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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.

Final lesion diagnosis	Subjects					Location		
	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.

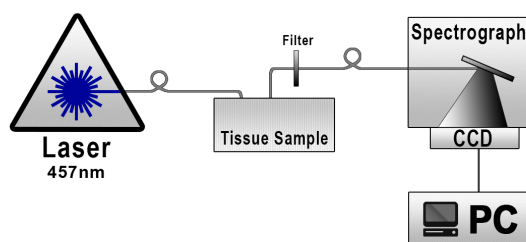


Fig. 1 Schematic diagram of the experimental setup.

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.

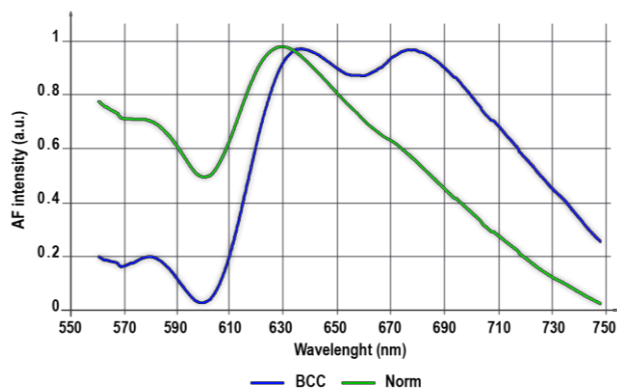


Fig. 2 Normalized AF spectra of normal skin and BCC.

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.

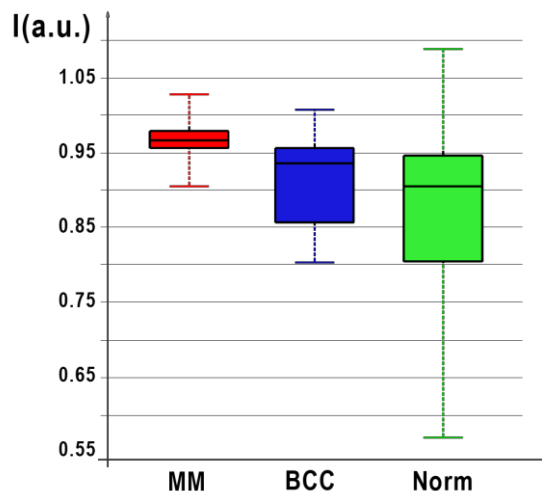


Fig. 3 Skin tissues classification based on intensities ratio $I = I_{610} / I_{570}$ (I_{570} and I_{620} are maximum AF intensities in 570 - 590 nm and 610 - 670 nm respectively).

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.

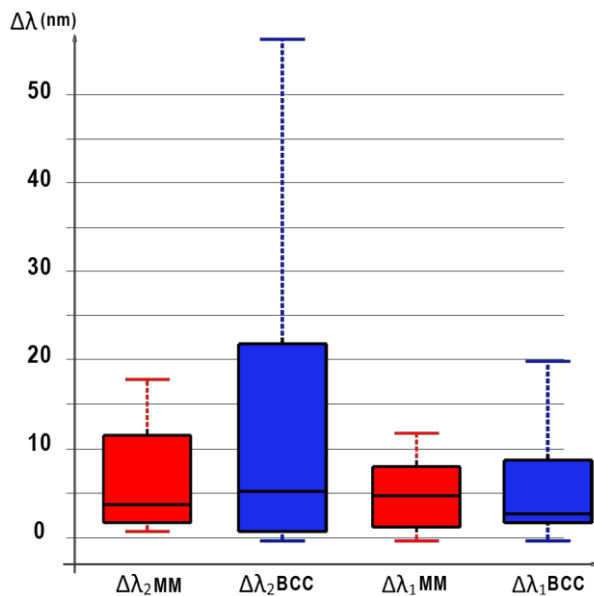


Fig. 4 MM and BCC classification based on wavelength shift between tumor and healthy skin: ($\Delta\lambda$ is the difference between the peaks of healthy skin and tumor in 570 – 590 nm and 610 – 670 nm areas $\Delta\lambda = |\lambda_{\text{healthy}} - \lambda_{\text{tumor}}|$).

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 line 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.

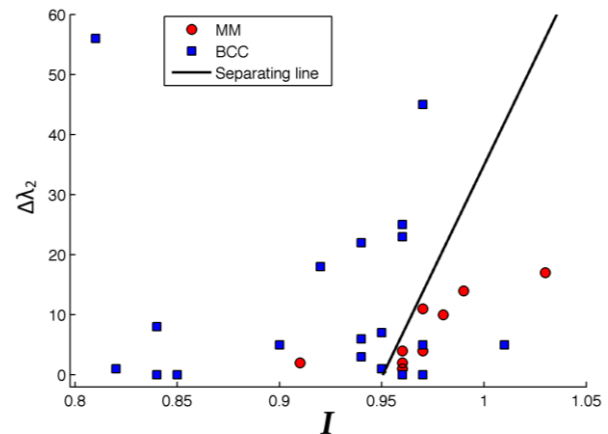


Fig. 5 Skin neoplasms separation on phase plane with linear discriminant analysis.

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, Stokes 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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Method of autofluorescence diagnostics of skin neoplasms in the near infrared region

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Abstract. We propose a method for the diagnostics of skin neoplasms, based on the analysis of the changes in the autofluorescence spectra in the near infrared range. The autofluorescence was excited by means of the laser radiation with the wavelength 785 nm for *ex vivo* and *in vivo* studies with subsequent exponential approximation of its spectrum. The quantitative and qualitative criteria for the neoplasm type differentiation by the change of the curvature and the rate of decrease of the approximating curve are found. It is shown that the proposed approach allows the diagnostics of malignant melanomas with the accuracy of 88.4% for *ex vivo* studies, and 86.2 % for *in vivo* ones. © 2015 Samara State Aerospace University (SSAU).

Keywords: autofluorescence, near IR range, dermatology, melanoma, exponential approximation, sensitivity, specificity.

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1 Introduction

The skin cancer is one of the most serious problems of the humanity, which is characterised by the high mortality index and treatment complexity. This disease can occur in any humans independent of sex, age and other specific features. Although the skin neoplasms can be revealed even by a visual examination, their diagnostics is very complex, and only 40% of the disease cases can be diagnosed during the first visit to an experienced physician [1]. The “golden standard” of unambiguous identification of the neoplasm type in the oncology is the histologic examination that requires the sample biopsy. This procedure can lead to such complications as bleeding, infection, persisting wounds, etc. If the skin melanoma is suspected, the pre-operational biopsy of the neoplasm is contra-indicated, since it can cause dramatic growth of the melanoma and active metastasis. In this regard, an urgent necessity exists in the development of instrumental methods of noninvasive diagnostics of skin neoplasms.

To increase the diagnostics efficiency and the quality of tumour treatment it is reasonable to use the optical noninvasive methods of the tissue diagnostics. The

modern method of confocal laser scanning microscopy is still not available in clinics because of high cost of the instrumentation [2]. The method of optical coherence tomography (OCT) offers the possibility to visualise the internal structure of the skin and provides high accuracy of diagnosing the basal cell carcinoma due to visualising the so called “nests” in the tumour structure, but it does not allow the diagnostics of melanoma with high accuracy [3]. Various imaging methods allow only the analysis of the spatial localisation of the neoplasm [4]. The Raman scattering is a high-sensitive method for identifying the melanoma [5]. However, since the Raman scattering probability is small, the signal acquisition takes much time and the diagnostics accuracy decreases in the mass screening. It is possible to increase the accuracy of the diagnostics using complex approaches combining a few optical diagnostic methods [6].

It is interesting to note that during the Raman scattering registration, the fluorescence component of the scattered radiation is usually eliminated as low-informative [7]. However, the fluorescence response of a tissue to the laser irradiation can be also used to identify the

neoplasm type [8], since the autofluorescence (AF) in the near infrared range carries information about the quantitative and qualitative changes of the composition of natural fluorophores in the area of neoplasms in comparison with the surrounding normal skin. This approach is free of the restrictions related to the time of the signal acquisition, inherent in the Raman scattering method, and admits the real-time operation without damaging the studied tissue, which allows for fast and economical solution of medical practice problems. The application of AF method was demonstrated in a number of papers for the cancer diagnostics in larynx [9] and lung [10]. The AF spectroscopy was also applied in wound healing monitoring [11] and eye tissue studies [12].

The concentration of natural fluorophores in the skin is very personal and can vary within a wide interval of values, which leads to an essential dispersion of the values of the AF intensity and the time of its decay from patient to patient. This does not allow the use of threshold methods of neoplasm diagnostics, which are characterised by low efficiency [13]. In the present paper, it is proposed to use the features of the AF spectra deformations in neoplasms as compared to the AF spectra of the surrounding normal skin as the informative criteria. The assessment of the AF curve behaviour was implemented using the exponential approximation of the studied curve.

2 Materials and methods

2.1 Experimental setup

The experimental studies were performed using the setup schematically presented in Fig. 1. The setup incorporates the thermally stabilised semiconductor laser unit LML-785.0RB-04 with the radiation wavelength 785 nm, the spectrograph Shamrock SR-303i with the integrated digital camera ANDOR DV-420A-OE, the optical unit, and the PC.

The probing laser radiation is passed through the bandpass filter BPF, directed by the mirror M and the dichroic reflector DM and focused by the lens L_2 onto the studied sample. The filter BPF served to protect the studied sample from the radiation of Raman scattering that occurs in the entrance optical fibre. The scattered radiation including the Rayleigh scattering, and the fluorescence signal was collected by the lens L_2 and sent to the spectrometer through a broadband filter LPF, aimed at filtering the probing laser radiation. The lenses L_1 and L_2 were used to match the optical unit with the entrance and collection optical fibre.

The signal was recorded within the spectral range 775–925 nm with the resolution 0.05 nm and low level of intrinsic noise. The radiation power did not exceed 200 mW. The used laser radiation of the near infrared region with the wavelength 785 nm provides deep penetration into the tissue by ~ 4 mm without damaging the integrity of the skin [14].

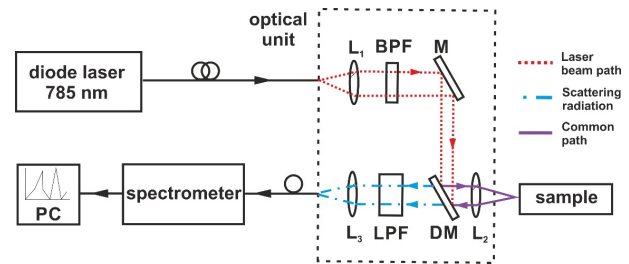


Fig. 1 Schematic diagram of the experimental setup.

2.2 Tissue samples

The *in vivo* studies of 28 human skin areas were carried out, including the AF spectra recording of tumours and surrounding healthy skin (14 melanoma areas, 9 areas of basal cell carcinoma, 1 area of squamous cell carcinoma, 3 areas of nevus, and 1 area of benign tumour). The *ex vivo* studies were carried out for 88 human skin samples with the registration of the tumour and surrounding healthy skin (34 samples of melanoma, 35 samples of basal cell carcinoma, 9 samples of benign tumours, 4 samples of squamous cell carcinoma, 3 samples of nevus, and 3 samples of skin cancer).

All tissue samples for the *ex vivo* studies were obtained as a result of surgical extraction from the patients of the Samara Regional Clinical Oncologic Dispensary and studied not later than in 4 hours after the resection. Each sample was a tumour surrounded by a healthy tissue. The size of the studied neoplasms varied in a wide interval from 0.5 cm to 3.5 cm. The diagnosis for each neoplasm was confirmed by the result of histologic examination. All *in vivo* and *ex vivo* studies were performed after the patients agreement. The ethic committee of Samara State Medical University approved the investigations.

2.3 Method of exponential approximation

The AF spectra of each sample were recorded in the neoplasm area and in the healthy skin area adjacent of pathology region. Within the spectral range 870 – 920 nm the intensity of AF decreases with the growth of the wavelength, and the most significant qualitative changes in the AF spectrum were observed within the range 810 – 870 nm. For the comparative analysis of the experimental data in the above range we approximate the AF spectrum with an exponential function:

$$I_{ap}(\lambda) = ae^{b\left(\frac{\lambda}{\lambda_{max}}\right)} + c, \quad (1)$$

where the sign of the coefficient a is responsible for the shape (convex/concave) of the approximating curve, the coefficient b characterises the curvature, and $\lambda_{max} = 870$ is the right boundary of the approximation interval. The approximation was implemented using the criterion of minimising the root-mean-square error. For the minimisation algorithm (the search for the global minimum of the functional) we used the Nelder-Mead simplex-method and the stochastic method of

differential evolution [15]. The limitation of 1000 iterations was imposed on each method. For the final result we took the values providing the minimal root-mean-square error of approximation. The mean relative error of approximation (with respect to the maximum of the spectrum in the approximated region) amounted to 2.3% for the melanoma spectra, 1.2% for the spectra of healthy skin, 1.9% for the spectra of basal cell carcinoma.

Generally, the curvature $k(\lambda)$ of the approximating curve of the spectral intensity (1) in the interval $\lambda \in [810; 870]$ is determined as [15]

$$k(\lambda) = \frac{l''_{ap}(\lambda)}{(1+[l'_{ap}(\lambda)]^2)^{3/2}}. \quad (2)$$

For convenience let us redefine the variable λ as $\lambda \stackrel{\text{def}}{=} 870\lambda$, $\lambda \in [810/870; 1]$. Then

$$k(\lambda) = \frac{ab^2 \exp(b\lambda)}{(1+a^2b^2 \exp(2b\lambda))^{3/2}}. \quad (3)$$

The function $a^2b^2 \exp(2b\lambda) \geq 0$ for any a, b, λ and is monotonic. Therefore,

$$\begin{aligned} |k(\lambda)| &< |\hat{k}(\lambda)|, \\ \hat{k}(\lambda) &= \frac{ab^2 \exp(b\lambda)}{(a^2b^2 \exp(2b\lambda))^{3/2}} = \\ &= \frac{\text{sign}(a)}{a^2|b|} \exp(-2b\lambda). \end{aligned} \quad (4)$$

The function $\hat{k}(\lambda)$ corresponds to the more stringent condition $a^2b^2 \exp(2b\lambda) \geq 1$, which was fulfilled for nearly all the neoplasms studied in this research. In this case, one can neglect the contribution of 1 in the denominator of Eq. (3), after which the function $k(\lambda)$ is reduced to $\hat{k}(\lambda)$ with similar properties.

3 Results and discussion

Due to the multilayer and multicomponent structure of the skin, its interaction with light is a complex process that includes the multiple scattering and absorption of photons. The quantum yield of the fluorescence of skin natural fluorophores varies from thousandth to hundredth parts of the absorbed radiation energy [17]. In healthy skin tissues, the main substances that actively absorb and scatter light are haemoglobin, water, lipids, and melanin [18]. The cell membranes, nuclei, organelles, melanin granules in the cells are the main scattering objects in a tissue. Depending on the malignant transformation of the tissue neoplasm, the randomness of the cell structure growth increases and the size of the cell nuclei grows. Thus, the mean value of the normal cell nucleus is 10 – 12 μm , for pathologic tissues it increases to 20 – 50 μm . The refractive index at the nucleus-cytoplasm boundary also increases [19].

All these features of the pathology development lead to the change of scattering and fluorescence characters in neoplasm tissues.

Beyond 800 nm, the AF spectrum is a nonlinear decreasing function. The characteristic AF spectra of different skin tissues are shown in Fig. 2. It is seen that the autofluorescence intensity is essentially different for different types of pathology and the healthy skin.

Since the AF intensity is determined by the skin phenotype of a particular patient, to get the unified results that could be used in practice we analysed the quantities, normalised to the maximal value of the intensity of spectrum of the surrounding healthy skin for each sample, rather than the absolute AF intensities.

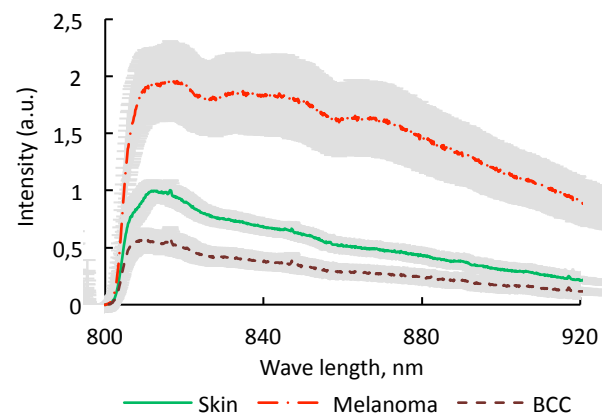


Fig. 2 AF spectra of healthy human skin and neoplasms.

In the course of the studies, it was found that the intensity of the AF spectrum in the area of basal cell carcinoma is reduced by 30-60% as compared to the intensity from the surrounding healthy skin area, and in the melanoma region, on the contrary, the AF intensity increases to 170-250%. The AF spectra of the studied tissues are characterised by the presence of expressed local maxima in the region 800 - 860 nm. These features of the AF spectra are related to the higher concentration of melanocytes and keratinocytes (containing melanin) in the tissues of melanomas and nevi as compared to the normal skin and basal cell carcinoma. The melanin intensely fluoresces in the near infrared region [20], which explains the high intensity of AF from pigmented formations.

In Fig 2 the plots of AF spectra are presented together with the confidence intervals of their measurement. One can see that the maximal spread of values of the AF spectrum intensity does not exceed 7% for healthy skin, 12% for basal cell carcinoma, whereas for the melanoma it is significantly higher and amounts to 30 – 40%. The decrease of the AF intensity is related to the continuous irradiation of the sample in the course of recording the AF spectrum, due to which the effect of the skin photobleaching occurs [21]. The photobleaching phenomenon arises due to the

degradation of fluorophores because of photochemical damage and modification of molecular bonds. As seen from Fig. 1, the areas of possible AF responses from the healthy skin, melanoma and basal cell carcinoma do not intersect, which allows their differentiation by the level of intensity. However the situation is opposite in the case of melanocyte neoplasms (melanoma, nevus, moles) for which a high level of AF is typical (Fig. 3). In this case, the accuracy of melanocyte neoplasms differentiation is essentially reduced, if the intensity values are used as a threshold diagnostic criterion. Thus in our studies the accuracy of differentiating melanomas and nevi by the intensity did not exceed 52%. In Ref. [13] the higher accuracy of differentiation of melanomas and nevi (about 80%) by the AF intensity level in the visible region of the spectrum was achieved due to using the high-intensity UV sources of excitation and careful positioning of the optical sensor to minimise the error of detecting the AF signals. Guaranteeing the unchanged geometry of the AF intensity measurement in the neoplasms from different part of body having different shape and size requires additional effort and is difficult for clinical applications, particularly in screening and prophylactic examinations. To overcome these difficulties we propose a method based on the skin neoplasm differentiation only on the shape of the AF spectrum, giving up the threshold criteria of AF radiation intensity. This allows essentially faster measurement, which is of particular importance for *in vivo* applications.

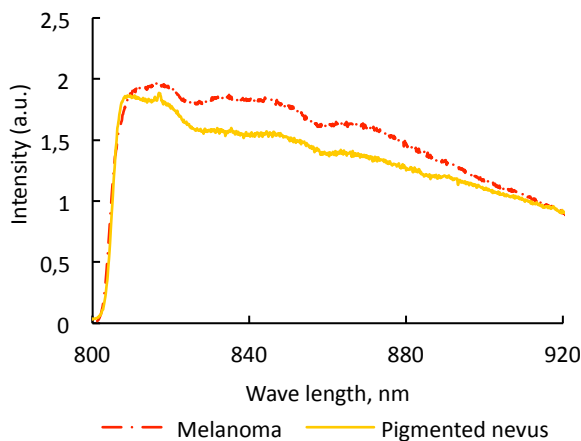


Fig. 3 AF spectra of malignant melanoma and pigmented nevus.

For the comparative analysis of the experimental data in the range 810 – 870 nm we approximated the AF spectral intensity by the exponential function (1):

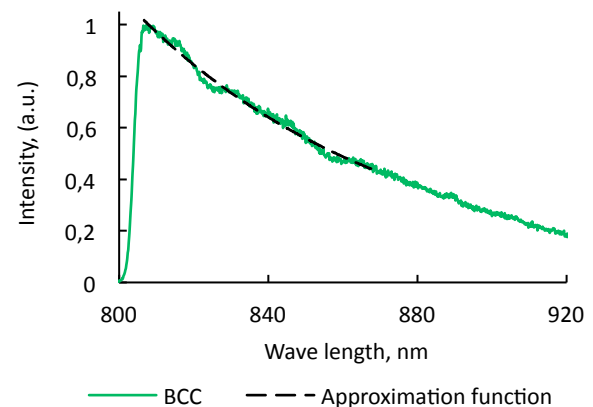
$$I_{ap}(\lambda) = ae^{b(\frac{\lambda}{\lambda_{max}})} + c.$$

As shown above, the sign (concave/convex) of the approximating function is completely determined by the sign of the parameter a from Eq. (1). The parameter c does not change the shape of the curve and, therefore, obviously is not involved in the diagnostic algorithm.

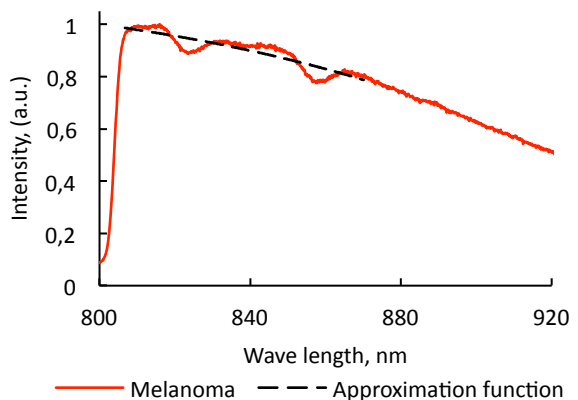
As follow from Eq. (1), the parameter b is responsible for the “degree of nonlinearity”. It should be noted that for the considered wavelength range in the case of exciting the skin fluorescence with the laser wavelength 785 nm only three cases are physically implementable (except all parameters are zero):

1. $a > 0, b < 0$ – concave monotonically decreasing function (1);
2. $a < 0, b < 0$ – convex monotonically increasing function (1);
3. $a < 0, b > 0$ – convex monotonically decreasing function (1).

The first case describes concave rapidly decreasing functions, typical for the AF spectra of non-melanocytic neoplasms (benign tumours, squamous cell cancer, and basal cell carcinoma). The case 3 ($a < 0, b > 0$) characterises the AF spectra of melanoma and nevus. At the expense of the increased melanin concentration the melanocytic neoplasms more intensely emit the radiation in the considered range, therefore, the AF spectra of the melanocytic neoplasms are characterised by the convex shape and slow decrease. For example, the results of approximating the AF spectra are presented for the basal cell carcinoma and the melanoma in Fig. 4.



a) $a = 6053.6; b = -0.64$



b) $a = -819.7; b = 1.82$

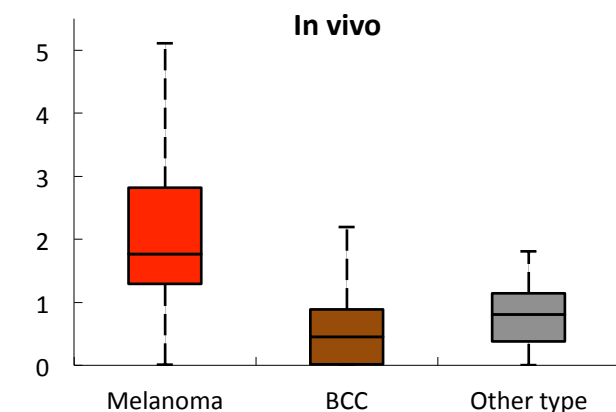
Fig. 4 Exponential approximation of AF spectrum of basal cell carcinoma (a) and melanoma (b).

Thus, as the informative criterion for the differentiation of tumour type we can consider the absolute value of the coefficient $|b| = \bar{b}$, that determines the curvature of the AF spectrum. It was found that the values of the coefficient $\bar{b}$ for melanoma are greater than 1 both for *ex vivo* and for *in vivo* measurements, whereas for other pathologies they are essentially smaller (see Fig. 5). Therefore, we can always introduce the criterial norm

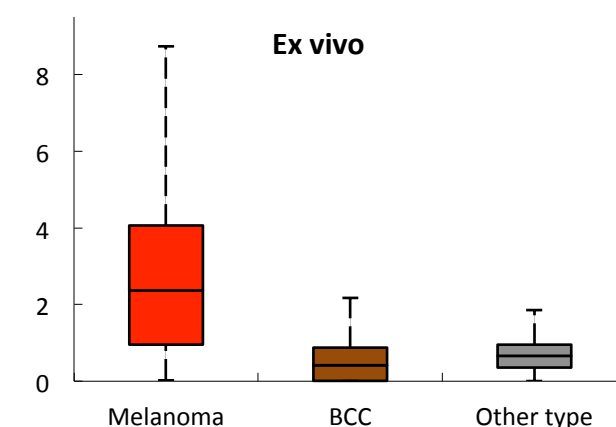
$$\bar{b} \geq 1, \quad (5)$$

that selects the melanoma among all possible neoplasms.

The second possible criterion for classifying the tumour type is the sign of the coefficient a before the exponential function. From the analysis of the obtained experimental data, it follows that the coefficient a is negative for all neoplasms with high concentration of melanin, in particular, for melanoma. Other types of tumours are characterised by positive values of a .



a)



b)

Fig. 5 Classification of melanoma, basal cell carcinoma and other types of tumours based on the curvature $\bar{b}$ of the spectral curve for *ex vivo* (a) and *in vivo* (b) studies.

Table 1 presents the values of the sensitivity and specificity of the proposed method of diagnostics, calculated from the data obtained *ex vivo* and *in vivo*. Here the diagnostic precision of the method was defined as

$$ACC = \frac{TP+TN}{TP+FP+FN+TN}, \quad (6)$$

where the terms in the right-hand side represent the number of true positive TP , false positive FP , true negative TN , and false negative FN decisions.

In the case of using the criterion (5) for the melanoma selection among other types of the studied pathologies the values of the sensitivity and specificity reached 67% and 83% for *ex vivo* and 78% and 80% for *in vivo* studies, respectively. When a combination of both criteria (a and $\bar{b}$) was used, the result was considered positive when one of the criteria showed that the neoplasm is melanoma. In this case, the values of the sensitivity and specificity for the diagnostics of melanoma increase to 96% and 83% for *ex vivo* and 92% and 80% for *in vivo* studies. The relatively low value of the specificity is due to the fact that the proposed criteria of the spectral curve shape allow one to select the pathologies with high content of melanocytes, which is typical both for melanomas and for nevi. This lead to the identification of some nevi as melanomas.

Table 1 Accuracy of melanoma diagnostics.

<i>Ex vivo</i>	Sensitivity, %	Specificity, %	Diagnostic accuracy, %
$\bar{b}$	67	83	75.5
a	64	66	65
$\bar{b} + a$	96	83	88.4

<i>In vivo</i>	Sensitivity, %	Specificity, %	Diagnostic accuracy, %
$\bar{b}$	78	80	79
a	61	50	55.5
$\bar{b} + a$	92	80	86.2

The melanoma diagnostic accuracy values for *ex vivo* (75.5%) and *in vivo* (79%) studies using the criterion $\bar{b}$ differ by 3.5%. This difference can be related to the relatively small sensitivity of detecting the melanomas in the *ex vivo* measurements (67%), because in the *ex vivo* measurements the number of melanoma samples was by 2.5 times greater than in the studies *in vivo*. On the other hand, when using the criterion a the observed difference between the values of diagnostic accuracy for melanomas *ex vivo* (65%) and *in vivo* (55.5%) studies is more essential (9.5%). This difference is explained by the low specificity (50%) of the diagnostics for *in vivo* measurements that can be associated with the larger fraction of nevi and other

non-melanoma pigmented neoplasms than in the *ex vivo* studies. Using the combination of both criteria of the AF spectrum shape allows the nearly similar accuracy of the melanoma diagnostics for *ex vivo* and *in vivo* cases at the level of 88.4% and 86.2%, respectively. However, the combination of the proposed criteria of the AF spectra shape appeared to be low-informative in the identification of the basal cell carcinoma, benign neoplasms, and nevi.

Comparing the results of the performed study with the data of other authors on the melanoma diagnostics one can conclude that the proposed method based on the criteria of the spectral curve shape change is comparable in the accuracy of melanoma diagnostics to the multimodal method [21] with the excitation of autofluorescence in the near infrared spectral region. In Ref. [21] it is shown that the diagnostic accuracy in the case of using the AF method reaches 90% in differentiating melanoma from healthy skin, however, the efficiency sharply falls in differentiating melanoma from nevus, with the values of sensitivity 67% and specificity 18%. Since both melanoma and nevus belong to the neoplasms of melanocytic type, their differentiation is difficult. In our work, we studied 3 nevi *ex vivo* and 3 nevi *in vivo*. Four of the six studied nevi were classified as melanoma basing on the proposed criteria, which is caused by the fact that both melanomas and nevi contain a large amount melanin fluorescing in the spectral region of interest. Apparently, this problem may be solved by operating in the visible or UV range [22, 23]. However, one cannot increase the accuracy of the melanoma diagnostics. Thus, according to the data of Ref. [23], the sensitivity and specificity of the melanoma diagnostics based on the measurement of the AF intensity under the UV laser excitation reached 77.8% and 93.3%, respectively. Thus, the efficiency of the proposed method is comparable with that of the method of the melanoma AF analysis in the visible spectral range.

4 Conclusions

The results obtained in our experimental study show the possibility of using the AF spectrum shape analysis in the near infrared region for the malignant melanoma diagnostics.

An important advantage of this method is its invariance with respect to the tumour size and the high speed of the information acquisition.

The best results in the melanoma diagnostics are achieved using the complex shape criteria, applied to the autofluorescence spectrum approximated by the exponential function. The accuracy of selecting melanoma among other types of tumours achieves 88.4% for *ex vivo* studies and 86.2% for *in vivo* studies. However, the method appeared inefficient for the diagnostics of non-melanocytic types of malignant tumours. In that the malignant melanoma is the most aggressive and dangerous pathology, the obtained results can be important for the medical practice. Since the proposed method is characterised by high speed of

processing the results and calculating the criterial norms and the method is independent of the fluorescence intensity level and the skin phenotype, it can be successfully applied in screening examinations. An alternative application is the fast primary instrumental testing with subsequent analysis of Raman scattering of the pathologies, detected at the first stage.

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Pulse wave digital processing and beat detection based on wavelet transform and adaptive thresholding

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Abstract. This paper considers the adaptive algorithm for pulse wave processing in the presence of various physiological interferences, such as motion artefacts and baseline wander. The proposed processing technique consists of two main steps: comprehensive filtering of pulse wave based on wavelet transforms and noise-resistant pulse beats detection based on the set of pass-band filtering, nonlinear transforms and adaptive thresholding. To eliminate baseline wander from pulse wave signal we introduced a new algorithm based on the principles of adaptive noise cancellation, while the reference signal for adaptive filtering was generated by using multiresolution wavelet transform. For reducing motion artefacts and others high frequency distortions of pulse wave we suggested an approach based on soft thresholding of detailed coefficients from wavelet decomposition. The efficiency of the developed algorithm for pulse wave signal processing was assessed in comparison with the existing approaches by using mathematical modeling of pulse wave signal and occurred contaminations. The performance of the pulse beats detector was further verified for different recordings of clinical pulse wave signals from the Physionet database. © 2015 Samara State Aerospace University (SSAU).

Keywords: pulse wave, wavelet transform, detector, fiducial point, error, motion artefact, baseline wander, adaptive threshold.

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1 Introduction

The recording and processing of the pulse wave signal finds wide application in the instrumental systems of cardiologic diagnostics, aimed at monitoring the heart rate and blood arterial pressure, assessing the saturation of arterial blood haemoglobin with oxygen, and studying haemodynamic processes in the human arterial bed [1-3].

In contrast to the ECG signal, for which the processing technique is a subject of substantial studies carried out by the researchers of different scientific profile during the last 40 years, the approaches to processing the pulse wave signals, in spite of their obvious diagnostic value, are much less known and presented in scientific literature. This fact is mainly due to the history and the field of application of the methods and instruments for pulse wave signal recording and processing in clinical practice, first of all, in clinical monitors and instruments for blood arterial pressure measurements, as well as in pulse oximeters. The software in these devices is proprietary, and this is just the reason why the algorithms used in them did not become available to the scientific community and, therefore, were not developed as intensely as the ECG signal processing techniques. The absence or insufficient development of open databases with real clinical records of this biological signal also caused reduced attention of researchers and clinical physicians to the problems of elaborating and comparative analysis of various methods for pulse wave signal automated processing.

However, there are a few publications and researches that describe algorithms for pulse wave filtering and morphological features detection [4-10]. The most successful approaches for pulse wave filtering from baseline wander and motion artefacts are based on different applications of multiresolution wavelet transforms [4-6]. For wearable devices and long-term monitoring using accelerometer for motions recording and principles of adaptive filtering for eliminating motion artefacts demonstrates very good results [7].

There are several publications concerning the challenge of pulse wave fiducial points detection [8-10].

At the same time there is a need to create the novel approach for comprehensive filtering of pulse wave from baseline wander and motion artefacts, as well as for noise-resistant pulse wave beat detection. The present paper is devoted to the development and thorough analysis of high-efficiency techniques for high quality processing of pulse wave signals contaminated by various types of distortions.

2 Theory

Pulse wave recording by means of plethysmography or sphygmography sensors is accompanied by disturbance and noise of different nature. The strongest signal distortions are due to the artefacts having the physiological origin, i.e., caused by the movements and breathing of the examined patient. Breathing trends, present in the arterial blood pulsation signal, distort the baseline and the shape of the biological signal. Motion artefacts are random and lead to the maximal distortions of the pulse wave morphology [1, 2].

The processing of the pulse wave signal against the background of physiologic artefacts faces a number of algorithmic difficulties. They are mainly due to the random character of the noise and the fact that their frequency components overlap with the fundamental band of the biosignal, which makes it difficult to use classical methods of linear frequency filtering and causes the necessity for creating nontrivial processing methods.

At present, the filtering based on the multi-resolution wavelet transforms is a promising line of research in the field of processing the biological signals, perturbed by broadband stochastic noises [4-6, 11, 12]. The signal processing procedure based on using discrete wavelet transforms includes the signal decomposition and signal reconstruction stage.

The signal decomposition is implemented in accordance with the Mallat algorithm and consists in the decomposition of the initial signal into a sequence of approximation and detailed coefficients. The signal

filtering to get rid of the noise can be implemented by direct modification of the detailed coefficients of the wavelet decomposition [12]. After the signal decomposition stage and the removal of noise components, the reconstruction of the initial signal is carried out.

3 Materials and methods

In the present paper, to make the pulse wave signal free of physiologic artefacts, we propose the wavelet filtering technique based on the discrete decomposition in terms of orthogonal wavelets that includes the following processing stages:

- 1) calculation of the direct discrete wavelet transform of the pulse wave signal;
- 2) modification of the detailed coefficients of the wavelet decomposition in correspondence with the threshold processing parameters;
- 3) reconstruction of the pulse wave signal basing on the initial approximation coefficients and modified detailed coefficients by means of the inverse wavelet transform.

The efficiency of the proposed pulse wave signal filtering technique based on the multi-resolution wavelet transforms depends upon the form of the mother wavelet function, the level of de-composition, the type of the threshold function for processing the specifying coefficients, and the algorithm for threshold determination.

The adaptive determination of the threshold level T for each level of the wavelet decomposition was performed using the Donoho formula [12]:

$$T = \sigma \sqrt{2 \lg M},$$

where σ is the root-mean-square deviation of noise, evaluated for the sequence of detailed coefficients at the given level of the wavelet decomposition; M is the total number of the signal samples.

In the threshold processing of detailed coefficients the hard (1) and the soft (2) threshold functions are distinguished [11, 12]:

$$y(x) = \begin{cases} x, & |x| \geq T, \\ 0, & |x| < T. \end{cases} \quad (1)$$

$$y(x) = \begin{cases} \text{sign}(x) \cdot (x - |T|), & |x| \geq T, \\ 0, & |x| < T. \end{cases} \quad (2)$$

where x is the input value of the detailed coefficients, y is their output value, $\text{sign}(x)$ is the function determining the sign of the number x , and T is the value of the adaptive threshold.

To study the efficiency of the pulse wave signal wavelet filtering technique we used the mathematical modelling of present distortions. The model of the pulse wave signal, distorted by the baseline wander and motion artefacts, was assumed to be additive. To get the model dependences of the pulse wave, we used the simulation model proposed by P.E. McSharry [13] that

allowed the formation of biosignal fragments with the required morphology and with the given values of amplitude-time parameters.

The analysis of the structure and factors, affecting the appearance of the pulse wave baseline wander, has shown that this type of noise is a low-frequency signal of mainly stochastic nature, which can be described in the form of additive combination of deterministic and random components:

$$W(k) = W_{\max} \cdot \left(\sum_{i=1}^4 \sin \left(2\pi f_i \frac{k}{f_s} \right) + \psi(k) \right)$$

where $W_{\max}$ is the amplitude of the baseline wander model signal; $\psi(k)$ is the random component, obtained by filtering the white Gaussian noise with zero mean value and unit variance with a low-frequency filter with the cut-off frequency 1 Hz; f_i is a set of harmonic signal frequencies, representing the deterministic component of the baseline wander. In the process of simulation, the following values were used: $f1 \dots f4 = 0.1$ Hz, 0.2 Hz, 0.4 Hz, and 0.6 Hz.

The experiments with the accelerometer sensor, fixed on the arm of the investigated human, followed by the spectral analysis of the recorded motion distortion signals, have shown that the frequency region of motion artefacts, accompanying the pulse wave recording at different motion activity (walking, gesticulation, running) ranges from 0 to 5 Hz [7].

As a mathematical model of motion artefacts, we used the Markov process model, or the first-order autoregressive model, described by the following recursion expression:

$$z(k) = z_{\max} \cdot (c + a \cdot z(k-1) + \varepsilon(k)),$$

where $z_{\max}$ is the amplitude of the motion artefact model signal; $\varepsilon(k)$ is a sequence of random numbers, distributed in accordance with the normal law with the zero mean value and the unit variance; a and c are constants; $z(k)$ is the current sample of the Markov process; $z(k-1)$ is the previous sample of the Markov process.

Since the spectral band of motion artefact frequencies observed under the real conditions is limited, the model signal $z(k)$ was processed with a low-frequency filter (LFF) having the cut-off frequency 5 Hz.

As one of the criteria for estimating the efficiency of filtering the pulse wave signal, we proposed the distortion coefficient δ of the biosignal after passing the relevant processing stages:

$$\delta = \frac{\sum_{i=1}^M [Y_f(i) - Y(i)]^2}{\sum_{i=1}^M Y^2(i)} \cdot 100\%,$$

where i is the signal sample's number; M is the numbers of samples in the considered signal fragments; $Y_f(i)$ is the sample of the distortion-free model pulse wave signal.

The optimal parameters for processing the pulse wave signals were chosen using the criterion of minimizing the value of the signal distortion coefficient within the range of variation of the signal-to-noise ratio, estimated as follows:

$$K_a = 10 \lg \frac{S_{\max}}{X_{\max}},$$

where $S_{\max}$ is the total spectral power of the initial model pulse wave signal; $X_{\max}$ is the total spectral power of the additive noise.

One of the basic problems of the pulse wave signal digital processing is to detect the fiducial points, mainly the systolic maxima, for further determination of heart rhythm parameters and measurement of the biosignal amplitudes for calculating the diagnostic indices of the cardiovascular system. Increasing the reliability of the performed diagnostics is possible at the expense of reducing the measurement error for the amplitude-time parameters of the biosignal, depending on the precision and efficiency of the implementation of the algorithms that detect the pulse wave fiducial points.

At present, the issues of minimizing the errors, caused by the inaccuracy of detecting the pulse wave fiducial points, remain to be insufficiently studied. The major requirement to the means of detecting the fiducial points is the possibility of efficient operation under the conditions of high-intensity noises and high variability of the biosignal shape. As fiducial points for the pulse wave signal, they usually choose the most recognizable points against the background of the noise, namely, the systolic maximum of the signal, the minimum of the pulse wave signal, and the maximum of the first derivative signal [8-10, 14].

To provide high-efficiency detection of the pulse wave beat detection, we propose here a detector with the adaptive threshold algorithm. At the stage of preliminary processing the operations of frequency band-filtering, differentiation, and nonlinear transformation are applied; the maximum of the biosignal first derivative is chosen to be a reference point for further localization of the pulse wave beats or systolic maximums.

To improve the accuracy of pulse wave signal processing and pulse beats detection we preliminary applied digital interpolation to the recorded biosignals by using cubic splines, thus the effective sampling frequency after interpolation was selected at 2000 Hz.

To implement the frequency filtering of the pulse wave signal we used the Butterworth digital filter of the 8-th order with the pass-band from 0.5 to 10 Hz. To linearize the phase characteristic, the signal output from the Butterworth filter was again passed through the filter, but in the reversed direction [15].

Then the pulse wave signal was differentiated using the operator:

$$P(n) = \frac{1}{\Delta_d} [Ppg(n-1) - Ppg(n)],$$

where n is the number of the signal sample; Δ_d is the sampling interval; $Ppg(n)$ is the pulse wave signal after the band-pass frequency filtering.

The signal obtained after the differentiation is raised to the third power, which additionally enhances the large differences of the signal $P(n)$. Then the samples with negative amplitude values are removed from the obtained signal.

Figure 1 presents the time dependences of the pulse wave signal at different stages of processing: curve 1 is the model signal of the pulse wave $Ppg(n)$, curve 2 is the signal after the differentiation $P(n)$, curve 3 is the signal raised to the third power $P_2(n)$, and curve 4 is the signal after selecting the positive samples $P_3(n)$.

The output signal $P_3(n)$ after passing all stages of the preliminary processing arrives at the input of the circuit executing the threshold pulse beat detection. The detection unit forms a moving window with the duration of 2 seconds; for each window the threshold unit determines the value of the threshold (Lev). Only those signal samples are input into the maximum detector, for which the condition $P_3(n) > Lev$ is fulfilled.

The running window duration is chosen such that at least one pulse beat should be present in the signal during this time. Since the periodicity of the pulse beats is determined by the heart rate, the minimal value of which is not less than 30 beats per minute, the appropriate detection interval should not exceed 2 seconds.

The detector of the maximum determines the position of the maximum of the pulse wave signal first derivative using the three-point detection method:

$$P_3(n) = \text{Max}, \text{ if } P_3(n) > Lev \ \& \\ \& P_3(n) > P_3(n+1) \ \& P_3(n) > P_3(n-1).$$

If this logical condition is fulfilled, the number of the sample n determines the temporal position of the pulse wave signal derivative maximum.

The efficiency of the proposed pulse beat detection scheme is largely dependent on the choice of the threshold value Lev . The threshold value is independently determined for each moving window basing on the following threshold function:

$$Lev(i) = \begin{cases} 0,4 \text{Max}(i), \Omega(i) \geq 0,2 \text{Max}(i) \ \& \text{Max}(i) < 2 \text{Max}(i-1); \\ 0,4 \text{Max}(i-1), \Omega(i) \geq 0,2 \text{Max}(i) \ \& \text{Max}(i) \geq 2 \text{Max}(i-1); \\ 1,6 \Omega(i), \Omega(i) < 0,2 \text{Max}(i), \end{cases}$$

where $\Omega(i)$ is the value of the root-mean-square deviation of the signal samples amplitude value within the limits of the i -th moving window; $\text{Max}(i)$ is the maximal value of the amplitude of the signal samples within the limits of the i -th moving window; $\text{Max}(i-1)$ is the similar maximum within the pre-ceding $(i-1)$ -th moving window.

The numerical values of the threshold function parameters were chosen empirically as a result of the preliminary processing of the noisy model signal using the criterion of maximizing the correct detections of pulse beats and minimizing the false detections and misses.

The described algorithm provides the detection of fiducial point, i.e. the maximum of the pulse wave's first derivative in our case. At the second stage detector uses the information provided by the previous step. A defined width window subset (± 100 ms form the

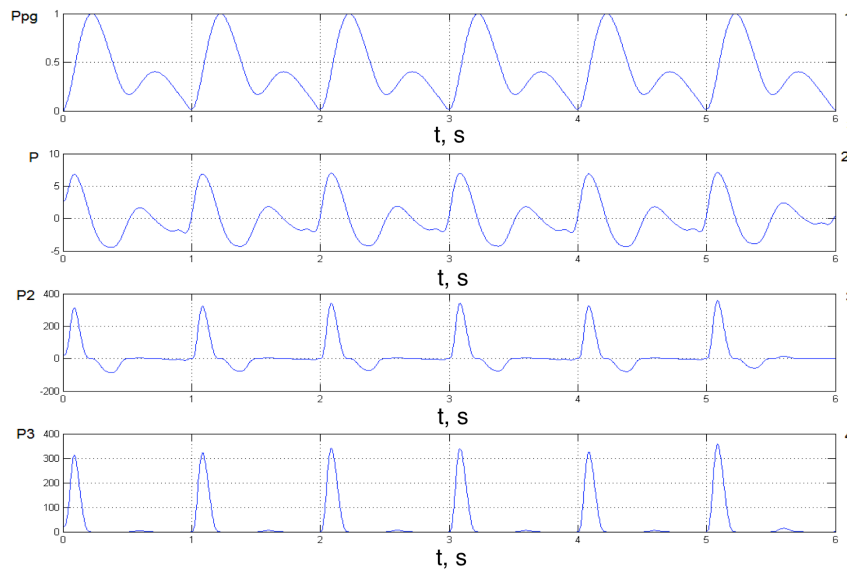


Fig. 1 Time dependences of the pulse wave signal at different stages of the pulse beat detection; amplitude values are expressed in relative units.

location of the peak found in the pulse wave's first derivative) is selected in the original filtered pulse wave to locate the real systolic peak. Once again a simple maximum peak locator in the values of this subset sequence is used.

The detection of pulse beats is implemented with a certain error, caused by the imperfection of the detection scheme, as well as by the distortions of the biosignal. This error may produce a random error in measuring the pulse wave amplitude and the duration of the beat-to-beat intervals. As one of the criteria for estimating the efficiency of the scheme for detecting the pulse beats the deviation of measuring beat-to-beat intervals from the true value with the confidence level $P=0.9$ could be chosen:

$$\Delta_u = 1,6 \cdot \sigma_u,$$

where Δ_u is the absolute error of measuring beat-to-beat intervals, σ_u is the root-mean-square deviation of the beat-to-beat intervals from the true value, determined as

$$\sigma_u = \sqrt{\frac{\sum_{i=1}^N (PP(i) - PP(i))^2}{N}},$$

where $PP(i)$ is the true value of the i -th beat-to-beat interval, $PP'(i)$ is the measured value of the i -th beat-to-beat interval, N is the total number of beat-to-beat intervals in the analyzed recording of the biosignal.

One more criterion for evaluating the efficiency of the pulse beat detection scheme is the random deviation of the measured amplitude from the true value, also determined at the confidence level $P=0.9$:

$$\delta_a = \frac{1,6 \cdot \sigma}{A_m} \cdot 100\%,$$

where δ_a is the relative error of the pulse wave amplitude measurement; A_m is the true value of the pulse wave amplitude; σ is the root-mean-square

deviation of the pulse wave amplitude from the true value, determined as

$$\sigma = \sqrt{\frac{\sum_{j=1}^N (A'_m(j) - A_m(j))^2}{N}},$$

where $A_m(j)$ is the true value of the j -th pulse wave amplitude; $A'_m(j)$ is the measured value of the j -th pulse wave amplitude; N is the total number of pulse wave fragments in the considered recording.

4 Results

The results of the studies were obtained using the standard functions of Signal Processing Toolbox MATLAB R2013a.

Table 1 presents the values of the pulse wave distortion coefficient δ for different levels of wavelet decomposition using the hard and soft threshold function. The signal-to-noise ratio is $K_a=8$ dB; the mother wavelet function is the Daubechies wavelet of the 6-th order (db6); the heart rate is 70 beats per minute, the pulse wave amplitude is 1; $f_s=2000$ Hz; $c=0$; $a=0.5$.

The analysis of the obtained results shows that the number of the wavelet decomposition levels, optimal from the point of view of minimizing the pulse wave distortions, should be equal to 6, when the soft threshold function is used for processing the detailed coefficients.

Figure 2 shows a fragment of the synthesized model pulse wave signal, distorted by strongly expressed motion artefacts, and the result of applying the technique of the noisy biosignal wavelet filtering. The time dependences were obtained for the following values of the model parameters: the heart rate is 70 beats per minute, the amplitude of pulse wave is 1; $f_s=2000$ Hz; $c=0$; $a=0.5$; $W_{max}=0$; $z_{max}=0.3$.

Table 1 The values of the pulse wave distortion coefficient δ for different levels of wavelet decomposition using soft and hard threshold function.

Wavelet decomposition level	Value of distortion coefficient δ	
	Soft threshold function	Hard threshold function
1	23.3 %	26.7 %
2	18.9 %	20.1 %
3	16.4 %	18.6 %
4	13.1 %	14.8 %
5	10.6 %	11.9 %
6	8.1 %	9.5 %
7	11.2 %	12.9 %

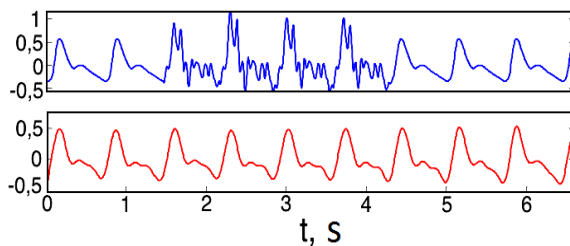


Fig. 2 A fragment of the pulse wave model signal with the motion artefacts (top); the result of wavelet filtering (bottom); amplitude values are expressed in relative units.

The obtained results demonstrate high quality of processing of the noisy model pulse wave signals using the multi-resolution wavelet transforms.

Figure 3 presents a fragment of clinical record of the finger pulse wave signal, distorted by the baseline wander and motion artefacts, caused by the patient's hand tremor. Figure 4 shows a fragment of the pulse wave signal processed using the proposed wavelet filtering technique.

The analysis of the pulse wave signal fragment shown in Fig. 4 demonstrates that the developed technique of wavelet filtering efficiently removes the motion artefacts, but has practically no effect on the baseline wander present in the signal. Additional studies have shown that the sequence of approximation coefficients, obtained at high levels of wavelet decomposition, has the frequency spectrum, similar to that of the pulse wave baseline wander.

The optimal parameters of wavelet decomposition for removing the pulse wave baseline wander were determined basing on the analysis of the coefficient of correlation between the model signal of baseline wander and the sequence of coefficients in the wavelet decomposition of the model pulse wave signal with the additive baseline wander present. The numerical values of the correlation coefficient are presented in Table 2.

The analysis of the results presented in Table 2 shows that in order to correct the baseline wander of the pulse wave it is optimal to use the sequence of approximation coefficients of the 6-th decomposition level.

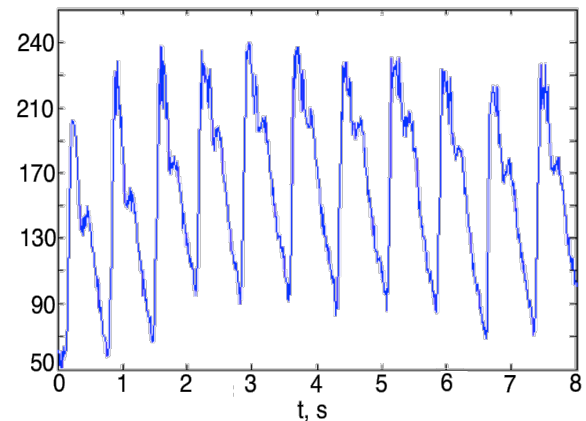


Fig. 3 A fragment of pulse wave signal, distorted by the baseline wander and motion artefacts; amplitude values are expressed in relative units.

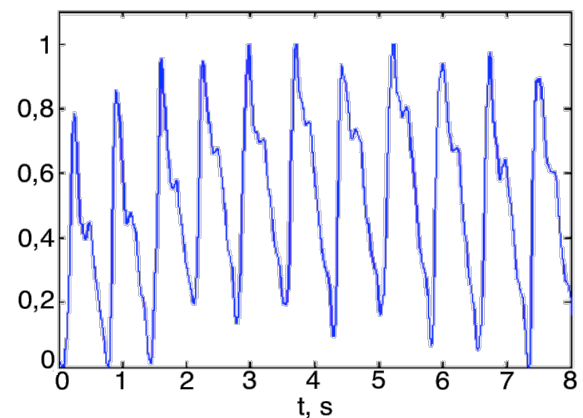


Fig. 4 The fragment of pulse wave signal after the application of wavelet filtering procedures; amplitude values are expressed in relative units.

Table 2 The values of the coefficient of correlation between the model baseline wander of the pulse wave and the sequence of wavelet decomposition coefficients.

Decomposition level	Detailed coefficients	Approximation coefficients
1	0.15	0.32
2	0.17	0.41
3	0.21	0.49
4	0.29	0.56
5	0.32	0.61
6	0.33	0.8
7	0.42	0.65

Figure 5 illustrates the correction of the pulse wave baseline wander basing on the subtraction of the interpolated sequence of approximation coefficients of the 6-th level of wavelet decomposition from the biosignal, distorted by the baseline wander (Fig. 4). The interpolation of the sequence of approximation coefficients is necessary due to the specific feature of the Mallat algorithm implementation, according to which at each stage of the wavelet decomposition the decimation with the factor 2 is carried out 2 [4].

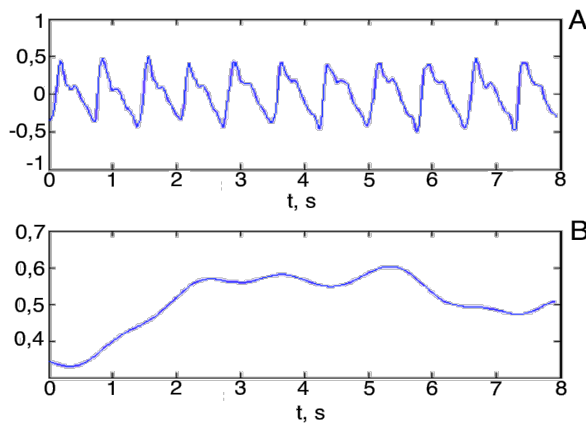


Fig. 5 A fragment of the pulse wave signal after correcting the baseline wander (A); the estimate of the baseline wander obtained basing on the wavelet decomposition (B); amplitude values are expressed in relative units.

The efficiency of the pulse beat detection was studied for different types of detectors, namely, the detector based on the moving average filtering (MAF) [12]; the detector based on band-pass filtering (BPF) and quantile detection thresholds [13]; the detector based on wavelet transforms (WT) [8]; the adaptive detector (AD) proposed in the present paper.

Figures 6 and 7 present the error of measuring the beat-to-beat intervals (Δu) and the relative error of measuring the pulse wave amplitude (δ_A), respectively, versus the signal-to-noise ratio K_a for different detectors of the pulse beats: 1 – BPF, 2 – MAF, 3 – WT, and 4 – AD. The dependences are obtained at the following parameters of the model: the heart rate 80 beats per minute; $f_p=50$ Hz; $f_s=2000$ Hz; $a=0.5$; $c=0$; the noise amplitudes W_{max} and z_{max} were specified in correspondence with the value of the signal-to-noise ratio K_a .

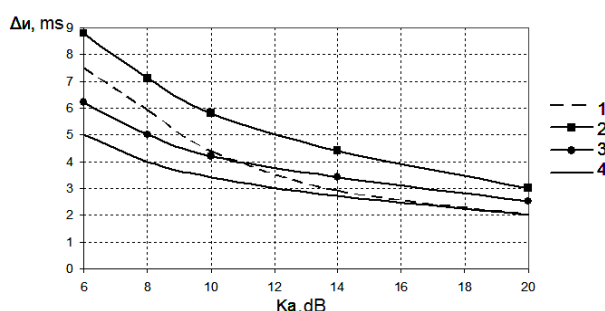


Fig. 6 The error of beat-to-beat intervals measurement versus the signal-to-noise ratio K_a (1 – BF, 2 – RAF, 3 – WT, and 4 – AD).

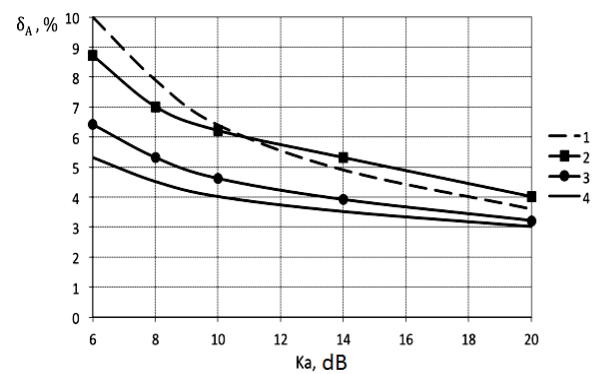


Fig. 7 The amplitude measurement error versus the signal-to-noise ratio K_a (1 – BPF, 2 – MAF, 3 – WT, and 4 – AD).

As follows from the obtained dependences, the reduction of the signal-to-noise ratio leads to the exponential growth of errors for the beat-to-beat intervals and pulse wave signal amplitude measurements. The minimal error of measuring the amplitude-time parameters of the pulse wave under the conditions of baseline wander and motion artefacts of different intensity is demonstrated by the proposed adaptive detector (AD), with preliminary processing based on the multi-resolution wavelet transforms.

To analyze the efficiency of the pulse beat detection in the processing of clinically recorded biosignals we used the Physionet open database of the Massachusetts Institute of Technology. The following statistical indicators were used for quantitative evaluation of the detection efficiency:

- 1) the probability of correct detection of pulse beats (PT):

$$P_T = \frac{N_T}{N} \cdot 100\%;$$

- 2) the probability of erroneous detection of pulse beats (PF):

$$P_F = \frac{N_F}{N} \cdot 100\%;$$

- 3) the index of detection error level Per:

$$P_{er} = \frac{N_m + N_F}{N} \cdot 100\%,$$

where N_T is the number of correctly detected pulse beats (systolic maxima); N_F is the number of false detected pulse beats; N_m is the number of missed pulse beats.

The computer processing of the signals was carried out for multichannel records of biological polysomnography signals from the database MIT-BIH Polysomnographic Database (<http://physionet.org>), containing 18 fragments of pulse wave signals, each 2 hours long. Separately, one recording with low noise (Slp45) and one recording with very intensive noise (Slp67x) were selected for the studies.

Table 3 presents the results of the quantitative evaluation of the efficiency of different detectors of systolic maxima in application to the processing of clinical pulse wave signals.

Table 3 Efficiency evaluation for the detection of pulse wave signal systolic maxima.

Biosignal recording	Slp45			Slp67x			Mean value for 18 recordings		
Detector	PT, %	PF, %	Per, %	PT, %	PF, %	Per, %	PT, %	PF, %	Per, %
1	98.9	0.1	0.1	97.2	0.3	0.95	97.1	0.09	0.44
2	99.3	0.07	0.06	97.5	0.28	0.8	97.6	0.08	0.29
3	100	0	0.07	98.1	0.17	0.78	99	0.04	0.23
4	100	0	0.05	98.7	0.16	0.57	99.3	0.03	0.2

Figure 8 presents the block diagram of the considered method of the pulse wave signal digital processing method. The notation is as follows: 1 – initial pulse wave signal; 2 – output pulse wave signal after the removal of physiological artefacts; 3 – array of detected fiducial points of the pulse wave; WDU – wavelet decomposition unit; AC – approximation coefficients; DC – detailed coefficients; TP – threshold processing; IWT – inverse wavelet transform; AC 6 – approximation coefficients of the 6-th decomposition level; A – adder; BF – band-pass filtering; DU – differentiation unit; NO – nonlinear operation unit; MWF – moving window formation; ATF – adaptive threshold formation unit; TU – threshold unit; MD – maximum detector.

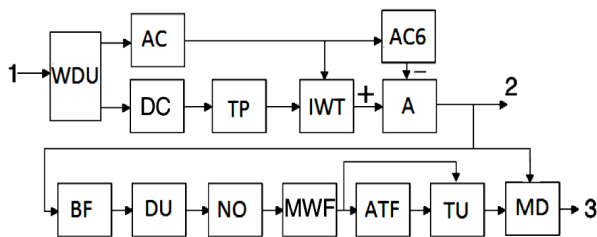


Fig. 8 Block-diagram of pulse wave signal processing

5 Conclusions

As a result of the performed studies, the method for complex digital processing of the pulse wave signal was developed and studied. The method is based on multi-resolution wavelet transforms and includes the filtering of baseline wander and motion artefacts, as well as the noise resistant fiducial point detection.

The studies have shown that the adaptive detector of systolic maxima, proposed here, provides higher accuracy as compared to the existing approaches, namely, the probability of correct detection amounts to not less than 99%, the probability of erroneous detection does not exceed 0.5%, the random error for the measurement of the pulse wave amplitude is less than 5%, and the random error for the measurements of beat-to-beat intervals is less than 5 ms.

The high quality of the pulse wave signal filtering and perfect accuracy of detecting the pulse beats can serve as a reliable base for developing high-efficiency algorithms and hardware-software cardiology diagnostic complexes.

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Application of Raman spectroscopy method to the diagnostics of caries development

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Abstract. The object of study were human teeth with caries cavities and healthy teeth extracted for orthopaedic indications. The main method of study was Raman spectroscopy. We revealed the specific features of Raman spectra for the healthy tooth tissues impaired ones and the tissues affected by caries. The optical criteria are established that allow caries detection and assessment, as well as the detection of pre-caries impairment of the tooth tissues. The results were analysed in comparison with the data of the chemical analysis performed using a raster electron microscope. © 2015 Samara State Aerospace University (SSAU).

Keywords: spectroscopy, Raman scattering, tooth, caries.

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1 Introduction

Like in any other branch of medicine in stomatology the successful and efficient treatment begins from high-quality teeth diagnostics. It is well known that caries is the most widespread and unpredictable problem in stomatology [1, 2]. At the first stages it is symptomless, but later the progressing caries can lead to the development of pulpitis and finally to the tooth extraction and the consequent patient's aesthetic problem. For the efficient and timely diagnosis the complete dental examination must include the investigation of hard tissues, caries leads to unnecessarily damage the organic matrix of tooth tissue, which subsequently loses its function of fixing mineral substrate, which leads to the formation caries defect, using a contactless and non-invasive diagnostic method.

Raman spectroscopy is one of possible methods of early diagnostics of the tooth hard tissue diseases. The advantage of the Raman spectroscopy is that this method is non-invasive, non-destructive and requires no sample preparation [3].

In [4] examined changes in the enamel mineral substrate using the method of Raman spectroscopy, cutting is perpendicular to the cross section of the tooth. The authors of [4] compared the enamel caries regions with the healthy regions of the tooth at 960 and 880 cm^{-1} corresponding to $(\text{PO}_4)^{3-}$ and $(\text{CO}_3)^{2-}$ respectively, since at these peaks the difference between the healthy and caries tooth regions is clearly seen. It was also shown experimentally that the enamel contains high concentration of mineral components.

In refs [5] the authors assess the ratio of cross-polarised (the polarisation of scattered light is perpendicular to that of the incident light) and co-polarised Raman spectra at 959 cm^{-1} . It was found that the ratio of co- and cross-polarised Raman scattering intensities can be used to assess the degree of demineralisation.

In [6,7,8], using the method of Raman spectroscopy determined that the difference between healthy and carious areas of enamel is well observed at wave number 959 cm^{-1} , as well as the study of various intensities PO_4^{3-} oscillations (1043, 590, and 431 cm^{-1}) revealed a consistent change in the intensity of the spectra of carious areas compared to healthy. The spectral changes associated with demineralization by

altering the elemental composition of the enamel. In stomatology there are a few instruments based on optical methods. One of them is the fluorescence-based device KaVo DIAGNOdent that can detect caries at early stages. However an essential drawback of this instrument is the false response, e.g. to the tooth plaque [9].

The tomography system ORTHOPHOS XG 3D possesses high resolution (160/100 μm), allows precise diagnostics of caries complications, and determination of efficient technique and tactics of channel treatment. However, the examination using this instrument is accompanied with the exposure to radiation of both the patient and the doctor [10].

On the basis of Raman spectroscopy instruments used in dentistry, it has not been developed. Therefore, one of the most urgent tasks is to develop a rapid instrument on the basis of Raman spectroscopy.

The aim of this work was to apply the Raman spectroscopy method to the diagnostics of caries and tooth tissue degradation at its early stage.

2 Materials and Methods of Study

As objects we used 11 samples of molar and premolar teeth of patients with the caries diagnosis (K02). The Raman spectroscopy method was implemented using the experimental stand described earlier [11], which includes a high-resolution digital spectrometer Shamrock with integrated refrigerated storage, fiber optic probe for Raman spectroscopy (RS), combined with the laser module LuxxMasterLML-785.0RB -04. Processing of the spectra made in the program Wolfram Mathematica 8.

The measurements were performed along the lines shown in Fig. 1. The power was being 300 mW in the healthy region and 100 mW in caries one the exposure time being 20 seconds and a wavelength of 785 nm.

In the course of the studies, we first carried out a series of test experiments to ensure that the action was non-invasive and to choose the optimal parameters of the setup varying the laser power from 50 to 300 mW. The variation of the probe height above the object within the interval of 6-8 mm exerted inessential influence on the values of Raman lines intensity ratio (the error amounted to 3%).

The comparative analysis of the chemical composition was performed using the raster electron microscope (REM) JED – 2300 AnalysisStation (Japan). The objects of study were different tooth regions (enamel, dentin, cement) both in the normal condition and with caries lesion.



Fig. 1 The studied tooth: the tooth section shows the dentin and enamel regions and the lines passing them. The caries lesion of dentin and enamel in the crown part of the tooth is seen.

3 Results

Figure 2 presents the characteristic Raman spectra of the tooth tissues obtained as a result of the studies in the regions of lesion and healthy tooth.

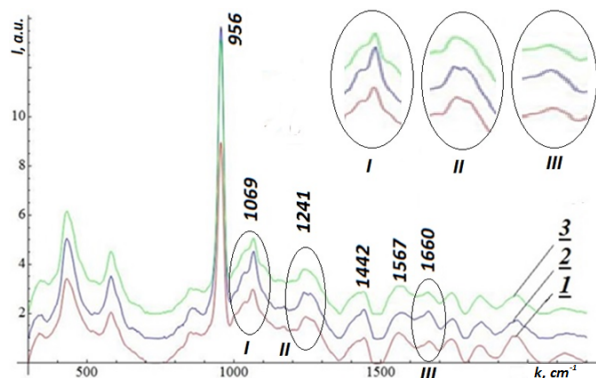


Fig. 2 Raman scattering spectra of healthy samples of the tooth cement (1), dentin (2), and enamel (3) tissues.

From Fig. 2 one can see the intensity decrease of the line at 1069 cm^{-1} corresponding to the vibration ν_1 (CO_3)²⁻ due to the B-type substitutions where this group replaces PO_4^{3-} and possesses less hardness. Besides that, the spectra differ in the intensities of lines at 1241 cm^{-1} and 1660 cm^{-1} corresponding to amide III and amide I respectively. The weakness of these lines is explained

by the fact that the enamel is the hardest tissue in the human organism due to high content of inorganic substances (up to 97 %) namely the hydroxyapatite [12].

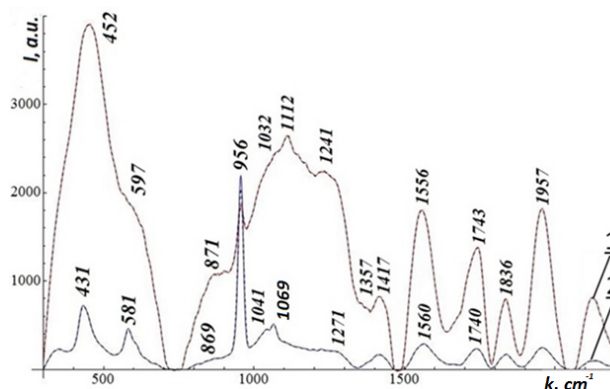
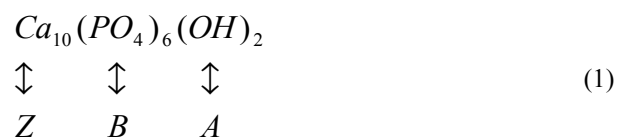


Fig. 3 Raman scattering spectra in the region of dentin with caries lesion (1) and healthy enamel (2) tooth tissues.

The analysis of Fig. 3 shows that for the samples of hard tissue affected by caries the main intensity changes are observed for the lines at 431, 581, 955, 1041, 1069 cm^{-1} corresponding to the phosphate and 1241 cm^{-1} , corresponding to amide III; in the caries-affected tissues the line 1660 cm^{-1} , corresponding to amide I manifests itself. For the samples with early stage of caries and weakened mineral component the spectra are transient. The early changes in the spectrum are mainly due to both the remineralisation of the tooth hard tissues and the change of the mineral component structure at the expense of replacement of more stable components with less stable ones.

The mineral component of the tooth is presented by various apatites possessing different properties:



Where the replacements of the following types are possible:

$\text{Z} = \text{Ca}^{2+}, \text{Na}^+, \text{Mg}^{2+};$
 $\text{B} = \text{PO}_4^{3-}, \text{CO}_3^{2-}, \text{HPO}_4^{2-};$
 $\text{A} = \text{OH}^-, \text{CO}_3^{2-}, \text{F}^-, \text{Cl}^-.$

The mineral component of the tooth is characterised by the vibration modes ν_1 , ν_3 , and ν_4 (PO_3)⁴⁻ in the hydroxyapatite molecule for which the Raman lines were recorded at 581, 647, 955, 1038 and 1169 cm^{-1} [13,14] as well as by the lines of the vibration modes ν_1 (CO_3)²⁻ of B-type substitutions, where (PO_4)³⁻ is replaced with (CO_3)²⁻ and A-type [14], where OH^- is replaced with (CO_3)²⁻ at 874, 1067, 1100, and 1435 cm^{-1} , respectively. Besides the Raman lines of proline and hydroxyproline the amide component is presented by the groups of amide III (in the region 1241-1271 cm^{-1}) [13,15], amide II (in the region 1520-1580 cm^{-1}) [13], and amide I (in the region 1630-1680 cm^{-1}) [13,15]. The

recorded Raman bands correspond to the vibrational modes presented in Table 1.

Table 1 Interpretation of Raman spectra.

Wavenumber, cm^{-1}	Fragment. vibration
431	ν_2 symmetric bending mode of $(\text{PO}_4)^{3-}$ [2]
581	ν_4 asymmetric bending mode of $(\text{PO}_4)^{3-}$ [2]
647	$\nu_4 (\text{PO}_4)^{3-}$ [2]
812	O–P–O stretch[16]
850	associated with proline[17]
874	$\nu_2 (\text{CO}_3)^{2-}$ [17]
955	ν_1 symmetric stretching mode of $(\text{PO}_4)^{3-}$ [2]
1004	phenylalanine [13]
1038,1169	ν_3 asymmetric stretching mode of $(\text{PO}_4)^{3-}$ [2]
1069	ν_1 symmetric stretching mode of type B $(\text{CO}_3)^{2-}$ [2]
1100	ν_1 symmetric stretching mode of type A $(\text{CO}_3)^{2-}$ [2]
1241,1271	Amide III (N-H) [2]
1435	CO_3 absorption of carbonate [17]
1567	amide II [17]
1660	Amide I (C = O) [2]
1741	C-O-C [17]

Since decay will damage the dental tissue organic matrix, which subsequently lose its fixing function mineral substrate, which leads to the formation of defect carious therefore been introduced for the diagnosis of the following factors:

$$D = I_{956}/I_{1069}$$

$$K = I_{1660}/I_{1241},$$

where I_i is the intensity of the line having the wavenumber i ; D is the proportionality factor of the variation of content of calcium carbonate ions in the tooth tissue (replacement of $(\text{PO}_4)^{3-}$ ions with $(\text{SO}_3)^{2-}$ B-type); K is the coefficient, characterizing the replacement of amide I with amide III.

The 2D dependences are plotted on Fig. 4.

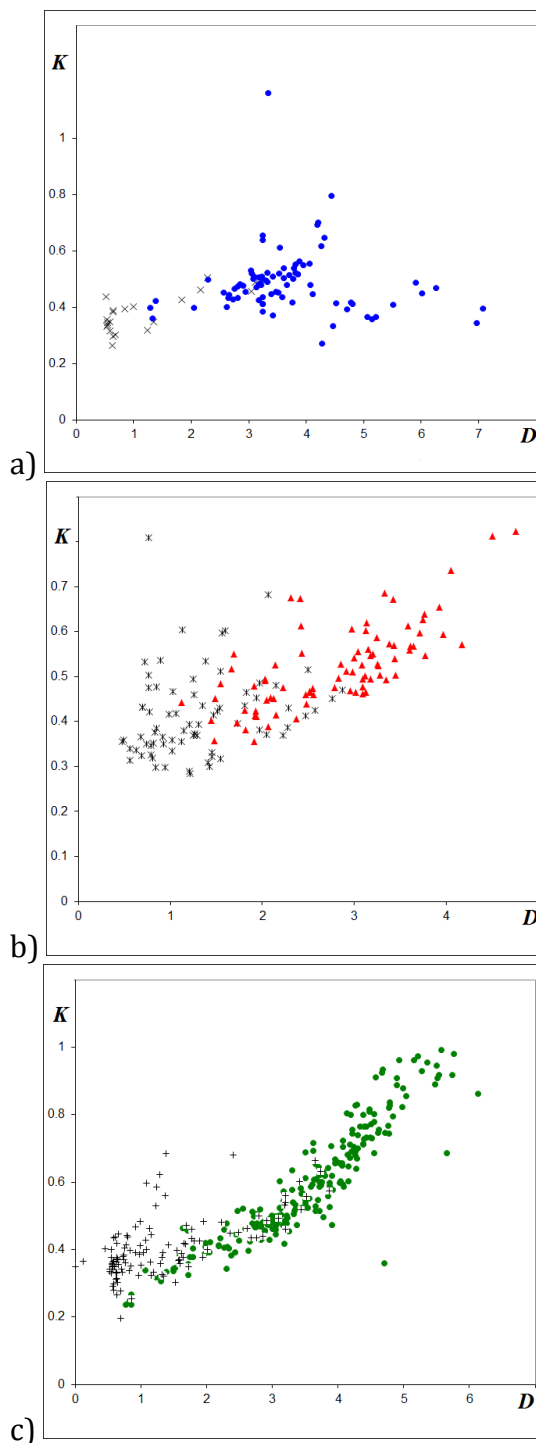


Fig. 4 2D presentation of the dependence of the coefficients D and K for the regions of (a) healthy enamel 1 and caries enamel 2; (b) healthy cement 1; (c) healthy dentin 1 and dentin with caries 2.

Figure 4 presents the 2D dependence of the coefficients D and K in different regions of the tooth tissues. It is seen that the caries is characterised by reduction of the optical coefficients D and K due to the decreased content of $(\text{PO}_4)^{3-}$ ions and amide I in the tooth tissue. From Fig. 4 (b, c) one observe the maximal relation of replacement of $(\text{PO}_4)^{3-}$ with $(\text{CO}_3)^{2-}$ (B-type substitution) with respect to the replacement of amide I

with amide III. In the case of pathological change like caries, the shift towards the B-type substitution occurs. Thus the 2D dependences in Fig. 4 allow the determination not only of the type of the tooth tissue, but also the presence of pathologic changes.

For control we used the method of raster electron microscopy (REM). Figure 5 presents the microphotographs of the surface structure for healthy and caries tooth tissue samples.

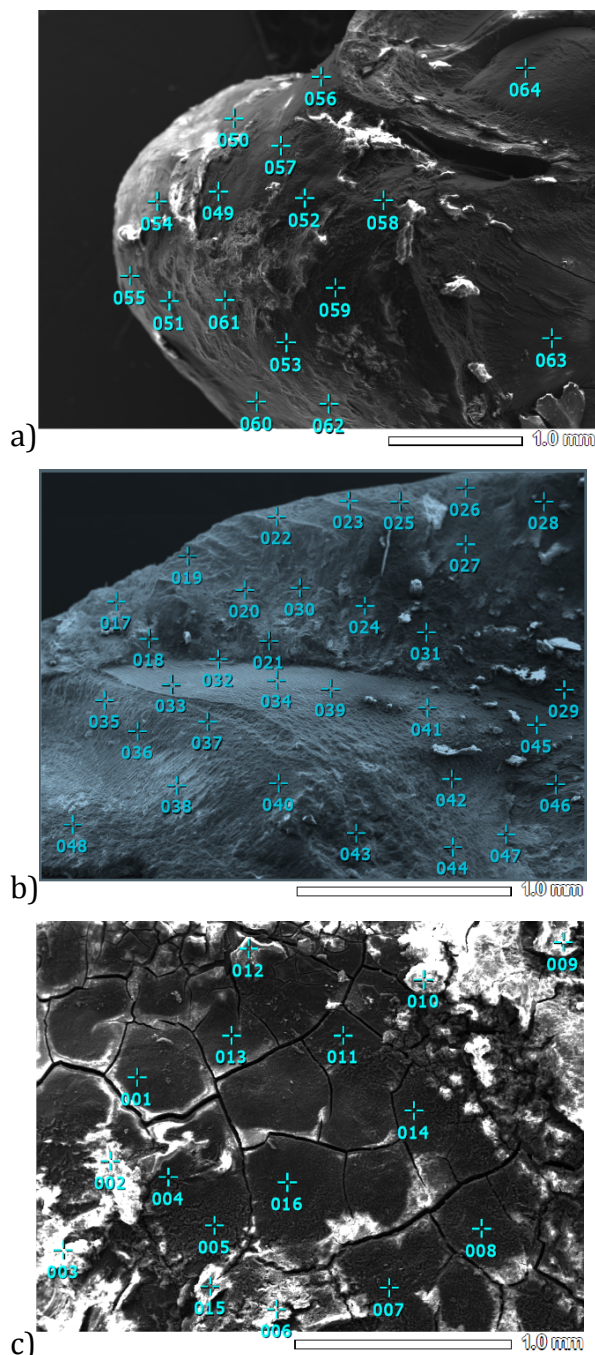


Fig. 5 Raster electron microscopy: a), b) - the healthy tooth tissue sections; c) - surface of the tooth with caries diagnosis.

According to the REM data on the element composition (Table 2) the caries samples are characterised by the reduced content of phosphorus and calcium and increased content of carbon and nitrogen, which confirms the obtained results and the reliability of the introduced coefficients.

Table 2 – REM data on the element composition.

	Enamel	Dentin	Cement	Dentin caries
C	29.49	33.07	50.72	59.63
N	1.22	3.34	6.50	13.71
O	37.42	37.15	18.22	17.38
Mg	0.17	0.42	0.92	0.04
P	10.99	8.83	2.38	2.38
Ca	16.62	13.12	4.34	4.72
I	2.42	1.94	0.73	0.75
Se	0.01	0.05	0.02	0.04
Br	0.52	0.73	15.62	0.05

4 Conclusions

We report the specific features of the Raman scattering spectrum for the healthy tooth tissues and those affected by caries. It is found that the caries is characterised by the change of the Raman spectrum at the lines with wavenumbers 956 cm^{-1} , 1069 cm^{-1} , corresponding to phosphate, and 1241 cm^{-1} , 1660 cm^{-1} , corresponding to amide III and amide I, respectively.

The 2D analysis of the introduced optical coefficients D and K is carried out that allowed the diagnostics of the beginning of the caries development in the hard tissues of the tooth. It is found that the caries is characterised by the reduction of $(\text{PO}_4)^{3-}$ ions content and the change of amide composition in the tooth tissue.

According to the REM data on the element composition, the caries samples are characterised by the reduced content of phosphorus and calcium and increased content of carbon and nitrogen, which confirms the obtained results and the reliability of the introduced coefficients.

Acknowledgments

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