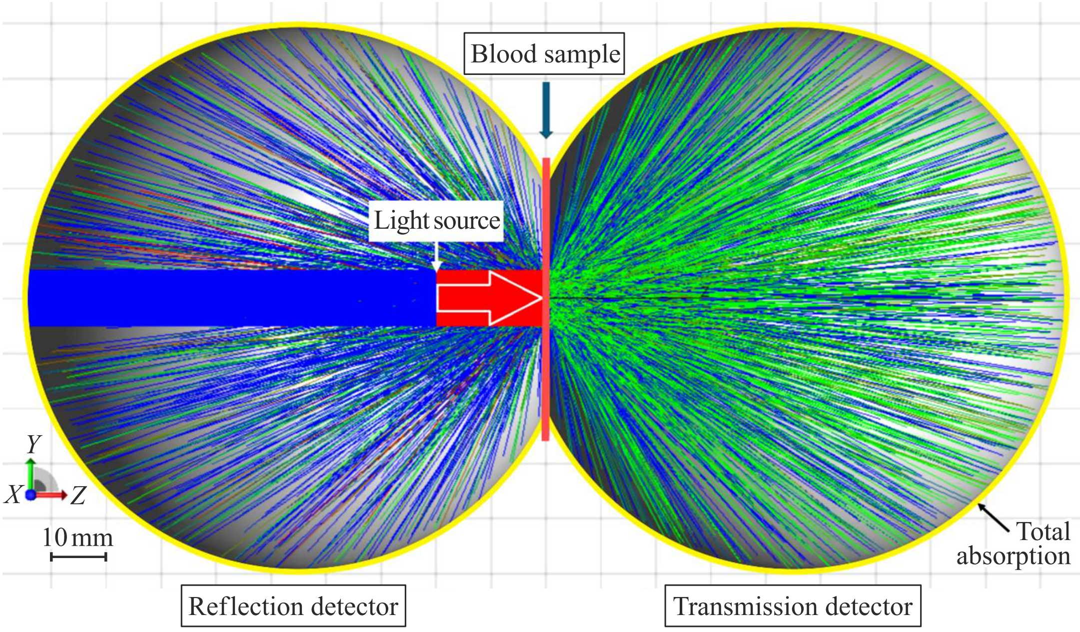

Figure 1. Ray path in the diagram of the computer model of the experimental setup for evaluating the power of reflected, absorbed and transmitted light streams through the human blood layer (for the combination No 1 according to the table, the wavelength is 800 nm). Red rays — for each such ray, the proportion of 1/10000 of the light source power ranges from 100.0 to 66.6%, green rays — from 66.6 to 33.3%, blue rays — from 33.3 to 0.1%.

$dA(\lambda)$ and $dT(\lambda)$:

$$dR(\lambda)_{\text{var}} = R(\lambda)_{\text{var}} - R(\lambda)_1, \tag{1}$$

where $R(\lambda)_{\text{var}}$ is the calculated power of the light reflected from the blood with parameters C_{HbO_2}, C_{Hb} and C_{MetHb} for combinations № 1−11 according to the table, $R(\lambda)_1$ is the calculated power of the light reflected from the blood layer for the combination № 1 according to the table;

$$dA(\lambda)_{\text{var}} = A(\lambda)_{\text{var}} - A(\lambda)_1, \tag{2}$$

where $A(\lambda)_{\text{var}}$ is the calculated power of the light absorbed by the blood with parameters C_{HbO_2}, C_{Hb} and C_{MetHb} for combinations № 1−11 according to the table, $A(\lambda)_1$ is the calculated power of the light absorbed by the blood layer for the combination № 1 according to the table;

$$dT(\lambda)_{\text{var}} = T(\lambda)_{\text{var}} - T(\lambda)_1, \tag{3}$$

where $T(\lambda)_{\text{var}}$ is the calculated power of the light transmitted through the blood with parameters C_{HbO_2}, C_{Hb} and C_{MetHb} for combinations № 1−11 according to the table, $T(\lambda)_1$ is the calculated power of the light transmitted through the blood layer for the combination № 1 according to the table.

The analysis of parameters $dR(\lambda)$, $dA(\lambda)$ and $dT(\lambda)$ was used to evaluate changes in the spectra of $R(\lambda)$, $A(\lambda)$ and $T(\lambda)$ with various combinations of oxyhemoglobin and deoxyhemoglobin concentrations and methemoglobin according to the table relative to the spectrum $R(\lambda)$, $A(\lambda)$ and $T(\lambda)$ of the blood layer consisting only of oxyhemoglobin (table, combination № 1) and allowed determining the wavelengths at which the intensities of reflected, absorbed and transmitted through the blood layer, the light changes as much as possible with changes in blood oxygen saturation and the concentration of methemoglobin in the blood, as well as the wavelengths at which the intensity of these light streams does not change with changes in the same parameters.

Results and discussion

Fig. 2 shows the spectra of reflection $R(\lambda)$ (Fig. 2, *a*), absorption $A(\lambda)$ (Fig. 2, *b*) and transmittance $T(\lambda)$ (Fig. 2, *c*) of a human blood layer with a thickness of 150 μm with different levels of oxyhemoglobin, deoxyhemoglobin and methemoglobin and spectra of parameters $dR(\lambda)$, $dA(\lambda)$ and $dT(\lambda)$, demonstrating changes in the reflection, absorption and transmittance of this layer of blood associated with changes in its composition relative to the initial one, for which the content of oxyhemoglobin is 100%, deoxyhemoglobin 0% and methemoglobin 0%.

It can be seen that when oxyhemoglobin is replaced by deoxyhemoglobin in a blood layer with a thickness of 150 μm, the most noticeable changes in reflection, absorption, and transmittance relative to the blood layer with $C_{\text{HbO}_2} = 100\%$ occur in the wavelength regions of 450−520, 590−780 and 780−1100 nm. Attention should be paid to the fact that the replacement of oxyhemoglobin with

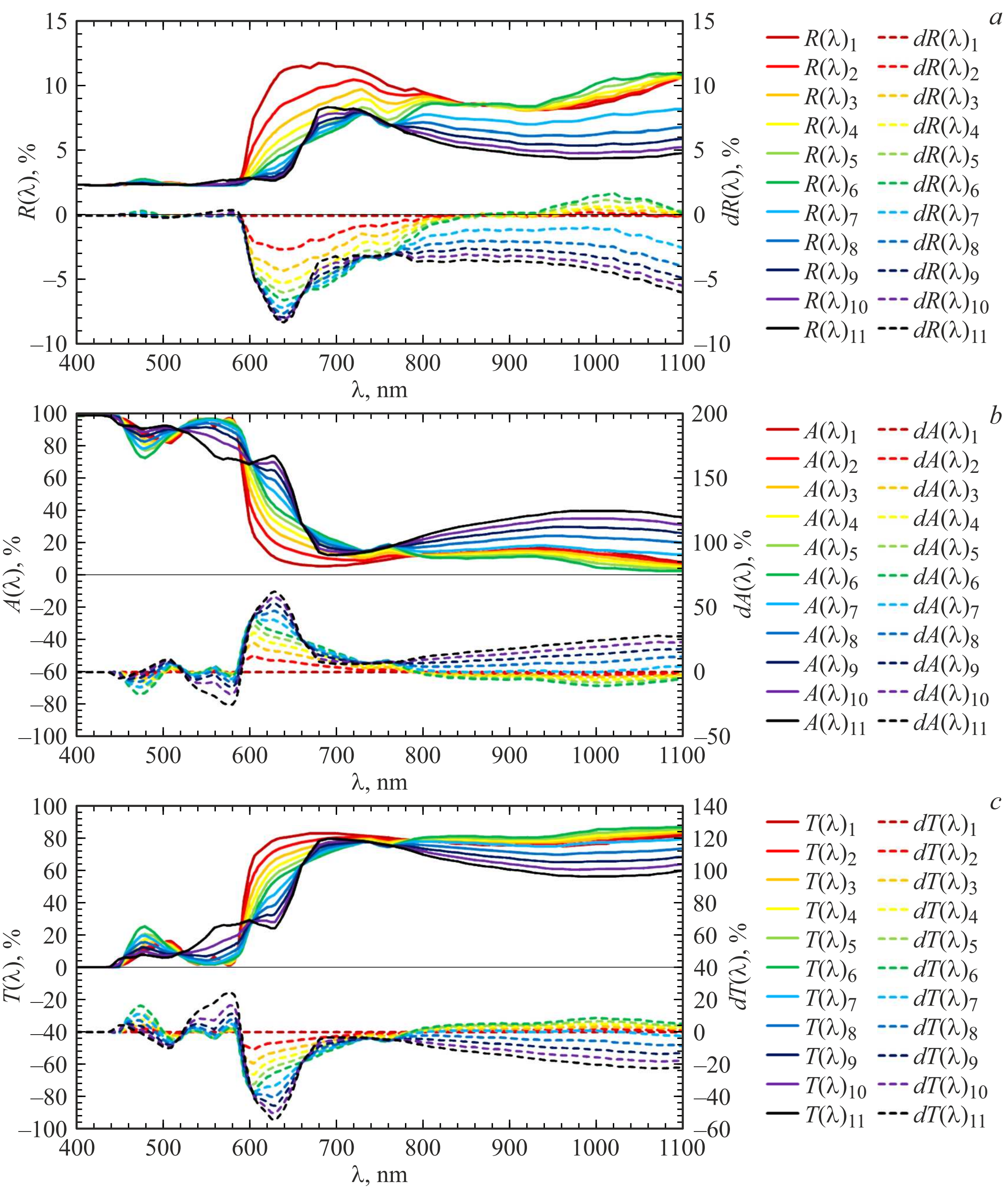

Figure 2. Spectra $R(\lambda)_{\mathrm{var}}$ and $dR(\lambda)_{\mathrm{var}}$ (a), spectra $A(\lambda)_{\mathrm{var}}$ and $dA(\lambda)_{\mathrm{var}}$ (b) and spectra $T(\lambda)_{\mathrm{var}}$ and $dT(\lambda)_{\mathrm{var}}$ (c) of a human blood layer with a thickness of $150\,\mu$m with different levels of oxyhemoglobin, deoxyhemoglobin and methemoglobin, where var is the combination number according to the table; $R(\lambda)_{\mathrm{var}}$, $A(\lambda)_{\mathrm{var}}$ and $T(\lambda)_{\mathrm{var}}$ — solid curves, $dR(\lambda)_{\mathrm{var}}$, $dA(\lambda)_{\mathrm{var}}$ and $dT(\lambda)_{\mathrm{var}}$ — dashed curves.

deoxyhemoglobin practically does not change the absorption of the blood layer at the wavelengths of 400–420, 430, 450, 500, 569, 586 and 795 nm, i.e. the absorption of oxyhemoglobin is equal to the absorption of deoxyhemoglobin at these wavelengths (isobestic points of blood). It is reported in Ref. [30] that the isobestic points of blood can include wavelengths 410, 430, 450, 500, 569, 586 and 805 nm. The authors of Ref. [31] attribute the wavelengths of 259.9, 339.5, 390.0, 422.0, 452.4, 500.1, 529.2, 545.3, 570.2, 584.1 and 796.8 nm to the isobestic points of blood. It should be noted that the results of simulation within the framework of the computer model developed in this work for assessing the power of light fluxes reflected, absorbed, and passing through a layer of human blood are in good agreement with the data of the above-mentioned works for isobestic points of blood, and minor discrepancies in the wavelengths of isobestic points of blood are associated with the contribution of scattering, which is taken into account in the computer model of the experimental setup and leads to a shift in the absorption band maxima in the blood layer when its thickness changes.

It should also be noted that when deoxyhemoglobin is replaced by methemoglobin, the absorption of the blood layer does not change at wavelengths of 430, 455, 520, 603, 660 and 760 nm, which also agrees well with the known isobestic points of methemoglobin [32,33].

The reflection exhibits a maximum increase by 0.34% ($\lambda = 476$ nm) in the region of 450–520 nm with an increase in the content of deoxyhemoglobin in the blood from 0 to 100%, it practically does not change in the region of 520–590 nm, it decreases as much as possible by 6.50% in the region of 590–780 nm, while the maximum corresponds to $\lambda = 640$ nm, while the reflection increases by a maximum of 1.73% ($\lambda = 1020$ nm) in the area of 780–1100 nm. This result is consistent with the data from Ref. [25], in which exposure to blood to laser radiation with wavelengths of 450 or 980 nm led to a decrease in reflection at wavelengths from the range of 590–780 nm, associated with a decrease in blood oxygen saturation, that is, with the replacement of oxyhemoglobin with deoxyhemoglobin. The absorption decreases maximally by 16.63% ($\lambda = 475$ nm) in the region of 450–520 nm with an increase in the deoxyhemoglobin content from 0 to 100%, it practically does not change in the region 520–590 nm, it is maximized by 41.76% ($\lambda = 606$ nm) in the region of 590–780 nm, and it exhibits a maximum decrease by 10.25% ($\lambda = 1000$ nm) in the region of 780–1100 nm. The transmittance exhibits a maximum increase by 16.30% ($\lambda = 475$ nm) in the region of 450–520 nm with an increase in the deoxyhemoglobin content from 0 to 100%, it practically does not change in the region of 520–590 nm, it exhibits a maximum decrease by 37.29% ($\lambda = 604$ nm) in the region of 590–780 nm, and it increases maximally by 8.71% ($\lambda = 1000$ nm) in the region of 780–1100 nm. Thus, the replacement of oxyhemoglobin with deoxyhemoglobin in human blood leads to a directly proportional change in reflection and transmittance relative to each other and an inversely proportional change in absorption. Obviously, when deoxyhemoglobin is replaced by oxyhemoglobin, changes in the spectra will demonstrate the reverse of the dynamics described above, i.e. its decrease will be observed in those wavelength regions where an increase in one or another of the above optical characteristics was observed.

It can be seen that when deoxyhemoglobin is replaced by methemoglobin in a blood layer with a thickness of 150 μm, the most noticeable changes in reflection, absorption, and transmittance relative to the blood layer with $C_{\mathrm{HbO_2}} = 100\%$ occur in the wavelength regions of 520–590, 590–780 and 780–1100 nm. The reflection practically does not change in the region of 450–520 nm, with an increase in the content of methemoglobin from 0 to 100%, it exhibits a maximum increase by 0.43% ($\lambda = 580$ nm) in the region 520–590 nm, it exhibits a maximum decrease by 8.23% ($\lambda = 640$ nm) in the region of 590–780 nm, and it decreases by a maximum of 5.91% ($\lambda = 1100$ nm) in the region of 780–1100 nm. The result obtained is in good agreement with the data presented Ref. [34], where a decrease in the intensity of light reflected by the skin with a wavelength of 980 nm was recorded during laser heating and an associated increase in the concentration of methemoglobin in the skin's blood. Also, similar changes in the reflection spectra of a blood solution associated with the conversion of oxyhemoglobin to deoxyhemoglobin and methemoglobin as a result of heating were demonstrated in Ref. [35], where the issue of reflection changes during liver surgery using a laser was studied. The absorption practically does not change in the region of 450–520 nm with an increase in the content of methemoglobin from 0 to 100%, it exhibits a maximum decrease by 24.90% ($\lambda = 578$ nm) in the region of 520–590 nm, it exhibits a maximum increase by 62.25% ($\lambda = 629$ nm) in the region of 590–780 nm, and it exhibits a maximum increase by 27.97% ($\lambda = 1070$ nm) in the region of 780–1100 nm. The transmittance practically does not change in the region of 450–520 nm with an increase in the content of methemoglobin from 0 to 100%, it increases by 24.47% ($\lambda = 578$ nm) in the region of 520–590 nm, it exhibits a maximum decrease by 54.50% ($\lambda = 629$ nm) in the region of 590–780 nm, and it exhibits a maximum decrease by 22.55% ($\lambda = 1070$ nm) in the region of 780–1100 nm. Thus, the replacement of deoxyhemoglobin with methemoglobin in human blood leads to a directly proportional change in reflection and transmittance relative to each other and an inversely proportional change in absorption. Obviously, when methemoglobin is replaced with deoxyhemoglobin, changes in the spectra will demonstrate the reverse of the dynamics described above, i.e. in those wavelength regions where an increase in one or another of the above optical characteristics was observed, its decrease will be observed.

Fig. 3 shows histograms reflecting the effect of the thickness of the blood layer on the reflection, absorption and transmittance of light by the layer in the wavelength regions noted above when oxyhemoglobin is replaced by deoxyhemoglobin and $C_{\mathrm{MetHb}} = 0\%$ in the blood.

The previously noted trends reflecting the effect of oxyhemoglobin replacement on deoxyhemoglobin in the blood persist and do not change with changing layer thickness. It can be seen that in this case, $dR(\lambda)$, $dA(\lambda)$ and $dT(\lambda)$ reach zero at a thickness of 1000 μm as a result of the increase in layer thickness in the wavelength regions of 450–520 and 520–590 nm. An increase in thickness leads to a decrease of $dR(\lambda)$ in the wavelength range of 590–780 nm, a nonlinear decrease of $dA(\lambda)$, and an increase in $dT(\lambda)$. An increase in thickness leads to an increase of $dR(\lambda)$, a decrease of $dA(\lambda)$, and an increase of $dT(\lambda)$ in the wavelength range of 780–1100 nm. The nonlinearity in the above dependencies can be attributed to the fact that with the increase of the wavelength thickness, the maxima of the studied ranges shift, for example, the maximum of the absorption band of 590–780 nm shifts from 606 nm with a thickness of the blood layer of 150 μm by 670 nm at a thickness of 1000 μm. It becomes clear from the

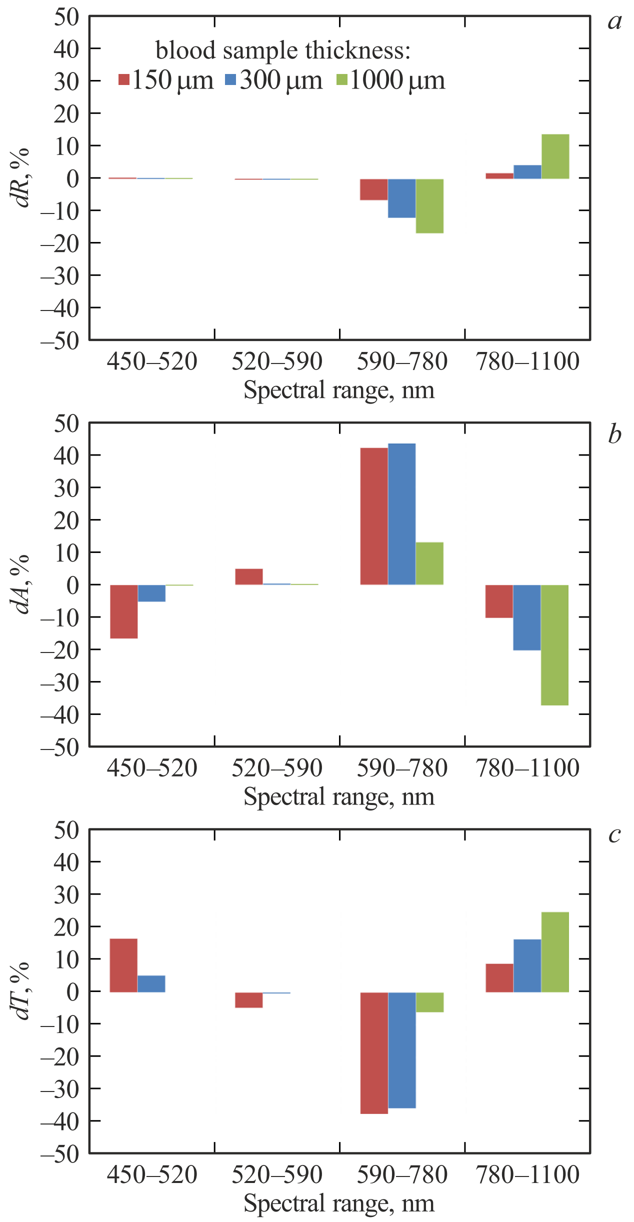

Figure 3. Effect of blood layer thickness on $dR(\lambda)$ (a), $dA(\lambda)$ (b) and $dT(\lambda)$ (c) in the wavelength regions of 450−520 (476/475/475), 520 −590 (560/560/560), 590 −780 (640/606/604) and 780−1100 nm (1020/1000/1000 nm) when oxyhemoglobin was replaced in the blood by deoxyhemoglobin and $C_{\mathrm{MetHb}} = 0\%$ (the wavelengths in the studied spectral region are indicated in parentheses, at which $dR(\lambda)$, $dA(\lambda)$ and $dT(\lambda)$, respectively, had maximum values with a blood layer thickness of 150 μm).

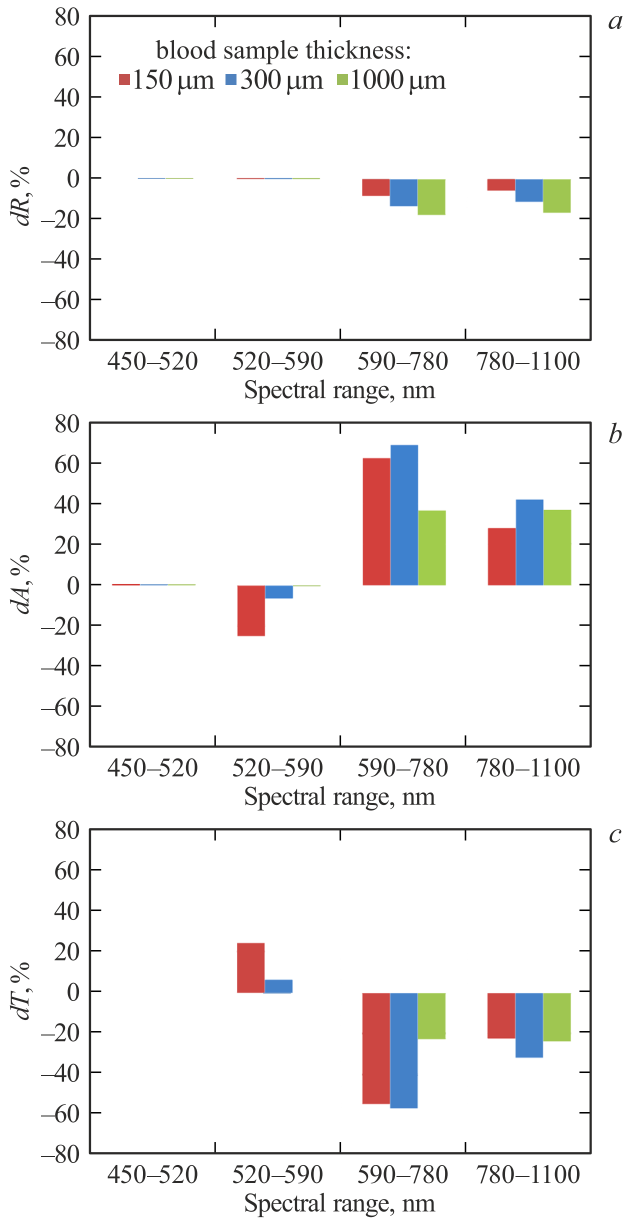

Figure 4. Effect of blood layer thickness on $dR(\lambda)$ (a), $dA(\lambda)$ (b) and $dT(\lambda)$ (c) in the wavelength regions of 450−520 (476/475/475), 520 −590 (580/578/578), 590 −780 (640/629/629) and 780−1100 nm (1100/1070/1070 nm) when deoxyhemoglobin was replaced in the blood by methemoglobin and $C_{\mathrm{HbO_2}} = 0\%$ (the wavelengths in the studied spectral region are indicated in parentheses, at which $dR(\lambda)$, $dA(\lambda)$ and $dT(\lambda)$, respectively, had maximum values with a blood layer thickness of 150 μm).

presented data that the wavelength ranges of 590−780 nm and 780−1100 nm are best suited for detecting the degree of blood oxygenation by analyzing the light reflected from it, since the value of $dR(\lambda)$ is significantly ($> 1\%$) above zero in these spectral regions. The decrease in oxygenation (replacement of oxyhemoglobin by deoxyhemoglobin) is accompanied by a decrease in $dR(\lambda)$ in wavelength range of 590−780 nm, and in the region of 780−1100 nm, on the contrary, it leads to an increase in $dR(\lambda)$ and these changes only increase with the increase of the thickness of the blood layer.

Fig. 4 shows histograms reflecting the effect of the thickness of the blood layer on the reflection, absorption and transmittance of light by the layer in the wavelength regions noted above when deoxyhemoglobin is replaced in the blood by methemoglobin and $C_{\mathrm{HbO_2}} = 0\%$.

The previously noted trends reflecting the effect of deoxyhemoglobin replacement on blood methemoglobin persist and do not change with changing layer thickness. It can be seen that in this case that an increase in layer thickness in the wavelength regions of 450−520 nm and 520−590 nm leads to the fact that $dR(\lambda)$, $dA(\lambda)$ and $dT(\lambda)$ reach zero at a thickness of $1000\,\mu$m. In the wavelength range of 590−780 nm, an increase in thickness leads to a decrease in $dR(\lambda)$, a nonlinear change in absorption $dA(\lambda)$ (an increase in the thickness of the blood layer from $150\,\mu$m to $300\,\mu$m and decrease with increasing thickness from 300 to $1000\,\mu$m) and nonlinear change $dT(\lambda)$ (decrease with increasing thickness of the blood layer from 150 to $300\,\mu$m and increase with the increase of the thickness from 300 to $1000\,\mu$m). An increase in thickness leads to a decrease in $dR(\lambda)$ in the wavelength range of 780−1100 nm, a nonlinear change in $dA(\lambda)$ (an increase in the thickness of the blood layer from 150 to $300\,\mu$m and a decrease in an increase in thickness from 300 to $1000\,\mu$m) and a nonlinear change in $dT(\lambda)$ (decrease with an increase in the thickness of the blood layer from 150 to $300\,\mu$m and increase with an increase in thickness from 300 to $1000\,\mu$m). The nonlinearities in the above dependences in this case, as well as with changes in saturation, can be attributed to the fact that with increasing wavelength thickness, the maxima of the studied ranges shift.

It becomes clear from the presented data that the wavelength ranges of 590−780 nm and 780−1100 nm are best suited for detecting the presence of methemoglobin in blood by analyzing the light reflected from it, since the value of $dR(\lambda)$ is significantly ($> 5\%$) above zero in these spectral regions. The replacement of deoxyhemoglobin with methemoglobin is accompanied by a decrease in $dR(\lambda)$ in the wavelength range of 590−780 nm, while a drop in $dR(\lambda)$ is observed in the range of 780−1100 nm, in contrast to the trend observed in case of replacement of oxyhemoglobin with hemoglobin, when hemoglobin is replaced by methemoglobin, and these changes only increase with the increase of the thickness of the blood layer.

Analysis of the presented spectra of the blood layer with a thickness of $150\,\mu$m allows identifying a band in the wavelength range of 520−590 nm with a maximum of 580 ± 5 nm, in which changes in the reflection, absorption and transmittance spectra of human blood are associated only with a change in the content of methemoglobin in it. In this range, according to the simulation presented above, replacing oxyhemoglobin with deoxyhemoglobin in the blood does not significantly change $dR(\lambda)$, $dA(\lambda)$ and $dT(\lambda)$, while $dR(\lambda)$ and $dT(\lambda)$ increase in case of replacement of deoxyhemoglobin with methemoglobin and $dA(\lambda)$ drops. Thus, the appearance of methemoglobin in the blood can lead to its brightening in the above wavelength range (520−590 nm), which can be used for diagnostic or therapeutic purposes in medicine, while the content of methemoglobin can be controlled by analyzing the dynamics of light reflected from biological tissue in this spectral range. At the same time, changes in blood oxygen saturation can also have a noticeable effect on blood transmittance in some cases, for example, when oxyhemoglobin is replaced with deoxyhemoglobin, leading to optical clearing in the wavelength ranges of 450−520 nm and 780−1100 nm.

Conclusion

As a part of a numerical optical model of human blood, the effect of blood oxygenation and the content of methemoglobin in it on the reflection, absorption and transmittance of light in the wavelength range of 400−1100 nm by a layer of blood of various thicknesses has been studied. As a result of numerical simulation, it was found that when oxyhemoglobin is replaced by deoxyhemoglobin, the blood light absorption does not change at 400−420, 430, 450, 500, 569, 586 and 795 nm, and when deoxyhemoglobin is replaced by methemoglobin the blood light absorption does not change at wavelengths of 430, 455, 520, 603, 660 and 760 nm, which is in good agreement with the data on isobestic points presented in the literature. It was found that when oxyhemoglobin is replaced by deoxyhemoglobin in a blood layer with a thickness of $150\,\mu$m, the main changes in reflection, absorption and transmittance occur in the wavelength regions of 450−520, 590−780 and 780−1100 nm, and when deoxyhemoglobin is replaced by methemoglobin the main changes in reflection, absorption and transmittance occur in the wavelength ranges of 520−590, 590−780 and 780−1100 nm. The replacement of oxyhemoglobin with deoxyhemoglobin and deoxyhemoglobin with methemoglobin in human blood leads to a directly proportional change in reflection and transmittance and an inversely proportional change in absorption. A thickness change of the blood layer from 150 to $1000\,\mu$m does not significantly affect the trends noted above although it leads to a deformation of the spectra. The replacement of oxyhemoglobin with deoxyhemoglobin in the blood does not significantly change reflection, absorption and transmittance in the wavelength range of 520−590 nm with a maximum of 580 ± 5 nm, while when the content of methemoglobin changes, the reflection and transmittance increase in this region of wavelengths and the absorption decreases. The results obtained in the study can be used in the development of feedback mechanisms in laser medical systems to optimize the treatment of biological tissues and organs.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] W.F. Boron, E.L. Boulpaep. *Medical Physiology E-Book* (Elsevier Health Sciences, Philadelphia, 2016).

[2] L. Dean. *Blood Groups and Red Cell Antigens* (NCBI, Bethesda, 2005).

[3] J. Mathew, P. Sankar, M. Varacallo. *Physiology, Blood Plasma* (StatPearls Publishing, Treasure Island (FL), 2018).

[4] O. Zhernovaya, V.V. Tuchin, M.J. Leahy. J. Biomed. Opt., **18** (2), 026014 (2013). DOI: 10.1117/1.jbo.18.2.026014

[5] V.V. Tuchin, X. Xu, R.K. Wang. Appl. Opt., **41** (1), 258 (2002). DOI: 10.1364/AO.41.000258

[6] T.L. Wall. Semin. Plast. Surg., **21** (3) (2007). DOI: 10.1055/s-2007-991183

[7] V.V. Tuchin et al. Opt. Express, **12** (13), 2966 (2004). DOI: 10.1364/opex.12.002966

[8] O. Zhernovaya, V.V. Tuchin, M.J. Leahy. J. Biomed. Opt., **18** (2), 026014 (2013). DOI: 10.1117/1.JBO.18.2.026014

[9] A.N. Bashkatov et al. J. Biomed. Photonics Eng., **3** (4), 040304 (2017). DOI: 10.18287/JBPE17.03.040304

[10] V.V. Tuchin, D. Zhu, E.A. Genina, eds. *Handbook of Tissue Optical Clearing: New Prospects in Optical Imaging* (CRC Press, 2022). DOI: 10.1201/9781003025252

[11] S. Kimel, B. Choi, L.O. Svaasand, J. Lotfi, J.A. Viator, J.S. Nelson. Lasers Surg. Med., **36** (4), 281 (2005). DOI: 10.1002/lsm.20154

[12] F. Khatun, Y. Aizu, I. Nishidate. Int. J. Mol. Sci., **22** (4), 1528 (2021). DOI: 10.3390/ijms22041528

[13] I.N. Yu. J. Biomed. Photonics Eng., **8** (4), 40513 (2022). DOI: 10.18287/JBPE22.08.040513

[14] H. Jia, B. Chen, D. Li. Lasers Med. Sci., **32**, 513 (2017). DOI: 10.1007/s10103-017-2143-8.

[15] M.A. Trelles, R. Weiss, J. Moreno-Moragas, C. Romero, M. Vélez, X. Álvarez. Lasers Surg. Med., **42** (9), 769 (2010). DOI: 10.1002/lsm.20972

[16] D.B. Smirnov et al. *Advances in 3OM: Opto-Mechatronics, Opto-Mechanics, and Optical Metrology*. V. 12170 (SPIE, 2022). DOI: 10.1117/12.2622983

[17] V. Belikov, F.I. Feldchtein, G.B. Altshuler. US Patent 2012/0123399 A1, N 13/379,916 (17 May, 2012).

[18] G.E. Romanos, A.V. Belikov, A.V. Skrypnik, F.I. Feldchtein, M.Z. Smirnov, G.B. Altshuler. Lasers Surg. Med., **47** (5), 411 (2015). DOI: 10.1002/lsm.22360

[19] L. Cummins, M. Nauenberg. Biophys. J., **42** (1), 99 (1983). DOI: 10.1016/s0006-3495(83)84373-2

[20] A.E. Pushkareva, I.V. Ponomarev, S.B. Topchiy, S.V. Klyuchareva. Laser Phys., **28** (9), 096003 (2018). DOI: 10.1088/1555-6611/aac903

[21] B. Yakimov, K. Buiankin, G. Denisenko, Y. Shitova, A. Shkoda, E. Shirshin. Sci. Rep., **14** (1), 22874 (2024). DOI: 10.1038/s41598-024-73084-6

[22] V. Perekatova, E. Sergeeva, M. Kirillin, A. Khilov, D. Kurakina, I. Turchin. Opt. Commun., **579**, 131440 (2025). DOI: 10.1016/j.optcom.2024.131440

[23] N. Bosschaart, G.J. Edelman, M.C. Aalders, T.G. van Leeuwen, D.J. Faber. Lasers Med. Sci., **29**, 453 (2014). DOI: 10.1007/s10103-013-1446-7

[24] D.M. Duffy. Cosmet. Dermatol., **25** (3), 126 (2012).

[25] V.Y. Chuchin, A.A. Masharskaya, A.V. Belikov. J. Biophotonics, e202400251 (2024). DOI: 10.1002/jbio.202400251

[26] M.P. Goldman. *Sclerotherapy, Treatment of Leg Telangiectasias with Laser and High-Intensity Pulsed Light* (Elsevier, 2017), p. 388–425.

[27] F. Bloos, K. Reinhart. Intensive Care Med., **31** (7), 911 (2005). DOI: 10.1007/s00134-005-2670-9

[28] L.L. Randeberg, J.H. Bonesrønning, M. Dalaker, J.S. Nelson, L.O. Svaasand. Lasers Surg. Med., **34** (5), 414 (2004). DOI: 10.1002/lsm.20042

[29] R.O. Wright, W.J. Lewander, A.D. Woolf. Ann. Emerg. Med., **34** (5), 646 (1999). DOI: 10.1016/s0196-0644(99)70167-8

[30] D.A. Rogatkin. Mes. fiz., (2), 97 (2012) (in Russian).

[31] W. Karlen, J. Lim, J.M. Ansermino, G. Dumont, C. Scheffer. *2012 Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 2448 (IEEE, 2012). DOI: 10.1109/EMBC.2012.6346459

[32] M. Nagai, A. Tomoda, Y. Yoneyama. J. Biol. Chem., **256** (17), 9195 (1981).

[33] F. Khatun, Y. Aizu, I. Nishidate. J. Biomed. Opt., **26** (3), 033708 (2021). DOI: 10.1016/S0021-9258(19)52527-3

[34] A.V. Belikov, V.Y. Chuchin. J. Biomed. Photonics Eng., **10** (2), 020303 (2024). DOI: 10.18287/JBPE24.10.020303

[35] J.W. Spliethoff et al. J. Biomed. Opt., **19** (9), 097004 (2014). DOI: 10.1117/1.JBO.19.9.097004

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