Upper epidermis autofluorescence dynamics under laser UV irradiation

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ABSTRACT

In present work the temporal behavior of autofluorescence of upper (30–40 μm) layer of human skin under continuous 337 nm laser exposure was investigated. The samples were prepared using epidermis stripping technique. Kinetic curves of autofluorescence decay at 420nm, 440nm, and 460nm have been obtained. In double exponential approximation ($I(t) = a \exp(-t/\tau_1) + b \exp(-t/\tau_2) + c$) the parameters of autofluorescence decay have been evaluated. Under the assumption that different terms in double exponential approximation correspond to different individual fluorophores the contribution of fast bleaching, slowly bleaching, and non-bleaching components to fluorescence spectrum of the sample at 420nm, 440nm, and 460nm have been calculated.

1. INTRODUCTION

Human skin autofluorescence is well-known phenomenon; for the first time it was reported about by R.Wood in 1919. Nowadays the fluorescence response of the skin forms the basis of some diagnostics of skin diseases[1]. On the other hand in measurements of skin optical parameters the autofluorescence of the sample interferes with the determination of tissue transmittance[2] or reflectance, especially over UV range.

In the fluorescence measurements in order to get high selectivity of excitation and to enhance the emission response CW or pulse lasers are now widely used. But due to the high density of laser irradiation the marked decrease of fluorescence intensity takes place during the experiment. We observed that when acquired fluorescence spectra of human epidermis with UV laser excitation. The same phenomenon was observed by Zeng et al.[3] in in vivo human skin under CW laser exposure. We supposed that the autofluorescence decay observed is connected with the photobleaching of epidermal fluorophore(s) under UV irradiation. In the frame of this hypothesis the investigation of temporal behavior of upper layer autofluorescence under continuous UV laser exposure may give additional information about fluorophores involved and mechanism of autofluorescence of such structurally and compositionally complex bioobject as human skin is.

2. MATERIALS AND METHODS

The experiments were carried out using commercially available spectroanalyzing system which was modified for this study.
The 337nm N_2 laser radiation was conducted by 350 μm core diameter quartz fiber to sample. The tip of the fiber was held by holder which allowed us to adjust the shape and size of illuminated spot on the surface of the sample. In the experiments the spot was nearly ellipsoidal with 4mm and 6mm semi axes. The light

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guiding system was adjusted in such a way as to obtain uniformly illuminated area on the sample. The average power (N_2 laser operated at the repetition rate near 1KHz) was measured at the tip of the fiber before and after experiment and showed no marked alterations. In our experiments this parameter had the value of 21 mW what gave the power density of 28 mW/cm^2 at the surface of the sample. UV filter cut off visible component of incident light. Fluorescence radiation was collected by quartz condenser and passed to entrance slit of grating monochromator. Between monochromator and photomultiplier filter was inserted which cut off scattered UV radiation.

To prepare the samples of upper layer of human skin we used epidermis strippings technique[4] modified for this study. We substituted polished aluminum plates for quartz slides for the latter showed too high fluorescence background under 337nm excitation; moreover this substitution allowed us to enhance the fluorescence signal. The drop of ethylcyanacrilate glue composition was applied to the inner surface of forearm then covered by aluminum plate which after approximately 30 seconds was torn off with 30-40μm upper layer of the skin.

3. RESULTS AND DISCUSSION

In experiments, under continuous UV laser exposure with the use of computer-aided spectroanalyzing system we acquired successive fluorescence spectra of the sample, the moment of the beginning of each spectrum scanning being marked. The temporal evolution of fluorescence spectra of epidermis stripping is shown in Figure 2.

To follow the dynamics of fluorescence decay we have chosen three wavelengths: 420nm, 440nm, and 460nm. From marked beginnings of every scanning and known speed of scanning we obtained times of registration at chosen wavelengths in every spectrum. Intensities at certain wavelength in successive spectra were divided by the intensity in initial spectrum to normalize these initial intensities to 1. So obtained kinetic curves of fluorescence decay for three chosen wavelength are shown in Figure 1.

We assumed that photobleaching of individual fluorophore obeys exponential low and that there exists a non-bleaching component which is responsible for residual constant fluorescence when the time of exposure exceeds 4000 seconds. We failed to approximate kinetic curves by single exponential and following Zeng et al.[3] tried a double exponential:

$$I(t) = a \exp(-t/\tau_1) + b \exp(-t/\tau_2) + c)$$

with fast process (a,τ_1), slow process(b,τ_2), and non-bleaching term (c). To evaluate the parameters of the processes we used modified technique of the analysis of complex decay curves[5]. The values obtained are shown in Table 1. The time constants of fast and slow processes differed by an order of magnitude. Zeng et al.[3] who investigated in vivo skin autofluorescence decay under 442nm laser exposure postulated for their results that the fast decay term corresponds to the photobleaching of the stratum corneum, while the slow decay term corresponds to the papillary dermis, but in our case of thin epidermis stripping we should suppose only the presence of different fluorophores responsible for the terms with so different decay times. This suggests that we may make an attempt to pick out the spectrum of individual fluorophore from the fluorescence spectrum of the sample, basing on the

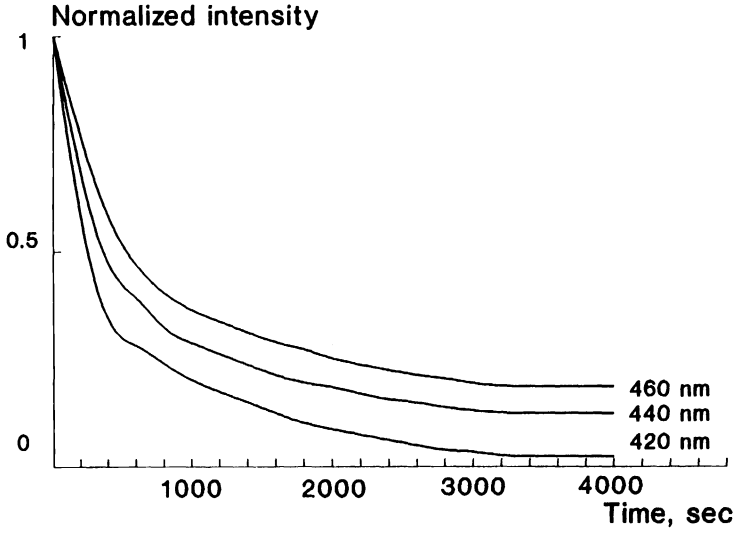

Fig.1 Kinetic curves of epidermis fluorescence decay.

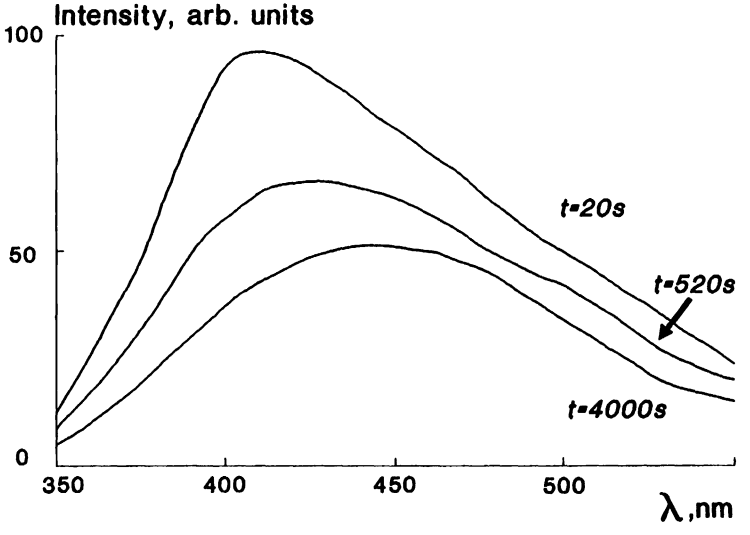

Fig.2 Evolution of the fluorescence spectra of epidermal stripping under UV laser exposure.

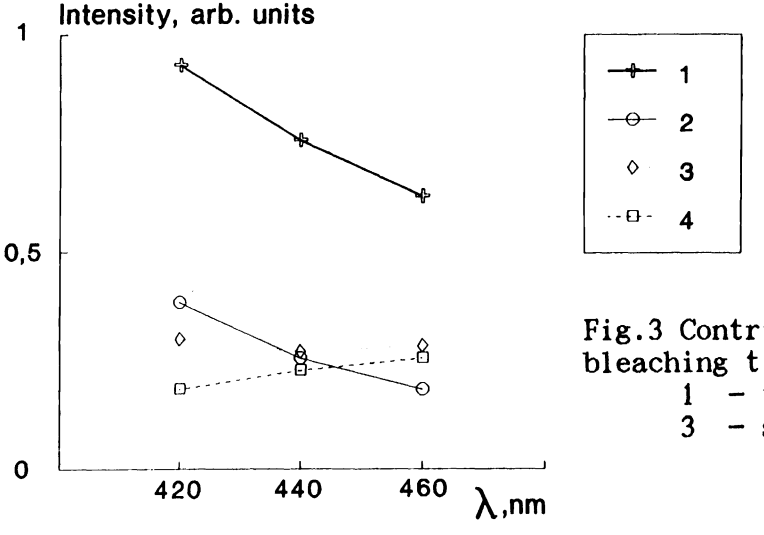

Fig.3 Contributions of the components with different bleaching time to sample fluorescence.

1 - total
2 - fast bleaching
3 - slowly bleaching
4 - non-bleaching

Table 1 Parameters of double exponential decay

λ,nm	420	440	460
a	0.44	0.33	0.26
b	0.33	0.35	0.36
c	0.23	0.32	0.38
τ1,sec	100	130	100
τ2,sec	1000	930	850

parameters of fluorescence decay. The contributions of fast bleaching, slowly bleaching, and non-bleaching components to initial spectra of human epidermis at 420nm, 440nm, and460nm were calculated by multiplying the total intensities by factors a, b, and c, respectively. As it can be seen from Figure 3, even such limited data provide insight into spectral behavior of these fluorophores.

4. CONCLUSIONS

Temporal behavior of autofluorescence of upper (30-40 μm) layer of human skin under continuous 337 nm laser exposure was investigated. Kinetic curves of autofluorescence decay at 420nm, 440nm, and 460nm have been obtained. In double exponential approximation ($I(t) = a \exp(-t/\tau_1) + b \exp(-t/\tau_2) + c$) the parameters of autofluorescence decay have been evaluated. Under the assumption that different terms in double exponential approximation correspond to different individual fluorophores the contribution of fast bleaching, slowly bleaching, and non-bleaching components to fluorescence spectrum of the sample at 420nm, 440nm, and 460nm have been calculated.

5. REFERENCES

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