Spontaneous Raman Spectroscopy for Intracranial Tumor Diagnostics

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Abstract. Surgical removal of glial tumors is a challenge due to their infiltrative growth. At the moment, the main way to solve this problem is fluorescent intraoperative navigation. However, in the absence of accumulation of a fluorescent marker in tumor tissues, other diagnostic parameters are required to find the boundaries of such tumors. Raman spectroscopy has the advantages of optical spectroscopy such as speed and non-invasiveness. With an exhaustive database of reference spectra of those components that can be expressed in glial tumors, Raman spectroscopy allows for diagnostics of such tumors and their intraoperative demarcation. The purpose of this work was to study healthy brain tissues and intracranial tumors of laboratory animals and humans in order to detect significant differences using spectroscopy of spontaneous Raman scattering. Also, the analysis of cultures of tumor and immunocompetent cells was carried out. The results of the study showed significant differences in the spectral lines related to lipids, proteins and nucleic acids, which can be used to develop an optical biopsy method of brain tumors based on Raman spectroscopy.

1. Introduction

Despite intensive study, glial brain tumors are a very serious disease and survival rates are low. The two most common brain tumors, medulloblastoma and glioblastoma, arise from neuronal and glial lines, respectively. Patients with WHO grade II tumors usually live less than 5 years, with WHO grade III tumors - 2-3 years, in the case of grade IV glioblastoma, the official survival prognosis is less than 15 months. Early diagnostics of glial tumors helps to identify tumors, effectively treat them and increase the life span. There are invasive and non-invasive methods for assessing the degree of malignancy of glial tumors. Currently, the diagnosis includes computed tomography, magnetic resonance imaging, electroencephalography. Unfortunately, surgery is not the final step of treatment, because cancer cells that are not removed during the operation can lead to a relapse. Also the removal of healthy tissues can lead to cognitive impairment in a patient. Thus, to increase the completeness of the removal of such tumors, tools that work in real time and are sensitive to tissue biochemistry are needed. Changes in biochemistry are reflected in the optical properties of tissues, which can be used during early diagnostics, as well as for intraoperative navigation. Therefore, in the last decade, optical methods for non-invasive and objective diagnosis of certain types of tumors, in particular Raman scattering (RS) spectroscopy and microscopy techniques, have become widespread. RS occurs due to the fact that light is inelastically scattered by a molecule, with a small amount of energy transferring

from photon to molecule (or vice versa). The difference between the energies of the incident and scattered photons is expressed in the form of a wavenumber shift (cm^{-1}). The spectrum of the scattered radiation contains spectral lines, the number and position of which can determine the molecular structure and composition of the substance. RS is an effective method of chemical analysis, the study of the composition and structure of substances. Due to minimal interference from water, which is the main component of living organisms, RS can serve as a tool for in vivo research.

The very first results of a brain tumor analysis based on RS were obtained by Tashiba [1]. In his work, he investigated the relative concentration of water in the normal and edematous tissues of the rat brain by analyzing the groups of CH and OH in the high wavenumber region. In works [2], [3] spectra of various brain tumors, characterized by a high content of lipids, were published. These early studies gave impetus to further study of human biopsy specimens. In work [4], pig brain tissue was characterized and the bands associated with proteins, DNA and phosphatidylcholine dominate in the analyzed Raman spectra of gray matter, while cholesterol and sphingomyelin dominate in the spectra of white matter.

In the above-mentioned work [3] the authors used RS and Fourier transform to study various samples of human brain tissue and obtained that the spectra from the normal, but edematous gray and white matter were similar to the spectra from the gray and white matter of the rat. It was observed that the spectra from gliomas and neurinomas are similar to the spectra of rat gray matter. In work [5], biochemical differences between necrosis and viable tumors were found. An elevated level of cholesterol and cholesterol ester has been detected in necrotic tissues. In publications [6], [7], 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. In [8], human glioma tissues were characterized by a higher content of water and a reduced content of lipids, and the results were similar to those observed in pig tissue. In addition, with the help of RS, it was found that the ratio of phosphatidylcholine and cholesterol was increased in gliomas compared with healthy tissues.The use of RS in [9] helped to distinguish neoplasms of the brain in children from normal brain tissue and similar types of tumors from each other. Thus, the RS can be used to differentiate normal and tumor tissues, as well as to determine the type and degree of malignancy of the tumor.

In recently published work related to intraoperative in vivo studies of brain tumors [10], the Raman spectra of healthy brain tissues, tumors and necrotic tissues (10 patients) were obtained under natural conditions, in real time. Differences in cholesterol, phospholipid, protein and nucleic acid levels were found.

2. Materials and metods

2.1. Experimental setup

Raman spectra were obtained using a system that included Raman-HR-TEC-785 spectrometer (StellarNet, USA), Ramulaser™ 785 laser source (StellarNet, USA), a fiber-optic confocal probe for delivering laser radiation and a RS signal, computer with SpectraWiz software. The spectrometer has a spectral resolution of 4 cm^{-1}. The spectral range corresponds to 200 - 2750 cm^{-1}. The laser peak width is 0.2 nm; power up to 500 mW.

2.2. Test specimens

As a model object, samples of the biological tissues of the brain of laboratory animals were examined: mouse and rat. Investigated intracranial tumors of the human brain - meningioma and glioma, which were provided by the N.N. Burdenko National Medical Research Center of Neurosurgery.

3. Result and discussion

3.1. Animal tissue samples

Based on the information obtained in [6-8], to determine these reference spectra in glial tumors, spectra of physical models of individual components of glial tissues, such as fat emulsion and serum spectra, were obtained. The proposed method for analyzing was tested on the brain of laboratory animals (Figure 1).

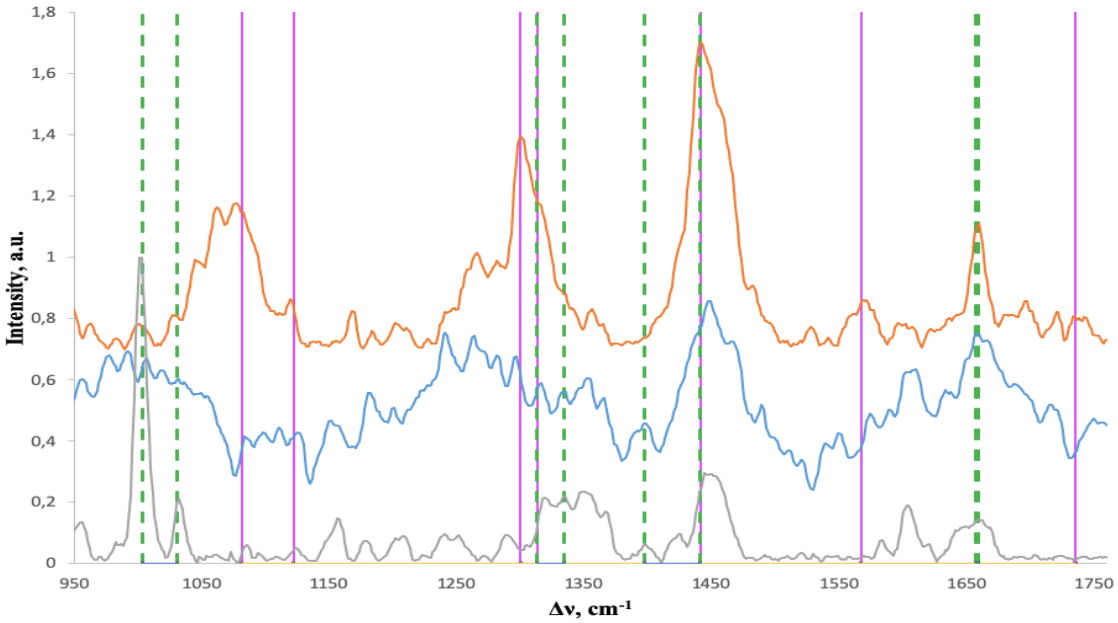

Figure 1. Raman spectra of healthy mouse brain (blue), intralipid (orange) and blood serum (gray). Markers of lipids (pink) and proteins (green dashed line).

Comparison of results showed the presence of characteristic peaks of proteins (1003, 1030, 1206, 1236, 1252, 1334, 1397 cm^{-1}) and fats (1061, 1081, 1122, 1300, 1313 cm^{-1}) in the Raman spectra of the mouse brain.

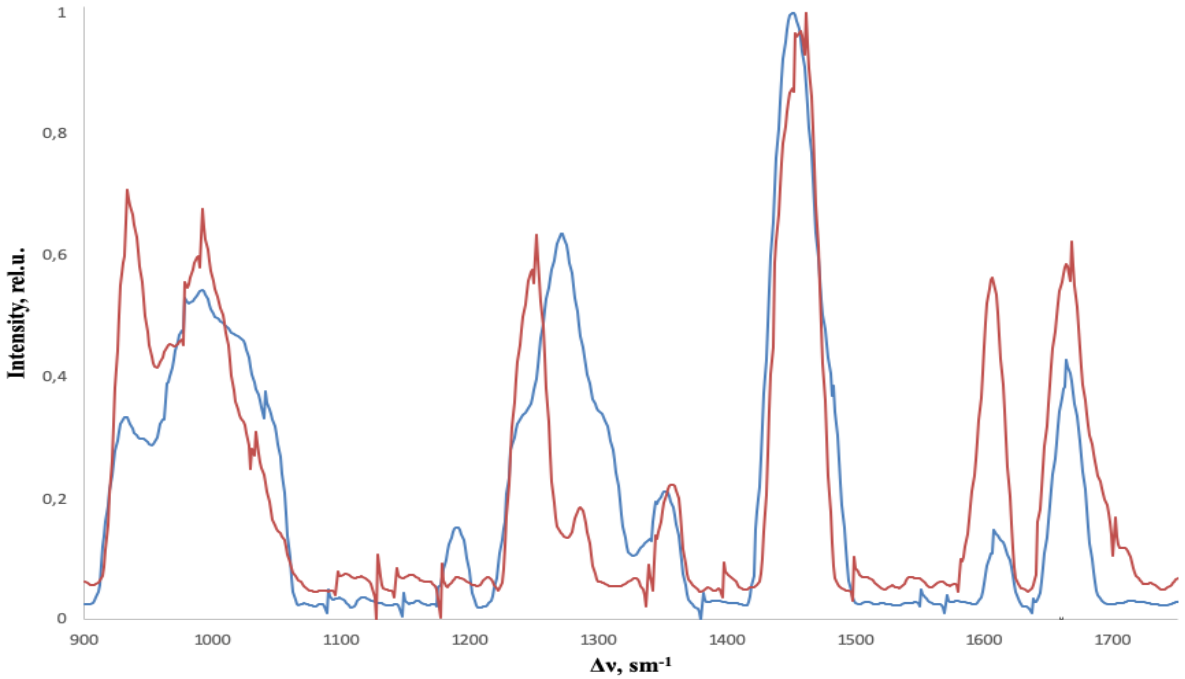

Figure 2. Raman spectra of rat brain (gray matter - red, white matter - blue). Markers of lipids (green), proteins (purple touches) and cholesterol (blue touches).

The Raman spectra of healthy rat brain from the side of white and gray matter are presented in Figure 2. Gray matter is characterized by a large contribution of proteins (1003, 1239, 1634 cm^{-1}) due to the predominance of the neuron bodies. White matter is characterized by a large contribution of lipids (1060, 1300 cm^{-1}) due to the membranes of the nerve tracts [11].

3.2. Meningioma

The RS spectra of meningiomas of the human brain in formalin were recorded and investigated. The obtained Raman spectra of meningiomas were compared with the Raman spectra of normal tissue (Figure 3). As a model object, instead of the norm, a healthy mouse brain in formalin was used. The main spectral features were observed for β-carotene (1157, 1520 cm^{-1}), amide bonds (1302, 1337 cm^{-1}), CH_2 deformation (1337, 1398 cm^{-1}), DNA (1080, 1340, 1420 cm^{-1}), tryptophan (1360 cm^{-1}), phenylalanine (1004, 1204 cm^{-1}) and lipids (1300 cm^{-1}) [12].

The spectra of both healthy tissue and meningiomas show a significant contribution of amino acids, proteins, DNA, lipids, β-carotene. Peaks of the main DNA chain (1080 cm^{-1}) and nitrogenous bases (1340 cm^{-1}) were significantly more intense in meningiomas compared to the healthy tissues. The presence of intense DNA peaks in meningioma may indicate a high level of cell-free DNA in tumors. Similarly, the peaks corresponding to the amide bonds (1337 cm^{-1}) were more intense in meningiomas. Peaks corresponding to CH_2 bending are more pronounced in meningioma, which indicates a high level of proteins and lipids. On the other hand, the peaks for phenylalanine (1204 cm^{-1}) were more intense in healthy tissue samples.

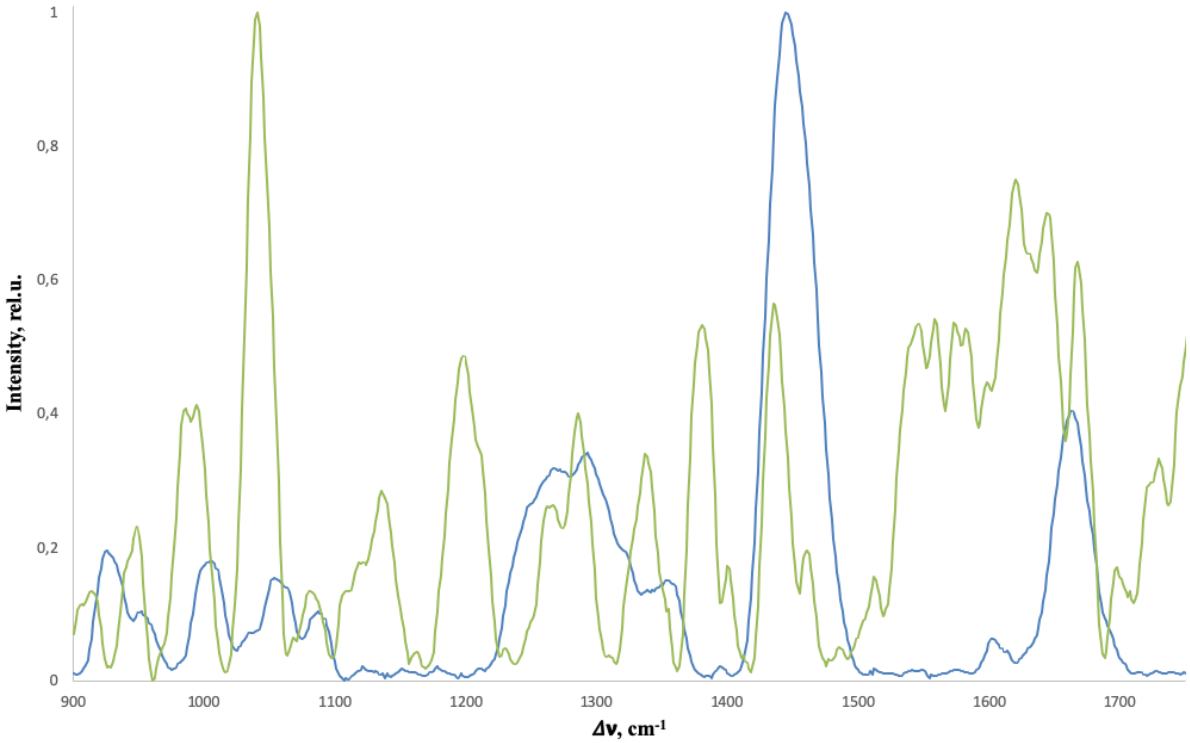

Figure 3. Raman spectra of meningiomas (green) and normal tissue (blue).

3.3. Microglia

Microglia cells are immune cells of the central nervous system (CNS), which serve as tissue-resident macrophages of the brain. Various pathological events in the CNS such as injuries, infections, or tissue damage lead to activation of the microglia cells. The induction of an inflammatory response by M1-like microglia is tightly regulated through a subsequent polarity transition to an anti-inflammatory M2-like phenotype [13]. Unfortunately a large proportion of immune cells in the tumour microenvironment are subverted by glioma cells for tumour growth through the process of M2-polarization. So, distinguishing the different states of microglia cells in the tumor microenvironment is of great importance for tumor diagnostics and therapy.

In [14], microglia were analyzed in the BV-2 cell line and it was shown that the main difference between the polarization types M1 and M2 was observed at peaks of 813 cm^{-1} and 1078 cm^{-1}, which may be due to the activity of RNA and lipid content. Also using bioimaging approach to raman scattering, it was shown that activation of microglia leads to accumulation of cellular proteins and lipids, apparently due to upregulation of synthesis of these biomolecules [15]. So the possibility of monitoring of these parameters is quite a good technique to analyze the activation of microglial cells and predict the tumor aggressiveness.

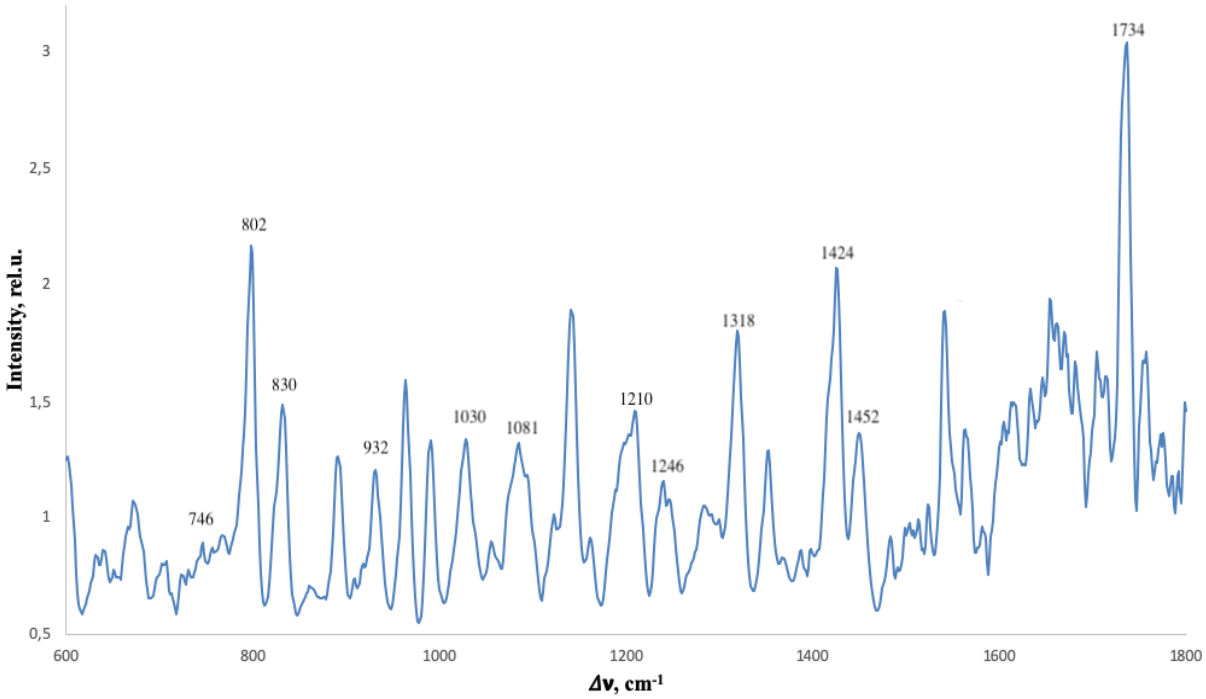

Figure 4. Raman spectra of microglia.

The measured in our work Raman spectra of glial cells had a developed structure in the spectral region of 600–1800 cm^{-1} (Figure 4). By focusing on the region of 700–830 cm^{-1}, one can observe various spectral signatures of base pairs of nucleic acids [16]: 746 cm^{-1} (ring breathing mode of DNA/RNA bases), 766 cm^{-1} (pyrimidine ring breathing mode), 802 cm^{-1} (uracil-based ring breathing mode) and 830 cm^{-1} (proline, hydroxyproline, tyrosine). There are characteristic peaks for carbonyl bonds (C=O, 1663 and 1736 cm^{-1}). The results showed the presence of characteristic peaks of proteins (932, 1030, 1206, 1210, 1246, 1318, 1452 cm^{-1}) and fats (1081, 1313 cm^{-1}). A noticeable peaks were observed at 1424 cm^{-1} (deoxyribose) and 1734 cm^{-1} (esters, C=O stretching).

3.4. Glioma

The Raman spectra of a glial tumor of the human brain have been recorded and investigated. We compared the obtained Raman spectra of glioma with the Raman spectra of normal tissue (Figure 5). Instead of the norm, a healthy rat brain was used as a model object. For this study, only the "gray matter" was used in the "normal" category (the white matter area was excluded). Previous studies have shown a clear biochemical and Raman difference between gray and white matter. Similarly, areas with heavy bleeding were excluded due to tissue damage, since the hemorrhage has its own RS signature.

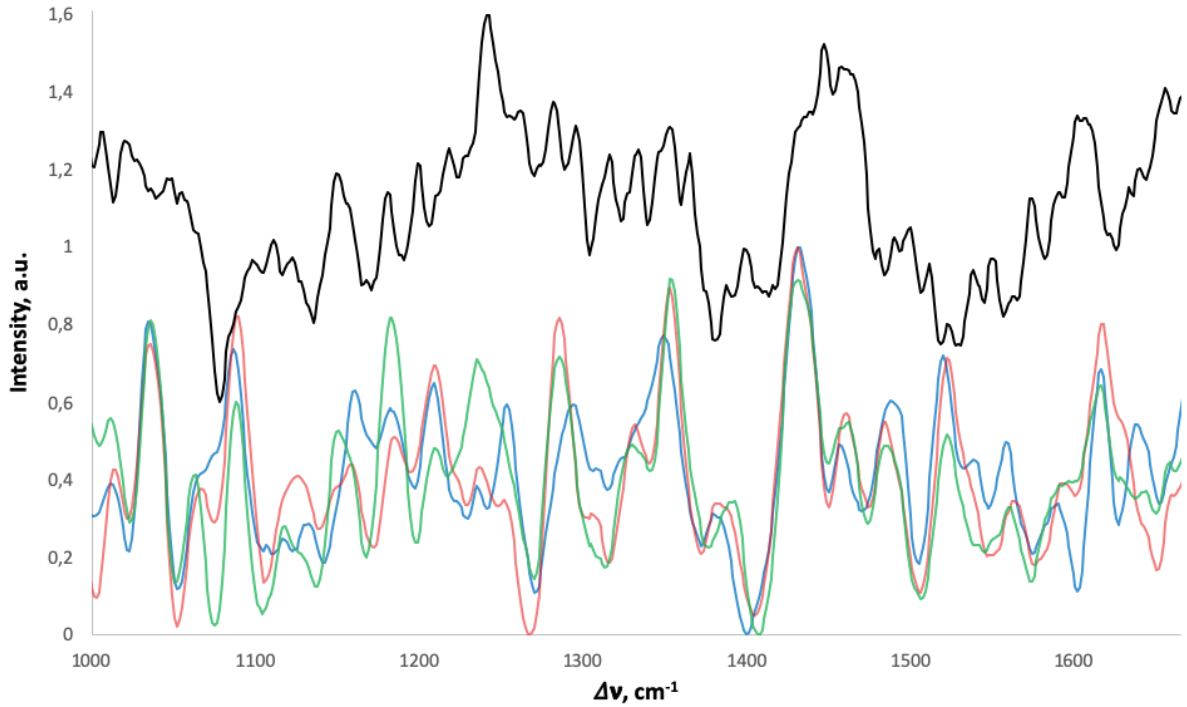

Figure 5. Raman spectra of a different areas glial tumor: GBL1 (red), GBL2 (green), GBL3 (blue) and normal tissue (black). The normal tissue spectrum was shifted vertically to highlight differences.

Gliomas are characterized by a higher content of water and hemoglobin, a reduced content of lipids and a low ratio of lipids to proteins [6-8].

In the RS 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 increased. The peaks corresponding to to CH_2 scissoring (1436 cm^{-1}), DNA (1490 cm^{-1}) and symmetric phosphate stretching vibrations (1090 cm^{-1}) were more intense in gliomas. The gliomas had a lower lipid concentration compared with normal tissue and an increased protein content (1035, 1206, 1441, 1560, 1616 cm^{-1}).

4. Conclusions

A set of reference RS spectra has been formed for models of glial tumors components. The Raman spectra of the nervous tissues of laboratory animals were obtained and spectral maxima typical of these components were detected. The Raman spectra of microglial cell lines were also obtained. The results of a comparison of the RS spectra of samples of the normal brain of laboratory animals and the RS spectra of samples of glial tumors and meningiomas showed a significant difference in the spectra, among others differences it was shown that gliomas had a lower lipid concentration compared with normal tissue and an increased protein content.

References

[1] Tashibu K Analysis of water content in rat brain using Raman spectroscopy 1990 *No to shinkei= Brain and nerve*, *42*(10), 999-1004.

[2] Mizuno A, Hayashi T, Tashibu K, Maraishi S, Kawauchi K and Ozaki Y Near-infrared FT-Raman spectra of the rat brain tissues 1992 *Neuroscience letters*, *141*(1), 47-52.

[3] Mizuno A, Kitajima H, Kawauchi K, Muraishi S and Ozaki Y Near-infrared Fourier transform Raman spectroscopic study of human brain tissues and Tumours 1994 *Journal of Raman spectroscopy*, *25*(1), 25-29.
[4] Koljenović S, Bakker Schut T C, Wolthuis R, Vincent A J P E, Hendriks-Hagevi G, Santos L and Puppels G J Raman spectroscopic characterization of porcine brain tissue using a single fiber-optic probe 2007 *Analytical chemistry*, *79*(2), 557-564.
[5] Koljenović S, Schut T C B, Kros J M, van den Berge H J and Puppels G J Discriminating vital tumor from necrotic tissue in human glioblastoma tissue samples by Raman spectroscopy 2002 *Laboratory Investigation*, *82*(10), 1265.
[6] C Krafft, L Neudert, T Simat & R Salzer. Near infrared Raman spectra of human brain lipids. 2005. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *61*(7), 1529-1535.
[7] C Krafft, S B Sobottka, G Schackert & R Salzer. Near infrared Raman spectroscopic mapping of native brain tissue and intracranial tumors. 2005. *Analyst*, *130*(7), 1070-1077.
[8] M Köhler, S Machill, R Salzer & C Krafft. Characterization of lipid extracts from brain tissue and tumors using Raman spectroscopy and mass spectrometry. 2009. *Analytical and bioanalytical chemistry*, *393*(5), 1513-1520.
[9] Leslie D G, Kast R E, Poulik J M, Rabah R, Sood S, Auner G W and Klein M D Identification of pediatric brain neoplasms using Raman spectroscopy 2012 *Pediatric neurosurgery*, *48*(2), 109-117.
[10] Desroches J, Jermyn M, Mok K, Lemieux-Leduc C, Mercier J, St-Arnaud K and Leblond F Characterization of a Raman spectroscopy probe system for intraoperative brain tissue classification 2015 *Biomedical optics express*, *6*(7), 2380-2397.
[11] Kast R, Auner G, Yurgelevic S, Broadbent B, Raghunathan A, Poisson L M and Kalkanis S N Identification of regions of normal grey matter and white matter from pathologic glioblastoma and necrosis in frozen sections using Raman imaging 2015 *Journal of neuro-oncology*, *125*(2), 287-295.
[12] Mehta K, Atak A, Sahu A and Srivastava S An early investigative serum Raman spectroscopy study of meningioma 2018 *Analyst*, *143*(8), 1916-1923.
[13] Roesch S, Rapp C, Dettling S and Herold-Mende C When immune cells turn bad-tumor-associated microglia/macrophages in glioma 2018 *International journal of molecular sciences*, *19*(2), 436.
[14] Schütze K, Gangnus R, Klein B, Aigner L Raman spectroscopy as tool for label-free identification of microglial activation *8th International Symposium on Neuroprotection and Neurorepair*, April 9th – 12th, 2014 in Magdeburg
[15] Dessai C V, Pliss A, Kuzmin A N, Furlani E P and Prasad P N Coherent Raman spectroscopic imaging to characterize microglia activation pathway 2019 *Journal of biophotonics*, *12*(5), e201800133.