

# Influence of low power CW laser irradiation on skin hemoglobin changes

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## ABSTRACT

Influence of low power laser irradiance on healthy skin using diffuse reflectance spectroscopy and multispectral imaging was studied. Changes of diffuse reflectance spectra in spectral range from 500 to 600 nm were observed after 405 nm, 473 nm and 532 nm laser provocation, leading to conclusion that the content of skin hemoglobin has changed. Peaks in spectral absorbance (optical density) curves corresponded to well-known oxy-hemoglobin absorbance peaks at 542 and 577 nm.

Key words: multispectral imaging, laser, diffuse reflectance spectra, optical density, normal skin, hemoglobin

## 1. INTRODUCTION

Laser irradiation is widely used for skin diagnostics and treatment. It is supposed that low power laser irradiation ( $<200 \text{ mW/cm}^2$ , exposition time up to  $10^3 \text{ s}$ ) is safe for the skin [1]. Higher power densities are used in surgery, dermatology and cosmetology to structurally change the skin [2].

Long-term impact of low-power laser irradiation on normal skin's autofluorescence photobleaching has been observed by means of multispectral imaging camera [3]. The autofluorescence recovery kinetics after preliminary laser irradiation was studied, and the skin autofluorescence images showed pronounced long-term changes –“signatures” of low power laser irradiation [3].

The multi-spectral imaging technique has been used for distant mapping of in-vivo skin chromophores by analyzing spectral data at each reflected image pixel and constructing 2-D maps of the optical density [4]. Point measurements of human skin diffuse reflectance spectra (DRS) contain both absorption and scattering characteristics of tissue and also could be useful for chromophore analysis.

Most probably, photobleaching is caused by degradation of the skin fluorophore molecules. The fluorophores that emit under blue-green excitation are NAD and keratin coferments (localized in epidermis), as well as the dermal collagen and elastin. The reconstructed (NAD-N) and bonded (NAD<sup>+</sup>) forms of NAD co-ferments have different fluorescence spectra (band maximum at 460 nm and 435 nm, respectively), quantum yields (for NAD-N it is considerably higher), and different times of fluorescence decay (for NAD-N it is lower). However, the mechanism of skin autofluorescence photobleaching is still under study. It is not yet 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. [1, 5, 6]. Role of hemoglobin in this process has not been studied so far.

## 2. EQUIPMENT AND METHODOLOGY

Two methods of skin diffuse reflectance spectra recording were used in the study: the single-spot irradiation/detection by means of fiber optic probe and spectrometer, and the non-contact method by means of multispectral imaging system.

## 2.1 Contact method

The contact DRS set-up included light source (10 W halogen lamp, AvaLight-HAL, Avantes BV, NL), detector (the dual channel AvaSpec-2048-2 spectrometer with 2048 pixel CCD detector array, spectral range 200 to 1100 nm, resolution 2.1 nm, Avantes BV, NL), cw low power lasers with wavelengths of 532 nm, 473 nm, 405 nm and fiber optic contact probe.

The contact probe was designed to provide the optimal distance of 3 mm between the skin surface and the optical fiber tip for the sample illumination and signal detection. The optical fibers were connected to illuminating halogen lamp, irradiating laser and detecting spectrometer. DRS of the skin surface was registered as the reference before the irradiation of laser. During 5 second interval reflected spectrum was recorded at 0.5 second integration time calculating the average value. Then skin surface was irradiated for 60 second by a selected laser via the same fiber. The optical density (OD) spectrum of the skin surface was registered immediately after lasers irradiation. Measurements were taken at different laser power density levels ranging from 20 mW/cm<sup>2</sup> to 120 mW/cm<sup>2</sup> each time of another “fresh” skin area.

## 2.2 Non-contact method

The non-contact method comprised of multi-spectral imaging system Nuance 2.4 (Cambridge Research & Instrumentation, Inc., USA) for response detection, illumination light source, cw low power lasers and PC. Skin areas of the forearm were measured for spectral analysis of normal and provoked skin. A 100 W tungsten incandescent lamp (intensity fluctuations less than ±2% during the measurement time) with linear polarization filter was used as illumination source. The polarizer was oriented orthogonally to the built-in polarizer of the camera, so significantly reducing the influence of skin specular reflection [4, 7].

The system was adjusted for spatial resolution 0.75x0.75 mm (the pixel size) and spectral resolution 10 nm (bandwidth of the Nuance 2.4 liquid crystal tunable filter).

The data were collected in an image cube – a stack of intensity images at numerous wavelength bands. Typical time required for creation of the image cube in spectral interval 450–750 nm was 10 s. The back reflected light intensity (*I*) values at each pixel were transformed to the optical density OD as

$$OD = -\log_{10} \left( \frac{I}{I_0} \right) \quad (1),$$

where *I*<sub>0</sub> – reflection intensity of the skin before the laser irradiation, *I* – intensity after laser irradiation.

Multispectral camera and optical fiber for laser beam delivery to skin surface were set stationary. Measurement lasted about 2 minutes, during this time patient held forearm fixed. Diffuse reflectance spectra were registered before and after laser irradiation of 60 second period. For irradiation lasers of 405 nm and 473 nm wavelength were used. Laser power density was about 120 mW/cm<sup>2</sup> for wavelength of 405 nm and about 100 mW/cm<sup>2</sup> for wavelength of 473 nm.

## 3. RESULTS

Results of the contact method measurements are presented in Fig.1 – normal skin relative optical density changed immediately after laser irradiation at all exploited wavelengths. One can see that the green 532 nm irradiation does not influence much the DRS signals, but both 473 nm and 405 nm irradiations cause significant signal changes. The most pronounced spectral changes were observed after 405 nm irradiation.

Temporal behavior of optical density changes demonstrated a fast return to the reference level in about 3 minutes after 532 nm laser irradiation. The same tendency was observed if 405 nm and 473 nm lasers were used, but the return time was slightly longer.

Non-contact spectral imaging results of the measurements are presented in Fig.2. The laser irradiated skin areas have less intense DRS resulting in relative optical density changes, as expected.

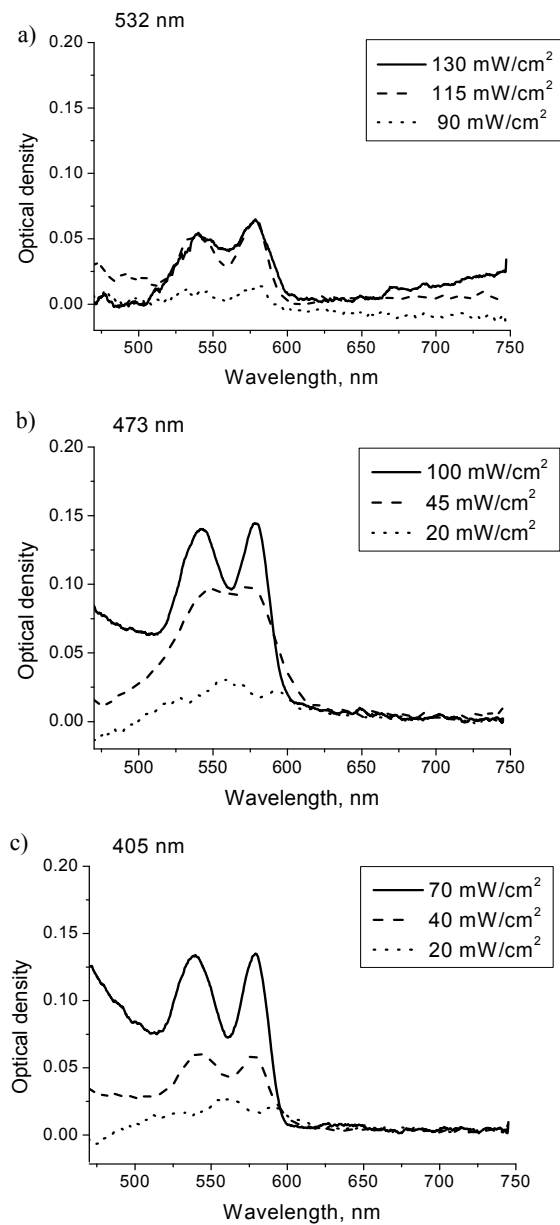


Fig.1. Changes of relative optical density depending on irradiation power density. a) skin was irradiated with cw laser of 532 nm, b) skin was irradiated with cw laser of 473 nm, c) skin was irradiated with cw laser of 405 nm

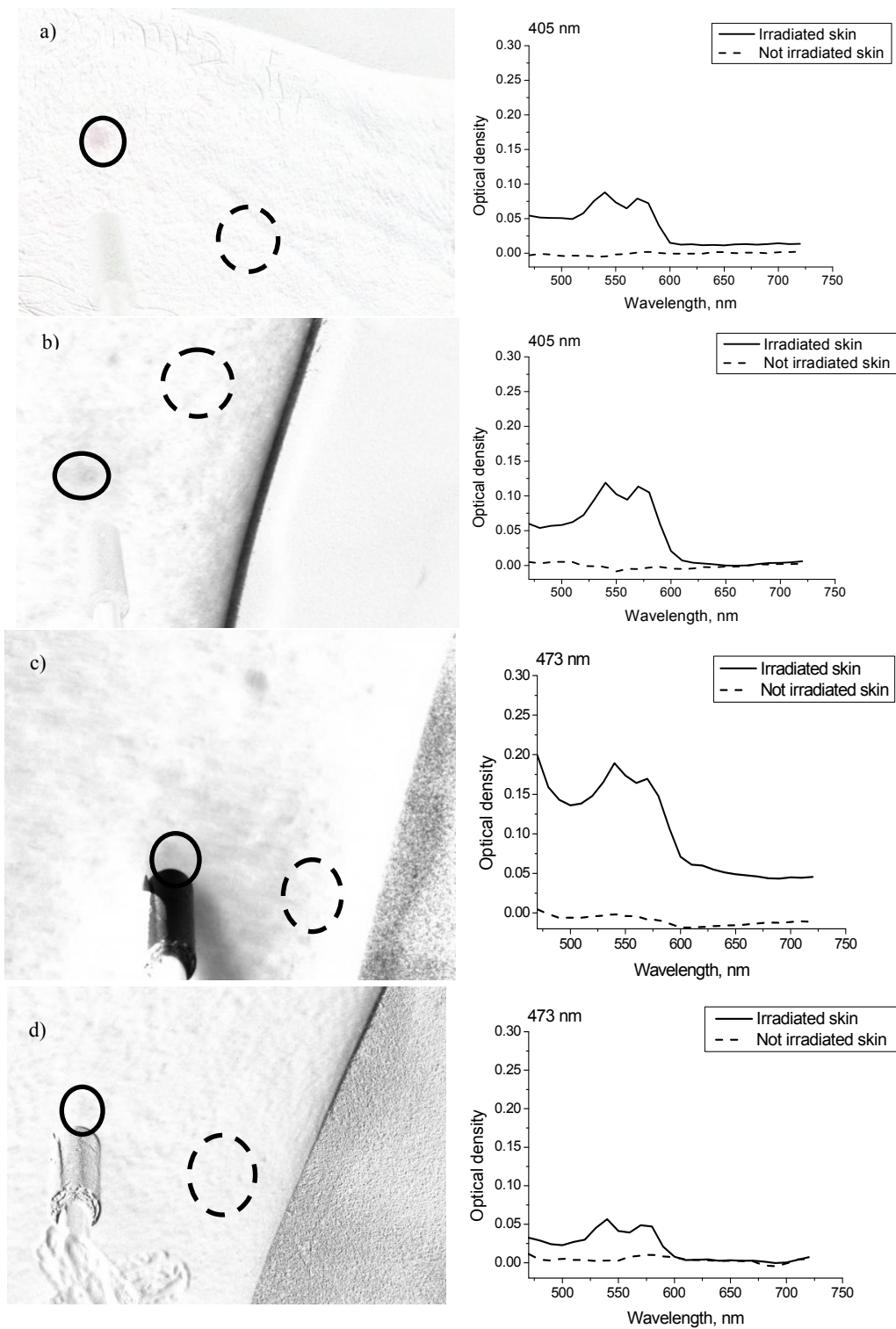


Fig.2. Left-side: multi-spectral images. Right-side: changes of relative optical density of selected areas in images. a), b) skin was irradiated with 405 nm laser; c), d) skin was irradiated with 473 nm laser.

## 4. DISCUSSION

Impact of low power laser irradiance on healthy skin shows up as dynamic changes of optical properties of tissue. This study provides insight into reflected optical information after AFPB effect, while our previous studies [3, 8] showed changes in optical properties of ongoing AFPB process in skin. The obtained results show that laser irradiation can affect the layers of human skin. Peaks at 542 nm and 577 nm in the relative optical density correspond to absorption of hemoglobin [9]. The same hemoglobin absorption impact were observed in previous study [8] indicating to some role of hemoglobin in the skin AFPB process at the 473 nm irradiation, and the 405 nm laser irradiation probably increases this role while 532 nm laser irradiation shows less significant spectral changes to autofluorescence spectra due to hemoglobin absorption (all laser output powers were adjusted to be approximately equal during the previous experiments).

Temporal behavior of relative OD shows that DRS absorption is a short-term effect – the DRS recovered to its initial level in a few minutes. Although the laser power densities per area unit were the same, for shorter laser wavelength the greater power density per volume unit were present, taking into account wavelength specific optical penetration depth into tissue. This resulted in longer return of OD values to the reference level after skin irradiation of 473 nm and 405 nm lasers. Our previous study [3] showed that autofluorescence recovery kinetics after laser irradiation is a long term effect. As relative restored intensity of autofluorescence after photobleaching is much less than fully restored DRS intensity in few minutes, this indicates to the DRS as a short-term coincidence with skin fluorophore degradation and as well as modification of skin absorption properties by laser irradiation.

Probably, complicate mechanisms of low power laser irradiation may cause direct skin fluorophore (for instance, porphyrin) degradation, as well as modification of absorption and quenching properties by both photo- and thermally-induced biophysical and biochemical processes inside the highly heterogeneous tissue structure. Appearance of hemoglobin absorption in the skin is caused by local fluorophore degradation during optical excitation, thereby increases the probability of light to penetrate to the deeper skin layers, where skin capillaries and vessels are located. Also it is assumed that laser irradiation causes inflammation by local heating and to this place comes more blood in regard to expanded capillaries, as an innate immune system defense of the organism to injurious stimulus. Increased blood volume fraction leads to intense optical absorption in oxyhemoglobin, after laser irradiation the skin locally cools down, so the capillaries narrow to initial size and oxyhemoglobin deoxygenates as the skin proceeds to heal itself.

The average change of the optical density depends on laser irradiation power and excitation laser wavelength. By contact method the change of the OD using laser with wavelength of 532 nm and power density of 120 mW/cm<sup>2</sup> is less than using laser with wavelength of 405 nm, 473 nm and power density of 70 mW/cm<sup>2</sup>, 100 mW/cm<sup>2</sup>.

Multispectral imaging camera was less sensitive to changes of OD due to spectral resolution of 10 nm and larger distance between skin surface and camera. However, non-contact methodology demonstrated the same features as OD peaks after low power laser-skin interaction compared to acquired results with single point methodology by using higher laser power densities for imaging than for contact method.

The obtained results coincide with the above postulated hypotheses. Fluorophore degradation might allow the light to penetrate the deeper layers of the skin as well as photochemical reactions can cause inflammation and locally increased blood volume as the immune defense.

## 5. CONCLUSIONS

Skin diffuse reflectance spectra showed increased hemoglobin level after 405nm, 473nm and 532nm cw laser irradiation at power densities around 100 mW/cm<sup>2</sup> (twice below the laser skin safety limit [3]). This indicates to some photo-inflammation; mechanism of erythema creation may be similar to that of sun-caused erythema. Accordingly to the obtained results, visible laser skin safety limits should be lowered at least for an order of magnitude to avoid any photo-biological effects.

Further studies are required to establish a general relationship between the mechanisms of the complex laser-skin interaction and the appearance of hemoglobin absorption after irradiation. Combined experiments involving parallel measurements of skin autofluorescence photo-bleaching and diffuse reflectance spectrometry are planned.

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