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Laser- and Light-Induced Autofluorescence Spectroscopy of Human Skin in Dependence on Excitation Wavelengths

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Human skin contains various types of native fluorophores and absorbers with unique absorption and emission spectra, different quantum efficiency, concentration and spatial distribution within the skin. Autofluorescence spectroscopy is applied as diagnostic tool for cutaneous tumor detection that increases the importance of evaluation of natural existing fluorophores and unification of data for given class of pathologies. In the current study, several excitation sources in the region 337–405 nm are applied, to achieve information about typical autofluorescent properties of normal human skin.

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1. Introduction

Optical spectroscopic techniques importance in the clinical practice is raised rapidly in the last two decades. With increase in knowledge about optical properties of different tissues and their pathologies, as well as with the improvement of spectroscopic equipment and the optimization of measurement methodologies new diagnostic approaches are introduced for the medical diagnosis purposes [1–4].

Biological tissues contain chromophores that absorb light, as well as fluorophores that absorb and reemit (fluoresce) light. Light absorption depends on the chromophores' content and their distribution within the organic matter. Biotissues are also structurally heterogeneous that promote strong light scattering [1, 2]. The diverse optical properties of biological tissues make their analysis quite complicate, but also allow achieving of highly sensitive and rapid spectroscopic methods for detection of small changes in their healthy status.

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Autofluorescence (AF) is one of the most sensitive spectroscopic techniques and it is used for diagnosis and follow-up of dermatological diseases, such as erythrasma and acne [5]. This method is also applied for skin cancer detection and lesion type determination [1, 6, 7].

In the current work there are presented some comparative results of using of N_2 laser (λ = 337.1 nm) and light emitting diodes (LEDs) (with emission in 365, 375, 385, and 405 nm) as excitation sources for cutaneous fluorescence investigations and the evaluation of autofluorescence properties of normal human skin *in vivo*.

2. Materials and methods

2.1. Fluorescent measurements

For these experiments, the fluorescence is stimulated by N_2 laser (λ = 337.1 nm) and LEDs with maximum emission at 365, 375, 385, and 400 nm. The signal is transmitted from the source of light to the investigated objects and from the investigated objects to the detector system by quartz optical fibers. The laser applied uses longitudinal pumping (N_2 laser, ILGI-503, Russia). The technical characteristics of the light diodes used in this experiment are: UVLED365-10 at λ = 365 nm, P = 1.4 mW, λ = 10 nm; UVLED380-10 with λ = 380 nm, P = 3.4 mW, λ = 10 nm ("Roithner-Laser Technik" GmbH, Austria); UV LED L4-1-U5TH15-1 (LED Supply Ltd., USA) with λ = 395 nm, P = 10 mW, λ = 15 nm; OTLH-0360-UV-400 (Rhopoint Components Ltd, UK) with λ = 405 nm, P = 120 mW, λ = 15 nm. In Fig. 1 there is presented schematically view of the experimental setup.

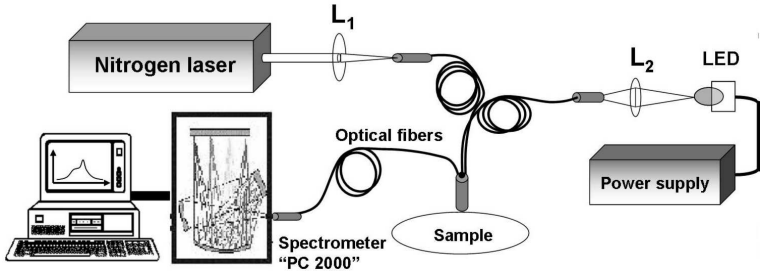

Fig. 1. Schematic view of the experimental setup used for measurements of fluorescent properties of human skin *in vivo*, using different excitation sources.

2.2. Skin samples

Healthy volunteers, representative of skin phototypes typical of Bulgarian region, namely phototypes I, II, and III were investigated on several anatomic sites: palm, medial, and lateral part of the forearm, using different excitation sources in the region 337–405 nm.

3. Results and discussion

The spectra, presented in this work, are smoothed and averaged in dependence of anatomic origin and skin phototype. Every autofluorescence spectrum detected *in vivo* is a superposition of fluorescence spectra of endogenous chromophores existing in the tissue under investigation distorted by re-absorption of tissue pigments, mainly blood and melanin. The spectral shape of normal skin fluorescence has no significant differences between volunteers, for a given excitation wavelength. Intensity changes are more pronounced due to different skin phototype and anatomic area, as in both cases different level of melanin and hemoglobin pigmentation could be observed. Detected slight differences in spectral shape are only for the case of palm skin fluorescence spectra versus other anatomic sites, where lack of melanin leads to deeper penetration of excitation and respectively for emission light. In this case influence of hemoglobin re-absorption of the fluorescence from deeper dermal layer is well pronounced. Strong changes are observed in the fluorescence spectra detected when using different excitation wavelengths as different fluorophores are included in the autofluorescence response of the tissue investigated.

3.1. Results — repeatability

To investigate repeatability of the measurements and possible differences from patient to patient, a comparison of the fluorescence spectra from several volunteers from one skin phototype and from the same anatomic area is carried out. The spectral shape observed is similar for all cases, but some slight differences in maximal intensity, related to pigmentation and blood content deviations are pronounced. Due to the same slight absorber concentrations differences from patient to patient differences in the spectral shape are observed.

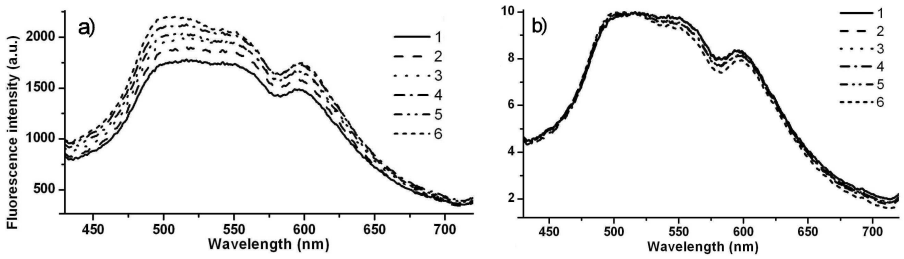

Fig. 2. Comparison of the fluorescence spectra obtained from lateral part of the forearm of six volunteers with phototype I, excitation used at 385 nm, unnormalized with respect to maximum (left) and normalized with respect to maximum (right).

However, these differences are relatively weakly pronounced and do not change general conclusions about good repeatability of the fluorescence spectra shape between patients, with additional condition about the same skin phototype and anatomic area investigated, as these two parameters have strong influence on

the spectra detected. The comparison of the fluorescence spectra from the lateral part of the forearm between the two curves shows a good repeatability of the received data in Fig. 2.

3.2. Influence of excitation wavelength

In comparison of fluorescence spectra using different excitation wavelengths there is clearly observed the appearance of new emission maxima and changes in fluorescence intensity, depending on absorption for given excitation wavelength of exact fluorophore. In Fig. 2 there are presented fluorescence spectra, normalized with respect to maximum for several excitation wavelengths. The normalization is applied to reveal better the spectral shape changes occurred.

With the increase in the excitation wavelength, new fluorophores are involved in the shape formation of fluorescence spectrum. This is related to deeper penetration of long-wavelength excitation [6], to higher absorption at given wavelength for some additional fluorophores and to differences in pigments influence, especially hemoglobin — see the minima detected at 545 and 575 nm in Fig. 3 [8, 9].

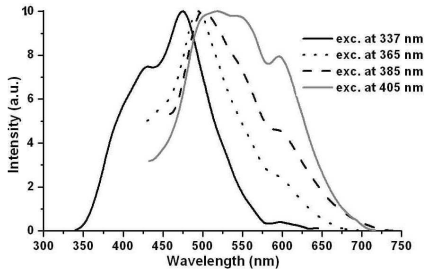

Fig. 3. Normalized with respect to maximum fluorescence spectra of normal human skin from one volunteer at different excitation wavelengths applied.

3.3. Influence of anatomic area

In the fluorescence spectra obtained several typical spectral features were observed, related to fluorescence of some endogenous chromophores on one side and to re-absorbance of the fluorescence by typical skin pigments on the other side.

These features are the maxima at the region of 470–500 nm, resulting from collagen and elastin cross-links fluorescence, maximally at the region 440–470 nm, related to NADH and FAD, which are main co-enzymes in cutaneous tissues.

The fluorescence peak at 550–600 nm is related to keratin and maximum obtained with excitation at 605 nm, which lies at the 800–830 nm is related to some lipids in the human skin. Minima in the fluorescence spectra, related to re-absorption from the cutaneous pigment — hemoglobin are observed at 420 nm and 540–575 nm. General decrease in the signals depending from skin phototype and anatomic area melanin pigmentation is also observed during measurements,

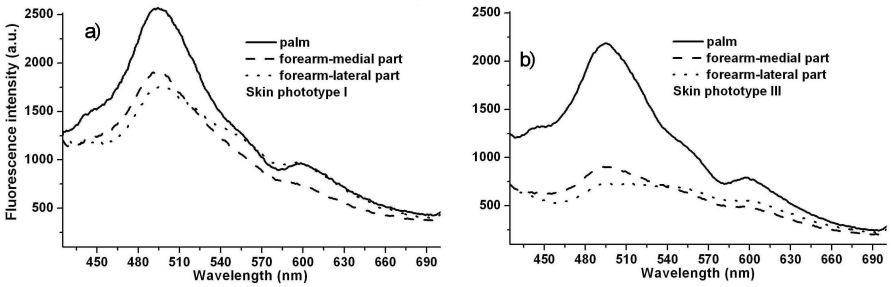

Fig. 4. Comparison of fluorescence spectra from different anatomic areas for phototype I (left) and phototype III (right), using excitation 365 nm.

Fig. 4. This decrease is related to non-selective absorption spectrum of the melanin pigment, which is characterized with slow rise from red to blue spectral region [1, 2, 10, 11].

TABLE

Fluorescent compounds and structures, typical of human skin with their absorption and emission maxima.

Skin chromophore	Description	Absorption maxima [nm]	Fluorescence maxima [nm]
Collagen	structural protein	325	400, 405
Collagen cross-links	structural proteins	370	460–490
Elastin	structural protein	290, 325	340, 400
Elastin cross-links	structural proteins	420–460	500–540
Keratin	structural protein	370	460–500
FAD, flavins	co-enzymes	450	535
NADH	co-enzyme	290, 350	440, 460
Phospholipids	lipids	340–440	430–460, 540–560

In Table there are classified the fluorophores with main contribution to the normal and abnormal skin fluorescence spectra with their absorption and emission maxima, respectively.

4. Conclusions

With the increase in the excitation wavelength, new fluorophores are involved in the shape formation of fluorescence spectrum. This is related to deeper penetration of long-wavelength excitation, to higher absorption at given wavelength for some additional fluorophores and to differences in pigments influence.

Optimization of fluorescence detection could give ones broad opportunities to increase the sensitivity and specificity for early diagnosis and differentiation of

cutaneous lesions. Application of cheaper light sources as LEDs could also help to spread the optical diagnostic methods in clinical practice.

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