Mathematical modelling of signals recorded in noninvasive medical laser fluorescence diagnosis

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Based on the Kinkelbach–Munk two-flux model modified by the authors, which makes it possible in one-dimensional problems to obtain exact analytical expressions for radiation fluxes at the boundary of a turbid medium, and Kokhanovsky’s solution for the radiation flux of fluorescence, questions are considered of modelling the spectrum of stimulated endogenous fluorescence of biological tissues as applied to problems of noninvasive medical diagnosis. An analytical expression is presented for the spectral distortion function, which depends on the scattering and absorption properties of cellular biological tissues and blood. It is shown that the model spectra agree well with the experimental data. © 2013 Optical Society of America.

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INTRODUCTION

Noninvasive in vivo, in situ laser fluorescence diagnosis (LFD) appeared as a new scientific diagnostic specialization in medicine around the mid-1980s. Today many scientific groups worldwide are undertaking intense attempts to develop LFD now at the level of practical health maintenance, including the creation of systems to visualize the internal structures of biological tissues, using the radiation of stimulated endogenous fluorescence (autofluorescence) of biological tissues, and to visualize the structure of so-called diffusion fluorescence tomographs based on this. However, a number of problems associated with the accuracy of such measurements, the quantitative determination of the concentration of active luminophores in the examination zone from the fluorescence intensity, the interpretation of the results of the total fluorescence spectra, etc., do not yet make it fully possible to implement these plans. One serious problem in LFD is the problem of allowing for distortions of the fluorescence spectra when the in vivo spectrum recorded by the diagnostic device does not always correspond to the original fluorescence spectrum of the endogenous luminophores. In the overwhelming majority of actual cases, such a situation arises because the tissue contains, besides the light-emitting luminophores, an even greater amount of substances that intensively absorb and scatter the light (for example, melanin in skin, hemoglobin in blood, etc.).

A number of authors have repeatedly attempted to construct theoretical models based on linear transport theory (TT) to calculate the spectra of endogenous fluorescence of biological tissues and to develop algorithms for correcting the spectra, taking into account plethora of the tissue and certain other factors (see, for example, Refs. 6–10). In particular, it has been shown that the fluorescence signal recorded in vivo from biological tissue depends in general not only on the fluorescence quantum yield and the concentration of fluorescent substance in the tissue but also has a complex and substantially nonlinear dependence on the overall optophysical properties of the tissue, formulated in terms of TT—the scattering transport coefficient $\mu_s$, the absorption coefficient $\mu_a$, etc. However, until recently, all such work was based on some approximate methods or other of solving the transport problem—the method of moments, the diffusion approximation, simplified heuristic algorithms, or on the basis of the well-known statistical method of random walk of a photon in a medium (the Monte Carlo method). Each of these approaches has definite drawbacks. Heuristic algorithms, as a rule, are applicable only for a chosen design of the diagnostic device. Approximate solutions possess low accuracy, while numerical methods like the Monte Carlo method require extensive calculations and do not provide a solution in the form of a closed analytical expression that could be easily analyzed on the subject of how one parameter or the other affects the final diagnostic result.

Recently A. Kokhanovsky proposed a rigorous analytical method of solving the fluorescence problem for the classical two-flux Kubelka–Munk (KM) approximation. Also, the author showed in Ref. 12 the main source of errors of the KM model and proposed a version of their equation that makes it possible in one-dimensional (1D) problems to obtain today exact values for radiation fluxes at the boundary of a medium (for the fluxes recorded by a device). This opens up prospects for constructing simple analytical real-time algorithms that make it easy to analyze the distortions of the fluorescence spectra without resorting to complex multistep computations and numerical methods. The goal of this article is to use a combination of the modified two-flux KM model and
Kokhanovsky’s solution as a basis to show the possibility of obtaining an exact analytical expression in terms of the 1D model for the distortion function of the fluorescence spectra, as well as to analyze this solution for agreement with typical experimental data.

THEORETICAL FORMULATION AND SOLUTION OF THE PROBLEM

This section discusses the well-known general formulation of the photometric 1D KM problem with fluorescence when the left boundary of the medium (x = 0) is illuminated by an external flux of radiation $\Phi_0$ with wavelength $\lambda_1$, and two oppositely directed fluxes $i(x)$ and $j(x)$ with initial wavelength $\lambda_1$ propagate inside the medium along the $x$ axis. Because light is absorbed and scattered in the medium, these fluxes are partially absorbed, are converted into each other, and also cause stimulated emission of fluorescence of the luminophores found in the medium. This causes additional similar fluorescence fluxes $I(x)$ and $J(x)$ with wavelength $\lambda_2 > \lambda_1$ to be formed in the medium (Fig. 1). Some wave properties of the radiation are neglected in this model because of its very simple photometric (energy) formulation and the one-dimensional calculational scheme.

When diagnostic measurements are made from the front (illuminated) surface of the biological tissue, a device records the output of fluxes $j(0)$ and $J(0)$ from the medium. This will interest us in the process of obtaining exact analytical solutions for them. In such a formulation of the problem, the fluxes $i(x)$, $j(x)$, $I(x)$, and $J(x)$ can be derived from the two connected systems of first-order linear differential equations:

$$\begin{align*}
\frac{dI_{j1}(x)}{dx} &= -\beta_1(\lambda_1)I_{j1}(x) + \beta_2(\lambda_1)J_{j1}(x) \\
\frac{dJ_{j1}(x)}{dx} &= \beta_1(\lambda_1)J_{j1}(x) - \beta_2(\lambda_1)I_{j1}(x)
\end{align*}$$

where we introduce the notation $\phi(\lambda) = (1 - (1 - R(\lambda)) e^{-2\mu_\lambda(\lambda)/\mu_p})/2$.

The mean density $\mu_p$ of inhomogeneities in the medium is determined from the mean number $N$ of inhomogeneities at which light is scattered divided by the thickness $H$ of the section of biological tissue under consideration (Fig. 1):

$$\mu_p = N/H.$$

The systems of Eqs. (1) and (2) are connected via the function $F_{12}(x)$ that describes the formation of the fluorescence radiation inside the medium,

$$F_{12}(x) = \frac{1}{2} [i_{11}(x) + j_{11}(x)]\mu_{af}(\lambda_1)\varphi_{12}\Phi_0,$$

where $\mu_{af}(\lambda_1)$ is the absorption coefficient of the initial radiation by the active luminophor inside the biological tissue at wavelength $\lambda_1$, and $\varphi_{12}$ is the quantum yield of fluorescence at wavelength $\lambda_2$ when the fluorescence is excited by radiation at wavelength $\lambda_1$ (the conversion coefficient of radiation $\lambda_1 \rightarrow \lambda_2$).

The system of Eqs. (1)–(4) with the corresponding very simple boundary conditions [left boundary $(x = 0)$ $i_{11}(0) = \Phi_0$ and $J_{12}(0) = 0$; right boundary $(x = H)$ $j_{11}(H) = J_{12}(H) = 0$] makes it possible to solve analytically the formulated problem for the fluxes $I_{j1}(0)$ and $J_{j1}(0)$.

In the case of the approximation of a semi-infinite medium ($H \rightarrow \infty$), the solution of the system of Eqs. (1) for the sum of fluxes $i_{11}(x)$ and $j_{11}(x)$ can be written in the form

$$i_{11}(x) + j_{11}(x) = \Phi_0(1 + r_{w\lambda}) e^{-\mu_\lambda x},$$

where $\beta_1(\lambda)$ and $\beta_2(\lambda)$ are called, respectively, the attenuation (extinction) coefficient and the radiation-backscattering coefficient. They determine the optical properties of the radiation-propagation medium (the biological tissue). According to Ref. 12, in the modified version, unlike the standard KM model, coefficients $\beta_1(\lambda)$ and $\beta_2(\lambda)$ are fairly complex functions of the absorption coefficients $\mu_\lambda(\lambda)$, the density $\mu_p$ of inhomogeneities in the medium, and the Fresnel reflectance $R(\lambda)$ at the boundaries of the inhomogeneities inside the medium:

$$\begin{align*}
\beta_1 = \omega \mu_\lambda(\lambda) - \mu_p \ln(1 - R(\lambda)) + \mu_p \ln(1 - \omega(\lambda)) + \sqrt{\omega(\lambda)^2 - R(\lambda)^2} e^{-2\mu_\lambda(\lambda)/\mu_p}, \\
\beta_2(\lambda) = R(\lambda)e^{-2\mu_\lambda(\lambda)/\mu_p} \mu_\lambda(\lambda) - \mu_p \ln(1 - R(\lambda)) + \mu_p \ln(1 - \omega(\lambda)) + \sqrt{\omega(\lambda)^2 - R(\lambda)^2} e^{-2\mu_\lambda(\lambda)/\mu_p}.
\end{align*}$$

FIG. 1. Formulation of the problem of radiation propagation in a medium with fluorescence. See text for explanation.
while the solution for \( f_{\lambda 1}(0) \) can be written as

\[
\gamma d \phi_{\gamma a 11}, \tag{6}
\]

where in the solutions we use the notation \( r_{a 0 11} = (\beta_2(\lambda_1))/((\beta_1(\lambda_2) + a_{j1}) \text{ and } \alpha_{11} = \sqrt{\beta_1(\lambda_1) - \beta_2(\lambda_2)}.\)

Making the substitutions (5) \( \rightarrow \) (4) \( \rightarrow \) (2) and solving the system of Eqs. (2) for \( J_{j2}(x) \) by the standard method of differentiation, we get for \( J_{j2}(x) \) the following second-order differential equation:

\[
\frac{d^2 J_{j2}(x)}{dx^2} - \alpha_{j2} \frac{d J_{j2}(x)}{dx} = -(\beta_1(\lambda_2) + \beta_2(\lambda_2)) F(x) - \frac{d F(x)}{dx}. \tag{7}
\]

As shown by Kokhanovsky,\(^{25}\) Eq. (7) is easy to solve analytically for \( J_{j2}(x) \). However, other boundary conditions were used in its solution, since in his problem the medium was simultaneously illuminated at both wavelengths \( \lambda_1 \) and \( \lambda_2 \), and therefore we cannot directly use that solution here. However, in our formulation of the problem and in the case of flux \( J_{j2}(0) \), which comes out of the biological tissue from its front surface, the solution by Kokhanovsky’s method with the boundary conditions indicated above in the approximation of a semi-infinite medium (\( H \rightarrow \infty \)) takes the form

\[
J_{j2}(0) = \Phi_0 \mu_{af}(\lambda_1) \phi_{12} \frac{(1 + r_{a 0 11})(1 + r_{a 0 12})}{2(\alpha_{j1} + \alpha_{j2})}. \tag{8}
\]

The product \( \Phi_0 \mu_{af}(\lambda_1) \phi_{12} \) determines the initial undistorted fluorescence spectrum. Thus, the distortion of the spectrum is determined by coefficient \( \gamma \), equal to

\[
\gamma = \frac{(1 + r_{a 0 11})(1 + r_{a 0 12})}{2(\alpha_{j1} + \alpha_{j2})}. \tag{9}
\]

where \( r_{a 0 12} = (\beta_2(\lambda_2))/((\beta_1(\lambda_2) + \alpha_{j2}) \text{ and } \alpha_{j2} = \sqrt{\beta_1(\lambda_2) - \beta_2(\lambda_2)}.\)

This coefficient is a function of the wavelength of the radiation and depends in a complex way on the optical properties \( \beta_1(\lambda) \) and \( \beta_2(\lambda) \) of the inhomogeneity medium of the radiation, so that there is no simple normalization of \( J_{j2}(0) \) to \( f_{\gamma a 11}(0) \) or \( \Phi_0 \) in Eq. (8) that makes it possible to eliminate it. Only in the ideal case of “grey” biological tissue, if all of its optical properties are independent of wavelength \( (r_{a 0 11} = \text{const} \text{ and } \alpha_{j1} = \text{const}) \), for the two wavelengths \( \lambda_2 \) and \( \lambda_3 \) and two luminophores \( f_1 \) and \( f_2 \) will the ratio of the signals for the undistorted spectra equal the ratio of the signals for the distorted spectra:

\[
\frac{J_{j2}(0)}{J_{j3}(0)} = \frac{\mu_{af 1}(\lambda_1) \phi_{12}}{\mu_{af 2}(\lambda_1) \phi_{13}} \tag{10}
\]

However, in the general case of actual clinical situations, to obtain the original (undistorted) spectrum, the value of \( \gamma \) needs to be accurately known in the entire working range of wavelengths, since the dependence of \( \gamma \) on the optical properties of the medium in principle is not linear and not monotonic.

As an illustration of this last statement, Fig. 2 shows how coefficient \( \gamma \) according to Eq. (9) depends on the density \( \mu_0 \) of the inhomogeneities (scatterers) inside the medium, while Fig. 3 shows how \( \gamma \) depends on the ratio \( \mu_0(\lambda_1)/\mu_0 \), also calculated from Eq. (9), using Eq. (3). Both dependences, as we shall see, have a nonlinear character and are far from obvious \textit{a priori}.

**MODELLING THE EXPERIMENTAL SPECTRA**

The advantages of Eqs. (6) and (8) consist of their obvious analytical form, which makes it easy to calculate the complete fluorescence spectrum and spectrum of radiation backscattered by biological tissue, recorded by the device in the process of diagnosis in the entire range of wavelengths of the recording and excitation of fluorescence. Figure 4 demonstrates such typical experimentally recorded spectra. Spectra that are similar in shape but with weaker resolution in wavelength are shown in Ref. 13. In this sense, they are fairly representative, and therefore we specially chose them as an example, since their interpretation, as we shall see below, can be erroneous. The spectra of Fig. 4 are recorded \textit{in vivo} on the LAKK-M diagnostic complex when fluorescence is excited by the narrow-band UV radiation of an LED with maximum at the line \( \lambda_1 = 365 \text{ nm} \).\(^{24}\) Fluorescence region I
corresponds to the fluorescence of such endogenous luminophores in biological tissue as elastin, collagen, keratin, NADH (nicotinamide), etc. NADH, flavines, and lipofuscin most actively fluoresce in region II. Region III most clearly reflects the presence of protoporphyrin IX, chlorophyll, and a number of other substances in biological tissue by fluorescence.

To equalize the amplitudes of backscattered radiation (the left peak in the graphs) and fluorescence radiation (in the longer-wavelength region), attenuating optical filters made from colored optical glass of type OS-13, KS-15, etc., are used in the optical layout of the LAKK-M diagnostic system to attenuate the recorded backscattered radiation by about a factor of 10^3. Therefore, the graphs of Fig. 4 show reduced amplitudes of the signals of backscattered radiation by comparison with their actual values, and the spectra was theoretically modelled in our paper using Eq. (8) and taking into account the spectral response of the filter that is used. The optical properties of the biological tissue were specified in the modelling by typical values of μ_s(λ), μ_a, and R(λ), taking into account the known spectral data on the optical properties of oxygenated and reduced hemoglobin of the blood. Different possible values of the volume plethora V_b of the tissue and tissue saturation of the oxyhemoglobin S_O2 in the blood were also allowed for. The biological tissue itself was represented as a macrohomogeneous semi-infinite continuous light-scattering and light-absorbing medium–matrix, in which the blood and various luminophores are distributed uniformly over the entire volume in different concentrations.

The results of modelling the experimental spectra shown in Fig. 4 are presented in Fig. 5. Only one principal maximum of protoporphyrin IX in the λ = 640 nm region was modelled for porphyrin. The tissue saturation of oxyhemoglobin is chosen to equal S_O2 = 95% for both cases. The total density of scatterers in the tissue is μ_s = 100 mm^{-1}. R(λ) was calculated for each wavelength as the Fresnel reflection coefficient at the water/air boundary. Both figures demonstrate extremely good qualitative and quantitative coincidence of the spectra for the chosen values of V_b. It is important to point out that the small visible increase of the amplitude in spectrum 2 in Fig. 4 in the λ = 604 nm region can be treated as an increased concentration of fluorescent flavines or lipofuscin in the tissue. Such a treatment is sometimes encountered in certain papers. However, this “increase” is only a consequence of the absorption of the fluorescence emission by the blood. Figure 6 shows model fluorescence spectra of biological tissue for various values of V_b and S_O2 of the blood when there is no change in the luminophore concentration. As the plethora and the tissue saturation of oxyhemoglobin in the blood increase, a distortion of the true spectrum just appears in the form of an overall nonlinear decrease of the amplitude of the recorded signals and the appearance of characteristic hemoglobin dips in the λ = 530–580 nm region.

Sometimes, when there are definite combinations of the emission spectrum of the source, the spectral attenuation function of the optical filter in a device, and the optical properties of a test object, it is possible to observe the appearance of cutouts (notches, gaps) in the backscattering spectrum of the radiation. An example of an experimentally recorded cutout in a spectrum is shown in Fig. 7 for the radiation of a He–Ne laser when an optical cutoff filter made from KS-17 colored optical glass is used in the device. Figure 8 shows a version of a theoretical model problem in which such a cutout also appears.

![FIG. 4. Experimental spectra of endogenous fluorescence, recorded in vivo on the LAKK-M diagnostic complex with fluorescence excited by the narrowband emission of a UV LED with a maximum at the line λ = 365 nm. 1—Rat tail skin, 2—mucous membrane of the human soft palate. I—the region where collagen, elastin, NADH, keratin, etc., fluoresce; II—the region where flavoproteins and lipofuscin fluoresce; III—the region where porphyrins fluoresce.](image)

![FIG. 5. The results of modelling the experimental spectra shown in Fig. 4. Only one principal maximum of protoporphyrin IX was modelled for porphyrin in the region λ = 640 nm, S_O2 = 95%. The total density of scatterers in the tissue was μ_s = 100 mm^{-1}.](image)

![FIG. 6. Dependence of the fluorescence spectra on the volume plethora of the tissue in the examination zone and the degree of tissue saturation of oxyhemoglobin.](image)
according to the results of the computations. Thus, a comparison of the experimental and theoretically computed fluorescence spectra and the spectra of backscattered radiation of the fluorescence-excitation source shows that the proposed model and the algorithm for calculating the spectra can describe the actual situations fairly adequately.

**CONCLUSION**

An analytically rigorous solution of the problem of theoretically modelling the excited endogenous fluorescence of biological tissues as applied to problems of noninvasive medical diagnosis has been proposed in this paper in terms of the two-flux Kubelka–Munk model and Kokhanovsky’s solution for fluorescence radiation. An adequate description of the experimentally recorded in vivo fluorescence spectra of actual biological tissues has been demonstrated. The resulting solution of Eq. (9) for the distortion function of the spectra shows that this function (coefficient $\gamma$) in principle nonlinearly and nonmonotonically depends on the optical properties of the radiation-recording medium. Therefore, in the general case of actual clinical diagnostic examinations for obtaining the initial (undistorted) spectrum, an accurate value of $\gamma$ is necessary in the entire working range of wavelengths for each patient and each of its regions of examination. Accordingly, all studies oriented to the solution of the problem of quantitatively processing fluorescence spectra in practical medicine must today be directed to methods of determining $\gamma$ and the errors of these methods.

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