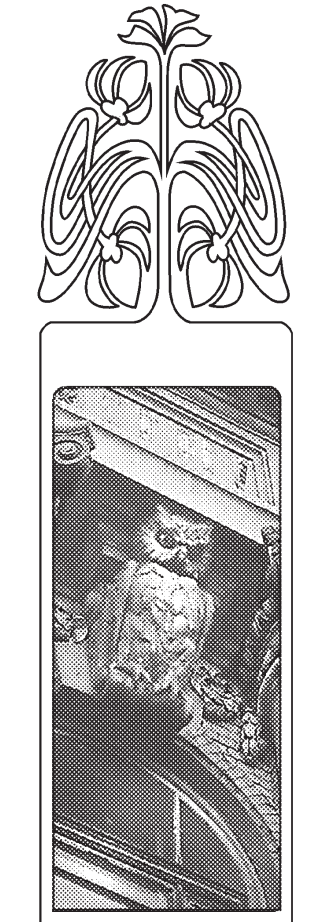

НАУЧНЫЙ ОТДЕЛ

# БИОФИЗИКА И МЕДИЦИНСКАЯ ФИЗИКА

## Measurement of Optical Properties of Human Gums and Dentin in the Spectral Range from 350 to 800 nm

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Knowledge of the optical properties of biological tissues is important for the development of optical diagnostics, photodynamic and photothermal therapy of various diseases. However, despite the significant number of works devoted to the determination of the optical properties of tissues, the optical properties of human gums and dentin remain currently poorly understood. In this work, we experimentally studied the optical properties of human gums and dentin in the spectral range from 350 nm to 800 nm. Basing on measured diffuse reflection and total transmission spectra and using the Inverse Adding Doubling (IAD) method, the spectral dependences of absorption and scattering coefficients of the studied tissue samples were calculated.

**Keywords:** gums, dentin, total transmission spectra, diffuse reflection spectra, absorption and scattering coefficients.

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**Introduction**

The scientific and technological progress of recent decades has taken medicine to a new higher level. Improvement of optical systems, the discovery of laser and LED sources, the widespread use of computers, microprocessors and the development of new technologies for obtaining three-dimensional images, have made it possible to make huge changes in the technology for obtaining images. The optical methods for tissue imaging and disease diagnosing in humans are increasingly used in different fields of medicine [1, 2]. An overview of emerging novel optical imaging techniques of tissues based on light scattering, nonlinear optics, and optical coherence tomography (OCT) is presented elsewhere [3].

For example, optical imaging modalities for the deep layers of tissues is used to differentiate malignant neoplasms in their early stages, including OCT, fluorescence and Raman spectroscopies, portable imaging systems based on fiber-optic technologies for delivering radiation to the pathological sites and back, and some other technologies [1, 3, 4]. Since therapeutic effects are achieved due to absorption of the optical radiation by tissue chromophores, a more detailed study of the optical properties of tissues and cellular structures at specific wavelengths are needed. One of the frequently used methods for calculating tissue scattering and absorption coefficients is the inverse addition-doubling (IAD) technique. It was developed by Dr. Scott Prahl et al. [5]. It is one of the most used algorithms for solution of inverse optical problem for reconstruction reduced (transport) scattering and absorption coefficients which is pretty fast and accurate. The IAD method is widely used for processing of the spectrophotometry data obtained with integrating spheres, including *in vitro* studies of pathologically altered mucous membrane of the human maxillary sinus in the spectral range of 350–2000 nm [6], sclera of the eye in the spectral range of 370–2500 nm [7], human eye lenses with various stages of cataract [8], peritoneal tissues in the spectral range of 350–2500 nm [9], human stomach mucosa in the spectral range of 400–2000 nm [10], human colon tissues in the spectral range 350–2500 [11], human colon mucosa and colon precancerous polyps in the spectral range of 400–1000 nm [12], and also a rather large number of other tissues: skin, muscles, skull, etc. [1, 13, 14].

For semi-quantitative assessment of the light permeability in photosensitized dentin *in vitro*, the method of photoacoustic spectroscopy was used [15]. An analysis of the fluorescence spectra recorded *in vivo* upon excitation by a continuous wave He-Cd laser (325 nm) made it possible to distinguish qualitatively normal, potentially malignant, and malignant sites of the oral mucosa [16]. When collecting the optimal amount of data and compiling an appropriate database, it can be used in clinical diagnostics. Authors of [17] proposed a survey algorithm for more accurate identification of the pathological process in the oral mucosa using the direct autofluorescence imaging of tissue and microscopy of biopsy material. Using the autofluorescence imaging method of the oral mucosa (autofluorescence stomatoscopy with AFS-400 LED illuminator (400 ± 10 nm)), the authors were able to detect verrucous leukoplakia, lichen planus and squamous cell carcinoma [18]. The use of color space technology, known as CIELab system, allowed one to evaluate the color coordinates of oral tissues in normal and pathological conditions [19–24]. Estimation of tooth color coordinates basing on patient age and gender indicated 75 shades grouped into 5 clusters of the maxillary central incisor color of 1361 Caucasian Spanish individuals aged 16 to 89 years measured on tooth surfaces through the Easyshade Compact spectrophotometer [25, 26].

Intravital noninvasive high-resolution imaging of the oral tissue structure using OCT demonstrates excellent ability to detect and diagnose precancer, early cancer, dysplasia and malignancy of the mucous membrane epithelium [27–30]. The microscopic OCT was used to determine the scattering coefficient of normal human oral epithelium from *ex vivo* measurements as $\mu_s = 27 \pm 11$ cm$^{-1}$ at 850 nm [31].

The optical thickness of the human gingival layers measured using OCT (1310 nm) with a probe for *in vivo* measurements (Asian female volunteer of 30 year old) was ~237 μm for epithelium (E) and ~830 μm for lamina propria (LP) (attached gingiva) [32]. The geometric thicknesses of tissue layers can be evaluated using the mean refractive index of these tissues $n \cong 1.4$ at 1310 nm [1] as ~169 μm for E and ~593 μm for LP, totally 762 μm.

The developed hybrid system of Raman spectroscopy and OCT is capable to provide simultaneous acquiring both morphological and biochemical information about the oral tissue, facilitating real-time, *in vivo* tissue diagnoses and characterization in the oral cavity [33].

OCT also makes it possible to diagnose caries in the stage of a white spot, to monitor an increase of optical penetration depth at usage optical clearing agents, and to improve the visibility of subsurface occlusal lesions and dentin-enamel compounds in the foci of demineralization [34–36].

An automated mobile microscope and simplified oral mucosal staining protocols have been used to screen for oral cancers, facilitating local digital imaging and remote evaluation of images by physicians, and demonstrated the compliance with existing histology and cytology methods [37].

Oral tissue optical transmittance was investigated by a few groups [38–42]. The scattering coefficient of a healthy oral mucosa epithelium was determined from *in vivo* studies using diffuse reflection spectroscopy as $\mu_s = 42$ cm$^{-1}$ at 810 nm and $\mu_s = 39$ cm$^{-1}$ at 855 nm [43].

The optical properties of the new organotypic substitute for the oral mucosa based on fibrin-agarose scaffolds were determined using the integrating sphere measurements and inverse adding doubling method (IAD) [44]. The optical properties of porcine gums were measured aiming the manufacturing of material for the human gums phantom for subsequent use in the prototype robotic system for laser maxillofacial surgery [45].

Significant interest in studying the optical properties of tissues of the oral cavity in humans is due to the trend of modern medicine and dentistry in particular, to use least invasive diagnostic and therapeutic techniques that can be associated with optical methods. The use of laser technologies, photodynamic therapy and light biomodulation in dentistry, including aesthetic dentistry, require accurate knowledge of the optical parameters of biological tissues for successful implementation in clinical practice. Despite a significant number of recent studies devoted to dental optical diagnostics and therapy, they are characterized by more qualitative than quantitative optical properties of the oral tissues. In this regard, the determination of the main optical parameters of gums and dentin, such as absorption and scattering coefficients, is an urgent task.

The aim of this study is to measure optical spectra of the human gums and dentin in the spectral range from 350 nm to 800 nm and to determine reduced scattering and absorption coefficients in this spectral range.

### Methodology

The materials for the *in vitro* study were cleaned tooth cuts (dentin) obtained from orthodontically extracted human teeth (molars) as well as sections of the human gingival mucosa obtained *after surgery*. The ***gingiva*** (gums) is the portion of oral mucosa covering the alveolar bone ridge surrounding the tooth. Before cutting, the teeth were stored in physiological saline in a dark place at a temperature of 4–6° C, and the gums was stored in a frozen state. The thickness of the samples was measured using a micrometer, placed between two glass slides, the measurement accuracy was ± 10 μm. The measurements were carried out at five points and the values were then averaged. The averaged thickness of the cuts of human dentin was (0.5 ± 0.07) mm and of the gums was (0.4 ± 0.08) mm. In total, the measurements were provided for 10 dentin samples and 10 gums samples obtained from different subjects. The area of dentin cuts was on average 150–210 mm$^2$, and the gingival sections was 150–220 mm$^2$.

To measure the total transmittance and diffuse reflectance of tissue samples in the spectral range of 200–800 nm, a Shimadzu UV-2550 dual-beam spectrophotometer (Japan) with an integrating sphere was used. A halogen lamp with radiation filtering in the studied spectral range served as a radiation source. The limiting spectrometer resolution was 0.1 nm. The spectra were normalized before the measurements using a $BaSO_4$ reference reflector, which has the best properties in UV [46]. All measurements were carried out at room temperature (~25° C) and normal atmospheric pressure. Each sample of the studied tissue was fixed in a special frame with a window of 0.5×0.5 cm, and fixed in a quartz cuvette so that the tissue sample was pressed against the wall of the cuvette and turned to an optical measurement. To measure the total transmission spectra, a quartz cuvette with a tissue sample was mounted directly in front of the integrating sphere collecting all the radiation transmitted through the tissue sample. When measuring the diffuse reflection spectra, a cuvette with a sample was placed behind an integrating sphere, which collected all the radiation backscattered by the sample. The light beam diameter falling onto a sample was of 3-mm. Prior measurements, a quartz cuvette with a fixed sample was filled up with saline to wet the sample and to bring the measurements closer to *ex vivo*.

To process the experimental results and determine the optical parameters of the human gums and dentin tissue, the combined method was used. At the first stage of which the measurement data were processed using the IAD [4, 5]. The IAD method allows one to determine the absorption coefficient $\mu_a$ and transport scattering coefficient $\mu'_s$ of tissue using experimental data for diffuse reflectance and total transmittance:

$$\mu'_s = \mu_s \cdot (1-g) \qquad (1)$$

here, $\mu_s$ is the scattering coefficient and $g$ is the scattering anisotropy factor. During calculations, the anisotropy factor is fixed. For the tissues (gums

and dentin of the human tooth) studied in this work, $g$ was assumed to be 0.9 [1]. However, this value of the anisotropy factor ($g$) is characteristic for only the visible and NIR spectral ranges [1], and in UV it strongly differs, therefore, we took a restriction when calculating the optical properties of the studied tissues over the range from 350 to 800 nm. The dentin refractive index of a human tooth was taken as 1.49 [47]. The refractive index of the human oral mucosa was taken as 1.45 [48]. The main limitation of the IAD method is related to the possible loss of scattered radiation through the sides of the tissue sample, which is possible in the case when the dimensions of the sample are relatively small compared to the dimensions of the beam incident on the tissue sample, or when the tissue is characterized by relatively low absorption and scattering coefficients. Not accounting the lateral losses of the probe radiation, leads to an overestimation of the determined absorption coefficient [1, 4 5]. For the correct application of the IAD method, it is necessary to require that the distance from the edge of the probe beam incident on the tissue sample to the nearest sample boundary to be greater than the transport mean free path of the photons, which is defined as $1/(\mu_a+\mu'_s)$ [1]. The calculation of the optical parameters was performed separately for each spectral point. The algorithm used includes the following steps:

1) Setting the initial values and using the following expressions [5]:

$$\frac{\mu'_s}{\mu_a+\mu'_s}=\begin{cases}1-\left(\dfrac{1-4R_d-T_t}{1-T_t}\right)^2, if\ \dfrac{R_d}{1-T_t}<0.1\\ 1-\dfrac{4}{9}\left(\dfrac{1-R_d-T_t}{1-T_t}\right)^2, if\ \dfrac{R_d}{1-T_t}\geq 0.1\end{cases}$$

$$(\mu_a+\mu'_s)\times l=\begin{cases}-\dfrac{lnT_t\ln(0.05)}{lnR_d}, if\ R_d<0.1\\ 2^{1+5(R_d+T_t)}, if\ R_d\geq 0.1.\end{cases}$$

Here $R_d$ and $T_t$ are the measured diffuse reflection and total transmission coefficients, $l$ is the thickness of tissue sample.

2) Calculation of diffuse reflection and total transmission coefficients basing on the initial values of $\mu_a$ and $\mu'_s$ and the «adding-doubling» method [4].

3) Comparison of the calculated $R_d$ and $T_t$ values with experimentally measured ones.

4) As a criterion for completing the iterative procedure, the following condition was used [4, 5]:

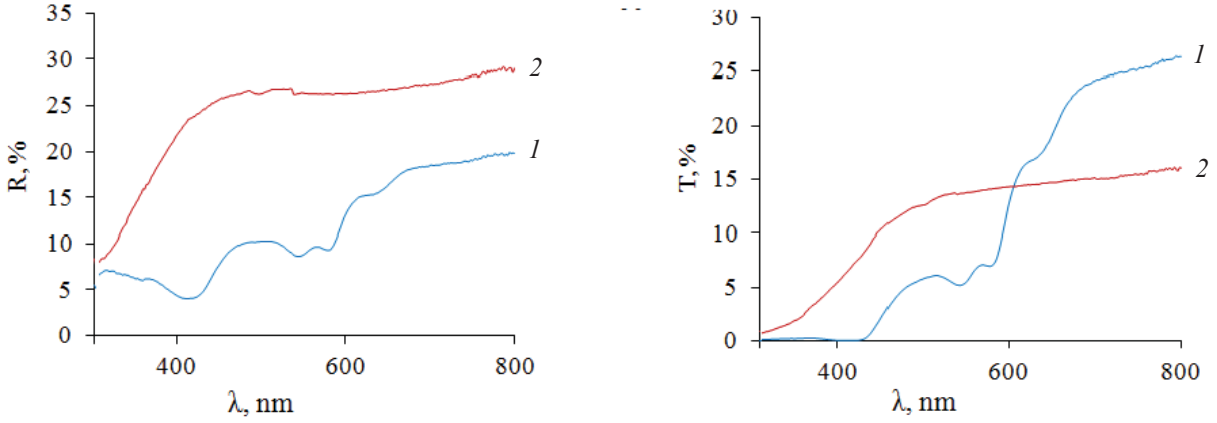
$$\frac{\left|R_d^{exp}-R_d^{calc}\right|}{R_d^{exp}}+\frac{\left|T_t^{exp}-T_t^{calc}\right|}{T_t^{exp}}<0.001,$$

where $R_d^{exp}$, $R_d^{calc}$, $R_t^{exp}$, $R_t^{calc}$ are the experimental (exp) and calculated (calc) values of the diffuse reflection and total transmission coefficients, respectively.

### Results and discussion

Dentin consists mainly of a collagen matrix. A structurer feature of dentin is the presence of dentinal tubules, penetrating the entire thickness of dentin. The own layer of the oral mucosa is a connective tissue which consists of fibrous structures, cellular elements and intercellular substance. Collagen and argyrophilic fibers of the own layer of the mucous membrane comprise fibrous structures, and there are especially many of them in the hard palate and gums. In the mucous membrane of the oral cavity there are more argyrophilic fibers, and less collagen fibers than in the skin. From an optical point of view, the gums and dentin of a human tooth can be attributed to optically turbid media in which, along with absorption, a strong light scattering is observed. During the propagation of optical radiation in tissue, chromophores – substances of endogenous or exogenous origin, are capable to absorb radiation energy (photons). Water absorption in the measured range of 350–800 nm is negligible and begins to affect in the range of 1200–2500 nm [15]. The gingival mucosa and dentin of a human tooth can be attributed to fibrous tissues, which are based on collagen and argyrophilic fibers and hemoglobins in the gums tissue. Figure 1*a* shows the diffuse reflectance spectra (DRS) of the gingival mucosa (curve *1*) and dentin of a human tooth (curve *2*).

In the region from 350 to 650 nm, the shape of the diffuse reflectance spectra correlates quite well with the shape of the transmission spectrum of the gingiva, since in this wavelength range, the shape of the spectra is determined by strong absorption bands of oxyhemoglobin and the effect of light scattering by the main scatterers of the gingival mucosa – collagen and elastin fibers. The DRS and total transmission spectra clearly show the dips corresponding to the absorption bands of oxyhemoglobin at wavelengths of 415, 542, and 576 nm. The presence of strong absorption bands reduces both the number of transmitted and backscattered photons within the absorption bands. Beginning from 650 nm and further up to 800 nm, the influence of the absorption bands of hemoglobin is no longer significant, the spectra of total transmission and back reflection are formed mainly due to scattering, since the contribution of absorption bands of all chromophores of soft tissues in this region is minimal, which corresponds to their “transparency window”.

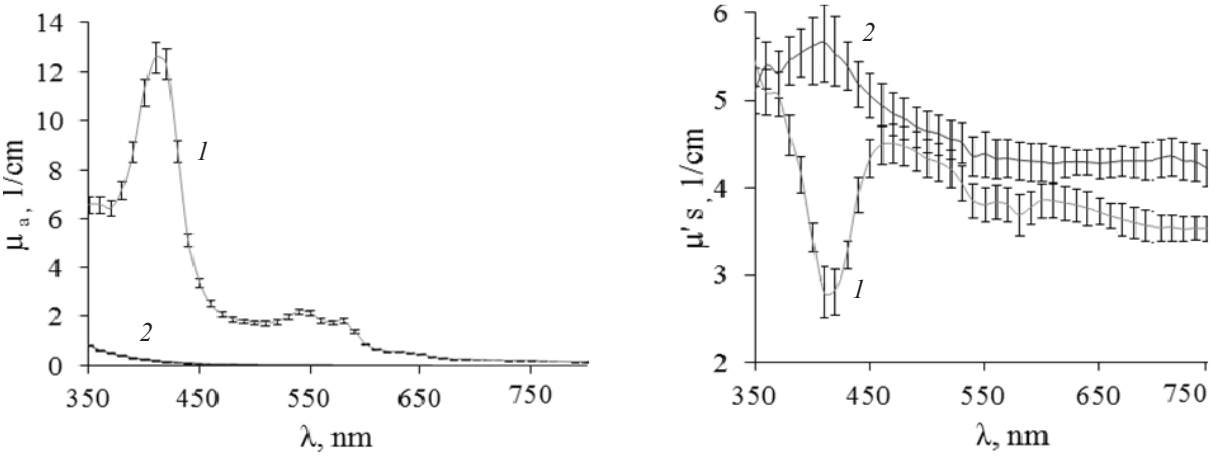



*a* *b*

Figure 1. Spectra of diffuse reflection (*a*) and total transmission (*b*) of human gums (curve *1*) and dentin (curve *2*) samples

For dentin, no such difference is observed, and the shapes of the diffuse reflectance and total transmission spectra complement each other in the entire investigated range (350–800 nm), since there are no characteristic endogenous chromophores in this range (amino acid residues of proteins have characteristic absorption bands in the range of 200–350 nm) and spectra are determined mainly by scattering by the matrix of hydroxyapatite and dentinal collagen (Fig. 1*a*, *b*).

Figures 2*a* and 3*b* show the absorption and transport scattering coefficients spectra calculated using the IAD method based on the measured values of the diffuse reflectance and total transmittance. Taking into account the dimensions of the probe beam incident on the surface of the tissue sample, the minimum sample size should be at least 9.5 mm, which is performed for the smallest of the studied samples with an area of about 120 mm$^2$ and having sizes of 12 × 10 mm.




*a* *b*

Figure 2. The absorption coefficient (*a*) and transport scattering coefficient (*b*) of the human gums (*1*) and dentin (*2*), calculated by IAD method using experimental data

Figure 2*a* shows the spectral dependences of the absorption coefficient of the gingival mucosa (curve *1*) and dentin of a human tooth (curve *2*) in the spectral range from 350 to 800 nm. The absorption bands of blood oxyhemoglobin (415, 542 and 576 nm) are clearly visible in the spectrum [1]. Figure 2*b* shows the spectral dependences of the transport coefficient of scattering of the gingival mucosa (curve *1*) and dentin of a human tooth (curve *2*) in the spectral range from 350 to 800 nm. These dependences were obtained by averaging the spectra of absorption and transport scattering coefficient of 10 samples of the mucous membrane and 10 samples of dentin cuts of a human tooth. Bars present standard deviations

of the absorption and reduced (transport) scattering coefficients. It is clearly seen that the transport scattering coefficient decreases rather smoothly towards large wavelengths, which corresponds to the general nature of the spectral behavior of the scattering properties of tissues [1]. However, in the region of strong absorption bands (i.e., 415, 542, and 576 nm), the shape of the scattering spectrum is distorted, i.e. it is deviated from monotonous dependence, that could be a manifestation of IAD algorithm drawback that allows for crosstalk between absorption and scattering, when absorption is strong.

The scattering coefficient of a healthy human oral mucosa measured in *in vivo* studies using diffuse reflection spectroscopy was determined as $\mu_s$= 42 cm$^{-1}$ at 810 nm and $\mu_s$= 39 cm$^{-1}$ at 855 nm [45]. For *ex vivo* studies, optical coherence microscopy gave $\mu_s$ = 27 ± 11 cm$^{-1}$ at 850 nm [33]. The scattering coefficient for the gingival mucosa equal to 36 cm$^{-1}$ at 800 nm, obtained using Eq. 1, correlates well with these results [31, 43].

Within the Soret band of hemoglobin at 415 nm reduced scattering coefficient has a dip (Fig. 2*b*), which can be associated with a so called "crosstalk" between absorption and scattering following from IAD algorithm. However some influence of physical reasons, which is related to influence of anomalous dispersion and light diffraction on particles with a high absorption, is also possible. Our estimations using Mie calculator for an ensemble of spherical particles [49] showed that drop of reduced scattering coefficient at 415 nm can be of 12–20% in comparison with no absorption case.

The penetration depth of light is one of the most important characteristics for the correct determination of the radiation dose during photochemical and photodynamic therapy of various diseases [1]. The penetration depth of tissue (δ) was estimated using the formula obtained in the diffusion approximation [6], $1/\sqrt{3\mu_a(\mu_a + \mu'_s)}$ (see Fig. 3).

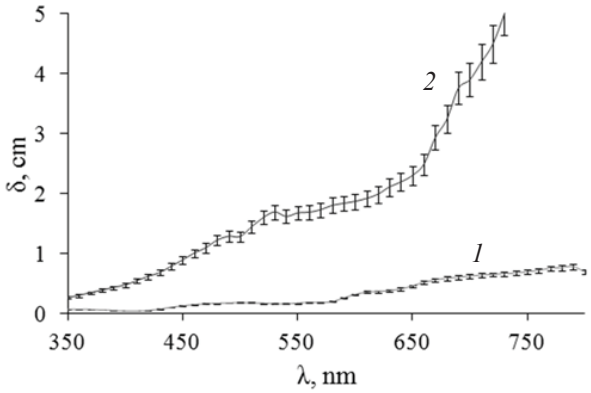



Figure 3. The penetration depth of the human gums (*1*) and dentin (*2*), calculated using data obtained from measurements by the IAD method

The determined absorption and transport scattering coefficients, as well as calculated using these data the penetration depth, of the human gums and dentin are given in the table.

*Table*

**The absorption and transport scattering coefficients, and the penetration depth of the human gums and dentin**

| λ,nm | 350 | 400 | 450 | 500 | 550 | 600 | 650 | 700 | 750 | 800 |
|---|---|---|---|---|---|---|---|---|---|---|
| Gums | | | | | | | | | | |
| $\mu_a$, cm$^{-1}$ | 6.6 ± 0.5 | 11.2 ± 0.2 | 3.4 ± 0.3 | 1.8 ± 0.4 | 2.1 ± 0.4 | 0.9 ± 0.2 | 0.5 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| $\mu'_s$, cm$^{-1}$ | 5.4 ± 0.8 | 3.4 ± 0.5 | 4.3 ± 0.4 | 4.3 ± 0.4 | 3.8 ± 0.3 | 3.9 ± 0.5 | 3.7 ± 0.4 | 3.6 ± 0.3 | 3.5 ± 0.4 | 3.6 ± 0.3 |
| $\mu_s$, cm$^{-1}$ | 54 ± 8 | 34 ± 5 | 43 ± 4 | 43 ± 4 | 38 ± 3 | 39 ± 5 | 37 ± 4 | 36 ± 3 | 35 ± 4 | 36 ± 3 |
| δ, cm | 0.10 ± 0.01 | 0.04 ±0.02 | 0.10 ±0.01 | 0.18 ± 0.02 | 0.17 ± 0.02 | 0.32 ± 0.02 | 0.46 ± 0.2 | 0.62 ± 0.03 | 0.69 ± 0.03 | 0.69 ± 0.03 |
| Dentin | | | | | | | | | | |
| $\mu_a$, cm$^{-1}$ | 0.8 ± 0.1 | 0.3 ± 0.1 | 0.08 ± 0.1 | 0.04 ± 0.1 | 0.03 ± 0.1 | 0.02 ± 0.1 | 0.02 ± 0.1 | 0.01 ± 0.1 | 0 | 0 |
| $\mu'_s$, cm$^{-1}$ | 5.1 ± 0.9 | 5.6 ± 0.9 | 5.1 ± 0.7 | 4.7 ± 0.5 | 4.4 ± 0.5 | 4.3 ± 0.6 | 4.3 ± 0.5 | 4.3 ± 0.5 | 4.2 ± 0.4 | 4.1 ± 0.4 |
| $\mu_s$, cm$^{-1}$ | 51 ± 9 | 56 ± 9 | 51 ± 7 | 47 ± 5 | 44 ± 5 | 43 ± 6 | 43 ± 5 | 43 ± 5 | 42 ± 4 | 41 ± 4 |
| δ, cm | 0.26 ± 0.02 | 0.47 ± 0.02 | 0.89 ± 0.02 | 1.28 ± 0.02 | 1.68 ± 0.04 | 1.87 ± 0.05 | 1.87 ± 0.06 | 3.9 ± 0.1 | 5.5 ± 0.3 | 5.5 ± 0.3 |

The penetration depth of radiation into the human mucous membrane of the gums (*1*) and dentin (*2*) was calculated using the values of absorption coefficients presented in Fig. 2*a*, and the transport scattering coefficient shown in Fig. 2*b*. From Fig. 3 it is clearly seen that, depending on the wavelength of the probe radiation, the depth of its penetration into the studied tissues varies significantly. The maximum effect is observed in the spectral range from 600 to 800 nm, where the radiation penetrates to a depth of 3–7 mm in the human gums and 19–55 mm in dentin.

We have received the penetration depth of light in dentin of 1.87 ± 0.06 cm at 650 nm and 5.5 ± 0.3 cm at 800 nm. As for cemented tooth root in healthy molars the penetration depth for a laser source at 780 nm was in the range from 0.43 cm to 1.33 cm [50]. The difference in light transmission can be attributed to significant differences in the structural organization of different parts of the tooth. Cement is a highly mineralized tissue and transmits less radiation, as dentin is a porous light-conducting tissue, where the dentinal tubular structures are waveguides [51].

## Conclusions

In this work, the optical properties of the human gums and dentin are experimentally studied in the spectral range from 350 to 800 nm. Based on the measured *in vitro* spectra of diffuse reflectance and total transmittance, the spectra of absorption and transport scattering coefficients of the gums and dentin and their optical penetration depths were calculated in the range from 350 to 800 nm using the IAD method. Knowledge of the optical characteristics of tissues is important for the development of optical diagnostic methods for the early diagnostics of pathological changes in the tissues of the gums and dentin *in vivo*, as well as for the preparation of the correct clinical protocols for the photodynamic and photothermal therapy of different dental diseases.

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**Измерение оптических свойств десны и дентина человека в спектральном диапазоне 350–800 нм**

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Знание оптических свойств биологических тканей важно для разработки методов оптической диагностики, фотодинамической и фототермической терапии различных заболеваний. Однако, несмотря на значительное количество работ, посвященных определению оптических свойств биологических тканей, оптические свойства десны и дентина человека в настоящее время остаются недостаточно изученными. В данной работе экспериментально исследованы оптические свойства тканей десны и дентина зуба человека в спектральном диапазоне от 350 до 800 нм. На основании измеренных спектров диффузного отражения и полного пропускания и с использованием метода обратного удвоения-добавления (IAD) были рассчитаны спектры коэффициентов поглощения и рассеяния исследуемых образцов ткани.

**Ключевые слова:** десна, дентин, спектры полного пропускания, спектры диффузного отражения, коэффициенты поглощения и рассеяния.

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