# Noise Influence on Low Contrast Image Correction for Soft X-Ray Projection Microscopy

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Abstract: Soft X-ray projection microscopy has been developed for high magnified imaging of hydrated biological specimens because water window region is available. The projection microscopy is a simple optical layout and has advantages over other types of microscopes particularly for biological specimens because of its wide viewing area, easy zooming function and easy extension to CT. However the projection image is blurred by the diffraction of X-rays, resulting in the deterioration of the spatial resolution. In this study, the blurred images have been corrected by an iteration procedure, i.e., Fresnel and inverse Fresnel transformations are repeated. The correction was found to be not effective for every image, especially for images with low contrast. A contrast enhancement method prior to the iteration procedure was installed to make the iteration procedure more effective, but it was not enough yet due to the influence of background noise. We evaluated dependency between the background noise level and iteration effect in the cases with or without the contrast enhancement prior to the iteration procedure by simulation. We also demonstrated upper limits of the background noises which chromosome images are effectively corrected by the iteration procedure.



## 1 INTRODUCTION

Soft X-ray microscopy covers wavelength region called water window. X-ray attenuation in this region is significantly smaller in water than in organic material. Therefore, it is possible to observe the biological specimens at cellular and sub-cellular levels with intact and/or in situ situation (Legall et al., 2013; Weigert et al., 2013; Schneider et al., 2000; Kirz et al., 1995).

Fresnel zone plate (FZP) has been frequently used for focusing optics in X-ray microscopy in combination with synchrotron radiation or a laser plasma X-ray source (Bertilson et al., 2009; Kirz et al., 1995), but there has been no approach to use FZP to produce a point X-ray source in a projection type X-ray microscopy. The projection microscopy has the following advantages.

A) It has a simple optical layout and wide viewing area.
B) Zooming is easily adjusted by changing distance between a specimen and a pinhole.
C) It is possible to extend to CT by installation of an additional part to rotate a specimen.

However the projection image is blurred by the diffraction of soft X-rays and contains diffraction fringes around the specimen image, leading the spatial resolution to be worse. In this study, the blurred images have been corrected by an iteration procedure, which has performed cycled calculations of Fresnel and inverse Fresnel transformations. Earlier studies confirmed the iteration effectiveness and also checked some additional methods such as contrast enhancement prior to the iteration procedure to make the iteration procedure more effective (Jamsranjav et al., 2015; Al-amri et al., 2010; Shiina et al., 2009; David et al., 2005). However, some images showing very low contrast such as chromosome images were not correctable probably due to influence of background noise, because the contrast of diffraction fringes and background noise distribution were compatible.

This study evaluated dependency between the iteration effect and the background noise level on simulation image with low contrast. The contrast of



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the simulation image was adjusted to be similar with that of projection images of chromosomes. It was about 1.6% of the image contrast. Upper limits of background noises that the images are corrected effectively were evaluated. The noise sizes were based on the noises of experimental projection images which the blur correction was not successful. The noise sizes were based on the noises of experimental projection images which the blur correction was not successful.

# 2 METHODS

## 2.1 Projection Experiment

The projection microscopy captures a magnified image of specimen by detection of monochromatic soft X-rays transmitted through and turned around specimen. In order to make a point source of monochromatic soft X-rays, the optics of microscopy are constructed with a grating monochromator, a zone-plate and a pinhole from bending magnet beam line BL-11A at the KEK (High Energy Accelerator Research Organization) Photon Factory in Tsukuba, Japan. The camera was a back-illuminated X-ray CCD with 24.8μm pixel-pitch (Hamamatsu Photonics C4880-30-26W). Optical layout of the microscopy was shown in Fig.1. Projection conditions were shown in Table 1.

Table 1: Projection conditions.

| Items | Values |
|---|---|
| X-ray energy | 700 eV |
| Pinhole diameter | 0.5 μm and 1 μm |
| Distance (Pinhole-CCD) | 329mm and 252 mm |
| Magnification | 47 – 658 times |
| Projection time | 40 sec – 10 min |

## 2.2 Iteration Procedure

By the iteration procedure, X-ray intensity distribution at the specimen surface are calculated and extracted as a correction image of specimen. For the calculation, we need phase and amplitude information of a projection image at a CCD screen. The amplitude information is possible to obtain from the projection image. X-ray intensity distributions were recorded on a CCD screen, while there was no information for the phase. Therefore, spherical wave propagations are calculated for initial phase information using equation (1).

$$\Phi(mT) = \frac{1}{\sqrt{r^2 + (mT)^2}} \exp\left\{\frac{i2\pi}{\lambda}\sqrt{r^2 + (mT)^2}\right\} \quad (1)$$

where $\Phi(mT)$: wave amplitude distribution on CCD screen, $\lambda$: wavelength, r: distance between pinhole and CCD screen, T: sampling interval on CCD screen, m: positive integer.

Subsequently, iteration procedure performs cycled calculations of Fresnel (FT) and inverse Fresnel (IFT) transformations taking into account of restriction condition (RC) using equation (2) to approach to proper phase distribution. The iteration procedure is shown in Fig.2.

$$\begin{aligned} FT: F(mT) &= \sum_{n=0}^{N-1} f(nT) \exp\left\{\frac{i\pi}{\lambda R}(mT - nT_o)^2\right\} \\ IFT: f(nT_o) &= \sum_{m=0}^{N-1} F(mT) \exp\left\{-\frac{i\pi}{\lambda R}(mT - nT_o)^2\right\} \\ RC: T_o T &= \frac{\lambda R}{N} \end{aligned} \quad (2)$$

where F(mT): X-ray intensity distribution on CCD screen, f(nT): X-ray intensity distribution on specimen surface, $\lambda$ : wave length, R: distance between specimen and CCD screen, N: sampling number, $T_0$: sampling interval on specimen surface, T: sampling interval on CCD screen, n, m: positive integer. The RC is required in order to make cycled calculation of FT and IFT with closed relation.

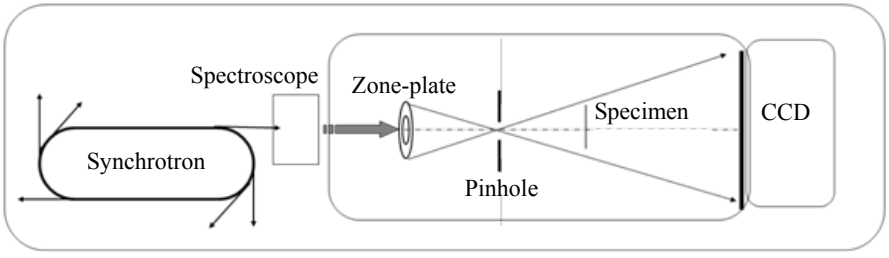



Figure 1: Optical layout of soft X-ray projection microscopy.

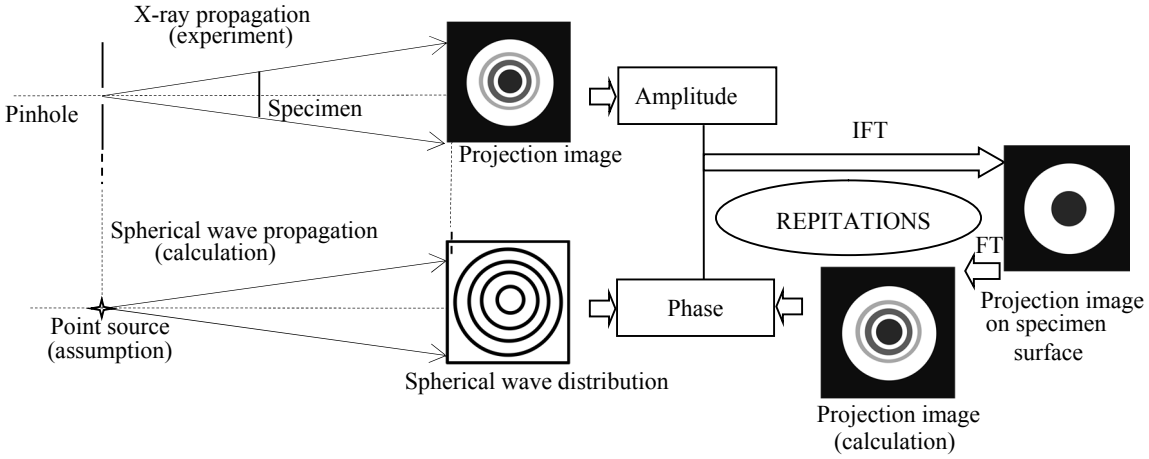



Figure 2: Iteration procedure.

## 2.3 Simulation of X-ray Beam Propagation

Simulation program performs FT calculation from specimen surface to CCD screen and produces an image on CCD screen instead of experimental projection image. Intensity distribution describing specimen figure is prepared as amplitude data on specimen surface. The phase distribution was produced by a calculation of spherical wave propagation to specimen surface. The simulation algorithm is shown in Fig.3.

The simulation effect was checked for an image with high contrast. The result is shown in Fig.4. Diffraction fringes were generated by the simulation and corrected by iteration procedure successfully.

For the evaluation of the influence of the background noise, the noise was set to distribute by using random number generator. Noise size was adjusted to small or large values as shown in Table 2. The sizes were based on the noise information of experimental projection images with low or very low contrasts respectively for which the blur correction was not effective. Noise numbers were set up as variables.

Table 2: Noise size for the simulation images.

| Size | Width (pixels) | Height (grayscales)[1)] |
|---|---|---|
| Small | 4 | $10^{-4}$~$10^{-2}$ |
| Large | | $3*10^{-2}$~$5*10^{-2}$ |

1) Normalized values with whole range of image grayscale

We adopted MSE (Mean Squared Error) as one of metrics in evaluating noise levels. It takes the noise numbers and sizes into account and calculated by equation (3).

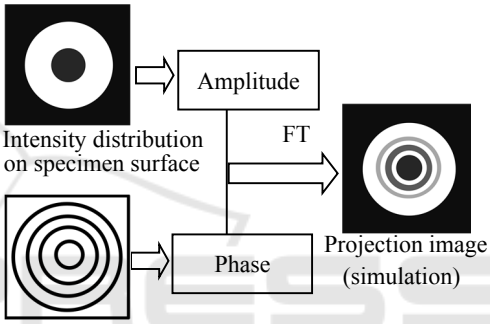



Figure 3: Simulation algorithm.

$$MSE = \frac{1}{N}\sum_{n\_1}^{N}(G_n - G_n^0)^2 \quad (3)$$

where $G_n$ : grayscale value of a pixel with No. n for the simulation image with noise, $G_n^0$ : grayscale value of a pixel with No. n for the simulation image without noise, N (=512*512): total number of the image pixels.

# 3 RESULTS AND DISCUSSIONS

## 3.1 Iteration Effect

Iteration effect was checked in the cases of many different patterns of projection conditions such as exposure time, magnification and pinhole diameter, etc. Representative results were shown in Fig.5 for chromosome, and Fig.6 (a) for latex and (b) for chromosome as the images with low contrast, high

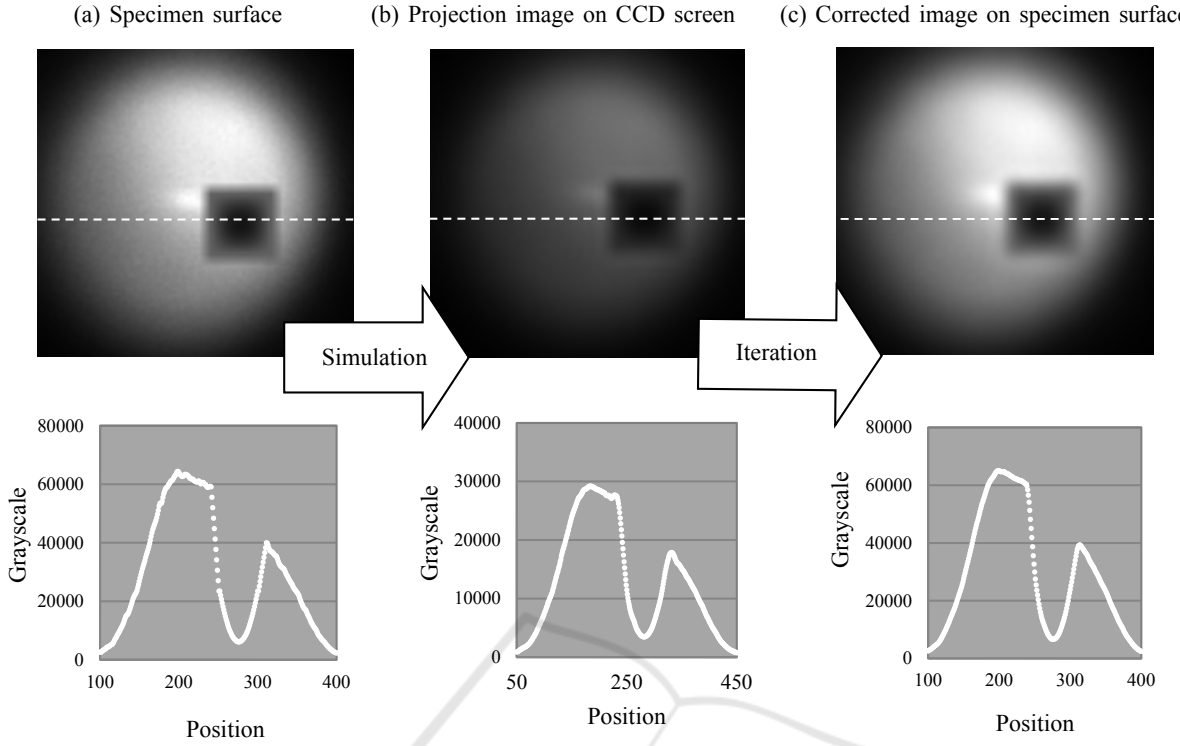



Figure 4: Simulation effects on high contrast image.

contrast and very low contrast, respectively.

Following 3 results were obtained.

A) Iteration effect was more effective for the latex particle than biological specimen (chromosome). The image contrast was also higher for the latex particle than chromosome. We considered the reason of poor iteration effects for the chromosome images; it may result from the decreased contrast of the diffraction fringes due to the high X-ray transmittance to the chromosome. Therefore the iteration process is more susceptible to CCD noise, the unevenness of the illumination intensity, and scattering from micro-fragments derived from inner-components of the specimen. (Fig. 6(a))

B) Some images with low contrast were corrected when the image contrast was enhanced prior to the iteration procedure. It is considered that the method could improve enough difference between the contrast of diffraction fringes and background grayscale distribution. (Fig. 5)

C) For the very low contrast images of chromosome, the iteration procedure was not effective in all of the cases with or without contrast enhancement prior to the iteration procedure. Some or whole parts of the images were lost by the iteration procedure, which was probably due to the low contrast for the iteration program. (Fig. 6(b))

To examine the above results, we have evaluated the influence of the background noise to the iteration effect with the simulation image. Some results are shown in the next section.

## 3.2 Noise Influence on Iteration Procedure

Projection image was produced by FT calculation as a simulation image. The contrast was adjusted to 1.6%, which was referred to projection images of chromosome. Background noises were set to distribute on the simulation images by using random number generator. The influence of the background noise to the iteration procedure was evaluated in many cases of the noise numbers and noise sizes as shown in Table 2.

Iteration results for the simulation images were shown in Fig.7, Fig.8 and Fig.9. The figures show correctable images in the cases without or with the contrast enhancement prior to the iteration procedure and an uncorrectable image for the both cases, respectively.

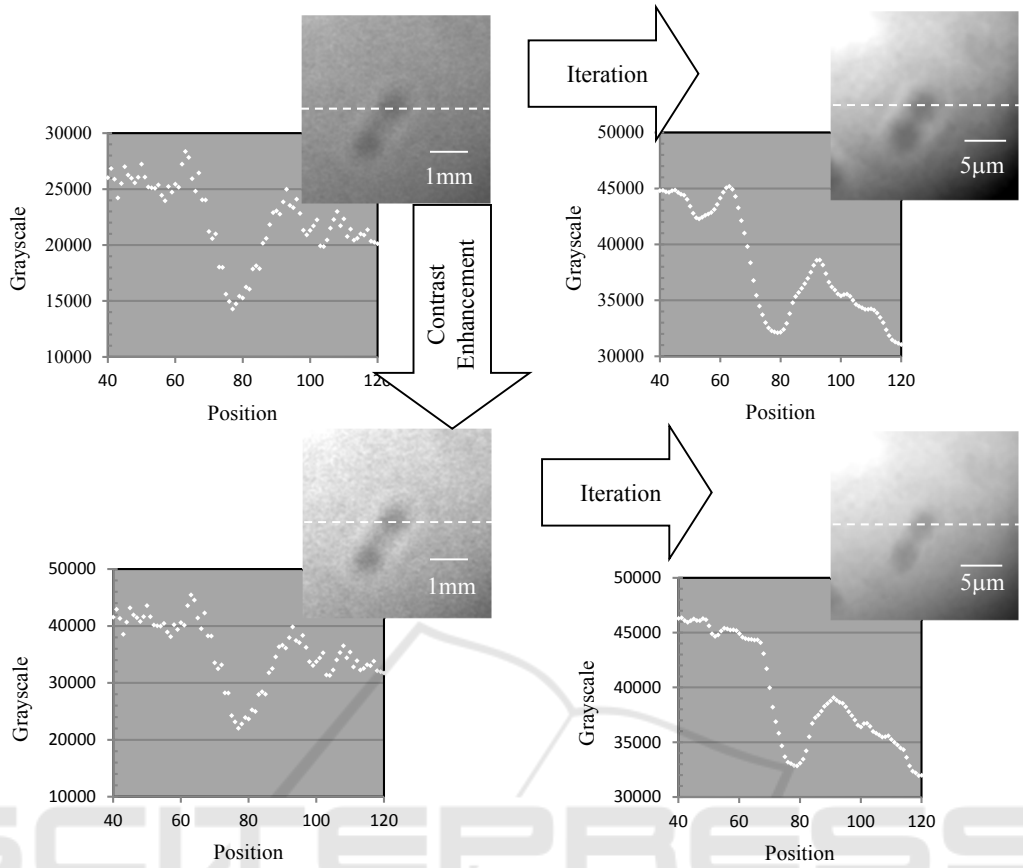
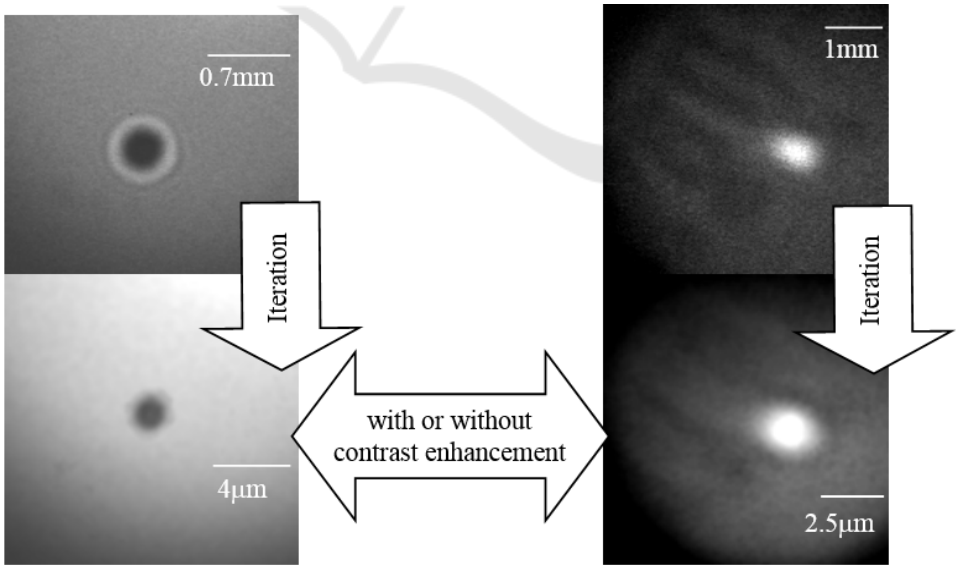



Figure 5: Iteration results for an image with low contrast. (Chromosome [Pinhole diameter: 0.5μm, Magnification: 219 times, Exposure time: 3 min]).






(a) (b)

Figure 6: Iteration results for the images with high and very low contrasts. (a) Latex particle [Pinhole diameter: 0.5μm, Magnification: 165 times, Exposure time: 3 min], (b) Chromosome [Pinhole diameter: 0.5μm, Magnification: 504 times, Exposure time: 3 min].

Upper limits of the background noises that the image is effectively corrected by iteration procedure were examined. The noise level was evaluated by MSE.

The following results were obtained.

A) The iteration correction was effective when the image did not contain any noise. (Fig.7)
B) For the image containing background noises with small sizes and high levels, diffraction fringes could not be corrected by the iteration procedure only. The upper limit of the background noise level was about $4*10^5$ of MSE underwhich the image was effectively corrected by the iteration procedure only. However the image was correctable in the case with the contrast enhancement prior to the iteration procedure for all of the noise level. (Fig. 8)
C) For the image containing background noises with large sizes and high levels, whole or some parts of the image were lost by the iteration procedure. The upper limit of the background noise level was about $10^6$ of MSE underwhich the image was effectively corrected by the iteration procedure. The image was not correctable in the both cases with or without contrast enhancement prior to the iteration procedure. (Fig. 9)

Future problems are suggested from the above results.

A) Noise sources should be identified to reduce the background noises by adjusting the noise sources.
B) Noise removal methods of image processing should be developed to improve the iteration effect with noise sources.

## 4 CONCLUSIONS

In the first step of this study, we aimed to demonstrate the iteration effectiveness and the iteration effect was checked for the projection images of the two types of latex particles with 2 and 10 μm diameters and chromosomes in the cases with or without the contrast enhancement prior to the iteration procedure.

The iteration procedure was not effective for all of the images. Especially, the effect was poor for low contrast images of chromosomes.

The contrast enhancement method was effective for correction of the images which were not correctable by the iteration procedure only. The method could produce enough difference between the contrast of diffraction fringes and the background grayscale distribution. However, further low contrast images could not correctable yet.

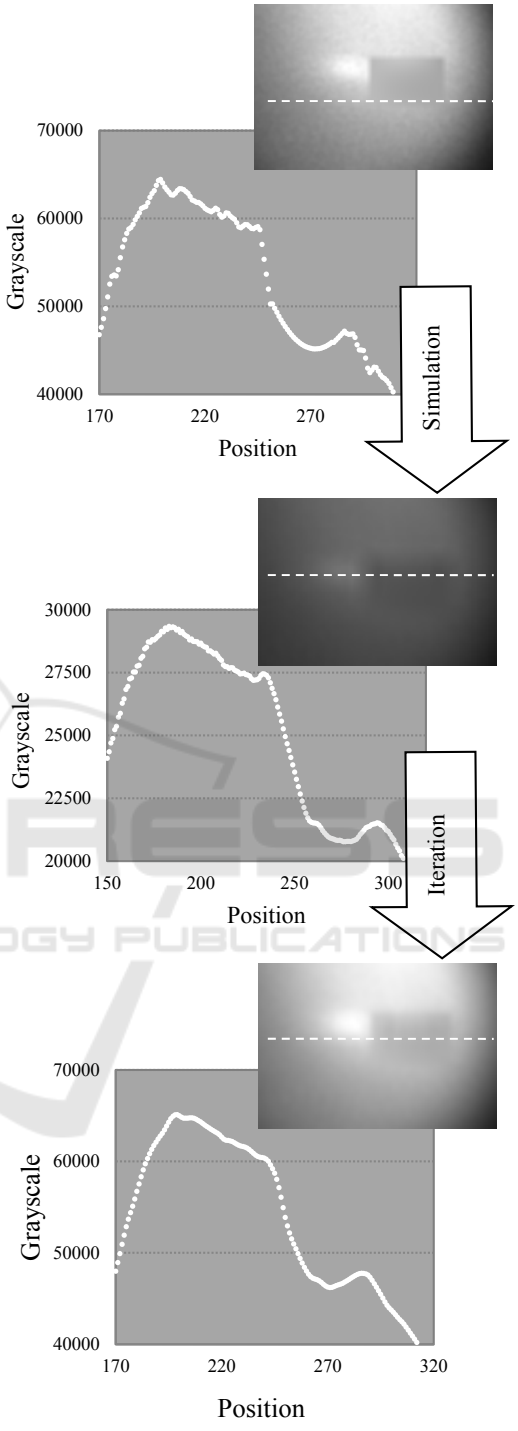



Figure 7: Iteration result for simulation image without noise.

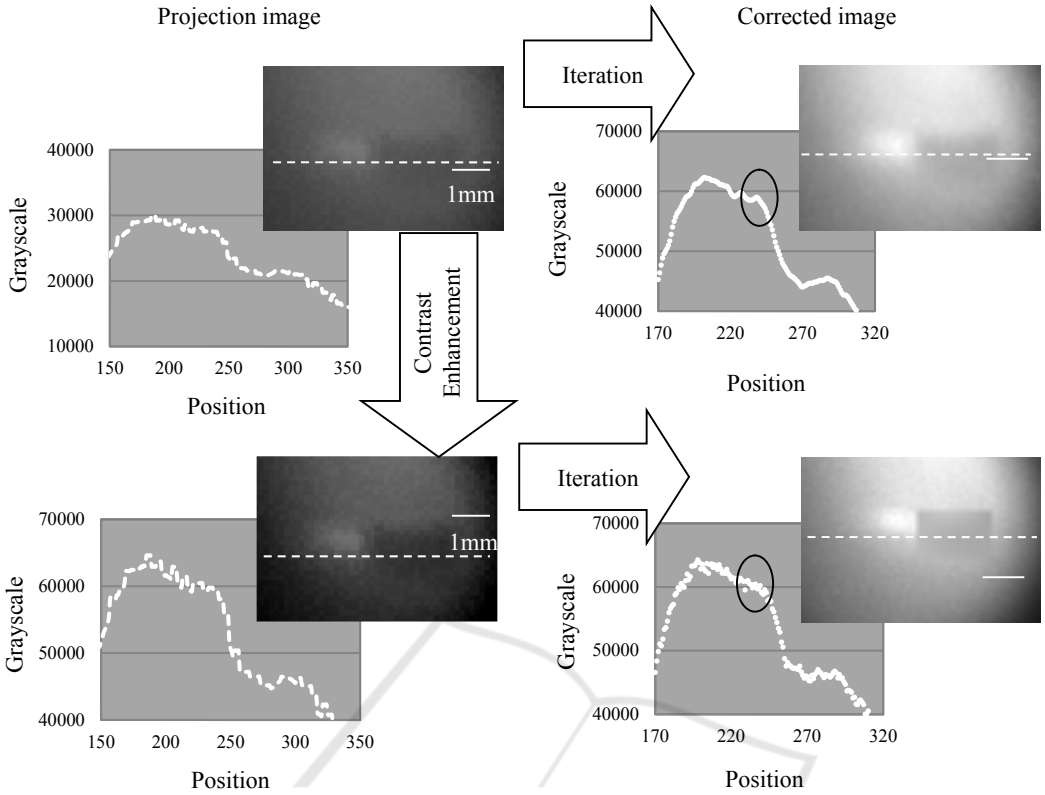



Figure 8: Iteration result for simulation image with small noises (MSE: $4*10^5$). (Circular marks on the corrected images show diffraction fringes situation).

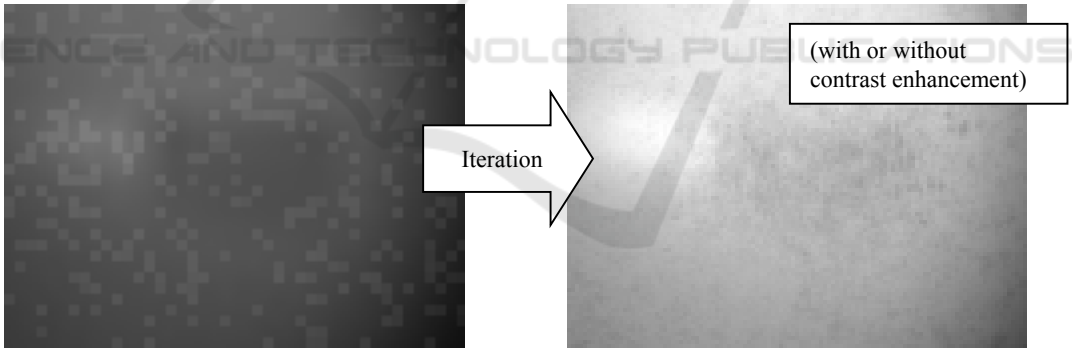



Figure 9: Iteration result for simulation image with large noises (MSE: $1.5*10^6$).

As the second step, influence of the background noise to the iteration effectiveness was evaluated using the simulating calculation. Iteration effect became worse as the background noise became larger. The uncorrected images showed two different characteristics depending on the noise sizes.

For the noises with small size, diffraction fringes were not correctable by the iteration procedure only. However it was correctable in the case with contrast enhancement prior to the iteration procedure.

For the noises with large size, whole or some parts of the image were lost by the iteration procedure. It was not correctable in the both cases with or without the contrast enhancement prior to the iteration procedure.

Upper limits of the background noises for the images which were effectively corrected by the iteration procedure were evaluated under the noise influence. MSE was calculated as an indicator of the noise level. The results are as follows.

- For small size noise: MSE=$4*10^5$
  (in the case of iteration procedure only)
- For large size noise: MSE=$10^6$

(in both cases with or without contrast enhancement prior to the iteration procedure)

Next, noise influences need to be checked for various cases of the noise sizes and the image contrasts. Development of noise sources and noise removal methods of image processing is another issue. An effective method to remove the background noise and to improve the iteration effectiveness is expected from these investigations.

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