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Visualizing and Correcting Dynamic Specimen Processes in TEM Using a Direct Detection Device

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Transmission electron microscopy (TEM) is a powerful technique for visualizing structure at nanometer or Angstrom resolution. However, TEM performance lags considerably behind its theoretical limit based on the physics of electron scattering, especially in cases requiring low-dose imaging. Multiple factors reduce the resolution and signal-to-noise ratio (SNR) of TEM images, including the microscope instrumentation, dynamic specimen processes (e.g., drift, beam-induced motion, charging, radiation damage, etc.), and inefficient electron detectors [1].

With the goal of overcoming many of these obstacles, Direct Electron (San Diego, CA, USA) introduced the first large-format Direct Detection Device (DDD®) in 2008, as the culmination of academic and industrial partnerships working through six generations of sensor development beginning in 2001 [2]. Recently, Direct Electron released its new 20-megapixel "DE-20" camera system, representing the ninth generation of DDD development and providing the largest field-of-view of any available TEM direct detection camera [3].

Performance evaluations of the DE-12 and DE-20 cameras show significantly improved performance compared to traditional electron detectors such as film or CCD cameras [4,5]. The Detective Quantum Efficiency (DQE) of each of these cameras exceeds 10% at Nyquist frequency. However, DQE measurements should be used with caution since they remain dependent on the DQE calculation method used and accurate dose measurement. Additionally, DQE does not include vital performance information such as the total field-of-view, frame rate, dynamic range, robustness of dark/gain correction, etc. Thus, we have also characterized our cameras using other metrics such as the spectral SNR from Thon rings in images of thin carbon film, as well as 2D analysis and 3D reconstructions of low-dose images. These reveal that the DDD cameras yield images with high SNR and retrievable information up to Nyquist frequency.

In addition to improved detection efficiency and resolution, the architecture of DDD cameras allows for continuous streaming of unbinned full-frame images at 30 frames per second, with no dead time between consecutive frames [2,3]. This "movie" acquisition provides a large field-of-view and improved resolution for visualizing dynamic specimens in experiments where this of interest, such as *in Situ* TEM.

However, many TEM methods require a static specimen image, such as low-dose electron cryo-microscopy of biological specimens. In these methods, dynamic specimen processes are detrimental, causing either non-isotropic resolution loss (i.e., specimen drift) or overall degradation of the SNR in each image (e.g., beam-induced motion, charging, radiation damage, etc.). We have developed methods and algorithms for exploiting the "movie mode" output from DDD cameras to correct for these dynamic processes and maximize the isotropic resolution and SNR of each image. Briefly, a "movie" is acquired of a specimen at 2-3× the normal total electron exposure. To correct specimen drift (which is consistent across the entire image), the frames from the movie are iteratively aligned, and to correct beam-induced specimen motion and charging (which are local effects that vary across the image), sub-regions for each

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frame are iteratively aligned [6]. To correct radiation damage, low-pass filters are applied to each frame based on expected damage rate of the specimen. For biological specimens, this damage rate can be estimated from previous radiation damage studies [7,8].

We have demonstrated the benefits of this method by using images of frozen-hydrated Brome mosaic virus (BMV). Images generated based on our method show improved isotropic high-frequency SNR along with significantly improved low-frequency contrast (*Fig. 1*). We expect the boost in low-frequency contrast to yield more accurate alignments of individual particles, while the improved SNR and resolution will help push the envelope of TEM performance ever-closer to its theoretical limit.

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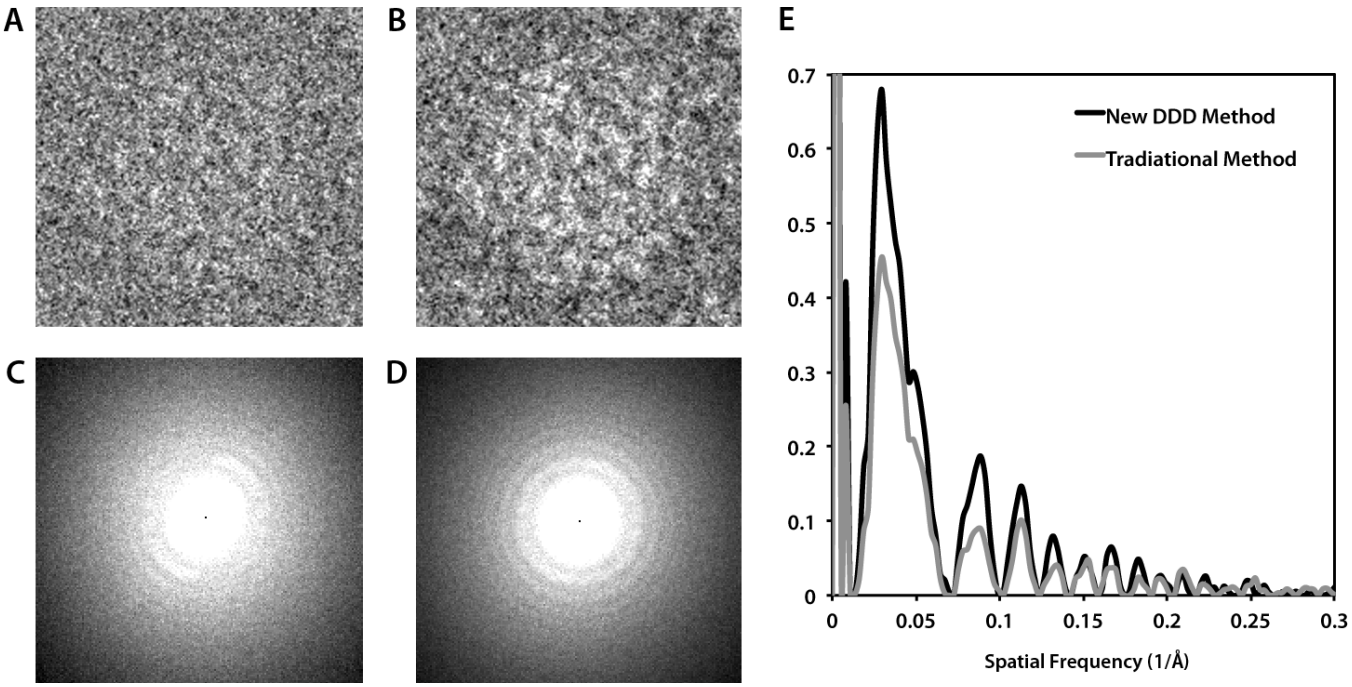

Figure 1. Frozen-hydrated BMV imaging on a 300 kV TEM at 1 μm underfocus. (A) An individual BMV particle from the image generated by the traditional method of imaging with a single 20 $e^-/Å^2$ exposure, and (B) the new method of imaging with a total exposure of 36 $e^-/Å^2$ using a DDD sensor with correction of dynamic specimen processes. (C) The average Fourier transform of all 96 BMV particles from the image with the traditional method, and (D) the new method. (E) Comparison of the spectral SNR based on the rotational averagesof the Fourier transforms in (C) and (D).

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