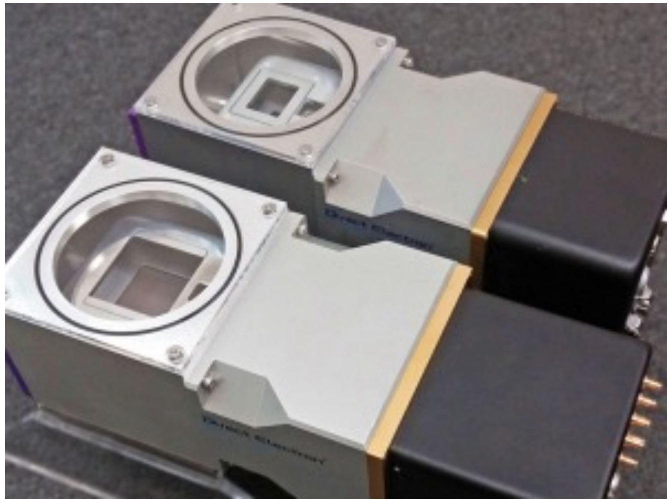
Cameras and Frames

Steps in a single particle reconstruction

- Collect data
- Align frames
- Estimate CTF
- Pick particles
- 2D Classification
- Generate initial model
- Refine data against initial model
- Estimate resolution

The revolution in cryo-EM

+



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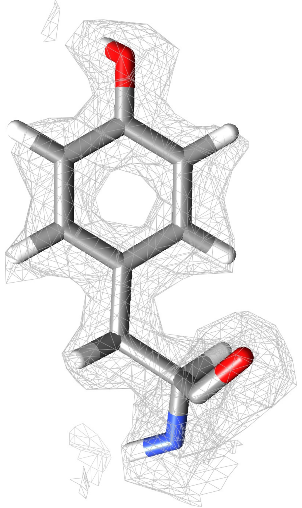


The Titan Krios
- Stable
- Automated
- Aberration corrected
- Bright coherent beam

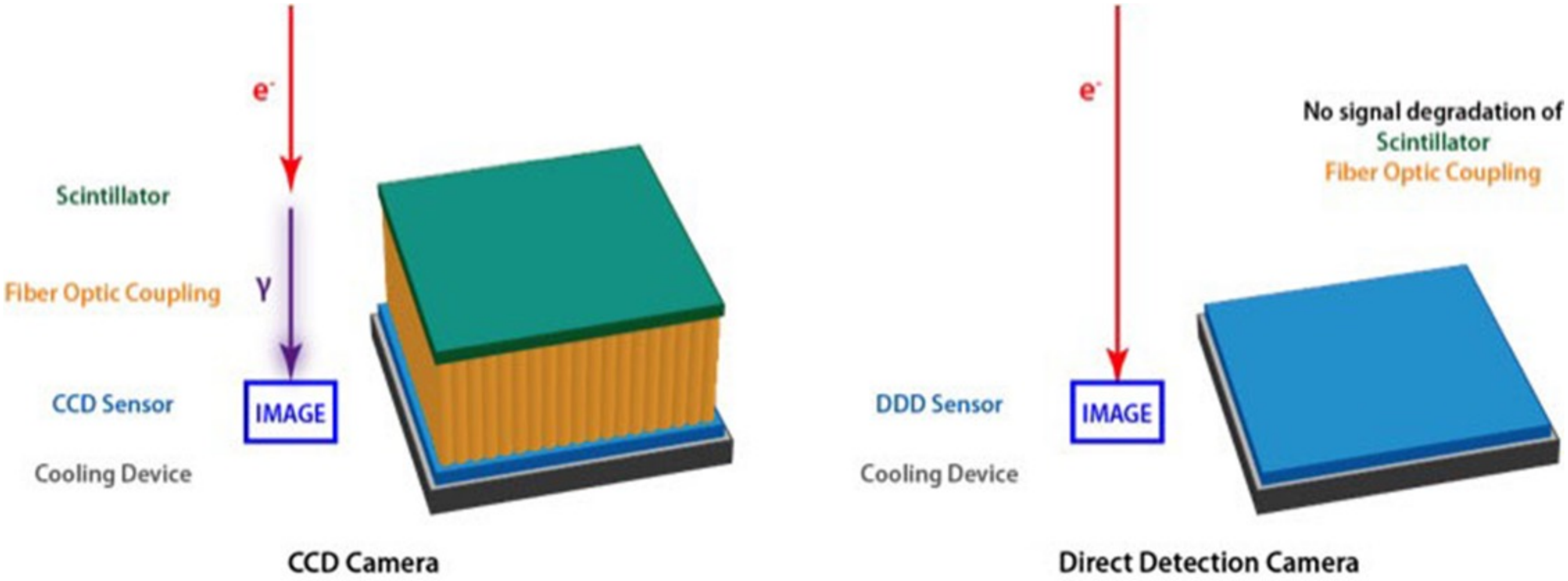
Direct electron detectors
- High DQE
- High frame rate

- HIgh resolution

History of Cameras in Cryo-EM

- **Film Cameras** (pre-1990s)
  - First used in electron microscopy.
  - Low sensitivity and limited dynamic range.
  - Cumbersome processing and low throughput.
- **Charge-Coupled Devices (CCDs)** (1990s-early 2000s)
  - Improved sensitivity over film.
  - Digital processing, enabling easier data handling and analysis.
  - Still had limitations in terms of resolution due to indirect electron detection.
- **Direct Electron Detectors** (2010s onwards)
  - Marked a revolution in cryo-EM.
  - Detect individual electrons – facilitated large improvement in resolution.
  - Facilitated many of the advancements we'll discuss, such as movie alignment, dose compensation, and DQE improvements.

CCD vs DED


e-
Scintillator
Fiber Optic Coupling
γ
CCD Sensor
IMAGE
Cooling Device
CCD Camera
e-
No signal degradation of
Scintillator
Fiber Optic Coupling
DDD Sensor
IMAGE
Cooling Device
Direct Detection Camera

Understanding DQE (Detective Quantum Efficiency)

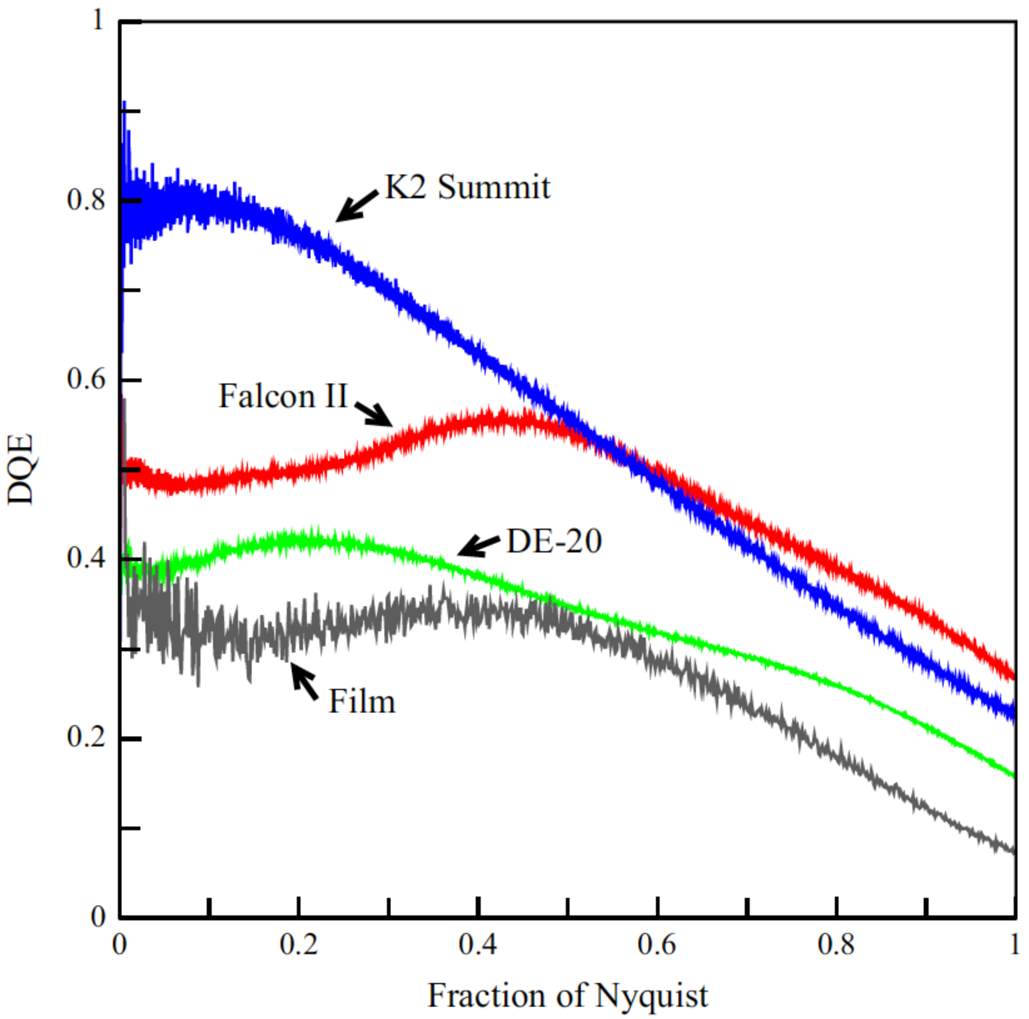
- **What is DQE?**
  - A metric for an imaging system's capability to capture information with high fidelity as a function of resolution.
- **Why DQE Matters:**
  - A higher DQE translates to clearer images with reduced noise.
  - Crucial for achieving high-resolution images in cryo-EM.
- **Evolving Tech & DQE:**
  - Direct electron detectors (DEDs) have dramatically improved DQE compared to older CCDs.
  - Result: Enhanced image clarity and information retention.

$$DQE(\omega) = \frac{SNR_{out}^2(\omega)}{SNR_{in}^2(\omega)}$$

DQE of 1 is good DQE of 0 is bad

DQE (Detective Quantum Efficiency)

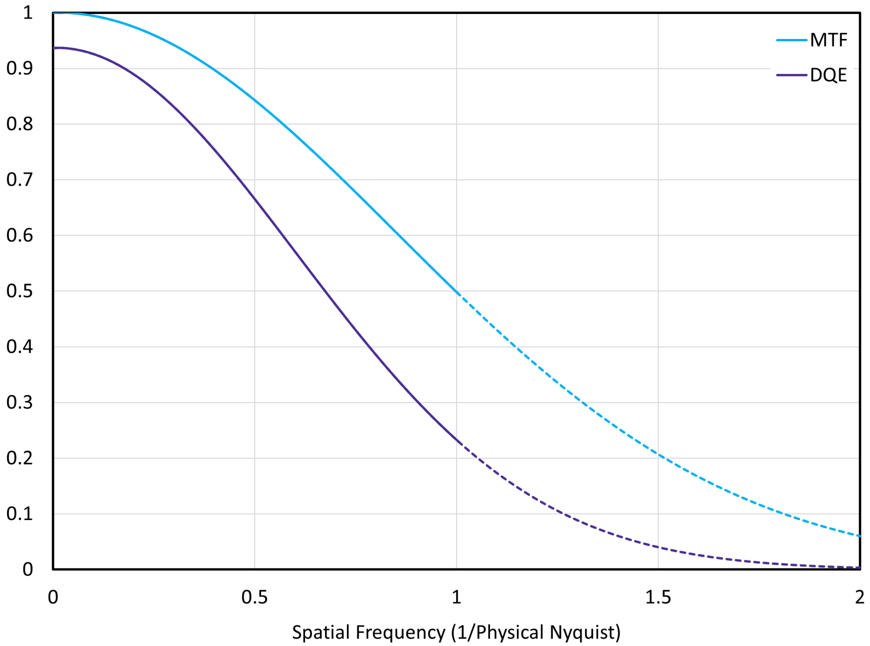
- **DEDs have higher DQEs than other detection methods**
  - With the advent of Direct Electron Detectors, there was a significant improvement in DQE over CCDs.
  - This meant that these newer cameras were better at capturing and representing the information from the electrons



McMullan et al., 2014

DQE (Detective Quantum Efficiency)

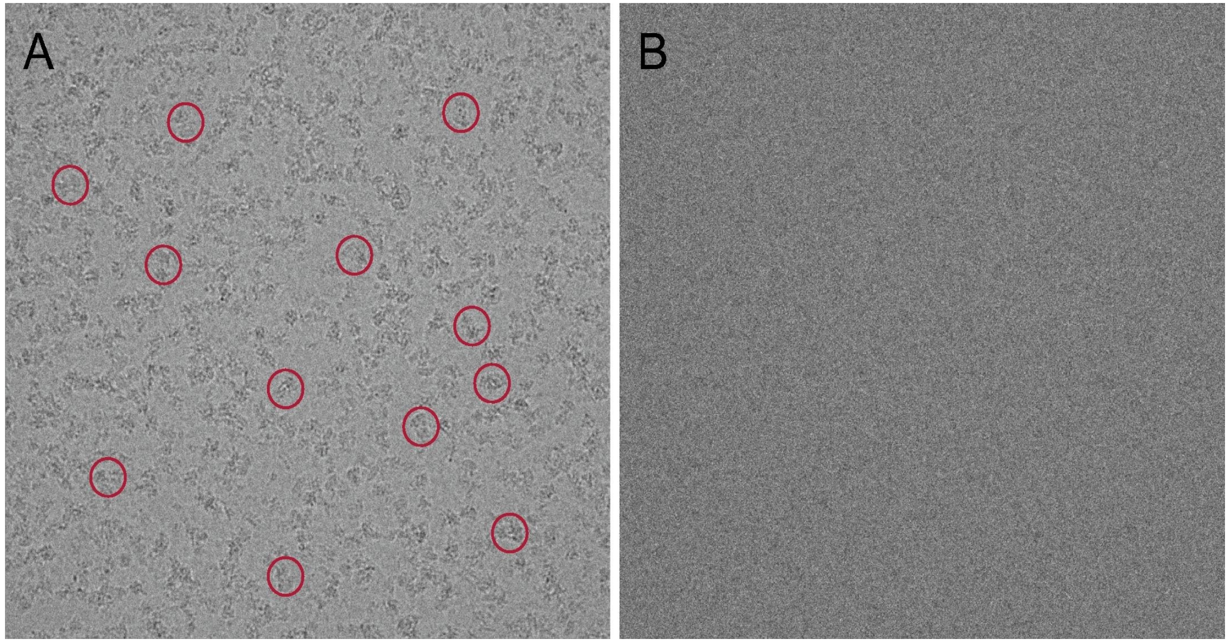
- **DQE Values and Interpretation**
  - If DQE(f)=1 or 100%: Perfect information preservation
  - If DQE(f)=0 or 0%: No preservation of information.
- **Why DQE Matters**
  - Resolution in single particle reconstruction depends on aligning particle images with high accuracy and precision
    - DQE, especially at low frequencies, is critical for image alignment accuracy and precision



DQE of the DE Apollo at FSU

Direct Detection and Frames

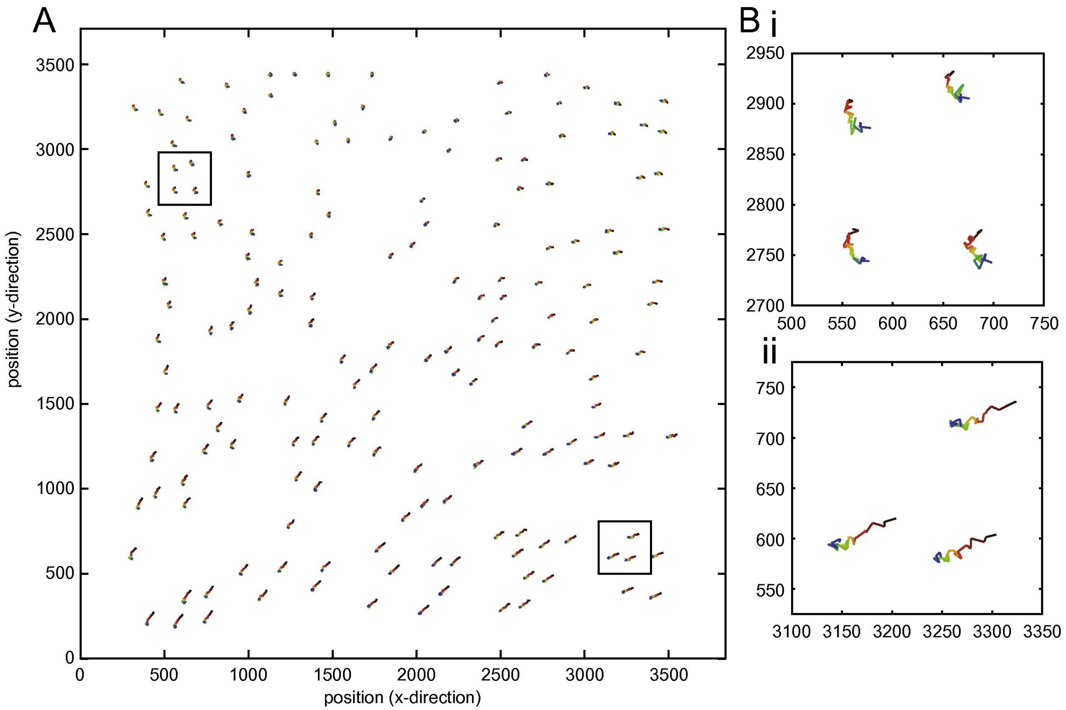
- **Movie Mode:**
  - Direct detectors capture rapid sequences of frames during exposure.



A) sum aligned high SNR

B) single frame low SNR

- **Benefits of Multiple Frames:**
  - Corrects specimen drift and movement.
  - Dose fractionation minimizes radiation damage.

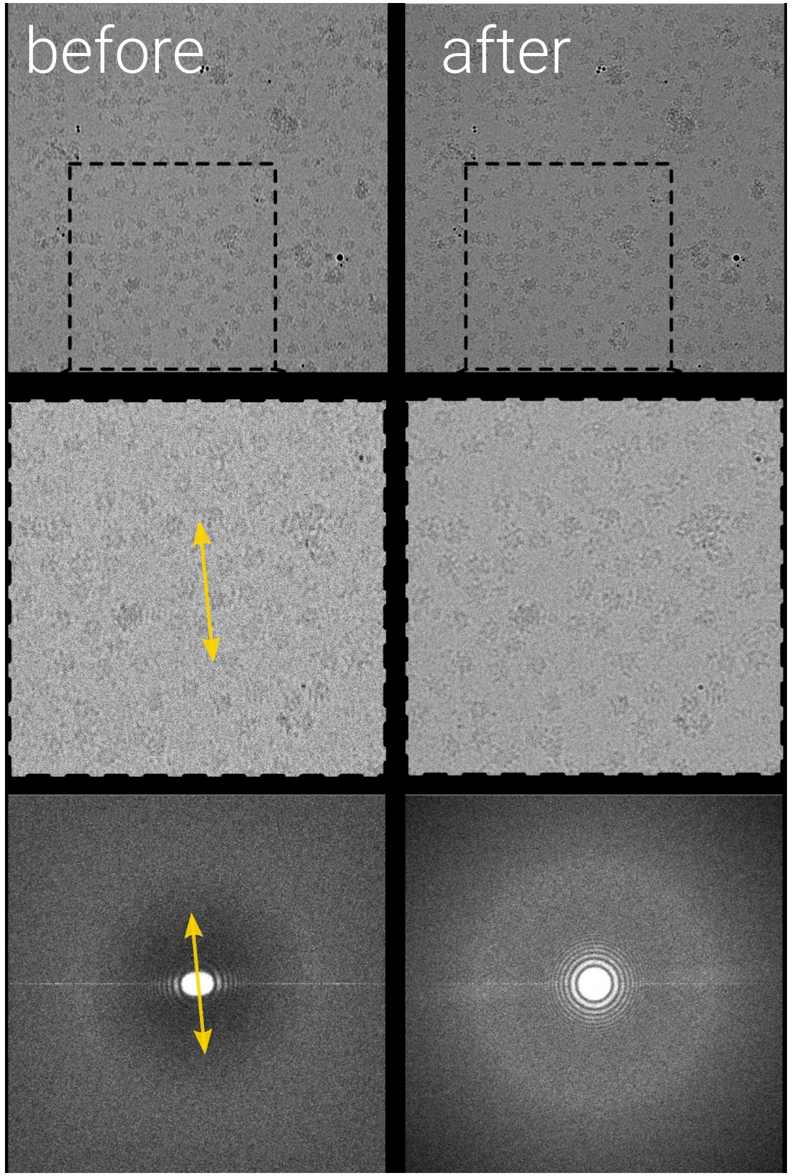


Particle motion/drift trajectories

https://doi.org/10.1016/j.jsb.2015.08.007

Motion Correction/Movie Alignment

- **Alignment Purpose:**
  - Align frames from direct electron detectors to counter specimen motion.
- **Importance:**
  - Beam induces specimen motion or stage causes drift.
    - Blurs images, reducing resolution.
  - Frame alignment enhances image clarity & signal-to-noise.
- **Techniques:**
  - **Cross-Correlation:** Aligns frames to determine shifts.
  - **Least-squares:** Minimizes frame differences.
- **Outcome:**
  - Clearer images with reduced drift.
  - Restores resolution for subsequent image processing stages.



Motion effects are especially noticeable by the directional loss of Thon rings in the power spectrum of the image

https://cryoem101.org/chapter-5/

Dose Compensation in CryoEM

Definition:

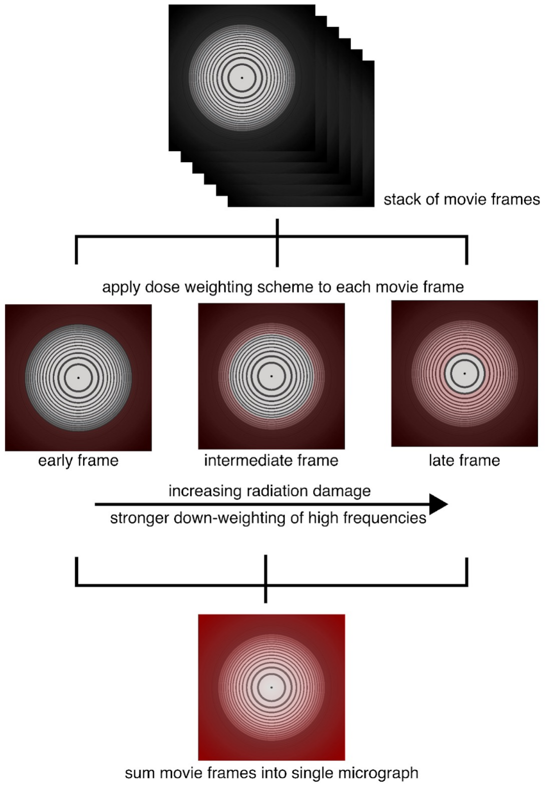
- Adjusts frame contribution based on received electron dose.
- Counters radiation damage's impact on successive frames.

Why it Matters:

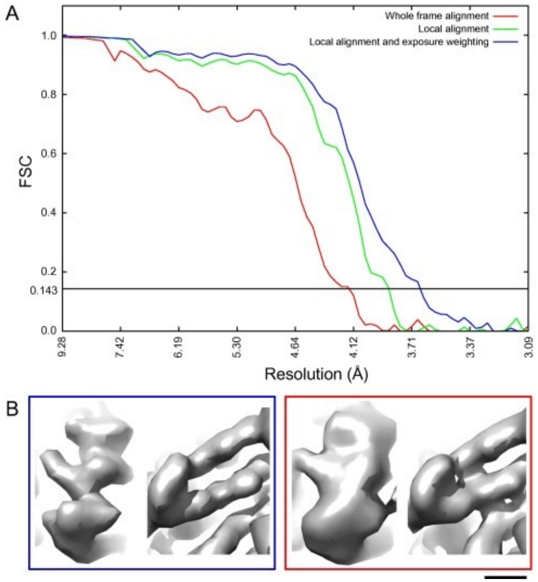
- **Radiation Damage:** Electron beams cause structural changes and information degradation in specimens.
- **Information Preservation:** Early frames have more high-res details; latter ones suffer from cumulative damage.

Impact on Image Quality:

- Blurred, artifacts, and lower resolution due to inconsistent frame weights.
- Frames weighted by reliability, improving signal-to-noise ratio, higher resolution.
- Ensures optimal data quality despite radiation damage.



Early frames retain content while later, high-exposure frames down-weight high frequencies, all summed into the final micrograph



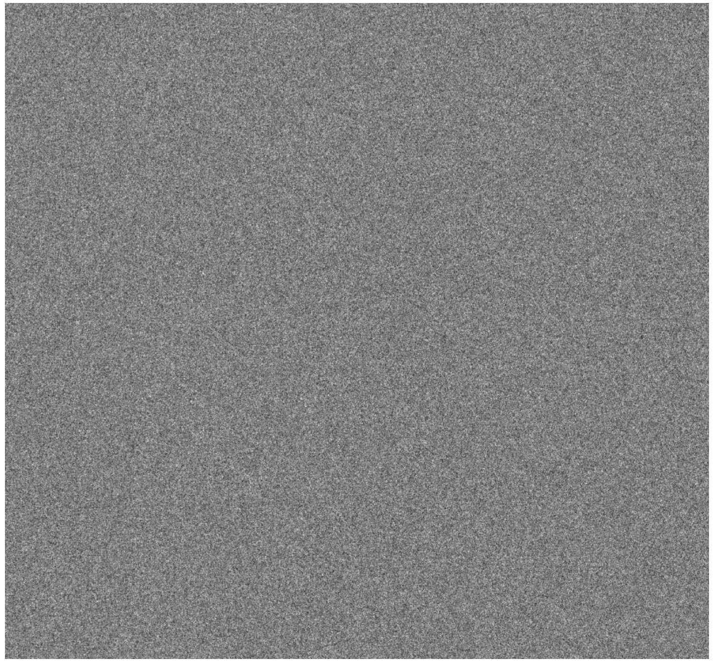
- individual particle motion corrected and exposure weighed map (blue box)
- whole frame alignment only (red box)

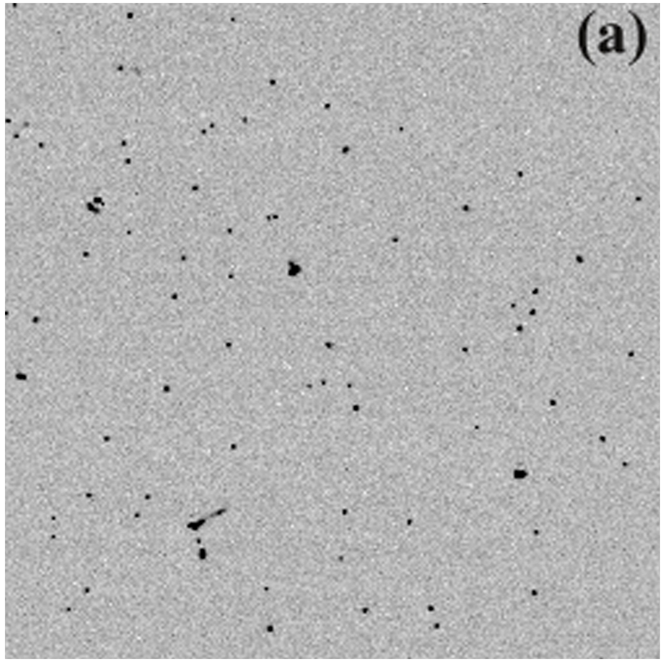
https://doi.org/10.1016/j.jsb.2015.08.007 https://cryoem101.org/chapter-5/

Features of DEDs

- Motion correction
- Beam damage compensation
- Improved image quality, i.e improved detective quantum efficiency
- Differences in throughput

Counting vs. integrating





- e^- detection results in a certain number of counts per frame
- Dozens of frames are summed up

- e^- hits are ”counted”
- Removes Landau noise due to e^- depositing different amounts of energy
- 1000s of counted frames sorted into bins then whole set of frames summed

McMullan et al., JSB, 2014

Detective Quantum Efficiency (DQE)

DQE measures how well a detector captures an image across spatial frequencies

$$DQE(\omega) = \frac{SNR_{out}^{2}(\omega)}{SNR_{in}^{2}(\omega)}$$

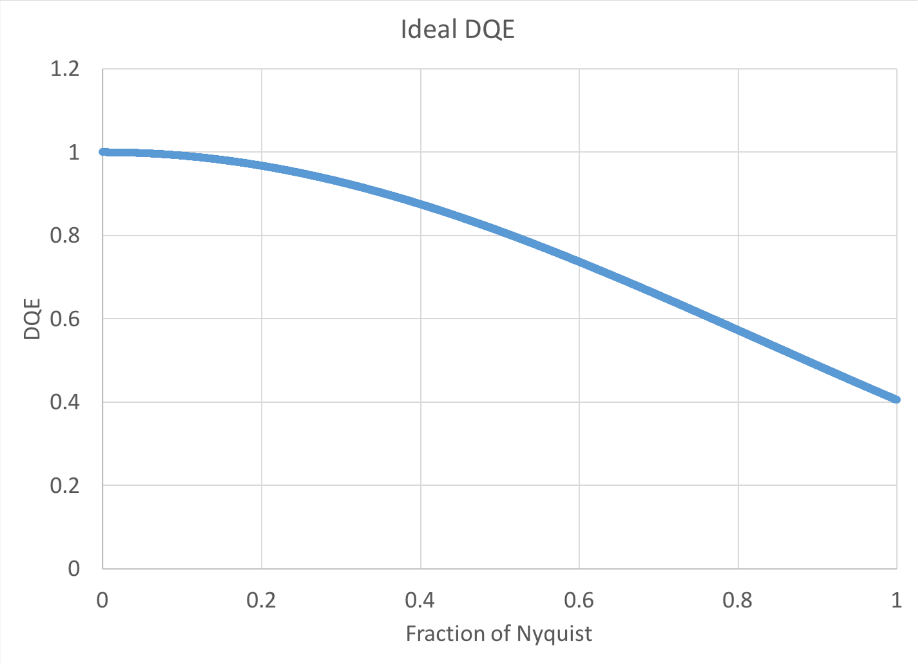
DQE of 1 is good DQE of 0 is bad

Measuring the DQE

$$DQE(\omega) = DQE(0)\frac{MTF^2(\omega)}{NPS_{norm}(\omega)}$$

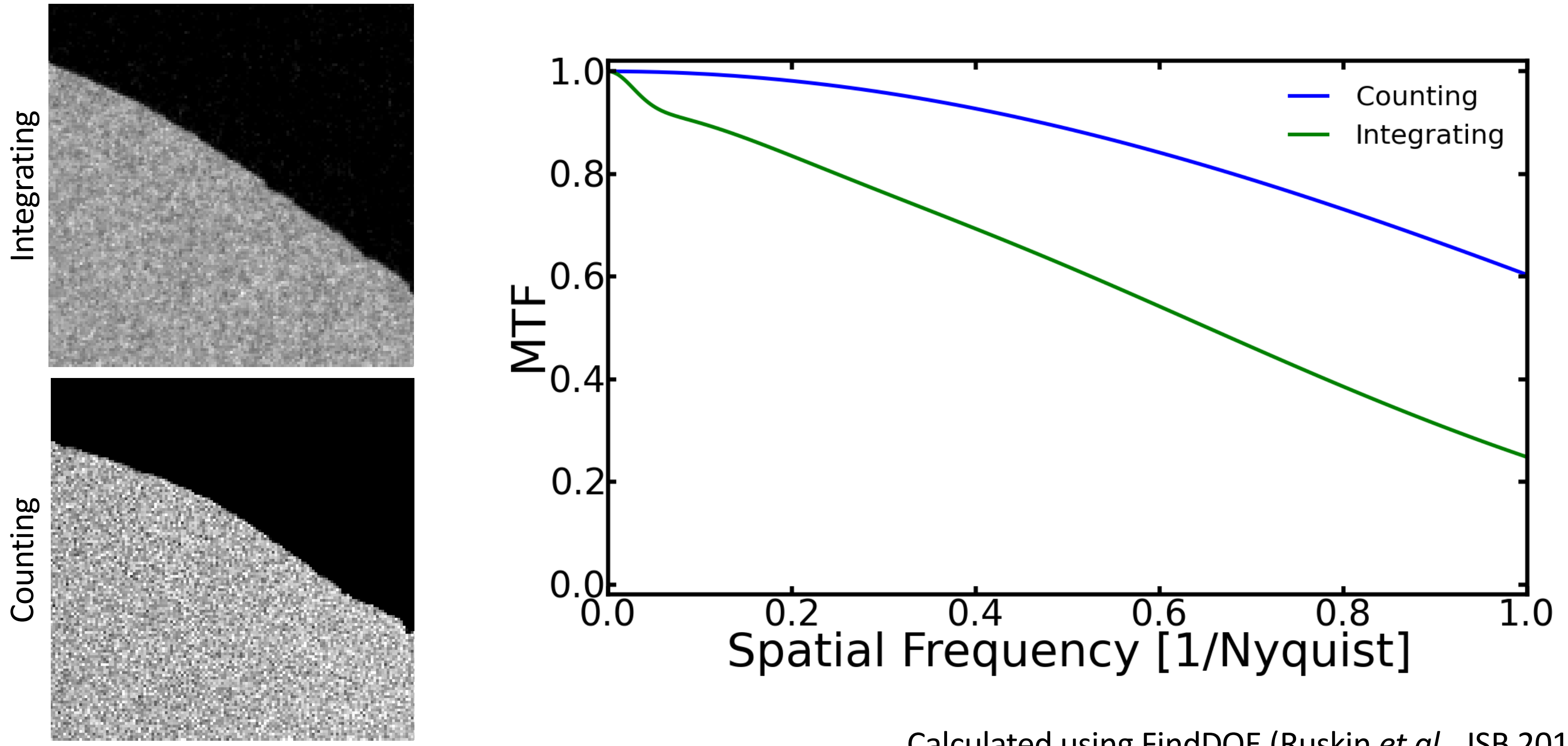
$$DQE(0) = \frac{{d_n}^2}{nNPS(0)}$$

$$DQE_{ideal}(\omega) = \text{sinc}^2\,(\pi\omega/2)$$



McMullan et al., JSB, 2014

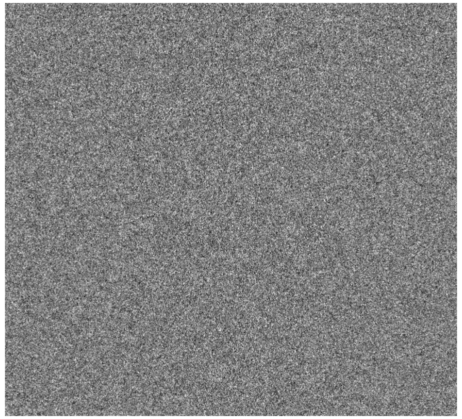
MTF for DE64



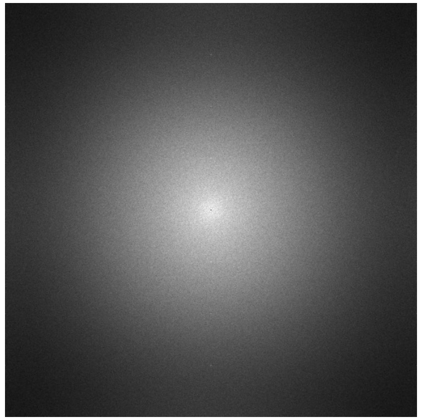
Calculated using FindDQE (Ruskin *et al.,* JSB 2013)

Noise Power Spectrum

Integrating

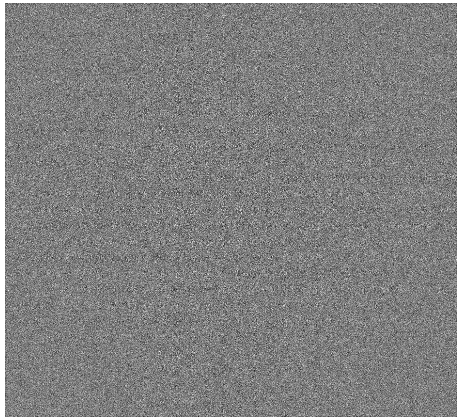


Noise image

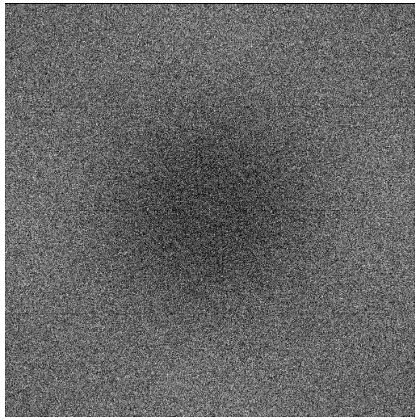


Noise power spectrum

Counting

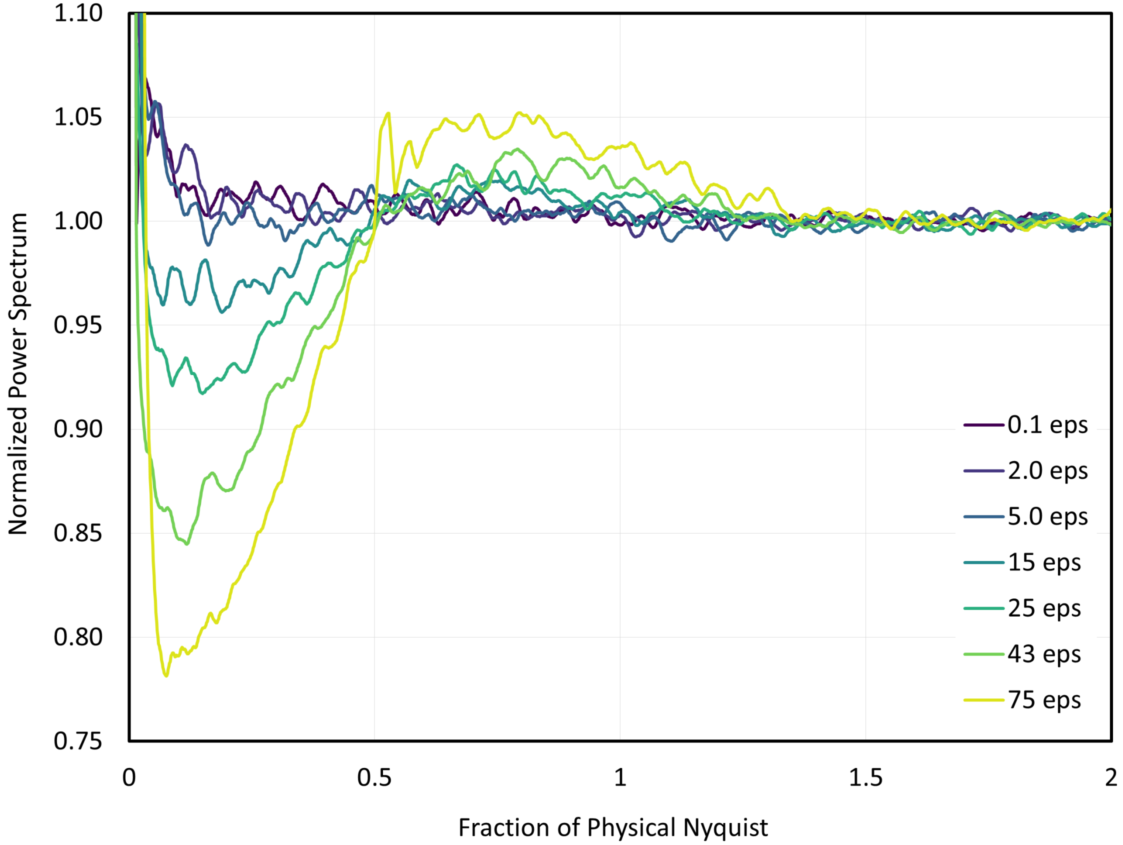


Noise image



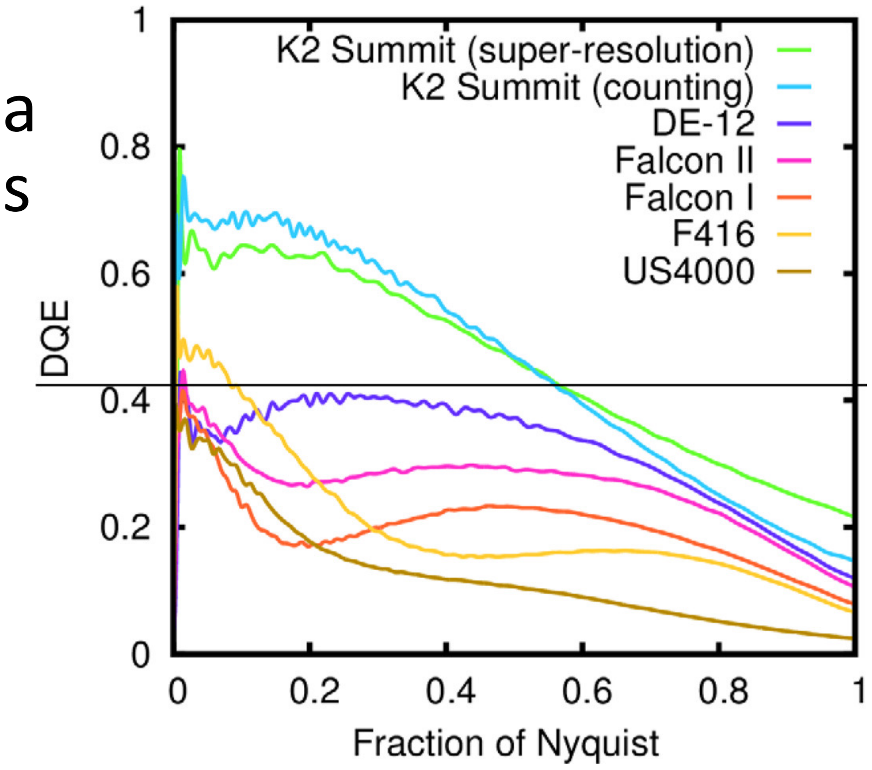
Noise power spectrum

Coincidence loss as function of dose rate



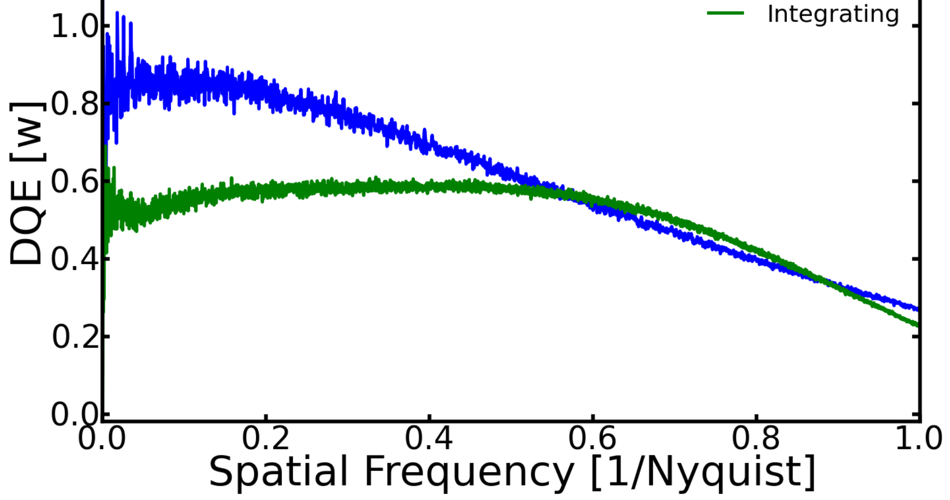
DQE comparison for various detectors

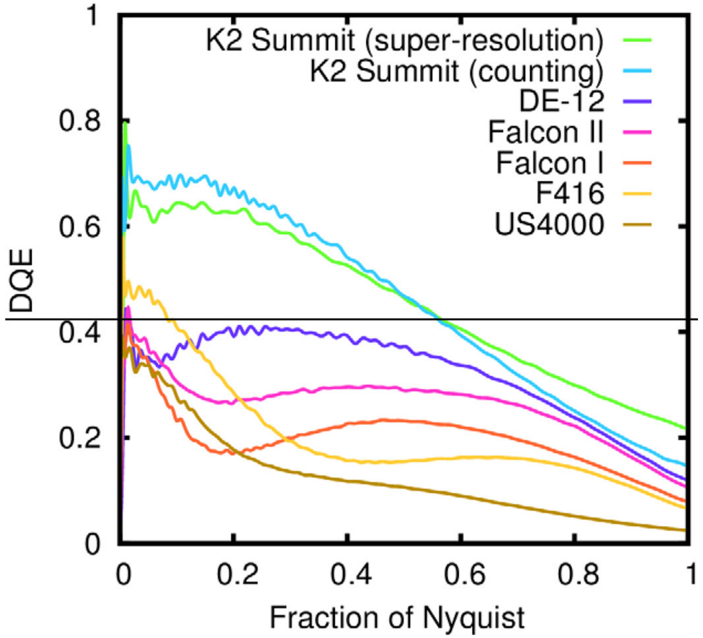
- Due to reduction in Landa noise, DQE for counting is dramatically better than integrating



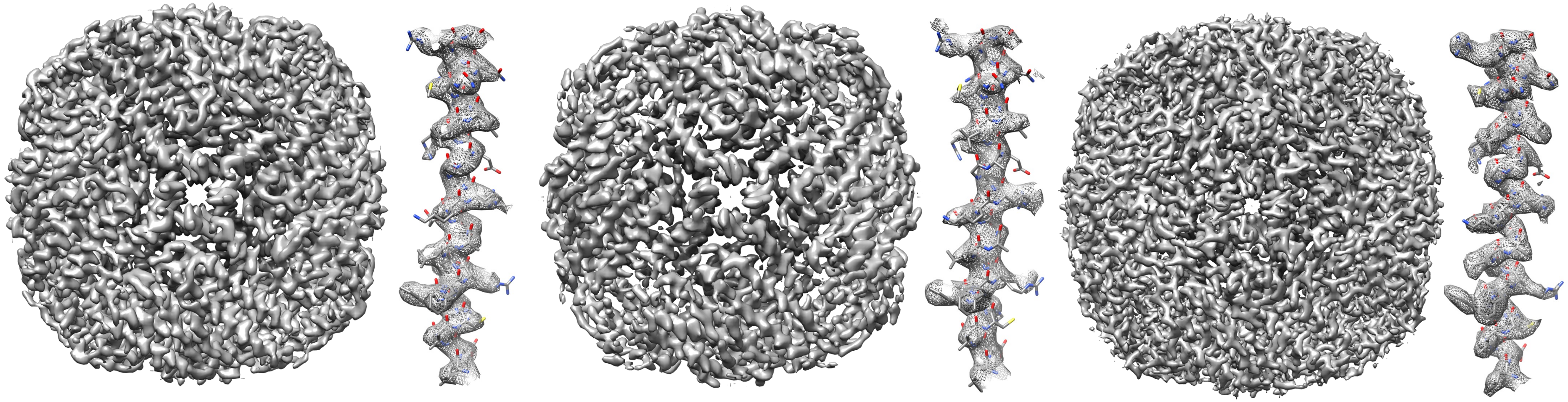
Ruskin *et al.*, JSB, 2013

Integrating/counting comparison for DE64





Comparison of apoferritin with integrating, integrating w/ VPP, and counting



<u>Integrating</u>
3.8 Å
127,621 ptcls

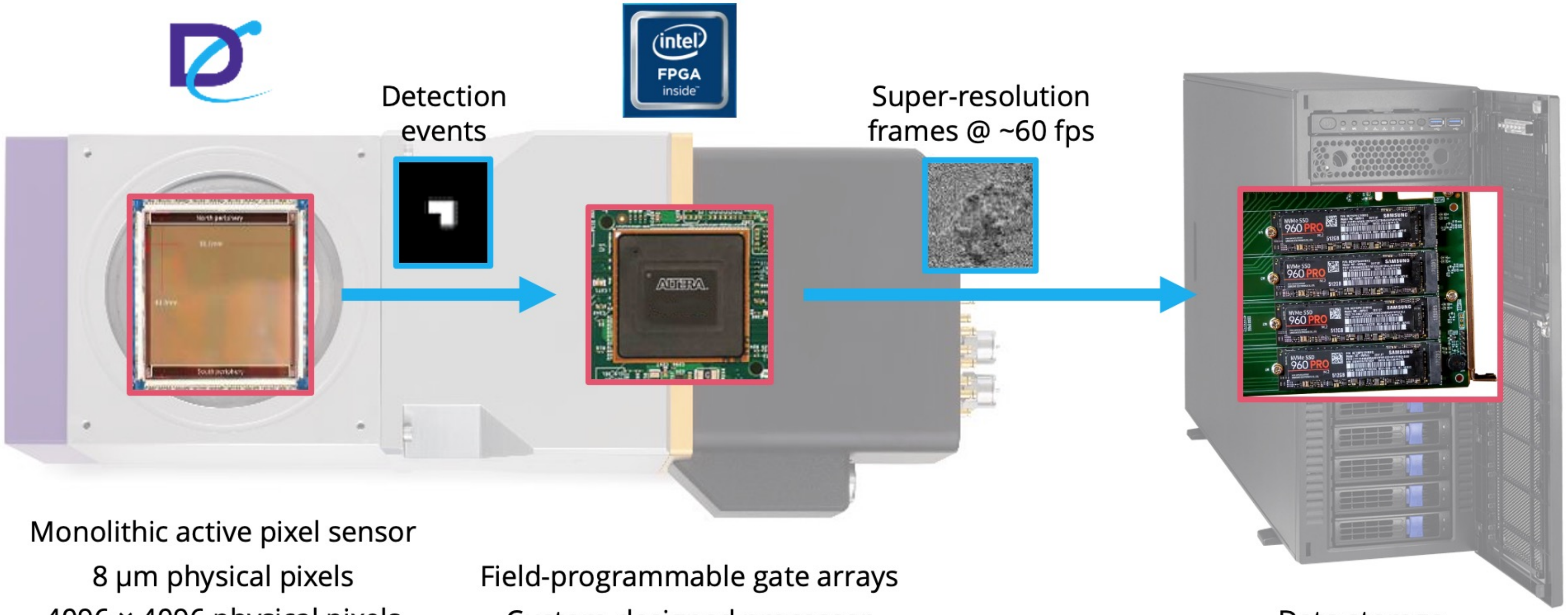
<u>Integrating w/ VPP</u>
4.3 Å
129,141 ptcls

<u>Counting</u>
2.9 Å
129,140 ptcls

Mendez et al., IUCRJ, 2019

DE Apollo Event-Based* Cryo-EM Camera

* Patented & patent-pending technology



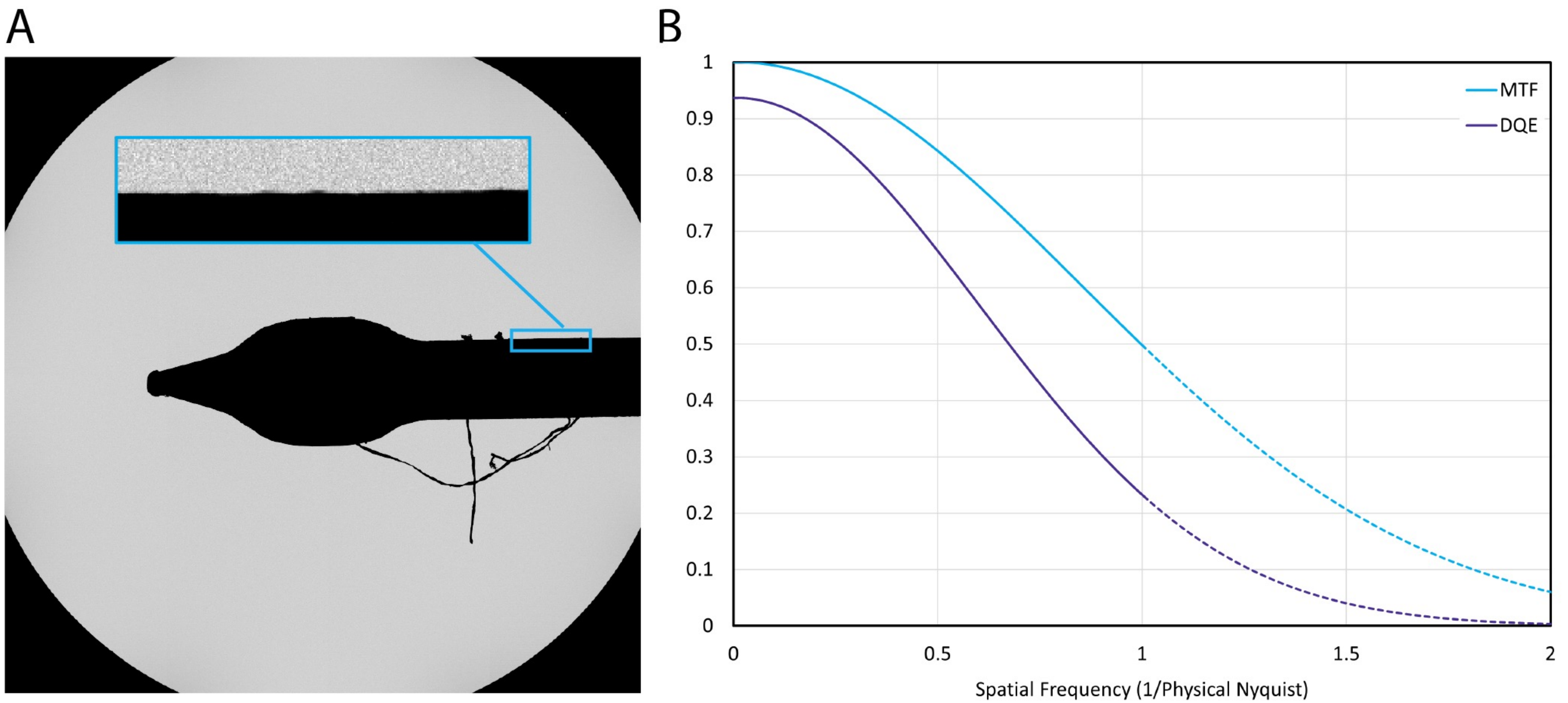
Monolithic active pixel sensor
8 µm physical pixels
4096 × 4096 physical pixels
On-chip CDS
On-chip thresholding
On-chip event detection
Event encoding

Field-programmable gate arrays
Custom-designed processor
Sensor control
Super-resolution centroiding
Generation of output frames

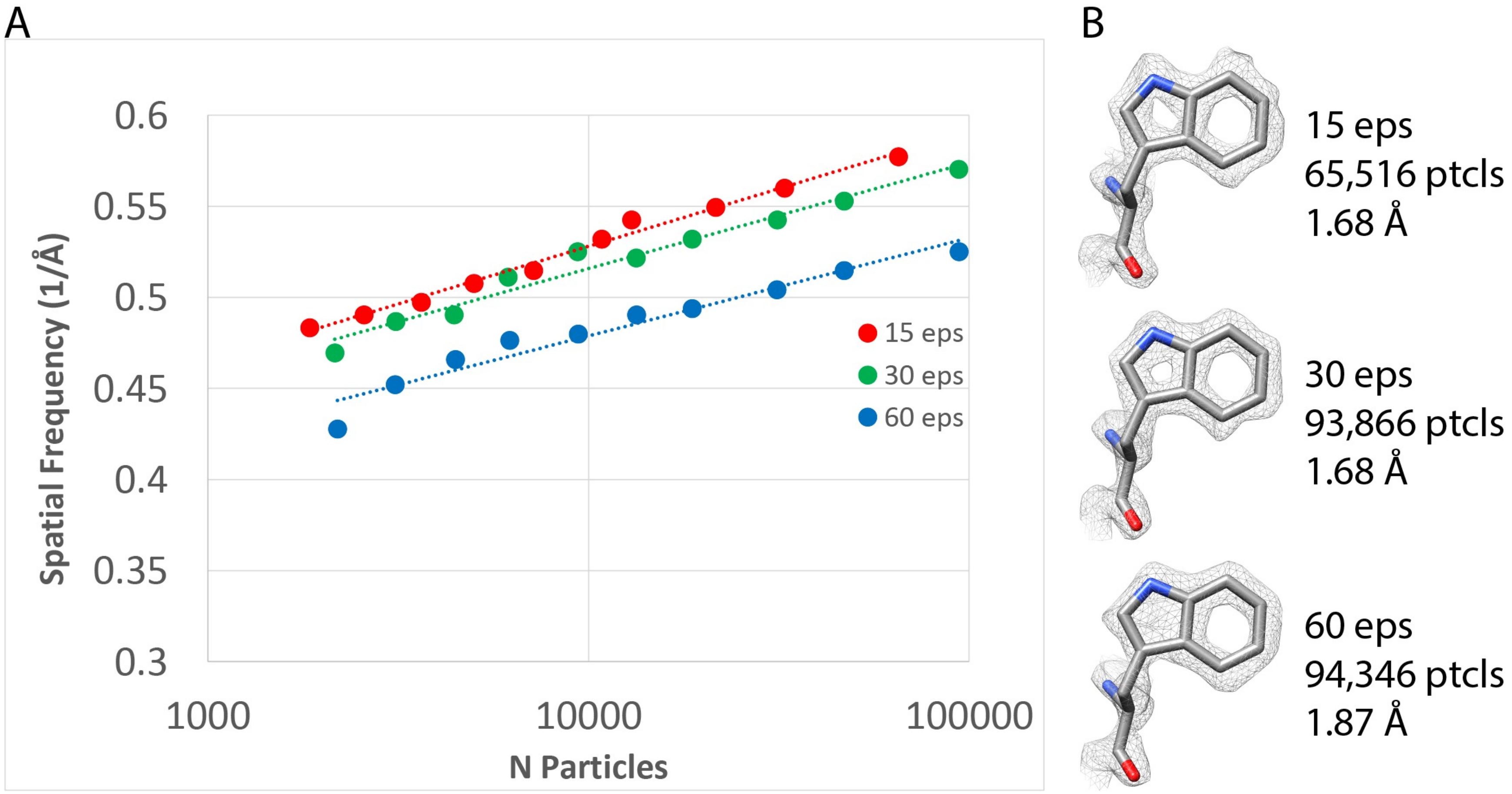
Data storage
User interface / TEM automation
Post-processing or network transfer

Slide courtesy Direct Electron

Apollo DQE

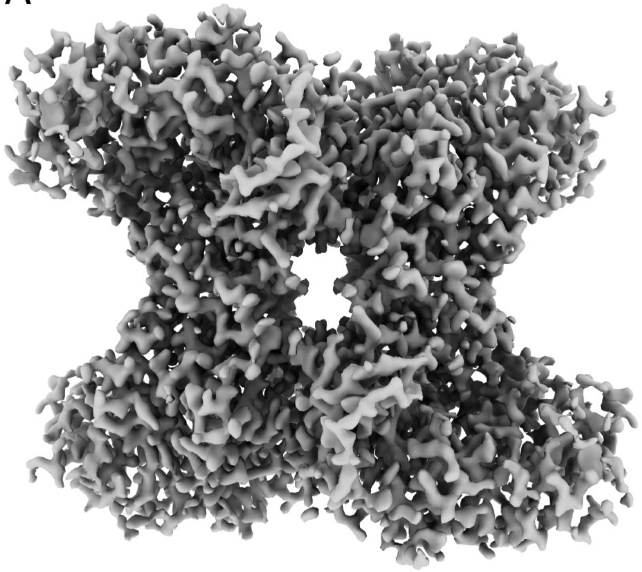

A
B
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.5
1
1.5
2
Spatial Frequency (1/Physical Nyquist)
MTF
DQE

Apollo performs well over a wide range of dose rates



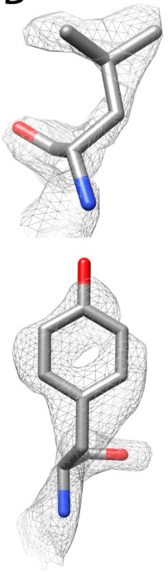
Other particles - aldolase

A



149 kDa, 2.24 Å

B



C