Prospects of combining SR micro/nano-XRF methods with optical tweezers based sample manipulation

Eva VERGUCHT[1*], Toon BRANS[2], Filip BEUNIS[2], Michiel VANDEGEHUCHTE[3], Bart VEKEMANS[1], Colin JANSSEN[3], Manfred BURGHAMMER[4], Christian RIEKEL[4] and Laszlo VINCZE[1]

1. Department of Analytical Chemistry, Ghent University, Krijgslaan 281 (S12), B-9000 Ghent, Belgium
2. Center for Nano and Biophotonics (NB-Photonics), Ghent University, Sint-Pietersnieuwstraat 41, B-9000 Ghent, Belgium
3. Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Jozef Plateaustraat 22, B-9000 Ghent, Belgium
4. European Synchrotron Radiation Facility (ESRF), F-38043 Grenoble, France

Introduction

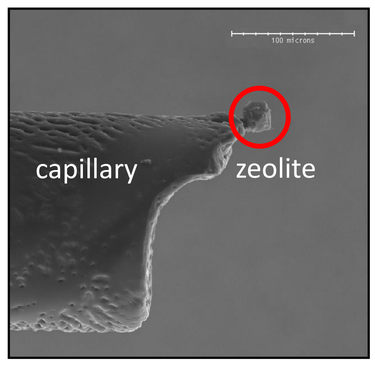
Owing to its high sensitivity and non-destructive nature, synchrotron radiation based X-ray fluorescence computed tomography (SR XRF-CT) and confocal XRF imaging are emerging methods that provide three-dimensional (3D) information on elemental distributions with trace level detection limits [1]. With the increased availability of nanoscopic X-ray beams provided by 3rd generation SR sources, these methods pose important methodological challenges concerning non-contact sample manipulation and positioning.

Methodological challenges related to XRF imaging

The **preservation of the structure of biological organisms** is a major challenge when preparing samples for **nano/micro** XRF experiments.

An optical image of a juvenile Daphnia magna (water flea). XRF imaging may reveal locations where toxic metals accumulate within the various tissues of this biological model organism that is used as a bio-indicator.

There is a special **need for delicate mounting** of microscopic samples onto a support that does not interfere with the XRF measurement.

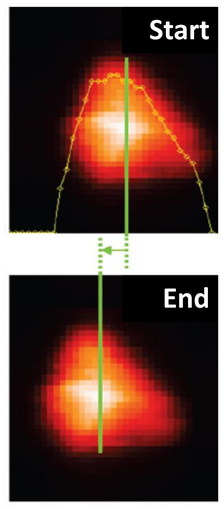


SEM image of a zeolite, the microscopic particle is mounted onto a glass capillary (Terzano et al.). This allows X-rays to only interact (directly) with the particle as if it was a 'free-standing' sample.

Nano/micro 2D/3D XRF imaging requests state-of-the-art and very expensive motor stages in order to **perform accurate and precise XYZθ movements** of the sample through the X-ray beam.

An example of a hexapod with nanoscopic positioning capabilities.

The example shows the nanoscopic Fe-K_α intensity distribution from the microscopic cometary sample (comet Wild2/81P). Both images should be identical, but a clear shift of the sample is observed in both horizontal and vertical direction mainly due to the imperfect nature of the motor system (Silversmit et al.). This imperfect data collection for nanoscopic XRF tomography requires appropriate software strategies in order to correctly reconstruct the requested 3D elemental distributions.



Proposed methodology: project objectives

Combining optical tweezers for non-contact sample manipulation with non-destructive micro/nano-XRF imaging

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

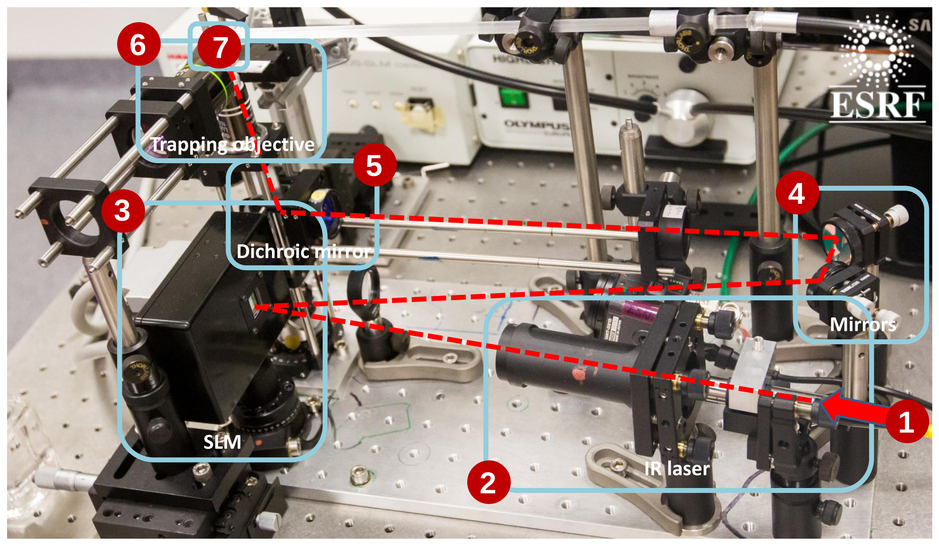
Compact Optical Tweezers Setup

⇨ Compact optical tweezers setup available from beamline ID13, ESRF [2].

1 **Start optical path (- - -)**

2 **IR laser coupled with fiber optics:**
- λ = 1070 nm, transparent wavelength for biological samples
- Beam expander in front of laser collimator
- IPG Photonics

3 **Spatial Light Modulator (SLM):**
- Bi-functional: beam splitter or mirror
- Control via holograms (pc)
- Hamamatsu Photonics



4 **Mirrors:**
- Manipulated for alignment

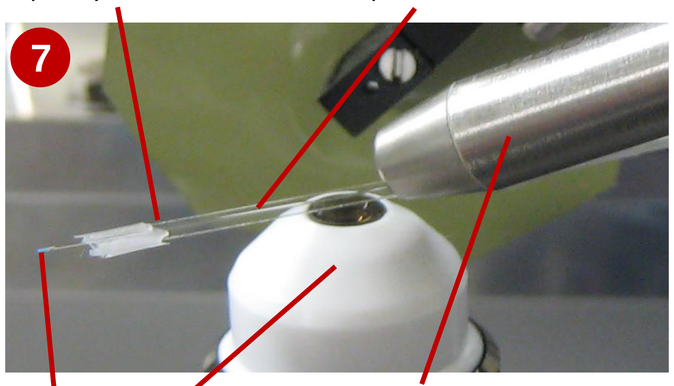
5 **Dichroic mirror:**
- ⇧ reflection IR light
- Transparent for visual light

6 **Microscope trapping objective (MO):**
- Focusses IR beam
- 100x, Olympus
- NA = 1
- Water immersion
- 1 mm working distance

7 **Sample area**

Cover glass: Interface between capillary and water

Glass capillary (CTS, UK): Filled with biological samples and medium

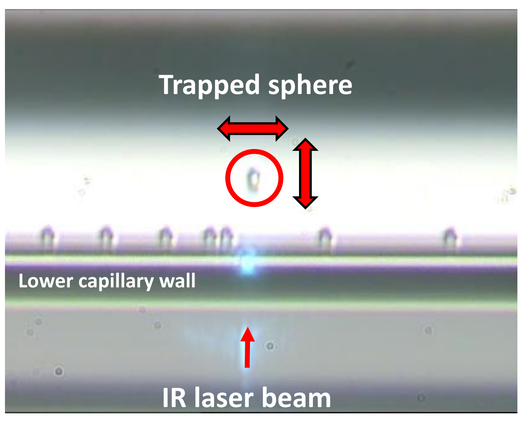


Plasticine: Capillary sealing

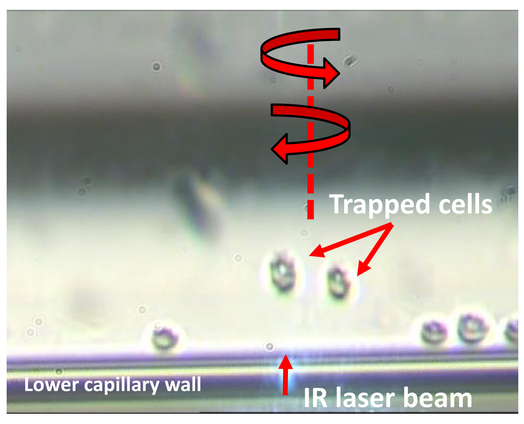
Confocal optic: Detector receives only fluorescent X-rays from small volume (100 µm³) inside the biological sample.

First results and prospects

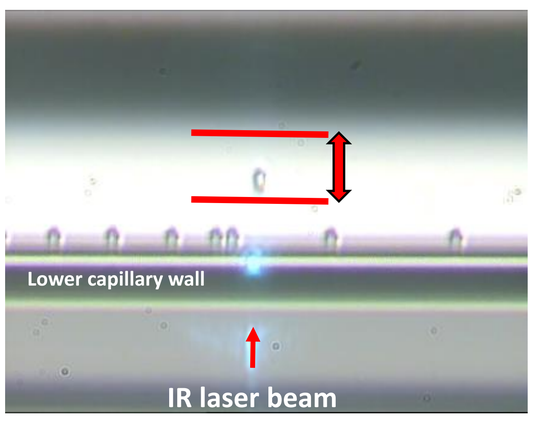
Laboratory optical trapping results



- Successful optical trapping of silica microspheres (Ø 3.5 µm, Bangs Laboratories, Inc.)
- Sample translation performed via manual micromanipulation stage.



- Optical trapping of *Chlamydomonas reinhardtii* (Ø 10 µm) micro algae.
- Sample rotations using SLM, showing the potential for XRF tomography by using multiple traps.



- Optical translation of silica microspheres using SLM.
- Set of holograms translated the sample in z-direction, showing the possibility for XRF scans without the need for beamline motor stages.

First OT + SR micro-XRF experiment

- First experiment performed at ID13 (microhutch).
- Poor trapping conditions for the micro algae at the beamline.
- Possible explanations: bad cell culturing conditions, slight misalignment of the OT setup.

Points of interest to work on:
- In general, improve on the optical trapping conditions at the beamline.
- Further search for a suitable (larger) biological model organism.
- Need for an optimized XRF confocal optic with a longer working distance (x > 4 mm).
- Look for other cover glasses/supports without traces of the elements of interest (e.g., quartz).
- Position XRF detector under an angle of 45° (instead of current 23°).

References

[1] Vincze, L., et al., Analytical Chemistry, 2004. 76(22): p. 6786-6791.
[2] Santucci, S.C., et al., Analytical Chemistry, 2011. 83(12): p. 4863-4870.

Corresponding author

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