Spectral Characteristics of Melanin-Pigmented Cutaneous Neoplasia

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Abstract. Optical spectral techniques for the detection of tissue pathologies, including cutaneous malignancies, are a powerful tool in medical diagnostic techniques' development. In the line of different skin neoplasia, malignant melanoma occupies a special place because of its rapid development and high metastatic activity leading to high mortality in patients. Therefore, new, sensitive and non-invasive methods are sought to properly diagnose and differentiate melanoma from other skin neoplasms, as well from dysplastic and benign melanin-pigmented skin lesions such as nevi.

Optical biopsy based on various spectral techniques, applied solely and/or in combination, incl. autofluorescence and diffuse-reflectance spectroscopy tools is one of the new approaches developed for an early diagnosis of melanin-pigmented malignant melanoma. In our study, we used autofluorescence spectroscopy of pigmented skin lesions at UV-VIS excitation, as well as broad-band diffuse-reflectance spectroscopy in the VIS-NIR (400-900 nm) range. Specific spectral characteristics for benign nevi, dysplastic nevi and for malignant melanoma were compared and determined for patients investigated *in vivo*. Characteristic spectral indicators for the fluorescence and reflectance characteristics of these tumors have been found. Differentiation algorithms have been developed to achieve a diagnostic accuracy of 93% for differentiation of dysplastic nevus from melanoma, which significantly improves this statistical parameter compared to the classic dermatoscopic observation.

Keywords: optical biopsy, fluorescence spectroscopy, diffuse reflectance spectroscopy, dysplastic nevi, malignant melanoma

1. INTRODUCTION

The optical spectral techniques for detection of tissue pathologies, including malignant neoplasms, are powerful tools in the development of medical diagnostics methodologies. The design, development and clinical application of the spectral techniques are among the major areas of biomedical photonics. The latter is the newest and the most quickly progressing field of physical technologies as applied to the life sciences.

Early diagnostics is considered as being a leading tool in improving the oncological patients' treatment, with many scientists endeavoring in the field of developing new systems and methodologies for early and precise detection and analysis of malignant neoplasms. The optical techniques allow non-invasive, real-time, high-precision, and objective work, so that the possibilities they offer are widely studied in view of their development and use as diagnostic techniques of analysis of tumour formations (Moy et al, 2016; Moy et al., 2017; Lihachev et al., 2018).

Among the cutaneous neoplasms, the malignant melanoma (MM) occupies a special place because of its fast development and high metastatic activity, both leading to a high mortality rate. Broadening the spectral and analytical methods applied brings about an increase in the diagnostic accuracy. The determination of tumour tissues' basic spectral parameters will lead to increasing the diagnostic accuracy and specificity in determining the type of pathology when spectral techniques are applied to the clinic. (Lihachev et al., 2018; Murphy et al., 2005)

The interaction of light with the skin is complex - in the detected reflected signal significant contribution has specular reflection itself of the surface and lower tissue layers, as well the back-scattered diffuse component. On the skin surface, due to the microscopic inhomogeneities at the boundary of the air-Stratum corneum layer, a collimated beam of incident light passes into diffuse reflection, reflecting 5-7% of the incident light. In diffuse backward scattering, some of the light is absorbed by the various tissue absorbers, resulting alteration in the spectrum of the back-scattered signal. Part of the backscattered photons fall at the boundary of the stratum corneum at angles greater than the limit and return back to the tissue (about 55%), others form the diffused backscattered light component, which is detected on the surface (Zhang et al., 2017; Petruk et al., 2016)). A schematic illustration of the interaction of light with the skin is presented in Fig. 1.

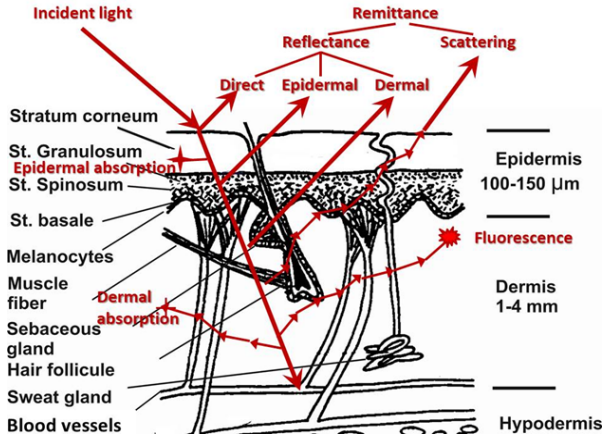

Fig. 1 Principal scheme of light-skin interaction in different cutaneous layers including reflectance, scattering, absorption and fluorescence.

On the Fig. 1 is presented principal scheme of light-skin tissues interaction and possibilities to obtain signal useful for diagnostic determination of the tissue state, based on its reflectance, scattering, absorption and/or fluorescence spectral properties.

2. MATERIALS AND METHODS

Skin melanin – pigmented lesions *in vivo* and were investigated spectroscopically using autofluorescence and diffuse-reflectance techniques to evaluate the differences in the spectral properties with diagnostic value. In table 1 are presented number of lesions evaluated and their diagnosis, according histological examination, used as a "gold standard" for comparison with spectral measurements carried out.

TABLE 1. Number of skin lesions evaluated *in vivo* and their diagnosis according "gold standard" histological verification

Lesions	Number of lesions
Compound nevus	17
Dysplastic nevus	12
Malignant melanoma	14

Measurements *in vivo* were obtained with the multiple wavelength excitation of the endogenous fluorescence of benign and malignant cutaneous lesions. Initially, lesions were classified visually by experienced dermatologist (P.T.) and dermatoscopically using ABCD scoring criteria Second step was detection of lesion' and surrounding normal skin fluorescence using different excitation wavelengths, namely 365, 385, 405, and 440 nm. Reflectance spectra are detected using broad-band white halogen lamp as a light source in the region 400-900 nm.

Optical fibers were used to deliver the light and to collect the fluorescence and reflectance sig-nals. The spectra were recorded and stored using a fiber-optic microspectrometer (USB4000, Ocean Optics, Dunedin, FL, USA).

A personal computer was used to control the system and to store and display the data using the specialized microspectrometer software Spectra-Suite ("Ocean Optics" Inc., Dunedin, FL, USA).

3. RESULTS AND DISCUSSION

Chromophores, related to the formation of autofluorescence signals observed are mainly structural proteins, their cross-links, co-enzymes and lipids. (Bachmann et al, 2006, Svanberg, 2004) The resultant spectrum detected *in vivo* is a superposition of these compounds with different level of expression for each excitation wavelength applied. Endogenous fluorophores, responsible for the signal detected are collagen type I – at 400-405 nm; its cross-links – at 460-490 nm; elastin – with maxima at 400-420 and 460 nm; NADH – at 440-470 nm; keratin – at 430-460, and around 520-550 nm, flavins –in the region of 510-540 nm. (Kollias et al, 2002; Bratchenko et al, 2015)

Influence of the hemoglobin and melanin pigments is observed in the received fluorescence and reflectance spectra related to bigger decrease of the short-wavelength intensity vs. long-wavelength intensity, as well as appearance of

minima at 420, 543 and 575 nm respectively, see Fig. 3.

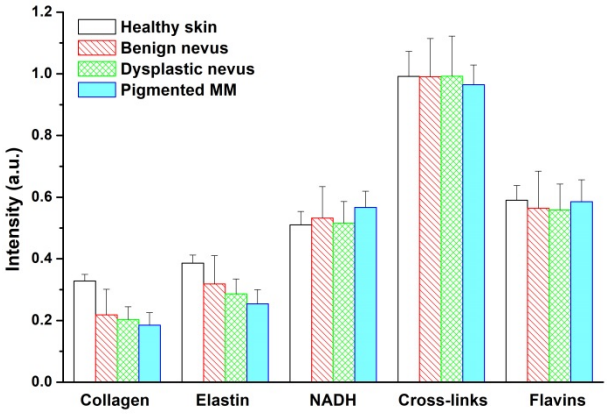

Fig. 2 Fluorescence emission for different endogenous fluorophores appeared in the normal skin and melanin-pigmented benign, dysplastic and malignant cutaneous lesions emission spectra. Protein cross-links emission signal was used for normalization of the fluorescence emission to obtain comparable values for the other types of endogenous fluorophores.

Structural proteins fluorescence signal fading was observed in malignant lesions in comparison with normal skin (see Fig. 2) and collagen and elastin emission values are significantly decreased. In opposite, small increase of the co-enzymes emission of NADH and flavins was observed that correlated to the increased metabolic activity in these tissues.

On Fig. 3 is presented comparison of diffuse-reflectance spectra of normal skin and melanin pigmented lesions. Significant decrease in the short wavelength region is related to the significant absorption of melanin in that spectral range. Hemoglobin absorption is pronounced with three minima, first situated at 400-420 nm, and second two – in the region of 543 and 575 nm for oxy-hemoglobin or one broad maximum in the region of 550-570 nm for deoxy-hemoglobin. The reduced level of oxygenation is typical for neoplastic lesions and such signal, related to deoxy-hemoglobin is observed in case of malignant neoplasia.

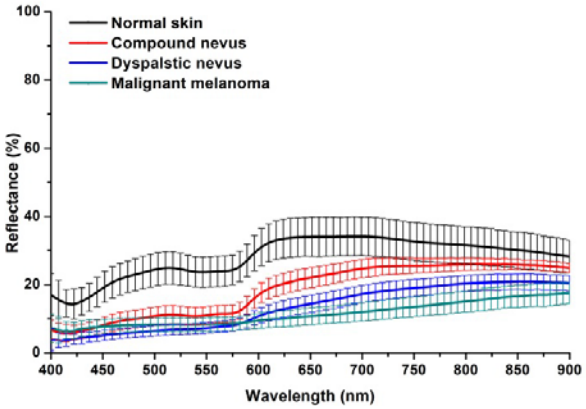

Fig. 3 Diffuse reflectance spectra of normal skin and melanin-pigmented benign, dysplastic and malignant cutaneous lesions. Reflectance spectra are averaged by type of lesions' diagnosis.

TABLE 2. Number of skin lesions evaluated *in vivo* and their diagnosis according "gold standard" histological verification

Statistical parameters	Value {%}
Sensitivity	100
Specificity	93
Positive predictive value	90
Negative predictive value	98
Index of suspicion	111
Diagnostic accuracy	93

Based on the spectral features detected for different pigmented lesions a discrimination algorithm was developed. In table 2 are presented statistical parameters of diagnostic discrimination for diagnosis of malignant melanoma. Diagnostic accuracy of 93 % is achieved for differentiation of nevi from malignant melanoma, which significantly improves this statistical parameter compared to the classic dermatoscopic observation.

4. CONCLUSIONS

It was received a good correlation between histological diagnosis of the pigmented cutaneous lesions and repeatability of the features of the spectral signals from patient to patient with one-type lesion obtained. Results of the present study suggest that the used approaches can provide useful information for the diagnosis of given types of pigmented lesions, that could be transformed into diagnostic algorithm for clinical usage.

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