

Optimized acquisition of off-axis holograms by dynamic computer control of the electron microscope

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In electron holography, the signal-to-noise of phase measurements increases with fringe visibility and electron dose (see [1] and references therein). Fringe visibility depends on the spatial coherence of the incident beam at the object plane, and hence the choice of overlap, and the transfer characteristics of the detector. Improving the visibility by spreading the beam will reduce the electron dose as constrained by the brightness of the electron gun. The choice of experimental parameters inevitably leads to a compromise. On the other hand, a longer exposure time would increase the number of electrons contributing to the electron hologram. This would benefit either hologram acquisitions at low beam intensities or where the total dose is not a constraint. However, holograms acquired over longer exposure times are deteriorated by biprism instabilities and specimen drift.

Instabilities in the biprism, due to charging or mechanical drift, cause the hologram fringes to shift over time. Simply increasing the acquisition time will therefore diminish the fringe visibility, counteracting the benefit of increased dose, and the image of the specimen will become blurred through specimen drift. A previously explored solution is to acquire image stacks of holograms [2]. Summing the phases of the individual holograms compensates for fringe shifts and numerical reregistration can compensate for specimen drift. Here we explore the possibilities of correcting both fringe instabilities and specimen drift by taking active control of the microscope deflectors and stage movements during the acquisition.

Experiments were carried out on the I2TEM a modified HF3300 60-300kV TEM (Hitachi) dedicated to *in situ* interferometry experiments. The microscope is equipped with multiple biprisms, an extra specimen stage above the objective lens for field-free imaging, an imaging corrector designed for wide fields of view (BCOR from CEOS) and a rapid 4K CCD detector (OneView from Gatan). Holograms were acquired at a microscope operating voltage of 300 kV, using two post-specimen biprisms to eliminate the Fresnel fringes, and the object placed in the upper field-free stage. Fringe spacings were typically 7 to 12 pixels with 600 fringes covering the 4K camera field of view. Holograms were acquired every 0.25 second and summed continuously. Computer analysis and control of the microscope was performed using in-house scripts written within Digital Micrograph 3.0 (Gatan).

We will show that unlimited acquisition times can be achieved without deterioration of fringe visibility or specimen image definition (Figure 1), and without any human intervention. The procedures are robust to low-dose conditions allowing accumulation of signal even when only 5-10 electrons per pixel contribute to individual holograms. The result being a single hologram with an optimized signal over noise ratio which reduces data storage compared with image stacks by several orders of magnitude and allows almost instant appraisal of phase quality.

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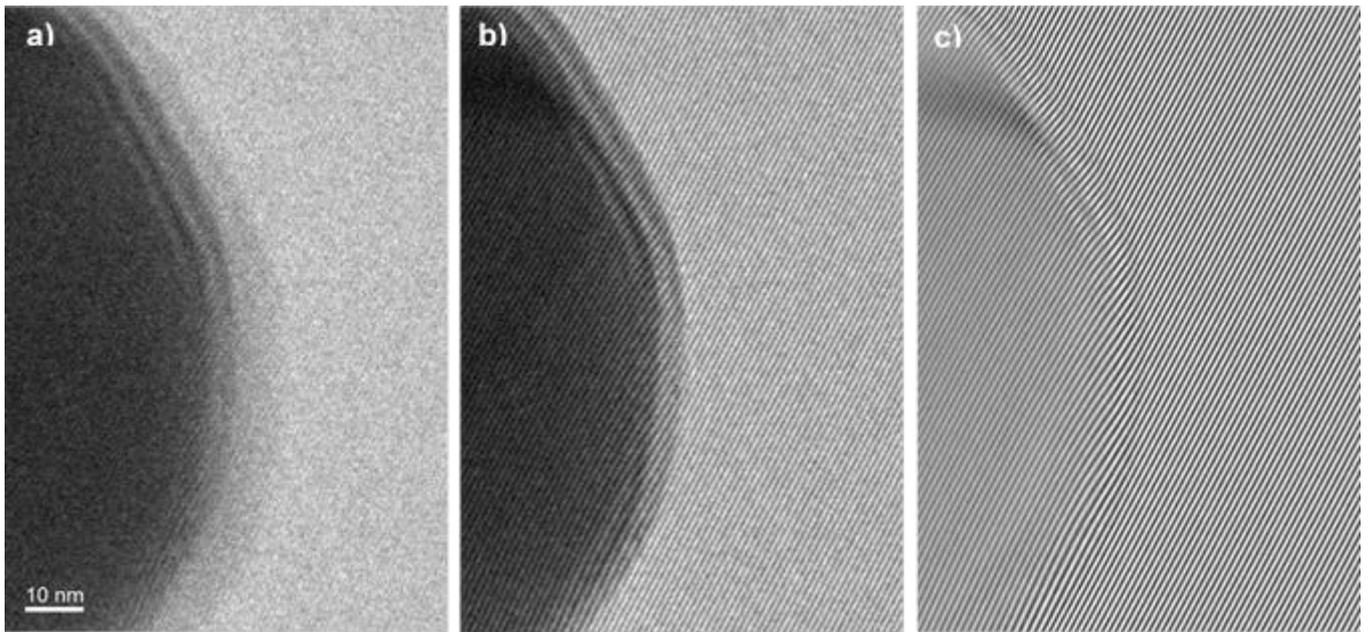


Figure1. Raw holograms after 10 mn of exposure time: a) No fringes and sample drift corrections, b) Fringes and sample drift corrections, c) Fringes and sample drift corrections with Fringe-Shifted method described in [3].