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IN VIVO FLUORESCENCE SPECTROSCOPY OF THE HUMAN SKIN: EXPERIMENTS AND MODELS

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ABSTRACT

The results of the experimental investigation of autofluorescence spectra of human skin *in vivo* caused by the UV radiation of the skin and by the external mechanical pressure applied to the skin are presented. These results are compared with results of Monte Carlo modeling of the autofluorescence of the skin with variable blood content. The proposed simple model of the skin gives the possibility of evaluation of changes of the blood and melanin content within the skin. © *1998 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(98)00602-9]

Keywords skin; autofluorescence; erythema; pressure; blood; melanin; Monte Carlo modeling.

1 INTRODUCTION

In vivo diagnostics of human skin status needs objective information about the content and spatial distribution of various biomolecules within the tissue. Quantitative evaluation of the human skin optical parameters provides dermatologists with this important information and is successfully used now in skin disease diagnostics, in investigation of the environmental factor impact (chemicals, UV irradiation, temperature, etc.), and for evaluation of treatment efficiency.

Among the optical methods of skin investigation, reflection and fluorescence spectroscopy are the most advanced.[1–15] These methods are being developed in two directions: (1) refinement of the methods for measuring the reflection and fluorescence spectra of the human skin, and (2) development of the methods of experimental determination of intrinsic optical parameters of the skin (μ_a, μ_s, g, n) and mathematical modeling of the processes of light propagation within the tissue.

These two directions are mutually related: the comparison of changes in skin diffuse reflectance (R) and autofluorescence (AF) spectra under the action of external factors with the results of mathematical simulation of light propagation within the skin with variable optical parameters allows an opportunity for the development of an adequate optical model of the human skin for practical purposes.

It is very important in the experimental investigation of R and AF spectra of the human skin that the contribution of the external factors that alter the concentration and distribution of the main skin chromophores be well known. This enables us to make corrections in the simulated optical model of the skin R and AF spectra.

In this paper, we show the results of the experimental investigation of changes in the AF spectra of human skin *in vivo* caused by UV irradiation of the skin and by external mechanical pressure applied to the skin. On the one hand, the changes of the blood and melanin content in the tissue as a result of the formation and development of UV erythema and the changes of the blood content as a result of external mechanical pressure give rise to alterations of the skin AF spectra and on the other, they allow the proper specification of the skin optical parameters in computer simulation.

The comparison of the results of the experimental investigation of the AF spectra of *in vivo* skin with the results of Monte Carlo modeling of excitation and fluorescence light propagation within the skin allows us to propose a simple optical model of the human skin for practical use in dermatology and pharmacology.

2 MATERIALS AND METHODS

2.1 INSTRUMENTS AND DATA PROCESSING

Two instrumental setups were used in the fluorescence measurements. One consists of a commercially available luminescence spectrometer (LS 50B,

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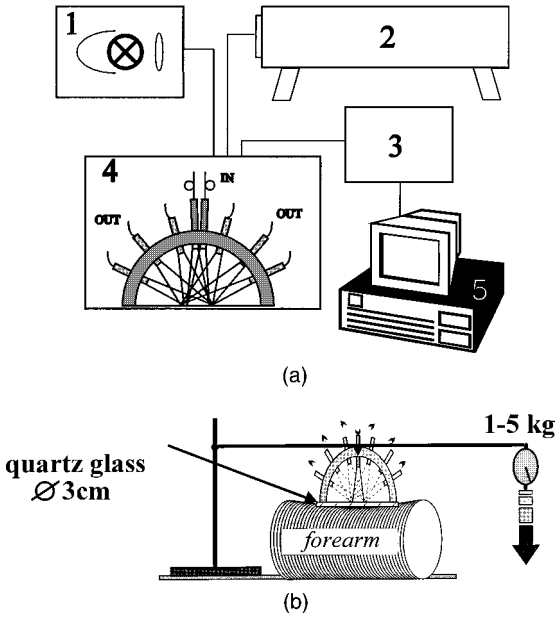

Fig. 1 (a) Experimental setup: (1) xenon arc lamp; (2) N_2 gas laser; (3) monochromator; (4) fiber-optical probe; and (5) PC. (b) Special device for external mechanical compression of the human skin *in vivo*.

Perkin Elmer, UK) with a spectral range of 200–900 nm. *In vivo* fluorescence measurements were performed using a Y-shaped flexible quartz fiber-optical guide. The distal end contained the fibers coming from both proximal arms, fibers being distributed in a random manner. The excitation light was transmitted to a skin area as a collimated beam of about 7 mm in diameter and the emitted light was collected and transported to the input slit of the spectrometer. To avoid the changes of the skin blood flow, caused by the pressure of the distal branch of the Y-shaped fiber optical guide, it was surrounded by an aluminum disk. The other instrument for *in vivo* remittance and fluorescence spectroscopy is presented in Figure 1(a) and described in detail in Ref. 16. Briefly, for diffuse reflectance and fluorescence measurements the fiber-optical probe was used: a semiring of 4 cm radius with seven fiber holders. The central fiber transmitted the excitation radiation to the skin, and the other six fibers collected the radiation scattered by the skin. The input fiber tips were arranged so as to collect the scattered radiation from the irradiated areas of the skin. The output tips of the fibers were placed near the entrance slit of the monochromator like a vertical structure. In this setup the fluorescence was excited by the UVA-laser radiation (nitrogen gas laser LGI-505, Rijazan, Russia), $\lambda = 337$ nm; maximum mean power 0.1 W; pulse duration 10 ns; repetition rate 1000 Hz) and in the R measurements a 250 W xenon arc lamp or 200 W halogen lamp with filtering of the radiation in the 400–800 nm range were used. The diffuse R spectra of the skin were measured against $BaSO_4$ as a reference.

On the volar forearm of ten volunteers (7 males and 3 females, aged 20 to 40 years, with skin phototypes II and III) erythema was induced by UV radiation. A circular area of the forearm skin (3 cm in diameter) was subjected to exposures of up to 4 MED (minimum erythema dose) UV exposure. Six commercially available irradiation lamps (UFO-L, Saratov, Russia) emitting in the main UVB were used. Six hours after exposure, the areas of erythema produced were analyzed by the R and fluorescence spectrophotometry. The measurements were performed every 12 h for 45 days. The volunteers sat comfortably in a chair with their forearms supported at the heart level. The room temperature was 22°C, and the volunteers were at rest for 10 min before the recordings began. Fiducial marks were placed on the skin with indelible ink so that the probe holder could be accurately relocated in succeeding hours and days.

We also performed R and AF measurements with a reduced blood volume in dermis, achieved by the application of the external mechanical pressure onto the probe [see Figure 1(b)]. Between the fiber-optical probe and the skin a thin quartz glass slide was placed (thickness 0.15 cm; diameter 3 cm). The pressure ranged from 0 to 1.4×10^5 Pa.

As in the case of changing the blood content in the dermis for optical measurements, Dr. Rudiger W. Sharenberg (*taberna pro* medicum, Luneburg, Germany, personal communication) has provided the measurements of the skin thickness while applying external mechanical pressure onto the probe using a digital 20-MHz ultrasound scanner (DUB-20, *taberna pro* medicum).

2.2 SKIN MODEL AND MONTE CARLO MODELING OF THE SKIN AUTOFLUORESCENCE

The light distribution within the skin tissue was evaluated using the software package presented in Ref. 6, in which a version of the Monte Carlo method is realized. This version enables us to take into account the multilayer structure of the skin (see Figure 2), the finite size of the incident beam, and reflection and refraction of light at the interfaces between the layers. A five-layer model of the skin, including epidermis and dermis was used. The optical parameters of the tissue[1,6] are presented in Table 1. The optical parameters (μ_a, μ_s, g, n) of the dermis with different blood content were calculated by adding the fractional contributions of their components.

At the first step, the spatial distribution of the excitation photon density within the skin was calculated. The density of the sources of fluorescence is proportional to the excitation photon density; and in the case of the homogeneous tissue, the distribution of the fluorescence sources is similar to the distribution of the excitation photon density. However, the real skin tissue contains various

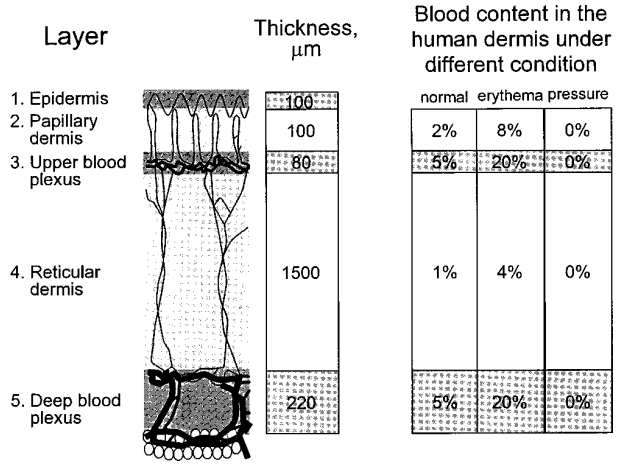

Fig. 2 Multilayered human skin model.

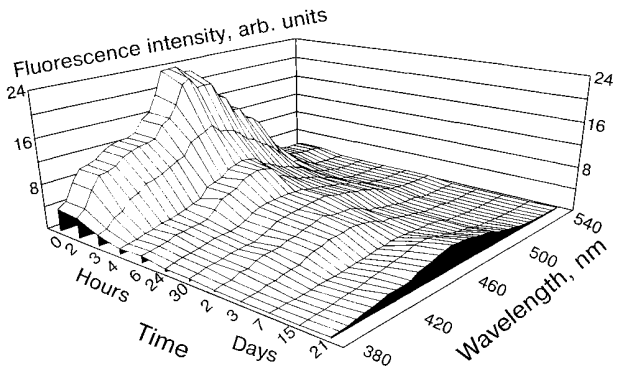

Fig. 3 Three-dimensional plot of the human skin AF after UV irradiation with 4 MED.

kinds of fluorophores with different spatial distributions and different quantum efficiencies of fluorescence. In our model we suggested that the main dermal component, collagen, is responsible for most of the AF of the skin (a discussion on the nature of the human skin AF will follow). Let us assume that there are no sources of fluorescence inside the epidermis, the distribution of the sources inside the dermis is homogeneous, and the fluorescence source irradiates isotropically with a quantum efficiency equal to 1.

At the second step, the distribution of the fluorescence photon density within the skin and the fluorescence escape flux from the tissue were calculated.

3 Results and Discussion

Figures 3 and 4 represent the temporal dynamics of the AF spectra of the skin in the process of UV-erythema formation. The main feature of the changes is a significant decrease of the fluorescence intensity during the first seven days. During the period from the seventh to the ninth day, the intensive upper stratum corneum desquamation leads to a sharp increase in the curve behavior.

Compressing the skin tissue causes a contrary effect. Figure 5 presents the AF spectra of the skin with different degrees of erythema formation at different values of pressure on the skin. As the pressure on the skin increases, the intensity of the AF increases. Figure 6 represents the fluorescence intensity at a wavelength of 460 nm as a function of the pressure on the skin before and after desquama-

Table 1 Biotissues optical parameters (Refs. 1 and 6).

Layer	λ (nm)	μ_a (cm^{-1})	μ_s (cm^{-1})	g	n
	337	32	220	0.72	
Epidermis	460	30	210	0.75	1.45
	575	10	200	0.79	
	650	4	100	0.89	
	337	23	500	0.72	
Dermis	460	1	216	0.75	1.4
	575	2.8	215	0.79	
	650	2	180	0.89	
	337	700	1000		
Blood	460	280	600	0.98	1.35
	575	324	500		
	650	1.5	300		

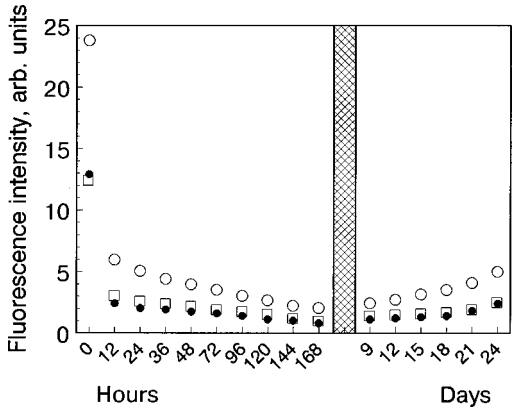

Fig. 4 Temporal dependence of the AF intensity for *in vivo* skin with developing erythema: (○) $\lambda = 460$ nm; (●) $\lambda = 420$ nm; and (□) $\lambda = 500$ nm.

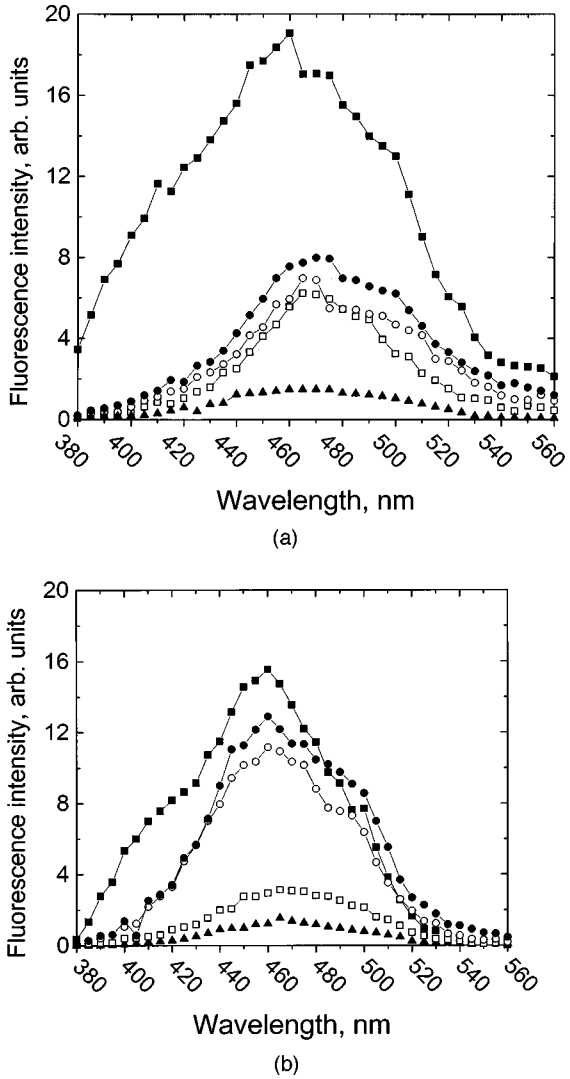

Fig. 5 The *in vivo* human skin AF with the UV erythema for different values of external mechanical pressure: (a) 7 days after UV irradiation; (b) 14 days after irradiation; (■) the AF of the normal skin; (□) 1.4×10^4 Pa; (○) 8.4×10^4 Pa; (●) 14×10^4 Pa; and (▲) erythema without pressure.

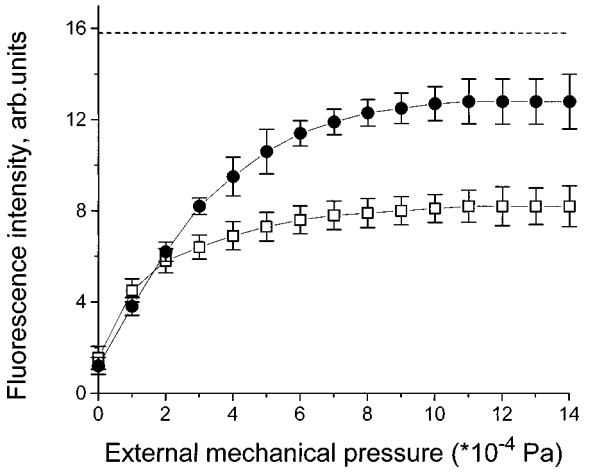

Fig. 6 The AF intensity of the human skin ($\lambda_F = 460$ nm) vs external mechanical pressure: (□) 7 days after UV irradiation; (●) 14 days after irradiation; and (dash line) normal skin with pressure 10^5 Pa.

tion. In other words, the increase of pressure causes the monotonous increase of fluorescence intensity, but at a pressure value of about 10^5 Pa the fluorescence intensity no longer increases (i.e., further compressing the dermis does not lead to an increase of fluorescence intensity).

The nature of the AF of the skin is still under discussion. Skin tissue consists of many kinds of fluorophores with different excitation and fluorescence spectra, different quantum efficiencies, and different spatial distributions, which have not been completely studied. Among the many endogenous skin fluorophores being investigated, the most promising are the different forms of NAD[17,18] and keratin[8] located in the epidermis and dermal collagen.[9] Reduced (NADH) and oxidized (NAD^+) forms of nicotinamide adenine dinucleotide take part in the cellular energy metabolism, and the intensity of the specific fluorescence is used not only for differential diagnostics of the metabolism disfunction,[19] but also in quantitative NADH detection.[20] Proliferative cells at the lower (basal) layer of the epidermis generate a continuous flow of cells that are released into the epidermis and transported to the upper (stratum corneum) layer. These cells change shape and chemical composition until they end up in the stratum corneum as flat pancake-shaped structures with a high keratin content. Sterenborg et al.[8] reported quite a similarity between the AF spectrum of human skin *in vivo* and the emission spectrum of keratin measured *in vitro*.

Among many skin fluorophores being investigated, collagen is one of the most attractive. Approximately 75% of the dry weight of the dermal tissue is composed of collagen fibers.[21] Collagen is the main structural component of connective tissue and accounts for about 90% of protein in human dermis. There are at least five types of collagen; types I (about 80%) and III (about 20%) are found in

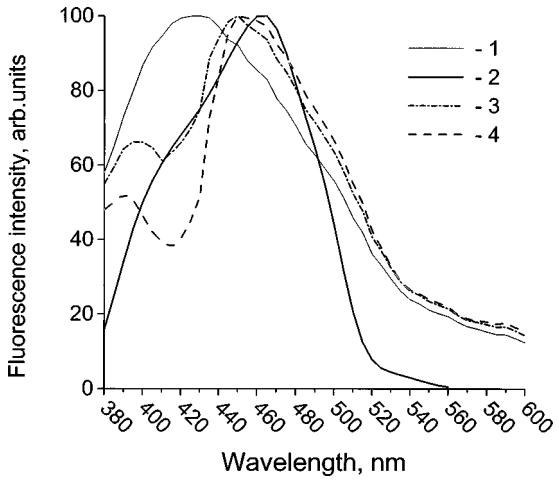

Fig. 7 The AF spectrum of the human skin (2) (*in vivo*), the AF spectrum of the collagen (1) (*in vitro*), and the AF spectra of the collagen after passage through dermal blood plexus [thickness: 50 μm (3) and 100 μm (4); oxygenation; 50%].

dermal collagen, and type IV is found in basement membranes.[22] Collagen fibers show a constant density throughout all dermal layers.[23]

The conclusion about the main role of the epidermal chromophores (keratin, NADH) in the formation of the AF spectrum of human skin is based on the fact that the *in vitro* fluorescence spectra of keratin and NADH are extremely similar to the *in vivo* AF spectrum of human skin.[8]

When we compared the fluorescence spectrum of collagen *in vitro* (collagen type I from human placenta, Pentapharm AG, Belgium) with the AF spectrum of *in vivo* human skin (Figure 7, λ_{EM}=460 nm) (both spectra were obtained employing a fiber-optical sensor under identical conditions) we found that the peak wavelengths were different.

However, it is necessary to take into account that the AF spectrum of the dermal collagen is much modified due to absorption by the blood. As presented in Figure 7, the AF spectrum of the human skin and the AF spectrum of the collagen after passage through the dermal blood plexus (thickness, 50 and 100 μm; blood content, 5%) practically coincide.

The collagen metabolism disturbance caused by aging leads to collagen deposition within the dermis[24] that provides an explanation for the observed significant increase of the skin AF in elderly persons, which is confirmed by the results of other authors.[9]

Our experimental investigations of the AF of the skin with different blood contents confirm the assumption that collagen is the main fluorophore that contributes to the AF of the skin.

Compressing the skin tissue may cause the alteration of the skin tissue geometry, the alteration of the chromophore content, determined spectrum of the AF of the skin, and the intensity of the AF spectrum. The measurements of the thickness of the skin under external mechanical pressure with the help of an ultrasound scanner show that the decrease of the human skin thickness is not very large at a pressure below 10^5 Pa. At high pressure (10^5 Pa) the decrease of the skin thickness ranges from 18% to 22%.

Rol[25] reported that sclera thickness can be reduced by about 10% under a pressure of 10^8 Pa exerted to the sample. When a pressure of 10^8 Pa is applied to the sclera, the total integrated transmission of the sclera sample at $\lambda = 488$ nm (Ar^+ laser) increases to about 50% of the initial value for 1 min (the time of our measurement of the AF spectrum), where the pressure is applied to the sample. The sclera is a strong fibrous tissue which is mainly made up from conjunctive fibers on the basis of collagen (75% of the dry weight of the sclera tissue is due to collagen) similarly to the skin dermis. At a pressure of about 10^5 Pa applied to the sclera the changes in scattering properties of the sclera are insignificant. Therefore, changes in the scattering properties of the skin cannot explain the substantial growth of the AF intensity under pressure (by 8–10 times for skin with erythema and by 1.4 times for normal skin).

In our opinion, the dermis becomes bloodless under external mechanical pressure, and the changes of the blood content in the dermis are the main reason for human skin AF alterations. Changes in the blood content within the skin tissue do not affect the fluorescence intensity of NADH and keratin that leads to the skin AF but are located mainly within bloodless epidermis.

UV irradiation of the skin causes various biologic effects, many of which can be involved in the formation of the skin AF. UV-radiation-induced erythema is an indication of the increased blood volume in the superficial and deep vascular plexi in the dermis. The initial photochemistry in the cascade leading to inflammation occurs in the epidermis with subsequent diffusion of the mediators into the neighborhood of the blood vessels. Injury of the dermal cells or structure or direct injury of the blood vessels may also be important.[26] Several mediators are believed to be important in the pathogenesis of UV-induced erythema. Histamine has been identified as a potential mediator in delayed UV-induced erythema. UV-radiation-induced lysosomal disruption, with the release of enzymes leading to cellular damage, may be an early step in the inflammatory process. A firm relationship between increased prostaglandin synthesis and UV-induced erythema has been demonstrated. Most cellular consistent mediators of the skin, including epidermis, blood cells, and vascular endothelium, are known to be capable of prostaglandin synthesizing. Prostaglandins directly cause vasodilatation, and prostaglandin synthesis is made active by the products of lipids peroxidation. Mediators may also be

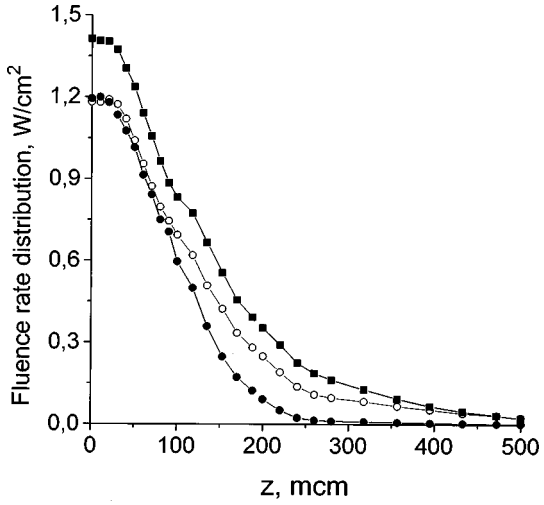

Fig. 8 Fluence rate distribution of the excitation light ($\lambda = 337$ nm) within the human skin: (○) normal skin; (●) human skin with erythema; and (■) human skin without blood.

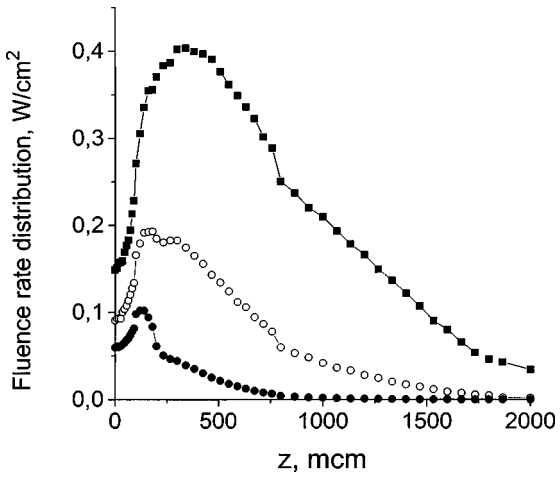

Fig. 9 Fluence rate distribution of the fluorescence light ($\lambda = 460$ nm) within the human skin: (○) normal skin; (●) human skin with erythema; and (■) human skin without blood.

formed in the plasma exudate that occurs during inflammation.

Coenzyme NAD does not directly act as a mediator in the formation of UV erythema. It is hardly probable that its quantity changes due to UV irradiation. UV irradiation alters the redox balance and can place the cells in a state of oxidative stress. In this tissue anoxia condition the free form of NADH is dominated, and the fluorescence intensity of NADH increases.[27–29]

Histologic changes can also be seen in human skin after UV irradiation. Sunburn cells are a characteristic feature of UV-induced epidermal damage.[26] They result from damage to keratinocytes. Sunburn cells characteristically occur in irregularly distributed clusters and are formed mainly in the lower half of the epidermis. After high doses, the cells may coalesce into a band of necrotic tissue that is eliminated upwards over a period of seven days or longer. Melanocytes also show vacuolization and swelling soon after irradiation of human skin. As it appears, such histological changes do not result in an essential change of the keratin content in the epidermis.

UV radiation causes changes in the kinetics of the epidermal cells. Macromolecular synthesis is greatly reduced 1–6 h after irradiation, but is most stimulated 1–2 days after irradiation. Changes in epidermal mitosis follow a similar time course.[26] As a consequence, it is possible to expect similar changes in the content of NADH in the epidermis.

In our opinion, the blood content in the superficial dermal vascular plexus can strongly affect the skin AF. In Figure 6 the increase of the fluorescence intensity of the skin at the increase of its compression originates from the decrease of the blood content within the dermis. [The AF intensity of the human skin without erythema and compression (a dot line) is marked as a reference value.] The difference in fluorescence intensity of normal skin and skin with erythema at a pressure value of $>10^5$ Pa is caused by and depends, in our opinion, on melanin absorption.

We have carried out calculations of the AF intensity of human skin without melanin with different contents of blood according to the model presented in Figure 2 with the assumption that the main fluorescence source is dermal collagen.

The results of Monte Carlo modeling of the AF of skin with erythema are represented in Figures 8–10. Figure 8 shows the excitation ($\lambda = 337$ nm) light distribution within the dermis with different blood content. The incident light is completely absorbed at a depth of 800 μm for normal skin and at 300 μm for skin with 4 MED erythema. According to our model, the distribution of the fluorescence sources has the same form at depths exceeding 100 μm. The distribution of the fluorescence photon density within the skin with different blood content is presented in Figure 9. Absorption and scattering of the

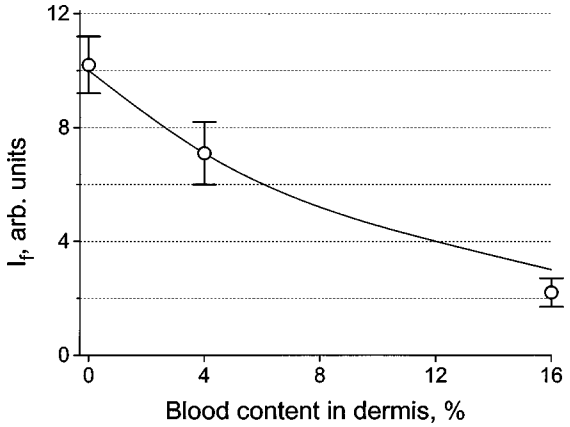

Fig. 10 The AF intensity vs blood content in dermis: (solid line) Monte Carlo simulation; and (○) experimental data.

fluorescence photons within the epidermis lead to a decrease of the fluorescence photon density near the skin surface. The calculated intensity of the AF of the skin with varying amounts of blood content is presented in Figure 10 (solid curve). The AF intensity of bloodless skin is higher by 1.4 times than that of normal skin and four times higher than that of skin with erythema. The results are in agreement with the experimental results (points in Figure 10), received for normal skin under external pressure, but the calculated AF intensity for skin with erythema is at least 1.5 times as high as that obtained experimentally. In this model we have ignored the epidermal fluorescence due to the low contribution of the fluorescence photons from the epidermis traveling through the dermis before it reaches the skin surface.

UV radiation leads to an increase in epidermal melanin pigmentation. Delayed tanning becomes visible about three days after irradiation and reflects an increase in the epidermal melanin content.[30] It is associated with an increased melanocyte population as well as melanocyte activity. Due to the transport of melanosomes into the top layers of the skin we have a dynamic screen against UV radiation, driven in due course to the top layers of the epidermis. The desquamation of human skin results in the reduction of melanin content that explains the increase of the AF intensity of compressed skin before and after desquamation (Figure 5).

NADH and keratin give certain contributions to the skin AF. Moreover, the results of our calculations[31] show a 2–2.5-fold decrease of the fluorescence of collagen at the surface of the skin compared with the dermal layers, but the fluorescence intensity of collagen stays sufficient to form the skin AF spectra. Nevertheless, in our investigation the major factors determining the change of the skin AF are blood and melanin.

For analysis of the changes in the skin AF spectra during UV-erythema development we have used a simple model based on the assumptions that the fluorescence of collagen is responsible for the skin AF and hemoglobin and melanin can exert a major influence on the fluorescence spectra.

By analogy with the simple optical model for the R spectra analysis[3,16] a simple model can be suggested for the skin AF spectra analysis. Skin tissue can be considered as consisting of distinct parts: epidermis, papillary dermis, and the layers beneath the papillary dermis (Figure 11). The fluorophore spatial distribution contributing to the human skin AF is supposed to be uniform within the limits of the epidermal (NADH, keratin) and dermal (collagen) layers. The contribution of the stratum corneum and other epidermal layers into the total skin AF slightly varies during erythema formation or under external mechanical pressure. The collagen fluorescence spectrum is affected by melanin and

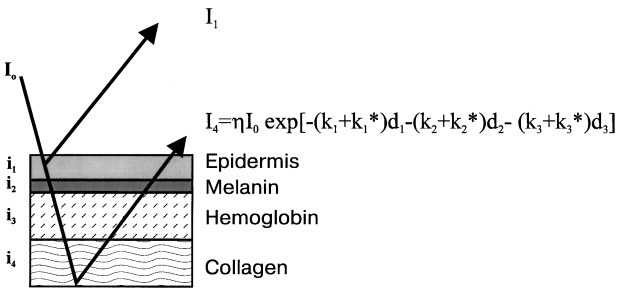

Fig. 11 Simple skin model for AF analysis.

hemoglobin, which absorb the emitted radiation escaping from the skin.

The intensity I_{AF} of the skin AF is defined by the total fluorescence intensity from the epidermal I_1 (NADH, keratin) and dermal I_4 (collagen) skin layers (see Figure 11).

In one-dimensional approximation, the flux of escaping fluorescence I_4 from the dermal layer can be presented as[32–34]

$$I_4(\lambda_{FL}) = \int_z \Phi(\lambda_{EX}, z)\, \eta(\lambda_{FL}, \lambda_{EX}) T(\lambda_{FL}, z)\, dz, \tag{1}$$

where λ_{EX} and λ_{FL} are the excitation and emission wavelengths; $\Phi(\lambda_{EX}, z)$ is the fluence distribution of excitation light within the fourth layer; and $\eta(\lambda_{FL}, \lambda_{EX})$ is the fluorescence quantum yield of dermal collagen.

The transfer function $T(\lambda_{FL}, z)$ governs the propagation of collagen fluorescence in upper layers and can be presented as

$$T(\lambda_{FL}) = \exp\left[-\sum_{i=1}^{3} k_i^*(\lambda_{FL}) d_i \right], \tag{2}$$

where $k_i^*(\lambda_{FL})$ is the absorption coefficient of the ith layer with thickness d_i.

The average value of the intensity of the excitation light in the fourth layer can be expressed as

$$I(\lambda_{EX}) = I_0(\lambda_{EX}) \exp\left[-\sum_{i=1}^{3} k_i(\lambda_{EX}) d_i \right], \tag{3}$$

where $I_0(\lambda_{EX})$ is the intensity of exciting radiation incident at the skin surface as a collimated beam, and $k_i(\lambda_{EX})$ is the absorption coefficient of the ith layer at the wavelength of the exciting radiation.

Then the intensity of the escaping fluorescence can be calculated as follows:

$$I_4(\lambda_{FL}) = \eta(\lambda_{FL}, \lambda_{EX}) I_0(\lambda_{EX}) \times \exp\left[-\sum_{i=1}^{3} [k_i(\lambda_{EX}) + k_i^*(\lambda_{FL})] d_i \right]. \tag{4}$$

In turbid tissue, such as the skin, the reflectance R can be expressed as

$$D = \log\left(\frac{1}{R}\right), \tag{5}$$

where D is the apparent optical density of the skin at the wavelength of incident radiation.[1,3]

Analogously, normalizing the fluorescence intensity $I_F(\lambda_{FL})$ to $\eta(\lambda_{FL},\lambda_{EX})\ I_0(\lambda_{EX})$ gives

$$R^* = \frac{I_4(\lambda_{FL})}{\eta(\lambda_{FL},\lambda_{EX})I_0(\lambda_{EX})}, \tag{6}$$

and the quantity D^* can be introduced:

$$D^* = \log\left(\frac{1}{R^*}\right) = \log(e)\sum_{i=1}^{3}\{[k_i(\lambda_{EX}) + k_i^*(\lambda_{FL})]d_i\}. \tag{7}$$

D^* can be called the ''effective optical density'' of human skin because it is directly proportional to absorption in the skin. As against D, the changes of D^* are defined by the variation of absorption properties of the skin at excitation and emission wavelengths and D^* permits one to define the changes of the absorbing properties of the skin, which in our case depend on the amount of skin chromophores (blood and melanin) at two wavelengths.

As it is extremely difficult from the AF measurements to receive absolute quantitative information about fluorophores, first of all because of the complexity of the determination of the fluorescence quantum yield η, fluorescence spectroscopy is usually used for analysis of the qualitative relative changes observed in biotissues. While the R spectroscopy, where reflected light normalizing is performed with $BaSO_4$ (or other standard objects) as a reference gives the possibility of determining the absolute values of R and D, the AF spectroscopy allows one to measure only the relative changes of skin absorption, mostly due to the unknown η value. So, ΔD^* is defined by the skin AF intensity changes only:

$$\Delta D^* = D_2^* - D_1^* = \log\left(\frac{\eta I_0}{I_{AF}^2}\right) - \log\left(\frac{\eta I_0}{I_{AF}^1}\right) = \log\left(\frac{I_{AF}^1}{I_{AF}^2}\right), \tag{8}$$

where I_{AF}^1 and I_{AF}^2 are the AF intensity values of erythematous skin or human skin under external mechanical pressure (under similar irradiation conditions).

Changes of D^* do not depend on ηI_0, and the ηI_0 value has been chosen in such a way, that R^* and R values did not dramatically differ.

The epidermal fluorescence brings the constant contribution to the skin AF and does not significantly modify the dependence of D^* on the blood and melanin content.

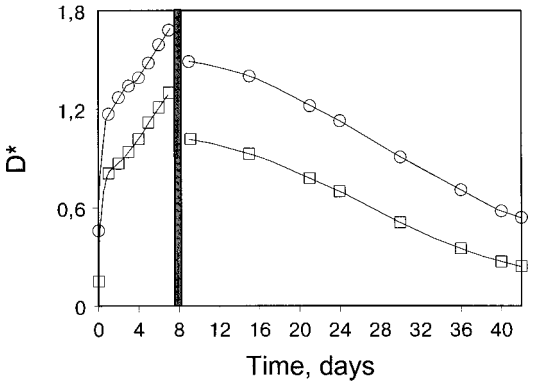

Fig. 12 The temporal dependence of D^* for *in vivo* human skin with developing erythema: (○) $\lambda = 460$ nm; and (□) $\lambda = 500$ nm.

From Figure 12 one can see that both the exciting light and the fluorescence absorption defined by the increase of the blood content rises by 3.2 times within the first day after UV irradiation. The further increase of D^*, and hence, an increase of absorption, results in a rise of the melanin content. The skin desquamation causes a loss in melanin content.

In Figure 13 the D^* spectra of normal skin and skin with 6-h and 6-day erythema obtained at pressure on the skin of 10^5 Pa are presented. These curves demonstrate the melanin effect on the fluorescence spectrum: as 6-h changes in the skin are caused mainly by alteration of blood content, the curves for normal skin and skin with 6-h erythema practically coincide; and so, the curve for skin with 6-day erythema lays beyond only due to the absorption rise owing to melanin formation.

The D^* spectra for skin with erythema, obtained at different pressures on the skin are illustrated in Figure 14. Figure 15 shows the dependence of D^* of

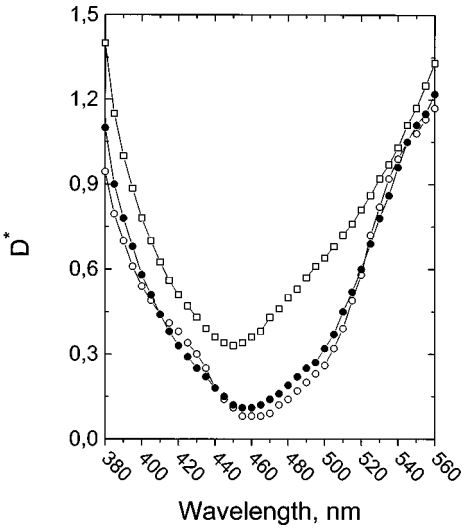

Fig. 13 The *in vivo* D^* spectral distribution for the human skin with the external mechanical pressure ($\times 10^5$ Pa): (○) normal skin; (●) 6 h after UV irradiation; and (□) 6 days after UV irradiation.

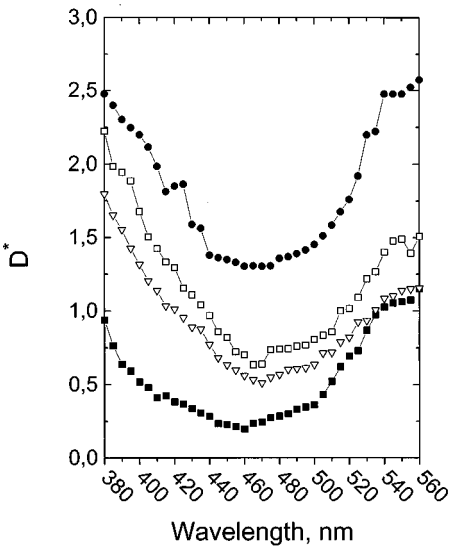

Fig. 14 The *in vivo* D^* spectral distribution for the human skin with UV erythema (after seven days of irradiation) for different values of external mechanical pressure: (■) normal skin; (△) 14×10^4 Pa; (□) 2.8×10^4 Pa; and (●) erythema without pressure.

skin with 7-day erythema on the pressure. The difference between the D^* values for erythematous skin at 10^5 Pa and normal skin at the same pressure (horizontal line in Figure 15) is equal to 0.36. Taking into account that the melanin content does not depend on the pressure, we get that the contribution of blood absorption into D^* is equal to 0.94 (1.30–0.36). The dermis of such erythematous skin contains 38% of blood. For normal skin, when the dermis contains 10% of blood, D^* is equal to 0.75; that is the skin with erythema looks like the normal skin at a pressure $\approx 2\times10^4$ Pa.

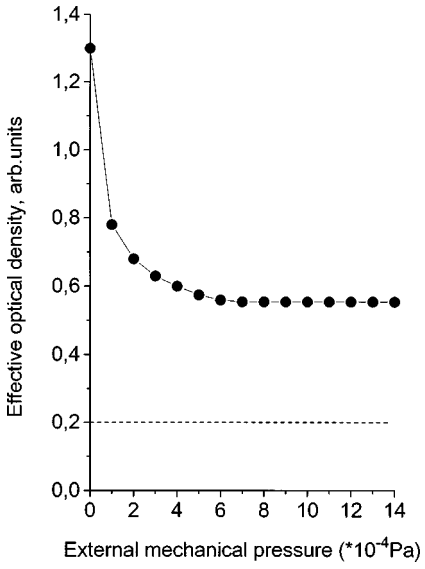

Fig. 15 D^* (λ_F=460 nm) for human skin (after 7 days of UV irradiation) vs external mechanical pressure: (dash line) normal skin under pressure 10^5 Pa.

Thus, the fluorescence spectra give comprehensive information about the changes in skin, and the method seems to be more sensitive than the reflection measurements.

The skin AF spectra may be used for determination of erythema and melanin skin indices.

The methods of erythema and melanin indices determination using *in vivo* R spectroscopy are well known. So, the erythema index is defined by the area under the spectral curve $D(\lambda)$ in the region with large blood absorption (510–610 nm)[3]:

$$E = 100[D_{560} + 1.5(D_{545} + D_{575}) - 2.0(D_{510} + D_{610})], \tag{9}$$

(the subscripts mean wavelength in nm) or by the difference of D's in the green ($\sim$560 nm) and red ($\sim$650 nm) spectral regions:[35]

$$E = 100[D_{560} - D_{650}]. \tag{10}$$

The melanin content in the skin is defined by the slope of the spectral dependence $D(\lambda)$ in the region above 620–640 nm.

If we apply *in vivo* skin fluorescence spectroscopy to erythema and melanin indices determination, we obtain expressions for D^* of normal (D^*_N) and erythematous (D^*_E) skin:

$$D^*_N = \log\left(\frac{\eta I_0}{I^N_{AF}}\right), \quad D^*_F = \left(\frac{\eta I_0}{I^F_{AF}}\right), \tag{11}$$

where λ_F=460 nm.

The erythema index that is proportional to absorption of fluorescence radiation (i.e., blood content) may be calculated from the expression

$$E \equiv D^*_E(\lambda) - D^*_N(\lambda) = \log\left(\frac{I^N_{AF}}{I^E_{AF}}\right). \tag{12}$$

The melanin index may be defined from the analogous expression, but the fluorescence intensities I^N_{AF} and I^E_{AF} should be measured under external mechanical pressure on the skin $\sim10^5$ Pa (bloodless skin). In Figure 16 the results of calculation of the index E on the basis of the R and AF spectra of the skin with developed erythema are presented.

4 CONCLUSION

Fluorescence spectroscopy, especially in the UVA range, opens many new possibilities to answer unsolved questions referring to skin erythema and pigmentation. This method gives comprehensive information about changes in the skin and seems to be very sensitive. Good agreement between the results of Monte Carlo simulation and experimental measurements has been obtained. These results confirm the main role of collagen in the forming of the AF spectra of the skin. Further investigations of the skin AF will give more detailed information on the spatial distribution of different chromophores,

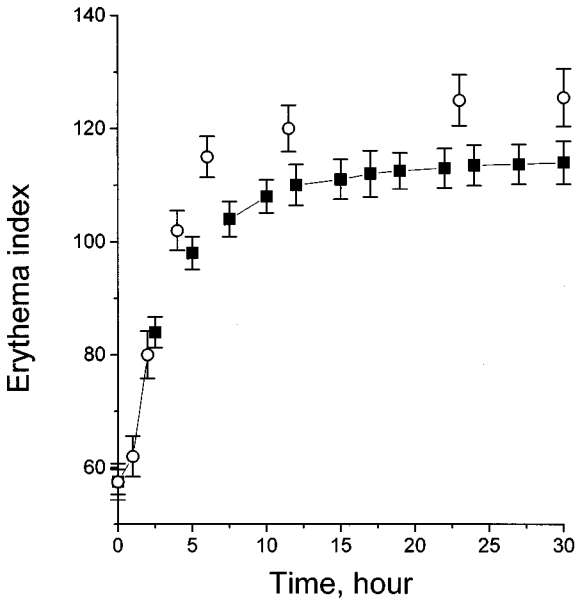

Fig. 16 The temporal dynamics of the erythema index, calculated from the reflectance (■) and the fluorescence (○) measurements.

including NADH and keratin, within skin tissue. Applications of these techniques to erythema and melanin quantification have opened up new measurement prospects and new applications in dermatology and pharmacology.

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