Fresnel Diffraction Correction by Phase-considered Iteration Procedure in Soft X-ray Projection Microscopy

Tatsuo Shiina¹, Tsuyoshi Suzuki¹, Toshio Honda¹, Atsushi Ito², Yasuhito Kinjo³, Hideyuki Yoshimura⁴, Keiji Yada⁵, Kunio Shinohara⁶

1. Graduate School of Advanced Integration Science, Chiba University
1-33 Yayoi-cho, Inage-ku, Chiba-shi, Chiba 263-8522, Japan
2. School of Engineering, Tokai University
1117 Kitakaname, Hiratsuka-shi, Kanagawa 259-1292 Japan
3. Tokyo Metropolitan Industrial Technology Research Institute
2-11-1 Fukazawa, Setagaya-ku, Tokyo 158-0081 Japan
4. School of Science and Technology, Meiji University
1-1-1 Higashimita, Tama-ku, Kawasaki-shi, Kanagawa 214-8571 Japan
5. Tohken Co., Ltd.
2-27-7 Tamagawa, Chofu-shi, Tokyo 182-0025 Japan
6. Advanced Research Institute for Science and Engineering, Waseda University
3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan

shiina@faculty.chiba-u.jp

Abstract. In soft X-ray projection microscopy, it is easy to alter the magnification by changing the distance between the pinhole and the specimen, while the image is blurred because the soft X-rays are diffracted through the propagation from specimen to CCD detector. We corrected the blurred image by the iteration procedure of Fresnel to inverse Fresnel transformation taking phase distribution of the specimen into account. The experiments were conducted at the BL-11A of the Photon Factory, KEK, Japan for the specimens such as glass-capillaries, latex-particles, dried mammalian cells and human chromosomes. Many of those blurred images were corrected adequately by the iteration procedure, though some images such as those which have high-contrast or are overlapped by small cells still remain to be improved.

1. Introduction

Projection microscope has advantages of wide field of view and simple optics in comparison with imaging microscope. It is easy to zoom in a specimen to obtain high-magnification images after capturing it with low magnification. Quality of the obtained image is often impaired at high resolution because it is blurred with fringes caused by Fresnel diffraction. Recently the high-resolution imaging microscope achieves good results [1] with a limited field of view, while various blur-correction methods for the projection microscopy have been developed for the improvement of processing capability [2]. An iterative algorithm for phase retrieval is effective since the phase of the X-rays is not recorded by the CCD detector and therefore a direct, algebraic back propagation is not possible. We also studied the blur-correction methods for the images obtained by the soft X-ray projection microscopy and developed the blur-correction method with an iteration of Fresnel to inverse Fresnel
transformation based on physical implication of the blur generation [3]. Here we improved the methods with respect to the restraint condition and the illumination condition in calculation procedure and applied to images of various specimens.

2. Projection microscope and iteration procedure

The system setup is illustrated in Fig.1. The light source was the monochromatic soft X-rays at BL-11A in KEK-PF, Japan. The wavelength was 1.5 to 2.5nm (0.83 to 0.50keV in energy). The detector is a back-illuminated X-ray CCD camera with the pixel pitch of 24.8μm (Hamamatsu C4880-30-26WS). A rotary stage was installed to obtain the CT-reconstruction images. All devices and specimens were in the vacuum chamber. The specimen was observed at x110.7 lateral magnification at the distance of 3mm between the post-pinhole and the specimen. The CCD’s FOV became 140μm square at this magnification. In that case, the Fresnel’s fringe was observed on the projection image when the diameter of the post-pinhole was less than 5μm. The size of the projection image per pixel was about 0.2μm. Each projection image was integrated in its intensity for 2 - 5 minutes to improve the signal-to-noise ratio. For the elimination of the blur on the projection image, the iteration process was applied. The process repeats the calculation between Fresnel to inverse Fresnel transformations under some restrictions such as addition of the intensity distribution of X-ray illumination in the CCD’s FOV, and the convergence condition in the calculation. A fixed sample to detector distance was used in the iteration process. In addition, the phase of a spherical wave was also considered as the radially-propagating beam. The specimens were glass-capillaries, latex-particles, dried mammalian cells (HeLa and MOLT-4) and human chromosomes.

3. Projection images and blur-correction image

Some of the blur-corrected results are shown in Figs.2-4. In these figures, magnification was x166 and the wavelength of the soft X-rays was 1.77nm (0.74keV in energy). In the blurred image of a HeLa cell (Fig.2a), there were bright fringes around the cell and white spots in the cell. They are due to the influence of the Fresnel diffraction. In the corrected image (Fig.2b), they were thoroughly taken out. The nucleus and the cytoplasm in the cell became clear. Figure 3 shows the images of a tapered hollow glass capillary. The apex was less than φ1μm. The several Fresnel fringes appeared around the capillary in the blurred image of Fig.3a. It was hard to distinguish the apex and width of the capillary. In view of the pixel-size of the CCD detector and the microscope magnification, the calculated resolution was estimated to be 0.2μm. The thickness of the glass capillary was estimated as about φ0.5μm. The hollow structure was reproduced in the corrected image of Fig.3b. Figure 4 shows the observation of the glass capillary with a latex particle on the apex. The magnification was x110.7. In Fig.4a, the several fringes appeared, the feature of the apex of the glass capillary was uncertain. The borders between the capillary and the latex particle were clearly recognized in Fig.4b. Both diameters of the latex particle and the apex of the capillary were 8μm. We performed the blur-correction for the images of various specimens including MOLT-4 cells and human chromosomes. As a result, it was confirmed that the blur-correction was adequate in many specimens. On the other hand, some of images cannot be corrected the blur properly. In those images, the edge of the object was sharp, or plural small samples were closely distributed. The correction failure of the former image was due to the restraint condition in the calculation. That of the latter was because the fringes from the small samples were overlapped and disappeared by the digitization. The partial coherence of the light source also made difficult to correct the above images because BL-11A is a bending magnet source.
Fig. 2 HeLa Cell (a) Projection image (b) Corrected image at the object position.

Fig. 3 Hollow glass capillary (a) Projection image (b) Corrected image at the object position.

Fig. 4 Glass capillary and a latex particles (a) Projection image (b) Corrected image at the object position.

4. Summary
The blur-correction method with the iteration procedure between Fresnel to inverse Fresnel transformation was developed for the soft X-ray projection microscopy and applied to various specimens. For many of the images the blur was removed adequately and the details of the image became clear. The size of the corrected image per pixel approximated to the physical limit defined by the CCD’s pixel size.

References
[1] Mark A Le Gros, Gerry McDermott and Carolyn A Larabell, “X-ray tomography of whole cells”, Current Opinion in Structural Biology, Vol.15, pp.193-600, 2005
[2] T. E. Gureyev, A.W. Stevenson, Ya. I. Nesterets, S.W. Wilkins, “Image deblurring by means of defocus”, Optics Communications, Vol.240, pp.81-88, 2004
[3] T. Shiina et al, “CT Reconstruction by Diffraction Correction in Soft X-ray Projection Microscopy”, Proc. 8th Int. Conf. X-ray Microscopy IPAP Conf. Series Vol.7, pp.363-365, 2005