Cryogenic Synchrotron Radiation X-ray Fluorescence Analysis of Biological Model Organisms Using a State-of-the-art Cryochamber at P06, PETRA III

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Synchrotron radiation X-ray fluorescence (SRXRF) imaging of cryogenically fixed biological organisms using a cryo-stream is limited to analysis with microscopic resolution due to icing and absorption effects in ambient sample environment [1]. To eliminate these problems, a new state-of-the-art cryochamber (Fig.1) has been developed which is currently being commissioned at the P06 X-ray Microprobe at PETRA III (DESY).

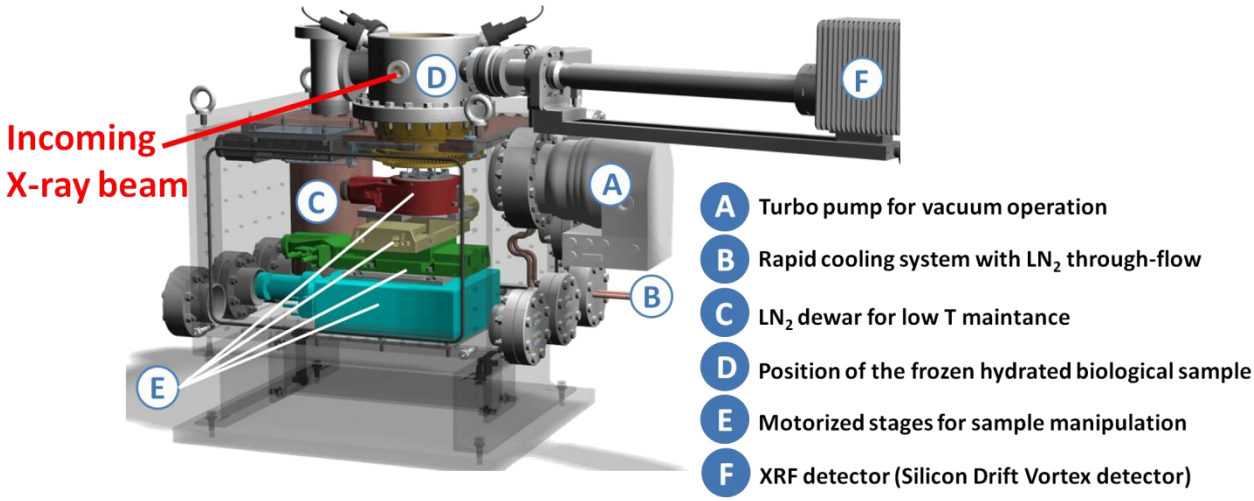

Figure 1: Technical drawing of the state-of-the-art cryochamber. High vacuum is generated using a turbo pump (A). Rapid cooling is achieved using LN_2 through flow (B) and cryogenic temperature is maintained using an internal LN_2 dewar (C). A frozen hydrated sample is inserted (D) and positioned into the nanoscopic X-ray beam using motorized stages (E). The fluorescent X-ray signal is collected using a Vortex Silicon Drift detector (F).

Due to the cryochamber design for static cooling and its vacuum environment, XRF imaging analysis of frozen hydrated microscopic samples can be performed at the sub-micron resolution level [2,3]. In December 2013, the cryochamber was tested for environmental science applications using biological microorganisms that were exposed to toxic concentrations of Zn (100 µg/L) and Cu (5 µg/L) for 24-72 hours. For this feasibility study, exposed samples were accumulated from growth media on a cellulose acetate filter membrane by mild suction (Fig.2-A), shock-frozen (Fig.2-B) and subsequently subjected to SRXRF analysis using the cryochamber (Fig.3). The selected samples were fresh water algae (*Chlamydomonas reinhardtti*, 10 µm diameter, Fig.2-C) and ocean water algae (*Prorocentrum lima*, 50 µm diameter, Fig.2-D). Two species of microalgae that act as valuable model organisms in ecotoxicology studies. In this report, an overview of the cryogenic SRXRF analysis on the exposed microalgae is presented and future prospects are discussed.

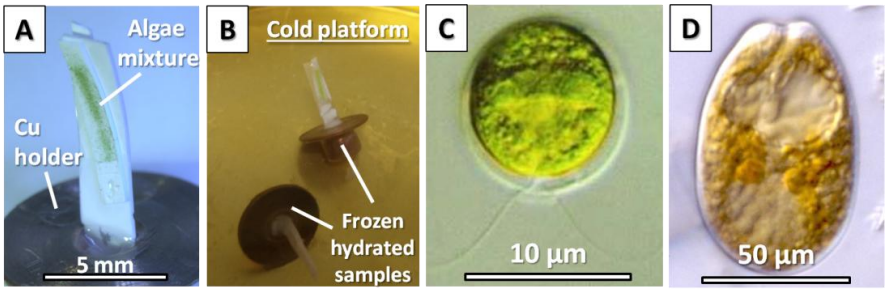

Figure 2: Overview of the investigated samples. Microalgae accumulated on cellulose acetate filter (A). After the plunge freezing procedure, shock-frozen samples are temporarily stored on a cold platform (B). *C. reinhardtti* (C) and *P. lima* (D), microalgae exposed to toxic concentrations of a mixture of transition metals.

The area corresponding to the rectangle indicated on the optical microscope image (Fig.3-A) was scanned in high-resolution mode. A 2D sweep scan was performed using 500 nm scanning step size and 0.55 s exposure per point. The elements of interest, zinc (Fig.3-E) and copper (Fig.3-F), are clearly accumulated within the exposed algae after 24-72 hours exposure time. Zn is homogeneously distributed, in contrast to Cu that is mainly accumulated at the exterior of *P. lima*. Copper is also present in areas that so far cannot be explained (possible leaching effects). The phosphorus (Fig.3-B), calcium (Fig.3-C) and iron (Fig.3-D) distributions give a good indication of the overall algae distribution. However, the phosphorus distribution indicates the presence of overlapping species of *C. reinhardtii* on top of *P. lima*.

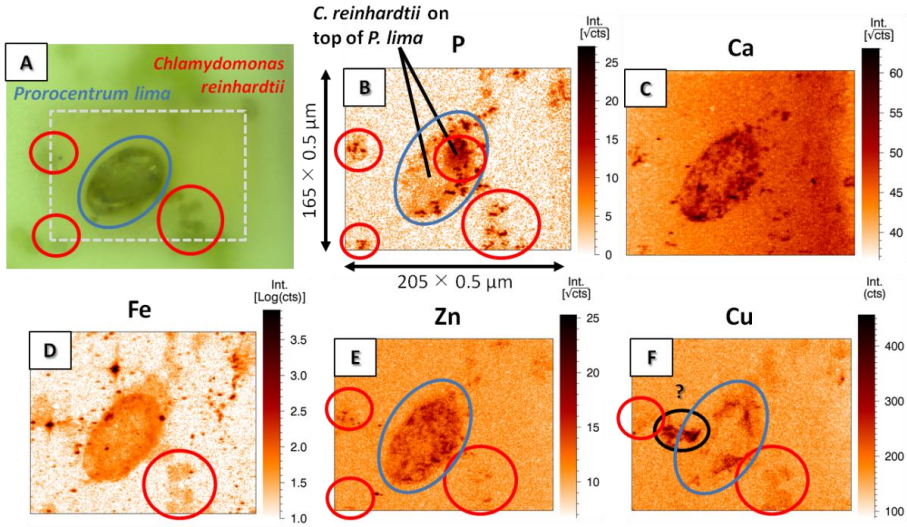

Figure 3: Optical image of the scanned sample area, blue circle indicates a *P. Lima* algae, red circles indicate *C. reinhardtii* (A). Phosphorus distribution corresponding to the scanned area, sample pile-up is indicated (B). Corresponding Ca (C) and Fe (D) elemental maps. Elements of interest Zn (E) and Cu (F) are clearly accumulated. Note: colour bars are differently scaled. Experimental details: 2D sweep scan, 205 (0.5 µm) × 165 (0.5 µm), 0.55 s/point, 10.5 keV.

The cryochamber proved to be a valuable sample environment for the analysis of microalgae under cryogenic conditions, having the potential to enhance significantly ecotoxicological research related to exposure effects of relevant model-species to transition metals at toxic concentrations [4]. In future experiments, cell pile-up should be avoided by working with lower sample concentrations and by investigating both algae separately. Quantification will be performed off-line based on a suitable standard reference material (NIST 1577c – Bovine Liver, NIST 1575a - Pine needle).

References

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