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Diagnostics of pigmented skin tumors based on laser-induced autofluorescence and diffuse reflectance spectroscopy

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Abstract. **Results of investigation of cutaneous benign and malignant pigmented lesions by laser-induced autofluorescence spectroscopy (LIAFS) and diffuse reflectance spectroscopy (DRS) are presented. The autofluorescence of human skin was excited by a 337-nm nitrogen laser. A broadband halogen lamp (400 – 900 nm) was used for diffuse reflectance measurements. A microspectrometer detected *in vivo* the fluorescence and reflectance signals from human skin. The main spectral features of benign (dermal nevi, compound nevi, dysplastic nevi) and malignant (melanoma) lesions are discussed. The combined usage of the fluorescence and reflectance spectral methods to determine the type of the lesion, which increases the total diagnostic accuracy, is compared with the usage of LIAFS or DRS only. We also applied colorimetric transformation of the reflectance spectra detected and received additional evaluation criteria for determination of type of the lesion under study. Spectra from healthy skin areas near the lesion were detected and changes between healthy and lesion skin spectra were revealed. The influence of the main skin pigments on the detected spectra is discussed and evaluation of possibilities for differentiation between malignant and benign lesions is performed based on their spectral properties. This research shows that the non-invasive and high-sensitive *in vivo* detection by means of appropriate light sources and detectors should be possible, related to the real-time determination of existing pathological conditions.**

Keywords: *laser-induced autofluorescence, diffuse reflectance, melanin, malignant melanoma, dysplastic nevi.*

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1. Introduction

Biomedical optics is one of the fast developing areas. The nonionizing nature of light used to study and detect abnormalities in human tissues makes this area very attractive for the development of new diagnostic techniques and modalities [1]. Optical spectra provide biochemical and morphological information about the tissue under study, based on its absorption, elastic and Raman scattering properties [1 – 3].

Optical biopsy is a relatively new term involving spectral techniques used in medical practice for early *in vivo* diagnostics of tissue pathologies. These forms of optical diagnoses are preferable to traditional diagnostics, which requires the removal of several square millimeters of the tissue surface, followed by delays while samples are sent for the histological analysis. Optical biopsy also could provide good diagnostic accuracy due to the high spectral sensitivity of techniques to small biochemical and morphological changes in the tissues under interest [4, 5]. The use of such equipment will only require rather little time to teach the staff several practical skills, compared to years of training needed for some more conventional techniques [2, 6].

Ones of the most promising optical techniques, proposed to be introduced in clinical diagnostics are fluorescence and reflectance spectroscopy.

Fluorescence spectroscopy is used to analyse many different types of samples, ranging from individual biochemical species to whole organs *in vivo* [7]. The fluorescence technique with or without exogenous fluorescent markers finds also many other applications such as monitoring of photoinactivation processes in pathogenic bacteria [8], dosimetry of photodynamic therapy procedures [2, 7, 9, 10], drug uptake analysis [10, 11].

Laser-induced autofluorescence spectroscopy (LIAFS) is very promising because of its possibility to use information from naturally present endogenous fluorophores, as well as it offers real-time detection and differentiation of lesions with a specified accuracy, selectivity and specificity [2, 3, 12, 13]. The autofluorescence spectroscopic technique is used for early detection and/or differentiation of many tissue pathologies – atherosclerotic plaques [14], aging, epidermal proliferation [5, 15]. The fluorescent technique is also widely used to study cutaneous lesions, including erythema [4], psoriasis, vitiligo [16], and skin cancer

[2, 3, 5, 7]. This method gives information about the biochemical composition of the tissue under study.

Diffuse reflectance spectroscopy on the other hand is mainly responsible for morphological information about tissues. Most of the tissue pathologies, including tumors, exhibit significant architecture changes in cellular and subcellular level [17]. Because the detected diffuse reflectance signal is a superposition of diffuse scattering and absorption from tissue pigments, the resultant spectrum also reveals information about main absorbers in the biological tissues, like hemoglobin and melanin in skin and skin pathologies [1, 6].

Diffuse reflectance and backward scattering spectroscopy have been applied to characterise ovarian tissues [18], esophagus [17], evaluation of skin colour and erythema doses [19], and also for colon cancer [20] and skin cancer diagnostics [21, 22]. Spatially resolved diffuse reflectance measurements are used to determine the optical scattering and absorption coefficients of biological tissues [23, 24], which fully describe the optical properties of the tissue under study. Skin colour measurements by optical reflectance spectroscopy are another important part of investigations of skin tissue properties. The skin colour has been used as an indicator for many pathological conditions for centuries. These measurements correlate the colour and the minimal erythema dose [25], as well as reflectance spectra with melanin and hemoglobin concentrations in skin [26, 27].

In several systems for *in vivo* and *in vitro* studies both autofluorescence and diffuse reflectance spectroscopy have been used to detect and study tissue lesions. A combination of these measurement methods has been employed for better understanding of the optical properties of normal and abnormal tissues [12, 28, 29]. The spectroscopic techniques are still being developed and one of the way to increase the statistical significance of the diagnostics algorithms developed on their base is to add more spectral features in the general differentiation algorithms applied.

In this paper we present the results of single and common application of diagnostic algorithms, based on spectral features from autofluorescence and diffuse reflectance spectra detected from melanin-pigmented benign, dysplastic and malignant cutaneous lesions. The colorimetric approach based on the extraction of chromaticity parameters from reflectance spectra is also used. This technique can be employed for the needs of automation evaluation procedures of pigmented skin lesions [30]. In this paper, the diagnostic feasibilities of techniques (LIAFS and DRS) and colorimetric evaluation, based on reflectance spectra received from skin lesions are compared to evaluate the applicability of the spectroscopic techniques in clinical practice.

When LIAFS and DRS are used together to determine pigmented melanoma lesions, the diagnostic accuracy (DA) achieved is 90 %, as long as DA of 70 % is achieved if only one of these techniques is applied. However, if one wants to expand the range of pigmented skin disorders, additional spectral features must be included in differential algorithms developed on basis of LIAFS and DRS techniques.

2. Methods and Materials

2.1 Clinical and dermatoscopic analysis

In the present study 24 patients of National Oncological Center with benign nevi (BN) (8 lesions), dysplastic nevi (DN) (7 lesions) and malignant melanoma (MM) (9 lesions) pathologies were included. All three kinds of lesions are highly melanin-pigmented pathologies, and the nevi are the group of skin pigmented pathologies, which can be misdiagnosed with MM lesions. Patients included in the current dataset are classified by the Fitzpatrick classification [31] with skin phototype II and phototype III, that are the most common cutaneous types for the region.

Initially, the lesions were classified visually by an experienced dermatologist (P.T.) and dermatoscopically (MoleMax II, DERMA Instruments). Each lesion was evaluated using ABCD scoring criteria as follows: Asymmetry (A), Border (B), Colour (C) and Dermoscopic structures (D). All lesions were excised. After excision materials were investigated histologically and that examination was used as a 'gold' standard for the determination of the lesion type; the final analysis of the feasibility of spectroscopic techniques was made by comparing histological results in all cases reported.

2.2 Fluorescence and reflectance measurements

The system applied for spectroscopic measurements of human skin is described in details in [6, 30, 32]. The excitation wavelength around 340 nm is found to be the most appropriate [2] for differentiation between autofluorescence signals from normal and neoplastic tissues. Therefore, a compact 14-μJ nitrogen laser emitting at 337 nm with a 10-Hz pulse repetition rate (ILGI-503, Russia) has been used as the most suitable excitation source. A halogen lamp with a broadband output spectrum (400 – 900 nm) was used to measure the reflectances. Quartz – polymer optical fibers were used to deliver light from the laser and lamp and to collect the fluorescence and reflectance signals. The optical fibers had a diameter of 600 μm and NA = 0.22. The spectra were recorded and stored using a PC2000 fibreoptic microspectrometer (Ocean Optics, USA). A personal computer was used to control the system and to store and display the data using the specialised microspectrometer OOI Base software (Ocean Optics, USA).

For the reflectance measurement regime, initial calibration was carried out. The intensity I_{pigm} of the light reflected from a sample was expressed as a percentage R relative to the intensity I_{norm} of a standard reflectance (in this case a block of barium sulphate – $BaSO_4$). Such normalisation eliminated the influence of the source and the detector response on the measurement of spectral dependences [33].

Spectroscopic measurements of normal skin and lesion areas were carried out after 5 – 10 minutes of rest for each patient at room temperature (23 to 25 °C). Several spectra were measured from each suspicious area and averaged to reduce the influence of the inhomogeinity of the lesions. Autofluorescence and diffuse reflectance spectra were detected also from the surrounding normal skin. These averaged spectra from the healthy skin were used as a baseline to determine spectral changes occurring in the pathological areas. The spectra were smoothed using the Savitzky – Golay algorithm in order to reduce the instrumental noise of the spectrometric system. The constant distance between the end tip of the optical fibres and the skin surface was maintained by using a mechanical stand to avoid the influence of the displacement on the intensity level. All spectra were obtained at normal incidence – at the 90° angle between the optical fibre end tip and the skin surface.

This geometry was used to minimise fluorescence and reflectance spectral uncertainties during measurements [34].

2.3 Colorimetric analysis

Application of the colorimetric approach in this investigation is based on the fact that each skin area, including lesions under study, has its own pigment contents with a corresponding colour, which affects the reflected light in the region of 380 – 780 nm. The colour features and calculation technique applied are specified in the standard CIE N15.2 base [35, 36]. The CIE defines the translation from the measured spectrum to integral features *XYZ*. There are relations based on the CIE *XYZ* between all colorimetric systems [37, 38] and consequently results obtained from spectral data can be compared with the images of the same lesions. Simple transformation of spectra such as scaling (amplifying by amplitude) or translation (adding of a constant value) changes significantly the lightness value in the both cases. However, the chromaticity remains constant after scaling and changes after translation. The direct comparison of the colour features of normal and abnormal skin areas of different patients is unsuitable for the classification of the real state of the pathology, due to large variations in pigmentation of normal skin (different skin phototypes, anatomic differences, etc.). The colour of the skin is usually estimated in the La^*b^* system that gives some independence on the colour temperature of the light source and a good resolution in the red – yellow area of chromaticity. After the analysis of possible steps for unification of the colorimetric approach in the great varieties of pigmentation and lesion types [30, 39], lightness was found to be not a very informative parameter for the colorimetric differentiation of pigmented pathologies and after spectral transformations – using the spectrum ratio between healthy area and lesion, chromaticity parameters are recognised as diagnostically significant parameters. However La^*b^* uses the ratio between integral *XYZ* parameters of the object and source, which 'glosses over' the differences in the spectrum indicating abnormality. Therefore, we prefer to use a colour presentation with uniform distances in the area of chromaticity as different ratios between parameters are absent in this presentation. In this research we used, from the variety of colorimetric coordination systems, only one – LSH (lightness, saturation, hue) because these three parameters describe the psychosomatic perception of colour by humans. Lightness (L) is the perception of light quantity, and the ratio between lightness of spectral components defines hue (H) and saturation (S) values. We assume below that S and H, which together are called chromaticity, are determined by the form of the reflectance spectrum.

The analytical approach applied in this comparison of colour features allows one, at first, to obtain results independent of the initial spectral distribution and, second, to use the uniform criterion for all estimates. The developed scheme of the classification includes finding the possible affiliation of the tested spectrum by deviation of its chromaticity features obtained after processing a new case in relation to the features of the created in advance library of ratio spectra with known diagnoses. The comparison with the existing patterns includes finding of the percentage of affiliation of the tested lesion to the patterned diseases, as the highest value of percentages gives the diagnosis for the current calculated spectrum. Three parameters are included in that comparison – S, H and $\Sigma = S + H$, and if two of these three features indicated the given type of the pathology, it is indicated as the final diagnosis of the lesion investigated by using the colorimetric analysis based on reflectance spectra.

3. Results and Discussion

The laser-induced autofluorescence and diffuse reflectance data presented in this paper are smoothed and averaged by the lesion type. Normal skin spectra, for comparison of the spectral changes occurring in presence of pathological conditions, are smoothed and averaged for all patients.

Each autofluorescence spectrum recorded *in vivo* is a superposition of fluorescence spectra of endogenous fluorophores existing in the tissue [3 – 5, 11, 12] distorted by photon reabsorption by the tissue pigments, mainly by blood and melanin. The spectral shape of normal skin fluorescence usually presents no significant differences from patient to patient. The intensity changes are more pronounced due to different skin phototypes and anatomic areas, as in both cases different levels of melanin pigmentation could be observed. Slight differences in the spectral shape are detected only for the case of palm skin fluorescence spectra versus other anatomic sites, where the lack of melanin leads to deeper penetration of excitation and respectively emission of light. In this case, the influence of hemoglobin reabsorption on the fluorescence from deeper dermal layers is well pronounced. This effect is discussed in detail in our work [40].

Figure 1 presents laser-induced autofluorescence spectra of normal skin, benign nevus, dysplastic nevus and malignant melanoma lesions, averaged by all patients and normalised with respect to the detected reflection signal from the excitation light. Spectra are presented with the standard deviation, obtained in the process of data averaging. The standard deviation is about 10 % for normal skin, while for the melanin-pigmented pathologies this deviation is higher, but does not correlate with the anatomic area or skin phototype of the patient. The standard deviation received due to averaging the autofluorescence spectra from the same kind of pathology gives values of the

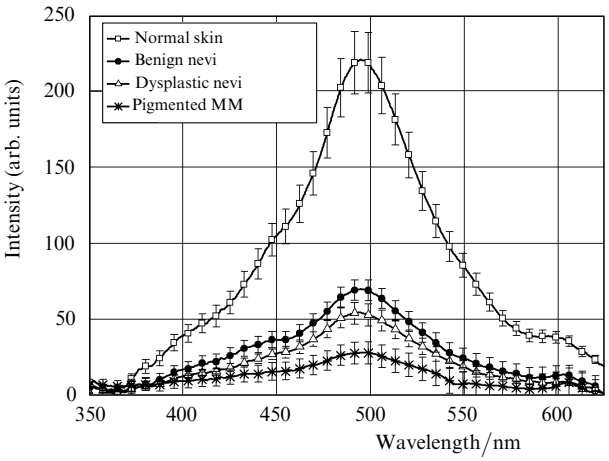

Figure 1. Laser-induced autofluorescence spectra of normal skin, benign and dysplastic nevi, and malignant melanoma, using excitation at 337 nm.

standard deviation less than 10 % for benign nevi, about 12 % for dysplastic nevi, and 18 % for pigmented melanoma lesions. No significant spectral shape differences between benign and malignant pathologies are observed, except the significant distinction in the intensity level of the fluorescence signal for the different skin status – normal/abnormal and benign/malignant.

The autofluorescence spectrum of human skin irradiated in the UV-A spectral range is a result of emission of several major fluorophores – collagen, elastin, NADH, flavins, proteins' cross-links [2–5, 11, 12, 41]. In the obtained spectra fluorescence signals from NADH, collagen, and collagen cross-links are easily recognised by using the reference data [3, 4, 5, 7, 11, 12].

A significant decrease in the fluorescence intensity correlated with the type of the pigment lesion was observed for all lesions. At least two types of nevi under study have very close values of the fluorescence intensities. The malignant melanoma fluorescence intensity is much lower than that of normal skin and nevi and could be separated from pigmented nevi spots. As no indicative discriminations of the fluorescence spectral shape between normal skin, nevi and MM are observed, a simple algorithm for discrimination between nevi and MM lesions is obtained, which uses only the intensity level as a criterion for differentiation between normal skin and pigmented pathologies. Some slight spectral shape differences in malignant melanoma lesions are related to metabolic changes (NADH fluorescence increase in the region around 430–460 nm) and blood content increases (deeper minima at 420, 540, 575 nm).

Figure 2 presents diffuse reflectance spectra of benign, pigmented MM lesions and normal skin. Spectra are presented with their standard deviation, obtained by averaging all spectra for the given skin status: 18 % for normal skin, less than 20 % in the blue part of the spectrum and about 6 % in the red part of the spectrum for benign nevi, about 18 % in the blue part of the spectra, and about 10 % in the red part of the spectra for dysplastic nevi, and about 20 % for pigmented melanoma lesions, respectively.

The reflectance spectrum of benign compound nevus shows a significant decrease in the entire spectral region, best pronounced in the blue region where melanin has stronger absorption than in the red region. Similar results

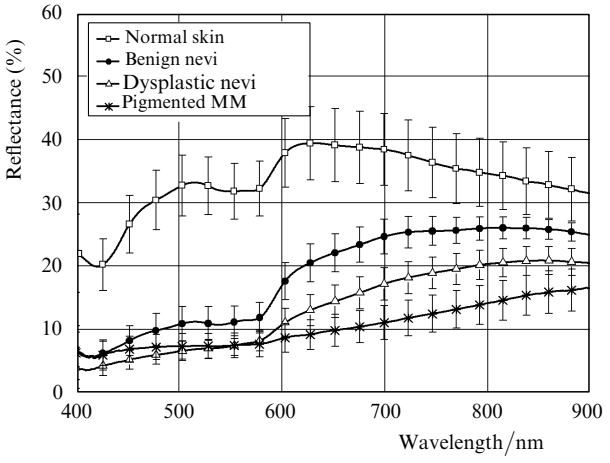

Figure 2. Diffuse reflectance spectra of normal skin, benign and dysplastic nevi, and malignant melanoma, obtained by using a broadband radiation source.

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 compared to all lesion types.

The reflectance spectra of compound nevus and dysplastic nevus are significantly different from those of melanoma. This difference in reflectance in blue and red spectral regions could be even more pronounced if an appropriate ratio between these spectral regions is applied. In some previous works [34, 40, 42] we proposed some spectral features extracted from the reflectance spectra to be applied as differentiation parameters for MM diagnostics. These spectral features include ratios of intensities at $\lambda = 500$, 575, 700 nm, related to the specific combination of absorption properties for hemoglobin and melanin at these wavelengths, as well as spectral slopes and area under the curves in different parts of the reflectance spectrum obtained from the lesion under study (see Fig. 3). Typical reflectance spectra of normal skin (for European patients) have a negative slope with increasing the wavelength in the region 700–900 nm. For melanin-pigmented pathologies, such as MM, that slope is positive, because the increase in the melanin content strongly affects the signal reflected from the lesion area. Because the melanin absorption is higher in the short wavelength region and lower in the red near-IR spectral regions [43], the influence in the both parts of the reflectance spectra is different and the slope of the curve changes from negative for non-pigmented normal skin to positive for high melanin-pigmented lesions.

Different authors used many spectral features extracted from fluorescence and diffuse reflectance spectra obtained from lesions *in vivo*. For example, if reflectance measurements are used to determine different pigmented skin lesions, such specific features of the spectra as the mean value for specific wavelength(s), the slope of the spectrum in one or more spectral regions, the integral value of the reflected signal for a specific wavelength region are used to differentiate benign and malignant pathologies [21, 22, 42]. Depending on various specific features used by different authors, the sensitivity (SE) and specificity (SP) of the reflectance spectroscopy technique can vary in a wide range. Thus, the values of SE : SP cited in different works are 76 : 87 [44], 80 : 46 [45], 83.6 : 90.8 [21], 89 : 88 [46], and 90.3 : 77.4 [47].

In our investigations we have also tried to find the most appropriate autofluorescence and reflectance spectral features, which will give the maximum deviation between different pathologies, and some algorithms have been proposed for these goals [6, 30, 34, 40]. Based on the spectroscopic differentiation algorithms developed previously, the diagnoses evaluated for the dataset of 24 patients are presented.

Tables 1–3 present the comparisons of diagnoses obtained with fluorescence and reflectance spectroscopy methods and colorimetric parameters as well as the histological results, i.e. the so-called 'real diagnoses'. The pathological condition data of 8 patients per pathology, summarised in accordance with their initial clinical and dermatoscopic examination are given, which allow us also to present some good examples of possible clinical misdiagnoses from the initial examination of the lesions and spectral diagnoses received in these cases.

In Tables 1–3, the sign '+' means the cases, when the histological diagnosis coincides with the diagnosis received

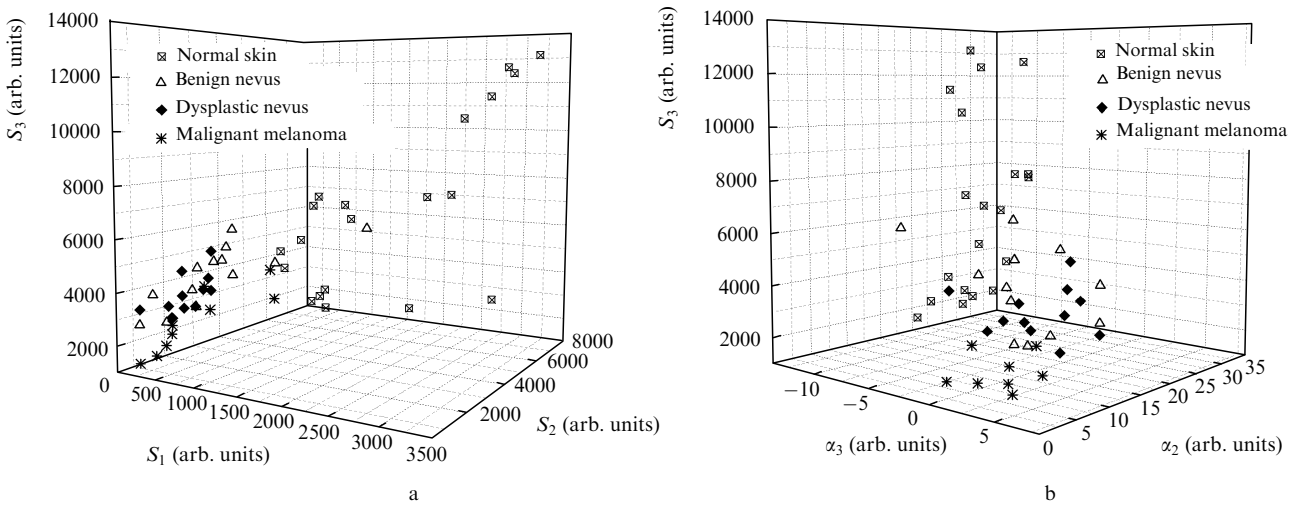

Figure 3. 3D distributions of the spectral features, chosen as the best differentiation characteristics for the diagnostics of normal skin, benign compound nevi, dysplastic nevi, and malignant melanoma lesions: S_1 is the area under the reflectance spectrum curve for the region 450 – 530 nm, S_2 – for the region 570 – 700 nm and S_3 – for the region 700 – 900 nm (a); slope α_3 of the spectral curve for 700 – 900 and α_2 – for 570 – 700 nm, as well as the area under the reflectance spectrum curve S_3 for the 700-900-nm spectral region (b).

by a particular method or parameter, the sign '–' means the cases, when the use of a particular method/parameter misdiagnosed the type of the pathology. The misdiagnosed cases are presented with the type of the false diagnosis obtained by the applied technique: BN – benign nevus, DN – dysplastic nevus, MM – malignant melanoma, BCC – base-cell carcinoma, HEM – heamangioma. The last two pathologies are not included in the general discussion in this article, which is focused on melanin-pigmented benign and malignant skin lesions; therefore, they just indicate the type of misclassification obtained for the given patients.

The tables present eight lesions diagnosed by the initial dermatoscopic examination with the help of the ABCD criteria for differentiation of benign compound and dermal nevi (Table 1) and dysplastic nevi (Table 2) as well as pigmented malignant melanoma (Table 3). After the initial clinical and dermatoscopic examinations, the spectroscopic evaluation was applied and diagnoses were independently established based on diagnostic algorithms previously developed by us [32, 40], which use fluorescence and reflectance spectral features of the lesions. After the optical examination, DN and MM were removed surgically due to their maliciousness by using standard clinical procedures, and benign nevi presented here were removed upon the request of patients because of cosmetic reasons. Then, all the pathalogies were histologically investigated after the removal.

In Table 1, patient M.B. is the most interesting example for discussion of the diagnostic accuracy of the spectral methods. Based on the initial clinical investigation, the lesion is classified as benign nevi, but the hue parameter of the colorimetric evaluation criteria classified it as dermal nevus. In this case, a very low level of autofluorescence signal and reflectance spectral features intermediate to those of dysplastic nevi or melanoma were observed. Therefore, the H parameter gives also improper diagnosis of BN. Other two parameters S and Σ gave the real pathological condition of the lesion under study. The ulcerous compound nevus (patient T.G.) was misdiagnosed by using only reflectance and its colorimetric derivatives. By using dimensionless ratios $R_1 = I_{\text{norm}}(500\ \text{nm})I_{\text{pigm}}(700\ \text{nm})/I_{\text{norm}}(700\ \text{nm})I_{\text{pigm}}(500\ \text{nm})$, $R_2 = I_{\text{pigm}}(500\ \text{nm})/I_{\text{pigm}}(575\ \text{nm})$, $R_3 = I_{\text{pigm}}(575\ \text{nm})/I_{\text{pigm}}(700\ \text{nm})$ [6, 34] from reflectance spectra of the lesion, an unusual conclusion was derived: the investigated lesion could be a heamangioma. This is related to the high influence of blood absorption on the diffuse reflectance from the pathology caused by the lesion bleeding. The BCC diagnosis obtained from the colorimetric features (S and Σ) is also caused by the hemoglobin influence on the diffuse reflectance spectra. Ones of the well pronounced features of BCC in the reflectance regime are the deeper minima of hemoglobin at 420, 543, and 575 nm, which correlate with the colour features observed in this particular case of ulcerous nevus. Patient M.D. also was misdiagnosed – a very low level of autofluorescence close to the average values for dysplastic nevi, and colorimetric features H and Σ also indicating the DN diagnosis. The lesion itself is very highly pigmented in visual observations;

Table 1. Diagnoses obtained with the LIAFS, DRS, and SHΣ methods, and also based on the histological examination of the lesions (real diagnosis). The initial diagnosis with the help of visual and dermatoscopic examinations was benign nevus (BN).

Patient, sex, age	LIAFS	DRS	S	H	Σ	Real diagnosis
K.P. (F, 31)	+	+	+	DN –	+	Compound nevus
M.D. (F, 20)	+	+	+	DN –	DN –	Compound nevus
S.S. (F, 40)	+	+	+	+	+	Compound nevus
T.G. (F, 20)	+	HEM –	BCC –	DN –	BCC –	Compound nevus
A.A. (F, 32)	+	+	+	DN –	MM –	Dermal nevus
K.T. (F, 29)	+	+	+	MM –	+	Dermal nevus
M.B. (M, 43)	MM –	MM –	MM –	+	MM –	Lentigo MM
M.D. (F, 20)	DN –	+	+	DN –	DN –	Dermal nevus

Note: F is female and M is male.

Table 2. Diagnoses obtained with the LIAFS, DRS, and SHΣ methods, and also based on the histological examination of the lesions (real diagnosis). The initial diagnosis with the help of visual and dermatoscopic examination was dysplastic nevus (DN).

Patient, sex, age	LIAFS	DRS	S	H	Σ	Real diagnosis
K.T. (F, 29)	+	+	BN +	+	+	DN
L.R. (F, 36)	BN –	+	BN –	+	+	DN
M.D. (F, 23)	MM –	MM –	BN –	MM –	MM –	DN
P.G. (M, 32)	+	+	+	MM –	+	DN
R.B. (F, 25)	MM –	MM –	MM –	MM –	MM –	Lentigo MM
R.D. (F, 29)	+	+	+	+	+	DN
R.T. (M, 42)	+	+	+	MM –	+	DN
T.G. (F, 20)	+	BN –	BN –	+	+	DN

Note: F is female and M is male.

therefore, this pigmentation strongly affects the level of the LIAF signal, as well as the H and Σ parameters in the colorimetric evaluation of the reflected signal.

Table 2 presents the results of diagnostics of patients with the initial dermatoscopic diagnosis DN with the help of LIAFS, DRS methods and colorimetry. The biggest part of the group is properly diagnosed, but two cases are interesting to be discussed. Patient M.D. with intensively pigmented nevus, which is misdiagnosed by using the spectroscopic techniques as a more malicious lesion – MM (therefore, the index of suspicion to the spectral diagnostic methods increased), and patient R.B., who was initially misdiagnosed by using the dermatoscopic observation as a DN lesion, while according to the results of the absolute majority of the methods, it was MM. Because the histological evaluation coincides with the diagnosis received from spectroscopic methods, only the first patient M.D. is misdiagnosed in the data presented.

Table 3. Diagnoses obtained with the LIAFS, DRS, and SHΣ methods, and also based on the histological examination of the lesions (real diagnosis). The initial diagnosis with the help of visual and dermatoscopic examination was pigmented malignant melanoma (MM).

Patient, sex, age	LIAFS	DRS	S	H	Σ	Real diagnosis
D.O. (M, 87)	+	+	+	+	+	Lentigo MM
H.H. (M, 31)	BN –	DN –	BN –	DN –	BN –	Congenital nevus
I.I. (M, 73)	+	+	+	+	+	Lentigo MM
I.J. (M, 71)	DN –	+	+	+	+	Lentigo MM
I.S. (M, 57)	+	HEM –	BCC –	+	+	Nodular MMM
K.C. (F, 52)	+	+	+	+	+	Lentigo MM
M.T. (F, 67)	+	BN –	BN –	DN –	BN –	Nodular MM
S.Y. (M, 68)	DN –	+	BCC –	+	BCC –	Nodular MM

Note: F is female and M is male.

In Table 3 eight patients with initially evaluated pigmented malignant melanoma pathologies are presented. After the initial dermatoscopic evaluation, the spectral measurements are made and additional independent diagnostic conclusions are obtained. The first conclusion, which could be made from the results obtained, is that lentigo melanoma lesions are better recognised by the spectral techniques than nodular lesions. In the case of the nodular lesion (patient M.T.), the determination of the pathlogy by the reflectance spectroscopy method gave an error. The case of patient H.H. was quite interesting, as the initial diagnosis by using the visual and dermatoscopic examination did not coincide with the clinician diagnosis. After that initial evaluation, the spectral observation did not differentiate between benign and dysplastic nevi.

The algorithms used to determine the type of the pathology are based on the previously collected database of spectral features for several types of skin benign and malignant lesions: intensity levels, dimensionless ratios, slopes and/or areas of parts of the spectra [42]. Thus, if any new type of the pathology appears, it can be misdiagnosed as another, if its features are near the already programmed one in the applied algorithm. The term 'new' is used from the point of view of the datasets collected and algorithms developed on the basis of the chosen spectral peculiarities of the autofluorescence and reflectance data existing in our database. In this particular case, the spectral algorithms applied give a mixture of misdiagnoses within the framework of benign and dysplastic nevi classifications. After the histological evaluation of the pathology, the lesion was determined as congenital nevus – benign melanin-pigmented pathology of human skin.

Based on the diagnoses presented in Tables 1–3, we could evaluate the statistical parameters of the diagnostic techniques compared to the 'gold standard' of the histological examination. Tables 4 and 5 present the sensitivity, specificity, diagnostic accuracy and other statistically important values for determination of the feasibility of the spectral and colorimetric techniques applied, separately (Table 4) and in combination (Table 5).

The use of a single spectroscopic technique such as laser-induced autofluorescence spectroscopy or diffuse reflectance spectroscopy of skin pigmented lesions gives sensitivities, specificities and the total diagnostic accuracy of the algorithms similar to values obtained by using the dermatoscopic examination of skin pathologies by trained dermatologists [48]. These results are much better than those of the initial visual examination of such kind of pathologies, which is the most common tool of diagnostics if a suspicious lesion exists on the patient skin [49]. However, these diagnostic values can be improved if additional features of spectral autofluorescence or reflectance are used, as well as if two or three techniques and algorithms for differentiation and evaluation of pigmented skin pathologies are applied.

Table 5 presents the results of diagnostic feasibility for determination of malignant melanoma lesions by using combinations of algorithms based on the fluorescence and reflectance techniques, reflectance and colorimetric algorithms, as well as by combining all the three types of measurements and evaluations – fluorescence, reflectance and colorimetry. For the first two cases, where two kinds of

Table 4. Statistical parameters of the correct diagnosis for the given type of the melanin-pigmented cutaneous pathology determined by using LIAFS, DRS methods, and SHΣ separately.

Diagnosis	Technique	SN (%)	SP (%)	PPV (%)	NPV (%)	IS (%)	DA (%)
	LIAFS	87.5	93.8	87.5	93.8	100	77.8
BN	DRS	75	87.5	75	87.5	100	60
	SHΣ	50	93.8	80	78.9	62.5	44.4
	LIAFS	71.4	82.4	62.5	87.5	114.3	50
DN	DRS	71.4	94.1	83.3	88.9	85.7	62.5
	SHΣ	85.7	88.2	75	93.8	114.3	66.7
	LIAFS	77.8	93.3	87.5	87.5	88.9	70
MM	DRS	77.8	93.3	87.5	87.5	88.9	70
	SHΣ	77.8	93.3	87.5	87.5	88.9	70

Note: SN is the sensitivity; SP is the specificity; PPV is the positive predictive value; NPV is the negative predictive value; IS is the index of suspicion; DA is the diagnostic accuracy.

algorithms are used to determine melanoma, the situation is considered to be a true positive result if one of the two algorithms indicates a malignant lesion. In the case, when all the three kinds of algorithms are used (LIAFS + DRS + SHΣ), three situations are evaluated – if only one of three approximations indicates MM as a true diagnosis, if two from three approximations give this result, and when all the three algorithms, based on different autofluorescence, diffuse reflectance, or colorimetric features give similar results about the pathology type.

When the three evaluation techniques are used separately, the diagnostic accuracy is about 70 % for malignant melanoma determination. When these techniques are combined much better results are achieved. When we combined DRS and SHΣ, the diagnostic accuracy is about 80 %, which is lower than in the case of combination of LIAFS and DRS. Because S, H, and Σ are obtained from the diffuse reflectance spectra of the lesions they bear some traces from their origin and are just complimentary to the reflectance spectrum itself. However, using these parameters we increase the diagnostic accuracy by 10 % when only one of the decision algorithms is applied; besides, the colorimetric approximation could be developed further for the needs of simplified devices in clinical applications. The best results are obtained, when the combination of LIAFS and DRS is commonly used. The achieved diagnostic accuracy is 90 %, the sensitivity and specificity also exceed 90 %, and their values are 100 % and 93.3 %, respectively. The same results are obtained when all the three techniques – fluorescence, reflectance and colorimetry are used and the lesion is determined as malignant if one of them indicates this diagnosis. This result is probably related to a small number of patients (24 patients with BN, DN, and MM pathologies) in the particular example. In the case of a larger dataset, the combined usage of all the three techniques must improve the diagnostic accuracy.

Note that when two of three or three of three indicators are taken into account simultaneously, the sensitivity, specificity and diagnostic accuracy decrease rapidly due to uncertainties in the lesion evaluation obtained by using particular techniques. The worst results are obtained when all the three techniques indicate the same diagnosis; the diagnostic accuracy in that case is about 50 %, which is not acceptable for clinical applications.

4. Conclusions

Melanoma incidence and mortality rates are increasing in many countries. It is obvious that the standard biopsy can be the reason for dissemination of the cancer cells and should not be used. In this context, the development of noninvasive, quick and reliable methods as optical biopsy is successful.

In this investigation we have demonstrated the potential of optical biopsy using the combination of LIAFS, DRS and colorimetric evaluation of chromaticity parameters from reflectance spectra of normal and abnormal skin areas, for the differential diagnostics of common benign and malignant melanin-pigmented cutaneous lesions. The best results are obtained, when the combination of laser-induced

Table 5. Statistical parameters of the pigmented malignant melanoma (real diagnosis) determined by using the combination of algorithms based on the LIAFS, DRS methods and SHΣ.

Technique	SN (%)	SP (%)	PPV (%)	NPV (%)	IS (%)	DA (%)
LIAFS + DRS	100	93.3	90	100	111.1	90
ДОС + SHΣ	88.9	93.3	88.9	93.3	100	80
LIAFS + DRS + SHΣ (3/3)	55.6	93.3	83.3	77.8	66.7	50
LIAFS + DRS + SHΣ (2/3)	77.8	93.3	87.5	87.5	88.9	70
LIAFS + ДОС + SHΣ (1/3)	100	93.3	90	100	111.1	90

autofluorescence and diffuse reflectance spectroscopy are commonly used to determine malignant melanoma lesions. The diagnostic accuracy is 90 %, the sensitivity and specificity are 100 % and 93.3 %, respectively. We have also discussed some of the origins of spectral features in fluorescence and reflectance spectra obtained from investigated lesions. These results can be used for better understanding of the optical biopsy applicability and give a range of possibilities related to early diagnostics and differentiation of cutaneous diseases.

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