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A cryogenic sample environment for the analysis of biological tissue: status 2012

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The cryogenic sample environment designed by Schroeder and Stamer in 2011 is the major instrument in the complete cryogenic workflow from tissue sample preparation to x-ray microscopy at the beamline PO6. It is based on a previously established analogue cryogenic workflow connecting sample preparation with SEM (scanning electron microscopy) and TOF SIMS (time of flight secondary ion mass spectrometry)[1,2].

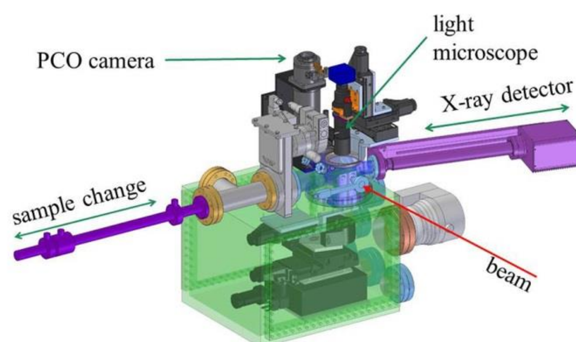


Figure 1 Schematic view of the cryogenic sample environment

The focus in 2012 was the assembly and testing of the major components. The components of the current cryogenic environment are 1) a two compartment vacuum system 2) the cooling system 3) an x-ray fluorescence detector for fluorescence tomography, the PCO camera for absorption and phase contrast tomography and 4) sample stages. Further components like a zoom-microscope for visual light observation (co-linear with the beam and top view), macroscopic TV cameras, multi-colour illumination are included in the design and are currently added. A shuttle system for loading and unloading samples at cryogenic conditions shall complete the system at a later stage.

1) **The vacuum system** was tested positive at ambient temperature with 10^{-6} mbar and at the cryogenic operating conditions at $3*10^{-8}$ mbar. This exceeds the required condition, especially at low temperature when cold internal surfaces support the pumping system, acting as cryogenic pumps.

2) **The cooling system** obtained a lowest temperature of -150°C at the sample holder. The time required for cooling from ambient temperature was ca. 5 hours. The thermal insulation and internal heat capacity of the system was large enough to sustain a useful working temperature of below -120°C for at least 6 hours without re-cooling (thus avoiding associated mechanical vibrations). The lowest temperature reached and the long-term temperature stability exceeds the original target specifications and allows long analytical runs. However, this comes in turn with the cost of long times for cool-down and warm-up. Therefore a supplementary quick cooling (and warming) system just for the initial cooling phase is currently added. This will be beneficial for installation and removal of the facility between user experiments, as well as in the event of system failure, that may require correction at room temperature.

3) An **ultrathin-window x-ray fluorescence detector** was selected that should be able to detect x-ray lines of low order number elements down to phosphorus. Of course the self-absorption within even thin samples would drastically reduce the signal of the lowest energy x-rays. Our tests show excellent sensitivity, as shown below.

4) The **long term mechanical precision and obtainable imaging quality** was tested in 3D full field tomography and element mapping by x-ray fluorescence tomography.

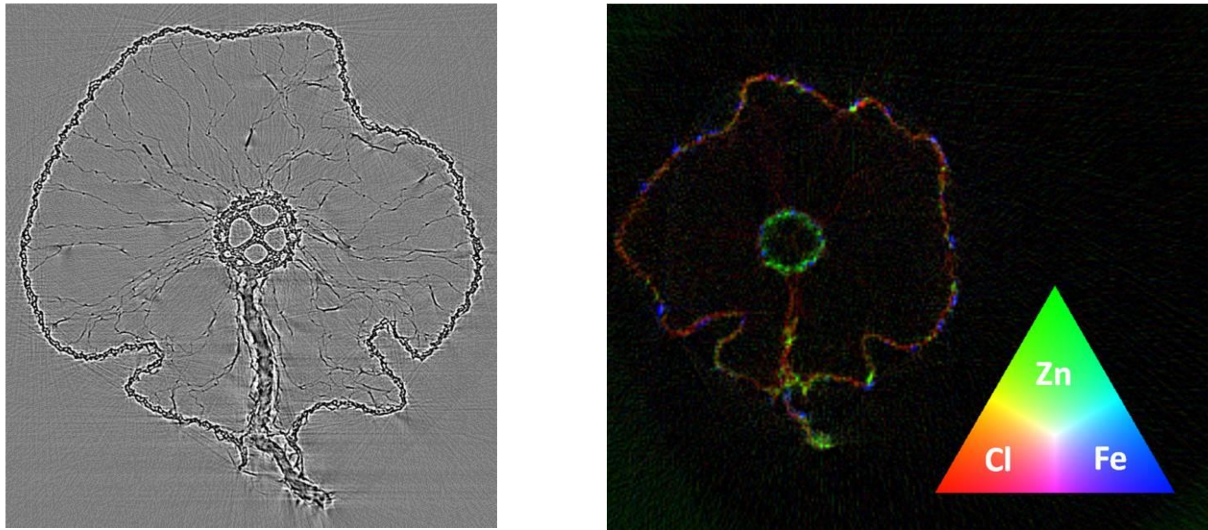
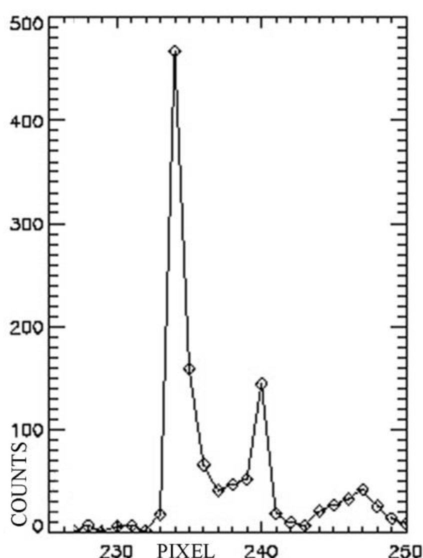


Figure 2 Single slice reconstruction of a rice root (a) and a 2D map of selected elements (b)

The test sample shown in Fig. 2 is part of a rice root with a diameter of ca. 500µm from hydroponic culture. The sample was shock frozen and subsequently freeze-dried. Due to the on-going optimization process during these initial measurements, tests were run at ambient temperature. The sample was chosen as a very stringent test for element detection and "worst case" for x-ray fluorescence tomography, since the selected sample is very sensitive and contains typically low amounts of inorganic nutrients with large air spaces (for aeration in the submerged root) and small cell walls and cell types. The phase contrast in full field tomography (Fig. 2a) seems excellent and the physiological relevant major nutrient elements could be well detected by x-ray fluorescence and mapped in reconstruction. At an detector sampling time of 100ms steps the 2D element map (Fig. 2b) shows as an example good sensitivity for Cl, a major constituent but also useful maps for Fe and Zn, typically present at low concentrations or only trace amounts in bulk samples.



The lateral resolution in the reconstructed element maps was close to the voxel size of 3µm, used in the data recording. A line scan obtained from a single iron deposit detectable in Figure 2b demonstrated a steep rise in signal between neighbouring pixels. At a good dynamic range the lateral resolution was apparently not limited by the cryogenic setup and can therefore be improved, once the overhead of the data collection is reduced and more data points with smaller voxels can be recorded. In addition, further tests shall be performed to demonstrate the limits of the technique at cryogenic conditions.

References

[1] Ralf Metzner, Heike Ursula Schneider, Uwe Breuer, and Walter Heinz Schroeder. Breakthrough Technologies, Plant Physiology, August 2008, Vol. 147, pp. 1774–1787