Differential diagnosis of paraffin-embedded tissues by IR-THz spectroscopy and machine learning

Yury V. Kistenev[1,3,*], Alexey V.Borisov[1,3], Anastasia I. Knyazkova[1,5], Viktor V.Nikolaev[1,5], Alica A. Samarinova[1], Nikita A. Navolokin[4], Daria K. Tuchina[1,2], Valery V. Tuchin[1,2,6]
[1]National Research Tomsk State University, Russian Federation;
[2]Saratov National Research State University, Russian Federation;
[3]Siberian State Medical University, Russian Federation;
[4]Saratov State Medical University, Russian Federation;
[5]Institute of Strength Physics and Materials Science SB RAS, Russian Federation;
[6]Institute of Precision Mechanics and Control of the RAS, Russian Federation;

ABSTRACT

The ability of diagnostics of melanoma and nevus based on spectral analysis of paraffin-embedded tissues in the 0.3-1.5 terahertz (THz) range has been carried out. The principal component analysis was applied to reduce the dimension of the feature space. A comparison of these spectra shows evident differences between samples.

The possibility of applying the optical clearing to paraffin-embedded tissue for improving the visualization of the internal structure of tissue for diagnostic and research purposes is shown. In the studies, a sufficiently strong effect of optical clearing of paraffin-embedded muscle was obtained (63%).

Keywords: paraffin-embedded tissue biopsy, cancer, MPM microscopy, THz spectroscopy, optical coherence tomography, tissue optical clearing, machine learning

INTRODUCTION

Annually, 2 million cases of non-melanoma skin cancer and more than 100 thousand cases of malignant skin melanoma are diagnosed in the world. Melanoma is a skin tumor characterized by a high degree of malignancy, a high level of metastasis and relapse, poor prognosis and high mortality. Correct and rapid diagnosis of melanoma is one of the decisive factors for the successful treatment of the patient and helps to reduce the likelihood of death significantly. The gold standard for cancer diagnosis is a histological analysis of tissue biopsy. Typically, pathologists visually assess histopathology slides using conventional microscopes, camera-equipped microscopes, etc. But similar estimation is time-consuming and may be biased. Recently, there is a fast development of computer-aided detection systems, based on the applications of artificial intelligence and computer vision, for interpretation of medical images.

There are restrictions on the direct computer vision analysis of histological slides. Thus, information about the spatial distribution of various molecular biomarkers (often called by chemical biopsy) is very important for more detail evaluation of pathology.

Methods based on IR and THz spectroscopy do not affect on tissue samples when the radiation incident on the sample has low power.[1],[2] IR spectroscopy is based on the absorption of infrared light by vibrational transitions in covalent bonds; the characteristic spectral features correlate with the biological properties of the sample. Thus, useful diagnostic information can be extracted from IR spectra for various pathologies.[1] IR spectroscopy allows obtaining spatially resolved chemical and structural information of a tissue sample; therefore, there is no need to stain samples and add chemical reagents.

Multiphoton microscopy is a kind of IR spectroscopy and imaging. This method is one of the options for laser scanning microscopy in which fluorochromes are excited by laser radiation in the IR or short-wavelength range.

*yuk@iao.ru; phone +7-913-828-6720

Tissue Optics and Photonics, edited by Valery V. Tuchin, Walter C. P. M. Blondel, Zeev Zalevsky, Proc. of SPIE Vol. 11363, 113630J · © 2020 SPIE
CCC code: 0277-786X/20/$21 · doi: 10.1117/12.2555632

The multiphoton microscopy is useful for three-dimensional visualization of subcellular structures and their changes with a high spatial and temporal resolution.[3] The structure of collagen and elastin, which well describe the condition of the skin, can be detected using the FLIM methods, second harmonic generation, and autofluorescence.[3]

Cell type identification can be achieved on formalin-fixed paraffin tissues, which gives access to a large bank of tumor tissue and allows direct comparison with gold standard histopathological procedures. Shown the ability of IR microspectroscopy to distinguish nevus from melanomas using sections of paraffinized tissue without prior dewaxing.[4]

THz spectroscopy, along with IR spectroscopy, is a promising method for the study of cancer tissues. THz waves have very low ionization possibility on cells, but strong absorption by water that limits their penetration in tissue. The content of various chemical compounds changes, such as tryptophan amino acids, and also structural changes in the affected areas of the skin were analyzed in the THz range.[2],[5] Skin cancer cells absorb more THz wave energy than healthy cells, which have a higher refractive index, absorption coefficient, and permittivity in the THz range, thereby demonstrating that the THz imaging can be useful for distinguishing cancer and healthy tissues, including those embedded in paraffin.[5],[6],[7] Due to the use of the THz time-domain system, the opportunity was realized not only to distinguish pathological tissue from healthy tissue clearly but also to identify differences between tumor tissues damaged by various degrees of adenocarcinoma.[8],[9]

The possibility of THz imaging of dehydrated tissue of malignant melanoma of the skin based on multiscale, multi-azimuthal and multi-structural mathematical morphology of elements is shown to reduce the effect of noise on edge detection and provide a relatively accurate definition of areas of normal and cancerous tissue.[10] A significant difference was found between sub-THz uptake in melanoma and nevus tissue embedded in paraffin.[11]

Typically, the penetration depth of IR and THz radiation into biological tissue does not exceed a couple of millimeters; therefore, increasing the penetration depth of radiation is an urgent task when conducting such studies.

One of the simple and effective methods for solving the problem of increasing the depth and quality of visualization of the interstitial structure, as well as improving the accuracy of spectroscopic information from the deep layers of the tissue, is a temporary decrease in light scattering by the tissue.[12],[13]

Most methods that reduce tissue scattering are based on aligning the values of the refractive index of tissue components due to the diffusion of the immersion agent with a specially selected value of the refractive index.[14],[15]

The tissue optical clearing method was proposed to increase the depth and resolution of optical diagnostic methods, such as optical coherence tomography, fluorescence and THz imaging.[15],[16]

The use of optical clearing agents can be especially important for improving two-photon imaging[17], since it was shown that the effect of light scattering greatly reduces the depth of light penetration to values smaller than the depth of fluorescence of a single photon, while the resolution usually remains unchanged.[18] Improving the depth of two-photon imaging using the tissue optical clearing method using hyperosmotic agents, such as glycerin, occurs mainly due to the combined effect of reducing scattering in the surface layers of a tissue sample, which reduces attenuation of the incident and detected radiation.[14]

We plan to discuss the abilities of automatic differential classification of the paraffin-embedded tissue samples using a combination of IR-THz molecular imaging, samples optical clearing, and machine learning.

MATERIALS AND METHODS

Paraffin-embedded, both melanoma (n=15) and nevus (n=13) tissue biopsy we analyzed in work were from the biobank of the Tomsk Cancer Research Institute. THz absorption spectra of paraffin-embedded tissue skin samples were measured using of Time-domain THz spectrometer (EKSPLA, Estonia) with tuning range 0.3-1.5 THz. Spatial 2D scanning of each paraffin-embedded tissue sample was performed with a step of 0.1 mm in vertical and horizontal directions. This step value is much smaller than THz beam diameter (about 4.2 mm) that ensures the ability to average random spatial fluctuations of the THz signal. The averaging over 128 spectrum scans in every spatial point also was used to improve the signal to noise ratio. The data obtained contain information on both the structure and spectroscopic characteristics of the sample.

The optical properties of paraffin sections of biological tissues were studied using an MPTflex two-photon tomography from JenLab (Germany) with a tunable titanium-sapphire femtosecond near-infrared laser (760 nm). Images 90 × 90 μm

in size are recorded with a 512 × 512 pixel detector array. The measurements were carried out in two stages: without an optical clearing agent and with the use of glycerin. At each stage, the paraffin block was scanned at 10 different spatial points; depth scanning was performed at each point (10 scans with a step of 5 μm).

Optical coherence tomography (Thorlabs Inc., USA) at a wavelength of 930 nm with the spectral band of 100 nm at 2 mW, scanning depth (in the air) - 1.6 mm, in-depth spatial resolution - 6.2 μm (on the air) was used as the reference method for estimation of the efficiency of optical clearing. The recording of OCT tomograms from the studied area of the sample was carried out before applying optical clearing agent to the surface of the sample, and then during the action of the agent (every 5 min) within 70 min. In these studies, propylene glycol (99.9%, Reactive, Russia) was used as an optical clearing agent.[19],[20],[21] The refractive index of the solution was measured using a multi-wave Abbe refractometer (Atago, Japan) at a wavelength of 930 nm as 1.4244 with the accuracy of ± 0.0002. The recorded OCT tomograms were used to calculate the kinetics of the light attenuation coefficient in the paraffin-embedded muscle in a region of 0.75 mm deep from the surface of the sample.

The time dependences of the light attenuation coefficient in the muscle were used to estimate the degree (*A*) of the optical clearing of the muscle under the action of propylene glycol on it, the kinetic curves were approximated by the equation[22]:

$$\mu_{norm}(t) = \frac{\mu(t)}{\mu(t=0)} = A \cdot \exp\left(-\frac{t}{\tau}\right) + y_0, \quad (1)$$

where $\mu(t=0)$ и $\mu(t)$ – the light attenuation coefficients in the sample at times t = 0 and t respectively; y_0 – residual value of $\mu_{norm}(t)$, which can be achieved.

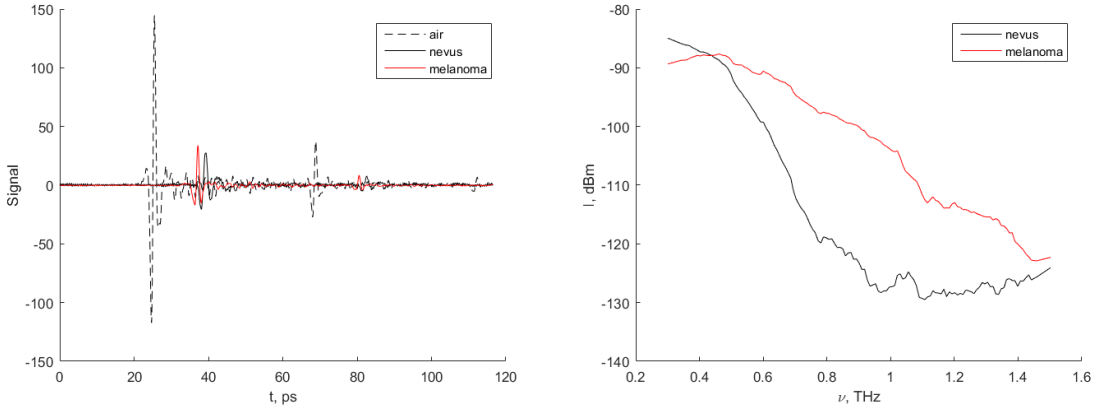
The efficiency of the muscle optical clearing was estimated as the ratio of the difference between the minimum and initial values of the light attenuation coefficient in the tissue to the initial value of it:

$$OC_{eff} = \frac{\mu_{t_0} - \mu_{t_{min}}}{\mu_{t_0}} \cdot 100\%.$$

We used the Principal Component Analysis (PCA) to estimate the spatial distribution of the paraffin-embedded samples under investigation, where the THz absorption spectra of paraffin blocks serve as feature vectors.[23],[24]

RESULTS

Fourier transform was used for each time signal (Figure 1a) to obtain the absorption spectrum (Figure 1b). The latter is represented in the range from 0.3 to 1.5 THz since signal-noise ratio was large enough in this range



a b

Figure 1. The example of the time signal (a) and corresponding absorption spectrum (b) for the paraffin block with nevus and melanoma tissues.

Preliminarily, the THz absorption spectra of the samples were smoothed using the Savitsky-Golay filter. For a set of spectra of each paraffin block, the procedure for selecting informative regions was carried out.[8] The PCA was used to reduce the dimension of the feature space. Figure 2 shows the projection of the objects understudy on the subspace of the first and fifth principal components. There is a good enough spatial separation of samples into two groups corresponding to melanoma and nevus tissues.

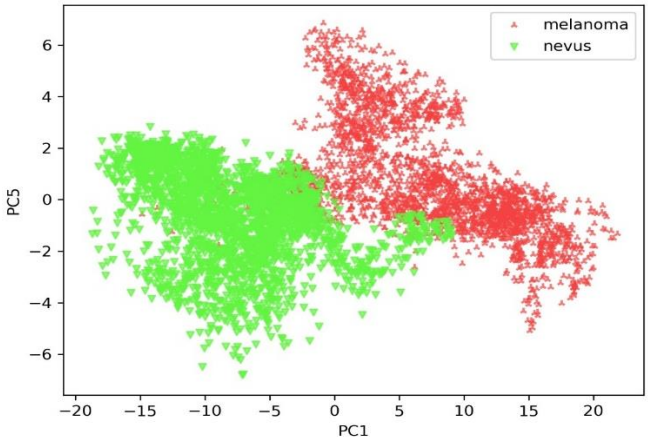
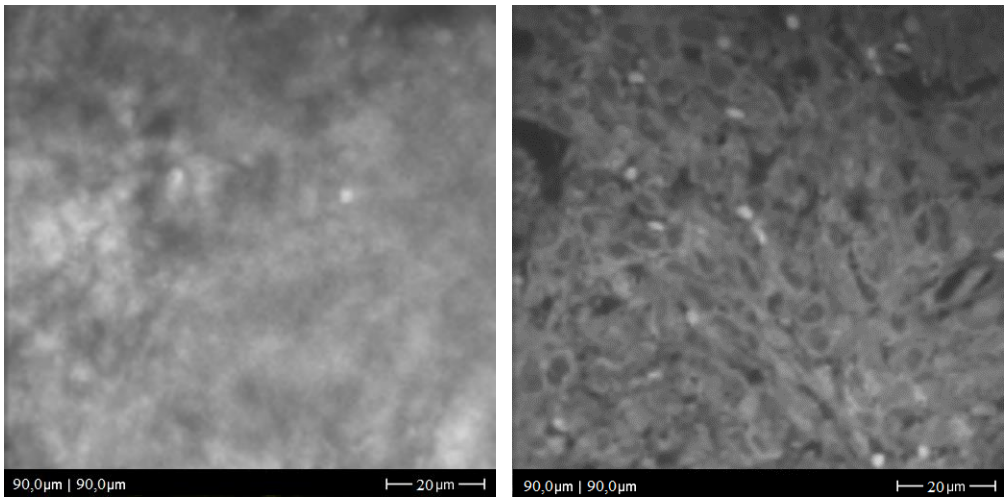


Figure 2. Projection of the studied absorption spectra of tissues with melanoma and nevus on the subspace of the first and fifth principal components.

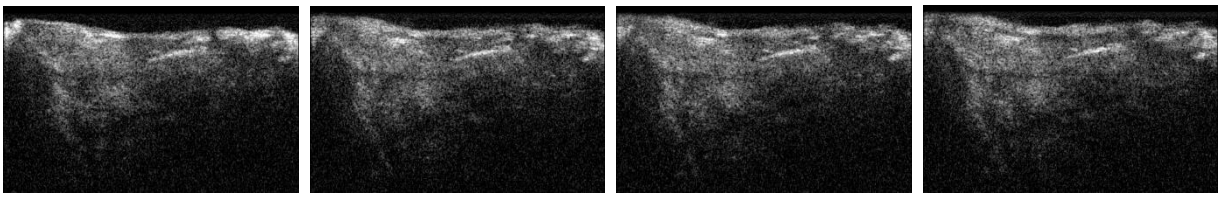
Figure 3 shows fluorescence images for a paraffin block with tissues without an optical clearing agent and after applying glycerin, to the surface of the block. From figure 3a, in the case of measuring a paraffin block without the use of an optical clearing agent, one can see the glow of individual tissue components; however, the structure of the tissue is poorly distinguishable. Figure 3b shows that after the action of glycerol on the surface of the paraffin block, the penetration depth of the laser beam increased and, as a result, the characteristic spatial structures of the biological tissue became visible.



a b

Figure 3. An example of a fluorescence image of a paraffin block with melanoma a) without an optical clearing agent, b) after glycerol is used.

Figure 4 shows typical OCT tomograms of muscle before and during exposure to propylene glycol. It can be seen an increase in the probing depth of the sample within the measured area during the impact of the solution on the tissue surface. The heterogeneity of the muscle structure that was not initially visible becomes more visible.



a b c d

Figure 4. OCT tomograms of paraffin-embedded muscle before the impact of propylene glycol (a), during 10 (b), 20 (c), 30 (d) min of exposure.

Figure 5 shows the dependence of the light attenuation coefficient in muscle tissue on the time of optical clearing by propylene glycol, which shows a fast decrease of the light attenuation coefficient in the tissue under the agent impact.

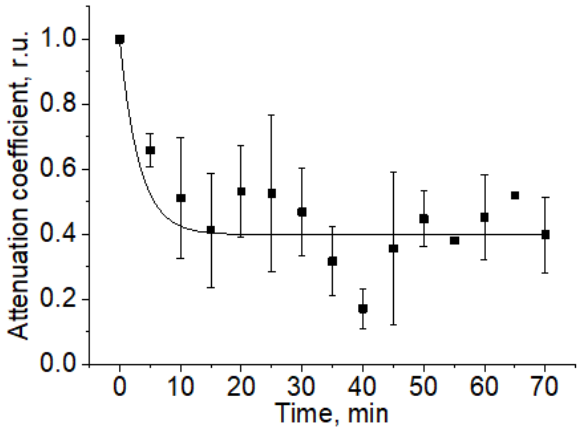


Figure 5. The dependence of the light attenuation coefficient in paraffin-embedded muscle tissue on the time of optical clearing by propylene glycol.

The characteristic efficiency of optical muscle cleaning under the influence of propylene glycol, obtained from the time dependence of the coefficient of light attenuation in muscles, is 63%. The decrease of the light attenuation coefficient in the tissue indicates the decrease of the light scattering. In tissues in vitro, ex vivo, in vivo, this is explained by an increase in the optical homogeneity of the tissue due to decrease in the difference between the refractive indices of the tissue components by partial dehydration of the tissue and the penetration of the optical clearing agent into the tissue.[22] The preparation of a paraffin-embedded tissue sample includes dehydration and fixation of the tissue, which implies a certain limitation of the optical clearing of the sample. Nevertheless, in the conducted studies a sufficiently strong effect of optical clearing of paraffin-embedded muscle was obtained (63%). The effect is probably caused by the outflow of residuary water from the tissue.

Thus, the ability of diagnostics of melanoma and nevus based on spectral analysis of paraffin-embedded tissues in the THz spectral range has been carried out. The principal component analysis was applied to reduce the dimension of the feature space. A comparison of these spectra shows evident differences between samples.

The possibility of applying the optical clearing to paraffin-embedded tissue for improving the visualization of the internal structure of tissue for diagnostic and research purposes is shown.

ACKNOWLEDGEMENTS

The work was carried out under partial financial support of the Russian Foundation for Basic Research (grants17-00-00186 and 17-00-00272 (17-00-00275 (K)) and 18-42-703012).

This work was performed within the frame of the Fundamental Research Program of the State Academies of Sciences for 2013-2020, line of research III.23.

We appreciate to Nikita Navolokin (Saratov State Medical University) for the preparation of paraffin-embedded tissue samples.

REFERENCES

[1] Benarda, A., Desmedt, C., Durbecq, V., Rouas, G., Larsimont, D., Sotirioub Ch., and Goormaghtigh, E., "Discrimination between healthy and tumor tissues on formalin-fixed paraffin-embedded breast cancer samples using IR imaging," Spectroscopy 24 (2010) 67–72, DOI 10.3233/SPE-2010-0406.

[2] Yu, C., Fan, Sh., Sun, Y., and Pickwell-MacPherson, E., "The potential of terahertz imaging for cancer diagnosis: A review of investigations to date," Quant. Imaging Med. Surg. 2, 33-45 (2012). DOI 10.3978/j.issn.2223-4292.2012.01.04.

[3] Shirshin, E. A., Yakimov, B. P., Darvin, M. E., Omelyanenk, N. P., Rodionov, S. A., Gurfinkel, Y. I., Lademann, J., Fadeev, V. V., and Priezzhev, A. V., "LabelFree Multiphoton Microscopy: The Origin of Fluorophores and Capabilities for Analyzing Biochemical Processes," Biochemistry (Moscow), 84(S1), 69–88 (2019). DOI 10.1134/s0006297919140050.

[4] Tfayli, A., Piot, O., Durlach, A., Bernard, P., and Manfait, M., "Discriminating nevus and melanoma on paraffin-embedded skin biopsies using FTIR microspectroscopy, " Biochim. Biophys. Acta. 1724(3), 262-269 (2005). DOI 10.1016/j.bbagen.2005.04.020.

[5] Nazarov, M.M., Shkurinov, A.P., Kuleshov, E.A., and Tuchin, V.V., "Terahertz time-domain spectroscopy of biological tissues," Quantum Electronics 38 (7) 647 - 654 (2008). DOI 10.1070/qe2008v038n07abeh013851.

[6] Meng, K., Chen, T., et al., "Terahertz pulsed spectroscopy of paraffin-embedded brain glioma," JBO 19(7), 077001 (2014). DOI 10.1117/1.JBO.19.7.077001.

[7] Wahaia, F., Kasalynas, I., et al., "Terahertz absorption and reflection imaging of carcinoma-affected colon tissues embedded in paraffin," Journal of Molecular Structure 1107, 214-219 (2016). DOI 10.1016/j.molstruc.2015.11.048.

[8] Knyazkova, A.I., Borisov, A.V., Spirina, L.V., Kistenev, Yu.V. "Paraffin-embedded prostate cancer tissue grading using THz spectroscopy and machine learning," Journal of Infrared, Millimeter, and Terahertz Waves (2019). DOI 10.1007/s10762-020-00673-7.

[9] Kistenev, Yu.V., Borisov, A.V., Knyazkova, A.I., et al., "Possibilities of cytospectrophotometry of oncological prostate cancer tissue analysis in the TGz spectral range," Proc. SPIE 10614, 106141T (2018). DOI 10.1117/12.2303625.

[10] Jiayu, L., Yijun, X., Ping, S., "Edge detection on terahertz pulse imaging of dehydrated cutaneous malignant melanoma embedded in paraffin, " Front. Optoelectron. 12(3), 317–323 (2019).

[11] Mitobe, K., et al., "Imaging of Epithelial Cancer in Sub-Terahertz Electromagnetic Wave," Proc. of IEEE 1, 199-200 (2005).

[12] Tuchin, V.V., [Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis] 3rd ed., Bellingham, WA, USA: SPIE Press, PM 254, 988 (2015).

[13] Tuchin ,V.V., "A clear vision for laser diagnostics Review," IEEE J.Sel. Top. Quantum Electron 13, 1621-1628 (2007).

[14] Bashkatov, A.N., et al., "Measurement of tissue optical properties in the context of tissue optical clearing," Journal of Biomedical Optics 23(9), 091416 (2018). DOI 10.1117/1.JBO.23.9.091416.

[15] Genina, E.A. et al., "Optical Clearing of Tissues: Benefits for Biology, Medical Diagnostics, and Phototherapy," in [Handbook of Optical Biomedical Diagnostics, Vol. 2: Methods], Bellingham, WA, USA: SPIE Press, 688 (2016).
[16] Smolyanskaya, O. A., Lazareva, E. N., Nalegaev, S. S., et al., "Multimodal Optical Diagnostics of Glycated Biological Tissues," Biochemistry (Moscow) 84(1), (2019).
[17] Cicchi, R., Pavone, F. S., Massi, D., and Sampson, D. D., "Contrast and depth enhancement in two - photon microscopy of human skin ex vivo by use of optical clearing agents," Opt. Exp. 13, 2337-2344 (2005).
[18] Tseng, S.-J., Lee, Y.- H., Chen, Z.-H., Lin, H.- H., Lin, C.- Y., and Tang, S.- C., "Integration of optical clearing and optical sectioning microscopy for three − dimensional imaging of natural biomaterial scaffolds in thin sections," J. Biomed. Opt.14(4) 044004 (2009).
[19] Zhi, Z., Han, Z., Luo, Q., and Zhu, D., "Improve optical clearing of skin in vitro with propylene glycol as a penetration enhancer," Journal of Innovative Optical Health Sciences 2(03), 269-278 (2009).
[20] Kolesnikov, A.S., Kolesnikova, E.A., et al., "THz monitoring of the dehydration of biological tissues affected by hyperosmotic agents," Physics of Wave Phenomena 22(3), 169-176 (2014).
[21] Yanina, I. Yu., Volkova, E.K., Tuchina, D. K., Konyukhova, Ju. G., Kochubey, V. I., and Tuchin. V. V., "Controlling of upconversion nanoparticle luminescence at heating and optical clearing of adipose tissue," Proc. SPIE 10417-5, 1-7 (2017).
[22] Tuchin, V. V. [Optical Clearing of Tissues and Blood], PM 154, SPIE Press, Bellingham, WA, (2006).
[23] Kistenev, Yu.V., Borisov, A.V., Kuzmin, D.A., Penkova, O.V., Kostyukova, N.Yu., and Karapuzikov, A.A. "Exhaled air analysis using wideband wave number tuning range infrared laser photoacoustic spectroscopy," J. Biomed. Opt. 22 (1), 017002 (2017).
[24] Kistenev, Yu.V., Kuzmin, D.A., Vrazhnov, D.A., and Borisov, A.V. "Classification of patients with broncho-pulmonary diseases based on analysis of absorption spectra of exhaled air samples with SVM and neural network algorithm application," Proc.SPIE 10035, 1003507 (2016).