ESRF	**Experiment title:** Fluorescence Microtomography of the Flower Stem of *Arabidopsis thaliana*: Studying Concentrations of Physiologically Relevant Ions	**Experiment number:** LS-1739
Beamline: ID22	**Date of experiment:** from: Nov. 8, 2000 to: Nov. 14, 2000	**Date of report:** March 2, 2001
Shifts: 18	**Local contact(s):** A. S. Siminovici	*Received at ESRF:*

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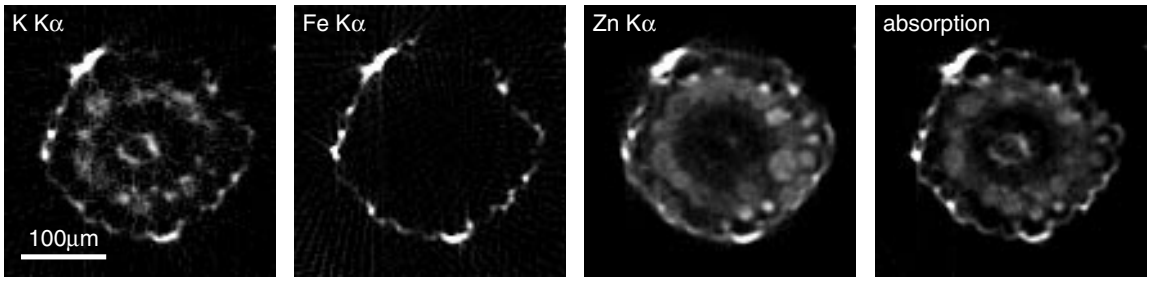
Report:

During the first part of the experiment, the x-ray fluorescence element microtomography (XFEMT) setup was built in EH1 of ID22. A parabolic compound refractive lens was used to produce the microbeam required for this scanning technique. The sample was mounted on a stage with three translations and a rotation about the vertical axis. A SiLi-Detector was placed at 90° to the beam in the horizontal plane to minimize scattering from the sample. Two PIN-Diodes, one before and one after the sample, were used to normalize the measured fluorescence intensities and to measure the transmission of the sample, respectively. A Detailed description of the general setup and the data acquisition procedure is given in [1,2]. In order to eliminate the scattering from the air, a sample chamber filled with helium specifically designed for the experiment was built into the setup, significantly improving the background count rate. In the future, the combination of a vacuum chamber and a detector with ultrathin window may allow to record low energy fluorescence lines.

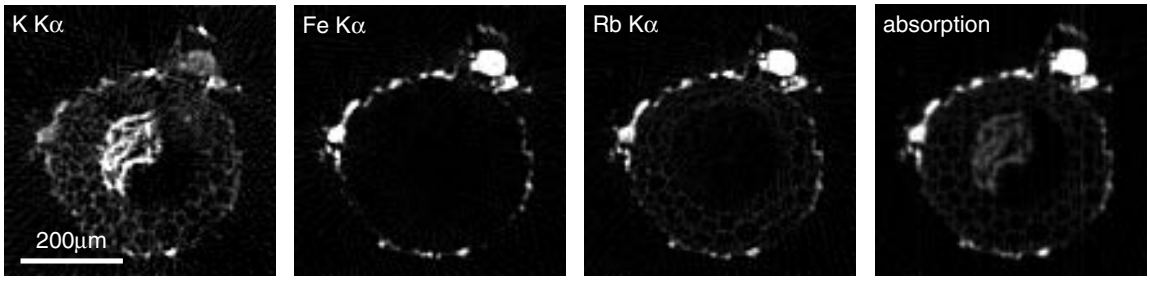
Since the source to sample distance is short compared to the previous experimental setup for LS-1422 at ID18F, a parabolic refractive lens with short focal distance was required to obtain a sufficiently small microbeam. For the excitation energy at 19.8keV, $N = 220$ single lenses were used to produce the microbeam 37cm behind the lens. The microbeam obtained had lateral extensions of 495nm vertically (smallest size ever measured for refractive lenses) by 6μm horizontally. Closing the primary slits of the beamline we were able to decrease the horizontal microbeam size down to 2μm. Depending on the sample size, spatial resolutions between 2μm and 5μm were possible in XFEMT scans, on average about a factor of two better than in the previous experiment (LS-1422).

Since the primary slits are 29m away from the undulator source, the spot size reduction is accompanied by an overproportional loss in flux. This could be avoided in the future, if horizontal slits could be installed near the undulator source. To compensate for the loss in flux, a polychromatic "pink" microbeam was used for the measurements generated by a Mo-absorber/Pd-Mirror filter to extract the 5th undulator harmonic at approx. 19.8keV. This way, one order of magnitude in flux was gained.
6 fluorescence tomograms were recorded, each of which took about 12 hours. For each sinogram 132 projections were recorded over 360° with 105 translational steps each. With 2s acquisition time per step required to obtain sufficient counting statistics for each fluorescence line of interest, the total acquisition time amounted to 7 hours and 40 minutes per tomogram. With over 1s of overhead per step (to be reduced in the future), 4 additional hours were spent on motor motion and readout of the detectors. To extend the useful range of elements, we have investigated two other types of plant samples besides *Arabidopsis*, a mycorrhizal root of the tomato plant (Figure A) and the root of the spruce (Figure B). Figure A depicts, for example, the distribution of Fe and Zn in the tomato root. Other heavy metals, such as Pb and Cd, were mapped as well. In the tomato root a resolution of 3μm was obtained, while the root of the spruce was imaged with a resolution of about 5μm.
The evaluation of the data with respect to the biological questions is in progress. Results will be published in Journal of Microscopy. As the method is now well established, we would like to apply it to a thorough biological investigation that is the subject of a long term proposal that is being submitted.

A) mycorrhizal root of tomato plant



B) root of spruce



Publications:

[1] A. Simionovici, *et al*, "X-ray fluorescence microtomography: experiment and reconstruction," in Developments in X-Ray Tomography II, U. Bonse, Editor, Proc. SPIE **3772**, 304 (1999)
[2] C. G. Schroer, *et al*, "Fluorescence Microtomography: External Mapping of Elements Inside Biological Samples", in Penetrating Radiation Systems and Applications II, F. P. Doty, *et al*, eds., Proc. SPIE **4142**, 287-296 (2000)