III. SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

1. Name and Surname: **Magdalena Szczerbowska-Boruchowska**

2. Diplomas / academic degrees
- Master of Science, Engineer, discipline: Technical Physics specializing in Medical Physics and Dissymmetry; 1997; AGH University of Science and Technology, Krakow

- Doctor of Philosophy in Physics; 2003, AGH University of Science and Technology, Krakow Ph.D. thesis: " X-rays in studies of the elemental composition of human central nervous system tissue "; supervised by Professor Marek Lankosz

3. Employment History
2003-2004: Teaching Assistant, AGH, Faculty of Physics and Nuclear Techniques, Department of Radiometric Analyses
2004 – present: Assistant Professor, AGH, Faculty of Physics and Applied Computer Science, Department of Medical Physics and Biophysics

4. Scientific achievement as grounds for the habilitation procedure

a) title:
The development of methods of qualitative and quantitative chemical micro-imaging of tissues using X-rays, and their use for the purposes of neuropathology

b) For achievement as grounds for the habilitation procedure I present a cycle of eight monothematic scientific publications. This series includes the following scientific articles:

H-1. **M. Szczerbowska-Boruchowska**. X-ray fluorescence spectrometry, an analytical tool in neurochemical research. *X-Ray Spectrometry*. 2008, 37, 21–31. (IF 1.390)

H-2. M. Z. Kastyak, **M. Szczerbowska-Boruchowska**, D. Adamek, B. Tomik, M. Lankosz, K. M. Gough. Pigmented creatine deposits in amyotrophic lateral sclerosis central nervous system tissues identified by synchrotron Fourier Transform Infrared microspectroscopy and X-ray fluorescence spectromicroscopy. *Neuroscience*. 2010, 166, 1119–1128. (IF 3.215)

H-3**. M. Szczerbowska-Boruchowska**, M. Lankosz, M. Czyzycki, D. Adamek. An integrated experimental and analytical approach to the chemical state imaging of iron in brain gliomas using X-ray absorption near edge structure spectroscopy. *Analytica Chimica Acta*, 2011, 699, 153-160. (IF 4.555)

H-4. **M. Szczerbowska-Boruchowska**, M. Lankosz, D. Adamek. First step toward the “fingerprinting” of brain tumors based on synchrotron radiation X-ray fluorescence and multiple discriminant analysis. *Journal of Biological Inorganic Chemistry*. 2011, 6, 1217–1226 (IF 3.289)

H-5. **M. Szczerbowska-Boruchowska**, Z. Stegowski, M. Lankosz, M. Szpak, D. Adamek. A synchrotron radiation micro-X-ray absorption near edge structure study of sulfur speciation in human brain tumors - a methodological approach – *Journal of Analytical Atomic Spectrometry* 2012, 27 (2), 239 - 247 (IF 3.220)

H-6. **M. Szczerbowska-Boruchowska**. Sample thickness considerations for quantitative X-ray fluorescence analysis of the soft and skeletal tissues of the human body. *X-Ray Spectrometry* 2012, 41, 328-337; DOI 10.1002/xrs.2407 (IF 1.445)

H-7. **M. Szczerbowska-Boruchowska**, A. Krygowska-Wajs, D. Adamek - Elemental micro-imaging and quantification of human substantia nigra using synchrotron radiation based X-ray fluorescence - in relation to Parkinson’s disease. *Journal of Physics: Condensed Matter* 24 (2012) 244104 (11pp). (IF 2.546)

H-8. **M. Szczerbowska-Boruchowska**, A. Krygowska-Wajs, A. Ziomber, P. Thor, P. Wrobel, M. Bukowczan, I. Zizak. The influence of electrical stimulation of vagus nerve on elemental composition of dopamine related brain structures in rats. *Neurochemistry International* 61 (2012) 156–165. (IF 2.857)

c) Description of the achievement as grounds for the habilitation procedure

Introduction

X-ray fluorescence (XRF) analysis is a multielement technique of high precision and accuracy. Over the years, various forms of the excitation of characteristic radiation of elements in analyzed materials have been used. Recently, however, incomparable opportunities of the XRF technique have arisen, which are related to synchrotron radiation (SR) as a source of photons. Among the main advantages of this type of excitation undoubtedly prominent are improving the detectability of elements, better spatial resolution, shortening the time of analysis, and the opportunity to adjust the energy of excitation radiation to the needs of the experiment. That is why X-ray fluorescence (micro)analysis has been widely used in biology and medicine. In these areas, there is a growing demand for analytical techniques providing either high detectability of trace elements or the possibility of qualitative and quantitative analysis at the single cell level. Often, the assessment of the elemental composition of tissues or cells alone is insufficient and a determination of the chemical forms of an element is also required. For this purpose X-ray absorption near edge structure spectroscopy (XANES) can be applied. The analysis of the elemental composition of biological material with the use of the XRF technique, as well as the study of oxidation states of elements based on the XANES technique requires the development of a number of procedures that are related to sample preparation, planning measurement conditions, and the evaluation of qualitative and quantitative results. The special nature of the biological material and especially clinical material also entails the need to find the relevant methods of statistical analysis to feed into the measurement results. The cycle of scientific articles submitted as the basis of my habilitation procedure includes a number of aspects related to the quantitative, topographic and quantitative analysis of both elemental composition and oxidation states of elements, using the XRF and the XANES techniques respectively, in studies of central nervous system tissue (CNS). The abovementioned publications propose methods to assist the chemical imaging of tissues, and theses procedures can be used as a set of instructions / guidelines for those who need to do quantitative elemental and speciation analysis of biological / clinical material.

Among the publications submitted as the basis of my habilitation procedure there are the scientific articles that, in addition to methodological aspects also present concrete applications of techniques based on X-rays for the purposes of neuropathology. The use of these non-conventional techniques in histopathological practice has two fundamental objectives. The first one is related to studies on the pathogenesis of selected neurological disorders (neurodegenerative disorders, brain tumors) and the evaluation of the potential role of chemical elements in biochemical processes accompanying pathological changes. The second essential aim of the studies is the opportunity to use X-ray fluorescence microanalysis in medical diagnostics. This involves both searching for biochemical markers in neurological disorders and supporting routine histopathological diagnostics. An important factor in histological examinations is that there is a subjective evaluation of the histopathologist. Often, in difficult or disputable cases, it would be useful to include additional analytical methods which could largely eliminate doubts in establishing the final diagnosis. This is an important factor in further clinical and therapeutic procedures. The presented works aimed to evaluate the feasibility of using the XRF technique, in a microscopic approach, to observe elemental changes in the pathological tissues and to assess the use of this technique as an analytical tool to aid modern medical diagnosis.

The influence of physical characteristic of a sample on the interactions of X-rays with matter - consequences for quantifications in the XRF technique

The physical characteristics of a sample, such as its density or thickness play an important role in quantitative elemental analysis using the X-ray fluorescence technique. This arises from the influence of such sample properties on the interactions of X-rays with the atoms present in the material analyzed. It should be emphasized that sample mass thickness is critical for quantitative XRF analysis. Therefore, different approaches to quantification procedures in the case of a thin sample, an intermediate-thickness sample or a thick sample have to be applied. In order to avoid any irregularities and spurious results, prior to quantification using XRF analysis a validation of the sample domain is absolutely necessary. In the case of biological specimens, the “dark matrix” is an additional problem. The most important constituents of a biological matrix are H, C, N, and O. None of them show sufficiently intense X-rays. What is more, they may have significant influence on the intensity of the characteristic X-rays of measured elements by primary and secondary absorption. Moreover, there is significant difficulty in XRF quantification, which is related to uncertain water content in tissues / organs, especially for intermediate-thickness samples. That is why, in the case of bio-medical applications, differences in the biochemical composition of various tissues require appropriate calculations using fundamental parameters (including X-ray matter interaction cross sections for X-rays and other critical data connecting the intensity of characteristic X-rays with the chemical composition of the sample) individually for each organ / tissue. My original contribution to the issue of the influence of the biochemical composition of bone and soft tissues on the interaction of X-rays with sample atoms and related aspects of the XRF quantitative analysis were described in paper [H-6].

Specimens for XRF analysis can be prepared following various procedures. At present, however, the most popular methods include:

- freeze-drying at a low temperature or lyophilization, followed by pulverization and pressing into pellets – the method designed for bulk XRF analysis,
- cryo-sectioning and freeze-drying – the most popular procedure for a microanalytical approach,
- the freezing of sections of a native tissue – a method destined for microanalysis or bulk analysis performed at a low temperature.

Due to such a variety of forms of samples used in the preparation of biological material, special emphasis was placed on a determination of sample thicknesses where the specimen can be regarded as of thin, thick, or intermediate thickness depending on the tissue type, the kinds of sample (dried or in natural form) and the element under consideration. This makes it necessary to apply an appropriate correction method in quantitative analysis. In calculations the published contents (mainly for the purposes of radiation protection) of H, C, N, O, and water in various tissues / organs were used. Due to the fact that the analysis was performed for main body organs or tissues, such us brain (white matter), brain (gray matter), lungs, heart, liver, kidneys, urinary bladder, testes, prostate glands, ovaries, uterus, breast (connective tissue), skeletal muscle, skin, bones (cortex), and teeth the work can be used by a wide range of potential researchers who apply the XRF technique for the purposes of medicine. A relatively simple method for a fully quantitative analysis allowing the determination of the masses per unit area (or mass fractions) of chemical elements in thin, thick, and intermediate thickness samples using the external standard method was proposed. As is known, when the mass per unit area of the sample analyzed does not exceed the threshold value of areal mass for a thin sample, the total detected fluorescence count rate depends linearly on the irradiated mass of the element under consideration and on elemental sensitivity (at the energy of excitation). This means that elemental sensitivities for measured elements may be obtained experimentally by measuring thin standard materials. Finally, the mass per unit area of an element (mass fraction) can be calculated using the simple relation given by the ratio of the normalized

count rate for the given element in the XRF spectrum and the fluorescence sensitivity for this element, determined based on the measurement of a standard sample. All the calculation procedures and their theoretical fundamentals were described in detail in paper [H-6]. In practice, however, the analyzed material does not always fulfil the criterion of a thin sample for all analyzed elements. Often, the specimen has to be regarded as a thick sample or a sample of intermediate thickness. In paper [H-6], calculation methods that enable quantitative analysis for both above mentioned sample domains were proposed. They include the use of the enhancements in the quantitative evaluation of intermediate thickness or thick sample which allow for the implementation of the above mentioned simple method of XRF quantitative analysis with the use of thin standard materials. The accuracy and usefulness of the theoretical evaluations presented in the paper were tested experimentally. For this purpose, I used the results of analysis that I performed in my doctoral thesis. This was to verify the usefulness of published elemental compositions of the dark matrix of various tissues / organs for XRF quantification. Moreover, experimental validation was necessary to determine the accuracy of the simple method of quantitative XRF analysis of thick and intermediate thickness biological samples described in paper [H-6]. In the study, samples of lyophilized tissue from white and gray human brain matter as well as NIST reference material SRM 1577b (bovine liver) were used. Based on the theoretical evaluation, it was found that pallet samples have to be considered as intermediate thickness samples for Fe, Cu, Zn, Br, Rb and Sr, while for K and Ca they fulfill the criterion of a thick sample. The measurements of tissue samples were carried out using an energy dispersive X-ray fluorescence (EDXRF) spectrometer. A Mo-anode diffraction X-ray tube was used as a source of primary X-rays. In addition, secondary Mo-target was applied. The spectrometer calibration was performed using thin standard samples of known masses per unit area of elements. The results of measurements of the samples were used in further quantitative analysis that was performed based on the calculation methods which took into account both fluorescence enhancements and the published composition of dark matrix as described in paper [H-6]. The results of such theoretical evaluations were compared with the elemental composition of tissues determined previously using the fundamental parameters method and the dark matrix composition of assessed using the ratio of coherently and incoherently scattered incident radiation. Additionally, the results of the XRF quantitative analysis were compared with those obtained using the Particle Induced X-ray Emission (PIXE) technique. Both in the case of elements for which the specimen was considered thick or those for which the analyzed material fulfilled the criterion of intermediate thickness there was high accordance of results obtained using various analytical methods. Only in the case of Fe mass fraction determined in brain white matter was there a difference of up to 15 % between theoretical and experimental methods. The experimental validation of the procedures described here confirms the high accuracy and usefulness of the proposed approaches to the XRF quantification of biomedical specimens (including the microanalytical approach).

Paper [H-6] also showe the discrepancies which result from omitting fluorescence enhancements in the quantification of various organs / tissues. This confirms the necessity of verifying whether a specimen designed for XRF analysis can be considered as thin, intermediate thickness or thick before the quantifications.

The samples of brain and brain tumors that were used in articles [H-2, H-4, H-7, H-8] met the thin sample criterion for the elements that were essential from the point of view of the research. Therefore, the approach appropriate for a thin sample was applied in quantification procedures. In theory, the samples used in the analyses had to be considered of intermediate thickness for P and S. However, taking into account the relatively small discrepancies (< 10 %) in the use of the thin sample approach for both elements, calculations appropriate for an intermediate thickness sample were omitted. This was also applied because in the case of microanalytical studies like those in papers [H-2, H-4, H-7, H-8], local variations of sample mass thickness may occur as a result of e.g. variations of tissue density or water content.

New standard materials for the XRF quantitative analysis of soft tissues using the external standard method

One of the main problems that arises in the quantification of XRF micro-analysis is the selection of appropriate standard samples. The typical thickness of tissue specimens studied using the SRXRF technique is about a few micrometers. That is why a thin film approximation that ignores the absorption effects of the exciting and the detected radiation is valid. It should be mentioned that the self-absorption effect of both primary and secondary X-rays is relatively small and can be safely ignored in such an approach. Detailed calculations relating to the self-absorption effect of Kα lines of the characteristic radiation of Fe and S in thin brain tissue slices were presented in [H-3 and H-5]. Standard samples of high elemental homogeneity are crucial, taking into account the beam size applied typically in the analysis. Some thin standard reference materials are commercially available (e.g. NIST SRM 1832, NIST SRM 1833, or MICROMATTERTM XRF calibration standards). However, they allow mainly for the determination of masses per unit area of elements. The standard reference materials of biological origin for which the certified values of elemental mass fractions are provided occur typically in lyophilized powder form, so they are not appropriate for microprobe analyses due to a large degree of heterogeneity. The results of the quantitative elemental analysis of thin brain tissue sections using special standard samples which I had developed were presented in papers [H-7 and H-8]. The new standard samples, in the form of thin films of known mass fractions of elements were prepared in such a way that their structure to the greatest extent was close to the research material (tissue slices). For this purpose, the mixtures of nitrates of selected elements (Cl, K, Sc, Ti, Mn, Fe, Cu, Zn, Se, Rb, Sr, and Y) in a Tissue Freezing Medium (Jung Leica) as typically used for tissue cryo-sectioning were prepared. To obtain flat, uniform and thin sections of standard samples each solution was frozen at -18 °C and cut with the use of a cryomicrotome into slices of the same thickness which was applied in the case of tissue sections. Afterwards, standard samples were freeze-dried at a low temperature, as in the case of tissue samples. The details concerning both the elemental composition and preparation procedures of the new kind of standard samples were described in [H-7]. To evaluate the usefulness of such prepared standards the homogeneity of the samples was tested using the SRXRF technique. Accordingly, the standard samples were measured at 100 different sample points using an SR beam 10 μm in diameter. The study showed a high degree of homogeneity (not less than 97 % for all analyzed elements) of the developed standard materials, which points to their usefulness in quantitative XRF analysis on a microscopic scale. Due to a lack of thin standard reference material of biological specimens it is not possible to precisely assess of the accuracy of the results of the quantitative analysis that was performed using the new standard samples. However, the elemental contents obtained for substantia nigra structures and presented in [H-7] are in good agreement with the results of elemental analyses presented in literature studies. As such, it provides grounds for optimism about the accuracy of the results of the analyses carried out using the developed standard materials.

Examples of applications of X-ray fluorescence microanalysis for the purposes of neuropathology

A number of the methodological aspects of X-ray fluorescence microanalysis, described in [H-2 - H-5, H-7, H-8], were always associated with specific uses of this technique for the purposes of neurobiology or neurooncology. A knowledge of the biochemical processes occurring at tissular or cellular level should influence routine diagnostic practice, including unconventional methods, for example, using the techniques of modern physics. I later continued the research which began with my doctoral thesis on two important neurodegenerative disorders i.e. Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). Selected aspects of this mainstream of research were presented in [H-2, H-7, H-8]. Moreover, there were several publications which I co-authored relating to this subject

matter which have not been included in the cycle of articles submitted as the basis for my habilitation procedure.

Paper [H-2] concerned studies of creatine deposits in the human central nervous system in relation to ALS. Possibilities were explored of using X-ray fluorescence analysis as a technique for obtaining new information about the biochemical composition of structures occurring in nervous tissue. The imaging of biochemical composition of the deposits observed in tissue sections was carried out with the use of both synchrotron infrared microspectroscopy (SRFTIR) and synchrotron radiation based X-ray fluorescence. What would be extremely valuable for uses not only for the biological or medical purposes is the possibility of measurements of the same sample with the use of techniques that provide complementary information. With this in mind, apart from the techniques that allow a study of the elemental composition or chemical forms of elements in tissues, in my research I use also Fourier transform infrared microspectroscopy. This technique provides, inter alia, information about functional groups of biological molecules. Research, which uses the techniques SRXRF and SRFTIR on exactly the same material requires, among other things, that the samples are appropriate for research using each of the techniques. It is therefore necessary to use the sample supports that will be transparent (or will reflect) for infrared radiation and which will also meet the conditions for the implementation of measurements by the SRXRF. The second important characteristic that should be emphasized in such studies is the thickness of the sample, which must also meet the requirements of both techniques. In infrared microspectroscopy, sample thickness is one of the critical parameters determining the success / failure of the analysis. The collection of research material and the development of an appropriate method of preparing it for study using both SRFTIR and SRXRF techniques was a part of my personal contribution to paper [H-2]. The precision of the location of analyzed areas of the samples also affects the usefulness of the results of analysis carried out with the use of these two techniques. An examination of creatine deposits by the SRFTIR technique (initiated by me before the work described in publication [H-2]) unambiguously confirmed the presence of this compound in brown deposits observed in brain tissue sections for cases of ALS. The application of the SRXRF technique was aimed at determing whether creatine in the observed deposits occurs in combination with some metals or other chemical elements. Attention should be drawn to the presence of the colour brown, which is not typical of creatine. The application of the SRXRF technique allowed precise imaging of the areas containing creatine deposits. Detailed topographic and quantitative analysis with the use of the SRXRF technique, which was also my work in paper [H-2], did not show an increased accumulation of any elements determined by this technique within creatine deposits, especially Fe. This excludes any relationship between the brown colouration and the presence of neuromelanin or erythrocytes, and confirming the participation of only organic components in creatine deposits.

As a continuation of my previous work on the role of trace elements in Parkinson's disease I conducted a study of the dopaminergic nerve cells of substantia nigra and extraneuronal space using synchrotron radiation based X-ray fluorescence microanalysis [H-7]. The analysis was carried out on four samples representing PD and four samples representing an age-matched control group taken from people who died due to non-neurological conditions. The SRXRF analysis led to findings of P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb in the thin samples taken from substantia nigra. Measurement conditions were adjusted in such a way as to allow the imaging of elemental composition at cellular level. The quantitative analysis, as mentioned previously, was carried out using an external standard, standard samples having been developed especially for this study. This uncovered the contents of the elements in neuron bodies and extraneuronal spaces. Complex statistical analysis, which includes, in addition to testing investigational groups, also multidimensional exploration techniques, was applied to the results of the research. It was found that contents of S, Cl, Ca, Fe, and Zn are significantly higher in PD neurons in comparison with a control group. A discriminant analysis showed that Cl, Fe, Ca and Zn are the most significant elements in the general discrimination between PD nerve cells and control. When the size of

the group representing Parkinson's disease was increased, it confirmed our preliminary observations on the elemental differences in PD, and findings were in agreement with the existing hypothesis about the contribution of chemical elements to the pathophysiology of PD. A clear cluster separation between PD and the control group was found. It was observed both for pericaryons or extraneuronal spaces. This result also confirms the presence of elemental abnormalities in the substantia nigra of brain in case of this neurodegenerative disorder.

One of the examples of the use of the techniques of modern physics for neurobiology, where my original contribution is the planning and implementation of experiments to discern elemental changes in dopamine-related structures of rat brains, relates directly to research on the early stage of Parkinson's disease. Actual clinical symptoms of this neurodegenerative disorder occur at advanced stages of the disease, so that present knowledge about the onset of the disease is insufficient to undertake the appropriate therapy or neuroprotective activities. There is therefore the need for update the state of knowledge of the biochemical processes that occur at the onset of PD. Recent studies of Parkinson's disease indicate that the dorsal motor nucleus of nerve vagus is one of the earliest brain areas affected by alpha-synuclein and Lewy bodies pathology. Both literature findings and our previous studies (e.g. presented in [H-7]) indicate the presence of elemental abnormalities in neurodegenerative processes in PD. Therefore, experimental work carried out in cooperation with departments of the Faculty of Medicine JU (Department of Neurology, Department of Pathophysiology, Department of Neurosurgery) was aimed to find an answer as to whether vagus nerve dysfunction may also affect biochemical changes (including metabolism of dopamine, serotonin and elemental composition) in dopamine-related brain structures in rats. As mentioned, the part of the work associated with the determination of elemental composition in the microstructures of brain samples from experimental animals was my personal contribution to this subject. It should be mentioned that both the size of the analyzed structures of rat brain motor cortex, striatum, nucleus accumbens, substantia nigra, ventral tectal area, and dorsal motor nucleus of vagus and their morphology require the application of an analytical technique allowing microscale studies on coronal sections of brain. Therefore, the analytical technique applied was synchrotron radiation based X-ray fluorescence. An additional difficulty in this type of research is the need to work with unstained material, due to the possibility of modifications to elemental composition during sample preparation, in the case of which adjacent structures of the brain are not clearly separated. This requires the acquisition of relatively difficult skills for the identification of anatomical structures of the brain in these types of samples, and then for using it on the measurement beamline, where typically any methods for improving the imaging in light microscopy (e.g. phase contrast) are not available. The use of the SRXRF technique made clear both the distribution and mass fractions of a wide range of elements in the studied rat brain areas. Based on the quantitative results appropriate statistical analyses were performed. They were aimed to verify and evaluate the significance of elemental changes observed in the dopaminergic system as a result of the dysfunction of the vagus nerve in experimental animals. Both the U Mann-Whitney test and cluster analysis pointed clearly to changes in the elemental composition in the striatum, caused by the electrical stimulation of the left vagal nerve. Moreover, the contents of Ca, Zn and Rb in the substantia nigra of right hemisphere are found to be significantly lower in the group with a stimulated vagus nerve than in control rats. Taking into account the fact that the inhibition of the dopamine system (and not of the serotonin system) was also observed as a result of vagal nerve stimulation, the role of chemical elements in the pathological processes at the early stage of Parkinson's disease is highly probable. A number of potential processes through which the chemical elements which show anomalies may participate in biochemical processes in PD was extensively discussed in [H-8]. The results obtained show the unique suitability of the SRXRF technique where is necessary to apply analytical methods of both a micrometer spatial resolution along with detectability in the range of a single mg/kg or less.

The participation of chemical elements in a number of pathological processes suggests that that certain elements may contribute, directly or indirectly, to the carcinogenic process. Due to the diversity of glioma brain tumor types, the question arises whether this morphological variability is related to the unique elemental fingerprint of different types of neoplastic tissues. Moreover, routine diagnostic tests often leave doubts as to the correct classification of a given case. Therefore, it is desirable to devise and use techniques which will support modern medical diagnostics.

One possible application of X-ray fluorescence microanalysis was to identify anything in the specific nature of the elemental composition of different brain gliomas. A diagnostic classifier for brain gliomas based on elemental composition of neoplastic tissues was also constructed [H-4]. Synchrotron radiation based X-ray fluorescence was used to find the elemental characteristics of seven types of brain tumors (6 gliomas, 1 meningioma) as well as of areas of tissue without malignant infiltration. Because the SRXRF technique provides information about the multi-element content of the specimens simultaneously it seemed to be useful in keeping all the data together for comparative studies. Multidimensional statistical analyses suit this purpose. In paper [H-4] discriminant analysis was used to find the elements from all those identified which most differentiate types of brain tumors. This method has also been applied to the creation of a brain tumor classifier which can support the routine histopathological examination, especially in difficult or disputable cases. It was found that S, Cl, Cu, Fe, K, Br, and Zn are the most significant elements in the general discrimination of tumor type. Findings showed that the discriminant function model which was constructed clearly separates classes which correspond to histopathologically diagnosed brain tumors and the control sample. The effectiveness of classification by the constructed model was also tested. The mean percentage of correct classifications estimated for the samples used for model construction was near 100 %, whereas a mean predictive pover of over 87 % was achieved for ten new cases previously excluded from the model construction. The results described in paper [H-4], clearly indicate the usefulness of X-ray fluorescence microanalysis in the elemental imaging of brain tumors. As shown there this usefulness can be substantially improved through the application of appropriate methods of statistical analysis. It should be mentioned that due to the required spatial resolution of the analysis, which should be about $20 \div 30$ μm, such studies can be carried out in the laboratory, without a synchrotron radiation source. This significantly extends the possible applications of such studies for the purposes of differentiation / classification of neoplastic tissues.

I am also the author of a review article [H-1] that popularizes the use of X-ray fluorescence, the XANES technique as well as other analytical methods of modern physics to studies of central nervous system tissue. The publication contains both an overview of work in the field of neurochemistry carried out by other researchers, as well as the results of my own research, including those that are not described in the cycle of articles submitted as the basis of my habilitation procedure. They concerned mainly the use of synchrotron radiation based X-ray microanalysis in studies of the elemental composition of the brain and spinal cord in relation to amyotrophic lateral sclerosis, preliminary research on Parkinson's disease as well as the bulk analysis of human brain samples with the use of the EDXRF and PIXE techniques.

Methodology of research of chemical forms of S and Fe in tissues with the use of X-ray absorption near edge structure spectroscopy

As demonstrated by the results of the research presented in paper [H-4] among the elements of greatest importance to the differentiation of brain tumor types are sulfur and iron. As is well known, in both physiological and pathological processes only particular forms of chemical elements may participate. To better establish the biochemical mechanisms at work tissues, it is important in addition to the determination of the total content of the element in the analyzed material also to know its chemical form. Due to the morphological variety of

tissues, it is necessary to use research techniques allowing a determination of the oxidation states of elements in microscale. The technique most commonly used to study the chemical forms of elements is X-ray absorption near edge structure spectroscopy. As in the case of x-ray fluorescence microanalysis, the use of the XANES technique in research into biological materials entails the need to develop appropriate procedures allowing the imaging and quantification of chemical forms of elements in tissue sections.

In article [H-5] both experimental and methodological aspects related to the imaging of sulfur oxidation states in brain tumor tissues with the use of the XANES technique were presented. The measurements were carried out in fluorescence mode, using the proportionality of mass absorption coefficient to the intensity of the Kα line of characteristic radiation of S. Full XANES spectra were measured inside the tumor cell, outside the cell and on its periphery. To find the dominant forms of S in the tissue structures, organic and inorganic compounds which contain sulfur in different oxidation states were measured as well. The results of measurements of the reference materials were used, in addition in the development of a database of the XANES spectra of sulfur at the beamline ID 21 / ESRF (European Synchrotron Radiation Facility). A comparative analysis for tissue samples and the reference materials was done using two approaches. In the first, from the spectrum of each reference compound, the position of the maximum of the white line peak was read and later compared with the position of the maximum of the tissue sample spectra. However, since the position of the white line also depends on the surroundings of the atom in question (here, sulfur) the energy of the first inflection point of the main edge of the XANES spectra was also found and used in the comparative analysis. Both methods indicated that in all the neoplastic tissue sites examined, sulfur is in the -2 oxidation state. However, differences in the shapes of the XANES spectra measured in various areas of the tissue were found. In particular, the XANES profiles for the intracellular area were more structured, suggesting the presence of various sulfur contributions inside the tumor cell. Furthermore, there was a pre-edge peak in the S XANES spectra measured inside the tumor cell. In general, pre-edge features are observed for transition metals and covalently bonded elements absorbing at low energy. In the case of S K-edge X-ray absorption spectra, intense pre-edge features occur due to the excitation of S *1s* electrons to the unoccupied valence orbitals formed by the interaction of the transition metal d-orbitals with the formally filled S *3p* orbital. The pre-edge peak in S XANES spectra in tissues may reflect the presence of iron–sulfur clusters in proteins. The XANES spectrum for extracellular tissue structures shows a smaller width of the K-edge peak, which indicates that sulfur occurs outside the tumor cells in a single chemical form.

To obtain more complete information on the distribution of sulfur at a certain oxidation state in larger areas of tissues, in paper [H-5] two-dimensional (2D) imaging of chemical forms of sulfur in tissue sections of brain tumors was presented for the first time. In the experimental approach, proposed by me and related to the selective chemical state imaging, the relationship between the differences in fluorescence yield for different chemical species of sulfur at various energies of excitation was used. This is directly related to the fact that with an increase in the energy of radiation incident in the sample, the probability of exciting sulfur atoms at increasingly higher oxidation levels increases. The physical fundamentals of this effect were discussed in article [H-5] as well. To excite sulfur in a given oxidation state i.e. -2, +4, +6, synchrotron radiation was used with photon energies determined experimentally based on the measurement of the full S XANES spectra of reference materials. As is known, the beam energy appropriate for the excitation of sulfur in a higher oxidation state also excites the more reduced chemical form of the element. Therefore, in order to obtain the distribution images of oxidized sulfur S^{4+} and S^{6+}, it is necessary to subtract the signal contributed by sulfur in lower oxidation states. This procedure is related to two important analytical aspects. The first one is related to the determination of the values of fluorescence intensity factors for sulfur in a particular oxidation state as a function of the photon energy of synchrotron radiation used. For this purpose the XANES profiles measured for selected reference materials were used. The second aspect concerning the procedure of

subtracting maps to produce a 2D map of the distribution of sulfur in specific oxidation states is related to the differences in the position of the synchrotron radiation beam on the sample at different exciting energies. Such an effect is observed typically when focusing the X-ray beam using Fresnel zone plates, as in the experiment described in article [H-5] . Therefore, in our study refocusing was applied to retain the focus during the S XANES imaging in each case when the energy appropriate for scanning was fixed. Although this procedure was applied in the experiment, a significant spatial shift of the maps of sulfur distribution obtained at different exciting energies was easy to notice. A detailed analysis of the differential maps showed that after changing the photon energy of the exciting radiation beam, not only did a permanent shift of its position in the XY plane occur, but also a change in the beam incidence angle on the sample. Although the cause of the observed effect was not known, in paper [H-5] the procedure that enables the correct determination of the distribution maps of chemical forms of sulfur were shown. It should be emphasized that the proposed measurement method based on 2D scanning of the sample as well as on both a beam size equal to 0.5 μm and a relatively short measurement time of single point (0.5 s) minimize the radiation damages induced by synchrotron radiation in biological material. Also, the results obtained allowed the identification of the presence of all three oxidation states of sulfur. This information is significant as there are controversies and doubts as to whether the sample preparation procedure used, storage of the samples and the influence of synchrotron radiation themselves lead to oxidation of the elements studied.

The important information from a medical point of view which emerged from the studies is a strong accumulation of sulfane sulfur in cancer cells, where its level was almost ten times higher than in the surrounding tissue. Moreover, findings have shown that sulfur in the -2 oxidation state is dominant and generally the only chemical form of sulfur in the cells of the brain tumors studied. This could confirm the hypothesis that this chemical form of sulfur plays a role in cancer cell proliferation. The presence of S^{2-} in homogenates obtained from human gliomas as well as on increase of sulfane sulfur in high-grade neoplasms was previously reported. Whereas the most important and the most meaningful finding found for the first time in [H-5] is precise indication of the tissue structures (here, tumor cells) where sulfane sulfur is highly accumulated. This observation was only possible by using a chemical micro-imaging method based on the XANES technique.

Integrated experimental and methodological aspects of the topographic and quantitative micro-imaging of iron oxidation states in tissue sections of brain tumors are presented in [H-3]. As in the case of research into the chemical form of sulfur in brain tumors [H-5] the determination of Fe oxidation states in neoplastic tissues was carried out in fluorescence mode in two ways. In the first one full XANES spectra were measured in selected structures of tissue and reference materials (Fe^{2+}, Fe^{3+}). The second one was the two-dimensional imaging of the chemical form of Fe in areas of brain tumors. The comparative analysis of the XANES spectra measured in tissue samples and reference materials showed that the ferric form of Fe is dominant in the case of high-grade gliomas (III and IV according to the World Health Organization). In the case of a brain tumor diagnosed of grade I of malignancy, the location of the main absorption edge of the XANES profile clearly indicated the presence of both chemical forms of Fe. However, because of the inhomogeneous structure of neoplastic tissues the analysis of single points would not be likely to yield reliable results. That is why it is essential to examine representative portions of tumors. For this purpose Fe XANES imaging was carried out on tissue areas of several hundred by several hundred μm^2. For studies of sulfur oxidation states, the selective excitation of Fe in the particular chemical form was applied. Based on the measurements of reference materials, the energies appropriate to excite iron in +2 and +3 oxidation states respectively were determined. Selecting the energy appropriate to excite a reduced form of Fe (7.1225 keV) a lower limit was chosen which was slightly above the Fe^{2+} absorption edge, in order to suppress the excitation of Fe^{3+}. Additionally, for the purposes of quantitative analysis, the total Fe content was determined using energy equal to 7.240 keV at which both chemical forms of Fe are excited comparably. The methodology of the preparation of the

distribution maps of Fe in a given oxidation state was analogous to that of the sulfur studies in [H-5]. In particular, imaging of the distribution of the reduced form of iron resulted directly from the scanning measurements with the use of the excitation energy equal to 7.1225 keV. At energies of 7.135 keV both Fe oxidation states (Fe^{2+} and Fe^{3+}) are excited. To image Fe^{3+} separately, the difference between the normalized Fe intensity obtained at an energy of 7.135 keV and the normalized Fe intensity at an energy of 7.1225 keV was calculated for each measurement point. Before the subtraction an appropriate factor allowing for the X-ray absorption coefficient was applied to the values of the normalized Fe^{2+} intensities. The applied method indicated the presence of microstructures where Fe^{2+} is dominant as well as ones with a high abundance of the oxidized form of Fe. In contrast with literature studies related to the XANES analysis of Fe in tissues, in the work [H-3] a method for a fully quantitative analysis allowing the determination of the masses per unit area of each chemical form of iron in tissue slices was proposed. Since the dried tissue sections used in this study can be considered a thin sample, the external standard method for quantitative analysis was used. In the calculations, presented in detail in [H-3], the Fe sensitivity factors at the appropriate excitation energies determined based on the XANES profiles of the reference materials were applied. In relation to brain gliomas, the analysis allowed for the determination of masses per unit area of Fe^{2+} and Fe^{3+} for various tumor types and control tissue. Moreover, the content of a particular chemical form of Fe in total Fe content in tissues for the analyzed groups was determined. To verify the statistical significance of the differences /similarities in Fe^{2+} and Fe^{3+} content between the cases analyzed the Kruskal–Wallis test was used. The quantitative analysis shows that for all cases the content of the oxidized form of Fe is significantly higher in comparison with Fe^{2+}. Moreover, the highest level of Fe^{3+} was found in the control sample and in gliomas of a low grade of malignancy. The lowest level of the oxidized form of Fe was observed for a glioma of the highest grade of malignancy. [H-3] proposes and describes in detail a method for quantitative analysis of the chemical forms of iron which is universal and which can be used by potential researchers dealing with similar subjects.

Scope of my personal contribution to the publications, submitted as the basis of my habilitation procedure

Articles [H-1] and [H-6] are my sole authorship. The other six articles were prepared with my significant involvement. My contribution to individual publications is set out below. In parentheses (in bold) there is a percentage assessment of my contributions to the articles.

- [H-2] – *This article is based on the results of research carried outunder a special research project of the Ministry of Science and Higher Education, of which I was the head (Synchrotron radiation in studies of the accumulation and chemical forms of iron in brain tissue structures in the case of selected disorders of the human central nervous system (DESY/304/2006) 2007-2010);*

  an initiation of studies on the presence of creatine in tissues of the human central nervous system in amyotrophic lateral sclerosis and the establishement of cooperation with professor K. Gough; the development for the methods of preparation of thin tissue samples appropriate to studies using both X-ray fluorescence microanalysis and infrared microspectroscopy; the collection of research material; the measurement of tissue samples using the SRXRF technique, qualitative and quantitative analysis (concerning the experiment carried out at HASYLAB / DESY); participation in both the discussion and evaluation of the results of studies on creatine deposits with the use of infrared microspectroscopy; participation in preparation of the manuscript. **(35 %)**

- H-3 – *This article is based on the results of research carried outunder a special research project of the Ministry of Science and Higher Education, of which I was the head (Synchrotron radiation in studies of the accumulation and chemical forms of iron in brain*

tissue structures in the case of selected disorders of the human central nervous system (DESY/304/2006) 2007-2010);

drawing up the assumptions and methodology of the research; the selection of research material and participation in sample preparation; preparation of the XANES experiment for its implementation in the synchrotron facility; the selection and optimization of the measurement conditions; participation in the XANES measurements of samples; a XANES spectral analysis of biological samples and reference materials including the determination of the fluorescence intensity factors for Fe^{2+} and Fe^{3+} depending on the energy of excitation; developing a method for the two-dimensional imaging of chemical forms of Fe and preparing of maps of distributions of Fe in a given oxidation state; the development and realization of a quantitative analysis concerning the determination of mass per unit area of a particular chemical form of iron; statistical analysis based on the results of the studies; interpretation of the results of the studies in relation to theoretical aspects of the XANES technique for Fe; the drawing and formulation of the conclusions of the study; preparation of the manuscript. **(75 %)**

- [H-4] – *This article is based on the results of research carried outunder a special research project of the Ministry of Science and Higher Education, of which I was the head (Synchrotron radiation in studies of the accumulation and chemical forms of iron in brain tissue structures in the case of selected disorders of the human central nervous system (DESY/304/2006) 2007-2010);*

  selecting research material and taking part in sample preparation; preparation of an experiment for its implementation using the SRXRF technique in the synchrotron facility; the selection and optimization of measurement conditions; participation in the SRXRF measurements of samples; the spectral analysis of biological samples and reference materials; realization of the topographic and quantitative elemental analysis of tissue samples; the selection of methods of statistical analysis including the realization of the statistical analysis; drawing and formulation of the conclusions of the study; interpretation of the results; preparation of the manuscript. **(80 %)**

- [H-5] – *This article is based on the results of the research carried out under a research project, headed by myself and entitled: "Investigation of sulfur oxidation states and biological molecules in brain glioma tissue." (MD-228), ESRF, Grenoble, France, 2006;*

  drawing up the assumptions and methodology of the research; the selection of research material and taking part in sample preparation; selection of the appropriate reference materials; preparation of a XANES experiment for its implementation in the synchrotron facility; the selection and optimization of measurement conditions; participation in the XANES measurements of samples; the XANES spectral analysis of biological samples and reference materials including a determination of the fluorescence intensity factors for sulfur in a given oxidation state depending on the energy of excitation; participation in the development of a method for the two-dimensional distribution of chemical forms of S including a method for eliminating the effect of a spatial shift of the maps of sulfur distribution obtained at different exciting energies; interpretation of the results of the studies in relation to theoretical aspects of the XANES technique for S; a determination of the absorption of the S-Kα line of fluorescence radiation for samples of various thicknesses in the context of the self-absorption effect; the drawing and formulation of the conclusions of the study; preparation of the manuscript in both theoretical and experimental aspects of the XANES technique for S. **(70%)**

- [H-7] – *This article is based on the results of research carried out under two research projects, headed by myself, i.e. a special research project of the Ministry of Science and*

Higher Education,(Synchrotron radiation in studies of the accumulation and chemical forms of iron in brain tissue structures in the case of selected disorders of the human central nervous system (DESY/304/2006) 2007-2010), and a research grant entitled "Synchrotron radiation - based chemical micro-imaging of human brain tissue in Parkinson's disease." (2010_1_91200) BESSY II, Berlin,Germany, 2010;

selectiing of research material and taking part in sample preparation; the development and preparation of reference materials for the quantitative analysis of tissue slices with the use of the external standard method; preparation of the experiment for its implementation using the SRXRF technique in the synchrotron facility; the selection and optimization of the measurement conditions; taking part in the SRXRF measurements of samples; spectral analysis of biological samples and reference materials; evaluation of the homogeneity of the reference materials; topographic and quantitative elemental analysis of substantia nigra tissues; selection of methods of statistical analysis including the realization of the statistical analysis; the drawing and formulation of the conclusions to the study; interpretation of the results; preparation of the manuscript. **(80 %)**

- [H-8] – *This article is based on the results of research carried out under a research project, headed by me and entitled: "Synchrotron radiation based investigation on biochemical changes of rat brain tissue in experimental model of Parkinson's disease." (2010_2_100175) BESSY II, Berlin, Germany, 2010.*

  drawing up the assumptions and methodology of the research of rat brain samples using the SRXRF technique; the selection of research material and taking part in sample preparation; the preparation of the experiment for its implementation using the SRXRF technique in the synchrotron facility; the selection and optimization of the measurement conditions; taking part in the SRXRF measurements of samples; the development and preparation of reference materials for the quantitative analysis of tissue slices with the use of the external standard method; spectral analysis of biological samples and reference materials; topographic and quantitative elemental analysis in rat brain areas; selection of methods for statistical analysis including the realization of the statistical analysis; the drawing and formulation of the conclusions of the study; interpretation of the results; preparation of the manuscript, excluding the description of physiological part of the experiment and grounds related to Parkinson's disease. **(60 %)**

Appropriate statements of co-authors of the articles which are included in the submitted publication cycle are attached as an annex.

5. Description of scientific research activity

5.1. Period before obtaining the Ph.D. degree

My first active scientific research was for my master's thesis. I was then a student of the Faculty of Physics and Nuclear Techniques AGH (now the Faculty of Physics and Applied Computer Science). My discipline was Technical Physics, specializing in Medical Physics and Dissymmetry. My master's thesis was entitled "Studies of the number and activity of macrophages in biopsies taken from tumors before therapy". I undertook it in cooperation with the Department of Biophysics at the Institute of Molecular Biology Jagiellonian University (JU). My supervisor was Professor S. Łukiewicz. After defending my master thesis I started Ph.D. studies at Faculty of Biology and Earth Sciences JU. At the Institute of Molecular Biology I extended the scope of the research begun while doing the thesis. I used Electron Paramagnetic Resonance technique to investigate nitrogen oxide as a source of the immunological response of the organism to cancer. After one year of Ph.D. studies at the Faculty of Biology and Earth Sciences JU I decided to change my research profile and return to the Faculty of Physics and Nuclear Techniques AGH, again as a doctoral student.

In 1998 I started studies related to the application of X-rays to the elemental analysis of brain tissues for the purposes of neuropathology. My supervisor was Professor Marek Lankosz. In 1998 I established cooperation with the Department of Neuropathology at the Institute of Neurology JU. This is still my main research area. The themes which were developed in studies conducted in cooperation with the Institute of Neurology JU, and carried out as part of my doctoral studies included, among others, the elemental analysis of relatively large brain areas (bulk analysis). The investigation was aimed at a comparison of element content in selected anatomical areas of the brain, and an evaluation of the differences in element content between white and gray brain matter, involving a study of the influence of age on the accumulation of elements in human brain. As analytical techniques I used X-ray fluorescence analysis (XRF) with an X-ray tube as a photon source as well as particle induced X-Ray emission (PIXE). The studies based on the PIXE technique I carried out in cooperation with the Institute for Nuclear Studies in Warsaw. The results of the studies performed using the XRF and the PIXE techniques showed that there is a higher accumulation of Ca, Fe, Cu, and Zn in gray brain matter than in white matter. This indicates that trace elements are connected mainly with the functioning of nerve cell bodies not with axons. Moreover, significant increase of zinc level with age was found in white matter. This may suggest the participation of this chemical element in processes of physiological ageing. For the purposes of neurology, appropriate methods were developed to allow a bulk analysis of samples of relatively large anatomical areas of the human central nervous system (CNS) using the XRF and the PIXE techniques. The quantitative elemental analysis of biomedical materials entails the need to eliminate matrix effects in the case of both techniques, so various methods of quantification were tested. The results of studies of human brain tissue in macroscale were described in detail in [1.1., 2.3., 2.6., 2.7.] (the numbering of the publications is given according to the list of the articles attached as an annex to this summary of professional accomplishments).

In the context of my doctoral studies I started to investigate of the role of trace elements in the pathogenesis of two neurodegenerative disorders i.e. Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). These studies were again carried out in cooperation with Institute of Neurology JU. Both diseases are characterized by the loss of nerve cells in selected areas of the central nervous system. In the case of Parkinson's disease degeneration and atrophy occur mainly in neuromelanin reach neurons in the substantia nigra (SN) of midbrain. In the case of ALS, neurodegeneration concerns mainly the motor neurons of the ventral roots of the spinal cord and motor cortex. The etiology of both disorders is as yet unknown. However, some closely related biochemical processes which accompany the process of cell loss are taken into account. Among the most important

of these are oxidative stress, excitotoxicity, protein aggregation, and mitochondrial dysfunction. Additionally, in the case of ALS, a certain percentage of morbidity is related to the mutation of the gene encoding Cu-Zn superoxide dismutase. It is significant that in all these processes trace elements such as Fe, Cu, Zn, Mn, and Ca play an important role. That is why to better understand pathogenesis of these disorders and to undertake appropriate neuroprotective treatment, studies including inter alia an assessment of elemental changes (including studies of the chemical forms of elements) are of great importance. An important aspect in this field is the determination of the elemental composition of tissues at the cellular level.

The possibilities of using the XRF technique with a polycapillary collimation of X-rays generated by an X-ray tube, for the purposes of the micro- and macroanalysis of brain tissues were also the subject of my doctoral thesis. In the light of this problem I developed procedures for the quantitative analysis of both tissue samples of several hundred milligrams and thin tissue slices. Findings concerning this subject area were described in scientific articles [2.2., 2.4.].

Laboratory conditions do not do not provide sufficient detectability of chemical elements in brain tissues at a satisfactory spatial resolution, so I included to my research synchrotron radiation. An indispensible tool that permits the imaging of the elemental composition of tissues at the cellular level is the synchrotron radiation based X-ray fluorescence technique (SRXRF). In 2000 I established scientific cooperation with the European Synchrotron Radiation Facility (ESRF) in Grenoble. One year later, I undertook the first measurement sessions at beamline ID 22 (ESRF) related to a qualitative and quantitative evaluation of changes in the elemental composition using the SRXRF technique in brain areas in the case of Parkinson's disease and amyotrophic lateral sclerosis in relation to a control group. The microbeam applied in the experiment was able to discern distribution of elements at the single cell level. The high intensity of the incident beam significantly improved the detectability of elements which are undetectable in the laboratory. Two-dimensional maps of elemental distribution in tissues allowed an answer to the question of whether a given chemical element is strongly accumulated in intra- or extraneuronal species. This is helpful in knowing whether an observed elemental abnormality has a neuronal or glial origin. These preliminary comparative studies carried out on control and pathological cases showed the presence of significant differences in the accumulation of selected elements in analyzed brain areas. These findings confirm the existing hypothesis about the role of trace elements in the pathogenesis of neurodegenerative disorders, providing information about the location of elemental abnormalities in tissue structures. The findings of these preliminary studies on elemental changes in central nervous system tissues in Parkinson's disease and amyotrophic lateral sclerosis were described in publications [1.2., 1.4., 1.5., 2.5.]. Paper [1.5.] held for a long time first place in the X-Ray Spectrometry ranking of highly cited papers.

In order to extend the research on the elemental composition of brain tissue on a microscale, in 2002 I started to cooperate with the synchrotron laboratory HASYLAB at DESY in Hamburg. There I continued the experimental part of my research after the completion of my doctoral thesis.

To conclude, in the years 2000-2003 I prepared four research projects carried out at ESRF (LS-2111, MD-32, MD-60, MD-103) and one three-year project that was performed in cooperation with HASYLAB / DESY (II-02-092). At the same time I started to set-up the inclusions of a new technique in my research, i.e. Fourier transform infrared microspectroscopy (FTIR-MS). This technique allows inter alia the determination of biological molecules in microareas of central nervous system tissues. For this purpose I established cooperation with LURE at the Super-ACO synchrotron in Orsay and prepared research project (IM 017-03), which was implemented in 2003.

In 2001-2004, I was the main participant in two research projects (7T11E02021, 4T11E02022 – promoter grant) funded by the State Committee for Scientific Research

(SCSR), Warsaw. These projects were related to the application of synchrotron radiation to studies of neurodegenerative disorders and preparation of analytical procedures for the X-ray fluorescence analysis (bulk analysis) of biological materials.

In research aimed to search for a biochemical marker for amyotrophic lateral sclerosis I participated in studies on the elemental composition of body fluids (cerebrospinal fluid, serum, total blood) using total reflection X-ray fluorescence (TXRF). The analyses did not show significant and clear differences in elemental composition between ALS and a control group. The results of this research were presented in [1.3., 2.4., 2.6.].

In 2003 I defended my doctoral thesis entitled "X-rays in studies of the elemental composition of human central nervous system tissue" at the Faculty of Physics and Nuclear Techniques AGH. The thesis was supervised by Professor Marek Lankosz. After the defense of my doctorate and the birth of my first child in June 2003, there was a short, formal break in my scientific research till September 2003.

5.2. Period after obtaining the Ph.D. degree

In October 2003 I was employed as Assistant at the Faculty of Physics and Nuclear Techniques, and in September 2004 as the Associate Professor. From 2003 I continued the research started during my doctoral thesis. In order to verify preliminary results concerning anomalies in the elemental composition of CNS tissues observed for single cases of PD and ALS new groups of cases were used for further SRXRF studies. In 2003 – 2005 I participated in special research project (SPB/DESY/P-05/DWM 728) funded by SCSR. An outstanding achievement from this period was the determination of significantly higher levels of Ca, Fe, and Zn in substantia nigra nerve cells and in the white matter area in the case of PD in comparison with the control. Moreover in the area of the motor cortex an increased level of Ca and Zn was found in PD. The results of the analysis of spinal cord samples, representing ALS, showed no significant differences in the elemental accumulation between the analyzed ALS cases either in nerve cells or in the white matter of the spinal cord. The results of quantitative analysis showed that there were no general abnormalities in the elemental accumulation between the ALS and the control group. However, for individual ALS cases such abnormalities were observed for nerve cells. The results of the studies were published in a series of scientific articles [1.6., 1.7., 1.8., 1.10., 1.13,1.15]. Based on the results of my research work related to the use of X-rays in neurobiology I was awarded the Professor Zbigniew Engel prize for the best work in the field of basic research.

I continued elemental micro-imaging of human brain tissues in relation to Parkinson's disease in 2009 – 2011 in a special research project headed by myself (DESY/304/2006) and funded by the Ministry of Science and Higher Education (MSHE). Moreover, Parkinson diseased brains were investigated in three experimental research projects carried out at BESSY II synchrotron at Helmholz-Zentrum in Berlin (2009_2_90056, 2010_1_91200, 2010_2_100136) of which I was the head. The result of the work from this period is, inter alia, scientific article [1.22.] that is attached to the cycle of publications submitted as the basis of my habilitation procedure.

As well as imaging the elemental composition of tissues, this research makes it also important to know the chemical forms of certain elements (e.g. S, Fe, Cu). Therefore, I also included X-ray absorption near edge structure spectroscopy (XANES) in the studies of neurodegenerative disorders. This allowed the determination of the chemical forms of elements.

In the case of Parkinson's disease the evaluation of Fe and Cu oxidation states was carried out for pigmented neurons of substantia nigra in selected points of tissues. However this point analysis does not show any significant differences in Fe and Cu oxidation states between Parkinson diseased and control cases [1.12., 1.14.]. Because these studies were carried out only for selected points of tissues I tackled this issue later using two-dimensional

imaging of chemical forms of Fe in whole tissue areas. The research was carried out in 2007 - 2010 in a special research project of MSHE of which I was the head (DESY/304/2006), and in an experimental project carried out at synchrotron BESSY II at Helmholz-Zentrum in Berlin (2009_2_90056). The analysis was carried out for samples representing PD, the early stage of PD (idiopathic form) and for samples representing a control group. It was found that in the case of PD both chemical forms of Fe are present in substantia nigra nerve cells. The maximum level of the oxidized form of Fe is, however, almost ten times higher than the reduced form of this element. Moreover, iron of a lower oxidation state is located additionally in extraneuronal spaces. Another characteristic of distribution was found in the case of early stage of PD. In nerve cell bodies it was mainly a reduced form of Fe that was accumulated. The distribution of Fe^{3+} was homogeneous in the whole sample area, and without any tendency for it to accumulate in neuron bodies. There was a significantly lower level of this form of Fe in the substantia nigra of the early stage of PD in comparison with Parkinson diseased cases. For the control, it was found that the dominant form in SN neurons is the oxidized form of iron. However, Fe of a lower oxidation state was present there as well. The level of Fe^{2+} in control SN was about 3, 4 times lower than in Parkinson's disease. The results of quantitative analysis indicate that there is a higher percentage of the reduced form of iron (and the same lower level of its oxidized form) in relation to total iron content in sample occurs in case of Parkinson's disease. The percentages of chemical forms of Fe for the early stage of PD and for the control were comparable.

In cooperation with the Department of Neurology at the Faculty of Medicine JU, the Department of Pathophysiology at the Faculty of Medicine JU, and the Helmholz-Zentrumin Berlin I carried out an investigation into elemental changes that occur in dopamine-related brain structures in rats as a result of the electrical stimulation of vagus nerve. This work is a part of studies on the early stage of Parkinson's disease, and is described in scientific article [1.20.], published this year. The part of the experiment headed by me was related to an elemental analysis of tissues and was carried out as part of three experimental projects (2009_2_90184, 2010_1_91201, 2010_2_100175) at synchrotron BESSY II in Berlin. The studies showed the influence of a dysfunction of the vagus nerve on elemental changes in dopaminergic structures. This suggests participation of certain elements in the processes which accompany the early stage of Parkinson's disease.

In 2006 I extended my research themes, bringing past experience to bear on studies of brain tumors, particularly gliomas. Work carried out on this subject has two main purposes. The first one is to assist medical diagnosis, while the second relates to obtaining additional information about the process of cancerogenesis. In 2007 – 2010 I was head of a special research project (DESY/304/2006) funded by MSHE, where I carried out a substantial part of the research on gliomas. Additionally, studies were performed in a three-year project (2006 – 2009) in cooperation with HASYLAB / DESY (II-20060029 EC) and one experimental project at ESRF (MD 228) in 2006. This work included, inter alia, a determination of the elemental distribution and content in neoplastic tissues diverse in terms of the type and grade of malignancy, the construction of a model for the classification of various types of brain gliomas, a determination of the elements of the highest importance for the differentiation of brain tumors, and a study of Fe and S chemical forms in brain tumors. The findings of this work were presented in a series of scientific articles [1.15., 1.17., 1.18., 1.19., 2.20.]. More precisely this research subject is presented in the description of publications submitted as the basis of my habilitation procedure.

As I mentioned previously, in 2002 I included studies using infrared microspectroscopy in my research. This is the other main theme of my scientific activity alongside the chemical imaging of brain tissues using X-rays. Because the infrared absorption spectrum provides unambiguous information about organic compounds (including biological molecules) and the secondary structures of proteins, the FTIR-MS technique is valuable as a complement to studies of elemental composition. This technique can also be useful for imaging the changes of functional groups of the main biological molecules in

pathological processes. Therefore, I used the FTIR-MS technique in my work investigating samples of brain tissues in neurodegenerative disorders (initially, as a research project at synchrotron Super-ACO, LURE in Orsay). It was very important to perform the measurements using both the FTIR-MS and the SRXRF on the same research material. This required the selection of sample preparation procedures using a sample support appropriate for both techniques. In the case of Parkinson's disease the FTIR-MS allowed a determination of changes in lipids, nucleic acids and in the secondary structure of proteins [1.8., 1.11., 1.13.]. The results confirm the multietiological nature of Parkinson's disease. Moreover, the findings clearly indicate that, in addition to a distinct visual observation, the diseased nerve cells exhibit a change of their biochemical composition. It suggests that disruption of the normal functioning of SN neurons appears before their morphological atrophy. In case of amyotrophic lateral sclerosis the use of infrared microspectroscopy was related among other things to microimaging creatine deposits in CNS tissues. This was described in [1.16] included in the cycle of articles submitted as the basis of the habilitation procedure, and is presented in more detail in appropriate part of this summary of professional accomplishments. Moreover this research subject was described in [2.18.].

A study of the macromolecular composition of human CNS tissue in neurodegenerative disorders was the subject of the master thesis of M.Sc. Eng. Marzena Kastyak. I was the supervisor of this thesis. The thesis was considered the best masters thesis in the field of application in the AGH Diamonds contest in 2006. This is one of my greatest successes in teaching. At a later period of my scientific research activity I introduced macromolecular imaging also to a series of studies on brain tumors. After the close of the Super-ACO synchrotron I continued the existing cooperation in carrying out studies on the new synchrotron SOLEIL in St. Aubin. There, in 2008 – 2009, I was project leader of two research projects (20080145, 20090103) related to the investigation of the molecular composition of brain gliomas. I also carried out the studies of brain tumors on beamline ID 21 at ESRF. The results have already allowed a characterization of the elemental and biomolecular features of various brain gliomas. Unlike the overall elemental composition which is different for various brain tumor types, in the case of the macromolecular composition of neoplastic tissues, the presence of unique features distinguishing the analyzed tissues was not observed.

In 2005 – 2008 I took a part in an EU-funded (6 FP) international research collaboration under the DASIM project (Diagnostic Applications of Synchrotron Infrared Microspectroscopy). Networking between biologists, clinicians and synchrotron scientists was aimed to further explore and advance the diagnostic possibilities of infrared microspectroscopy.

As I mentioned, I initially carried out measurements using the FTIR-MS technique at the synchrotron facilities LURE, ESRF, and SOLEIL. In 2009, with financial support from the Fund for Polish Science and Technology I created a specialized laboratory of infrared microspectroscopy at the Faculty of Physics and Applied Computer Science AGH. Highly sophisticated equipment allows measurements in both a macro- and microscale, including ATR-FTIR (attenuated total reflection), the grazing angle method, etc. The instrumentation is used for both research and teaching (laboratory activities, studies used in diploma theses, supervised by me) purposes. The creation of this laboratory of infrared microspectroscopy with unrestricted access to very high quality equipment has significantly improved the quantity of samples that can be measured. This is of great importance in studies on clinical material.

In 2011 I extended my research activity in the field of neurology to embrace issues related to the biochemical microimaging of brain tissues in physiological ageing. Up to now I have the structure of studied substantia nigra. The degeneration of the nerve cells of the substantia nigra pars compacta of the midbrain, and which is present in brain ageing processes, leads to significant dopamine depletion mainly in the nigrostriatal pathway. A dysfunction of neuronal physiology in the dopaminergic system causes, in the end, motor

dysfunctions that are an important problem in human life and in intense cases disorders such as Parkinson's or Alzheimer's. The processes of physiological ageing are accompanied by numerous histopathological phenomena that are often related to the atrophy of nerve cells. Many factors may cause neuronal atrophy either in physiological ageing or neurodegenerative disorders. The most important are protein aggregation, oxidative stress or genetic or environmental factors. The research I have begun is directed towards a knowledge of the potential abnormalities in the elemental and biomolecular composition of brain tissues which accompany histopathological phenomena in processes of physiological ageing. The studies headed by me are conducted in cooperation with the Department of Patomorphology, Faculty of Medicine JU and the Helmholz-Zentrum in Berlin where I carry out measurement work. In the future I will continue to build on the work already done in this area of research.

In conclusion, the results of my scientific research have been published in 43 research articles and presented at 54 scientific conferences. Three times I was invited to give lectures at prestigious scientific meetings. My publications have found recognition in the international scientific community, which results, in no small part from international cooperation established with scientists form the Department of Neurology - University Medicine, Göttingen, CNRS – the University of Bordeaux, DESY (PETRA III), the Department of Physics - Institute for X-Ray Physics, Göttingen. Under the umbrella of this collaboration we have worked on research related to the use of techniques based on X-rays and infrared in the studies on Parkinson's disease. This area, alongside ageing processes in general will be one of the main strands of my scientific research activity in the near future.

A detailed list of my scientific research achievements, teaching experience and organizational activity is presented in the annex.

M. Szczerbowska-Boruchowska