

2026 SPA short course

Anatomy of an EM

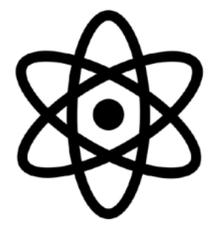
March 17, 2026



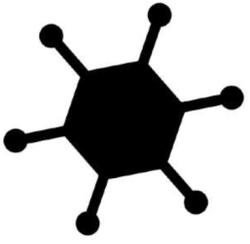
NYSBC SEMC



Illustration by David S. Goodsell
RCSB Protein Data Bank.



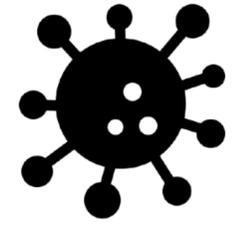
atoms
1 Å



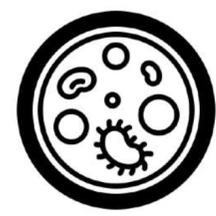
Small molecules
1 nm



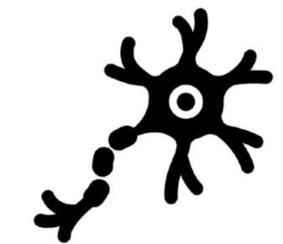
Biological macromolecules
10-100 nm



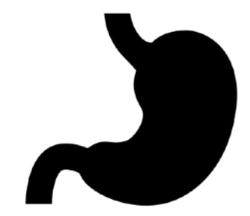
Organelles
prokaryotic cells
0.1-5 μm



Eukaryotic cells
5-100 μm



Neurons
mm



organs & organisms
cm-m

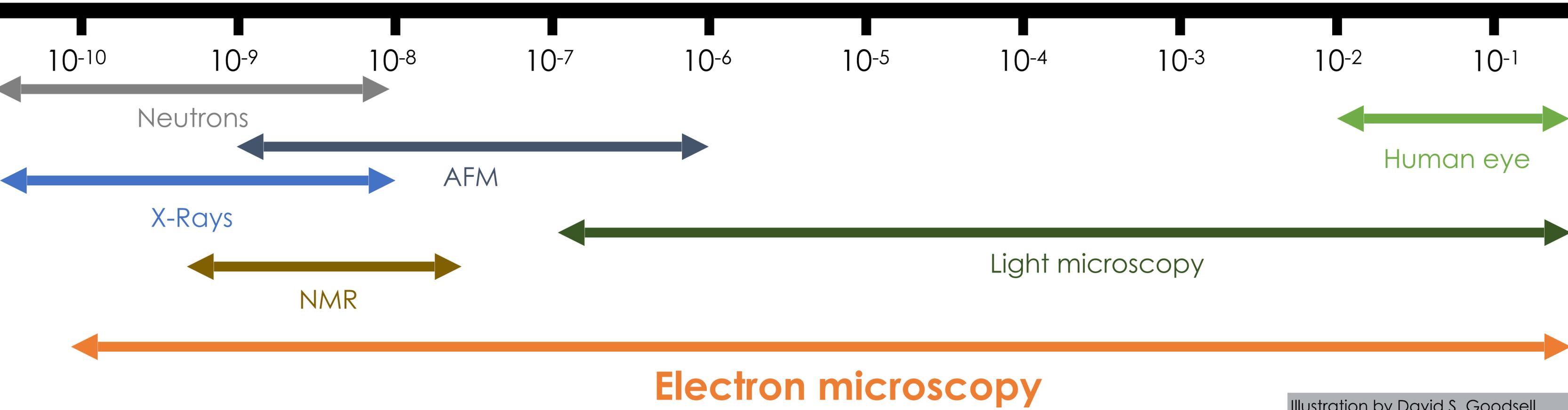
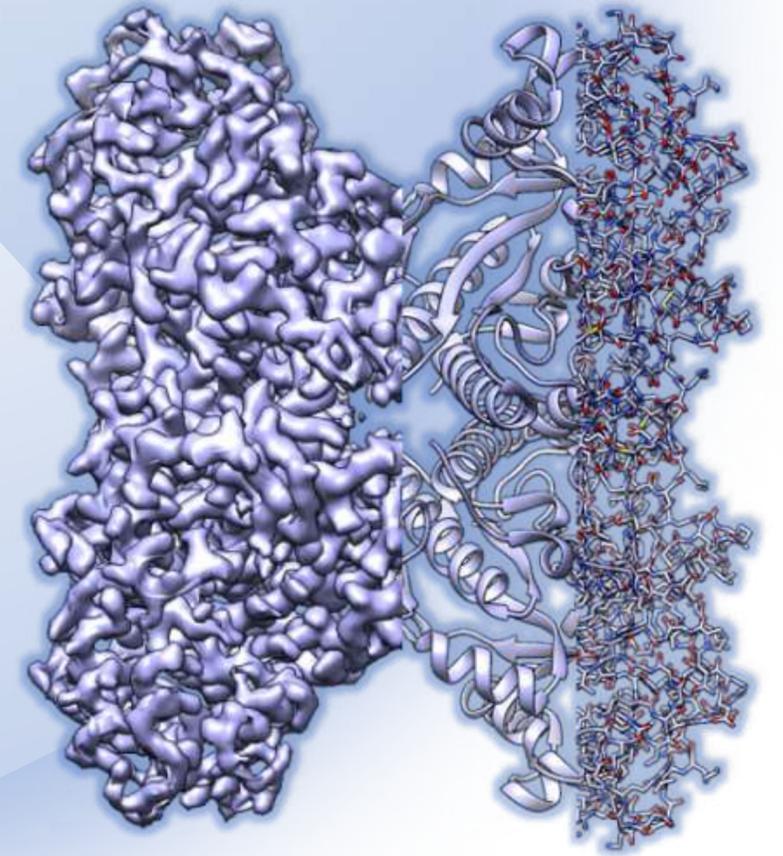
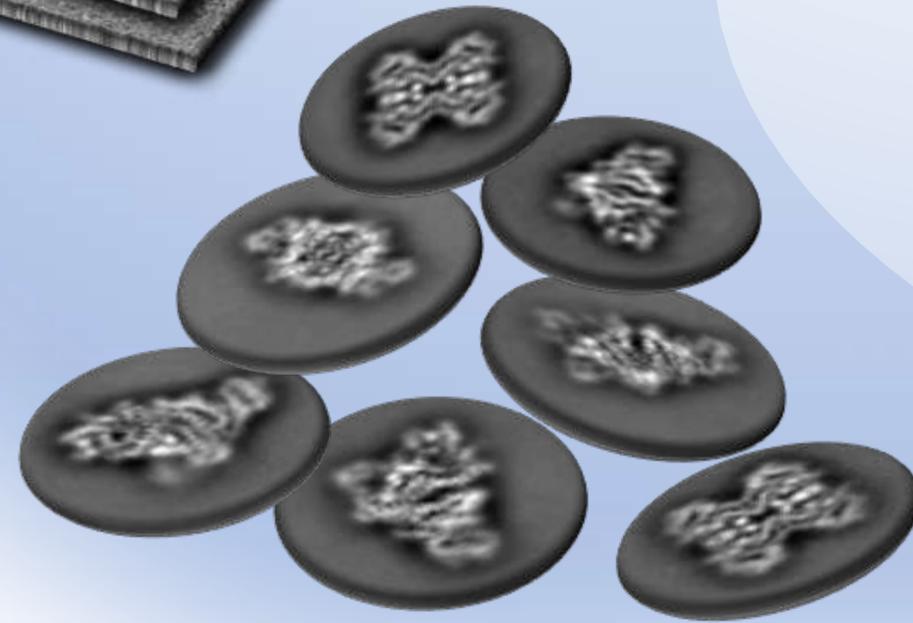
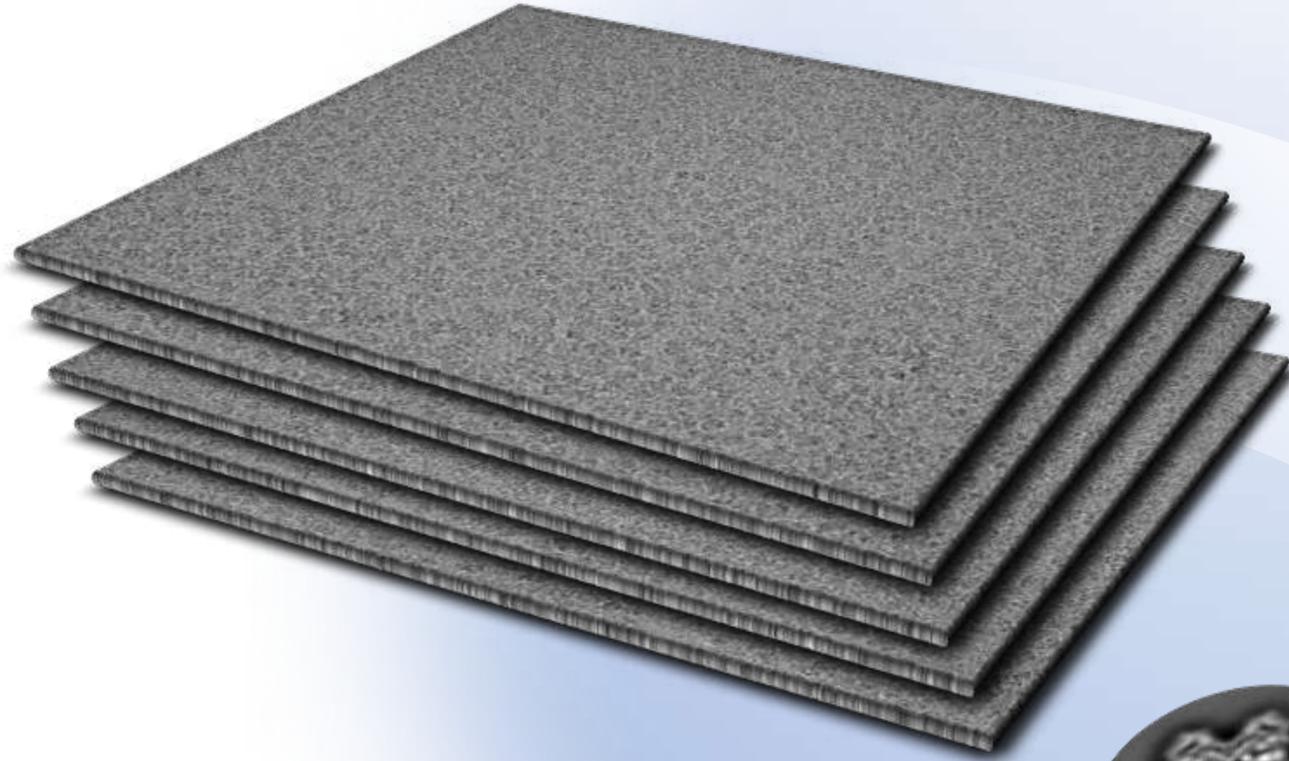


Illustration by David S. Goodsell
RCSB Protein Data Bank.

What is possible today?



What brought about the resolution revolution?

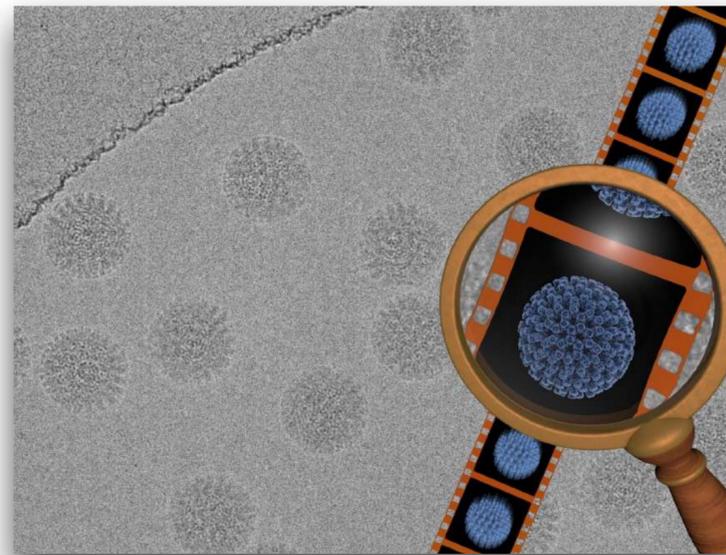
(~2012-2014)

Hardware

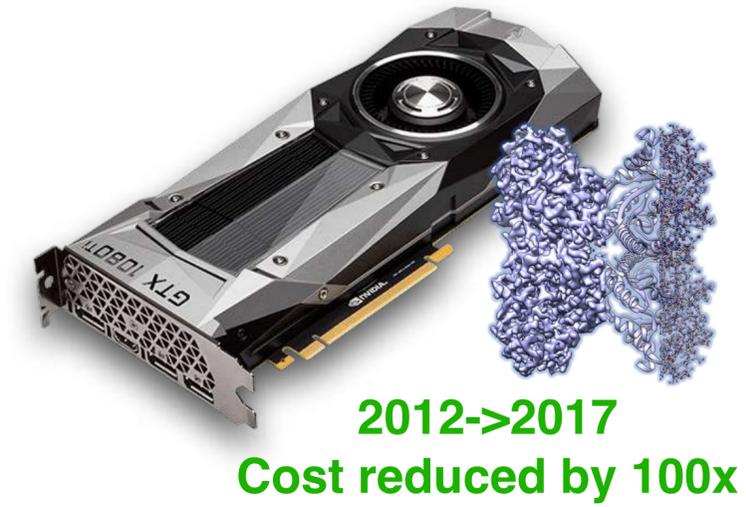
Microscopes



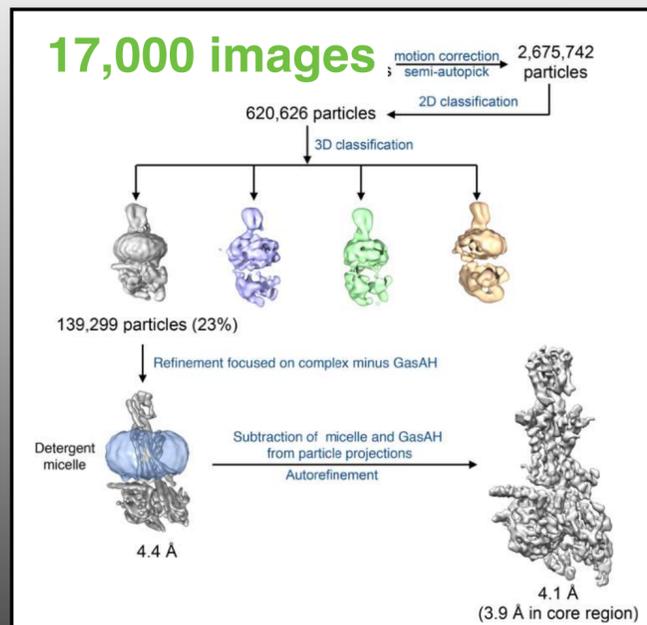
Direct Detectors



Computers



Software



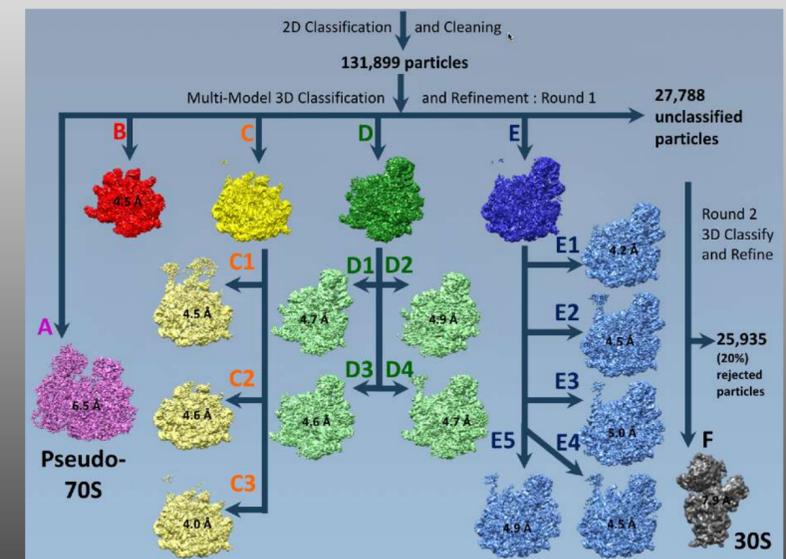
5% used in map!

Leginon / SerialEM / EPU, ...

MotionCorr2, Unblur, ...

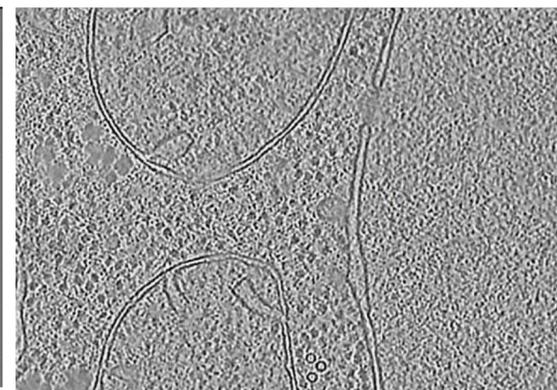
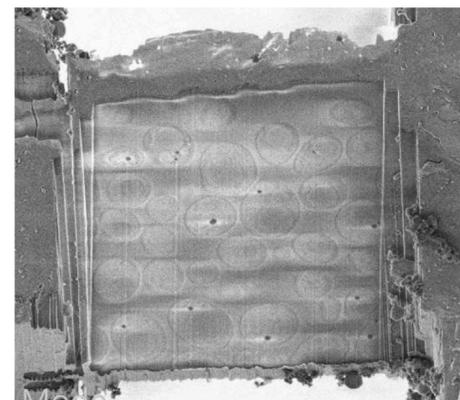
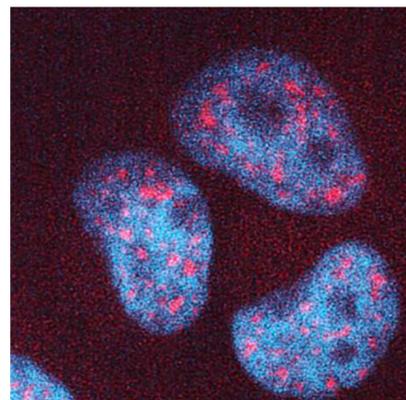
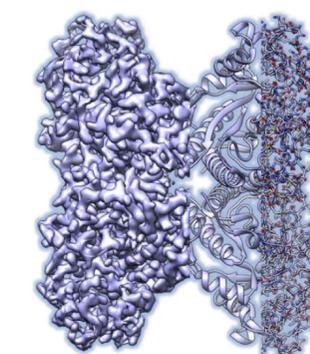
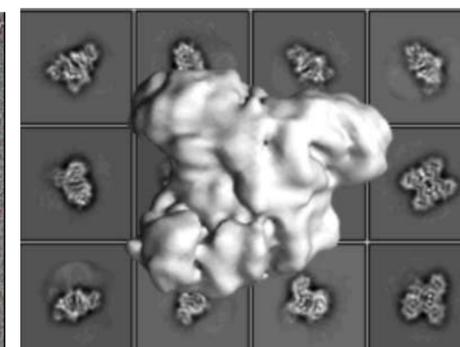
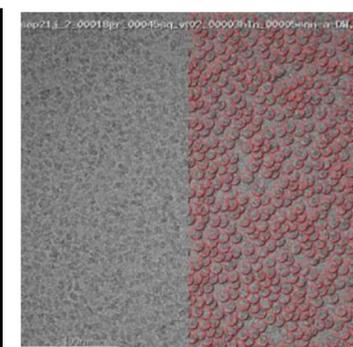
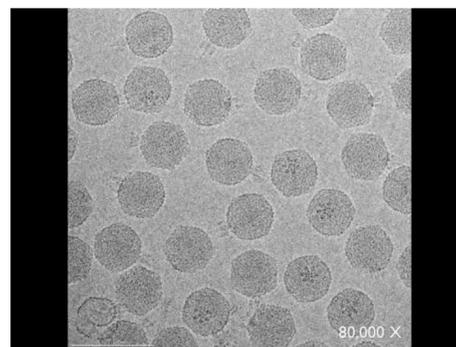
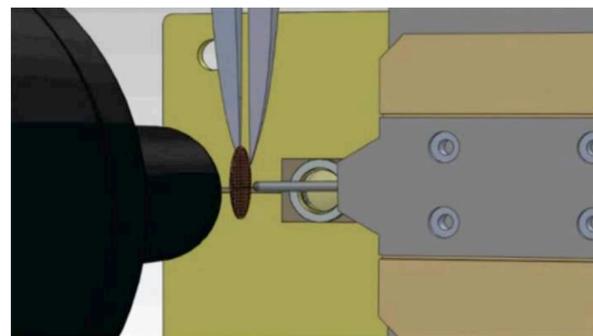
RELION, FREALIGN/cisTEM, cryoSPARC
EMAN, Sparx, SPHIRE, XMIPP, ...

14 independent structures



cryoEM: a technology on the rise

Single particle cryoEM

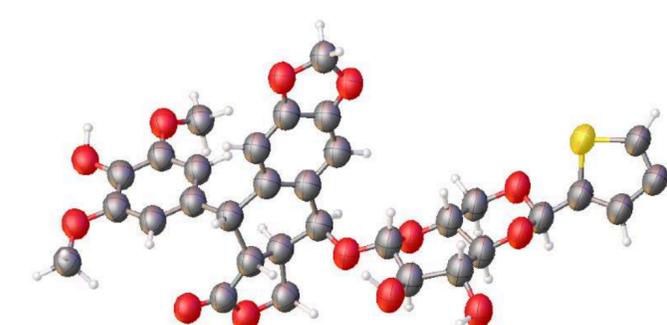
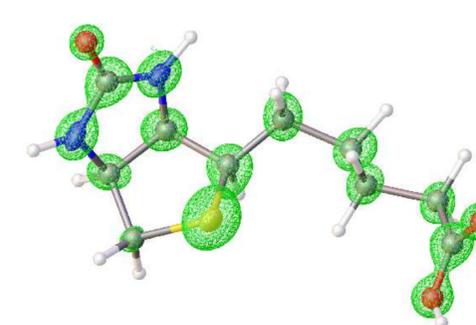
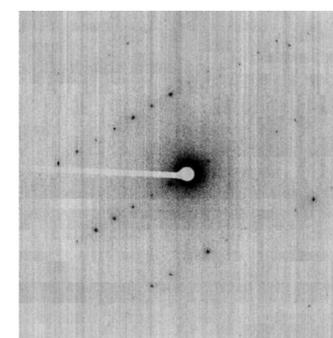
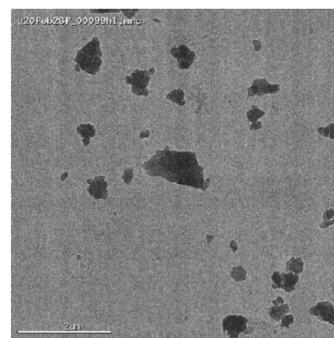


Cryo Electron Tomography (cryoET)

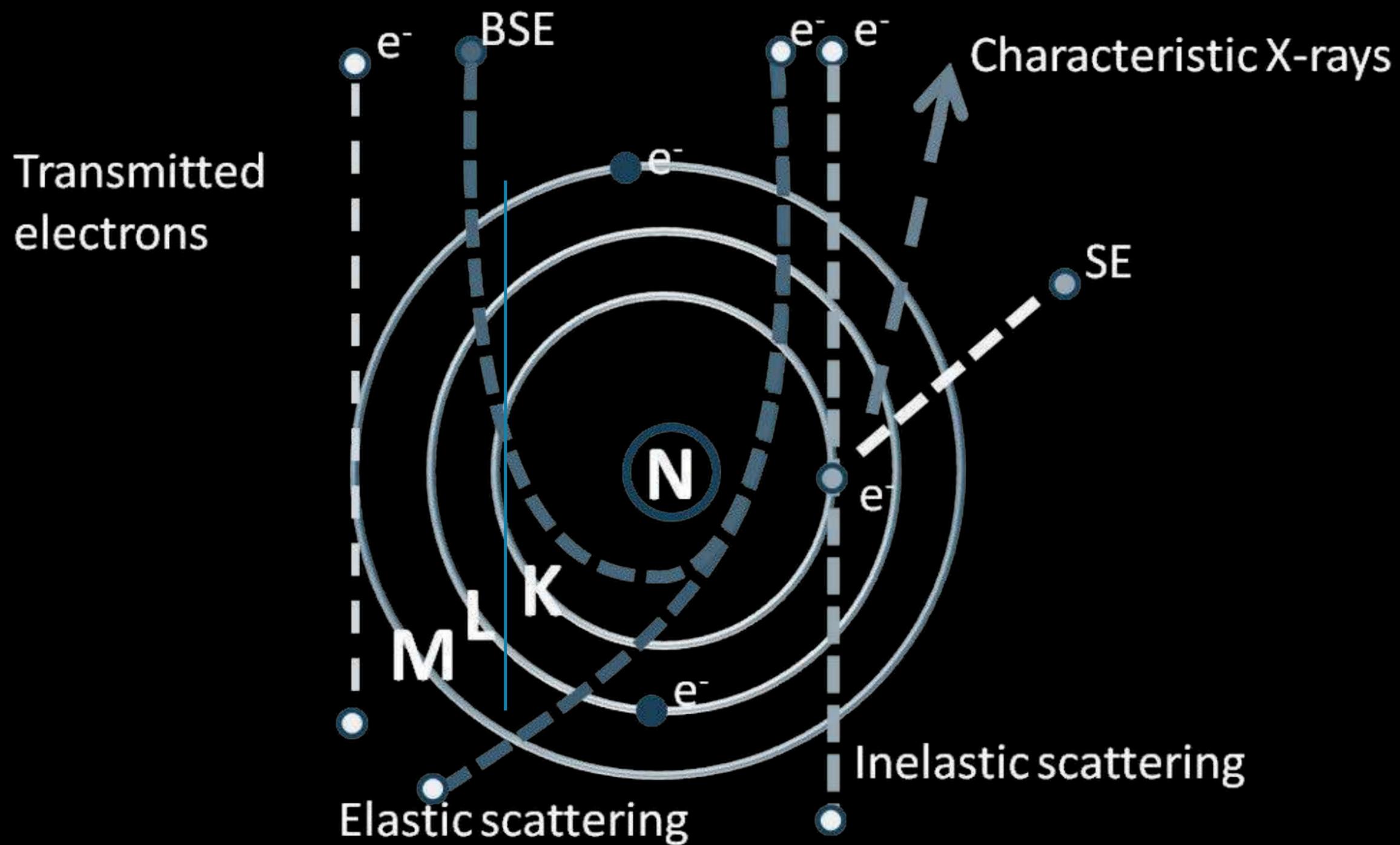
Micro crystal electron diffraction (microED)



2 μm



Why electrons?



● Main beam electrons

Why electrons?

Pros

Small wavelength

Can be focused

Cons

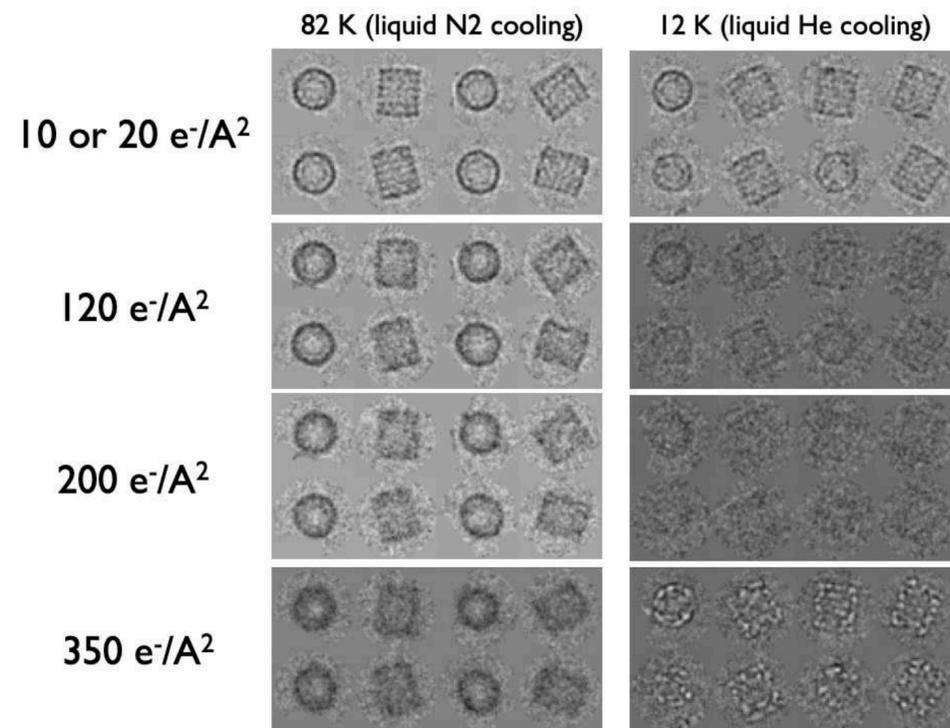
Damages sample
worse with faster electrons

Poor penetration
better with faster electrons

Why electrons?

Ideal dose for cryoEM?

Radiation damage



<https://cryo-em-course.caltech.edu/>

Specimen Behavior in the Electron Beam

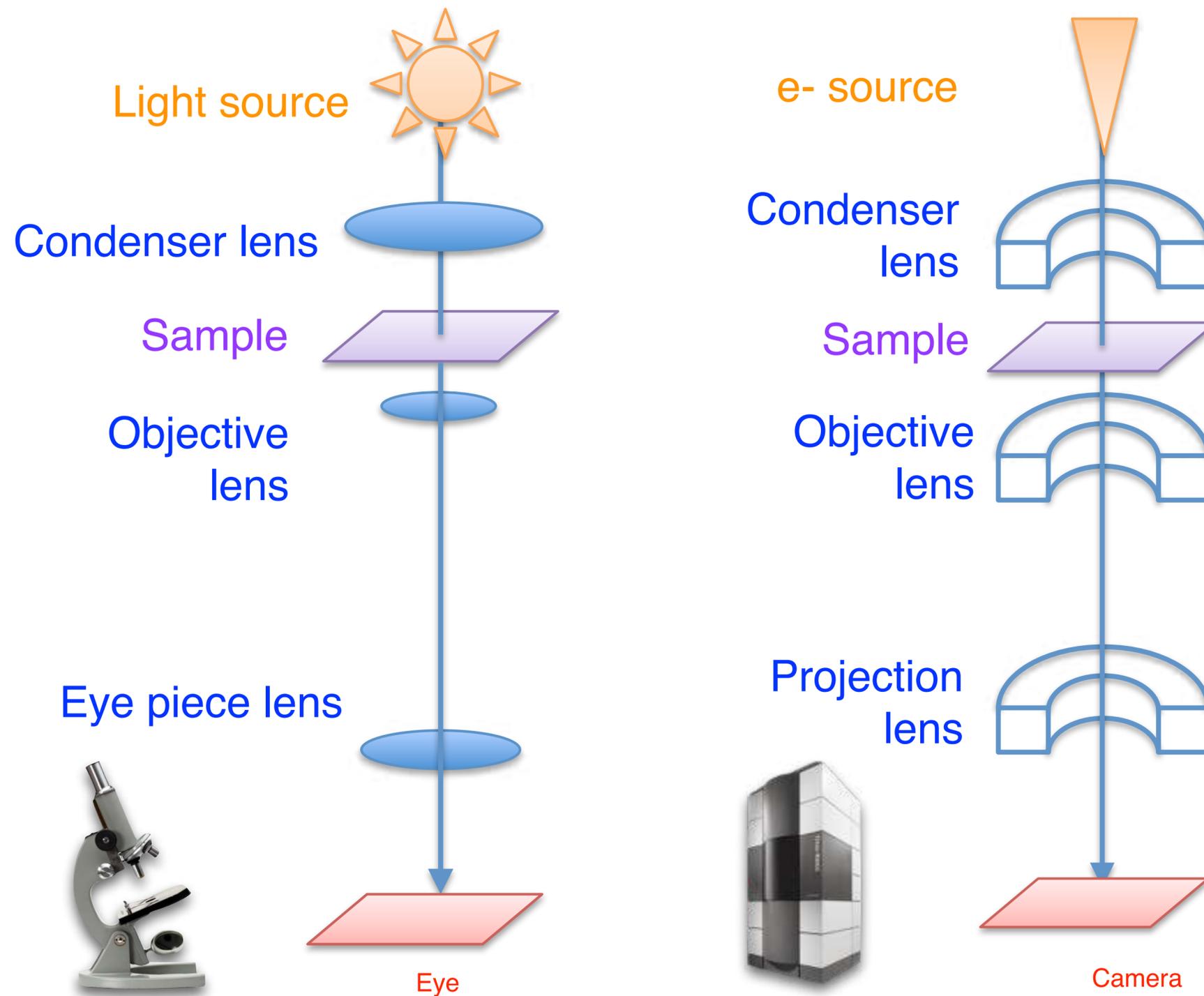
R.M. Glaeser¹

Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, United States

¹Corresponding author: e-mail address: rmglaser@lbl.gov

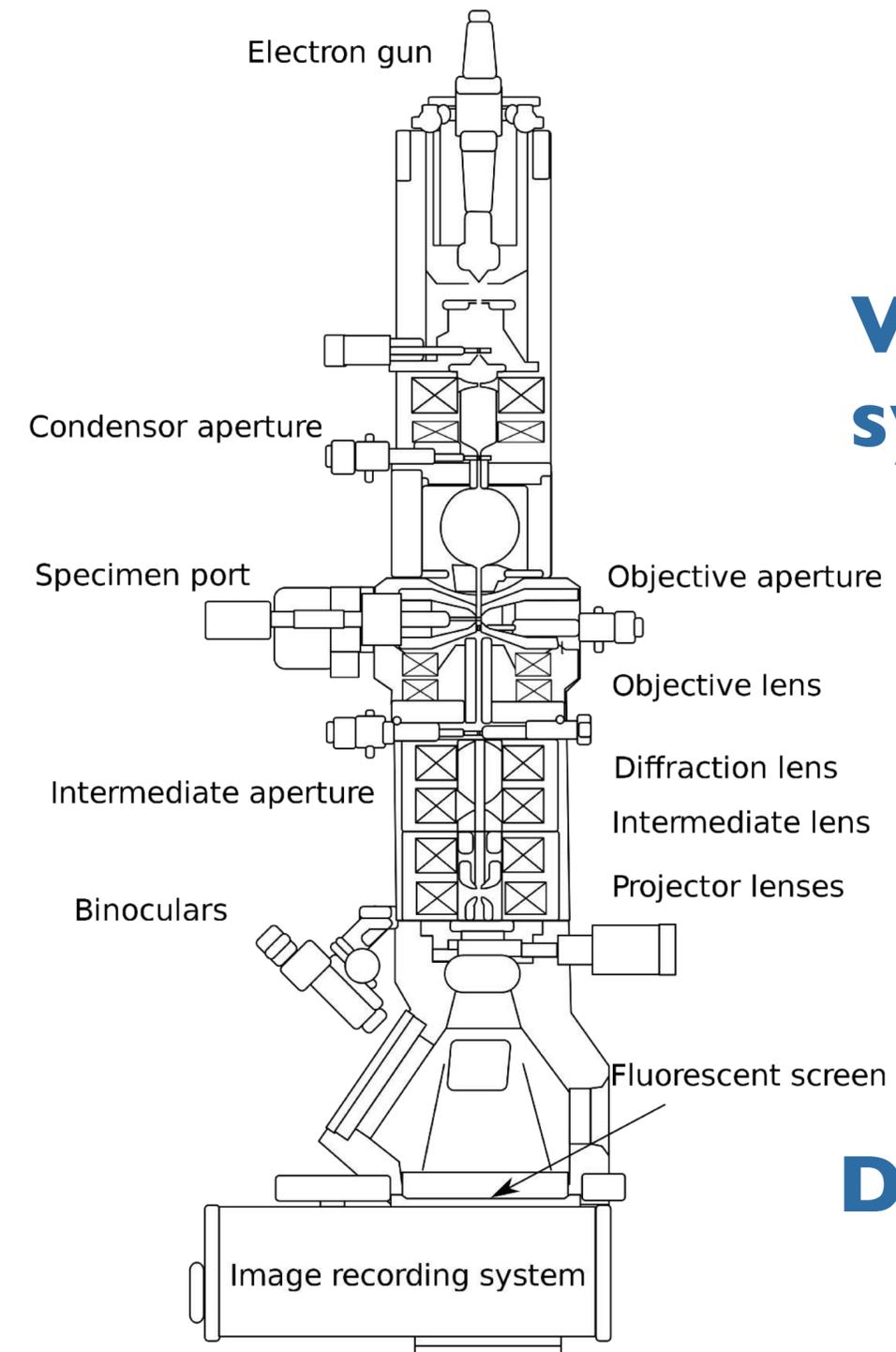
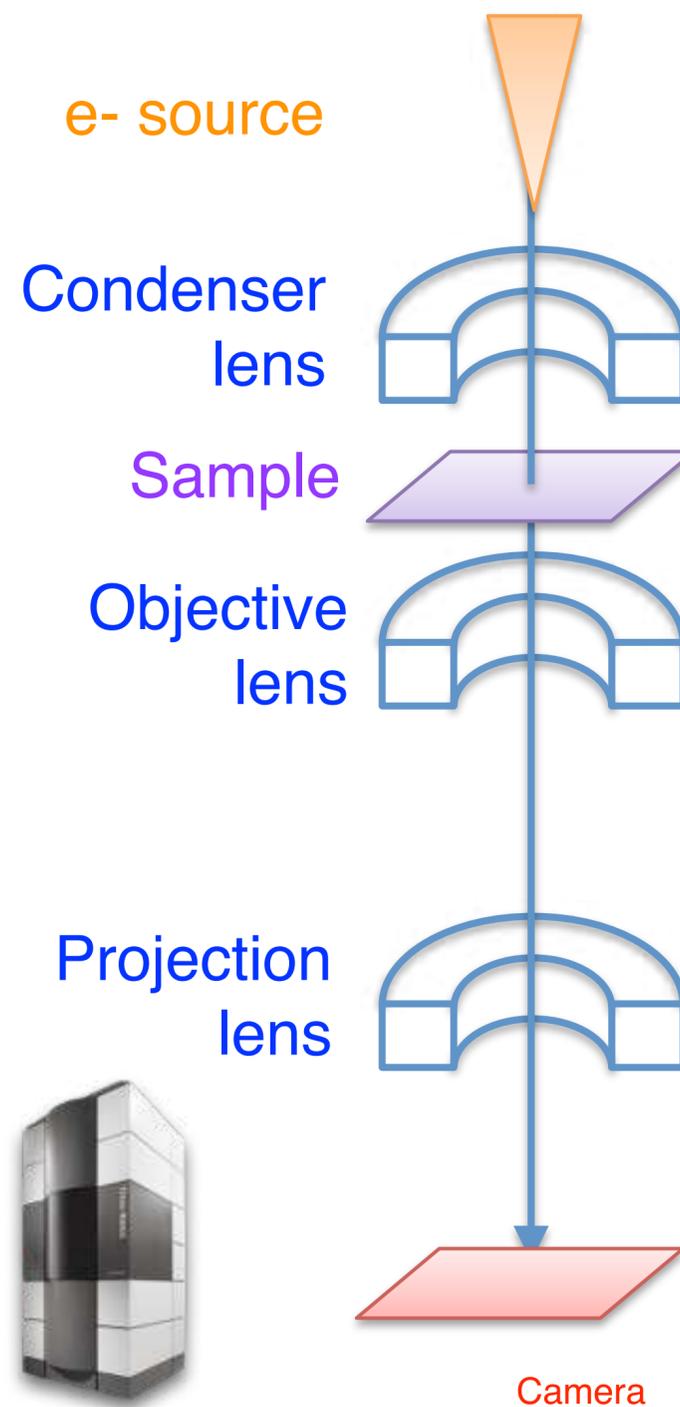
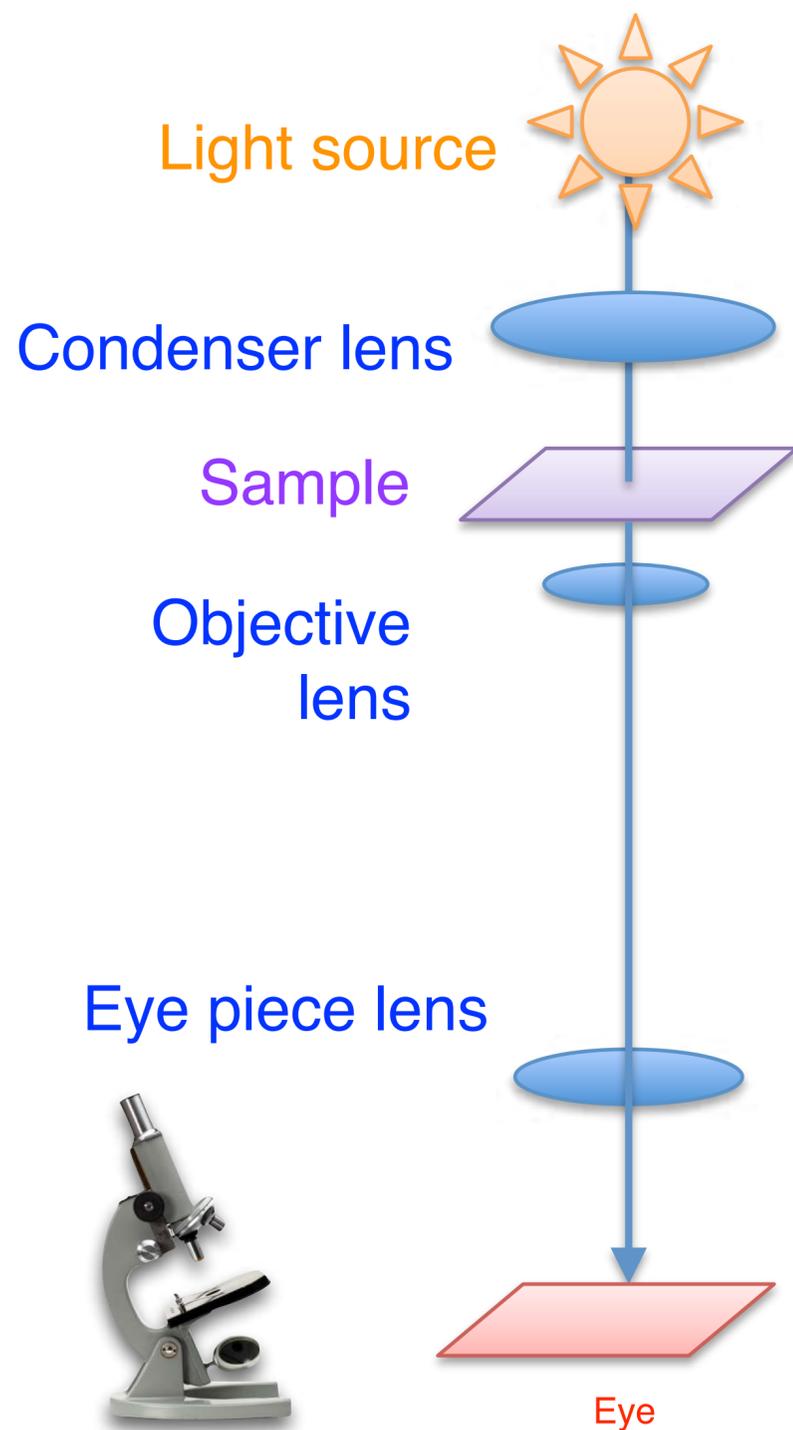
- The first noticeable bubbles appear after the accumulated exposure (for 300 keV electrons) is approximately 150 e/A . At this high exposure, high-resolution features would long since be destroyed, of course, but the macromolecular particles might still be visible.

The electron microscope



Light microscope	Transmission electron microscope
Visible light	Electrons
Glass lenses	
Electron-magnetic lenses	
450-650 nm	3.70 pm (100 keV) 2.51 pm (200 keV) 1.96 pm (300 keV)

The electron microscope



Electron sources



Vacuum systems



Lenses



Detectors



The electron microscope

- e- gun** produces e-
- accelerator** accelerate e- to high energy
- condenser** control illumination on sample
- objective** sample and main imaging lens
- Intermediate projection** controls mag and image/diffraction mode
- Flu-screen** Flu-screen image via camera
- TEM camera** TEM detector

e- source

Condenser lens

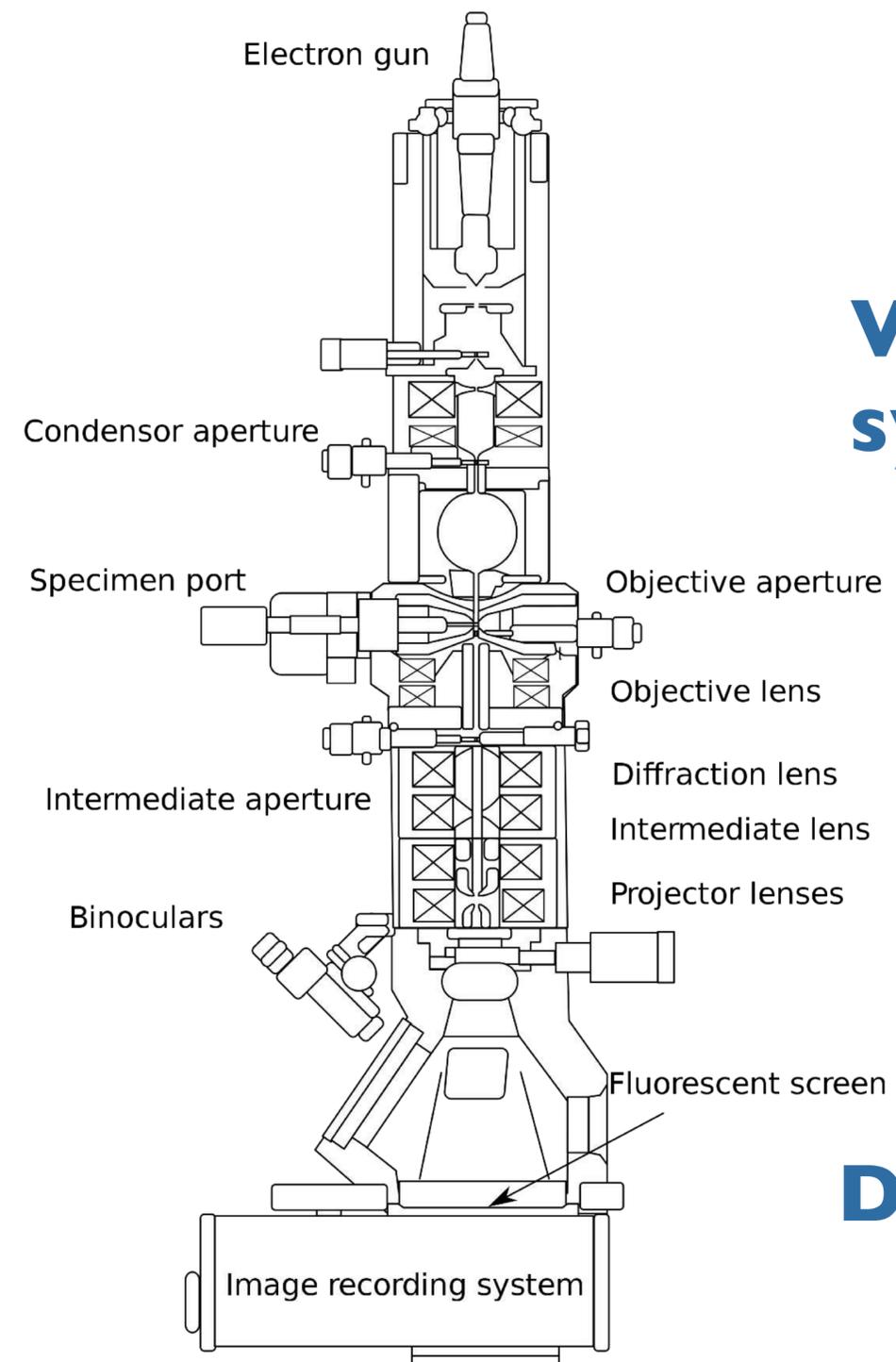
Sample

Objective lens

Projection lens

Eye

Camera



Electron sources



Vacuum systems



Lenses



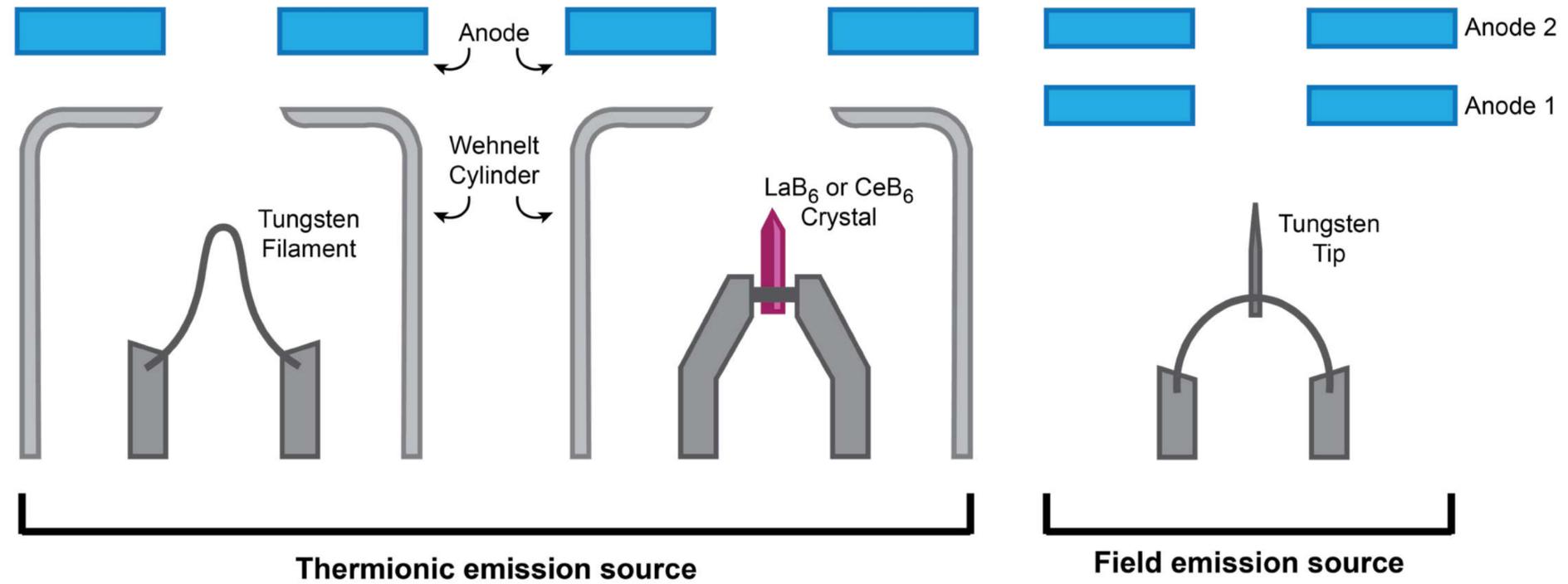
Detectors



Electron sources

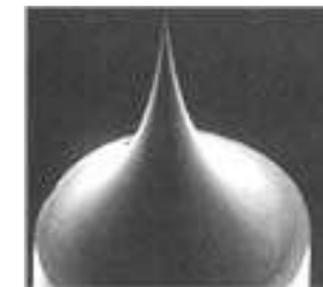
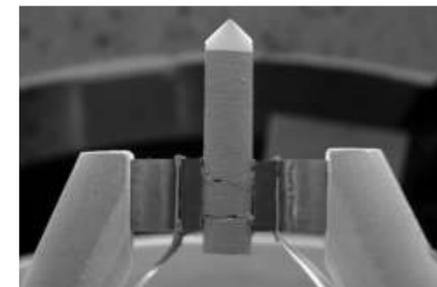
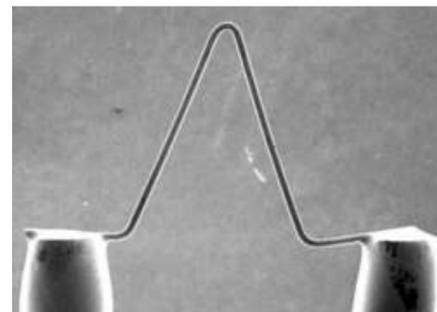


What are the 3 main kinds of electron sources?



www.thermofisher.com

nanoscience.com

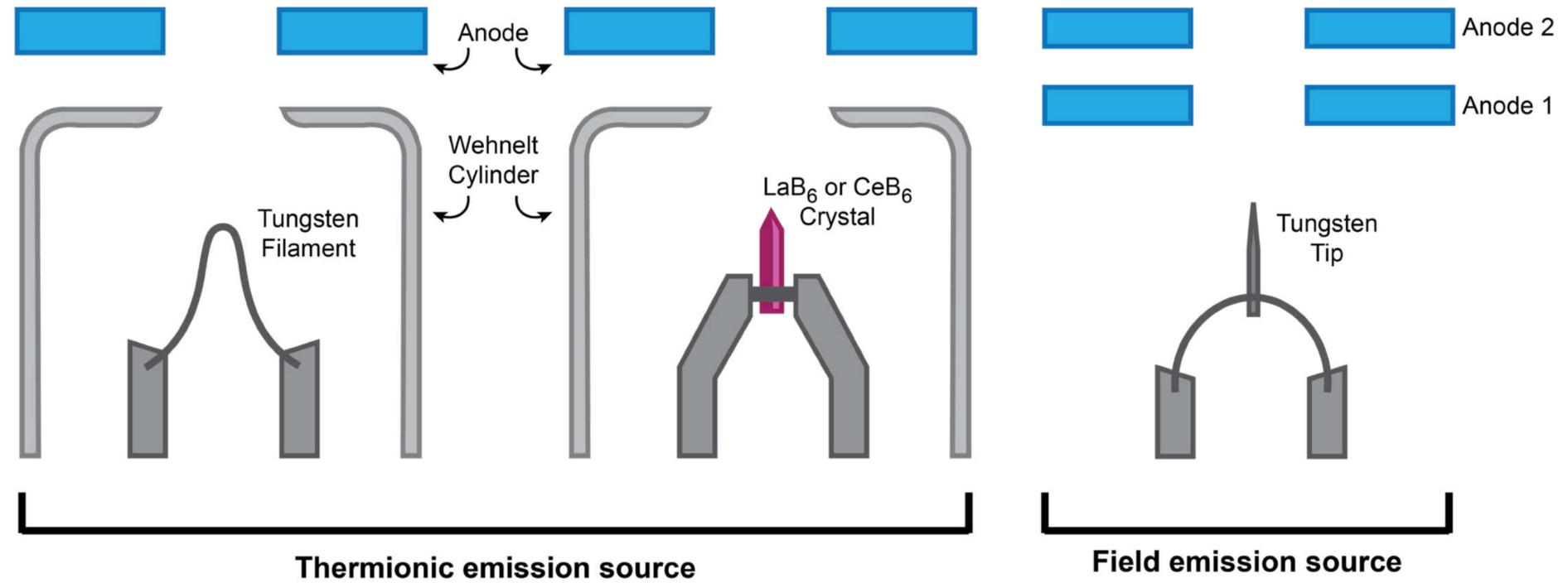
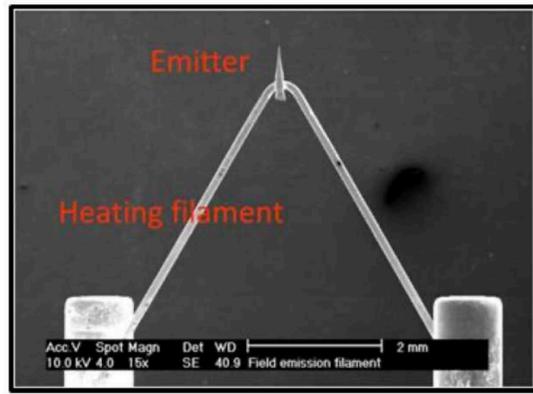


Electron sources

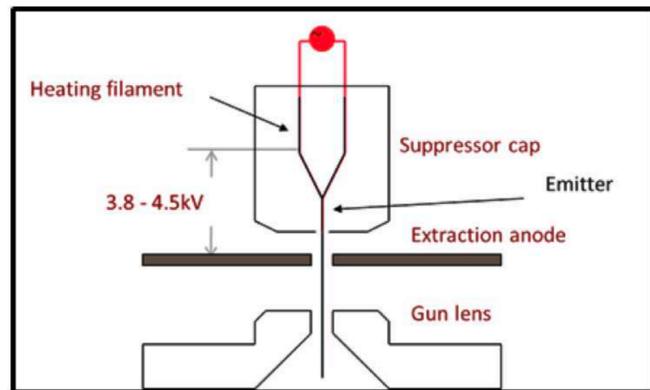


What are the 3 main kinds of electron sources?

Thermionic

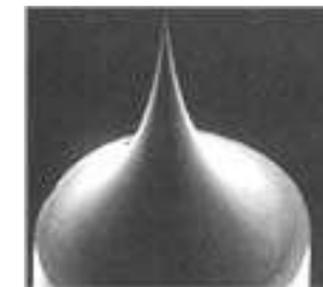
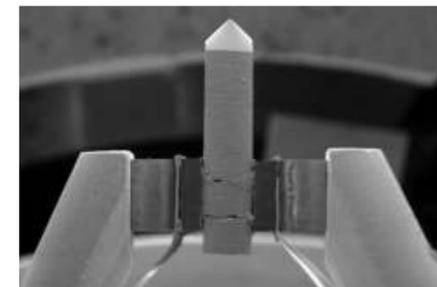
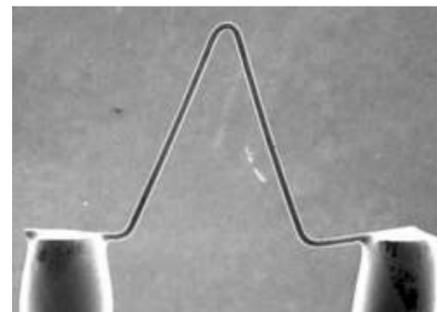


Field Emission Gun (FEG)



thermofisher.com

nanoscience.com



www.thermofisher.com

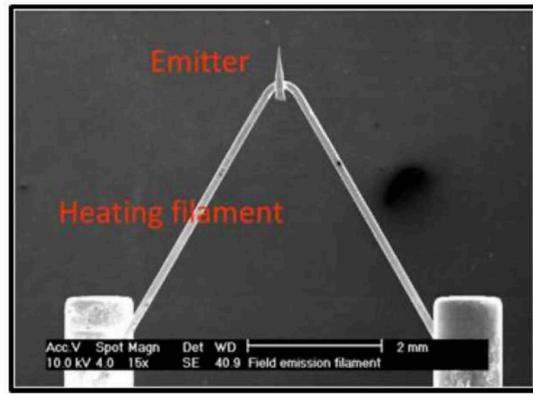
X-CFEG is 1.0×10^8 A/m² sr V

Electron sources

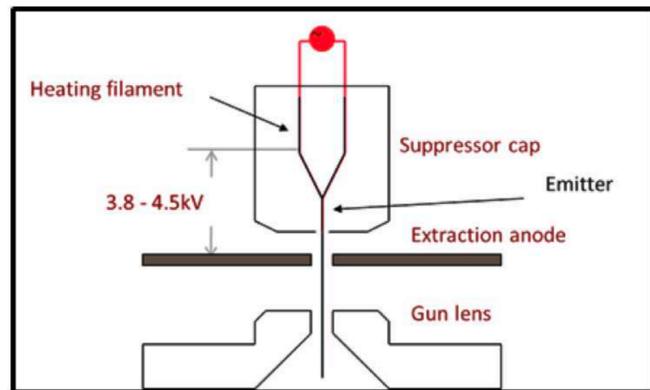


What are the 3 main kinds of electron sources?

Thermionic



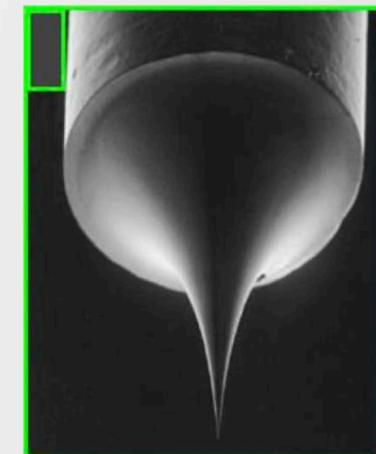
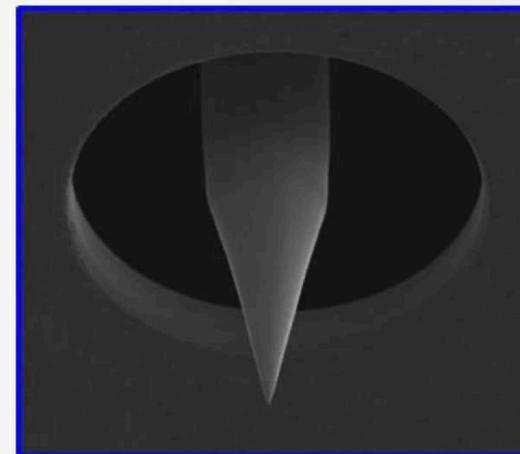
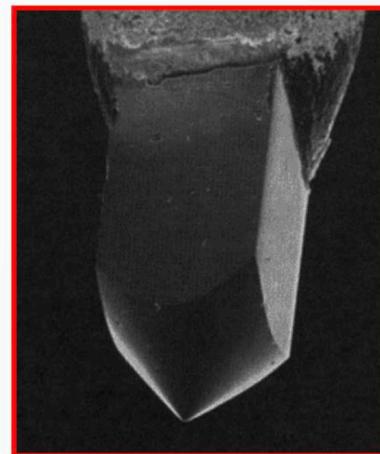
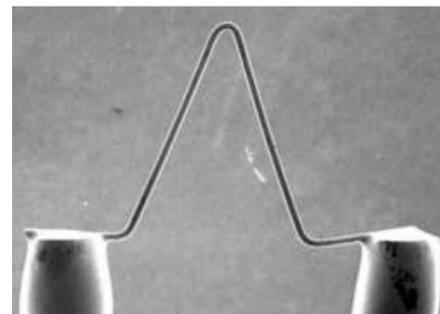
Field Emission Gun (FEG)



thermofisher.com

nanoscience.com

	W Filament	LaB6 or CeB6	S-FEG (Schottky FEG)	X-FEG (extreme high-brightness field emission gun)	C-FEG (low-energy-spread cold FEG)
Relative brightness	1	1-3	250	1250	1625
Energy spread	2.5 eV	1-1.5 eV	<1 eV	0.6-0.8 eV	<0.3 eV
Source size	50-100 μm	25 μm	<30nm	<20nm	<5nm
Lifetime	100 h	1,500 h	5,000 h+	1 yr+	1 yr+

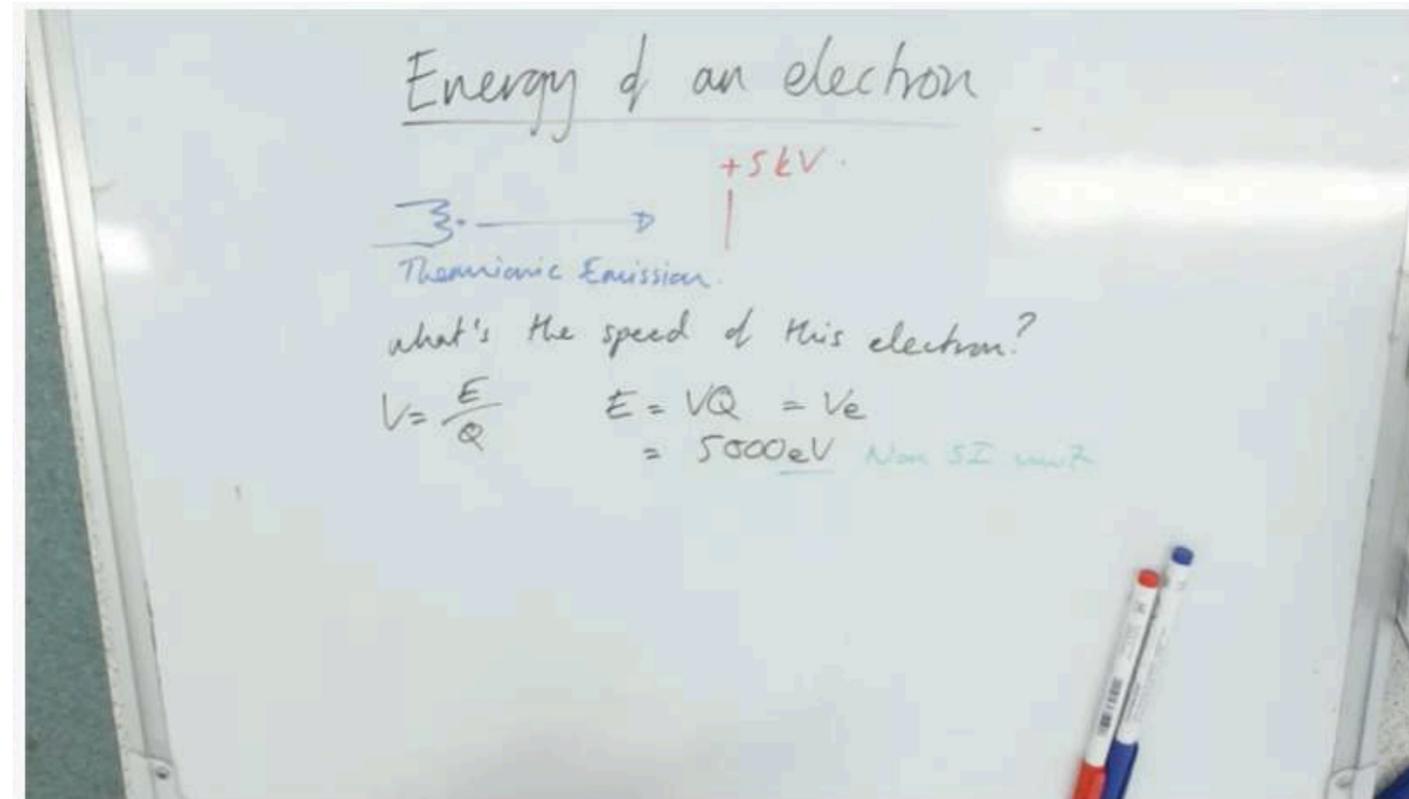
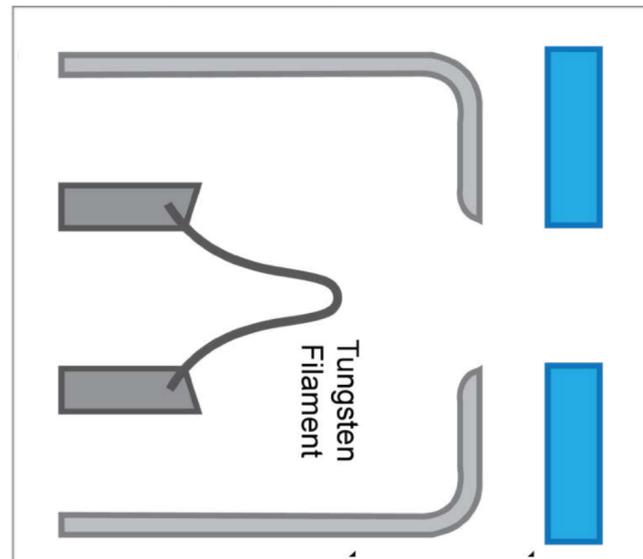


X-CFEG is 1.0×10^8 A/m² sr V

Electron sources



How fast are the electrons moving?

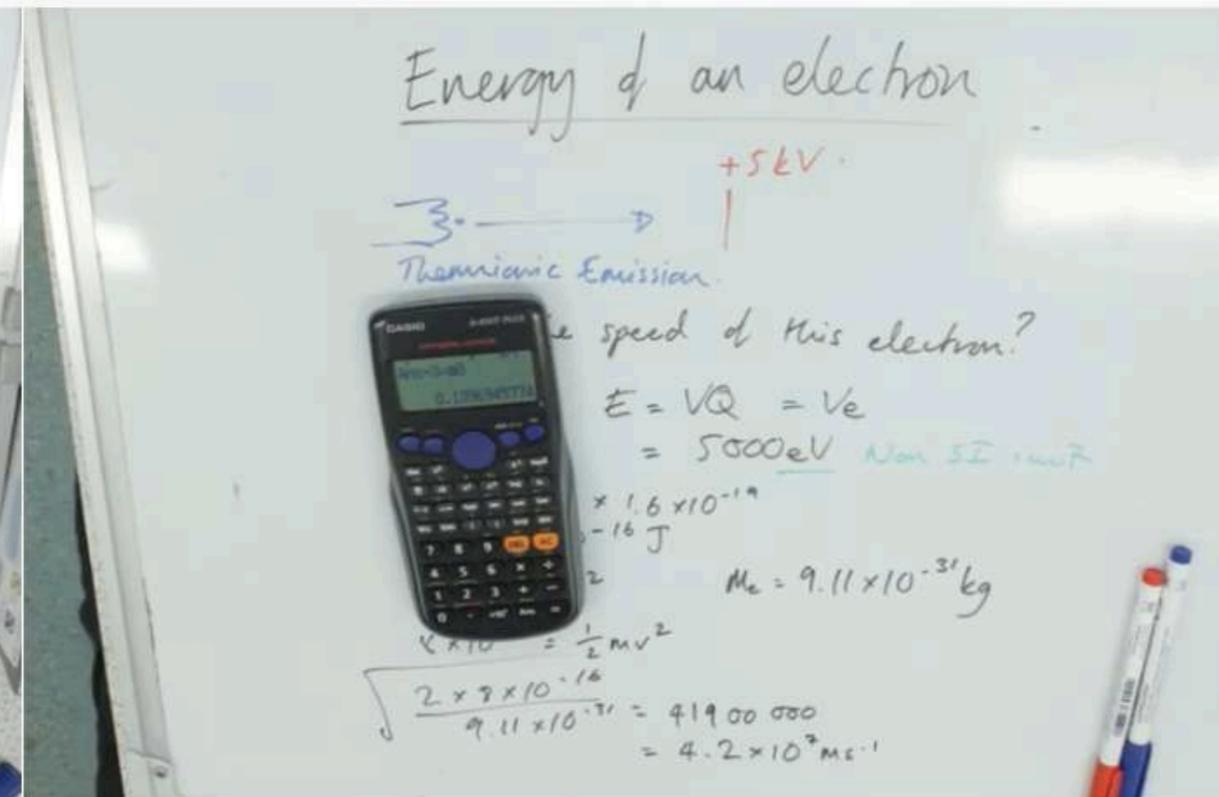
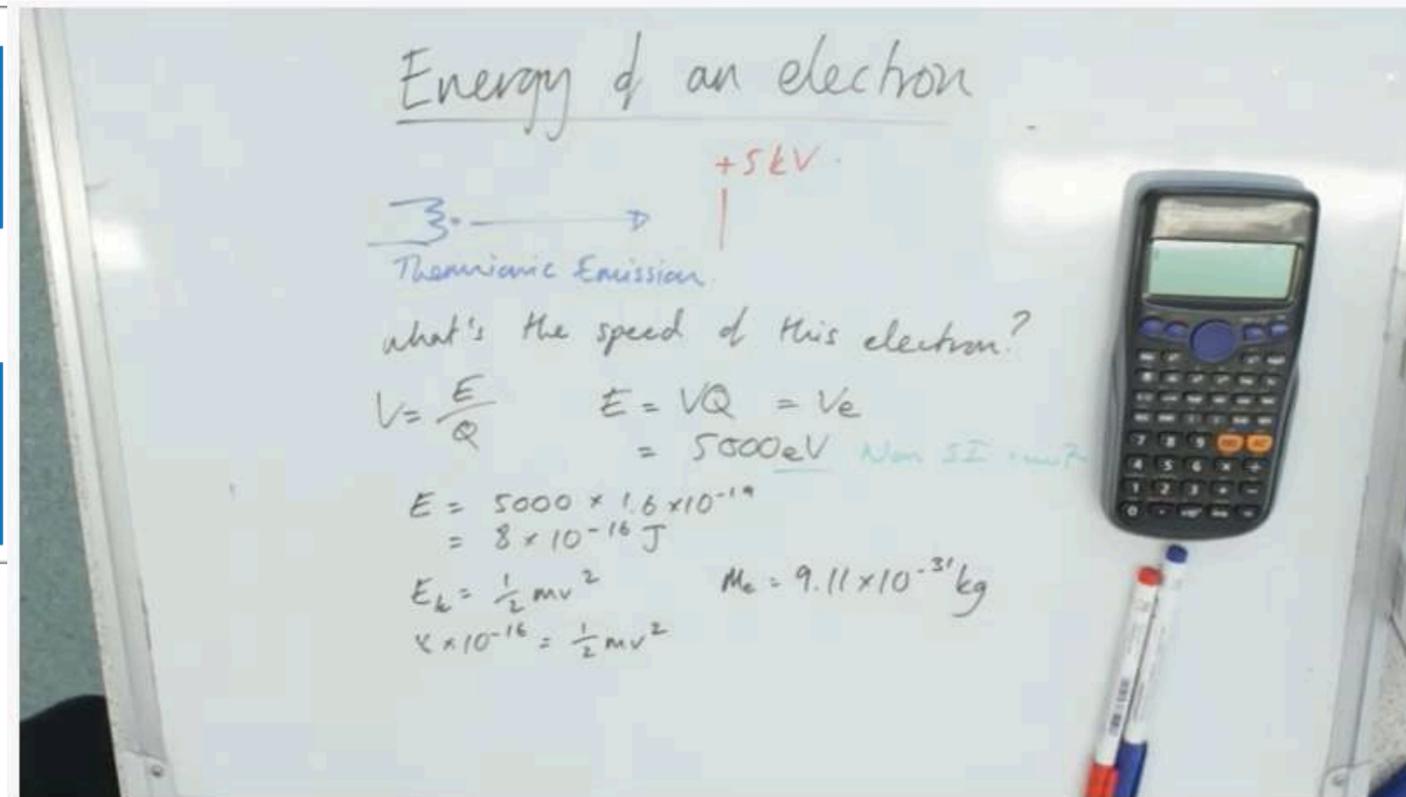
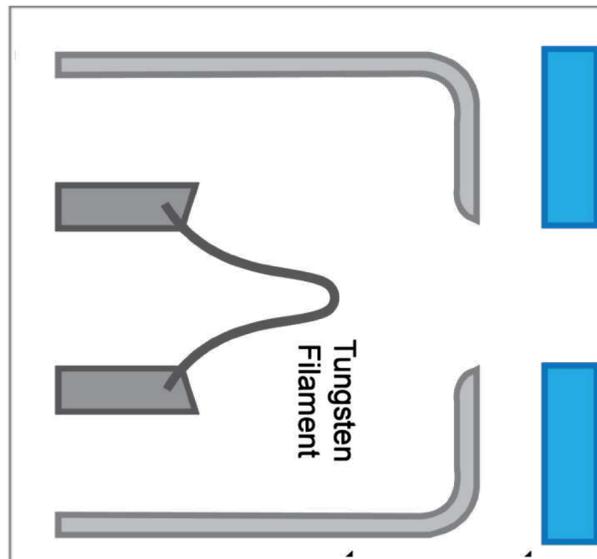


<https://www.youtube.com/watch?v=tYCET6vYdYk>

Electron sources



How fast are the electrons moving?

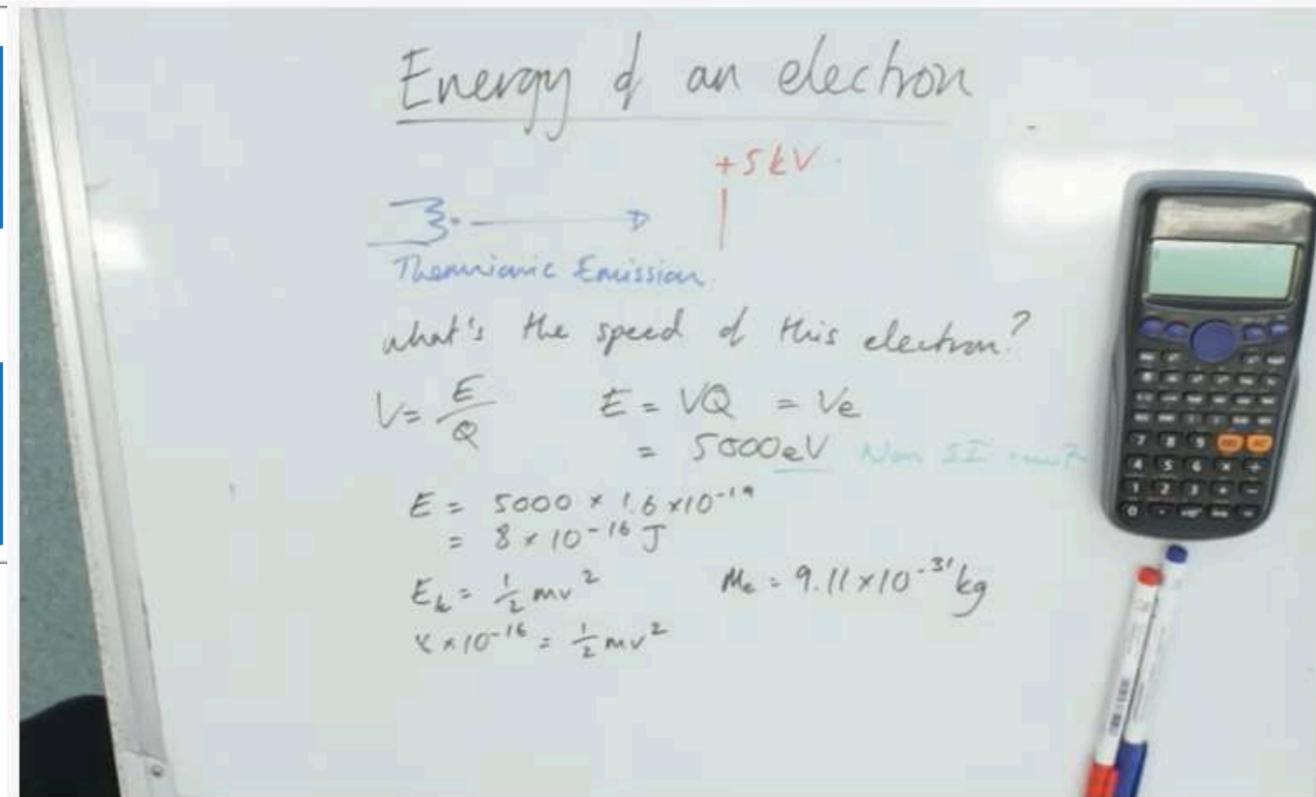
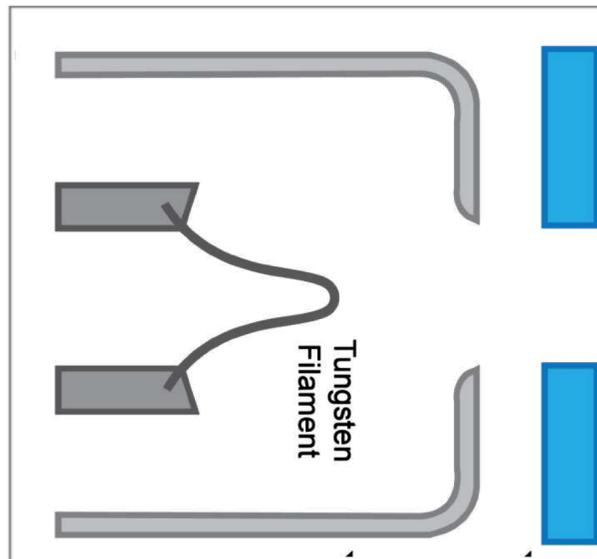


<https://www.youtube.com/watch?v=tYCET6vYdYk>

Electron sources



How fast are the electrons moving?



Light microscope	Transmission electron microscope
Visible light	Electrons
Glass lenses	Electron-magnetic lenses
450-650 nm	3.70 pm (100 keV) 2.51 pm (200 keV) 1.96 pm (300 keV)
speed of light in vacuum c	0.548c (100 keV) 0.695c (200 keV) 0.776c (300 keV)

<https://www.youtube.com/watch?v=tYCET6vYdYk>

Electron sources



80-120 kV: Hitachi 7800, JEOL J400, TFS Talos I 20 W or LaB6

High contrast & robust
sub-nm resolution

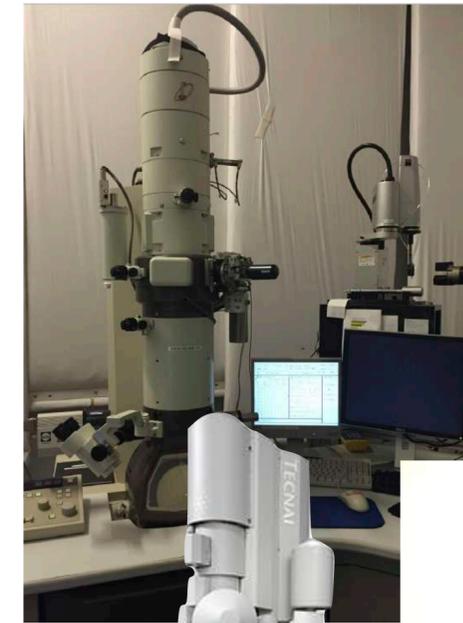
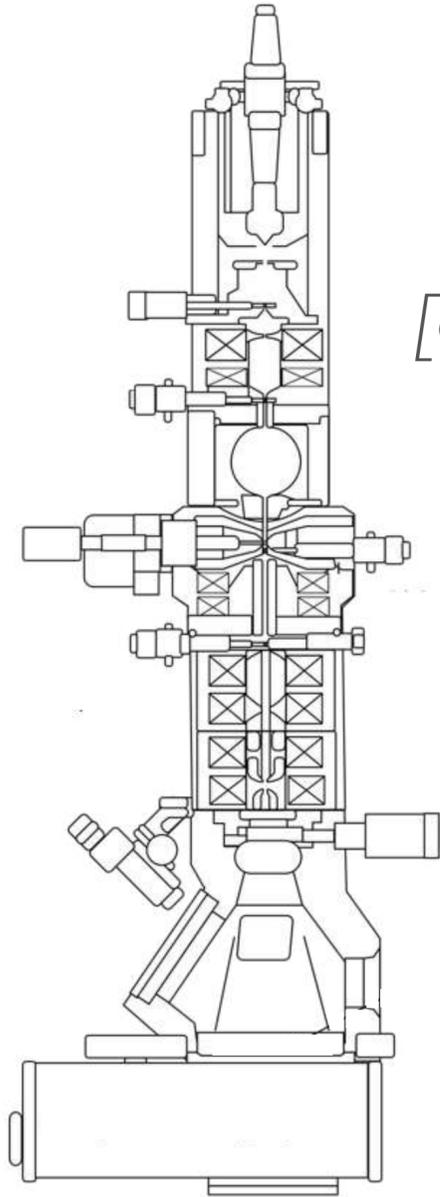
[developments ongoing to push resolution with FEG systems]

200 kV: JEOL J2100F; TFS Tecnai, Glacios, Arctica
FEG

2+ Å resolution (3.5-4 Å)

300 kV: JEOL J3200FSC, cryoARM; TFS Krios, Halo
FEG

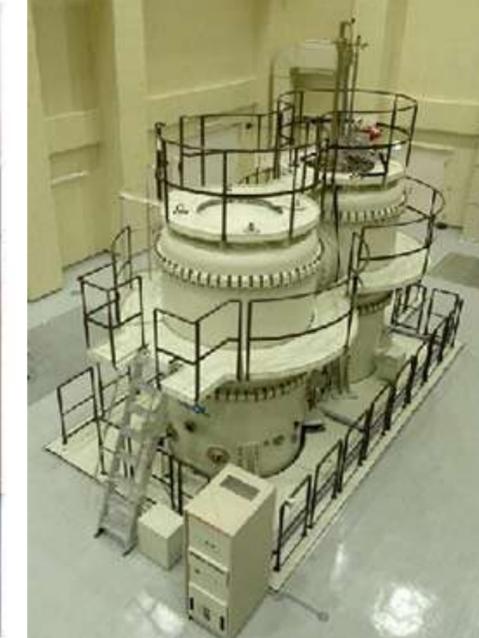
Smaller effect on unwanted lens aberrations
1.5-3 Å resolution



Electron sources

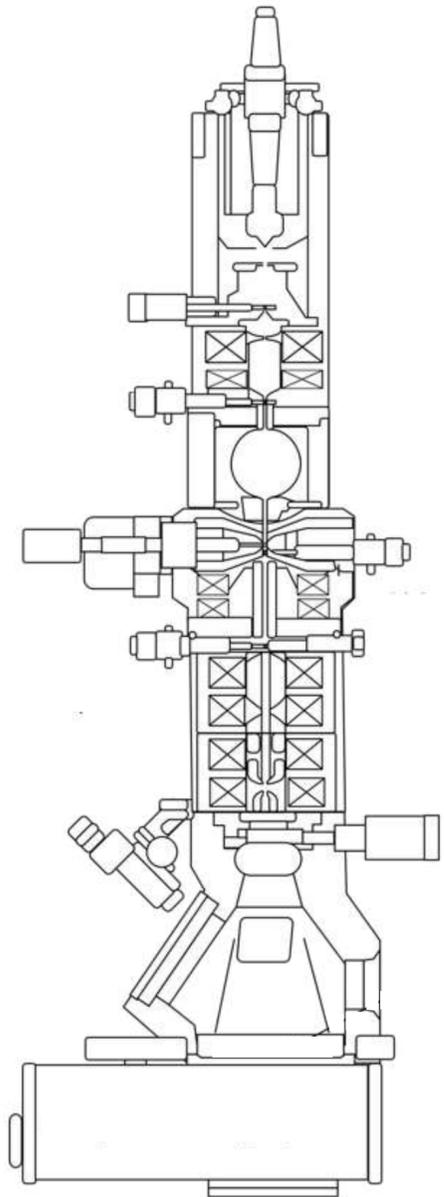
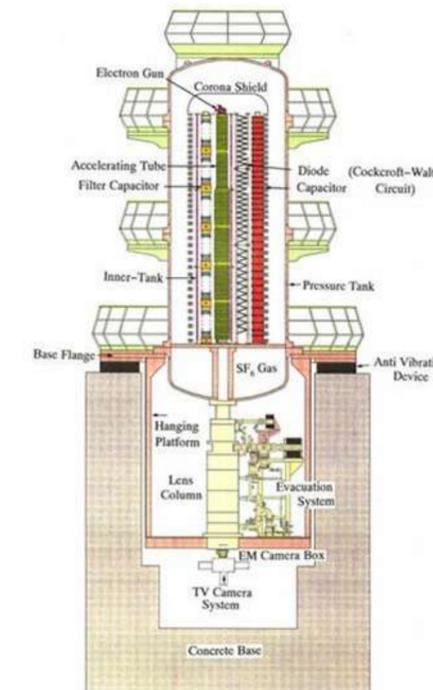


1-1.2 MV: Hitachi, JEOL
LaB6



uhvem.osaka-u.ac.jp

3 MV: Hitachi H3000
LaB6



Vacuum systems



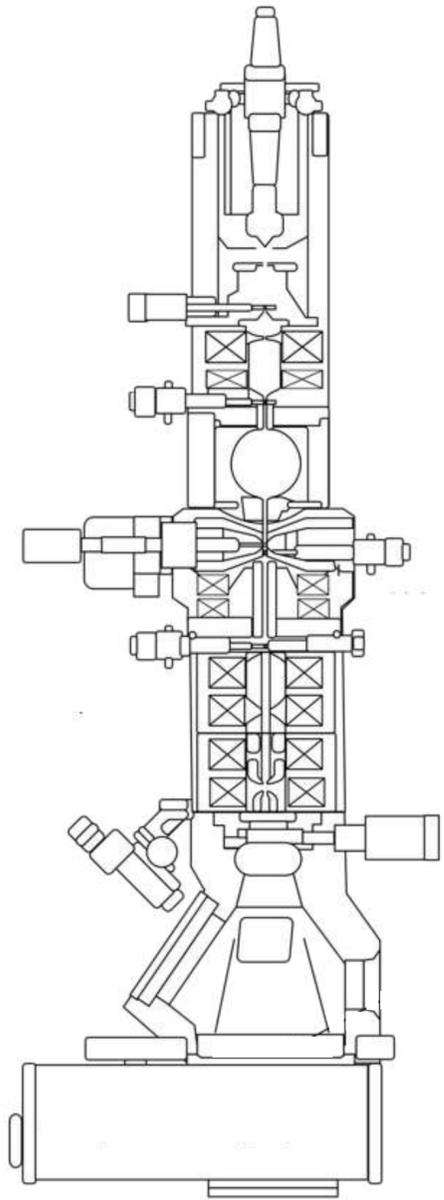
Why do we need a vacuum?

Beam coherence - at STP mean free path ~ 1 cm

Insulation - interaction between e- and air

Filament - O₂ will burn out source

Contamination - reduce interaction gas, e-beam and sample

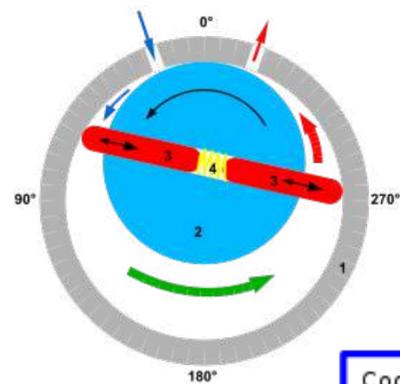
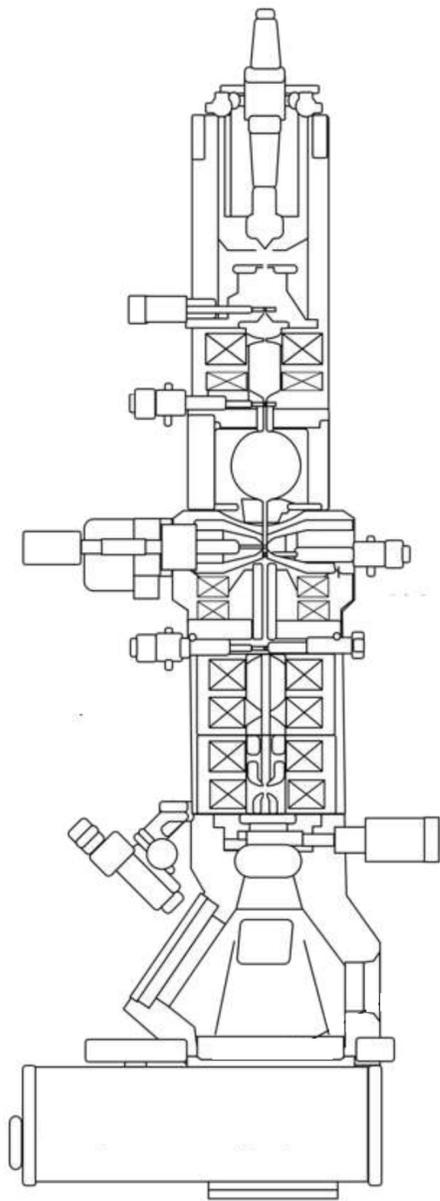


Vacuum systems



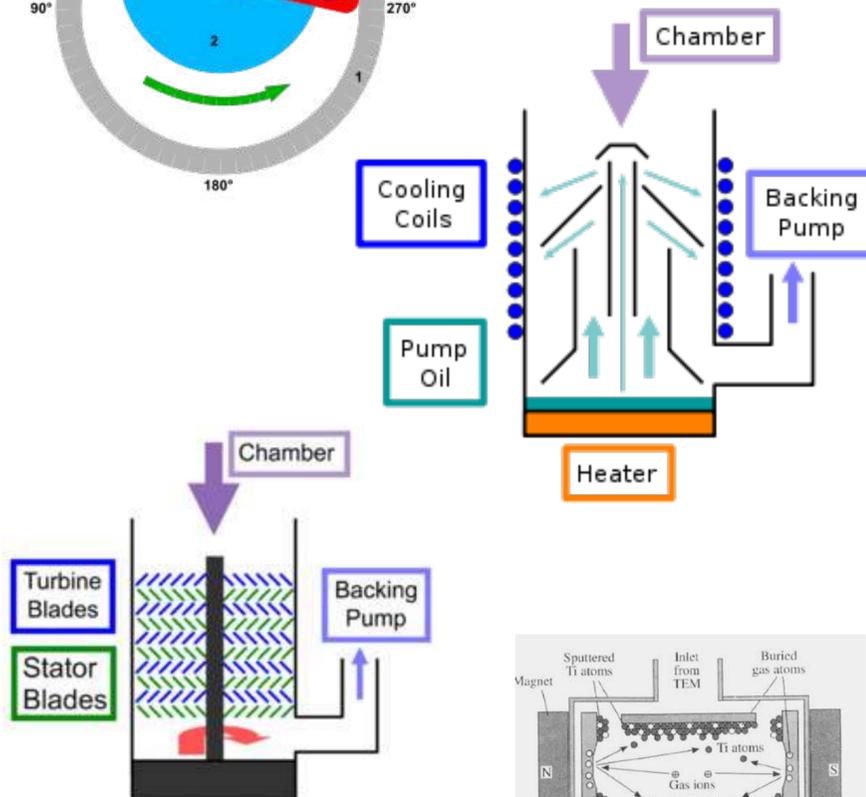
What types of pumps do we have?

1 mm Hg = 1 Torr = 10^2 Pa
 1 atm = 760 Torr = 7.5×10^4 Pa



PVP / Rotary

$1 - 10^{-3}$ Torr | > 0.1 Pa

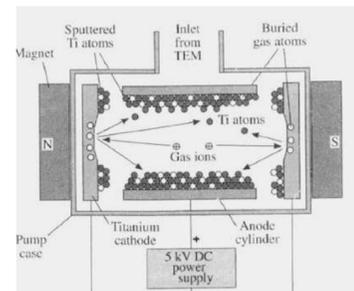


Diffusion

$10^{-3} - 10^{-6}$ Torr | $0.1 - 10^{-4}$ Pa

Turbo

$10^{-6} - 10^{-9}$ Torr | $10^{-4} - 10^{-7}$ Pa



IGP

$10^{-9} - 10^{-12}$ Torr | $10^{-7} - 10^{-9}$ Pa

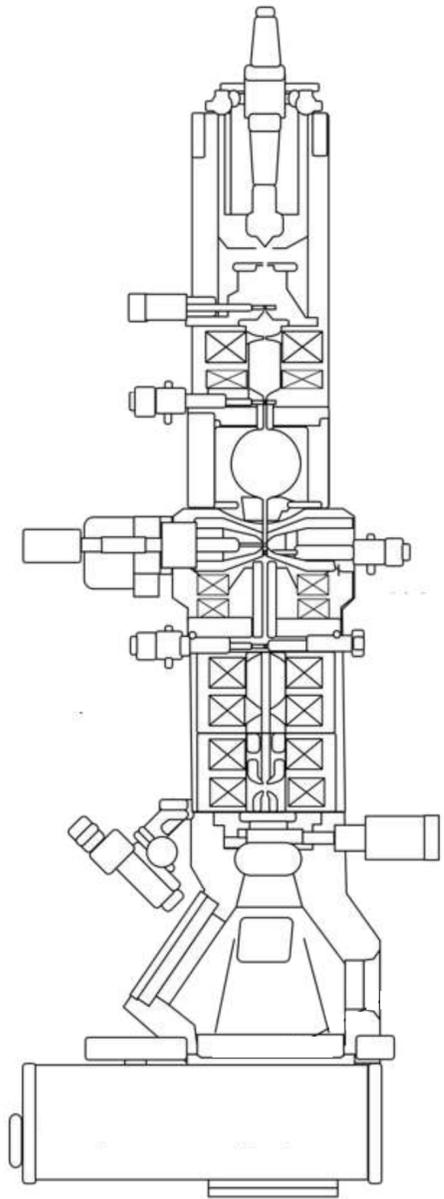
wikipedia.com

Vacuum systems



What types of pumps do we have?

1 mm Hg = 1 Torr = 10^2 Pa
 1 atm = 760 Torr = 7.5×10^4 Pa



Gun

10^{-9} Torr

Specimen

10^{-6} - 10^{-7} Torr

Chamber and Camera

10^{-5} - 10^{-6} Torr

Vacuum (Supervisor) Cryo Settings Control

Status: COL. VALVES

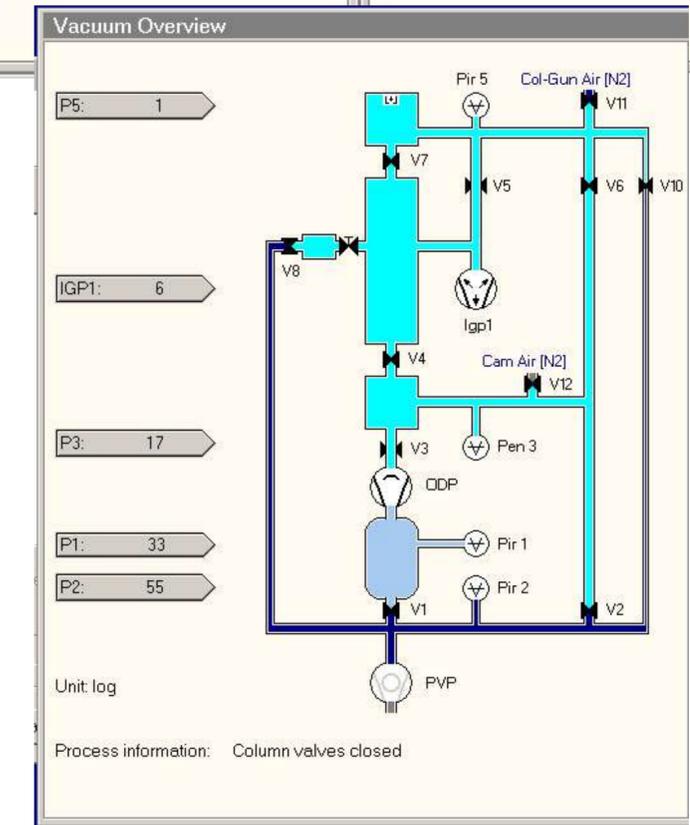
Pressure

Gun/Col	6	Log
Camera	17	Log
Buffertank	33	Log
Backing line	55	Log

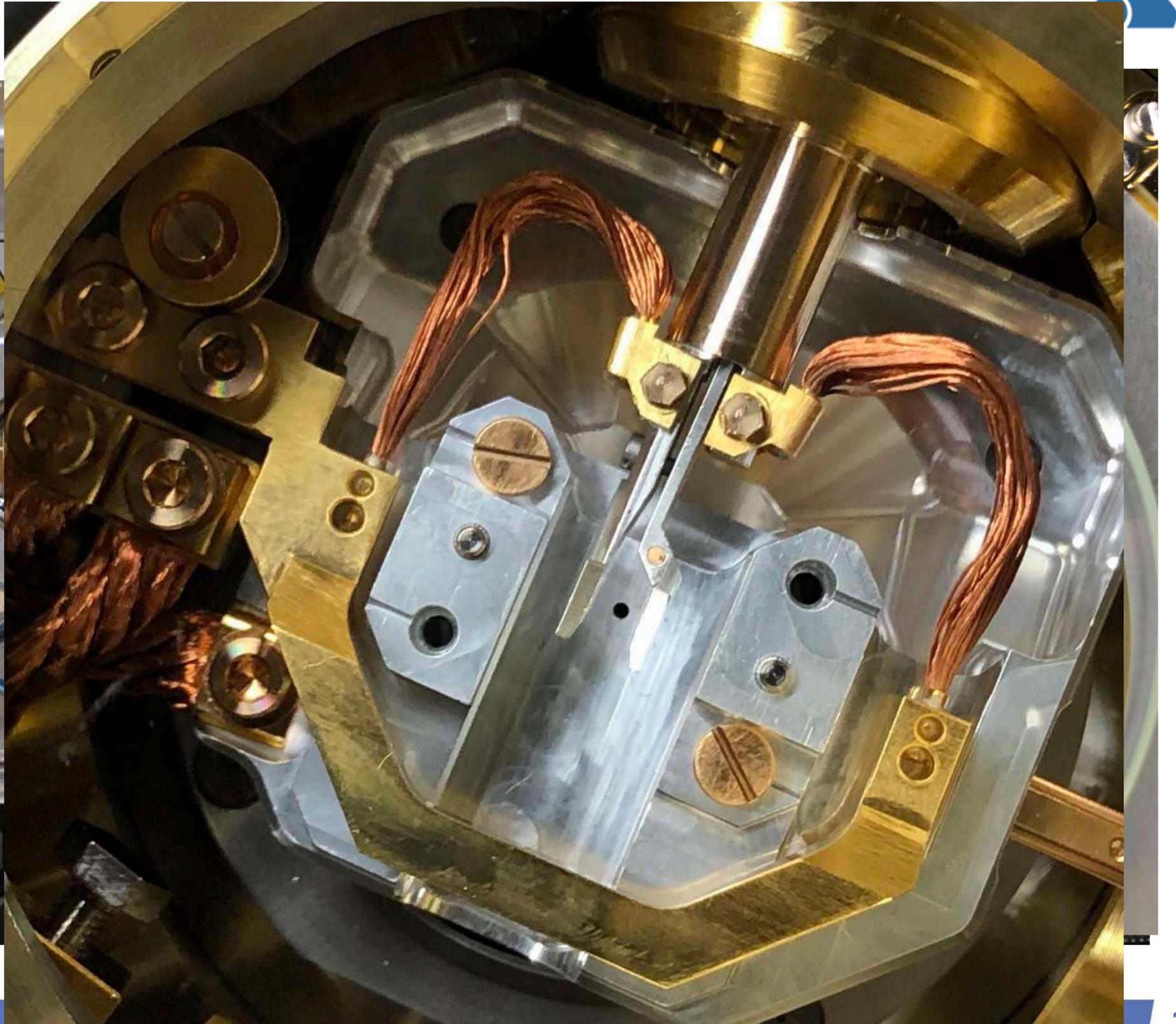
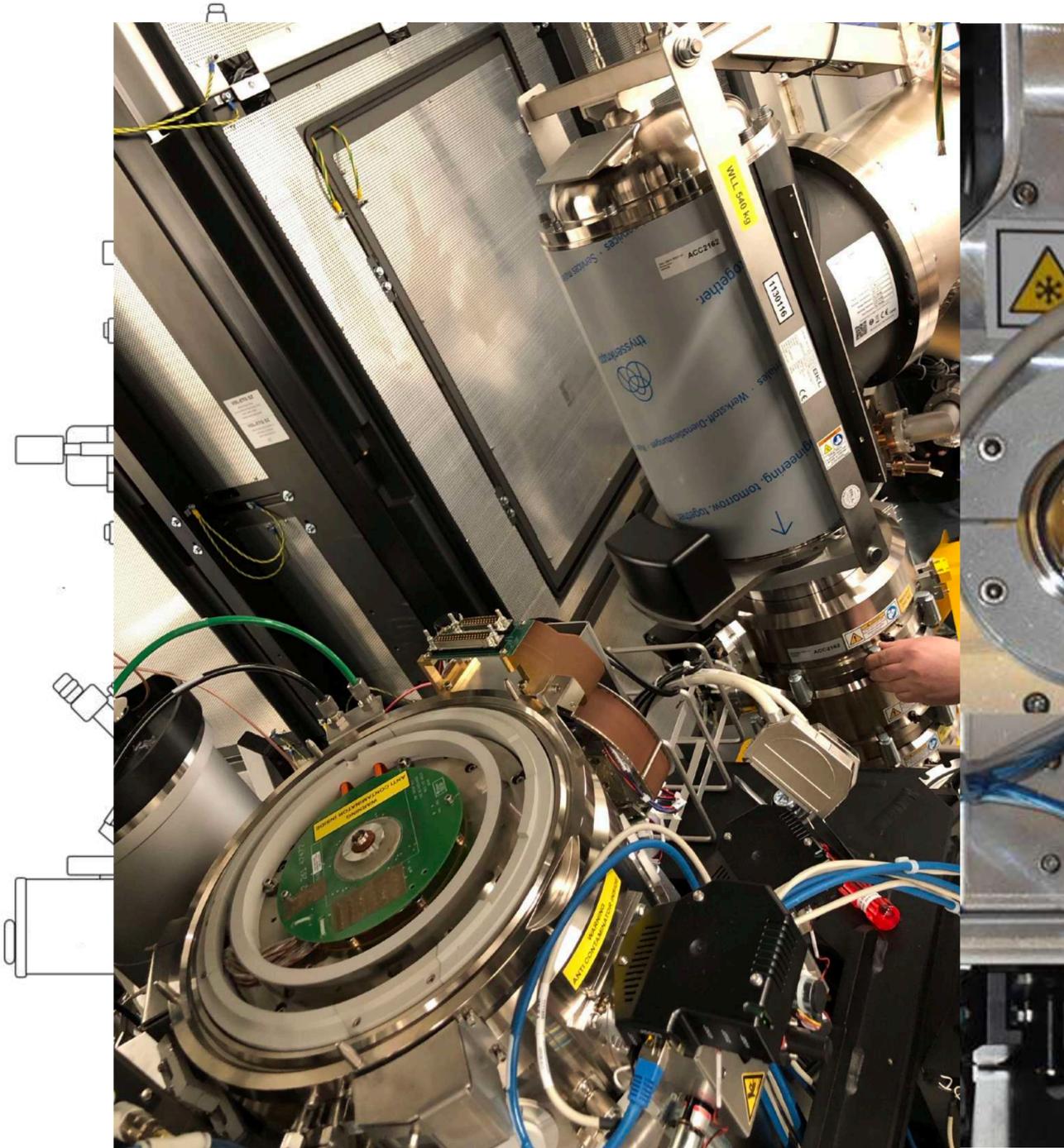
Col. Valves Closed

Default pressure unit: Log
 Default airlock time: 120 s

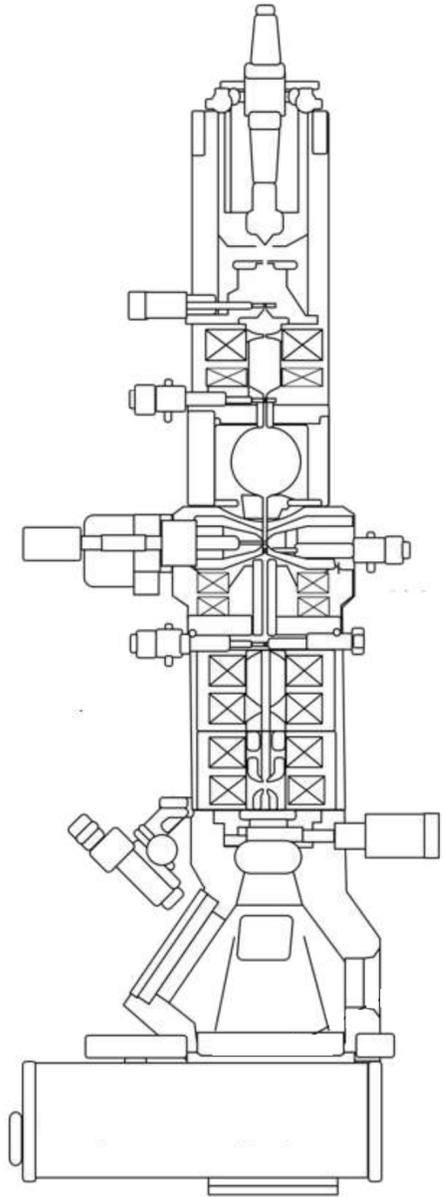
Pressure	Torr	Pascal	Log
Gun/Col	88.29 e-9	11.77 e-6	6
Camera	0.35 e-6	46.05 e-6	17
Buffertank	0.19	25.85	33
Backing	3.86	514.32	55



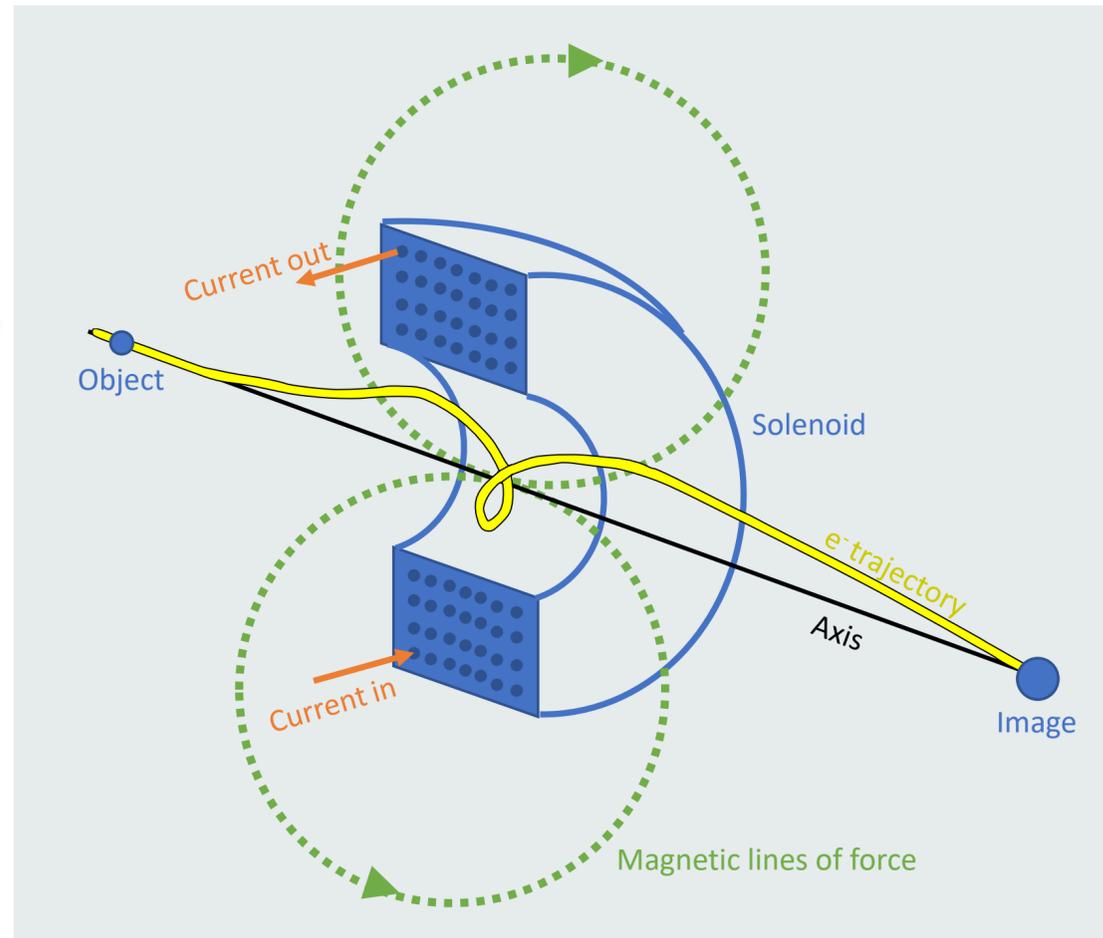
Vacuum systems



Lenses



What types of lenses do we have?



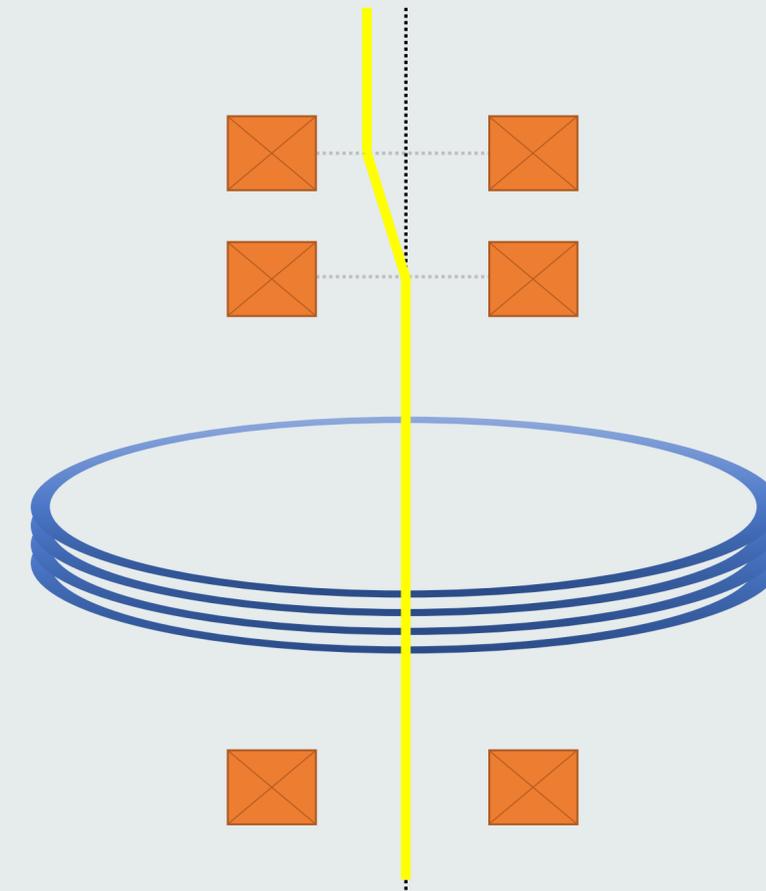
- Focus
- Magnify
- Rotate

Deflector 1 (shift)

Deflector 2 (tilt)

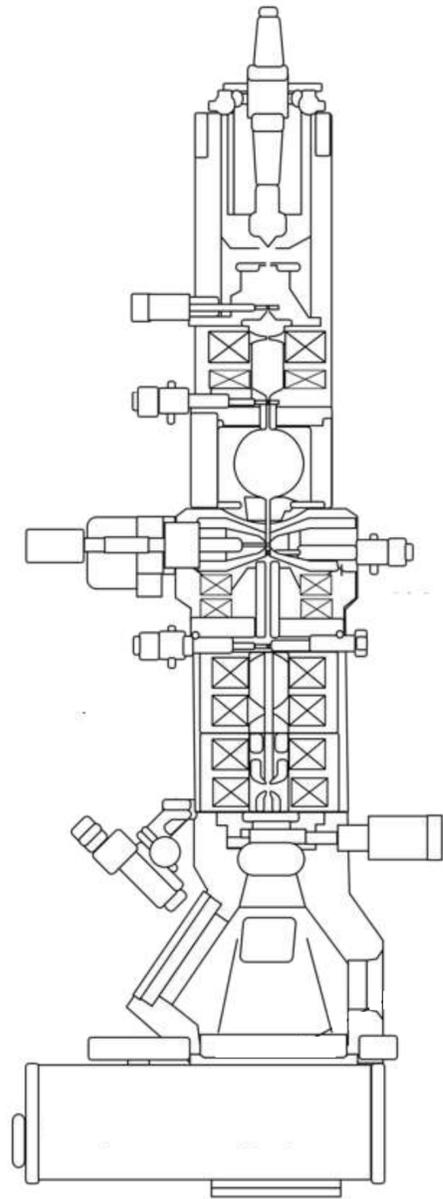
Lens

Stigmator

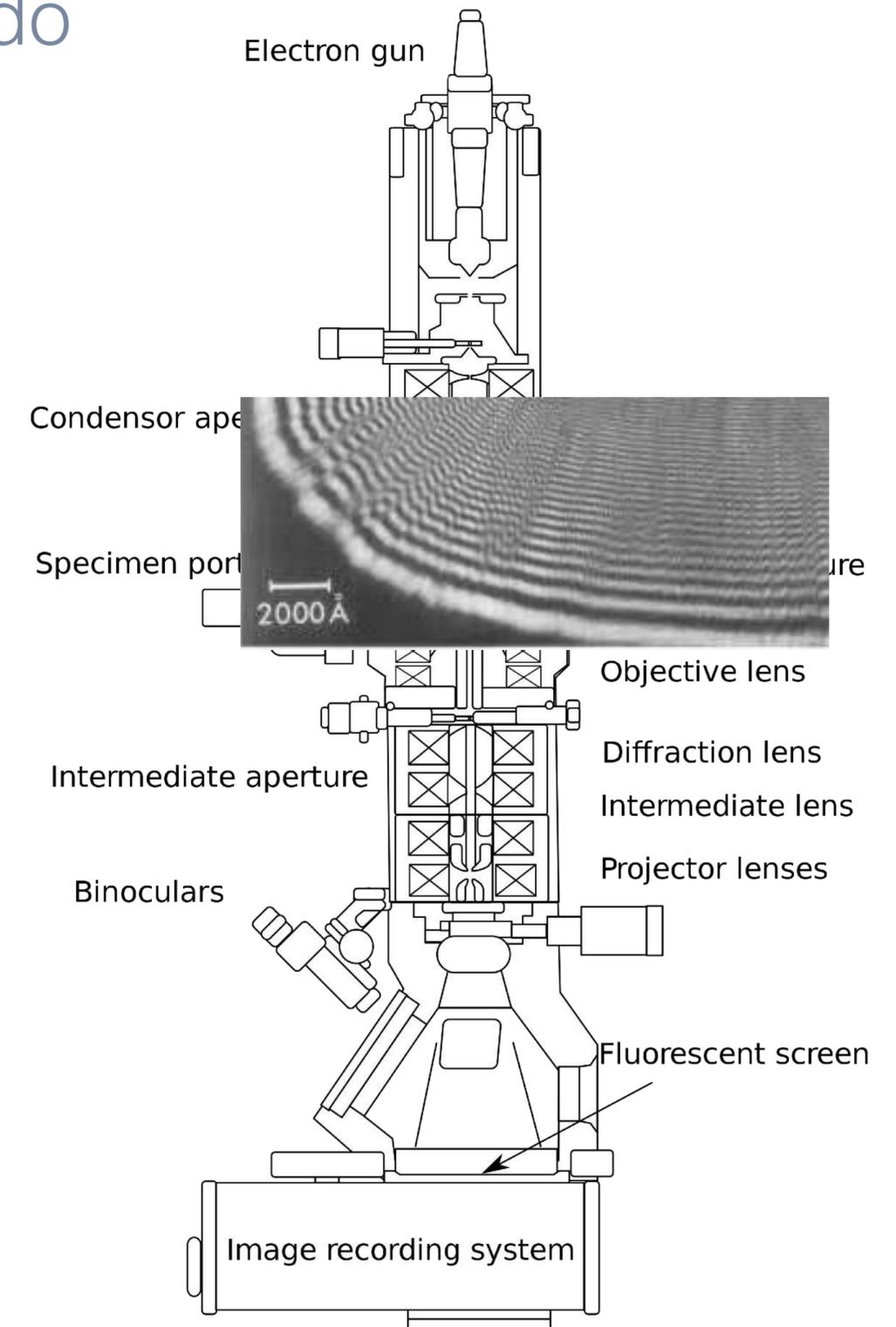


Lenses

Microscope Alignments What to do & what not to do



- Do:
 - Start at eucentric height and focus
 - Check if it is already good before attempt
 - Align from top to bottom
- Not to do:
 - ~~Align without a way to undo~~
 - ~~Align when TEM is not stable (i.e., temperature)~~

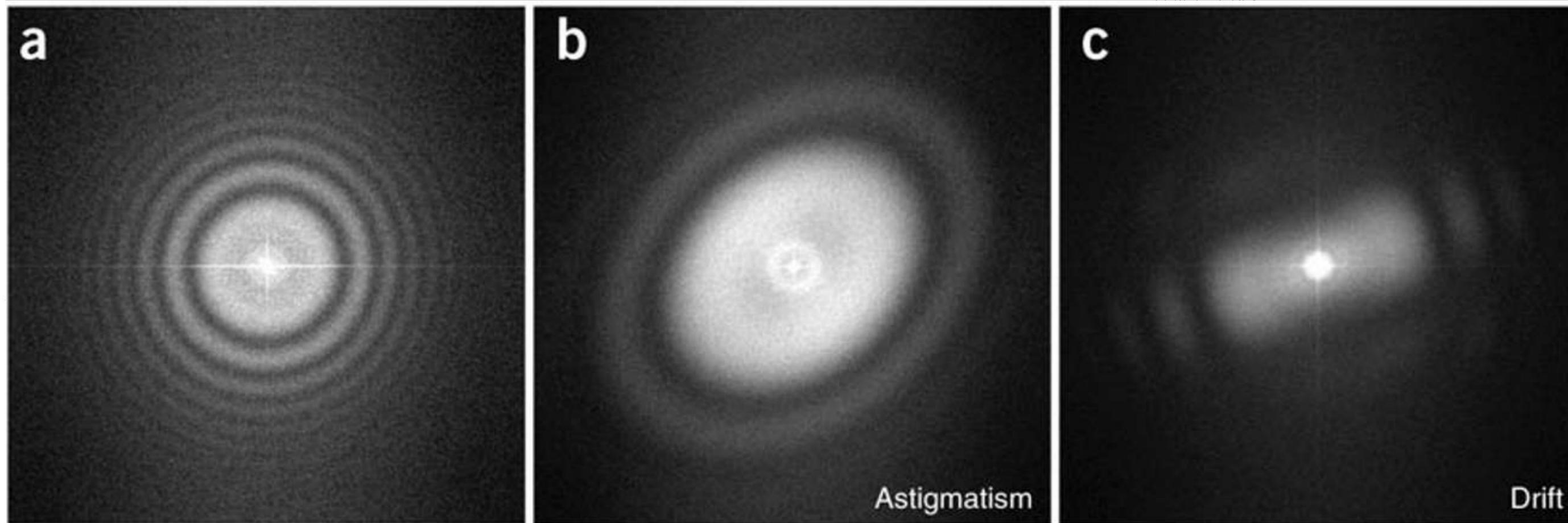
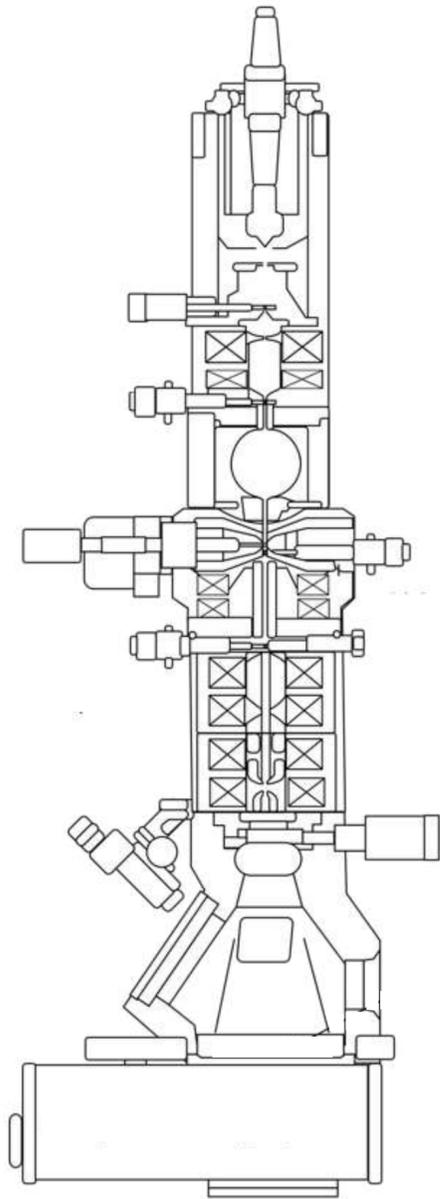


Lenses

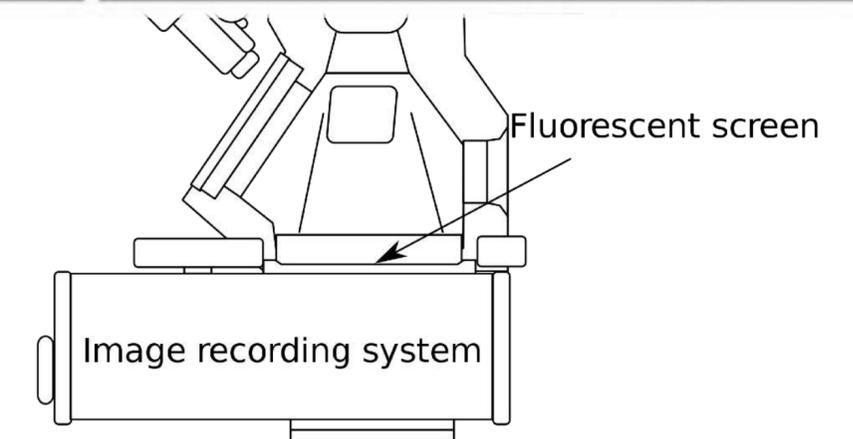
Microscope Alignments What to do & what not to do

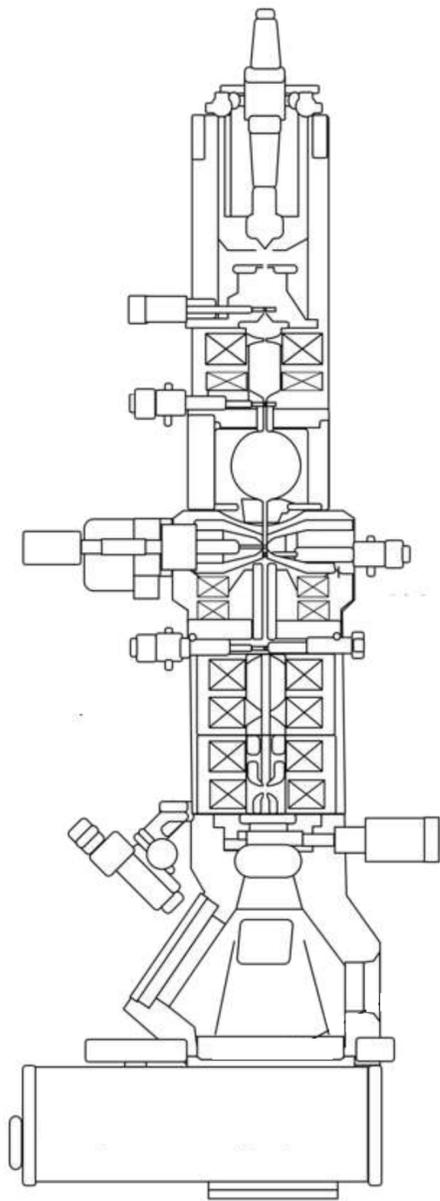


- Do:



- ~~Align without a way to undo~~
- ~~Align when TEM is not stable (i.e., temperature)~~





a

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,

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

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

where,

C_s -- The spherical aberration coefficient, defining the quality of objective lens,

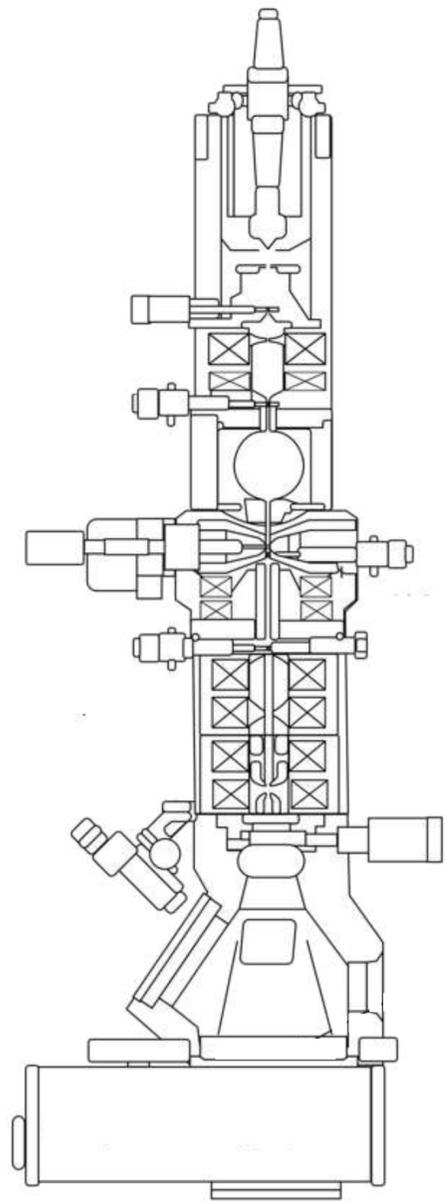
λ -- The wave-length,

Δf -- The defocus value,

$|g|$ -- The spatial frequency,

α -- The convergence semi-angle.

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



a

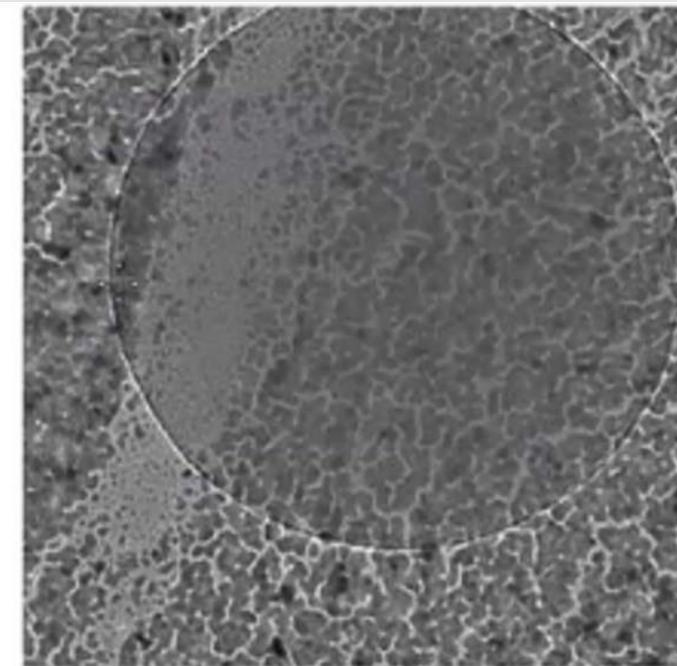
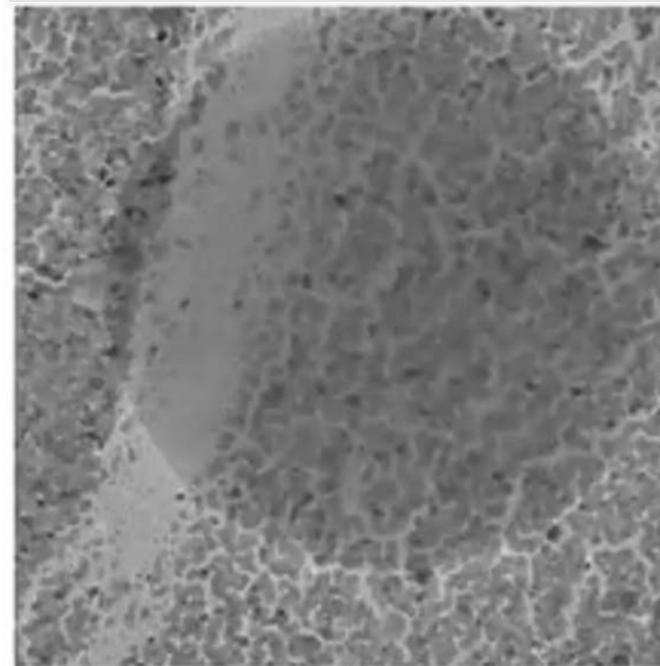
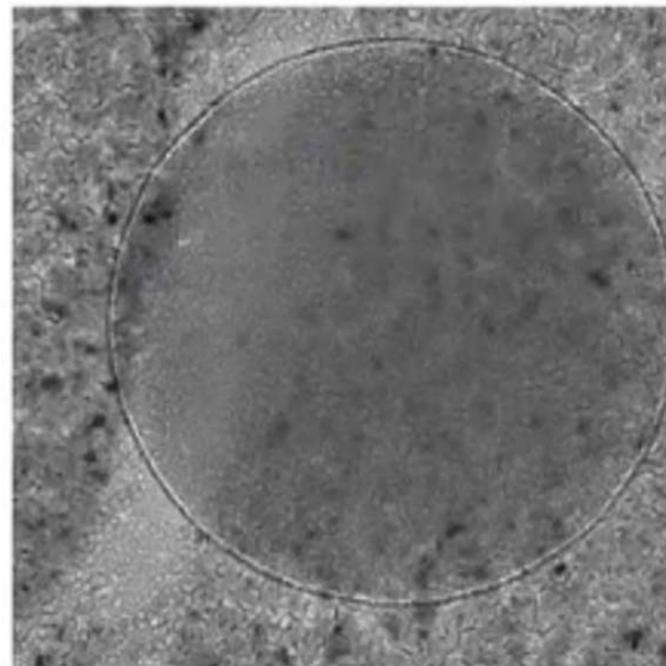
The **contrast transfer function (CTF)** mathematically describes how aberrations in a transmission electron microscope (TEM) modify the image of a sample

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

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

where



Lenses

How to increase efficiency?



1 target/setup
80 s/image
~1000 images/day

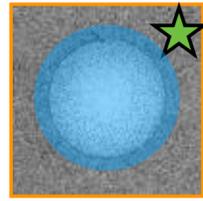
5 targets/setup
35 s/image
~2500 images/day

30 targets/setup
22 s/image
~3800 images/day

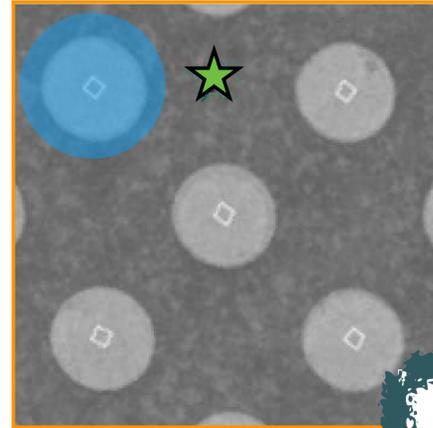
70 targets/setup
18 s / image
~ 4800 images/day



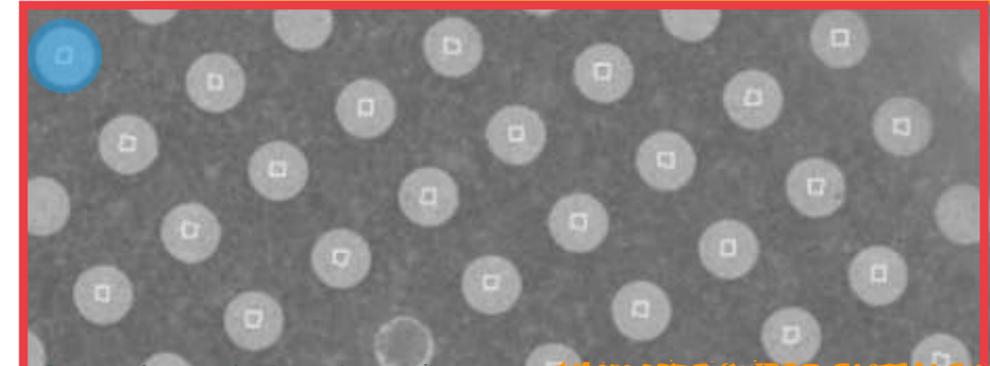
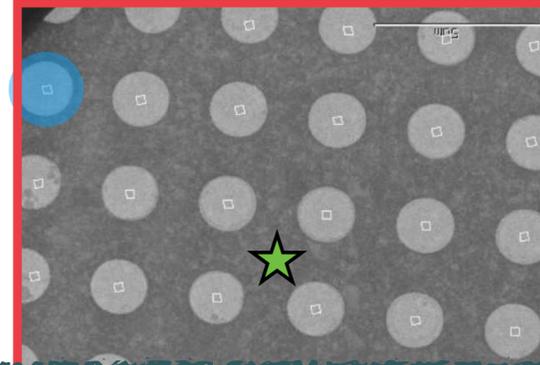
13.6MP



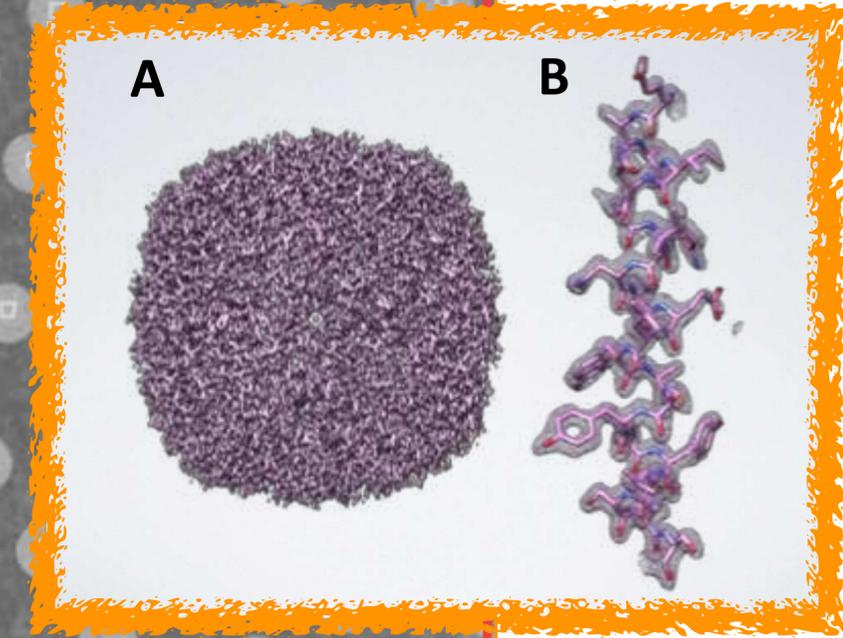
beam tilt
0 mrad



beam tilt
0.5 mrad



Upgrade to K3
24MP
3.75x the
framerate of K2



beam tilt
~3 mrad

But... image shift induced
so... implement hardware

Overhead

30 s stage move and settling
30 s focus and drift check
20s for K2 40 frame movie to save

Cheng A, Eng ET,
Alink L, Rice WJ,
Jordan KD, Kim LY,
Potter CS, Carragher
B. High resolution
single particle cryo-
electron microscopy
using beam-image
shift. J Struct Biol.
2018;



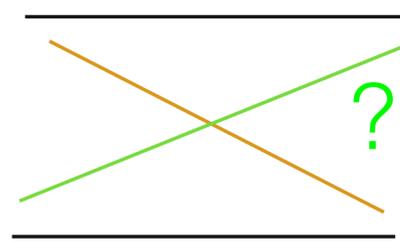
Anchi Cheng

Detectors

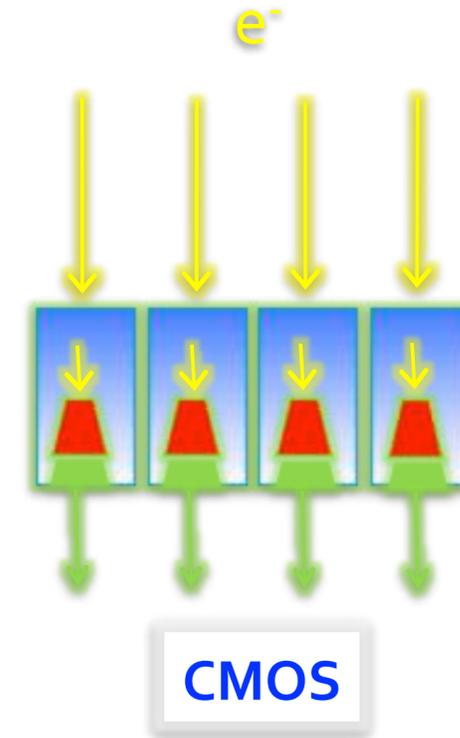
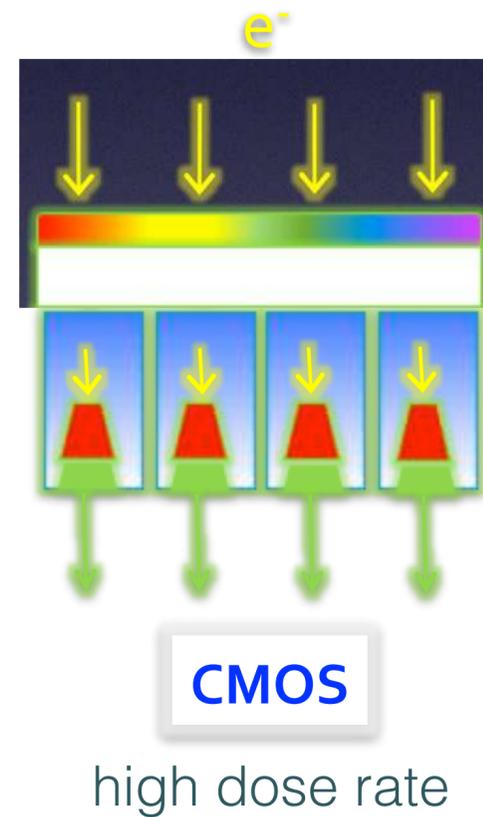
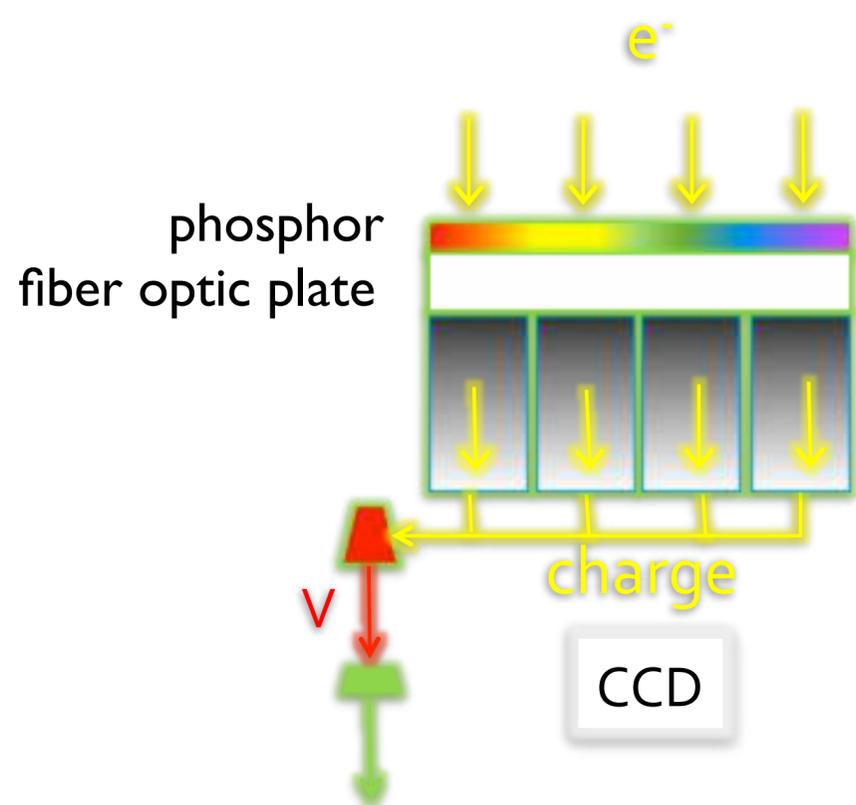
Digital Cameras for TEM



- Photon converted
- Direct sensing

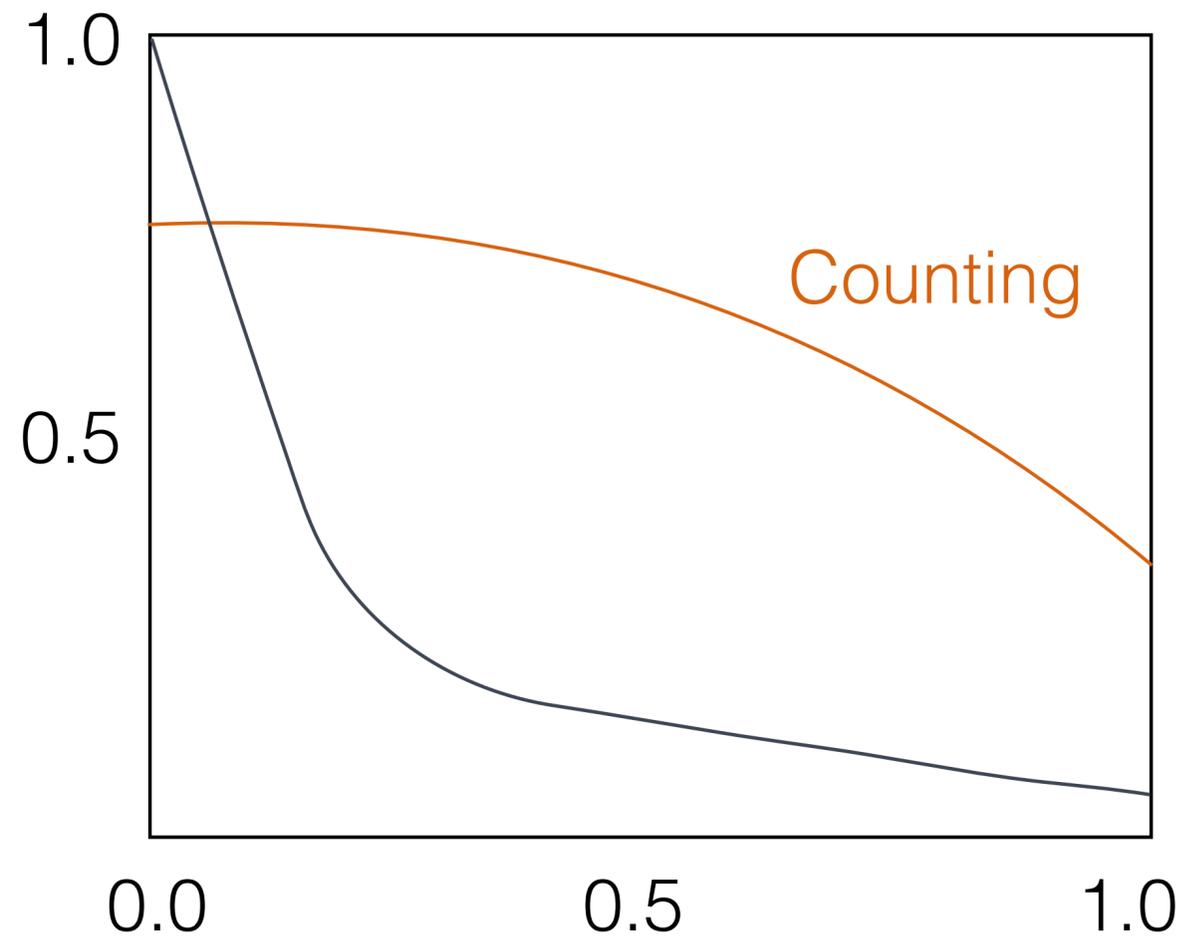


- **CCD** Charge Coupled Device
- **CMOS** Complementary Metal Oxide Semiconductor

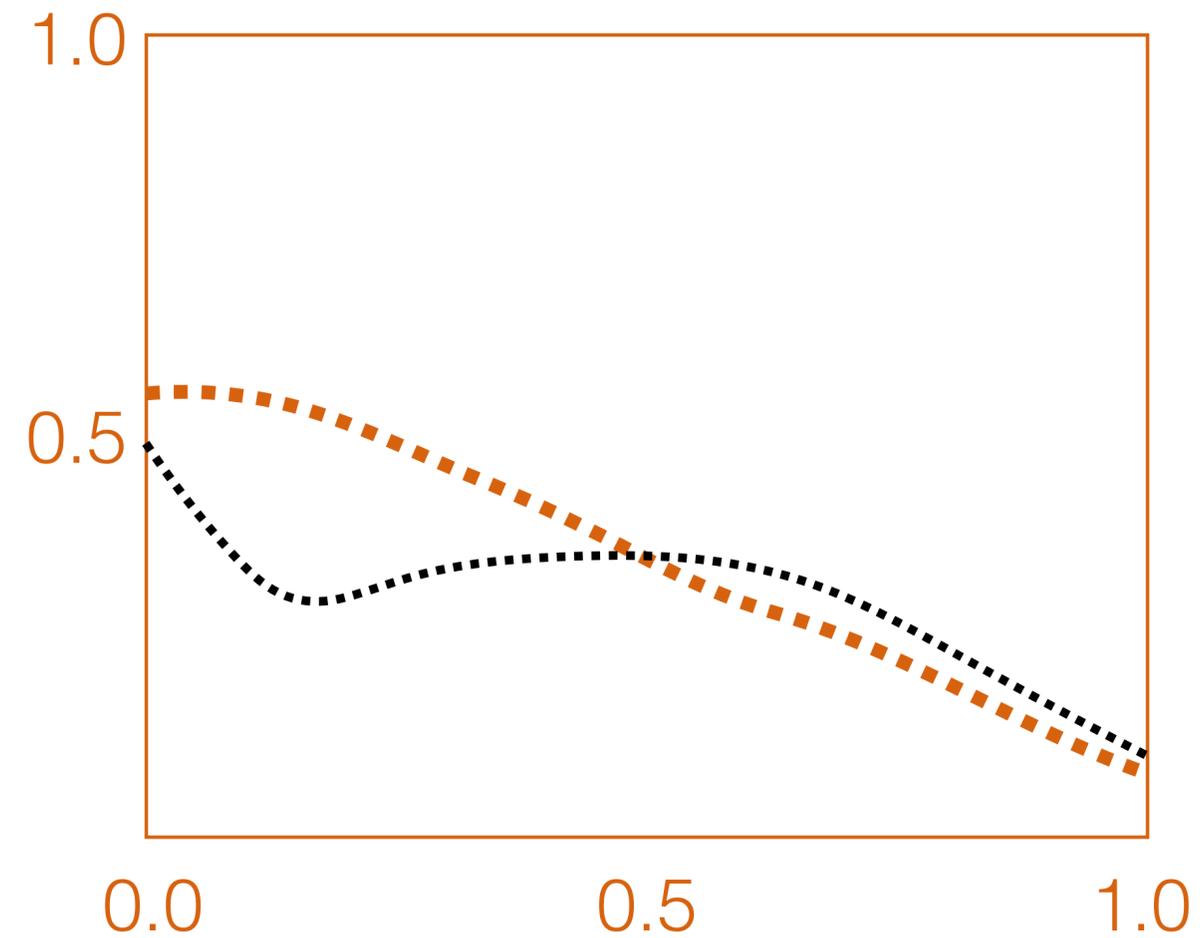




- MTF (Modulation Transfer Transform)
- contribute to signal envelope

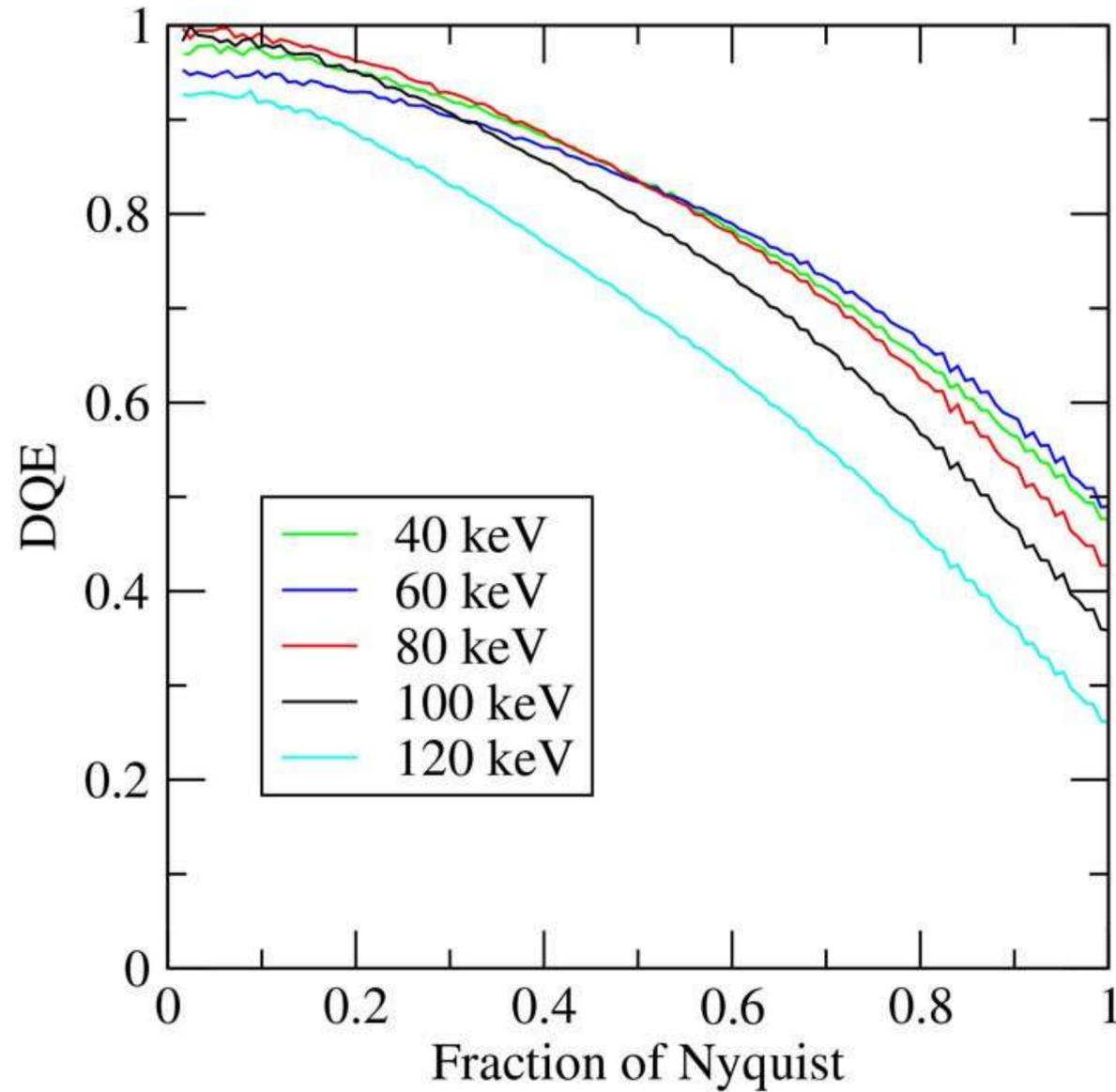


- DQE (Detector Quantum Efficiency)
- S/N over spatial frequency range

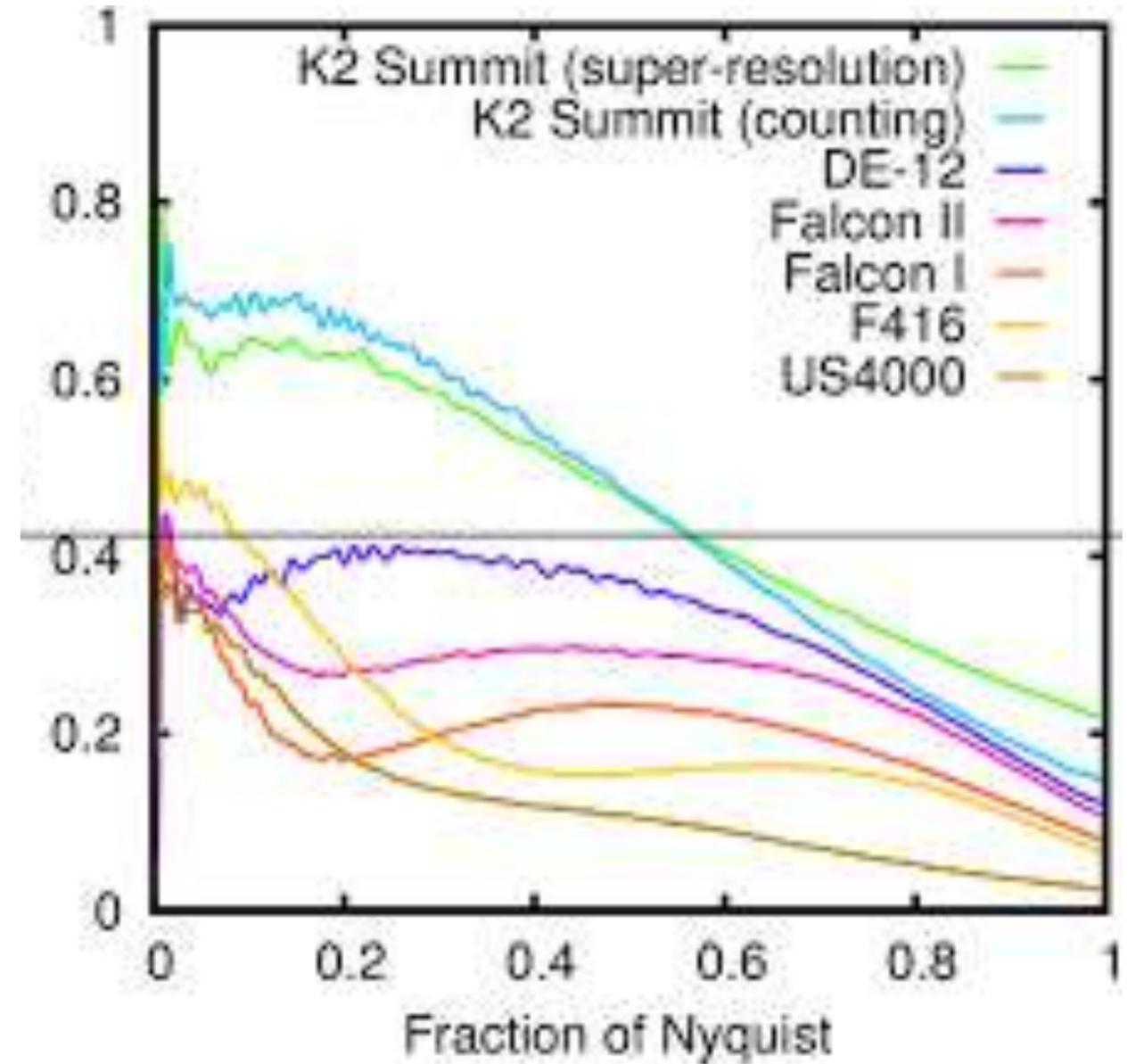


Detectors

Detector Performance Characterization



dectris.com



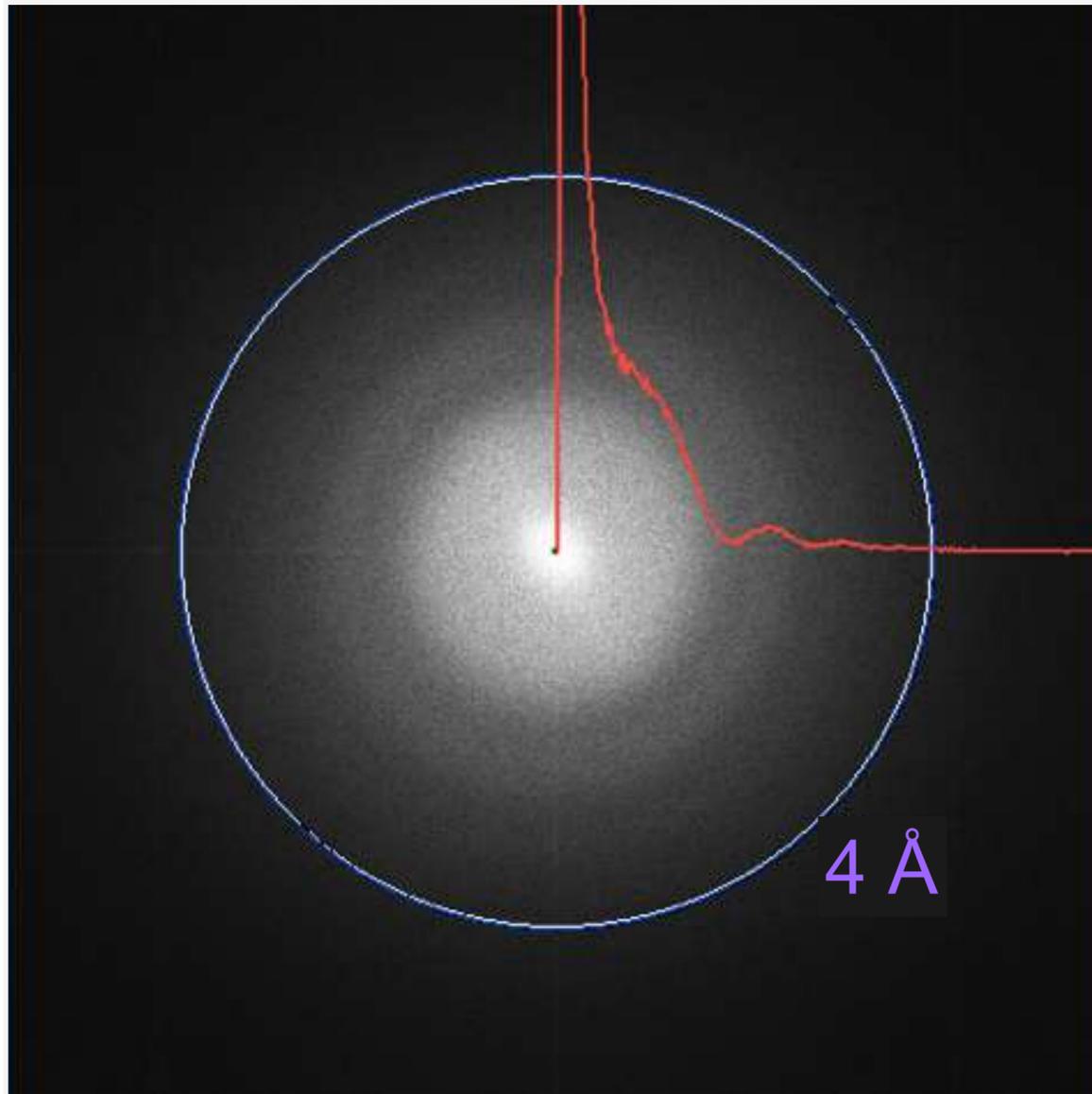
Ruskin, et al JSB

Detectors

Improving the resolution:
Detecting electrons instead of photons

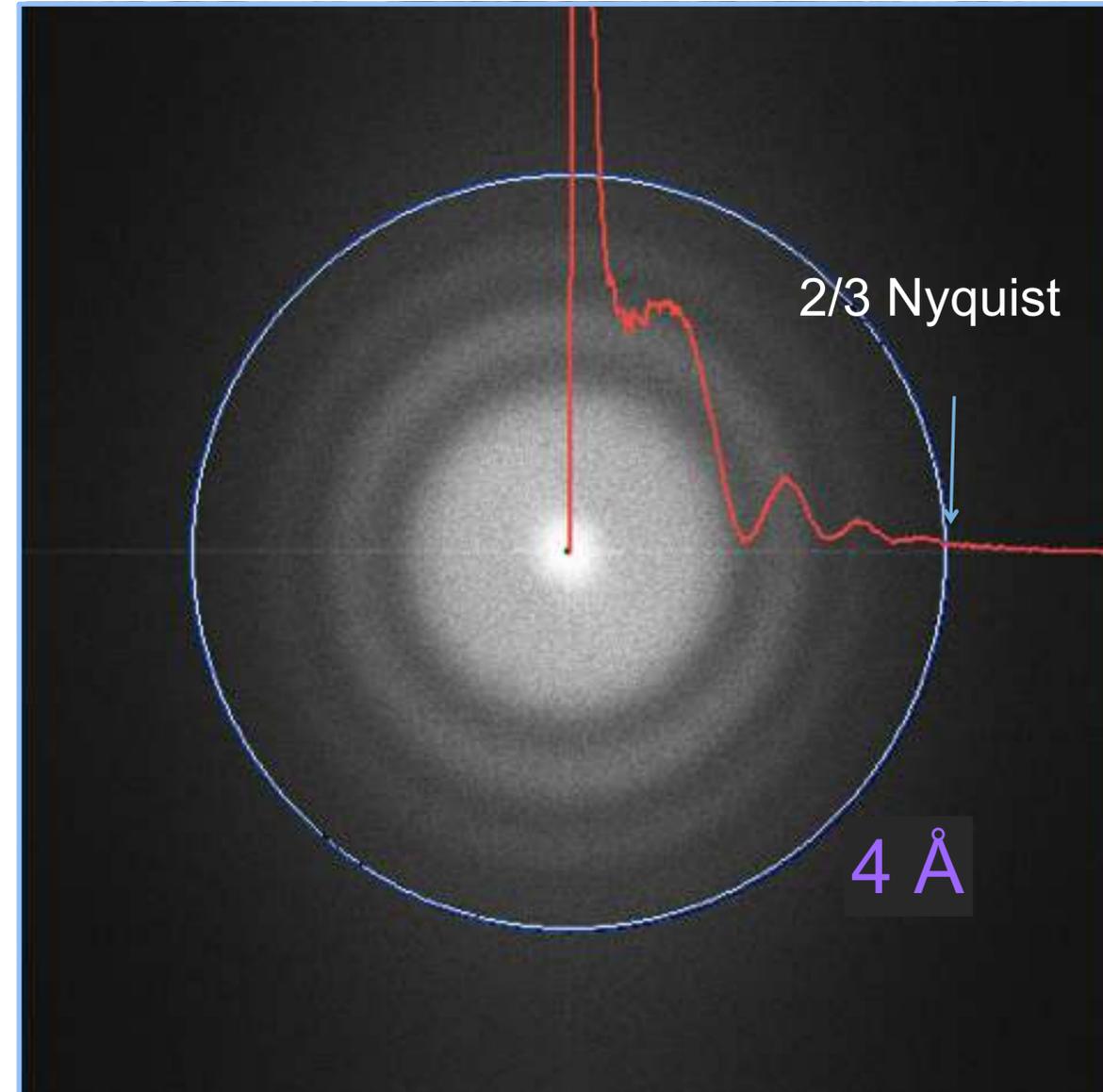


CCD



1.37 Å/pixel

DDD



1.38 Å/pixel

200KeV ; 20 e-/Å² ; carbon film ;
3k x 3k image

Detectors

Improving the resolution:
Detecting electrons instead of photons



K3 specs



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.

<https://www.gatan.com/K3>

Detectors

Improving the resolution:
Detecting electrons instead of photons

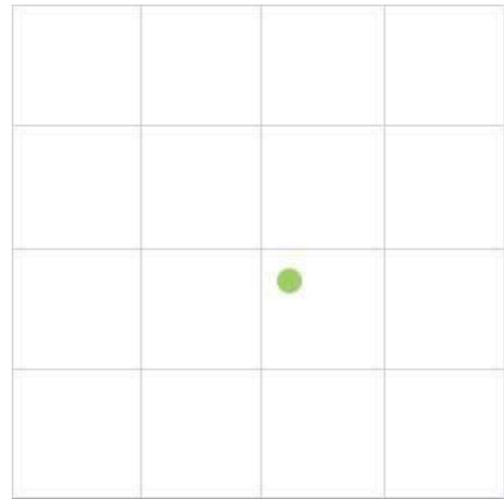


Counting mode

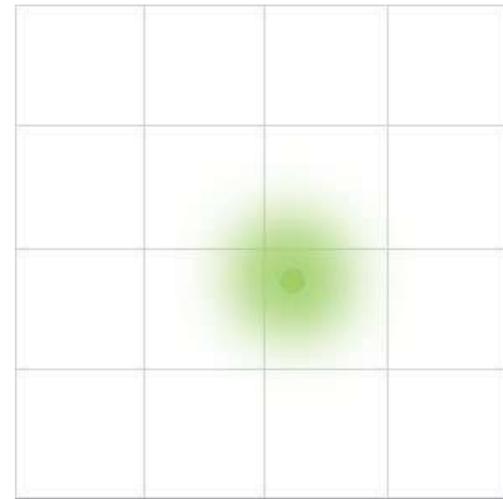
5,760 x 4,096 px



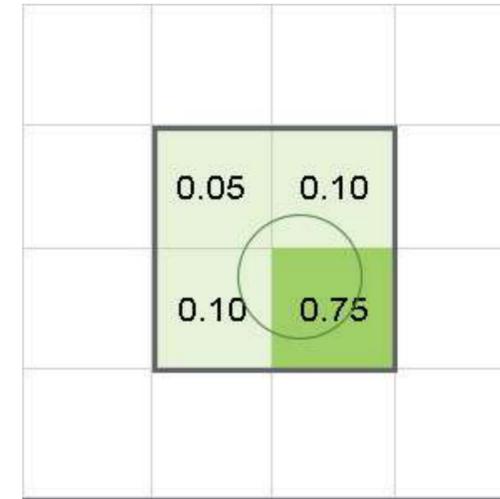
11,520 x 8,184 px



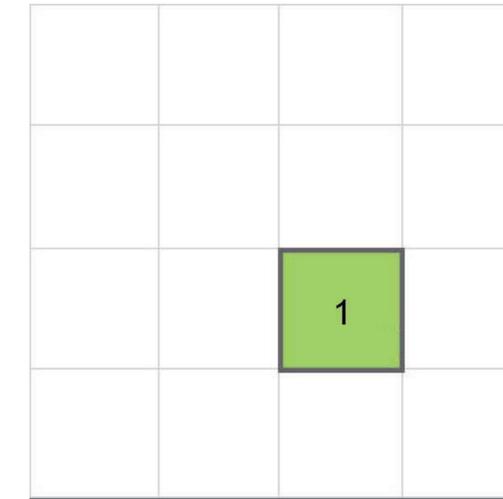
Electron enters detector.



Electron signal is scattered.



Charge collects in each pixel.

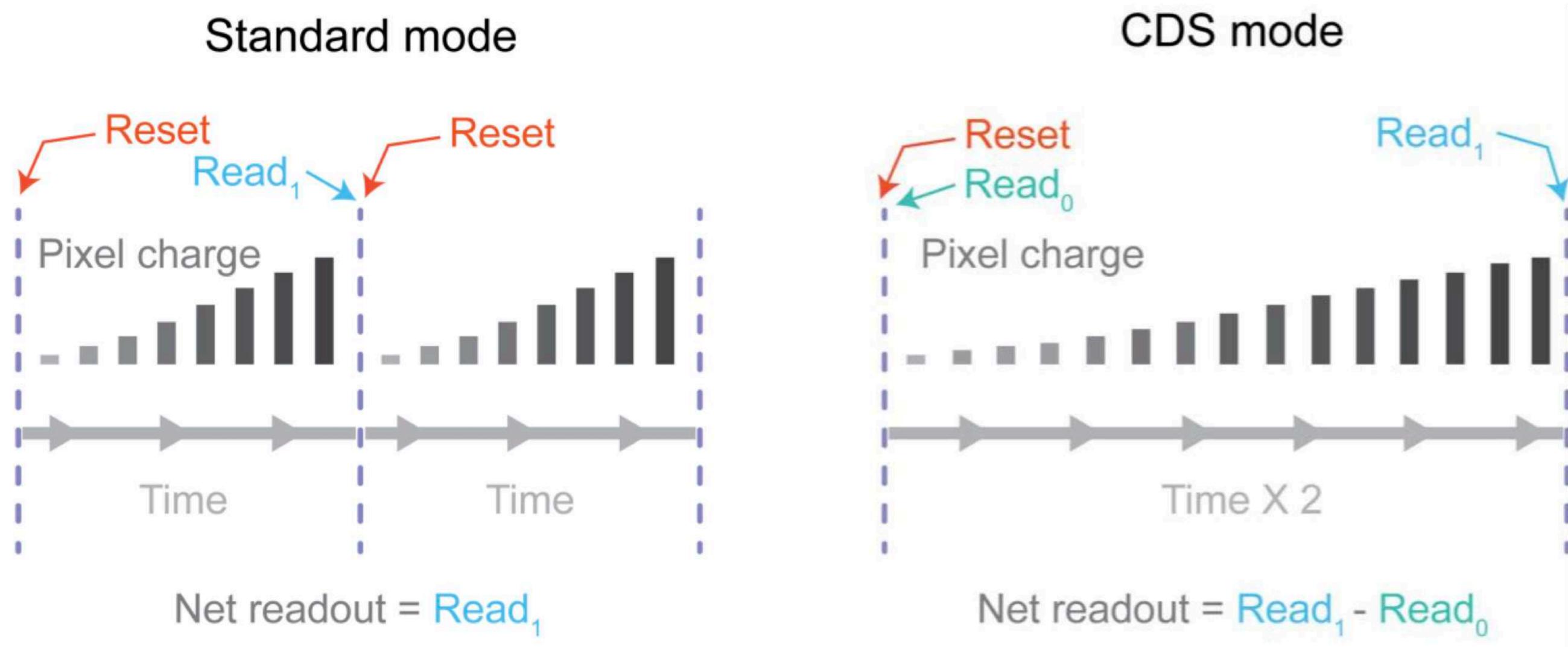


Events reduced to highest charge pixels.

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



K3 lowers Read Noise with Correlated Double Sampling (CDS)



<https://www.gatan.com/>

Detectors

Falcon4 specs

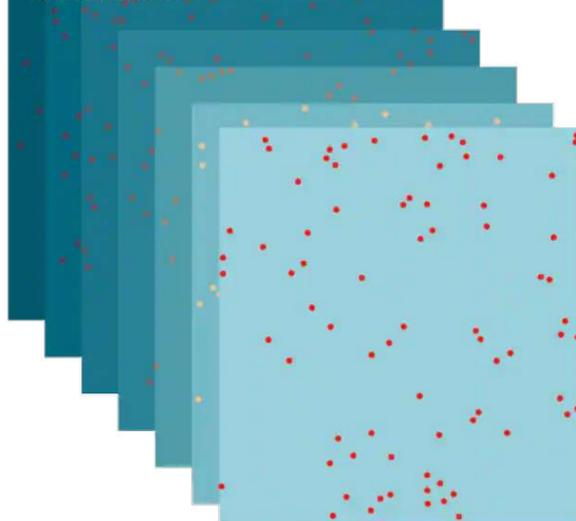


Improving the resolution:
Detecting electrons instead of photons



Camera architecture	Direct electron detection
Sensor size	4,096 × 4,096 pixels, ~ 5.7 x 5.7 cm ²
Pixel size	14 x 14 μm ²
TEM Operating voltage	200 kV, 300 kV
Internal frame rate	320 fps
Frame rate to storage	320 fps (EER mode) Electron-event representation (EER)
Camera Overhead time	0.5 s per acquisition
File formats	EER (native), MRC, TIFF, LZW TIFF
Lifetime (<10% DQE degradation)	5 years in normal use (1.5Ge/px)
Detection Modes	Electron counting mode Survey mode (fast linear mode)
Imaging performance in EER mode (4k x 4k)	300 kV 200 kV
DQE (0)	0.92 0.91
DQE (½ Nq)	0.72 0.62
DQE (1 Nq)	0.50 0.33

Full Temporal Resolution
Record all single frames,
no fractionation



Full Spatial Resolution
All localized events

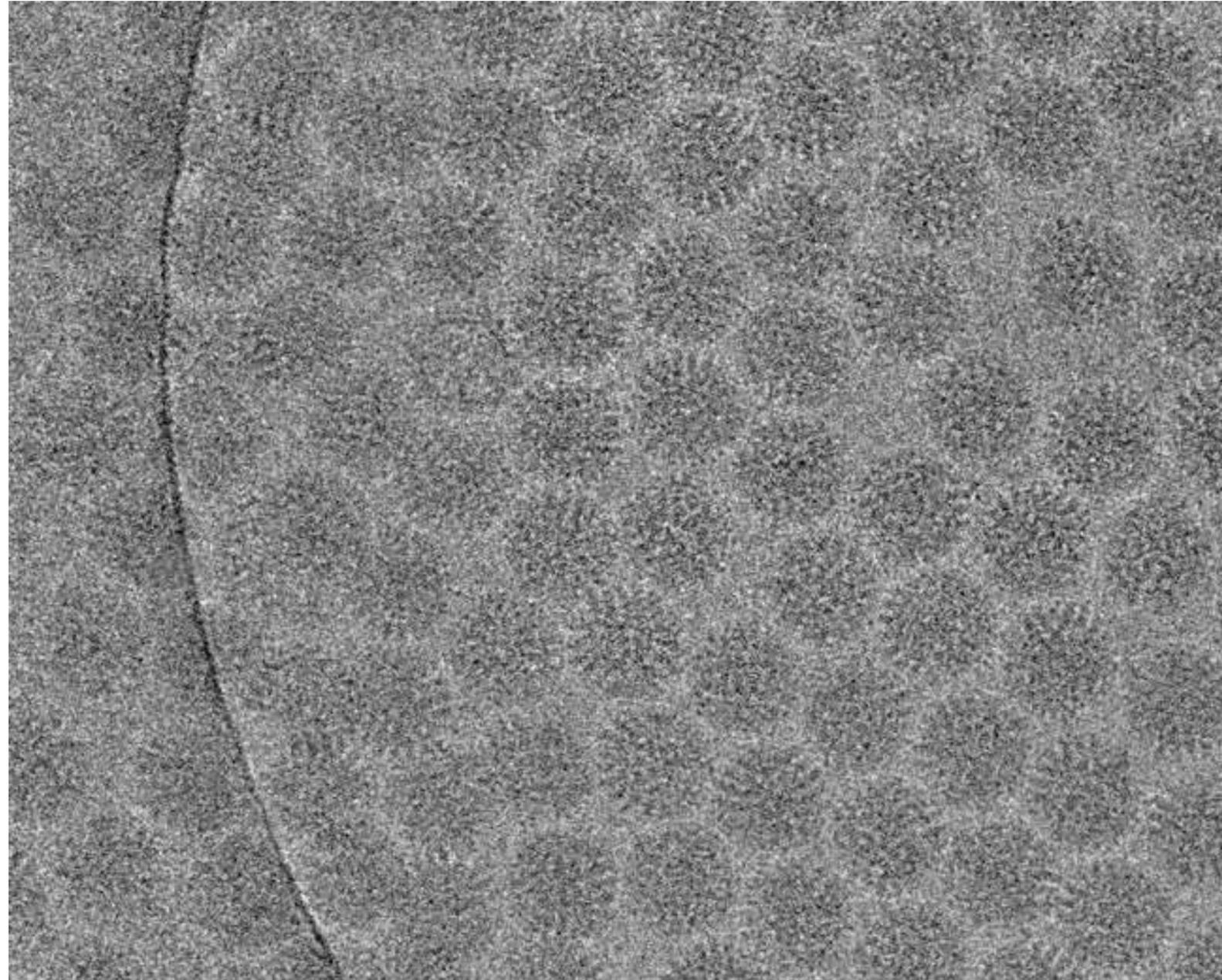
Coordinates	
x	y
3953.24	2845.63
919.78	1447.39
3864.43	348.13
3606.05	1539.54
1758.86	2971.55
...	...
3983.58	531.96

Counted events of all raw frames with full temporal resolution (320 fps) and spatial resolution (events are localized to one-sixteenth of a pixel).

<https://www.thermofisher.com/us/en/home/electron-microscopy/products/accessories-em/falcon-detector.html>

Detectors

Images are movies



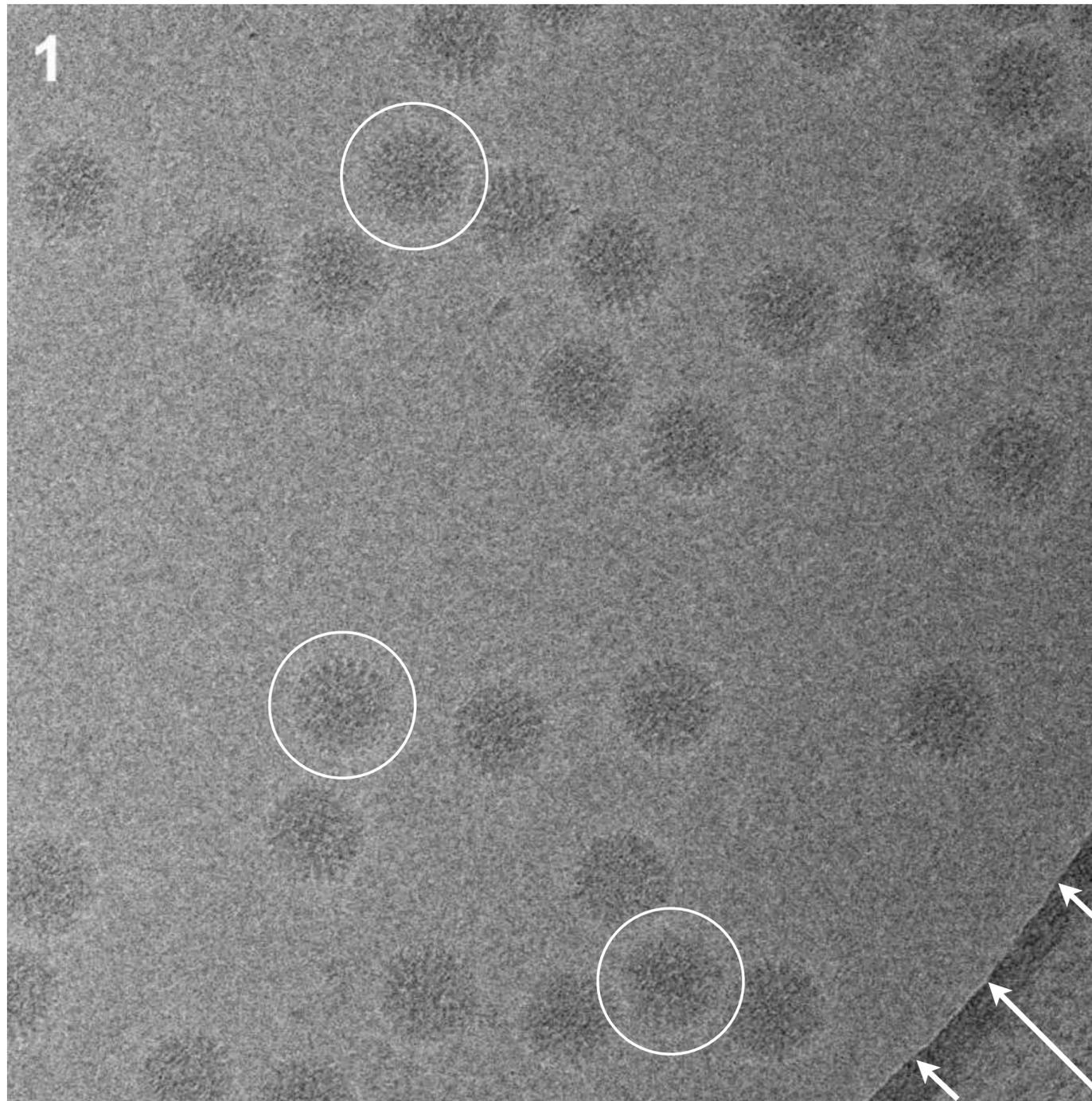
$0.5 \text{ e}^-/\text{\AA}^2/\text{frame}$

Image = Frame1 + Frame2 + Frame3 + Frame4 + Frame5

We can use DDD movies to examine (and correct) “beam induced motion”

Detectors

Images are movies



Each averaged frame
corresponds to 0.25 s.

Dose/frame = $5 \text{ e}^-/\text{\AA}^2$

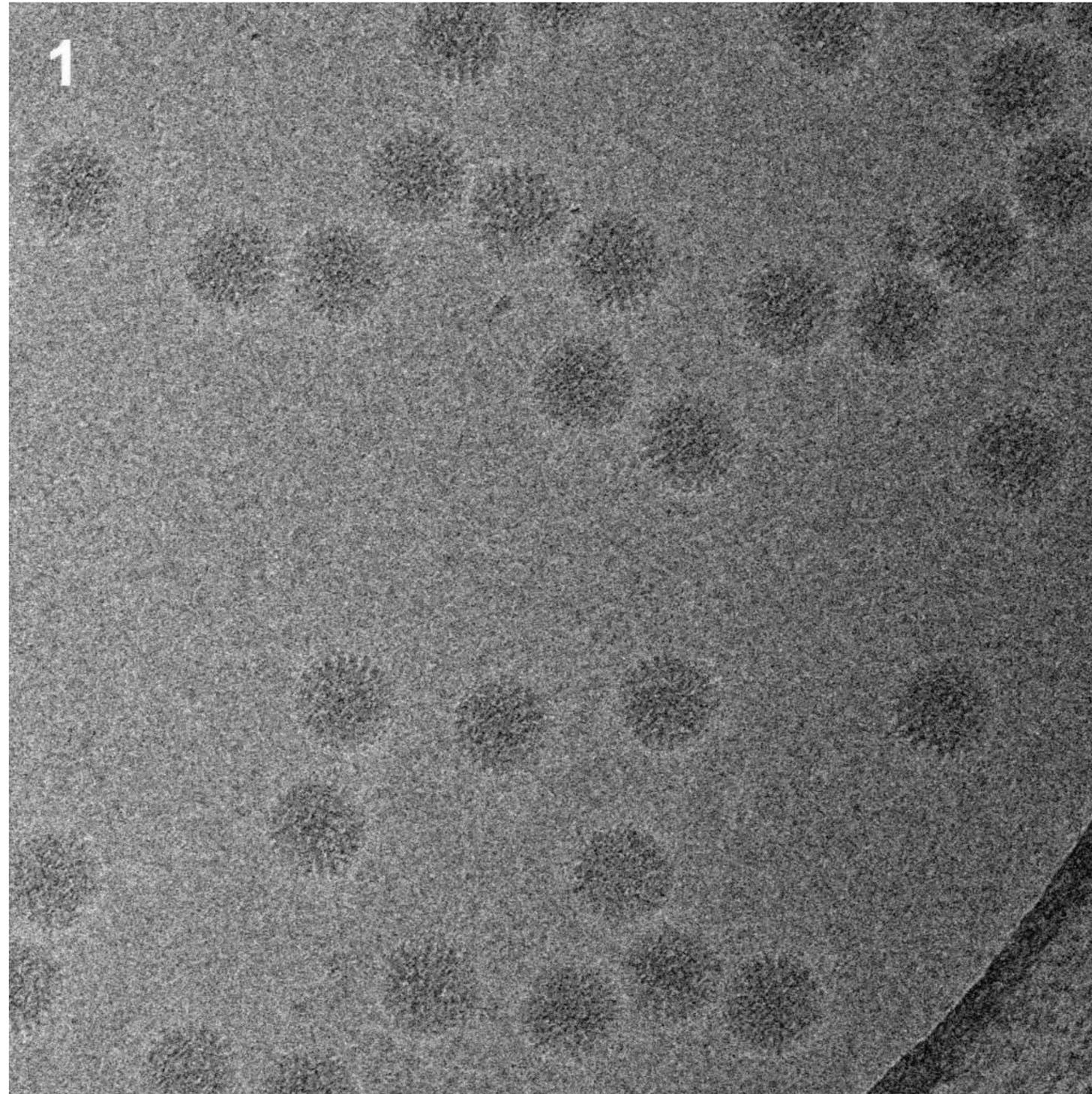
**A “movie” of rotavirus
exposed to electron beam**

10 frame averages

Brilot C.F. et al. (2012) J Struct Biol.

Detectors

Images are movies

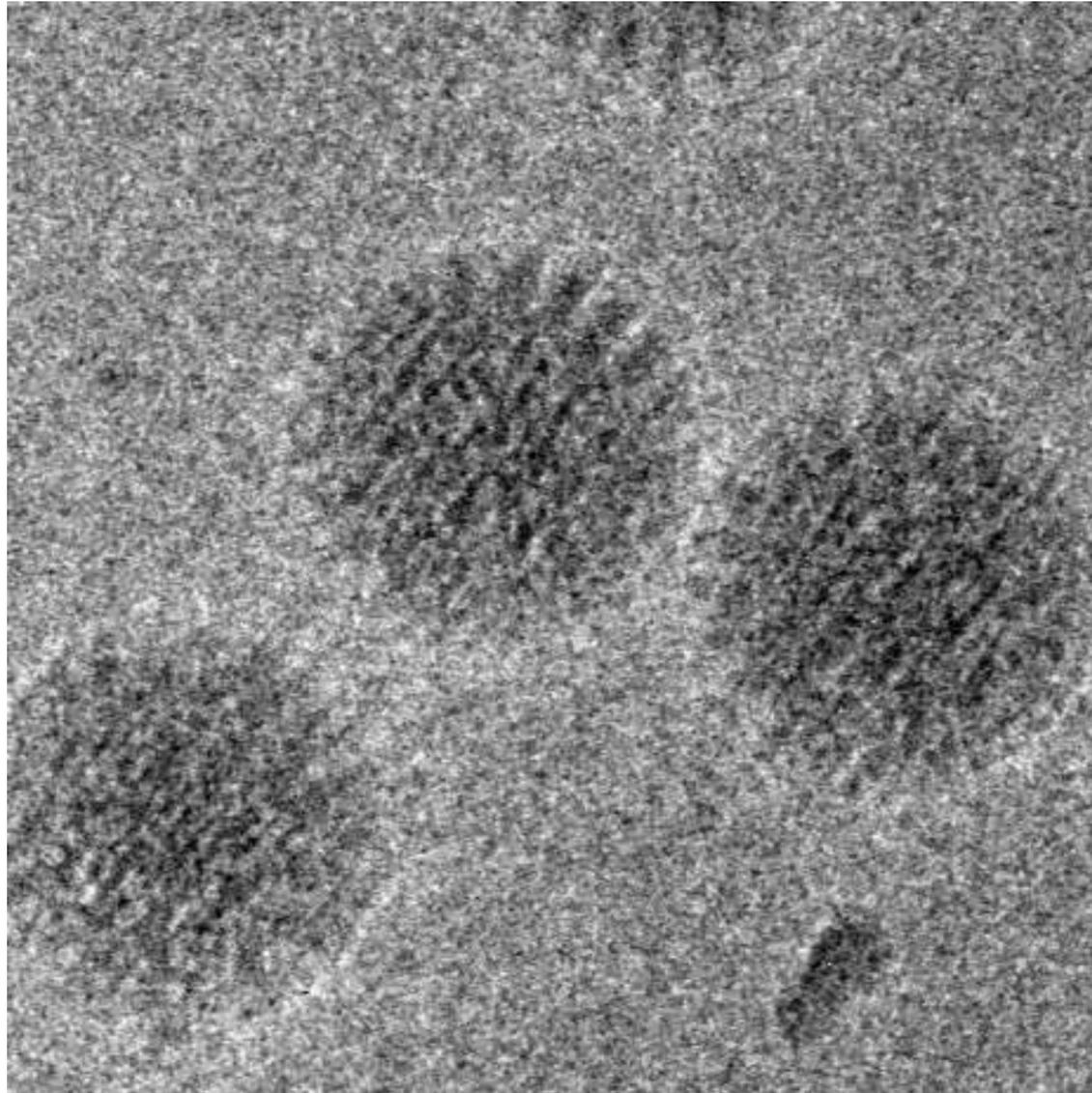


2.5nm

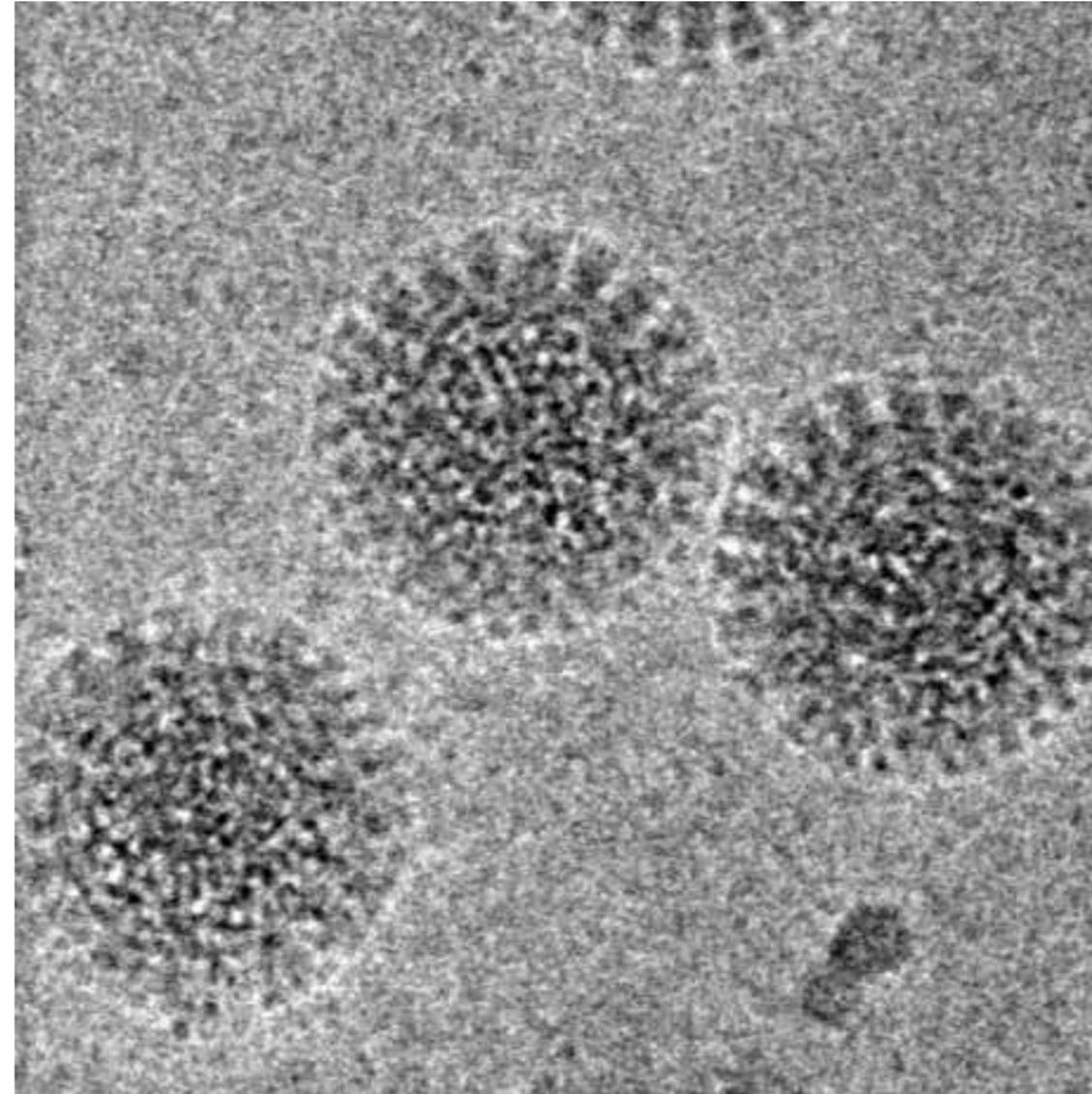
Translations over time



60-frame average
(no alignment)



60-frame average
(translational alignment)



Brilot C.F. et al. (2012) J Struct Biol.

What brought about the resolution revolution?

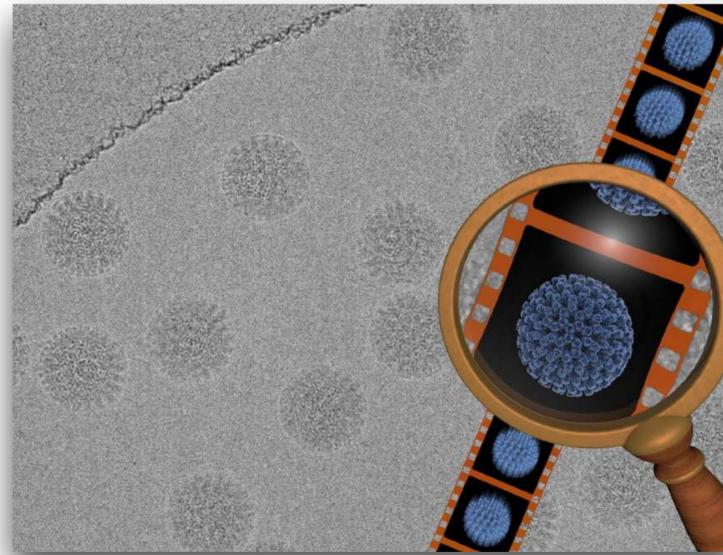
(~2012-2014)

Hardware

Microscopes



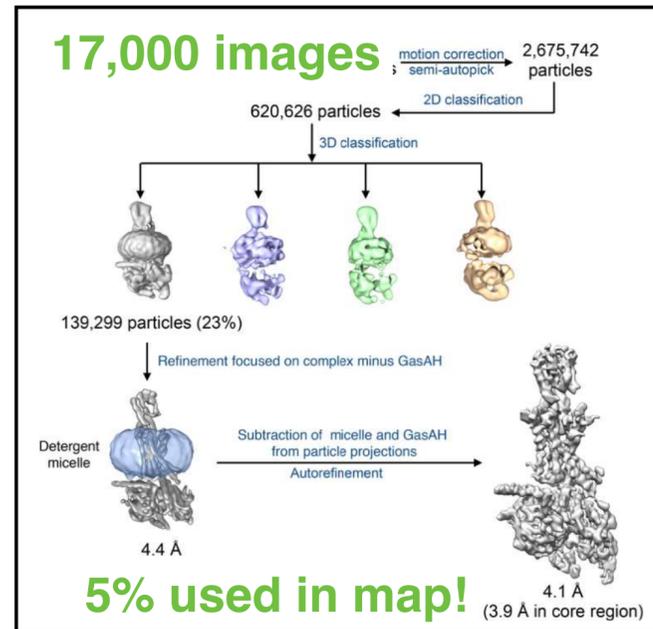
Direct Detectors



Computers



Software

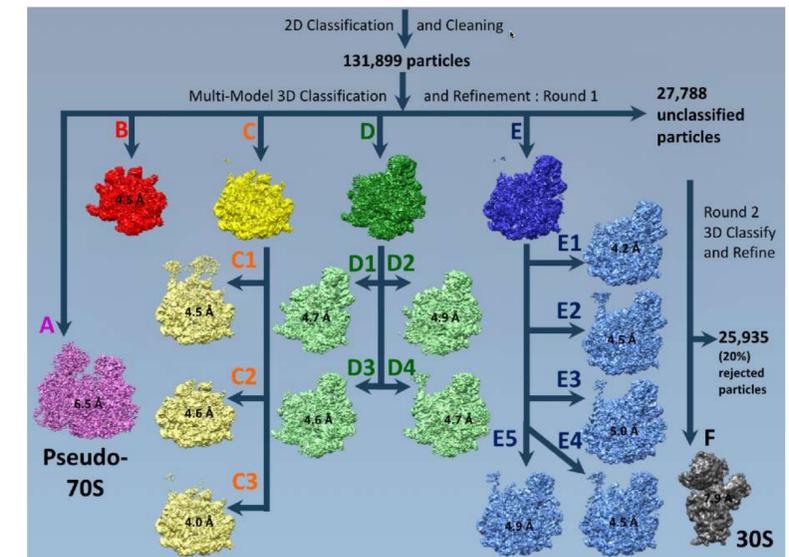


Leginon / SerialEM / EPU, ...

MotionCorr2, Unblur, ...

RELION, FREALIGN/cisTEM, cryoSPARC
EMAN, Sparx, SPHIRE, XMIPP, ...

14 independent structures



What brought about the resolution revolution?

Hardware



Patrick Sexton, PhD

Monash Institute of Pharmaceutical Sciences, Monash University



Denise Wootten, PhD

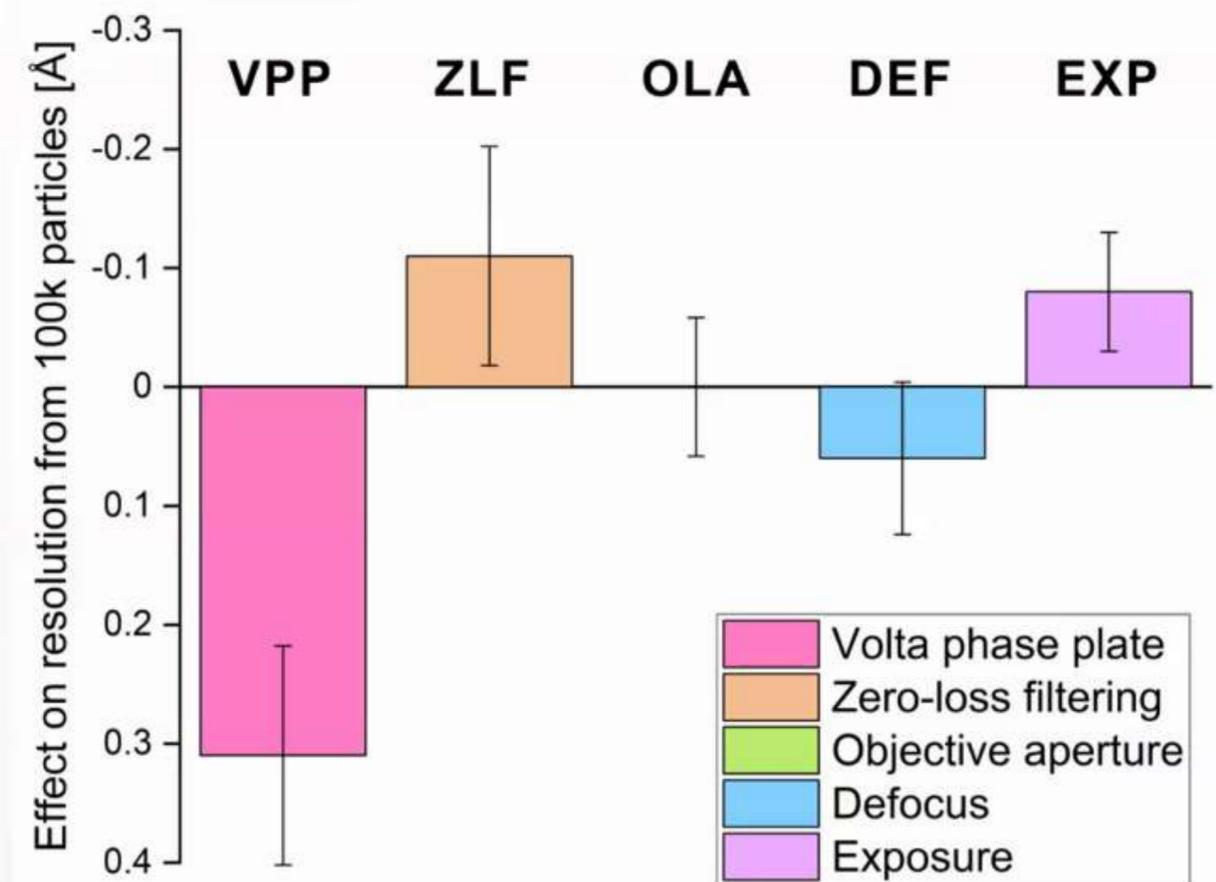
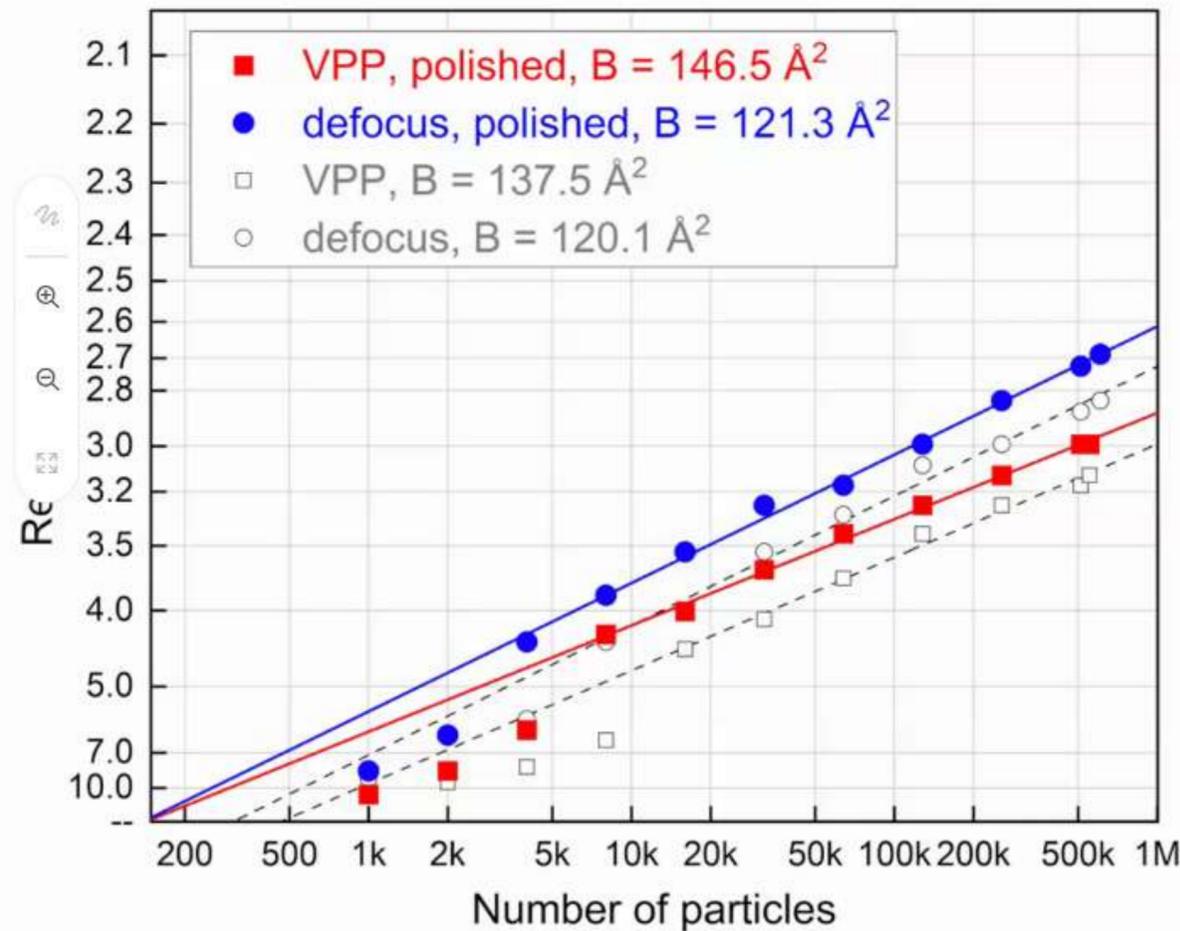
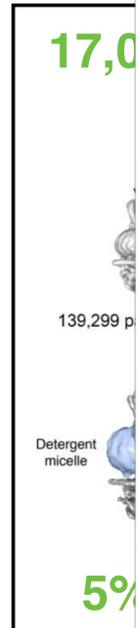
Monash Institute of Pharmaceutical Sciences, Monash University



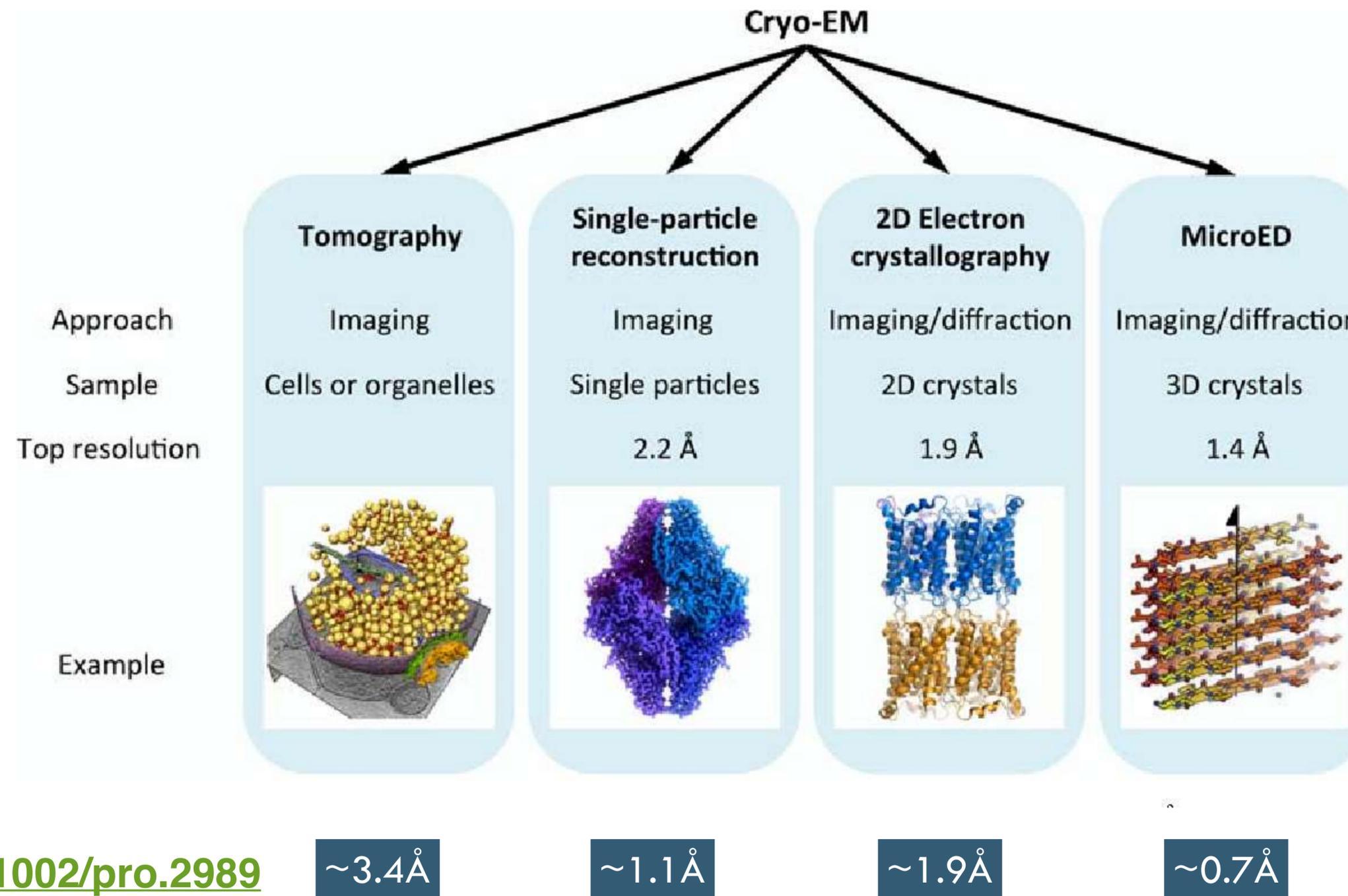
Radostin Danev, PhD

Graduate School of Medicine, The University of Tokyo

Software



Cryoem modalities and tools



<https://doi.org/10.1002/pro.2989>

~3.4Å

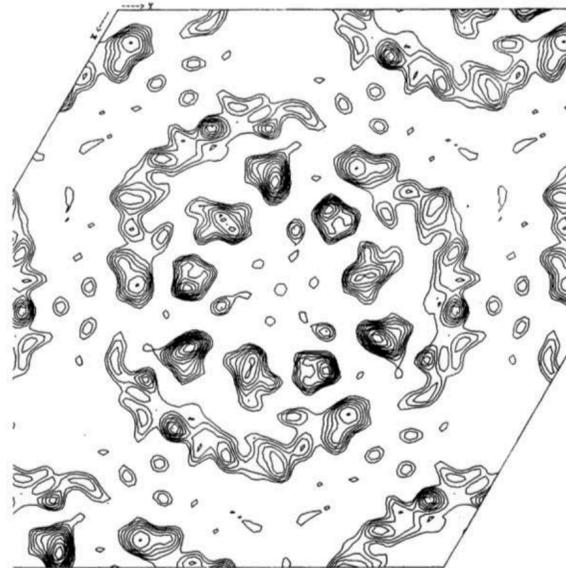
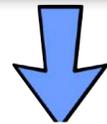
~1.1Å

~1.9Å

~0.7Å

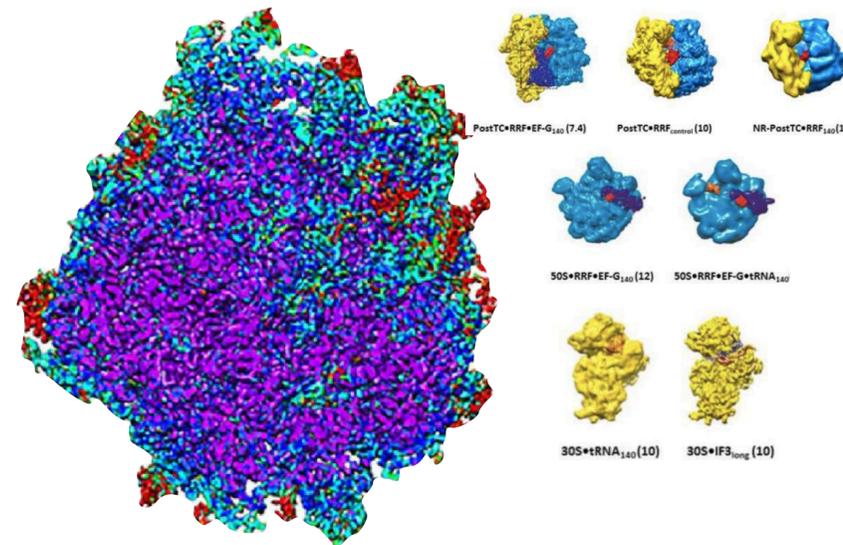
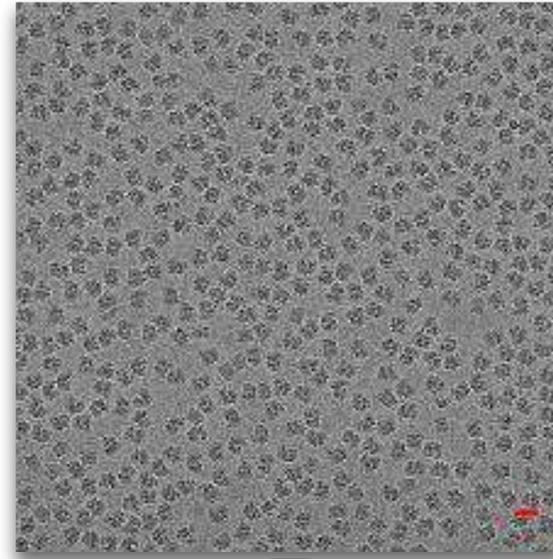
cryoEM: technology on the rise

1986



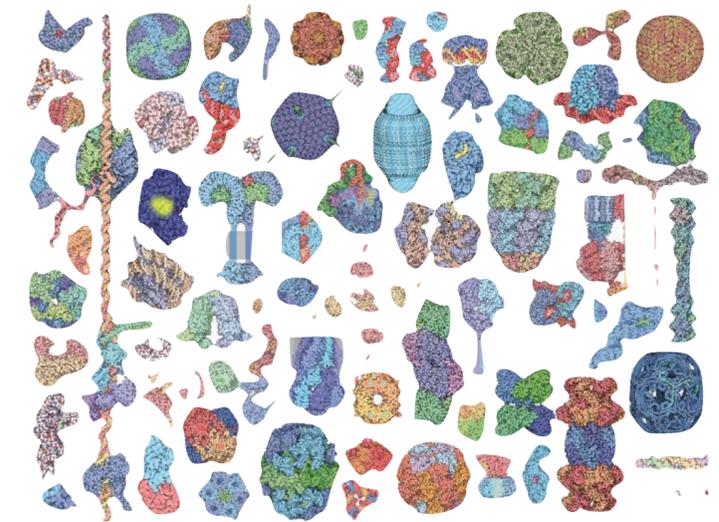
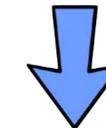
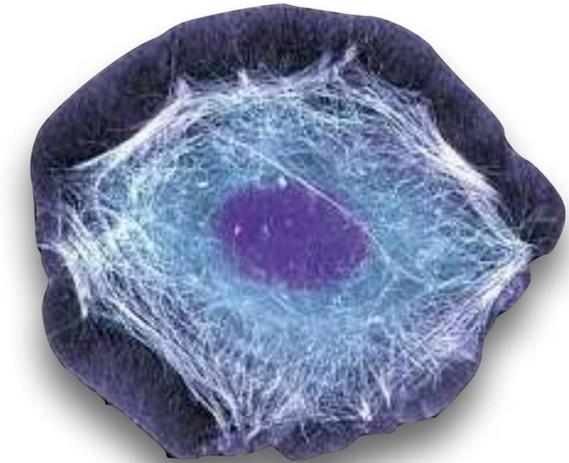
Henderson et al. (1986)

2017



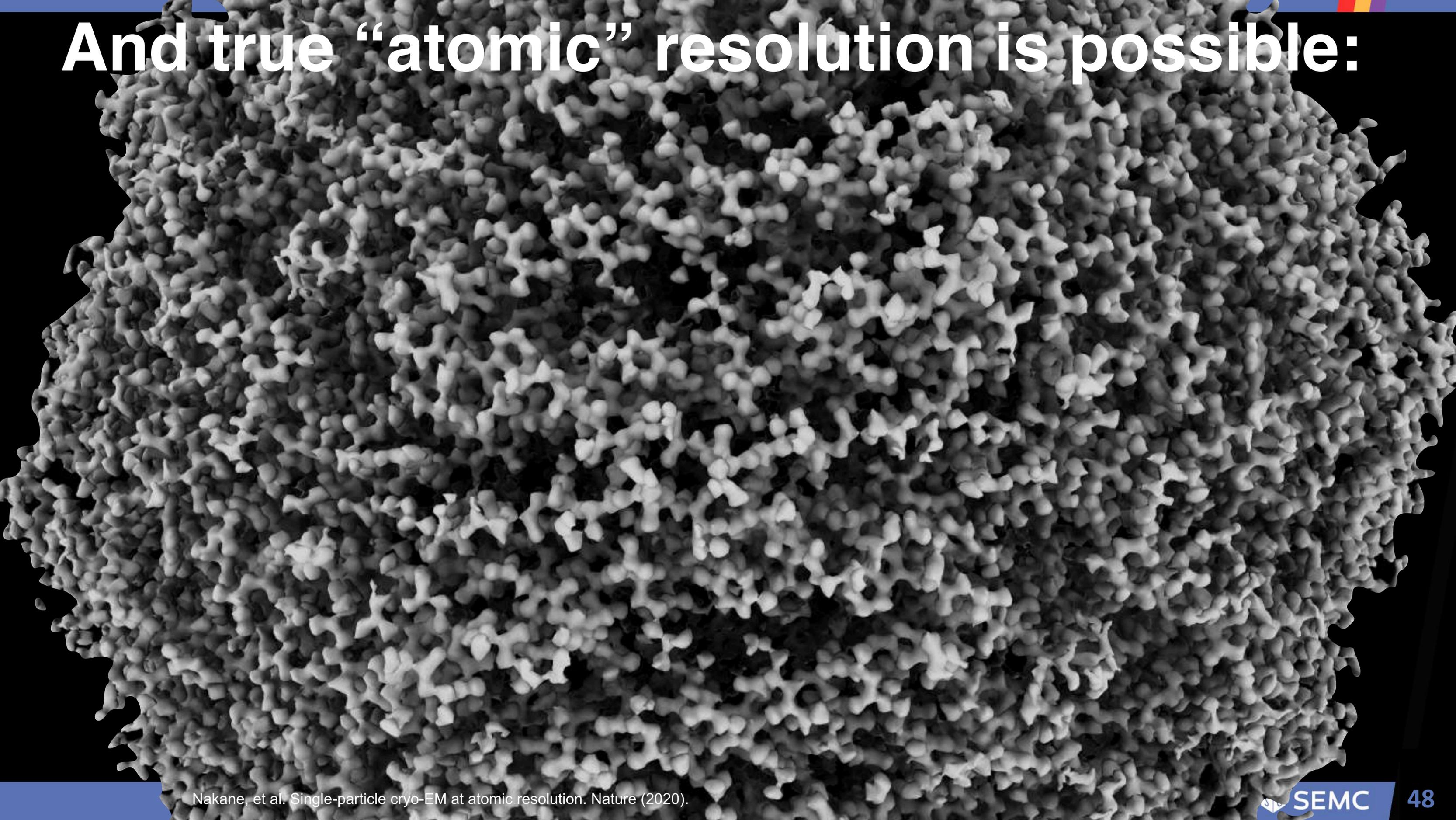
Frank et al. (2017)

in progress

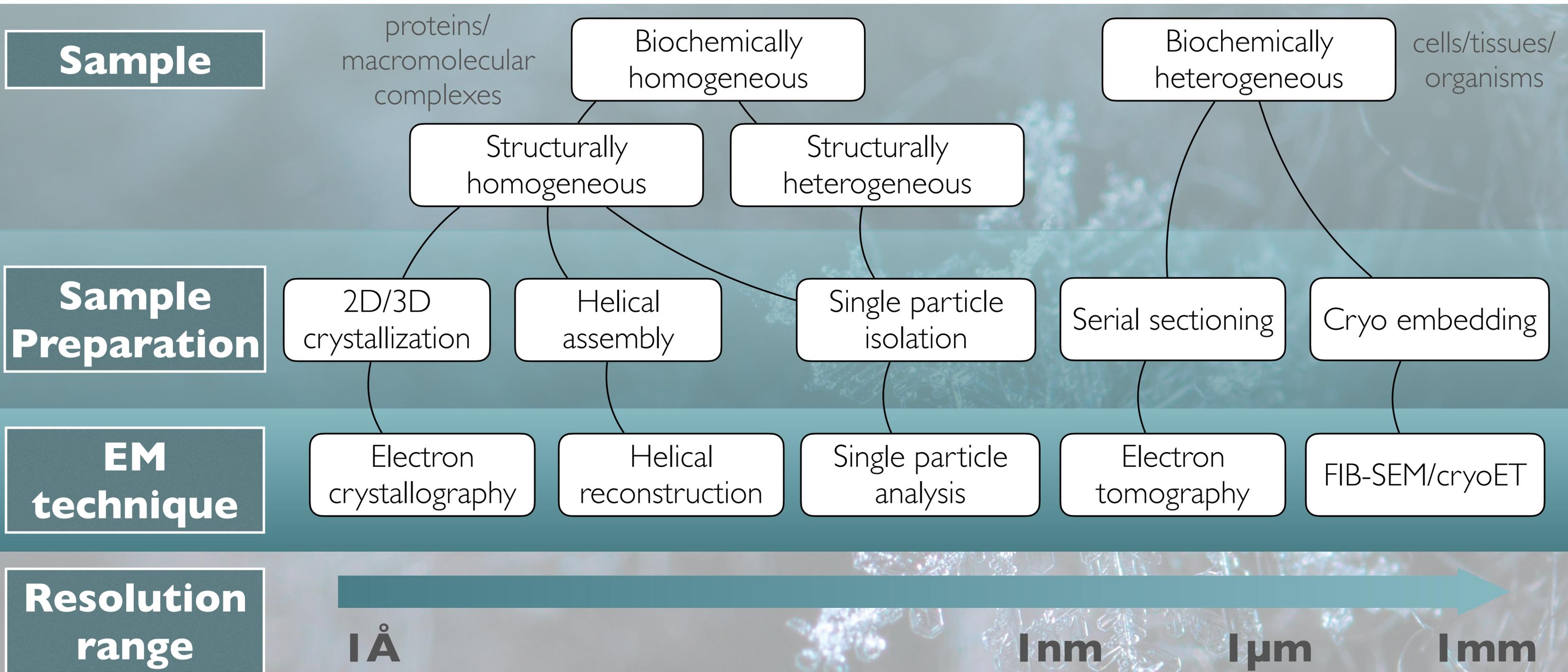


the next chapter

And true “atomic” resolution is possible:



How are samples prepared for cryoEM?





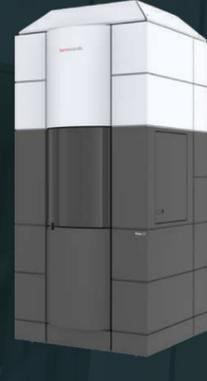
**Krios1
G2**



**Krios2
G2**



**Krios3
G2**



**Krios4
G3i**



**Krios5
G3i**



**Krios6
G3i**



**Krios7
G4**



**Krios8
G3i**



**Krios9
G3i**



**Glacios1
G1**



**Glacios2
G1**



**Glacios3
G2**



**Glacios4
G1**



**Hitachi
7800**



Tundra



Aquilos2



**Helios5
Hydra**



Arctis



CUBEII

Support: NIH NIGMS. Common Fund; NYSTAR

SIMONS FOUNDATION

Simons Resource for Automated Molecular Microscopy nramm.nysbc.org
National Center for CryoEM Access and Training nccat.nysbc.org
National Center for In-situ Tomographic Ultramicroscopy ncitu.nysbc.org
Simons Machine Learning Center smlc.nysbc.org
Simons Electron Microscopy Center semc.nysbc.org

The start

Questions?