Cryo-CT Observation using a New Cryogenic System at a Soft X-ray Microscope

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The full field imaging soft X-ray microscopy system that was installed at the SR center in Ritsumeikan University (BL-12) uses soft X-ray region between (1.7 - 4.4 nm corresponding to a photon energy between 282 eV – 729 eV) to observe biological specimens. Three-dimensional structure and elemental distribution were observed by the microscope with computed tomography (CT) [1-3]. A sample can be put in air to observe under wet condition. The X-ray wavelength between carbon K-absorption edge (4.36 nm) and oxygen K-absorption edge (2.28 nm), which is called water window, produces a good contrast of the specimens in water. In CT observation, it is difficult to collect tilt series data set because specimens move freely in water. Hydrated electron and free radicals such as ·OH are developed by dissociation of water molecules exposed to X-rays. The hydrated sample is destroyed by the radiation damage. A cryogenic fixation is an excellent technique to prevent radiation damage [4]. In the previous work, a cryogenic system was developed to reduce radiation damage [5]. In this report, we report the improved cryogenic system and its application to observe polystyrene spheres in water.

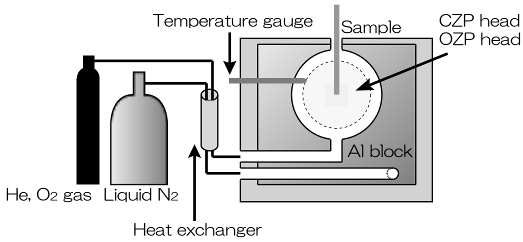

Fig. 1 Schematic of a new cryogenic system

The improved cryogenic system is shown in Fig. 1. The cryogenic system consists of a cooling Aluminum (Al) block and a cryo-gas injector. The sample stage, the tip of condenser zone plate chamber (CZP head) and the tip of objective zone plate chamber (OZP head) are covered with the Al block. A pipe coupled with the Al block supplies a cooling dry gas to the inner space of the Al block. The Al block is also cooled by liquid nitrogen, and then the sample is cooled. A flow of dry gas prevents from the frost build up inside the Al block. Helium and oxygen gases have relatively high X-ray transmittance to the range of soft X-ray wavelength, 1.7 – 4.4 nm. Two improvements were carried out. A new optional attaching tool was provided to facilitate attachment/detachment operation in installing the Al block. A pressurized supply tank was provided to introduce liquid nitrogen. The temperature in the Al block was controlled by the pressure of liquid nitrogen. A sample-cooling test was carried out using the new cryogenic system. The temperature was monitored near the sample position as shown in Fig. 1. Fig. 2 shows temperature change record from the start of cooling. The lowest temperature depends on the gas type and the pressure. The lowest temperature with helium was -160°C, whereas the lowest temperature with oxygen was -110°C. The freezing of the sample was confirmed by motion-stop of polystyrene spheres in water.

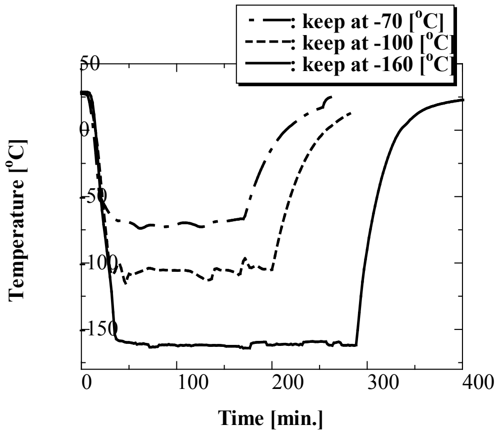

Fig. 2 Temperature change during cooling. The lowest temperatures achieved were –70°C (chain), -100°C (dash) with O_2, and -160°C (solid) with He.

An imaging test was carried out using oxygen gas because a strong vibration of sample tube of unknown origin occurred with using helium gas. Aqueous solutions with polystyrene spheres were inserted into a glass capillary tube. The glass capillary tube was set onto the sample stage and then the cooling was started. The temperature reached to -105°C and was kept during CT observation. A tilt series was obtained each 4° in 180°. Cross sectional images were reconstructed by convolution-backprojection method. A three-dimensional image was prepared by volume rendering method.

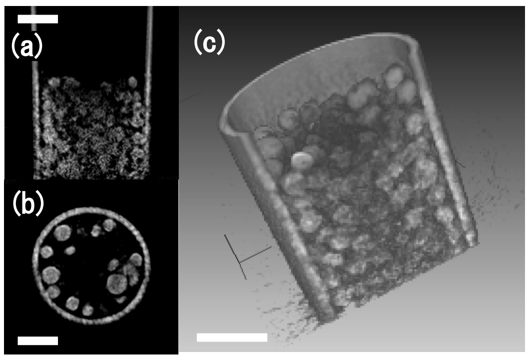

Fig. 3 Three-dimensional X-ray micrograph of polystyrene spheres in frozen water. Cross sectional images of side (a) and top (b). The volume-rendering image (c). Wavelength was 2.33 nm, Exposure time was 120 seconds, projection angle was each 4° in 180°, sample space temperature was –105 °C, the scale bar is 3 μm.

Fig. 3 shows the cross sectional image of side view (a), top view (b) and volume rendering image (c) of polystyrene spheres in water. The vibration of the capillary tube was sufficiently low to obtain tilt series data. The spherical structure of polystyrene spheres was well reproduced since the polystyrene spheres fixed by cryogenic fixation during exposure.

We improved a cryogenic system to cool down samples. The cryogenic setup provides quick and simple cryogenic X-ray observations of the samples. Using this system, a three-dimensional observation of polystyrene sphere was successfully done. We will try to obtain a three-dimensional elemental mapping of a specimen in water using X-ray absorption edge.

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