ESRF	**Experiment title:** Hard x-ray fluorescence microtomography with sub-micrometer resolution using refractive x-ray lenses	**Experiment number:** MI-704-5
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Report:

The aim of this beamtime was to use our scanning microscope for two-dimensional fluorescence element mapping and fluorescence microtomography with a lateral resolution below 100 nm.

The scanning microscope had been aligned and characterized during the experiment MI-817 that was carried out one week before this experiment. All measurements in this beamtime were done with a focus of size slightly below 100 nm FWHM and a flux of $1.5 \cdot 10^8 \frac{\text{ph}}{\text{s}}$. The relatively high flux was achieved with prefocussing CRLs (cf. report of MI-817). Several microstuctured test objects, a tip of a trichome of *Arabidopsis thaliana*, a particle from the stardust space mission, and a micro meteorite have been investigated. Besides creating a stable focus of sub-100 nm size, the most challenging task was the preparation and alignment of the sample. Much time was spent finding the region of interest. Another difficulty was a slow drift of the sample while scanning. We hope to come up against this thermal drift with interferometric methods in the future.

To characterize the setup, two-dimensional mappings were recorded of two small gold structures showing the emblem of the TU Dresden and a spiral, respectively. The flat sample was scanned horizontally and vertically while the fluorescence signal was recorded by the energy dispersive detector (SiLi) and the transmission signal was monitored by a pindiode. In order to avoid saturation of the detector and to reduce background from Compton and elastic scattering, the fluorescence detector was directed orthogonally to the x-ray beam.

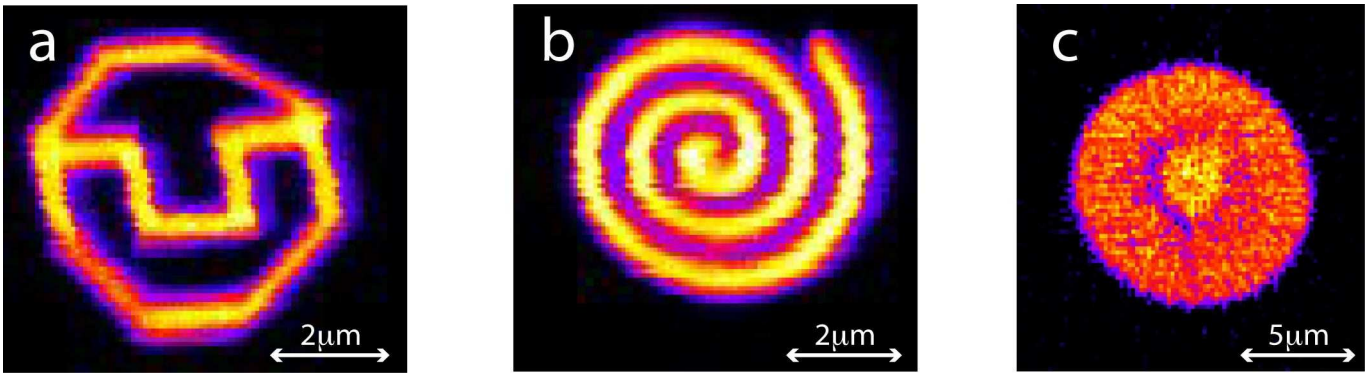

Figure 1a) Map of the TU Dresden emblem (sum of Au K_α and Au K_β emission lines)

Figure 1b) Map of the spiral structure (sum of Au K_α and Au K_β emission lines)

Figure 1c) Reconstructed tomogram of the calcium distribution of the tip of a trichome of *Arabidopsis thaliana*.

The scanning step size was 40 nm in both directions fulfilling Nyquist's theorem for the given focus size of about 100 nm. The resulting maps of the fluorescence signal of both, the emblem and the spiral structure, are shown in figures 1a and 1b (sum of the Au K_α and Au K_β channels). The images reveal a lateral resolution of 2-3 pixels, corresponding to about 100 nm. The map of the emblem structure is slightly distorted, which is due to slow drifts of the sample with respect to the optical axis.

Besides two-dimensional maps, we recorded a fluorescence microtomogram of the tip of a trichome of *Arabidopsis thaliana* (provided by W. Schröder, FZ Jülich). The challenge in microtomography is the rotational stage which must assure a concentric circular orbit free of excentricity for each point of the sample around the rotational axis. As every part of the sample must stay inside the lateral scanning range for any angle of rotation and the scanning range must not become too large, the geometric center of the sample must coincide with the rotational axis. In contrast to the gold structures used in the first experiment, the fluorescence signal of the elements in the biological sample is not very large, leading to long exposure times of about 5 seconds per scan point. Because of this, the sample drift due to thermal instabilities becomes practically relevant and must be considered in data analysis. The integrated fluorescence spectrum (sum over all scanning points of the tomogram) revealed that our biological sample gave rise to significant signal only for the Calcium K_α and K_β emission lines. Other in principle detectable elements were present (if at all) only on very low concentrations. Figure 1c shows the reconstructed Ca distribution in a central section through the trichome with a pixel size of 100 nm.

As a conclusion we have proved the feasibility of performing scanning experiments (two-dimensional mapping as well as microtomography) with x-ray beams focussed down to a size of below 100 nm, resulting in images with lateral resolutions of slightly below 100 nm. A challenge for future experiments will be stability. In the first place we will have to improve stability of the sample using more precise and stable stages for sample positioning. Thermal drifts of the sample will be coped with monitoring the sample position relative to the focussing optics (horizontally and vertically) with interferometric methods. This data could be used as additional feedback information for closed-loop movements of the sample while scanning, or it may just be incorporated in the data analysis.