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Application of Zernike Phase-Contrast Electron Microscopy for Vitrified Complex Biological Specimens

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In order to acquire phase-contrast images with an adequate contrast, conventional TEM requires large amount of defocus. Increasing the defocus enables to recover low-frequency components but attenuates high-frequency ones. On the other hand, Zernike phase-contrast TEM (ZPC-TEM) can recover low-frequency components without losing high-frequency ones under in-focus conditions [1]. ZPC-TEM is able to successfully visualize unstained vitreous ice-embedded biomolecules and biological specimens (e.g., proteins, viruses, and liposomes) [2], [3], [4]. ZPC-TEM however, has another problem, especially in imaging of complex biological specimens such as cells and tissues; strong halos appear around specimen structures, and these halos make the interpretation of images difficult. Due to this problem, the application of ZPC-TEM has been restricted to imaging of smaller particles. In order to restrain the halo appearance, we fabricated a new phase-plate with a smaller central hole and tested it on vitreous biological specimens. ZPC-TEM with the new plate could successfully visualize the intracellular fine features of cultured cells and brain tissues. This result indicates that reduction of the central hole diameter makes ZPC-TEM applicable to specimens ranging from protein particles to tissue sections. The application of ZPC-TEM to vitreous biological specimens will be convenient to advance the new field of imaging ultrastructures in close-to-physiological state.

References

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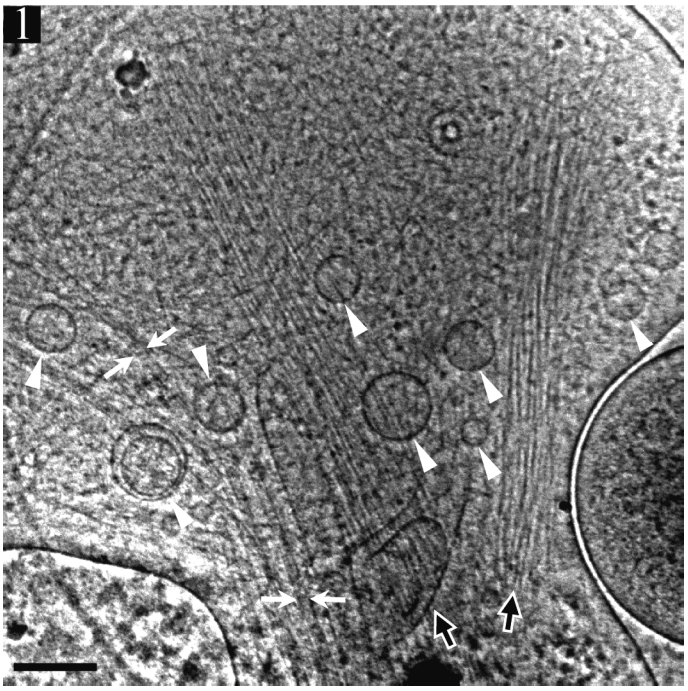

FIG. 1. A Zernike phase-contrast zero-loss image of vitreous cultured cells acquired in in-focus. Black arrows, straight filamentous structures; facing white arrows, microtubules; white arrowheads, vesicles. Scale bar is 200 nm.

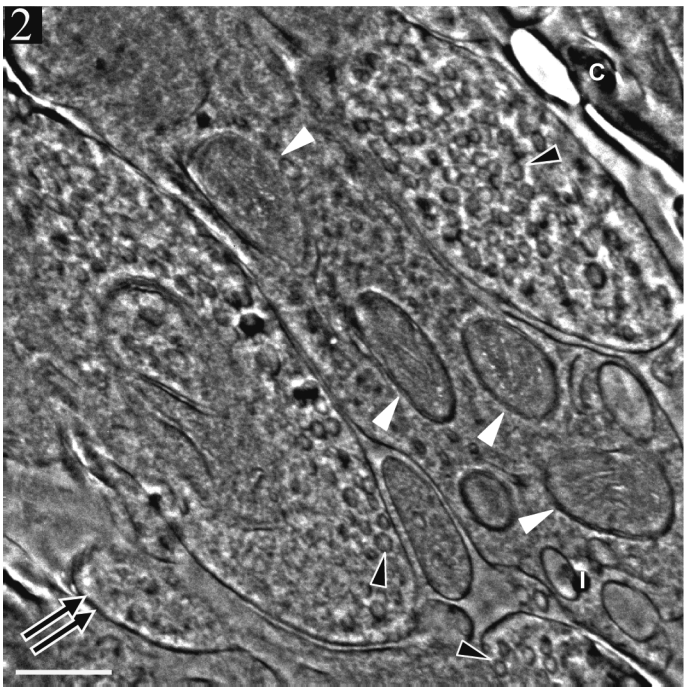

FIG. 2. A Zernike phase-contrast zero-loss image acquired in in-focus of a vitreous section of mouse brain. White arrowheads, mitochondria; black arrowheads, vesicles; C, crevasses; I, ice crystal; double black arrows, cutting direction. Scale bar is 200 nm.

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