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Volume Imaging By Tracking Sparse Topological Features In Electron Micrograph Tilt Series

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Accurate three-dimensional (3D) reconstruction from electron micrograph tilt series of strongly scattering specimens has long been demonstrated using back-projection algorithms, for measured intensities that vary monotonically with the specimen scattering strength or thickness. For electron tomography of crystalline specimens, incoherent imaging techniques such as high angle annular dark field scanning transmission electron microscopy (HAADF-STEM) have been favored to satisfy such back-projection requirements [1], and refined iterative algorithms have now established 'atomic electron tomography' [2, 3].

Subtle information arising from coherent electron wave interference can be revealed in micrographs using phase-contrast techniques such as bright-field (BF) TEM or BF-STEM. While the former technique is routinely suitable for non-crystalline cryo-EM specimens, carefully accounting for contrast-transfer of the microscope [4], such micrographs can be problematic for back-projection from inorganic solids, as the requisite Radon transform is difficult to reconcile with the non-linear and often oscillatory image contrast. However, for thin specimens or small nanocrystals, including amorphized surfaces, multiple scattering can be forward-simulated from detailed models with consistent 3D refinement [5].

Often one might be interested in the mere *shape* of an object, rather than a measure of the complete electron scattering density, as might be the case for rapid surveying or in-situ measurements using BF-TEM imaging [6]. Using tilt series, are there ways to harness useful electron phase-contrast information to reconstruct 3D morphology, without recourse to weak scattering approximations or model refinement? For suitably sparse features, geometric tracking-based approaches can address this question. Related stereoscopic reconstruction methods from electron micrographs of biological specimens were dismissed in the first inception of back-projection based TEM tomography in this context [7]. However, such triangulation methods have continued to be employed for other 3D electron microscopy techniques, such as quantifying surface relief in scanning electron microscopy [8, 9].

By modifying an algorithm originally designed to assess atom probe lenses [10], here we show that sparse features exhibited in tilt-series micrographs can be reliably tracked as a smooth function of specimen orientation or rocking of the incident beam tilt, the continuous variation of which can be harnessed for differential triangulation to recover shape information. The interior and exterior morphology of scattering surfaces is then reconstructed by fitting polynomials to the angular variation of pertinent features, to recover the intersection points of local ray bundles with these surfaces and also estimate the surface normal. The localized persistence of such features across several micrographs need only be nascent for sufficient tracking.

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We shall present 3D reconstructions for electron microscope tilt series ranging from BF-STEM and ADF-STEM micrographs of polyhedral nanoparticles, to BF-STEM images of steel dislocation networks, cryo-EM cellular structures using TEM phase-contrast data, and topological descriptors for 3D diffuse scattering of a relaxor ferroelectric measured from selected area diffraction patterns. By assuming sparsity and non-overlapping detail in suitable micrographs, and segmenting connected topological points in the image contrast from the outset, we assess the reliability of tracking in our differential geometric form of stereoscopy and reconstruct 3D morphology irrespective of whether a Radon transform projection law holds or not.

In Fig. 1a, the unstructured point-cloud of differentially tracked and geometrically reconstructed topological features was structured onto a Cartesian grid and a 3D Gaussian filter was applied to highlight the two projections of the Pt nanocrystal shapes. The bright/dark contrast pertains to the differences between the combined independent ridge and edge reconstructions, which were sensitive to different features in the BF-STEM tilt series. The 3D reconstruction visualized in Fig. 1b was calculated using electron micrograph tilt series from the extensive Caltech ETDB resource [11], and the 3D projection was processed in a similar manner to that of Fig. 1a. Topologically linked valleys that outlined the bacterium morphology in the phase-contrast images in Fig. 1b were tracked in the tilt series, amidst the obfuscating dimpled contrast from the gold fiducial makers (appearing here as white dots) and amorphous speckle in each bright field image (creating some systematic streaking errors about the almost-vertical tilt axis).

We conclude that topological features can be robustly tracked in electron micrographs tilt series, using several different imaging modalities [12]. For this form of electron volume imaging, the angular variation of these features yields 3D point-clouds representing subtle specimen structure across a wide variety of different specimen types, with room for further development to ameliorate systematic errors by optimising the imaging conditions [14].

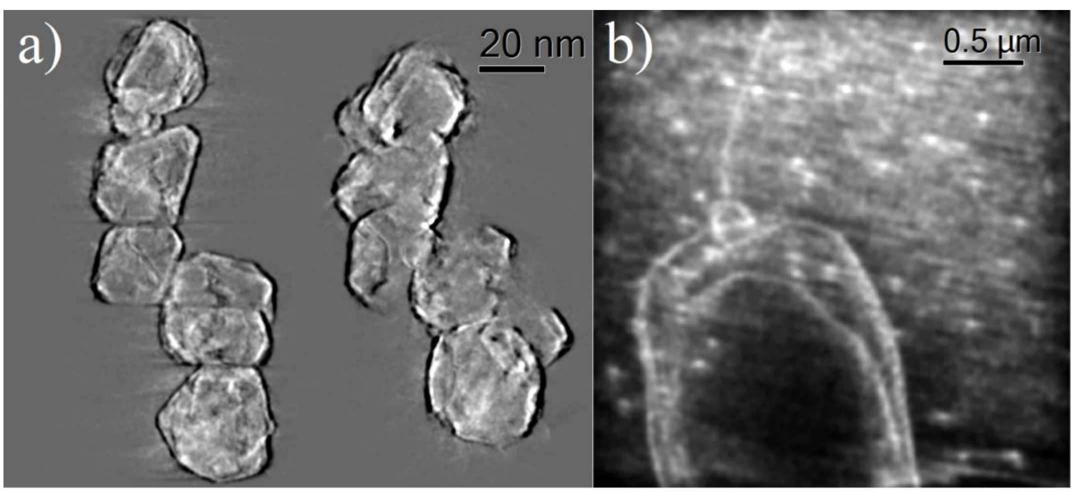

Figure 1. a) 3D reconstructions of Pt nano-crystals (two different views) from a BF-STEM tilt series. b) Similarly-processed [13] 2D projection of 3D points reconstructed from Vibrio delta Cholera bacterium phase-contrast images (Caltech ETDB resource [11])

References:

[1] M Weyland, PA Midgley and JM Thomas, J. Phys. Chem. B **105** (2001), p. 7882.
[2] Y Yang et al., Nature **542** (2017), p. 75.
[3] J Zhou et al., Nature **570** (2019), p. 500.
[4] J Frank in "Electron tomography: three-dimensional imaging with the transmission electron microscope" (Plenum Press, New York, 1992).
[5] D Ren et al., Ultramicroscopy **208** (2020), p. 112860.
[6] S Hata et al., Curr. Opin. Solid State Mater. Sci. **24** (2020), p. 100850.
[7] DJ De Rosier and A Klug, Nature **217** (1968), p. 130.
[8] JE Hilliard, J. Microsc. **95** (1972), p. 45.
[9] P Podsiadlo and G Stachowiak, Wear **230** (1999), p. 184.
[10] D Haley et al., J. Microsc. **244** (2011), p. 170.
[11] D Ortega et al., PLoS ONE **14** (2019), p. e0215531.
[12] TC Petersen et al., Ultramicroscopy (2022) p.113475, https://doi.org/10.1016/j.ultramic.2022.113475.
[13] DRG Mitchell and B Schaffer, Ultramicroscopy **103** (2005), p. 319.
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