Development of Cryogenic System for Soft X-ray Microscope

M. Kimura, K. Takemoto, Y. Ohashi[1], K. Nakanishi[2], H. Namba[1], and H. Kihara

Abstract

In order to reduce radiation damage in observing biological specimens, a cryogenic sample stage for soft X-ray microscope BL12 has started. The system was designed to image hydrated biological specimen at a temperature below glass transition temperature. The cryostat allowed the specimen to be treated both by cooled in nitrogen vapor and by cooled with heat conduction through liquid nitrogen. Using this system, the temperature of the sample stage was kept below 113 K for 50 min. Based on the cooling system, the cryogenic chamber was improved. This improved cryogenic chamber is compactly and high-performance device. Cooling power, stability, and cryogenic temperature range are sufficient for observing biological and polymer specimens. After performing at a test vacuum system, the chamber was installed in BL12. Under cooling condition with the cryogenic chamber, both upper stream and lower stream heads realized non-leak and the chamber vacuum was $< 4 \times 10^{-5}$ Pa.

Department of Physics, Kansai Medical University, 18-89 Uyamahigashi, Hirakata, Osaka-pref. 573-1135, Japan.

[1]Department of Physical Science, Ritsumeikan University, 1-1-1, Noji-Higashi Kusatsu, Shiga, 525-8577, Japan

[2]SR Center, Ritsumeikan University, 1-1-1, Noji-Higashi, Kusatsu, Shiga, 525-8577, Japan

1. Introduction

Soft X-ray microscope beam line at BL-12 has been opened to researchers of university, corporation, and any laboratories (medicine, chemical and engineering etc) since 1996 [1]. In order to improve this X-ray microscope to a full automatic observation system, a new project started at 2002. As the first step in this project, an automatic multi-wavelength X-ray microscopy system has been introduced [2]. This system can be controlled by a single finger and performs multi-wavelength imaging. Now, it is used for daily observation and shows good performance [3, 4]. And as the next step, a cryogenic sample chamber was designed. Since there is serous radiation damage during observation of biological and polymer specimens at room temperature, high resolution and high quality imaging is difficult. Basic considerations of image contrast indicate that doses of ~10^6 Gy are involved in 50 nm resolution imaging with soft X-rays (Sayre *et al*.,) [5]. It is well known that cooling biological specimens till cryogenic area can greatly reduce radiation damage. Schmahl's group in the BESSY reported that to freeze algae became 1000 times strong against radiation damage [6]. Therefore the cryogenic sample chamber has been demanded to replace a sample stage of BL12. In this year, new cryogenic sample chamber was installed at BL-12. When used for low temperature work, the chamber is connected to a transfer line from a dewar that contains the cryogen for low-temperature operation at observation temperatures. In this report, details of this cryogenic system are described. Cooling power and the thermal stability of this cryogenic system are also reported.

2. Cryogenic Sample Chamber

Design concept of cryogenic sample chamber is described in the followings:

1) The cooling equipment is designed to maintain the cryo-condition for 100 minutes without frost forming.

2) The equipment is able to adapt to X-ray microscope at BL-12.

3) The equipment contains the sample holder which can be easily mounted.

In order to achieve these, the cryogenic sample chamber was improved. A new cryogenic sample chamber was designed and produced. Figure 1 is the new cryogenic sample chamber. The chamber is connected to a test vacuum system. A constitution of the new chamber is the same as that of a previous one [7]. However new chamber is more compact than previous one. In order to avoid frost forming, the sample stage is enclosed with a cryo-house. The cryo-house consists of a thermal insulation block and stainless steel (SUS) metal box. In addition, specific temperature stability is also improved. Cooling power has improved remarkably (See

Fig. 2). Cooling speed of it is three times faster than previous one, and the sample chamber is cooled quite rapidly from room temperature to 173 K in 10 minutes. In order to observe forest-forming, a gate was opened and sample stage in the chamber was observed. Figure 3 shows the inside of the chamber of 7 minutes after opening the gate. Forest free is achieved. Cryogenic temperature range is 113 K - 203 K.

When cryo-observation is performed, frozen specimens must be installed rapidly in the cryogenic sample chamber without melting. New sample holder that can be mounted and removed easily with hand was designed. Figure 4 (a) shows the new sample holder. It consists of two thin plates (thickness: 1.5 mm, diameter: 50 mm) with a center hole (diameter: 20 mm). Thin film cultivated biological specimen or polymer film is sandwiched between these two plates.

Using a handle (handy guide bracket), the sample holder is mounted on a sample stage in the cryogenic sample chamber (Fig. 4 (b)). Two little slide guide pins are on the sample holder. The sample holder and the handy guide bracket are connected with screw pins.

In addition, since the sample stage of the cryogenic chamber has two guides, the sample holder is inserted in rapidly to their terminals. After arriving at the terminal, the sample holder is locked on by turning to the right. Finally removing the screw pins, handy guide bracket is removed easily.

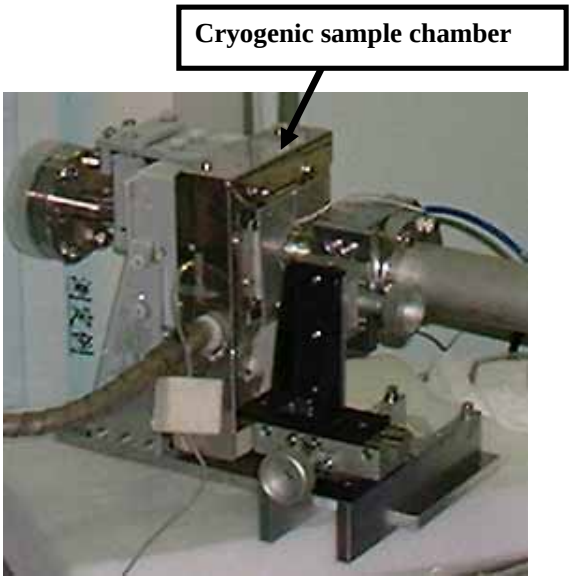


Fig. 1 Cryogenic sample chamber. It is connected to a test vacuum system. To avoid frost-forming, the sample stage is enclosed with a cryo-house. The cryo-house consists of a thermal insulation block and SUS metal box.

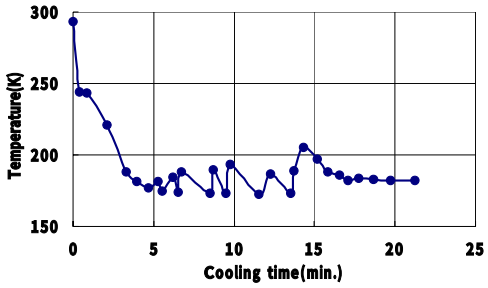


Fig. 2 Cooling test of a new cryogenic sample chamber. Setting temperature: 173 K.
The longitudinal axis represents temperature at the sample position, and the transverse axis represents cooling rate.

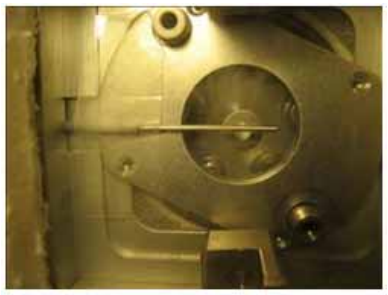
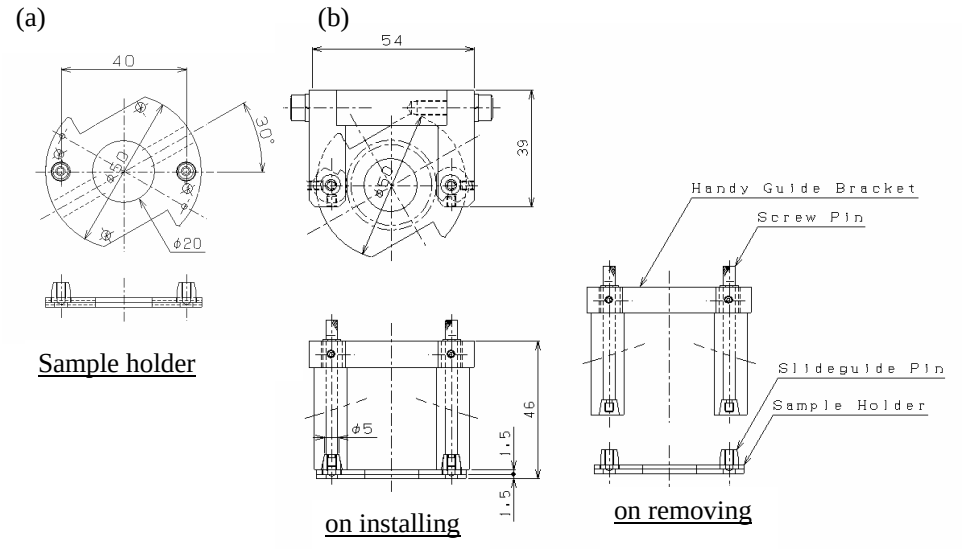


Fig. 3 Inside Photograph of the cryogenic sample chamber of 7 minutes after opening the gate. The gate was opened at 14 minutes. Frost-free is achieved. Center plate with a center hole is sample holder.



Sample holder with handy guide bracket

Fig. 4 Sample holder and handy guide bracket. (a) Sample holder, (b) sample holder handy guide bracket.

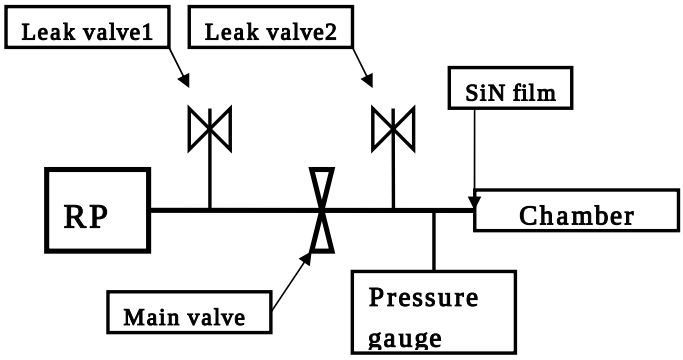
3. Cryogenic Test under Low Vacuum Condition

Before installing the cryogenic chamber to the X-ray microscope beamline, cryogenic test was performed at low vacuum condition, 4 Pa. This optical system except is mounted on an optical bench [8]. The bench is separated into two parts: one is the condenser part (CZP chamber) and the other is the imaging part (OZP chamber). The condenser part and an optical microscope are placed on the same moving stage that is set on the optical bench. They can be used alternately by means of a pneumatic cylinder. The specimen cell is mounted at atmospheric pressure. Since the Si_3N_4 films of 250×250 μm^2 and 100 nm thickness isolated shield the vacuum part from atmospheric pressure, the cryogenic stability of the Si_3N_4 film was evaluate under low vacuum condition. Under room temperature, a silicon substrate with Si_3N_4 films is attached to a device using an epoxy resin (Torr-Seal). The seal materials and the vacuum pipes were also evaluated.

3.1 Vacuum Test Chamber

Figures 5 show a schematic diagram of vacuum test chamber (a) and a test vacuum system (b). This vacuum system consists of a main valve (manual moving), two leak valves, a pressure gauge, and an oil rotary pump (RP). Main valve is installed between two leak valves. One leak valve is installed on the RP side and another is on the chamber side. The RP is used for evacuating the vacuum test chamber with Si_3N_4 film from the atmospheric pressure to 4 Pa. Since the Si_3N_4 film is thin, the vacuum operation required prudently. After starting the RP for about 1 minute, the main value was opened carefully and slowly. During this operation, the pressure of the chamber was monitoring the pressure gauge. When the pressure was achieved at 4 Pa, the main valve was closed. And cryogenic test was performed under this condition.

(a)



(b)

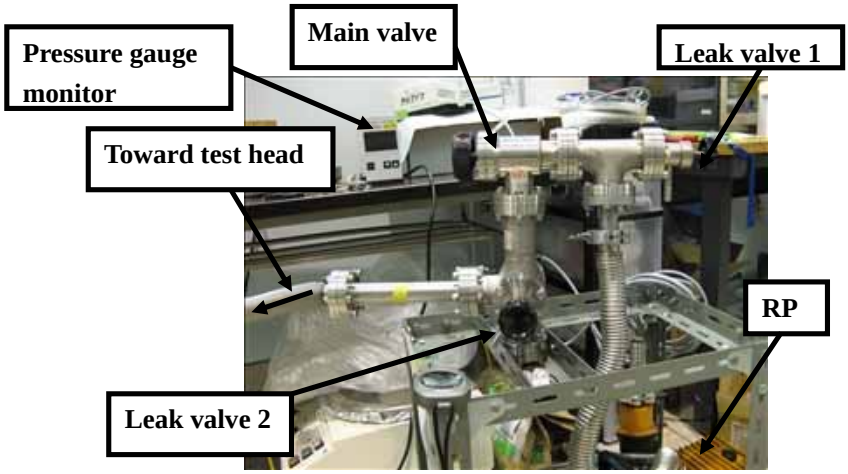


Fig. 5 (a) Schematic diagram of vacuum test chamber. RP: oil rotary pump. Chamber: vacuum test chamber with the Si_3N_4 film. Leak valve1: leak valve for RP. Leak valve2: leak valve for the chamber. (b) Photograph of vacuum system. Pressure gauge is installed behind leak valve 2.

3.2 Cryogenic Stability of Si_3N_4 film, Seal Materials and Vacuum Pipes

To evaluate the cryogenic stabilities of the Si_3N_4 film, seal materials and vacuum pipes under vacuum condition, the following testing was conducted by. For the cryogenic application, upper stream pipe (CZP head) and lower stream pipe (OZP head) made from Macor [7]. Thermal conductivity of it is more than about ten times higher than that of SUS which is conventional material for vacuum equipment (Table 1). And then both heads material were changed from SUS to Macor.

At room temperature, Viton O-ring used as a sealing of the head in BL12. Generally, in high vacuum system, Viton O-ring and/or acrylonitrile butadiene rubber (NBR) are used as a sealing for the vacuum. Especially in high temperature conditions, Viton shows good performance. However, on cryogenic temperature conditions, Viton didn't work well. Teflon is known as one of the best materials used at low temperature. Ductile-brittle transition temperature (DBTT) of Teflon is about 173K. However, elastic property of Teflon is worse than that of NBR and Viton. At the BL12, sample observation is performed at $<10^{-4}$ Pa. For maintaining such a high vacuum condition, NBR O-ring or Viton O-ring is adequate to the operation. The DBTT of NBR is about 223 K and the DBTT of Viton is about 263 K. In light of these considerations it can be

easily concluded that the NBR is determined as an O-ring material.

Figure 6 shows the cooling test result of Macor head, Si_3N_4 film, Torr-Seal and NBR O-ring set. The longitudinal axes represent temperature of sample position (left side) and vacuum pressure of the chamber (right side). The transverse axis represents measurement times. Cryostat setting temperature is 173 K. After 20 minutes preliminary cooling, the Macor head is inserted in the cryogenic test chamber. When the head was inserted in the chamber, there is a light increase. However the chamber pressure is maintained at < 9.2 Pa. The vacuum property of these set shows good performance.

Table 1 Thermal conductivity of head materials.

Head Material	Thermal conductivity（W/m・K）
SUS	19
Macor	1.6

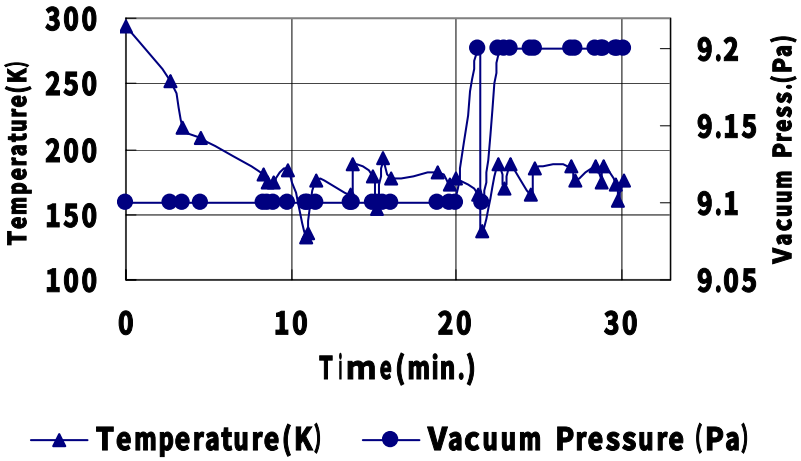


Fig.6 Cooling test of the cryogenic sample chamber. Macor head, Si_3N_4 film, Torr-Seal and NBR O-ring set. The longitudinal axes represent temperature of sample position (left side) and vacuum pressure of the chamber (right side). The transverse axis represents measurement times. Setting temperature is 173K. After 20 minutes preliminary cooling, the Macor head is inserted in the cryogenic test chamber.

4. Installation of Cryogenic Sample Chamber in BL-12

The SUS heads were exchanged to Macor heads. Viton O-rings were also exchanged to NBR O-rings. The silicon substrate with Si_3N_4 film was attached to the head using Torr-Seal. After improving these on the X-ray microscope, the improved cryogenic sample chamber was installed in BL-12. Figure 7 shows the cryogenic system in BL-12. LN2 dewar vessel is located about 20 cm higher than cryogenic sample chamber position.

Figure 8 shows cooling property of the cryogenic chamber. Vacuum pressures of CZP chamber (G3) and OZP chamber (G4) are also shown in Fig. 8. Setting temperature is 203 K. Only 10 minutes are required for the preliminary cooling. The low-temperature stability is also good. Both vacuum pressures of OZP chamber and CZP chamber is stable without leak. These vacuum levels are sufficient for X-ray observation of bio-specimens.

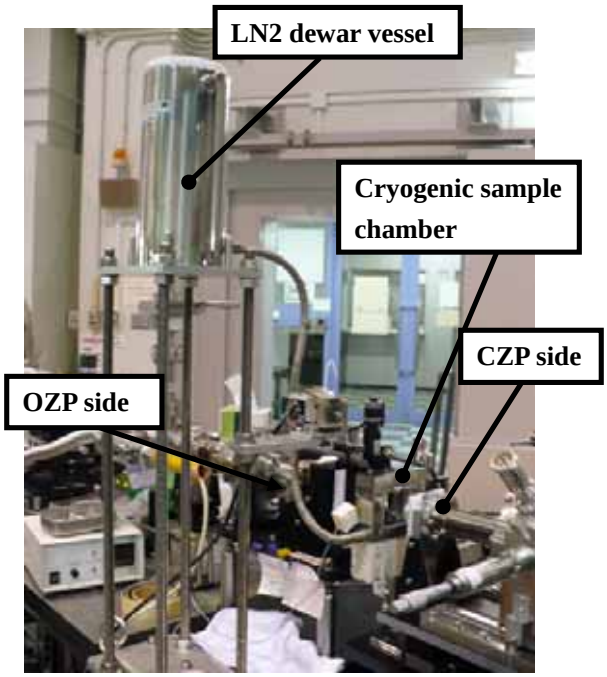


Fig.7 Present cryogenic chamber in BL-12. In this system, the sample chamber is cooled in nitrogen vapor and by heat conduction toward the cold metal wall, and keeps the sample cryogenic temperatures [7]. LN2 dewar vessel is located about 20 cm higher than cryogenic sample chamber position.

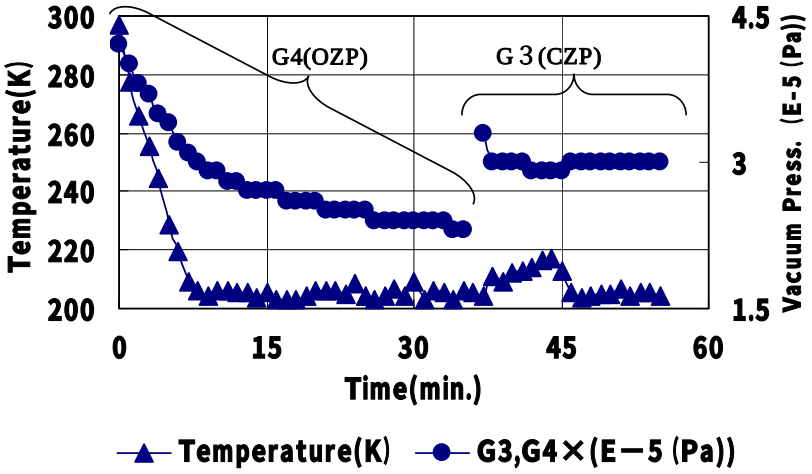


Fig. 8 Properties of cryogenic sample chamber temperature and vacuum of CZP chamber (G3) and OZP chamber (G4). The longitudinal axes represent temperature of sample position (left side) and vacuum pressure of the chambers (right side). The transverse axis represents measurement times. Setting temperature is 203 K. Only 10 minutes are required for the preliminary cooling. These vacuum levels and stabilities are sufficient for X-ray observation.

5. Summary

Soft X-ray microscope beam line at BL-12 has been improved to be user-friendly for several years since 2002. As the first step in this project, an automatic multi-wavelength X-ray microscopy system has been introduced. Now, it is used for daily observation and shows good performance. And as the next step, a cryogenic sample chamber was designed.

Design concept of cryogenic sample chamber is described in the followings:

1) The cooling equipment is designed to maintain the cryo-condition for 100 minutes without frost forming.

2) The equipment is able to adapt to X-ray microscope at BL-12.

3) The equipment contains the sample holder which can be easily mounted.

In order to achieve these, the cryogenic sample chamber repeated improvement. At last a new cryogenic sample chamber was designed and produced. The new chamber

is quite compact. In order to avoid frost forming and maintain temperature stability, the sample stage is enclosed with a cryo-house. Before installing the cryogenic sample chamber, the cryogenic stability of the Si_3N_4 film, seal materials and vacuum pipes under vacuum condition were evaluated. The combination of the most powerful parts and materials was determined. After improving on the X-ray microscope, the cryogenic sample chamber was installed. Only 10 minutes are required for the preliminary cooling. The cryogenic stability is sufficient for observing biological and polymer specimens. Both vacuum pressures of OZP chamber and CZP chamber is stable without leak ($< 4\times10^{-5}$ Pa) .

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