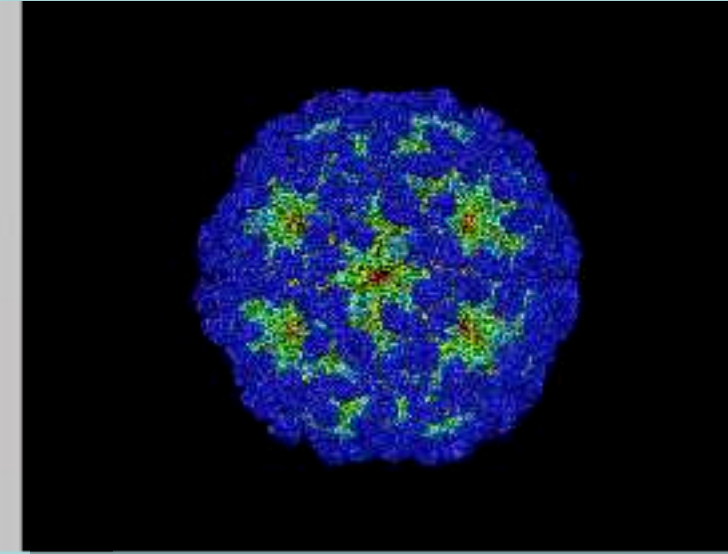
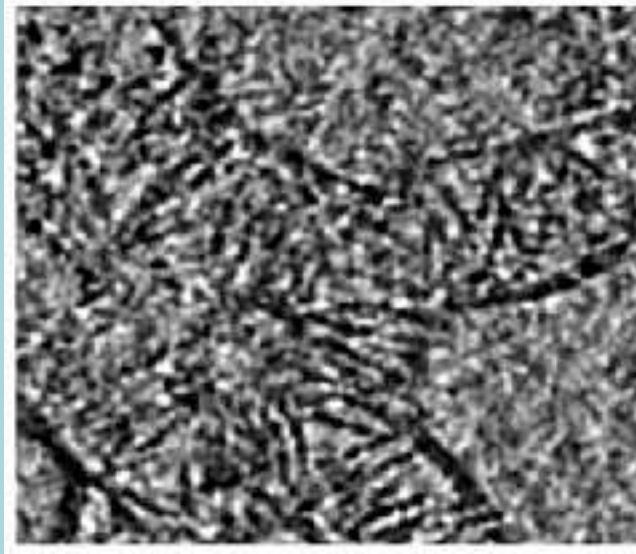


# Microscopes and tools of the trade



NCCAT SPA  
Short-  
course

Edward T Eng  
March 3, 2020



Simons Electron Microscopy Center

NEW YORK STRUCTURAL BIOLOGY CENTER



# Course logistics

## DAY 2: TUESDAY, MARCH 3, 2020

09:00 – 09:30	<b>Registration/Information desk</b> – SEMC conference room
09:45 – 10:45	<b>Lecture 3</b> – NYSBC conference room <i>Microscopes and tools of the trade</i> SEMC Staff
10:45 – 11:00	<b>Break</b>

## DAY 3: WEDNESDAY, MARCH 4, 2020

09:00 – 09:30	<b>Registration/Information desk</b> – SEMC conference room
09:45 – 10:35	<b>Lecture 4</b> – NYSBC conference room <i>Algorithms and foundational math PartI: derivation/explanation of the CTF, FTs.</i>
10:35 – 11:30	<i>Algorithms and foundational math PartII: reconstruction, classification, maximum likelihood.</i> Fred Sigworth (Yale University)
11:30 – 11:45	<b>Break</b>
11:45 – 12:30	<b>Lecture 5</b> – NYSBC conference room <i>Data Analysis and reconstruction workflow</i> Amedee des Georges (ASRC/CUNY)



# Course logistics

## DAY 4: THURSDAY, MARCH 5, 2020

09:00 – 09:30

**Registration/Information desk** – SEMC conference room

09:45 – 10:45

**Lecture 6** – NYSBC conference room  
*Interpretation and Limitations of SPA*  
Rich Hite (Memorial Sloan Kettering Cancer Center)



10:45 – 11:45

**Lecture 7** – NYSBC conference room  
*Validation Methods*  
Tom Walz (Rockefeller University)



11:45 – 12:15

**Roundtable 4** – NYSBC conference room  
*EM challenges and new frontiers*  
Rich Hite, Tom Walz & others

## DAY 5: FRIDAY, MARCH 6, 2020

09:00 – 17:00

**Registration/Information desk** – SEMC conference room  
*room available all day except for practicals*

09:45 – 10:45

**Lecture 9** – NYSBC conference room  
*Fitting Atomic Models*  
Oli Clarke (Columbia University)



10:45 – 11:45

**Lecture 10** – NYSBC conference room  
*Coordinate Refinement and Validation*  
Gira Bhabha & Damian Ekiert (New York University)



11:45 – 12:15

**Roundtable 4** – NYSBC conference room  
*Making biological conclusions from cryoEM reconstructions.*  
Gira Bhabha, Damian Ekiert, Oli Clarke & others



# Course logistics



The screenshot shows the NCCAT website homepage. At the top, there are social media icons and logos for NYSBC, SEMC, and NIH CommonFund. The main header features the NCCAT logo and the text "National Center for CryoEM Access". Below this is a navigation menu with "HOME", "ABOUT", "NEWS", "ACCESS", and "TRAINING". The main content area has a large text block describing the mission: "The mission of NCCAT is twofold: to provide nationwide access to advanced cryoEM technical capabilities, and to assist users in the development of cryoEM skills needed for independent research. NCCAT provides access to state-of-the-art equipment required to solve structures to the highest possible resolution using cryoEM methods. Our staff provides direct support, guidance and assistance. A cross-training program provides training across a wide variety of skill levels and career goals. We are committed to an open and transparent application process for nationwide access. [Learn more](#)". A green button labeled "ACCESS NCCAT" is positioned below the text. To the right, there is a partial view of a brick wall with a sign that says "NEW YORK STRU".



The screenshot shows a Twitter post from the account "NCCAT Info" (@NCCATInfo). The profile picture is the NCCAT logo. The bio states: "Our mission is to provide nationwide access to cryoEM instrumentation and training needed for independent EM research. Contact us at [nccatinfo@nysbc.org](mailto:nccatinfo@nysbc.org)". The location is "Manhattan, NY" and the website is "[nccat.nysbc.org](http://nccat.nysbc.org)". The account was joined in October 2018, has 131 following, and 355 followers. The tweet, dated Feb 16, says: "At #BPS20? Come by the exhibit hall to learn about the national #cryoEM service centers and the Transformative High Resolution Cryo-Electron Microscopy Program ([commonfund.nih.gov/cryoem](http://commonfund.nih.gov/cryoem)). Booth 129 - @SLAClab #S2C2 @CryoEM\_PNCC @NCCATInfo @NIH\_CommonFund". The tweet includes a photo of a booth at a conference with people and banners for "Cryo-EM" and "S'C".

## News and Events

2020 Annual Meeting - Biophysical Society

Graphene Grids Workshop

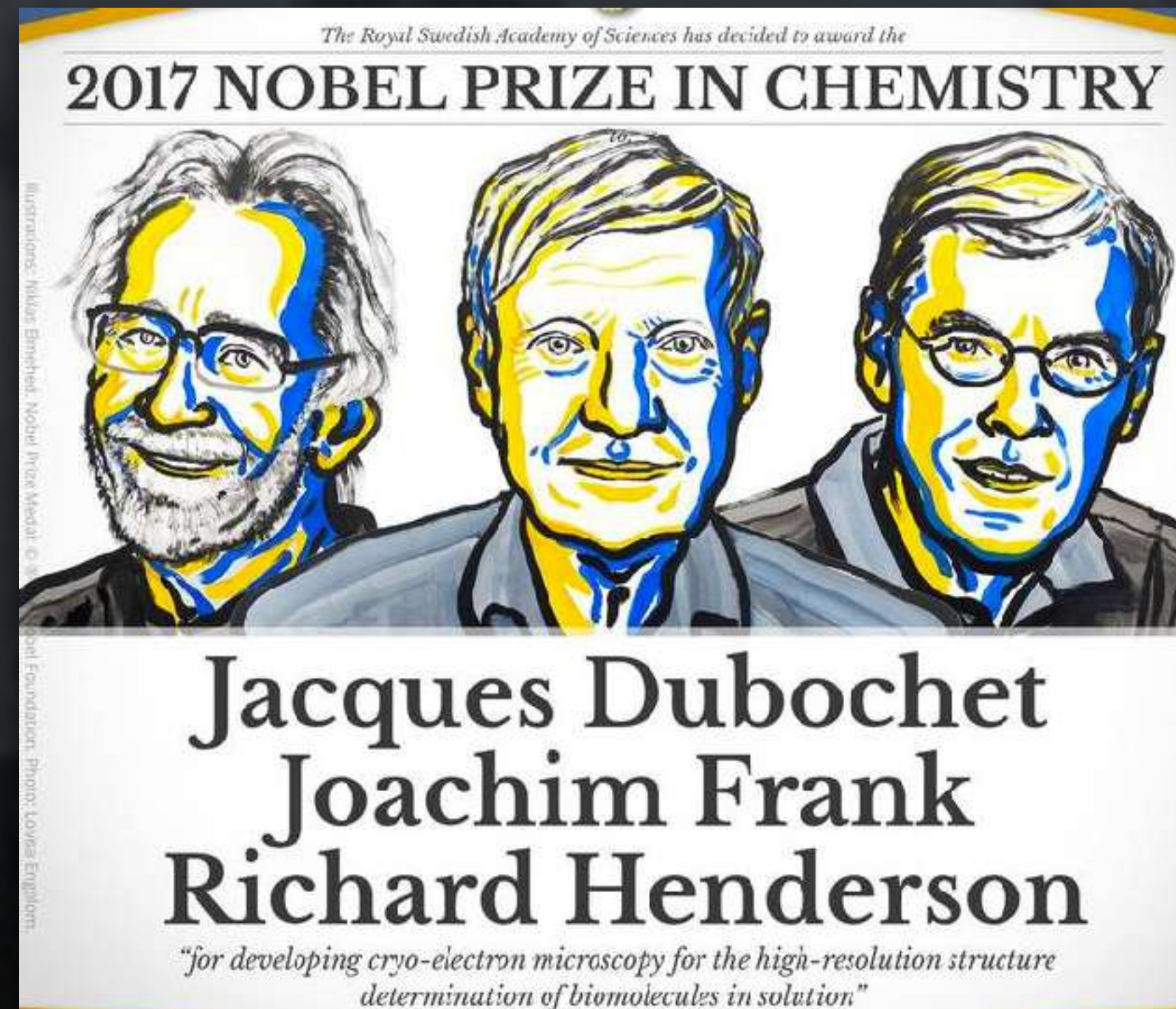
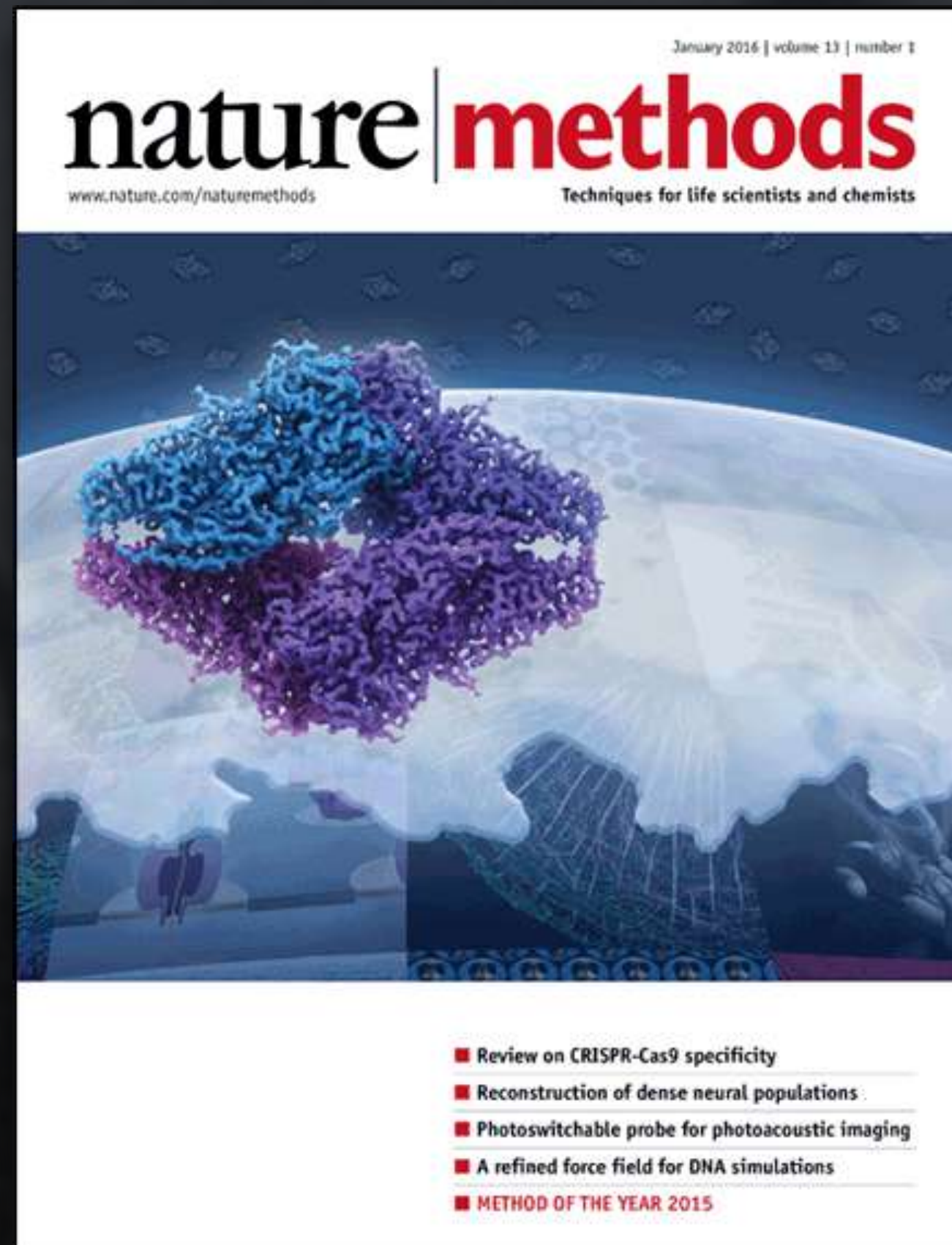


# CRYOEM: TECHNOLOGY ON THE RISE

Single-particle cryo-electron microscopy (cryoEM) is the Method of the Year 2015

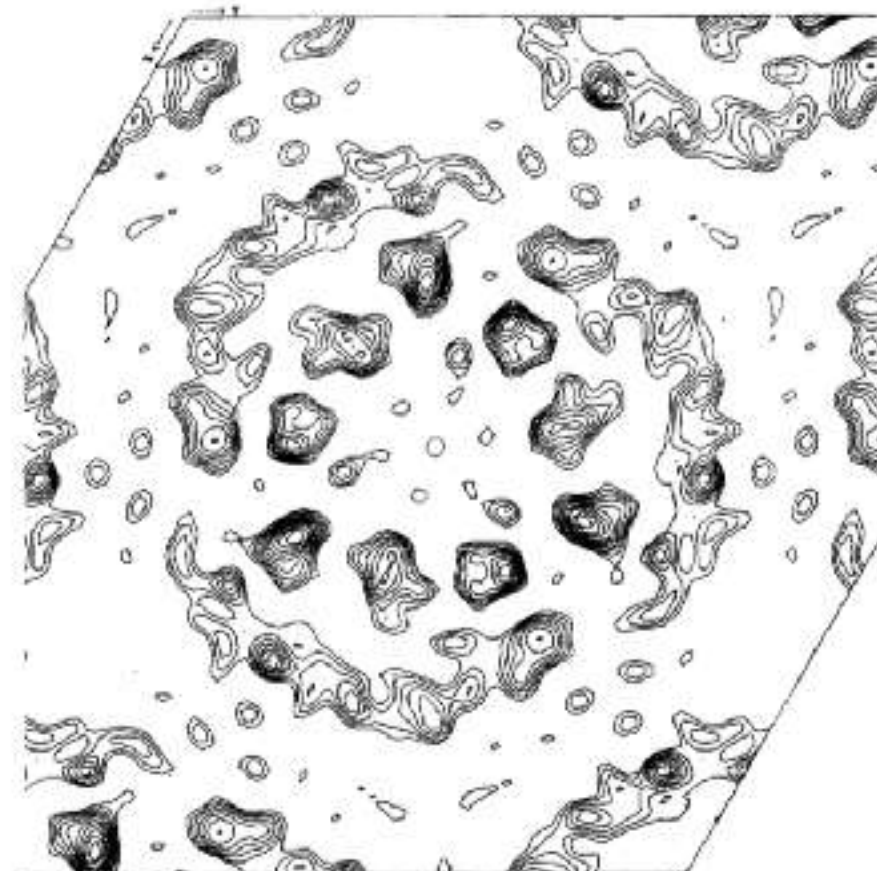
Chemistry Nobel prize 2017

microED  
Science breakthrough of the year runner-up 2018



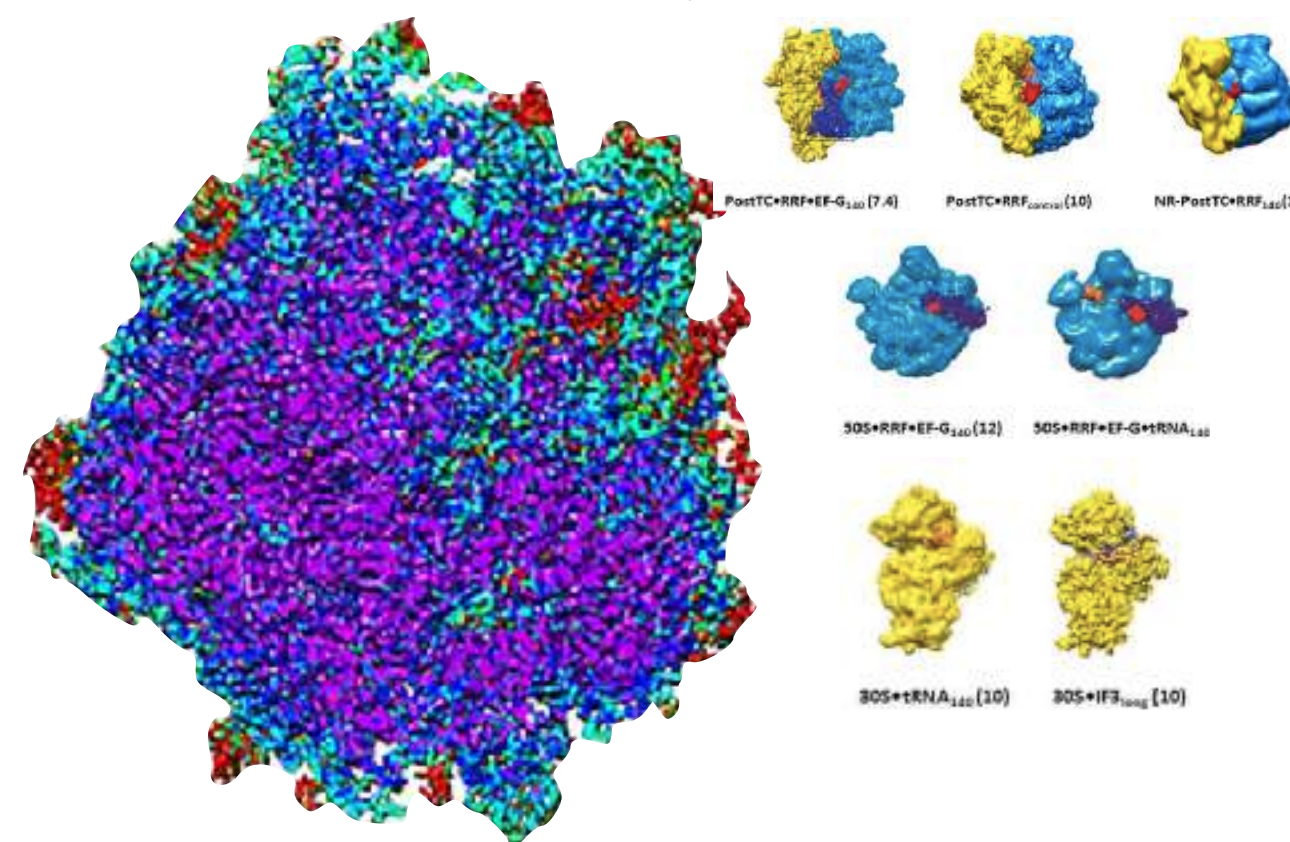
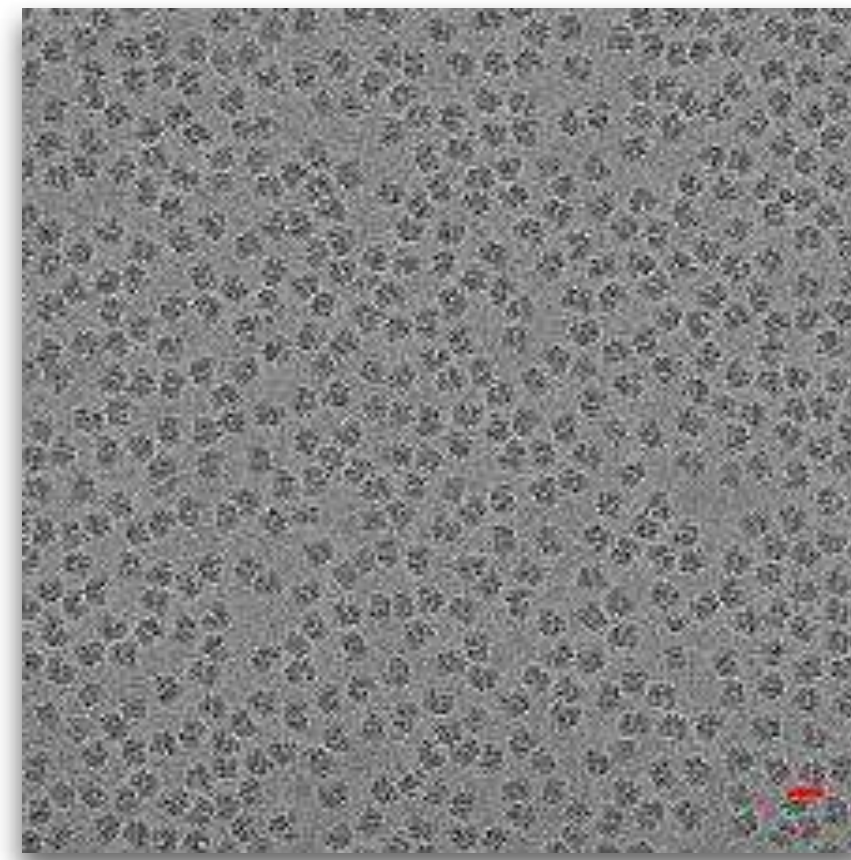
# CRYOEM: TECHNOLOGY ON THE RISE

1986



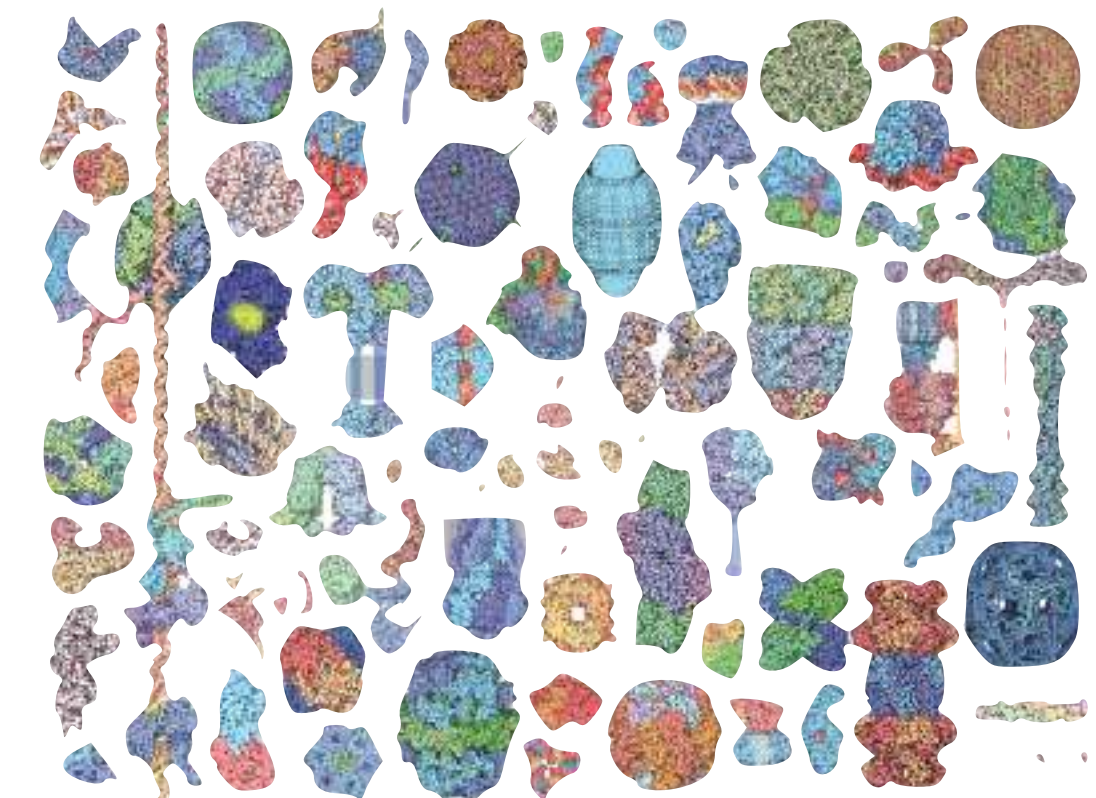
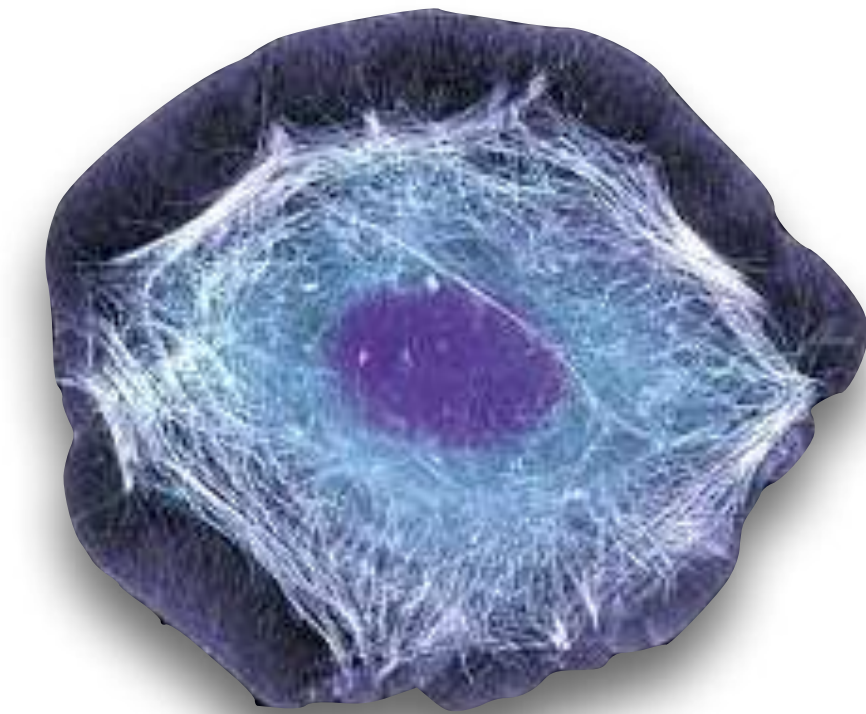
Henderson et al. (1986)

2017



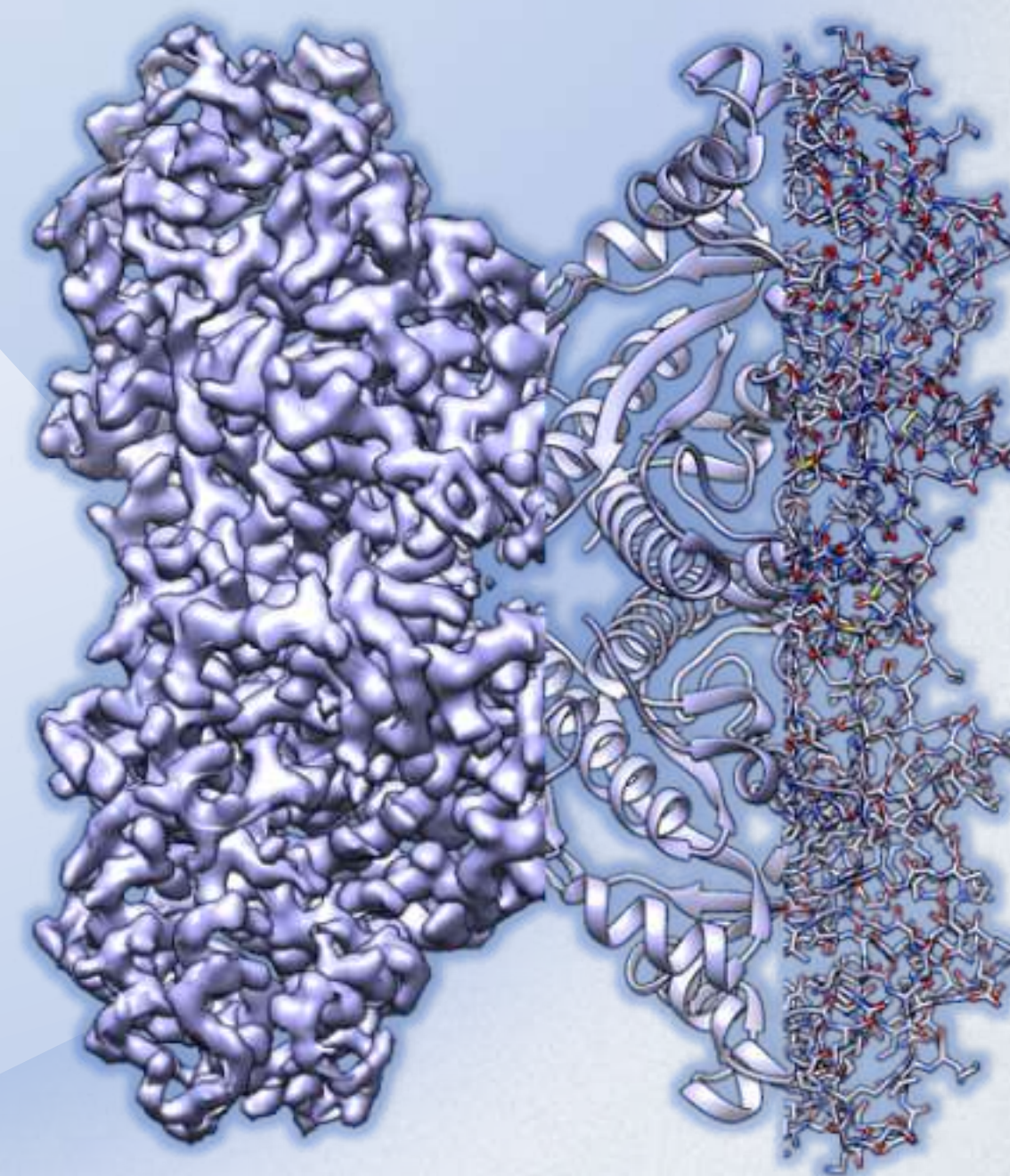
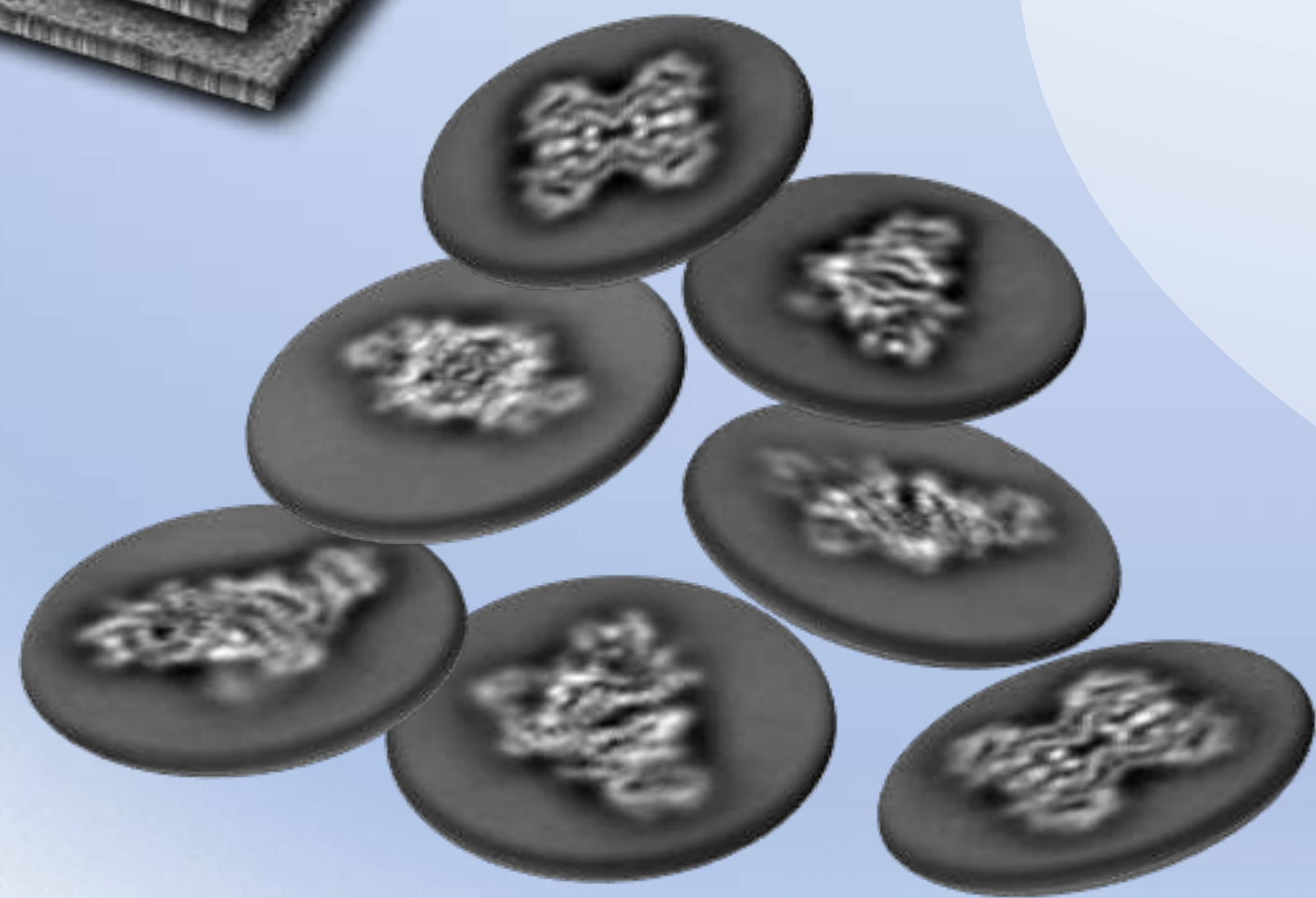
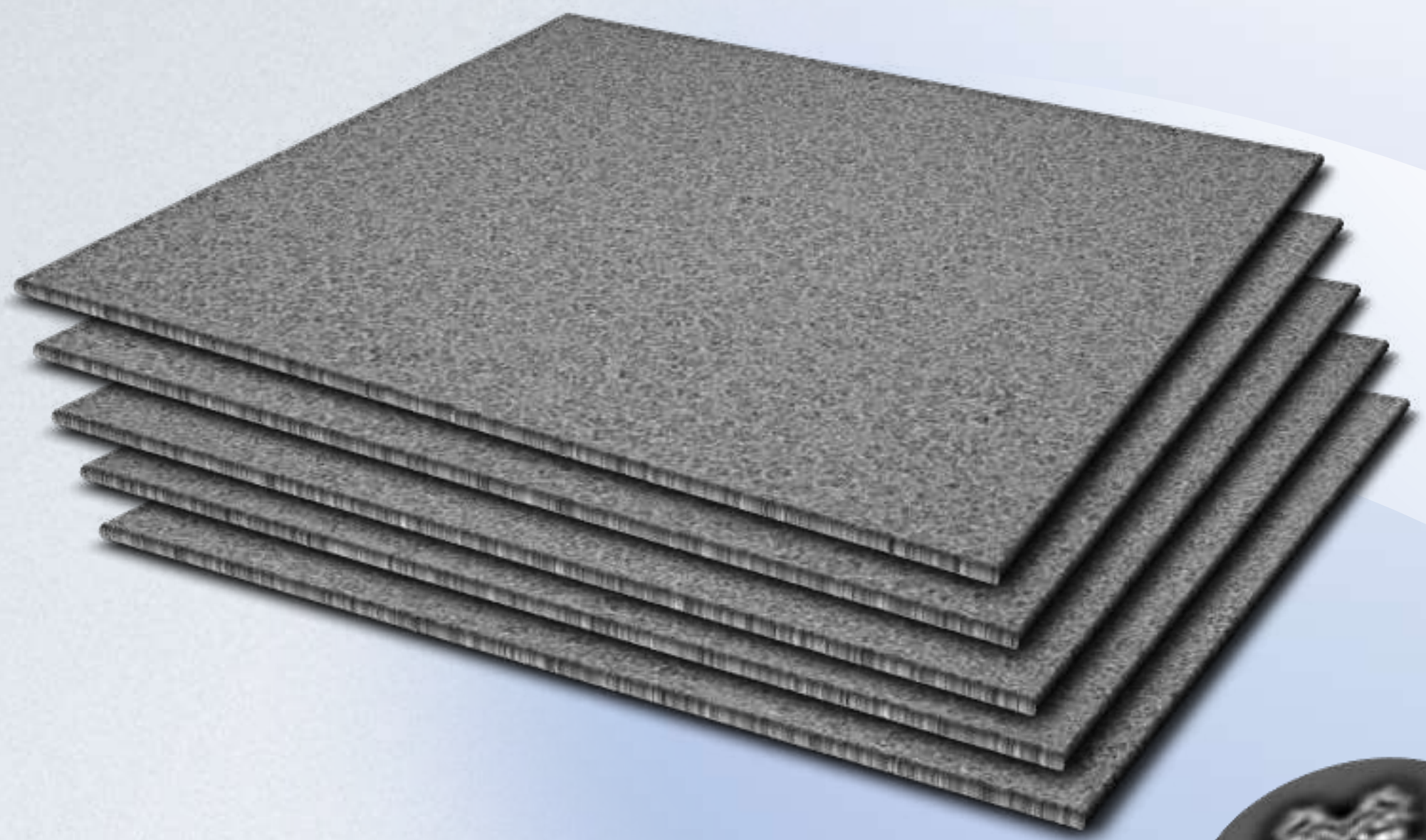
Frank et al. (2017)

????



TBD (20???)

# WHAT IS POSSIBLE TODAY?



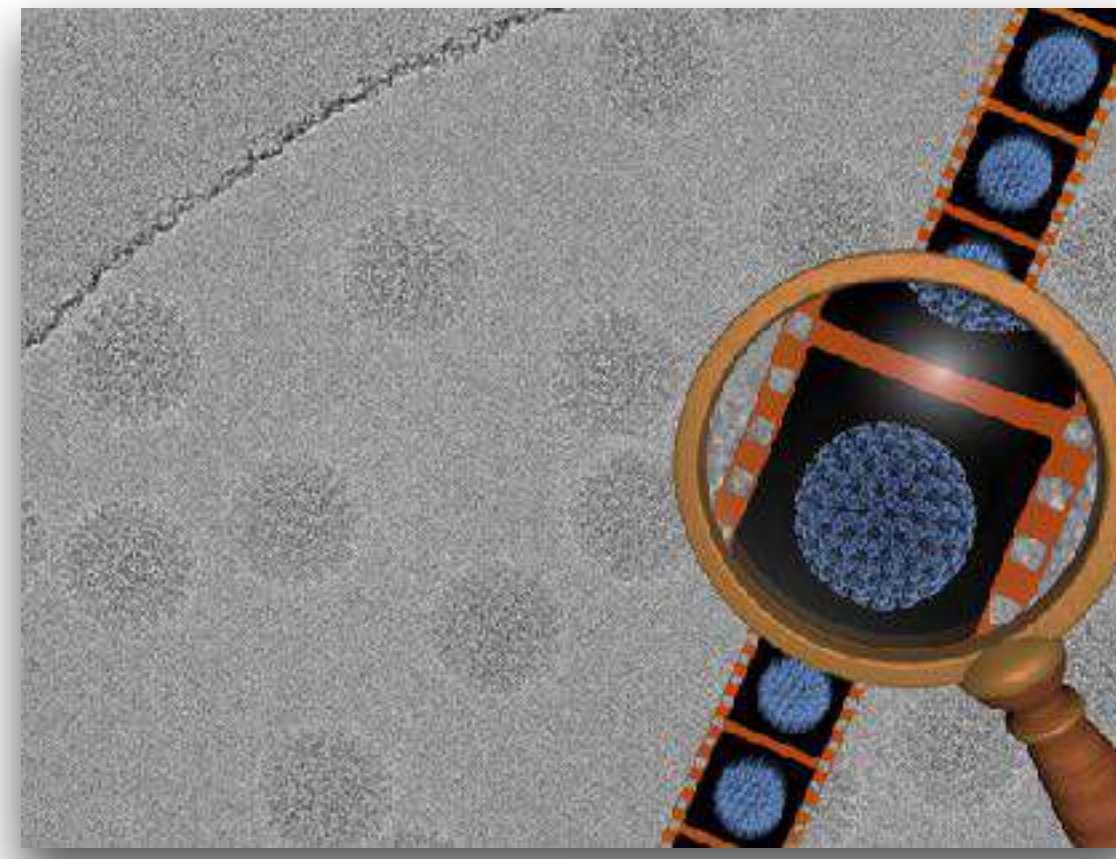
# WHAT BROUGHT ABOUT THE RESOLUTION REVOLUTION? (~2012-2014)

Hardware

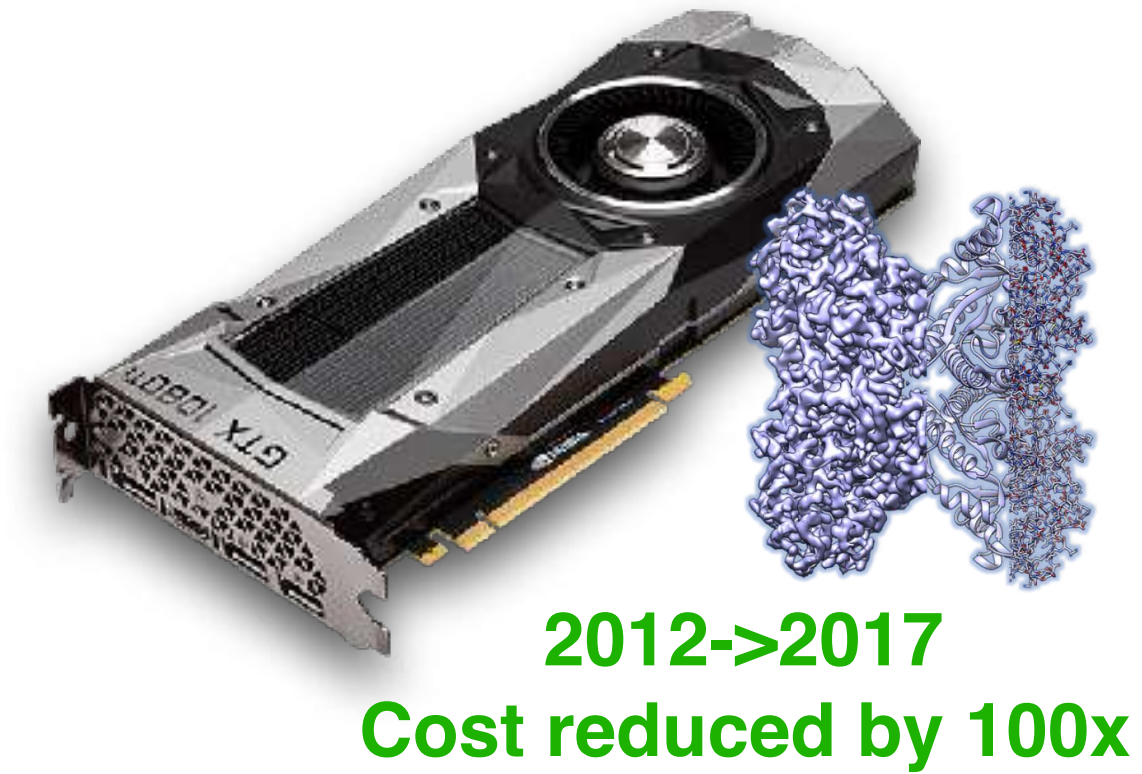
Microscopes



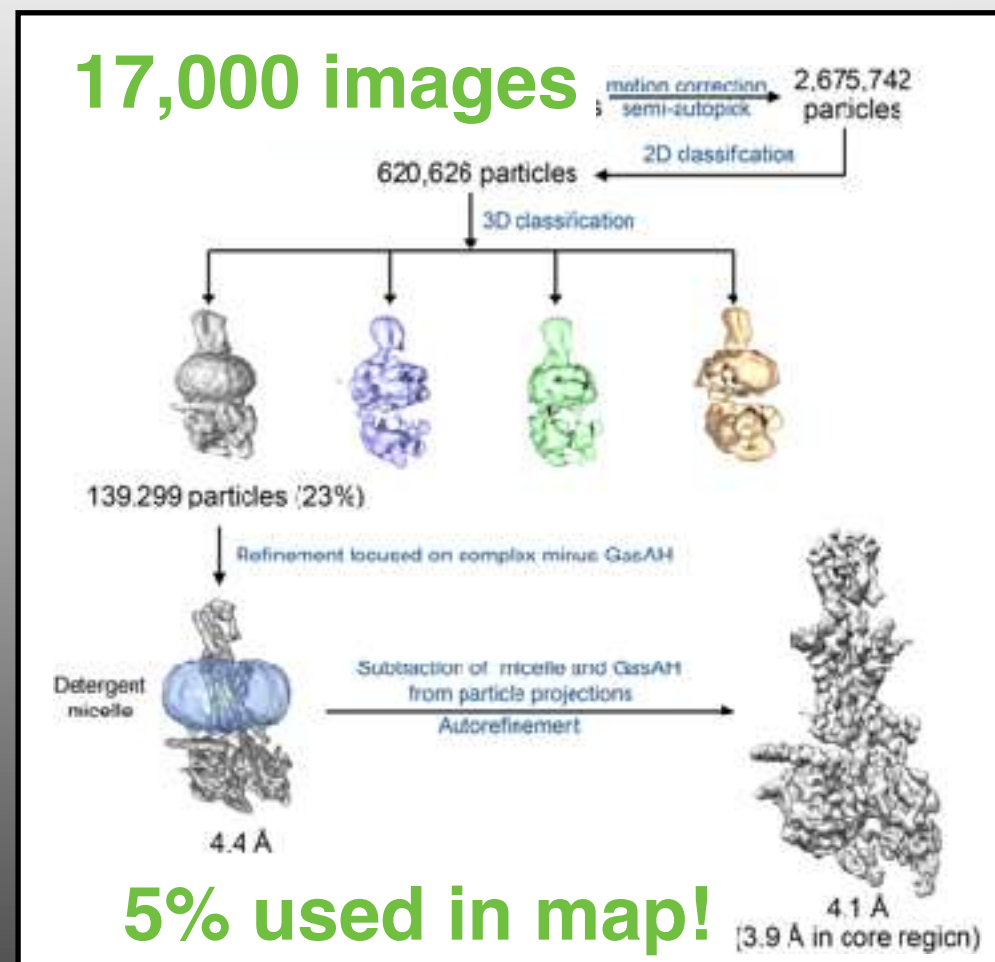
Direct Detectors



Computers



Software

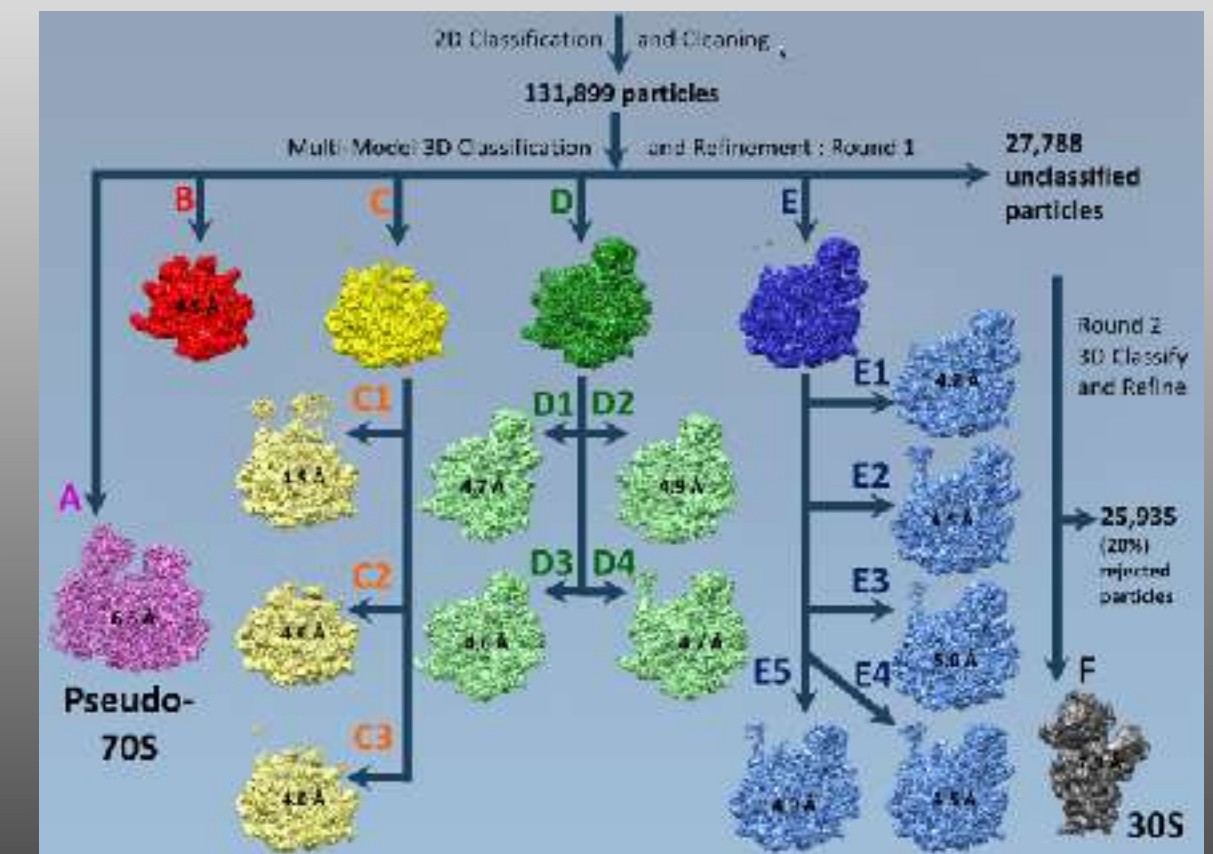


Leginon / SerialEM / EPU, ...

MotionCorr2, Unblur, ...

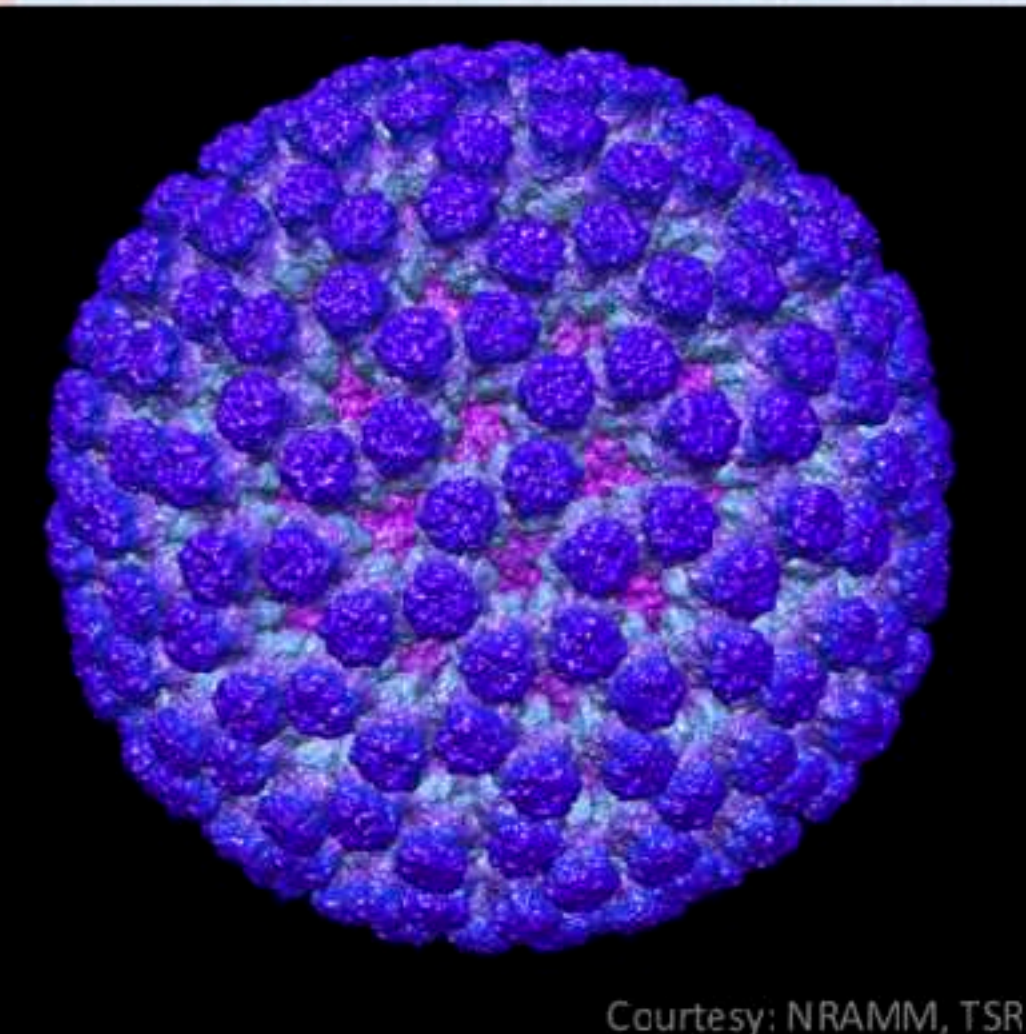
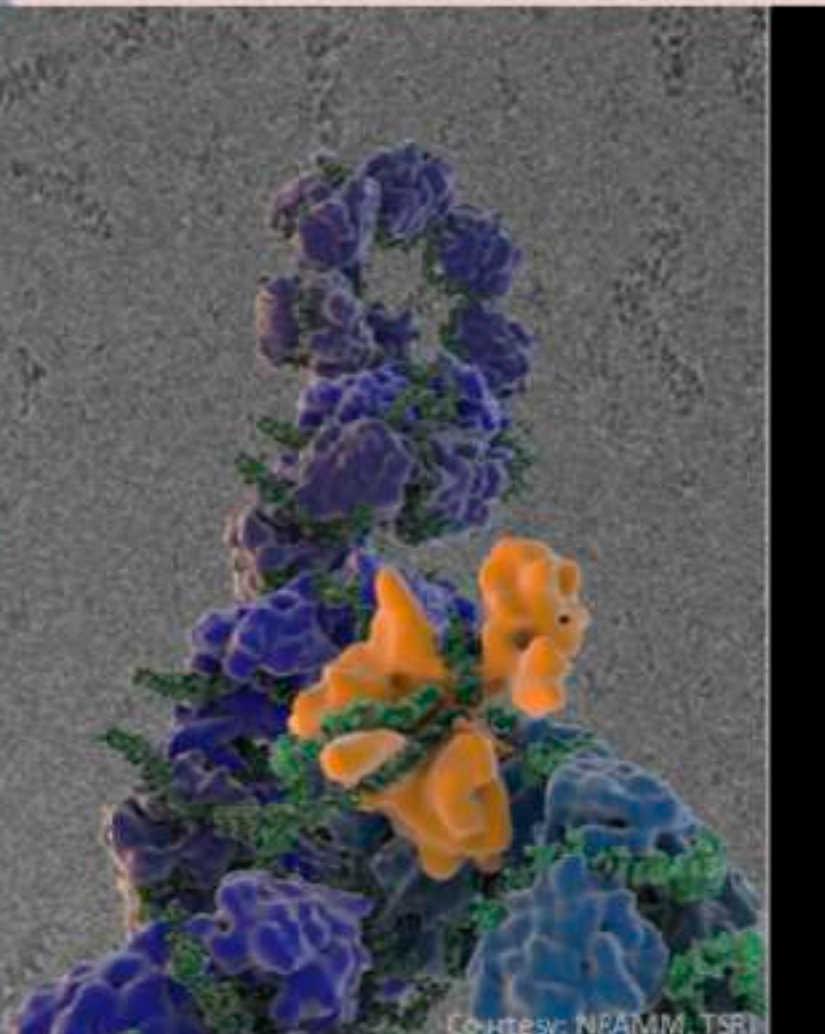
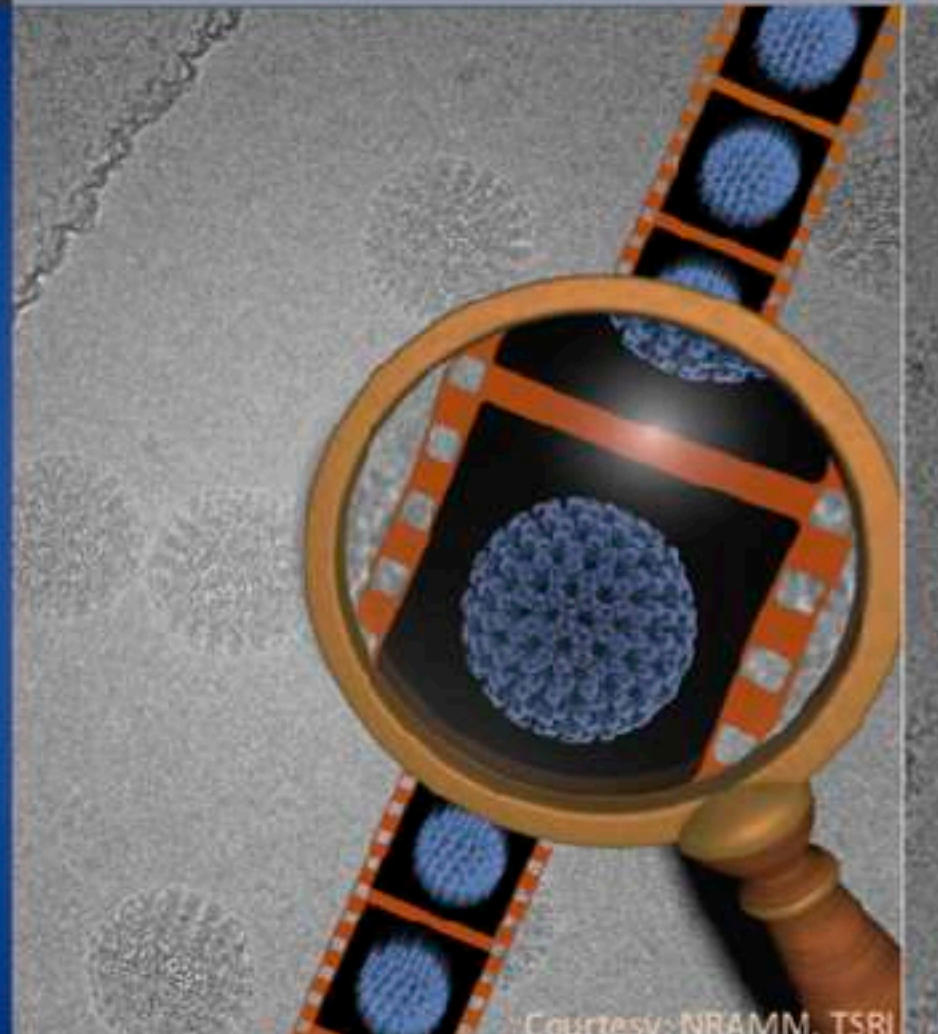
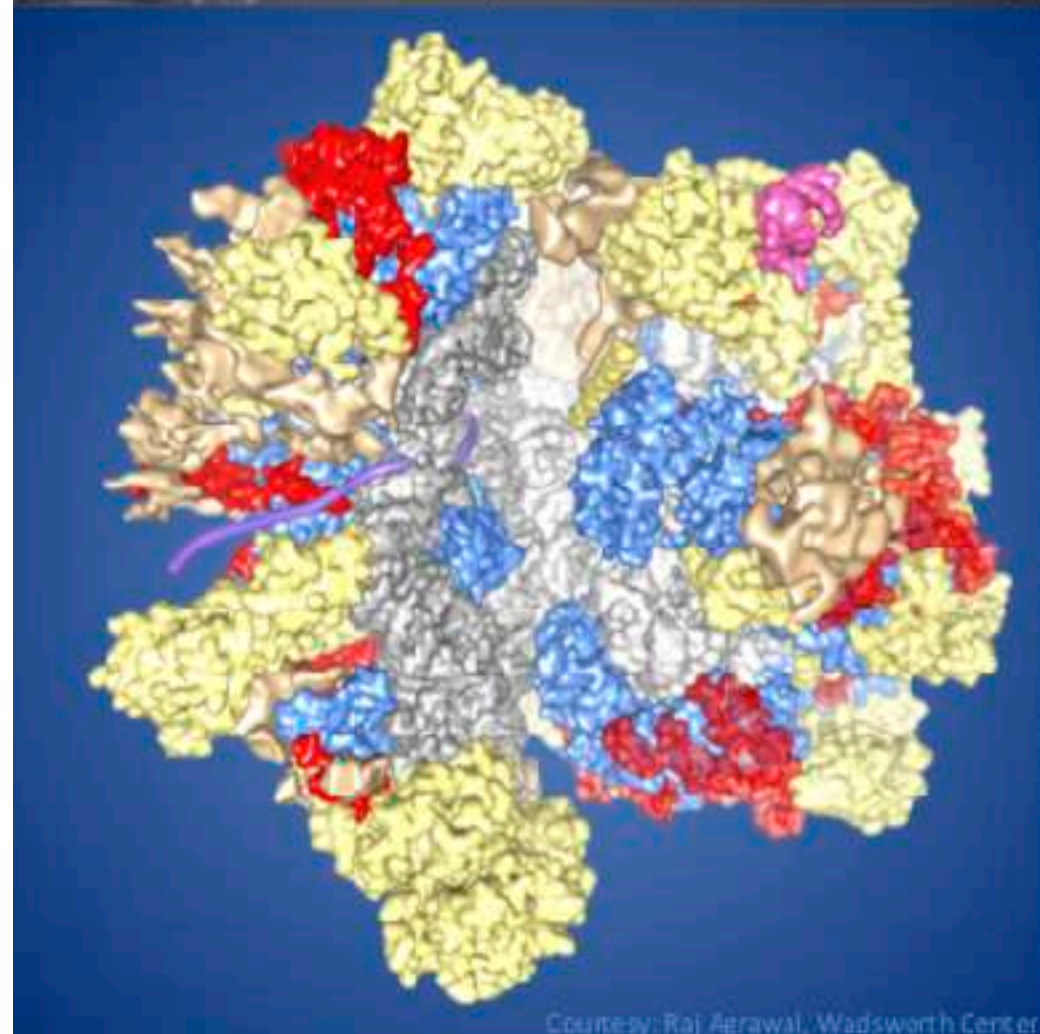
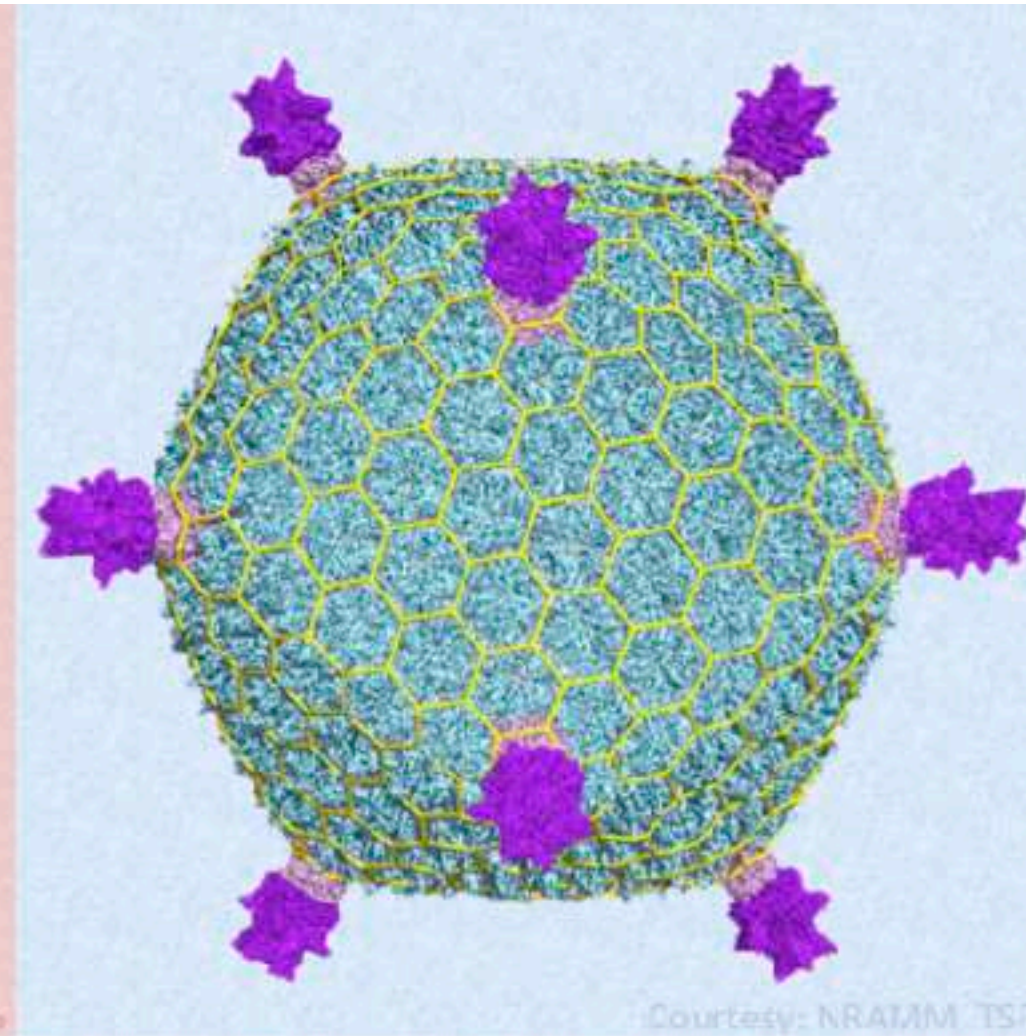
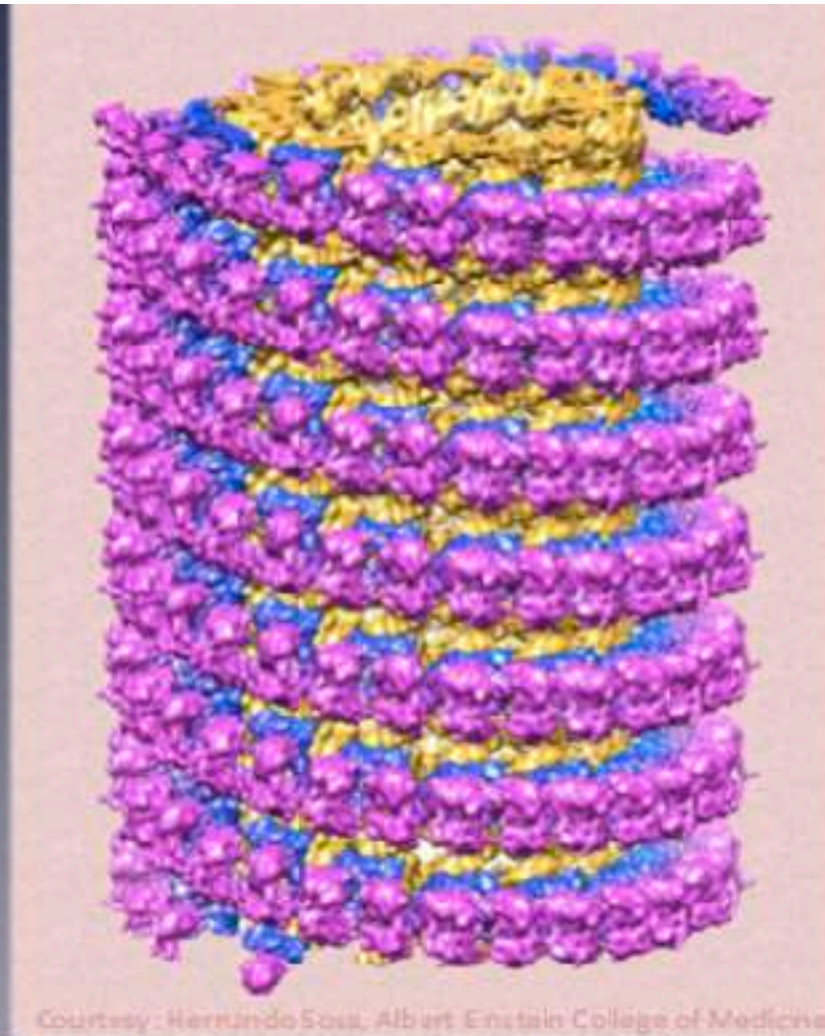
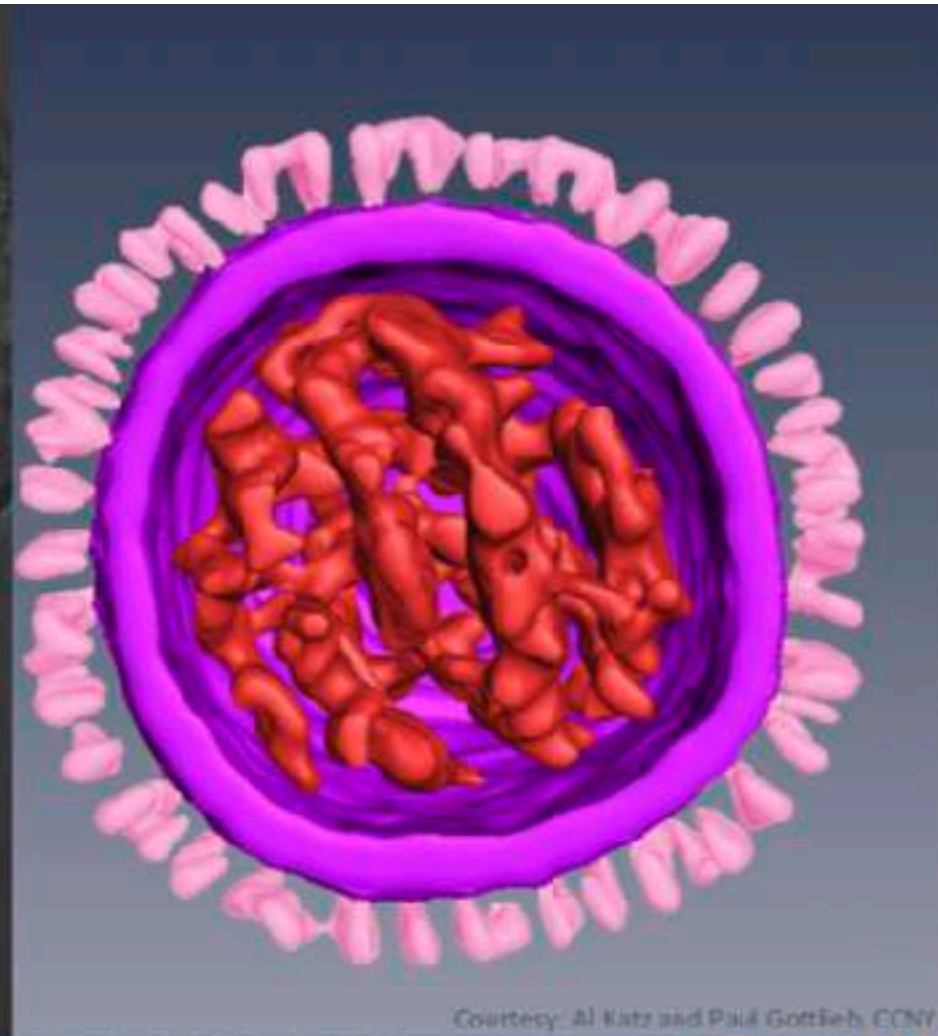
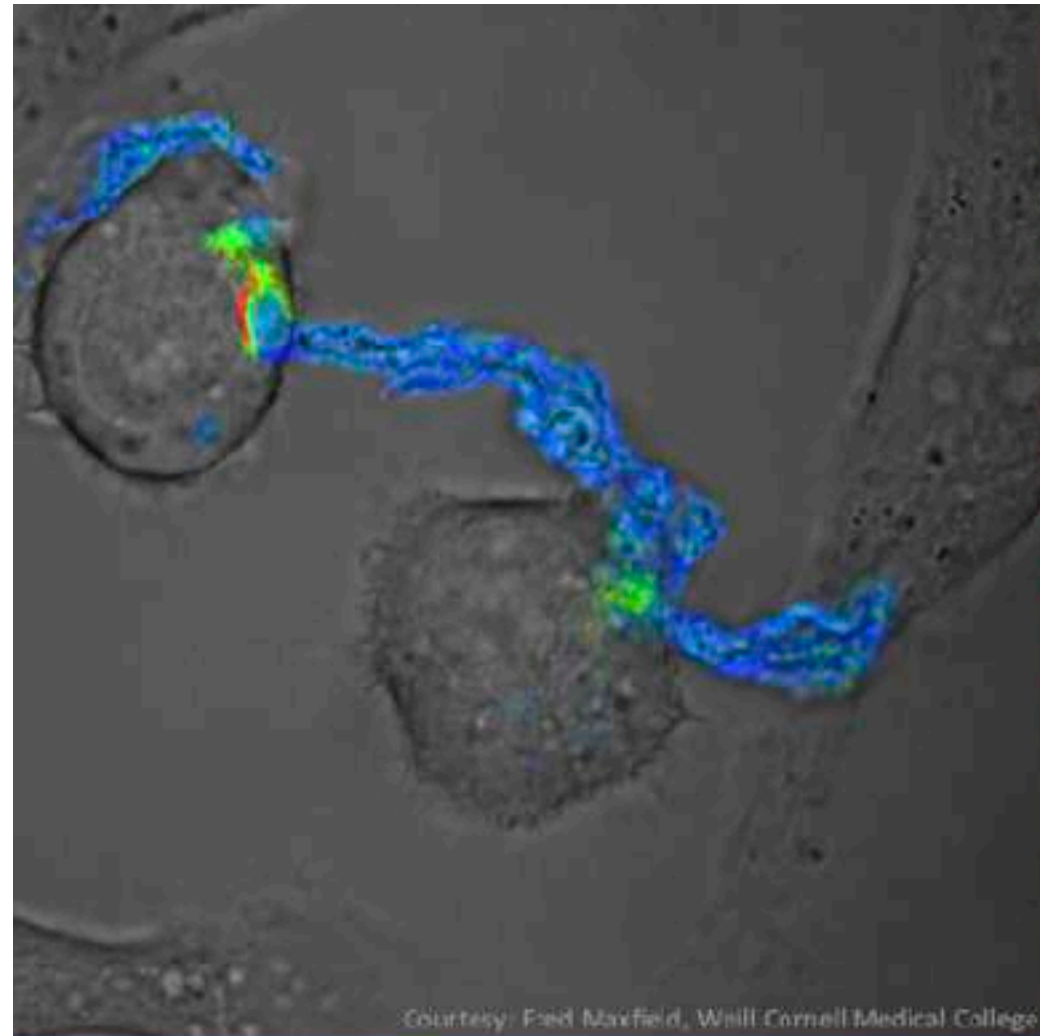
RELION, FREALIGN/cisTEM, cryoSPARC  
EMAN, Sparx, SPHIRE, XMIPP, ...

14 independent structures

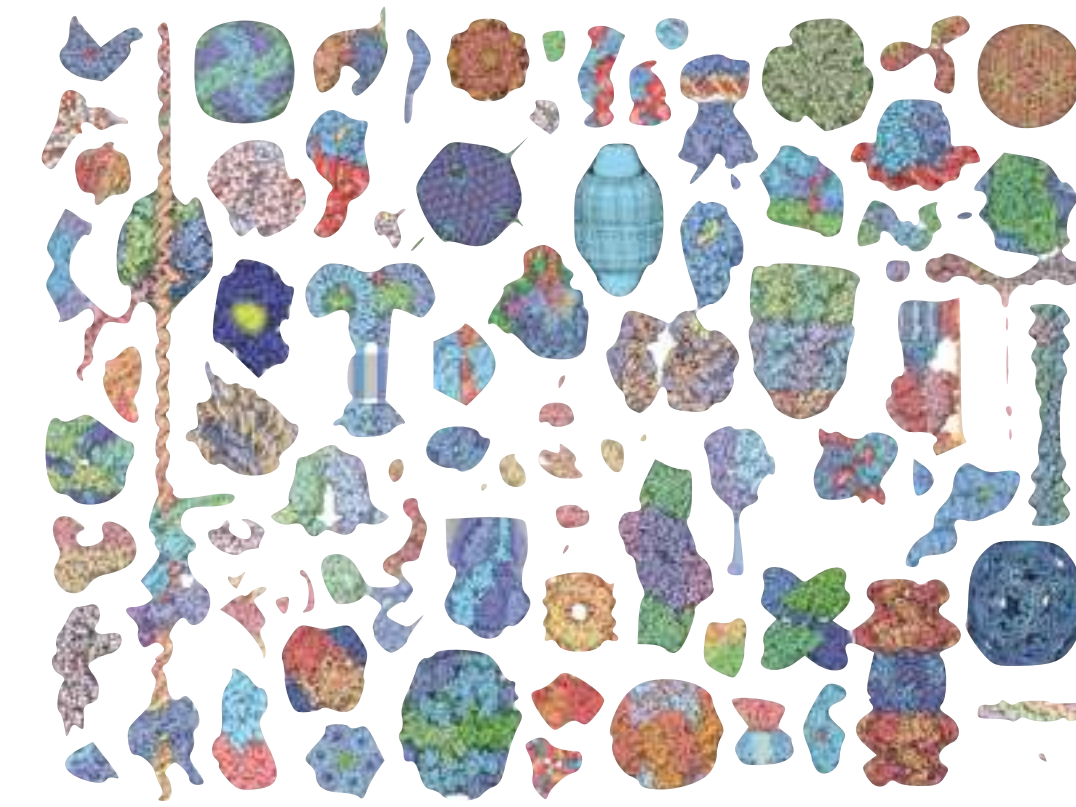
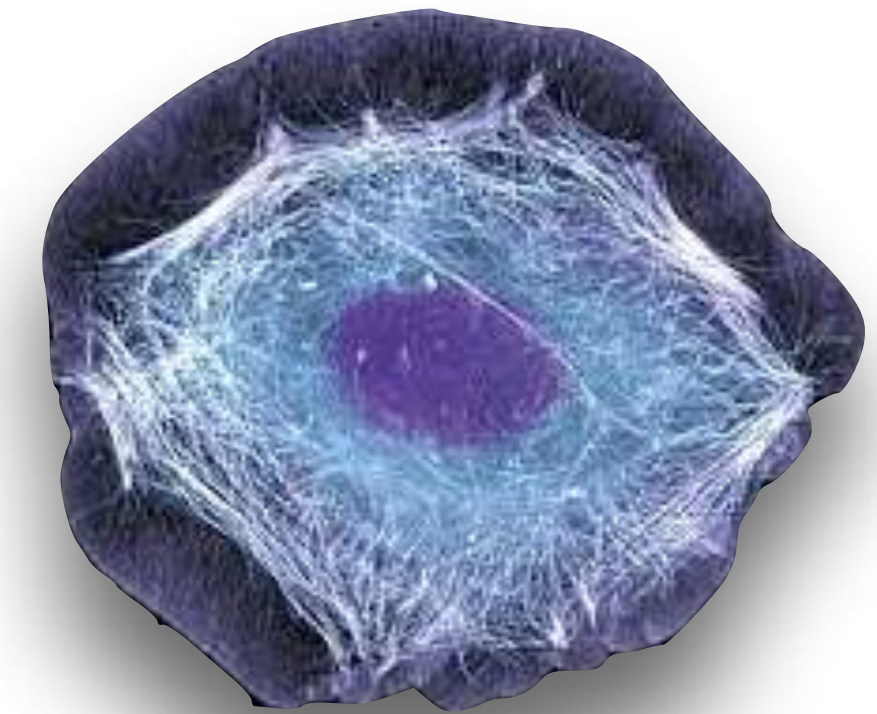




# CRYOEM: TECHNOLOGY ON THE RISE



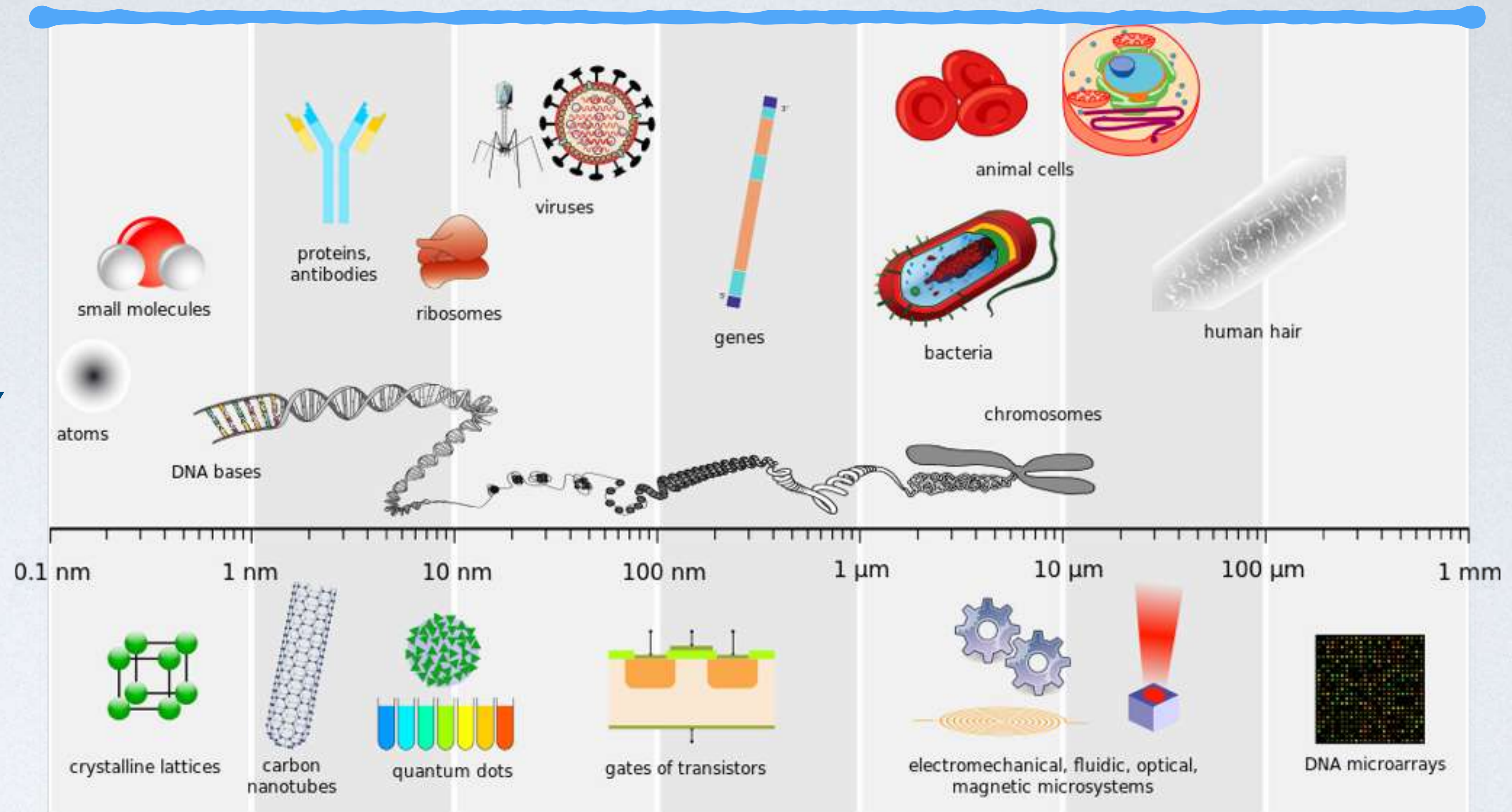
????



TBD (20??)

# CRYOEM: SCALE WITHIN BIOLOGY

## Electron Microscopy

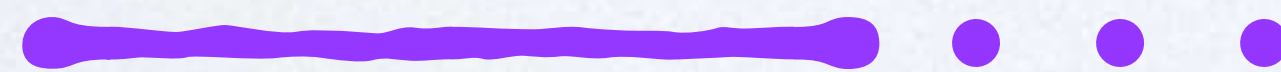


[https://en.wikipedia.org/wiki/Nanoscope\\_scale](https://en.wikipedia.org/wiki/Nanoscope_scale)

X-ray



NMR



AFM



Light microscopy



Naked eye



# CRYOEM: WHY ELECTRONS?

## Pros

Small wavelength

Can be focused

## Cons

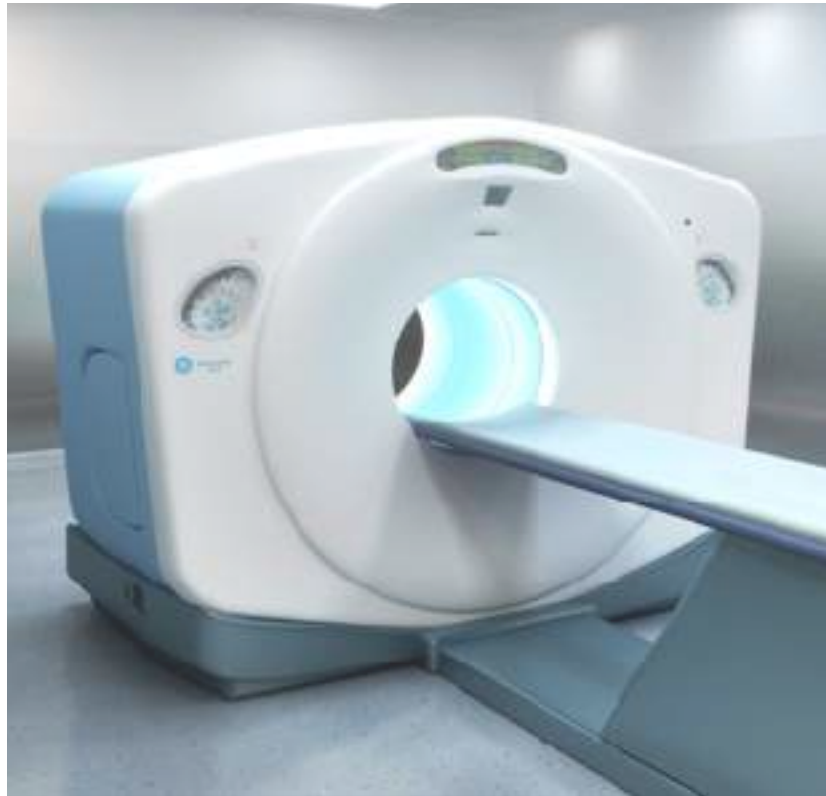
Damages sample  
worse with faster electrons

Poor penetration  
better with faster electrons

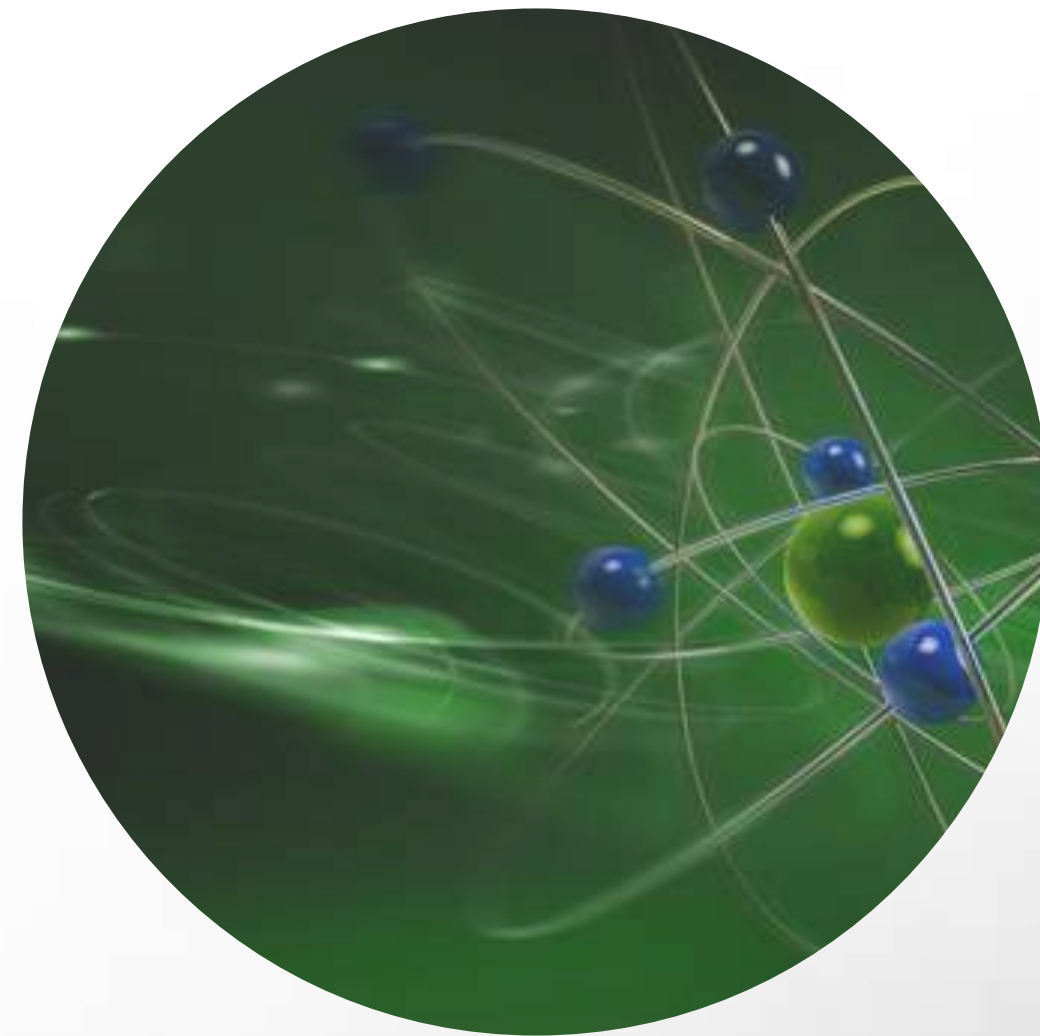
# CRYOEM:

# MODALITIES | TOOLS

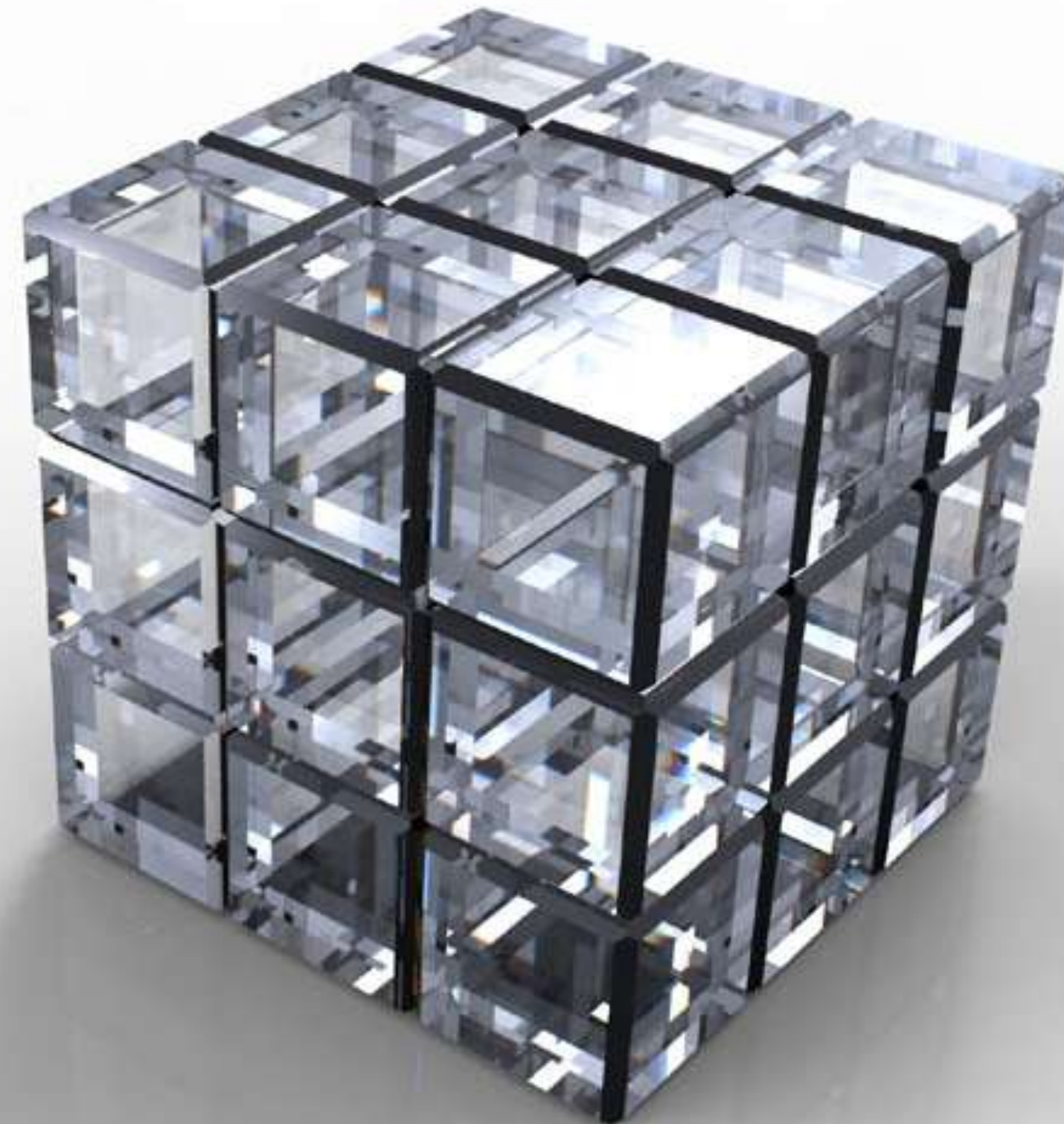
Tomography



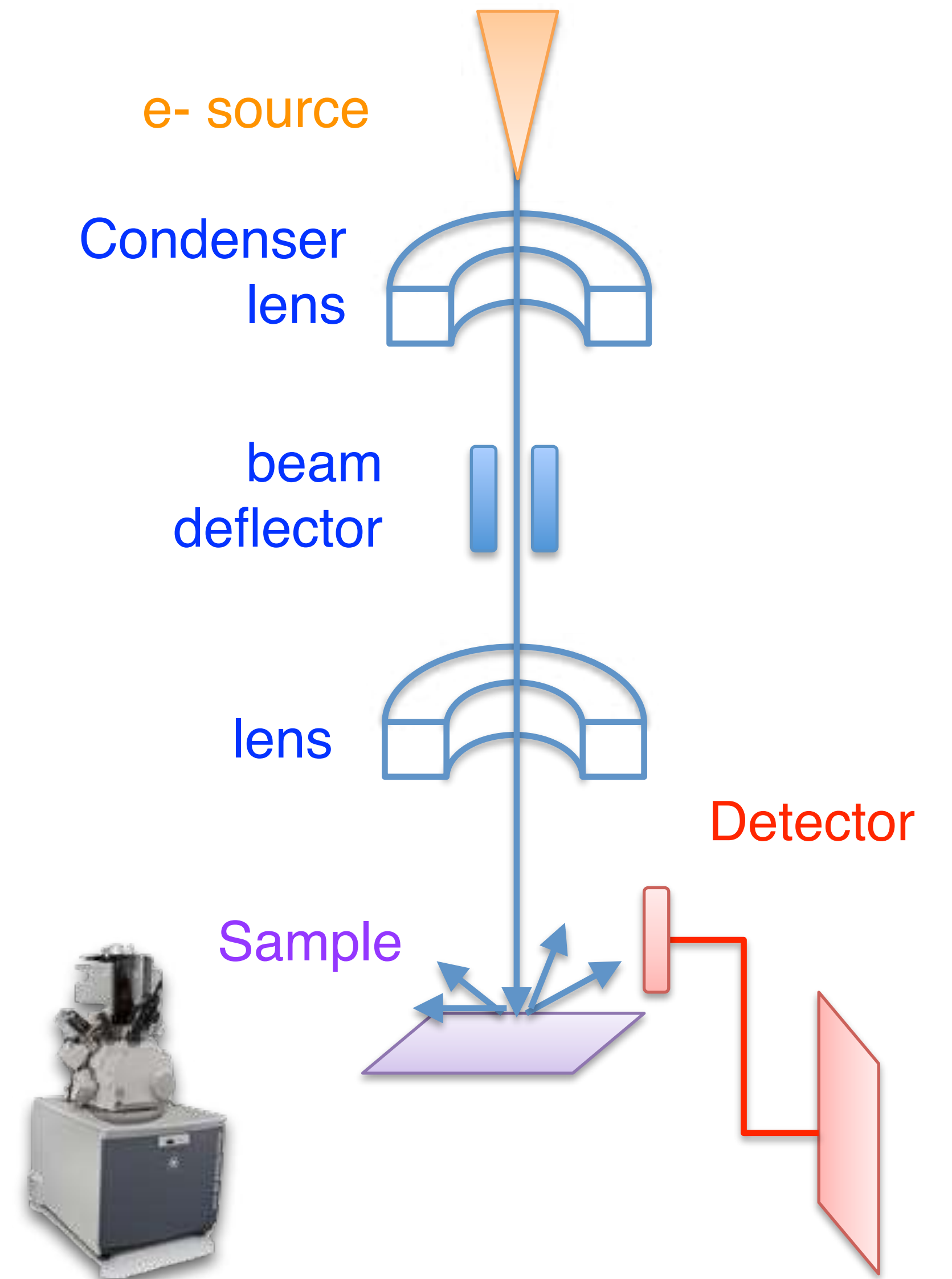
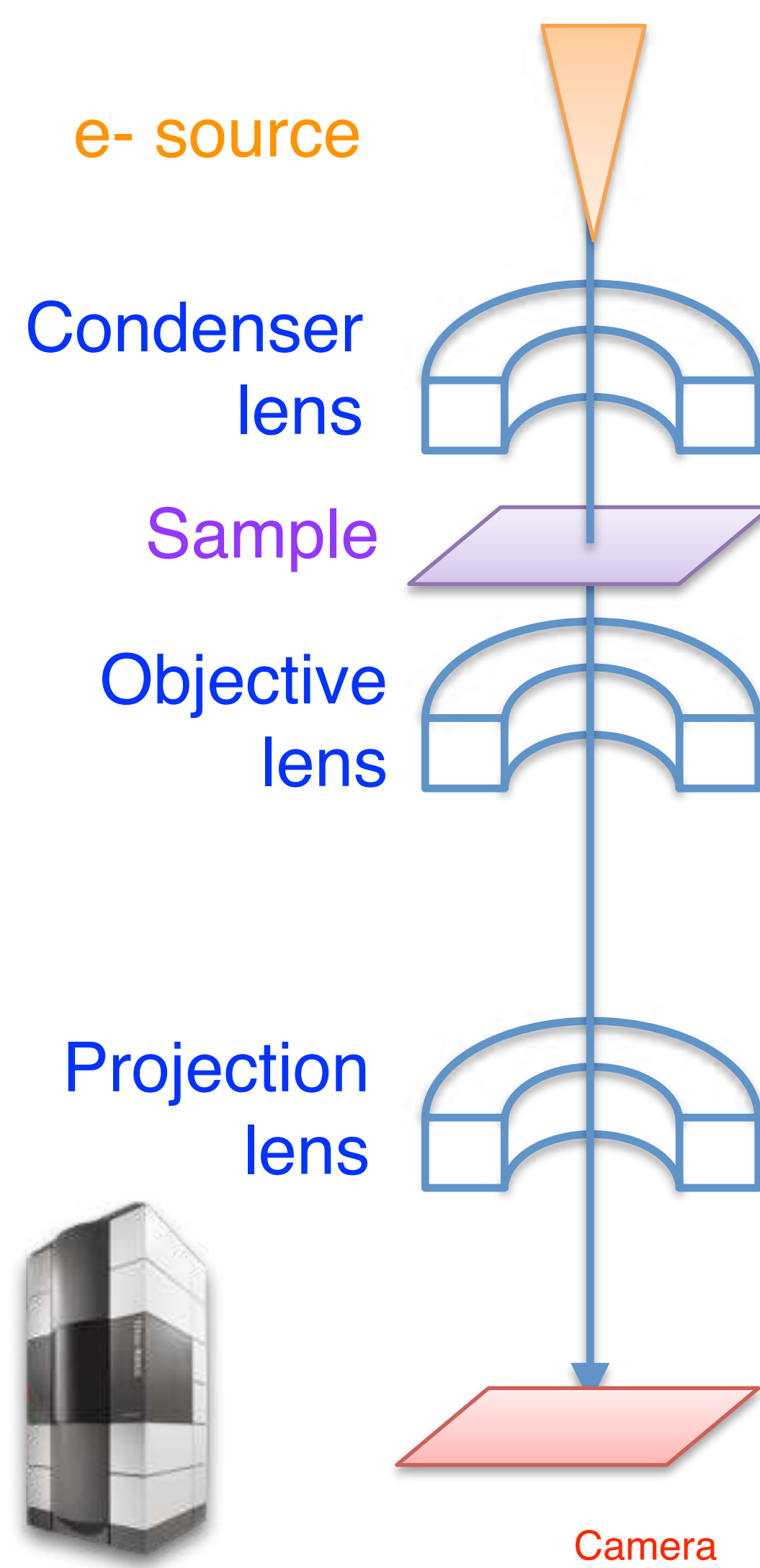
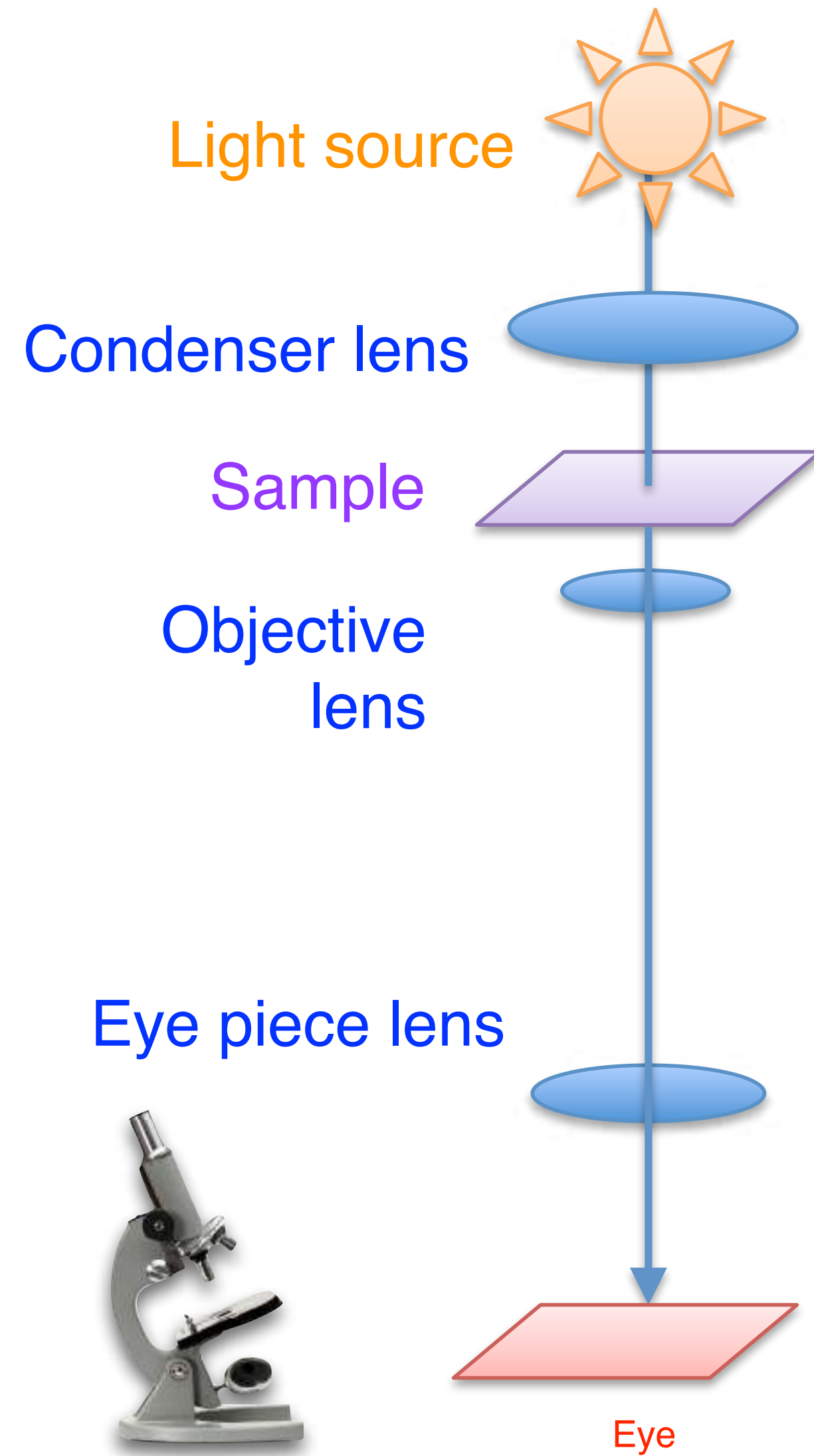
Single-particle



2D arrays

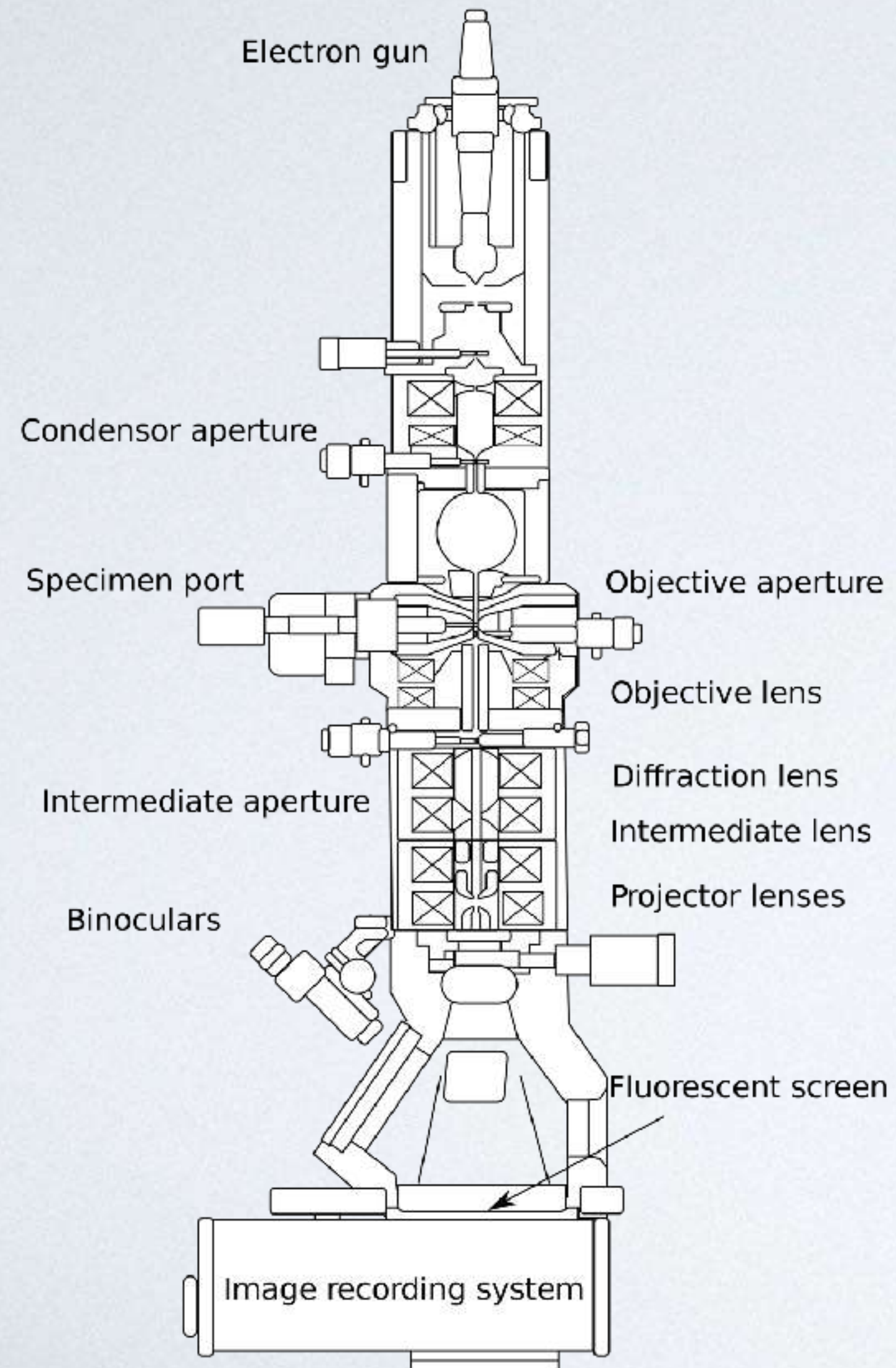


# CRYOEM:



# TOOLS

# MAIN PARTS OF AN EM



**Electron sources**



**Vacuum systems**



**Lenses**

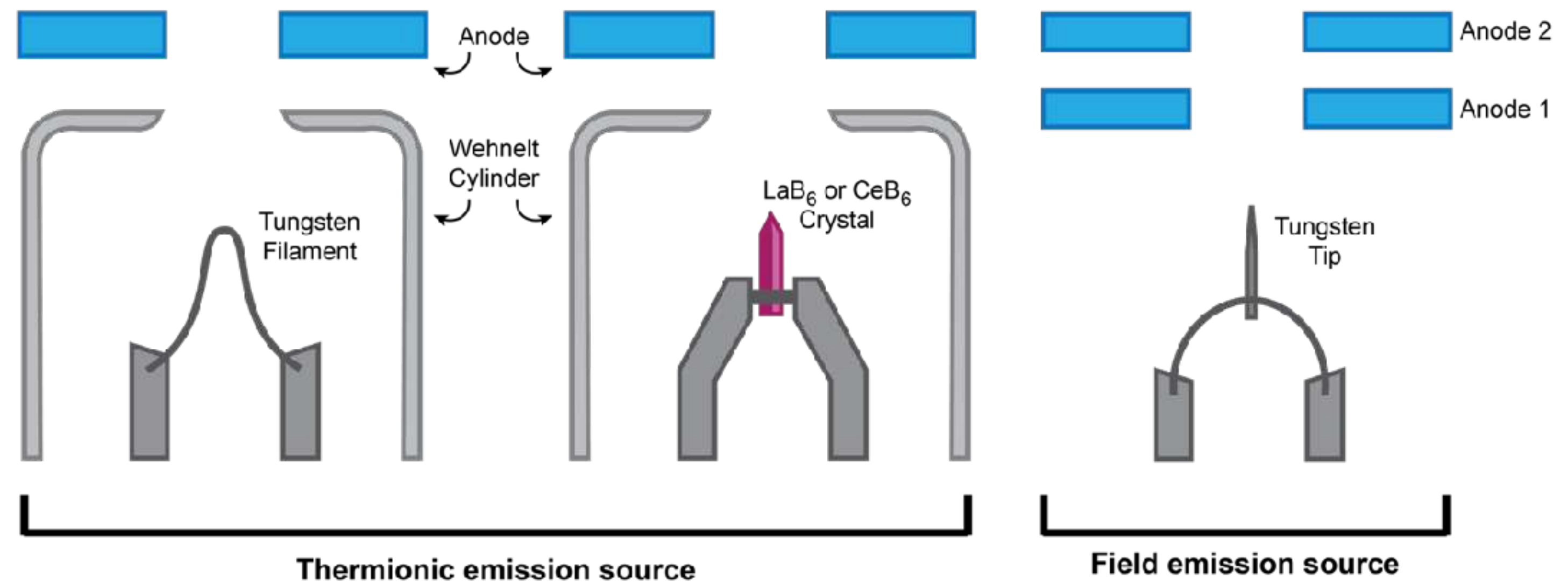


**Detectors**



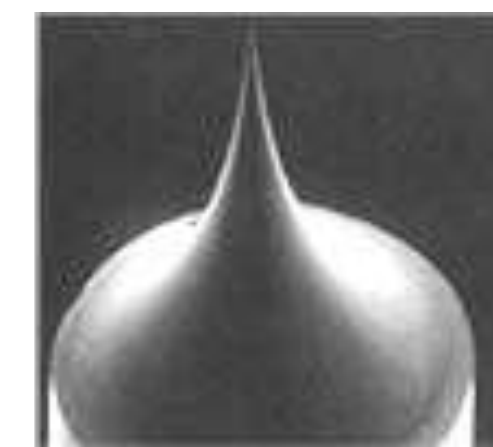
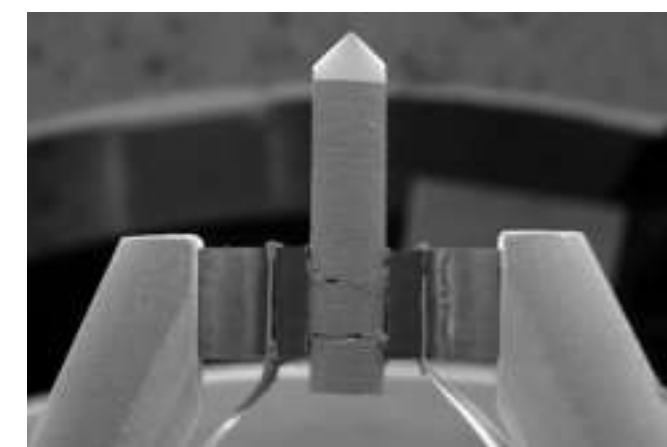
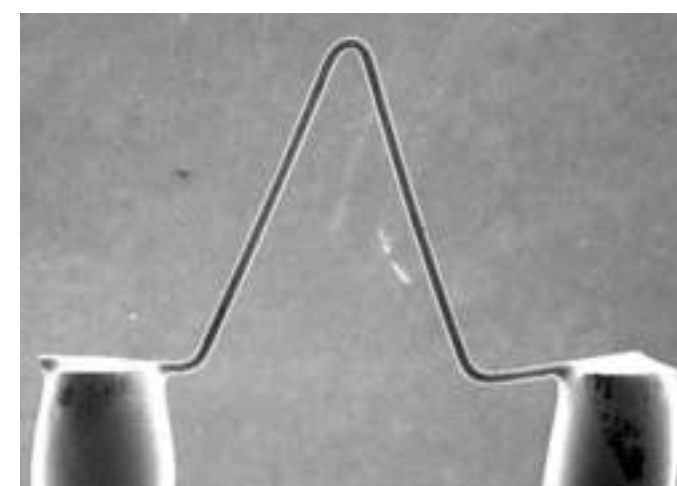
# ELECTRON SOURCES

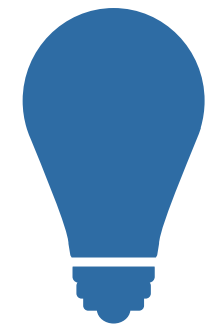
What are the 3 main kinds of electron sources?



[www.thermofisher.com](http://www.thermofisher.com)

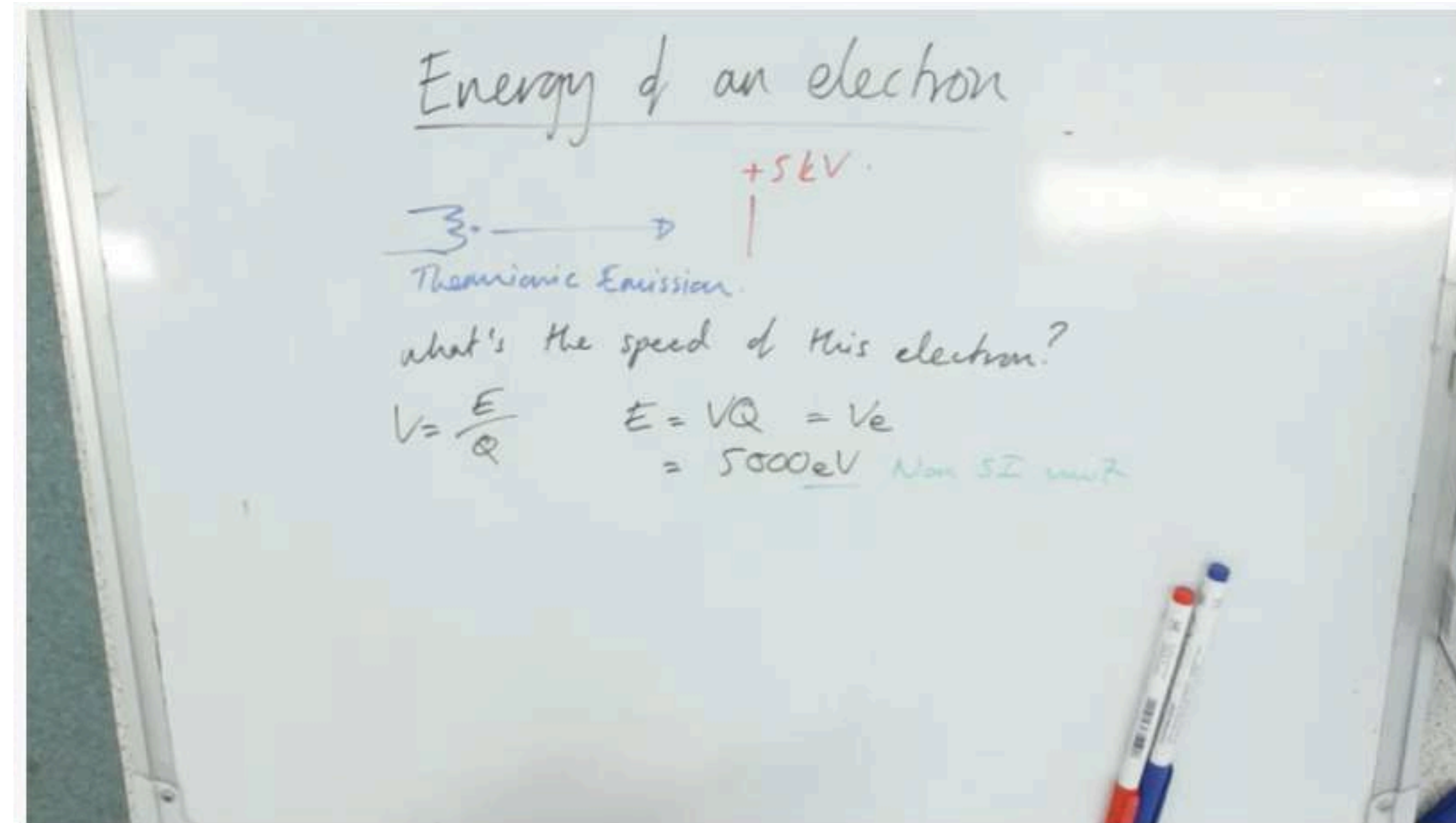
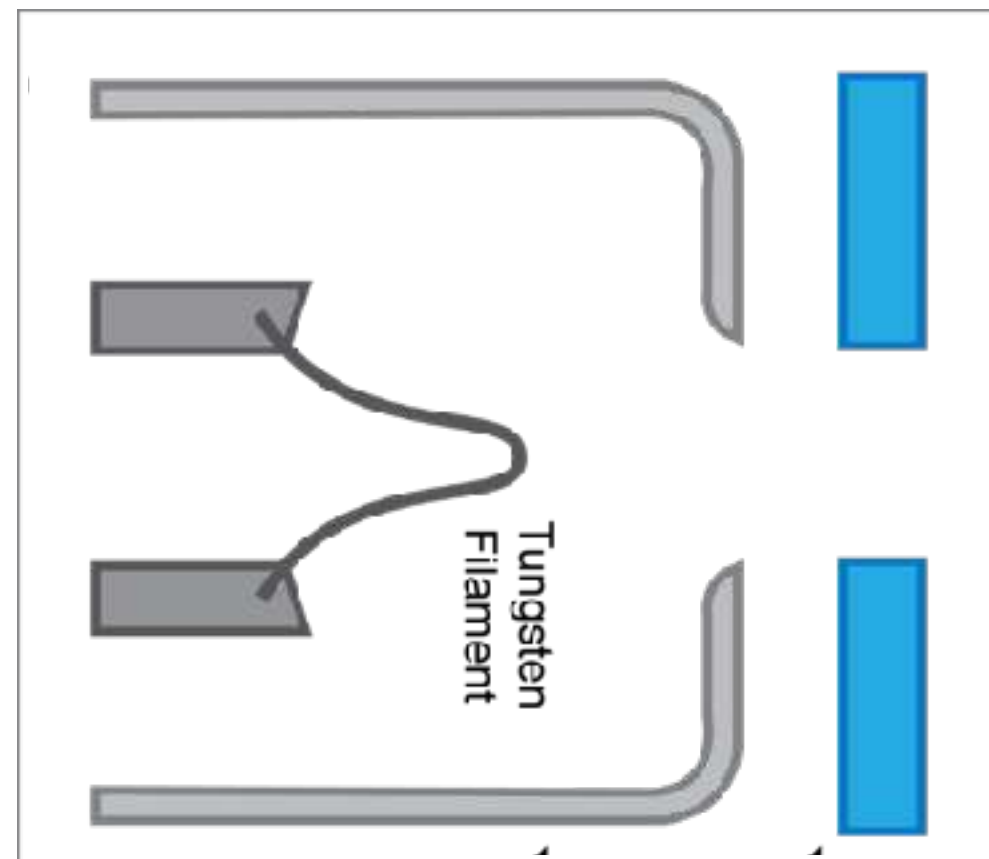
[nanoscience.com](http://nanoscience.com)





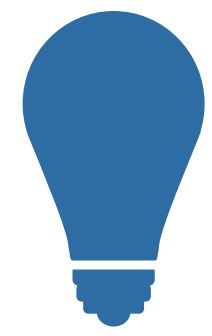
# ELECTRON SOURCES

How fast are the electrons moving?



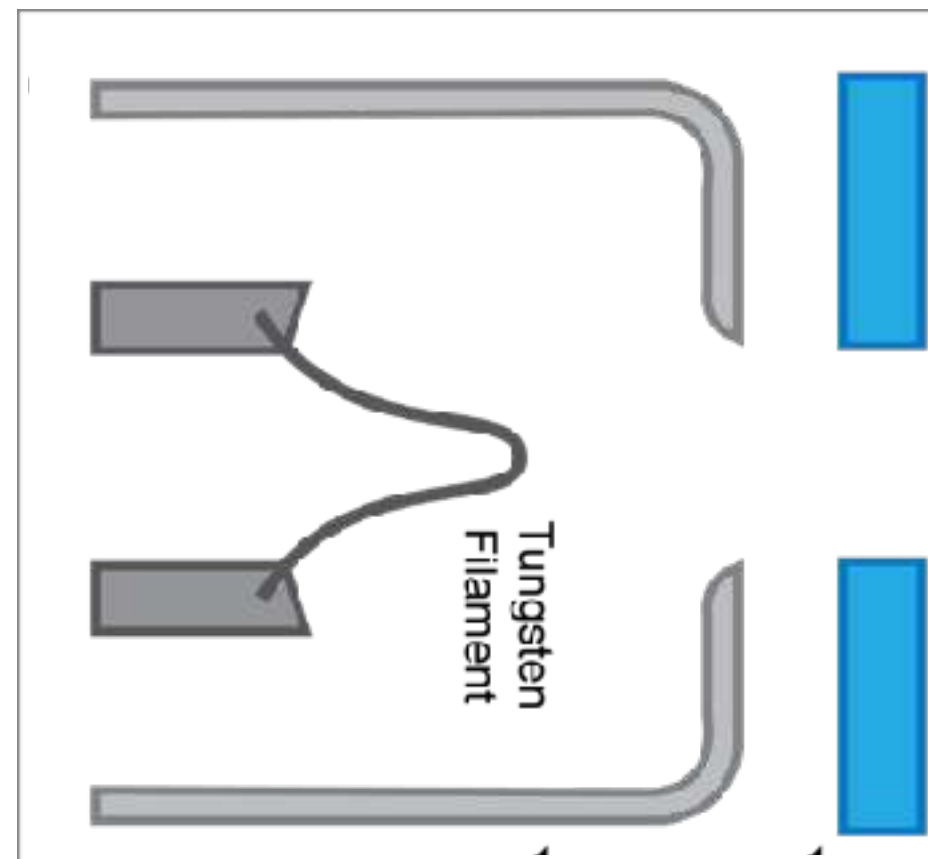
<https://www.youtube.com/watch?v=tYCET6vYdYk>





# ELECTRON SOURCES

How fast are the electrons moving?



Energy of an electron

$+5kV$

Thermionic Emission

what's the speed of this electron?

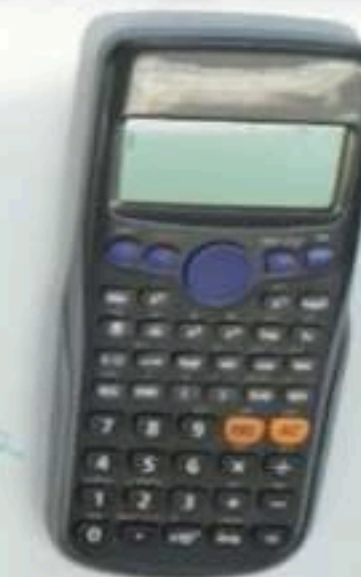
$$V = \frac{E}{Q} \quad E = VQ = Ve$$

$$= 5000eV \text{ Non SI unit?}$$

$$E = 5000 \times 1.6 \times 10^{-19}$$

$$= 8 \times 10^{-16} J$$

$$E_k = \frac{1}{2}mv^2 \quad m_e = 9.11 \times 10^{-31} kg$$

$$8 \times 10^{-16} = \frac{1}{2}mv^2$$


Energy of an electron

$+5kV$

Thermionic Emission

what's the speed of this electron?

$$E = VQ = Ve$$

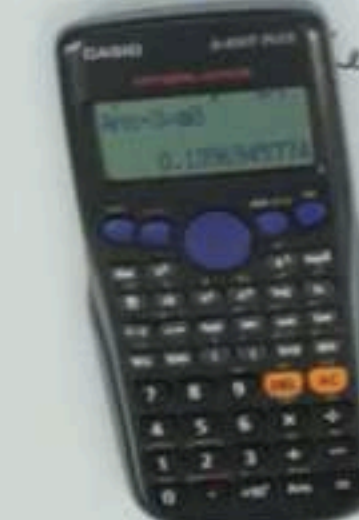
$$= 5000eV \text{ Non SI unit?}$$

$$\times 1.6 \times 10^{-19}$$

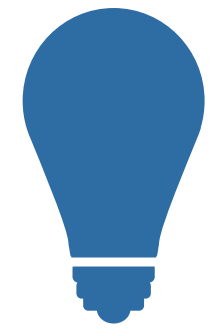
$$= 8 \times 10^{-16} J$$

$$8 \times 10^{-16} = \frac{1}{2}mv^2 \quad m_e = 9.11 \times 10^{-31} kg$$

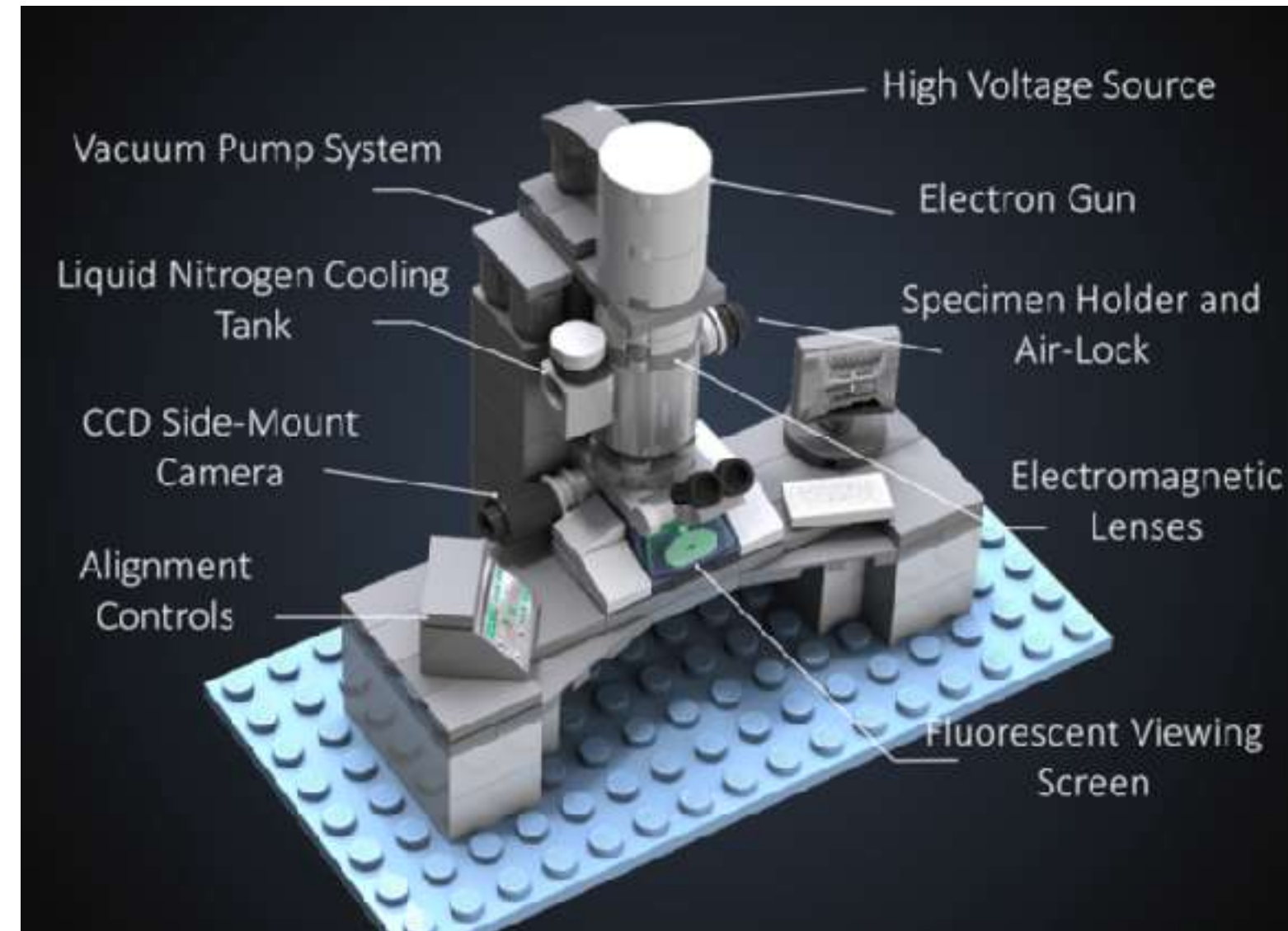
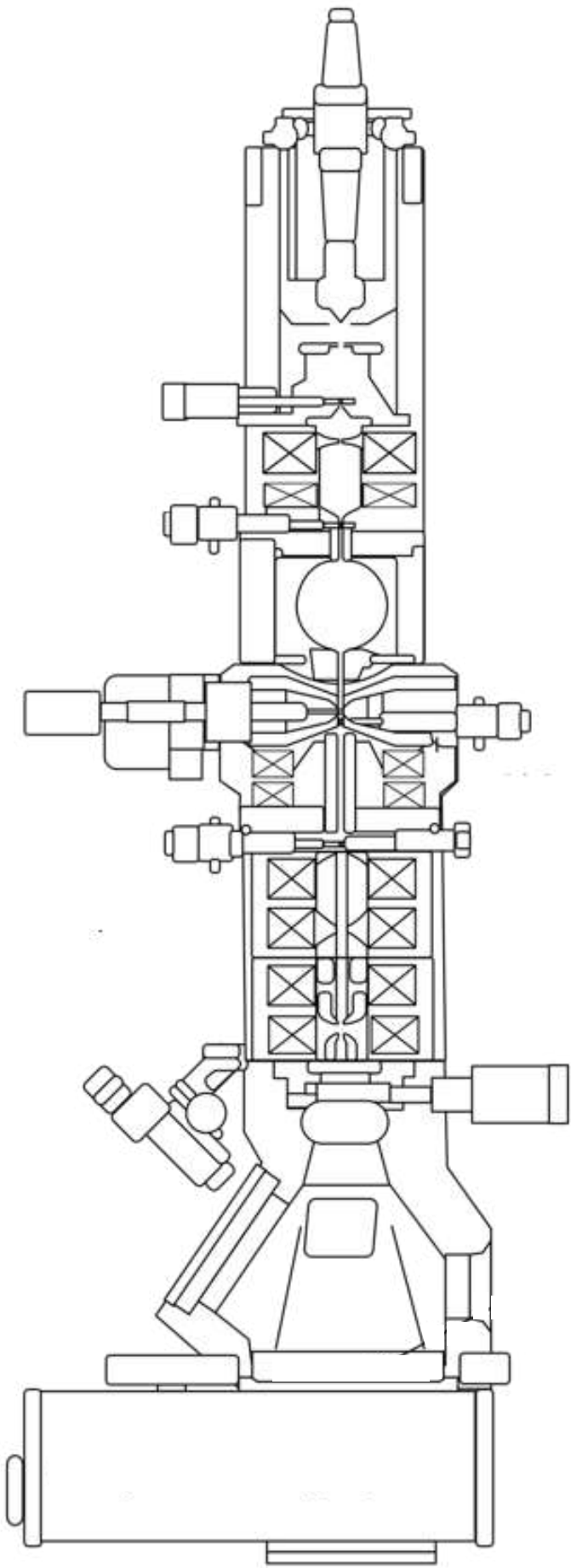
$$\sqrt{\frac{2 \times 8 \times 10^{-16}}{9.11 \times 10^{-31}}} = 41900000$$

$$= 4.2 \times 10^7 m/s$$


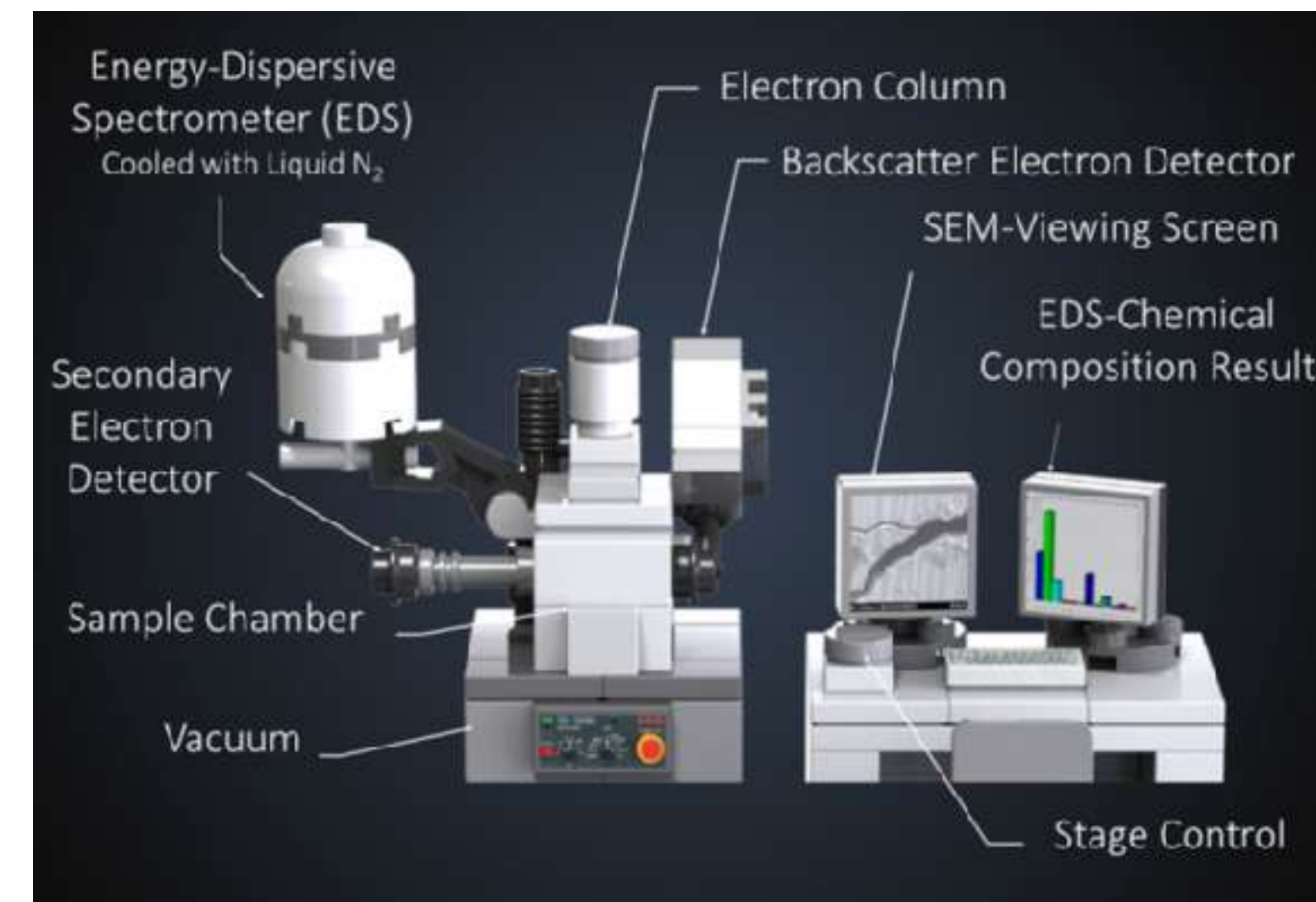
<https://www.youtube.com/watch?v=tYCET6vYdYk>

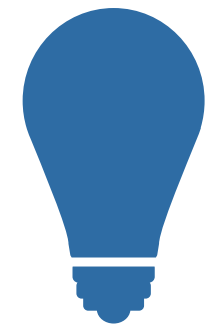


# ELECTRON SOURCES & TYPES OF CRYOEMs

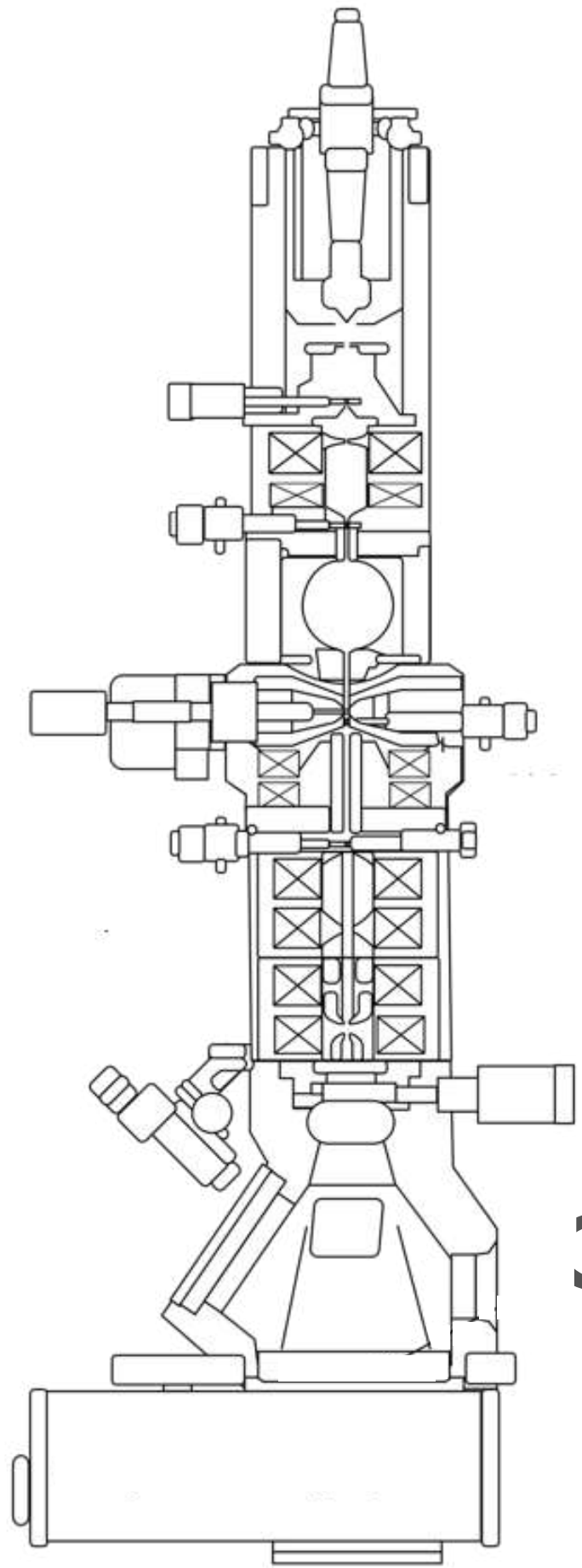


<https://ideas.lego.com/projects/102281>





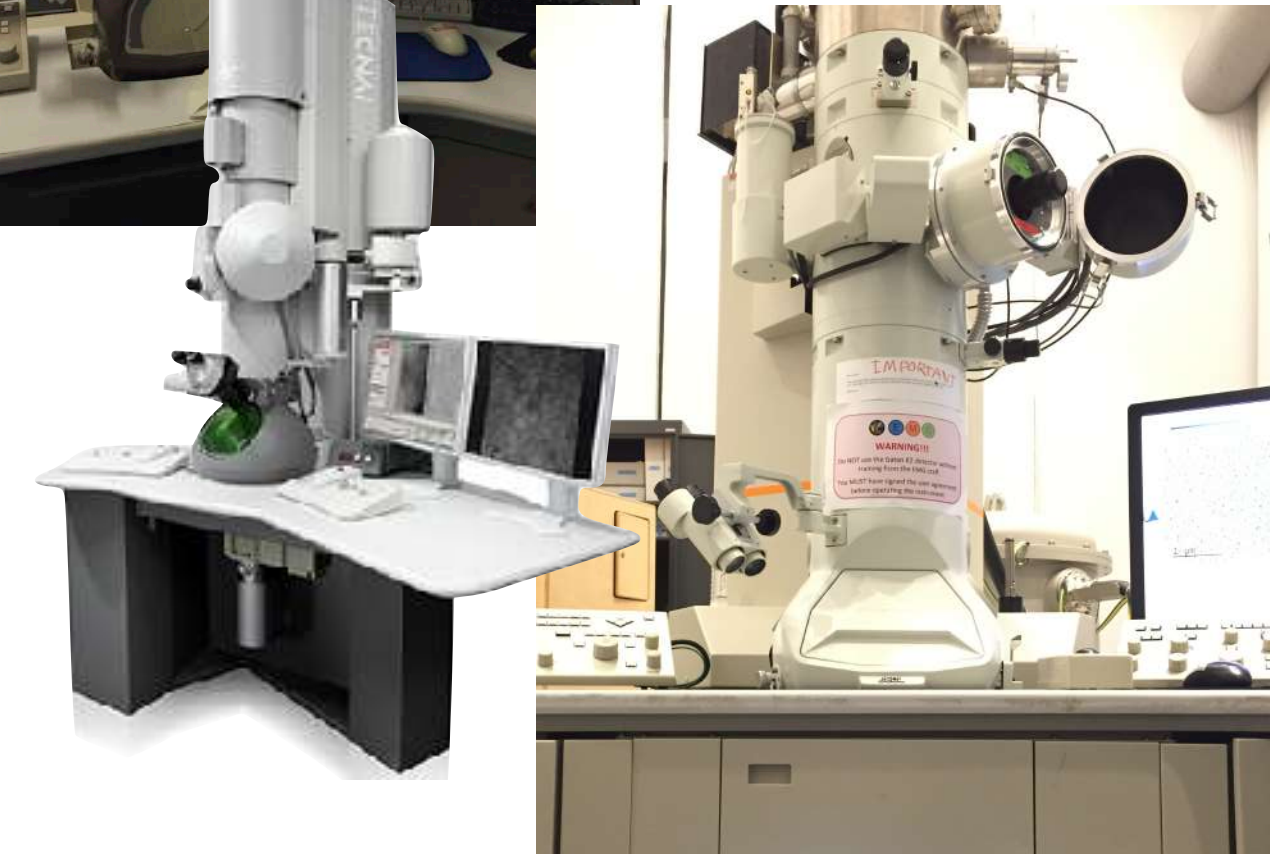
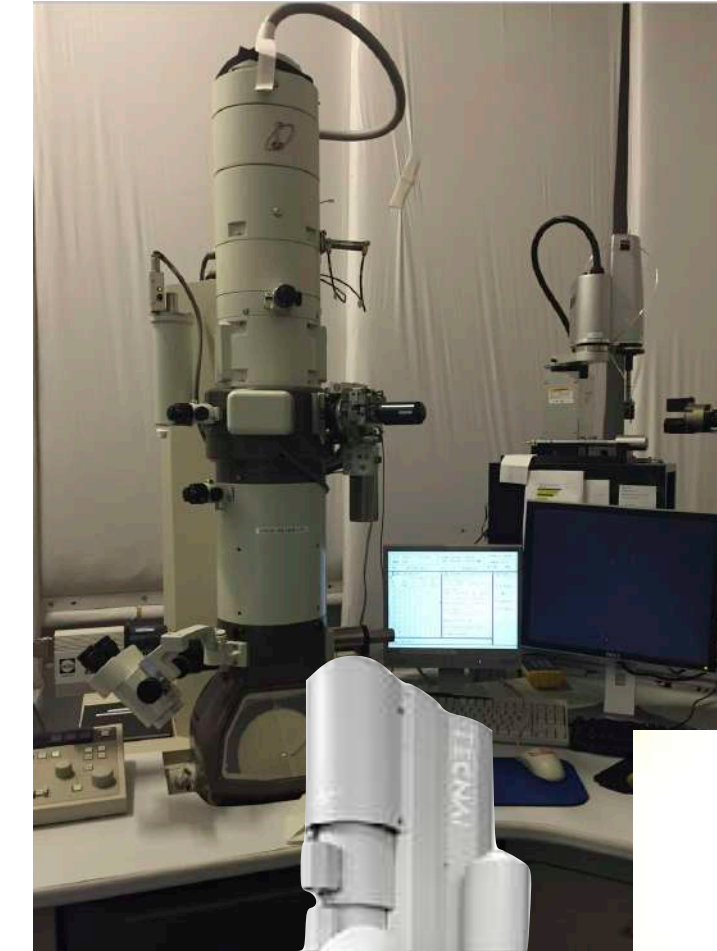
# ELECTRON SOURCES & TYPES OF CRYOEM

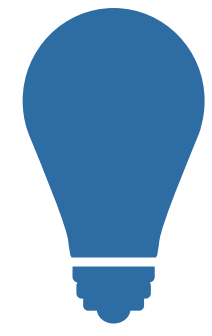


**80-120 kV:** JEM 1230; Tecnai T12  
W or LaB6  
High contrast & robust  
sub-nm resolution

**200 kV:** JEM 2100F, Tecnai F20, Talos, Artica  
FEG  
2+ Å resolution (3.5-4 Å)

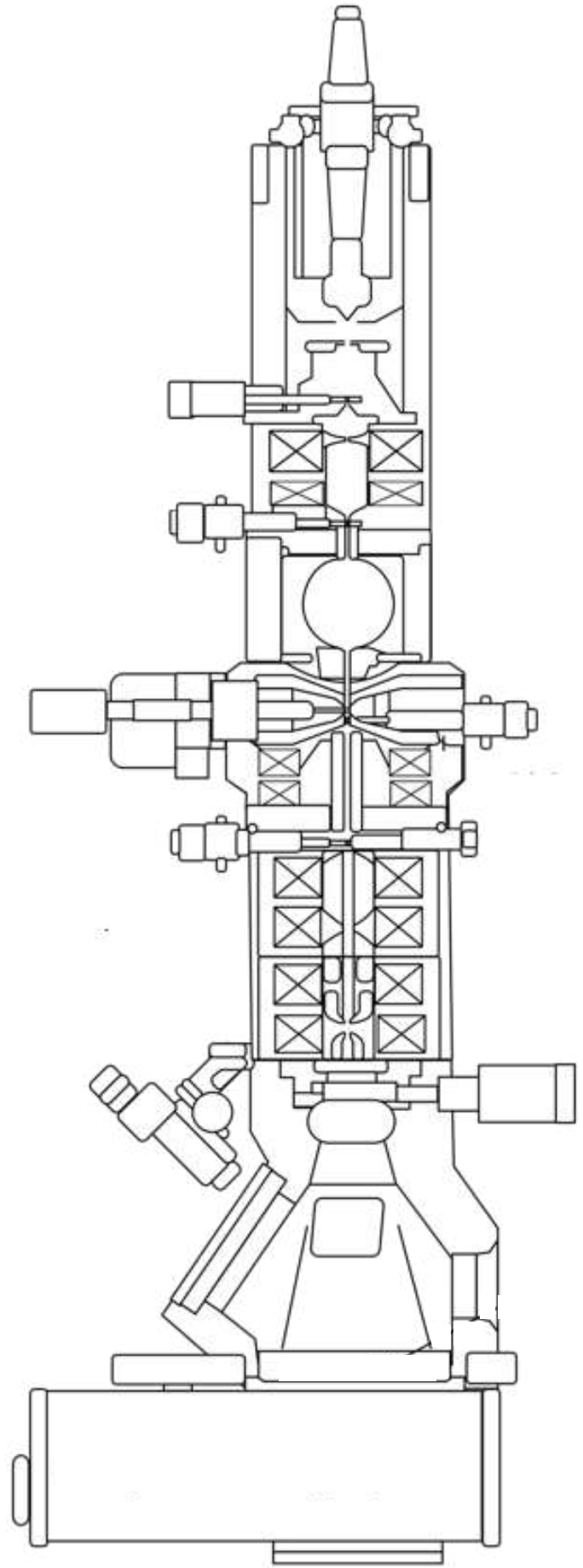
**300 kV:** JEM 3200FSC, cryo-ARM, Krios, Polara  
FEG  
Smaller effect on unwanted lens aberration  
1.5-3 Å resolution





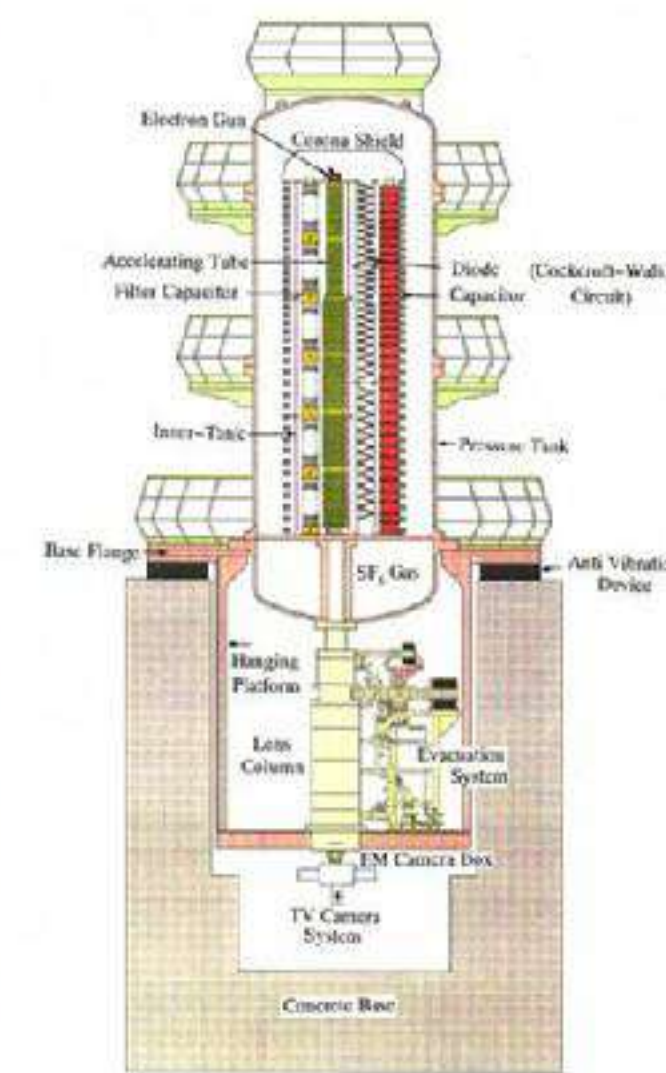
# ELECTRON SOURCES & TYPES OF CRYOEM

**1-1.2 MV:** Hitachi, JEOL  
LaB6

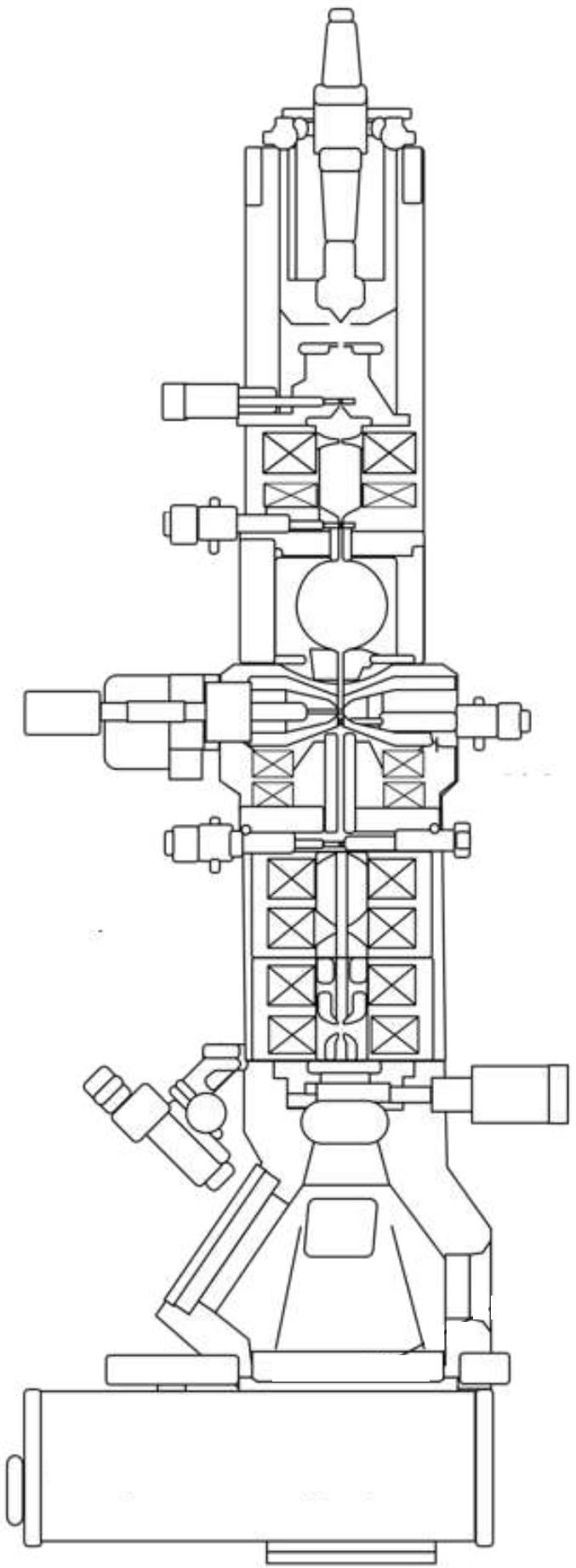


[uhvem.osaka-u.ac.jp](http://uhvem.osaka-u.ac.jp)

**3 MV:** Hitachi H3000  
LaB6



Why do we need a vacuum?



**Beam coherence** - at STP mean free path  $\sim 1$  cm

**Insulation** - interaction between e- and air

**Filament** - O<sub>2</sub> 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 =  $7.5 \times 10^4$  Pa

**PVP / Rotary**

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

**Diffusion**

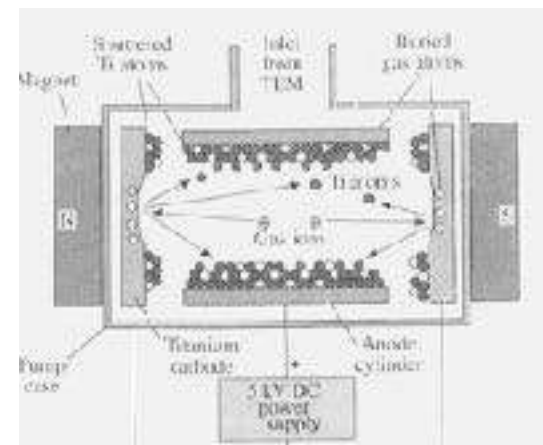
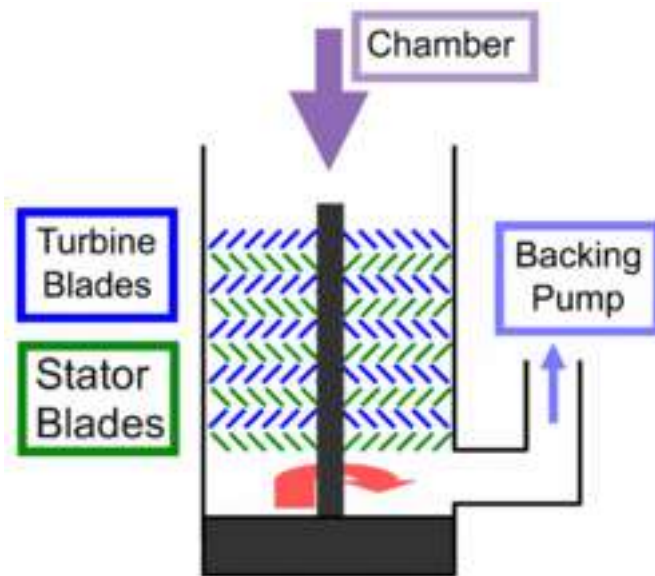
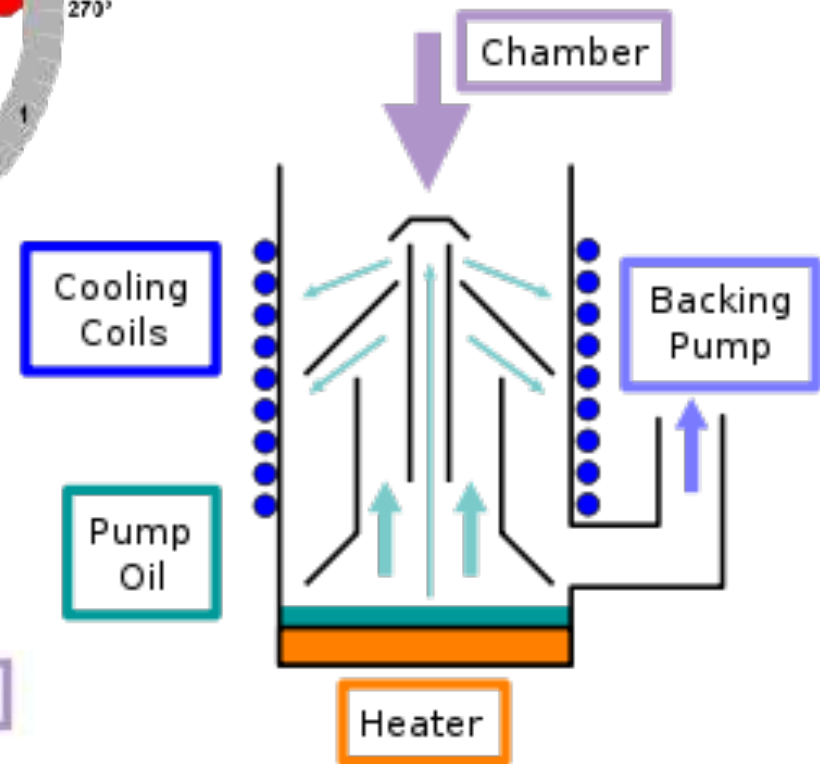
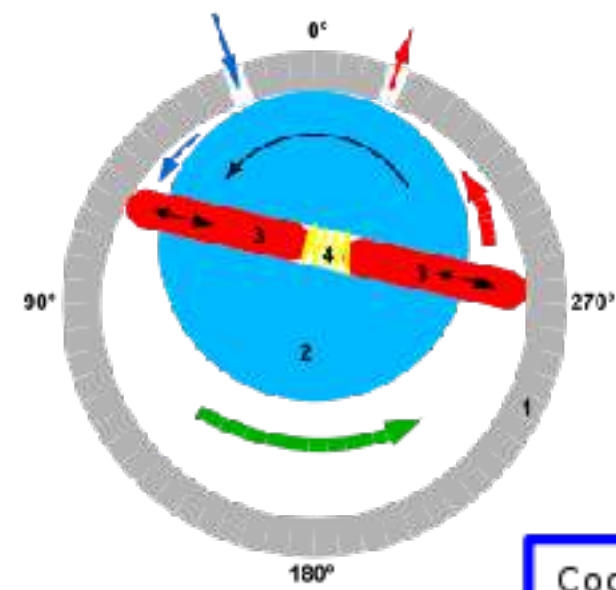
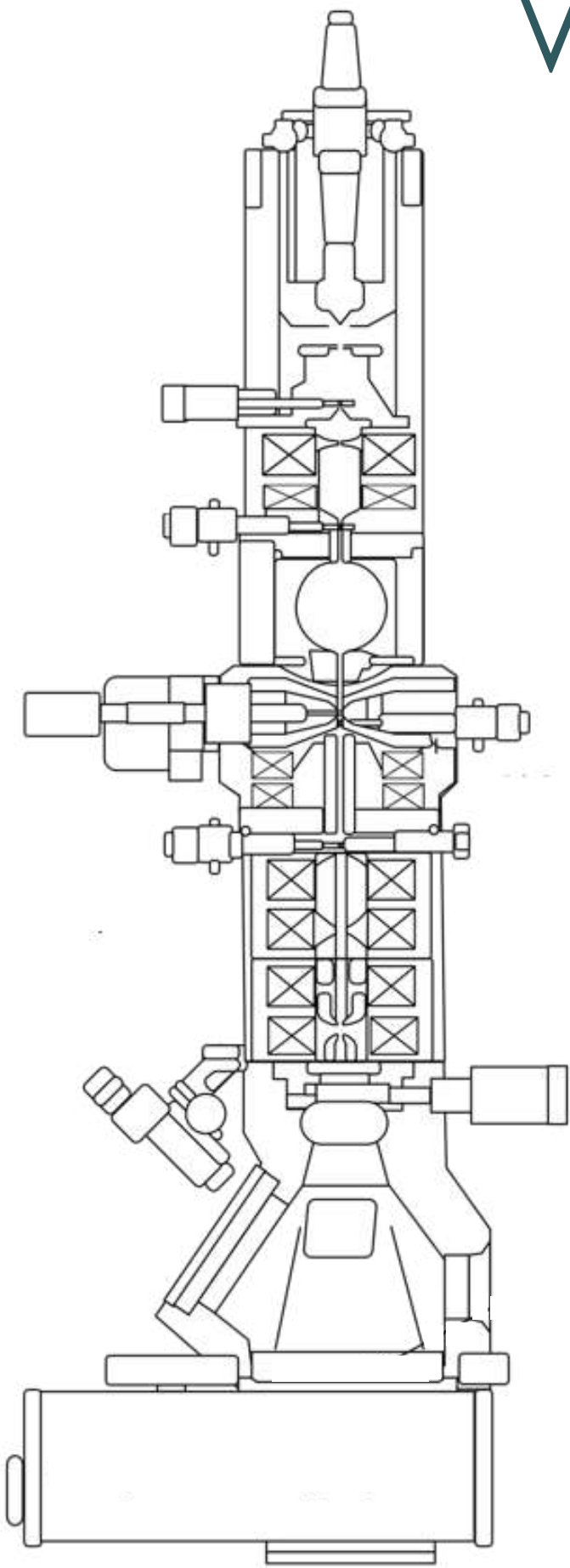
$10^{-3} - 10^{-6}$  Torr |  $0.1 - 10^{-4}$  Pa

**Turbo**

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

**IGP**

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



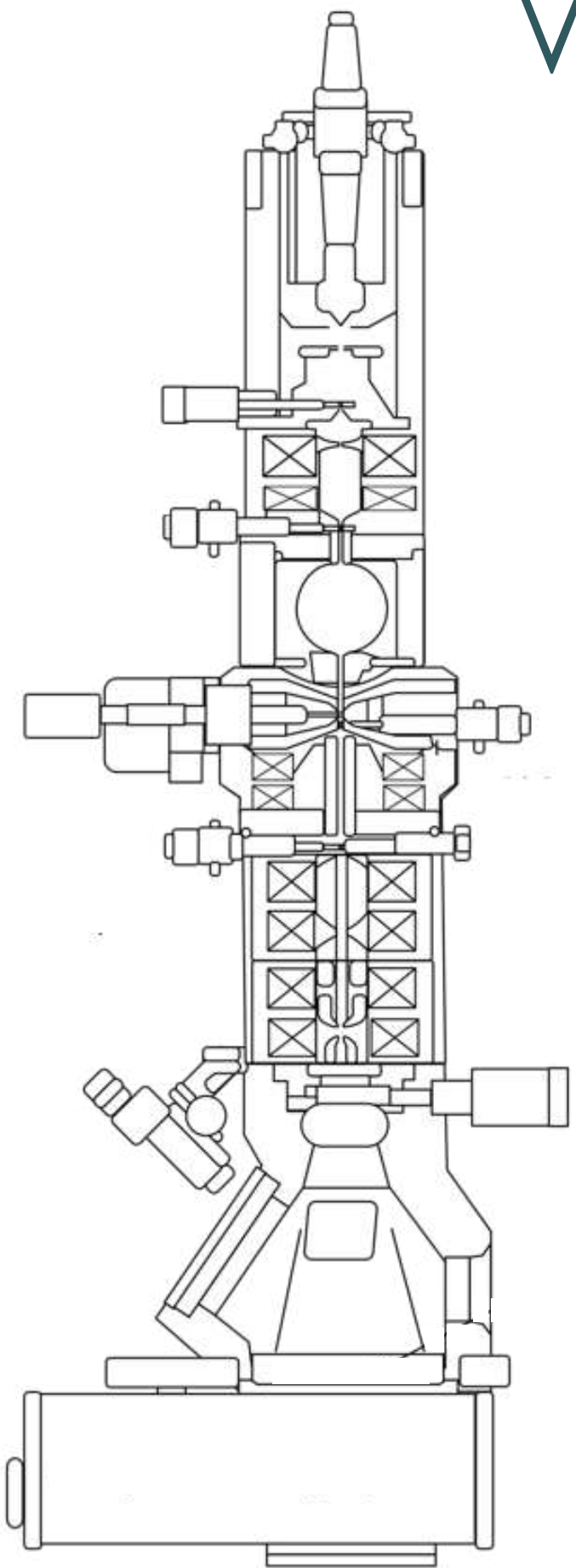
wikipedia.com



# VACUUM SYSTEMS

What types of pumps do we have?

1 mm Hg = 1 Torr =  $10^2$  Pa  
 1 atm = 760 Torr =  $7.5 \times 10^4$  Pa



Gun

$10^{-9}$  Torr

Specimen

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

Chamber and Camera

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

Vacuum (Supervisor) Cryo Settings **Control**

**Status: COL. VALVES**

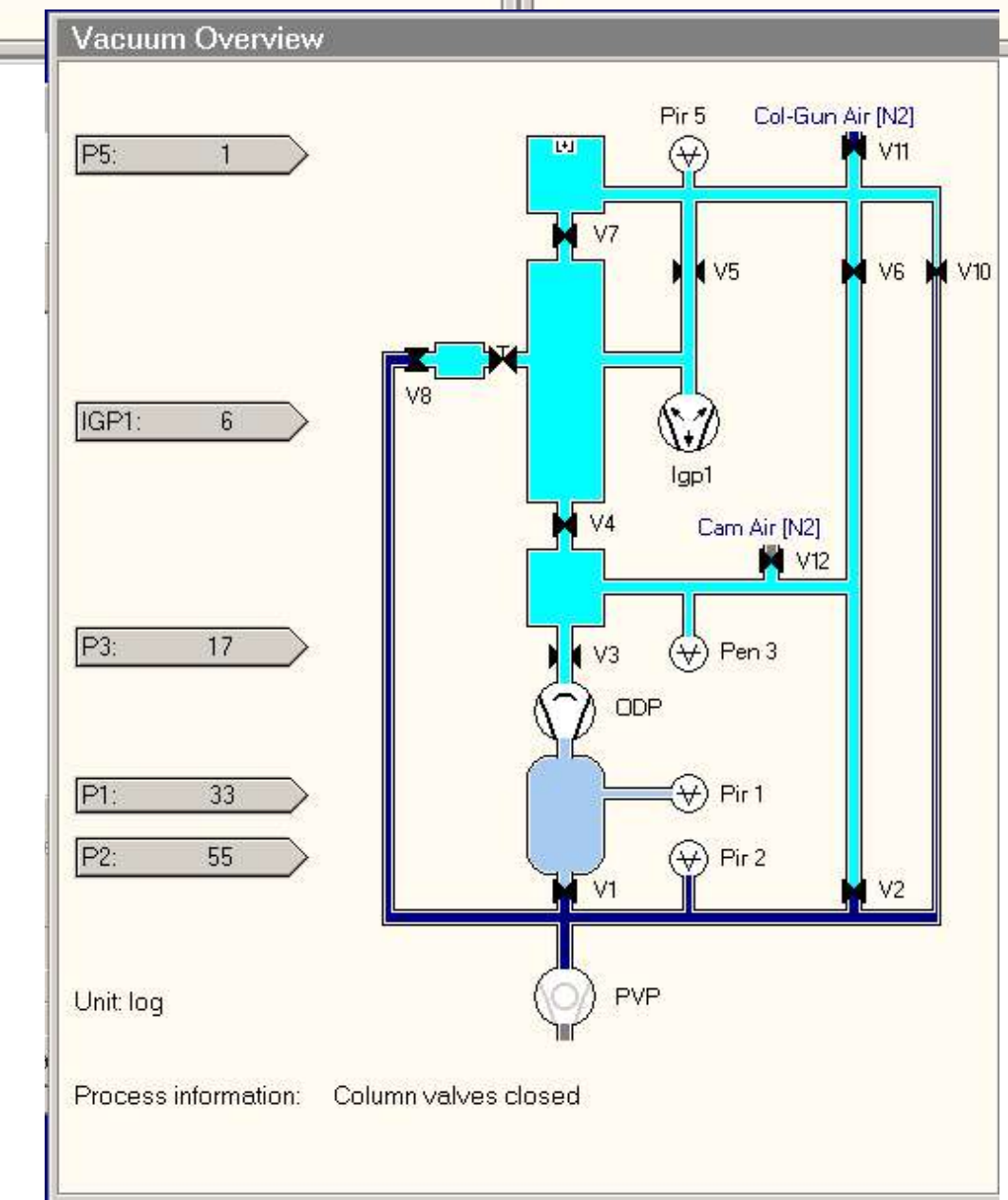
Pressure

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

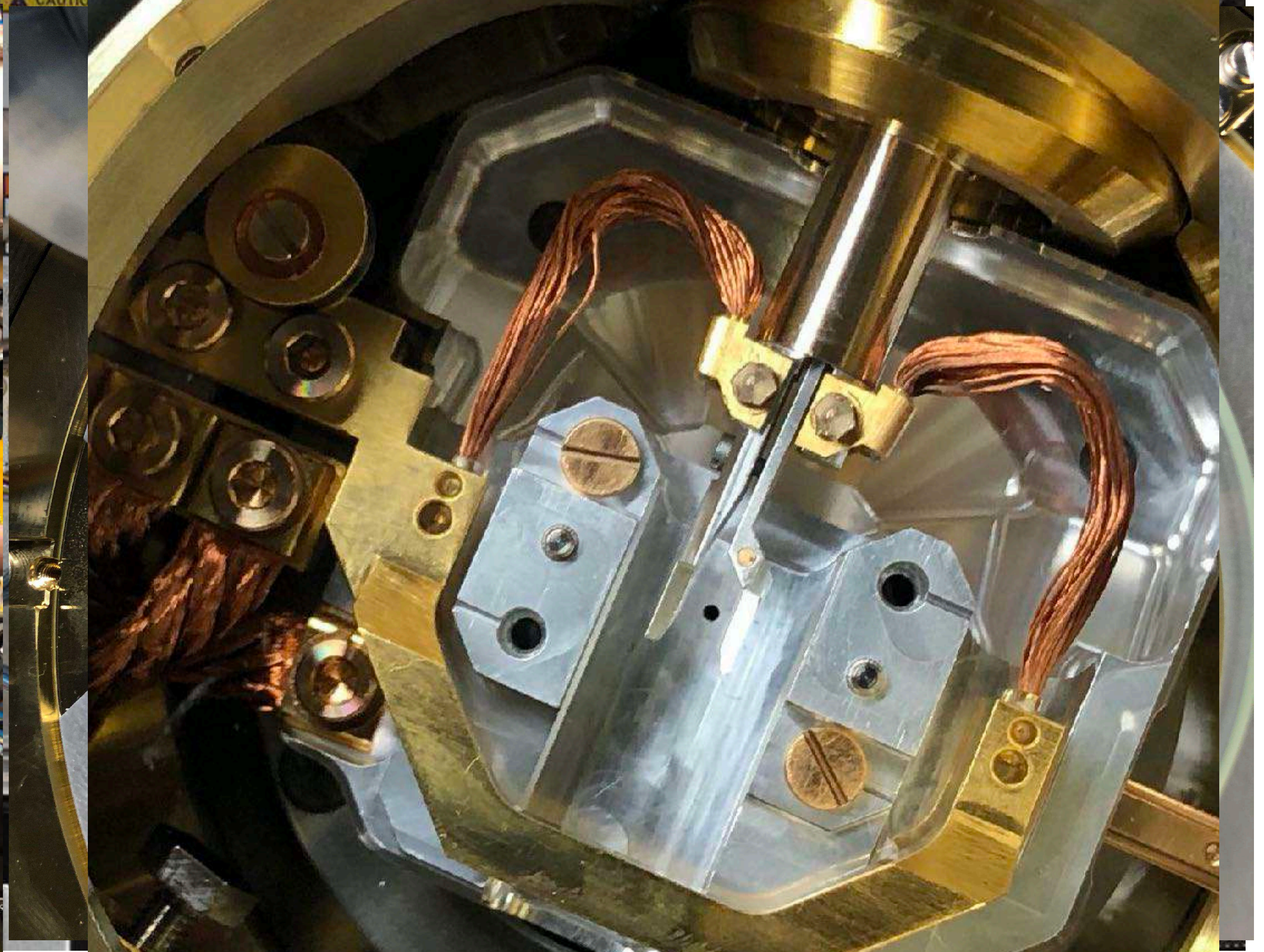
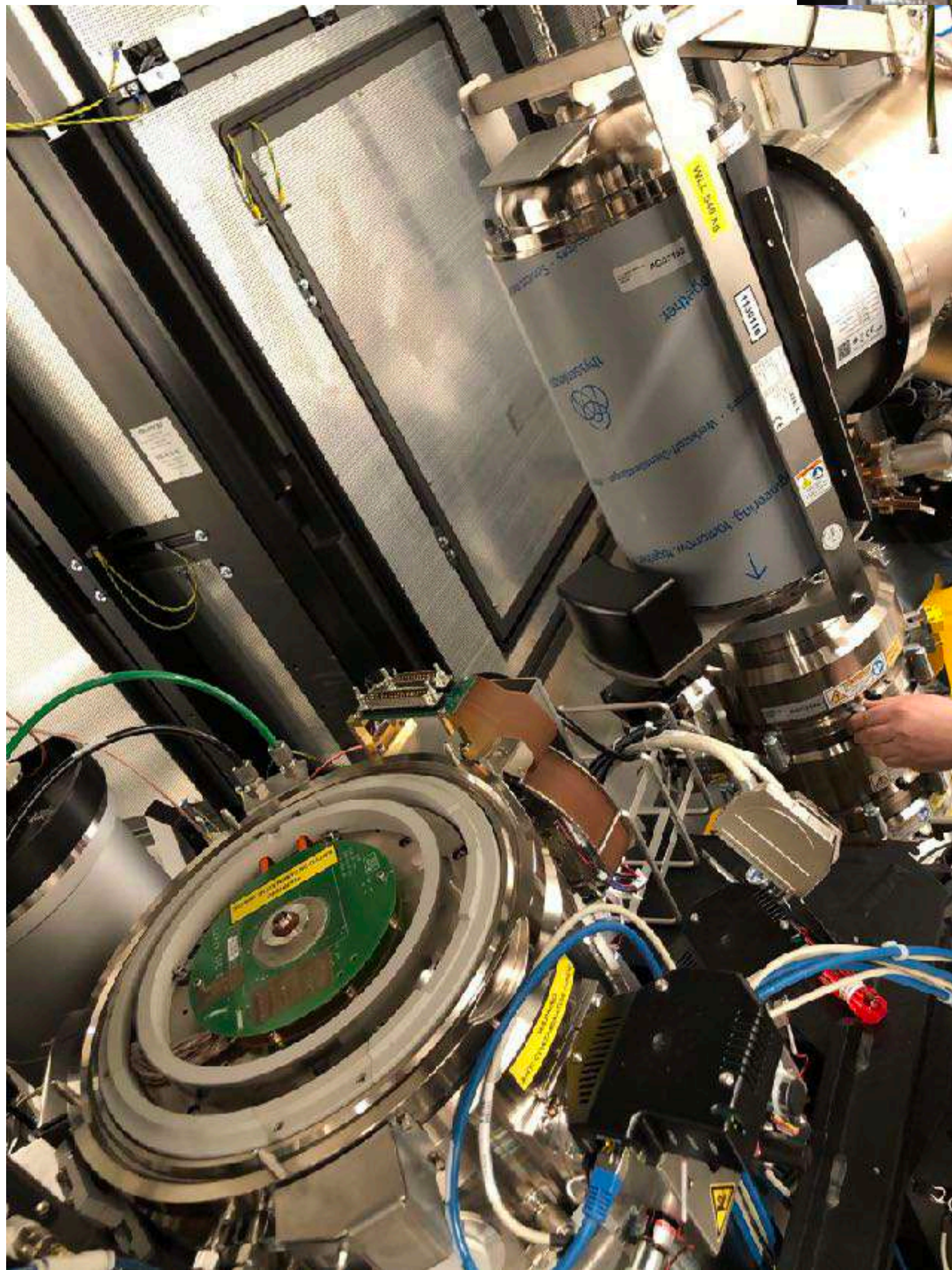
Col. Valves Closed

Default pressure unit: Log  
 Default airlock time: 120 s

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



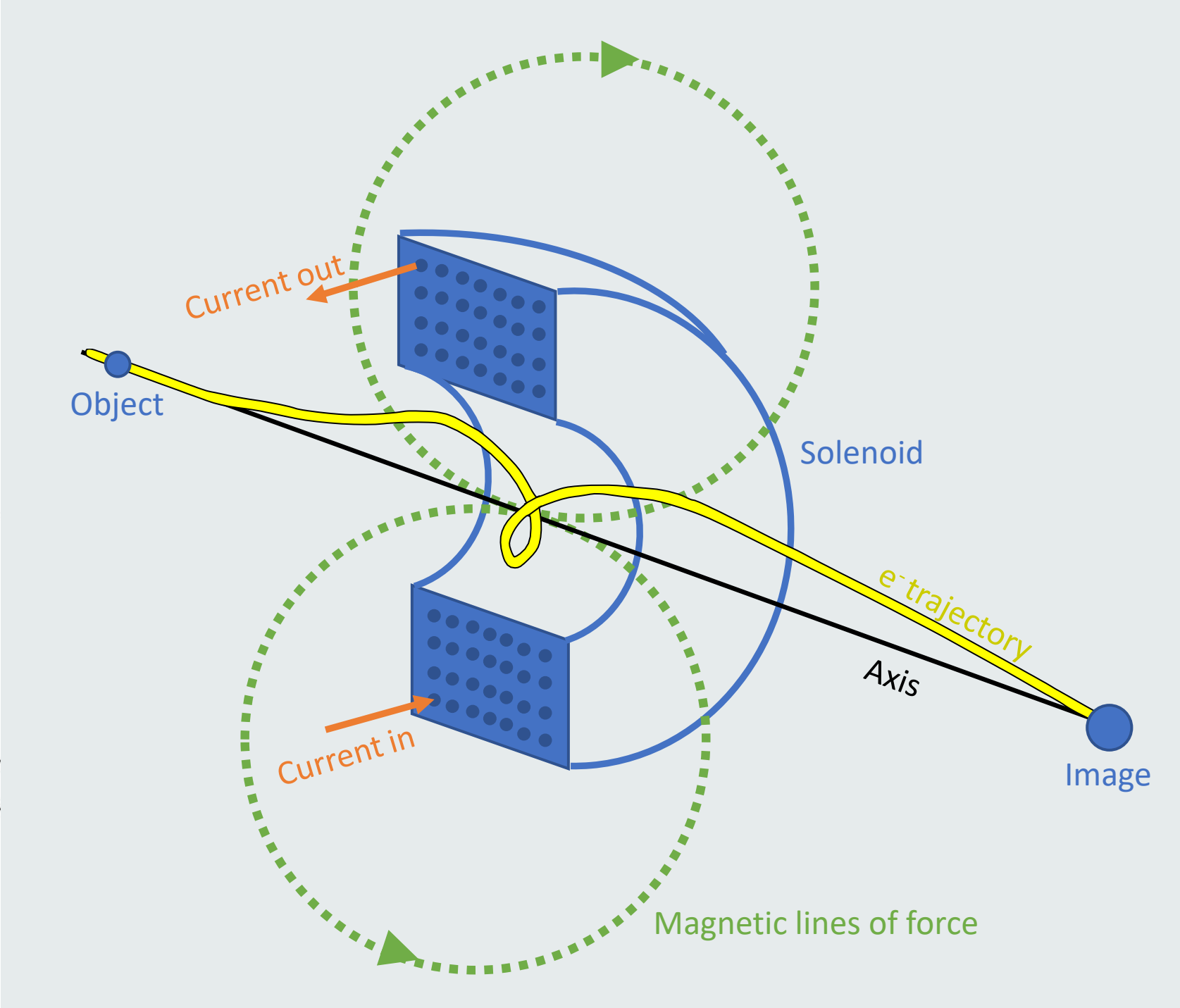
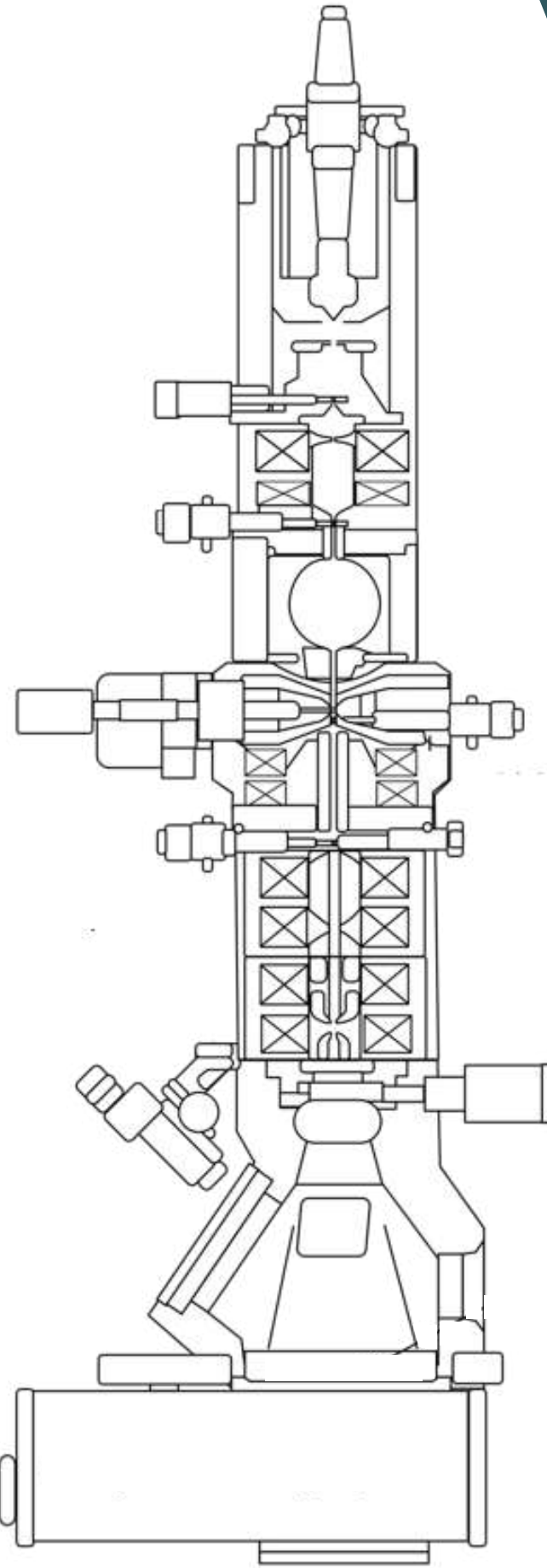
# VACUUM SYSTEMS



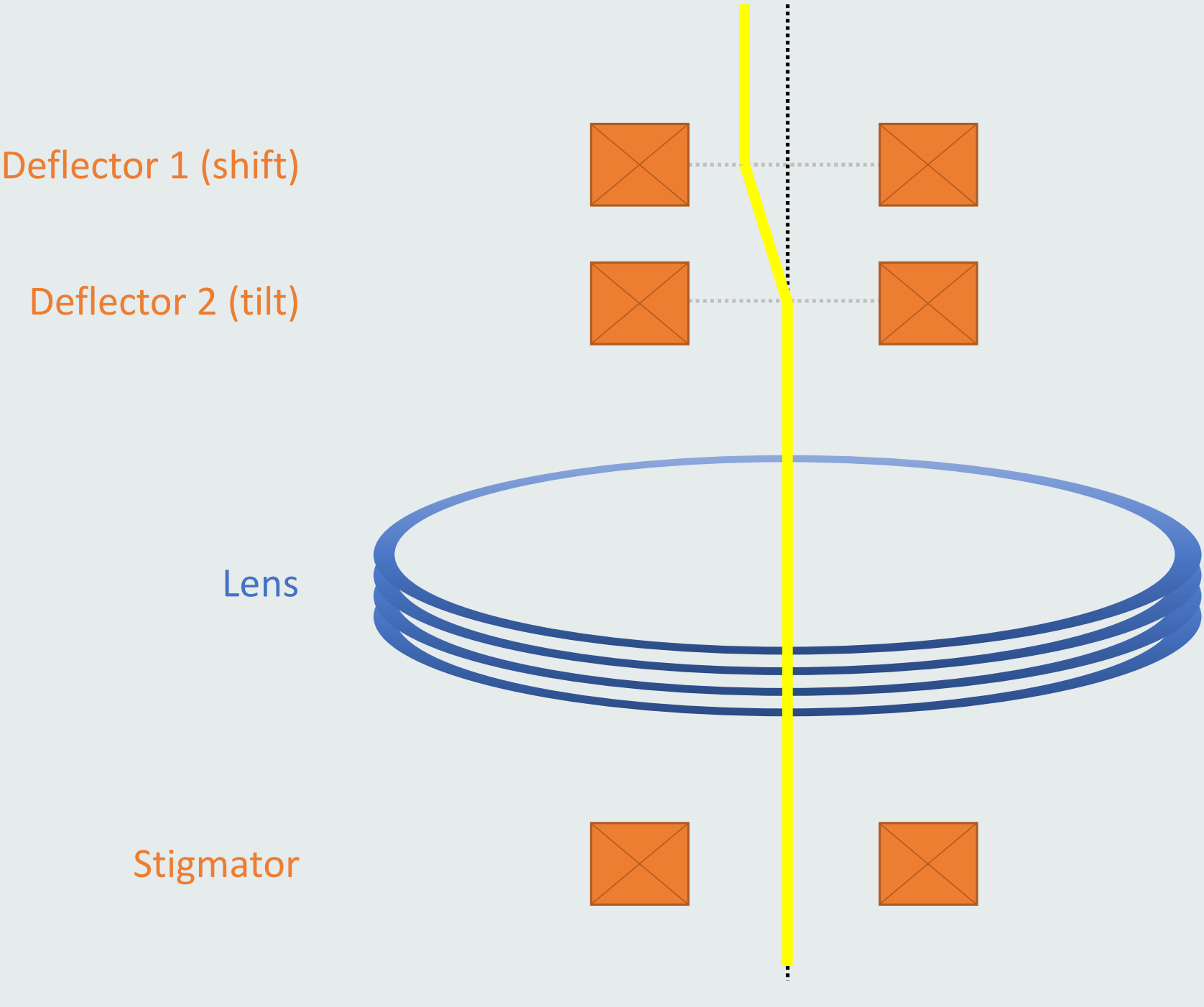


# LENSSES

What types of lenses do we have?



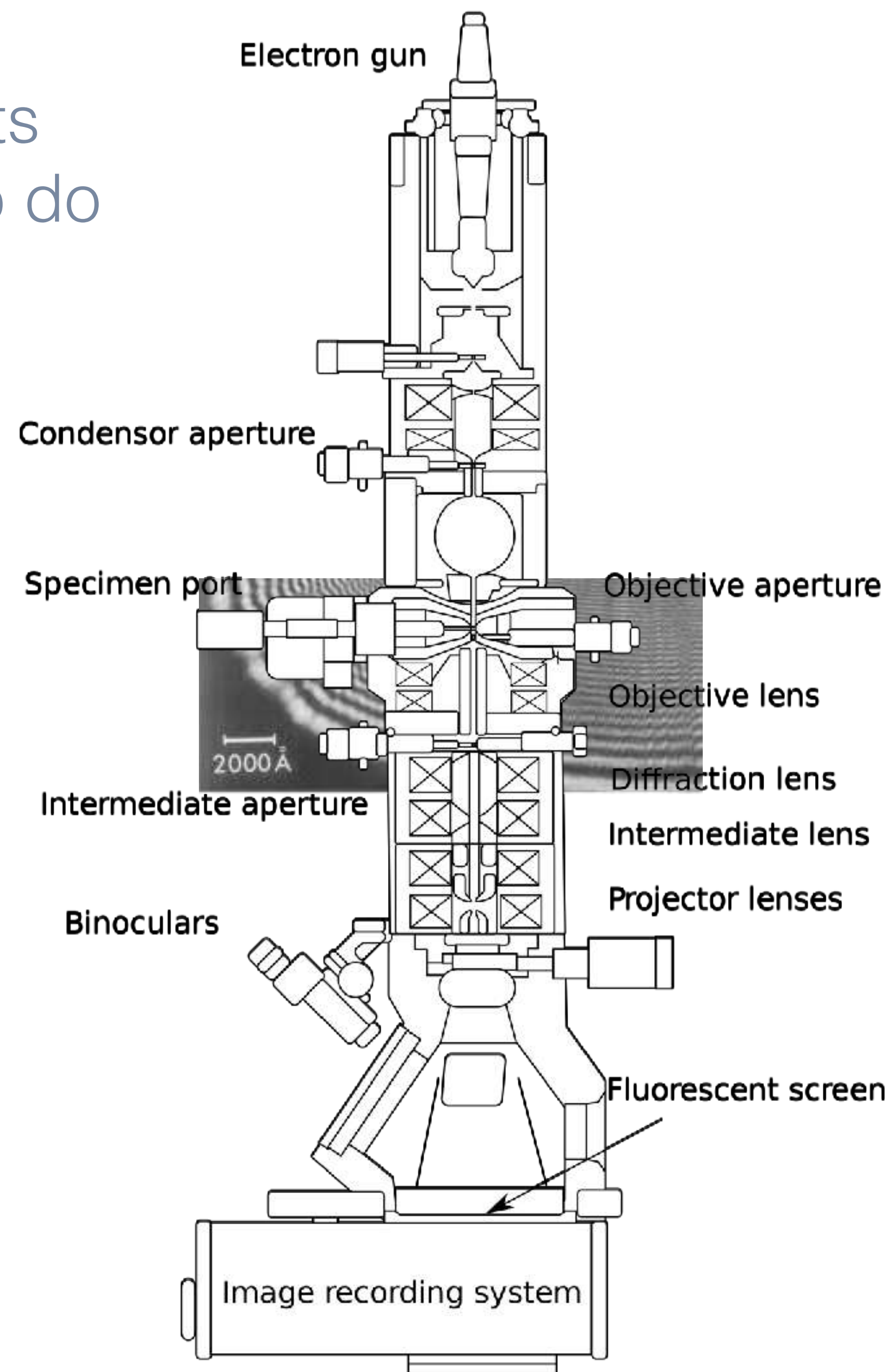
- Focus
- Magnify
- Rotate



# Q LENSES

## Microscope Alignments What to do & what not to do

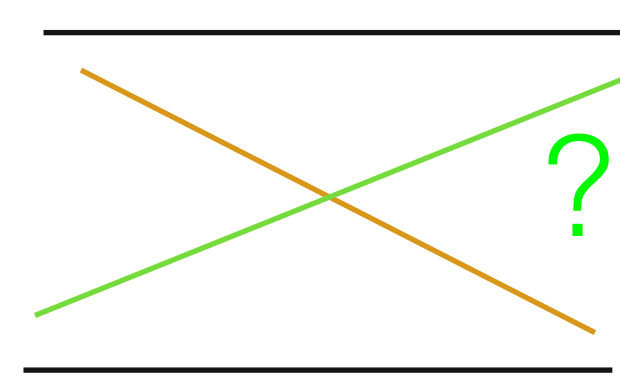
- Do:
  - Start at eucentric height and focus
  - Check if it is already good before attempt
  - Align from top to bottom
- Not to do:
  - ~~Align without a way to undo~~
  - ~~Align when TEM is not stable (i.e., temperature)~~



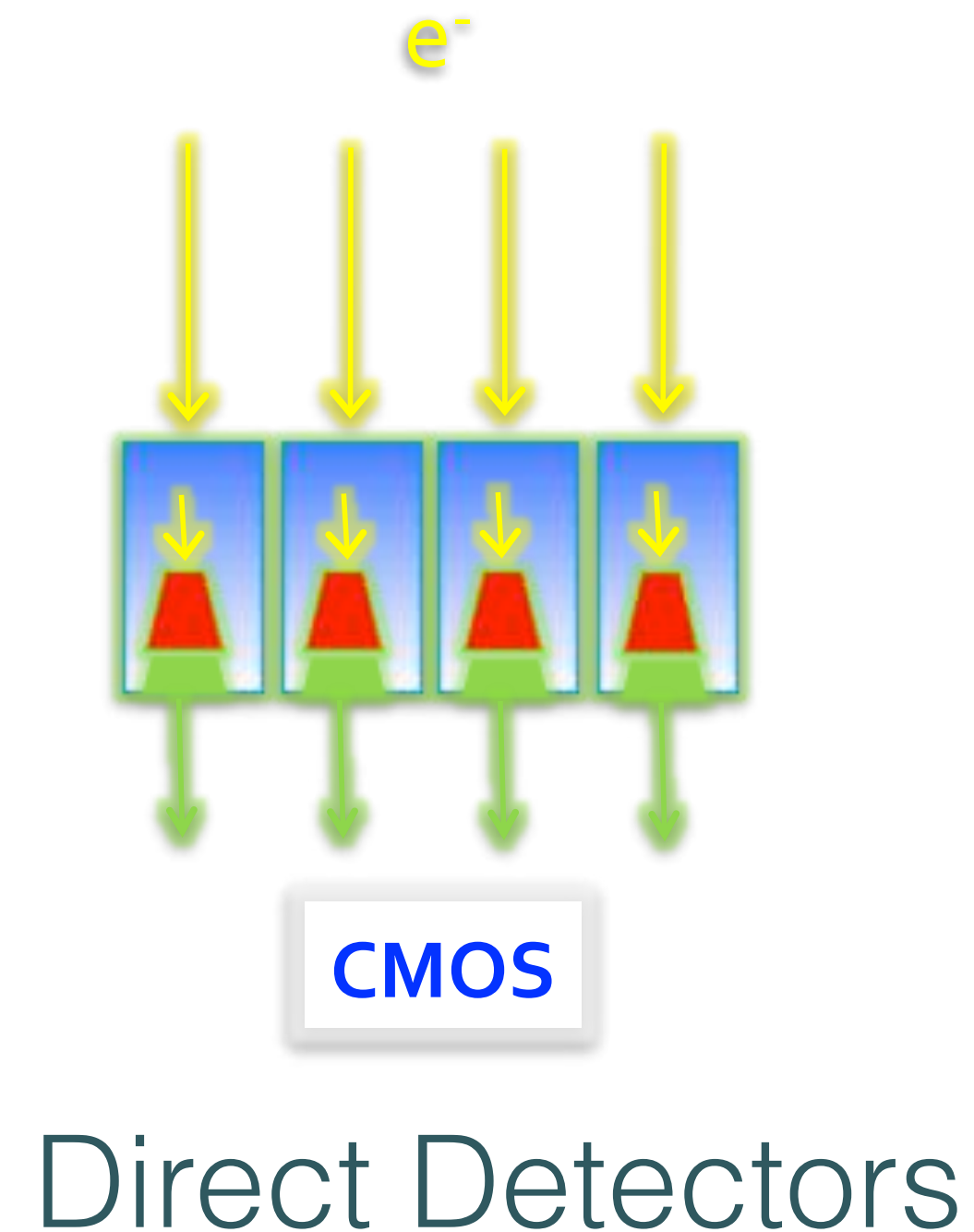
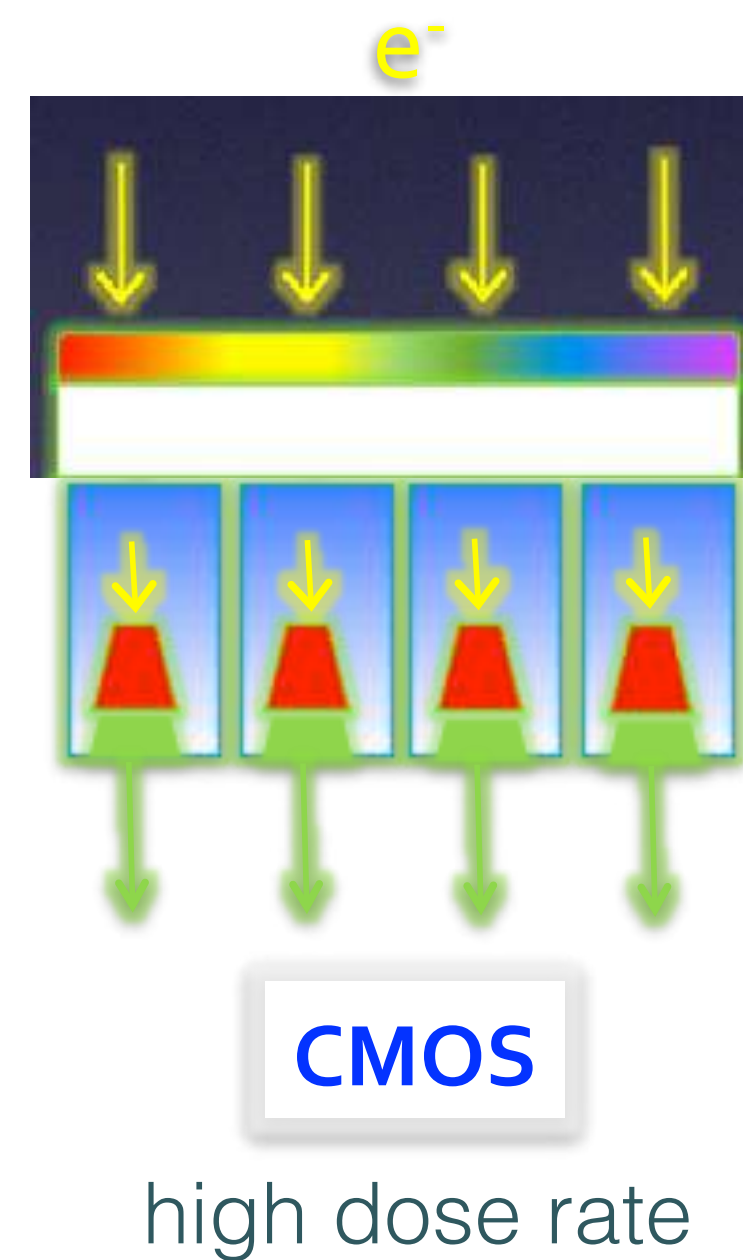
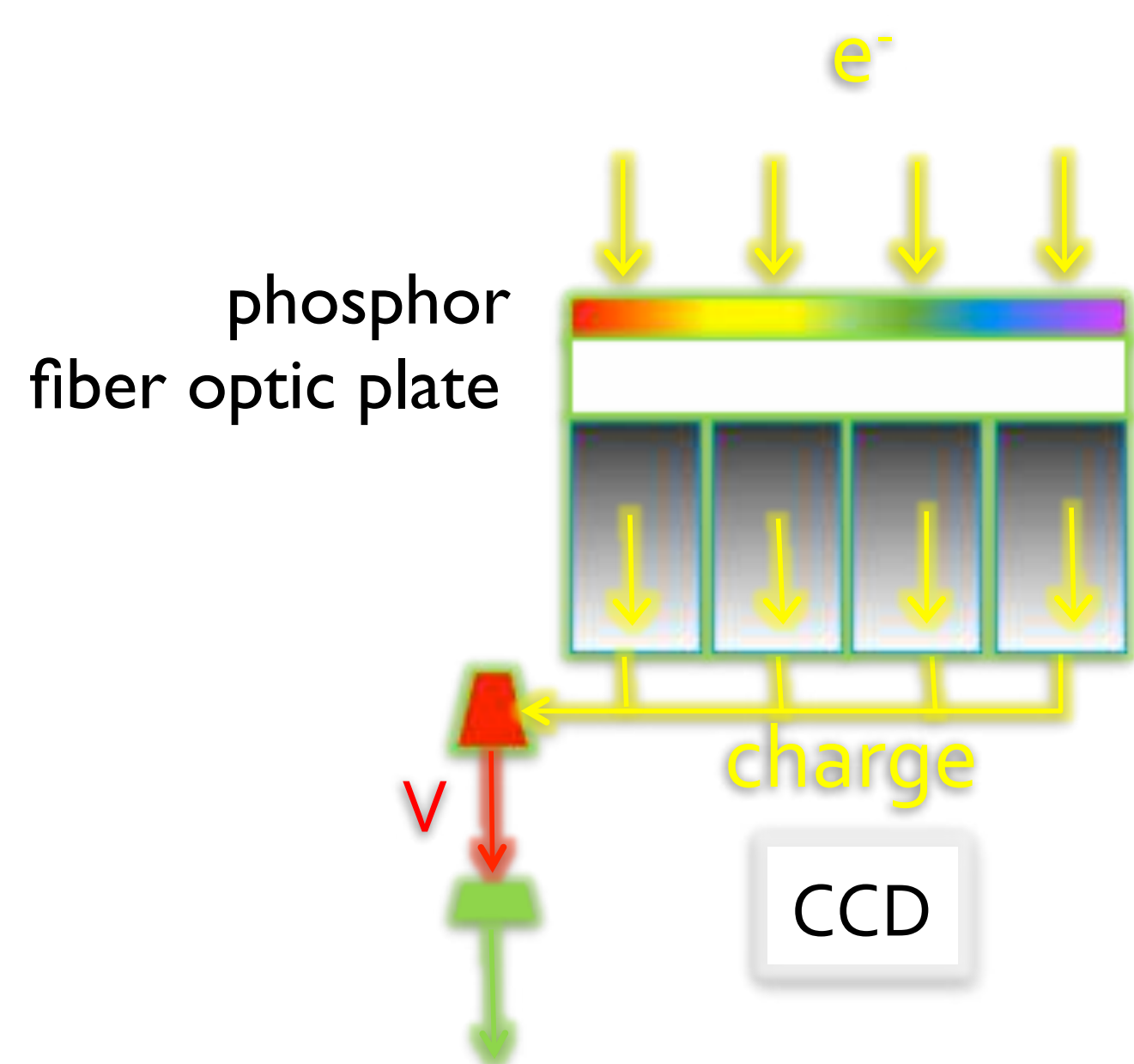
# DETECTORS

## Digital Cameras for TEM

- Photon converted
- Direct sensing



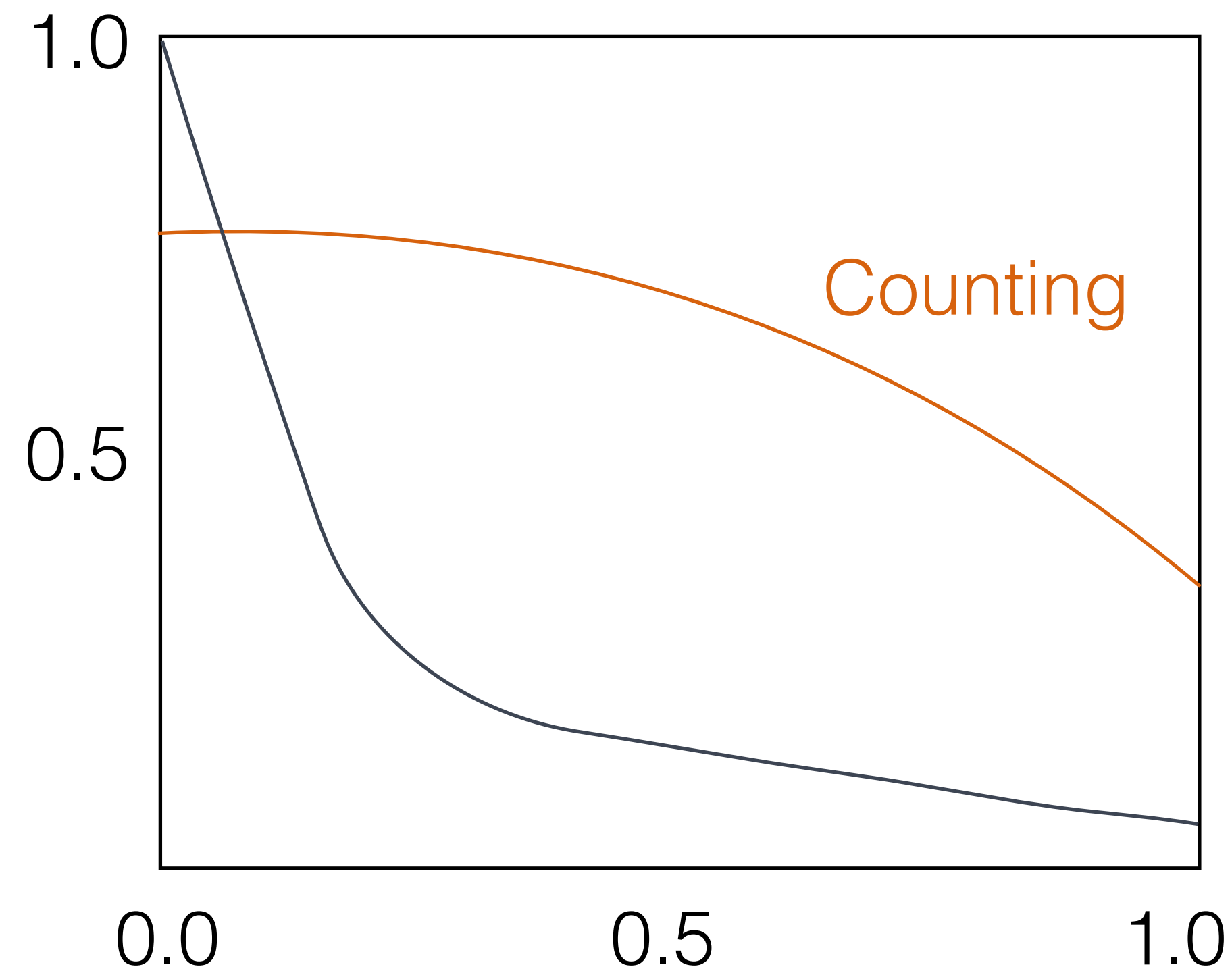
- **CCD** Charge Coupled Device
- **CMOS** Complementary Metal Oxide Semiconductor



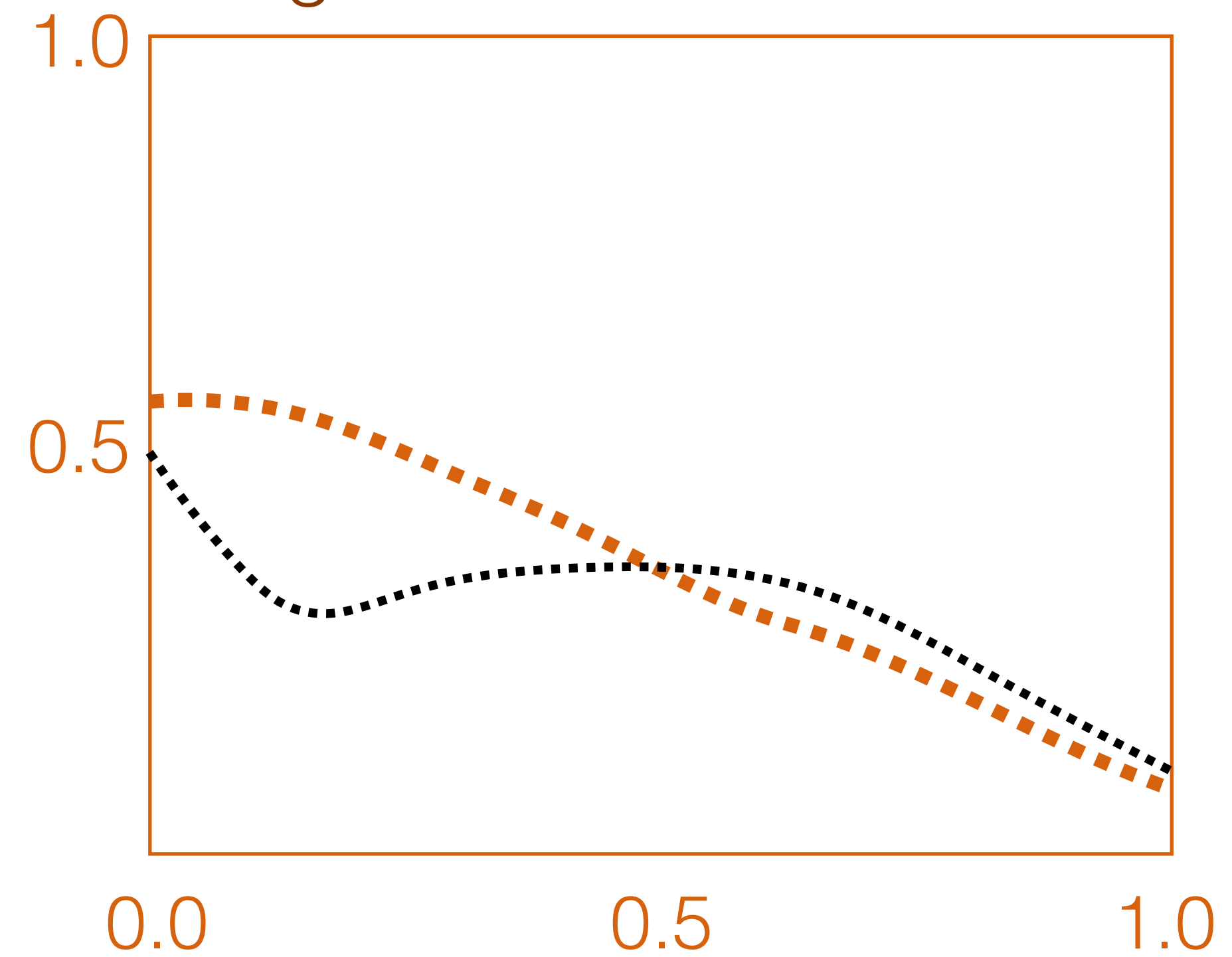
# DETECTORS

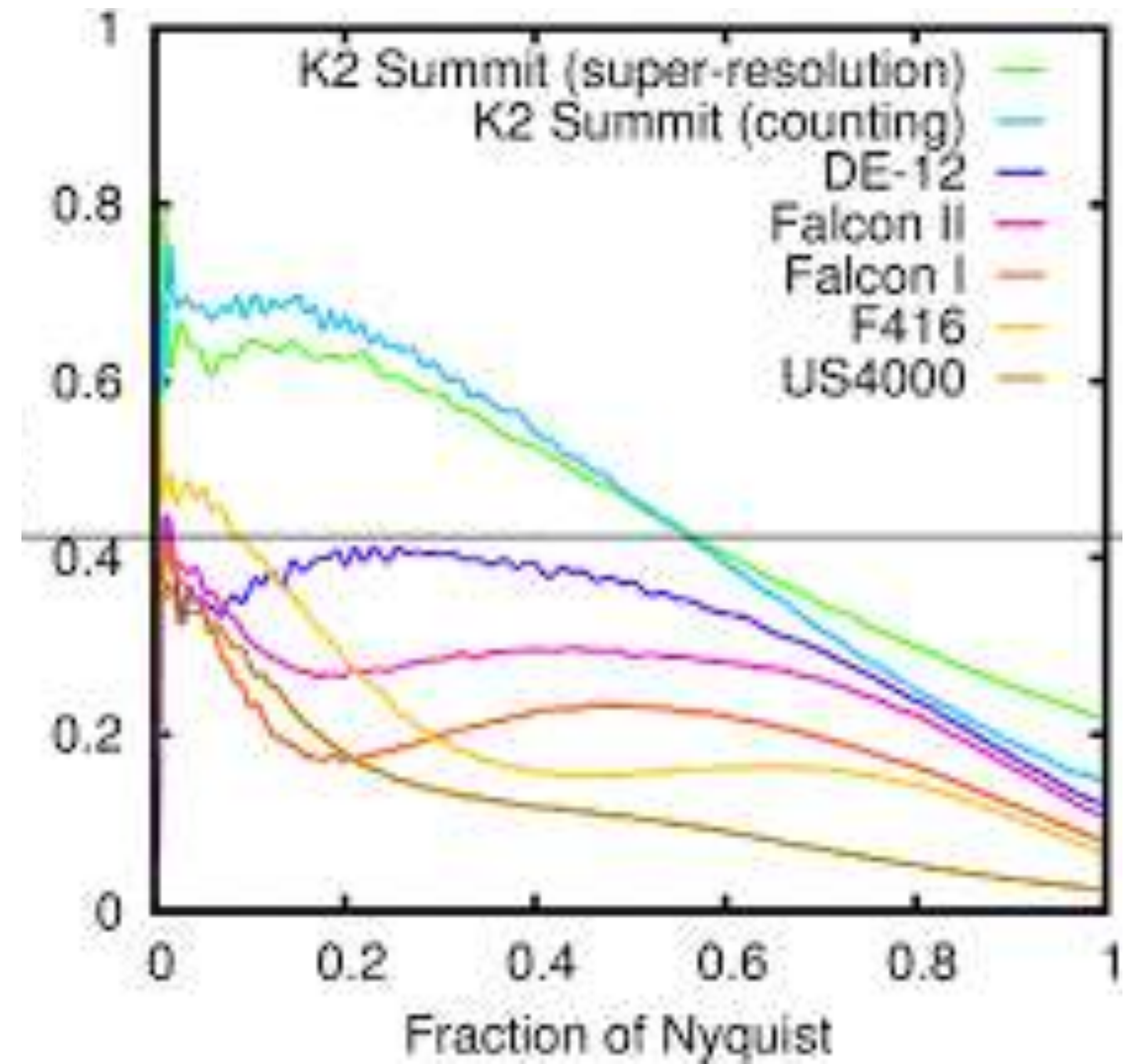
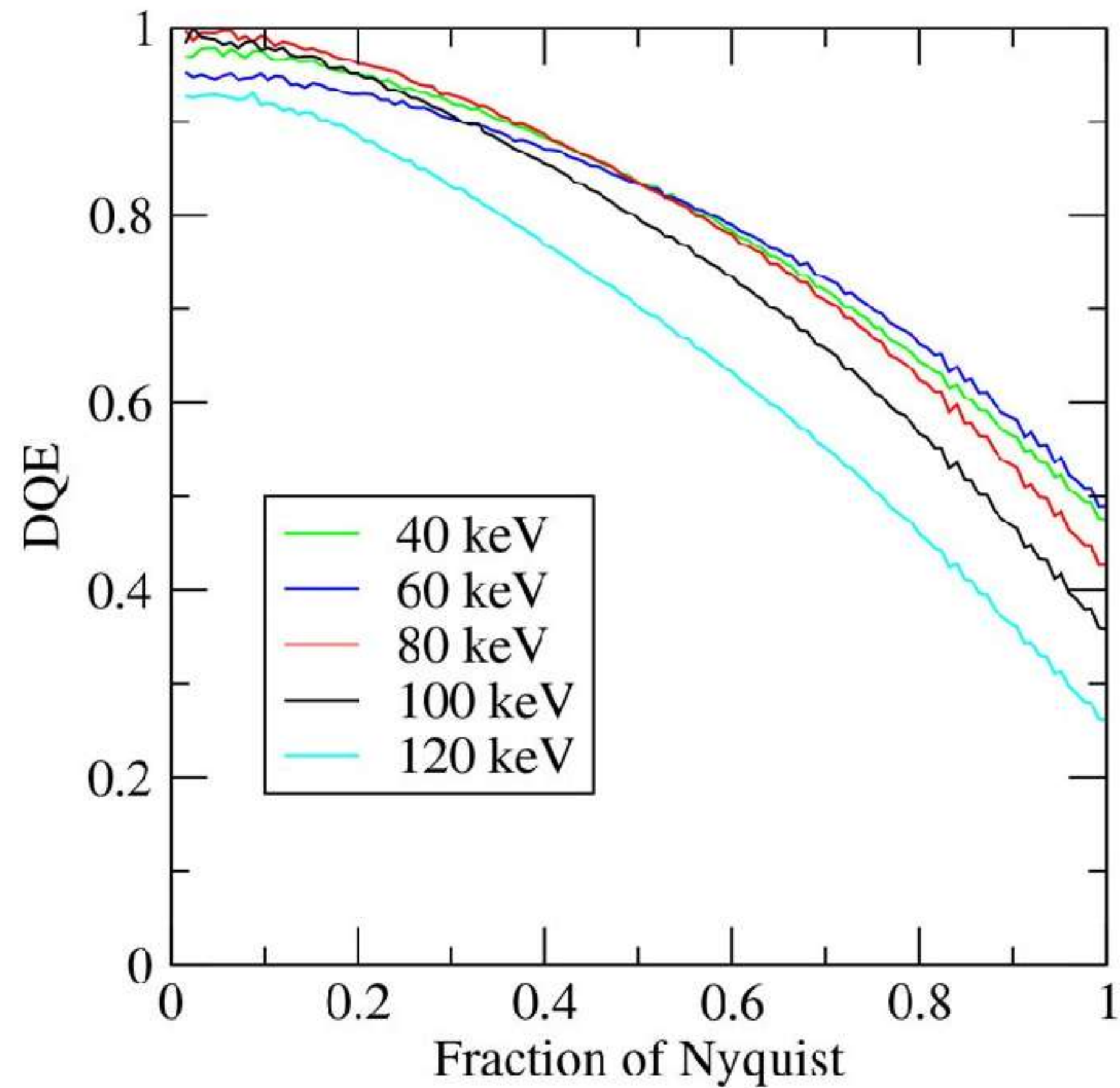
## Detector Performance Characterization

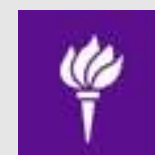
- MTF (Modulation Transfer Transform)
- contribute to signal envelope



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







Department of Health



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**Krios1**



**Krios2**



**Krios3**



**Krios4**



**Krios5**



**Krios6**



**Krios7  
2020**



**Tecnai F20**



**Tecnai12**



**JEOL1230**



**Helios650**



**Hitachi 7800**



**Glacios**



**Chameleon**

