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A hardware and software system of fluorescence and reflectance spectroscopy for intraoperative diagnosis of tissue metabolism

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Abstract

A multimodal device for assessing the metabolic status of tissues during minimally invasive surgeries is proposed. The hardware and software system and specially developed fine-needle fiber-optic probes support assessment of the fluorescence of the main tissue fluorophores, as well as tissue saturation. The approach proposed here addresses a number of clinical problems, including the differentiation of healthy and tumor tissues and determination of the metabolic status of neoplasms and the functional state of organs. The system can be used for intraoperative diagnostics in various areas of minimally invasive surgery.

Introduction

The field of minimally invasive surgical interventions (MISI) is currently developing rapidly. Optical biopsy, based on optical spectroscopy techniques, has great potential for improving the diagnostic capabilities of MISI [1, 2]. The advantage of spectroscopic investigation methods is that they can be implemented through small-diameter optical fibers, which can be integrated into MISI instruments. This supports spectroscopic analysis based on the results of measurements of tissues for which pathomorphological investigation is planned. The ultimate goals of introducing optical diagnostic methods into MISI instruments are to assess the condition of tissues and to diagnose pathological changes in real time based on spectroscopic measurements.

Current proposals for devices for optical biopsy using standard MISI instruments are based on the use of technologies such as Raman spectroscopy [3], diffuse reflectance spectroscopy (DRS) [4], fluorescence spectroscopy (FS) [5], and laser Doppler flowmetry [6]. Most studies primarily address autofluorescence spectroscopy, because important biochemical changes associated with pathological metabolic rearrangements alter the intrinsic fluorescence spectrum of tissues. The DRS method yields data on the tissue oxygen saturation level, which can also be used to analyze tissue metabolism.

The proposed combined use of FS and DRS methods in a single device with the ability to record spectra through a fine-needle probe during MISI can provide the ability to carry out comprehensive analysis of the state of tissues and pathological changes within them, which will potentially increase the information content and reliability of the diagnostic results obtained using multimodal approaches [7].

The aim of the present work was to develop a hardware and software system (HSS) based on fluorescence-reflectance spectroscopy with a set of fiber-optic probes for intraoperative diagnosis of tissue metabolism to address clinical medical problems in various areas of minimally invasive surgery.

Description of the hardware and software system

The hardware and software system for fluorescence and reflectance spectroscopy was developed in the following main stages:

1. evaluation of the specialized medical and technical requirements for the device and original fiber-optic probes for various clinical tasks;
2. design and engineering processes involved in creating the device, including the selection of optoelectronic components, design and manufacture of the housing and manufacture of fiber-optic probes of various diameters and lengths;

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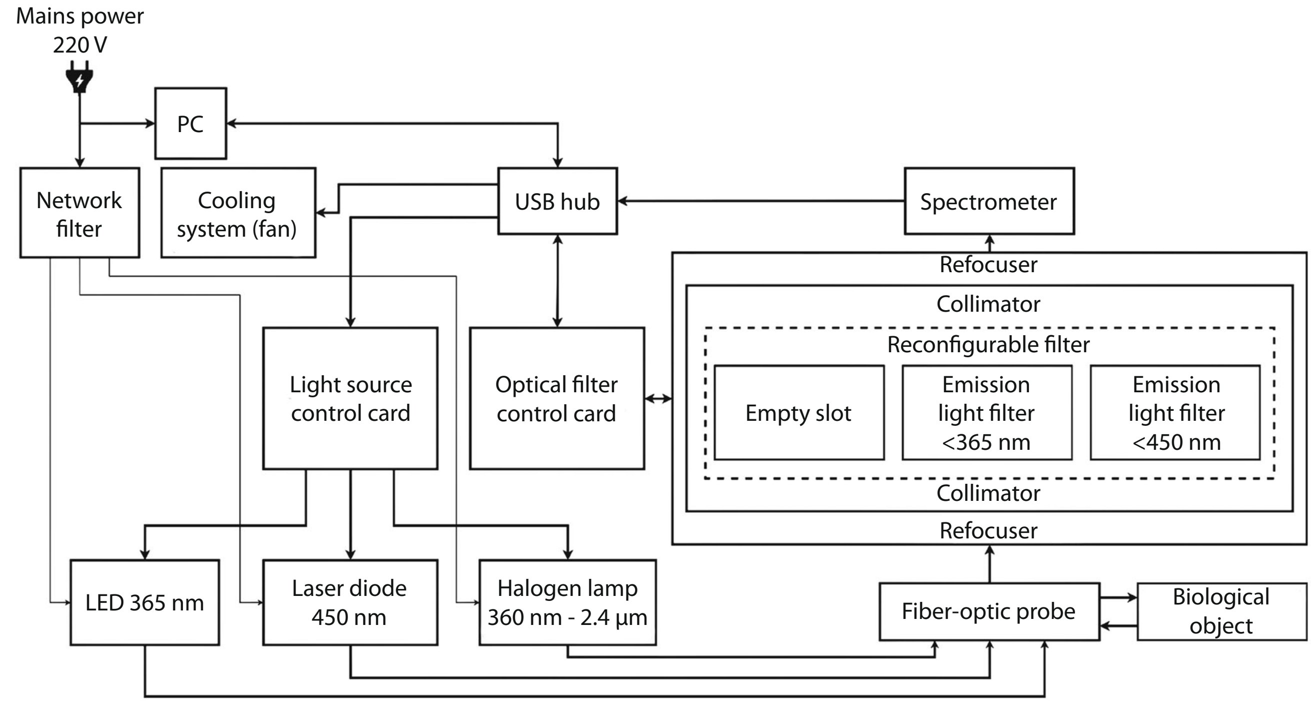

Fig. 1 Functional diagram of the HSS for intraoperative diagnostics of tissue metabolism

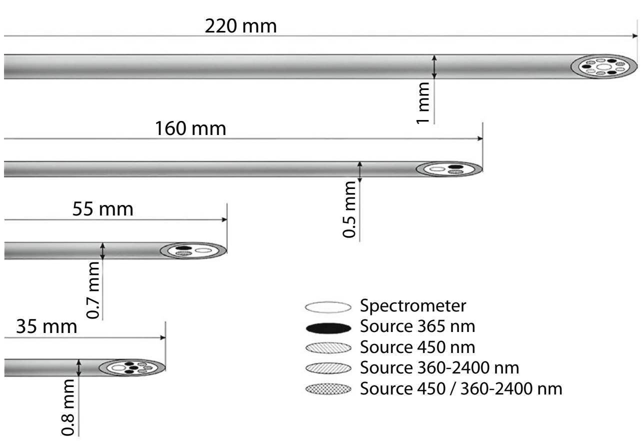

Fig. 2 Different types of fine-needle fiber-optic probes

3. verification of the layout of the HSS by assessment of the sensitivity of the fine-needle optical probe to changes in the fluorescence of cellular respiration coenzymes and changes in tissue saturation;
4. testing of the HSS in clinical conditions.

The functional diagram of the HSS developed here for intraoperative diagnostics of tissue metabolism (RU Patent No. 2709830) is shown in Fig. 1.

Two radiation sources are used to excite the endogenous fluorescence of biological tissues: a light-emitting diode with a wavelength of 365 nm and a laser diode with a wavelength of 450 nm. The output power levels of these sources are no more than 1.5 and 3.5 mW respectively, which ensures compliance with safety requirements and reduces the impact of photobleaching. These wavelengths were selected for the FS channel in relation to the maximum quantities of endogenous fluorophores excited, including the energy metabolism coenzymes NADH and FAD. In the DRS channel, tissue is illuminated with polychromatic halogen lamp radiation (360–2400 nm). A small CCD spectrometer (working wavelength range 350–1000 nm) is used to record fluorescence and diffuse reflection spectra. Acquisition and transmission of optical radiation is carried out using a fine-needle fiber-optic probe of small diameter, which can be inserted into standard medical needles or drainage systems and can be sterilized. Light from the sources is passed through the fine-needle fiber-optic probe to the biological object, is reflected or re-emitted as fluorescence, is collected by the optical fiber, and is passed to the refocuser, which consists of two collimators with emission filters between them. Emission filters are installed in the optical path of the measuring channels automatically and are designed to weaken the back-reflected radiation from the monochromatic light sources in the FS channel. The user communicates with the control unit through a specialized program developed for the purpose and installed on a personal computer.

Depending on the medical tasks, fiber-optic probes of various diameters (0.5–1 mm), lengths, and configurations of transmitting and receiving optical fibers can be used (Fig. 2). The end bevel of the probes (20°) corresponds to the bevel of the puncture needle and ensures the largest area

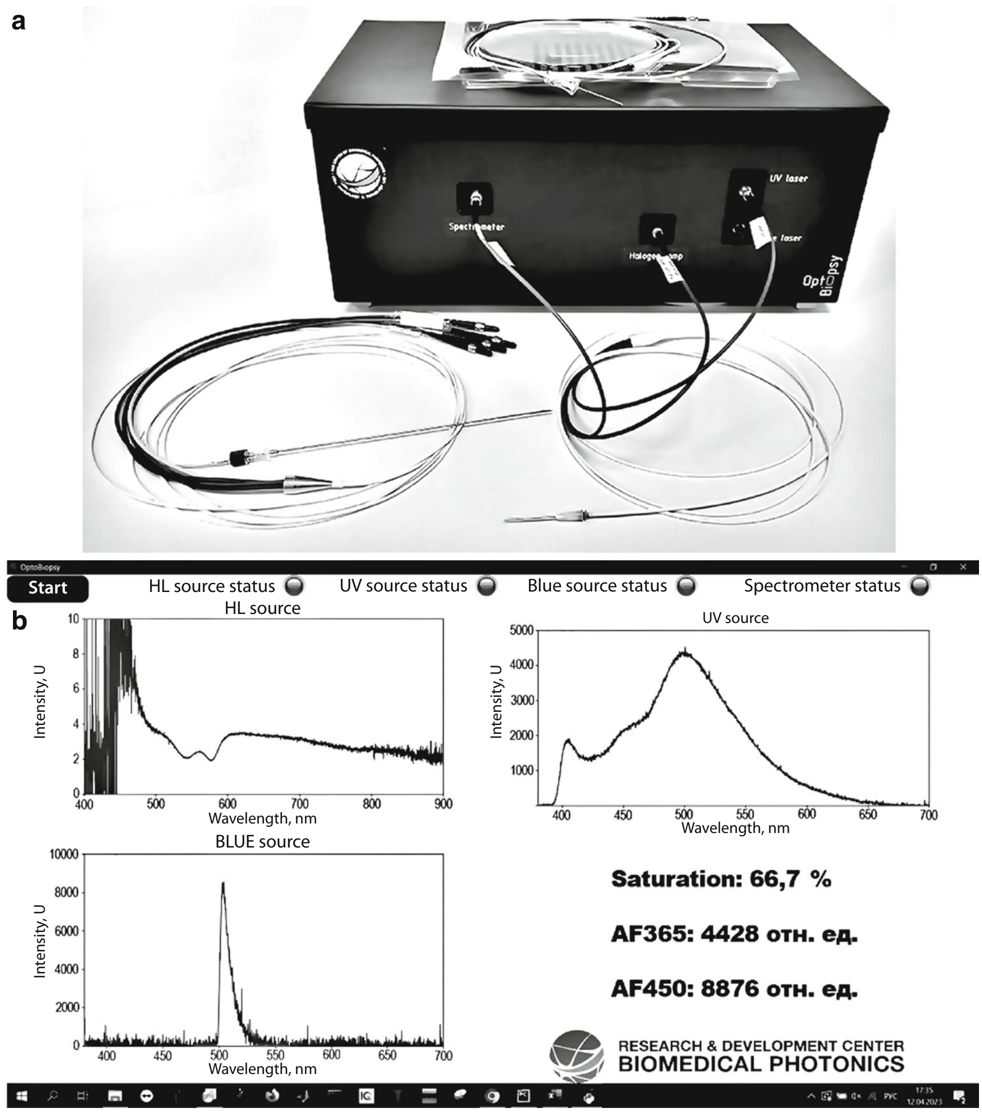

Fig. 3 View of the working model of the HSS for intraoperative diagnosis of tissue metabolism (**a**) and the control interface (**b**)

of contact with tissues and, if there is need to change probe position (for dynamic measurements), it provides the ability to take measurements along the trajectory of the instrument in the tissue such that the surgeon can take sequences of measurements in the area being diagnosed.

Results

The appearances of the working model of the HSS and a set of fine-needle fiber-optic probes are shown in Fig. 3a. The system is controlled using a graphical interface developed in the Python programming language (Fig. 3b). The interface includes the following elements: indicators of the alternating operation of the radiation sources, diffuse reflectance and fluorescence spectra excited at wavelengths of 365 and 450 nm, the calculated value of tissue saturation, and amplitudes of fluorescence AF_{365} and AF_{450}.

To verify the HSS developed here, experimental measurements of the fluorescence intensity of the internal organs of a laboratory rat were carried out *in vivo* on exposure of the tissue surface to a mitochondrial inhibitor to induce changes in cellular respiration. The results demonstrated that the channel developed here could record changes in fluorescence intensity caused by changes in the processes of mitochondrial oxidative phosphorylation. Studies also addressed changes in tissue saturation as a result of an occlusion test on the fingers of presumptively healthy volunteers.

The HSS was tested in clinical settings. All studies were conducted in accordance with the principles of biomedical ethics and were approved by the Ethics Committee; participants were familiarized with the content of the study and signed an informed consent form.

The fluorescence and reflectance spectroscopy HSS was used in studies seeking to increase the diagnostic efficiency of puncture biopsy (PB) of the liver. The clinical studies involved 20 patients at the surgical department of the Orel Regional Clinical Hospital. Spectra were recorded during the standard PB procedure under ultrasound control along the trajectory of the puncture needle in the parenchymal tissues of the liver and neoplasms, the nature of which was confirmed by pathomorphological examination. A fine-needle fiber-optic probe with diameter 1 mm and length 220 mm was used. Analysis of fluorescence spectra amplitudes showed that the fluorescence intensity peaks of tumor tissue at both excitation wavelengths (365 and 450 nm) differed from the peaks of the unaltered liver parenchyma in displaying a red shift in the spectral wavelengths. This is probably due to the fact that unaltered liver tissues contain more than five times more bile than tumors. A statistically significant difference in the oxygen saturation levels of the two tissue types (tumor and unaltered liver parenchyma) was also recorded. Classifiers proposed on the basis of the support vector machine method based on the parameters obtained (the shift in the peak amplitudes of the FS and tissue saturation) allow neoplasms and presumptively healthy liver tissues to be differentiated with sensitivity and specificity of 0.90 and 0.95 respectively [8]. Thus, the HSS developed here is a promising tool for differentiating tumor tissue and unaltered liver parenchyma during fine-needle PB.

Another possible application of the HSS is in the assessment of the functional state of the liver in patients with obstructive jaundice (OJ) syndrome. A study of 20 patients with OJ syndrome was conducted at the Orel Regional Clinical Hospital. Measurements were taken during the formation of primary access to the biliary system to achieve antegrade biliary decompression using the FS method at wavelengths of 365 and 450 nm. The same fiber-optic probe, with diameter 1 mm and length 220 mm, was used to transmit optical radiation. The captured spectra were analyzed using the deconvolution method to calculate a combination of Gaussian curves reflecting the contribution of individual fluorophores [9]. The results were compared with data from 11 patients without OJ obtained during PB of space-occupying liver lesions [8]. Analysis of the curves revealed statistically significant increases in the contributions of NADH, bilirubin, and flavin fluorescence in the group of patients with OJ. In terms of potential markers of impairments to the functional state of the liver, changes in the contribution of vitamin A were noted as an indirect sign of decreased excretory function; in some cases, patients with negative dynamics showed increases in the contributions of porphyrins and lipofuscin [10]. This technology is currently being moved towards development of an algorithm for determining the degree of liver failure by comparing the captured data with results from clinical laboratory tests and instrumental diagnostic methods (ultrasound and magnetic resonance imaging).

Another clinical example of the use of the HSS developed here is in determination of the metabolic status of breast tumors. These studies were conducted at the Smolensk Regional Oncology Clinical Dispensary with the participation of 21 patients with breast tumors, who, in addition to standard examination methods, underwent assessment of tumor metabolism using the FS and DRS methods using this HSS and a specially developed fine-needle fiber-optic probe with diameter 0.7 mm and a length 55 mm. Malignant breast tumors were found to have lower tissue saturation levels than benign neoplasms of the mammary glands. It was also found that malignant tumors had higher fluorescence intensities at an excitation wavelength of 365 nm than benign tumors, which may be due to the accumulation of the coenzyme NADH or changes in the collagen matrix. The proposed method for assessing the metabolism of breast neoplasms can be used to improve the results of ultrasound-guided PB and to assess the therapeutic pathomorphism of breast cancer.

Conclusions

The HSS developed here for real-time intraoperative assessment of tissue metabolism, based on optical diagnostic methods (FS and DRS), is consistent with modern trends in personalized medicine and high-tech healthcare. The system has a convenient interface for recording FS and DRS signals and is equipped with a set of fiber-optic probes of various diameters and lengths for the convenience of conducting investigations in different areas of minimally invasive surgery.

The performance of the proposed HSS has been tested clinically by comparing the data collected with clinical laboratory tests and the results of pathomorphological examination. Further work on improving the system will seek to refine classifiers with implementation of these in the HSS interface to operate a system for supporting medical decision-making and to obtain a registration certificate.

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