Microscopes and Cameras for Cryo-EM

Paul Mooney
Gatan, Inc.
June 3, 2025

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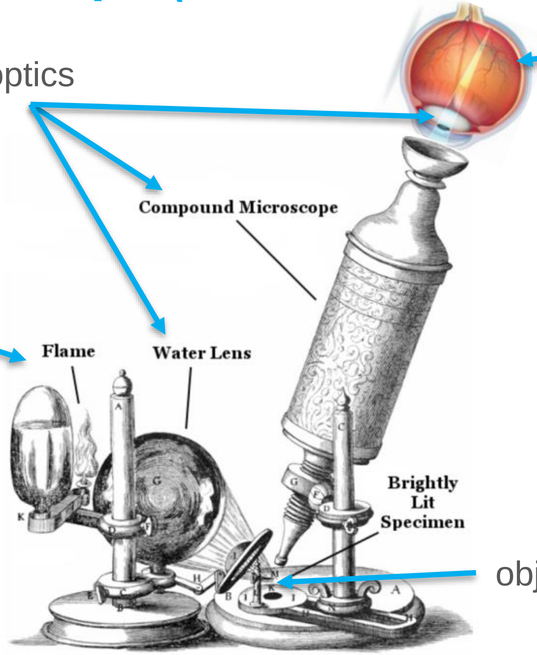
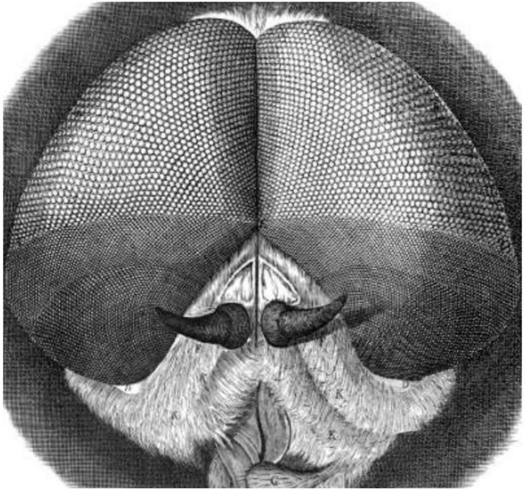
A microscope (Hooke's own in his drawing)



Robert Hooke's own illustration of his compound microscope, with labels added by this website.



Robert Hooke's drawing from *Micrographia* of a dronefly's head, showing detailed eye-structure. Images like this were too worryingly alien for some people to believe genuine.

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https://www.famousscientists.org/robert-hooke/

This talk

The microscope

- The illumination, which puts the **electron** in Cryo-Electron Microscopy
- The optics, which puts the **microscope** into Cryo-Electron Microscopy
- Specimen handling, which put the **Cryo** into Cryo-Electron Microscopy

The camera

- Counted direct detection, with a signal to noise ratio enabling in-silico orientation classification and alignment to enable correlated signal averaging without crystallization.

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Why electrons are good (1)

They are waves! De Broglie, 1925

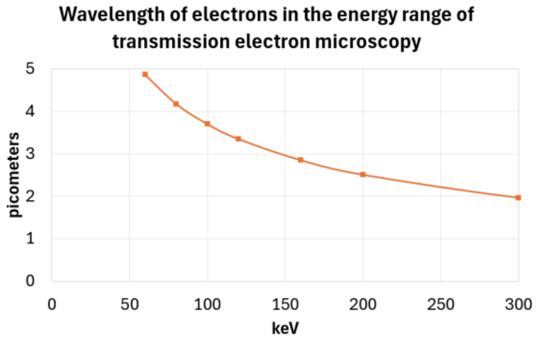
- Small wavelength ~ 160,000 times smaller than visible light's wavelength
- Can do phase contrast

They can be focused by a magnetic lens!

- Hans Busch theory, 1926

They can be used in a microscope!

- Ernst Ruska and Max Knoll, 1932



Data source:
https://www.jeol.com/words/emterms/20121023.071258.php#gsc.tab=0

ERNST RUSKA (RIGHT) AND MAX KNOLL (LEFT) PICTURED WITH THEIR INVENTION, THE FIRST MAGNETIC ELECTRON MICROSCOPE, BERLIN, 1932.

https://www.nobel.mpg.de/en/ernst-ruska

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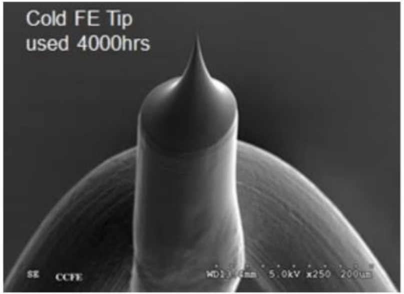
Why electrons are good (2)

Cold or warm electrostatic extraction

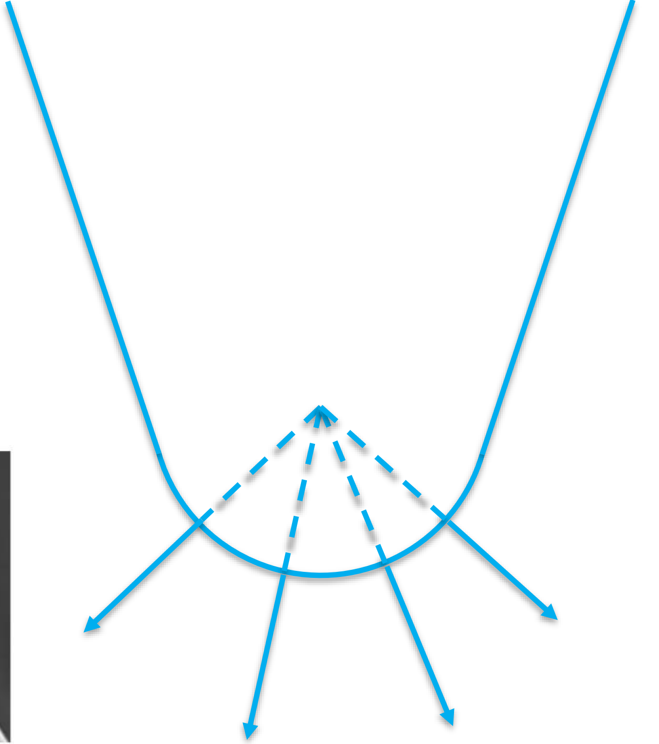
→Good source coherence

- →Small effective spot size
- →Narrow energy distribution

Albert Crewe introduced cold field emission to electron microscopy



https://www.hitachi-hightech.com/global/en/knowledge/microscopes/sem-tem-stem/fe-sem/cold-fe.html



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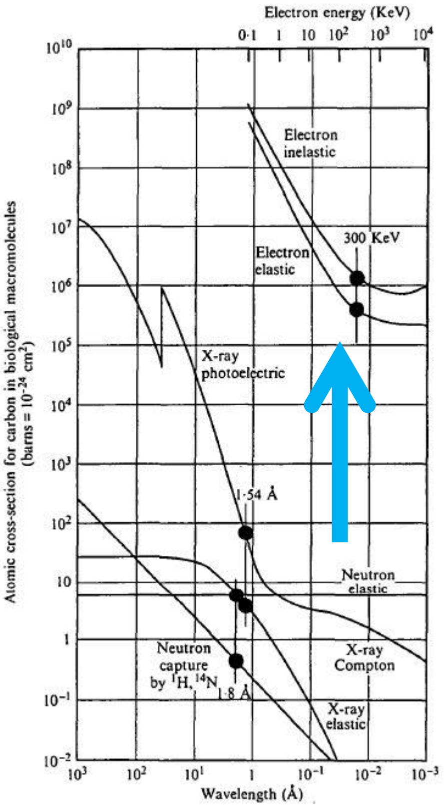
Why electrons are good (3)

Strong interaction

→Use of small samples

→Easy to detect



Henderson, QRB 1995, Figure 2

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Why electrons are good (4)

Less damage (by inelastic scattering) per elastic event

	Electrons 80–500 keV	X-rays 1·5 Å	X-rays 30 Å
Ratio † (inelastic/elastic) scattering events	3	10	10^3–10^4
Mechanism of radiation damage	Secondary e^- emission	Photoelectric e^- emission	
Energy deposited per inelastic event	20 eV	8 keV	400 eV
Energy deposited per elastic event**	60 eV	80 keV	400 keV
Energy deposited relative to electrons			
(inelastic)	1	400	20
(elastic)	1	1000	10000

Henderson, QRB 1995, Table 1 (excluding nuetrons)

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Why magnetic lenses?

- Magnetic force, **F** = q(**v** x **B**) is proportional to the velocity of the electron.
- Electric force, F=qE is not.
- The result is that magnetic lenses are chosen for higher kV and electrostatic for lower kV with the transition being in the 30-50kV range.

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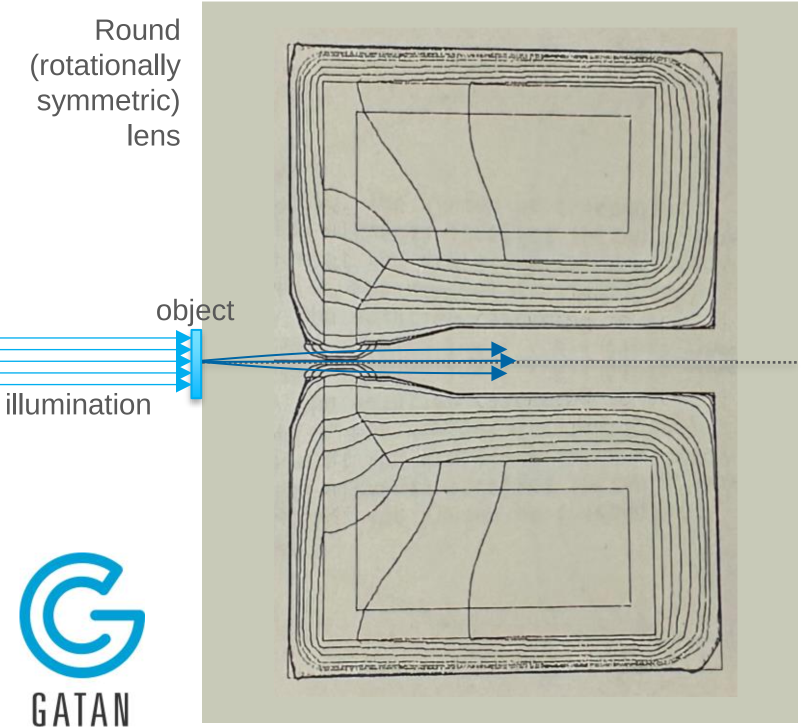
How do magnetic lenses work?

Round (rotationally symmetric) lens



$$\vec{F} = q(\vec{v} \times \vec{B})$$

- Mechanism:
  - Near-axis beam motion interacts with magnetic fringe fields to experience azimuthal forces and develop rotational motion.
  - The rotational motion in turn interacts with the axis-parallel components of the magnetic field to experience a converging force.
- Scherzer theorem: any kind of round static electron lens (including electrostatic) has same-sign spherical aberration.
  - Spherical aberration is minimized by concentrating the field at the pole piece with as narrow a gap as possible
  - And worked around by limiting the effective aperture to ~ 1-10 mRadians (1/20th to ½ degree)

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Illustration constructed rom fig 2, page 82, E. Kasper, Magnetic Field Calculation and the Determination of Electron Trajectories, Magnetic Electron Lenses, Ed. P.W. Hawkes, 1982

Abbe's resolution criterion

$$\text{Resolution} = \frac{\text{wavelength}}{2 \sin(\text{aperture angle})}$$

$$= \frac{2.5 \text{ pm}}{2 \sin(.01)}$$

$$= 125 \text{ pm} \quad \sim 1.25 \text{ Å}$$

Without aberration correction

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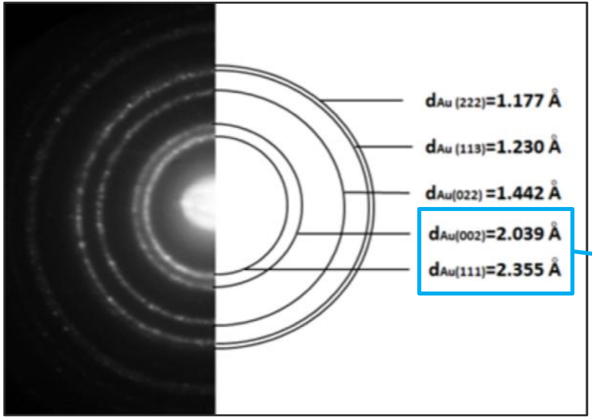
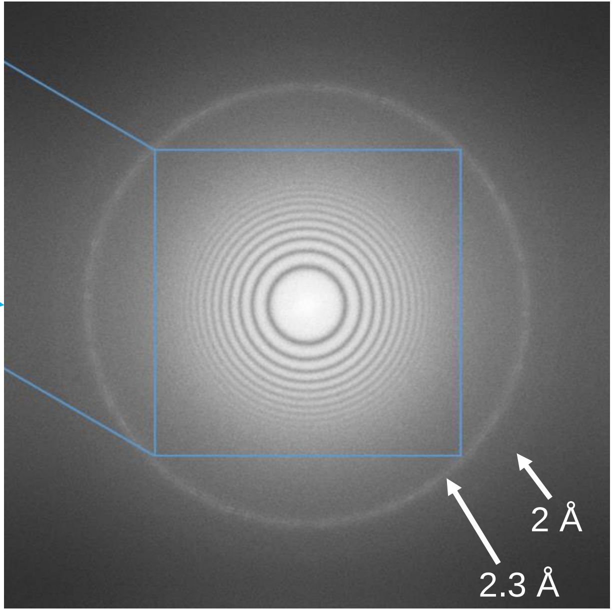
Low-order gold lattice fringes can be seen on non-aberration-corrected microscopes



Polycrystalline gold ***diffraction*** rings of various orders, for reference.
https://www.intechopen.com/chapters/49537, figure 6



Polycrystaline gold (or PtIr) used to calibrate microscope resolution tests in imaging mode – typically just the first two in non-aberration-corrected microscopes (PtIr is normally used but gold shows up in many test specimens like gold-shadowed waffle grids)

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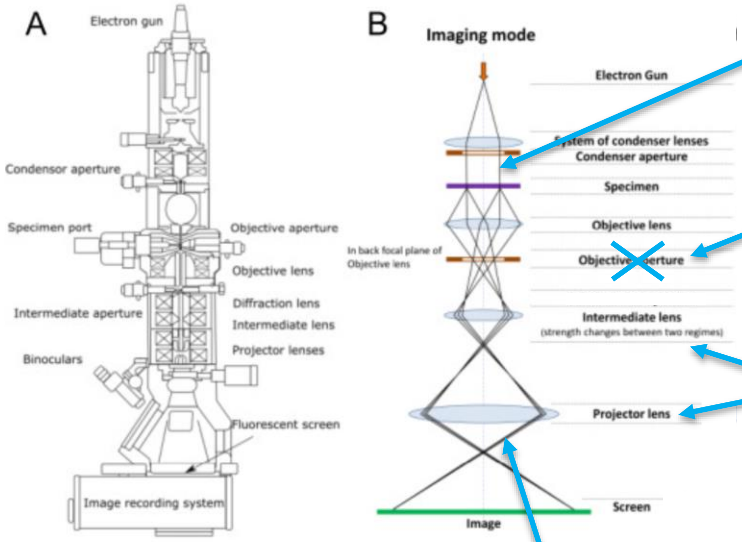
Illumination and imaging optics of a transmission electron microscope



Angles exaggerated for illustration

- Parallel illumination so that full field of view is similarly imaged wave-optically.
- The objective aperture is not used for contrast generation in phase-contrast imaging.
- A projection system instead of ocular puts a real image onto the detector or viewing screen. Multiple lenses enable independent rotation and magnification control.

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https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/transmission-electron-microscopes

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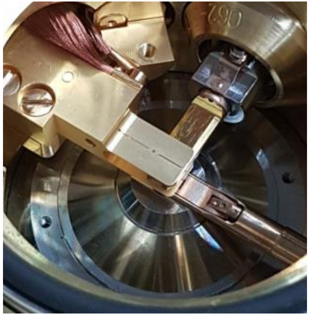
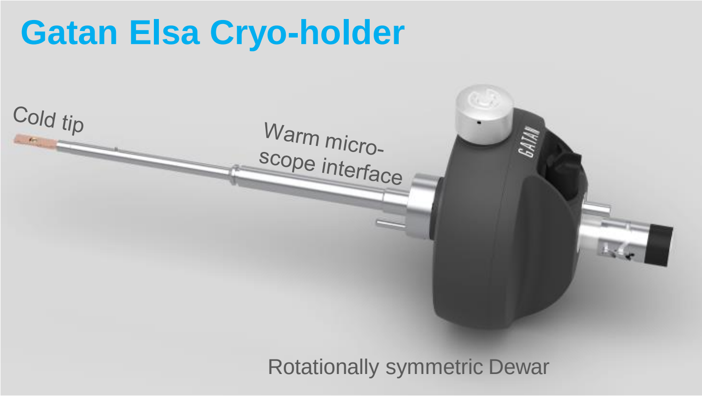
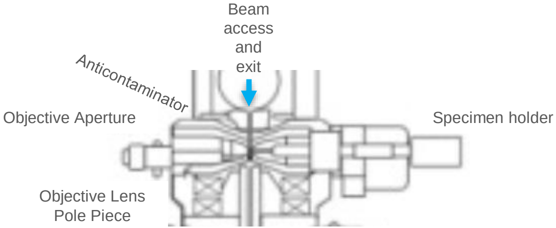
Holding the specimen – the macroscopic part



Anti-contaminator: blades which are colder than the specimen keep the vacuum nearby cleaner





(Snip of diagram from earlier slide)

…quite crowded

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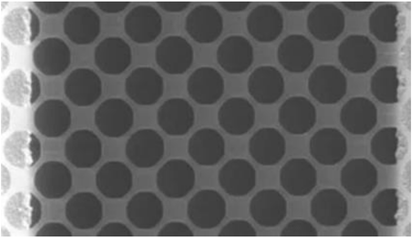
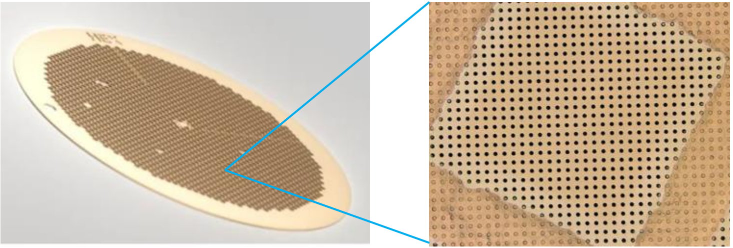
Holding the specimen – the microscopic part

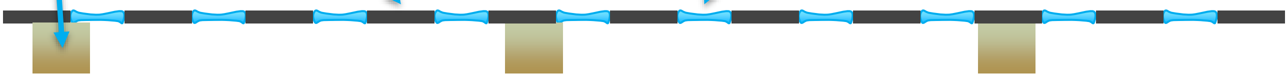


O(1) to O(10 µm) holes in a carbon film on a secondary square grid of copper



Gold grids developed by Chris Russo and Lori Passmore at the LMB

Last level of “holder” is vitrified ice….



All images here are from https://www.quantifoil.com/products

What illumination, specimen and optics produce

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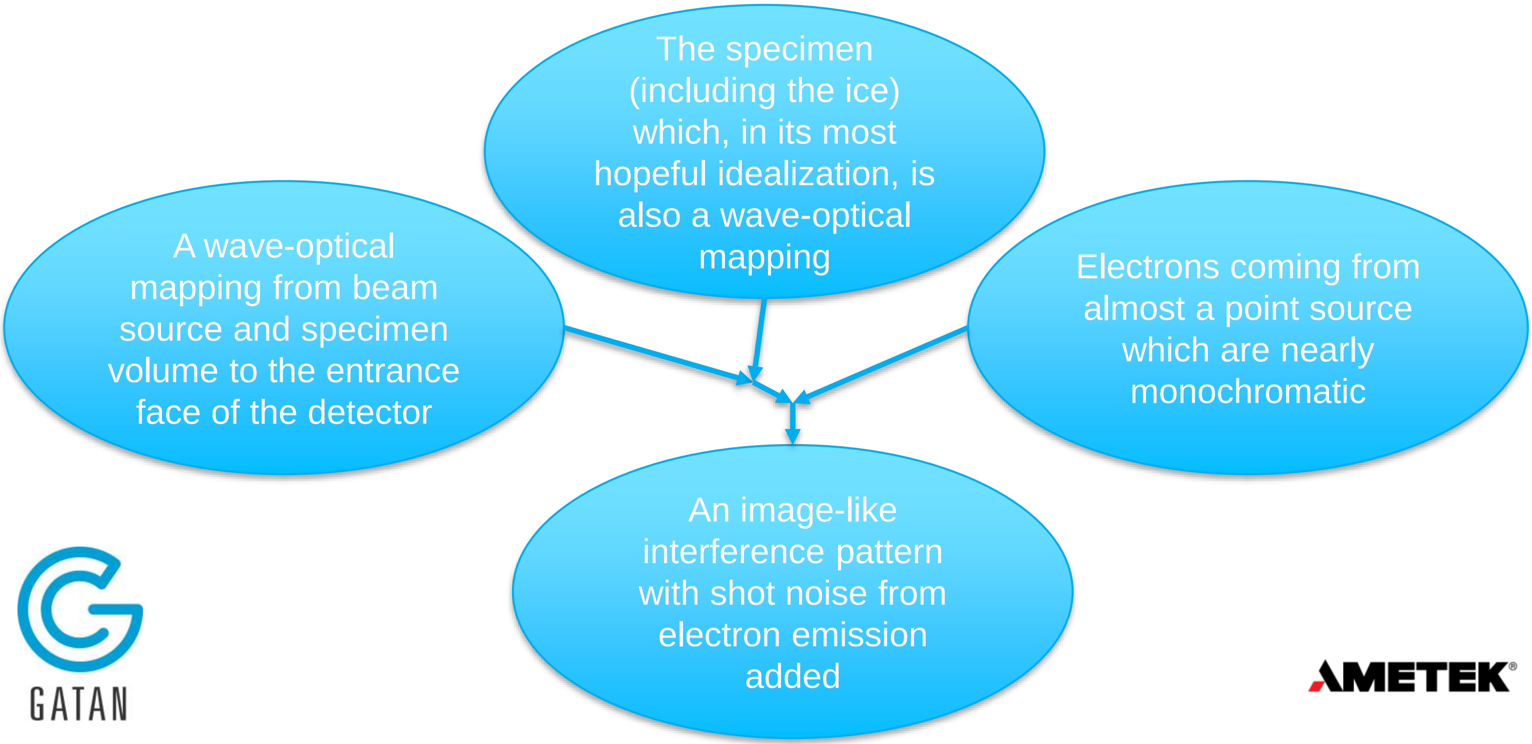
And what the detector detects



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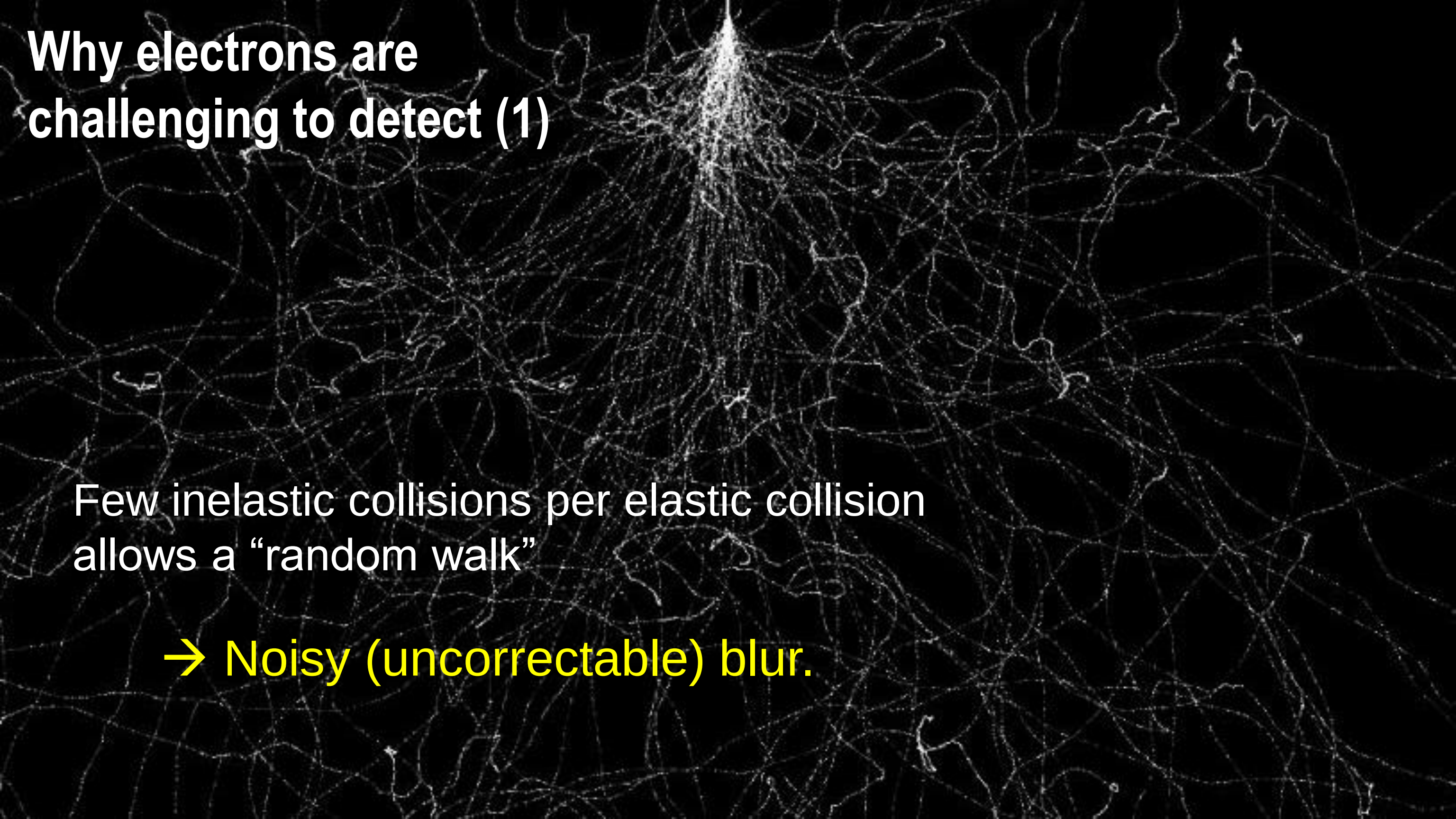
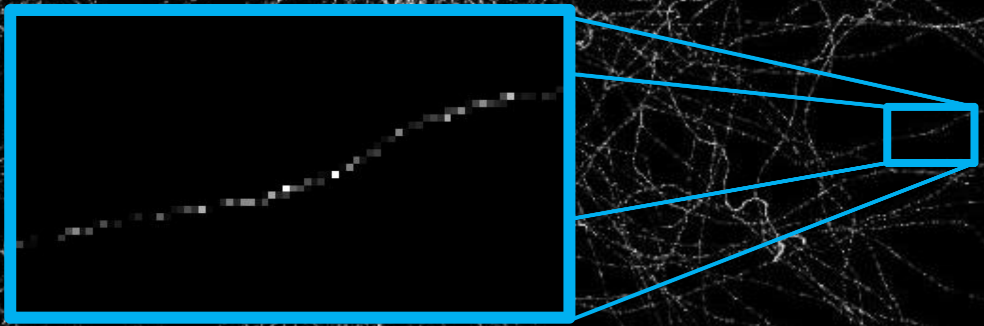


Why electrons are challenging to detect (1)

Few inelastic collisions per elastic collision allows a “random walk”

→ Noisy (uncorrectable) blur.

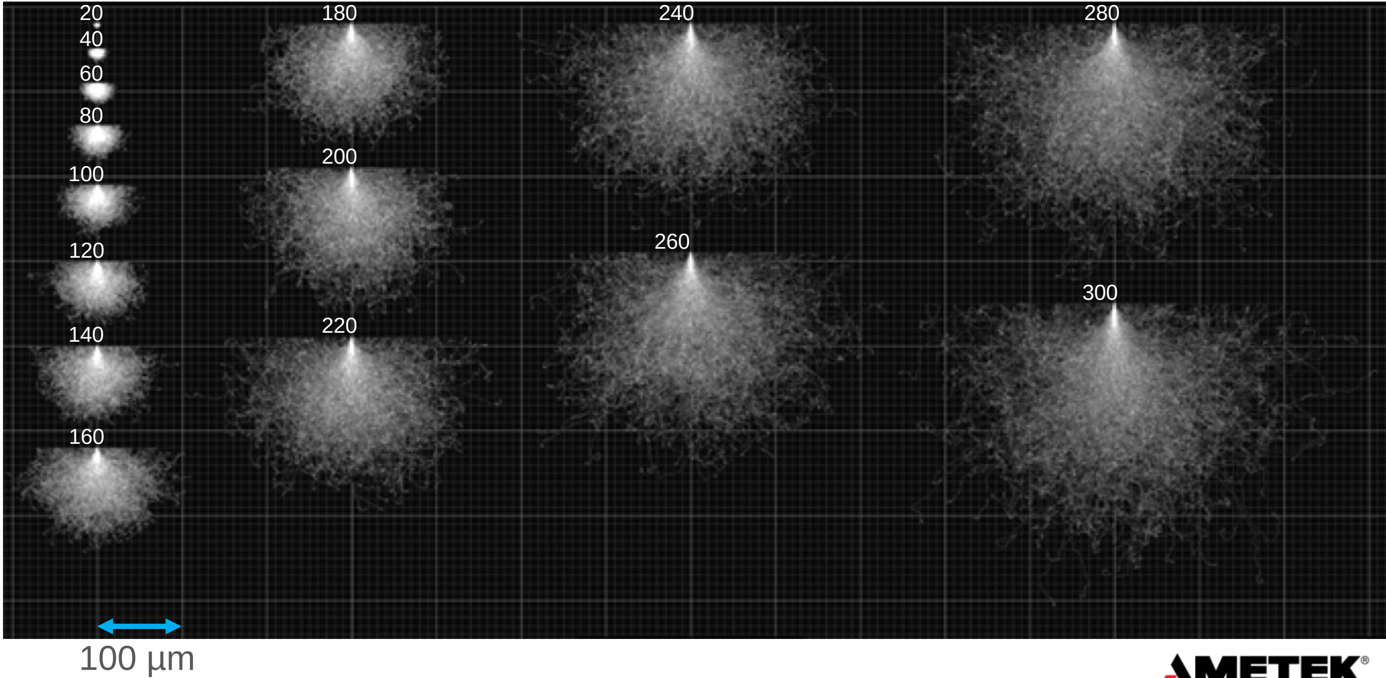
Why electrons are challenging to detect (2)



Randomness of inelastic events called "Landau noise"

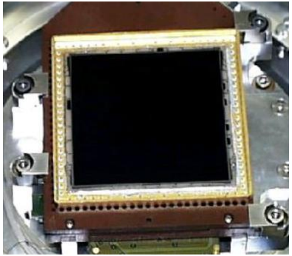
→ Detection made even noisier

Why electrons are challenging to detect (3)

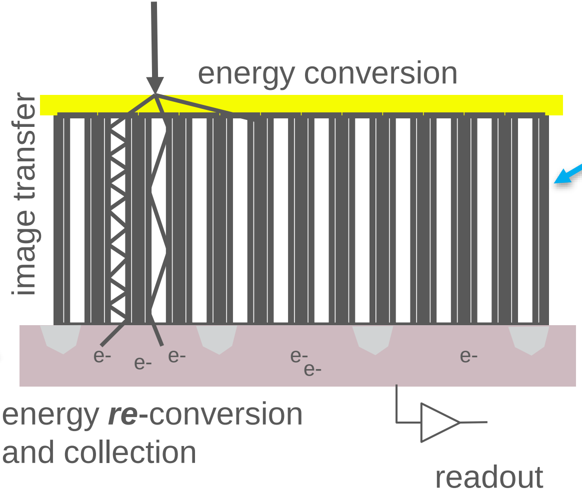


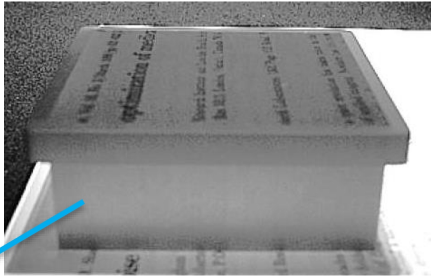
Higher kV electrons go a long way before they stop.

Indirect Detection: A partial fix

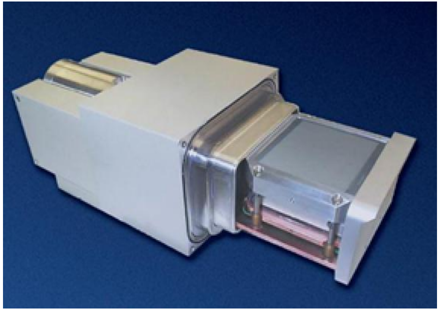


Astronomy CCDs





Hi-angle fiber-optic light capture



Gatan UltraScan 4000

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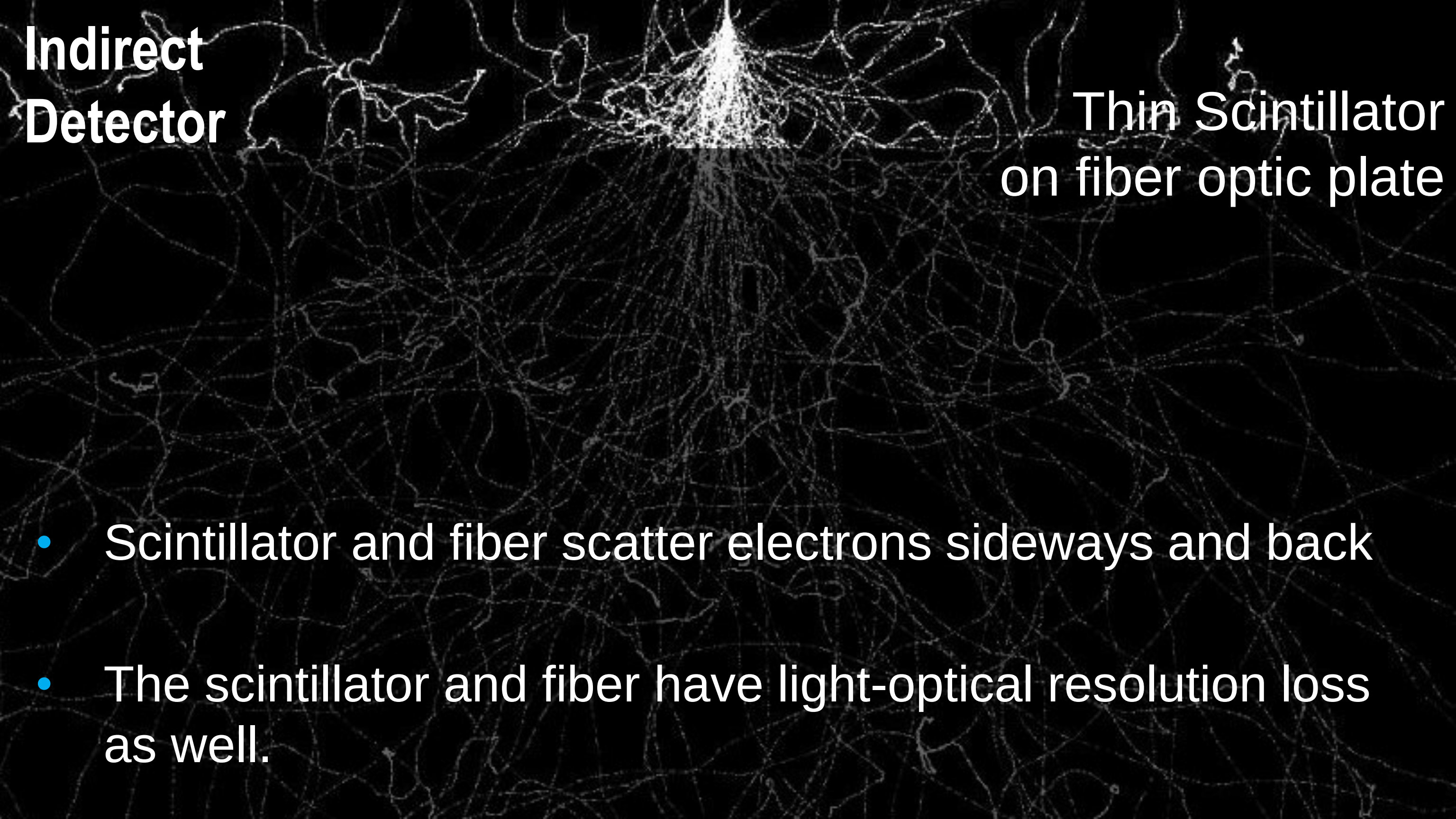
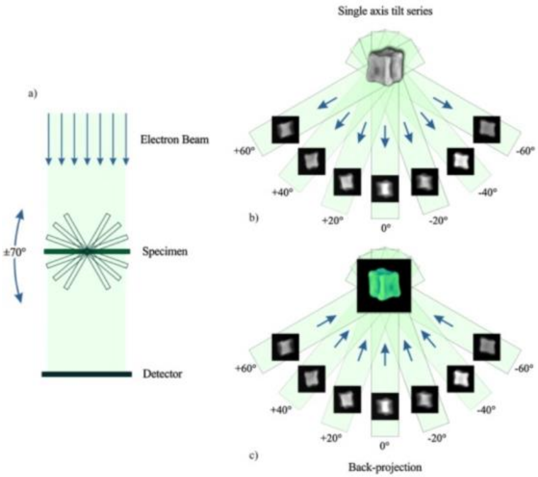


Indirect Detector

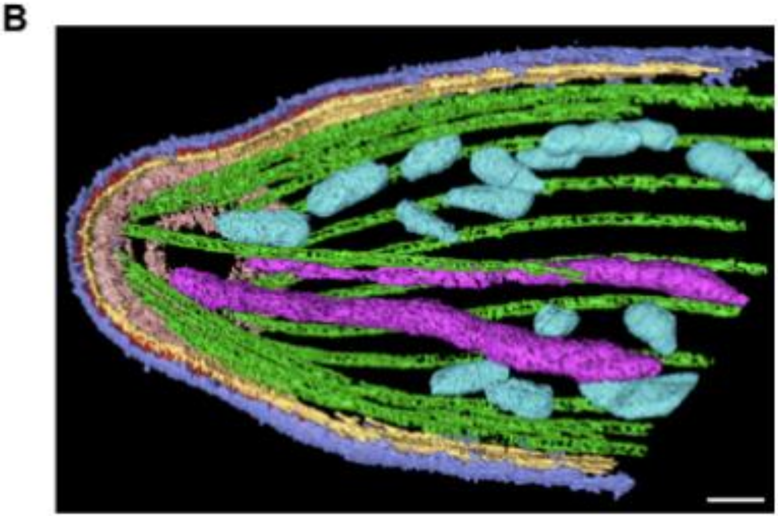
Thin Scintillator on fiber optic plate

- Scintillator and fiber scatter electrons sideways and back
- The scintillator and fiber have light-optical resolution loss as well.

Online acquisition allows automated tomography



https://doi.org/10.1016/j.crhy.2013.09.015, Bals et al.



Apical pole of a P. berghei sporozoite showing, among others, microtubules (green), rhoptries (magenta), micronemes (cyan), plasma membrane (blue), and the inner membrane complex (yellow).
Kudryashev et al., 2010.
Scale bar: 100 nm.

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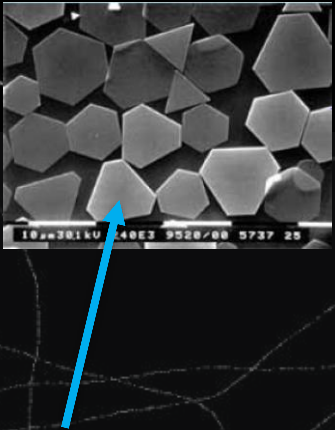
Film



All-or-nothing grain development acted as discriminator/counter.

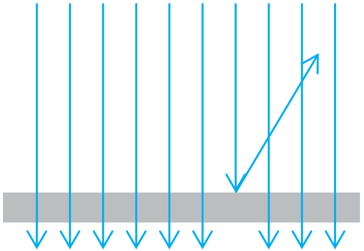
Plastic substrate minimized scatter.

Also thin to further reduce scatter.

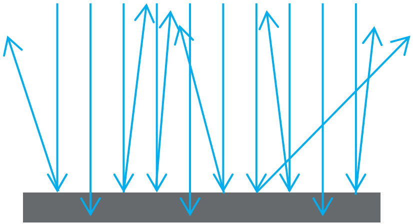
Film-based EM image capture was quite good in resolution and sensitivity at low dose.

Understanding noise in an electron camera: ~~Quantum Efficiency~~

QE = 90%



QE = 30%



Siméon Denis Poisson

15% contrast imaged with 222 quanta with 10% lost before detection - net 200 quanta

The same contrast imaged with 222 quanta with 70% lost – net 67 quanta

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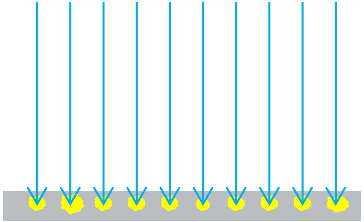
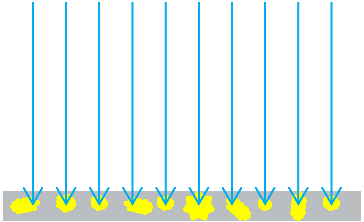
Detective Quantum Efficiency = SNR_{out} / SNR_{in}

DQE
= 90%



15% contrast imaged with 222 quanta with 11% variation in deposited signal

DQE
= 30%



The same contrast imaged with 222 quanta with 333% variation in deposited signal

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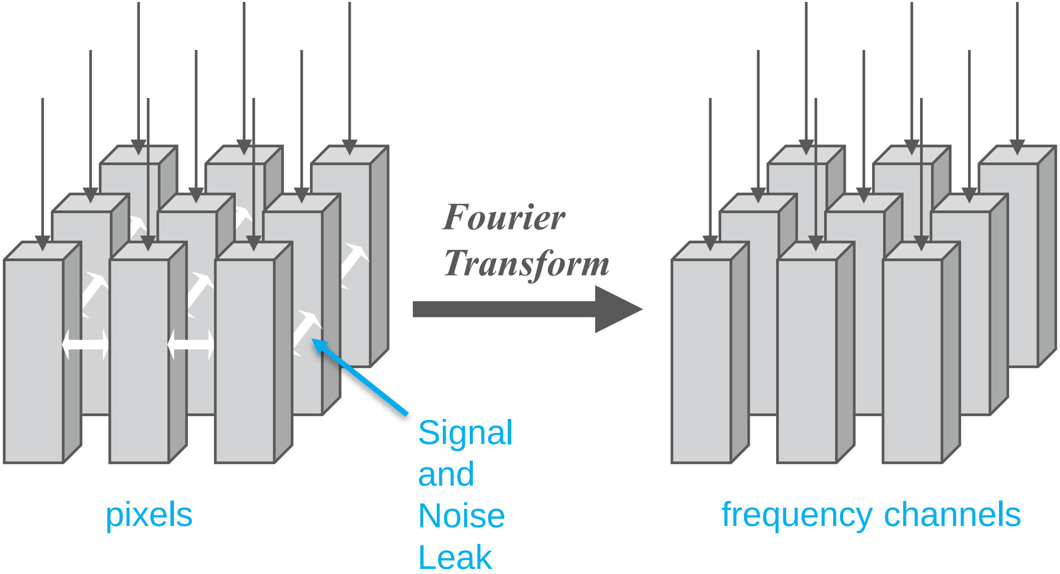
But pixels leak signal and noise amongst neighbors



Fourier transformation isolates channels and allows the SNR ratio to be defined.

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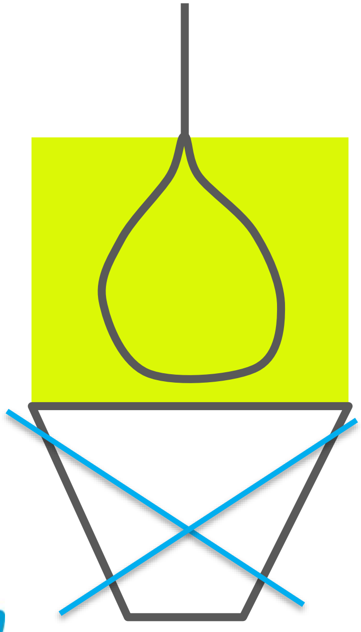
Full DQE Definition

$$DQE(N, \boldsymbol{s}) = \frac{SNR_{out}(N,\boldsymbol{s})}{SNR_{in}(N,\boldsymbol{s})}$$

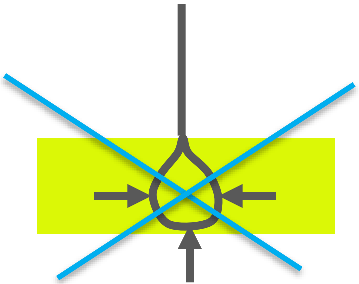
Dose in electrons

Spatial frequency

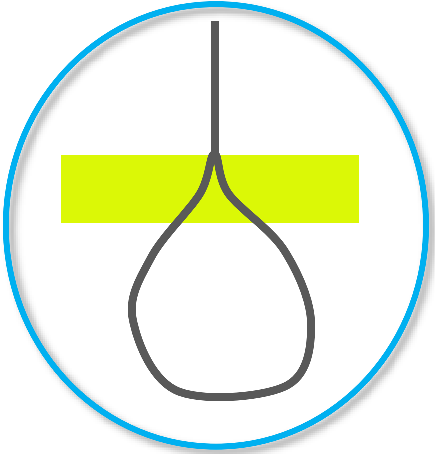
Three ways to improve indirect detector resolution



Bigger pixels



Deceleration



Transmission

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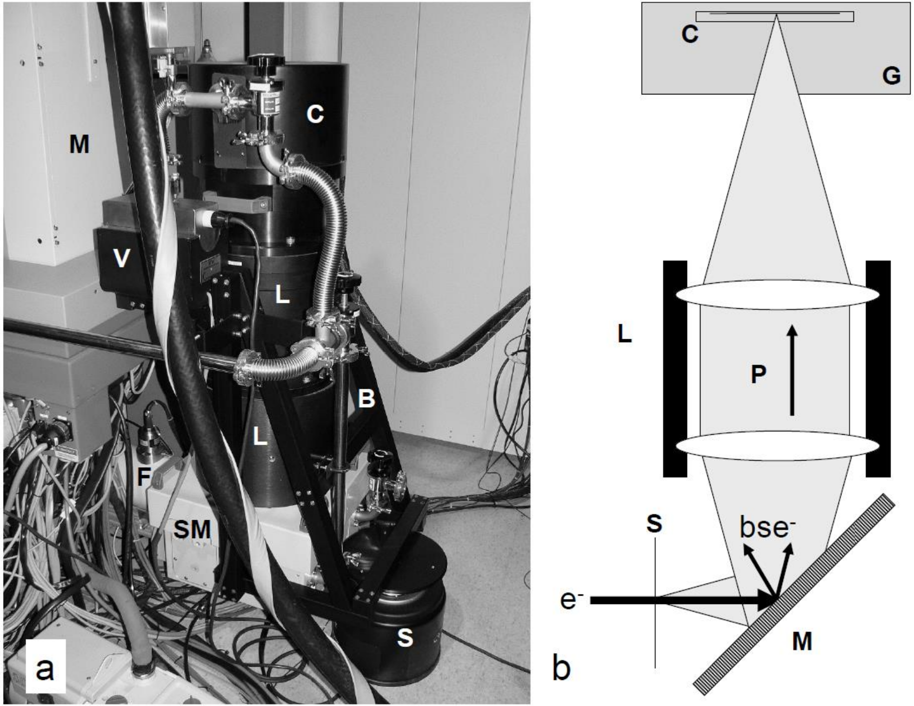
A thin scintillator without back-scattering substrate



Mooney, Methods Cell Bio, **79** (2007).

Gatan DiffCam – a small commercial version

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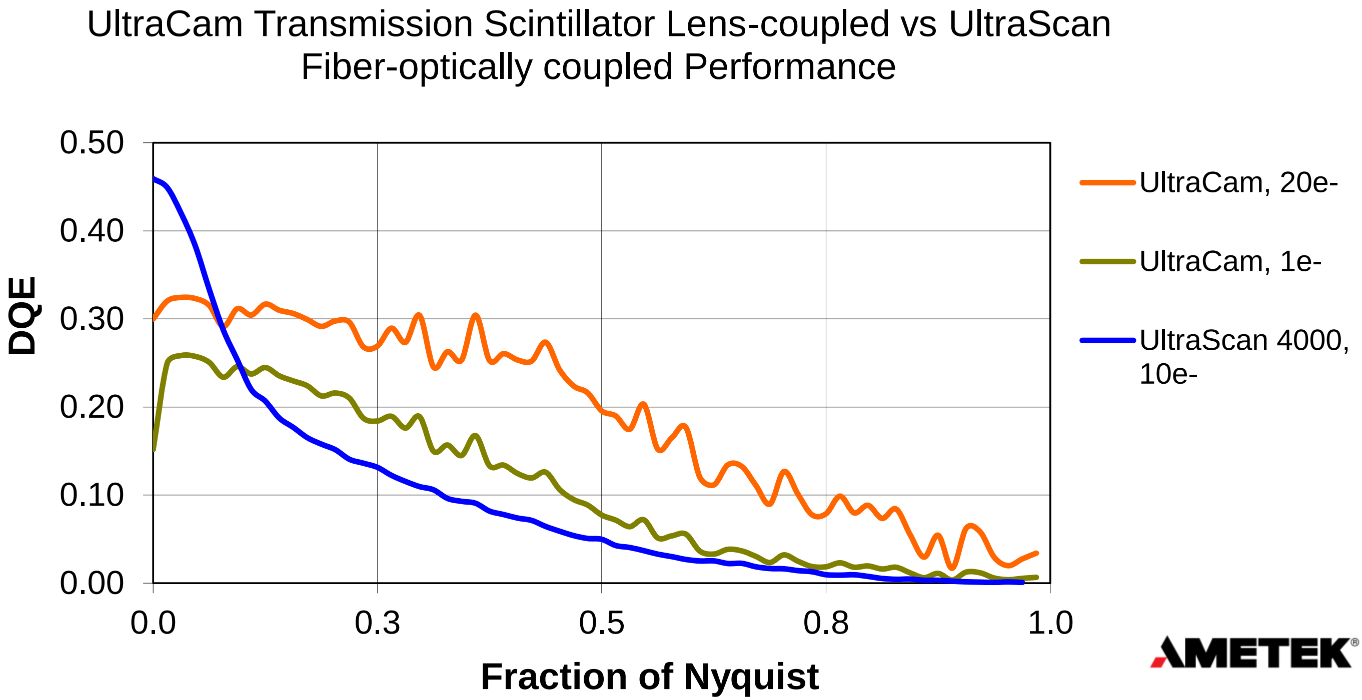
Landau noise reduces benefit from transmission scintillator



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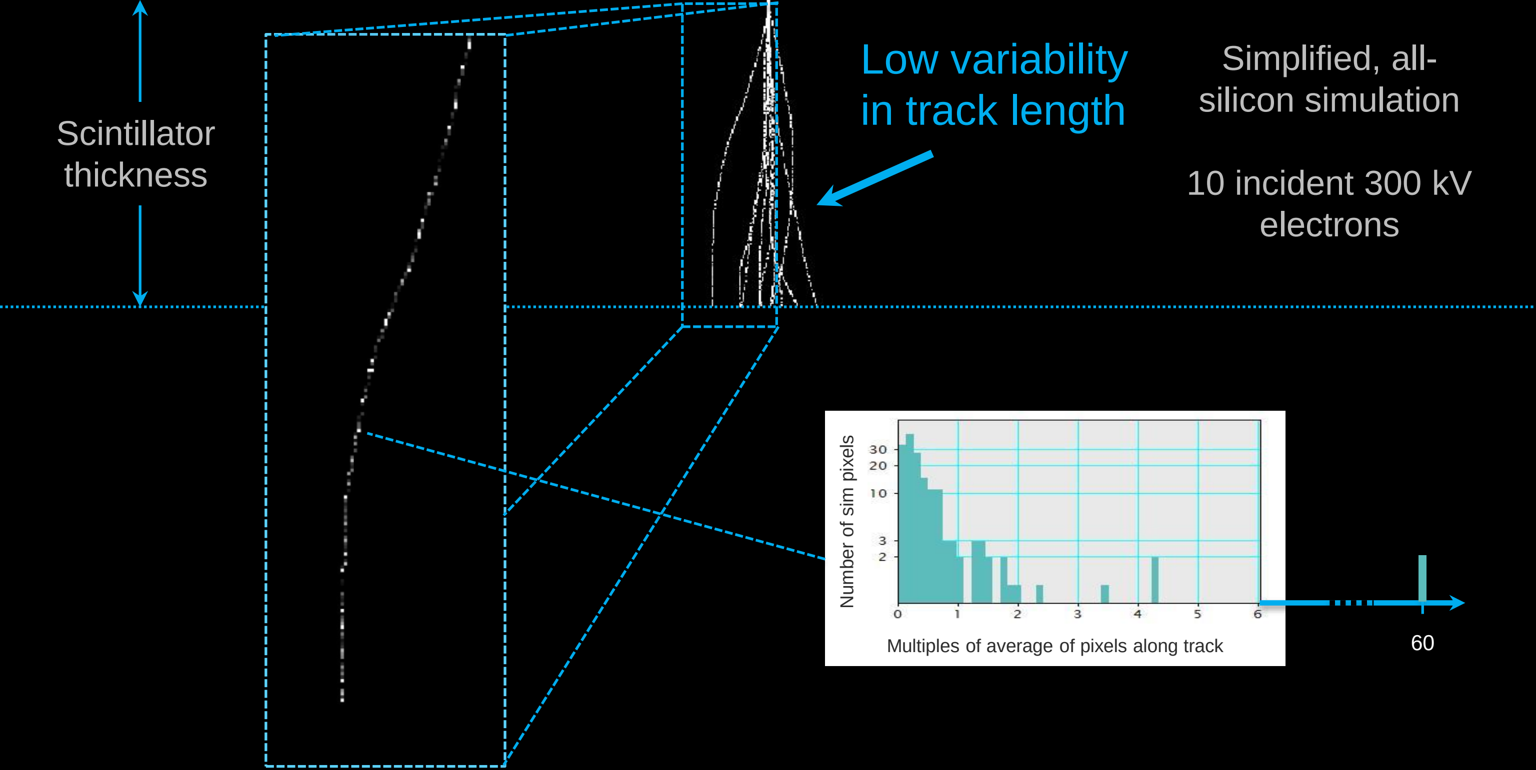
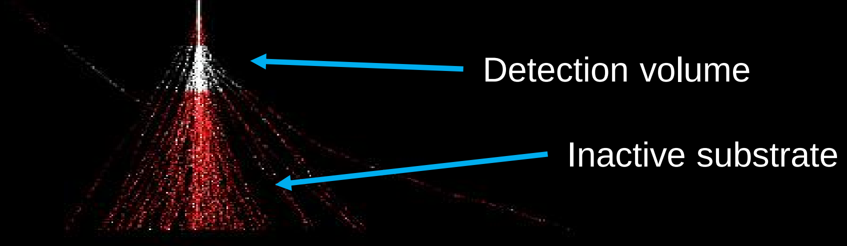


Noisy Inelastic Scattering is a Problem Even when Elastic Scatter is Not

Silicon APS



- Same transmission arrangement as film and transmission lens-coupled camera
- But high SNR → which allows discrimination

"Counting" is done by discrimination

What we thought happened

What happened	Electron counted	Electron not counted	
Electron hit	True positive	False negative	"sensitivity"
No electron hit	False positive	True negative	"selectivity"

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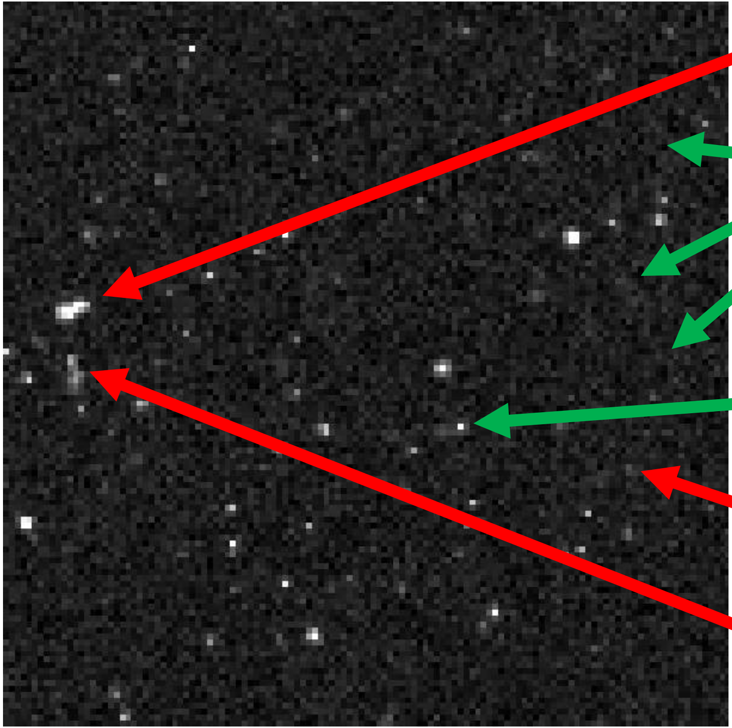
And discrimination is non-trivial



One electron or two?

Probably no electron –but have to get this right many many times

Probably one electron

Maybe an electron

Possibly a scattered electron

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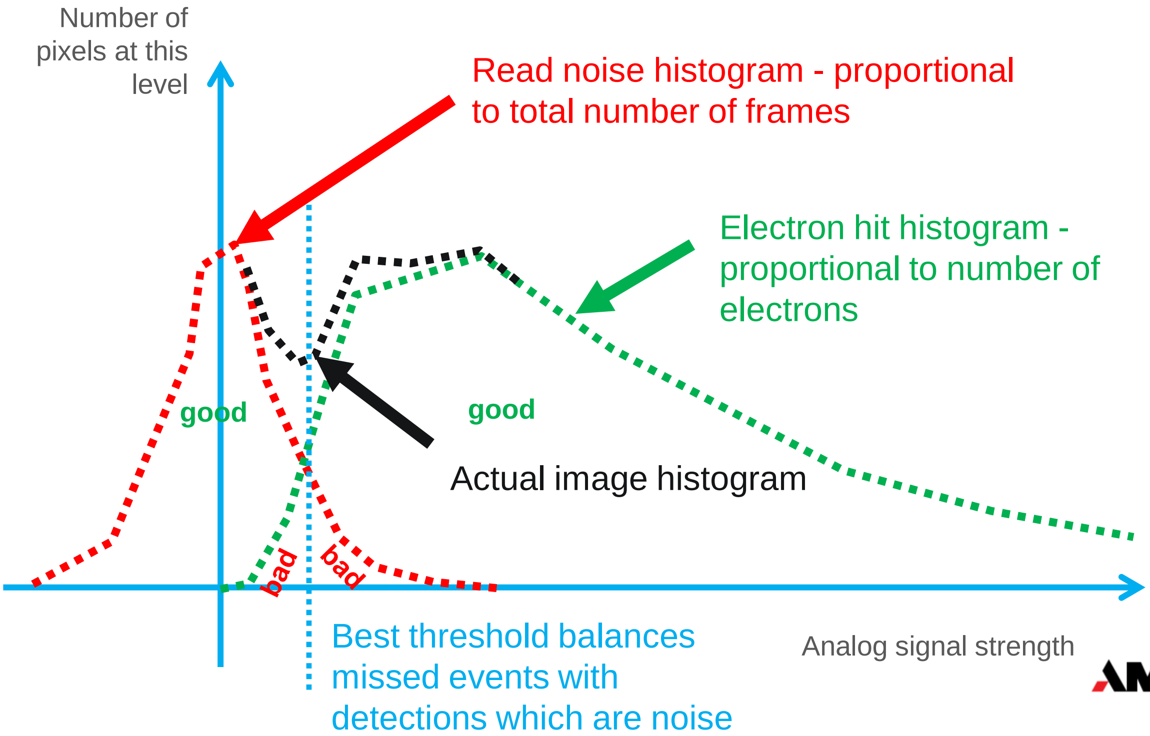
Discriminating electrons from background



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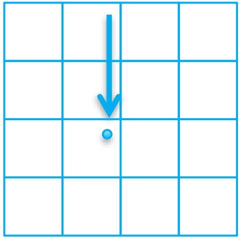
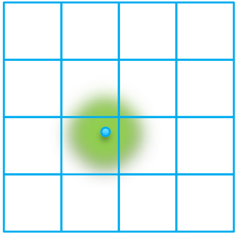
Super-resolution by centroiding extracts more location information from each event

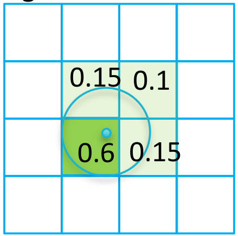
1. Electron enters detector



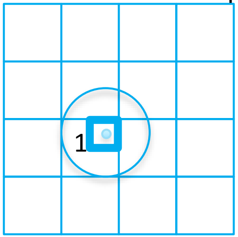
2. Signal is scattered



3. Charge collects in each pixel



4. Events are localized with sub-pixel accuracy



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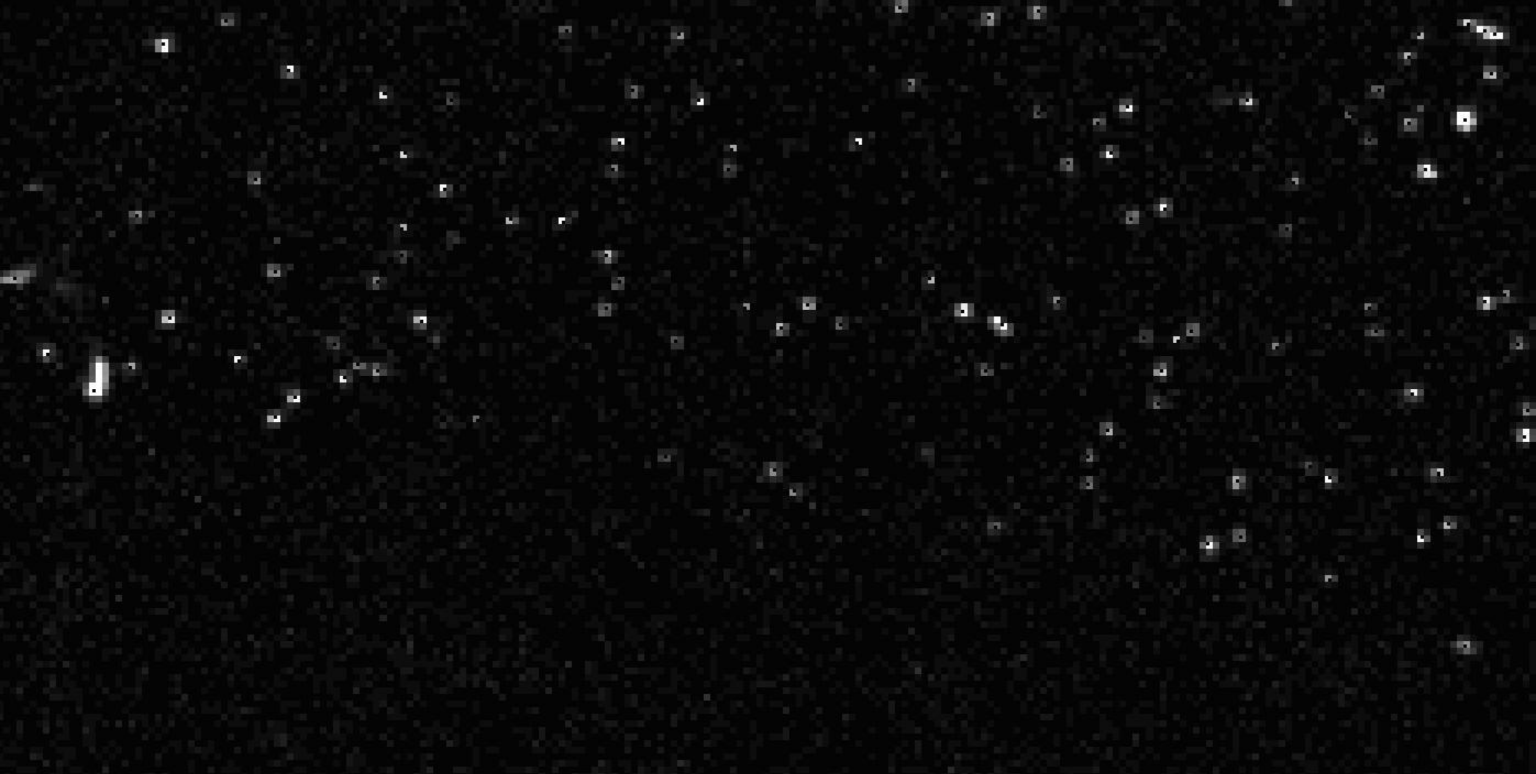
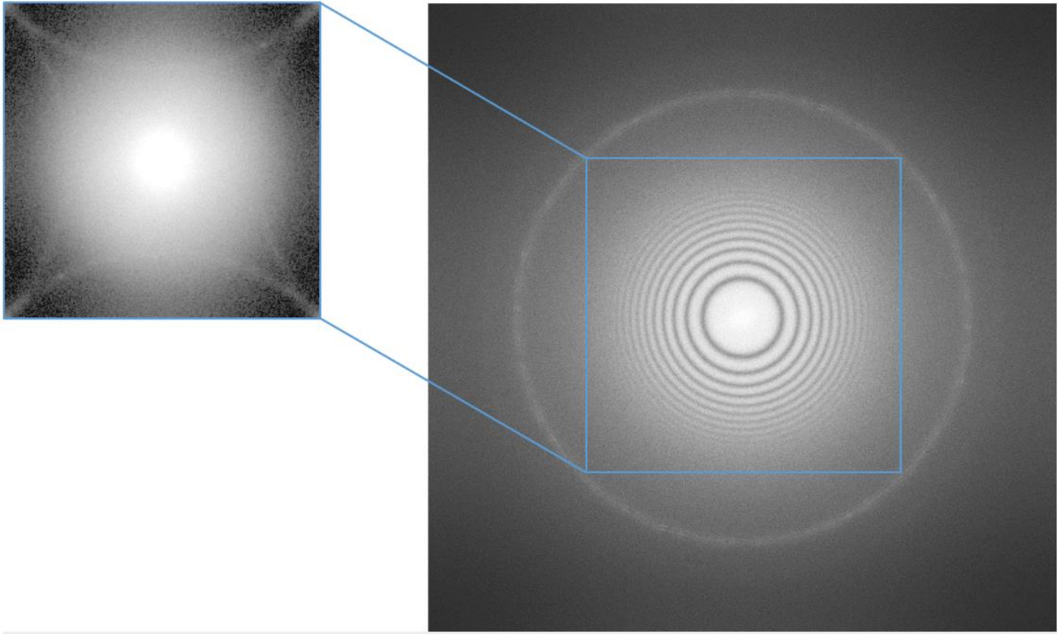
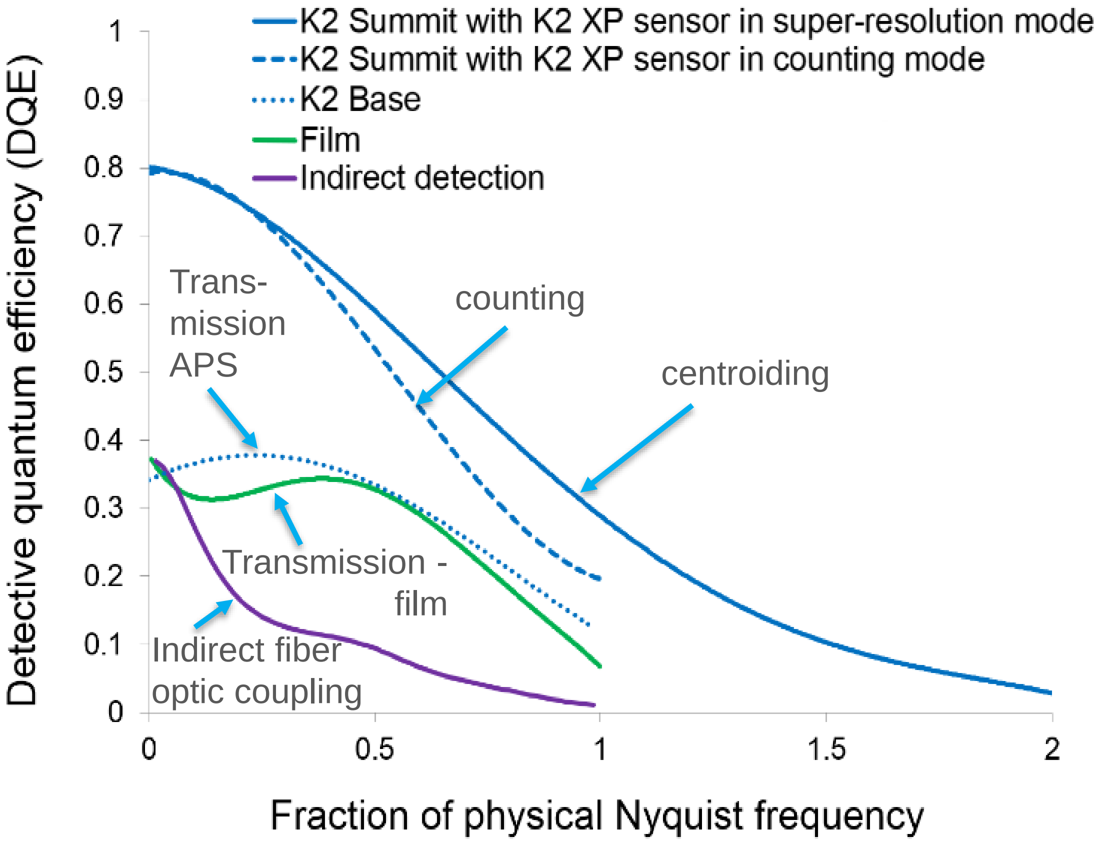


Events with centroided (super-resolution) counts marked

Super-resolution eliminates aliased signal and noise



DQE benefits of transmission, counting and centroiding



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With the more that 2x jump in SNR, come related benefits

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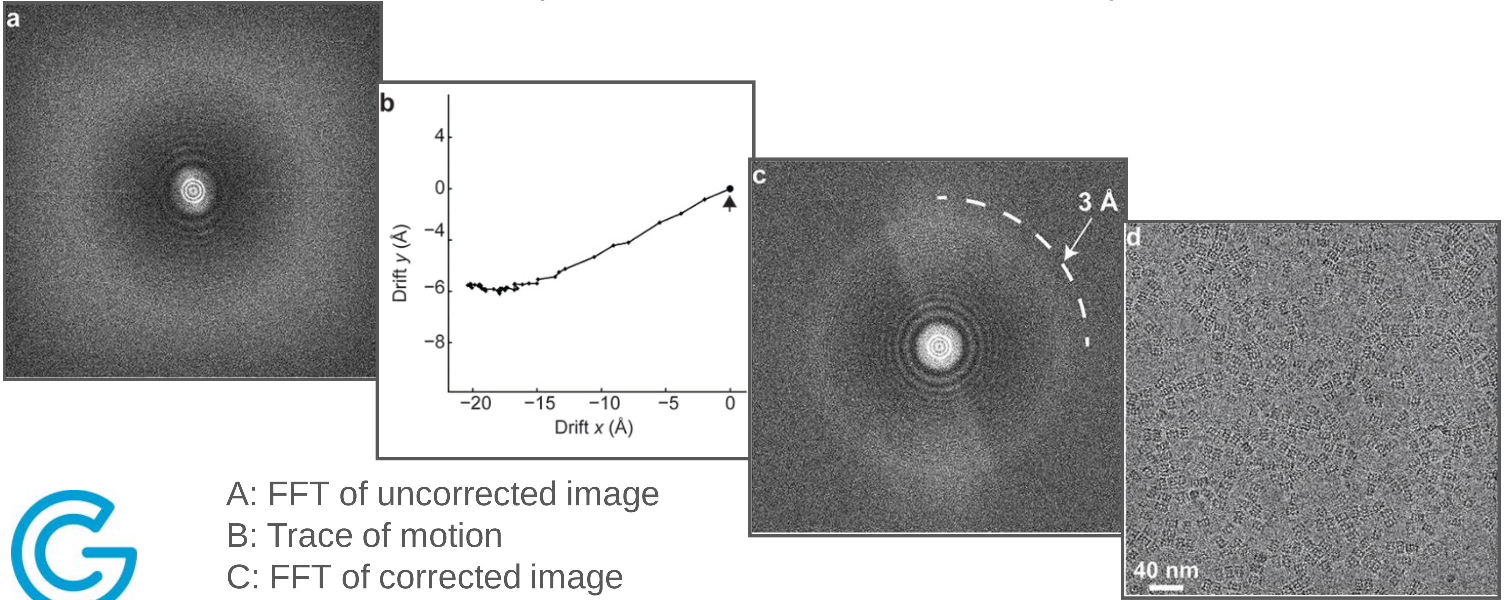
Counted dose-fractionation enables motion correction

Total 5 s exposure with 0.1 s subframes and -1.4 µm defocus



A: FFT of uncorrected image
B: Trace of motion
C: FFT of corrected image
D: Corrected image

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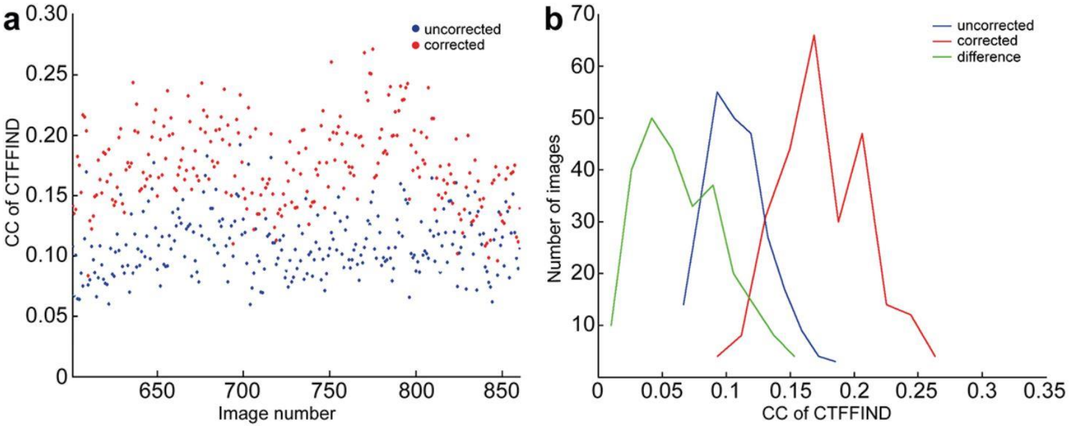
Li et al. 2013 Nature Methods

Better CTF determination



Li et al, Nature Methods 10, pp 584–590 (2013)
Supplementary Figure 3

The cross correlation coefficient output from *CTFFIND*1 was used to assess the Thon ring quality within the resolution range of 10 ~ 5 Å before and after drift correction.

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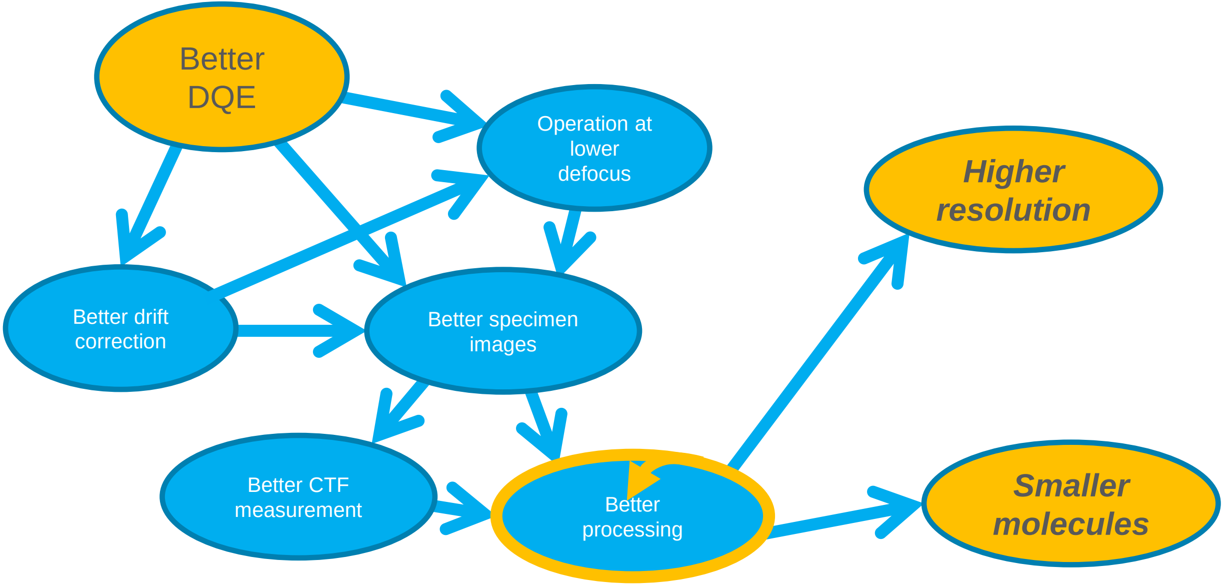
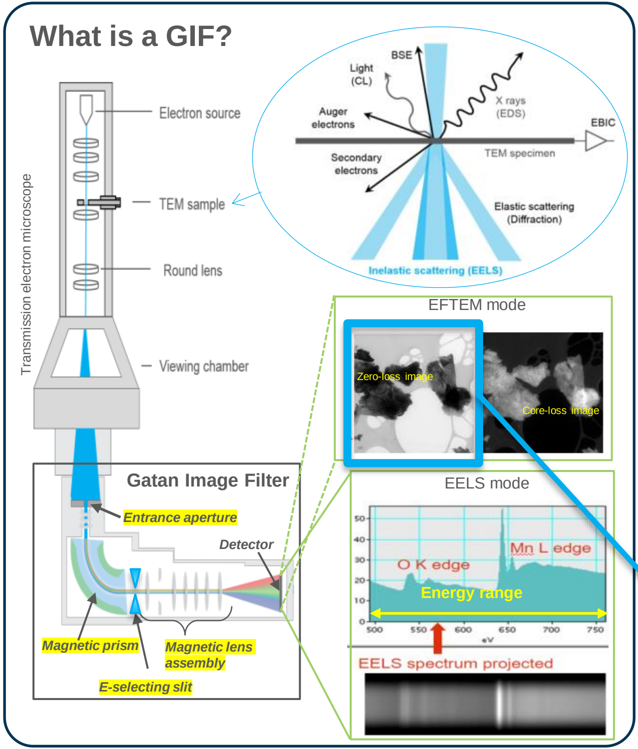
Cryo-EM methods leverage DQE



Combining counted Direct Detection with an Energy Filter



GIF Continuum K3

Contrast-enhancing mode which is useful for cryo-EM and indispensable for cryo-tomography with thicker specimens

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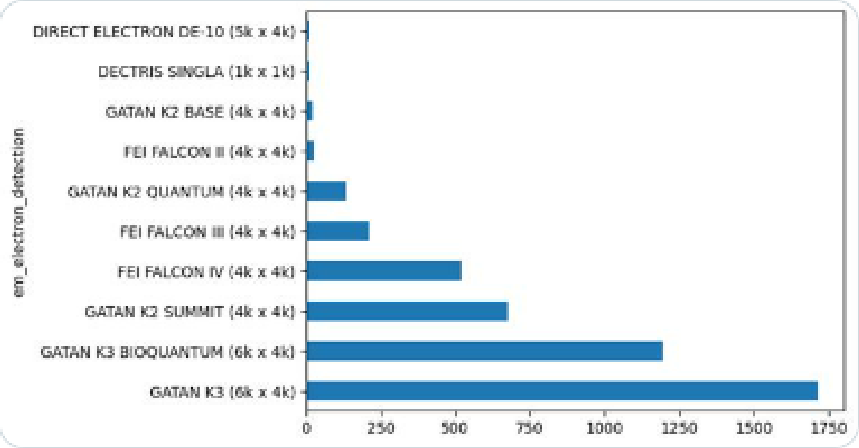
With the now well-known result

Protein Data Bank @PDBeurope · Jan 5

Replying to @PDBeurope @NatureComms and 7 others

For cryoEM PDB structures, 2023 was again the year of the @GatanMicroscopy K3, with ~3000 entries containing 'GATAN K3' in EM detector name. The GATAN K2 had around 700 entries, while the FEI Falcon detectors (@thermofisher) account for around 700 entries in total. 6/10



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Newer developments

- Alpine Vista and Alpine for Cryo-EM at lower kV (80 – 200 kV)

- K3IS - first counting, high-speed, large-format electron camera for *in-situ* microscopy

- Metro and Metro 300 for Material sciences and *in situ*

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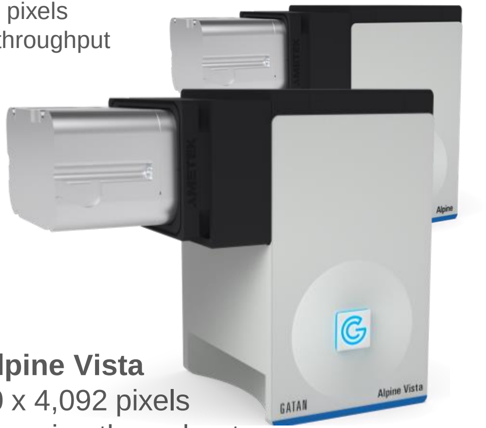
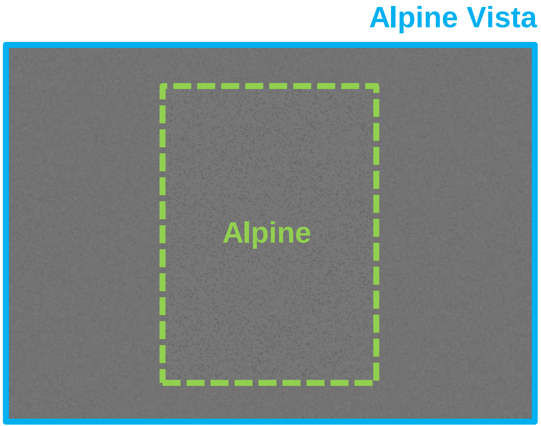
Alpine direct detection cameras

Two models available to match the field of view with your application needs:

Alpine
2,304 x 3,240 pixels
Medium imaging throughput



Alpine Vista
5,760 x 4,092 pixels
Highest imaging throughput



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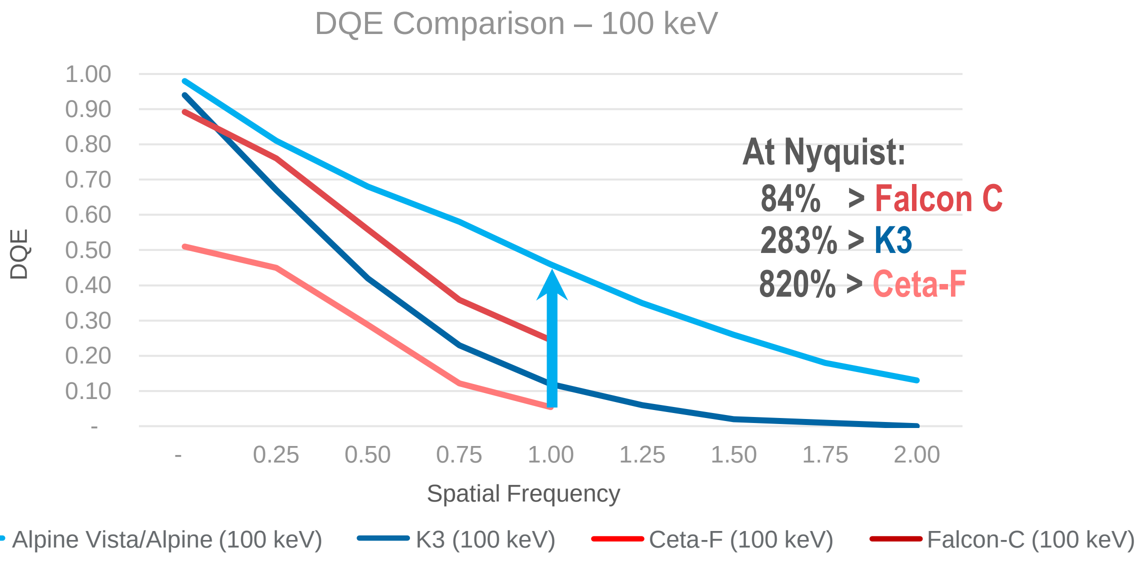

Delivers remarkable DQE results at 100 keV

- Proprietary sensor rejects read noise and variability associated with electron scattering
- Dramatic DQE improvement across all spatial frequencies
- Alpine Vista and K3 deliver super-resolution readout

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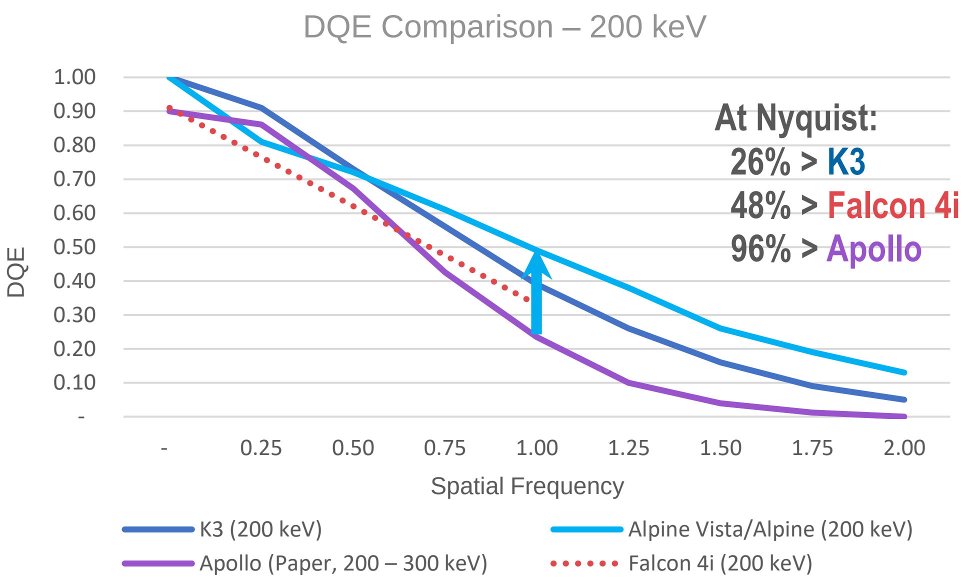

Delivers remarkable DQE results at 200 keV

Proprietary sensor rejects read noise and variability associated with electron scattering

Dramatic DQE improvement across all spatial frequencies

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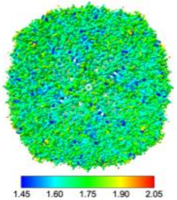
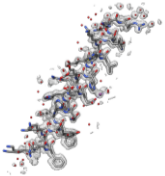
Structures solved with Alpine (100,120, and 200 keV)

* Difficult sample

Apoferritin
120 keV Glacios

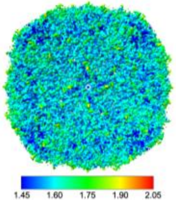


1.92 Å

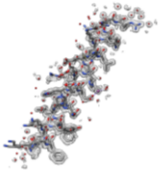


Q score: 0.85
~1.51 Å

Apoferritin
200 keV Glacios

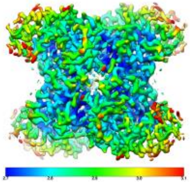


1.76 Å



Q score: 0.88
~1.35 Å

Aldolase
100 keV Talos F200C

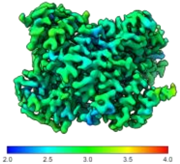


3.10 Å

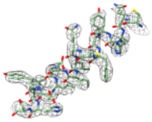
Hemoglobin
120 keV Tecnai G2-spirit (LaB6) *

4.33 Å

LKB1 heterocomplex
120 keV Glacios

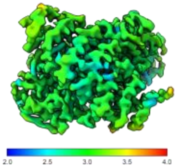


3.19 Å

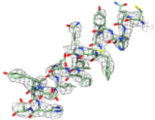


Q score: 0.59
~2.96 Å

LKB1 heterocomplex
200 keV Glacios

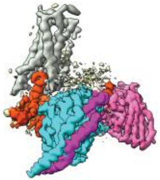


3.37 Å



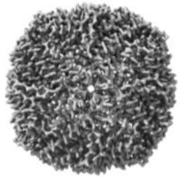
Q score: 0.51
~3.41 Å

M4R in complex with Gi
120 keV Tecnai G2-spirit (LaB6) *



4.40 Å

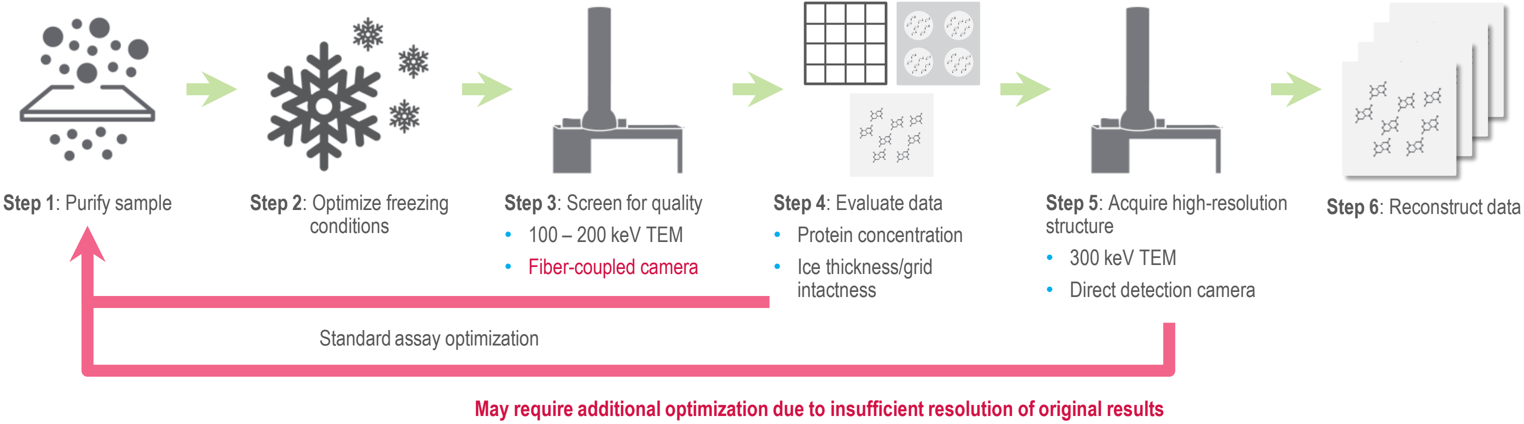
Apoferritin
120 keV Tecnai G2-spirit (LaB6)



2.65 Å

The larger FOV of Alpine Vista will yield similar results but **3x Faster**
Will maximize imaging throughput for mid-tier to high-tier screening TEMs

https://doi.org/10.1101/2024.05.26.595910
https://doi.org/10.1101/2024.02.14.580363



Requires fewer TEM sessions – Internal 300 keV

- Fiber-coupled cameras provide insufficient data to determine sample quality
- See **more failures** when transfer to 300 keV system

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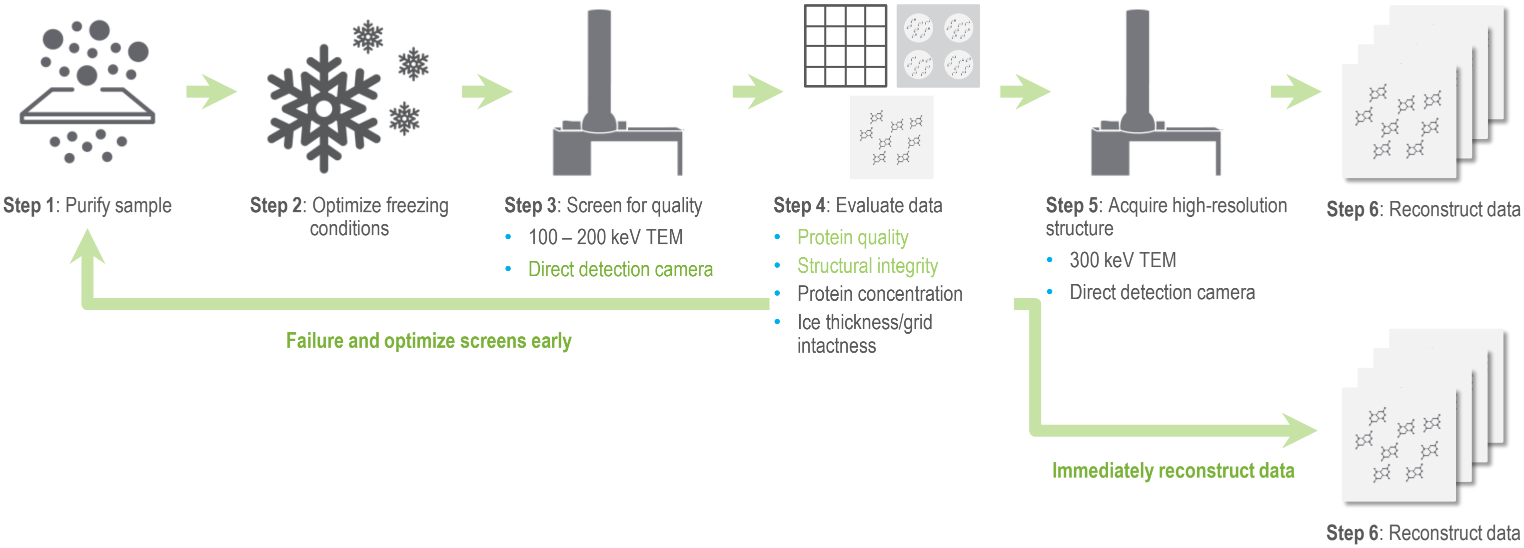

Requires fewer TEM sessions – Internal 300 keV

- Early direction detection screens allow you to **fail and optimize early**
- Frees up time on 300 kV for high-resolution reconstructions
- Overall, less expensive to fail early than later

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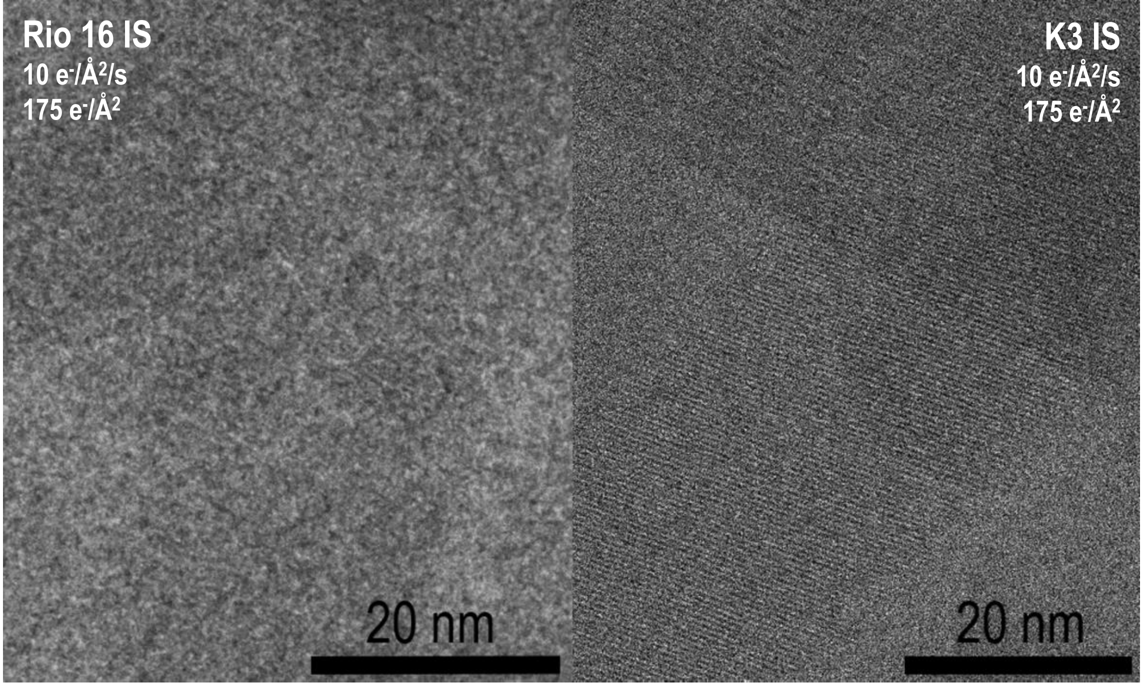

Acquire Higher Quality Images with a Counting Camera

- Capture more detail with less noise
- Direct comparison of the Rio™ 16 IS and K3 IS cameras with a dose rate of 10 $e^-/Å^2/s$

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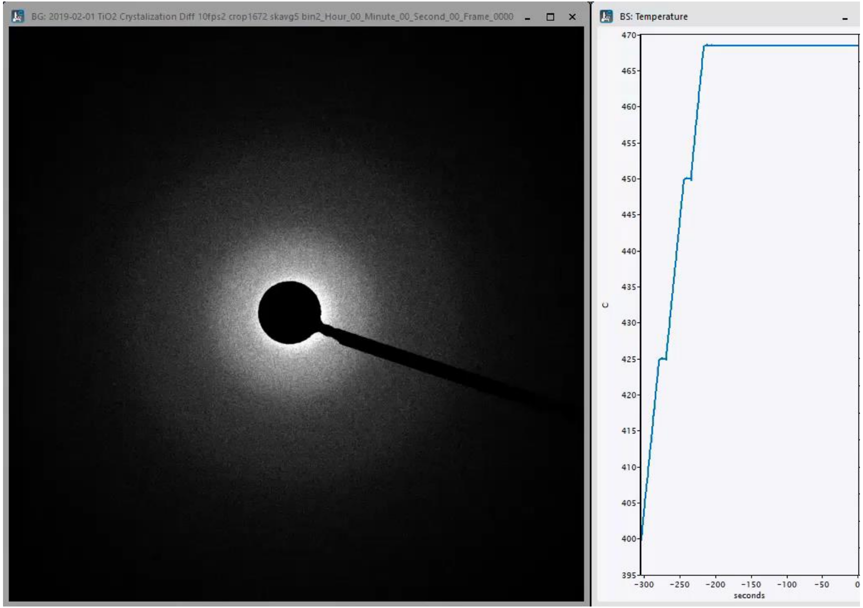
Hydroxycancrinite zeolite sample courtesy of Shery Chang, ASU



Observe Dynamic Behavior With Fast Counted Diffraction

- Observe sample dynamics with high temporal resolution – e.g., crystallization, phase transformations, lattice expansions
- Utilize counting to detect faint diffraction spots in this video of TiO_2 nanoparticle crystallization

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Sample courtesy of Shuo Chen, University of Houston

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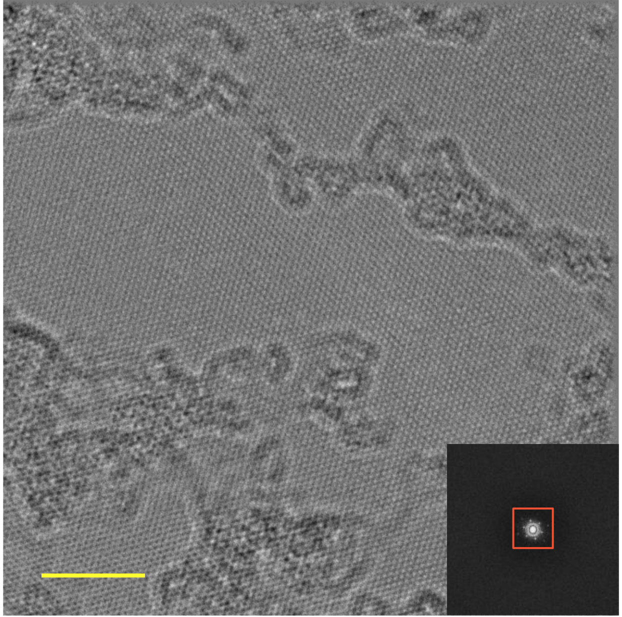
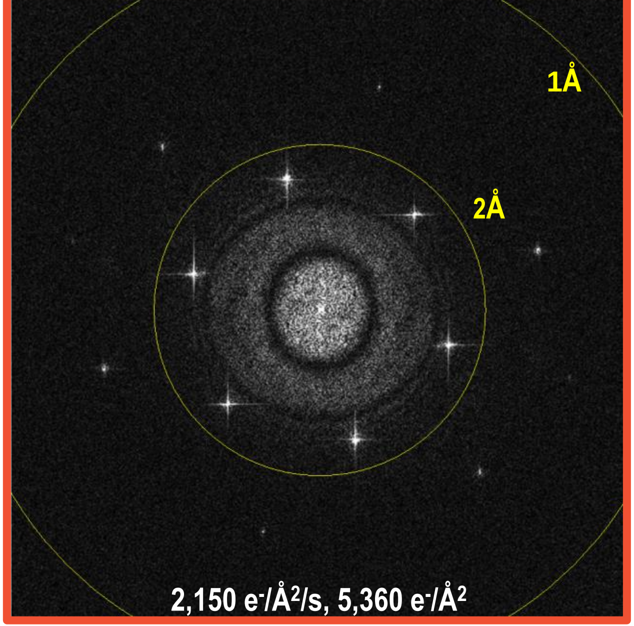
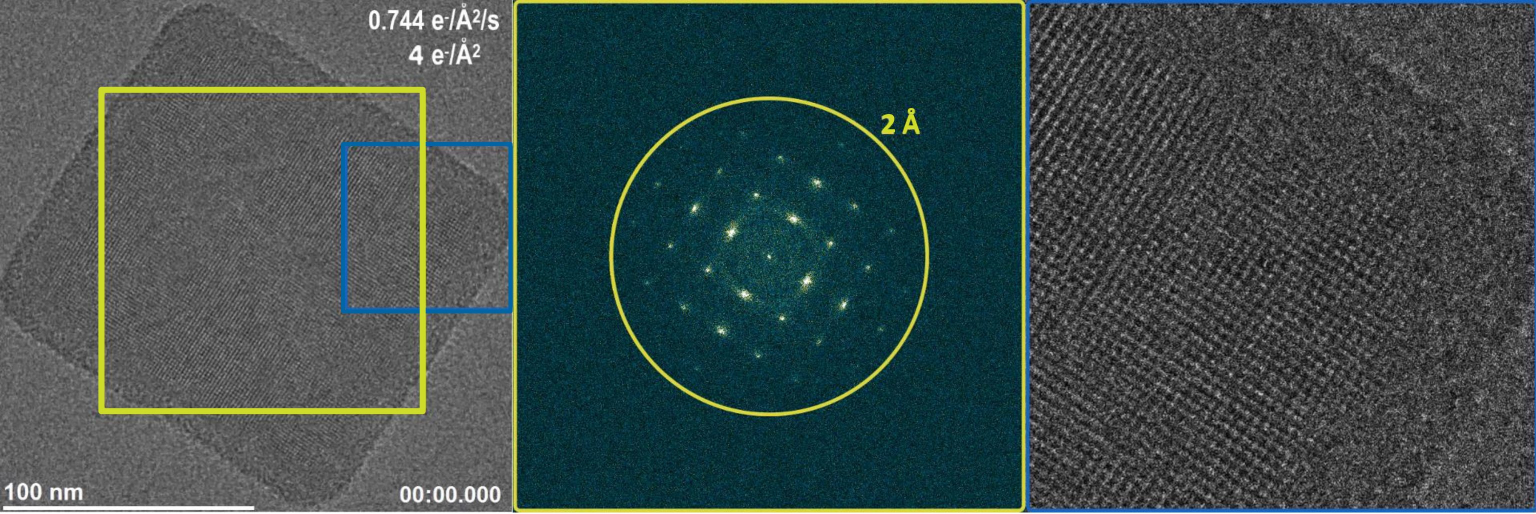




High DQE at low KV makes Metro ideal for 2D materials

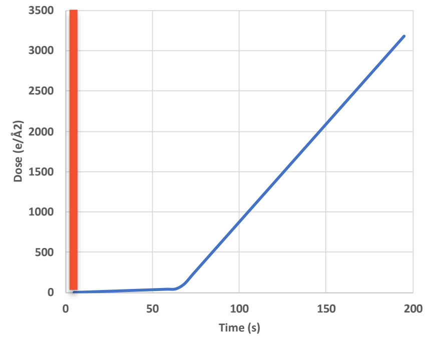
MoS_2 is imaged clearly, even on a non-aberration corrected TEM at 80 KV

Data courtesy of Rachael Keneipp, Parisa Yasini, and Marija Drndic



Sample courtesy Changhao Xu & Wei Gao, Caltech; video courtesy of Mingjie Xu, UC Irvine

[Metro Data]



Metro 300 images the most sensitive specimens at 300kV

Counting electrons one by one means the dose rate on this MOF sample can be kept low enough to image the material for extended periods with minimal damage

Can discern structural changes and quantify damage over time

Counting enables observation of the pristine lattice structure in MOF samples under low-dose conditions

GATAN Metro

Thanks for listening!

Any questions?

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