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Abstract. Optical fiber contact probe diffuse reflectance spectroscopy and remote multispectral imaging methods in the spectral range of 400 to 1100 nm were used for skin vascular malformation assessment and recovery tracing after treatment by intense pulsed light. The results confirmed that oxy-hemoglobin relative changes and the optical density difference between lesion and healthy skin in the spectral region 500 to 600 nm may be successfully used for objective appraisal of the therapy effect. Color redness parameter $a^* = 2$ is suggested as a diagnostic border to distinguish healthy skin and vascular lesions, and as the indicator of phototreatment efficiency. Valuable diagnostic information on large area (>5 mm) lesions and lesions with uncertain borders can be proved by the multispectral imaging method. [DOI: 10.1117/1.3569119]

Keywords: multispectral imaging; diffuse reflectance spectroscopy; skin vascular lesions.

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Fast and painless noninvasive assessment of skin lesions is very important in medical treatment. Such assessment has been performed by optical coherence tomography[1] and pulsed photothermal radiometry.[2] Diffuse reflectance spectra of human skin contain both absorption and scattering characteristics of tissue and also could be useful from this point. Optical parameters of skin vascular malformations are mainly related to modified hemoglobin absorption.[3]

Diffuse reflectance spectroscopy (DRS) measurements can be taken in contact or noncontact modes. Several authors[4,5] have used optical fiber-based skin contact probes, where light is delivered at a specific point on the skin surface and the remitted signals are collected at some distance from the source point by another optical fiber.

DRS data can be converted into skin color parameters.[3] Tri-stimulus analysis converts intensity versus wavelength data (i.e., spectral information) into three numbers that indicate how a color of an object appears to a human observer: $L^* = 116(Y/Y_0)^{1/3}–16$, $a^* = 500[(X/X_0)^{1/3}–(Y/Y_0)^{1/3}]$, $b^* = 200[(Y/Y_0)^{1/3}–(Z/Z_0)^{1/3}]$, where X_0, Y_0, Z_0 are nominally white object-color stimuli given by CIE standard[6] C, $Y_0 = 100$, $X = \sum_{380\,\mathrm{nm}}^{780\,\mathrm{nm}} R_d(\lambda)\bar{x}(\lambda)\Delta\lambda$, $Y = \sum_{380\,\mathrm{nm}}^{780\,\mathrm{nm}} R_d(\lambda)\bar{y}(\lambda)\Delta\lambda$, $Z = \sum_{380\,\mathrm{nm}}^{780\,\mathrm{nm}} R_d\bar{z}(\lambda)\Delta\lambda$—is tristimulus values, R_d–the total skin diffuse reflectance, $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, $\bar{z}(\lambda)$–three color matching parameters, which represent the spectral sensitivity of a standard observer, $\Delta\lambda = 5$ nm. L^* indicates light intensity and takes values from 0 (black) to 100 (white). Parameter a^* indicates the color of the object on a scale that goes from green (negative values) to red (positive values). Criterion b* indicates the color of the object on a scale from blue (negative values) to yellow (positive values). Several authors have investigated color of different skin phototypes, pigmentation,[7] and post-inflammatory hyperpigmentation (hypermelanosis).[8] To the author's knowledge, vascular lesion recovery studies have not been performed by such analysis so far and could be tried as an alternative assessment method.

Multispectral imaging (MSI) is a contactless method based on subsequent image acquiring of a fixed object area at different wavelength bands, usually covering the visible (VIS) and near-infrared (NIR) region (400 to 900 nm). The MSI technique has been used for studies of skin tumors, cutaneous inflammations, healthy and bruised skin.[9–11]

Vascular diseases are widely phototreated by intense pulsed light (IPL). Such treatment is based on the absorption of photons by endogenous and exogenous chromophores within the skin, heating them up and destructing the target structures.[12] In order to monitor the skin recovery processes, reliable quantitative methodologies have to be developed, and DRS technology seems to have good potential for it.

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), and two types of skin contact probes. Probe A with six source fibers surrounding one detector fiber (source-detector distance D=0.4 mm), all with 400 μm core diameter, was placed orthogonally to the skin surface for contact DRS measurements with shallow penetration, and at a 45 deg angle without direct contact—for skin color measurements. The dual fiber probe B with a 600 μm core source fiber and a 7×200 μm detector fiber bundle (D=2 mm) was used for measurements in deeper layers of the skin. The AvaSoft-Basic software[13] performed the data storage and processing.

The MSI experimental set-up included a multispectral camera Nuance EX (CRi, USA). The *Nuance* imaging module contained a high-resolution, scientific-grade CCD imaging sensor, solid-state polarizing liquid crystal filter, wavelength tuning element, and an spectrally optimized objective.[14] The illuminating white light emitting diode (450 to 700 nm) ring source with a

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polarizing film (oriented orthogonally to polarizer in the camera) was mounted around the objective. Images were acquired within the wavelength range 500 to 700 nm with the scanning step ~10 nm and were stored in the laptop for further processing.

The contact measurements of local diffuse reflectance were taken from diseased and healthy skin in the spectral range 400 to 1100 nm. The detection depth (d) was evaluated according to reported results of the studies of probe geometry influence on vessel detection and Monte Carlo simulations:[4,5] the mainly superficial level (d~1 mm) was detected by probe A (D=0.4 mm), the deeper level (d~2 mm) was reached by probe B (D=2 mm). The spectra were normalized at 500 nm for the VIS spectral region, and at 700 nm for the NIR spectral region. The typical measurement error of the spectra was 5 to 8%.

The multispectral image of a white reference tile with flat diffuse reflectance 99% (WS-2, Avantes) was taken before each measurement. The optical density (OD) was automatically calculated by the CRi Nuance program: OD (λ) = – log [$I(\lambda)/I_0(\lambda)$], where $I(\lambda)$ is the intensity of light reflected from the skin, and $I_0(\lambda)$ is the intensity of light reflected from the white reference.

To compare the DRS and MSI techniques, the OD values were calculated for the lesion and nearby healthy skin. In the case of MSI the corresponding mean OD values were taken. Then the OD spectrum of healthy skin was subtracted from the OD spectrum of the lesion. The area under the curve of optical density spectral difference (OD_{lesion}–OD_{skin}) was calculated in the range 500 to 600 nm (where oxy-hemoglobin features are most pronounced), and the obtained results before and after treatment were compared.

Overall 20 patients with 20 cases of vascular lesions were inspected. Eight cases of port-wine stains (PWS) were inspected before, immediately after, and about 1 month after phototreatment by means of the contact DRS method. The lesions were treated by the IPL VascuLight^{TM} system (515 to 1200 nm; 3 to 90 J/cm^2; repetition rate: 0.11 Hz; pulse duration: 0.5 to 25 ms). Twelve cases (telangiectasia, hemangioma, PWS) were inspected by applying the MSI technique before, immediately after and 14 to 35 days after the laser treatment. Lesions were treated by the IPL Quantum^{TM} system (560 to 1200 nm; 15 to 45 J/cm^2; pulse sequence: 2 to 3 pulses; pulse duration: 6 to 26 ms).

Diffuse reflectance spectra from both healthy skin and PWS clearly showed hemoglobin and oxy-hemoglobin absorption bands at 414 nm and between 500 and 600 nm. The OD-ratio of the normalized spectra for the superficial skin layer was calculated (Fig. 1). PWS demonstrated stronger absorption than healthy skin over the whole spectral range, which can be related to higher blood content in the damaged tissue.

Figure 1(a) illustrates the recovery pattern of average spectra for PWS. High blood content in tissue before and immediately after the treatment was confirmed by the reduced ratio in the region 530 to 590 nm. Stronger absorption peaks at 542 and 577 nm denoting the presence of oxy-hemoglobin lead to the lower intensity ratio "lesion/healthy tissue." After 2 months the diffuse reflection ratio values were much closer to 1.0 over the whole spectral range.

Diffuse reflectance spectra were studied in the NIR range as well. Figure 1(b) shows averaged spectral ratio curves of the PWS before and 1 month after treatment. Spectral differences in

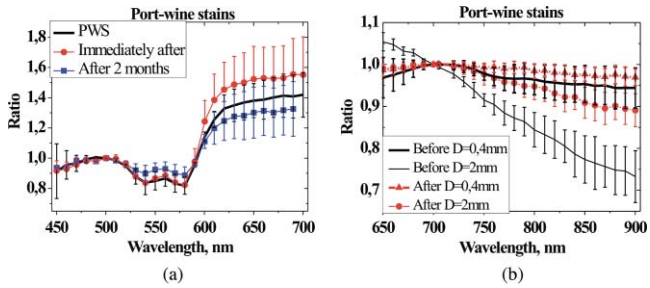

Fig. 1 Recovery pattern of PWS (a) in the VIS range, normalized at 500 nm (the spectral ratio of PWS to healthy skin before, immediately after, and 2 months after the treatment at superficial layer, source-detector distance D=0.4 mm) and (b) in the NIR range, normalized at 700 nm (the spectral ratio of PWS to healthy skin before and 1 month after treatment at D=0.4, 2 mm).

deeper skin layers (detected by the D=2 mm probe) were more pronounced than those in the superficial layer (D=0.4 mm). The results obtained with both probes confirmed the lesion recovery after 1 month—the spectral ratio curves were getting notably closer to the healthy skin level 1.0.

Figure 2(a) shows the OD difference of PWS and healthy skin at the superficial layer (D=0, 4 mm) before, immediately after treatment and after 2 months. Figure 2(b) shows how the recovery process influences the area under the curve ($\Delta OD = OD_{lesion} - OD_{skin}$) in the range 500 to 600 nm. Before treatment and immediately after it the change is negligible; however, after 2 months the area has dramatically decreased, indicating the recovering of skin.

Figure 3 illustrates the potential for recovery monitoring of PWS by color parameters. The diagnostic criterion $a^* = 2$ may be used to mark the difference between lesion and healthy skin. Lesions before (circles) and after (up triangles) treatment corresponded to $a^* > 2$ (higher red color values), while healthy skin or almost recovered lesions corresponded to $a^* < 2$. Besides, integral reflected light intensity difference ΔL between lesion and healthy skin before and after treatment was compared [Fig. 3(b)]. There are obvious changes after treatment where the difference ΔL approaches the zero level.

Decomposition of each MSI image set gives a feeling on the lesion at different penetration depths corresponding to the specific wavelengths. Immediately after the treatment the optical density in the region of lesion increased at the wavelength range 450 to 550 nm, but less pronounced changes could be detected

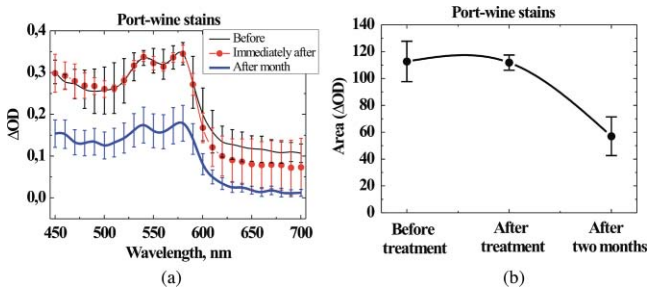

Fig. 2 (a) The differential OD spectra of PWS and healthy skin at superficial layer (D=0.4 mm); (b) the areas under the spectra curves ($\Delta OD = OD_{lesion}$–OD_{skin}) of PWS before treatment, after IPL treatment and after 2 months in the 500 to 600-nm range.

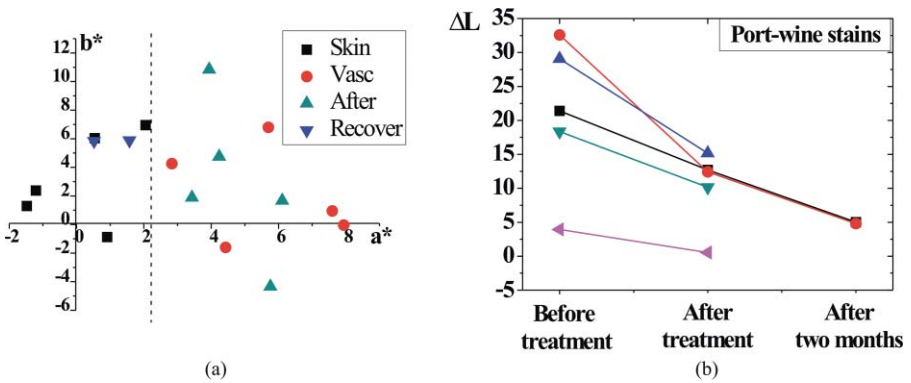

Fig. 3 Lesions' recovery monitoring by (a) color parameters a^* and b^* (squares—healthy skin, circles—PWS, up-triangles—PWS after treatment, down triangles—recovered skin) and (b) light intensity difference ΔL between healthy skin and PWS for five volunteers.

at 700 nm. Two weeks after the treatment at the range 450 to 550 nm obvious recovery was observed if compared to the images before treatment; at 700 nm there was a full recovery.

Figure 4 shows the OD spectral differences of healthy skin and vascular lesions before, immediately, after, and 2 to 3 weeks after the IPL treatment. In the case of telangiectasia immediately after treatment the area under the curve increased due to the increment of oxy-hemoglobin. After 2 to 3 weeks the areas slightly decreased, but entire recovery had not yet been reached.

The clinical studies have confirmed the potential of two non-invasive techniques (DRS and MSI) for IPL therapy efficiency assessment of skin vascular lesions. The OD difference of lesion and healthy skin in the spectral region 500 to 600 nm can be recommended for follow-up of vascular malformations and objective estimation of the therapy effect. The DRS method is more advisable for lesions of small size (2 to 5 mm); this method also enables the assessment by color parameter values. Color redness parameter $a^* = 2$ is suggested as a marker to distinguish healthy skin and vascular lesions, and as a criterion for lesions' recovery monitoring. The MSI technique is more convenient for analysis of larger lesions (>5 mm) or those with uncertain borders (e.g., PWS and telangiectasias) and provides information on spatial distribution of skin damages at specific wavelength bands.

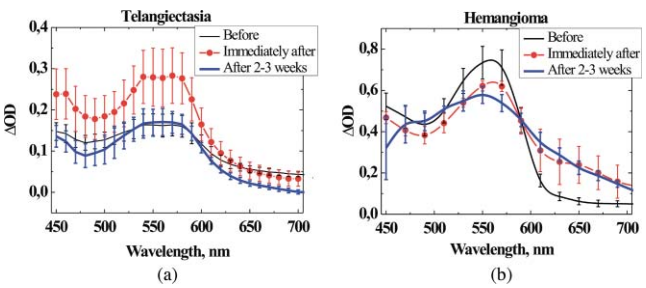

Fig. 4 (a) Lesions' recovery monitoring by MSI: the OD difference spectra of vascular lesion and healthy skin before, immediately after, and 2 to 3 weeks after IPL treatment for telangiectasia and (b) hemangioma.

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