Analysis of correlation between Raman and autofluorescence human skin response in visible and NIR region

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Abstract. In current study we used Raman spectroscopy and autofluorescence analysis in visible and NIR region for the analysis of human skin spectral characteristics in the presence of various influencing factors. We performed the comparative research of Raman experimental data and visible autofluorescence analysis results. The processing of experimental data was performed on the bases of regression analysis. We estimated correlations between Raman and autofluorescence signals and also find informative Raman and autofluorescence bands that may be used as predictors of general condition of the body. The use of Raman spectroscopy of humanskin made it possible to forevealkidney failure with a diagnostic accuracy more than 90%. When constructing the regression for analysing the correlation of Raman spectral features of skin in the NIR and the arithmetic average of the fluorescence intensity of the visible region $R^2=0.69$. A combined skin analysis based on Raman and fluorescence in the visible region may improve the performed skin analysis.

1. Introduction

The study of the advanced glycation end-products (AGEs) has a long-term history. In 1910, the French scientist and physician Louis Camille Mayard began research on the reactions between amino acids and sugars in the food industry [1]. In the 1980s, it was found that the protein-sugar reactions (so-called glycation) occur in the living body. The result of the glycation reaction is the AGEs formation. They affect almost every type of cells in the body and are considered one of the aging factors and some chronic diseases associated with aging. Proved that AGEs accumulation in tissues has a dependency with the development of atherosclerosis [2], diabetes [3], kidney damage [1], eye [1, 4] and nervous system.

The investigation of aging processes and aging-associated disease process (such as type II diabetes, arterial hypertension, atherosclerosis and kidney disease) demonstrated the accumulation of modified proteins and lipids in the vascular wall, tissues of vital organs. The human skin is a kind of
products accumulator, including AGEs, resulting from the processes occurring in the body. At the same time, the skin is affordable, and therefore it is competitive for in vivo diagnosis.

Consequently, the state of body internal homeostasis, namely various physiological and pathological conditions of internal organs - the endocrine system, the digestive tract, the nervous, hematopoietic, cardiovascular and other systems, are closely related to the skin condition and affect its component composition. Changes in the skin biochemistry are a representation of the human body internal state. Therefore, analysis of changes in the composition of human skin different layers is part of therapeutic disciplines. In addition to the laboratory analysis methods used today, various physical methods can be successfully used to study the biochemistry of the human skin [5]. Methods of Raman spectroscopy and autofluorescence analysis can detect changes in the skin component composition at the molecular level. Therefore, in our study we utilized a combination of Raman spectroscopy and autofluorescence analysis in the visible and near infrared region to analyze the spectral characteristics of the human skin in the presence of various influencing factors.

Optical diagnostic methods are instrumentally well-equipped, operationally simple, cost-effective, highly sensitive, and have been used for more than half a century in dermatology for diagnosis. When experimental confirmation of the efficiency of AGEs fluorescence excitation in the near ultraviolet (UV) spectral range and the relationship of the AGEs accumulated in the skin with its fluorescence was obtained [6], the scope of in vivo skin optical studies for diagnostic purposes went beyond the framework of dermatology; and the optical diagnostics itself the skin began to be intensively introduced into the medical practice of various specialties doctors. It should be noted that the optical control of the AGEs content in the skin assumes only the spectrum registration, i.e. does not require additional costs and traumatizing patient procedures, and the such analysis takes no more than 3-5 minutes.

Therefore, the aim of this work is to investigate the skin spectral features in the visible and near infrared region at various influencing factors, such as age and pathological conditions.

2. Materials and methods

2.1. Portable fluorimeter for skin analysis

Register skin AF spectra under investigation realized by the portable fluorimeter for medical purposes the optical scheme of which is shown in Figure 1. The excitation AF of AGEs products is performed to ultraviolet (UV) EOLD-365-525 LED radiation with a peak wavelength of 365 nm, which passes through the wavelength filter of the optical color glass UFS6 with a 2 mm thickness and cover glass (a microscope slide 1 mm thick). The purpose of the wavelength filter installed in the exciting branch of the optical circuit is to suppress the parasitic long-wave radiation, the spectrum of which is superimposed on the AF skin spectrum [7]. The inner side of the forearm – the tested fluorescing object - is applied to the cover glass from the outside. The fluorescence measurement geometry is assumed to be 45°/0°. A part of the skin-scattered exciting and fluorescent radiation falls on the silicon photodiode BPW21R through a cut filter FGL435 from Thorlabs made of glass GG435 Schott Glass 2 mm thick. The other part of the scattered radiation falls on the photodiode SFH 229, which is part of theelastic scattering channel. All elements of the optical scheme are placed inside the light-proof metal casing. Inside the casing there is an electronic circuit board.

The fluorometer diagnostic parameter is the ratio of the output signals of the AF measuring channel and the elastic scattering.

\[
AUF = k \left( \frac{I_f}{I_b} \right),
\]

where \( k \) - the proportionality coefficient, \( AUF \) - fluorometer diagnostic parameter, \( I_f \) - output signal of AF measuring channel, \( I_b \) - elastic scattering output signal.

Integration an elastic scattering channel and selecting the signal ratio as a diagnostic parameter is necessary to compensate for individual differences in the skin optical properties.
2.2. Raman spectroscopy for skin analysis

In order to make studies of the skin state by optical methods as informative as possible, it is required to add complement chromophores to the study of AF in the visible region. Such as study can be carried out using RS.

To study the spectral characteristics of the skin using RS, the experimental setup shown in Figure 2 was utilized. The detected signal was decomposed into a spectrum using spectrometer Ocean optics QE65Pro. Excitation of the collected spectra was carried out by radiation from the LuxxMaster Raman Boxx laser module from PD-LD (central wavelength 785 nm). Raman probe RPB785 from InPhotonics allows for focusing the exiting radiation, collecting and filtering the scattered radiation. The spectra were recorded in the spectral range 780-1050 nm. The exposure time was 20 seconds. Consequent recording of three spectra for each sample was performed, the final spectrum was constructed by averaging all three recorded spectra.

2.3. Skin samples and experimental preparation

In this study, in vivo samples of human skin were investigated. The 350 volunteers aged 16 to 86 years were enrolled in the study (55 patients with kidney insufficiency, 84 volunteers without systemic diseases and 211 patients with benign and malignant skin tumour). The performed studies were approved by the ethical committee of Samara State Medical University.
The processing of experimental data were performed on the bases of regression analysis. Prior to regression analysis, the raw spectral data were centered, smoothed by the Savitsky-Golay filter, and normalized 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 [8]. The analysis of experimental data were performed by PLS-DA method for pathology determination and PLS for comparative research of the Raman data with autofluorescence analysis results. Definition of spectrum informative bands during the regression model constructing was performed by the analysis of the variable importance in projection (VIP) [9]. Multivariate analysis was carried out with using the TPTcloudbeta software module [10].

3. Results and discussion

There are many factors, such as the life quality, the past diseases, the immunity state, etc., during life affecting the skin condition of a person, provoking changes in its biochemical parameters. In study [11], the authors demonstrated and described the approach to the skin component composition analysis of different ages people. The authors using the Raman microscopic system analyzed 5 skin samples and described the age features of each sample. The proposed approach is asserted as an opportunity to accurately determine a person's age from the features of the Raman skin spectra. In current study the correlation of the 350 volunteers spectral characteristics and their ages is analyzed. This spectra set was a subject to the multivariate analysis for constructing regression model. The PLS analysis of the experimental data obtained utilizing the experimental setup without a microscopic system allows for detection that the correlation coefficient of skin Raman spectral characteristics and age was 0.47. Figure 3 shows the VIP-scores of Raman spectra matrix of skin samples for the constructed regression model [11].

![Figure 3. VIP-scores of Raman spectra matrix of skin samples. Correlation of age and spectral characteristics. Correlation coefficient R²=0.47.](image)

Analysis of Figure 3 allows to identify the most informative spectral bands (480-530 cm⁻¹), (1500-1560 cm⁻¹) in constructing the regression model for determining age-related changes in skin. The next phase of the current study was the analysis of the skin spectral features in the presence of pathologic behavior in human body, such as kidney failure. Multivariate analysis of experimental data allowed to construct a regression model for discriminating kidney failure from the volunteers group without
systemic diseases. The regression model enables to reveal kidney failure with a diagnostic accuracy, specificity and sensitivity at the level of 0.98, 0.98 and 1, respectively. It should be noted that the AGEs accumulation is associated with various pathological processes, including various tumors development. The refore for assess the specificity of this analysis and determination of the most informativespectral bands for kidney failure, there were constructed a regression model for discriminating kidney failure from group consisting of patients with skin neoplasms and volunteers without systemic diseases. VIP-scores of skin Raman spectra matrix and themultivariate analysis results are presented in Figure 4[11]. The constructed regression model enables to discriminate the kidney failure from patients with skin neoplasms and volunteers without systemic diseases withcharacteristics shown in Figure 5.

Figure 4. VIP-scores of Raman spectra matrix of skin samples. Model for identifying kidney pathologies among the entire sample.

Figure 5. Characteristics of constructed regression model for discrimination of the kidney failure from patients with skin neoplasms and volunteers without systemic diseases.

Presently, the utilization of skin fluorescence analysis for diagnostic purposes is progressing in medicine. For example, study of the skin fluorescence level in the range of 420-600 nm allows for detection of the changes in the AGEs concentration [12]. We analyzed the correlation of the Raman
characteristics and fluorescence in the visible region. The correlation coefficient in the construction of the regression for analyzing the correlation of Raman spectral features of skin in the NIR and the arithmetic average of the fluorescence intensity in the visible region was $R^2=0.69$. There is no significant correlation. Moreover, the human skin is a multi-component biological tissue; and the various skin chemical compounds may have partially overlapping spectra. Therefore, the evaluation of the correlation between the AF analysis results in the visible region and the Raman spectral characteristics allowed for inference that the combined study will expand the analysis of the human skin component composition and the description of the various chromophores spectral contribution. The correlation coefficients, diagnostic accuracy, specificity and sensitivity of constructed regression models are presented in Table 1.

### Table 1. Characteristics of the constructed regression models.

| Analysis                                      | Correlation coefficient | Sensitivity (%) | Specificity (%) | Diagnostic accuracy (%) |
|-----------------------------------------------|-------------------------|----------------|-----------------|-------------------------|
| Correlation of age and Raman                  | 0.47                    |                |                 |                         |
| Discriminating kidney failure from the volunteers group |                        | 100            | 98              | 98                      |
| Discriminating kidney failure from group consisting of patients with skin neoplasms and volunteers |                        | 92             | 90              | 90                      |
| Correlation of Raman and AF                  | 0.69                    |                |                 |                         |

4. Conclusion

Separated and studied features of the Raman and AF spectra of the skin can be the basis of methods for analyzing the physiological processes of the human body. Raman spectroscopy, based on changes in the tissue biochemical composition, demonstrate effective results in determining kidney pathology without injury and invasive. Therefore, the introduction of kidney failure screening by Raman skin analysis allow more accurate identification of patients in need of further treatment. A comparative study of the Raman experimental and the results of AF analysis in the visible region was performed. The correlation between Raman and AF signals was estimated in our work, as well as we identified Raman band, which can be used as predictors of the organism general state. The use of Raman spectroscopy of human skin made it possible to reveal kidney failure with a diagnostic accuracy more than 90%. When constructing the regression for analyzing the correlation of Raman spectral features of skin in the NIR and the arithmetic average of the fluorescence intensity in the visible region $R^2=0.69$. A combined skin analysis based on Raman and fluorescence in the visible region may improve the performed skin analysis.

5. References

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