# High-resolution X-ray Imaging of Frozen Hydrated Samples in Amplitude and Phase Contrast

G. Schneider, B. Niemann, P. Guttmann, and G. Schmahl
*Forschungseinrichtung Roentgenphysik, Georg-August-Universitaet Goettingen, Goettingen, Germany*

X-ray microscopy provides higher resolution than optical microscopy and higher penetration power than electron microscopy. Therefore, x-ray microscopy allows high-resolution imaging of thick hydrated samples. The two dominating processes determining the contrast in x-ray microscopy are photoelectric absorption and phase shift. For this reason x-ray microscopy can be performed in amplitude and phase contrast. The Goettingen x-ray microscope at the BESSY electron storage ring in Berlin is operating in both contrast modes and is used for different application fields, for example in biology, biophysics, medicine, colloid chemistry, and soil sciences. Biological objects are especially sensitive to ionizing radiation. Theoretical investigations show that x-ray images of frozen hydrated specimens can be obtained without radiation-induced artifacts. Therefore, an object stage for cryogenic specimens was developed and implemented on the Goettingen transmission x-ray microscope (TXM) at the electron storage ring BESSY. It allows objects to be imaged at temperatures below 120 K in cryogenic nitrogen gas at atmospheric pressure. This system was used to perform experiments with initially living biological objects, e.g., cells, chromosomes and algae. Amplitude and phase contrast x-ray images show details inside the frozen hydrated objects as small as 30 nm with high contrast and the cryogenic samples show no structural changes. The preparation of the samples was accomplished by shock freezing in liquid ethane to cryogenic temperatures. Furthermore, it was demonstrated that at cryogenic temperatures the structural stability of hydrated biological objects is increased by about four orders of magnitude compared to unfixed wet specimens at room temperature. It is now apparent that multiple imaging of a frozen hydrated sample done at different viewing angles will reveal images of the three-dimensional morphology, e.g., of whole cells, which are close to the natural, functional state.