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GEOMETRICAL AND APPLIED OPTICS

Optical Properties of Mucous Membrane in the Spectral Range 350–2000 nm

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Abstract—The optical characteristics of the mucous membrane from the human maxillary sinus are studied experimentally. The experiments were carried out in vitro in the spectral range 350–2000 nm. On the basis of the measured total transmittance and diffuse reflectance spectra, the absorption and transport scattering coefficients are calculated in the entire range in terms of the inverse adding–doubling method. *© 2004 MAIK “Nauka/Interperiodica”.*

INTRODUCTION

The development of new optical methods used in different fields of biology and medicine for the study of the permeability of cell membranes and photodynamic and photothermal destruction of cells and tissues, as well as for the elaboration of new approaches in photodynamic therapy, optical tomography, optical biopsy, etc., determines the necessity of measuring the optical characteristics of cell structures and biological tissues [1–4], which plays a key role in the construction of mathematical models adequately describing light propagation in these media.

Among the various methods of determination of the optical parameters of scattering media, spectrophotometry with the use of integrating spheres is a precise method that is the most widely used in optics of biological tissues [4].

In modern rhinology, the treatment of purulent maxillary sinusitis remains an important problem, despite the wide application of modern antibacterial agents and novel surgical methods [5, 6]. One of the new methods of treatment of this disease is associated with photodynamic therapy of suppurative inflammation of the mucous membrane of the maxillary sinus [5]. However, despite numerous investigations into the optics of biological tissues, at present, the optical parameters of the mucous membrane of the maxillary sinus remain insufficiently studied. At the same time, knowledge of these parameters is fundamentally important for reliable sectional dosimetry of laser radiation used in photodynamic therapy of acute and chronic maxillary sinusitis. In addition, the study of the optical characteristics of the mucous membrane in a wide spectral range allows researchers to extend the possibilities for development of new methods and optimization of the existing ones for photodynamic therapy of this disease. It should also be noted that, since the structural and morphological properties of mucous membranes of different internal organs, which represent the stratified epithelium, are sufficiently similar to each other, the study of the optical characteristics of the mucous membrane of the maxillary sinus is also of interest for other fields of modern medicine in addition to otorhinolaryngology.

The objective of this study is to determine the scattering and absorption characteristics of mucous membrane in the spectral range 350–2000 nm by using the mucous membrane of the human maxillary sinus as an example.

THE STRUCTURE OF THE MUCOUS MEMBRANE

The mucous membrane plays a leading role in the physiology and pathophysiology of the nose and paranasal sinuses [6, 7]. It is covered with a pseudostratified epithelium, which consists of ciliated, columnar, as well as short and long inserted epithelial cells. The membrane called the basic membrane divides the epithelial and proper layers of the mucosa. This membrane consists of reticular fibers located in an interstitial homogeneous medium and is not a formation of a constant thickness. In the case of hyperplasia of the mucous membrane, the basement membrane considerably thickens [8].

The proper layer of the mucous membrane is similar in structure to connective tissue, consisting of collagen and elastic fibers. The interstitial fluid of the mucous membrane contains proteins and polysaccharides and is similar in composition to the interstitial fluid of most of connective tissues. The proper layer of the mucous membrane consists of three sublayers. A subepithelial (or lymphoid) layer contains a great amount of leukocytes. In the intermediate sublayer of the proper layer, tubuloalveolar glands are contained. In the deep sublayer of the proper layer, venous plexuses are arranged, which consist of a surface network of smaller vessels and a deeper network of larger vessels. Normally, the total thickness of the mucous membrane varies from 0.1 to 0.5 mm [6, 7]. In the presence of pathology (maxillary sinusitis, rhinitis, or other rhinological disease), the thickness of the mucous membrane considerably increases and can reach 2–3 mm [6]. It should be noted that the proper layer of the mucous membrane is the main layer protecting against microorganisms causing infectious diseases [7]. The optical properties of the mucous membranes of the nose and paranasal sinuses are mainly determined by the optical properties of the proper layer since this layer is much thicker than the epithelial layer.

MATERIALS AND METHODS

As material for study, we used ten biopsy samples of the mucous membrane of the maxillary sinuses obtained from ten patients with chronic maxillary sinusitis by maxillary sinusotomy. The area of the samples varied from 100 to 240 mm^2. Immediately after the operation, the samples were placed in a 0.9% solution of NaCl and were stored in it for 2–3 h at room temperature (about 20°C) prior to spectral measurements. To measure the thickness of the samples, they were sandwiched between two cover glasses; the measurements were performed with a micrometer at several points of each sample. The accuracy of a single measurement was within ±50 μm. The values obtained were averaged. The thickness of the experimental samples varied from 1 to 2 mm and, on the average, amounted to 1.5 ± 0.5 mm. To perform the spectral measurements, each sample was fixed in a special clamp in the form of a frame with a square window of 5 × 5 mm.

The optical properties of the mucous membrane were studied in the spectral range 350–2000 nm on a Cary-2415 spectrophotometer (Varian, Australia) with an integrating sphere. This instrument is a two-channel monochromator with a built-in system of control and signal recording. As a radiation source, a halogen incandescent lamp was employed. The cross section of the light beam incident on the sample was 5 × 5 mm; the scanning rate was 2 nm/s.

For data processing and determining the optical parameters of the mucous membrane, we employed the inverse adding–doubling method [9], widely used in optics of biological tissues for data processing in integrating sphere spectrophotometry [10–16]. This method allows one to determine the absorption coefficient (μ_a) and the transport scattering coefficient ($\mu_s' = \mu_s(1-g)$) of a biological tissue on the basis of the total transmittance and diffuse reflectance. Here, μ_s is the scattering coefficient and g is the anisotropy scattering factor. In calculations, the value of the latter parameter is fixed. In this study, g was assumed to be equal to 0.9 since this value is the most typical for the majority of biological tissues in the visible and near-IR spectral ranges [4].

The main restriction of the inverse adding–doubling method is associated with possible losses of scattering radiation through lateral surfaces of the sample [17], which is possible when the size of the sample is small in comparison with the size of the incident beam or when the absorption and scattering coefficients of the biological tissue are comparatively small. Neglect of lateral losses of the incident radiation (if they occur) leads to an overestimation of the absorption coefficient [17]. For the correct application of the inverse adding–doubling method, it is necessary that the distance from the edge of the light spot from the probing light beam to the nearest edge of the sample be greater than the transport mean free path of photons, which is determined as $1/(\mu_a + \mu_s')$ [1].

The optical parameters were calculated separately for each spectral point. The algorithm used involves the following steps:

(1) Setting of the initial values of μ_a and μ_s' with the aid of expressions [9]

$$\frac{\mu_s'}{\mu_a + \mu_s'} = \begin{cases} 1 - \left(\dfrac{1 - 4R_d - T_t}{1 - T_t}\right)^2, & \text{if} \quad \dfrac{R_d}{1 - T_t} < 0.1, \\[2ex] 1 - \dfrac{4}{9}\left(\dfrac{1 - R_d - T_t}{1 - T_t}\right)^2, & \text{if} \quad \dfrac{R_d}{1 - T_t} \geq 0.1, \end{cases}$$

$$(\mu_a + \mu_s')l = \begin{cases} -\dfrac{\ln T_t \ln(0.05)}{\ln R_d}, & \text{if} \quad R_d \leq 0.1, \\[2ex] 2^{1 + 5(R_d + T_t)}, & \text{if} \quad R_d > 0.1. \end{cases}$$

Here, R_d and T_t are the experimentally measured diffuse reflectance and total transmittance and l is the thickness of the sample of biological tissue.

(2) Calculation of the diffuse reflectance and total transmittance on the basis of the initial values of μ_a and μ_s' by the adding–doubling method [18].

(3) Comparison of the calculated and experimentally measured values of these parameters.

(4) Performance of the iteration procedure until the calculated data match the experimental ones within a specified accuracy.

As an iteration procedure, we employed the Nelder–Mead simplex method, described in detail in [19]. As a criterion of the completion of this procedure, we used the condition $|R_d^{\text{exp}} - R_d^{\text{calc}}|/R_d^{\text{exp}} + |T_t^{\text{exp}} - T_t^{\text{calc}}|/T_t^{\text{exp}} < 0.001$, where R_d^{exp}, R_d^{calc}, T_t^{exp}, and T_t^{calc} are, respectively, the experimentally measured and calculated diffuse reflectance and total transmittance.

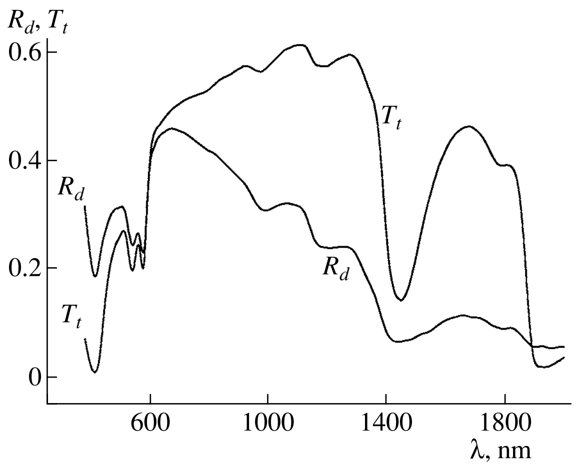

Fig. 1. Spectra of the total transmittance (T_t) and diffuse reflectance (R_d) of a sample of the mucous membrane of the human maxillary sinus. The thickness of the sample is 1.5 mm.

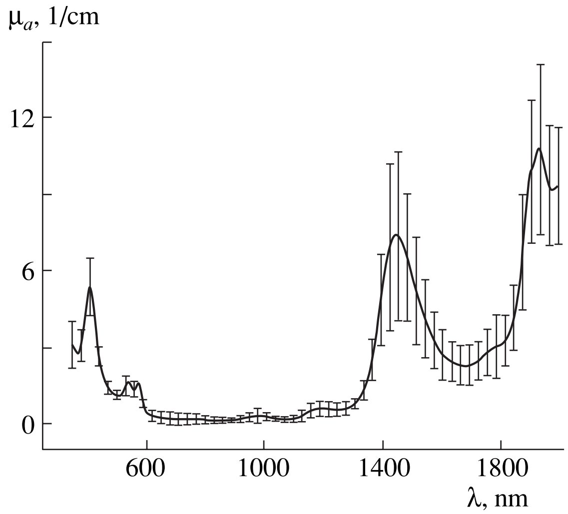

Fig. 2. Spectral dependence of the absorption coefficient μ_a of the mucous membrane of the maxillary sinus calculated from the experimental data by the inverse adding–doubling method. The vertical lines show the root-mean-square deviation.

RESULTS AND DISCUSSION

Figure 1 shows the typical spectra of the total transmittance and diffuse reflectance of a sample of the mucous membrane of the human maxillary sinus measured on the Cary-2415 spectrophotometer in the range 350–2000 nm. The thickness of the sample is 1.5 ± 0.1 mm. The shape of the spectra in the visible wavelength range is determined by the absorption bands of blood hemoglobin localized in the venous plexus of the proper layer of the mucous membrane and by the spectral dependence of the scattering coefficient. In the IR spectral range, the shape of the spectra is determined by the absorption bands of water. In both the visible and IR spectral ranges, collagen and elastin fibers of the proper layer of the mucous membrane play the role of the main scatterers. In the range from 350 to 650 nm, both spectra correlate well with each other in shape; i.e., the diffuse reflectance and the total transmittance simultaneously increase with increasing wavelength, showing sharp minima in the range of the hemoglobin absorption bands. However, upon further increase in the wavelength, the behavior of the reflectance and transmittance spectra is diametrically opposite; i.e., in the range 650–1300 nm, the transmittance of the mucous membrane increases, whereas its diffuse reflectance decreases. Such a behavior of the reflectance and transmittance spectra is typical of the majority of biological tissues in this spectral range (the so-called transparency window [3]) since, in the range 650–1300 nm, absorption in biological tissues is virtually absent and the shape of the reflectance and transmittance spectra is determined by the spectral dependence of the transport scattering coefficient. Upon a still further increase in the wavelength (from 1300 to 2000 nm), both spectra correlate with each other again; i.e., both the total transmittance and the diffuse reflectance decrease with increasing wavelength. The absorption of water in this spectral range manifests itself in the form of rather strong minima in the spectrum of the total transmittance; the shape of the diffuse reflectance spectrum is affected considerably less and the minima due to the water absorption are much less pronounced.

At present, the absorption spectra of hemoglobin and water have been well studied. In the visible wavelength range, hemoglobin (in the oxygenated state) is characterized by three absorption bands, with their maxima being located at 415, 540, and 575 nm [20]; the absorption of water in this range is negligibly small [21]. In the IR spectral range, the main chromophore is water, whose absorption bands are located at 976, 1197, 1450, 1787, and 1930 nm [22, 23]. It is clearly seen from Fig. 1 that the minima corresponding to the absorption bands of water and hemoglobin are observed both in the total transmittance spectrum of the sample of the mucous membrane and in its diffuse reflectance spectrum. At the same time, it should be noted that the absorption minima are most pronounced in the transmittance spectrum, which is especially clearly seen in the range of the strongest absorption bands, i.e., in the range of the Soret absorption band (415 nm) and in the range of the absorption bands of water at 1450 and 1930 nm.

Figures 2 and 3 show the spectra of the absorption and transport scattering coefficients calculated by the inverse adding–doubling method from the experimentally measured diffuse reflectance and total transmittance. An analysis of the absorption and scattering spectra presented shows that the inverse adding–doubling method can be applied in determining the optical parameters of these samples of the mucous membrane.

The maximum value of the transport free path length of photons observed at a wavelength of 1284 nm amounts to 2.2 mm. Taking into account the cross section of the probing beam incident on the sample of the biological tissue (5 × 5 mm), we obtain that the minimal size of the sample should be no less than 9.5 mm, which is fulfilled for the smallest of all the samples studied (whose area and size are about 100 mm^2 and 10 × 10 mm, respectively).

Figure 2 shows the absorption spectrum of the mucous membrane from the maxillary sinus in the spectral range from 350 to 2000 nm. The vertical lines indicate the standard deviation (*SD*) calculated by the formula

$$SD = \sqrt{\frac{\sum_{i=1}^{N}(\bar{\mu}_a - \mu_{ai})^2}{N(N-1)}},$$

where N is the number of samples under measurement, μ_{ai} is the absorption coefficient of the ith sample of biological tissue, and $\bar{\mu}_a$ is the average absorption coefficient at each spectral point calculated by the formula $\sum_{i=1}^{N}\mu_{ai}/N$. In the spectrum, absorption bands of blood oxyhemoglobin (415, 540, and 575 nm) and water (1450 and 1930 nm) are clearly seen. The absorption bands of water located at 976, 1197, and 1787 nm are considerably less pronounced. An increase in the standard deviation from the average values observed in the range of the absorption bands indicates that the blood and water contents in different samples of biological tissue are different.

Figure 3 presents the spectral dependence of the transport scattering coefficient of the mucous membrane of the maxillary sinuses of the nose. This dependence was obtained by averaging of the spectra of the transport scattering coefficient of the ten samples of mucous membrane. The vertical lines indicate the values of the standard deviation of the scattering characteristics of the mucous membrane obtained in the measurements. It is clearly seen that, with increasing wavelength, the transport scattering coefficient decreases fairly smoothly, which, on the whole, corresponds to the general spectral behavior of the scattering characteristics of biological tissues [4, 24–26]. However, in the range of the strong absorption bands (415, 1450, and 1930 nm), the shape of the spectrum is distorted; i.e., it deviates from a monotonic dependence. At the same time, the absorption bands of water at 976, 1197, and 1787 nm are not observed in the spectrum of scattering of the mucous membrane.

The deviation of the spectrum of the transport scattering coefficient from a monotonic dependence is explained by the fact that the absorption bands of water significantly differently affect the spectrum of the scattering coefficient of the biological tissue μ_s and the spectral dependence of the scattering anisotropy factor g, which form the spectrum of the transport scattering coefficient μ_s'. By using the liver as an example, it was experimentally shown in [27] that, in the range of the absorption bands of water at 1450 and 1930 nm, the spectrum virtually does not deviate from a monotonic dependence. At the same time, in the range of these bands, a considerable decrease in the scattering anisotropy factor is observed, which inevitably leads to an increase in the transport scattering coefficient $\mu_s' = \mu_s(1-g)$ and to the manifestation of the water absorption bands in its spectrum. In this case, a decrease in the scattering anisotropy factor in the range of the absorption bands is proportional to the intensity of these bands. Thus, from Fig. 4 in [27], it is clearly seen that the anisotropy factor is decreased significantly more greatly in the range of the more intense absorption band at 1930 nm than in the range of the band at 1450 nm (see also Fig. 2 of the present study). This explains the absence of deformation of the spectrum of the transport scattering coefficient of the mucous membrane in the range of the relatively weak absorption bands located at 976, 1197, and 1787 nm. A similar effect was also observed in [28] in the range of the absorption band of water at 1450 nm in studying the optical characteristics of the dermis. The shift of the band maxima in the spectrum of the transport scattering coefficient of the mucous membrane with respect to the absorption bands of water and hemoglobin is seemingly related to an anomalous dispersion of the real part of the complex refractive index in the range of the absorption bands.

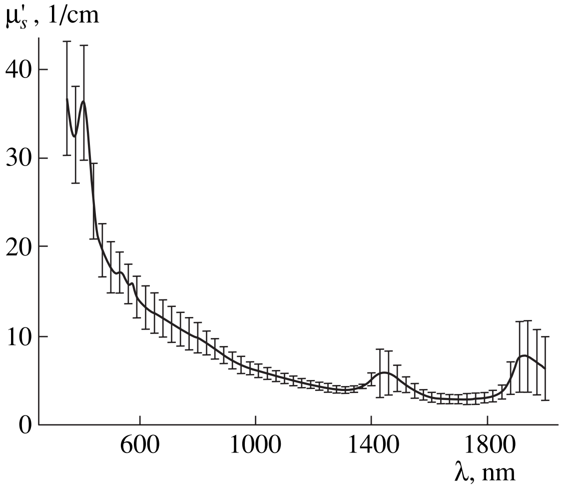

Fig. 3. Spectral dependence of the transport scattering coefficient μ_s' of the mucous membrane of the maxillary sinus calculated from the experimental data by the inverse adding–doubling method. The vertical lines show the standard deviation.

The penetration depth of light into a biological tissue is an important characteristic for the correct determination of the irradiation dose in photochemical and photodynamic therapy of various diseases [2]. We esti-

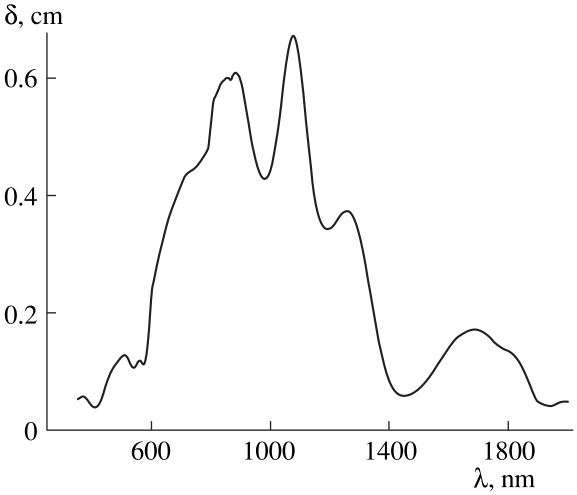

Fig. 4. Penetration depth of radiation (δ) into the mucous membrane as a function of the wavelength calculated from the experimental data presented in Figs. 2 and 3.

mated the penetration depth of light (δ) using the relation reported in [27]:

$$\delta = 1/\sqrt{3\mu_a(\mu_a + \mu_s')}.$$

The result of the estimation is shown in Fig. 4. The expression used is valid when the surface of a biological tissue is uniformly illuminated by radiation from a point source placed at some distance from the surface, which corresponds to the real conditions of photodynamic therapy of purulent maxillary sinusitis since, in this case, the irradiating probe is introduced directly into the maxillary sinus without any contact with the surface of the mucous membrane.

The penetration depth of radiation into the mucous membrane of the maxillary sinus was calculated using the absorption coefficients shown in Fig. 2 and the transport scattering coefficients presented in Fig. 3. It is seen from Fig. 4 that, depending on the wavelength of the probing radiation, the penetration depth varies considerably. The value of this parameter is maximal in the spectral ranges 800–900 nm and 1000–1100 nm, in which the radiation penetrates to depths of up to 6–6.5 mm, which significantly exceeds the thickness of the mucous membrane both in the normal and in the pathological state. In the range of radiation of a He–Ne laser (633 nm) and a diode laser (660 nm), which are most frequently used in photodynamic therapy [2, 4], the penetration depth amounts to 3–3.5 mm, which also exceeds the thickness of the mucous membrane.

CONCLUSIONS

The development of methods of photodynamic therapy of acute and chronic maxillary sinusitis requires knowledge of the optical characteristics of mucous tissues of the nasal cavity in a wide wavelength range. In this paper, the optical parameters of the mucous membrane of the maxillary sinus of the nose are experimentally studied. The experiments were performed in vitro on a Cary-2415 spectrophotometer in the spectral range 350–2000 nm. On the basis of the experimentally measured diffuse reflectance and total transmittance spectra, the spectra of the absorption and transport scattering coefficients are calculated in terms of the inverse adding–doubling method.

The appearance of bands in the spectrum of the transport scattering coefficient in the range of the absorption bands of water and hemoglobin is explained. The penetration depth of optical radiation into the mucous membrane is estimated.

Our results can be used for the development of new methods and optimization of the existing ones for photodynamic therapy of rhinological diseases, in particular, acute and chronic maxillary sinusitis.

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