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Effect of laser UV radiation on the eye scleral tissue in patients with open-angle glaucoma

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Abstract. **We report the results of an experimental study of the effect of short-pulse laser UV radiation on the eye scleral tissue. As samples, we used isolated flaps of the eye scleral tissue from the patients with open-angle glaucoma of the second and third stages. The impact was implemented using the radiation of an excimer XeCl laser with a wavelength of 308 nm and a laser with a wavelength tunable within from 210 to 355 nm. Depending on the problem to be solved, the energy density on the surface of the irradiated tissue varied from a fraction of mJ cm^{-2} to 15 J cm^{-2}. For the first time we studied the optical properties of the intraocular fluid in the UV and blue spectral range. The study of the ablation process under the action of radiation with a wavelength of 308 nm showed that the rate of material evaporation can vary within 24 %–30 % at an energy density above 7 J cm^{-2}, depending on the glaucoma stage and the individual features of a patient. The excitation–emission matrices of laser-induced fluorescence (LIF) of the eye scleral tissue were studied experimentally using a laser with a wavelength tuned in the range 210–355 nm. We found the differences in the LIF spectra caused by the excitation wavelength and the open-angle glaucoma stage.**

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1. Introduction

Presently, the use of lasers is an essential part of up-to-date medical practice [1–4]. Most laser applications include diagnostics, therapy, and surgery. The main advantages of laser methods are low invasiveness, high operation rate, short rehabilitation period, and reduced number of post-operative complications. The lasers that operate in different spectral regions (UV, visible, and IR) find application in different fields of medicine. In ophthalmology, pulsed lasers that radiate in the UV spectral range (mainly excimer lasers, as well as the fourth and the fifth harmonics of Nd:YAG lasers) are being extensively used today. Different methods have been developed and a variety of laser systems have been constructed to implement them and have become widely used [5]. The main fields of laser application in ophthalmology are the correction of refraction anomalies, the treatment of virus diseases, and anti-glaucoma operations.

The laser treatment of glaucoma proved itself long ago to be one of the most painless and efficient methods in the world [6–11]. Three stages of open-angle glaucoma are commonly distinguished. As a rule, the first stage is treated using medicaments. In the second and third stages of the disease, a number of procedures can be applied [12], including the use of lasers. Independent of the laser type, the treatment of glaucoma is aimed at normalising the intraocular fluid drainage and, therefore, decreasing the intraocular pressure. The laser can be used both as an independent method of glaucoma treatment and in combination with microsurgical anti-glaucoma operation [13]. According to the method of execution, the operations can be ab interno or ab externo, when the appropriate sections and tissues of the eye are affected from inside or from the outside of the eye, respectively. Laser trabeculotomy [14] and laser trabeculoplastics [15] that are rather widely used today can be considered as operations of the first kind. These operations are commonly performed using argon or diode lasers of the visible spectral range. Laser nonpenetrating deep sclerectomy belongs to the second kind [16]. Earlier we have proposed and implemented a new method of open-angle glaucoma laser surgery based on this technique [17].

The execution of laser anti-glaucoma operations is related to the impact of high-power laser radiation on different parts of the eye, which are biological tissues. Therefore, in the development of the operation technologies it is necessary to

determine optimal characteristics of the radiation, such as the wavelength, the operation mode (pulsed or continuous-wave), as well as the energy density and the radiation intensity at the surface of the processed tissue, depending on its optical properties. In papers [18, 19] we have shown that in the course of laser anti-glaucoma operations *ab externo* it is optimal to use the radiation of an excimer XeCl laser with a wavelength of 308 nm. Preliminary estimates were made for the ablation threshold and evaporation rate of the human eye scleral tissue depending on the laser energy density at the surface.

At the same time, a number of questions related to the change of optical properties of eye scleral tissue under the development of open-angle glaucoma still remain open. In this connection, the aim of the present paper is to study the optical properties of the eye scleral tissue experimentally in patients with open-angle glaucoma at its different stages.

2. Experimental setup

We studied the impact of high-power short-pulse laser UV radiation on the scleral tissues of human eye, as well as the optical characteristics of these tissues subjected to the low-intensity pulsed laser UV radiation that has already proved its efficiency in the diagnostics of the eye tissue condition [20].

In the first case, we used an excimer XeCl laser with λ = 308 nm, a pulse energy up to 100 mJ and a FWHM duration of 15±2 ns. We studied the dependence of the evaporation rate for the isolated flaps of scleral tissue from two patients with open-angle glaucoma of the second stage. The optical scheme of the experimental setup is presented in Fig. 1.

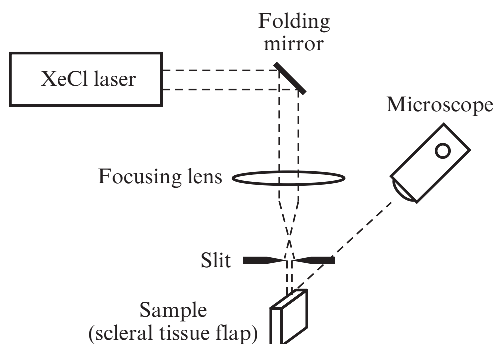

Figure 1. Optical scheme of the experimental setup for studying the impact of high-power short-pulse UV laser radiation on the eye scleral tissue.

By means of a folding mirror, the radiation of the XeCl laser was directed onto the focusing lens. Then through the slit having a width of ~0.05 mm the radiation arrived at the sample (scleral tissue), the face of which was perpendicular to the laser beam and located at a minimal distance from the slit to exclude a change in the laser radiation intensity due to the beam divergence. The observation of the ablation process was implemented using a microscope. The evaporation rate was determined by counting the number of laser pulses necessary to evaporate the sclera flap to the given depth.

Figure 2 presents a typical profile of the cavern produced by laser-induced evaporation, the so-called laser wound.

In the second case, we recorded the spectra of laser-induced fluorescence (LIF) of the eye scleral tissue affected by open-angle glaucoma of the second and third stage. To study the LIF in the sample, we used a spectroscopic measurement stand (Fig. 3) based on a repetitively pulsed Vibrant HE 355 II+UV laser (*1*) (Opotek, USA) with optical parametric frequency conversion that generated the laser radiation tunable within the wavelength range 210–355 nm. After a spatial optical filter [elements (*2–4*)], part of radiation (~5%) was directed to a photodiode (*7*) by means of a silica plate (*5*). After passing through the plate (*5*), a mirror (*8*) directed the laser beam to a studied sample (*9*), placed in the cuvette made of a weakly fluorescing material (stainless steel). A spherical mirror (*10*) focused the sample fluorescence light at the slit of an Action SP2300 spectrometer (*12*) (Princeton Instruments, USA). The spectrum was detected by a cooled CCD matrix with open Pixis 256 electrodes (Princeton Instruments, USA). The input slit of the spectrometer was located in the focal plane of a mirror (*10*), so that the measured signal was averaged over the sample surface. The LIF spectra of the samples were recorded at fixed wavelengths of the exciting laser radiation, varying in the range 210–350 nm with a step of 10 nm. It was not necessary to perform measurements with higher resolution, since the characteristic exciting linewidth for biological substances is at least 40 nm. For each exciting wavelength, the spectrum recording was repeated 5 times. Then each spectrum was normalised to the total absorbed energy of laser radiation, averaged and normalised to the spectral sensitivity of the device, measured using deuterium and tungsten lamps. The energy density of the laser pulse was restricted to a value

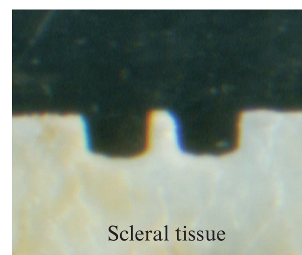

Figure 2. Profile of a cavern produced by laser radiation with λ = 308 nm in the eye scleral tissue.

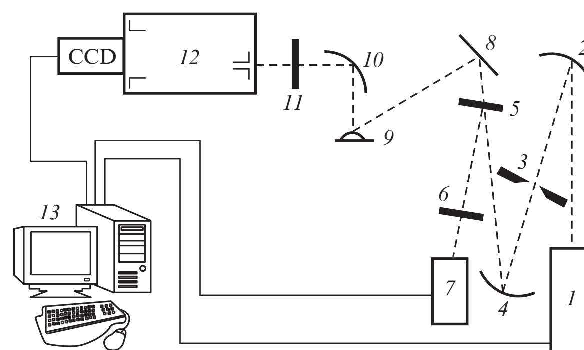

Figure 3. Schematic of the experimental setup for measuring LIF excitation–emission matrices:
(*1*) laser; (*2*) spherical mirror condenser; (*3*) aperture; (*4*) spherical mirror collimator; (*5*) semitransparent mirror; (*6*) diffusing plate; (*7*) FD-24 photodiode; (*8*) mirror; (*9*) sample; (*10*) focusing spherical mirror; (*11*) filter cutting off laser radiation; (*12*) spectrometer; (*13*) computer.

of 200 J cm^{-2}, since above this value the dependence of the maximum in the fluorescence intensity spectrum on the laser pulse energy becomes nonlinear, thus making the normalisation to the laser pulse energy incorrect. In our case after the normalisation to the reference signal, the root-mean-square deviation of intensities of the measured fluorescence did not exceed 5%.

To measure the excitation–emission matrices we used dried resected sclera fragments. Each sample was a flap having irregular shape with a characteristic size of 3–4 mm and a thickness of a few tenths of a millimetre. The spectra of each fragment were measured from each side two times, smoothing it out between the measurements to minimise the fluctuations caused by the variability of the radiation collection site.

3. Results and discussion

The effect of high-power UV laser radiation on the scleral tissue was studied by assuming that the evaporation rate of the dried and humid tissue flaps does not differ strongly, since the fluid wetting the scleral tissue consists mainly of water. To confirm the assumption, we preliminarily studied the dependence of the absorption coefficient of the intraocular fluid (IOF) solution in the distilled water (miliQ) in the proportion 1 : 10 on the laser radiation wavelength by means of a Cary 300 bio spectrometer (Varian Inc., USA). From the results of the study, the IOF transmission spectrum in the UV and blue spectral regions was obtained for the first time. It was found that at a wavelength of 308 nm it practically does not absorb the laser radiation (Fig. 4). Thus, the possible presence of the liquid, wetting the scleral tissue, does not affect the ablation process.

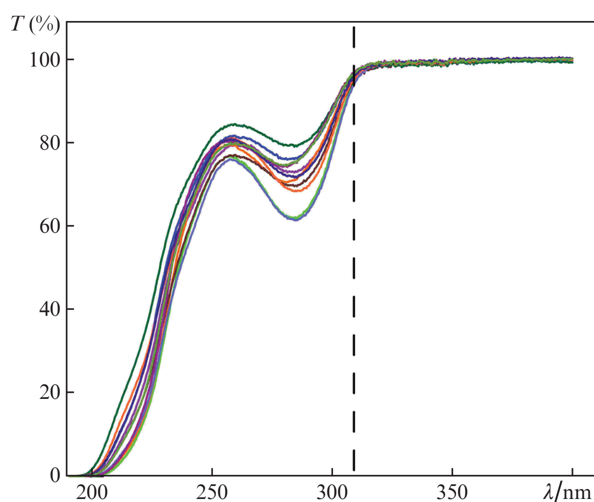

Figure 4. Transmission spectra of several samples of intraocular fluid. The vertical dashed line indicates the wavelength 308 nm. The cuvette thickness is 10 mm.

The ablation threshold of the scleral tissue for λ = 308 nm was determined by us previously [18, 19] as nearly 260 mJ cm^{-2}; therefore, in the present case the energy density was chosen obviously greater, from 1 to 20 J cm^{-2}. The results of the studies are presented in Fig. 5. It is seen that at an energy density increasing from 2 J cm^{-2} the thickness of the evaporated layer of the scleral tissue continuously grows. In two studied

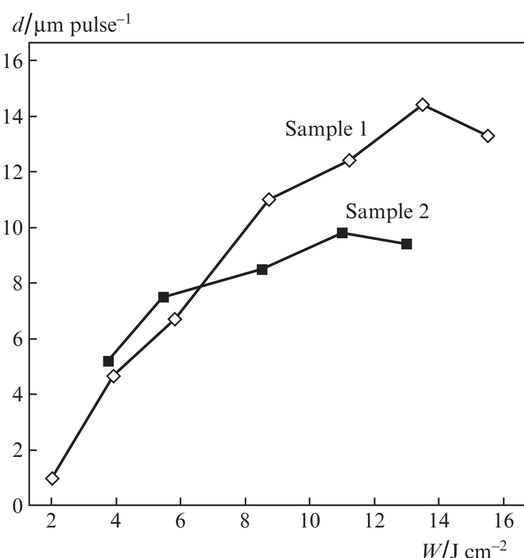

Figure 5. Dependences of the evaporation rate on the laser energy density for two different samples of the eye scleral tissue from a patient with the second stage of open-angle glaucoma.

samples, the maximal evaporation rates were strongly different. For the first sample, this rate was 14 μm pulse^{-1} in the range of energy densities 13–14 J cm^{-2}. When the energy density exceeded 15 J cm^{-2}, the thickness of the layer evaporated by a single pulse began to decrease. For the second sample the maximal evaporation rate did not exceed 9–10 μm pulse^{-1} at an energy density of 11 J cm^{-2}. This fact can be explained by the dependence of biotissue optical properties on the individual difference between patients. Therefore, for getting a more complete and reliable picture we plan additional studies aimed at accumulating the statistical data, from which it would be possible to determine the mean rates of tissue evaporation and the corresponding energy density for the second stage of open-angle glaucoma. For anti-glaucoma operations it seems sufficient to deal with the energy densities 6–11 J cm^{-2}, since such values already provide a relatively high rate of material evaporation that will weakly affect the total operation time.

The study of the scleral tissue LIF was aimed to reveal the effect of open-angle glaucoma development on the LIF spectrum. For the first experiments, we used six samples of the scleral tissue from the patients with open-angle glaucoma, three with the second stage and three with the third stage. Typical LIF spectra are presented in Fig. 6. The spectra were analysed using the principal component analysis. All spectra could be well described by a sum of four components, presented in Fig. 7. Since the components are sign-alternating, the spectra of real fluorophores are their linear combinations, and since any positive definite linear combination of principal component spectra can represent a fluorophore spectrum, their choice is determined by certain additional factors [21]. As a determining factor, we have chosen the peak width of the resulting function (characterised by the ratio of the function maximal value to its integral). The continuous spectra result from the broadening of individual lines, and so the narrowest peaks are expected to be most alike the initial ones. Using the enumerative techniques we obtained the spectra in the form of narrow peaks presented in Fig. 7b.

Typical contributions of the components to the LIF spectra depending on the excitation wavelength are presented in Fig. 8.

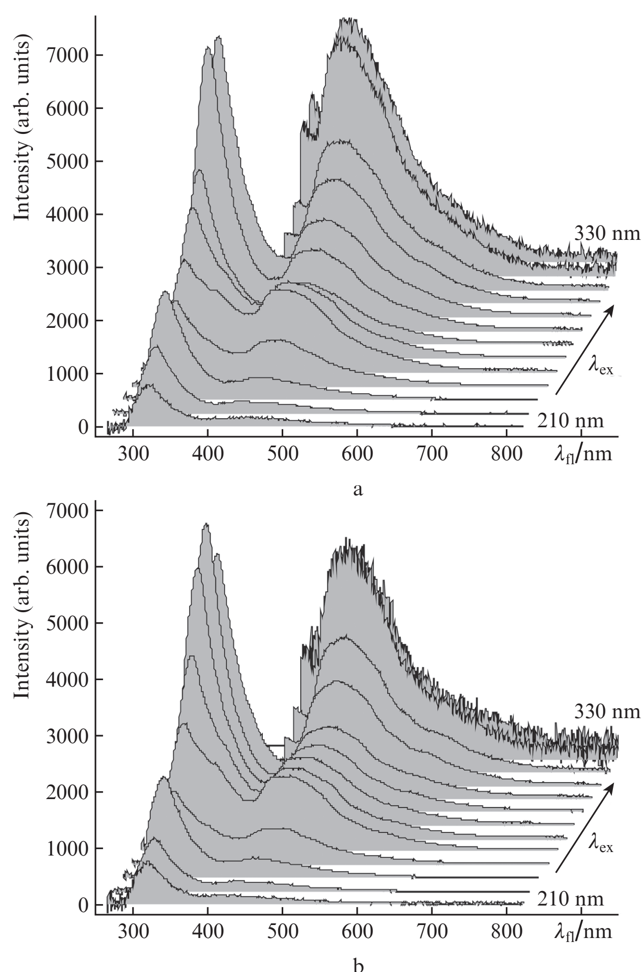

Figure 6. Examples of excitation–emission matrices of scleral tissues for the open-angle glaucoma of (a) the second and (b) the third stages.

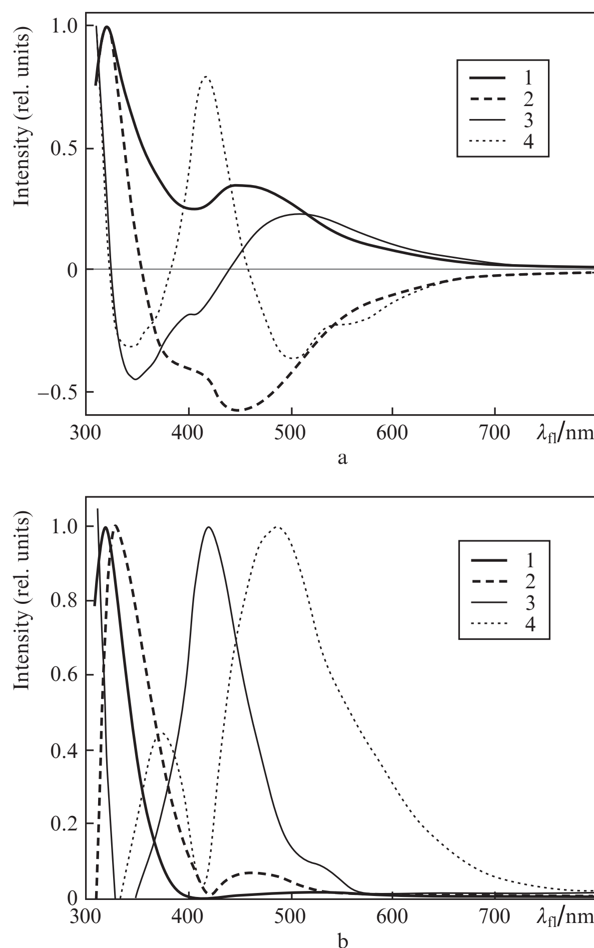

Figure 7. (a) Spectra of principal components and (b) narrow peaks of the fluorescence intensity obtained from them.

It is seen that the excitation spectra for the third and the fourth components are close enough, the dips in the fluorescence spectra for the second and the fourth components coinciding with the peak of the third component. Based on these facts and the literature data, one can attribute this peak (the third one) to the reabsorption of fluorescence in oxyhaemoglobin. The reabsorption is a nonlinear effect, so that the LIF spectrum is a result of multiplying the absorption and fluorescence spectra. For each sample, the presence of reabsorption should lead to the appearance of an independent component. However, in the present case the contribution from the reabsorption to the LIF spectra is small and yields only an averaged correction similar for all spectra in the first approximation. Thus, the total contribution of the third component must be negative, and the spectra of real fluorophores are linear combinations of the third component with any of the rest ones. However, since these linear transformations are unable to affect the ratio of other components and the reabsorption is related to the presence of residual blood in the samples rather than pathology, the third component can be simply excluded from consideration.

Since the contribution of each of the remaining components can be practically zero with respect to the contribution of the others, one can conclude that their spectra coincide with those of real fluorophores to the accuracy determined by reabsorption. The maxima of the first and second components are located at $\lambda = 321$ nm and $\lambda = 332$ nm, respectively, and are caused by different states of tryptophan (depending on the environment, the tryptophan fluorescence peak can be at any wavelength within the range 320–360 nm). The fourth component is likely to belong to the elastic component of the tissue. Its spectrum is not like the spectra of the known transverse bonds in collagen and elastin (with the maximum at 380 nm); however, it is also met in the spectra of elastic tissues [22].

Since the exciting laser radiation in biological tissues is also absorbed by nonfluorescent chromophores, the excitation spectra of every chromophore depend on the contributions both from chromophores and other fluorophores and, therefore have no simple representation. Thus, it is reasonable to compare only the relative contribution of the components. As seen from Fig. 8, the maximal difference of contributions from the components to the LIF spectra of the samples affected by the disease at different stages takes place at excitation wavelengths of 230 nm and 280 nm. Assuming the elastic carcass to be the base tissue component, we compare the contributions of the fourth component with those of two other components. It is simple to presented it graphically using the ratios of the intensity of component 4 (I_4) to those of components 1 (I_1) and 2 (I_2) as coordinates, describing the LIF spectrum (Fig. 9).

Using this representation, one can distinguish the LIF spectra reliably enough between the stages of open-angle

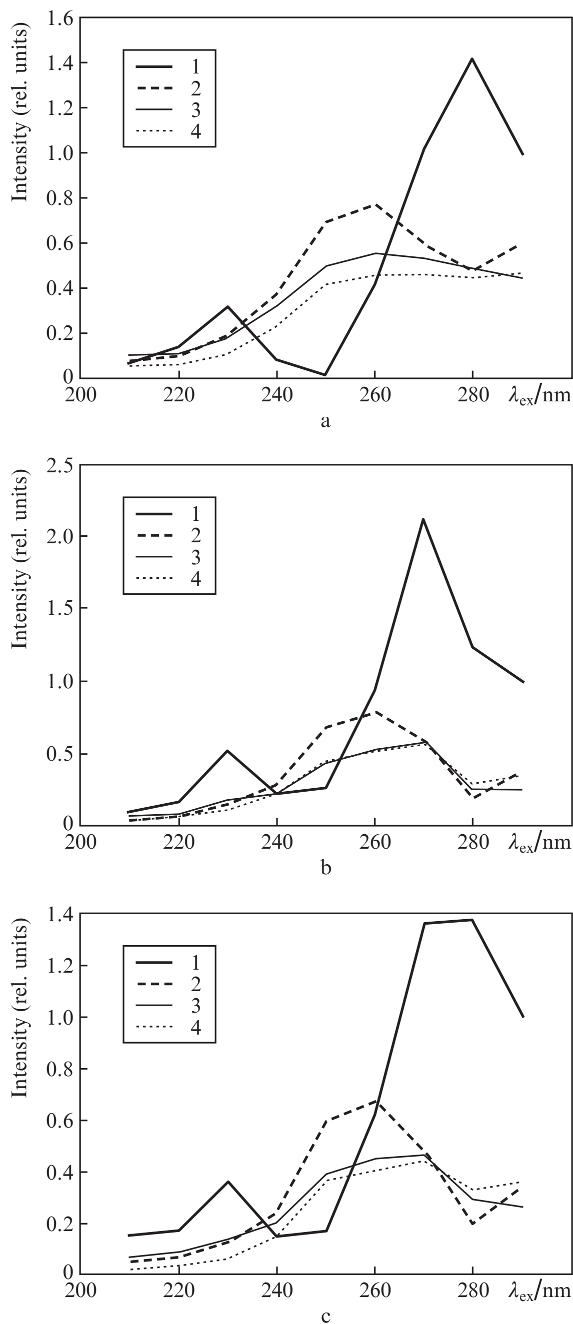

Figure 8. Examples of contributions of narrow peaks to the LIF spectra of the samples of scleral tissues taken from three patients with open-angle glaucoma of (a) the second and (b, c) the third stages as functions of the excitation wavelength.

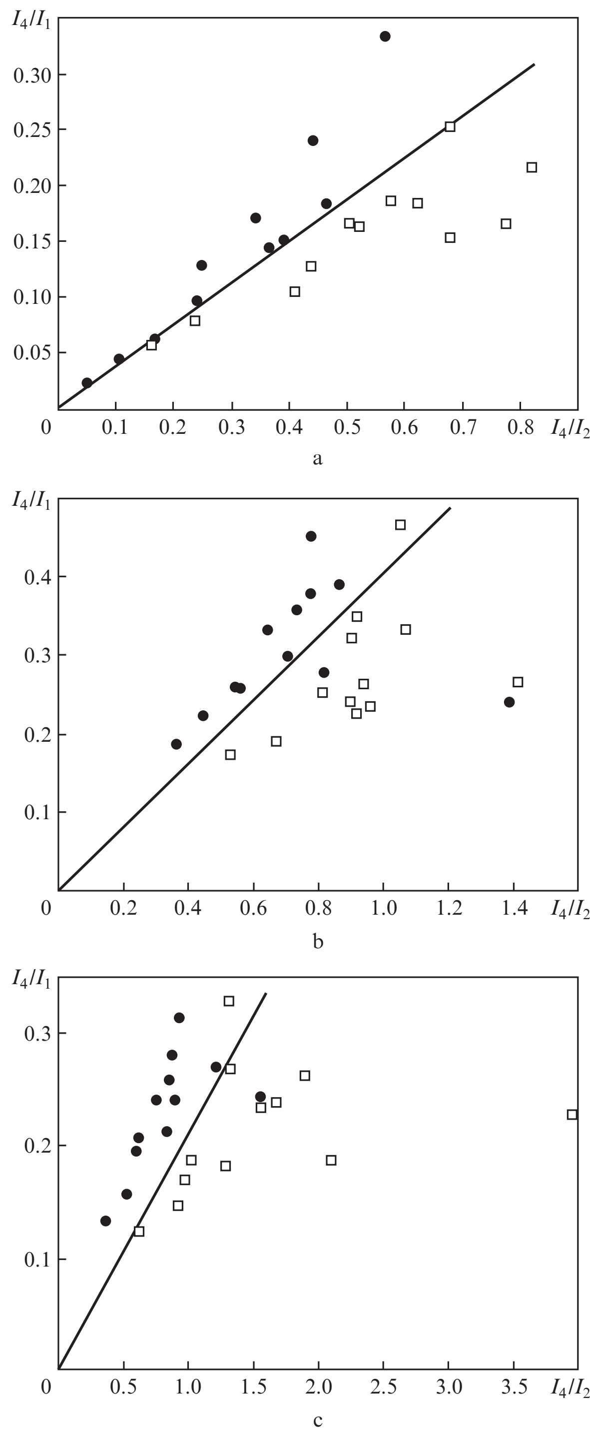

Figure 9. Relative contribution of fluorescent components to the LIF spectra of the scleral tissues of the patients suffering from the glaucoma of (●) the second and (□) the third stages at λ_{ex} = (a) 230 nm, (b) 270 nm, and (c) 280 nm.

glaucoma (in the present case between the second and the third stage). Moreover, the obtained data on the difference in the LIF spectra allow an assumption that during the development of open-angle glaucoma the structural changes occur in the scleral tissue, too. To improve the reliability of the used method we plan to perform further studies for accumulating statistical data.

4. Conclusions

We have studied the optical properties of the eye scleral tissue in patients with open-angle glaucoma of different stages. For the first time the spectrum of intraocular fluid transmission is obtained in the UV and blue spectral regions. It is shown that at a wavelength of 308 nm the IOF practically does not absorb laser radiation. Thus, the possible presence of the fluid wetting the scleral tissue does not affect the process of tissue ablation by the radiation of the excimer XeCl laser.

The studies of the human eye scleral tissue ablation by the radiation at a wavelength of 308 nm have shown that depending

on the glaucoma stage and the patient's individual features the evaporation rate can vary within 25%–30% at an energy density above 7 J cm^{-2}.

The LIF excitation–emission matrices of the human eye scleral tissue have been experimentally studied using the laser radiation with a wavelength tuned within the range 210–355 nm. The differences in the LIF spectra as a function of the excitation wavelength and open-angle glaucoma stage have been demonstrated, which can be an evidence of structural changes in the scleral tissue.

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