EUROPEAN SYNCHROTRON RADIATION FACILITY

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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Studies of Fe, Cu and Zn oxidation state and chemical environment in brain cancers with the use of cryo-XAS	**Experiment number**: MD-676
Beamline: BM23	**Date of experiment**: from: 27.06.2012 to: 3.07.2012	**Date of report**: 14.09.2012
Shifts: 18	**Local contact(s)**: Olivier Mathon	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Brain tumours are still one of the main causes of death. In order to successfully combat the disease, it is extremely important to learn about the mechanism of its formation. It is believed that trace elements such as Fe, Cu and Zn play a significant role in neoplastic processes. Although present in the human body at low concentrations, trace elements are necessary for its proper functioning. These elements are present in enzymes and hormones and thus affect the metabolic processes that occur in cells. For this reason, information on the differences in their levels and forms in healthy and cancerous tissues with various malignancy grades may significantly contribute to the knowledge of biochemical reactions involved in oncogenesis.

The samples under study were collected intraoperatively and contained brain tumours with different malignancy grades. The tissues were placed in specially designed polypropylene rings in diameter of 10 mm and thickness of 1 mm. The front and the end of the ring are flat and covered by ultralene foil. Afterwards, the samples were immediately frozen to -80°C, which prevents all biological and chemical processes, e.g., oxidation. Altogether six samples of brain gliomas with various malignancy grades, including glioblastoma multiforme, oligodendroglioma anaplasticum, meningeoma monstrocellulare, meningeoma meningotheliale, astrocytoma diffusum and astrocytoma anaplasticum were analysed. Additionally, one metastasis and one non-cancerous control sample were measured. Inorganic reference materials (Fe, Cu and Zn foils, $Fe_2(SO_4)_3 \cdot nH_2O$, $FeSO_4 \cdot 7H_2O$, CuO, Cu_2O and ZnO and organic reference materials (hemoglobin and transferrin) were examined.

The experiment was performed at beamline BM23 dedicated for X-ray absorption spectroscopy. The beam was monochromated with a Si(111) monochromator. The final beam spot size on the sample was about 7x1 mm. Special conditions were designed to avoid radiation damage. Containers with samples were placed in a liquid-helium cryostat (temperature about 20K) in a helium atmosphere. Absorption spectra of Fe were taken using incident radiation energies ranging from 7.0 to 7.7 keV, the spectra of Cu – from 8.9 to 9.6 keV and those of Zn – from 9.6 to 10.3 keV. The measurements were performed in fluorescent mode. The fluorescence radiation was collected by a 13-element Ge detector.

The first part of the measurements was intended to study the effect of sample preparation on the results obtained. For this purpose, metastatic cancer (adenocarcinoma metastaticum) material was used to prepare two identical samples, one of which was cleaned of blood. In both samples, EXAFS spectra were measured for Fe, Cu and Zn. Analysis of the spectra showed that the absorption edge positions for each of the elements did not significantly differ between the rinsed and unrinsed samples (Fig. 1). Neither were any significant differences found in the chemical environment of the analyzed elements between the two samples. It can be concluded that the contribution of the signal from blood present in the sample is negligible as compared to the signal from the tissue, which is important [from the point of view of/for] further preparation of samples.

The key findings from these measurements relate to the oxidation state and the chemical environment of iron in the samples. Figure 2 shows the energies at which the absorption edges for the tumour samples with different malignancy grades were found. These energies were estimated from the XANES spectra using a mathematical "centre of gravity" of the absorption curve. A trend can be observed that the higher the tumour grade, the more Fe^{2+} as compared to Fe^{3+} the sample contains. Analysis of EXAFS spectra showed that the distance at which the atoms of the first coordination zone are located slightly increases with the increase in the tumour malignancy grade (Fig. 3). Further analysis will attempt to model the chemical environment of iron in tumour samples.

Analysis of EXAFS spectra of Cu and Zn showed differences in the chemical environment of these elements. As very few samples have been measured so far further studies are needed.

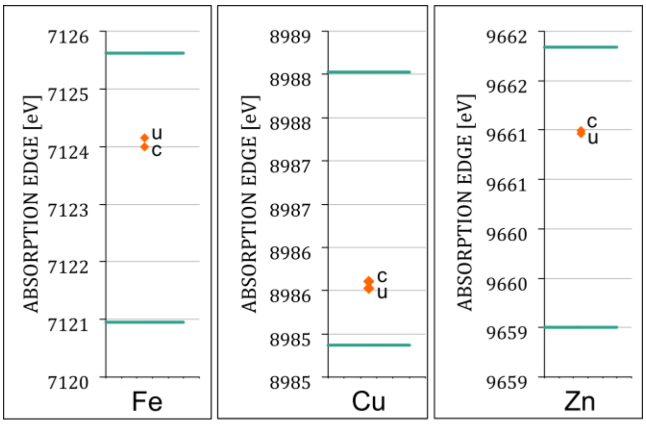

Figure 1: Absorption edge energies of Fe-K, Cu-K and Zn-K for an untreated sample (u) and a sample cleaned of blood (c).

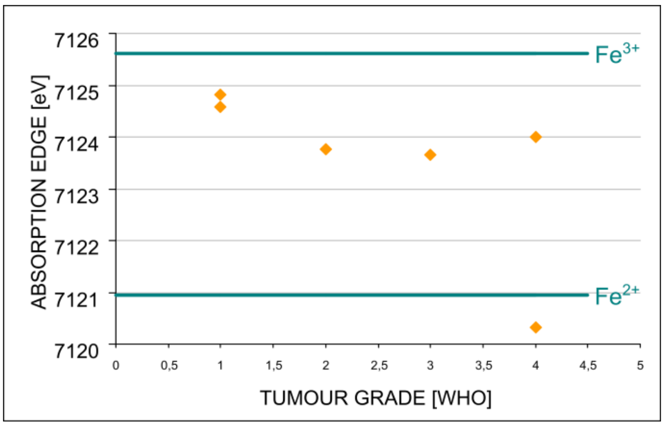

Figure 2: Absorption edge energies of Fe K for various malignancy grades. Energies of Fe^{2+} and Fe^{3+} from inorganic iron standards are also shown.

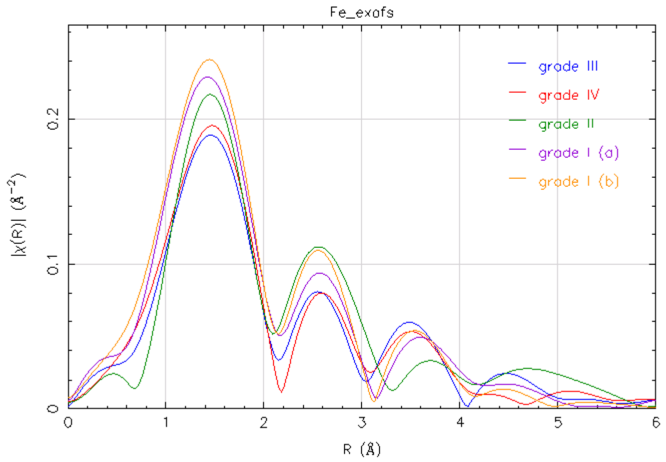

Figure 3: Fourier transform of Fe EXAFS data for tumour samples with various malignancy grades as a function of the radial coordinate

Acknowledgements:

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