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DALIA KAŠKELYTĖ

APPLICATION OF FLUOROMETRIC TECHNIQUES FOR DIAGNOSTIC OF TISSUE PATHOLOGIC CHANGES

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Scientific supervisor:
prof. dr. Roaldas Gadonas (Vilnius University, physical sciences, physics – 02P)

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Chairman – prof. dr. Vida Kirvelienė (Vilnius University, physical sciences, biochemistry – 04P)

Members:

prof. habil. dr. Juozas Vidas Gražulevičius (Kaunas University of Technology, chemistry – 03P)
prof. habil. dr. Ričardas Rotomskis (Vilnius University, physical sciences, physics – 02P)
dr. Virgilijus Vaičaitis (Vilnius University, physical sciences, physics – 02P)
prof. habil. dr. Gediminas Gaigalas (Lithuanian University of Educology, physical sciences, physics – 02P)

Opponents:

doc. dr. Saulius Bagdonas (Vilnius University, biomedical sciences, biophysics – 02B)
prof. habil. dr. Eugenijus Šatkovskis (Vilnius Gediminas Technical University, physical sciences, physics – 02P)

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Address: Saulėtekio ave. 9, bldg. 3, LT-10222 Vilnius, Lithuania.

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DALIA KAŠKELYTĖ

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Pirmininkas – prof. dr. Vida Kirvelienė (Vilniaus universitetas, fiziniai mokslai, biochemija – 04P)

Nariai:

prof. habil. dr. Juozas Vidas Gražulevičius (Kauno technologijos universitetas, fiziniai mokslai, chemija – 03P)
prof. habil. dr. Ričardas Rotomskis (Vilniaus universitetas, fiziniai mokslai, fizika – 02P)
dr. Virgilijus Vaičaitis (Vilniaus universitetas, fiziniai mokslai, fizika – 02P)
prof. habil. dr. Gediminas Gaigalas (Lietuvos edukologijos universitetas, fiziniai mokslai, fizika – 02P)

Oponentai:

doc. dr. Saulius Bagdonas (Vilniaus universitetas, biomedicinos mokslai, biofizika – 02B)
prof. habil. dr. Eugenijus Šatkovskis (Vilniaus Gedimino technikos universitetas, fiziniai mokslai, fizika – 02P)

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INTRODUCTION

Fluorescence spectroscopy is one of the most widely used spectroscopic techniques in the fields of biochemistry and molecular biophysics today. These techniques become quite popular because of its simplicity and acute sensitivity to changes in the structural and dynamic properties of biomolecules or biomolecular systems. It helps to track small amounts of fluorescing compounds, to identify them by spectral features, to assess the chemical composition or to identify structural and functional peculiarities of the object. Fluorescence spectroscopy techniques do essential to study complex biomolecular systems, such as biological tissue, to assess valuable information on tissue architecture and laying processes within for diagnostic purposes. Though the fluorescence phenomenon has been known since the middle of 19 century, however it has not paid any attention for biomedical applications until the end of 20 century, hence, reaching useful approach in various fields of biomedicine especially for non-invasive, real-time in situ assessment of tissue pathology.

Demarcation of tumor margins in situ, assessment of initial biochemical composition changes at the early stages of cancer development are the main problems in practical biomedical diagnostic nowadays directed to applications of fluorescence spectroscopy techniques [1,2]. The development of reliable diagnostic method is not possible without knowledge of the main fundamental physical, chemical and biological processes initiating and controlling the progression of the disease, as all physical disorders might be related to biochemical, physiological or morphological changes in cell or tissue level, reflecting the peculiarity of light - tissue interaction for particular case. Knowledge's of the main optical properties of the tissue (such as absorption, scattering, fluorescence) are important not only for the implementation of any novel optical diagnostic technique but also to convey diagnostic information useful for clinical needs and not requiring expert interpretation.

Considering complex nature of biological tissue a fair amount of experience has been stored by analyzing neoplastic tissue fluorescence spectra and fluorescence images from different body sites and various cells or tissue layer levels working with experimental model systems [3,4]. Main attention has been paid for early cancer and pre-cancer fluorescence diagnostic aiming to identify specific differences of tissue absorption, architectural or physiological state which indicates the early steps of the disease [5-6]. Fluorescence techniques are often attributed as early optical biopsy methods and are based on fluorescence sensitivity and selectivity to tissue state [7-9]. By analyzing differences of tissue fluorescence spectral shape or relative intensity it is possible to identify the initial tissue abnormalities at the early stages of malignancy form while fluorescence imaging is useful for remote screening and proper demarcation of growth extension margins. The same tissue site can be examined many times without any damage to the surrounding normal tissue and that is attractive adjuvant help to collect proper doubtful tissue samples in situ for further morphological examination. A benefit of fluorescence spectroscopy methods is their potential to improve tumor demarcation ability and to guide to detect early tissue abnormalities regions not only in oncological applications but also in other diagnostic applications in practical medicine.

A growing attention for the use of fluorometric methods in biomedicine shows a rising number of performed studies devoted to delineation of tumor extension margins in the surrounding normal tissue, especially when other conventional methods are unhelpful or theirs facilities are limited [10]. One of the relevant practical diagnostic challenges nowadays is surgical management of malignant high grade gliomas extension margins intraoperatively. In the brain, viable, marginal tumor is difficult to distinguish during the surgery, thus these highly invasive tumors determines to incompleteness of theirs resection and, ultimately,

determinates low patients survival [11]. During the surgery brain tumor extension normally is guided by the visual inspection of the surgeon using white-light microscope evaluating brain tissue external features such as color, elasticity, alterations in blood circulating system or analyzing preconceived information estimated by neuroimaging techniques (e.g. computer tomography, nuclear magnetic resonance imaging, ultrasound imaging). The experience of a neurosurgeon has significant influence on the thoroughness of malignancy surgical removal and thus the patient's lifespan. The construction of experimental spectrofluorometric diagnostic system suitable for intraoperative detection of malignant brain tumours in rat's brain and validation of its applicability for experimental malignant glioma fluorescence localization in vivo was one of the main topics of this thesis. By implementing the Lithuanian State Science and Studies Foundation supported scientific program "Light in biomedicine: diagnostic and therapy" the collaboration of scientific colleagues of Oncology Institute of Vilnius University, Institute for Biomedical Research Kaunas University of Medicine revealed the construction of experimental spectrofluorometric detection system and leaded to practical implication of its performance for accurate identification of experimental malignant gliomas in vivo.

Another topic presented in this thesis is devoted to fluorescence detection of the initial structural composition changes in the artery wall caused by physical inactivity - as one of important risk factors for developing coronary heart disease, by use of autofluorescence spectroscopy. The tissue autofluorescence spectrum contains useful information about the biochemical composition or (and) the structure of the tissue and might be used as diagnostic feature. A benefit of this study has practical implementation by assessment of initial biochemical composition changes and early disorders caused by disease develop or other external factors in blood vessel wall by use of non-invasive, real-time diagnostic technique when the preventive pharmaceutical treatment is applicable. For the first time the ability of autofluorescence technique to detect the initial biochemical composition changes in the blood vessel wall inner surface caused by physical inactivity has been assessed.

The aim of this work is to investigate fluorescence spectral features of different biological tissues under specific structural/metabolic disturbance level with the intension to assess the diagnostic conveyance and the potential of fluorescence spectroscopy techniques – spectrofluorometric and fluorescence imaging – for malignant brain tumours (glioma 101.8 and C6) intraoperative identification in vivo in rat's brain model system and for detection of the initial structural composition changes in blood vessels wall of rabbit model system due to hypodynamic stress.

The following tasks were carried out in this work:

1. **To evaluate fluorometric techniques applicability for experimental malignant glioma fluorescence localization in vivo:**
   - ✓ to construct experimental a single point measurement spectrofluorometric diagnostic system suitable for intraoperative detection of malignant brain tumours in rat's brain;
   - ✓ to investigate 5-aminolevulinic acid induced protoporphyrin IX (PpIX) selective accumulation in experimental malignant gliomas (101.8 and C6 glioma models);
   - ✓ to ascertain autofluorescence features of malignant gliomas (101.8 and C6) and to identify specific spectral differences for rat brain tissues differentiation;
   - ✓ to determine marginal fluorescence intensity ratios for optimal identification of 5-ALA sensitized malignant 101.8 glioma in vivo;

- ✓ based on fluorescence spectra analysis, to ascertain a single point measurement spectrofluorometric method reliability parameters for intraoperative detection of malignant gliomas;
- ✓ to evaluate fluorescence imaging technique to visualize malignant C6 glioma infiltration margins into surrounding normal brain tissue intraoperatively in rat's brain.

2. **To evaluate possibility to assess initial structural changes in inner surface of blood vessel wall due to hypodynamic stress by use of autofluorescence spectroscopy:**
   - ✓ to investigate autofluorescence spectral features of aorta and peripheral artery of experimental animals over a range of different excitation wavelengths;
   - ✓ to determine the optimal excitation/emission wavelengths for fluorescence guided identification of initial structural changes of normal and altered blood vessel wall.

NOVELTY AND IMPORTANCE OF THE THESIS

The main theme of this thesis is the use of fluorescence spectroscopy techniques to convey diagnostically important information in the fields of biomedicine. Tissue fluorescence spectroscopy is a new area in the field of medical diagnostics which might ultimately provide non-invasive, real-time in situ assessment of tissue structural or histochemical information. The fluorescence spectrum of the examined tissue contains information about biochemical or structural composition and might be used as indicator for remote screening of the disease with high sensitivity. Increasing interest of fluorescence spectroscopy techniques is based on their ease of implementation, low cost and compatibility with other instruments.

Considering the complexity of information contained in a tissue fluorescence spectrum depending on various technical aspects used for fluorescence detection and collection systems, it is necessary to examine particular clinical problem individually and to provide complete characterization of tissue spectroscopic peculiarities. Thus further development of fluorometric techniques, especially those which are devoted for in situ tissue detection, are still on evaluation path depending on variety not only of neoplastic tissue types and their unique characteristics but also on different variations in fluorescence excitation and detection units applied.

The work presented in this thesis involves the use of fluorometric techniques to detect malignant gliomas intraoperatively in rat's brain. In this study we explored the constructed experimental a single point measurement spectrofluorometric diagnostic system suitable for intraoperative detection and showed its applicability for experimental malignant glioma identification in vivo. The achieved optical contrast and fluorescence spectral features make this system convenient for in situ glioma detection guided by fluorescent endogenous porphyrins spectral fingerprints. The obtained data demonstrate that spectrally we can identify water soluble endogenous porphyrins which are mainly attributed to normal brain tissue sensitization in tumour bearing animals and protoporphyrin IX which is specific for experimental malignant glioma and to estimate quantitative theirs content. Therefore, a single point measurement spectrofluorometric method allows providing quantitatively important information for in situ fluorescence guided tumour identification in comparison with fluorescence imaging technique and might be useful adjuvant technique for glioma resection control during surgical procedure.

Common intraoperative brain tumour detection has been mainly directed to observation of glioma fluorescence and fluorescence image guided resection. Here we demonstrate the advantage of fluorescence spectra measurement approach and present our first clinical experience for spectrally guided glioma resection intraoperatively. This was implemented for the first time in Lithuania by collaboration with neurosurgeons of the Department of Neurosurgery at Vilnius University Emergency Hospital.

The second part of investigation of this thesis is devoted to assessment of autofluorescence spectral signature differences of initial structural changes in the experimental aorta and peripheral artery inner wall surface due to physical inactivity. We were the first to ascertain hypodynamics altered tissue autofluorescence spectral peculiarities.

PROPOSITIONS TO DEFEND

1. The designed experimental a single point measurement spectrofluorometric system allows selective excitation of fluorescence of endogenous porphyrin type pigments in rat brain tissues and provides accurate identification of malignant gliomas in vivo by estimating the integral intensity ratio $I_{620-660}$(normal tissue)/$I_{620-660}$(tumour) that indicates the difference of the amount of endogenous protoporphyrin IX in case of malignant glioma.
2. 5-ALA induced PpIX selectively accumulates in experimental 101.8 malignant gliomas by providing optical contrast ratio from 1:1,4 to 1:8,7 between normal brain white-matter and malignant glioma. Spectrofluorometric method allows diagnosing brain tumour with 83 % sensitivity, 84 % specificity and 87 % accuracy. A single point measurement spectrofluorometric method is suitable as adjuvant technique for white light surgical microscope to examine and control brain tumour removal in real-time during surgical procedure. A portable fibre optic system is introduced as adjuvant technique for clinical use in neurosurgery.
3. The designed fluorescence imaging system is optimized for selective endogenous porphyrins excitation and theirs fluorescence visual detection with exploited commercially available blue light emitting diodes (emission at 405 nm) and colour digital camera for brain tissue fluorescence image detection. Such system is suitable for intraoperative experimental C6 malignant glioma fluorescence remote visual inspection in vivo and different brain tissue type assessment in rat's brain in real time.
4. Autofluorescence spectral features peculiarity analysis is applicable to detect structural changes in the experimental aorta and peripheral artery walls caused by physical inactivity.

APPROBATION

On the results presented in this thesis 4 papers were published, 8 reports presented at various scientific national and international conferences (A1-A7 presented by the author personally) and two chapters were prepared for the book "Visualization of tumors of the central nervous system" concerning basic approaches of light and tissue interaction, tissue optical properties and its applicability for the malignancy detection, the benefit and personal experience of the use of fluorescence spectroscopy techniques for gliomas detection and fluorescence guided tumor resection in neurosurgery.

ACKNOWLEDGEMENT

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AUTHOR'S PUBLICATIONS

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[P1] A.Čiburys, D.Gadonas, R.Gadonas, J.Didžiapetrienė, I.Gudinavičienė, G.Graželienė, D.Kaškelytė, A.Piskarskas, K.Skauminas, V.Smilgevičius, A.Sukackaitė, 5-Aminolevulinic acid induced protoporphyrin IX fluorescence for detection of brain tumor cells in vivo, Experimental Oncology 25, 51-54 (2003).

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(Book was prepared by implementing EU supported structural funds project „Medical physics nad senior nanophotonics studies realization" (contract No.ESF/2004/2.5.0-03-385/BPD-210/ES2006-01))

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[A1] D.Kaškelytė, R.Gadonas, M.Kušleikaitė, S.Stonkus. Periferinių kraujagyslių sienelių struktūros pažaidų, sukeltų hipodinaminio streso, atpažinimas fluorescencinės spektroskopijos metodu. 36-oji Lietuvos nacionalinė fizikos konferencija: programa ir pranešimų tezės, 2005 m. birželio 16-18, Vilnius, p.87.

[A2] D.Kaškelytė, R.Gadonas, V.Smilgevičius, K.Skauminas, I.Gudinavičiene, J.Didžiapetrienė, G.Graželienė, A.Sukackaitė. Fluorescence guided intraoperative detection of malignant gliomas. Acta Bio-optica et Informatika Medica 10 (1-2): International Symposium on New Trends in Photodynamic Therapy and Diagnosis, 21-22 June, Wroclaw, Poland, p.32 (2004).

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[A4] R.Gadonas, M.Kušleikaitė, D.Kaškelytė, V.Smilgevičius, V.Karabanovas, S.Stonkus, S.Kušleika. Autofluorescence study of hypodynamic stress affected aorta intima, XVI Lithuanian-Belarussian seminar „Lasers and optical nonlinearity", 27-29 October 2004, Vilnius, Lithuania.

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[A7] R.Gadonas, M.Kušleikaitė, R.Smilgevičiūtė, D.Kaškelytė, V.Smilgevičius, S.Stonkus, V.Karabanovas, L.Slavinskaitė. Kraujagyslių sienelės fluorescencijos tyrimas taikant atrankųjį žadinimą derinamo bangos ilgio spinduliuote. 35-oji jubiliejinė Lietuvos nacionalinė fizikos konferencija : programa ir pranešimų tezės, Vilnius, 2003 m. birželio 12-14 d., Vilnius, p. 258.

[A8] K.Skauminas, J.Šidiškis, J.Didžiapetrienė, R.Gadonas, G.Graželienė, D.Kaškelytė, V.Smilgevičius. Experimental assessment of sensitivity and specificity of the spectrofluorimetric method for intraoperative diagnosing of malinant gliomas, Proceedings of the Latvian academy of sciences: 1 st Scandinavian and Baltic congress on neurosurgery. ISSN 1407-009X, 2003, Vol. 57, No. ½, p. A6, No. Abstr. SC5.

SUMMARY OF DOCTORAL DISERTATION

The dissertation consists of: **Introduction** - the motivation of the relevancy of the investigation, main tasks and propositions to defend are formulated. The list of author's publications is also included; **Literature review** – a short history on fluorescence techniques for diagnostic applications, the description of pathways of molecular excitation and deactivation, overview of optical properties of tissue is presented, principles and examples of tissue derived fluorescence and autofluorescence techniques for identification and demarcation of malignant and pre-malignant lesions are explained; **Chapters I and II describes main experimental investigations of two different topics of applications of fluorometric techniques** – it starts with the short description of the motivation for a given problem under investigation, explains the aspects of the designed fluorescence detection system used and obtained results. The discussion is presented at the end of each chapter. The final part of the thesis is dissertation **Conclusions**. Each part of the thesis has its separate **List of references**.

The total number of pages is 146, including 44 figures.

CHAPTER I. STUDY OF EXPERIMENTAL GLIOMA FLUORESCENCE GUIDED IDENTIFICATION IN VIVO IN RAT BRAIN

One of the relevant practical diagnostic challenges nowadays is surgical management of malignant gliomas extension margins during the surgery. In the brain, viable, marginal tumor is difficult to distinguish intraoperatively thus these highly invasive tumors determines to incompleteness of theirs resection and, ultimately, determinates low patients survival [11]. Currently, intraoperative brain tumor detection and tumor extension margins delineation relies on the visual inspection of the surgeon using white-light microscope, analyzing preconceived image information gained by different neuroimaging techniques, assessment of ex vivo tissue samples diagnostic or 5-ALA induced protoporphyrin IX fluorescence observation and its guided tumor resection using expensive, specifically adjusted surgical microscope. The use of 5-ALA induced glioma fluorescence for delineation of malignant tumor was initiated by German scientists in 1998 [12]. They were the first to present specifically adapted neurosurgical operating microscope for screening porphyrin fluorescence and demonstrated its use for first clinical operation. However, tumor fluorescence is not homogenous and this complicates to discriminate marginal tumor that predominantly indicates infiltration boarder and fluoresce less as the density of viable tumor cells is small. Thus fluorescence imaging technique has some limitation to localize and successive removal of those less fluorescent tumor affected regions and to reach better survival prognosis.

In our work we demonstrate the advantage of fluorescence spectra measurement approach and present our first clinical experience for intraoperative spectrally guided glioma resection.

In this chapter the results of investigation of experimental malignant brain tumour - glioma fluorescence guided identification and brain tissue differentiation in vivo in rat brain are presented. We used two different experimental brain tumour cells lines – rats glioma 101,8 cells line and human glioma C6 cells line - to assess a single point measurement fluorescence spectral method and fluorescence imaging method diagnostic suitability for experimental glioma intraoperative identification in animal model systems.

Two experimental systems were designed and applied for our study. First, we designed a single point measurement spectrofluorometric system for selective excitation and fluorescence detection of endogenous porphyrin type pigments used for intraoperative brain tissue examination (Fig. 1).

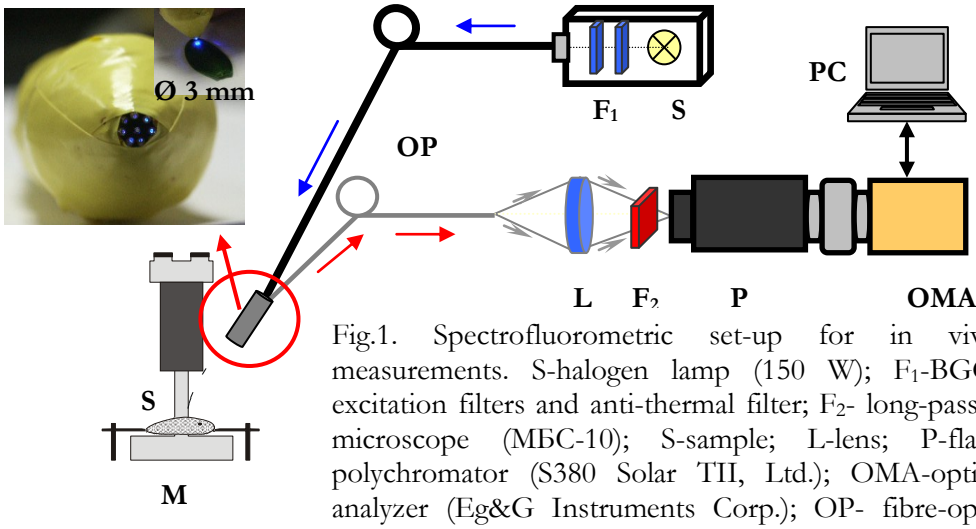

Fig.1. Spectrofluorometric set-up for in vivo fluorescence measurements. S-halogen lamp (150 W); F_1-BGG22 and BG15 excitation filters and anti-thermal filter; F_2- long-pass filter OG13, M-microscope (MBC-10); S-sample; L-lens; P-flat field grating polychromator (S380 Solar TII, Ltd.); OMA-optical multichannel analyzer (Eg&G Instruments Corp.); OP- fibre-optic probe: seven fibers to deliver excitation light and one central fiber to collect fluorescence emission

It consists of light source (halogen lamp (150W)), fiber-optic probe and registration system (flat field grating polychromator with cryogenically cooled CCD camera). Experimental surgical operation was performed using a microscope. The spectrum of light source was filtered to provide violet-blue light for excitation of endogenous porphyrin (375 – 500 nm spectral range). Seven fibers were used to guide the excitation light to the site of examination in rat brain and the fluorescence was collected by a separate fiber located in the probe centre. To eliminate stray light at the wavelengths below 550 nm, the long-pass filter OG13 was used. All brain tissue fluorescence measurements were carried out in the darkened laboratory room with the probe head hold perpendicularly at a 0,8 – 1 mm distance from the surface.

Second, we designed fluorescence imaging system presented in Fig. 2 used for intraoperative C6 glioma fluorescence image guided detection. Commercially available 31 blue light emitting diodes (LEDs) (emission peak at 405 nm, power 5mW) were used to design a ring shape excitation source. A BGG-22 filter has been placed for excitation light filtering to avoid LEDs emission enhancement in the red spectral range. At the distance of 10 cm we could obtain even distribution of excitation light intensity field (~ 3mW/cm^2). The recording of tissue fluorescence images has been performed through the hole in the centre of LEDs ring supplemented with a long-pass filter RG10 to eliminate signal below 600 nm and a colour digital camera (Minolta Dimage 7). The detection channel was optimized for endogenous pophyrin fluorescence detection (characteristic porphyrin emission peaks are near 636 nm and 706 nm) and exploiting suitable camera sensitivity in the wavelength range above 600 nm. The camera enabled automatic adjustable target integration of the porphyrin signal with exposure periods of 4 and 10 seconds. The obtained images were processed with Matlab program.

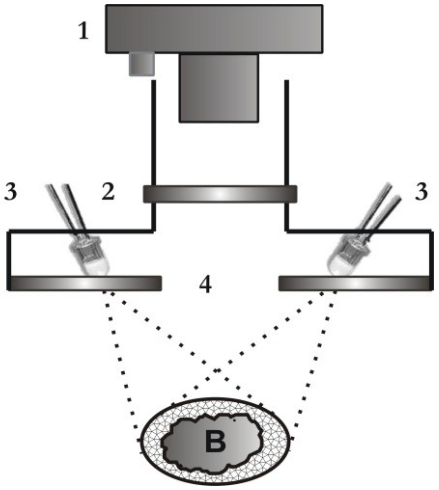

Fig.2. The experimental fluorescence imaging system set-up. 1 - colour digital camera Dimage7; 2 - RG10 filter; 3-LEDs (emission peak at 405 nm); 4 - BGG22 filter; B - a brain section under investigation

The experiments were carried out using Wistar rat's bearing experimental malignant brain tumours. Intraoperative detection of glioma fluorescence has been performed after 12 days (for 101,8 and C6 gliomas) after tumour cells implantation into rat brain. Measurements were performed at four selected brain tissue sites: tumour, doubtful normal brain tissue, doubtful brain tumour and as a control site – normal brain tissue from the opposite hemisphere. That enabled to assess accuracy of a single point measurement spectrofluorometric technique and its applicability to identify doubtful brain tissue – marginal tumour.

The first part of the results is devoted to the tissue autofluorescence study. The in vivo brain tissue autofluorescence study using a single point measurement spectrofluorometric system revealed that registered spectra of normal brain tissue (control site) of tumour bearing animal and that of animal without inoculated tumour are different in 600 – 720 nm spectral range (Fig. 3). While the experimental malignant glioma (101,8) showed slightly more expressive difference in this spectral range comparing with normal brain tissue. The observed alterations of spectral shape are attributed to fluorescence of endogenous porphyrin type pigments: water soluble endogenous porphyrins are mainly predominating and responsible for normal brain tissue sensitization in tumour bearing

animals while protoporphyrin IX is predominate for experimental malignant gliomas. The increase of porphyrins level in organism might be a result of disturbances of heme biosynthesis. It is known that the activity of heme biosynthesis enzymes vary in the case of pathological conditions, inflammatory processes, inherited diseases (such as porphyrias) or other physiological stresses [13]. Thus in our case, tumour growth, its inoculation procedure and the disturbance of blood-brain barrier, the induced stress to the animal might be the reasons for observed sensitization of normal brain tissue (white-matter) and selective protoporphyrin accumulation in the tumour.

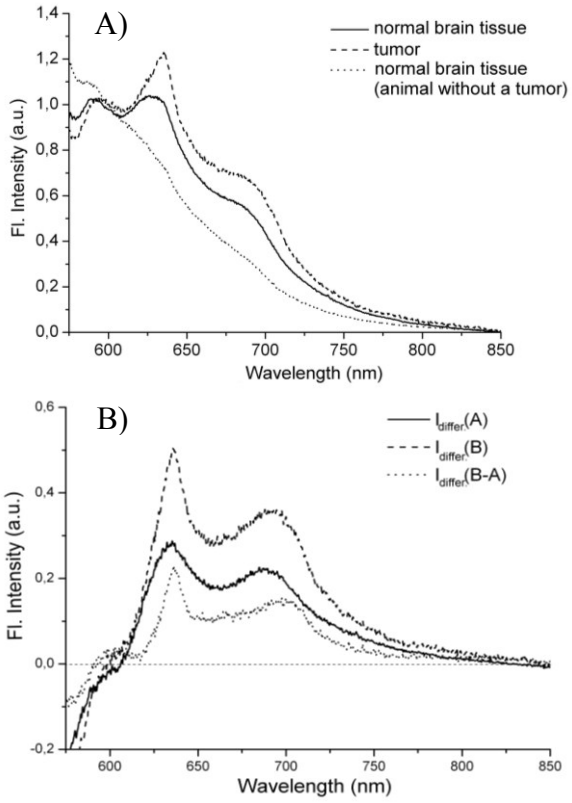

Fig.3. A) Autofluorescence spectra of normal rat brain tissue and 101,8 malignant glioma in vivo. B) Difference spectra reflecting spectral changes resulted by various endogenous porphyrins: $I_{differ.}(A)$ – difference spectrum obtained subtracting spectra of normal brain tissue from the normal brain tissue of the animal without a tumour; $I_{differ.}(B)$ – difference spectrum obtained subtracting spectra of tumour from the normal brain tissue of the animal without a tumour; $I_{differ.}(B-A)$ – difference spectrum of $I_{differ.}(B) - I_{differ.}(A)$

Importantly, protoporphyrin IX does not accumulates in the normal brain tissue thus for the identification of malignant gliomas in vivo and for brain tissue differentiation it is expedient to estimate the integral intensity ratio $I_{620\ -\ 660}$(normal tissue)/$I_{620\ -\ 660}$(tumour) that indicates the difference of the amount of endogenous protoporphyrin IX, exceptionally, in case of malignant glioma.

From the view of practical applications of autofluorescence techniques for brain tissue differentiation it is necessary to obtain sufficient optical contrast for tumour identification. It was shown that analysis of difference spectra in the 620 – 660 nm spectral range can be used to obtain significant optical contrast between normal brain tissue and experimental glioma that might be used as fluorescence rate indicator for brain tissue differentiation using autofluorescence spectroscopy.

There were also performed ex vivo measurement in some other rat tissues and the presents of endogenous porphyrins accumulation was found. The highest fluorescence signal from endogenous protoporphyrin has been observed in skin and digestive tract while smaller signal was detected in liver and kidney. Urinary bladder fluorescence spectrum showed the presents of only water soluble porphyrins (peak near 619 nm). The main fluorescence peaks were identified at 636 nm (endogenous protoporphyrin IX), 620 nm (uroporphyrin III) and 625 nm (coproporphyrin III) [13,14]. Hence, because of particular amount of endogenous protoporphyrin in different tissues the evaluation of its relative fluorescence intensity is possible only for the same tissue type to compare. The increased level of different porphyrins in rat organism is explained by the presents of natural (inborn) and specific regulation mechanism of heme biosynthesis of these albino animals as they possess a complex photo-protective system maintained by the Harderian gland produced

porphyrins [15-17]. It was reported that environmental lighting might affect porphyrin metabolism by raising ALA-synthase enzyme that governs the rate-limiting step in heme biosynthesis, activity in the rat. Due to very low ferrochelatase activity the appearance of porphyrins is observed as in case of porphyria [18].

The differences in autofluorescence spectra were demonstrated for experimental C6 glioma ex vivo. Dependably on the excitation wavelength the fluorescence of normal brain tissue is more intense in 420 – 580 nm spectral range (with exceptions at 400 and 410 nm excitation wavelengths) while tumour fluorescence is more intense in 600 - 700 nm spectral range (Fig. 4A,B). Similar spectroscopic changes in the red spectral range as for 101,8 glioma, were observed: endogenous protoporphyrin mainly fluoresce in C6 glioma (highest signal reached at 400 – 420 nm excitation) while water soluble porphyrins fingerprints detected in normal brain tissue under 380 – 400 nm excitation wavelengths (Fig. 4C). New fluorescence peak near 590 nm was identified mainly for C6 glioma fluorescence induced by 400-430 nm excitation wavelengths. This suggested to be resulted by the endogenous metaloporphyrin – Zn-protoporphyrin (ZnPp) [19-21]. It is known that small amount of ZnPp is present in erythrocytes and the ex vivo sample preparation, caused disorders in blood supply, could influence the observed emission. Furthermore, ZnPp also reflects the disorders in porphyrin biosynthesis. Tissue metabolic activity, local environment conditions can influence the appearance of metaloporphyrins [22-24].

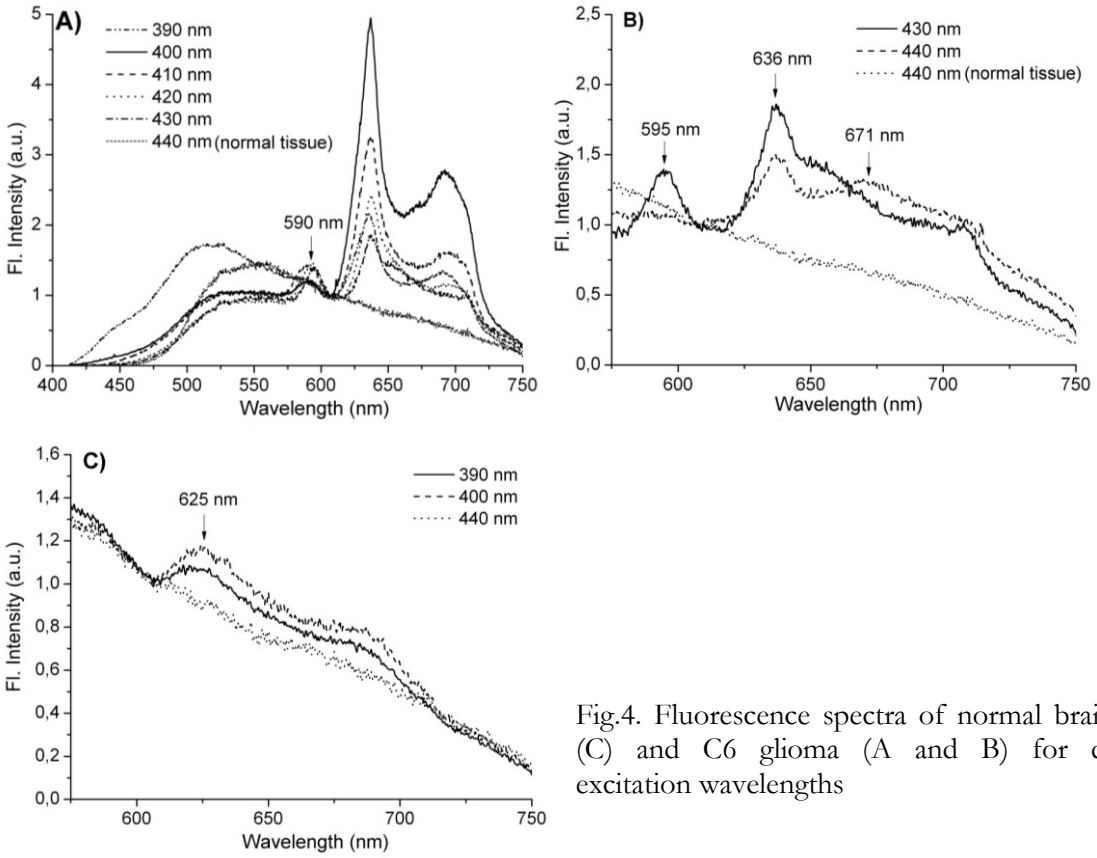

Fig.4. Fluorescence spectra of normal brain tissue (C) and C6 glioma (A and B) for different excitation wavelengths

Also, we have found fingerprints of fluorescent pigment near 670 nm observed in C6 glioma tumour and some other rat tissues fluorescence spectra ex vivo with exception to normal brain tissue. It is known that spectroscopic changes in this spectral region might be attributed to food pigment chlorophyll-a, or its photo-degradation products (pheophorbide-a and/or pheophytin-a) that have been shown to cause the 674 nm fluorescence peak in mouse skin [25-26].

In conclusion, the tissue natural spectroscopic changes might be used to estimate protoporphyrin content evaluated by integral fluorescence intensity at 620-660 nm spectral range in vivo which is a major feature of diagnosis of various disorders resulting from altered porphyrin metabolism. As their might exist a differences not only between tumour and normal brain tissue but also between different types of brain tumour distinctive porphyrin content to increase the sensitivity of autofluorescence method it is necessary to optimize the excitation and detection conditions of measurements and collect as much as possible experimental data to identify peculiarities of different types of brain tumours. Still, our findings have an important practical implication because it clearly shows the applicability of a single point measurement spectrofluorometric method to estimate porphyrin content in vivo and might be used for brain tissue differentiation in further researches.

The second part is devoted to investigation of 5 - aminolevulinic acid (5-ALA) induced protoporphyrin IX (PpIX) guided intraoperative brain tumour (rat glioma 101,8) identification by use of a single point measurement spectrofluorometric system. It was shown that 5-ALA induced protoporphyrin IX selectively accumulates in experimental glioma (Fig.5) while fluorescence spectra of normal brain tissue remains unchanged. Malignant brain tissue is characterized by expressive dual-peak (636 nm and 706 nm) fluorescence of PpIX. Thus, a selective uptake of PpIX and its specific fluorescence in red spectral range provided a possibility to identify 101,8 glioma from normal brain tissue in vivo by their spectroscopic patterns.

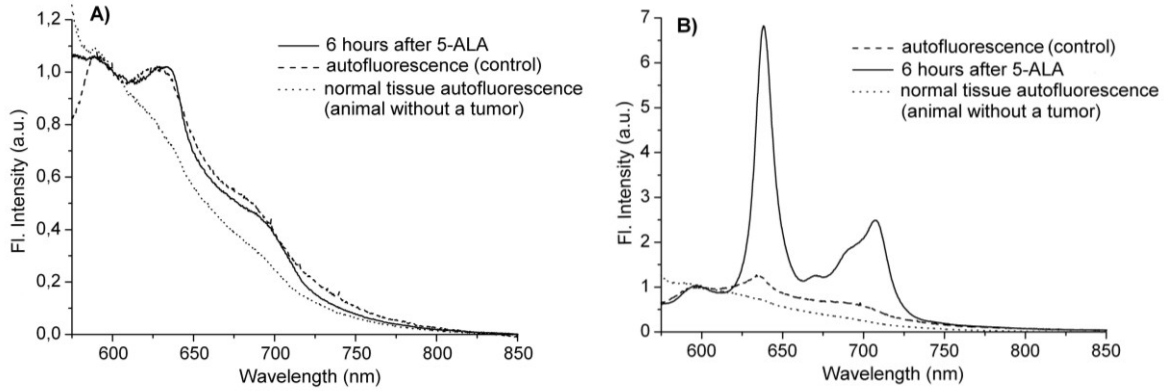

Fig.5. Autofluorescence and PpIX fluorescence spectra of 101,8 glioma and normal brain tissue 6 hours after administration of 5-ALA dose 300mg/kg. The autofluorescence spectrum of normal brain tissue of animal without a tumour is illustrated. Spectra are normalized to 600 nm

Spectral features of other different rat tissues have been shown to vary in ability to accumulate PpIX. Quantitative protoporphyrin content has been estimated by calculating integral fluorescence intensity value over the spectral range corresponding to the most intense fluorescence peak of PpIX (in the spectral range 620 – 660 nm). It was found that the highest fluorescence signal corresponds to the skin. The fluorescence intensity of periosteum and dura mater was higher than glioma and normal brain tissue thus indicating the precautions locating tissue sites for spectroscopic examination during the surgery.

Experimentally collected fluorescence spectra were analyzed for quantitative determination of 5-ALA induced PpIX fluorescence in rat brain tissues. It was shown that the obtained optical contrast ratio varies from 1:1,4 to 1:8,7 between normal brain tissue (white-matter) and malignant 101,8 glioma which was estimated by calculating the integral fluorescence intensity values in 620 – 660 nm spectral range. The obtained results prove that

the assessed spectral features difference and estimated optical contrast is sufficient for intraoperative detection of malignant glioma in rat brain.

It should be notice that not always recorded fluorescence spectra showed significant differences in porphyrin emission intensities between tumour and normal brain tissue after 5-ALA i.v. administration. Fig. 6 illustrates one of experimental example. As we can see, in case when we don't have a properly growth tumour (tumour cell density is small) the enhance fluorescence signal in the red spectra region indicates a small uptake of PpIX (peak at 636 nm) in the doubtful brain tissue (Fig.6, indications 1). However, spectrally we might clearly indentify the spectral shape modification by presents of water soluble endogenous porphyrins (Fig.6A indication 2) or endogenous protoporphyrin IX (Fig.6A indication 1). Difference spectra, obtained by subtracting the spectrum of normal brain tissue of an animal without a tumour, reveals bigger contrast level between normal brain tissue (control site) and doubtful brain tissue in 620 – 660 nm spectral range. Furthermore, the spectral shift between two selected doubtful tissue sites is originated and resulted of different porphyrins emission impact. Morphological findings showed that doubtful tissue indicated as 1 is small density brain tumour (localized exceptionally at the tumour inoculation site) and indication 2 represents normal brain tissue adjacent to the tumour. In Figure 6 (B,b) presented an example when no tumour was obtained after 12 days after inoculation procedure to the animal. As we can see, just slight spectral shape variation is observed in spectral range corresponding to PpIX (Fig.6 B, indication 1). The increase of PpIX

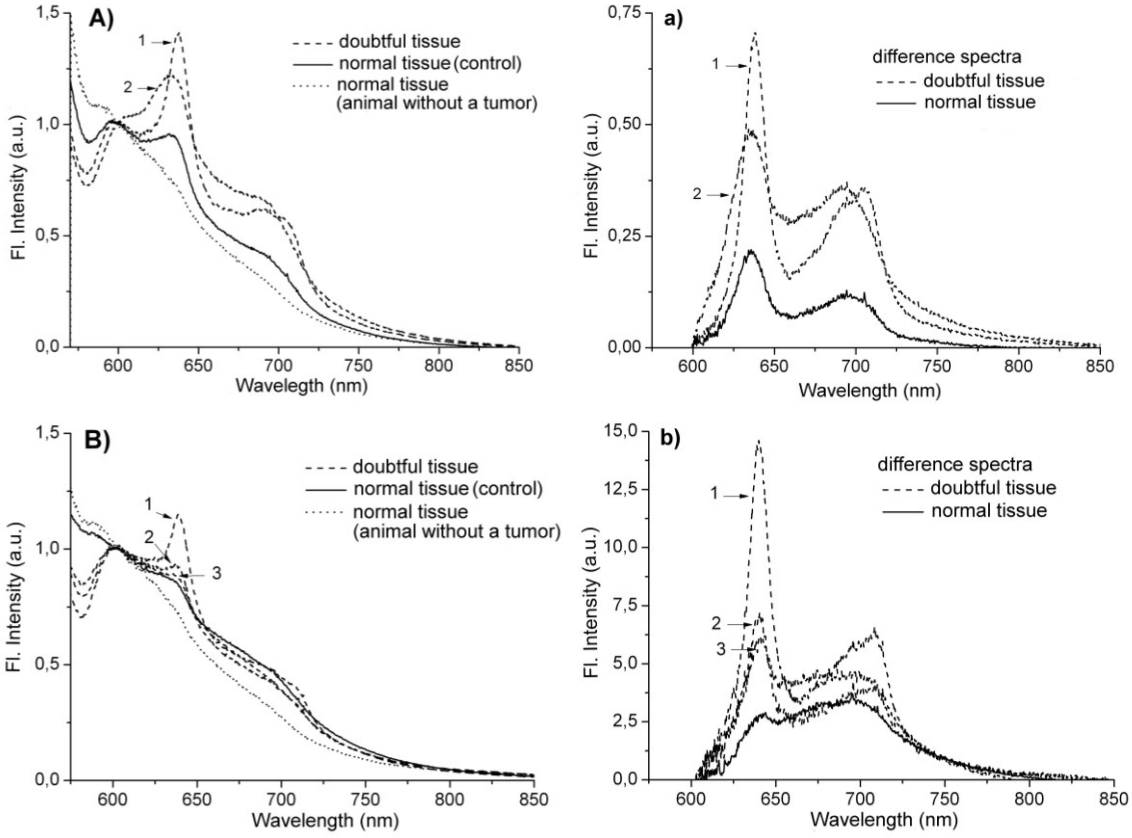

Fig.6. Fluorescence spectra detected at doubtful brain tissue site (tumour or normal brain tissue) and normal brain tissue detected in control site 6 hours after 5-ALA administration (300mg/kg). (a)-(b) are difference spectra indicating spectral changes resulted by the porphyrins. (A,a) – pathological biopsy showed brain tumour; (B,b) - pathological biopsy showed normal brain tissue. Spectra are normalized to 600 nm

fluorescence has been detected at the tumour inoculation site and that might be the result of

altered blood-brain barrier during the inoculation procedure and possible porphyrin uptake. Our findings have an important practical meaning to explore a single point measurement fluorescence technique because fluorescence imaging technique in this case would provide false positive evaluation of marginal tumour and only spectral shape patterns clearly enables differentiate doubtful brain tissue.

For quantitative reliability of a single point measurement spectrofluorometric method for in situ 5-ALA induced PpIX fluorescence guided 101,8 glioma identification we evaluated integral fluorescence intensity values over the 620 – 660 nm spectral range for 99 examined brain tissue sites of 27 animals and compared them with surgeon judgement under white-light microscope examination. For the statistical data analysis ROC curve (receiver operating characteristic) was used in order to evaluate method's applicability for diagnostic [27]. The obtained results revealed that spectrofluorometric method allows diagnosing brain tumour with 83 % sensitivity, 84 % specificity and 87 % accuracy. While malignant tumours identification under white-light microscope showed 83 % sensitivity, 69 % specificity and 73 % accuracy.

Thus, we might conclude that, applied a single point measurement spectrofluorometric method allows obtaining quantitatively important information of porphyrin content for in situ fluorescence guided tumour identification and might have important practical implementation as adjuvant technique for more accurate glioma resection control during surgical procedure using 5-ALA induced tumour fluorescence.

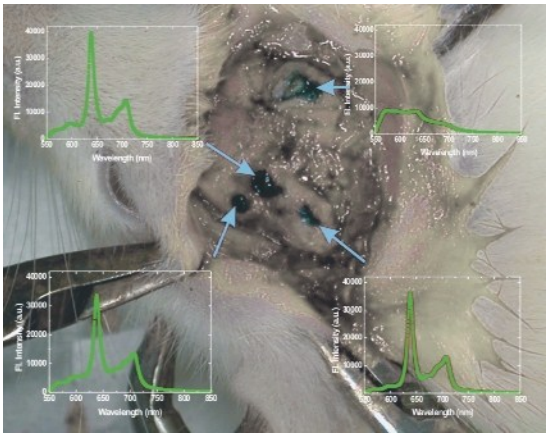

Fig.7. An example of experimental surgical intervention field with marked selected brain tissue sites and recorded fluorescence spectra indicating fingerprints of protoporphyrin IX for 101,8 glioma. In this case tumour affected whole left brain hemisphere

The third part of this work is devoted to the application of fluorescence imaging technique for C6 glioma in vivo demarcation in rat brain. It was shown that 5-ALA induced protoporphyrin IX selectively accumulates in experimental C6 glioma and allows intraoperative visualization of tumour fluorescence. The recorded fluorescence images information provided in a digital data format was separated into three RGB channels data. The obtained data indicated that only R channel possess the diagnostically important information thus all images were analyzed getting information depicted on the R channel. Here we presented two approaches for glioma tumour extension margins demarcation in vivo. First, we can extract fluorescence intensities along an axis selected in the obtained fluorescence image (Fig.8, green line). As it is illustrated in Figure 8 using two different signal accumulation times (4 s (B) or 10 s (C)) we might get a section view reflecting fluorescence intensity distribution and enhancement in the rage of 400 - 550 resulted by the protoporphyrin IX within a tumour. Changing the position or angle of depicted green line (Fig.8.A) we might estimate the tumour localization within normal brain tissue. However, for estimation of tumour extension margins we need to know marginal fluorescence intensity values that is difficult to achieve having in mind tumour infiltration manner, no

homogenous fluorescence pattern, bleeding, and surface roughness. It could be estimated just for particular situation and thus complicates its practical implementation utility requiring estimate marginal calibration. Consequently, all doubtful tissue sites should be confirmed morphologically.

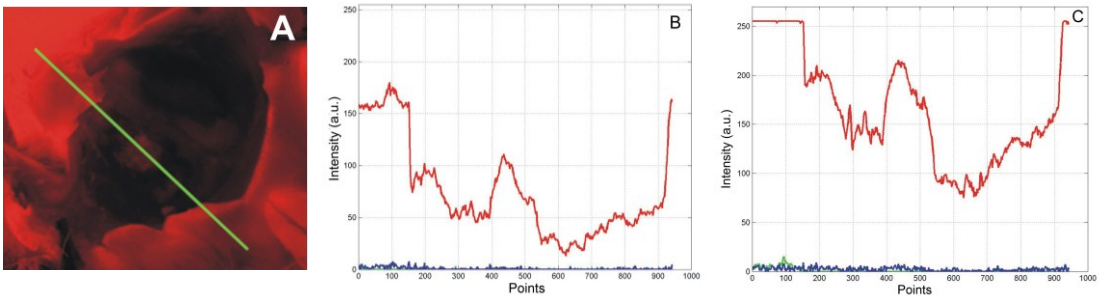

Fig.8. A) Experimentally obtained fluorescence image of surgical field with fluorescing C6 glioma and extracted RGB channels fluorescence intensities data along green line depicted in A image using 4 s signal accumulation time (B) or 10 s (C). Fluorescence image recorded at 6 h after 5-ALA i.v. administration (300mg/kg)

The second approach is based on 2D fluorescence intensity maps plotting of the selected operative area for C6 glioma extension visualization (Fig.9). The fluorescence intensity maps plotting procedure provide information of tumour localization at once in the selected section area (Fig.9 C,D) and might be used to proper guidance of doubtful tissue biopsies selection.

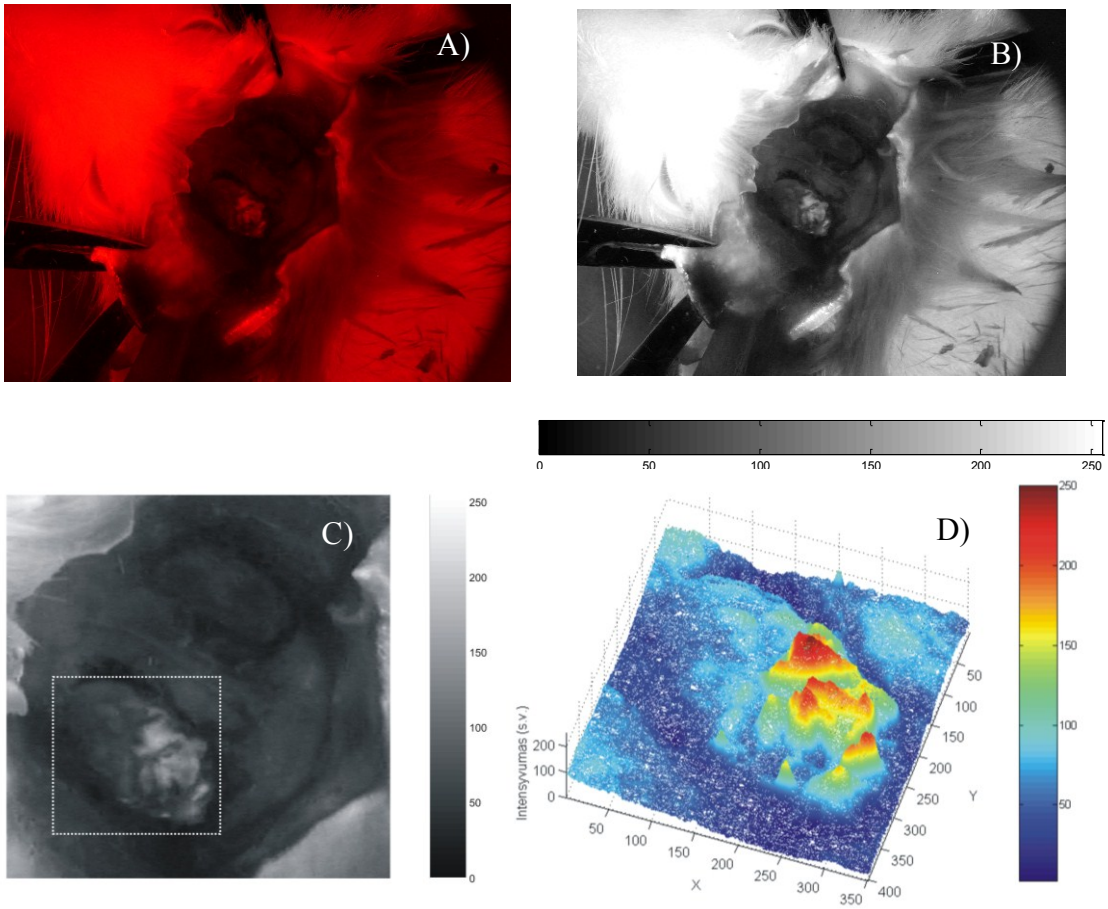

Fig.8. A) Experimentally obtained fluorescence image of surgical field with fluorescing C6 glioma; B) extracted R channel fluorescence image; C) enhanced surgical field area with indicated box section were 2D fluorescence intensity map is obtained (D). Fluorescence image recorded at 6 h after 5-ALA i.v. administration (300mg/kg)

In conclusion, experimental C6 malignant glioma fluorescence imaging examination revealed that the analysis of the recorded fluorescence images by composing the fluorescence intensities maps of the brain section area of interest, provides more accurate identification of doubtful tissue because of the difference of fluorescence intensity and this might be explored as effective indicator to guide tissue biopsy collection. Therefore, although fluorescence image gives assessment of alterations of brain tissue by fluorescence intensity mapping of a broad area of interest at once, however, provides less spectroscopically valuable information for tissue characterization and for the reliability of diagnosis it is necessary to perform morphological examination.

The final part of this work is devoted to practical implementation of a single point measurement spectrofluorometric method for high grade glioma resection extension control during the surgery in clinic. A designed portable single excitation-detection channel fiber-optic system is presented and first collected human glioma fluorescence spectra guiding tumour resection extension were achieved. The structural arrangement of a system is presented in Fig. 9. The constructed fluorescence excitation/emission collection unit was specifically designed to explore single fiber probe for selective porphyrin excitation and emission collection in brain tissue. The excitation and collection realized through the same optical fiber (600 μm in diameter, length 2 m) the tip of which was kept distantly during the measurements. We used GaN diode laser operating in cw regime at the wavelength 405 nm with output power up to 16 mW. A spectrometer was used to register the fluorescence spectra detected at the tested doubtful brain tissue site during the surgery. The fluorescence signal coupling time during each spectrum recording was 50 ms.

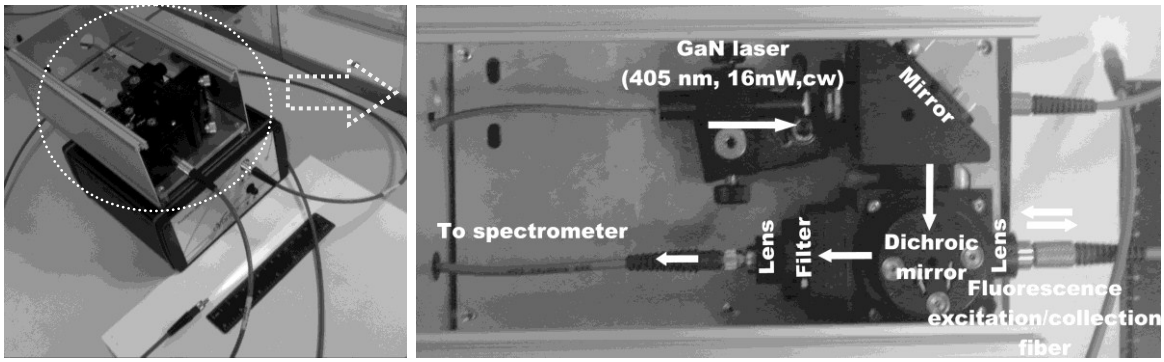

Fig.9. The structural arrangement of designed portable single excitation-detection channel fiber-optic system. Left – the basic view; right – the configuration of fluorescence excitation/emission collection unit. Dichroic beamsplitter for 405 nm laser (Semrock, Inc.) was used to ensure separation of excitation and fluorescence emission channels.

During the surgery a laser light at 405 nm was delivered to the tissue through a hand-held optical fiber. We used larger diameter fiber on purpose to have the possibility to overlook the tumour fluorescence prospect by keeping the fiber further from the tissue surface. Thus for the spectrum measurement the fiber was aimed closer at the doubtful tissue site.

PpIX in human glioblastoma tumour assigned with peaks at 636 nm and 706 nm was detected and allowed to distinguish marginal tumour during the surgery in reference to fluorescence spectral shape profiles. Figure 10 shows the spectral signatures measured at tumour, normal tissue site and marginal tumour. The surgeon performed tumour resection guided by spectroscopy results until no PpIX signal remained. Noticeable, the marginal tumour also shows spectral differences in 450 – 600 nm spectral range which means that tumour cells are infiltrating and surrounded by normal brain tissue.

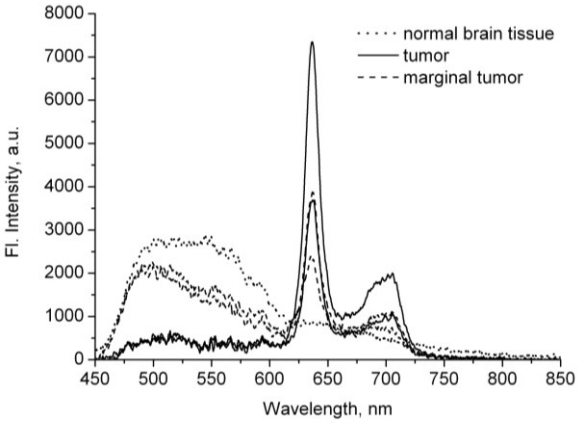

Fig.10. A typical fluorescence spectra of human glioblastoma and normal brain tissue measured intraoperatively (Gliolan dose 20mg/kg). Peaks at 636 nm and 706 nm are representative of PpIX and the spectral differences in 450 – 600 nm are due to normal brain. Pathological biopsy showed a glioma grade IV (glioblastoma multiforme)

In conclution, we were able to effectively detect 5-ALA induced PpIX fluorescence in the human glioblastoma during surgical resection. The designed portable fiber-optic system for fluorescence measurements could provide the real-time intraoperative targeting of fluorescent tissue sites in the brain. Our first obtained results are promising and this system is undoubtfully very usefull adjuvant technique for remote screening of brain tissues peculiarities and glioma removal control for clinical use in neurosurgery.

CHAPTER II. AUTOFLUORESCENCE STUDY OF ALTERED EXPERIMENTAL AORTA AND PERIPHERAL ARTERY WALL

Physical inactivity is an important risk factor for developing coronary heart disease [28]. It has been confirmed that physical inactivity contributes to population risk more than hypertension, elevated cholesterol levels or obesity. In tissues, physical inactivity provokes changes in the concentration of bio-elements that participate in the metabolism of lipids, carbohydrates, secretion of hormones, stability of membranes and contractility of cardiomyocytes [29-30]. This allows considering the possible early injuries in blood vessels specifically to the inner (intima) surface and the importance of detecting initial surface structural composition changes caused by physical inactivity. The surface structural composition changes in the vessel wall, induced by physical inactivity, have not yet been studied by fluorescence spectroscopy. As there is a close functional connection between coronary and peripheral arteries [31], the latter are expedient to use for monitoring the development of atherosclerotic disease.

A benefit of this study has practical implementation by assessment of initial surface biochemical composition changes and early disorders caused by external factors by use of non-invasive real-time diagnostic technique when the preventive pharmaceutical treatment is applicable. For example, the findings might be important not only for early atherosclerotic lesions spectroscopic changes screening but also for stents implantation control as it can also affect blood vessel structure adjacent to implant site [32-33] or for adjuvant guidance system design for laser angioplasty procedure control [34].

In this chapter, the preliminary results of assessment of fluorescence spectroscopy for surface structural changes in aorta and peripheral artery wall induced by hypodynamic stress [35] was investigated in rabbit model systems. Tissue fluorescence excitation was performed using tuneable radiation of parametric generator ("Topas", Light Conversion Ltd.) pumped by 2nd harmonic of a picosecond Nd:glass laser ("Twincle", Light Conversion Ltd.) in the range of excitation wavelengths 340 – 420 nm for aorta samples and 320 – 360 nm for peripheral arteries. The collected spectroscopic results were contrasted against ultrastructure studies of the arterial wall by electron microscopy.

The analysis of autofluorescence spectral peculiarities was used to investigate spectroscopic signatures of blood vessels ex vivo under excitation in the spectral range of the absorption of structural proteins (elastin and collagen). Differential spectral feature analysis might provide useful information about separate tissue constituents [1-4]. The principal components in blood vessels are collagen and elastin in the adventitia and media, respectively [36-38]. However, in the case of elastin and collagen the emission spectra overlap significantly, thus rendering their characterization a difficult task [39]. Thus, this study is based on preliminary comparison of fluorescence spectral shape variations between normal and hypodynamic stress altered inner blood vessel wall surfaces to evaluate the possibility to identify initial structural composition changes due to 48 days rabbit subjection to a confined space.

Slight differences in spectral shape signature, peaks positions and their relative intensities has been observed between the fluorescence spectra of normal and altered blood vessel walls being specific to a particular type of tissue (aorta or peripheral artery). Mean fluorescence spectra of aorta samples ex vivo revealed that relative fluorescence intensity is slightly higher in normal aorta than in hypodynamic stress affected aorta inner wall surface and spectral shape signature varies depending on excitation wavelength applied. Furthermore, a broad autofluorescence spectrum indicates the possible impact of excited fluorescing endogenous fluorophores other than structural proteins. Our inspection for the

spectral differences in the aorta samples revealed that normal aorta (Fig.11 A) is characterized by dominating fluorescence peak at 440 nm under 340-400 nm excitation, and peak at 480 nm which starts to dominate under 410-420 nm excitation. A slight fluorescence enhancement near 390 nm (under 340 – 360 nm excitation) and the band near 550 nm was also detected. A small jog of spectral shape near 410 nm and valley near 540 nm might be caused by the blood absorption. Whereas the fluorescence spectra of altered aorta (Fig.11 C) shown some spectral differences in comparison to normal tissue. Firstly, by changing the excitation wavelength from 340 nm to longer wavelengths the fluorescence peak near 480 nm arise faster and the peak near 410 nm drops down. Higher fluorescence enhancement reached at 390 nm for 340 nm excitation and bigger relative broadening of spectral shape towards red spectral region with the band near 550 nm was observed. The obtained fluorescence spectral signatures dependency on excitation wavelength is plotted as fluorescence excitation-emission matrixes (Fig. 11 B, D) for normal and hypodynamic stress altered aorta samples, respectively. Difference excitation-emission matrix indicated the spectral regions most informative for detection of structural injuries caused by hypodynamic stress. We have estimated that altered aorta has specific fluorescence enhancement at 480 nm under excitation 370-410 nm, and at 390 nm for 340 nm excitation wavelength what might be used as distinguishing feature for aorta inner wall surface structural alterations identification ex vivo.

The fluorescence spectra peculiarities of peripheral artery samples in comparison with the aorta were different but exhibited a similar tendency: higher relative fluorescence intensity for normal tissue, dual peak spectral shape signature and specific spectral broadening to the longer wavelengths in case of altered artery (Fig. 12). The fluorescence peak at 410 nm is dominating for normal artery with higher relative fluorescence intensity than for altered tissue. The two shoulders of indicated peak might be identified: one at 390 nm and the second at 440 nm and, similarly, slight variation of spectral shape near 480 nm and 550 nm which are observed for normal and altered artery wall with latter more expressed for injury site.

Thus, the fluorescence measurements of blood vessel samples ex vivo indicates that several fluorophores with overlapping fluorescence bands results the autofluorescence and their relative amount is altered by hypodynamic stress. Both examined blood vessel samples has specific and unique structural arrangement so is hard to compare. However, in agreement with other researchers, the observed spectral shape variations might be attributed to structural proteins content alterations (not identifying an impact of particular one) and possible impact of lipids in case of hypodynamics.

It is known that, the fluorescence spectrum of normal artery wall is dominated by elastin (peak at 410 nm), which mainly is localized in intima and media, with a fingerprint of collagen impact near 390 nm [36-38]. In case of alteration (usually attributed to atherosclerotic plaque formation) the enhancement of collagen fluorescence is observed. The predominant fibrillar collagen types in the aorta are types I and III [40-41]. The collagen type ratio changes with atherosclerotic plaque formation and this could be reflected in differences of collagen crosslinking level, therefore, and fluorescence spectral response. Both collagen and elastin possess a broad fluorescence band in the 360-440 nm and 370-560 nm, respectively, and the peak position shall be depended on excitation wavelength [39]. Hence, because of an overlap of fluorescence spectra of these natural compounds, resulted by similar crosslinks responsible for the fluorescence, the direct assessment of structural tissue composition can't be obtained and needs more precise morphological and spectroscopic data comparison.

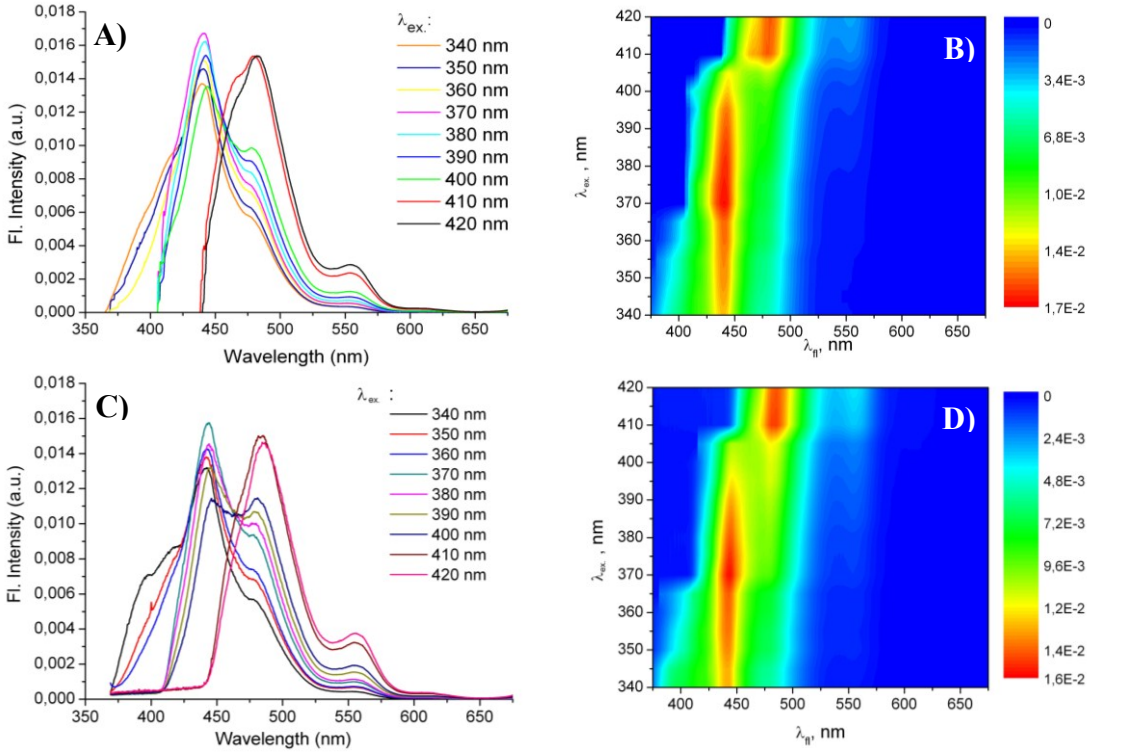

Fig.11. Mean autofluorescence spectra of the inner surface of intact aorta (A) and altered by hypodynamic stress (C) under different excitation wavelengths. Fluorescence spectra are normalized to unit area under the curve. (B), (D) – excitation-emission matrices of intact and altered aorta inner wall surface respectively

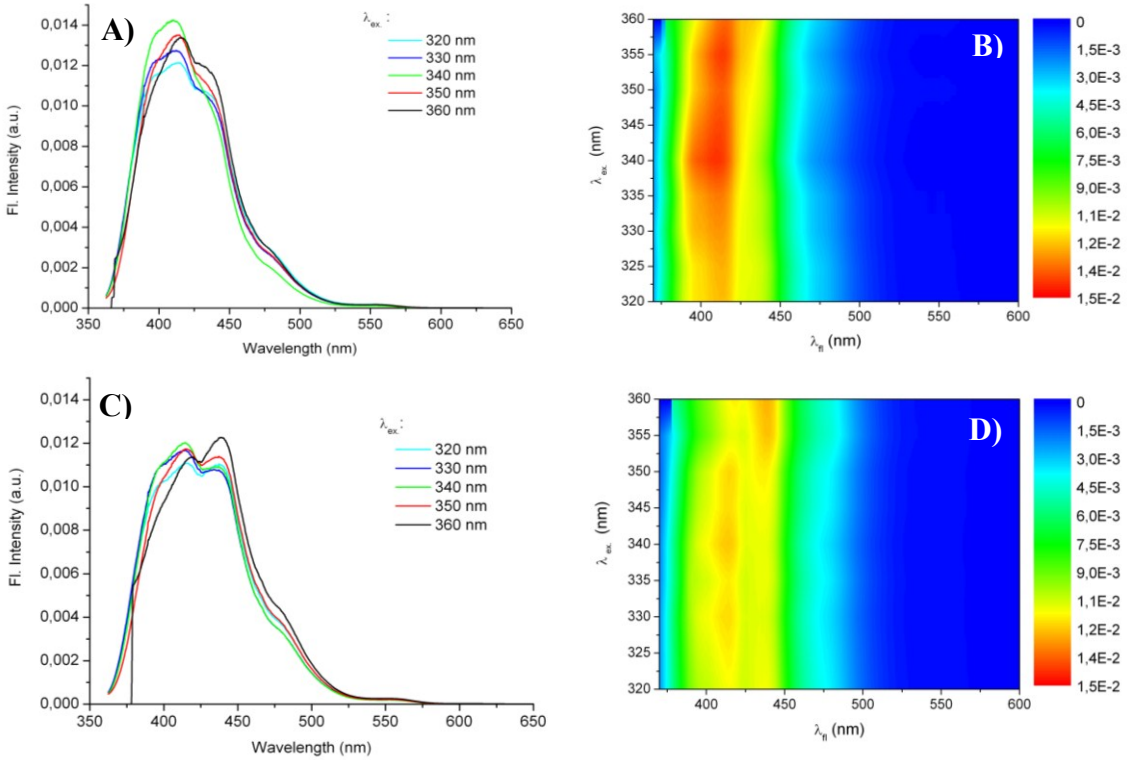

Fig.12. Mean autofluorescence spectra of the inner surface of intact peripheral artery (A) and altered by hypodynamic stress (C) under different excitation wavelengths. Fluorescence spectra are normalized to unit area under the curve. (B), (D) – excitation-emission matrices of intact and altered peripheral artery inner wall surface respectively

In conclusion, the obtained data confirms that the analysis of autofluorescence spectral features is applicable to detect initial structural changes in the experimental aorta and peripheral artery inner wall surface caused by physical inactivity. The ultrastructure examination revealed the thickening of intima, incorporation of lipids, and the disruption of basement membrane which indicates the disintegration of blood vessel wall structural components content. We were able to identify the spectral regions for injury assessment; however, it is necessary to provide more precise investigation devoted to morphological and spectroscopic data identification for particular type of tissue with intension to determine the specific endogenous fluorophore quantitative impact.

This work has continuation maintaining the human artery samples investigation (realistic system) with intension to identify particular spectral shape signatures of various level of disease injury with specifically designed fluorescence scanning system for remote screening of sample surface and 4D data collection (x, y coordinates, fluorescence intensity and emission wavelength).

CONCLUSIONS

1. Autofluorescence measurements of malignant gliomas (101,8 and C6 glioma cell lines) revealed that observed alterations of spectral shape in the red spectral region are attributed to fluorescence of endogenous porphyrin type pigments: water soluble endogenous porphyrins are mainly predominating and responsible for normal brain tissue sensitization in tumour bearing animals, while protoporphyrin IX is predominate for experimental malignant gliomas. Whereas protoporphyrin IX does not accumulates in the normal brain tissue thus, for the identification of malignant gliomas in vivo and for brain tissue differentiation it is expedient to estimate the integral intensity ratio $I_{620-660}$(normal tissue)/$I_{620-660}$(tumour) that indicates the difference of the amount of endogenous protoporphyrin IX in case of malignant glioma.
2. Fluorescence spectra analysis revealed that 5-ALA induced PpIX selectively accumulates in experimental 101.8 malignant gliomas by providing optical contrast ratio from 1:1,4 to 1:8,7 between normal brain white-matter and malignant glioma that has been estimated by calculating the integral intensity values within 620 – 660 nm spectral range. The obtained results indicate the applicability of a single point measurement spectrofluorometric technique to assess malignant glioma in vivo.
3. The obtained results of calculation of fluorescence integral intensities revealed that spectrofluorometric method allows diagnosing brain tumour with 83 % sensitivity, 84 % specificity and 87 % accuracy. Single point measurement spectrofluorometric method is suitable as adjuvant technique, for white light surgical microscope, to examine and control brain tumour removal in real-time during surgical procedure. A portable fibre optic system is introduced as adjuvant technique for remote screening of brain tissues peculiarities and brain tumour removal control for clinical use in neurosurgery.
4. Experimental C6 malignant glioma fluorescence imaging examination revealed that the analysis of the recorded fluorescence images by composing the fluorescence intensities maps of the brain section of interest, provides more accurate identification of doubtful tissue because of the difference of relative fluorescence intensity and this might be explored as effective indicator to guide tissue biopsy collection. Thus, although fluorescence image gives assessment of alterations of brain tissue fluorescence intensity mapping of a broad area of interest at once, however, provides less spectroscopically valuable information for tissue characterization and for the reliability of diagnosis it is necessary to perform morphological examination.
5. Physical inactivity induces structural alterations in inner surface of aorta and peripheral artery wall which might be identified by analyzing differences of autofluorescence spectral shape in comparison to normal tissue. Comparative spectral analysis revealed that we might distinguish emission and excitation wavelengths sensitive for the biochemical composition of inner blood vessel wall. Spectral shape differences in the blue spectral range might be associated with a change in the relative amount of collagen and elastin, while enhanced fluorescence emission at longer wavelengths could be a result of the lipid components emission.
6. The obtained data confirms that the analysis of autofluorescence spectral features is applicable to detect initial structural changes in the experimental aorta and peripheral artery wall due to physical inactivity. As there is a close correlation between inner aorta wall state and small arteries, thus small arteries model system might be used for assessment of initial structural alterations due to hypodynamics.

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FLUORIMETRINIŲ METODŲ TAIKYMAI BIOLOGINIŲ AUDINIŲ PATOLOGINIŲ PAKITIMŲ DIAGNOSTIKOJE

Fluorimetriniai metodai suteikia svarbios informacijos apie audinio sandarą, morfologiją ir biochemiją įvairiu aspektu, todėl neatsitiktinai yra itin patrauklūs ir plačiai taikomi daugelyje praktinės medicinos sričių. Nors kiekvienai metodikai paprastai taikomi skirtingi algoritmai kiekybinių pokyčių paieškai (parenkant charakteringiausius tiek žadinimo, tiek ir emisijos bangos ilgius), naviko identifikavimui ar jo išplitimo ribų vaizdinimui aplinkiniuose sveikuose audiniuose, tačiau fluorimetrinių metodų jautrumas biologinio objekto būsenai, jų įvairiapusiškumas bei adaptyvumas yra pagrindiniai kriterijai apsprendžiantys jų patrauklumą ir tinkamumą klinikai. Kombinuojant esamas metodikas kartu su optinėmis galima pasiekti daug geresnių rezultatų, padidinti chirurgo galimybes audinių klasifikacijai, sukaupti papildomos informacijos apie skirtingų navikų fluorescencijos savitumus, bei kontroliuoti ir vaizdinti navikinio darinio ribas in situ, net ir tais atvejais, kai spektriniai skirtumai yra nežymūs.

Viena aktualesnių praktinės diagnostikos problemų yra galvos smegenų glijos navikų išplitimo ribų nustatymas operacijų metu. Iki šiol pagrindinė mokslinių tyrimų kryptis buvo orientuota į fluorescencijos vaizdinimo metodo panaudojimą gliomos intraoperaciniam vaizdinimui ir šalinimo kontrolei. Tačiau pilnutinis naviko pašalinimas pasitelkiant fluorescencinį vaizdą siekia iki 65 proc. atvejų, lyginant su 20 proc. pasiekiamu operuojant įprastu būdu (baltoje mikroskopo šviesoje). Fluorescencijos vaizdo panaudojimas galvos smegenų navikų šalinimui turi tam tikrų trūkumų ir šiai dienai vis dar yra intensyvių tyrimų eigoje.

Daugelio eksperimentinių ir klinikinių tyrimų rezultate buvo įsitikinta, kad vien tik fluorescencijos vaizdo nepakanka ir būtinos papildomos priemonės glijos naviko ribų kontrolei operacijos metu. Mažų naviko infiltracijos židinių aptikimui yra siūlomas šviesolaidinis zondas, kuris suteiktų papildomos informacijos audinių spektrinių savitumų analizei. Atlikome pirmuosius taškinio matavimo spektrofluorimetrinės sistemos panaudojimo galimybių įvertinimą sensibilizuotų eksperimentinių galvos smegenų navikų lokalizacijai in vivo. Šio darbo rezultate sukonstruota mobili fluorescencijos taškinio registravimo sistema, kuri sėkmingai panaudota klinikoje – piktybinio glijos naviko atpažinimui operacijos metu.

Darbe taip pat atliktas organizmo fizinio aktyvumo suvaržymo sąlygotų kraujagyslės sienelės struktūrinių pakitimų, atitinkančių biocheminių komponentų disbalansą, savosios fluorescencijos tyrimas. Organizmo fiziologinių sąlygų pokyčiai įtakoja fluorescencijos skirtumus, kurie galėtų būti informatyvūs vertinant audinio morfologinę būklę. Šie tyrimai turi praktinės reikšmės vertinant pradinius biocheminius pokyčius bei nustatant ankstyvuosius ligos formavimosi pakitimus, kuomet galimas prevencinis medikamentinis gydymas. Papildomos spektroskopinės informacijos gavimas leistų sudaryti pirminių struktūrinių pažeidimų paveikslą ir atitinkamai identifikuoti pagrindinius biocheminius komponentus. Šio darbo pradžiai tyrimų susijusių su fizinio aktyvumo, kaip vieno iš aterosklerozės riziką sukeliančių veiksnių, sąlygojamų kraujagyslės sienelės struktūrinių pažaidų, panaudojant fluorimetrinius metodus, mokslinėje literatūroje nebuvo skelbta.

Pagrindinis šio darbo tikslas yra ištirti skirtingų biologinių audinių biocheminių/metabolinių procesų sąlygojamas fluorescencijos spektrinius ypatumus, siekiant įvertinti fluorimetrinių metodų – spektrofluorimetrinio ir fluorescencinio vaizdinimo - pritaikymo piktybinių galvos smegenų navikų ir kraujagyslių vidinės sienelės struktūrinių pažaidų diagnostikoje galimybes.

Pagrindinės darbo išvados:

1. Savosios fluorescencijos tyrimai parodė, kad piktybinių galvos smegenų glijos navikų (žiurkių gliomos 101.8 linija ir žmogaus gliomos C6 linija) savosios fluorescencijos spektrams charakteringa endogeninių porfirino tipo pigmentų fluorescencija: navikų fluorescencijos spektruose dominuoja endogeninis protoporfirinas IX, tuo tarpu gyvūnų su naviku sveikų smegenų audinių spektre – vandenyje tirpūs endogeniniai porfirinai. Kadangi sveikose smegenyse endogeninis protoporfirinas IX nesikaupia, navikinių darinių lokalizavimui ir smegenų audinių diferenciacijai yra tikslinga vertinti integrinio intensyvumo santykį $I_{620-660}$(sv. aud.)/$I_{620-660}$(nav.) apibrėžiantį endogeninio protoporfirino IX santykinio kiekio pokytį glijos naviko atveju.
2. Fluorescencijos spektrų analizė parodė, kad 5-ALA indukuotas protoporfirinas IX selektyviai kaupiasi eksperimentinėse gliomose (101.8). Nustatyta, kad stebimas optinis kontrastas tarp sveikų smegenų audinio ir naviko, gautas apskaičiavus integrinių intensyvumų vertes 620 – 660 nm spektrinėje srityje, atitinkančioje PpIX intensyviausios smailės padėtį, siekia nuo 1:1,4 iki 1:8,7, o tai įgalina panaudoti spektrofluorimetrinį metodą glijos navikų atpažinimui in vivo.
3. Atlikti integrinių fluorescencijos intensyvumų skaičiavimai parodė, kad intraoperaciniu spektrofluorimetriniu būdu žiurkių galvos smegenų piktybinės gliomos gali būti identifikuojamos 83 % jautrumu, 84 % specifiškumu ir 87 % tikslumu. Taškinė fluorescencijos spektrų registravimo sistema yra tinkama papildoma priemonė baltos šviesos chirurginiam mikroskopui, kontroliuoti naviko šalinimą operacijos metu. Pasiūlyta mobili šviesolaidinio zondo sistema klinikiniams taikymams neurochirurgijoje, galvos smegenų audinių spektrinių savitumų analizei bei naviko šalinimo apimties kontrolei.
4. Galvos smegenų C6 gliomos fluorescencijos vaizdinimo eksperimentinių tyrimų metu nustatyta, kad fluorescencijos vaizdų analizė, sudarant tam tikros smegenų srities fluorescencijos intensyvumo skirstinių žemėlapius, įgalina tiksliau identifikuoti abejotino audinio sritį remiantis santykinai didesne fluorescencijos intensyvumo verte bei galėtų būti tinkamu rodikliu audinių biopsijoms paimti. Taigi nors fluorescencijos vaizdas įgalina išsyk įvertinti fluorescencijos intensyvumo pokytį dideliame apžvalgos plote, tačiau spektroskopiniu požiūriu suteikia mažiau naudingos informacijos ir diagnozės patikimumui būtina atlikti morfologinius tyrimus.
5. Tiriant hipodinaminio streso paveiktų širdies aortos ir periferinių kraujagyslių sienelių savąją fluorescenciją taikant atrankų žadinimą nustatyta, kad fizinio aktyvumo stoka indukuoja aortos bei periferinių kraujagyslių struktūrines pažaidas, dėl ko stebimi fluorescencijos spektrų formos pokyčiai lyginant su sveika kraujagyslės sienele. Lyginamoji skirtuminių fluorescencijos spektrų analizė įgalina identifikuoti žadinimo ir emisijos bangos ilgius atspindinčius vidinės sienelės biocheminės sudėties pokyčius dėl pažaidos. Manome, kad spektrinius pokyčius mėlynoje spektro srityje sąlygoja elastino ir kolageno santykinio kiekio pokytis, tuo tarpu santykinį fluorescencijos intensyvumo padidėjimą bei spektro praplatėjimą į ilgabangę pusę sąlygoja lipidai.
6. Remiantis eksperimentinio tyrimo rezultatais, galime teigti, kad hipodinaminio streso sukelti struktūriniai pokyčiai kraujagyslių vidinėje sienelėje (intimoje) gali būti atpažinti remiantis savosios fluorescencijos spektrų analize. Kadangi egzistuoja koreliacija tarp aortos vidinės sienelės būklės ir mažesnių arterijų, todėl hipodinaminio streso sukeltų ankstyvų pažaidų atpažinimui galima būtų naudoti periferinių kraujagyslių modelį.

Curriculum Vitae

Name, surname DALIA KAŠKELYTĖ
Date and place of birth 9 August 1978, Vilnius
Work and position Junior Research Associate in Department of Quantum Electronics, Vilnius University
E-mail: dalia.kaskelyte@ff.vu.lt

Education

1996 M.Daukšos Secondary School, Vilnius, Lithuania
1996 - 2000 Bachelor degree in Physics, Vilnius University, Faculty of Physics
2000 - 2002 Master degree in Laser Physics and Optical Technologies, Vilnius University, Faculty of Physics
2002–2006 PhD studies, Department of Quantum Electronics, Vilnius University

Work experience

1998 – 2002 Laboratory assistant, Department of Quantum Electronics, Vilnius University
Since 2002 Junior research fellow, Department of Quantum Electronics, Vilnius University

Educational activity

Since 2006 Lector of „Laser applications in biomedicine“ at Vilnius University Faculty of Physics

Research Interests

Laser fluorescence tumour diagnostic, fluorescence imaging, laser applications in biomedicine, laser spectroscopy