Optical biopsy: fundamentals and applications in neurosurgery

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Abstract. Currently, there is a significant increase in the incidence of cancer of the central nervous system. Determination of the boundaries of intracerebral and intramedullary tumors is especially difficult. The urgency of the problem of determining the boundaries of astrocytic tumors is due to the peculiarities of their growth along myelinated nerve fibers and vessels, leading to the infiltration of healthy white matter by tumor cells, which affects the high frequency of postoperative relapses. The complexity of surgery for intramedullary tumors of the spinal cord is that the tumor does not always have a clear border and the risk of injury is high due to the smaller size of the operated area compared to the brain. Reliable information regarding the volume of the resected tumor should be obtained by intraoperative imaging. The solution to this problem is implemented mainly in three directions: the use of intraoperative computed tomography, magnetic resonance imaging and ultrasound scanning, and various combinations of these methods. Unfortunately, all these methods of intraoperative diagnostics do not allow real-time examination of tissues in an operating wound and/or do not provide a simultaneous analysis of both structural and metabolic changes. The limitations of intraoperative navigation methods in neurosurgery have led to the relevance of the development of an accurate spectroscopic method for in vivo determination of the content of specific metabolic markers and structural changes accompanying the development of the tumor process in the nervous tissue. Various approaches to intraoperative navigation based on optical spectroscopy are called optical biopsy. In this article, we present the methods and tools developed in recent years for spectroscopic guidance in neurooncology. First of all, this, of course, concerns the analysis of spectral dependences recorded before, during and after tumor removal. We have used such modalities of optical spectroscopy as fluorescence, diffuse reflectance spectroscopy and spontaneous Raman scattering. An equally important issue on the way to increasing the efficiency of tumor resection is the development of new instrumentation; therefore, we have developed a number of new devices, which are a combination of well-known neurosurgical instruments and laser and fiber-optic technologies. Last but not least is the issue of rapid classification of the studied tissues based on the recorded signals, which was solved by us using machine learning methods.
1. Introduction

In modern clinical practice, in the treatment of intracranial tumors, the most important is the accurate diagnosis of the localization and borders of the tumor and, as a consequence, ensuring maximum organ preservation during surgical removal of the neoplasm. In view of the differences in the morphology of intracranial tumors, an effective solution to increasing the sensitivity and specificity of intraoperative diagnostics is the simultaneous monitoring of several characteristics of tumor tissue. At the moment, several different approaches to such an analysis have been implemented, which can be conditionally divided into approaches based on the sequential registration of various parameters [1] and on the simultaneous one [2].

Studies have shown that the content of 5-ALA-induced Protoporphyrin IX (PP IX) in glial brain tumors is a highly specific criterion for demarcating tumor boundaries, as well as determining the degree of its malignancy [3]. PP IX also has properties of photosensitizer leading to the generation of reactive oxygen species upon absorption of radiation with a characteristic wavelength, which makes it possible to use it both for intraoperative navigation and for photodynamic therapy [4]. However, in 30% of cases, only a slight accumulation of the PP IX in tumor cells was observed, only 10–20% of low-grade gliomas exhibited visible fluorescence with 5-ALA, for example [5], which is due to a number of factors, such as glucose level, tissue oxygen saturation, acidity level, stage of the cell cycle [6]. Due to the above factors that complicate intraoperative navigation using 5-ALA-induced Pp IX, additional techniques are required for intraoperative diagnostics of glial tumors.

A tumor and a healthy brain are characterized by differences in tissue structure and in the degree of blood circulation and oxygenation. Structural changes can be observed at the subcellular and tissue levels.

One of the most widely used prognostic criteria in determining the degree of malignancy of a tumor are the vascular structure changes and, as a consequence, the blood supply of the tumor, usually determined by preoperative MRI. The same parameters can be determined intraoperatively with diffuse reflectance spectroscopy, which makes it possible to extend the conclusions based on preoperative MRI diagnostics to the results of spectral analysis obtained in vivo. Blood supply correlates with the level of vascularization and the degree of malignancy of gliomas. The density of microvessels in glioma can be an independent prognostic factor. The level of hypoxia also correlates with the degree of tumor malignancy.

In [7], several degrees of hypoxia were introduced (pO\(_2\) under physiological conditions is 10% or 75 mm Hg, in this case, moderate hypoxia corresponds to pO\(_2\) equal to 2.5% or 19 mm Hg, average hypoxia 0.5% or 4 mm Hg, severe hypoxia 0.1% or 0.75 mm Hg) and it was shown that tumors of the second degree of malignancy (according to the WHO classification) are characterized by moderate cellular hypoxia (pO\(_2\) ≈ 10%), third degree - moderate to average hypoxia (pO\(_2\) ≈ 10 - 2.5%), while severe hypoxia was observed in 5 out of 12 cases of grade 4 tumors (pO\(_2\) ≈ 0.1%).

When considering the structural changes occurring in nerve tissues with the development of glial tumors, it is necessary to take into account the fact that their main characteristic is the growth along myelinated nerve fibers and blood vessels without the formation of a capsule, which leads to the infiltration of the normal white matter with tumor cells. At the tissue level of organization, glial tumors consist of central and perifocal zones, and are characterized by processes that intensify with the development of the tumor, such as displacement, deviation and destruction of nerve tracts. The central zone of glioblastoma multiforme (the most malignant form of glial tumors) is characterized by the development of necrosis with destructive changes in myelinated nerve fibers. Tumors without necrosis in the center and edema in the perifocal zone, such as benign gliomas (WHO Grades I-II) or anaplastic astrocytoma (WHO Grade III), usually have a more uniform structure throughout the volume.

The number and shape of cell membranes should also be taken into account. About 50% of the white matter is made up of the myelin sheaths of the nerve tracts, which are multilayer lipid membranes (70-85% of dry matter) with protein inclusions (15-30%), which causes a high refractive index of this
structure. It also matters how developed the surface of the astrocyte membranes is, since this factor changes with the densification of cells in the tumor and also affects scattering.

Significant variations in the shape and size of mitochondria, as well as their internal structure, force us to consider in more detail the nature of the change in these parameters during the development of the tumor process. In normally functioning cells, the ratio of the matrix that fills the mitochondria and is characterized by a low content of proteins, that is, with a low refractive index, and membrane structures depends on the amount of ADP, that is, on respiratory activity. So, two states are distinguished – a state of rest (state IV) and a state of oxidative phosphorylation (state III). In the state of oxidative phosphorylation the volume of the matrix decreases by 30%, which thickens the membrane structures and the relative content of proteins in the mitochondria increases. Since the metabolism of tumor cells proceeds mainly along the pathway of glycolysis, mitochondria in it are in the third state. Part of the mitochondria is degraded and destroyed. Works devoted to the structural analysis of mitochondria in astrocytic tumor cells by means of electron microscopy [8] showed a high heterogeneity of their location in the cell, partial or complete destruction of cristae and a decrease in the number of mitochondria.

In light of the above, it becomes obvious that tumors differ significantly from normal tissues not only in structure but also in molecular composition, which can be estimated using such an optical-spectral method as Raman spectroscopy. Raman spectroscopy is inelastic light scattering, which leads to a change in the energy and, consequently, in the frequency of the scattered radiation relative to the incident one. This change is due to the vibrational movements of the molecules, which, in turn, directly depend on the chemical structure that moves.

Raman spectroscopy in neurooncology is an actively developing field, the first studies in which showed encouraging results in the search for molecular differences between tumor and normal tissues. In work [8], biochemical differences between necrosis and viable tumors were found. An elevated level of cholesterol and cholesterol ester has been detected in necrotic tissues. Higher levels of lipids in normal tissues and a higher content of hemoglobin, but a lower ratio of lipids to proteins in intracranial tumors, have been reported [10]. In [11], human glioma tissues were characterized by a higher content of water and reduced content of lipids.

The discussed above structural and biochemical features of brain tumors suggest a broad capabilities of optical spectroscopy for their detection.

2. Materials and methods

2.1. Combined fluorescence and diffuse reflectance spectroscopy

As already mentioned, glial tissues are characterized by complex changes occurring in them during the development of the tumor process, and affecting both the metabolic status of tissues and structural changes at various levels of organization. For a quantitative analysis of these changes by means of optical spectroscopy, it is necessary to compare the spectroscopic characteristics of normal tissues and tumors of various degrees of malignancy.

A similar approach to spectroscopic analysis is indicated in [1]. However, it involves the sequential recording of the background light spectrum, two diffuse scattering spectra and a fluorescence spectrum. The total time required to record all spectral dependencies at each point is about three seconds. Measurements carried out sequentially also do not allow us to reliably state that all of them were obtained from the same point under the same conditions. Another disadvantage of this method is the use of short-wavelength radiation (405 nm) for the excitation of fluorescence, where biological tissue characterized by the low transparency, which leads to fluorescent sensing of tissue volume not exceeding several micrometers and requires preliminary washing of tissue from blood before conducting optical measurement. These disadvantages complicate the procedure for tissue analysis by optical biopsy.

In this work we describe the spectroscopic surgery guidance technique we developed which provides an increase in the reliability of measurements, a decrease in the time of their implementation, an increase in the probing depth, and a simplification of the process of recording spectra in vivo.
For the simultaneous recording of diffuse reflectance and laser-induced fluorescence spectra, a setup was developed that consists of a spectrum analyzer (LESA-01-BIOSPEC), two light sources, helium-neon laser 632.8 nm and a halogen lamp filtered to limit the transmission to the range between 500 and 600 nm (band-pass interference filter FF01-550/88-25, Semrock), fiber-optic probe for delivering light to and from tissue, as well as a personal computer with special software for registration and analysis of spectra in real-time. At the input of the spectrum analyzer, a band-pass filter was installed (“NIOPIK”), which suppressed the backscattered laser light by three orders of magnitude, which made it possible to register the latter in the same dynamic range with laser-induced fluorescent light.

With the aim of a deeper understanding of the processes leading to the formation of recorded spectral dependences, and giving the method predictive power, we have implemented mathematical simulation of the propagation of laser radiation in the tissues of tumors of various degrees of malignancy. The scatterers and absorbers in tissues in various states discussed above were taken into account, both in healthy and in malignant tissues.

In order to evaluate the optical properties of the white matter, the electro-magnetic light theory was used. In 1908 Gustav Mie has published a solution to the problem of light scattering by homogeneous spherical particles [12]. In the present study scattering by rounded-shape tissue components with finite appropriate diameters (cell nuclei and mitochondria), was analyzed by the Mie theory. Another affecting scattering components with a high refractive index – myelinated axons with a more complex structure – for mathematical approximation can be considered as infinite cylinders [13]. In that case, expansions of incident electric and magnetic fields are made in vector cylindrical harmonics [14].

For this study the characteristics of the main scatterers were to be determined: size, refractive index and density. For cell nuclei we can estimate the refractive index (n = 1.39 [15]) as well as mean sizes and density of them for the normal, astrocytoma and glioblastoma tissues (from the histological studies). Mitochondria in glial cells appear with possible size variations from 0.2 to 1.5 µm [16]. Average diameters and density of the myelinated axons of normal white matter were taken from the studies of von Keyserlingk et al. [17], Evangelou et al. [18] and Biedenbach et al. [19], respectively. Stepwise growth of the number of demyelinated axons, leads to the diminution of mean diameter of the fibers up to axonal diameter, related to the thickness of the myelin sheath. The value of the refractive index is also reduced due to the non-myelin axonal consistence, similar to the neuron body, and is about 1.375 [20] in contrast to the refractive index of the myelin sheath (n = 1.455 [21]). Besides, most studies revealed an increase in the average cosine (g) of scattering from normal tissues to tumorous, and raised absorption coefficient at 530-700 nm in the glial tumors due to the higher blood content [22]. The average values of the absorption coefficients and g were taken according to the data presented in [22], and their growth was performed at a gradual form from normal tissues to tumorous. The surrounding media for the main scatterers is represented by microglia, extracellular matrix and cellular cytoplasm with relatively low refractive indices due to their consistence. The simulation results were compared with clinical data on the scattering of laser light by the tissues of glial tumors.

Monte Carlo modeling of light transport in the brain tissue was used in the present study in order to restore the physiological correlates of the spectral changes that we can observe during the development of the tumor process. In mathematical model we considered the back-scattering from semi-infinite media, containing scatterers, absorbers and fluorophores. The propagation of light with two wavelengths – laser and fluorescent – was modeled. Parameters obtained by the Mie calculations were used as the input parameters of the Monte Carlo simulations. The source and collecting fibers had a numerical aperture of 0.37 (in air) at a distance of 250 µm between their centers.

The study of combined spectroscopic technique involved 19 patients (10 men and 9 women aged 29 to 63 years, the average age was 41 years): 14 patients with a diagnosis of glioblastoma, 1 patient with a diagnosis of gliosarcoma, 3 patients with a diagnosis of oligoastrocytoma. 90 patients with Grade II-III-IV intracranial glial tumors were studied by two of 4 parameters (fluorescence and scattering).

After obtaining informed consent, patients took orally a solution of 5-aminolevulinic acid hydrochloride (drug Alasens, manufactured by NIOPIK) at the rate of 25 mg/kg body weight 2-4 hours before the start of tumor removal. For the purpose of intraoperative video fluorescence guidance, a Carl
Zeiss Pentero microscope with a fluorescent module was used, which allows to qualitatively determine the degree of accumulation of 5-ALA-induced PP IX in tumor tissue as compared to normal tissue. From 1 to 8 tissue samples were taken from each patient for subsequent histological analysis and comparison of its results with the data of spectroscopic examination. Thus, 53 tissue samples were analyzed, of which 41 samples from patients with glioblastoma, 1 sample from a patient with gliosarcoma, 5 samples from patients with anaplastic astrocytoma, 4 samples from anaplastic oligoastrocytoma, 2 samples from a patient with oligoastrocytoma. For the proper comparison the intraoperative spectroscopic analysis was carried out in vivo in the area of tumor removal and after that the tissue sample from the exactly same place was subjected to histological examination.

2.2. **Spontaneous Raman scattering spectroscopy**
Raman spectra of the biological material extracted during neurosurgery were obtained from 8 patients with a diagnosis of glioblastoma (WHO Grade IV). Of the measured samples, only those that were morphologically classified as either brain tissue, tumor center or tumor edge were included.

The spectra were measured ex vivo no more than 4 hours after material removal. To obtain Raman spectra, a laser with a radiation wavelength of 785 nm (Ramulaser-785, StellarNet, USA) and a Raman-HR-Tec spectrometer (StellarNet, USA) with fiber-optic radiation delivery were used. The used spectrometer makes it possible to measure Raman spectra in the range of 300-2700 cm\(^{-1}\).

The output power of the laser radiation was set at 150 mW. Each spectrum was measured with an exposure of 30 sec. Before measuring the Raman spectra, the background signal was measured five times for subsequent subtraction. The Raman spectra from each sample were also recorded 5 times. After that, those spectra were excluded, the values of individual pixels of which exceeded 80% of the dynamic range of the spectrometer in order to avoid "flare" and distortion of the signal shape.

For each sample, the spectra were processed as follows: the averaged dark spectrum was subtracted from each Raman spectrum in the series. To smooth out the remaining noise, a third-order Savitsky-Golay filter with a running window width of 21 pixels was used. The filter approximates the data inside the window with a polynomial function and returns a value equal to the value of the polynomial in the middle of the running window.

The next step was to exclude the fluorescent signal that arose when the material under study was irradiated. The fluorescent signal is characterized by a high intensity compared to the Raman signal, and also has a bell-shaped shape that is much wider than the Raman peaks. To subtract it, we used the algorithm described in [23], which approximates the background signal by a set of Morlet wavelets of various widths.

To classify the processed Raman spectra, the data was dimensionally reduced using Principal component analysis (PCA) where each successive principal component seeks to describe the maximal variation between the data points. The first two principal components were used to visualize and classify the data. The classification was done with support-vector machines (SVM) learning model with radial basis function kernel by taking 70% of the measurements as a training dataset.

2.3. **Neurosurgical aspirator with fiber optics for spectroscopic guidance**
To simplify the procedure of spectroscopic navigation during tumor removal, the authors proposed a technical solution that combines the functionality of a standard neurosurgical aspirator tip and a fiber optic probe. Several technologies were considered for embedding optical fibers into the aspirator tip: both into the thickness of the tip wall evenly or in groups along the perimeter, and into an additional channel parallel to the aspiration channel. All of these designs have been tested and proven to be beneficial for a variety of applications. The most ergonomic option is with fibers built into the wall thickness of the aspirator tip (Fig. 1), since such a tip is almost the same as the usual one, that is, it does not require the surgeon to get used to the new instrument.

For spectroscopic analysis the laser fiber optic spectrometer LESA-01-BIOSPEC was used. Two light sources and a system of optical filters provided the simultaneous registration of fluorescence and diffuse reflectance spectra. The concentration of 5-ALA induced protoporphyrin IX, total hemoglobin
and level of oxygen saturation of hemoglobin were calculated in real-time from these spectra during the operation. The proposed method was appribrated in a laboratory with optical phantoms containing protoporphyrin IX, hemoglobin and scattering media in physiologically relevant concentration and in clinical conditions ex vivo on samples of biological tissues obtained during the removal of glial tumors.

![Figure 1. Draft of working part of the spectroscopic device coupled with the cannula of neurosurgical aspirator.](image)

Based on the results of the approbation, the ability of simultaneous detection of spectral dependencies from biological tissues and aspiration of tissues was shown. Thus, the device provides the simultaneous spectroscopic analysis of tissues with their aspiration, which leads to an increase in the accuracy of spectroscopic diagnostics in the area of neurosurgical aspiration, and also improves the quality of the surgeon's work.

2.4. Stereotactic biopsy cannula with fluorescence guidance

A traditional biopsy method has a significant percentage of false-negative results. The reason for this can be in various grades of malignancy of different parts of a tumor, a mismatch between the true location of the tumor and the location calculated from the MRI or CT images. In addition, the stereotactic biopsy may be accompanied by life-threatening complications such as intracerebral hemorrhage vascular lesions on the path of biopsy cannula. Currently, there is no possibility of identifying the vessels during the movement of the biopsy cannula.

We have developed new stereotactic biopsy needle that combines stereotactic biopsy and spectroscopic control procedures (Fig. 2a). New device helps to eliminate such disadvantages.

In current clinical practice, spectroscopic control with protoporphyrin IX as a tumor marker is widely used. Protoporphyrin IX accumulation in tumors induced by injection of 5-aminolevulinic acid, which selectively accumulates in tumor tissues due to enzymatic disorders in rapidly proliferating tumor cells. Simultaneous applying stereotactic biopsy and spectroscopic control could help to increase the efficiency and safety of stereotactic biopsy.

We developed a special stereotactic biopsy cannula with a laser spectroscopic control, which allows evaluating the type of tissue through which passes biopsy cannula, to identify the most informative area for biopsy, and to promptly change the trajectory of the cannula with the appearance of blood vessels. Thus, the developed system can increase diagnostic accuracy (sensitivity) of the method of stereotactic biopsy of brain tumors and reduce the risk of complications associated with this procedure.
The new stereotactic needle includes two fibers with a diameter of 200 and 600 μm (Fig. 2b). The 200mcm fiber transmits laser radiation from the laser to a tissue. After leaving the fiber, the laser radiation is reflected 90 degrees by a mirror towards the tissue. The 600 μm fiber transmits fluorescence light to the spectrometer.

The developed device was used in stereotactic biopsy procedure and helped to achieve a positive result [24]. Application of the device allowed to obtain new information about brain tissue state and to correct data for further tumor resection.

2.5. Fiber-optic scaffold for targeted tumor growth with fluorescence control

For targeted tumor growth and the possibility of postoperative monitoring of tissue condition and local photodynamic exposure, an implant with a fiber optic probe for postoperative installation was developed.

To study the process of bio-integration of the scaffold a series of experiments was carried out on sexually mature female Wistar rats weighing 200-220 g at the beginning of the experiment, in which glioblastoma multiforme was simulated by stereotaxic implantation of 5x10^5 C6 glioma cells into the striatum region. As a photosensitizer, we used the drug Phthalosens (FSUE SSC NIOPIK, Russia) based on a metal-free sulfonated phthalocyanine derivative, which is a good photocatalyst for oxidation processes, which is necessary for effective fluorescence diagnostics and photodynamic therapy. The photosensitizer was administered to experimental animals intravenously at a dose of 5 mg/kg.

Fluorescence diagnostics was carried out by exposure to laser radiation with a power density of 100 mW/cm² and λ = 632.8 nm. Using laser scanning confocal microscopy (Zeiss LSM 710 NLO), the processes of growth of C6 malignant glioma cells and their localization on the surface of optical fibers
were visualized. Photodynamic therapy using a metal-free sulfonated phthalocyanine derivative \( c = 5 \text{ mg/kg} \) was carried out by exposure to laser radiation \( \lambda = 675 \text{ nm} \), a dose of \( 200 \text{ J/cm}^2 \).

3. Results and discussion

3.1. Results of using combined fluorescence and diffuse reflectance spectroscopy in neurooncology

An analysis of the diffuse reflectance and fluorescence spectra from different tumors, carried out on a sample of 19 patients, is shown in Table 1. Blood supply and oxygenation are calculated on the basis of the analysis of the diffuse reflectance spectra in the range of 500-600 nm by decomposing the spectral dependence of diffusely reflected light into components corresponding to the absorption spectra of reduced and oxygenated hemoglobin and the background due to the contribution of scattering. The calculated values for the studied tissues are represented relative to the values for the normal cortex. The scattering properties of tissues are estimated by the intensity of backscattered laser light (in the biological transparency window) and are given relative to the value for the normal cortex. The fluorescence index is calculated as the ratio of the fluorescence intensity of PP IX in the range of 690-730 nm to the intensity of backscattered laser light. The fluorescence contrast is calculated as the ratio of the fluorescence index of the studied tissue to the fluorescence index of the normal cortex.

Table 1. Intraoperatively measured mean values for hemoglobin content and saturation and median values (with interquartile ranges) for the scattering and fluorescence of tumor tissues (in relation to the corresponding values for normal tissue).

| Diagnosis                                | Hemoglobin content | Saturation | Scattering | Fluorescence |
|------------------------------------------|--------------------|------------|------------|--------------|
| Glioblastoma multiform                   | 4.9±4              | 0.8±0.4    | 0.37 (0.3) | 17.6 (19.1)  |
| Anaplastic astrocytoma                   | 1.3±0.8            | 0.4±0.3    | 0.19 (0.21) | 12.2 (15.6)  |
| Grade II gliomas (diffuse astrocytoma and oligoastrocytoma) | 0.7±0.5            | 0.4±0.3    | 0.32 (0.24) for diffuse astrocytoma 0.52 (0.55) for oligoastrocytoma | 1.46 (3.3) |

Figure 3. The distribution of relative fluorescence (a) and relative scattering (b) values for glial tumors of different grades.

For all types of astrocytic tumors, spectral analysis showed an increase in the fluorescence level of 5-ALA-induced protoporphyrin IX. Since determining the degree of tumor malignancy is one of the key points of intraoperative spectroscopic guidance, additional diagnostic criteria must be used. The most
informative criterion for differentiating low-grade and high-grade gliomas is a change in the scattering properties of tumor tissue compared to normal. The results of the analysis of the scattering properties of the studied tissues showed a tendency for a decrease with local minimum for anaplastic astrocytoma. For low-grade glial tumors with an oligocomponent this indicator is on average higher than for the other types of tumors. The blood supply of tumor tissue is also an important diagnostic parameter, since it has been shown to correlate with the degree of malignancy. This sample showed a significant difference between the blood supply of glioblastoma tissues from the blood supply of the tissues of anaplastic astrocytoma and oligoastrocytoma.

Table 2. Comparison of simulation data of the diffuse reflectance of altered tissues in relation to normal tissues and the results of in vivo spectroscopy.

| Diffuse reflectance signal relative to normal tissue | WHO Grade II-III | WHO Grade IV |
|---------------------------------------------------|-----------------|--------------|
|                                                   | Tumor center    | Tumor border | Tumor center | Tumor border |
| Monte-Carlo modelling                              | 0.22±0.001      | 0.41±0.002   | 0.56±0.001   | 0.28±0.001   |
| in vivo                                           | 0.21±0.02       | 0.37±0.02    | 0.53±0.02    | 0.26±0.02    |

Both in a numerical experiment and in in vivo spectroscopic studies, differences in the light scattering properties were observed for the center and edge of the tumor of various degrees of malignancy (Table 2).

3.2. Results of neuroscaffold approbation on laboratory animals

Polymeric constructions of the scaffold with an internal fiber-optic structure were fixed subcutaneously on the cranium so that the base of the microsensor and the end of the fiber-optic internal part were located in the tumor bed of experimental animals. The scaffold installed in the tumor bed served as a port for local delivery of diagnostic and therapeutic laser radiation, which made it possible to monitor the state of experimental animals after implantation using fluorescent diagnostics based on a metal-free sulfonated phthalocyanine photosensitizer, excitation by radiation with $\lambda_{ex} = 632.8$ nm. It was shown with spectral methods that the internal fiber structure contributes to the tracking of cell growth.

The high sensitivity and efficiency of spectral methods for the early detection of glioma cells in close proximity to the microsensor structure using a fluorescent signal has been experimentally established. Hollow polymer constructs were also tested on experimental animals and were fixed subcutaneously on the cranium so that the base of the scaffold and the cavity were located in the tumor bed of rats, which was monitored by MRI. As a result of photodynamic therapy (with metal-free sulfonated phthalocyanine, excitation by radiation with $\lambda = 675$nm), a high therapeutic effect was achieved (assessed in terms of survival in comparison with the control group) and constant access to the tumor bed was provided for monitoring the condition.

The results obtained on the brain of rats with induced tumors (C6 glioma) after the installation of the working part of the neuroscaffold demonstrate rather intense fluorescence in the tumor bed after intravenous injection of a photosensitizer and a pronounced photodynamic effect, which led to total destruction of the tumor, and also suggest effective monitoring of the process through micro-optical detection.
Figure 4. 3D reconstruction of fluorescent images of cell growth along the optical fiber: a) single cell of C6 glioma (highlighted area); b) clusters of C6 glioma cells stained with acridine orange (AO) (green - image of living cells) and propidium iodide (PI) (red - image of dead cells).

3.3. Results of using spontaneous Raman spectroscopy in neurooncology

Based on the work [25], it was decided to consider the range 900-1800 cm\(^{-1}\), in which the peaks of biological molecules characteristic of the substance of the brain, as well as of malignant tissue, are located. In the range of fingerprints, the Raman spectra are well developed, which complicates visual analysis. However, with some experience, it is possible to visually highlight the most pronounced components, such as peaks of lipids or nucleic acids.

In order to better visually distinguish the spectra of glioblastoma from brain tissue, the averaged spectra for each tissue type are presented. This, of course, levels out individual differences but allows us to highlight those peaks that are characteristic for all spectra in the class (Fig. 5).

We see in such averaged spectra the main maxima in the vicinity of 1000 cm\(^{-1}\), 1100 cm\(^{-1}\), 1250 cm\(^{-1}\), a pronounced multiplet in the vicinity of 1450 cm\(^{-1}\).

Figure 5. Averaged Raman spectra of various types of tumors.
Gliomas are characterized by a higher content of water and hemoglobin, reduced content of lipids and a low ratio of lipids to proteins. In the Raman spectrum of normal brain tissue, strong peaks of lipids (1255, 1259, 1465, 1657 cm\(^{-1}\)) and cholesterol (1659 cm\(^{-1}\)) can be seen. Similarly, in the normal tissue, carotenoid (1008 cm\(^{-1}\)) and glycogen peaks (1022, 1048, 1150 cm\(^{-1}\)) were higher. The peaks corresponding to CH2 scissoring (1436 cm\(^{-1}\)), DNA (1490 cm\(^{-1}\)) and symmetric phosphate stretching vibrations (1090 cm\(^{-1}\)) were higher in gliomas. The gliomas had a lower lipid concentration than normal tissue and an increased protein content (1035, 1206, 1441, 1560, 1616 cm\(^{-1}\)).

After reducing the dimensionality of the Raman spectra data and training the SVM model, the PC-space separation was obtained (Fig. 6). The reclassification of the data by the trained model showed 86% accuracy. But when considering that both the tumor center and the edge are subject to surgical removal, the accuracy of determining malignant tissue was 93%.

![Figure 6. Visualization of classification results for different sites of glial tumors.](image)

This work presents the results of using principal component analysis for the classification different sites of glial tumors using Raman spectroscopy data obtained \textit{ex vivo} with intermediate processing of the spectra to minimize possible errors from the fluorescence of both endogenous fluorophores and photosensitizers used in fluorescence intraoperative guidance. As a result, differences were found corresponding to tissue samples from central and peripheral areas of tumors in the same fashion as it was done for combined spectroscopic analysis described earlier. It is shown that this approach can serve as a basis for constructing a system for automatic intraoperative tissue classification based on the analysis of Raman spectra.

4. Conclusion
The proposed method for the simultaneous analysis of the concentration of hemoglobin in oxygenated and reduced forms, the concentration of a tumor marker (5-ALA induced protoporphyrin IX), as well as changes in the scattering properties of the studied tissues, implemented due to the spectral division of the visible spectrum into intervals in which these chromophores and fluorophores have characteristic peaks of absorption and fluorescence, showed the possibility of differentiation of tumors similar in the degree of fluorescence manifestation of 5-ALA-induced protoporphyrin IX, but differing in other parameters available for analysis using this method.

Thus, based on the statistical analysis of spectroscopic data, depending on the degree of tumor malignancy, as well as on the degree of infiltration of healthy tissue by tumor cells, carried out in comparison with the results of the morphological examination, it can be concluded that this technique is applicable for intraoperative analysis of structural and metabolic changes occurring with the development of glial tumors.
The using of principal component analysis for the classification of Raman spectra obtained \textit{ex vivo} from different sites of glial tumors showed the possibility to distinguish between tissues with different lipid and protein content what is extremely important for tumors which do not accumulate a photosensitizer.

The presented results of the classification of spectroscopic data when compared with the morphology results, showed the possibility of intraoperative demarcation of glioblastoma boundaries, since in the space of the studied characteristics, the classes of necrosis, the tumor itself, and the zone of infiltration have statistically significant differences.

The developed special instruments with the possibility of optical-spectral control before, during and after neurosurgical operations will make it possible to introduce the optical biopsy technique into wide clinical practice to assist in making a diagnosis, intraoperative navigation and postoperative monitoring.

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