Summary of professional accomplishments

1. First and Last Name: **Marzena Kastyak-Ibrahim**

2. Diplomas and Academic degrees

- Certification in Higher Education Teaching (CHET) Program; 2011; The University of Manitoba, Winnipeg, MB, Canada

- PhD in Physics, 2009, University of Science and Technology; Title of the doctoral dissertation: Chemical characterization and imaging of creatine deposits in the human central nervous system tissue with infrared and X-Ray fluorescence spectromicroscopy; supervisors: Dr. Hab. Eng Marek Lankosz and Dr. Kathleen Gough (University of Manitoba)

- MSc in Chemistry (Biological Chemistry), 2009, Jagiellonian University, supervisor: Professor Dr. Hab. Grażyna Stochel

- MSc. Eng. in technical Physics (Medical Physics and Dosimetry), 2005, AGH University of Science and Technology, supervisor Dr. Hab. Eng. Magdalena Szczerbowska-Boruchowska

3. Professional experience in Academia:

Aug 2015 – present: Undergraduate Learning Coordinator, the University of Calgary, Department of Physics & Astronomy, Calgary, AB, Canada

Sep 2013 – Jul 2015: Laboratory coordinator; the University of Manitoba, Department of Physics & Astronomy, Winnipeg, MB, Canada

Jan 2013 – Aug 2014: Instructor for Physics Class; International College of Manitoba, Winnipeg, MB, Canada

Sep 2011 – Aug 2013: Postdoctoral Fellow (funded by Alzheimer Society of Canada), the University of Winnipeg, Department of Physics, Winnipeg, MB, Canada

Jul 2010 – Aug 2011: Instructor for Biochemistry class, the University of Manitoba, Winnipeg, MB, Canada

Jun 2009 – Apr 2011: Research Associate/ Postdoctoral Fellowship; the University of Manitoba, Department of Chemistry, Winnipeg, MB, Canada

4. Scientific contribution according to art. 16 ust. 2 ustawy z dnia 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz. U. nr 65, poz. 595 ze zm.)

a) Title of the scientific contribution: Publication series: **Testing the limits of micro-imaging: Optimization of methods and protocols for micro-imaging of changes in the Central Nervous System underlying neurodegeneration.**

b) The publication series includes the following:

H-1. **Kastyak, M.Z.,** Szczerbowska-Boruchowska, M., Adamek, D., Tomik, B., Lankosz , M. and Gough, K.M. (2010) Pigmented creatine deposits in Amyotrophic Lateral Sclerosis central nervous system tissues identified by synchrotron FTIR and X-Ray Fluorescence spectromicroscopy. *Neuroscience* 166: 1119–1128.

H-2. Kuzyk, A., **Kastyak, M.Z. (co-first author)**, Agrawal, V., Gallant, M., Sivakumar, G., Rak, M., Del Bigio, M.R., Westaway, D., Julian, R., and Gough, K.M. (2010) Association between amyloid plaque, lipid, and creatine in hippocampus of TgCRND8 mouse model for Alzheimer disease, *Journal of Biological Chemistry* 285(41): 31202-31207

H-3. Gough, K.M., Tzadu, L., **Kastyak, M.Z.**, Kuzyk, A.C., and Julian, R.L. (2010) Theoretical and Experimental Considerations for Interpretation of Amide I Bands in Tissue, *Vibrational Spectroscopy*, 53(1): 71-76

H-4**.** **Kastyak-Ibrahim M.Z.**, Nasse M.J., Rak M., Hirschmugl C., Del Bigio M.R., Albensi B.C., and Gough K.M. (2012) Biochemical label-free tissue imaging with subcellular-resolution synchrotron FTIR-FPA. *NeuroImage* 60(1):376-83

H-5. Stitt D., **Kastyak-Ibrahim M. Z.**, Liao C. R., Morrison J., Albensi B. C., Gough K.M. (2012) Tissue acquisition and storage associated oxidation considerations for FTIR microspectroscopic imaging of polyunsaturated fatty acids. *Vibrational Spectroscopy* 60:16-22, 2012

H-6. **Marzena Kastyak-Ibrahim**, Domenico Di Curzio, Sheryl Herrera, Richard Buist, Marc R Del Bigio, Benedict C Albensi, Melanie Martin (2013) Neurofibrillary tangles and plaques are not accompanied by white matter pathology in aged triple transgenic-Alzheimer disease mice. *Magnetic Resonance Imaging* 31(9):1515-21. doi: 10.1016/j.mri.2013.06.013

c) Description of goals of the publication series and its possible applications

"Only those who will risk going too far can possibly find how far one can go." T.S. Eliot

Introduction

The impact of neurodegenerative diseases on societies, especially in developed countries, is constantly increasing. Having a family member or a friend affected by Alzheimer's disease or other neurodegenerative disorder became rather common. At present there is no cure; therefore, efforts supporting the development of the early diagnostics and prevention are crucial. In order to develop a diagnostic tool or to establish which factors can slow down neurodegeneration, the level of understanding of the processes underlying neurodegeneration needs to improve. The family of neurodegenerative disorders is very complex. It is known that there are several processes involved in disease progression, such as oxidative stress, excitotoxicity, protein aggregation or mitochondrial dysfunction, but why and how neurons die remains a mystery. Imaging techniques can be used to study biochemical and structural changes in the Central Nervous System (CNS). They can be applied to analyze post mortem tissues, both human (taken during autopsies) and from animal models of a neurodegenerative disorder. Additionally, certain techniques, such as Magnetic Resonance Imaging (MRI) allow detection of changes in living organisms (*in vivo*).

The challenge is that the changes observed in the neurodegenerative disorders can be very subtle, especially when the disease starts to develop, and each technique has its limitations related to the sample preparation, storage, size of the imaged area, spatial resolution, to the time it takes to image and to the approach chosen to analyze the data.

The publication series, presented in my habilitation application, focuses on testing the limits of the imaging techniques (by developing and optimizing imaging methods and protocols) at the microscopic level (micro-imaging) in order to study the changes underlying neurodegeneration.

Fourier Transform Infrared Spectroscopy (FTIR) applied for biological samples

Vibrational spectroscopy enables identification of the functional groups and molecular conformation and is gaining recognition as a valuable tool in biomedical research. Fourier Transform Infrared Spectroscopy (FTIR) permits two-dimensional mapping of the principal biomolecules of tissue with sub-cellular spatial resolution[1] and also enables investigation of differences in protein secondary structure, an important consideration for research and diagnostic. Each IR spectrum can be analyzed for multiple components (lipid, protein, sugar).

Synchrotron FTIR microspectroscopy combines FTIR microspectroscopy with the bright light emitted by synchrotron source, and enables analysis of the tissue composition at the sub-cellular level (5 microns or less). All major types of macromolecules have their characteristic peaks in infrared. Amide I and II band envelopes are assigned to various vibrations of the protein backbone and can be used to assess conformational changes (they are sensitive to molecular structure and local environment of the molecules) based on the peak position, intensity and shape (H-3).

The table below shows characteristics peaks most commonly used for the analysis of the FTIR maps, in the series of the publications (H-1 – H-5).

[1] Dumas P, Miller L (2003) The use of synchrotron infrared microspectroscopy in biological and biomedical investigations. Vib Spectrosc 32: 3–21.

Peak analysis shows distribution of	Used for visualization of	Peak name	Peak maximum [cm^{-1}]	Range [cm^{-1}]	Base line [cm^{-1}]	Publication
Lipids (saturated fatty acids)	White and grey matter	symmetric CH_2 band envelope	2850	2860-2840	3012-2750	H-1, H-2, H-4
	Layers in retina	Lipid carbonyl bond	1735	1750-1720	1760-1710	H-4, H-5
Lipids (polyunsaturated fatty acid esters, PUFA)	Lipid bilayers	PUFA olefin functional group	3012	3026-3000	3026-3000	H-4, H-6
Proteins	Proteins	α - helix amide I band	1655	1662-1652	1806-900	H-1, H-2, H-3, H-4
Dense core plaques	Dense core plaques	β - sheet amide I band (shoulder)	1630	1630-1620	1806-900	H-1, H-2, H-3, H-4
Creatine	Creatine deposits	Creatine 1390	1390	1410-1384	1410-1384	H-1, H-2
		Creatine 1304	1304	1309-1298	1317-1282	H-1
		Creatine 2800	2800	2809-2787	2820-2760	H-1
Nucleic acids	Neurons	Nucleic acid marker	1712	1725-1700	1725-1700	H-4
Collagen	Layers in retina	Collagen 1204	1204	1214-1197	1214-1197	H-4

FTIR analysis (H-1 – H-5) was always performed on unprocessed, original spectra in order to avoid the introduction of artifacts due to post processing such as smoothing.

Several applications of FTIR have been described in details in the series of the publications presented here, including: analysis of diffuse or dense core plaques (H-2, H-3, H-4, H-3 references 6-7), characterization of neuronal layers in retina, based on the analysis of saturated and unsaturated fatty acids esters (H-4, H-5) and visualization of individual hippocampal neurons (H-4),

Applications of FTIR to neurodegenerative diseases

Using FTIR, described in the publication series, my research focuses on three types of components: lipids, creatine deposits and amyloid plaques.

FTIR images processed for lipid distribution (2850 cm^{-1} peak) are used to show tissue morphology in CNS tissue. Neurons are revealed by lowest lipid intensity, while white matter is much higher in lipid content. FTIR maps can therefore reveal the same information as standard staining protocol, used to visualize white matter, such as the stain for myelin: Luxor Fast Blue (H-1, H-6). Loss of myelin often accompanies the neurodegenerative processes.

Creatine is a small molecule which plays an important role in metabolism. In the spectrum from the tissue containing creatine, there are very distinctive sharp peaks (due to creatine being in crystallized form) much different from other broader peaks originating from macromolecules. Creatine monohydrate was used to collect reference spectra for the study evaluating the occurrence and distribution of creatine deposits in the human CNS tissue samples (motor cortex, spinal cord (ventral horns), brain stem) taken during autopsies from Amyotrophic Lateral Sclerosis and non-neurodegenerative control cases (H-1). Amyotrophic Lateral Sclerosis (ALS) is an untreatable, neurodegenerative disease affecting motor neurons in the ventral horns of the spinal cord, the motor nuclei in the brain stem, the large pyramidal neurons of motor cortex and the large myelinated axons of corticospinal tracts. Creatine was found to be focally elevated and it formed deposits in human ALS CNS tissue. Focally elevated creatine may be a marker of the disease process, indicative of disturbed energy metabolism or inflammatory response to the disease progression. No correlation of creatine deposit location and elevated concentration of the selected metal distribution (calcium, potassium, iron, copper and zinc) have been found using the synchrotron micro-X-ray fluorescence technique (H-1).

Amyloid plaques are characteristic feature for Alzheimer's disease (AD). AD is a progressive neurodegenerative disorder characterized by memory loss and dementia. The pathological hallmarks include extracellular deposits of amyloid peptides (plaques) and neurofibrillary tangles within the neurons. The hippocampus is affected significantly by neurodegeneration, even in early AD; therefore, hippocampal tissues were selected for studies (H-2, H-3). The biochemical pathways of AD are complicated and not fully understood. My research findings described in (H-2, H-3, H-4) focus mainly on biochemical changes occurring in the brain tissue of Alzheimer disease mouse models.

Two mouse models have been used:

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- The TgCRND8 mice (H-2 - H-4) carrying two mutations (Swedish K67ON/M671L in APP and Indiana V717F) and developing florid deposition of dense-core amyloid plaques from an early age.
- The 3xTg mice (H-4 – H-6) carrying three mutations (Swedish K67ON/M671L in APP, preselin mutation PS1 (M146V) and human four-repeat Tau harboring the P301L) and developing both plaques and tangles. They are excellent subjects for evaluating potential AD therapeutics.

FTIR two-dimensional analysis was used to revel the spatial correlation between dense core plaques, creatine deposits and lipid distribution in AD mouse model (H-2). A study of the TgCRND8 AD mouse model and non-transgenic controls showed that lipid distribution throughout the hippocampus is not affected by AD (H-2), because there were no differences between the lipid distribution in transgenic (Tg) and non-Tg littermates at ages from five to seventeen months. The analysis of the serial sections from the region containing dense core plaques revealed that a thin (30-50 micrometers thick) lipid envelope always surrounds the dense core plaques which can be evidence of inflammation process. Creatine deposits are not co-localized with dense core plaques or lipid envelopes. Elevated creatine was also found in the hippocampus of the AD mouse model. The number of deposits increases with age. In the control group, fewer and smaller deposits were only found in the hippocampus of the oldest animals (H-2). Moderate increase of creatine in the older controls might be a result of normal aging.

Creatine forming the deposits can be extracellular or intracellular. It is possible that creatine is released when tissue is frozen, sectioned, or both. Crystalline long and branched creatine deposits might be formed when the tissue is drying after cryosectioning. It is interesting that in the AD mouse models, creatine deposits are found predominantly in the gray matter (H-2) while in the ALS human tissue larger deposits are located in the gray matter, but they could also be found in white matter (H-1). Large creatine deposits were found in human tissue affected by ALS (spinal cord, brainstem and motor neuron cortex), but not in tissue samples from regions that are not affected by ALS (hippocampus or substantia nigra) or controls. Only few punctate deposits could be seen in the controls (H-1).

Creatine deposits have been found in the tissue samples from the Amyotrophic Lateral Sclerosis (ALS) patients and Alzheimer's disease mouse model brains (H-1, H-2) which indicates that elevated creatine may be a more general indicator of neurodegeneration.

Consideration of IR band complexity

Understanding the origin of the bands in the FTIR spectrum is crucial for data analysis. Amide I band envelope is present in most of the FTIR and Raman spectra from biological samples. This broad and complex band is assigned to various vibrations of the protein backbone and contains information about conformation of the protein based on peak positions associated with the changes in molecular structure and local environment. It is important to remember that Amide I envelope, in tissue analysis, is the sum of all Amide I vibrations from every protein and modes originating from other tissue components within the IR microscope field of view.

Biomolecules are difficult to model due to their size. In order to analyze spectra and determine the protein confirmation or larger proteins, an understanding of how the Amide I band looks for a simple model of protein in alpha or beta confirmation, is needed. The basic features of the Amide I profile have been explored (H-3) using the computational modeling of spectra (Hartree-Fock calculations with B3LYP/6-31) for 2-9 amino acids glycine peptides arranged as straight chain, α-helix, parallel and anti-parallel β- sheets. FTIR vibrations are due to the molecular dipole variation. It was found that while it is true that the intensity increases with the number of residues, the intensity of the peak is a function of the entire structural arrangement and not just the number of residues of particular conformation, because of the complexity of the modes. When a straight chain

and α- helix were compared, the identity of the most intense band has changed, because of the change of the conformation. For β- sheets structures, the most intense mode is the same form as in the straight chain, but the overall intensity has increases significantly for comparable number of residues.

Theoretical (calculated for glycine peptides) and experimental (data collected for CNS tissues using FTIR and FTP-FTIR) results are compared. Only good quality FTIR tissue spectra (no scattering artifacts) were used. From the point of view of my research the discussion of the secondary protein structure associated with AD plaques in the TgCRND8 mouse model is of most interest. One has to be very careful when analyzing biological samples, and consider which spatial resolution (pixel size) has been used to collect the data. Inappropriate spatial resolution might blur or dilute a specific signature (for example β- sheets structure) when many proteins are present (which is more likely when using a larger pixel size).

Achieving subcellular spatial resolution

Factors that determine how successful data analysis is include: physical size of the sample, tissue heterogeneity, chemical composition and molecular conformation differences between different regions of the sample or between pathological and control samples and the presence of the spectrally resolvable, distinguishing marker peaks (H-4). Each FTIR spectrum collected from a human or animal CNS tissue sample is the sum of spectra of myriad components. The larger the pixel size, the larger the area from which the spectrum was collected; therefore, larger pixel size increases the probability of having of information from more than one cellular structure collected. Focal Plane Array FTIR (H-1 – H-5) allows fast data collection using 64 x 64 pixel FPA whit a pixel size from 8 by 8 to 25 by 25 microns squared. It is a recommended technique, when white light microscope images alone are not sufficient to determine which region of the tissue sample contains the desired information and should be imaged. Due to the time constriction, only a carefully selected, representative region of the tissue sample can be imaged using higher resolution FTIR methods.

FTIR spectrometers using synchrotron radiation as the IR source (sFTIR) allow imaging using smaller pixel size (usually of the order of 5 by 5 to 8 by 8 microns squared H-1- H-3). Higher spatial resolution for sFTIR is possible, because the synchrotron light is much brighter and allows data collection with smaller pixel size (aperture size) while keeping the spectrum intensity (high signal-to-noise ratio) high and data time collection reasonable.

The mid-infrared beamline built in Madison, Wisconsin, called IRENI (**I**nfra**R**ed **EN**vironmental **I**maging) is the first synchrotron-based wide-filed IR imaging system, uniquely combining twelve synchrotron beams to homogenously illuminate a large are of the sample. It allows imaging using FPA of 128 by 128 and pixel size as small as 0.54 by 0.54 microns squared (H-4). Pixel size of the order of half micron square is smaller than the size of most of the cells imaged in the CNS tissues, meaning that IRENI creates a possibility of FTIR imaging at sub-cellular spatial resolution. Acquisition time with IRENI is 10^4 faster than could be achieved with thermal source FTIR. Characteristic IR absorption peaks from all tissue components are detected simultaneously at each pixel, in situ, eliminating the need of staining the tissue.

Spectra from 3xTg and TgCRND8 AD mouse model hippocampus and cortex tissue as well as control mouse retina tissue were collected with a 0.54 by 0.54 micron squared resolution (H-4). The location of single neuronal cell bodies has been shown possible for the first time. In

comparison FTP-FTIR data can be used to show only general location of group of neurons. A new band at 1390 cm^{-1}, previously unreported, was discovered with IRENI. This band is associated with the dense core plaques. Amide I band (β- sheets), traditionally used for imaging of dense core plaques, located at 1630 cm^{-1}, is often distorted when really dense core plaques are imaged, while the 1390 cm^{-1} band is located in the spectral region which is not as affected by scattering as the Amide I band. A distinctive lipid envelope was found to surround the dense core plaques as shown before (H-2), but this time it was also confirmed that a small amount of lipids infiltrates within the dense core plaques. IR band, characteristic for the nucleic acids (1712 cm^{-1}) was very apparent in the individual neuron spectra.

Each FTIR method allows achieving specifically selected goals. While results using IRENI beamline are impressive, each of the methods has both strengths and limitations. A combined approach of multi-modal imaging, using many different, complementary techniques, is usually the most successful (H-4). The quality of the FTIR spectra always depends on the quality of the sample, but the spatial resolution should be chosen depending on the goal of the experiment.

Sample preparation and storage for FTIR analysis

Sample preparation differs depending on the goal of the experiment and the technique used. The FTIR data collection is performed on unfixed, snap-frozen tissue sections, permitting simultaneous in situ detection of multiple tissue components, including metabolites that would be lost in tissue preparation for staining. For FTIR micro-imaging tissues were collected during the autopsy (human CNS tissues, H-1) or brains are collected post mortem, in case of Alzheimer's disease mouse models. In both cases tissues were snap-frozen, stored at -70^{0}C, cryosectioned and mounted on an appropriate substrate. For samples imaged in transmittance mode, the substrate has to be transparent for IR radiation (H-1, H-4). For samples imaged in trans-reflectance mode (H-2 – H-5) gold coated microscope slides or glass IR reflective slides are used. Tissue sections cannot be too thick or too thin. In the first case the tissue would strongly absorb IR radiation. Also, the spectrum would not be collected only from the area as seen when looking at the sample (pixel size would be much smaller than the thickness of the sample). In the latter case the intensity of the spectra would be too low. It is also very difficult to ensure that the sample has no folds or tears when it is very thin. I found the optimal thickness to be between eight and ten micrometers.

When studying biological samples one needs to realize that sample storage can make a difference for the experimental results, especially when tissues containing biomolecules that can be easily oxidized, such as polyunsaturated fatty acids (PUFA; containing olefin functional group C=C-H), are investigated. Series of time-lapse experiments (H-5) were performed in order to assess the influence of sample preparation and storage on imaging of the PUFA which are highly concentrated, for example, in membrane phospholipids of retina and brain. Mouse brain and retina samples were stored under three different conditions:

1) Samples were stored at -70^{0}C for a short period of time (1 week) and an extended period of time (8 months)
2) Freshly cryosectioned samples were imaged before, during and after the exposure to 4 cycles (24h) of sunlight
3) Freshly cryosectioned samples were imaged before, during and after being stored in darkness at room temperature for 12h, 3, 7, 15, 31 and 72 days.

FTIR maps were processed for 3012 cm^{-1} peak and carbonyl 1750 cm^{-1} peak (see Table.1) in order to assess the degree of tissue oxidation. As expected, sun exposure (in front of the closed window, with no UV light) caused tissue oxidation after 24 hours. 3012 cm^{-1} peak could no longer be observed after four days of exposure. Carbonyl peak intensity was significantly reduced. Samples stored at -70^0C for an extended period of time and samples stored in the darkness at room temperature were also affected but to a lesser extent. Based on the observations (H-5), it is recommended to image the samples as soon after cryosectioning as possible. When storing is required, they should then be stored in darkness for a period of time not longer than one month. The protocols described in H-5 permit more accurate assessment of PUFA in biological tissues by FTIR analysis.

In vivo brain imaging versus *ex vivo* tissue imaging

Imaging using the FTIR offers tremendous potential for investigating the appearance and distribution of abnormal proteins and classifying disease stage, but can only be used in tissue sections from biopsies or autopsies. I have been always interested in using other non-destructive spectroscopic methods that enable imagining *in vivo*. This is why I have chosen the Magnetic Resonance Imaging (MRI) to continue the studies of the 3xTg AD mouse model.

MRI is widely used in medical imaging in humans, but there is an increasing need for MRI imaging designed for mouse models used to study human disease. MRI gives the unique possibility of repeated measurements within a single animal over time in order to study a disease progress and hallmarks and to test new therapeutic approaches.

Amyloid plaques, the most know features of AD, are not always present and their appearance may occur in the late stage of the disease when no available treatment would be effective. Moreover, plaques are difficult to detect with MRI, because of the limited spatial resolution of this imaging method. Therefore, targeting early and easier to detect changes, preceding plaque formation is crucial. The overall aim of the study (H-6) was to measure white matter changes in the brain of the 3xTg AD mice in order to determine if they precede grey matter changes as it was previously suggested[2].

Two MRI methods were used; Diffusion Tensor Imaging, DTI, with the sequence allowing faster data acquisition (Echo Planar Imaging, EPI) and T_2-weighted Imaging which shown white matter as dark and grey matter brighter. Diffusion Tensor Imaging has been shown to be very sensitive to diffusion restriction and useful to detect connectivity of different regions in brain (white matter tracks), whereas T_2-weighted imaged show lipid density. Both *in vivo* and *ex vivo* (post mortem) images of mouse brain were collected. Distinctive on MRI images (due to its size), white matter structures were chosen for imaging: corpus callosum and fornix.

MRI techniques are based on physical interaction of electromagnetic radiation with matter. Diffusion Tensor Imaging (DTI) detects the restriction of diffusion of water. In tissues, diffusion is restricted due to the packing and orientation of cells and extracellular components. Diffusion in

[2] Desai MK et al. (2009) Triple-transgenic Alzheimer's disease mice exhibit region-specific abnormalities in brain myelination patterns prior to appearance of amyloid and tau pathology. Glia 57(1):54-65

tissues such as white matter is anisotropic, as a consequence of the orientation of the axons and the surrounding multi-layered myelin sheath, which are thought to restrict water diffusion. Diffusion anisotropy means that each image voxel containing white matter has different diffusion rates along different spatial direction. As a result, the signal intensity of a given voxel will vary with the application of gradient pulses of comparable strength applied along different directions. Mathematically, diffusion anisotropy is described by a symmetric 3 by 3 tensor. A convenient shorthand for the diffusion anisotropy is the ratio of the diffusion rates parallel and perpendicular to the fiber orientation. In white matter tracts, the anisotropy should range from about 2 to 10. Empirically, diffusion anisotropy in human brain white matter has been measured to be about 3, whereas in grey-matter the diffusion anisotropy is nearly 1 (essentially isotropic).

DTI enables visualization of white matter tracks in two and three dimensions and can be also used to show tissue integrity. DTI measurements can be conducted *in vivo,* but in case of small mouse brain and required high resolution, they might be too slow. Echo planer imaging (EPI), is a diffusion-weighting method to a fast imaging sequence, to collect diffusion MR data rapidly. Diffusion-weighted EPI can be performed allowing for faster measurements, so that at least seven directions of gradients can be obtained and thus the full diffusion tensor calculated.

3xTg AD mice and matching controls were imaged periodically *in vivo* and then *ex vivo*. (Details can be found in H-6). Histological and immunohistochemical labelling methods (sensitive to changes in white matter, presence of plaques and tau pathology) were used in order to ensure any changes in white matter would be detected, even if they would not be visible on MRI images.

After detailed analysis of the results obtained using sensitive MRI methods, no statistically significant changes were found between the AD mouse model and control group (H-6). It was concluded that the 3xTg-AD mouse model does not develop MRI detectable changes prior to plaque formation. No changes in the white matter were found using even higher resolution MRI methods for fixed mouse brains *ex vivo*. The results of the histological stains were consistent with the MRI data analysis.

Testing the limits

Developing and optimizing imaging methods and protocols for studying the changes underlying neurodegeneration requires understanding of the limits of imaging techniques. It is often worth to test the limits for yourself in order to find out how far you can go.

The goal of the study often determines the type of samples to be used. They have to be representative with respect to the neurodegenerative disorder in question, including the region of the CNS. For example, hippocampal tissues are used in studies related to AD, as the hippocampus is the region affected by neurodegeneration. Sample preparation for *ex vivo* imaging involves span-freezing a sample block from an autopsy or part of the mouse CNS harvested after an animal has been sacrificed. Shortly before imaging, samples for FTIR are cryosectioned and mounted on an appropriate substrate. Sample storage can significantly affect the results of the study (H-5); therefore, it is recommended to prepare tissue sections as close to the time they would be imaged as possible.

A typical tissue section usually has a size of up to one centimeter squared. In order to determine the areas of the samples should be imaged with sFTIR, series of white light microscope photographs are taken prior to imaging. In many cases the photographs are sufficient, but not always. A decision made very early in my career significantly affected the way my research was conducted. While imaging samples containing creatine deposits, many of the deposits could be seen on the photographs due to pigmentation (H-1), but I was not sure how many more deposits (maybe not pigmented) could be there. Only imaging of the entire sample could give the certainty. Despite being told that using a non-synchrotron source would not yield the quality results, I have decided to give the 16 element linear array FTIR instrument located at the synchrotron facility (NSLS) a try. Taking advantage of the fact that the creatine deposits I have imaged so far were large (over 50 microns in length), and had very distinctive peaks, I have decided to test the limits of the instrument by increasing the pixel size to 25 by 25 microns square and decreasing number of scans from 128 to 4. The combination of the three factors: number of pixels imaged at once: 16 (instead of one); larger pixels (25 microns squared compared to 8 microns squared (25/ 8 = 3.125) and lower number of scans (128/4 = 32) decreased the imaging time by the factor of 1600. It made possible the imaging of 26 entire samples and discovering many more creatine deposits (H-1). My approach was quickly adapted in the research group I worked with and imaging with the FTIR instrument using an array detector is done before using higher resolution sFTIR techniques (H-2 – H-4).

Each FTIR spectrum contains information about multiple tissue components. Molecular level information is buried in overlapping bands whose spectral separation is often less than the spectral resolution. Therefore caution should be excercised when it comes to the interpretation of the seemingly simple spectra of complex tissues. While several post-processing algorithms are provided by manufactures or the FTIR spectometers (e.g. zero-fill, smoothing), from my experience, it is best to analyze the original spectra (with no additional artifacts acidentally introduced by the algorithms).

The types of analyses that can be chosen for a data set are affected by the spectral quality, the amount of data and the goal of the study (H-3). Challenges in the application of FTIR to microscopic biological tissue analysis include the fact that spectra contain molecular level information while the typical spatial resolution is on the order of few microns. Decreasing the pixel size can provide more detailed information about a selected tissue area (H-4). Optimum parameters for visualizing the same biological feature may be radically different when data originate from different instruments and caution should be used when setting up the parameters for analysis.

A new challenge arises as one moves from the analysis of the mouse brain tissue to *in vivo* mouse brain imaging, the imaging time is limited to approximately two hours (maximum time an animal should stay under anesthesia); therefore, imaging protocols have to be optimized in order to fit this tight time window and still allow achieving the spatial resolution required. Another challenge related to using MRI for imaging white matter in the mouse brain is the fact that the mouse brain contains only 10% of white matter and 90% of grey matter. Due to the MRI spatial resolution and low white matter content in mice for the study (H-6) only large white matter structures (corpus callosum, external capsule and fornix) were selected for analysis.

MRI imaging of the mouse brain requires higher spatial resolution and therefore higher magnetic field strength than the one produced in the magnets used for clinical MRI imaging (7 T compared to 1.5 T). Methods developed at lower fields, such as 1.5 T might not apply at higher field strengths used to study animal models. Often new, more suitable methods have to be developed and/ or optimized. The need to use the higher field strength with mice lies in the ability to obtain the higher-resolution images.

The complexity of the neurodegenerative disease makes studying the changes occurring during the disease process very challenging. Depending on the approach and technique chosen, different kinds of information can be obtained. The series of the publication presented here is my contribution to a big puzzle to be solved, called neurodegeneration.

My contribution to the series of publications presented in the habilitation application

My contribution to the series of publications (with an estimated percentage contribution to each of publications) is as follows:

- H-1 – I prepared the white light microscopy photographs of the samples and selected the areas for analysis, optimized the data collection parameters and protocols, collected data using FTIR, sFTIR and sXRF (Synchrotron radiation Center (SRC), Wisconsin and National Synchrotron Light Source (NSLS), Brookhaven National Laboratories, Upton, NY), I selected and optimized the parameters for analysis, I prepared the first version of the manuscript, including the figures, I acted as link between two research groups during the discussion of the co-authors regarding the interpretation of the results. I prepared the final version of the manuscript for the submission to the journal, and was a corresponding author. I estimate my contribution to be 65%.
- H-2 – I was working (and supervising the summer student in Dr. Gough's research group: Alexandra Kuzyk) during the selection of the areas of the samples for analysis (white light microscopy), optimization of the imaging protocols, data collection an analysis using FTP-FTIR (University of Manitoba) and sFTIR (Synchrotron radiation Center (SRC), Wisconsin) and the preparation of the first draft of the manuscript. I prepared the final version of the manuscript for the submission to the journal, was a corresponding author, and addressed the comments of the reviewers. I estimate my contribution to be 75%.
- H-3 – I was working (and supervising the summer student in Dr. Gough's research group: Alexandra Kuzyk) during the selection of the areas of the samples for analysis (white light microscopy), optimization of the imaging protocols, data collection an analysis, and I prepared the experimental part of the manuscript. I estimate my contribution to be 40%.
- H-4 – I participated in the sacrificing of the mice, bi-sectioned the brains and snap-froze the samples; I prepared the samples and selected the areas for analysis using the FTP-FTIR (University of Manitoba) and sFTIR (Synchrotron radiation Center (SRC), Wisconsin). I optimized imaging protocols, collected data (Dr. Rak and Dr. Nasse also collected data), found the best parameters for the data analysis (spectra collected with IRENI) and analyzed the data. I have also performed staining of the tissues, prepared the first version of the manuscript, including the figures. I prepared the final version of the manuscript for the submission to the journal, was a corresponding author, and addressed the comments of the reviewers. I estimate my contribution to be 65%.

- H-5 – I was working (and supervising the summer student in Dr. Gough's research group David Stitt) during the preparation of the samples, optimization of the imaging protocols, data collection and analysis of FTP-FTIR data (University of Manitoba) and sFTIR (Synchrotron radiation Center (SRC), Wisconsin), I prepared the final version of the manuscript for the submission to the journal I estimate my contribution to be 50%.
- H-6 – I prepared the project and applied for funding with the Alzheimer's Association of Canada. After consultation with the neuropathologist, Dr. DelBigio, I selected the mouse model, optimized of the imaging protocols, performed all MRI imaging and most of the animal handling, participated in sacrificing of the animals, and prepared mouse brains for *ex vivo* imaging; collected and analyzed post mortem MRI data. I prepared tissue sections for histological analysis, performed basic histological stains (H&E, SC) and helped with the immunohistochemical labelling (amyloid beta). I prepared the first version of the manuscript, including the figures, participated in the discussions. I prepared the final version of the manuscript for the submission to the journal, was a corresponding author, and addressed the comments of the reviewers I estimate my contribution to be 90%.

Statements of the co-authors (in alphabetical order of their last names) of the series of the publications are included as an attachment (Załącznik 5) to this application.

5. Description of other scientific (artistic) achievements:

Summary of publications

Year	Journal	Impact factor (year of publication)	Number of citations (Jul 2, 2016 Scholar Google)	Total
2005	X-Ray Spectrometry	1.372	25	1
2007	Arch Biochem Biophys	2.578	45	1
2010	JBC	5.328	30	1
2010	Neuroscience	3.357	19	1
2010	Vibrational Spectroscopy	1.747	15	1
2011	Vibrational Spectroscopy	1.747	13	1
2012	NeuroImage	6.132	33	1
2013	Magnetic Resonance Imaging	2.788	3	1
		Ogółem	183	8
		Indeks Hircha	**7**	

Time period prior to the PhD defense

My interests have always been broad, multidisciplinary and medically-oriented. I chose to study Medical Physics and Dosimetry (MSc followed by PhD) and later Biological Chemistry (MSc). My solid science background has been systematically enriched by anatomy, physiology,

pathology, histology, cell biology, biochemistry, neuroscience and radiology courses and work experience. The common denominator for all my interests is imaging of Central Nervous System. Neuroimaging allows me to use my diverse skills and knowledge.

I have completed my first research project in high school as a preparation for the competition at the provincial level (Science Olympics). When I think about it now, my first project was already imaging related, because it focused on determining the preferred location of Gladiolus imbricatus in the area of Bialka Tatrzanska based on the growth conditions, such as soil profile and sun exposure.

As a student of the Department of Physics and Nuclear Techniques (currently the Department of Physics and Applied Computer Science AGH), majoring in Technical Physics, and specializing in Medical Physics and Dosimetry, at the end of the fourth year of my studies I started a research project leading to the MSc desideration "Biochemical analysis of central nervous tissue using synchrotron infrared microscopy," under the supervision of Magdalena Szczerbowska-Boruchowska in cooperation with the Department of Neuropathology, Institute of Neurology, Jagiellonian University. At that time, I was already a student at the department of Chemistry of the Jagiellonian University, which allowed me to better understand the basics of infrared spectroscopy. A few months later, I went to the University of Bordeaux I as a Socrates-Erasmus student, where I continued learning about the infrared spectroscopy by taking the courses and completing my research project entitled "Imaging alamathicin-phospholipids bilayers with Attenuated total reflectance FTIR."

Upon my return from France, I completed the FTIR analysis of the sFTIR spectra and defended my MSc Thesis in July 2005. One year later, I have received an award for my thesis: Award for the Best Master's Thesis (AGH Diamonds).

It was during the data analysis for my MSc thesis that I came across an unknown spectral features: sharp, intense peaks overlaid on the familiar spectrum of a biological sample. It was not until much later, during my PhD research, when I found out the unusual spectral peaks are characteristic for creatine.

After defending my MSc thesis, I started doctoral studies at the Department of Physics and Applied Computer Science using synchrotron infrared spectroscopy. In the summer 2006, I took my first trip to the synchrotron facility in Grenoble (ESRF) in France.

From September 2006 until December 2007, I was a visiting PhD student at the University of Manitoba in Winnipeg, Canada. During my stay in Winnipeg, I took numerous trips to the two synchrotron facilities (SRC, Synchrotron Radiation Center in Madison , Wisconsin and NSLS , the National Synchrotron Light Source, Brookhaven National Laboratories, Upton, NY), where I continued the study of creatine deposits, In October 2009 I defended my PhD dissertation entitled : "Chemical characterization and imaging of creatine deposits in human central nervous system tissue with infrared and X -ray fluorescence spectromicroscopy ."

Due to the numerous research trips, I continued my master studies at the Department of Chemistry at the Jagiellonian University in a custom program. In May 2009, I defended my MSc thesis entitled: "Selected transition metal ions impact on Ataxin - 3 structure and aggregation".
In the period before the defense of my doctoral thesis, the following publications were written with my participation:

- Szczerbowska-Boruchowska, M., Dumas, P., Kastyak, M.Z., Chwiej, J., Lankosz, M., Adamek, D., and Krygowska-Wajs, A. (2007) Bimolecular investigation of human substantia nigra in Parkinson's disease by synchrotron radiation Fourier transform infrared microspectroscopy. Arch Biochem Biophys 459(2): 241-248.

- Szczerbowska-Boruchowska, M., Chwiej, J., Lankosz, M., Adamek, D., Wojcik, S., Krygowska-Wajs, A., Tomik, B., Bohic, S., Susini, J., Simionovici, A., Dumas, P., and Kastyak, M. (2005) Intraneuronal investigations of organic components and trace elements with the use of synchrotron radiation. X-Ray Spectrom. 34(6), 514-520.

During this period, I also participated in the following conferences and scientific meetings:

- Kastyak, M.Z., Szczerbowska-Boruchowska, M., Lankosz, M., Adamek, D., Tomik, B., and Gough, K.M.: Study of Amyotrophic Lateral Sclerosis brain tissue with FTIR Microspectroscopy, Synchrotron Radiation Center Users Meeting, Stoughton, WI, October 12-13, 2007

- Agrawal, V., Gallant, M., Wiens, R., Kastyak, M., and Gough, K.M.: Evaluation of Creatine Deposits In TgCRND8 Mouse Brain Tissue By Synchrotron FTIR Spectromicroscopy, Synchrotron Radiation Center Users Meeting, Stoughton, WI, October 12-13,2007

- Kastyak, M.Z., Szczerbowska-Boruchowska, M., Lankosz, M., Adamek, D., Tomik, B., and Gough K.M.: Study of Amyotrophic Lateral Sclerosis brain tissue with FTIR Microspectroscopy and X-Ray Fluorescence, 1st International Workshop on Spectral Diagnosis (SD-1), Northeastern University, Boston, MA, June 21– 23, 2007

- Gallant, M., Rak, M., Kastyak, M., Del Bigio, M.R., Westaway, D., and Gough, K.M.: Imaging Focally Elevated Creatine in APP Transgenic Mice, Biophysical Chemistry Symposium, Toronto, April 21-23, 2007

- Kastyak, M.Z.: SR-FTIR microscopy studies of biological macromolecules changes in neurodegenerative disorders, Conference SPEC2006, Optical Diagnosis for the New Millennium at the German Cancer Research Center, Heidelberg, Germany, May 20- 24 2006

Time period after the PhD defense

After obtaining a doctoral degree, as a postdoc at the University of Manitoba in Winnipeg I continued my research started during the preparation of my doctoral thesis. I started to study mouse CNS tissue of mice and I continued the IR analysis of many areas of tissue obtained before the defense of the doctoral thesis. I also supervised summer students: Alexandra Kuzyk and David Stitt. Consequently, I also completed a two-year post-doctoral research at the University of Winnipeg. The funding for my research was granted (after I prepared and submitted an application of a research project) in a competition organized by Alzheimer Society of Canada (Postdoctoral Fellowship: Alzheimer Society of Canada).

My research form that period is described in a series of publications presented in the habilitation application: H - 1 - H- 6. My doctoral thesis was also published in book form:

- **Kastyak M.Z.:** Imaging of creatine deposits in human CNS tissues with FTIR and XRF microspectroscopy. LAP Lambert Academic Publishing AG & Co. KG. ISBN: 978-3-8383-8483-2

In the period after obtaining a doctoral degree participated in the following conferences and scientific events:

- Kastyak-Ibrahim, M.Z., Richard Buist, Domenico L Di Curzio, Marc R Del Bigio, Benedict C Albensi, Melanie Martin: Neurofibrillary tangles and plaques are not accompanied by white matter pathology in older 3xTg-AD mice. Manitoba Neuroscience Network Meeting, Winnipeg, MB, June 10, 2013

- Kastyak-Ibrahim, M.Z., Richard Buist, Domenico L Di Curzio, Sheryl L. Herrera, Benedict C Albensi, Marc R Del Bigio, Melanie Martin: Region specific changes in 3xTg Alzheimer's disease mouse model evaluated by volume and DTI metric values. Canadian Association of Physicist Meeting, Montreal, QC, May 27-31, 2013

- Kastyak-Ibrahim, M.Z., Richard Buist, Domenico L Di Curzio, Sheryl L. Herrera, Benedict C Albensi, Marc R Del Bigio, Melanie Martin Imaging of selected brain regions in 3xTg Alzheimer's mouse model by Magnetic Resonance Microscopy. Alzheimer's Society of Manitoba Meeting, Winnipeg, MB, Jan 24, 2013

- Kastyak-Ibrahim, M.Z. Imaging of white matter changes in AD mice by MR microscopy - a continuous challenge. Pharmacology Seminar Series, University of Manitoba, Winnipeg, MB, Oct 5, 2012.

- Kastyak-Ibrahim, M.Z., Richard Buist, Domenico L Di Curzio, Marc R Del Bigio, Benedict C Albensi, Melanie Martin Imaging of selected brain regions in 3xTg Alzheimer's mouse model by Magnetic Resonance Microscopy. Manitoba Neuroscience Network Meeting, Winnipeg, MB, June 4, 2012

- Kastyak-Ibrahim, M.Z., M.J. Nasse, M. Rak, C. Hirschmugl, M.R. Del Bigio, B. C. Albensi and K.M. Gough: Biochemical Label-Free Tissue Imaging with Subcellular-Resolution

Synchrotron FTIR-FPA, Synchrotron Radiation Center Users Meeting, Stoughton, WI, Sep 16-17, 2011

- Stitt D., Kastyak-Ibrahim M.Z., Suh M, Albensi B., Gough K.M.: FTIR Microspectroscopic Imaging of Polyunsaturated Fatty Acids in Biological Tissues, Undergraduate Life Science Research Conference, Winnipeg, MB, November 20, 2010

- Kastyak, M.Z., Nasse, M.J., Albensi, B.C., Del Bigio M., Hirschmugl, C., and Gough, K.M.: High definition sFTIR imaging of tissue from Alzheimer disease mouse models, Manitoba Neuroscience Network Meeting, Winnipeg, MB, June 14, 2010

- Kastyak, M.Z., Szczerbowska-Boruchowska, M., Lankosz, M., Adamek, D., Tomik, B., and Gough, K.M.: Study of Amyotrophic Lateral Sclerosis brain tissue with FTIR Microspectroscopy, Synchrotron Radiation Center Users Meeting, Stoughton, WI, October 12-13, 2007

- Agrawal, V., Gallant, M., Wiens, R., Kastyak, M., and Gough, K.M.: Evaluation of Creatine Deposits in TgCRND8 Mouse Brain Tissue By Synchrotron FTIR Spectromicroscopy, Synchrotron Radiation Center Users Meeting, Stoughton, WI, October 12-13,2007

Teaching and popularization of Science

I believe that teaching is an integral part of the career in Academia. It does not matter whether you are meeting students during the lecture, in a laboratory setting or if you supervise their research project. There is always a dynamic that results in new ideas, new approaches and that brings creativity.

Teaching has been an integral part of my life. I used to watch my mother teach at school and spend hours at home with me and my sister, helping us discover our own way of learning. She was the first person who led me to understand that teaching is not about yourself, it is about others and doing your best to help them understand. I started to apply her methods while tutoring my colleagues at school who had trouble with passing exams or understanding a particular topic. Tutoring continued during my years at university. During my PhD I started teaching university classes and my adventure with teaching began.

While I have always loved classroom teaching, I was a little skeptical about laboratory work, until I was given an opportunity to develop new experiments. It was then when I finally started to appreciate the value of laboratory experiments as they give students an opportunity to discover by themselves and to think independently. Experiments, performed in a laboratory setting as well as those preformed in the classroom, are an integral part of teaching science.

I had an opportunity to be involved in teaching in the following roles:

- **Instructor for Physics Course** at the International College of Manitoba, Winnipeg, Manitoba, Canada from January 2013 until Aug 2014. As an instructor, I had the opportunity to develop a method of teaching that allowed students from different countries to understand the material. I introduced a new way of teaching in groups (Team Based Learning, TBL), which allows teachers to better understand ways of thinking of their students. TBL also introduces to students an element of preparation of the material before coming to class. It also allows you to spend more time on deeper learning, not only on the superficial understanding. After successfully introducing TBL, I was invited to give a workshop about this method.
  - Kastyak-Ibrahim, M.Z., Yamchuk A.: Team Based Learning workshop. Centre for the Advancement of Teaching and Learning, University of Manitoba, Winnipeg, MB, March 25, 2015
- **Laboratory coordinator** at the University of Manitoba, Department of Physics & Astronomy, Winnipeg, MB, Canada from September 2013 until July 2015. During that time, I prepared laboratory manuals, introductions (Power Point presentations with images and videos embedded), and additional materials needed for first year Physics experiments (Mechanics, Wave & Modern Physics, both calculus and non-calculus based courses) and Optics Second Year course. I was setting up and managing the learning management system (Desire2Learn). My duties included assigning, training and supervising teaching assistants as well as making sure that grading of the lab reports was fair and correct. During that time, I submitted two successful applications and obtained funding to modernize the laboratories (over $100,000 each), and to design new or update existing experiments. My idea of introducing templates in Excel for electronic data analysis/ reporting changed the way the laboratories are run and was presented at the conference in June 2015.
  - Kastyak-Ibrahim, M.Z., Kunkel H., Sharma, K: A New format for Reporting in a First Year Physics Laboratory. Canadian Association of Physicist Meeting, Edmonton, AB, June, 15-19, 2015
- **Undergraduate Learning Coordinator** at The University of Calgary, Department of Physics & Astronomy, Calgary, AB, Canada, starting from Aug 2015. This position allows me to oversee multiple Physics courses as a course coordinator. I am responsible for development of common policies and learning objectives for the course, determining, in consultation with course instructors, and the Undergraduate Program Director, selection of educational resources, such as textbooks. During the academic year (the Fall and Winter terms), I work with over 3000 students and develop new teaching methods and laboratory experiments during the summer. I manage on-line learning systems, including coordinating assignments; posting educational materials, grades for laboratories, tutorials, in class activities, examinations, and the calculation of the final course grades. As Laboratory and Teaching Assistants (TAs) Coordination, I am in charge of evaluating the role and effectiveness of the laboratory components in achieving the course and program objectives; developing and implementing laboratory experiments to meet the course and program objectives as well as the needs of the students; coordinating and participating in the updating and revising of laboratory exercises and manuals; serving as the primary resource in providing orientation, mentorship and meeting regularly with the TAs to provide ongoing training; preparing and coordinating TA evaluation and feedback. I am actively involved in testing innovative teaching approaches for the multi-section courses. During

the Winter 2016 term, in-class activities helping students in understanding course material were introduced. I presented this new approach in May 2016 during the meeting of the instructors interested in improving their teaching methods.

- Kastyak-Ibrahim, M.Z.: Increasing student engagement by introducing problem solving activities and modifying TA training. Strategies for Success Event, Toronto, ON, May, 13, 2016

During the course of my career, I have also had the opportunity to get involved in activities popularizing Science. For example, I volunteered as a judge for a high school poster competition (Winnipeg School Division's science fair).

While working on my postdoctoral project at the University of Winnipeg, I was invited to give a talk to seniors about MRI and Alzheimer's disease:

- Kastyak-Ibrahim, M.Z. How can magnetic resonance imaging help to diagnose Alzheimer's disease? Charlswood Senior Center Seminar Series, Winnipeg, MB, Jan 28, 2013

I was also interviewed about my research in order to make it known to the public. Please see the link to the article and a short movie: http://alzheimer.mb.ca/researchmb/marzena.html

M. Kastyak-Ibrahim

Calgary, Kanada dn. 8 lipca 2016 roku