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Trial for soft X-ray imaging of cultured mammalian cells in water environment by contact microscopy with an electronic zooming tube

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Introduction

The advantage of soft X-ray microscopy has been well recognized in its high resolution imaging of biological specimens in the natural physiological state particularly by using photon energies situated in the "water window" region. However a specimen chamber has to be carefully installed in vacuum environment of soft X-ray optics. In imaging and scanning types of soft X-ray microscopes very thin layer of atmospheric pressure that contains wet specimens is inserted in the beam path [1]. Hydrated human chromosomes have been also imaged by contact microscopy using specimen sandwiched between SiN window and PMMA used as a photoresist [2].

In the present study we applied hydrated specimen to the contact microscope with an electronic zooming tube as a two dimensional high resolution detector to obtain light element maps of thin biological specimens such as cultured cells [3]. The most serious problem to fit hydrated specimens to the electronic zooming tube is in the possible disruption of vacuum that results in the breakdown of photoelectric surface and MCP. We have made a special specimen chamber to hold hydrated cells, tested the adaptability to vacuum environment, and taken images of human cells in wet condition.

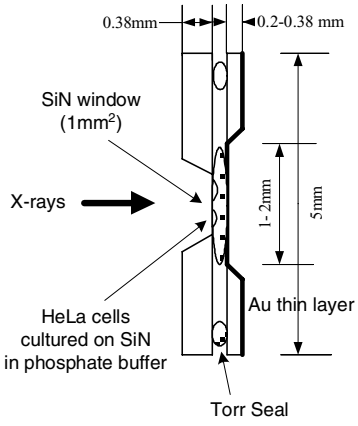

Fig. 1. A specimen chamber for hydrated cells in vacuum environment.

Materials and Methods

Contact X-ray microscopy with an electronic zooming tube was used to obtain soft X-ray images of wet human cancer HeLa cells at the BL-12A beamline. Fig. 1 shows a specimen chamber for hydrated cells. HeLa cells cultured on SiN membrane were fixed with glutaraldehyde, and kept in phosphate buffer saline (PBS). The SiN window was placed beneath another SiN window coated with Au for a photocathode of the electronic zooming tube. The size of SiN window and the thickness of Si base were changed to examine the effect of flatness of photocathode on the image distortion. Both SiN windows were sealed with Torr Seal.

Results and Discussion

Fig. 2 shows the soft X-ray images of HeLa cells with different SiN window size and Si thickness. The flatness of photocathode improved cell images. The absorption spectra of Fig. 3 indicate the evidence for the existence of water in the chamber. The absorption jump at the O-K edge observed for the spectrum of the extracellular space represents the presence of H_2O in contrast with the spectrum for a dried specimen as a reference.

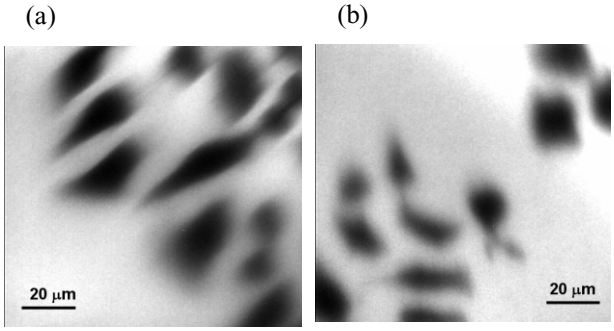

Fig. 2. Fixed human HeLa cells in PBS imaged at the wavelength of 3.14nm. (a) SiN window: 1mm, Si: 0.38mm; (b) SiN window: 2mm, Si: 0.2mm

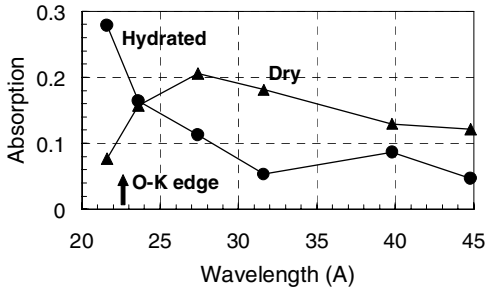

Fig. 3. Absorption spectra of extracellular area for hydrated and dried specimens.

References

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