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Biomedical Photonics Methods in Solving Diagnostic Tasks

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Modern photonics methods are considered in relation to solving a number of diagnostic problems in practical medicine. The basic principles of the application of optical noninvasive diagnostic methods are described and examples are given: laser Doppler flowmetry, digital diaphanoscopy, Raman spectroscopy, spectrophotometry, time-resolved fluorescence spectroscopy, and video capillaroscopy. The advantages and prospects of using photonics methods in clinical practice, making it possible to detect various pathological conditions of biological tissues, including those at the early stages of diseases, are demonstrated.

Introduction

Biomedical photonics technologies provide unique opportunities for studying various pathological disorders of biological tissues, including non-invasive detection of diseases at earlier stages [1]. One object of research in optical non-invasive diagnostics is the human body's microcirculatory tissue system (MTS) — the smallest functional unit of the vascular system — in which microvessels are in close relationship with the surrounding tissue and regulatory elements. Disorders of the MTS play a key role in the pathogenesis of the complications of a variety of diseases, so their timely diagnosis is the subject of extensive research [2]. There is currently a surge of interest in portable diagnostic tools, as daily monitoring (of, for example, MTS parameters) promises a new quality of diagnostics. One of the first attempts to use such devices with success is their introduction into endocrinology, in the form of a distributed system on the bodies of patients with diabetes mellitus, to assess microcirculatory disorders [3]. Portable analyzers have also shown their effectiveness in assessing the effects of yoga hypo- and hyperventilation breathing exercises on peripheral blood flow parameters, as well as in monitoring rehabilitation measures in patients who have had COVID-19.

Another example of the promising implementation of photonics methods in clinical practice is the instrumental implementation of digital diaphanoscopy technology for diagnosing inflammatory diseases of the maxillary sinuses, taking account of the anatomical, age, and gender characteristics of patients. The proposed approach may have promise as a screening method for assessing the state of the maxillary sinuses.

Raman scattering and spectrophotometry methods are used to study the composition, molecular structure, and chemical identification of samples; these are promising technologies for studying the composition of bile in patients with obstructive jaundice of various etiologies and assessing the severity of liver failure. It should be noted that the last decade has seen the active introduction of photonics methods into minimally invasive surgery for the diagnosis of tumor diseases [4]. One example is the use of time-resolved fluorescence spectroscopy to improve the diagnostic performance of percutaneous liver puncture biopsy.

One example of the application of photonics methods as an approach to objective monitoring in solving problems in basic medicine and determining the effectiveness of therapeutic procedures is the use of video capillaroscopy to assess the regulation of the vascular bed, using direct optical generation of singlet oxygen during photodynamic therapy (PDT) of tumor diseases and the correction of non-oncological vascular anomalies.

Thus, the aim of the present article was to demonstrate the advantages and unique aspects of spectroscopy and

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diagnostic imaging methods for detecting and evaluating various diseases and pathological conditions, as well as for monitoring therapeutic procedures in clinical practice.

Portable Devices for the Diagnosis of Adaptive Reserves and Pathological Changes in the Blood Microcirculation System

Laser Doppler flowmetry (LDF) provides for evaluation of the functioning of the mechanisms regulating peripheral blood flow (endothelial cell and precapillary sphincter functions, innervation of the study area, as well as respiration and heartbeat [2]).

A series of studies to diagnose adaptive reserves and pathological changes in the blood microcirculation system used LAZMA-PF portable laser blood microcirculation analyzers combined to form a distributed system with wireless data transmission. The analyzers were used to assess the effects of yoga hypo- and hyperventilation breathing exercises on peripheral blood flow parameters [5] and their relationship with gas exchange parameters. The index of microcirculation was recorded in the basins of the supraorbital arteries, middle fingers, and big toes in volunteers practicing full breathing. Spirometry, gas analysis, and pulse oximetry data were recorded using a MAC-2C spirometer.

This study showed that there are significant correlations between blood microcirculation parameters and gas analysis during free breathing and hypoventilation. Hypoventilation leads to a greater increase in the contribution of the myogenic component to the overall modulation of blood flow when measured on the forehead and in the fingers, while in the toes the contribution of the neurogenic component increases significantly after hyperventilation. These results my be useful in studying the properties of the mechanisms of oxygen delivery to biological tissues and will also facilitate the development of an instrumental method for assessing the effectiveness of breathing exercises during rehabilitation.

LAZMA-PF portable devices have also been used to assess peripheral blood flow parameters during the rehabilitation period after COVID-19 and to study the dynamics of processes occurring in the blood microcirculation system during coronavirus infection. Studies have demonstrated a tendency to dilation of blood vessels after the acute phase of coronavirus disease as the patient's body gradually adapts to the influence of the infection. Recovery after disease takes more than a month, and during this period the body activates compensatory mechanisms to supply cells with nutrients and oxygen, gradually stabilizing the functioning of all systems.

Application of Digital Diaphanoscopy in Otorhinolaryngology for the Diagnosis of Pathological Changes in the Maxillary Sinuses

Digital diaphanoscopy is used in various fields of medicine, in particular in otorhinolaryngology to determine pathological changes in the maxillary sinuses (MS). The method is based on optical probing of the maxillary sinuses, with recording of light scattering patterns (diaphanograms) and digital processing of the latter [6].

The experimental arrangement developed in this work includes an applicator probe with LEDs at wavelengths of 650 and 850 nm, a CMOS camera for recording diaphanograms, and a unit for adjusting the brightness of the LED applicator. Power levels are set for each of the wavelengths for diagnostics in patients of different sexes [7].

Presumptively healthy volunteers and patients with maxillary sinus pathologies were examined using the arrangement developed for the study. The results were compared with CT and MRI imaging results. The diaphanograms reflected the dependence of light absorption in the region of the maxillary sinus on the presence of pathological changes. Thus, in the case of the investigation of a patient with chronic left-sided maxillary sinusitis, light absorption associated with the strong absorbing properties of the pathological change at the probing wavelengths was observed [8]. The CT data revealed a picture of chronic left-sided maxillary sinusitis, confirming the reliability and accuracy of the data obtained by digital diaphanoscopy, as well as the correctness of the preliminary diagnosis.

The following indicators were calculated for quantitative confirmation of the presence of pathological changes in the maxillary sinus: the intensity parameter, characterizing the amount of radiation that reached the camera detector after absorption by biological layers and various pathological changes, and the K coefficient, which is the ratio of the intensity in the orbit to the intensity in the area of the MS. Analysis of the data revealed a statistically significant difference between the calculated values for diaphanograms of apparently healthy volunteers and patients with pathological changes in the maxillary sinuses. The mean intensity parameter for healthy volunteers was 98.5 ± 3.3 rel. units, compared with 45.4 ± 26.1 rel. units for sinuses with pathology. The calculated coefficient K was 1.4 ± 0.8 and 15.8 ± 13.9 rel. units in these groups respectively.

Application of this approach for recording diaphanograms allows two states of the maxillary sinus to be differentiated (healthy and with pathological changes) and has potential for diagnosing patients, as well as for population screening.

Optical Studies of Bile Samples from Patients with Obstructive Jaundice Syndrome

Spectrophotometry and Raman spectroscopy methods have proven themselves in studies seeking to assess changes in the molecular and morphological structure of biological fluids [9]. Application of these optical technologies is promising in addressing the challenge of improving differential diagnosis and timely detection of liver failure in patients with obstructive jaundice.

Optical measurements were made of bile samples from three patients diagnosed with choledocholithiasis and three patients with malignant tumors obtained during antegrade decompression of the biliary tract.

Spectrophotometric measurements were carried out using a Shimadzu UV-2600 spectrophotometer with an ISR-2600Plus integrating sphere in the range of 220–1400 nm. Raman spectra were obtained using a QEPRO-RAMAN spectrometer, a 785-LAB-ADJ-FC laser (wavelength 785 nm), and a RIP-RPB-785-FC-SMA fiberoptic probe. Differences were found in the spectral compositions and amplitudes of parameters recorded by both optical methods for different groups. The total transmission and reflection spectra showed higher values in the near infrared range and significant decreases in the range 350–500 nm due to the bilirubin content [10]. The shapes of spectra in the near infrared range were determined mainly by the lipid and water contents. Raman spectra exhibited two main bands, near 1611 and 1255 cm^{-1}, which are due largely to the presence of bilirubin and its derivatives [11]. Analysis of measurement results for bile samples obtained separately from the biliary systems of the right and left lobes of the liver in a patient with a tumor at the confluence of the right and left bile ducts revealed significant differences in the spectral characteristics of the bile samples. The cause of the differences seen in the spectra may be the different sizes of the lobes, the volumes of the ducts within them, and the extent of blockade of each lobe, affecting the chemical composition of the bile produced, including its bilirubin content.

Raman spectra, along with functions of the total transmission, reflection, and absorption coefficient, revealed the presence of typical bile components. This approach demonstrates the promise and wide potentials both for acquiring new knowledge regarding the optical properties of bile in various pathologies and for further use in developing new multimodal diagnostic technologies to establish the etiology of obstructive jaundice and the degree of liver failure.

Application of Time-Resolved Fluorescence Spectroscopy to Improve the Diagnostic Efficiency of Percutaneous Puncture Liver Biopsy

Measurement of fluorescence lifetime is an effective approach to studying biological tissues and detecting shifts in energy supply from mitochondrial oxidative phosphorylation to aerobic glycolysis, which occurs in tumor cells [12]. Modern technologies make it possible to combine optical measurements with standard instruments for minimally invasive surgery [13, 14], including the use of needles for percutaneous needle biopsy (PCNB) procedures [15]. This provides ample opportunities for acquiring data on the state of the tissue during the standard PCNB procedure and may form the basis of a technology for differentiating healthy parenchyma and liver tumors.

An instrument was developed for measuring fluorescence lifetime parameters through a fine-needle probe compatible with standard Chiba 17.5G biopsy needles [16]. The device recording the parameters of fluorescence intensity and lifetime was built on the basis of the time-correlated single photon counting method and included an SPC-130-EMN photon counting module, a BDS-SM-375-FBC-101 (375 nm) laser source, a HPM-100-40-CMOUNT hybrid photodetector, and an MF445 (445 ± 25 nm) bandpass filter.

The studies were carried out on BDF (C57Bl6 × DBA) laboratory mice with a liver tumor model. Optical signals were recorded within the tumor and in the adjacent areas of the liver, and also in the livers of healthy mice. Data processing yielded the following parameters: fluorescence intensity, I_f; the intensities of the amplitudes of the short- and long-lifetime components α_1 and α_2; the ratio α_1/α_2; and parameters of short and long fluorescence lifetimes τ_1 and τ_2 respectively. Linear discriminant analysis was used to analyze classifiers built on pairs of independent parameters, i.e., (τ_1, I_f), (τ_2, I_f), (τ_1, α_1/α_2), and (τ_2, α_1/α_2). Sensitivity (*Se*), specificity (*Sp*), and the areas under the curve (AUC) for tissue discrimination results are given in Table 1.

TABLE 1. Effectiveness parameters of proposed classifiers. hcc — hepatocellular carcinoma

Parameter	Control liver — murine HCC			Adjacent liver — HCC		
	Se	*Sp*	AUC	*Se*	*Sp*	AUC
τ_1, I_f	0.97	0.91	0.86	0.92	0.72	0.96
τ_2, I_f	0.92	0.99	0.94	0.99	0.99	0.99
τ_1, α_1/α_2	0.9	1.0	0.98	0.65	1.0	0.99
τ_2, α_1/α_2	0.87	0.97	0.97	0.79	0.96	0.99

These results demonstrate the high sensitivity and specificity of this optical biopsy technique, whose use will improve the diagnostic efficiency of the liver PCNB procedure.

Application of Video Capillaroscopy for Visualization of Vascular Changes during Direct Optical Generation of Singlet Oxygen

Interest in studying the role of singlet oxygen (SO) as the main mediator of therapeutic effects during PDT is currently growing. In addition to generating singlet oxygen in the presence of photosensitizers, the possibility of its direct optical generation has been demonstrated [17, 18]. Studies at the cellular level have shown the effectiveness of light-induced SO in the regulation of cellular life processes [19, 20]. The results showed that this approach to generating SO has promise for correcting angiogenesis and triggering the mechanism of tumor tissue cell death, as well as for correcting non-oncological vascular anomalies.

An approach for in vivo studies of the effects of direct optical generation of SO on changes in the parameters of the vascular bed was proposed, along with a corresponding experimental arrangement for generating SO and visualizing vascular changes. SO was generated at a wavelength of 1267 nm using an SM-1267-PM-500 diode laser. The diode laser was powered and its operating modes were changed using a driver board with an SF8150-ZIF14 power supply and Maiman BenchSoft software. A fiberoptic cable and an F280FC-C collimator were used to deliver laser radiation from the source to the object.

Visualization of the vascular bed was carried out by video capillaroscopy (VCS), with simultaneous recording of images of the intensity of backscattered radiation under illumination with incoherent (525 nm) and coherent (660 nm) light sources using a 300X Zoom C-mount Lens high-aperture optical system and UI-3060CP-C-HQ R2 color CMOS cameras. A powerful side illumination subsystem with different wavelengths ensured uniformity of the illumination field and contrasting of moving erythrocytes in superficial and deep blood vessels.

Sequential processing [21] of the acquired series of VCS images yielded photoplethysmography (PPG) signals. Fourier analysis of spectra was used to construct blood vessel maps (525 nm) and blood vessel filling maps (660 nm) [21]. In addition, spectral analysis of PPG signals was performed to identify the contributions of periodic signal changes associated with various mechanisms of blood flow fluctuations to the resulting signal.

During the investigation, images of the vascular network of the femoral and gluteal regions were continuously recorded in rats at a frame rate of 250 fps at a magnification of ×3.5 before irradiation, during irradiation, taking the selected dose into account, and after irradiation with a 1267-nm laser with a power of 50 mW.

Analysis of changes in the PPG signal and the contributions of the myogenic, respiratory, and cardiac components to the signal showed that direct optical generation of SO (at a dose of 50 J/cm^2) leads to changes in the vascular bed. PPG and frequency analysis demonstrated decreased blood filling (a decrease in the pulse amplitude at the heart rate), as well as vasoconstriction and arrest of blood flow, as shown by analysis of processed speckle images. The decrease in blood flow may be associated with noradrenaline-mediated vasoconstriction due to Ca^{2+}-independent noradrenaline release from the presynaptic element of adrenergic neurotransmission induced by SO [22].

Thus, the approach proposed and the experimental arrangement to implement it provide continuous and long-term high-speed recording of backscatter intensity images with the ability to reconstruct PPG signals, acquire maps of vessels and their blood supply, and study rapidly changing processes in the vascular bed during direct optical generation of SC.

Conclusions

These examples of the application of various photonics methods in clinical practice, which also serve as instrumental methods for monitoring for research in fundamental medicine, reflect new concepts in optical diagnostics and bring it closer to the gold standard. It should be noted that the introduction of medical decision support systems in optical diagnostics is promising and that this will expand the scope of photonics methods in practical healthcare challenges.

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REFERENCES

1. Dunaev, A. V., Multimodal Optical Diagnostics of Microcirculatory-Tissue Systems of the Human Body. A Monograph [in Russian], TNT, Staryi Oskol (2022).
2. Krupatkin, A. I. and Sidorov, V. V., Functional Diagnostics of the State of Microcirculatory-Tissue Systems: Fluctuations, Information, Nonlinearity. Guidelines for Doctors [in Russian], Librokom Publishers, Moscow (2013).
3. Biomedical Photonics for Diabetes Research, A. V. Dunaev, and V. V. Tuchin, (eds.), CRC Press (2022).
4. Multimodal Optical Diagnostics of Cancer, V. V. Tuchin, J. Popp, and V. P. Zakharov, (eds.), Springer (2020).
5. Frolov, A. V., Loktionova, Yu. I., Zharkikh, E. V., Sidorov, V. V., Krupatkin, A. I., and Dunaev, A. V., "Studies of changes in skin microcirculation of blood during the implementation of the respiratory technique of hatha yoga," Regionarn. Krovoobrashch. Mikrotsirkulyats., **20**, No. 4, 33–44 (2021).
6. Stoelzel, K., Szczepek, A. J., Olze, H., Koss, S., Minet, O., and Zabarylo, U., "Digital diaphanoscopy of the maxillary sinuses: A revival of optical diagnosis for rhinosinusitis," Am. J. Otolaryngol., **41**, No. 3, 102444 (2020).
7. Bryanskaya, E. O., Novikova, I. N., Dremin, V. V., Gneushev, R. Y., Bibikova, O. A., Dunaev, A. V., and Artyushenko, V. G., "Optical diagnostics of the maxillary sinuses by digital diaphanoscopy technology," Diagnostics (Basel), **11**, No. 77, 1–13 (2021).
8. Bashkatov, A. N., Genina, E. A., and Tuchin, V. V., "Optical properties of skin, subcutaneous, and muscle tissues: A review.," J. Innov Opt. Health Sci., **4**, (01), 9–38 (2011).
9. Jang, E., Vu, T. D., Choi, D., Jung, Y. K., Lee, K. G., and Chung, H., "Feasibility study for rapid near–infrared spectroscopic identification of different gallbladder diseases by direct analysis of bile juice," Analyst, **144**, No. 24, 7236–7241 (2019).
10. Maitland, D. J., Walsh, J. T., and Prystowsky, J. B., "Optical properties of human gallbladder tissue and bile," Appl. Opt., **32**, No. 4, 586–591 (1993).
11. Vu, T. D., Jang, E., Lee, J., Choi, D., Chang, J., and Chung, H., "Feasibility of voltage-applied SERS measurement of bile juice as an effective analytical scheme to enhance discrimination between gall bladder (GB) polyp and GB cancer," Anal. Chem., **92**, No. 12, 8159–8169 (2020).
12. Becker, W., Bergmann, A., Hink, M. A., König, K., Benndorf, K., and Biskup, C., "Fluorescence lifetime imaging by time correlated single photon counting," Microsc. Res. Tech., **63**, No. 1, 58–66 (2004).
13. Kandurova, K., Dremin, V., Zherebtsov, E., Potapova, E., Alyanov, A., Mamoshin, A., Ivanov Yu., Borsukov, A., and Dunaev, A., "Fiber–optic system for intraoperative study of abdominal organs during minimally invasive surgical interventions," Appl. Sci., **9**, No. 2, 217 (2019).
14. Zherebtsov, E., Zajnulina, M., Kandurova, K., Potapova, E., Dremin, V., Mamoshin, A., Sokolovski, S., Dunaev, A., and Rafailov, E., "Machine learning aided photonic diagnostic system for minimally invasive optically guided surgery in the hepatoduodenal area," Diagnostics (Basel), **10**, No. 11, 873 (2020).
15. Dremin, V., Potapova, E., Zherebtsov, E., Kandurova, K., Shupletsov, V., Alekseyev, A., Mamoshin, A., and Dunaev, A., "Optical percutaneous needle biopsy of the liver: A pilot animal and clinical study," Sci. Rep., **10**, No. 1, 1–11 (2020).
16. Zherebtsov, E. A., Potapova, E. V., Mamoshin, A. V., Shupletsov, V. V., Kandurova, K. Y., Dremin, V. V., Abramov, A. Y., and Dunaev, A. V., "Fluorescence lifetime needle optical biopsy discriminates hepatocellular carcinoma," Biomed. Opt. Express, **13**, No. 2, 633–646 (2022).
17. Zakharov, S. D. and Ivanov, A. V., "Light–oxygen effect in cells and prospects for its application in tumor therapy," Kvantov. Élektron., **29**, No. 3, 192–214 (1999).
18. Blázquez–Castro, A., "Direct $^1O_2$ optical excitation: A tool for redox biology," Redox Biol., **13**, 39–59 (2017).
19. Sokolovski, S. G., Rafailov, E. U., Abramov, A. Y., and Angelova, P. R., "Singlet oxygen stimulates mitochondrial bioenergetics in brain cells," Free Radic. Biol., **163**, 306–313 (2021).
20. Novikova, I. N., Potapova, E. V., Dremin, V. V., Dunaev, A. V., and Abramov, A. Y., "Laser-induced singlet oxygen selectively triggers oscillatory mitochondrial permeability transition and apoptosis in melanoma cell lines," Life Sci., **304**, 120720 (2022).
21. Volkov, M. V., Margaryants, N. B., Potemkin, A. V., Machikhin, A. S., Khokhlov, D. D., Batshev, V. I., and Danilychev, M. V., "Blood vessel visualization method in human skin based on video recording of blood flow using a laparoscope," J. Commun. Technol., **65**, No. 7, 806–814 (2020).
22. Yoshino, F., Shoji, H., and Lee, M.-C., "Vascular effects of singlet oxygen ($^1O_2$) generated by photo-excitation on adrenergic neurotransmission in isolated rabbit mesenteric vein," Redox Rep., **7**, No. 5, 266–270 (2002).