Observation of biological specimens using soft X-ray microscope

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Abstract

Biological specimens were observed using a soft X-ray microscope constructed at the Ritsumeikan synchrotron radiation center BL12. The spatial resolution of the microscope using an X-ray wavelength of 2.4nm was estimated to be 75nm from the edge response. Three kinds of diatoms were observed and ultrastructures of approximately 100nm could be clearly resolved. Two kinds of bacteria and red blood cells of a chicken were observed and their intensity distributions of whole cells were clearly observed. Furthermore, the image contrast of red blood cells was compared by taking the X-ray image using an iron L-absorption edge.

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1. Introduction

In the biological and medical science, it is very important to observe biological specimens without destruction. Although electron microscopes have been used as a high-resolution microscope, it is impossible to observe biological specimens in their natural state, i.e. in the water environment because their sample preparations need many procedures such as a stain and dehydration. On the contrary, optical microscopes enable us to observe biological specimens dynamically. However, the spatial resolution is limited to several hundred nanometers because of the diffraction limit of the visible light.

Recent advances of micro and nano machining techniques make it possible to fabricate optical devices which are used in the X-ray region. Since X-ray microscopes can be used to observe biological specimens without destruction and have better spatial resolution than optical microscope, they are expected to become a complement to the electron microscope and the optical microscope[1–3]. Especially, soft X-ray microscopes using the water window X-rays which are from an oxygen K-absorption edge (2.3nm) to a carbon K-absorption edge (4.4nm) are mainly developed to observe biological specimens in the water environment with a relatively high contrast[4]. Soft X-ray microscopes are mainly constructed at the synchrotron radiation facilities and the Fresnel zone plates are used as an optical device. The spatial resolution has been achieved better than 20nm and the applications such as a computed tomography (CT) have been performed[5,6].

In this paper, results of observation of biological specimens using a soft X-ray microscope, which is constructed at the Ritsumeikan synchrotron radiation center BL12 (RITS SR center, Ritsumeikan University, Kusatsu, Shiga, Japan), are shown.

2. Optical System

A schematic diagram of the optical setup is shown in Fig.1. A soft X-ray microscope was constructed at the RITS SR center BL12 and the SR from a storage ring operated at 575MeV with 300mA was used as an X-ray source[7]. The plane mirror installed at the upstream of a microscope cut off the higher energy than approximately 900eV. A sample, which was set in air, was illuminated by condenser zone plate (CZP) and the transmitted and diffracted X-rays were magnified by the objective zone plate (OZP). The numerical parameters of a CZP and an OZP are shown in Table.1. The CZP served as a linear monochromator combined with a ϕ 20μm pinhole.

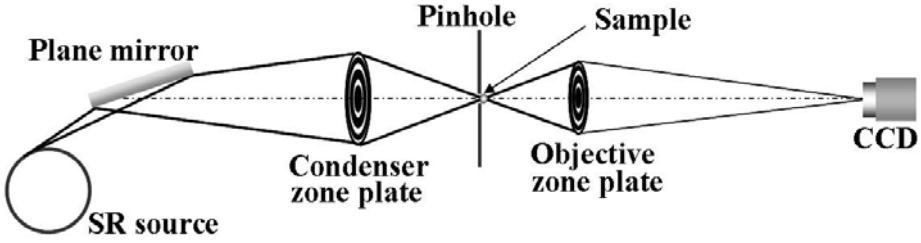

Fig.1 Schematic diagram of a RITS soft X-ray microscope

The theoretical resolution Δ of the microscope is represented as,

$$\Delta = 1.22 \times \delta r_n ,$$

where δr_n is the outermost zone width of an OZP. In this microscope, since the outermost zone width of an OZP is 45nm, the resolution of 55nm will be expected.

The magnified image was detected by the back illuminated CCD camera whose pixel size was 24μm (512*512 pixels, Hamamatsu Photonics K.K.).

Table 1 Numerical parameters of CZP and OZP.

	CZP	OZP
Diameter	9mm	50μm
Number of zones	41890	277
Outermost zone width	53.7nm	45nm
Material	0.3μm Ge	0.13μm Ni
Support material	0.1μm Si	0.1μm SiN

3. Experiments and Results

3.1 Spatial resolution

To estimate the spatial resolution of the microscope, the copper mesh (#2000, 12.7μm/pitch) was observed. The X-ray image of a mesh is shown in Fig.2 (a). The magnification of the microscope is estimated to be approximately 1110× from a mesh image when using an X-ray wavelength of 2.4 nm. Therefore, the effective pixel size at the objective plane becomes approximately 20nm. The intensity profile of a line A is shown in Fig. 2(b). The spatial resolution which is defined by the drop of the intensity from 80% to 20% is approximately 75nm.

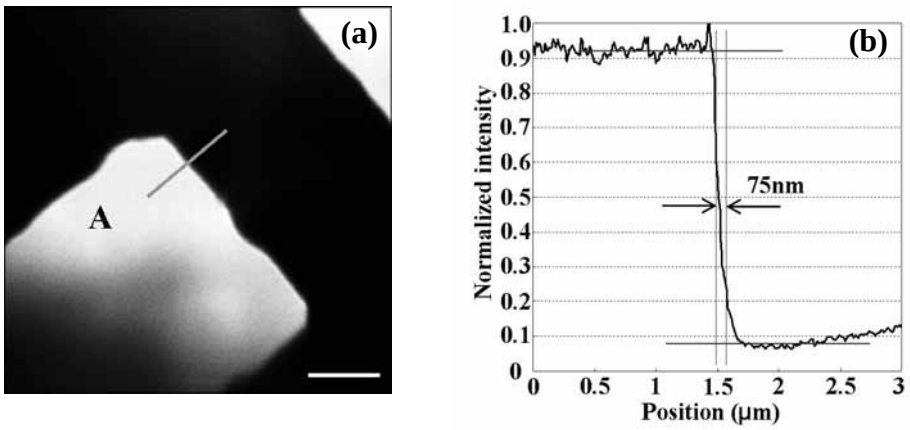

Fig.2 (a) X-ray image of a copper mesh (12.7μm/pitch). (b) Intensity profile of a line A. Bar=2μm.

3.2 Observation of biological specimens

To observe biological specimens with the soft X-ray microscope, they were prepared on the copper mesh covered with a collodion membrane. In this case, biological specimens were dried naturally after setting on the membrane.

First, diatoms were observed. Since diatoms were typically more than 10μm in size, a montage image was made from several images. Three kinds of diatoms are shown in Fig. 3(a)-(c). A diatom shown in Fig. 3(a) was observed with an X-ray wavelength of 2.4nm which is the water window X-ray. The enlarged image of a white square region is shown in the upper left of the image. Ultrastructures of approximately 100nm, which are called *areolae*, can be observed. Diatoms shown in Fig. 3(b) and (c) were observed with an X-ray wavelength of 1.9nm. Since diatoms have hard shells made of SiO_2, considerably high contrast image can be obtained even in the shorter wavelength than water window X-rays. As well as Fig. 3 (a), their grating like structures and ultrastructures can be clearly observed.

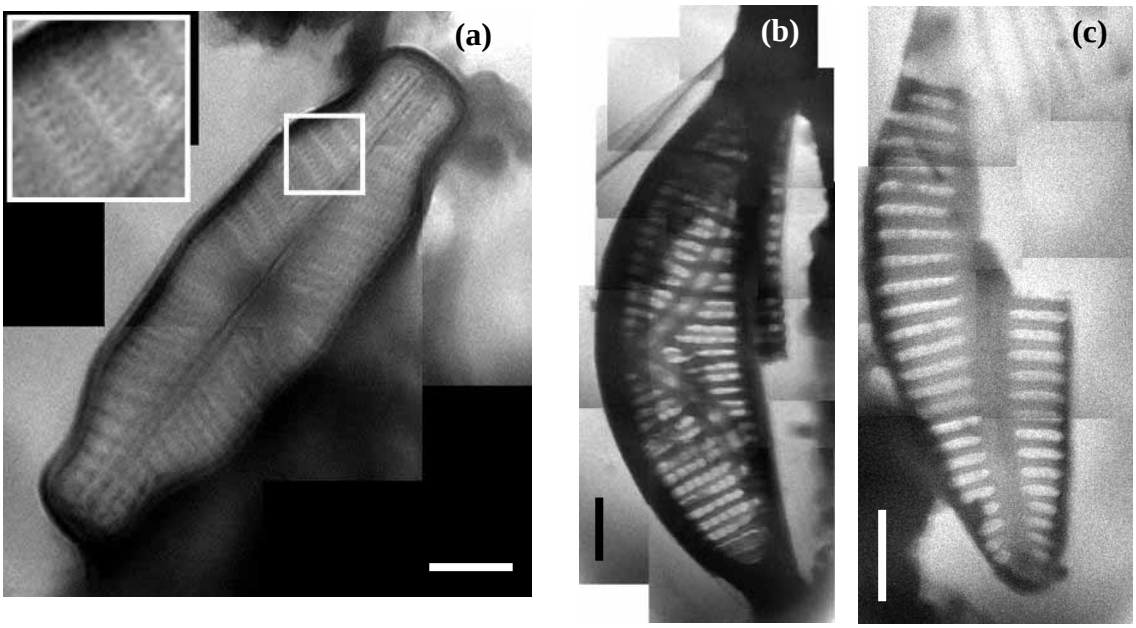

Fig.3 X-ray images of diatoms. (a) λ=2.4nm. Exposure was 10 minutes for each image. (b)(c) λ=1.9nm. Exposure was 30 seconds for each image. Bar=2μm.

Second, two kinds of bacteria were observed. They were observed with an X-ray wavelength of 1.9nm. The X-ray images of *Acaryochloris marina* (*A. marina*) and *Rhodosphirillum rubrum* (*R. rubrum*) are shown in Fig. 4(a) and (b), respectively. In the X-ray image of *A. marina*, the intensity distribution can be observed in each bacterium cell. In the X-ray image of *R. rubrum*, we can find the strong absorption areas.

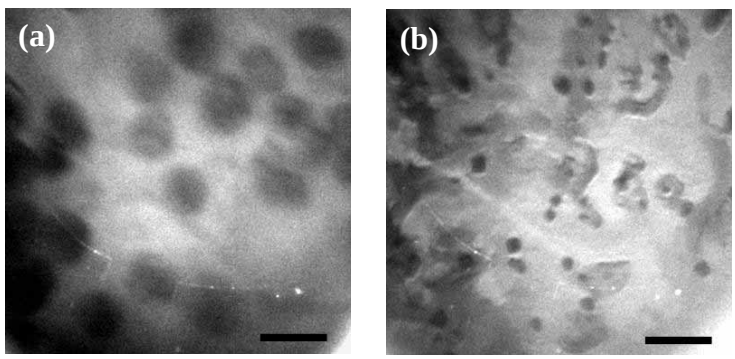

Fig.4 X-ray images of two kinds of bacteria. (a) Acaryochloris marina. Exposure is 1 minute×5 integration. (b) Rhodosphirillum rubrum. Exposure is 1minute×5 integration. Bar=2μm.

Last, red blood cells of a chicken were observed. The X-ray image of them observed with an X-ray wavelength of 2.4nm is shown in Fig.5. The central part of the cell which absorbed X-ray considerably is a nucleus of the cell. Unlike the mammals, birds have a nucleus in the red blood cell. We can also observe the intensity distribution around the nucleus.

Since the red blood cells have hemoglobin which includes iron, they were observed with an iron L-absorption edge which was 1.75nm in wavelength. The X-ray images taken with X-ray wavelengths of 1.77nm and 1.71nm are shown in Fig. 6(a) and (b), respectively. In this case, an aperture was inserted before the CCD to eliminate the stray X-rays. Since the focal length of a FZP became long as the X-ray wavelength became short, the magnification of the images at the image plane were changed. Comparing Fig. 6(a) with (b), the contrast of a whole image deteriorates. This means that iron distributes over the whole cell. Especially, the deterioration of the contrast at the nucleus is remarkable. Therefore, it seems that iron mainly distributes in the nucleus. Since the same blood cell was imaged to compare the contrast accurately, the dosage of the X-ray to the cell increased and then the shrinkage of the cell appeared, which is shown in the images (black arrows).

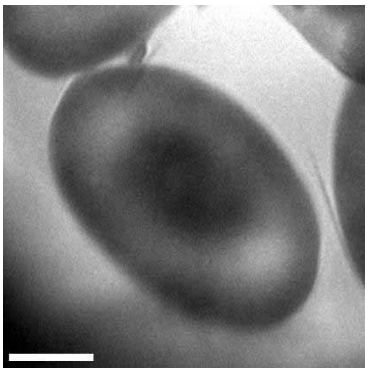

Fig.5 X-ray image of red blood cells of a chicken. Exposure is 10 minutes. Bar=2μm.

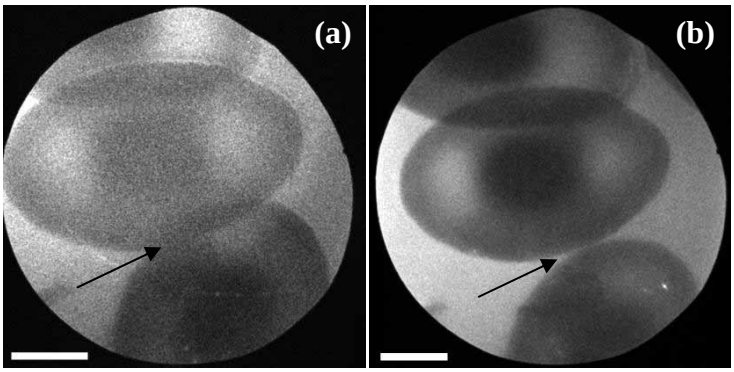

Fig.6 X-ray images of red blood cells of a chicken. (a) λ=1.77nm. Exposure is 2 minutes. (b) λ=1.71nm. Exposure is 2 minutes. Bar=2μm.

4. Discussion

Biological specimens were observed with a better resolution than 100nm using a soft X-ray microscope constructed at the RITS SR center BL12. However, as the exposure time became long, the apparent radiation damage was observed. This is a significant problem to observe the biological specimens. To overcome this problem, a cryogenic method is one of the prominent candidates[8]. By freezing a hydrated sample instantly, the ultrastructures of a sample are preserved and the maximum tolerable dosage is increased by a factor of 10^3 [9]. If a biological sample withstands the multiple exposures, the computed tomography (CT) will be applied to obtain the whole three dimensional image of the biological cells.

References

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