AMTC Letters
Vol. 1 (2008)

Observation of unstained ferritin molecules by in-line Fresnel electron holography

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Electromagnetic structures of weak phase objects can be observed with high precision by in-line Fresnel electron holography [1]. Since Fresnel holograms have higher contrast than Fraunhofer holograms, they are of advantage to higher-resolution and -precision observation. However, the reconstructed image has a serious problem that the reconstructed image is superposed by the defocused conjugate image and then phase information of the object is modulated [2-3]. In the present study, observation of a faithful phase shift image of isolated unstained ferritin bio-molecules by the procedure of a sequential inference method is discussed with computer simulation and experiments. The retrieval result by the sequential inference procedure depends on the original phase image and the initial phase image, where the initial image is evaluated from the original image to start the iteration process. The closer to the exit objects wave they are estimated, the better inference image is expected [4]. We combined two conjugate phase images with a different weight to make the phase flat all over a ferritin molecule. This is convenient for finding the good original image and initial image in the sequential inference procedure.

Figure 1(a) shows a simulated phase image in an exit plane for a ferritin molecule at an accelerating voltage of 150kV. Figures 1(b) and (c) show the conjugate phase image and the reconstructed phase image from a Δf =15μm (over-focus) hologram, respectively. The mean inner potentials of a Fe hydrate micelle of 5.5nm in diameter and a protein shell of 11nm in diameter were assumed to be 12.5V and 3.0V, respectively. Experimentally, Fresnel holograms of the defocus of $\Delta f \leq 15$μm is desired for higher resolution less than 1nm. Figure 1(d) shows phase distribution profiles of the conjugate image for Fresnel holograms of Δf =3-15μm, which are advanced phases in 0th order and the phase image of object in an exit plane, which is a delayed phase. The conjugate phase profiles of $\Delta f > 8$μm show concave and those of $\Delta f < 6$μm convex. Figure 1(e) shows that the combination phase profile of conjugate image of Δf =15μm and δ times amplified conjugate images of Δf =6μm to be flatly broadened all over a ferritin molecule, where parameter δ was selected 0.3. In this case, the combined original image will give a better inference image.

Figure 2 shows a flow of procedure of a sequential inference to retrieve phase image by in-line Fresnel electron holography. The experimental holograms and phase images are shown in Figure 3. Figures 3(a) and (b) show the hologram of Δf =6μm and that of Δf =15μm, and Figures 3(c) amd (d) show the corresponding reconstructed phase images of Δf =6μm, and Δf =15μm, respectively. The image of Δf =6μm (FIG.3(c)) is reconstructed with a higher resolution and is further strongly influenced with the conjugate image than that of Δf =15μm (FIG.3.(d)) is. Figure 3(e) shows the original phase image, *i.e.*, the combination of the phase image of Δf =15μm and 0.3times amplified phase image of Δf =6μm. Figure 3(f) shows the initial phase image for Figure 3(e), which is extracted using an edge filter. Figure 3(g) shows a 20 time processing image of a sequential inference, for an error coefficient α of 0.1 The phase line profile is shown in Figure 3(h) for the ferritin molecule indicated by the dotted line. These results indicate that the combination image has deduced an inference phase image with good agreement with the phase of the exit wave from the specimen and the faithful phase image for ferritin molecules can be observed by in-line Fresnel electron holography.

References

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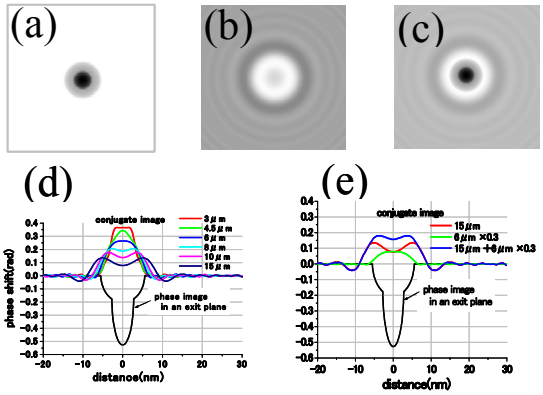
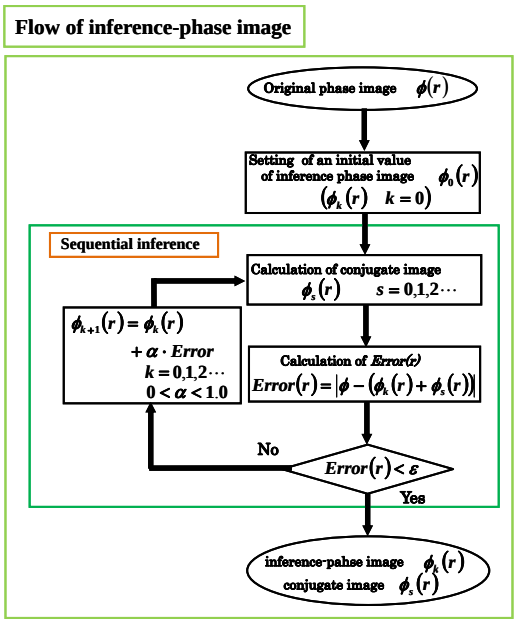
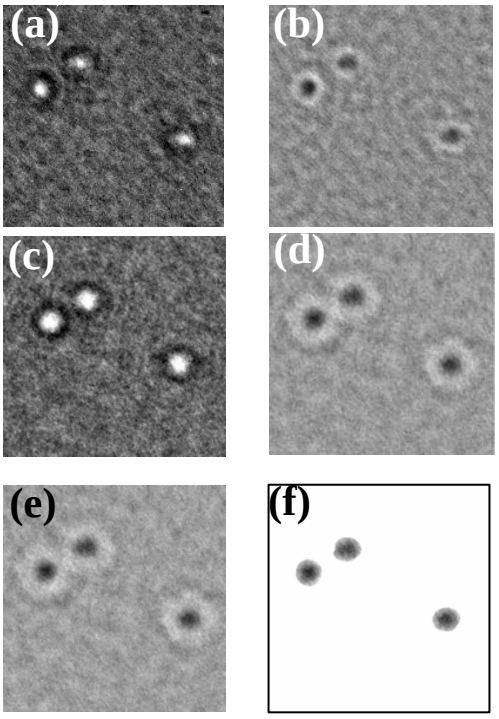


FIG.1. Simulated phase images and phase profiles for a ferritin molecule at 150kV. Phase image in an exit plane (a), conjugate image and reconstructed phase image for Δf =15μm (b), (c), phase profiles for conjugate image of Δf =3-15μm, phase image in an exit plane (d), and phase profile of combination of conjugate (e).





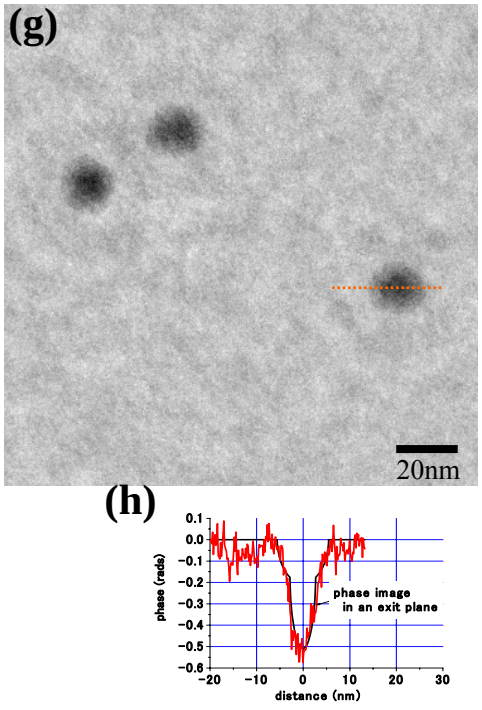


FIG.3. Experimental holograms and phase images. Holograms of Δf =6μm (a) and Δf =15μm (b), and the corresponding reconstructed phase images of Δf =6μm (c) and Δf =15μm (d), original phase image, i.e., combination image of Δf =15μm and 0.3times amplified phase image of Δf =6μm (e) and initial image for original image (f), inference phase image by 20 times sequential processing (g), and line profile for the ferritin molecule indicated by a dotted line (h).