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Conceptual Design of Soft X-ray Microscopy for Live Biological Samples

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This study describes the conceptual design of a soft x-ray microscope system based on a laser-based source for biomedical application with high resolution ($\leq$50nm). The laboratory scale soft x-ray microscope consists of high power laser plasma x-ray source and grazing incidence mirrors with high reflectivity. The laser plasma source used for developing this system employs Q-switched Nd-YAG pulsed laser. The laser beam is focused on a tantalum (Ta) target. The Wolter type I mirror was used as condenser optics for sample illumination and as objective mirror for focusing on a detector. The fabrication of the Wolter type I mirror was direct internal cutting using ultra-precision DTM. A hydrated biological specimen was put between the two silicon wafers, the center of which was Si_3N_4 windows of 100nm thickness. The main issues in the future development work are to make a stable, reliable and reproducible x-ray microscope system.

OCIS codes : 170.3880, 170.7440, 180.7460, 340.7460.

I. INTRODUCTION

Soft X-ray microscopy provides a unique set of capabilities in-between those of visible light and electron microscopy [1,2]. It has long been recognized that nature provides a 'water window' spectral region between the K shell x-ray absorption edges of carbon ($\sim$284eV) and oxygen ($\sim$543eV), where organic materials show strong absorption and phase contrast, while water is relatively non-absorbing [3,4]. This enables imaging of specimens that are several microns thick with high intrinsic contrast using x-rays with a wavelength of 2.3$\sim$4.4 nm [5].

The most direct advantage of x-ray microscopes is their high spatial resolution when compared with far-field visible light microscopes, combined with an ability to image hydrated specimens that are several microns thick with a minimum of preparation. In recent years, the promise of high-resolution imaging has been realized, thanks to advances in x-ray sources, focusing optics and specimen preparation methods, and several microscopes can now image biological specimens at about 50-nm resolution. Many works have been performed using synchrotron radiation sources and zone plates, and resolutions better than 50nm were achieved.

Although synchrotron radiation is a powerful source, it is not necessarily convenient for routine work because the machine time is severely limited and the cost is very high. Soft x-ray microscopes on a laboratory scale overcome these problems. There are some groups developing a soft x-ray microscope on a laboratory scale. The cooperative group between Goettingen and Aachen universities has been developing a microscope using a plasma source and zone plates, and the resolution of about 0.1μm was obtained [6]. On the contrary, our work has been developing a microscope using a laser-produced plasma source and a Wolter mirror.

Although most of the experimental arrangement of a synchrotron x-ray microscope is of table-top scale, the x-ray source is obviously not. This means that all users have to bring their samples to the microscope to do experiments, which in many cases is neither convenient nor possible. The development of a compact soft x-ray microscope would make it possible to place the instrument in the laboratory of the biological or other researcher. Thus, we try to develop a compact soft x-ray microscopy based on a laser plasma source and Wolter optics.

II. OPTICAL SYSTEM WITH LASER PLASMA SOFT X-RAY

The optical system of the grazing incidence soft x-ray microscope with a laser-produced plasma source is schematically shown in Fig. 1 [8]. The X-ray microscope consists of a laser-produced plasma source, a condenser mirror, a Wolter type objective mirror and a back-illuminated type CCD detector to record a soft x-ray image. The laser plasma x-ray source is produced by focusing laser pulses 1,064nm wavelength from a Q-switched Nd-YAG laser onto a tantalum target. The laser operates at 20Hz with pulse duration of 8ns and pulse energy up to 1.2J. The laser was focused to a spot about 80μm in diameter on the target at an angle of 45° to the normal. The area of the plasma emitting X-rays was about 150μm in diameter. The wavelength region of X-rays to be radiated was mainly between 3 and 7nm. Between the target and the condenser a filter of titanium foil (100nm thickness) was put to eliminate visible and infrared radiation from the plasma source. Fig. 2 shows the transmission according to thickness of a titanium filter [9].

The x-ray source was focused on a specimen plane by a condenser Wolter mirror. X-rays transmitted through a specimen were focused on a detector by the objective Wolter mirror.

The back-illumunated CCD camera was used as a detector for the alignment of the optical elements. The high sensitivity and the wide dynamic range of the CCD make it possible to reduce the soft x-ray dose to a specimen and to observe the specimen with minimum radiation damage. However the spatial resolution was restricted by the pixel size of the CCD (13×13μm) resulting in the resolution becoming worse than 0.7μm. In order to accomplish the resolution of 50nm, we introduced an image intensifier to magnify(×10) the x-ray signal coming from the objective optics.

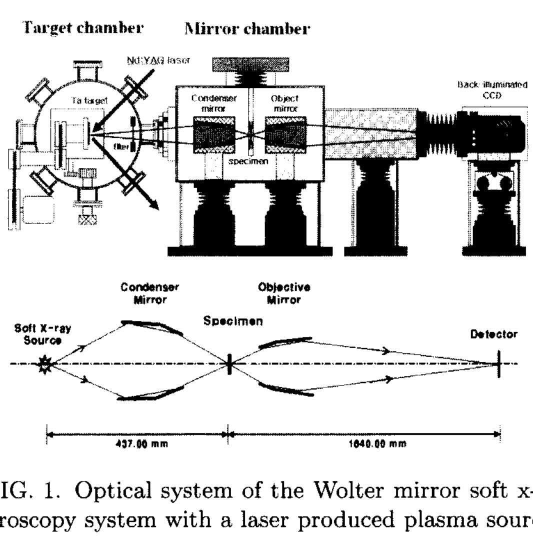

FIG. 1. Optical system of the Wolter mirror soft x-ray microscopy system with a laser produced plasma source.

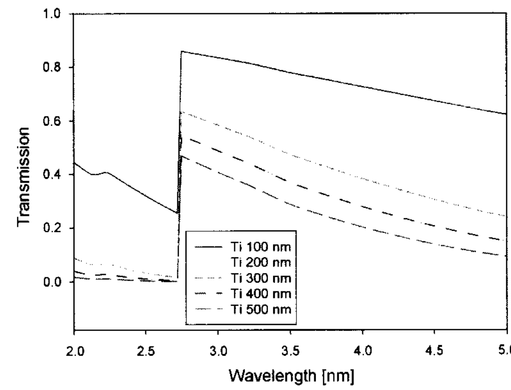

FIG. 2. Transmission of Titanium filters with variation thickness

III. OPTICAL SYSTEM DESIGN

In this present study, we used Wolter type I, ellipsoid-hyperboloid pair, mirrors for both, although others like spherical and elliptical mirrors as a condenser mirrors, can be combined with x-ray focusing devices. Wolter showed that a grazing incidence x-ray imaging system must have an even number of reflecting surfaces in order to get good image quality [7]. Wolter type I optics for an x-ray microscope is composed of two co-axial, confocal ellipsoidal and hyperboloidal surfaces of revolution. When the reflected x-rays reach the hyperboloidal surface, they are reflected again to form a real image at another hyperboloid focus. This grazing incidence mirror has essential advantages such as no spherical aberration and a high x-ray collecting efficiency.

It is possible to design the condenser and the objective mirrors independently, however what is important is to match and to determine the numerical apertures of two mirrors. We impose the condition, the numerical aperture matching, to them. Fig. 3 and Table 1 show the parameters of mirror design for a soft x-ray microscope. The mirror design was calculated under the conditins of the reflector material was the nonelectrolytic nickel, demagnification of 4 and magnification of 32, and the grazing incidence angle for the condenser mirror was 35 *mrad*, which was evaluated at

TABLE 1. Design Specifications of the Condenser and Objective Wolter Mirror

Parameter		Condenser mirror	Objective mirror
Magnification		1/4 X	32 X
Focal length(mm)		437.0000	1640.0000
Eccentricity	Ellipsoid	0.9994115	0.9999048
	Hyperboloid	1.0023512	1.0030416
Semimajor axis(mm)	Ellipsoid	283.0810369	844.1774565
	Hyperboloid	64.2633115	24.0240261
Length(mm)	Ellipsoid	26.0000	15.9899
	Hyperboloid	22.8530	13.0751
Diameter(mm)	Front	19.2195	8.7053
	Intersection	18.8746	10.8924
	Rear	15.3433	11.6884

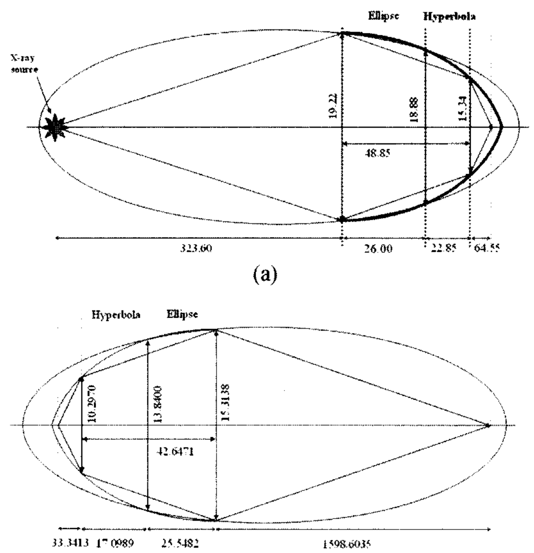

FIG. 3. Design parameters of the condenser mirror and objective mirror (Wolter type I, unit : mm) (a) Condenser mirror (Magnification: ×1/4, Glancing angle: 35 *mrad*, wavelength: 2.3 nm, roughness: 2 nm RMS) (b) Objective mirror (Magnification: ×32 Glancing angle: 28.86 *mrad*, wavelength: 2.3 nm, roughness: 2 nm RMS).

the 2.3 nm x-ray wavelength and 2 nm RMS surface roughness. The grazing incidence angle for the objective mirror is 28.86 *mrad* which comes close to the average angle in the hyperboloid or ellipsoid. The average reflectivities of condenser and objective mirrors are respectively 51.87% and 61.24% and points near the intersection of two surfaces still give the highest reflectivity.

IV. SAMPLE HOLDER FOR HYDRATED BIOLOGICAL SPECIMENS

To observe a hydrated biological specimen, an environmental chamber was necessary to keep the specimen in a wet state under vacuum surroundings. The chamber consisted of two silicon wafers, the centers of which were Si_3N_4(100nm thickness) windows of a 200μm square. The windows were made by a photo lithographic method. The transmission of a window at 400eV energy is about 70% (Fig. 4). A hydrated biological specimen was put between the two silicon nitride windows (Fig. 5). With syringes connected to the chamber by tubes, liquid can be pumped in and

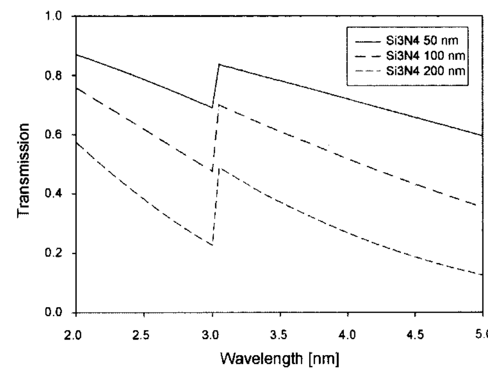

FIG. 4. Transmission of silicon nitride (Si_3N_4) windows with variation thickness

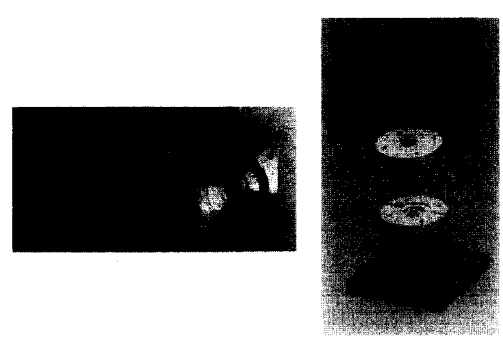

FIG. 5. Photograph and 3-D modeling of the sample holder for hydrate living cell with two tube and syringes attached. Two silicon wafers with thinned silicon nitride central parts have been placed in the center of the image.

out and the liquid layer thickness can be adjusted. Thus, sample can be kept under wet conditions in this chamber for a relatively long time. Cells have been kept alive in the holder for more than a day.

V. CONCLUSION

In this paper, we reported the conceptual design of a soft x-ray microscope system for biomedical application with high resolution and shorter exposure time. The laboratory scale soft x-ray microscope consists of high power laser plasma x-ray source and grazing incidence mirrors (Wolter type mirror) with high reflectivity. The laser plasma source used for developing this system employs Q-switched Nd-YAG pulsed laser. To achieve high reflectivity of the mirror, we have designed Wolter type I mirrors as condenser optics for sample illumination and as objective mirror for focusing on a detector. A hydrated biological specimen will be put between the two Si wafers, the center of which was Si_3N_4 windows of 100nm thickness. We considered, for the observation of hydrated living cells, the concept and design of a soft x-ray microscope, including the importance of different design variables.

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