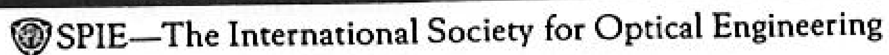


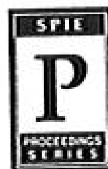
PROCEEDINGS REPRINT

SPIE—The International Society for Optical Engineering

Reprinted from

Soft X-Ray Microscopy

19–21 July 1992
San Diego, California



Volume 1741

©1992 by the Society of Photo-Optical Instrumentation Engineers
Box 10, Bellingham, Washington 98227 USA. Telephone 206/676-3290.

Observation of human chromosome fibers in a water layer by laser-plasma X-ray contact microscopy

Kunio Shinohara[1], Yasuhito Kinjo[2], Martin C. Richardson[3], Atsushi Ito[1], Noboru Morimoto[4], Yasuhiro Horiike[4], Makoto Watanabe[2], Keiji Yada[1], and Kazuo A. Tanaka[5]

[1]Department of Radiation Research, Tokyo Metropolitan Institute of Medical Science, Bunkyo-ku, Tokyo 113, Japan,
[2]Radiation Biology Division, Tokyo Metropolitan Isotope Research Center, Setagaya-ku, Tokyo 158, Japan,
[3]Center for Research in Electro-Optics and Lasers, University of Central Florida, 12424 Research Parkway, Orlando, Florida 32826, USA,
[4]Department of Engineering, Hiroshima University, Higashihiroshima-shi, Hiroshima 724, Japan,
[5]Institute of Laser Engineering, Osaka University, Suita-shi, Osaka 565, Japan.

ABSTRACT

Soft X-ray contact microscopy was applied to hydrated human chromosomes. Chromosomes of human lymphocytes were spread on a clean surface of distilled water, attached on a X-ray resist, polymethylmethacrylate (PMMA), immediately covered with silicon nitride window, and mounted in a simple hydrated chamber. The specimens were exposed to a single shot of laser-produced gold plasma X-rays (600 ps) in a vacuum chamber. The developed images were observed with transmission electron microscope using the replica method with a plasma polymerization-film in a glow discharge. The results show that we have imaged the complicated entanglement of chromosome fibers in a hydrated condition. The thickness was estimated as 10 nm in average of four narrow parts of these fibers. Particle like structures were observed in many places. The present results prove that hydrated biological specimen is observable with the contrast produced by their components themselves by soft X-ray microscopy at the resolution of 10 nm.

During this imaging exposure, however, silicon nitride (SiN) window was broken. We have studied the reason for this evidence and found that the energy absorbed by the SiN window or water layer was very high. The estimated temperature increase were 870-1470 °C for SiN and 43 °C for water layer. These results suggest that the temperature increase may be responsible for the breakage of SiN window.

1. INTRODUCTION

Application of soft X-ray microscopy to hydrated biological specimens has been demonstrated in recent years [1]. Accumulation of these results will present the new findings in biology.

Chromosomes are composed of DNA, an essential genetic material in life, and proteins. In addition, chromosome fibers have a unit structure called nucleosome, a disc-like structure of 11 nm in diameter and 5.5 nm in height. Nucleosomes are made of DNA and eight core proteins called histone. They are packed to form 30 nm chromatin fibers in a interphase cell and folded into chromosome at mitosis. During a cell cycle, nucleosomes are folded to form chromosomes for cell division and unfolded to 30 nm fibers and a single nucleosome for replication and transcription. Therefore, it is of great interest to understand this highly organized process of nucleosomes to form chromosomes and chromosome fibers, especially in situ. As a step to this goal, we have imaged dry nucleosomes in chromosome fibers in a previous paper [2]. In the present report, we have tried to observe hydrated chromosome and obtained the images of unfolded chromosome fibers. The thickness of the fibers was estimated as 10 nm in narrow parts, which means that we have imaged the size of nucleosomes and suggest that X-ray microscopy will give

0-8194-0914-6/92/$4.00

us the new method to understand the detailed configuration of nucleosomes in chromosome fibers.

2. MATERIALS AND METHODS

Preparation of human chromosomes are as described elsewhere [3,4]. Briefly, chromosomes from mitotic human lymphocytes (RPMI 1788) were spread on a clean surface of distilled water and whole-mounted directly on a X-ray resist, polymethylmethacrylate (PMMA). The PMMA with a water droplet was immediately mounted in a simple hydrated specimen chamber [5]. The chamber was composed of silicon nitride window (0.25 mm x 0.25 mm; thickness, 0.4 μm) and PMMA supported by silicon bases (see, Fig. 7), and covered with a sticky tape (Scotch Sealing Tape #483). The thickness of water layer was 1-5 μm. The chamber was placed at the specimen-target distance of 28.8 mm and exposed to laser-produced gold plasma X-rays (laser wavelength, 527 nm; energy, 15.9 J) with a pulse length of 600 ps at Gekko IV, Institute of Laser Engineering, Osaka University [6]. Exposed specimens were treated as described previously [3]: (1) Removing the specimen from PMMA with sodium hypochlorite (chlorine concentration, 0.5%), (2) developing the PMMA with mixed solution of methylisobutylketone and isopropanol (3:1) for 250 s, and (3) observing with transmission electron microscope using the replica method with plasma polymerization-film in a glow discharge made of a mixture gas of ethylene and methane.

3. RESULTS AND DISCUSSION

3.1. Imaging chromosome fibers

Figure 1 shows an image of stretched part of human chromosome fibers. Since replica method was used for the observation of developed PMMA, white line surrounded by dark lines corresponds to X-ray image [2] in Fig. 1. Figure 2 shows the negative image of Fig. 1. Complicated entanglement of dark lines was observed in the whole area of Fig. 2. The image with weak contrast may be the image for the entangled chromosome fibers unfolded and dispersed from a chromosome, the remainning core part of which was imaged with clear contrast. Figure 3 shows another image of a remaining chromosome core and chromosome fibers. The negative image of Fig. 3 is shown in Fig. 4. The image shown in Figs. 3 and 4 may be the image of chromosome fibers sprung out from one chromosome core.

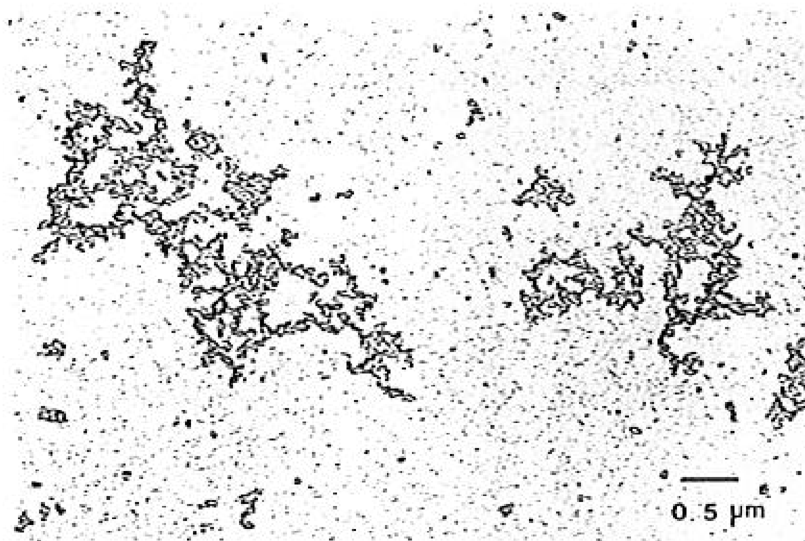


Fig. 1. X-ray image of entangled chromosome fibers with remaining core of chromosomes.

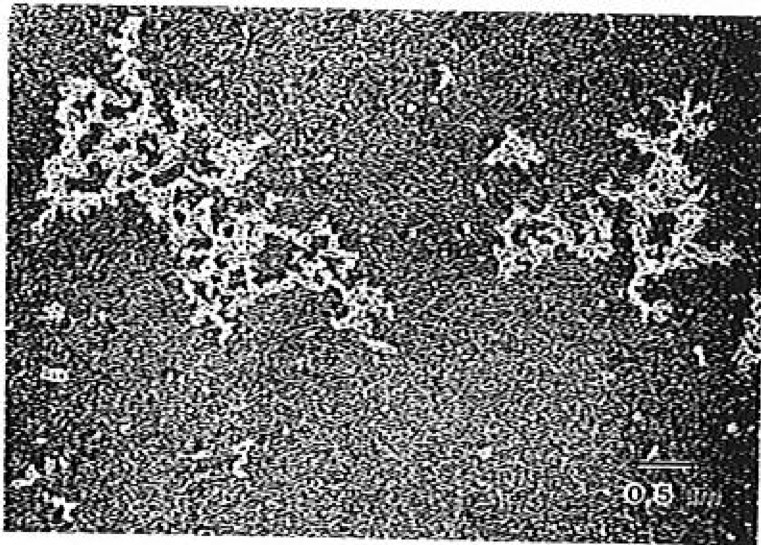


Fig. 2. Negative image of Fig. 1.

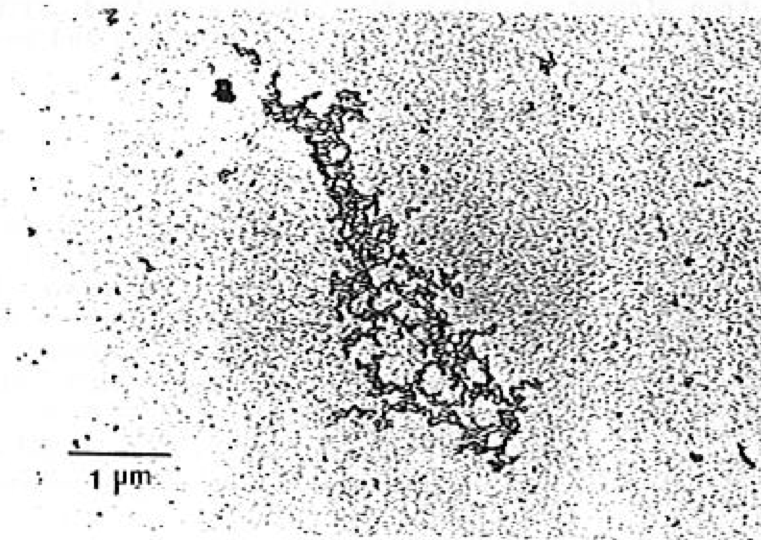


Fig. 3. Another image of entangled chromosome fibers with a remaining core of a chromosome.

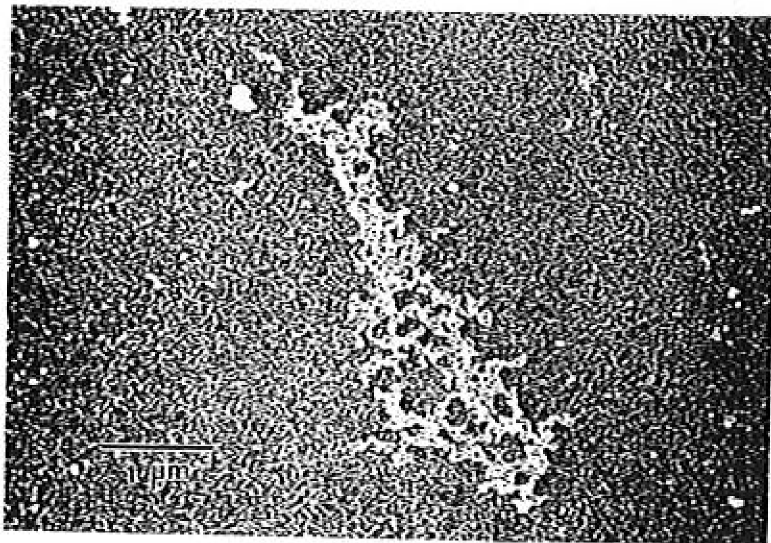


Fig. 4. Negative image of Fig. 3.

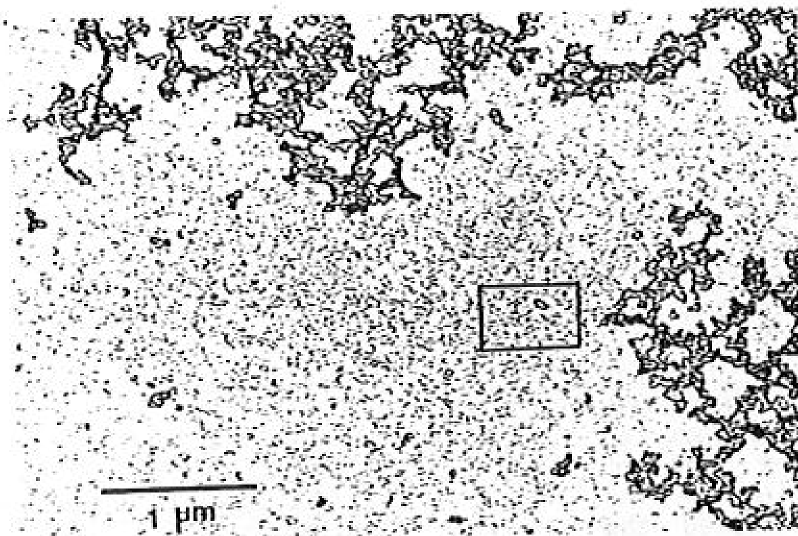
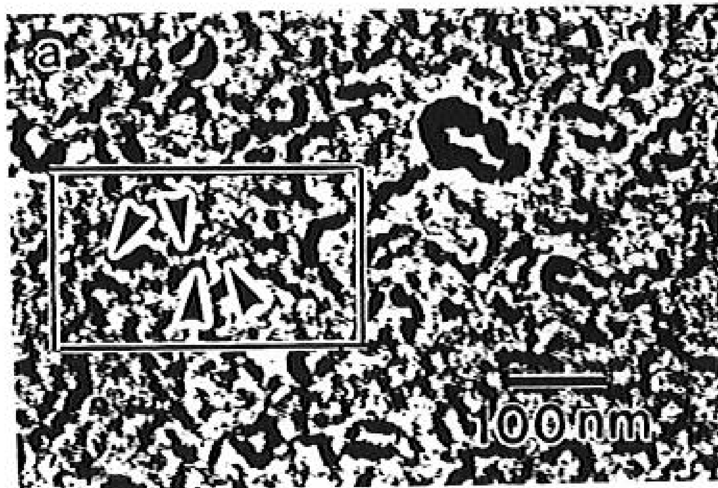


Fig. 5. Different image of an entangled chromosome fibers.



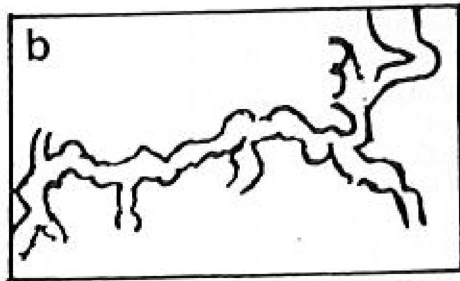


Fig. 6a. Enlarged image of a part of Fig. 5. (surrounded by a rectangle line)
6b. Illustration of a rectangle part of Fig. 6a.

Different part of the image of chromosome fibers was shown in Fig. 5. The rectangle part surrounded by a line was enlarged and shown in Fig. 6a. Figure 6b illustrates a part of the image in Fig. 6a. The thickness of the four narrow parts of the image of a chromosome fiber shown by arrows were estimated as 9.9 nm, 9.9 nm, 9.5 nm, and 10.7 nm (10.0±0.5 nm in average). These narrow parts show particle shapes suggesting the image of nucleosomes.

The present results indicated that chromosome fibers and their unit structure, nucleosomes in a hydrated condition were observed by soft X-ray contact microscopy using pulsed (600 ps) laser-produced plasma X-rays with the contrast of their components and

suggest that soft X-ray microscopy will be a new method to study the structure of hydrated biological specimens at the resolution of as high as 10 nm.

3.2. X-ray spectra and the dose estimates

Figure 7 illustrates the exposure system. Laser (wavelength, 527 nm) was focused to a gold target. The X-rays emitted from the gold plasma show a broad spectrum with a peak wavelength of 6.2 nm [7]. The X-rays were, however, exposed to the specimen in water after passing through SiN window. Therefore, the actual spectrum of the exposed X-rays to the specimen should be changed from that of the source because of the absorption by the SiN window and water. We have estimated the X-ray spectra of various stages from level 0 to level 2 as shown in Fig. 7 with the following equation:

$$I = I_0 \cdot \exp(-\mu \cdot d) \quad \ldots\ldots\ldots\ldots\ldots(1)$$

where I_0 and I are X-ray fluences before and after the substance (i.e., SiN window, water, or water and a nucleosome), μ and d are the linear absorption coefficient and the thickness of the substance. Since μ is dependent on the energy of a photon (wavelength of X-rays), fluence I was calculated for each photon energy by the equation (1).

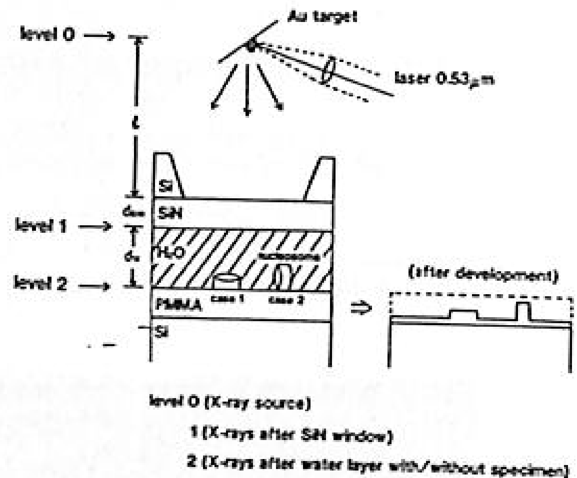


Fig. 7. Illustration of the experimental system.

Figure 8 shows the spectra of the source (gold plasma; broken line), of the X-rays immediately after the 0.4 μm SiN window (level 1 in Fig. 7), and of the X-rays after passing through 1 μm water layer (level 2 without nucleosome in Fig. 7). Total photon flux (F) after the SiN window was estimated by the following equation:

$$F = \Sigma I_{Ei} \cdot \delta Ei = \Sigma[I_0 \cdot \exp(-\mu_{SiN} \cdot d_{SiN})]_{Ei} \cdot \delta Ei \quad \ldots(2)$$

where suffix Ei means the value at the photon energy of Ei. With the exposure condition for the Figs. 1-6 (laser energy, E_L=15.9 J; target-specimen distance=28.8 mm; thickness of SiN window d_{SiN}=0.4 μm), the total photon flux in the energy range of 0.114-1.254 keV (0.99 nm-10.9 nm) was estimated as 4.0×10^{14} photons/cm^2. Figure 9 shows the comparison of the spectrum of the X-rays after the water layer (1 μm) with that after the water (1 μm) and a long axis (11 nm in depth) of a nucleosome (level 2 of the case 2 in Fig. 7). The difference of these two spectra was shown in Fig. 10. This difference should cause the production of the image contrast of a nucleosome. The percent contribution to imaging a nucleosome of the wavelength of longer than 4.37 nm, 4.37-2.33 nm (water window), and shorter than 2.33 nm were 1.1%, 82.0%, and 16.9%, respectively. The results indicated that X-rays in water window were the main photons contributing to to the contrast formation of a nucleosome in water.

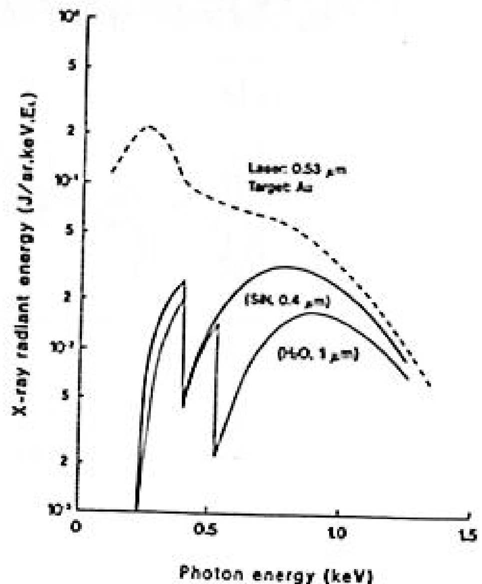


Fig. 8. X-ray spectra of a source, level 1 (after the SiN window, and level 2 (after the water layer).

The results were in good agreement with the expectation for X-ray microscopy of hydrated biological specimens. It should be noted, however,

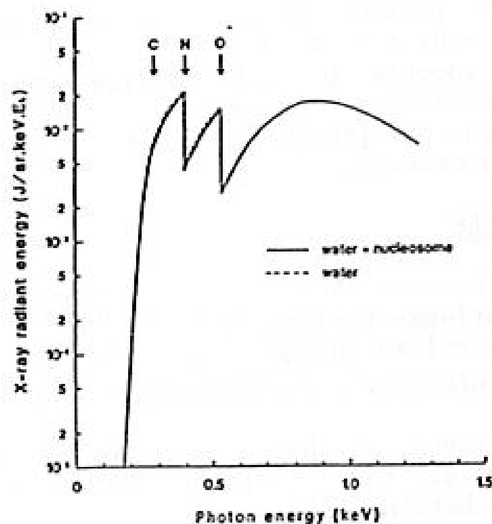


Fig. 9. X-ray spectra of level 2 (after the water with/without a nucleosome).

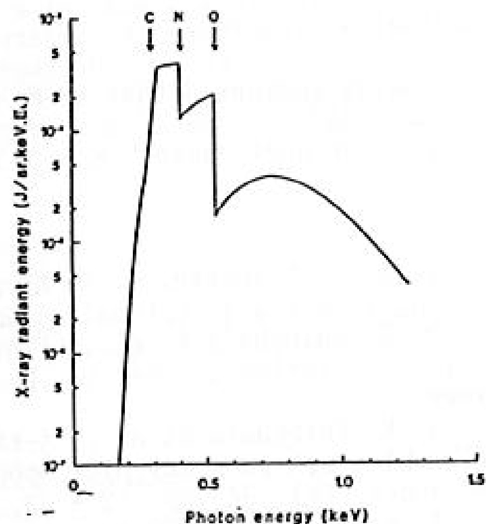


Fig. 10. Difference in X-ray spectrum after water layer with nucleosome from the spectrum after water layer alone.

that there existed a significant amount (16.9%) of contribution by X-rays shorter than 2.33 nm to the image contrast.

We have noticed that the SiN window was broken by a single shot of exposure. To study the cause for this evidence, we have estimated the energies absorbed by the SiN window and by the water with the following equations:

$$E_{SiN} = \Sigma[I_8 \cdot \{1-\exp(-\mu_{SiN} \cdot d_{SiN})\}]_{Ei} \cdot \delta Ei \quad \text{..........(3)}$$

for SiN window and

$$E_{H2O} = \Sigma[I' \cdot \{1-\exp(-\mu_{H2O} \cdot d_{H2O})\}]_{Ei} \cdot \delta Ei \quad \text{..........(4)}$$

for water layer where I' is X-ray fluence after SiN window. In the present exposure condition for Figs. 1-6 (laser energy, E_L=15.9 J; target-specimen distance=28.8 mm; thickness of SiN window, d_{SiN}=0.4 μm), estimated energies were as follows:

E_{SiN} = 0.134 J/cm^2 at 0.4μm SiN, and

E_{H2O} = 0.018 J/cm^2 at 1 μm H_2O.

The results may correspond to the temperature increase (δT) for SiN window of,

δT_{SiN} = 1470 °C if we use C_p = 99.89 J/K·mol at 25 °C or

= 869 °C if we use C_p = 169 J/K·mol at 727 °C, and for water layer of,

δT_{H2O} = 43 °C

where C_p is a molar heat at constant pressure assuming that there is no thermal loss during the exposure time of 600 ps. Therefore, the cause for the breakage of SiN window may be attributed to the temperature increase in SiN window and water in addition to the physical damage caused by plasma particles. The use of monochromoatic X-rays in water window may reduce these temperature increase remarkably and prevent the breakage of SiN window.

4.CONCLUSION

Human chromosome fibers were observed in hydrated condition with a sigle shot of flush X-ray contact microscopy with a laser-produced gold plasma X-rays. The image showed the complicated entanglement of chromosome fibers unfolded and dispersed from a chromosome. The thickness of the narrow parts of unfolded chromosome fibers was estimated as 10 nm

corresponding to the size of a nucleosome. The present results prove that hydrated biological specimen is observable with the contrast produced by their components themselves by X-rays. The studies on the X-ray spectra of the imaging and the dose estimates indicated that there may be a big temperature increase in SiN window and recommended the use of monochromatic X-rays at the wavelength in water window for the imaging of nucleosomes, a unit structure of a chromosome.

5.REFERENCES

1. A. G. Michette, G. R. Morrison, and C. J. Buckley (Eds.), "X-Ray Microscopy III", Springer Series in Optical Sciences, Vol. 67, Springer-Verlag, Berlin, 1992.
2. K. Shinohara et al., "Fine structure of unstained human chromosome fibres dried with no fixative as observed by X-ray contact microscopy", J. Microscopy, 158, 335-342, 1990.
3. K. Shinohara et al., "X-ray contact microscopy of human chromosomes and human fibroblasts," in X-Ray Microscopy II, D. Sayre et al. Eds., Springer Series in Optical Sciences, Vol. 56, pp. 429-432, Springer-Verlag, Berlin, 1988.
4. K. Shinohara, "Observation of human chromosomes with soft X-ray contact microscopy", SPIE Proceedings, 1140, 196-200, 1989.
5. K. Shinohara et al., "A simple chamber for hydrated specimens in a vacuum and its application to X-ray contact microscopy with laser-produced plasma X-rays", in X-Ray Microscopy III, A. G. Michette, G. R. Morrison, and C. J. Buckley Eds., Springer Series in Optical Sciences, Vol. 67, pp. 347-349, Springer-Verlag, Berlin, 1992.
6. M. Richardson et al., "Pulsed X-ray microscopy of biological specimens with laser plasma sources", SPIE Proceedings, 1741, in press.
7. R. Kodama et al., "Soft X-ray emission from ω_0, $2\omega_0$, and $4\omega_0$ laser-produced plasmas", J. Appl. Phys., 59, 3050-3052, 1986.