ESRF	**Experiment title:** **Distribution of organic components and light elements inside nerve cells in cases of selected neurodegenerative disorders.**	**Experiment number**: MD103
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Report:

Three methods were applied to study of biochemical composition in single neurons of human central nervous system (CNS) tissue for selected neurodegenerative disorders (Parkinson's disease -PD and Amyotrophic Lateral Sclerosis -ALS). The synchrotron radiation microbeam x-ray fluorescence (micro-SRXRF) was used for analysis of selected elements in the tissue slices. The evaluation of Fe oxidation state was performed with the use of microscopic X-ray absorption near edge structure spectroscopy (micro-XANES). Fourier transform infrared microspectroscopy (FTIRM) was applied to investigation of main organic components of the tissues.

For the experiment, samples were taken during the autopsy of patients deceased with PD, ALS and from patient died due to non-neurological conditions. Two areas of CNS i.e. substantia nigra (SN) of brain and thoracic spinal cord (SC) were sampled. The specimens were frozen and cut into sections of 20 micrometers thickness in a cryomicrotome. From each section one slice was taken for routine histopathological investigation and the contiguous one for spectroscopic analysis. The slices designed for biochemical analyses were mounted immediately onto AP1 or Ultralene foil and freeze-dried. The measurements of NIST standard reference materials (SRM 1833 and SRM 1832) were performed for spectrometer calibration. For each standard the measurement was performed in 9 points. The acquisition time was equal to 10 s per pixel.

The micro–SRXRF analysis was carried out for SN of PD and the control group samples. A 7.18 keV monochromatic photons beam, focused to the dimension of 1 μm x 0.5 μm (horizontal (H) x vertical (V)) and flux at the sample of about $1*10^{11}$ photons/s was used. For single neurons scanning areas were 60 x 60 μm^2 and were mapped by steps of 1 μm (H) by 1 μm (V). Time of measurement was equal to 3 s per point. The 2D maps of elemental distribution in the tissue slices were obtained after normalization of the counts number to the incident photon flux.

The elements such P, S, Cl, K, Ca and Fe were identified in CNS tissue. The present results are in good agreement with the results obtained in the previous experiments. We observed that significantly higher fluorescence intensities of selected elements in micro–SRXRF images reflect

position of the neurons in SN. The Figure 1 shows elemental maps of S, Cl, K, Ca and Fe in PD tissue in comparison with the control group. Topographic analysis of neuron pericarial parts showed differences in intraneuronal distribution between the analyzed elements. The inhomogeneous intensities of characteristic x-rays of selected elements for the sample areas may result either from nature of the tissue or inhomogeneous mass per unit area of the sample. It was suggested by the fact that the intensities of the x-ray transmitted through the sample are significantly lower in the areas of the neuron bodies in comparison with the surrounding tissue.

The absorption spectra of near Fe K-edge were measured in melanized neurons from substantia nigra of control and Parkinson's disease samples. Additionally for PD sample, one point having about ten times higher intensity of Fe than in the nerve cell was investigated in respect to iron oxidation state. The energy of microbeam used for iron excitation was changed from 7.05 to 7.30 keV. The time of measurement was equal to 2 s for each energy point of the single XANES spectrum. For every measurement point from 10 to 20 spectra were collected. Then they were normalized to the incident photon flux and summarized. The sum spectra were smoothed with the use of Savitzky-Golay algorithm, normalized to the intensity of "white line" peak and then compared (see Figure 2). The preliminary results showed that the spectrum measured for PD neuron is shifted towards the higher energies in comparison with the spectra obtained for the neurons representing three controls, the early stage of PD case and for the point of high Fe fluorescence intensity. Further investigations are necessary, especially more cells form each sample should be examined.

The FTIRM measurements were performed for the nerve cells of SN (PD and the control tissue) and for motor neurons of SC (ALS and control tissue). The IR spectra were collected in absorption mode using Atlμs software. The spot size of the IR beam was equal to 25 μm x 25 μm. The spectra were collected in the spectral range between 650 and 4000 cm^{-1}, at the resolution of 8 cm^{-1} and with 128 scans per spectrum. The comparison of the absorption IR spectra obtained in the area of control SN neuron and the outside tissue was presented in Figure 3. Application of FTIRM enabled to perform two-dimensional maps of main organic components distribution in the specimens (Figure 4). The preliminary studies revealed differences in composition of the tissue taken from areas of SN and SC. Moreover, they suggest changes in the main organic components for the analyzed pathological cases. Especially, in the spectra obtained for PD SN neurons differences in the region characteristic for nucleic acids were observed. It is worth to mention that in spite of the cell degeneration in ALS, that was observed histopathologically, the infrared spectra still revealed strong peaks characteristic for nucleic acids.

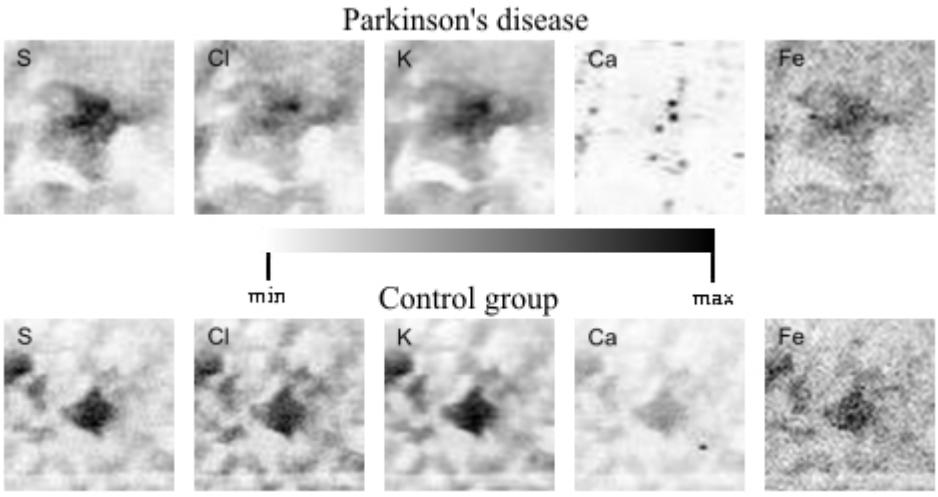

Figure 1. Micro–SRXRF elemental maps of S, Cl, K, Ca and Fe in PD tissue and in the control group.

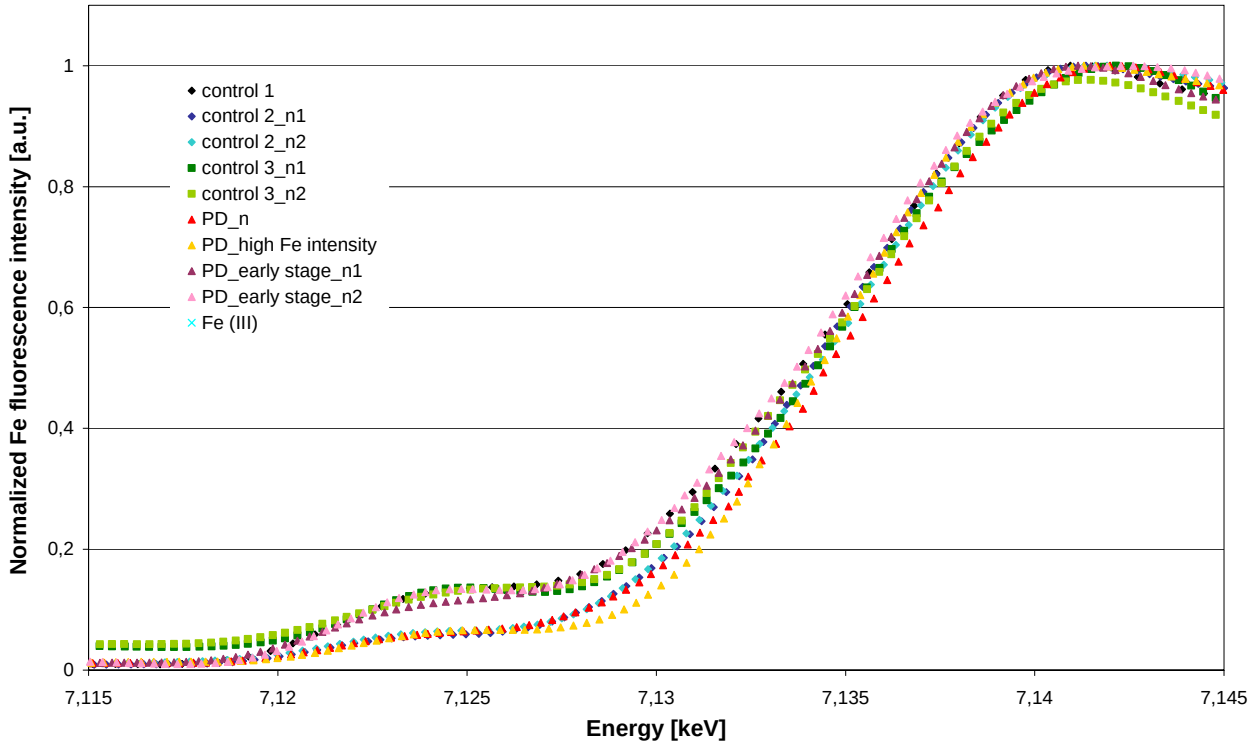

Figure 2. Absorption spectra near Fe K-edge measured in melanized neurons from substantia nigra of control and Parkinson's disease samples (date obtained inside nerve cells, excluding "PD_high Fe intensity").

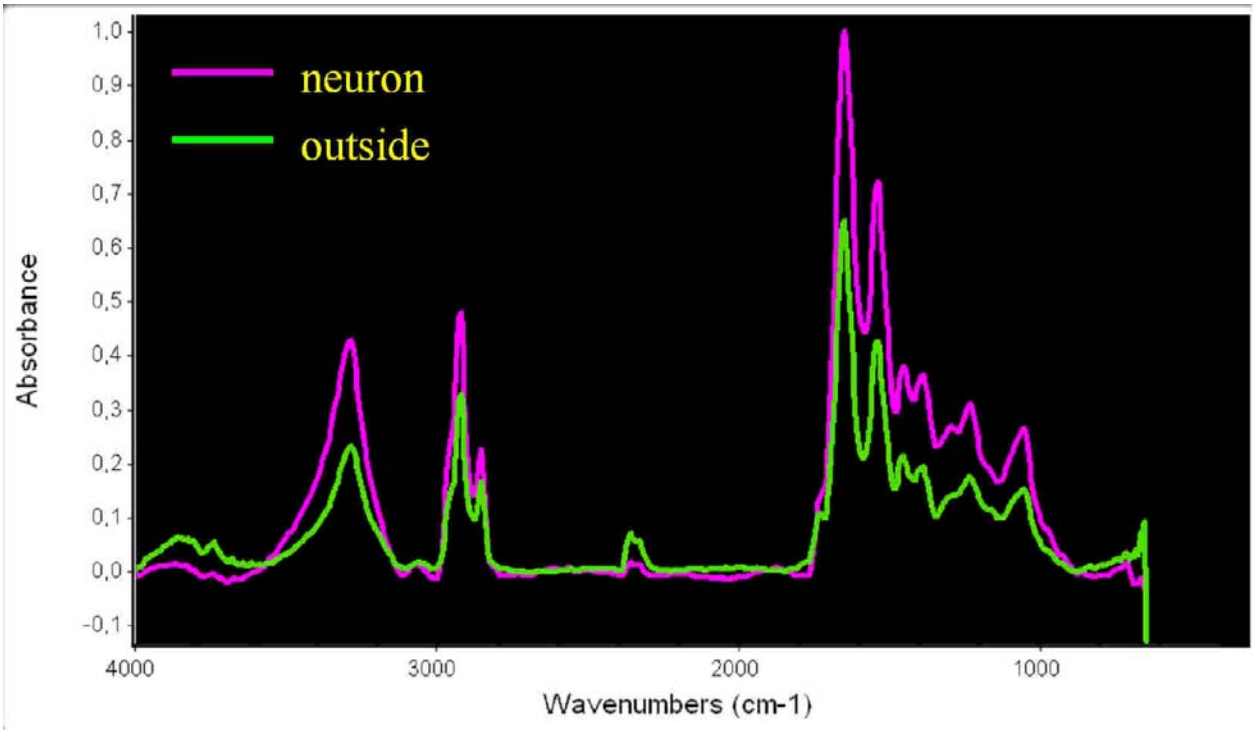

Figure 3. The typical IR spectra excited in the area of substantia nigra neuron and the outside tissue.

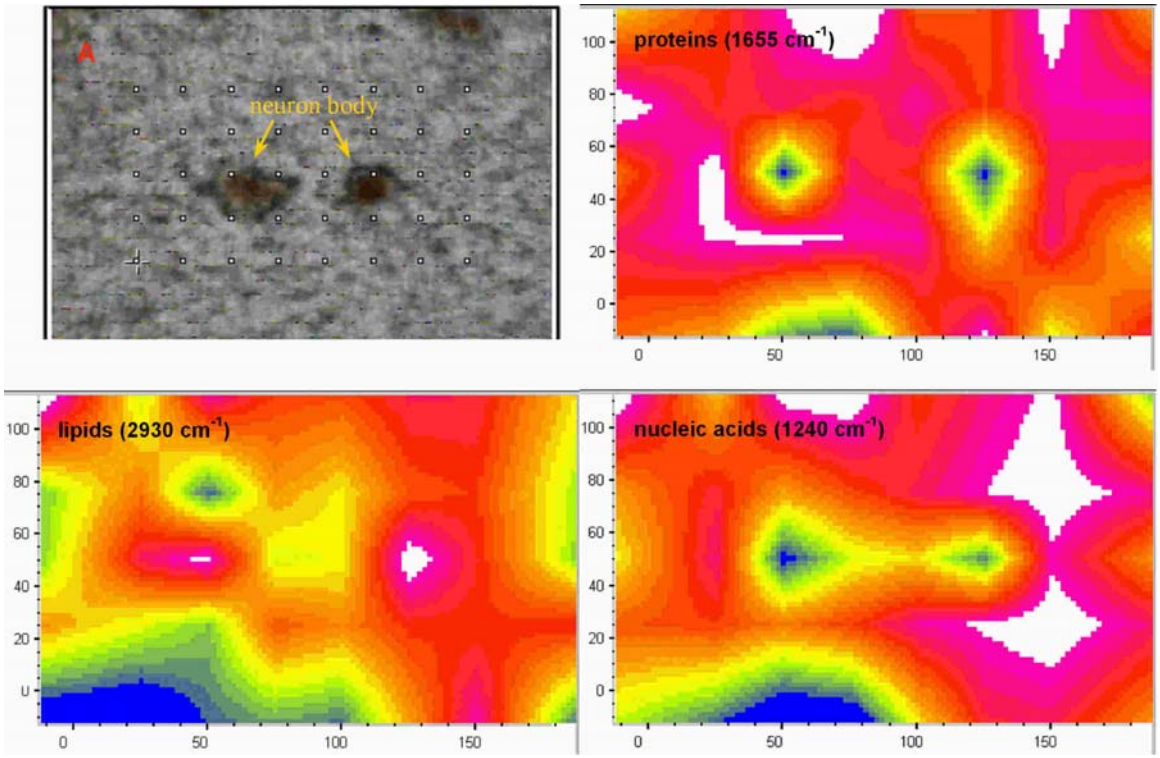

Figure 4. Distributions of selected biomolecules in the area of substantia nigra in comparison with the microscopic view of the scanned area of the tissue (A).