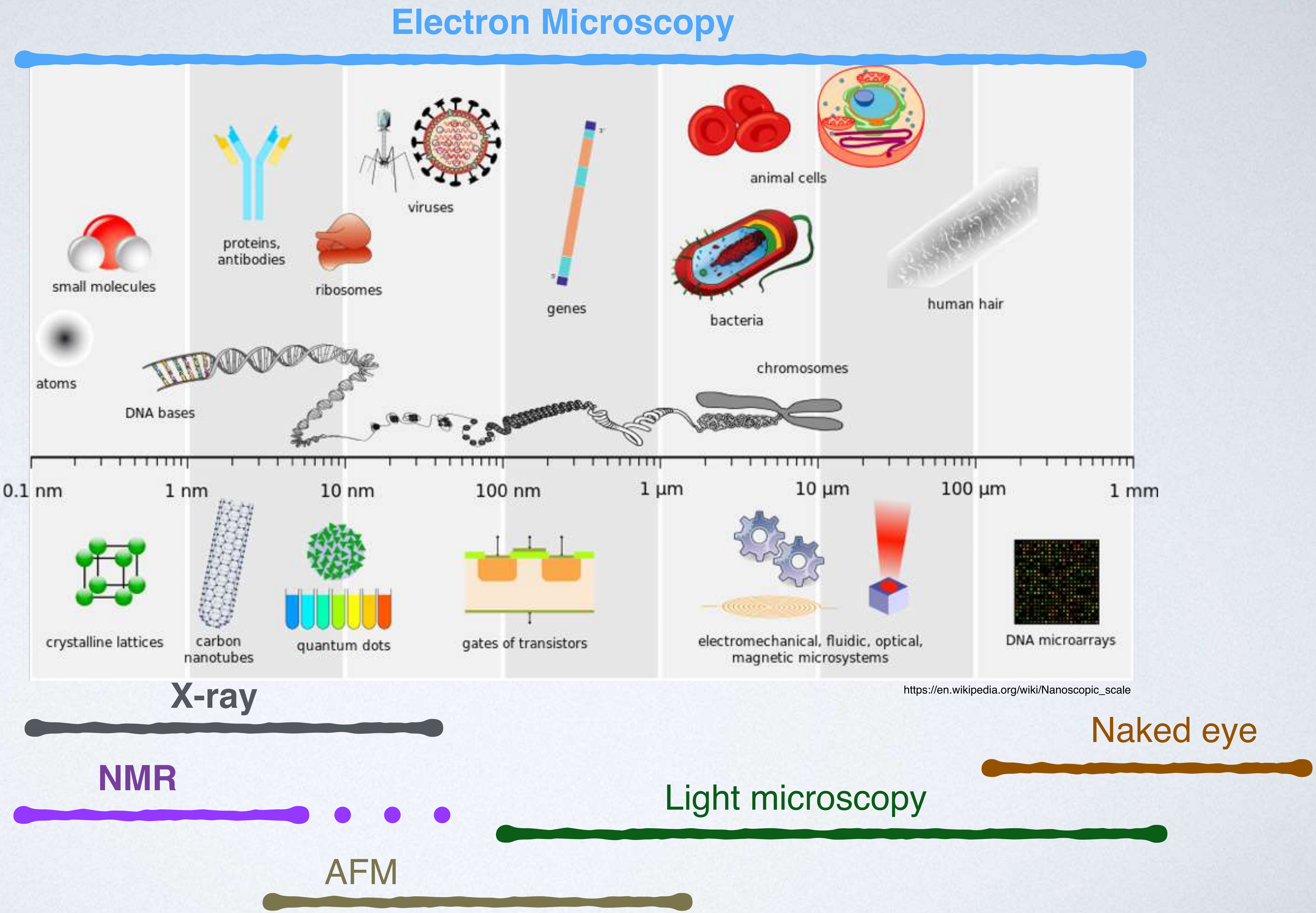
2023 Winter-Spring EM Course

anatomy of an EM

JANUARY 12, 2023

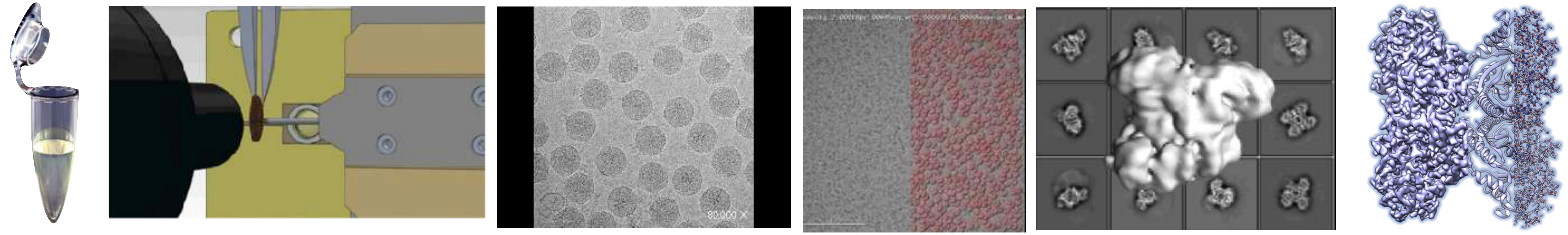
EDWARD T ENG

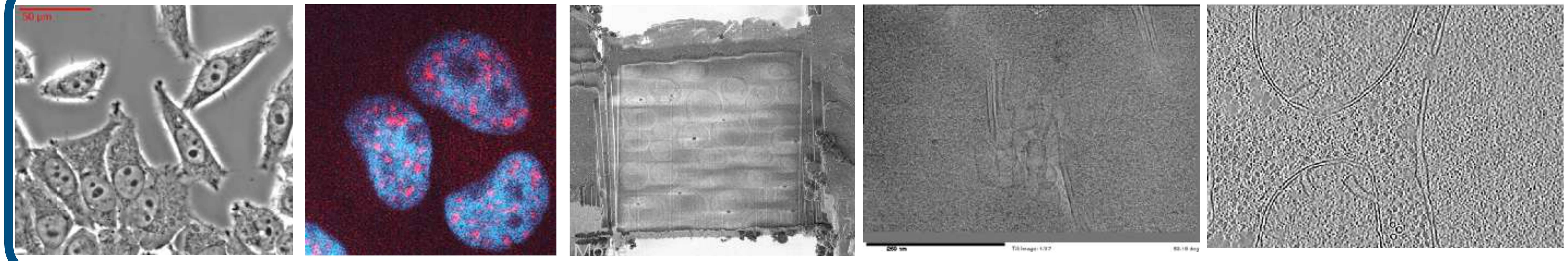
CRYOEM: SCALE WITHIN BIOLOGY



CRYOEM: TECHNOLOGY ON THE RISE

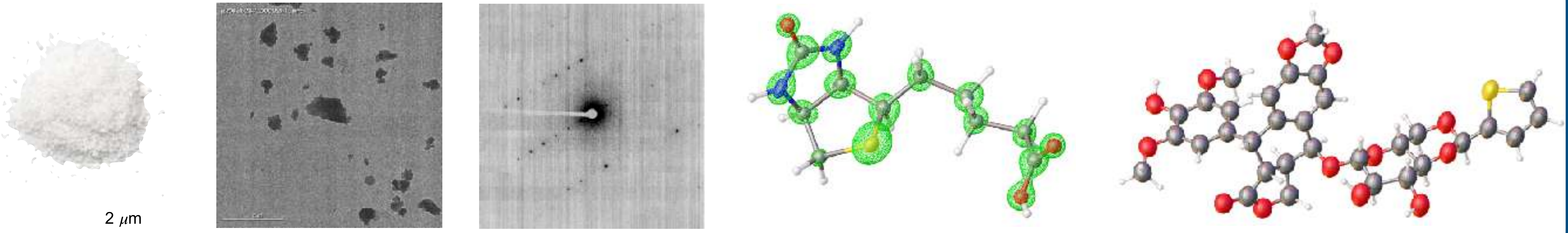
Single particle cryoEM



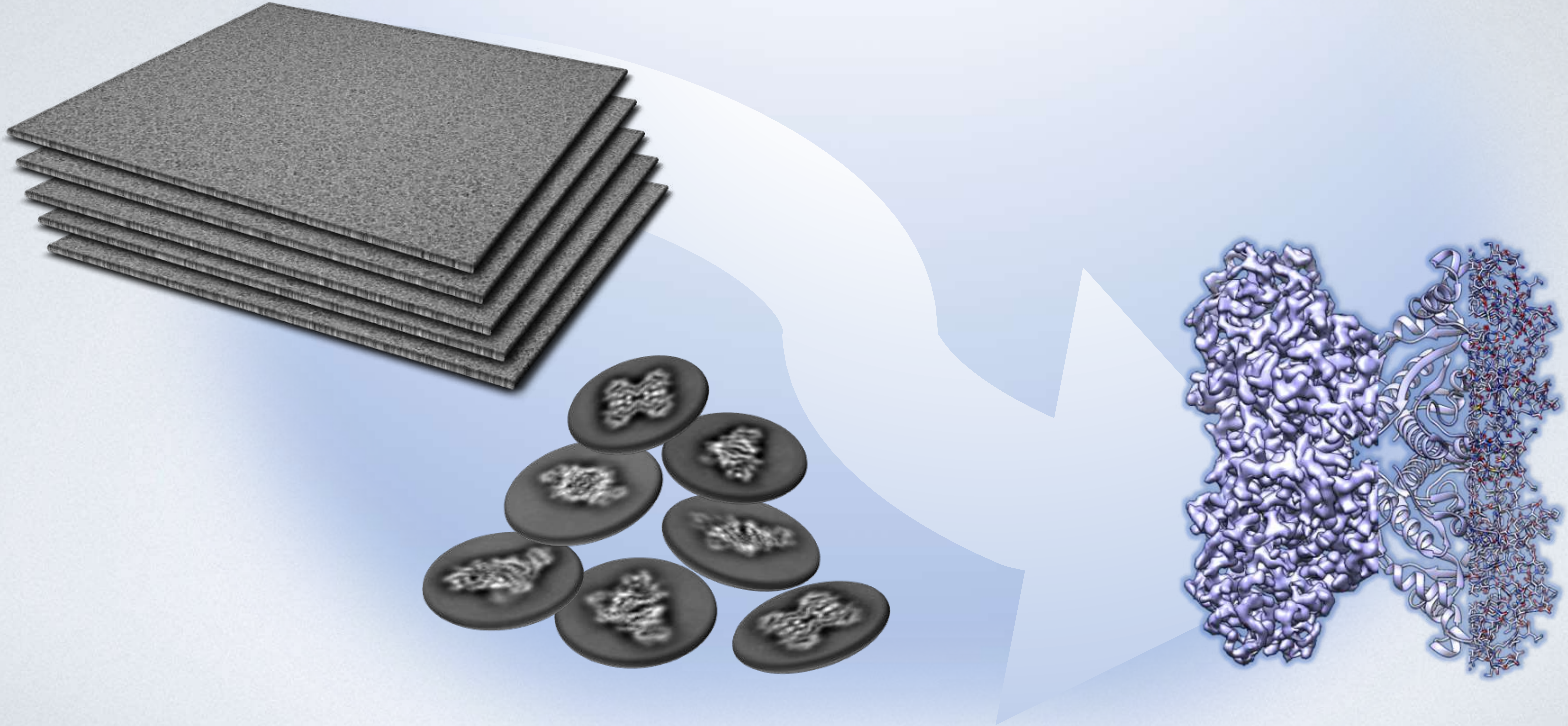


Cryo Electron Tomography (cryoET)

Micro crystal electron diffraction (microED)



WHAT IS POSSIBLE TODAY?



WHY ELECTRONS

CRYOEM: WHY ELECTRONS?

Pros

Small wavelength

Can be focused

Cons

Damages sample

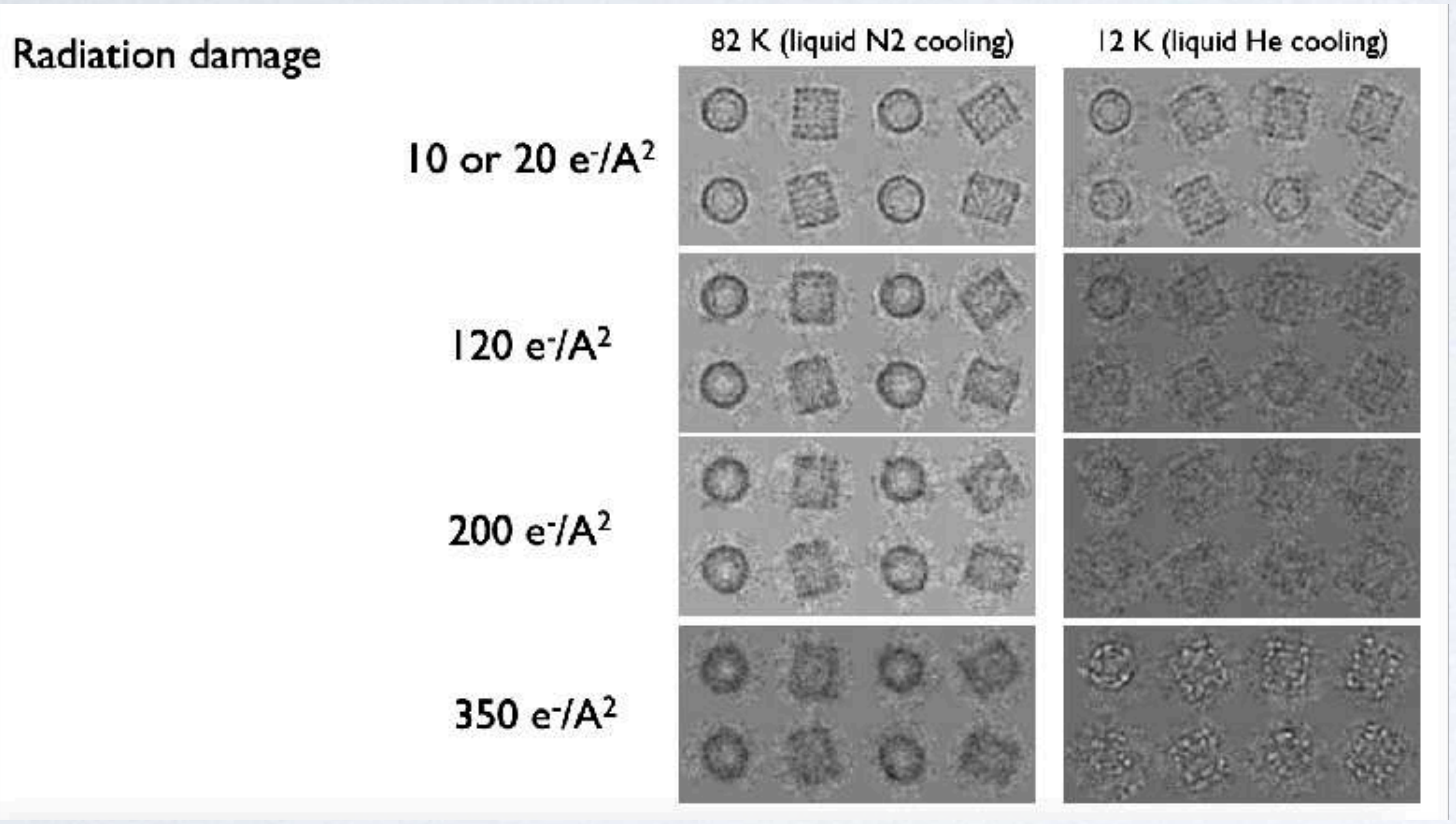
worse with faster electrons

Poor penetration

better with faster electrons

CRYOEM: WHY ELECTRONS?

Ideal dose for cryoEM?



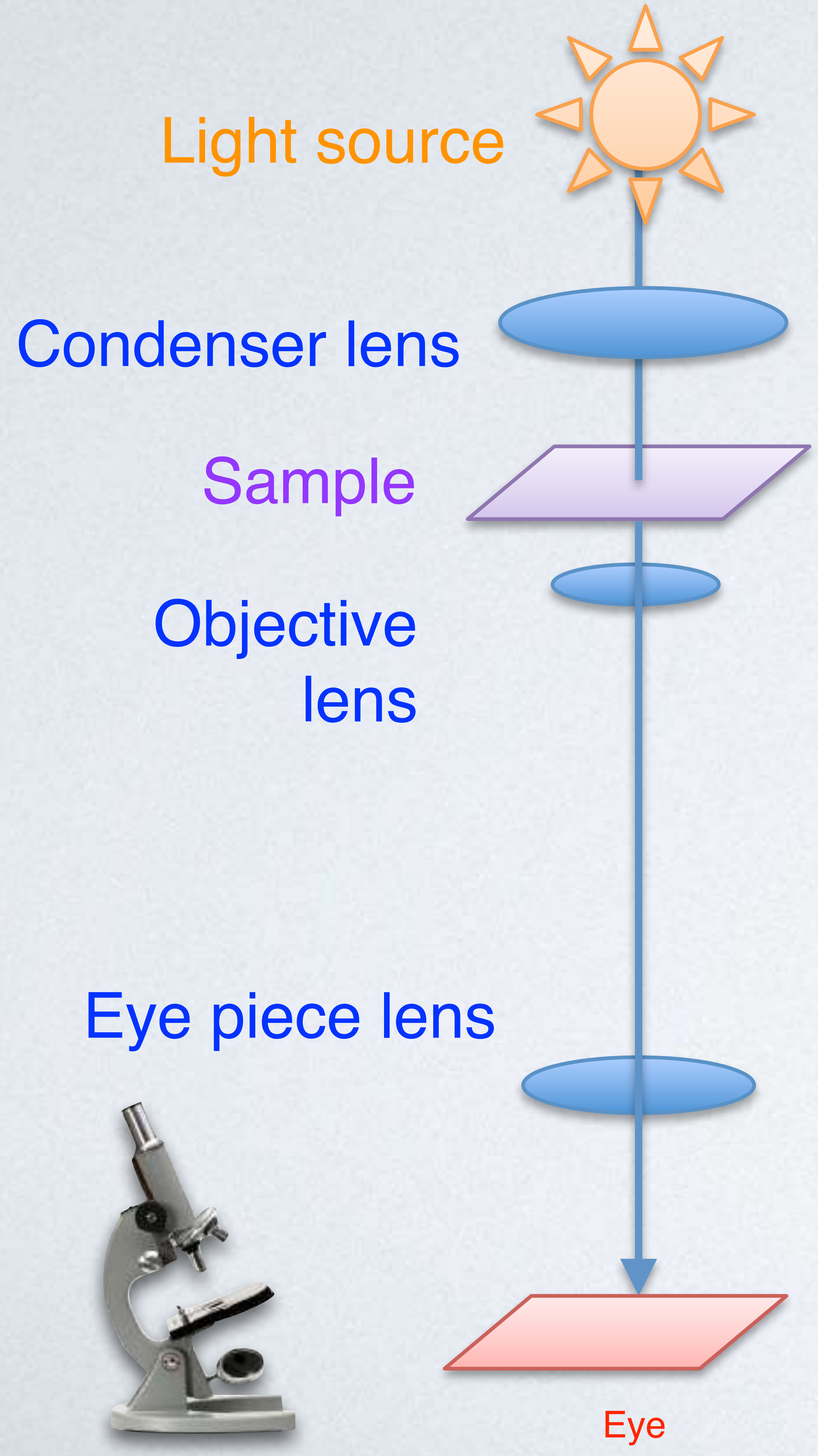
https://cryo-em-course.caltech.edu/

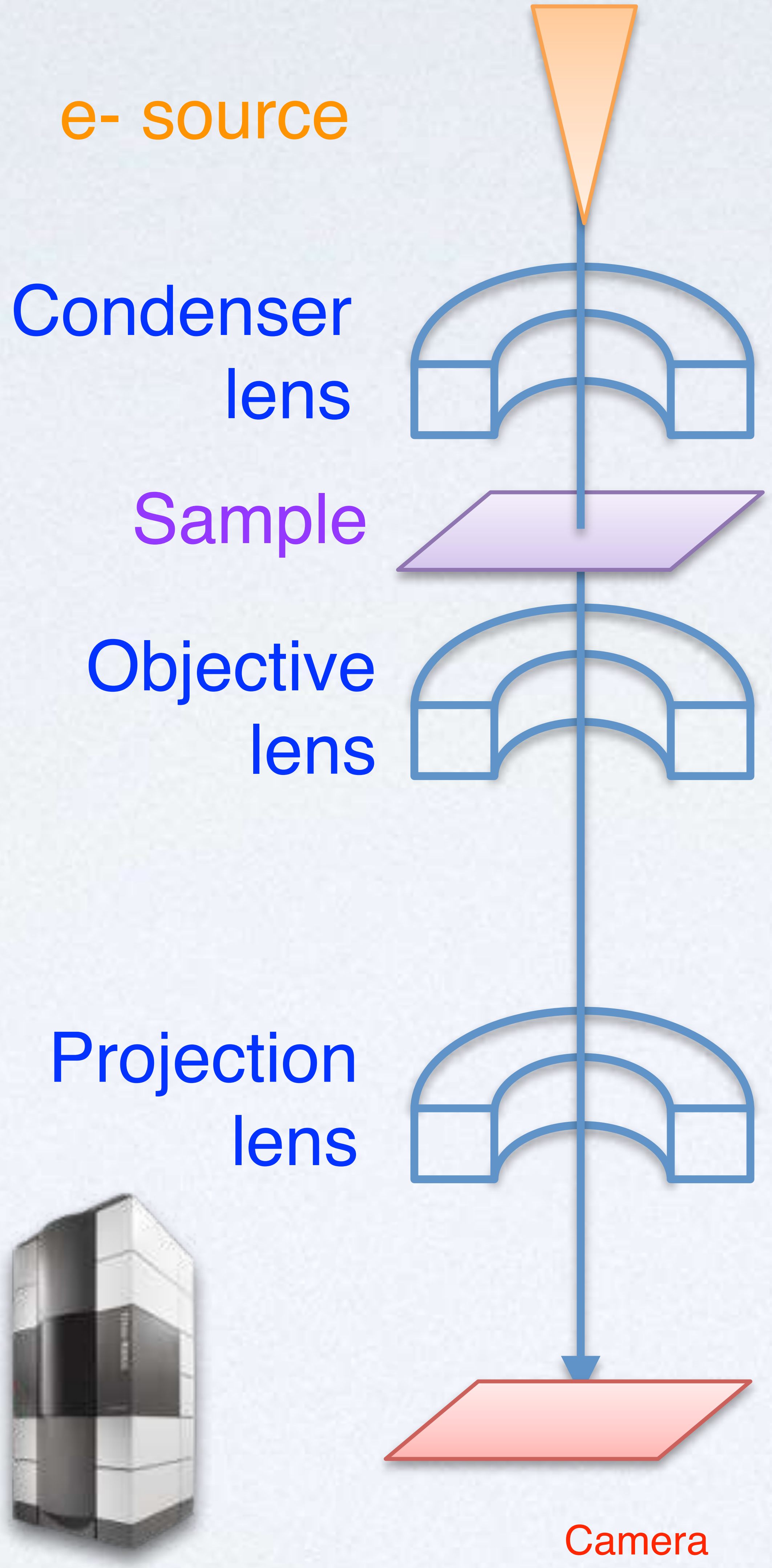
Specimen Behavior in the Electron Beam

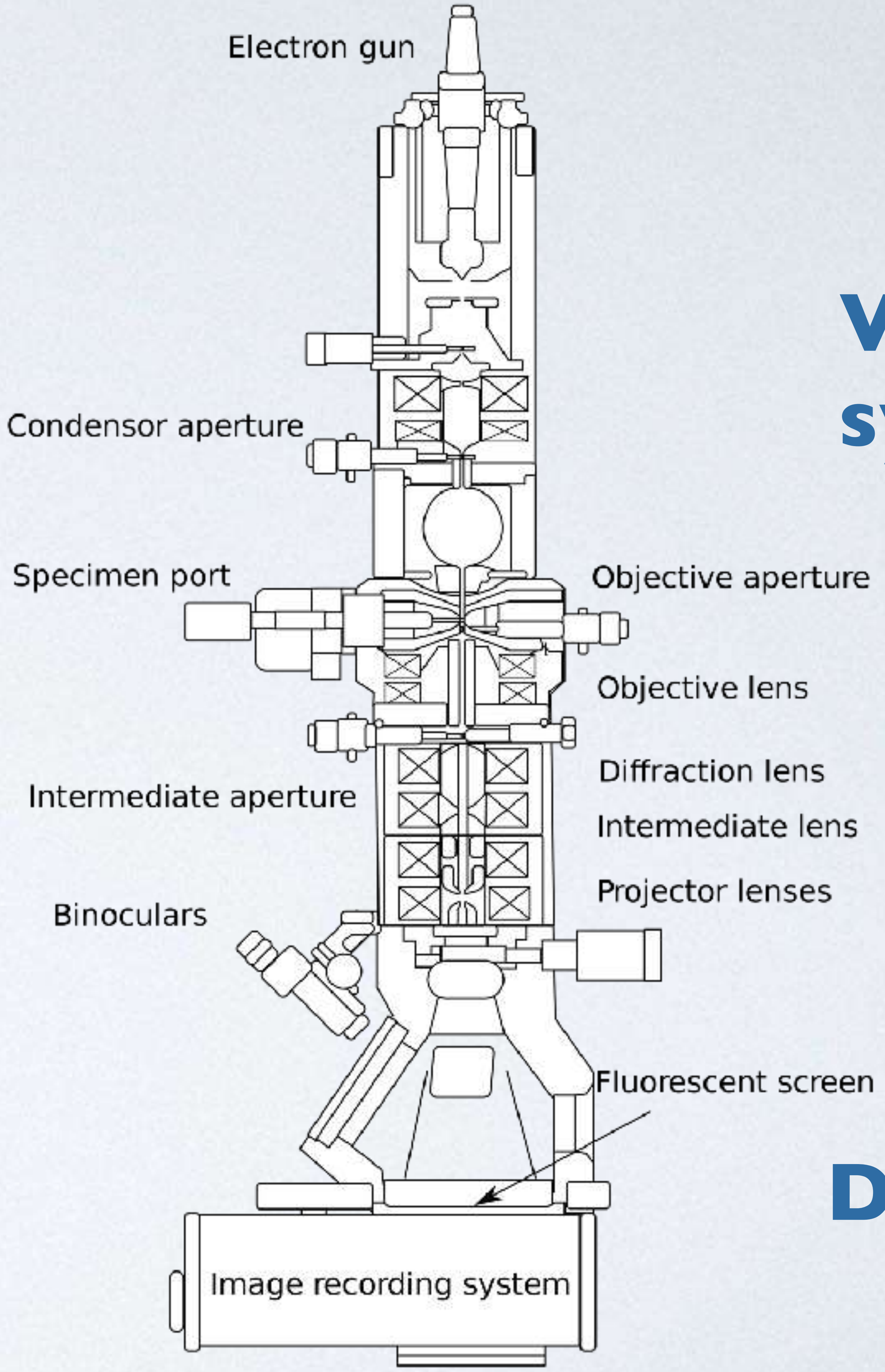
R.M. Glaeser[1]
Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, United States
[1]Corresponding author: e-mail address: rmglaeser@lbl.gov

- 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.

CRYOEM: THE MICROSCOPE







Electron sources

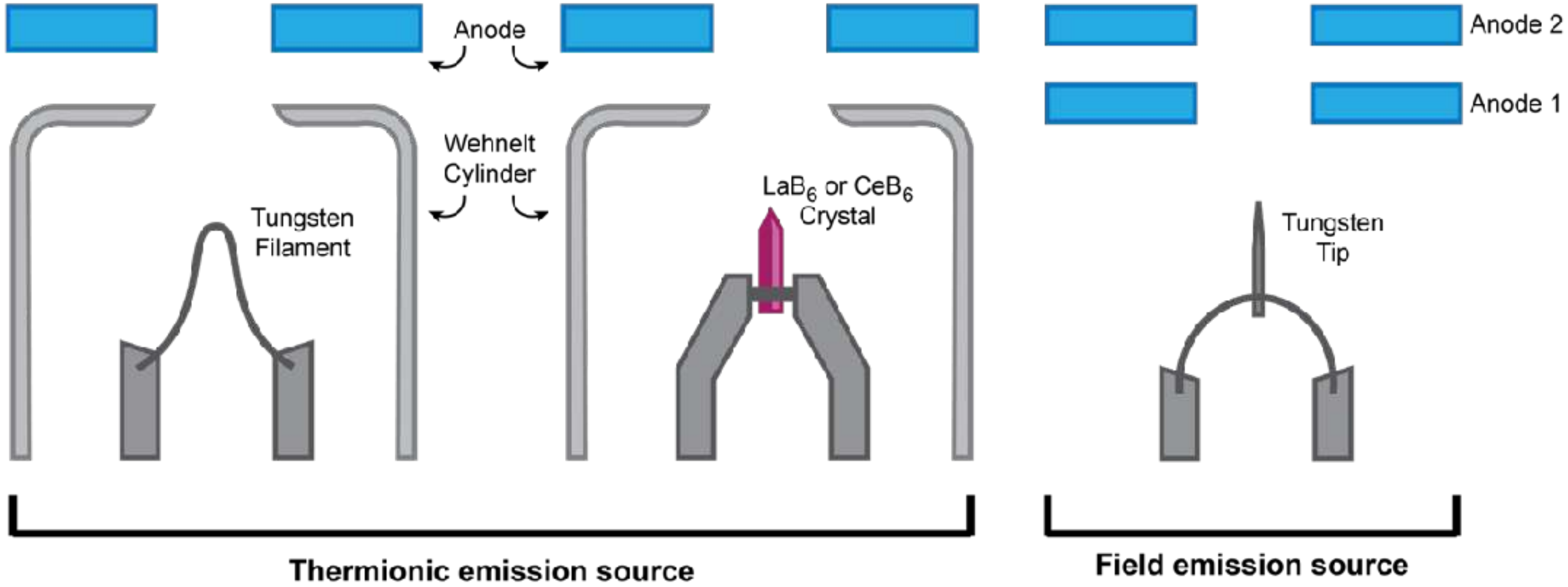
Vacuum systems

Lenses

Detectors

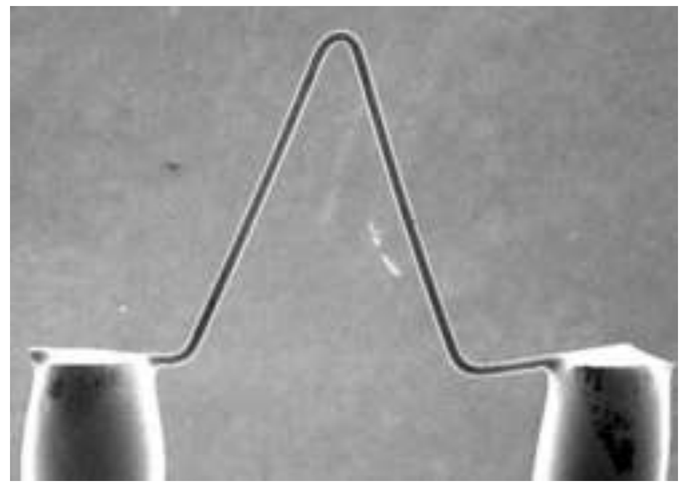
ELECTRON SOURCES

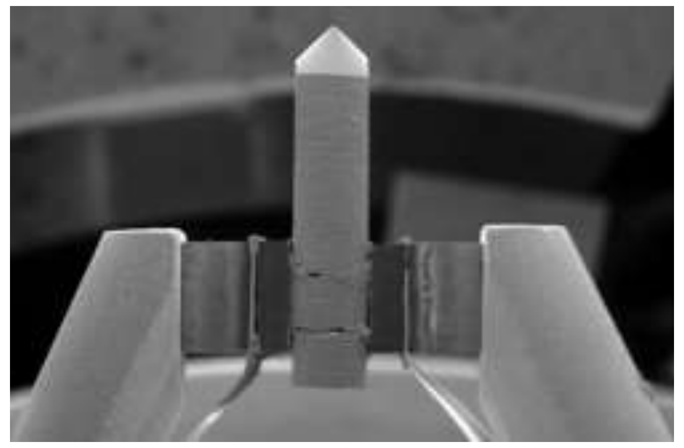
What are the 3 main kinds of electron sources?

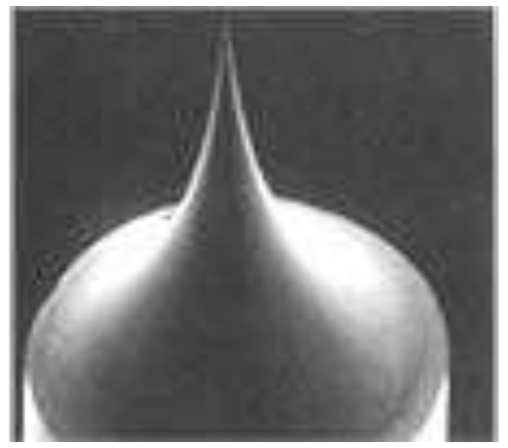


www.thermofisher.com

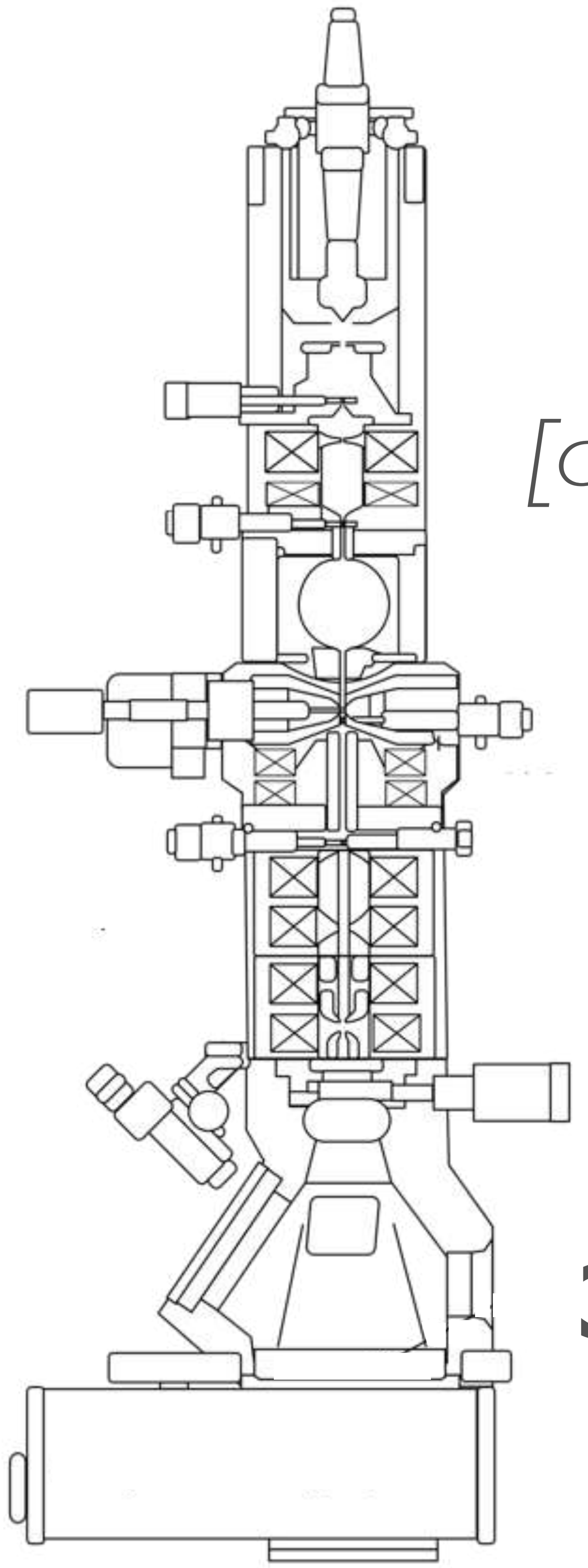
nanoscience.com



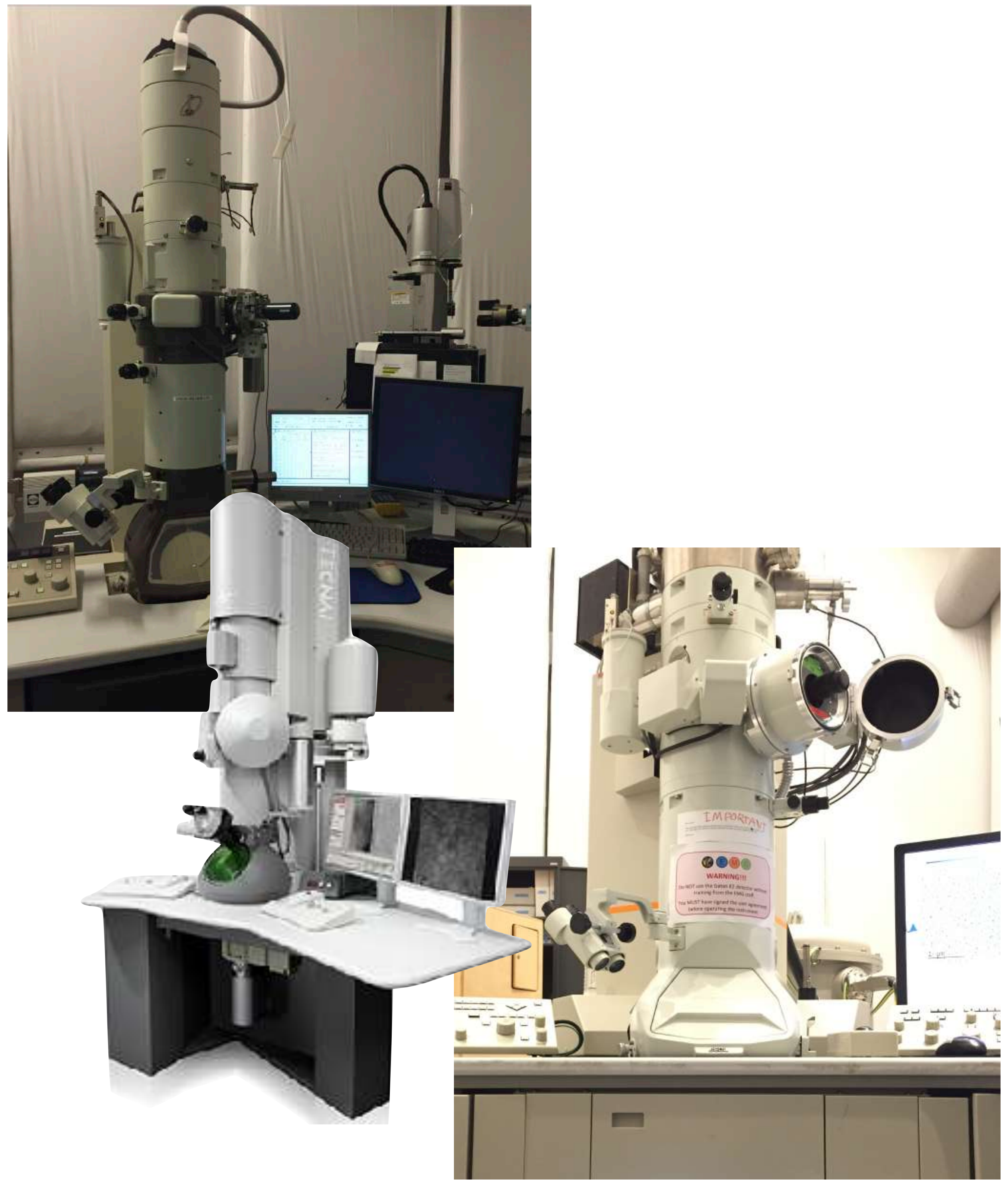




ELECTRON SOURCES & TYPES OF CRYOEM

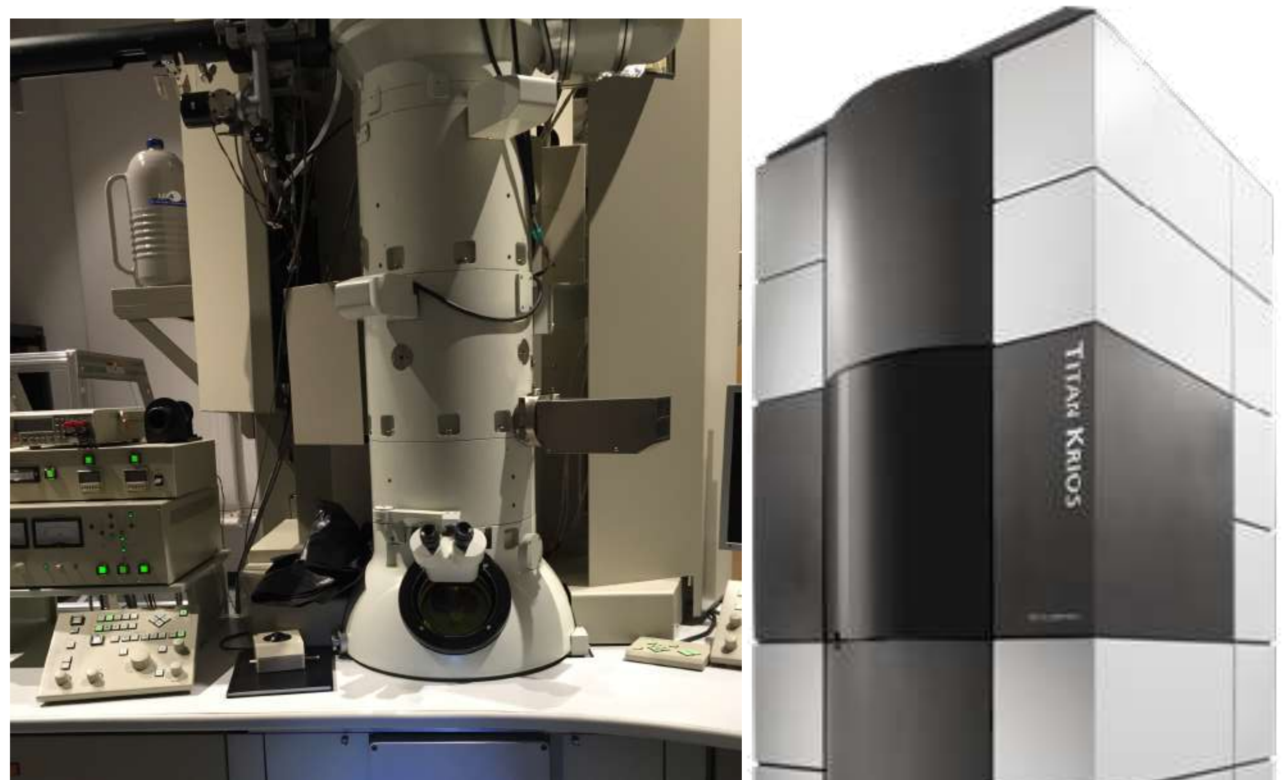


80-120 kV: Hitachi 7800, JEOL1400, TFS Talos120
W or LaB6
High contrast & robust
sub-nm resolution
[developments ongoing to push resolution with FEG systems]

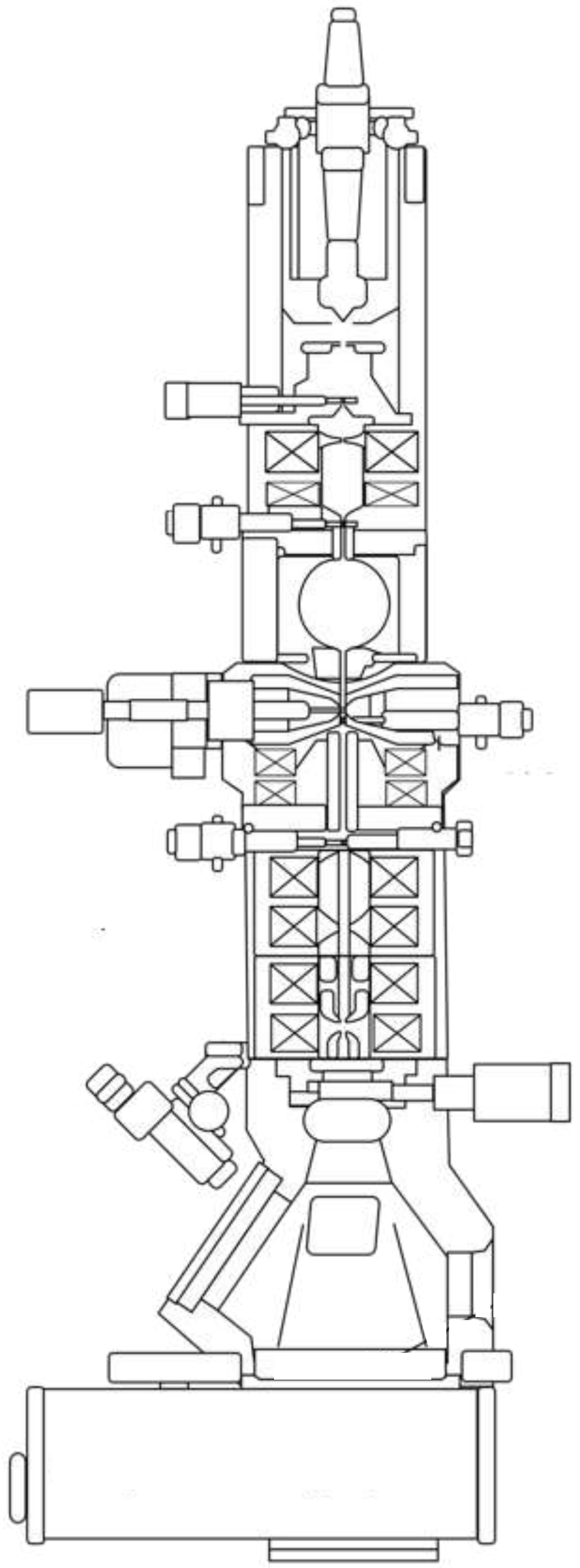


200 kV: J2100F; TFS Tecnai, Glacios, Arctica
FEG
2+ Å resolution (3.5-4 Å)

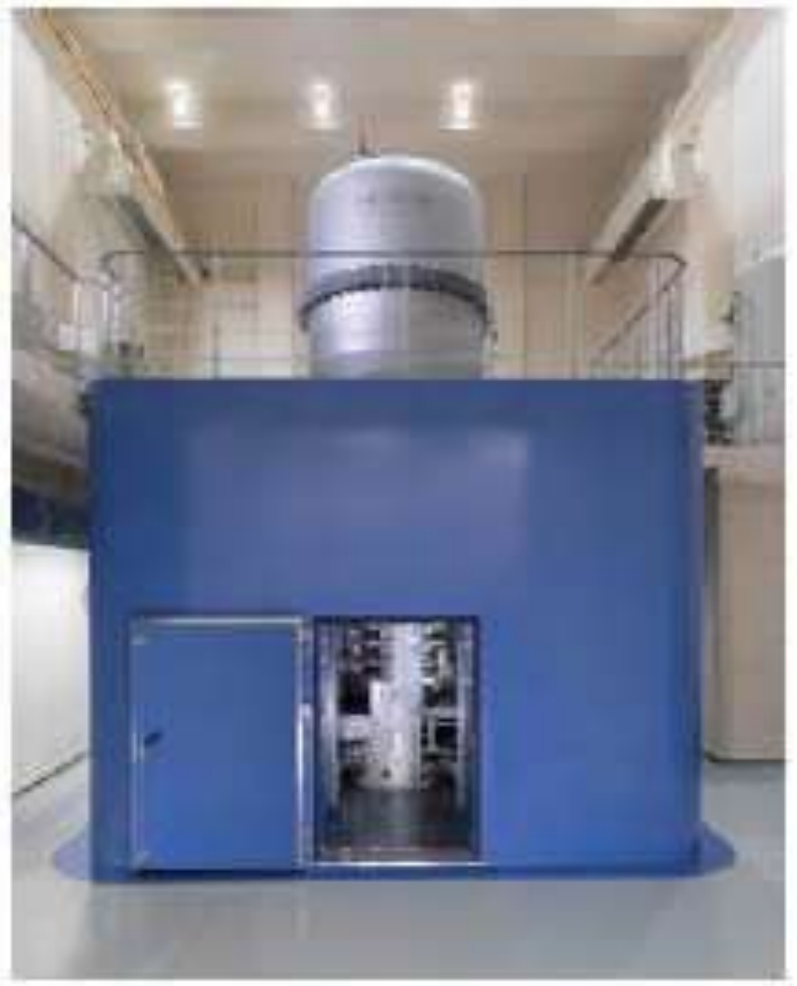
300 kV: JEOL3200FSC, cryoARM; TFS Krios, Halo
FEG
Smaller effect on unwanted lens aberrations
1.5-3 Å resolution

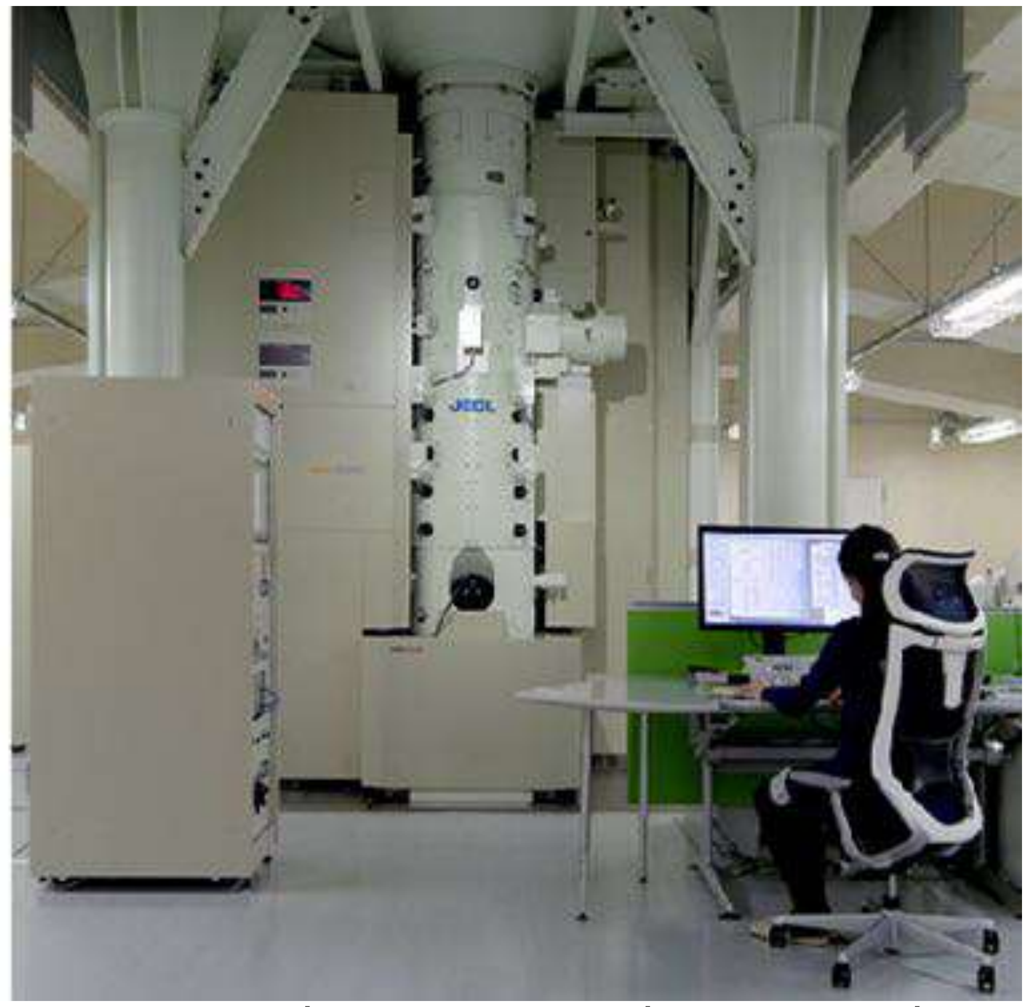


ELECTRON SOURCES & TYPES OF CRYOEM



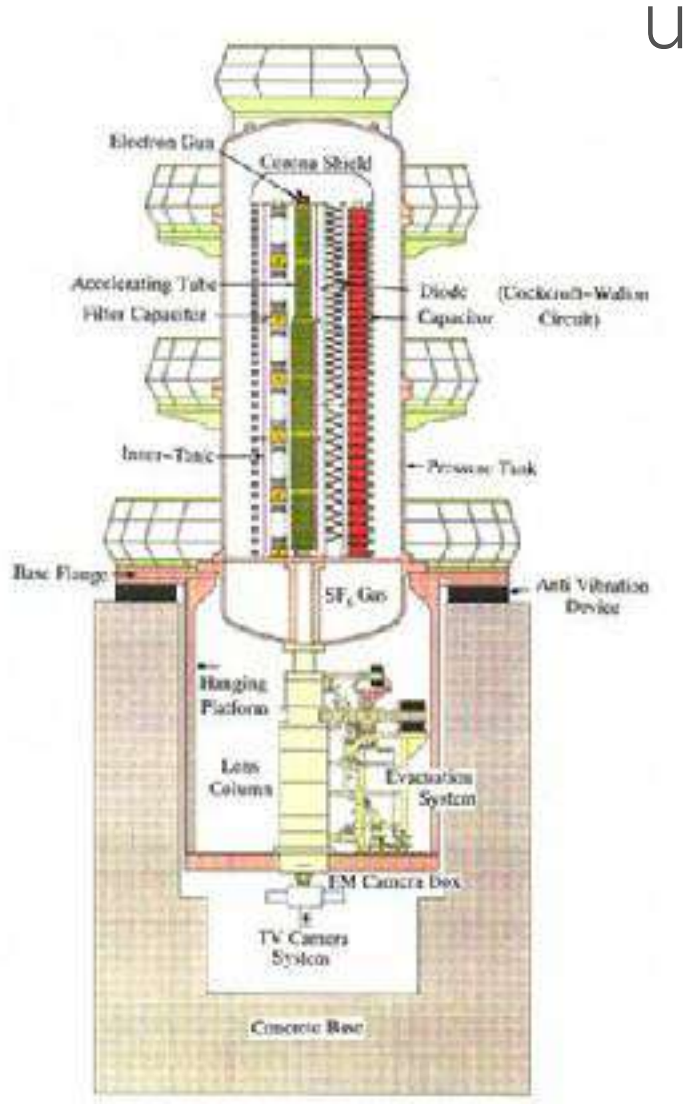
1-1.2 MV: Hitachi, JEOL
LaB6





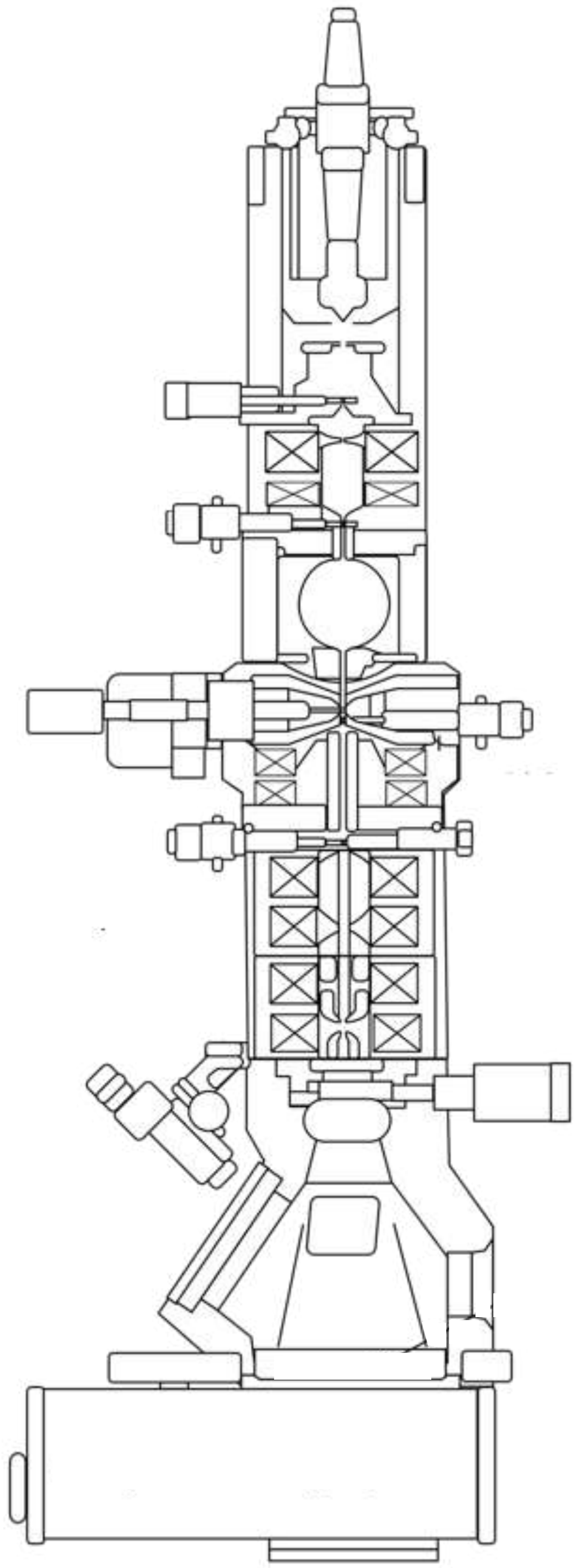
uhvem.osaka-u.ac.jp

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

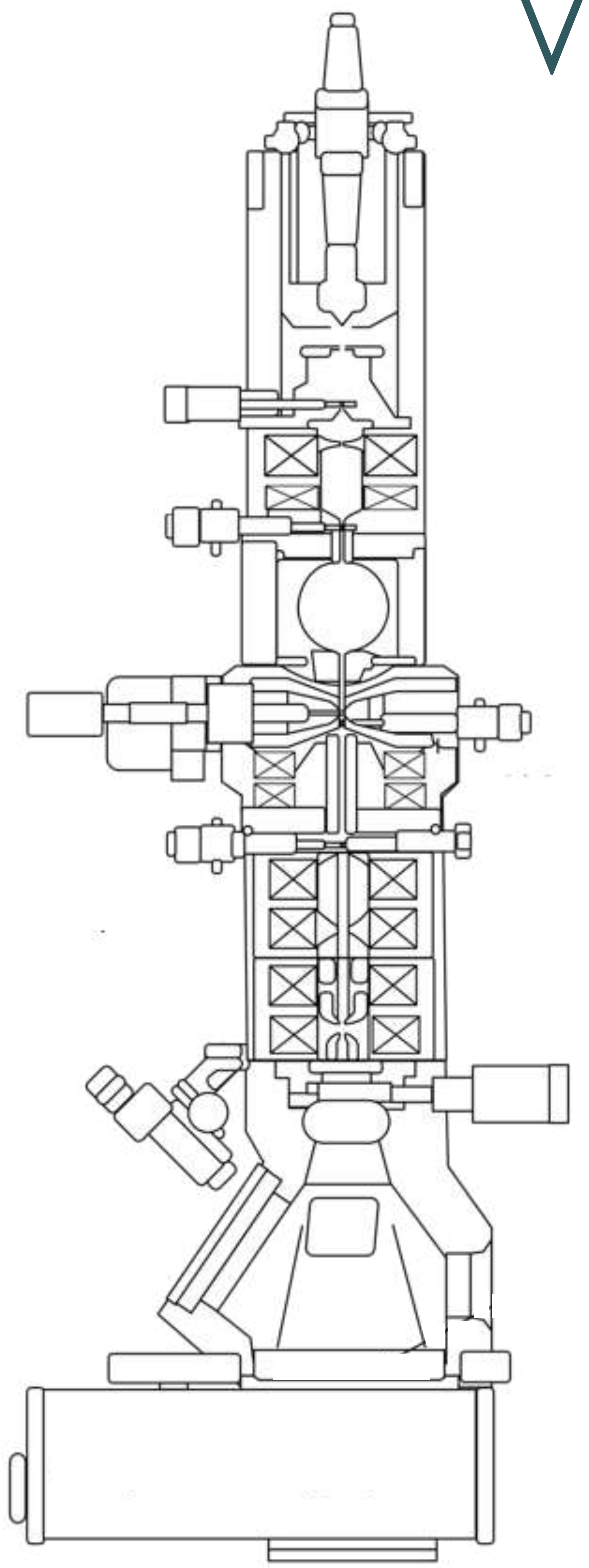
Contamination - reduce interaction gas, e-beam and sample

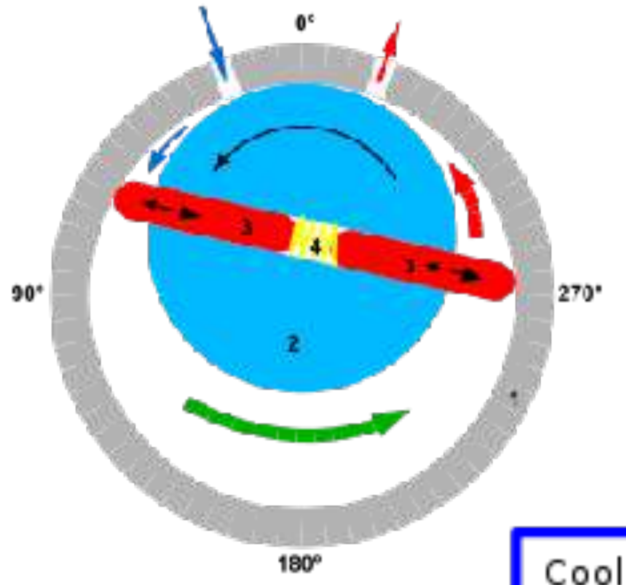
VACUUM SYSTEMS

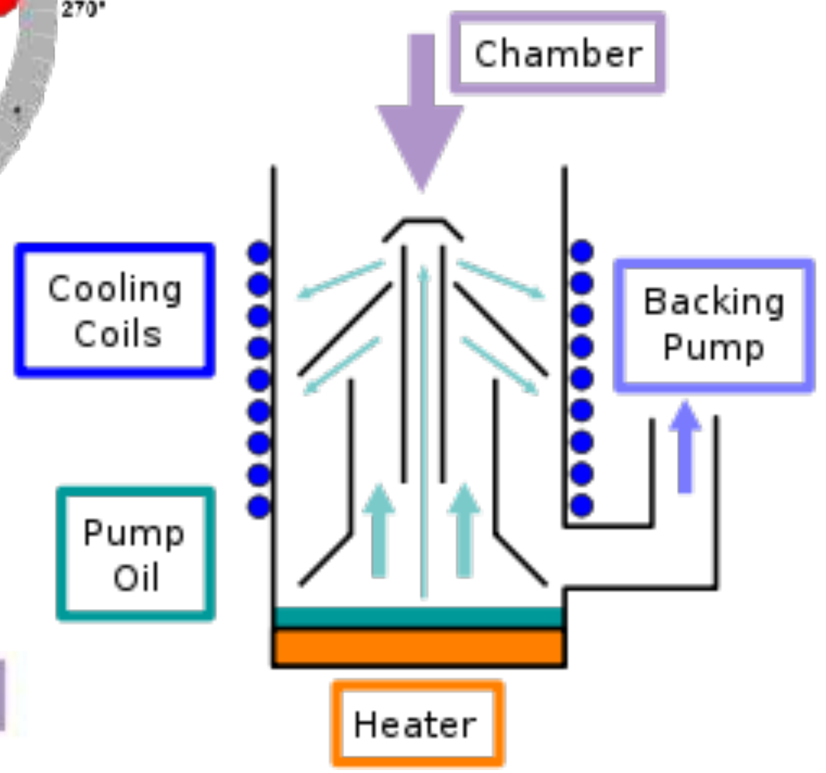
What types of pumps do we have?

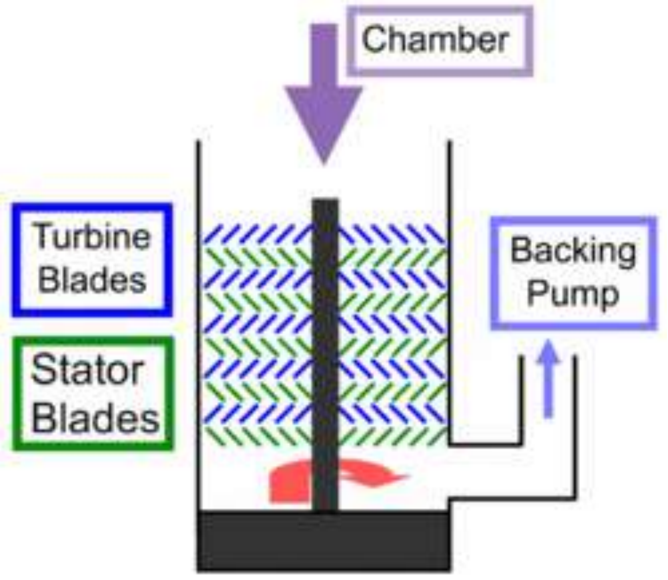
$1 \text{ mm Hg} = 1 \text{ Torr} = 10^2 \text{ Pa}$

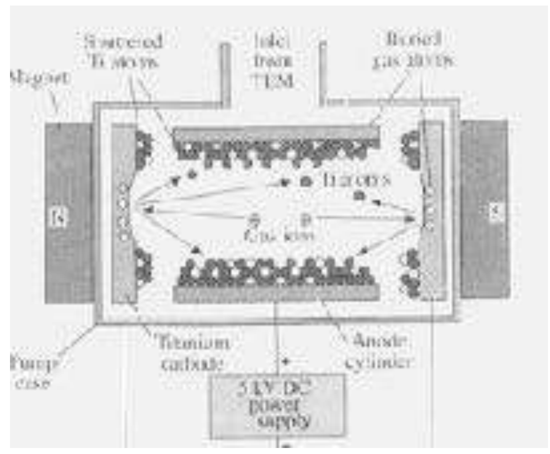
$1 \text{ atm} = 760 \text{ Torr} = 7.5 \times 10^4 \text{ Pa}$











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

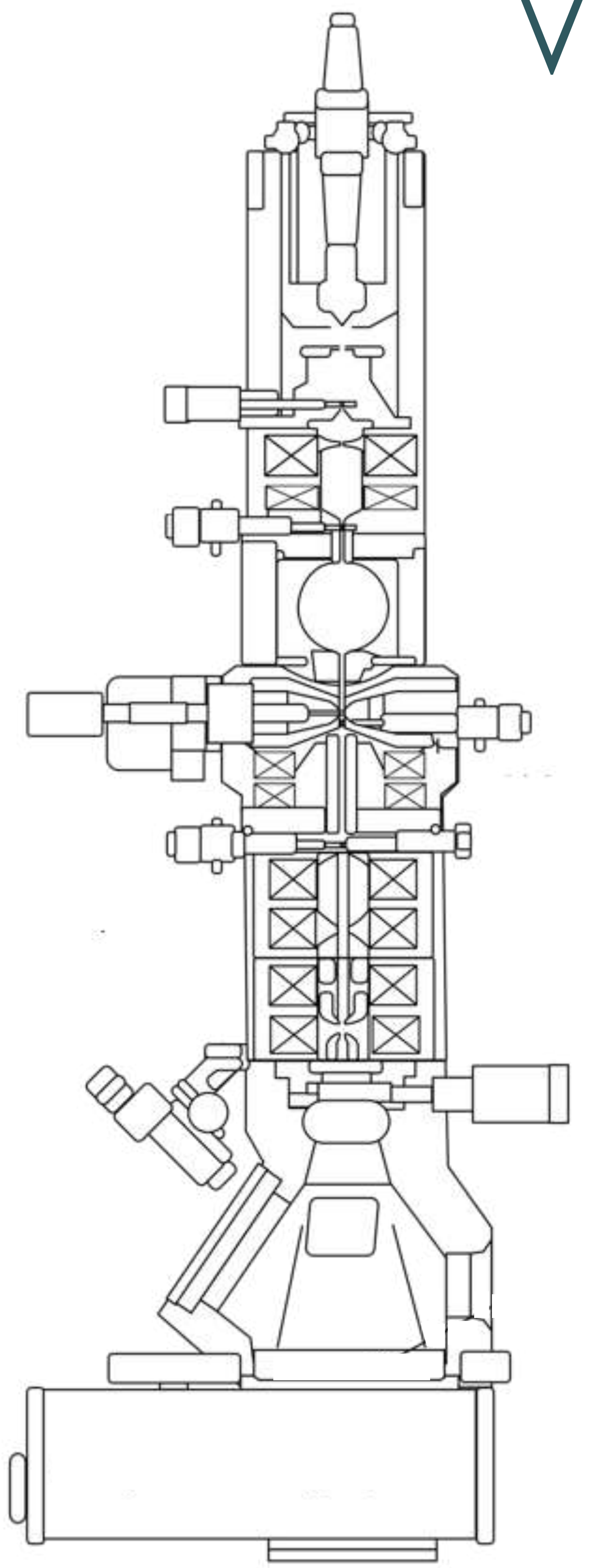
wikipedia.com

VACUUM SYSTEMS

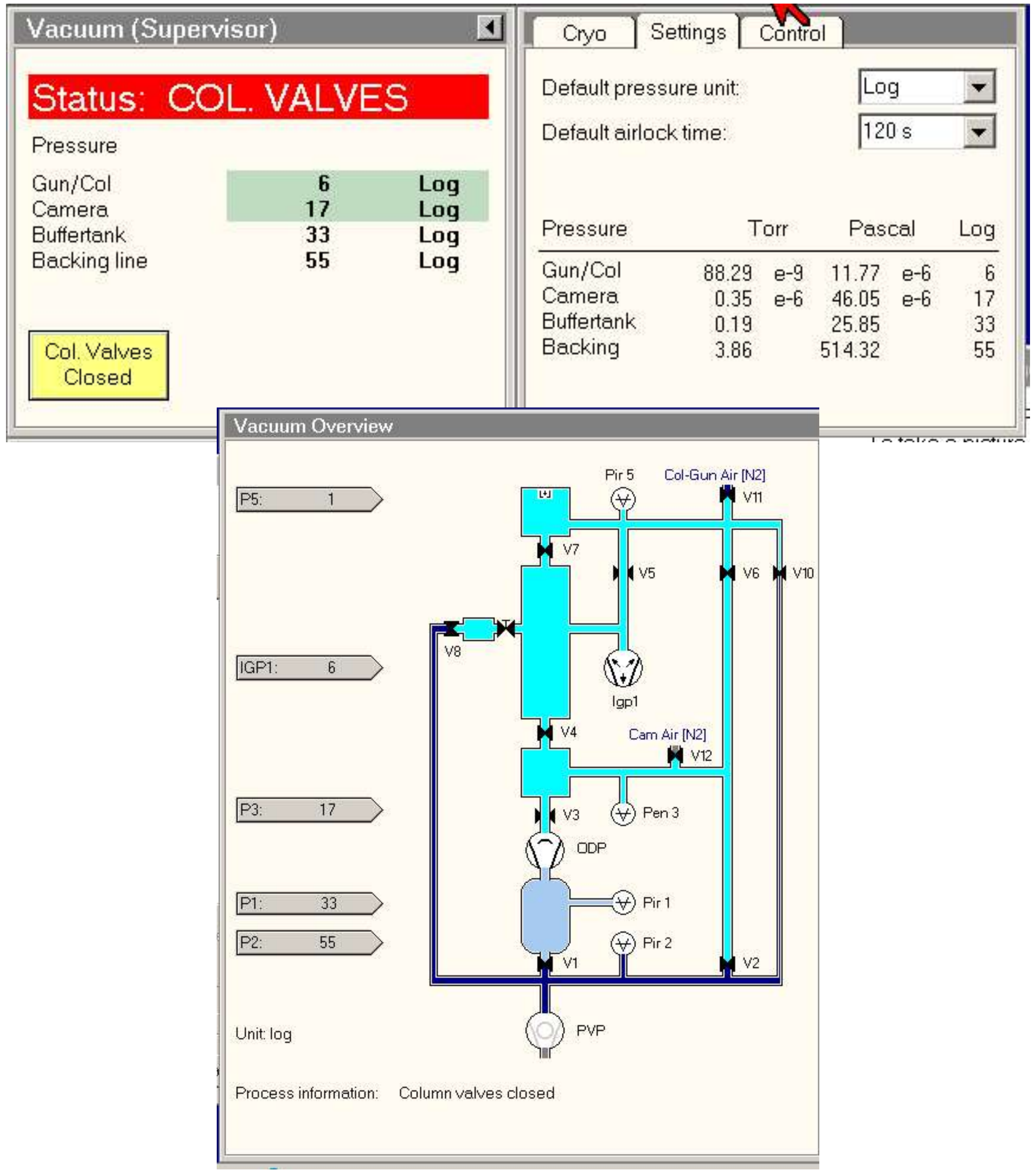
What types of pumps do we have?

$1 \text{ mm Hg} = 1 \text{ Torr} = 10^2 \text{ Pa}$

$1 \text{ atm} = 760 \text{ Torr} = 7.5\times10^4 \text{ Pa}$

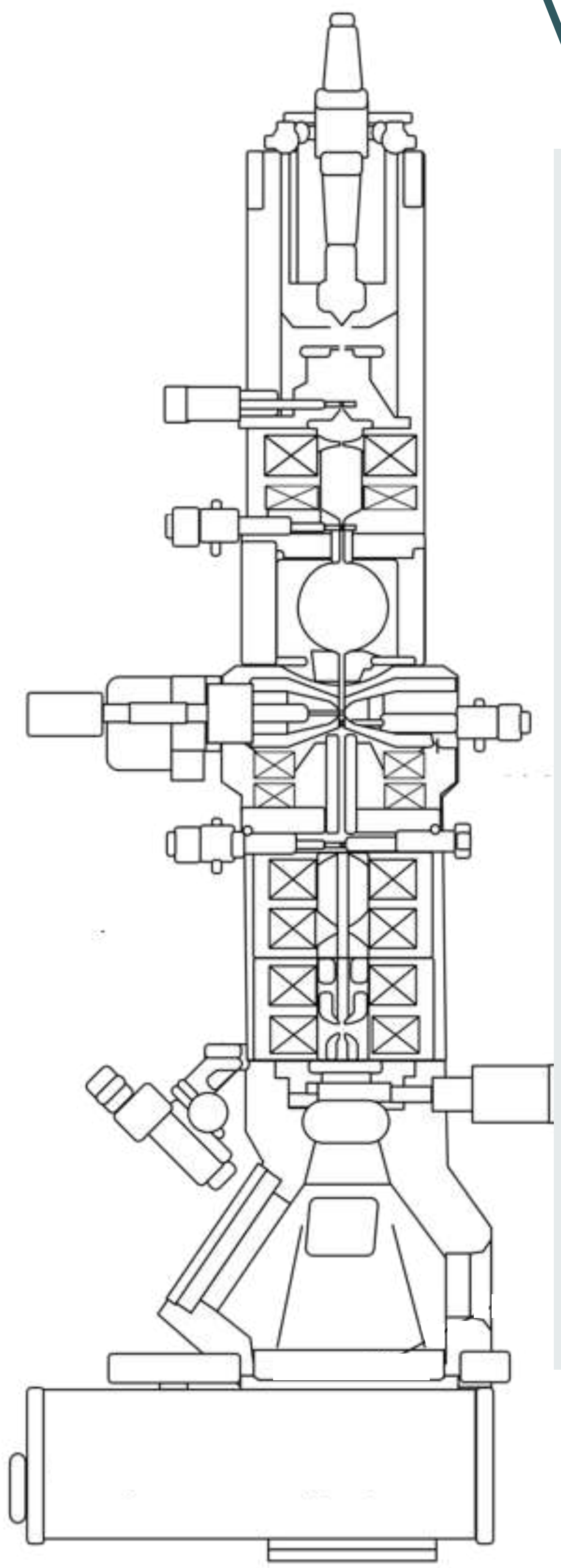


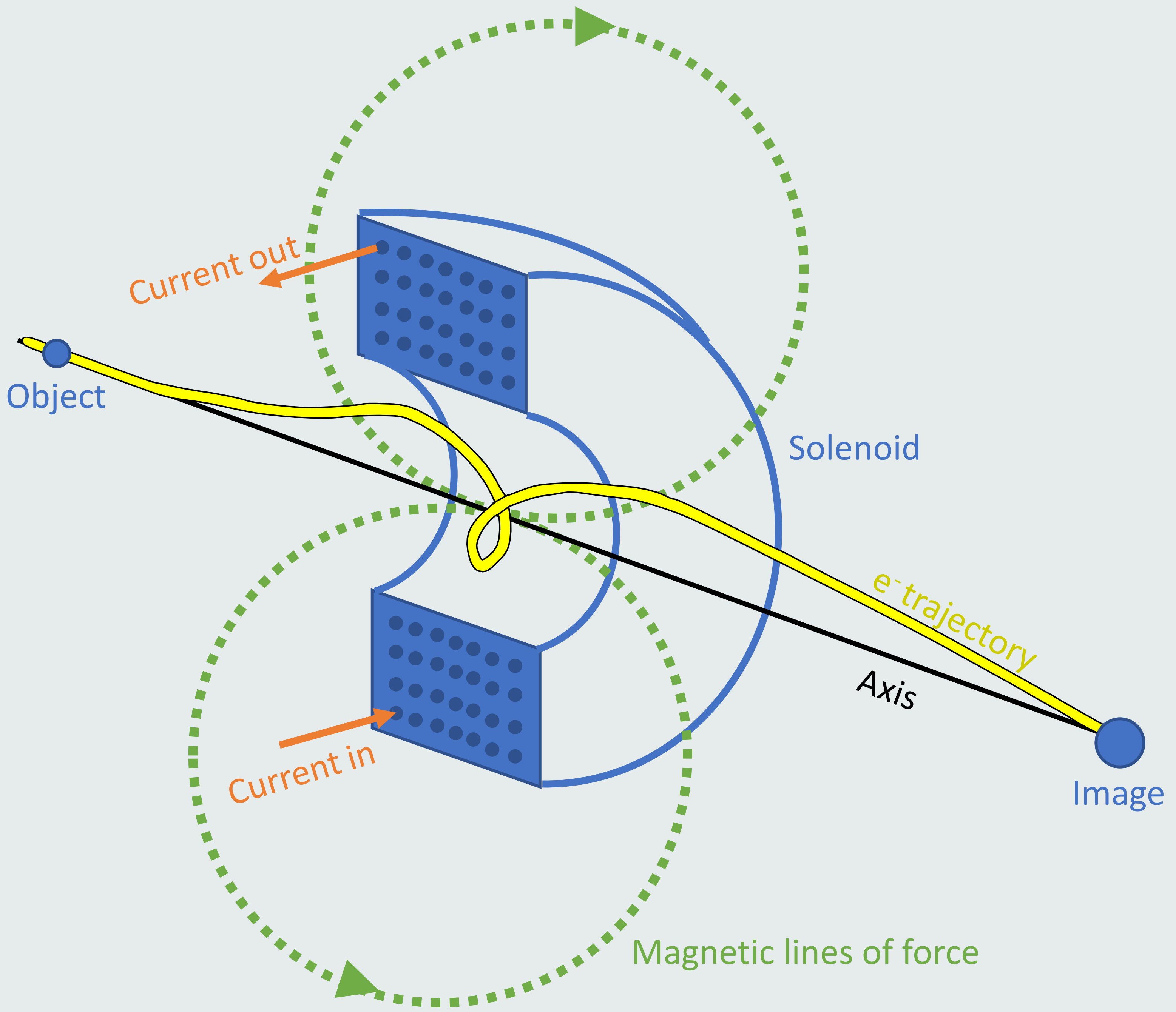
Gun	10^{-9} Torr
Specimen	10^{-6} - 10^{-7} Torr
Chamber and Camera	10^{-5} - 10^{-6} Torr



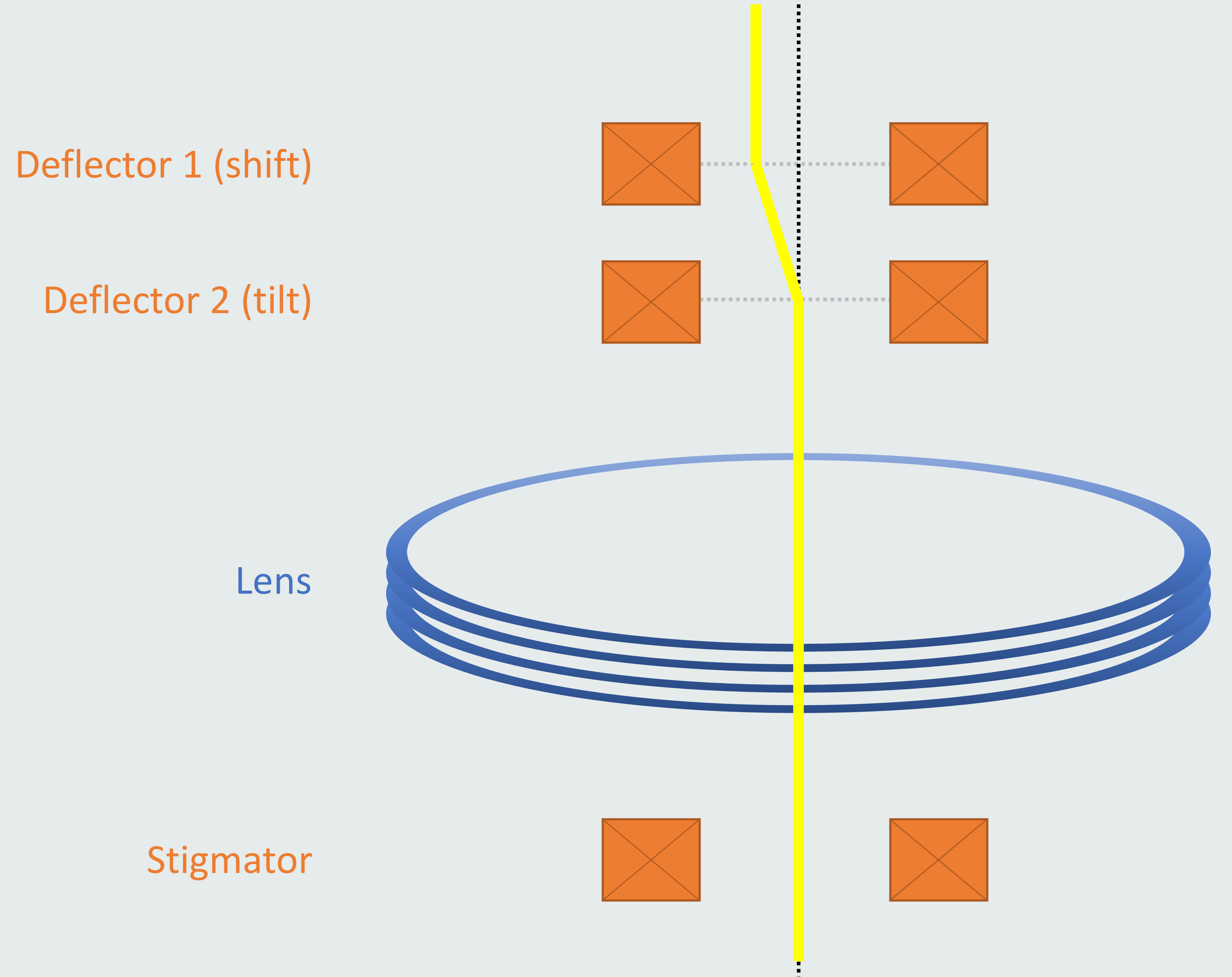
LENSES

What types of lenses do we have?





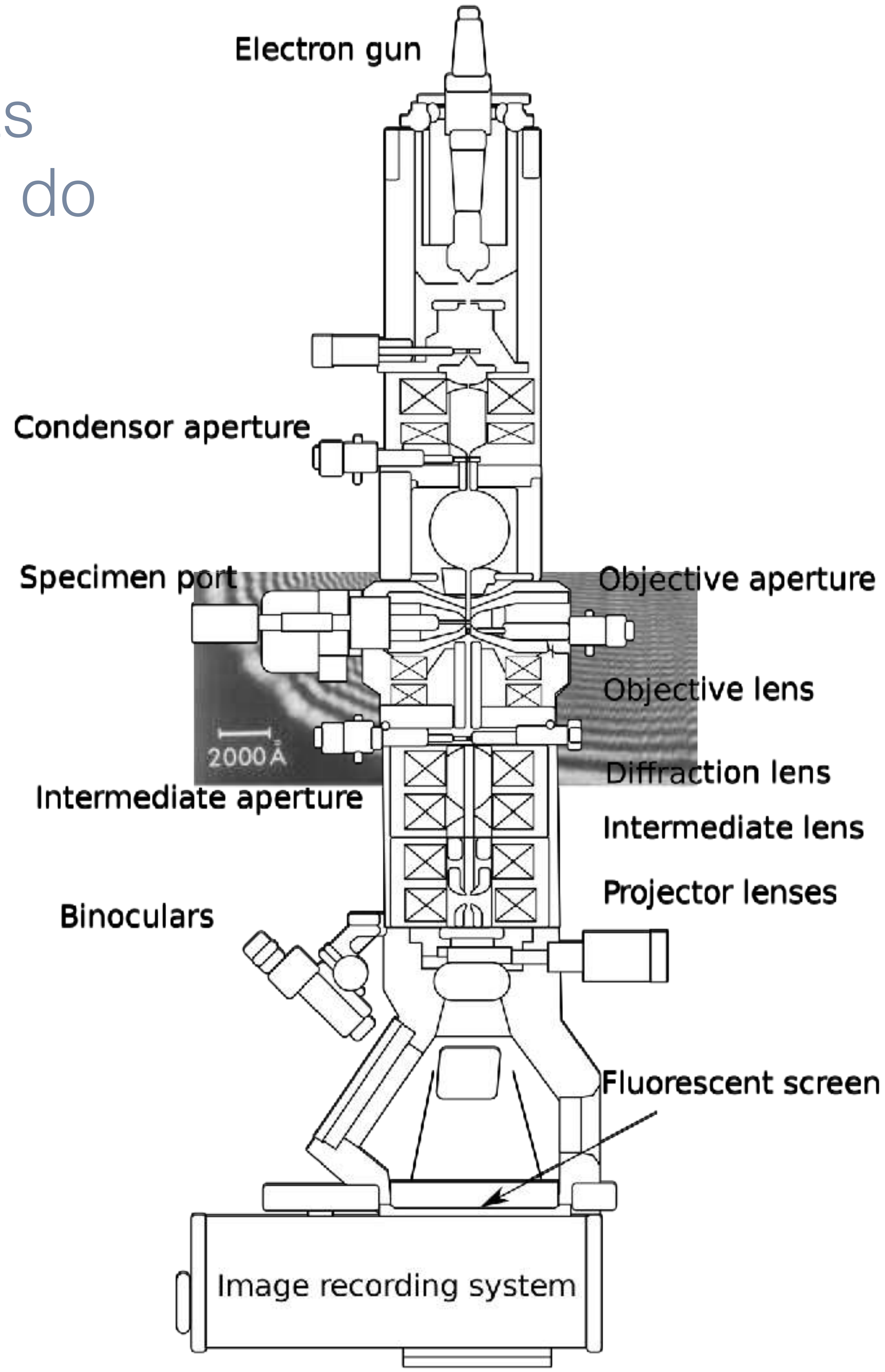
- Focus
- Magnify
- Rotate



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)~~

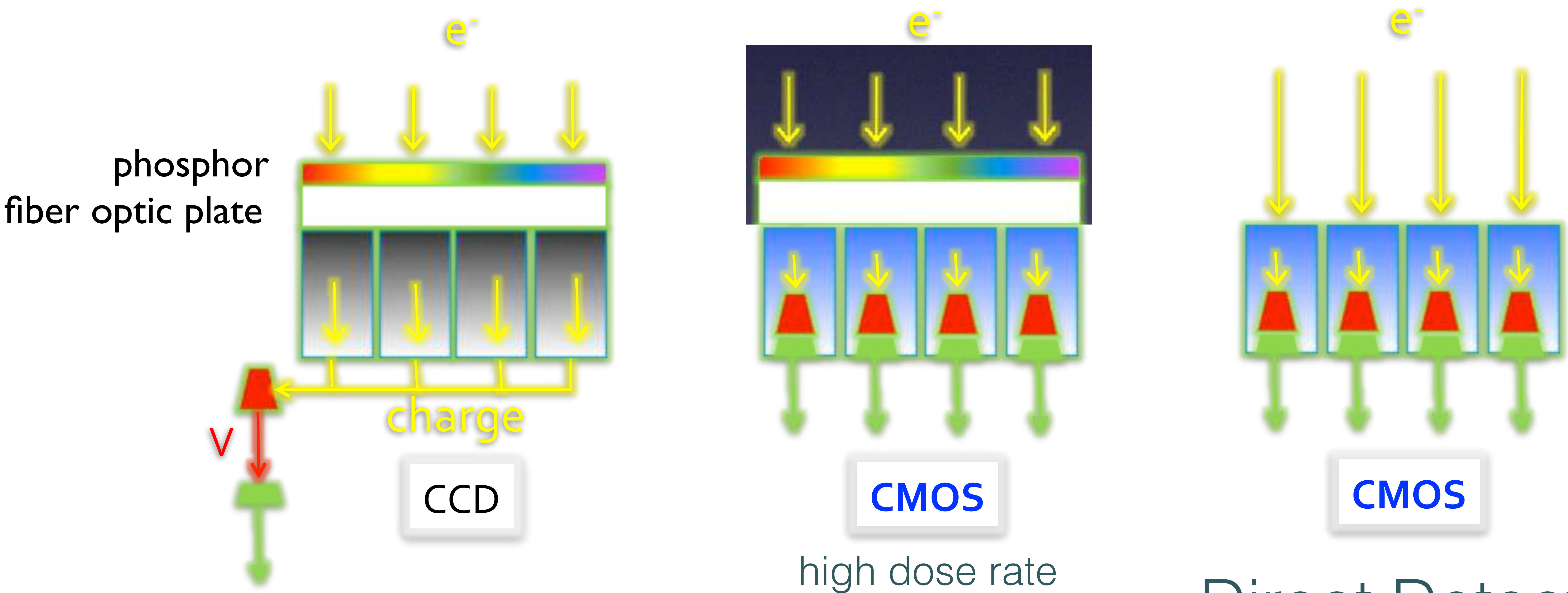


DETECTORS Digital Cameras for TEM

- Photon converted
- Direct sensing

?

- CCD Charge Coupled Device
- CMOS Complementary Metal Oxide Semiconductor

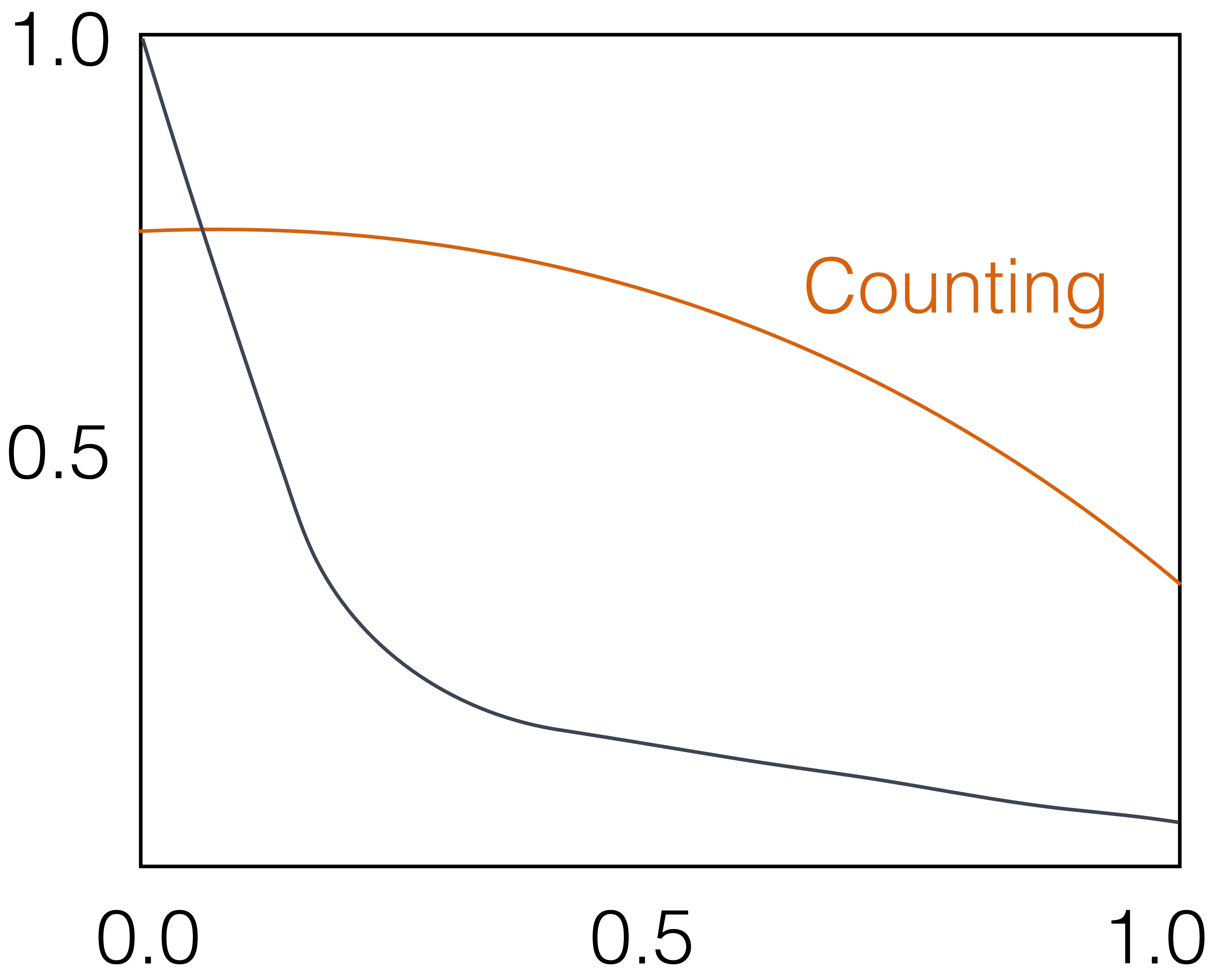


high dose rate

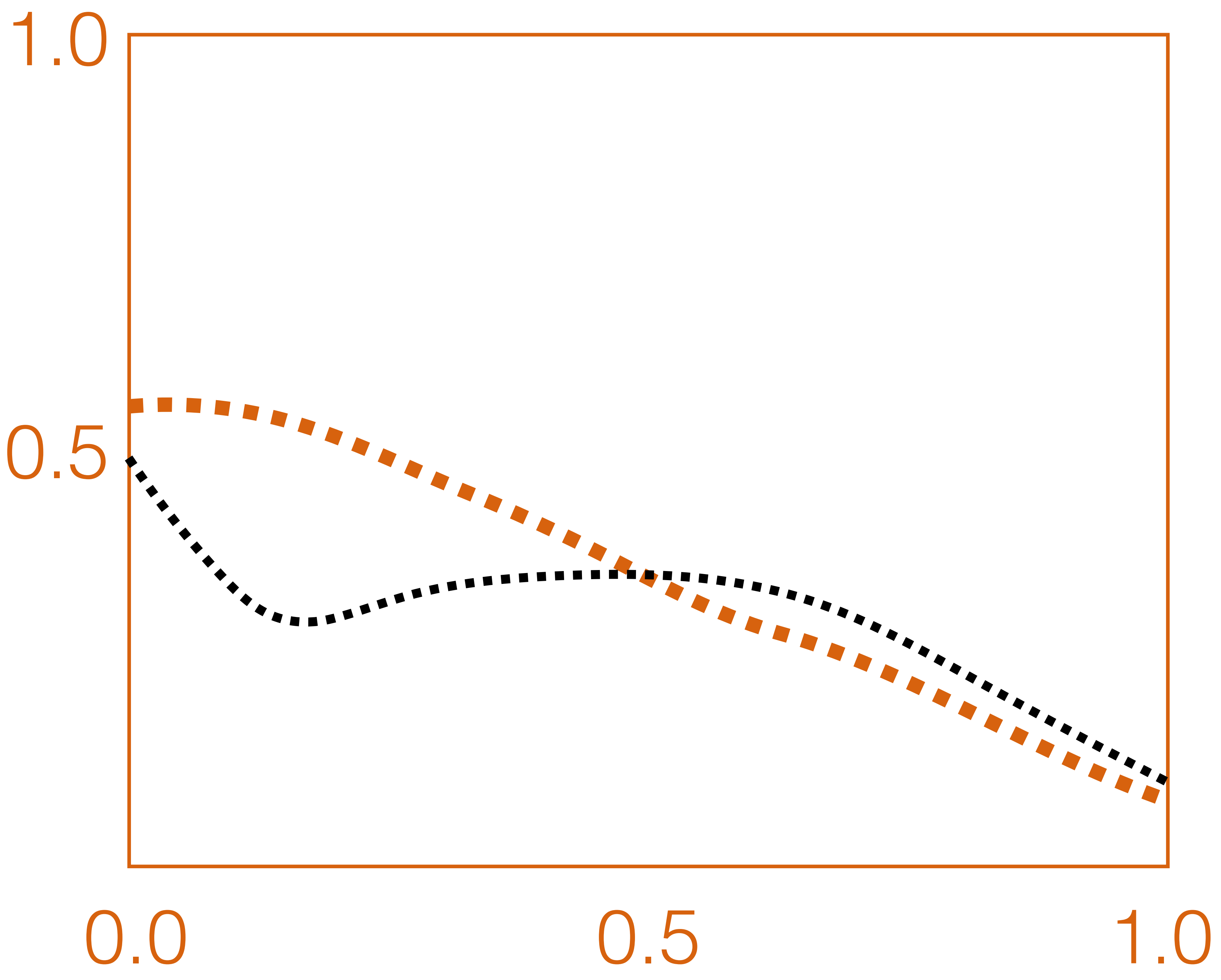
Direct Detectors

DETECTORS Detector Performance Characterization

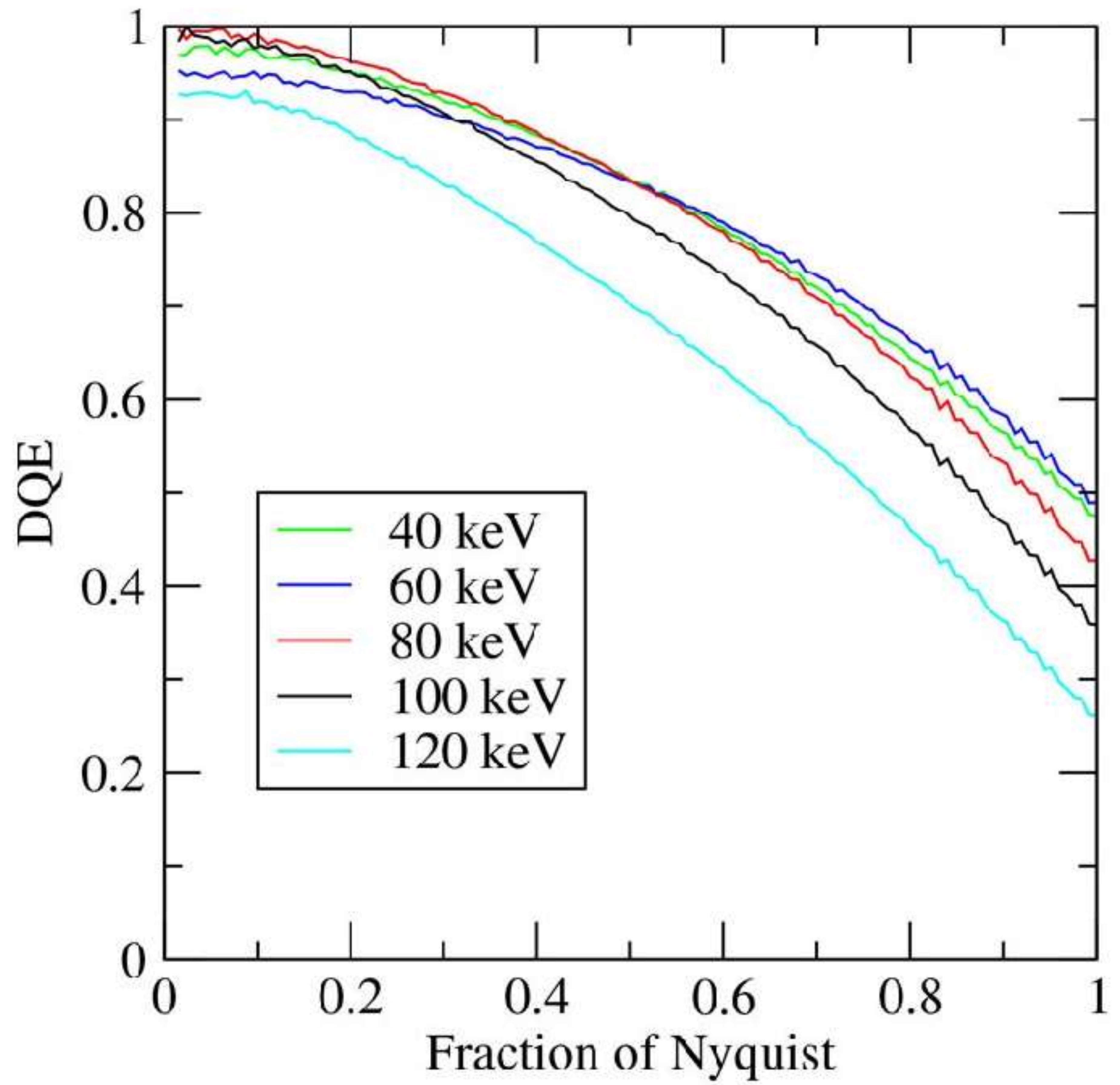
- MTF (Modulation Transfer Transform)
  - contribute to signal envelope



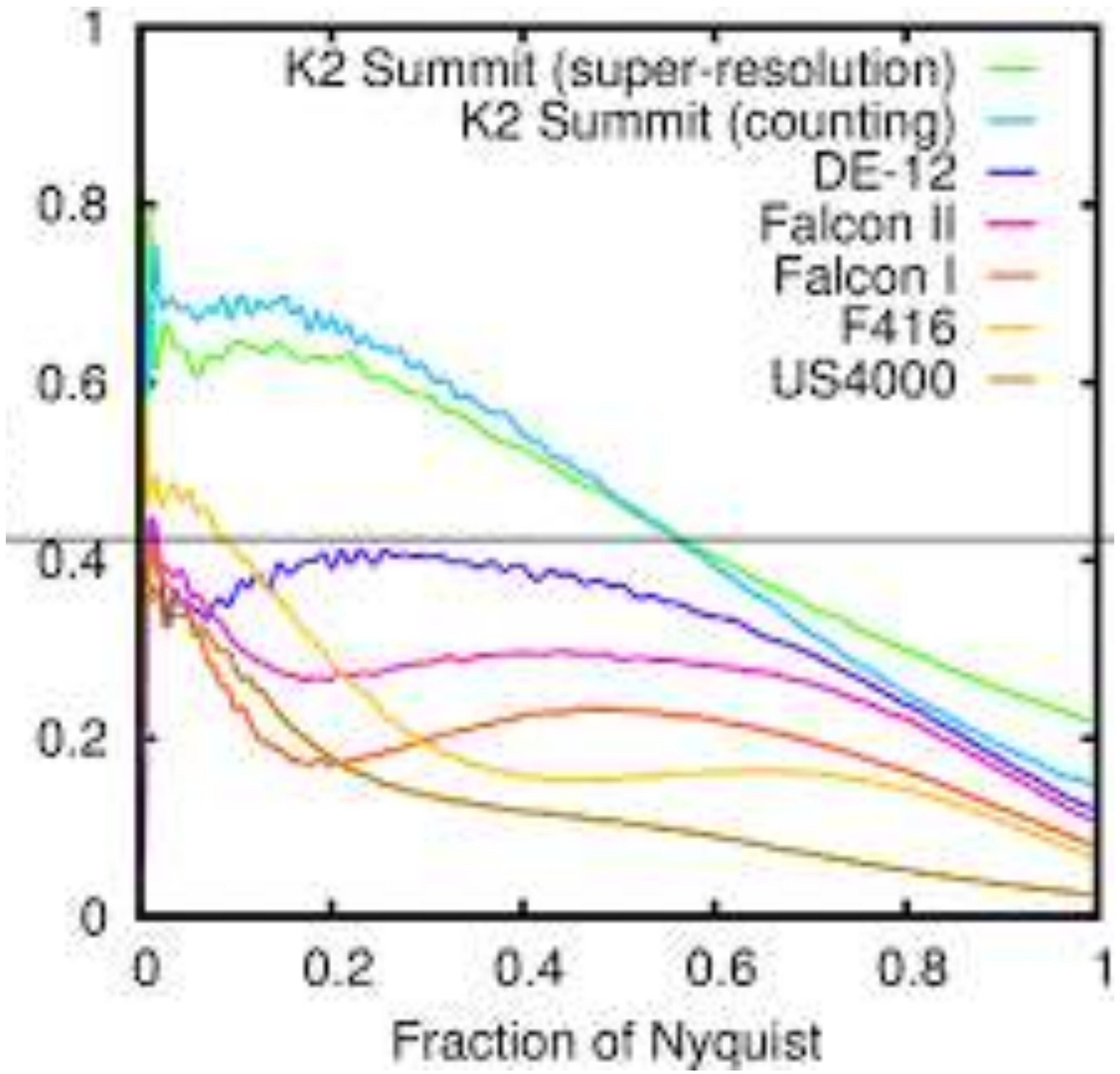
- DQE (Detector Quantum Efficiency)
  - S/N over spatial frequency range



DETECTORS Detector Performance Characterization



dectris.com



Ruskin, et al JSB

K3 specs

https://www.gatan.com/K3

Specifications

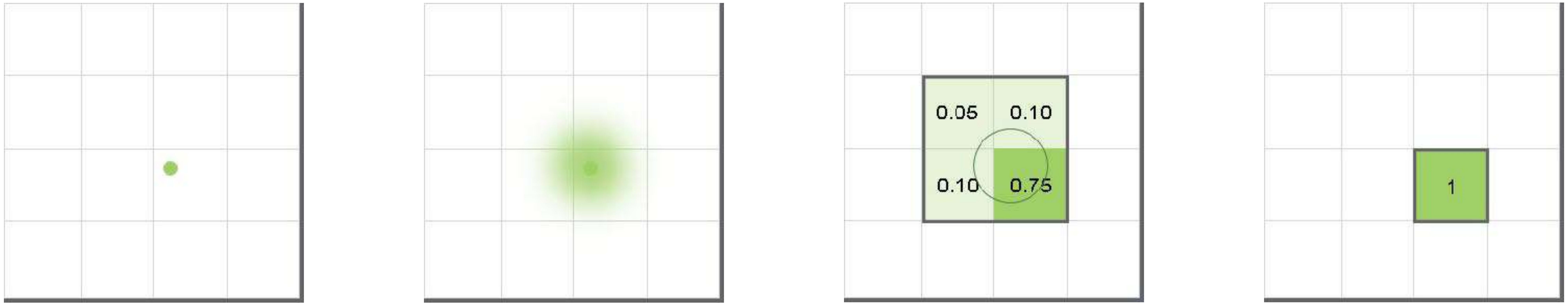
	K3	K3 Base
TEM operating voltage (kV)	200 / 300	
Sensor size (pixels)	5,760 x 4,096	3,456 x 4,096
Readout modes	Counting Super-resolution	Counting
Max. image size (pixels)	11,520 x 8,184 Super-resolution	3,456 x 4,096
Performance relative to physical Nyquist (DQE)		
Peak	>0.87 / >0.83	>0.8
0.5	>0.53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

Specifications are subject to change without notice.

Counting mode

5,760 x 4,096 px

11,520 x 8,184 px



Electron enters detector.

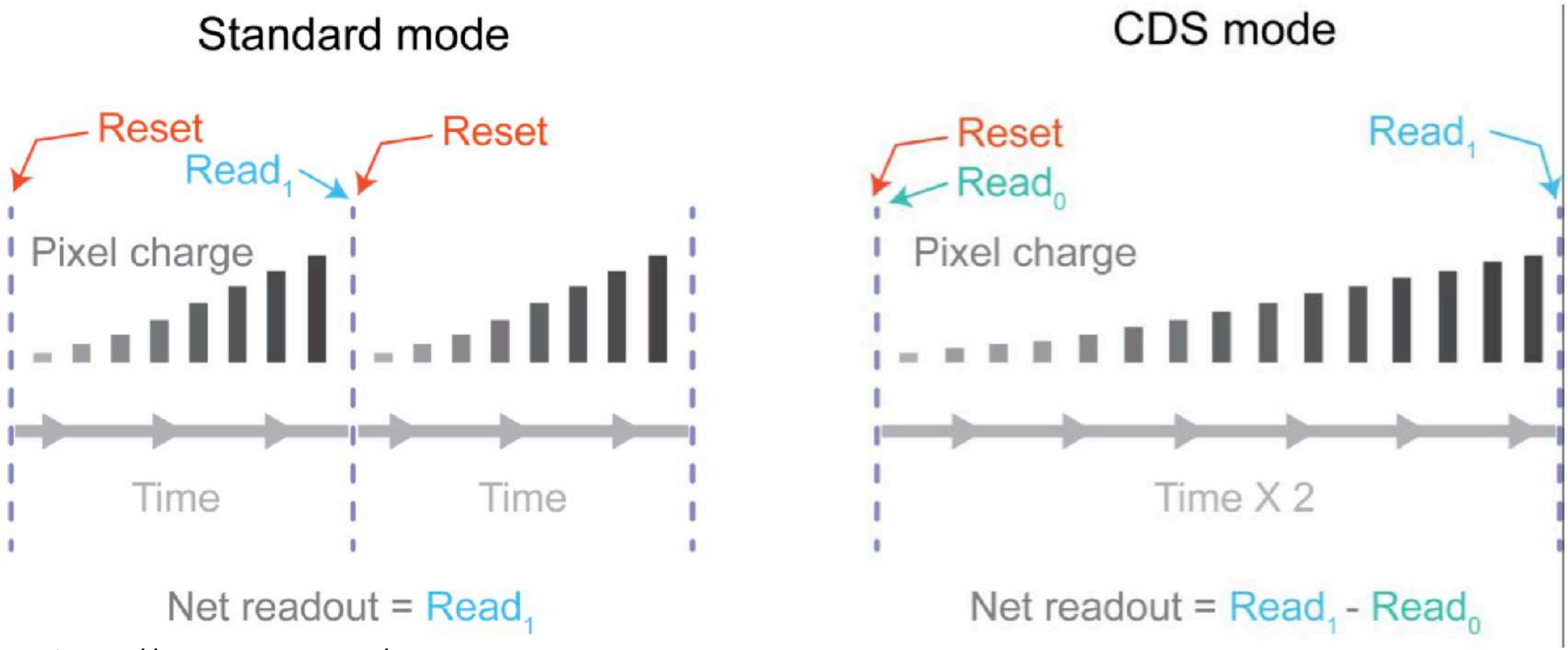
Electron signal is scattered.

Charge collects in each pixel.

Events reduced to highest charge pixels.

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

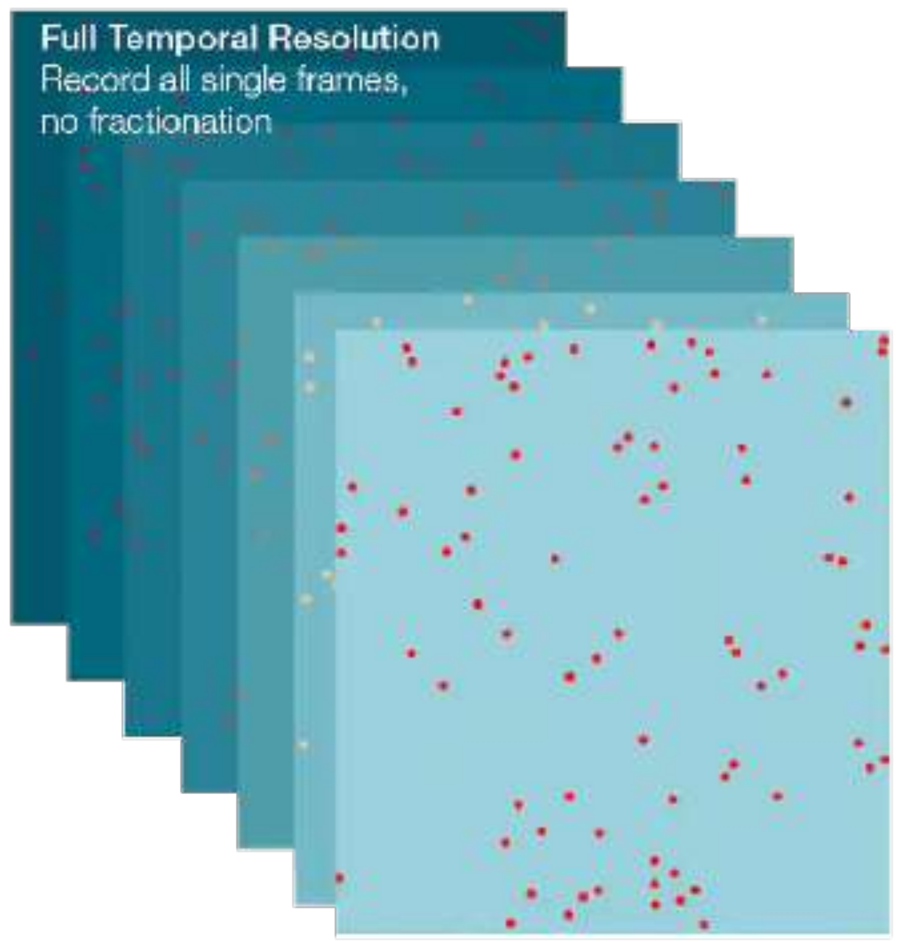
K3 lowers Read Noise with Correlated Double Sampling (CDS)

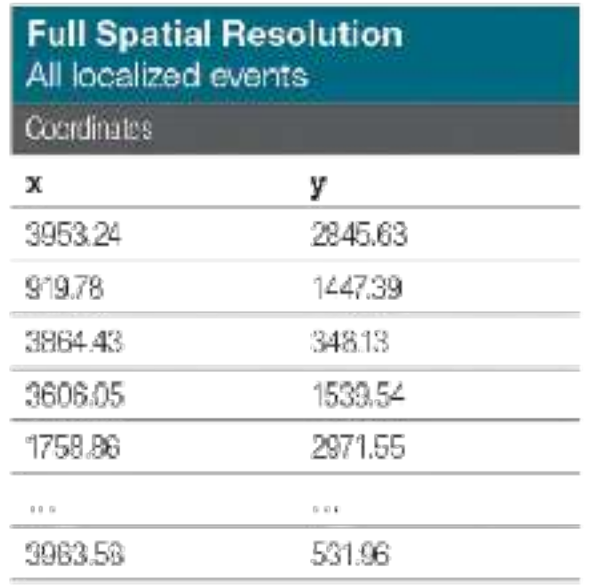


https://www.gatan.com/

Falcon4 specs

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	Electron-event representation (EER) 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 Spatial Resolution
All localized events

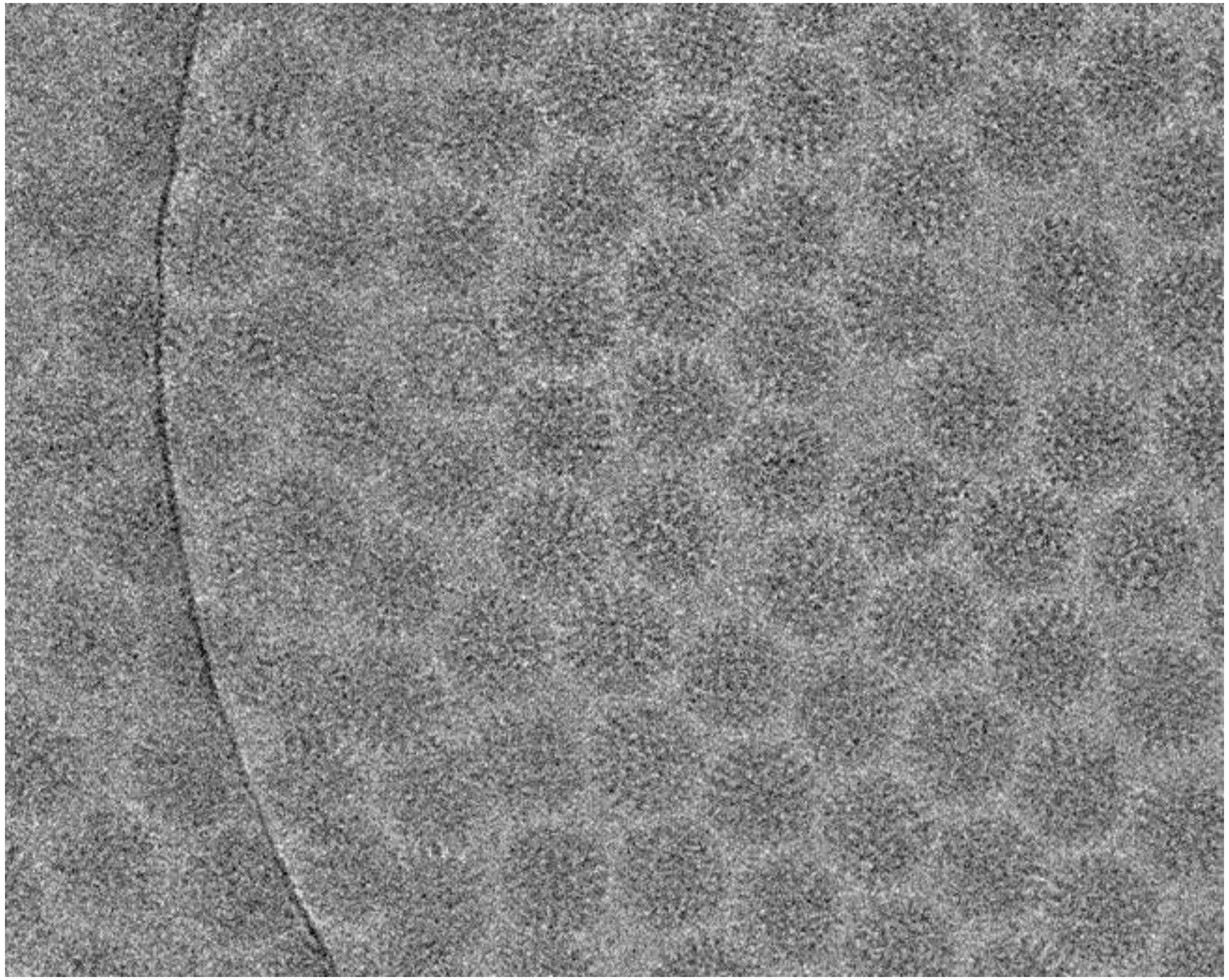
Coordinates	
x	y
3953.24	2845.63
919.78	1447.39
3864.43	348.13
3606.05	1539.54
1758.86	2971.55
...	...
3963.58	531.96

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 e^{-}/$Å^2$/frame

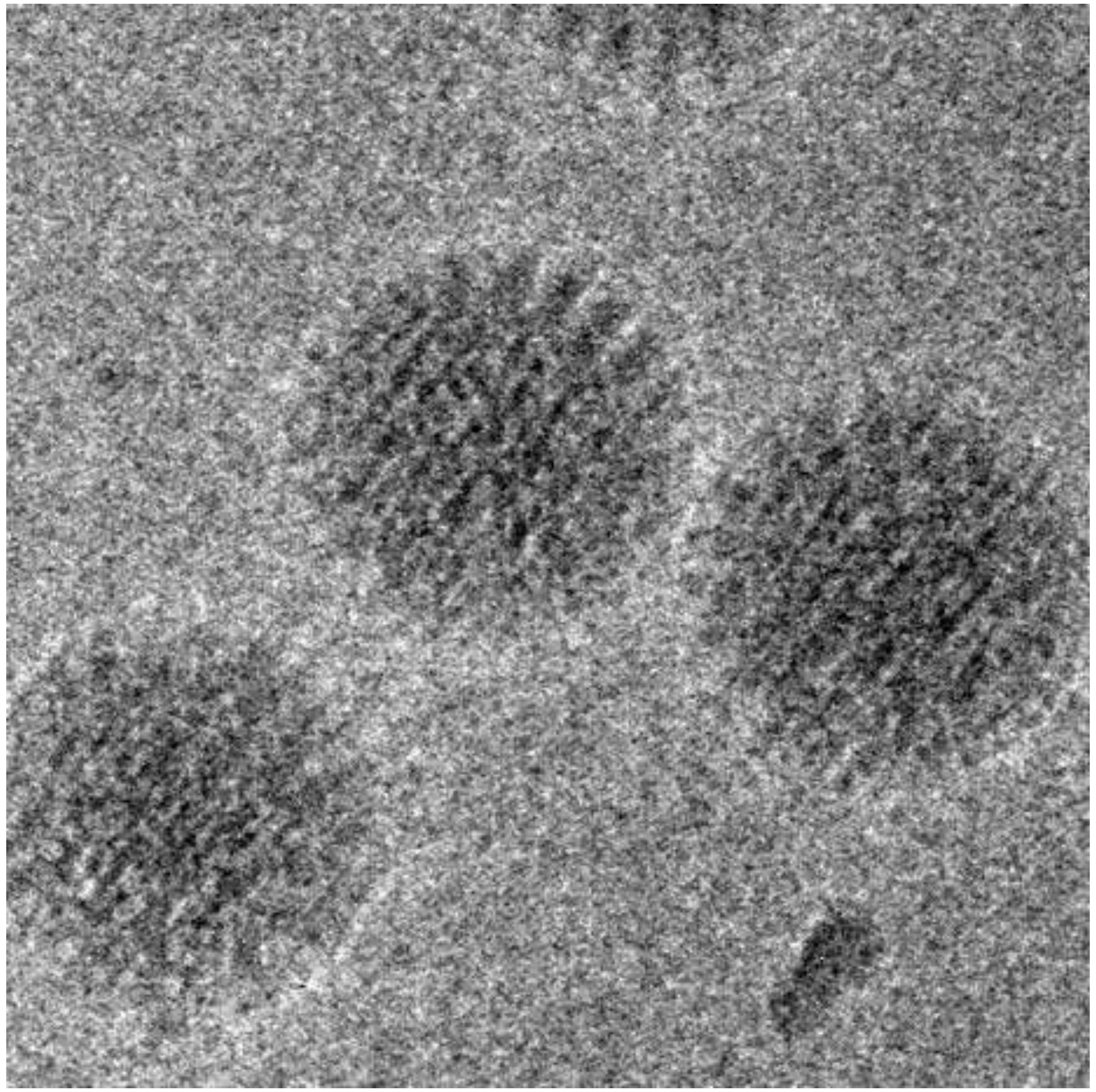
Image = Frame1 + Frame2 + Frame3 + Frame4 + Frame5

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

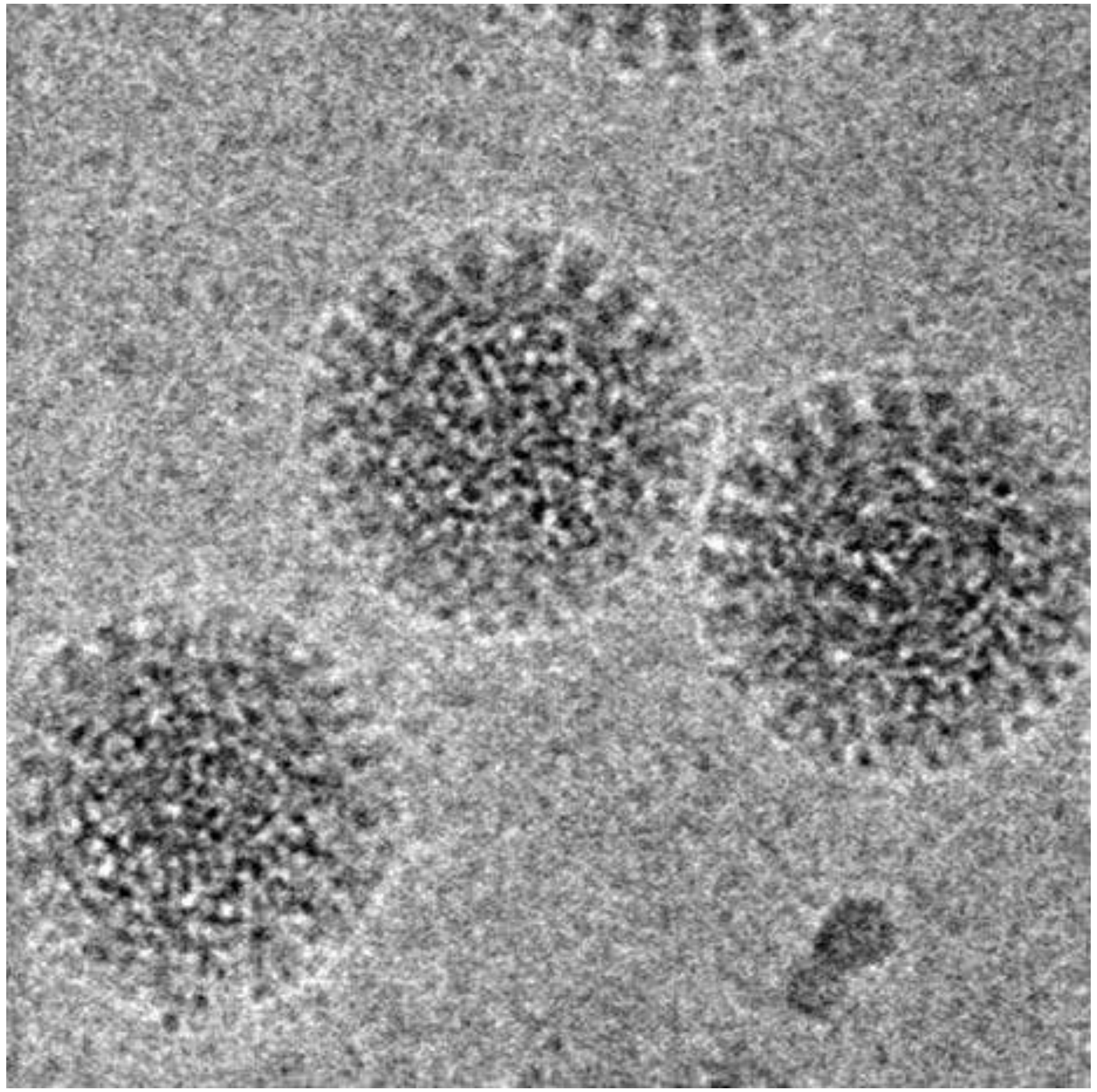
DETECTORS

Correcting for movement

60-frame average
(no alignment)



60-frame average
(translational alignment)



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

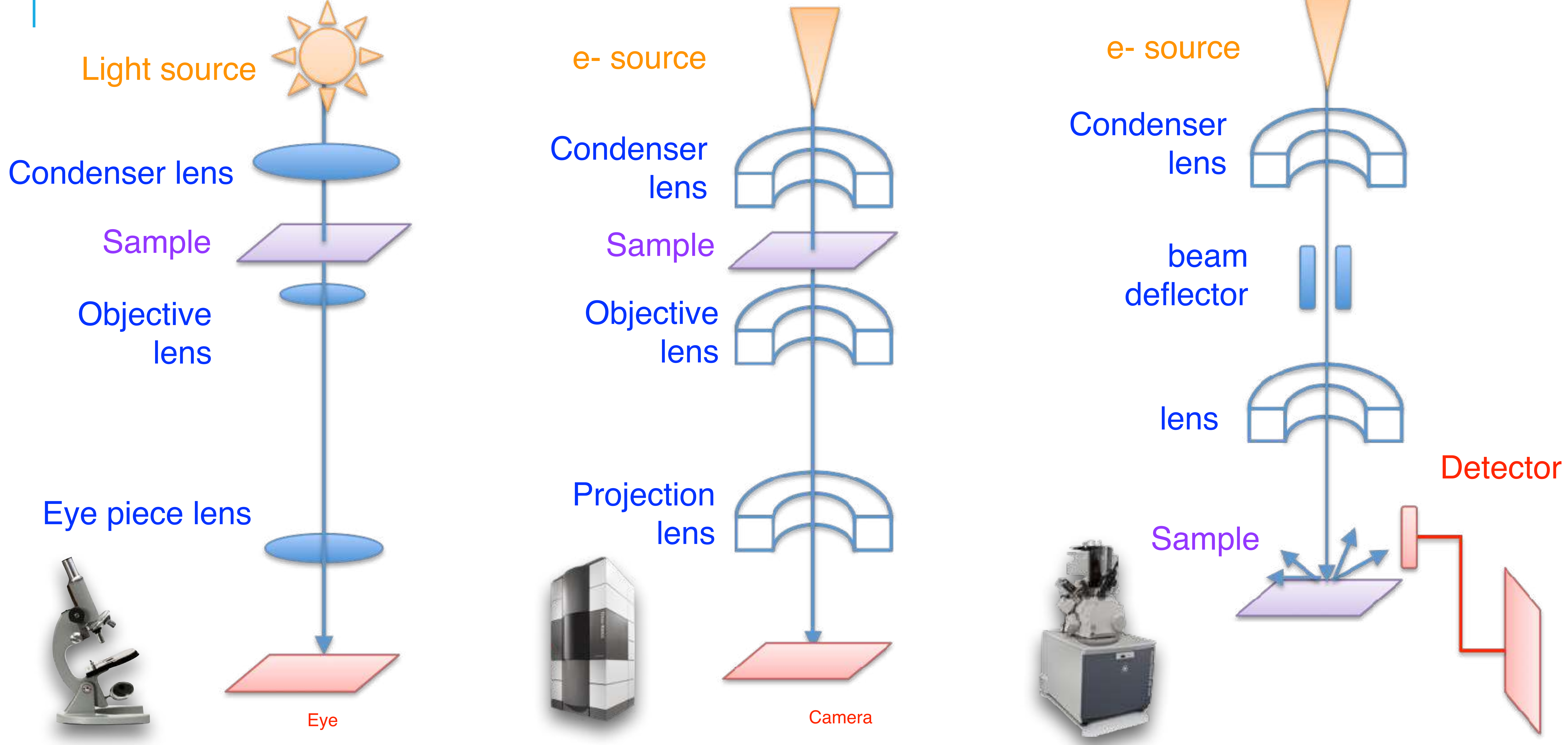
And true “atomic” resolution is possible:

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

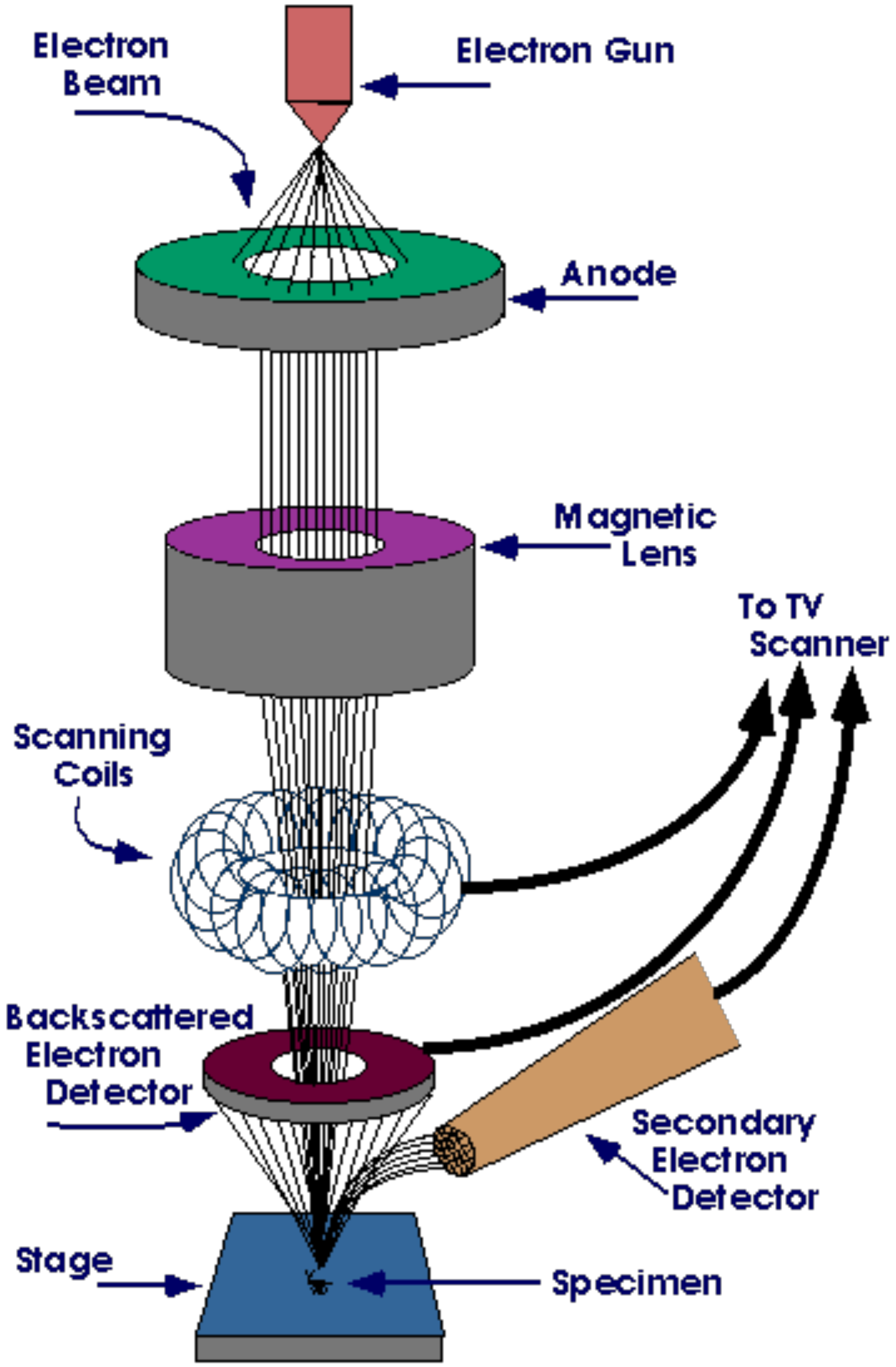
HOW ARE SAMPLES PREPARED?

WHY ELECTRONS

CRYOEM TOOLS



ANATOMY OF AN SEM



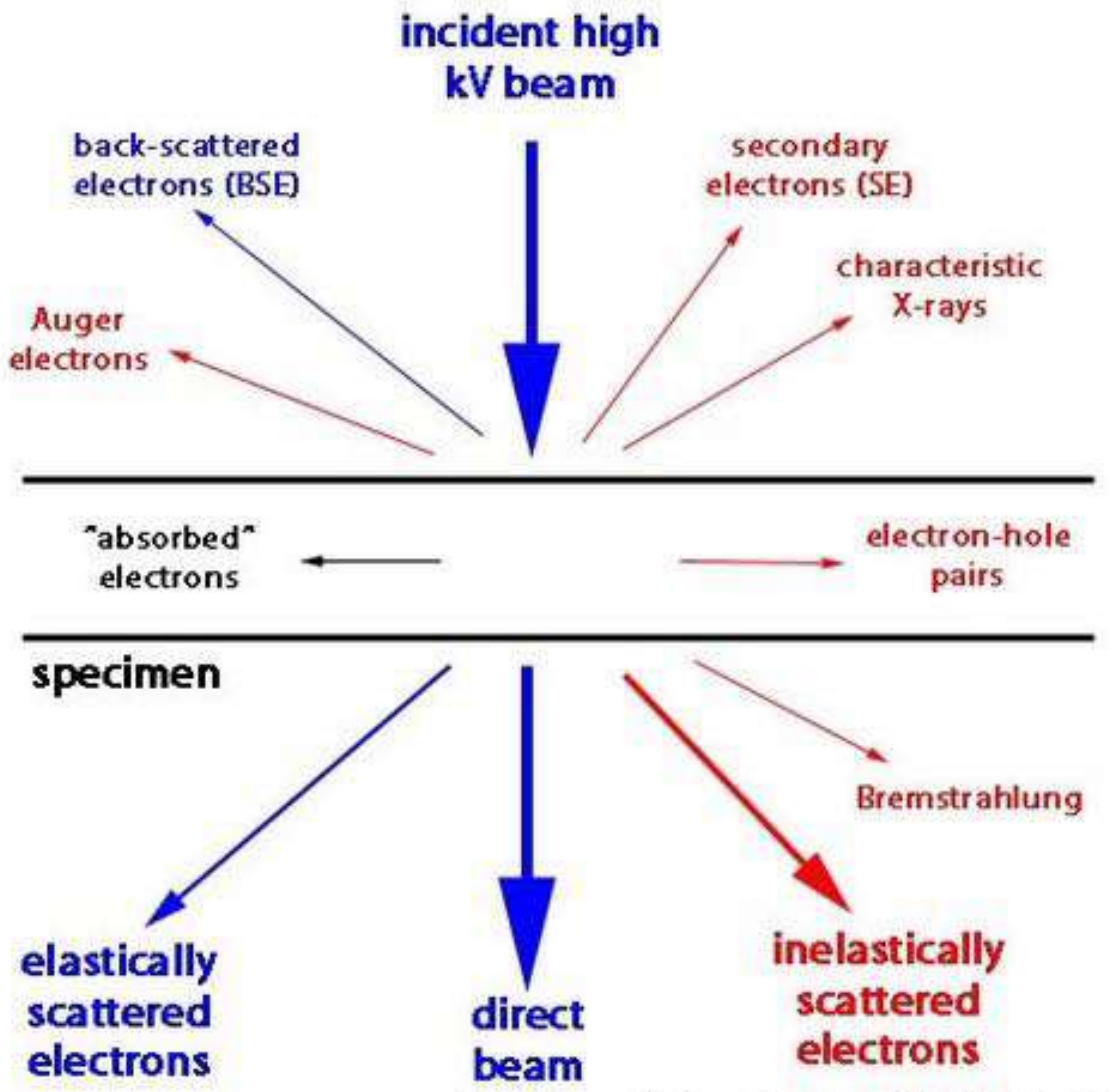
Electron gun: range from tungsten filaments in lower vacuum SEMs to FEGs which need modern high vacuum SEMs

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

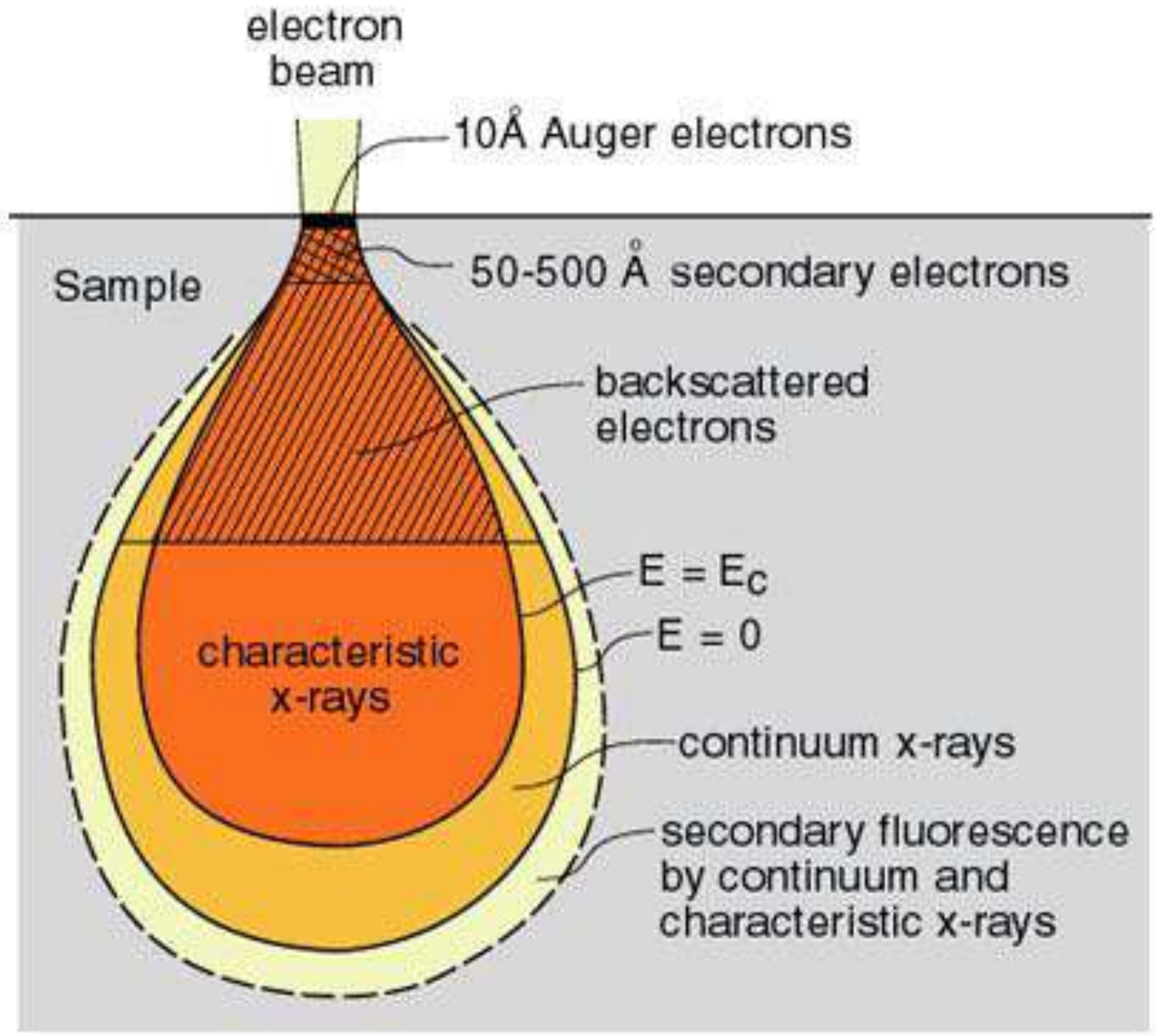
Typical resolution of SEM is between 1 and 20 nm where the record is 0.4 nm

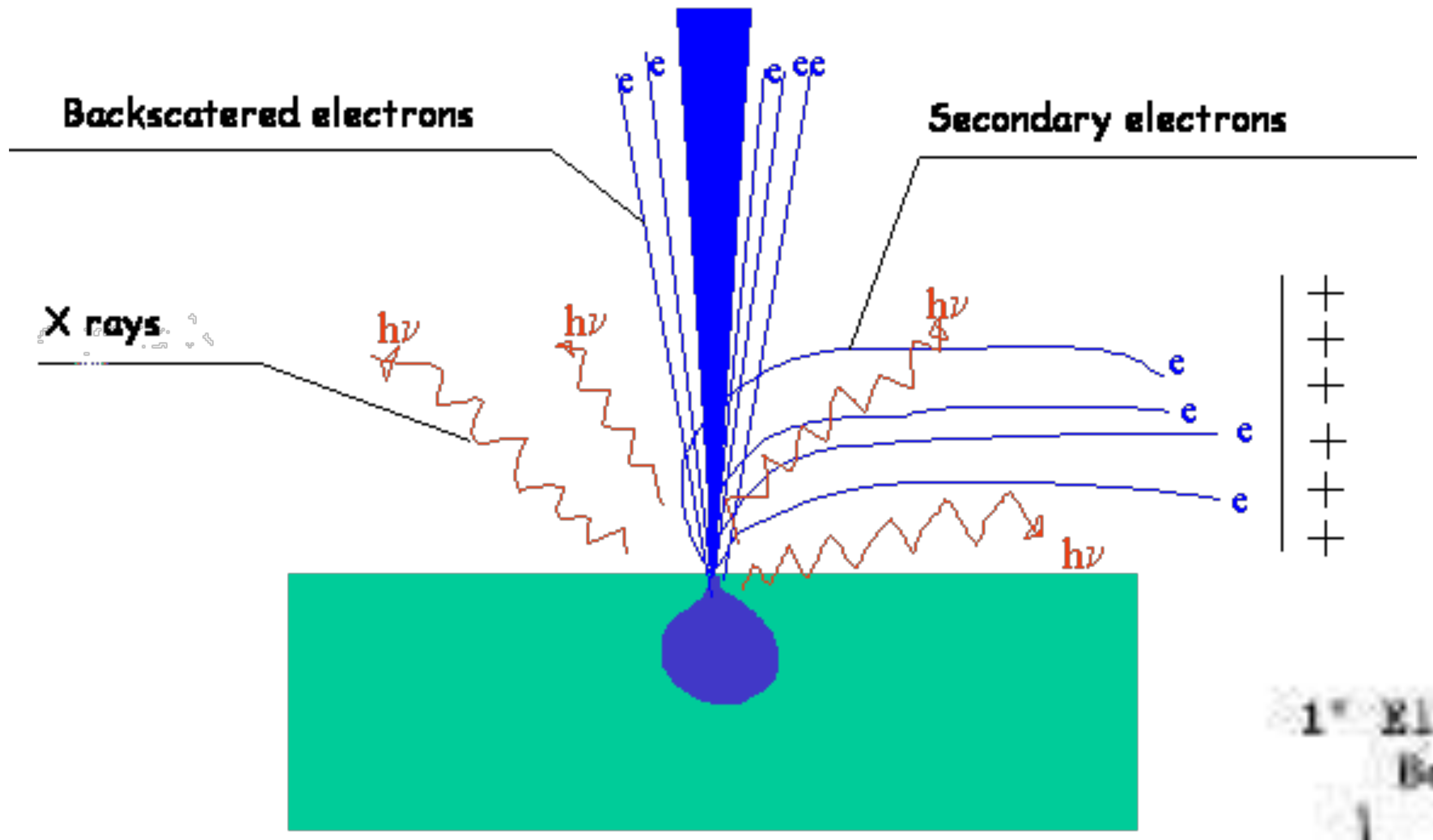
ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS

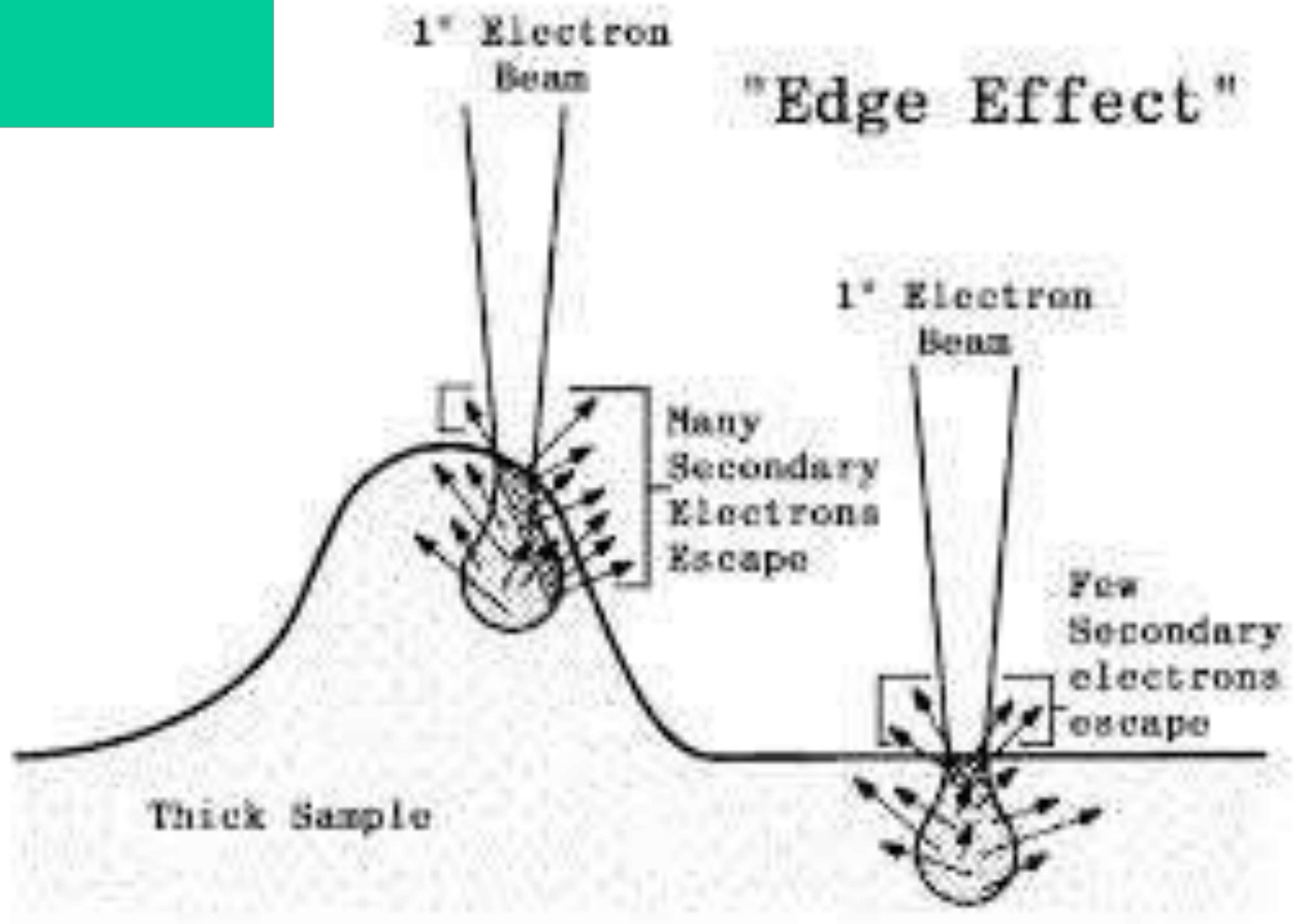


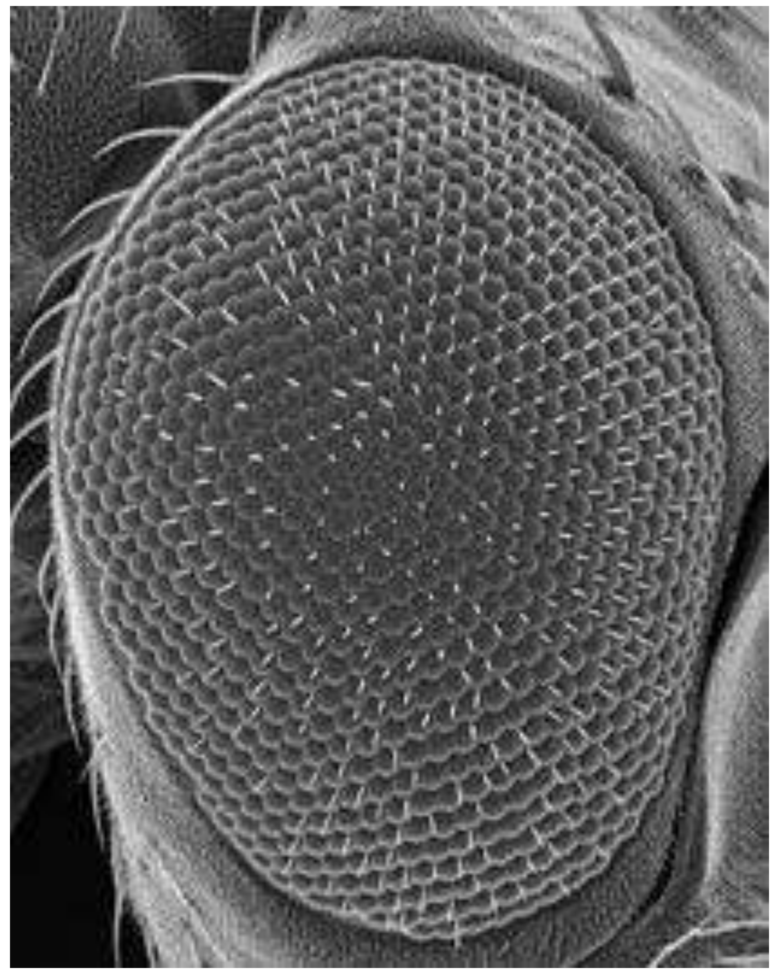
modified from Williams & Carter (1996) Fig. 1.3

ANATOMY OF AN SEM – BEAM SAMPLE INTERACTIONS &IMAGE FORMATION









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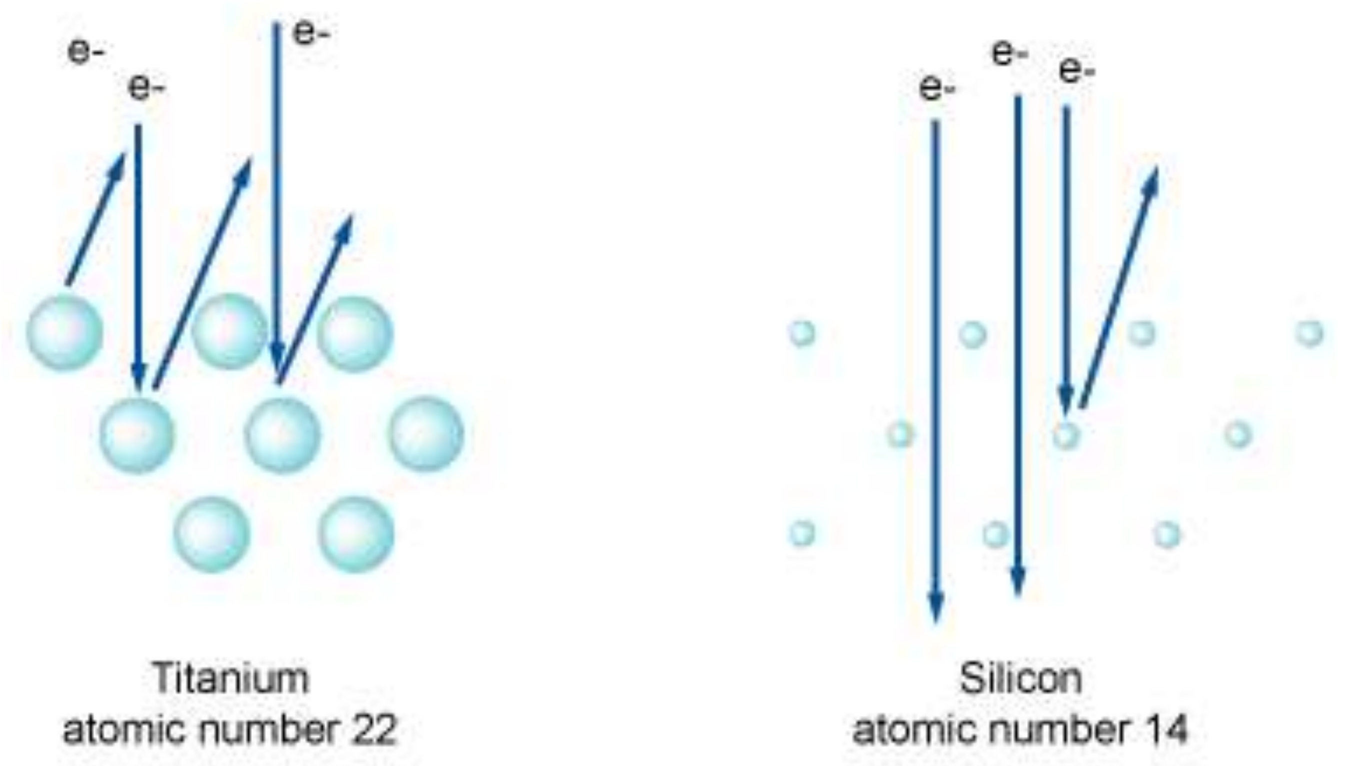

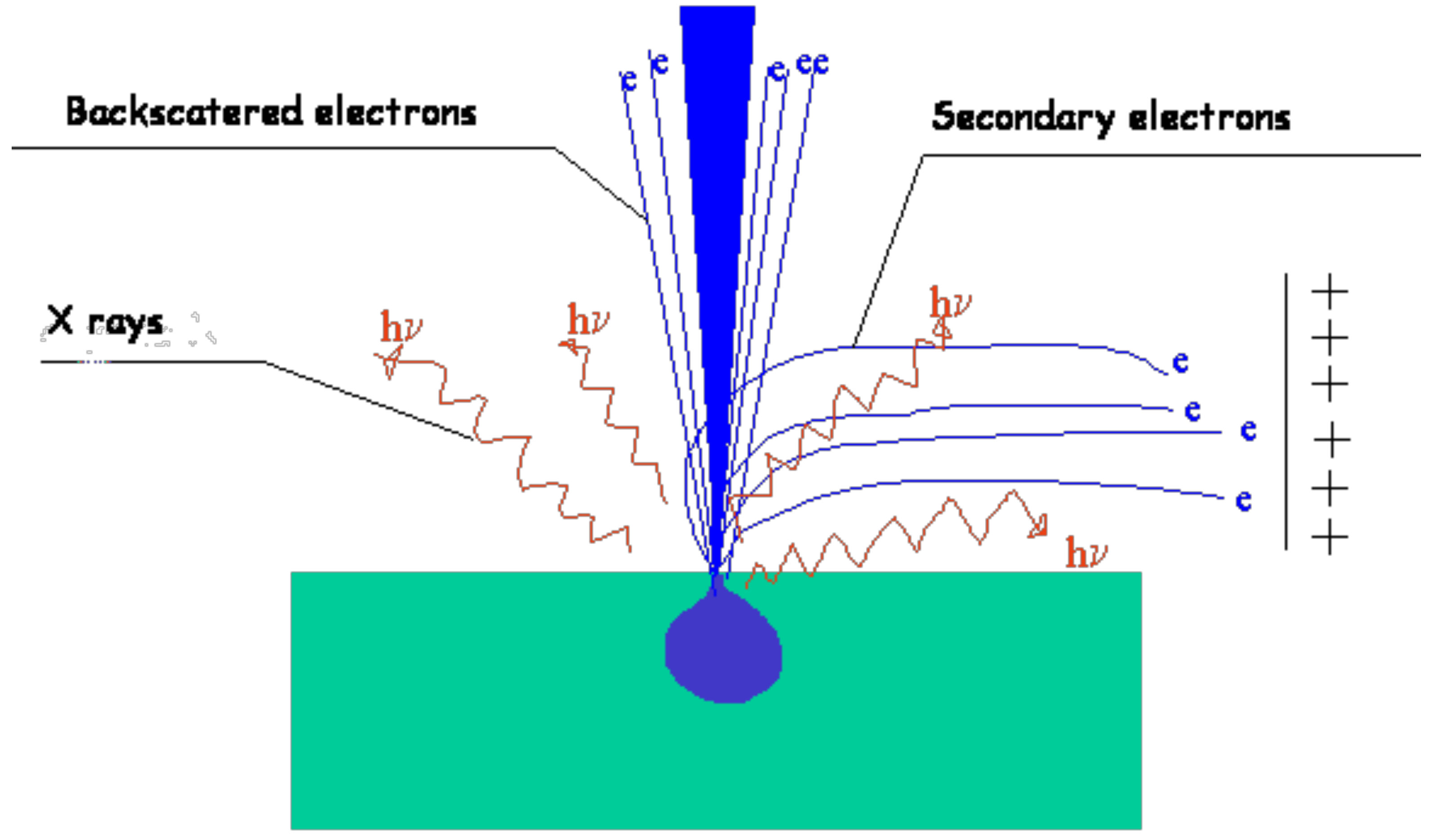

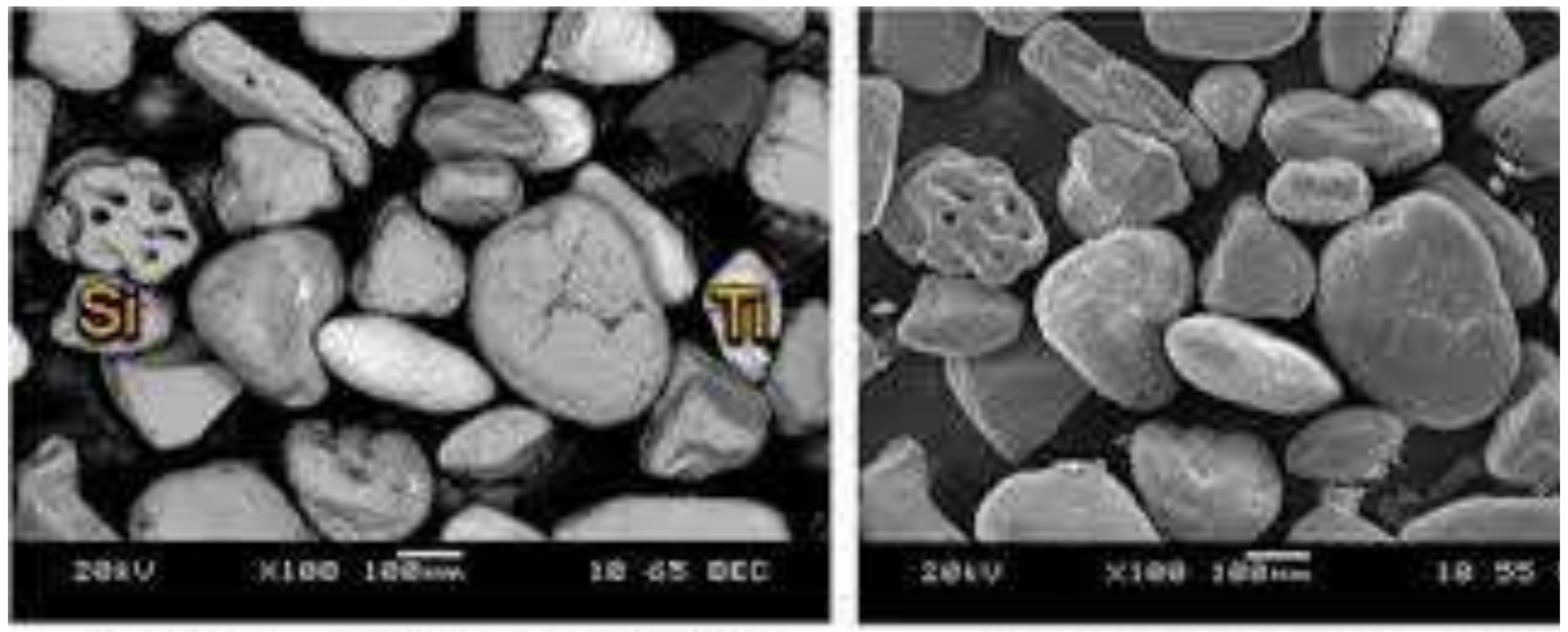

THE START

Questions?