Application of the LIF method to studying the UV-radiation effect on biological tissues

P.M. Larionov,[1] A.N. Malov,[2] N.A. Maslov,[2] and A.M. Orishich[2]

[1] *Institute of Blood Circulation Pathology, Ministry of Public Health of the Russian Federation*
[2] *Institute of Theoretical and Applied Mechanics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk*

Received January 27, 2000

The method of laser-induced fluorescence (LIF) is widely used in investigating biological tissues. It uses pulsed UV-laser radiation to excite fluorescence in the objects under study. But the radiation itself may cause some changes in the tissues irradiated. The results of our experiments have shown some changes of LIF spectrum of a cross-striated muscle under its exposure to the UV-radiation. In particular, a noticeable attenuation of intensity within tryptophan fluorescence band (330 nm) takes place. The intensity is shown to depend on the number of pulses. At lower energy of the exciting radiation the intensity falls off more slowly. A quantitative information is obtained on the tryptophan inactivation cross section. The conclusion on commensuration of the inactivation cross sections of tryptophan and cystine within 248 nm wavelength is proved. Nonetheless, although some changes take place in the tissues exposed to laser radiation, they have not time to exceed the magnitude of random scatter for the period needed for the LIF spectra recording (~ 30 pulses), so these changes do not influence the final result.

At present, pulsed UV-lasers are widely used in clinical practice as surgery and diagnostic means. The excimer lasers play therewith particularly important part. They are much promising tools in therapy of some ophthalmologic diseases,[1,2] angioplasty at treating coronary atherosclerosis,[3] cardiosurgery at laser revascularization of myocardium,[4] treating bones,[5] and so on. In devising the treatment methods with the use of UV radiation, the photobiological processes in macromolecules (biopolymers – proteins and DNA), cells, and other materials of the biological tissues, exposed to direct and scattered light should be taken into account.[6]

The possibility of using laser-induced fluorescence excited by the excimer laser radiation in diagnosis of the human heart tissue calcinosis of various degree was investigated in Ref. 7. The use of shortwave (λ = = 248 nm) radiation for excitation allows one to study the luminescence in a wide spectral range and observe significant changes in LIF spectra of cardiomuscle attacked by calcinosis. The LIF-spectra of calcium-saturated tissues vary depending on the saturation degree, what opens a new prospects for the LIF application to diagnose the degree of calcinosis injury, when conducting the open-heart or plastic operations.

In the experiments described in Ref. 7 the laser radiation intensity was 20–50 times lower than the photoablation threshold, so the radiation did not cause macroscopic changes. But the energy of UV radiation quantum at 248 nm wavelength is rather high and can initiate the destruction-modifying reactions,[6] resulting in changes of some tissue composition and, correspondingly, its LIF spectrum.

The goal of this work was studying the effect of UV radiation (λ = 248 nm) on biological tissue.

To investigate the fluorescence spectra of some biological tissue, the experimental setup was designed. A gas-discharge KrF excimer laser emitting at the wavelength λ = 248 nm (or XeCl one at λ = 308 nm) and pulse energy of 10–15 mJ was used as the radiation source. The radiation pulse length was 5 ns and pulse power ~ $2 \cdot 10^6$ W. The sensing radiation was attenuated with a set of filters. Laser radiation was directed by means of optical system to a table made of weakly fluorescent material. The tissues under study were placed on the table. The investigation area was ~ 10 mm^2. By means of a mirror and a lens this fragment of the tissue was imaged on the input slit of a monochromator equipped with a photodetector at its output. A DMR–4 double prism monochromator with the inverse linear dispersion about 4 nm/mm at 400 nm wavelength and photoelectric multiplier FEU–106 sensitive in the 170–950 nm spectral range were used in the setup. A portion of laser radiation was directed by means of a beam splitter to the reference receiver, i.e., coaxial photocell FEK–22 sensitive in the 220–650 nm spectral range. The photoelectric currents were integrated in capacitors, and then entered into the signal processing system CAMAC, and then recorded in a personal computer.

The experiments were conducted in two operation modes. The first one included the spectrum scanning at 0.5–5 nm drop-out time. The spectral width of the monochromator entrance provided for spectral resolution of 0.5–5 nm in the scanning range (the drop-out time). The signal from the PMT was normalized to the reference signal in order to eliminate the effect of the laser power pulsing. In processing the spectra, the signal was normalized by the PMT sensitivity depending on the wavelength and corrected for the

0235-6880/00/03 279-04 $02.00

nonlinearity of the monochromator dispersion characteristics over spectrum.

In the second operation mode the monochromator is tuned to a fixed wavelength and then the intensity of fluorescence is being recorded as a function of time (number of sounding pulses).

Experimental results

The effect of UV radiation dose on the spectrum of laser-induced fluorescence of a biological material exposed to the radiation was studied using a cross-striated muscle as an example.

Figure 1 presents the LIF spectra of the muscle before (*1*) and after (*2*) its irradiation by 2000 laser pulses. The irradiation dose Q was 10 mJ/cm^2 per pulse. Different spectral regions responded to the irradiation in different ways. Noticeable (by more than 2 times) attenuation of the fluorescence band centered at 330 nm took place. At the same time, the LIF intensity at 450 nm increased.

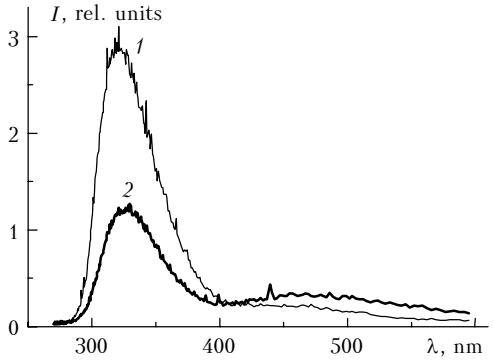

Fig. 1.

We have studied the variation of the fluorescence intensity of the cross-striated muscle at 330 nm wavelength depending on the number of radiation pulses. Figure 2 presents the variations of the LIF relative intensity for this muscle exposed to laser radiation at $\lambda = 248$ nm and $Q = 10$ (*1*) and 2.5 mJ/cm^2 (*2*) per pulse. As is seen, the rate of LIF intensity decrease depends on the irradiation dose.

We have measured the rate of cells destruction as a function of the irradiation dose. The results are shown in Fig. 3. High rate of reduction of the number of living cells (by e times) during first 500 pulses followed by its deceleration is well seen in the figure. Simultaneously, we measured the natural reduction of the number of living cells occurred during the experiment. Thus, in the beginning of the experiment the concentration of living cells was 10200 per 1 ml. In 24 hours it became 6100 per 1 ml in the sample free of the UV irradiation. In the samples exposed to the radiation the concentration of living cells has decreased by more than 5 times for 2.5 hours. This allows us to separate the processes of natural destruction of the cells and that caused by the UV-irradiation.

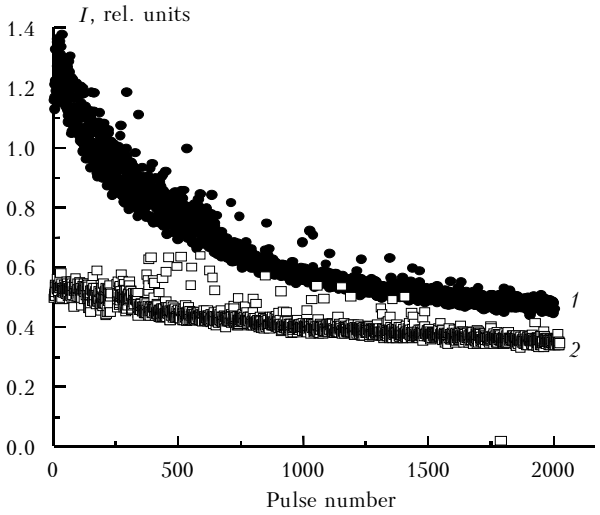

Fig. 2.

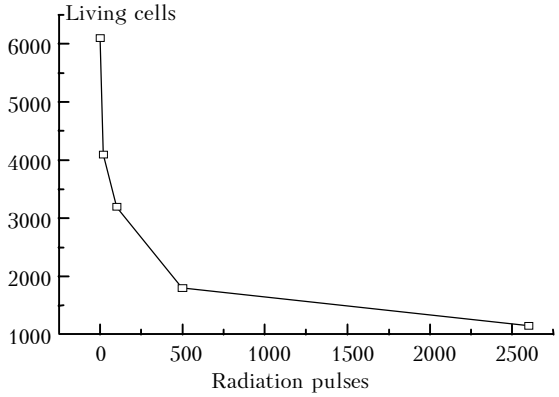

Fig. 3. The amount of living blood lymph cells as a function of the number of radiation pulses. The samples were exposed to laser radiation at $\lambda = 248$ nm and energy density of 10 mJ/cm^2.

Discussion

Analysis of LIF spectra of some normal tissue (Fig. 4) shows that three bands can be distinguished in the fluorescence of a cross-striated muscle: 330, 370, and 450 nm, where the 330 nm short-wave one prevails. The 450 nm band is quite distinct but relatively weak, whereas the 370 nm band is damped by more strong adjacent 330 nm band.

It is well known that the 330 nm band intensity is caused by the luminescence of tryptophan or proteins containing the tryptophan.[6,8,9] When studying the LIF spectra of aorta excited by 308 nm radiation, it was found[10] that the 370 and 450 nm bands are related to the fiber connective structures – collagen and elastin present in the tissue; and a dip between these bands can be explained by the fluorescence reabsorption by hemoglobin. Collagen and elastin are the protein connective tissues containing many other chromophores in addition to tryptophan.[11] The quantum yield of

fluorescence of these tissues is comparable with that of tryptophan, and it contributes significantly to the LIF spectra of the tissues that contain those. The LIF spectra of collagen and elastin are rather similar and have maxima at 383 and 379 nm, respectively. But they have noticeably different bandwidths (40 and 70 nm). Oxyhemoglobin has the absorption maximum in 418 nm region, which well coincides with the LIF intensity minimum (Fig. 4).

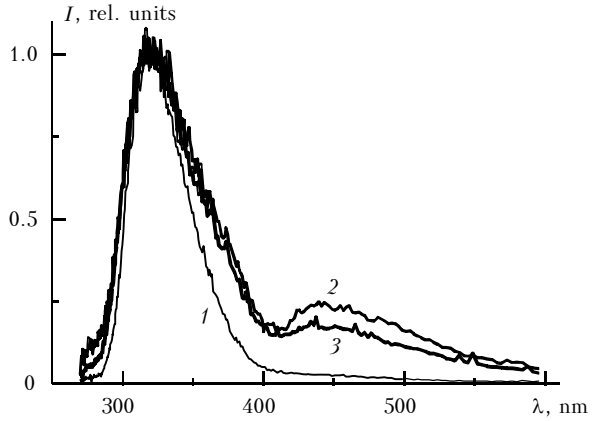

Fig. 4. The LIF spectra of sound tissues of an animal (pig). The wavelength λ of LIF excitation is 248 nm: (*1*) the heart muscle; (*2*) the aorta wall; (*3*) the cardiac valve.

Thus, evidently, the LIF spectra of a sound tissue (Fig. 4) are defined by the fluorescence contribution from tryptophan, collagen, and elastin, as well as absorption by oxyhemoglobin.

It is well-known that destructive-modifying reactions are initiated by UV light and that nucleic acids and proteins play an important part of acceptors of biologically active light.[6]

Basic chromophores of the nucleic acids are purine (adenine and cytosine) and pyrimidine nitrous bases of nucleotides. The absorption of light is mainly due to the electron system of rings with the band whose maximum is in the 255–270 nm range. Interaction of UV light with nucleic acids results in appearance of electronically excited states of different bases, which practically do not luminesce, i.e., the rate constant of the energy thermal dissipation is high for all bases.

Basic protein chromophores are the residuals of aromatic amino acids, first of all of tryptophan, to significantly less degree – tyrosine, phenylalanine, and cystine. The cystine photochemistry manifests itself in augmentation of quantum yield of inactivation of the cystine-rich proteines. In this case the tryptophan photoinactivation dominates under exposure to longwave irradiation (270–310 nm), and the cystine one – under exposure to shortwave (240–260 nm) irradiation. In the region of our interest (248 nm) the absorption by cystine is comparable with that of tryptophan. But cystine does not fluoresce and can not transfer the absorbed energy to tryptophan via migration. This conclusion on decisive role of tryptophan in the LIF-diagnosis of biotissue is proved by our experimental results. We have shown that under exposure to UV light the tryptophan deactivation takes place (Fig. 1, 330 nm band).

The tryptophan inactivation goes on according to single-quantum single-impact mechanism. In this case the number of survived molecules is described by exponential function derived from the Poisson distribution

$$N = N_0 \exp(-\sigma D),$$

where N_0 is the number of initial molecules; N is the number of survived molecules; σ is the inactivation cross section; D is the dose. $D = Qn/h\nu$, where n is the number of laser pulses; $h\nu$ is the energy of light quantum; Q is the radiation dose per pulse, J/cm^2.

The agreement between the above expression and dependence of the LIF intensity on dose (Fig. 2) allows us to come to a conclusion that the tryptophan photoinactivation in our experiments goes on in accordance with the single-quantum single-impact mechanism. Comparison of the experimental and theoretical dependences (Fig. 2) yielded the estimate of the tryptophan inactivation cross section to be $\sigma \cong 3.2 \cdot 10^{-19}$ cm^2. The cystine inactivation cross section value from Ref. 12 ($3.4 \cdot 10^{-19}$ cm^2 at 248 nm wavelength) well agrees with our experimental results. Similar mechanism of the cells destruction may be connected with the single-quantum single-impact absorption by tryptophan, which is in the cell composition. Qualitative interconnection between the radiation dose, under exposure to which a decrease in the number of living cells is observed (Fig. 3), and the cross-striated muscle LIF intensity at 330 nm wavelength (Fig. 2) has been observed in our experiments.

The LIF of the collagen and elastin exposed to UV light (370 and 450 nm bands) does not experience a reduction, but, on the contrary, it increases. Some peculiarities in the excitation of 370 and 450 nm fluorescence bands also manifests itself in an additional increase of the fluorescence time. The LIF mechanism of these bands needs further investigation. Although the collagen and elastin fluorescence significantly contributes to the LIF spectrum of some tissue, it can not be used for investigation of the biotissue photoinactivation.

Conclusion

Our experiments allowed us to obtain quantitative information on tryptophan inactivation cross section. We have proved the conclusion from Ref. 12 on commensurability of inactivation cross sections for tryptophan and cystine within 248 nm wavelength range. It is seen that the intensity falls with reduction of the number of pulses, and it falls slower at a decrease in the exciting radiation power.

Although some changes take place in the tissue, under the action of laser radiation, these are not that essential to exceed the magnitude of random scatter of the data for the period of spectrum recording (about 30 pulses). This magnitude, as is seen from the plots presented, is about 10%. Thus, the LIF spectra changes caused by laser radiation only slightly influence all the above-mentioned results.

References

1. S.N. Bagaev, A.M. Razhev, and A.A. Zhupikov, SPIE **3564**, 94–100 (1999).
2. S.N. Bagaev, A.M. Razhev, and A.A. Zhupikov, Laser Physics **8**, 794–798 (1998).
3. J.A. Baittle, N-E J-l Med. **335**, 1282–1289 (1996).
4. M. Klein, H.D. Schulte, and I. Gams, *Transmyocardical Laser Revascularisation* (Springer, Berlin, 1998).
5. J. Lustman, M. Ulmannsky, A. Fuxbruner, and A. Levis, Lasers Surg. Med. **11**, 51–57 (1991).
6. S.V. Konev and I.D. Volotovskii, *Introduction into Molecular Photobiology* (Nauka i Tekhnika, Minsk, 1971), 229 pp.
7. P.M. Larionov, A.N. Malov, A.M. Orishich, and V.S. Shchukin, J. Appl. Spectrosc. **64**, 539–541 (1997).
8. A.V. Priezzhev, V.V. Tuchin, and A.P. Shubochkin, *Laser Diagnostics in Biology and Medicine* (Nauka, Moscow, 1986), 199 pp.
9. E.A. Chernitskii and E.I. Slobozhanina, *Spectral Luminescent Analysis in Medicine* (Nauka i Tekhnika, Minsk, 1989).
10. J.J Baraga, R.P. Rava, P. Taroni, C. Kittrell, et al., Lasers Surg. Med. **10**, 245–261 (1990).
11. Z. Deyl, K. Macek, M. Adam, and Vancikova, Biochim. Biophys. Acta. **625**, 248–254 (1980).
12. R. Setlow and B. Doyle, Biochim. Biophys. Acta. **24**, 27–41 (1957).