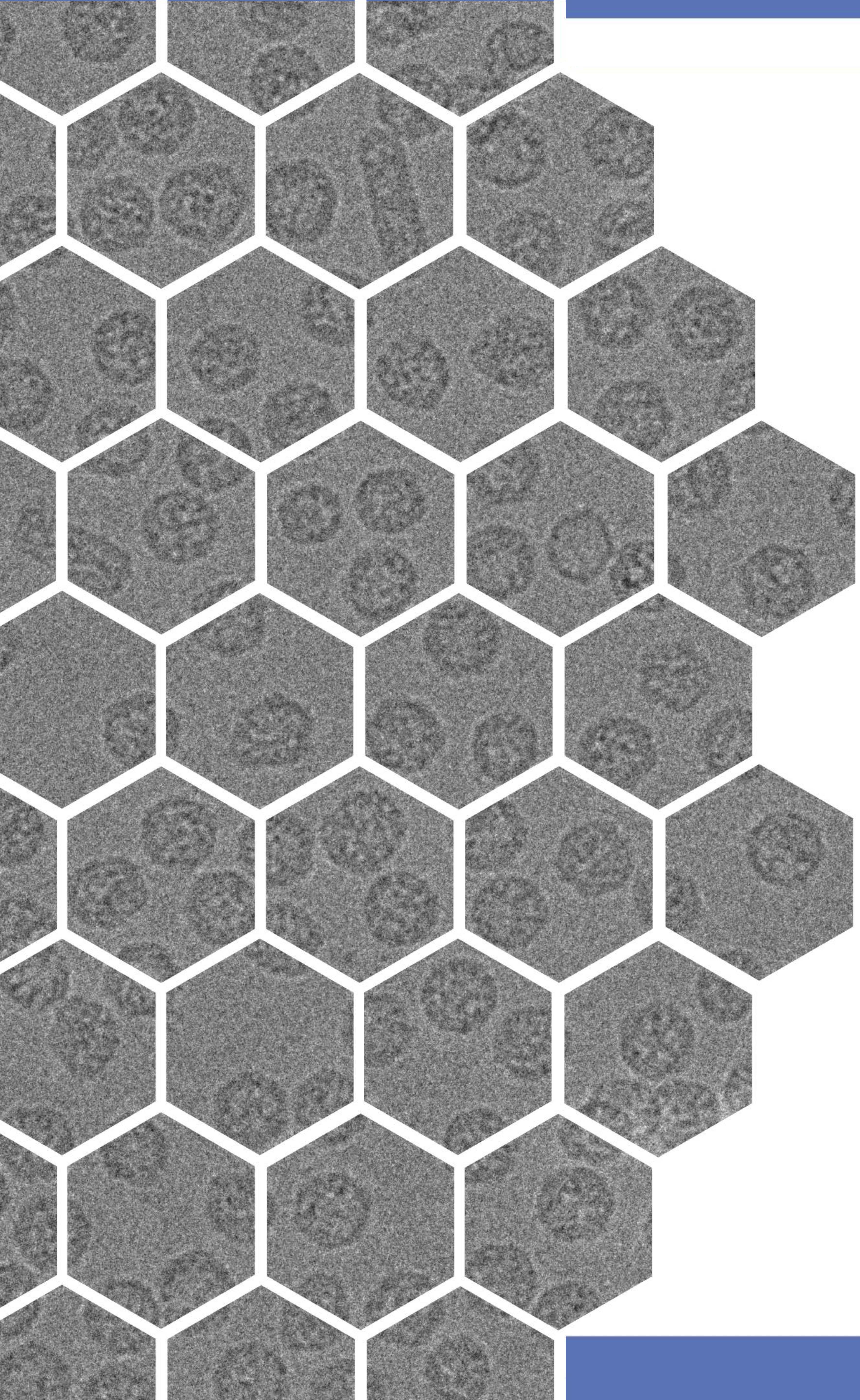
2026 Spring cryoEM course

Welcome and Anatomy of an EM

January 22, 2026

NYSBC SEMC



- Course outline
- Student survey
- Anatomy of an EM

Welcome to SEMC

21st year of the course

SIMONS ELECTRON MICROSCOPY CENTER • GUILD OF MICROSCOPISTS •

Course logistics: main website

https://semc.nysbc.org/workshops/2026-em-course/

SIMONS ELECTRON MICROSCOPY CENTER

HOME ABOUT PEOPLE PUBLICATIONS RESOURCES COURSES JOBS FIND US

WORKSHOPS & COURSES

CURRENT/UPCOMING COURSES | PAST COURSES

EM Courses:

2026
2025
2024
2023
2022
2021
2020
2019
2018
2017
2016

The Winter-Spring 2026 EM Course

About the course

Electron microscopy in combination with image analysis is increasingly powerful in producing 3D structures of individual molecules and large macromolecular complexes that are unapproachable by other methods. This course is focused on the concepts and theories behind electron microscopy. Each week guest lecturers and SEMC staff lead discussions on the practice of solving molecular structures by electron microscopy. Students will be responsible for watching relevant sections from Getting Started in Cryo-EM and cryoEM101 ahead of attending the lectures.

The course will be held at the New York Structural Biology Center at 89 Convent Ave (133rd St).

Course Schedule

Classes in NYSBC A-11 seminar room (Mondays 3:30-5pm and select Wednesdays 3:30-5pm)

EM fundamentals section
Jan 19: Martin Luther King Holiday (no class)
Jan 21: Lecture – Introduction & Basic anatomy of the electron microscope (Ed Eng – NYSBC/SEMC & SEMC staff)
Jan 26: Lecture – New cryoEM hardware and supporting a facility (Michael Alink – NYSBC/SEMC)
Jan 28: Practical – TEM use (SEMC staff [worksheet])
Feb 2: Lecture – Considerations for biological cryoEM (Ed Eng – NYSBC/SEMC & SEMC staff)
Feb 4: Practical – Sample Preparation & Support films (SEMC staff [worksheet])

EM crystallography section
Feb 9: Lecture – MicroED (Bill Rice – New York University)
Feb 11: Practical – Journal club (On sample vitrification)
Feb 16: President's Day Holiday (no class)
Feb 18: Practical – Journal club (On the best keV for biological samples)
Feb 23: Lecture – Helical reconstruction (Hernando Sosa – Albert Einstein College of Medicine)
Feb 25: Practical – Journal club

Tomography section
Mar 2: Lecture – Tomography (Alex de Marco, NYSBC/SEMC)
Mar 4: Lecture – Tomography software (Jake Johnston — NYSBC/Columbia University)
Mar 9: Lecture – FIB-SEM (Bill Rice – New York University)
Mar 11: Practical – Journal Club

- Course Administrator:
  - Ed Eng (eeng@nysbc.org)

- Teaching Assistants:
  - Mahira Aragon (NYSBC)
  - Ricky Pegeuro (NYSBC)
  - and others

Course logistics: Slack channel

Make sure you're on the email list!

Class Slack channel
#semc-2026-cryoem-course

https://join.slack.com/t/nccatworkshops/shared_invite/zt-1aayp8pok-sgO3cB9oeifBfrl6IsTTyw

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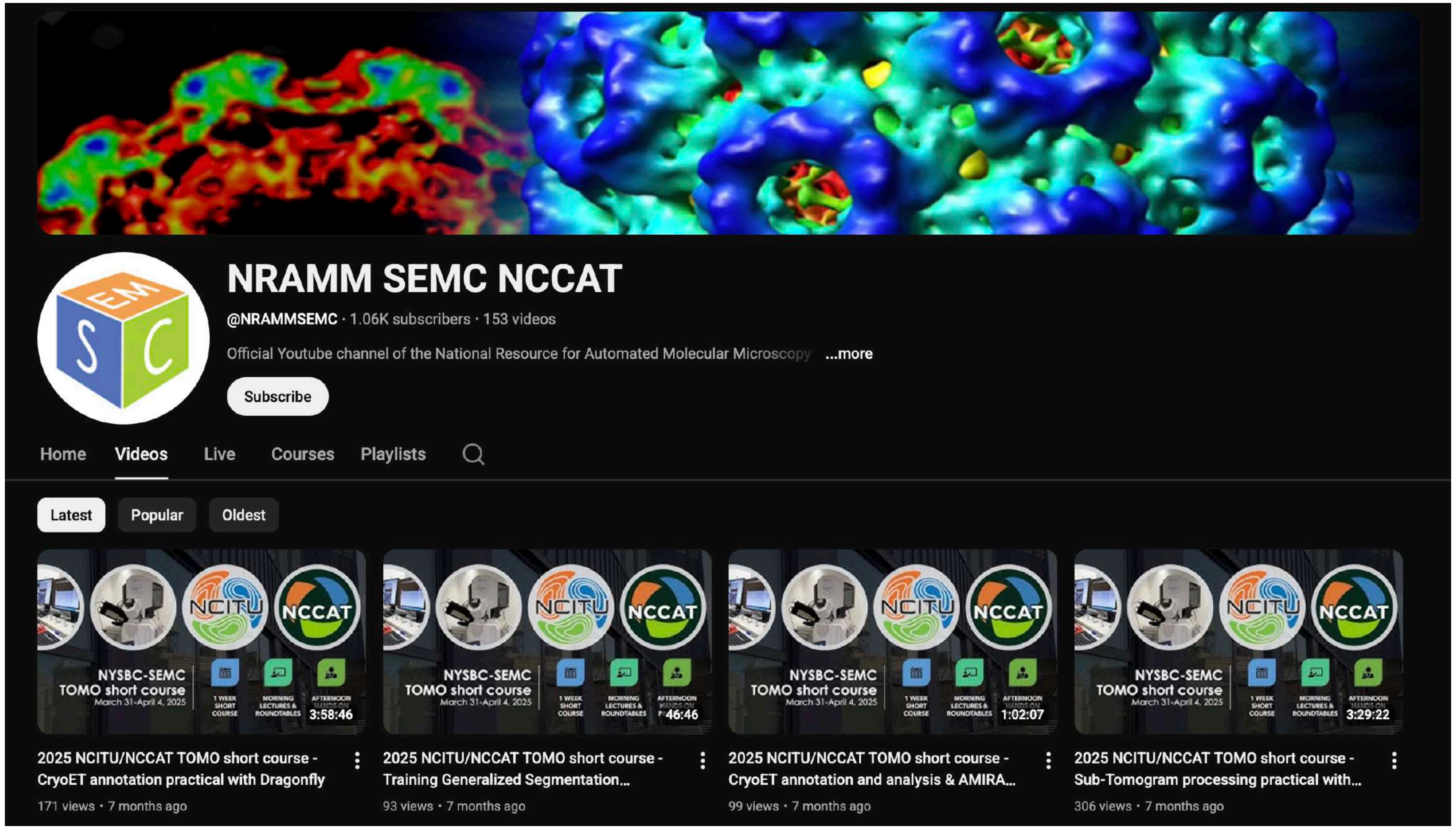
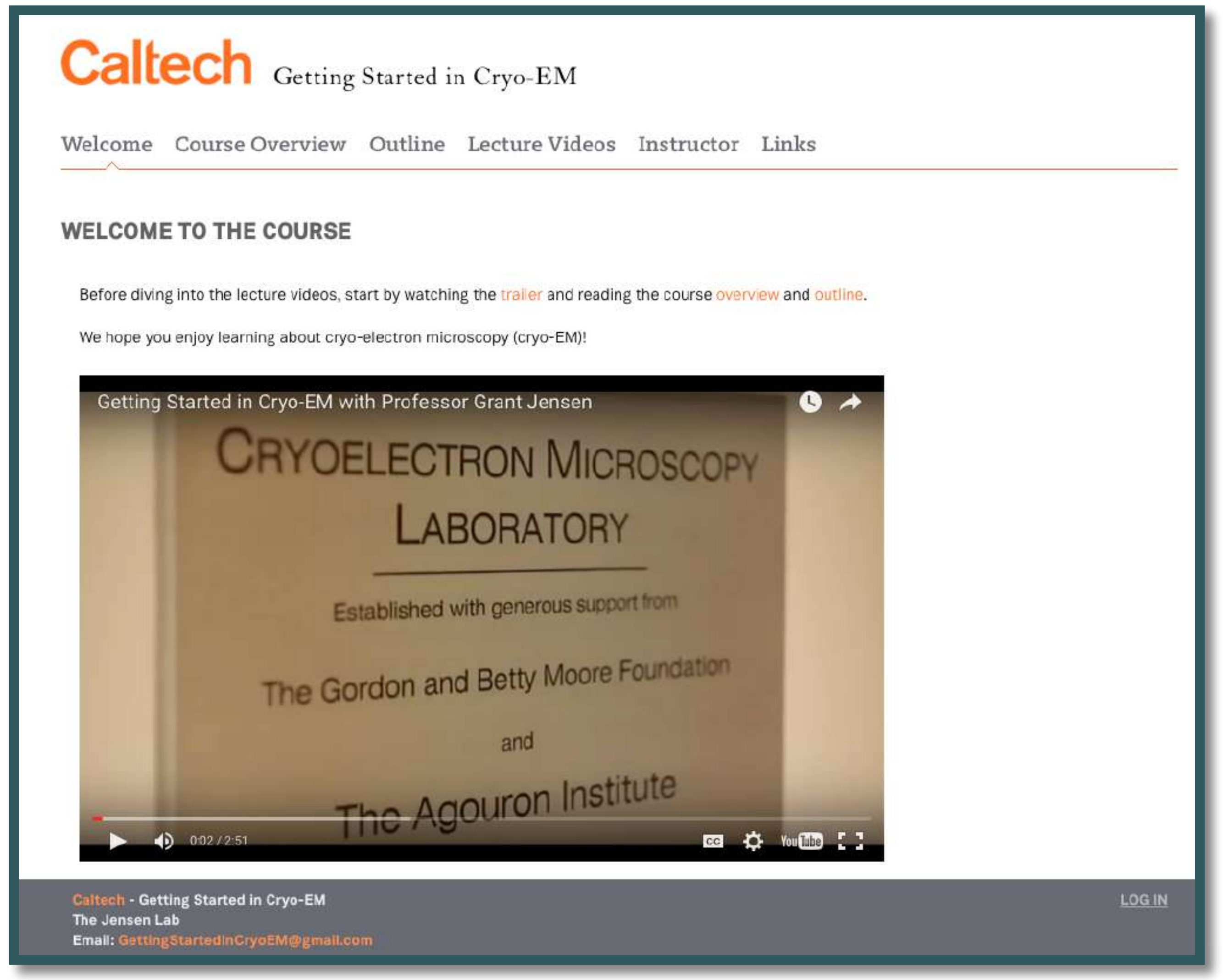
Course logistics: additional resources

cryo-em-course.caltech.edu/videos

youtube.com/nrammsemc





cryoem101.org

Course logistics: main topics

Section 1 : EM fundamentals

Section 2 : EM crystallography

Section 3 : Tomography

Section 4 : Single Particle Analysis Short Course
March 16-20

Section 5 : Future perspectives

Course logistics: main topics

Sect
Sect
Sect
Sect
Sect

1 week.

Morning lectures.

Afternoon practicals.

March 16-20, 2026.

students/postdocs $375.
staff/PIs/professionals $450.
industry $625.

Room/Accomodations NOT included.

JOIN THE WAITLIST.

Standard applications will close December 15, 2025. Late applications will be considered up to January 2, 2026.

Accepted applicants will be notified early January 2026.

For more information email nccatuseroffice [at] nysbc.org

Single Particle Short Course
March 16-20, 2026

NCCAT will offer a 1 week workshop focused on the theory and practice of single-particle analysis. The mornings are filled with lectures and stimulating round table discussions. The afternoons provide hands-on practicals to reinforce fundamental concepts and topics covered earlier in the day.

Short-course Agenda

Monday, March 16, 2026

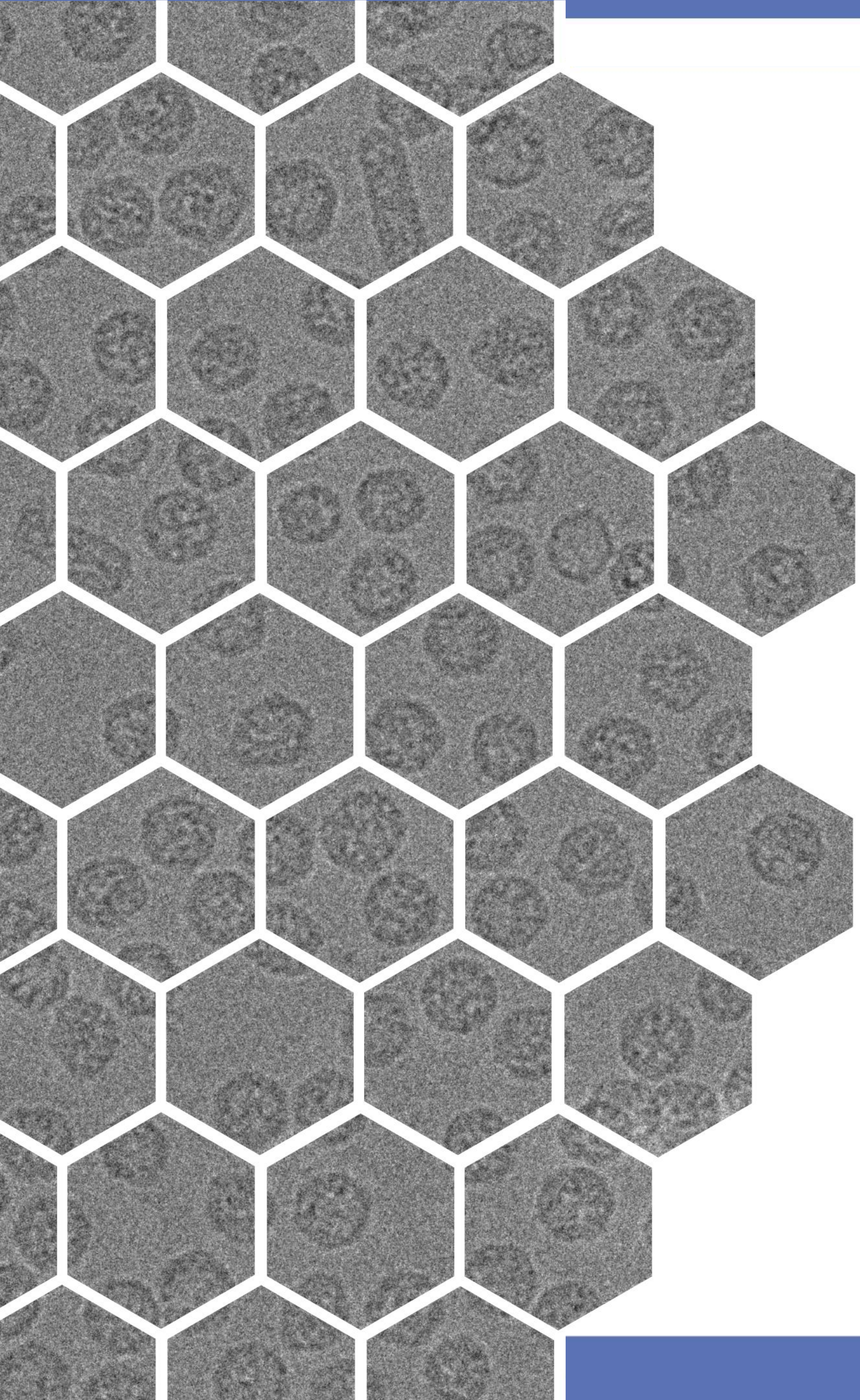
Session	Type	Lecturer	Topic
Morning	Lecture 1 – Keynote	Joachim Frank (Columbia University)	Intro and overview of SPA
Morning	Roundtable 1	students	Flashtalks from students
Afternoon	Lecture 2	SEMC staff	Sample preparation for biological samples
Afternoon	Practical 1	SEMC staff	cryoEM sample prep and grid prep stations

Tuesday, March 17, 2026

Session	Type	Lecturer	Topic
Morning	Lecture 3a	SEMC staff	Microscopes and tools of the trade
Morning	Lecture 3b	SEMC staff	Maintenance of cryoEM facilities

Course logistics: class for credit

Component	Percentage
Recitation/Participation	50%
- *JC/HW/questions*	
Practicals	10% x 3
Attendance	20%



- Course outline
- Student survey
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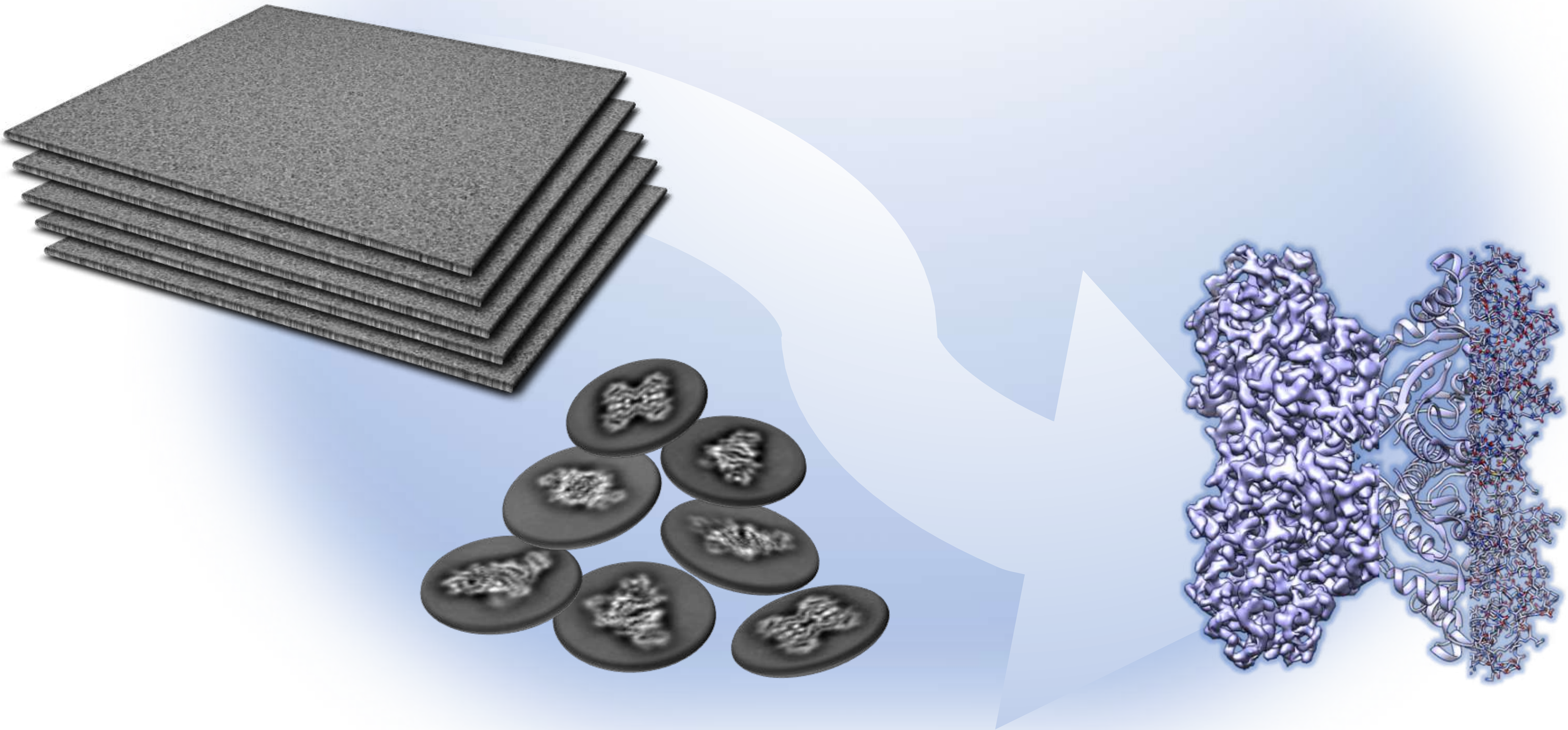
Illustration by David S. Goodsell
RCSB Protein Data Bank.

Illustration by David S. Goodsell RCSB Protein Data Bank.

What is possible today?



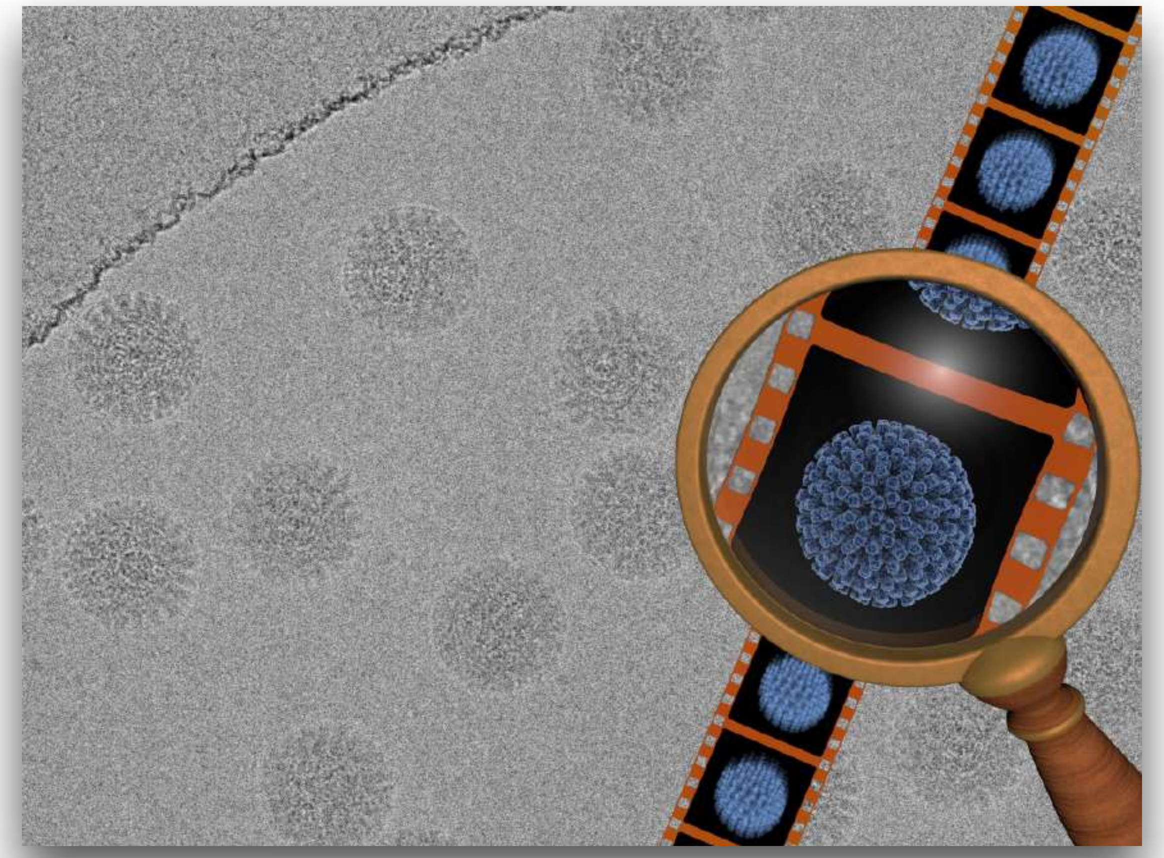
What brought about the resolution revolution?

(~2012-2014)

Hardware

Microscopes

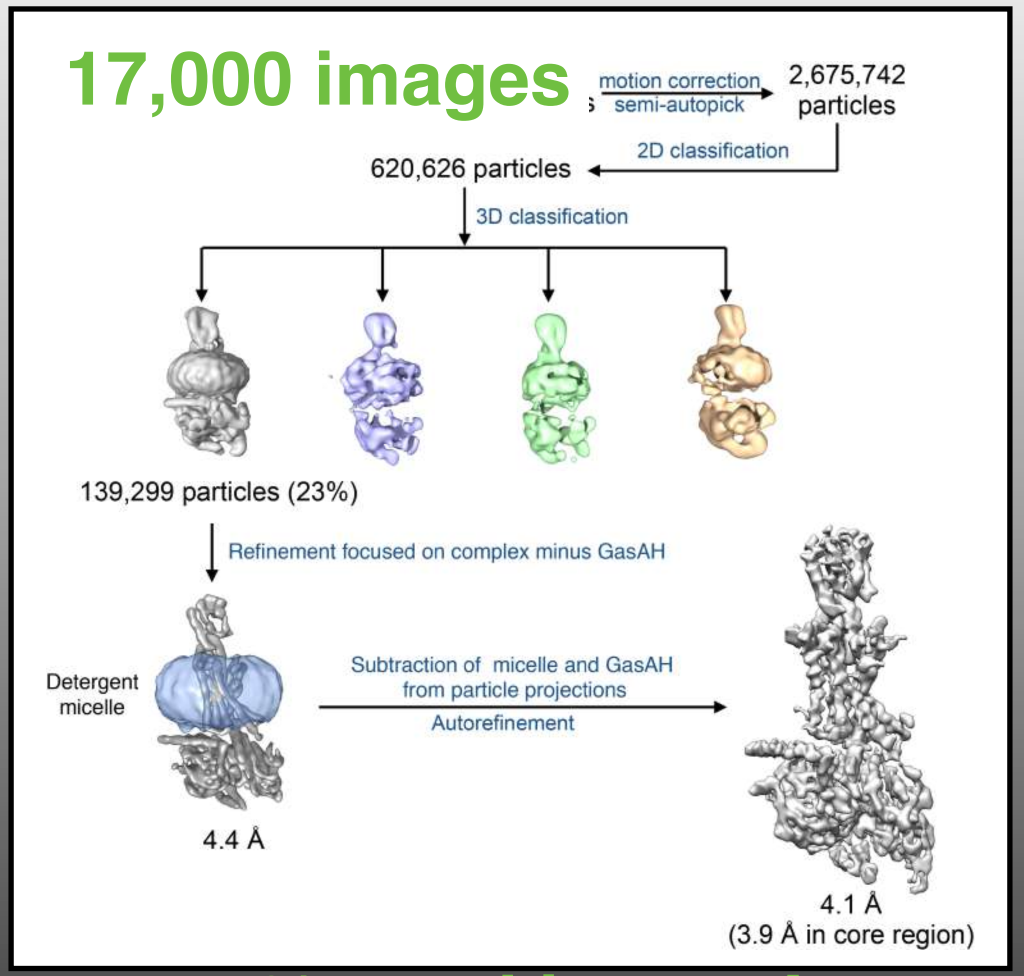
Direct Detectors



Computers

2012->2017
Cost reduced by 100x

Software



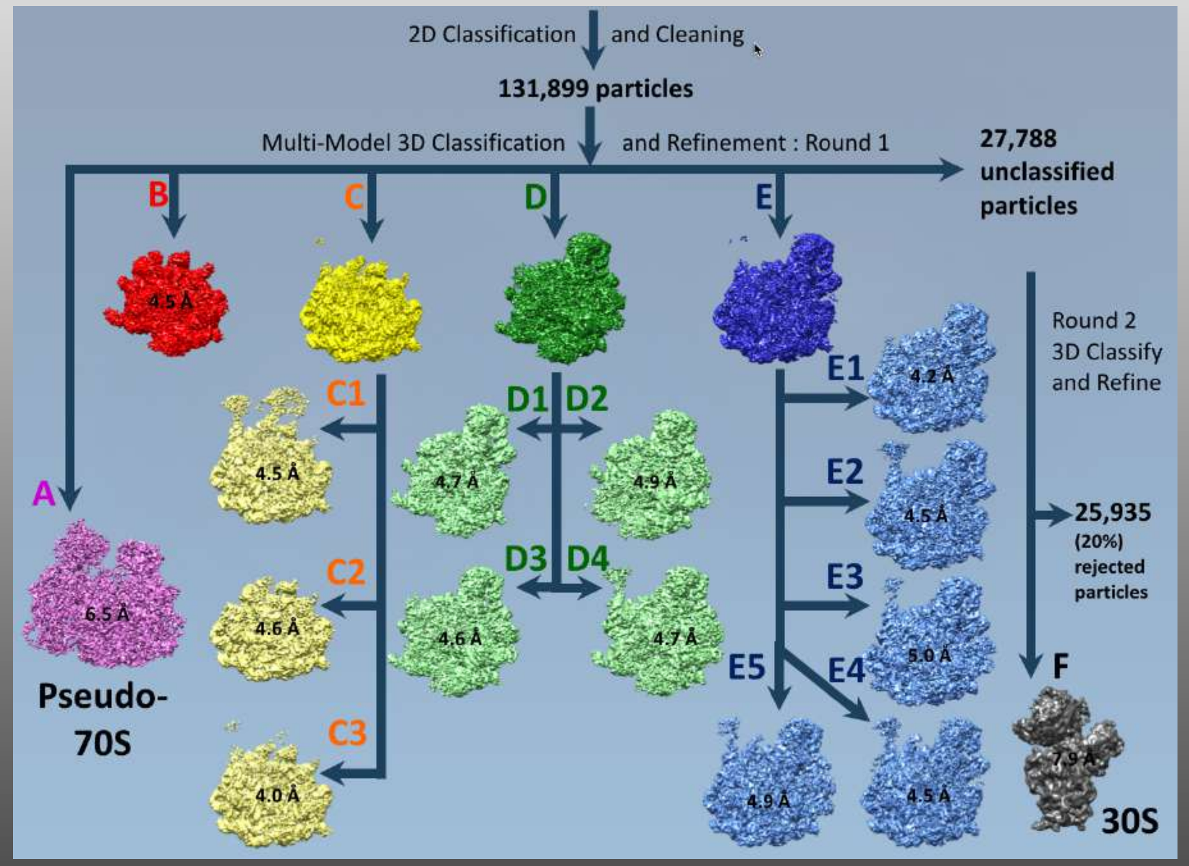
5% used in map!

Leginon / SerialEM / EPU, …

MotionCorr2, Unblur, …

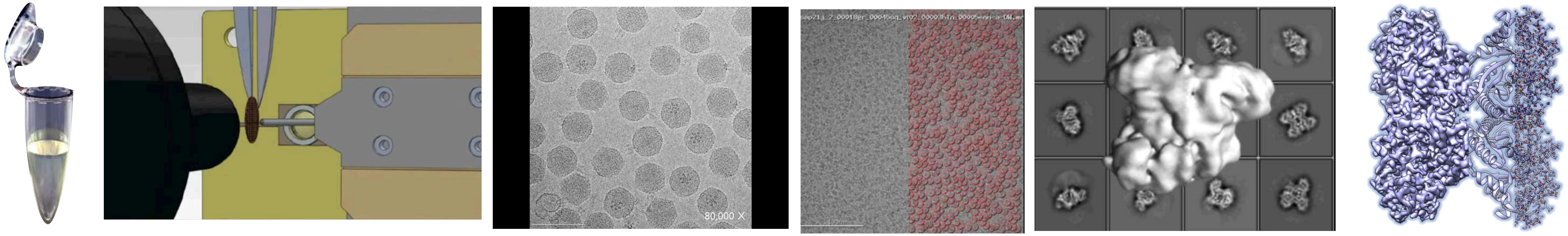
RELION, FREALIGN/cisTEM, cryoSPARC
EMAN, Sparx, SPHIRE, XMIPP, …

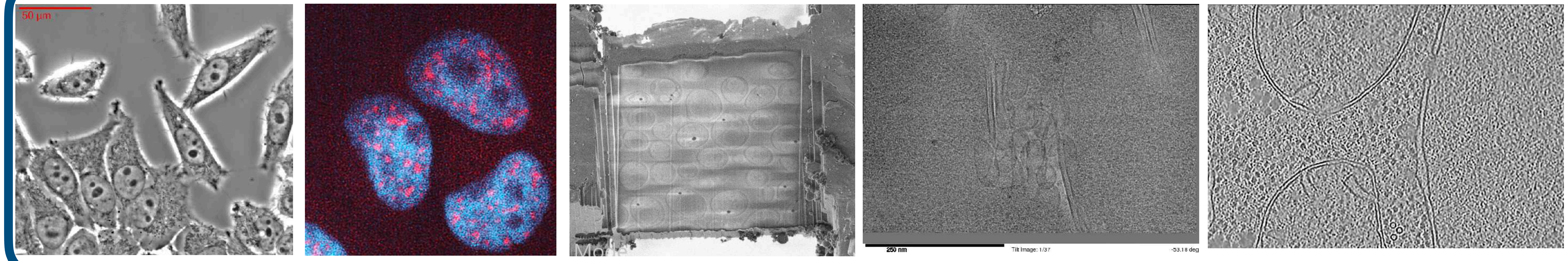
14 independent structures



cryoEM: a technology on the rise

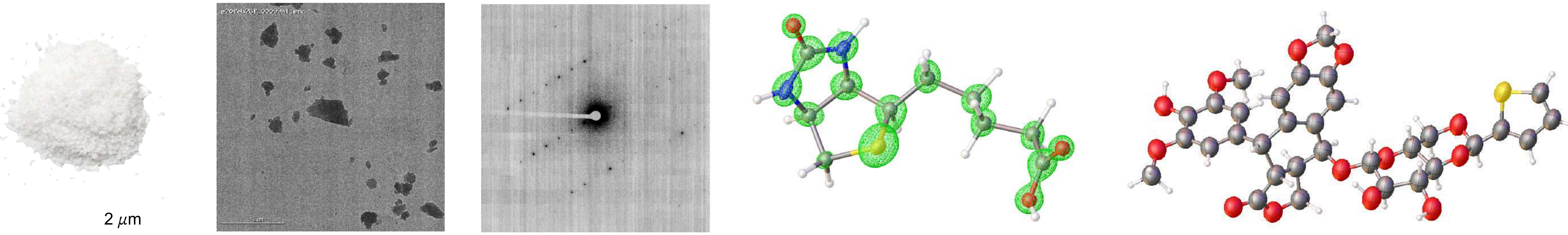
Single particle cryoEM





Cryo Electron Tomography (cryoET)

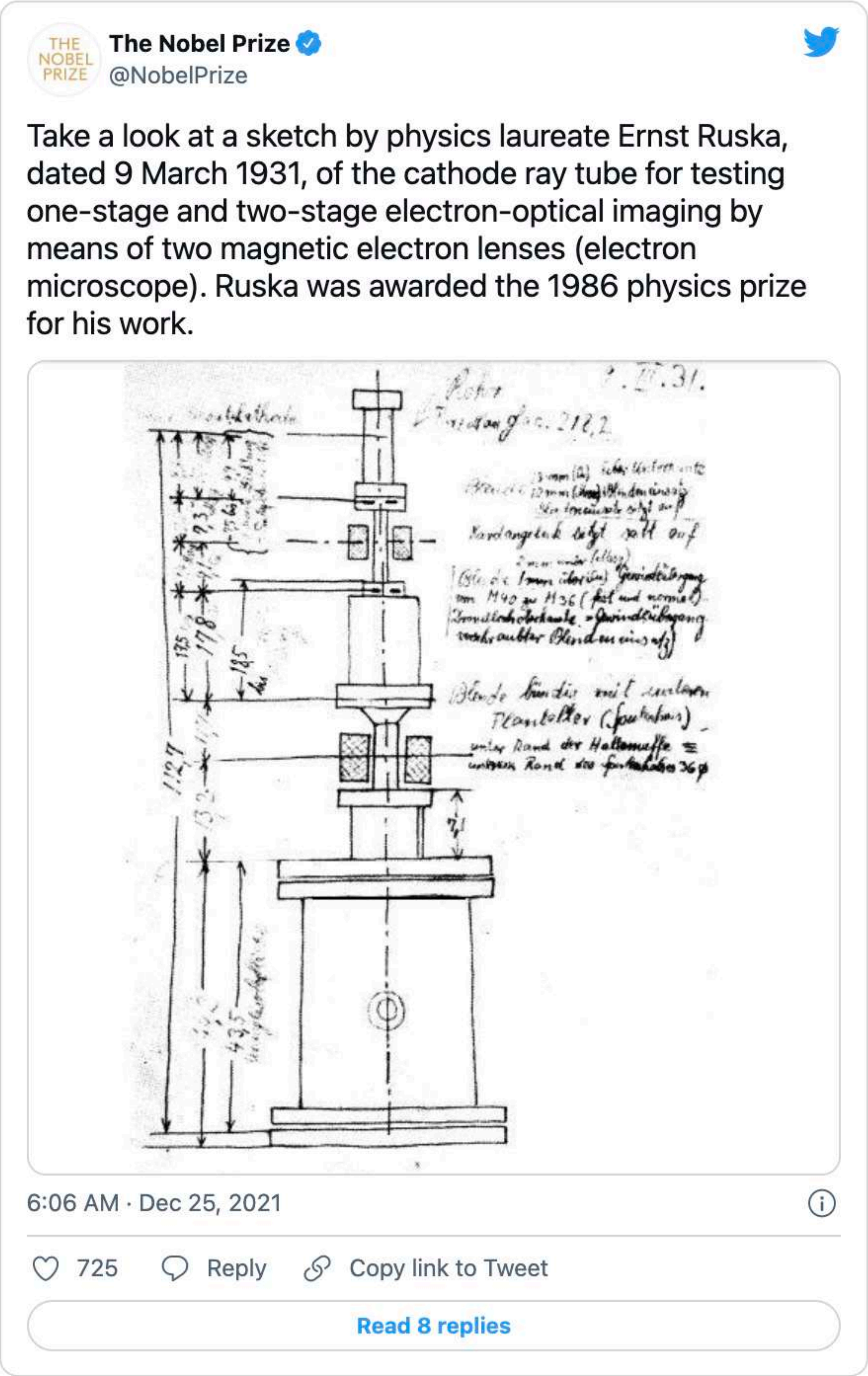
Micro crystal electron diffraction (microED)

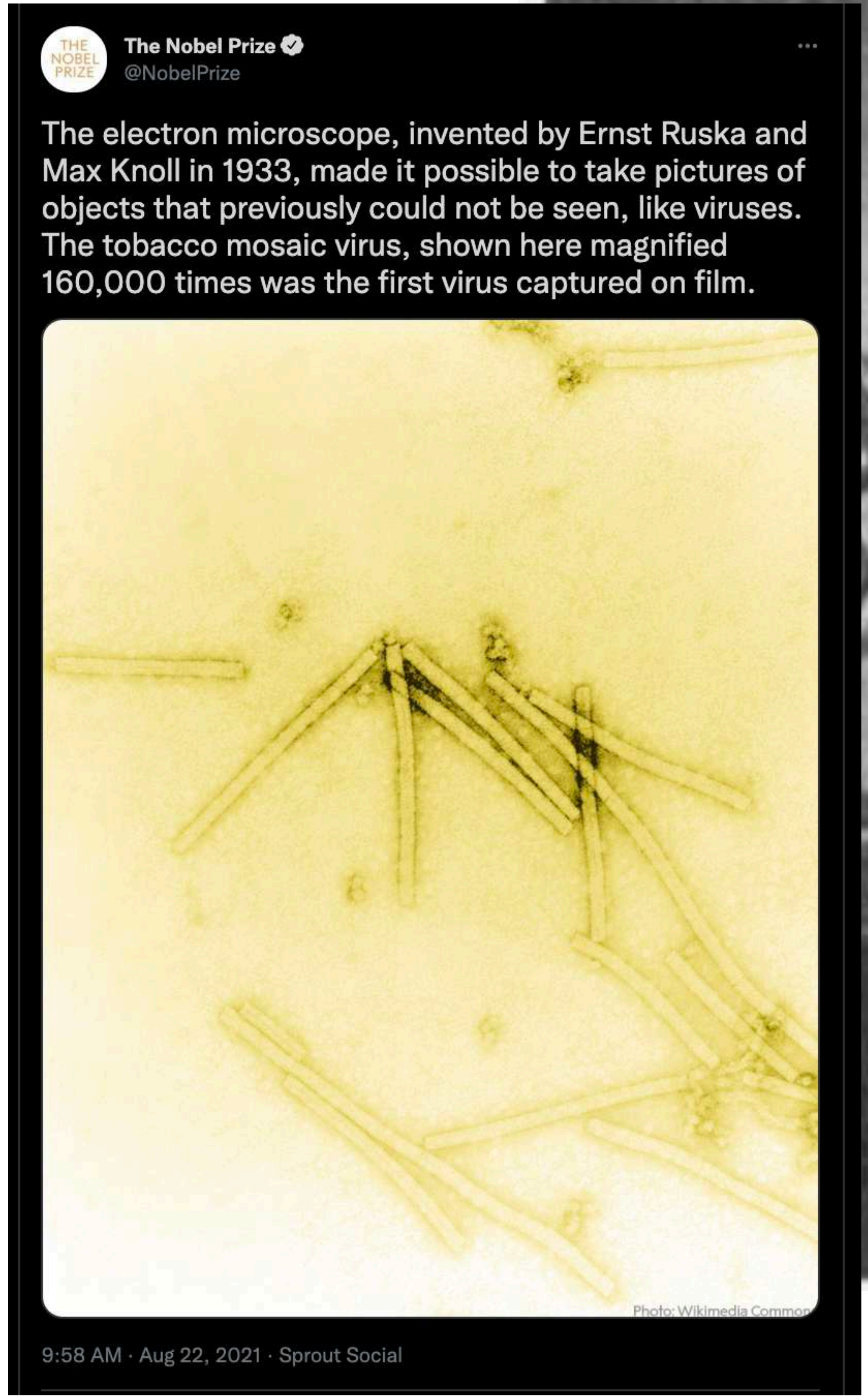


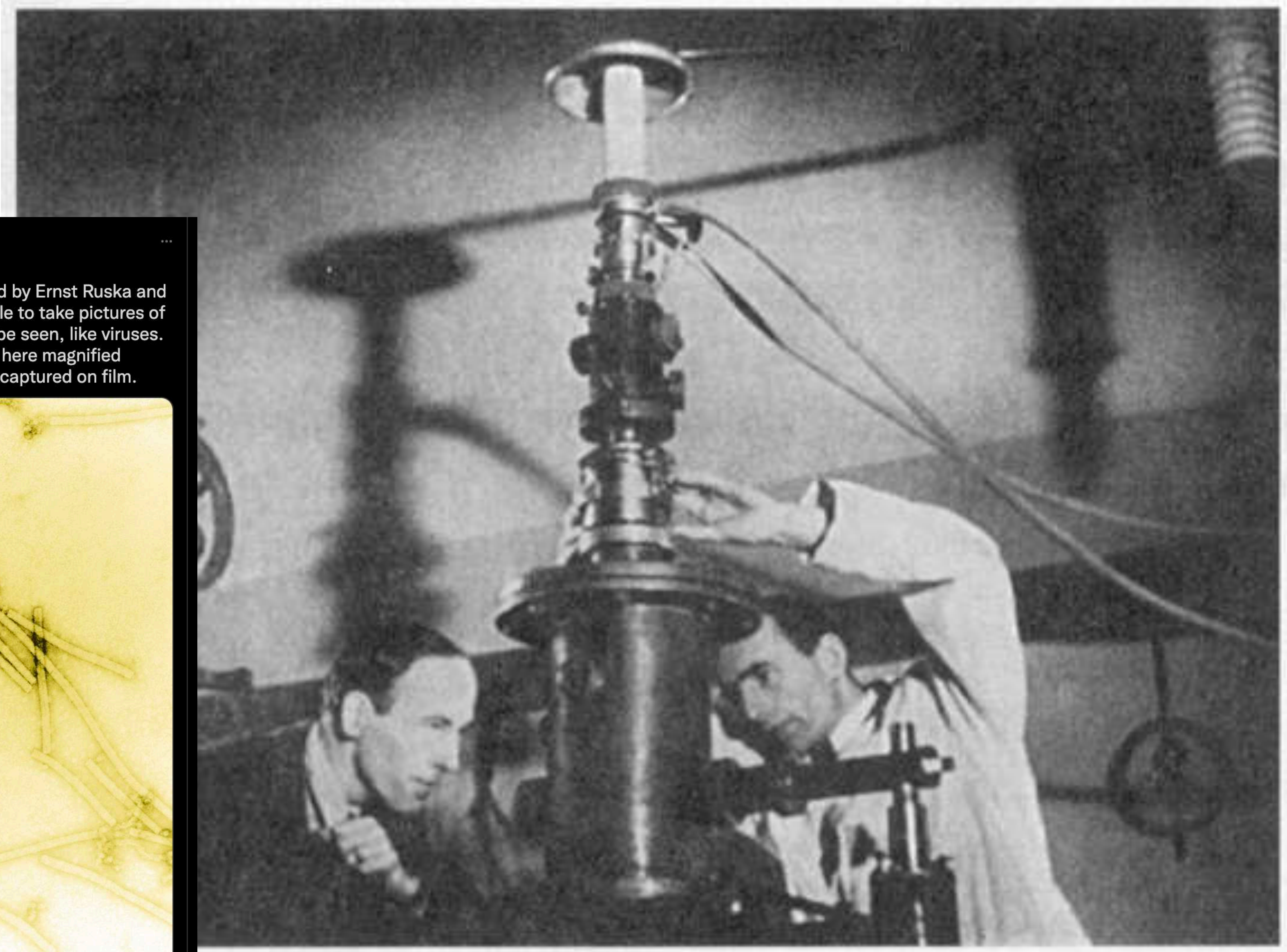
The tool of our trade: EM

Ruska and Knoll in Berlin in the early 1930s

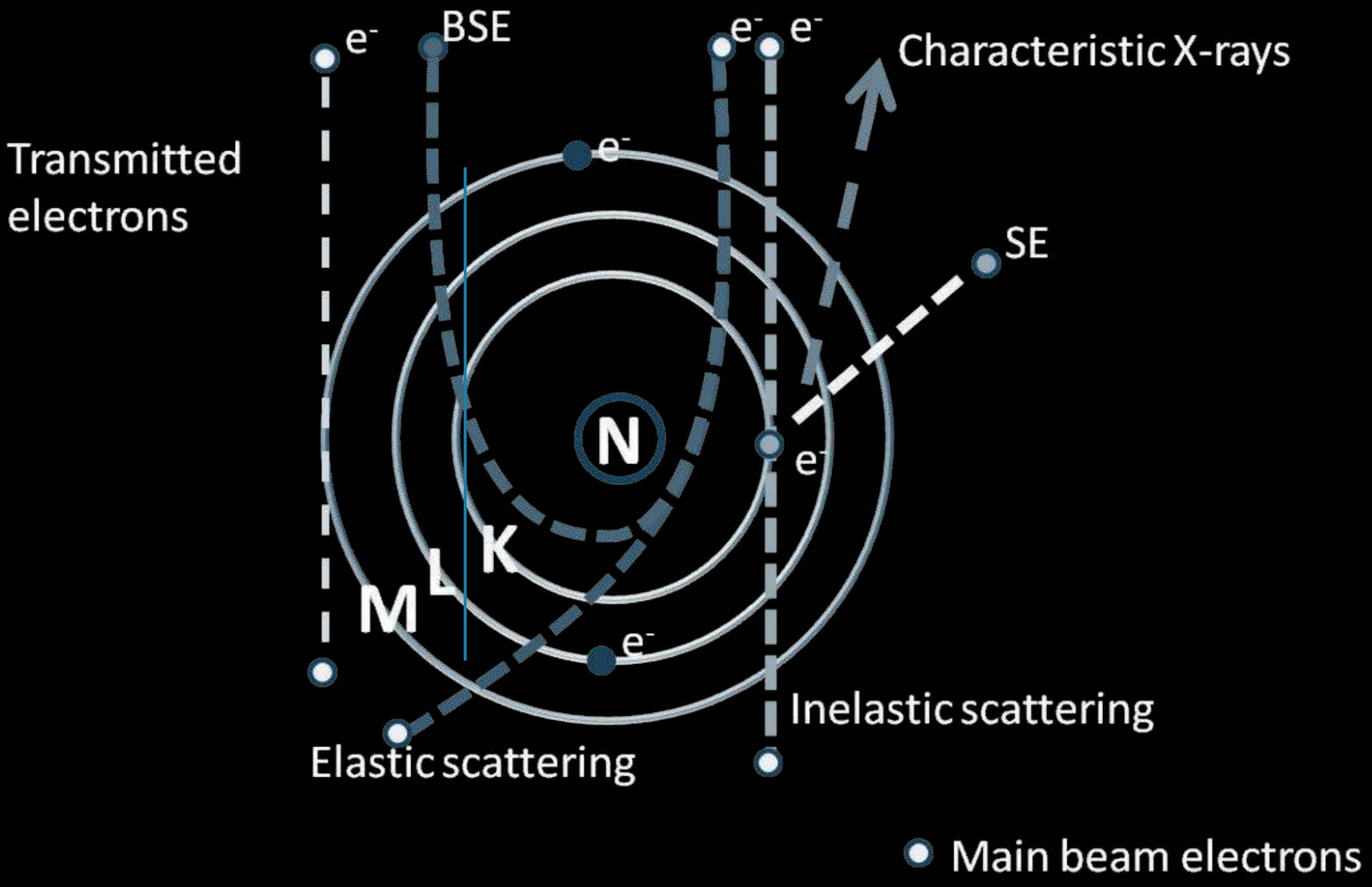
-Wikipedia







Why electrons?



Why electrons?

Pros

Small wavelength

Can be focused

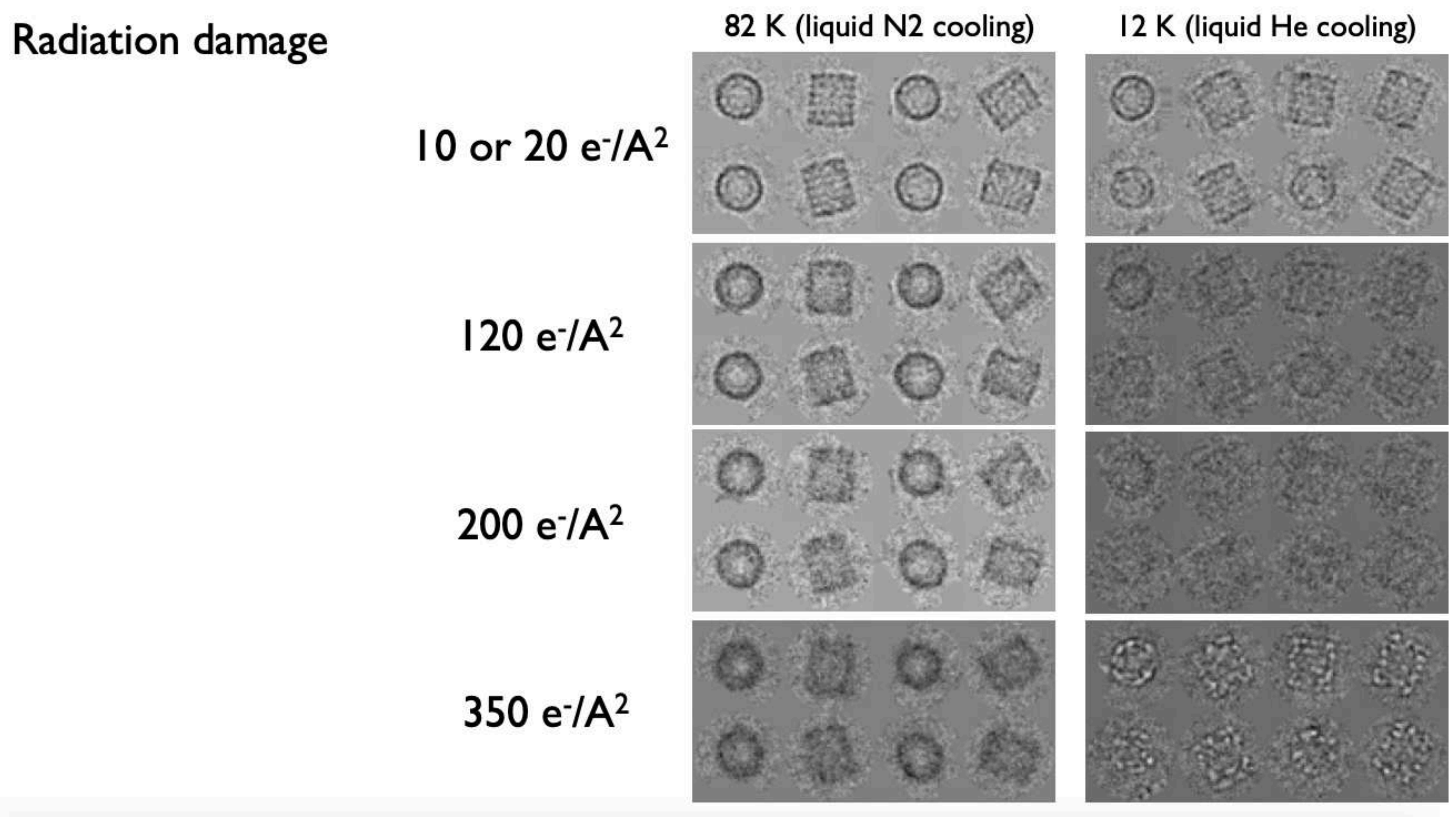
Cons

Damages sample
worse with faster electrons

Poor penetration
better with faster electrons

Why electrons?

Ideal dose for cryoEM?



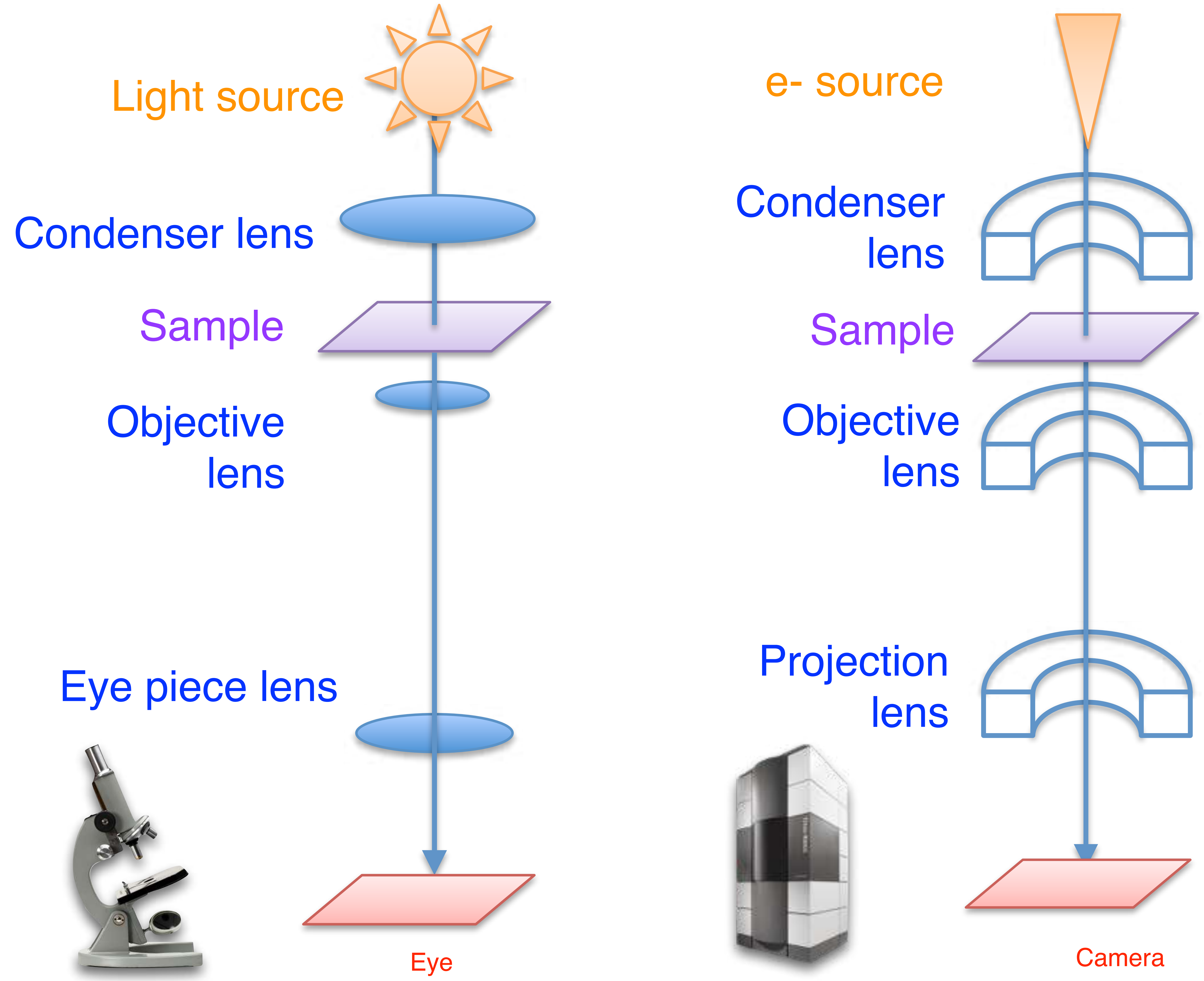
https://cryo-em-course.caltech.edu/

Specimen Behavior in the Electron Beam

R.M. Glaeser[1]
Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, United States
[1]Corresponding author: e-mail address: rmglaeser@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.

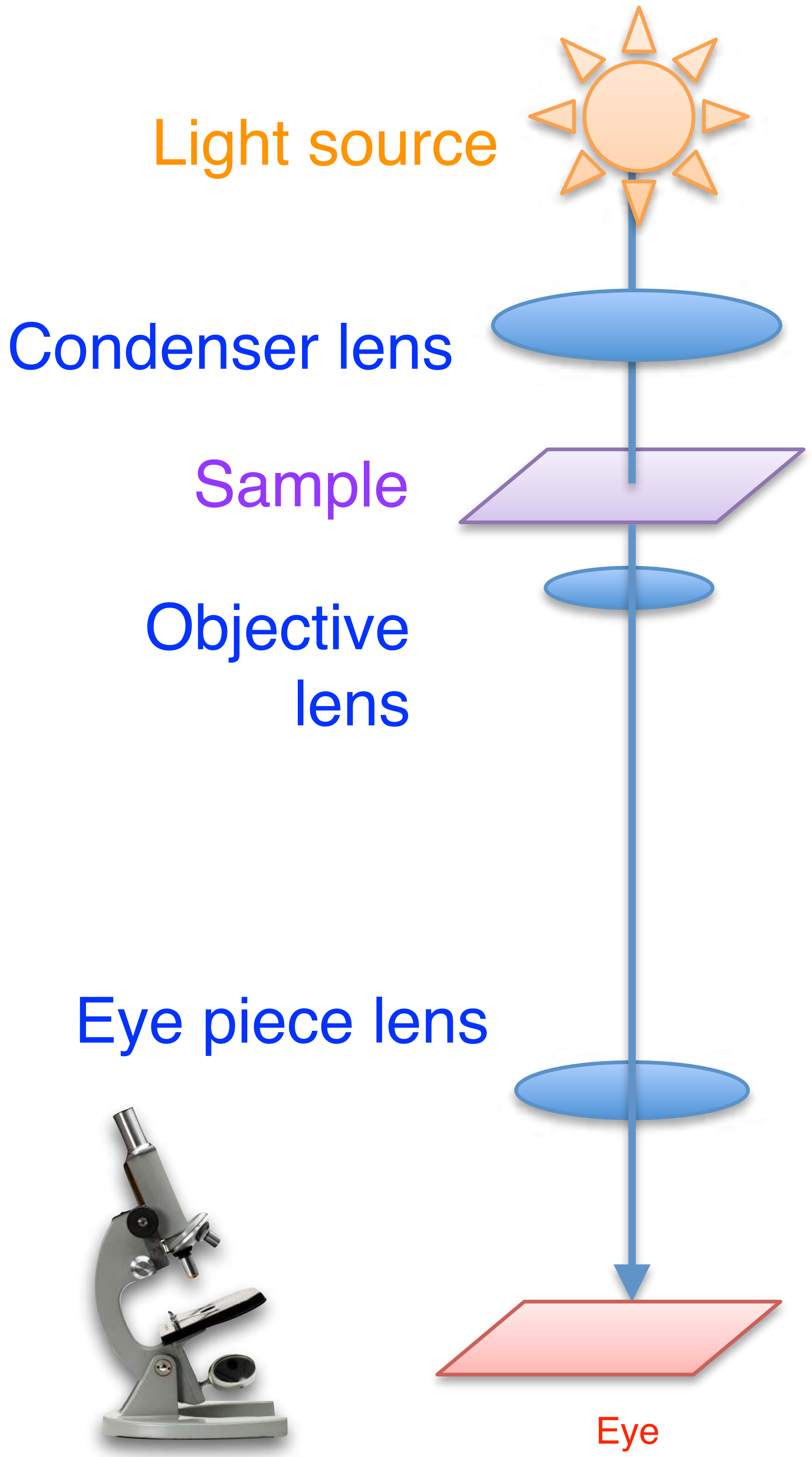
The electron microscope

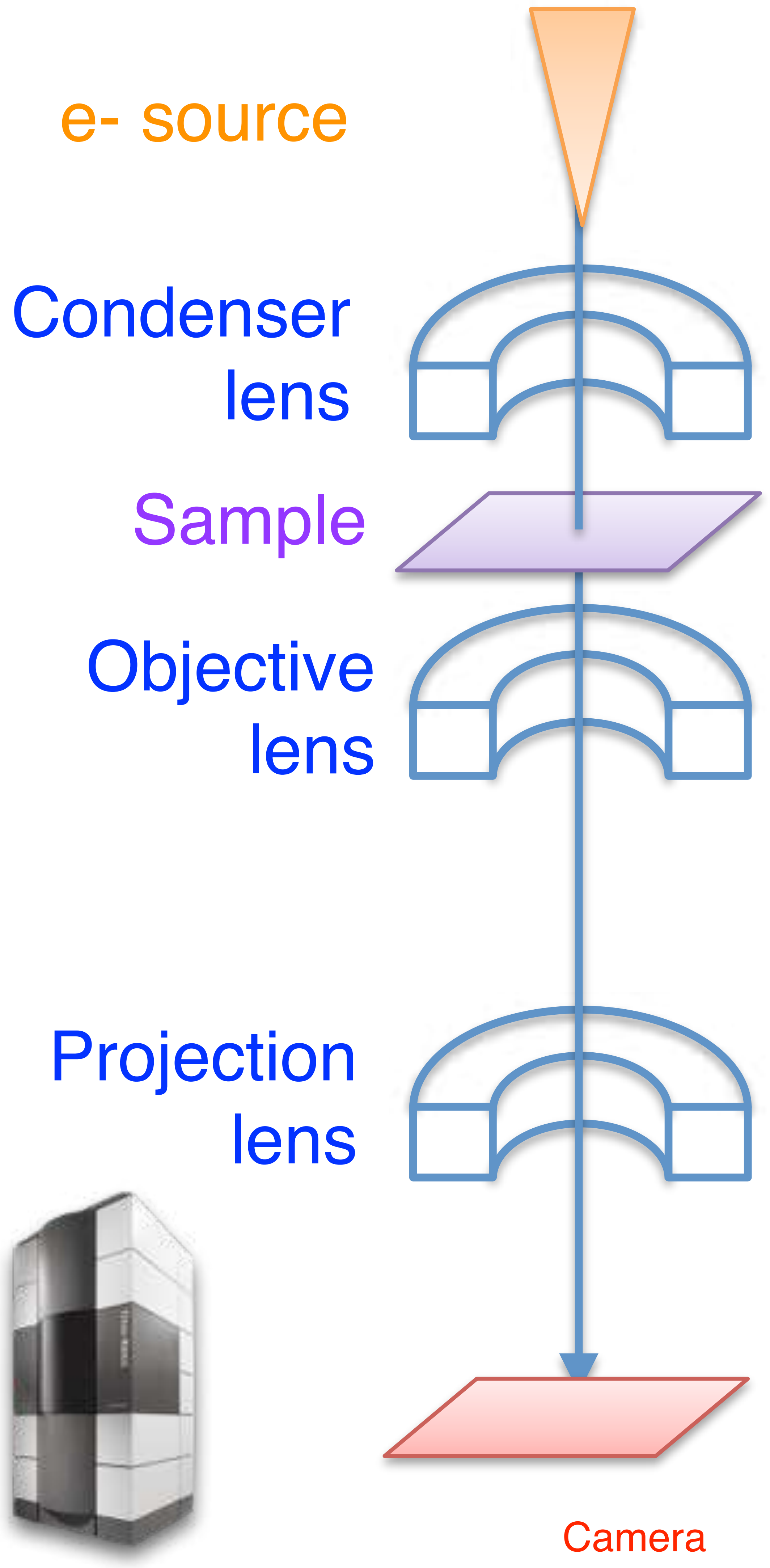


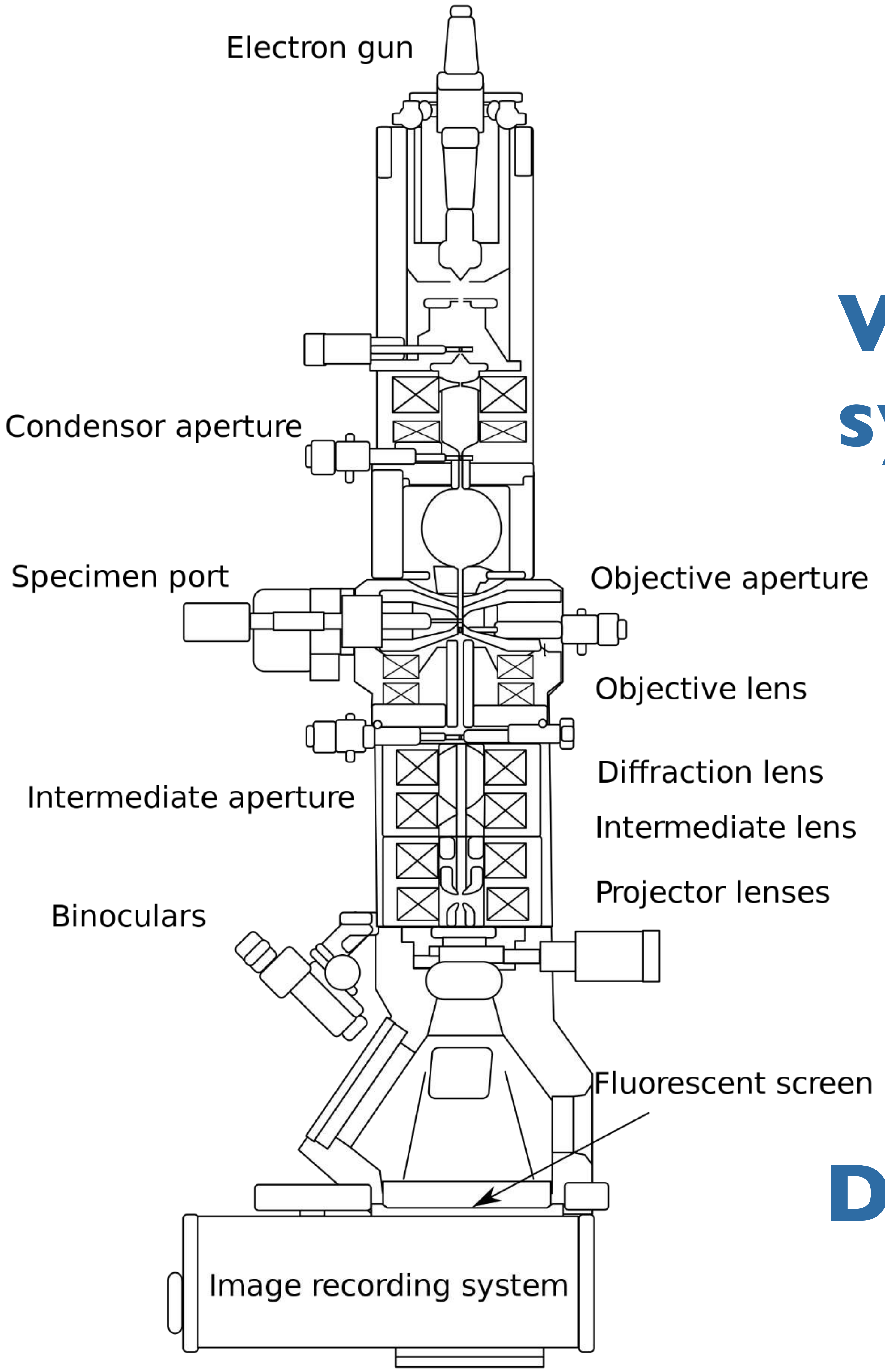
Light microscope	Transmission electron microscope
Visible light	Electrons
Glass lenses	Electron-magnetic lenses
450-650 nm	3.70 pm (100 keV) 2.51 pm (200 keV) 1.96 pm (300 keV)

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

The electron microscope







Electron sources

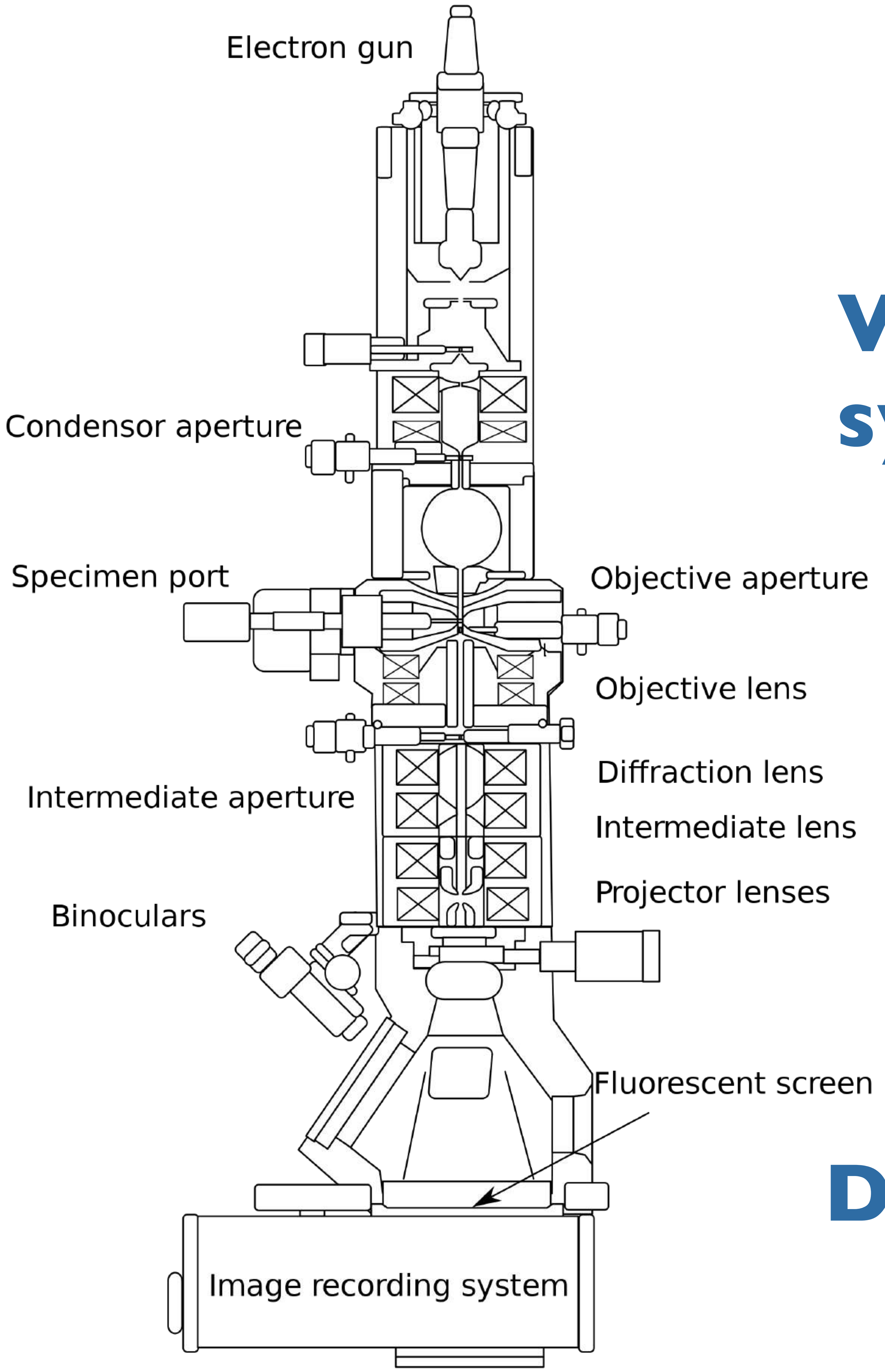
Vacuum systems

Lenses

Detectors

The electron microscope

e- gun	produces e-
accelerator	accelerate e- to high engergy
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



Electron sources

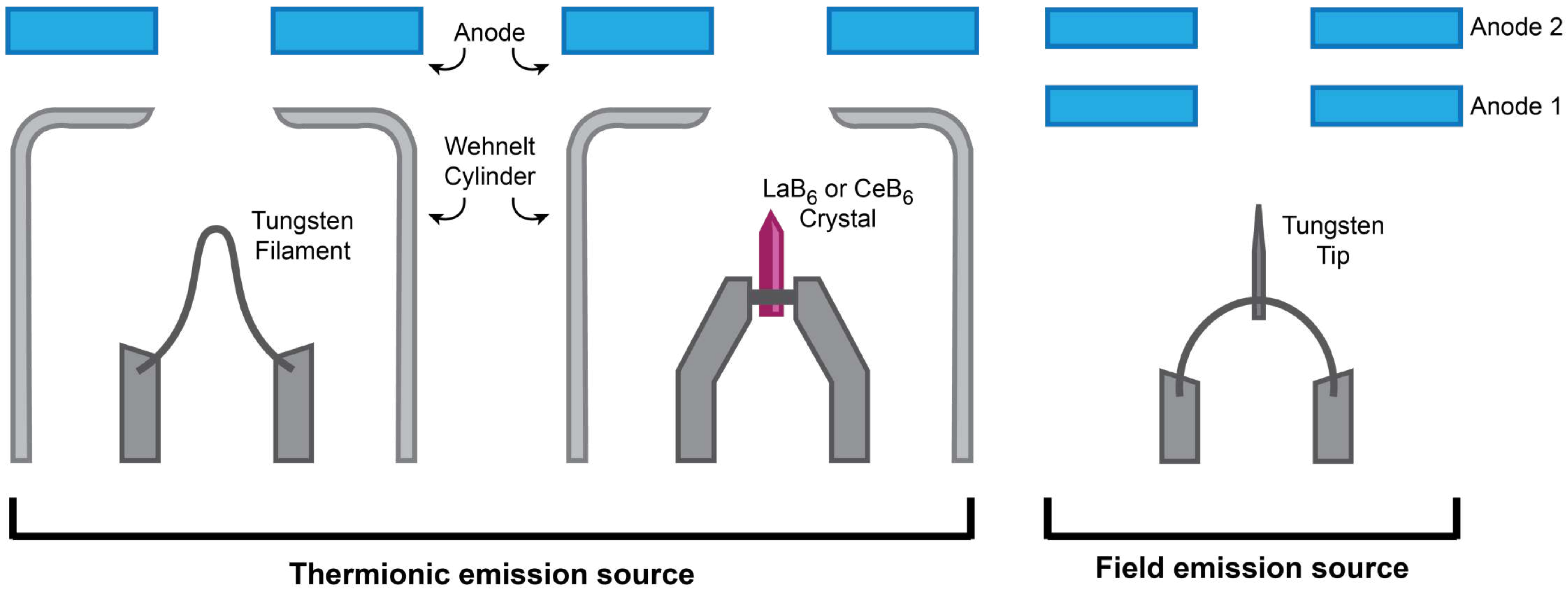
Vacuum systems

Lenses

Detectors

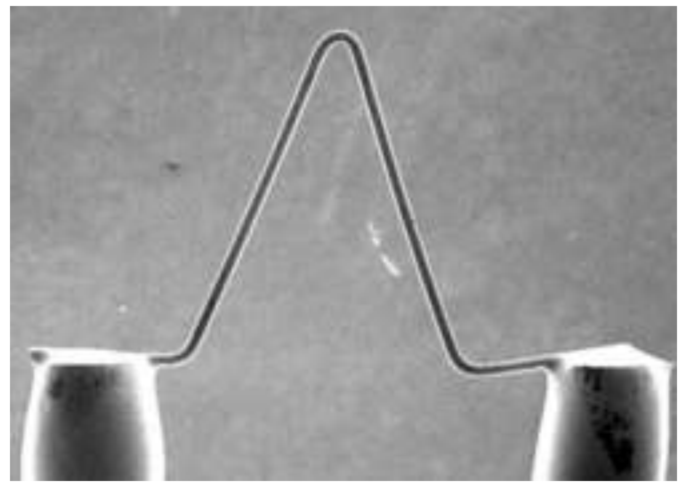
Electron sources

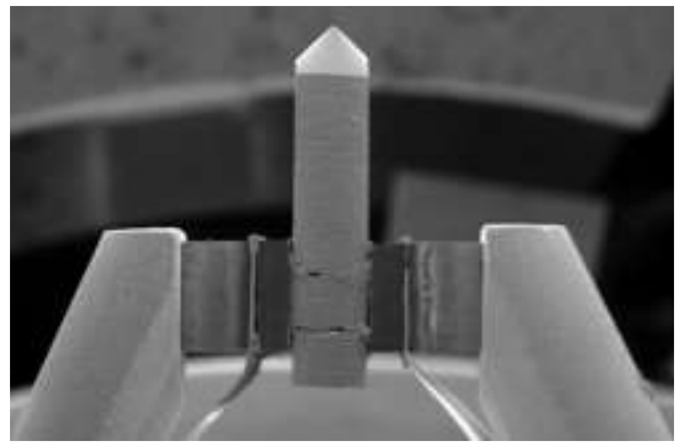
What are the 3 main kinds of electron sources?

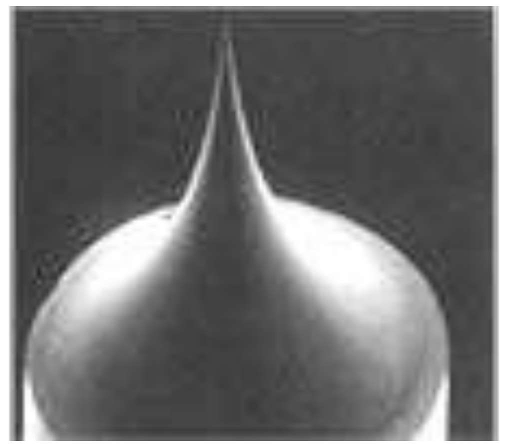


www.thermofisher.com

nanoscience.com



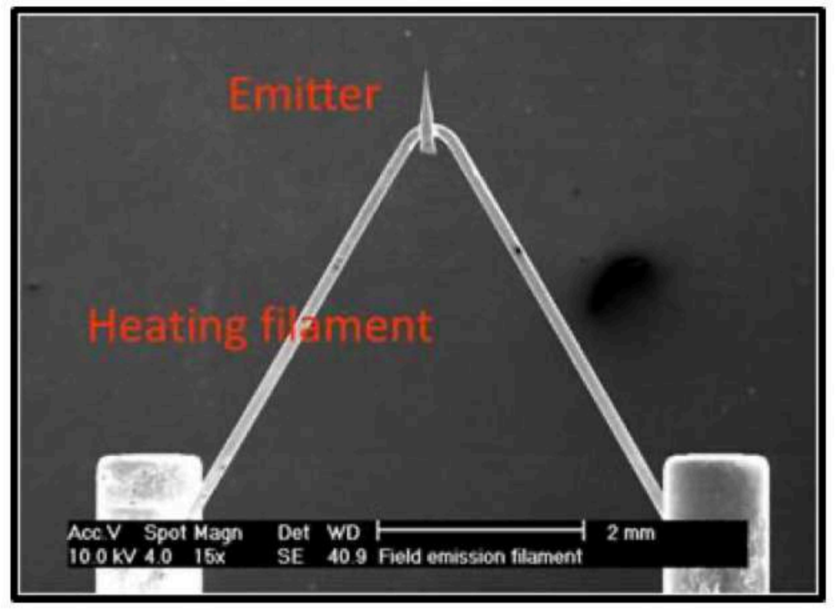




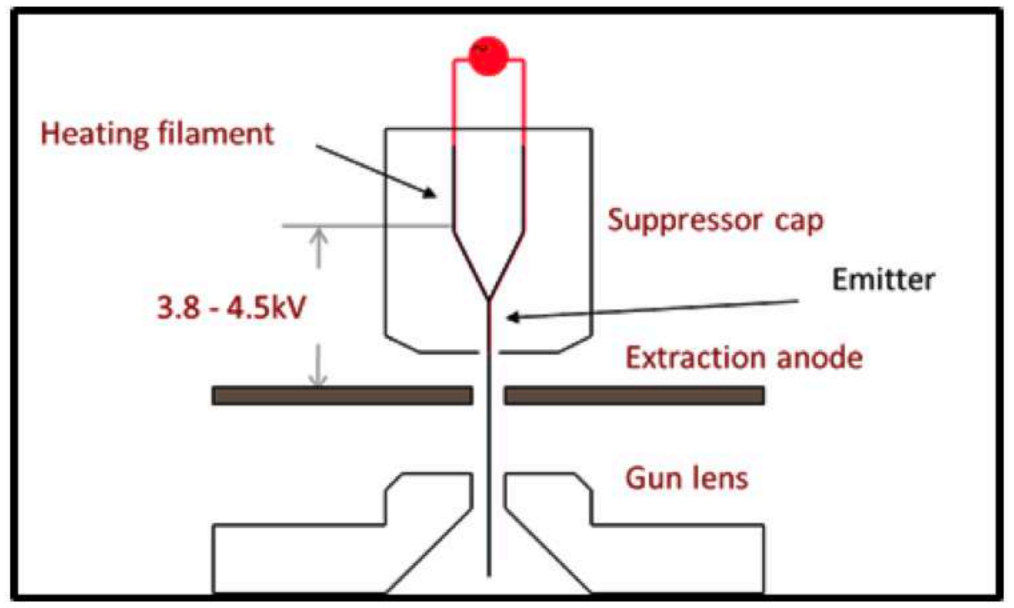
Electron sources

What are the 3 main kinds of electron sources?

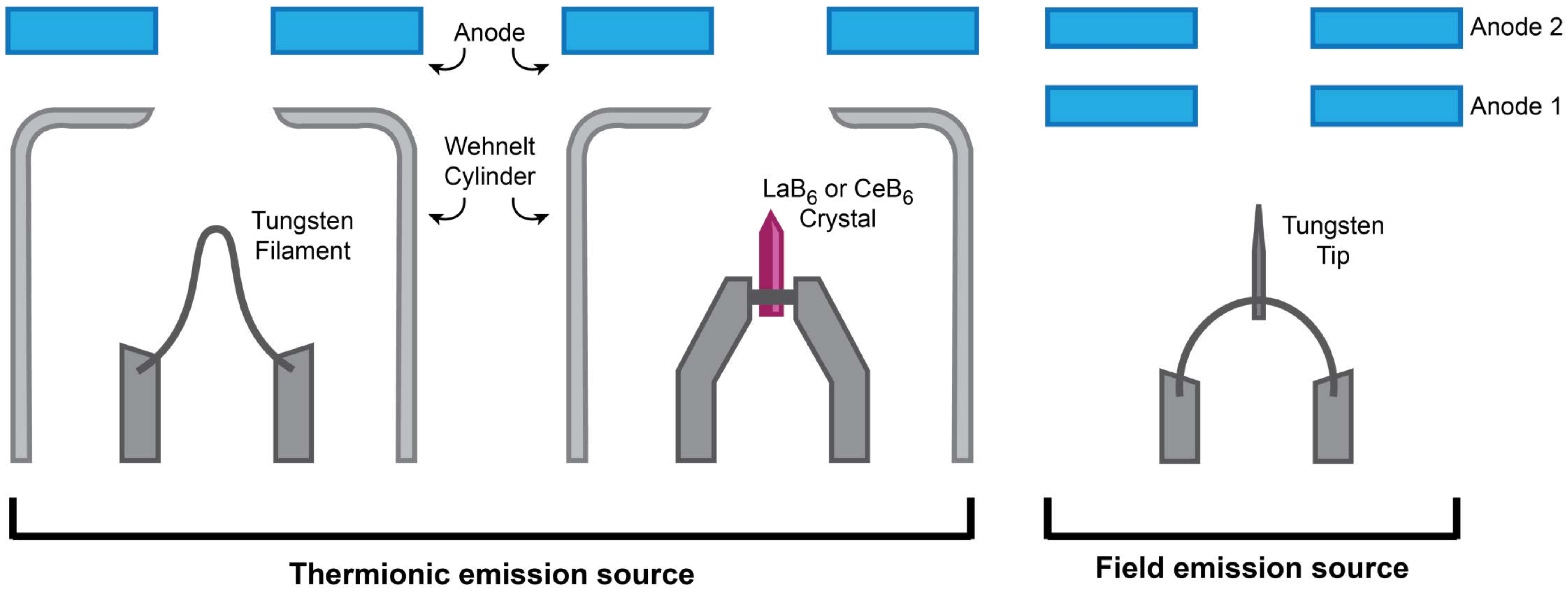
Thermionic



Field Emission Gun (FEG)

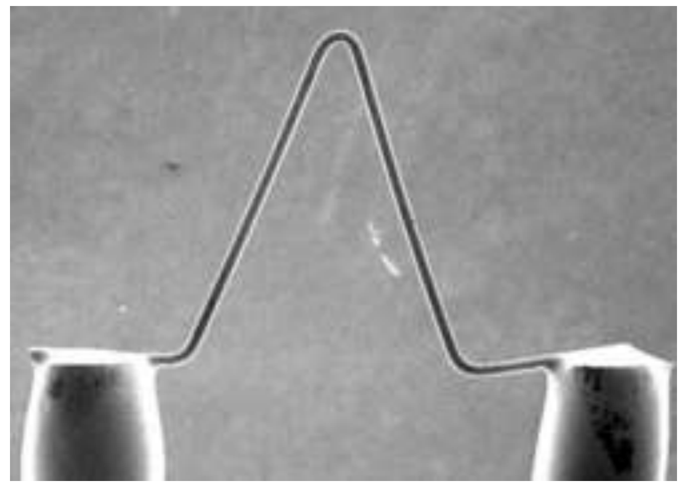


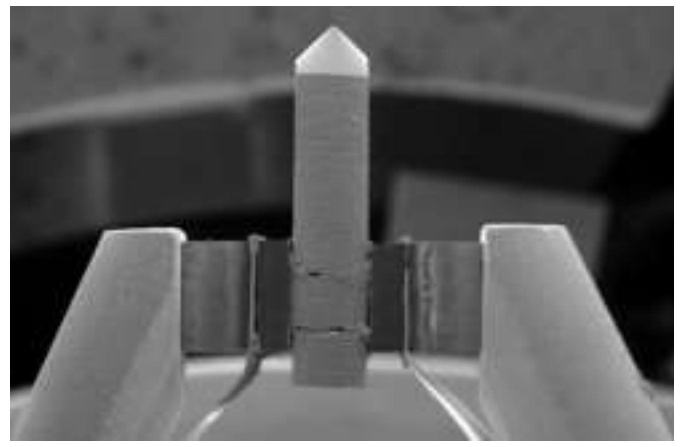
thermofisher.com

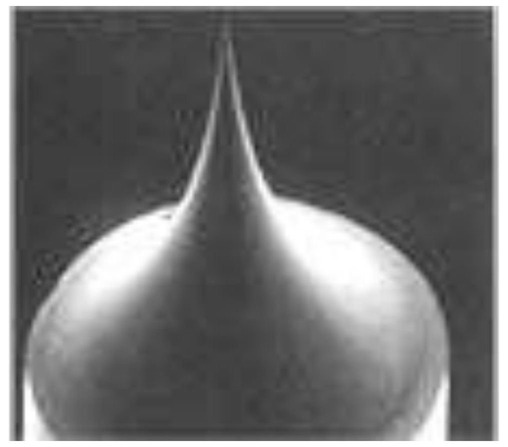


www.thermofisher.com

nanoscience.com





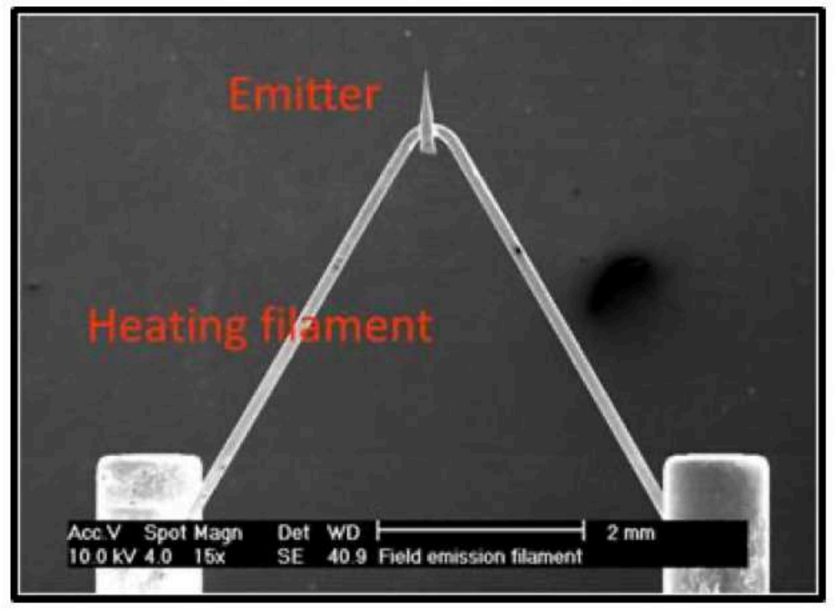


X-CFEG is 1.0x10^8 A/m2 sr V

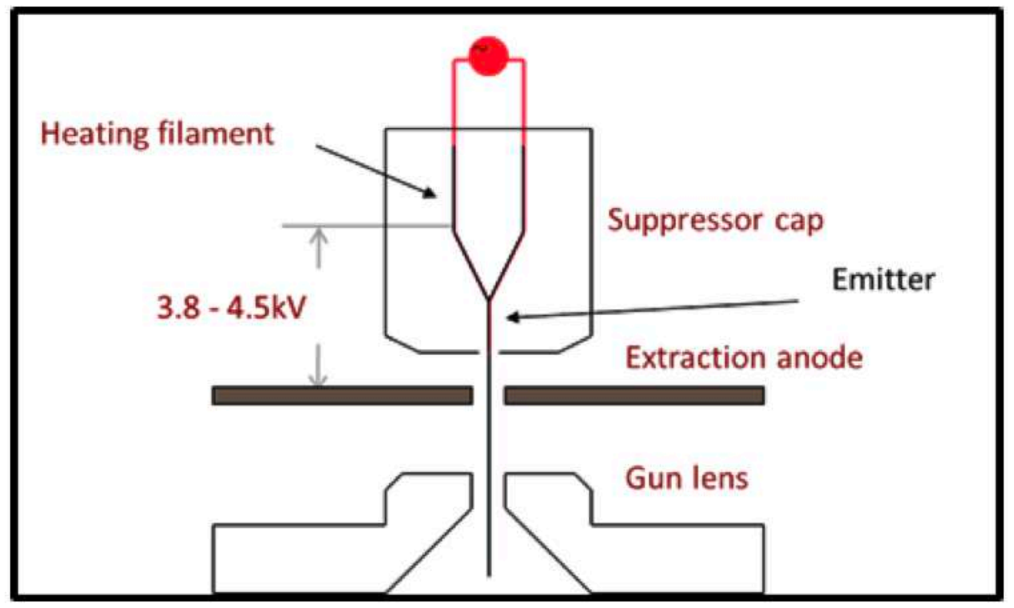
Electron sources

What are the 3 main kinds of electron sources?

Thermionic



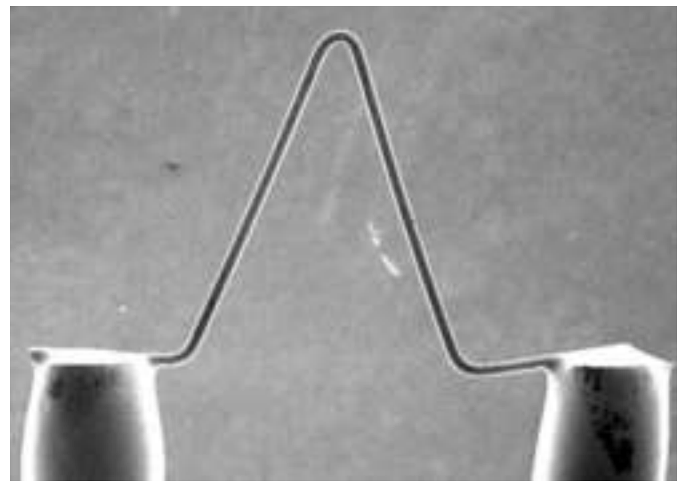
Field Emission Gun (FEG)

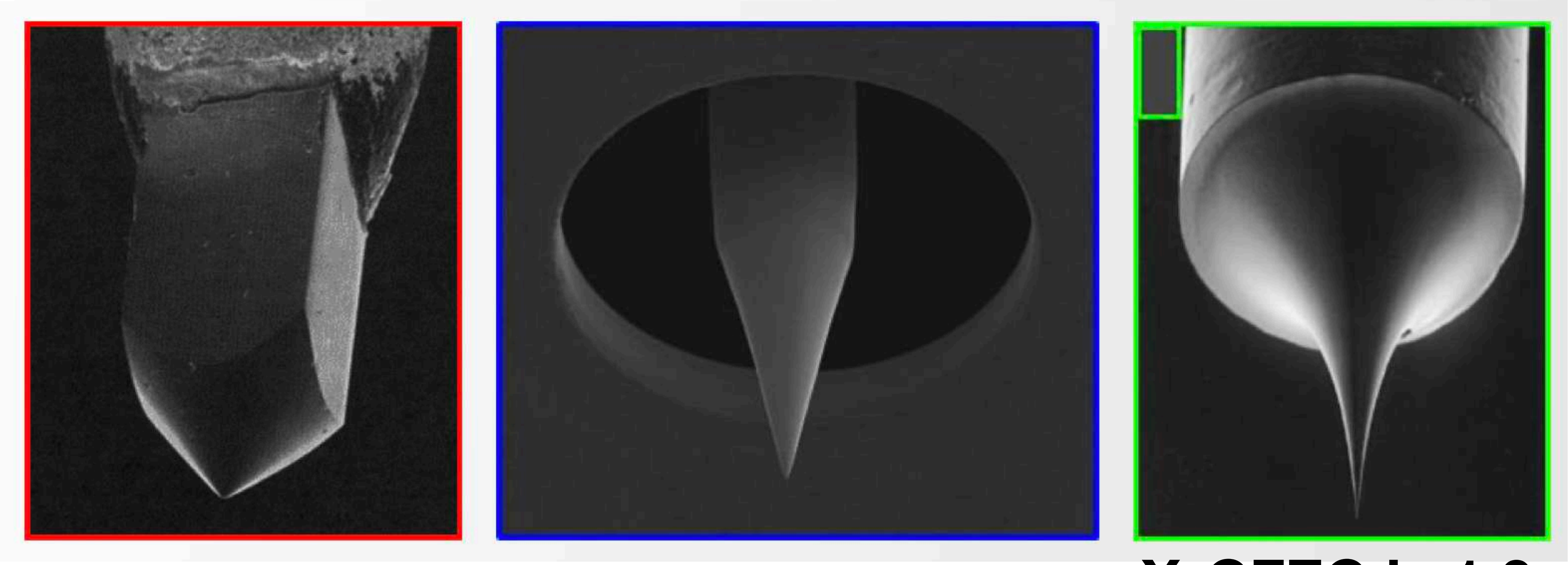


thermofisher.com

	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+

nanoscience.com

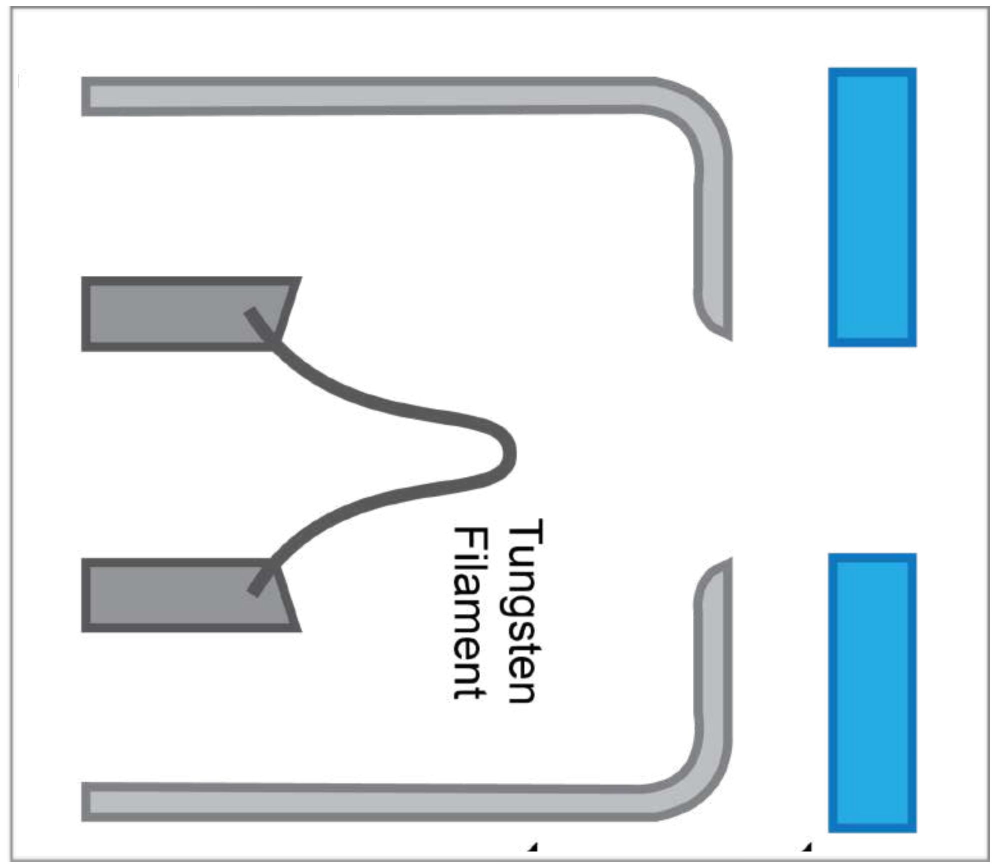


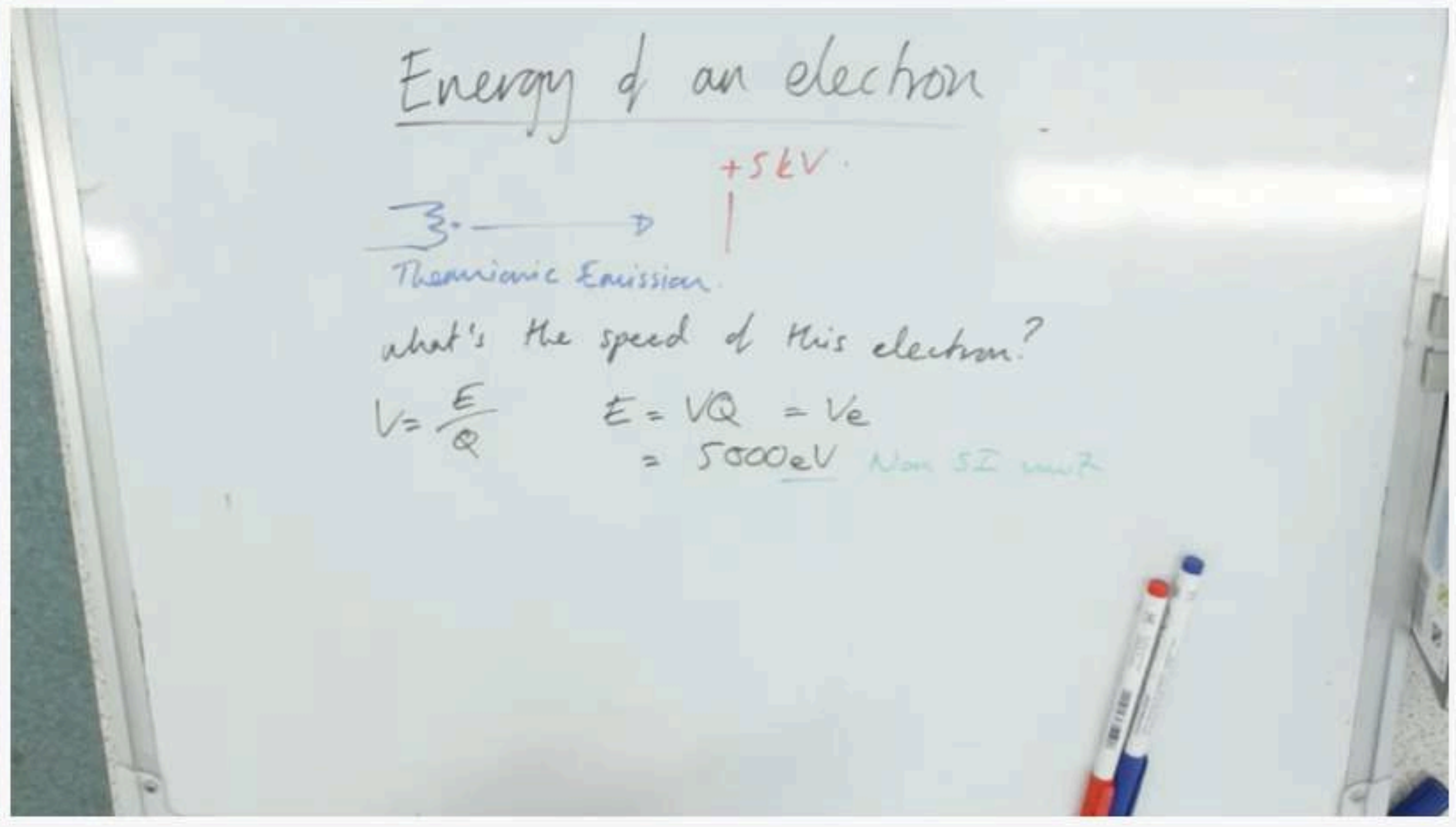


X-CFEG is 1.0×10^{8} A/m2 sr V

Electron sources

How fast are the electrons moving?

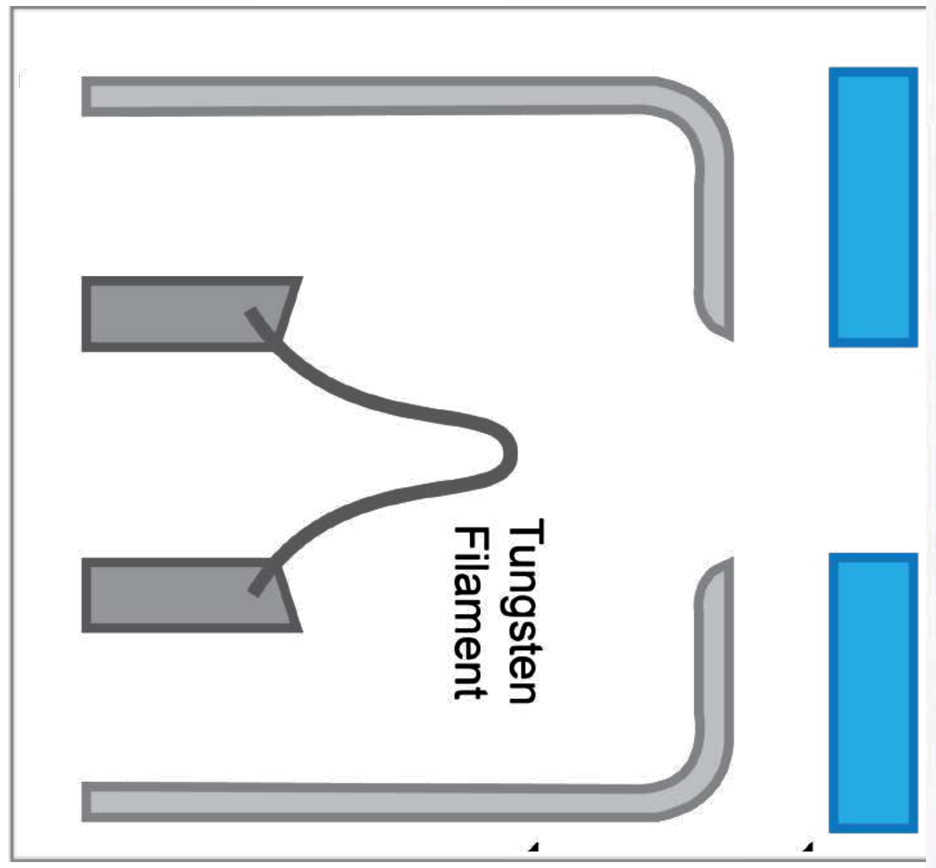


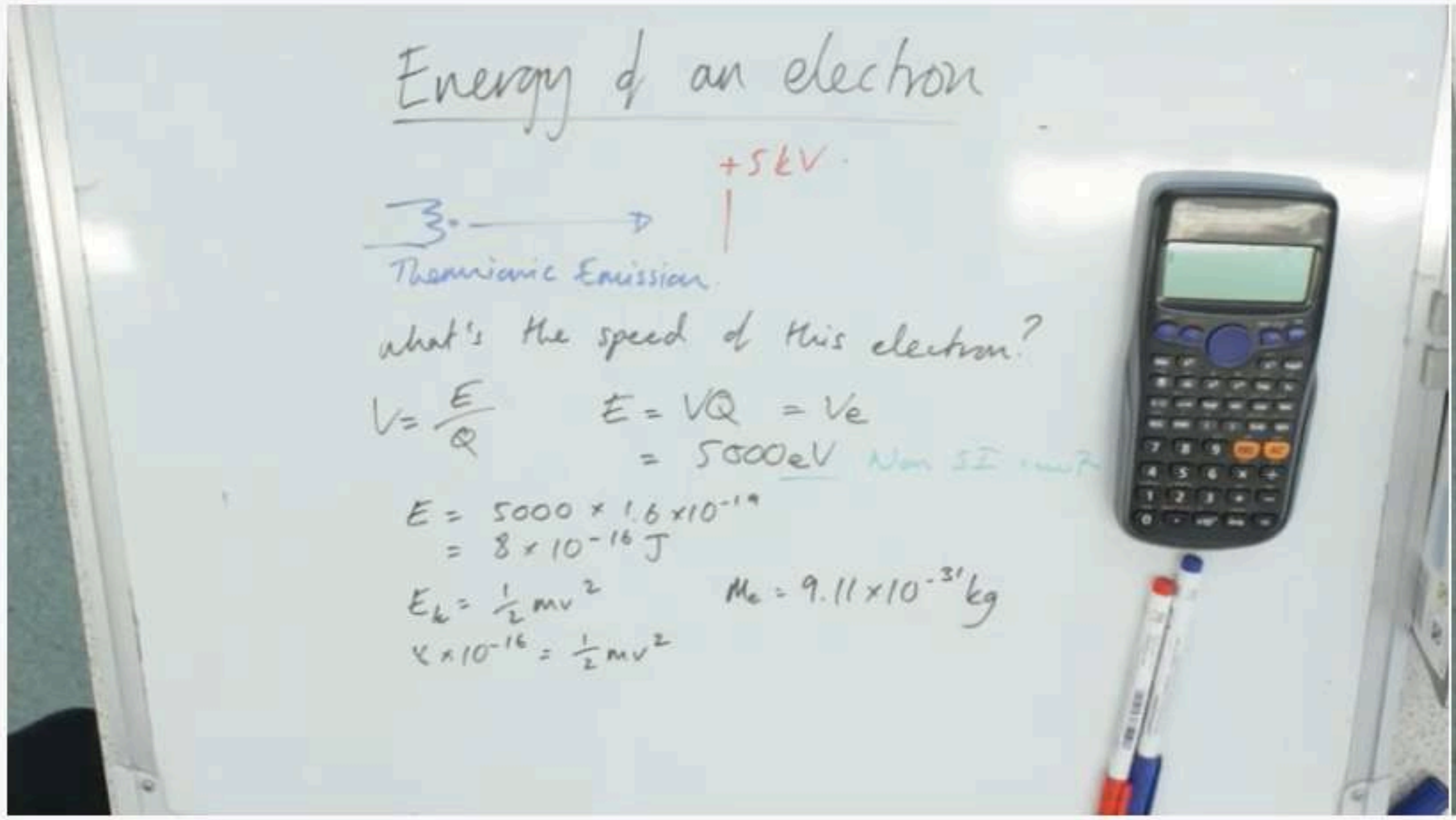


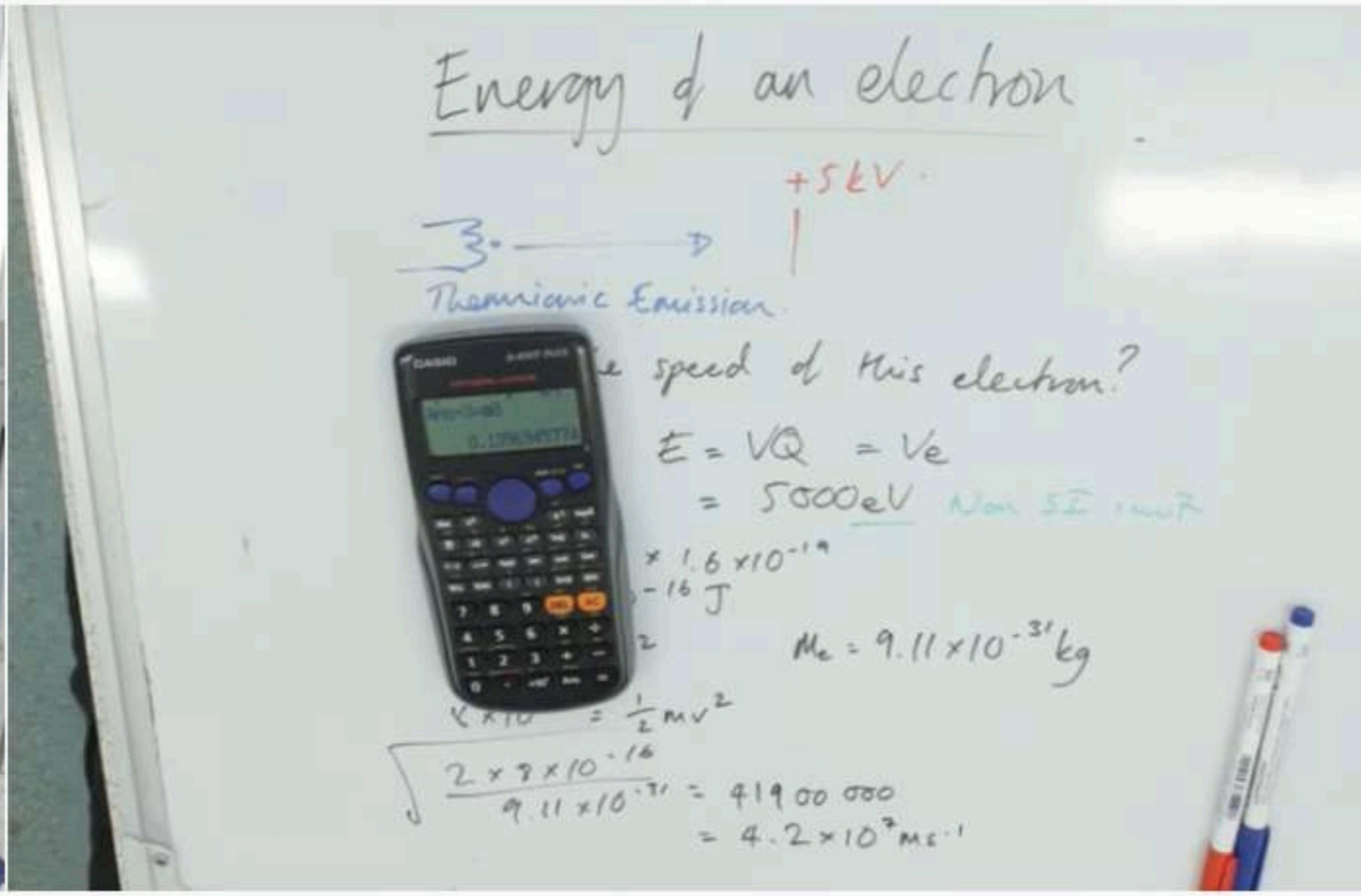
https://www.youtube.com/watch?v=tYCET6vYdYk

Electron sources

How fast are the electrons moving?



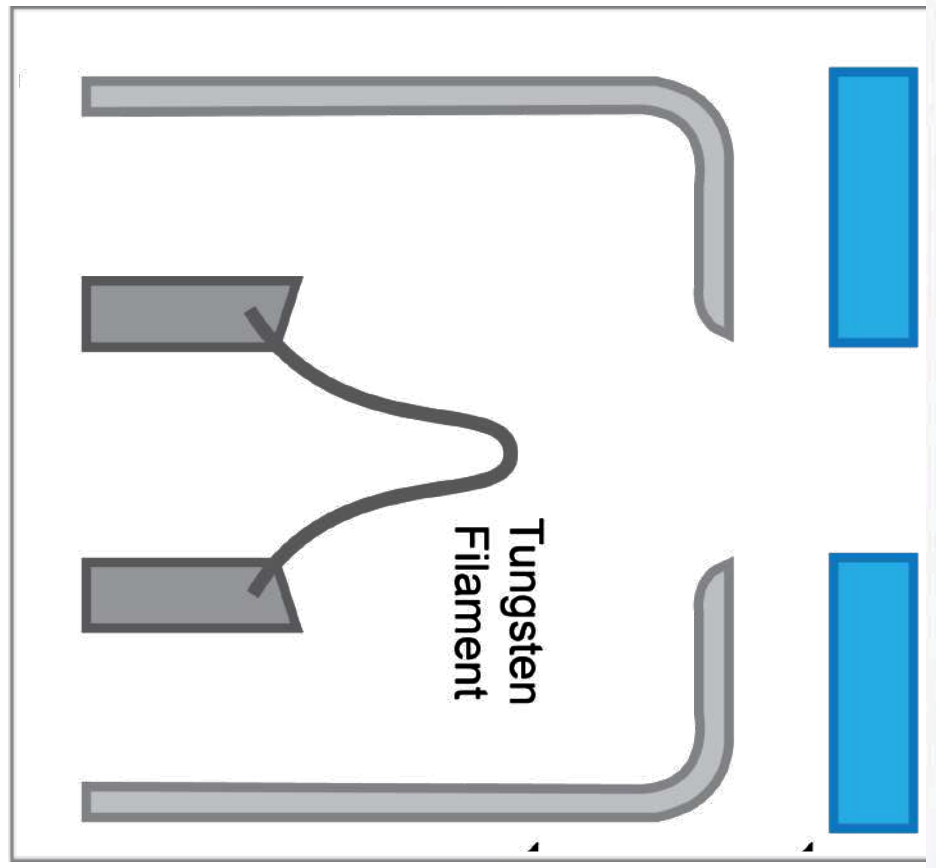


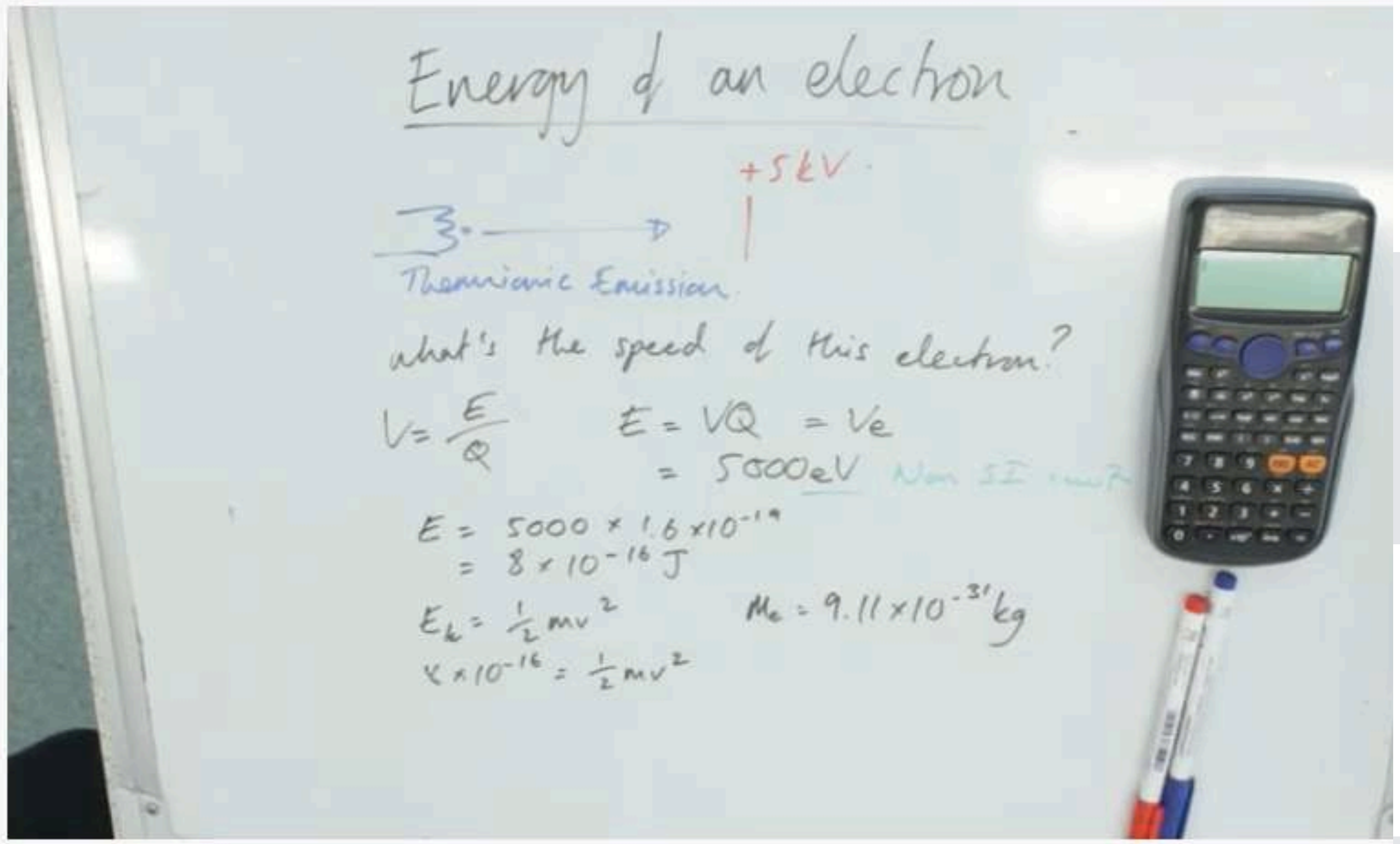


https://www.youtube.com/watch?v=tYCET6vYdYk

Electron sources

How fast are the electrons movi

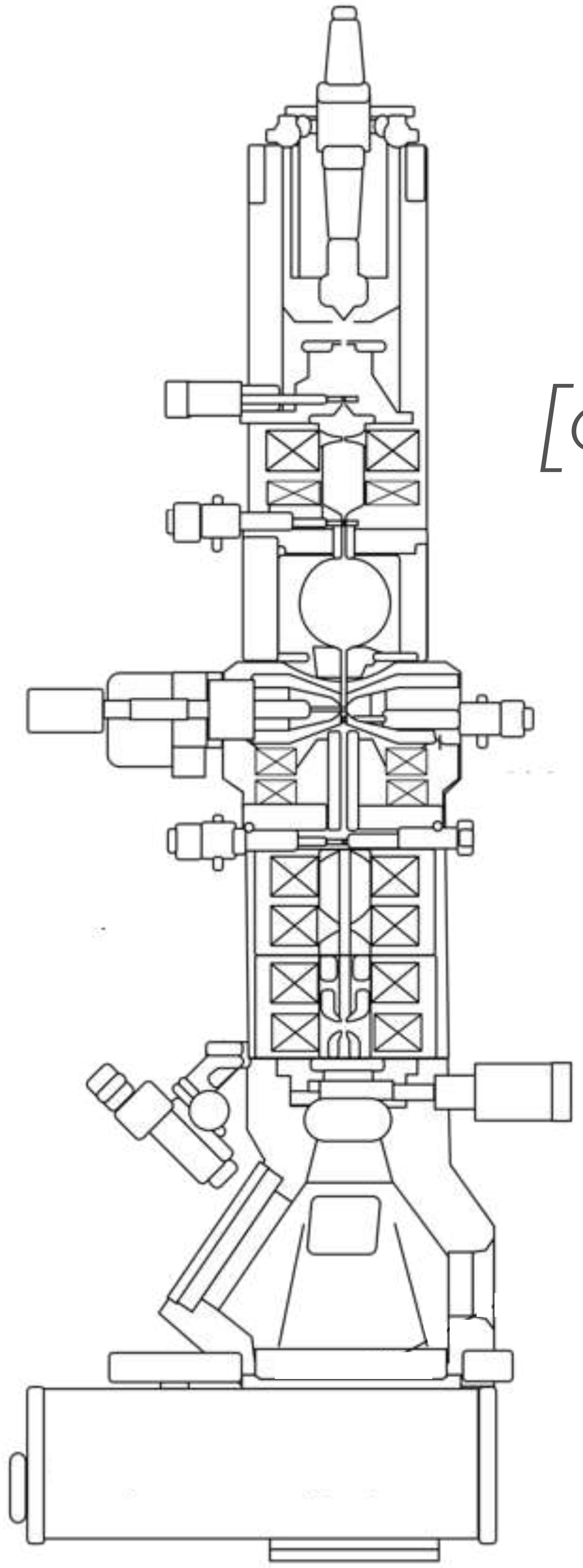




Light microscope	Transmission electron microscope
Visible light	Electrons
Glass lenses	Electron-magnetic lenses
450-650 nm	3.70 pm (100 keV) 2.51 pm (200 keV) 1.96 pm (300 keV.)
speed of light in vacuum c	0.548c (100 keV) 0.695c (200 keV) 0.776c (300 keV.)

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

Electron sources

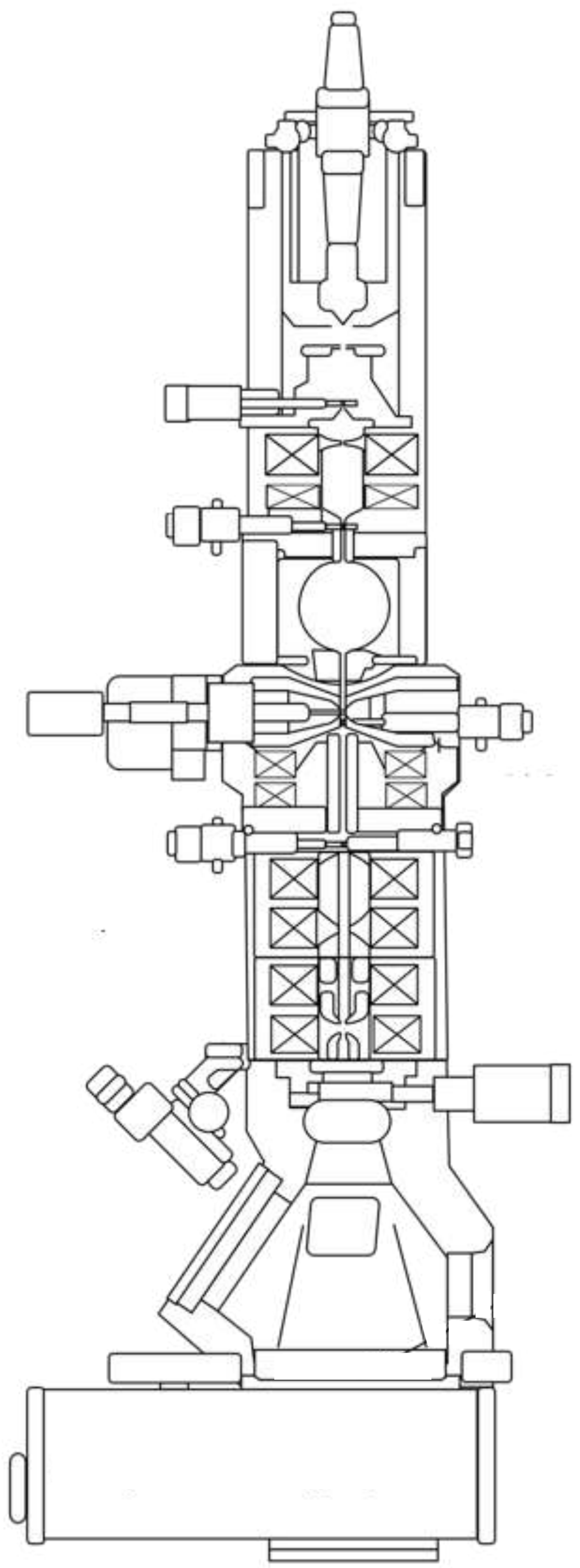


80-120 kV: Hitachi 7800, JEOL1400, TFS Talos120
W or LaB6
High contrast & robust
sub-nm resolution
[developments ongoing to push resolution with FEG systems]

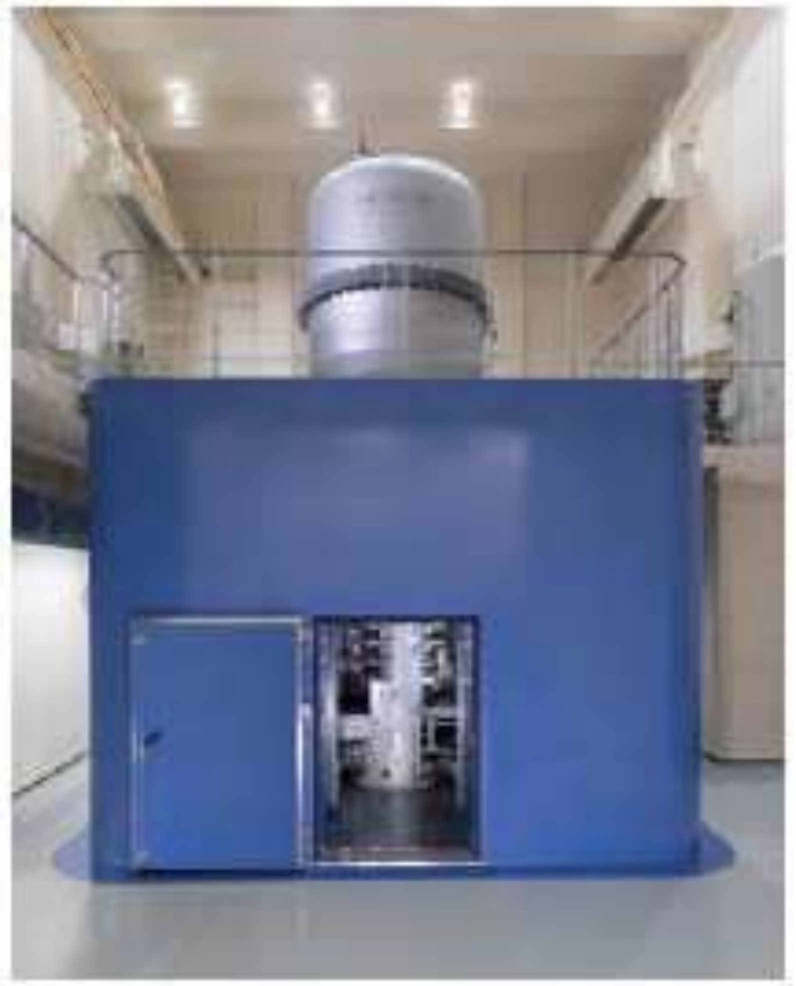
200 kV: J2100F; TFS Tecnai, Glacios, Arctica
FEG
2+ Å resolution (3.5-4 Å)

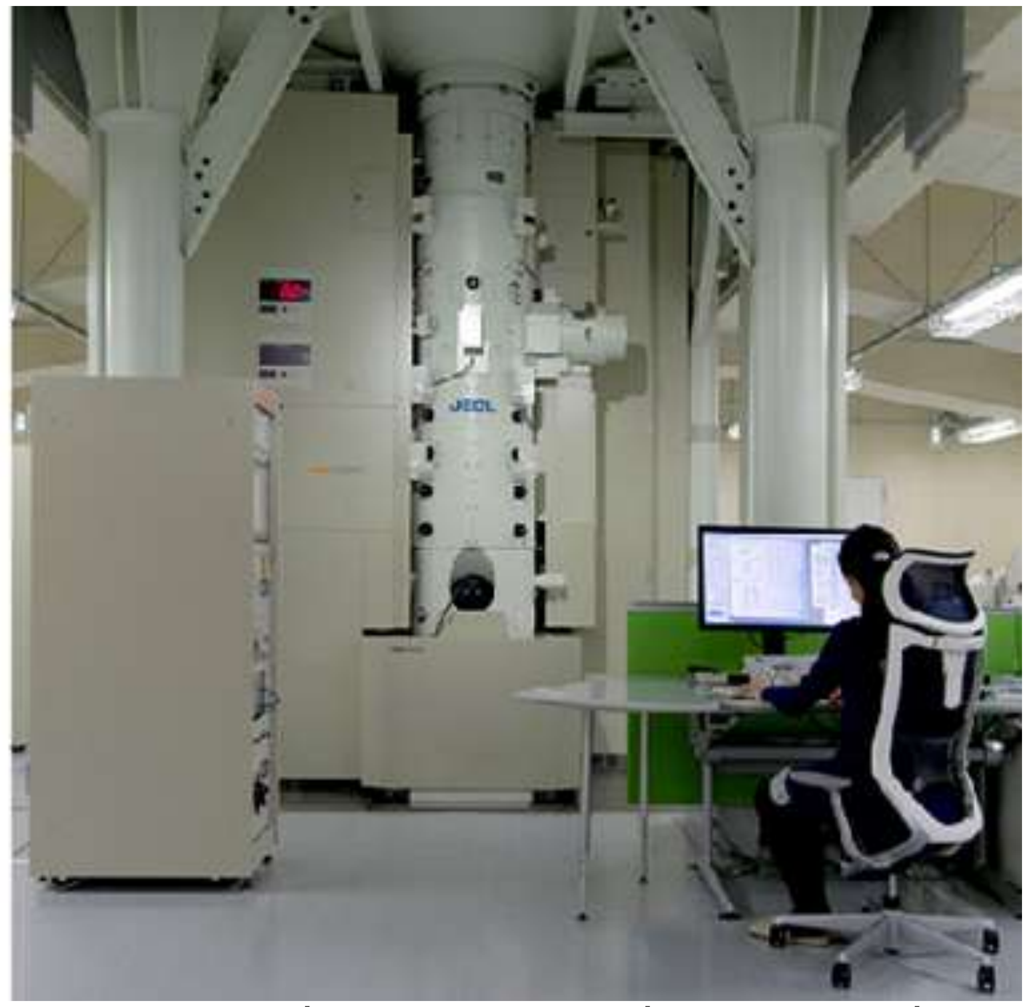
300 kV: JEOL3200FSC, cryoARM; TFS Krios, Halo
FEG
Smaller effect on unwanted lens aberrations
1.5-3 Å resolution

Electron sources



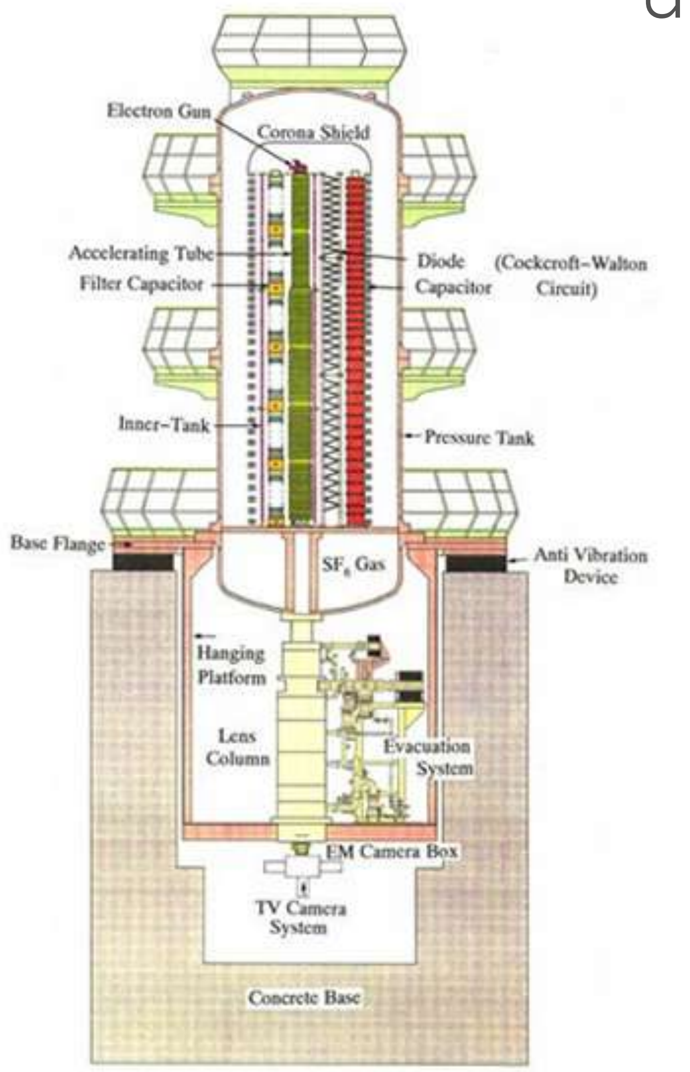
1-1.2 MV: Hitachi, JEOL
LaB6



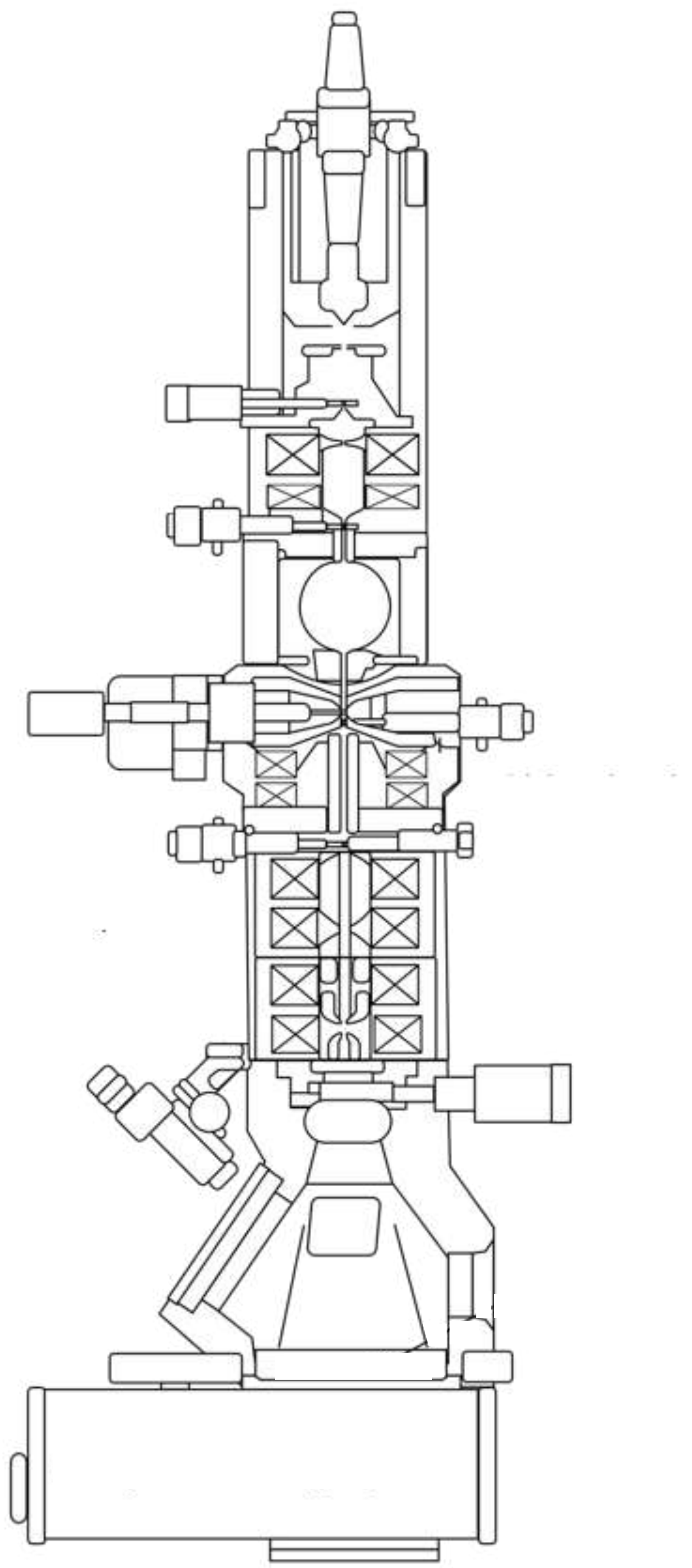


uhvem.osaka-u.ac.jp

3 MV: Hitachi H3000
LaB6



Vacuum systems



Why do we need a vacuum?

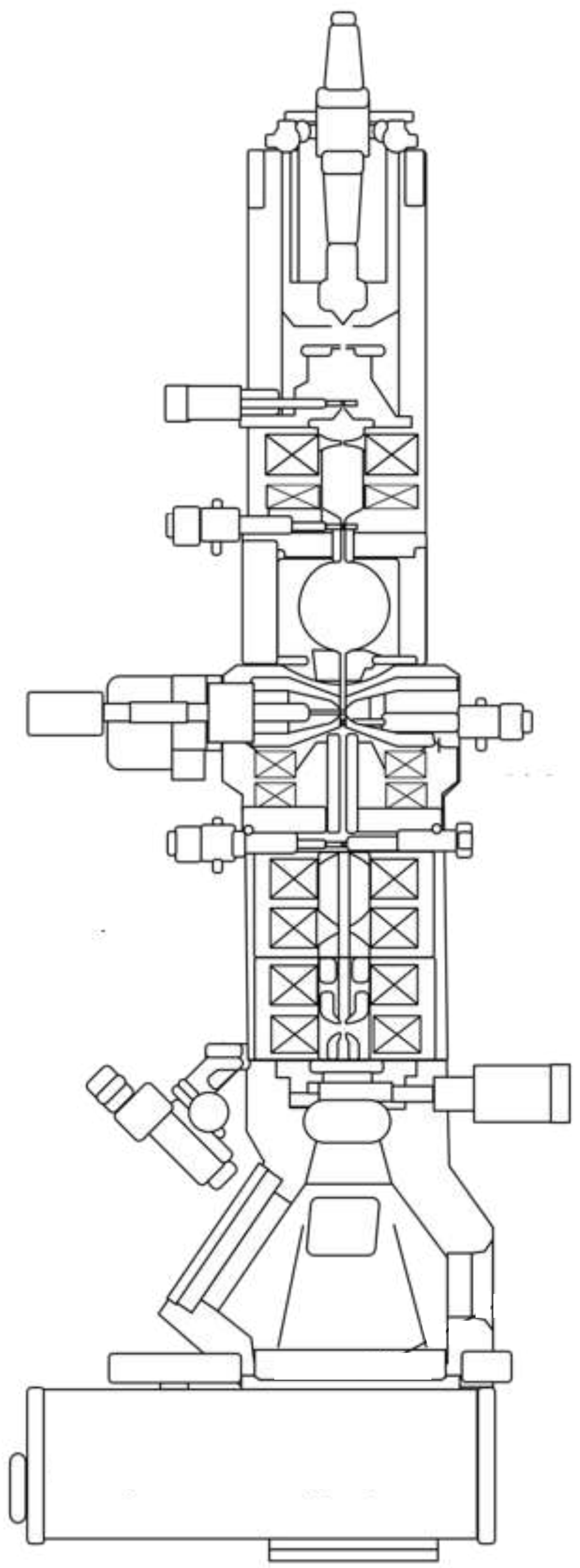
Beam coherence - at STP mean free path ~1 cm

Insulation - interaction between e- and air

Filament - O2 will burn out source

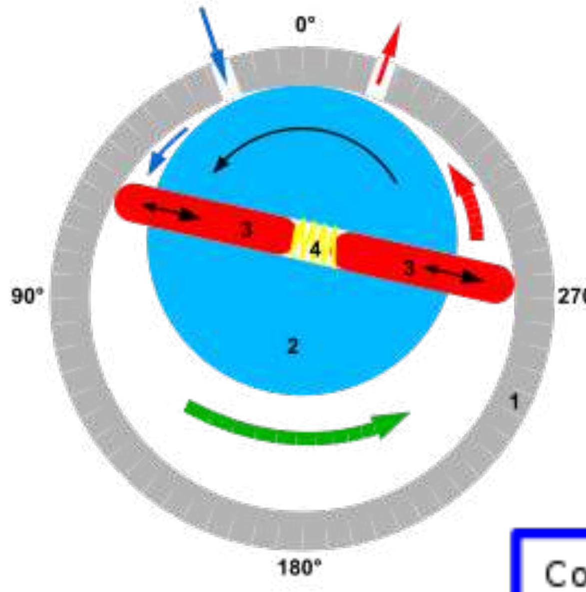
Contamination - reduce interaction gas, e-beam and sample

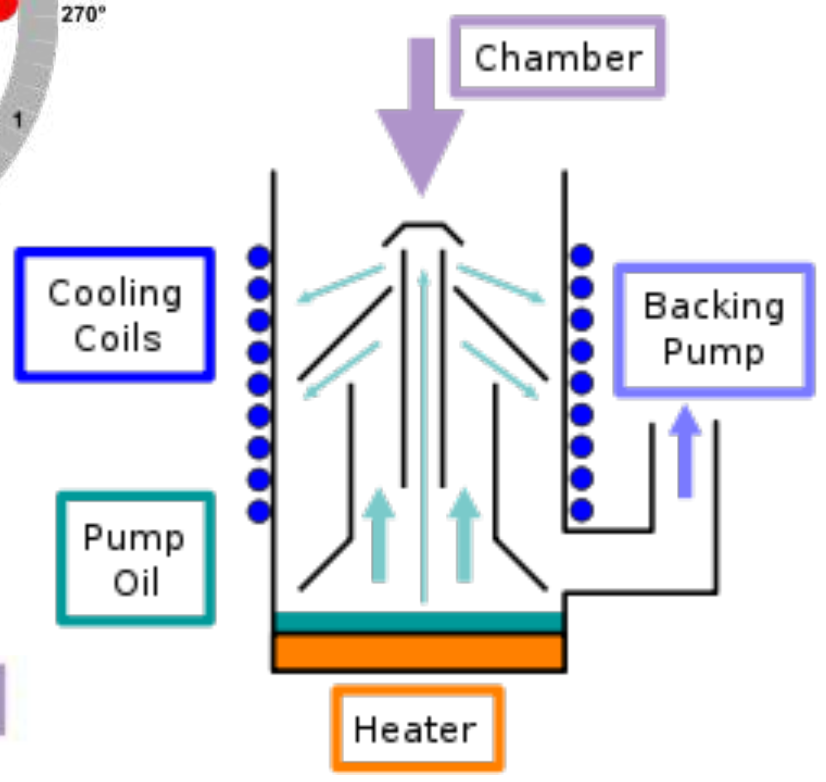
Vacuum systems

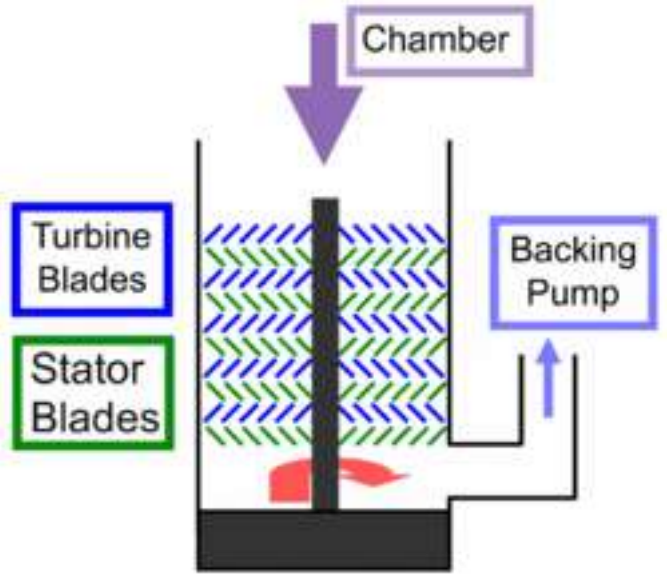


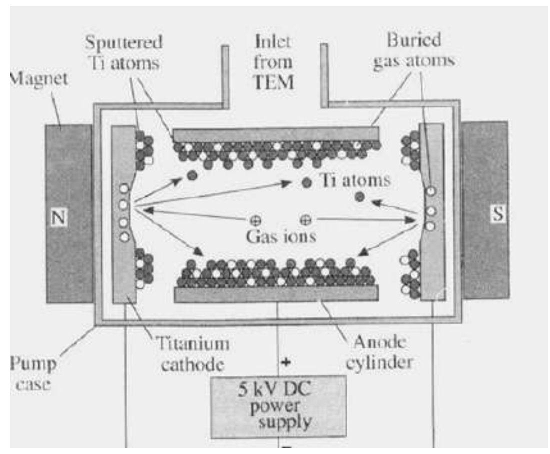
What types of pumps do we have?

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





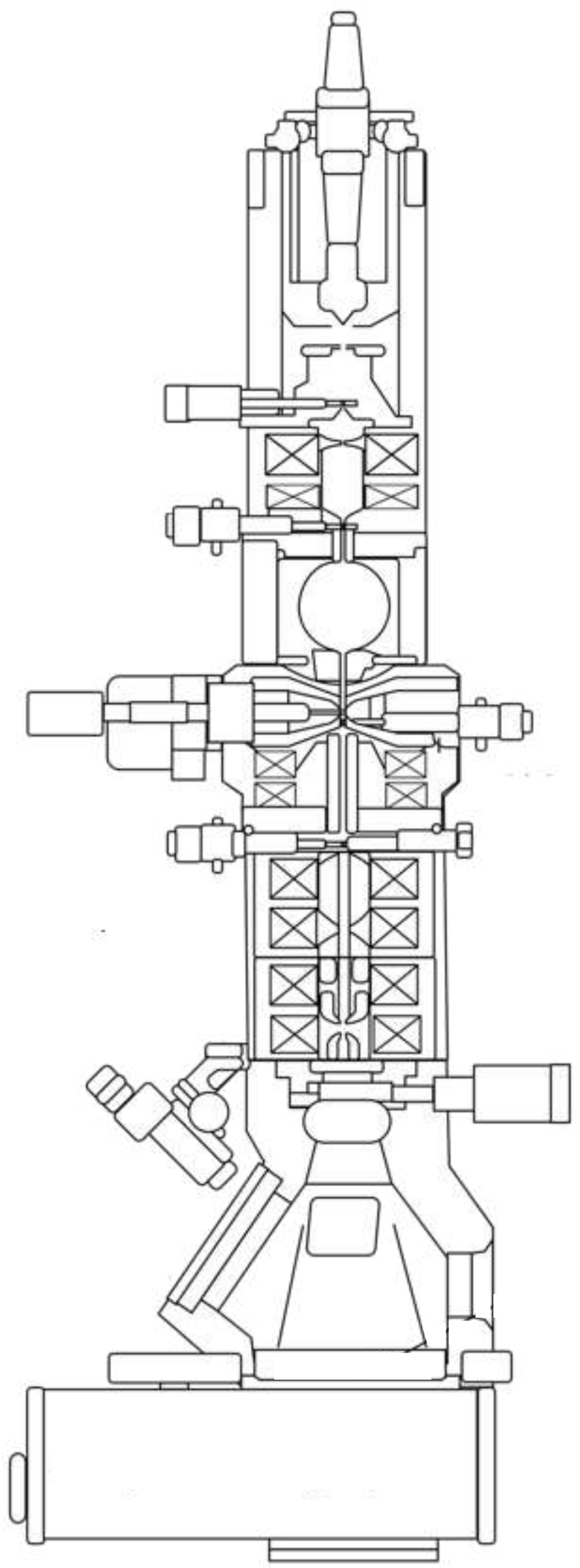




wikipedia.com

PVP / Rotary	$1\text{-}10^{-3}$ Torr	>0.1 Pa
Diffusion	$10^{-3}\text{-}10^{-6}$ Torr	$0.1\text{-}10^{-4}$ Pa
Turbo	$10^{-6}\text{-}10^{-9}$ Torr	$10^{-4}\text{–}10^{-7}$ Pa
IGP	$10^{-9}\text{-}10^{-12}$ Torr	$10^{-7}\text{-}10^{-9}$ Pa

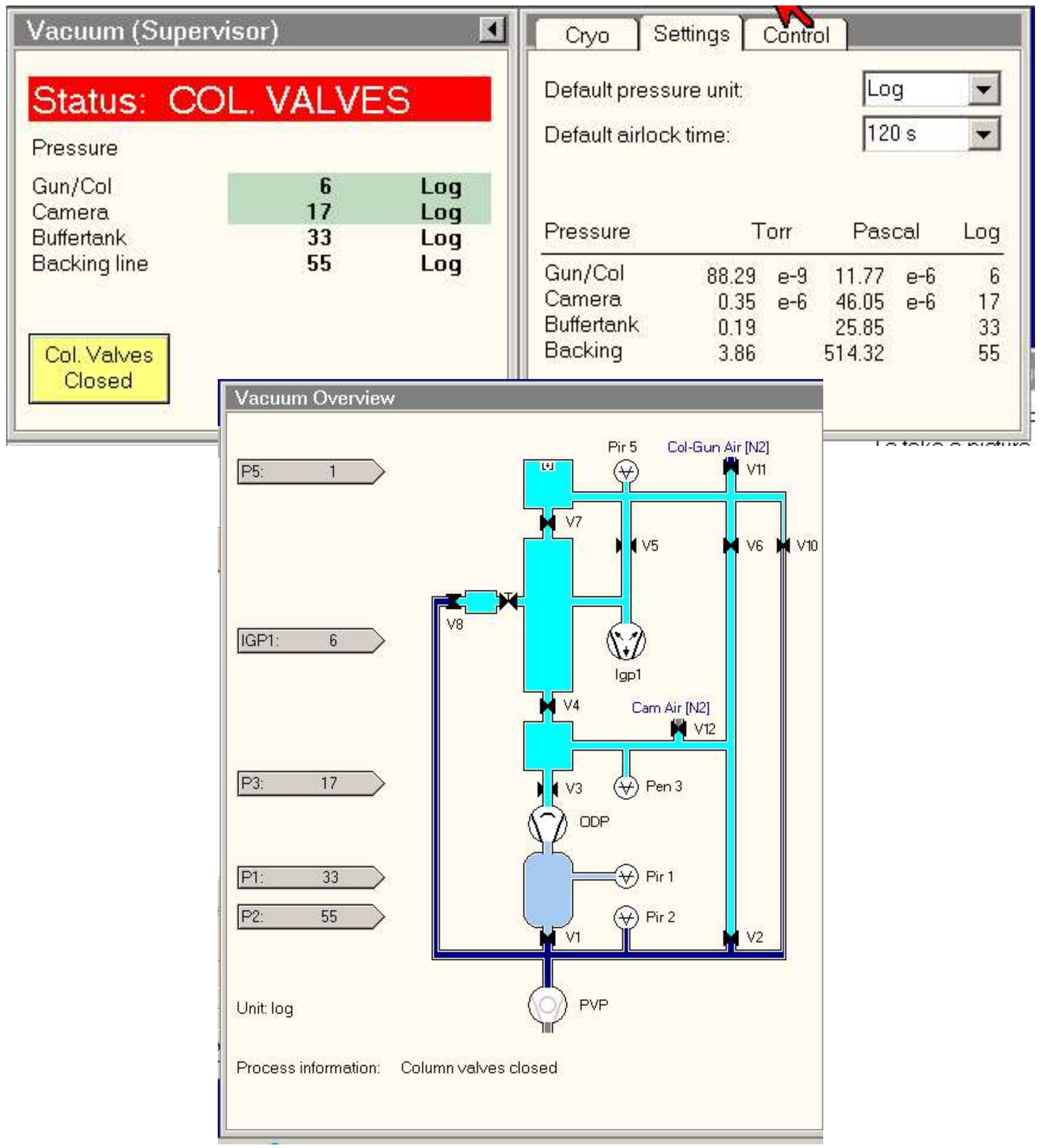
Vacuum systems



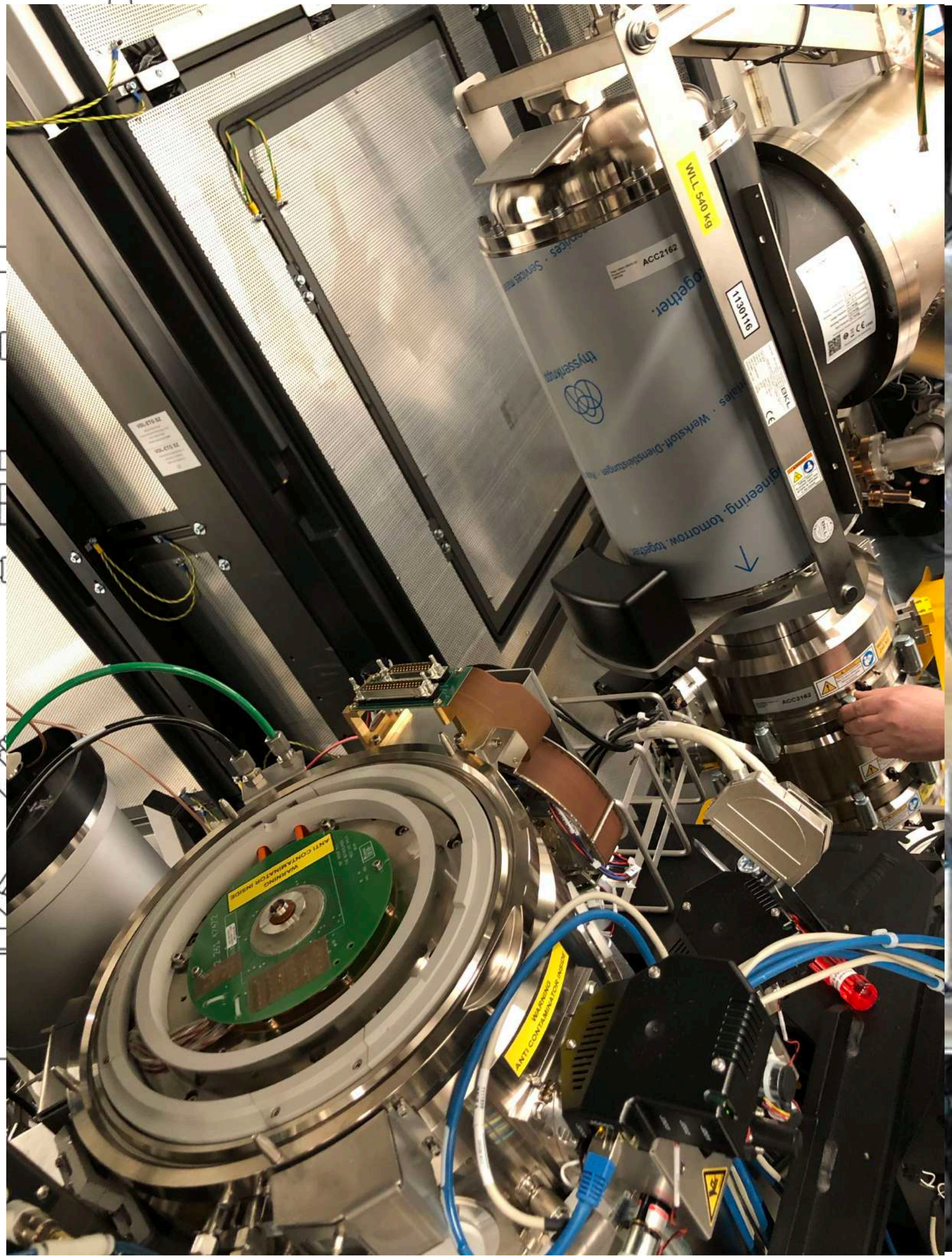
What types of pumps do we have?

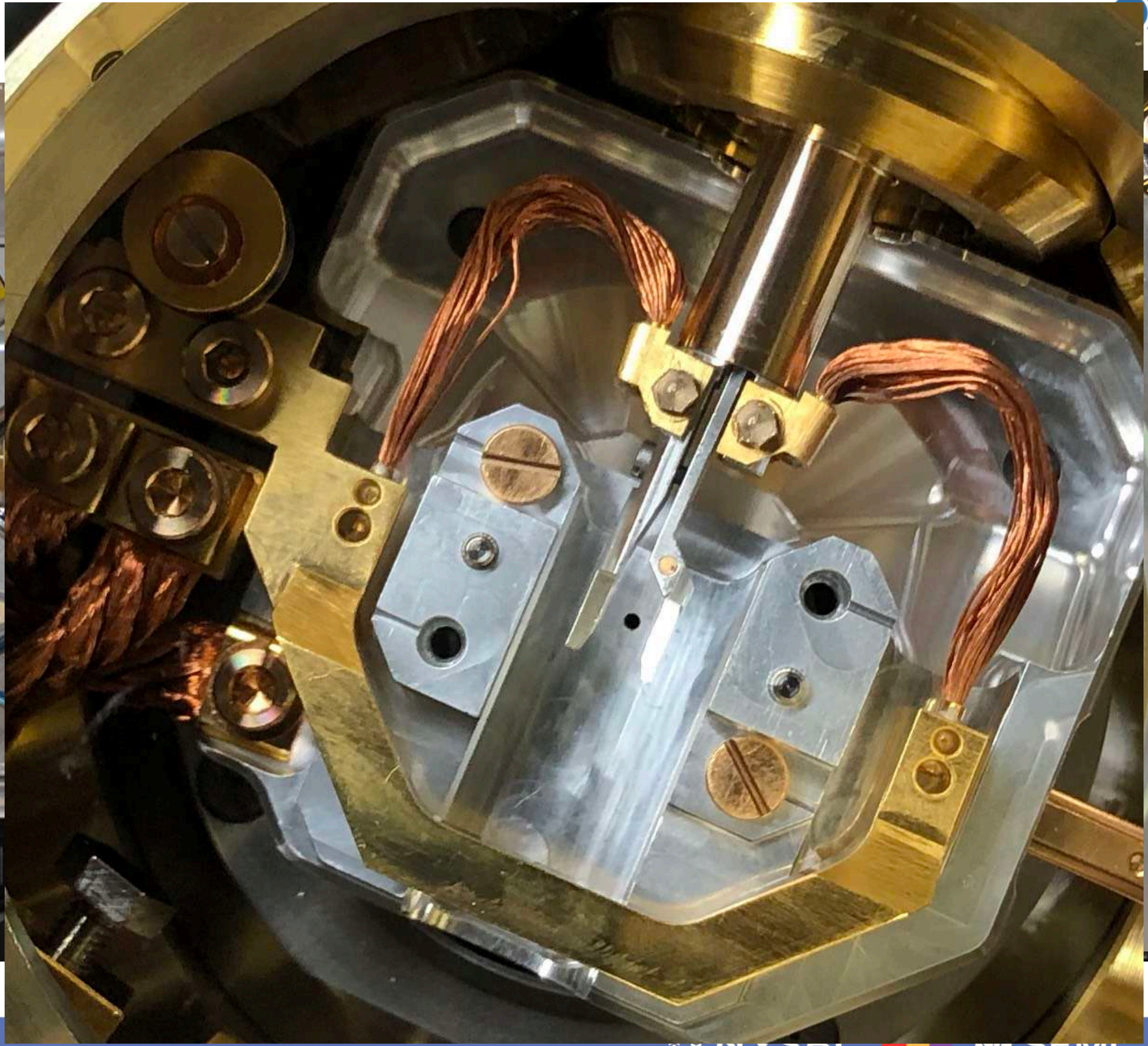
$1\ \text{mm Hg} = 1\ \text{Torr} = 10^2\ \text{Pa}$
$1\ \text{atm} = 760\ \text{Torr} = 7.5\times10^4\ \text{Pa}$

Gun	10^{-9} Torr
Specimen	10^{-6} - 10^{-7} Torr
Chamber and Camera	10^{-5} - 10^{-6} Torr

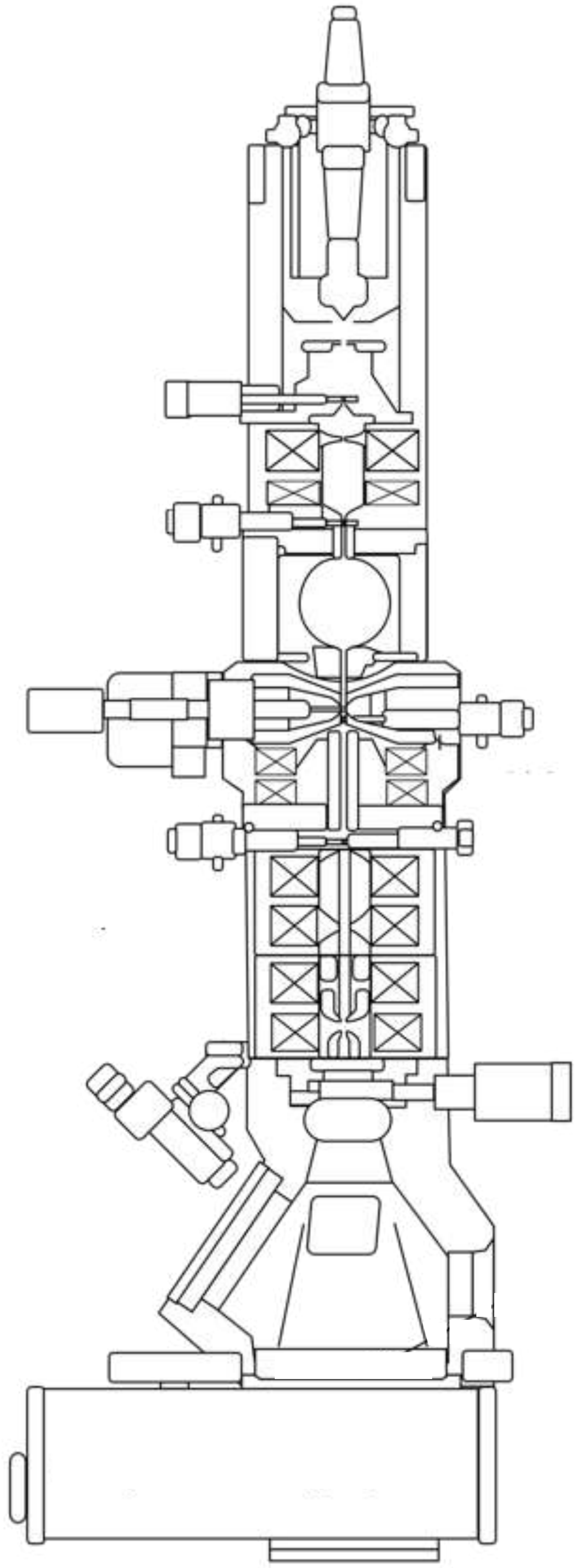


Vacuum systems

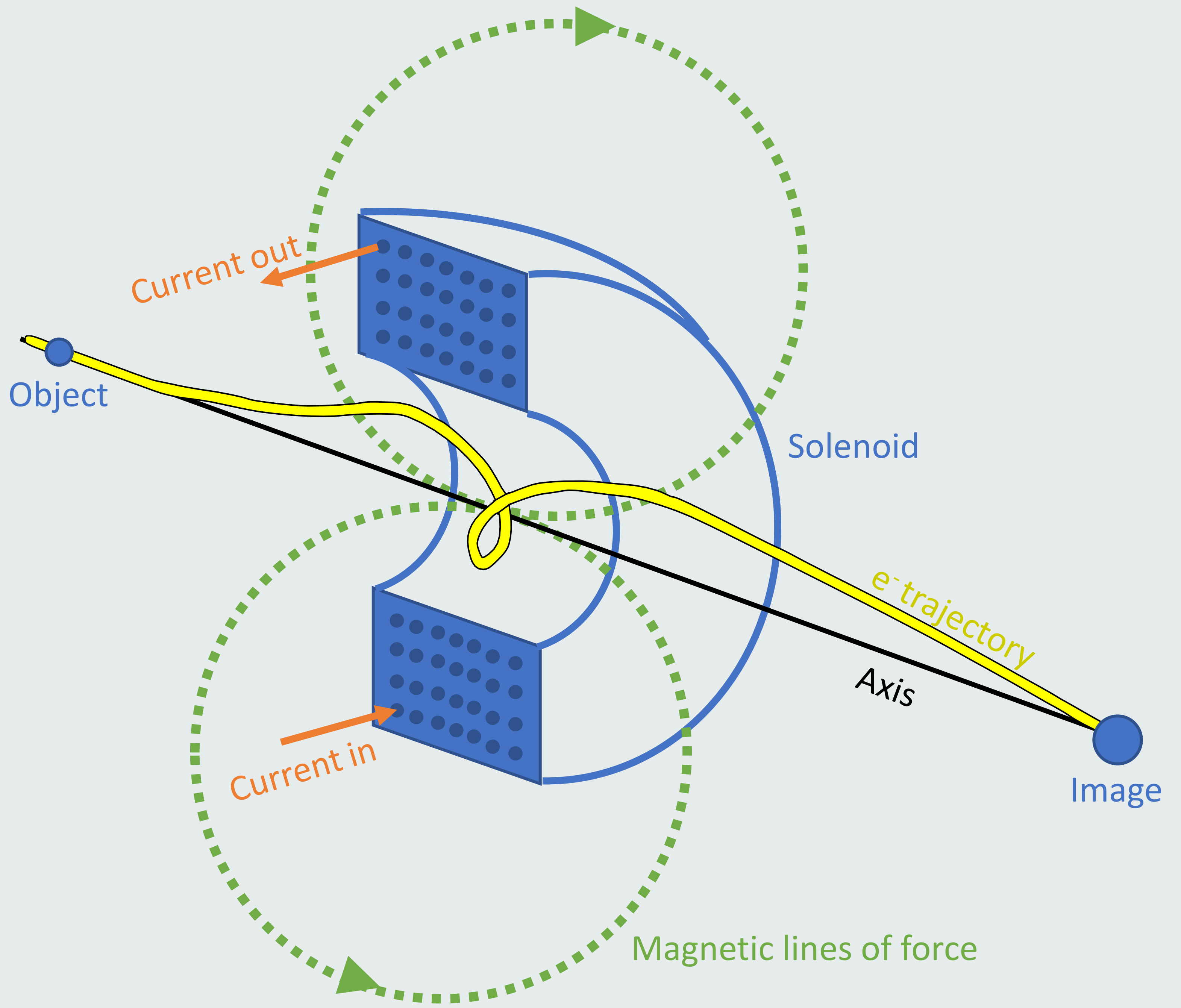




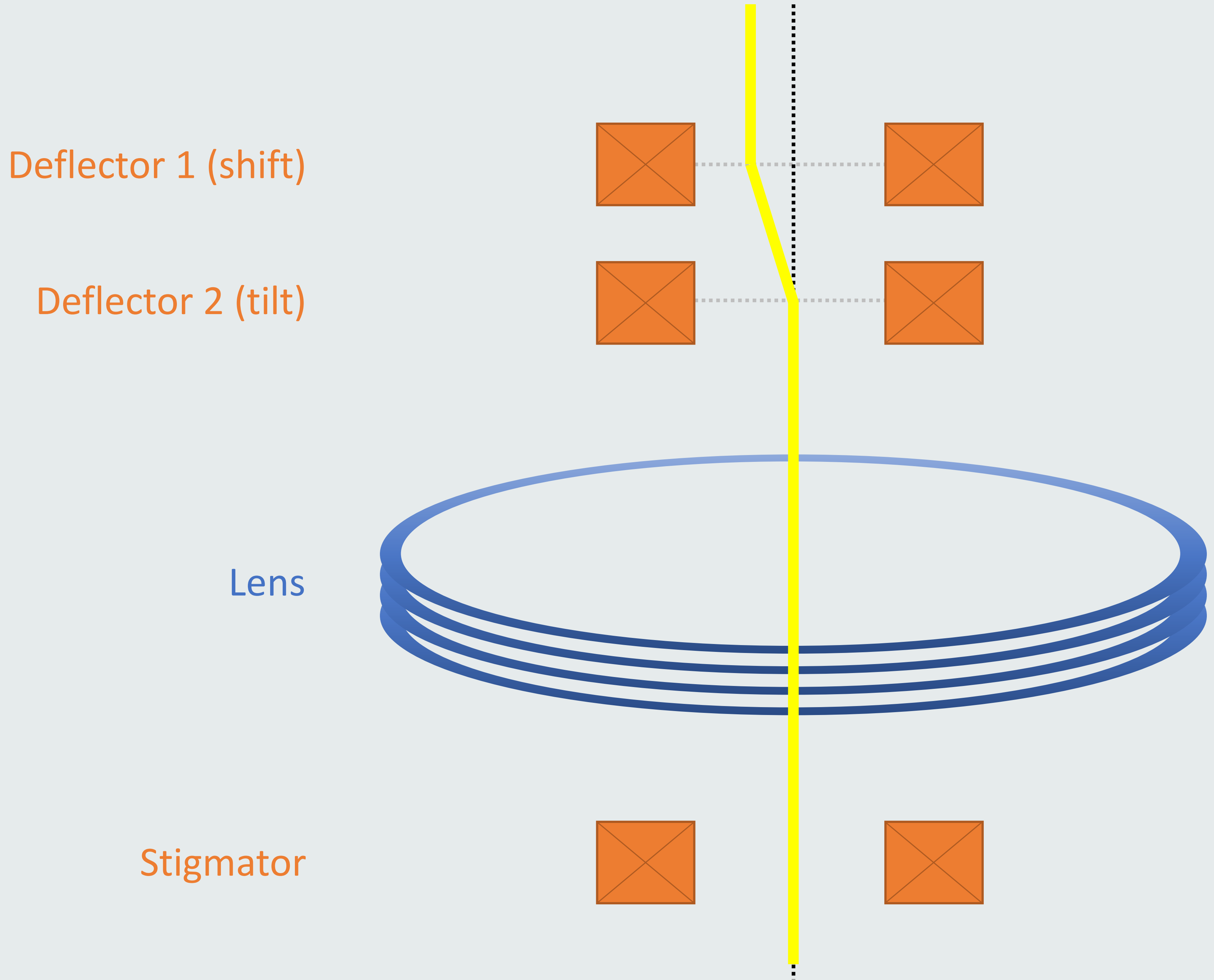
Lenses



What types of lenses do we have?

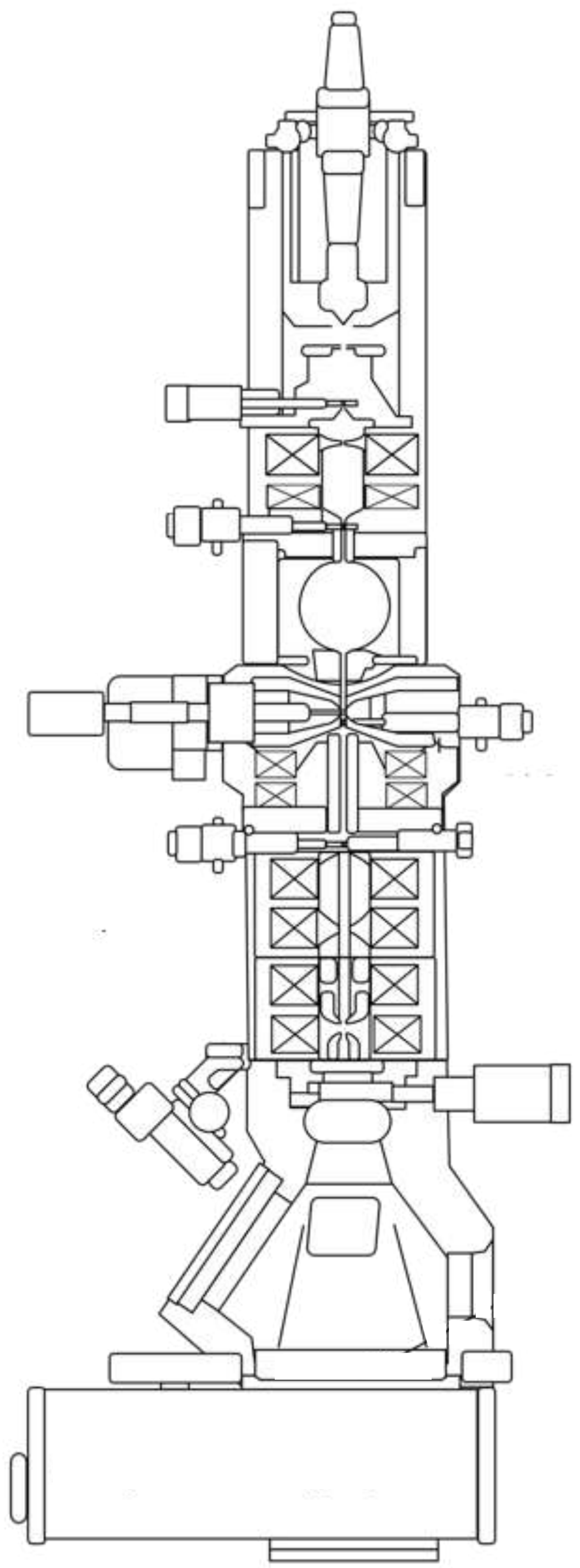


- Focus
- Magnify
- Rotate

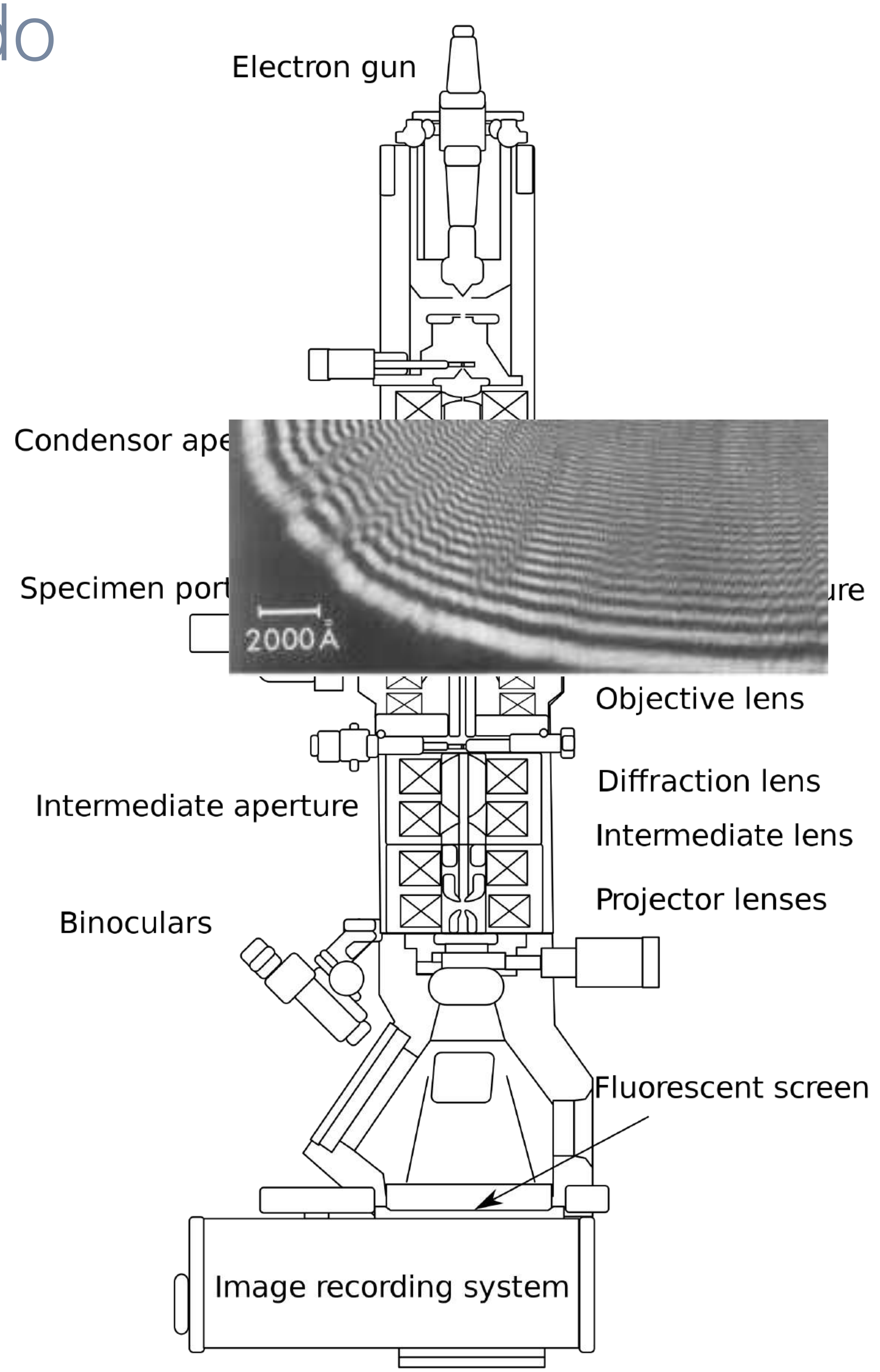


Lenses

Microscope Alignments
What to do & what not to do



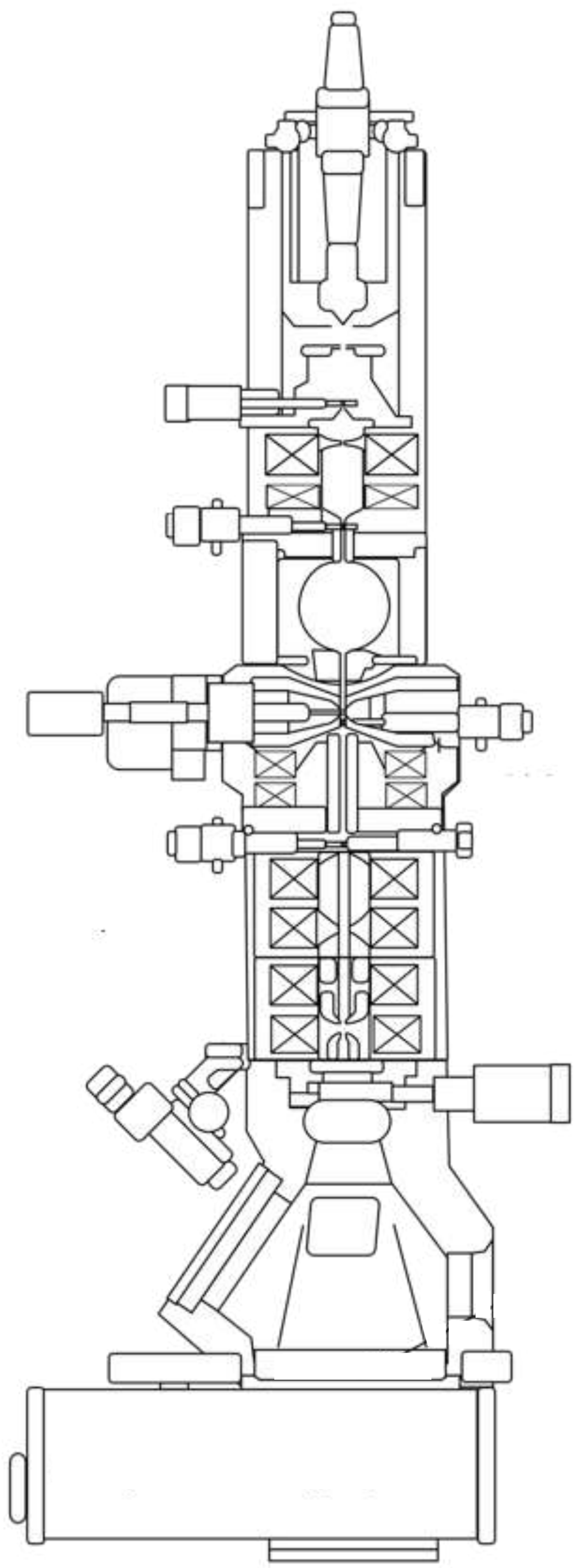
- Do:
  - Start at eucentric height and focus
  - Check if it is already good before attempt
  - Align from top to bottom
- Not to do:
  - ~~Align without a way to undo~~
  - ~~Align when TEM is not stable (i.e., temperature)~~

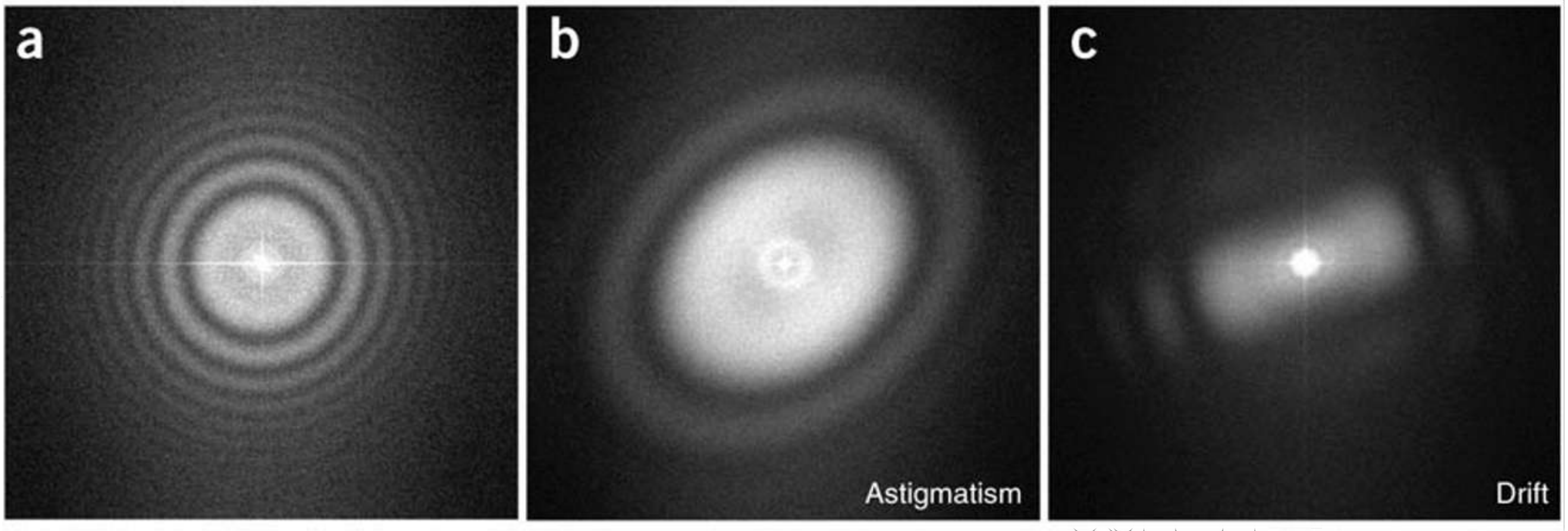


Lenses

Microscope Alignments
What to do & what not to do

- Do:





- ~~Align without a way to undo~~
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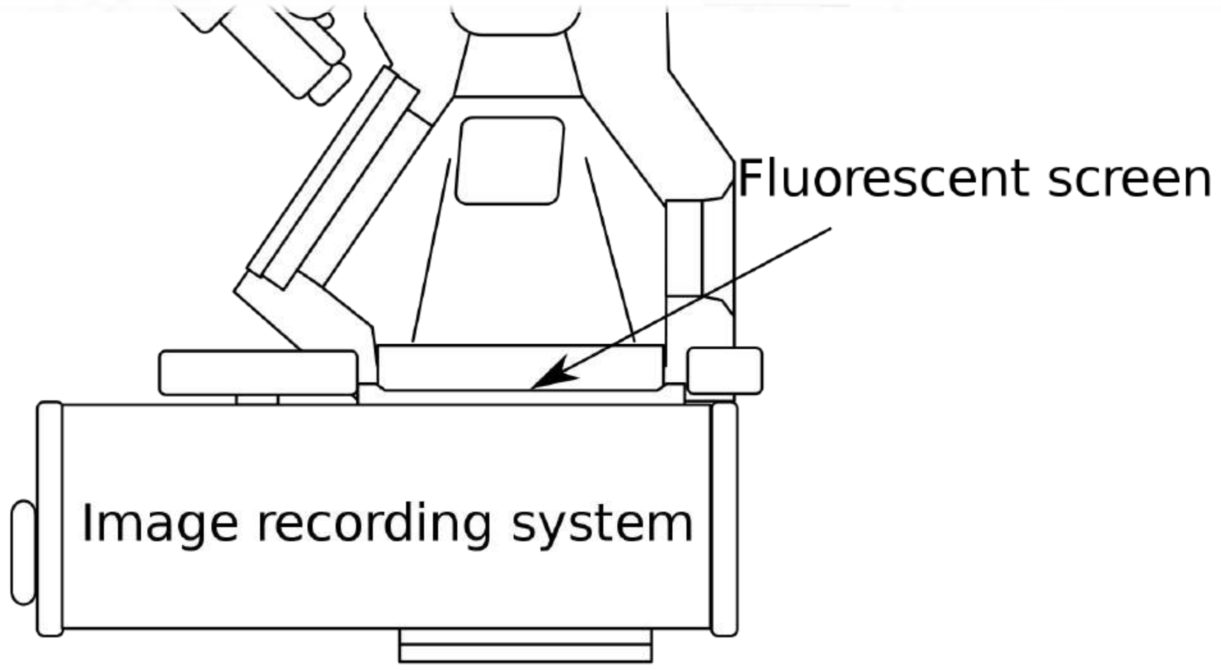
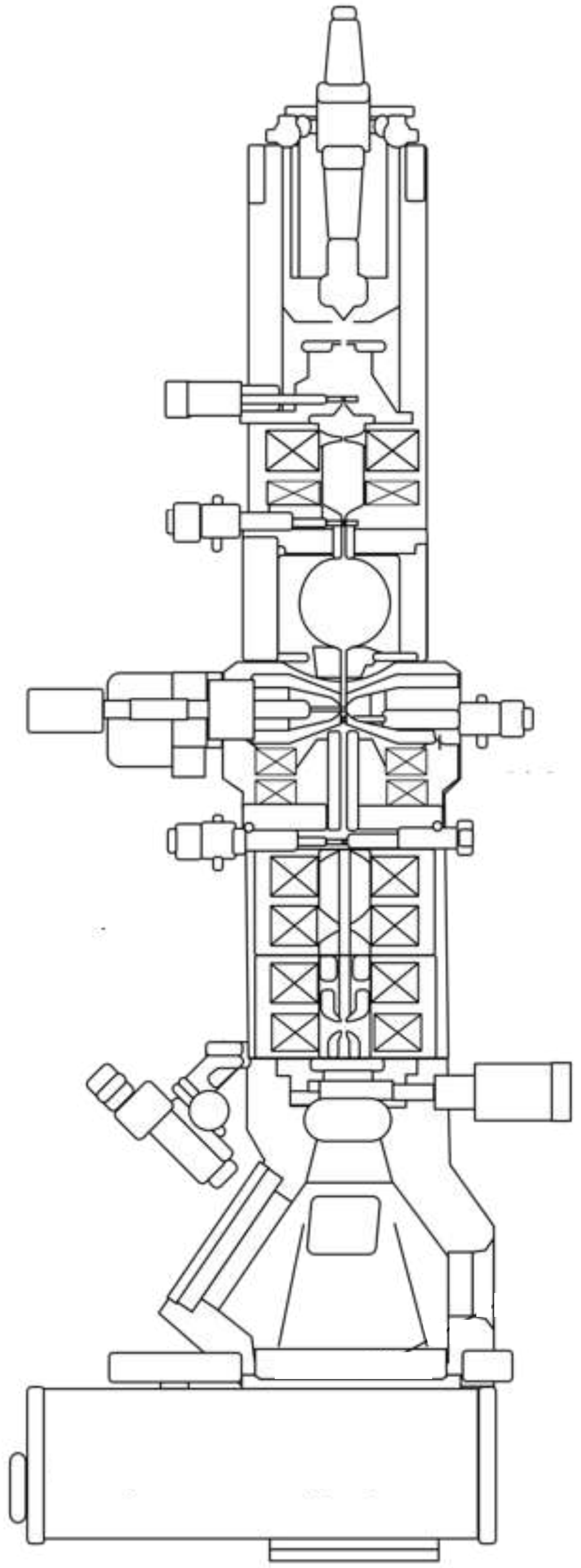


CTF



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,

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

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

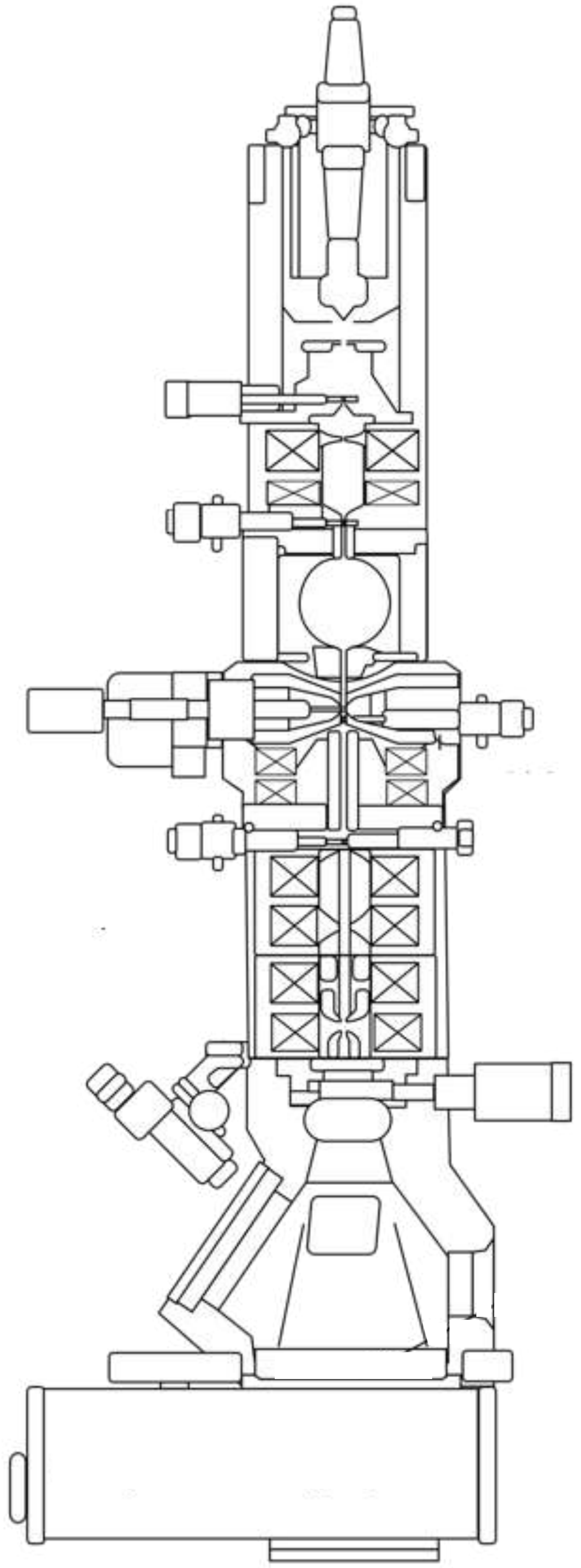
where,

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

Lenses

CTF



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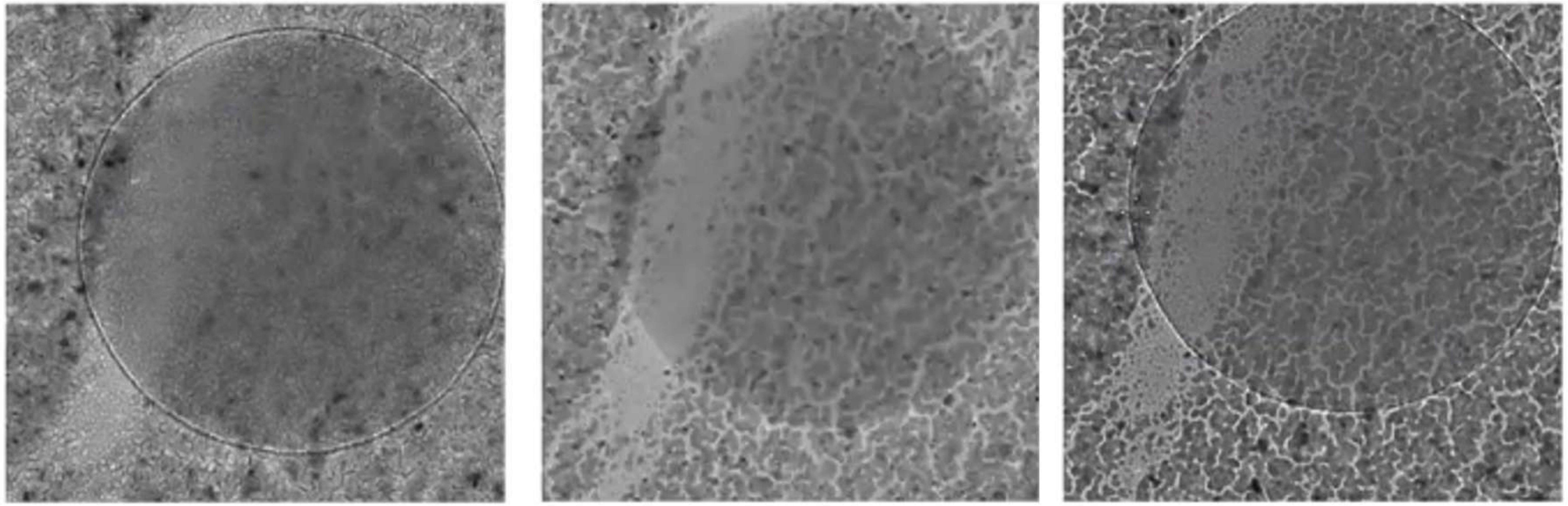
where



Lenses

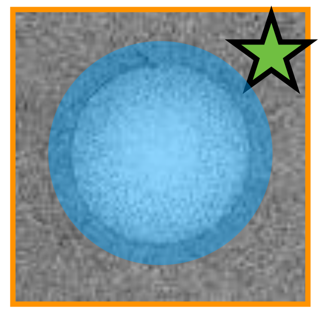
How to increase efficiency?

13.6MP

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;

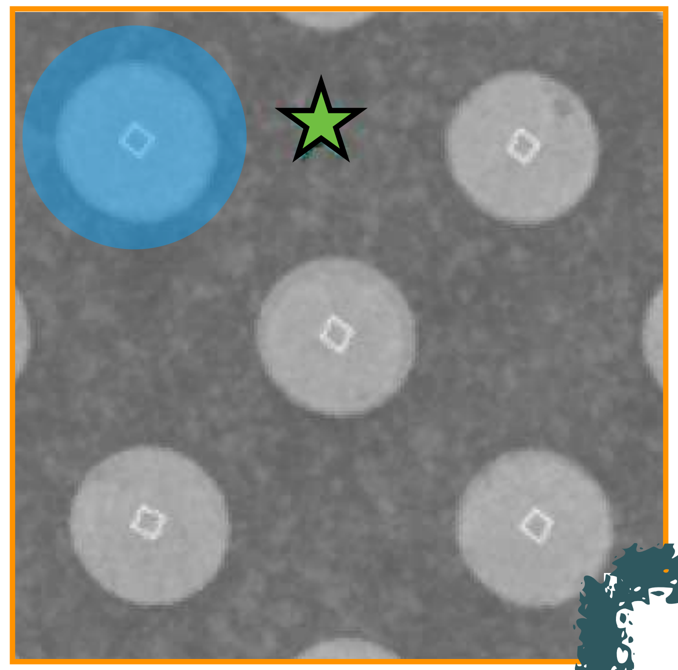
Anchi Cheng

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



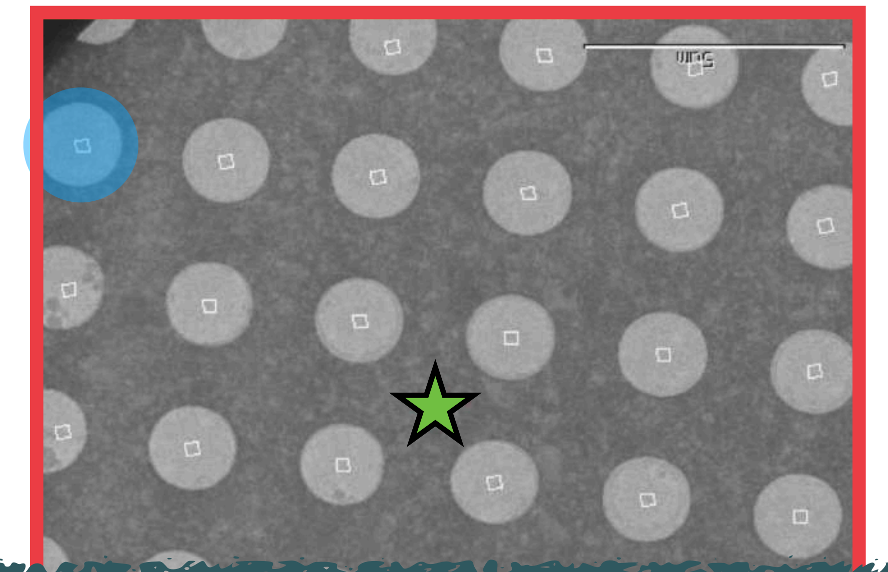
beam tilt
0 mrad

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

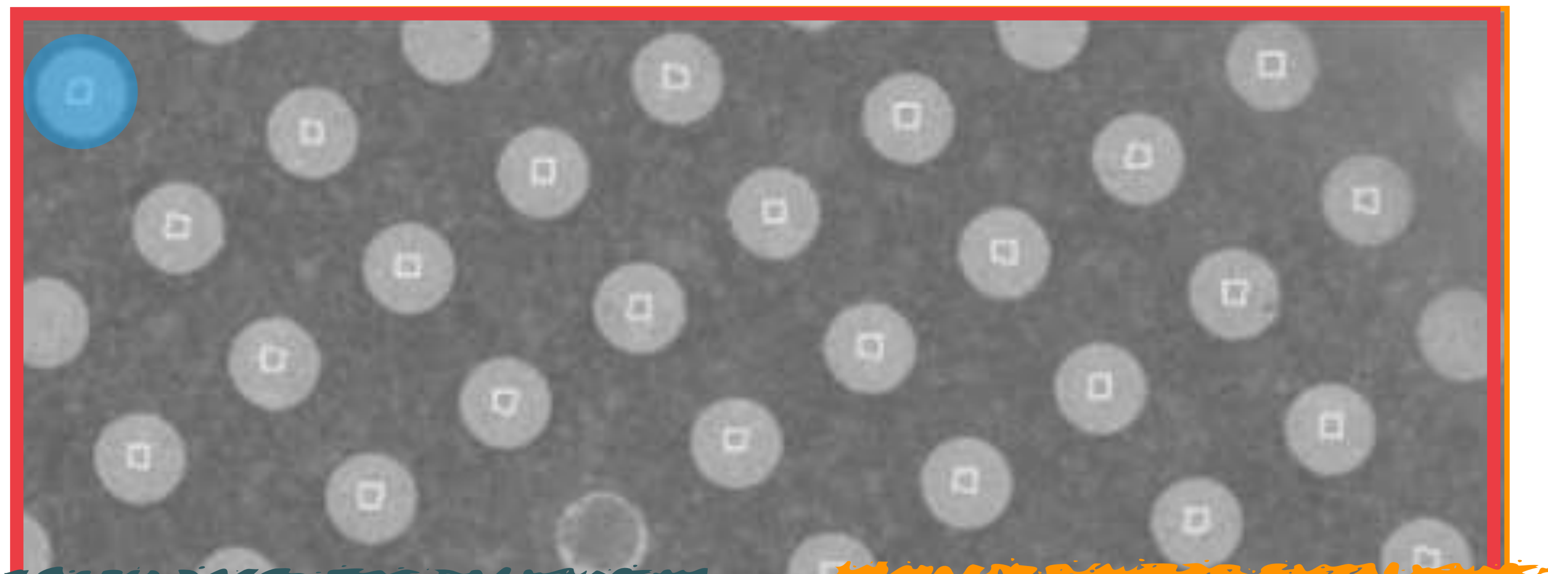


beam tilt
0.5 mrad

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



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

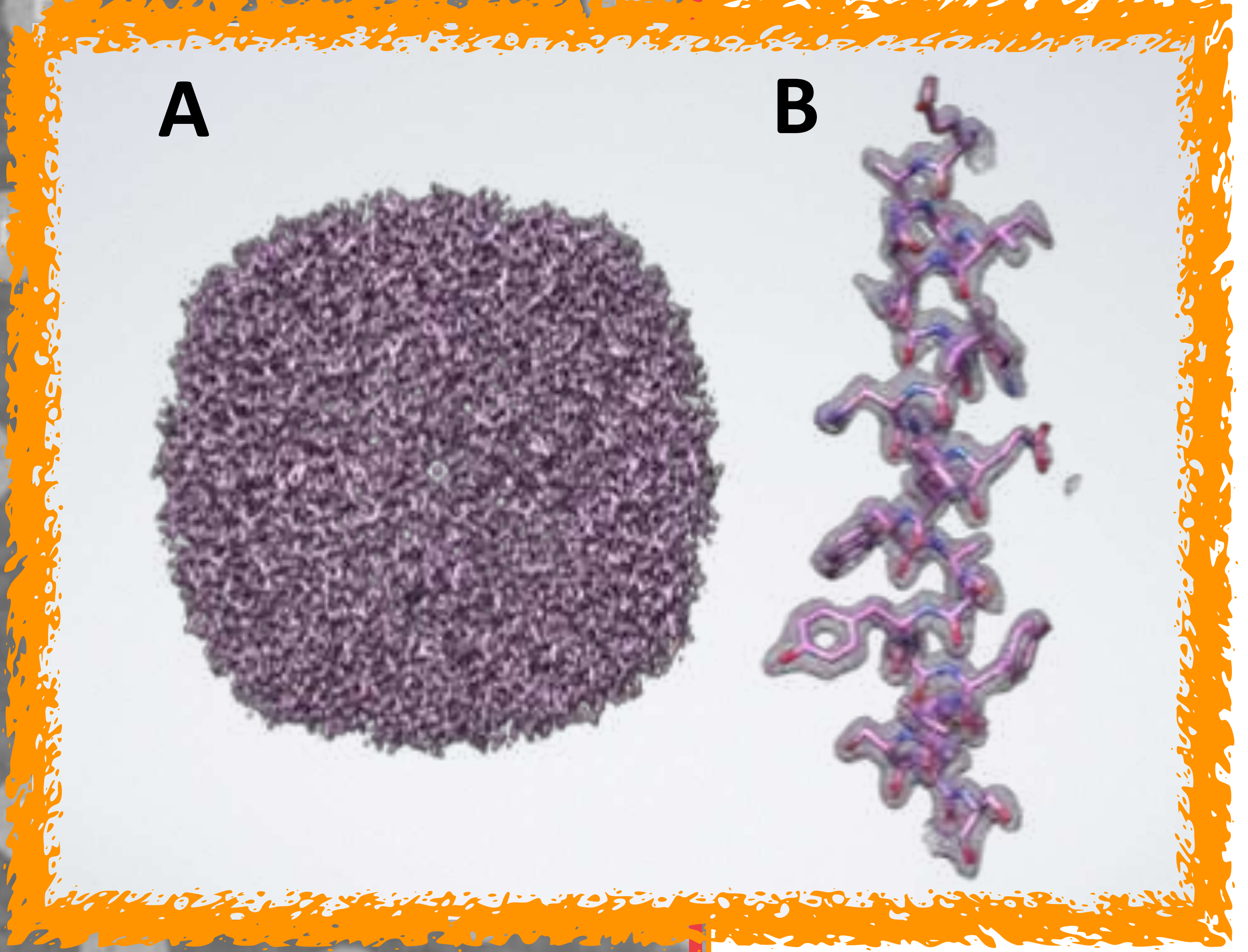


beam tilt
~3 mad

But… image shift ind

so… implement hardwar

Upgrade to K3
24MP
3.75x the
framerate of K2



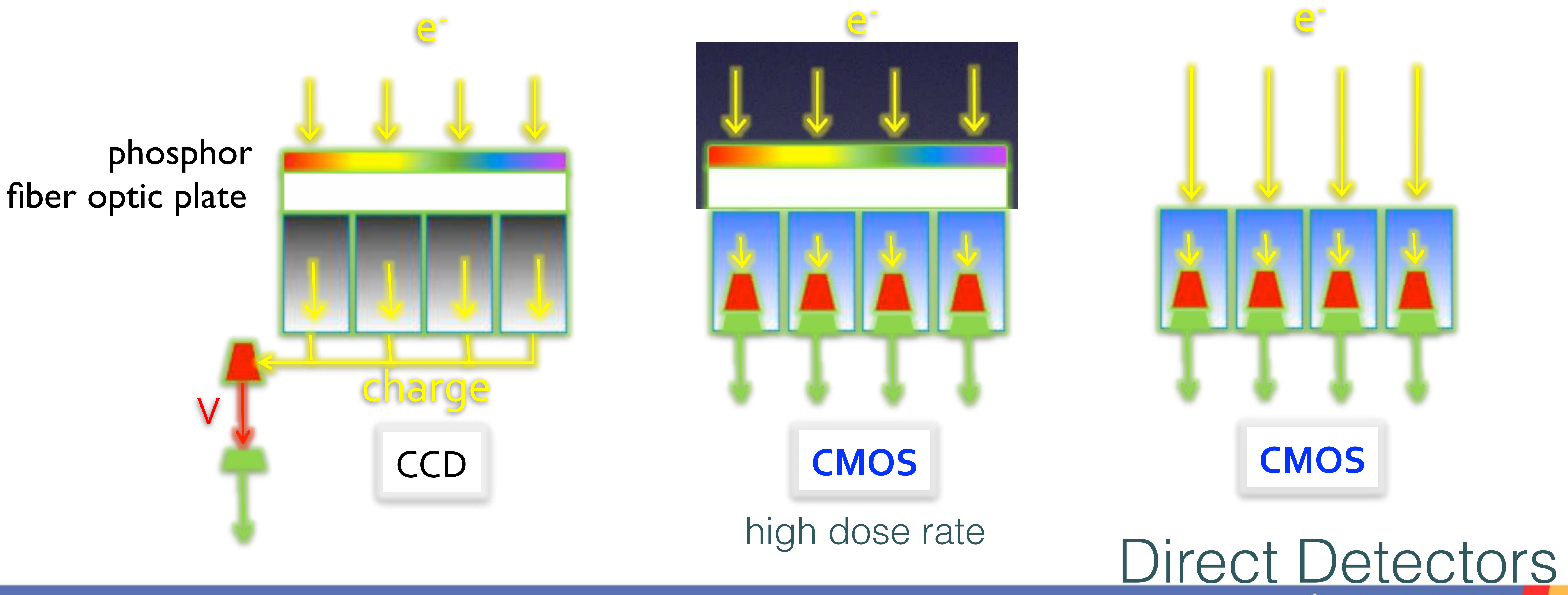
Overhead
30 s stage move and settling
30 s focus and drift check
20s for K2 40 frame movie to save

Detectors

Digital Cameras for TEM

- Photon converted
- Direct sensing

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

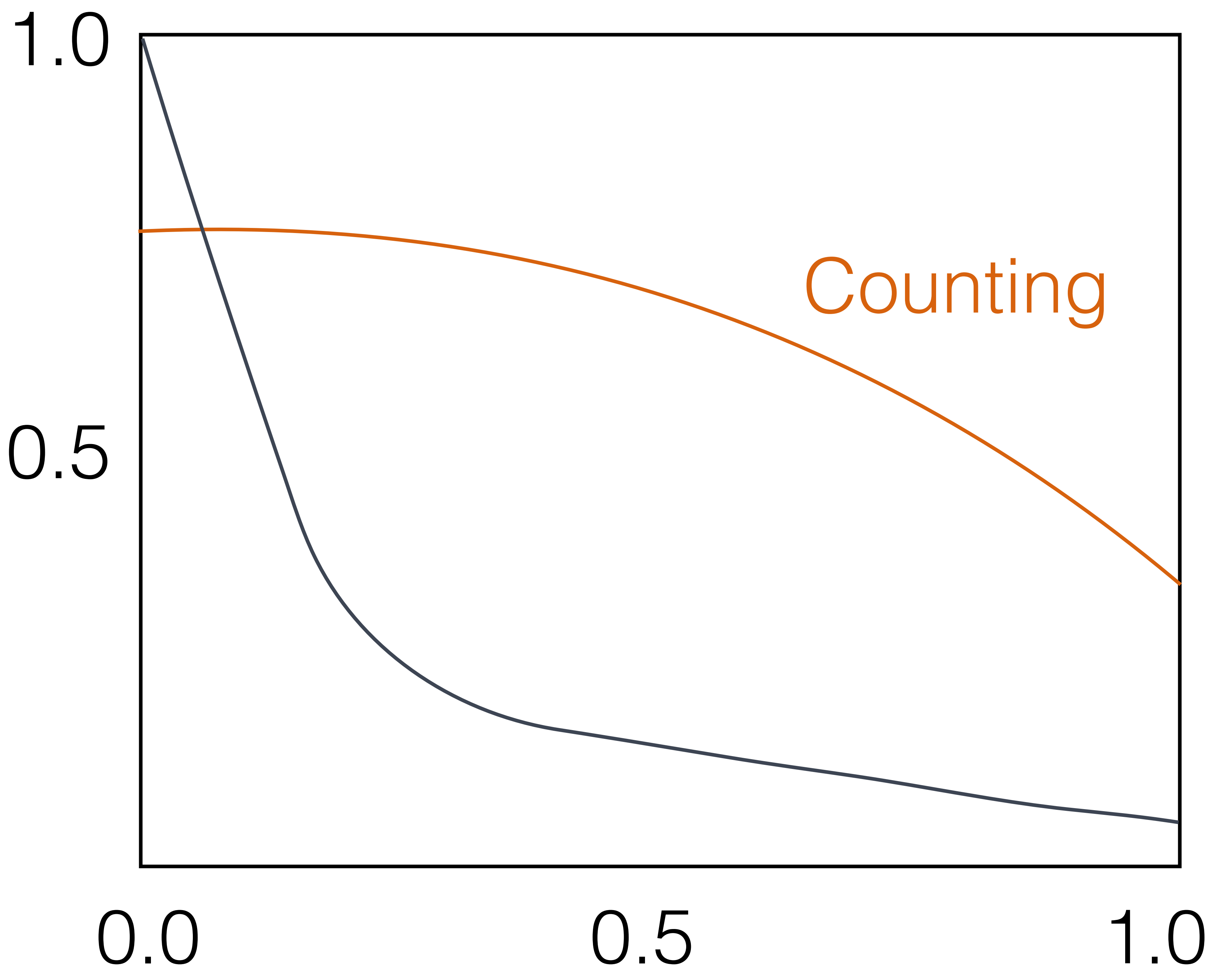


Anchi Cheng

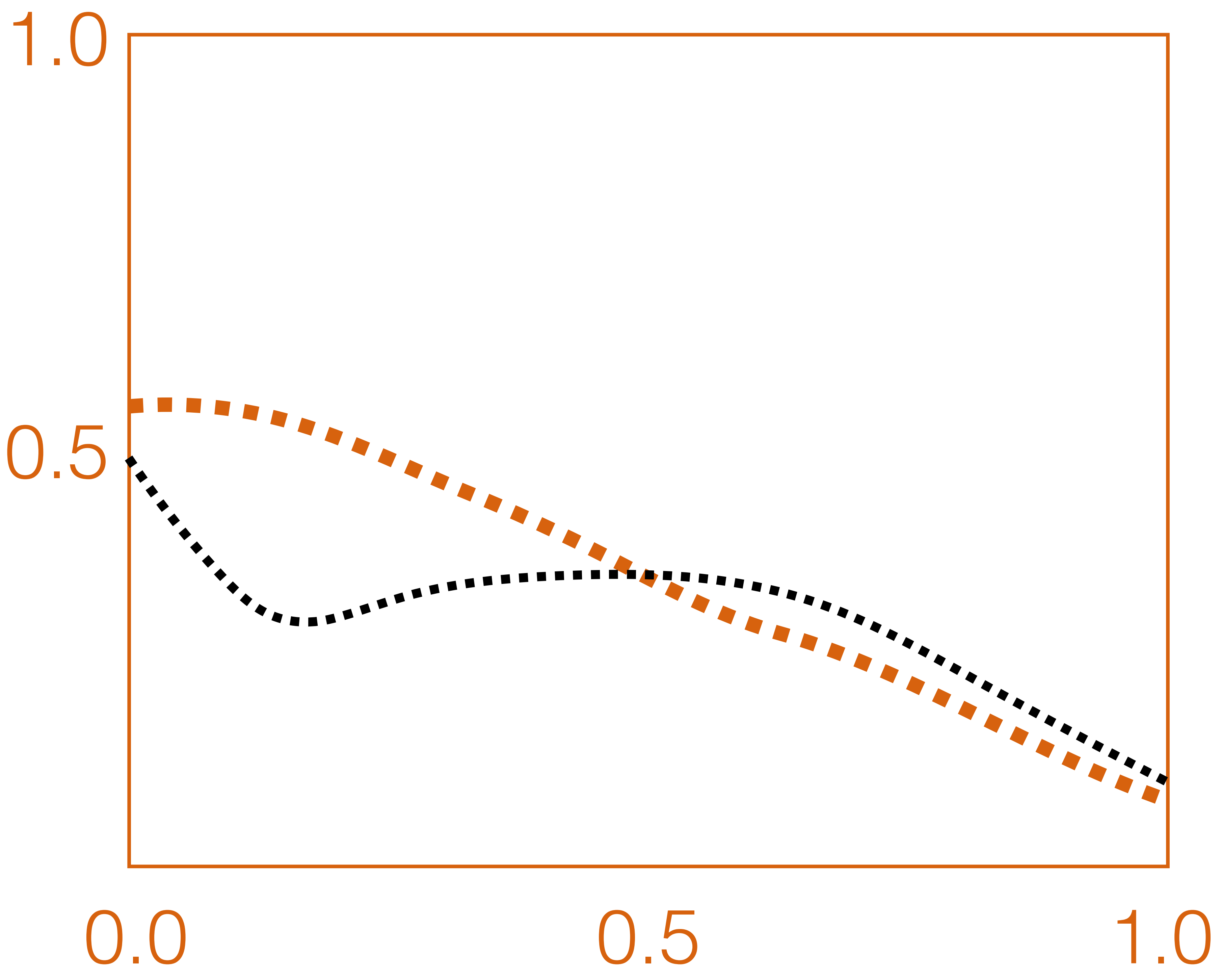
Detectors

Detector Performance Characterization

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

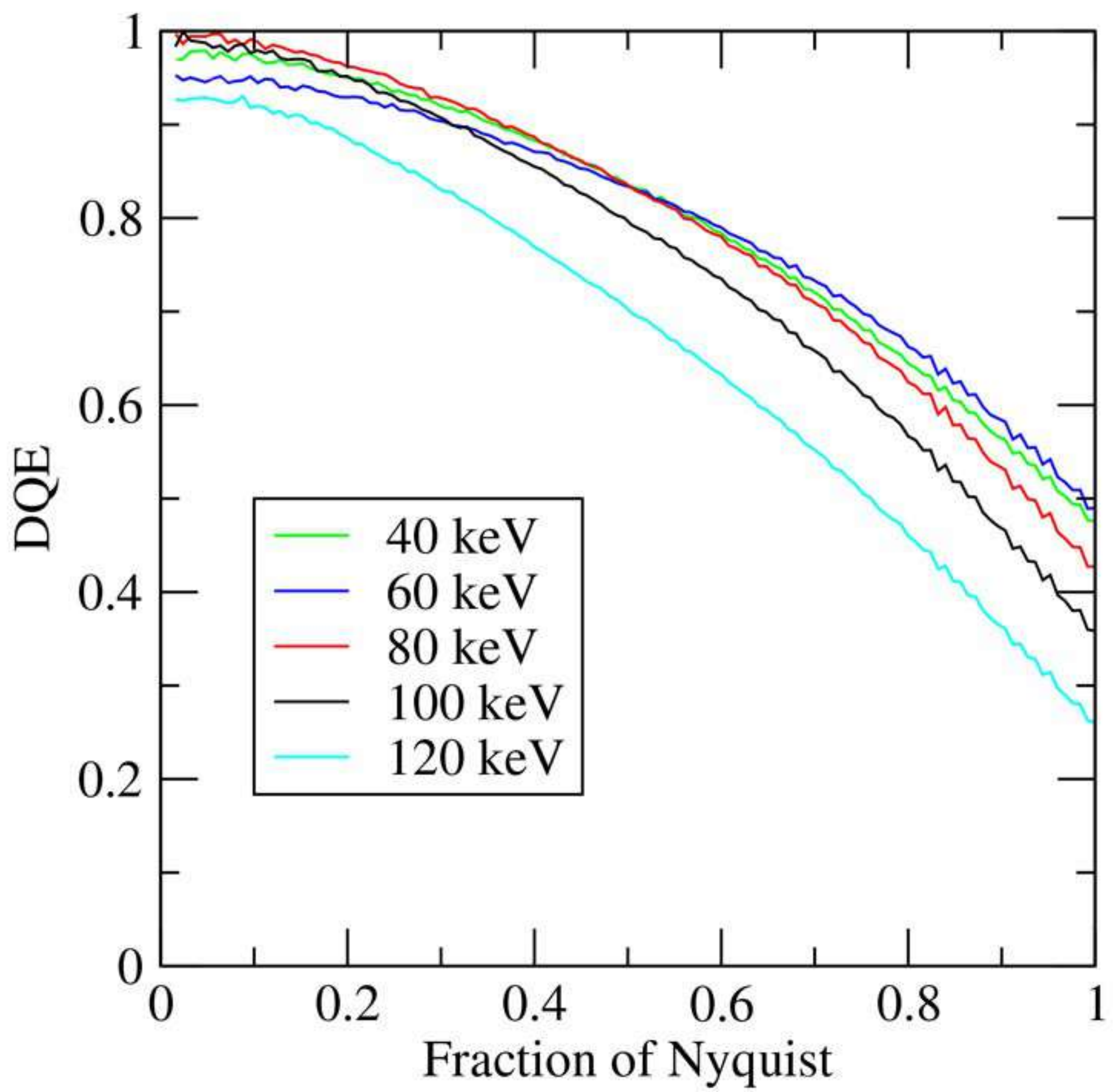


- DQE (Detector Quantum Efficiency)
  - S/N over spatial frequency range

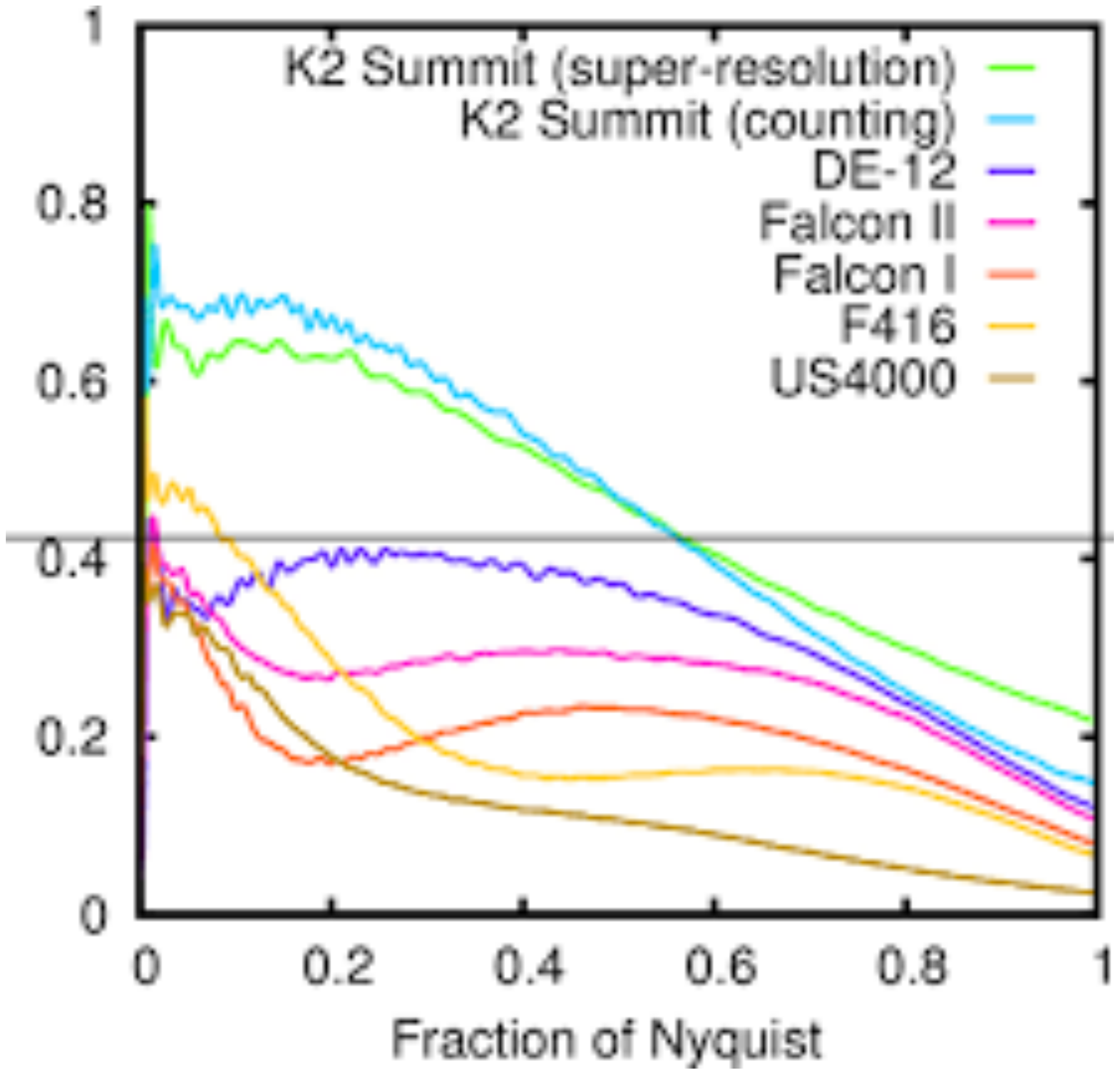


Detectors

Detector Performance Characterization



dectris.com

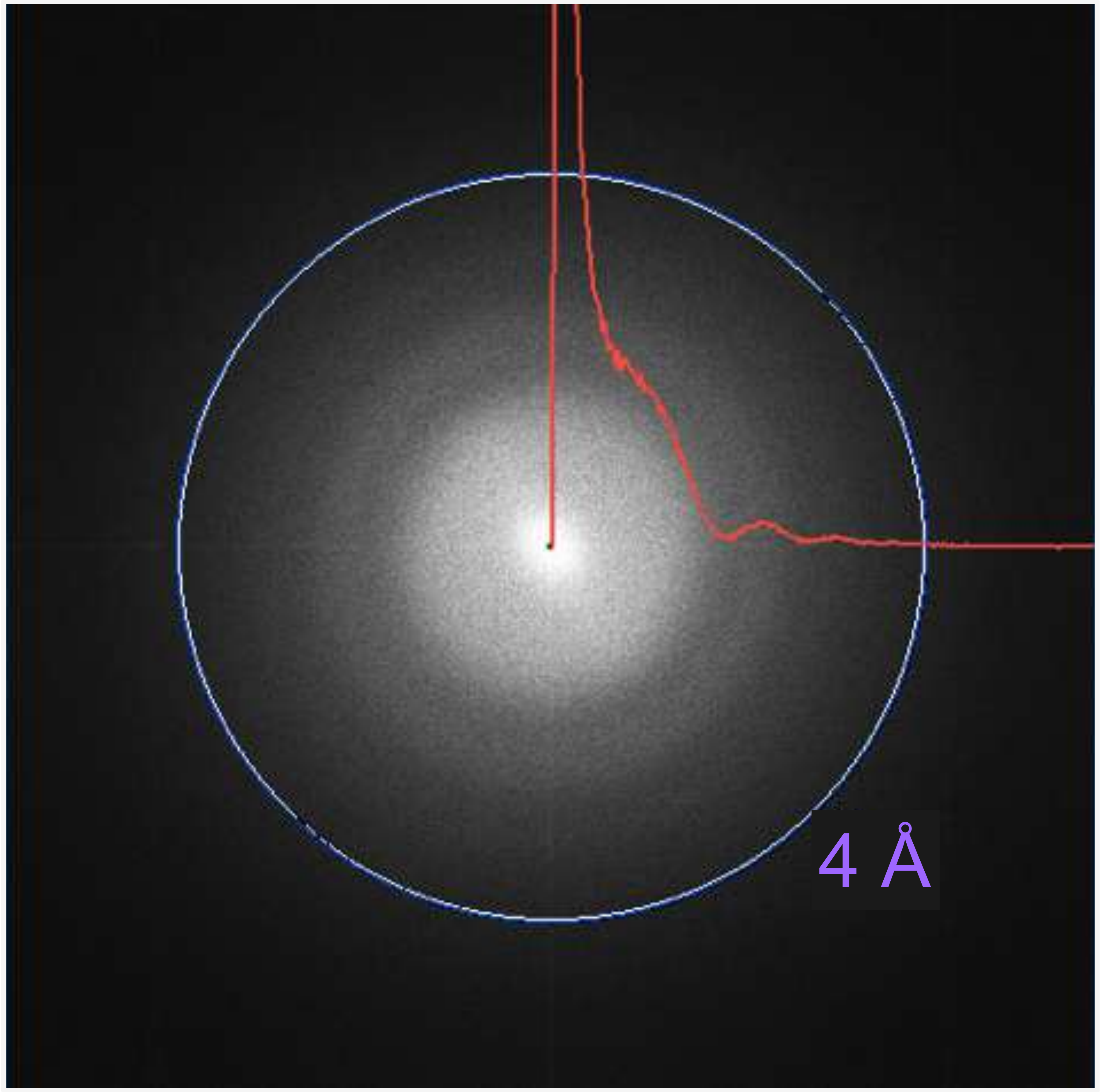


Ruskin, et al JSB

Detectors

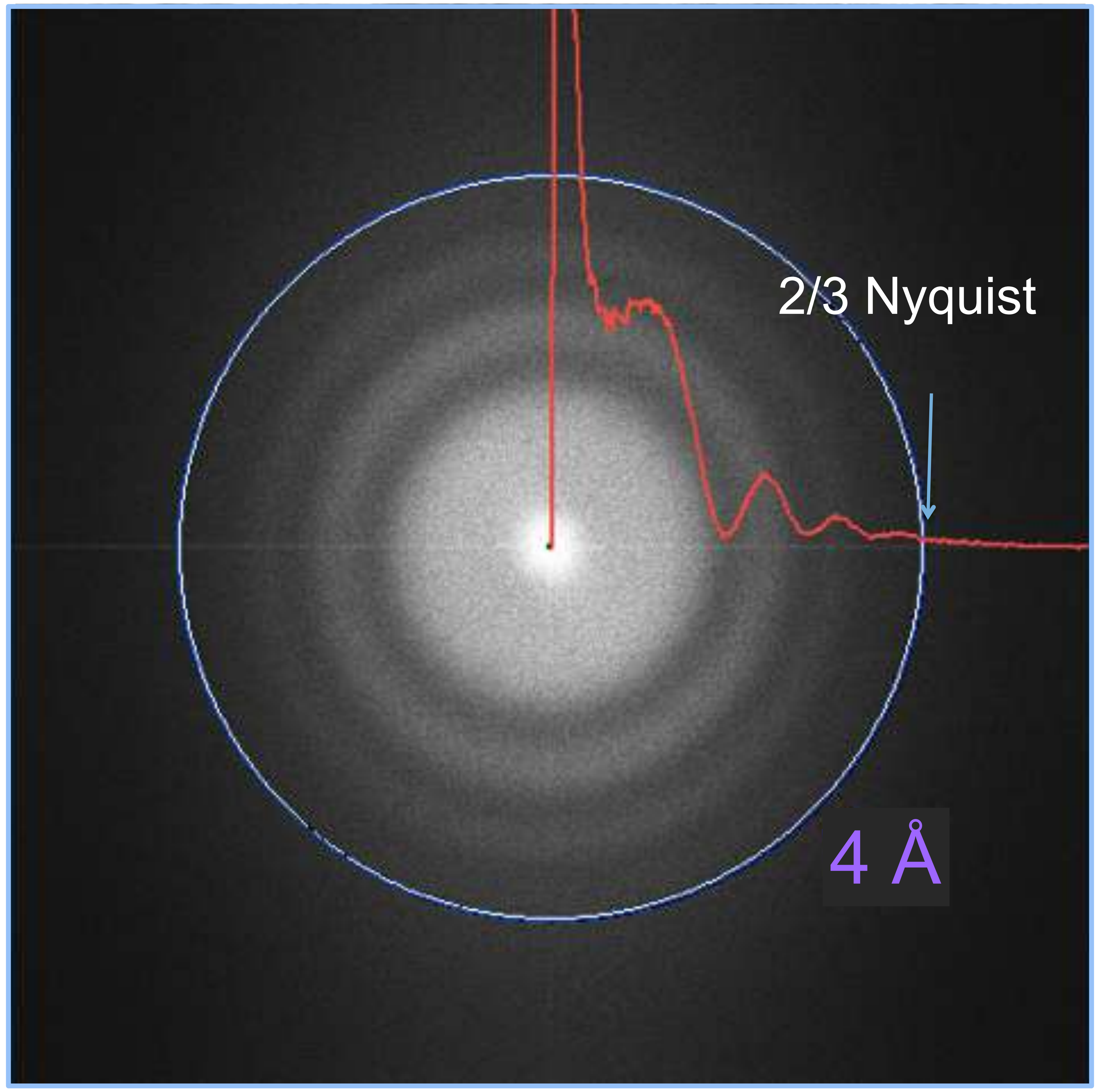
Improving the resolution:
Detecting electrons instead of photons

CCD



1.37 Å/pixel

DDD



1.38 Å/pixel

200KeV ; 20 e-/Å^2 ; carbon film ;
3k x 3k image

Detectors

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 0.5	 >0.87 / >0.83 >0.53 / >0.53	 >0.8 >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.

Detectors

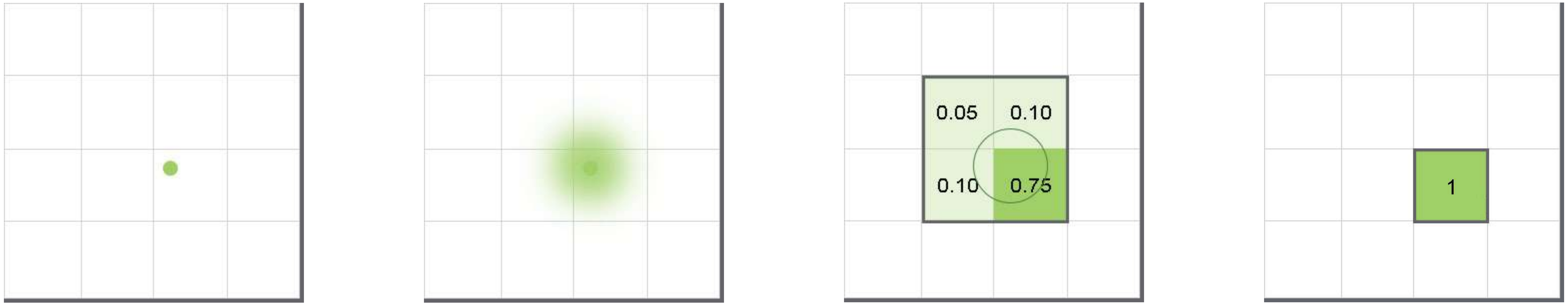
Improving the resolution:
Detecting electrons instead of photons

5,760 x 4,096 px

↓

11,520 x 8,184 px

Counting mode



Electron enters detector.

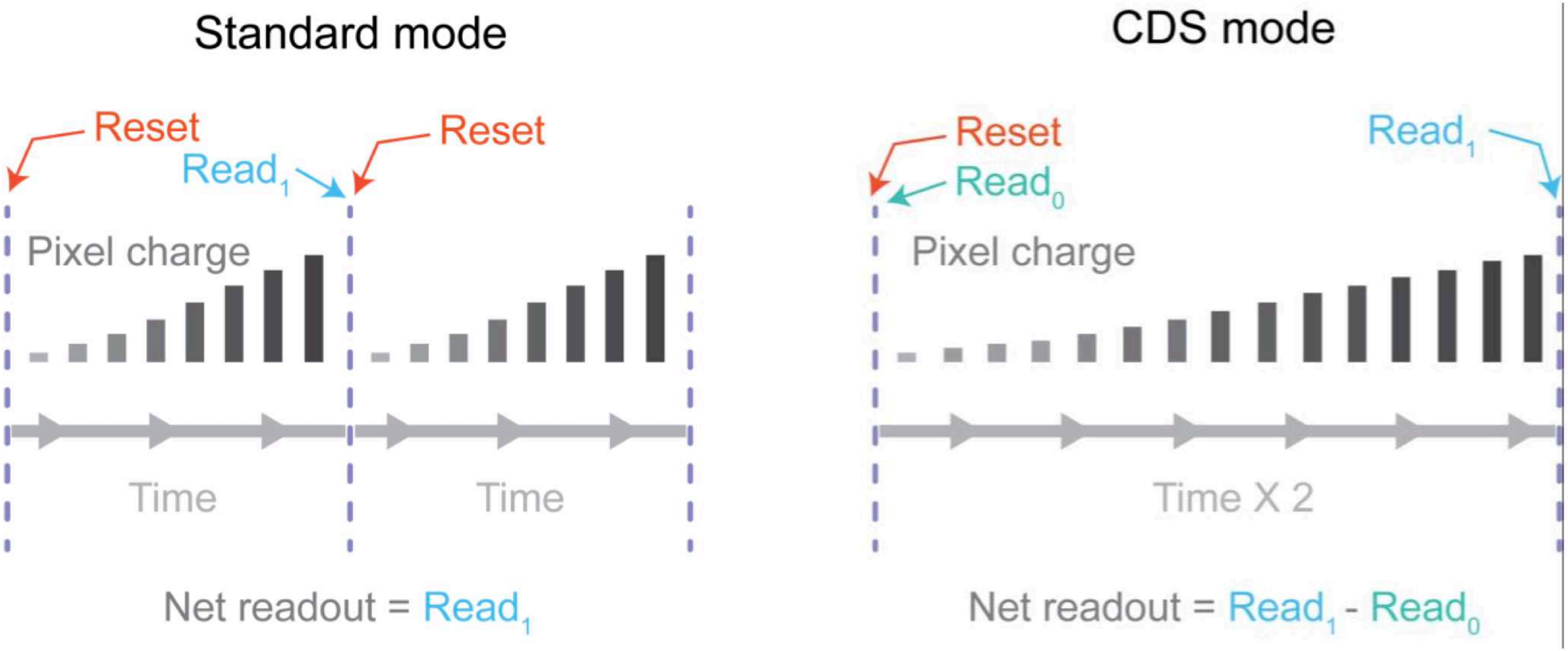
Electron signal is scattered.

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)

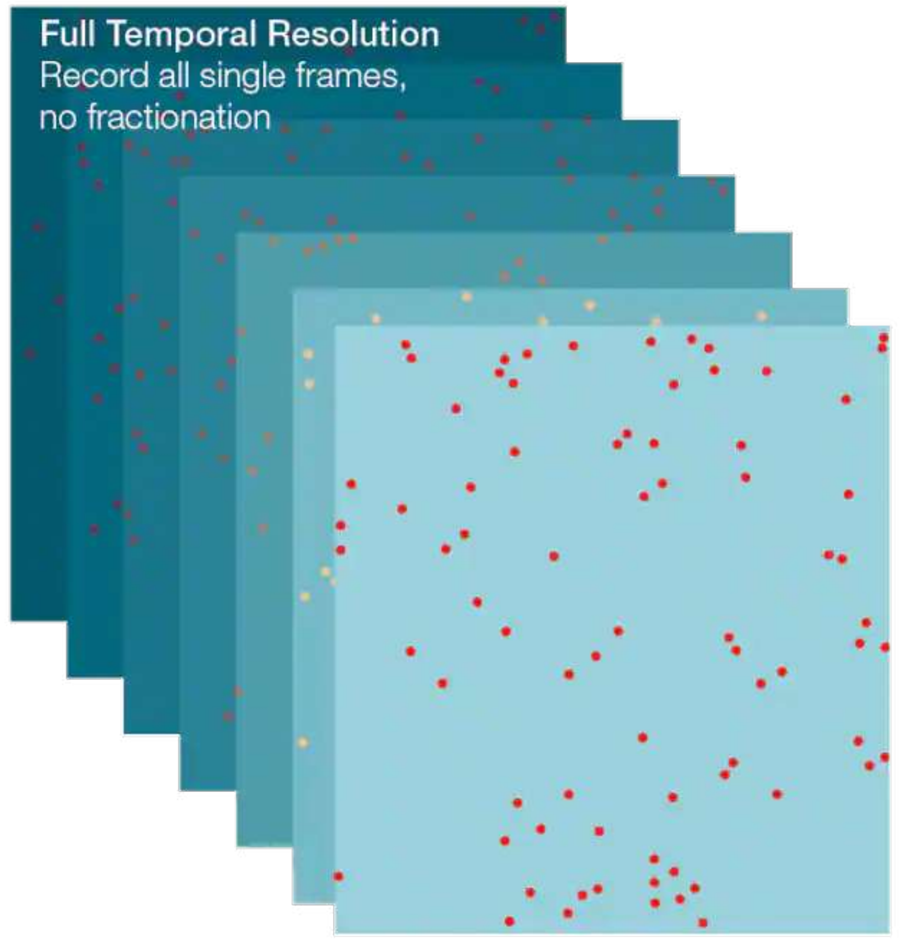


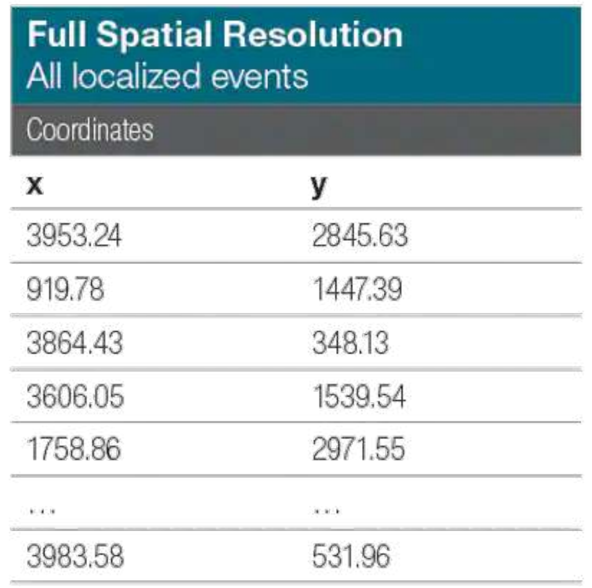
https://www.gatan.com/

Detectors

Improving the resolution:
Detecting electrons instead of photons

Falcon4 specs





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

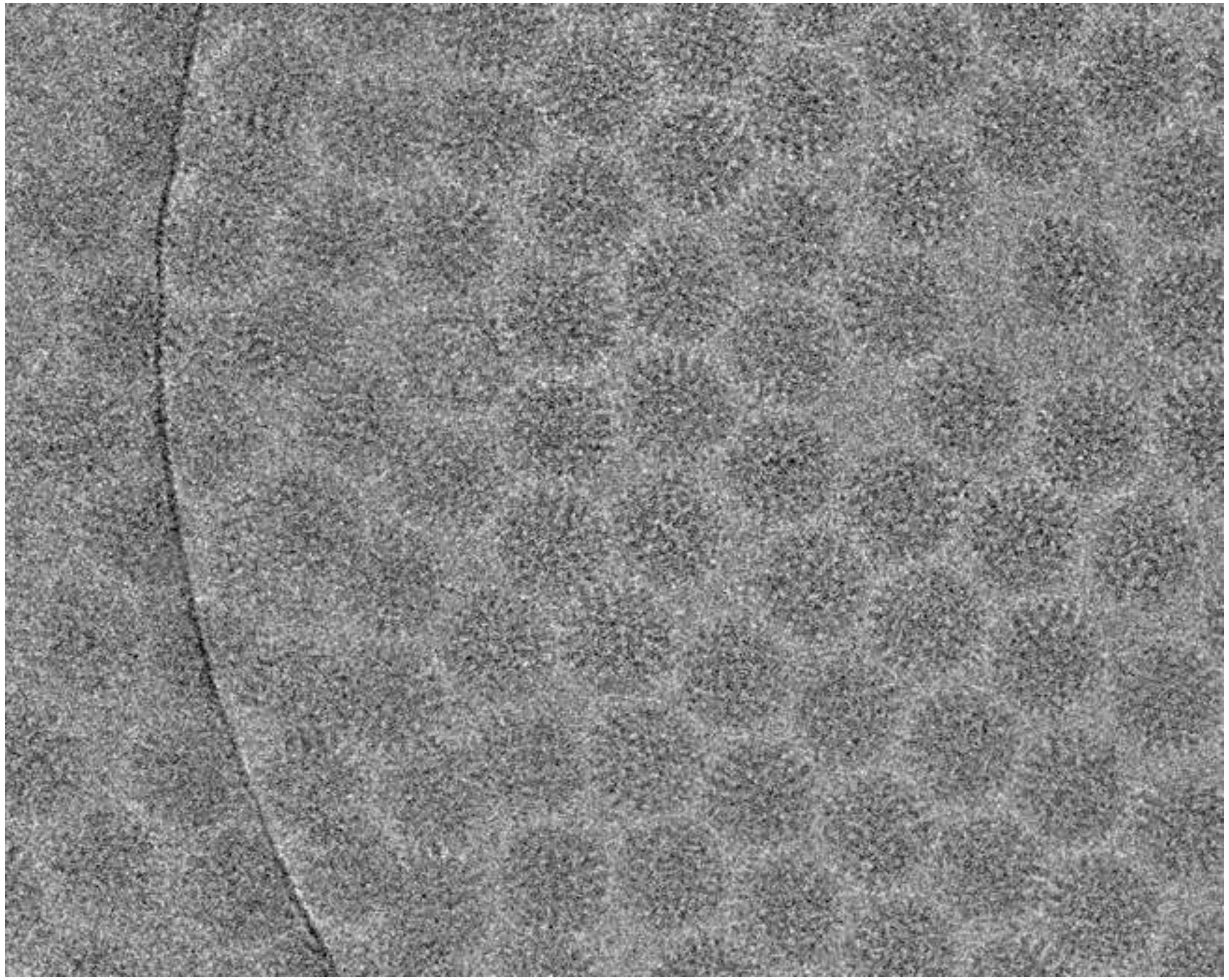
Camera architecture	Direct electron detection	
Sensor size	4,096 × 4,096 pixels, ~ 5.7 x 5.7 cm^2	
Pixel size	14 x 14 μm^2	
TEM Operating voltage	200 kV, 300 kV	
Internal frame rate	320 fps	
Frame rate to storage	320 fps (EER mode)	
Camera Overhead time	0.5 s per acquisition	
File formats	EER (native), 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

Electron-event representation (EER)

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

Detectors

Images are movies



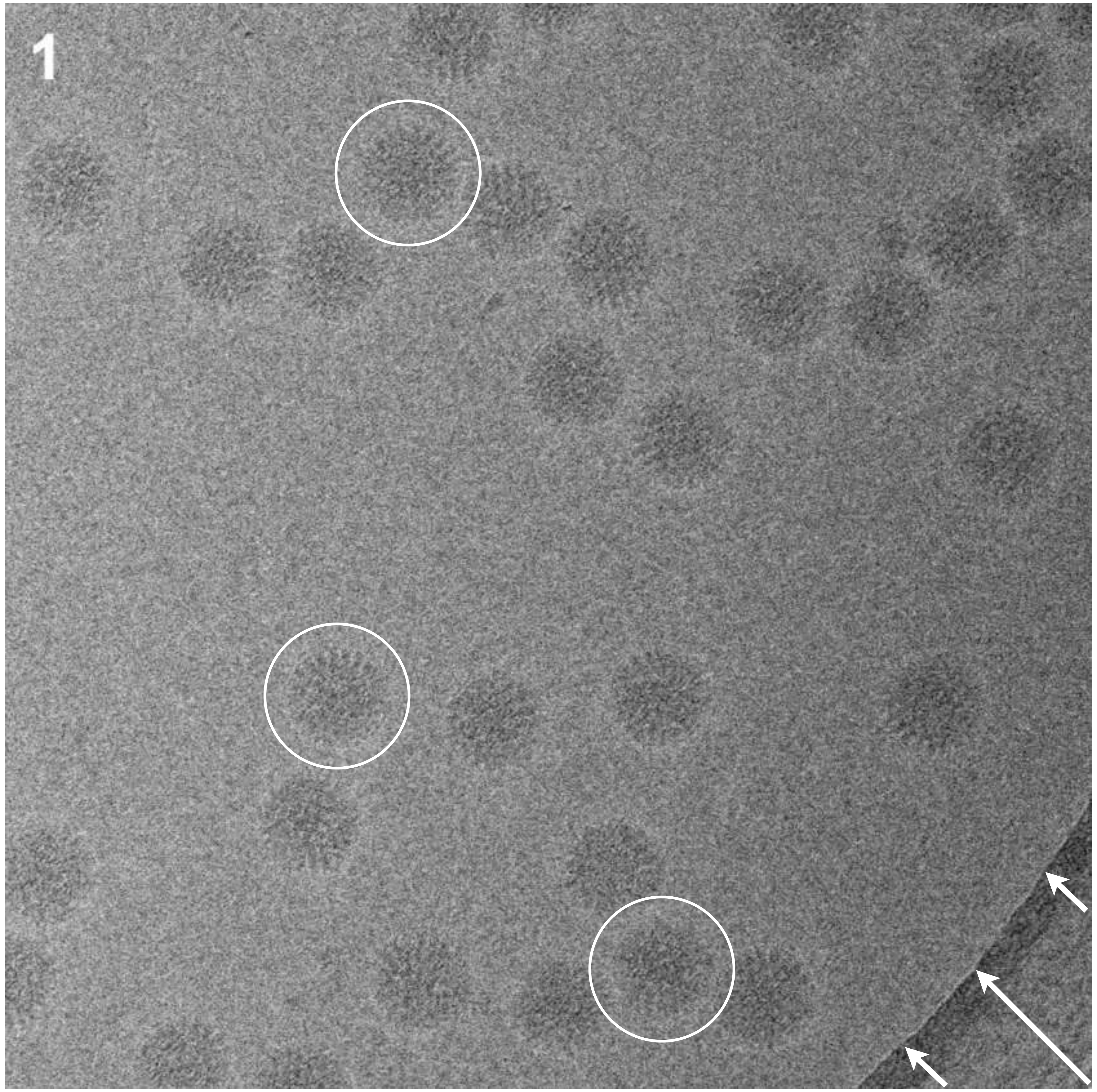
0.5 e-/Å2/frame

Image = Frame1 + Frame2 + Frame3 + Frame4 + Frame5

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

Detectors

Images are movies



10 frame averages

Each averaged frame corresponds to 0.25 s.

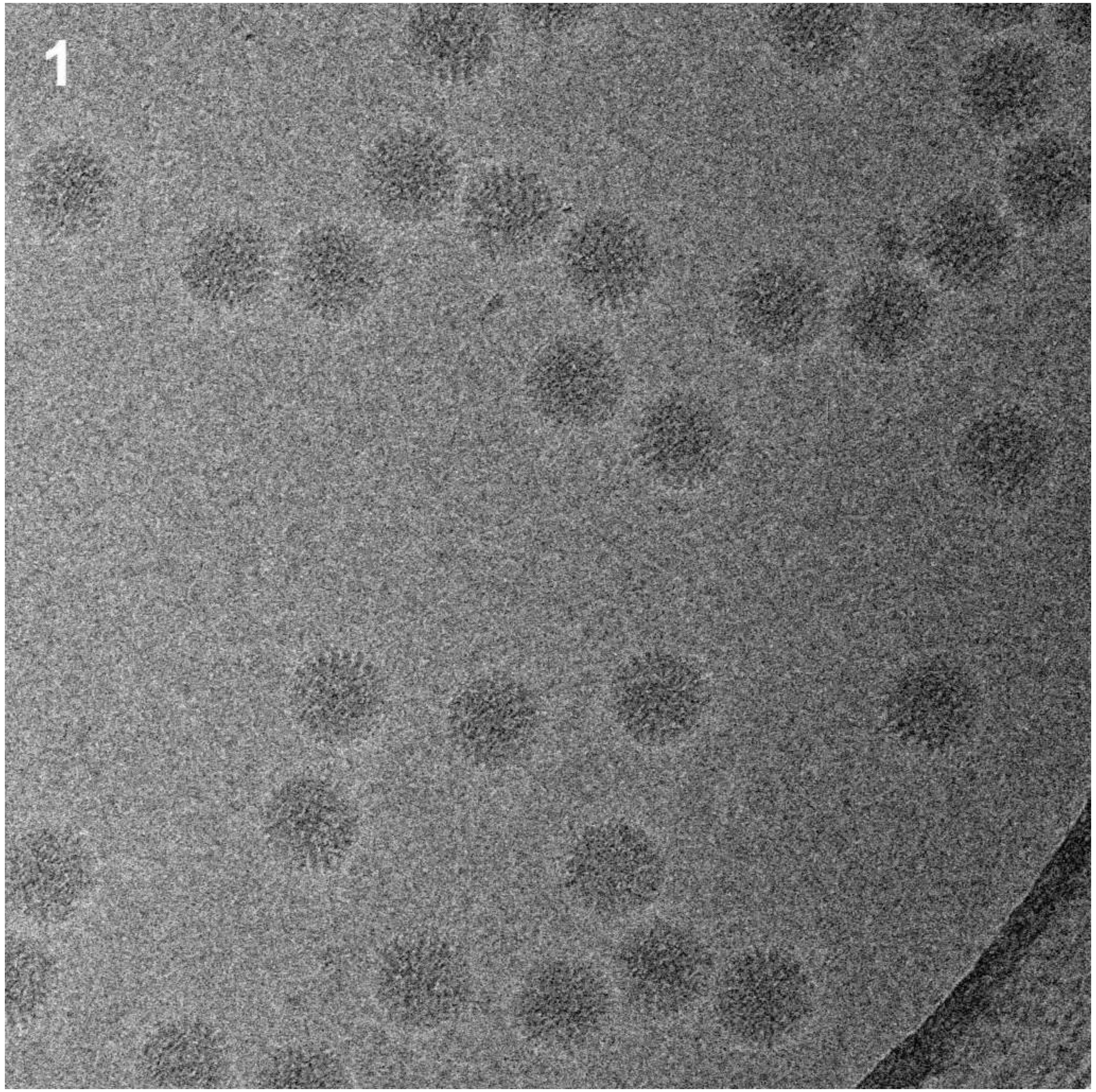
Dose/frame = 5 $e^-/Å^2$

A “movie” of rotavirus exposed to electron beam

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

Detectors

Images are movies



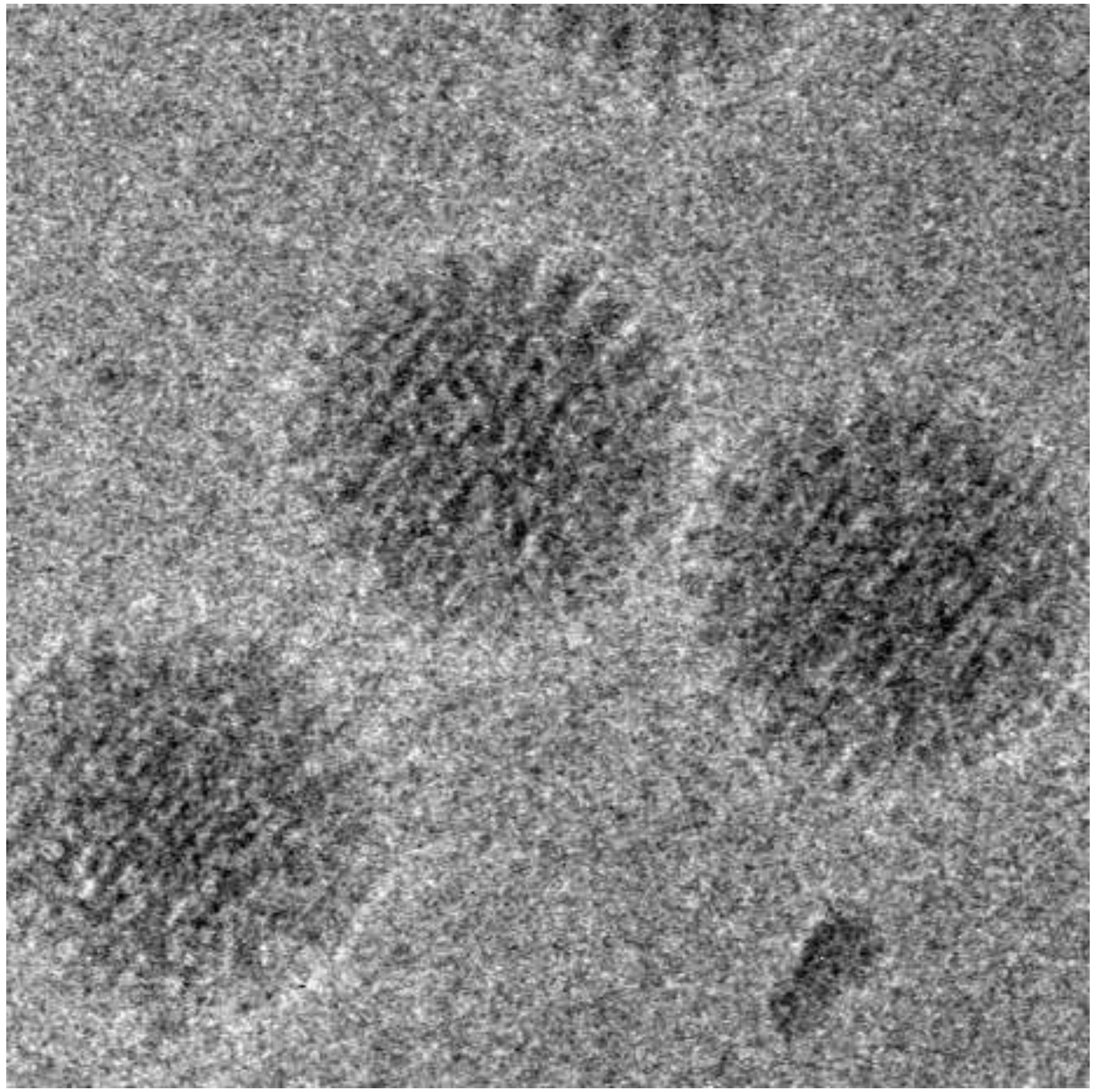
2.5nm

Translations over time

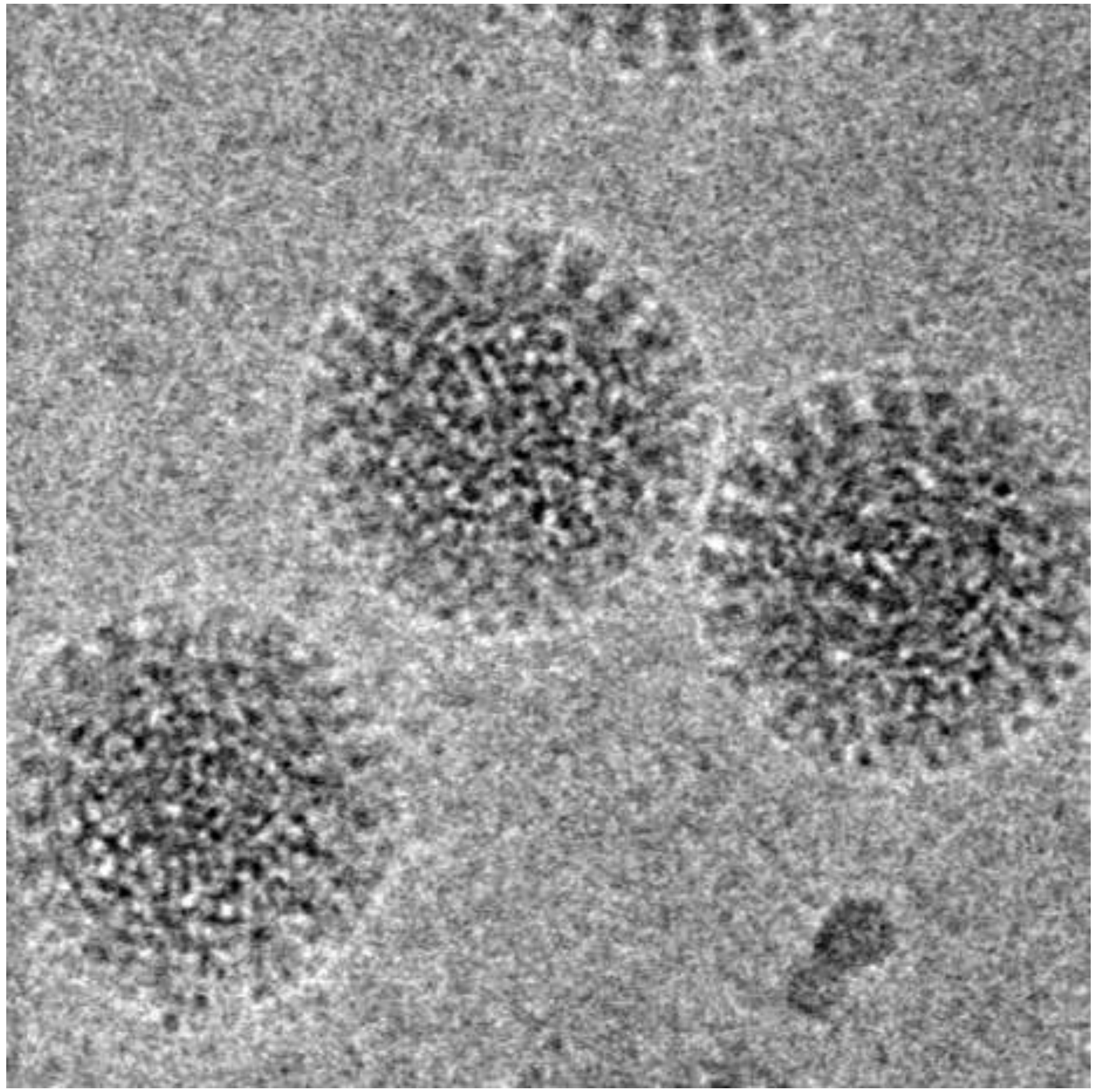
Detectors

Correcting for movement

60-frame average
(no alignment)



60-frame average
(translational alignment)



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

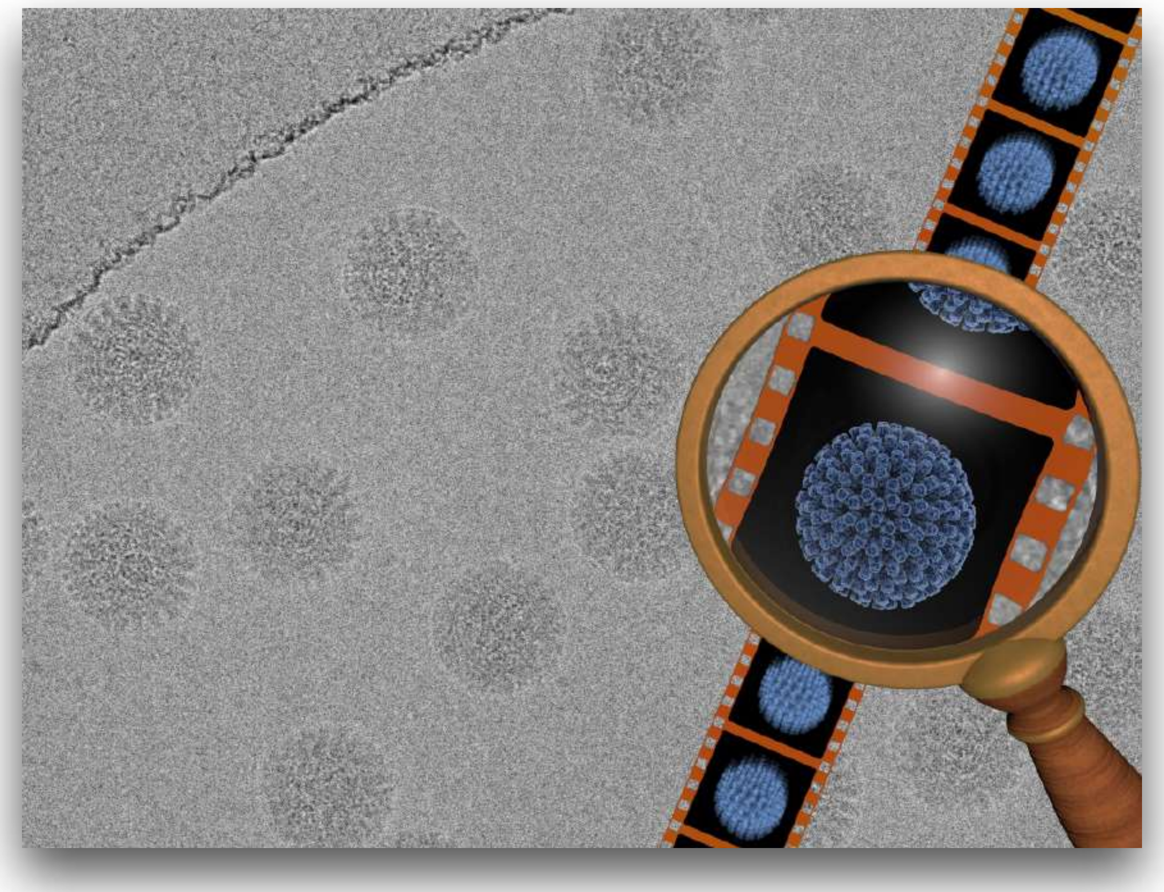
What brought about the resolution revolution?

(~2012-2014)

Hardware

Microscopes

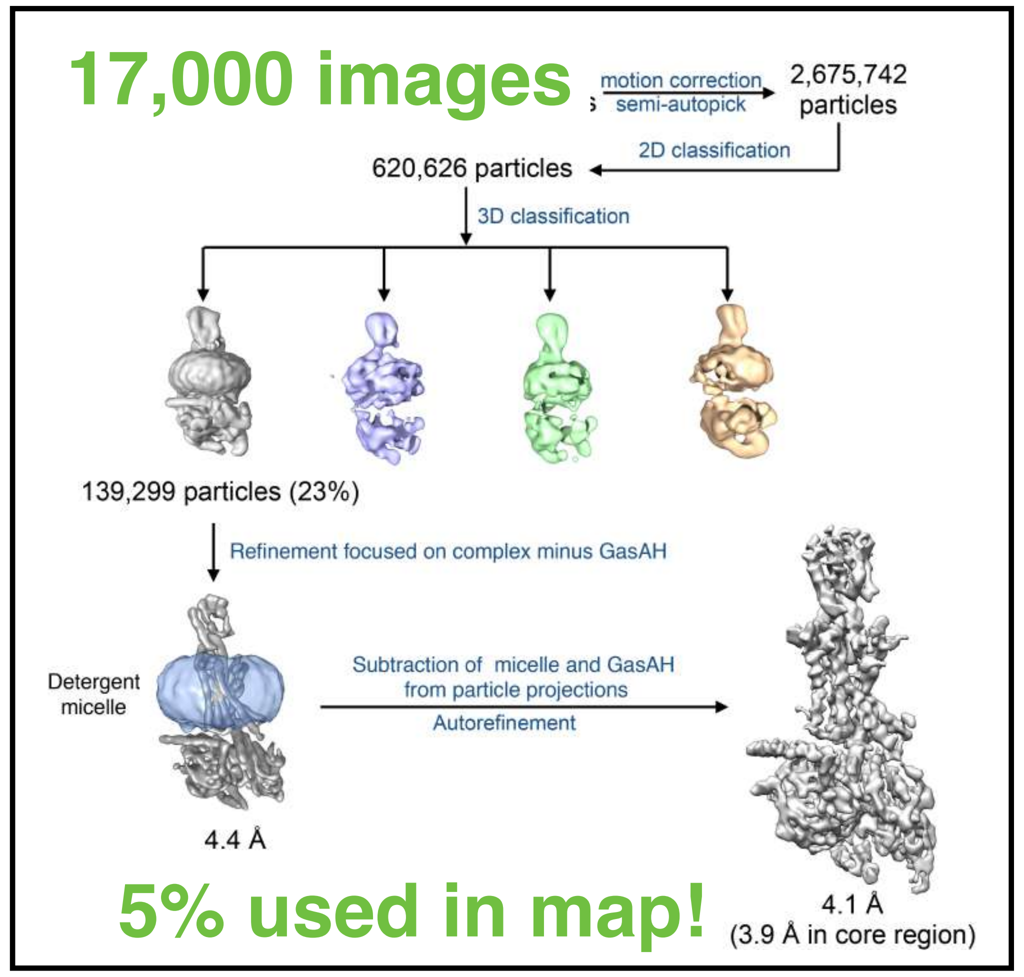
Direct Detectors



Computers

2012->2017
Cost reduced by 100x

Software

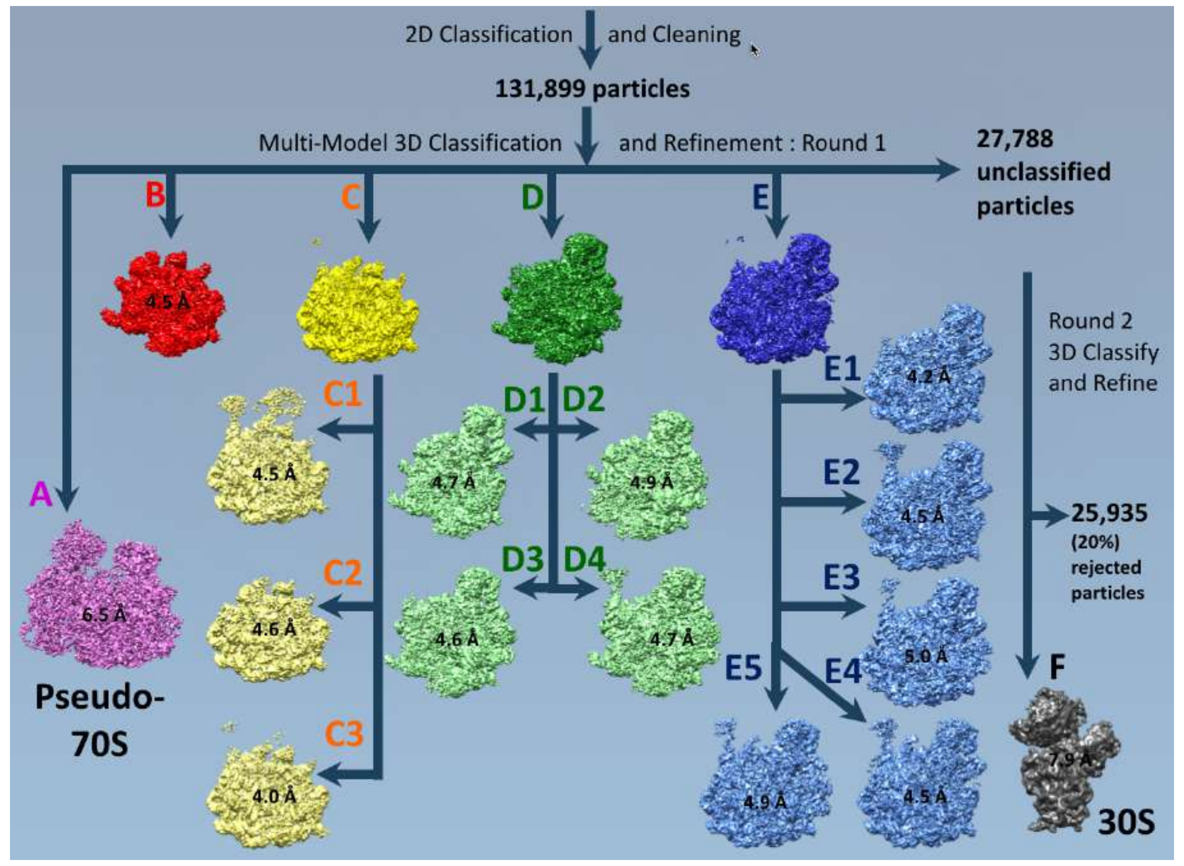


Leginon / SerialEM / EPU, …

MotionCorr2, Unblur, …

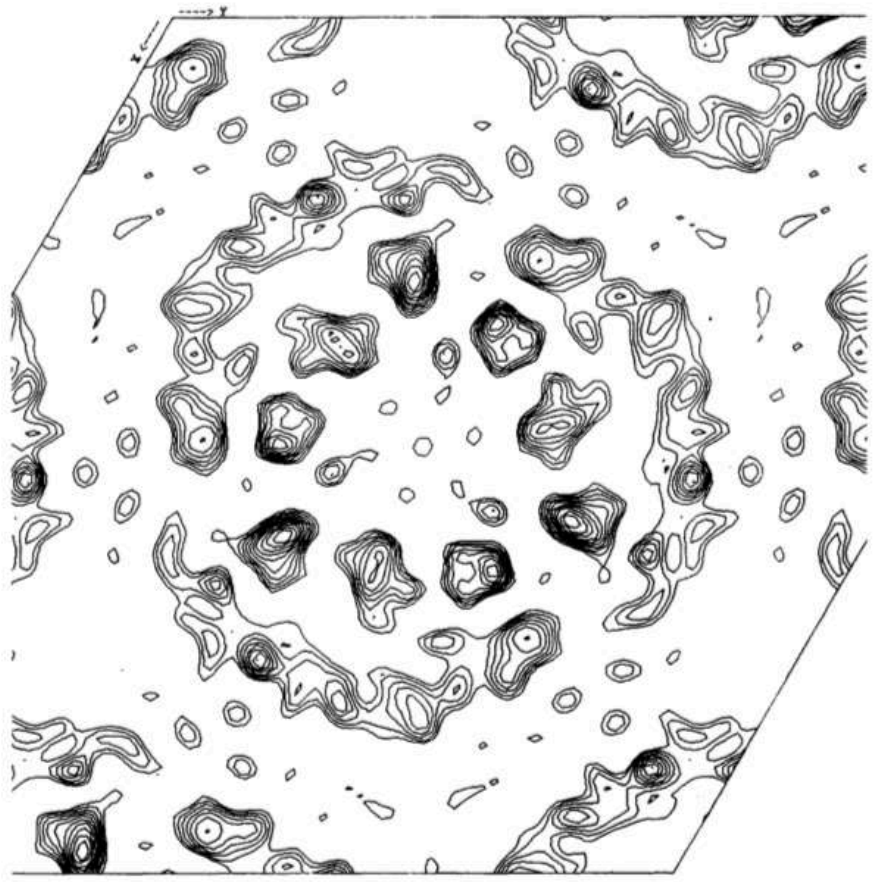
RELION, FREALIGN/cisTEM, cryoSPARC
EMAN, Sparx, SPHIRE, XMIPP, …

14 independent structures



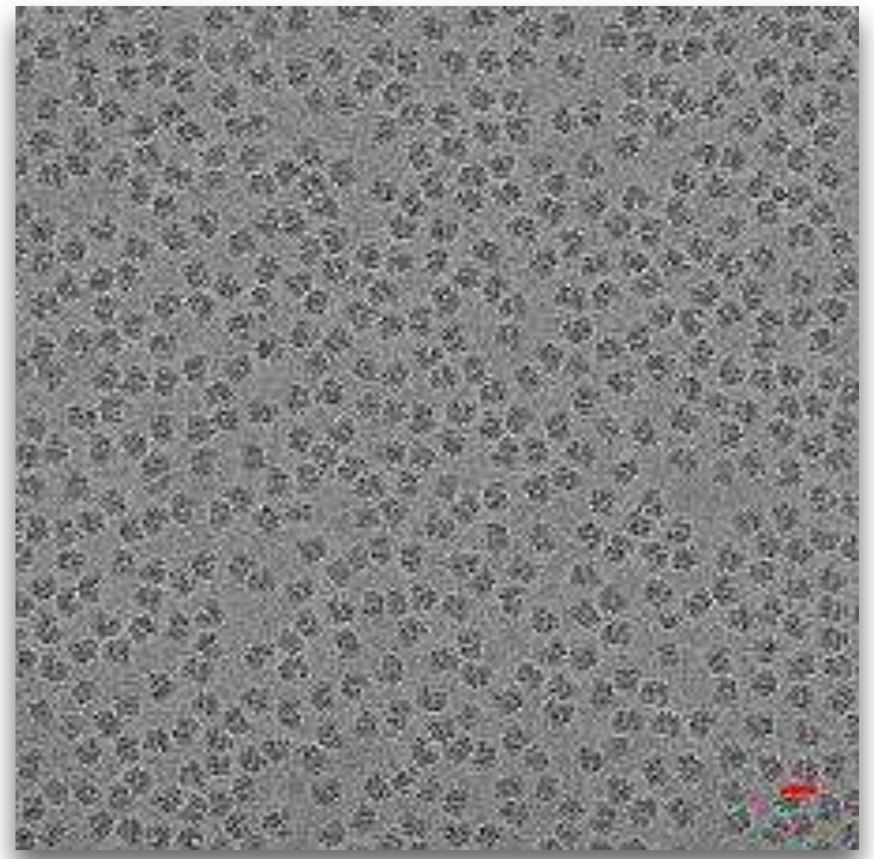
cryoEM: technology on the rise

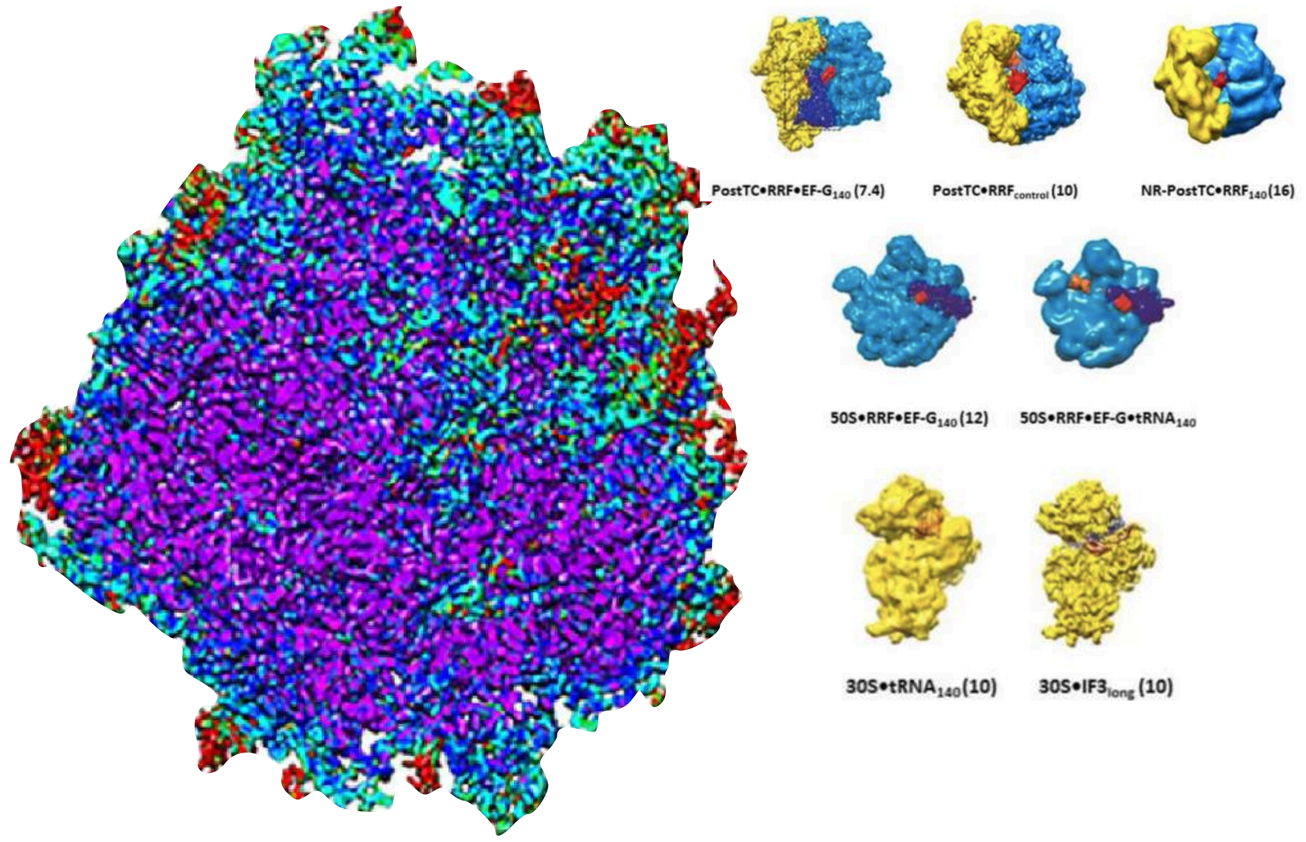
1986



Henderson et al. (1986)

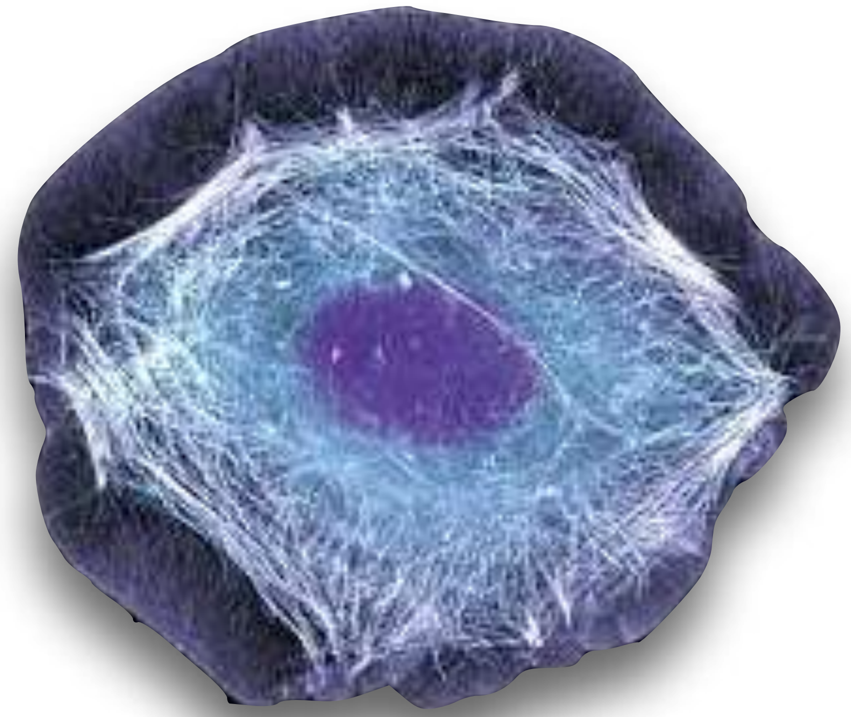
2017

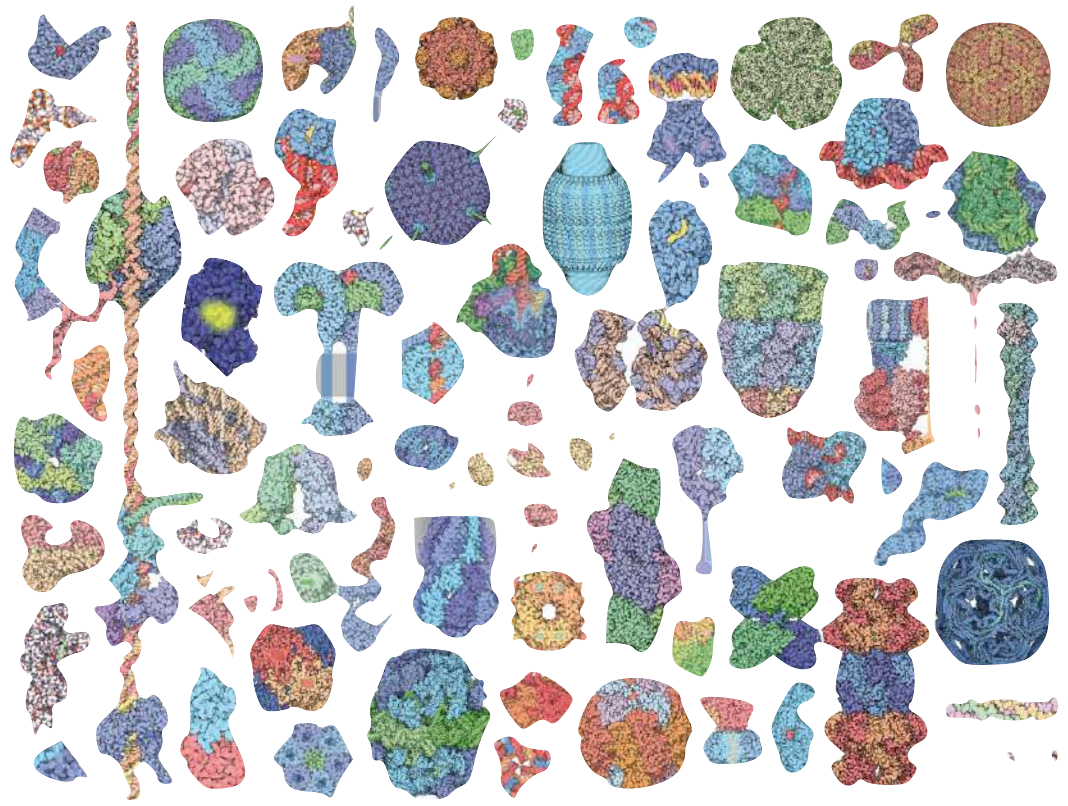




Frank et al. (2017)

in progress





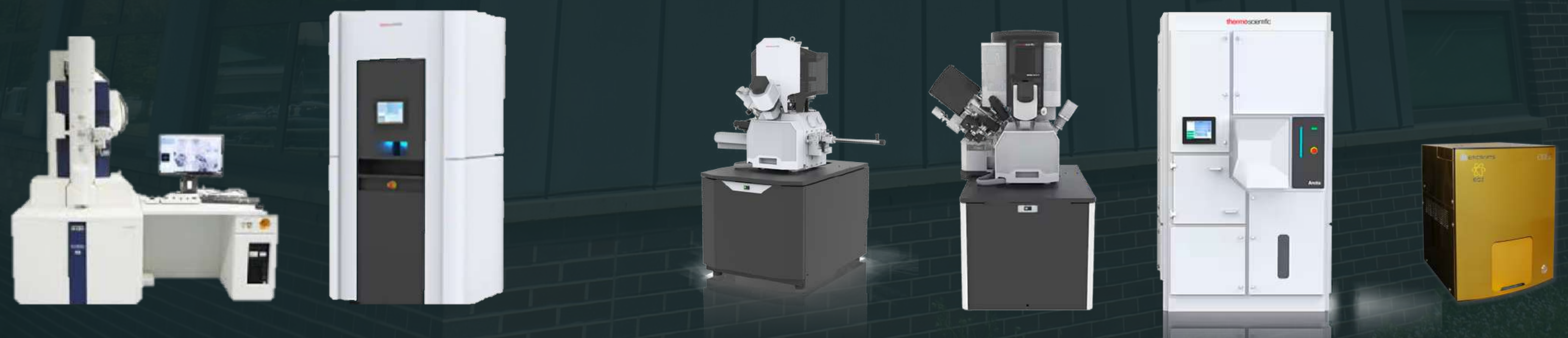
the next chapter

And true “atomic” resolution is possible:

Nakane, et al. Single-particle cryo-EM at atomic resolution. Nature (2020).

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?