In vivo X-ray fluorescence microimaging of biological model organisms manipulated by laser-based optical tweezers

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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 methodological challenges related to XRF imaging

- **Preservation of the structure of biological organisms** is a major challenge when preparing biological samples for nano/micro XRF experiments.
- Special **need for delicate mounting** of microscopic samples onto a support that does not interfere with the XRF measurement.
- State-of-the-art and very expensive motor stages to **perform accurate and precise XYZθ movements** of the sample through the X-ray beam.

Proposed methodology for solving challenges

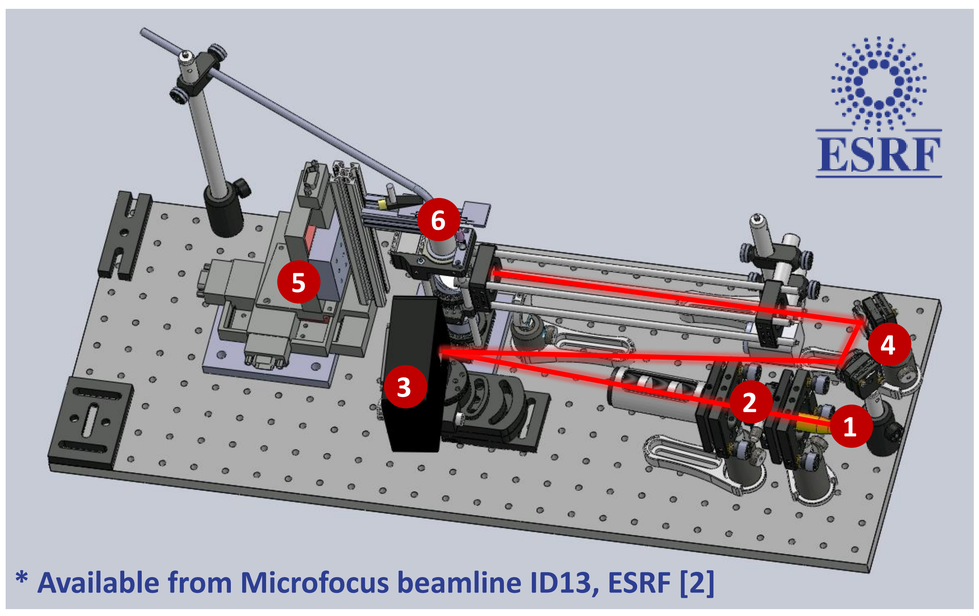
Optical tweezers for non-contact sample manipulation

combined with

Highly sensitive, multi-elemental micro-XRF imaging

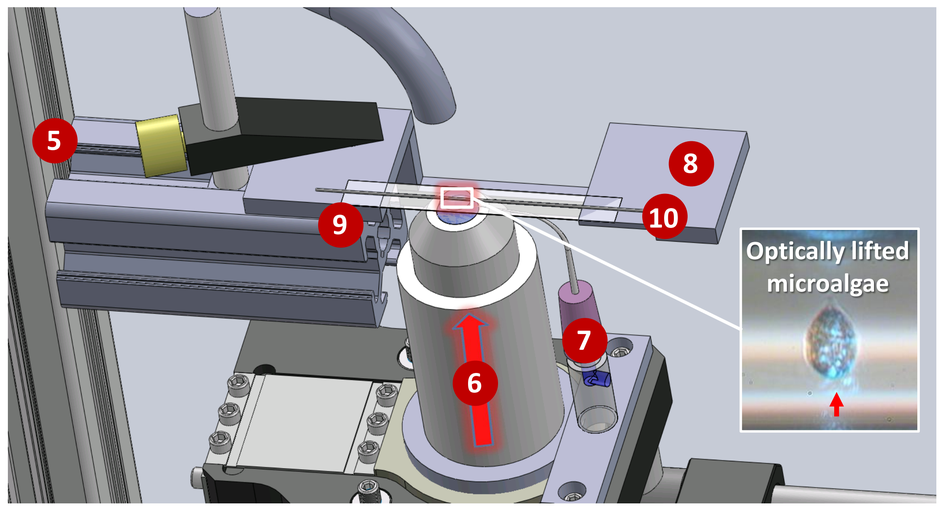
- ⇨ Organisms close to their **natural, *in vivo* state**
- ⇨ **Free-standing samples** in their natural, aqueous environment
- ⇨ **Non-contact sample positioning** and manipulation
- ⇨ **Eliminate** time-consuming and error prone **sample preparation**
- ⇨ Possibility of **XRF tomography** using multiple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

Compact Optical Tweezers Setup



* Available from Microfocus beamline ID13, ESRF [2]

1. **Start optical path (——)**
2. **IR laser coupled with fiber optics:**
   - λ = 1070 nm, transparent wavelength for biological samples
3. **Spatial Light Modulator (SLM):**
   - Bi-functional: beam splitter or mirror
   - Computer-controlled via holograms
4. **Mirrors** (manipulated for alignment)
5. **Motorized stages**
6. **Microscope trapping objective:**
   - Focusses IR beam
   - 100x magnification, NA = 1
   - Water immersion

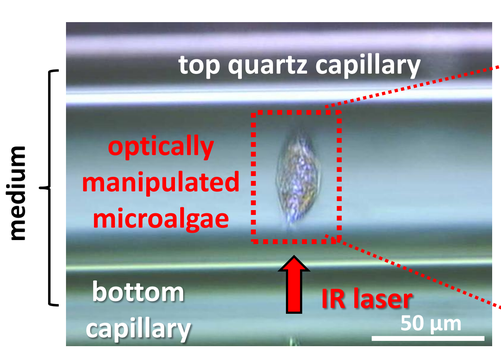


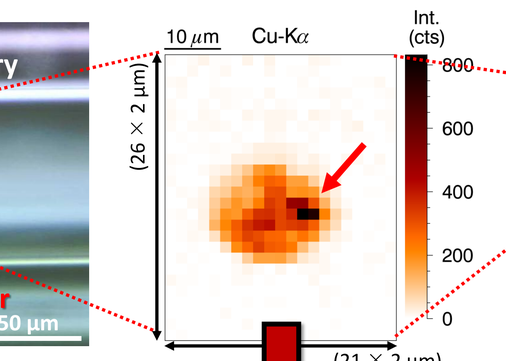
7. **Water droplet refill system**
8. **Aluminum sample holder**
9. **Quartz coverslip** (100 µm thickness)
10. **Quartz capillary with samples & medium** (100 µm diameter, 10 µm wall thickness)

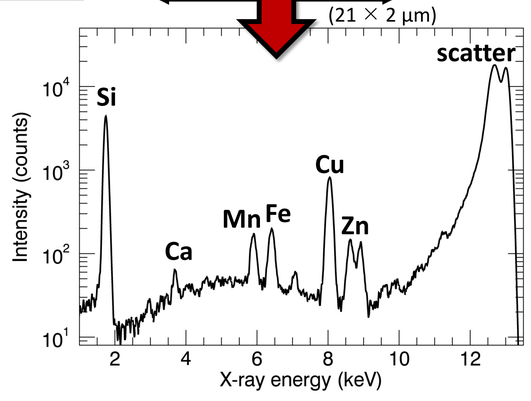
OT micro-XRF imaging at Microfocus beamline ID13 (ESRF)

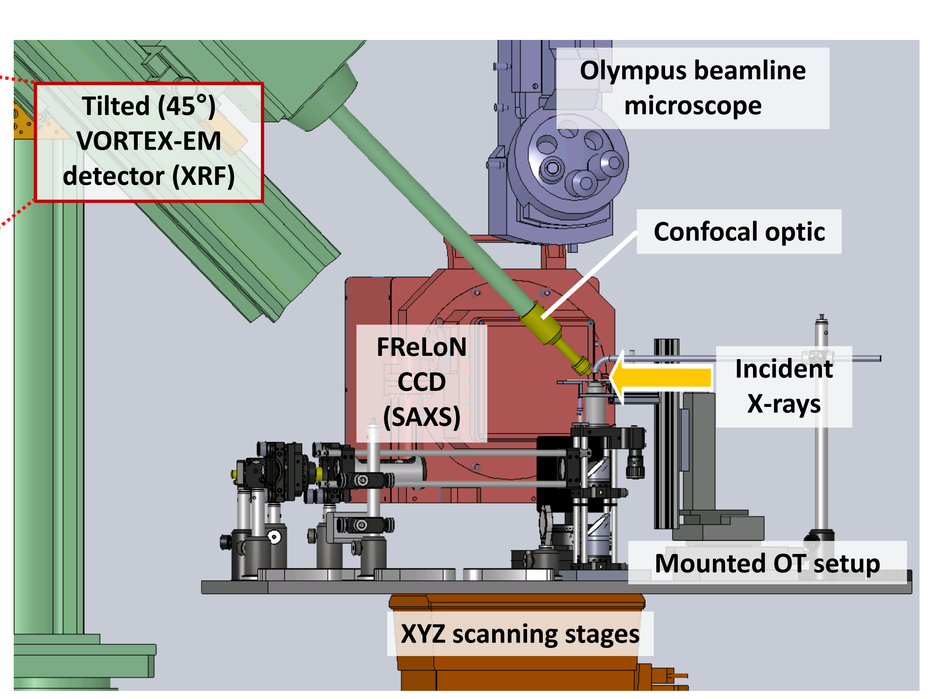
Experimental conditions

- ***Scrippsiella trochoidea* microalgae**
- Exposed to elevated, toxic concentrations of transition metals (Ni, Cu, Zn, 0-2700 µg/L, 96 h).
- 2.10^{10} photons/s at 13 keV, 0.5 s/pixel









Experimental results

- Significant amounts of Mn, Fe, Cu and Zn are detected within reference samples, reflecting their essential nature in photosynthesis processes [3].
- **Inhomogenous** subcellular **bioaccumulation of Cu** (675 µg/L).
- Average scanning time of 5-10 minutes demonstrates **high-throughput potential** of the OT XRF methodology.

Conclusions and prospects

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*. In future experiments, the possibilities of direct sample positioning and scanning using the SLM will be explored. Moreover, we propose ultra fast scans on a **variety of biological organisms/single cells** with a wide range of applications in all disciplines where *in vivo*, spatially resolved and **highly sensitive multi-element analysis** is of relevance on the microscopic scale.

References

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