ORIGINAL ARTICLES

APPLICATION OF THE KUBELKA-MUNK MODEL FOR FAST INTRAOPERATIVE ANALYSIS OF INTESTINAL OPTICAL PROPERTIES USING A FIBER OPTIC SPECTROMETER

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Abstract

Intraoperative determination of optical properties of biological tissues is an important task of medical physics, in particular, for photodynamic therapy, because it allows personalizing photodynamic treatment by accurately calculating the required light dose. In this work, we propose a new approach to simultaneous measurement of diffuse reflectance and transmission spectra of the colon wall tissue during intestinal anastomosis, based on the use of two fiber-optic devices to deliver broadband radiation both from the side of the intestinal lumen and from the outer side of the intestinal wall, from which diffuse scattered light is also recorded. To restore the optical properties of these tissues it was proposed to use the Kubelka-Munk model with transformation of the optical parameters of the model into the optical parameters of the diffusion approximation theory with custom equations based on the results of numerical modeling. The proposed approach have been tested *ex vivo* on the biological samples of GIT tissues, allowing us to conclude on its applicability in clinical conditions.

Key words: optical spectroscopy, optical properties, light transport model, gastrointestinal tract.

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ПРИМЕНЕНИЕ МОДЕЛИ КУБЕЛКИ-МУНКА ДЛЯ БЫСТРОГО ИНТРАОПЕРАЦИОННОГО АНАЛИЗА ОПТИЧЕСКИХ СВОЙСТВ СТЕНКИ КИШЕЧНИКА С ПОМОЩЬЮ ОПТОВОЛОКОННОГО СПЕКТРОМЕТРА

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Резюме

Интраоперационное определение оптических свойств биологических тканей является важной задачей медицинской физики, в частности, при планировании фотодинамической терапии, поскольку позволяет персонализировать фотодинамическое воздействие за счет точного расчета необходимой световой дозы. В данной работе предложен новый подход к одновременному измерению спектров диффузного отражения и пропускания света тканью стенки толстой кишки при формировании кишечного анастомоза, основанный на использовании двух волоконно-оптических устройств для подачи широкополосного излучения как со стороны просвета кишки, так и с наружной стороны кишечной стенки, с которой также регистрируется диффузно рассеянный свет. Для восстановления оптических свойств этих тканей предложено использовать модель Кубелки-Мунка с преобразованием оптических параметров модели в оптические параметры теории диффузионного приближения с использованием собственных уравнений на основе результатов численного моделирования. Предложенный подход апробирован *ex vivo* на биологических образцах тканей желудочно-кишечного тракта, что позволяет сделать вывод о его применимости в клинических условиях.

Ключевые слова: оптическая спектроскопия, оптические свойства, теория переноса излучения, желудочно-кишечный тракт.

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Introduction

Intraoperative determination of optical properties is an important task of medical physics, in particular, for photodynamic therapy [1]. Optical properties also influence the interpretation of the results of diagnostic methods [2]. In connection with the development and widespread use of phototheranostics methods using laser and optical technologies in surgery and therapy, various instrumental and algorithmic approaches are proposed to study the propagation of light in biological tissues, whose complex structure and the natural variability of properties make this task non-trivial. In particular, the effectiveness of photodynamic therapy (PDT) largely depends on the content of the photosensitizer and the distribution of light in the irradiated tissue, and an accurate assessment of the radiation dose absorbed by the tissue makes it possible to determine the mode of photodynamic exposure.

From the point of view of light propagation, biological tissues of the human body are turbid media, i.e. heterogeneous structures with spatial fluctuations in optical properties. These fluctuations and their density cause strong scattering of light by biological tissue. It is important to take into account the effects of multiple scattering which complicate the direct application of electromagnetic theory. Therefore, to describe the propagation of light in such media, the radiation transfer theory (RTT) is used, which ignores some of the wave properties of light, such as interference and polarization, considering only the transfer of light energy in the medium. However, the model implicitly takes into account the wave properties of light due to the optical properties of the tissue included in the basic equation. These properties represent a generalization of optical parameters to an ensemble of particles and were obtained by solving the Maxwell equations proposed by Gustav Mie in 1908. For this purpose optical characteristics averaged over a certain elementary volume are used, such as the absorption coefficient (μ_a), which is the inverse of the photon's free path before the act of absorption, the scattering coefficient (μ_s), which is the inverse of the photon's free path between scattering events, and the anisotropy coefficient (g), which represents the average cosine of the scattering angle. For media characterized by anisotropic scattering, the so-called reduced scattering coefficient ($\mu'_s = \mu_s (1-g)$) is introduced. This coefficient takes into account how many more collisions must occur in an anisotropic medium compared to an isotropic one in order for the light to turn at the same angle as in an isotropic medium. A significant simplification of the theory of radiation transfer is the equation for a stationary state in which the intensity at any point of the medium does not change with time. The diffusion approximation of the RTT also proves useful in the case of small values of the single scattering anisotropy factor and large albedos (close to one). For most biological tissues, the anisotropy coefficient ranges from 0.6 to 0.9, and for blood it reaches 0.995, which significantly limits the applicability of the diffusion approximation [3].

To numerically solve the RTT equation, the Monte Carlo numerical simulation method based on the simulation of photon transport in a scattering medium is widely used. The initial conditions are set and each act of photon scattering on inhomogeneities of the medium is traced until its absorption or exit from the sample. The main disadvantage of the Monte Carlo method is the large expenditure of computer time due to the need of solving the inverse problem consisting of the selection of values of optical properties that correspond to the measured values of transmission and reflection, which complicates the intraoperative determination of the optical properties of tissues. To solve the inverse problem, it is necessary to run the simulation several times for a given set of optical properties; in this case, the search for initial values, optimization method and stopping criteria is also non-trivial [4].

One of the common methods for solving the RTT equation is the Kubelka-Munk method, which has already been used to analyze the optical properties of intestinal tissues [5]. However, in that work, bloodless samples were studied, measured within 2 hours after resection of human small intestine tissue. In work [6], the optical properties of normal and adenomatous human colon tissues were obtained by the method of two integrating spheres in vitro. The paper [7] presents the optical characteristics of the mucosa and submucosa of human colon tissue obtained in vitro on a spectrophotometer with an integrating sphere LAMBDA 950. The authors of the work [8] in order to assess the kinetics of the optical properties of biological tissues under conditions of optical clearing with glycerol, proposed a simple and indirect method , based on the collimated transmittance (Tc) and thickness d(t) which was measured during processing. The Tc of the sample depends on both the thickness of the sample (d) and the light attenuation coefficient, μt. Assuming that the absorption coefficient remains constant throughout the process, they derive the scattering coefficient from Tc.

As can be seen from the works mentioned above, measurements with two integrating spheres make it possible to determine the optical properties of samples in vitro and ex vivo, but they seem too cumbersome to be used during surgery. At the same time, it is important to know the optical properties of tissues under light-induced exposure directly at the moment of exposure, not for a similar type of tissue sample measured in the laboratory, since the state of the tissue is variable and critically important for planning and monitoring photodynamic therapy. Therefore, in this work it is proposed to use as measured parameters the values of diffuse transmission and reflection obtained by measuring with a fiber with a given aperture in soft contact with the surface of a biological object. The inevitable decrease in the accuracy of the model in this case is a payment for the ability to analyze optical properties in real time. The purpose of this study was to analyze the possibility of intraoperative assessment of the optical properties of intestinal wall tissues in both diffusely reflected and diffusely transmitted light.

Materials and Methods

Spectroscopic setup for in vivo measurements

The experimental setup (Fig. 1) consisted of a white light source, a spectrum analyzer with a fiber-optic input (LESA-01-BIOSPEC), optical fibers and a personal computer for recording measurements. The diffuse reflection and transmission spectra were recorded in the spectral range of 500-600 nm. An LED lamp with a fiber-optic output was used as a broadband radiation source. The power of the light source is adjustable up to 1 W, the color temperature is 5000K-6500K. The spectrum of the light source in the range of 500-600 nm has sufficient uniformity for analysis. To calculate the diffuse reflectance coefficient $R_d = I_r/I_0$ and the diffuse transmission coefficient $T_d = I_t/I_0$ measurements of the diffuse reflection and transmission spectra were carried out relative to a standard sample ($BaSO_4$) with a reflection coefficient close to unity in the selected spectral range.

Two measurement geometries were used for illumination and registration of radiation: in diffusely reflected (green arrows on Fig. 1) and transmitted light (purple arrows on Fig. 1), therefore, two fiber-optic devices were used as previously for hemoglobin oxygen saturation analysis of colonic wall [10]. A fiber optic diffuser placed inside the Foley catheter was used to uniformly illuminate the intestinal wall from the inside in order to measure the transmitted light passing through the intestinal wall to the outside. The Foley catheter was filled with a fat emulsion (Intralid MCT/LST 10%) diluted to 1% with NaCl 0.9% to ensure a uniform scattering phase function. The length of the diffuser was 10 mm.

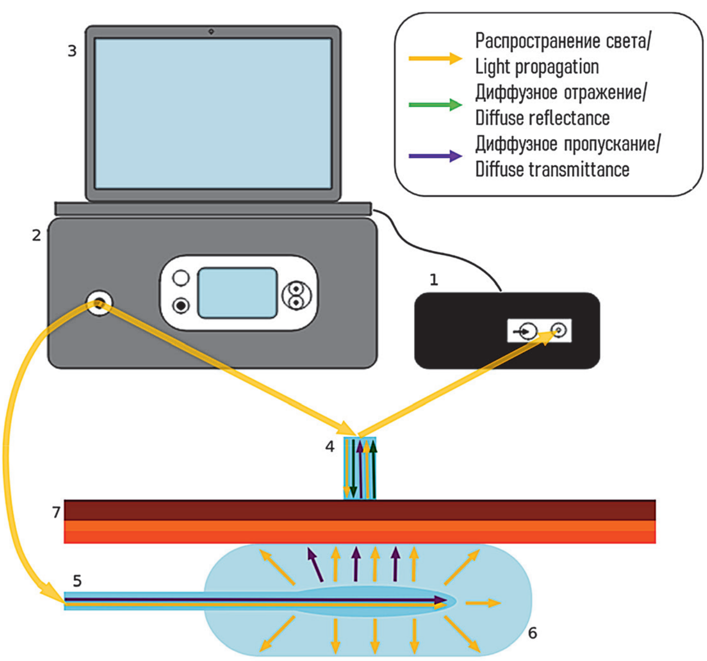

Рис. 1. Схема спектроскопической установки. В состав оборудования входят: 1 – спектрометр «ЛЭСА-01-БИОСПЕК», 2 – широкополосный источник излучения, 3 – компьютер с программой «Uno Momento», 4 – Y-образный оптический световод, 5 – волоконно-оптический диффузор, 6 – катетер Фолея. Исследуемая ткань – стенка кишечника (7).

Fig. 1. Scheme of the spectroscopic setup. The equipment includes: 1 – a "LESA-01-BIOSPEC" spectrometer, 2 – a broadband radiation source, 3 – a computer with the program "Uno Momento", 4 – a y-shaped optical fiber, 5 – fiber-optic diffuser, 6 – Foley catheter. The investigated tissue is the intestinal wall (7).

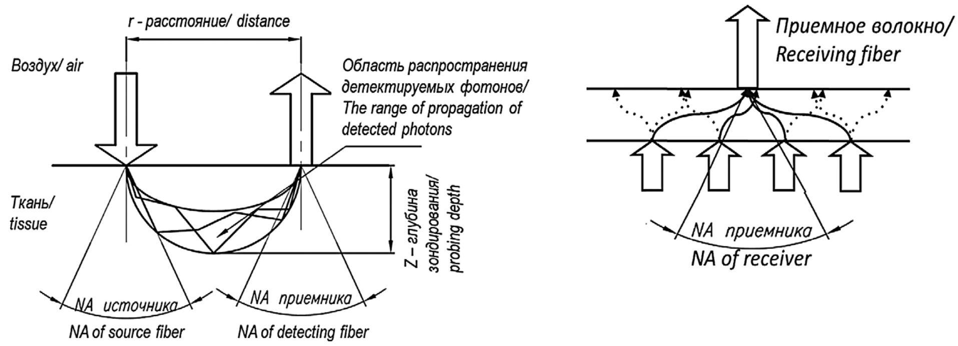

Рис. 2. Две конфигурации источника и приемника при проведении численного эксперимента.
Fig. 2. Two configurations of the source and receiver when conducting a numerical experiment.

The receiving fibers at the entrance to the spectrometer form a line that serves as the entrance slit of the monochromator, hence they are located perpendicular to the diffraction plane of the dispersing element. The diameter of each fiber is 125 microns, which determines the distance between the centers of closely spaced fibers. The diameter of each fiber including the sheath is 250 microns. The numerical aperture of each fiber is 0.22. The measurements were carried out using an optical fiber with an aperture of 0.22 in contact with biological tissues for both illumination and registration of diffuse reflection spectra and transmission spectra. The light detected in this way is the part of the diffusely scattered light, which can be measured using an integrating sphere. A hypothetical receiving fiber with a numerical aperture of NA = 1 would provide detection of light scattered by tissue at any angle, which can be compared to the total diffuse reflectance signal recorded using an integrating sphere. To estimate the proportion of light received by a real optical fiber with a numerical aperture different from 1, numerical modeling of light propagation in a multilayer medium was performed, the optical parameters of samples corresponded to the optical properties of the intestinal wall layers according to works [6-9].

Monte Carlo simulation of light propagation in tissues

In our work, numerical simulation using the Monte Carlo method was used to model the trajectories of photons. Its acceptability is based on the assumption of a large number of acts of light scattering on fluctuations of the refractive index, which makes it possible to neglect the consideration of wave effects. Boundary conditions were set and every act of photon scattering on inhomogeneities of the medium was monitored, up to its absorption or exit from the sample. The value of free path length and the angle of rotation, which are random variables distributed according to a certain law, were determined using the coefficients g, μ_s and μ_a. The Henyey-Greenstein (HG) scattering phase function was used to determine the scattering angle. Reflection, refraction, and total internal reflection were taken into account at the boundaries of the media. The receiver with a given numerical aperture was positioned relative to the sample both from the source side (to take into account diffusely reflected photons) and from the opposite side (to account for transmitted photons) (Fig.2).

The optical properties of the intestinal wall layers were used as input parameters for modeling according to works [6-9] (Table 1). The method was implemented using the C# language in the Visual Studio 2019

Таблица 1.
Оптические свойства исследуемых тканей стенки кишечника как входные данные для моделирования распространения света методом Монте-Карло, литературные данные [6-9]

Table 1.
Optical properties of the studied intestinal wall tissues as input data for modeling the propagation of light by the Monte Carlo method, literature data from [6-9]

Характеристики Characteristics	Слой Layer		
	Мышечный Muscular	Подслизистый Submucosal	Слизистый Mucosal
Коэффициент поглощения Absorption coefficient μ_a	6.5 cm^{-1}	1 cm^{-1}	3.5 cm^{-1}
Коэффициент рассеяния Scattering coefficient μ_s	100 cm^{-1}	100 cm^{-1}	300 cm^{-1}
Показатель анизотропии Anisotropy factor g	0.89	0.9	0.9
Коэффициент преломления Refractive index n	1.36	1.36	1.38
Толщина Thickness d	1 – 3 mm	0.2 mm	0.8 mm

Таблица 2.
Содержание цельной крови в оптических фантомах и соответствующие значения коэффициента поглощения на длинах волн 542, 560, 576 нм

Table 2.
The content of whole blood in optical phantoms and the corresponding absorption coefficient values at wavelengths of 542, 560, 576 nm

Коэффициент поглощения μ_a (см$^{-1}$) на длине волны / Absorption coefficient μa (cm^{-1}) at wavelength	Содержание крови в ткани (% или мл/100 г) / Blood in tissue (% or ml/100 g)					
	20	8	4	2	1	0,5
542 nm	53,29	21,32	10,66	5,33	2,67	1,33
560 nm	32,61	13,05	6,52	3,26	1,63	0,82
576 nm	55,35	22,14	11,07	5,55	2,77	1,38

development environment based on the algorithm proposed in [11].

Principle of customization of Kubelka-Munk model

As a rule, during studying of biological tissues in a living organism, the illuminator and light receiver are located on the one side of the sample. Therefore, in most cases, in vivo spectral measurements of biological tissues are performed in diffuse reflectance geometry. Considering the heterogeneity of biological tissues and variations in their physiological characteristics, in the general case the problem of restoring their optical properties does not have a strict solution.

To restore all three optical properties (absorption and scattering coefficients and anisotropy coefficient), it is necessary to measure any three of the following five parameters: total (or diffuse) transmission, total (or diffuse) reflection, absorption of a sample placed in an integrating sphere, collimated transmission coefficient for non-scattered light, angular distribution of light scattered by the sample [3]. Many methods for estimating optical parameters of tissue are also based on a combination of several diffuse reflection measurements with spatial resolution, for example, at several distances from the source.

If in considered case it is enough to determine the absorption coefficient and the reduced scattering coefficient (without decomposing it into the anisotropy factor and the scattering coefficient), then it is enough to measure two of the parameters mentioned above. This paper proposes a method for measuring diffusely scattered light in reflected and transmitted geometry with subsequent restoration of information about the optical properties of an object using the two-stream Kubelka-Munk model with empirically determined conversion coefficients from the optical parameters of the Kubelka-Munk model to the optical parameters of the RTT.

The Kubelka-Munk method is widely used to separate light attenuation into contributions due to absorption and scattering based on measured values of total diffuse reflectance R_d, diffuse transmittance T_d, and collimated transmittance T_c in turbid media such as biological tissues [12]. This method is based on considering two radiation fluxes propagating inside the tissue: one flow is in the direction of the incident beam L_1, and the other is in the backscattering direction L_2, which can be described using the following equations [13, 14]:

$$\frac{\partial L_1}{\partial z} = -S \cdot L_1 - A \cdot L_1 + S \cdot L_2, \quad (1)$$

$$\frac{\partial L_2}{\partial z} = -S \cdot L_2 - A \cdot L_2 + S \cdot L_1, \quad (2)$$

where S and K are special parameters of the Kubelka-Munk model describing scattering and absorption, which can be calculated as:

$$S = \frac{ln\left(\frac{1-R_d\left(a-b\right)}{T_d}\right)}{b \cdot d}, \quad (3)$$

$$A = S\left(a-1\right), \quad (4)$$

where Rd is the diffuse reflectance coefficient, Td is the diffuse transmittance coefficient, d is the layer thickness measured in cm, a and b are coefficients defined as:

$$a = \frac{1-T_d^2+R_d^2}{2R_d}, \quad (5)$$

$$b^2 = a^2 - 1. \quad (6)$$

In this case, it is possible to associate these absorption (A) and scattering (S) parameters of the model with the absorption (μa) and reduced scattering (μ's) coefficients used in the radiation transfer theory, in accordance with the following dependencies [15]:

$$A = 2\mu_a, \quad (7)$$

$$S = \frac{3}{4}\mu_s' - \frac{1}{4}\mu_a. \quad (8)$$

Expression (8) for the scattering parameter in the Kubelka-Munk model through the optical parameters of the RTT has a physical meaning only in the case of an absorption coefficient less than a threefold reduced scattering coefficient. Our work proposes alternative conversion formulas based on the results of numerical modeling of light propagation in a medium with optical parameters corresponding to ones of the intestinal wall. These conversion formulas were tested on optical phantoms, the optical properties of which are relevant to the intestinal wall.

Therefore, knowing T_d and R_d, it is possible to calculate which optical parameters (μ_a and μ'_s) correspond to values of the observed transmittance and reflection coefficients in scattered light through the scattering and absorption parameters of the Kubelka-Munk model (A and S).

Empirical connections of the results of the Kubelka-Munk method and radiative transfer coefficients were used to derive the relationships between them. From the measured total diffuse reflectance (R_d) and diffuse transmittance (T_d), the relation between Kubelka-Munk coefficients and the radiation transfer coefficients of the tissue phantom (μ_a and μ'_s) were calculated.

Fig. 3 schematically shows the empirical relationship between the results of the Kubelka-Munk method and radiative transfer coefficients.

Biological samples

Clinical testing was carried out *ex vivo* on a specimen of intestine obtained as a result of surgical operation of gastrointestinal tumors on the basis of the University Clinical Hospital no. 1 of the I.M. Sechenov First Moscow State Medical University. The reflectance and transmittance spectra of specimen were registered at a distance of + and - 5 cm from the tumor. The thickness of the sample was measured using a caliper.

Results and discussion

The result of using the Monte Carlo method is R_d and T_d values at different concentrations of fat emulsion and blood for each wavelength. The simulation was carried out with values similar to those that were obtained in the experiment (the values of diffuse reflectance and diffuse transmission) for the optical parameters from Table 2. Based on the obtained values of transmission and reflectance, the dependences of the Kubelka-Munk coefficients were calculated at three different wavelengths: 542 nm, 560 nm and 576 nm. Primarily, the dependences of the obtained Kubelka-Munk coefficients for absorption A and scattering S on the known values of the reduced scattering coefficient μ'_s and the absorption coefficient μ_a were calculated.

Fig. 4 shows, as an example, the calculated dependences for a wavelength of 560 nm and an anisotropy factor of

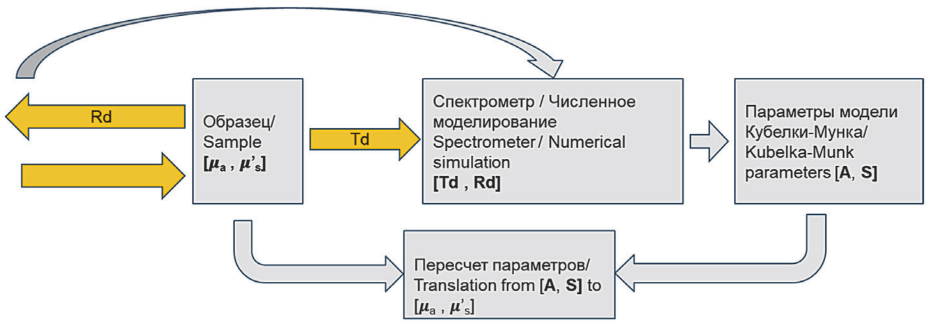

Рис. 3. Схема построения формул для пересчета параметров модели Кубелки-Мунка и оптических параметров теории переноса излучения.

Fig. 3. Scheme for constructing formulas for recalculating the parameters of the Kubelka-Munk model and the optical parameters of the radiation transfer theory.

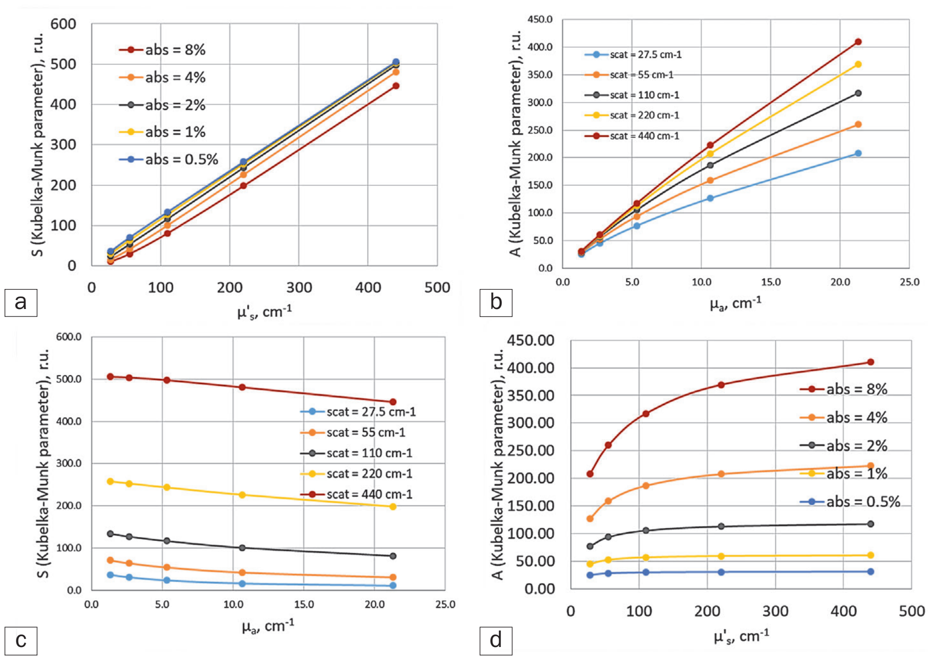

Рис. 4. Зависимости параметров модели Кубелки-Мунка от значений μ'_s и μ_a для длины волны 560 нм: a – взаимная зависимость двух коэффициентов рассеяния; b – взаимная зависимость двух коэффициентов поглощения; c – зависимость параметра рассеяния модели Кубелки-Мунка от коэффициента поглощения; d – зависимость параметра поглощения модели Кубелки-Мунка от приведенного коэффициента рассеяния.

Fig. 4. Dependences of the parameters of the Kubelka-Munk model on the values of μ'_s and μ_a for a wavelength of 560 nm: a – mutual dependence of two scattering coefficients; b – mutual dependence of two absorption coefficients; c – dependence of the scattering parameter of the Kubelka-Munk model on the absorption coefficient; d – dependence of the absorption parameter of the Kubelka-Munk model on the reduced scattering coefficient.

0.71: scattering S from the reduced scattering coefficient μ_s', scattering S from the absorption coefficient μ_a, absorption A from the reduced scattering coefficient μ_s' and absorption A from the absorption coefficient μ_a.

These results show us the linear dependences of the scattering parameter S of the Kubelka-Munk model on the reduced scattering coefficient μ_s' and the absorption parameter A of the Kubelka-Munk model on the absorption coefficient μ_a, which generally corresponds to the most commonly used conversion formulas (7) and (8). But in the range of optical properties, we are interested in, the other conversion formulas are needed for our ratio of parameters and geometry of measurements.

As a first approximation, we propose the following type of dependence for the parameters under consideration:

$$A(\mu_a.\mu_s') = k(\mu_s')*\mu_a + l, \quad (9)$$

$$S(\mu_a.\mu_s') = m*\mu_s' + n(\mu_a). \quad (10)$$

This type of dependence (9) is due to the fact that, as we see in Figure 3, the slope of the mutual absorption dependence depends on the scattering, and not the shift (Figure 3 (b)). For cross-scattering dependence (10), we observe, on the contrary, that its shift depends on absorption rather than its slope (Figure 3 (a)). For example, for a wavelength of 560 nm, reciprocal terms can be neglected due to the smallness of the coefficients in front of them, in which case more direct relationships will remain:

$$A(\mu_a) = 15.8\mu_a + 35.6, \quad (11)$$

$$S(\mu_a.\mu_s') = 1.2*\mu_s' - \mu_a - 14.4. \quad (12)$$

This simplicity of the dependencies allows us to reverse them in order to obtain recalculation formulas for solving the inverse problem - restoring optical properties from known transmittance and reflectance through the Kubelka-Munk parameters:

$$\mu_a(A) = (A - l)/k, \quad (11)$$

$$\mu_s'(A,S) = (S + n1(A - l)/k + n0)/m. \quad (12)$$

On Fig. 5 we can see the example of restoration of optical properties which were used as input parameters for numerical simulation. We see a tendency for the absorption values to be overestimated at higher scattering coefficients, however, this is a second-order effect that we cannot eliminate using the fast linear model. However, the scattering coefficient is recovered with this approximation with a fairly high accuracy. The problem with the upward

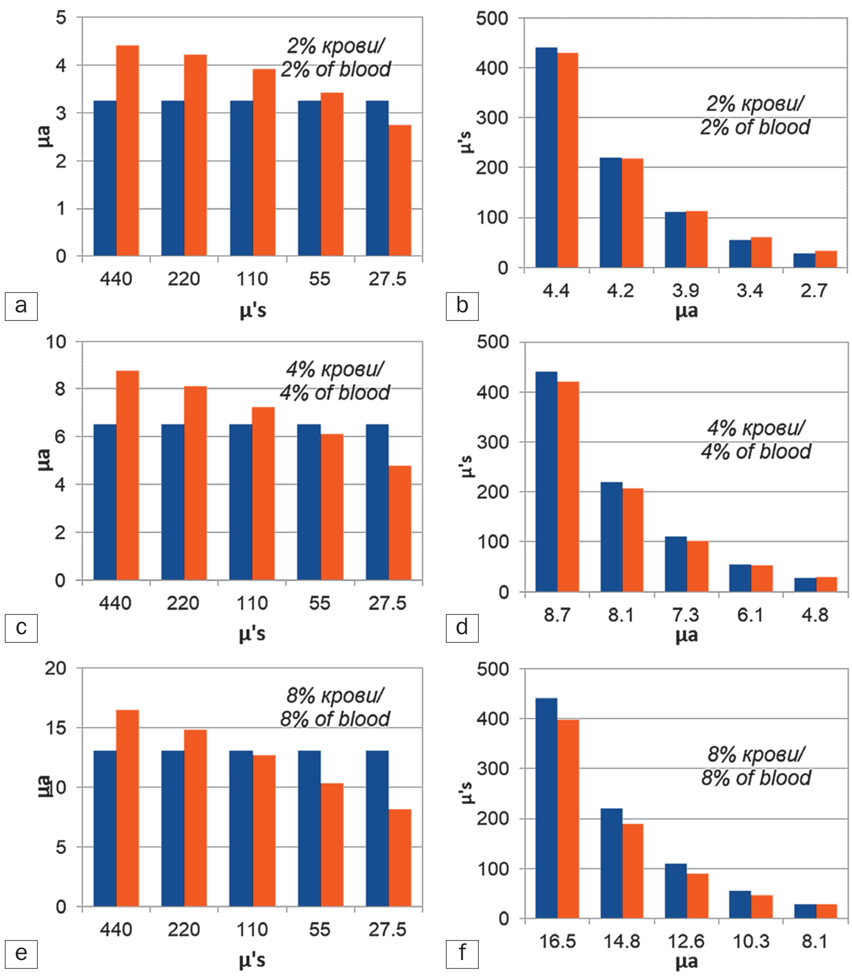

Рис. 5. Результаты восстановления оптических свойств оптических фантомов, содержащих рассеивающие среды (жировую эмульсию) и кровь для 560 нм (красным отмечены восстановленные значения, синим – заданные): a – восстановление μ_a при 2% крови в образце; b – восстановление μ_s' при 2% крови в образце; c – восстановление μ_a при 4% крови в образце; d – восстановление μ_s' при 4% крови в образце; e – восстановление μ_a при 8% крови в образце; f – восстановление μ_s' при 8% крови в образце.

Fig. 5. Results of restoration of optical properties of optical phantoms containing scattering media (fat emulsion) and blood for 560 nm (recovered values are marked in red, specified values are marked in blue): a – μ_a recovery at 2% blood in sample; b – μ_s' recovery at 2% blood in sample; c – μ_a recovery at 4% blood in sample; d – μ_s' recovery at 4% blood in sample; e – μ_a recovery at 8% blood in sample; f – μ_s' recovery at 8% blood in sample.

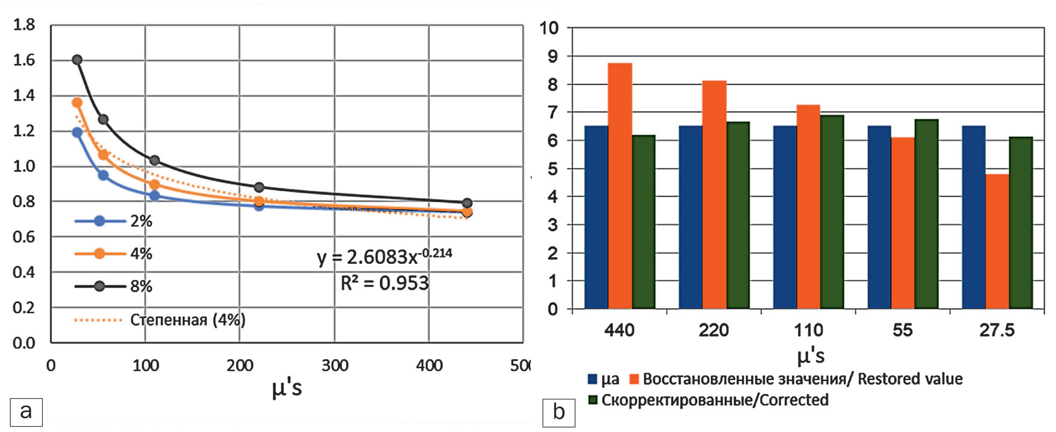

Рис. 6. Зависимость поправочного коэффициента поглощения от рассеяния: a – аналитическое выражение поправочного коэффициента; b – результат применения поправочного коэффициента.
Fig. 6. An absorption correction factor dependence on scattering: a – the analytic expression of correction factor; b – the result of implication of correction factor.

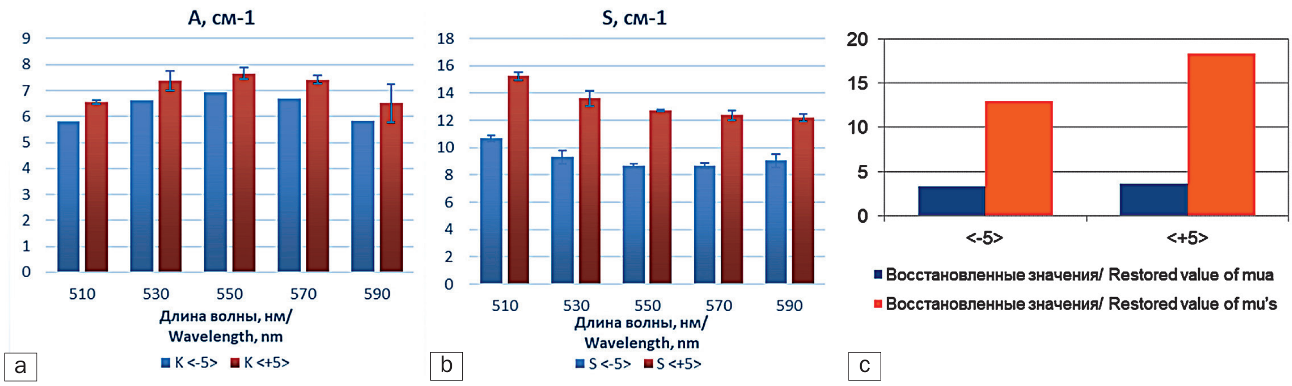

Рис. 7. Клинические испытания модифицированной модели Кубелки-Мунка применительно к восстановлению оптических свойств: a – коэффициент поглощения модели Кубелки-Мунка; b – коэффициент рассеяния модели Кубелки-Мунка; c – восстановленные по значениям коэффициентов модели Кубелки-Мунка оптические свойства тканей.
Fig. 7. Clinical testing of modified Kubelka-Munk model applied to restoration of optical properties: a – absorption coefficient of the Kubelka-Munk model; b – scattering coefficient of the Kubelka-Munk model; c – optical properties of tissues reconstructed from the values of the coefficients of the Kubelka-Munk model.

trend of the reconstructed absorption coefficient can be circumvented either by complicating the algorithm for recalculating the Kubelka-Munk parameters into optical properties according to the RTT, or by introducing a correction factor that takes into account the reconstructed value of the scattering coefficient (Fig. 6).

Results of *ex vivo* colonic wall (thickness 0.2 cm) optical properties assessment with customized formulas are presented on the Fig. 7.

Conclusion

An approach has been proposed for measuring the spectra of diffuse reflection and diffuse transmission of light through the wall of hollow organs intraoperatively. An algorithm for reconstructing the values of the optical properties of the intestinal wall from the measured spectral dependences is proposed, based on the two-stream Kubelka-Munk model with customized formulas for converting into optical properties based on numerical modeling. The method was tested in a clinical setting, which showed the promise of its use for personalizing the calculation of the dose of light exposure in laser-induced methods of therapy.

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