Development of a soft X-ray microscope with Wolter mirrors for the observation of biological specimens in the atmospheric state

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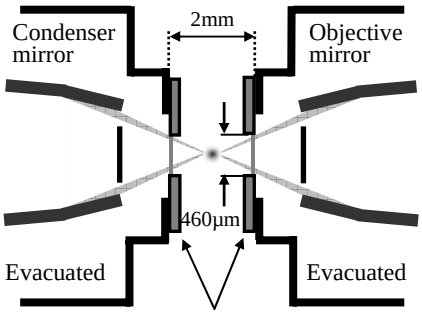
Soft X-ray microscopes using water window X-rays are powerful tools for the observation of biological specimens in the natural state. Most of them are constructed at the synchrotron radiation facilities with zone plates and approximately 20nm resolution is achieved[1]. In the laboratory, a laser plasma soft X-ray microscope with Wolter mirrors has been developed as one method of these microscopic techniques. Relatively narrow band spectrum around 3.2nm soft X-rays is obtained using a tantalum target and a titanium filter. In our previous microscope, specimens were put in a vacuum to avoid the absorption of soft X-rays by the air[2]. However, this method was very laborious because the sample preparation was difficult to preserve the natural state of the specimens in a vacuum. So, a new environmental sample chamber was developed so that biological specimens can be set in the atmospheric state. The schematic diagram around a specimen is shown in Fig.1. A silicon nitride membrane (460×460μm, 100nm thickness) on the silicon substrate was used as a transparent window between a vacuum chamber and the atmosphere. As the window is also transparent for visible rays, the alignment of a Wolter mirror is possible with visible rays before using soft X-rays. The atmospheric layer is approximately 2mm, so the working distance of a sample is 1.2mm. Therefore, the application to various sample holders can be expected. The transmission of 3.2nm soft X-rays of a silicon nitride membrane and the atmospheric layer are 67% and 60%, respectively. So, the net transmission except a specimen is 25% compared with a previous microscope. The resolution of this microscope is estimated to be better than 100nm and biological specimens are observed using a renewed microscope. The X-ray micrograph of a diatom and red blood cells of a cow are shown in Fig.2 (a) and (b), respectively. A photographic plate was used as a high resolution detector. In the X-ray image of a diatom, approximately 100nm fine structures are observed with relatively high contrast. In the case of red blood cells, each cell and crystal like structures are clearly observed. Exposure was less than 10seconds.



SiN membrane on the Si substrate

Fig.1 The schematic diagram of a new sample chamber

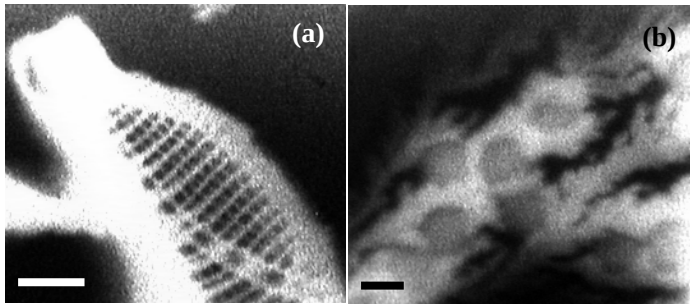


Fig.2 X-ray micrographs of biological specimens: bar 2μm
(a) A diatom (b) red blood cells of a cow

(1) W. Chao et al. J.Vac.Sci.Technol. B **21**(6), 3108-3111 (2003)
(2) T. Ogata et al. J. Electron Spect. Rel. Phenom., **80**, 357-360 (1996)