

# X-ray microscope

Tamio Hara,\* Kozo Ando, and Yoshinobu Aoyagi\*\*  
*Laser Science Research Group*

The development of a soft-x-ray microscope is anticipated for biological application in the wavelength region of 2.4 to 4.3 nm. Biologists can hope to see, in a near-native aqueous environment, structural features far beyond the resolving power of a visible-light microscope. From these points of view, a laser-produced plasma can be the most suitable flash x-ray source for x-ray microscopy. The x-ray lasers are also characterized by very high spectral brightness and interesting coherence properties. We have tried to observe hydrated chromosome by a contact soft-x-ray microscope and obtained its image. These results suggest that soft-x-ray microscopy will be a new method to study the structure of hydrated biological specimens at the resolution as high as 50 nm.

The soft-x-ray spectral region, nominally extending from wavelength of several angstroms to several hundred angstroms and including photon energies from tens of electron volts to several thousand electron volts, is providing many new research and development opportunities in the physical and life sciences and in industry. The move toward shorter wavelengths is driven in part by the desire to see and write smaller features.

The development of a soft-x-ray microscope is anticipated for biological application in the wavelength region of 2.4 to 4.3 nm, which is called the water window region. With soft-x-ray microscopes, biologists can hope to see, in a near-native aqueous environment, structural features far beyond the resolving power of a visible-light microscope, and perhaps even motion. However, the practical soft-x-ray microscope for the water window region has not yet been realized. With recent soft-x-ray images discerning features less than 50 nm, we can envision the day when we gain new insights into the expression of genetic information encoded in DNA through observations of the higher-order packing and dynamics of chromatin in a near-native environment. The soft-x-ray microscope requires development of thin windows, high-resolution optics, optical coatings, x-ray sources that generate x-rays of high brightness which are now becoming available.

A laser-produced plasma can emit strong x-ray with high conversion efficiency. For high resolution imaging with a high signal-to-noise ratio, quite high x-ray flux exposure is required. Furthermore, extremely short duration exposure is essentially important for high-resolution x-ray imaging because of the thermal expansion of the specimen heated by the x-ray absorption. Allowable x-ray exposure time is considered to be shorter than 1 ns to observe specimens at a high resolution by x-ray microscopy. From these points of view, a laser-produced plasma can be the most suitable flash x-ray source for x-ray microscopy. The x-ray lasers are also characterized by very high spectral brightness and interesting coherence properties. Of particular interest are their single-pulse, subnanosecond nature and the relatively narrow spectral width ( $\Delta\lambda/\lambda \approx 10^{-4}$ ) of their emissions. In addition, the very short pulse reduces the likelihood of

blurring due to motion. Researchers hope to pursue high-resolution, subnanosecond imaging of biological objects using multiview two-dimensional microscopy as well as holographic techniques.

Much attention is being given to developing soft-x-ray microscopy as an extension of visible-light techniques but with a resolution between those of visible-light and electron microscopy. There are some of the major techniques being developed for high-resolution soft-x-ray microimaging such as a magnified imaging x-ray microscope, a scanning x-ray microscope and a contact x-ray microscope. A magnified imaging x-ray microscope requires radiation of very high brightness to illuminate small samples optimally. It is an analog of the visible-light microscope in which only a portion of the radiation passing through the sample contributes to image formation. Spatial resolution is set primarily by the wavelength  $\lambda$ . A major advantage of this type of soft-x-ray microscope is its simplicity and its ability to produce high-resolution images. A scanning x-ray microscope achieves its spatial resolution by focusing radiation to the smallest possible spot size and then raster scanning the sample to obtain a two-dimensional image. The necessity to build a scanning x-ray microscope results from the advantageously reduced radiation dose of such a system compared with an imaging x-ray microscope.

In the present report, we have tried to observe hydrated chromosome by a contact soft-x-ray microscope and obtained its image. These results suggest that x-ray microscopy will give us the new method to understand the detailed configuration of human chromosomes.

Chromosomes of human lymphocytes were spread on a clean surface of distilled water, attached on an x-ray resist, polymethylmethacrylate (PMMA). The PMMA with a water droplet was immediately mounted in a simple hydrated specimen chamber. The chamber was composed of a silicon nitride window (0.5 mm  $\times$  0.5 mm; thickness, 0.1  $\mu$ m) and PMMA supported by silicon bases. The thickness of water layer was 1-5  $\mu$ m. Figure 1 illustrates the present exposure system. The chamber was placed at the specimen-target distance of 10 mm and exposed to a single shot of laser-produced titanium plasma x-ray source pumped by a pulse train YAG laser (laser wavelength, 1.06  $\mu$ m; energy, 1 J; 16 pulses; each pulsewidth,

\* Present address: Toyota Technological Institute

\*\* Joint appointment in Semiconductors Laboratory

100 ps; interpulse time, 200 ps). The x-rays emitted from the titanium plasma is a broad spectrum with a range of 1.8–2.7 nm. In this spectrum, the spectrum of 2.3–2.7 nm is inside the water window region and highly transparent to water. X-rays in the water window were the main photons contributing to the contrast formation of chromosome. Exposed specimens were treated as following: (1) Removing the specimen from PMMA with sodium hypochlorite, (2) developing the PMMA with a mixed solution of methylisobutylketone and isopropanol (3:1), and (3) observing with an atomic force microscope.

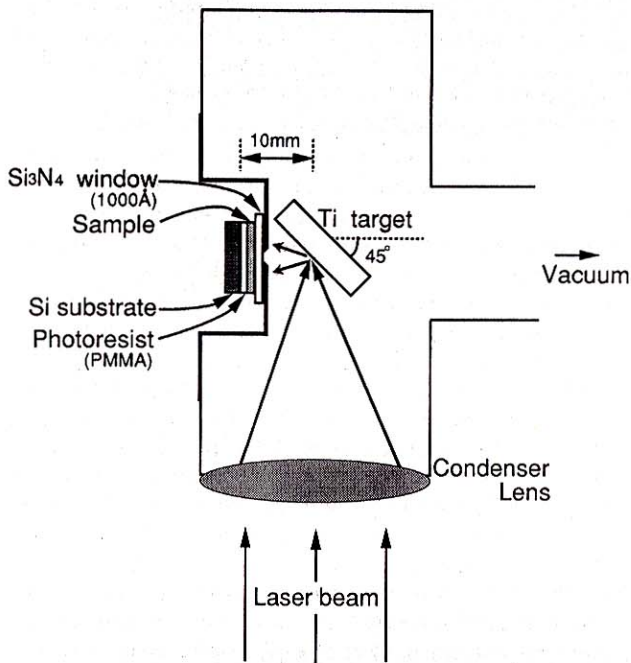


Fig. 1. Schematic diagram of the contact x-ray microscope.

Figure 2 shows an image of human chromosome. The image with weak contrast may be the image for the entangled chromosome fibers unfolded from a chromosome, the remaining core part of which was imaged with clear contrast. From Fig. 2, the present resolution is estimated to be as high as 50 nm. These results indicated that chromosome fibers in a hydrated condition were observed by contact soft-x-ray microscopy

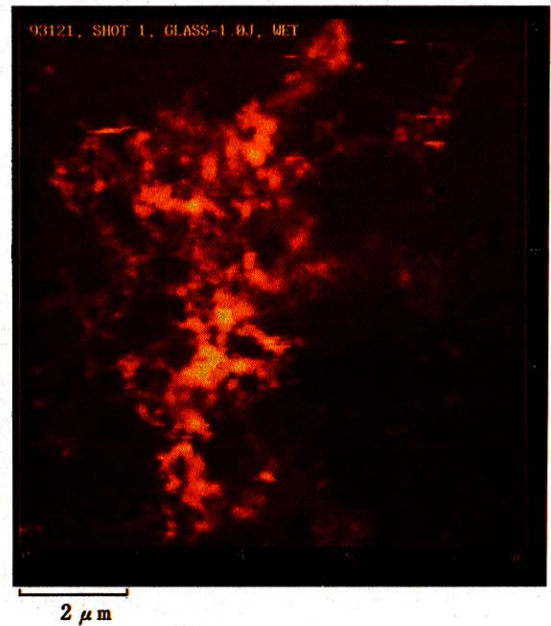


Fig. 2. Single shot x-ray image of a hydrated human chromosome.

using pulse train laser-produced plasma x-ray sources 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 as high as 50 nm.

We have noticed that the  $\text{Si}_3\text{N}_4$  window was broken by a single shot of exposure. The cause for the breakage of  $\text{Si}_3\text{N}_4$  window may be attributed to the temperature increase in the  $\text{Si}_3\text{N}_4$  window and water in addition to the physical damage caused by plasma particles.<sup>1)</sup> The use of x-ray lasers in the water window may reduce this temperature increase remarkably and prevent the breakage of  $\text{Si}_3\text{N}_4$  window.

#### References

- 1) K. Shinohara, Y. Kinjo, M. Richardson, A. Ito, N. Morimoto, Y. Horiike, M. Watanabe, K. Yada, and K. A. Tanaka: SPIE Proc. **1741**, 386 (1992).