CRYOEM 001 :
COLLECTING DATA AND OTHER MATTERS

February 8, 2023

New York Structural Biology Center

SIMONS ELECTRON MICROSCOPY CENTER

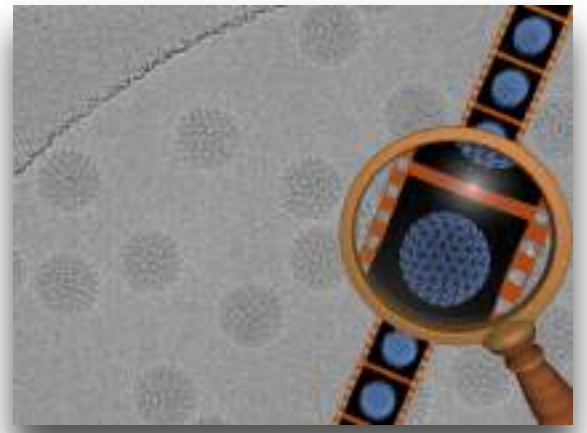
WHAT BROUGHT ABOUT THE RESOLUTION REVOLUTION

(~2012-2014)

Hardware

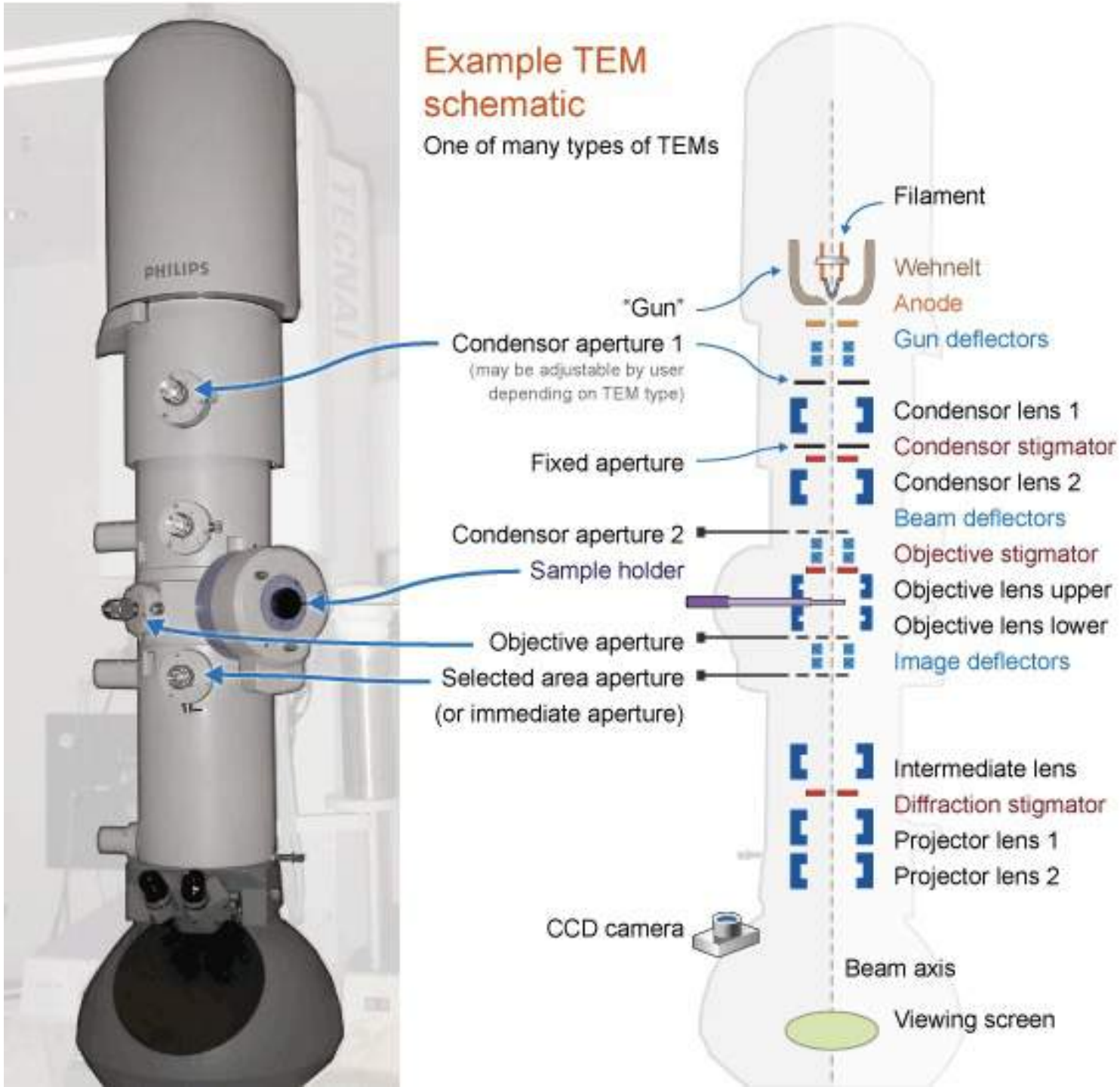
Microscopes

Direct Detectors



Computers

2012->2017
Cost reduced by 100x



Example TEM schematic

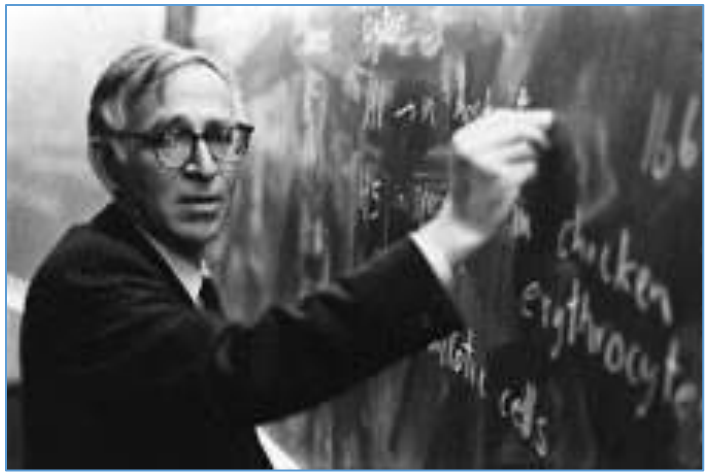
One of many types of TEMs

Filament
Wehnelt
Anode
"Gun"
Gun deflectors
Condensor aperture 1
(may be adjustable by user depending on TEM type)
Condensor lens 1
Condensor stigmator
Fixed aperture
Condensor lens 2
Beam deflectors
Condensor aperture 2
Objective stigmator
Sample holder
Objective lens upper
Objective lens lower
Objective aperture
Image deflectors
Selected area aperture
(or immediate aperture)
Intermediate lens
Diffraction stigmator
Projector lens 1
Projector lens 2
CCD camera
Beam axis
Viewing screen

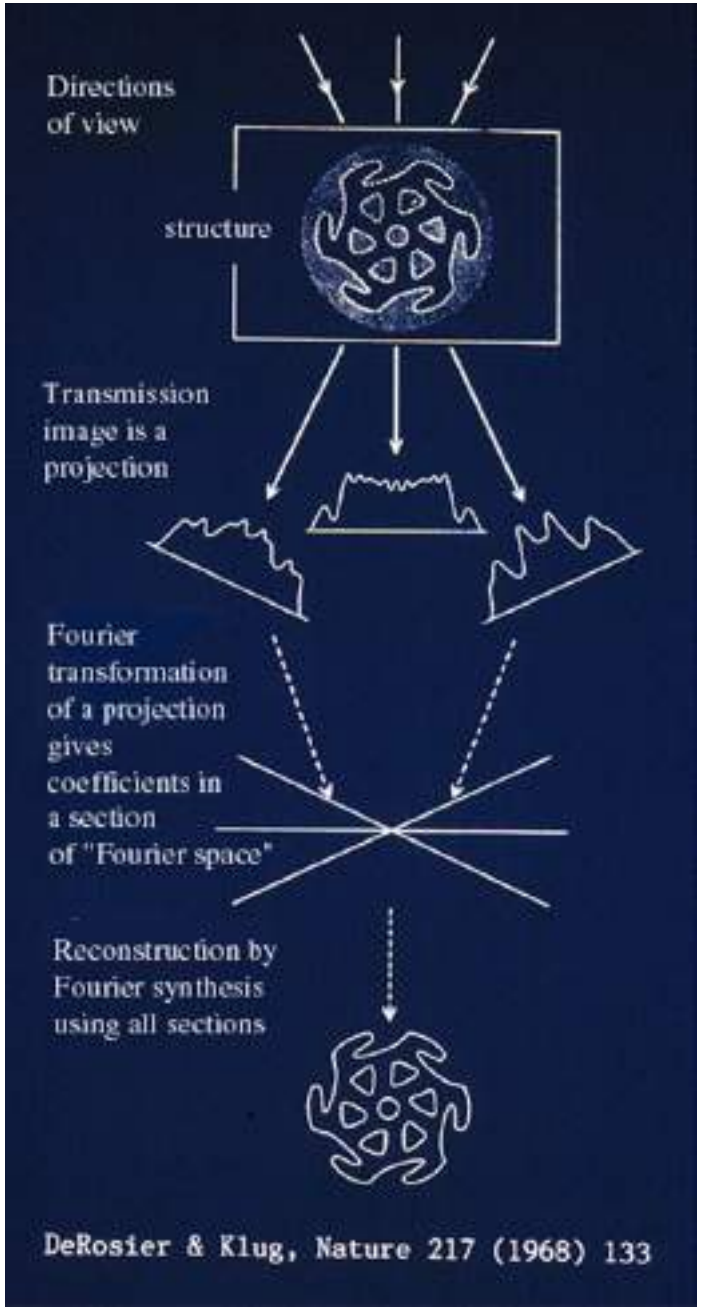
THREE-DIMENSIONAL RECONSTRUCTION:
STRUCTURES WITH HELICAL SYMMETRY. 1968
(sample prep: negative staining)

Pioneering work: 3D reconstruction of a bacteriophage tail using the Fourier-Bessel approach, 1968

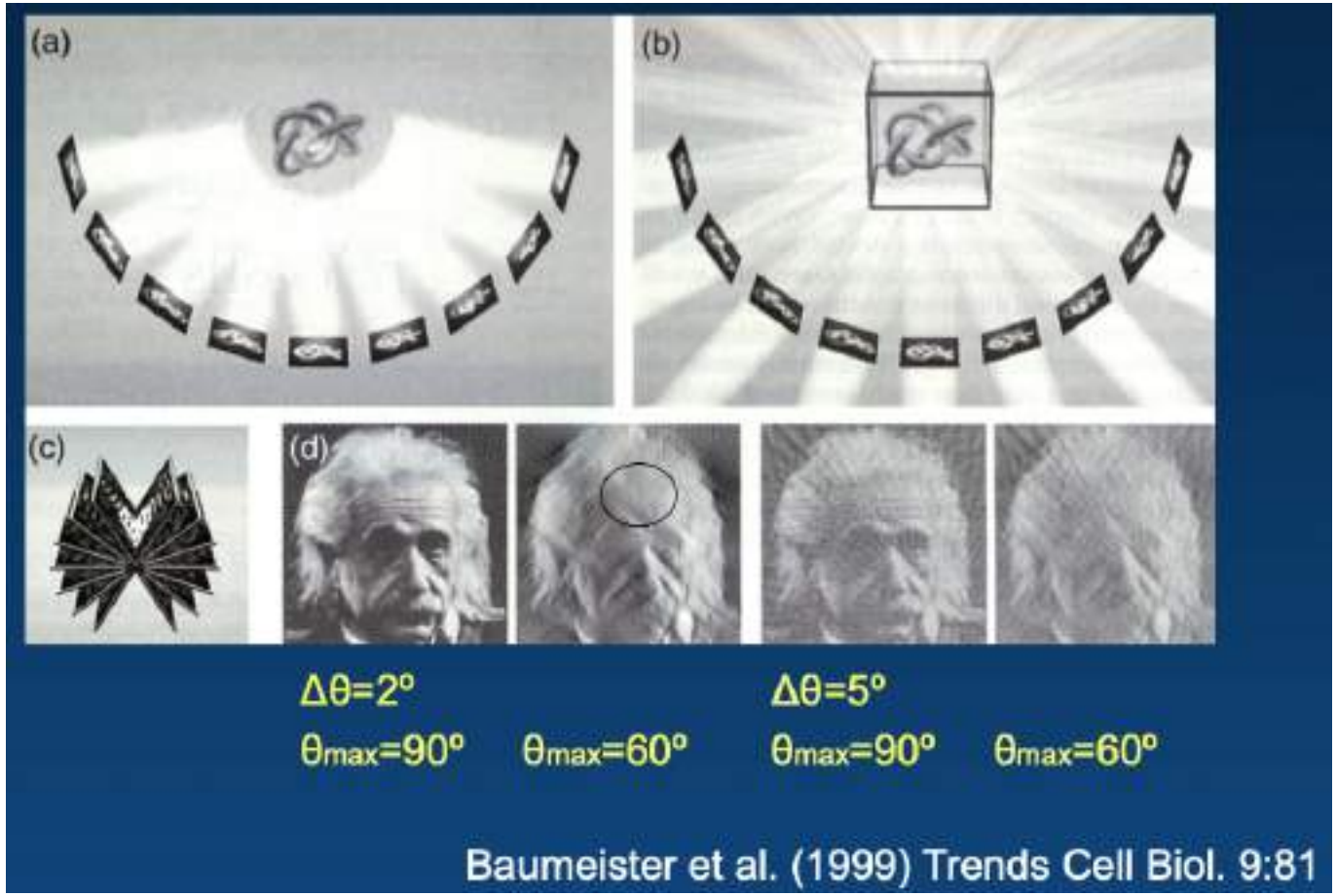
Application of the Projection-Slice Theorem



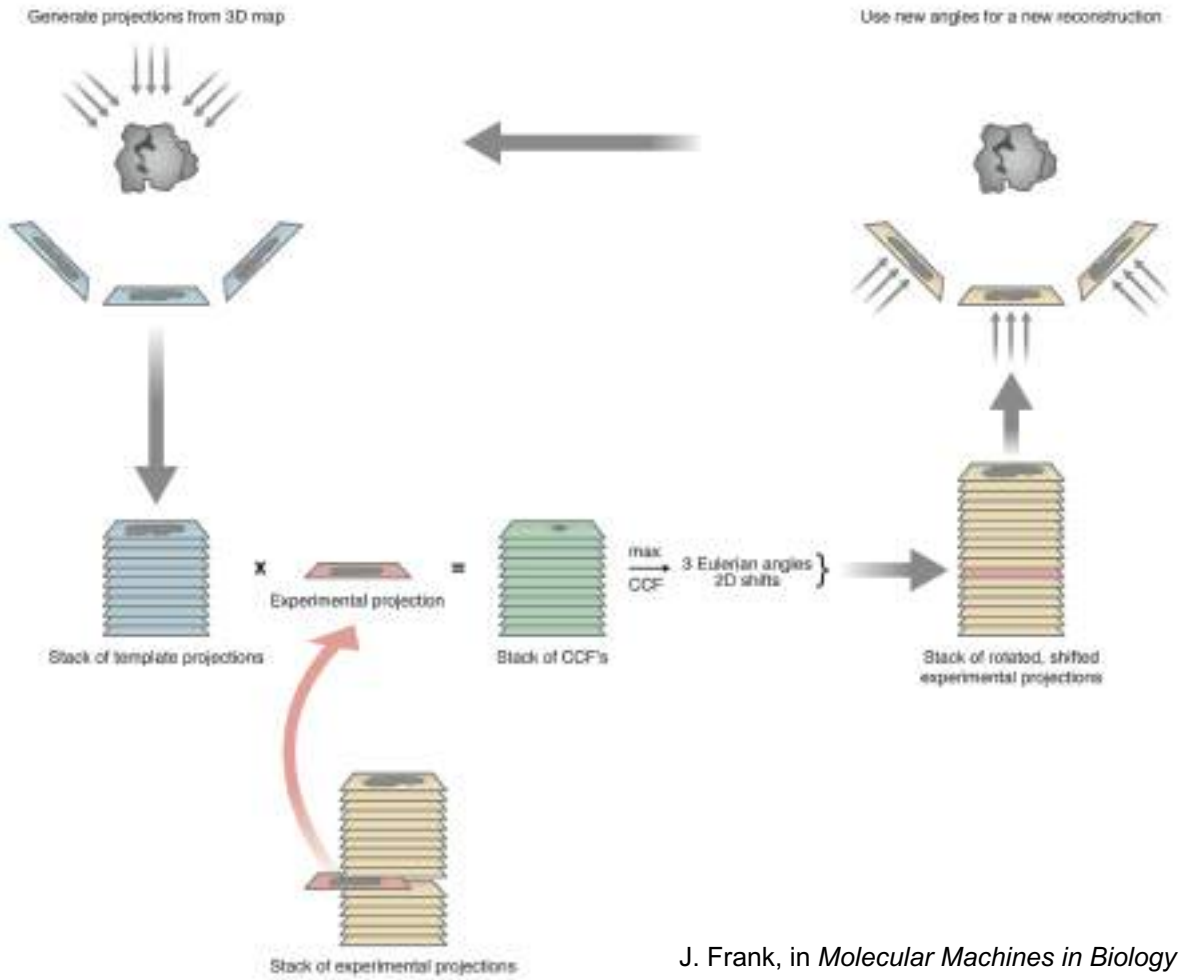
Aaron Klug and David DeRosier, LMB/MRC Cambridge



DIRECTIONAL INFORMATION LOSS

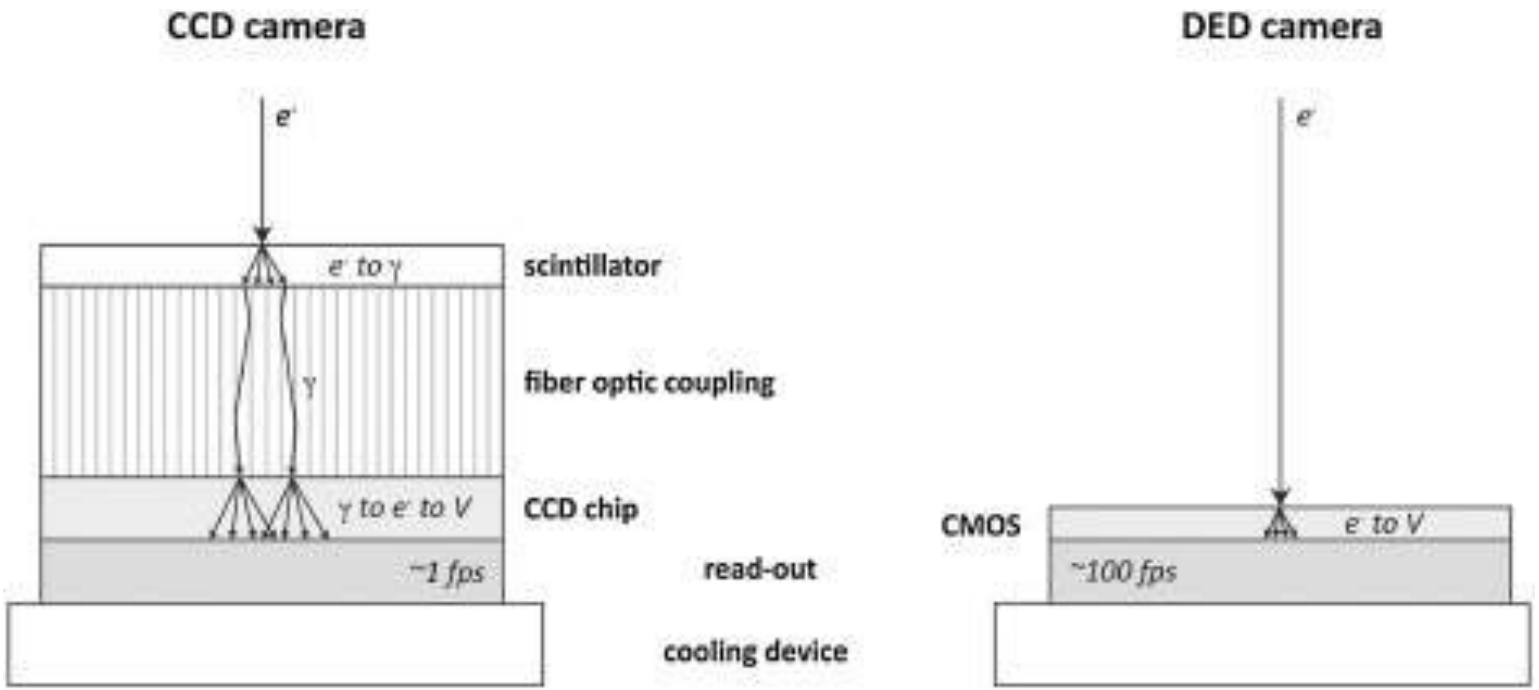


Iterative angular refinement



J. Frank, in *Molecular Machines in Biology* 2011

NEW ERA (SINCE 2012): DIRECT ELECTRON DETECTING CAMERAS



Koning et al. Ann. Anatomy 2018

DETECTORS

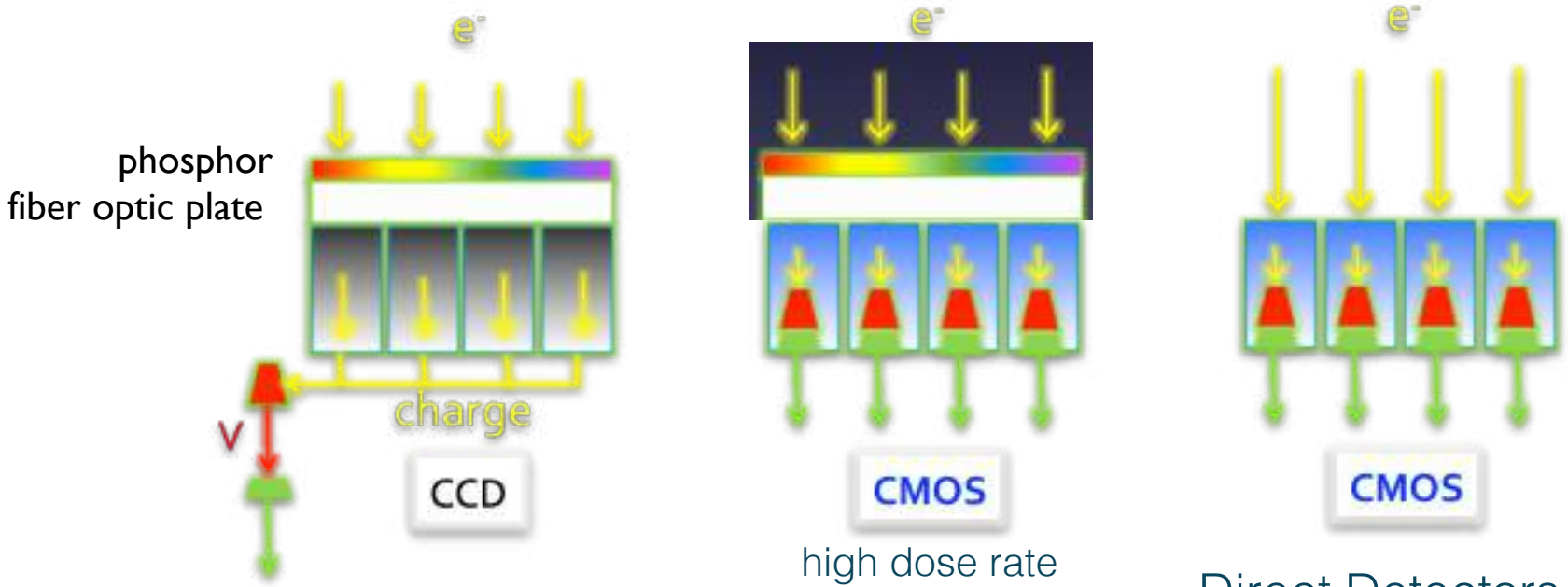
Digital Cameras for TEM

Photon converted

Direct sensing

?

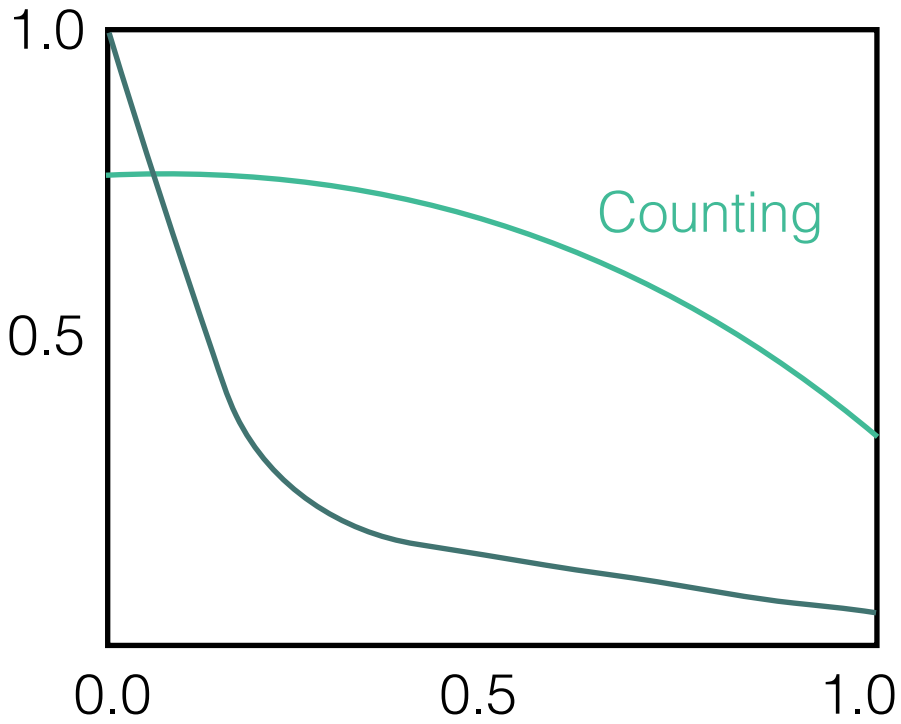
- CCD — Charge Coupled Device
- CMOS — Complementary Metal Oxide Semiconductor



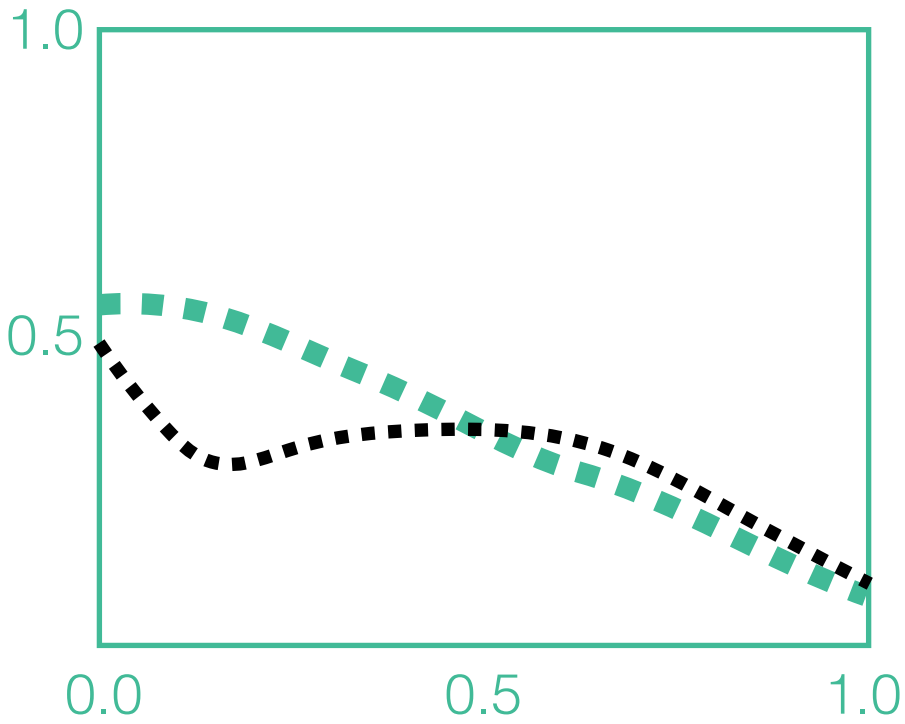
DETECTORS

Detector Performance Characterization

MTF (Modulation Transfer Transform)
contribute to signal envelope

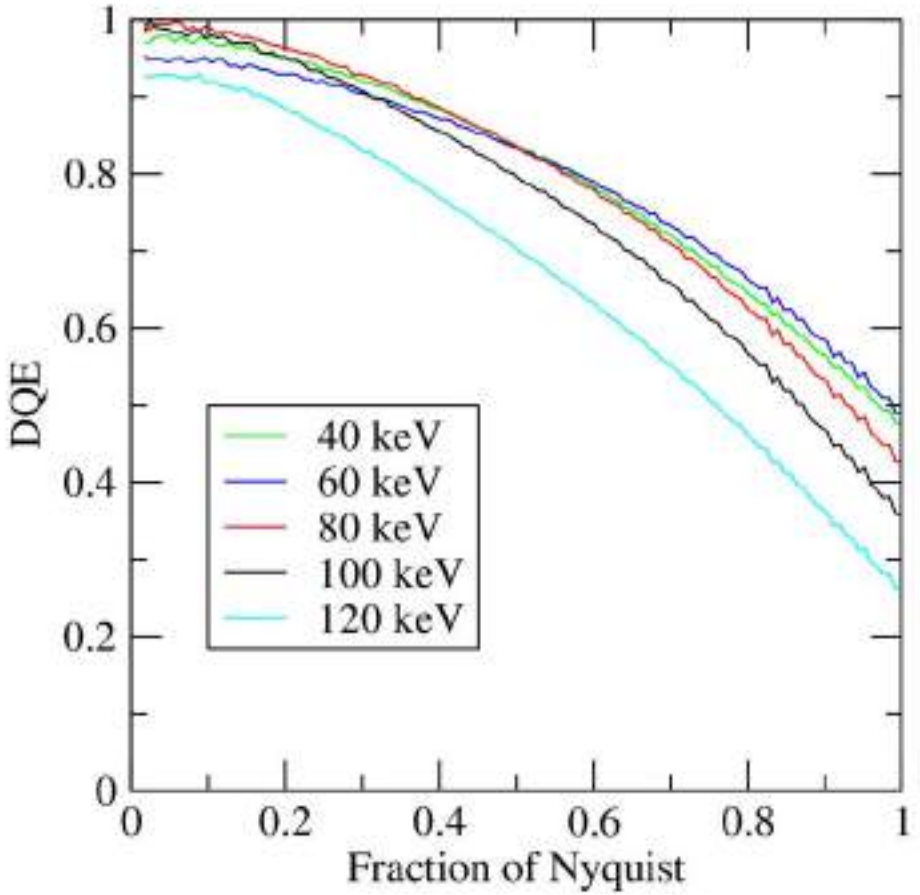


DQE (Detector Quantum Efficiency)
S/N over spatial frequency range



DETECTORS

Detector Performance Characterization



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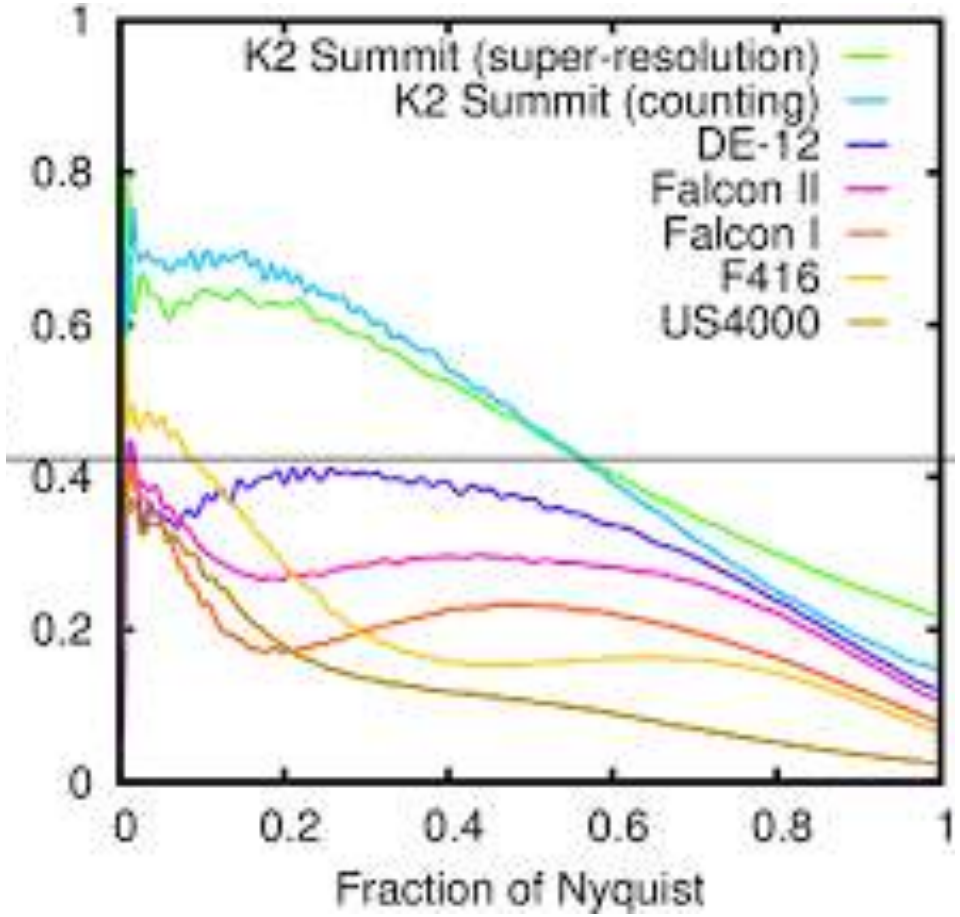


Ruskin, et al JSB

K3 SPECS

https://www.gatan.com/K3

Specifications

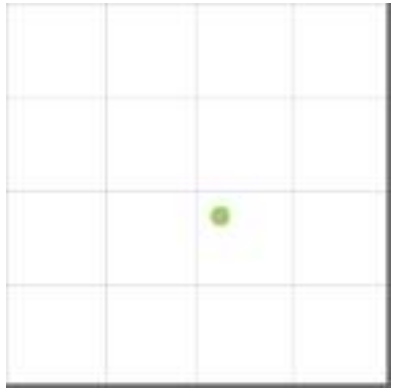
	K3	K3 Base
TEM operating voltage (kV)	200 / 300	
Sensor size (pixels)	5,760 x 4,096	3,456 x 4,096
Readout modes	Counting Super-resolution	Counting
Max. image size (pixels)	11,520 x 8,184 Super-resolution	3,456 x 4,096
Performance relative to physical Nyquist (DQE)		
Peak	>0.87 / >0.83	>0.8
0.5	>0.53 / >0.53	>0.5
Sensor read-out (full fps)	>1500	
Transfer speed to computer (full fps)	>75	>25
Motion correction	Inline	
Gatan Microscopy Suite® software	Included	
Automation support	Latitude and other third-party software	

Specifications are subject to change without notice.

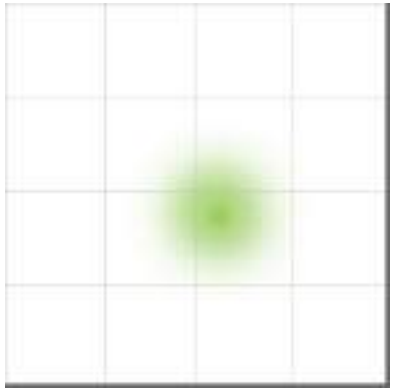
COUNTING MODE

5,760 x 4,096 px

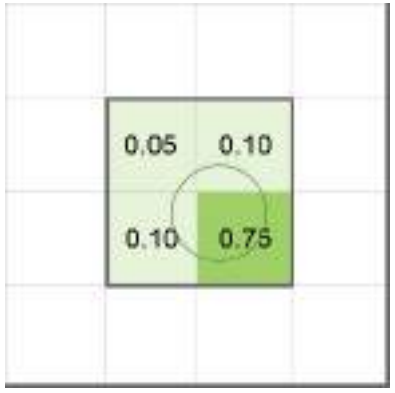
11,520 x 8,184 px



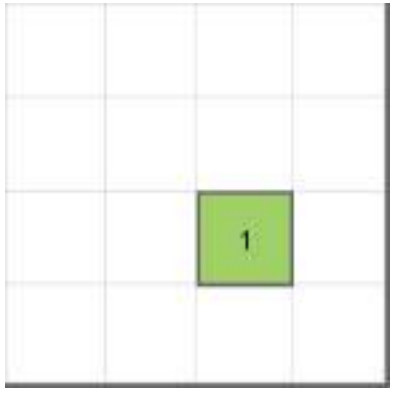
Electron enters detector.



Electron signal is scattered.



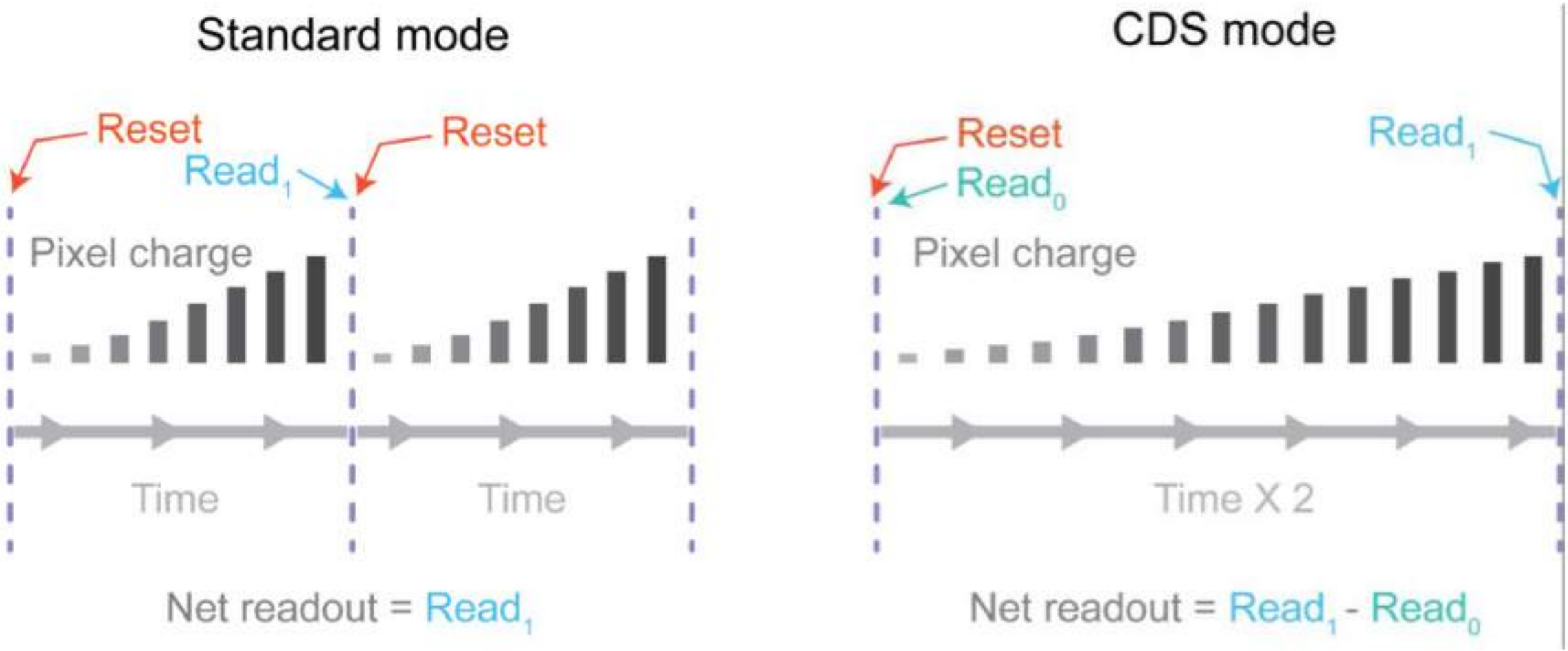
Charge collects in each pixel.



Events reduced to highest charge pixels.

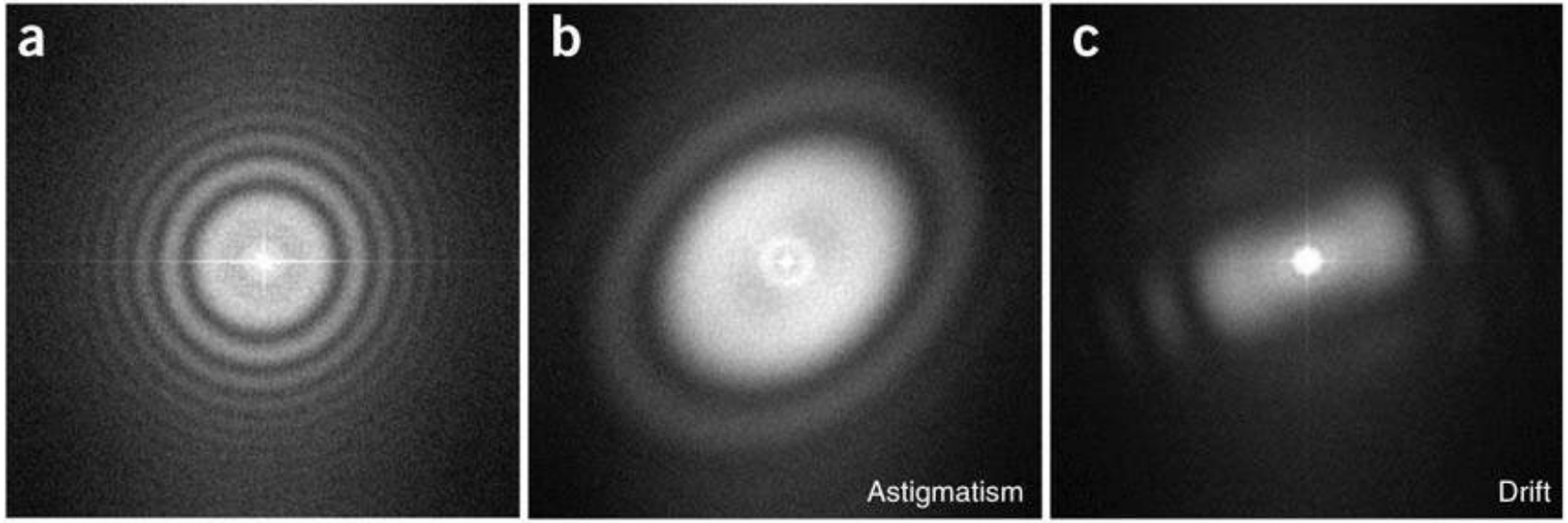
https://www.gatan.com/improving-dqe-counting-and-super-resolution

K3 lowers Read Noise with Correlated Double Sampling (CDS)



https://www.gatan.com/

CTF: WHY IS MONITORING THE CTF IMPORTANT IN OUR DATA COLLECTION?



CTF

The **contrast transfer function** (**CTF**) mathematically describes how aberrations in a transmission electron microscope (TEM) modify the image of a sample

The phase shift (phase distortion function) due to the objective lens can be combined into a single phase factor χ, given by,

$$\chi(|g|)=\left(\frac{1}{2}\pi C_s\lambda^3|g|^4-\pi\Delta f\lambda|g|^2\right)$$ ---------- [4236a.a]

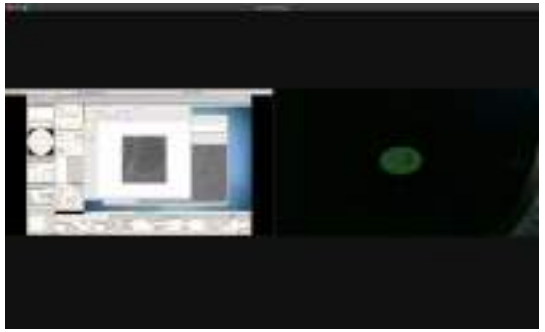
$$=\frac{2\pi}{\lambda}\left(\frac{1}{4}C_s\alpha^4-\frac{1}{2}\Delta f\,\alpha^2\right)$$ ---------- [4236a.b]

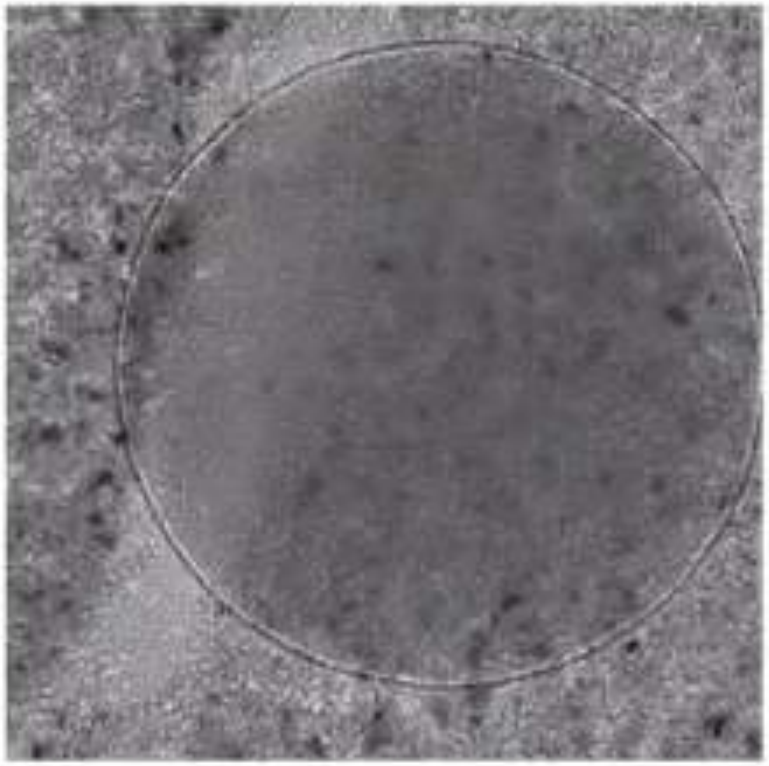
where,

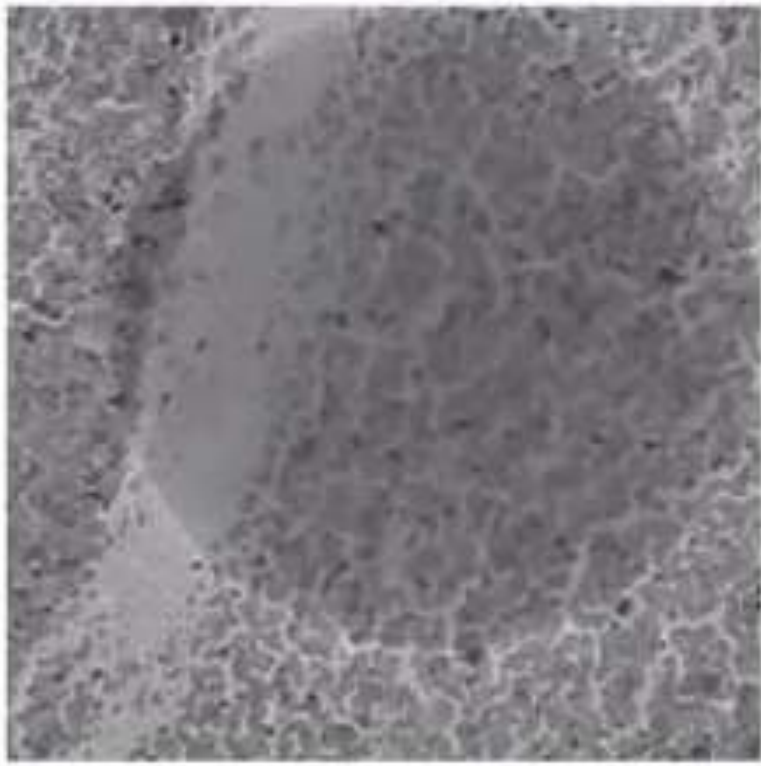
C_s -- The spherical aberration coefficient, defining the quality of objective lens,
λ -- The wave-length,
Δf -- The defocus value,
$|g|$ -- The spatial frequency,
α -- The convergence semi-angle.

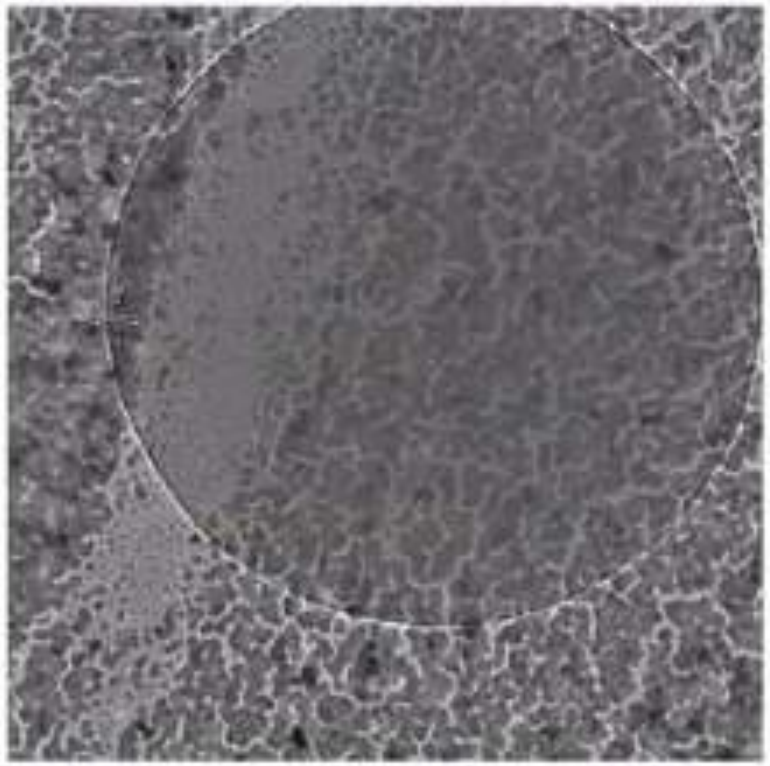
https://www.globalsino.com/EM/page4236.html

FOCUS

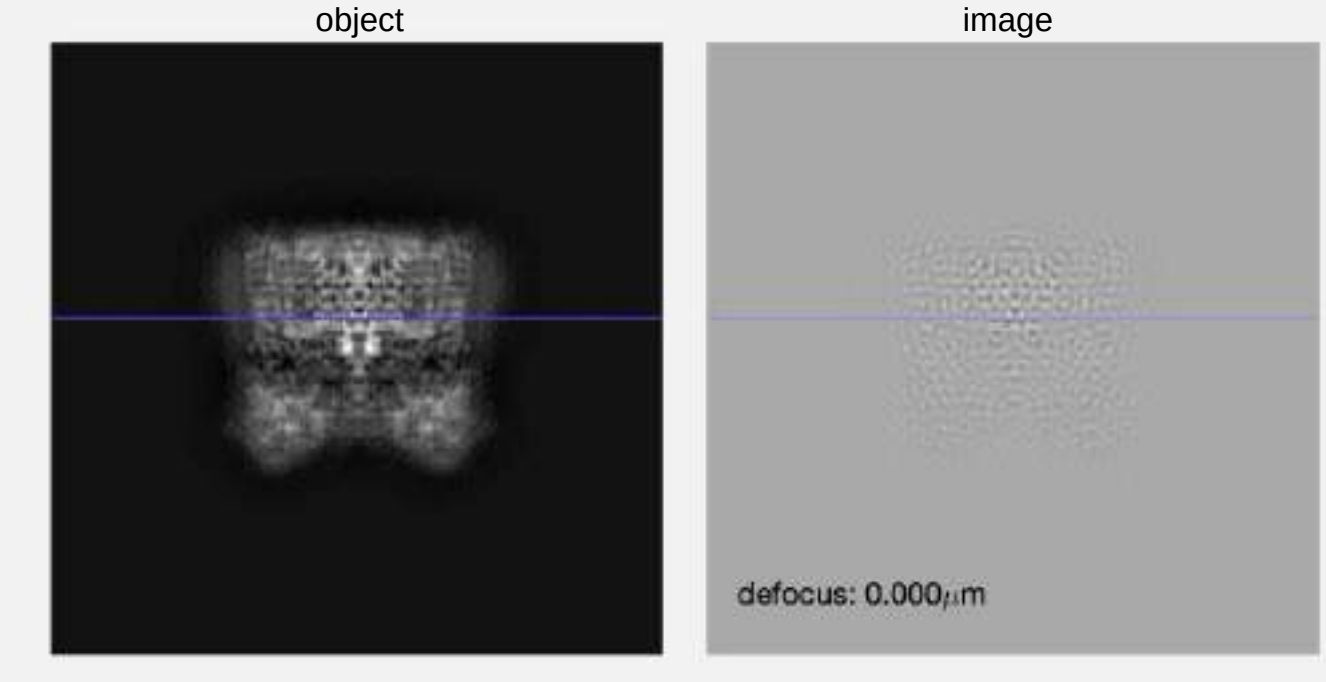








Most cryo-EM data are acquired using defocus contrast

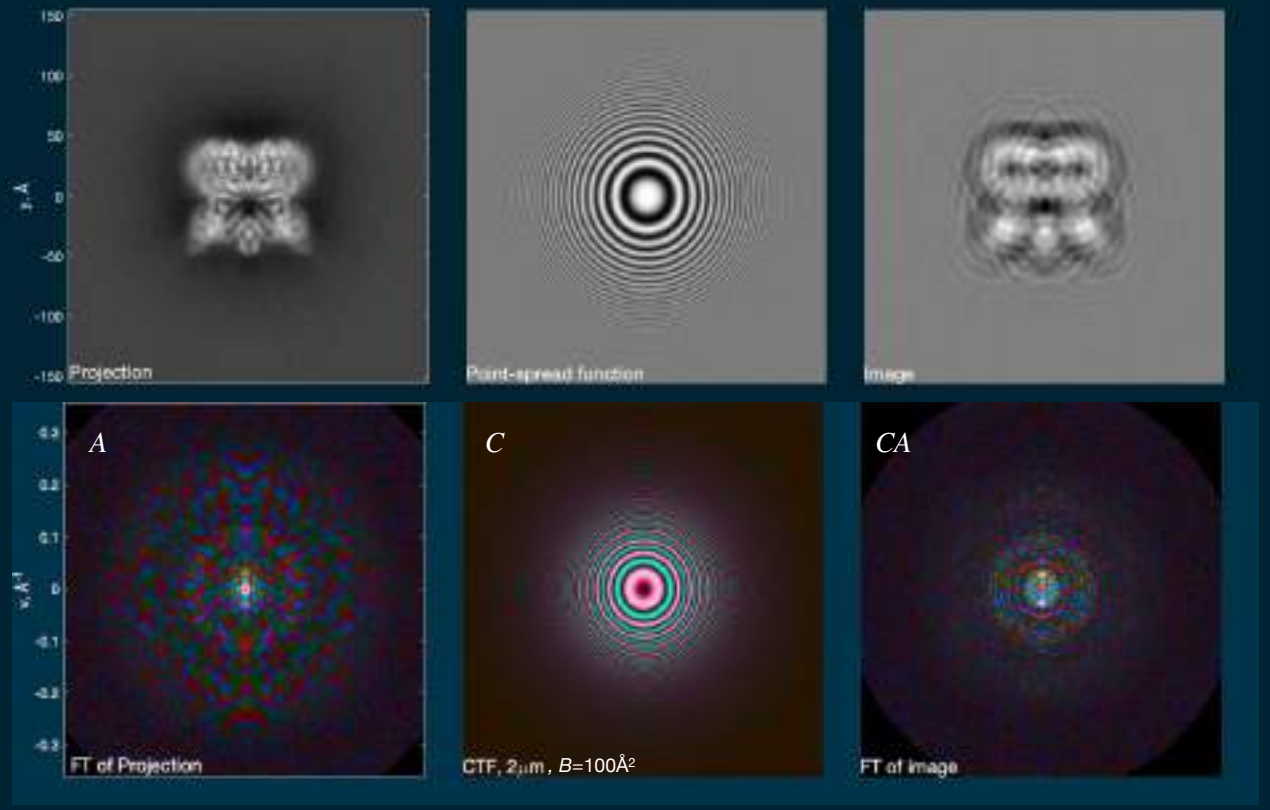


- At high defocus, high-resolution information in the image is strongly delocalized.
- Image processing can re-localize the signals, but at most only about half of the theoretical contrast is preserved by defocusing.
- “Underfocus” means decreasing the strength of the objective lens, effectively focusing above the specimen.

Modeling an image

$X = CA + N$

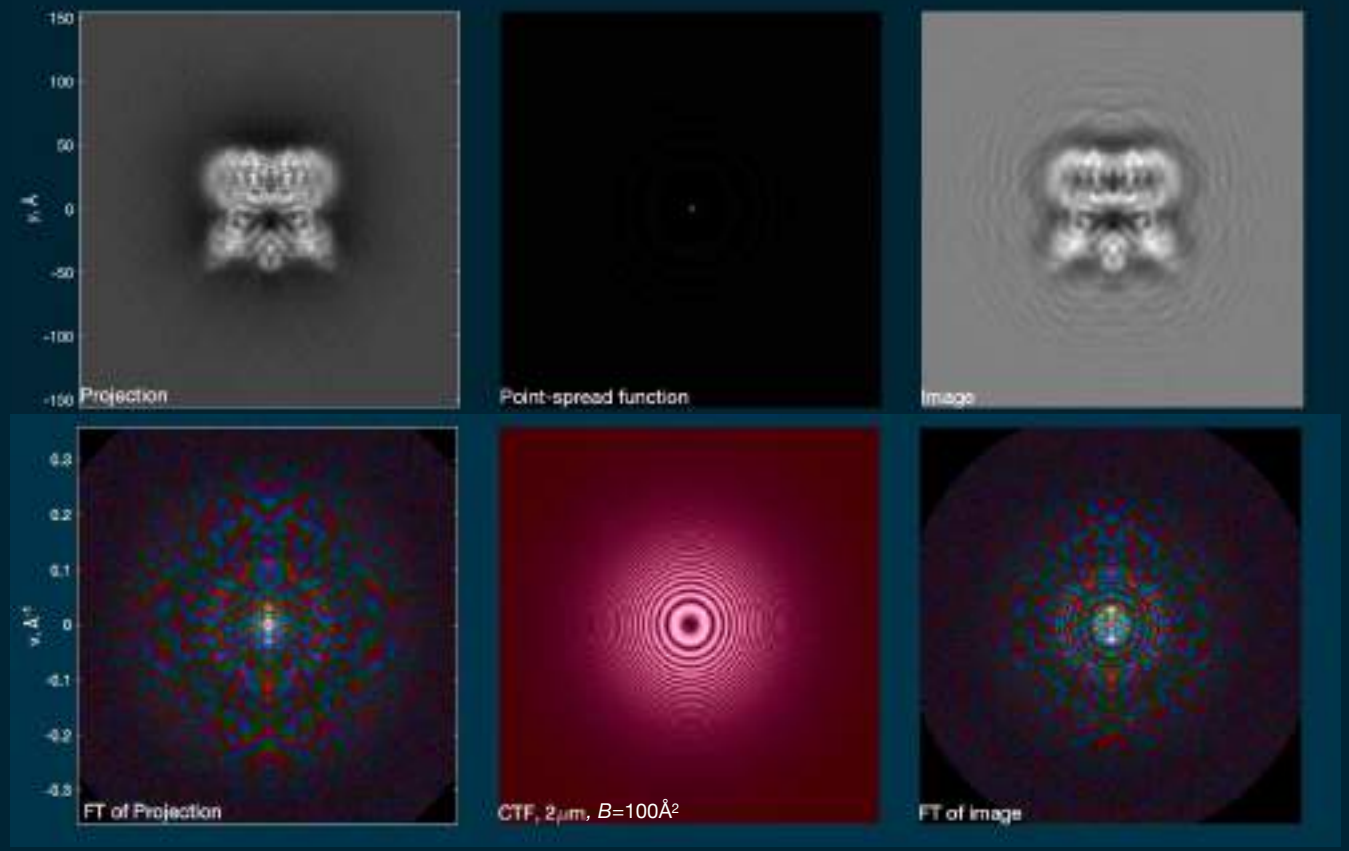
Deconvolution:
$\tilde{A} = X/C$??



How to undo the CTF effects?

1. **Phase flipping**

$$\tilde{A} = \mathrm{sgn}(C)X$$



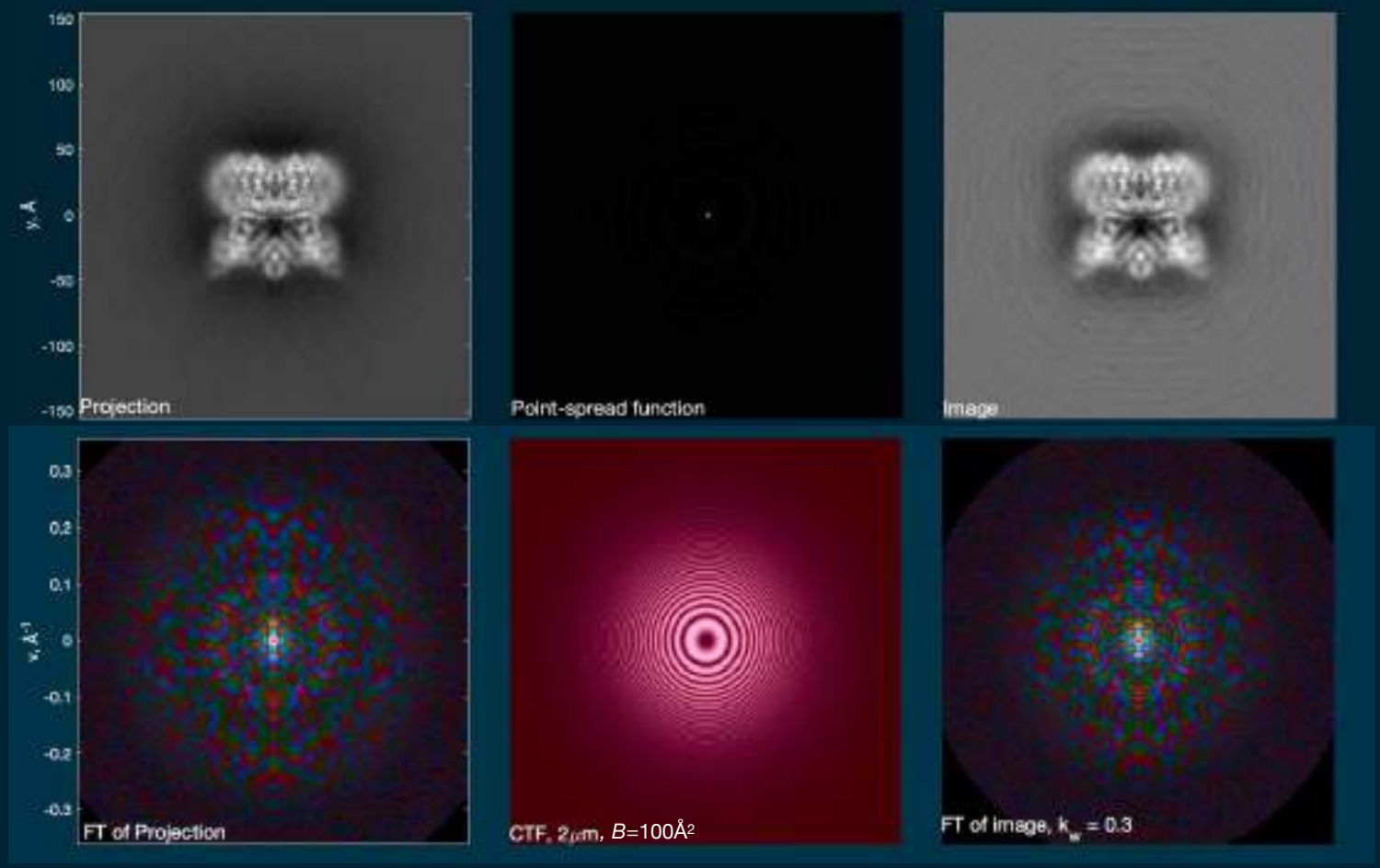
How to undo the CTF effects?

1. **Phase flipping**

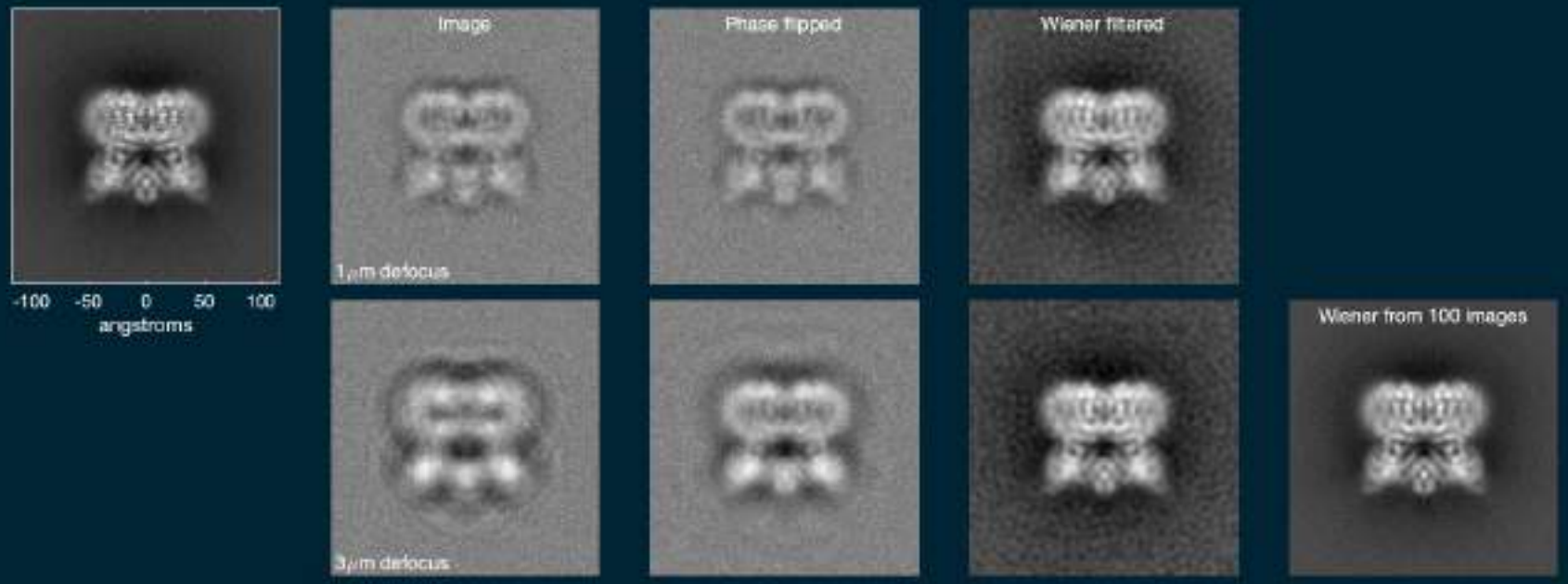
$$\tilde{A} = \mathrm{sgn}(C)X$$

2. **Wiener filter**

$$\tilde{A} = \frac{CX}{C^2 + k}$$

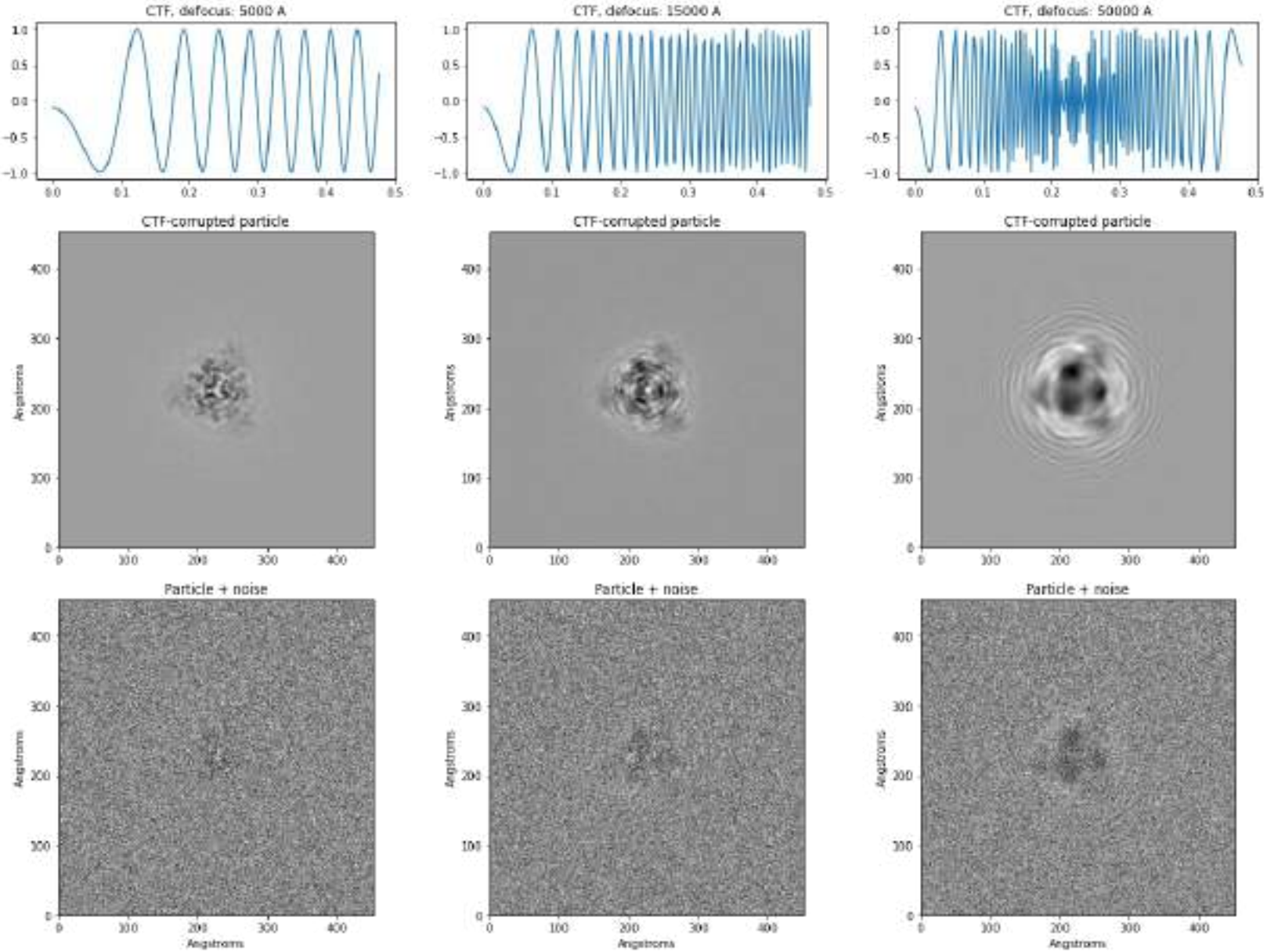


How to undo the CTF effects in noisy images?



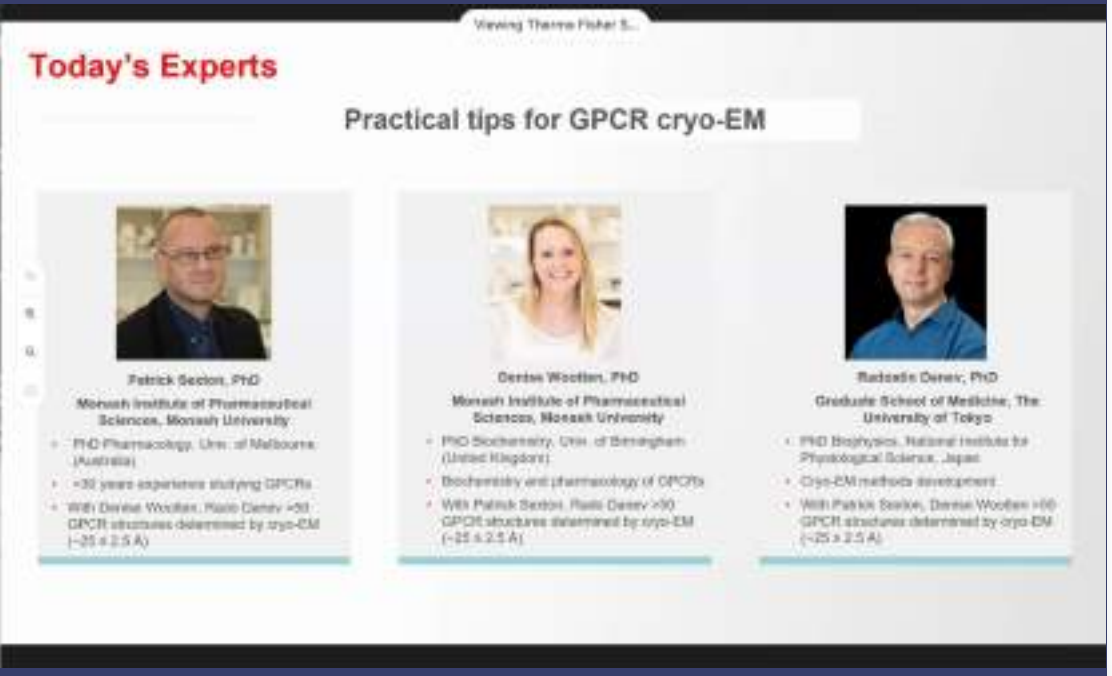
3. Wiener from multiple images

$$\tilde{A} = \frac{\sum_i^N C_i X_i}{k + \sum_i^N C_i^2} \qquad k = 1/\mathrm{SNR} = \frac{|N|^2}{|A|^2}$$

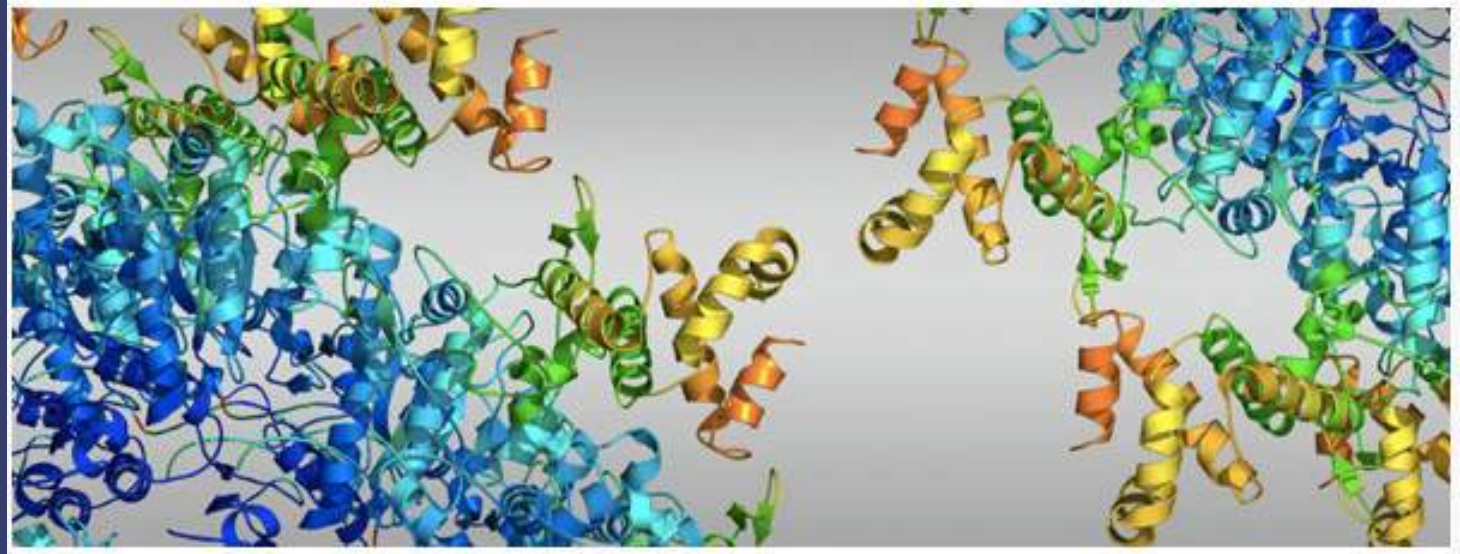


https://guide.cryosparc.com/processing-data/all-job-types-in-cryosparc/ctf-estimation

ROUTINE USE OF SPA



ThermoFisher SCIENTIFIC



Join a discussion on sub-2.5 Å cryo-EM structure determination of GPCRs for drug design

Wed, Sep 9, 2020, 8 p.m. EDT | 5 p.m. PDT | 10 a.m. AEST | 9 a.m. JST

Attend our upcoming *Ask the Experts* Q&A session on routine sub-2.5 Å cryo-EM structure determination of GPCRs for drug design. This rapidly developing field is constantly producing new and exciting biological and pharmacological discoveries. Ask questions and get answers from leading academic investigators in the field.

You'll learn about:

- GPCR biochemistry and purification for cryo-EM
- GPCR sample preparation for cryo-EM
- High-resolution single-particle cryo-EM imaging and 3D reconstruction of GPCRs

TIPS AND TRICKS FOR

Viewing Thermo Fisher S...

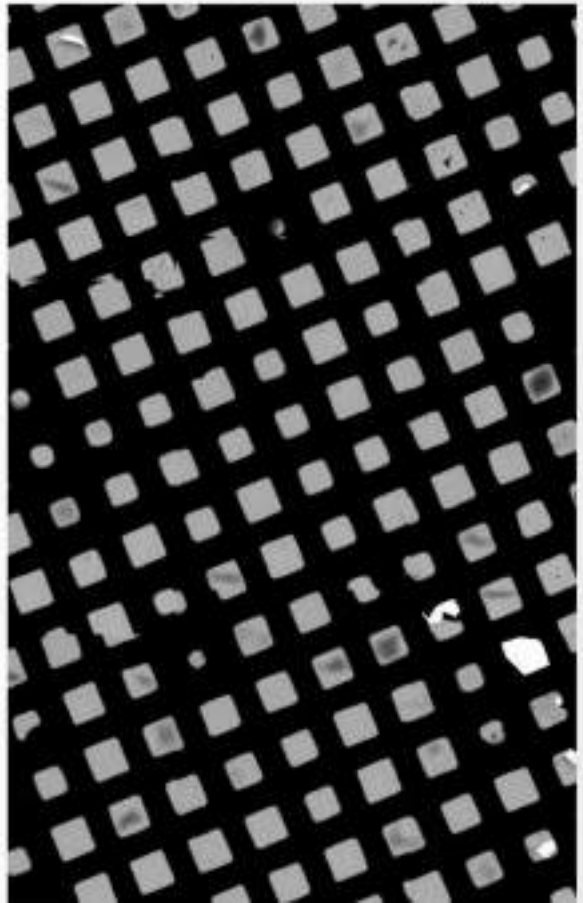
Cryo-EM sample preparation

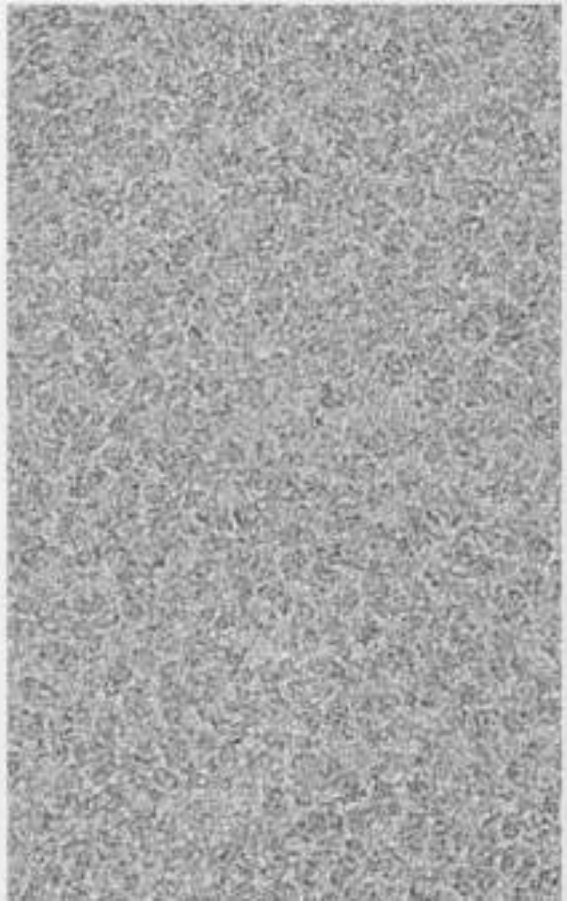
MONASH University

東京大学 THE UNIVERSITY OF TOKYO

- **The quality of the cryo-EM sample governs the outcome of the experiment!**

- We optimized the plunging parameters for ice thickness consistency and grid coverage
- Our blot time is relatively long: 10 s
- For every new sample, depending on the initial concentration, we prepare 2-3 grids with 2x dilution in-between
- GPCR sample concentrations in the range 3 – 7 mg/ml work best.
- Avoid as much as possible lower concentrations!
- Gold foil grids (UltrAuFoil) improved the consistency of getting uniformly thin ice and reduce beam-induced motion.





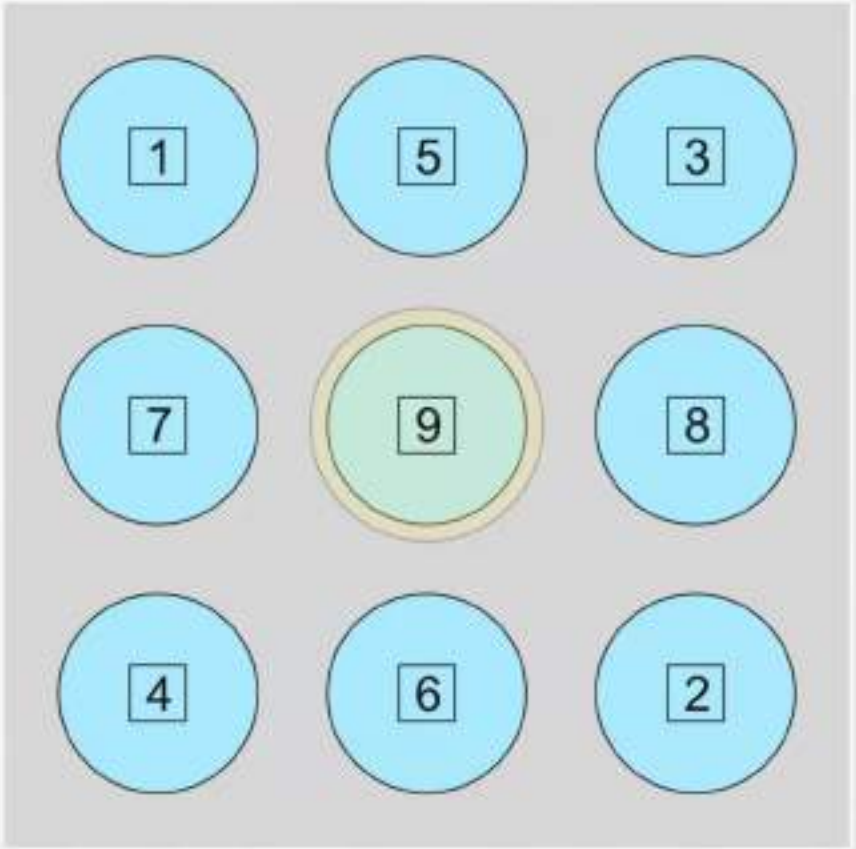
DATA COLLECTION

Viewing Thermo Fisher S...

Cryo-EM data acquisition strategy

- **Collect on the thinnest possible ice that still has good particle coverage !**

- We used 3 x 3 = 9-hole beam-image shift data acquisition scheme, 1 image/hole, realized with home-made scripts in SerialEM
- Defocus range: 0.5 – 1.5 µm. Start at the high end on the first hole and reduce the defocus step-wise for each hole in the pattern, e.g. 1.4 → 1.3 → 1.2 → … → 0.6
- Use an energy filter with <15 eV slit
- Do not use super-res (K3), select pixel size ≈ 1/3 the resolution you are hoping to get. Use EER with Falcon 4.
- Throughput: 1 sample/day ≈ 1 structure/day; ~5,500 movies
- Collect non-gain-normalized compressed TIFFs/EER. prepare your own gain reference with Relion



WHAT FACTORS MATTER

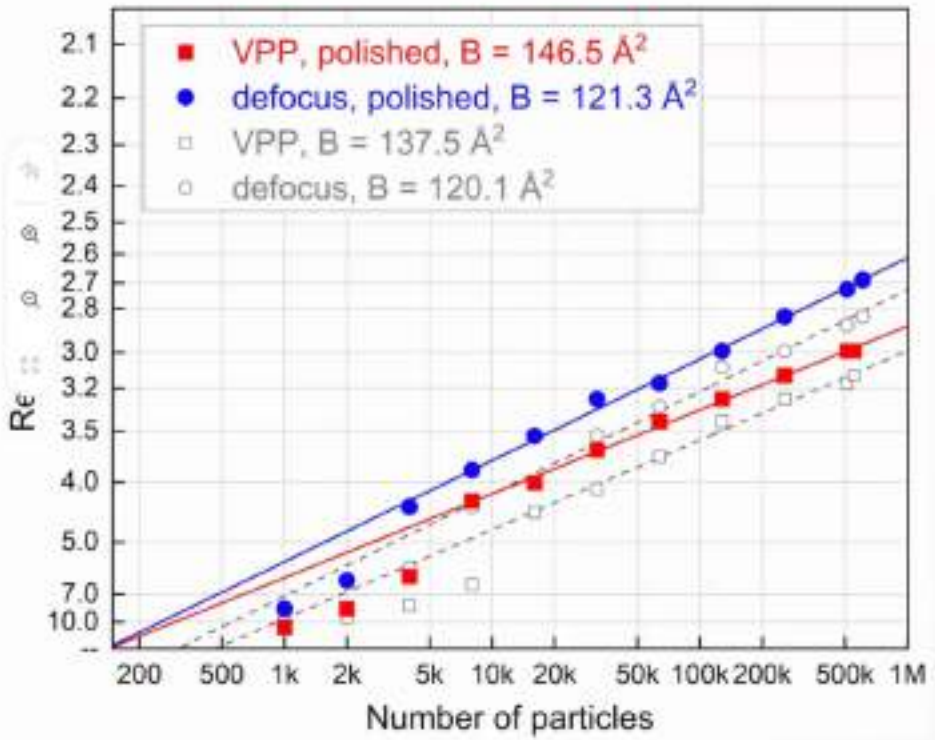
Viewing Thermo Fisher S...

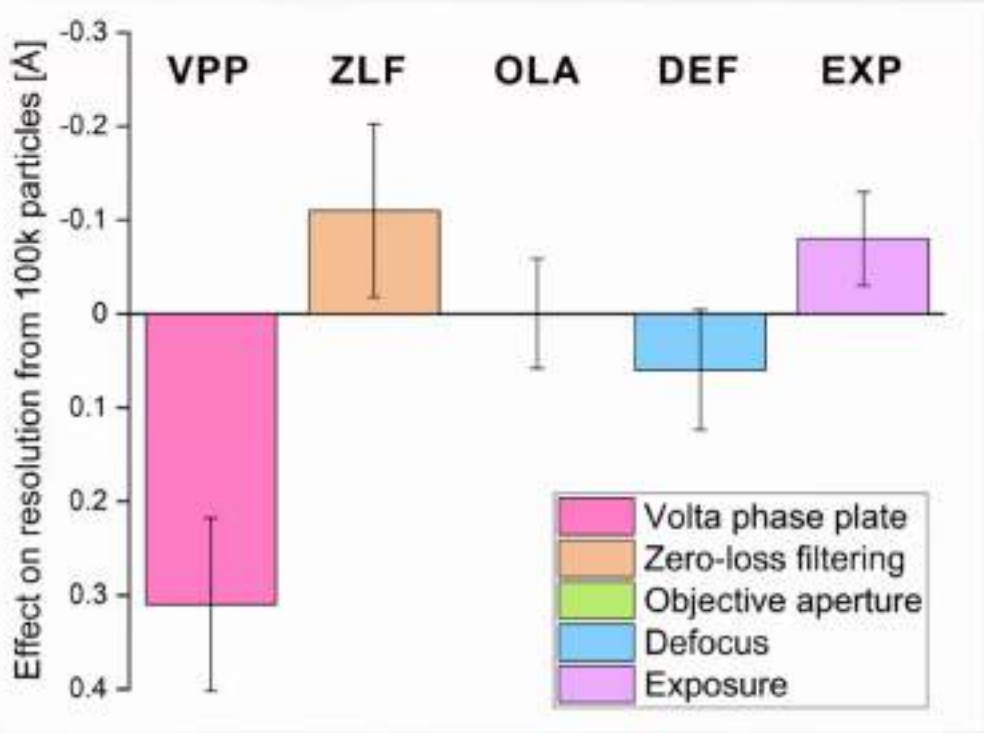
MONASH University

東京大学 THE UNIVERSITY OF TOKYO

Performance factors

- Do not use VPP; use zero-loss filtering; defocus <1.5 µm; total exposure ≥60 e/Å²





IMPACT OF GRIDS