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# Three-Dimensional Aberration-Corrected Scanning Transmission Electron Microscopy of Biological Specimens

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Tilt-series transmission electron microscopy (TEM) is the traditional methodology to resolve unique parts of the three-dimensional (3D) cellular ultra-structure [1]. A cubic volume is reconstructed from projections obtained by mechanically tilting the sample stage. The resolution of the reconstruction is in the range of 1–20 nm, thereby filling a critical length scale between atomic-resolution X-ray crystallography and single-particle electron tomography, and, at the other end of the scale, high-resolution confocal light microscopy [2]. A new development in electron microscopy is the use of aberration-corrected scanning transmission electron microscopy (STEM) to record 3D datasets for cell- and structural biology [3]. Aberration-corrected STEM is capable of high-resolution 3D imaging without a tilt stage. In a manner similar to confocal microscopy, the sample is scanned layer-by-layer by changing the focus so that a focal-series is recorded. The technique is possible with high vertical resolution because of the greatly reduced depth of field in an aberration-corrected STEM [4]. 3D STEM compares to 3D wide-field light microscopy, where the 3D image is reconstructed by deconvolution with a 3D point spread function (PSF). 3D STEM datasets were recorded of a metal replica of the cytoskeleton and a clathrin-coated pit of mammalian cells [5]. Fig. 1 shows two images of the 3D dataset differing 67 nm their focal position, recorded with an aberration-corrected STEM/TEM (JEOL 2200FS). The total imaging time for 220 images of 512x512 pixels was 5 minutes. The top surface of the clathrin-coated pit is in focus in Fig. 1A, while the cytoskeleton is in focus in Fig. 1B, demonstrating the depth-sensitivity. The images contained both the in-focus information from the focal plane as well as out-of-focus contributions. An iterative blind deconvolution using a maximum-likelihood approach was adapted for 3D STEM to resolve the vertical information. Fig. 2A-C show three images of the deconvolved 3D dataset, each differing 20 nm in focal position. The top surface of the clathrin-coated pit is visible in Fig. 2A, the sidewall of the pit in Fig. 2B and the cytoskeleton in Fig. 2C. The horizontal resolution of the 3D STEM dataset was 1.1 nm, set by the pixel size. The vertical resolution was different for different positions in the sample due to shadowing effects of the metal grains of the sample. After deconvolution, the vertical resolution for small grains amounted to 10 nm on average. The combined horizontal- and vertical resolution is already comparable with tilt-series TEM. The deconvolution algorithm used a position invariant PSF, which neglects changes of the PSF due to interaction of the electron-beam with the specimen. For specimens containing regions of a high-stain density, or for thicker samples, electron scattering leads to a position-dependent broadening of the probe size, and to a loss of the number of electrons in the probe. The deconvolution could possibly be improved by introducing a spatially variant PSF. The convergence of a deconvolution algorithm with a variant PSF can be helped by prior knowledge of the interaction of the electron

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beam with the specimen. For this purpose, experiments were conducted on test samples of different materials and geometries, from which experimental data of the beam broadening was obtained. In parallel, Monte Carlo simulations were performed for various sample geometries. The CASINO software was modified to include the full functionality of STEM imaging. The simulations were then calibrated using the experimental data. 3D STEM exhibits several advantages over tilt-series TEM due to the absence of mechanical tilt that can potentially be used to image very thick samples and even whole cells. Of importance is also the order of magnitude shorter time required to record the data compared to recording a tilt-series, making it suitable for high-throughput imaging. Finally, the contrast mechanism of STEM can be used to detect nanometer-sized particles of materials of a high atomic number inside an embedding medium of a low atomic number. [6]

References

[1] H. Stahlberg and T. Walz, *ACS Chem. Biol.* 3 (2008) 268.
[2] S.W. Hell, *Science* 316 (2007) 1153.
[3] N. de Jonge et al., N., in "Nanotechnology in Biology and Medicine-Methods, Devices and Applications", T. Vo-Dinh, Ed., CRC Press, Boca Raton (2007), p. 13.1.
[4] K. van Benthem et al., Appl. Phys. Lett. 87 (2005) 034104.
[5] N. de Jonge et al., *Microsc. Microanal.* 16 (2010) 54.
[6] We thank L.F. Allard, D. Blom, J.F. Deatherage, A.R. Lupini, S.J. Pennycook, and R. Sougrat for discussions and help. This work was supported by the High Temperature Materials Laboratory, sponsored by the U. S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Vehicle Technologies Program and by NIH grant R01GM081801 (NJ).

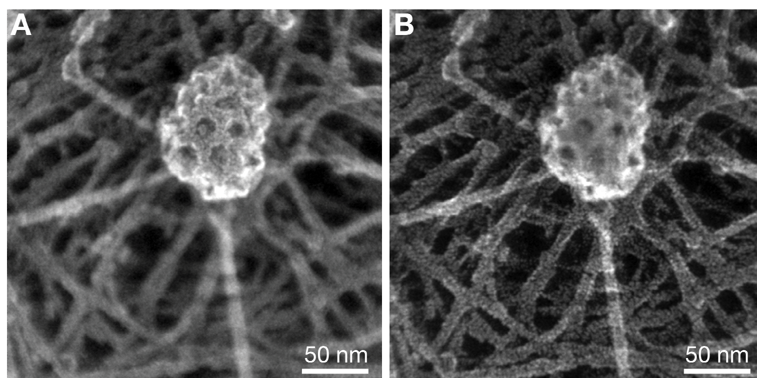


Fig. 1. 3D STEM images of a clathrin-coated pit and parts of the cytoskeleton of MDCK cells. (A) and (B) are at the same lateral (horizontal) position, but differ by 67 nm in focus (vertical position). The beam semi-angle was 41 mrad, the pixel-dwell time was 5 ⌠s, and the beam voltage was 200 kV. From [5].

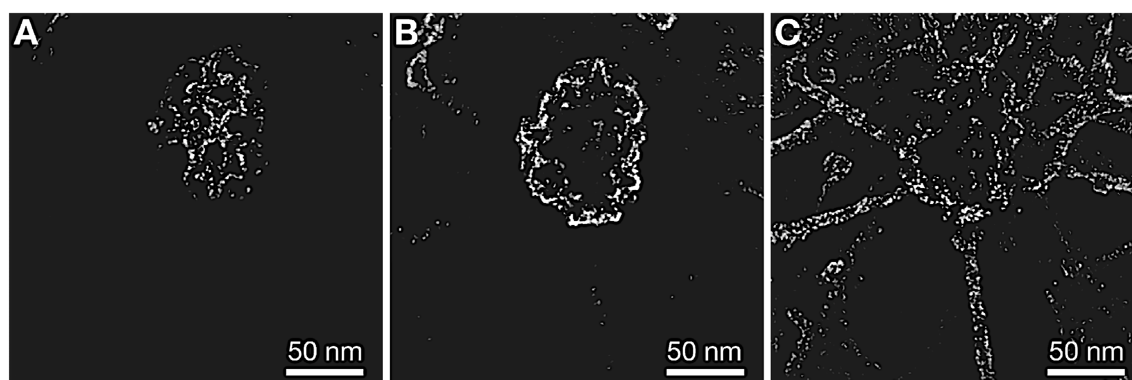


Fig. 2. 3D reconstruction of a clathrin-coated pit and parts of the cytoskeleton of MDCK cells obtained via deconvolution. The signal level is color-coded. (A) - (C) Horizontal slices of the 3D dataset differing by 20 nm in vertical position. From [5].

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