New Improvements in Direct Detection
Benjamin Bammes¹, Michael Spilman¹, Liang Jin¹
¹ Direct Electron, Sunnyvale, CA

DE-64 Camera: Massive Field-of-View

The DE-64 direct detection camera has 8192 x 8192 real pixels. The camera has a maximum frame rate of 42 frames per second for full-frame unbinning readout. A low magnification image at 120x of a fine-grating grid is shown above. The entire 8k x 8k field-of-view is shown at the left, and the middle and right images show digital zoom to display the level of detail in this massive image with individual leaflet beads visible even at this low magnification.

The Fourier transform (FFT) of a higher magnification (37,000x) image of the fine-grating is shown at the left. The sampling at this magnification is 1.61 Å/pixel. The 2.3 Å and 2.0 Å gold lattice spacing are clearly visible in the FFT, even at Nyquist frequency (the theoretical best resolution of a camera). Data collection courtesy of Scott Stagg, Florida State University (Tallahassee, FL, USA).

Near-Perfect MTF: Counting with Large Pixels

With 2x hardware binning, the DE-64 camera has a pixel pitch of 13 μm, which is well-matched to the size of the point-spread function of primary electrons incident on the sensor. Under these conditions, the DE-64 runs at 146 frames per second (fps) with a 4096 x 4096 area; frames are processed using a GPU-based electron counting algorithm to generate images with a near-perfect modulation transfer function (MTF) and an extraordinary detective quantum efficiency (DQE).

Above: A cropped field of view of a single frame from the DE-64 with no binning (left), showing electron events after thresholding. 40% of the events are larger than 4 unbinned pixels (which corresponds to the size of one binned 2x pixel). The distribution of counts from incident primary electrons is shown at the right. The most probable signal-to-noise ratio (SNR) is ~50, and the average SNR is ~98. Based on the full-frame Landau distribution, only ~1% of incident electrons will be missed due to low signal. Right: A cropped region of a beamstop edge with 2x-24 counting. Below: The MTF and DQE of the DE-64 with 2x-24 counting.

Fast, High-Resolution at 200 kV

A representative cryo-EM micrograph and selected 2D class averages of Thermotoga maritima 20S Proteasome imaged on a DE-20 direct detection camera on a 200 kV FEI Titan T20 TEM without an objective aperture. The micrograph shown is at 1.3 μm resolution. A total of 350 micrographs were collected using automation in Serafim, at a rate of approximately 80 acquisitions per hour. The pixel size was 0.31 Å/pixel. Data courtesy of Matthias Danner, Max Planck Institute of Biochemistry (Martinsried, Germany).

Each acquisition contained 20 frames, which were motion corrected and dose filtered using MotionCor2 (D‘Hondt et al., 2017) with 5x6 patches. CTF parameters were determined using FTFM (Babcock & Grigorieff, 2015). Binning was completed using EMAN2 (Tsai et al. 2007). 2D class averages and the 3D reconstruction were generated using Molsoft (Scheres, 2012). All image processing was completed on a desktop computer with a single NVIDIA GeForce GTX 1060 GPU.

A total of 43,637 particles were boxed, of which 31,372 particles were retained after selecting reasonable-looking 2D class averages (examples shown above). The final 3D reconstruction (shown below) reached 3.76 Å resolution by gold-standard FSC. An α-helix and beta-sheet from the reconstruction are shown below with the X-ray crystal structure fits into the cryo-EM density map.

Electron Diffraction “Motion Correction”

Three selected frames from a 1 second movie at 25 fps of electron diffraction of frozen-hydrated catalase crystal collected on a DE-12 Camera mounted on a JEOL 2010F microscope. For reference, the diffraction pattern from the final frame is overlaid in light green. Data collected in the lab of Wei Chu, Baylor College of Medicine (Houston, TX, USA).

The integrated 1 second diffraction pattern from above, without (left) and with (right) motion correction. Motion correction used the Qil process frame Python script, which uses cross-correlation to align prominent features in a movie acquired with a DE camera. In this case, instead of correcting specimen motion, we were correcting the motion of the beam during diffraction, which noticeably improved the signal-to-noise ratio (SNR) of diffraction spots.

Biggest: Massive 8k x 8k Field-of-View

DE-64 Camera System

Best Performance: Near-Perfect MTF

DE-16 or DE-64 with 2x-Binning Counting