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

Eva VERGUCHT1, Walter H. SCHROEDER2, Jan GARREVOET3, Björn DE SAMBER1, Michiel VANDEGEHUCHTE1, Ulrike BOESENBERG2, Philip ALRAUN², Mateusz CZYZYCK2, Matthias ALFELD2, Thorsten CLAUSSEN3, Colin JANSSEN4, Gerald FALKENBERG2 and Laszlo VINCZE1.

1. X-ray Microscopy and Imaging Group (XMI), Ghent University, Krijgslaan 281 (S12), B-9000 Ghent, Belgium
2. Deutsches Elektronen-Synchrotron (DESY), Notkestraße 85, D-22607 Hamburg, Germany
3. Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Jozef Plateaustraat 22, B-9000 Ghent, Belgium

Abstract

Up to now, Synchrotron Radiation X-ray Fluorescence (SR XRF) imaging on cryogenically fixed biological organisms using a cryo-stream was limited to analysis with microscopic resolution due to vibration effects induced by the applied gas flow [1]. To circumvent this problem, a new state-of-the-art cryochamber is currently being developed at the P06 X-ray Micro/Nanoprobe at PETRA III (DESY), a 3rd generation synchrotron located in Hamburg (Germany). Due to the cryochamber design for static cooling and its vacuum environment, XRF nano-imaging analysis of frozen hydrated microscopic samples can be performed at the sub-micron resolution level. In December 2013, microalgae were exposed to toxic concentration levels of Zn and Cu. The exposed samples were accumulated on cellulose acetate filter paper, shock-frozen and subsequently subjected to SR nano-XRF elemental analysis using the cryochamber.

State-of-the-art Cryochamber

- State-of-the-art instrument developed by Walter H. Schroeder.
- Analysis of frozen hydrated biological samples:
  - Samples are shock-frozen by plunge freezing to obtain a frozen hydrated state.
  - Frozen hydrated state = closest to the natural state, all the sample water content remains present.
  - Obtain vitrified ice (below -137°C) by rapid cooling, avoid slow cooling below 0°C.

Sample Preparation Procedure

Sample accumulation on cellulose acetate filter paper:

A. A mixture of both exposed microalgae was accumulated on cellulose acetate filter paper by mild suction. Part of the filter paper was cut and positioned into a clean Cu holder.

Obtaining shock-frozen samples by plunge freezing procedure:

B. Liquefied propane is obtained by bringing propane gas in contact with a low T surface. The temperature should be below the melting point of propane (-187.7°C), the latter is achieved by cooling the surface with LN2 (-196°C). Polystyrene boxes are used for insulation purposes.
C. Take a sample using tweezers and put it quickly in the liquid propane, keep it there for 2 to 3 seconds. Withdraw the sample from the propane and remove the remaining propane by tapping the tweezers.
D. Put the sample quickly in the LN2 surrounding the propane reservoir.
E. Transfer the sample to a second polystyrene box filled with LN2.
F. Put the sample on the cold platform in the 2nd polystyrene box, this platform is used for temporary sample storage. Later, the frozen hydrated samples are stored in small plastic boxes (G) and positioned into a large LN2 dewar for long term storage.

sample area:

Vacuum maintenance
1 Turbo pump for vacuum operation (10⁻⁶ mbar at ambient T, 10⁻⁵ mbar at cryogenic conditions)
2 Rapid cooling system with LN2 through-flow
3 LN2 dewar for low T maintenance
4 Finger cameras for sample observation
5 Position of the frozen hydrated biological sample
6 XRF detector (Silicon Drift Vortex detector)

References


Results and Conclusions

Microscopic images

- 2D sweep scan
- Incident energy: 10.5 keV
- Scan dimensions: 205 (0.5 µm) × 165 (0.5 µm)
- Exposure time: 0.55 s/pixel

Elemental distributions

C. reinhardtii on top of P. lima
- Prorocentrum lima
- Chlamydomonas reinhardtii
- Prorocentrum lima

Conclusions and future prospects

- The cryochamber is a valuable sample environment for the analysis of microalgae under cryogenic conditions.
- Zn and Cu are clearly accumulated within the exposed algae. Zn is homogeneously distributed, in contrast to Cu that is mainly accumulated at the borders of P. lima. Copper is also present in areas that so far cannot be explained (leaching?).
- The P, Ca and Fe distributions give a good indication of the overall algae distribution. However, the P distribution indicates sample pile-up of C. reinhardtii on top of P. lima.
- In future experiments, sample pile-up should be avoided by working with lower sample concentrations or by investigating both algae separately.

Corresponding author

* Eva Vergucht, X-ray Microscopy and Imaging Group (XMI), Department of Analytical Chemistry, Ghent University, Krijgslaan 281 (S12), B-9000 Ghent, Belgium. Eva.Vergucht@UGent.be

*1 Eva Vergucht, X-ray Microscopy and Imaging Group (XMI), Ghent University, Krijgslaan 281 (S12), B-9000 Ghent, Belgium. Eva.Vergucht@UGent.be

Figures:

- Sample area:
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  - Rapid cooling system with LN2 through-flow
  - LN2 dewar for low T maintenance
  - Finger cameras for sample observation
  - Position of the frozen hydrated biological sample
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- Elemental distributions:
  - C. reinhardtii on top of P. lima
  - Prorocentrum lima
  - Chlamydomonas reinhardtii

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- Corresponding author:
  - Eva Vergucht, X-ray Microscopy and Imaging Group (XMI), Department of Analytical Chemistry, Ghent University, Krijgslaan 281 (S12), B-9000 Ghent, Belgium. Eva.Vergucht@UGent.be