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# Analytical model of diffuse reflectance spectrum of skin tissue

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*Abstract.* **We have derived simple analytical expressions that enable highly accurate calculation of diffusely reflected light signals of skin in the spectral range from 450 to 800 nm at a distance from the region of delivery of exciting radiation. The expressions, taking into account the dependence of the detected signals on the refractive index, transport scattering coefficient, absorption coefficient and anisotropy factor of the medium, have been obtained in the approximation of a two-layer medium model (epidermis and dermis) for the same parameters of light scattering but different absorption coefficients of layers. Numerical experiments on the retrieval of the skin biophysical parameters from the diffuse reflectance spectra simulated by the Monte Carlo method show that commercially available fibre-optic spectrophotometers with a fixed distance between the radiation source and detector can reliably determine the concentration of bilirubin, oxy- and deoxyhaemoglobin in the dermis tissues and the tissue structure parameter characterising the size of its effective scatterers. We present the examples of quantitative analysis of the experimental data, confirming the correctness of estimates of biophysical parameters of skin using the obtained analytical expressions.**

***Keywords:*** *skin, biophysical parameters, diffuse reflectance, analytical model.*

## 1. Introduction

Among the methods of optical diagnostics of human tissues, the simplest and most effective are the methods of diffuse reflectance spectrophotometry [1–7], based on measurements of the spectrum of light reflected from the tissue employing data collection with an optical fibre probe in fixed delivery and collection geometry. An important advantage of these methods is the relative cheapness and availability of the necessary equipment, because the measurements can be performed on the basis of commercial optical elements – a source of white light, spectrometer and optical fibres to deliver the exciting radiation from the source to the tissue and to collect the radiation diffusely reflected from the tissue. The detected optical signals carry important information about the biophysical parameters of tissue, affecting the light field in the medium, – the scattering coefficient, the diameter of the capillaries, the concentrations of major tissue chromophores (oxyhaemoglobin, reduced haemoglobin, melanin, bilirubin, etc.). Quantitative estimates of these parameters are based on the modelling of the transport of light in tissue and comparison of theoretical calculations of the reflection spectrum of tissue with the experimental data. The majority of researchers rely on a simple analytical model [1–5], describing the radiative transfer in the tissue under study by two optical parameters – absorption coefficient and transport scattering coefficient. However, the narrow range of applicability of the models used, the lack of account for the layering of the tissue and other variations in its optical parameters, such as the scattering anisotropy factor and the refractive index, lead to rather rough estimates of biophysical parameters. Basically, the only method that allows one to perform a correct comparison with the experimental data obtained with the fibre-optic probes is currently the Monte Carlo (MC) method [8]. However, despite broad capabilities of modern computers, this method still fails to process the experimental data in real time.

The aim of this work is to improve the efficiency of the method of diffuse reflectance spectroscopy of biological tissues through the use of a new model of the diffuse reflectance spectrum of the tissue, allowing one to calculate the experimentally measured optical signal in a simple analytic form with the accuracy of the MC method. The model is based on the approximating equation for calculating the dependence of reflectance of a two-layer medium mimicking human skin on the optical and structural parameters of the medium. The reflectance of the medium is $R = P/P_0$, where $P_0$ is the power of collimated light incident on the medium, and $P$ is the power of diffuse light coming from the site on the surface of the medium outside the illuminated region. The initial data are the results of numerical calculations of reflectance by the MC method. The error in retrieval of biophysical parameters of human skin is assessed from the reflectance spectrum using the approximations obtained. The theoretical calculations of the skin reflectance are compared with experimental data.

## 2. Method for calculating the skin reflectance

We will calculate the reflectance spectrum of the skin tissue in the framework of the model [9], which describes skin in the form of a two-layer medium (epidermis and dermis) with the same parameters of light scattering and different absorption coefficients of the layers. The stratum corneum, because of low optical thickness, plays a minor role in the diffuse reflection of light, and so it is conditionally included in the epidermis. Anatomical regions of the dermis (papillary, reticular,

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superficial and deep vascular plexus) have neither clear physical boundaries, nor fundamental morphological differences, and hence they are all replaced by a single homogeneous layer. The deeper layers of skin (fat layer and muscle tissue) do not participate in the process of reflection of light with $\lambda = 450-800$ nm due to its strong attenuation by overlying layers.

The model parameters of the problem are: $n_{sk}$ is the refractive index of skin; $\beta'(\lambda_0)$ is the transport scattering coefficient of connective tissue at $\lambda_0 = 400$ nm; $\rho_{Mie}$ is the fraction of Mie scattering in the total scattering of tissue at $\lambda_0 = 400$ nm; $x$ is the parameter of the spectral dependence of the Mie scattering coefficient; $L_e$ is the epidermal thickness; $f_m$ is the volume concentration of melanin in the epidermis; $C_{bil}$ is the concentration of bilirubin in the dermis (g L$^{-1}$); $f_{bl}$ is the volume concentration of blood capillaries in the dermis; $d_v$ is the average diameter of the capillaries; $C_{tHb}$ is the concentration of total haemoglobin in blood (g L$^{-1}$); and $S$ is the degree of blood oxygenation. The model [9] also takes into account the content of water in skin; however, the effect of water on the attenuation of radiation with $\lambda \leqslant 800$ nm can be safely neglected. In this case, the optical parameters of skin are calculated by the formulas

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

$$\beta'(\lambda) = \beta'(\lambda_0)\left[\rho_{Mie}\left(\frac{\lambda_0}{\lambda}\right)^x + (1-\rho_{Mie})\left(\frac{\lambda_0}{\lambda}\right)^4\right], \quad (2)$$

$$k_e(\lambda) = f_m k_m(\lambda) + (1 - f_m)k_t(\lambda), \quad (3)$$

$$k_d(\lambda) = f_{bl}\alpha k_{bl}(\lambda) + (1 - f_{bl})k_t(\lambda) + \frac{C_{bil}}{\mu_{bil}}\ln 10\varepsilon_{bil}(\lambda), \quad (4)$$

$$k_{bl}(\lambda) = \frac{C_{tHb}}{\mu_{tHb}}\ln 10\left[S\varepsilon_{HbO_2}(\lambda) + (1 - S)\varepsilon_{Hb}(\lambda)\right] + \frac{t_{dif}C_{bil}}{\mu_{bil}}\ln 10\varepsilon_{bil}(\lambda), \quad (5)$$

where $\beta'$ and $g$ are the transport scattering coefficient and the scattering anisotropy factor for the epidermis and dermis; $k_e$, $k_d$, $k_t$ and $k_{bl}$ are the absorption coefficients of epidermis, dermis, connective tissue and blood; $\varepsilon_{HbO_2}$, $\varepsilon_{Hb}$ and $\varepsilon_{bil}$ are the molar absorption coefficients of oxyhaemoglobin, deoxyhaemoglobin and bilirubin [mm$^{-1}$ (mol L$^{-1}$)$^{-1}$] [10, 11]; $\mu_{tHb} = 64500$ g mol$^{-1}$ is the molar mass of haemoglobin; $\mu_{bil} = 585$ g mol$^{-1}$ is the molar mass of bilirubin; $t_{dif} = 5$ is the ratio of concentrations of bilirubin in the blood and in the surrounding tissue, which depends, in general, on the coefficient of bilirubin diffusion through the blood vessel wall; and

$$\alpha = \frac{2\sqrt{3}}{\pi k_{bl} d_v}\{1 - \exp[-\pi k_{bl} d_v(1 - 0.043k_{bl}d_v)/2\sqrt{3}]\} \quad (6)$$

is the correction factor that takes into account the effect of localised light absorption by blood vessels [12].

Lisenko and Kugeiko [9] modelled the radial distribution of the diffuse radiation flux $\Phi(\rho)$ reflected by a skin segment separated by a distance $\rho$ from the illuminated point upon illumination of skin along the normal to the surface by a single luminous flux at point $\rho = 0$. The distribution $\Phi(\rho)$ was calculated by the MC method for 30 wavelengths from the range of 450–800 nm at a wide variation in the model parameters. Knowing the distribution $\Phi(\rho)$, it is easy to calculate the reflectance $R$ of any illuminated and receiving sites on the surface of skin. For circular illuminated and receiving sites with radii $r_0$ and $r$, respectively, the distance between the centres of which is equal to $L$, the reflectance can be calculated as a convolution:

$$R = \int_{-r_0}^{r_0} l\arccos\left(\frac{l^2 + (l+x)^2 - r_0^2}{2l(l+x)}\right)dx \times \int_{l-r}^{l+r}\Phi(\rho)\arccos\left(\frac{\rho^2 + l^2 - r^2}{2l\rho}\right)\rho d\rho, \quad (7)$$

where $l = L - x$.

We assume that the light incident on skin forms a spot with a diameter of 400 μm, and the light diffusely reflected by skin is collected on a circular site with a diameter of 400 μm at a distance 430 μm from this spot. This delivery and collection geometry is typical of optical fibre probes produced by a number of manufacturers and is widely used in practice. On the basis of the simulated ensemble of distributions $\Phi(\rho)$, we calculated by formula (7) the reflectances corresponding to the measurement geometry in question. The spectral values of reflectances and the corresponding structural and optical parameters of skin are combined into a single data set comprising the following ranges: $n_{sk} = 1.4-1.5$, $L_e = 0.05-1.50$ mm, $k_e = 0.09-18$ mm$^{-1}$, $k_d = 0.02-3.0$ mm$^{-1}$, $\beta' = 0.5-8$ mm$^{-1}$, $g = 0.75-0.85$, $\beta'/k_e = 0.15-35$, $\beta'/k_d = 0.7-150$, $R = (3-300)\times10^{-4}$. Using the data obtained we searched for the analytic expression approximating the dependences $R(n_{sk}, L_e, k_e, k_d, \beta', g)$. The search was carried out in a class of polynomial functions of the form:

$$-\ln R = \sum_{m=1}^{3} a_{1m}(y_1)^m + \ldots + \sum_{m=1}^{3} a_{nm}(y_n)^m, \quad (8)$$

where $y_i$ ($i = 1, \ldots, n$) are the structural and optical parameters of skin, as well as derivatives of these quantities; and $a_{ij}$ are the coefficients, which are determined by the least squares method. Ease of use of polynomials (8) consists in the fact that they allow one to evaluate the significance of any parameter of skin in the total variation of its reflectance, and thus to select the optimal combination of the parameters corresponding to the minimum approximation error. In searching for such combinations we arrived at the expression:

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

**Table 1.** Coefficients $a_{ij}$ in expression (9) for calculating the reflectance of the medium at 400-μm diameters of the illuminated and receiving sites spaced by 430 μm on the medium surface.

| $i, j$ | $a_{ij}$ | $i, j$ | $a_{ij}$ | $i, j$ | $a_{ij}$ |
|---|---|---|---|---|---|
| 1, 1 | −0.5845 | 5, 1 | −10.785 | 9, 1 | −1.1647 |
| 1, 2 | 0.1205 | 5, 2 | 26.881 | 9, 2 | 0.2458 |
| 1, 3 | −0.0074 | 5, 3 | −19.757 | 9, 3 | −0.0162 |
| 2, 1 | −0.0193 | 6, 1 | 0.2185 | 10, 1 | −2.4387 |
| 2, 2 | 0.0037 | 6, 2 | −0.0150 | 10, 2 | 1.0495 |
| 2, 3 | −0.0002 | 6, 3 | 0.0019 | 10, 3 | −0.1722 |
| 3, 1 | 1.3624 | 7, 1 | 5.8379 | 11, 1 | 3.7004 |
| 3, 2 | −0.3327 | 7, 2 | −1.8591 | 11, 2 | −5.1912 |
| 3, 3 | 0.0410 | 7, 3 | 1.1101 | 11, 3 | 2.0525 |
| 4, 1 | 10.512 | 8, 1 | 9.3417 | 12, 1 | −8.8913 |
| 4, 2 | −2.7548 | 8, 2 | −34.946 | 12, 2 | 23.444 |
| 4, 3 | −3.5737 | 8, 3 | 58.744 | 12, 3 | −27.775 |

where $\delta_d = [3k_d(k_d + \beta')]^{-1/2}$ is the depth of light penetration into the dermis (in the diffusion approximation), whereas the approximation coefficients $a_{ij}$ are given in Table. 1. The reflectances of skin, calculated by the MC method ($R_{MC}$) and the approximate formula (9) are compared in Fig. 1. It is seen that the resulting formula with high accuracy approximates the results of numerical calculations of the reflectance. The average error of approximation $R_{MC}$ is 0.85%, the correlation coefficient between $R_{MC}$ and $R$ is equal to 0.9998. Note that the coefficients $a_{ij}$ listed in Table 1 correspond to the particular geometry of the experiment; however, similar coefficients can be obtained for other conditions of measurement of skin reflectances. Thus, for circular illuminated and receiving sites with a diameter of 0.4 mm, the centres of which are separated by a distance $L = 0.86$ mm, formula (9) allows one to approximate the results of numerical calculations of the reflectances with an error of 2.1%. At $L = 1.29$ mm a similar error is 3.4%. An increase in the approximation error with increasing $L$ is due to the statistical 'noise' of the MC method, which grows with distance between the illuminated surface area and the surface area through which the collected reflected radiation is coupled out.

By substituting (1)–(6) into (9), we obtain the analytical dependence of the reflectance of a two-layer medium mimicking the skin tissue on $\lambda$ and model parameters $\boldsymbol{b} = (n_{sk}, \beta'(\lambda_0), \rho_{Mie}, x, L_e, f_m, f_{bl}, d_v, C_{bil}, C_{tHb}, S)$. Thus, we can explicitly calculate the reflectance of skin from the given values of its model parameters, the accuracy of the calculation being close to the accuracy of the MC method. Then, using the known techniques of minimisation, we can calculate the values of the model parameters corresponding to the minimum difference between the experimental and calculated spectra of the diffuse reflectance of skin.

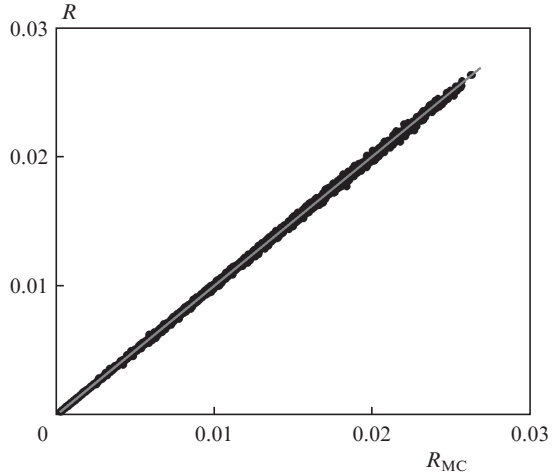


**Figure 1.** Comparison of the skin reflectance calculated by the MC method and formula (9) for the same optical parameters.

## 3. Analysis of the inverse problem solution

Calibration measurements of reflectance of a scattering medium at spatially separated illuminating and collecting fibres are rather cumbersome; therefore, in practice the reflectance of a medium is usually measured up to some constant factor. In fact, the measured characteristic is the spectral variation of the reflectance, i.e., $r(\lambda) = R(\lambda)/R(\lambda_{ref})$, where $\lambda_{ref}$ is the normalisation wavelength. Since such measurements do not allow one to separate the contributions of scattering and absorption of the medium to the measured spectrum, it is of interest to determine what parameters of skin (and with what accuracy) can be retrieved from the experimental spectra $r(\lambda)$ in the approximation of the above analytical model. This analysis was conducted on the basis of skin reflectance $R_{MC}(\boldsymbol{b}, \lambda)$ (30 values of $\lambda$ in the range 450–800 nm), calculated by the MC method for $10^3$ random realisations of model parameters $\boldsymbol{b} = (b_m)$. As a solution to the inverse problem we chose a set of parameters $\boldsymbol{b}^* = (b_m^*)$, corresponding to the minimum residual:

$$\sigma^2 = \frac{1}{N_\lambda - 1} \sum_{i=1}^{N_\lambda - 1} [r_{MC}(\boldsymbol{b}, \lambda_i) - r(\boldsymbol{b}^*, \lambda_i)]^2, \qquad (10)$$

where $N_\lambda = 30$ is the number of points in the reflectance spectrum; and $r_{MC}(\boldsymbol{b}, \lambda_i)$ and $r(\boldsymbol{b}^*, \lambda_i)$ are the normalised reflectance spectra calculated numerically and analytically, respectively ($\lambda_{ref} = 800$ nm). The retrieved values of $b_m^*$ were compared with the exact values of $b_m$, and conclusions were drawn about the informativeness of the considered measurements of each $b_m$. As a result we found that the spectrum $r(\lambda)$ makes it possible to reliably find the concentrations of haemoglobin $F_{tHb} = f_{bl}C_{tHb}$ and bilirubin $C_{bil}$ in the dermis tissues, the degree of oxygenation of the arterio-venous blood $S$ and the tissue structure parameter $v$, which determines the spectral variation of its transport scattering coefficient in the range of 600–700 nm as $\beta' \sim \lambda^{-v}$. Exact and retrieved values of these parameters are compared in Fig. 2. Average retrieval errors in the parameters $F_{tHb}$, $C_{bil}$, $S$ and $v$ are 9.5%, 8.4%, 2.3% and 4.0%, respectively. The correlation coefficients between the exact and retrieved values of these parameters are 0.990, 0.985, 0.992 and 0.986, respectively. As for other important biophysical parameters of skin, in particular integral epidermal melanin content $\Phi_m = f_m L_e$, the scattering coefficient of tissue $\beta'$ and the capillary diameter $d_v$, then the spread in their retrieved values $b_m^*$ with respect to exact ones $b_m = b_m^*$ is comparable with an *a priori* uncertainty $b_m$. To determine $d_v$, it is apparently necessary to extend the spectral range to smaller $\lambda$, where the effect of localised absorption of light in blood vessels is more pronounced [12]. The estimates of $\Phi_m$ and $\beta'$ can be performed with acceptable accuracy for practice only on the basis of absolute measurements of skin reflectances $R(\lambda)$ – the correlation coefficients between the exact values of $\Phi_m$ and $\beta'$ (632 nm) and those retrieved from $R(\lambda)$ were equal to

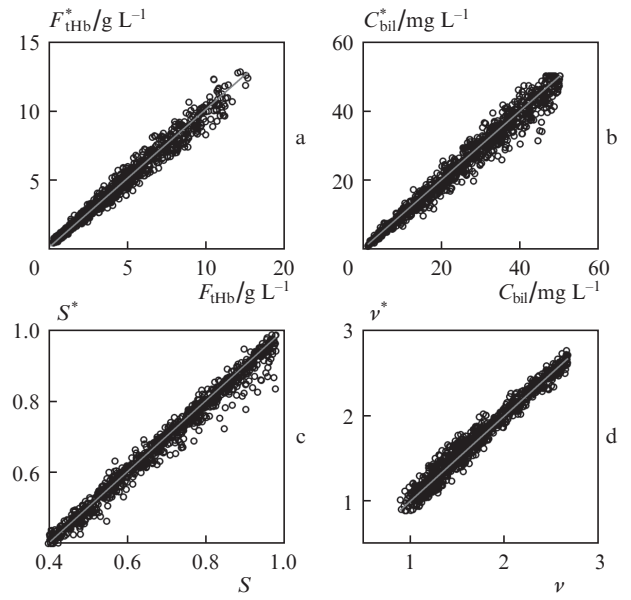


**Figure 2.** Results of numerical experiments on retrieval of the parameters (a) $F_{tHb}$, (b) $C_{bil}$, (c) $S$ and (d) $v$ from the reflectance spectra of skin.

0.999 and 0.984, respectively. However, this requires a calibration sample with known optical parameters that do not change with time.

## 4. Experimental results

To test the developed analytical model, we used the reflectance spectra of skin from 30 volunteers, measured in the range 450–800 nm. Anatomical areas under study were ring finger, palm, earlobe and nose bridge. The measurements were performed using a standard spectrophotometer, the main elements of which are a broadband light source, fibre-optic catheter and fibre-optic spectrometer. As a source of light we used a deuterium/halogen lamp emitting in the range of 215–2500 nm. Delivery of radiation from the lamp and collection of the reflected signal was carried via a fibre-optic catheter consisting of a central illuminating fibre and six receiving fibres (diameters of all fibres were equal to 400 μm). Reflectance spectra were recorded with 0.5-nm resolution by a diffraction-grating fibre-optic CCD spectrometer. The signal from the CCD matrix was fed via a USB-port to a PC for storage and processing. Skin reflectance spectra were normalised to the reflectance spectra of a WS white diffusing reference tile (Avantes, Netherlands), which made it possible to eliminate the influence of the radiation intensity of the source and the receiver sensitivity on the measured signals. However, the reflectance of the reference tile specified in data sheets (over 98% in the wavelength range from 350 to 1800 nm) corresponds to the measurement geometry when the photon collection aperture covers the entire area of the tile from which diffusely reflected light comes out. In our case, we registered only those photons that escaped the region of irradiation and fell on the receiving site. The reflectance of the reference tile, corresponding to this measurement geometry, is not known; therefore, the skin reflectance can be measured up to some constant factor. In processing the experimental data, the value of this factor is not essential since the analysed characteristic of the tissue is the relative spectral variation of its reflectane: $r(\lambda) = R(\lambda)/R(800\ \text{nm})$.

The experimental reflectance spectra of skin, $r_{exp}(\lambda)$, were quantitatively analysed in the approximation of the above analytical model by fitting the model parameters corresponding to the minimum residual (10) [with replacement of $r_{MC}(\boldsymbol{b}, \lambda_i)$ by $r_{exp}(\lambda)$ and $N_\lambda = 700$]. The spectra $r_{exp}(\lambda)$ for four anatomical areas of skin from four volunteers are shown in Fig. 3. Virtually all the measured spectra have a slight dip in the range of 475–480 nm, which is presumably due to absorption of light by beta-carotene contained in small quantities in the skin tissue and having a local absorption maximum in this spectral region [13]. In processing the experimental data, absorption was taken into account by introducing an additional term, $C_\beta\varepsilon_\beta(\lambda)$, into formula (4), where $C_\beta$ and $\varepsilon_\beta$ are the molar concentration and the molar absorption coefficient of beta-carotene. As can be seen from Fig. 3, the reflectance spectra of skin, $r(\boldsymbol{b}^*, \lambda)$, chosen by solving the inverse problem well reproduce the experimental data. The rms deviation $\sigma$ calculated by formula (10) does not exceed 0.007 for both presented and all other measured reflectance spectra of skin. In this case, the average time needed to solve the inverse problem for a spectrum $r_{exp}(\lambda)$ is about 1 s, indicating the operation speed of the developed method for calculating the skin reflectance.

The average values and rms deviations of the biophysical parameters of skin, calculated from the experimental reflectance spectra, are listed in Table 2. Taking into account the information capabilities of the considered measurements, Table 2 shows the parameters $F_{tHb}$, $S$ and $v$ ($C_{bil} \leqslant 0.2$ mg L$^{-1}$ for all volunteers) and retrieved values of the concentrations of beta-carotene $C_\beta$, which give an idea of the range of variation of this parameter for normal light skin. For comparison with literature data, the parameter $F_{tHb}$ should be converted to the volume concentration of capillaries in the tissue: $f_{bl} = F_{tHb}/C_{tHb}$, where $C_{tHb} = 150$ g L$^{-1}$ is the average density of haemoglobin in blood. The range of $f_{bl}$ variations, obtained from the analysis of 120 reflectance spectra of skin (30 volunteers and 4 anatomical areas) is 0.2%–5.1%, which is in full accordance with the results of independent studies [14–16]. The found values for arterial-venous haemoglobin saturation are also reasonable and consistent with the literature data [17, 18].

The most reliable data on the parameter $v$ were obtained in [19, 20] by photometry using integrating spheres. According to these data, $v = 1.21$ and 1.27, respectively, which is sufficiently close to the values of $v$ indicated in Table 2. Thus, the developed analytical model of the diffuse reflectance spectrum of skin produces quite correct assessment of its biophysical parameters in real time.

It should be noted that the considered measurements performed for skin areas with pulsatile changes in the vascular volume (due to heart contractions) also allow one to determine the saturation of arterial haemoglobin. The heart blood flow in the arteries modulates detectable signals; therefore, by

**Table 2.** Biophysical parameters (mean value ± rms deviation) calculated from experimental reflectance spectra of various anatomical areas of 30 volunteers.

| Parameters | Biophysical parameters | | | |
|---|---|---|---|---|
| | Ring finger | Palm | Earlobe | Nose bridge |
| $F_{tHb}$/ gL$^{-1}$ | 1.91 ± 1.18 | 0.87 ± 0.52 | 2.02 ± 1.23 | 2.31 ± 2.09 |
| $S$ | 0.66 ± 0.11 | 0.39 ± 0.19 | 0.70 ± 0.21 | 0.65 ± 0.19 |
| $v$ | 1.37 ± 0.13 | 1.47 ± 0.24 | 1.39 ± 0.14 | 1.24 ± 0.18 |
| $C_\beta$/μmolL$^{-1}$ | 2.40 ± 1.05 | 1.78 ± 0.94 | 2.45 ± 1.11 | 2.54 ± 1.88 |

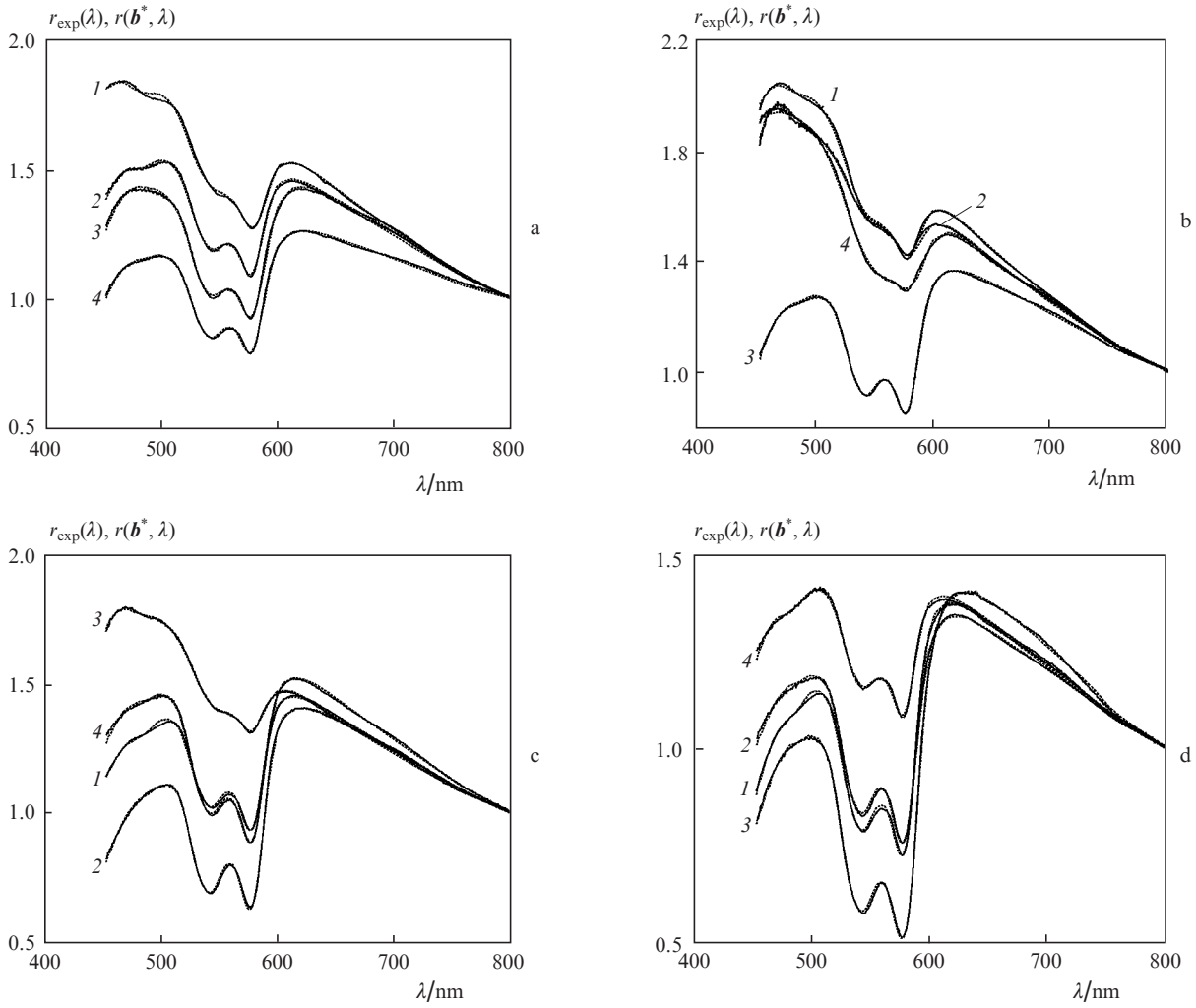


**Figure 3.** Experimental (solid curves) and theoretical (dashed curves) spectral dependences of reflectances of (a) ring finger, (b) palm, (c) earlobe and (d) nose bridge of four volunteers (*1–4*).

measuring the reflectance spectra of light at different times $t$, one can monitor the dynamics of the parameters $F_{tHb}(t)$ and $S(t)$ in the tissue under study. The parameter $S$ can be represented as a linear combination of saturation of venous ($S_v$) and arterial ($S_a$) haemoglobin:

$$S(t) = \frac{S_v F_{tHb}^v + S_a(t) F_{tHb}^a(t)}{F_{tHb}^v + F_{tHb}^a(t)}, \tag{11}$$

where $F_{tHb}^v$ and $F_{tHb}^a$ are the venous and arterial concentrations of haemoglobin in the tissue. From equation (11) for times $t_0$ and $t_i$ we obtain

$$\Delta(SF_{tHb})_i = S_a \Delta(F_{tHb})_i, \tag{12}$$

where $\Delta(F_{tHb})_i = F_{tHb}(t_i) - F_{tHb}(t_0)$ and $\Delta(SF_{tHb})_i = S(t_i)F_{tHb}(t_i) - S(t_0)F_{tHb}(t_0)$. In order to improve the stability of the method for determining $S_a$ to errors of optical measurements and the influence of contact of the fibre-optic catheter with the tissue under study, $F_{tHb}$ and $S$ should be preferably measured for $N > 2$ instants of time $t_i$. In this case, the system of equations (12) is solved using the least squares method:

$$S_a = \sum_{i=1}^{N} \Delta(F_{tHb})_i \Delta(SF_{tHb})_i \Big/ \sum_{i=1}^{N} \Delta(F_{tHb})_i^2. \tag{13}$$

In the practical implementation of the proposed method the skin reflectance spectra were recorded with a time resolution of 4.5 ms within 5 s. The ring finger was used in the measurements. The spectrometer was controlled by a PC via the USB interface using special software. Figure 4a allows one to observe the influence of arterial pulsation on the detected optical signals and shows the time-base of the signal at the wavelength of maximum difference between the coefficients of oxy- and deoxyhaemoglobin absorption. Along with distinct periodic oscillations of the signal, also visible is a small systematic shift of the overall signal level, caused by the pressure exerting by the probe on the skin of the volunteer. This shift is the main source of errors in the measurement of arterial blood oxygenation; therefore, it is needed to fix in measurements the fibre probe in the investigated area of skin. Another source of errors is the noise of the measuring device.

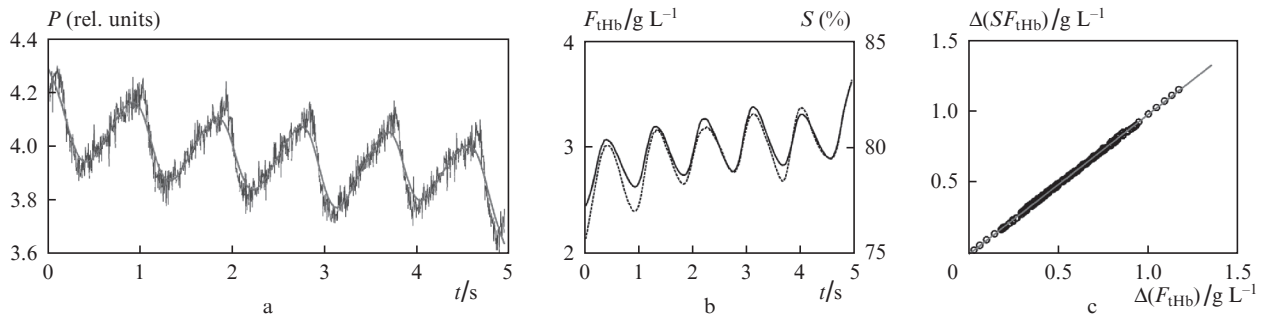


**Figure 4.** (a) Results of the experiment on determination of the degree of oxygenation of arterial blood – timebase of the diffuse reflectance signal of skin at $\lambda = 577$ nm and its approximation by cubic splines, (b) time dependences of the retrieved parameters $F_{tHb}$ (solid curve) and $S$ (dashed curve) and (c) approximation of the aggregation of experimental points $[\Delta(F_{tHb}), \Delta(SF_{tHb})]$ by expression (12).

To weaken their influence on the measurement results, time values of the signals, $P_i$, were smoothed by cubic splines $f(t_i)$ minimising the functional of form

$$\gamma \sum_{i=1}^{N} |P_i - f(t_i)|^2 + (1-\gamma) \int_{t_{min}}^{t_{max}} \left| \frac{\partial^2 f(x)}{\partial x^2} \right|^2 dx, \qquad (14)$$

where $\gamma$ is the smoothing parameter ($\gamma = 0-1$) and $[t_{min}, t_{max}]$ is the time interval. The first term in (14) is a discrepancy between $P_i$ and $f(t_i)$, and the second term is responsible for the smoothness of the function $f(t)$ within the interval $[t_{min}, t_{max}]$. The parameter regulates the relationship between the smoothness of the unknown function and the residual of the measurements. The choice of $\gamma$ is generally determined by the level of measurement errors. In our case, the optimal smoothing of experimental signals without significant loss of information contained in them is achieved at $\gamma = 0.99$.

The smoothing was performed for all spectral regions in the range from 450 to 800 nm. The resulting functions $f(\lambda, t)$ allow one to calculate the reflectance spectrum of skin at any instant of time. The parameters $F_{tHb}$ and $S$ were retrieved for 200 values of $t$, uniformly distributed in the interval 0–5 s. In searching for the model parameters providing a minimum residual (10), as an initial approximation we used the solution of the minimisation problem for the previous instant of time, thereby significantly reducing the computational cost and time of processing of the experimental data. The retrieved dependences $F_{tHb}(t)$ and $S(t)$ are presented in Fig. 4b. Because the inflow/outflow of the arterial blood (with a high degree of oxygenation) into the analysed volume of the tissue leads to an increase/decrease in haemoglobin concentration and degree of oxygenation of the mixed (arterio-venous) blood, the variations of the parameters $F_{tHb}$ and $S$ are synchronous, which is confirmed by the presented dependences.

Using the dependence $F_{tHb}(t)$ we selected the instant of time $t_0$, corresponding to the minimum content of haemoglobin in the tissue, and calculated with respect to it the increments $\Delta(F_{tHb})$ and $\Delta(SF_{tHb})$. These increments are compared in Fig. 4c. All the points are located close to the straight line (12) corresponding to $S_a = 0.97$. It should be noted that, despite the considerable spread of parameters $F_{tHb}$ and $S$ for the skin of volunteers, the values of $S_a$, calculated by dependences $F_{tHb}(t)$ and $S(t)$ for each volunteer, lie in a narrow range corresponding to the physiological data of a healthy person, i.e., 0.94–0.99. This fact confirms the correctness of the estimates of biophysical parameters of skin using the developed model.

## 5. Conclusions

We have developed the model of the diffuse reflectance spectrum of human skin, enabling highly accurate calculation of optical signals, detected outside the area of skin exposure, by taking into account its refractive index, scattering anisotropy factor and different coefficients of absorption of epidermis and dermis. This model is the basis for quantitative interpretation of experimental data obtained by using commercially available spectrophotometers with a fixed distance between the radiation source and detector. Such optical measurements allow one to reliably determine the concentration of bilirubin and basic derivatives of haemoglobin (oxy- and deoxyhaemoglobin) in the skin tissues, and the structure parameter of the tissue, characterising the size of its effective scatterers. In this case, the diagnostic possibilities of the considered measurements can be expanded by increasing the measuring base, i.e., the distance between the illuminating and collecting optical fibres. This will increase the sensitivity of the detected signals to small variations in both major forms of haemoglobin and dishaemoglobin (carboxy-, met- and sulfhaemoglobin), which, in turn, will make it possible to identify and diagnose various pathological conditions of the human body.

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