Low-dose off-axis holography for biological specimens

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The need of biochemists to know the structure of biological crystals and particles in the greatest possible detail continues and seems likely to increase. These structures are determined despite the susceptibility of specimens to damage, which may occur at accumulated electron dose as low as one electron per Angstrom^2. When low-dose methods are used, it is clearly important to collect the maximum possible information by optimizing the imaging method.

Direct detectors, as described in [1], have shown great benefit in conventional cryo-electron microscopy. This sparked consideration of whether they can enhance other types of imaging, such as off-axis holography (OAH) which can measure the specimen's phase shift directly.

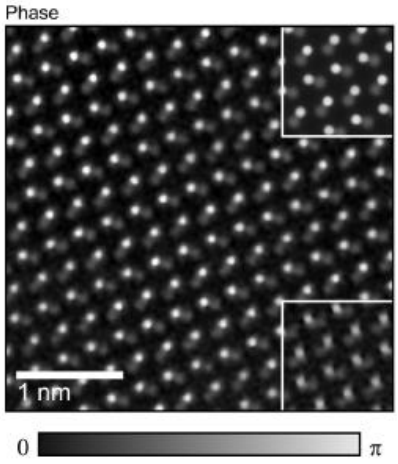

Fig. 1 Phase part of image by OAH of GaN film at focus, from stack of images [3].

OAH of biological specimens reported up to 2006, using CCD detectors, showed that spatial resolution was limited by the conflicting needs for visibility and minimum fringe spacing. More recent work [2] showed that fringe spacing and interference width can be optimized separately while eliminating the image of the biprism wire. An image of GaN film (Fig. 1) obtained in this way with a CCD detector [3] contains information with a spatial period of 75 pm.

The standard deviation (SD) of measurement of phase has been discussed in the literature. For CCD detectors, it is found to vary as $1 / (V\, n_p^{1/2})$, where V is the fringe visibility and n_p is the number of electrons per pixel at the specimen. For a given allowable dose and with a CCD, the SD of phase appears to be proportional to magnification.

Direct detectors offer the further benefit of counting mode. With an optimum arrival rate of the order of one electron per pixel per second, this mode clearly merits investigation for low-dose measurement. At this low rate, it is desirable to divide the allowable dose over a stack of n images, enabling some drift correction between frames. Some re-statement of the precision of phase measurement is also desirable, to account for the variation in shot noise.

The question also arises of what performance OAH can achieve in conditions for conventional cryomicroscopy. Some practical work is needed to resolve this question. When all possible improvements in phase resolution are needed, it seems well worth investigating what can be achieved by OAH in combination with direct detectors.

References

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