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Low power cw-laser signatures on human skin

A. Lihachev, J. Lesinsh, D. Jakovels, J. Spigulis

Abstract. **Impact of cw laser radiation on autofluorescence features of human skin is studied. Two methods of autofluorescence detection are applied: the spectral method with the use of a fibreoptic probe and spectrometer for determining the autofluorescence recovery kinetics at a fixed skin area of ~ 12 mm^2, and the multispectral visualisation method with the use of a multispectral imaging camera for visualising long-term autofluorescence changes in a skin area of ~ 4 cm^2. The autofluorescence recovery kinetics after preliminary laser irradiation is determined. Skin autofluorescence images with visible long-term changes – 'signatures' of low power laser treatment are acquired.**

Keywords: *autofluorescence, skin, photobleaching, laser*.

1. Introduction

Laser radiation is widely used in dermatology for skin diagnostics and treatment of skin diseases, using a wide range of laser wavelengths and radiation powers. The structural changing of skin by high power laser irradiation is used in surgery, dermatology and cosmetology [1]. However, it is supposed that low power laser irradiation (<200 mW cm^{-2}, exposition time up to 10^3 s) is safe for the skin [2].

Photobleaching is the process of decreasing the fluorescence intensity during long-term optical excitation; it can be clinically used in photodynamic therapy for tracking the concentration changes in light sensitising agents (exogenous fluorophores). The decrease in the sensitised fluorescence intensity indicates a decrease in the concentration of light sensitising agents that are able to produce singlet oxygen, which is very important for successful therapy. Photobleaching during the photodynamic therapy reflects destruction of photosensitising agents due to oxidation: the excited sensitiser produces singlet oxygen that triggers destruction of the sensitiser. Such destruction results in a dramatic decrease in the therapy effectiveness [3 – 5]. Purposeful use of the photobleaching effect increases the measurement precision of the keratinoid concentration in the skin and is beneficial in non-invasive Raman spectroscopy [6].

Autofluorescence photobleaching (AFPB) of the skin has been studied in a wide spectral range under ultraviolet (337 nm), violet (405 nm), blue (442 nm), green (532 nm), and red (632 nm) excitation. This phenomenon was observed under pulsed and cw excitation within the power density range 1 – 500 mW cm^{-2} [6 – 10].

The temporal behaviour of the skin AFPB can be well described using the double exponential equation [6 – 10]. Under continuous excitation, the main decrease in the intensity I occurs in the first 10 – 15 s, followed by a relatively slow decrease which strives to a constant intensity level A:

$$I(t) = a\exp(-t/\tau_1) + b\exp(-t/\tau_2) + A. \quad (1)$$

Here, the parameters τ_1 and τ_2 characterise the fast and slow phases of AFPB; a, b and A are the constants; t is the time. Experimental validations of this approximation are described in more details in our previous papers [11, 12].

Previous research on skin photobleaching under 405- and 532-nm laser excitation proved that changes in the excitation power mostly affect the A-value. Spigulis et al. [12] also demonstrated that the temporal AFPB indices at different areas of the skin may be different, and the index distribution over the skin surface is uneven. The potential of photobleaching for skin clinical diagnostics is of particular interest. Previous investigations revealed that the fast photobleaching parameter τ_1 is the most sensitive from the viewpoint of diagnostics. The results of clinical studies showed that the parameter τ_1 significantly differs for healthy skin and skin with pathologies of the same person, in some cases differing even by five times. Photobleaching of hyperpigmented pathologies, however, has not been observed [11].

Most probably, photobleaching is caused by degradation of the skin fluorophore molecules. The fluorophores that emit under blue-green excitation are NAD and keratin co-ferments (localised in epidermis), as well as the dermal collagen and elastin. The reconstructed (NAD-N) and bonded (NAD+) forms of NAD co-ferments have different fluorescence spectra (band maxima at 460 nm and 435 nm, resepectively), quantum yields (for NAD-N it is considerably higher), and different times of fluorescence decay (for NAD-N it is lower) [6, 13].

However, the mechanism of skin AFPB is not yet completely understood. It is also not clear how exactly continuous excitation influences endogenous fluorophores of skin. We can assume that cw irradiation of the skin causes a photochemical process that leads to degradation of endogenous fluorophores.

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In this paper we study the impact of low power cw laser radiation on the characteristics of healthy skin autofluorescence. The effect of skin 'photo-memory' is observed and investigated.

2. Experimental

The purpose of this experiment is to determine the kinetics of the recovery of skin autofluorescence and display long-term changes in autofluorescence properties of previously irradiated skin sectors. Two methods of autofluorescence recording were used in the study: the spectral registration method and multispectral imaging method, which are described below.

2.1 Spectral registration method

To achieve this goal, we used the setup shown in Fig. 1. The setup consisted of a commercial laser (BWT-532-15-SMA, B&WTek, USA) emitting at 532 nm, a fibreoptic probe containing seven fibres (6×400 μm for delivering radiation, 1×400 μm for registration), a spectrometer (AvaSpec-2048, Avantes, The Netherlands), and a computer.

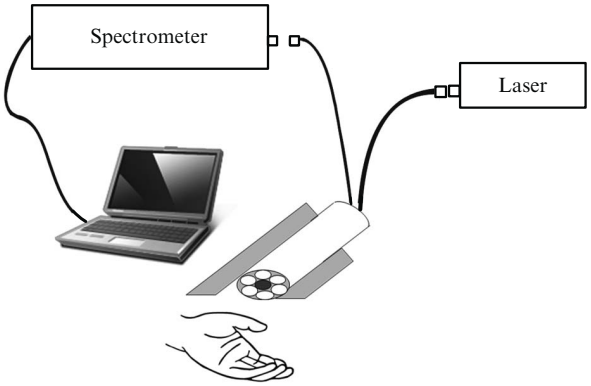

Figure 1. Scheme of the setup for detecting the skin AFPB by the spectral registration method.

Ten round spots 4 mm in diameter were selected on healthy skin of the inner forearm, and the spots were pre-irradiated by a 532-nm laser with a power density 85 mW cm^{-2} for 3 min. The skin autofluorescence intensity at the beginning and at the end of irradiation was registered. Since the intensity decreases under cw excitation due to photobleaching, the value of the intensity at the beginning of irradiation was higher than after three minutes of irradiation. Autofluorescence was excited and registered at a distance of 3 mm from the skin surface. To calculate the recovery kinetics, the maximum intensity of the autofluorescence band corresponding to 600 nm was used.

Thus, ten areas with a decreased fluorescence intensity were obtained on the skin. After a certain control time, the fluorescence was excited again in all ten areas (each of them had a different control time) with the same power density as during the pre-irradiation, and the intensity of the excited fluorescence was recorded. Because each fluorescence excitation initiated the bleaching process, the control measurements were performed only once for each of the 10 areas. As a result, ten average values of the fluorescence intensity versus time were obtained. The study involved three volunteers, the results presented are the arithmetic mean value.

2.2 Multispectral imaging method

To visualise changes in autofluorescence after pre-irradiation, we used a multispectral imaging system shown in Fig. 2. The system consisted of a multispectral camera (Nuance 2.4, Cambridge Research and Instrumentation, USA), two lasers emitting at 405 nm (BWB-405-40-PIG-200-0.22-SMA, B&W Tek, USA) and 532 nm (BWT-532-15-SMA, B&W Tek, USA), and a fibreoptic cable for delivering laser radiation to the skin surface. Two areas of 4 cm^2 were selected on the skin surface, and a mask of a non-transmitting material was imposed on these surfaces. In the case of excitation at 405 nm, the mask had a cross-shaped opening, while in the case of excitation at 532 nm, the mask was in the form of an opaque cross. Each region under the mask was irradiated by a laser with the power density of 35 mW cm^{-2} for two minutes. As a result, two skin areas

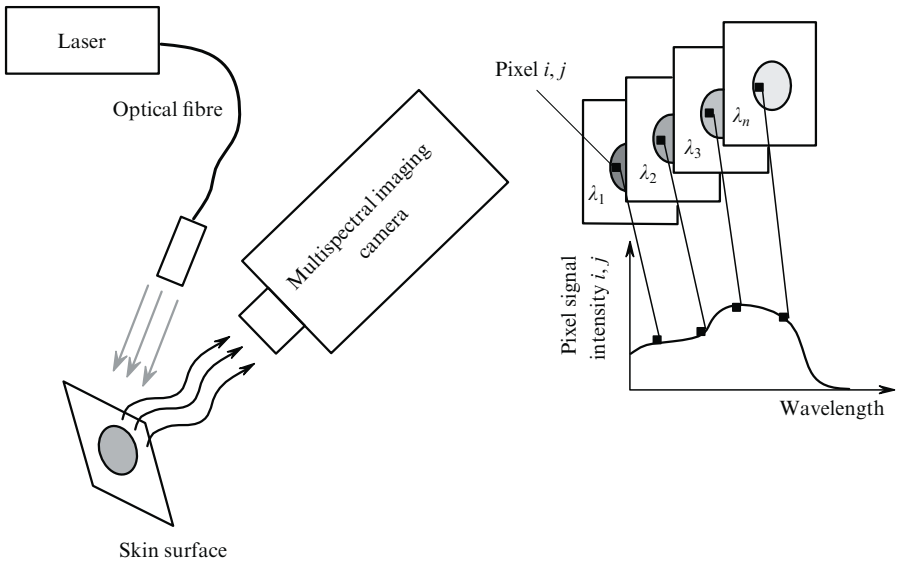

Figure 2. Scheme of the setup for the skin autofluorescence multispectral imaging.

were obtained: one pre-irradiated at 405 nm and the other – at 532 nm. Then, the shielding material was removed from the skin surface, and 24 hours later these areas were irradiated again and the fluorescence images were recorded. To obtain fluorescence images, two narrow-band filters corresponding to the autofluorescence maxima were used: a filter passing radiation in the wavelength range 500 – 520 nm to record the fluorescence excited at 405 nm, and a filter passing radiation in the wavelength range 600 – 620 nm to record the fluorescence excited at 532 nm.

3. Results

Figure 3 illustrates the changes of the skin autofluorescence intensity during repeated excitations. Each excitation leads to a decrease in the intensity caused by photobleaching. After switching off the excitation for 2 min and switching it on again, the autofluorescence intensity never restores back to its initial value. Each subsequent excitation leads to a deeper reduction in the autofluorescence intensity.

Figure 4 presents the kinetics of skin autofluorescence intensity recovery after pre-irradiation by the 532-nm laser. These results show that even after 125 hours of relaxation, the autofluorescence intensity of the skin was recovered for only about 80 % of its initial value.

Figure 5 shows fluorescence images of healthy skin after laser pre-irradiation via cross-shaped masks. Laser 'fingerprints' are visible on the image reproducing the outlines of the shielding material. The preliminary irradiated skin areas fluoresce with a lower intensity.

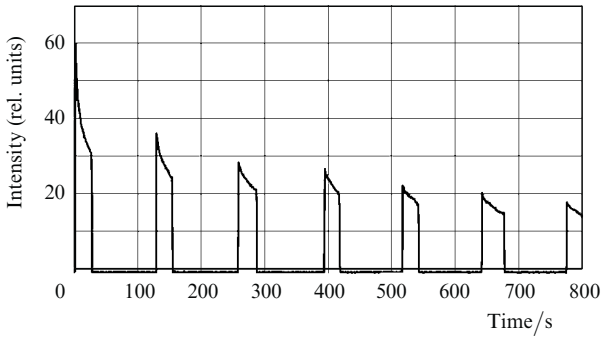

Figure 3. Changes in the skin autofluorescence intensity during repeated excitations. The interval between excitations is 2 min, the power density is 85 mW cm^{-2}, the excitation wavelength is 532 nm, and the registration wavelength is 600 nm.

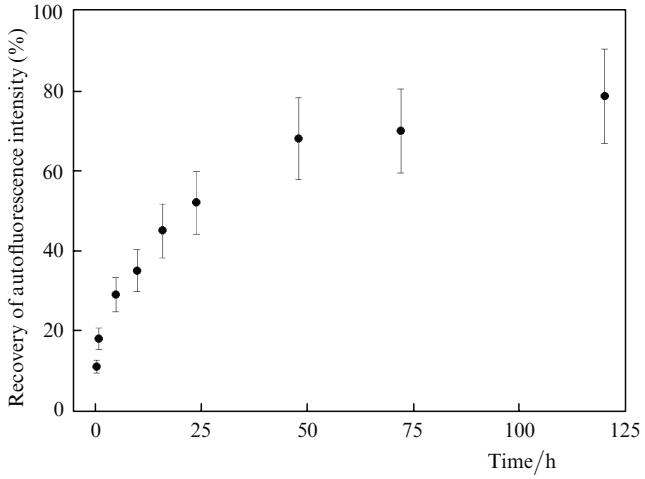

Figure 4. Kinetics of skin autofluorescence recovery after 532-nm excitation. Dots on the graph indicate the mean arithmetic value of the data for three volunteers, vertical lines indicate deviations from this value. Kinetics was obtained at a wavelength of 600 nm.

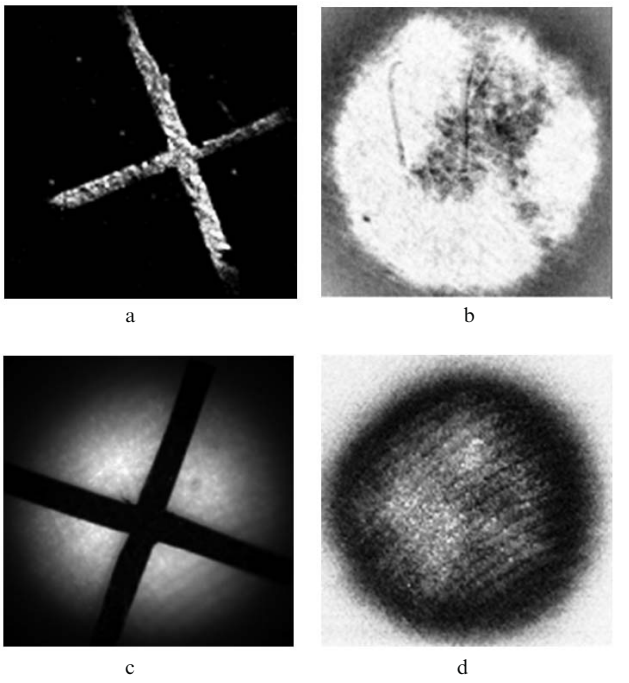

Figure 5. Skin autofluorescence images after pre-irradiation via cross-shaped masks: (a) fluorescence image with a permeable cross mask under 405-nm excitation, (b) fluorescence image of the same skin area without a mask 24 hours after irradiation, (c) fluorescence image with an impermeable cross mask under 532-nm excitation, (d) fluorescence image of the same skin area without a mask 24 hours after irradiation.

4. Discussion

The obtained results show that the autofluorescence intensity from the areas of the pre-irradiated skin is much lower than from 'fresh' skin, indicating long-term changes in the surface structure of the skin. This reveals an interesting property of the skin which can be described as 'photo-memory': the skin 'stores' the previously received low-power irradiation like a photosensitive material, for example, such as a photographic film. The restoration of the autofluorescence intensity is relatively slow – most likely, it indicates long-term changes in the composition (concentration) of fluorophores of the skin, or the reduction of the skin chromophore absorption caused by laser irradiation. The mechanism of these processes should be further explored in more details. An interesting fact is that photobleaching was not observed during direct contact between the skin and the fibreoptic probe; in the case of the excised skin, the autofluorescence intensity also decayed, but never restored.

The results presented here may initiate further review of skin laser safety standards. In this study, exposures of the laser irradiation (15.3 J at the spot irradiation and 4.2 J at

the surface irradiation) were well below the minimum permissible exposures as stated in the European Standard of Laser Safety (200 mW cm^{-2}, exposure time to 10^3 s, i.e., dose of 20.6 J) [2].

5. Conclusions

In this study, we have experimentally demonstrated the effect of 'photo-memory' of the skin resulting after low-power (100 mW cm^{-2}) cw laser irradiation. The effect is manifested as the decreased autofluorescence intensity of pre-irradiated areas of healthy skin. The mechanism of this phenomenon is probably associated with a decrease in the concentration of endogenous fluorophores in the superficial layers of the skin. Reduced absorption of skin chromophores under the influence of laser irradiation is not excluded, as well. The effect of 'photo-memory' requires additional studies to determine the exact mechanism of photobleaching and its impact on the structure and physiology of the skin.

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