Optical UV-VIS-NIR spectroscopy of benign, dysplastic and malignant cutaneous lesions ex vivo

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ABSTRACT

Optical spectroscopic measurements with a few different modalities have been performed namely autofluorescence, transmission and diffuse reflectance spectroscopies in ultraviolet, visible and near-infrared spectral ranges.

The investigated samples were cutaneous tumours ex vivo, obtained after surgical removal and kept in a formalin solution and histological sections from biopsy tissue samples, which were routinely processed for histological analysis. Comparative spectral data for benign, dysplastic nevi and pigmented malignant melanoma lesions, as well as for non-melanoma skin tumour – basal cell carcinoma, squamous cell carcinoma and benign non-melanin pigmented pathologies – heamangioma and seboreic veruca are presented in the current report.

Fluorescence spectra obtained reveal statistically significant differences between the different benign, dysplastic and malignant lesions by the level of emission intensity, as well by spectral shape, which are fingerprints applicable for differentiation algorithms. In reflectance and absorption modes the most significant differences are related to the influence of skin pigments – melanin and hemoglobin, less pronounced is the influence of structural proteins, such as collagen and keratin. Transmission spectroscopy mode gives complementary optical properties information about the tissue samples investigated to that one of reflectance and absorption spectroscopy.

Keywords: fluorescence spectroscopy, skin cancer, diffuse reflectance spectroscopy, transmission spectroscopy

1. INTRODUCTION

Due to their high sensitivity in detection of small changes, optical techniques are widely used for detection of early changes in biological tissues. One of the promising directions of optical spectroscopy modalities is for a development of differential diagnostic algorithms of cutaneous neoplasia. The human skin is a complex, multilayered and inhomogeneous organ with spatially varying optical properties. Since analysis of cutaneous optical spectra could be a very complicated task; then researchers apply complex mathematical tools for data evaluation, or apply several spectroscopic modalities in multimodal detection and search for specific optical fingerprints in the spectra obtained.

Skin neoplasm detection and evaluation of the sub-type and stage of development of the lesion investigated is still a significant challenge for the contemporary oncology. Nevertheless of the easy access to the cutaneous tissues under interest the long list of benign and dysplastic pathologies, different anatomic localization of the pathology, variety of the patients' skin phototypes, and the clinical similarity between different types of malignancies by their structure and clinical appearance lead to relatively low diagnostic accuracy under clinical evaluation.[1]

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Only highly experienced dermatologists could achieve significant diagnostic accuracy values, the general practitioners, or not specialized in oncology medical specialists could miss some of malicious lesions on early stage of the tumour' growth that would lead to a high number of misdiagnosed patients.[2] Last, but not least problem is related to the procedure of histological sampling. The tissue removal during such procedure could lead to spreading of the tumour cells by blood flow, which in the case of doubt for malignant melanoma lesion is a contradictive indication for taken of a biopsy sample at all.

Optical techniques are very perspective to overcome most of the disadvantages, related to skin lesions examination, but each of the spectral techniques has strong and weak sides, depending from the type of lesions investigated and/or compared benign and malignant forms. All of the spectral modalities are highly-sensitive, could work in non-contact real-time regime of detection, presents different non-invasive and feasible instruments, easy applicable into the clinical environment.[3,4]

Autofluorescence spectroscopy (AFS) is based on re-emission of endogenous fluorophores in the tissue, after optical excitation. The most typical endogenous sources of fluorescence signal are amino acids, structural proteins and their cross-links, co-enzymes, vitamins and lipids. In a case of tumour lesion development the biochemical content is changed, changes in the general concentration of a given fluorophore, appearance or fading of their emission due to these concentration alterations, or due to chemical interactions lead to changes in the autofluorescence properties of the tissues investigated. These changes are specific for each type of pathology and could be used as fingerprints for lesion type evaluation. The highest problem of autofluorescence spectroscopy application as a clinical diagnostic tool is the requirement of high sensitive detection technique due to low-level fluorescence signals detected and the moderate even low specificity in melanin-pigmented pathologies, where the pigment re-absorb the fluorescence itself and no reasonable signal could be detected and examined to evaluate the lesion type.[5-7]

Diffuse reflectance spectroscopy (DRS) is based on back-scattered signals obtained from the biological tissues investigated and it is responsible mainly about morphological information, which could be received from the tissues. Scattering intensity and spectral distribution of the signals allow receiving information about scatterers' size and distribution (cells, nuclei, etc.). As the detected diffuse reflectance signal is superposition from diffuse scattering and absorption from tissues' pigments, the resultant spectrum also reveal information about main absorbers in the biological tissues, like hemoglobin and melanin in the skin and its pathologies. DRS is very sensitive to melanin pigmented pathologies and reveal moderate sensitivity in the cases of comparison of non-melanoma skin neoplasia.

While the autofluorescence and diffuse-reflectance technique could be applied in vivo, for the other two modalities-absorption and transmission spectroscopies, which requirements include operation with optically thin samples (several tens of micrometers thickness), the ex vivo modality is only applicable. However, these two modalities are very interesting, as they allow receiving the absolute optical properties of the samples investigated – their absorption and scattering coefficients, as well they provide information about the major tissue scatterers and absorbing pigments, which allow to interpret better the results obtained from in vivo measurements.

The clinicians search for single or combined objective and non-invasive detection technique, which could lead to a fast and precise diagnostics of different cutaneous neoplasia. A lot of groups worldwide work on development of significant database for the optical and spectral features, specific to the whole set of cutaneous pathologies, in the field of pigmentation disorders, chronic (diabetes, eczema, dermatitis, etc.) and autoimmune (psoriasis, vitiligo) skin conditions, and especially for neoplasia detection and evaluation.

Combination of autofluorescence, absorption, transmission, and diffuse-reflection spectroscopies in a multimodal diagnostic instrument could allow achieving high diagnostic accuracy for a broad set of skin pathologies. This type of diagnostic tool could be extremely useful in the case of primary examination of novel patients, where the diagnosis is not-known yet and a primary observation of suspicious skin spots during dermatological examination has place. To benefit fully from combined spectroscopic techniques advantages, the spectral data and specific optical features received are needed to be connected with the morphology and biochemical composition of the cutaneous tissues investigated.[3-9]

In the current study are used several optical spectroscopy techniques, including autofluorescence, diffuse-reflectance, transmission and absorption spectroscopies in a broad spectral area - covering ultraviolet, visible and near infrared spectral ranges. The spectroscopic measurements were made on different cutaneous lesions, namely basal cell carcinoma, squamous cell carcinoma, malignant melanoma, and dysplastic (dysplastic nevi, keratoacantoma) and benign (benign pigmented nevi, seboreic keratosis) samples.

2. METHODS AND MATERIALS

Measurements were made on two types of skin samples – surgically excised cutaneous tumour tissues and prepared for histology analysis glass slices but without eosin or hematoxylin staining - to not distort the optical properties of the samples investigated by these typical colorants used in pathology practice for tissue examination. The different skin tumour lesions were obtained from University Hospital "Tsaritsa Yoanna-ISUL", Sofia, in the frames of collaboration with the Institute of Electronics, Bulgarian Academy of Sciences. All ethical issues and approvals for work with these tissue samples were received from the ethical committee of the hospital.

Shimadzu spectrophotometer system (UV-3600, Japan) with integrating sphere LISR-3100 was used for the measurements of total transmission and diffuse reflectance at the spectral range from 250 to 700 nm. Diffuse reflectance measurements were performed using USB4000-Vis-NIR (Ocean Optics Inc., Dunedin, USA) spectrometer in the spectral range from 400 nm to 950 nm, and the collimated transmittance measurements were performed using NIRQuest 512-2.2 (Ocean Optics Inc., Dunedin, USA) fiber-optic modular system in the range of 900-2150 nm.

Autofluorescence measurements were carried out with a spectrofluorimeter FluoroLog 3 (HORIBA Jobin Yvon, France). The system is equipped with a light source – xenon lamp with output light power of 300 W, performance in the 200-800 nm region and photomultiplier (PMT) detector with performance range of 220-850 nm for fluorescence detection. The samples were measured using additional fibro-optic module, which allows investigation of samples outside of the sample chamber, since the surgically excised tissue samples vary in size and shape. During the experiment the optical fiber of the used fiber optic module (F-3000 HORIBA Jobin Yvon Inc., France) was positioned perpendicularly to the sample with fixed geometry and the tip of the fiber was brought in contact with the tissue, in order to reduce the fluorescence signal interference by the backscattered excitation light, as well as the area of illumination of the sample corresponds with the surface of the tip of the optical fiber, which allow easier addressing of the signal detected from the tissue investigated. The autofluorescence of the safe-keeping solution used for surgically removed tissue samples investigated ex vivo was measured and well and found negligible by signal level in comparison with the investigated tissue endogenous fluorescence intensity. The shape and dimensions of the tissue samples vary depending on the size and pattern of the excised tumor, however the thickness of the investigated samples do not exceed 7 mm.

A block fiberoptic system, with a broad-band light source and detector - microspectrometer USB4000 (OceanOptics Inc., Dunedin, USA) in the region from 345 nm to 1040 nm was also applied for diffuse reflectance measurements of the tissue samples.

The spectral measurements of the glass slides with tissue slices were compared with the spectral data obtained from ex vivo tissue samples for the same set of cutaneous neoplasia investigated.

3. RESULTS AND DISCUSSION

The investigated samples are skin bulk lesions ex vivo or glass slices with thin tissue samples from these lesions, obtained after surgical removal and kept in a safe-keeping solution. The glass slides with histological sections from tissue samples were routinely processed as for histological analysis, but without the step including staining with hematoxylin and eosin, to not distort the optical parameters of the tissues investigated by these colorants. The completed measurements provided spectral data with specific characteristics, which diagnostic value could be evaluated through comparison and analysis of the optical properties of the tissues investigated. The spectral data are averaged by set of samples with the same histological diagnosis, which is used as a "golden standard" indicator during all comparative wok on spectral data received.

Autofluorescence spectra (fig.1) allow observing significant differences between the different benign, dysplastic and malignant lesions by the level of emission intensity, as well as by spectral shape, These features are like fingerprints for each type of pathology detected, which is observable due to low values of the standard deviations obtained during averaging of the spectra from different tissue samples obtained from different patients but with same histological diagnosis. The AFS data are useful for development of differentiation algorithms for detection and evaluation of non-melanoma cutaneous neoplasia investigated. In some of the pathologies the endogenous porphyrins signals even appear, but in general the fluorescence signals are a result of the emission of the co-enzymes, such as NADH, flavins; structural proteins collagen, elastin, and their cross-links, as well keratin for basal cell lesions. Pigments, such as hemoglobin and melanin distort the fluorescence signal due to re-absorption of the part of the autofluorescence from intrinsic

fluorophores in the tissues investigated, which has to be taken into account when the algorithms for discrimination of the pathology types are developed. [3-6, 10] The melanin – pigmented pathologies, such as dysplastic nevi and malignant melanoma lesions have very similar AFS features, which do not allow their differentiation with high diagnostic accuracy and AFS is suboptimal in use for such type of lesions.

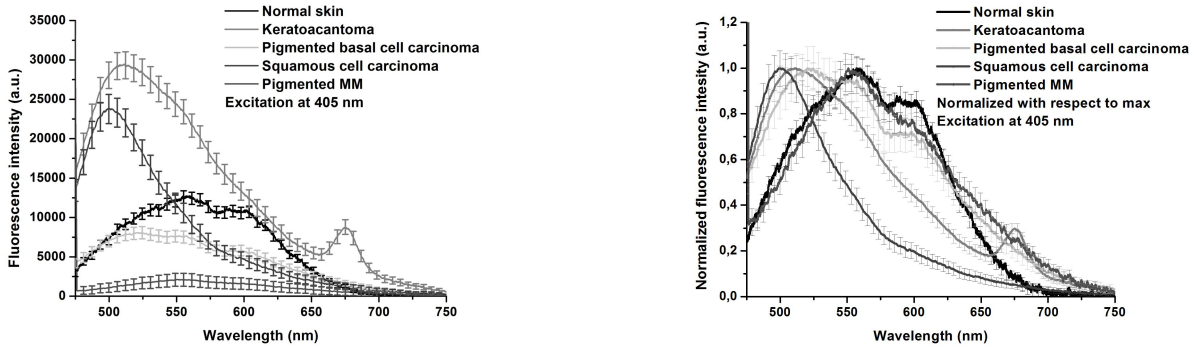

Figure 1. Fluorescence spectra of non-melanin and melanin pigmented lesions, including comparison with the fluorescence signal from healthy skin tissue (left) and the spectra normalized with respect to maximum (right). Spectra are averaged by the set of samples of different pathologies investigated using excitation at 405 nm and the standard deviation is presented as error bars.

In diffuse reflectance mode the most prominent effect came from the influence of skin pigmentation. The most common absorbers are melanin in epidermis and hemoglobin from the blood vessels in dermal layer of the skin. For non-melanoma lesions no significant spectral peculiarities were observed, that could be used for diagnostics and differentiation from normal skin and between each other. More pronounced there were the minima at the region of 545-575 nm, which are related to the increased neo-vascularization of the lesions and inflammatory processes, which often accompany the cancerous changes in the tissues. The hemoglobin and melanin pigments are responsible for the shape of the reflectance spectra observed in the region of 350-1000 nm for all types of skin neoplasia.

On figure 2 are presented averaged, compared by pathology type non-melanoma lesions and melanin-pigmented lesions vs. normal skin reflectance. The benign compound nevus reflectance spectrum shows a significant decrease in the entire spectral region, best expressed in the blue region where melanin has stronger absorption than in the red region. Similar results are observed in the case of dysplastic nevus, but the intensity of the reflectance signal is lower. The malignant melanoma spectrum has the lowest total reflectance of all lesion types in VIS-NIR region. The dysplastic nevi reflectance is similar by intensity levels and could not be easily separated by values from MM, but the spectral shape differences observed, related to a negative slope in the region of 600-900 nm which is typical for the non-melanoma pigmented lesions, as well as for normal skin significantly differ from the malicious melanocytes neoplasia - MM.[4, 5]

The reflectance spectra of pigmented lesions presented a gradual decrease in the hemoglobin absorption peaks at 420, 540 and 575 nm in passing from normal skin to benign and dysplastic nevi to malignant melanoma. Another characteristic decrease is related to alterations of the reflectance spectra slope in the 420-500 nm region for all pigmented lesion types (benign, dysplastic nevi and malignant melanoma) in comparison to the normal skin.

The spectra obtained from normal skin in identical anatomic sites of different patients have similar spectral shape features, but differ in what concerns the reflectance intensity at different wavelengths, depending on the particular patient skin phototype as well, when normal skin area are compared. However, spectra obtained from normal skin sites near the pigmented lesion investigated change significantly from patient to patient as a result of the different spectral properties of the skin in the various anatomic sites (different melanin pigmentation, respectively absorption of the epidermal layer, or varied values of oxy- and deoxy-hemoglobin concentrations in dermal blood vessels).

These individual differences are related to the skin type, patient age and the particular measurement area on the skin surface and may affect the cutaneous lesion spectra, especially in the cases of not highly pigmented moles and spots. Thus, when developing a diagnostic algorithm, the normal skin features that are specific for each patient and each position of the lesion investigated must be also included. As a possible differentiation criterion, a dimensionless ratio of the reflectance intensities at 500 nm and 700 nm could be applied for melanin-pigmented pathologies, which allow to

differentiate with a high accuracy nevi from malignant melanoma lesions, see fig.2 (right). Transmission spectra of the thin tissue slices from different skin pathologies in the ultraviolet and visible spectral range are presented on figure 3.

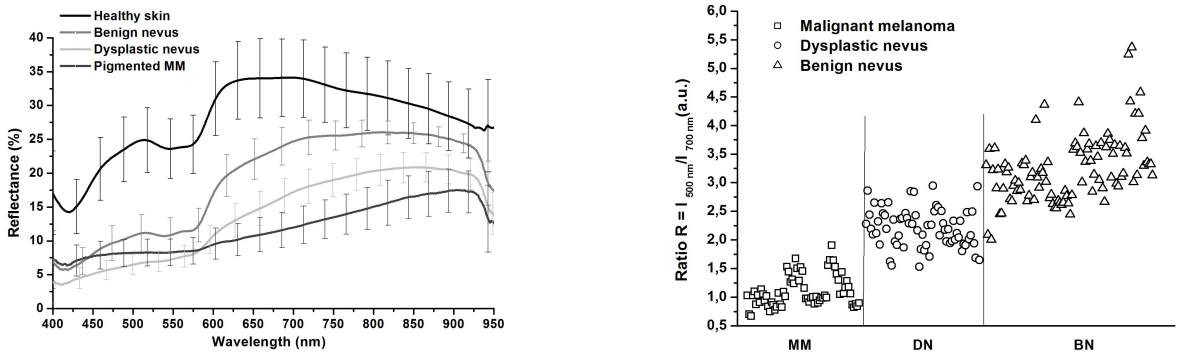

Figure 2. Diffuse reflectance spectra of melanin-pigmented lesions vs. healthy skin tissue reflectance (left). Spectra are averaged by the set of samples of different pathologies investigated and the standard deviation is presented as error bars. Ratio of the intensities at 500 nm and 700 nm for the reflectance ofbenign, dysplastic and malignant melanin-pigmented skin lesions (right).

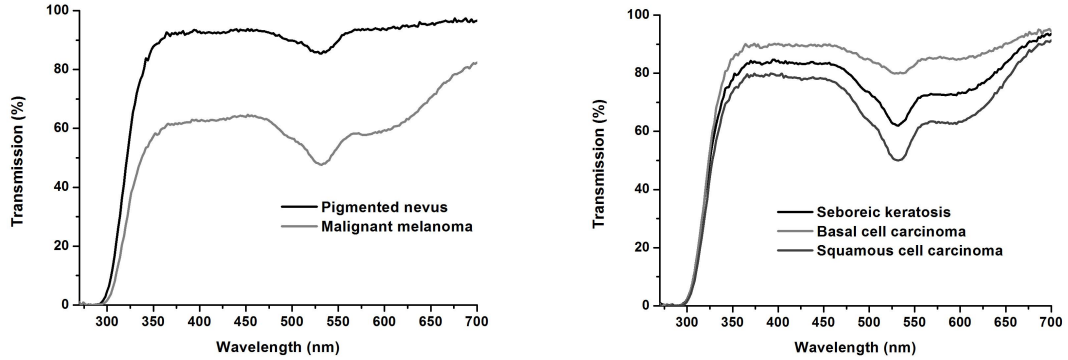

Figure 3.UV-VIS transmission spectra in the 250-700 nm spectral region of melanin-pigmented benign and malignant lesions (left) and non-melanoma skin benign and malignant neoplasia (right). Spectra are averaged by the set of samples of different pathologies investigated.

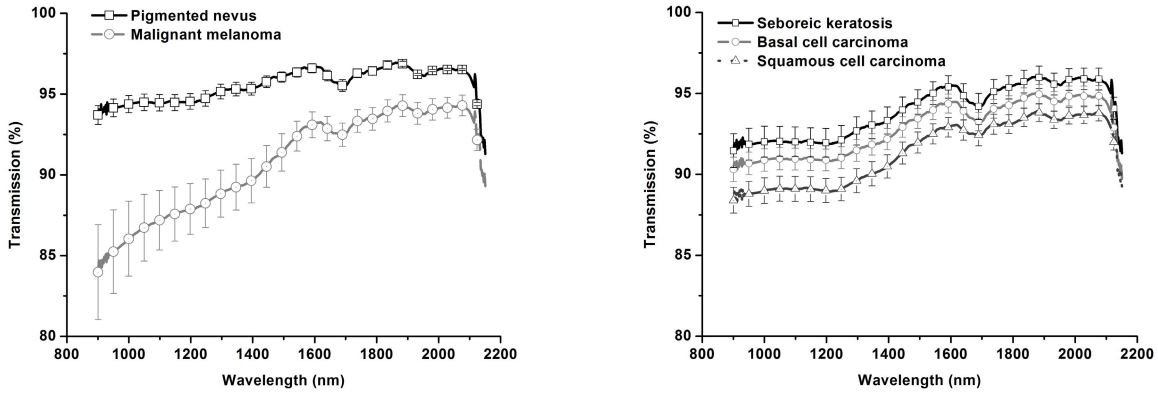

Figure 4. Diffuse NIR transmission spectra in the 900-2200 nm spectral region of melanin-pigmented benign and malignant lesions (left) and non-melanoma skin benign and malignant neoplasia (right). Spectra are averaged by the set of samples of different pathologies investigated.

NIR-transmission spectroscopy results are presented on figure 4 in comparison melanin-pigmented nevi-melanoma and non-melanoma skin lesions groups. This mode gave complementary optical properties information about the tissue samples investigated to that one of absorption spectroscopy. It could be used in the cases of ex vivo and histological samples investigations – for better evaluation of the absorption and scattering coefficients for the given NIR spectral range. However, it is not very convenient for in vivo tissue examination in the case of cutaneous tumour detection and diagnostics.

4. CONCLUSIONS

Using autofluorescence detection of skin benign and malignant lesions we obtain very good diagnostic performance for distinguishing of non-melanoma lesions from other simulating benign and malignant pathologies. Using diffuse reflectance and transmission spectroscopy we obtain significant tool for pigmented pathologies differentiation. Therefore, the possibility to obtain new knowledge about the optical and spectral properties for skin benign, dysplastic and malignant tissues, based on multimodality optical detection, improve the quality of the performed differentiation and could contribute to a better understanding of the observed spectral peculiarities of the cutaneous neoplasia.

When several spectral detection techniques are applied in common tool and multispectral algorithms for diagnosis and differentiations are applied we could rapidly increase the diagnostic accuracy of the received combined "optical biopsy" method. Results obtained in the current study would be used for development of such combined diagnostic algorithms for skin neoplasia differentiation in vivo.

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