ESRF	**Experiment title:** X-ray fluorescence imaging of single-cell organisms by means of non-contact sample manipulation by laser-based optical tweezers.	**Experiment number**: EV-118
Beamline: ID13	**Date of experiment**: from: 01/10/2014 to: 05/10/2014	**Date of report**: 16/02/2015
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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]. 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. Here, we report on the subcellular complementary nature of OT-based XRF imaging combined with integrated SAXS spectroscopy and specific confocal XRF-related challenges.

In 2011, Santucci *et al.* reported on a dedicated optical tweezers (OT) setup for SR microdiffraction experiments of soft matter objects in their natural, aqueous environments at ESRF-ID13 [2]. During the past years, we further optimized the compact OT setup and extended its application towards SR confocal XRF imaging of biological model organisms and single cells in the *in vivo* state [1]. Detection of the fluorescent photons is performed by a Vortex-EM detector (50 mm² active area, 350 µm crystal thickness, 2 µs peaking time, HITACHI, USA, Fig. 1a) that is equipped with confocal optics (XOS, X-ray Optical Systems Inc., Albany, USA) [3]. A schematically overview of the sample area (Fig. 1b) shows the direction of the primary X-ray beam and the confocal optic positioned under 45°. The scattered X-ray patterns are collected downstream by a MAXIPIX 2D photon-counting X-ray detector (TAA22PC, 55×55 µm² pixel size) used for sample outline and internal structure visualisation [4].

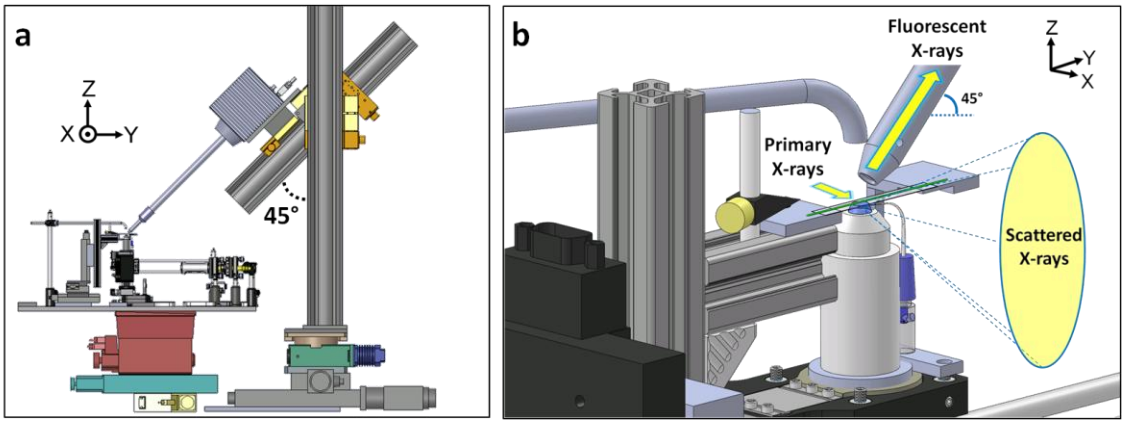

Figure 1: OT XRF technical overview. (a) Mounted compact OT setup with the Vortex-EM detector positioned under 45°. (b) Detail of the sample area.

An overview of the combined OT XRF and integrated SAXS experimental results of a scanned *S. trochoidea* microalgae is shown in Fig. 2. The selected microalgae was exposed to Ni-contaminated medium (50 µg/L, 48 h exposure), optically manipulated to the upper capillary wall using a 0.5 W laser power and scanned using a 3×2 µm² primary beam at a flux of approximately 3×10^{11} photons/s (2 µm step size, 0.2 s/point). Due to the relatively long exposure time, an extra beam stop was positioned onto the MAXPIX detector for protective purposes, resulting in a significant reduction of the detected scattered signal. Consequently, a first data processing strategy involved identifying the number of illuminated pixels of each scattered pattern, from which the outline of scanned specimen can be clearly derived (Fig. 2a). Next, a threshold of the mean and the standard deviation was set as a basis for the segmentation into algal outline and background regions (Fig. 2b). The algal region was subsequently projected onto the integrated SAXS distribution thereby indicating a subcellular structure that generated significantly more scattering signal (Fig. 2c).
The Mn, Fe and Zn elemental distributions corresponding to the scanned area are respectively shown in Fig. 2d-f and display an accumulation centre which perfectly corresponds to the subcellular region derived from the integrated SAXS pattern. These findings were confirmed by multiple scanned microalgae and demonstrate the subcellular complementary nature and potential of combining OT-based XRF imaging with integrated SAXS spectroscopy. Note that this accumulation centre may indicate an important organelle (e.g. nucleus, Golgi apparatus etc.), however further experimental evidence is essential in this respect. Although that the selected microalgae culture was exposed to Ni-contaminated medium, no significant Ni accumulation was observed which corresponds to the findings described in our earlier work [1].

For the OT XRF experiments performed in this study, the confocal optic is typically aligned to an area of approximately 50×50 µm², resulting in high transmission efficiency, however posing limitations on the repeatability of the measurements [5]. One potential cause involves the size of the selected microalgae (≈ 35 µm width) which is of the same size order as the confocal volume dimensions. Secondly, optical manipulation is not an absolute sample fixation device, meaning that residual mobility of the algae can still manifest in the sub-micrometer range, even after the HEPES buffer treatment. Furthermore, both the beamline microscope and the confocal optic can become slightly misalignment or/and drift in the course of SR experiments. All of the above-mentioned factors can give rise to the alignment issue schematically depicted in Fig. 3. In view of this potential problem, it is therefore recommended to slightly defocus the confocal optic resulting in larger confocal volume (e.g., 100×100 µm²) and thereby resulting in increased OT XRF repeatability. Note that a somewhat larger confocal volume will naturally generate a larger

background in the XRF spectra, combined with a more intense Si fluorescent signal since a larger fraction of the capillary walls will be captured.

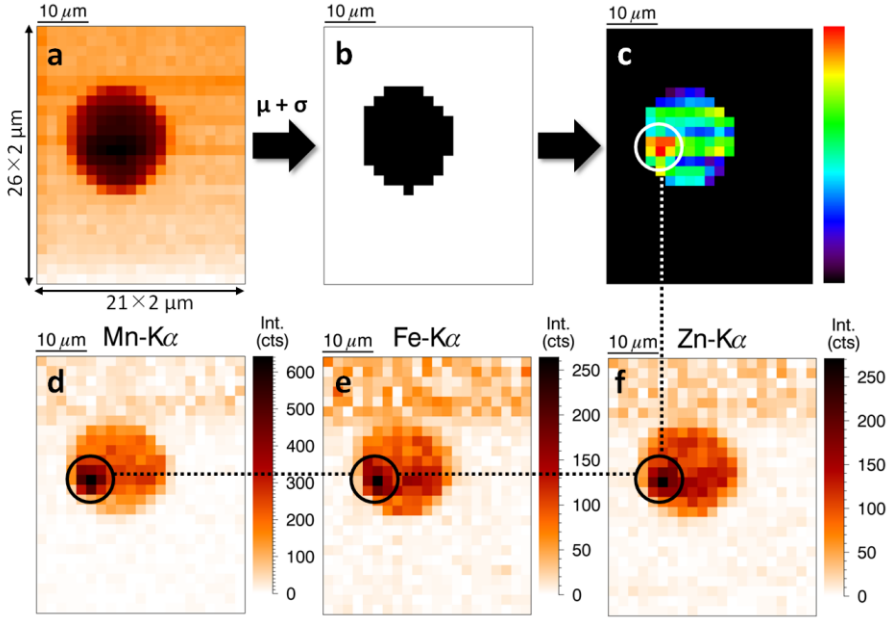

Figure 2: Combined XRF and integrated SAXS experimental results. (a) Distribution map of a scanned algae based on the number of illuminated pixels of each scattering pattern (0.2 s exposure/point). (b) Algae isolated from (a) using μ + σ as a threshold. (c) Algae isolated from the integrated SAXS map based on (b). (d-f) Mn, Fe and Cu distributions maps of a scanned Ni-exposed algae with an indication of the accumulation centre.

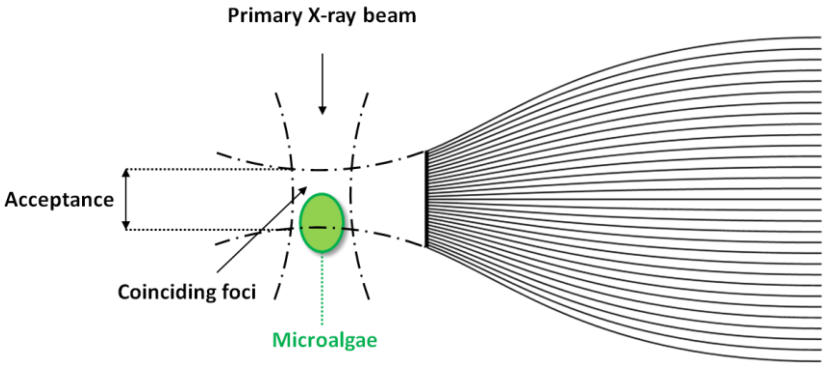

Figure 3: Schematically overview of potentially reduced OT XRF repeatability due to a tightly aligned confocal volume.

References:

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