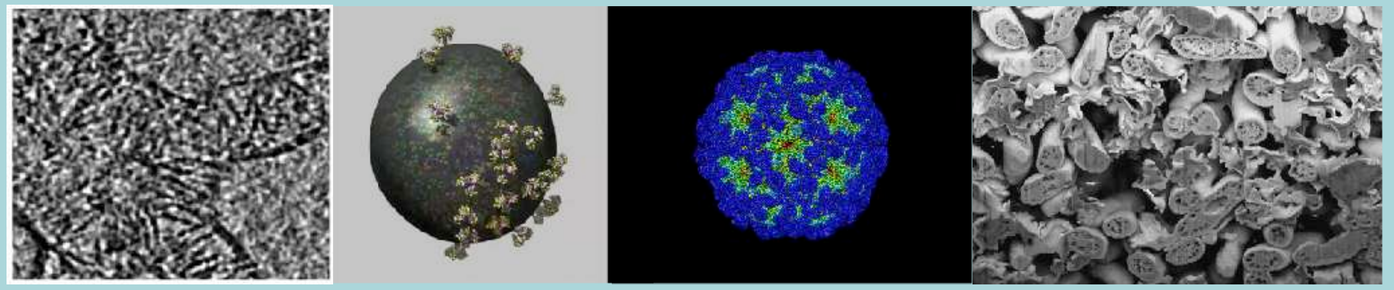
Microscopes and tools of the trade



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

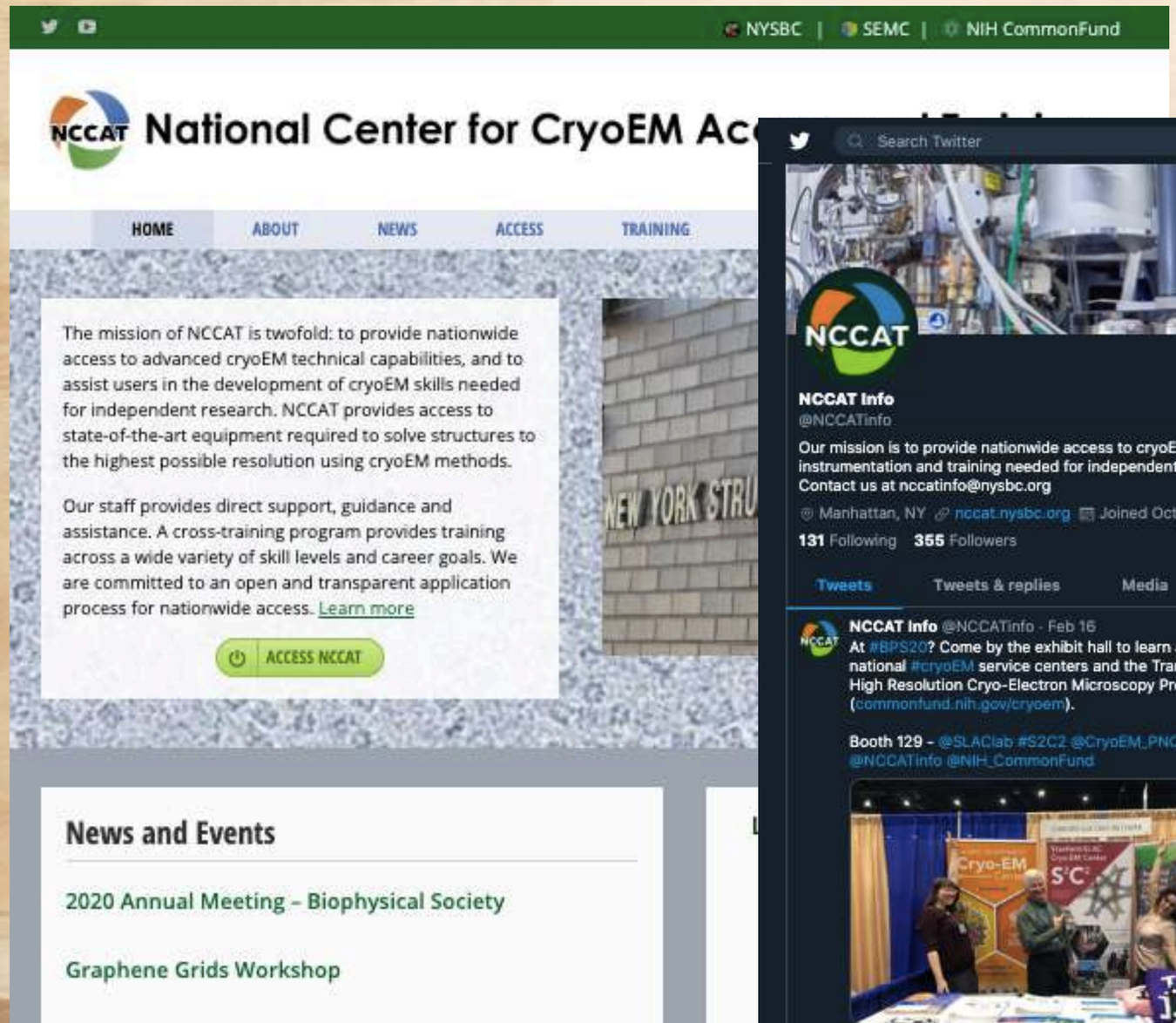
DAY 3: WEDNESDAY, MARCH 4, 2020		
09:00 – 09:30	**Registration/Information desk** – SEMC conference room	
09:45 – 10:35	**Lecture 4** – NYSBC conference room *Algorithms and foundational math PartI:* *derivation/explanation of the CTF, FTs.*	
10:35 – 11:30	*Algorithms and foundational math PartII:* *reconstruction, classification, maximum likelihood.* Fred Sigworth (Yale University)	
11:30 – 11:45	**Break**	
11:45 – 12:30	**Lecture 5** – NYSBC conference room *Data Analysis and reconstruction workflow* 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

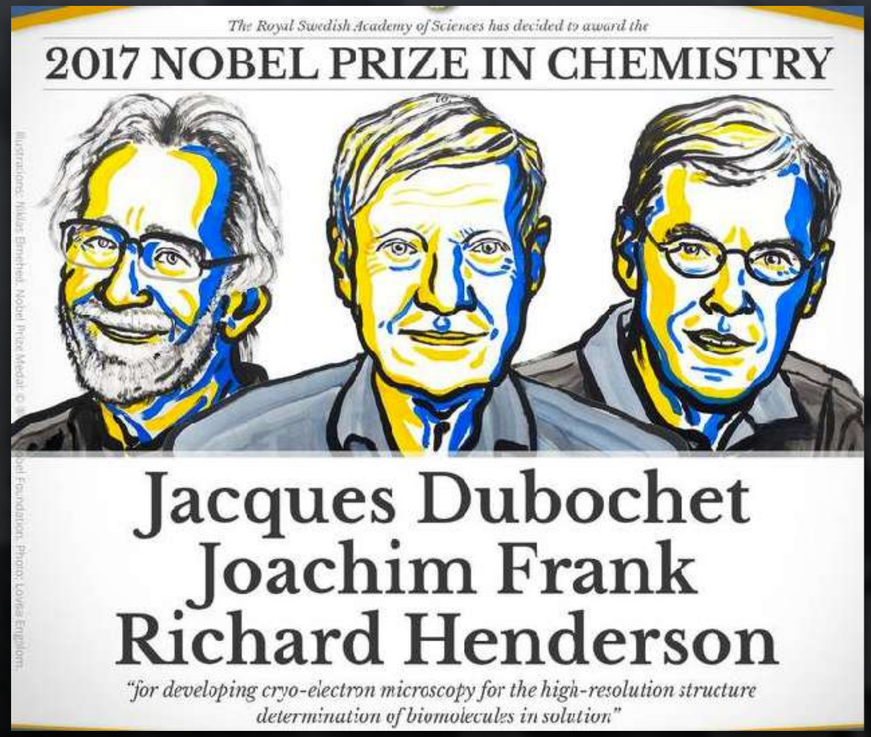
Course logistics



CRYOEM: TECHNOLOGY ON THE RISE

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

Chemistry Nobel prize 2017

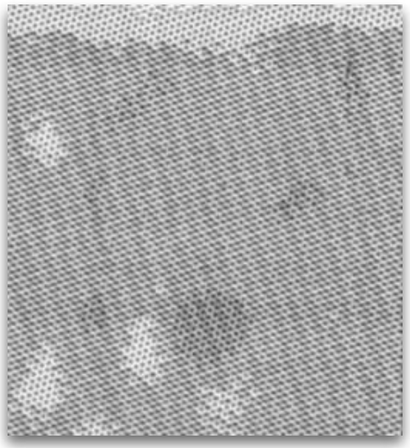


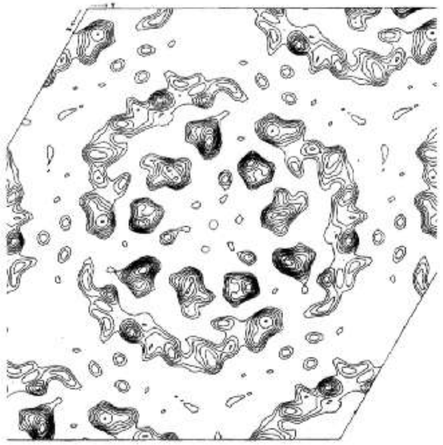
microED
Science breakthrough of the year runner-up 2018

Ruska and Knoll in Berlin in the early 1930s

CRYOEM: TECHNOLOGY ON THE RISE

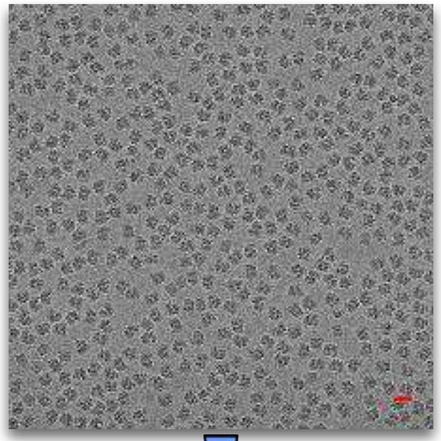
1986

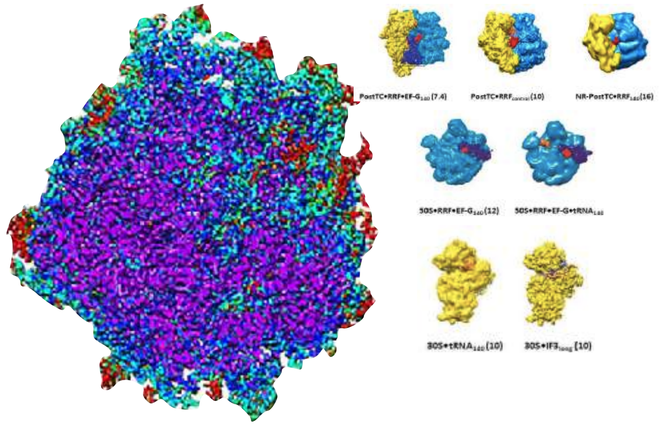




Henderson et al. (1986)

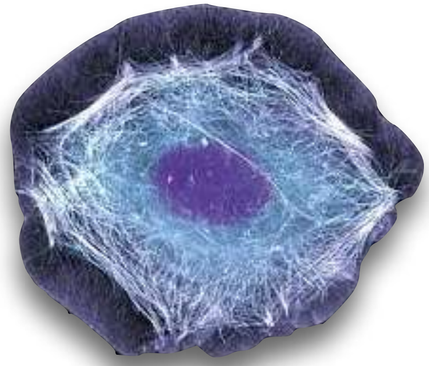
2017

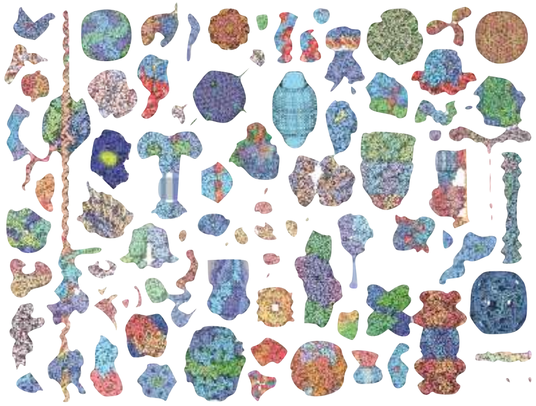




Frank et al. (2017)

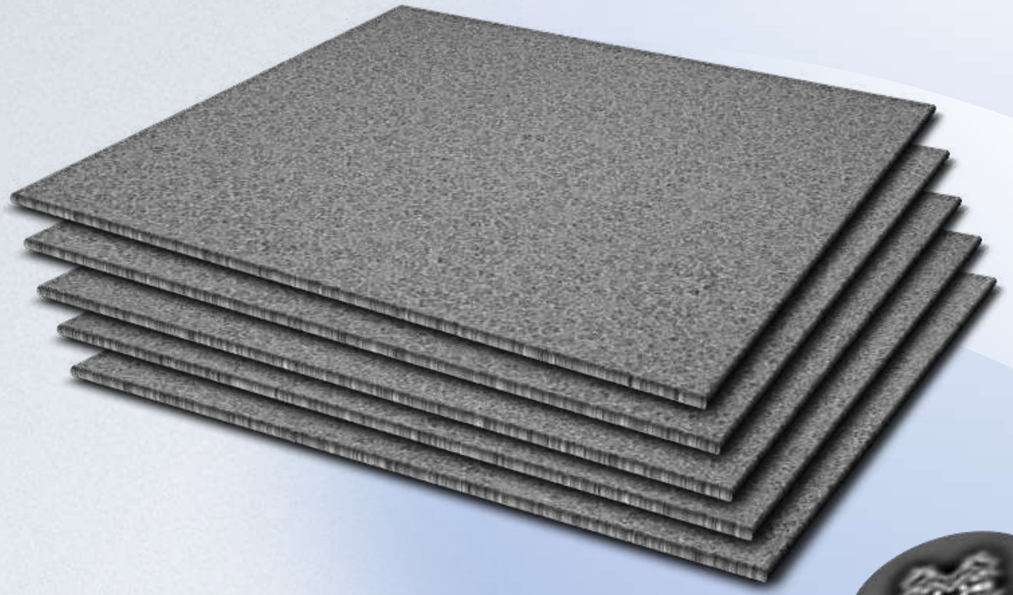
????

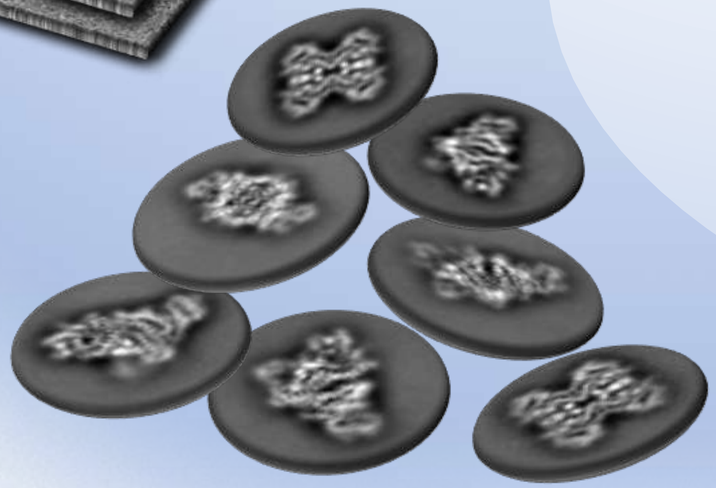


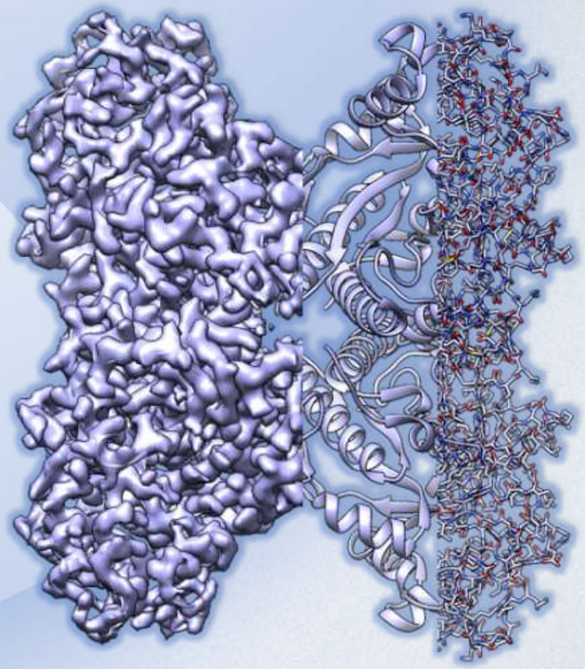


TBD (20??)

WHAT IS POSSIBLE TODAY?





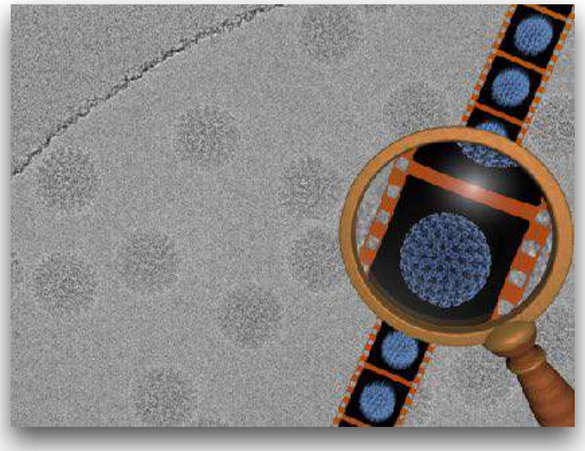


WHAT BROUGHT ABOUT THE RESOLUTION REVOLUTION? (~2012-2014)

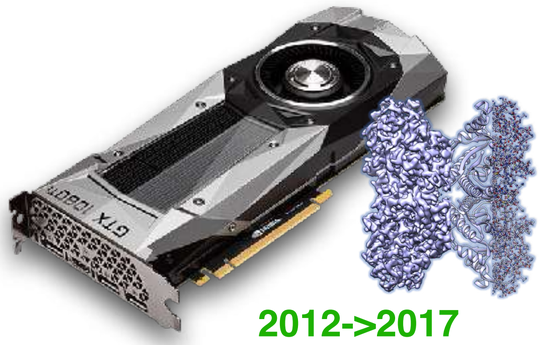
Hardware

Microscopes

Direct Detectors

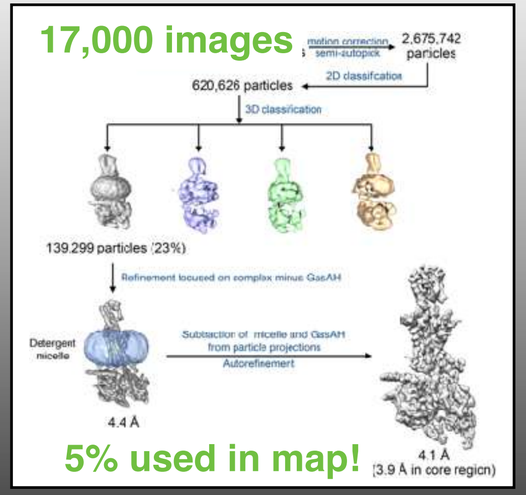


Computers



2012->2017
Cost reduced by 100x

Software

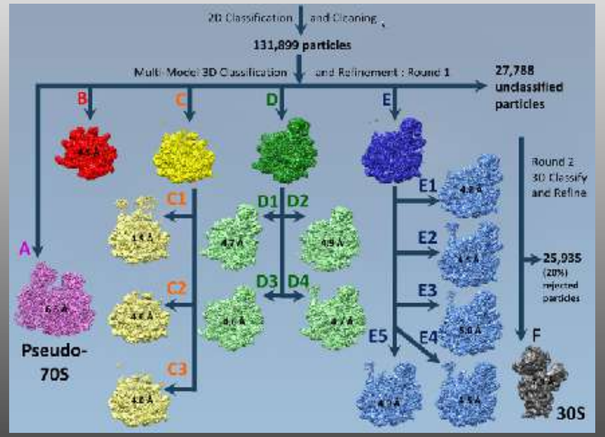


Leginon / SerialEM / EPU, …

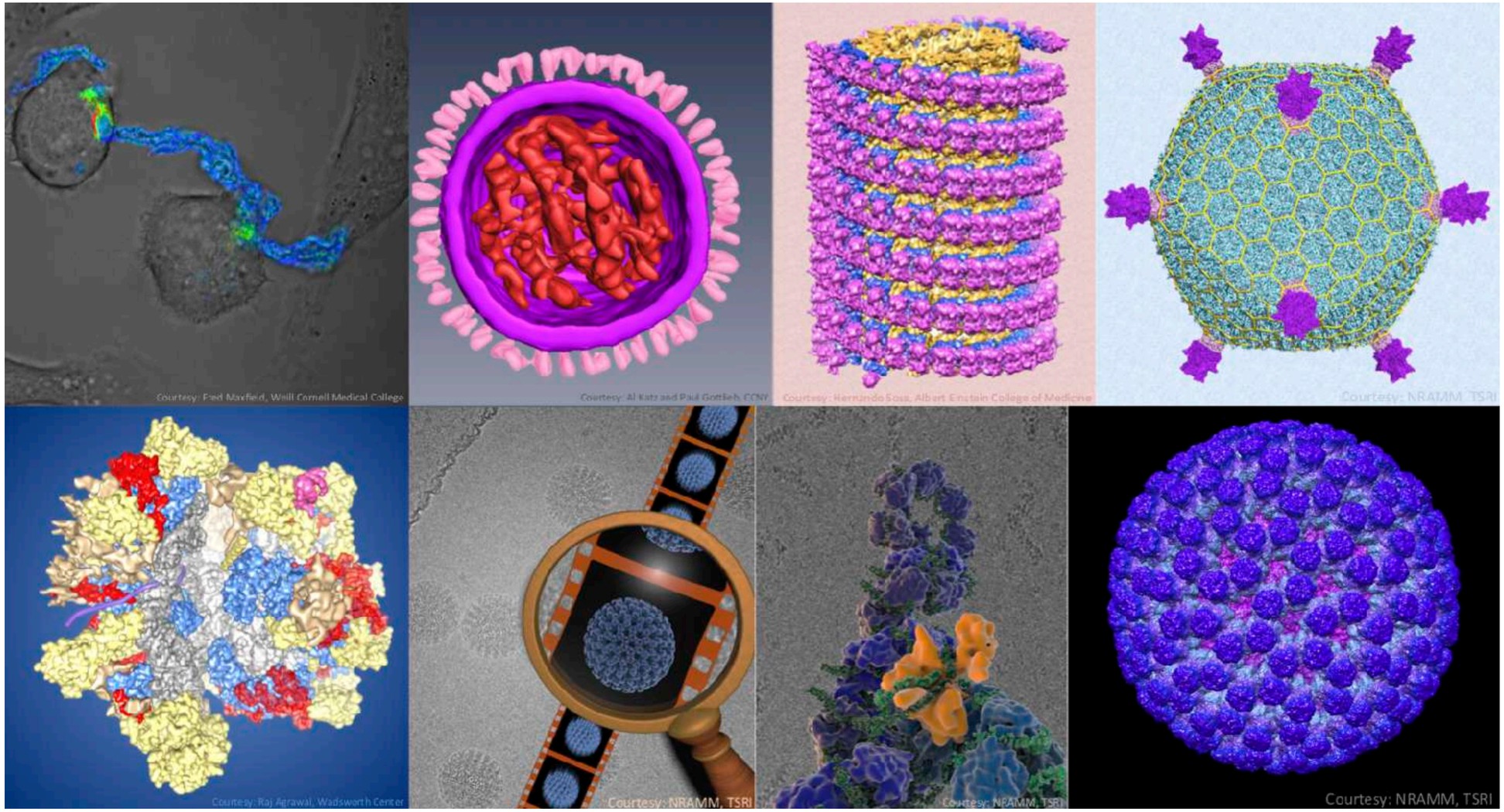
MotionCorr2, Unblur, …

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

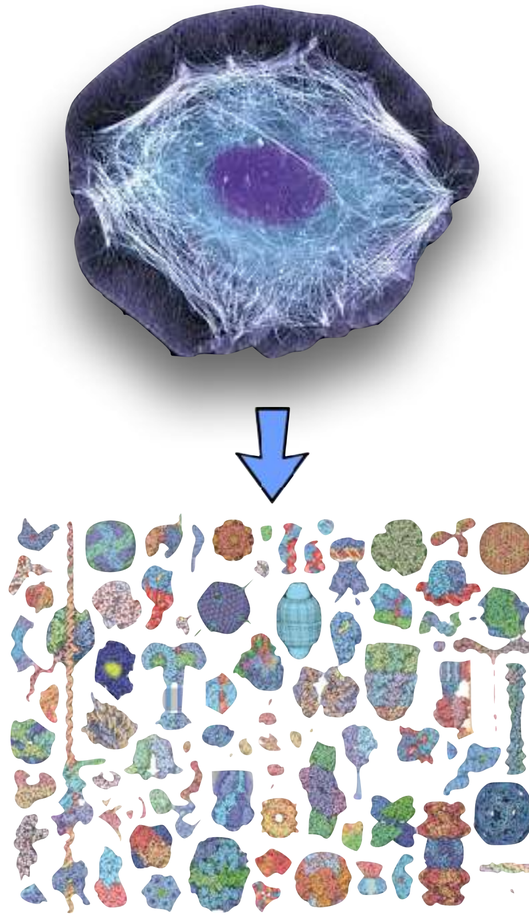
14 independent structures



CRYOEM: TECHNOLOGY ON THE RISE



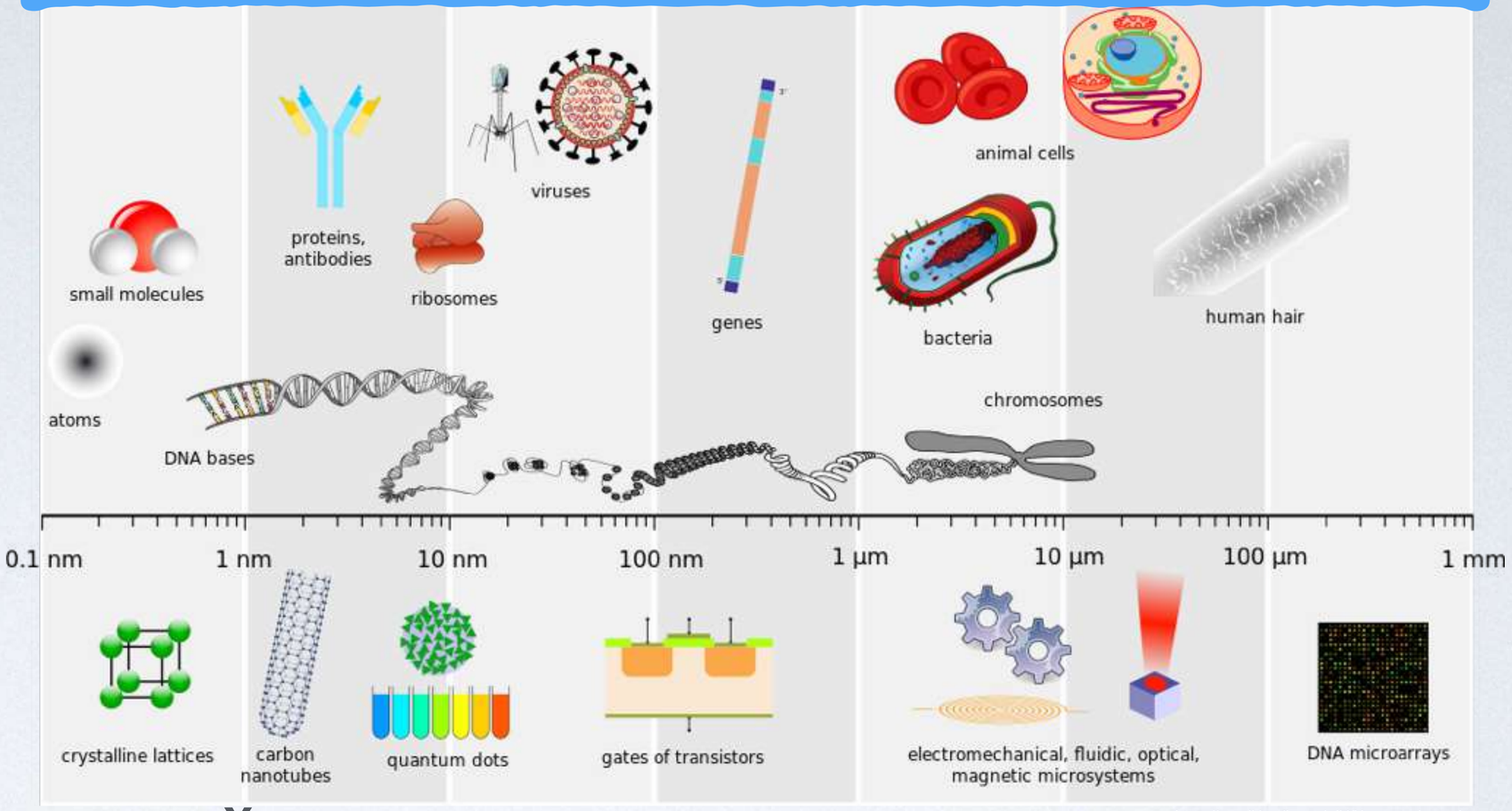
????



TBD (20??)

CRYOEM: SCALE WITHIN BIOLOGY

Electron Microscopy



https://en.wikipedia.org/wiki/Nanoscopic_scale

X-ray

Naked eye

NMR

Light microscopy

AFM

CRYOEM: WHY ELECTRONS?

Pros

Small wavelength

Can be focused

Cons

Damages sample

worse with faster electrons

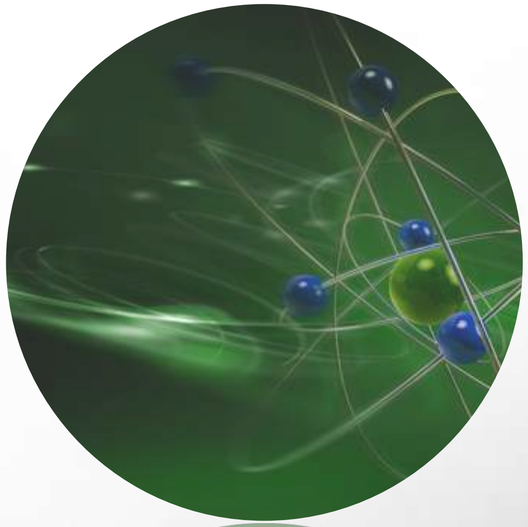
Poor penetration

better with faster electrons

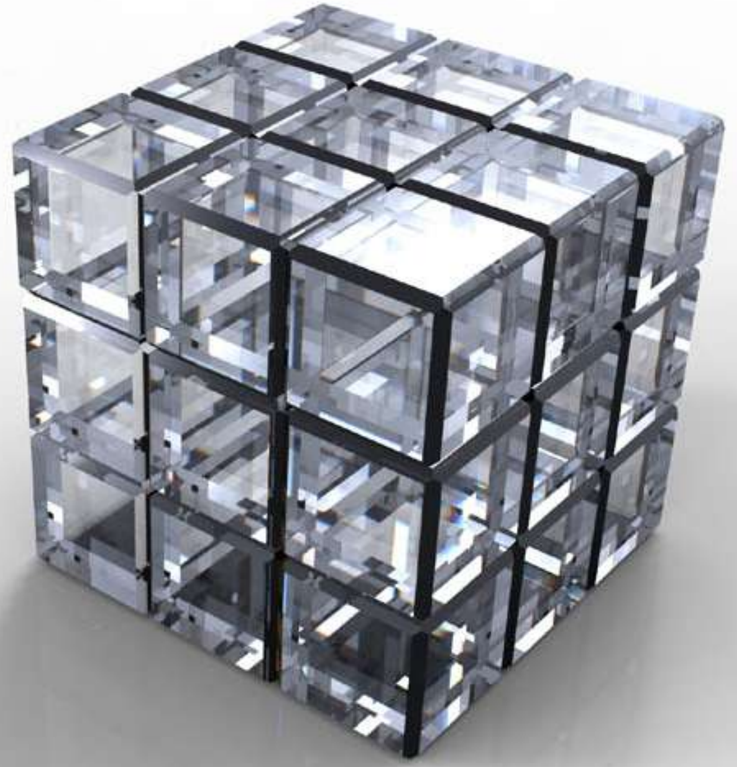
CRYOEM: MODALITIES | TOOLS

Tomography

Single-particle

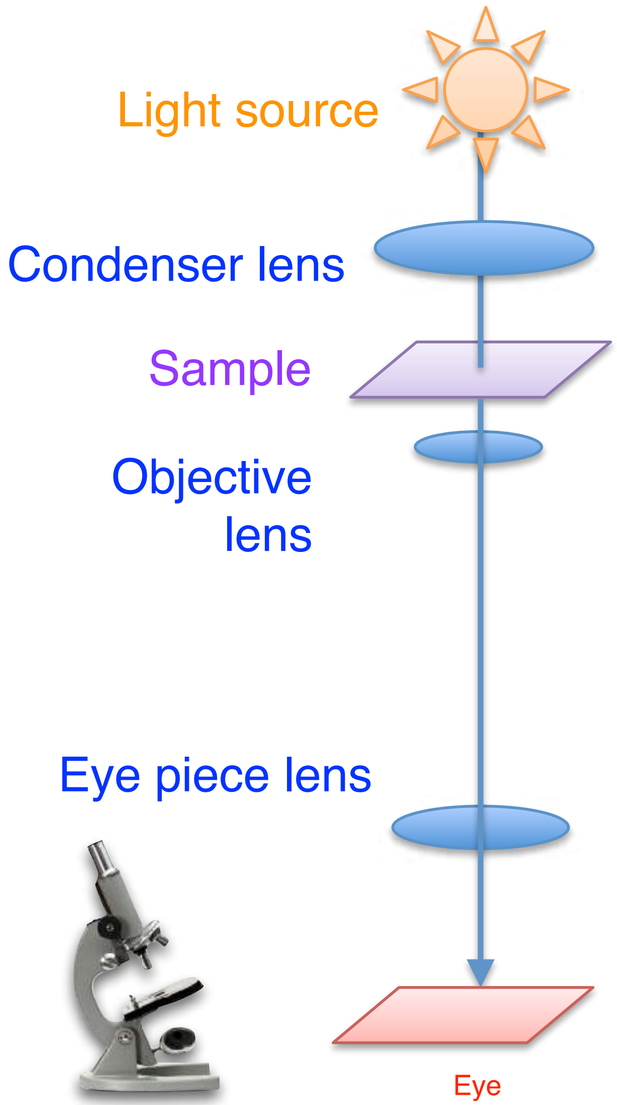


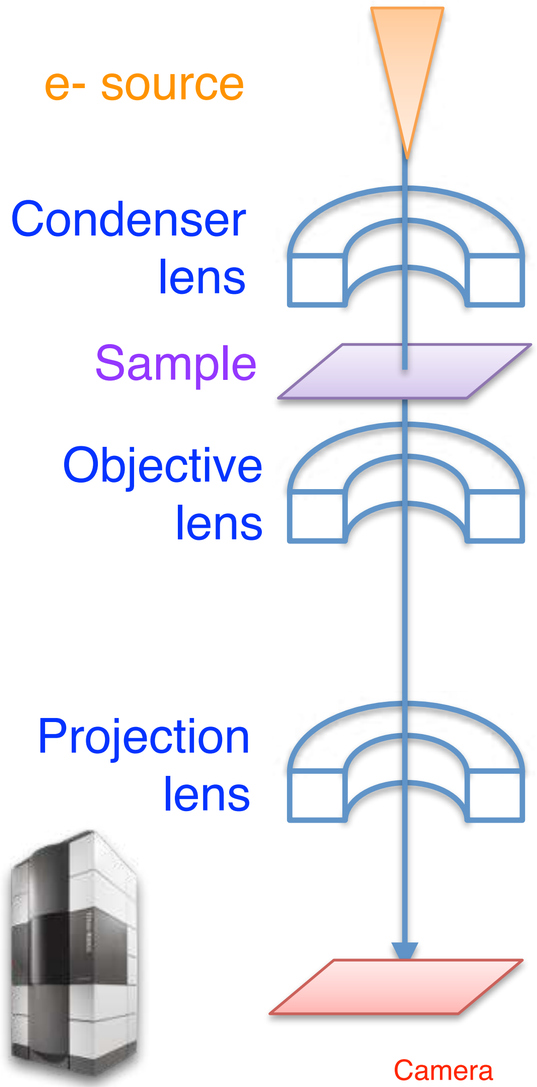
2D arrays

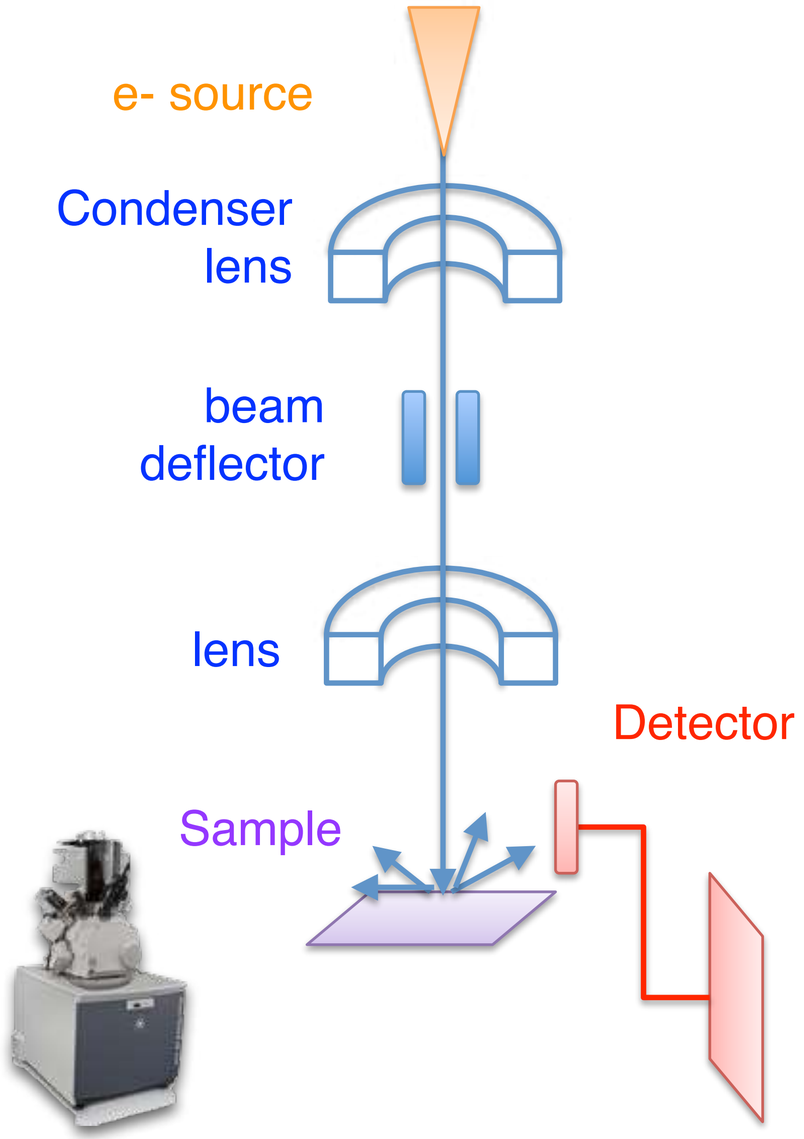


CRYOEM:

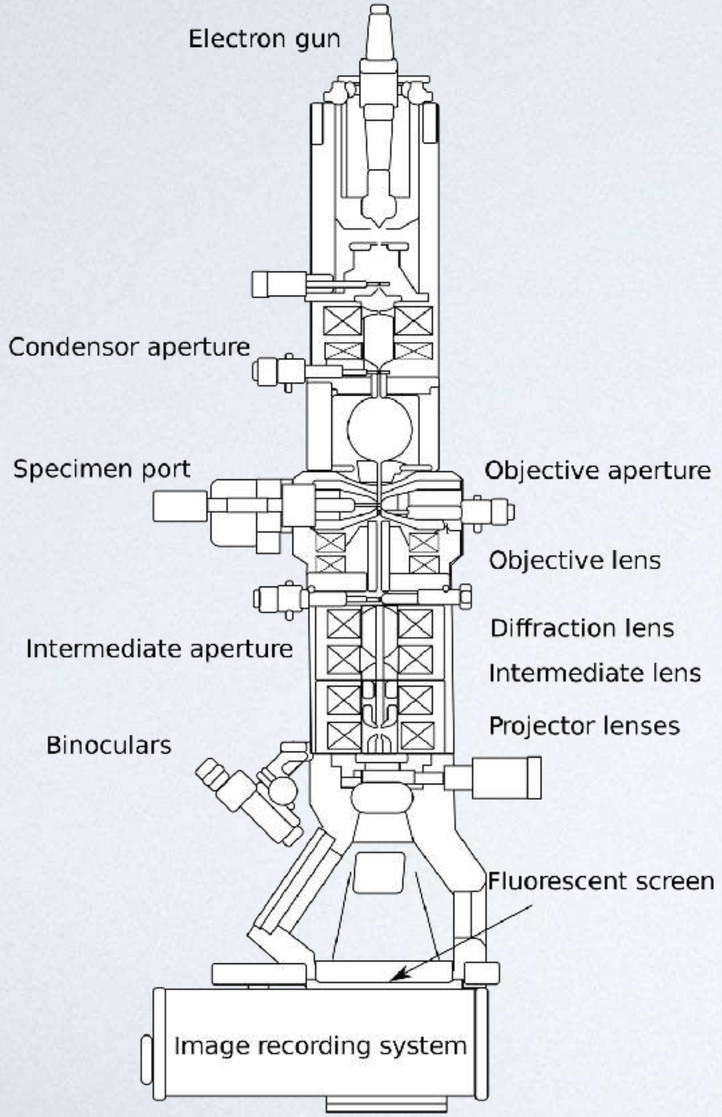
TOOLS







MAIN PARTS OF AN EM



Electron sources

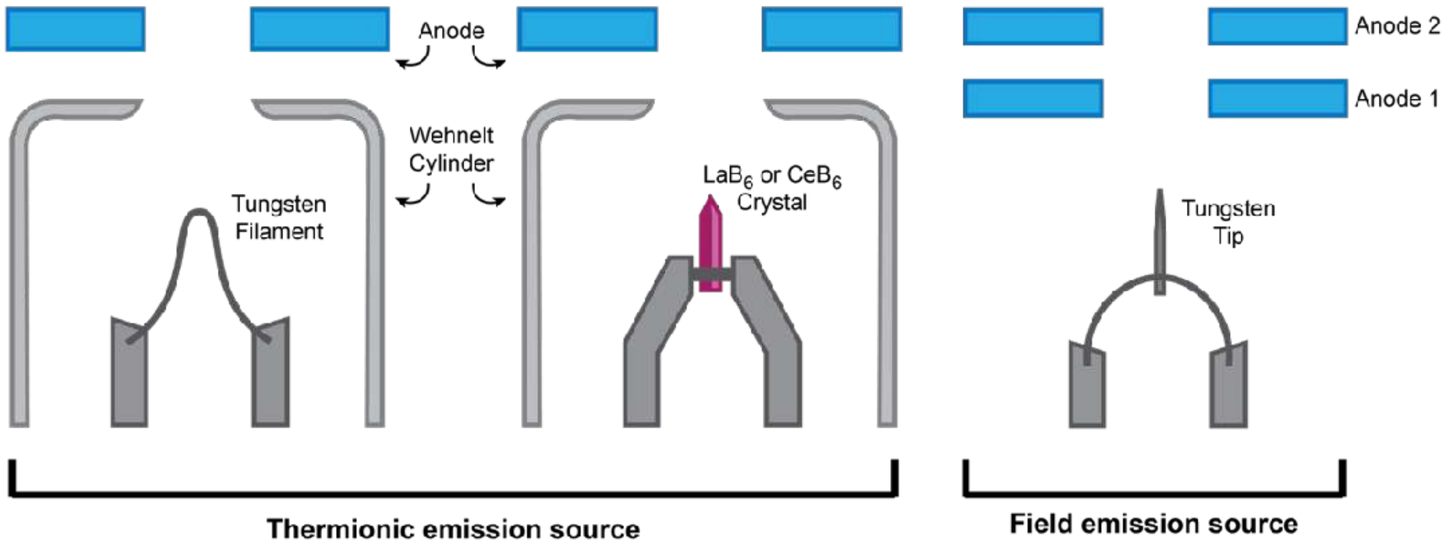
Vacuum systems

Lenses

Detectors

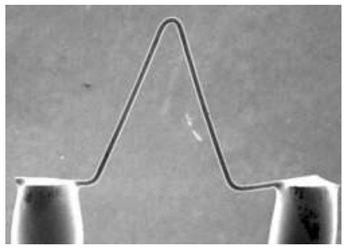
ELECTRON SOURCES

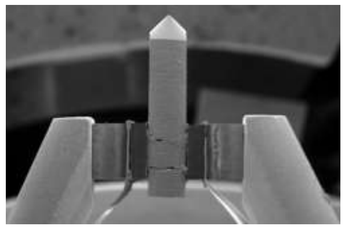
What are the 3 main kinds of electron sources?

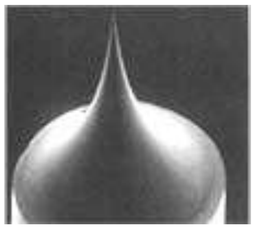


www.thermofisher.com

nanoscience.com

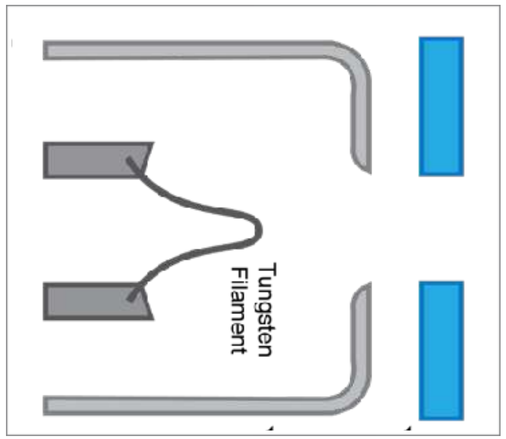


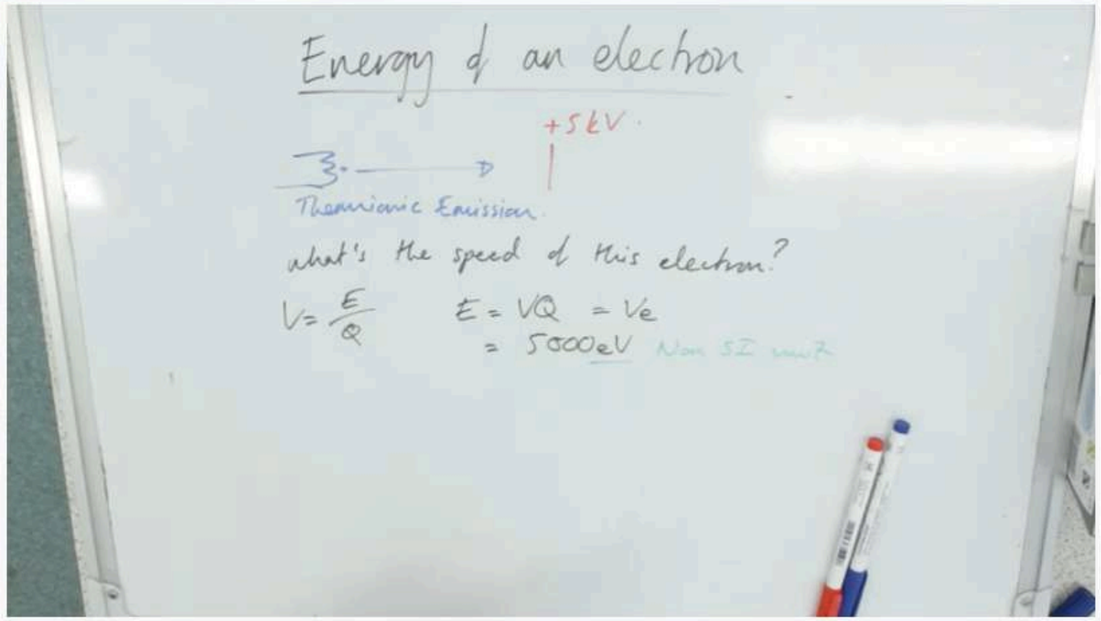




ELECTRON SOURCES

How fast are the electrons moving?

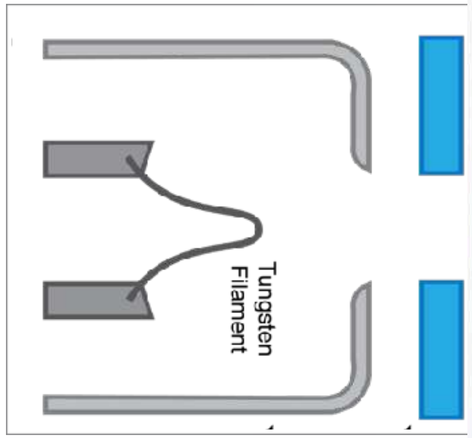


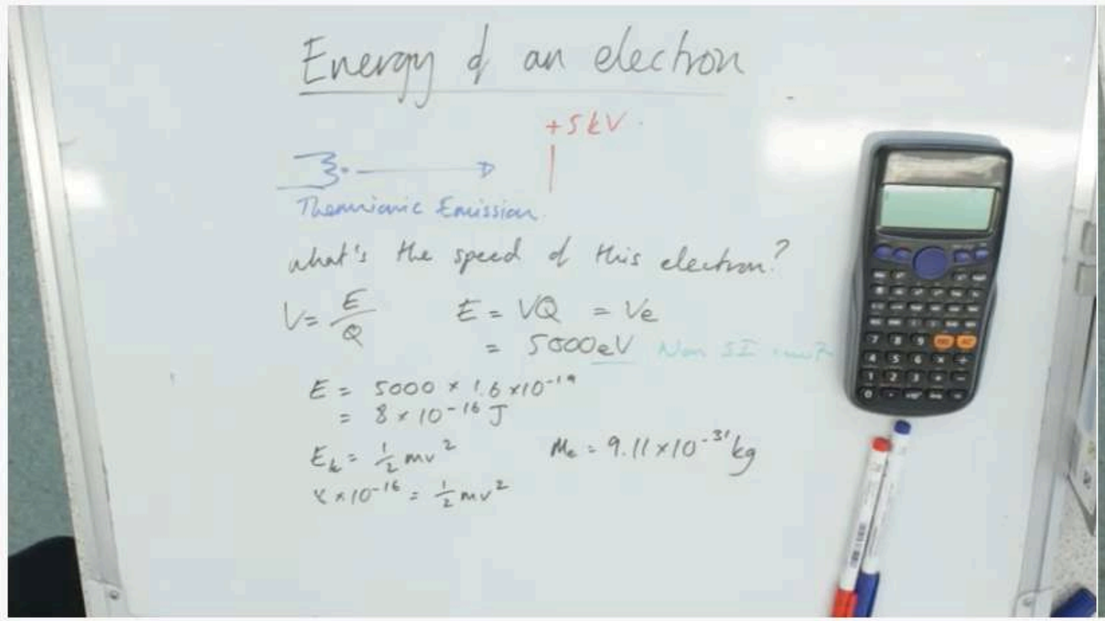


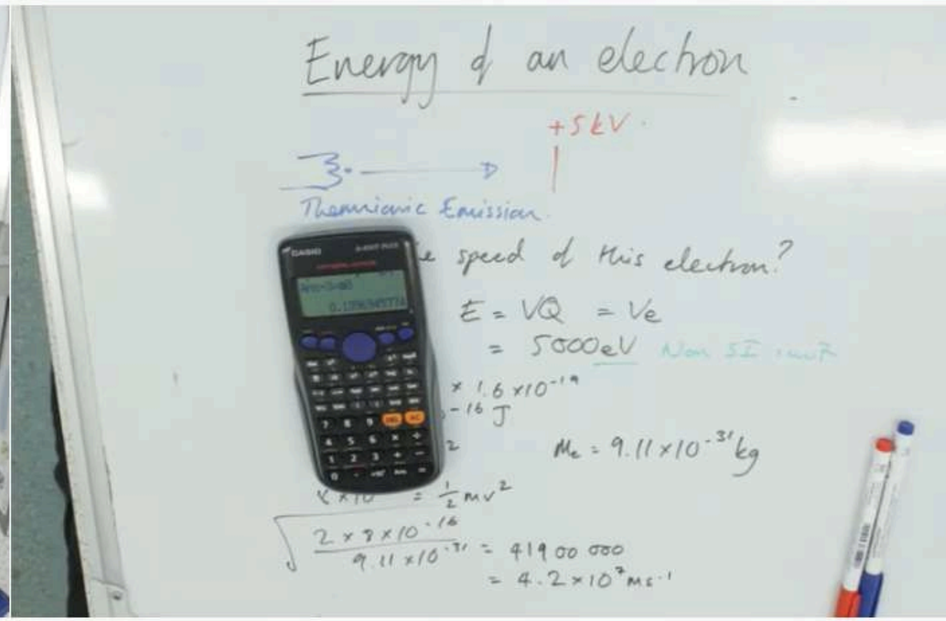
https://www.youtube.com/watch?v=tYCET6vYdYk

ELECTRON SOURCES

How fast are the electrons moving?

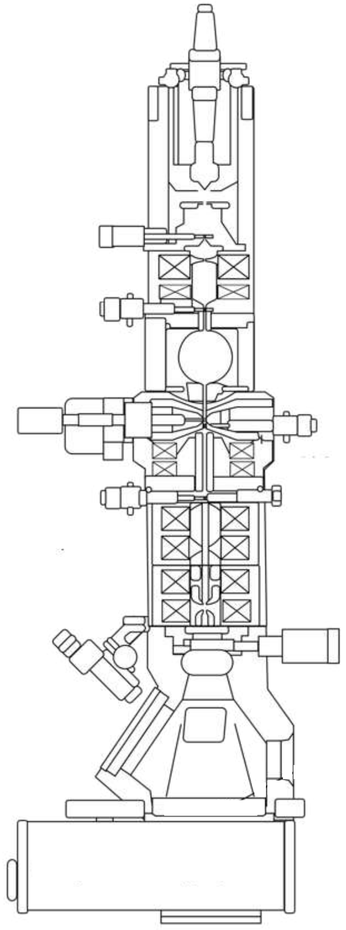


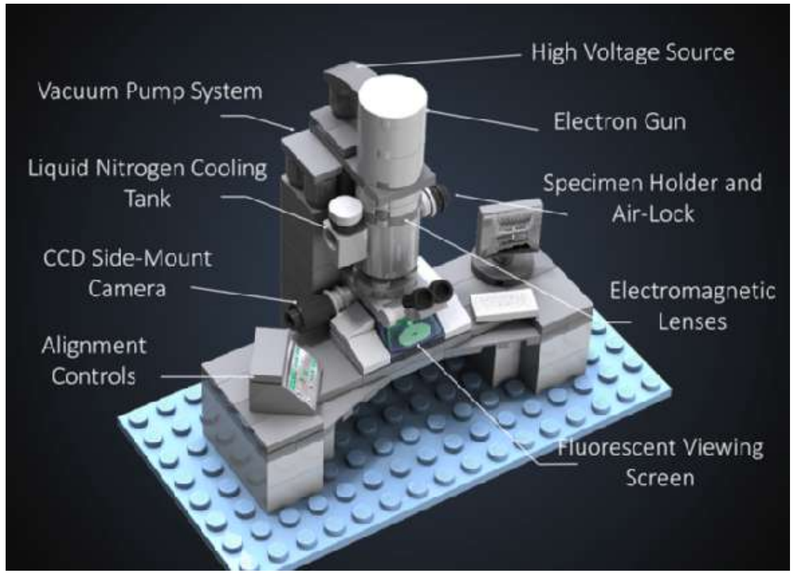




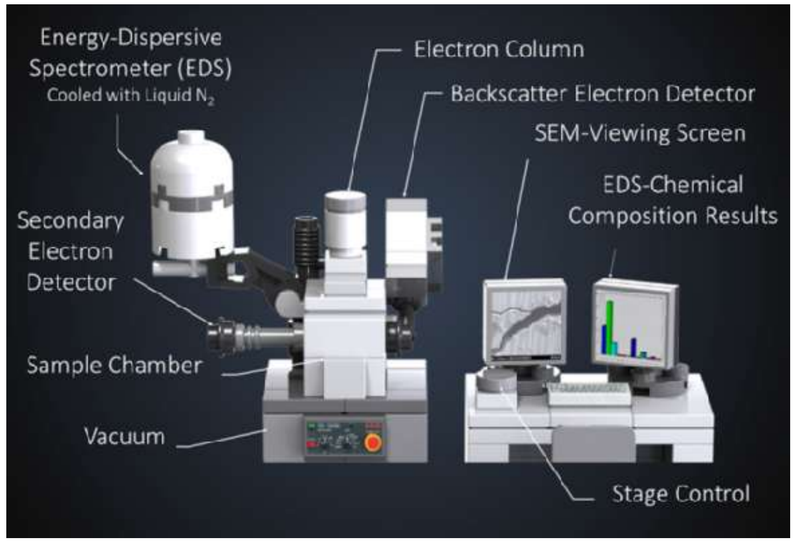
https://www.youtube.com/watch?v=tYCET6vYdYk

ELECTRON SOURCES & TYPES OF CRYOEMs

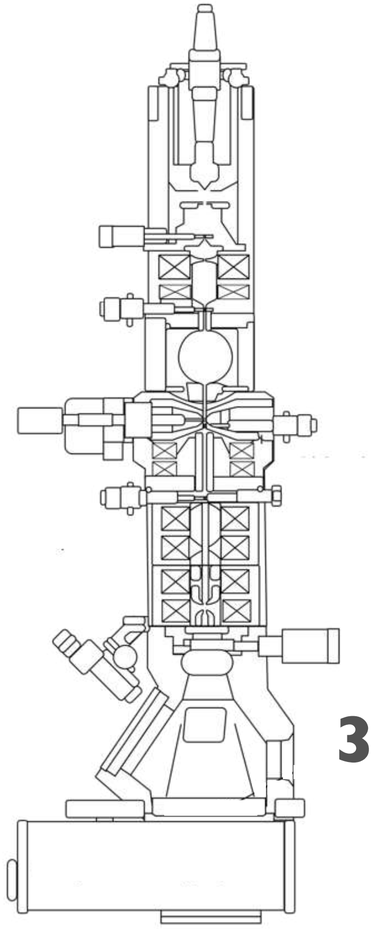




https://ideas.lego.com/projects/102281



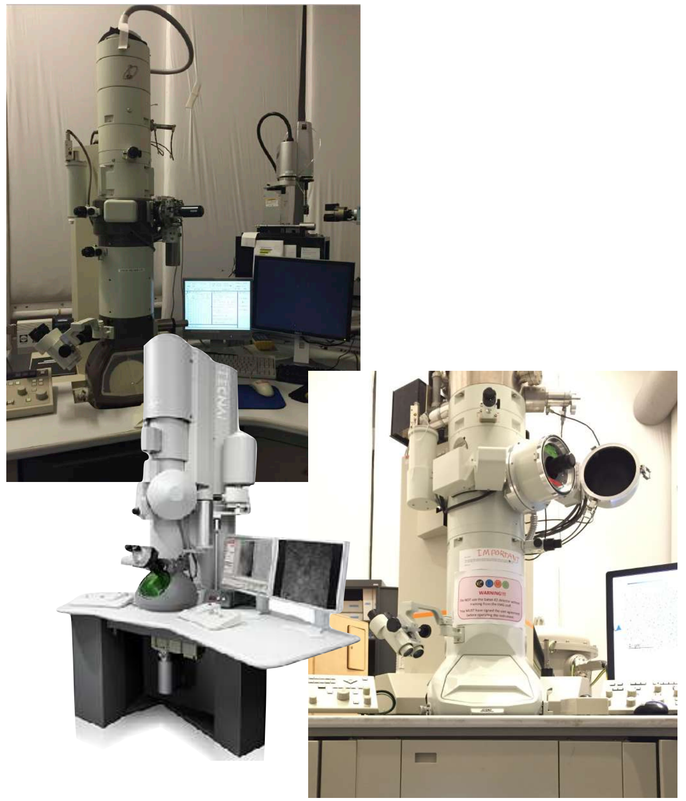
ELECTRON SOURCES & TYPES OF CRYOEM

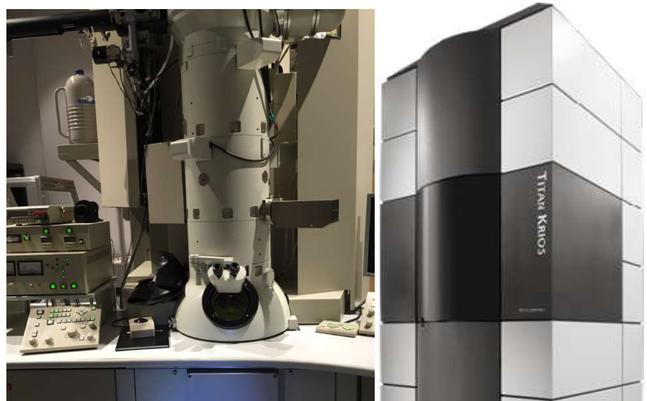


80-120 kV: JEM 1230; Tecnai T12
W or LaB6
High contrast & robust
sub-nm resolution

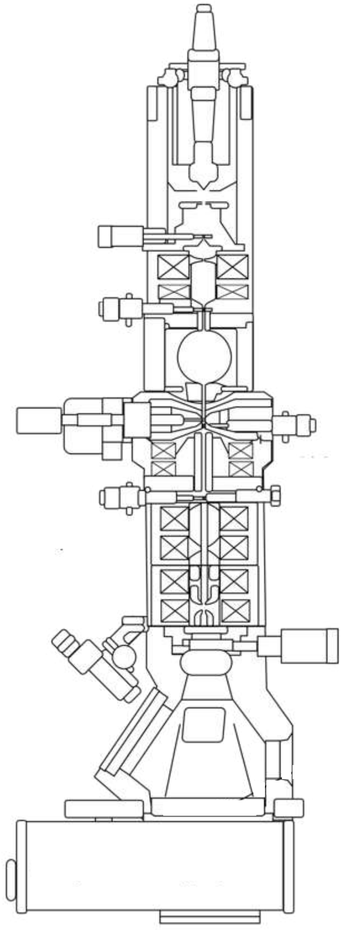
200 kV: JEM 2100F, Tecnai F20, Talos, Artica
FEG
2+ Å resolution (3.5-4 Å)

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

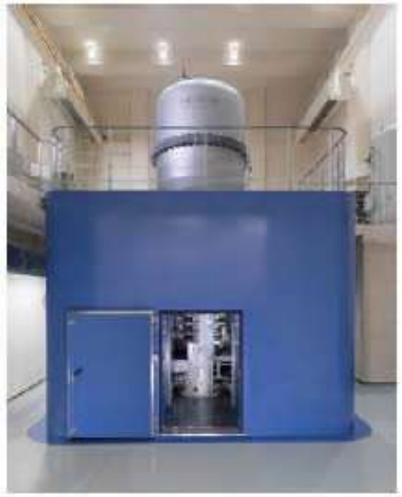


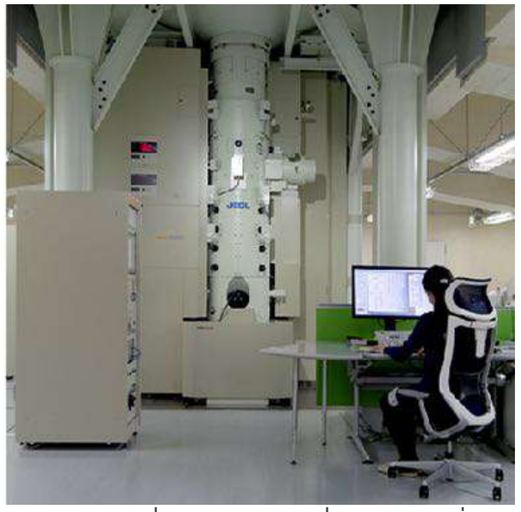


ELECTRON SOURCES & TYPES OF CRYOEM



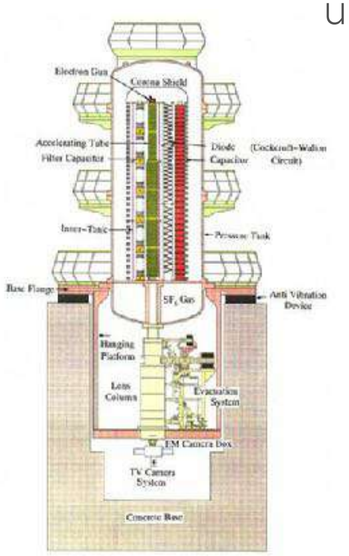
1-1.2 MV: Hitachi, JEOL
LaB6



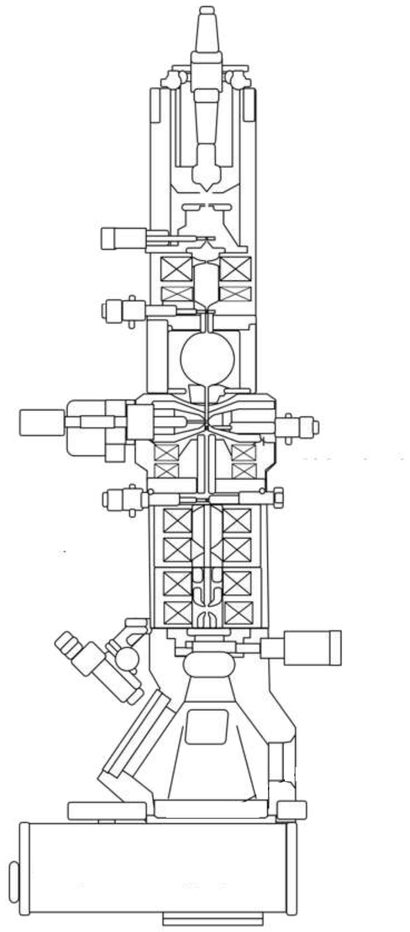


uhvem.osaka-u.ac.jp

3 MV: Hitachi H3000
LaB6



VACUUM SYSTEMS



Why do we need a vacuum?

Beam coherence - at STP mean free path ~1 cm

Insulation - interaction between e- and air

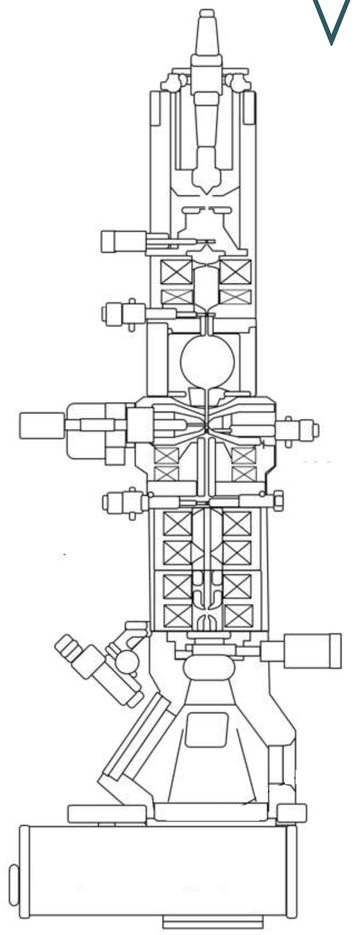
Filament - O2 will burn out source

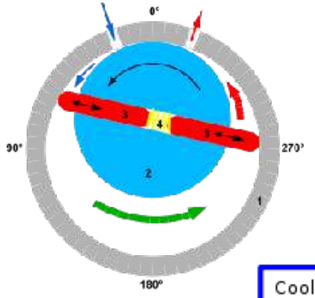
Contamination - reduce interaction gas, e-beam and sample

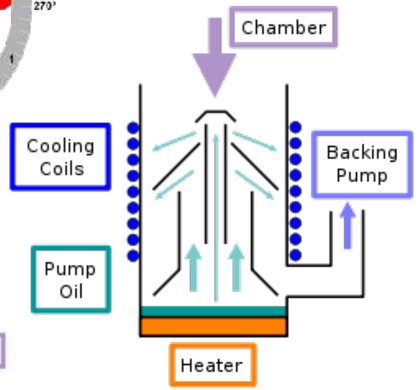
VACUUM SYSTEMS

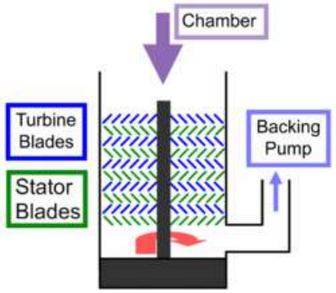
What types of pumps do we have?

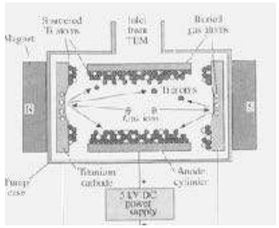
1 mm Hg = 1 Torr = 10^2 Pa
1 atm = 760 Torr = 7.5×10^4 Pa











wikipedia.com

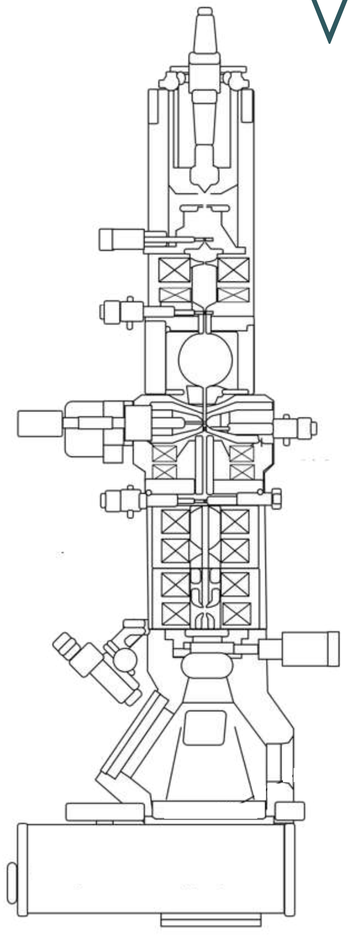
PVP / Rotary 1-10^{-3} Torr | >0.1 Pa

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

VACUUM SYSTEMS



What types of pumps do we have?

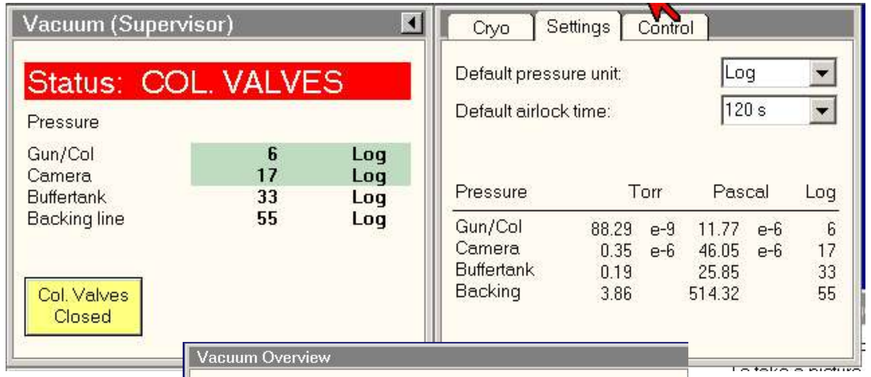
1 mm Hg = 1 Torr = 10^2 Pa

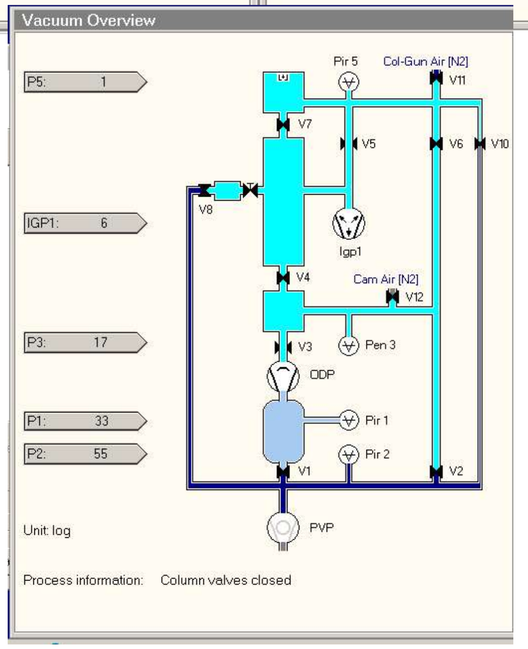
1 atm = 760 Torr = 7.5×10^4 Pa

Gun 10^{-9} Torr

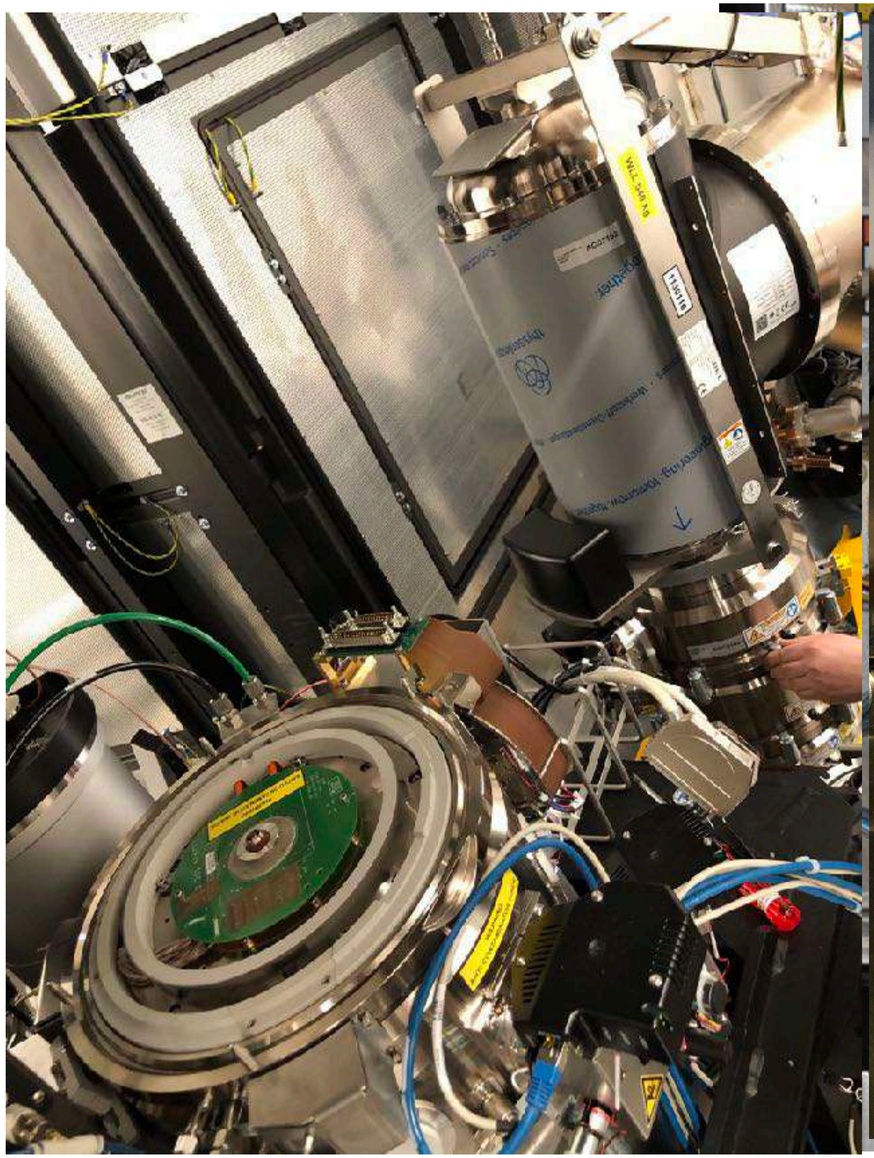
Specimen 10^{-6} - 10^{-7} Torr

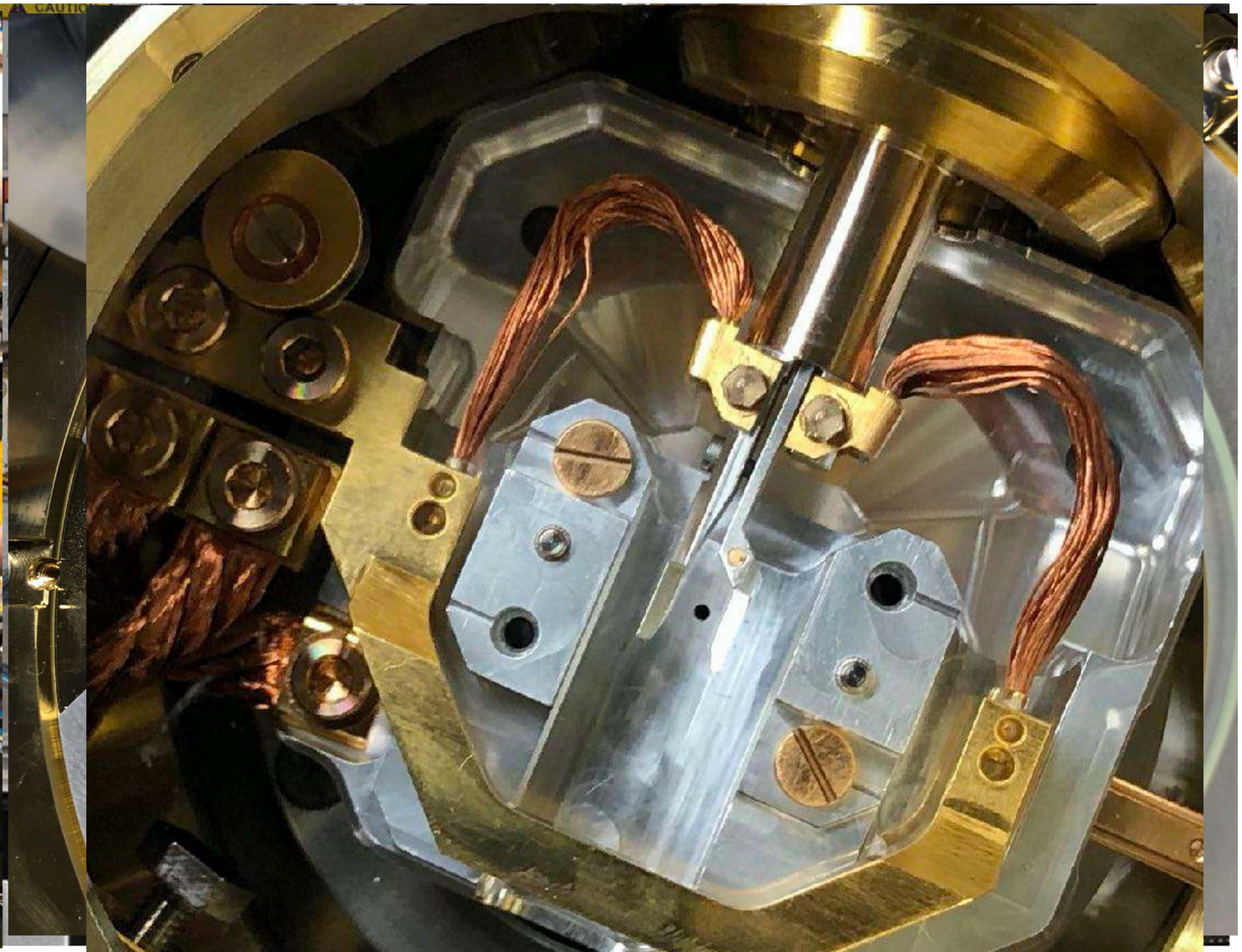
Chamber and Camera 10^{-5} - 10^{-6} Torr





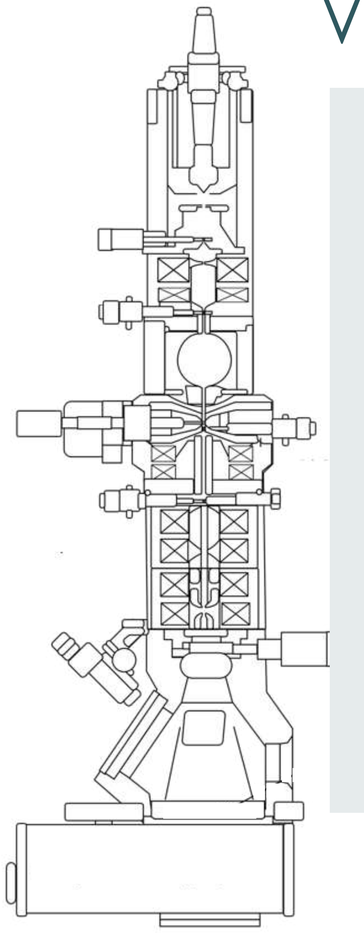
VACUUM SYSTEMS

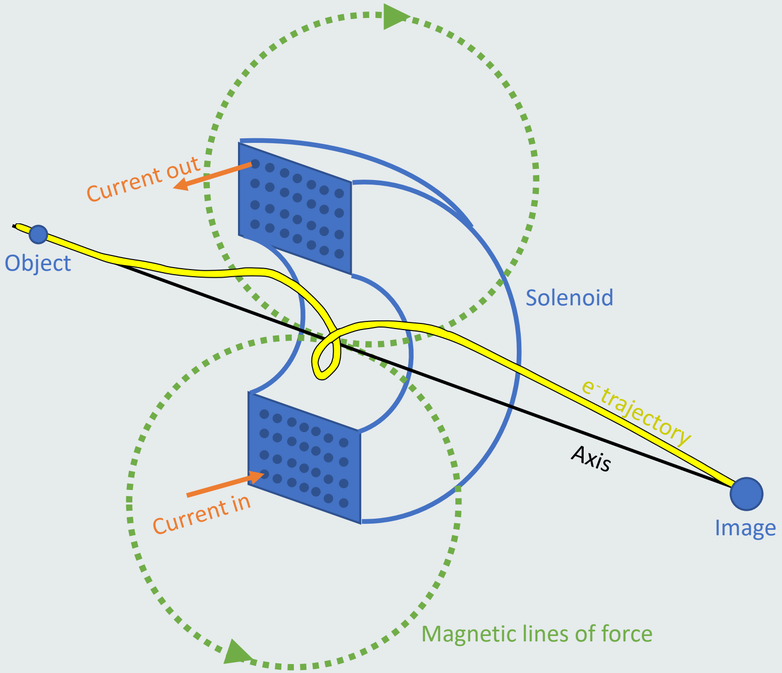




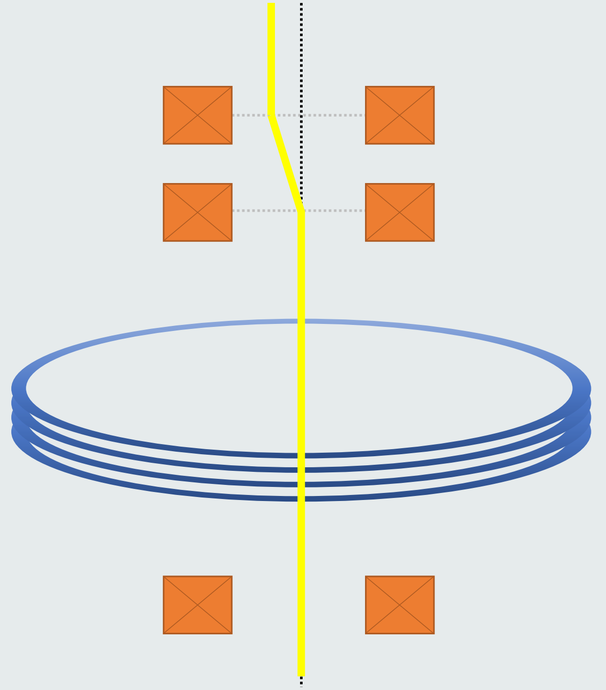
LENSES

What types of lenses do we have?





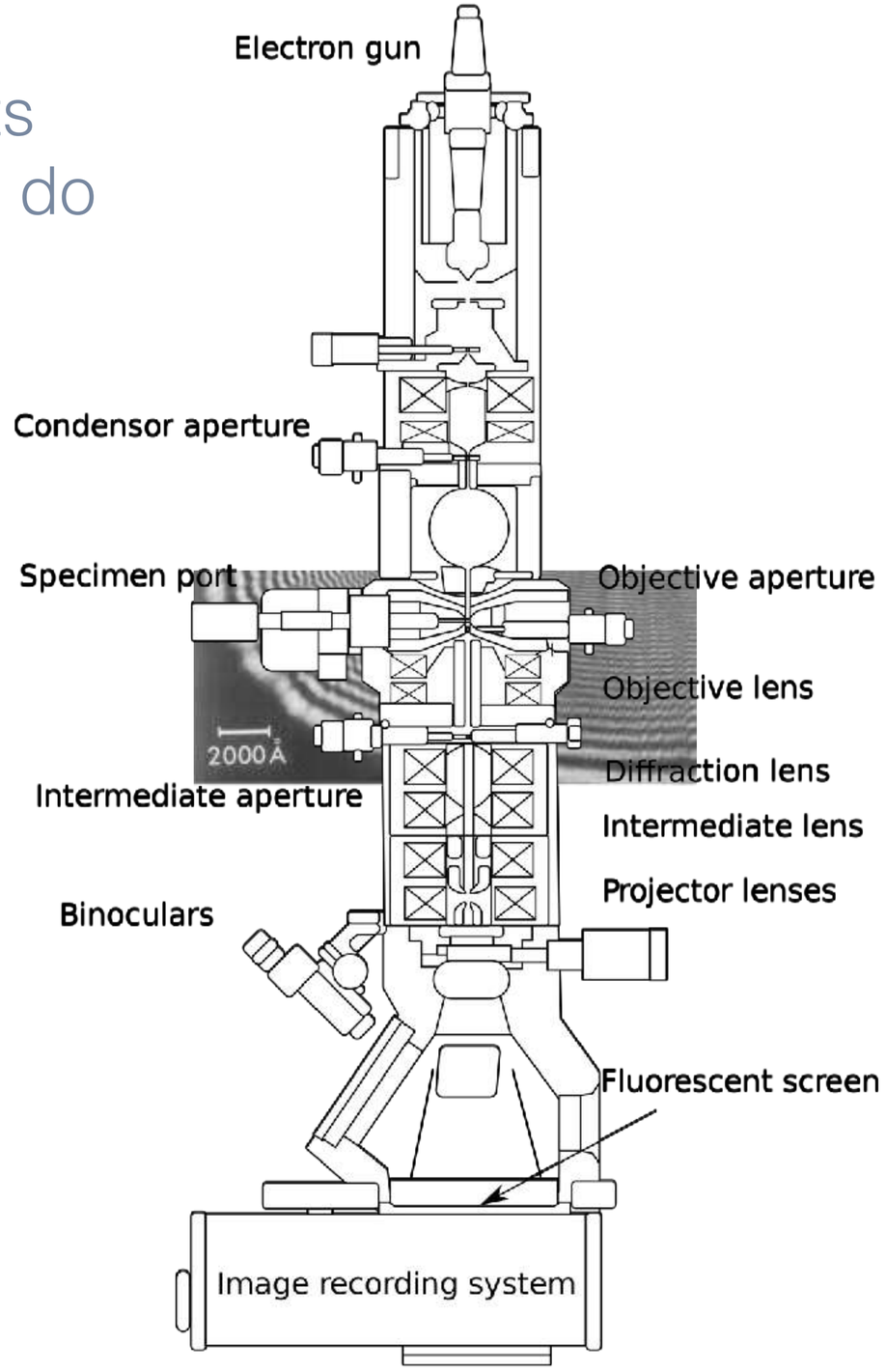
- Focus
- Magnify
- Rotate



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)~~

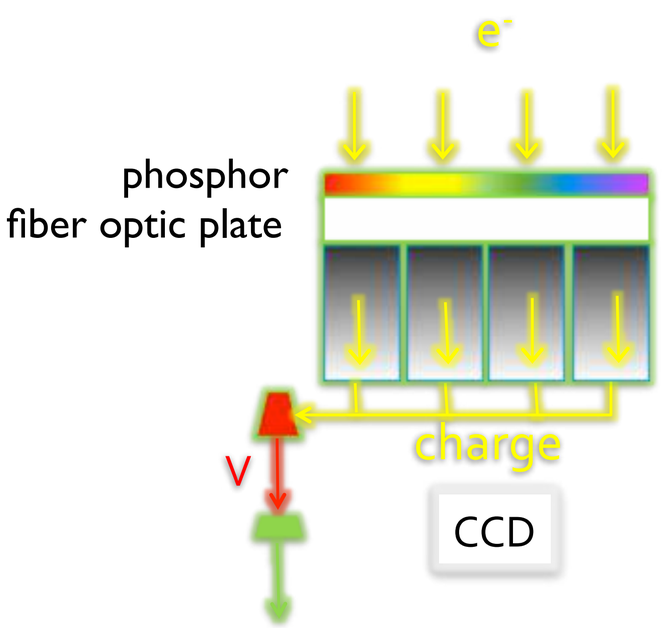


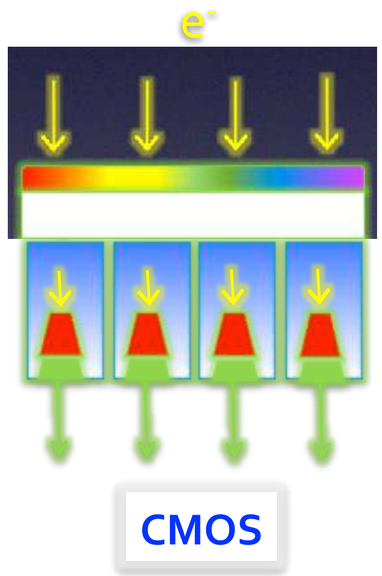
DETECTORS Digital Cameras for TEM

- Photon converted
- Direct sensing

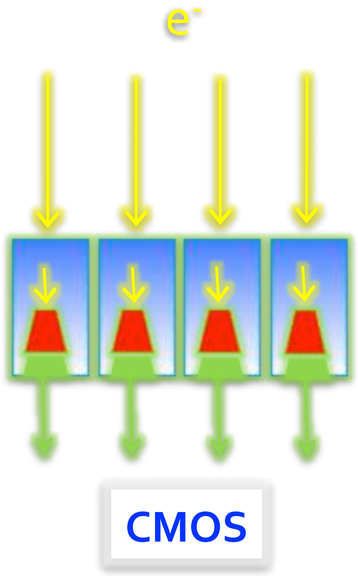
?

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





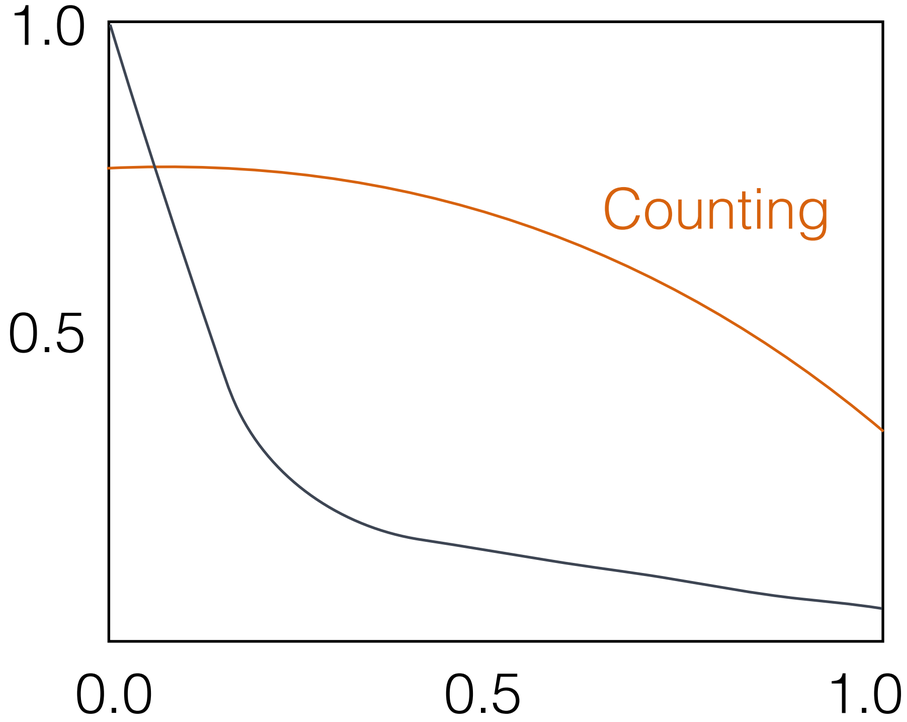
high dose rate



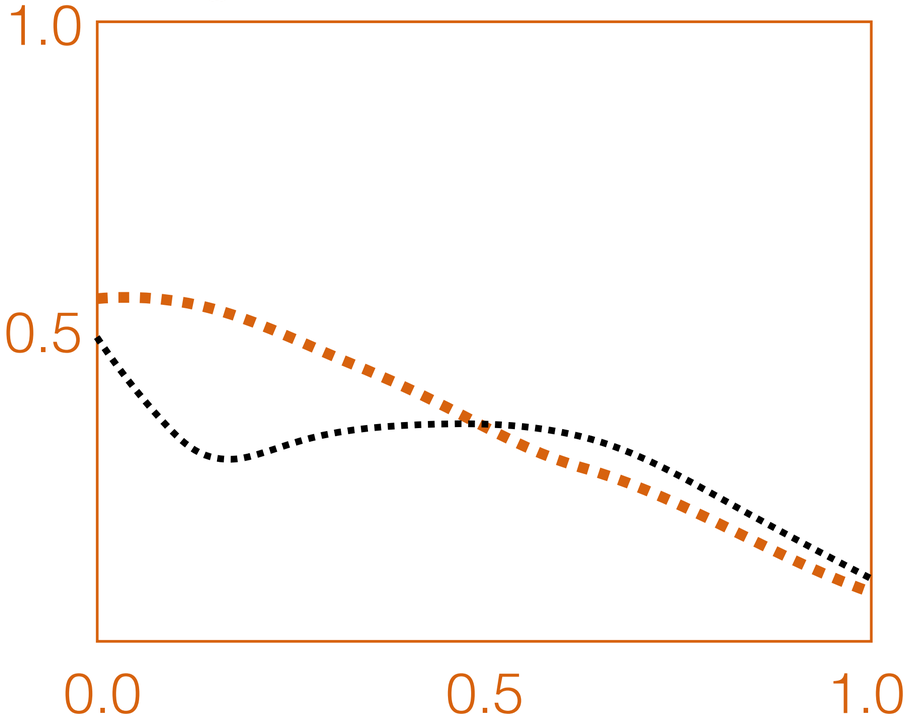
Direct Detectors

DETECTORS Detector Performance Characterization

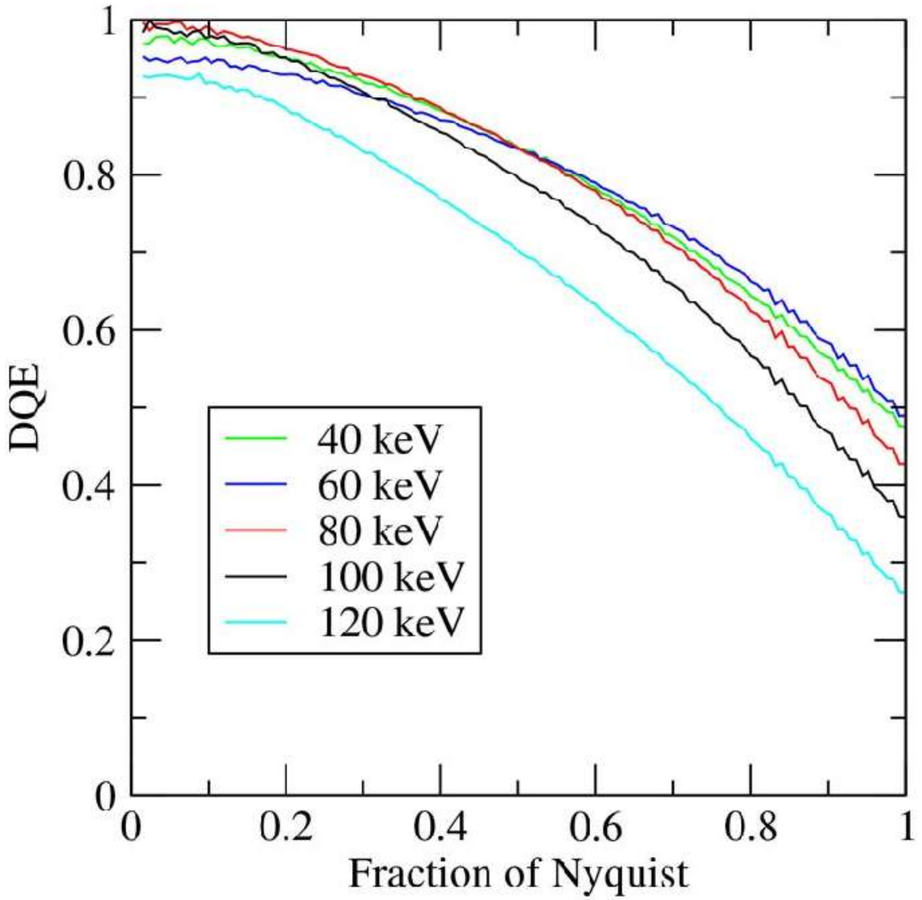
- MTF (Modulation Transfer Transform)
  - contribute to signal envelope



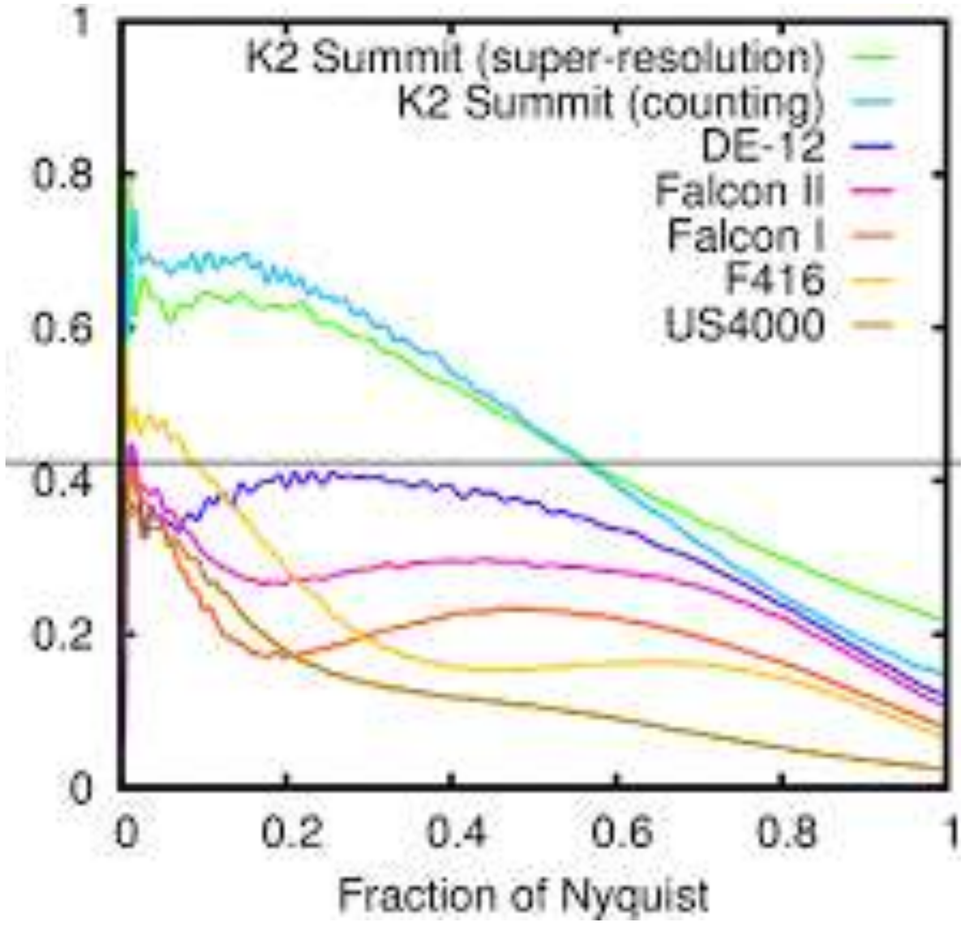
- DQE (Detector Quantum Efficiency)
  - S/N over spatial frequency range



DETECTORS Detector Performance Characterization



dectris.com



Ruskin, et al JSB

NIH P41 - National Biomedical Technology Research Resources (BTRR)


Krios1


Krios2


Krios3


Krios4


Krios5


Krios6


Krios7
2020


Tecnai F20


Tecnai12


JEOL1230


Helios650


Hitachi 7800


Glacios

Chameleon