ESRF	**Experiment title:** Investigation of sulfur oxidation states and biological molecules in brain glioma tissue.	**Experiment number**: MD 228
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Shifts:	**Local contact(s)**: Dr Jean SUSINI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Magdalena SZCZERBOWSKA-BORUCHOWSKA*, Slawomir WOJCIK*, Marzena KASTYAK*, Zdzislaw STEGOWSKI*, Joanna CHWIEJ, Marek LANKOSZ, Anna MANKA – Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Krakow, Poland

Dariusz ADAMEK - Institute of Neurology, Medical College, Jagiellonian University, Krakow, Poland

Maria WROBEL - Institute of Medical Biochemistry, Medical College, Jagiellonian University, Krakow, Poland

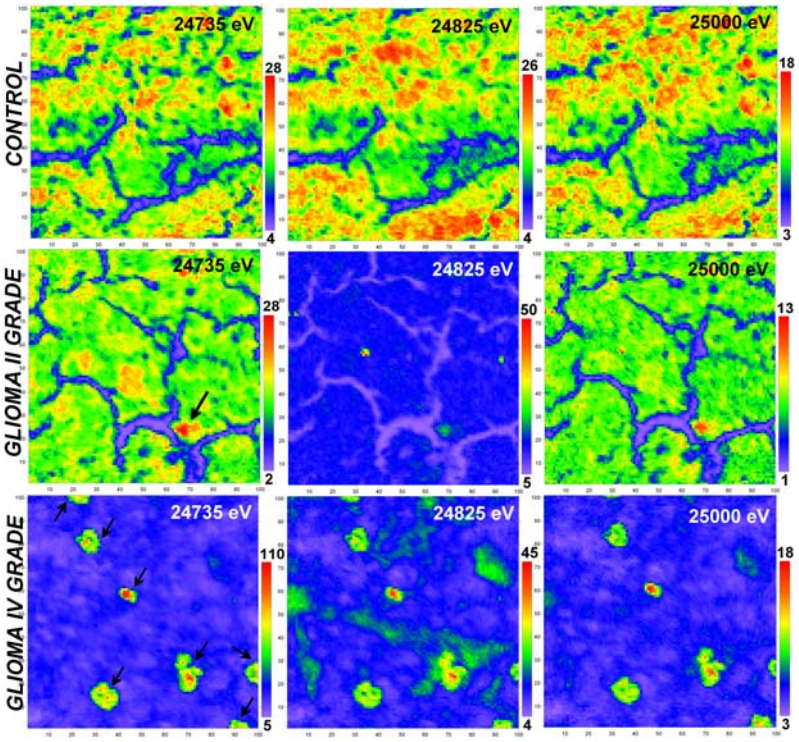
Report:

The main goal of the experiment was preliminary investigation of sulfur oxidation states and distribution of bio-organic components in human brain gliomas tissue. In the Institute of Medical Biochemistry (MC UJ) the investigations of the level of sulfane sulfur (S^{2-}) and the activity of the enzymes involved in sulfane sulfur metabolism (MPST, g-cystathionase, rhodanese) in homogenates obtained from human gliomas were undertaken. The level of sulfane sulfur, determined be the method of Wood, increased significantly with the increasing grades of malignancy [1]. Since biochemical changes may be related to the growth rate of cancer cells, they can be thought of as markers of tumor cell proliferation.

The experiment was carried out on the undulator beamline ID21. X-ray absorption near edge structure (XANES) spectroscopy at the sulfur K-edge was applied using the scanning X-ray microscope in fluorescence mode. The microscope used a Fresnel zone-plate as a focusing lens and delivered a microbeam of 0.5 x 0.5 μm^2. The thin tissue sections of human brain gliomas with different grades of glioma malignancy as well as tissue without malignant infiltration were probed. As the reference materials the following organic compounds were applied: glutathione (oxidized and reduced form), cysteine, cystine, methionine, dimethyl sulphoxide, cysteic acid, zinc sulphate, sodium tetrathionate, sodium thiosulfate.

Oxidation states imaging involved scanning a sample in two dimensions with spatial resolution equal to 0.5 μm on each direction. Typical area of scanning was 100 x 100 μm^2. The step size was equal to the incident photons beam i.e. 0.5 μm. The monochromator was set to the appropriate energy for the edge of S oxidation states. Mapping of the different forms of sulfur was performed at energies of 2.4735 keV (S^{2-}), 2.4764 keV (S^{4+}), 2.4825 keV (S^{6+}) and 2.5000 keV (total S). The results obtained for cancerous tissue (II and IV stage of glioma malignancy) and control sample were illustrated in Fig. 1. As it is shown cancer cells accumulate sulfur mainly as sulfide (S^{2-}) form. The preliminary results indicated also higher accumulation of this form of sulfur in glioma of IV grade of malignancy in comparison with the samples of II grade neoplasms. The presence of sulfate (S^{+6}) species was revealed in histological structures outside the cancer cells. The sulfite (S^{+4}) form of sulfur was not detected in the scanned areas of tissue.

Additionally, for selected points the structure of S K absorption edge was studied. Micro-XANES spectra at the sulfur K-edge collected inside cancer cell, its boundary and surrounding tissue in comparison with the spectra obtained for zinc sulfate and methionine were presented in Fig. 2.



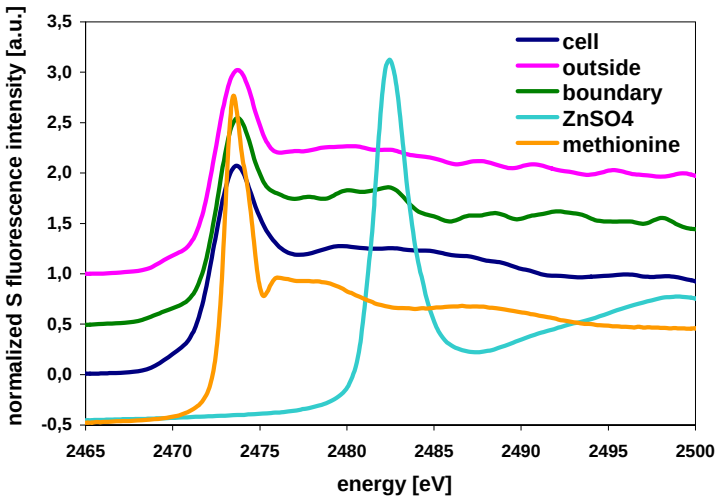


Fig.1 (left). Scanning X-ray mocroprobe determination of sulfur oxidation state distribution in brain glioma and control samples (area: 100 x100 μm^2). The arrows show cancer cells. Data presented in arbitrary units.
Fig. 2 (right). The S micro-XANES spectra acquired inside glioma cell, cell boundary, surrounding tissue, methionine and zinc sulfate.

Apart from determination of sulfur oxidation states, the investigation of changes in main biological molecules in case of brain gliomas was performed. The IR microspectroscopic maps were collected in transmission mode using an infrared microscope coupled to a FTIR spectrometer. The applied IR microbeam has the size between 5 and 8 µm following the wavelength probed. A comparison of the IR absorption spectra collected inside cancer cell and tissue area without malignant infiltration was illustrated in Fig. 3. The major spectral differences between control and cancerous tissues were identified at the following vibrational frequencies: 3300 cm^{-1}, 2925 cm^{-1}, 2830 cm^{-1}, 1655 cm^{-1} and 1545 cm^{-1}. The tissue areas were mapped to generate two-dimensional images of the main biological molecules of interest. Integrated intensities of selected bands were extracted after raster scanning of the sample. The infrared maps of the selected functional groups enabled to find precise distribution of biomolecules in cancer cells that was presented in Fig. 4.

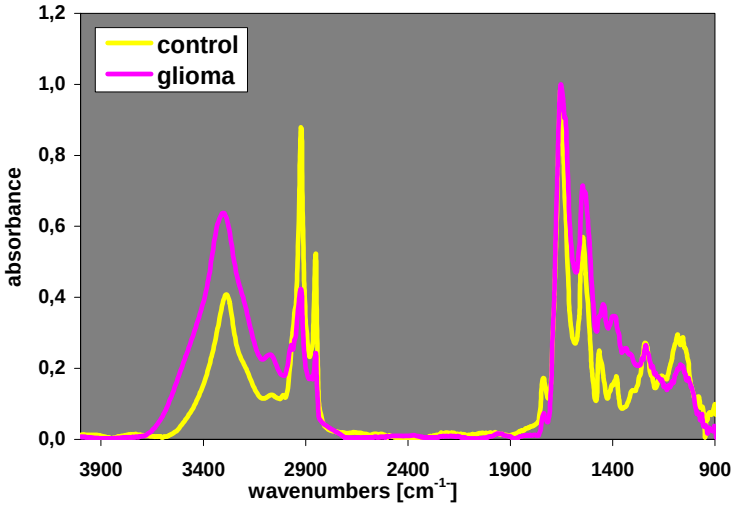


Fig. 3. The comparison of FTIR absorption spectra acquired in glioma cell and control tissue.

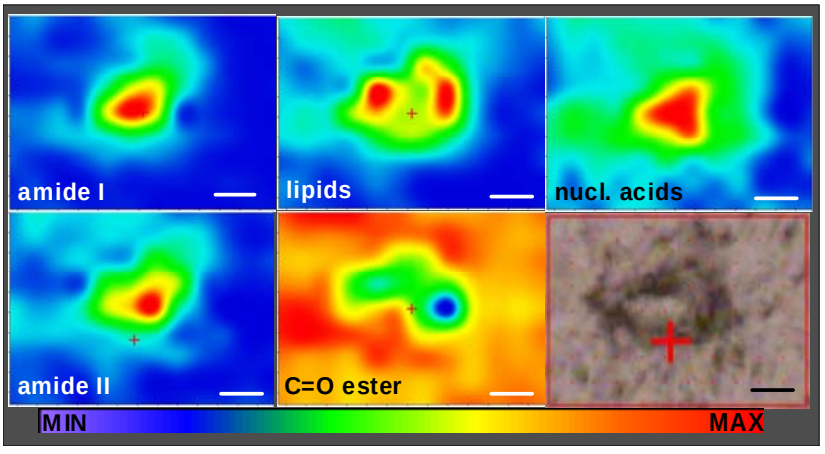


Fig. 4. Infrared maps of glioma cell in comparison with microscopic view of scanned area of the tissue. Scale bar: 10 µm.

The obtained results of our work are in good agreement with the previous observations reported by Wrobel et al. [1]. However, the application of micro-XANES technique in opposite to previously used bulk analysis allowed to find that high accumulation of sulfane sulfur is localized just in cancer cell. A confirmation of the increased level of reduced sulfur atoms (-2 valence) in malignant gliomas as well as determination of distribution of biological molecles in cancer cell is of great importance.

References:

1. Wrobel M., Czubak J., Adamek D., Bronowicka P., Czepko R. Can the level of sulfane sulfur be related to the grades of glioma malignancy? Folia Neuropatologica 2005, 43/3: 224-225.