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# Online determination of biophysical parameters of mucous membranes of a human body

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*Abstract.* **We have developed a method for online determination of biophysical parameters of mucous membranes (MMs) of a human body (transport scattering coefficient, scattering anisotropy factor, haemoglobin concentration, degrees of blood oxygenation, average diameter of capillaries with blood) from measurements of spectral and spatial characteristics of diffuse reflection. The method is based on regression relationships between linearly independent components of the measured light signals and the unknown parameters of MMs, obtained by simulation of the radiation transfer in the MM under conditions of its general variability. We have proposed and justified the calibration-free fibre-optic method for determining the concentration of haemoglobin in MMs by measuring the light signals diffusely reflected by the tissue in four spectral regions at two different distances from the illumination spot. We have selected the optimal wavelengths of optical probing for the implementation of the method.**

***Keywords:*** *endoscopy, mucous membrane, optical parameters, biophysical parameters, diffuse reflectance, optical measurements, inverse problem, multiple regressions.*

## 1. Introduction

The diagnosis of tumours is one of the most urgent problems of modern oncology and surgery. Early diagnosis and effective treatment at early stages of the disease can be important factors for a favourable outcome of the disease, increase the survival rate and improve the quality of life of cancer patients.

The primary means of diagnosis of malignant diseases of the respiratory tract and the gastrointestinal tract is endoscopy during which pieces of a mucosa are taken for a biopsy. The results of the study provide information about the pathological changes in the mucous membranes (MMs) and help to determine the structure of the tumour, which is important for a decision on further treatment strategies. The disadvantages of surgical biopsies are invasiveness, high cost, inability to multiple repetitions of the procedure, and delay in the appointment of therapy. In addition, surgical biopsy is not for every patient because of the severity of their condition.

It is known that the optical properties of the tumour and surrounding normal tissues are significantly different. Optical methods for the study of biological tissues [1–3] can reduce the number of cases of an invasive biopsy and the cost of its implementation. Among these methods, the simplest and most effective are the methods of diffuse reflectance (DR) spectroscopy [3–6]. The DR spectra of mucous membranes are typically measured by using fibre mini-probes introduced or embedded in the instrument channel of the endoscope. In the simplest case, the fibre probe may comprise two fibres placed at a fixed distance from each other. One optical fibre is used for delivering radiation to the tissue (excitation channel), the other – to receive the backscattered light (detection channel) [4–6]. Biophysical parameters of a tissue that characterise its structure and component composition are assessed by comparing the calculated and experimental DR spectra of the tissue. The main drawback of these measurements is the impossibility of separating the contributions of absorption and scattering in the measured spectral signals, which leads to ambiguity of the solution to the corresponding inverse problem.

Measurements of scattered light at several distances from the illumination spot [3, 7, 8] in principle allow one to determine independently the absorption and transport scattering coefficients of the tissue under study. To this end, using one of the methods of the theory of radiative transfer we may solve the inverse problem on the interpretation of the measured spatial profiles of the intensity of radiation scattered by the tissue. Generally, for these purposes, either the Monte Carlo method [9] or the diffusion approximation [10] is employed. The first method requires large computational efforts and is therefore not suitable for online quantitative interpretation of the experimental data of endoscopic studies (especially in the case of many optical probe wavelengths). The second method imposes restrictions on the geometry of the scheme for measuring scattered radiation (the distance between the illuminating and receiving fibres should exceed the length of the transport free path of a photon, which, as a rule, is larger than the diameter of the endoscope instrument channel) and is applicable only in the spectral region of low absorptions of tissue chromophores ($\lambda$ = 600–900 nm). In this regard, oncology and surgery still lack a simple and reliable optical method, which makes it possible to quickly and accurately identify biophysical parameters of a suspicious tumour and its surrounding normal tissue in endoscopic studies.

A new method of interpretation of the data of optical probing of light-scattering media has been proposed in [11–13]. The essence of the method is to find the solutions to the inverse problem using multiple regressions between the measured optical characteristics and sought-for parameters of the medium obtained by simulating radiative transfer in the medium in question under conditions of their general variability. This method allows one to determine the parameters of the medium in real time without solving the equation of

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radiative transfer and without the use of complex mathematical algorithms for solving inverse problems. In this paper, on the basis of this method we solve the problem of an online determination of biophysical parameters of mucous membranes by spectral and spatial characteristics of their DR measured during endoscopy.

## 2. Measuring the diffuse reflection coefficient

Figure 1 shows the scheme of a device for spectral measurements of the DR of biological tissues with a spatial resolution. The scheme includes a light source unit (*1*), a monochromator (*2*), a fibre-optic probe (*3*) equipped with the illuminating and receiving fibres, a detection unit (*5*) and a unit processing the measured information (*6*). Light from the light source (*1*), passed through the monochromator (*2*), is coupled into the fibre-optic probe (*3*) and delivered to the tissue (*4*) through this probe. In one scenario, shown in Fig. 1b, the fibre-optic probe (*3*) can comprise two illuminating fibres (excitation channels) (*7*) between which are sandwiched the receiving fibres (detection channels) (*8*). The spacings between the illuminating and receiving fibres should not exceed the diameter of the endoscope instrument channel, the typical values of which are 2–3 mm. Radiation diffusely reflected by the tissue (*4*) is coupled into the receiving fibres (*8*) and through them into the photodetectors or is focused by the microlens (*9*) onto the CCD-sensor (*10*). Thus, the radiation is recorded simultaneously in all spatial detection channels. A comparison of the DR signals from symmetrically spaced excitation channels allows us to estimate the degree of homogeneity of the illuminated volume and thus to choose the best tissue site for measurements. After measuring the signals the data obtained are processed by unit (*6*), where they are analysed to determine the biophysical parameters of the tissue.

The measured signals $U(L,\lambda)$ in the fibre-optic scheme under consideration depend on the spectral and spatial profile of the DR of the tissue, $U(L,\lambda)$, as well as on the instrumental constants and spectral light source power $P_0(\lambda)$:

$$U(L,\lambda) = P_0(\lambda)\, R(L,\lambda)\, \tau(\lambda)\, S(\lambda), \tag{1}$$

where $L$ is the distance between the centres of the illuminating and receiving fibres; $\lambda$ is the wavelength of light; $S(\lambda)$ is the spectral sensitivity of the receiver; and $\tau(\lambda)$ is the transmission function of the optical system. Profiles $R(L,\lambda)$ are found by comparing the signals $U(L,\lambda)$ with the signals $P_{\text{std}}(L,\lambda)$ corresponding to the standard sample:

$$R = R_{\text{std}} \frac{U - U_{\text{dark}}}{U_{\text{std}} - U_{\text{dark}}}, \tag{2}$$

where $R_{\text{std}}$ is the DR of the standard sample and $U_{\text{dark}}(L,\lambda)$ is the signal power in the absence of illumination, determined by the dark current. It should be noted that the coefficient $R_{\text{std}}$ depends not only on optical parameters of the sample, but also on the collecting ability of the fibres [4, 6], and therefore its calculation must take into account the numerical aperture of the fibres and their geometric configuration.

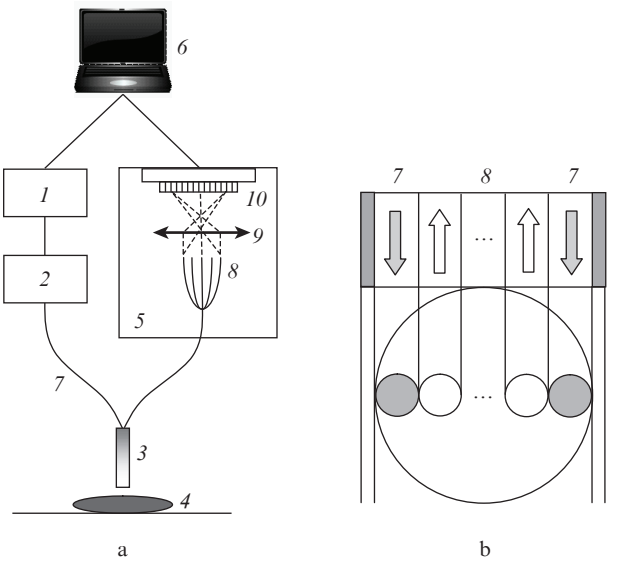


**Figure 1.** (a) Block diagram of the experimental setup for spectral measurements of the DR of biological tissues with a spatial resolution and (b) scheme of location of excitation and detection channels in a fibre-optic probe.

## 3. Interpretation of the measurement results

The measured profiles $R(L,\lambda)$ are conveniently represented as a vector of measurements of $\boldsymbol{r}$ from $N_{\text{mes}} = N_\lambda N_L$ components, where $N_\lambda$ and $N_L$ are the number of spectral and spatial channels of DR signal. For the convenience of working with numbers of the same order we will use a logarithmic representation of the experimental data, i.e., the components $\boldsymbol{r}$ will be $r_n = -\ln R(L_i, \lambda_j)$, where $1 \leqslant i \leqslant N_L$, $1 \leqslant j \leqslant N_\lambda$, and $n = (i-1)N_\lambda + j$. Using principal component analysis [14, 15], the vector $\boldsymbol{r}$ can be decomposed into a system of orthogonal vectors, where the best approximation for $\boldsymbol{r}$ is its expansion in the eigenvectors of its covariance matrix

$$S_{nm} = \frac{1}{\sigma_m \sigma_n} \sum_{k=1}^{K} (r_n^k - \bar{r}_n)(r_m^k - \bar{r}_m), \tag{3}$$

where $\bar{\boldsymbol{r}} = (\bar{r}_n)$ and $\boldsymbol{\sigma} = (\sigma_n)$ are the mean value and the variance of the vector $\boldsymbol{r}$, defined on the basis of the $K$ ensemble of realisations of $\boldsymbol{r}$. In this case, the expansion of any realisation of $\boldsymbol{r}$ has the form:

$$\boldsymbol{r} = \bar{\boldsymbol{r}} + \boldsymbol{V}\boldsymbol{\xi}, \tag{4}$$

where $\boldsymbol{V} = (\boldsymbol{v}_1, ..., v_P)$ is the matrix of size $N_{\text{mes}} \times P$, whose columns are the eigenvectors $\boldsymbol{v}_n$ of matrix (3); $P$ is the number of used eigenvectors ($P \leqslant N_{\text{mes}}$); and $\boldsymbol{\xi} = (\xi_1, ..., \xi_P)$ are linearly independent components, which are found as the projections of $\boldsymbol{r}$ on the space of the vectors $\boldsymbol{v}_p$:

$$\boldsymbol{\xi} = \boldsymbol{V}^{\text{t}}(\boldsymbol{r} - \bar{\boldsymbol{r}}), \tag{5}$$

where the superscript 't' denotes transposition.

Due to the rapid convergence of the expansion in question, the first eigenvectors corresponding to the largest eigenvalues of the matrix (3) make the greatest contribution to the change in the vector $\boldsymbol{r}$. Thus, it is possible to significantly reduce the dimensionality of the original data and to select several linearly independent components, which contain as

much information as in the original data. In this case, the required parameters of the tissue $\boldsymbol{x} = (x_k)$ can be found directly from the linearly independent components, for example using polynomial regressions:

$$\ln x_k = a_{00}^k + \sum_{p=1}^{P} \sum_{m=1}^{M} a_{pm}^k (\xi_p)^m, \tag{6}$$

where $a_{pm}^k$ are the regression coefficients that depend on the design parameters of the measuring device (optical fibre diameters, their geometrical configuration, numerical apertures) and the wavelengths of the probe radiation; and $M$ is the degree of the polynomial which is selected based on the value of residual dispersion (usually for statistical approximation of relationship between $\boldsymbol{\xi}$ and $\boldsymbol{x}$ it is sufficient to use $M = 3$). The use of the logarithm in (6) allows one to achieve approximately equal approximation error throughout the $x_k$ range and eliminates the possibility of obtaining negative values of $x_k$.

Thus, the inverse problem should be solved by using analytical expressions relating the required parameters of the tissue with the linearly independent components of the $R(L, \lambda)$ profile. These analytical expressions can be derived by regression analysis of the results of the calculation of $R(L, \lambda)$ by the Monte Carlo method for different parameters of a medium simulating a biological tissue under investigation. Let us consider in more detail the process of obtaining the vectors $\bar{\boldsymbol{r}}$, $\boldsymbol{v}_p$ and multiple regressions (6) using the optical MM model.

## 4. Optical model of a mucous membrane

It is known that the main parameters characterising the propagation of optical radiation in a scattering medium are the absorption coefficient $k$, the scattering coefficient $\beta$ and the scattering indicatrix or its average cosine $g$. In the optics of biological tissues which are very turbid media, to describe the light fields it is needed to know not the values of $\beta$ and $g$ but their combination – the transport scattering coefficient $\beta' = \beta(1 - g)$.

The spectrum of the MM absorption coefficient $k(\lambda)$ is modelled as a linear combination of the absorption spectra of the bloodless (connective) tissue $k_{\text{tis}}(\lambda)$ and capillaries with blood $k_{\text{bl}}(\lambda)$:

$$k(\lambda) = k_{\text{tis}}(\lambda) + \alpha(\lambda) f_{\text{bl}} k_{\text{bl}}(\lambda), \tag{7}$$

where $f_{\text{bl}}$ is the volume fraction of capillaries; and $\alpha$ is the correction factor that takes into account the difference between the absorptive capacity of the blood uniformly distributed over the tissue volume and the blood localised in the capillaries. Barun and Ivanov [16] studied the effect of localised absorption of light in detail and showed that for randomly distributed capillaries of diameter $D_{\text{v}}$ the factor $\alpha$ with a reasonable degree of accuracy can be defined by the expression:

$$\alpha(\lambda) = 2\sqrt{3}\, \frac{1 - \exp[-\pi k_{\text{bl}}(\lambda) D_{\text{v}} (1 - 0.043 k_{\text{bl}} D_{\text{v}})/2\sqrt{3}]}{\pi k_{\text{bl}}(\lambda) D_{\text{v}}}. \tag{8}$$

In the visible region of the spectrum the main light-absorbing chromophores of blood are oxy- ($HbO_2$) and deoxyhaemoglobin (Hb). In this connection, the expression for its absorption coefficient is:

$$k_{\text{bl}}(\lambda) = (C_{\text{tHb}} \ln(10)/\mu_{\text{tHb}})[S\varepsilon_{\text{HbO}_2}(\lambda) + (1 - S)\varepsilon_{\text{Hb}}(\lambda)], \tag{9}$$

where $C_{\text{tHb}}$ is the concentration of haemoglobin in blood; $\mu_{\text{tHb}} = 64\,500$ g mol$^{-1}$ is the molar mass of haemoglobin; $\varepsilon_{\text{HbO}_2}$ and $\varepsilon_{\text{Hb}}$ are the molar absorption coefficients of $HbO_2$ and Hb [17]; and $S$ is the degree of blood oxygenation.

One can see from (7) and (9) that the absorption of radiation by the blood is determined by the product $f_{\text{bl}} C_{\text{tHb}}$ rather than by the values $f_{\text{bl}}$ and $C_{\text{tHb}}$ separately. Consequently, from the optical experiments we can determine only the given product. In this connection, for the parameter $C_{\text{tHb}}$ we use below a fixed value, corresponding to the normal level of haemoglobin in blood, i.e., 150 g L$^{-1}$.

The dependence of the absorption by a bloodless tissue on the wavelength $\lambda$ in the visible region of the spectrum is well approximated by an exponential function

$$k_{\text{tis}}(\lambda) = A \exp[-B(\lambda - \lambda_0)], \tag{10}$$

where $\lambda_0 = 600$ nm, and $A$ (mm$^{-1}$) and B (nm$^{-1}$) are empirical parameters [4, 18–20].

The spectral dependence of the transport scattering coefficient of the tissue can be described with a good degree of accuracy by a smooth power-law function [3, 4, 8, 20–24]

$$\beta'(\lambda) = C(\lambda_0/\lambda)^{\nu}, \tag{11}$$

where $\lambda_0 = 600$ nm, and $C$ and $\nu$ are the structural parameters of tissue characterising the volume fraction and the size of its 'effective' scatterers. To describe the single scattering indicatrix of the tissue, use is made of a one-parameter Henyey–Greenstein function [9] with an average cosine of the scattering indicatrix $g$.

Thus, the optical MM model is determined by the parameters $g$, $A$, $B$, $C$, $\nu$, $f_{\text{bl}}$, $C_{\text{tHb}}$, $D_{\text{v}}$ and $S$. In addition, to account for the reflection of the incident light from the tissue surface and multiple reflections of the induced diffuse radiation between the inner layers and the surface of the tissue, it is necessary to have information about its refractive index $n_{\text{tis}}$. Below we present following ranges of variations in model parameters selected by analysing the results of various authors [3–5, 18–27] for normal and tumoured MMs of the oral cavity, oesophagus, gastrointestinal tract and lungs.

The ranges of variations in the optical MM model

| | |
|---|---|
| $n_{\text{tis}}$ | 1.35–1.45 |
| $A$ (mm$^{-1}$) | 0.01–0.1 |
| $B$ (nm$^{-1}$) | $(1–28)\times10^{-3}$ |
| $C$ (mm$^{-1}$) | .0.5–3.0 |
| $\nu$ | .1.0–2.5 |
| $g$ | 0.5–0.95 |
| $f_{\text{bl}}$ (%) | 0.5–20 |
| $D_{\text{v}}$ (μm) | 5–30 |
| $S$ (%) | 40–98 |

We will vary the model parameters independently of each other, but we will make sure that in every combination the values of the optical parameters of the tissue derived from formulas (7)–(11) lie within the ranges observed in the experiment. On the basis of the above data, we have selected the following limitations: $\beta'(700\text{ nm}) \geqslant 0.2$ mm$^{-1}$, $k(632\text{ nm}) = 0.02–0.5$ mm$^{-1}$, $k_{\text{tis}}(450\text{ nm}) \leqslant 0.35$ mm$^{-1}$, $\Lambda'(632\text{ nm}) = 0.5–0.98$, where $\Lambda' = \beta'/(k + \beta')$ is the transport single-scattering albedo of a tissue.

## 5. Scheme of the simulated experiment

In this paper, we consider the following scheme for fibre-optic measurements of the DR of the tissue. Radiation is administered into the medium by an illuminating optical fibre. Radiation scattered by the tissue into the back half-space is coupled into the receiving optical fibres spaced by the distance $L = 0.23$, 0.46, 0.69, 0.92 and 1.15 mm from the centre of the illumination spot. The core diameter of all the fibres is equal to 0.2 mm. The detection channels with $L > 1.15$ mm are not considered because of the smallness of the corresponding optical signals and also because of the high probability of horizontal inhomogeneity of the corresponding volumes of light–tissue interaction.

The ensemble of realisations of the biophysical parameters of the tissue and its spectral and spatial DR characteristics corresponding to the above-described scheme of optical measurements is formed by multiple solutions of the direct problem by the Monte Carlo method for different combinations of parameters of the optical model of the tissue. The profiles $R(L,\lambda)$ are calculated numerically as follows. The values of the model parameters of the above ranges are randomly selected. For each realisation of the parameters we calculate $k(\lambda)$ and $\beta'(\lambda)$ by (7)–(11) at 26 wavelengths characterising the main features of the spectral curves of the MM chromophore absorption in the range from 450 to 700 nm. In accordance with the obtained values of $k(\lambda)$, $\beta'(\lambda)$, $g$ and $n_{tis}$ by the Monte Carlo method we simulated the profile $R(L,\lambda)$. The tissue phantom is a cylinder with a radius of 10 mm and a height of 5 mm. The dimensions are chosen so that on the one hand, to eliminate the effect of the boundaries and on the other, to reduce the calculation time. In the calculation we assumed that the radiation is introduced into the medium in the direction of the normal to its surface and uniformly distributed over the cross-section area of the illuminating fibre. The numerical apertures of all the fibres are conventionally assumed equal to 1.0 [of course, in solving the problem of interpreting the real fibre-optic measurements, the profiles $R(L,\lambda)$ must be calculated strictly in accordance with the values of the apertures of the fibres and the angular distribution of radiation injected into the medium). The number of photons used in the Monte Carlo method was chosen as a function of the transport single-scattering albedo and was varied from $6\times10^6$ at $\Lambda' \leqslant 0.3$ to $2\times10^5$ at $\Lambda' \geqslant 0.9$. This procedure was repeated for $10^3$ realisations of $R(L,\lambda)$, which, as will be shown below, is sufficient to obtain statistically significant results.

## 6. Retrieval of the biophysical parameters of mucosa

The spectral and spatial profile $-\ln R(L,\lambda)$, obtained with the help of the scheme for optical measurements, is presented in Fig. 2. The set of points in the profile forms a measurement vector $\boldsymbol{r}$, corresponding to particular values of the biophysical parameters of the tissue. On the basis of an ensemble of similar representations of $\boldsymbol{r}$, corresponding to the widest possible variations in the biophysical parameters of the tissue, we calculated the average measurement vector $\bar{\boldsymbol{r}}$ and eigenvectors $\boldsymbol{v}_p$ of the covariance matrix (3). Next, using formula (5) we found the linearly independent components ($\boldsymbol{\xi}$) of all the realisations of $\boldsymbol{r}$ and using the least-squares method we derived multiple regression equations between $\boldsymbol{\xi}$ and model parameters $\boldsymbol{x}$. The thus obtained vectors $\bar{\boldsymbol{r}}$, $\boldsymbol{v}_p$ and regressions between $\boldsymbol{x}$ and $\boldsymbol{\xi}$ can be used for online determination of bio-

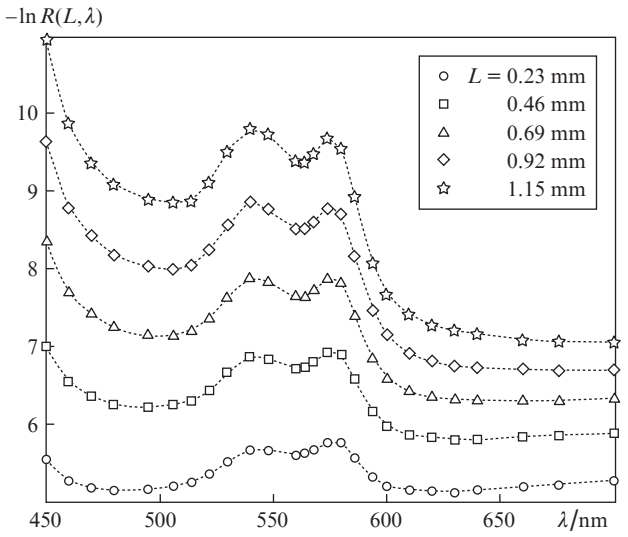


**Figure 2.** Profile $-\ln R(L,\lambda)$ calculated by the Monte Carlo method for the following values of the model parameters: $n_{tis} = 1.4$, $g = 0.73$, $A = 0.02$ mm$^{-1}$, $B = 0.012$ nm$^{-1}$, $C = 1.64$ mm$^{-1}$, $\nu = 1.6$, $f_{bl} = 3.2\%$, $C_{tHb} = 150$ g L$^{-1}$, $D_v = 25$ μm, $S = 70\%$.

physical parameters of the tissue by the measured characteristics of its DR.

The optimal number of biophysical parameters in the regression formula (6) for each of the model parameters $x_k$ is determined by the closed numerical experiment as follows. Realisations of all the model parameters are searched for and $x_k$ is calculated for each realisation with the help of (5), (6) (using $P$ vectors $\boldsymbol{v}_p$) by superimposing on $R(L,\lambda)$ the random deviations within $\delta R$, modelling errors of optical measurements. The resulting values of $x_k^*$ are compared with the values of $x_k$, corresponding to the realisation in question, and the retrieval errors of $x_k$ are calculated. After searching all the realisations the average retrieval error of $x_k$ is calculated. The optimal value of $P$ corresponds to the minimum of this error.

The above numerical experiments were performed for the following biophysical parameters: $F_{tHb} = f_{bl}C_{tHb}$ is the haemoglobin concentration in the tissue (g L$^{-1}$); $S$ is the oxygen saturation; $C$ and $\nu$ are the parameters of the spectral dependence of the transport scattering coefficient of the tissue; $g$ is the average cosine of the scattering indicatrix; and $D_v$ is the average diameter of capillaries. The dependences of the average errors ($\delta x_k$) of the parameters' retrieval from the profiles $R(L,\lambda)$, when the errors are superimposed on $R(L,\lambda)$ within 1%, 5% and 10%, are shown in Fig. 3. One can see that at small errors of optical measurements ($\delta R = 1\%$), errors in $\delta x_k$ decrease with increasing $P$. When $\delta R = 5\%$–$10\%$, 9 to 12 linearly independent components of the profile $R(L,\lambda)$ are optimal for the retrieval of the parameters under study.

The values of the biophysical parameters of the tissue, for which the profiles of $R(L,\lambda)$ were calculated, and the values of these parameters, retrieved from $R(L,\lambda)$ at $P = 11$ and $R = 0\%$, are compared in Fig. 4. Also are shows the average retrieval errors of the parameters. Since the parameters were retrieved without the superimposition of the errors on the profiles of $R(L,\lambda)$, the spread of the points on the plots with respect to the straight line $x_k^* = x_k$ allows one to judge the sensitivity of the results of the $x_k$ parameter retrieval to the variations of all the other parameters of the tissue, i.e., the impact of an *a priori* uncertainty.

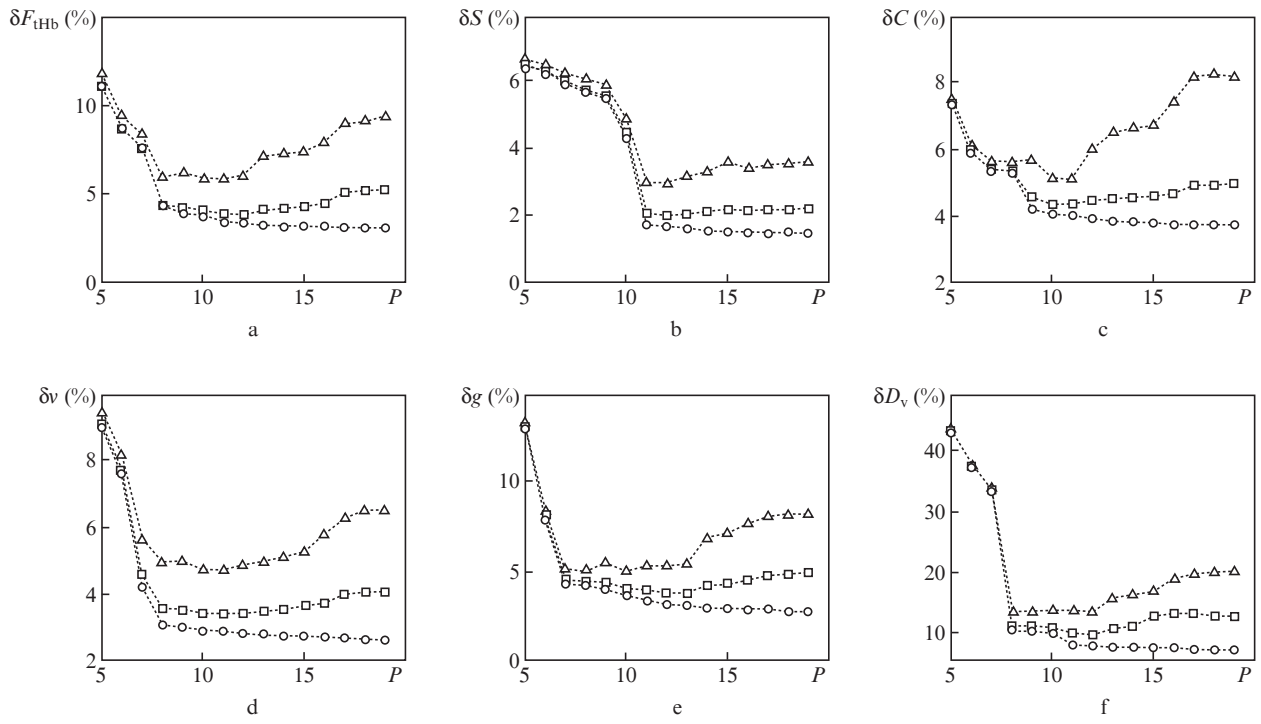


**Figure 3.** Dependences of the average retrieval of the parameters on the number of vectors $\nu_n$ when superimposing on $R(L,\lambda)$ random deviations in the range of 1% (○), 5% (□) and 10% (△).

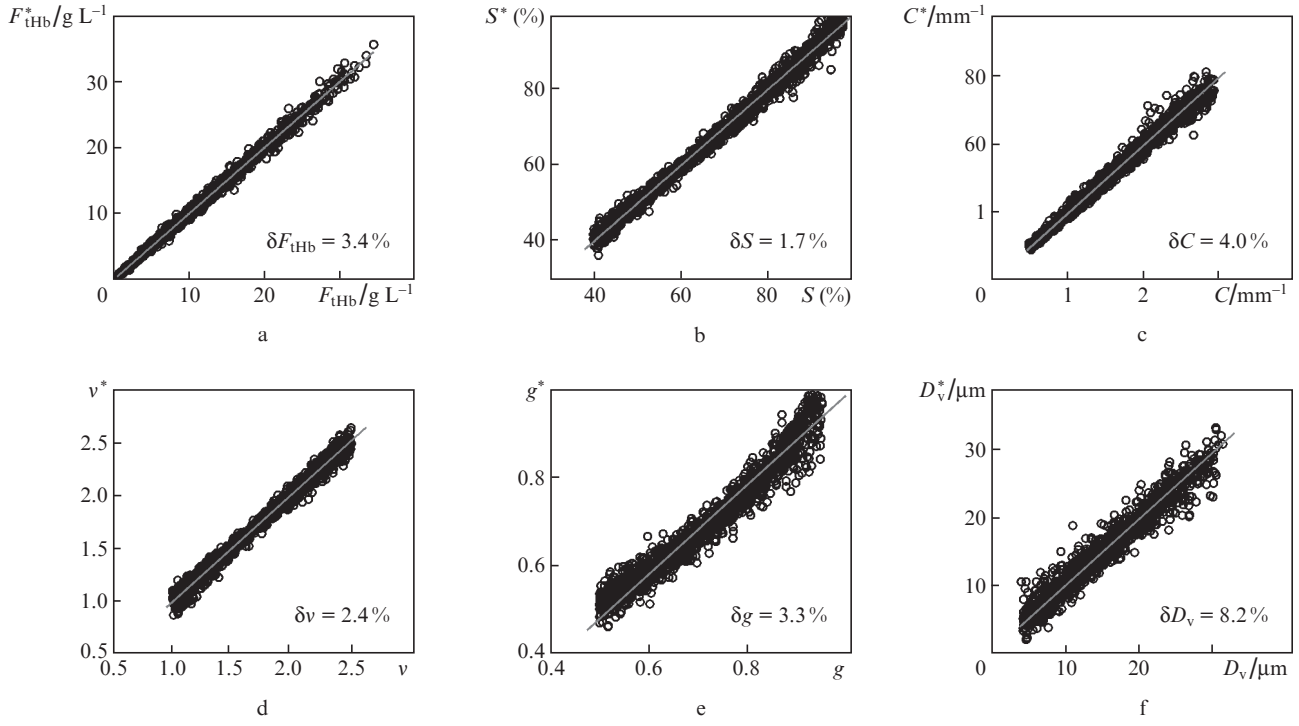


**Figure 4.** Results of retrieval of the parameters $F_{tHb}$, $S$, $C$, $\nu$, $g$ and $D_v$ using 11 linearly independent components of the profile $R(L,\lambda)$.

## 7. Calibration-free method for determining the concentration of haemoglobin in the tissue

It is known that the cancer tissues are characterised by enhanced blood circulation in comparison with normal tissues; therefore, the data on the amount of haemoglobin can help distinguish benign from malignant neoplasms. However, an increase in tissue capillary hyperaemia cannot always be detected visually or by analysing the colour endoscopic image because the light scattering characteristics of the tissue change due to pathological changes in its structure. In this regard, the development of a simple and reliable method of noninvasive determination of haemoglobin concentration in biological tissues is an extremely important task.

Above, to determine the biophysical parameters of the tissue, we used quite a large number of spectral and spatial channels for detecting radiation reflected by the tissue. This, on the one hand, makes it possible to increase the stability of the inverse problem solution to the errors of optical measurements and, on the other hand, increases the measurement time, requires the use of expensive and complex spectrometers and complex optics in the radiation detection unit. Moreover, for finding the coefficients $R(L,\lambda)$ it is necessary to perform the calibration measurements. From the standpoint of economic feasibility, ease of implementation and convenience of practical application, of greatest interest is the calibration-free method for determining the haemoglobin concentration in the tissue ($F_{\text{tHb}}$), based on the detection of radiation reflected by the tissue at several spectral regions.

We can eliminate the need for calibration of the measuring device by determining the parameters of the tissue from the difference DR signals: $r(\lambda) = \ln[P(L_2,\lambda)/P(L_1,\lambda)]$. As follows from (1), the signals $r(\lambda)$ do not depend on the instrumental constants and the power of radiation incident on the tissue; they are are determined only by the effective optical thickness of the path travelled by radiation between two receiving optical fibres. In this regard, we consider the possibility of retrieving $F_{\text{tHb}}$ from spectral measurements of $r(\lambda)$.

The minimum number of spectral regions needed to retrieve $F_{\text{tHb}}$ can be determined on the basis of closed numerical experiments. The components of the vector $\boldsymbol{r}$ are the spectral values of $r(\lambda)$; therefore, the number of linearly independent components of $\boldsymbol{r}$ corresponds to the number of independent probe wavelengths $N_\lambda$. The dependence of the $F_{\text{tHb}}$ retrieval error on $N_\lambda$ was analysed for the difference signals corresponding to the maximum optical path between the receiving optical fibres in the above scheme of optical measurements, i.e., $L_1 = 0.23$ mm and $L_2 = 1.15$ mm. The results of such an analysis show that at the measurement error $r(\lambda)$ equals to 5%–10%, $F_{\text{tHb}}$ can be determined by using only four probe wavelengths. Thus, the problem of planning $F_{\text{tHb}}$ measurements is reduced to a choice of four optimal spectral regions. This choice was made by a computer search of all possible combinations of the original set of $\lambda$ (26 values) and the evaluation of the corresponding error in the $F_{\text{tHb}}$ retrieval.

It is obvious that the interpretation of $r(\lambda)$ measurements in four spectral regions is not possible with the use of rigorous mathematical methods for solving inverse problems, since the number of measurements is less than the number of model parameters. However, the regression method [11–13] allows us to solve the inverse problem even in the case of a small number of optical measurements. In this case, as the operator of the inverse problem [transformation operator of $r(\lambda)$ into $F_{\text{tHb}}$] we used the polynomial regression of the form:

$$\ln F_{\text{tHb}} = a_{00} + \sum_{n=1}^{N_\lambda} \sum_{m=1}^{6} a_{nm}[r(\lambda_n)]^m. \qquad (12)$$

The optimal set of $\lambda_n$ in equation (12) depends on the $F_{\text{tHb}}$ retrieval accuracy and stability of this equation to random deviations $\delta r$ of the corresponding spectral values of $r(\lambda_n)$. Influence of $\delta r$ on the result of the $F_{\text{tHb}}$ retrieval is evaluated on a model ensemble of realisations of $F_{\text{tHb}}$ and $r(\lambda_n)$ by performing a closed numerical experiment in which for all realisations of $r(\lambda_n)$ $F_{\text{tHb}}$ is calculated using (12) with the superimposition of random deviations in within $\delta r = 5\%$ on $r(\lambda_n)$. After the search through all the realisations we calculated the ensemble-averaged error of the $F_{\text{tHb}}$ retrieval, which serves as a criterion for selecting the optimum combination of $\lambda_n$. For the considered set of 26 wavelengths, such a combination is $\lambda_1 = 480$ nm, $\lambda_2 = 574$ nm, $\lambda_3 = 586$ nm and $\lambda_4 = 630$ nm. The corresponding error of the $F_{\text{tHb}}$ retrieval is 9.2% and 10.8% at $\delta r = 1\%$ and 5%, respectively. The regression coefficients (12) corresponding to the selected wavelengths are shown in Table 1.

**Table 1.** Regression coefficients $a_{nm}$ between $\ln F_{\text{tHb}}$ and $r(\lambda_n)$ ($a_{00}$ = –4.1742).

| $n$ | $m$ | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| 1 | 2.1791 | 0.3204 | 0.0183 |
| 2 | –7.5811 | –1.2871 | –0.0677 |
| 3 | 4.6361 | 1.1887 | 0.0706 |
| 4 | –3.1531 | –1.5916 | –0.1688 |

The presented results are the basis of a simple method for determining the haemoglobin concentration in tissues of a human body. The method is based on measuring the signals of light (at wavelengths of 480, 574, 586 and 630 nm) reflected by the tissue at two different distances from the illumination spot and on the use of multiple regression (12) between the spectral values of the difference signals and the haemoglobin concentration.

Thus, regression relationships between the biophysical parameters of the tissue and spectral and spatial characteristics of the DR make it possible to process in real-time the experimental data, and thus to conduct an online diagnosis of the mucous membranes directly during their endoscopy. Knowledge of biophysical parameters of a suspicious neoplasm and the surrounding normal tissue may be helpful to improve the diagnosis of a malignant process and to determine its prevalence and severity, which in turn will reduce the use of invasive biopsy and its cost.

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