

PACS numbers: 42.25.Fx; 87.64.Cc; 87.53.Tf
DOI: 10.1070/QE2015v045n04ABEH015381

Method for estimating optimal spectral and energy parameters of laser irradiation in photodynamic therapy of biological tissue

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Abstract. **We have solved the problem of layer-by-layer laser-light dosimetry in biological tissues and of selecting an individual therapeutic dose in laser therapy. A method is proposed for real-time monitoring of the radiation density in tissue layers *in vivo*, concentrations of its endogenous (natural) and exogenous (specially administered) chromophores, as well as in-depth distributions of the spectrum of light action on these chromophores. As the background information use is made of the spectrum of diffuse light reflected from a patient's tissue, measured by a fibre-optic spectrophotometer. The measured spectrum is quantitatively analysed by the method of approximating functions for fluxes of light multiply scattered in tissue and by a semi-analytical method for calculating the in-depth distribution of the light flux in a multi-layered medium. We have shown the possibility of employing the developed method for monitoring photosensitizer and oxyhaemoglobin concentrations in tissue, light power absorbed by chromophores in tissue layers at different depths and laser-induced changes in the tissue morphology (vascular volume content and ratios of various forms of haemoglobin) during photodynamic therapy.**

Keywords: *biological tissue, photodynamic therapy, layer-by-layer dosimetry of laser light, diffuse reflectance, light action spectrum, photodissociation of oxyhaemoglobin.*

1. Introduction

Photodynamic therapy (PDT) is the least aggressive of cancer treatments, which is based on the use of light and a chemical compound known as a photosensitizer (PS). The physical mechanism of PDT can be represented as follows [1]. A PS administered intravenously into a human body selectively accumulates in tumour tissues. Once a lesion tissue is irradiated with light of a particular wavelength, the PS absorbs photons and activates a photochemical reaction, which generates from molecular triplet oxygen singlet oxygen that is toxic to biological tissue and causes the tumour destruction. In addition, PDT damages blood vessels feeding and delivering oxygen inside the tumour, which further enhances the death of cancer cells.

Because PDT is accompanied by continuous utilisation of molecular oxygen in photochemical reactions, it is necessary to maintain the required level of the tumour tissue oxygenation throughout the therapy session to cause a maximum damage to cancer cells. An effective method to eliminate the lack of oxygen (hypoxia) in the tumour area is the laser-induced photodissociation of oxyhaemoglobin (HbO_2) with the release of oxygen [2]. Dzhagarbov et al. [3] describe a method of photodynamic therapy of cancer, which consists in PS administration to tumour tissue and its simultaneous irradiation at two wavelengths. The first wavelength coincides with the absorption maximum of the photosensitizer, and the second is used for photodissociation of HbO_2. This method involves using the same wavelengths of laser illumination and irradiation doses for all patients, which can lead to low PDT efficacy and to a number of undesirable consequences associated with excessive heating of the patient's tissue and its irreversible changes (coagulation, destruction of capillaries, etc.).

A quantitative measure of the effectiveness of photoprocesses in irradiated tissue, caused by the absorption of light by its chromophore X, is the action spectrum of light on this chromophore [4]:

$$K_X(z,\lambda) = C_X(z)k_X(\lambda)\Phi(z,\lambda), \quad (1)$$

where λ is the wavelength of light; C_X and k_X are the concentration and absorption coefficient of the chromophore; and $\Phi(z,\lambda)$ is the power density spectrum at depth z in tissue (spatial illumination). To select individual spectral and energy parameters of PDT it is necessary to know the action spectra of light on the PS and HbO_2 in the patient's tissue, because the desired therapeutic effect is achieved only at a certain power absorbed by the target chromophore.

The development of an optimal strategy of the therapeutic effect of light on biological tissue is considered in many publications. For example, the authors of papers [4–9], based on the results of numerical modelling of the light field in human skin at average or sample values of its parameters, present general guidelines for spectral optimisation of the methods of light therapy of various diseases. However, these recommendations are qualitative in nature, and their practical use for the selection of optimal spectral and energy characteristics of laser light in each case is hindered by the lack of reliable data on optical parameters of irradiated tissue and on their changes in the interaction of light with tissue. According to the experimental studies [10–12], PDT is accompanied by a partial destruction of the walls of blood vessels (with release of haemoglobin) and by a significant increase in the concentration methaemoglobin in tissue (up to 60% of total haemoglobin concentration), which leads to a change in tissue optical parameters.

Thus, in order to increase the PDT efficacy and to avoid adverse side effects, it is necessary to control the PS and HbO_2

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Received 13 January 2014; revision received 2 June 2014
Kvantovaya Elektronika **45** (4) 358–365 (2015)
Translated by I.A. Ulitkin

concentration in the tumour, the in-depth distribution of the action spectra of light on these chromophores and the local changes in tissue morphology (volume content of blood vessels, and ratios of various forms of haemoglobin).

The amount of PS in tissue is commonly determined by the standard sample technique [13–15], based on a comparison of diffuse reflectance (DR) or fluorescence spectra of real tissue with similar spectra of model solutions in which the amount of PS is known. However, in real biological tissue, light is absorbed and scattered by a large number of optically active chromophores, whereby the absorption line of PS in the reflectance or fluorescence spectrum of tissue is shifted, and its shape is modified in comparison with that of the model solutions [11, 16]. Therefore, an attempt to interpret the measured spectrum using similar spectra of model samples with specific optical parameters is not always justified. As mentioned in Ref. [14], the PS concentration in tissue and the degree of haemoglobin oxygenation can be also found on the basis of a quantitative analysis of the DR spectrum of tissue in the framework of diffusion approximation of the radiative transfer theory. Unfortunately, the proposed method can be only applied at low concentrations of PS when its contribution to the attenuation of light in tissue at $\lambda = 975$ nm is less than that a similar contribution of water. Austwick et al. [17] solved a similar problem using the Beer–Lambert law and showed that the correlation coefficient between the results of spectral and biochemical analyses amounts to 0.77–0.88.

A number of studies [18–20] are known, in which PS and HbO_2 concentrations in tissue and depths of light penetration into tissue are found (in the diffusion approximation) from the tissue diffuse reflectance spectrum measured with a spatial resolution. However, the complexity of necessary experimental equipment, large dynamic range of detected signals and severe limitations on the measurement geometry and tissue optical parameters, imposed by diffusion approximation, significantly complicate the use of these methods in clinical practice. In addition, these methods do not allow one to evaluate the effectiveness of light absorption by photosensitizer and oxyhaemoglobin in tissue layers at different depths and to select optimal therapeutic doses.

Known also is the method of determining the PS concentration in a scattering medium, based on the measurement of fluorescence and diffuse reflectances at distances of 0.65 and 1.35 mm from the excitation source [21, 22]. As shown in [21], the ratio of these signals is weakly influenced by the optical parameters of the medium and characterises the concentration of the fluorophore in it. The disadvantages of the method are the need for a calibration sample with a known concentration of the fluorophore and the effect of differences in the optical parameters of the medium at the wavelengths of exciting light and fluorescence on the measurement result.

The present paper is aimed at solving the problems of layer-by-layer laser light dosimetry in biological tissue, improving PDT efficacy and minimising adverse side effects of the treatment. This is achieved by controlling the concentrations of PS and HbO_2 in tissue and the light absorption efficiency of these chromophores in tissue layers at different depths, as well as by monitoring morphological changes in the irradiated tissue (volume content of blood vessels and the ratio of various forms of haemoglobin). The source of the initial information is the DR spectrum of tissue, measured at a fixed distance from the region of delivery of exciting light. The mathematical apparatus used for the measurement data processing includes approximating functions for the fluxes of light diffusely reflected by tissue [23, 24] and rapid method for calculating the light flux distribution in the depth of a multilayer, optically dense medium [25].

2. Determination of morphological parameters of skin tissue with an administered photosensitizer

Let us examine the possibility of determining parameters important for PDT of human skin using a compact system measuring the diffuse reflectance of light from tissues. The system consists of a broadband emitter, a spectrometer and a fibre-optic probe [26]. The probe has seven closely packed optical fibres protected by an outer jacket (six illumination fibres around a read fibre). The central (read) fibre is connected to the spectrometer. Six illumination fibres are connected to a light source and transport the light to the object under study.

Determining structural and morphological parameters of tissue is based on a comparison of the model $[\omega(\boldsymbol{x},\lambda_i)]$ and experimental $[\omega_{\exp}(\lambda_i)]$ DR spectra of tissue and on a selection of model parameters $\boldsymbol{x} = (x_p)$, providing the minimum of the functional

$$\sigma^2 = \frac{1}{N_\lambda - 1} \sum_{i=1}^{N_\lambda - 1} [\omega_{\exp}(\lambda_i) - \omega(\boldsymbol{x},\lambda_i)]^2, \tag{2}$$

where N_λ is the number of points in the measured spectra. The spectrum $\omega_{\exp}(\lambda_i)$ is determined by comparing the detected signals from tissue (P) and white diffuse reflector (P_{ref}):

$$\omega_{\exp}(\lambda_i) = \frac{P(\lambda_i)/P_{\text{ref}}(\lambda_i)}{P(\lambda_0)/P_{\text{ref}}(\lambda_0)}, \tag{3}$$

where λ_0 is the normalisation wavelength. The spectrum $\omega(\boldsymbol{x},\lambda_i) = R(\lambda_i)/R(\lambda_0)$ is calculated using the analytical model [22, 23] of tissue reflectance $R = P/P_0$, where P_0 is the power of collimated light incident on the medium, and P is the power of the light diffused by the medium surface outside of the illuminated region. Normalisation (3) allows one to rule out the effect of the light source intensity, the receiver sensitivity and the reference reflector reflectance on the accuracy of estimates of the tissue parameters.

We will calculate the reflectance of skin tissue by using a skin model in the form of a two-layer medium (epidermis and dermis) with the same light scattering parameters and different absorption coefficients of the layers [24, 27]. We believe that the PS is localised in dermal tissues where blood vessels are located. Then, the model parameters of $\boldsymbol{x}$ are as follows: n_{sk} is the refractive index of skin; B_{sca} is the transport scattering coefficient of connective tissue at $\lambda = 400$ nm; ρ_{Mie} is the fraction of Mie scattering in the total scattering by tissue at $\lambda = 400$ nm; x is the parameter of the spectral dependence of the transport Mie scattering coefficient; L_e is the epidermis thickness; f_m is the volume concentration of melanin in the epidermis; f_{bl} is the volume concentration of capillaries in the dermis; d_v is the average diameter capillaries; C_{tHb} is the concentration of total haemoglobin in blood (g L^{-1}); S and C_{MetHb} are the relative concentrations of oxyhaemoglobin (HbO_2) and methaemoglobin (MetHb) in the total haemoglobin; C_β is the molar concentration of β-carotene; and C_{PS} is the molar concentration of PS. For L_e and C_{tHb} we will use fixed values: $L_e = 60$ μm and $C_{\text{tHb}} = 150$ g L^{-1} (average value for human skin). Optical parameters of skin are calculated by the formulas [24, 27]

$$g(\lambda) = 0.7645 + 0.2355[1 - \exp(-(\lambda - 500)/729.1)], \quad (4)$$

$$\beta'(\lambda) = B_{\text{sca}}\left[\rho_{\text{Mie}}\left(\frac{\lambda_0}{\lambda}\right)^x + (1-\rho_{\text{Mie}})\left(\frac{\lambda_0}{\lambda}\right)^4\right], \quad (5)$$

$$k_e(\lambda) = f_m k_m(\lambda) + (1 - f_m)k_t(\lambda), \quad (6)$$

$$k_d(\lambda) = f_{bl}\alpha(\lambda)k_{bl}(\lambda) + (1 - f_{bl})k_t(\lambda) + C_\beta\varepsilon_\beta(\lambda) + C_{PS}\varepsilon_{PS}(\lambda), \quad (7)$$

$$k_{bl}(\lambda) = \ln 10\,\frac{C_{tHb}}{\mu_{tHb}}\,[S\varepsilon_{Hb}(\lambda) + C_{MetHb}\varepsilon_{MetHb}(\lambda) + (1 - S - C_{MetHb})\varepsilon_{HbO_2}(\lambda)], \quad (8)$$

where β' and g are the transport scattering coefficient and the scattering anisotropy factor of the epidermis and dermis; k_e and k_d are the absorption coefficients of the epidermis and dermis; k_t is the absorption coefficient of connective (bloodless) tissue; k_{bl} is the absorption coefficient of blood, taking into account the laser-induced formation of methaemoglobin in blood vessels of irradiated tissue; ε_{Hb}, ε_{HbO_2}, ε_β and ε_{PS} are the molar absorption coefficients of Hb, HbO_2, β-carotene and PS in $mm^{-1}/(mol\ L^{-1})$; $\mu_{tHb} = 64{,}500$ g mol^{-1} is the molar weight of haemoglobin; and α is the correction factor that takes into account the effect of localised light absorption by blood vessels [28]:

$$\alpha(\lambda) = \frac{1 - \exp(-d_v k_{bl}(\lambda))}{d_v k_{bl}(\lambda)}. \quad (9)$$

We use a dye-based PS, 'Fotosens' (State Research Center 'Organic Intermediates and Dyes Institute'), which is a mixture of aluminium phthalocyanine factions with varying degrees of sulfonation. The optical density of 'Fotosens' is given in [29]. Its molar absorption coefficient ε_{PS} reaches its maximum at $\lambda = 675$ nm, and, according to [14], is 0.25 $cm^{-1}\ \mu M^{-1}$.

The reflectance of a two-layer medium modelling human skin is related with high accuracy to the optical parameters of the medium by the equation [24]:

$$-\ln R = \sum_{m=1}^{3} a_{1,m}(\beta')^m + \sum_{m=1}^{3} a_{2,m}k_e^m + \sum_{m=1}^{3} a_{3,m}k_d^m + \sum_{m=1}^{3} a_{4,m}g^m + \sum_{m=1}^{3} a_{5,m}(n_{sk} - 1)^m + \sum_{m=1}^{3} a_{6,m}\delta_d^m + \sum_{m=1}^{3} a_{7,m}(k_e L_e)^m + \sum_{m=1}^{3} a_{8,m}(k_d\delta_d)^m + k_e L_e \sum_{m=1}^{3} a_{9,m}\left(\frac{k_e}{\beta'}\right)^m + L_e \sum_{m=1}^{3} a_{10,m}\left(\frac{k_e}{\beta'}\right)^m + \delta_d \sum_{m=1}^{3} a_{11,m}\left(\frac{k_d}{\beta'}\right)^m + \frac{L_e}{\delta_d}\sum_{m=1}^{3} a_{12,m}(k_d\delta_d)^m, \quad (10)$$

where $a_{i,m}$ are the approximation coefficients; and $\delta_d = [3k_d(k_d + \beta')]^{-1/2}$ is the depth of light penetration into the dermis (in diffusion approximation). Consider the geometric configuration of the fibre-optic probe where the illumination and read fibres having a diameter of 0.8 mm are spaced apart from each other by 0.83 mm. The coefficients presented in expression (10) and corresponding to the geometry of the experiment are listed in Table 1. These coefficients of the medium parameters are measured in the following units: $[L_e]$ = mm, $[\beta'] = mm^{-1}$, $[k_e] = mm^{-1}$ and $[k_d] = mm^{-1}$. The rms error of approximation of the results of numerical calculations, R, by expression (10) is 0.85%. The correlation coefficient between the values of R, obtained by the Monte Carlo (MC) method and formula (10), is equal to 0.9998.

Table 1. Coefficients of formula (10) for calculating the reflectance of skin.

i, m	$a_{i,m}$	i, m	$a_{i,m}$	i, m	$a_{i,m}$
1, 1	0.0847	5, 1	–8.1287	9, 1	–1.1419
1, 2	0.0413	5, 2	21.329	9, 2	0.2703
1, 3	–0.0038	5, 3	–16.079	9, 3	–0.0223
2, 1	–0.0154	6, 1	0.3936	10, 1	–2.0241
2, 2	0.0011	6, 2	–0.0282	10, 2	0.5741
2, 3	0.0000	6, 3	0.0010	10, 3	–0.0730
3, 1	1.4851	7, 1	6.5092	11, 1	2.9094
3, 2	–0.4444	7, 2	–2.7701	11, 2	–4.8814
3, 3	0.0606	7, 3	1.6099	11, 3	1.6287
4, 1	–2.1242	8, 1	16.925	12, 1	–9.2751
4, 2	15.478	8, 2	–55.178	12, 2	23.034
4, 3	–11.822	8, 3	90.164	12, 3	–27.204

The algorithm for retrieving the model parameters is based on the minimisation of the discrepancy between the results of the calculation of the normalised DR spectrum of skin by formulas (4)–(10) and the experimental data. The accuracy of the retrieval of model parameters important for PDT (f_{bl}, S, C_{MetHb}, C_{PS}) was estimated based on the results of a numerical calculation of the DR spectra of skin by the MC method. The calculation was carried out for 70 values of λ, uniformly distributed on the interval [450 nm, 800 nm], with the following spread of model parameters: f_m = 1%–10%, f_{bl} = 0.4%–14%, d_v = 5–90 μm, S = 20%–98%, C_{MetHb} = 1%–60%, C_β = 0.2–5.0 μM, C_{PS} = 0.2–2.0 μM, B_{sca} = 4–11 mm^{-1}, ρ_{Mie} = 0.1–0.6, x = 0.5–1.0 and n_{sk} = 1.4–1.5.

Figure 1 shows the parameters f_{bl}, S, C_{MetHb} and C_{PS}, corresponding to 550 random realisations of the DR spectrum of skin, modelled by the MC method and retrieved from these spectra. The correlation coefficients between the known and retrieved values of f_{bl}, S, C_{MetHb} and C_{PS} are equal to 0.996, 0.991, 0.994 and 0.980, respectively. The rms errors in the retrieval of these parameters are: f_{bl} = 0.26%, S = 2.4%, C_{MetHb} = 1.7% and C_{PS} = 0.1 μM. Consequently, the DR signals recorded at the measuring base under consideration (0.83 mm) have an adequate (for practical use) sensitivity to the amount of methaemoglobin in blood and to the additional exogenous chromophore of the tissue, i.e., photosensitizer.

3. Determination of the action spectra of light on the photosensitizer and dissociation efficiency of oxyhaemoglobin in the tissue layers

Consider the possibility of determining the action spectrum of light on PS, $K_{PS}(z,\lambda)$, and oxyhaemoglobin dissociation efficiency, $n(z,\lambda)$, by the normalised DR spectrum of skin tissue, $\omega(\lambda) = R(\lambda)/R(800\text{ nm})$. The function $K_{PS}(z,\lambda)$ is defined as the total radiation power absorbed by PS per unit volume of the medium when monochromatic light with unit power density is incident on its surface:

$$K_{PS}(z,\lambda) = C_{PS}\varepsilon_{PS}(\lambda)\Phi(z,\lambda). \quad (11)$$

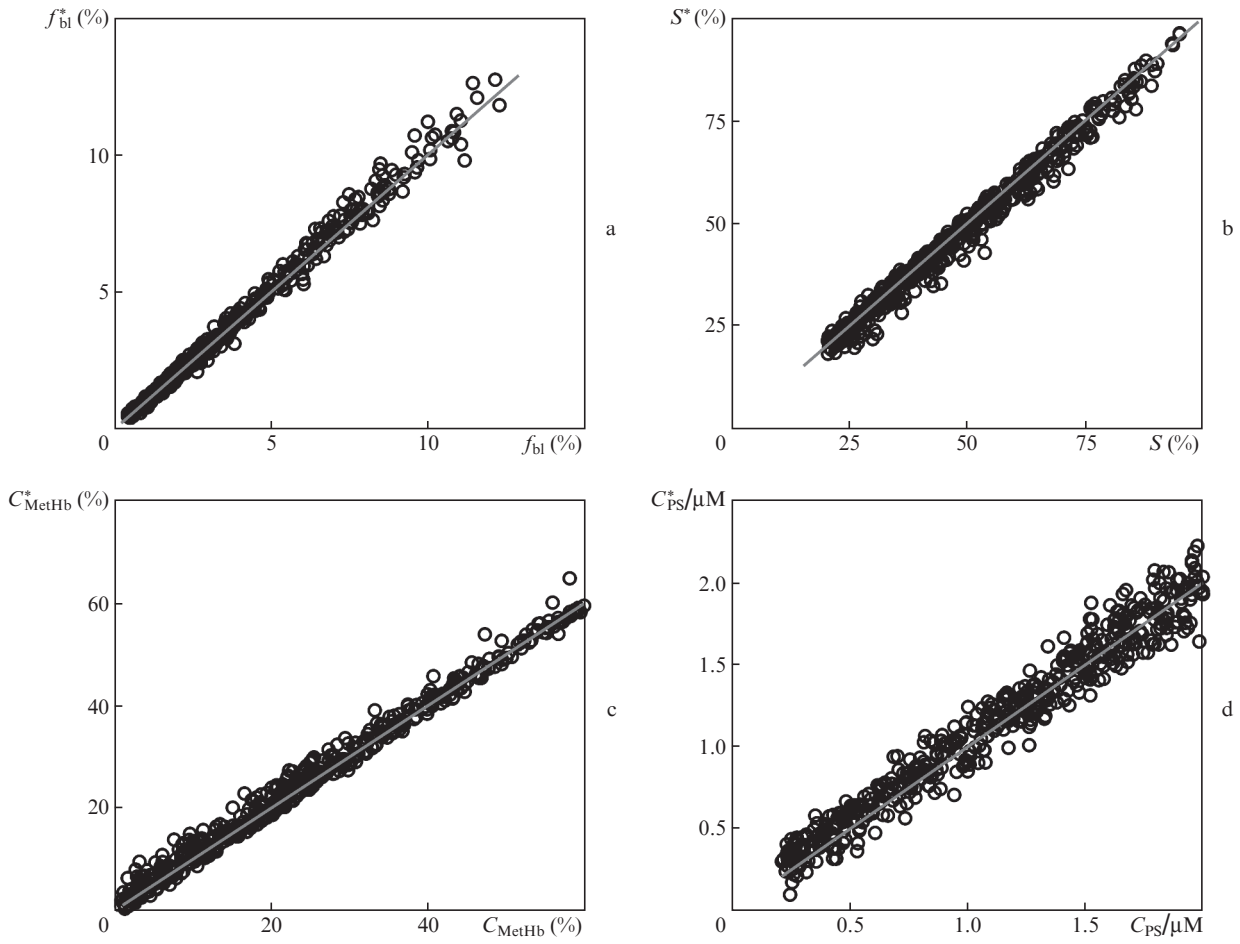

Figure 1. Results of numerical experiments on retrieval of morphological parameters of skin (a–c) and concentration of PS in skin (d) from the spectral DR signals normalised to λ = 800 nm. The straight lines in the figures correspond to the exact equality of known (x axis) and retrieved (y axis) parameters.

The function $n(z,\lambda)$ is defined as the number of oxygen molecules produced per unit time and per unit volume of tissue at depth z when light with unit power density is incident on the tissue surface [30]:

$$n(z,\lambda) = f_{bl} S (C_{tHb}/\mu_{tHb}) \ln 10 \varepsilon_{HbO_2}(\lambda) \Phi(z,\lambda)(q\lambda/hc), \quad (12)$$

where h is Planck's constant; c is the speed of light in the medium; and q is the quantum photodissociation yield, which, upon excitation of HbO_2 with light at λ = 300–650 nm, is approximately equal to 3%–5% [31].

Let the DR signal be measured for circular illuminated and receiving sites with a diameter of 0.8 mm on the medium surface, the centres of the sites being separated by a distance of 0.83 mm. The spectrum $\omega(\lambda)$ corresponding to this measurement geometry is presented in Fig. 2a. This range is calculated by the MC method for a random combination of model parameters (x_p): f_m = 1.85%, f_{bl} = 3.84%, d_v = 32.2 μm, S = 82.5%, C_{MetHb} = 11.4%, C_β = 0.47 μM, C_{PS} = 1.04 μM, B_{sca} = 6.46 mm^{-1}, ρ_{Mie} = 0.19, x = 0.94 and n_{sk} = 1.45. The model parameters (x_p^*), obtained by minimisation of the discrepancy between this spectrum and the spectrum calculated by formula (10), have the following values: f_m^* = 0.74%, f_{bl}^* = 3.80%, d_v^* = 43.9 μm, S^* = 83.3%, C_{MetHb}^* = 11.4%, C_β^* = 0.25 μM, C_{PS}^* = 1.02 μM, B_{sca}^* = 8.14 mm^{-1}, ρ_{Mie}^* = 0.12, x^* = 0.56, n_{sk}^* = 1.44. It can be seen that the parameters f_{bl}, S, C_{MetHb} and C_{PS} are retrieved with high accuracy, which is consistent with the results of the analysis of the diagnostic capabilities of the measurements in question. Meanwhile, the retrieved values of other parameters differ significantly from their specified values. The reason for this is the ambiguous dependence of the normalised DR spectrum of the medium modelling the skin tissue on the above parameters. There are an infinite number of solutions of the inverse problem, which equally well reproduce the spectrum $\omega(\lambda)$ within the framework of the model used. The range of the parameter f_{bl}, S, C_{MetHb} and C_{PS}, corresponding to one and the same spectrum $\omega(\lambda)$, is quite narrow (see the scatter of points in Fig. 1 with respect to straight lines $x_p = x_p^*$), which allows one to satisfactorily evaluate these parameters by measuring $\omega(\lambda)$. Similar ranges of other model parameters are comparable with the *a priori* uncertainty of the latter.

Nevertheless, all the combination of the model parameters, leading to the same calculated spectrum $\omega(\lambda)$, correspond to approximately identical optical conditions inside the medium. In support of this, Fig. 2b shows the distribution of light in the depth of the medium, $\Phi(z,\lambda)$, at λ = 575 and 675 nm, calculated by the MC method for the given values of the model parameters and analytically – with their retrieved values (the calculation method is described in [25]). Indeed, despite significant differences between the specified and retrieved parameters of the medium, they both are responsible for almost the same spatial illumination in the medium. The retrieved profiles $\Phi(z,\lambda)$ differ from the true distributions of

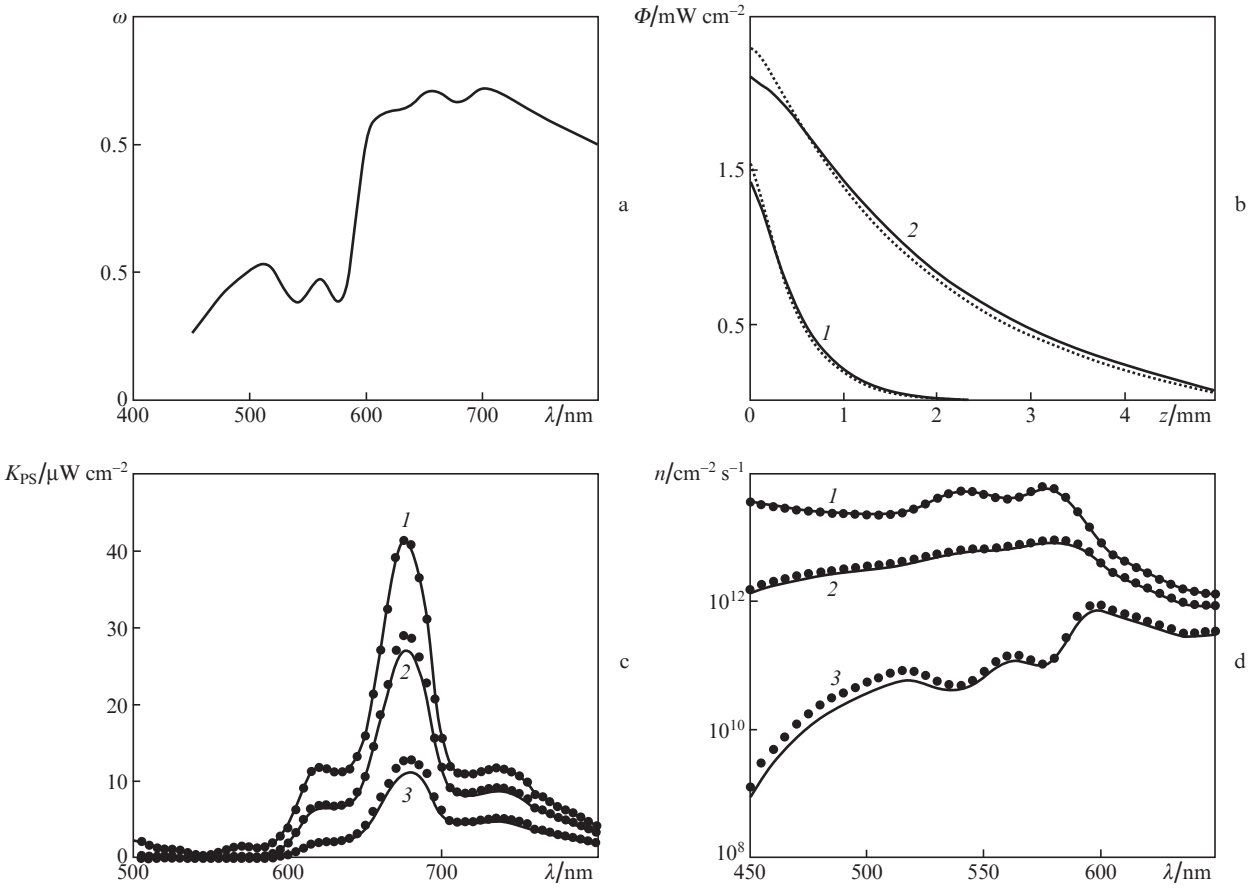

Figure 2. Results of a numerical experiments on retrieval of the spectral density of light absorbed by the PS 'Fotosens' and oxyhaemoglobin in the skin layers at a depth (*1*) $z_1 = 0.1$ mm, $z_2 = 1.0$ mm; (*2*) $z_1 = 1.0$ mm, $z^2 = 2.0$ mm; and (*3*) $z_1 = 3.0$ mm, $z_2 = 5.0$ mm from the DR spectrum of skin; panel (a) shows the spectral signal of the diffuse light, normalised to $\lambda = 800$ nm; (b) – true (solid curves) and retrieved (dotted curves) in-depth distributions of the total illumination at $\lambda =$ (*1*) 575 and (*2*) 675 nm; (c) – true (points) and retrieved (solid curves) spectral efficiencies of light absorption by PS; (d) – true (points) and retrieved (solid curves) spectral efficiencies of HbO_2 photodissociation.

the illumination in the medium (corresponding to the given parameters of the medium) within the error of the method of calculation of $\Phi(z,\lambda)$. The functions $K_{PS}(z,\lambda)$ and $n(z,\lambda)$, obtained by using the retrieved parameters f_{bl}, S, C_{PS} and distribution $\Phi(z,\lambda)$ also quite well reproduce the true action spectrum of light on PS and efficiency of HbO_2 photodissociation. As an example, Figs 2c and 2d illustrate the functions

$$K_{PS}(\lambda) = \int_{z_1}^{z_2} K_{PS}(z,\lambda)\,dz \text{ and } n(\lambda) = \int_{z_1}^{z_2} n(z,\lambda)\,dz,$$

calculated for the three layers of the medium with the depth $[z_1, z_2]$. One can see that nonessential quantitative differences between true and retrieved profiles $K_{PS}(\lambda)$ and $n(\lambda)$ take place only for deep layers of the medium.

To confirm the correctness of our conclusions, we have performed a series of similar numerical experiments. We have used an ensemble of 550 realisations of $\omega(\lambda)$ and $\Phi(z,\lambda)$, obtained by the MC method for random combinations of model parameters. The spectra $K_{PS}(\lambda)$ and $n(\lambda)$, retrieved from $\omega(\lambda)$, were compared with similar known spectra corresponding to the given parameters of the medium. The correlation coefficients between the true and retrieved spectra $K_{PS}(\lambda)$ and $n(\lambda)$ are shown in Fig. 3. It can be seen that the proposed method allows one to determine with a high degree of reliability the spectra $K_{PS}(\lambda)$ and $n(\lambda)$ in the tissue layers at a depth of up to 2.0–2.5 mm for the considered spread of the tissue optical parameters. For deeper layers one can obtain correct estimates of the spectral features of light absorption by PS and HbO_2 (for example, the position of the absorption maximum); however, the absolute values of $K_{PS}(\lambda)$ and $n(\lambda)$ in some cases (for example, in the case of high pigmentation of skin) can be determined with high errors. This fact is not a limitation of the proposed method, but results from the final depth of light penetration into tissue. To obtain information about deeper layers of tissue one should complement the measurements of $\omega(\lambda)$ with the near-IR range. At the same time, one should, of course, take into account additional chromophores of tissue – water and lipids, which absorb radiation at $\lambda > 900$ nm. Otherwise, the processing algorithm remains unchanged.

4. Effect of the anatomical structure of tissue

The above results were obtained in the approximation of a skin tissue model with a thin upper layer (epidermis) of a semi-infinite homogeneous lower layer (dermis). This model, despite its simplicity, describes well the experimental DR spectra to skin and allows some average-volume parameters of the epidermis and dermis to be evaluated [24, 27]. However, the actual structure of skin is much more complicated than it is assumed in the model used. The dermis consists of several anatomical regions (layers) with a different amount of blood vessels. In this regard, a question arises: to what degree is the

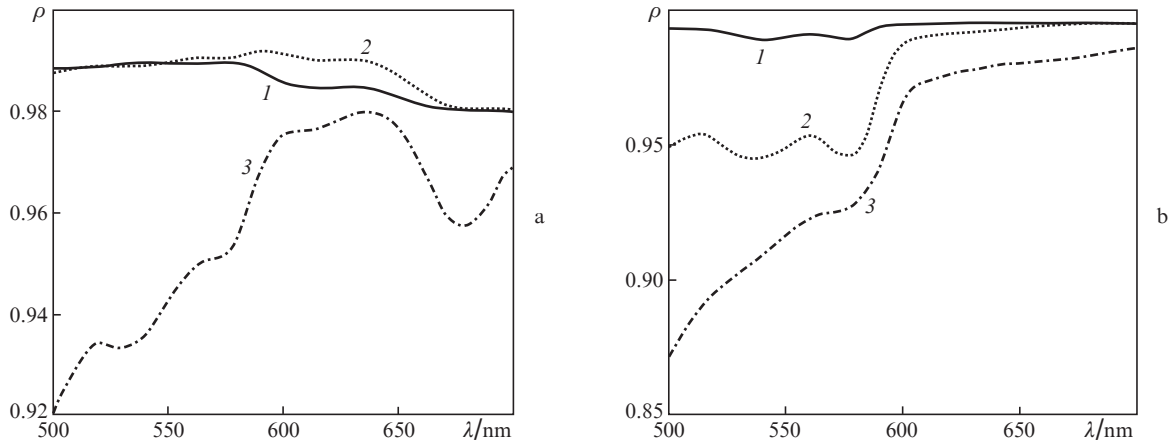

Figure 3. Correlation coefficients between the true and retrieved spectra $K_{PS}(\lambda)$ (a) and $n(\lambda)$ (b) for the three layers of tissue at (*1*) $z_1 = 0.1$ mm, $z_2 = 1.0$ mm; (*2*) $z_1 = 1.0$ mm, $z_2 = 2.0$ mm; and (*3*) $z_1 = 3.0$ mm, $z_2 = 5.0$ mm.

relation of the spectrum of skin with the characteristics of the light field in its multilayer dermis reproduced correctly within the model in question?

To answer this question, we consider a more realistic model of the human skin, as proposed in [32]. In the original version the model is presented in the form of skin layers with a geometric thickness L and optical parameters (absorption coefficient k, scattering coefficient β, scattering anisotropy factor g) at $\lambda_1 = 337$ nm and $\lambda_2 = 633$ nm. Assuming that the main absorbers of light in skin are melanin, Hb and HbO_2, for given absorption coefficients of each layer at λ_1 (isosbestic point of the absorption spectra of Hb and HbO_2) one can easily obtain the concentration of melanin, f_m, and capillaries, f_{bl}, in the respective layers (Table 2). We assume that in all layers of the dermis $S = 70\%$, $C_{tHb} = 150$ g L^{-1} and $d_v = 10$ μm, and the background absorption of tissue $k_t(\lambda)$ corresponds to the experimental data [33]. The total absorption coefficient of each layer is calculated by formulas (6)–(8) at $C_\beta = 0$, $C_{PS} = 0$ and $C_{MetHb} = 0$. To calculate $\beta(\lambda)$ and $g(\lambda)$ in the range $\lambda = 450–800$, we respectively use the power $[A(\lambda_2/\lambda)^\nu]$ and linear $(B + C\lambda)$ functions with coefficients A, ν, B and C obtained for each layer of tissue at the corresponding values of β and g at λ_1 and λ_2 (Table 2). The refractive index of all skin layers is assumed the same and equal to 1.45.

Table 2. Parameters of the skin layers used in the simulation.

Layer	L/mm	f_m (%)	f_{bl} (%)	A/mm^{-1}	ν	B	C/10^4 nm^{-1}
1	0.1	1.3	0	10.7	0.687	0.64	2.36
2	0.2	0	4	18.7	0.307	0.61	3.38
3	0.2	0	7	19.2	0.393	0.61	3.38
4	0.9	0	4	18.7	0.687	0.61	3.38
5	0.6	0	8	19.4	0.421	0.61	3.38

The DR spectrum of skin with a multilayer dermis, $\omega_m(\lambda)$, calculated by the MC method (Fig. 4a), was interpreted in the approximation of the skin model with homogeneous dermis. The parameters of a two-layer skin model, retrieved from the spectrum $\omega_m(\lambda)$, are as follows: $f_m L_e = 0.84$ μm, $f_{bl} = 5.13\%$, $d_v = 12.5$ μm, $S = 68\%$, $B_{sca} = 7.59$ mm^{-1}, $\rho_{Mie} = 0.95$, $x = 1.97$ and $n_{sk} = 1.37$. It is seen that the retrieved values of the integral content of melanin in the epidermis, the degree of blood oxygenation and capillary diameters are sufficiently close to

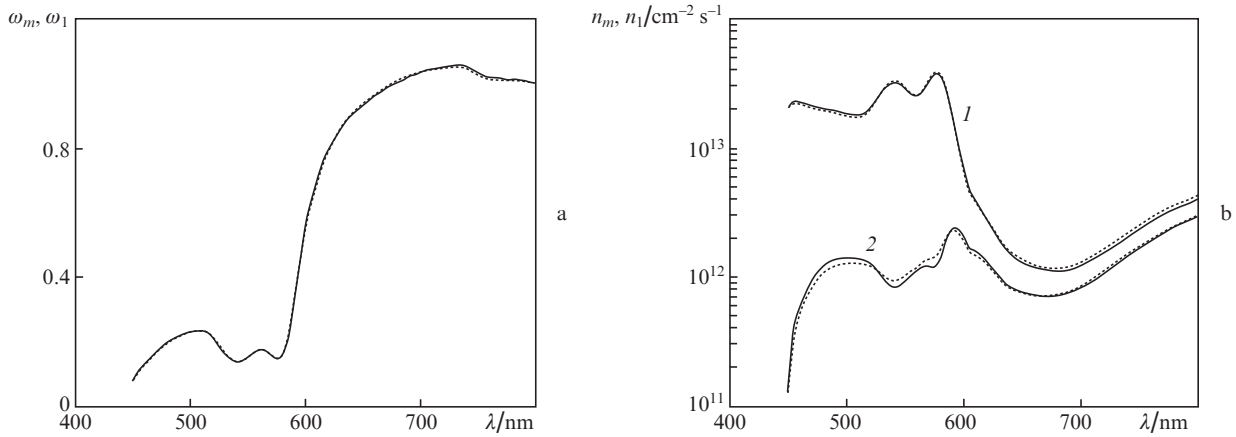

Figure 4. Results of a numerical experiment on retrieval of the spectral efficiency of the HbO_2 photodissociation from the DR spectrum of a multilayer medium simulating skin tissue; panel (a) shows the normalised DR spectra of skin, calculated in the framework of skin models with a multilayer (solid curve) and homogeneous (dotted curve) dermis (virtually coincide); (b) – HbO_2 photodissociation efficiency spectra at $z = 0.1–1.0$ mm (*1*) and 1.0–2.0 mm (*2*), calculated with the true parameters of a five-layer medium (solid curves) and from the retrieved parameters of a two-layer medium (dotted curve).

the corresponding values for the multilayered skin model. The retrieved parameter f_{bl} is approximately equal to the concentration of capillaries in the depth-averaged multilayer dermis,

$$\sum_{i=2}^{5} L_i f_{bl,i} / \sum_{i=1}^{5} L_i = 5.58\%.$$

The DR spectrum of skin, $\omega_1(\lambda)$, calculated by formulas (4)–(10) for the retrieved model parameters, is virtually indistinguishable from the spectrum $\omega_m(\lambda)$.

Consider the results of the retrieval of the light field characteristics inside a multilayer tissue by its DR. The physical basis of this retrieval is the dependence of the depth of light penetration into the tissue at λ. Probing light at different λ penetrates different layers of tissue and therefore contains information about these layers. Figure 4b presents the spectra of the HbO_2 photodissociation efficiency, $n_m(\lambda)$, at different depths of tissue, which were calculated by the MC method at the tissue parameters borrowed from Table 2. The same figure also shows similar spectra $n_1(\lambda)$, calculated in the framework of a two-layer skin model by the method described in [25]. Calculation of $n_1(\lambda)$ was performed using the model parameters retrieved from $\omega_m(\lambda)$. The difference between $n_1(\lambda)$ and $n_m(\lambda)$ spectra does not exceed the error of $n_1(\lambda)$ retrieval, predicted on the basis of a skin model with a uniform dermis, and is not essential for the practice.

Thus, we can conclude that to determine the spatial illumination in a multilayer dermis and action spectra of light on its chromophores, one can successfully use the skin tissue model with a uniform dermis. The reason for this is the optical equivalence of these media, i.e., the spectral-spatial characteristics of the light field inside and outside a multilayer medium can be accurately reproduced in the framework of a two-layer medium. It should be noted that this principle lays the basis of data processing methods, obtained by means of the AERONET (AErosol RObotic NETwork), which is a global ground-based sun and sky scanning photometer network monitoring aerosol optical properties [34, 35]. Aerosol microphysical parameters are obtained in the approximation of the model of homogeneous atmosphere by the angular distributions of the sky radiance in almucantar and principal planes.

5. Experimental evaluation of the efficiency of oxyhaemoglobin photodissociation in blood vessels of skin

The method for determining the HbO_2 photodissociation efficiency in tissues *in vivo* was tested on the skin of 30 volunteers (25 men and 5 women aged 20 to 65). In the experiments, we used an Avantes fibre-optic spectrophotometer [26] with illuminating and receiving fibres 0.4 mm in diameter (a more detailed description of the experiments is given in Ref. [24]). Structural and morphological parameters of skin, found form the DR spectrum, were used to calculate the optical parameters of skin and the in-depth distribution of the illumination $\Phi(z,\lambda)$. The spectrum of the HbO_2 photodissociation efficiency was calculated by formula (12) in accordance with the retrieved values of the model parameters f_{bl}, S and spatial illumination $\Phi(z,\lambda)$.

As an example, Fig. 5a shows the experimental $[\omega_{exp}(\lambda)]$ and model $[\omega(\lambda)]$ DR spectra of the ring finger of three volunteers (back side). The spectra of the HbO_2 photodissociation efficiency throughout the entire thickness of the dermis, retrieved from $\omega_{exp}(\lambda)$, are shown in Fig. 5b. It is seen that the amount of molecular oxygen released from the blood vessels of the dermis into the surrounding tissue depends on optical parameters of the tissue and, at the same irradiation dose, may vary by several times. In general, the efficiency of oxygen generation at the wavelength of maximum light absorption by HbO_2 (λ_{max} = 577 nm) for the same anatomical area of skin varies in different volunteers by two–three times. For different anatomical areas, the variation in $n(\lambda_{max})$ is even more significant – the values of $n(\lambda_{max})$ for the skin of the volunteers' finger, palm and nose can differ by more than 10 times. Obviously, this fact should be taken into account when conducting laser therapy sessions in order to improve efficiency of oxygen generation and to avoid undesirable effects of laser therapy.

6. Conclusions

The developed methods for noninvasive determination of concentrations of endogenous and exogenous chromophores in tissue (capillaries, oxyhaemoglobin, methaemoglobin, photo-

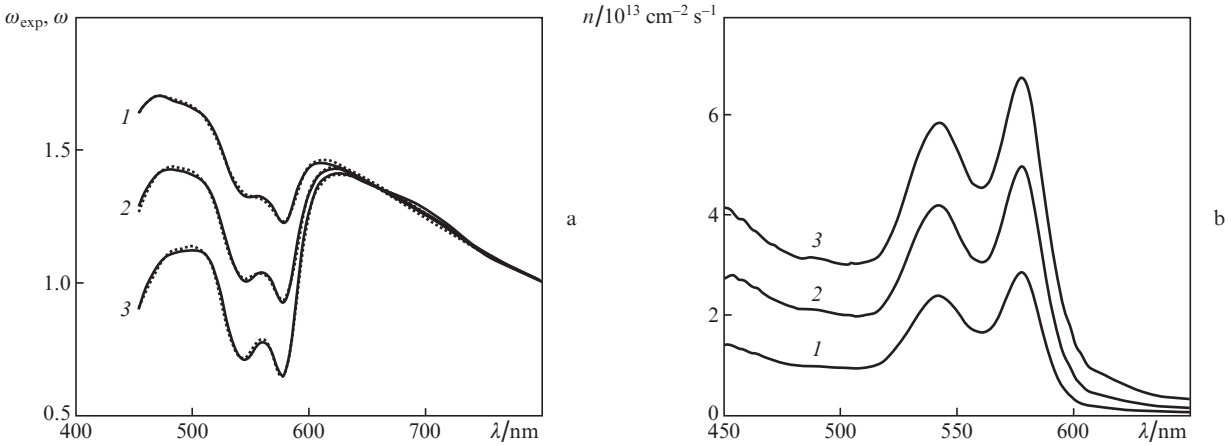

Figure 5. Results of an experiment on non-invasive determination of the HbO_2 photodissociation efficiency in the skin of the ring finger; panel (a) shows the experimental (solid curves) and model (dashed curve) spectra of the diffuse reflectance of light from skin; (b) – dependences of the HbO_2 photodissociation efficiency throughout the entire thickness of the dermis on the wavelength of the exciting light at (*1*) f_{bl} = 0.6%, S = 54%; (*2*) f_{bl} = 1.6%, S = 64%; and (*3*) f_{bl} = 3.2%, S = 83%.

sensitizer) and the light absorption efficiency in the tissue layers that are at different depths allow one to take into account the individual characteristics of the patient's tissue in PDT. This, in turn, will make it possible to select the best (for a particular patient) laser light sources and therapeutic dose to ensure the highest absorption of light by photosensitizer and the best generation of oxygen at the depth of the tissue site with pathology or throughout its thickness at minimum laser irradiation of the healthy tissue. Thus, it is possible to increase the efficacy and to reduce the duration of laser therapy sessions as well as to avoid side adverse effects and standardise the treatment methods of patients with the same pathology.

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