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

Eva VERGUCHT1*, Toon BRANS2,3, Filip BEUNIS2,3, Jan GARREVOET3, Maarten DE RIJCKE4, Stephen BAUTERS1,3, Michiel VANDEGHEUCHT5, Colin JANSEN6, Manfred BURGHAMMER5,1 and Laszlo VINCZE2

1. X-ray Microspectroscopy and Imaging Group (XMI), Ghent University, Krijgslaan 281 (S12), B-9000 Ghent, Belgium
2. Department of Electronics and Information Systems (ELIS), Ghent University, Sint-Pietersnieuwstraat 41, B-9000 Ghent, Belgium
3. Center for Nano and Biophotonics (NB-Photonics), Ghent University, Sint-Pietersnieuwstraat 41, B-9000 Ghent, Belgium
4. Laboratory of Environmental Toxicology and Aquatic Ecology (GhEnToxLab), Ghent University, Jozef Plateaustraat 22, B-9000 Ghent, Belgium
5. European Synchrotron Radiation Facility (ESRF), 71 avenue des Martyrs, F-38043 Grenoble, France

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 XY2D 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

### Experimental conditions

- **Scrippsiella trochoidea** microalgae
- Exposed to elevated, toxic concentrations of transition metals (Ni, Cu, Zn, 0-2700 µg/L, 96 h).
- 2.10¹⁰ 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].
- Inhomogeneous subcellular bioaccumulation of Cu (675 µg/L).
- Average scanning time of 5-10 minutes demonstrates high-throughput potential of the OT XRF methodology.

### Conclusion 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


* Eva Vergucht, X-ray Microspectroscopy and Imaging Group (XMI), Department of Analytical Chemistry, Ghent University, Eva.Vergucht@UGent.be