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Zernike phase-contrast x-ray microscope with pseudo-Kohler illumination generated by sectored (polygon) condenser plate

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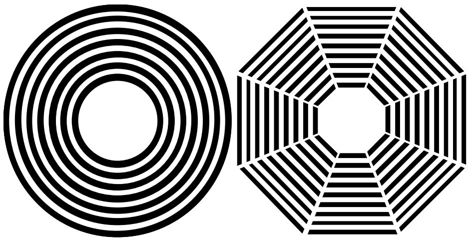
Abstract. Zernike phase contrast x-ray microscope has been developed at the undulator beamline 20XU and 47XU of SPring-8. The system consists of a pseudo-Köhler-illuminating system, a Fresnel zone plate objective with outermost zone width of 100 nm, a Zernike phase plate (0.96-μm-thick tantalum, $\lambda/4$ or $3\lambda/4$ phase-shifter at 8 keV) installed at the back-focal plane of the objective, and a visible-light conversion type cooled CCD camera as an image detector. A sectored (polygon) condenser plate is employed as the condenser in order to secure a large and flat field of view. Details and experimental results of the system will be shown.

1. Introduction

Full-field transmission x-ray microscope (TXM) with Fresnel zone plate (FZP) objective has been developed at the undulator beamline 20XU and 47XU of SPring-8 aimed for 100-nm-resolution x-ray CT [1-4]. Since the field of view of CT system determines the measurable size of object, large and flat image field is required for the TXM optics as well as the spatial resolution. From this point of view, employing of a Köhler-illumination is one of the best choices. Therefore, in order to produce a (pseudo-) Köhler-illumination, we have previously developed a circle condenser plate (CCP) which has concentric diffractive grating patterns with even pitch (Fig. 1(a)). The spatial resolution of the system has been reached to 160 nm, and the CT using this system is now in operation constantly showing the spatial resolution of better than 300 nm in three-dimension [4].

However, obtained field of view had an extremely inhomogeneous intensity distribution proportional to the inverse of transverse distance from optical axis (gray line in Fig. 2). In order to solve this problem, we newly developed a sectored (polygon) condenser plate (SCP) which consists of



(a) (b)

Figure 1. Schematic drawing of condensers, (a) circle condenser plate (CCP) and (b) sectored condenser plate (SCP).

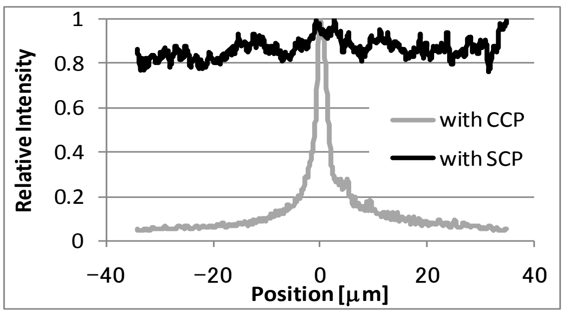


Figure 2. Intensity profiles of image field, gray line: with CCP, black line: with SCP.

plural diffractive gratings with even pitch (Fig. 1(b)). As shown in the figure, these gratings are arranged in a radial manner so that all the +1st diffractions are gathered at object plane. The use of SCP successfully produces even intensity distribution over the field of view of 50-100 µm (black line in Fig. 2). Calculated modulation transfer functions (MTF) of the TXM system using a CCP and using an octagon SCP with same optical parameters are shown in Fig. 3. Because the illumination system with the SCP gives eight oblique illuminating beams, the MTF shows a step-function. Therefore, the MTF curve has different shape from that of CCP system. However, they have the same diffraction-limit being superior to the case of parallel illumination.

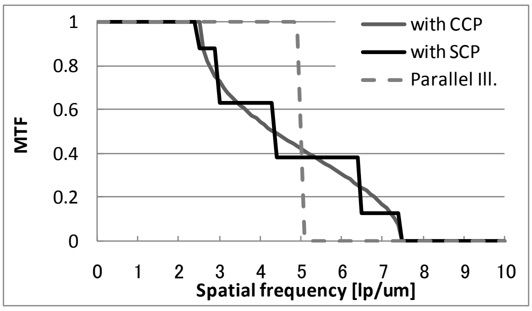


Figure 3. Calculated MTFs of TXM system, gray line: with CCP, black line: with SCP, broken gray line: parallel illumination.

On the other hand, in the user-experiment, requirement of use of phase-contrast imaging has become higher and higher because of the miniaturization of volume of object related to the improvement of spatial resolution. As an optional use of the TXM system, a Zernike phase contrast x-ray microscope (ZXM) coupled with the Köhler-illuminating system has been developed (Fig. 4). Principle of the Zernike phase contrast method is that the filtering of Fourier spectrum of the object formed at the back-focal plane. It is well-known that the Köhler-illumination is very suitable for the Zernike phase contrast method because the diffracted rays of the object and non-diffracted rays (0th-order) are spatially sorted at the back-focal plane.

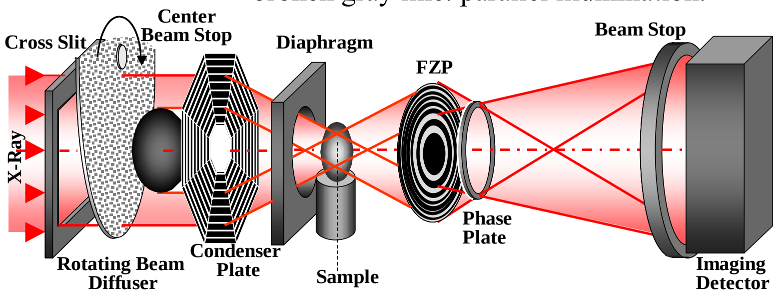


Figure 4. Schematic diagram of Zernike phase-contrast x-ray microscope (ZXM) optical system.

2. Experimental setup

A SCP consists of 8 sectors of diffractive gratings with even pitch of 400 nm (200 nm line width) is used as the condenser. Total width of the SCP is 1000 µm, and the pattern is drawn with tantalum (1.65 µm thick). A FZP with outermost zone width of 100 nm is used as the objective. The zone material is tantalum with 1 µm thick. A Zernike phase plate (ZPP) made from tantalum with 0.96 µm thick is placed at the back-focal plane of the objective FZP. In this case, the ZPP works as a $\lambda/4$ phase shifter for 8 keV x-rays. A pair of positive and negative ring patterns is drawn in the ZPP, and one can choose between the bright phase contrast mode and dark phase contrast mode by selecting these ring patterns. Line width of each ring pattern is 4 µm. Used SCP, FZP and ZPP are fabricated by NTT-AT, Japan. A cooled CCD camera (C4880-41S, Hamamatsu Photonics, Japan) coupled with relay lens and scintillator (P43) screen is placed at the image plane. The CCD camera is used under the 2×2 binning mode and in this case the effective pixel size and the camera format are 3.4 µm and 2000×1312 pixels, respectively. A rotating beam diffuser (letter paper) is installed in front of the SCP in order to eliminate speckle noise. Angular spread of the beam behind the diffuser is about 13 µrad in FWHM.

Black line in Fig. 5 shows a calculated contrast transfer function (CTF) of the ZXM system under the dark phase contrast mode. Periodic rectangular tantalum pattern with 0.5 µm thick is assumed as the object. Although the CTF in high frequency region is the same as that of TXM mode, as shown in Fig. 3 and Fig. 5, it drastically decreases in the low frequency region because of the finite width of the phase ring (in this case, the line width is 4 µm). It is because there is no phase shift if the distance at the Fourier space between the diffracted beam and the non-diffracted beam is smaller than the line width of phase ring. Therefore, image contrast of the lower frequency region is almost same as the TXM mode. In this case, the image contrast at the frequency region lower than 0.129 μm^{-1} corresponding to the 4 μm in real space is approximately 0.067, that is just same as transmission image contrast of tantalum 0.5 μm thick. Since the CTF of ZXM mode has characteristics of a band-pass filter as shown in Fig. 5, therefore, edge-enhance-like contrast modulation is observed in the image.

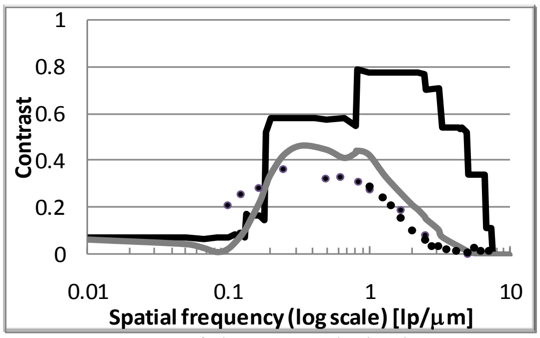
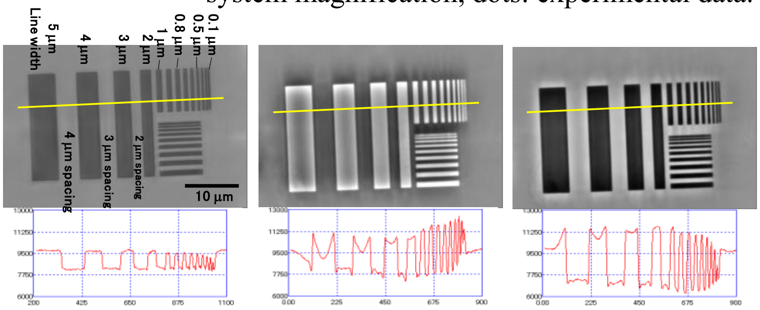


Figure 5. CTFs of the ZXM dark phase contrast mode. Black line: calculated value, gray line: calculated value considering with beam spread with diffuser, detector MTF and system magnification, dots: experimental data.

3. Results

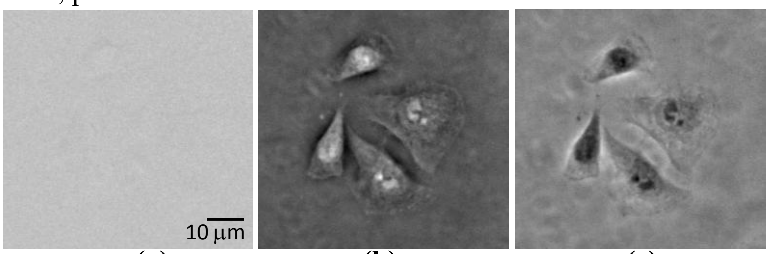
Figures 6(a)-6(c) show x-ray images (above) and intensity profiles (below) of tantalum test pattern with 0.5 μm thick observed with TXM mode, ZXM bright phase contrast mode, and ZXM dark phase contrast mode, respectively. Although clear image is obtained even with the TXM mode, the ZXM mode images show several times higher contrast than the TXM mode. There is no difference in spatial resolution between three modes, however, edge-enhance-like contrast modulation is seen in the ZXM mode images due to the band-pass filtering effect. Image contrast under the ZXM dark phase contrast mode is plotted in Fig. 5. This shows a good agreement with the calculated CTF (gray line in Fig. 5).



(a) (b) (c)

Figure 6. X-ray images and intensity profiles of tantalum test pattern (thickness: 0.5 μm), (a) TXM image, (b) ZXM bright phase contrast, and (c) ZXM dark phase contrast image. Beamline: BL47XU, x-ray energy: 8 keV, exposure time: 0.15 sec, pixel size: 47 nm.

Figure 7 shows x-ray images of human cervical cancer HeLa cells. The samples are cultured on a Si_3N_4 window, fixed with gultaraldehyde and then dried by critical point drying. Although the sample is hardly seen with the TXM mode, detailed structures such as the nucleolus in the nucleus are clearly observed with both of ZXM modes.



(a) (b) (c)

Figure 7. X-ray images of human cervical cancer HeLa cells (dry), (a) TXM image, (b) ZXM bright phase contrast, and (c) ZXM dark phase contrast image. Beamline: BL20XU, x-ray energy: 8 keV, exposure time: 30 sec, pixel size: 130 nm.

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