Optimizing data quality with an event-based direct detector

Benjamin Bammes[1*], Erik D. Anderson[2], Steven J. Ludtke[2]

[1] Direct Electron LP (San Diego, CA USA) [2] Department of Biochemistry and Molecular Biology, Baylor College of Medicine (Houston, TX USA) * Corresponding Author (bbammes@directelectron.com)

Direct Electron INNOVATION PROPELLING DISCOVERY

Baylor College of Medicine

5th International Symposium on Cryo-3D Image Analysis 2024

Apollo's unique, optimized design for counting

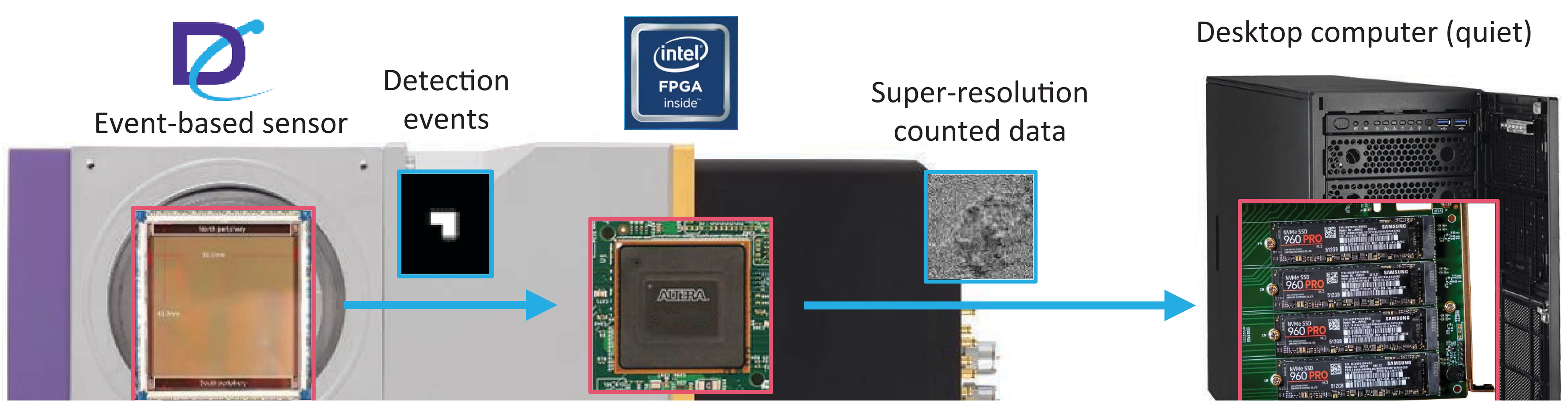


Figure 1: The Apollo is based on a novel (US Patent #10,616,521) ultra-fast, event-based, direct detection device (DDD®). Instead of outputting analog frames, the sensor detects incident electrons and outputs the location and shape of each detection "blob" registered on the sensor. On-chip correlated double sampling (CDS), on-chip thresholding, noise-free digital output, and ultra-fast counting speed maximize data quality.

Better contrast for tomography

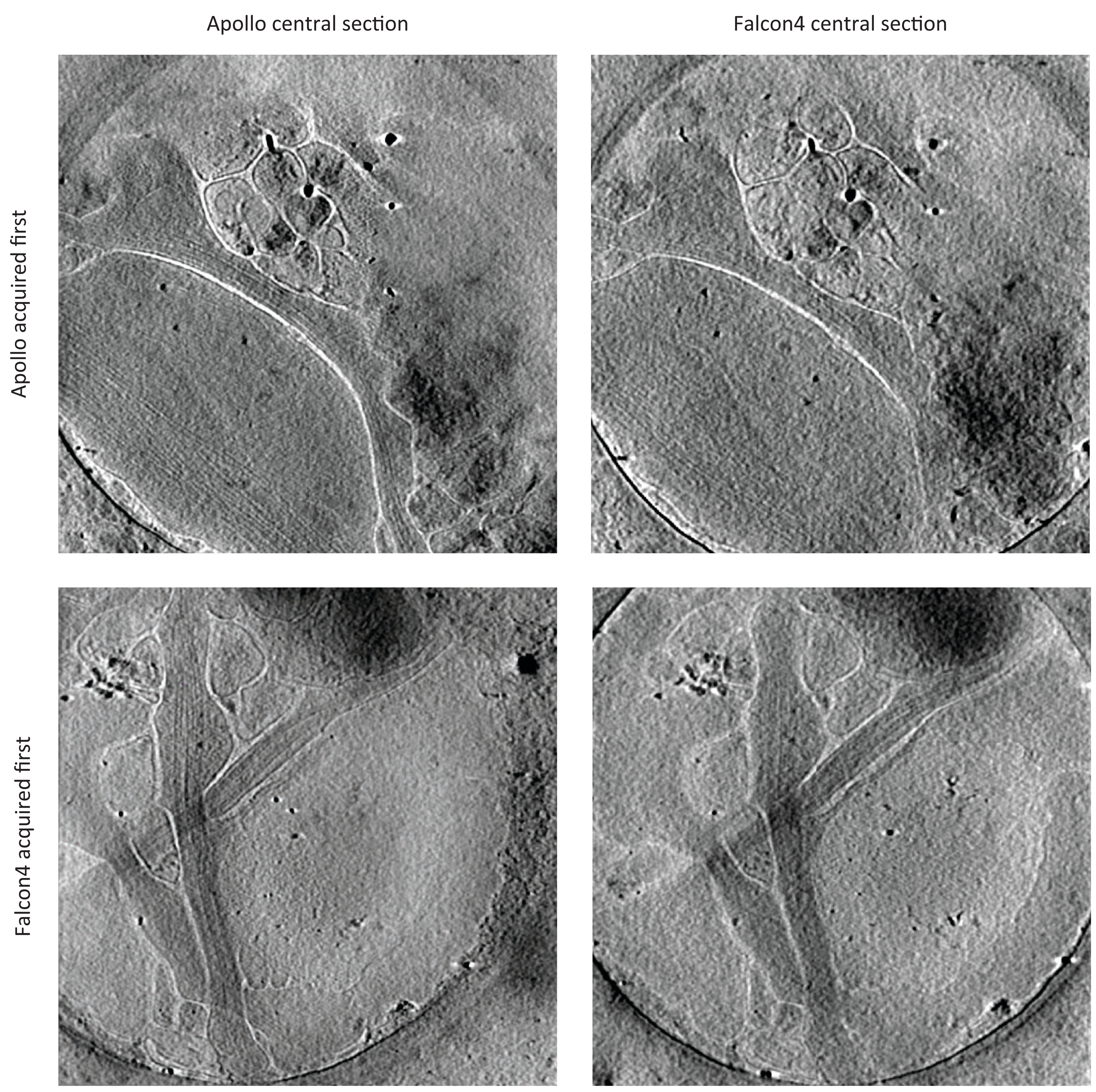
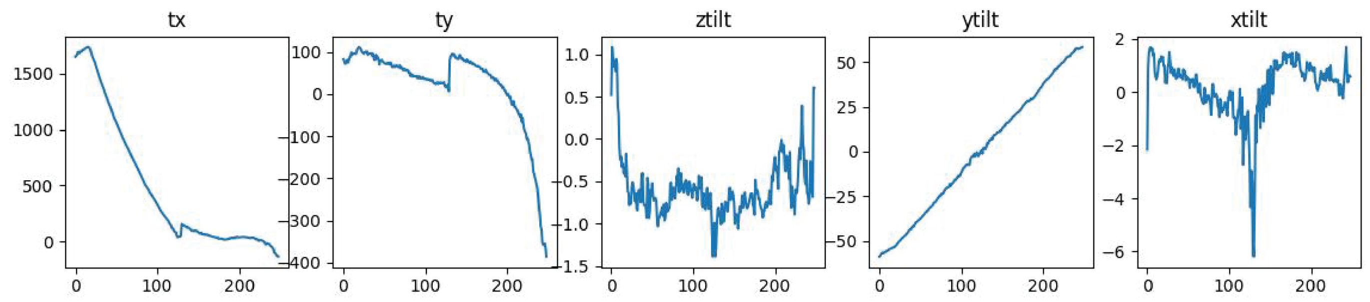
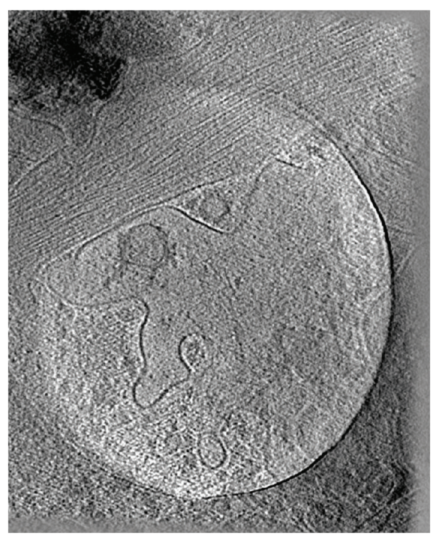


Figure 2: Tilt series were collected of rat cortical neurons on a Thermo Fisher Glacios (200 kV). All tilt series were dose symmetric, spanning -45° to 45° with 3° steps. Falcon4 data was acquired using Tomo (Thermo Fisher Scientific) with a pixel size of ~5 Å/pixel, while Apollo data used SerialEM with ~4.6 Å/pixel. The specimen exposure rate and C2 lens were kept constant (~1.6 e⁻/Å²/tilt, C2 = 38.87%). Acquisitions were alternated between using the Apollo first or the Falcon first. EMAN2 was used for 3D reconstruction.

Continuous rotation tomography

Figure 3: Bidirectional low-dose continuous-rotation tomography acquired in ~60 seconds on a Thermo Fisher Glacios. Frames were averaged over 0.5 s intervals to facilitate analysis. Plots of the alignment of each frame are shown below. A cropped region of the central section of the reconstruction also shown.





Centroided Event Streaming (CES)

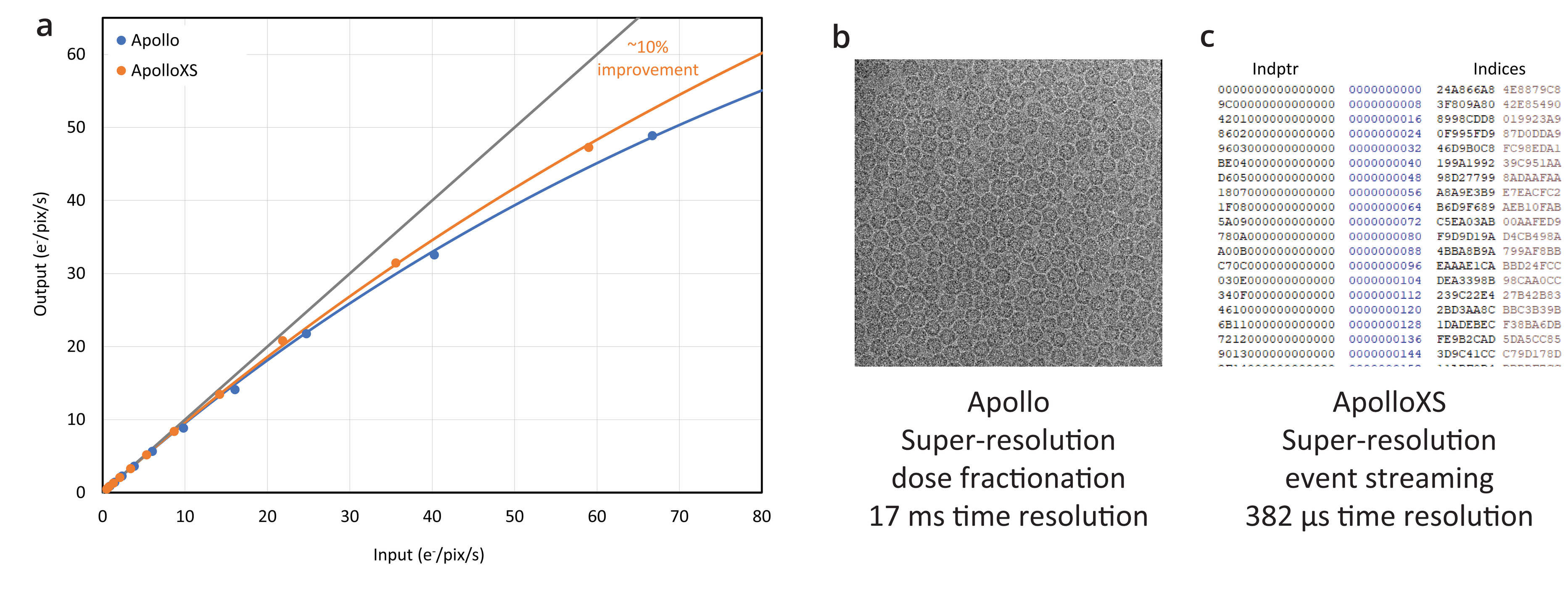


Figure 4: The new "ApolloXS" version of the camera includes faster sequencer timing for running the sensor, a new strategy in FPGA for identifying the boundaries of single detection events (which are connected groups of pixels output from the sensor), and the new "Centroided Event Streaming (CES)" readout mode. **(a)** Coincidence loss curves at 200 kV for Apollo and the new ApolloXS models, showing ~20% less coincidence loss in the new model. **(b)** The Apollo camera delivers a stream of super-resolution dose fractionated movie frames with 17 ms time resolution. **(c)** The new ApolloXS camera delivers a compressed stream of coordinates of centroided events with 382 µs time resolution.

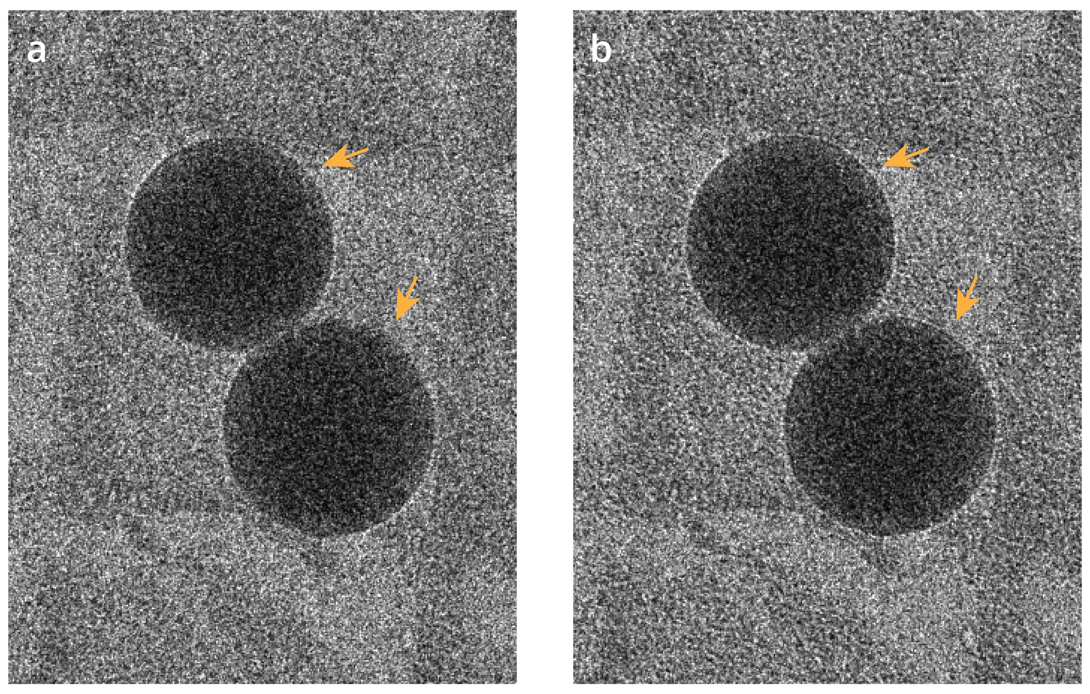
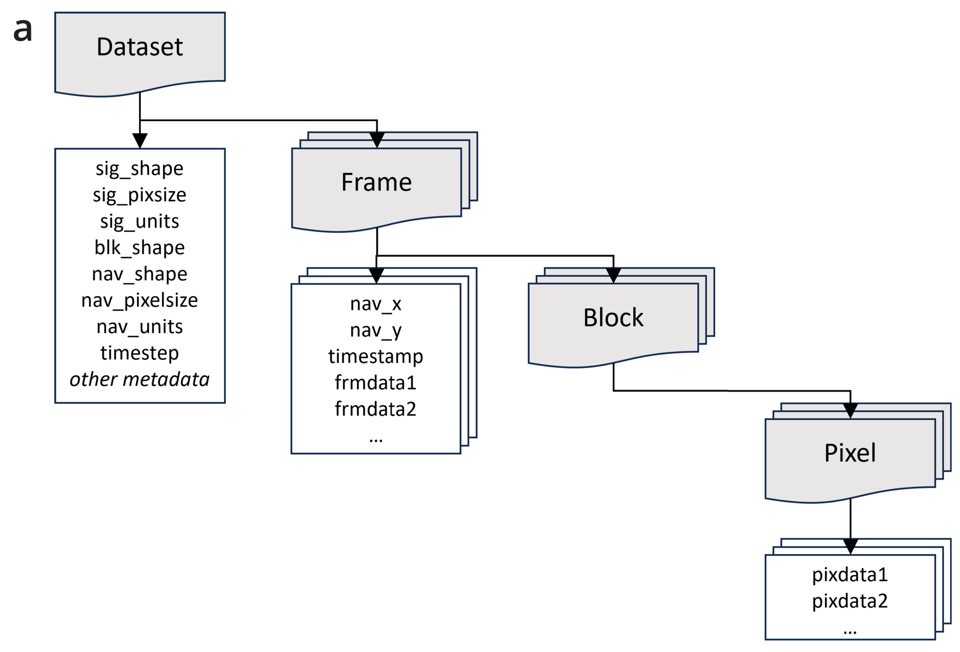


Figure 5: The first demonstration of CES mode from the ApolloXS camera. Data was acquired on a JEOL 2200FS at 200 kV. The detection rate was ~48 e⁻/physical pixel/second. The images show a 4×-binned cropped regions of a line grating grid with latex beads. Each image represents the sum of the events detected over just 19 ms. **(a)** The sum of events without attempting to correct for specimen motion. **(b)** The same data but with shifts applied to correct the specimen motion. The total detected motion over the 19 ms was ~2 and ~8 physical pixels in the x and y directions, respectively.

Event Detection CSB (EDC) File Format



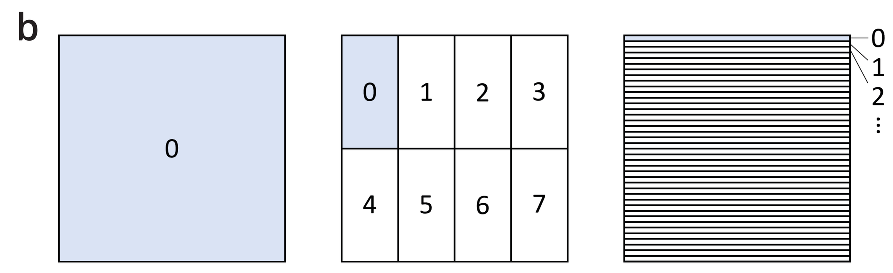


Figure 6: We propose to store event data in a compressed sparse block (CSB) format (Buluç, et al., 2009; Tinney & Walker, 1967). **(a)** Each data set contains a hierarchy of values and arrays, enabling wide-ranging flexibility. **(b)**. The area of a sensor/image can be segmented arbitrarily into rectangular blocks. For efficiency, each block should contain 65,536 coordinate locations.

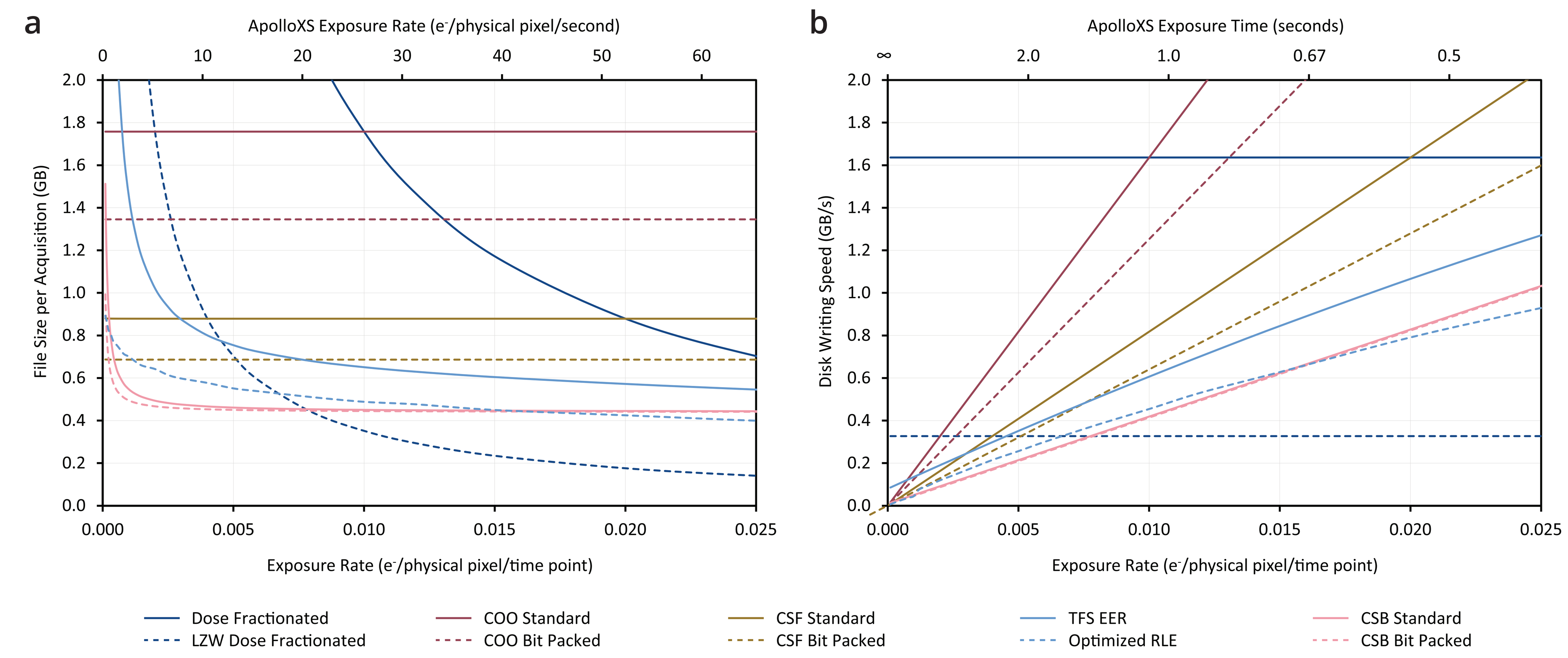


Figure 7: Comparison of several possible file formats for saving event detection data. Each acquisition represents a typical single-particle cryo-EM acquisition, with 4096 × 4096 physical pixels, 2×-super-resolution electron counting, 0.75 Å/pixel sampling, and 50 e⁻/Å² total exposure. The horizontal axis at the top of each plot shows the corresponding exposure rate and exposure time on the ApolloXS camera. (Note that the horizontal ranges of both plots are identical, so that the horizontal axes correspond to each other.) The dose fractionated formats assume a 50× reduction in time resolution. Dashed lines show compressed or optimized versions of each file format. Across a broad range of exposure rates, the compressed sparse block (CSB) format adopted in our proposed "EDC" file format offers the smallest file sizes **(a)** and lowest required disk writing speed **(b)**.

TO LEARN MORE ABOUT APOLLO OR CELERITAS, VISIT www.directelectron.com

Copyright © 2024 Direct Electron, LP. All rights reserved. *Printed on March 13, 2024.*