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NOTE

Compact soft x-ray transmission microscopy with sub-50 nm spatial resolution

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Abstract

In this paper, the development of compact transmission soft x-ray microscopy (XM) with sub-50 nm spatial resolution for biomedical applications is described. The compact transmission soft x-ray microscope operates at $\lambda = 2.88$ nm (430 eV) and is based on a tabletop regenerative x-ray source in combination with a tandem ellipsoidal condenser mirror for sample illumination, an objective micro zone plate and a thinned back-illuminated charge coupled device to record an x-ray image. The new, compact x-ray microscope system requires the fabrication of proper x-ray optical devices in order to obtain high-quality images. For an application-oriented microscope, the alignment procedure is fully automated via computer control through a graphic user interface. In imaging studies using our compact XM system, a gold mesh image was obtained with 45 nm resolution at $\times 580$ magnification and 1 min exposure. Images of a biological sample (*Coscinodiscus oculoides*) were recorded.

(Some figures in this article are in colour only in the electronic version)

Introduction

Soft x-ray microscopes are becoming able to provide information to complement that obtained from optical and electron microscopy. Soft x-ray microscopy can deliver 15 nm resolution images of hydrated cells up to ∼10 microns thick, and efforts towards obtaining higher resolution are under way (Jacobsen 1999, Chao *et al* 2005). The few full-field transmission x-ray microscopes currently in operation rely on high brightness synchrotron radiation sources in order to achieve short exposure times. Since transmission x-ray microscopes (TXRM) which use zone plates as objective lenses for high resolution imaging were first developed at the University of Göttingen in Germany and at the SUNY at Stony Brook (USA) (Schmahl *et al* 1993, Kirz *et al* 1995), various applications in, for example, studies of malaria (Magowan *et al* 1997), sperm (Abraham-Peskir *et al* 1998), and cytoskeletal elements (Scherfeld *et al* 1998) have been found. Although the synchrotron based XM can produce high resolution images within short exposure times, it is very inconvenient for its users. Therefore, compact non-synchrotron x-ray microscopy has been developed since the first report of Da Silva *et al* as it uses a single-shot x-ray laser source outside the water window (Da Silva *et al* 1992).

Compact, non-synchrotron based x-ray microscopes employ compact laser-produced plasma (LPP) or pinch plasma sources. In the water window (figure 1), attempts at compact high resolution transmission microscopy have been based on zone plate optics and LPP or pinch-plasma sources. A solid carbon target LPP has been combined with an elliptical condenser and a zone plate objective to perform imaging of dry test objects (Nakayama *et al* 1994). The XM system image thus produced was magnified about 286 times, and the resolution was slightly better than that of an optical microscope. However, the debris emission from non-regenerative character of the solid carbon target results in limited operability of this system. A low repetition rate pinch plasma source has subsequently been combined with an elliptical condenser and zone plate optics for wet and dry imaging. The low repetition rate and instability of the source make this system less operative, although 100–150 nm features were detectable on dry objects with a low signal-to-noise ratio. For both sources, the somewhat large line width (typically $\lambda/\Delta\lambda = 100$–$300$), producing chromatic aberrations in the diffractive zone plate optics, results in limited extension towards very high-resolution x-ray microscopy.

In this paper, we demonstrate that high spatial resolution (sub-50 nm) water window transmission x-ray microscopy can be performed with reasonable exposure times and good contrast with a tabletop arrangement using zone plate imaging. It allows long term operation due to the negligible debris emission from the source, has high average power due to the regenerative target type, and produces the narrow line width allowing extension to very high resolution imaging. The major results presented in this paper are the development and performance of the system on gold mesh and diatom samples.

Materials and methods

Due to the development of high-power laser technology, LPP sources become excellent compact sources for hard x-ray, soft x-ray and EUV lights. LPP x-ray sources can be broad band or quasi-monochromatic with a proper choice of material and filter. Carbon and nitrogen have suitable line transitions in the water window region. Solid targets, such as carbon and boron nitride that contain carbon and nitrogen, are cheap and easily accessible. One problem with the LPP x-ray source from a solid target is the production of debris which can coat and scratch delicate x-ray optics, thereby degrading their performance. To overcome these problems of solid targets, various ideas including gas target, liquid target, gas-shielding,

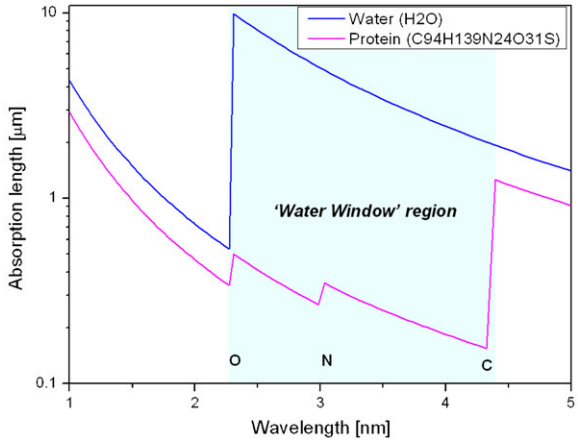


Figure 1. The absorption length for soft x-rays in water and protein as a function of wavelength. The spectral range between carbon and oxygen absorption edges is called the *'water window'* and offers a natural contrast for biological materials.

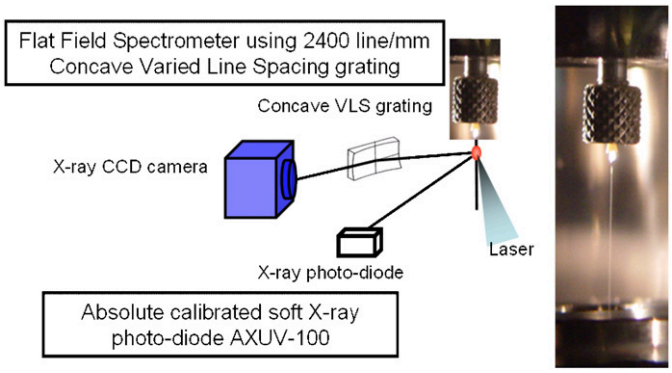


Figure 2. Experimental set-up for radiation characteristics of x-ray sources.

etc were suggested and tested. Considering the radiation intensity and the amount of debris produced, a liquid target seems to be the best choice.

We have endeavoured to develop a quasi-monochromatic source at 2.88 nm (N VI 1s2-1s2p transition) using a liquid nitrogen jet. The experimental apparatus used in this study is shown schematically in figure 2. Figure 3 shows a typical quasi-monochromatic spectrum obtained with a Ti 200 nm/Al 150 nm filter (the inset shows the transmission of such a filter). The strong single line is N VI 1s2-1s2p line transition at 2.88 nm (430 eV) and the weak one at 2.48 nm (500 eV) N VII 1s-2p line. The spectral resolution at 2.88 nm bandwidth was measured as $\lambda/\Delta\lambda = 1000$. This monochromatic radiation is well suited to imaging with zone plates.

High average power is very important for short exposure time in imaging and may be obtained by using high-repetition-rate lasers. For the imaging experiment, a 300 Hz rep-rate Nd:YAG-laser at 1064 nm with a pulse duration of 800 ps and a pulse energy of 40 mJ/pulse (JMAR, San Diego, USA) was used. The laser was focused on liquid nitrogen with a spot of about 10 μm in diameter on the target at an angle of 45° to the normal plane. The source size was measured by an x-ray pinhole camera. The source was vertically elongated because the

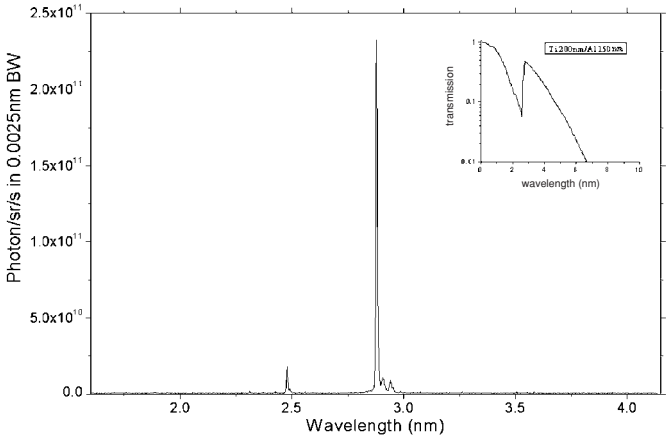


Figure 3. Typical spectrum obtained with a Ti/Al filter, showing a quasi-monochromatic source suitable to imaging with zone plates. The spectral resolution at 2.88 nm bandwidth was measured as $\lambda/\Delta\lambda = 1000$.

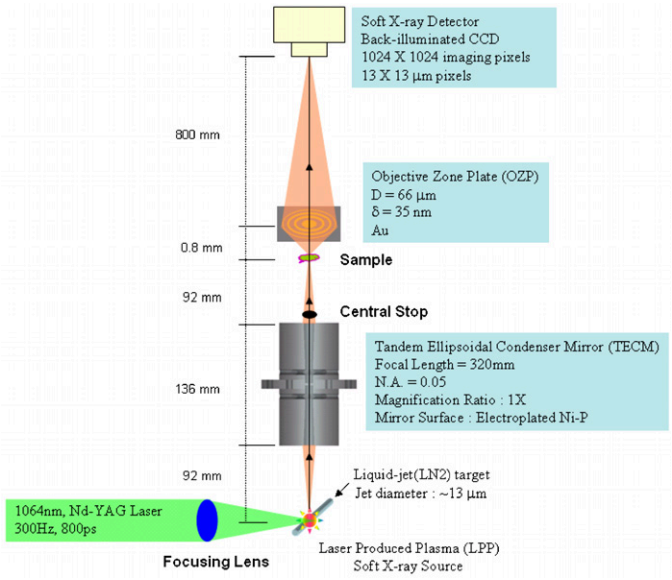


Figure 4. Optical system layout of the compact vertical soft x-ray microscope system using a laser-produced plasma source.

laser spot size at the focus was larger than the liquid jet diameter in this experiment. The source size was measured to be 20 micron in vertical dimension. Between the target and the condenser mirror a filter of Ti/Al thin film was put in place to eliminate visible and infrared radiation from the plasma source, as well as scattered laser light. The absolute intensity of the source was measured using an XUV spectrometer and an absolutely-calibrated x-ray photodiode. The estimated maximum photon number is 4×10^{11} photons/sr/pulse at 1.5×10^{14} W cm^{-2} of a high power laser.

The optical system of the compact, laboratory-scale soft x-ray microscope is schematically shown in figure 4. The optical set-up of the x-ray microscope is equivalent to visible light

transmission microscopes. Radiation from a liquid-jet target LPP x-ray source is focused by a condenser reflective mirror onto the sample. A micro-zone plate as an objective lens behind the sample forms a transmission image on an x-ray detector. Between the LPP x-ray source and the condenser mirror a filter of titanium (200 nm) and aluminium (150 nm) thin film was put to eliminate not only visible and infrared radiation but also scattered laser light from the plasma source.

The optical system of the soft x-ray microscope system was designed for 50 nm spatial resolution and an exposure time of less than 1 min. We use a grazing incidence water window tandem ellipsoid condenser mirror (TECM). The TECM has the advantage of increasing the photon density in the objective plane. This is necessary for high spatial resolution imaging with a diffractive zone plate lens. The TECM design was calculated under the condition that the reflector material was electroless nickel, magnification of $1\times$, the grazing incidence angle was 4.03°–5.04°, and the geometrical solid angle of the condenser mirror was 48.1803×10^{-4} sr, which was evaluated at the 2.88 nm x-ray wavelength and 2 nm root-mean-square (RMS) surface roughness. The designed TECM has a focal length from the plasma source to the mirror front end of 92 mm, a mirror length of 29.1 mm, a maximal diameter of 5.2 mm, and a minimal diameter of 4.1 mm. The objective optics we used included a Fresnel zone plate (Xradia, CA, USA) for high spatial resolution imaging in our soft x-ray microscopy, which was designed under a magnification of 1000 times, an outmost zone width of 35 nm, a diameter of 66 μm and an object-to-image distance of about 800 mm. The transmission efficiency of the zone plate was 3–10% at 2.88 nm. We used a back-illuminated charge coupled device (CCD) camera (Princeton Instruments, NJ, USA) with an array of 1024×1024 pixels ($13\ \mu\text{m} \times 13\ \mu\text{m}$ pixel size). With the CCD camera at magnification of 580 times, each pixel corresponds to 22.5 nm and the resolution is limited to about 45 nm.

To obtain a soft x-ray microscope image, the optical elements must be aligned very accurately in the designed position. Their position errors cause deterioration in the image quality and make the image invisible at worst. Because the designed optical system has high magnification of 1000 times and the size of the x-ray source and OZP are small, an alignment requires a few micron tolerances. However, this is not so easy as x-ray is not visible and the microscope is operated in a vacuum condition. Therefore, a proper method is required, and all of the optical elements are designed to be controlled having a proper degree of freedom (DOF). As the sensitive errors of the optical elements are all different, the DOF of each optical component was determined by optical simulations. The source, OZP and CCD have three DOFs of X, Y and Z directions and the condenser mirror has five DOFs of X, Y, Z and tilt (θ_X, θ_Y) directions. (The Z axis is the vertical optical axis.) The alignment of the condenser mirror is the most tedious work and we designed the special alignment stage for this purpose. It is composed of the cross-roller-guided XY stage and the kinematic mount based tilt and the Z stage.

In this paper, we present some new ideas for making the alignment easier. First, the magnification was reduced by moving the CCD downward to loosen the alignment tolerance between the illumination and the objective zone plate because the high magnification magnifies this tolerance. Second, scattered laser light was used in order to align the reflective condenser mirror. If the condenser mirror is aligned, the ring pattern by its optical properties is irradiated at the CCD plane, and as the size of the ring pattern is larger than that of the CCD field, nothing is visible at the CCD. In the case of scattered light, it is possible to let the pattern be visible in the CCD by using a normal lens and we can align the condenser mirror using this normal lens without any additional equipment. But, in the case of x-rays, this is impossible due to optics problems. The filter to let only x-rays pass was designed to be removed with a linear motion component. Third, the alignment optics of the OZP and the specimen were used. As

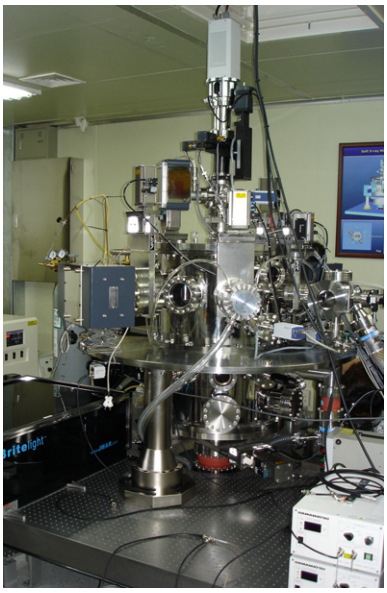


Figure 5. Photograph of a vertical type compact soft x-ray transmission microscope system for biomedical application.

the visible light microscope was used for this, we can measure the relative distances between them in the x, y and z directions. An aspheric objective lens (NA is 0.5) was used with a hole to prevent interference with the x-rays.

Results

The development of compact soft x-ray transmission microscopy would make it possible to place the instrument in the laboratory of biological or other researchers. The new compact x-ray microscopy system offers a very stable mechanical construction, easier alignment of the optics, and an easy-to-use sample handling system. These types of requirements must be fulfilled to enable the system to be used for biomedical applications. Figure 5 shows a photograph of a compact soft x-ray microscope.

The principal goal of compact x-ray microscopy is to pursue hydrated applications within the life sciences and hence the choice of a laser-plasma source operating within the water window. Although the sample handling for soft x-ray imaging is generally quite simple, the preparation and characterization of the samples can be quite complicated and require special equipment not accessible at the few existing soft x-ray microscopy facilities.

The compact soft x-ray microscope has demonstrated gold mesh imaging with a 7.5 μm hole and a 5 μm bar width. Figure 6 is a gold mesh (#2000, pitch size of 12.5 μm) image measured by $\times$580 magnification and 1 min exposure. The spatial resolution which was measured using the line spread function was about 45 nm. The image quality can be improved by finer alignment and increased magnification.

In order to test the performance of the compact soft x-ray transmission microscope for biological application, a diatom (*Coscinodiscus oculoides*) was used as a standard test specimen. In figure 7, a diatom was imaged with $\times$530 magnification and an exposure time of 3 min. With sub-100 nm resolution, the hexagonal structures of the diatom were clearly seen but could not be seen with the optical microscope.

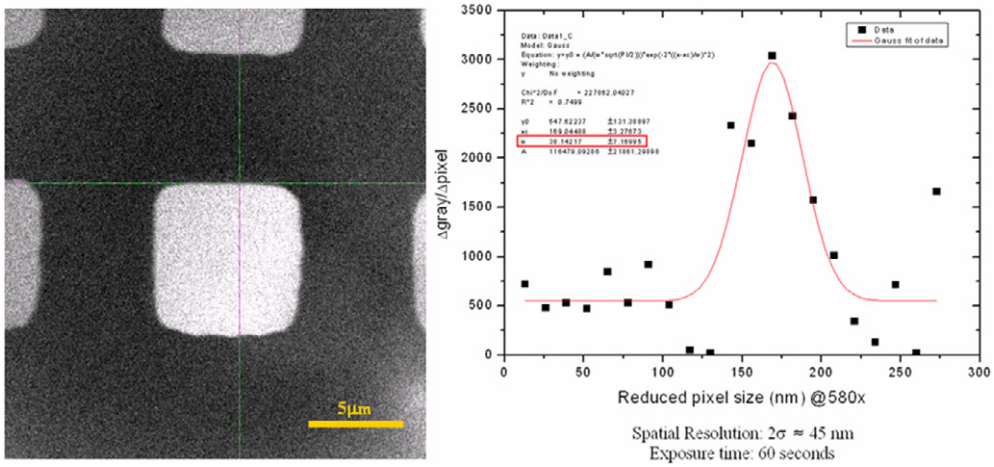
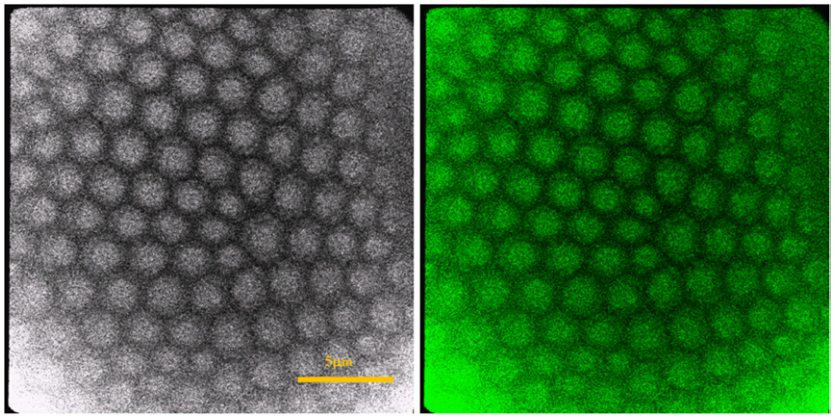


Figure 6. An x-ray microscopy image of gold mesh (12.5 μm pitch) obtained with $\times$580 magnification at a 1 min exposure time. The spatial resolution is approximately 45 nm.



(a) (b)

Figure 7. An x-ray microscopy image of a diatom (*Coscinodiscus oculoides*) with $\times$530 magnification. The inner hexagonal structure of the *Coscinodiscus oculoides* can be clearly seen in both the x-ray microscope image (a) and the pseudo colour image (b).

Discussion and conclusion

The compact x-ray microscope can fill the gap between electron microscopy and optical microscopy. In addition, as it has a natural contrast between water and carbon-based substances in the water window region, it is suited for research in biology, medicine, material science and colloidal chemistry.

In the 1990s, the non-synchrotron-based soft x-ray microscope employing laser-produced plasma was developed for imaging cells. Johansson *et al* developed compact x-ray microscopy which consisted of a liquid-jet x-ray source, a normal incidence condenser multilayer mirror and an objective zone plate (Berglund *et al* 2000, Johansson *et al* 2002). Their microscope demonstrated approximately 50 nm spatial resolution, but the major drawbacks of their system

were transmission efficiency of the condenser mirror, source brightness, long exposure time, and low signal-to-noise ratio. Also, Hoshino *et al* have fabricated and examined the Wolter mirror for the x-ray microscope using a solid target LPP source (Hoshino *et al* 2004). They were experiencing difficulties in mirror fabrication to obtain high resolution. Until now, compact x-ray microscopes have not been able to show the specimen images with reasonable resolution, signal-to-noise ratio and exposure time.

In this paper, we presented an optical layout of an application-oriented laboratory-scale compact soft x-ray microscope using the LPP source, TECM, OZP and the alignment method. The liquid-jet source has enough spectral resolution ($\lambda/\Delta\lambda = 1000$) to use a zone plate, is debris-free and suitable for high average power operation. This monochromatic radiation is well suited to imaging with zone plates. TECM has essential advantages such as no spherical aberration and a high x-ray collecting efficiency. The fabrication of TECM was direct internal cutting using an ultra-precision diamond turning machine with polishing processes. The precisions of TECM fabricated were 2 nm RMS surface roughness and 50 nm P-V surface figure errors.

As a result, we obtained a 1 min exposure image having about 45 nm resolution measured at $\times$580 magnification and obtained a diatom *(Coscinodiscus oculoides)* image with 3 min exposure time. We will continue to improve the image quality such as resolution and constrast and reduce the exposure time by increasing the magnification and the x-ray source intensity and solving the mechanical instability and vibration problems.

For future biomedical application, testing of biomedical specimens is currently being performed to provide for the instrument development of the compact soft x-ray transmission microscope. Presently, most of the development with biological applications concerns dry specimens, as no wet cell has yet been implemented. With the current wavelength and photon flux, only relatively thin biological samples can be imaged. For a wet cell application, a thin chamber which protects the hydrated specimen from the vacuum system is under development. To learn more about the function of a structure or about a specific protein inside a cell, it is essential to localize them during various times of the cell cycle. A common way to do this is by immunogold labelling, which has been found very attractive for soft x-ray microscopy. In the near future, we will image several biological samples and will present our results.

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