Development of a cryogenic sample environment for the analysis of biological tissue

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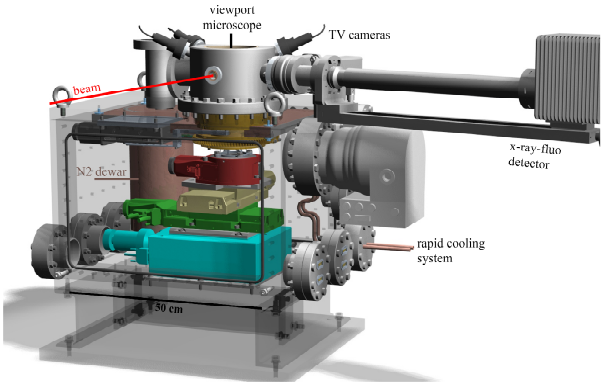
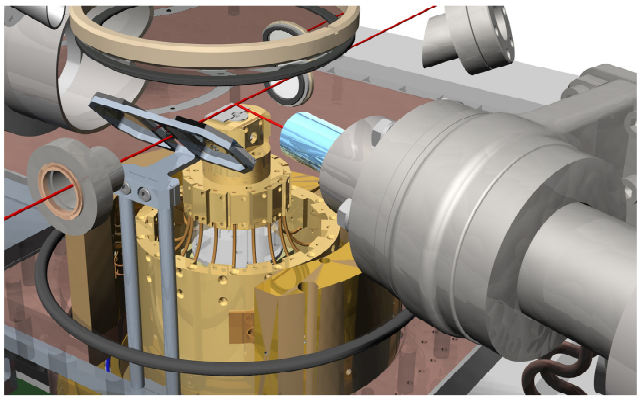
We are developing a complete cryogenic workflow, consisting of **shock freezing**, cryogenic **transfer** into **vacuum** and sample environment for **CRYO- X-ray microscopy** of **frozen hydrated tissue**. Here we report **first test data** to characterize our cryogenic sample environment that will be the major instrument for low temperature analytical x-ray microscopy at the PETRA III beamline PO6. Currently we can obtain 3D absorption and phase contrast computed tomography images, as well as 2D element mappings, „virtual cross sections" of selected image planes obtained by 2D X-ray fluorescence computed tomography, as well as surface scans.



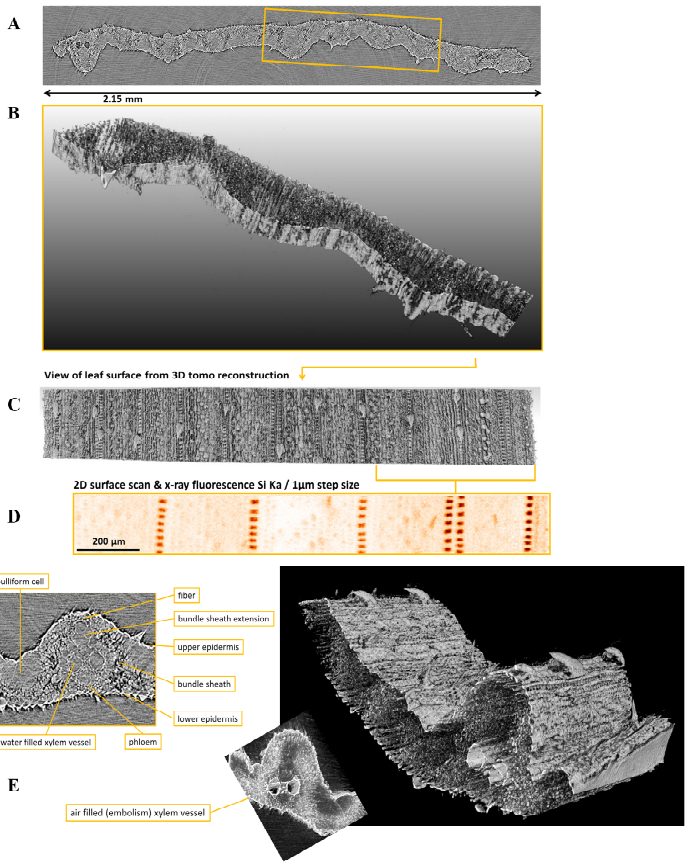
The cryogenic environment consists of a two compartment vacuum system, two cooling systems, an X-ray fluorescence detector with ultrathin polymer window for e.g. fluorescence tomography, a PCO camera for full field absorption and phase contrast tomography, sample stages as well as components like a zoom-microscope for visual light observation (co-linear with the beam and top view), and TV cameras. A shuttle system for cryogenic sample loading in vacuum is currently being added.

The vacuum system: Typical pressure ranges are at ambient temperature 10^{-6} mbar, at cryogenic operation $3*10^{-8}$ mbar. **Two cooling systems** are installed. A rapid cooling system with liquid N_2 through-flow is intended for initial rapid cooling. During operation cooling is maintained by an internal dewar and a Cu metal block of large heat capacity. This allows low vibration cooling of the sample environment and a cryo-shield (blanked out below) at temperatures between -120°C and -150°C. A useful working temperature of below -120°C can be sustained for at least 6 hours without refilling.

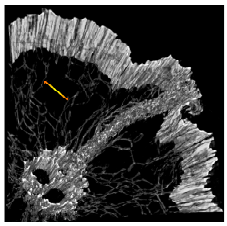


To minimize interfering mechanical stress on the sample holder, especially during movements, it is mechanically decoupled from the cold reservoir. The sample holder is cooled by a circular array of highly flexible Cu wires between the sample stage and an outer cold cylinder that moves in synchrony with the sample rotation stage in order to keep the strings slack. The cooling cylinder itself is cooled by spring loaded sliding connectors attached to the cold reservoir.

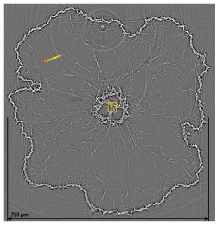
Test sample: Shock frozen rice leaf. A 3D absorption/phase contrast **CRYO-CT** was recorded from a leaf mounted free standing at 12 keV with 0.3° steps 2s exposures within 100 minutes. The top image **(A)** shows a virtual cross section of the leaf, obtained from a single slice reconstruction. A selected 3D volume obtained by cropping a complete 3D volume model of the indicated area is shown in **(B)**. The alignment of the 3D volume model to the leaf surface allows a more detailed view of leaf surface structures **(C)**. Scanning the leaf surface using a focused beam of 0.8µm, $<10^{10}$ photons/s and recording the X-ray fluorescence allowed us to obtain 2D element maps . The map of Si, primarily present in lines of Si enforced „saw-teeth", presumably a defense against herbivores, is shown as an example **(D).** See some details in **(E)**

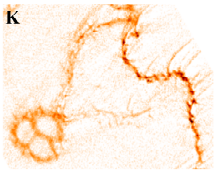


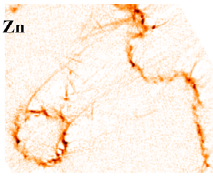
Test sample: Shock frozen freeze dried root of rice.

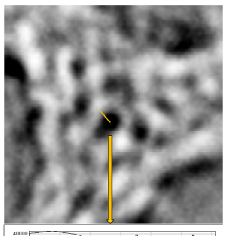


A 3D **Cryo-CT** was recorded at 12 keV from a fine root, mounted vertically, free standing. A clipped quadrant from a 3D reconstruction showing the emergence of a side root is depicted left. A „virtual cross section", obtained from a single slice reconstruction can be seen right. The very fragile sample was selected as a worst case object. Cortex cell walls are very thin (⟵) and element content is very low due to hydroponic growth. The indicated region of interest is magnified right to visualize small structures that are suitable for an estimate of spatial resolution by a line scan across the indicated edge.
For this CT rotational steps of 0.1° were used for recording images by the PCO camera at 175 ms exposure.









CRYO-2D x-ray fluorescence tomography reveals element maps in virtual sections (above left). Maps of two elements (quadrants of total maps), K and Zn, are shown. Potassium is the major cation in plant cells and Zn is a minor nutrient present in trace amounts in bulk samples. X-ray spectra were recorded at 0.2° steps of rotation with the transverse-axis in sweep mode at 70 ms / virtual step. (Artefacts may be due to a trivial motor problem.)

Conclusion.

We demonstrate a first preliminary application of the cryogenic sample environment to correlate 3D structure with 2D element maps of biological tissue samples. The desk top size, relatively low cost instrument is part of a complete uninterrupted cryogenic workflow from sample preparation including shock freezing to x-ray microscopy. Operation at high vacuum and low temperature appeared stable for several days. Tissue samples may be as large as 2 mm on the long axis. The spatial resolution is limited by sample size and beamtime, but below 1mm size a resolution in the µm range was obtained. Sensitivity for element mapping is high, however limited by self-absorption. Resolution and sensitivity may possibly be further improved, but are always limited by a number of factors, including sample preparation, sample size, beamtime and beam damage.
Next challenges are a pile of technical bugs, major improvements in data handling and processing as well as first applications and the transformation from demonstrator into user friendly operation.