ESRF	**Experiment title:** Sub-cellular elemental imaging and Fe speciation in human ovarian cancers and their potential as a tissue classifier	**Experiment number**: MD-935
Beamline: ID21	**Date of experiment**: from: 24.02.2016 to: 1.03.2016	**Date of report**: 5.03.2016
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Report:

The goal of this experiment was the quantitative, chemical imaging of minor and trace elements in human ovarian cancers, coupled with the analysis of Fe oxidation states. The hypothetical role of Fe, and other low-Z elements, suspected to participate in the carcinogenesis in ovarian tissue, will be verified. This experiment will explain if the concentration of elements, detectable with XRF micro-spectroscopy, as well as Fe speciation in malignant tissues can be utilized for the differentiation and the classification of ovarian cancers. Ovarian cancer specimens were provided from the Chair of Pathomorphology, Medical Faculty, Jagiellonian University in Krakow, Poland. The selection of specimens was from an intra-operative resection, followed by the quick histopathological assessment of its malignant character. Tissue material for the experiment was cryo-sectioned to slices of 5 μm thick, mounted on a square silicon nitride membrane window (2x2 mm, 200 nm thick) on silicon frame and stored frozen at temperature of liguid nitrogen. Altogether therteen samples of ovarian tumors including endometroid cystadenocarcinoma, borderline serous tumor, high grade serous carcinoma, endometrioid adenocarcinoma, poorly differentiated carcinoma, borderline mucinous tumor, fibrothecoma, carcinosarcoma, adenocarcinoma and fibrothecoma were analysed. The total number of eighteen areas were scanned.

The experiment was performed at beamline ID21 dedicated for X-ray fluorescence microscopy and X-ray absorption microspectroscopy. This experiment was performed at a vacuum environment with a cryo-stage. The X-ray beam monochromatized by Si-111 crystal was focused with KB mirrors to the spot size of ca. 0.5×0.9 μm^2. The fluorescence radiation was collected by a SDD detector with thin berylium window. For XRF imaging of element distribution X-rays of 7.3 keV were used. The areas varying from about 40×40 μm^2 to 600x300 μm^2 were scanned in a continuous (raster) mode with a counting time of 100 ms-400 ms over a distance of 1 μm. AXO and home made multielemental samples were used as concentration standards of the elements studied. Absorption spectra of Fe were taken using incident radiation energies ranging from 7.05 to 7.30 keV with the step of 0,33 eV, The measurements were performed in fluorescent mode from a few interesting tissue points. Inorganic reference materials $Fe_2(SO_4)_3 \cdot nH_2O$, $FeSO_4 \cdot 7H_2O$ and organic reference materials hemoglobin, myoglobin, transferrin, ferritin were examined. Such elements as P, S, Cl, K, Ca, Fe were present in all neoplastic tissues analyzed. The maps of Fe, S and P distribution in malignan and controlt tissues are presented on Fig. 1. The XANES spectra of reference materials and ovarian cancer sample were presented on Fig.2. The higher concentration of Fe, S and P in cancer cells in comparison to control area was observed.The

position of Fe K-edge suggests that ovarian tumors contain both Fe(II) and Fe(III), however a substantial is fraction of Fe(III).

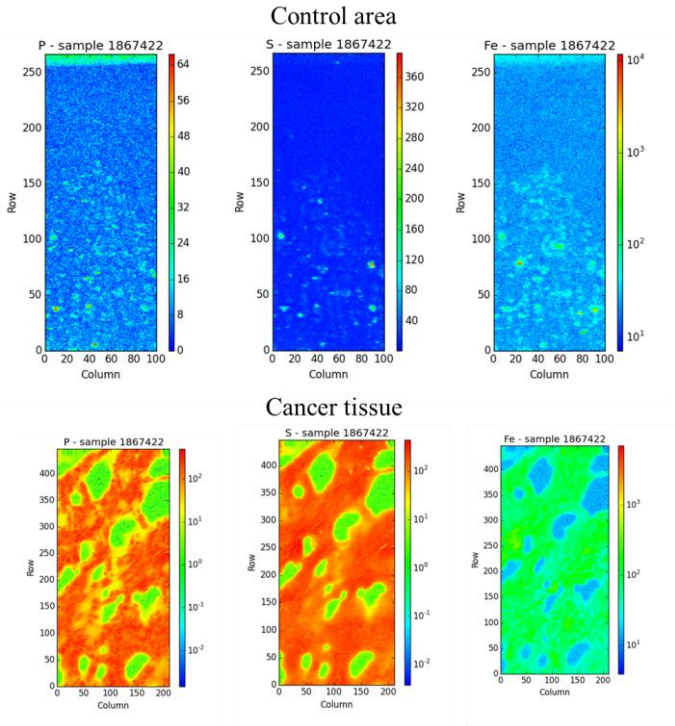

Fig 1. SR-XRF maps of P, S and Fe distribution in cancer tissue and control area

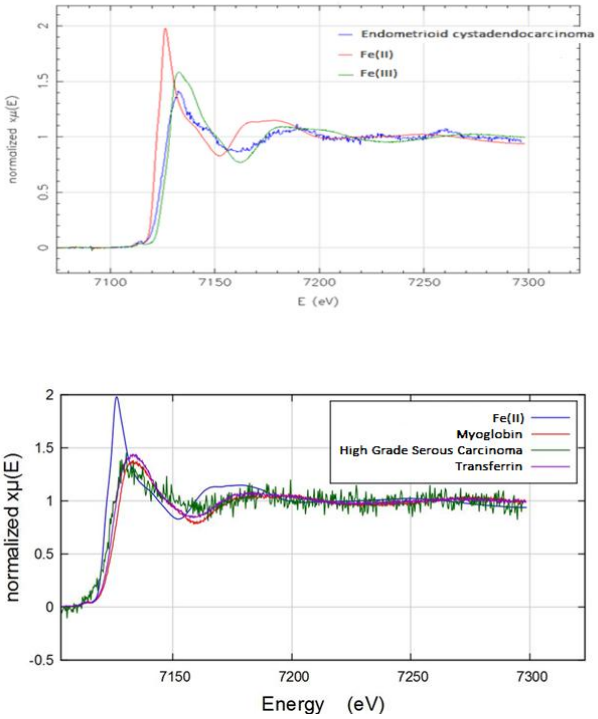

Fig. 2 XANES spectra of ovarian cancer samples and reference materials

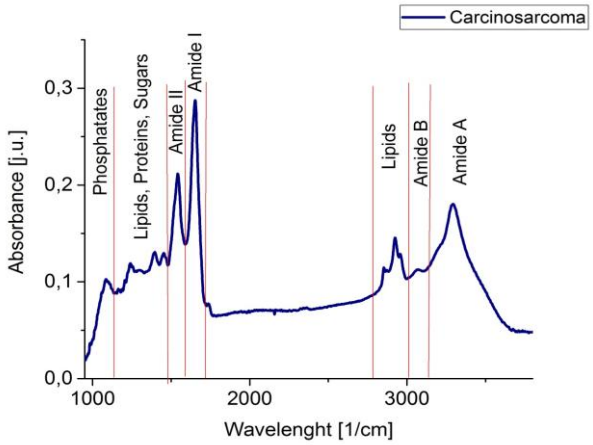

Fig. 3. Infrared absorbance spectrum of ovarian caner tissue

Apart from elemental chemica imaging, the investigation of changes in main biological molecules in case of ovarian cancers 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 of 10 μm. The IR absorption spectra collected inside cancer cell was illustrated in Fig. 3. The differences in the IR spectra for various types of ovarian tumors were observed.

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