ESRF	**Experiment title:** **Mapping of elements in cancer cell clusters (spheroids)**	**Experiment number:** **LS-995**
Beamline: ID 22	**Date of experiment:** from: 18th Sep 1998 to: 22nd Sep 1998	**Date of report:** 26th Feb 1999
Shifts: 12	**Local contact(s):** Alexandre Simionovici – beamline ID22 μFID	*Received at ESRF:*

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Report:

In this preliminary experiment, the micro-XRF technique has been applied for the first time to map the elemental distribution in human cancer cell clusters (spheroids). Spheroids are considered to be a biological model for micrometastases and for testing their response to a wide variety of antitumoral drugs such as immunotoxins. An immunotoxin is composed by a carrier protein bound to a toxin whose antitumor potential strongly depends on the ability to penetrate and distribute within cancer tissues. Some beamtime was spent to assess the radiation damage sensitivity of the tissue and to set the working conditions using different sample preparations. The optimization was achieved with a monochromatic 15 keV photon beam focused by a Fresnel Au/Si Zone Plate for a final beamspot dimension of 1 μm x 10 μm (vert. x hor.) and a flux of about 2_10^9 photons/s at the sample. Spheroids, included in polyacrylamide gel and placed into quarz capillaries were accurately positioned in transmission mode using a high resolution CCD camera located behind the sample.

The 2D scanning was made on a 300 μm by 200 μm (vert. x hor.) region. The X-ray fluorescence signal was collected for typically 50 seconds per point by a Si(Li) detector placed at 2-5 cm from the sample in the 90° configuration. The great part of the beamtime was used to acquire the elemental maps of four groups of spheroids grown under different conditions: untreated, treated with carrier only, treated with toxin only, and treated with the complete immunotoxin molecule (carrier+toxin). The 2D elemental maps were obtained after normalization to the incident photon flux and to the acquisition time per point. Our results indicate that Zn (Fig.1) and to some extent Cu (Fig.2) are concentrated in the spheroids independently on the treatment; moreover, they result homogeneously distributed in the tissue when normalized to the thickness of the sample region crossed by the photon beam taking into account the almost ellipsoidal shape of the spheroids (Fig.3) and the tubular shape of the capillary (Fig.4), too. The map of Fe, an indicator of the transferrin, was unexpectedly masked by a contamination of the quartz as well as the presence of Pb and Ti mainly in the capillary. However, the information on the elemental composition of the spheroids allow us to optimize our plans for future measurements: i.e. following the diffusion of the immunotoxins labelled with not naturally occurring elements (in particular Ga and I) used as tracers within the spheroids, and performing a quantitative analysis by enriching the cancer cells with an internal standard (Rb) added to the grown medium.

Fig.1

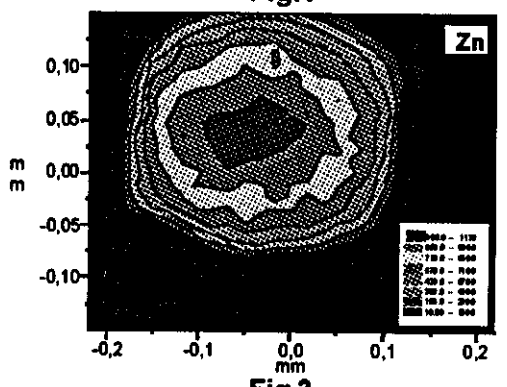

Fig.2

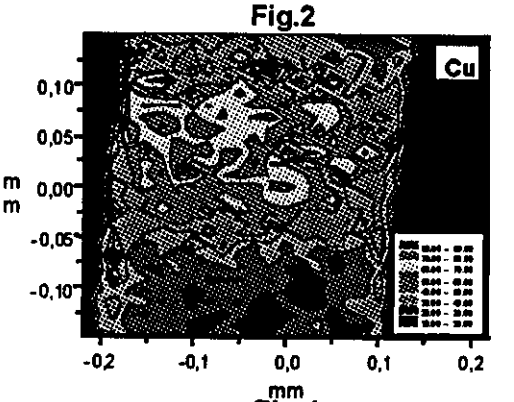

Fig.3

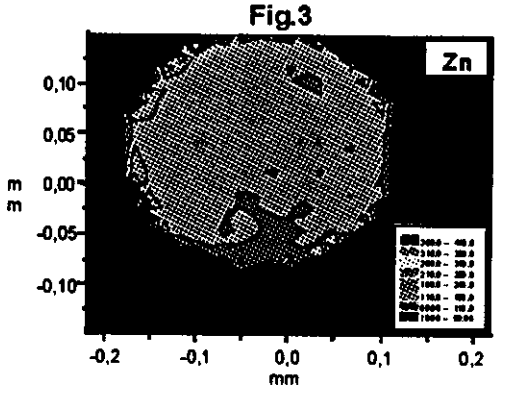

Fig.4