Investigation of the Influence of Thermally Induced Methemoglobin on the Human Skin Optical Reflection

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Abstract. The relationship between the reflection coefficient of human skin at a wavelength of 980 nm and its temperature during exposure to laser radiation of the same wavelength has been measured for the first time in an in vivo experiment. An interpretation of the experimentally obtained results is provided within the framework of a seven-layer optical computer model of human skin. Association of the observed alteration in the reflection coefficient at the wavelength of 980 nm to the conversion of skin blood hemoglobin into methemoglobin is demonstrated. The results of this research may find application in the development of novel laser systems with feedback mechanisms and technologies for treating dermatological conditions, including telangiectasias. © 2024 Journal of Biomedical Photonics & Engineering.

Keywords: diode laser; reflection; human skin; computer model; heating; temperature.

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1 Introduction

Laser systems with feedback capability enable real-time adjustment of laser exposure parameters on biological tissue, depending on the desired therapeutic outcome [1]. The biological tissue condition monitoring allows to adapt to non-standard conditions during the operation and optimization of laser intervention timely. The thermal-optical feedback is most prevalent, which measures the temperature in treated area by the radiance of the heated fiber tip [2, 3]. However, ability of such feedback is limited to register changes occurring within the depth of the biological tissue. This limitation becomes particularly crucial in non-contact interstitial laser procedures in dermatology, where the delivery of maximum energy into deeper layers of the skin is imperative without compromising the integrity of superficial layers. This non-contact procedure usually applicable for the treatment of telangiectasias through laser sclerotherapy.

During laser sclerotherapy, the laser radiation is absorbed by the pathological blood vessel, elevating vessel temperature to approximately +80 °C [4]. Consequently, the vessel walls adhere together, leading to irreversible damage [5]. Various types of lasers are employed for laser sclerotherapy, including neodymium lasers with second harmonic generation, krypton, copper vapor, alexandrite, diode lasers (with wavelengths ranging from 800 nm to 1000 nm), neodymium, and others [5, 6].

Laser treatment of telangiectasias can be categorized into single- and dual-wavelength approaches [5, 7–10]. Single-wavelength laser therapy presents several drawbacks, such as hyper- and hypopigmentation, treatment efficacy variability based on skin type, discomfort during the procedure, and limitations concerning the depth and size of treatable vessels [5, 8]. Dual-wavelength laser treatment of telangiectasias involves the use of a combination of wavelengths at 585 nm and 1064 nm for example [7]. This method relies on altering the skin optical properties through the dermis preliminary heating with a laser pulse at a wavelength of 585 nm. The dual-wavelength approach is characterized by higher efficacy compared to the single-wavelength method, absence of hyper- and hypopigmentation, independence from the skin type for treatment effectiveness, as well as the mitigation of other side effects. Furthermore, larger blood vessels exhibit better responsiveness to dual-wavelength treatment [7].

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However, in adopting such an approach, it is crucial to possess knowledge and capability in measuring changes in the skin optical characteristics resulting from concomitant laser-induced effects, such as its laser-induced heating, to optimize the sclerotherapy process with minimal side effects.

The alteration of the skin optical properties during laser-induced heating may be influenced by changes in the concentration of its major chromophores, such as water and hemoglobin, as heating leads to an increase in blood flow and intensifies water evaporation from its surface [11, 12]. It is also known that hemoglobin begins to transform into methemoglobin at a temperature of approximately +50 ºC [13]. Theoretical analysis of the hemoglobin and methemoglobin absorption spectra, as well as the human skin absorption spectrum, reveals that with a complete hemoglobin replacement with methemoglobin in human skin, the most significant increase in absorption occurs at wavelengths of 629 nm (by 112.5%) and 1105 nm (by 96.2%), while the greatest increase in transmission occurs at wavelengths of 441 nm (by 3.7%) and 574 nm (by 24.4%) [14]. The increase in absorption arises from the fact that in the near-infrared region of the electromagnetic spectrum, methemoglobin exhibits a higher absorption coefficient than hemoglobin. The elevation of methemoglobin concentration in the blood of the skin leads to change light absorption, both at the previously mentioned wavelengths [15−17]. In this context, a dual-wavelength approach, different from that described above, to increase the efficiency of sclerosis of deep-lying telangiectasia we can proposed. It involves preheating the skin with an infrared laser pulse to achieve maximal transformation of hemoglobin into methemoglobin. This alteration of the skin optical properties is designed to increase its transparency in the visible spectrum. Subsequently, a visible laser pulse is used for effectively sclerosis deep-seated pathological vessels. It is evident that the efficacy of any dual-wavelength approach can be augmented through the monitoring of temperature and optical characteristics of the skin during the impact of the first wavelength. This involves determining the conditions under which the impact of the second wavelength will be optimal. A diode laser with a wavelength of 980 nm can be employed as a source of infrared laser emission, as its radiation adequately penetrates the skin (penetration depth ~1.6 mm at the e^{-1} level [18]). Notably, the human skin absorption at this wavelength significantly intensifies, by 50.4%, when replacing hemoglobin with methemoglobin [14].

Unfortunately, authentic *in vivo* data on the measured dynamics of optical properties of human skin under the influence of 980 nm wavelength radiation and the ensuing laser-induced heating are absent in the literature. Consequently, experimental acquisition of these data is a pertinent task for enhancing the efficacy of laser sclerotherapy for deep-seated telangiectasias.

It is worth noting that non-invasive and contactless *in vivo* measurement of the dynamics of optical properties of human skin is simplest realized during the assessment of the reflected signal at the wavelength of the laser radiation stimulating skin heating, in this case, at the wavelength of 980 nm including because that this approach eliminates the need to inject an optical radiation receiver into the biotissue.

Thus, the primary objective of this study is to experimentally assess the influence of laser-induced heating on the human skin optical properties *in vivo*. This influence will be studied by measuring the dependency of the intensity of light reflected by human skin at the wavelength of 980 nm on the heating of the skin stimulated by laser radiation with the same wavelength. Furthermore, the obtained results will be interpreted, and through optical computer modeling, we aim to evaluate the influence of changes in the concentration of major chromophores in human skin on its reflection of radiation at wavelength of 980 nm.

2 Materials and Methods

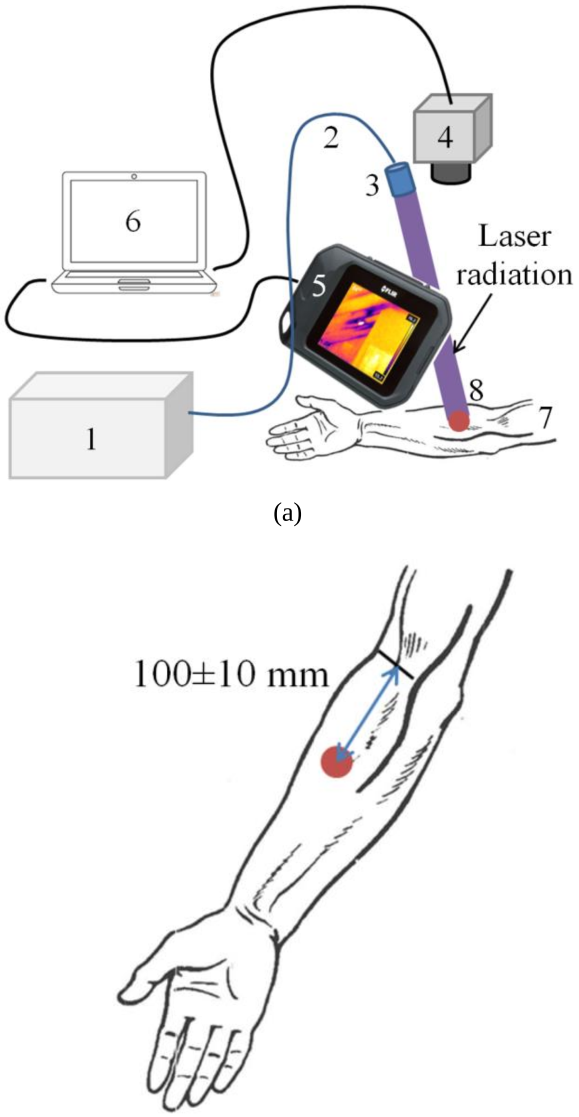
To perform *in vivo* measurements of the intensity of light reflected by human skin at a wavelength of 980 nm, an experimental setup was created (see Fig. 1(a)). The setup included a continuous wave laser source ALPH-01-“DIOLAN” (NPP VOLO LLC, Russia), operating at a wavelength of 980 nm with an average power of up to 25 W. The laser radiation was directed into a quartz fiber and focused on the volunteer’s skin using a collimator. The setup also comprised an infrared video camera BCK-756-USB (EVS LLC, Russia), capturing variations in the intensity of reflected light, thermal imaging camera FLIR C2 (Teledyne FLIR LLC, USA) for monitoring the skin surface temperature, and a computer. The laser radiation spot diameter on the skin surface was 17 ± 1 mm.

Twelve volunteers participated in the experiment, where the same area of skin on the arm underwent laser exposure (see Fig. 1(b)). Written consent for the experiment was obtained from all volunteers.

The laser exposure was conducted continuously, with an average output power of 15 ± 0.3 W at the collimator, continuing until the volunteer reported pain, at which point the laser was switched off. The infrared video camera and thermal imaging camera recorded the video of the laser radiation spot at a wavelength of 980 nm and the skin surface temperature, respectively. Subsequently, the video data were analyzed on a computer, synchronized in time, and each frame of the thermal imaging camera video was correlated with a frame from the infrared camera video. The maximum temperature in the frame was determined for each n frame of the thermal imaging camera video. The total intensity of all pixels was computed for each n frame of the infrared camera video. The intensity of light reflected by human skin at a wavelength of 980 nm was estimated using the equation:

$$I_R = I_n/I_1, \tag{1}$$

where I_1 is the total intensity of all pixels for the $n = 1$ frame, corresponding to the onset of laser radiation.



(a)

(b)

Fig. 1 Assessment of the impact of laser-induced heating on the optical properties of human skin *in vivo* by measuring the dependence of the skin reflection at a wavelength of 980 nm on the heating of the skin stimulated by laser radiation of the same wavelength: (a) experimental setup (1 – 980 nm diode laser, 2 – quartz fiber, 3 – collimator, 4 – infrared video camera, 5 – thermal imaging camera, 6 – computer, 7 – volunteer's arm, 8 – laser exposure site); (b) schematic diagram illustrating the location of the laser exposure site (red dot).

Through the analysis of correlated frames from the video recordings of the infrared camera and thermal imaging camera, dependencies of I_R on the maximum skin surface temperature at the laser exposure site were obtained for each volunteer.

Numerical model of the experimental setup (see Fig. 2) was created using the TracePro Expert ver. 7.0.1 software package (Lambda Research Corporation, USA) to interpret the experimentally obtained results. Subsequently, modelling was performed to determine the simulated dependence of I_R at a wavelength of 980 nm on the concentration of methemoglobin in the blood of human skin, as well as on the concentrations of water and blood in the skin layers.

Seven-Layer Optical Computer Model of Human skin [10] was used during the simulation (see Fig. 3).

The methemoglobin concentration was set at 0%, 25%, 50%, 75%, and 100% relative to the total hemoglobin content in the skin layer in assessing the impact of methemoglobin concentration in the blood on the intensity of light reflected by human skin I_R at a wavelength of 980 nm.

The water concentration varied in the range of −20% to +20% relative to the initial value in evaluating the influence of water concentration in the entire skin on the intensity of light reflected by human skin I_R at a wavelength of 980 nm. This corresponds to changes in water content in individual skin layers according to Table 1. Meanwhile, the concentrations of other chromophores were adjusted proportionally to the changes in water concentration.

The blood concentration was varied within the range of 0% to +100% relative to the initial value when assessing the impact of varying the concentration of blood in the entire skin on the intensity of light reflected by human skin I_R at a wavelength of 980 nm. This corresponds to changes in the blood content in individual skin layers according to Table 2. Meanwhile, the concentrations of other chromophores were proportionally reduced with the increase in blood concentration. In the stratum corneum (SC) and living epidermis (LE) layers, the blood concentration remained at 0%.

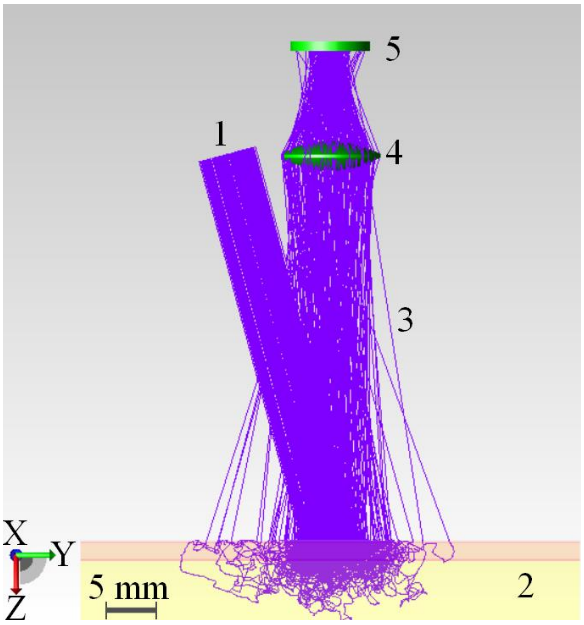


Fig. 2 Numerical model of the experimental setup (1 – a collimated beam of 980 nm laser radiation with diameter of 17 mm, 2 – a seven-layer optical computer model of human skin, 3 – light reflected by the skin with a wavelength of 980 nm, 4 – lens, 5 – receiving platform (100% absorber)).

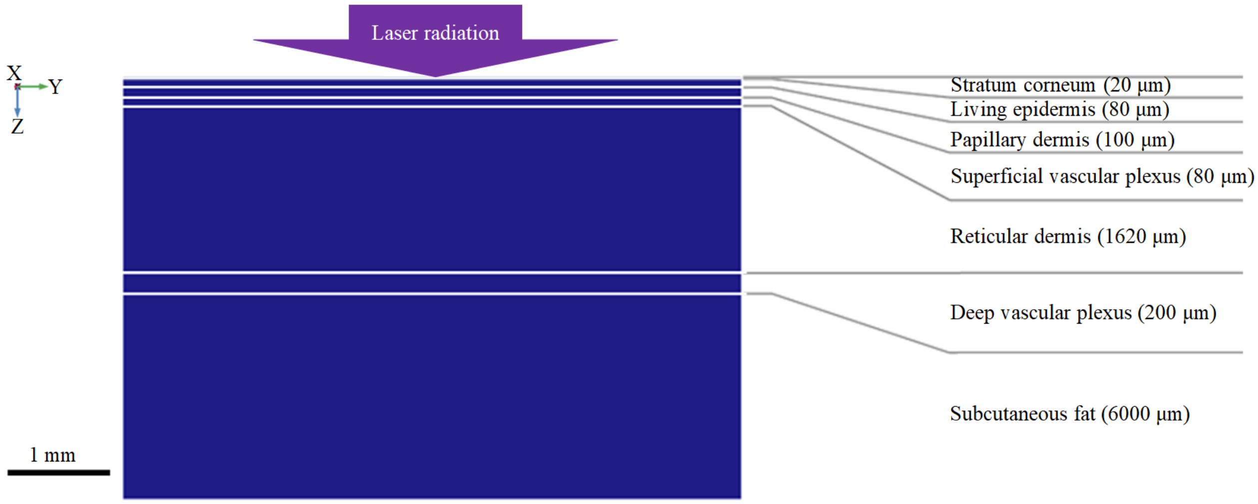


Fig. 3 Seven-layer optical computer model of human skin [10].

Table 1 Water concentrations in the layers of the seven-layer optical computer model of human skin used in the simulation.

Skin layer	Water concentration								
	−20%	**−15%**	**−10%**	**−5%**	**0%** (initial)	**+5%**	**+10%**	**+15%**	**+20%**
SC	4%	4.25%	4.5%	4.75%	5%	5.25%	5.5%	5.75%	6%
LE	16%	17%	18%	19%	20%	21%	22%	23%	24%
PD	40%	42.5%	45%	47.5%	50%	52.5%	55%	57.5%	60%
SVP	48%	51%	54%	57%	60%	63%	66%	69%	72%
RD	56%	59.5%	63%	66.5%	70%	73.5%	77%	80.5%	84%
DVP	56%	59.5%	63%	66.5%	70%	73.5%	77%	80.5%	84%

where SC – stratum corneum layer, LE – living epidermis layer, PD – papillary dermis layer, SVP – superficial vascular plexus layer, RD – reticular dermis layer, DVP – deep vascular plexus layer.

Table 2 Blood concentrations in the layers of the seven-layer optical computer model of human skin used in the simulation.

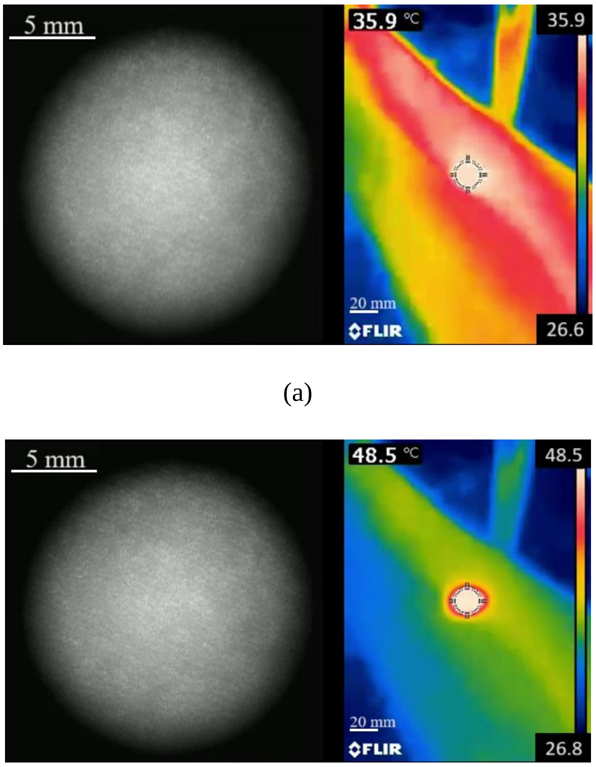
Skin layer	Blood concentration				
	+0% (initial)	**+25%**	**+50%**	**+75%**	**+100%**
PD	4%	5%	6%	7%	8%
SVP	30%	37.5%	45%	52.5%	60%
RD	4%	5%	6%	7%	8%
DVP	10%	12.5%	15%	17.5%	20%

where PD – papillary dermis layer, SVP – superficial vascular plexus layer, RD – reticular dermis layer, DVP – deep vascular plexus layer.

3 Results and Discussion

It was determined that the skin surface temperature at the laser exposure site reached +48.8 ± 0.6 °C at the moment volunteers reported pain within the *in vivo* experiment.

Examples of frames from the infrared video camera and their corresponding frames from the thermal imaging camera, obtained during the *in vivo* experiment before and immediately after laser exposure (when pain occurred), are shown in Fig. 4.



(a)

(b)

Fig. 4 Frames from the infrared video camera and their corresponding frames from the thermal imaging camera obtained during the *in vivo* experiment (a) before and (b) immediately after laser exposure (when pain occurred).

The dependency of the intensity of light reflected by human skin I_R at a wavelength of 980 nm on the skin surface temperature T_{skin}, derived from the analysis of the sequence of frames from the infrared video camera and thermal imaging camera, is presented in Fig. 5.

From the dependences presented in Fig. 5 it is clear that with increasing temperature, the average intensity of light reflected by human skin I_R at a wavelength of 980 nm decreases with increasing skin surface temperature T_{skin} and by the end the decrease in I_R reaches 3.6 ± 1.0%.

Optical computer simulation of skin reflection was performed to interpret the experimental results and assess the influence of skin chromophore concentrations on the intensity of light reflected by human skin I_R at a wavelength of 980 nm. Simulated dependencies of I_R at a wavelength of 980 nm from methemoglobin concentration C_{MetHb}, changes in water concentration ΔC_{water}, and changes in blood concentration ΔC_{blood} in the skin layers was determined and represented on Fig. 6.

The dependences presented in Fig. 6 reveal that alterations in the concentration of major chromophores in the skin exert an influence on the intensity of light reflected by human skin I_R at the wavelength of 980 nm. Specifically, as the methemoglobin concentration in the skin increases, the intensity of reflected light I_R at 980 nm decreases (see Fig. 6(a)). This reduction is attributed to the fact that methemoglobin possesses a higher absorption coefficient than hemoglobin at the wavelength of 980 nm. Simultaneously, an elevation in the concentration of water within the skin leads to a decrease in the concentration of other light-absorbing components, resulting in an augmentation of the intensity of light reflected by human skin I_R, at 980 nm (see Fig. 6(b)). Nevertheless, it is worth noting that variations in water concentration within the range of ±20% in the skin layers lead to a marginal alteration in the intensity of light reflected by human skin I_R at the wavelength of 980 nm, amounting to only ±0.5%.

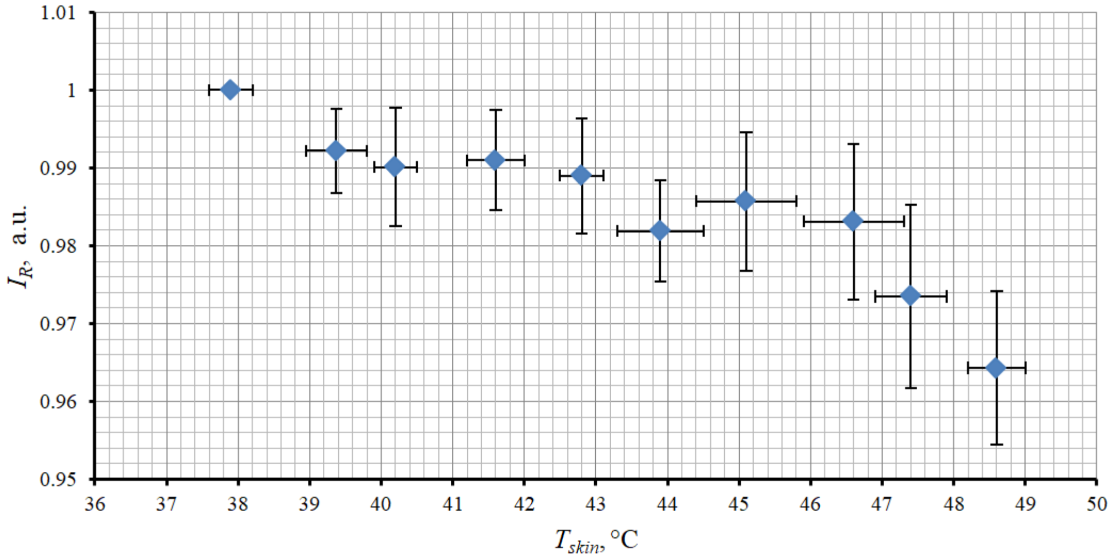
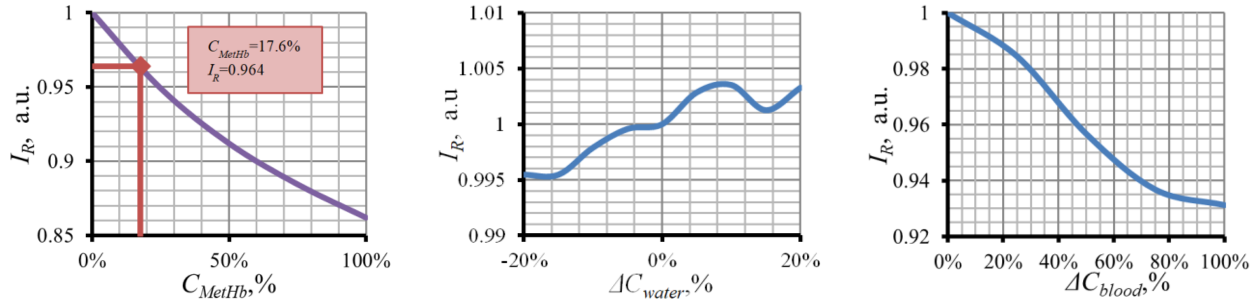


Fig. 5 The experimental dependence of the intensity of light reflected by human skin I_R at a wavelength of 980 nm (blue dots) with standard deviation (black bars) on the surface temperature of the volunteers' skin T_{skin} in the *in vivo* experiment.



(a) (b) (c)

Fig. 6 Simulated dependencies of the intensity of light reflected by human skin I_R at a wavelength of 980 nm on the (a) methemoglobin concentration C_{MetHb}, (b) changes in water concentration ΔC_{water}, and (c) changes in blood concentration ΔC_{blood} (c) in the skin layers.

Given this negligible change, when interpreting the results of *in vivo* experiments, the influence of water concentration fluctuations in skin can be disregarded. With an increase in blood concentration within the skin, the intensity of light reflected by human skin I_R at 980 nm decreases (see Fig. 6c). This reduction is attributed to the heightened presence of hemoglobin in the skin, which efficiently absorbs light at the wavelength of 980 nm. However, it is crucial to acknowledge that, in accordance with literature findings [7], an increase in blood flow in human skin occurs several minutes after the onset of heating. Therefore, when interpreting the results of this particular *in vivo* experiment, the impact of changes in blood concentration within the skin can be neglected.

Thus, by excluding the influence of changes in water and blood concentration within the layers of human skin, it can be inferred that the observed variation in the intensity of light reflected by human skin IR at 980 nm, during the *in vivo* experiment is primarily determined by the formation of methemoglobin in the blood of the skin resulting from its laser-induced heating. Based on the simulated dependence of the intensity of light reflected by human skin IR at 980 nm on the concentration of methemoglobin (see Fig. 6(a)) the change in the I_R is 3.6 ± 1.0% when the concentration of methemoglobin in the skin layers reaches a value equal to 17.6 ± 4.9%.

4 Conclusion

It has been demonstrated that continuous diode laser radiation with a wavelength of 980 nm, heating the surface of human skin to a temperature of +48.8 ± 0.6 °C, leads to a reduction in the intensity of light reflected by human skin I_R at 980 nm by an average of 3.6 ± 1.0% for the first time in an *in vivo* experiment. Using the numerical model of the experimental setup and a seven-layer optical computer model of human skin, the influence of the concentration of the major chromophores (water, blood) in the layers of human skin on the intensity of light reflected by human skin I_R at 980 nm was investigated. Additionally, the impact of the transformation of hemoglobin into methemoglobin on the I_R was studied. It was found that changes in the concentration of water in the layers of human skin in the range of ±20% do not lead to a significant change in the intensity of light reflected by human skin I_R at 980 nm, doubling the blood volume (100% relative to the initial) results in a 7% reduction in the I_R, and complete replacement of hemoglobin with methemoglobin in the skin leads to a 14% reduction in the I_R. Considering experimental results and simulated dependence of the intensity of light reflected by human skin I_R at 980 nm on the concentration of methemoglobin in the skin obtained from optical computer simulation, at a skin surface temperature of +48.8 ± 0.4 °C, the concentration of methemoglobin in the skin reaches 17.6 ± 4.9%. This effect can be used to monitor changes in skin's condition during laser exposure, which will allow one optimizing the parameters of laser for improvement of treatment of skin in dermatology and cosmetology.

Disclosures

The authors declare that they have no conflict of interest.

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