Recent Observation of Biospecimens by Soft X-Ray Microscope at BL12

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Abstract

The high resolution soft X-ray microscope (BL12) was used to observe various biospecimens. In 2004, an automatic multi-wavelength X-ray microscopy system was installed. The achieved resolution is about 70 nm, and energy resolution (E/ΔE) is about 200. Using this improved system, we observed the following whole cells: blood cell of chicken, *Dictyostelium discoideum* cell, and NIH 3T3 cell. These observations indicate that biospecimens such as subcellular structures can be analyzed in vivo.

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1. Introduction

One of the important advantages of soft X-ray microscopy is its capability of observing a live cell. X-ray microscopy is especially well suited to investigate a whole, unsectioned cell, since its resolution is higher than that of light microscope. Soft X-ray microscope at BL12 achieved resolution was about 70 nm, judging from the edge analysis [2]. Many samples were observed; latex spheres of 0.23 μm diameter and fine structures of 0.1 μm size in diatom cells could clearly be observed [3].

The X-ray microscope has made important progress through last year. In order to improve this X-ray microscope to a user-friendly system, an automated wavelength scanning system was introduced [4]. Now, it is used for daily observation and shows good performance.

In this report, we describe the specifications and recent results of this improved X-ray microscope.

2. X-ray Microscope Beamline (BL12)

The present optical configuration of the X-ray microscope is the same with the previous one [1-2]. It consists of two parts: a condensing part and an imaging part. The X-rays reflected by the SiC plane mirror are condensed and monochromatized by a CZP through a pinhole of 20μm diameter, and then focused on a specimen in air. The transmitted photons are magnified by an OZP to form an image on a new CCD camera. Images are focused with the first-order diffraction. The new CCD system is a cooled CCD (C4880-21-24WD, Hamamatsu K.K.) The effective pixel size is 24 μm **x** 24 μm and the field of view is 512 pixel **x** 512 pixel.

The CZP is a Göttingen KZP 7 type (diameter: 9 mm, outermost zone width: 53.7 nm, number of the zones: 41,890) [5]. The OZP was fabricated at IBM/LBNL (diameter: 50 μm, outermost zone width: 45 nm, number of the zones: 277) [6]. In 2004, an automated CZP positioning system was introduced [4]. Usable wavelength range is 1.6 – 3.3 nm. Now, it is used for daily observation and shows good performance.

3. Experimental results

3.1 Evaluation of resolution

The system resolution was experimentally evaluated by observing a Cu 2000 mesh illuminated by 2.4 nm wavelength, as shown in Fig. 1. The image was taken with 60 s exposure time, which corresponded to 1.5 x 10^{11} photons/μm^2 on the CZP. Its resolution was estimated to be 71 nm (20 – 80 %) from the intensity gradient of a knife edge of the mesh. It is as good as the previous system [3].

3.2 Latex sphere

Latex spheres (Dow Chemical Co.) of 1.09 μm diameter and 0.5 μm diameter were observed and are shown in Fig. 2 (A) and (B), respectively. As clearly seen in the figures, the

latex spheres show good contrast, which demonstrates that specimens of organic materials can be observed with reasonable contrast using the X-ray microscope in the water window region.

3.3 Diatom

Figs. 3 are X-ray micrographs of diatoms. Images were obtained using a wavelength of 2.4nm and exposure time of 60 s. (A) is a montage image of a diatom. Since the sample stage can be scanned, we can observe a sample which is bigger than a view field. In Fig. 3 (B), an image of a part of the other diatom is shown. The image in Fig. 3 (B) was digitally magnified, and shown in Fig. 3 (C). Fine structures of 0.1 μm size in the diatom cell can be clearly observed.

3.4 Chicken red blood cell

Fig. 4 is an X-ray micrograph of red blood cells of chicken. Observed wavelength was 2.4 nm and exposure time was 60 s. It shows an ellipse form. The long axis of the ellipse is approximately 10 μm and the short axis is approximately 6 μm. A nucleus is clearly observed at the center of the cell. Such a nucleolus is not observed in a red blood cell of mammal. In case of human, some disease causes a special form induced by the structural change of the constituent of the blood. This result shows that X-ray microscopy is quite effective in observing blood cells.

3.5 *Dictyostelium discoideum*

Dictyostelium discoideum is a soil-living amoeba. It is widely used for model organisms in biology since its culture is easy and the patterning and differentiation in the development are simple.

Fig. 5 is an optical micrograph (A) and X-Ray micrograph (B) of *Dictyostelium* cell. Without any staining treatment, microstructure of nucleus and cytoplasm are seen.

For the understanding of physiologically important processes in live cells, special labeling is useful. In order to develop a new labeling technique, *Dictyostelium* cell is used as a sample cell and vanadyl sulfate is used as a staining solution agent. A detailed description of it is given in an accompanying paper in this volume [7].

3.6 NIH3T3 cell

Although generally labeling is not required for X-ray microscopy, a special labeling technique is quite useful. It can enhance contrast at the specific site, which gives us a clue to observe functionally important structures. In addition, highly-resolved images are obtainable with lower doses with the use of heavy atom labeling, which also contributes to reduce radiation damages.

We show silver-enhanced immunogold labeling which can be used to localized both

cytoplasmic and nuclear proteins in whole of cultured cells. Fig. 6 shows the image of a tubulin stained mouse fibroblast cells NIH3T3. Although under light microscope, silver enhancement procedure stained tubulin brown, it is difficult to distinguish each fiber structure (Fig 6 A). X-ray microscopy clearly imaged many microtubules as fibers (Fig. 6 B, white arrows). A detailed description of this experiment is given in an accompanying paper in this volume [8].

4. Summary

With the newly introduced CZP chamber, automatic multi-wavelength X-ray microscopy system, we could obtain 71 nm resolution (20 % -80 %) from the knife edge test at 2.4 nm. Several specimens such as latex spheres of 0.5 μm diameter and diatoms of 0.1 μm inner structures could be clearly resolved. Using the X-ray microscope, various biospecimens have been observed: blood cell of chicken, *Dictyostelium discoideum* cell, and NIH3T3 cell. These observations indicate that biospecimens such as subcellular structures and cellular mechanisms will be analyzed in vivo.

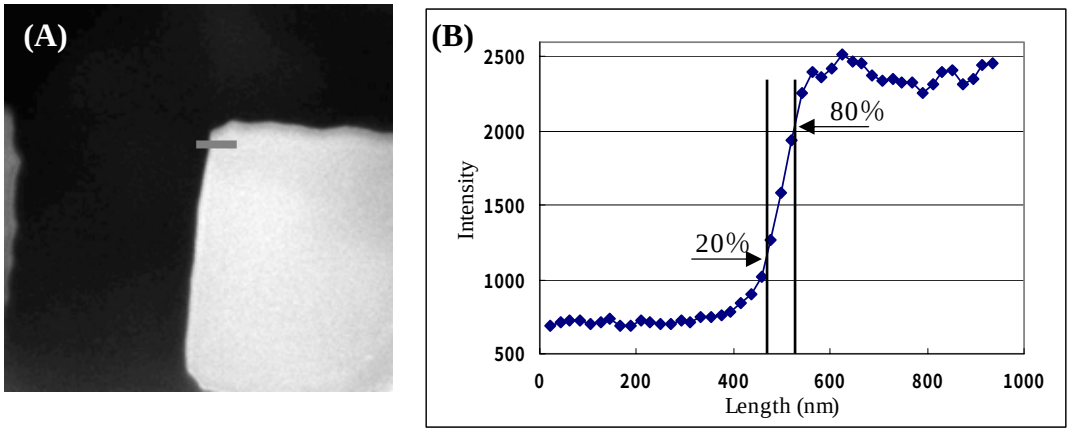

Fig.1 (A) X-ray microscopic image of Cu 2000 mesh taken at 2.4 nm. Exposure time was 60 s. (B) Knife-edge response obtained from the mesh image at gray line. The resolution is evaluated to be at 71 nm at 20 - 80%.

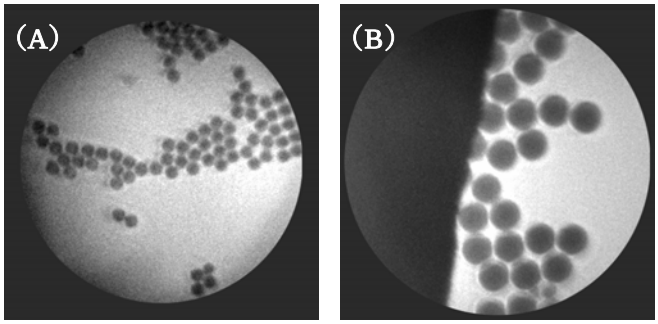

Fig. 2. X-ray microscopic images of poly-styrene latex spheres taken at 2.4 nm. Exposure time was 180 s. (A) Image of the latex spheres of 0.5 μm diameter, (B) image of the latex spheres of 1.09 μm diameter. Each scale bar is 2 μm.

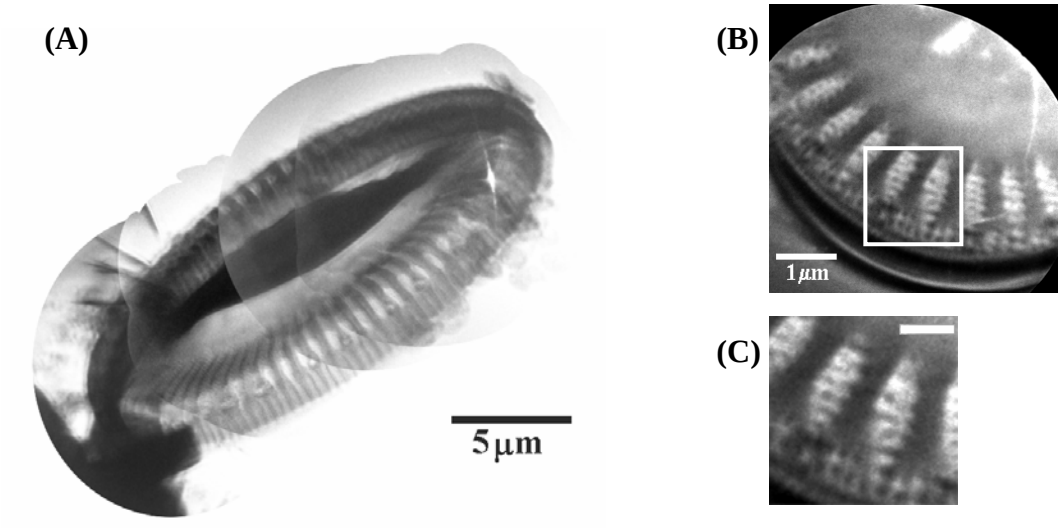

Fig. 3. X-ray microscopic image of diatom taken at a wavelength of 2.4 nm. (A) A montage image of a diatom. (B) An image of a part of another diatom. (C) A digitally magnified image of (B). Scale bar is 0.5 μm.

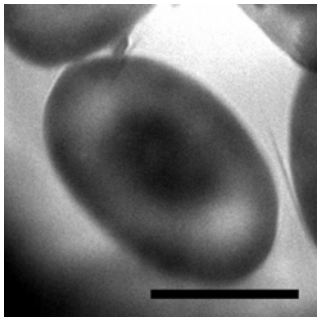

Fig. 4. X-ray microscopic image of red blood cells of chicken, taken at 2.4 nm. Exposure time was 60 s. The scale bar is 5 μm.

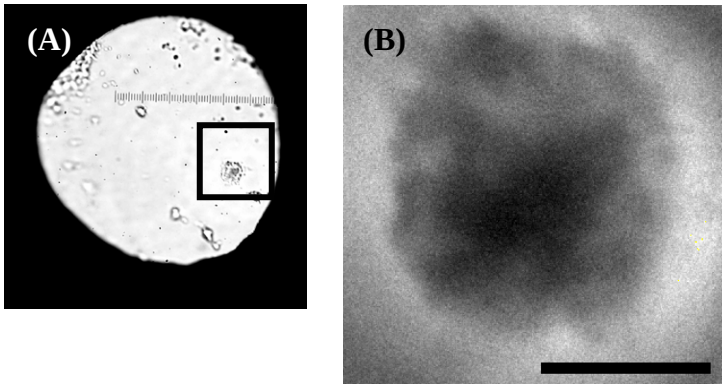

Fig. 5 Unstained *Dictyostelium discoideum* cell. (A) light microscopic image, (B) X-ray microscopic image at 2.3 nm. (A). The exposure time was 60 s. Image (B) is an image of square in (A). The scale bar is 5 μm.

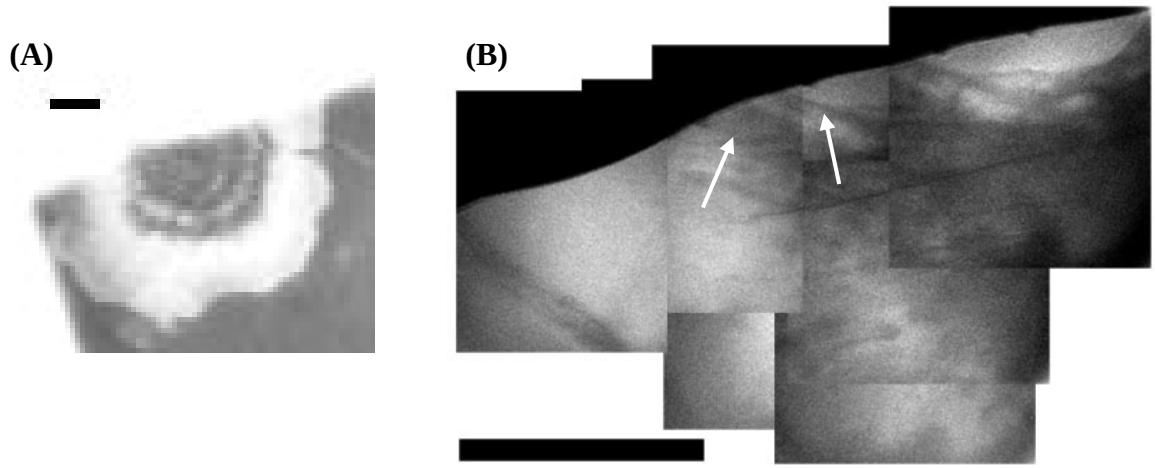

Fig. 6 Light and X-ray microscopic images of a NIH3T3 cell immuno-labeled by anti-tubulin antibody using immunogold-silver enhancement technique. (A) light microscopic image, (B) X-ray microscopic image at 2.4 nm. The exposure time was 60 s. The scale bar is 10 μm.

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