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Practical and Theoretic Development of Phase Contrast Devices for Advanced Biologic Imaging

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Low temperature transmission electron microscopy (TEM) known as cryo-EM has emerged as a powerful tool in biological imaging. Cryo-EM images of protein usually exhibits low conrast and presents noise due to low electron exposure as proteins are radiation sentitive. In conventional TEM, large defocusing is necessary to gain the contrast, however, special care in correcting contrast transfer function is needed to retrieve the high resolution structural information. One soultion to obtain in-focus image with high signal to noise (S/N) for non-stained protein is implementation of the Zernike phase plate for TEM [1-6]. There are two types of EM phase plate have been demonstrated, the carbon film and electrostatic based phase plates. As shown in Fig. 1, the basic structure of an electrostatic phase plate consists of 5 layer structure Au/Si_3N_4/Au/Si_3N_4/Au. The electric field in the center hole can be tuned to produce desired phase shift to transmission beam.

The development of electrostatic phase plate has theoretic and practical difficulties to overcome. From theoretic point of view, the current imaging theory for EM phase plate has two basic assumptions, 1) the electron beam is a parallel and perfect coherent source 2) the phase plate is a pure phase contrast device. Based on these two assumptions, the imaging theory predicts a misleading expectation that near one order magnitude of contrast may gain from “phase” contrast. Although electron source can be set up to be so-called Kohler illumination by adjusting the condenser lens, practically, we have a partial conherent with slightly convergent beam illuminating on the sample. As shown in fig. 2, it leads to a broaden transmission spot in reciprocal plane instead of a sharp delta function for the case of perfect parallel illumnation. It is not only that partial conherence of source degrades the phase contrast, but also the “phase efficiency” is reduced as the size of transmission spot is larger than that of the central hole in phase plate. In the case of Fig. 2, only 10% of transmission beam has been phase shifted and therefore lower phase contrast is expected. Furthermore, as pointed out in our previous publications [2, 7], the phase plate is, in fact, not only a pure phase lens, practically, it is also an absorption device that it acts as a role of objective aperture. The images recorded with electrostatic phase plate contains mixture of “absorption” and “phase” contrasts, as has been discussed in [2].

Besides, the theory of partial coherent imaging for EM eelctrostatic phase plate will be discussed in detail in the conference, the practical issues on fabricating high phase efficiency, low absorption and stable phase device will be addressed.

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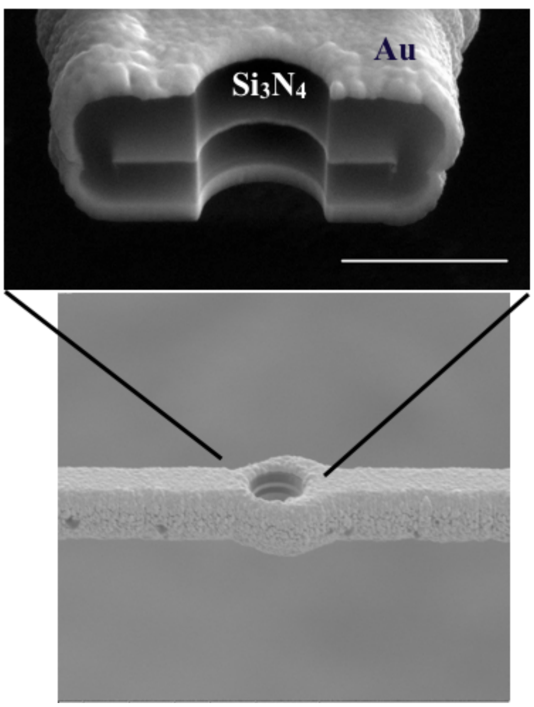

Fig. 1 shows five-layer structure of Zernike electro-static phase plate

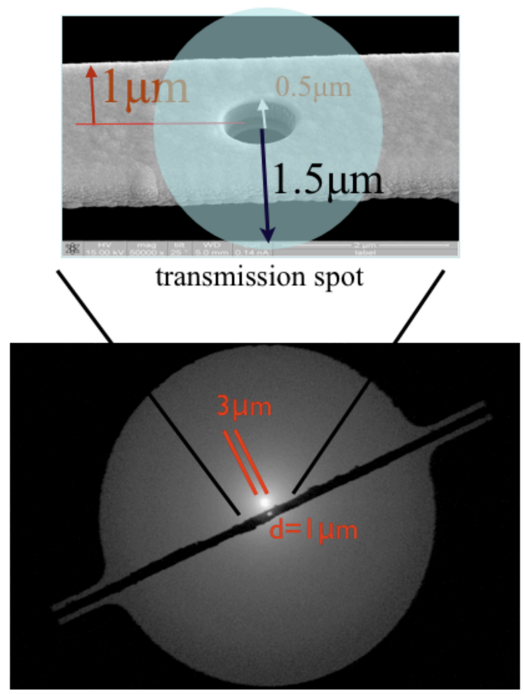

Fig. 2 shows transmission spot from a partial coherent convergent beam. The green circle represents the transmission spot. In this case, transmission spot is larger than the size of central hole

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