Non-contact optical tweezers-based single cell analysis through *in vivo* X-ray elemental imaging

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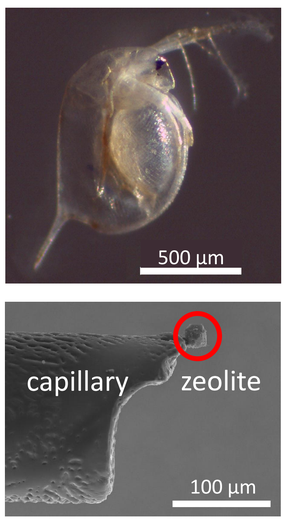
Introduction

Owing to its high sensitivity and non-destructive nature, synchrotron radiation (SR) based confocal X-Ray Fluorescence (XRF) imaging offers the unique potential of providing two- and three-dimensional information on the sample composition and elemental distributions with trace level detection limits [1]. With the increased availability of nanoscopic X-ray beams provided by 3rd generation SR sources, SR X-ray imaging methods pose important methodological challenges concerning sample preparation, non-contact sample manipulation and non-contact positioning.

Current XRF-related methodological challenges

- **Preservation of the structure of biological organisms**
- Special **need for delicate mounting** of microscopic samples onto a support that does not interfere with the X-ray measurement itself.
- Offline and **time-consuming sample preparation** procedure prior to analysis at a synchrotron facility.
- **Accurate and precise XYZθ movements** of the sample through the X-ray beam.



Proposed methodology for solving challenges

Optical tweezers (OT) for non-contact sample manipulation

combined with

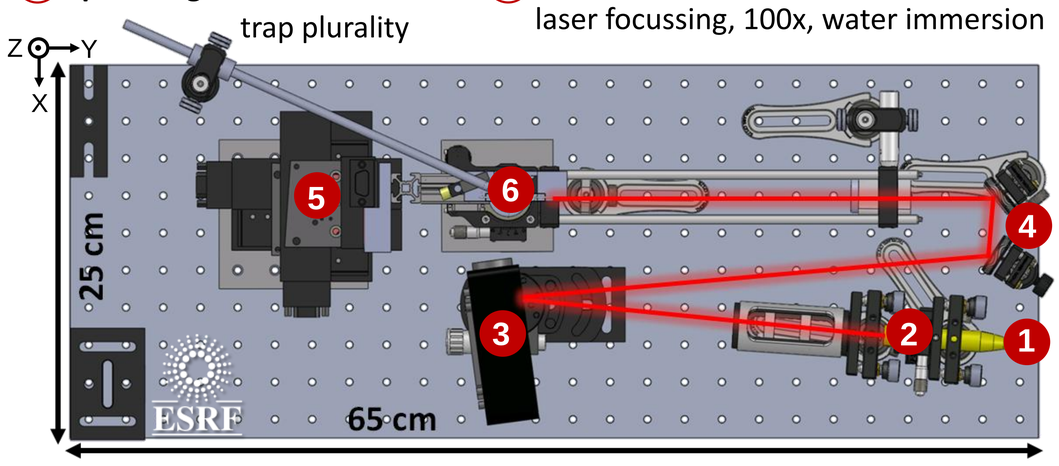
Highly sensitive, multi-elemental micro-XRF imaging

⇨ Organisms close to their **natural, *in vivo* state**
⇨ **Free-standing samples** in their natural, aqueous environment
⇨ **Non-contact sample positioning** and manipulation
⇨ **Eliminate** time-consuming and error prone **sample preparation**
⇨ Possibility of **XRF tomography** using multiple optical traps

Optical Tweezers Setup for X-ray Imaging at Synchrotron Facilities

- Compact OT setup available from Microfocus beamline ID13, ESRF [2]:

① **Start optical path** (——)
② **IR laser**, λ = 1070 nm
③ **Spatial Light Modulator** for trap plurality
④ **Mirrors**, manipulated for laser alignment
⑤ **Motorized stages** for sample positioning
⑥ **Microscope trapping objective** for laser focussing, 100x, water immersion

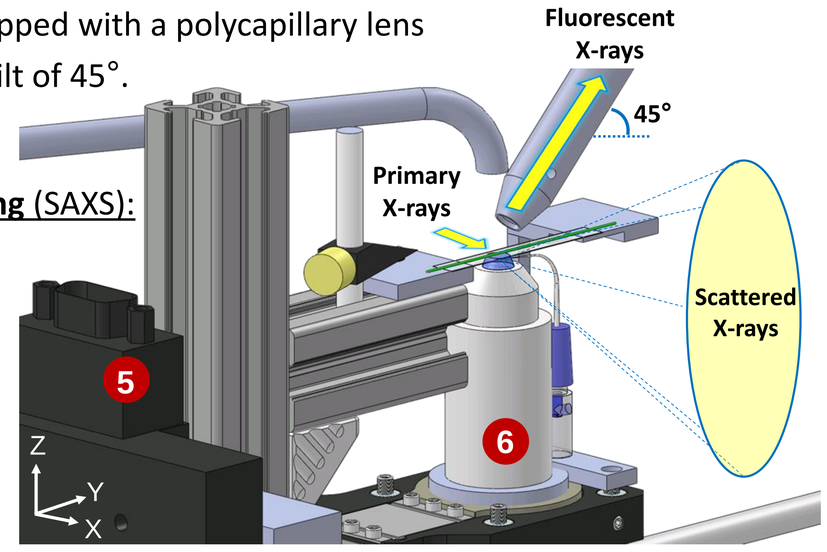


Confocal XRF imaging:

⇨ Provides **highly sensitive, multi-elemental information** on the sample composition.
⇨ Nozzle of Vortex-EM detector equipped with a polycapillary lens **confocal optic**, consequent detector tilt of 45°.

Integrated Small Angle X-ray Scattering (SAXS):

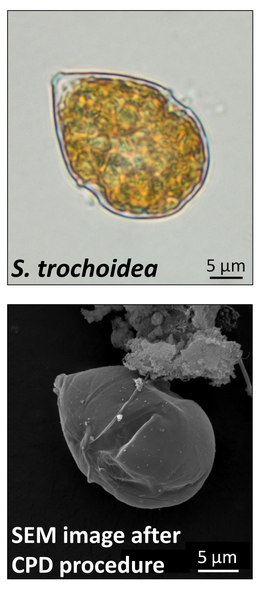
⇨ Visualize the **sample outline** and **internal sample structure**.
⇨ Allows for improved OT XRF data processing strategies.
⇨ Applied CCD cameras include: FReLoN, MAXIPIX, Eiger 4M.

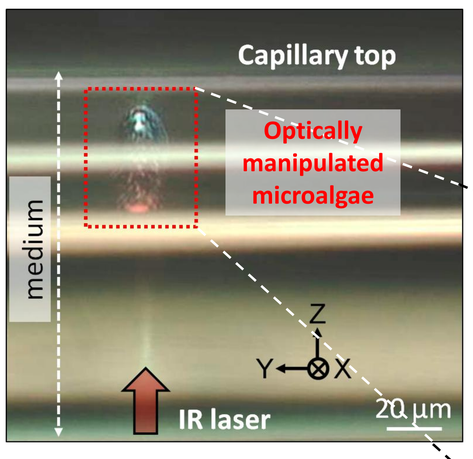


OT micro-XRF imaging at Microfocus ESRF-ID13

Sample experimental conditions

- ***Scrippsiella trochoidea* microalgae** (≈35 µm width)
- Exposed to elevated, toxic concentrations of transition metals (Ni, Cu, Zn, 96 h exposure).
- Sample container consists of a quartz capillary filled with specimens & medium (Ø 100 µm, 10 µm wall).

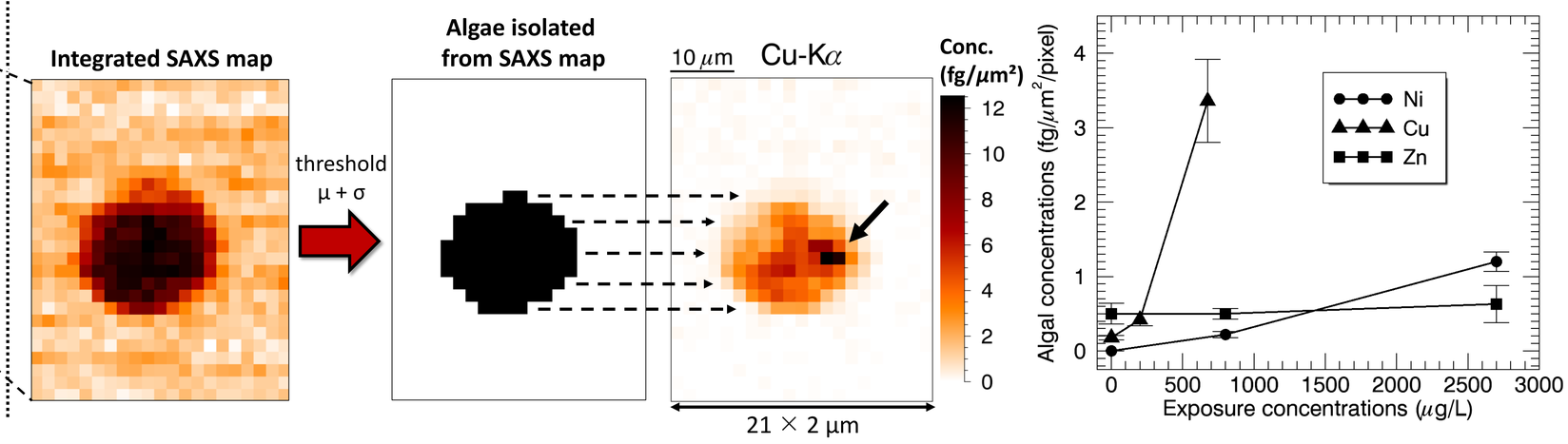




Optically levitated *S. trochoidea* microalgae in aqueous medium, translated to the upper capillary wall to prevent X-ray induced vertical sample movement during a progressing scan [3].

Experimental results

- Significant amounts of Mn, Fe, Cu and Zn are detected within the reference and exposed samples, reflecting their essential nature in photosynthesis processes [3].
- Average scanning time of 5-10 min demonstrates the **high throughput potential.**
- Algae outline derived from the SAXS distribution, followed by projection onto the XRF elemental maps.
- **Inhomogenous** subcellular **bioaccumulation of Cu** in highly concentrated exposure medium (675 µg/L Cu).
- Large differences in algal sensitivity towards the bioaccumulation of metals: **Cu >> Ni > Zn**.
- Experimental details: 2.10^{10} photons/s at 13 keV, 0.5 s/pixel, NIST SRM 1577c for quantification purposes.



Conclusions

We report on the radically **new elemental imaging approach** for the **analysis** of biological model organisms and **single cells in their natural, *in vivo* state**. The methodology **combines optical tweezers** (OT) technology for non-contact, laser-based sample manipulation **with synchrotron radiation confocal XRF** micro-imaging *for the very first time*. Several successful test experiments focussing on applications in environmental toxicology have been performed at ESRF-ID13, demonstrating the feasibility, repeatability and high throughput potential of the OT XRF methodology [3,4].

References

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