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Apollo: A Novel Event-Based Direct Detector for Cryo-EM

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Over the past 10 years, electron counting with direct detection cameras [1, 2, 3] has become the de facto standard for cryo-EM data acquisition. However, since its initial demonstration in 2009, the technology for electron counting has remained fundamentally unchanged: Electron counting is performed computationally, by thresholding and centroiding blobs on each of many integrating mode frames acquired under a strictly limited TEM beam current.

One of the most significant bottlenecks for cryo-EM is the restrictive imaging conditions imposed by electron counting. Maintaining sparse illumination within each frame from the camera is necessary to avoid coincidence loss stemming from the inability to discriminate multiple coincident electrons as separate events [3]. The limited exposure rate imposed by current cameras has two consequences: it places an upper limit on throughput, and it eliminates the microscopist's flexibility to optimize imaging conditions for new methods.

A new TEM camera, Apollo, is based on new direct detection technology that—for the first time—performs electron counting in hardware with a large-format sensor, enabling high-quality image acquisition across a wide range of exposure rates with minimal coincidence loss. Apollo's novel sparse-binary-readout direct detection sensor performs correlated-double sampling (CDS), thresholding, and identification of event "blobs" automatically on-chip, at a significantly higher readout rate than has been achieved with conventional direct detection sensors. Subsequently, super-resolution centroiding is performed in FPGA hardware in real-time, forming 8192×8192 dose-fractionated frames for motion correction and dose filtering [4, 5] at 60 frames per second (fps).

To evaluate the coincidence loss rate on Apollo, we acquired images with constant beam intensity at various magnifications, so that the relative electron exposure incident on the camera could be calculated based on the calibrated pixel size at each calibrated magnification. The resulting coincidence loss curve (Fig. 1) was fit using the model described by Li, et al. [3]. Apollo's ultra-fast counting enables high-quality imaging with minimal coincidence loss up to ~60 e^-/pixel/s. The measured coincidence loss rate is only ~4% at 30 e^-/pixel/s and only ~10% at 60 e^-/pixel/s, which is significantly better than the other conventional electron counting cameras for cryo-EM [6, 7].

As an initial demonstration for single-particle cryo-EM, we acquired images of frozen-hydrated apoferritin on a Titan Krios (Thermo Fisher Scientific, Waltham, MA) operated at 300 kV. The total exposure for each acquisition was 56.7 $e^-/Å^2$ acquired over ~0.67 seconds, corresponding to a high exposure rate of ~30 e^-/pixel/s. A total of 1478 images were used to generate a 1.76 Å reconstruction (Fig. 2), demonstrating low-coincidence-loss electron counting cryo-EM data acquisition at significantly higher exposure rates than feasible for other counting direct detectors [8].

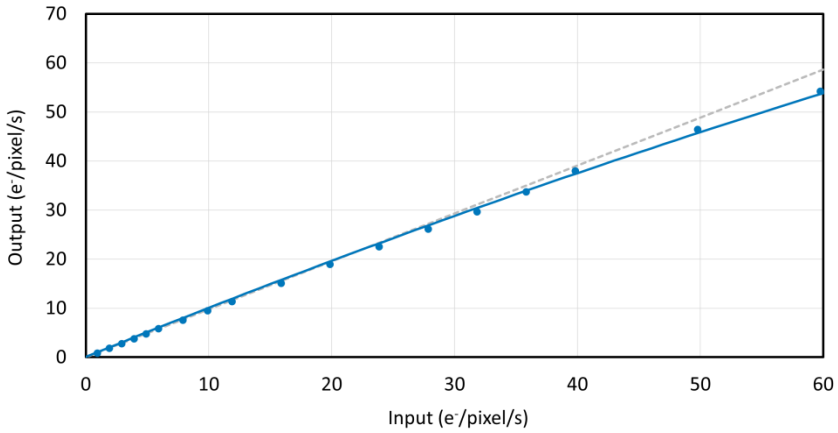

Figure 1. Coincidence loss curve for Apollo.

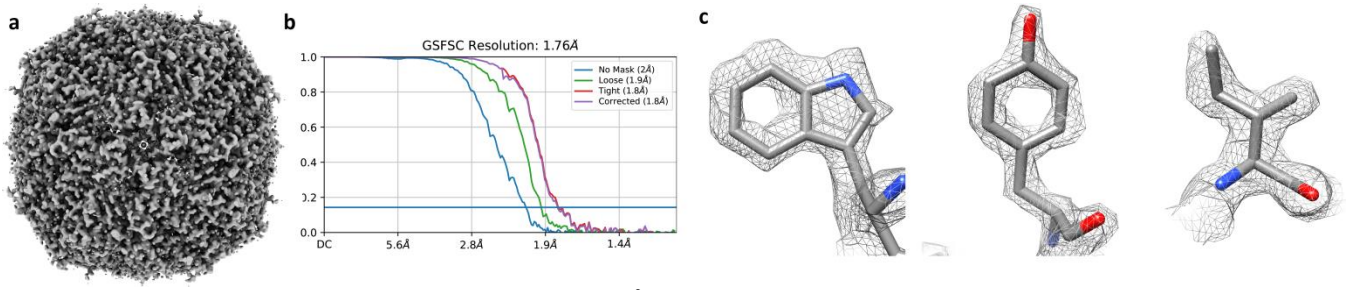

Figure 2. Reconstruction of apoferritin at 1.76 Å resolution. (a) The entire map. (b) The Fourier-shell correlation curve. (c) Example side-chains from the map, including the fitted atomic model.

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