Development of Cryogenic Sample Chamber in Soft X-Ray Microscope at BL-12

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Abstract

In order to reduce radiation damage in observing biological specimens, new cryogenic sample stage for soft X-ray microscope BL12 was designed. The system was designed to image hydrated biological specimen at temperature around 110 K. The cryostat allowed the specimen to be treated both by cooling in nitrogen vapor and by cooling with heat conduction through the liquid nitrogen. Using this system, the temperature of the sample stage was kept below 113K for 50 min.

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1. Introduction

Soft X-ray microscope at the beam-line BL-12 is a unique tool for observing bio-specimens and polymer specimens. Observable wavelength range is set at "water-window" and the spatial resolution has reached as small as 70 nm [1, 2]. The microscope has been improved; a pre-observing optical microscopy system [3], an automatic wavelength scanning system [3, 4], an automatic sample scanning system, a new CCD camera [3], and a new condenser zone plate [1]. However, one of the most serious problems, the radiation damage problem, has remained. The radiation damage problem is a limiting factor in imaging of living biological specimens at high resolution. Basic considerations of image contrast indicate that doses of $\sim 10^6$ Gy are involved in 50 nm resolution imaging with soft X-rays [5]. These doses are sufficient to cause immediate changes in living cells. As a result, it produces noticeable mass loss and shrinkage in some specimens. It is well known that cryo-methods can greatly reduce radiation damage in biological specimens. Cryo-X-ray microscopy experiments at 113K have shown essentially no observable mass loss at the 50nm spatial resolution level with radiation doses up to $\sim 10^{10}$ Gy [6]. Thus cryo-X-ray microscopy experiments system is necessary to observe biological specimens. To improve the stability especially of hydrated biological specimens, cryogenic sample chamber has been developed and tested. In this article the design concept of cryogenic sample chamber and the results of cooling test are presented.

2. New Cryogenic Sample Chamber

In order to simplify the handling and cooling effectively, a cooling system was designed [4]. The cryo-system is based on the X-ray beamline at BESY [7]. Design concept of the new cryogenic sample chamber is as follows: 1) The new cooling equipment is designed to maintain temperatures around 110 K for 30 min without frost formation. 2) The equipment is able to adapt to X-ray microscope at BL-12.

Figure 1 shows a schematic diagram of the new cryogenic sample chamber system. The cryogenic sample chamber system consists of liquid nitrogen (LN2) dewar vessel with a pumping system and a sample stage. A metal tray is put at the bottom of the sample stage. The dewar vessel and the metal tray are connected by a silicon tube directly and, LN2 flows from the dewar vessel to the metal tray. The LN2 flow rate is controlled by a temperature sensor set on a sample mounting position. The sample stage is cooled in nitrogen vapor and by heat conduction toward the cold metal wall, and keeps samples cryogenic temperatures. Limitation of a setting temperature is 77 K. To avoid frost from forming, the sample stage is enclosed in a thermally insulation

cryo-house. The cryo-house obtains two gates for inserting CZP and OZP heads. Each nipple to which the head is attached is equipped with an airlock flange. These airlock flanges are attached to the cryo-house by magnetic power, and wet air is shut out from cryogenic sample chamber.

Several improvements for cryogenic application were also done at the X-ray microscope. New CZP and OZP heads made from Macor. Its thermal conductivity is more than ten times higher than that of stainless steel which is conventional material for vacuum equipment. Viton O-ring was exchanged to silicone O-ring. Torr seal (Varian) was also exchanged to CAF4 silicone sealant (Rockgate).

3. Cryogenic Experiment

The system adapted to a test chamber with the same sample stage. The test chamber is enclosed a same type cryo-house. Experimental cryogenic test chamber is shown in Fig.2. Results of the cooling test are shown in Fig.3. Side axis represents temperatures of sample position and bottom axis represents measurement times. Owing to take advantage of effective cooling, 2 liter LN2 is used for preliminary cooling. The temperature of the sample stage reached below 273 K with 30 min preliminary cooling. The cryogenic test experiment started after the preliminary cooling. The setting temperature was 77 K. During 23 – 46 min, the temperature is steady about 180 K. LN2 supply increased so as to decrease the temperature. After the rapid drop at 46min, the temperature is almost steady around at 110 K. It is reported that the required temperature to reduce the radiation damage of the living cell is enough at 113 K [6]. In this experiment, the cryo-condition contained for 50 min without frost.

4. Summary

The X-ray microscope at BL12 has been expanded to allow imaging of bio-specimens at cryogenic temperatures. New cryogenic system was designed. In this system, the sample stage is cooled in nitrogen vapor and by heat conduction toward the cold metal wall, and keeps the sample in cryogenic condition. With the experimental cryogenic test device, direct measurement of sample position temperatures were performed. The temperatures were kept below 113 K for 50 min. At the next beam time, we plan to adapt it to the X-ray microscope at BL-12 and observe living cells.

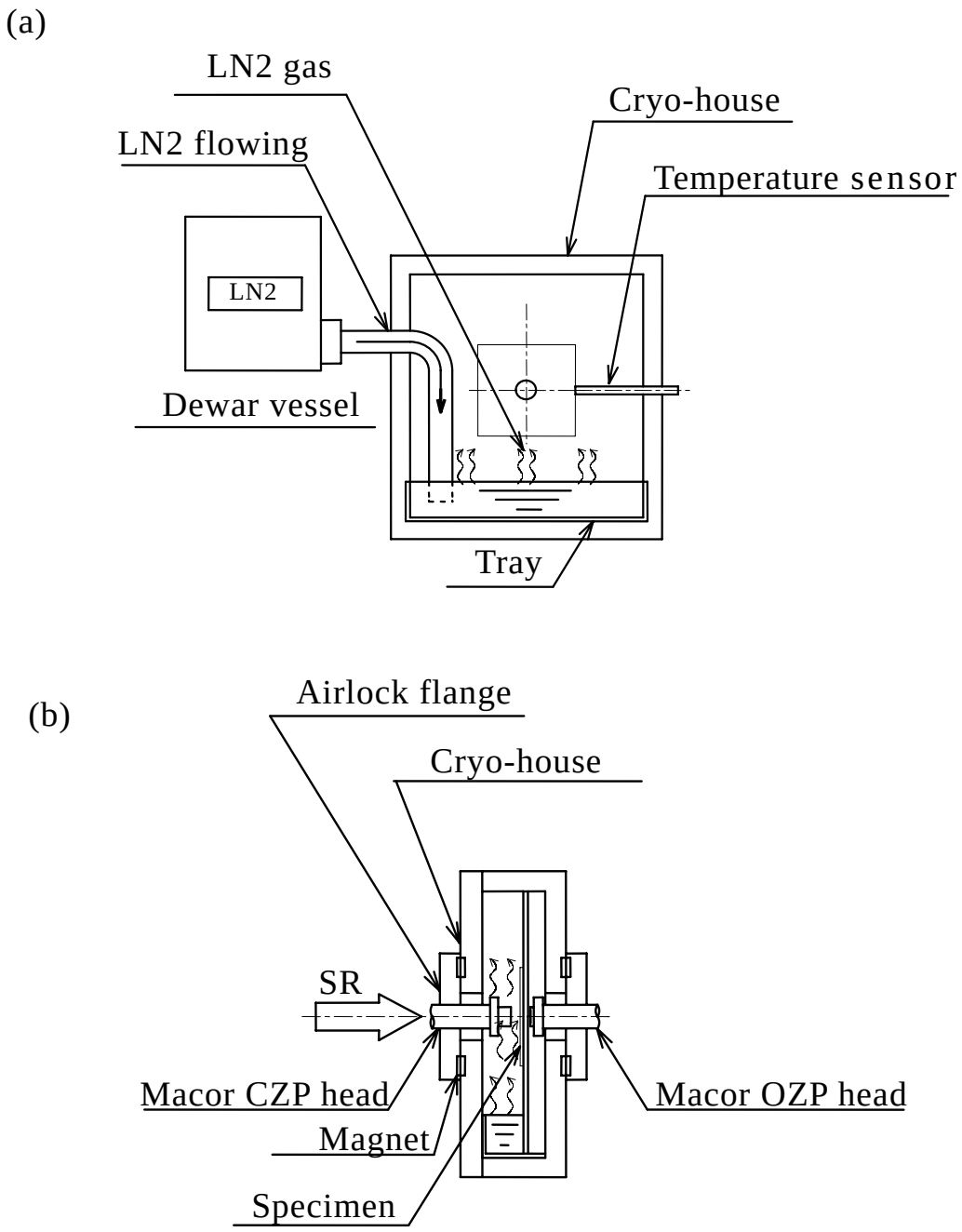

Fig. 1 Schematic diagram of the cryogenic chamber system adapted to the X-ray microscope. (a) Front section view. The X-rays impinge perpendicular to the plane of the figure. (b) Side section view. The sample is mounted on a LN2-cooled sample holder. The sample stage is enclosed in a thermally insulation cryo-house. Using magnetic power, airlock flanges are attached to the cryo-house.

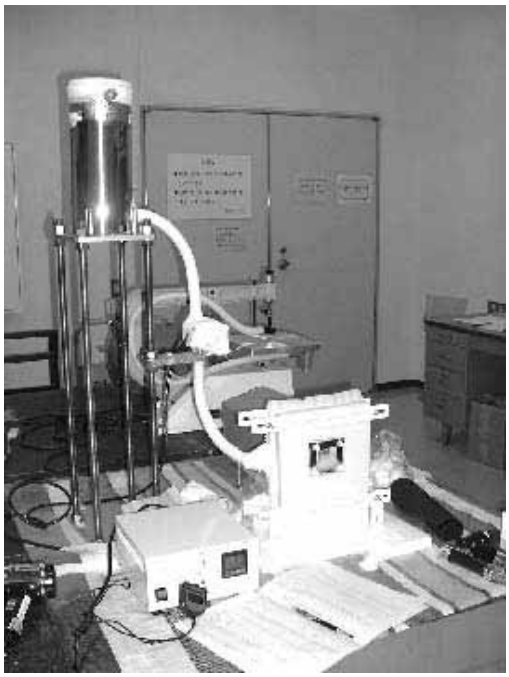

Fig. 2 Experimental cryogenic test chamber.

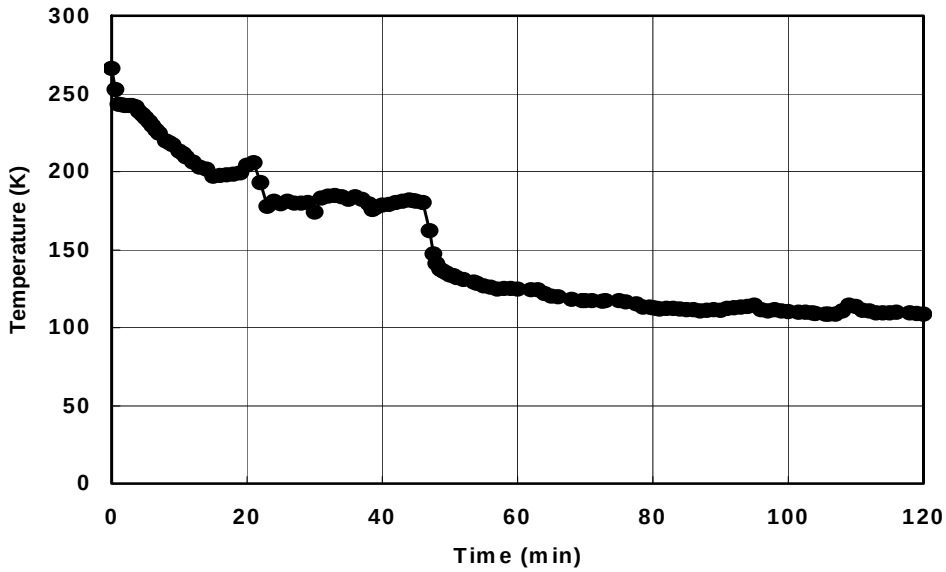

Fig. 3 Cooling test of the experimental cryogenic test chamber. Temperature: 300 K, setting temperature: 77 K, humidity: 40 %(AC), weather: fine.

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