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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** X-ray fluorescence imaging of biological model organisms trapped by laser-based optical tweezers	**Experiment number**: LS-2300
Beamline: ID13	**Date of experiment**: from: 16/03/2014 to: 19/03/2014 from: 20/05/2014 to: 24/05/2014	**Date of report**: 12/08/2014
Shifts: 2x 15 shifts	**Local contact(s)**: Manfred BURGHAMMER (burgham@esrf.fr) Britta WEINHAUSEN (britta.weinhausen@esrf.fr)	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

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 X-ray fluorescence (XRF) micro-imaging *for the very first time*. Two subsequent experiments were conducted at the ESRF-ID13 Microfocus beamline showing the potential of the new OT micro-XRF methodology. In a first proof of principle (POP) experiment, we examined the possibilities of OT micro-XRF imaging on *Scrippsiella trochoidea* microalgae exposed to Ni, Cu and Zn (96 h). In a second experiment, the OT XRF methodology was successfully combined by complementary Small Angle X-ray Scattering (SAXS) scanning techniques that enable visualizing the sample outline and the internal sample structure. In addition, the mixture toxicity of Cu-Ni and Cu-Zn on *Scrippsiella trochoidea* microalgae was investigated in order to retrieve a possible synergistic or antagonistic effect after 96 h exposure time. Moreover, the use of SR confocal micro-XRF provides a better insight in the cellular distribution of metals.

In 2011, Santucci *et al.* reported on a dedicated optical tweezers (OT) setup for SR scanning micro-diffraction experiments on proteins trapped within their natural, aqueous environments. Optical tweezers use a focused laser beam for optically manipulating a sample within an aqueous environment, enabling non-contact sample manipulation and positioning. The initial compact OT setup developed by Santucci *et al*. was modified to match with the spatial

requirements for SR confocal micro-XRF experiments. All optical components are mounted on a THORLABS optical breadboard that can be fixed to the Micos UPL-160 elevation stage of beamline ID13. A CAD design in SolidWorks (Fig. 1a, corresponding laser path in blue) and a detail of the sample area (Fig. 1b) of the modified OT setup are shown in Figure 1. The samples consist of quartz capillaries (filled with algae and medium, QGCT 0.1, CTS Ltd, UK, 100 µm Ø, 10 µm wall thickness) taped onto quartz coverslips and onto an aluminum holder (as a stable support). It should be noted that all glass wear in the vicinity of the trapping objective is high purity quartz to minimize spectral contributions.

Detection of the fluorescent photons was performed using a VORTEX-EM detector (50 mm^2 active area, 2 µs peaking time, HITACHI, USA) tilted to an angle of 45° (with respect to the plane of polarization) and perpendicular to the incoming X-ray beam. The VORTEX-EM detector was equipped with polycapillary optics (X-ray Optical Systems Inc., Albany, USA) in a confocal XRF detection geometry with an acceptance of 50-100 µm in order to minimize the fluorescent/scatter signal from the surrounding sample environment.

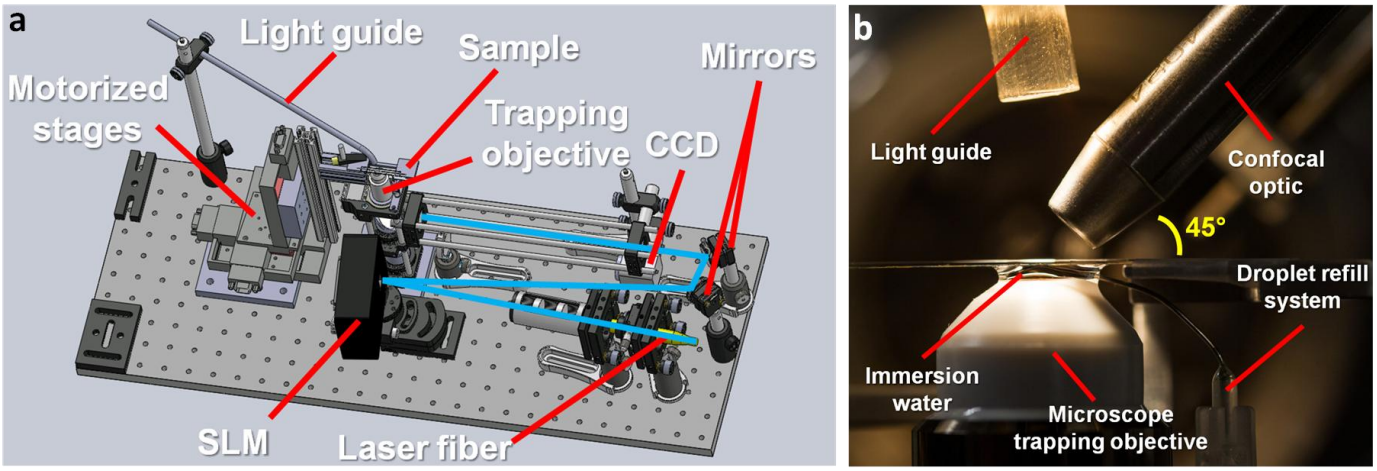

Figure 1: Overview of the compact OT setup. (a) CAD design in SolidWorks, IR laser path in blue. (b) Detail of the sample environment, the confocal optic is positioned under 45°.

Within the POP experiment, four cultures of *Scrippsiella trochoidea* microalgae were prepared (Zn, Cu and Ni, 100 µg/L, L1 medium, 96 h) and one reference. The microalgae are initially lifted using a 0.2-0.3 W power (one trap or multiple traps depending on the algae size), positioned into the X-ray beam and scanned with SR confocal micro-XRF. The area corresponding to the dotted rectangle on the microscopic image (Fig. 2a) is scanned with high resolution (1 µm step size, 0.1 s/point, top to bottom). The total time per scan varied between 5 and 10 min demonstrating the high-throughput potential of the OT micro-XRF methodology. The elemental distributions in Fig. 2 show that significant amounts of Mn (Fig. 2b), Fe (Fig. 2c), Cu (Fig. 2d) and Zn (Fig. 2e) are detected within the Zn-exposed algae. These transition metals are essential micronutrients for the growth and metabolism of the microalgae and fulfill critical roles in photosynthesis and the assimilation of essential macronutrients (e.g., N, P).
From the Ni and Cu exposed cultures, similar elemental distributions were obtained, however the reference measurements showed no significant Ni signal. Since Ni is an essential micronutrient, playing an important role in nitrogen assimilation; it is naturally present in algae however in amounts below the XRF limit of detection.

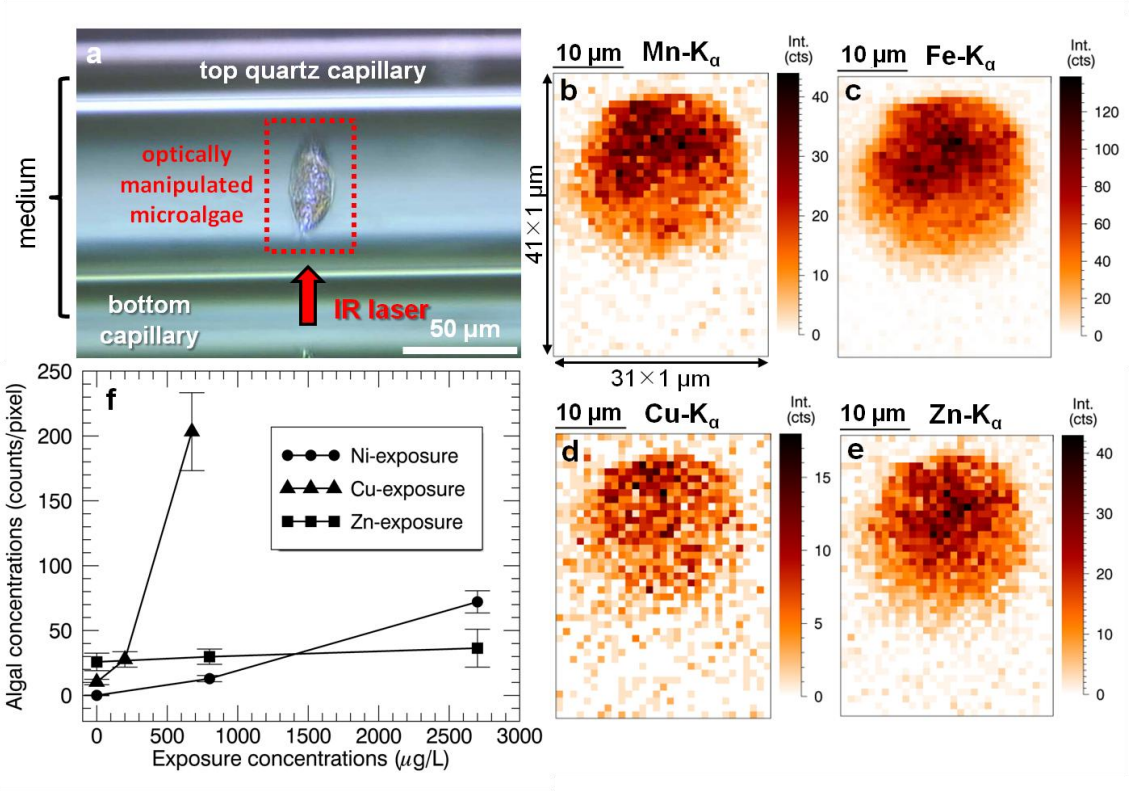

Figure 2: Experimental results of scanned *Scrippsiella trochoidea* microalgae. (a) Microscopic image of an optically manipulated algae in a quartz capillary, dotted rectangle indicates scanned area. (b-c-d-e) Mn, Fe, Cu and Zn elemental distributions corresponding to the area scanned with high resolution. (f) Tendency towards the bioaccumulation of Ni (case 1, filled dot), Cu (case 2, filled triangle) and Zn (case 2, filled square).

In a second experiment, the mixture toxicity of Cu-Ni and Cu-Zn on *Scrippsiella trochoidea* microalgae was investigated in order to retrieve a possible synergistic or antagonistic effect. Moreover, we examined the combination of the new the OT micro-XRF methodology with complementary Small Angle X-ray Scattering (SAXS) scanning measurements for sample outline and the sample structure visualization. Within two cases, the combined exposure of Cu/Ni (case 1) and Cu/Zn (case 2) was tested using six exposure conditions (1x reference, 2x Cu, 2x Ni/Zn, 1x mix, L1 medium, 96 h). For each exposure condition, three algae were selected and subjected to OT XRF-SAXS analysis. The recorded SAXS patterns give a clear indication of the algae outline and were used for improved semi-quantitative XRF data analysis. The semi-quantitative XRF data indicate synergistic effects after 96 h exposure time. Moreover, the binary mixture experimental results indicate that large differences exist in algal sensitivity towards the bioaccumulation of metals. The graphical representation in Fig. 2f shows the following trend in accumulation behavior: Cu >> Ni > Zn.

In future experiments, the possibilities of direct sample positioning and scanning using the SLM will be explored, ultimately making the complex high-resolution motor stage systems obsolete. Moreover, the sample can be slowly rotated by the SLM chip, resulting in the possibility for OT XRF tomographic imaging on microscopic samples. For future OT XRF experiments 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.