Fresnel zone-plate based X-ray microscopy in Zernike phase contrast with sub-50 nm resolution at NSRL

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Abstract. A transmission X-ray microscope using Fresnel zone-plates (FZPs) has been installed at U7A beamline of National Synchrotron Radiation Laboratory (NSRL). The objective FZP with 45 nm outermost zone width delivers a sub-50 nm resolution. A gold phase ring with 2.5 μm thickness and 4 μm width was placed at the focal plane of the objective FZP at 8 keV to produce a negative Zernike phase contrast. A series of samples were used to test the performance of the Zernike phase contrast X-ray microscopy.

1. Introduction
The key power of the FZP based multi-keV X-ray microscopy is the large penetration of the X-rays and the several tens of nanometers resolution. It is potential to image the interior nanoscale features in thick objects, such as microprocessor chip, micro/nano-sized materials, and biological samples. However, the absorption contrast of the image diminished rapidly with the photon energy, especially for objects consisted of light elements. Therefore, methods are desired to improve the image contrast. One method is the Zernike phase contrast microscopy proposed by Zernike [1]. A similar method was pioneered in the soft X-ray region [2] and was then applied to multi-keV X-ray microscopy.

Here we report a FZP based transmission X-ray microscopy in Zernike phase contrast at 8 keV with sub-50 nm resolution at NSRL. Samples such as Au spoke pattern, Au lines-and-spaces pattern, carbon microtube, and wheat straw were used to test the performance of the microscope.

2. Optical layout
The transmission X-ray microscope was constructed at beamline U7A of NSRL. The detailed schematic experimental setup of this x-ray microscope is described elsewhere [3, 4].

3. Experimental results
The performance of the microscope was first tested with an Au spoke pattern with 600 nm thickness and 50 nm finest line widths at the center. Figure 1(a) presents the Zernike phase contrast image of the pattern. The measured Zernike phase contrast is about 30%. It is better than the calculated absorption
contrast 12% of 600 nm thick gold at 8 keV. Figure 1(b) and (c) show the absorption contrast image and Zernike phase contrast image of an Au lines-and-spaces pattern respectively. The Au lines appear dark. The measure absorption contrast is about 22% while the measured Zernike phase contrast is about 33%. We found bright boundaries of the lines in Fig.1 (c), which is the so-called halo effect corresponding to negative phase contrast. More details about the halo effect can be found in [5]. The halo effect is especially apparent around the lower spatial frequency lines, since the halos would overlap around the high spatial frequency features such as the spoke pattern in Fig. 1, which is little blurred by the halos.

![Figure 1](image)

**Figure 1.** (a) Zernike phase contrast image of spoke pattern. (b) Absorption contrast and (c) Zernike phase contrast images of lines-and-spaces pattern.

The halo effect is pronounced when the indices of refraction between the neighbouring regions are highly different. Therefore, Zernike phase contrast would be more suitable for low-Z materials and thin specimens. Carbon microtubes with 1 µm diameter were used to test at 8 keV. The carbon microtubes were prepared by optimal floating chemical vapor deposition (CVD) method [6]. Compared with the absorption contrast image of a carbon microtube in Fig. 2(a), the phase contrast image in Fig. 2(b) shows better contrast. The dark spheres are reference gold particles.

Concerns about global warming, the soaring cost of gasoline and national security issues have rekindled interest in producing liquid transportation fuels from renewable resources, particularly those derived from biomass (cellulose, lignin, and hemicellulose). Recalcitrance to saccharification is a major limitation for conversion of lignocellulosic biomass to ethanol. Anaerobic fermentation of lignocellulosic wastes is attractive due to environmental benefits, such as reducing the greenhouse effect, generating useful products, and increasing resource availability. Wheat straw was collected from a farm in the suburb of Hefei city, China. After it was pretreatment, mixed rumen microorganisms, composed of bacteria, fungi, and protozoa (all needed enzyme components with high enzyme activity), were used for the degradation of lignocellulosic wastes. More details can be found elsewhere [7]. Figure 2(c) is a phase contrast 3x3 mosaic image of wheat straw before anaerobic degradation. It shows detailed microstructures of lignin and cellulose fibers in the straw. X-ray tomography had been performed on the red square area in Fig. 2(c). The dark spheres in the images are gold particles used as alignment mark. The total 71 sequential tomographic images were automatically collected from -70° to +70° in 2° intervals at 8.0 keV. Subsequently, these projections were aligned and reconstructed by a standard filtered-back-projection algorithm. Figure 2(d) presents the three-dimensional (3D) rendering of the straw. Figure 2(e-f) are two single phase contrast images of wheat straw after anaerobic degradation of lignin in waste straw by ruminal microbes after 12 days. Tunnelings can be clearly seen perpendicular to the lignins and fibers, which might be one of the ways for rumen microorganisms to attack the straw. It is very interesting that the conversion processes can be observed on nanoscale by the x-ray microscopy. Further investigation will be focused on the correlation between the microstructures and the conversion mechanism.
4. Conclusions
We have demonstrated a FZP based transmission X-ray microscope in Zernike phase contrast at 8 keV. A 50 nm resolution test spoke pattern was imaged. Halo effect was found in the lines-and-spaces pattern. Low-Z materials, such as carbon microtube, could be observed. In addition to the results of the wheat straw and nanoscale materials in [3], all these performance highlights the potential of its applications in environmental science, and materials science.

Acknowledgment
The authors are grateful for the financial support from the 985 project of the State Ministry of Education, and the National Science Foundation of China (10675113, 10734070).

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