Zoological Studies 34, Supplement I (1995)

Zoological Studies

Applications of X-ray Microscopy with Hydrated Specimens in Biomedical Research

H. Spring[1], P. Guttmann[2], D. Rudolph[2], G. Schneider[2], G. Schmahl[2] and M.F. Trendelenburg[1]

[1]*Biomedical Structure Analysis Group, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany*

[2]*Institute of X-Ray Physics, University of Göttingen, Geiststr. 11, D-37073 Göttingen, Germany*

INTRODUCTION

In the last years, the development of zone plates with high efficiency as optics for high resolution X-ray microscopy of hydrated specimens has undergone great steps towards an application of such a microscope in biomedical research. Concomitant with the development of zone plates for high resolution and high magnification it was also necessary to develop stable environmental ('wet') chambers as support for the hydrated specimen during observation. With such an equipment we were able to observe different biomedical specimens at high resolution in the hydrated state using amplitude contrast and/or phase contrast X-ray microscopy.

MATERIALS AND METHODS

For the experiments described, we used the Göttingen X-ray microscope situated at the BESSY (Berlin, Germany) electron storage ring facility operated with a wavelength of 2.4 nm (for more details, see Rudolph et al. 1984, Niemann et al. 1994). Different zone plates as objective lenses were constructed with electron beam lithography (David et al. 1992) and had outermost zone widths in the range of 50-20 nm and numerical apertures of NA 0.06, thus allowing theoretical resolutions of up to 25 nm and primary magnifications of up to 2400 X. The specimens were observed in special environmental chambers (Schneider and Niemann 1992, see also Rudolph et al. 1992) consisting of polypropylene foils with a layer of polyimide and a stabilizing layer of silicon H evaporated on the surface (for the 'slide' foil) and a layer of aluminium oxide (for the 'coverslip' foil). The metal carrier rings of the foils were separated by a spacer ring and due to a special syringe evacuation system the distance of the foils could be calibrated to produce an inner chamber width of about 10 μm or less for sufficient transmission of the X-rays and structural preservation. Such foils could be sterilized by immersion into 70% ethanol before the cultivation of cell culture cells on the surface of the foil acting as the 'microscope slide'. We used PTK2 epithelal cells grown on the foil for 24 hours. In other experiments we used freshly isolated nucleoli and nuclear contents of oocytes of the newt Pleurodeles waltlii or a dispersal of a culture of E. coli bacteria. All specimens were slightly fixed with a solution of 1-2.5 % glutaraldehyde made up in appropriate buffers. After fixation the specimen chamber was closed with the spacer ring and the second plastic foil and mounted into a special holding system (for details, see Schneider and Niemann 1992) for observation in the X-ray microscope. Prior to the observation the thickness of the chamber was adjusted and calibrated with syringes to obtain a chamber of about 10 μm in depth. Interesting regions of the specimen could be selected by a built-in light microscope (Carl Zeiss, Oberkochen/Jena, Germany) and prefocussed for X-ray microscopy to minimize H radiation damage. Specimen images generated by the microscope were taken with a high resolution CCD-camera with a thinned backside illuminated chip. They were stored on magneto-optical disks and photographed and printed with appropriate equipment.

RESULTS AND DISCUSSION

Fig. 1 shows an image of a PTK2 cell culture cell as seen in the X-ray microscope in the slightly fixed hydrated state using the phase contrast mode. The nucleus and especially the densely packed nucleolus are clearly visible. At closer inspection also the nuclear membrane and some cytoplasmic structures like vesicles, mitochondria and stress fibers can be detected. Fig. 2 gives an example of a cell division in a PTK2 cell at metaphase with the amplitude contrast mode of the X-ray microscope. The paired chromosomes and one centriole (arrow) are in the focal plane. Numerous microtubules (some are denoted by arrowheads) are connecting the chromosomes with the centrioles. Fig. 3 gives an example of nuclear contents of the oocyte of the newt Pleurodeles waltlii as seen in amplitude contrast. Three individual nucleoli can be seen in the image. Interestingly, the internal structure of the nucleoli with more ore less densely packed constituents and nucleolar caverns can easily be detected, even though the average of diameters of such nucleoli is 12-15 μm. In other images (data not shown) we could observe parts of the lampbrush chromosomes with the characteristic transcriptional aspects of the individual genes. Further experiments will also deal with spread defined amphibian oocyte ribosomal genes located in the nucleoli (for images of such genes in light and electron microscopy, see Spring and Trendelenburg 1990). Fig. 4 gives an example of hydrated Escherichia coli bacteria. Individual flagella as well as the bacterial nucleoid can be detected.

In summary we can show, that the X-ray microscope is a useful tool to investigate biomedical hydrated specimens, especially for observations at resolutions higher than offered by light microscopy . It should be noted, that the exposure time of the images we have shown was in the range of several seconds. Further investigations with

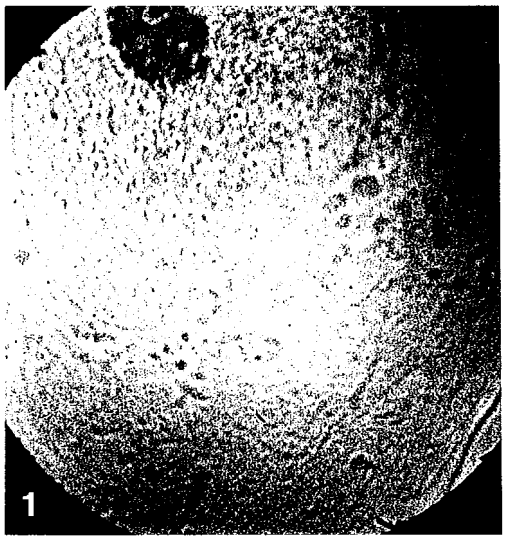

Fig. 1. PTK2 cell in interphase as seen in the phase contrast imaging mode of the X-ray microscope in the wet chamber.

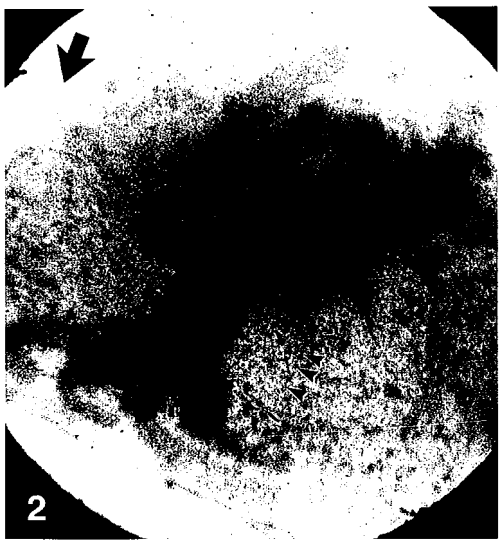

Fig. 2. PTK2 cell in metaphase of mitosis imaged in amplitude contrast. The arrow denotes the centriolar region, several microtubules are demarcated by arrowheads.

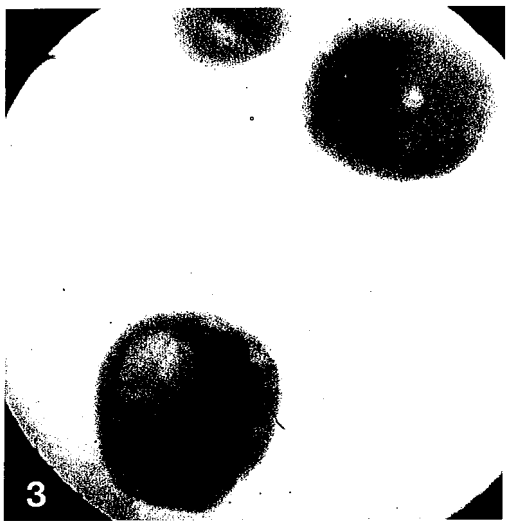

Fig. 3. Nucleoli from the nuclear contents of the oocyte of the newt Pleurodeles waltlii. Note that even from such thick specimens (10-15 μm) a transmission of the beam can be achieved.

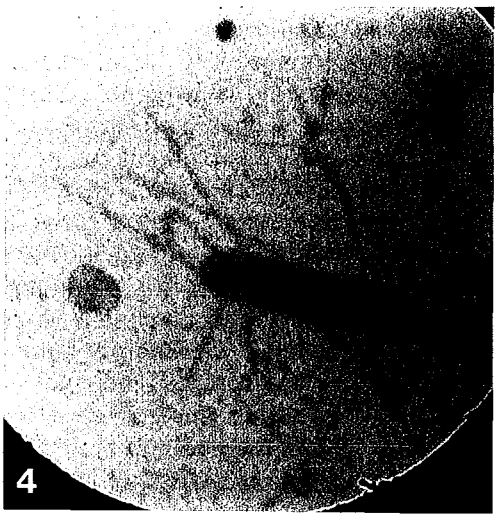

Fig. 4. Bacterial suspension of a culture of Escherichia coli. Numerous bacterial flagella as well as the nucleoid can be seen.

hydrated biomedical specimens will be undertaken with a laboratory X-ray microscope with a plasma X-ray source (for details see Schmahl et al. 1992) with the advantage of short beam exposure times avoiding image blurring due to molecular (Brownian) movements in the specimens. It will also be possible to observe living, unfixed cells.

REFERENCES

David C, J Thieme, P Guttmann, G Schneider, D Rudolph, G Schmahl. 1992. Electron-beam generated x-ray optics for high resolution microscopy studies. Optik **91:** 95-99.

Niemann B, G Schneider, P Guttmann, D. Rudolph, G Schmahl. 1994. The new Göttingen x-ray microscope with object holder in air for wet specimens. In: X-Ray Microscopy IV, V.V. Aristov, AJ Erko, eds.; Bogorodski Pechatnik Publishing, Moscow, in press.

Rudolph D, B Niemann, G Schmahl, O Christ. 1984. The Göttingen x-ray microscope and x-ray microscopy experiments at the BESSY storage ring. In: X-ray Microscopy, Springer Series in Optical Sciences, Vol. 43, pp 192-202, G.Schmahl, D. Rudolph, eds.; Springer-Verlag Berlin.

Rudolph D, G Schneider, P Guttmann, G Schmahl, B Niemann, J Thieme. 1992. Investigations of wet biological specimens with the x-rax microscope at BESSY. In: X-Ray Microscopy III, Springer Series in Optical Sciences, Vol. 67, pp 392-396; AG Michette, GR Morrison, CJ Buckley, eds.; Springer-Verlag Berlin.

Schmahl G, B Niemann, D Rudolph, M Diehl, J Thieme, W Neff, R. Holz, R Lebert, F Richter, G Herziger. 1992. A laboratory x-ray microscope with a plasma x-ray source. In: X-Ray Microscopy III, Springer Series in Optical Sciences, Vol. 67, pp 66-69; A.G. Michette, G.R. Morrison, C.J. Buckley, eds.; Springer-Verlag Berlin

Schneider G,, B Niemann. 1992. Environmental chamber for x-ray imaging of wet biological specimens. In: X-Ray Microscopy III, Springer Series in Optical Sciences, Vol. 67, pp 350-354; AG Michette, GR Morrison, CJ Buckley, eds.; Springer-Verlag.

Spring, H., MF Trendelenburg. 1990. Towards light microscopic imaging of hydrated 'native' ribosomal RNA genes. J. Microscopy **158:** 323-333.