In vivo Raman and autofluorescence study of the pigmented skin neoplasms

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Abstract. This paper studies classification of the pigmented skin neoplasms on the basis of the spectral Raman and autofluorescence features. The spectra registration was performed by means of the portable spectroscopic system with 785 nm laser radiation. The analysis of the spectral data was carried out using a projection on the latent structure with linear discriminant analysis. Using regression analysis the correlations between Raman and autofluorescence data were estimated. The variable importance in projection analysis allows one to find out important spectral information to differ spectra of the different neoplasm types. The use of combination of the Raman/autofluorescence data and projection method made it possible to classify pigmented skin neoplasms with 81% accuracy.

1. Introduction

Raman spectroscopy is useful technique to analyze total chemical composition of samples in different areas due to its ability to register a spectral “fingerprints” of molecules. Raman spectroscopy bases on the change of the excitation wavelength after the interaction with molecules of the tested sample. The registered spectrum allows one to determine chemical bonds in the sample. In particular, Raman spectroscopy is able to detect changes in biological tissues during malignization because of its sensitivity to the molecular composition [1, 2]. During the excitation of the skin neoplasms by the laser wavelength in the near-infrared region Raman and autofluorescence signals register simultaneously. So, the analysis of the entire registered tissue spectrum included Raman and autofluorescence signals allows one to analyze both biochemical and structural features of the different skin neoplasms [3].

However, there are not specific Raman and/or autofluorescence bands that correspond to the definite skin neoplasms. Moreover, the same chemical components contribute to the different spectral bands that causes analyzed spectral data have multiple correlations. For this reasons, to extract important differentiating spectral information it is need to use projection statistical methods. The progression on the latent structure with discriminant analysis (PLS-DA) is the most popular method for this task [4]. Set of the spectral data is the matrix consisting of the wavenumbers and corresponding intensity values for each spectrum. PLS method applies to the spectral matrix to build
The regression models identify the useful spectral data and separate them from less important data and dark noise.

The using spectroscopic methods in combination with statistical analysis allows one to differentiate different neoplasms types [5, 6]. In particular, the task of skin neoplasm differentiating is useful because of not effective enough tumor diagnostics in clinical application. In our previously work [7] we considered four differentiating models: malignant versus benign tumors, melanomas versus other tumors, melanomas versus basal cell carcinomas and basal cell carcinomas versus other tumors. In current work, we focused on the detection of spectral differences between pigmented skin neoplasms.

The studies of melanoma using optical noninvasive methods are presented in works [1, 8, 9]. Melanoma and benign pigmented neoplasms especially pigmented nevus have the similar external signs making them complicated to differ [10]. It should be noted that malignant melanoma is the most aggressive skin cancer that causes more than 76% death among all skin cancer cases. Taking into account importance the study of the pigmented tumors the aim of this work is to apply Raman and autofluorescence spectroscopy to collect informative spectral data for differentiating pigmented skin neoplasms using statistical method.

2. Materials and methods

2.1. Experimental setup

The experimental setup (Fig. 1) includes thermally stabilized diode laser module LML-785.0RB-04 (785±0.1 nm central wavelength) for excitation of Raman and autofluorescence signals of skin neoplasms in the near-infrared region (NIR). Laser radiation is delivered to the optical Raman probe InPhotonics, passes through the lens L1 and bandpass filter BPF, which cuts off Raman and fluorescence components of the optical fibers. Then, mirror M transmits laser radiation to the lens L2 which focuses exciting radiation onto the sample. The same L2 lens collects backscattering radiation of the skin sample. Dichroic mirror DM transmits only NIR components of the collected radiation, longpass filter LPF blocks the reflected excitation laser light from the tissue. By matching lens L3 and collection fibers (200 μm diameter, 0.22 NA) Raman and NIR fluorescence signals of the skin sample are focused and propagate to the portable QE 65 Pro spectrometer. The portable spectrometer cooled up to –15 °C allows one to register low-noise spectral signals. Spectra registration was carried out in the 780–1000 nm region with the spectral resolution of 0.2 nm. The each spectrum was acquired with 1.65 W/cm² laser density on the skin surface and 60 seconds exposure time.

Using experimental setup the 63 spectra of the different skin neoplasms (32 spectra of malignant melanomas (MM) and 31 – benign pigmented neoplasms: 11 melanocytic dysplasia, 20 pigmented nevi) were registered. The protocols of the in vivo experiment were approved by the ethical committee of Samara State Medical University.

![Figure 1. The scheme of the experimental setup.](image)

2.2. Data processing and statistical analysis of the spectral data

The registered spectra of the skin neoplasms are a combination of the Raman and autofluorescence signals in the NIR. The analysis of the spectral data was performed in the 804-914 nm that
corresponds to the 300–1800 cm\(^{-1}\). The registered spectra were preprocessed with baseline removal, smoothing by the Savitzky-Golay method, data centering and normalization by using standard deviation of a normal variate method (SNV). Data centering decreases the model rank by one, and is applicable in uniform model cases. The SNV method subtracts mean value from each spectrum and divides each signal value by the standard deviation of the whole spectrum. The SNV method is used for leveling the experimental data dispersion.

Because a priori information about belonging of the spectra to the neoplasm types is available it is need to meet a challenge of spectra classification with training. PLS-DA analysis allows one to build regression models that can determine on the basis of spectral differences what neoplasm type each spectrum belongs. The built regression is able to predict a belonging of the new spectra further. PLS method allows one to find the latent factors that describe covariations between dependent and independent variables. At the regression stage the value of the dependent variables are predicted using decomposition of the independent variables. The importance of the variables for the prediction ability of the model was determined using by variable importance in projection analysis VIP [11]. The higher VIP scores correspond to the more important variables. Thus, VIP scores allow one to find out informative spectral bands in regression model to classify different tumor types. On the basis of the highlighted spectral differences PLS-predictor values were calculated for each spectrum. The classification of the tumors was performed on the basis of distribution of the PLS-predictors by the linear discriminant analysis. The efficiency of the instrumentational diagnostics was expresses by the sensitivity and specificity calculated by comparing with the histological analysis of skin tumors in the clinics. Sensitivity is a proportion of the correctly classified melanomas among all melanomas while specificity is a proportion of benign pigmented neoplasms that are correctly identified among all benign pigmented neoplasms. The receiving operating curve analysis (ROC analysis) clearly demonstrates the sensitivity against 1–specificity on the plane. The area under the ROC curve is a numeric measure to estimate classifier performance. AUC ROC is independent of a threshold criterion at classification. The closer AUC ROC value to 1 the better classification.

The multivariate analysis of the spectral data was performed using TPTcloudbeta software (https://tptcloud.com).

3. Results and discussion

Figure 2 shows the typical melanoma and nevus spectra included Raman and fluorescence signals. The Raman spectra of the skin are characterized the presence of the peaks: 1248 cm\(^{-1}\) (Amide III and CH\(_2\) wagging vibration), 1290–1300 cm\(^{-1}\) (CH\(_2\) twisting and wagging of lipids; CH\(_2/CH_3\) bands in triolein), 1336 cm\(^{-1}\) (CH\(_2\), CH\(_3\) twisting in collagen), 1450 cm\(^{-1}\) (CH\(_2\) bond in proteins and lipids), 1573 cm\(^{-1}\) (in-plane stretching of the aromatic rings in melanin), 1645–1655 cm\(^{-1}\) (Amide I, v(C=O) in lipids, v(C=O) in proteins and lipids), 1745 cm\(^{-1}\) (v(C=O) in phospholipids).

The PLS model for differentiation between the melanomas (n=32) and melanocytic neoplasms spectra (n=31) was performed. The VIP analysis of this PLS model are presented in Figure 3. According to the intensity of the VIP scores the most important spectral differences of this model are observed in the 800–860 nm region. In this region autofluorescence background overlaps weak Raman signal [2] because spectra of the pigmented skin tumors characterized by the high level autofluorescence of the melanin in the near infrared region [12]. So, the high level of the autofluorescence intensity are not important diagnostics parameter to differentiate pigmented skin neoplasms between them. The important VIP scores in autofluorescence region (300–1200 cm\(^{-1}\)) are associated with spectral features of the autofluorescence curve. Autofluorescence curves of melanoma and pigmented neoplasms characterize an evident wide local maxima around 475, 740, 915 cm\(^{-1}\) and minima around 645, 1100 cm\(^{-1}\) in comparison with the more monotonic autofluorescence curve of the nonpigmented skin area (normal skin, basal cell carcinoma, benign neoplasms). Thus, highlighted spectral differences can be associated with different concentration of melanocytes cells in the studied skin neoplasms.

The VIP scores in the 1200–1800 cm\(^{-1}\) region are less significant in this classification. However, it should be noted, VIP scores in this region correspond to the tissue Raman peaks: 1300, 1550, 1650 cm\(^{-1}\). It is interesting to note that differences between melanoma and benign pigmented neoplasms are
not associated with changes in the key tissue Raman peaks 1450 cm\(^{-1}\) and Raman peaks 1573 cm\(^{-1}\) reflected melanin contribution.

Figure 2. The registered spectra of melanoma and pigmented nevus.

Figure 3. VIP analysis of the differentiating between melanoma and benign pigmented neoplasms.

Figure 4 shows classification by the linear discriminant analysis of the melanoma and benign pigmented neoplasms on the basis of the PLS-predictors calculated for the each spectrum. According to the distribution of the PLS-predictors sensitivity and specificity for this classification model are 84\% and 77\% respectively. ROC analysis presented in Figure 5 shows ROC AUC of this classification is equal to 0.91.

Figure 4. Box-plot analysis of the melanoma and pigmented neoplasms classification.  
Figure 5. ROC analysis of the melanoma and pigmented neoplasms classification.

In our recent work [13] differentiating accuracy of the melanoma and pigmented nevi does not exceed 52\% on the basis of analysis only autofluorescence signal. In this work, we propose joint autofluorescence and Raman spectroscopy in the near-infrared region to optimize the efficiency of the differentiating pigmented skin neoplasms. We achieved 81\% accuracy to classify melanoma from benign pigmented skin neoplasms using multivariate analysis of the AF and Raman features. In contrast, Cicchi et.al. [3] using diagnostic capability of the NIR Raman and AF spectroscopy achieved
56% sensitivity and 89% specificity for classification melanoma and melanocytic nevi. They improved diagnostic capability to 89% sensitivity and 100% specificity due to the combination of the several techniques: 378 nm fluorescence, 445 nm fluorescence, 785 nm fluorescence – Raman).

4. Conclusions
The combined analysis of the Raman and autofluorescence spectroscopy of the pigmented skin neoplasms using PLS method is sensitive to the wide set of the changes of the structural tissue components. Due to the high concentration melanocytes cells in the pigmented skin neoplasms autofluorescence by melanin significantly overlaps the contribution of the other skin chromophores to the spectrum. Thus, proposed approach allowed us to achieve 81% accuracy in differentiation of melanoma and benign pigmented tumors.

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