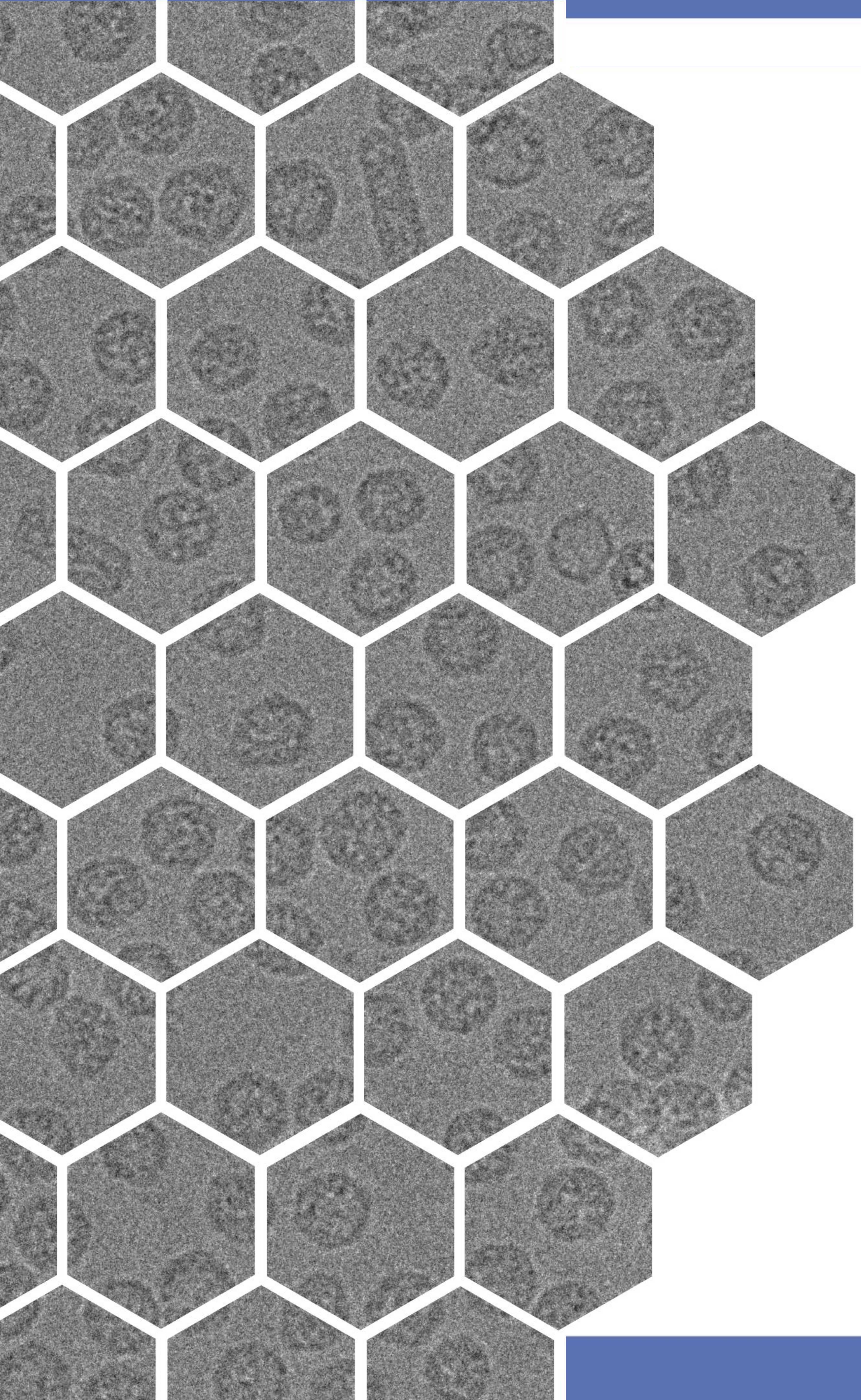
2024 Winter cryoEM course

Welcome and Anatomy of an EM

January 17, 2024

NYSBC SEMC



- Course outline
- Student survey
- Anatomy of an EM

Welcome to SEMC

19th year of the course

SIMONS ELECTRON MICROSCOPY CENTER • GUILD OF MICROSCOPISTS •

Course logistics: main website

https://semc.nysbc.org/workshops/2024-em-course/


SIMONS ELECTRON MICROSCOPY CENTER

HOME ABOUT PEOPLE PUBLICATIONS RESOURCES COURSES JOBS FIND US

WORKSHOPS & COURSES

CURRENT/UPCOMING COURSES | PAST COURSES

EM Courses:
2023
2022
2021
2020
2019
2018
2017
2016

The Winter-Spring 2024 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 SEMC seminar room (Mondays 3:30-5pm and select Wednesdays 3:30-5pm)

EM fundamentals section
Jan 17: Lecture – Introduction & Basic anatomy of the electron microscope (Ed Eng – NYSBC/SEMC & SEMC staff)
Jan 22: Lecture – New cryoEM hardware and supporting a facility (Michael Alink – NYSBC/SEMC)
Jan 24: Practical – TEM use (SEMC staff)
Jan 29: Lecture – Considerations for biological cryoEM (SEMC staff)
Jan 31: Practical – Sample Preparation & Support films (SEMC staff)

EM crystallography section
Feb 5: Lecture – MicroED (Bill Rice – New York University)
Feb 7: Practical – Journal club

- Course Administrator:
  - Ed Eng (eeng@nysbc.org)
- Teaching Assistants:
  - Mahira Aragon (NYSBC)
  - Aaron Owji (NYSBC)
  - Jessalyn Miller (NYSBC)
  - Alex Flynn (NYSBC)

Course logistics: Slack channel

Make sure you're on the email list!

Class Slack channel
#semc-2024-cryoem-course

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

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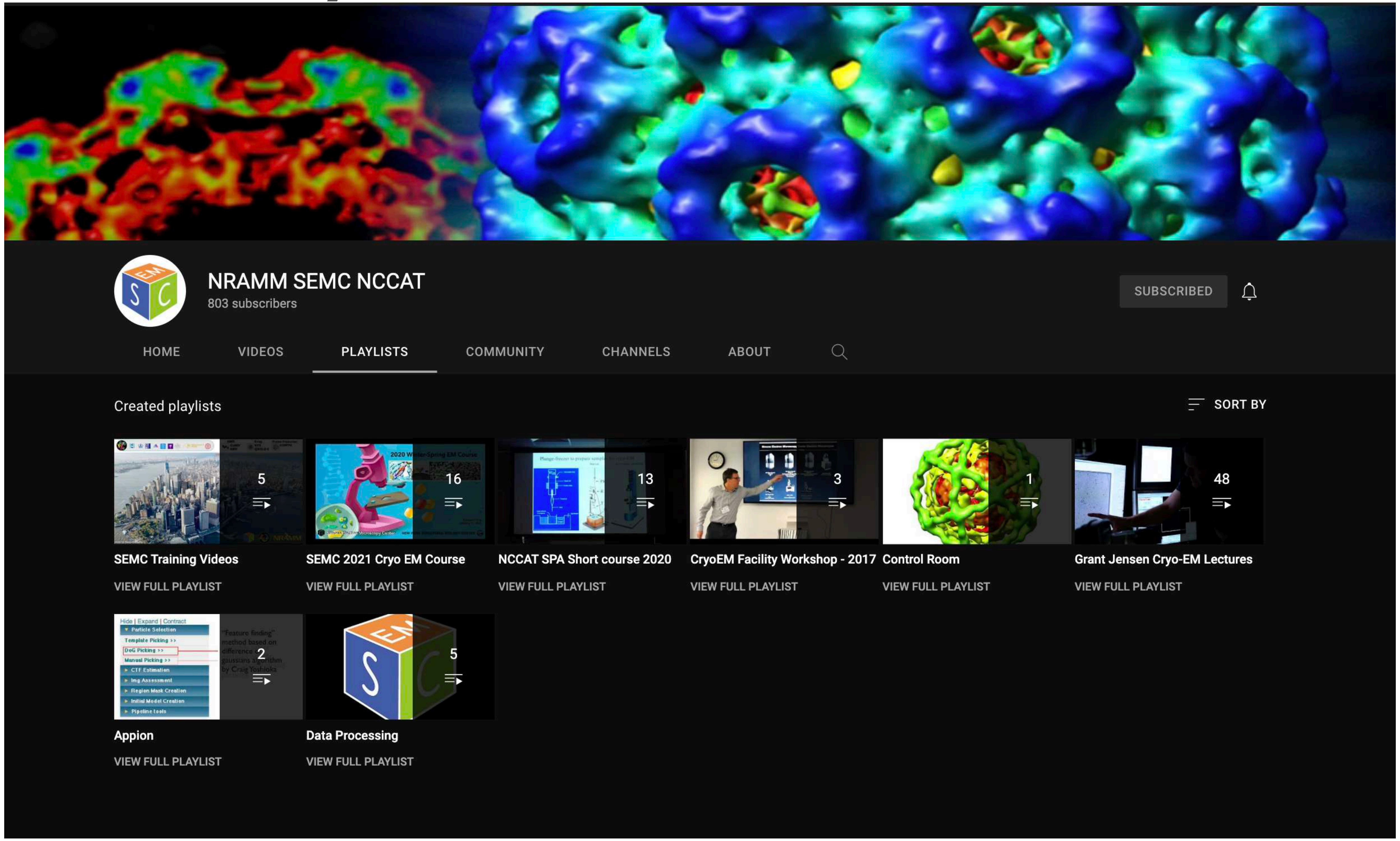
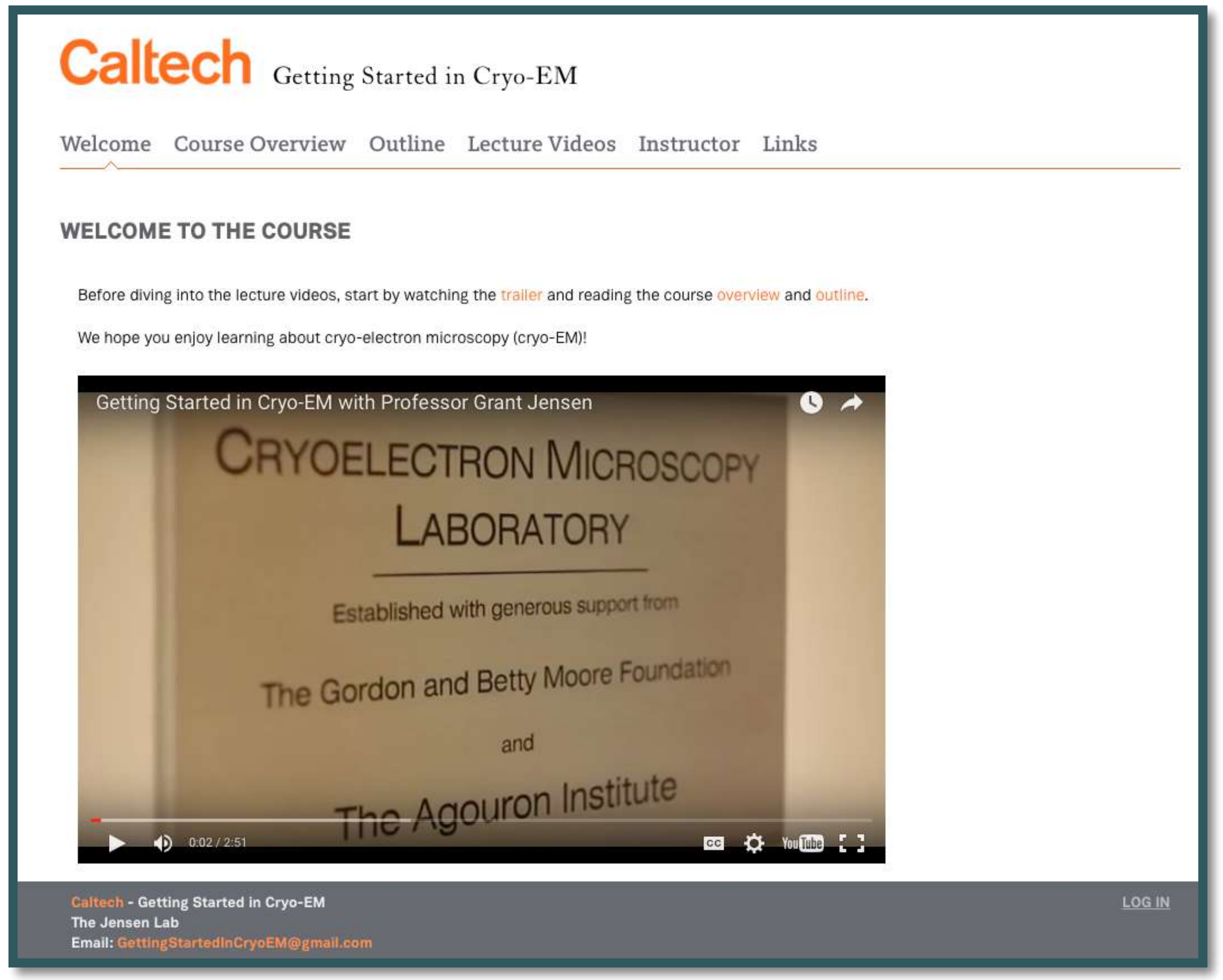
Course logistics: additional resources

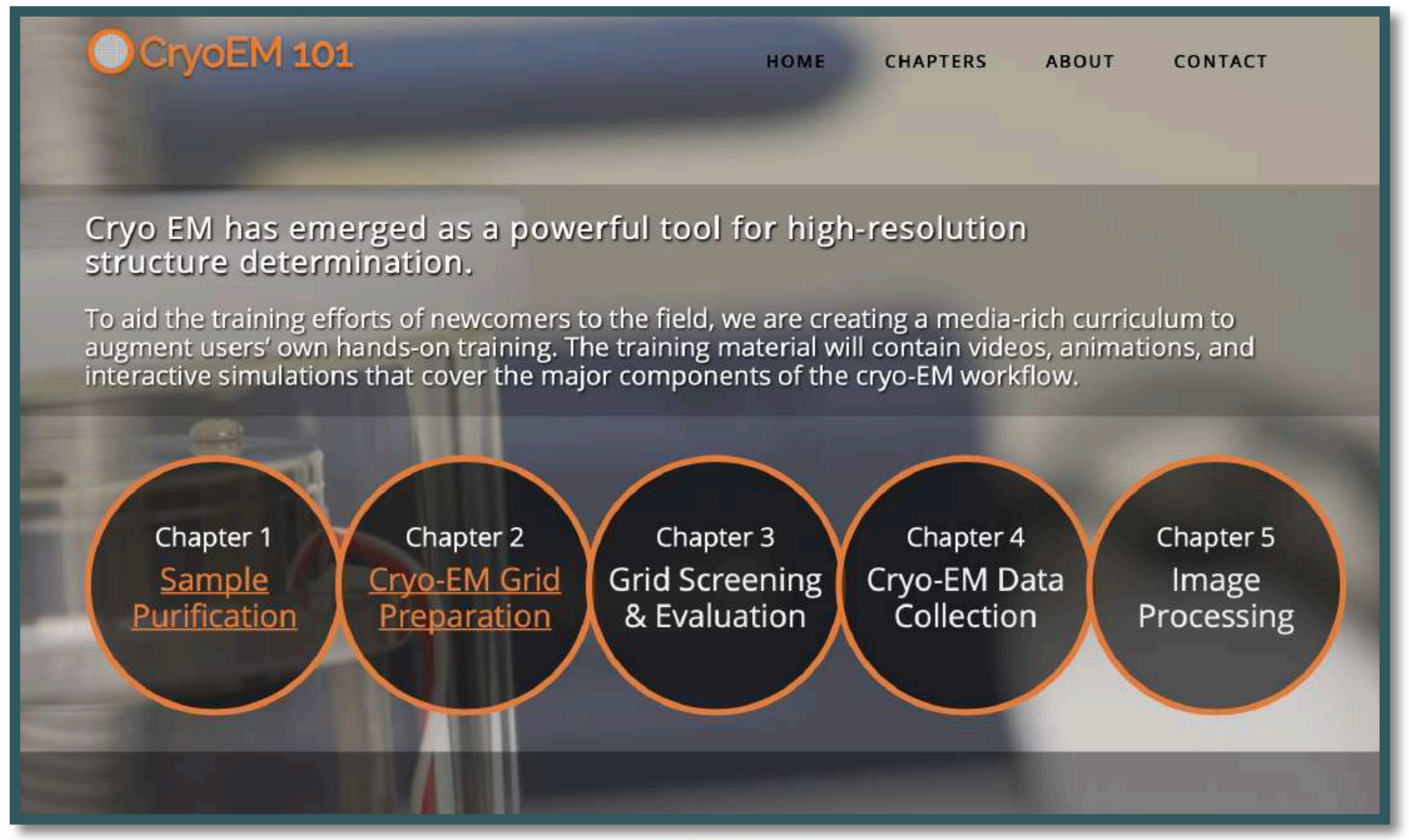
youtube.com/nrammsemc



cryo-em-course.caltech.edu/videos



cryoem101.org



Course logistics: main topics

Section 1 : EM fundamentals

Section 2 : EM crystallography

Section 3 : Tomography

Section 4 : SPA short course*
March 11-15

Section 5 : Future perspectives

Course logistics: main topics

Course logistics: class for credit

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


START

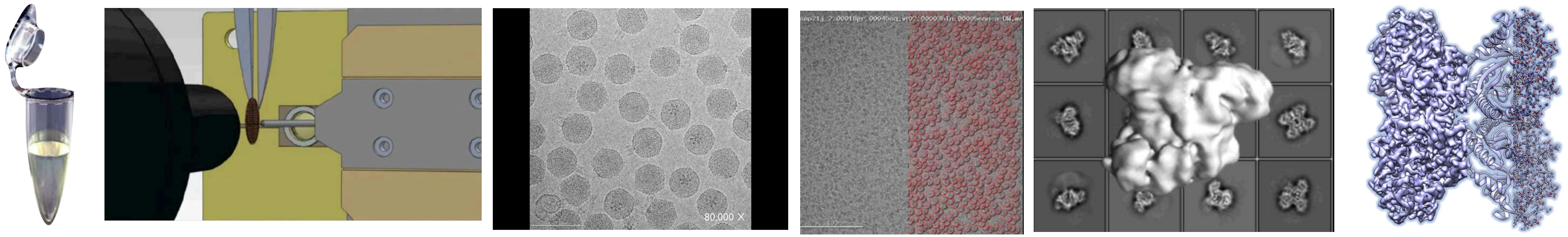
http://etc.ch/bBLE

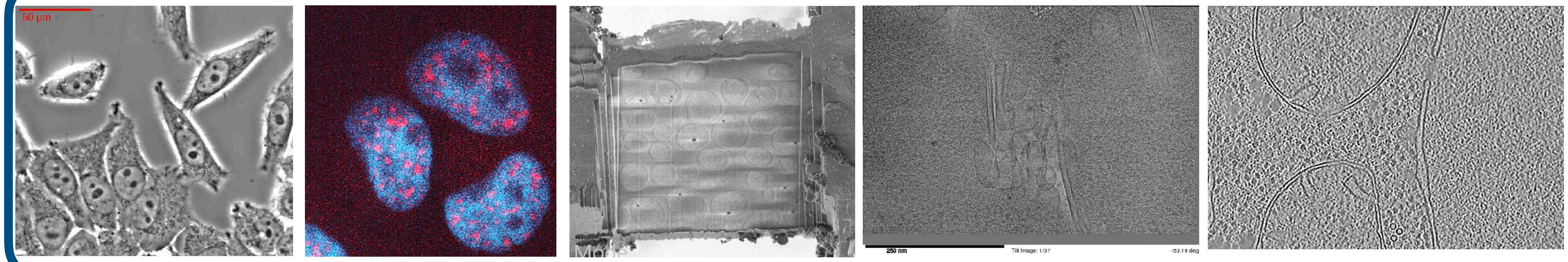
Illustration by David S. Goodsell
RCSB Protein Data Bank.

Illustration by David S. Goodsell
RCSB Protein Data Bank.

cryoEM: a technology on the rise

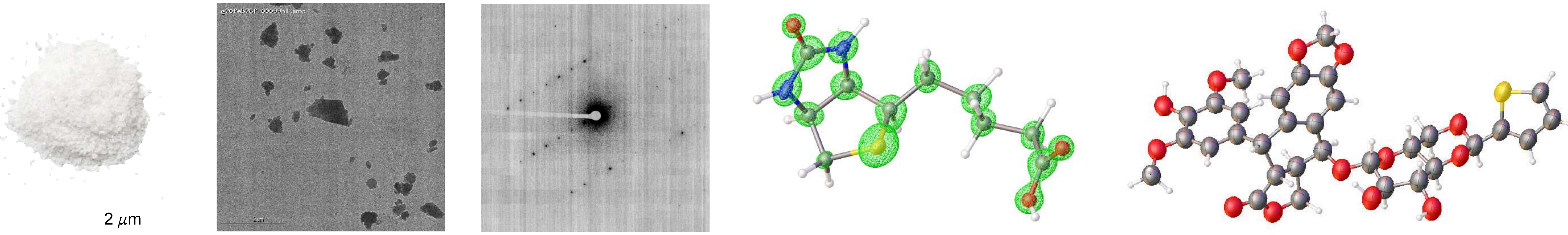
Single particle cryoEM



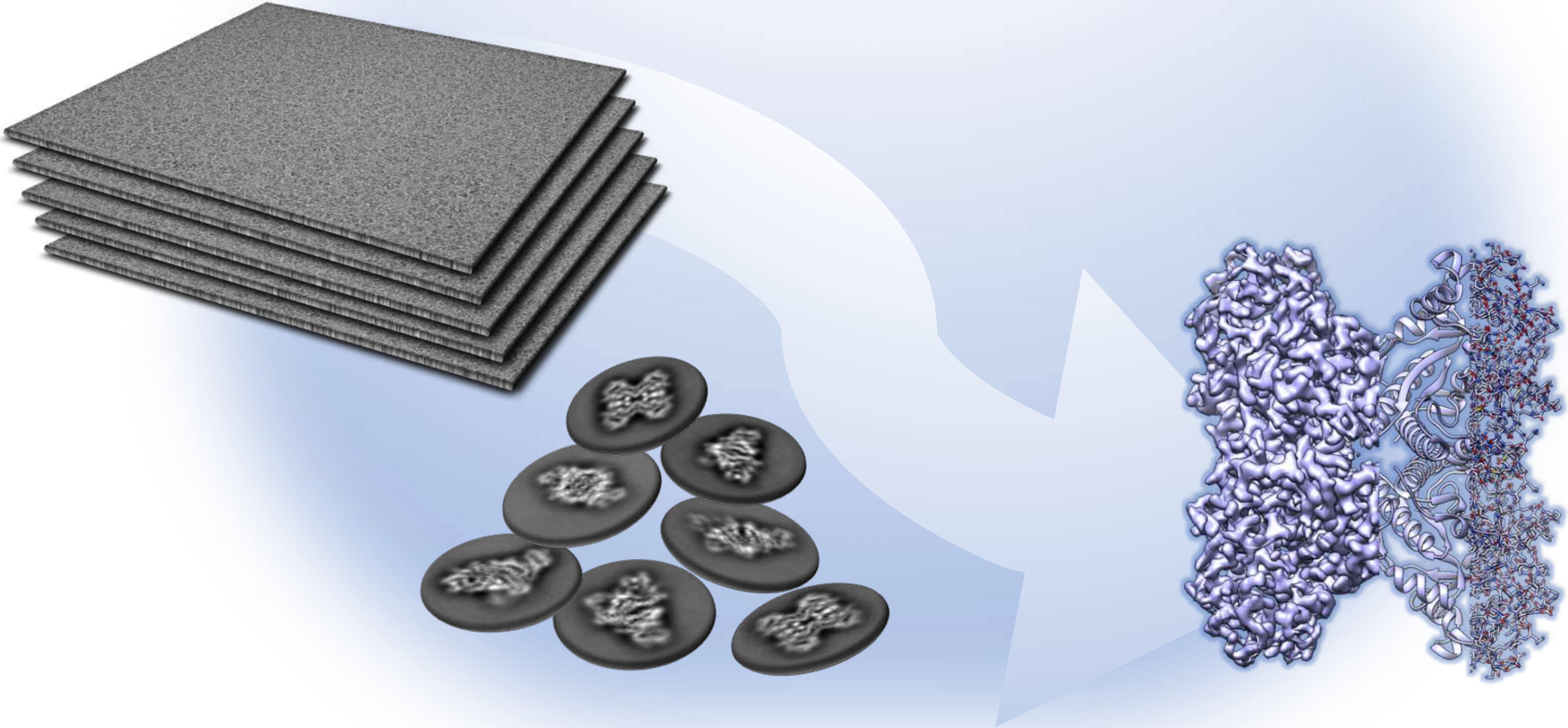


Cryo Electron Tomography (cryoET)

Micro crystal electron diffraction (microED)



What is possible today?



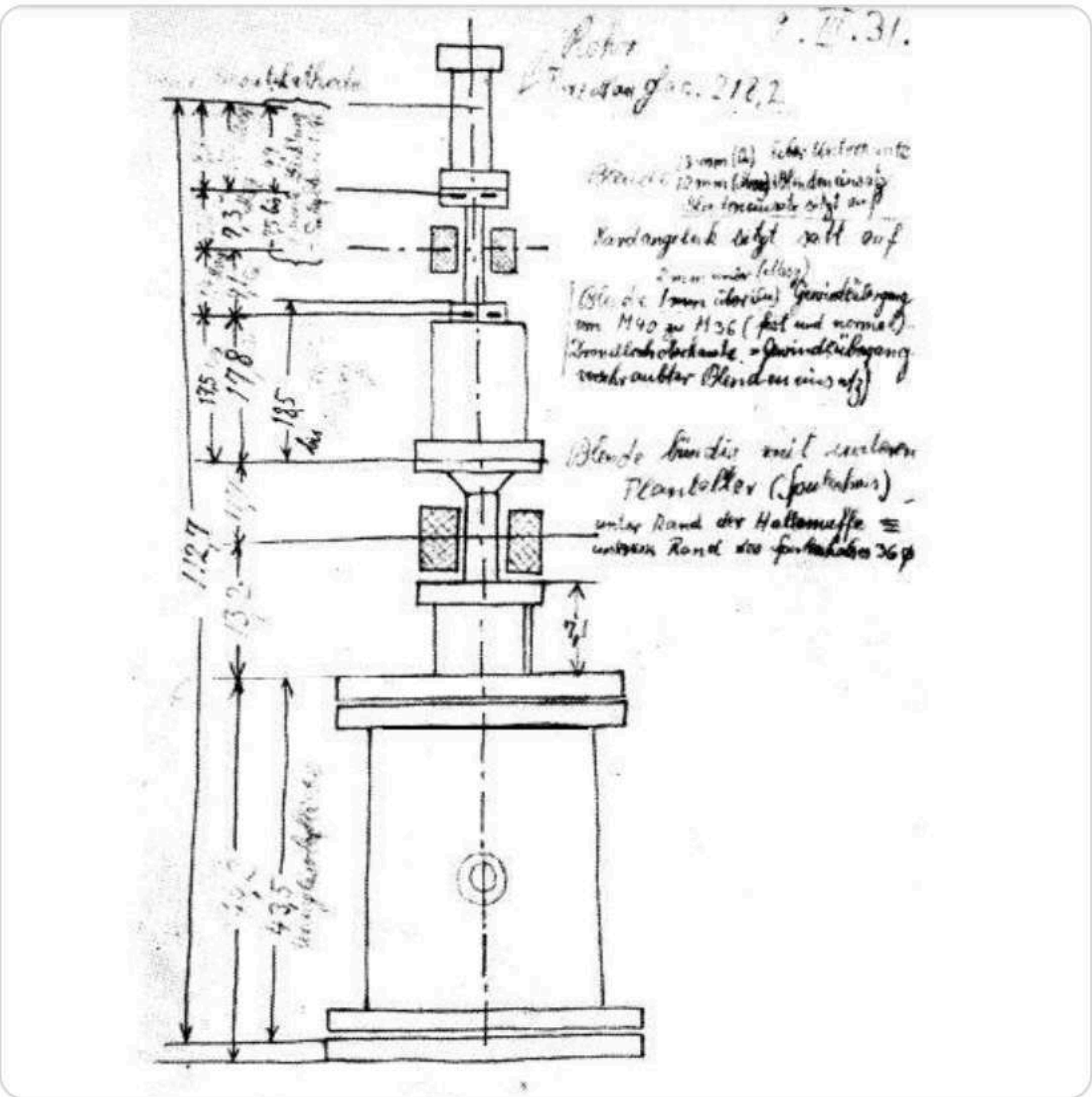
The tool of our trade: EM

Ruska and Knoll in Berlin in the early 1930s

-Wikipedia

The Nobel Prize @NobelPrize

Take a look at a sketch by physics laureate Ernst Ruska, dated 9 March 1931, of the cathode ray tube for testing one-stage and two-stage electron-optical imaging by means of two magnetic electron lenses (electron microscope). Ruska was awarded the 1986 physics prize for his work.



6:06 AM · Dec 25, 2021

725 Reply Copy link to Tweet

Read 8 replies

The Nobel Prize @NobelPrize

The electron microscope, invented by Ernst Ruska and Max Knoll in 1933, made it possible to take pictures of objects that previously could not be seen, like viruses. The tobacco mosaic virus, shown here magnified 160,000 times was the first virus captured on film.

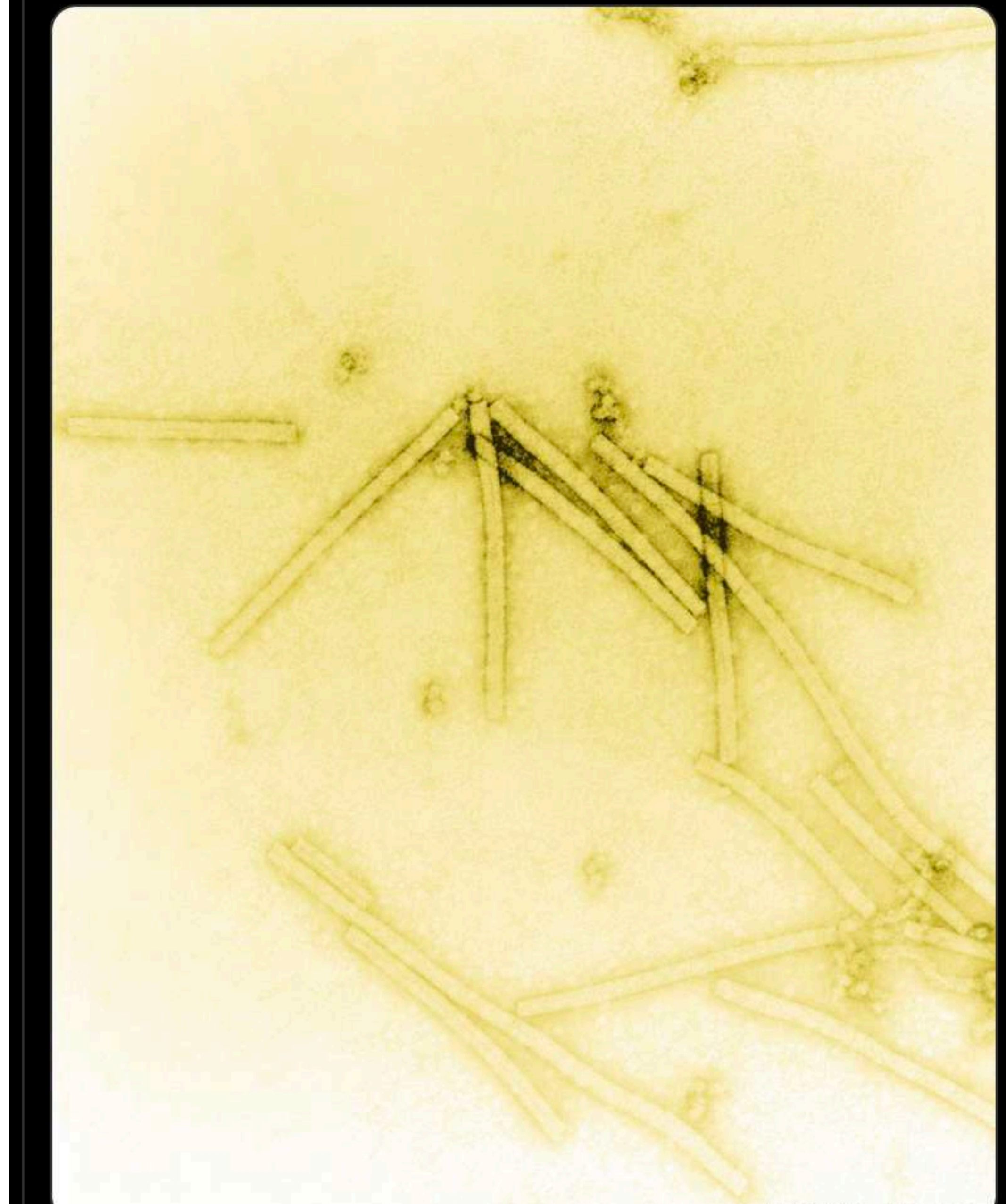
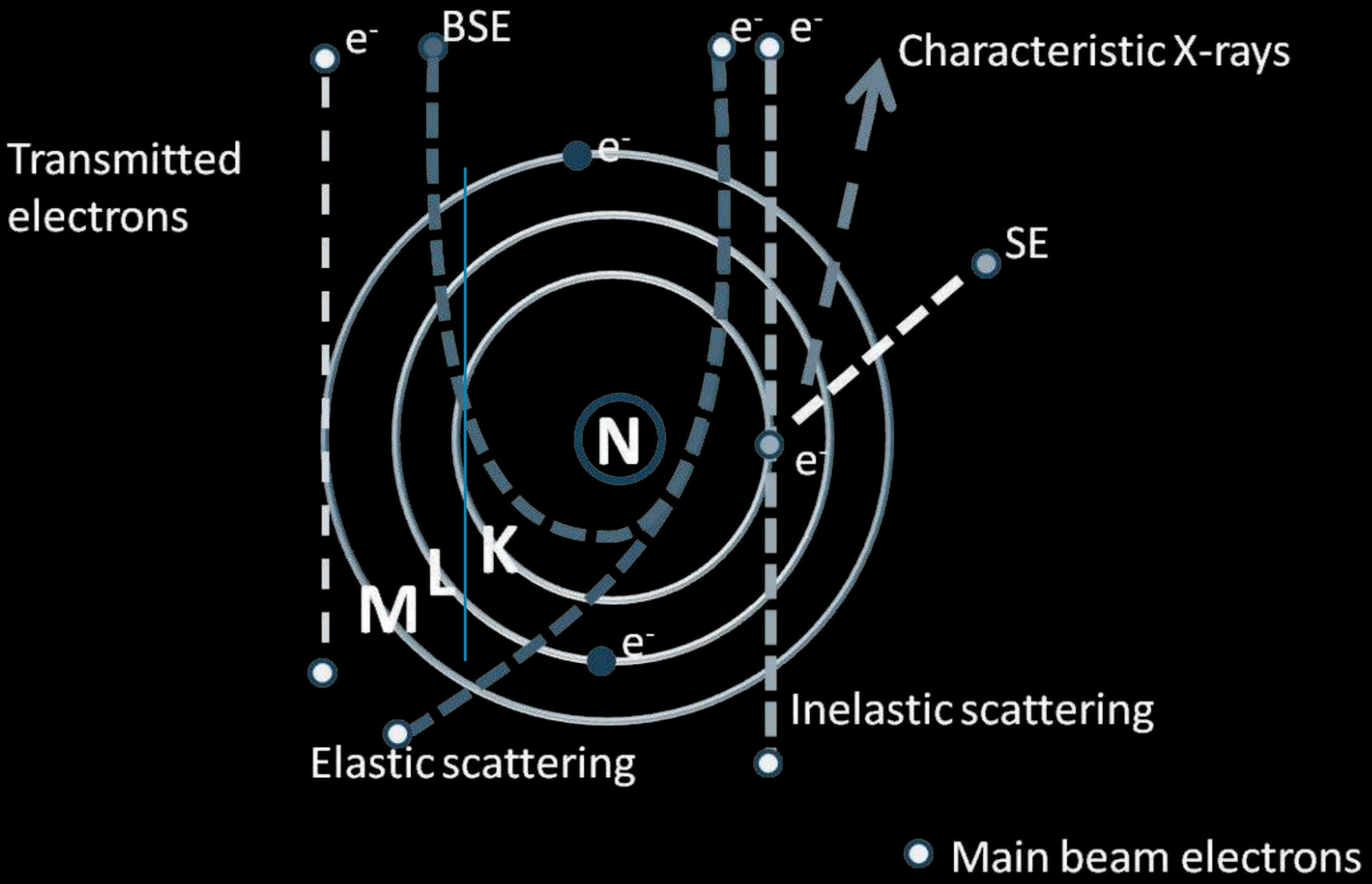


Photo: Wikimedia Commons

9:58 AM · Aug 22, 2021 · Sprout Social

Why electrons?



Why electrons?

Pros

Small wavelength

Can be focused

Cons

Damages sample

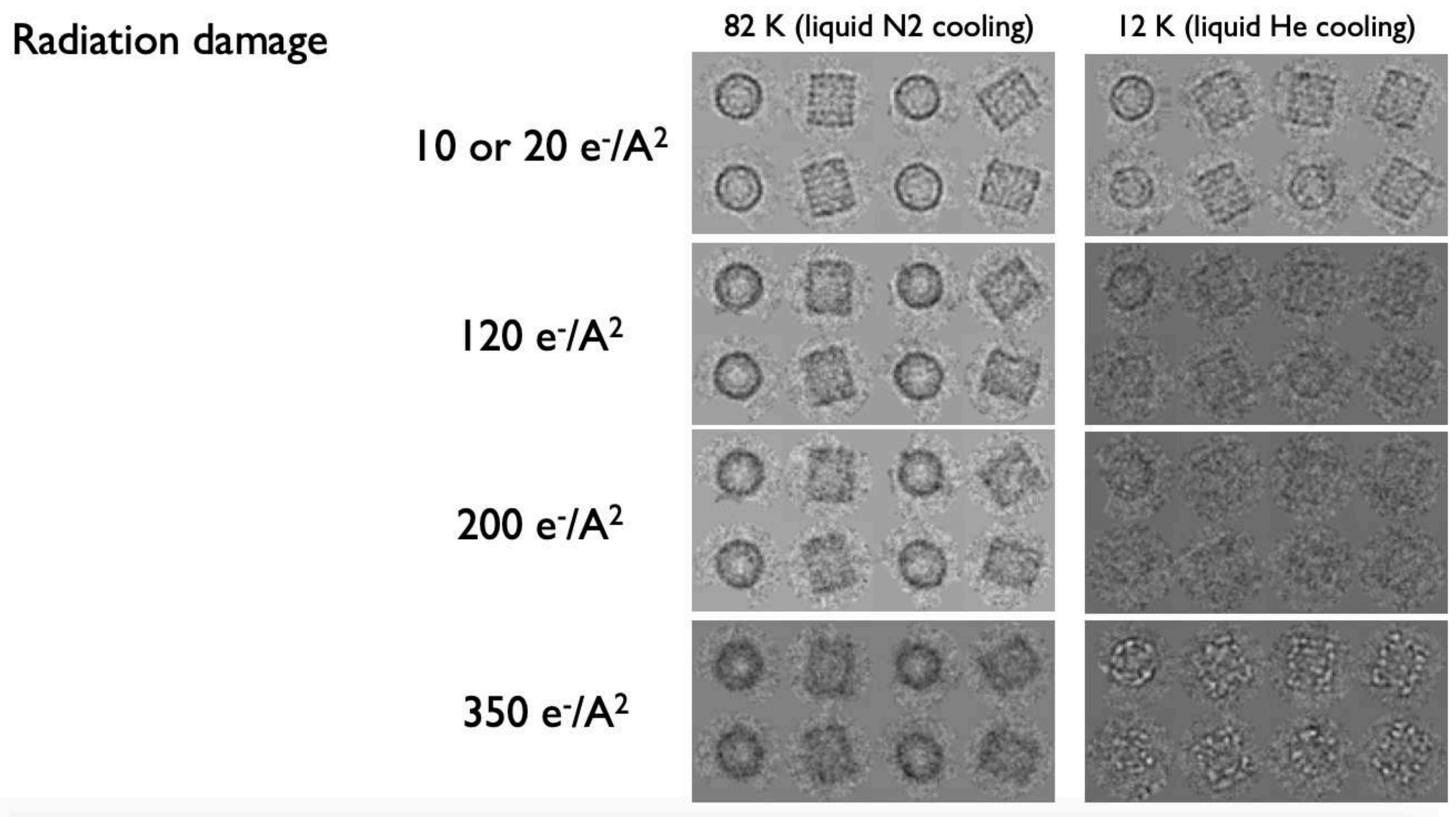
worse with faster electrons

Poor penetration

better with faster electrons

Why electrons?

Ideal dose for cryoEM?



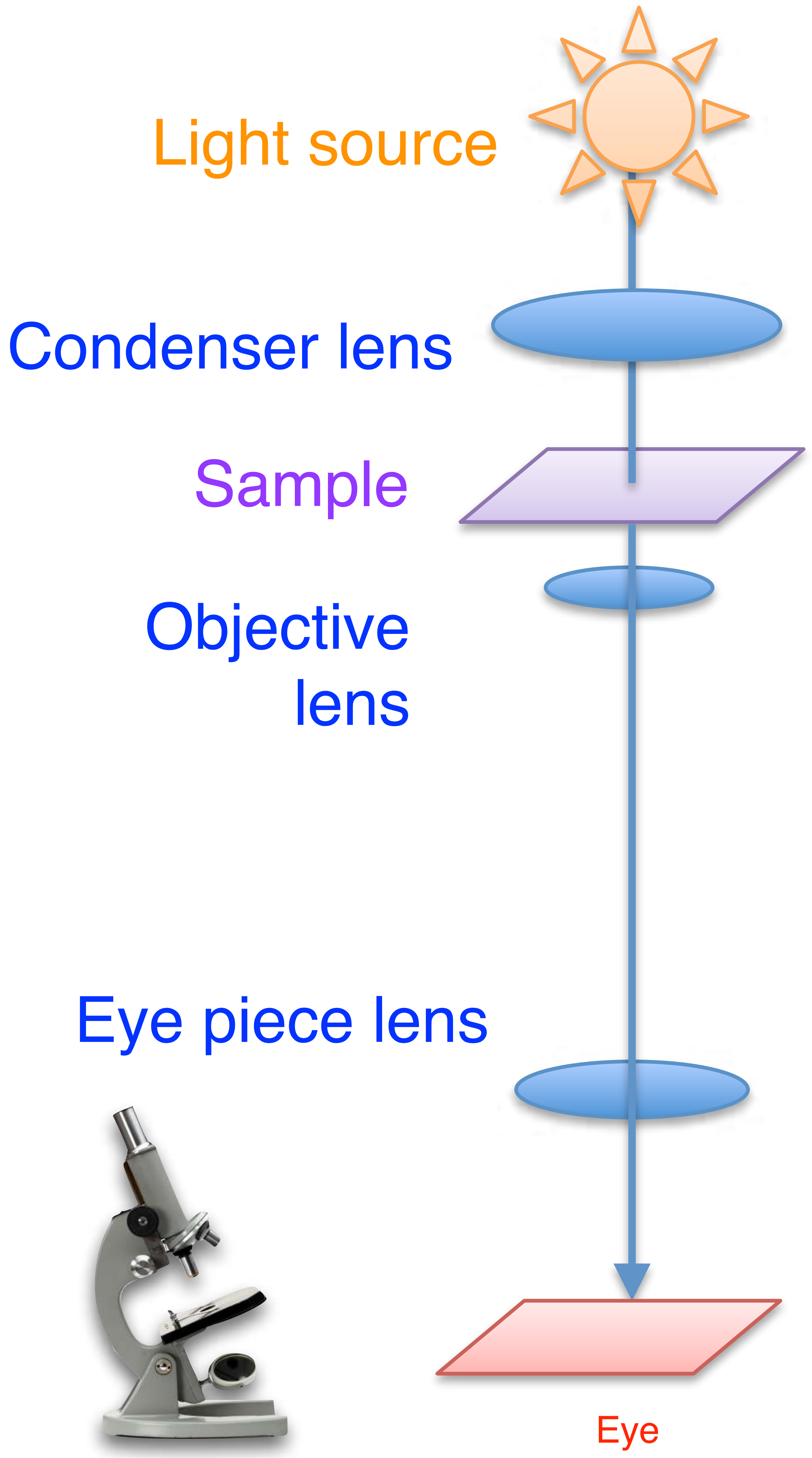
https://cryo-em-course.caltech.edu/

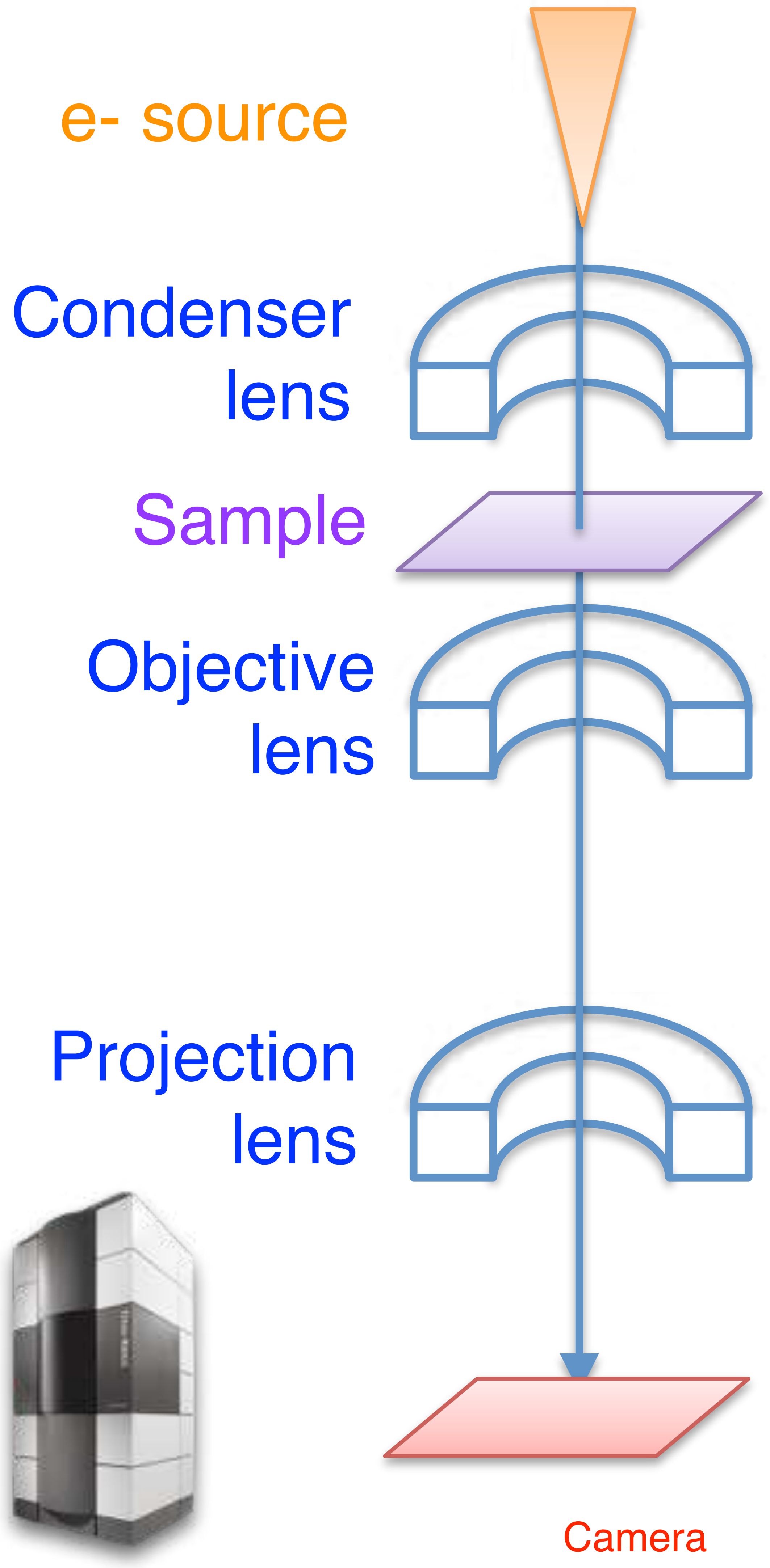
Specimen Behavior in the Electron Beam

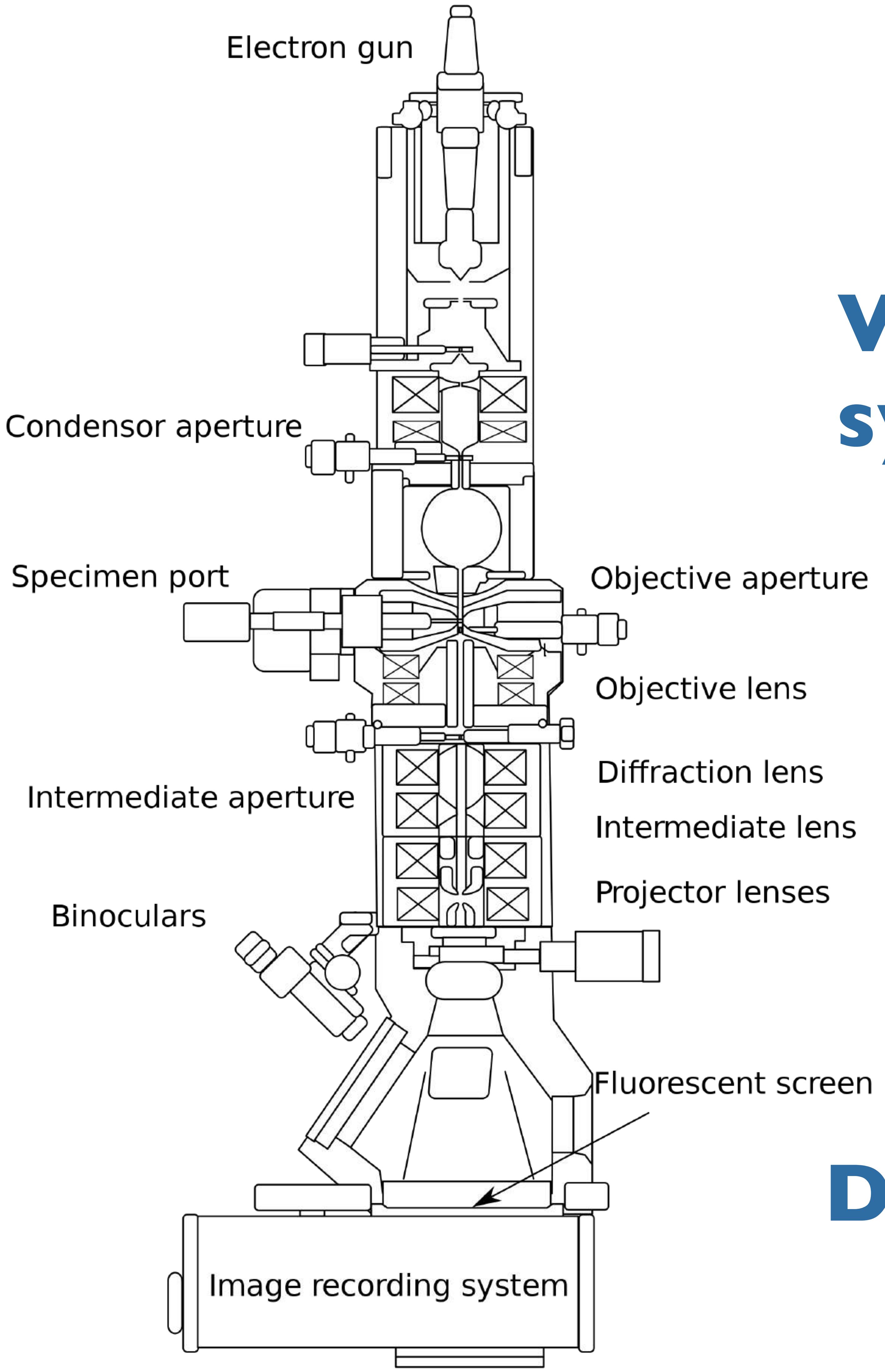
R.M. Glaeser[1]
Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, United States
[1]Corresponding author: e-mail address: rmglaeser@lbl.gov

- 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







Electron sources

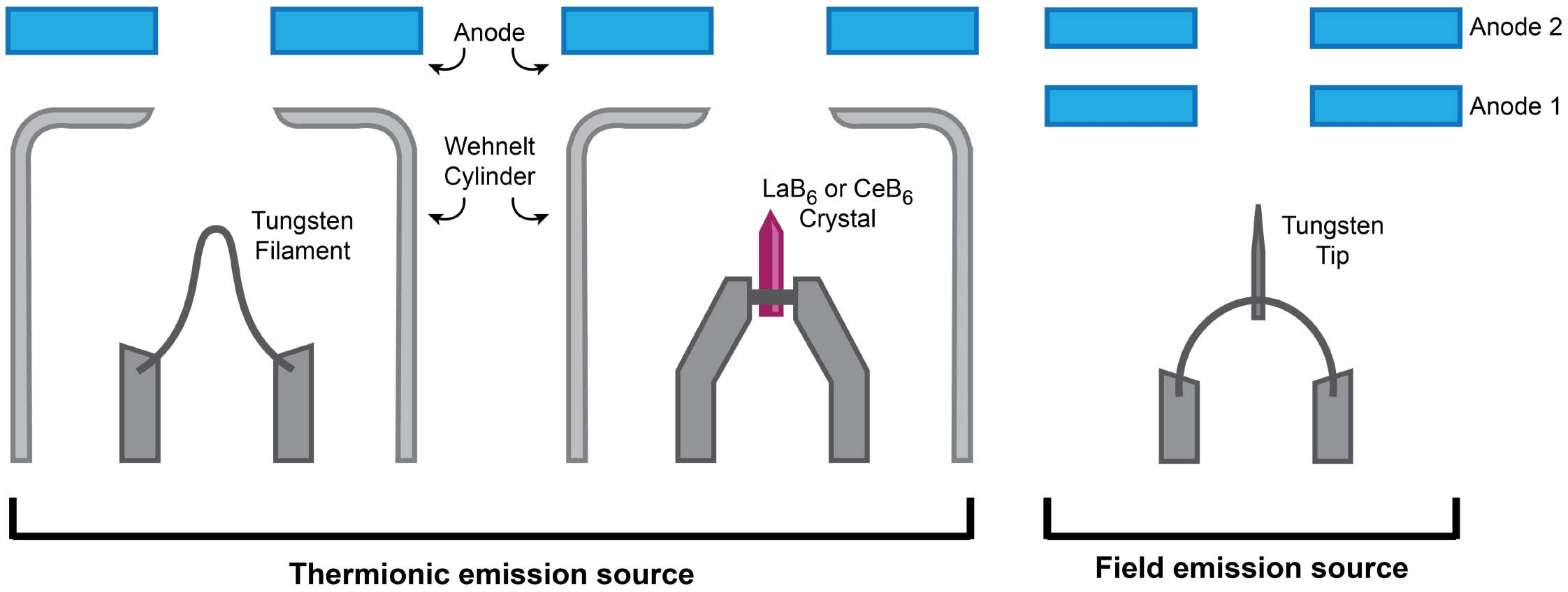
Vacuum systems

Lenses

Detectors

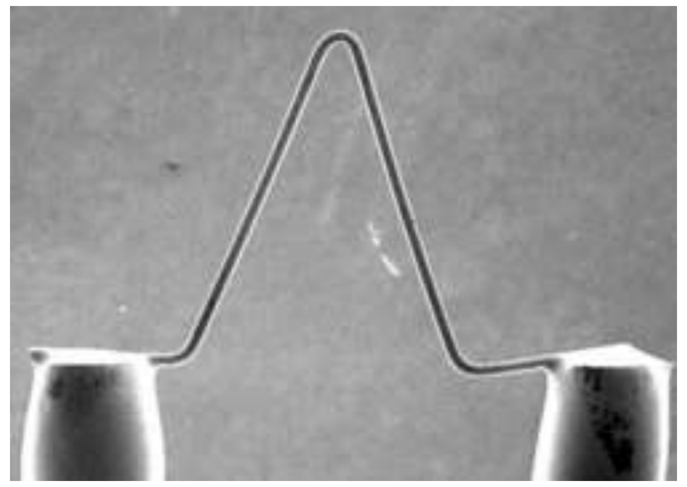
Electron sources

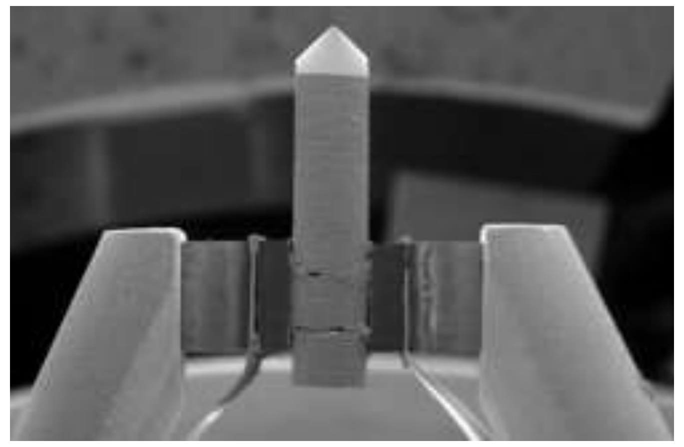
What are the 3 main kinds of electron sources?

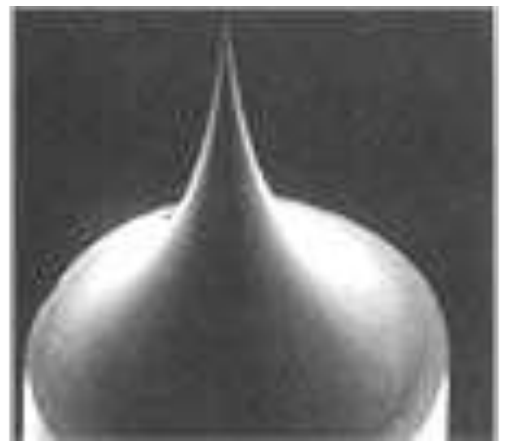


www.thermofisher.com

nanoscience.com

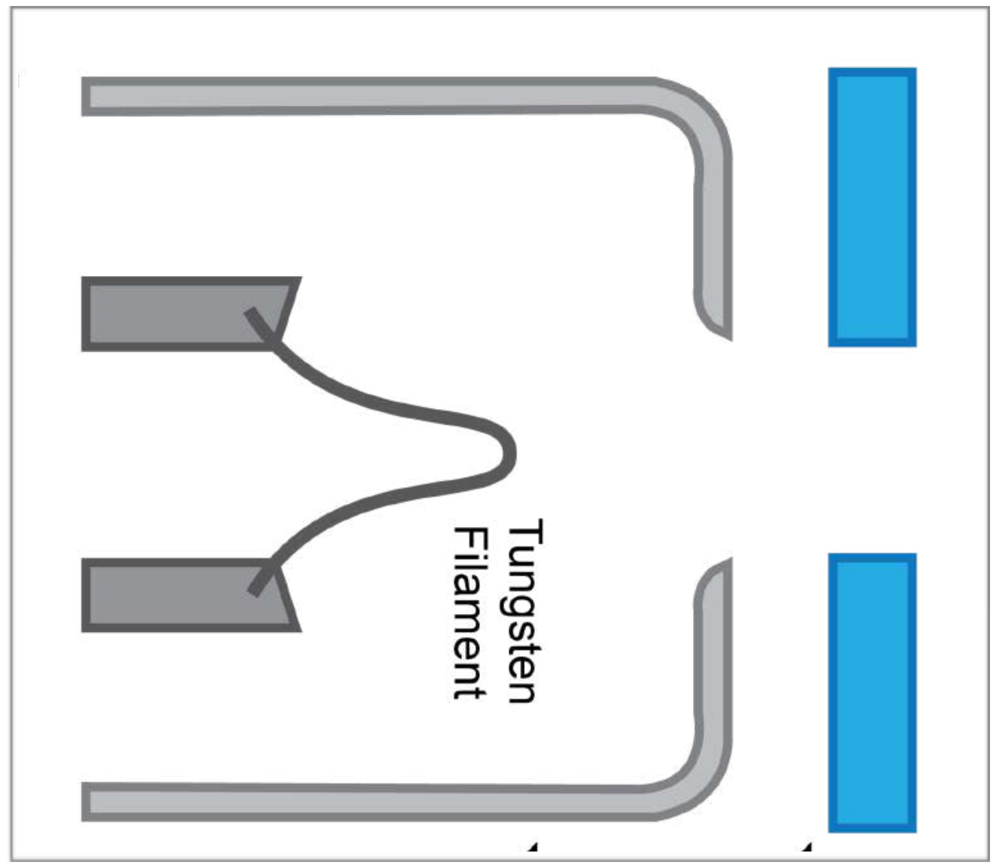


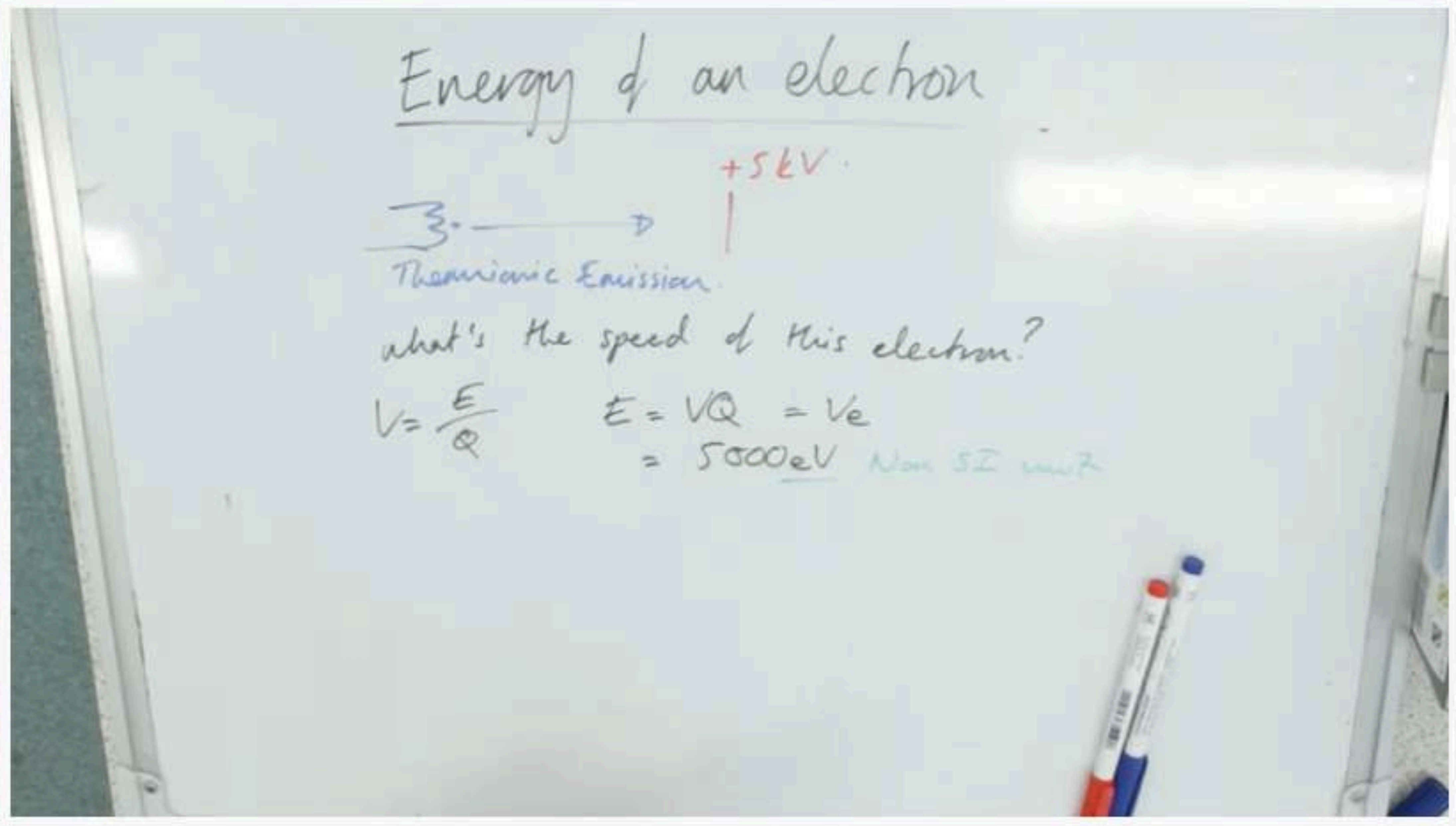




Electron sources

How fast are the electrons moving?

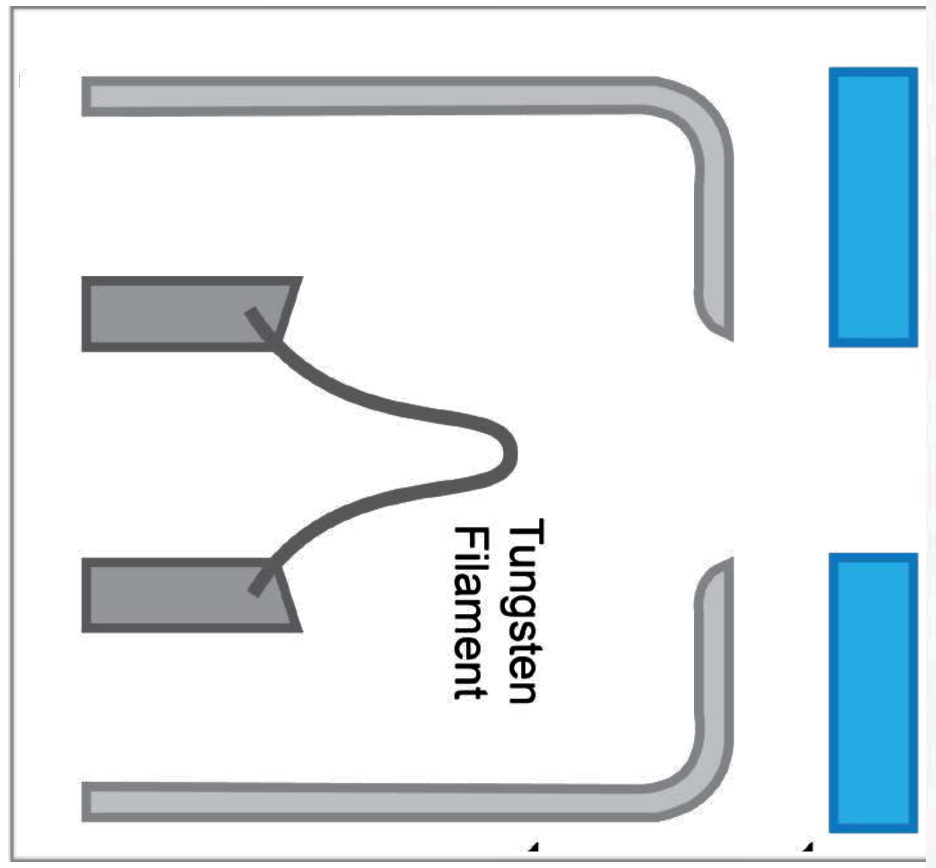


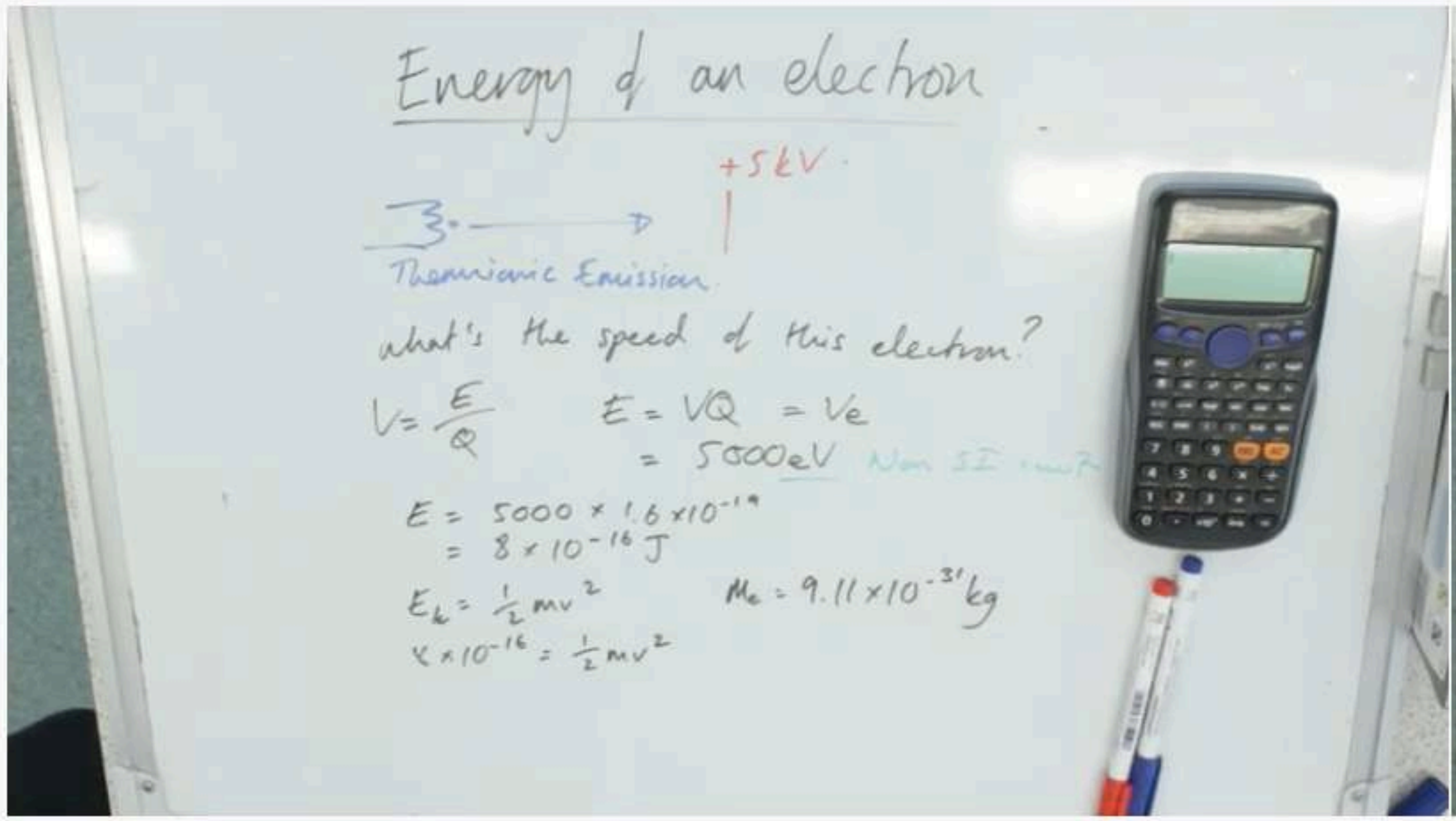


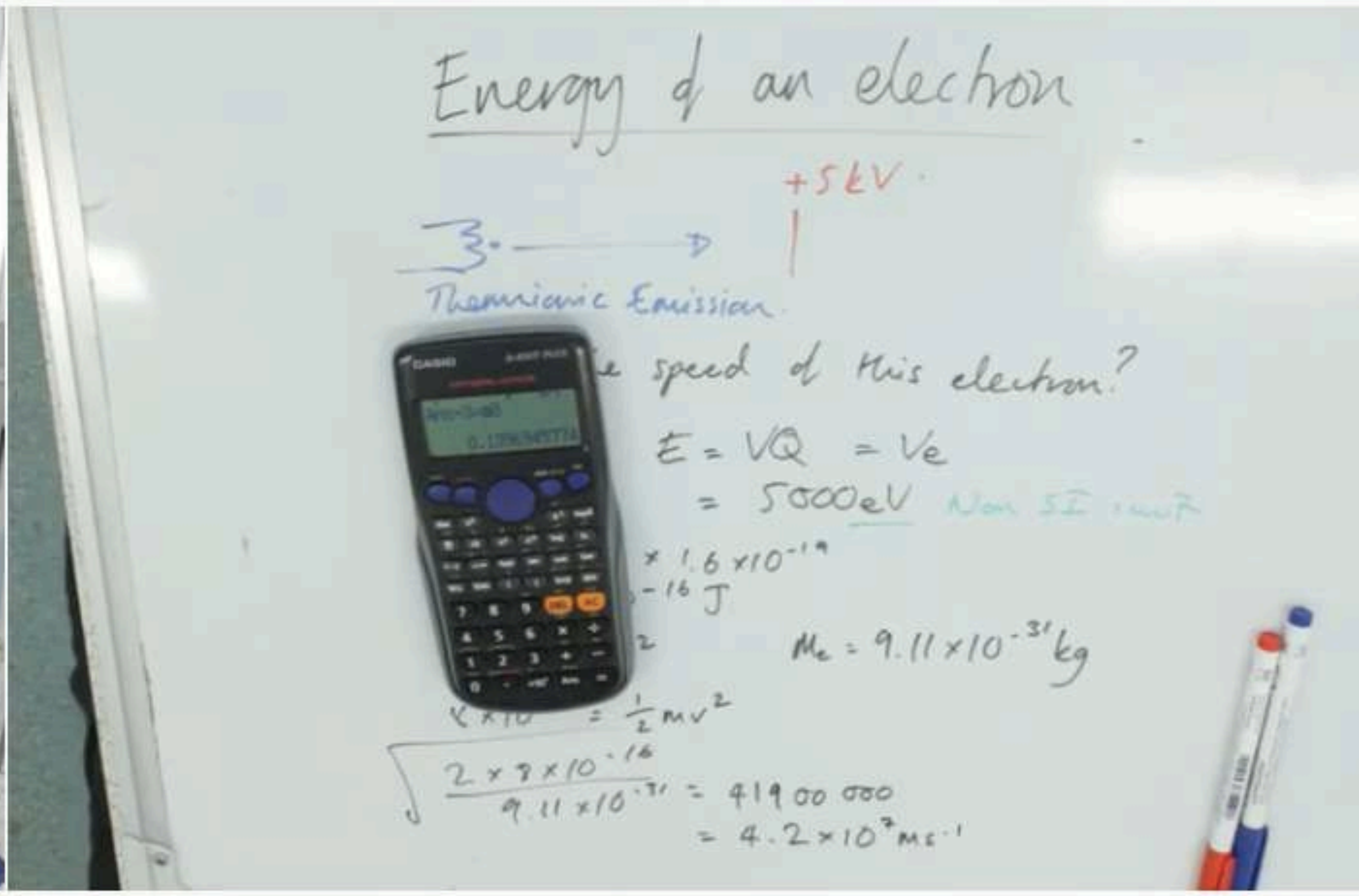
https://www.youtube.com/watch?v=tYCET6vYdYk

Electron sources

How fast are the electrons moving?

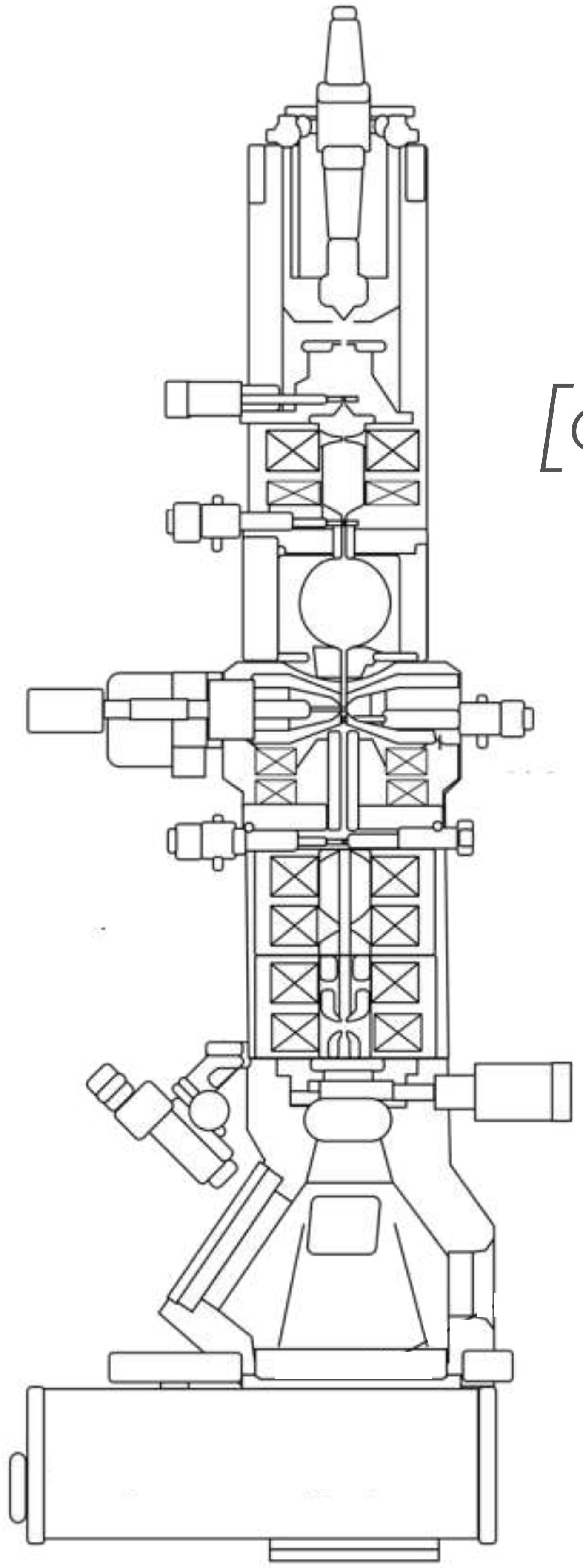






https://www.youtube.com/watch?v=tYCET6vYdYk

Electron sources

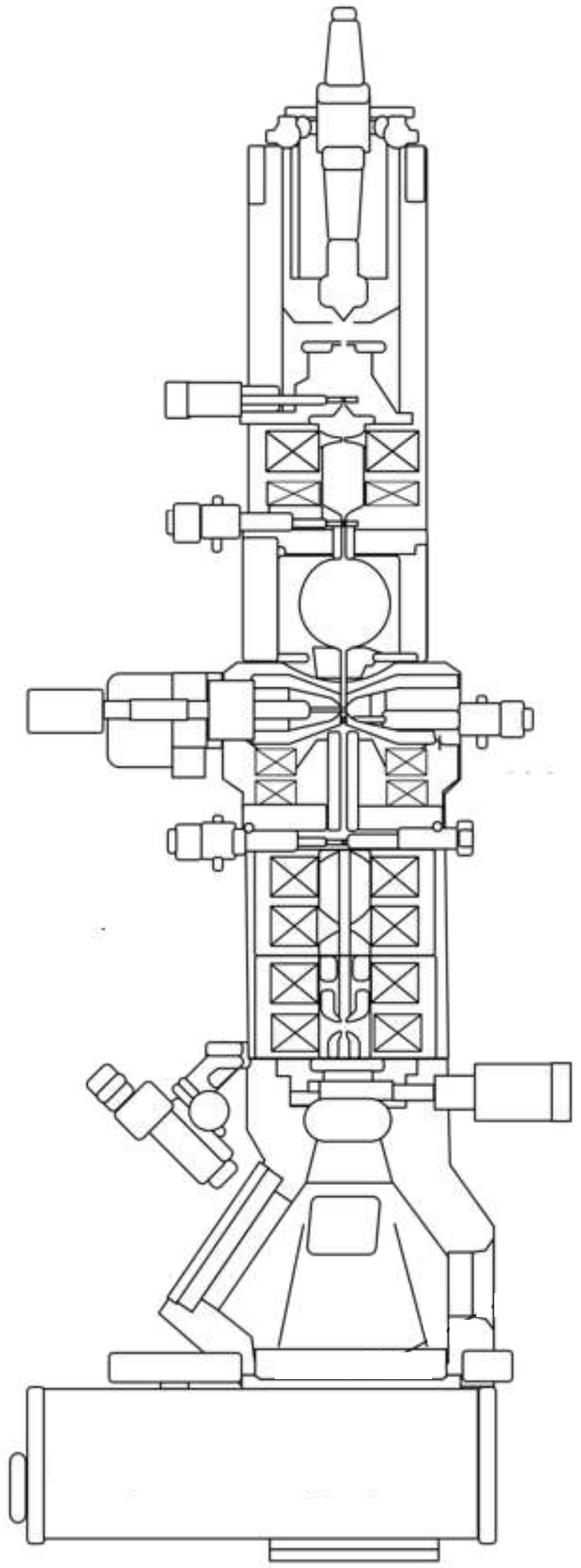


80-120 kV: Hitachi 7800, JEOL1400, TFS Talos120
W or LaB6
High contrast & robust
sub-nm resolution
[developments ongoing to push resolution with FEG systems]

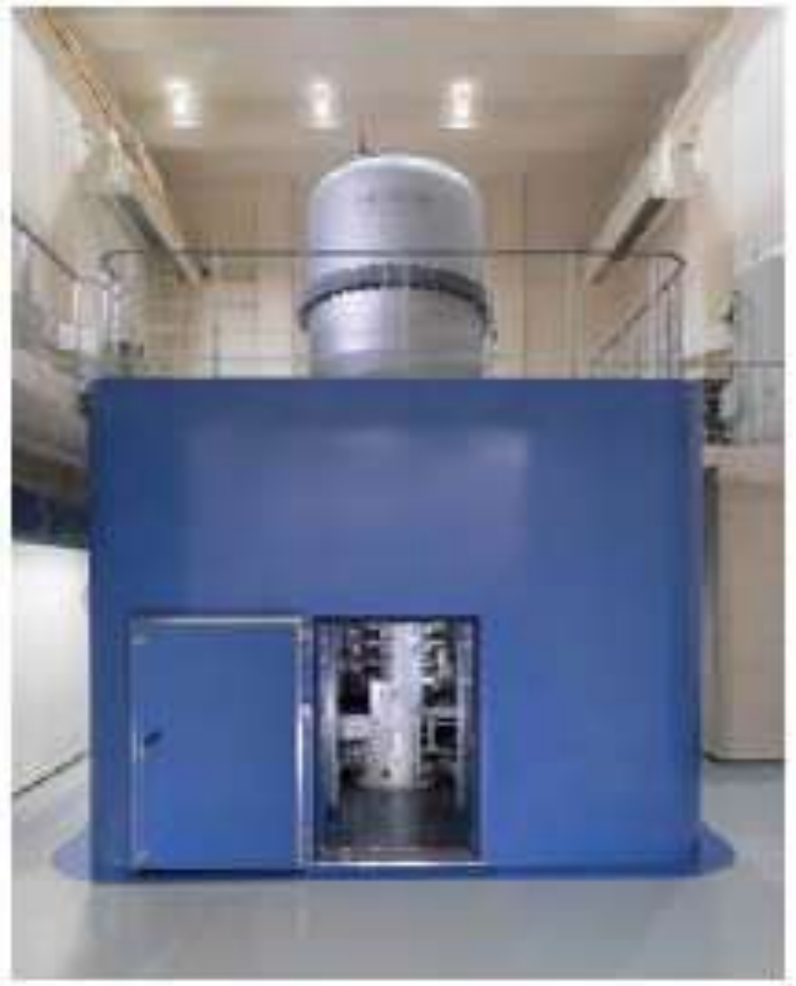
200 kV: J2100F; TFS Tecnai, Glacios, Arctica
FEG
2+ Å resolution (3.5-4 Å)

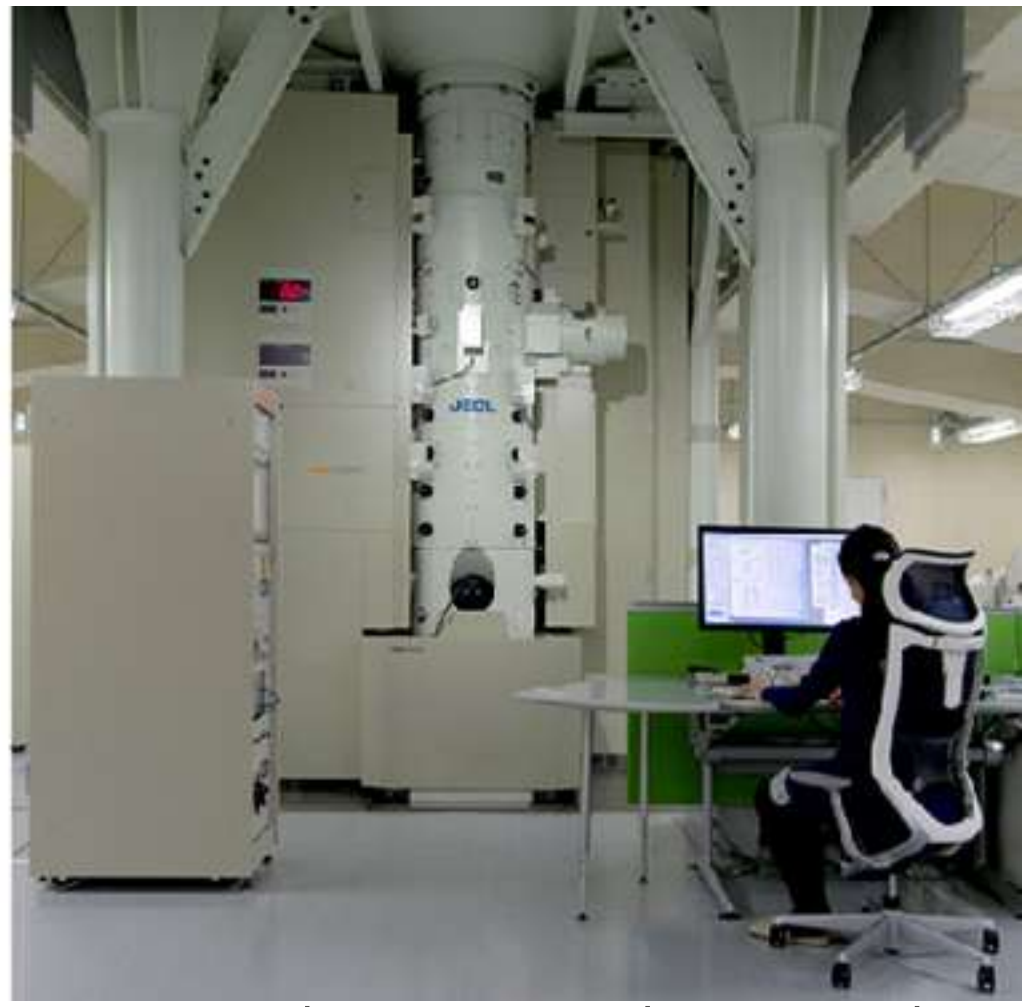
300 kV: JEOL3200FSC, cryoARM; TFS Krios, Halo
FEG
Smaller effect on unwanted lens aberrations
1.5-3 Å resolution

Electron sources



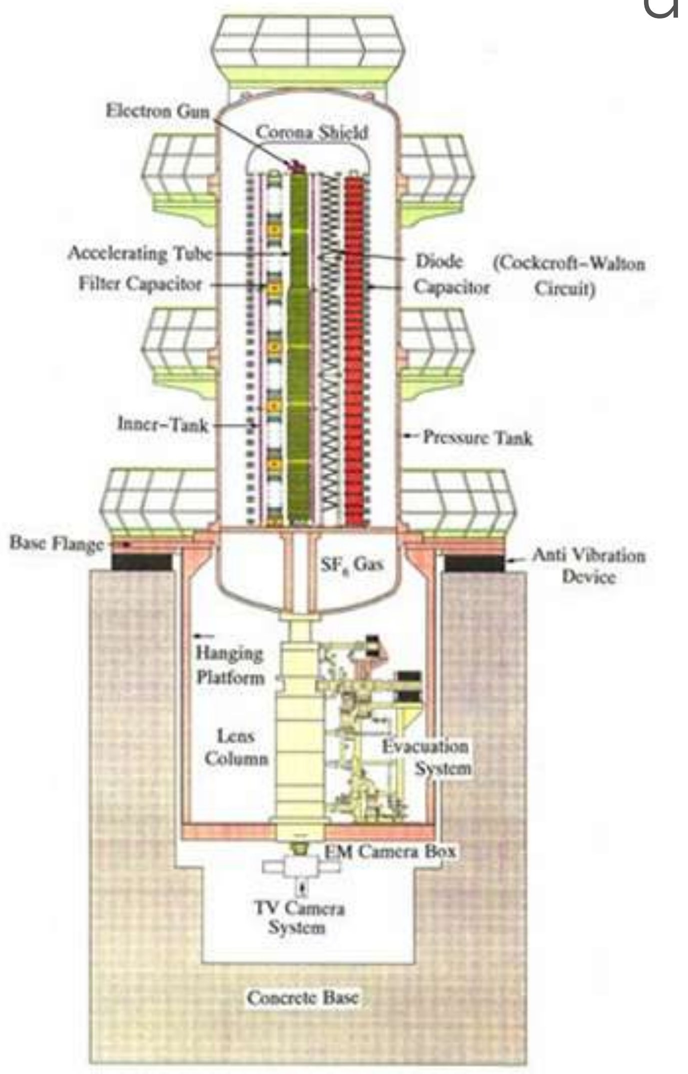
1-1.2 MV: Hitachi, JEOL
LaB6



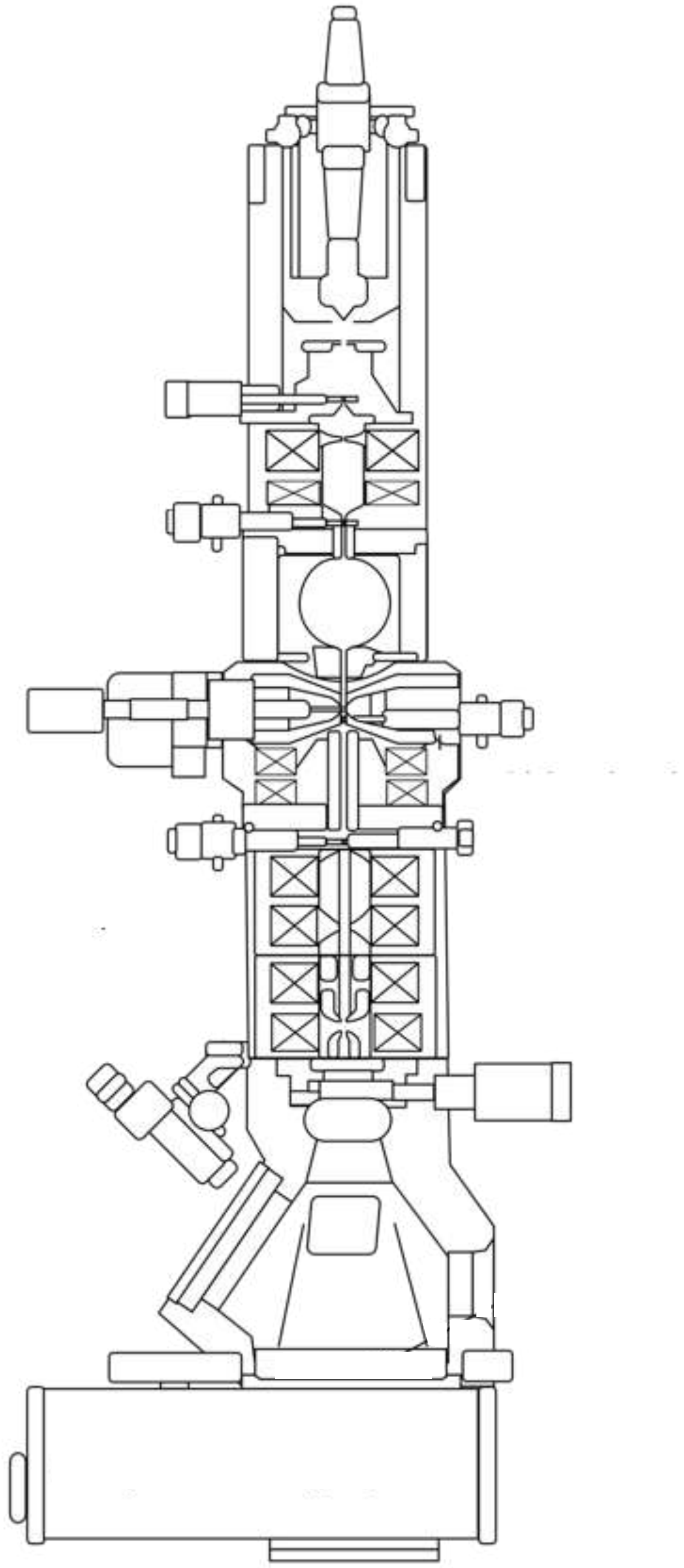


uhvem.osaka-u.ac.jp

3 MV: Hitachi H3000
LaB6



Vacuum systems



Why do we need a vacuum?

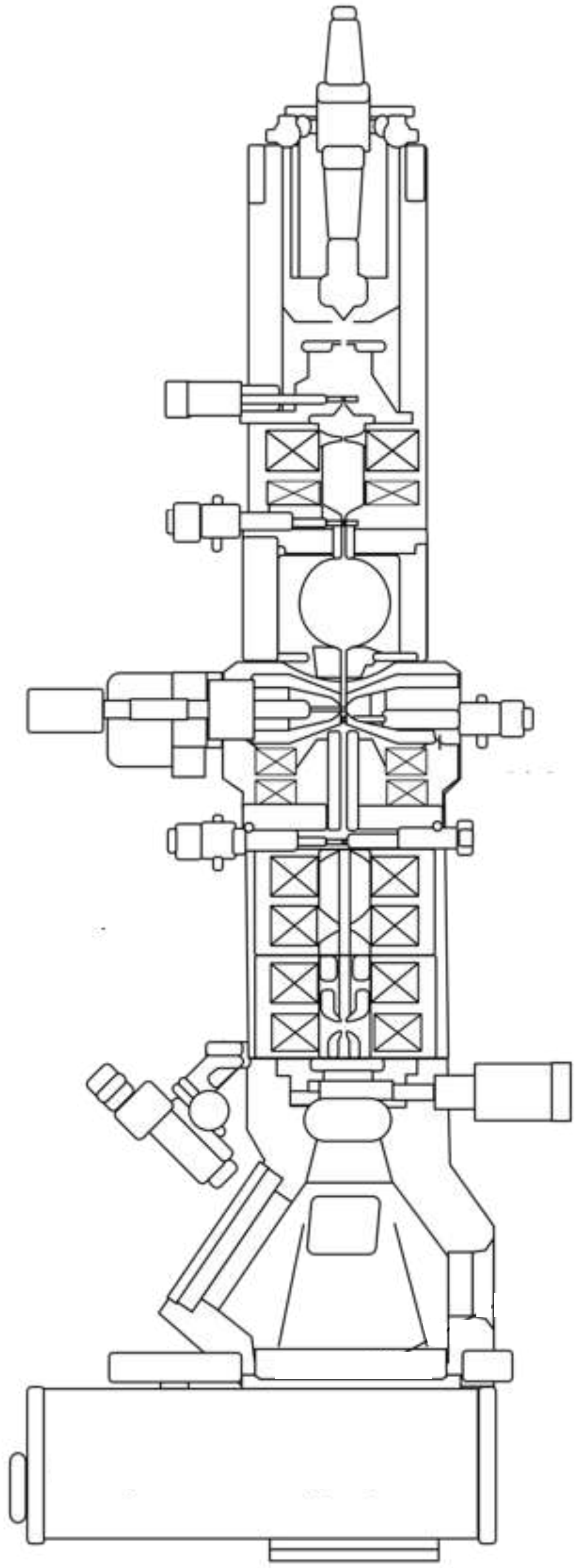
Beam coherence - at STP mean free path ~1 cm

Insulation - interaction between e- and air

Filament - O_2 will burn out source

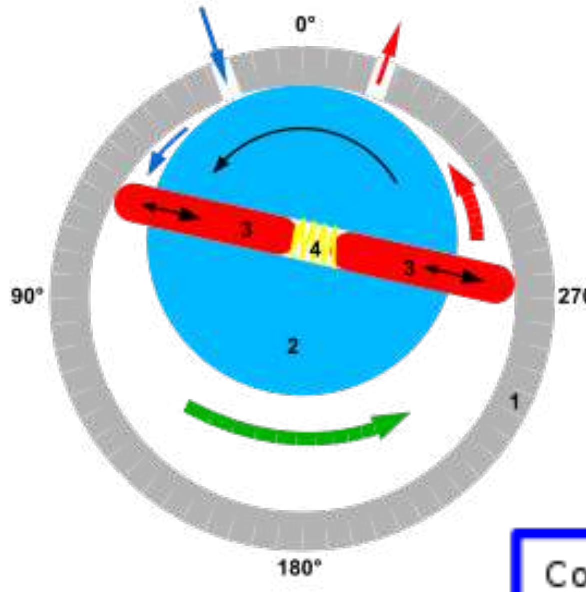
Contamination - reduce interaction gas, e-beam and sample

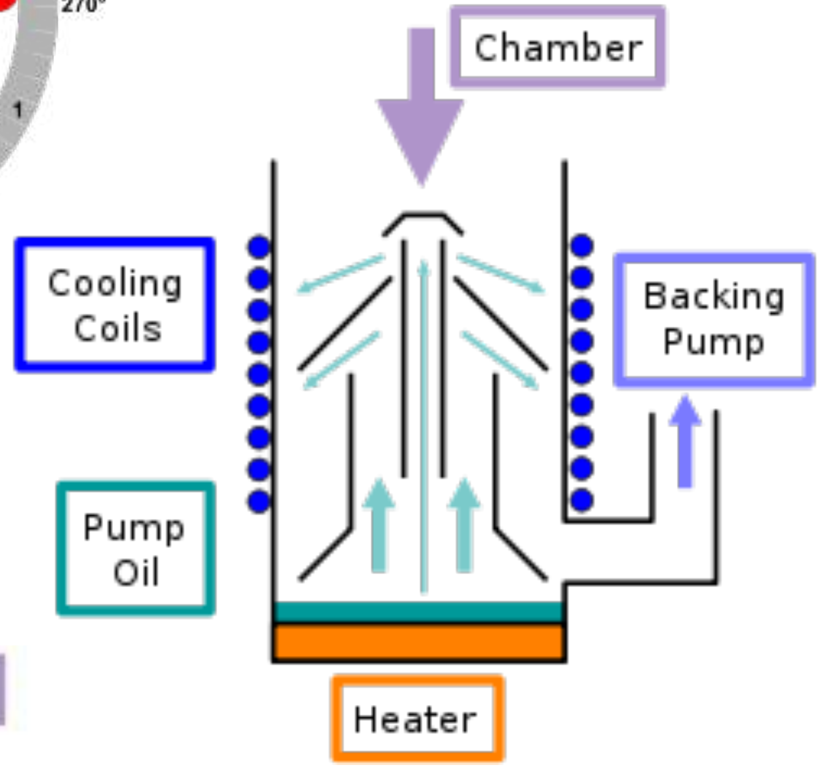
Vacuum systems

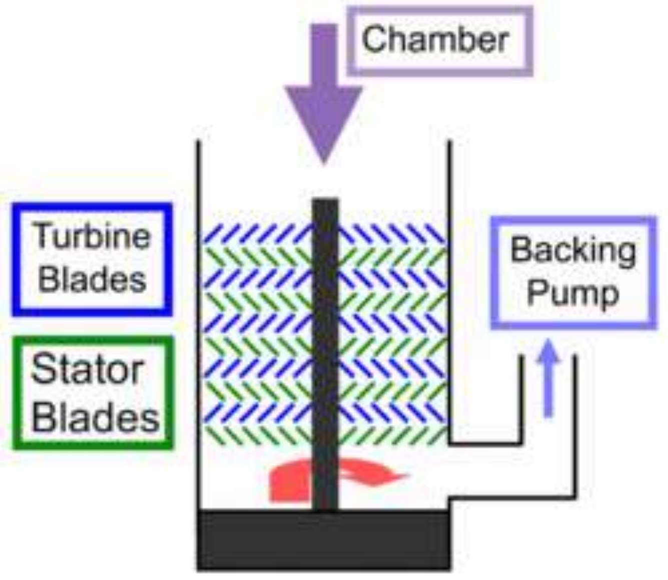


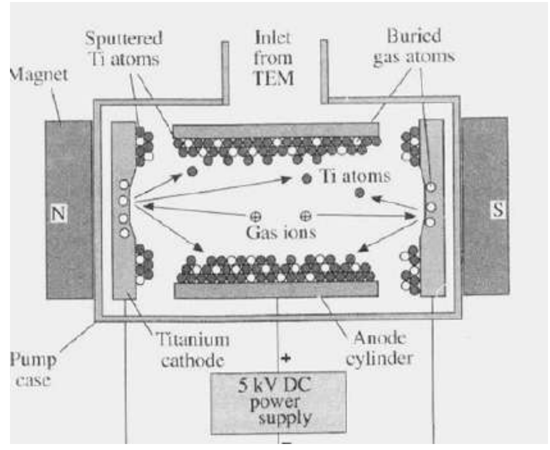
What types of pumps do we have?

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





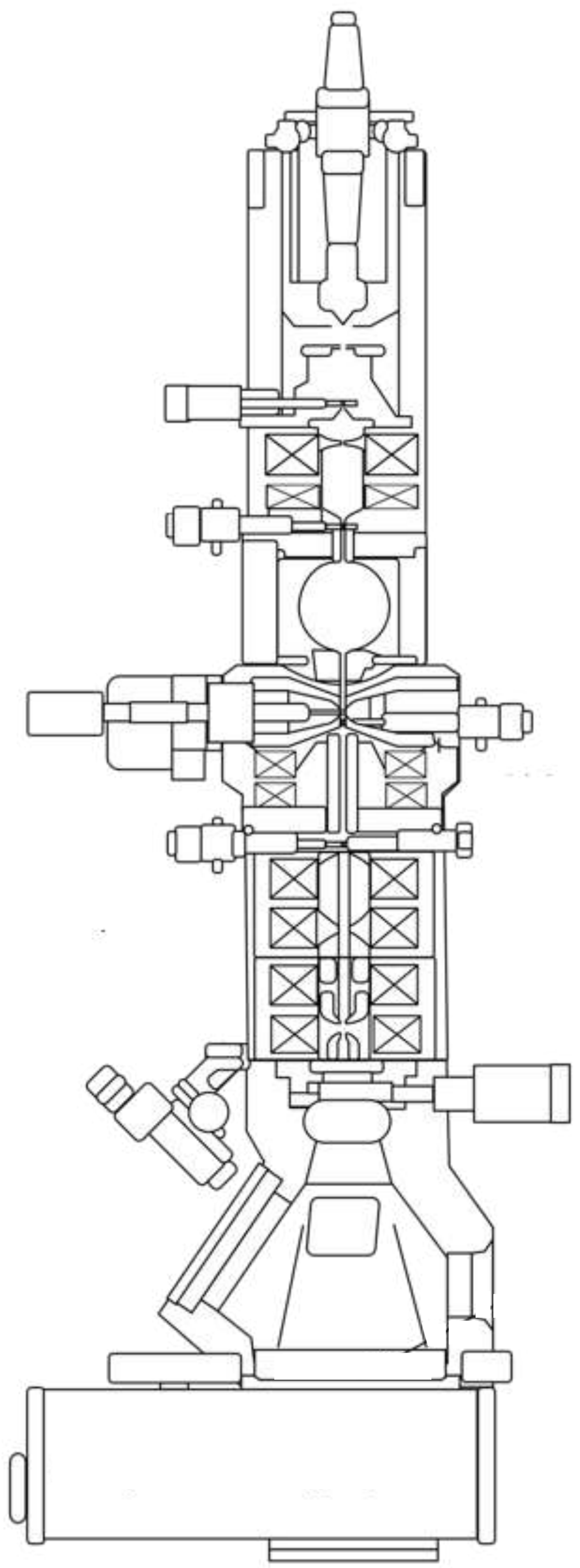




wikipedia.com

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

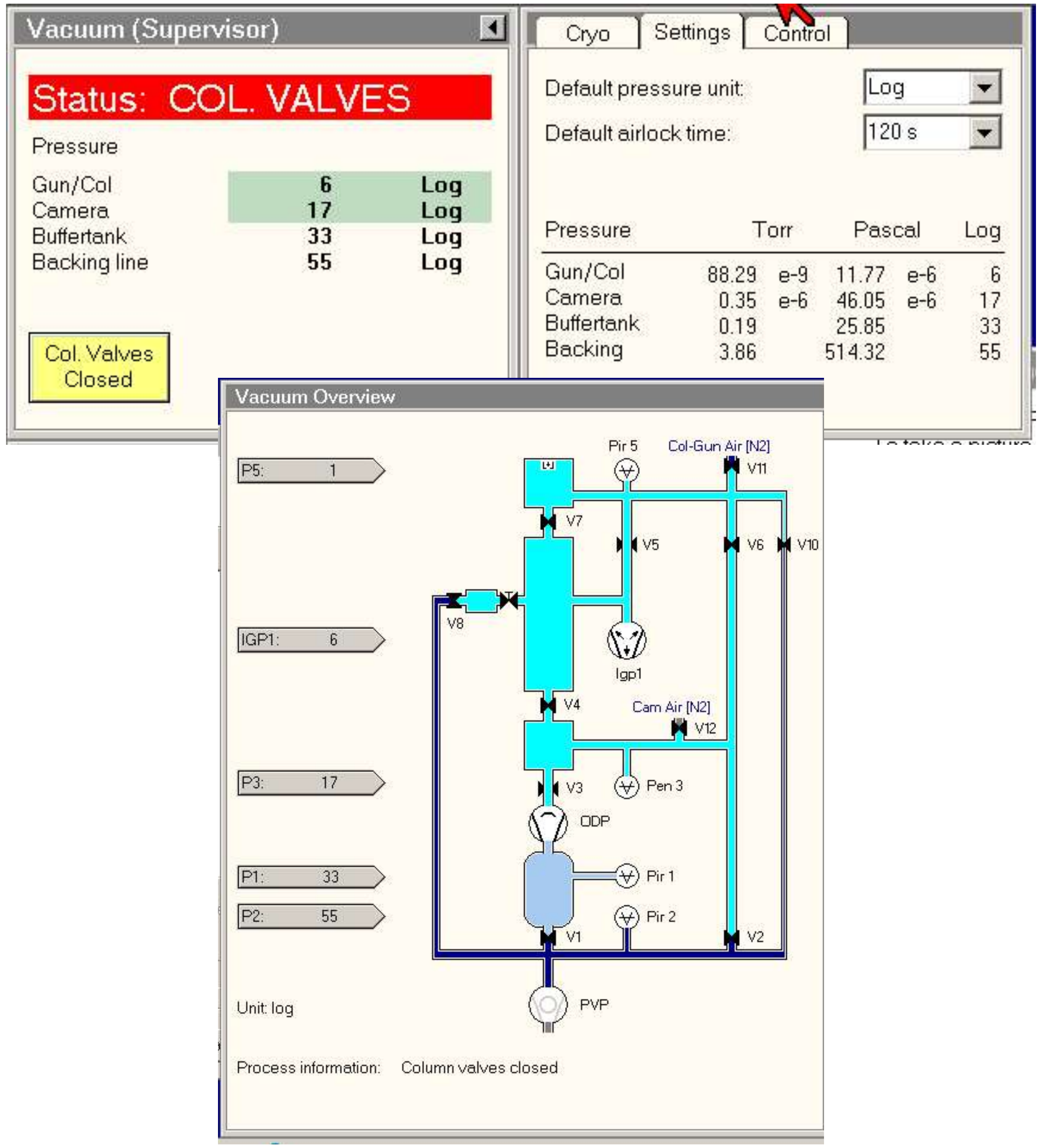
Vacuum systems



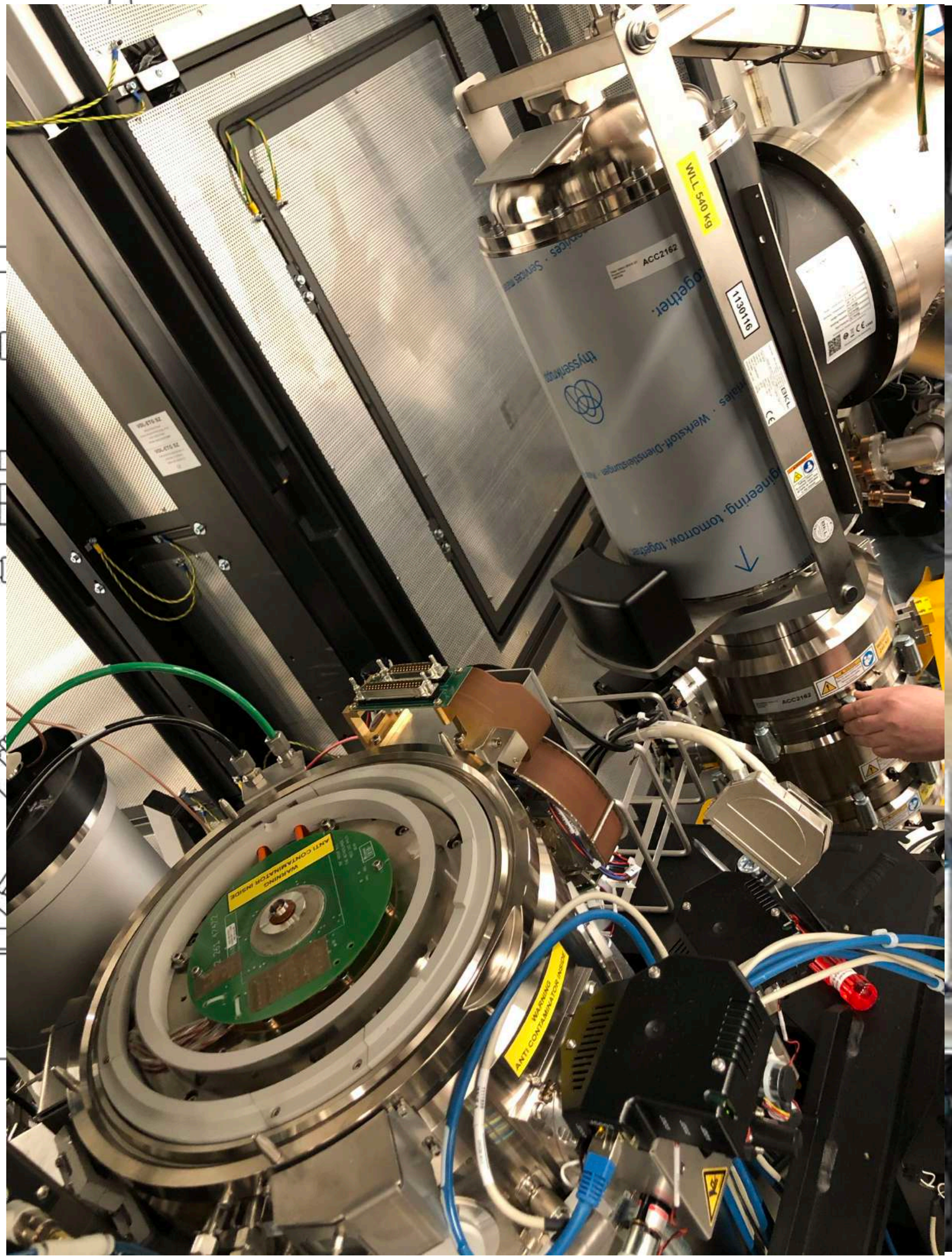
1 mm Hg = 1 Torr = 10^2 Pa
1 atm = 760 Torr = 7.5×10^4 Pa

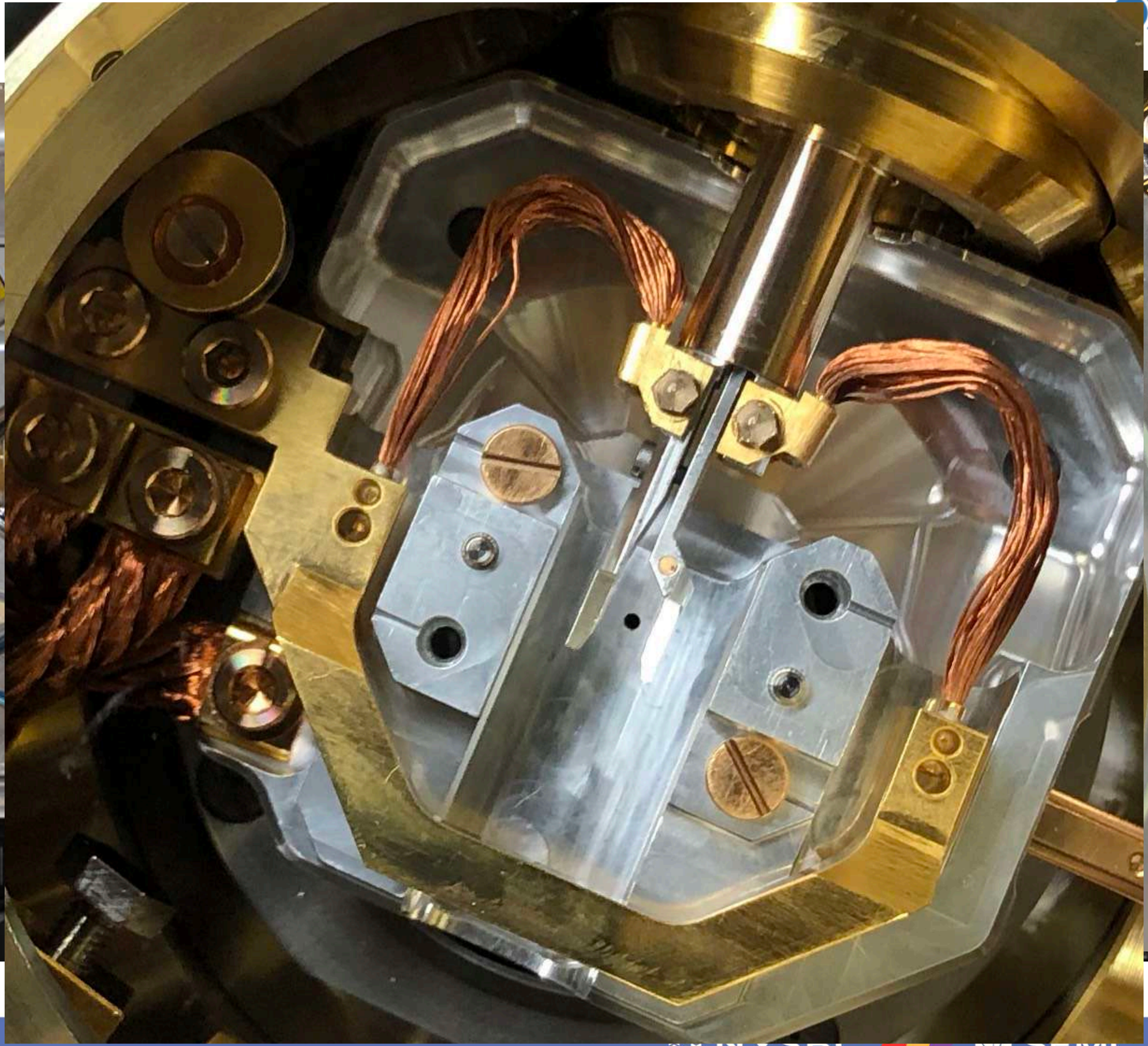
What types of pumps do we have?

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

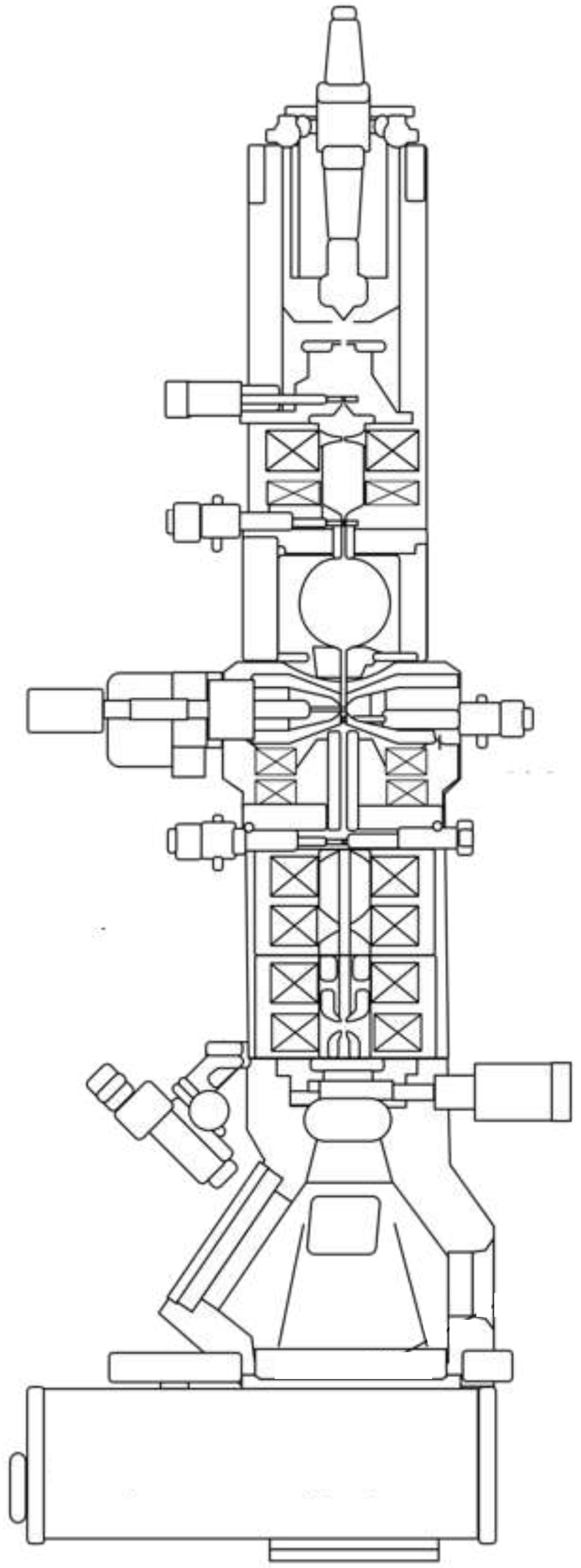


Vacuum systems

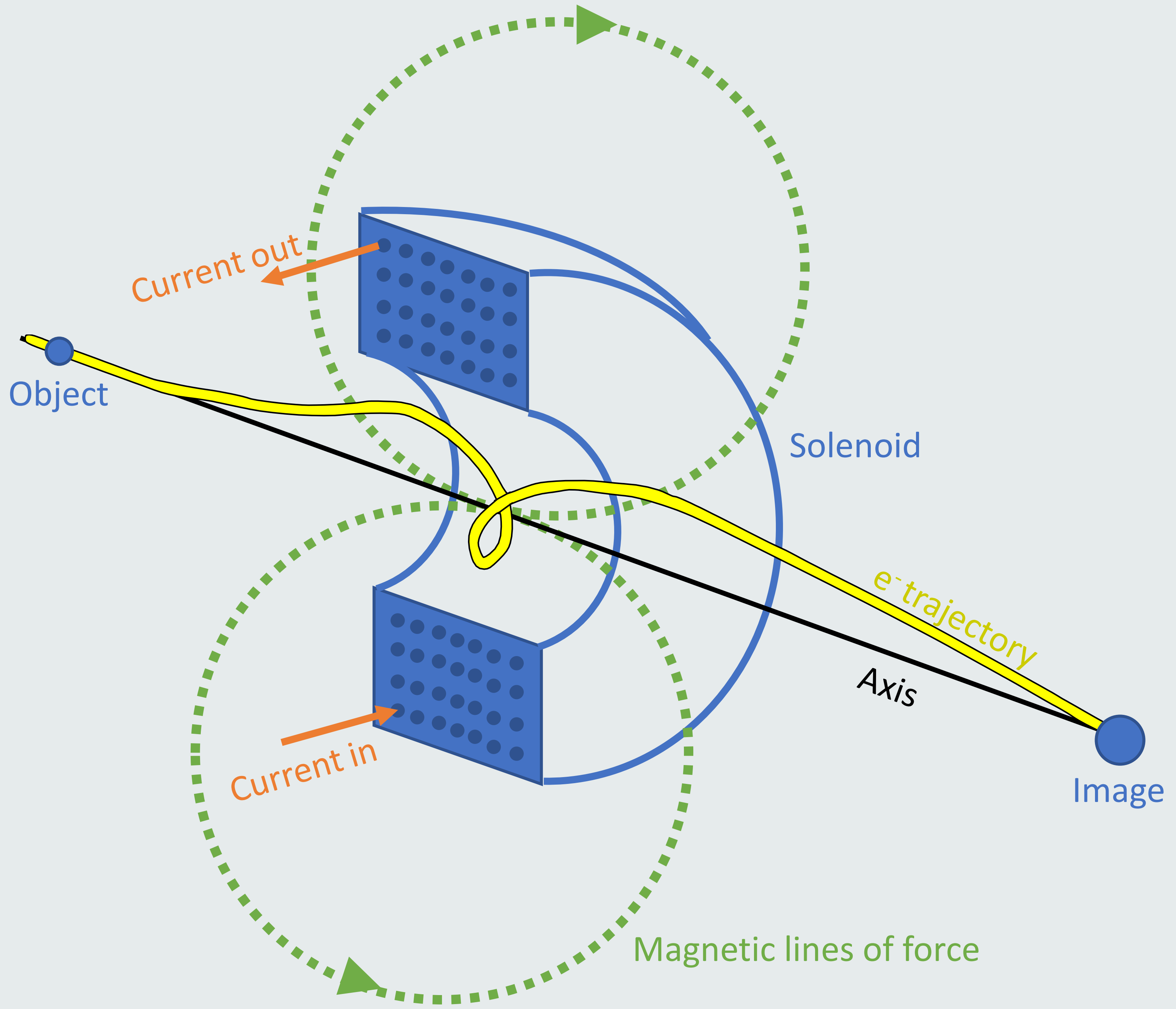




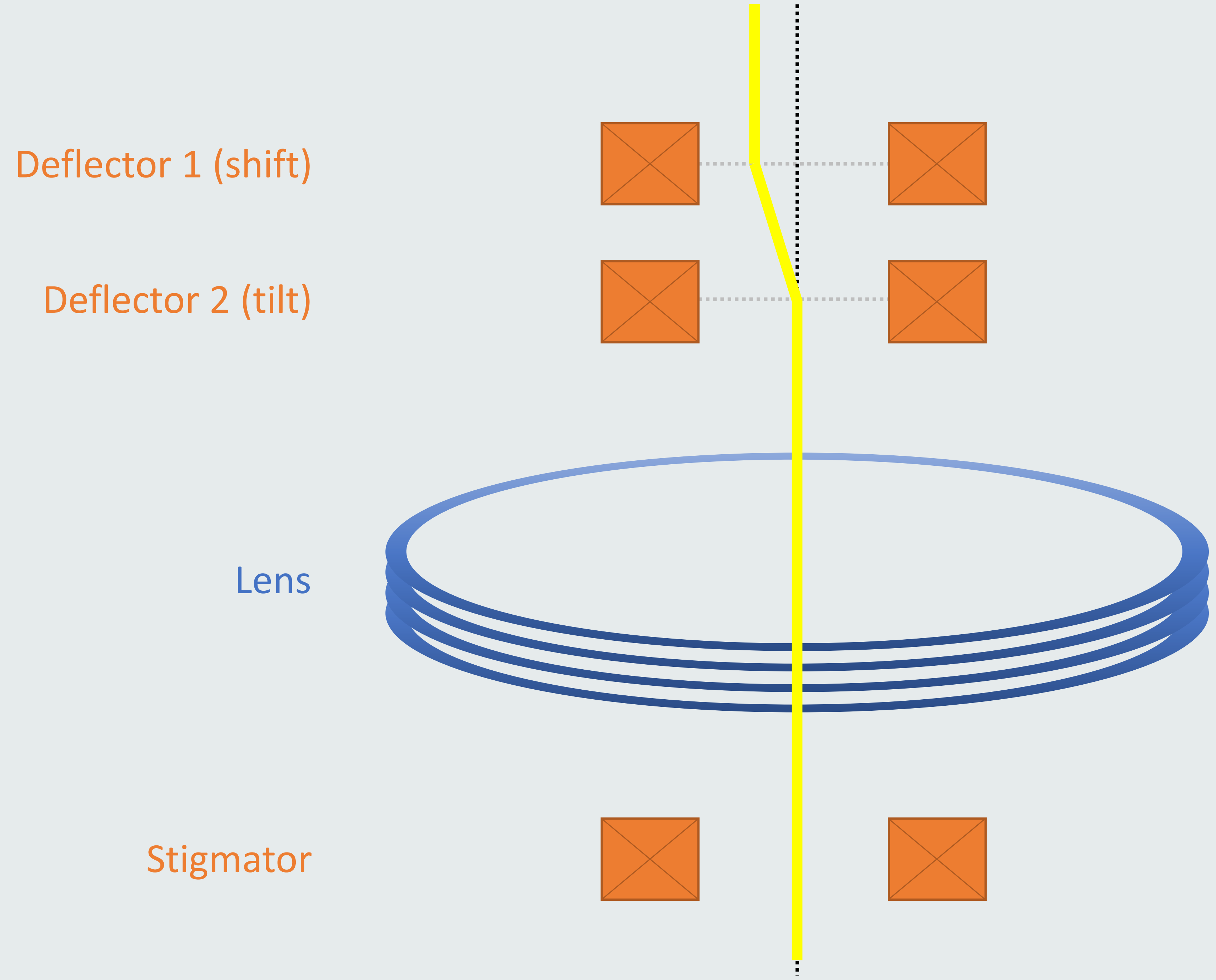
Lenses



What types of lenses do we have?

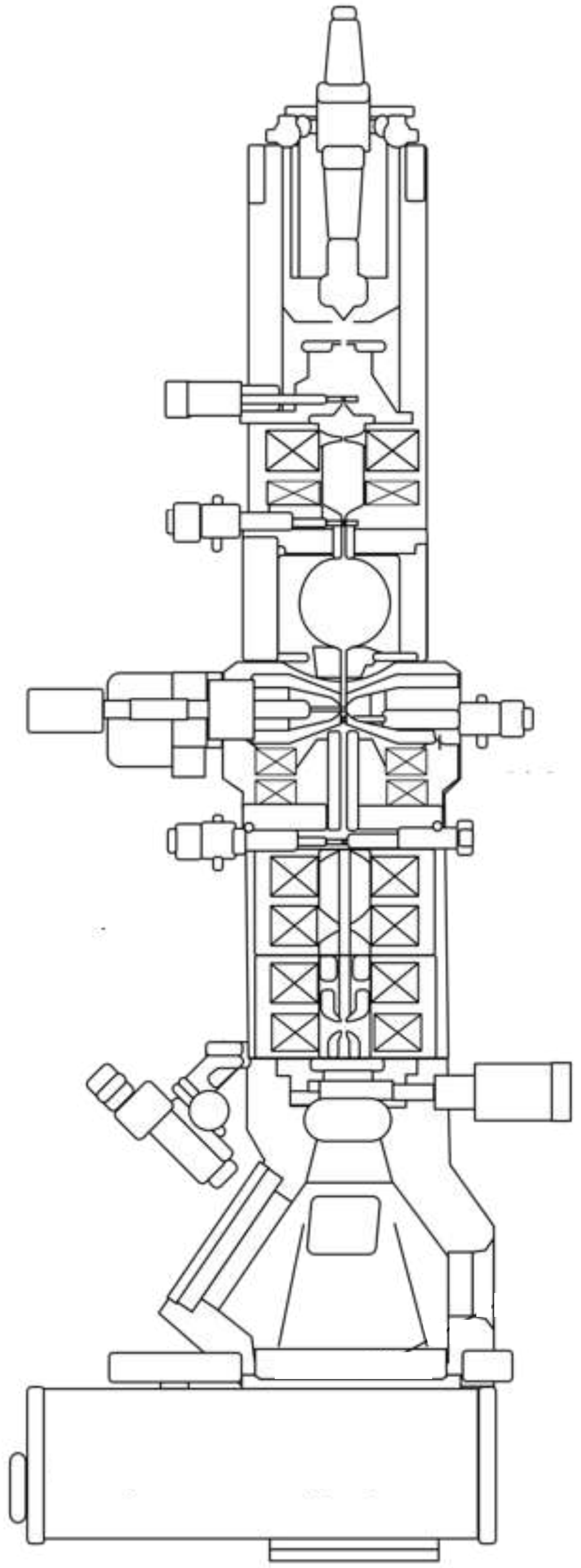


- Focus
- Magnify
- Rotate

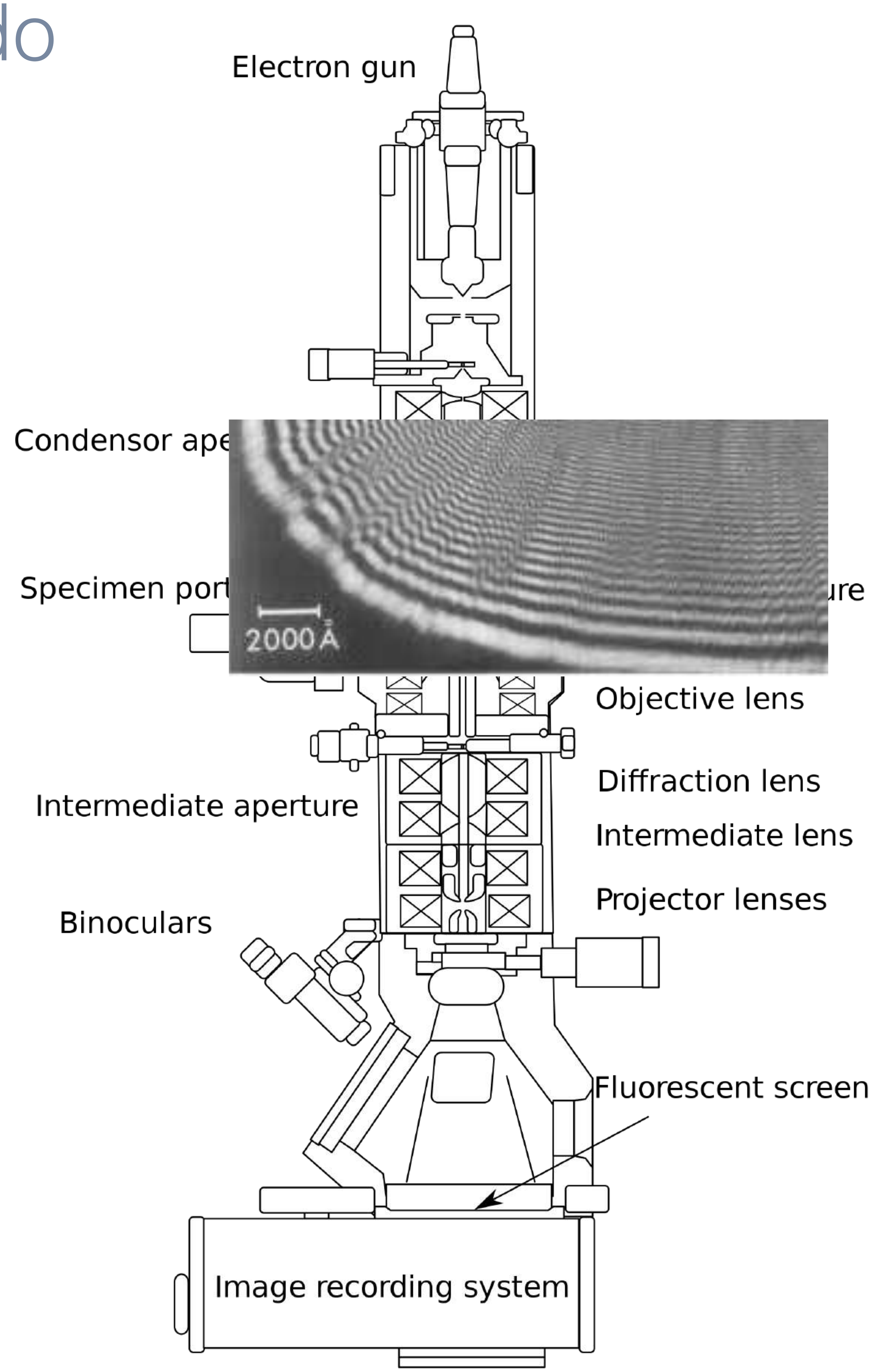


Lenses

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

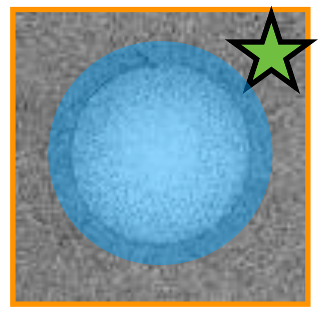
How to increase efficiency?

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;

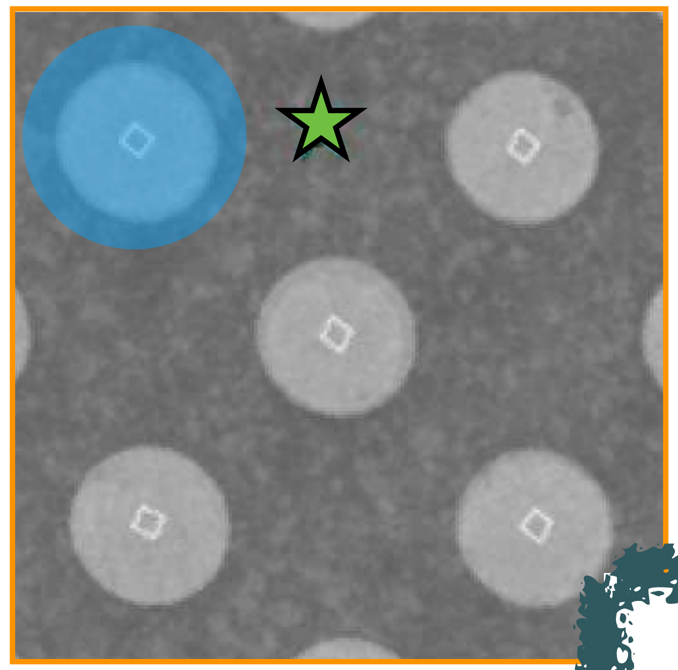
Anchi Cheng

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



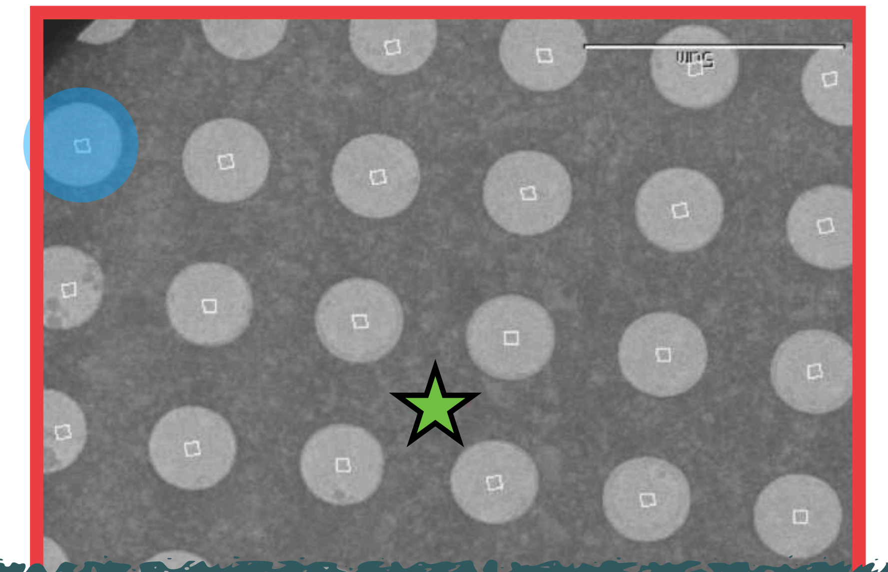
beam tilt
0 mrad

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

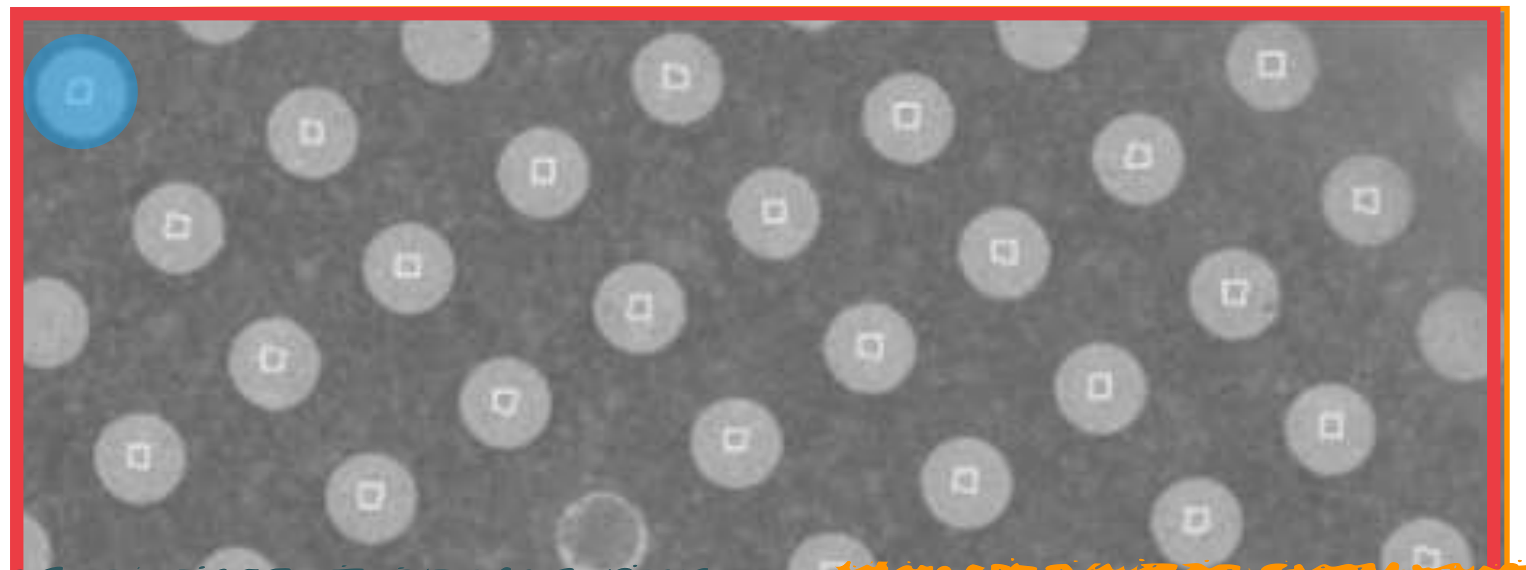


beam tilt
0.5 mrad

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



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

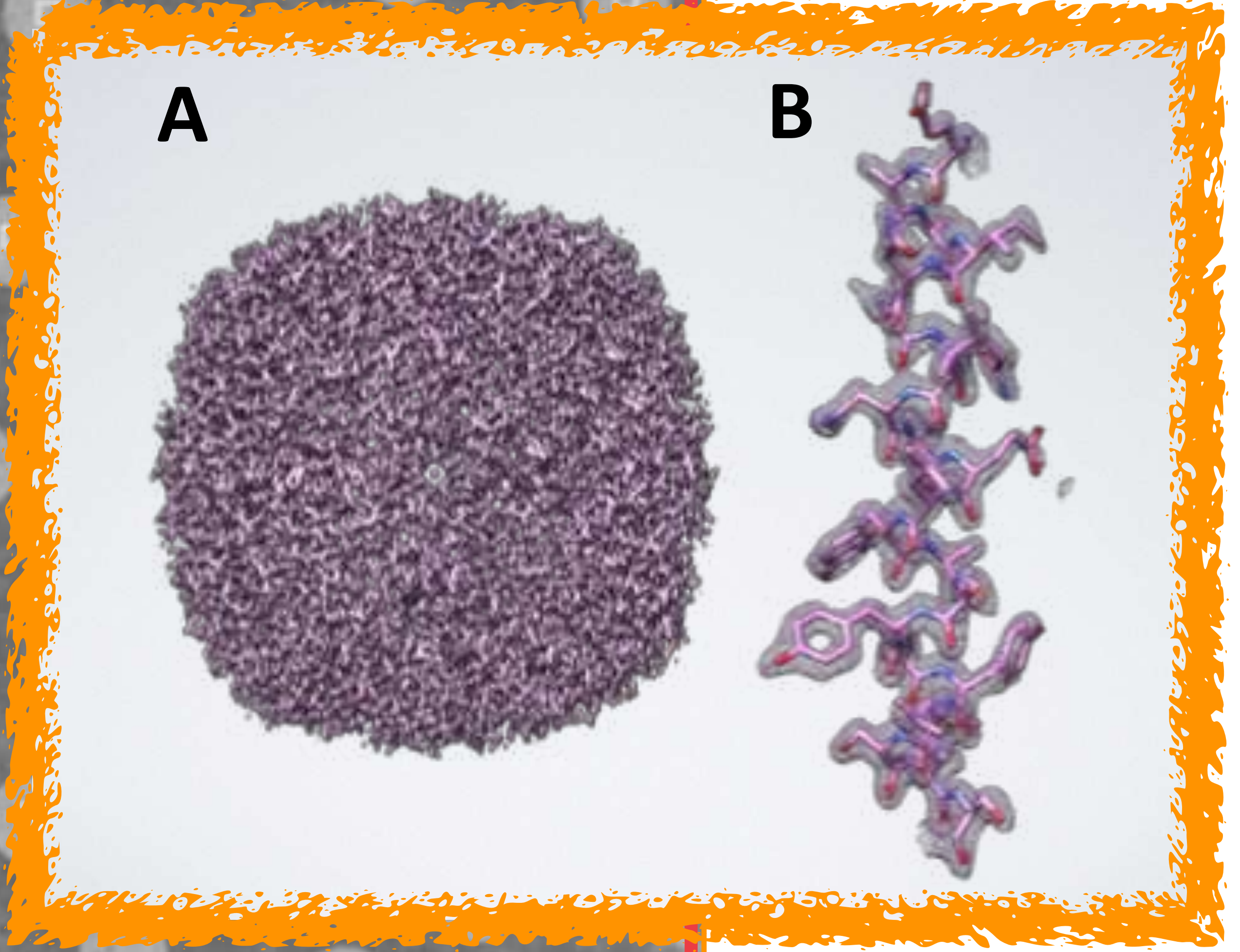


beam tilt
~3 mad

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

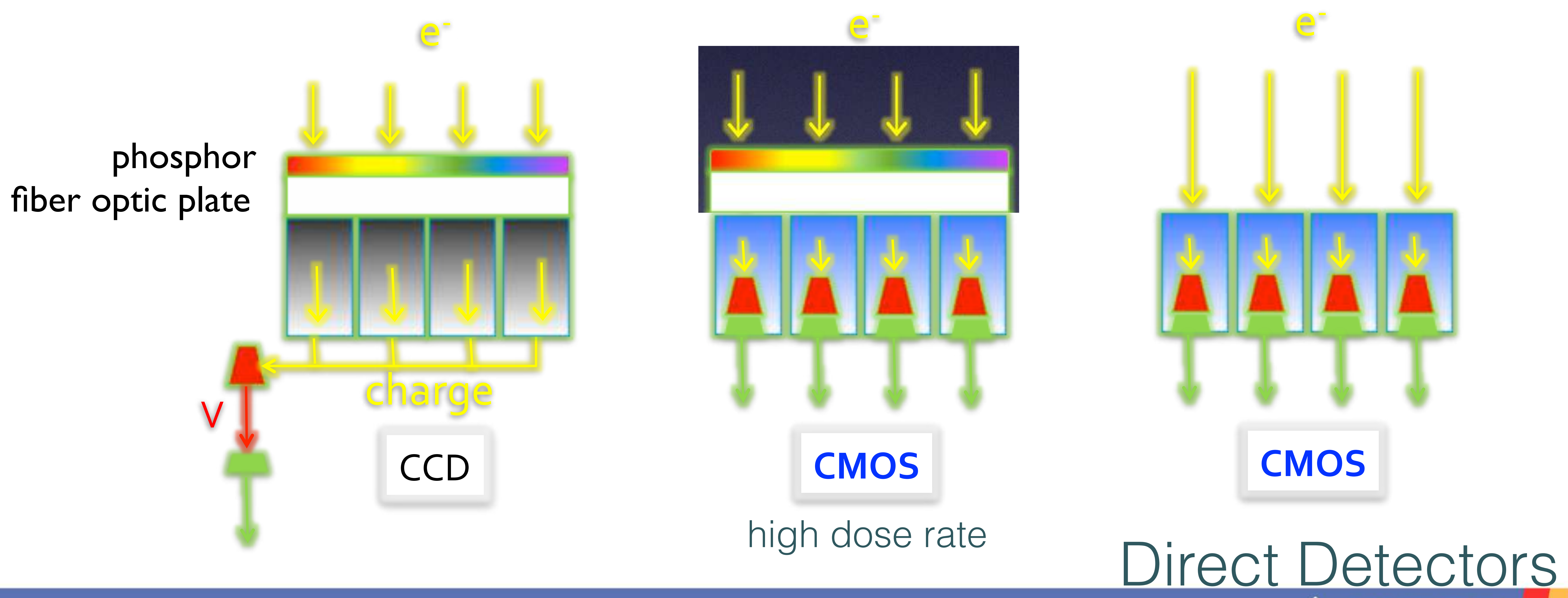
Detectors

Digital Cameras for TEM

- Photon converted
- Direct sensing

- CCD Charge Coupled Device
- CMOS Complementary Metal Oxide Semiconductor

?

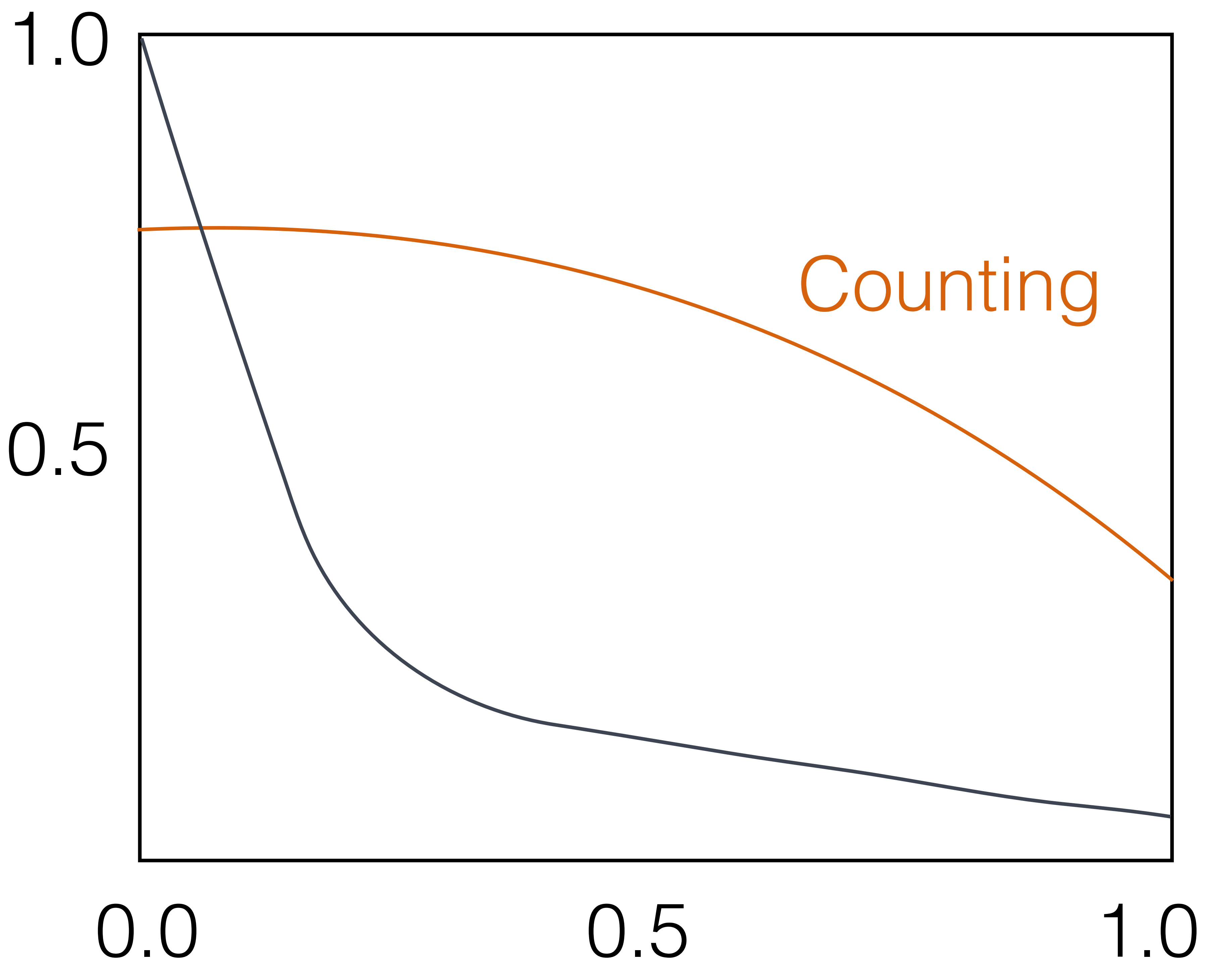


Anchi Cheng

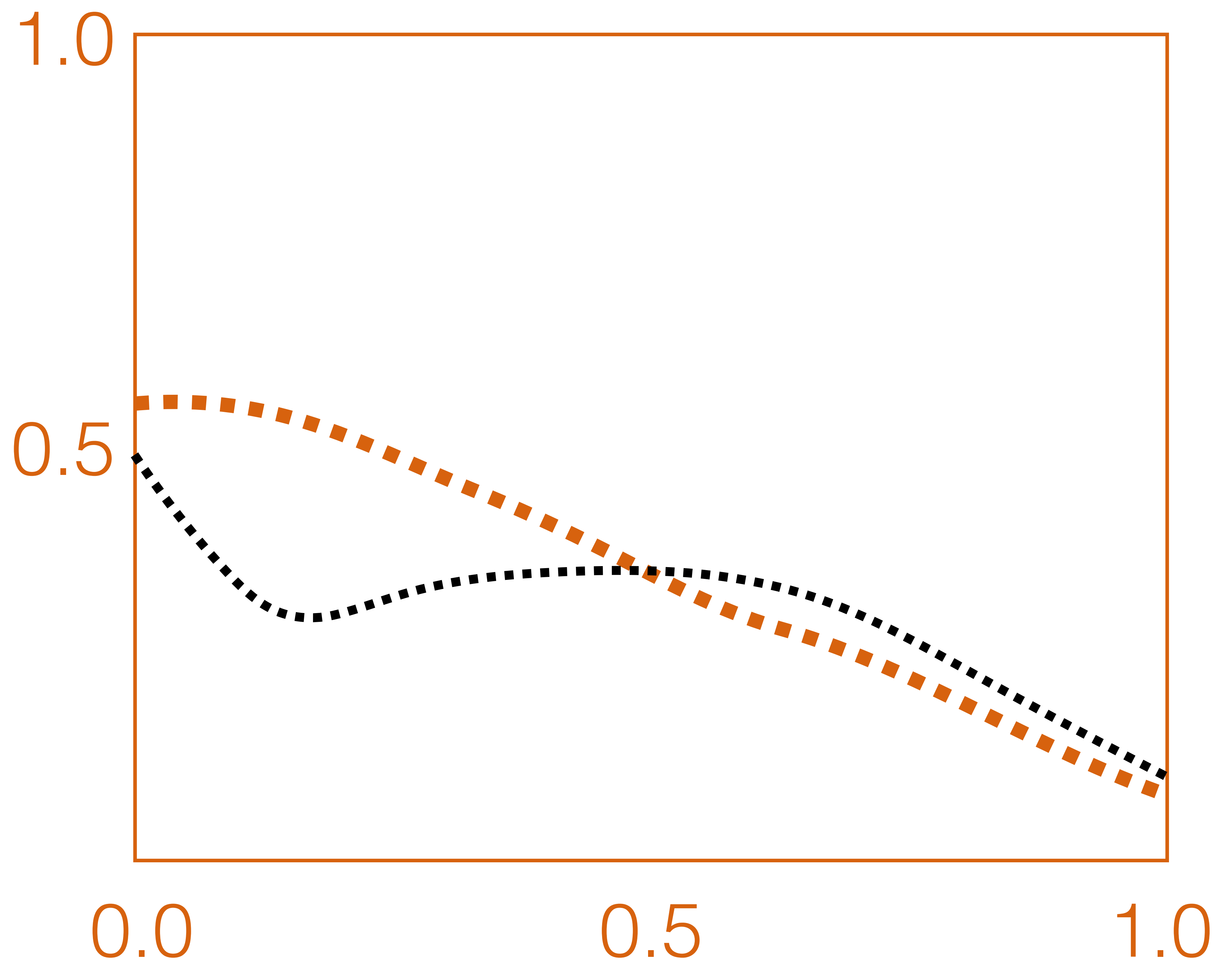
Detectors

Detector Performance Characterization

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

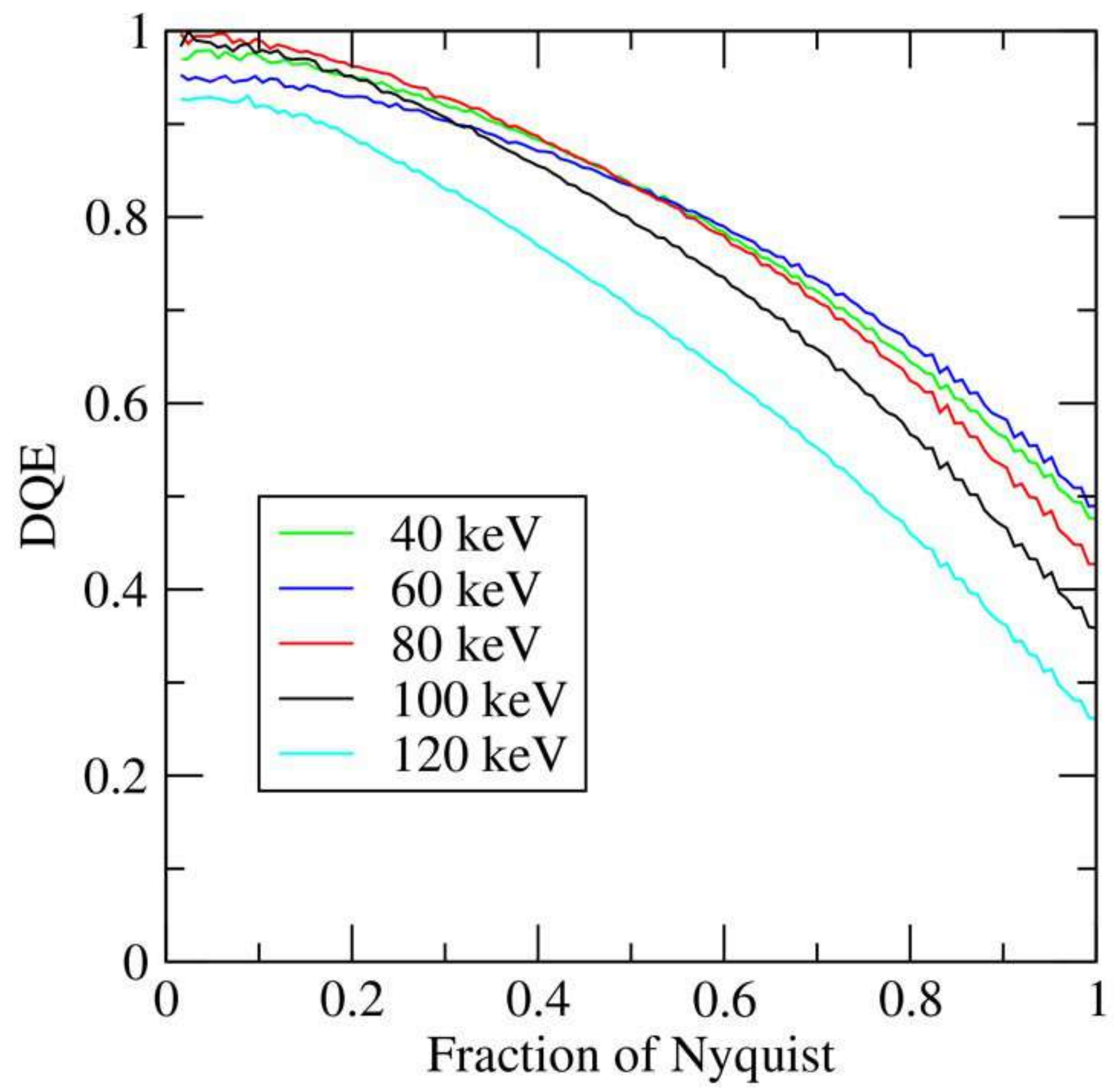


- DQE (Detector Quantum Efficiency)
  - S/N over spatial frequency range

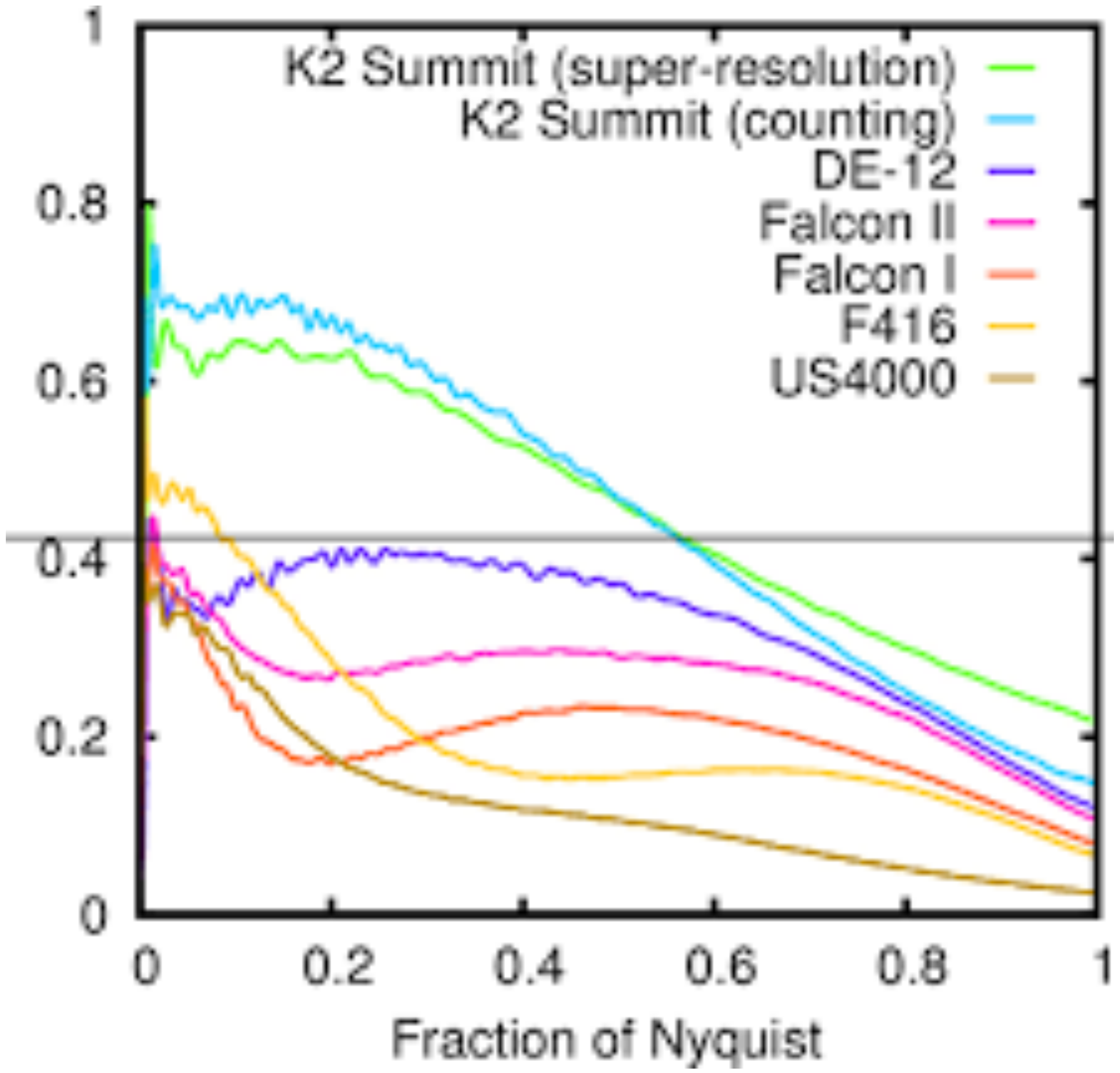


Detectors

Detector Performance Characterization



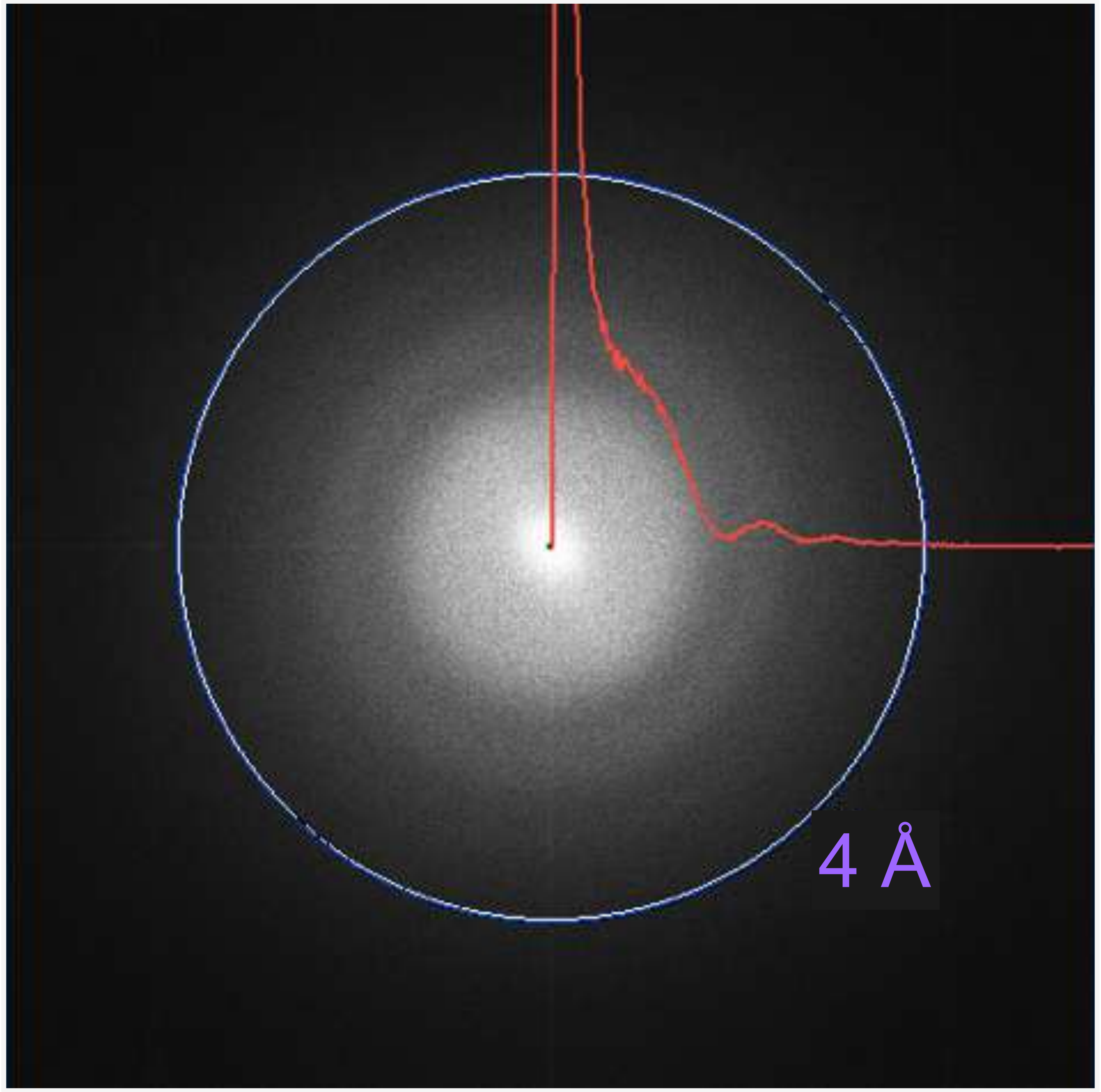
dectris.com



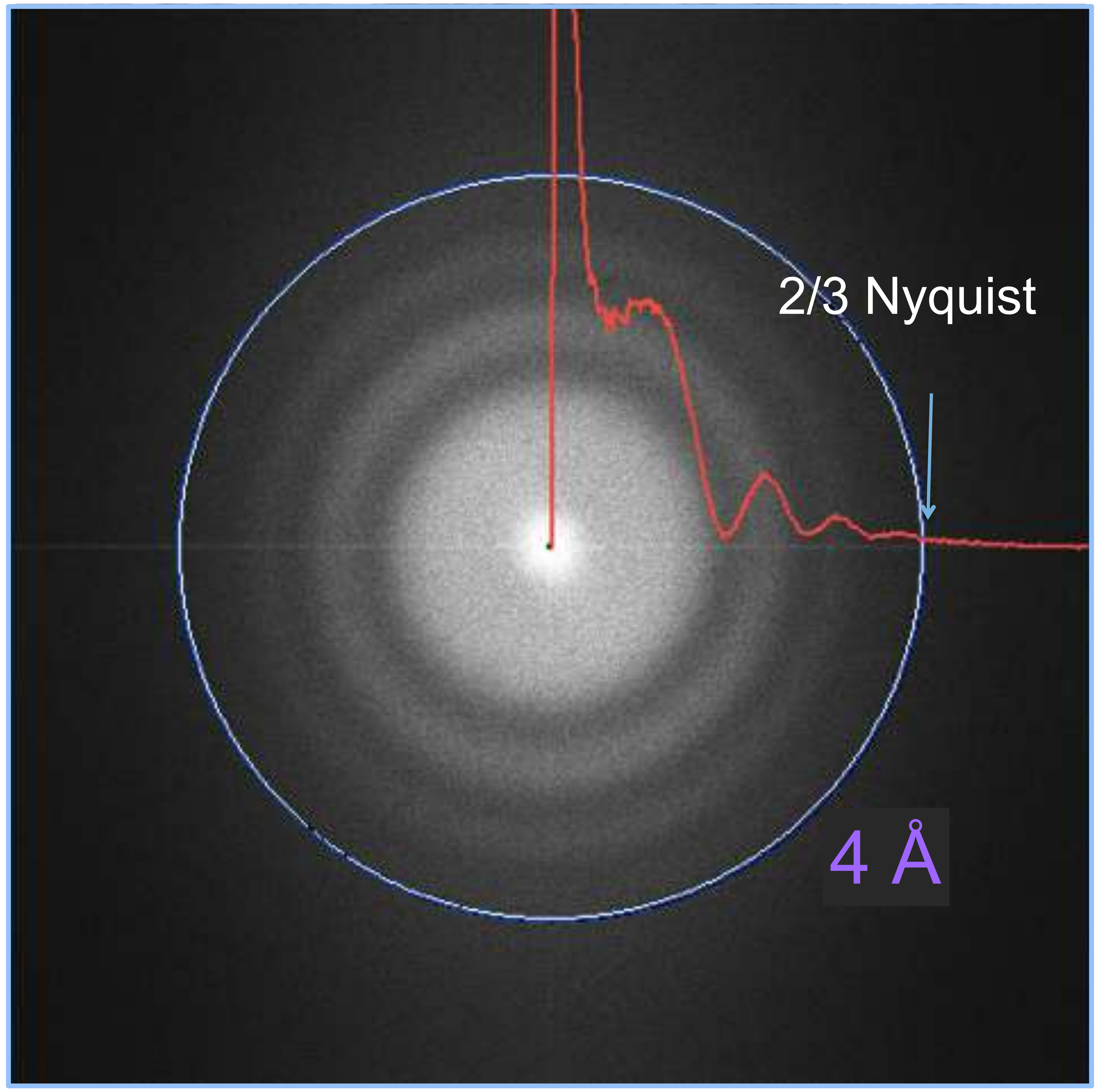
Ruskin, et al JSB

Detectors

Improving the resolution:
Detecting electrons instead of photons



1.37 Å/pixel



1.38 Å/pixel

200KeV ; 20 e-/Å² ; 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.

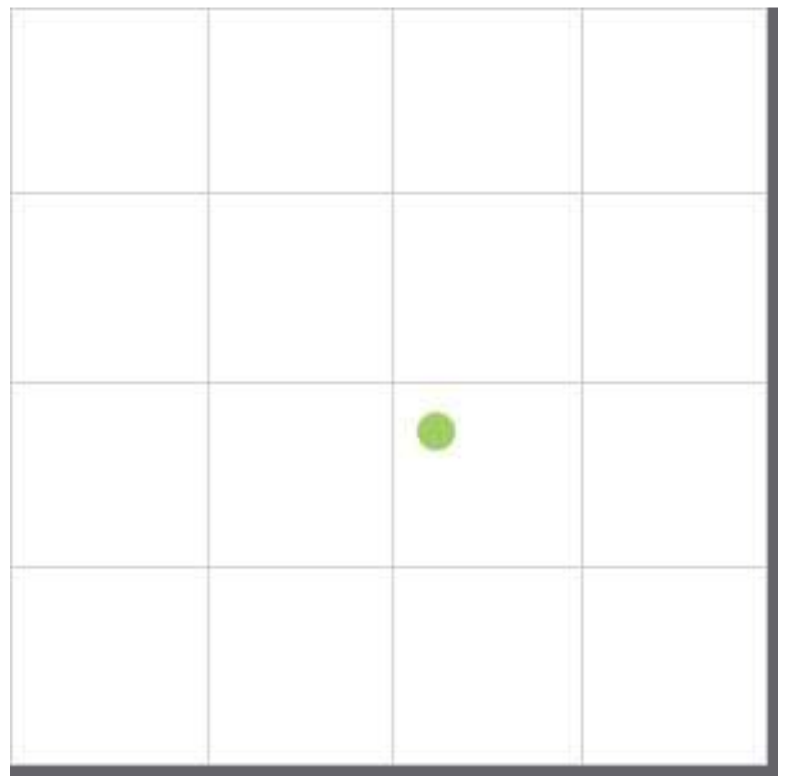
Detectors

Improving the resolution:
Detecting electrons instead of photons

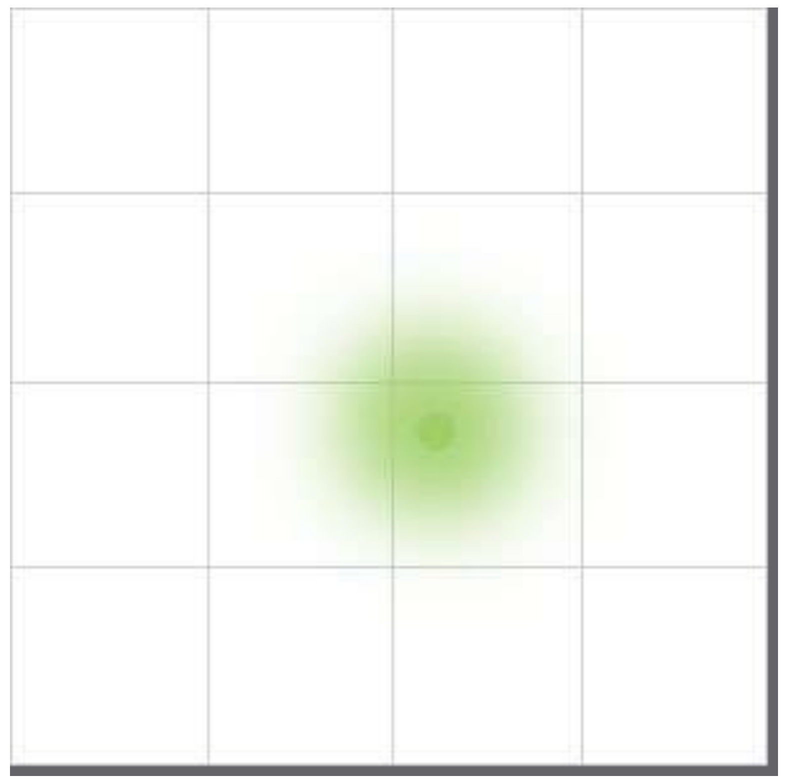
5,760 x 4,096 px

11,520 x 8,184 px

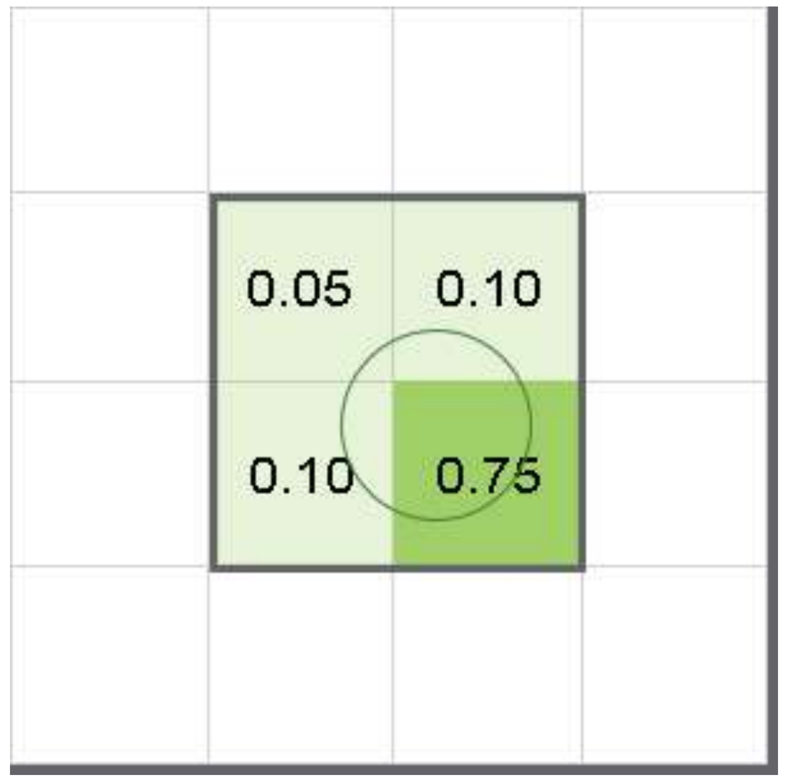
Counting mode



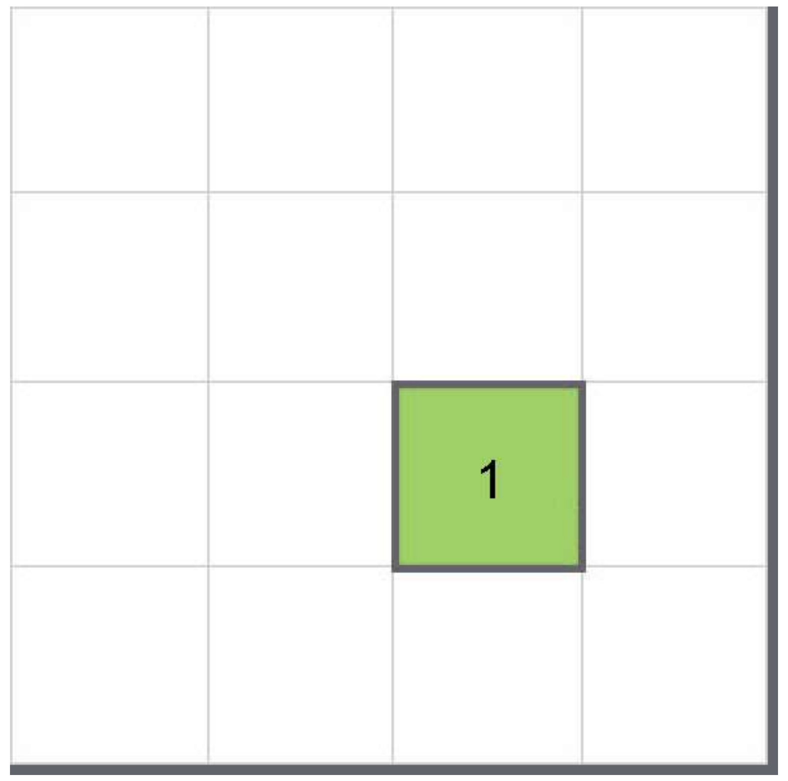
Electron enters detector.



Electron signal is scattered.



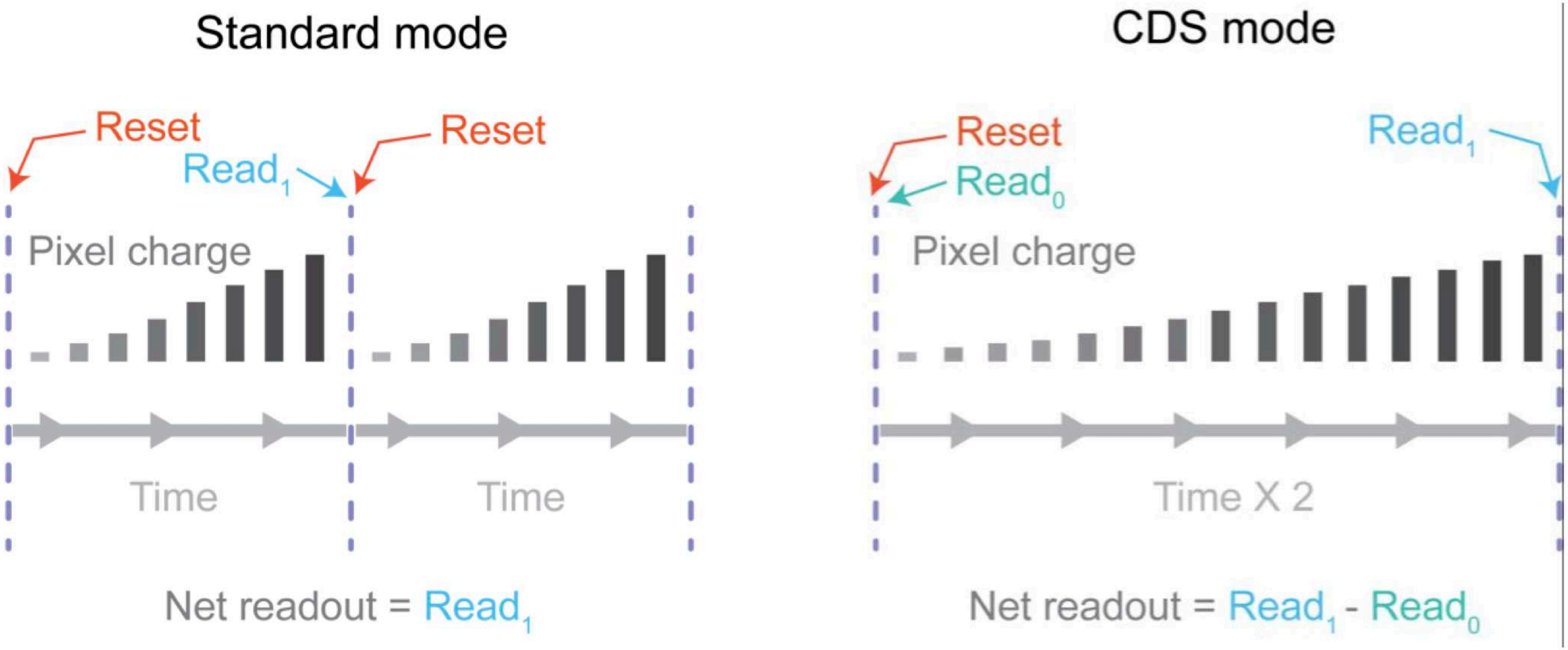
Charge collects in each pixel.



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)

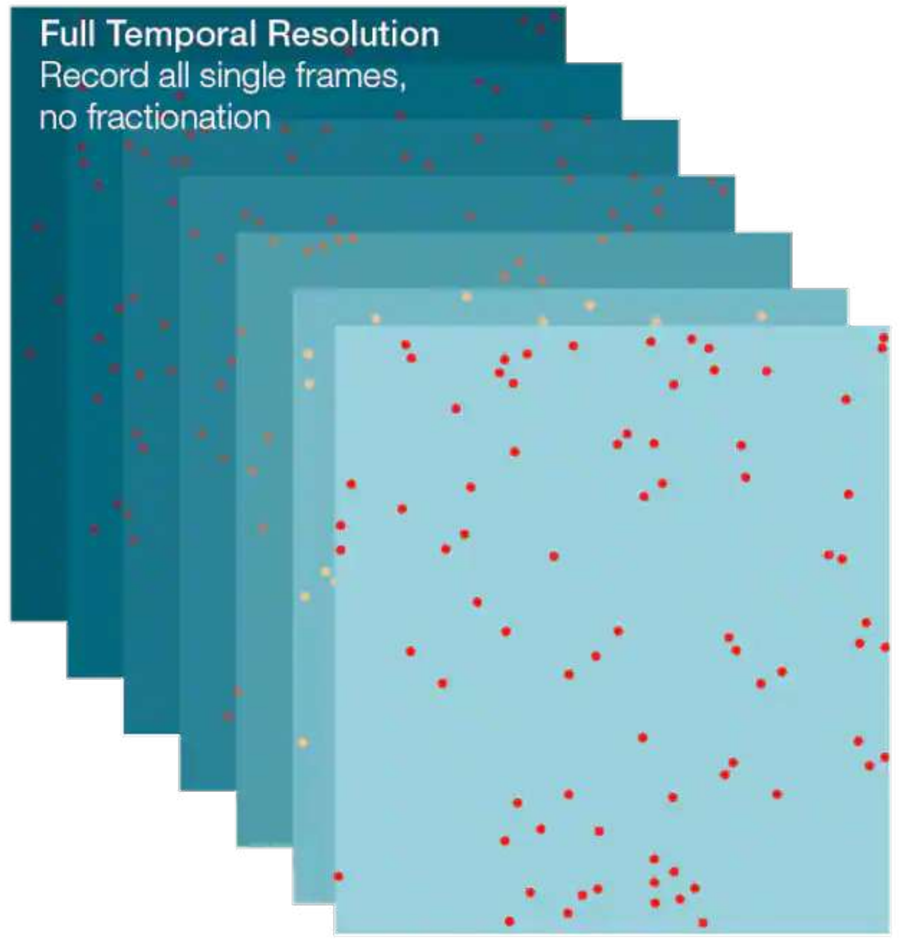


https://www.gatan.com/

Detectors

Improving the resolution:
Detecting electrons instead of photons

Falcon4 specs



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).

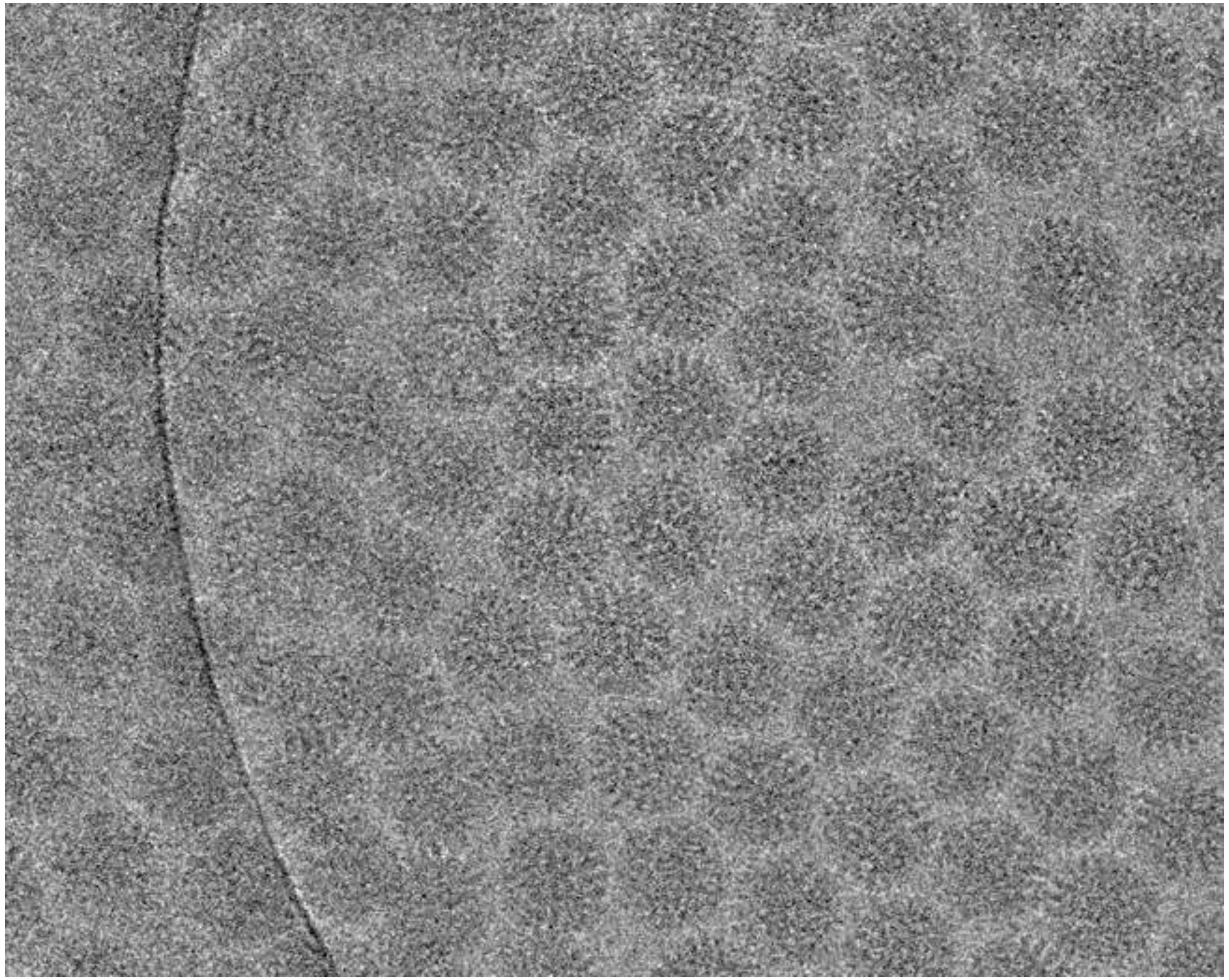
Camera architecture	Direct electron detection	
Sensor size	4,096 × 4,096 pixels, ~ 5.7 x 5.7 cm^2	
Pixel size	14 x 14 μm^2	
TEM Operating voltage	200 kV, 300 kV	
Internal frame rate	320 fps	
Frame rate to storage	320 fps (EER mode)	
Camera Overhead time	0.5 s per acquisition	
File formats	EER (native),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



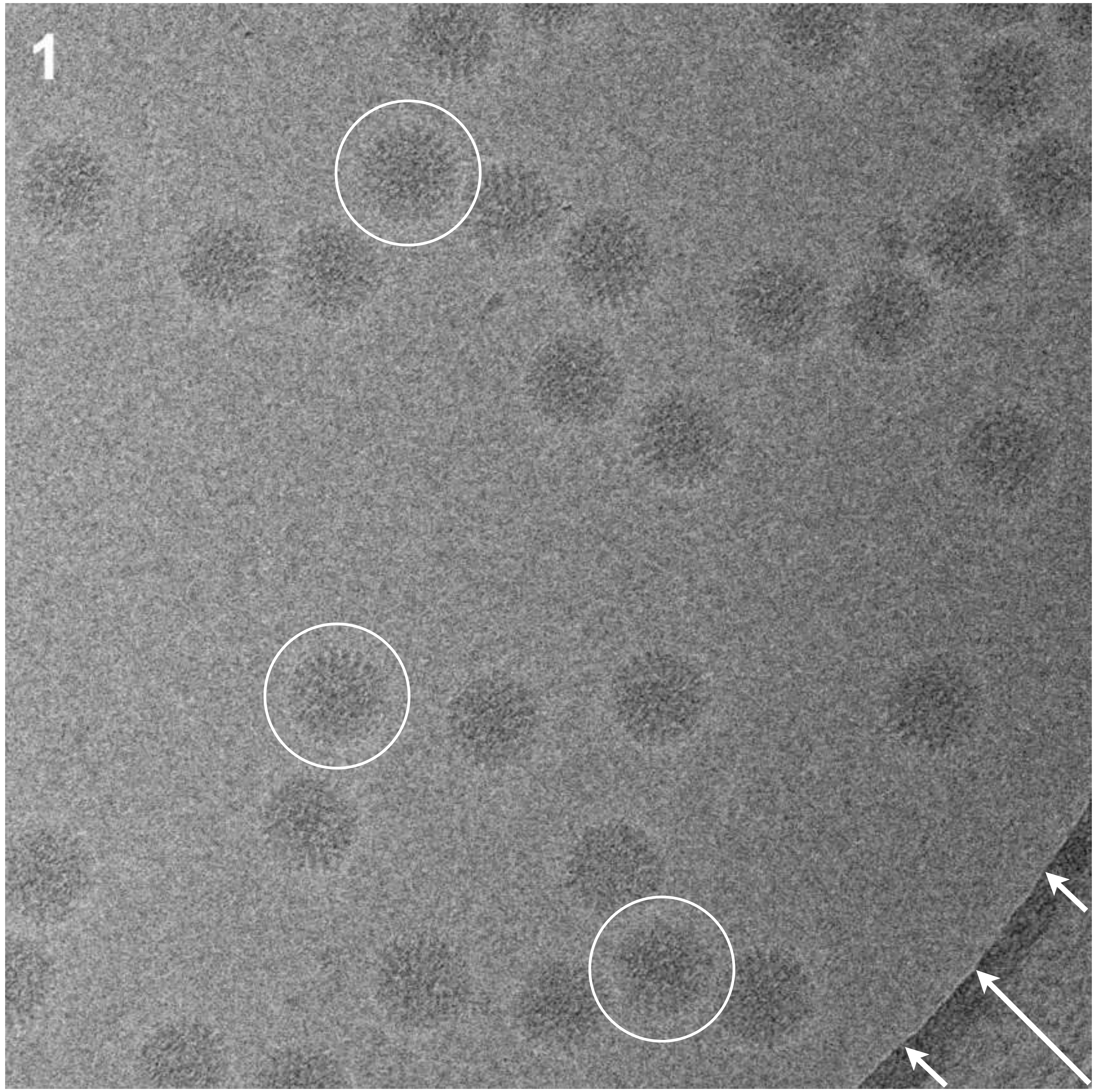
0.5 $e^-/Å^2$/frame

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.

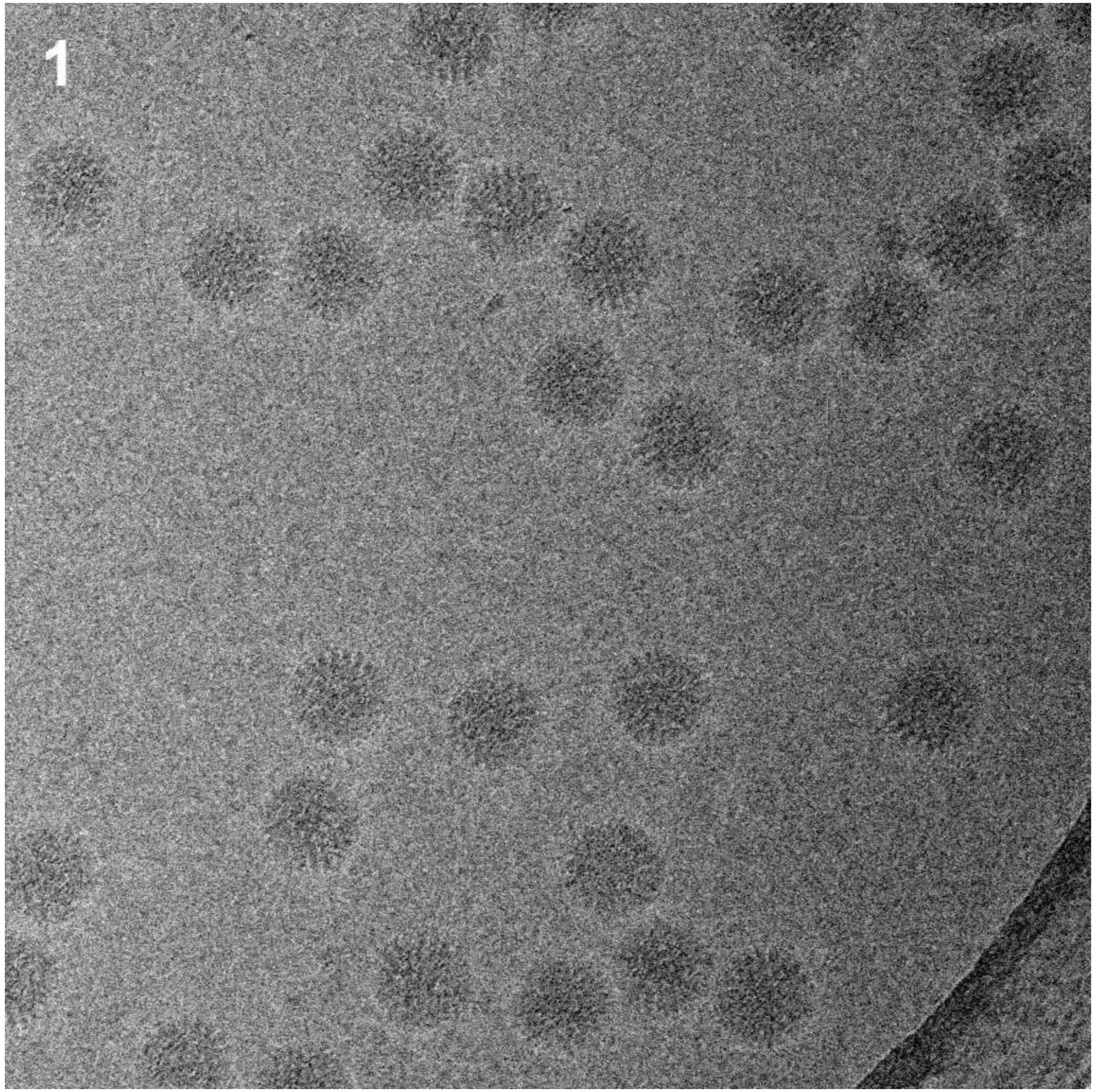
Dose/frame = 5 $e^{-}/Å^2$

A “movie” of rotavirus exposed to electron beam

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

Detectors

Images are movies



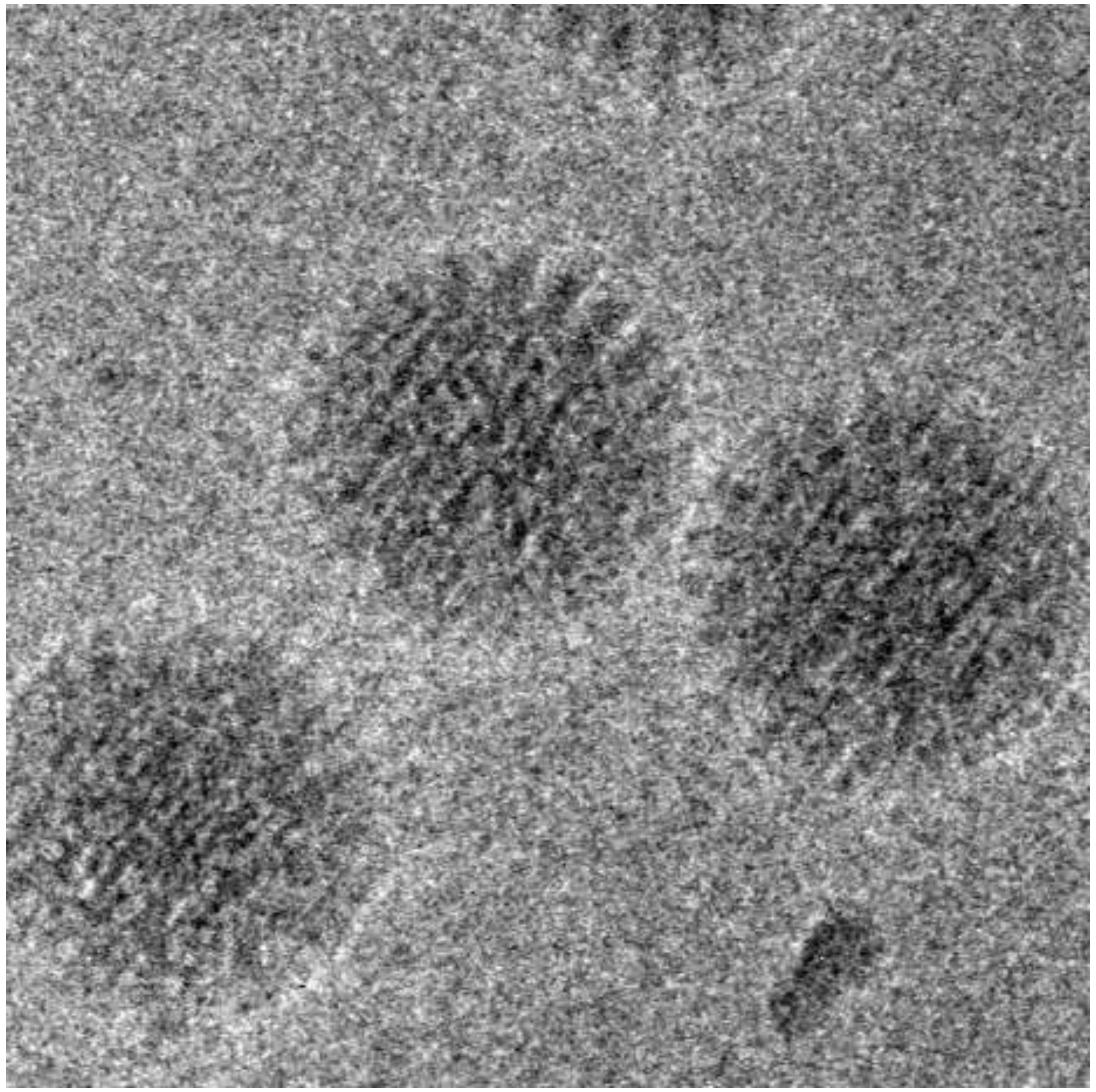
2.5nm

Translations over time

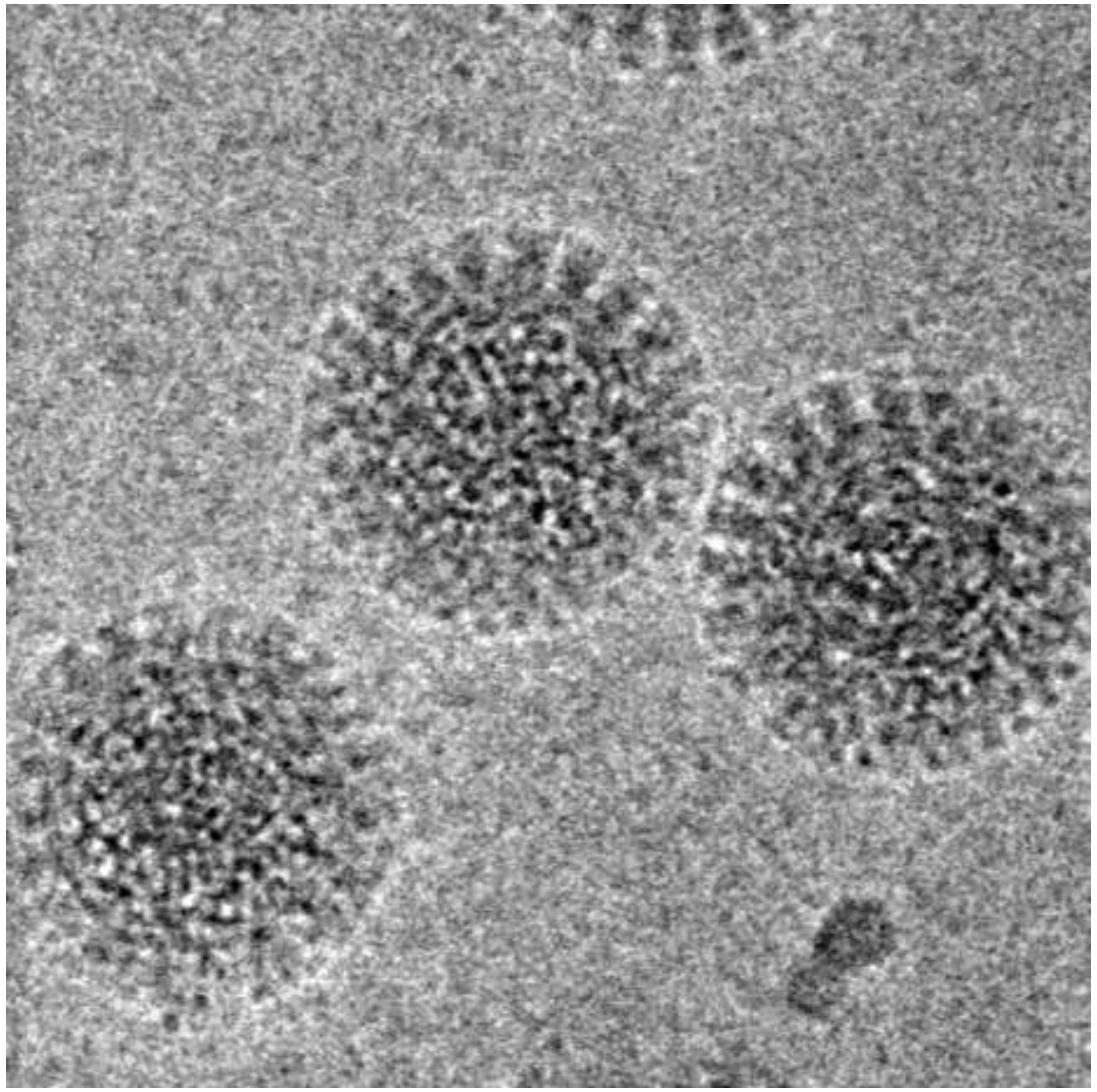
Detectors

Correcting for movement

60-frame average
(no alignment)



60-frame average
(translational alignment)



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

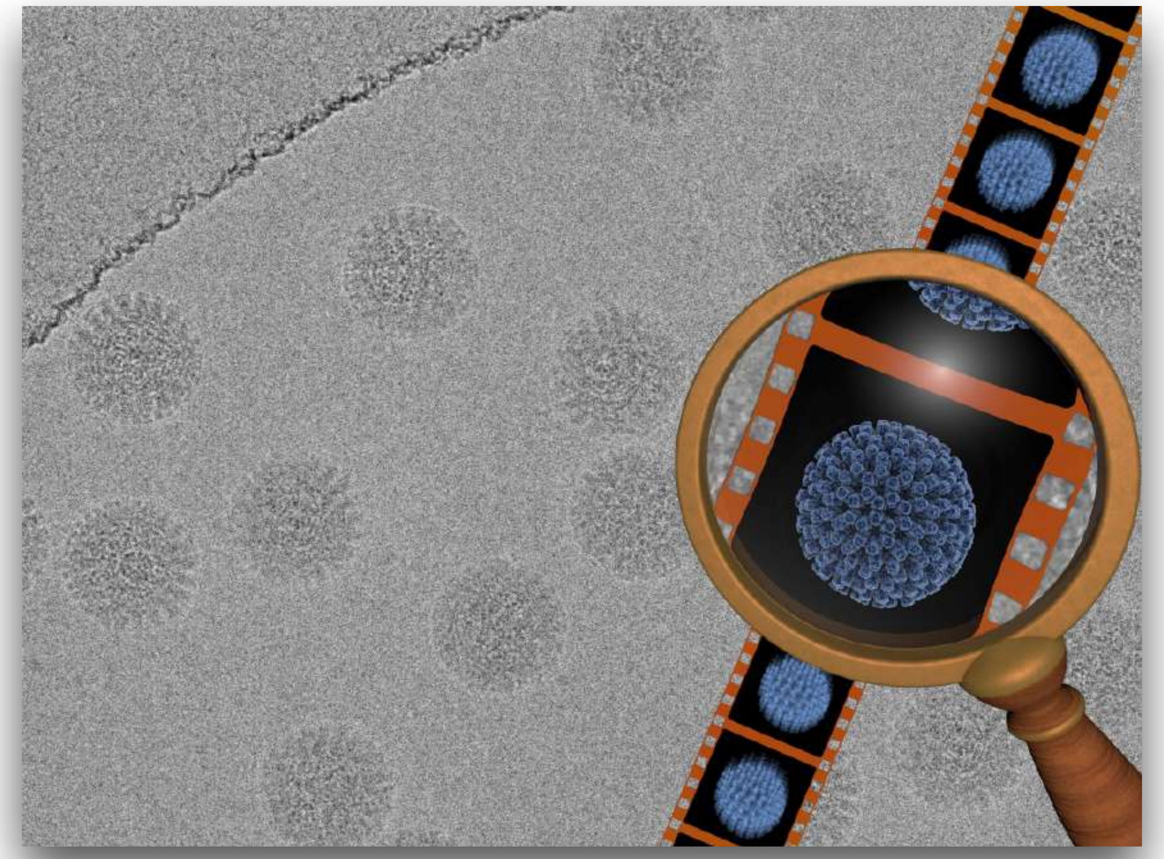
What brought about the resolution revolution?

(~2012-2014)

Hardware

Microscopes

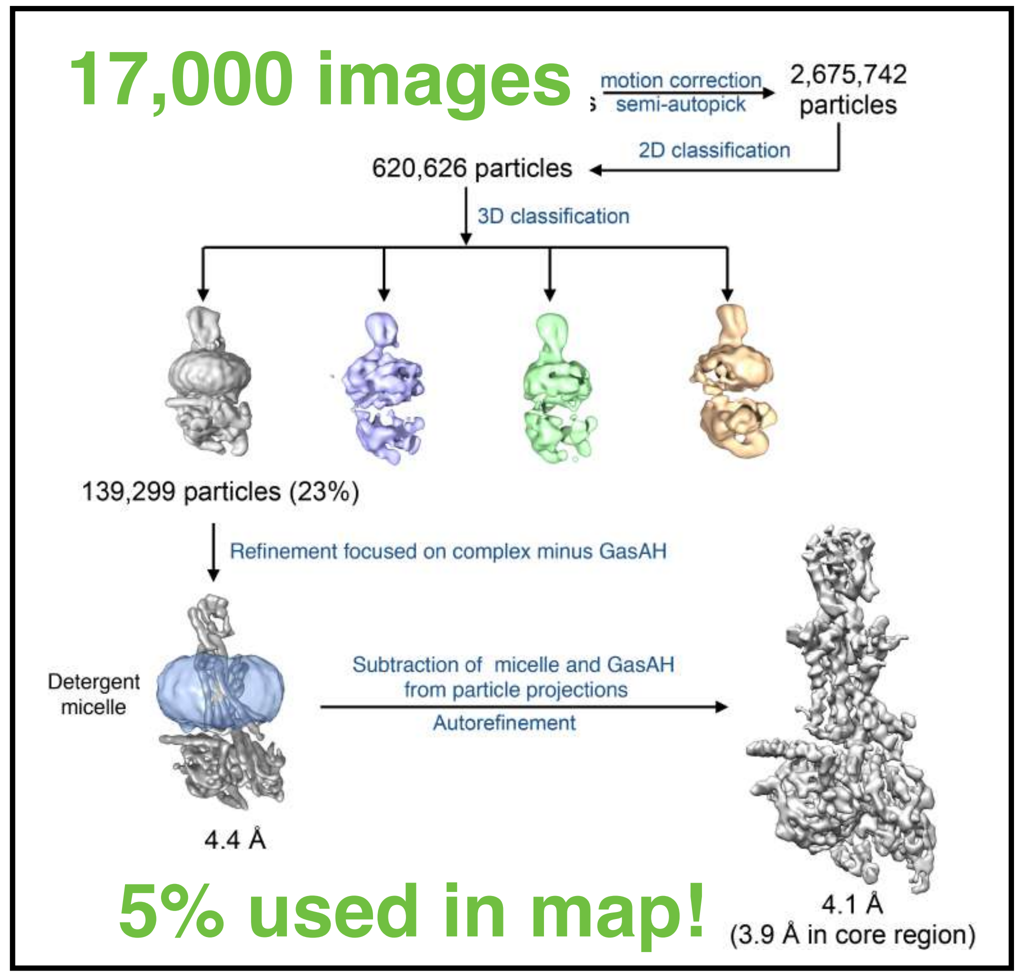
Direct Detectors



Computers

2012->2017
Cost reduced by 100x

Software

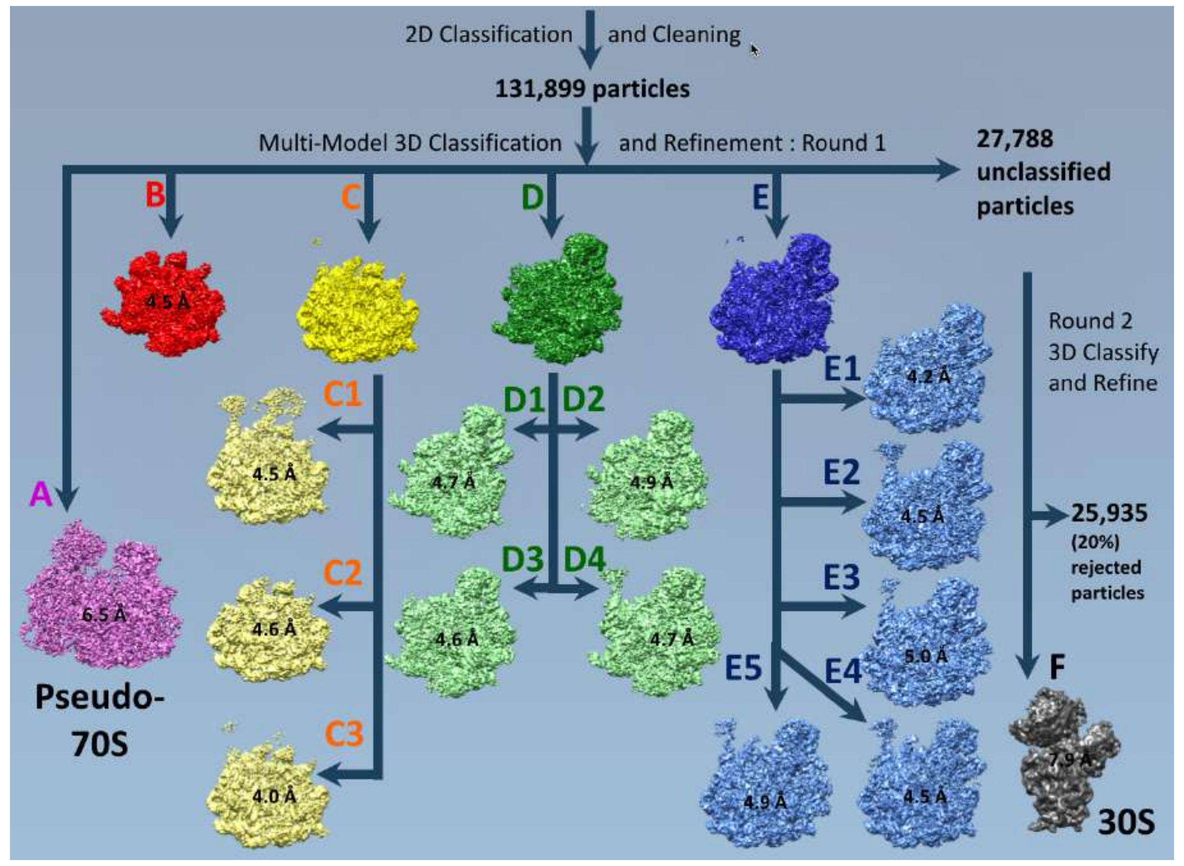


Leginon / SerialEM / EPU, …

MotionCorr2, Unblur, …

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

14 independent structures



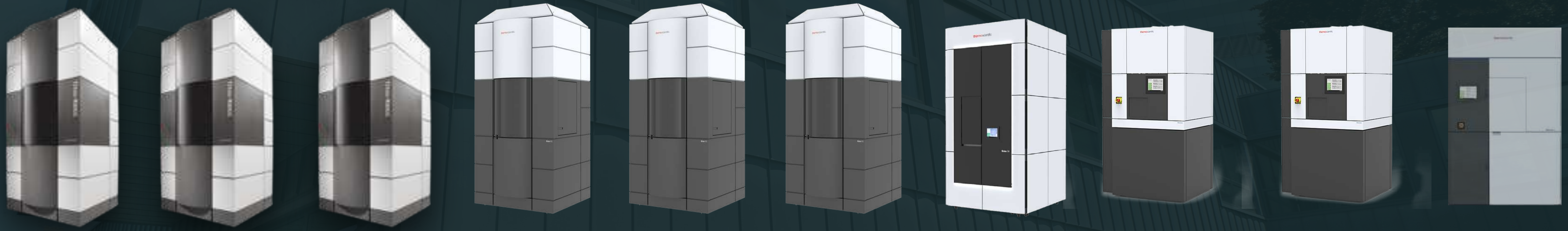
And true "atomic" resolution is possible:

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

SEMC

How are samples prepared for cryoEM?

How are samples prepared for cryoEM?



Krios1 G2

Krios2 G2

Krios3 G2

Krios4 G3i

Krios5 G3i

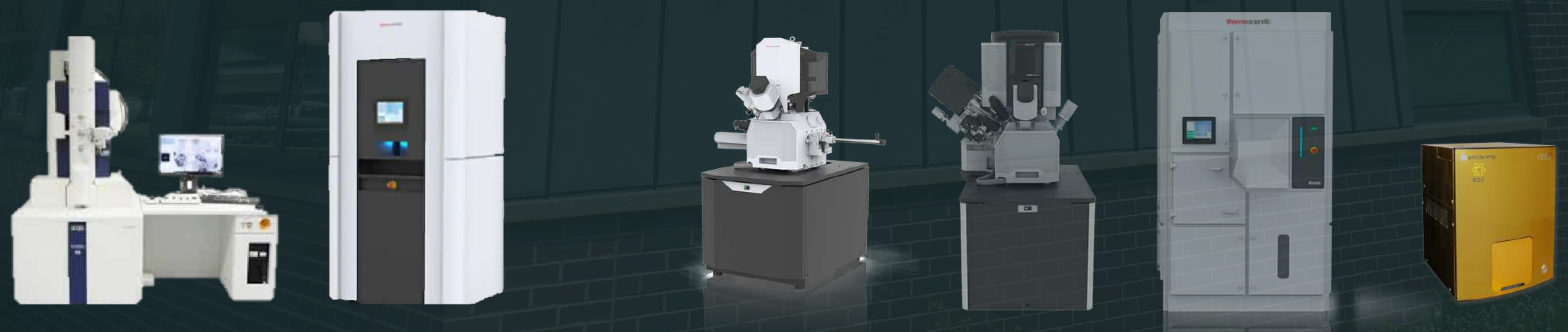
Krios6 G3i

Krios7 G4

Glacios1 G1

Glacios2 G1

Glacios3 G2 *Q1 2024*



Hitachi 7800

Tundra

Aquilos2

Helios5 Hydra 2024

Arctis 2024

CUBEII

Support: NIH NIGMS. Common Fund; NYSTAR

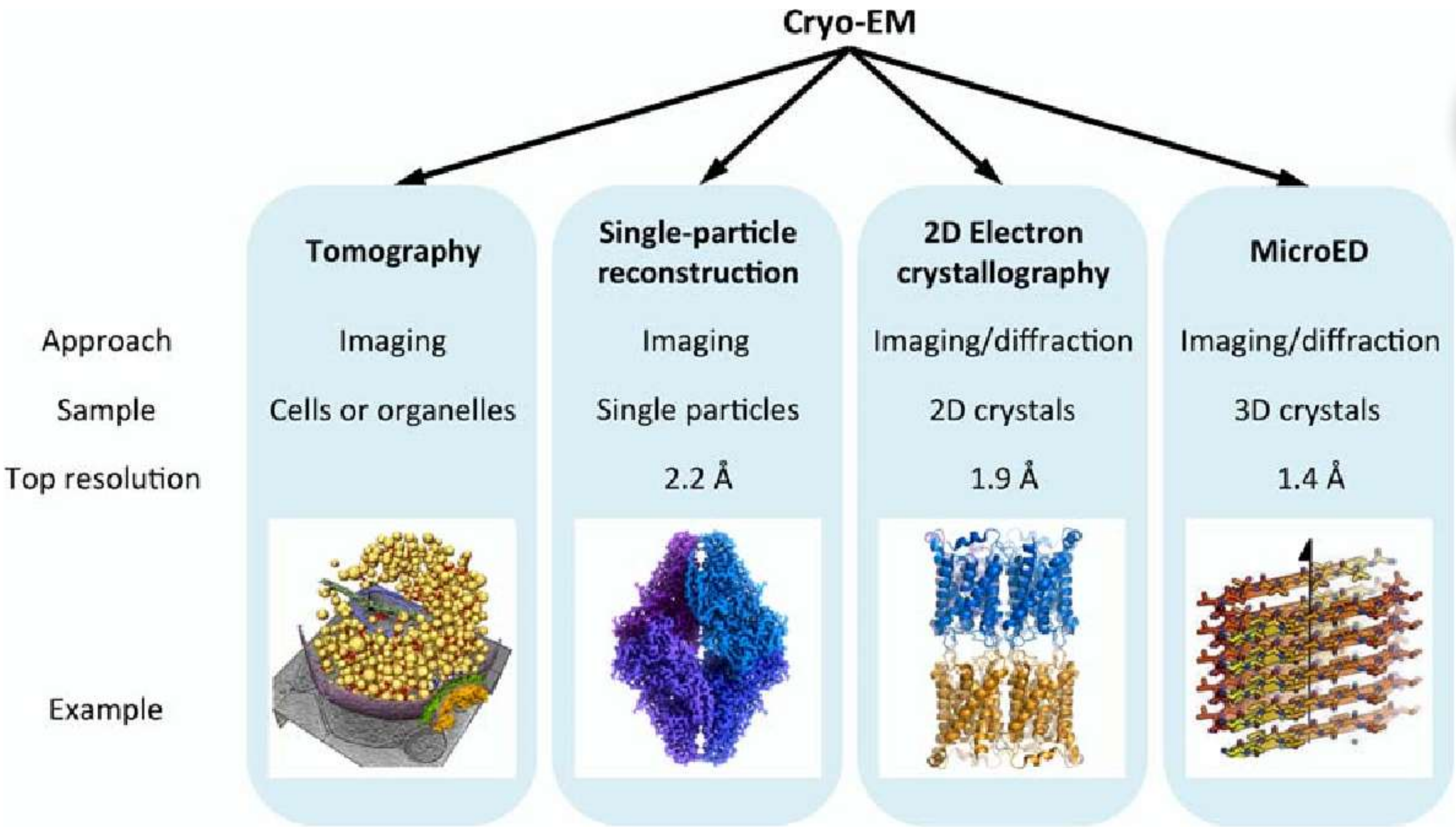
SIMONS FOUNDATION

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?

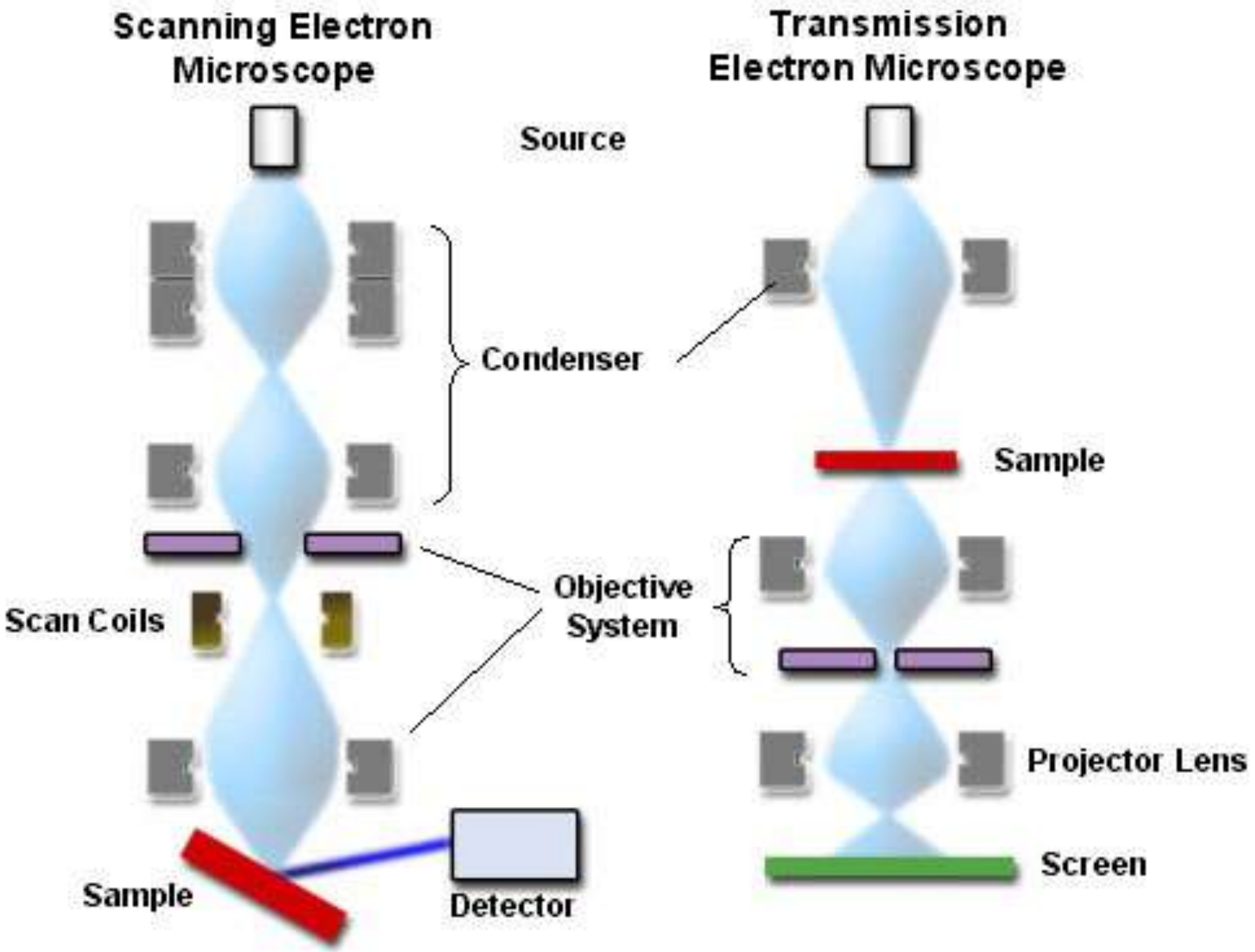
Cryoem modalities and tools



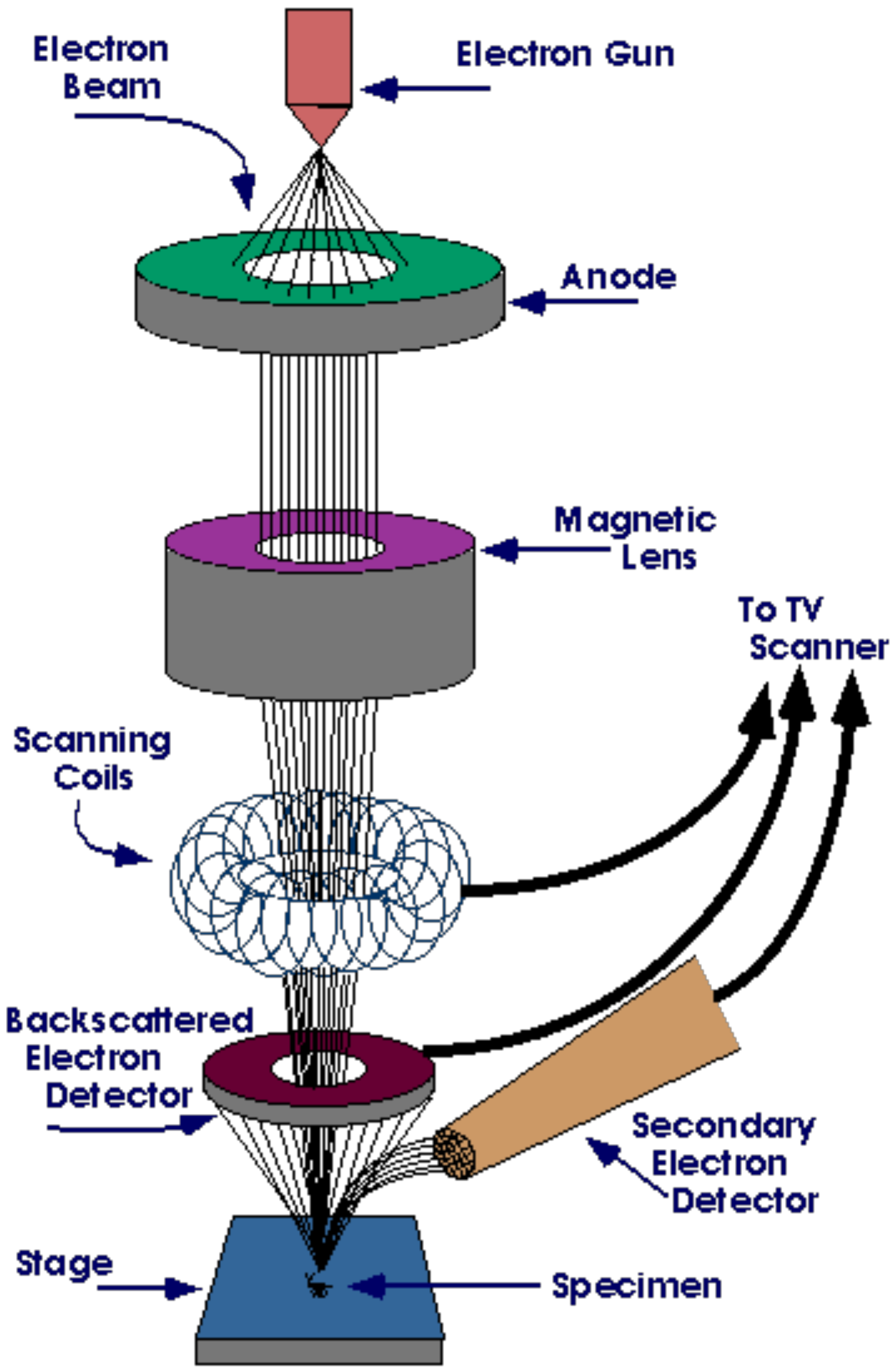
https://doi.org/10.1002/pro.2989

~3.4Å ~1.2Å ~1.9Å ~0.7Å

Anatomy of an SEM



Anatomy of an SEM



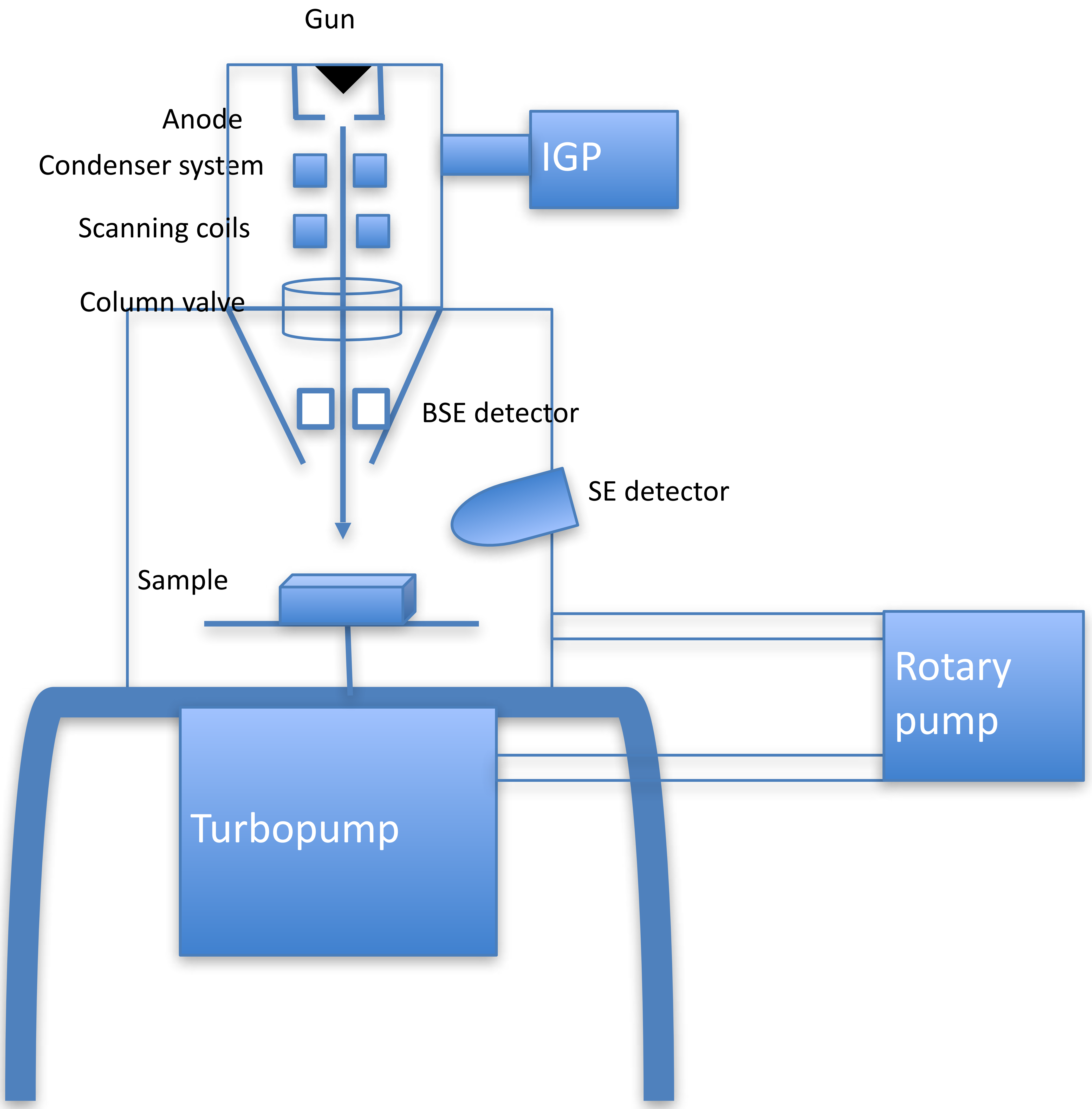
Electron gun: range from tungsten filaments in lower vacuum SEMs to FEGs which need modern high vacuum SEMs

Beam energy: 0.2 – 40 keV is focused by a condenser lens system into a spot of 0.4 – 5 nm

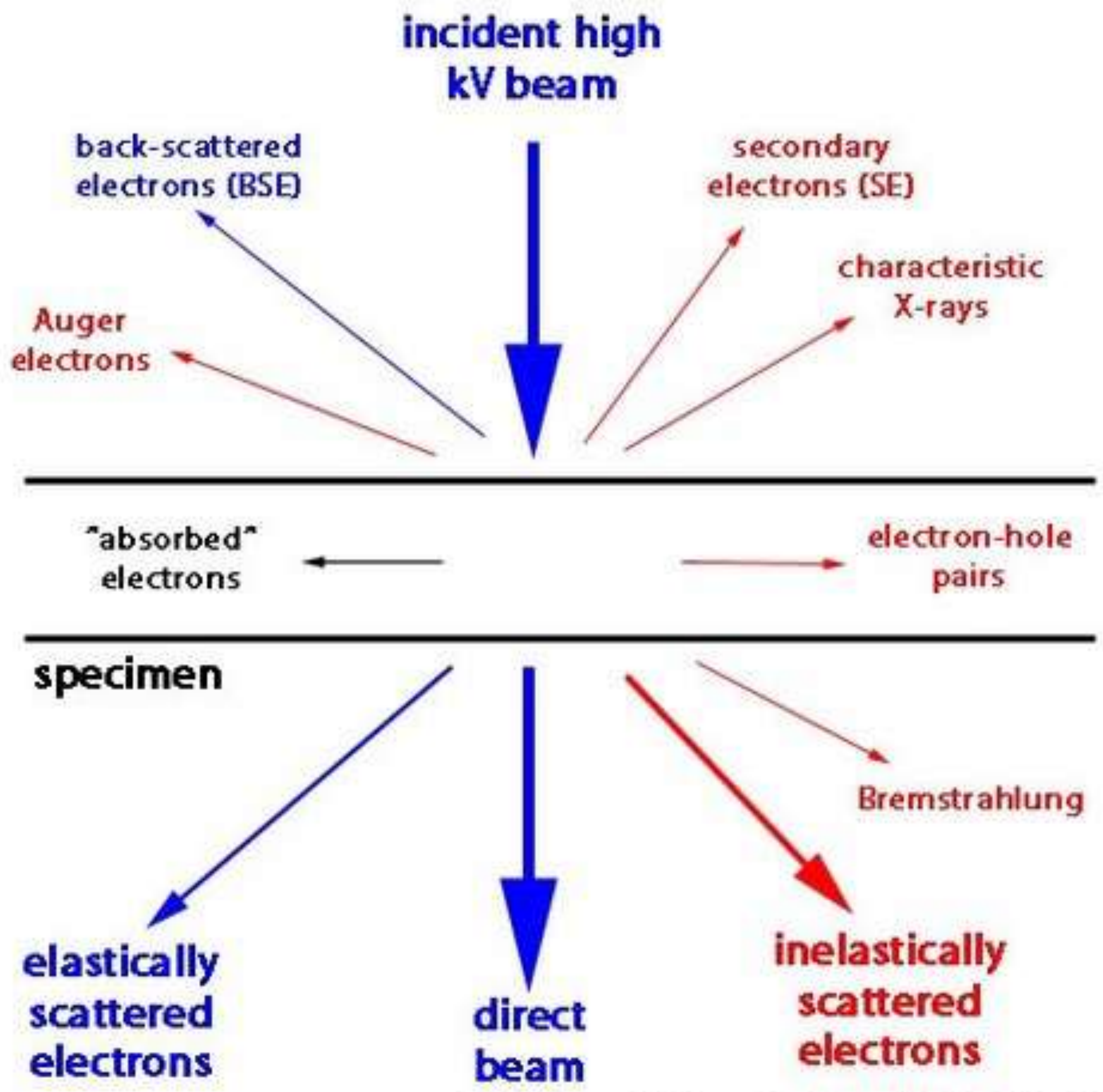
Beam is deflected by very fast scanning coils and rasters the sample surface

Typical resolution of SEM is between 1 and 20 nm where the record is 0.4 nm

Anatomy of an SEM - Vacuum systems

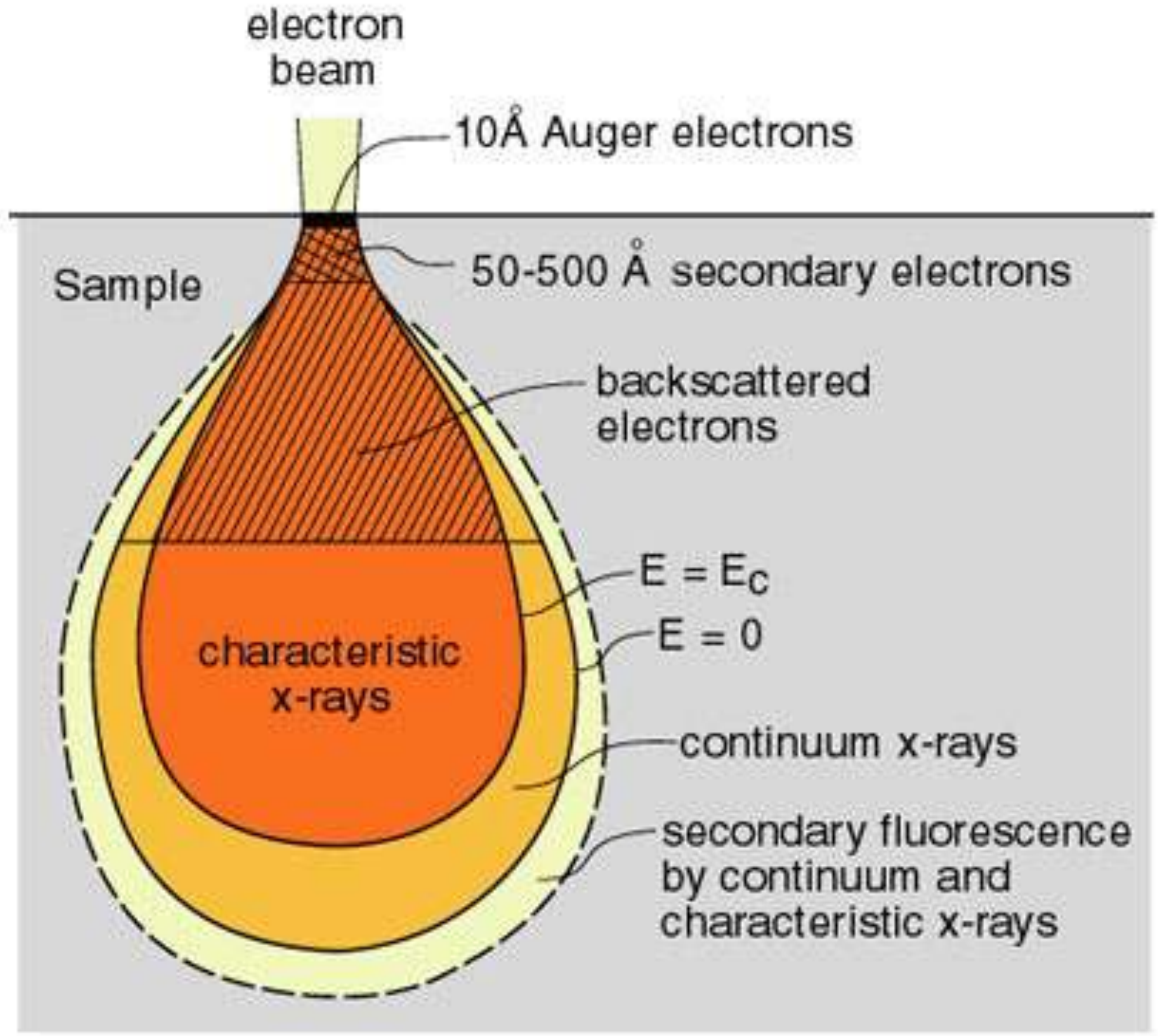


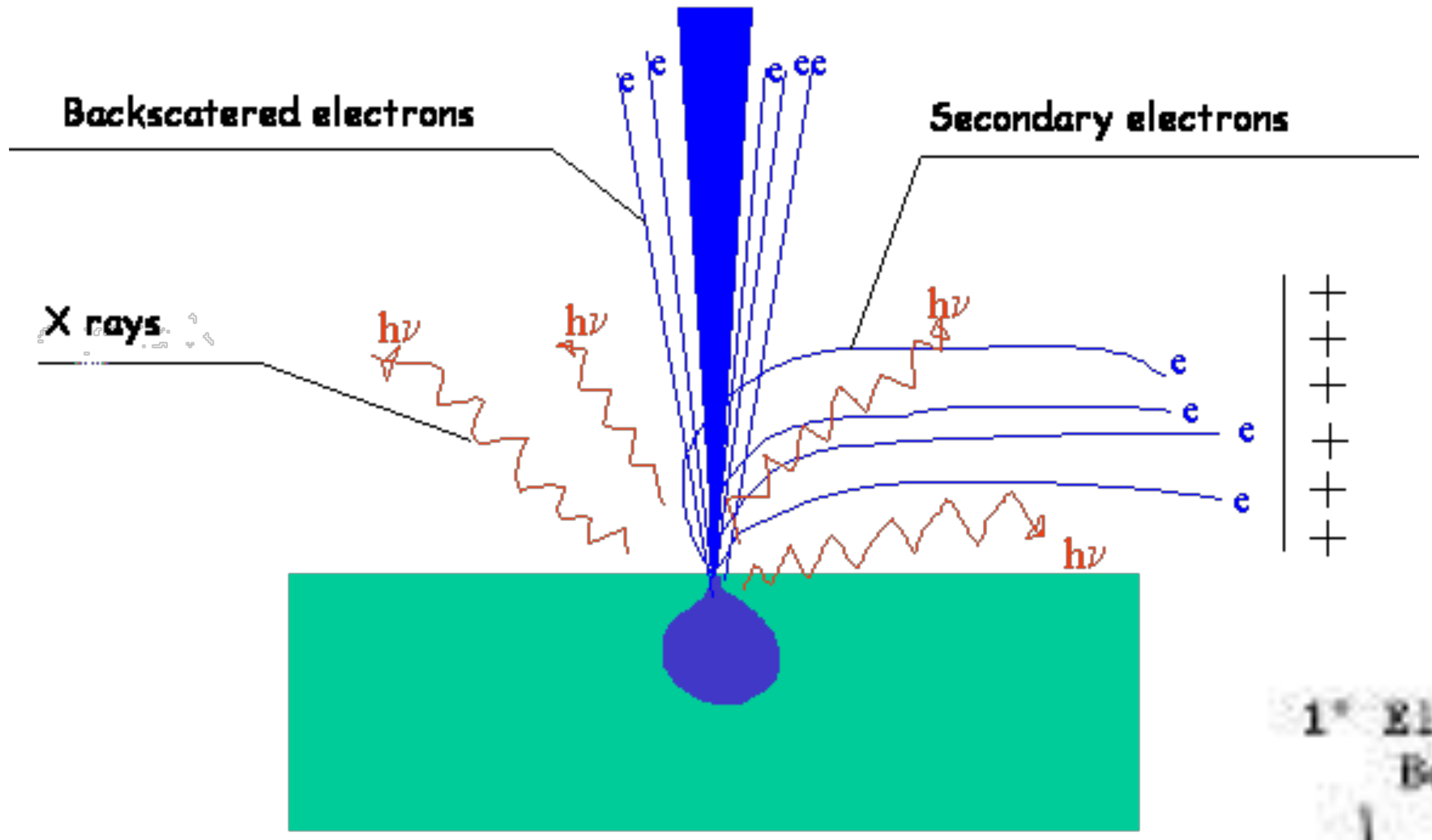
Anatomy of an SEM - e- beam interactions

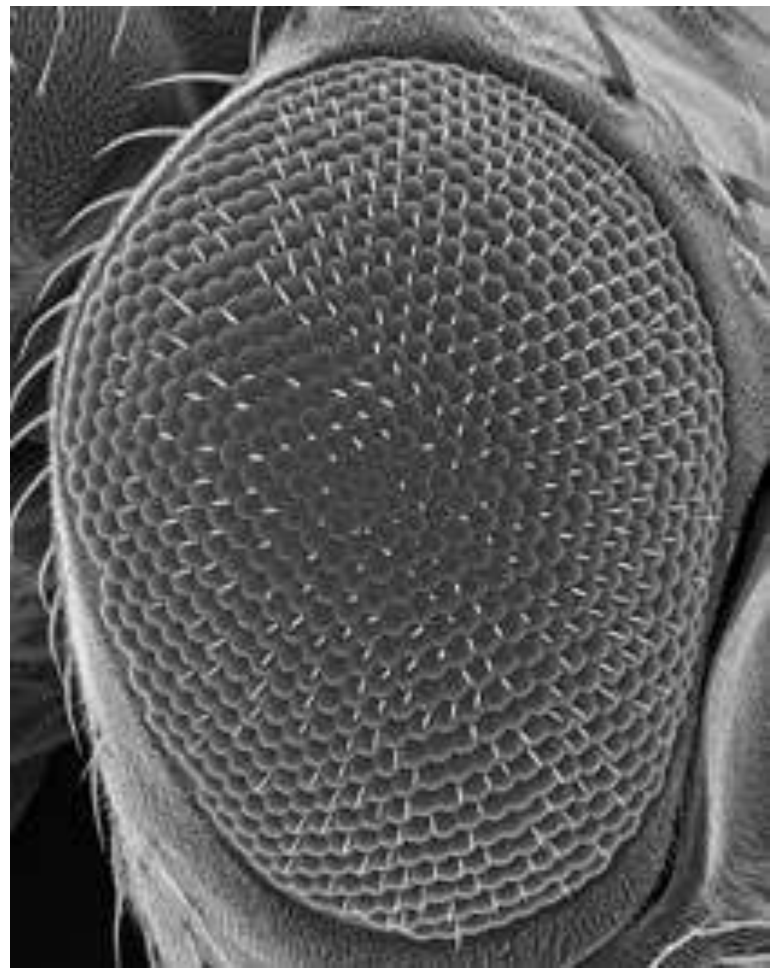


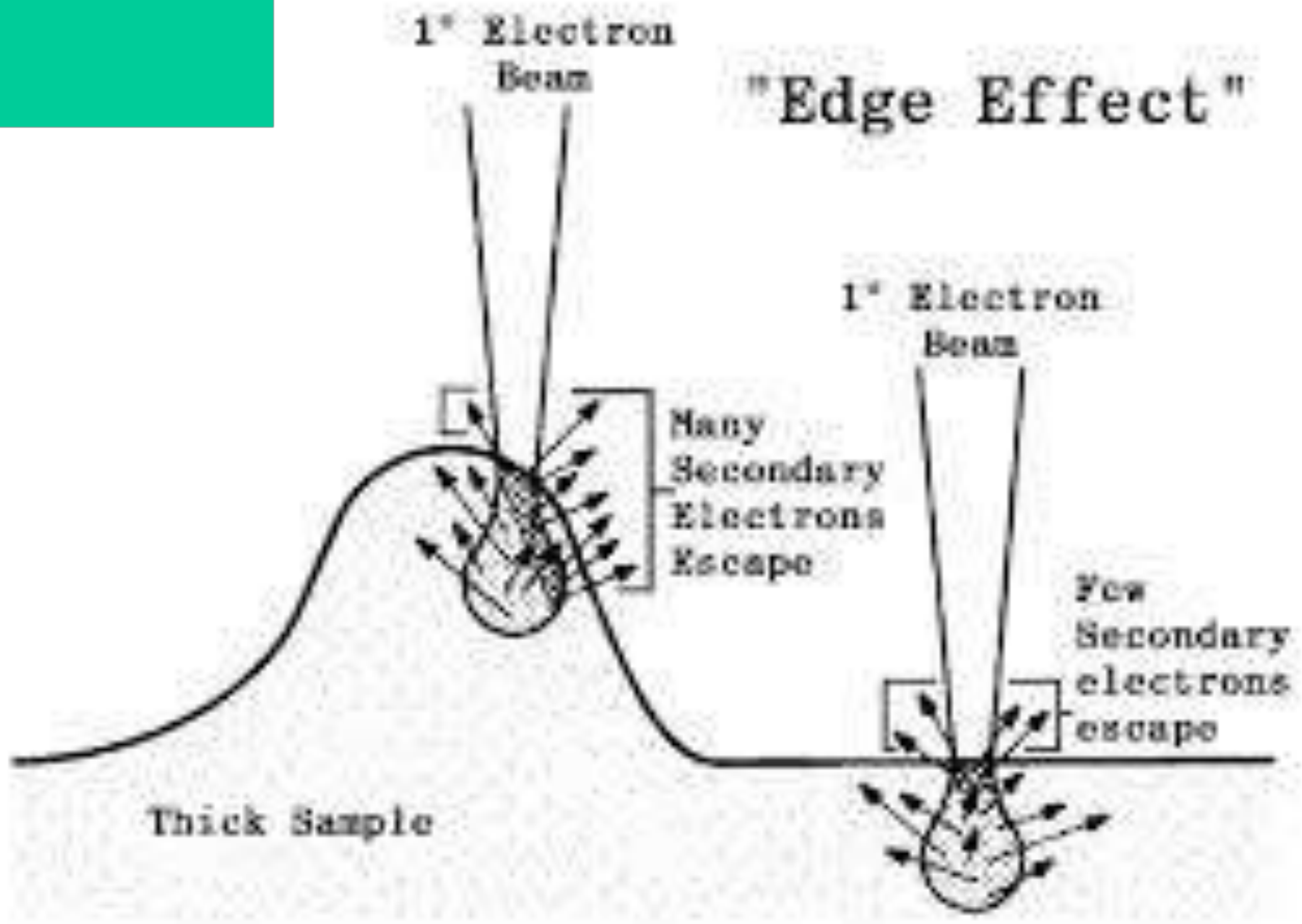
modified from Williams & Carter (1996) Fig. 1.3

Anatomy of an SEM - e- beam interactions

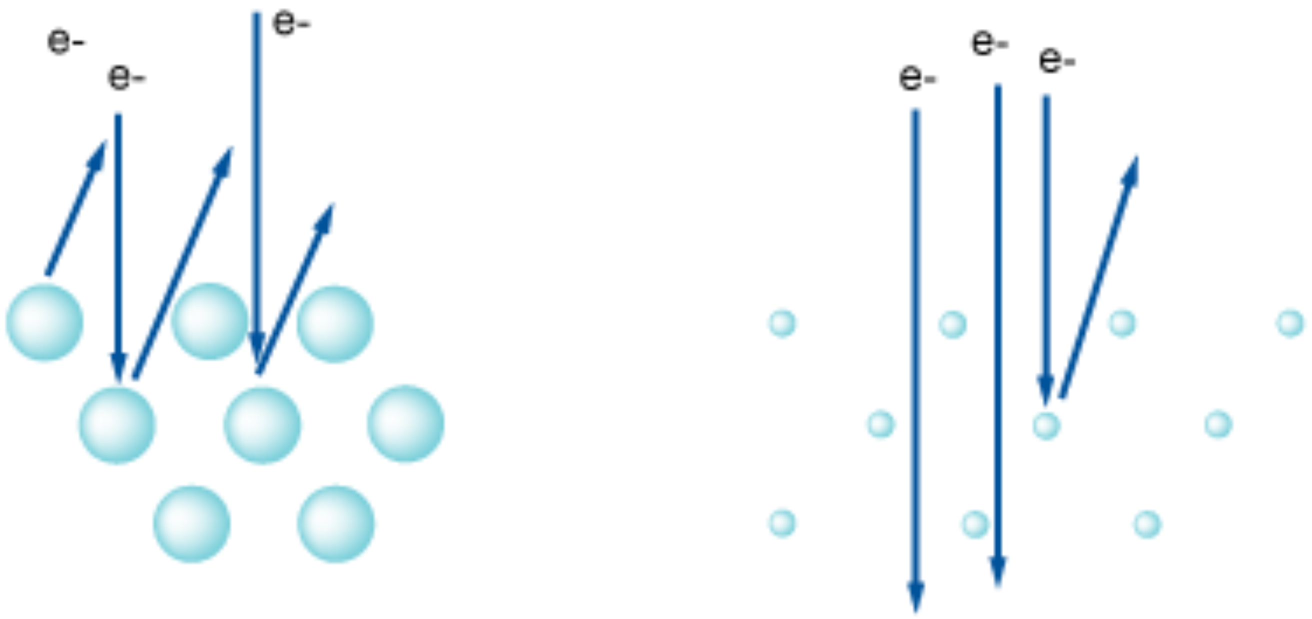








Anatomy of an SEM - e- beam interactions



Titanium
atomic number 22

Silicon
atomic number 14