OFF-AXIS ELECTRON HOLOGRAPHY IN THE TEM AND ITS APPLICATION TO MAGNETOTACTIC BACTERIA

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Off-axis electron holography in the transmission electron microscope (TEM) allows the amplitude and phase of the electron wave that has passed through a sample to be recorded. Here, we outline how holography can be used to characterize the magnetic microstructure of magnetite nanocrystals in magnetotactic bacteria (DUNIN-BORKOWSKI et al., 1998).

Figure 1a shows a bright-field image of a single cell of the marine vibrioid strain MV-1, recorded at 200 keV in a Philips CM200 field-emission-gun TEM. A chain of magnetite crystals, which are each ~60 nm in length, can be seen next to a hole in the C film. The presence of the hole allows a reference wave from vacuum to be overlapped onto the cell to form a hologram (Figs. 1b and 2a). This is achieved by applying a positive voltage to a biprism (a 0.6 μm Au-coated quartz wire) located in the selected-area aperture plane of the microscope. The amplitude and phase of the electron wave leaving the sample are determined by extracting one 'sideband' from the Fourier transform of the hologram. This sideband is inverse-Fourier-transformed and the amplitude and phase of the resulting complex image are calculated. The phase is 'unwrapped' to remove discontinuities that result from the fact that it is initially calculated modulo 2π.

Neglecting dynamical diffraction, the measured phase is given in one dimension by

$$\phi(x) = \left(\frac{2\pi}{\lambda}\right)\left(\frac{E+E_0}{E(E+2E_0)}\right)\int V(x,z)dz - \left(\frac{e}{\eta}\right)\iint B_{\perp}(x,z)dxdz \qquad (1)$$

where z is the incident beam direction, x is a direction in the plane of the sample, $B_{\perp}$ is the component of the magnetic induction perpendicular to both x and z, V is the mean inner potential (MIP), λ is the wavelength and E and E_0 are, respectively, the kinetic and rest mass energies of the incident electron. Equation (1) shows that the phase is sensitive to both the MIP of the sample and the magnetic induction. For magnetic nanocrystals of this size, the MIP contribution dominates the phase and must be removed to characterize the magnetic microstructure of the sample. This can be achieved by recording two holograms with the chain of crystals magnetized in opposite directions. If the magnetic field in the sample reverses exactly, the magnetic and MIP contributions are given by half the difference and half the sum of the phases of the two holograms, respectively.

Figures 2b and 2c show two phases images obtained from the region in Fig. 2a, between which the magnetization direction of the chain was reversed using the field of the microscope objective lens. The two images are almost identical as they are dominated by the MIP contribution to the phase. The magnetization of the crystals produces a slight change in background across the chain; as a result, the top right of Fig. 2c is very slightly darker than in Fig. 2b. Figures 3a and 3b show the MIP and magnetic contributions to the phase determined from four pairs of holograms. The MIP contribution to the

phase of each crystal is proportional to its thickness and can be used to provide information about its morphology. The magnetic contribution, which is more slowly-varying, can be used to measure the magnetic moment of the cell. Alternatively, contours can be added to it to represent magnetic field lines, which are most closely-spaced along the line of crystals.

References

DUNIN-BORKOWSKI, R.E., McCARTNEY, M.R., FRANKEL, R.B., BAZYLINSKI, D.A., PÓSFAI, M. & BUSECK, P.R. (1998). Science, **282:** 1868–1870.

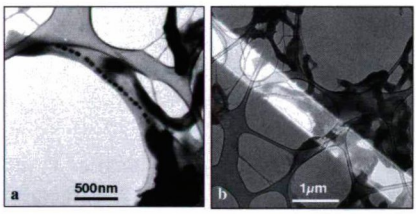

Figure 1: (a) TEM bright-field image of single cell of magnetotactic bacterium strain MV-1 located next to hole in carbon film. Chain is ~1600 nm in length and contains 15 magnetite crystals that are elongated along [111] and ~60 × ~35 nm in size. (b) Low magnification bright-field image obtained in field-free conditions showing effect of applying 120 V to biprism wire placed over region shown in (a).

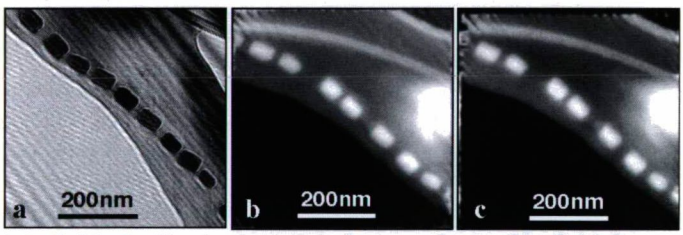

Figure 2: (a) Individual off-axis electron hologram obtained in field-free conditions by increasing magnification from condition shown in Fig. 1b, after tilting sample and applying large vertical field using conventional objective lens along axis of cell. Coarse fringes are Fresnel fringes from edge of biprism wire and fine fringes are holographic interference fringes which carry information about amplitude and phase of electron wave at exit surface of sample. (b) Phase image reconstructed from hologram shown in (a). (c) Phase image obtained after magnetizing chain in opposite direction to (b).

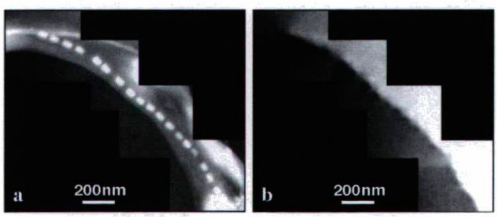

Figure 3: Composite images obtained by combining four pairs of phase images such as those shown in Figs. 2b and 2c. (a) and (b) show mean inner potential and magnetic contributions to phase and are formed by calculating half of the sum and half of the difference of each pair of images, respectively.