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**Report:**

The use of genetically modified strains of single cell organisms corresponds to a promising strategy concerning the development of sensitive and highly selective biosensors for measuring bioavailable toxic metal ions at environmentally relevant concentrations. However, to fully understand the cell functioning under natural conditions it is necessary to utilize a variety of biochemical, molecular, and imaging techniques. In this report we summarize results obtained by examining single cells of various unmodified and transgenic strains of the eukaryotic green alga *Chlamydomonas reinhardtii* by means of X-ray microprobe techniques.

*Micro X-ray fluorescence* (μ–XRF) and *micro X-ray absorption spectroscopy* (μ-XAS) investigations were performed to gain information on the cellular distribution and on the localized speciation and oxidation state of metals within the organism. Qualitative elemental distribution maps and μ-XANES spectra were obtained for *single intact organisms* under *in-situ condition* by using the microprobe setup installed at beamline ID22 at the ESRF. Freeze-dried organisms deposited on ultra-pure polymer support films as well as cryo-cooled organisms trapped in micro-capillaries were analysed.

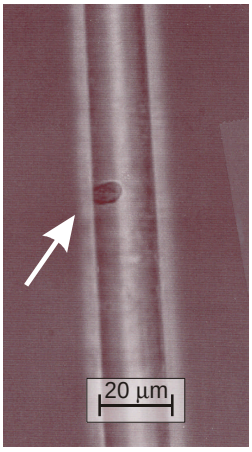

Fig. 1: Intact single cell organism within micro capillary.

We have been successful in recording μXRF maps of *single intact organisms* under *in-situ condition* for both types of techniques employed, polymer support films as well as cryo-cooled micro-capillaries (Figure 1).

Later corresponds to a pioneering implementation of this new sampling strategy, demonstrating highest promises for future investigation of single cells or single cell organisms.

Furthermore, we have succeeded in recording µ-XANES spectra of intracellular metal species.

Our combined µ-XRF and µ-XANES investigations revealed a close link between the internalized toxic metal concentrations and the metabolism of the macro nutrients as well as trace elements. For illustration, Figure 2 shows a comparison between qualitative elemental distribution maps (µ-XRF maps) for control cultures and for *Chlamydomonas reinhardtii* exposed to aqueous nickel. Clearly, an accumulation of Ni within the cell can be established. Further, the internalization of Ni is accompanied by a simultaneous uptake of Cu and by the expelling of K.

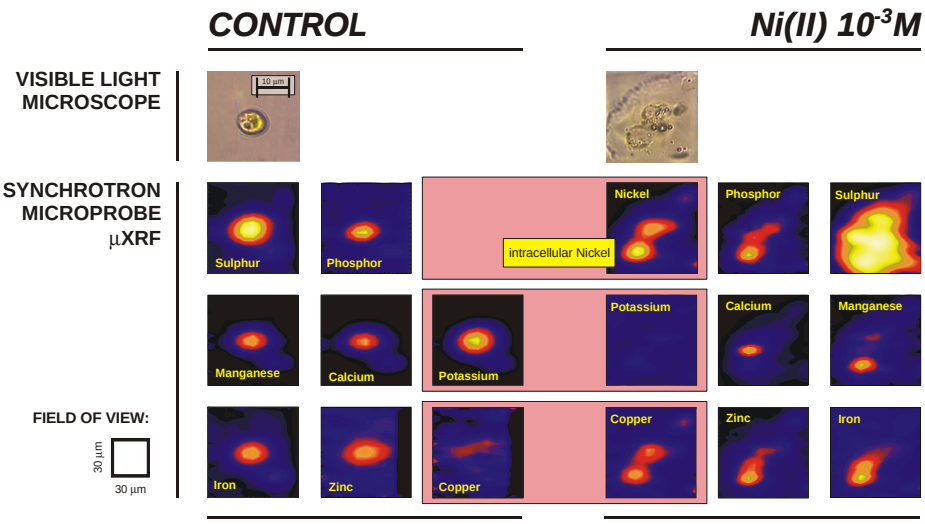


Fig. 2: µ-XRF maps of single *Chlamydomonas reinhardtii* organisms.

Figure 3 shows µXANES spectra of intracellular metal species. The observed metal speciation within the cell is evidently different from the original speciation present within the growth media. Most noticable, for the redox-sensitive As anion electron transfer reactions induced by *Chlamy. reinh.* were observed.

Investigating transgenic organisms, we observed similar responses.

The present study represent a first important step towards a biochemical and molecular-level understanding of adaptive responses of single-cell organisms.

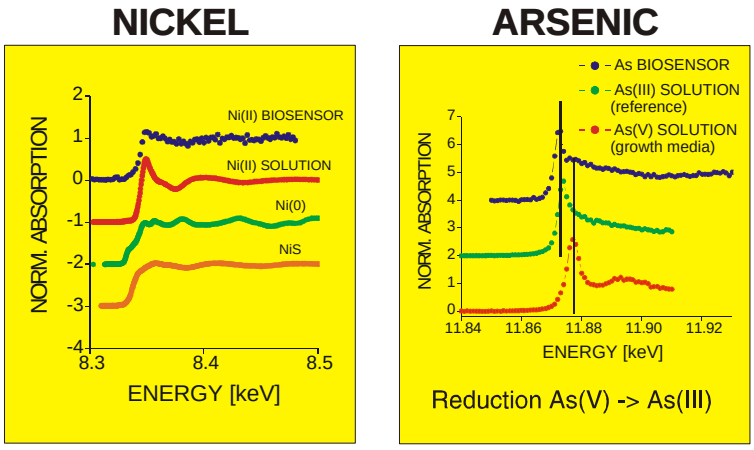


Fig. 3: µXANES spectra of intracellular metal species.

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D. Grolimund, A. M. Scheidegger, A. Simionovici, S. Bohic, I. Worms, H. Kola, D. Simon, K. Wilkinson, *Enlightening the Functionality of Single Cell Biosensors by micro-XRF and micro-XANES*, accepted as poster presentation, 17th International Congress on X-Ray Optics and Microanalysis (ICXOM 17), Chamonix, France, September, 2003.

D. Grolimund, A. M. Scheidegger, A. Simionovici , S. Bohic, I. Worms, H. Kola, D. Simon, K. Wilkinson, *Inner Life of a Biosensor: New Insights by X-ray Microprobes*, oral presentation, 12´th International Conference on X-ray Absorption Fine Structure (XAFS 12), Lund/Malmö, Sweden, June, 2003.