ESRF	**Experiment title:** In vivo X-ray fluorescence microimaging of biological model organisms manipulated by laser-based optical tweezers.	**Experiment number**: EV-141
Beamline: ID13	**Date of experiment**: from: 29/04/2015 to: 03/05/2015	**Date of report**: 14/10/2015
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Recently, we introduced a radically new synchrotron radiation-based elemental imaging approach for the analysis of biological model organisms and single cells in their natural, *in vivo* state [1,2]. The methodology combines optical tweezers (OT) technology for non-contact, laser-based sample manipulation with synchrotron radiation confocal X-ray fluorescence (XRF). Using the model organism *Scrippsiella trochoidea* (marine microalgae), several successful OT XRF experiments have been performed at ESRF-ID13 demonstrating the feasibility, repeatability and high throughput potential of the OT XRF methodology with a strong focus on the research field of environmental toxicology. Within this continuation experiment, emphasis was placed on the combination of optical tweezers-based sample manipulation with SAXS spectroscopy by applying an ultrafast CCD pixel detector and the integration of a flight tube for improved scattered data quality. More specifically, the OT XRF methodology was combined with a DECTRIS EIGER 4M single photon counting detector that provides frames with 2070×2167 pixels (75×75 μm^2 pixel size) at a rate up to 750 Hz and hence allows for *in situ* dynamic X-ray measurements at a high spatial resolution level.

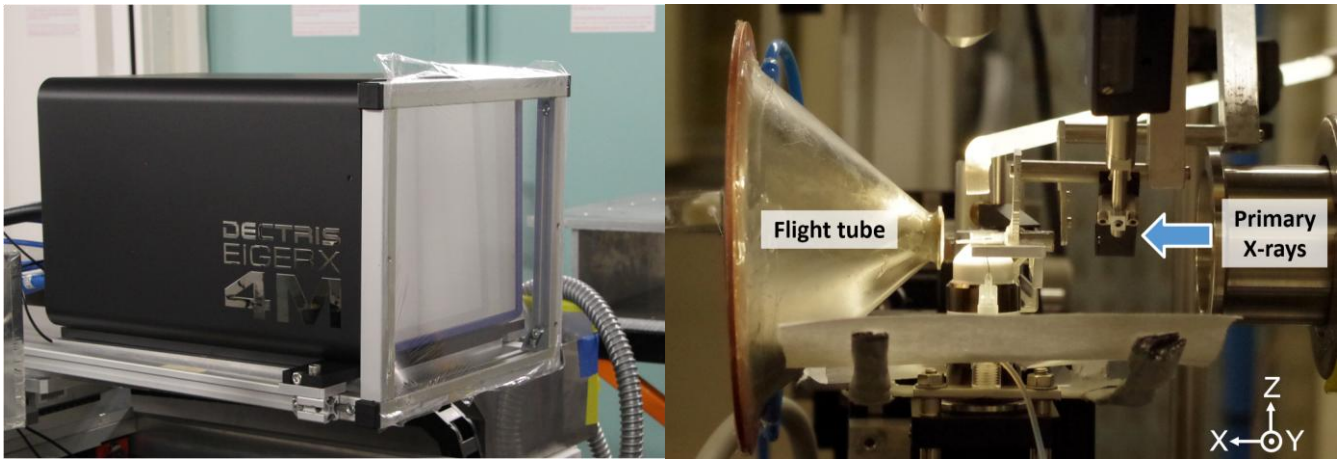

Figure 1. Photographs of the DECTRIS EIGER 4M detector installed at ESRF-ID13 (left) and the integrated flight tube flushed with helium gas to reduce the absorption and scattering by air [3] and hence resulting in improved scattered data quality (right).

Similarly to our previous studies, the marine microalgae *Scrippsiella trochoidea* was exposed to high concentrations of transition metals. In particular, emphasis was placed on copper toxicity by preparing three cell cultures with increasing Cu concentrations and one non-exposed culture serving as a reference (0-20-65-80 µg/L Cu, 96 hours exposure, L1 medium). Furthermore, the sample preparation was consistent with the procedure previously outlined, including the HEPES buffer treatment for decreasing the background signal and the microalgae motility. For confocal detection of the fluorescent signal, a 10 mm working distance confocal optic was aligned to an approximate 100 µm acceptance (FWHM) for increased repeatability. For each exposure condition, five replicates were optically trapped and positioned to the upper capillary wall using a 0.5-0.7 W laser power throughout the confocal XRF scan. The cells were subsequently scanned using a 2×1.7 µm^2 primary beam at a flux of approximately 2×10^{11} photons/s (2 µm step size, 0.7 s/point).

For demonstration purposes, the combined XRF and integrated SAXS distribution maps of a 65 µg/L Cu-exposed cell are presented in Figure 2. The combination of a high speed single photon counting detector and a He-flushed flight tube resulted in straightforward sample outline and internal structure visualisation of optically manipulated microalgae. In good agreement with our previous findings, a subcellular structure is found that generates significantly more scattering and transition metal fluorescent signal.

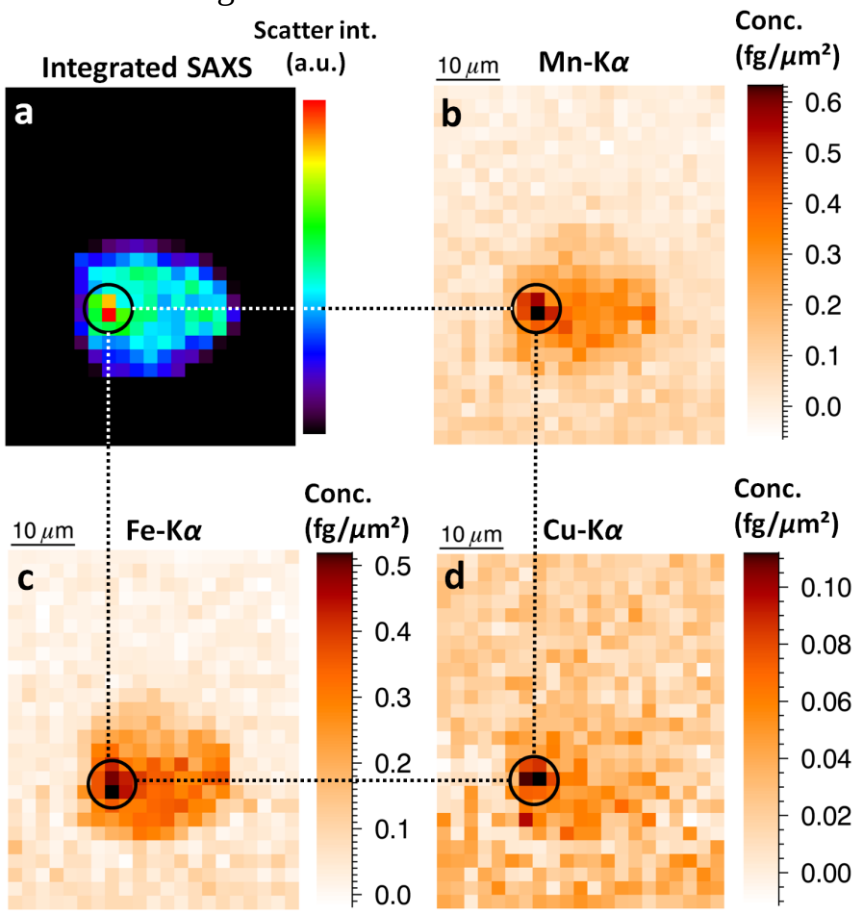

Figure 2. Combined XRF and integrated SAXS experimental results. (a) Integrated SAXS distribution map. (b-d) Mn, Fe and Cu areal concentration maps of a scanned Cu-exposed algae with an indication of the accumulation centre (65 µg/L Cu, 96 hours exposure). Scanning details: 2 µm step size, 0.7 s/point, bottom to top scan.

Besides the sample outline and internal structure visualisation, the quality-improved SAXS data perfectly enabled to visualize the cell boundaries. In particular, the composite image in Figure 3 shows the scattering signal around the beam stop (for each pixel) on the lower half of the

scanned algae. As indicated by the red squares, the borders of the cell clearly indicate a strongly ordered structure pointing towards the cellulose plates of which the cell wall is composed. Moreover, it can be seen that the preferred scattering direction naturally changes with the position on the scanned cell. Within this study, this distinct scattering phenomena was observed for the *very first time* due to the application of an improved SAXS setup and hence opens opportunities for more detailed OT SAXS analysis of microalgae cells under *in vivo* conditions.

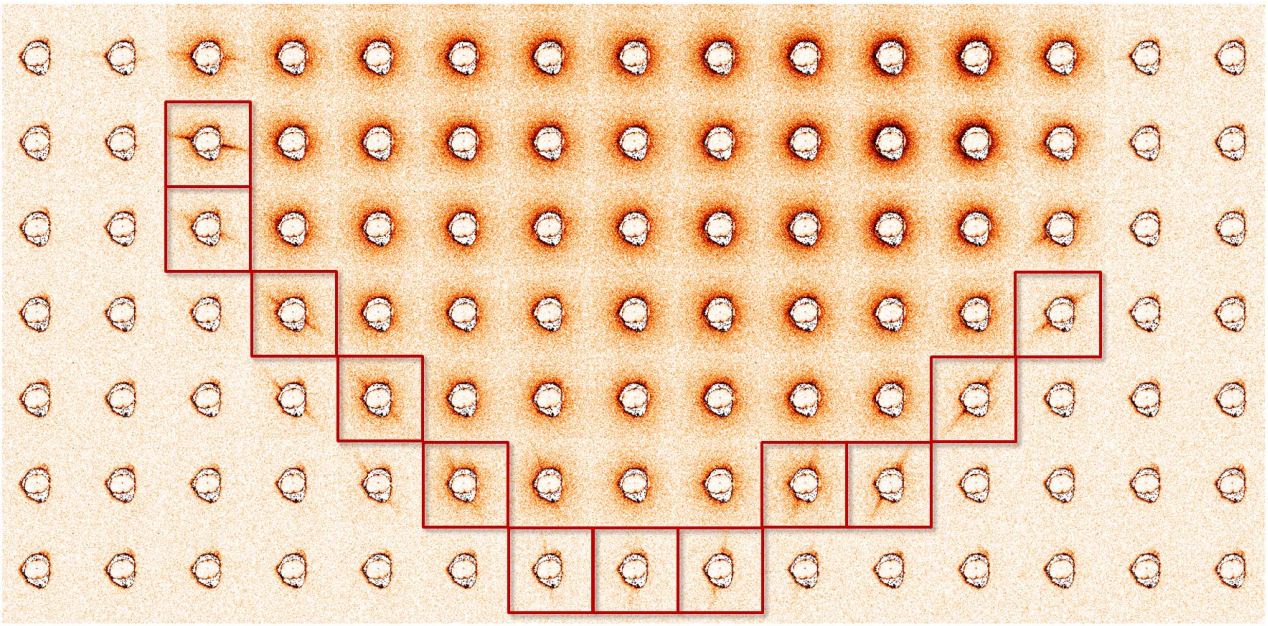

Figure 3. Composite image of the scattered signal from the lower half of a scanned cell (65 µg/L Cu, 96 hours exposure). Scanning details: 2 µm step size, 0.7 s/point, bottom to top scan.

References:

[1] Vergucht, E. *et al.*, *In vivo* X-ray elemental imaging of single cell model organisms manipulated by laser-based optical tweezers (2015). *Sci. Rep.*, Nature Publishing Group, **5**, 9049.

[2] Vergucht, E. *et al.*, Methodological challenges of optical tweezers-based X-ray fluorescence imaging of biological model organisms at synchrotron facilities (2015). *J. Synchrot. Radiat.*, **22**, 1096-1105.

[3] T. Narayanan. Synchrotron Small-Angle X-Ray Scattering. Springer-Verlag Berlin Heidelberg, 2008.