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Opto-acoustic measurement of the local light absorption coefficient in turbid media: 2. On the possibility of light absorption coefficient measurement in a turbid medium from the amplitude of the opto-acoustic signal

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***Abstract*. The second part of this work describes the experimental technique of measuring the local light absorption in turbid media. The technique is based on the measurement of the amplitude of an opto-acoustic (OA) signal excited in a turbid medium under the condition of one-sided access to the object under study. An OA transducer is developed to perform the proposed measurement procedure. Experiments are conducted for the turbid media with different optical properties (light absorption and reduced scattering coefficients) and for different diameters of the incident laser beam. It is found that the laser beam diameter can be chosen so that the dependences of the measured OA signal amplitude on the light absorption coefficient coincide upon varying the reduced scattering coefficient by more than twice. The obtained numerical and experimental results demonstrate that the OA method is applicable for measuring the local light absorption coefficient in turbid media, for example, in biological tissues.**

Keywords: *laser opto-acoustic method, optical biomedical diagnostics, light absorption coefficient, scattering coefficient, laser fluence.*

1. Introduction

The second part of the work is based on the characteristic properties of the laser fluence distribution in turbid media obtained by the Monte-Carlo method in the first part and is aimed at the development of the OA method for the local light absorption coefficient measurement in such media

The principle of the OA method used to measure the optical properties of strongly scattering media consists in the following. If a short laser pulse is incident on a plane surface of a turbid medium, this leads to a nonuniform and nonstationary heating of the medium and to its subsequent thermal expansion, which results in the excitation of pressure pulses – the so-called OA signals. When the relation $\mu_{\rm eff}c_0\tau_{\rm las} \ll 1$ is fulfilled [c_0 is the sound velocity; $\tau_{\rm las}$ is the laser pulse duration; $\mu_{\rm eff} = (3\mu_{\rm a}\mu_{\rm s}')^{1/2}$ is the effective light attenuation coefficient; $\mu_{\rm a}$ and $\mu_{\rm s}'$ are the light absorption and reduced scattering coefficients, respectively], the temporal profile of the excited OA signals repeats the in-depth distribution of the heat release, i.e. the in-depth laser fluence distribution [1]. Therefore, the OA method allows one to perform the direct measurement of the in-depth distribution of the laser fluence in a turbid medium, revealing its optical inhomogeneity at the same time [2, 3]. This method has another important advantage, namely, the information on the optical properties of the medium is delivered by the acoustic waves, for which the absorption and scattering in biological tissues are much smaller than those for light [4].

The method for determining both the light absorption and scattering coefficients with the help of the temporal profile of the OA signal excited in a turbid medium was proposed in [1]. This method was employed in [2] to measure *in vitro* the optical properties of biological tissues. Unfortunately, the optical coefficients obtained in *in vitro* measurements may be inaccurate due to the loss of blood by the tissue and possible modification of its structure. Moreover, in the forward detection mode used in [1] laser irradiation of the medium and the detection of excited OA signals are performed at the opposite sides of the medium, therefore restricting the applications of this method to *in vitro* studies.

The possibility of measuring the light absorption coefficient by using the temporal profile of OA signals, detected in the backward mode (the OA signals are both excited and detected at the same surface of the medium) was demonstrated in [5] for the case of a uniformly absorbing and nonscattering medium. The advantage of the backward detection mode is the opportunity to perform *in vivo* diagnostics having only a one-side access to the tissue. However, in case of a strongly scattering medium, the use of the backward mode for the OA signal detection to determine the optical absorption coefficient requires special consideration because the OA signal profile is determined, in this case, both by the absorption and scattering coefficients. On the other hand, the amplitude of the OA signal is proportional to the light absorption coefficient. Therefore, the OA tomography (see, for example [6]) and microscopy [7, 8] are based on this fact. For example, the backward detection mode was used for visualisation of blood vessels in mouse and human skin [7, 9]. Evaluation of the light absorption coefficient in some biological tissues from the OA signal amplitude was performed in [2] without rigorous justification of the method.

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The aim of this paper is to obtain experimental confirmation of the applicability of the OA signal detection in the backward mode for the measurement of the local light absorption coefficient in turbid media.

2. Method

Consider a laser beam irradiating the boundary $z = 0$ of a turbid medium from the side of a transparent medium. The laser fluence in the incident beam can be written in the form:

$$I_{\text{ref}}(r_\perp, \tau) = I_0 L(\tau) R(r_\perp), \tag{1}$$

where $R(r_\perp)$ and $L(\tau)$ are the dimensionless distributions of the laser fluence over the transverse coordinate $r_\perp$ and time τ, respectively. For definiteness, the function $R(r_\perp)$ is assumed Gaussian:

$$R(r_\perp) = \exp\left(-\frac{4r_\perp^2}{d^2}\right), \tag{2}$$

where d is the laser beam diameter. The distribution of the laser fluence along the beam axis inside the turbid medium can be written in the form:

$$I(z, r_\perp = 0, \tau) = I(z)L(\tau). \tag{3}$$

In case of an infinitely short laser pulse, $L(\tau) = \tau_{\text{las}}\delta(\tau)$, and the heating of a turbid medium can be considered as instantaneous and the thermal sources as 'frozen'. The profile of the OA signal excited in the turbid medium and detected in the backward mode in a transparent medium can be expressed as [5]:

$$p_0(\tau, r_\perp = 0) = \begin{cases} 0, & \tau<0, \\ R_{\text{ac}}\Gamma\mu_{\text{a}}E_0 \dfrac{I(z = c_{\text{tr}}\tau)}{I_0} = R_{\text{ac}}\Gamma\mu_{\text{a}}E(z = c_{\text{tr}}\tau), & \tau>0, \end{cases} \tag{4}$$

where $R_{\text{ac}} = 2\rho_{\text{tr}}c_{\text{tr}}/(\rho_{\text{tr}}c_{\text{tr}} + \rho_0 c_0)$; ρ_{tr}, c_{tr} and ρ_0, c_0 are the density and sound velocity of the transparent and turbid media, respectively; Γ is the efficiency of the OA conversion; $E_0 = I_0\int_{-\infty}^{\infty} L(\tau)\,\mathrm{d}\tau$ and $E(z) = I(z)\int_{-\infty}^{\infty} L(\tau)\,\mathrm{d}\tau$ are the laser fluence in the incident laser beam and in a turbid medium.

In the case of a short, but finite laser pulse ($\mu_{\text{eff}}c_0\tau_{\text{las}} \ll 1$), it is necessary to take into account the change in the heat release distribution within the laser pulse duration, resulting in spreading of the OA signal front:

$$p(t, r_\perp = 0) = R_{\text{ac}}\Gamma\mu_{\text{a}}\int_0^\infty L(\tau)I[c_{\text{tr}}(t-\tau)]\vartheta(t-\tau)\mathrm{d}\tau, \tag{5}$$

where $\vartheta(\tau)$ is the Heaviside function.

The temporal profile of the acoustic signal described by (5) is shown in Fig. 1 (dashed curve). One can see that the pulse profile in the region $\tau > 0$ repeats with an accuracy to the multipler the in-depth distribution of the heat release. In the case of a wide laser beam, $d/l_{\text{tr}} \gg 1$ ($l_{\text{tr}} = 1/\mu_{\text{s}}'$ is the photon transport mean free path in a turbid medium), the in-depth distribution of laser fluence at the distances $z > (2-3)l_{\text{tr}}$ has the form

$$E \sim \exp(-\mu_{\text{eff}}z). \tag{6}$$

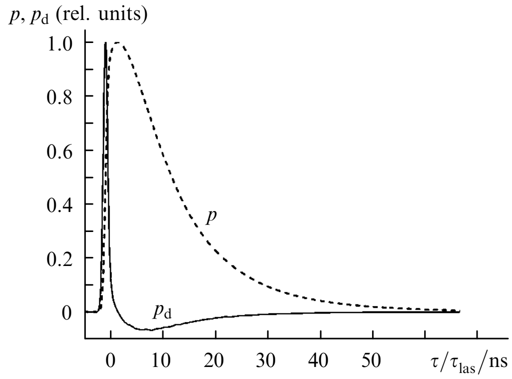


Figure 1. A typical temporal profile of the OA signal excited in a turbid medium and detected in the backward mode by neglecting the diffraction distortions of the signal (dashed curve), and in the case of strong diffraction (solid curve).

Therefore, if the laser beam is wide, the light attenuation coefficient μ_{eff} can be determined by fitting the exponential function to the trailing edge of the OA signal within the range $\tau > (2-3)l_{\text{tr}}/c_0$. However, this procedure is practically difficult to perform in the *in vivo* setting. First, because the reduced light scattering coefficient $\mu_{\text{s}}' \approx 10\ \text{cm}^{-1}$ is typical for most biological tissues in the visible and near-IR range [10], the laser beam diameter must exceed 1 cm in order to provide the one-dimensional regime of light diffusion into the medium. This leads to a substantial decrease in the locality of the measurements of optical properties. Second, if the laser beam is wide, the determination of the light absorption coefficient from the OA signal amplitude is also difficult because the laser fluence amplification coefficient $k = E_{\max}/E_0$ ($E_{\max}$ is the maximum laser fluence in a medium) in the near-surface layer of a turbid media depends on the ratio $\mu_{\text{a}}/\mu_{\text{s}}'$ of optical coefficients and on d/l_{tr} (see Figs 2, 3 in Part 1).

If the laser beam is narrow, i.e. $d/l_{\text{tr}} \lesssim 1$, the coefficient $k \to 1$ and does not depend on the ratio of optical coefficients $\mu_{\text{a}}/\mu_{\text{s}}'$ (see Fig. 2 [11]). However, in the case of a narrow laser beam, strong diffraction of the acoustic waves [12] propagating in a transparent medium leads to suppression of low frequency harmonics of the OA signal and, consequently, to the initiation of the rarefaction part in the OA signal profile [2, 13]. If the signal is strongly diffracted, i.e. $\omega_{\text{d}} \gg \omega_{\text{a}}$ ($\omega_{\text{d}} = 8Lc_{\text{tr}}/d^2$ is the acoustic wave frequency at which the diffraction length is equal to medium depth L, $\omega_{\text{a}} = \mu_{\text{eff}}c_0$ is the characteristic frequency of the OA signal spectrum), the OA signal temporal profile can be represented as [13]

$$p_{\text{d}}(t, r_\perp = 0) = \frac{d^2}{8c_{\text{tr}}L}\frac{\partial p}{\partial t} = \frac{d^2}{8c_{\text{tr}}L}R_{\text{ac}}\Gamma\mu_{\text{a}} \times\left[\frac{I_0}{E_0}E(z=0)L(t) + \frac{\partial E(c_{\text{tr}}t)}{\partial t}\vartheta(t)\right] \tag{7}$$

and is the temporal derivative of (5). The normalised temporal profile of $p_{\text{d}}(\tau)$ is shown in Fig. 1 by a solid curve. The first term of (7) repeats the laser pulse temporal profile and has a maximum at $t = 0$. Because the laser fluence $E(z)$ inside a turbid medium is a smoothly varying function, the second term of (7) is many times smaller than the first one.

Therefore, if the laser beam is narrow, the OA signal amplitude is proportional to the maximum value of the absorbed laser energy density $\mu_a E_{max}$ in the medium which depends linearly on the light absorption coefficient μ_a and is almost independent of the reduced scattering coefficient μ_s' (see Fig. 4c in [11]).

Thus, the local light absorption coefficient can be measured from the amplitude of the OA signal excited by the narrow laser beam, $d/l_{tr} \lesssim 1$, in a turbid medium.

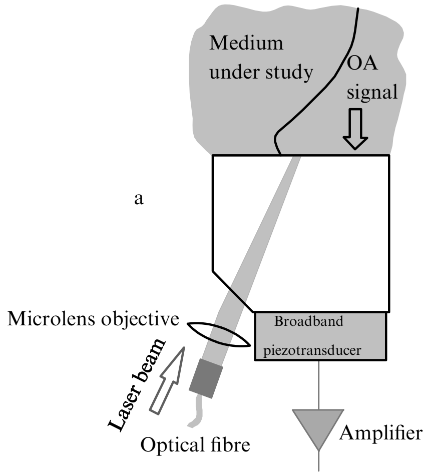
3. Media under study

To confirm experimentally the results of numerical simulations [11] and the theoretical approach presented in section 2, we performed a series of experimental measurements by using strongly scattering media with known optical properties. Milk with different fat content – 1.5 %, 3.5 %, and 6 % – was used in experiments which provided three different values of the reduced scattering coefficient μ_s'. The variation in the light absorption was achieved by adding different volumes of black Indian ink (0.02 – 1.2 ml) into the fixed volume of milk (100 ml). Milk is a medium well simulating human biological tissues. Optical properties (μ_a and μ_s') of milk with different fat contents and solutions of milk and black Indian ink were measured by the OA method in the forward mode [1], which was calibrated by measuring the optical properties of polystyrene spheres in water solutions and comparing these results with those of the Mie theory. The variations in the light absorption coefficient were within the range of 0.18 – 3 cm^{-1}, the reduced scattering coefficient – 11 – 24 cm^{-1} for all tested solutions. These values were used to obtain the media with optical properties typical of human tissues within the so-called therapeutic window of laser wavelengths [10]. Before performing measurements in the backward mode of the OA signal detection, the optical properties of each solution were measured by using the calibration technique [1], which involved the forward detection mode. The inaccuracy in determining the light absorption coefficient of the solutions did not exceed 2 %.

4. Experimental setup

The dependences of the OA signal amplitude on the optical properties of turbid media for different laser beam diameters were measured in the backward detection mode [5]. A specially designed OA transducer was developed to perform these measurements. The principle scheme of the OA transducer and its photo are shown in Fig. 2. A diode-pumped Nd : YAG laser emitting 10-ns pulses with the pulse energy of $W = 30 - 100$ mJ and repetition rate of 500 Hz operated at the main harmonic, and was used to irradiate the turbid media under study. The radiation was delivered to the OA transducer with an optical fibre and was incident onto the surface of the turbid medium through a quartz prism. The angle of incidence was about 18°, and could not affect the measurement results because of the high light scattering inherent in the media under study. Excited OA signals propagated backward through the same prism and were detected at its rear surface by a broadband piezoelectric transducer. The processed electric signal was then amplified and digitised by an analogue-to-digital converter. Finally, the data were processed on a personal computer.

a



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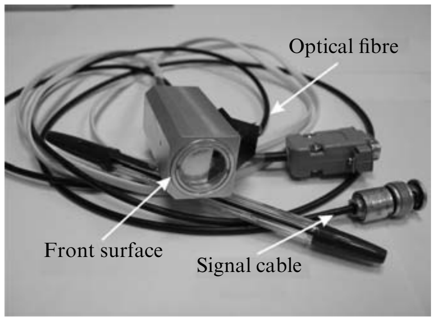

Figure 2. Principle scheme (a) and photo of the OA transducer operating in the backward mode.

The laser beam was focused on the surface of a turbid medium by a microlens objective inbuilt into the OA transducer housing. Measurements were performed for two different laser beam diameters determined experimentally: $d_1 = 3.0 \pm 0.2$ mm and $d_2 = 0.6 \pm 0.2$ mm.

5. Results

The experimentally obtained dependences of the OA signal amplitude on the light absorption coefficient μ_a for the laser beam diameters d_1 and d_2 are shown in Figs 3a, b, respectively. The different symbols on the plots correspond to experimental data for the media with $\mu_s' = 11$, 17, and 24 cm^{-1} (solutions of milk with various fat contents of 1.5 %, 3.5 %, and 6 %, respectively). Note that the same dependences numerically calculated with the Monte-Carlo method were presented in Fig. 4 of paper [11]. The experimental and numerically calculated dependences were obtained in different units that were proportional to each other. The proportionality coefficient depends on a number of parameters, such as the transducer sensitivity, the efficiency of OA conversion Γ, the laser beam diameter, and the PMMA prism length. Therefore, for direct comparison of theoretical and experimental results the normalisation procedure was performed as follows. The slope coefficients of the experimental (Fig. 3, symbols) and theoretical lines (Figs 4a, c in [11]) for the reduced scattering coefficient $\mu_s' = 17$ cm^{-1} were calculated within the range of $\mu_a < 1$ cm^{-1}. Then, we determined the ratios of these slope coefficients for the experimental and theoretical

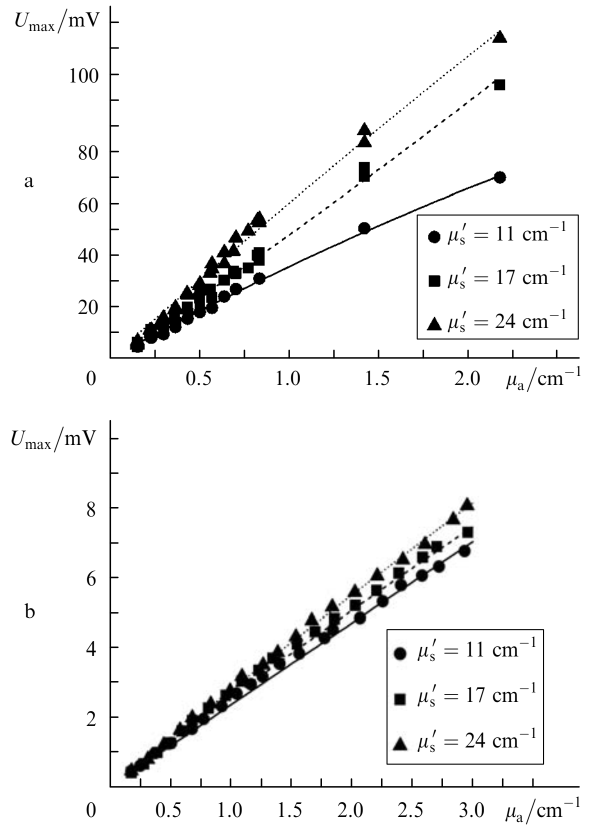

Figure 3. Dependences of the OA signal amplitude on the light absorption coefficient μ_a of a turbid medium for different reduced scattering coefficients μ_s' and for two laser beam diameters: 3 (a) and 0.6 mm (b). Symbols corresponds to experimental data and solid lines are the results of Monte-Carlo simulations.

dependences ($\gamma_1 = 0.059$ mJ cm^{-3}mV^{-1} and $\gamma_2 = 0.49$ mJ cm^{-3} mV^{-1}) obtained at the laser beam diameters $d_1 = 3$ mm and $d_2 = 0.6$ mm, respectively. Finally, all the dependences obtained in Monte-Carlo simulations (Fig. 3, solid lines) for $\mu_s' = 11$ and 24 cm^{-1} were normalised by γ_1 and γ_2 (Figs 3a and b), respectively.

At $d_1 = 3$ mm, the dependence of the OA signal amplitude on the absorption coefficient has different reduced scattering coefficients, which differ by 15 % from the slope coefficients. As the laser beam diameter decreases down to $d_2 = 0.6$ mm, the slopes of the approximating lines for different μ_s' approach each other, which was obtained in numerical simulations. For the dependences shown in Fig. 3b, the difference in the slope coefficients is no more than 8 % of the average value. Therefore, it is possible to plot a mean universal curve to determine the light absorption coefficient in turbid media with *a priori* unknown values of μ_s' from the OA signal amplitude.

6. Discussion and conclusions

By using a compact measurement system, we have demonstrated the possibility of measuring the light absorption coefficient in the media with optical properties inherent in biological tissues in the so-called therapeutic window of laser wavelengths. The use of a diode-pumped Nd:YAG laser makes it possible to perform noninvasive diagnostics and achieve a high productivity of measurements, that is necessary for biomedical applications. The specially designed OA transducer is the essential component of the measurement system. The mean universal curve, which can be obtained from the dependences illustrated in Fig. 3b and used for determination of light absorption coefficient in turbid media, is calibration one for this particular OA transducer. It allows one to determine the local light absorption coefficient of biological tissue *in vivo* from the OA signal amplitude. The smaller the incident laser beam diameter, the higher the accuracy and locality of the measurement. For example, the accuracy of 8 % in determining the light absorption coefficient can be achieved if the laser beam diameter is $d_2 = 0.6$ mm. The advantages of the developed method are the absence of complicated data processing and the absence of the *a priori* assumptions concerning the medium structure, for example, homogeneity.

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