ESRF	**Experiment title:** X-ray fluorescence imaging of biological model organisms trapped by laser-based optical tweezers	**Experiment number**: EV-22
Beamline: ID13	**Date of experiment**: from: 12/06/2013 to: 18/06/2013	**Date of report**: 29/08/2013
Shifts: 18	**Local contact(s)**: Manfred BURGHAMMER	*Received at ESRF:*

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Report:

The main objective of this project was to realize a novel X-ray fluorescence (XRF) microscopy methodology by combining confocal-XRF and XRF-tomographic techniques with an IR laser-based optical tweezers (OT) setup for non-contact sample manipulation/positioning, which allows elemental imaging of biological micro-samples essentially in their natural state. The coupling of the laser-based optical tweezers (OT) technology with 2D/3D XRF micro/nano-imaging techniques represents a new milestone concerning the development of non-invasive elemental imaging of biological systems in their native environment. The use of the OT technology not only provides real 'freestanding' samples, but may also allow translation and rotation of the trapped samples via multiple traps, ultimately making X-ray fluorescence tomography in a non-contact environment possible. We intended to explore these remarkable capabilities of the OT setup to demonstrate, for the very first time, XRF microimaging of trapped (biological) micro-samples.

Experiments were performed in the micro-hutch of beamline ID13 where the OT setup was mounted onto the beamline motor stages. An overview of the setup is shown in Figure 1 and Figure 2.

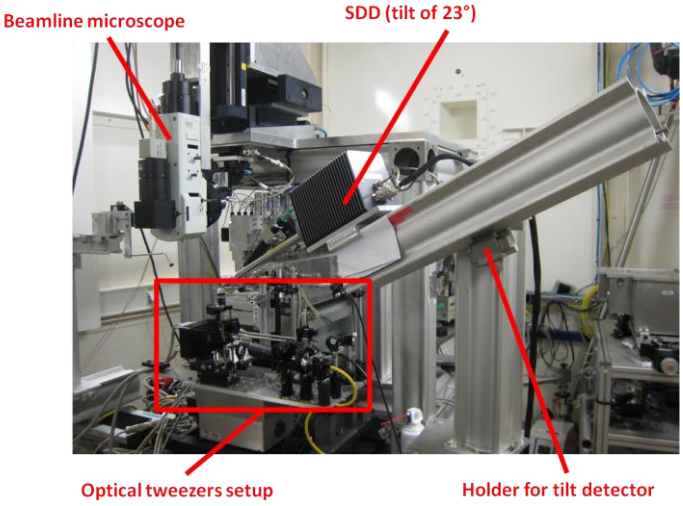

Figure 1. Overview setup at micro-hutch ID13.

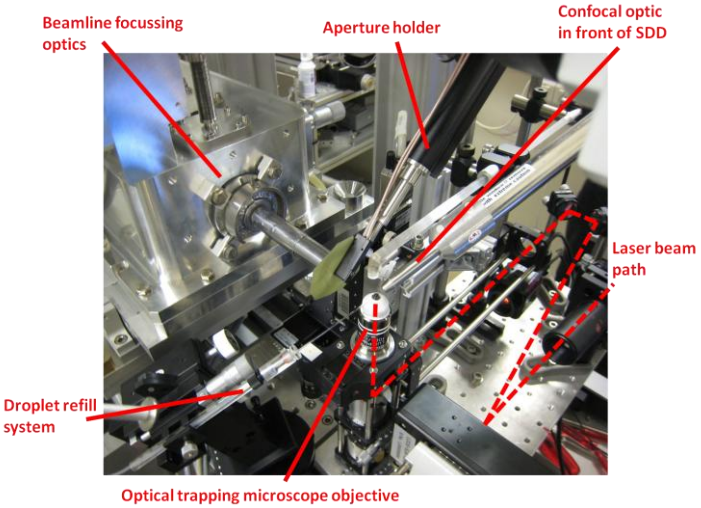

Figure 2. Detail of the OT setup on the beamline stages.

Silica microspheres (3.5 µm diameter, Bangs Laboratories, Inc.) were used for testing the optical trapping conditions at the beamline. The microspheres were suspended in deionised water in a cylindrical glass capillary container (200 µm diameter, 10 µm wall thickness, CTS Ltd). The capillary was sealed at both ends with plasticine and subsequently taped onto a cover glass (Figure 3). The cover glass ensured sample stability and was necessary for sample observation using the beamline microscope (Figure 4).

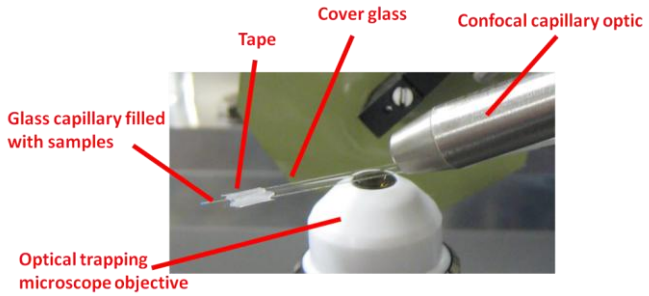

Figure 3. Detail of a sample on the optical trapping MO.

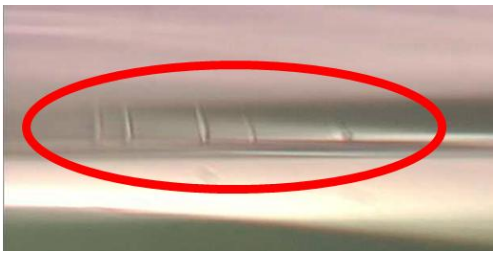

Figure 4. Silica spheres at the bottom of a glass capillary.

During the initial tests of the OT-setup, a series of silica microspheres were optically trapped (Figure 5) and scanned via conventional (non-confocal) micro-XRF detection using a silicon drift detector (SSD) that was mounted horizontally. The sum spectrum of the scans is shown in Figure 6. As expected, a considerable Ar signal is present due to the ambient conditions. The Si signal originates from the silica microspheres, as well as the Ge signal. The Pb and Ti signal can probably be explained by the surrounding or shielding material. Finally the Zn signal deserves special attention since it originates from the cover glass that supports the glass capillary. This contribution from the cover glass is highly undesirable since Zn is one of the elements of interest in biological samples. As a consequence, the option of installing a confocal polycapillary optic in front of the detector was considered even for this preliminary experiment. Using this optic, the fluorescent signal of only a very small volume inside the sample container is captured, thereby eliminating the fluorescent signal from the cover glass and surrounding materials (OT-objective, shielding, etc.) completely.

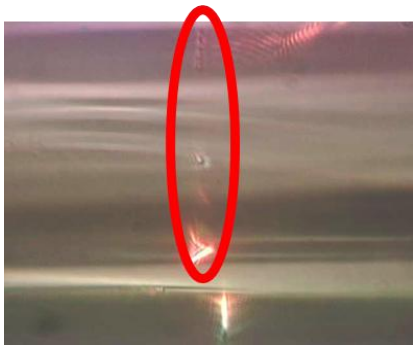

Figure 5. Optically trapped silica microspheres.

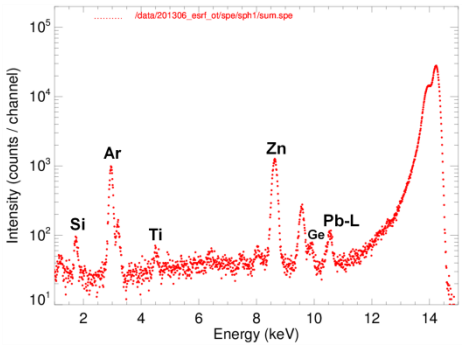

Figure 6. Sum spectrum of an optically trapped silica microsphere.

Installing the confocal polycapillary optic (XOS, Figure 7) required tilting the detector at an angle of 23° because of two reasons. First, the working distance of the optic was very limited being only 4 mm and secondly the optical trapping microscope objective has a certain curvature as well (Figure 3). For aligning and calibration purposes, a NIST SRM 2066 microsphere standard was used mounted on a glass capillary tip (Figure 8).

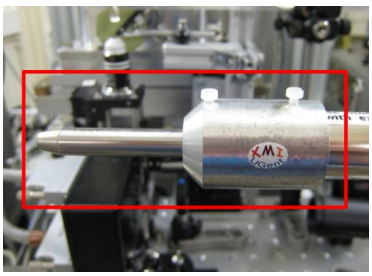

Figure 7. Confocal optic.

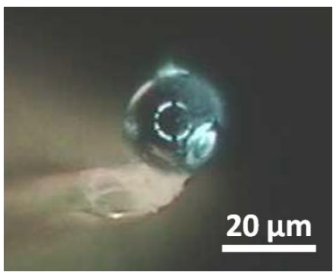

Figure 8. A mounted NIST SRM 2066 sphere.

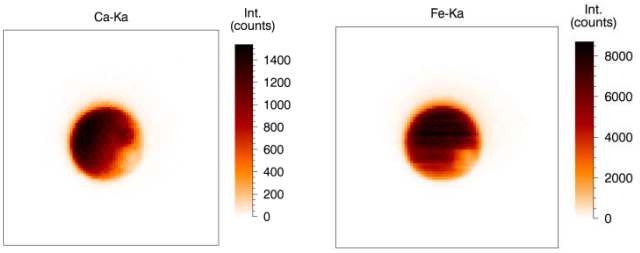

Figure 9. Ca (left) and Fe (right) distribution in a NIST SRM 2066 microsphere.

The NIST SRM 2066 is a glass microsphere standard containing well-known concentrations of calcium and iron that could be perfectly visualised as shown in Figure 9. While these microspheres were suitable for alignment/calibration purposes, this standard does not fulfil the requirements of optical trapping due to the strong absorption of both visible and infrared light, resulting in a fast upward movement of the microspheres without being optically trapped.

Next to realizing the combined confocal-XRF/OT setup, the ultimate goal of this experiment was to determine the elemental composition of optically trapped biological model organisms that were exposed to metal gradients. The biological samples used for this purpose were *Chlamydomonas reinhardtii* cells (Figure 10) and *Pseudokirchneriella subcapitata* cells (Figure 11). Prior to experiment EV22 at beamline ID13, these cell types were extensively tested for their trapping capabilities. The results of these preliminary studies showed that both cell types can be perfectly trapped for extended periods of time.

Figure 10. Microscopic image of a *Chlamydomonas reinhardtii* cell.

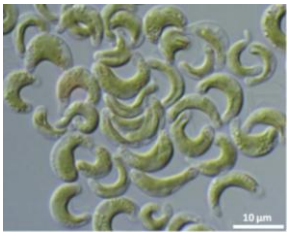

Figure 11. Microscopic image of *Pseudokirchneriella subcapitata* cells.

Unfortunately, under the measuring conditions at ID13, the measurements suffered from very poor trapping conditions since most of the cells appeared in clusters that were sticking to the walls of the glass capillary. This hampered the optical trapping enormously and an increase in the applied laser power did not cause detachment the cells from the glass walls. Probably bad culturing conditions in the optical tweezers laboratory created the abnormal sticking behaviour. Only a very limited number of times a biological sample was actually optically trapped, typically for only a few seconds. Possibly the OT setup was also not perfectly aligned which hampered the extended trapping periods.

For future comparison between OT-trapping based and conventional scanning micro/nano-XRF, high resolution measurements were performed on relevant biological model organisms that were mounted on Si_3N_4 membranes. Figure 12 shows a cluster of *Chlamydomonas reinhardtii* cells that were exposed to high metal concentrations (5 µg/l Cu and 500 µg/l Zn) and mounted onto a Si_3N_4 membrane. A combined RGB image of the Zn, Cu and Fe distributions (respectively) shows that both Fe and Zn are fairly homogenously distributed. In specific cell parts Cu and Zn appear similarly distributed indicated in yellow.

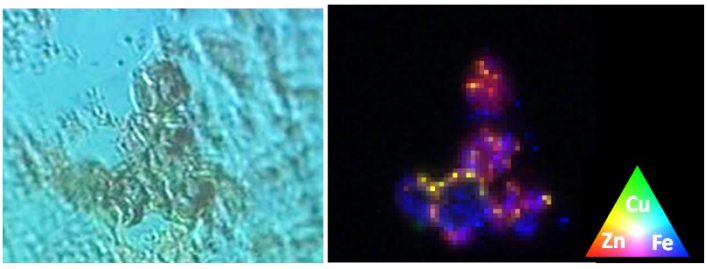

Figure 12. RGB image of a cluster of Chlamydomonas reinhardtii cells.

We can conclude that the novel methodology of combing confocal-XRF spectroscopy with IR laser-based OT has certainly a high potential and a new experimental setup for this combination was realized. However, certain problems still need to be tackled and future experiments are necessary to improve on the realized setup. Especially the optical trapping conditions at the beamline need to be improved and better culturing conditions at the ESRF for the biological samples are necessary as well. For quantification and aligning purposes, microspheres with a well-defined elemental composition and good optical trapping capabilities should be available. In addition, a confocal polycapillary optic with a longer working distance would simplify the measurements considerably.