Improvement of cryogenic 3-dimensional observation system of soft x-ray microscope at the SR center of Ritsumeikan University

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Abstract. The improvements of a soft x-ray microscope beamline (BL-12) at the SR center of Ritsumeikan University are reported. A wedge-shaped slit and Si plane mirror were newly introduced. The better energy resolution was expected and the +2nd order diffraction from the CZP (1.2 nm at 2.4 nm observation) was suppressed. A new sample holding fixture allows the sample to be replaced quickly and accurately. A new sample cooling system allowed a stable cryogenic x-ray imaging.

1. Introduction

The full-field imaging soft x-ray microscopy (XM) system was installed at BL-12 of the SR center in Ritsumeikan University (575 MeV, 300 mA) [1, 2]. In 2009, the x-ray microscope was improved to perform the nano computed tomography (nano-CT), and we reported the 3-dimensional structures of the brain cortex of the mouse in XRM2010 [3]. After the previous conference, improvements of an illumination optics and a sample holding fixture were carried out, and better nano-CT images were observed.

Cryogenic x-ray microscopy is now accepted as one of the most useful tools for the direct observation of bio-specimens because cryogenic technique promises reduction of their radiation damages. We have developed and installed a cryogenic sample chamber system to the soft XM at BL-12. The temperature of the specimens could be regulated continuously from 273 K down to 173 K. The reduction of the radiation damage was experimentally achieved and the cryogenic images of biospecimens have been taken. However, because of the thermal drift, the spatial resolution of the image was lower than that of the room temperature observation [4, 5]. In addition, the sample stages of the XM at BL-12 have been improved to perform the CT. Therefore, we also developed a new cryogenic sample chamber that improved the cryogenic system. Using this new system, we pursue the...
goal of the achievement of reducing radiation damage experimentally and the acquirement of x-ray image of biospecimens.

In this paper, we report the overview of the illumination optics, the sample holding fixture, and the new cryogenic system.

2. Improvement of illumination optics

The schematic of the full-field imaging soft XM is shown in Figure 1. In the full-field imaging soft XM with two Fresnel zone plate optics, the combination of a plane mirror, a condenser zone plate (CZP) and a pinhole work not only as an illumination to the sample but also as a linear monochromator [1,2]. The linear monochromator uses difference of focal lengths by chromatic aberration of the CZP. Then, the plane mirror works to suppress the higher diffraction from the CZP, which has the same focal length with the main wavelength. The performance of this optics essentially determines the contrast and the spatial resolution of the image. However, in our XM, the CZP produces +2nd order diffraction of half wavelength of the target wavelength because of its figure error [6]. Illumination optics has been improved to suppress the +2nd order diffraction in order to obtain proper energy resolution for better imaging.

The XM was designed to use the wavelength of the water window region (2.3-4.4 nm) mainly. For the improvement of the performance of the illumination optics in this region, especially the wavelength of 2.4 nm, just below the K-edge of oxygen, a wedge-shaped slit and a silicon (Si) plane mirror were installed. The energy resolution of the linear monochromator is determined by the size of the CZP and the pinhole approximately. Then, the demagnetized light source image by the CZP should be smaller than the size of the pinhole for the expected monochromatic performance [7]. The slit, whose horizontal size varies from 0.37 to 1.9 mm, set downstream the light source was used as a virtual source and reduces the horizontal size of the light source. The energy resolution, \( E/\Delta E \), of 300 was expected by using the pinhole of 15 \( \mu m \) diameter.

On the other hand, the Si plane mirror was installed to suppress the +2nd order diffraction from the CZP, which was the wavelength of 1.2 nm in use of 2.4 nm. The cut-off wavelength changes from 1.0 to 1.2 nm by changing the current silicon carbide (SiC) mirror to the Si one. The ratios of +2nd order reflectance to +1st order one is expected to decrease from 0.16 to 0.05 (32%) at the wavelength of 2.4 nm [8].

![Figure 1. Schematic of the full-field imaging soft XM. The wedge-shaped slit set downstream the light source as a virtual source. Si and SiC plane mirrors are installed. The CZP is a Göttingen KZP 7 type (diameter: 9 mm, outermost zone width: 53.7 nm, number of the zones: 41,890) [9]. The pinhole diameter is 20 \( \mu m \). The objective ZP (OZP) was fabricated in NTT-AT (diameter: 84 \( \mu m \), outermost zone width: 38 nm, number of the zones: 550). The images are detected by a back-illuminated CCD camera (C4880-21-24WD, Hamamatsu K.K.).](image-url)
disciform Neodymium magnet and three steel balls are attached on a backside of the base plate of the head. Three receptacle dimples are formed on the sample stage stainless steel plate (SUS403). Positioning is accomplished by the steel balls and dimples, and rigid fixing is accomplished by the magnet and stainless steel plate. The quick-release fixture allows the sample to be replaced quickly and accurately. The head is also used for wet sample which is contained in glass capillary tube. When 2-dimensional imaging is performed, the head is changed for a dedicated head for it.

Figure 2. (A) XM sample stage. (B) Sample holding fixture head for CT and wet sample.

3. New cryogenic system
We developed a new cryogenic sample chamber that improved the sample cooling system (SCS). The schematic image and the photo of the SCS are shown in Figure 3. The SCS consists of a sample chamber made of aluminum block and Dewar vessel. Liquid nitrogen stored in the Dewar vessel flows into a metal tube in the aluminum block. In order to avoid frost forming, the sample is mounted in cryogenic oxygen gas environment. The oxygen gas flow rate is about 0.15L/min. The sample chamber is encompassed high heat insulating material (STYRO FORM™, EK-2, The Dow Chemical Co., the thermal conductivity coefficient: 0.028 W/m⋅k). The temperature stability was improved by introducing non-contact cooling system with the use of liquid nitrogen and cryogenic oxygen gas. The lowest sample chamber temperature reached -170 °C. X-ray micrographs of polystyrene (PS) latex spheres with x-ray irradiation at room temperature and at cryogenic temperature are shown in Figure 4. Alleviation of radiation damage in PS latex spheres at cryogenic temperatures was verified.

Figure 3. Schematic diagram (A) and photograph (B) of cryogenic sample chamber.

Figure 4. X-ray micrographs of PS latex spheres suspension. (A) was taken at room temperature and (B) was at -160 °C.

4. X-ray images
X-ray images are shown in Figure 5. (A) is air-dried Scenedesmus and (B) is air-dried Skeletonema potamos. Both images were taken at 2.33 nm wavelength with exposure time of 120 s. The imaging fields are quite good and image contrasts are also good.

Figure 6 presents an x-ray integrated image of cryogenic S. potamos. A sealed glass capillary tube 10 µm in diameter containing the laboratory-cultured S. potamos was mounted on sample holding
fixture head. During exposure time, each cell maintains its position. CT imaging that uses this system is scheduled to be carried out soon.

5. Conclusion
We have improved an illumination optics, sample holding fixture, and new cryogenic system of the soft x-ray microscope beamline (BL-12) at the SR center of Ritsumeikan University. The wedge-shaped slit and the Si plane mirror were newly introduced. The energy resolution, $E/\Delta E$, of 300 was expected and the $+2$nd order diffraction from the CZP which was the wavelength of 1.2 nm in use of 2.4 nm was suppressed. The new sample holding fixture allows the sample to be replaced quickly and accurately. The new sample cooling system allowed a stable cryogenic x-ray imaging, and alleviation of radiation damage at cryogenic temperatures was also verified.

Figure 5. X-ray micrographs of phytoplanktons. (A) *Scenedesmus* and (B) *S. potamos*. Wavelength: 2.0 nm, exp. time: 120 s, air-dried, room temperature.

Figure 6. X-ray micrograph of *S. potamos*. Wavelength: 2.33 nm, exp. time: 12 s, wet, cryo-condition. This is an integrated image of three microphotographs.

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