19pD1-4

Intense laser-plasma soft-X-ray sources and its application to biological X-ray microscopy

レーザー生成プラズマ軟X線源の高輝度化と軟X線顕微鏡による生きている細胞の観察

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Soft X-ray microscope has a potential to observe live biological cells. But the cells had to be frozen in order to avoid radiation effects onto the biological cells or to be made a short pulse imaging with an intense X-ray source. Otherwise live cell imaging was not realized. Laser-plasma soft X-ray source was extremely bright and had short pulse duration. Hence it makes possible to capture soft X-ray images of biological cells before the cells damaged. Since the spatial resolution of the soft X-ray microscope depends on the photon flux irradiated onto the samples, it is important to increase brightness of the soft X-ray sources to observe inner structures of biological cells. We have increased the brightness of the soft X-ray source and succeeded in observing inner structures of live biological cells.

1. Introduction

Soft X-ray microscope using water window X-rays (2.3 nm~4.4 nm) has ability to observe biological specimens in hydrated environment without any artificial treatment. There have been many works [1-7] to develop several types of soft X-ray microscope such as imaging type, scanning type, projection type and contact type using also various X-ray sources such as synchrotron radiations, laser plasmas, discharge plasmas and X-rays produced by a focused electron beam. The most important issue to be solved to develop a soft X-ray microscope is to avoid radiation effects onto the biological specimens since extremely high X-ray dose resulting in over 10^5 Gy was necessary to image biological specimens [8]. The most common approach to avoid radiation effects is to freeze the biological specimens, which keeps the original structure even after the cells have been damaged [2]. Another approach to avoid radiation effects is to capture an X-ray image of the biological specimens before the specimens have developed structural changes [3-7]. For this purpose extremely bright X-ray sources were required. We have studied a soft X-ray microscope to image live biological cells using very intense laser-produced plasmas as the soft X-ray source.

2. Laser-produced plasma soft X-ray source

Plasma was generated focusing a high power laser pulse, one of the 12 beams of GEKKO XII at the Institute of Laser Engineering, Osaka University, onto a gold foil target. The pulse duration of the laser was 500 ps and the laser energy was set to 30 J, 60 J and 120 J. The wavelength of the laser was also selected to be 1.053 μm, 0.527 μm and 0.351 μm. The laser focused size and the x-ray spectrum were measured with an X-ray pinhole camera and a flat-field soft X-ray spectrometer, respectively. The results of the X-ray spectrometer showed strong dependence on the laser energy and wavelength in spectral shape and total X-ray flux in water window region.

3. Imaging of hydrated biological cells

In order to observe hydrated biological cells we

have fabricated special specimen holder that keeps the biological cells hydrated in vacuum. Leydig cells from mouse testis were directly cultivated on PMMA photoresists for a few days, fixed with formaldehyde, covered with a 200 nm-thick silicon nitride membrane, and enclosed into the specimen holder. The gold-foiled target was attached to the specimen holder. The specimen holder with the gold-foiled target was placed at the center of the target chamber of GEKKO XII and the laser was focused onto the gold target to generate laser-produced plasma. After irradiating X-rays onto the specimen, the cells were removed and the PMMA photoresist was developed with a mixed solution of MIBK and IPA. X-ray images of the biological cells recorded on the PMMA photoresist were read by an atomic force microscope.

Shown in figure 1 is one of the soft X-ray images of a Leydig cell. Parameters of the laser pulse used to obtain the data were 1.053 μm in wavelength, 120 J in energy and 500 ps in pulse duration. Nucleus was clearly recognized at the center of the cell and bright spots surrounding the nucleus were recognized to be mitochondria. Looking at the image carefully very fine hair-like structures were also recognized on the surface.

Such hair-like structures were more obvious in figure 2. Since those hair-like structures were not found before, those images indicated that the soft X-ray microscope combined with an extremely bright plasma X-ray source was a very powerful tool to investigate cellular structures, although very careful discussion was necessary.

4. Summary

Imaging of hydrated biological cells has been conducted with a soft X-ray microscope coupled with a bright laser-plasma soft X-ray source generated by one of the 12 beams of GEKKO XII. Using an extremely bright soft X-ray source improvement of the spatial resolution has been confirmed. In the images of Leydig cells from mouse testis cellular organelles such as mitochondria have been observed. In addition heir-like structures on the surface of the cell were apparently found.

Acknowledgments

This work was performed under the joint research project of the Institute of Laser Engineering, Osaka University. The authors would like to acknowledge the dedicated technical support by the staff at the GEKKO-XII facility and we also acknowledge Prof. Sakawa and Prof. Fujioka of Osaka University and Prof. Morita of Kyushu University for their valuable support. This research was partially supported by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research (C), 25390134, 2014.

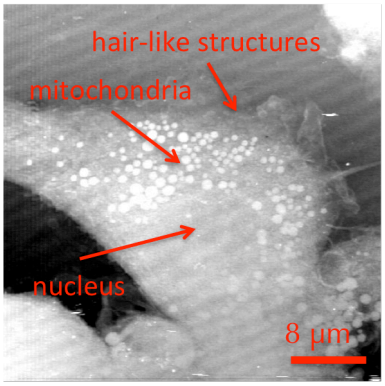

Fig. 1 Soft X-ray image of a Leydig cell showing cellular organelles

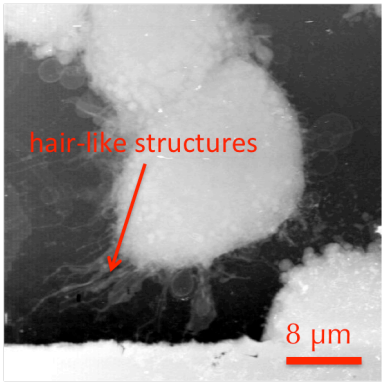

Fig. 2 Soft X-ray image of Leydig cell with hair-like structures

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