Malignant melanoma and basal cell carcinoma detection with 457 nm laser-induced fluorescence

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Abstract. In this study we propose a several methods of autofluorescence signal analysis for skin cancers control. Autofluorescence spectra of normal skin and oncological pathologies stimulated by 457 nm laser were registered for 56 skin tissue samples. Spectra of 9 melanomas and 19 basal cell carcinomas were registered *ex vivo*. Estimation of tissue malignancy was made on the basis of autofluorescence spectra intensity and shifts of local maxima in 570 – 590 nm and 610 – 670 nm area. Separation of melanomas and basal cell carcinomas was performed with linear discriminant analysis. Overall accuracy of tissue type determining in current study reached 82.1%. © 2015 Samara State Aerospace University (SSAU).

Keywords: autofluorescence, spectroscopy, cancer detection, malignant melanoma, basal cell carcinoma, discriminant analysis.

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1 Introduction

Skin cancer is one of the most common forms of cancer in the world. Skin is a leading localization in the total structure of cancer incidence for both sexes in Russia [1]. There are 3 major types of skin cancer: basal cell carcinoma (BCC), squamous cell carcinomas and malignant melanoma (MM). MM is the most dangerous type of skin cancer. And it's incidence and mortality increases in most countries all over the world [2]. Taking into account low (not exceeding 50%) diagnostic accuracy of melanocytic tumors at an early stage by a general practitioner a new methods of tumors control should be designed. Other unwanted sides of tumors diagnosis associated with the complexity in the interpretation of clinical marks of the tumor at an early stage, and the inability to use invasive techniques, such as biopsy with histological or cytological examination, due to increased risk of lesions progression. In this regard, optical methods have the potential to identify and monitor neoplastic changes in skin tissues noninvasively using instrumental methods.

One of the promising methods for noninvasive analysis of skin lesions is Raman spectroscopy (RS) [3]. This method was approved for skin cancer *in vivo* and *ex vivo* research [4,5]. The diagnosis of malignant melanoma by the RS method was made with more than 85% accuracy. But, the accuracy of diagnostics significantly decreases in mass screening studies [3]. The increase in diagnostic accuracy is possible with combination of optical techniques. The combined diagnostic methods help to scan large tissue areas using autofluorescence (AF) or backscattering analysis for large tissue areas scanning with subsequent RS examination of suspicious tissues [6,7]. The applicability of the AF methods for skin cancer analysis in visible and near infrared (NIR) regions has been demonstrated in many studies [8,9]. But overall accuracy of AF implementation still remains under consideration.

In current study we demonstrate possibility of AF application in visible region to find MM and separate them from BCC. We propose to use AF spectra features associated with significance of intensities in local maxima and shifts of local maxima positions between tumor and normal skin. We present a calculation of sensitivities and specificities values for two types of tumors (MM and BCC) diagnosis on the basis of selected AF spectra features. Accuracy of visible range

Table 1 Summary of patients and lesions.

	Subjects					Location		
Final lesion diagnosis	Mean age, y (range)	Mean diameter, cm (range)	Male	Female	Number of lesions	Head and Neck	Body	Arms and Legs
MM	64.6 (45–77)	1.82 (0.2–5)	2	7	9	1	4	4
BCC	69.8 (51–85)	2,17 (1.5–3.5)	13	6	19	5	10	4

AF tumors diagnosis is compared with RS and NIR AF diagnostical accuracy for skin neoplasms control.

2 Materials and methods

2.1 Experimental setup

The laboratory setup is presented in Fig.1. The setup includes a DPSS laser module (457 nm, 200 mW) for AF stimulation in visible region, a Shamrock SR-500i-D1-R spectrograph and an Andor iDus DU416A-LDC-DD digital camera for low-noise recording of AF spectra with 0.05 nm resolution. The AF signal was passed through a broadband filter to cut off the probing radiation signal. All of collected AF spectra were recorded from healthy skin and neoplasms. The irradiation of tissue samples before spectra collection was performed with exposure time about 3-4 minutes to decrease the photobleaching effect on the experimental results [10]. All spectra were registered with 15 second exposure time. Three spectra were collected in every probing point and averaged with subsequent smoothing to reduce random noises.
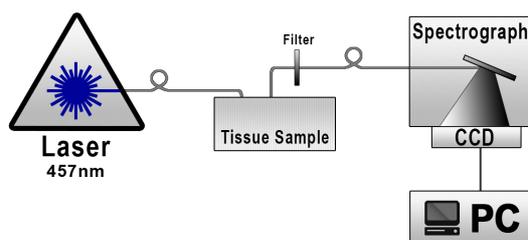

Fig. 1 Schematic diagram of the experimental setup.

2.2 Tissue samples

In a current study *ex vivo* samples of human skin were tested. Tissue samples were taken after surgical removal from the patients of Samara Regional Clinical Oncology Dispensary. Specimens tested in experiments were not altered before spectra collection and were stored not more than 4 hours at +2 - +6 C ° temperature. Tissue samples were taken from people with Caucasian skin type from different genders and age groups. Information about skin samples is gathered in Table 1. Tested samples were approximately 1.8 – 2.2 cm in diameter and contained healthy tissue and tumor.

In a series of experiments registration of AF spectra of 56 skin tissue samples was carried out. In the skin tissues analysis 9 MM samples and 19 BCC samples were studied. Also 28 sample of healthy skin were studied in the experiments. The diagnosis of each studied tissue sample was confirmed by histological analysis. Performed studies were approved by the Ethics Committee of Samara State Medical University (Russia Ministry of Health).

2.3 Discriminant analysis of the collected data

Discriminant analysis was used to designate tissue classes on the phase planes. Discriminant analysis is a classification method which can separate two or more classes based on different statistical parameters of Gaussian distributions. Discriminant analysis has the ability to flexibly change priority to favor sensitivity or specificity. As such, the efficiency of proposed approaches is characterized by their sensitivity and specificity and the ability to select defined classes in different volumes of phase plane. Availability in many mathematical applications is another advantage of discriminant analysis. The analysis of skin-tumor data allocation was performed using a linear discriminant analysis classifier [11]. Linear discriminant (or Fisher discriminant) has some principal assumptions like normally distributed data sets and equal covariance matrix (characterization of data dispersion on coordinate axes) for both classes and separates classes on the phase plane with straight lines.

3 Results and discussion

The typical normalized laser-induced fluorescence spectra of normal skin and BCC from one tested tissue sample are presented on Fig. 2. Three main local maxima may be observed on these spectra in 570 – 590 nm, 610 – 630 nm and 650 – 670 nm areas. The maximum of skin tissues AF spectra may be located in area 610 – 670 nm, as it is a combination of two local maxima. Redistribution of AF intensity in 610 – 630 nm and 650 – 670 nm maxima leads to the main maximum position shift. Also this redistribution in AF intensity may lead to the appearance of one or two strong maxima as demonstrates Fig. 2 for BCC. Here normal

skin has only one maximum in 629 nm position, while BCC has two strong maxima of comparable intensity in 635 nm and 678 nm positions. Two given examples of spectra are common for AF spectra of normal skin, MM and BCC and may differ from sample to sample. All of these skin tissues may have one or two maxima in 610 – 670 nm area and main maximum position may be located in any spot of this area.
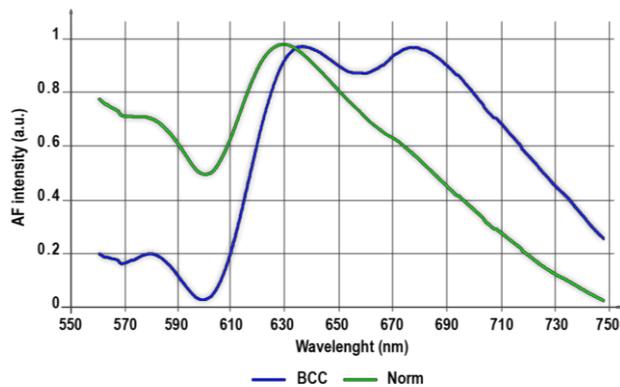

Fig. 2 Normalized AF spectra of normal skin and BCC.

Positions and intensities of maxima in AF spectra may provide information about chemical composition of tested samples and thus give information about changes in pathological formations in comparison to healthy skin. The main fluorophores emitting in orange and red areas of visible spectrum are lipo-pigments, flavins and porphyrins [12,13]. Peak in 570-590 nm area is formed by lipids and flavins. Lipo-pigments have maximum of absorption near 340 nm and maximum of emission near 560 nm. Flavins are characterized by strong absorption in wide area from 200 nm to 500 nm with strong maxima of absorption at 220 and 260 nm and less strong absorption at 380 and 460 nm. Maximum of flavins emission is at 555 nm band [14]. Porphyrins are characterized by wide absorption in 300 – 470 nm area with maxima at 400 nm. Emission of porphyrins has complicated form with two maxima near 615 - 630 and 660 - 670 nm. [12,13,15]. Thus local maximum of AF spectra observed at 570 nm is characterized of flavins and lipo-pigments presence in skin tissues and porphyrins determine the form of AF spectra in red area of spectra.

Epidermal surface lipids contribute to normal skin functions as the barrier function and the maintenance of healthy skin and hair. Consequently, they contribute to aging and to the conditioning and defense of this organ. Moreover, some lipids found on skin's surface make the skin unfriendly to fungi and bacteria [16]. On the other hand role of flavins for living organisms is in dehydrogenation function in metabolism reactions and in coloring of tissues, as flavins play role in controlling photostimulated generation of melanin within the specialized cells [17]. Porphyrins also plays a significant role in metabolism processes, and not only in human skin, but also in bacteria living on human skin (such as *Propionibacterium acnes*) [18, 19]. This makes

possible to assume presence of significant changes in total content of flavins, lipo-pigments and porphyrins in different skin tissues during the nucleation and growth of tumors. Tumors are characterized by increased rate of metabolism processes, thus this tissue restructuring cause changes in flavins and porphyrins concentrations. Also changes of flavin concentrations are explained by changes of tumor color. Moreover, growth of tumor damages normal skin functions that causes decrease of skin defense role and leads to the colonization of skin tissues with new porphyrins producing bacteria [20]. All the facts show possibility of skin cancers control with AF stimulated by the blue laser radiation.

First possible feature of AF spectra which may be useful in determination of skin tissues type is the ratio of AF spectra intensities in 570 - 590 nm and 610 - 670 nm bands. Results for MM, BCC and normal skin separation on the basis of $I = I_{610}$ / I_{570} criterion are showed on box and whisker plot at Fig. 3. One may see that melanomas differentiation is hardly possible with high accuracy. Assuming necessity of 100% sensitivity of MM detection ratios of the I coefficient for MM detection must lie in 0.91 – 1.03 range. In these conditions specificity of MM detection is 47.2%, and accuracy of MM detection is only 53.1%. Such accuracy is not sufficient for clinical implementation and other features of AF spectra must be used for tissues classification.
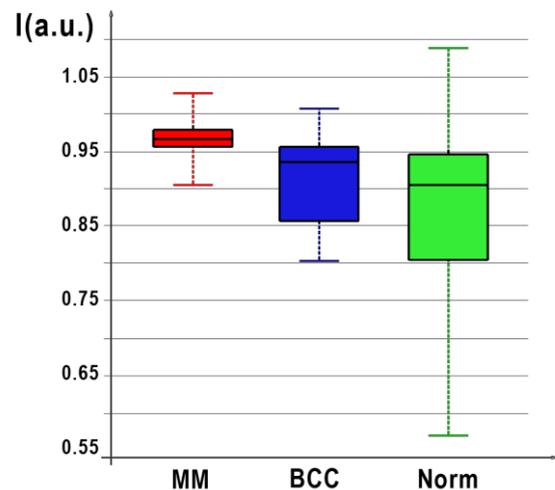

Fig. 3 Skin tissues classification based on intensities ratio $I = I_{610}$ / I_{570} (I_{570} and I_{620} are maximum AF intensities in 570 - 590 nm and 610 - 670 nm respectively).

Second possible way of skin tissues classification found in this research is a tracking of local maxima position shift between tumor and healthy skin. This feature uses not only information about tumor AF spectra, but also includes spectral information from healthy skin. Shift of local maxima in AF spectra uses information both from tumor and normal skin. Such information is helpful as during the tumor growth metabolic processes changes the chemical composition of tumor in comparison with normal tissue. Fig. 4

represents box and whisker plot for maxima positions shifts between MM or BCC and normal skin. Here $\Delta\lambda_1$ and $\Delta\lambda_2$ are coefficients of tumors local maxima shifts relatively to normal skin in 570 – 590 nm and 610 - 670 nm areas respectively.
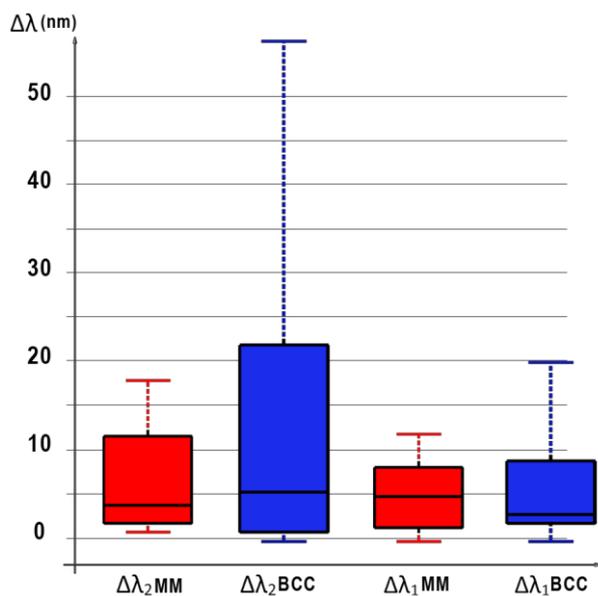

Fig. 4 MM and BCC classification based on wavelength shift between tumor and healthy skin: ($\Delta\lambda$ is the difference between the peaks of healthy skin and tumor in 570 – 590 nm and 610 - 670 nm areas $\Delta\lambda = |\lambda_{healthy} - \lambda_{tumor}|$).

Again assuming necessity of MM detection at 100% level specificities for $\Delta\lambda_1$ and $\Delta\lambda_2$ shows rather low values: 10.6% and 31.6% respectively. This leads to the total accuracy of 39.3% and 53.6% for MM detection with shift criteria in case of $\Delta\lambda_1$ and $\Delta\lambda_2$ calculation. The $\Delta\lambda_1$ criterion demonstrates rather weak potential for MM and BCC separation, while the $\Delta\lambda_2$ criterion shows accuracy comparable with I criterion implementation.

Further increase of MM and BCC classification accuracy is possible with joint implementation of two and more criteria. This approach may be implemented with phase plane analysis. In separation of phase plane classes axes of the plane are criterions of tissues classification, and every tested sample is a point on the phase plane with coordinates corresponding to the criterion values for this tissue sample [5]. Phase plane analysis was performed for three pairs of AF spectra criteria: $\Delta\lambda_2 - I$, $\Delta\lambda_1 - I$ and $\Delta\lambda_2 - \Delta\lambda_1$. Example of phase plane analysis is shown on Fig. 5. Separation of BCC and MM was performed with linear discriminant analysis, axes of phase plane on Fig. 5 are I and $\Delta\lambda_2$ criteria. Thus, Fig. 5 presents the possibility of MM and BCC differentiation on the phase plane, and separation line designates areas predominantly containing BCC and MM to the left and to the right from the separating ling respectively.

Accuracy of phase plane analysis presented in Table 2 to compare them with accuracy of one criteria of AF spectra analysis applying. The highest accuracy of MM

and BCC separation shows phase plane analysis with I and $\Delta\lambda_2$ criteria. It allows for MM determination with 88.9% sensitivity, 78.9% specificity and 82.1% accuracy. Option of I and $\Delta\lambda_1$ criteria demonstrates 77.8% sensitivity, 68.4% specificity and 71.4% accuracy. Pair of $\Delta\lambda_1$ and $\Delta\lambda_2$ criteria shows the lowest potential of MM determination with phase plane analysis. Accuracy of joint $\Delta\lambda_1$ and $\Delta\lambda_2$ criteria implementation is only 57.1%. In general phase plane analysis demonstrates 15 – 30% higher potential for MM detection in comparison with only one criterion of skin AF spectra analysis.
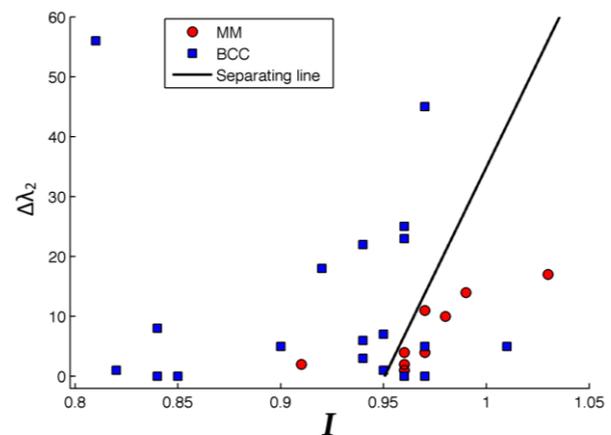

Fig. 5 Skin neoplasms separation on phase plane with linear discriminant analysis.

Comparison of achieved accuracy with other methods of melanomas detection gives ambiguous results. For example in [8] achieved accuracy of melanomas and non-melanoma skin cancers separation was 93.6%. But in research [8] AF analysis was supplemented with backscattered radiation analysis. Joint application of two spectroscopic techniques increased the information rate of AF study, while only AF analysis provided about 60 - 80% accuracy of cancers separation that is quite close to the results of current study (for example BCC separation in [21] or overview [22]). AF skin properties control in non-visible region uses information about other skin components and may provide additional information about tested skin tissue type. For example Zeng et al. [23] studied AF spectra of skin in NIR region with 785 nm laser excitation. In NIR region main fluorophor is melanin, thus AF of melanin may be useful in pigmented skin lesions control. Study of skin AF properties in NIR region helps to reach about 80 - 90% accuracy in skin cancer diagnosis [9] that is once again close to the results shown in current study. Significant improvement of AF diagnosis of skin tumors is possible with exogenous fluorophores control [22]. However, implementation of exogenous fluorophores is expensive and requires their injection in patient's body that makes such AF study not acceptable for mass screening.

Table 2 Melanomas diagnosis accuracy.

Method of analysis	Sensitivity	Specificity	Accuracy
I	100%	47.2%	53.1%
$\Delta\lambda_1$	100%	10.6%	39.3%
$\Delta\lambda_2$	100%	31.6%	53.6%
$I - \Delta\lambda_1$	77.8%	68.4%	71.4%
$I - \Delta\lambda_2$	88.9%	78.9%	82.1%
$\Delta\lambda_1 - \Delta\lambda_2$	77.8%	47.4%	57.1%

4 Conclusions

The first results of AF skin neoplasms analysis demonstrate high potential of the proposed method. Analysis of AF spectra is simple and may be used in mass screening applications, as AF registration is possible within a few seconds. Therefore this method can be a basis of a complex method of oncological pathologies diagnosis. In order to do this proposed method of AF analysis should be combined with other spectroscopic techniques. This will help to increase the sensitivity of malignant tumor detection and overall accuracy of tissue type determining. In this way improvement in tumor diagnosis is possible with AF analysis combining with methods such as diffuse reflectance spectroscopy, Raman spectroscopy, Stocks shift spectroscopy and many others.

The efficiency of the proposed method is comparable with similar methods of AF skin analysis in visible and NIR regions [9,22]. In proposed method we use not just spectral information from tumor but also characteristics of healthy tissue surrounding tumor. Such an approach helps to individualize studies and track the changes in tumor chemical composition during the process of growth.

Further studies of AF spectra stimulated by 457 nm laser should include larger number of tested specimens for precise evaluation of proposed method diagnostic accuracy. Another possible way of further research is implementation of developed method for analysis of other tissues. Further research may include analysis of larger skin tissues types including seborrheic keratosis, squamous cell carcinomas and benign tumors, or may include analysis of other tissue types including lung and gastric tissues analysis. Also this method may be useful in specific analysis of porphyrins, flavins and lipids content in biological tissues as AF spectra stimulated by 457 nm laser contains information about these fluorophores.

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