

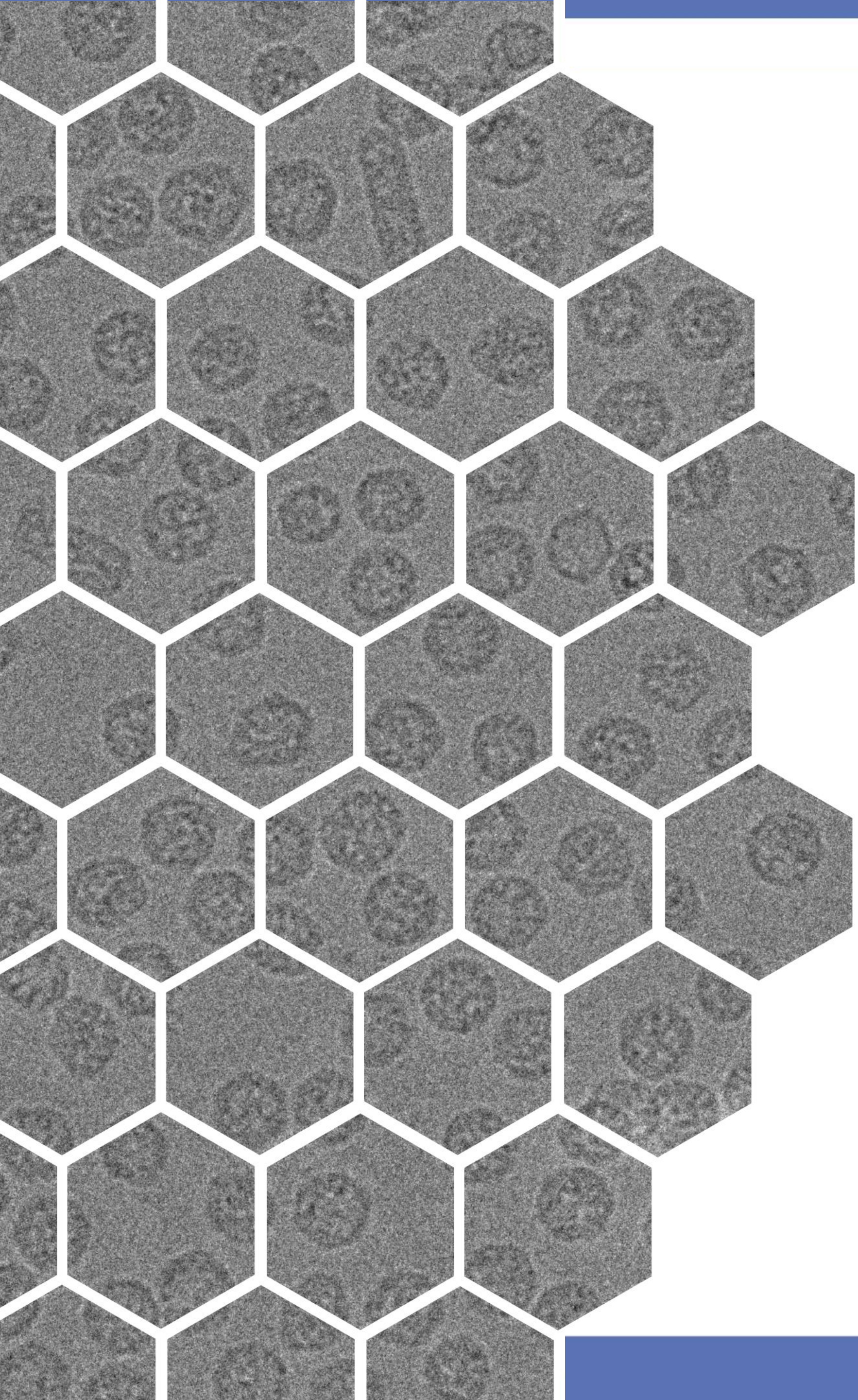
2025 Spring cryoEM course

Welcome and Anatomy of an EM

January 22, 2025

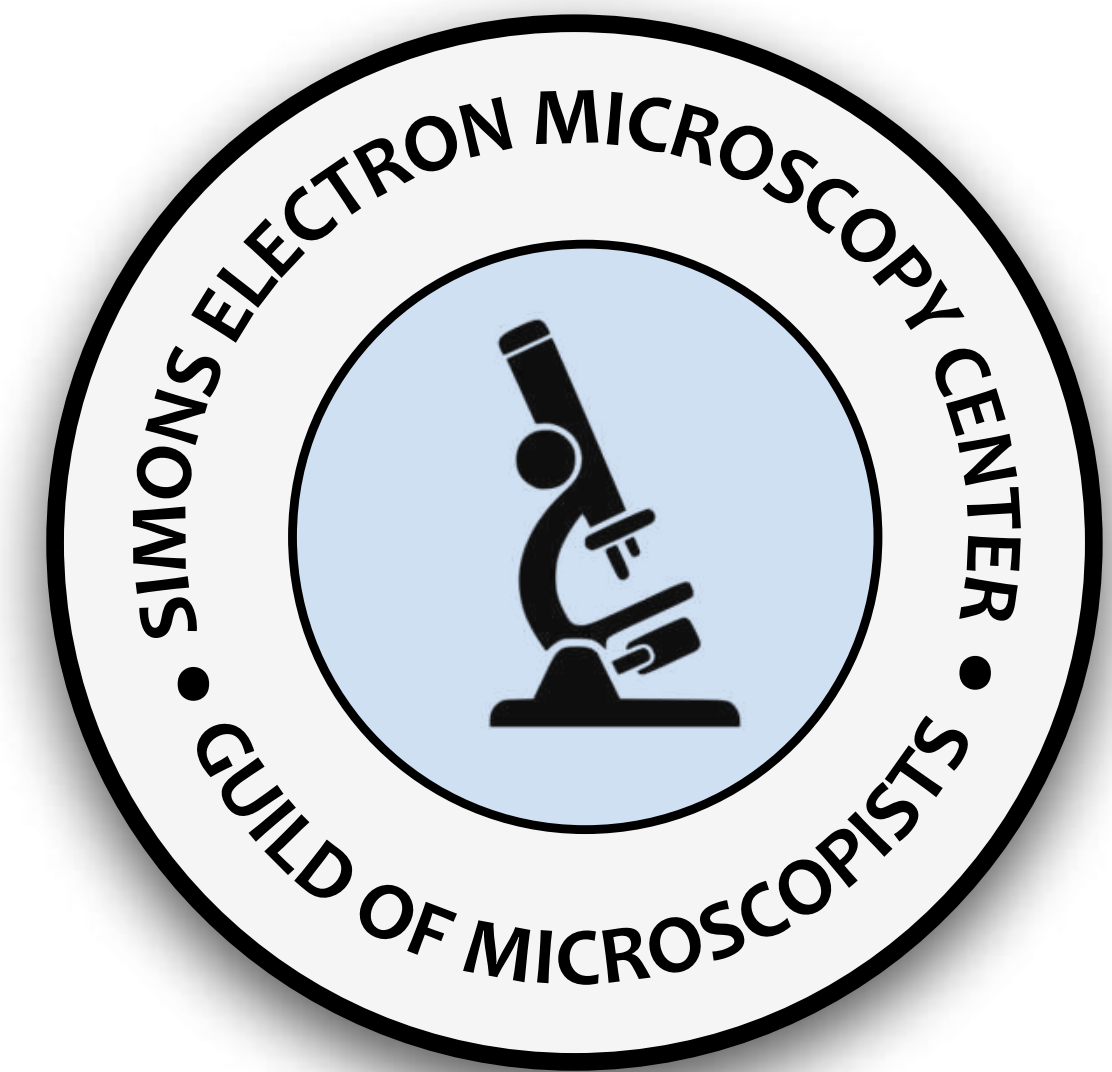


NYSBC SEMC

- 
- ◆ Course outline
 - ◆ Student survey
 - ◆ Anatomy of an EM

Welcome to SEMC

20th year of the course



Course logistics: main website

<https://semc.nysbc.org/workshops/2025-em-course/>



The screenshot shows the website header with the logo and navigation menu (HOME, ABOUT, PEOPLE, PUBLICATIONS, RESOURCES, COURSES, JOBS, FIND US). Below is a banner for 'WORKSHOPS & COURSES'. The main content area is titled 'CURRENT/UPCOMING COURSES | PAST COURSES' and lists 'EM Courses' for the years 2024 through 2016. The 2024 entry is 'The Winter-Spring 2025 EM Course', which includes a description, location, and a detailed course schedule.

EM Courses:

2024 The Winter-Spring 2025 EM Course

2023 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.

2022

2021

2020

2019 The course will be held at the New York Structural Biology Center at 89 Convent Ave (133rd St).

2018

2017 Course Schedule

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

2016

EM fundamentals section

Jan 20: Martin Luther King Holiday (no class)

Jan 22: Lecture – Introduction & Basic anatomy of the electron microscope (Ed Eng – NYSBC/SEMC & SEMC staff)

Jan 27: Lecture – New cryoEM hardware and supporting a facility (Michael Alink – NYSBC/SEMC)

Jan 29: Practical – TEM use (SEMC staff [worksheet])

Feb 3: Lecture – Considerations for biological cryoEM (Ed Eng – NYSBC/SEMC & SEMC staff)

Feb 5: Practical – Sample Preparation & Support films (SEMC staff [worksheet])

◆ Course Administrator:

- ◆ Ed Eng (NYSBC)

◆ Teaching Assistants:

- ◆ Mahira Aragon (NYSBC)
- ◆ Alex Flynn (NYSBC)
- ◆ Shubhangi Agarwal (NYSBC)
- ◆ Kasahun Neselu (NYSBC)

Course logistics: additional resources

youtube.com/nrammsemc

NRAMM SEMC NCCAT
@NRAMMSEMC · 995 subscribers · 125 videos
Official Youtube channel of the National Resource for Automated Molecular Microscopy ...more

Subscribe

Home Videos Live Playlists

Created playlists

- NCCAT - NCITU TOMO short course April 10-14, 2023 5 videos
- NCCAT SPA short course March 14-18, 2022 12 videos
- SEMC 2022 Winter-Spring EM Course 10 videos
- SEMC Training Videos 8 videos
- SEMC 2021 Winter-Spring EM Course 16 videos

cryo-em-course.caltech.edu/videos

Caltech Getting Started in Cryo-EM

Welcome Course Overview Outline Lecture Videos Instructor Links

WELCOME TO THE COURSE

Before diving into the lecture videos, start by watching the [trailer](#) and reading the course [overview](#) and [outline](#).

We hope you enjoy learning about cryo-electron microscopy (cryo-EM)!

Getting Started in Cryo-EM with Professor Grant Jensen

CRYOELECTRON MICROSCOPY LABORATORY

Established with generous support from
The Gordon and Betty Moore Foundation
and
The Agouron Institute

cryoem101.org

CryoEM 101 **CryoET 101**

HOME CRYOEM 101 CHAPTERS CRYOET 101 CHAPTERS ABOUT CONTACT

Cryo-EM and Cryo-ET are emerging methods to image biological specimens at ever-improving resolutions.

The purpose of CryoEM and CryoET 101 is to teach the principles of both techniques using a media-rich approach with videos, animations, interactive simulations, and real data that cover relevant steps along a typical project workflow. If you are working with purified proteins or protein complexes, we invite you to start with CryoEM 101. If you are interested in imaging cells, subcellular components, or otherwise heterogeneous biological materials, CryoET 101 may be more suitable for you.

- CryoEM 101**
 - Ch. 1: Sample Purification
 - Ch. 2: Cryo-EM Grid Preparation
 - Ch. 3: Grid Screening & Evaluation
 - Ch. 4: Cryo-EM Data Collection
 - Ch. 5: Image Processing
- CryoET 101**
 - Ch. 1: Is Cryo-ET for You?
 - Ch. 2: From Sample to Cryo-ET Grid
 - Ch. 3: Zoning into Regions of Interest
 - Ch. 4: Data Collection
 - Ch. 5: Image Processing & 3D Reconstruction
 - Ch. 6: Tomogram Annotation & Subtomogram

Course logistics: main topics

Section 1 : EM fundamentals

Section 2 : EM crystallography

Section 3 : Single Particle Analysis

Section 4 : Tomography Short Course
March 31 -April 4

Section 5 : Future perspectives



Course logistics: main topics



**NYSBC-SEMC
TOMO short course**
March 31-April 4, 2025

NCITU

NCCAT

 **1 WEEK
SHORT
COURSE**

 **MORNING
LECTURES &
ROUNDTABLES**

 **AFTERNOON
HANDS-ON
PRACTICALS**

Course logistics: class for credit

Component Percentage

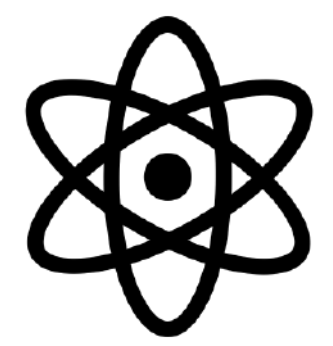
Recitation/Participation 50%

- *JC/HW/questions*

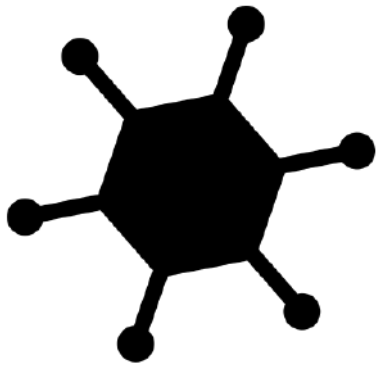
Practicals 10% × 3

Attendance 20%

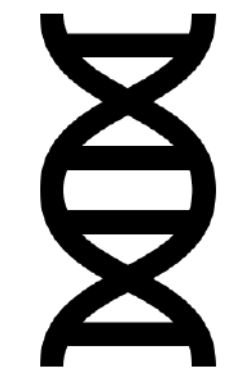




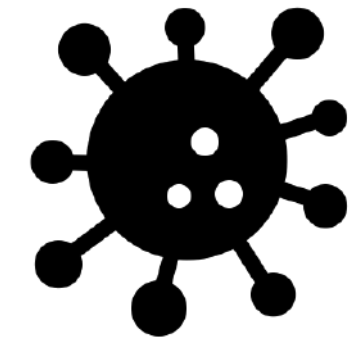
atoms
1 Å



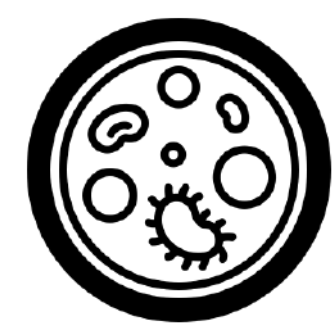
Small molecules
1 nm



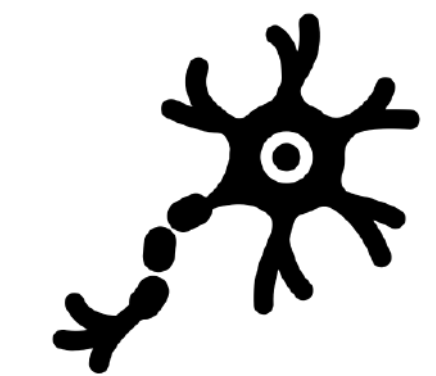
Biological macromolecules
10-100 nm



Organelles
prokaryotic cells
0.1-5 μm



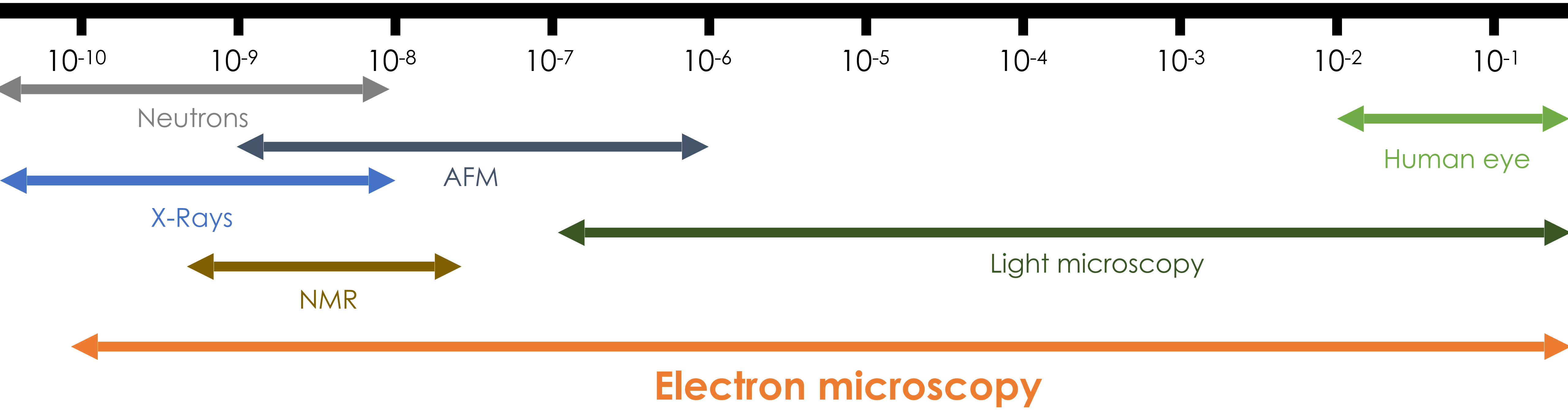
Eukaryotic cells
5-100 μm



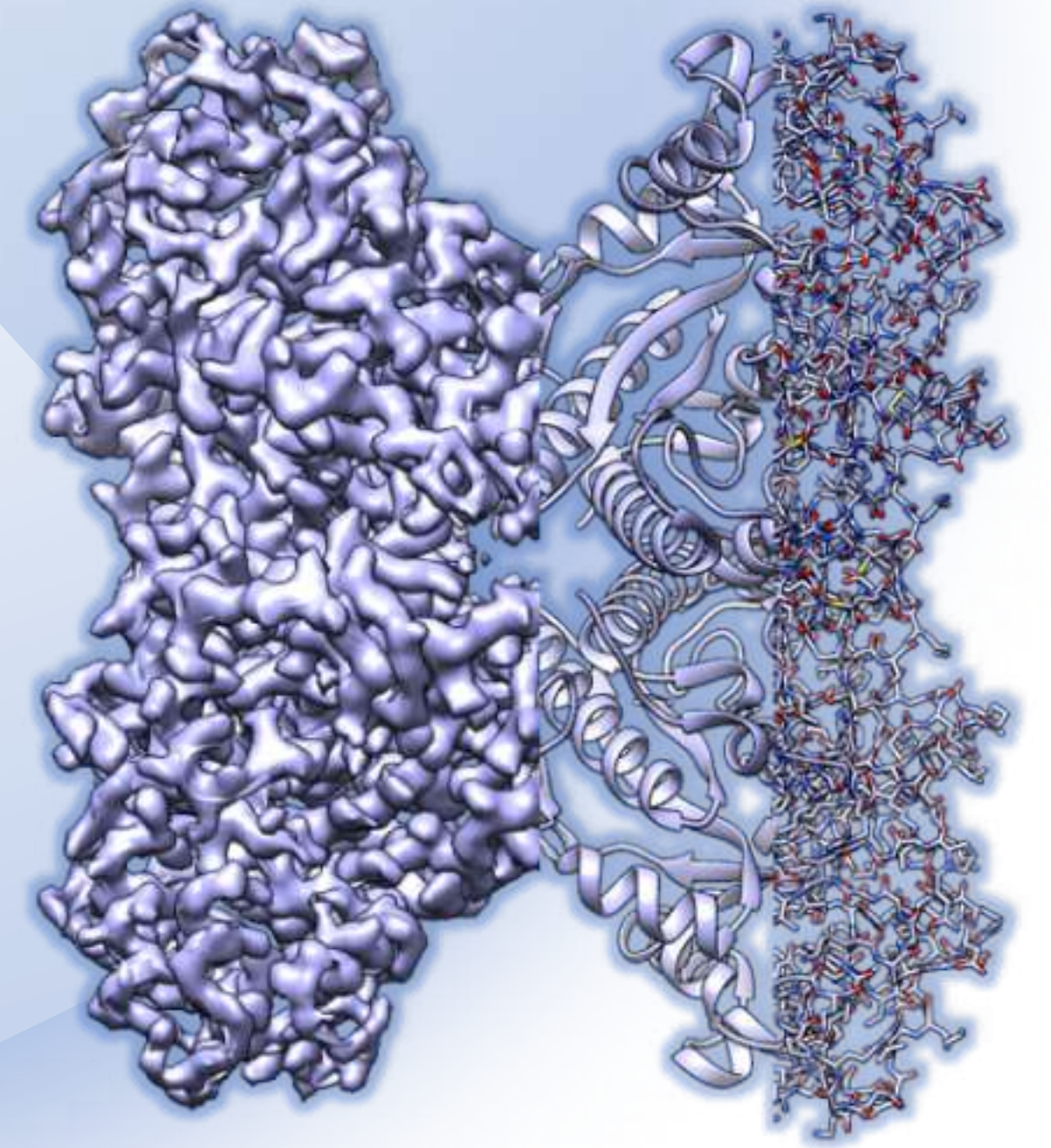
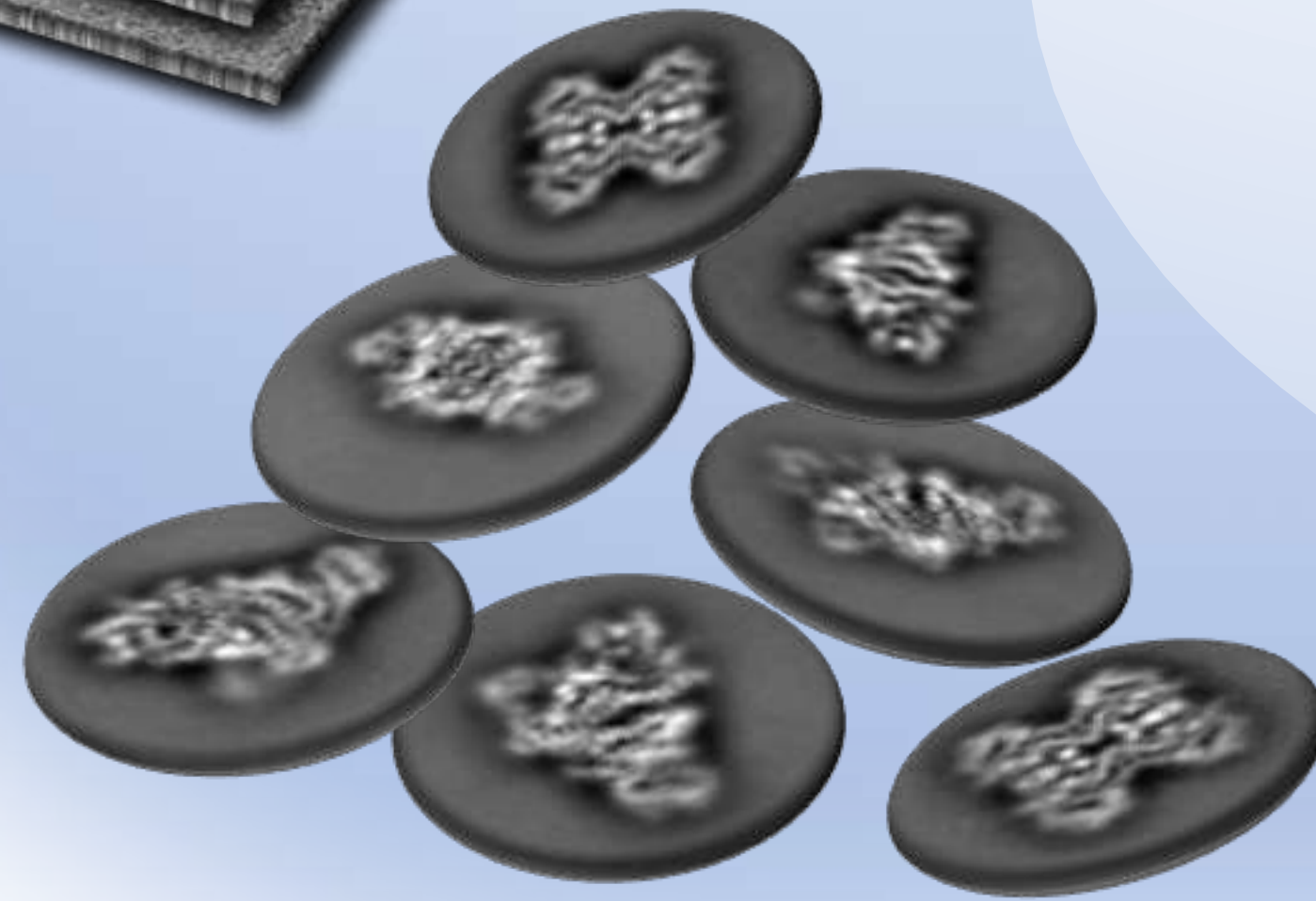
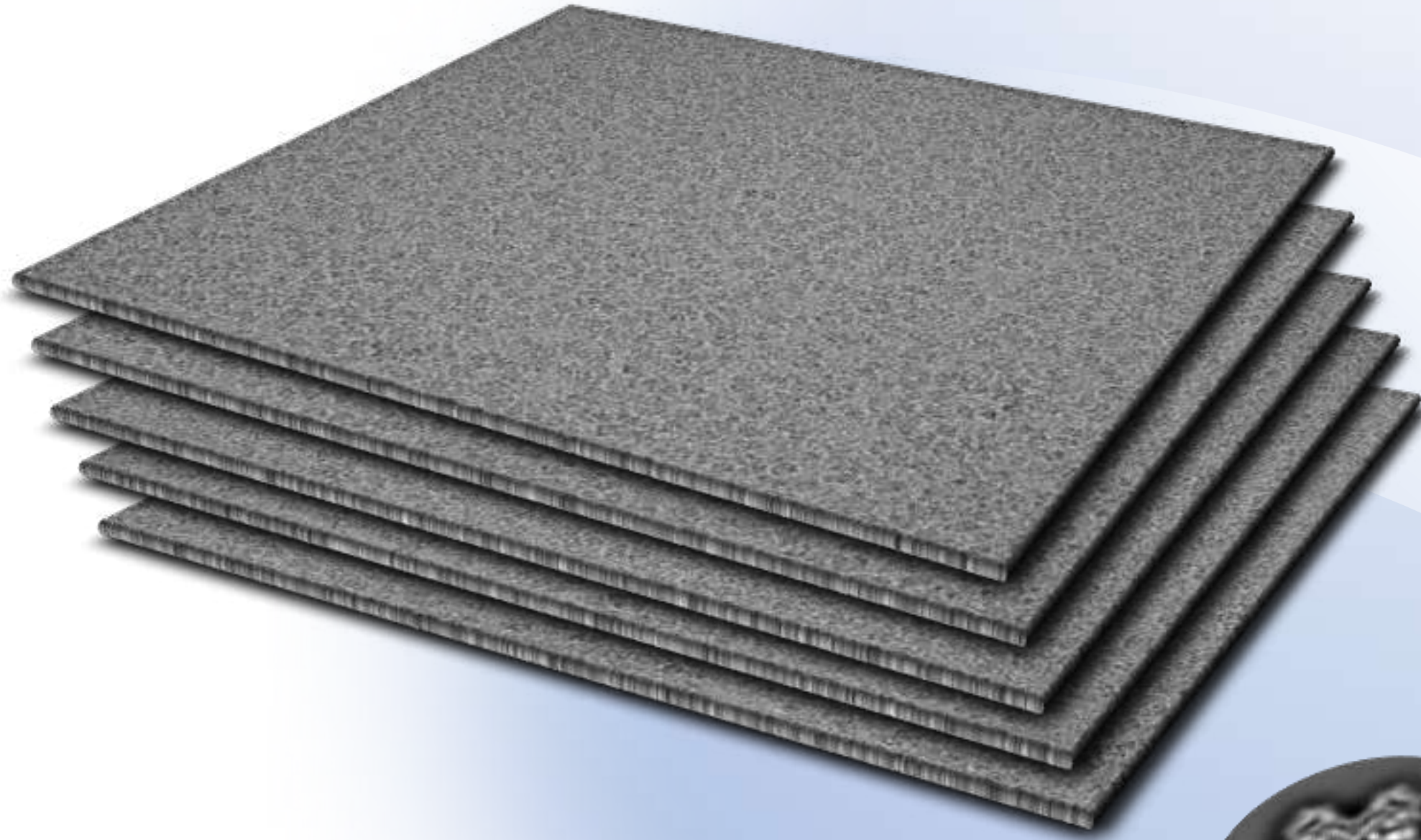
Neurons
mm



organs & organisms
cm-m



What is possible today?



What brought about the resolution revolution?

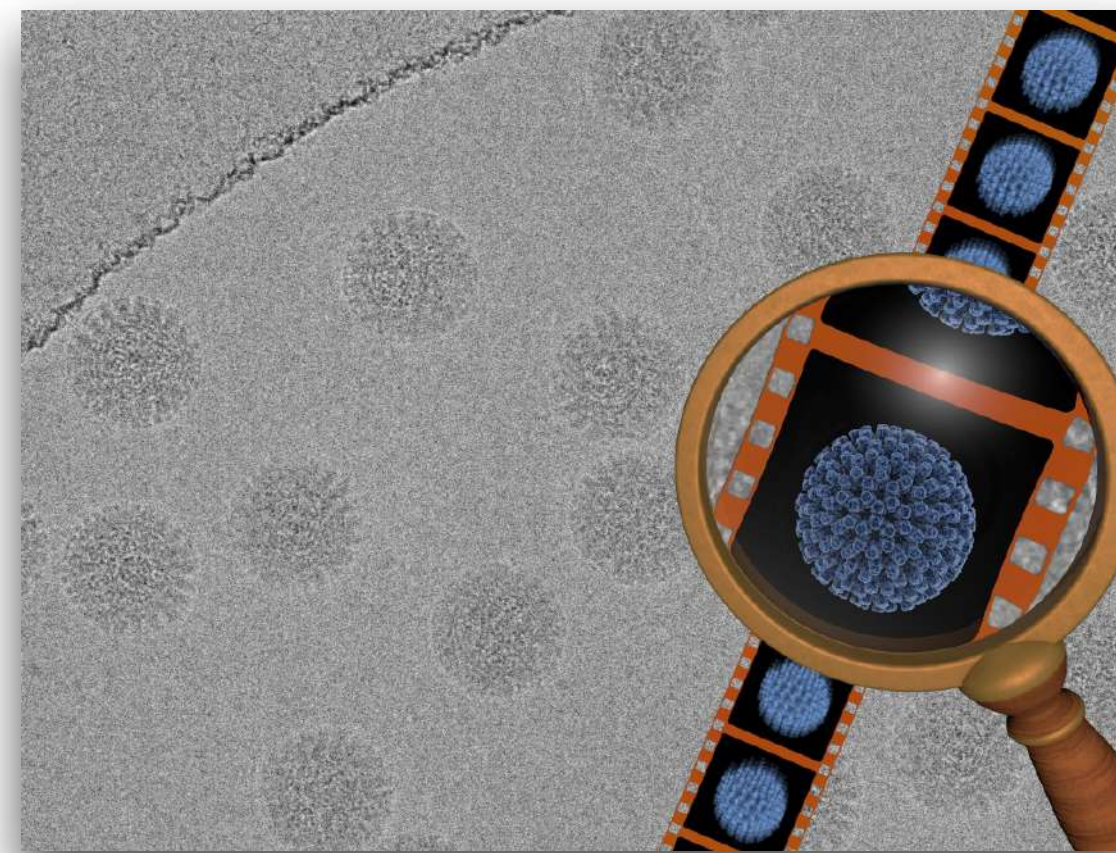
(~2012-2014)

Hardware

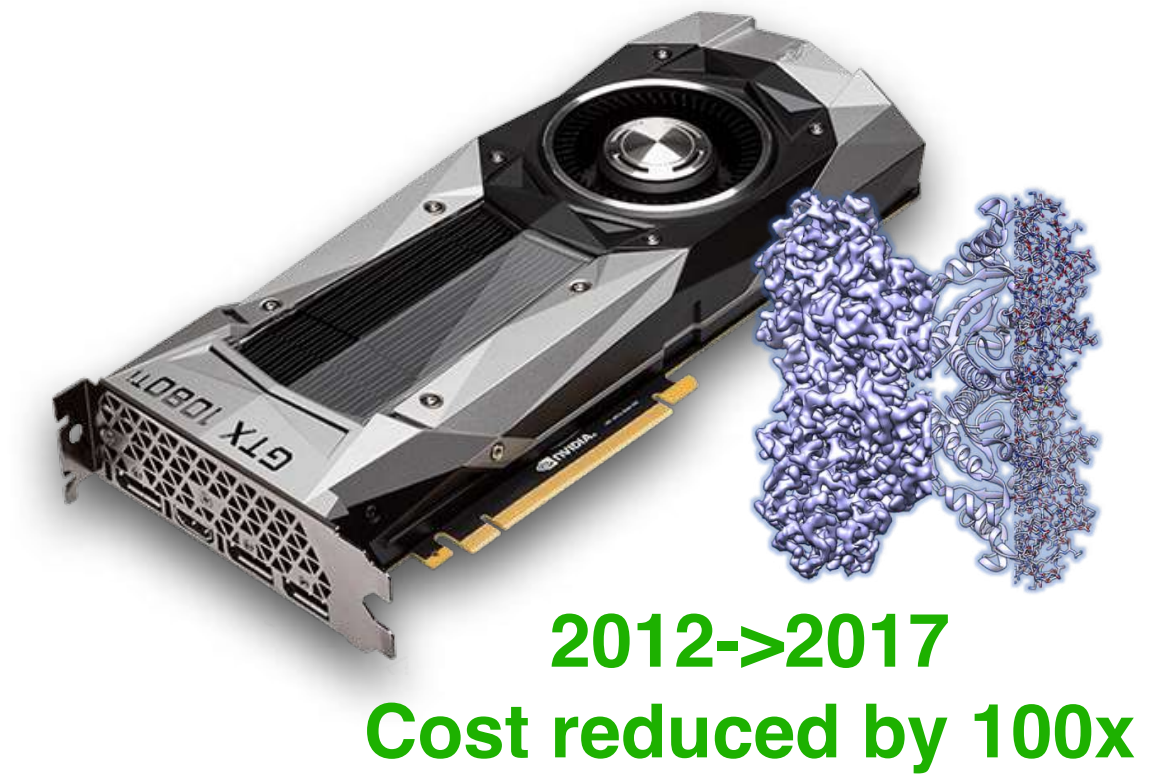
Microscopes



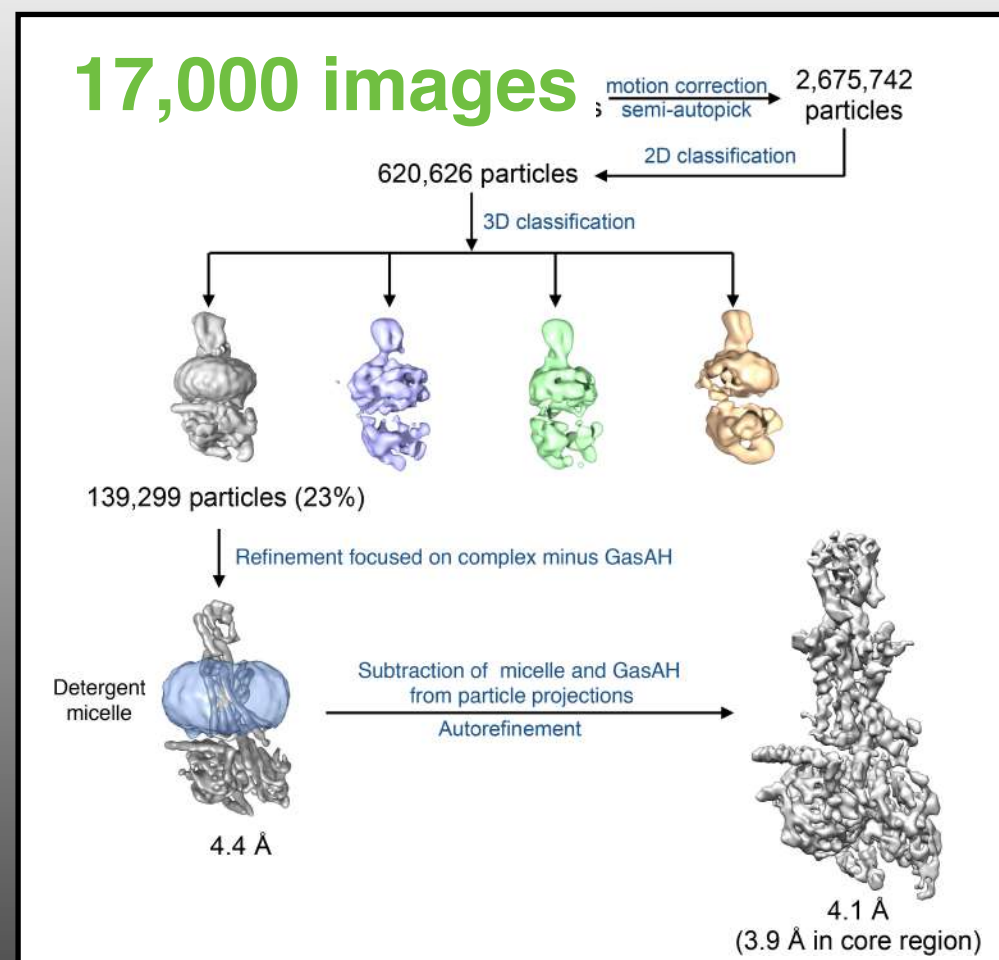
Direct Detectors



Computers



Software



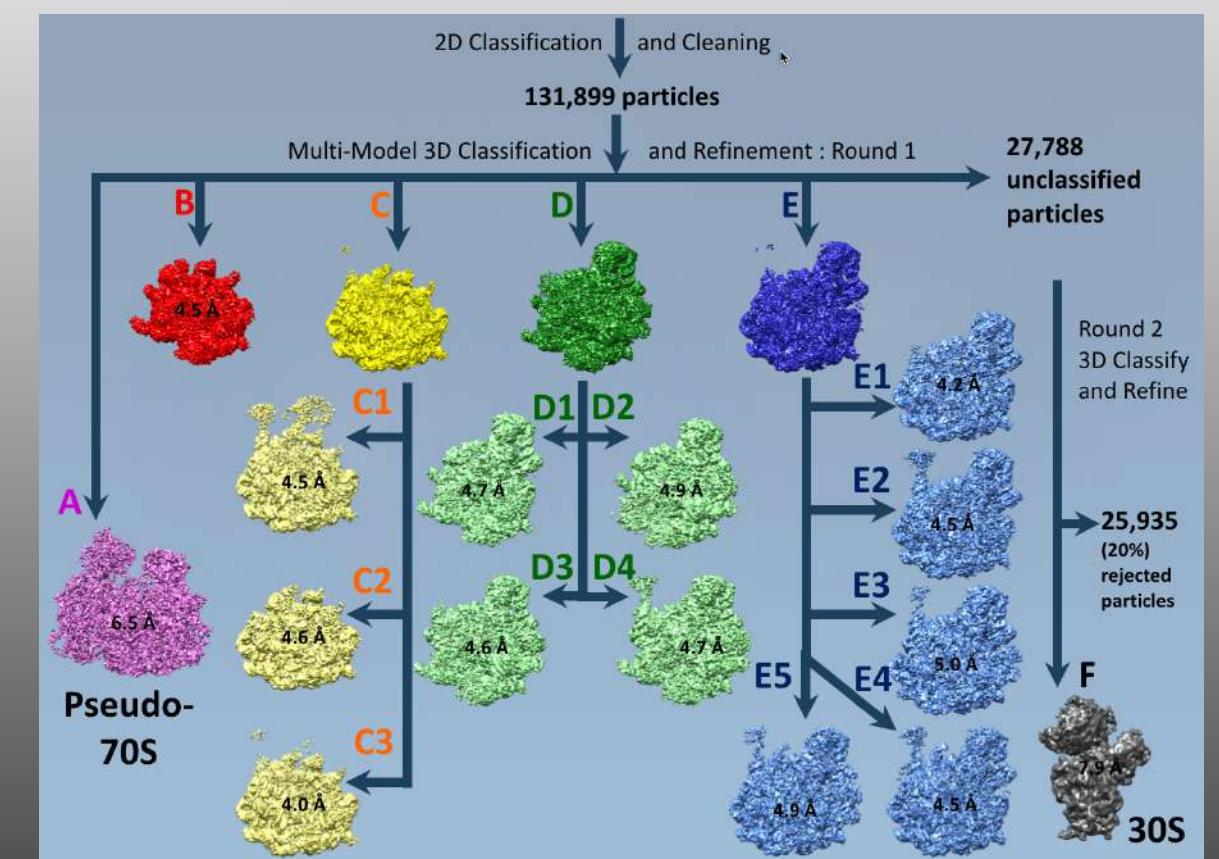
5% used in map!

Leginon / SerialEM / EPU, ...

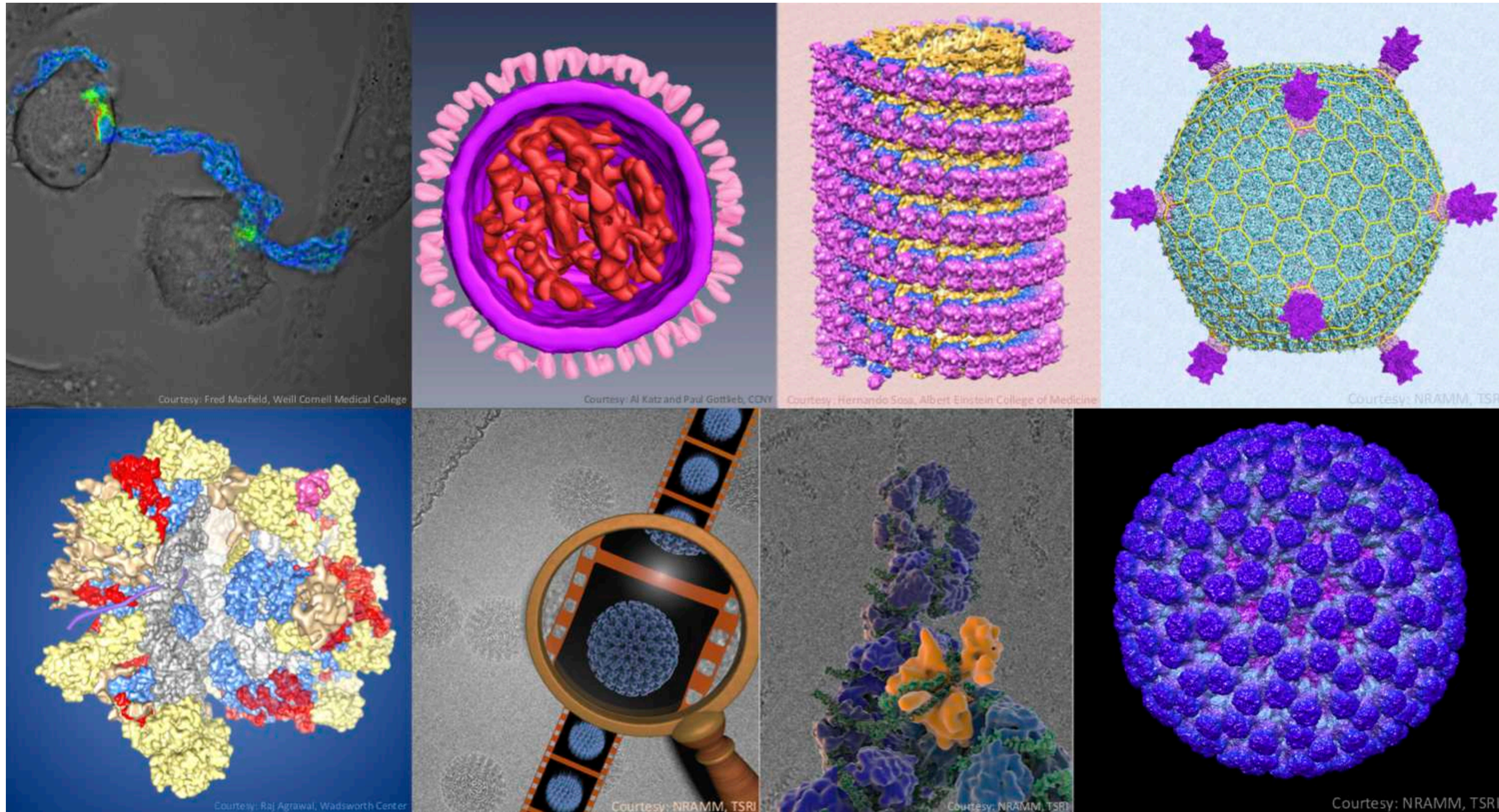
MotionCorr2, Unblur, ...

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

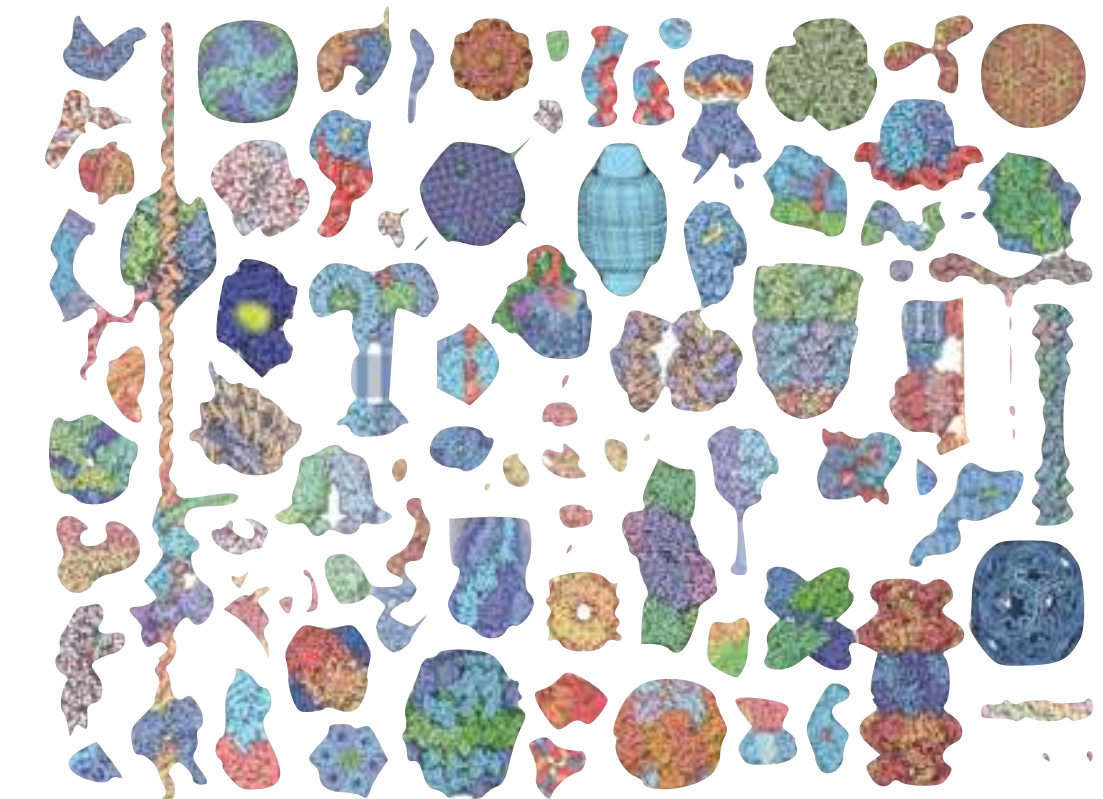
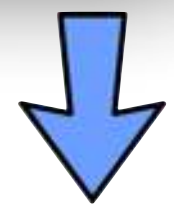
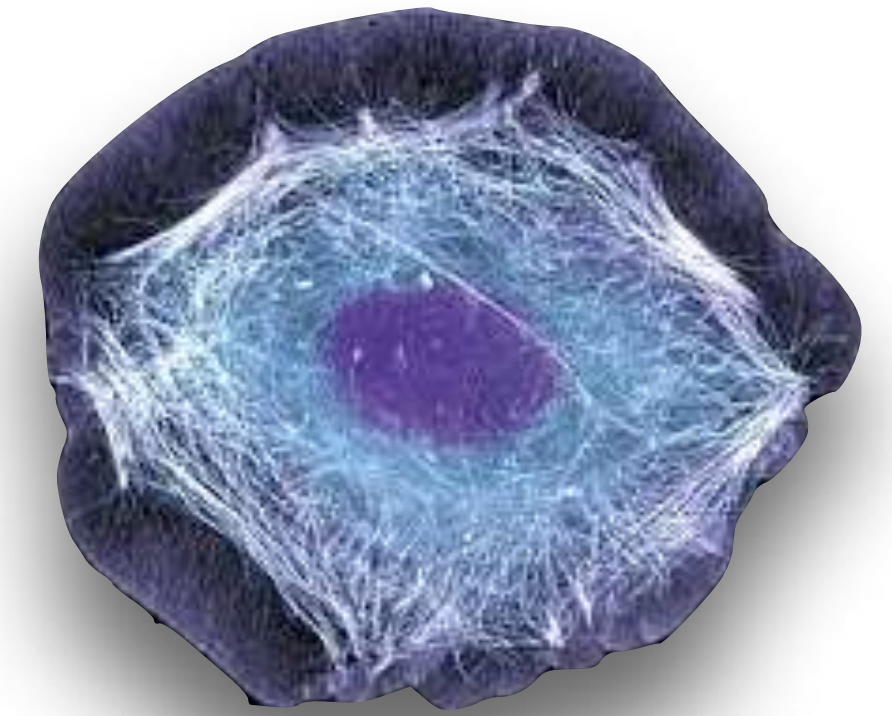
14 independent structures



cryoEM: a technology on the rise



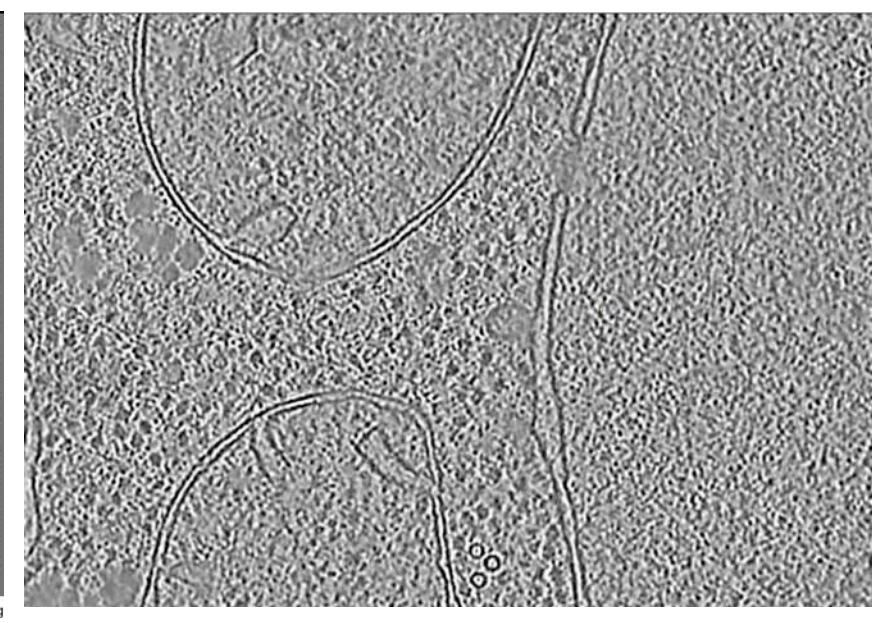
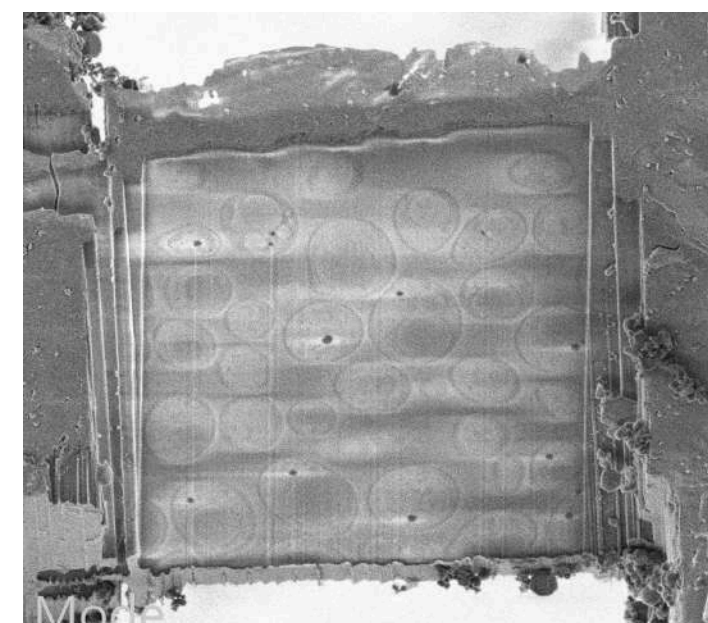
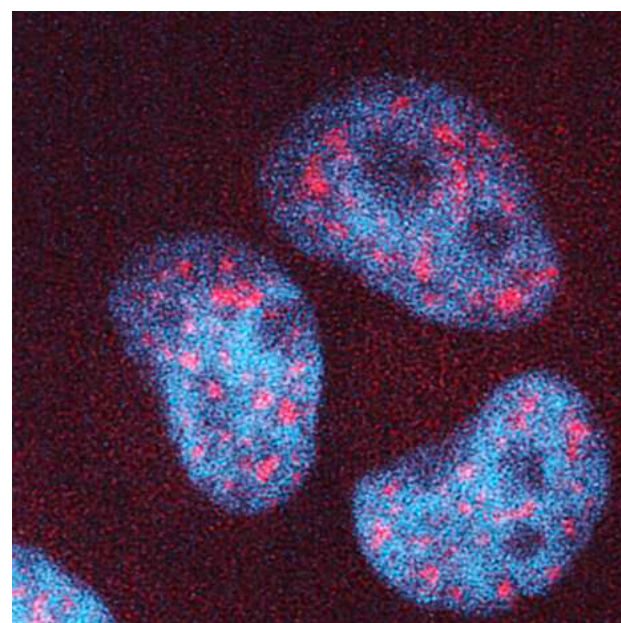
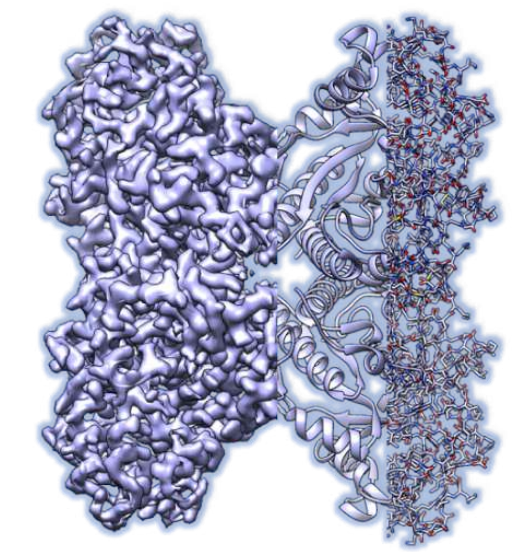
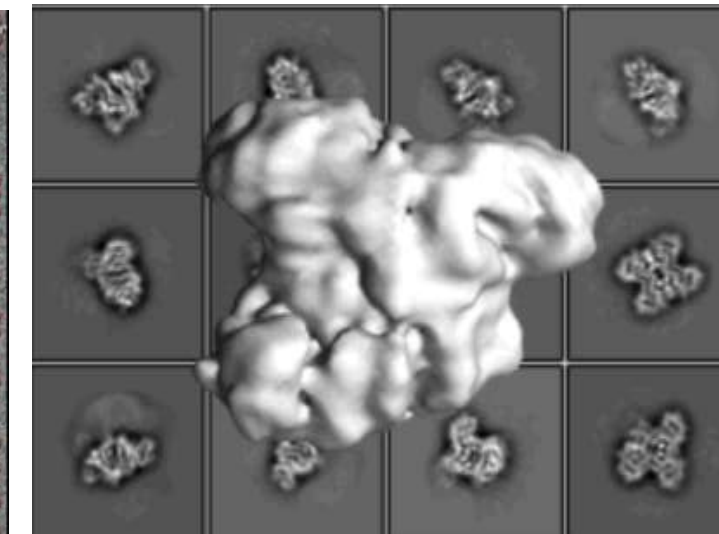
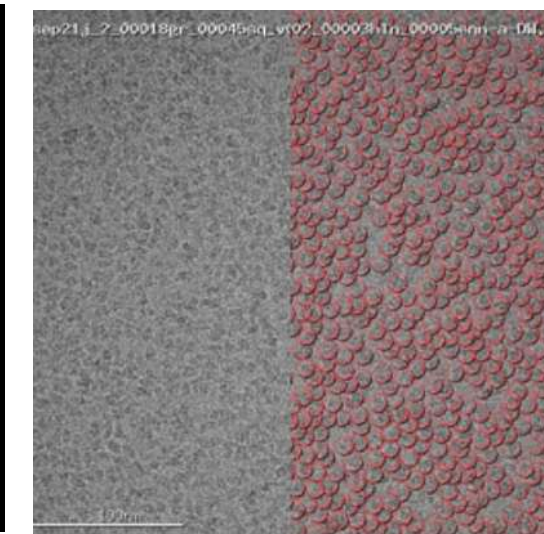
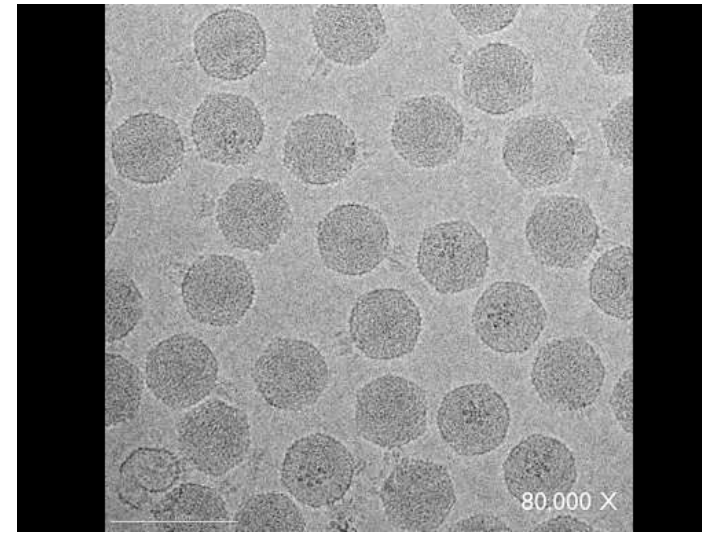
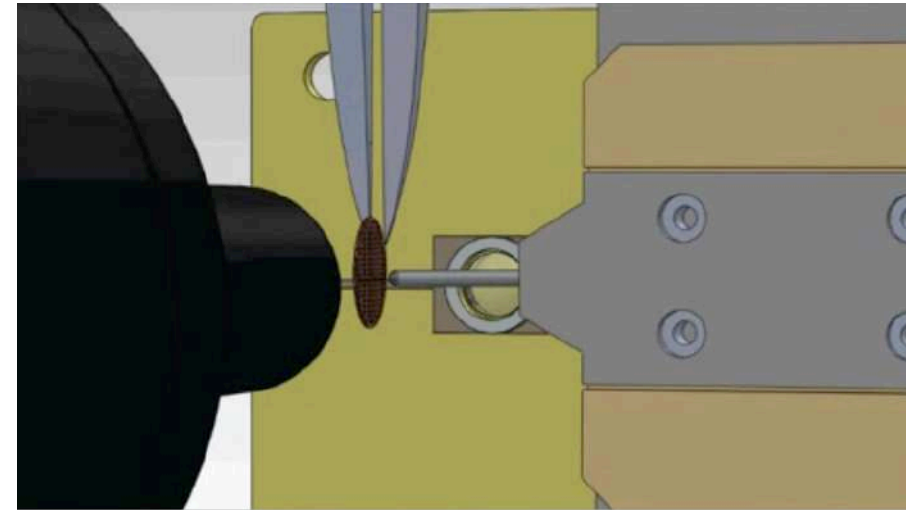
next steps



coming soon

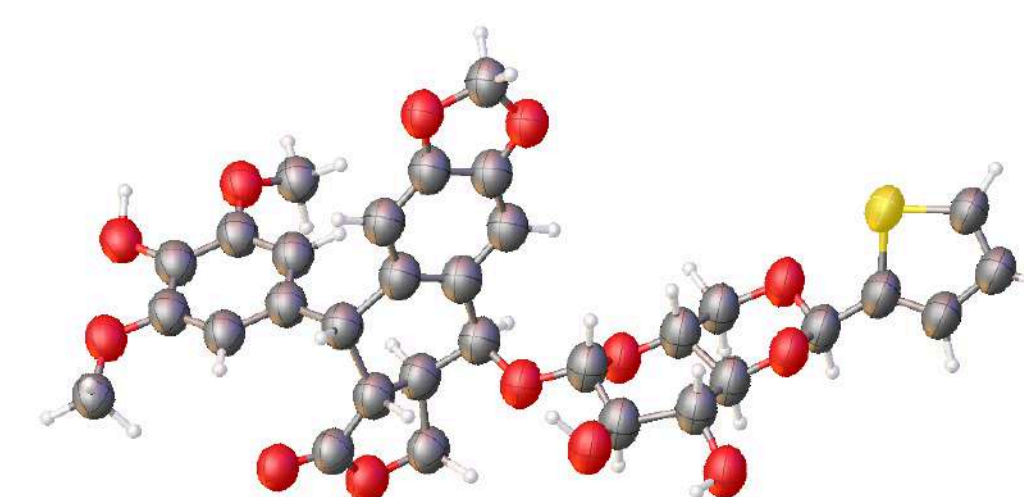
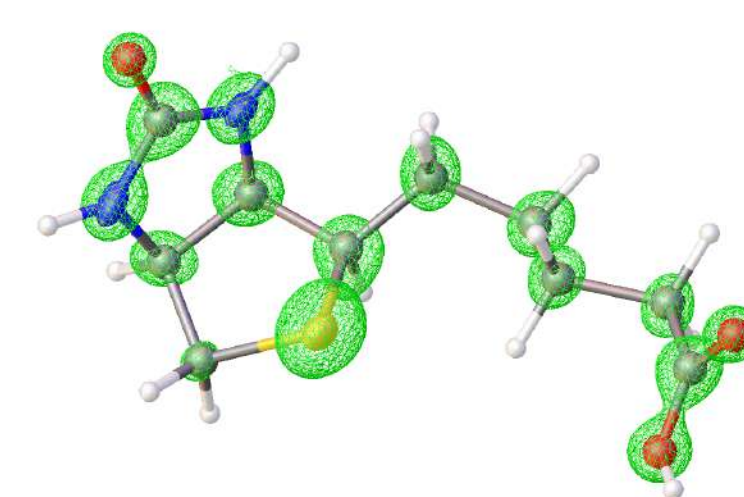
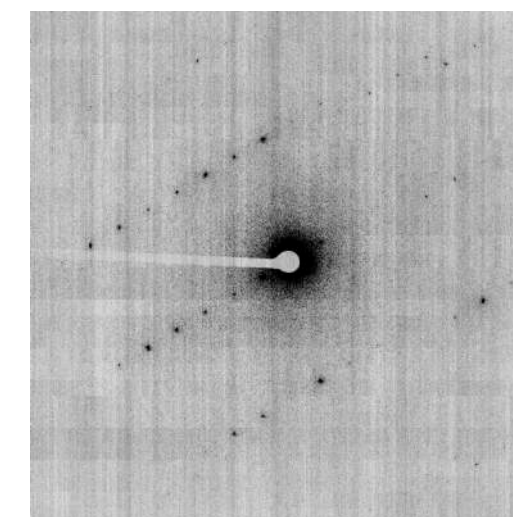
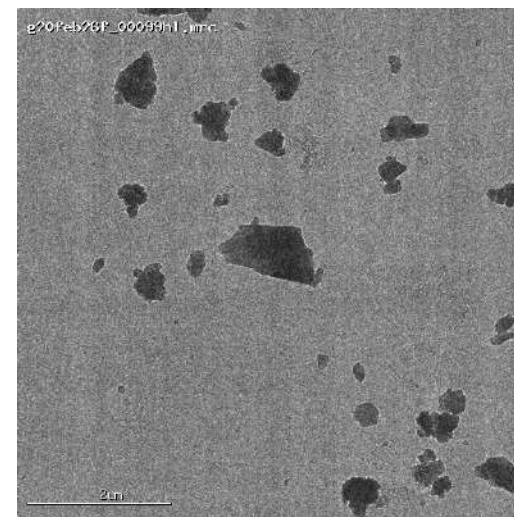
cryoEM: a technology on the rise

Single
particle
cryoEM



Cryo Electron
Tomography
(cryoET)

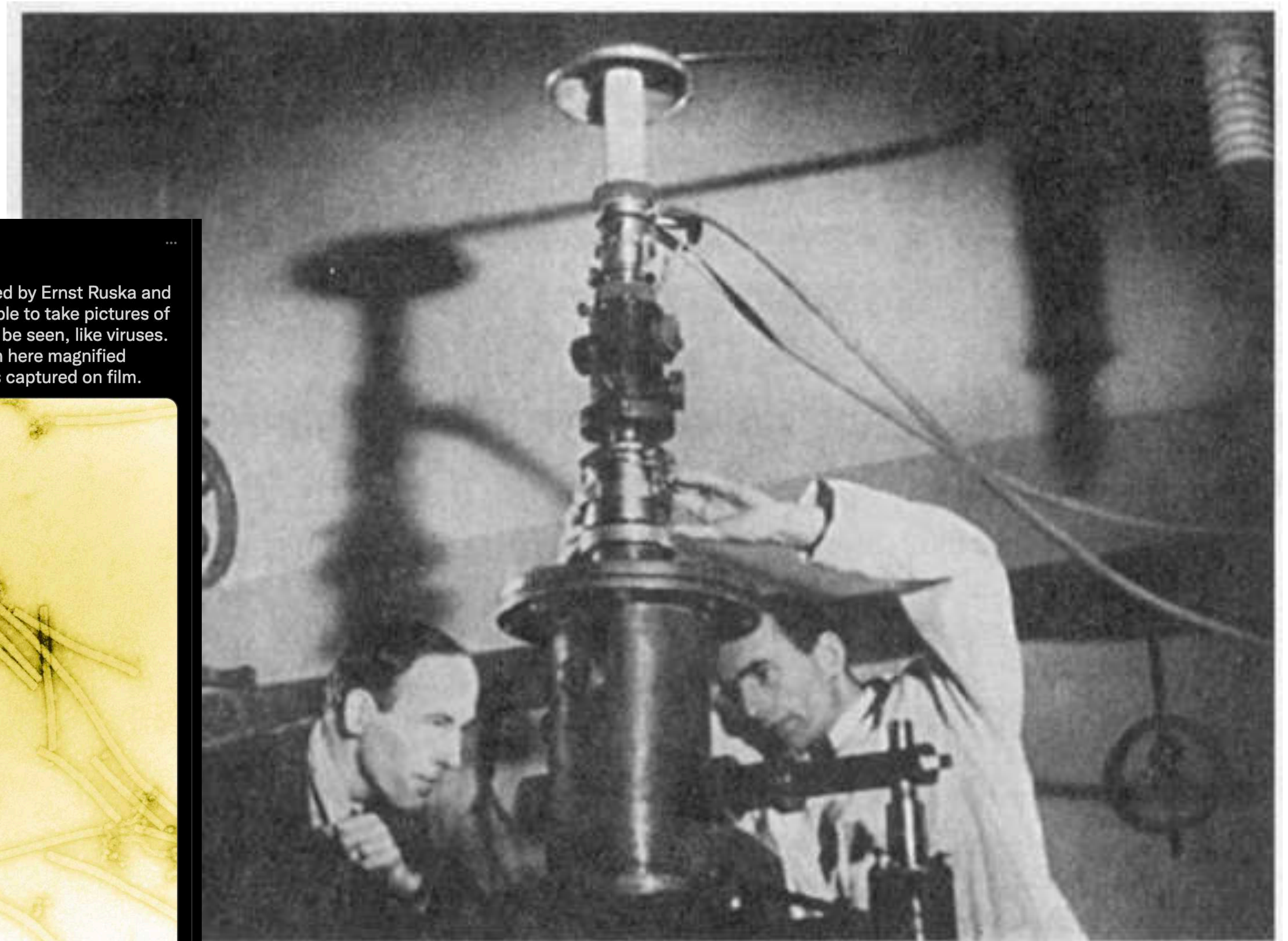
Micro crystal electron
diffraction (microED)



The tool of our trade: EM

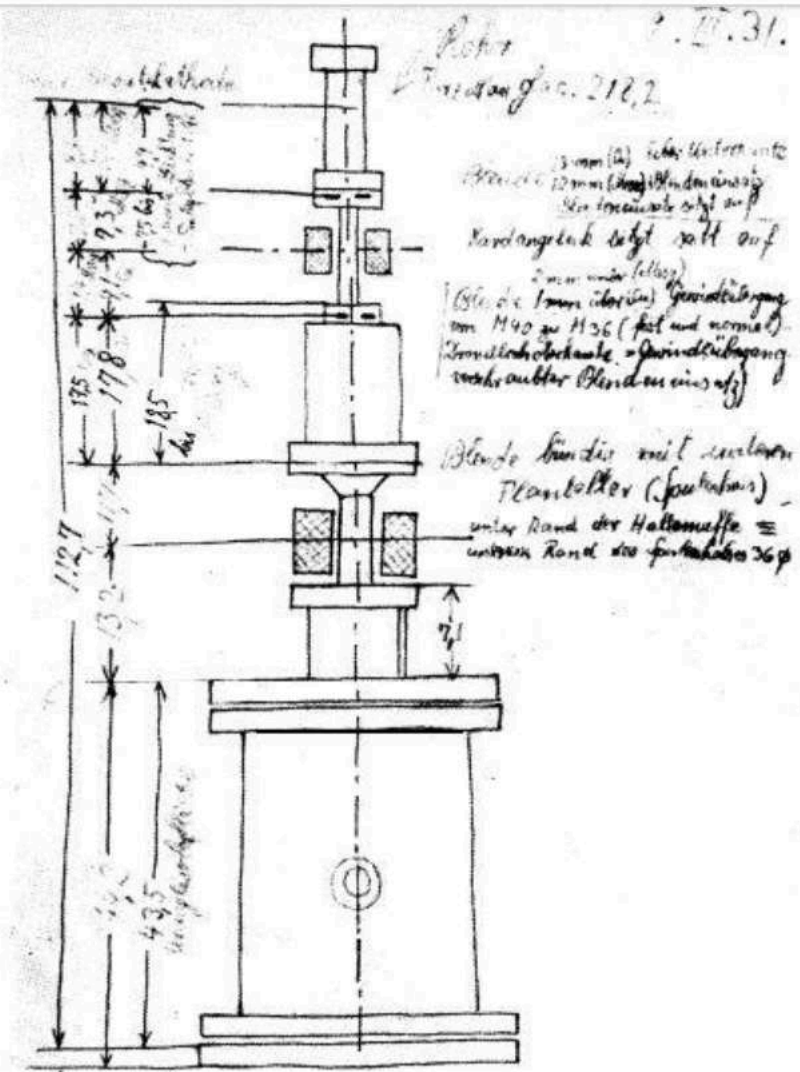
Ruska and Knoll in Berlin in the early 1930s

-Wikipedia





 **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.

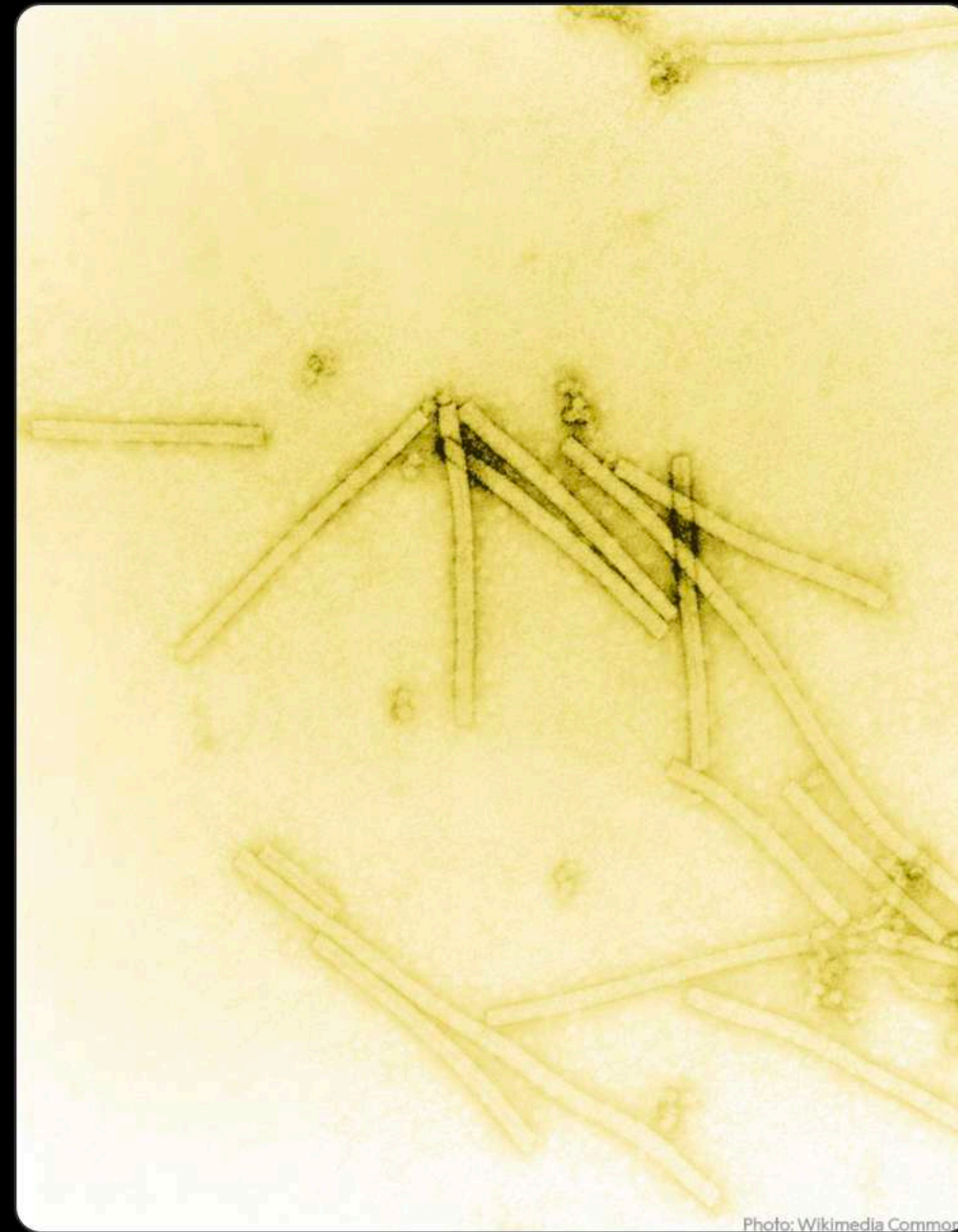


6:06 AM · Dec 25, 2021
725 Reply Copy link to Tweet

[Read 8 replies](#)

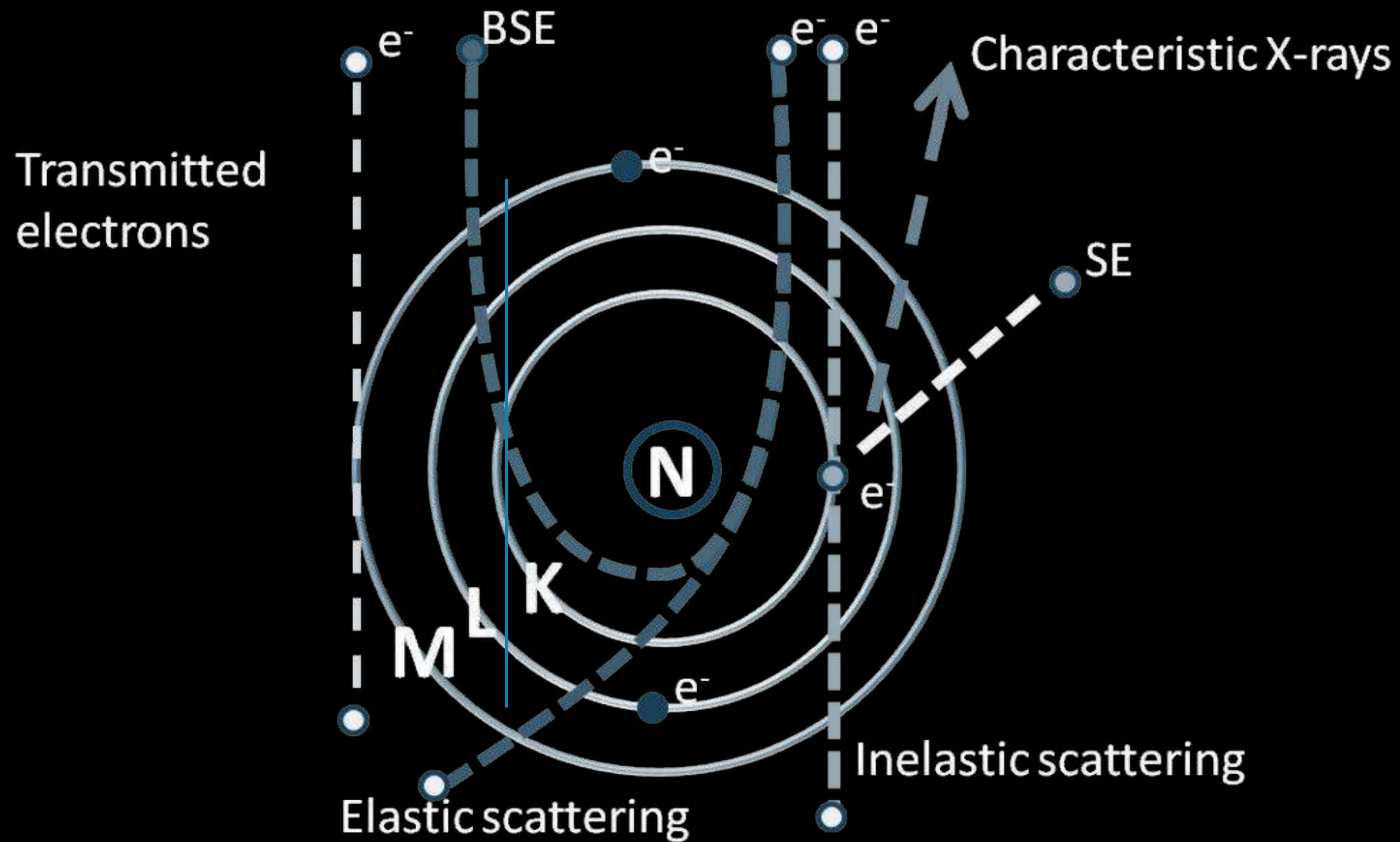
 **The Nobel Prize** 
@NobelPrize

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.



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

Why electrons?



● Main beam electrons

Why electrons?

Pros

Small wavelength

Can be focused

Cons

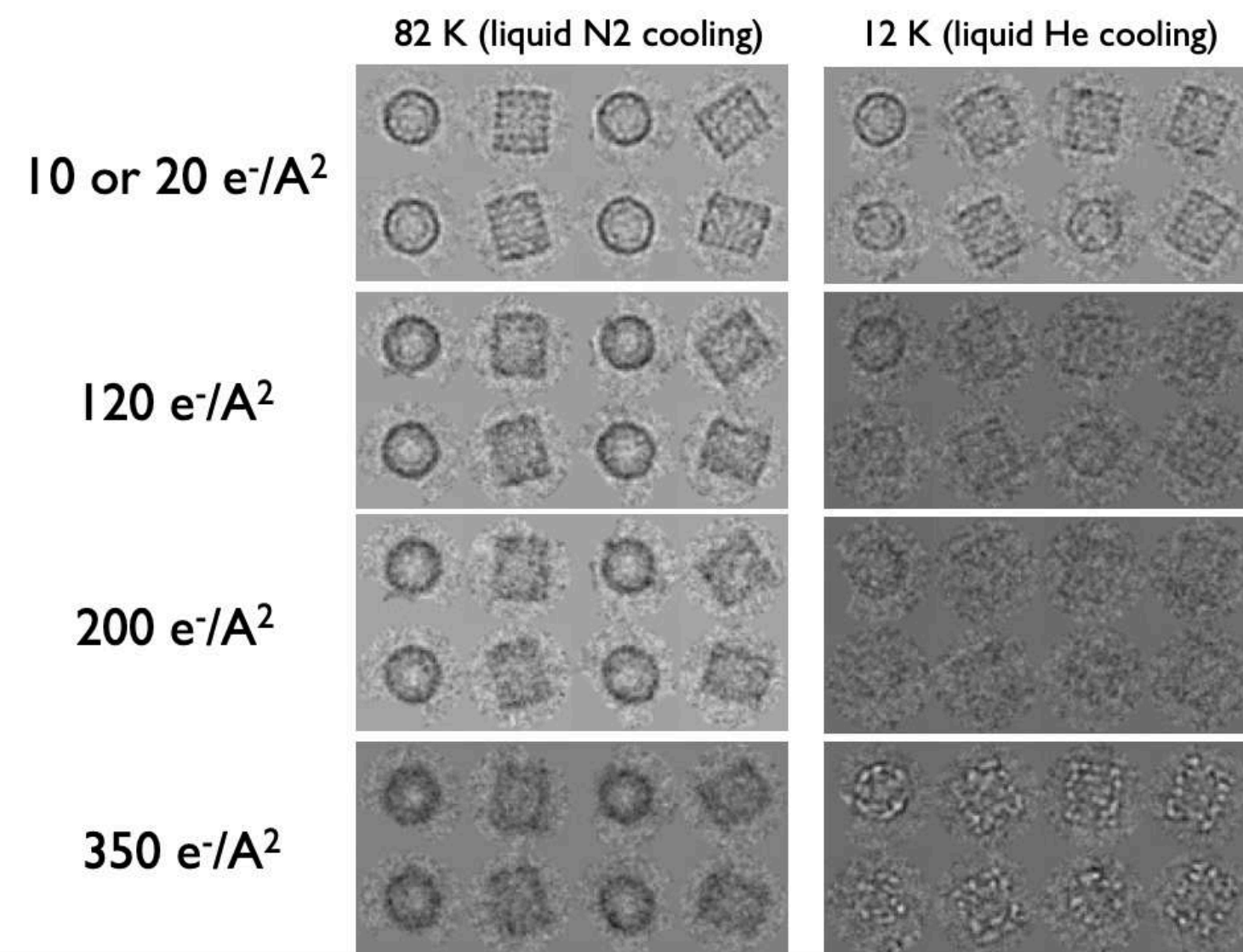
Damages sample
worse with faster electrons

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

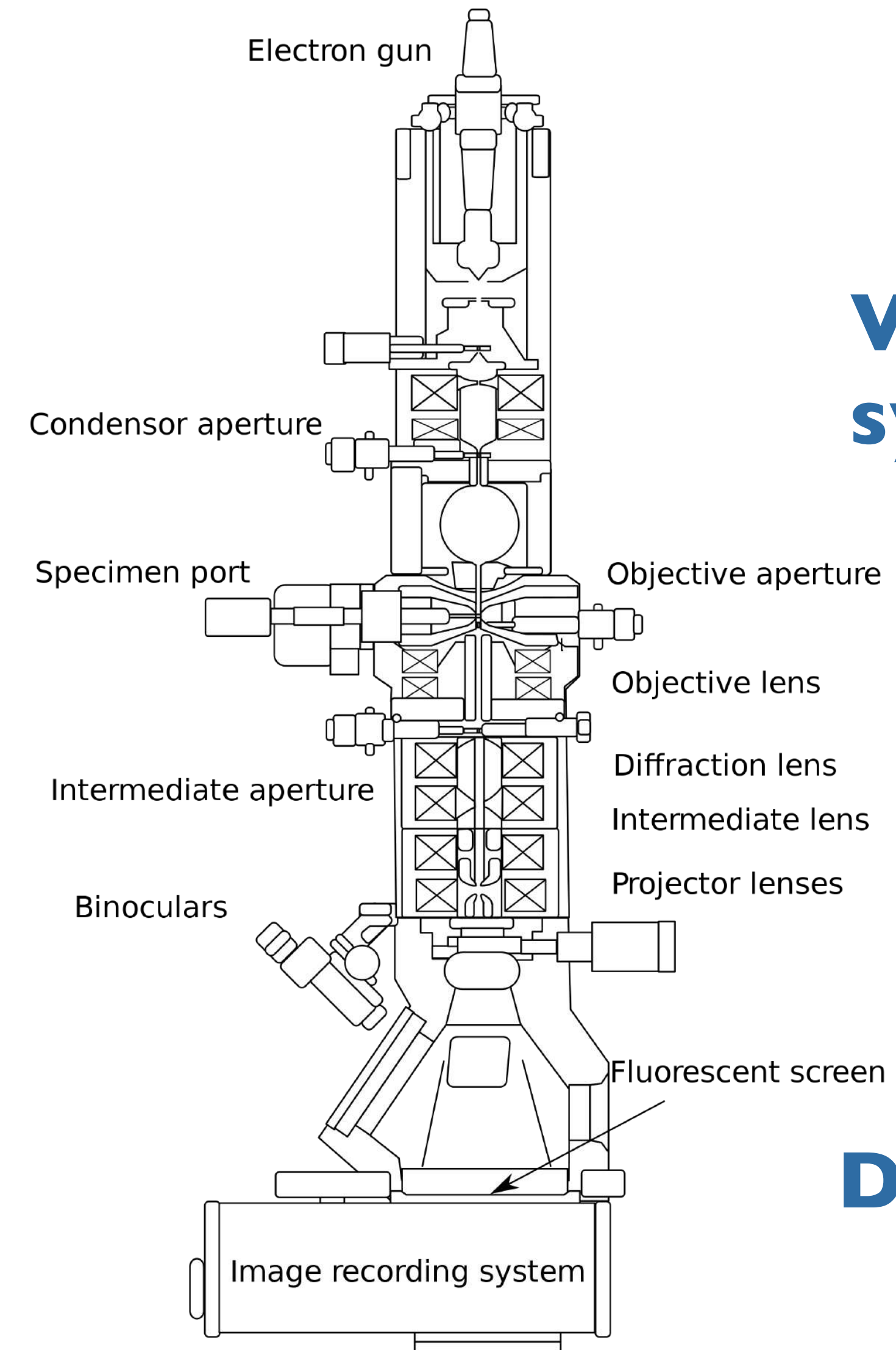
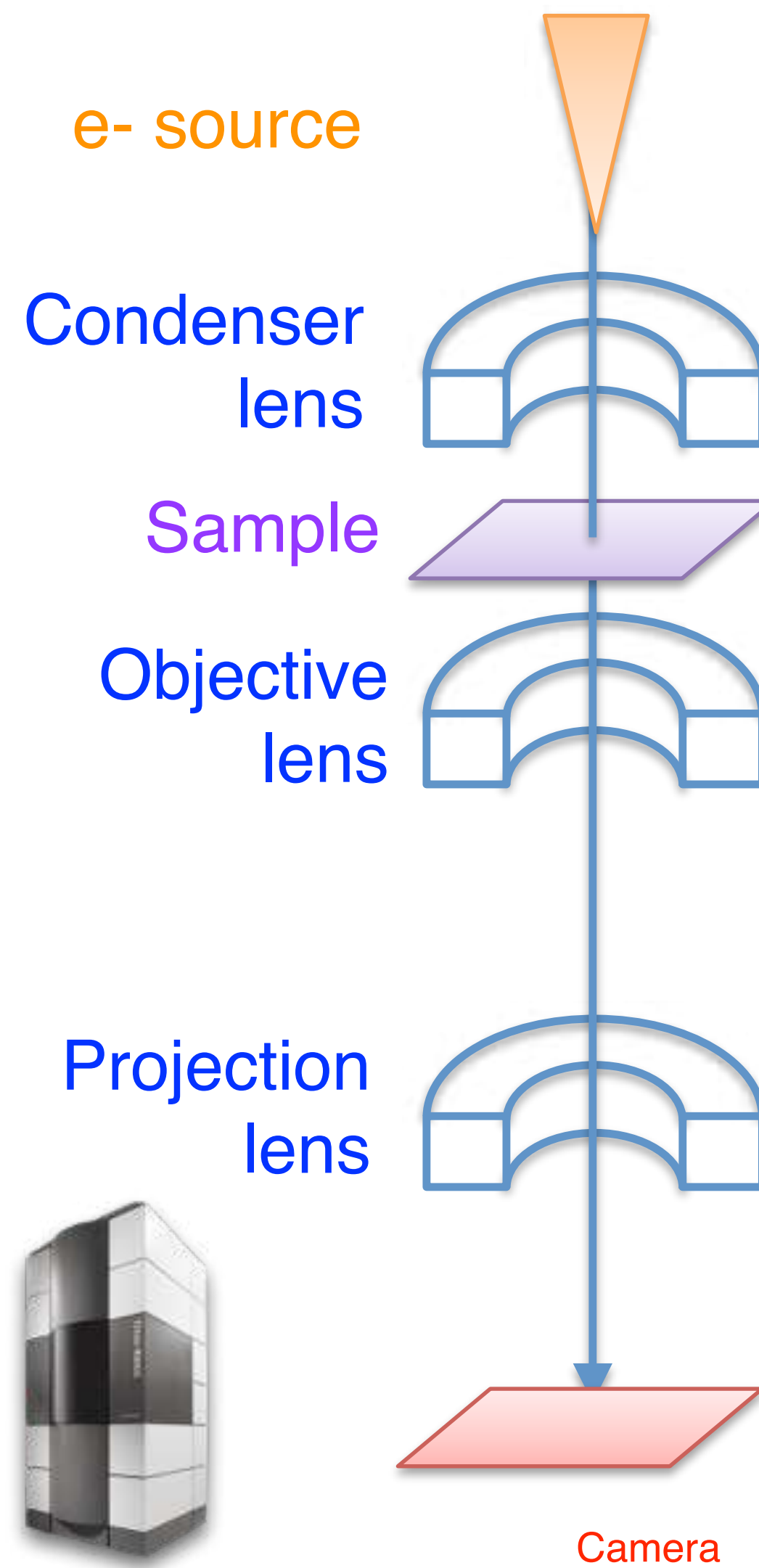
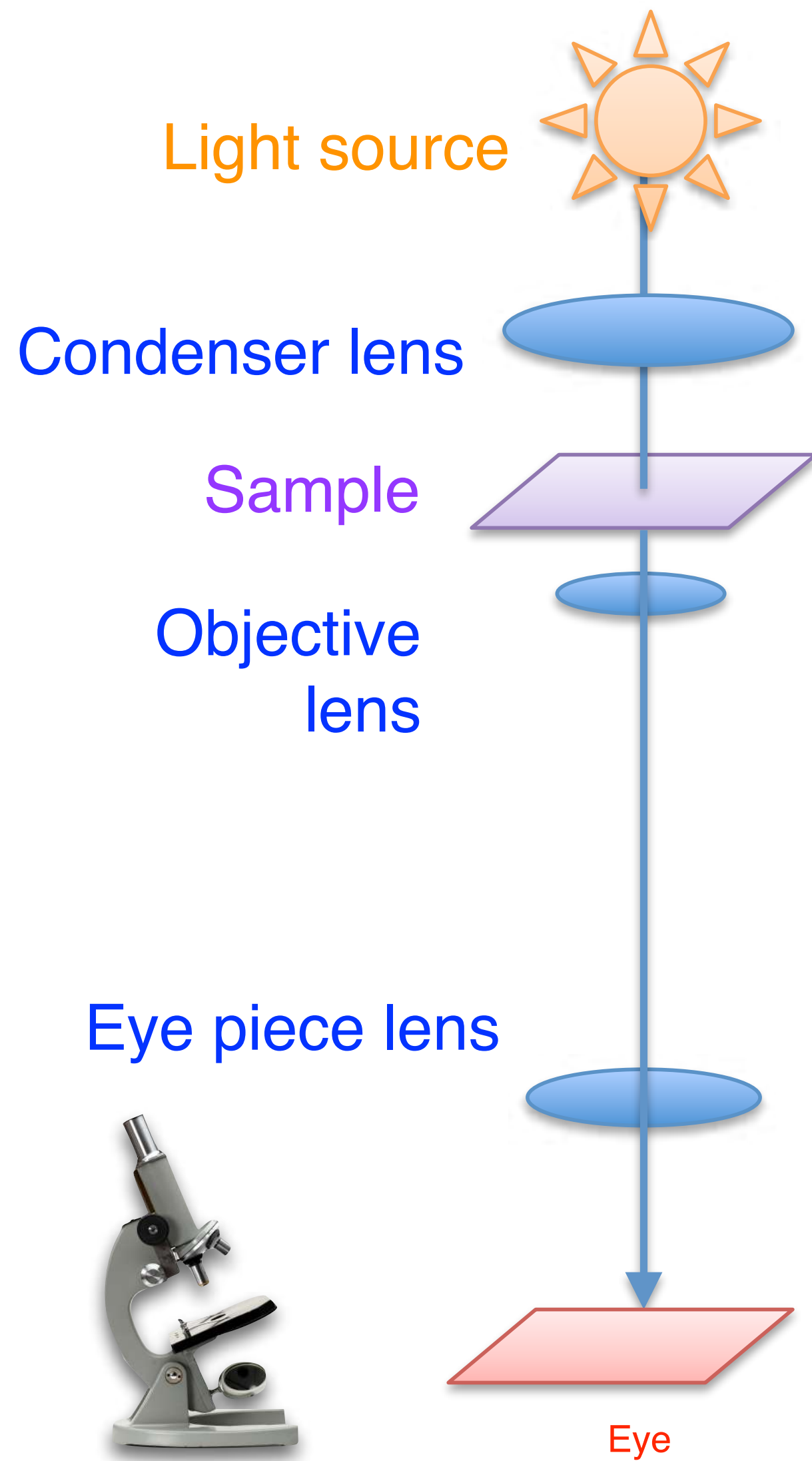
R.M. Glaeser¹

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

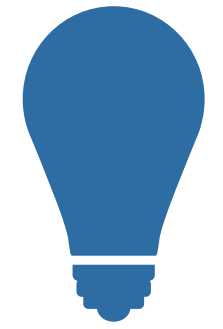
¹Corresponding author: e-mail address: rmglaser@lbl.gov

- 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



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

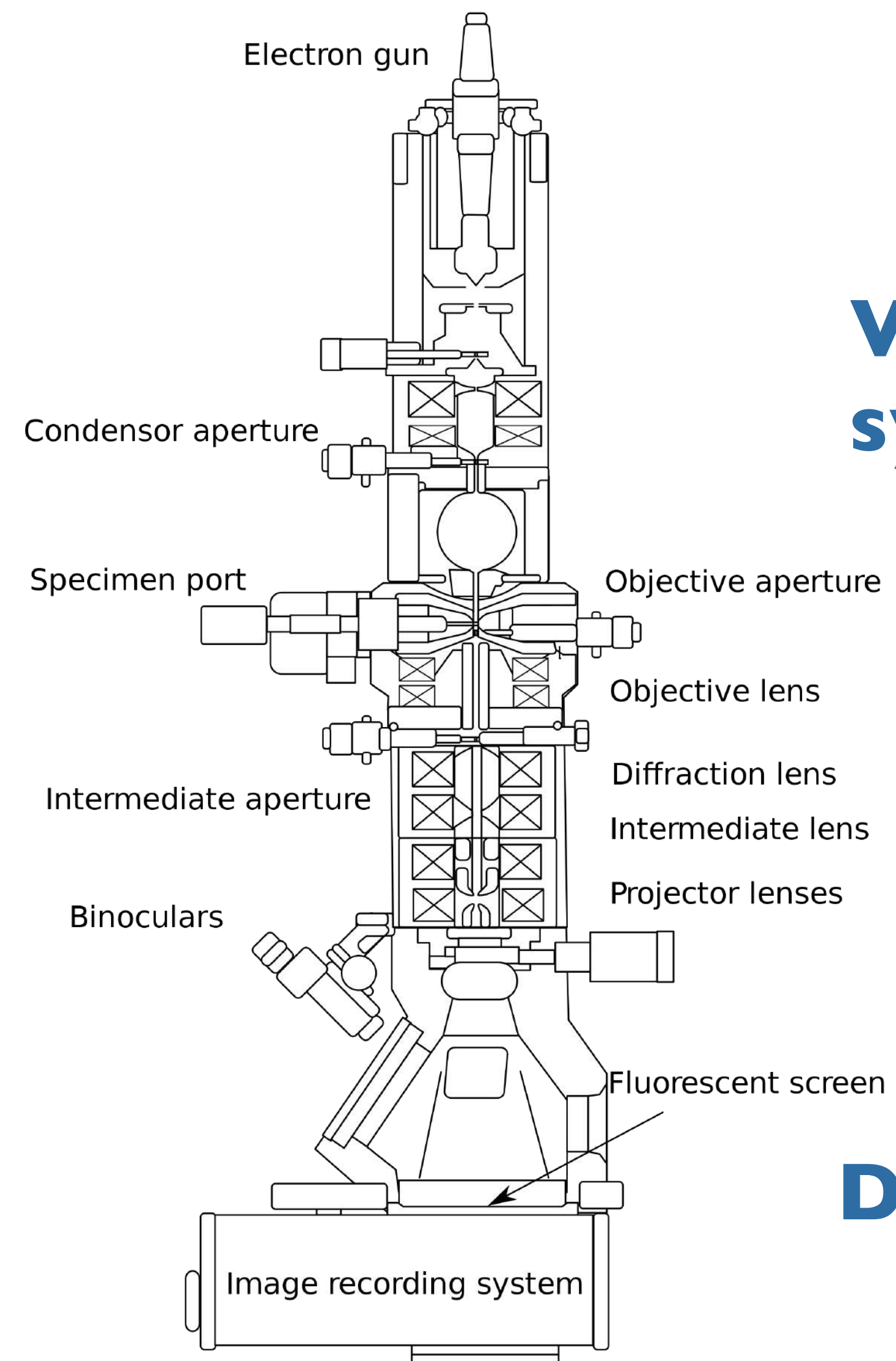
Sample

Objective lens

Projection lens

Eye

Camera



Electron sources



Vacuum systems



Lenses



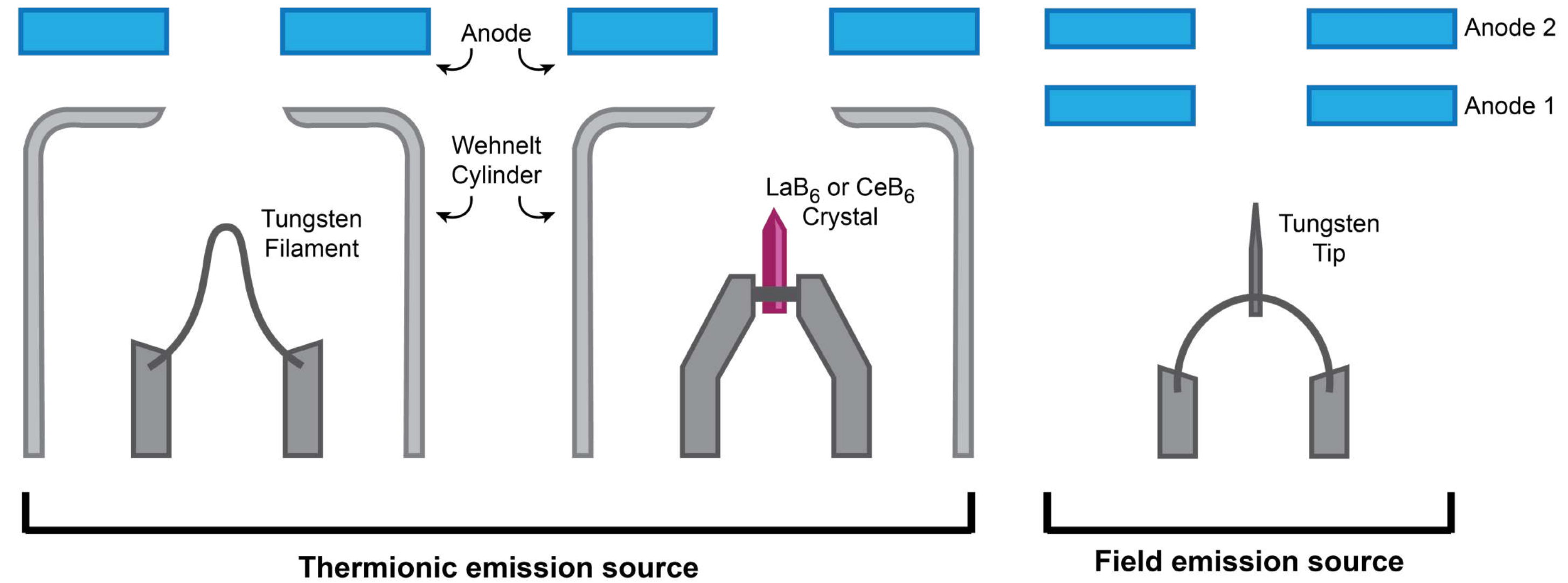
Detectors



Electron sources



What are the 3 main kinds of electron sources?

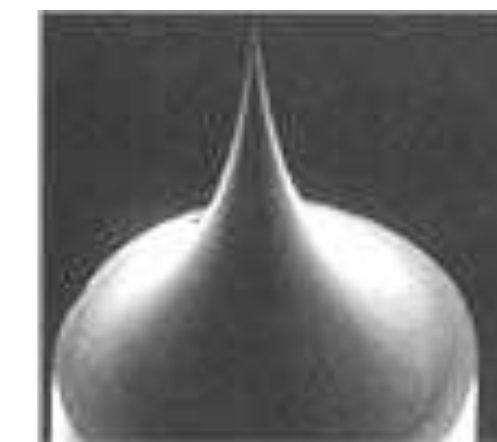
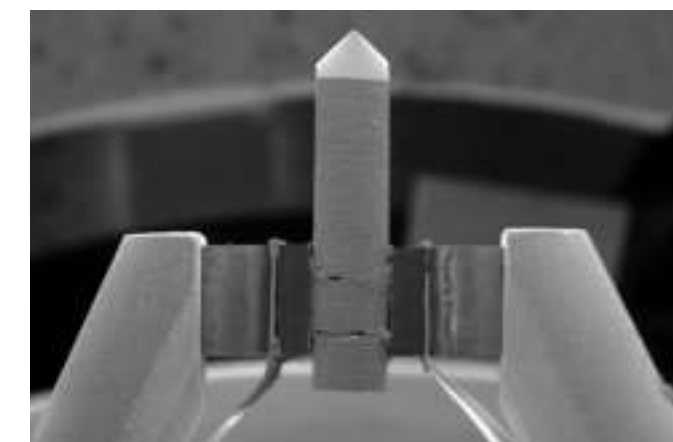
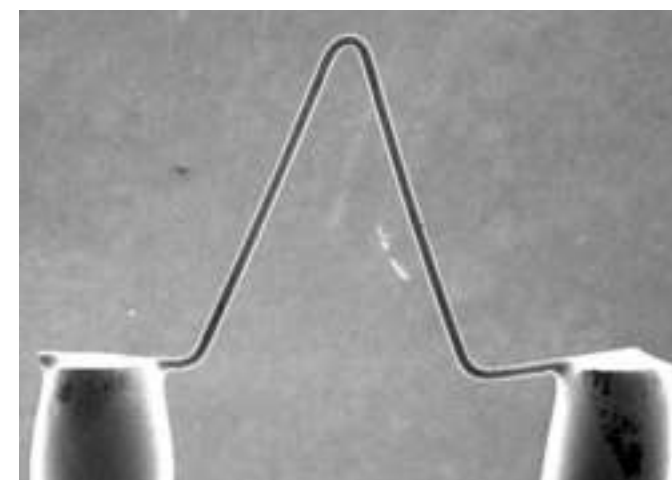


Thermionic emission source

Field emission source

thermofisher.com

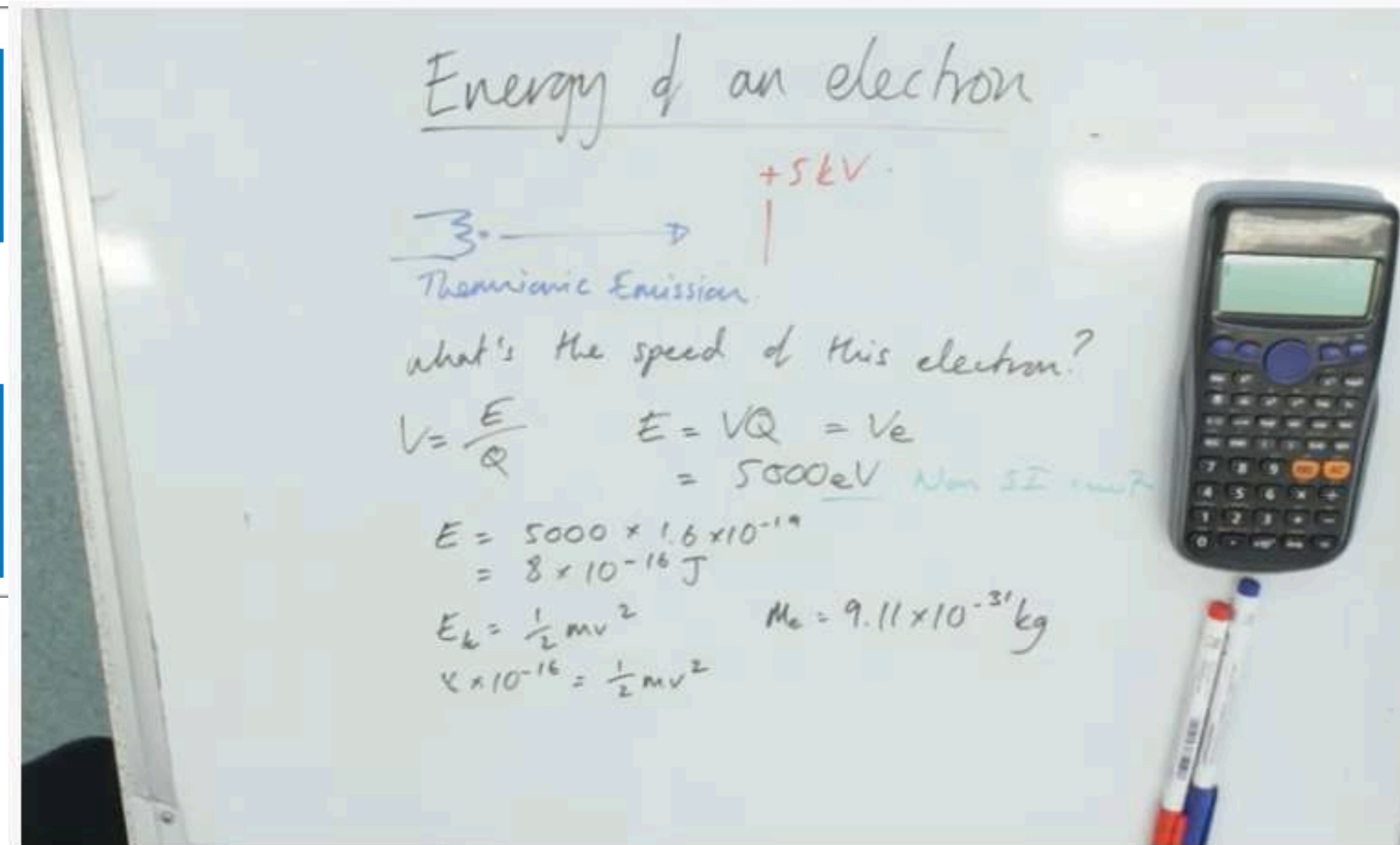
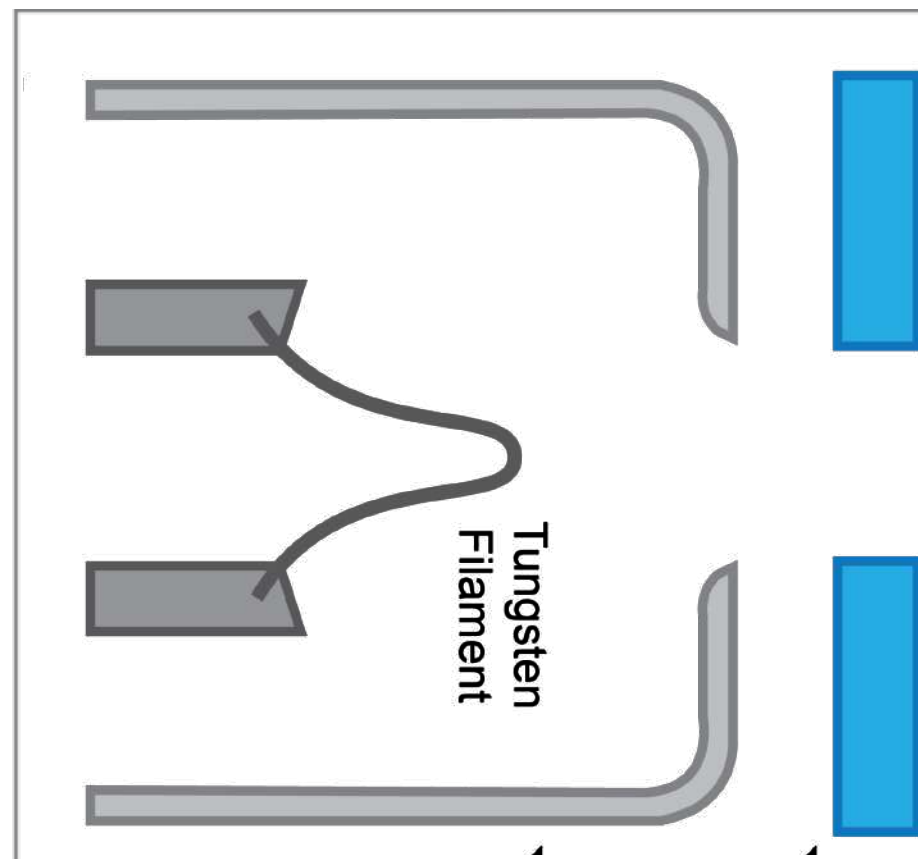
nanoscience.com



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

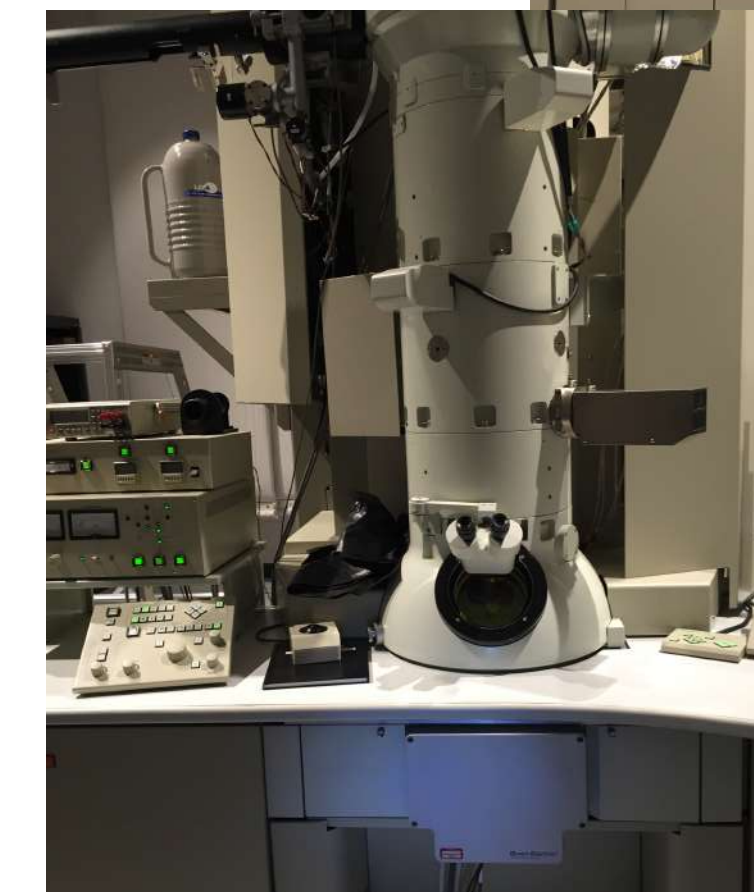
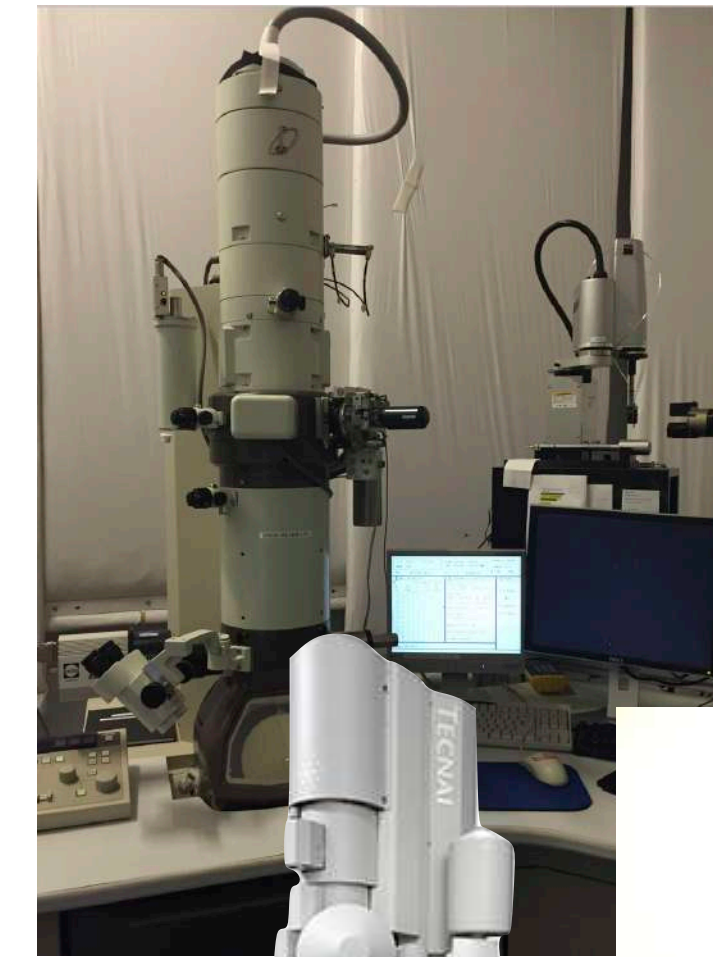
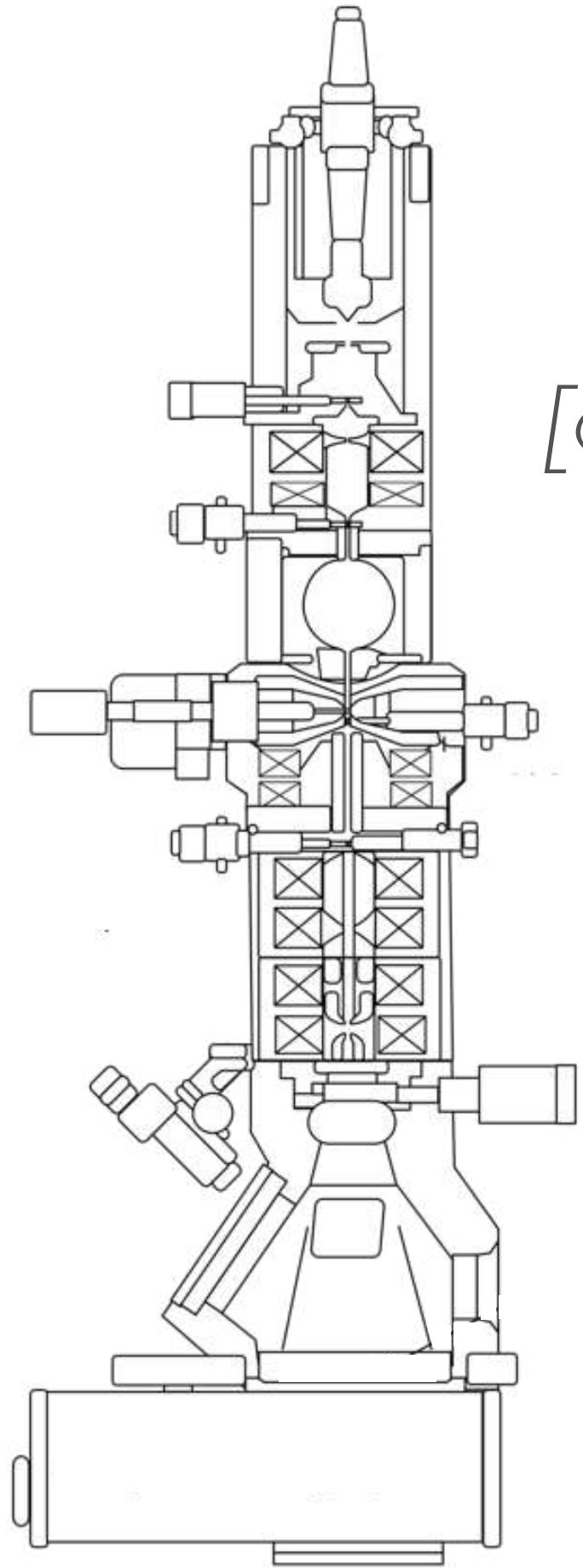
[developments ongoing to push resolution with FEG systems]

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

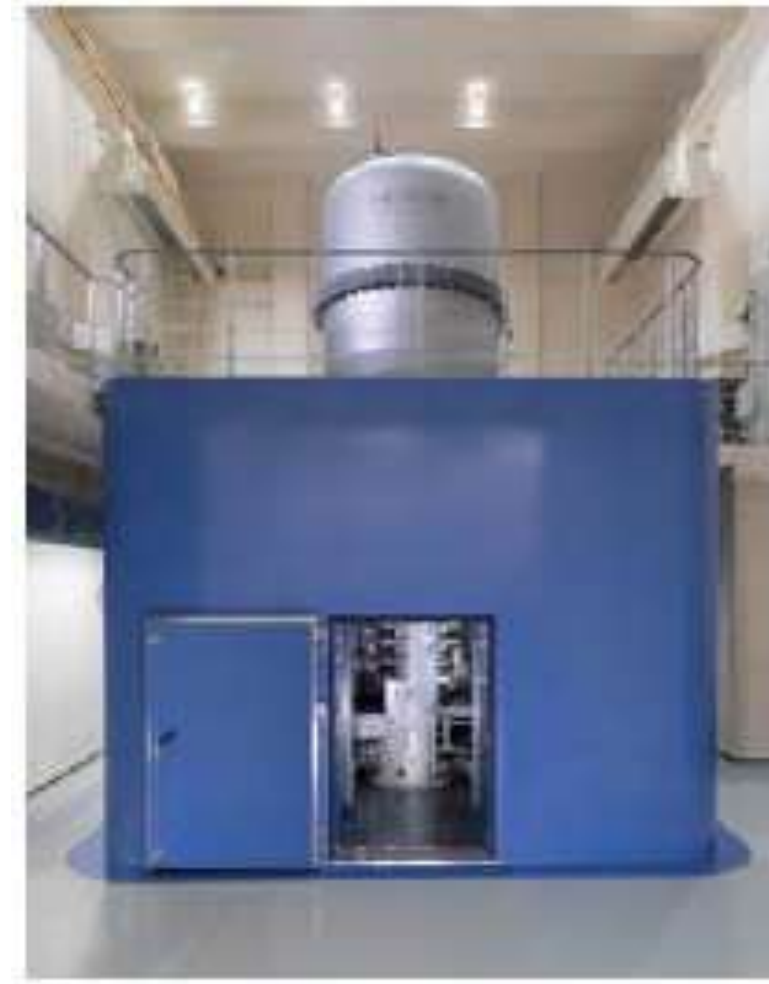
Smaller effect on unwanted lens aberrations
1.5-3 Å resolution



Electron sources

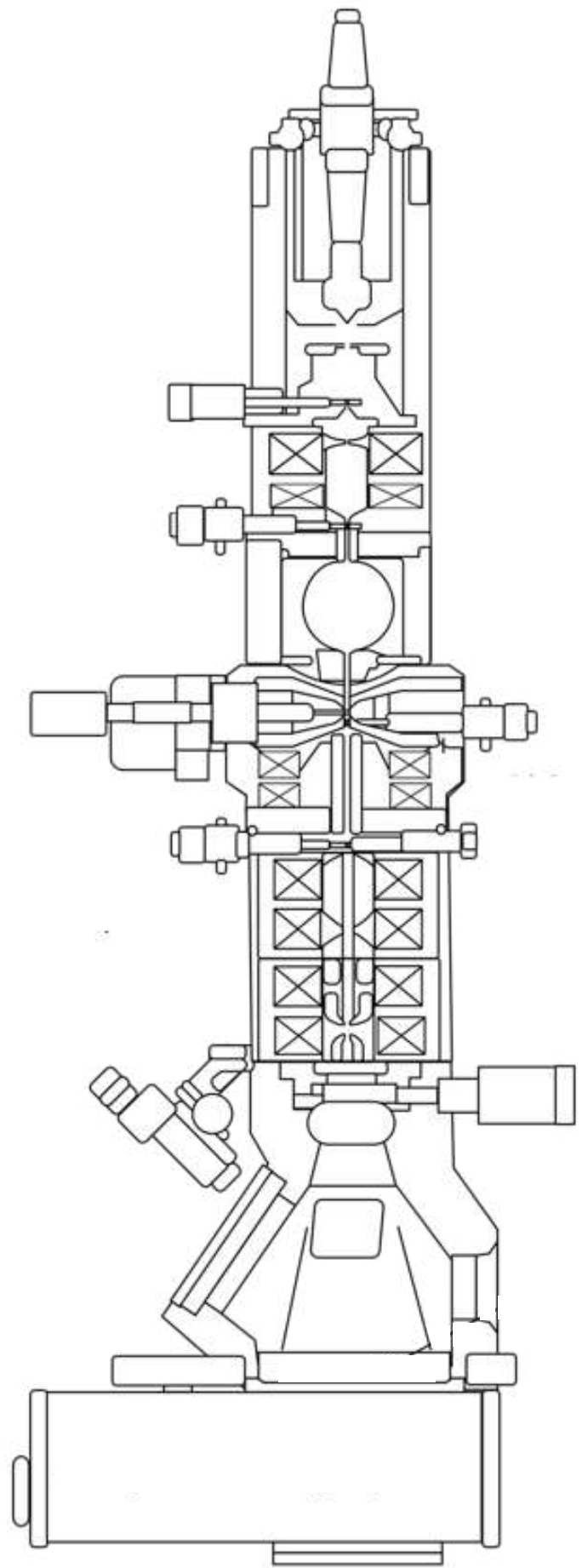
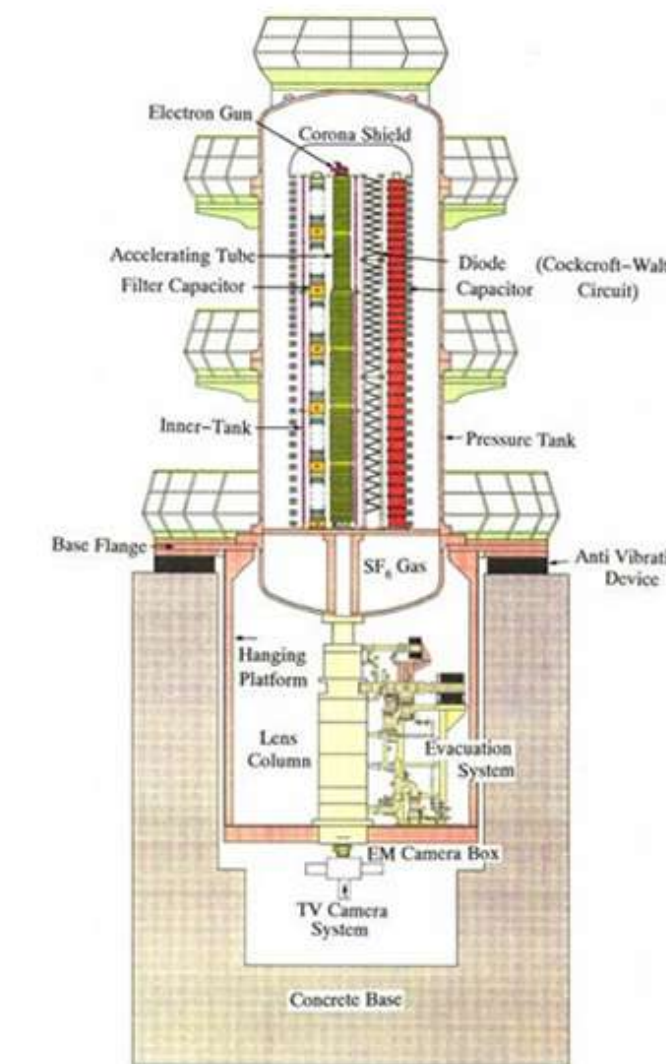


1-1.2 MV: Hitachi, JEOL
LaB6



uhvem.osaka-u.ac.jp

3 MV: Hitachi H3000
LaB6



Vacuum systems



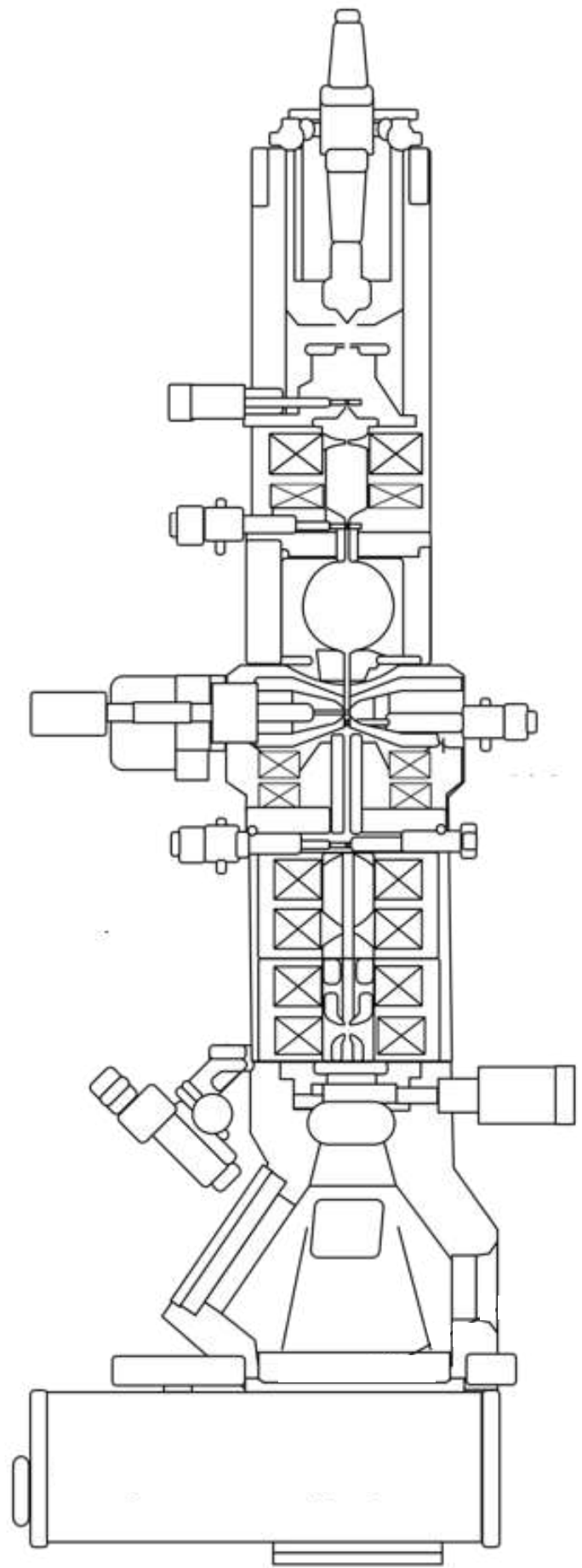
Why do we need a vacuum?

Beam coherence - at STP mean free path ~ 1 cm

Insulation - interaction between e- and air

Filament - O_2 will burn out source

Contamination - reduce interaction gas, e-beam and sample

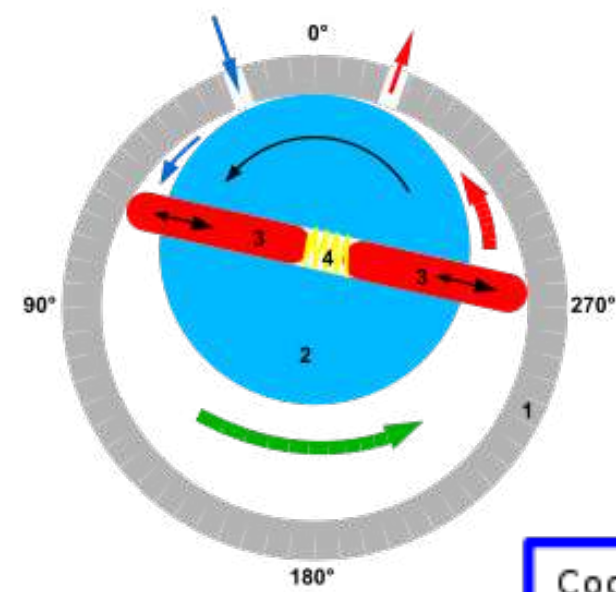
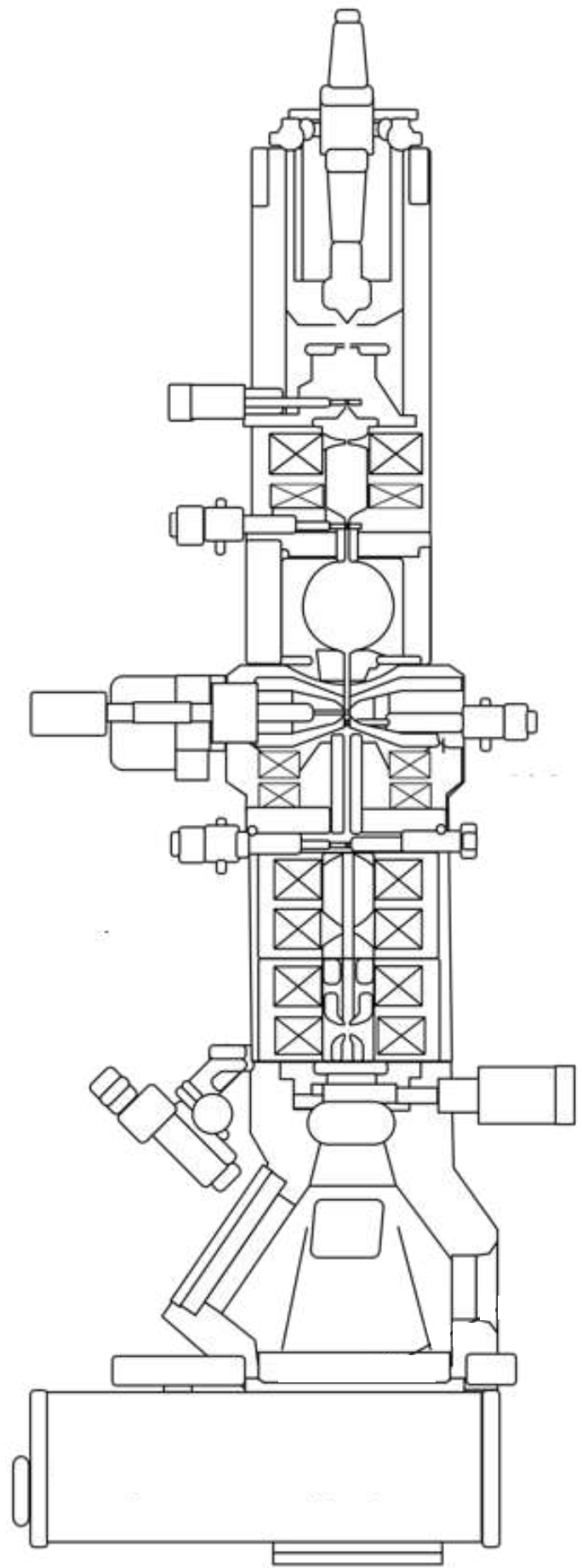


Vacuum systems



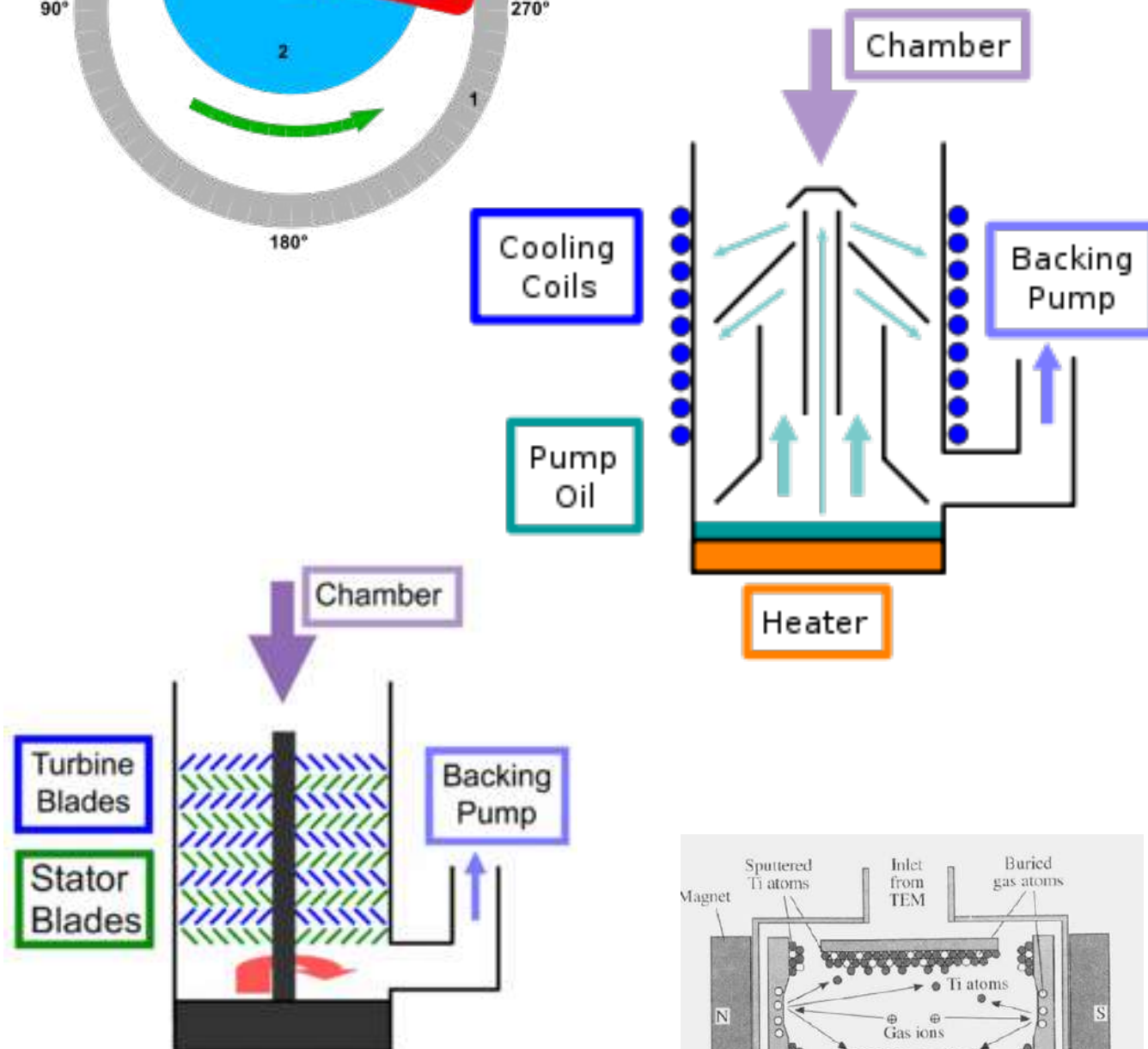
What types of pumps do we have?

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

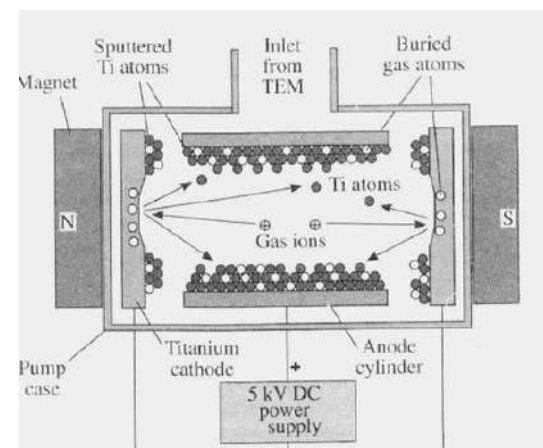


Diffusion

$10^{-3} - 10^{-6}$ 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

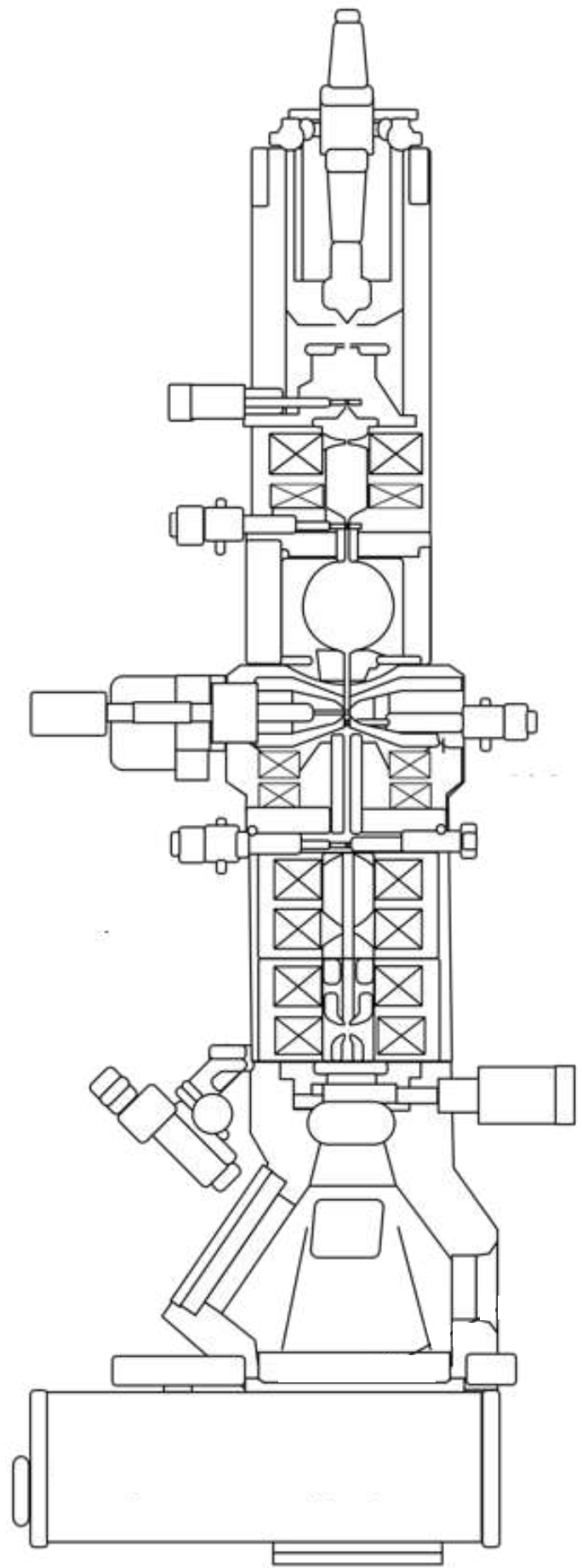
wikipedia.com

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

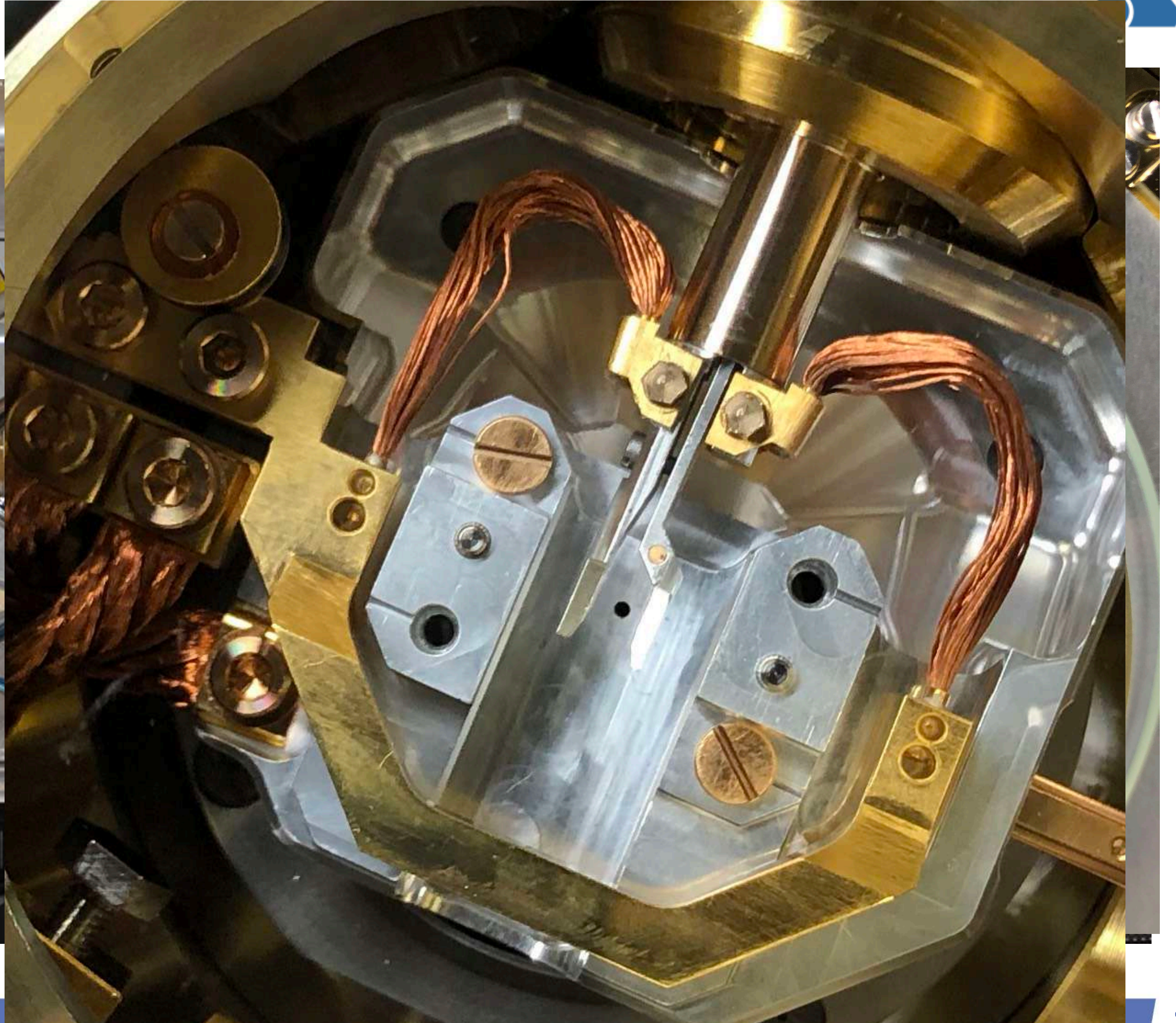
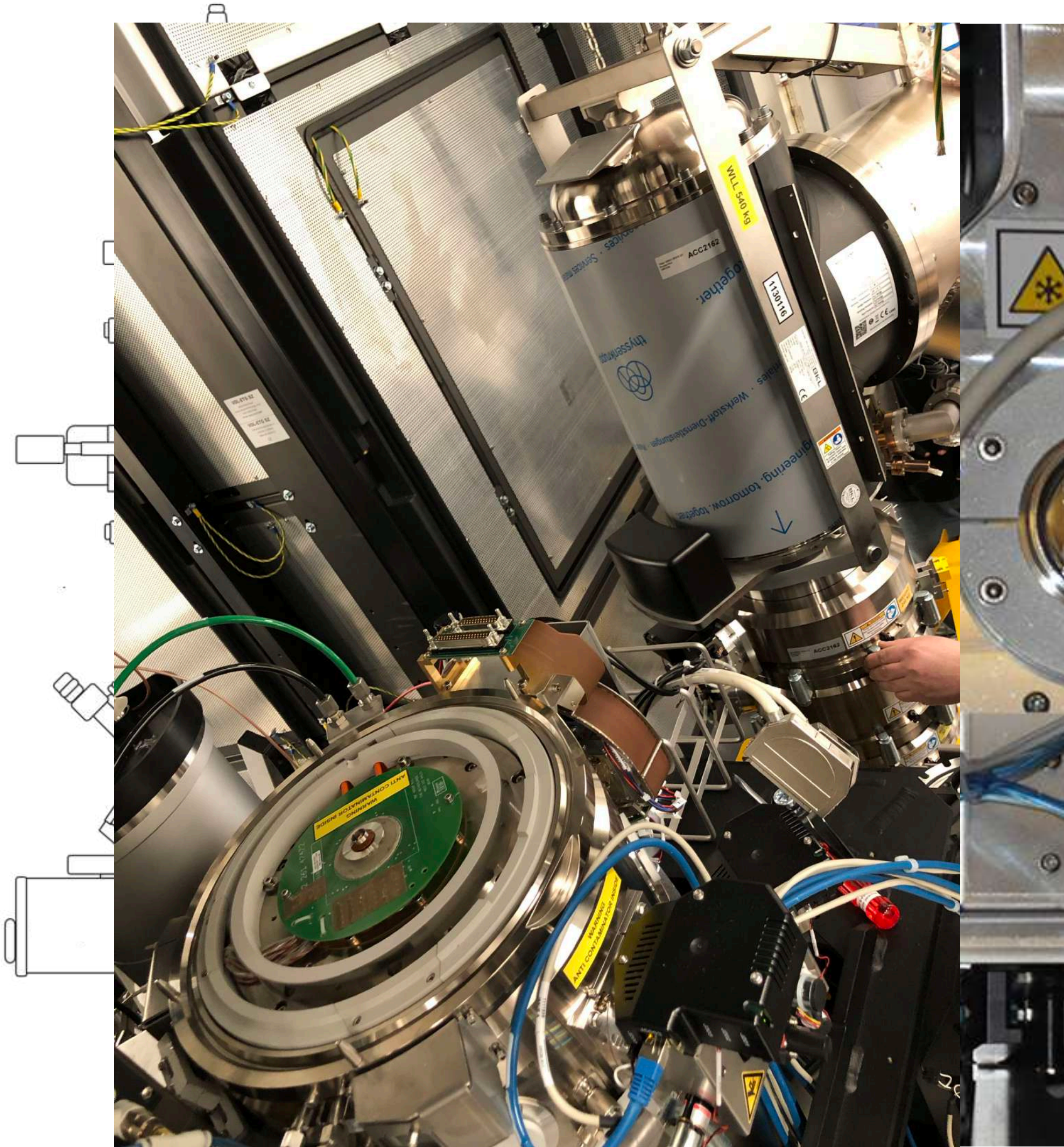
Pressure	Torr	Pascal	Log
Gun/Col	88.29 e-9	11.77 e-6	6
Camera	0.35 e-6	46.05 e-6	17
Buffertank	0.19	25.85	33
Backing	3.86	514.32	55

Vacuum Overview

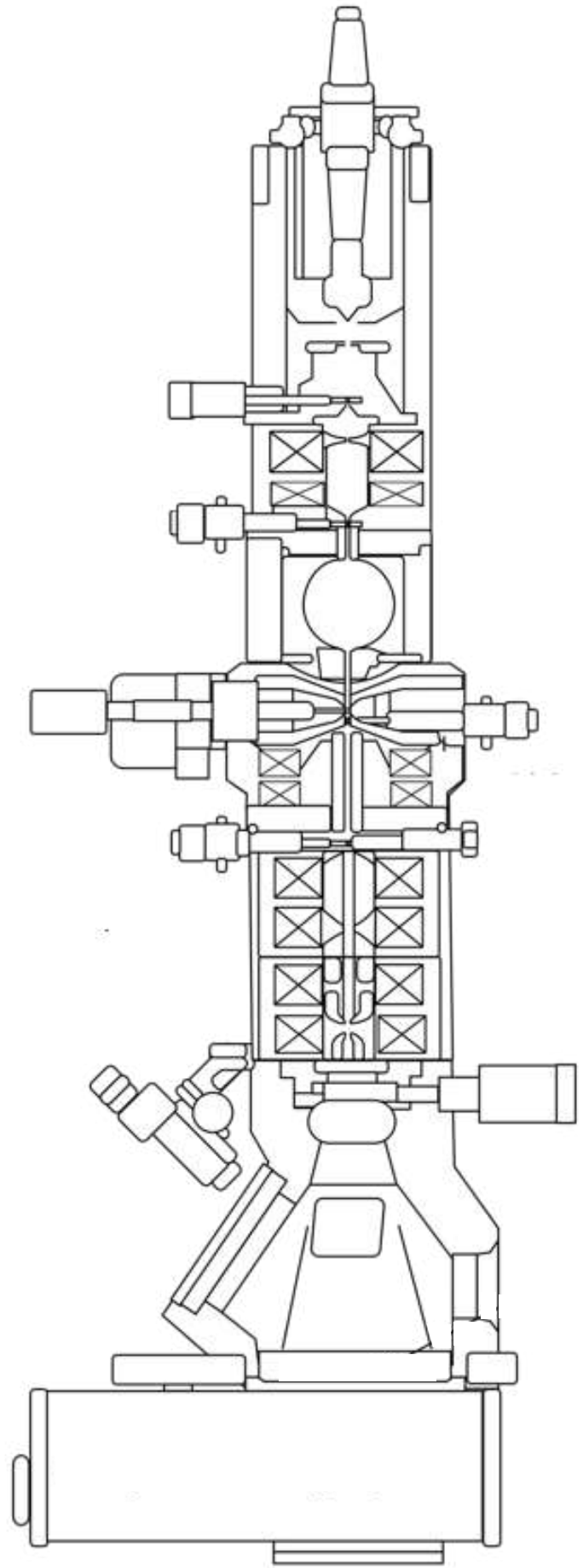
Unit log

Process information: Column valves closed

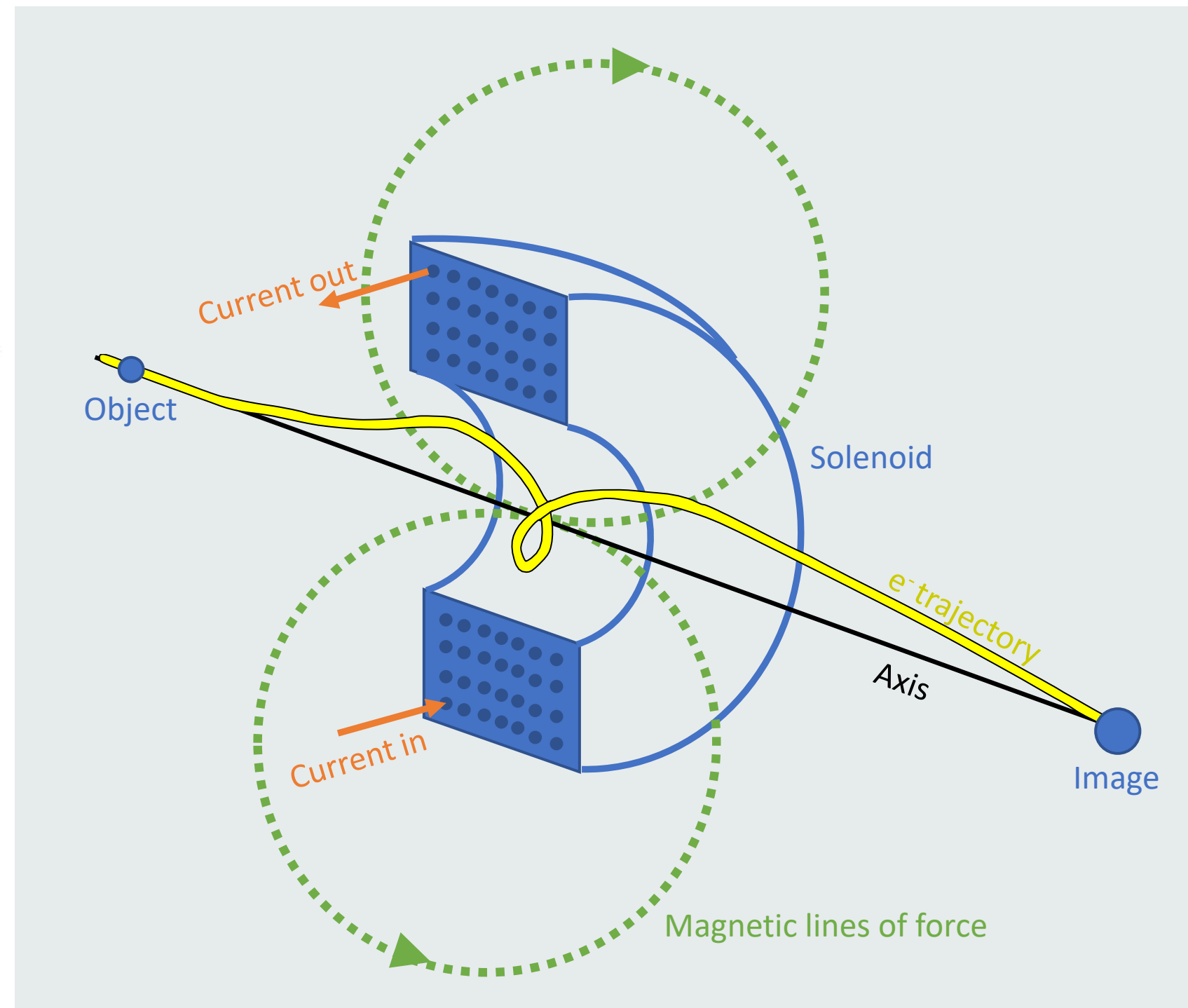
Vacuum systems



Lenses



What types of lenses do we have?



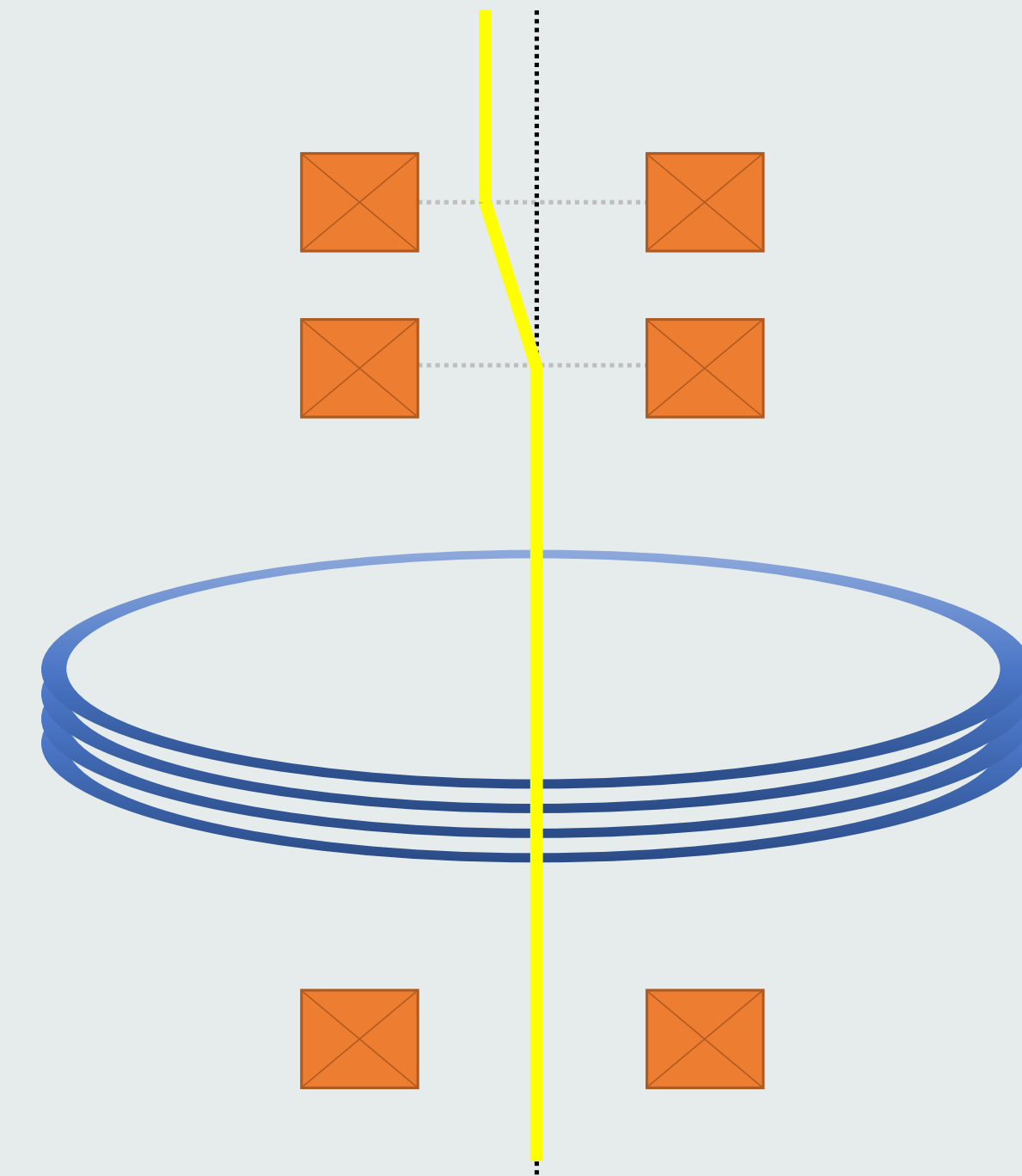
- Focus
- Magnify
- Rotate

Deflector 1 (shift)

Deflector 2 (tilt)

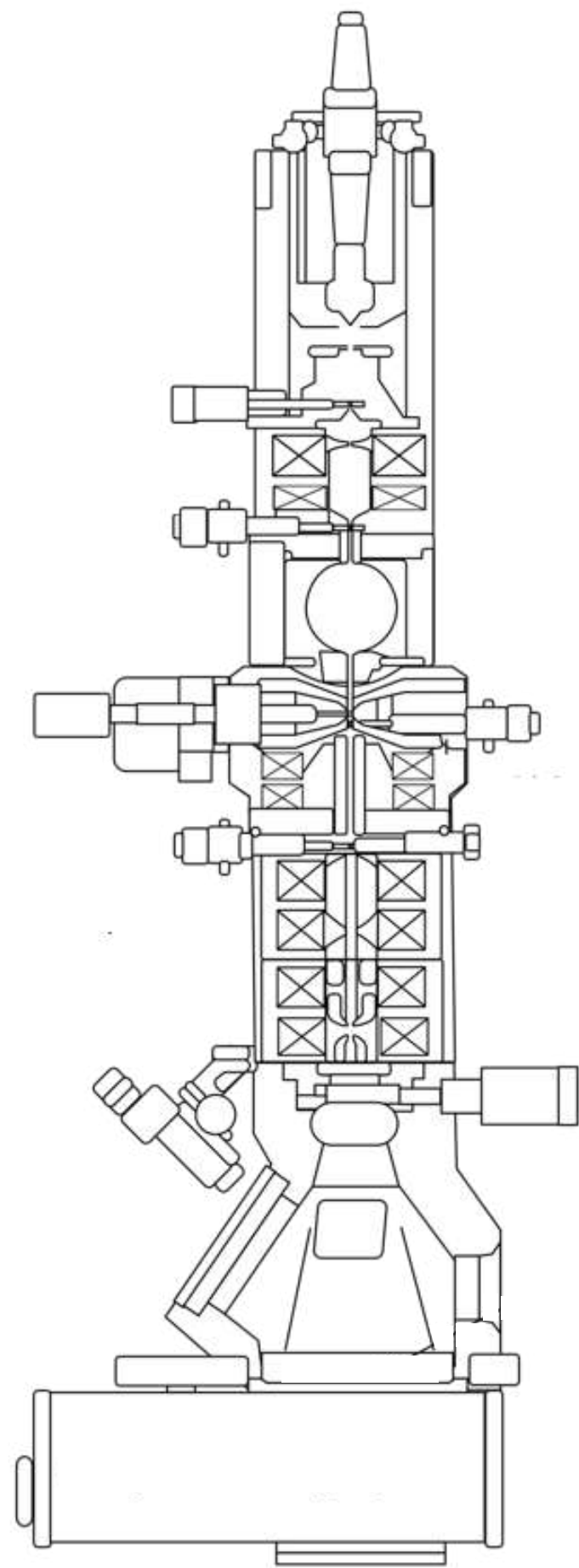
Lens

Stigmator

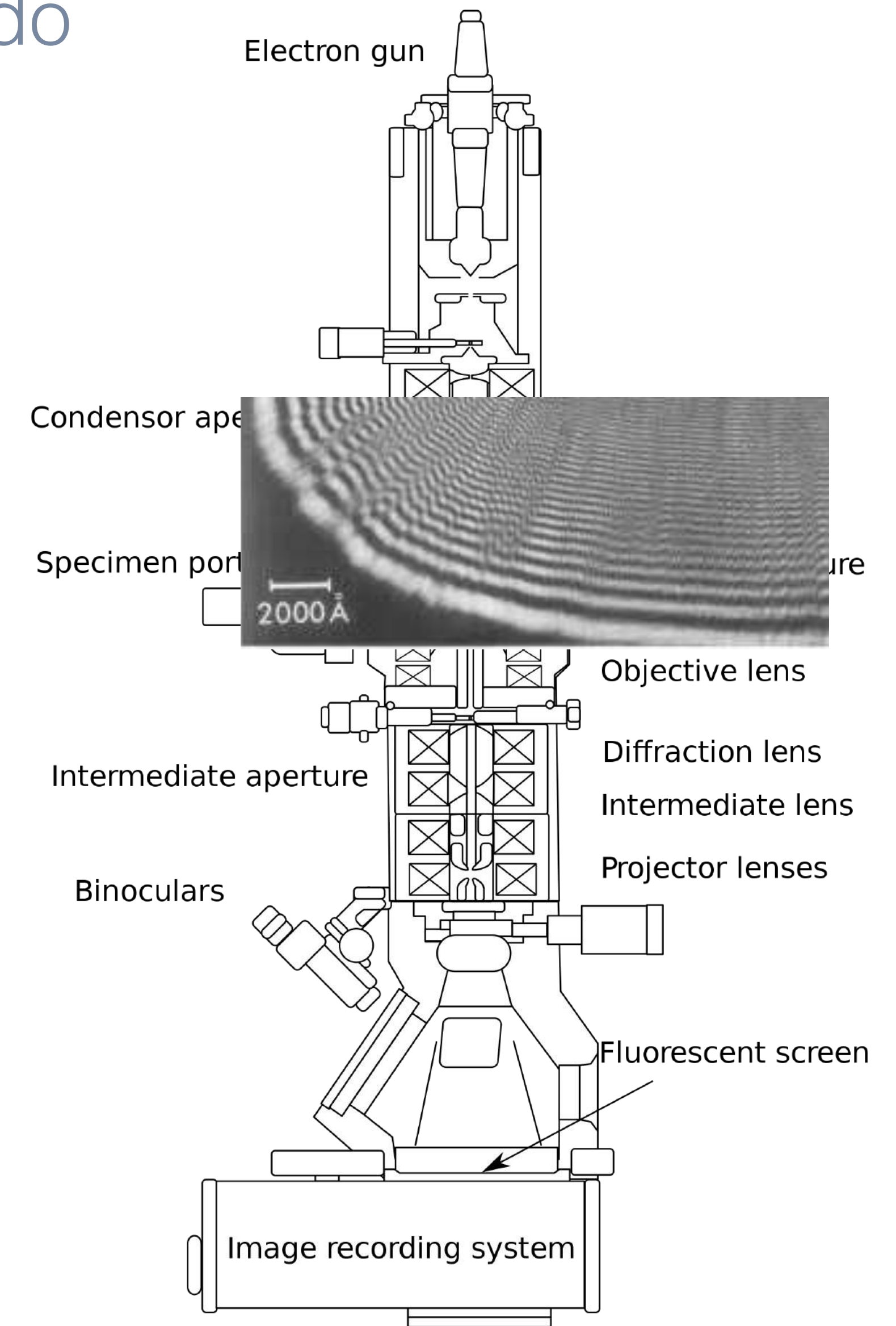


Lenses

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)~~

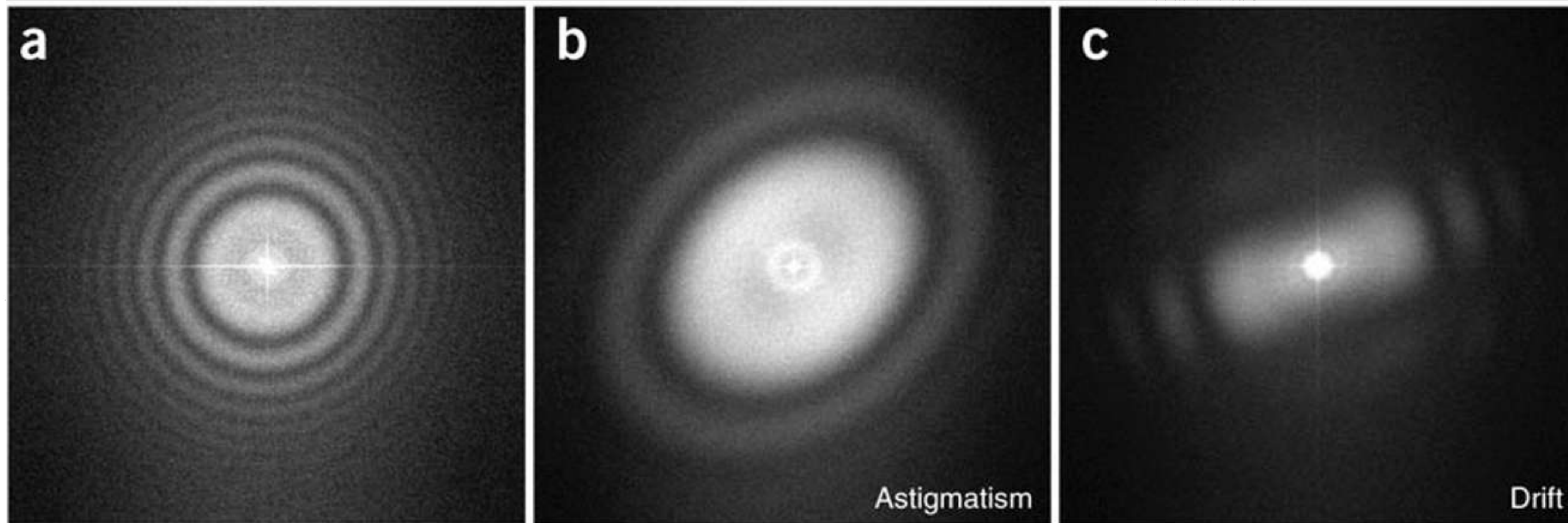
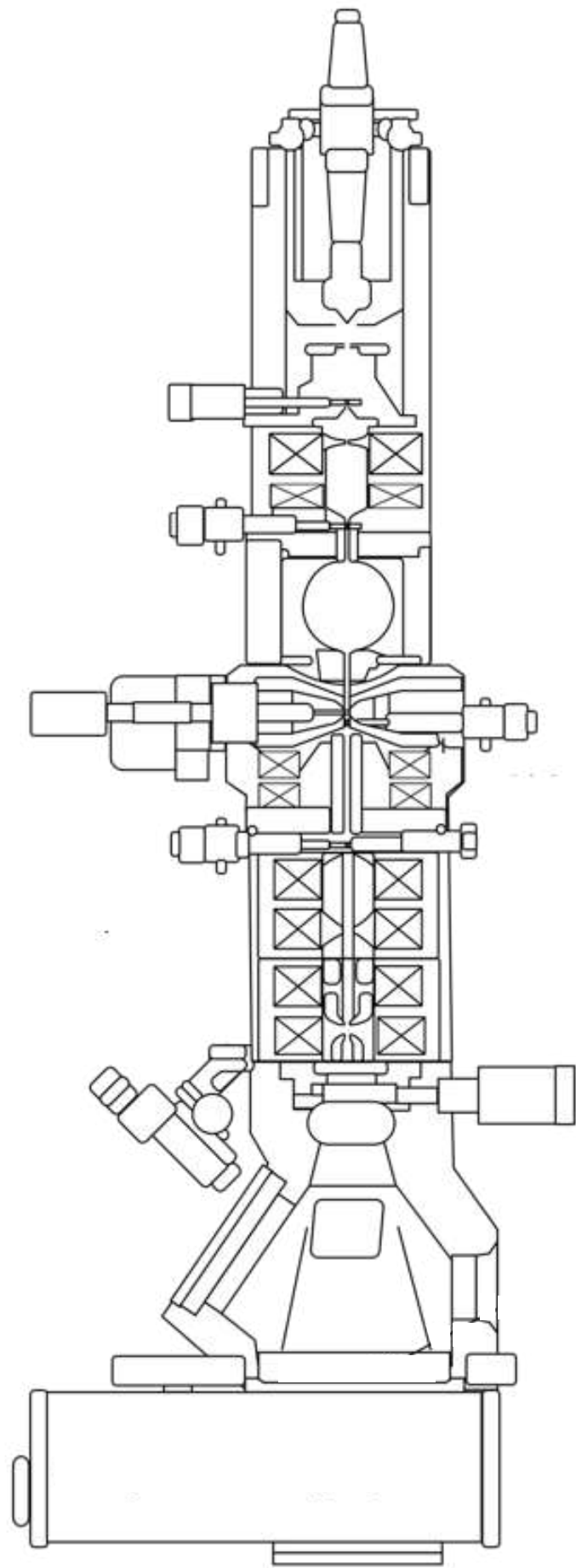


Lenses

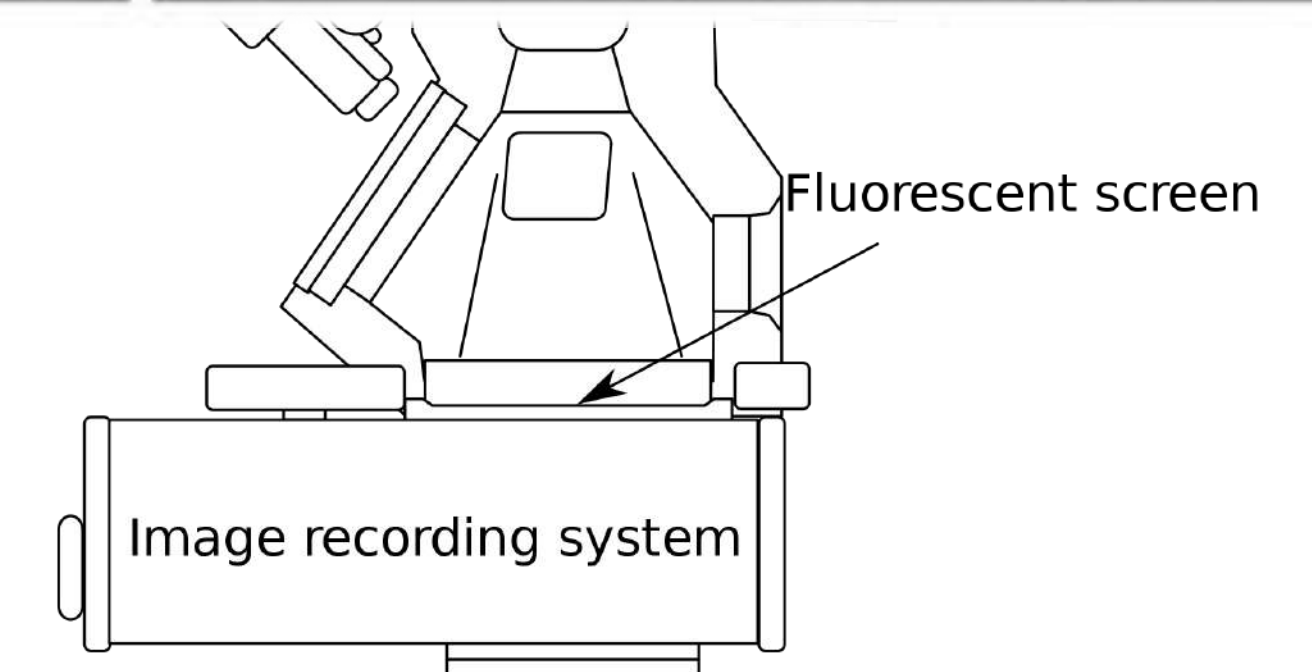
Microscope Alignments What to do & what not to do

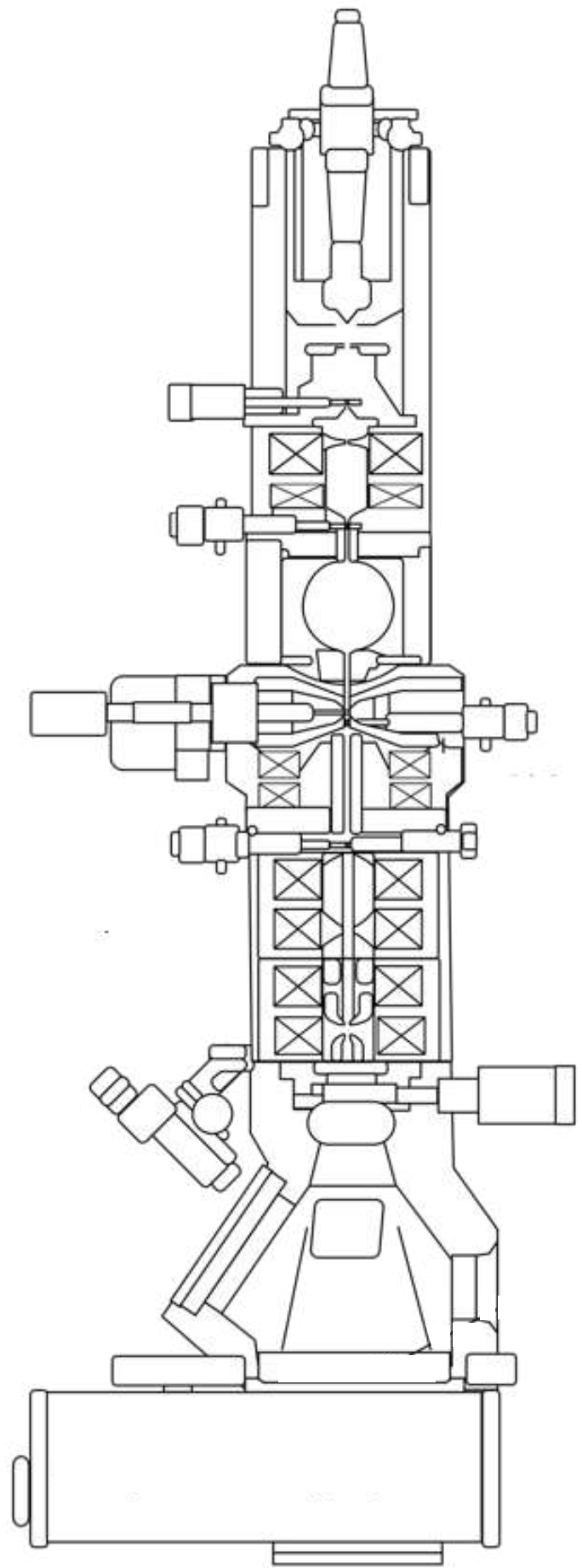


- Do:



- ~~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,

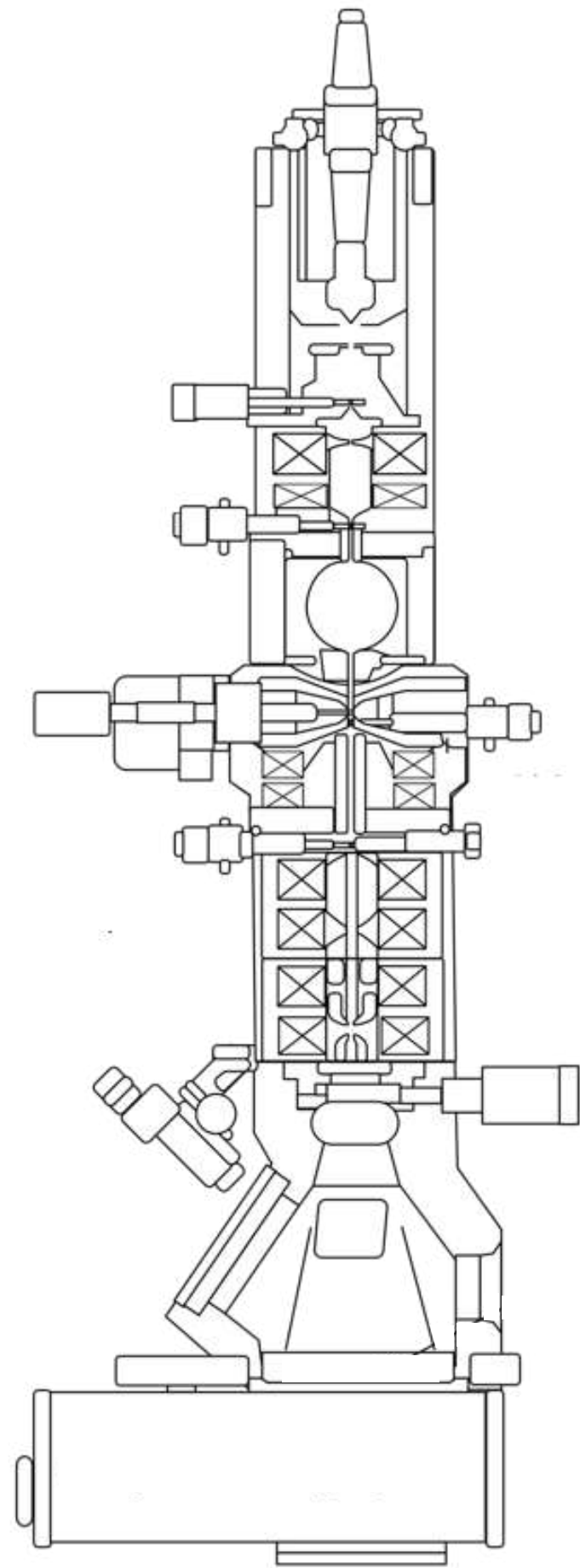
λ -- The wave-length,

Δf -- The defocus value,

$|g|$ -- The spatial frequency,

α -- The convergence semi-angle.

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



a

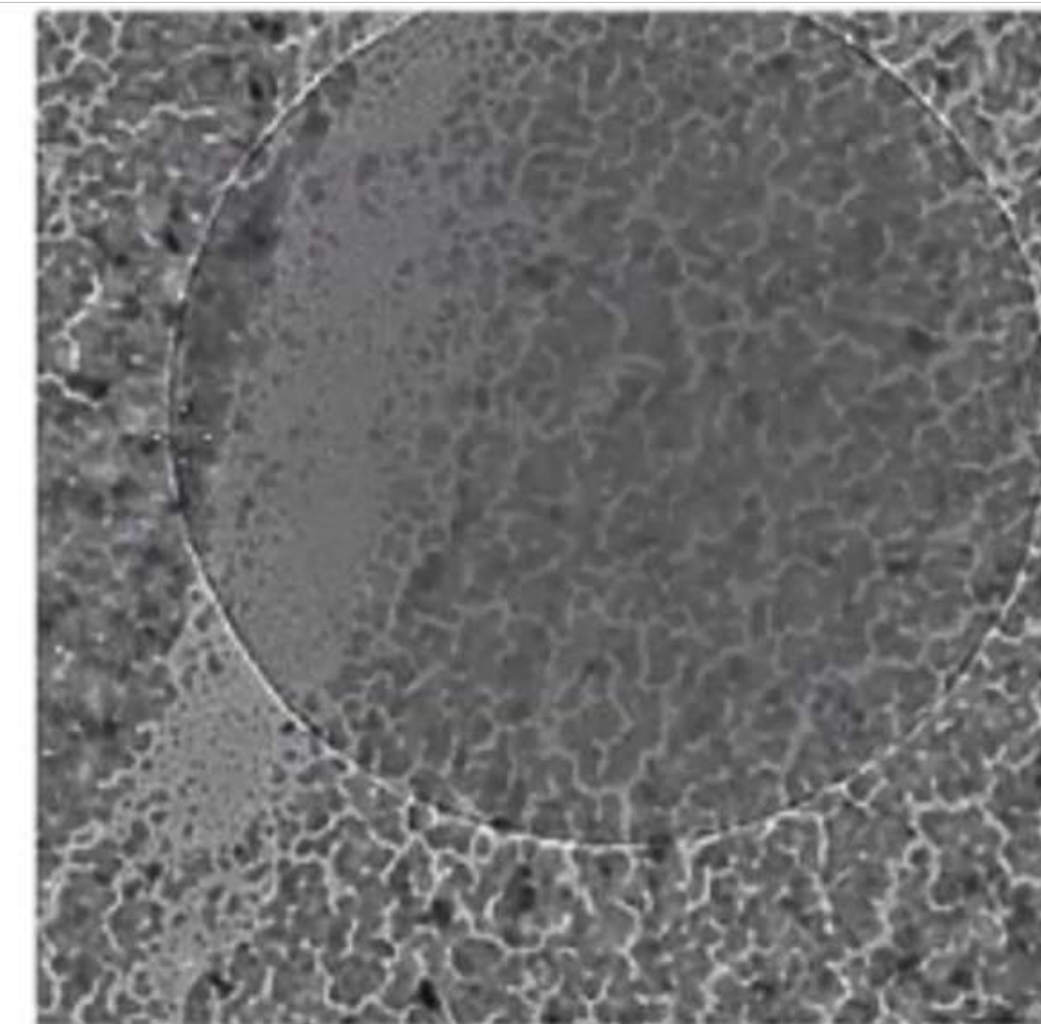
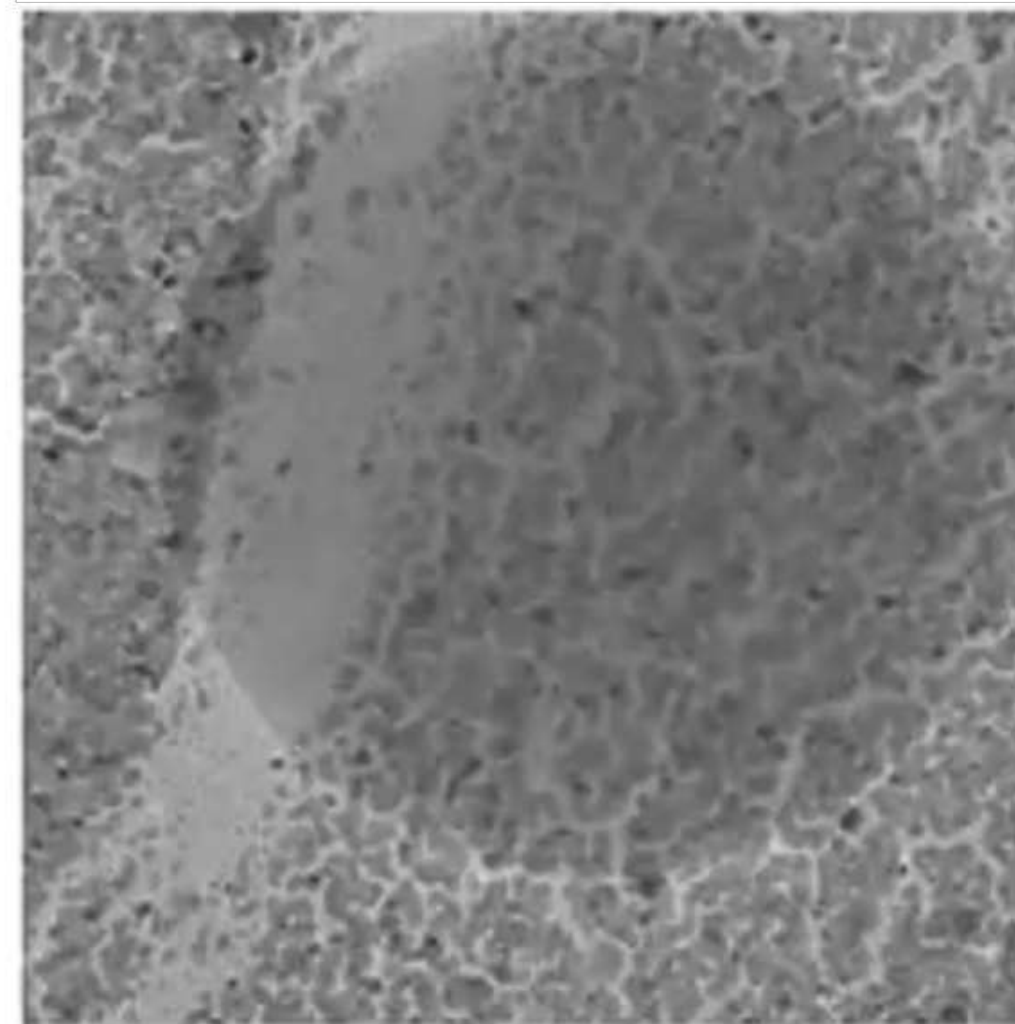
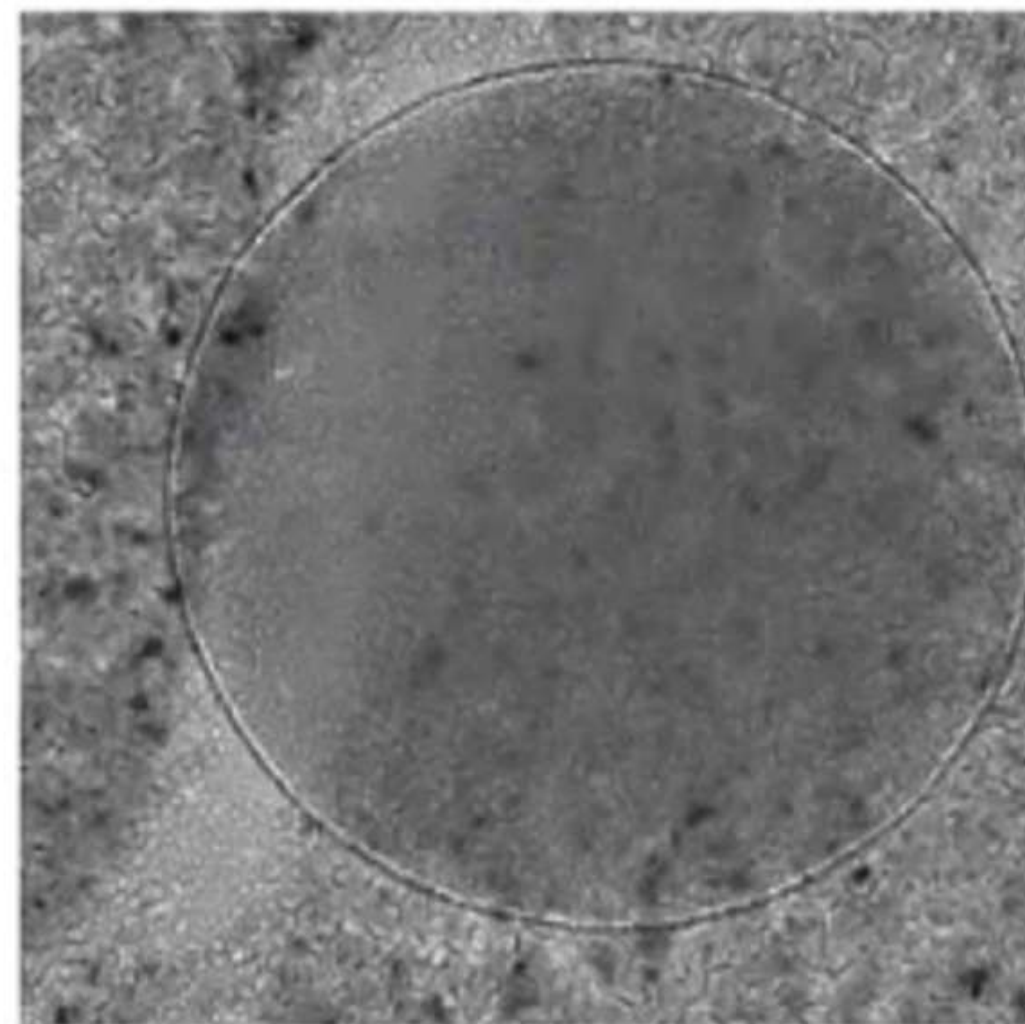
The **contrast transfer function (CTF)** mathematically describes how aberrations in a transmission electron microscope (TEM) modify the image of a sample

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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

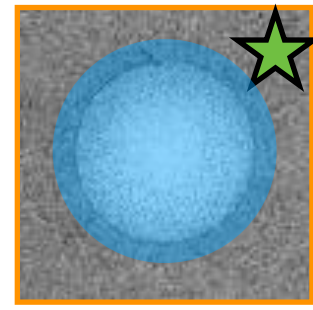
5 targets/setup
35 s/image
~2500 images/day

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

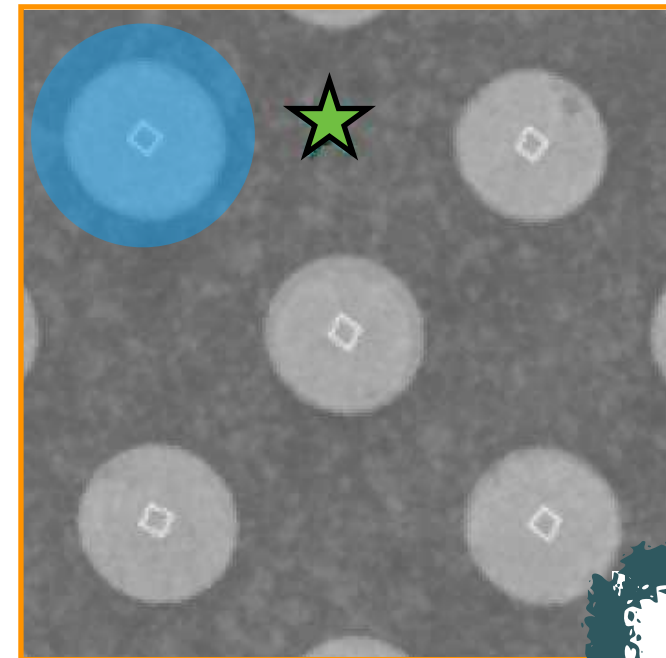
70 targets/setup
18 s / image
~ 4800 images/day



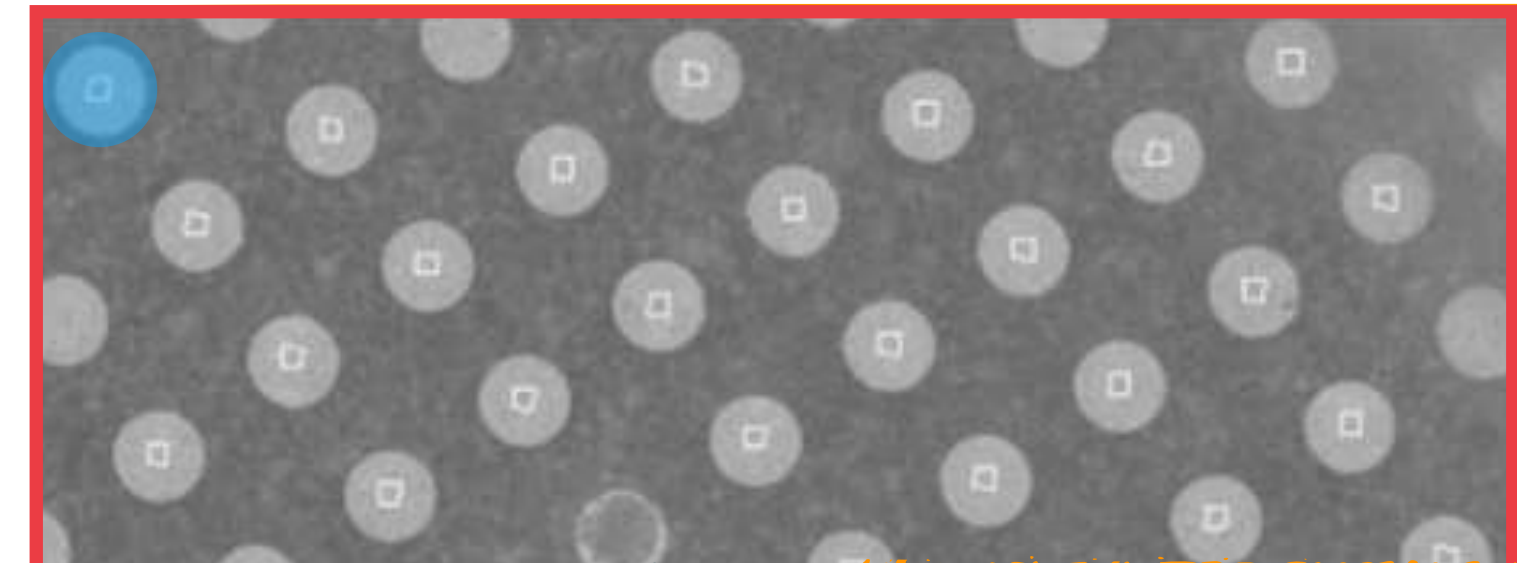
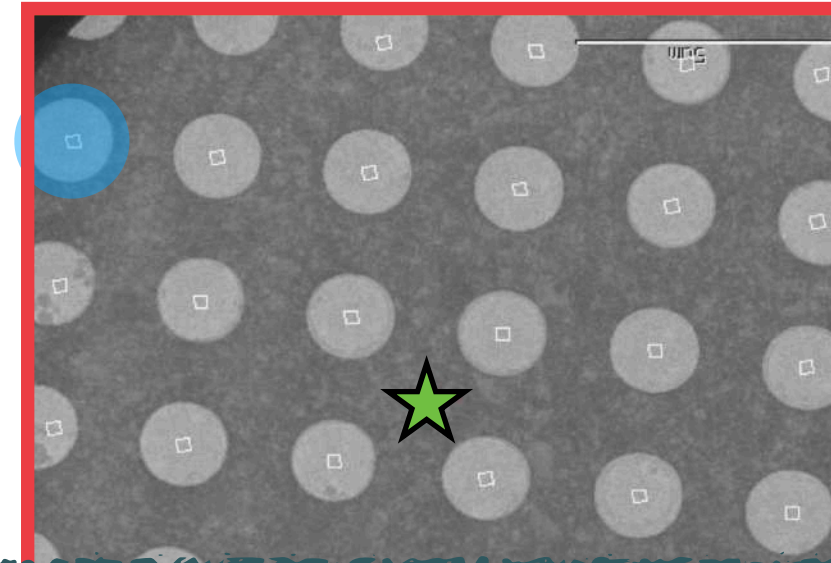
13.6MP



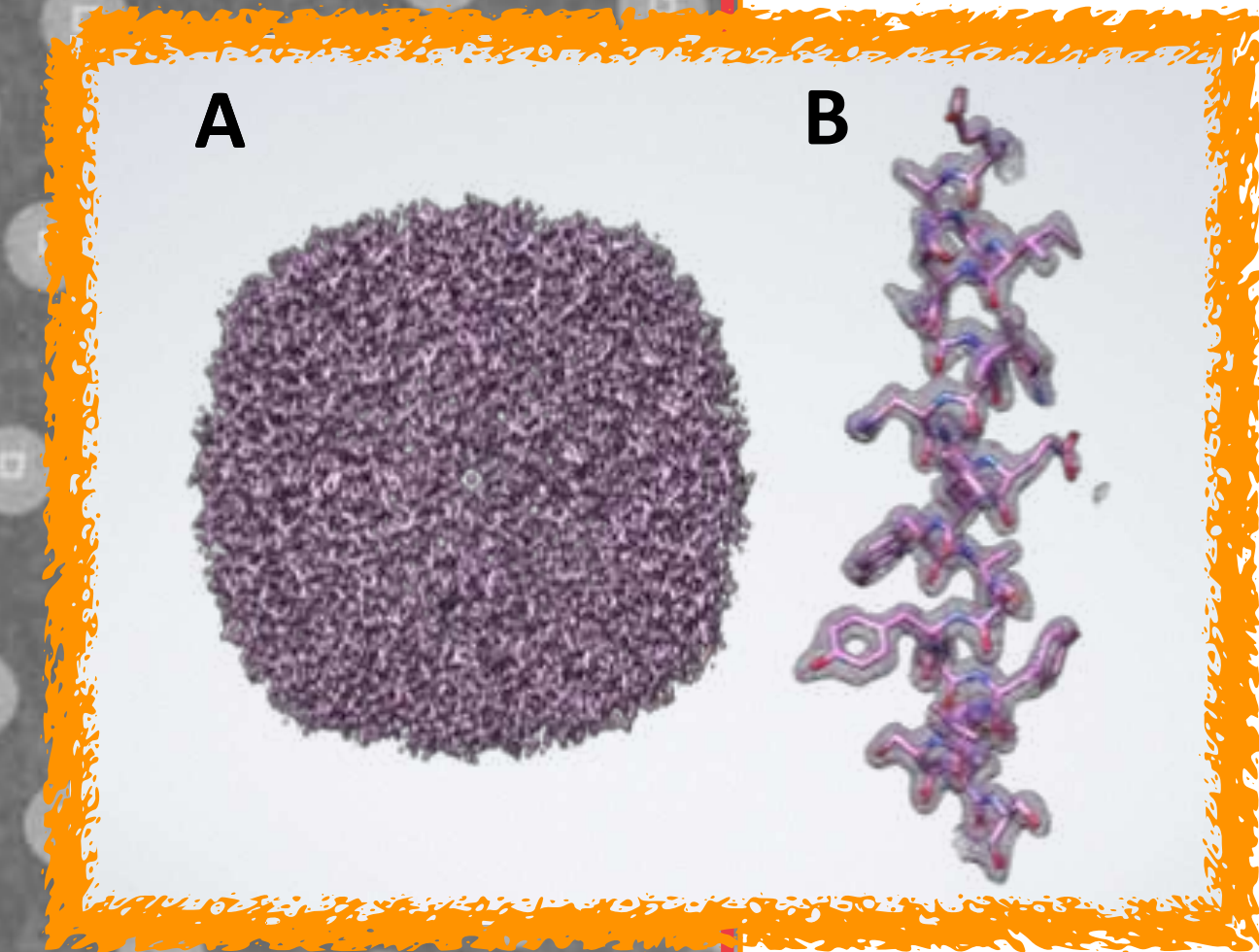
beam tilt
0 mrad



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;



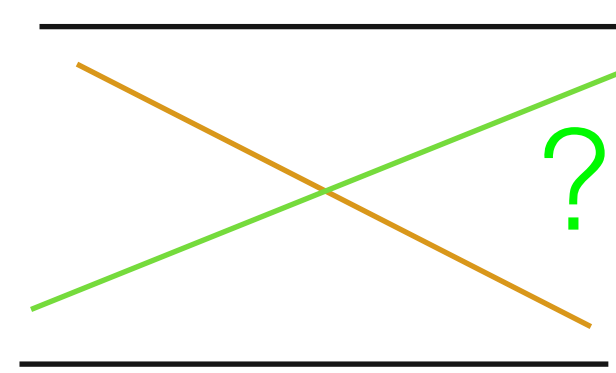
Anchi Cheng

Detectors

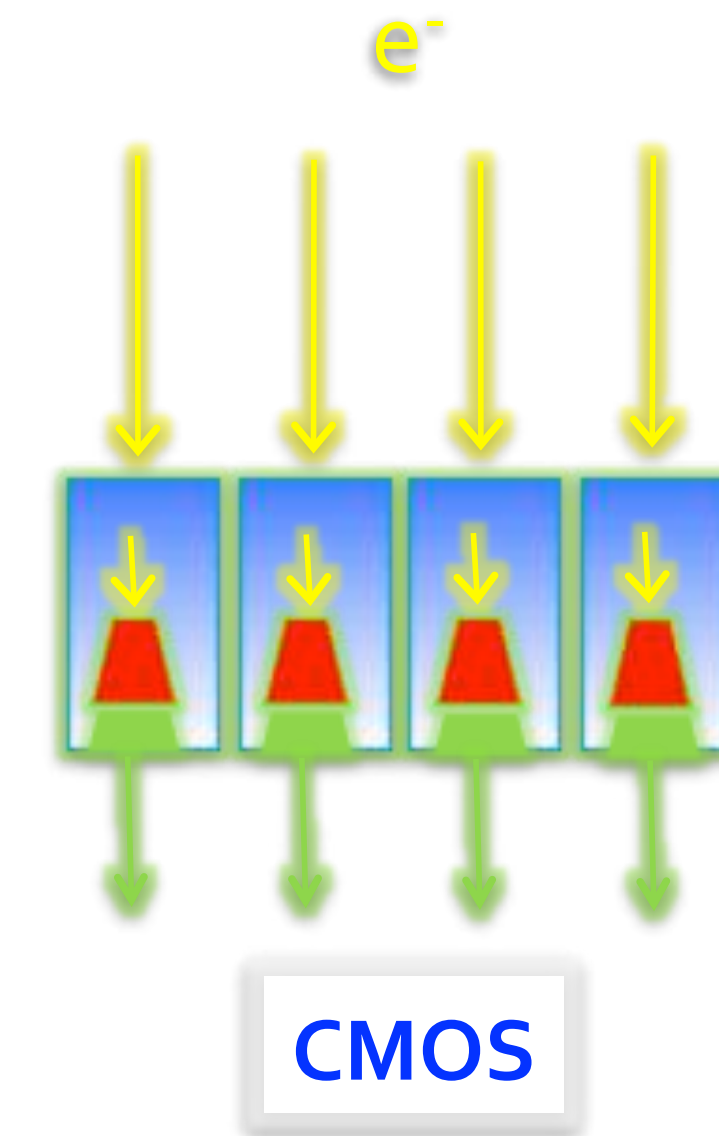
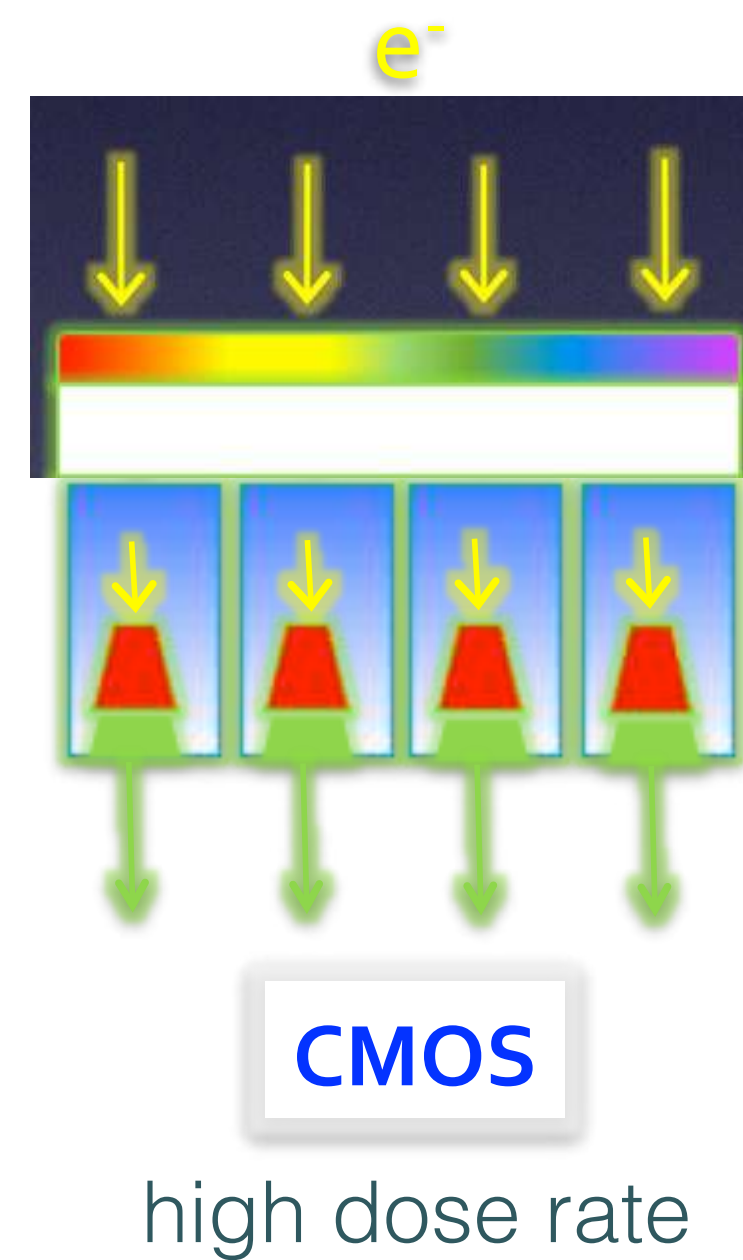
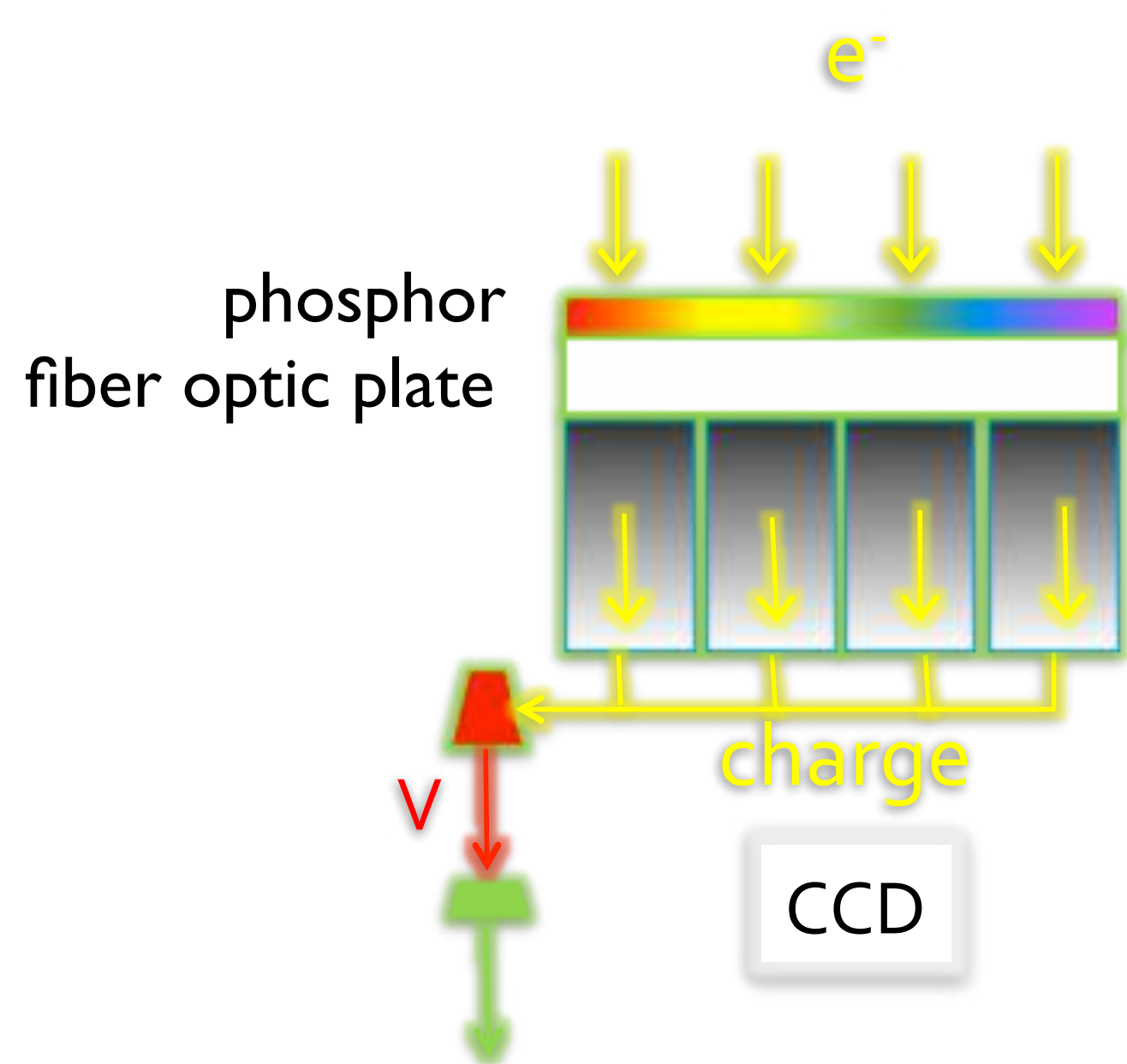
Digital Cameras for TEM



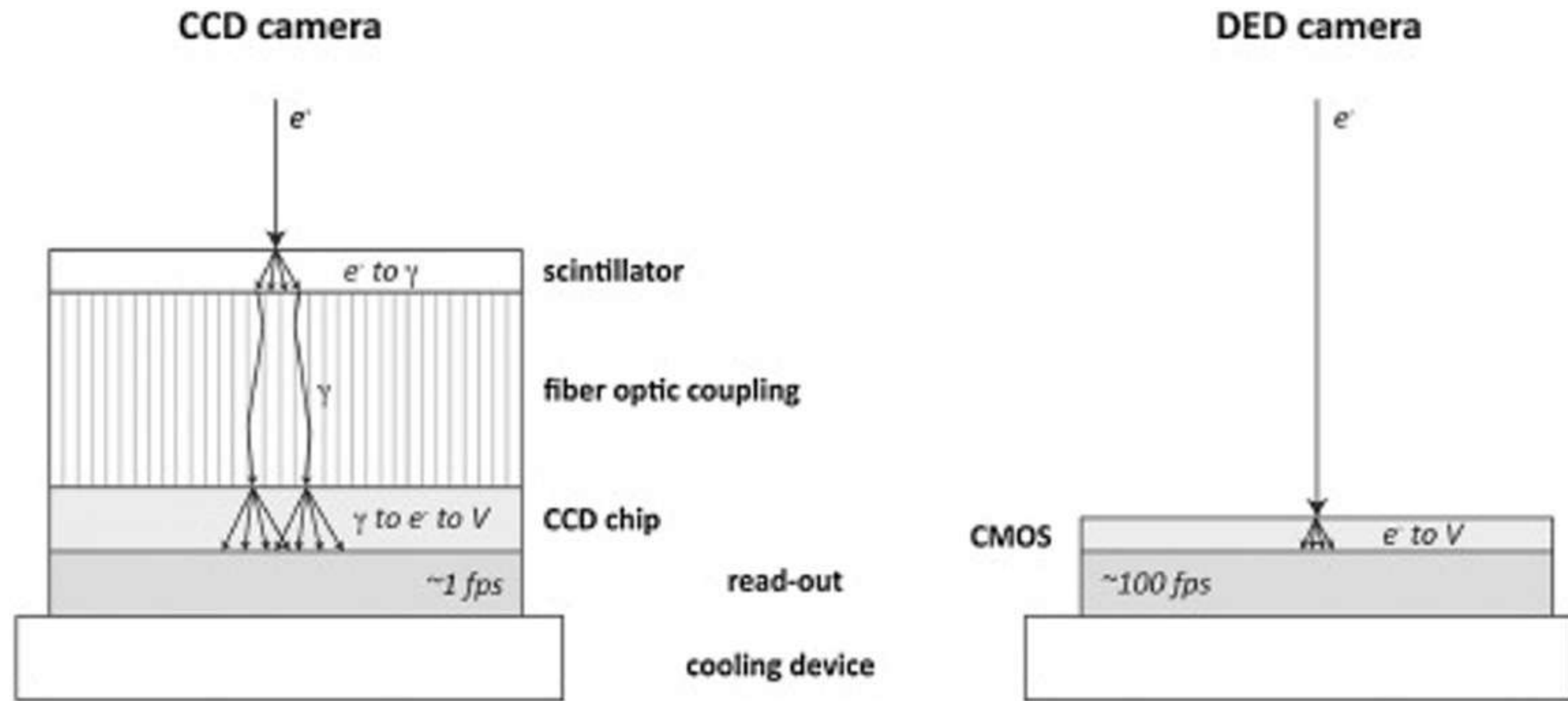
- Photon converted
- Direct sensing



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



Direct Detectors



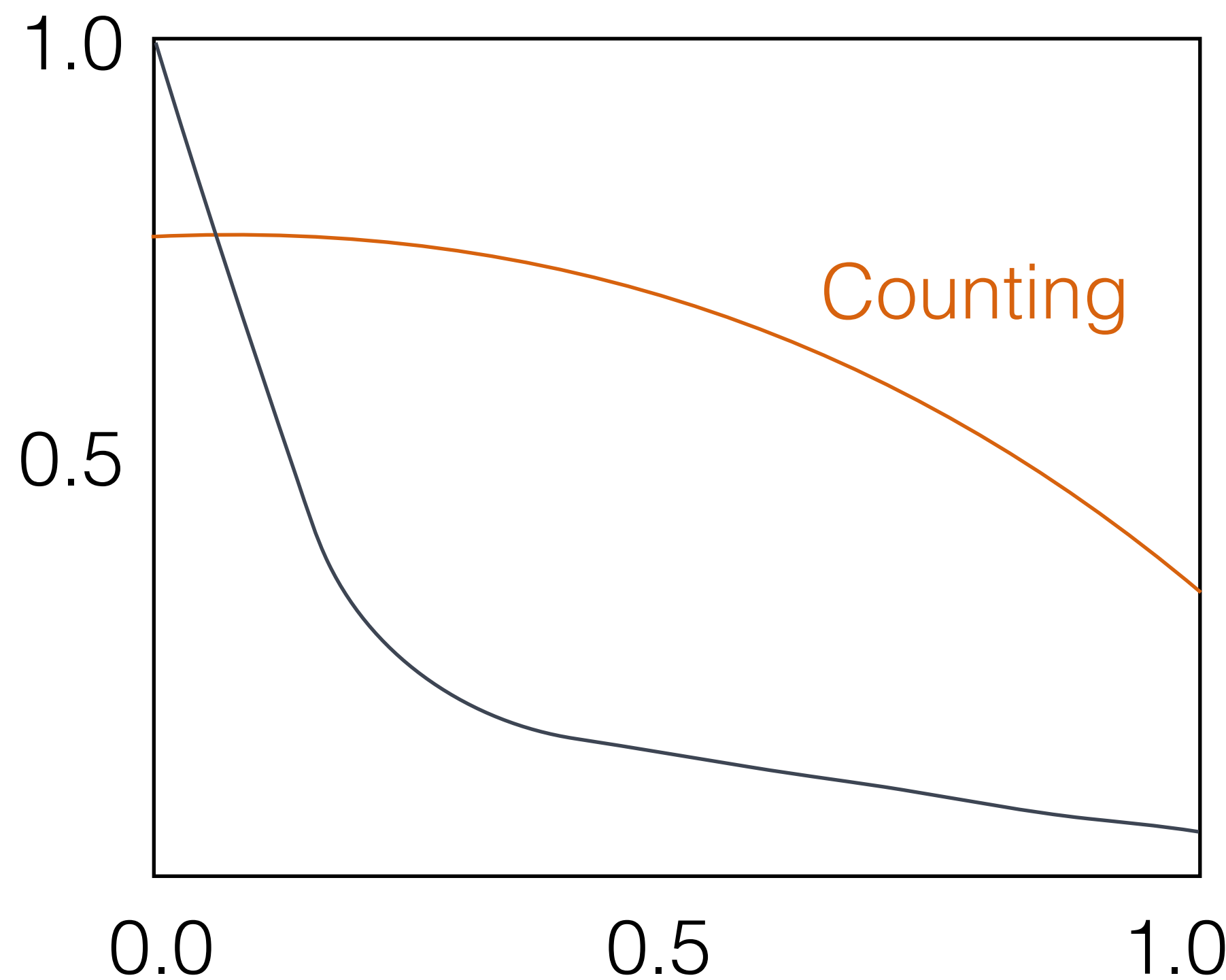
Koning et al. Ann. Anatomy 2018

Detectors

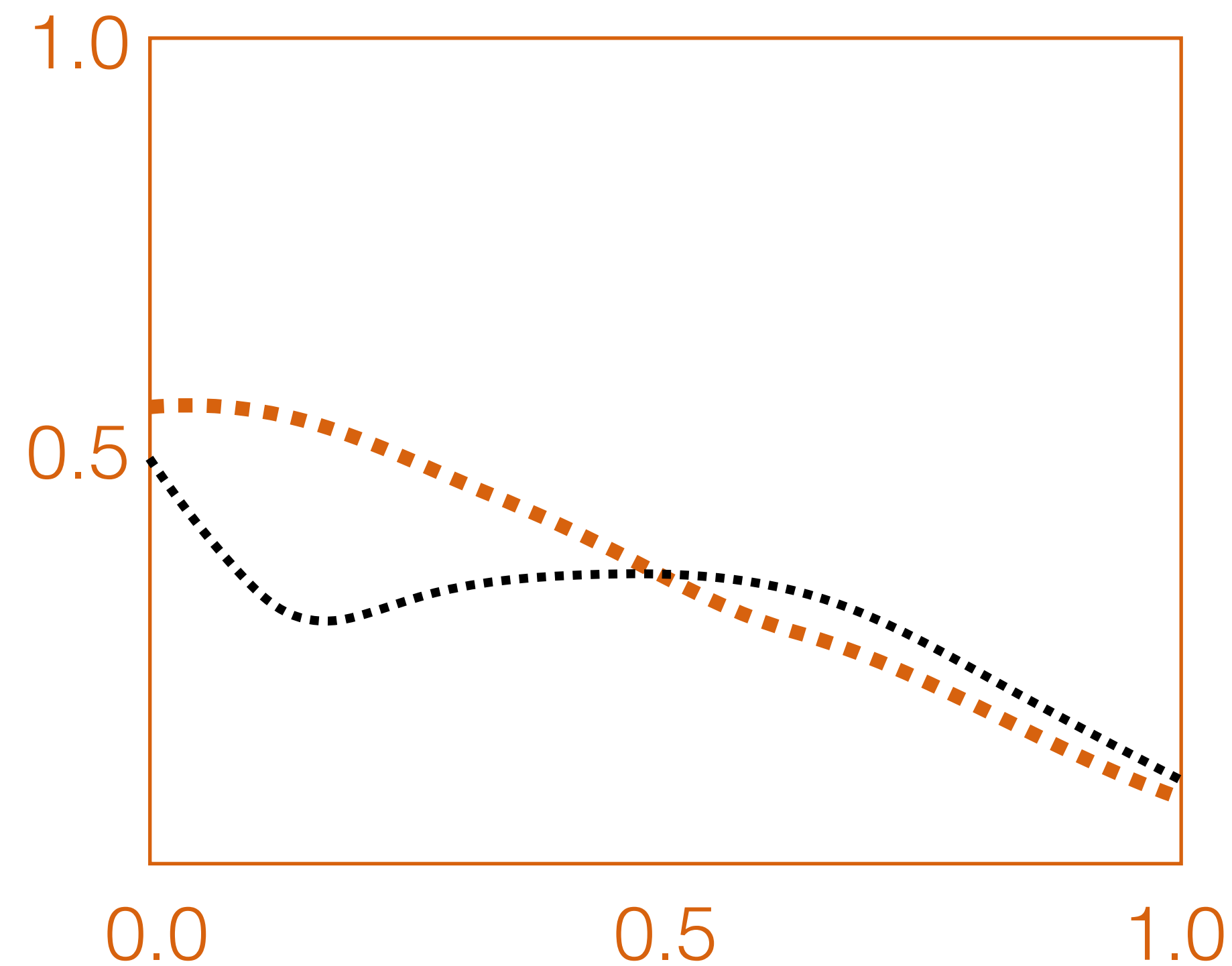
Detector Performance Characterization



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



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

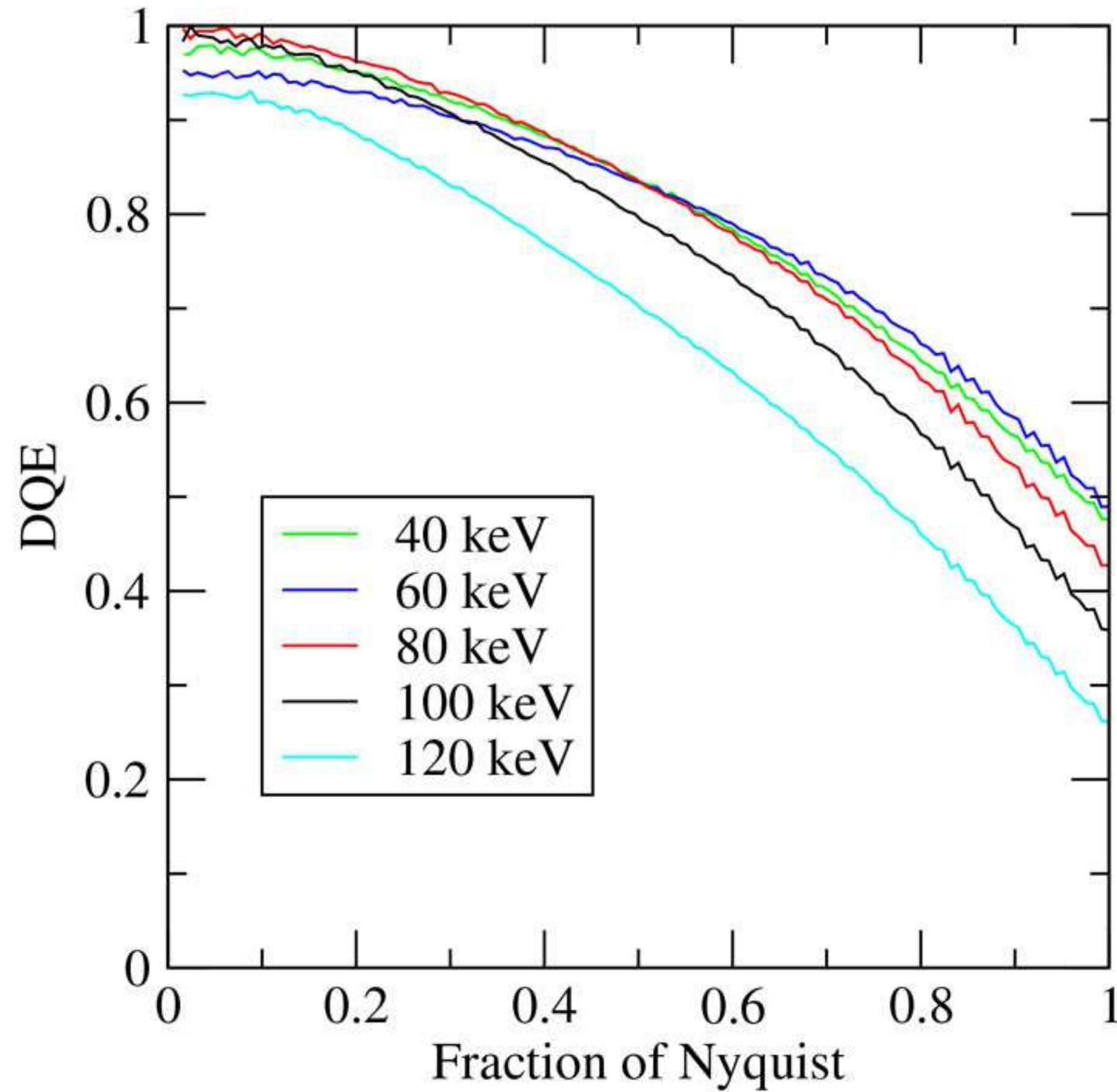


PSF: the point spread function describes the response of an imaging system to a point source or point object.

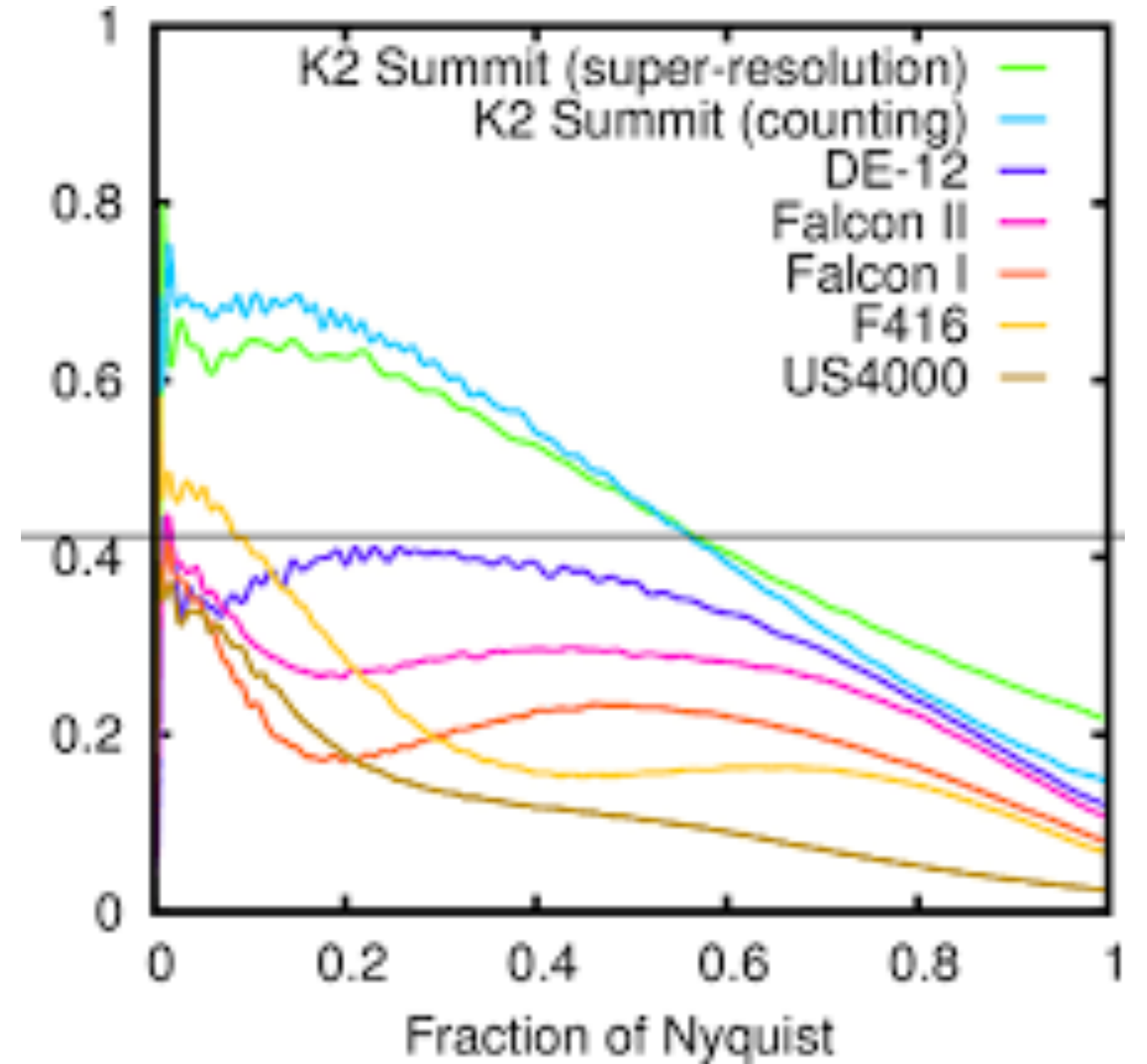
MTF: the modulation transfer function, is defined as the Fourier transform of the point spread function

Detectors

Detector Performance Characterization



dectris.com



Ruskin, et al JSB

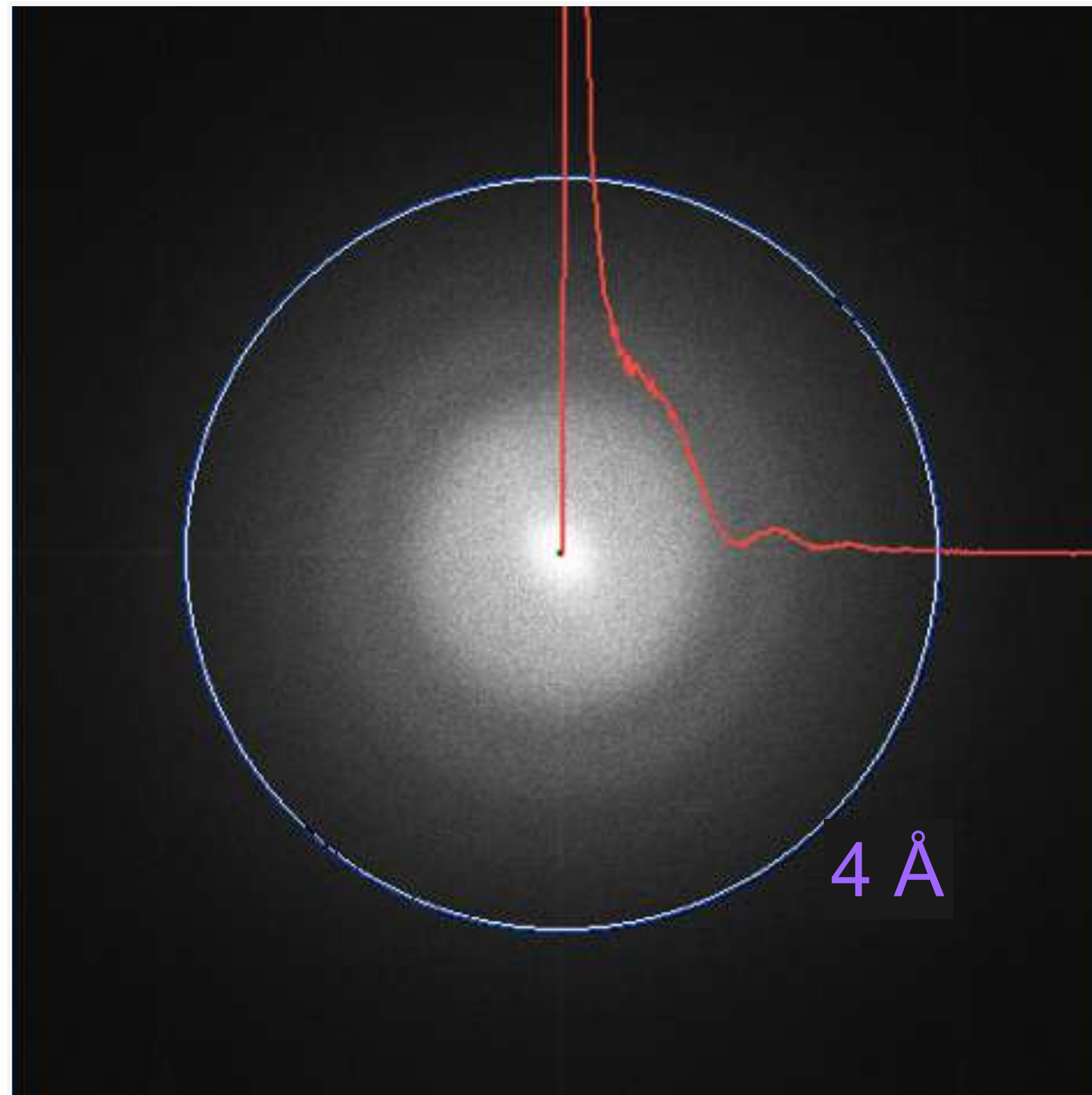
Detectors

Improving the resolution:
Detecting electrons instead of photons

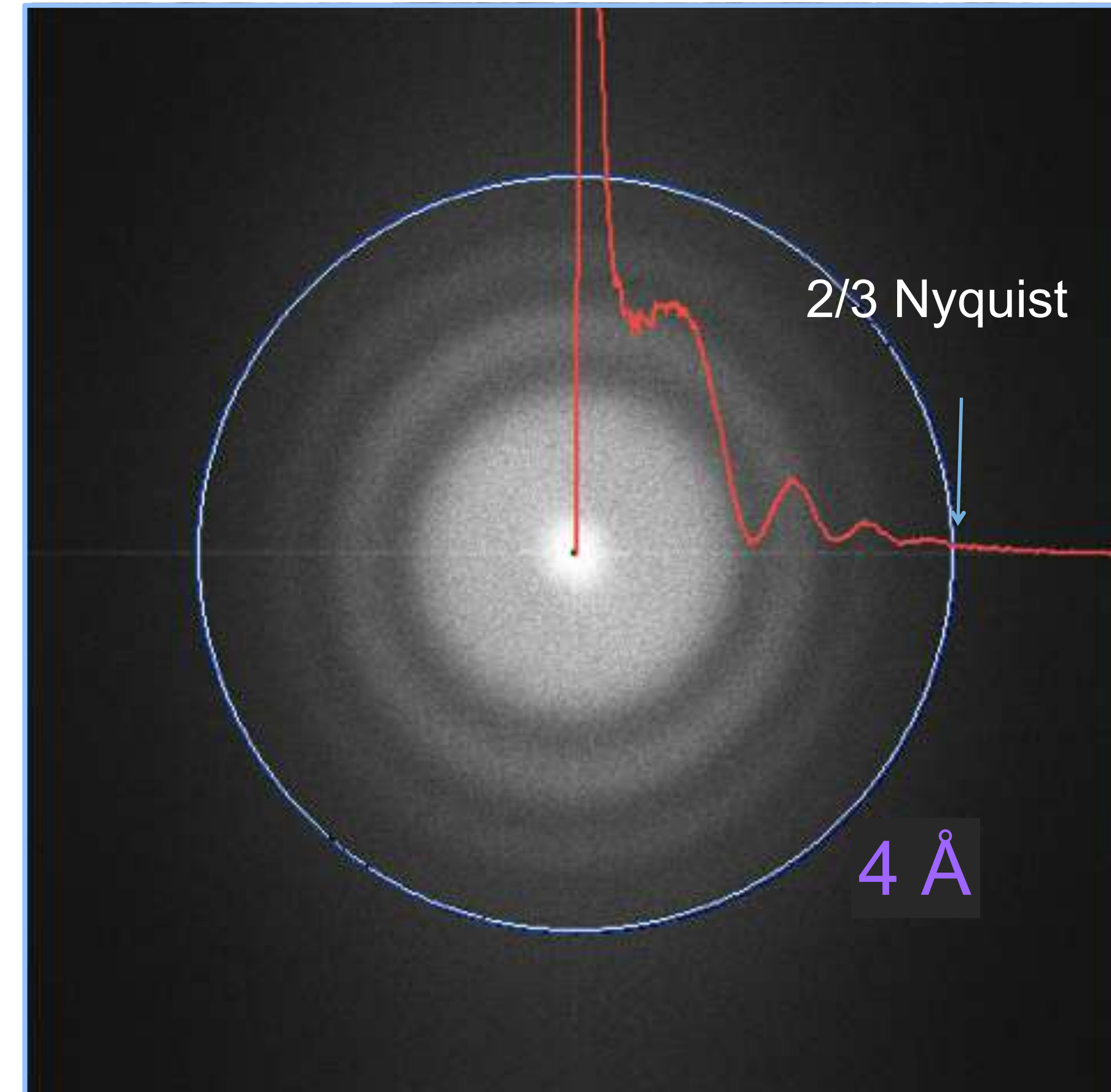


CCD

DDD



1.37 Å/pixel



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	

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

Specifications are subject to change without notice.

Detectors

Improving the resolution:
Detecting electrons instead of photons

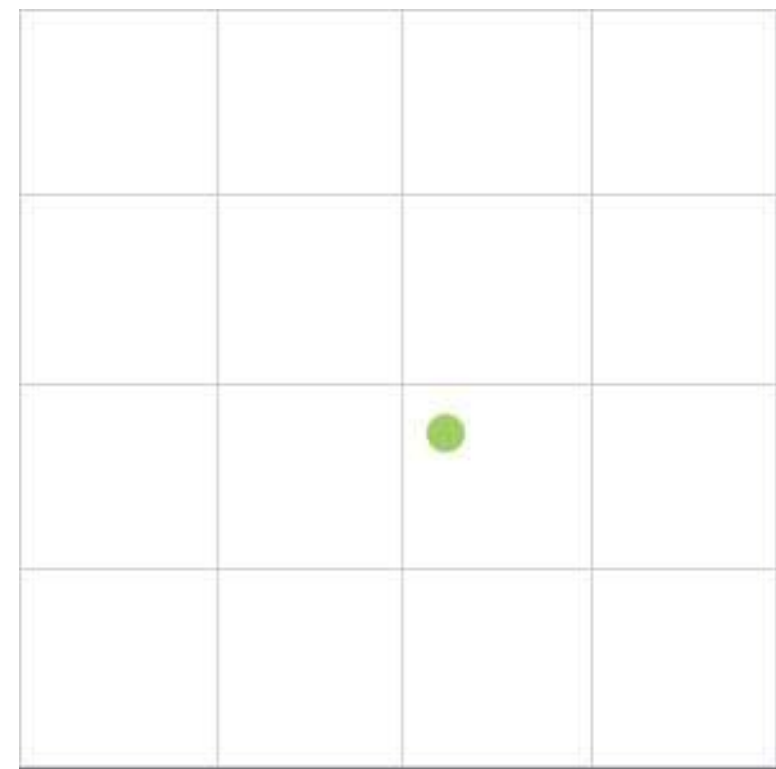


Counting mode

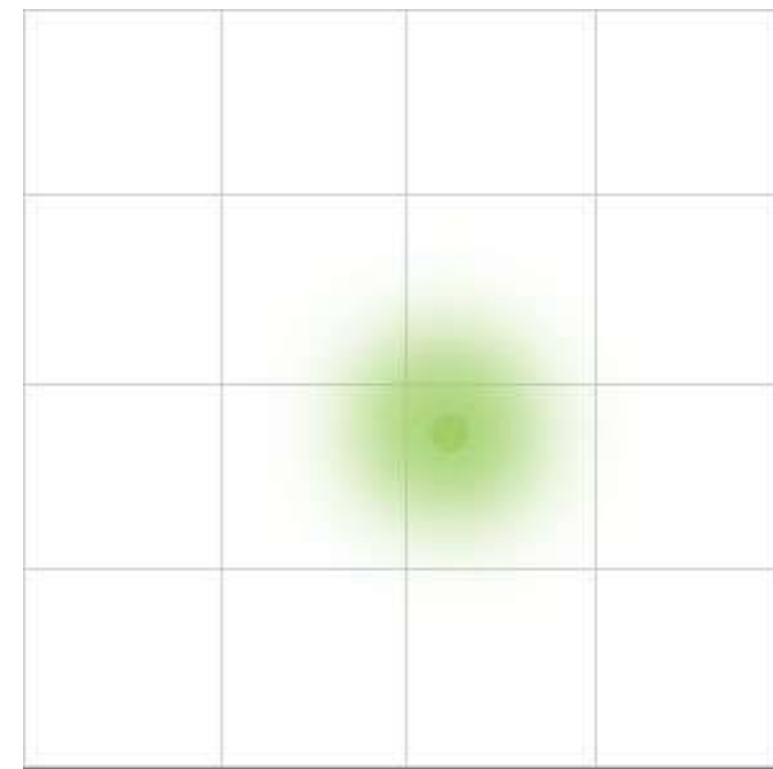
5,760 x 4,096 px



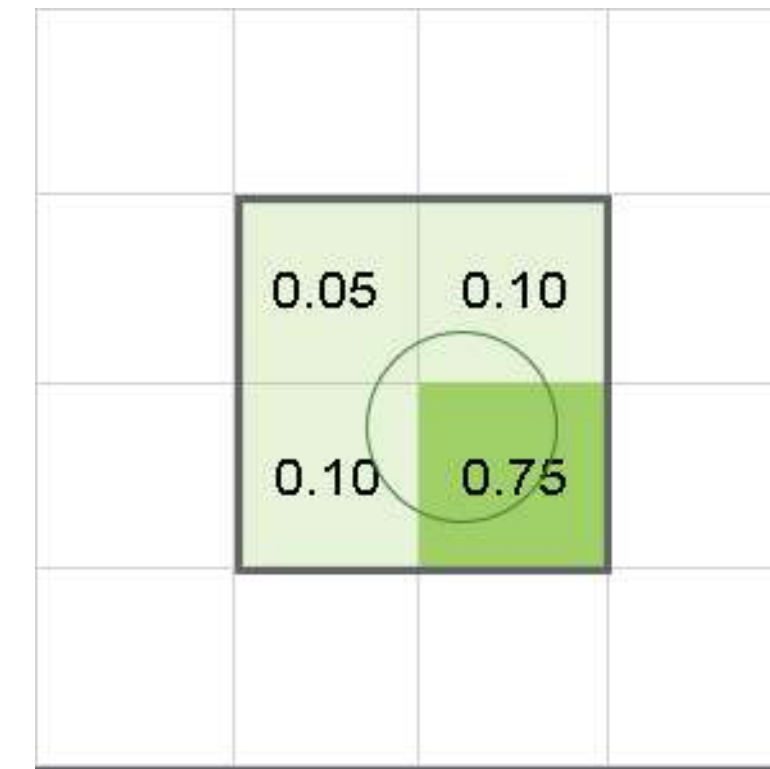
11,520 x 8,184 px



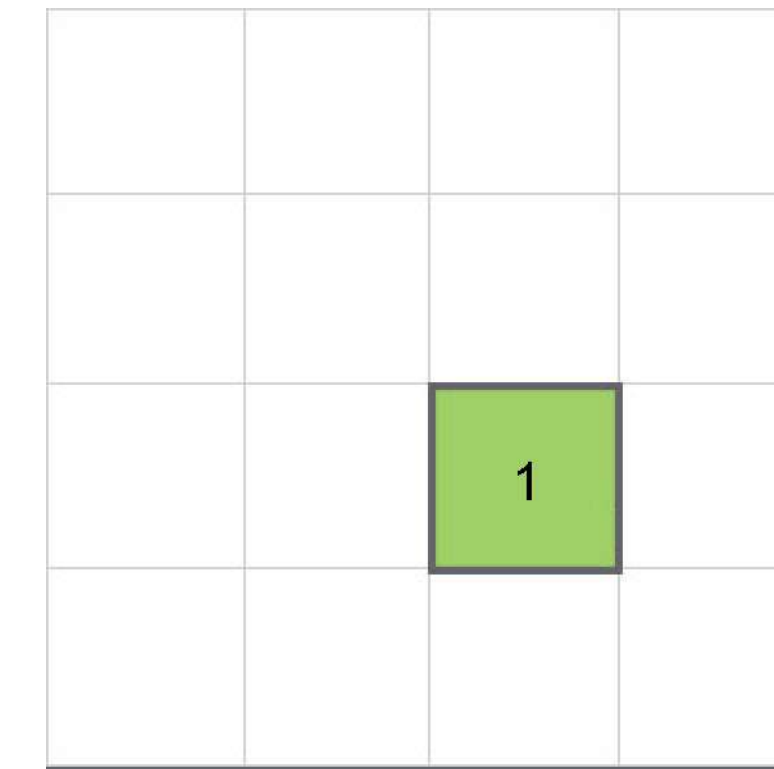
Electron enters detector.



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

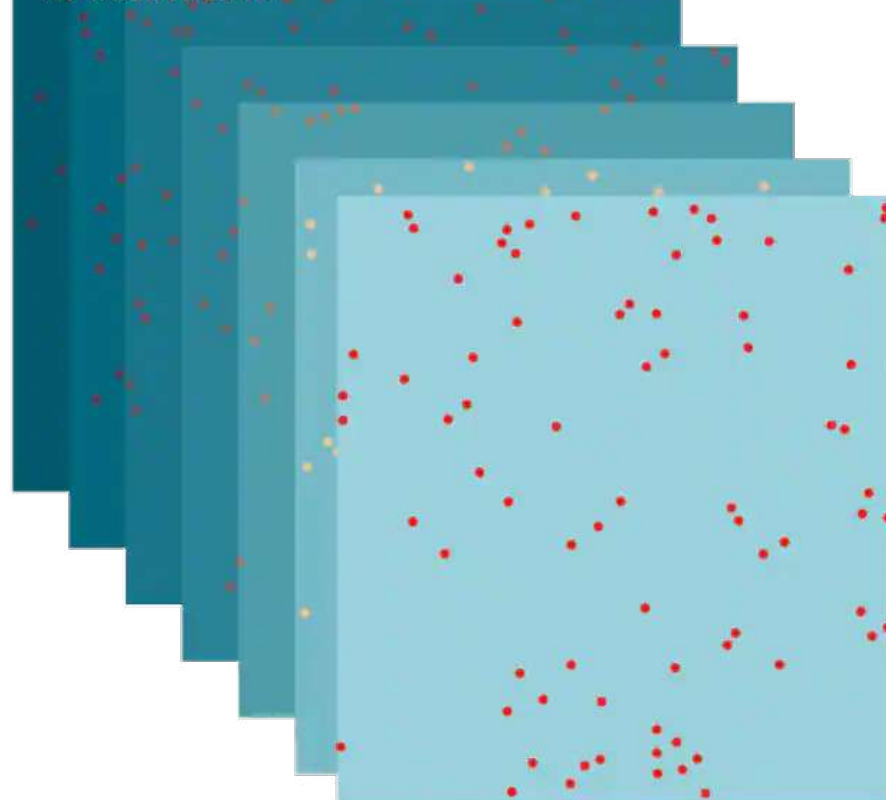


Improving the resolution:
Detecting electrons instead of photons



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

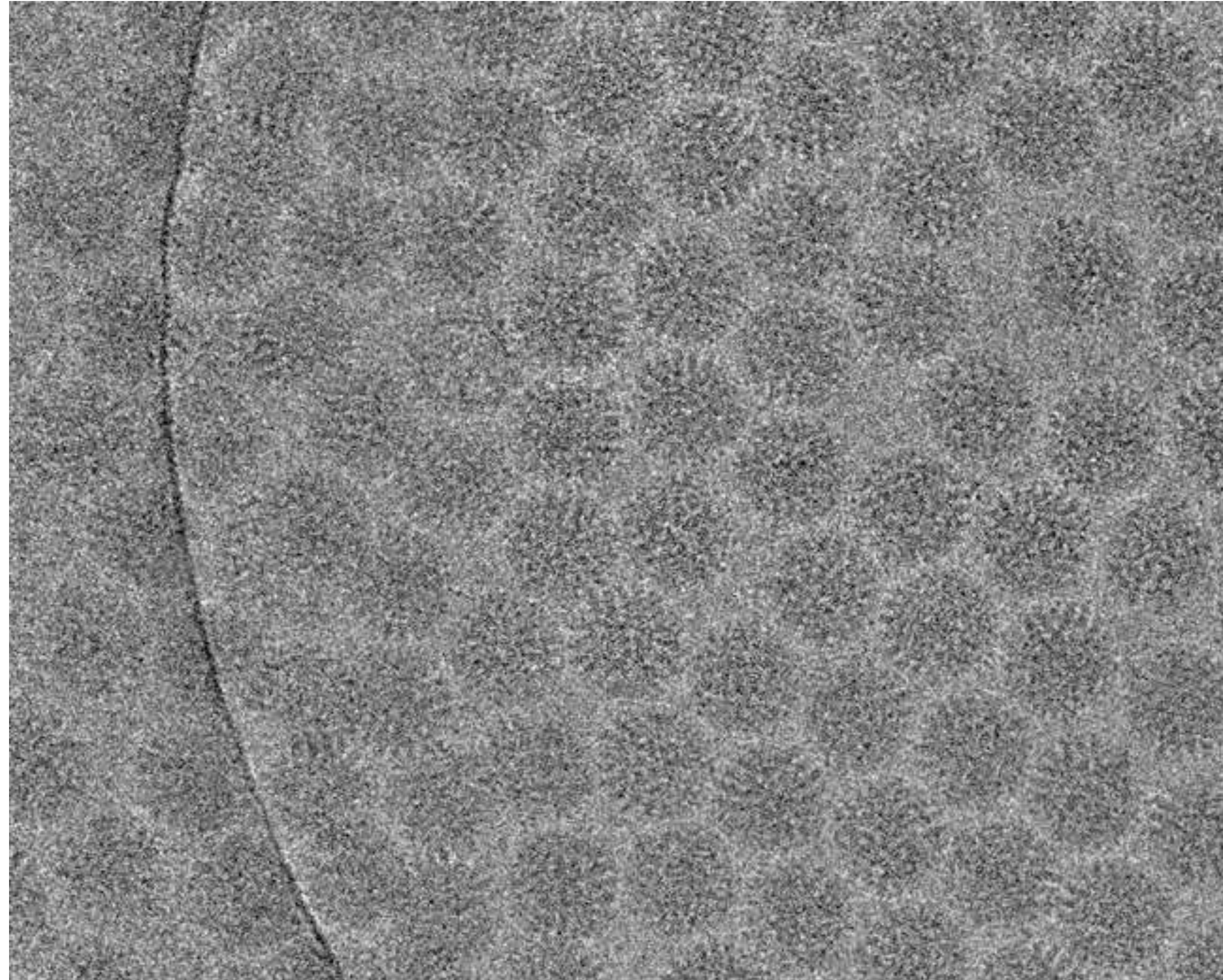
Coordinates	
x	y
3953.24	2845.63
919.78	1447.39
3864.43	348.13
3606.05	1539.54
1758.86	2971.55
...	...
3983.58	531.96

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



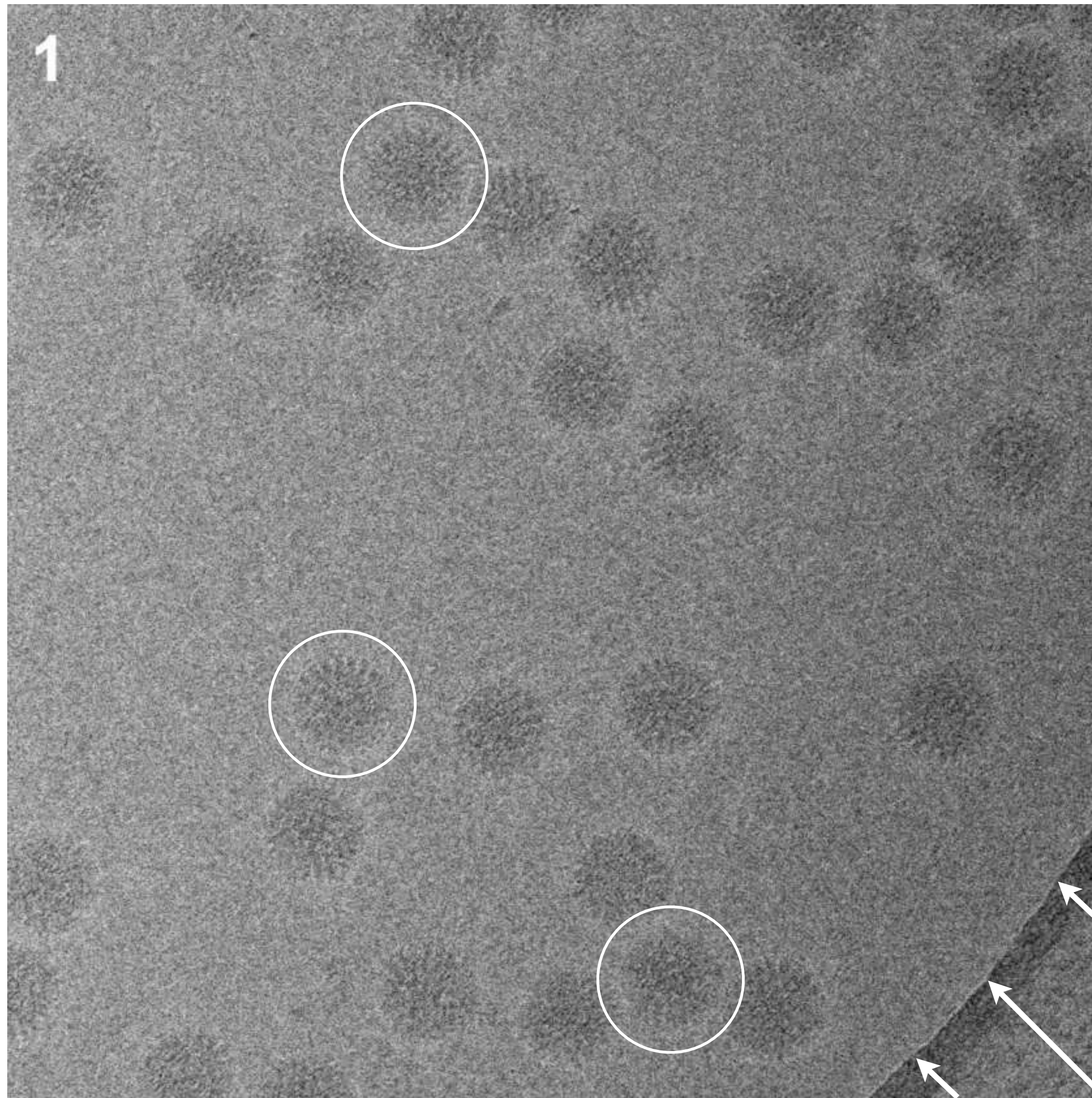
$0.5 \text{ e}^-/\text{\AA}^2/\text{frame}$

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

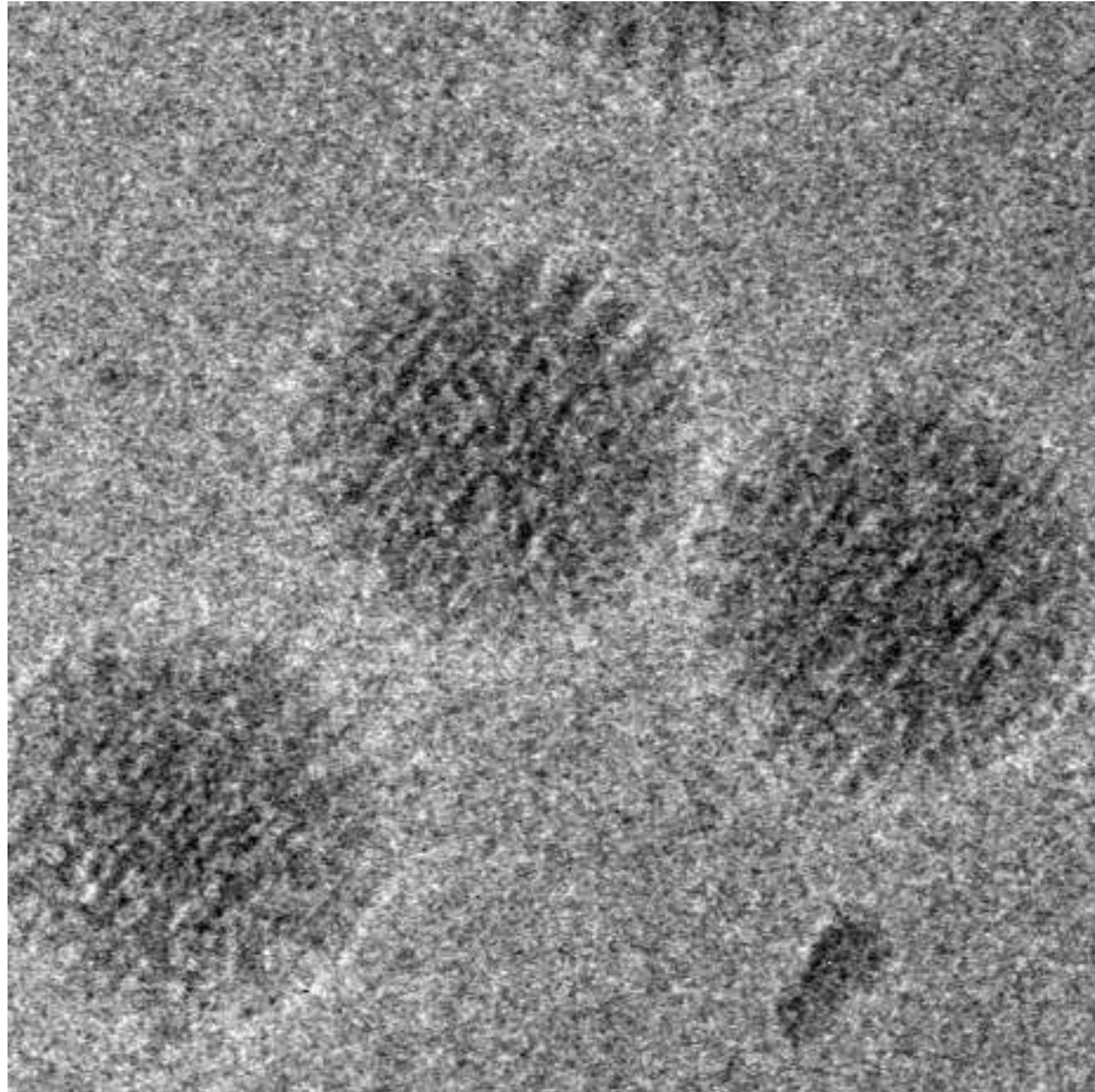
**A “movie” of rotavirus
exposed to electron beam**

10 frame averages

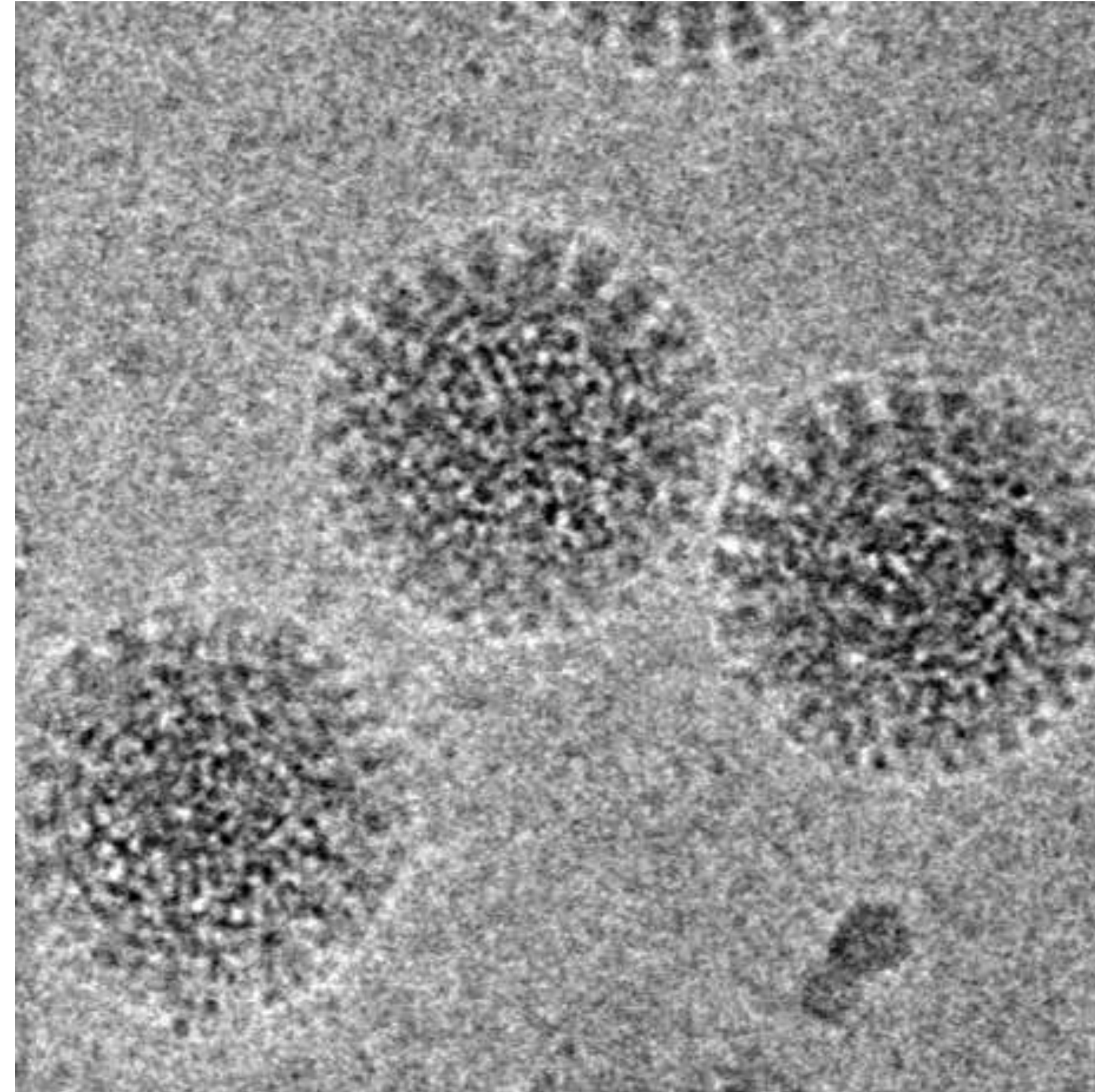
Brilot C.F. et al. (2012) J Struct Biol.



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?

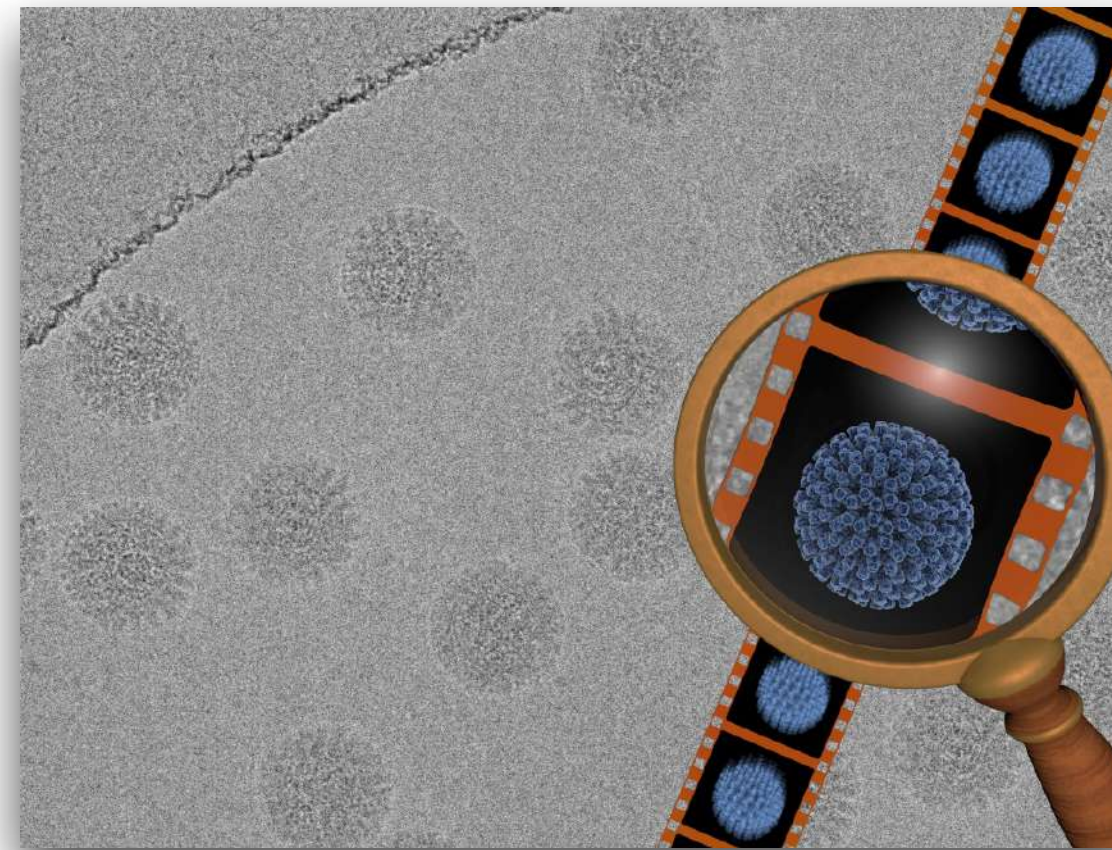
(~2012-2014)

Hardware

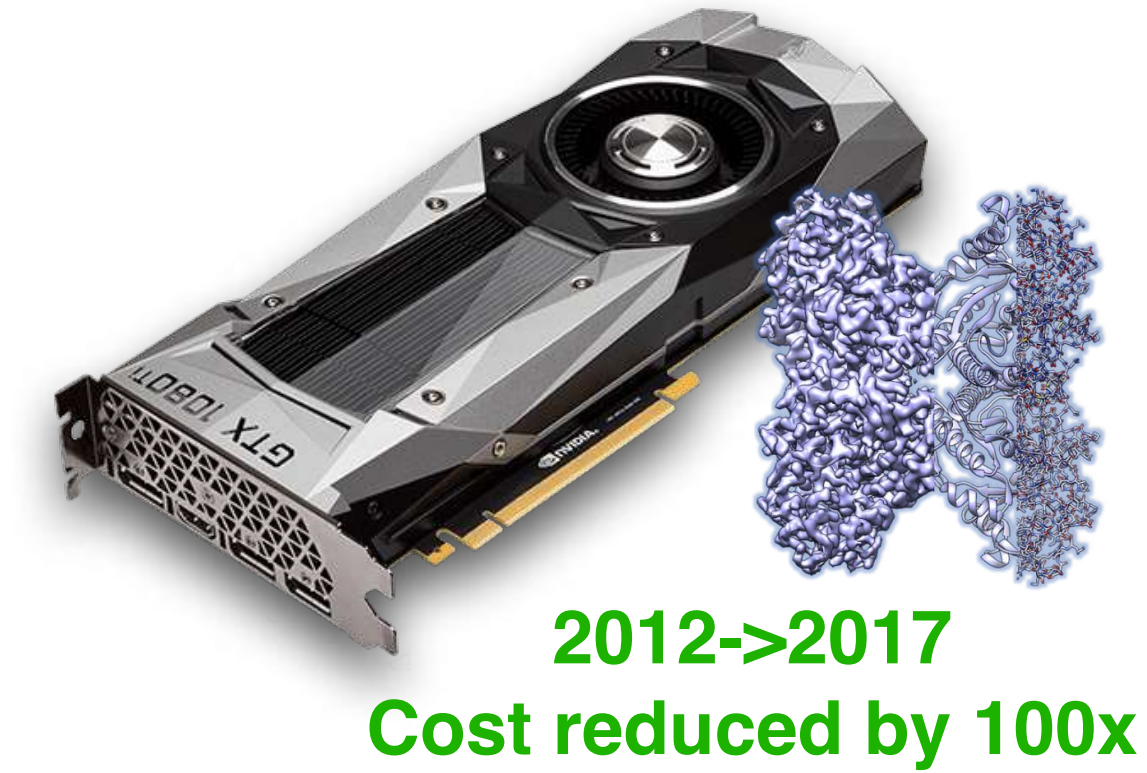
Microscopes



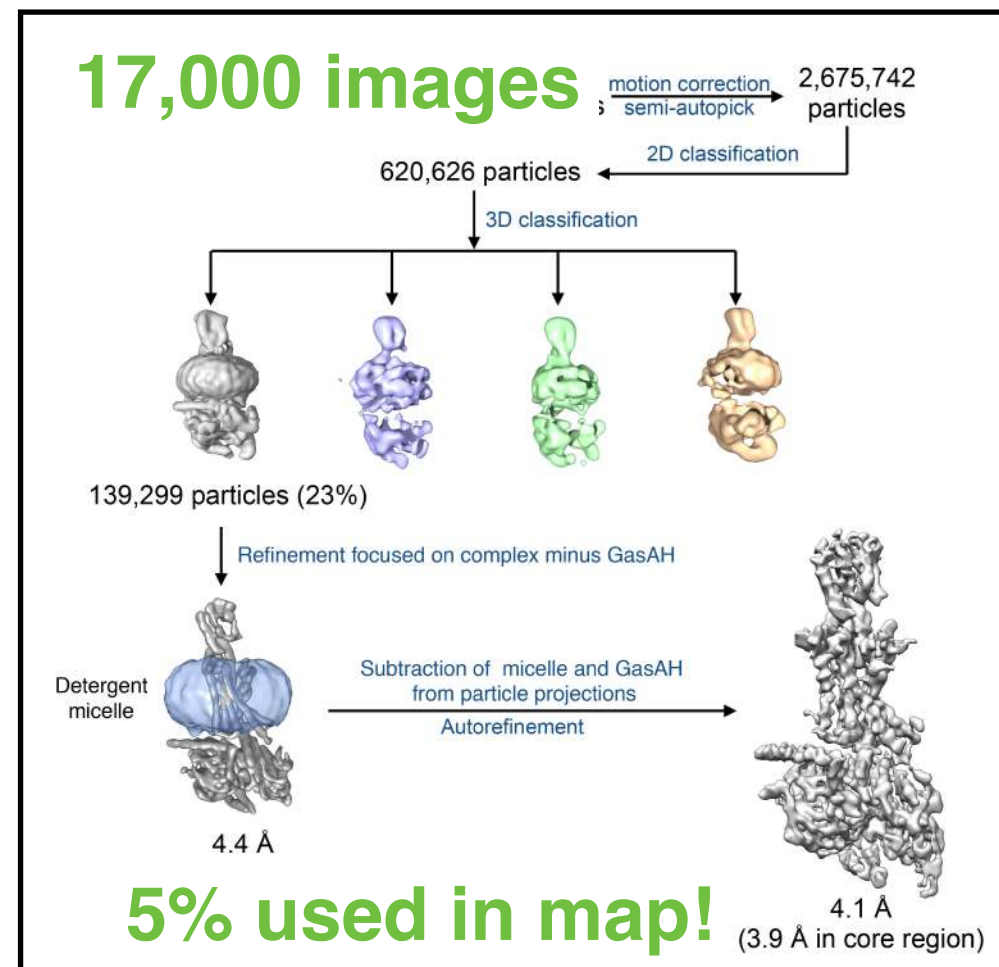
Direct Detectors



Computers



Software

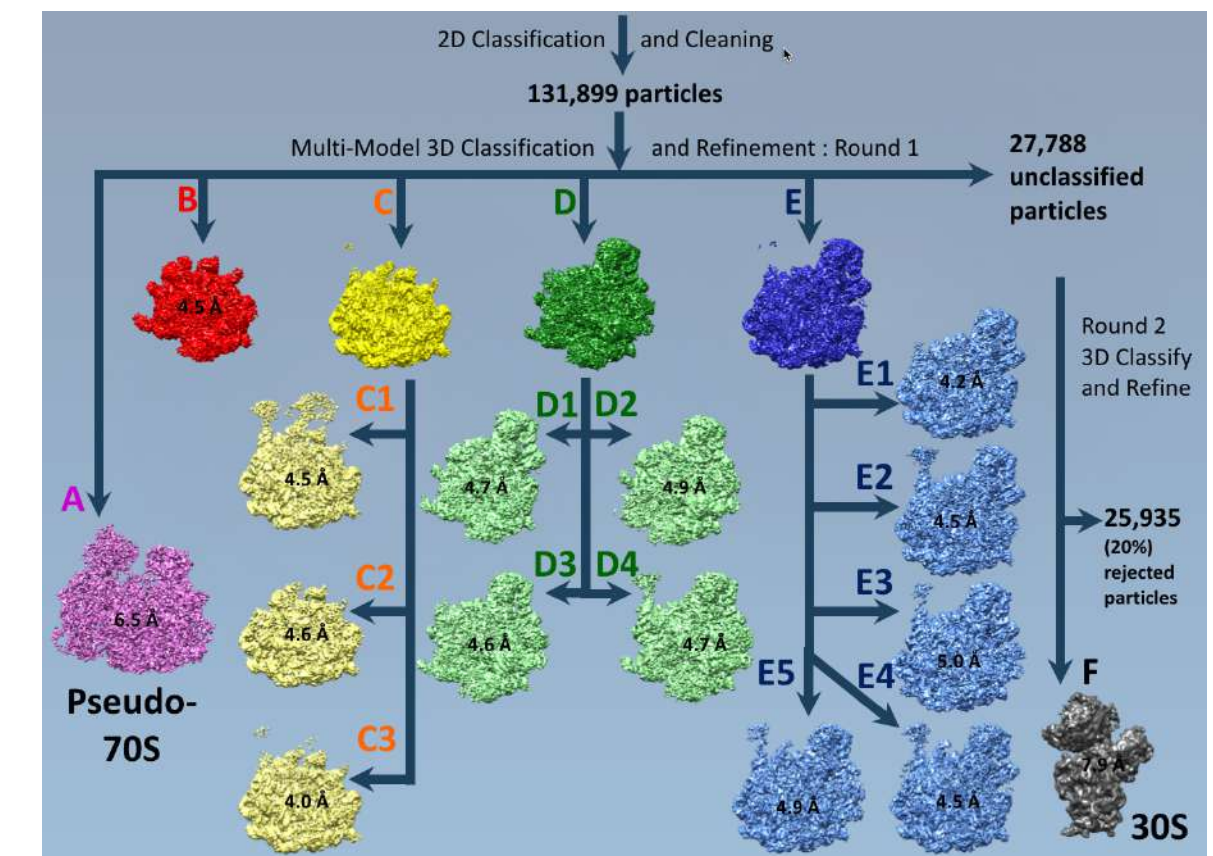


Leginon / SerialEM / EPU, ...

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

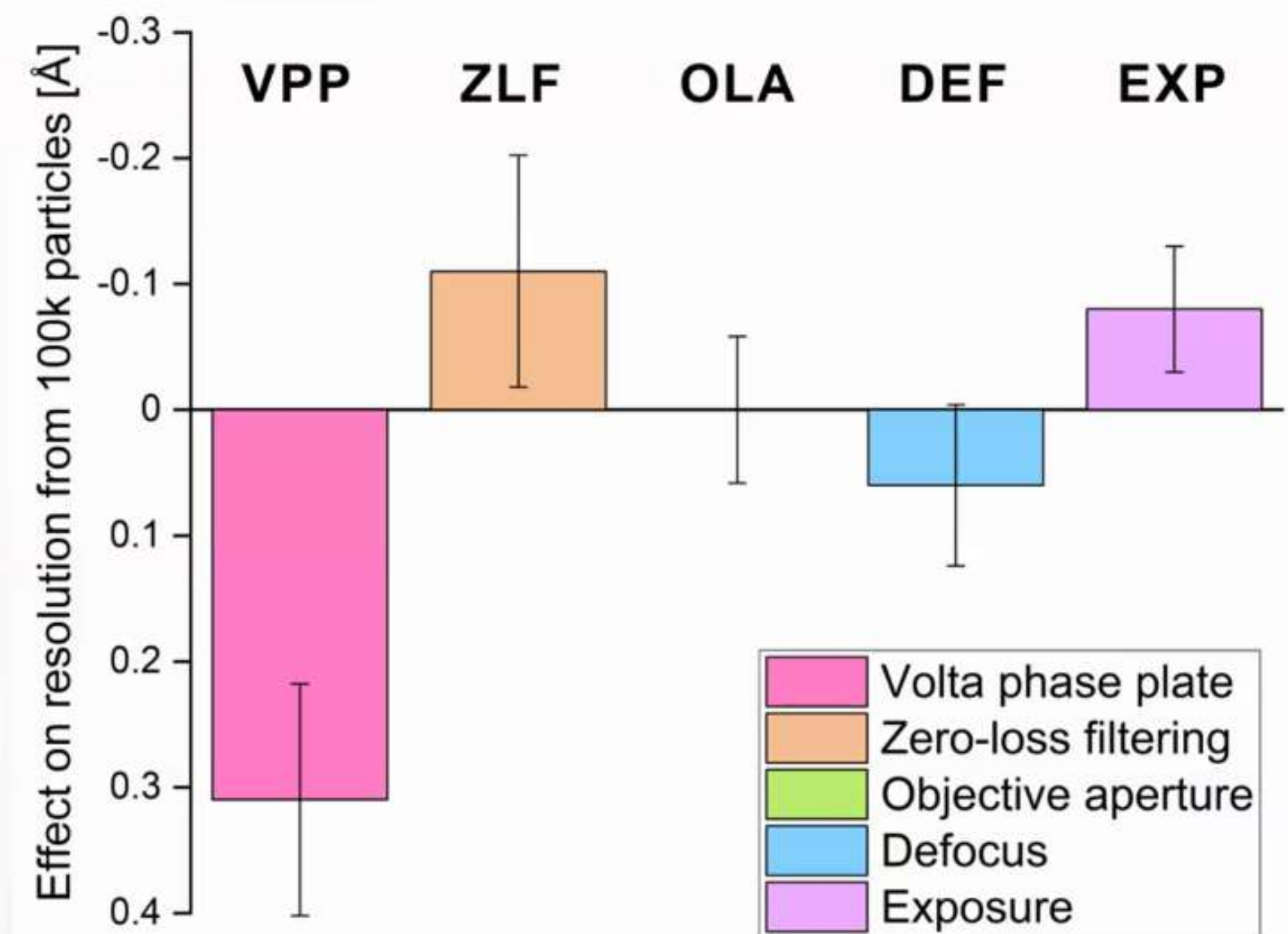
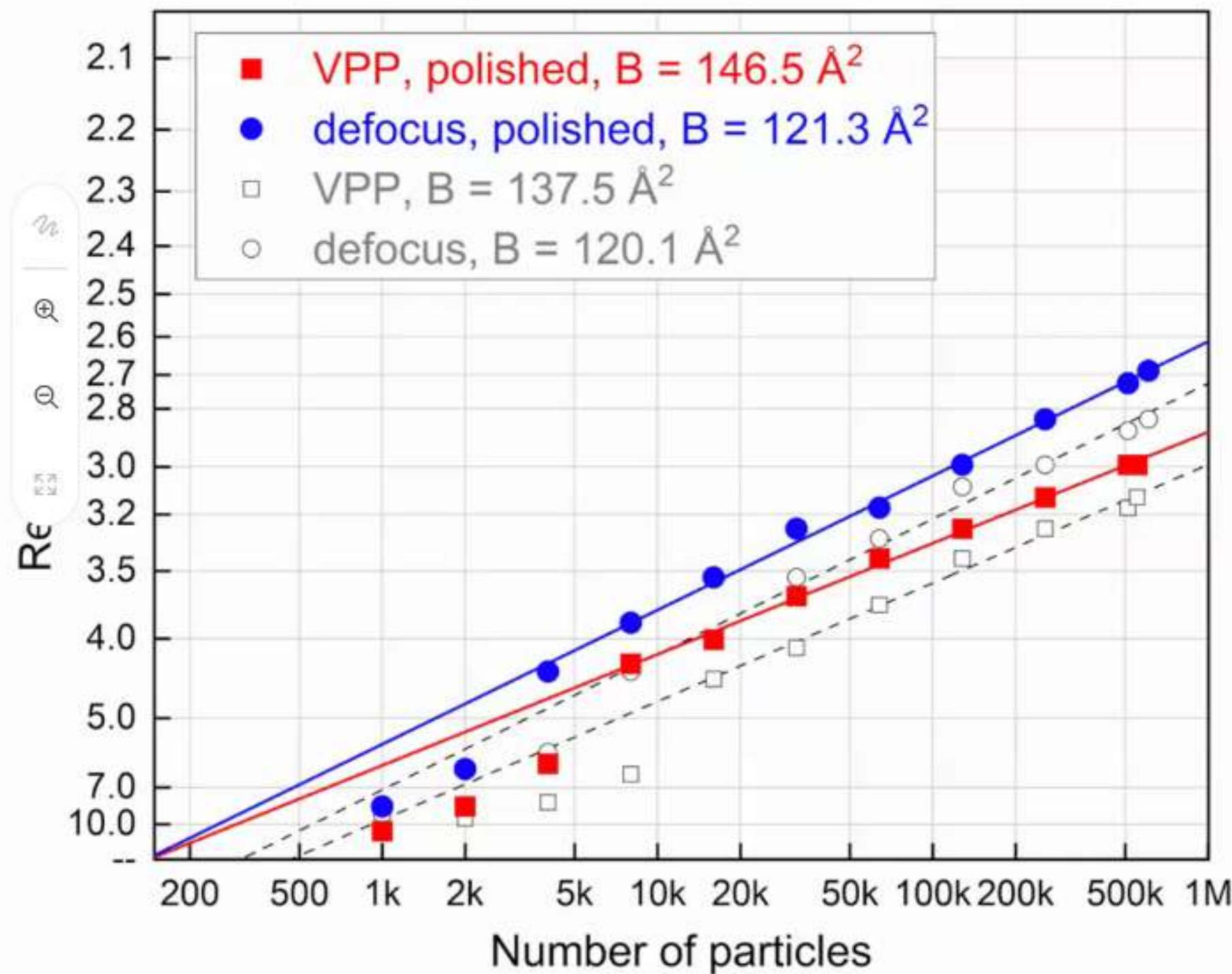
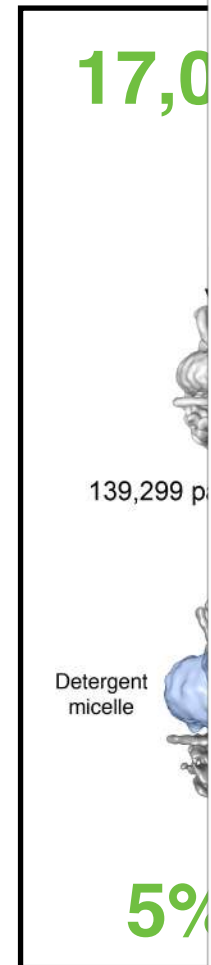
Monash Institute of Pharmaceutical Sciences, Monash University



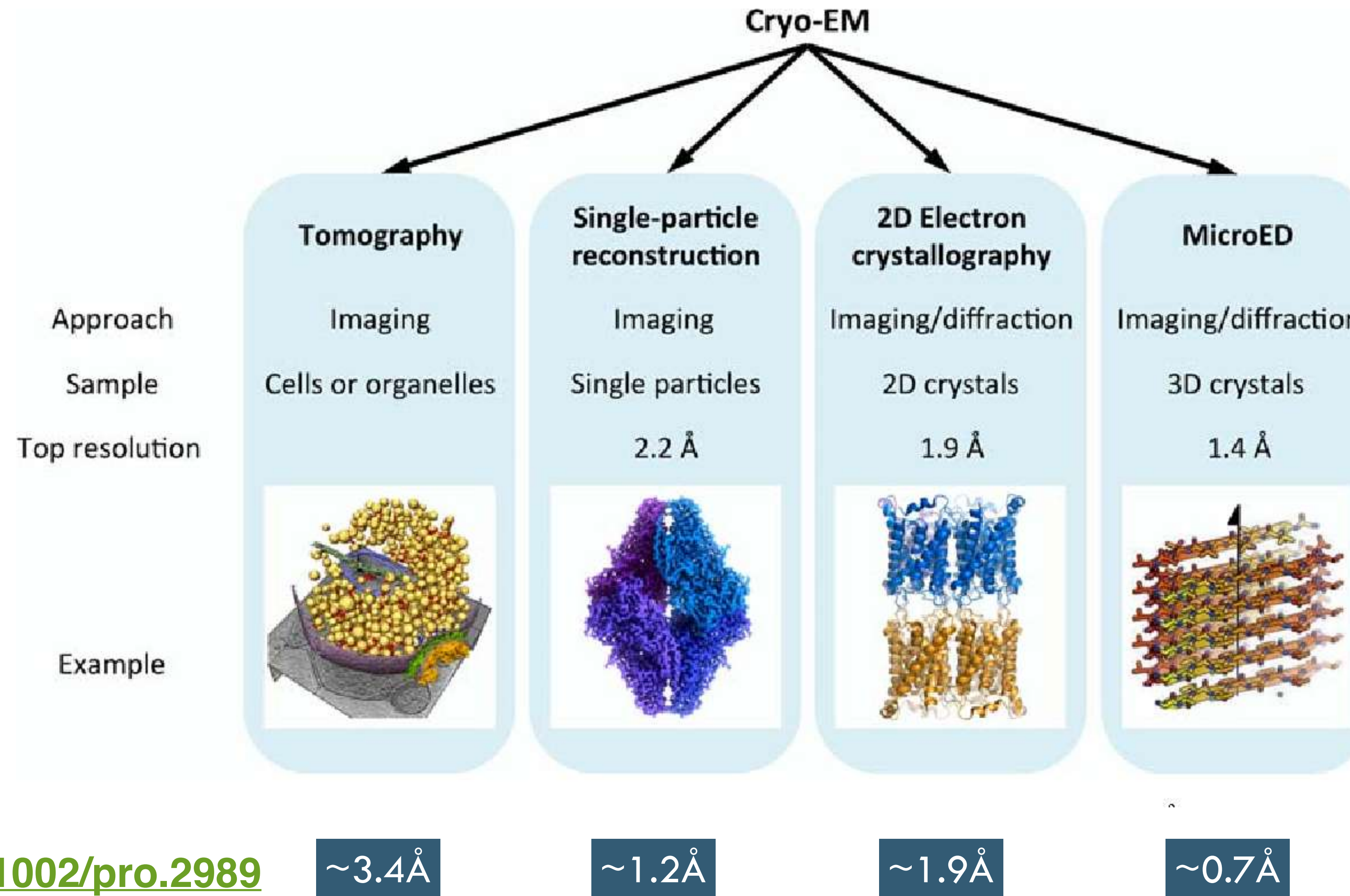
Radostin Danev, PhD

Graduate School of Medicine, The University of Tokyo

Software



Cryoem modalities and tools



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

~3.4Å

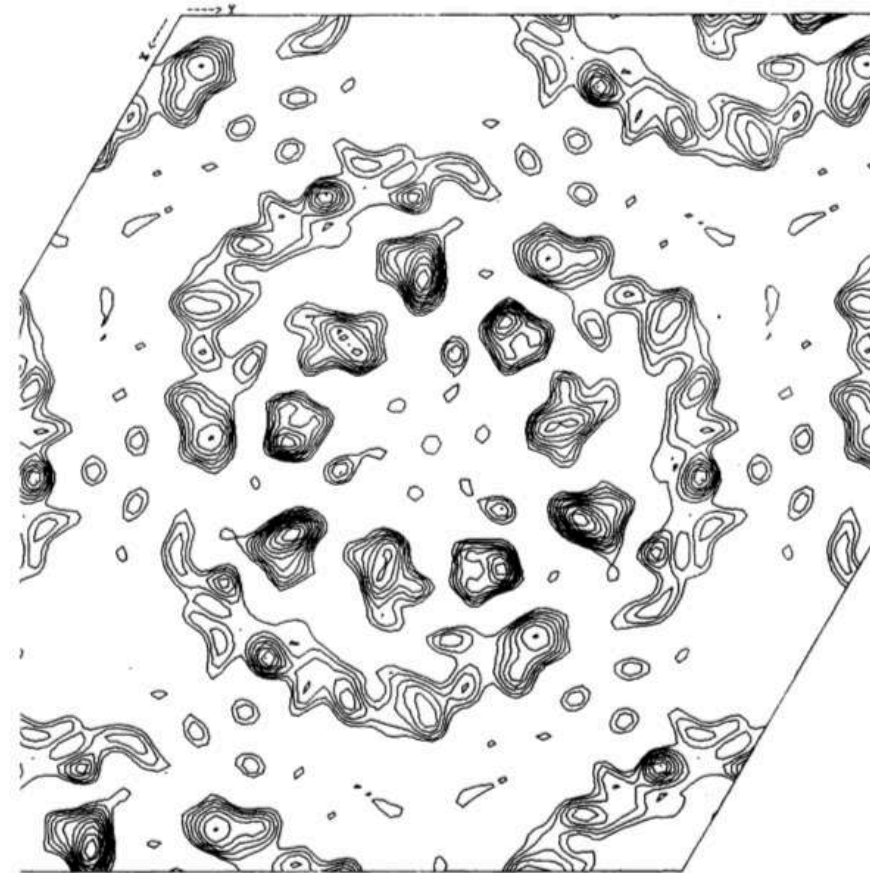
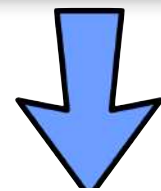
~1.2Å

~1.9Å

~0.7Å

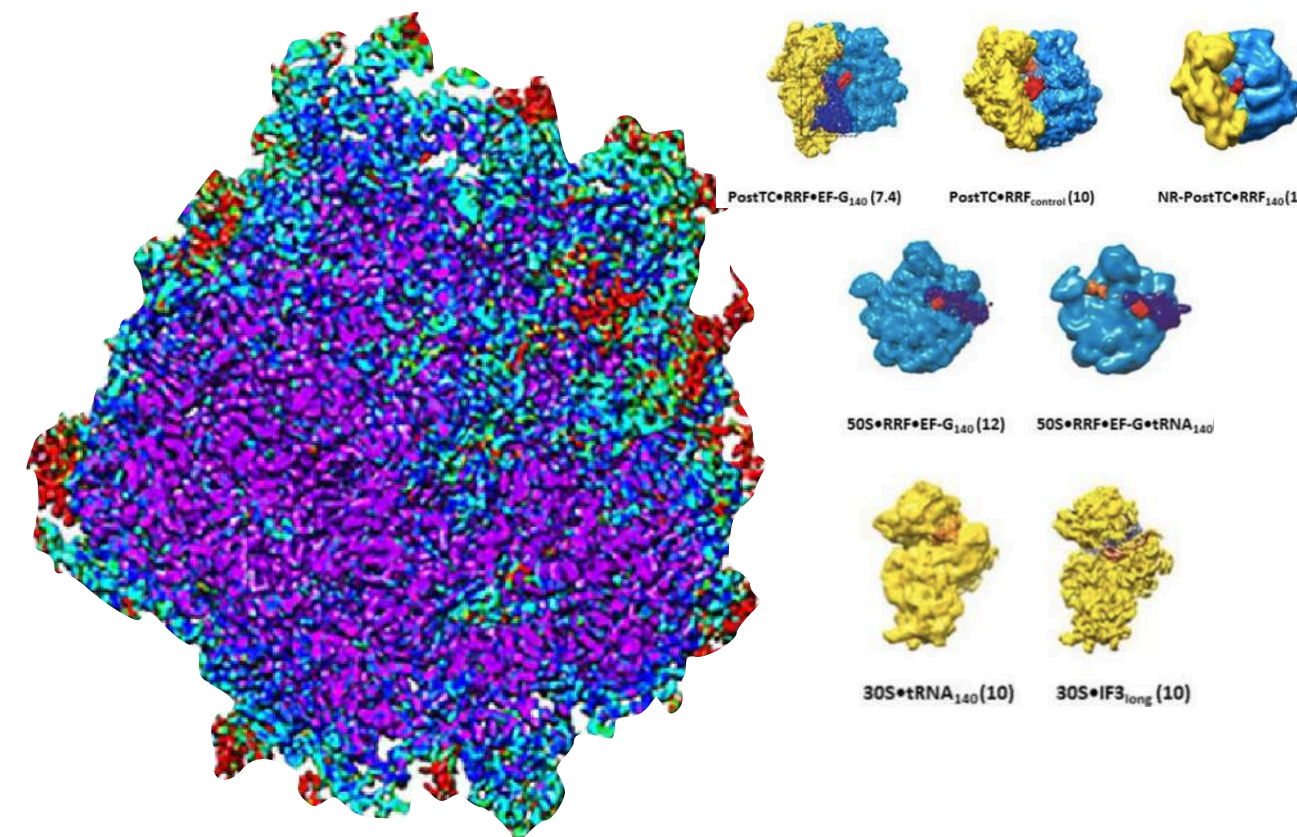
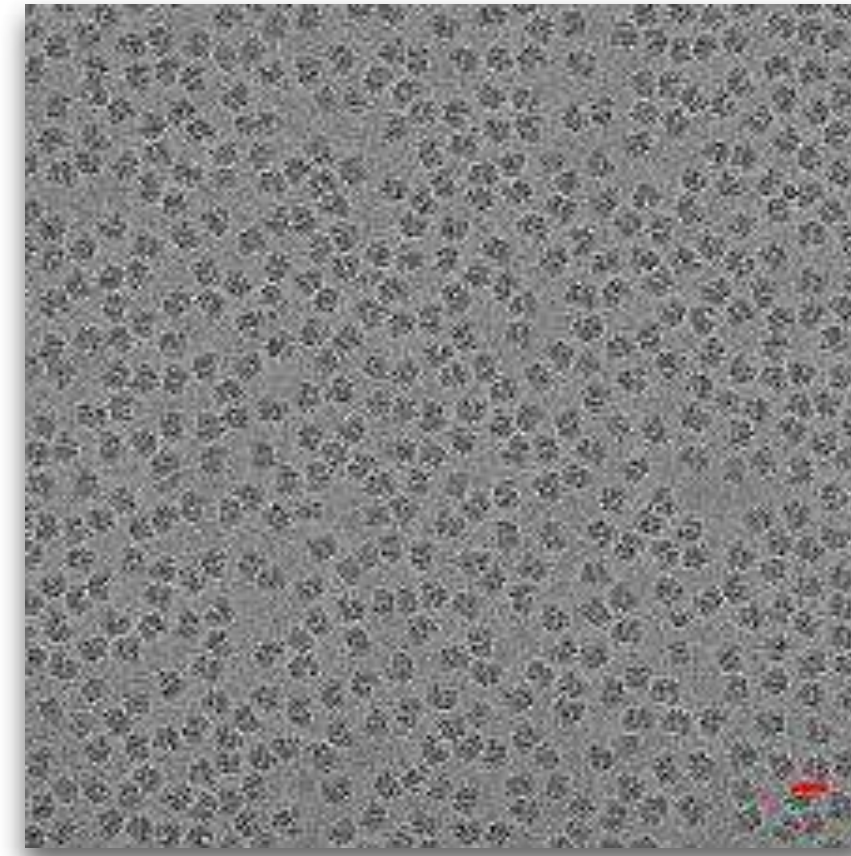
cryoEM: technology on the rise

1986



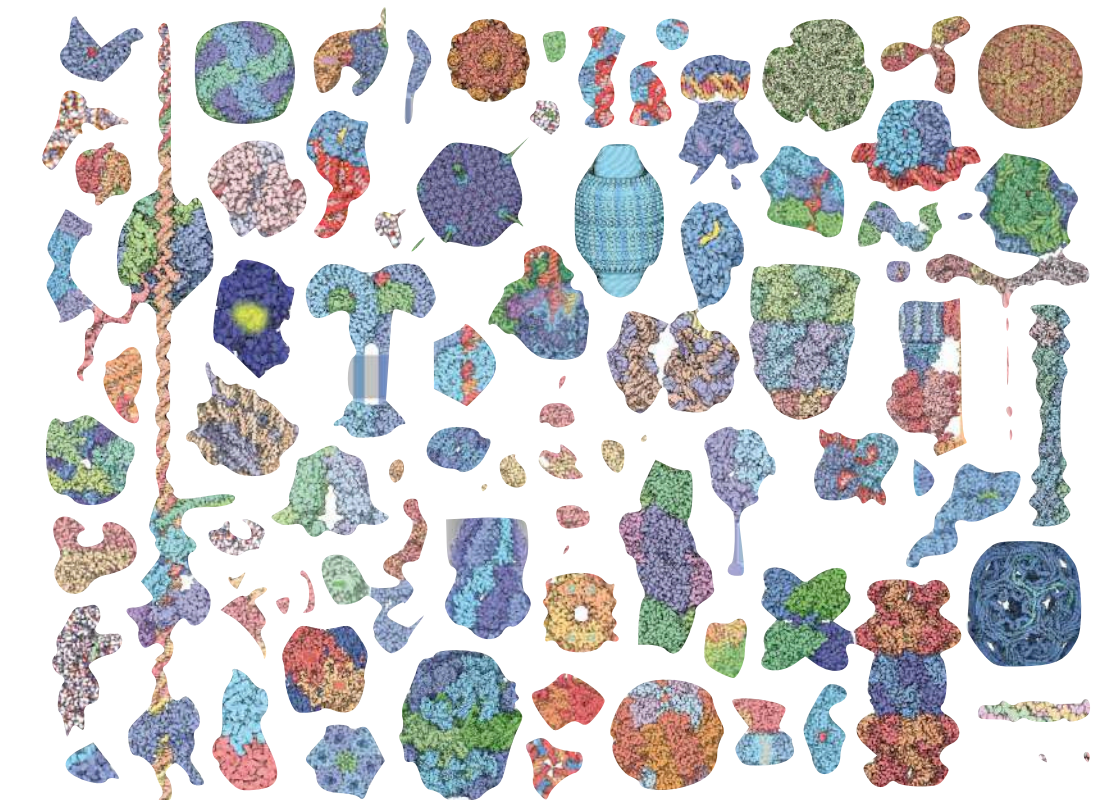
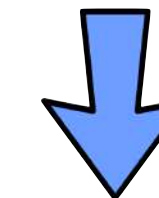
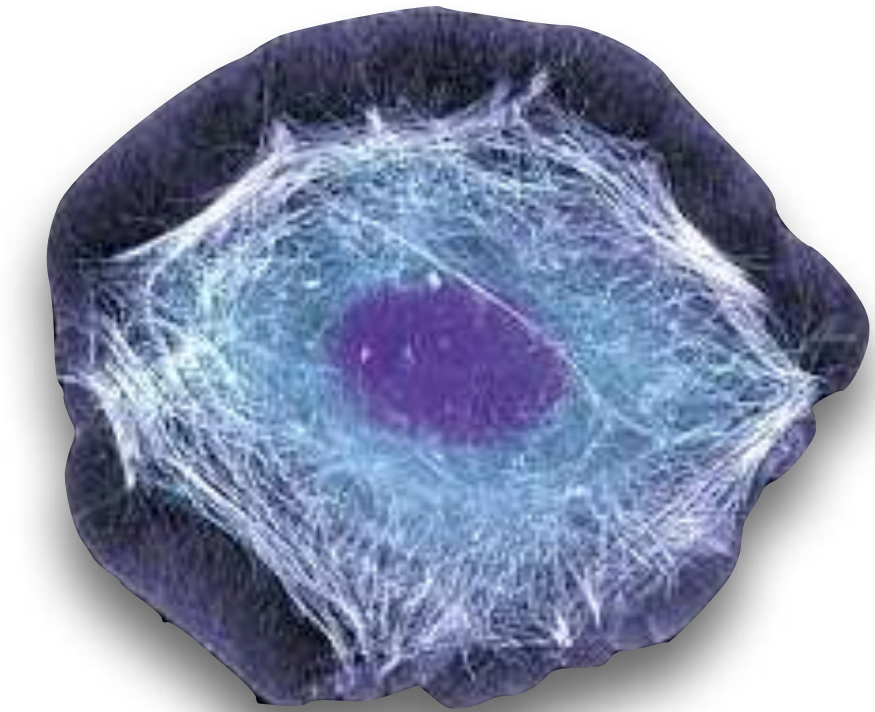
Henderson et al. (1986)

2017



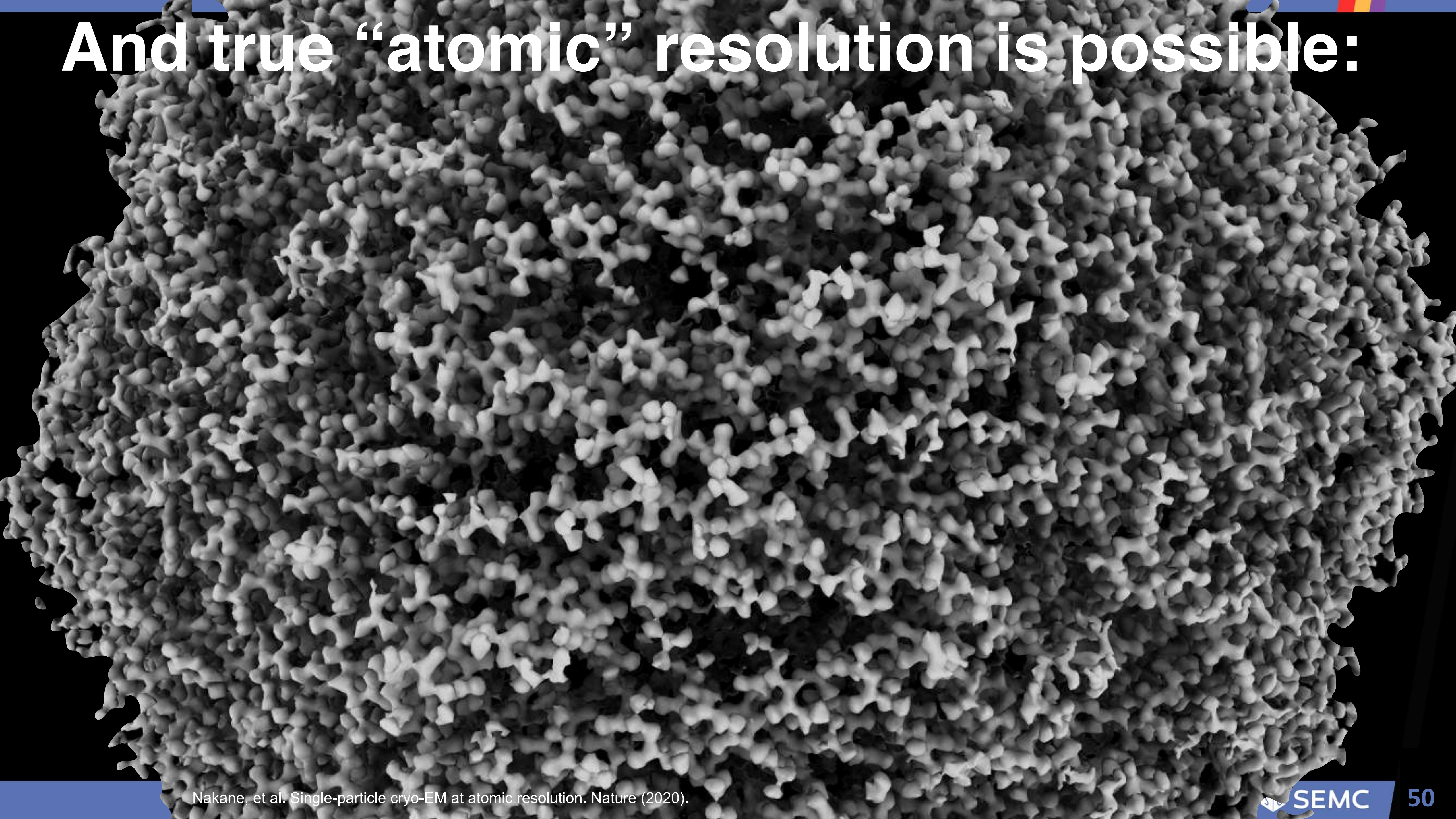
Frank et al. (2017)

in progress

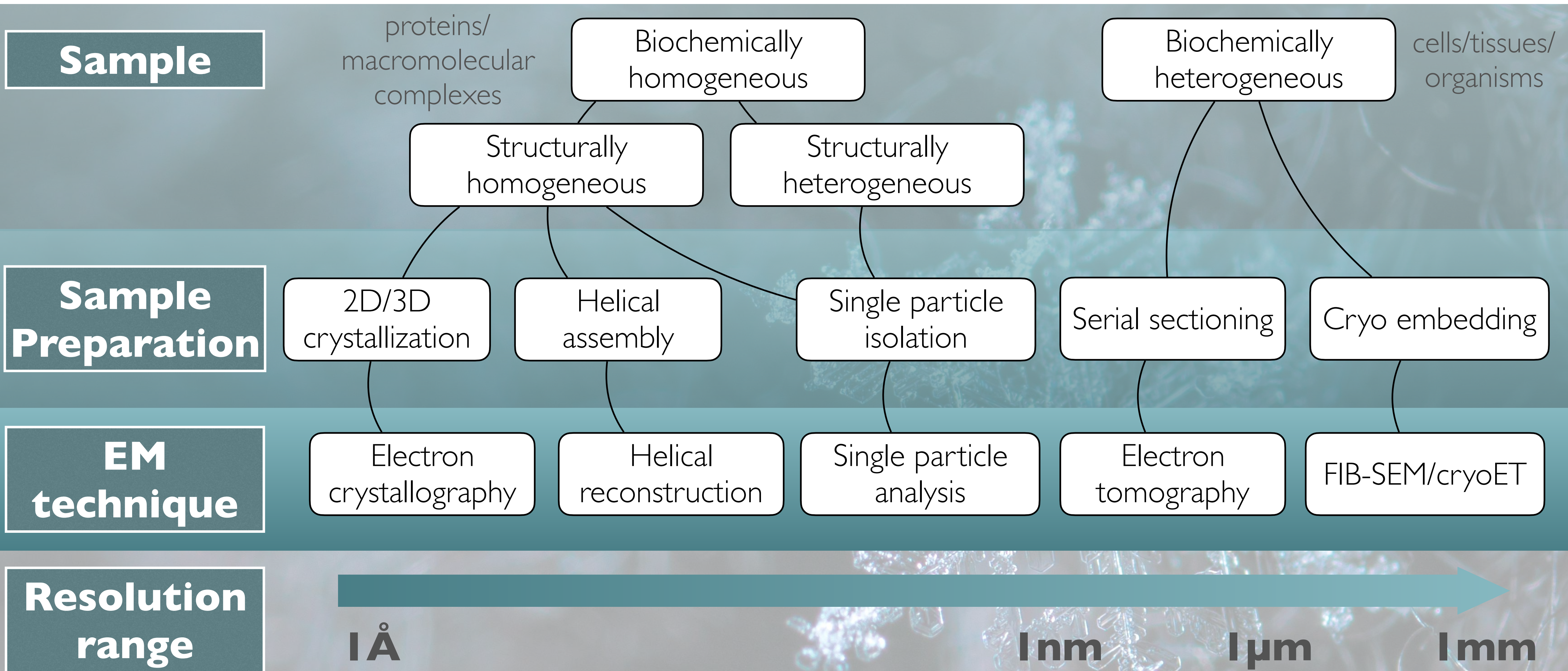


the next chapter

And true “atomic” resolution is possible:



How are samples prepared for cryoEM?



The start

Questions?