2024 SPA shortcourse

Microscopes and tools of the trade

March 12, 2024

NYSBC SEMC

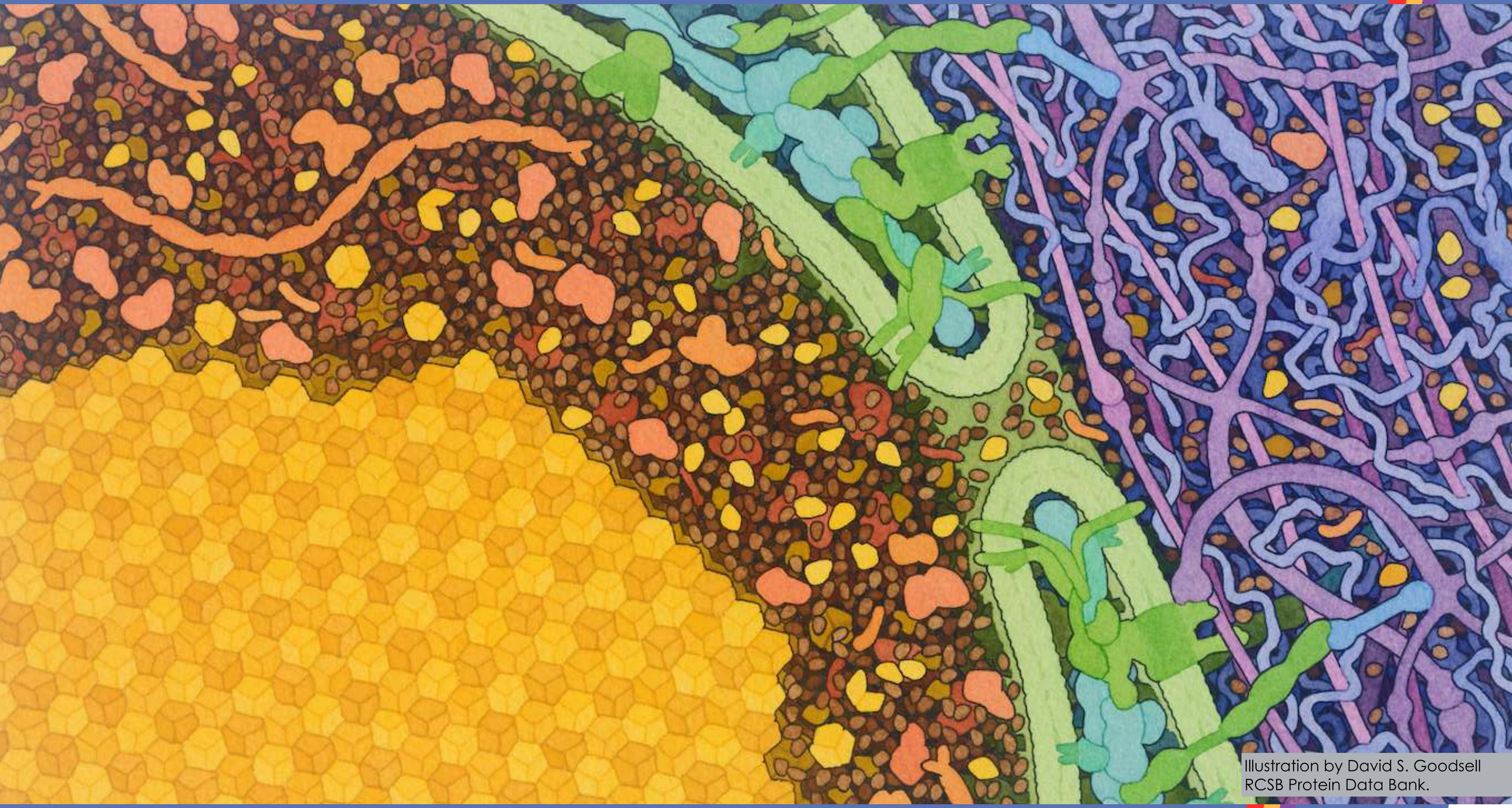


Illustration by David S. Goodsell
RCSB Protein Data Bank.

doi: 10.2210/rcsb_pdb/goodsell-gallery-044

Insulin release, 2022

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

10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1}

Neutrons

AFM

Human eye

X-Rays

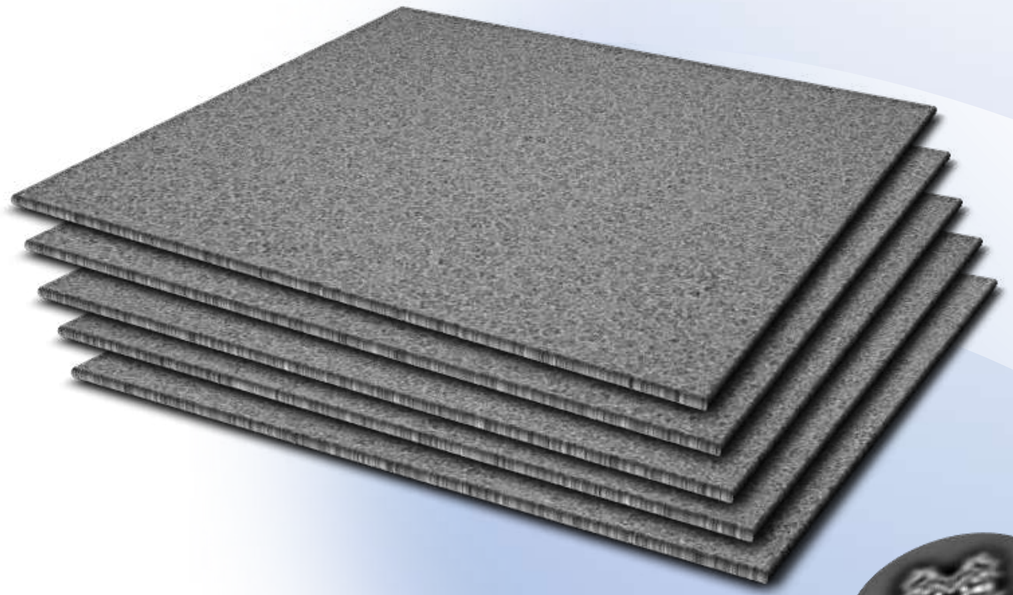
Light microscopy

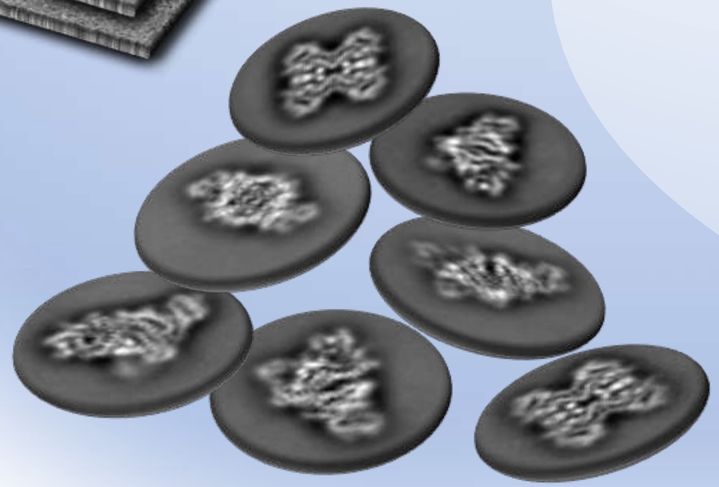
NMR

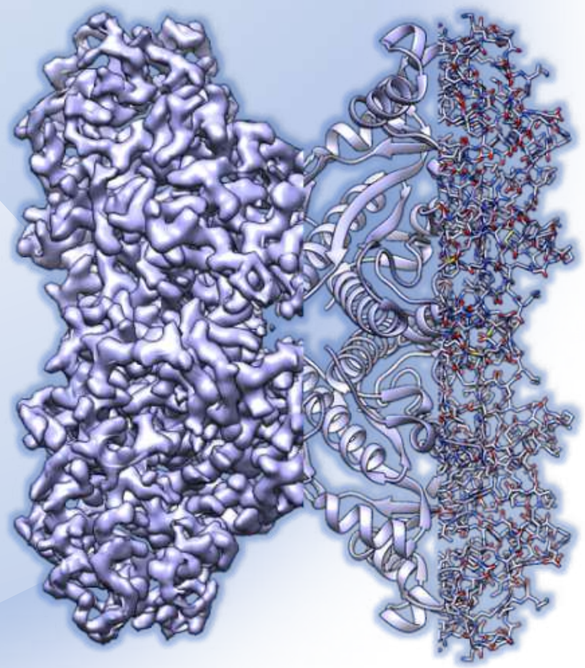
Electron microscopy

doi: 10.2210/rcsb_pdb/goodsell-gallery-044

What is possible today?







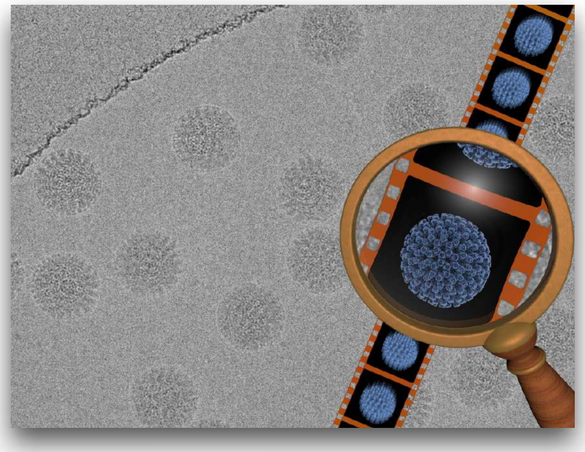
What brought about the resolution revolution?

(~2012-2014)

Hardware

Microscopes

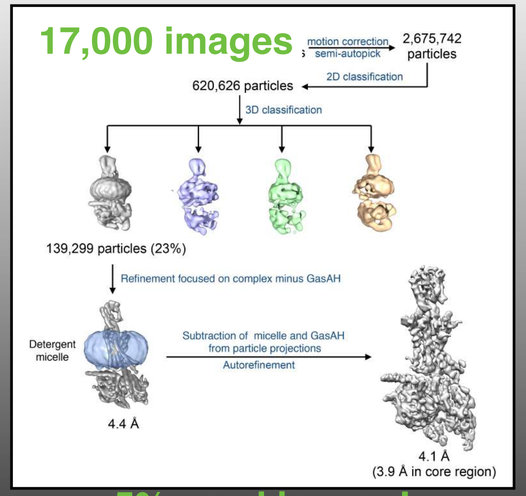
Direct Detectors



Computers

2012->2017
Cost reduced by 100x

Software



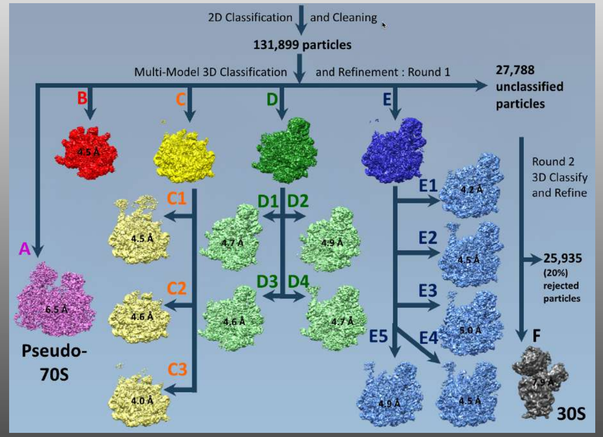
5% used in map!

Leginon / SerialEM / EPU, …

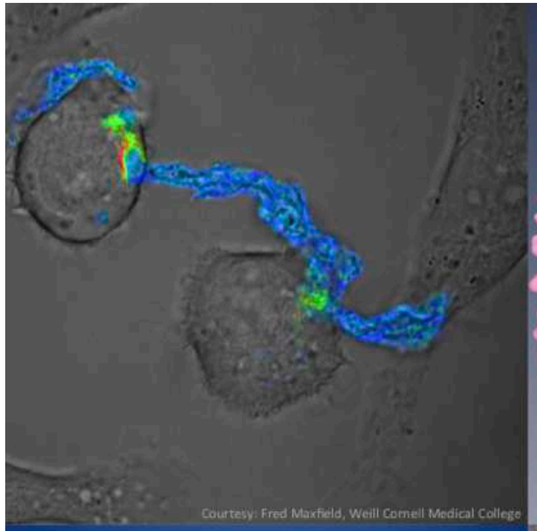
MotionCorr2, Unblur, …

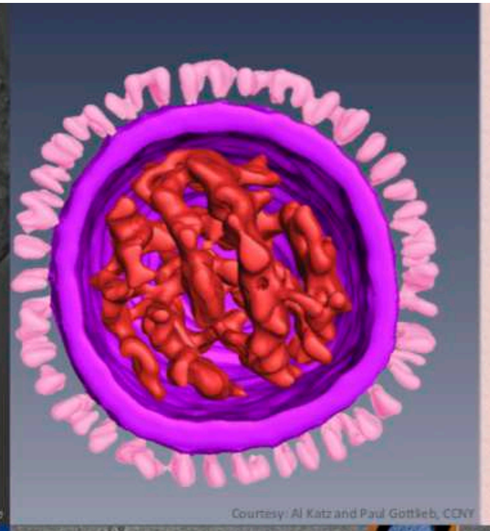
RELION, FREALIGN/cisTEM, cryoSPARC
EMAN, Sparx, SPHIRE, XMIPP, …

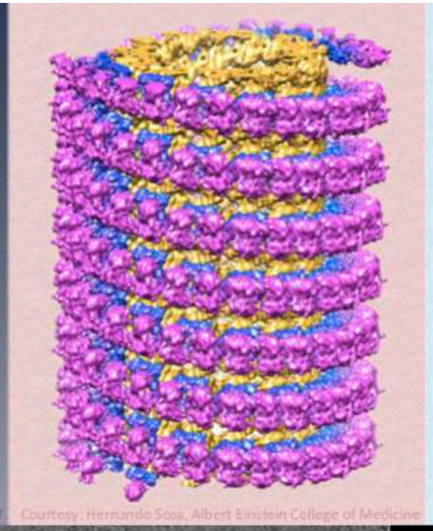
14 independent structures

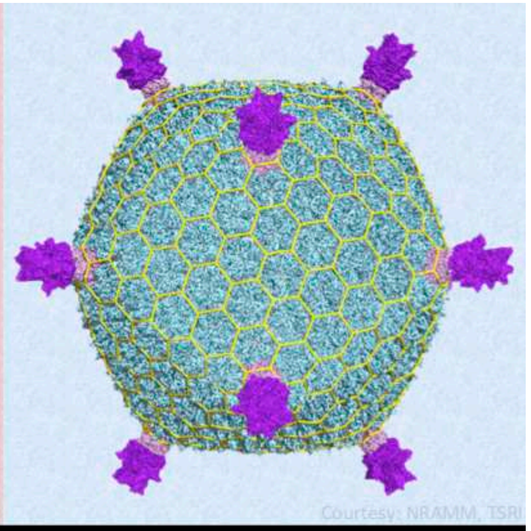


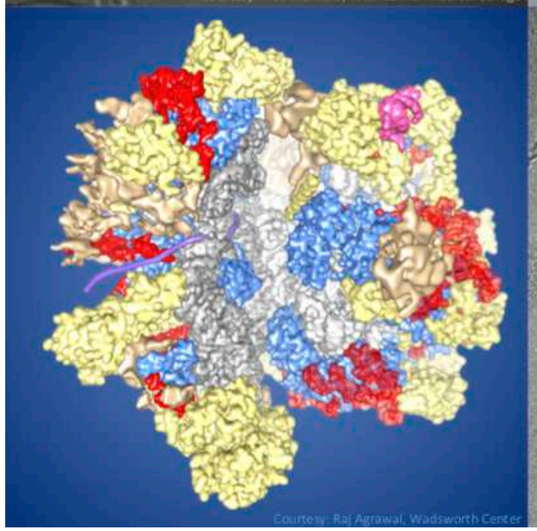
cryoEM: a technology on the rise

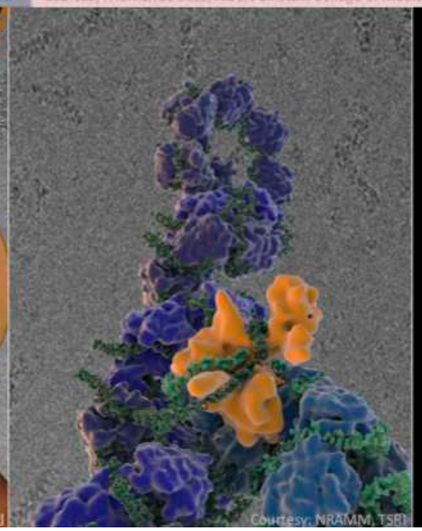


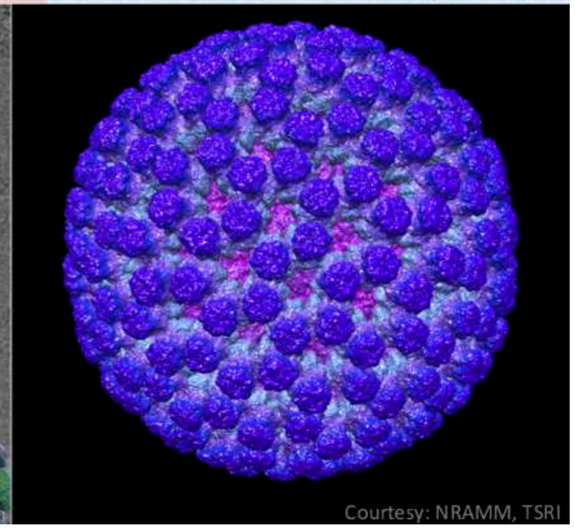




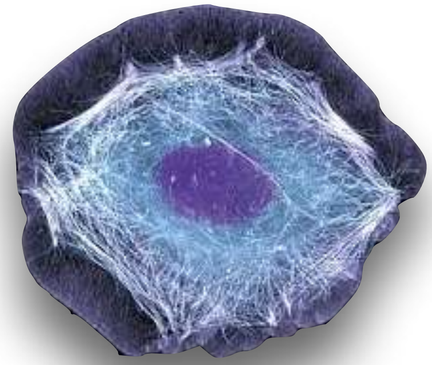


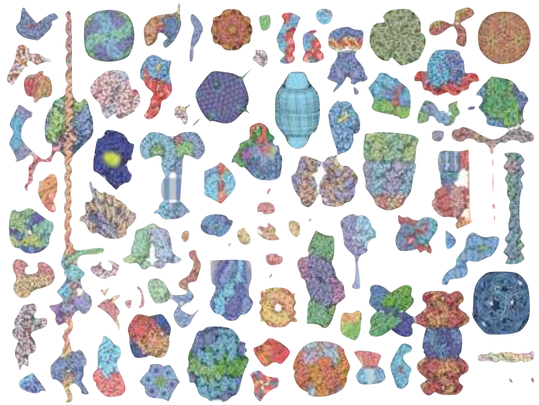






next steps

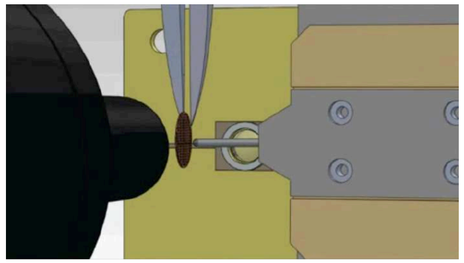
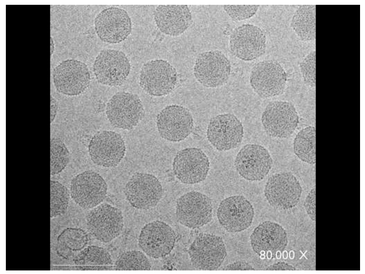


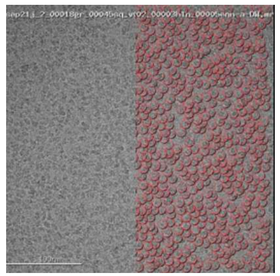
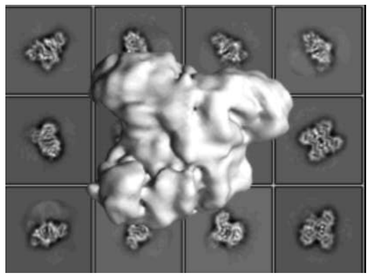
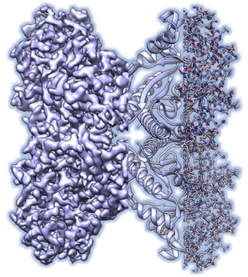


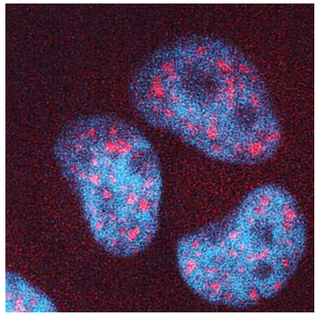
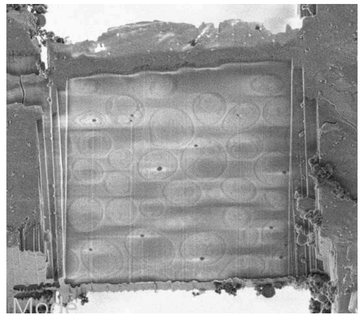
coming soon

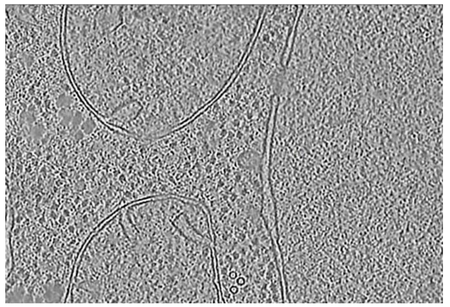
cryoEM: a technology on the rise

Single particle cryoEM

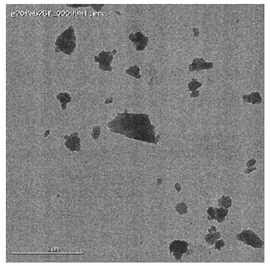
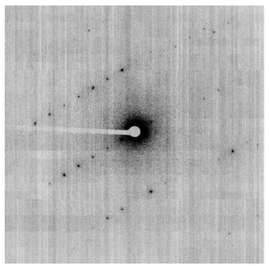
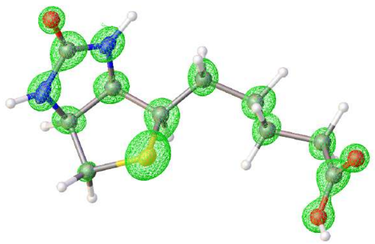
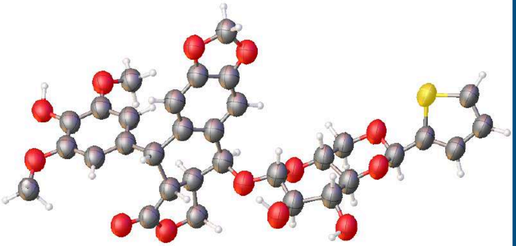




Cryo Electron Tomography (cryoET)

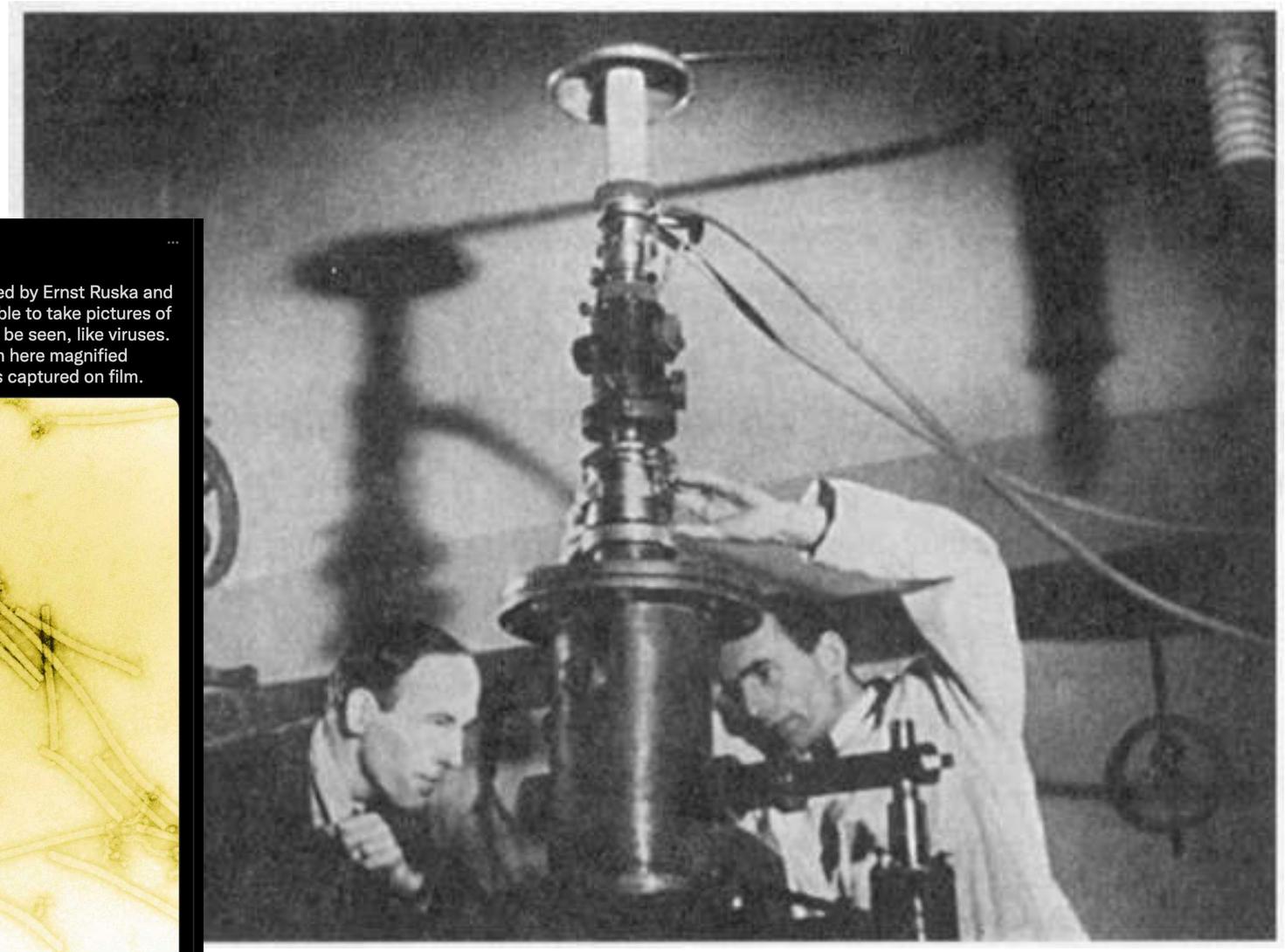
Micro crystal electron diffraction (microED)

The tool of our trade: EM

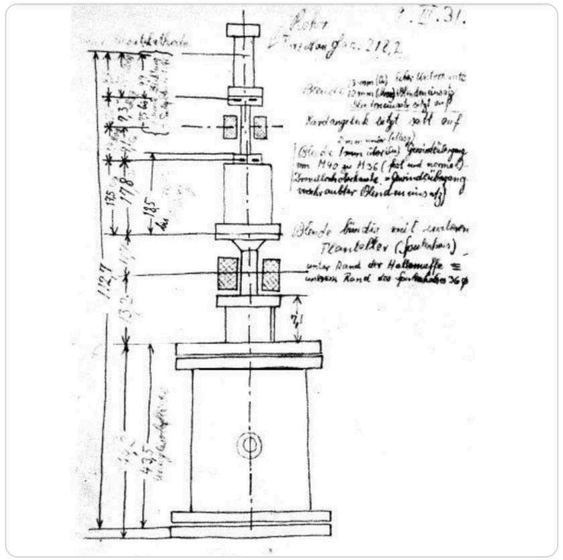
Ruska and Knoll in Berlin in the early 1930s

-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.



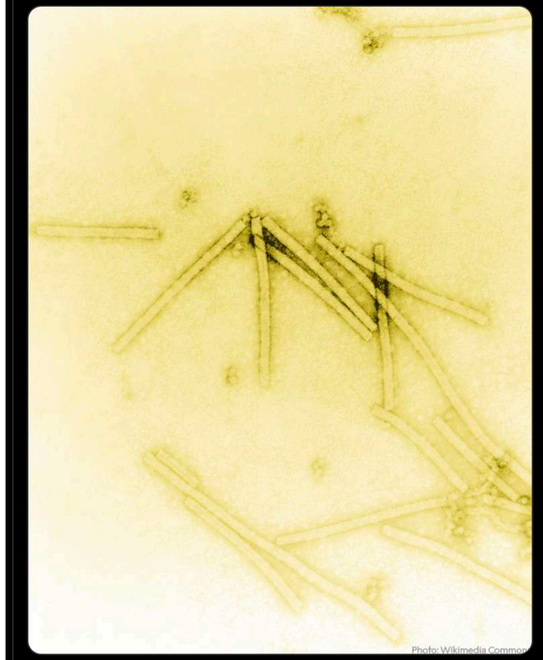
6:06 AM · Dec 25, 2021

725 Reply Copy link to Tweet

Read 8 replies

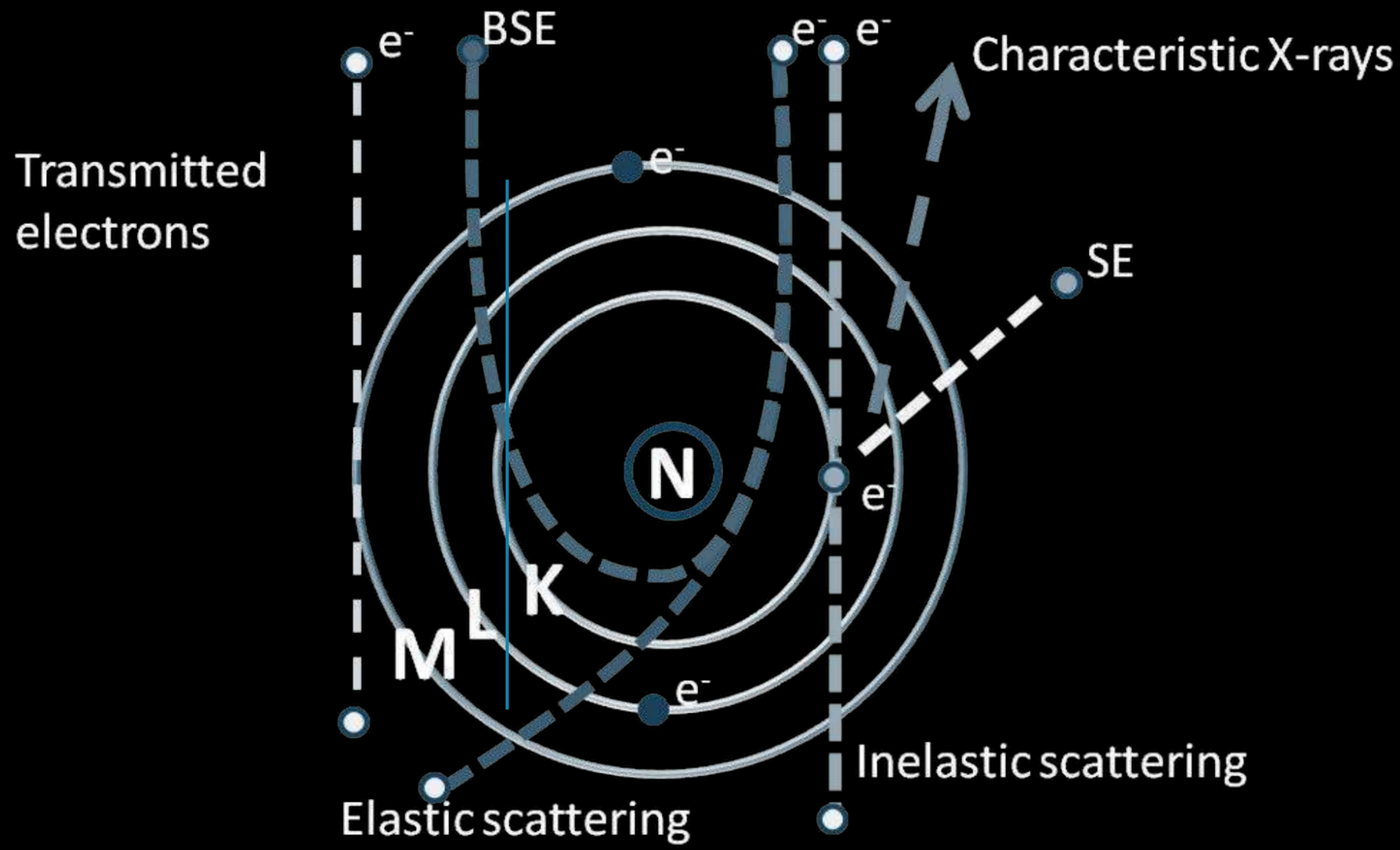
The Nobel Prize
@NobelPrize

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?



Why electrons?

Pros

Small wavelength

Can be focused

Cons

Damages sample

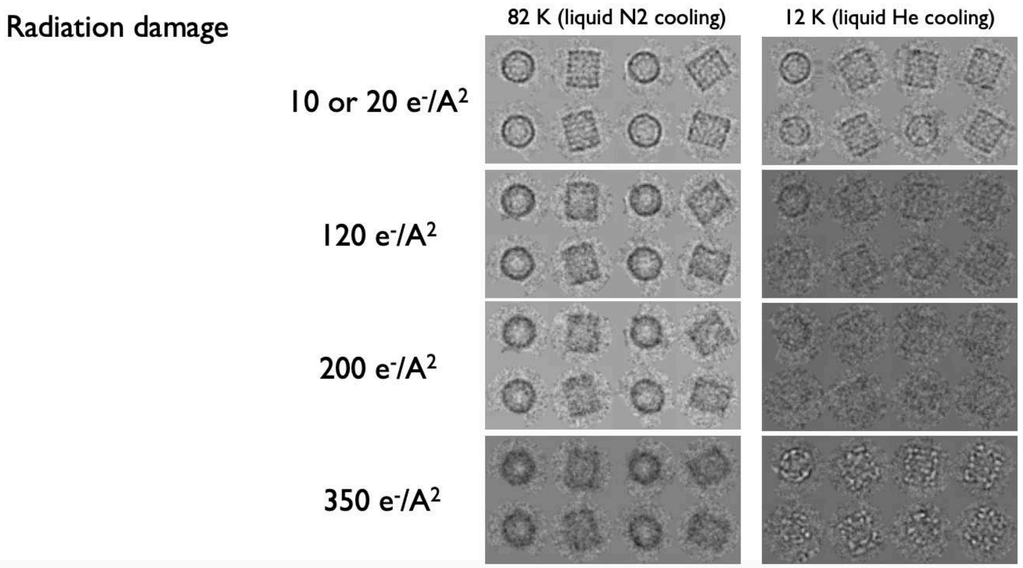
worse with faster electrons

Poor penetration

better with faster electrons

Why electrons?

Ideal dose for cryoEM?



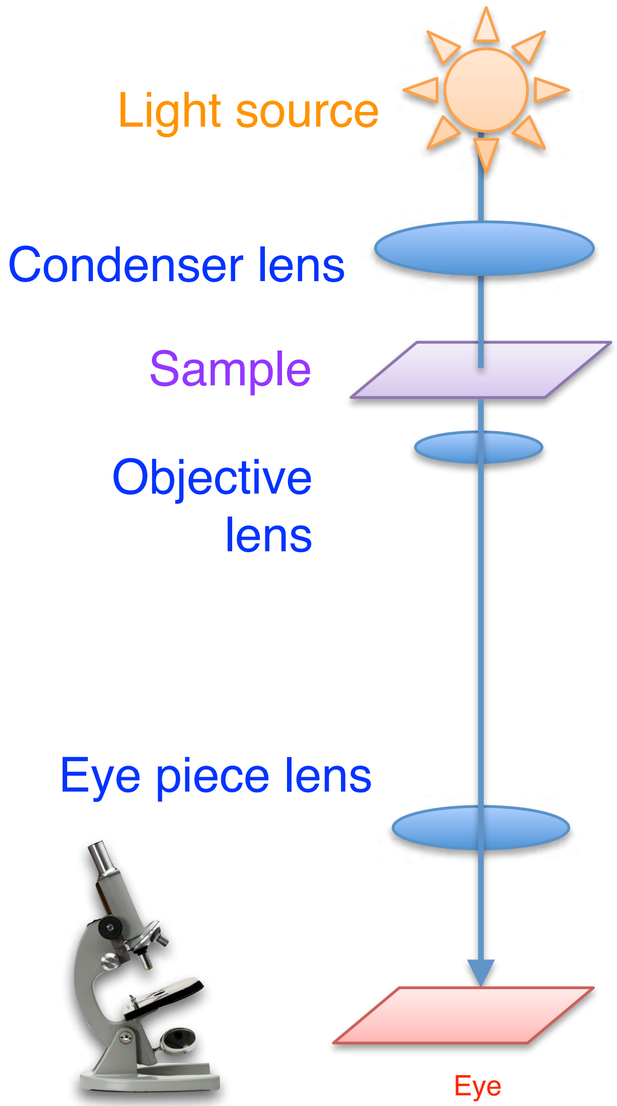
https://cryo-em-course.caltech.edu/

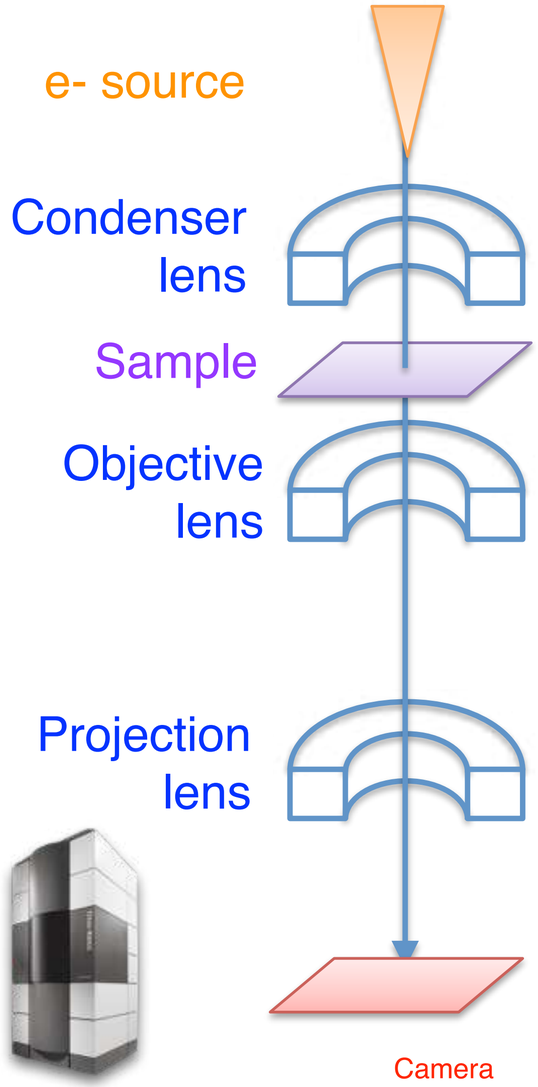
Specimen Behavior in the Electron Beam

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

- The first noticeable bubbles appear after the accumulated exposure (for 300 keV electrons) is approximately 150 e/A . At this high exposure, high-resolution features would long since be destroyed, of course, but the macromolecular particles might still be visible.

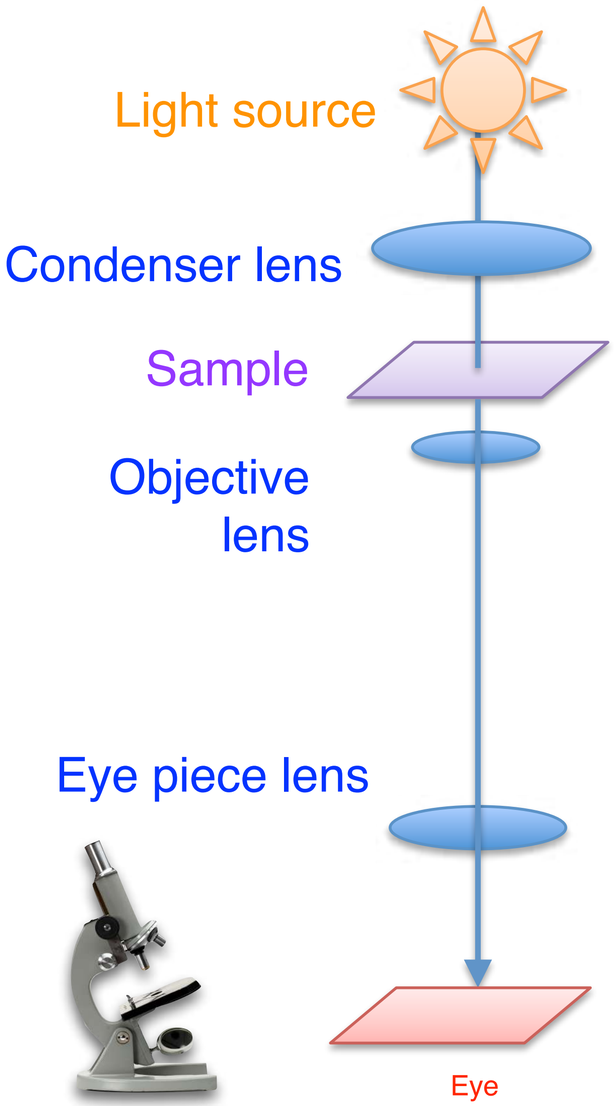
The electron microscope

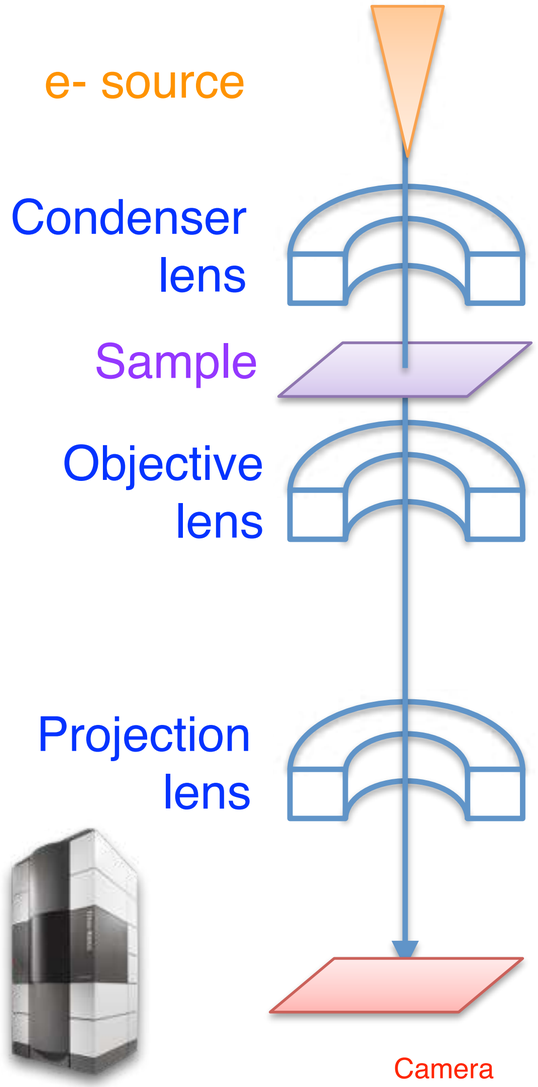


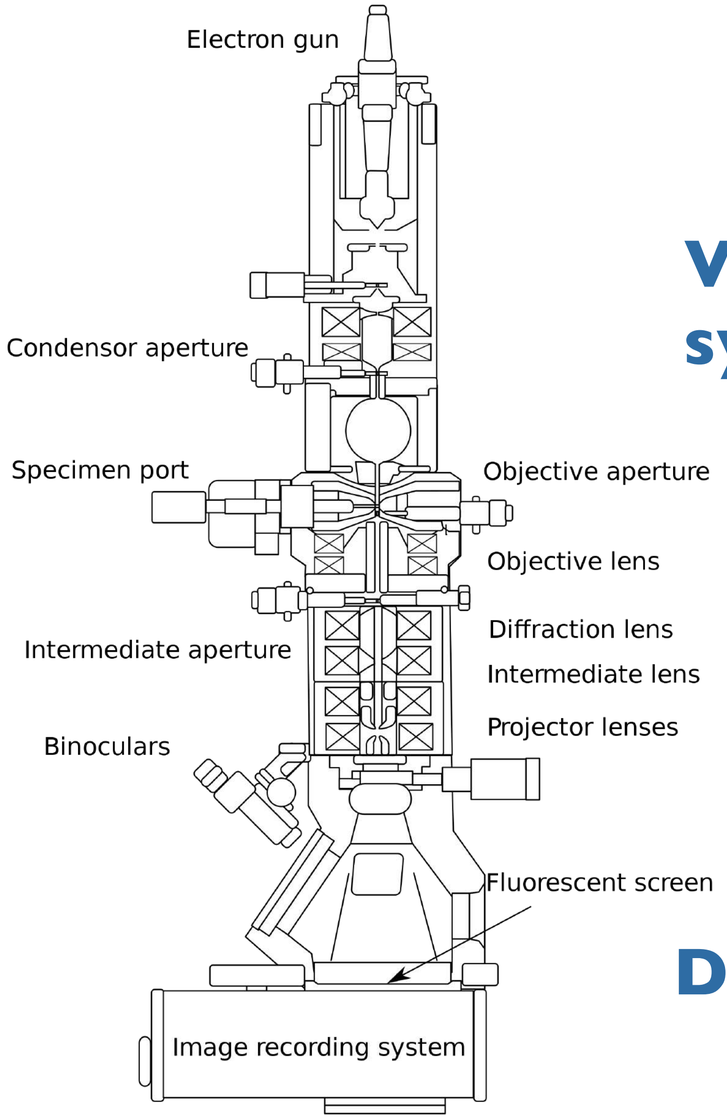


Light microscope	Transmission electron microscope
Visible light	Electrons
Glass lenses	Electron-magnetic lenses
450-650 nm	3.70 pm (100 keV) 2.51 pm (200 keV) 1.96 pm (300 keV)

The electron microscope







Electron sources

Vacuum systems

Lenses

Detectors

The electron microscope

e- gun produces e-

accelerator accelerate e- to high engergy

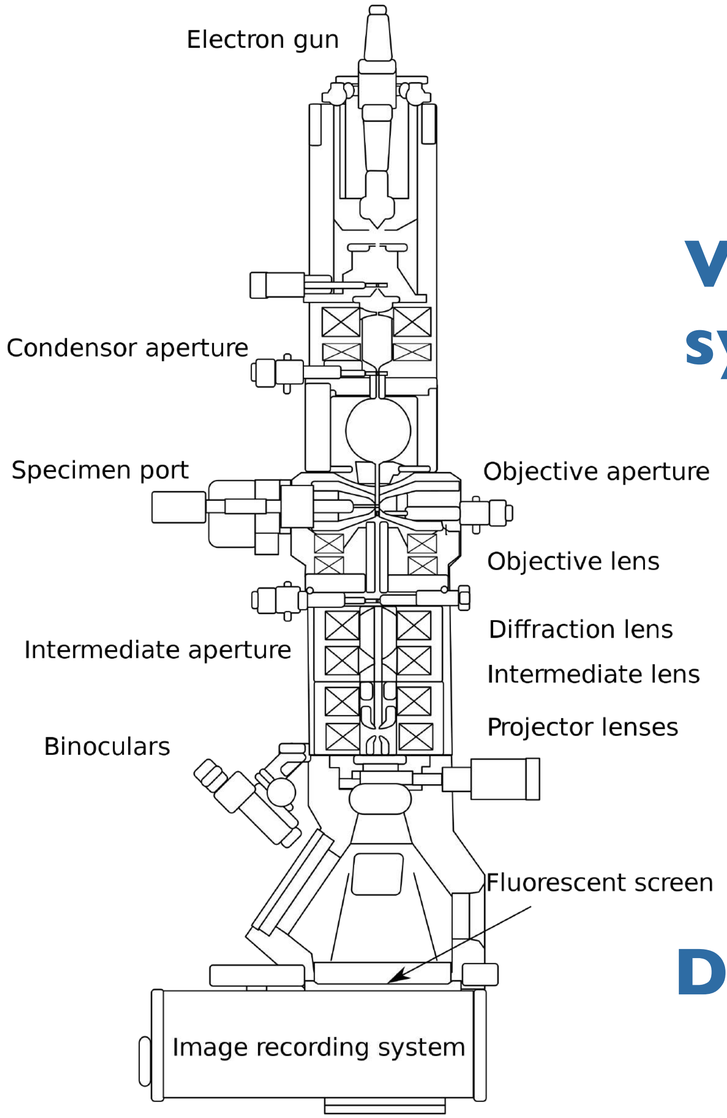
condenser control illumination on sample

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



Electron sources

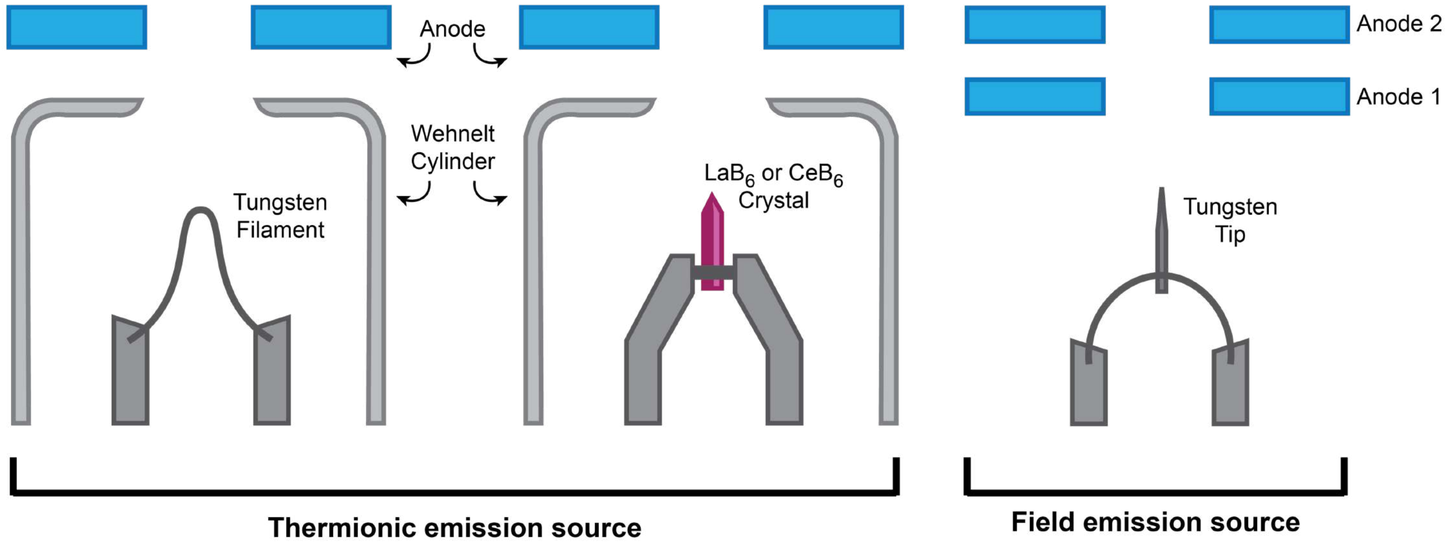
Vacuum systems

Lenses

Detectors

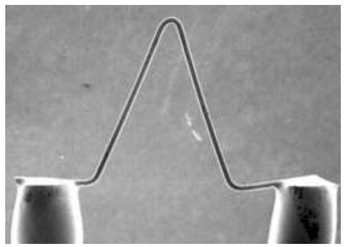
Electron sources

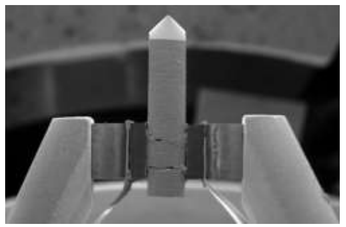
What are the 3 main kinds of electron sources?

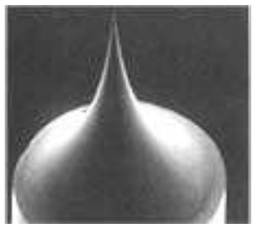


thermofisher.com

nanoscience.com



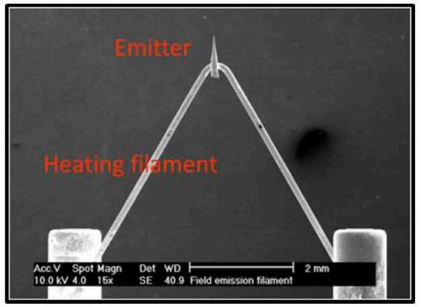




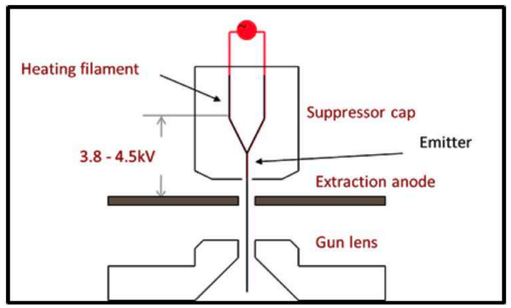
Electron sources

What are the 3 main kinds of electron sources?

Thermionic

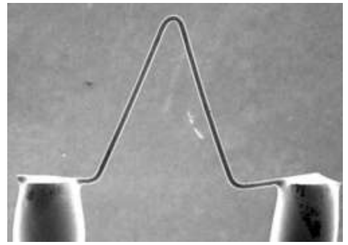


Field Emission Gun (FEG)



thermofisher.com

	W Filament	LaB6 or CeB6	S-FEG (Schottky FEG)	X-FEG (extreme high-brightness field emission gun)	C-FEG (low-energy-spread cold FEG)
Relative brightness	1	1-3	250	1250	1625
Energy spread	2.5 eV	1-1.5 eV	<1 eV	0.6-0.8 eV	<0.3 eV
Source size	50-100 µm	25 µm	<30nm	<20nm	<5nm
Lifetime	100 h	1,500 h	5,000 h+	1 yr+	1 yr+



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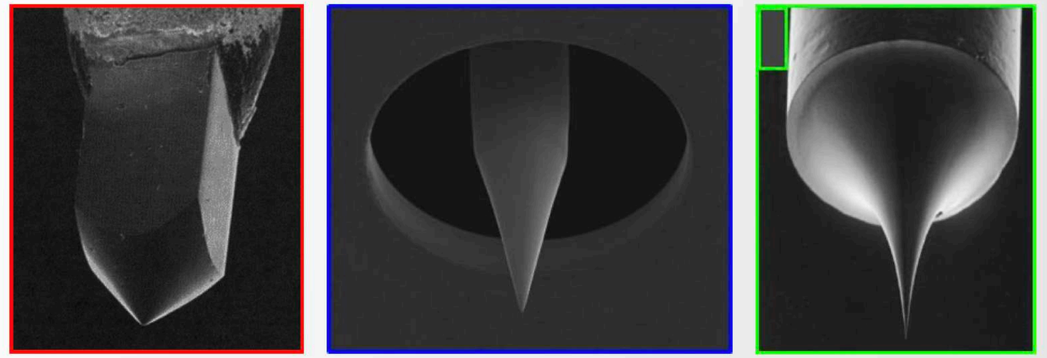
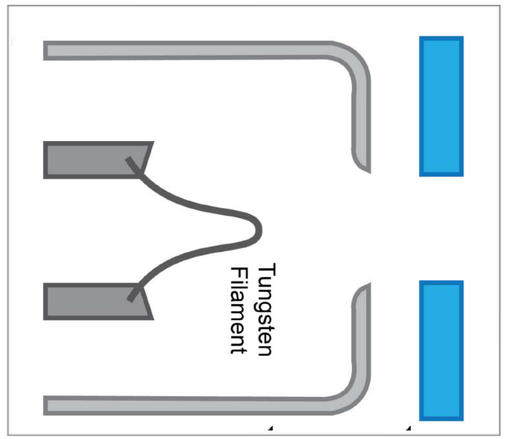
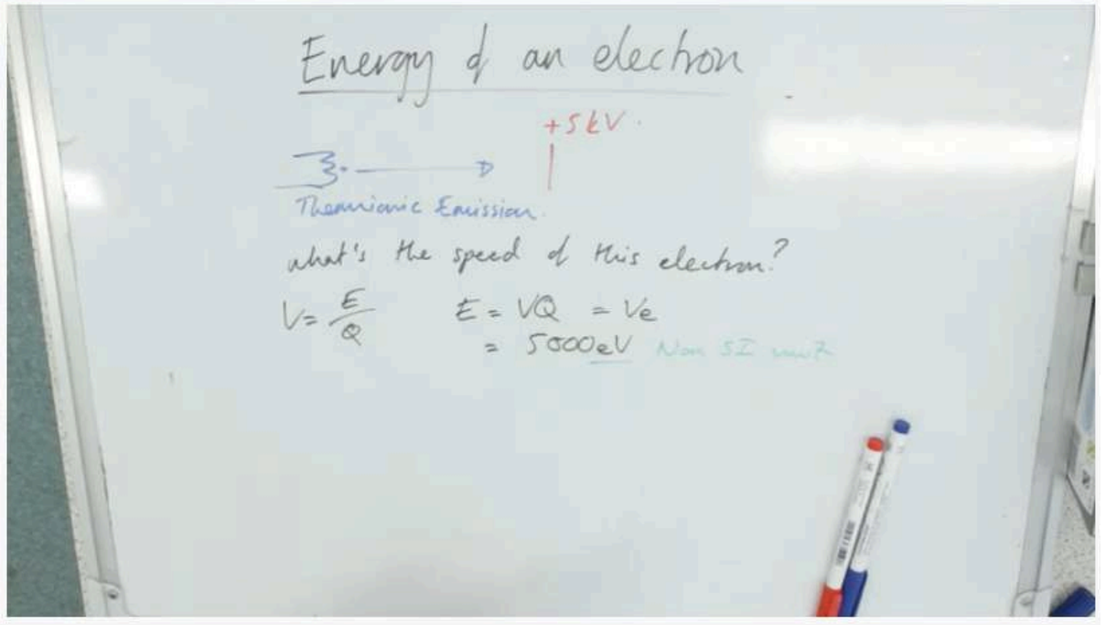


X-CFEG is 1.0x10^8 A/m2 sr V

Electron sources

How fast are the electrons moving?

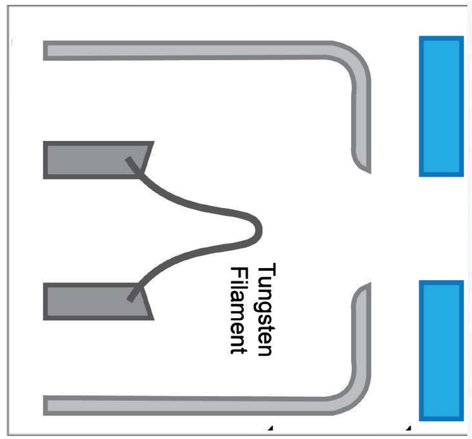


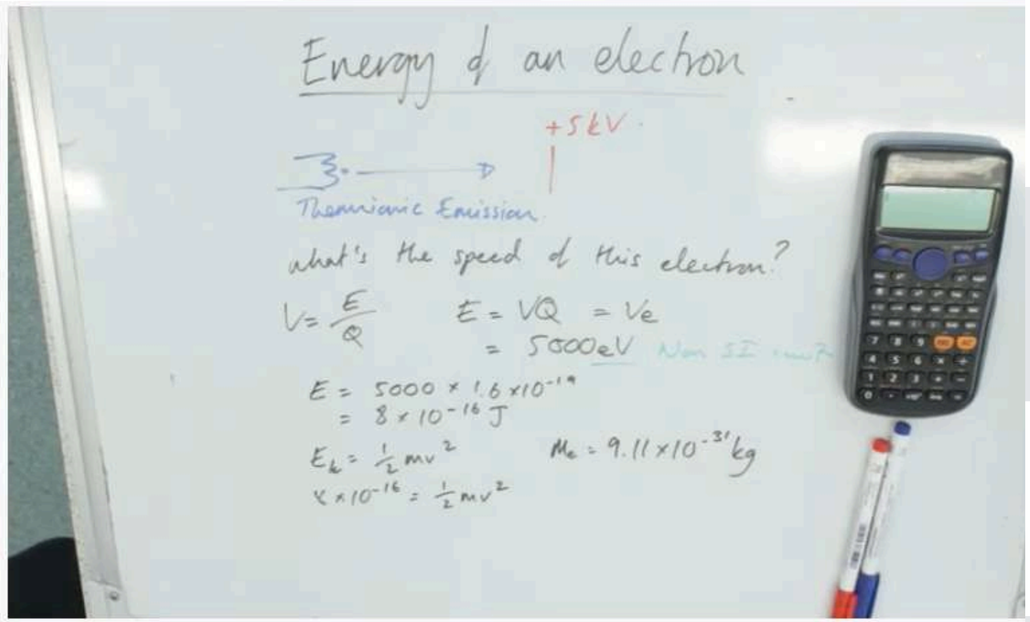


https://www.youtube.com/watch?v=tYCET6vYdYk

Electron sources

How fast are the electrons movi

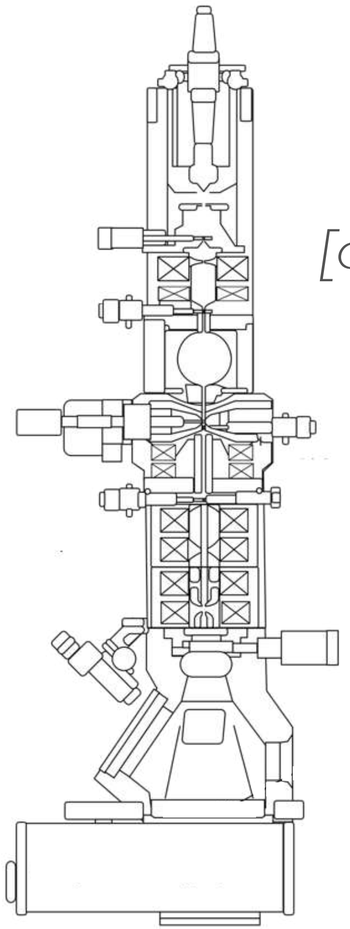




Light microscope	Transmission electron microscope
Visible light	Electrons
Glass lenses	Electron-magnetic lenses
450-650 nm	3.70 pm (100 keV) 2.51 pm (200 keV) 1.96 pm (300 keV.)
speed of light in vacuum c	0.548c (100 keV) 0.695c (200 keV) 0.776c (300 keV.)

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

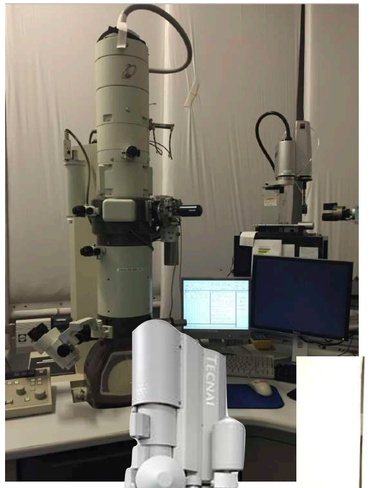
Electron sources

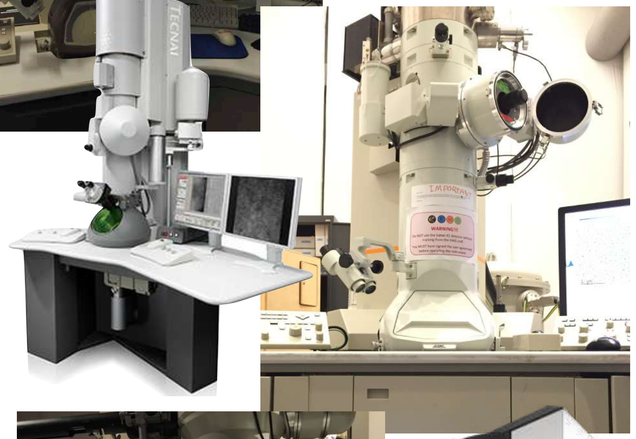


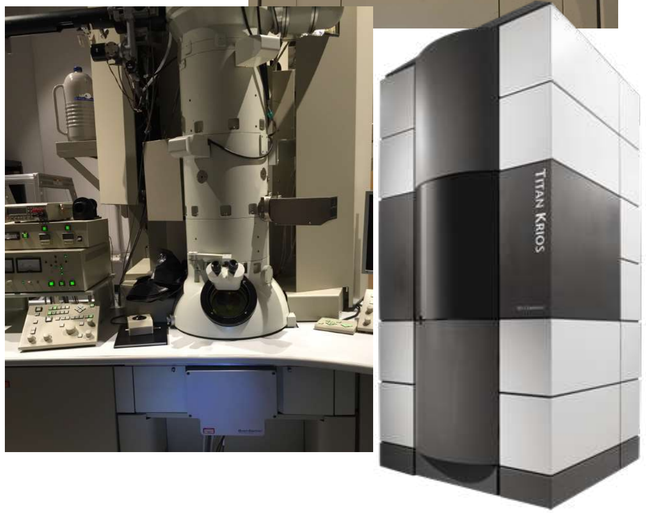
80-120 kV: Hitachi 7800, JEOL1400, TFS Talos120
W or LaB6
High contrast & robust
sub-nm resolution
[developments ongoing to push resolution with FEG systems]

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

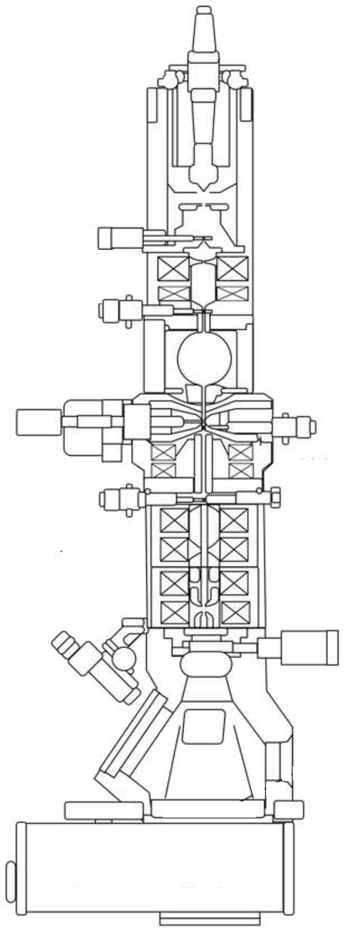
300 kV: JEOL3200FSC, cryoARM; TFS Krios, Halo
FEG
Smaller effect on unwanted lens aberrations
1.5-3 Å resolution



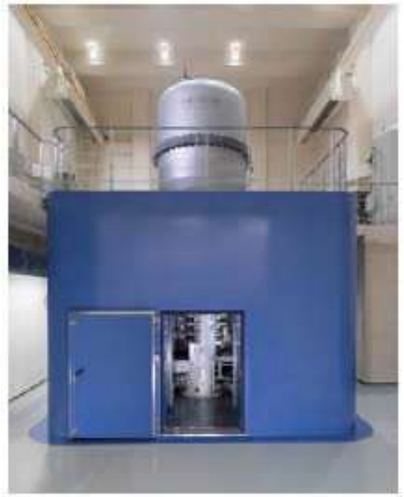




Electron sources

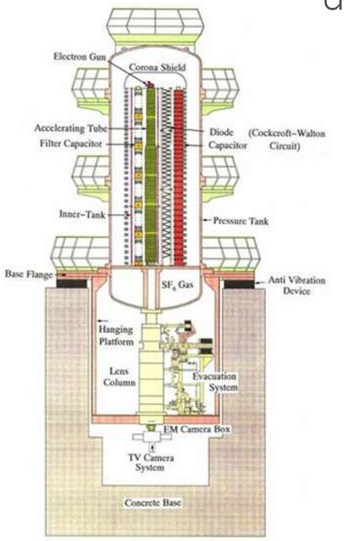


1-1.2 MV: Hitachi, JEOL
LaB6

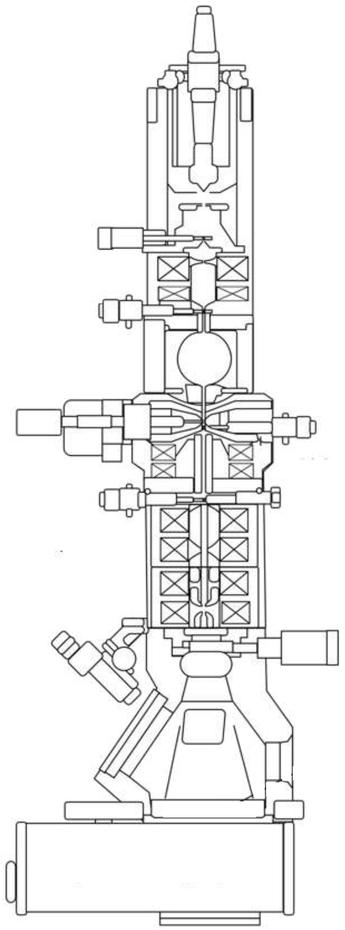


uhvem.osaka-u.ac.jp

3 MV: Hitachi H3000
LaB6



Vacuum systems



Why do we need a vacuum?

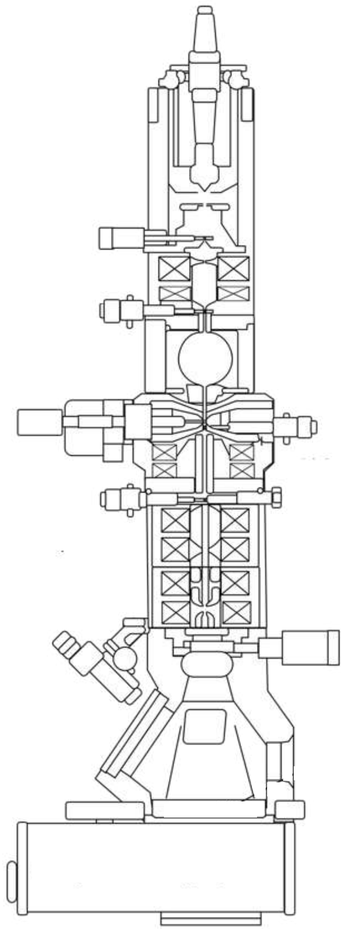
Beam coherence - at STP mean free path ~1 cm

Insulation - interaction between e- and air

Filament - O2 will burn out source

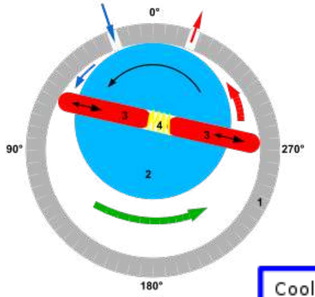
Contamination - reduce interaction gas, e-beam and sample

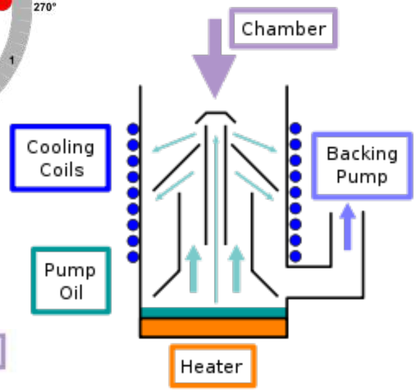
Vacuum systems

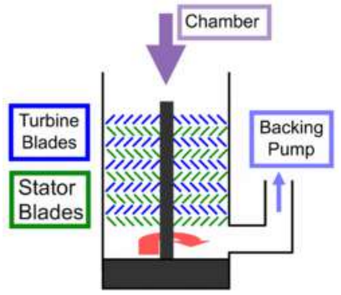


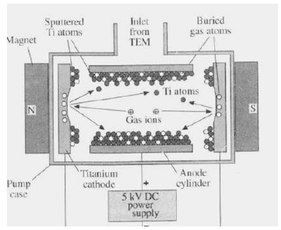
What types of pumps do we have?

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









wikipedia.com

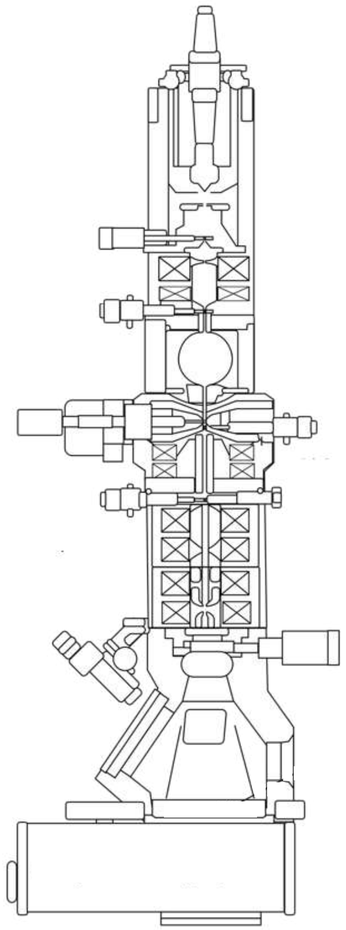
PVP / Rotary — $1\text{-}10^{-3}$ Torr | >0.1 Pa

Diffusion — $10^{-3}\text{-}10^{-6}$ Torr | $0.1\text{-}10^{-4}$ Pa

Turbo — $10^{-6}\text{-}10^{-9}$ Torr | 10^{-4}–10^{-7} Pa

IGP — $10^{-9}\text{-}10^{-12}$ Torr | $10^{-7}\text{-}10^{-9}$ Pa

Vacuum systems



What types of pumps do we have?

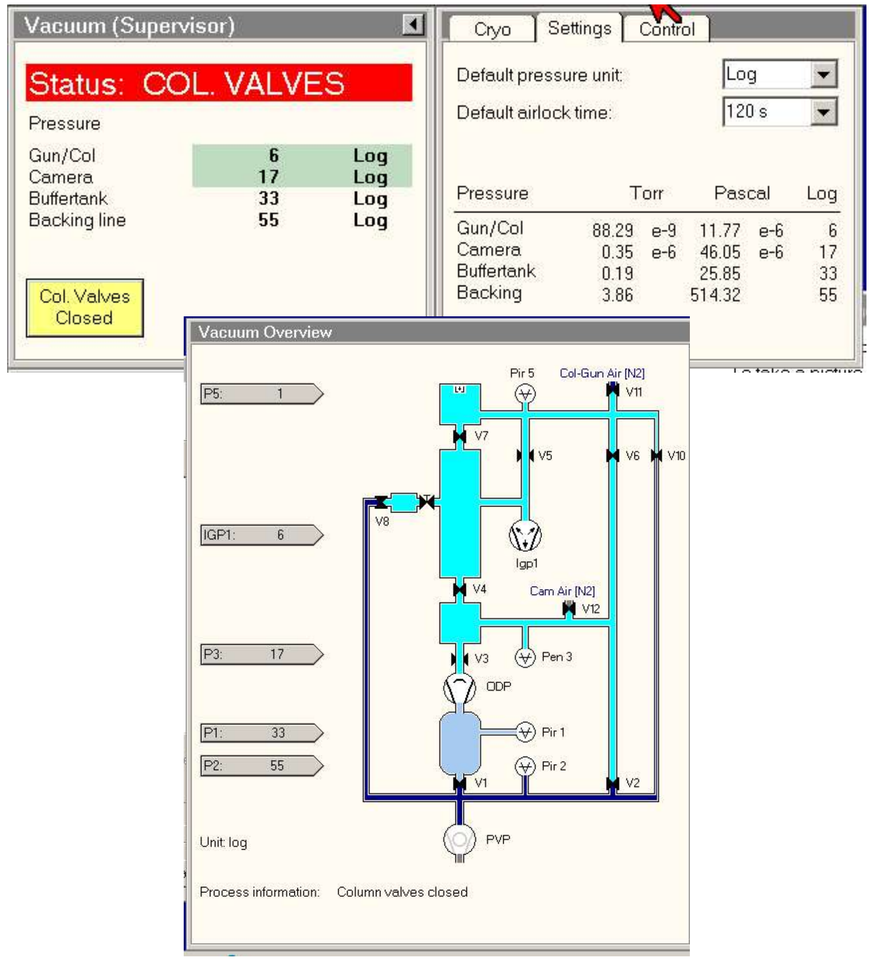
1 mm Hg = 1 Torr = 10^2 Pa

1 atm = 760 Torr = 7.5×10^4 Pa

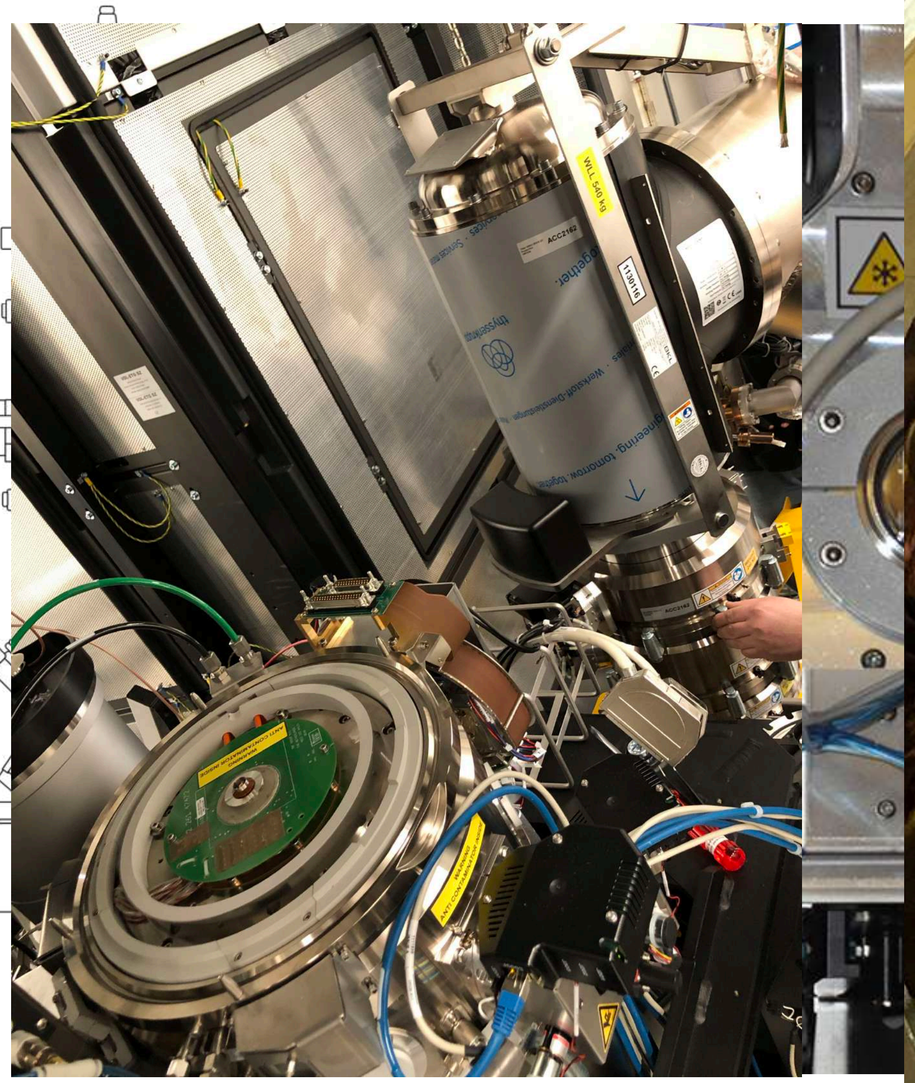
Gun 10^{-9} Torr

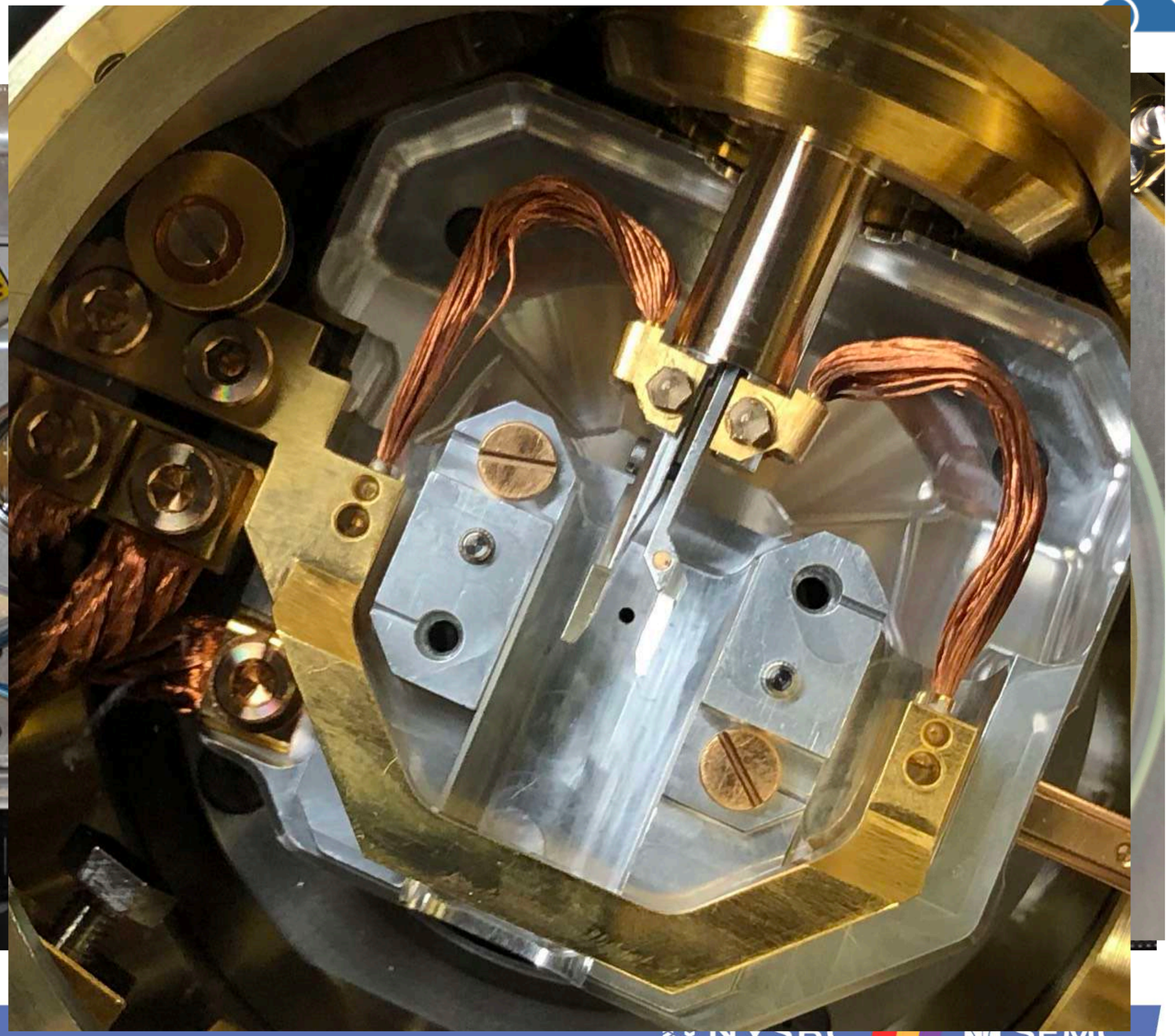
Specimen 10^{-6} -10^{-7} Torr

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

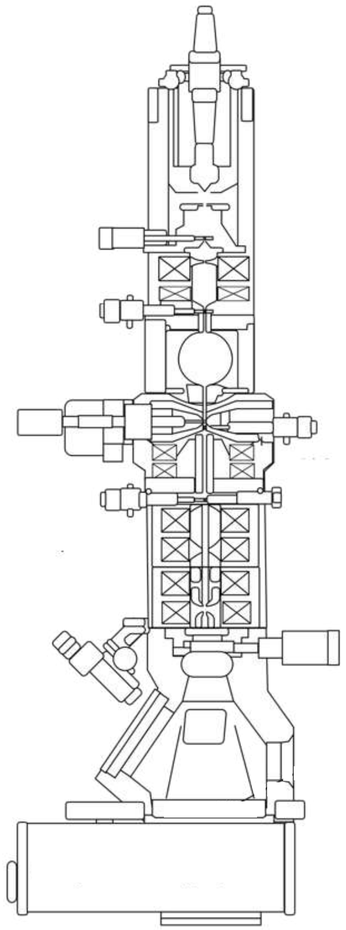


Vacuum systems

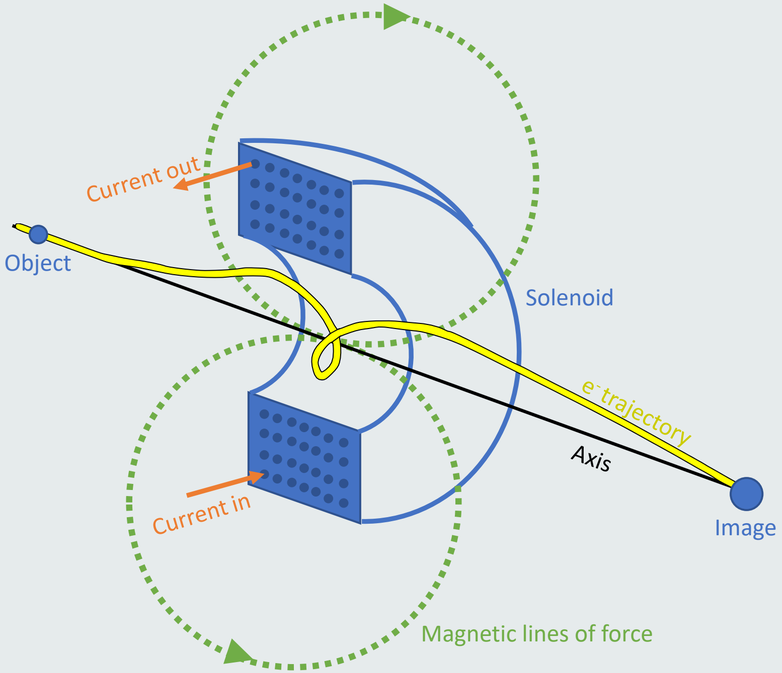




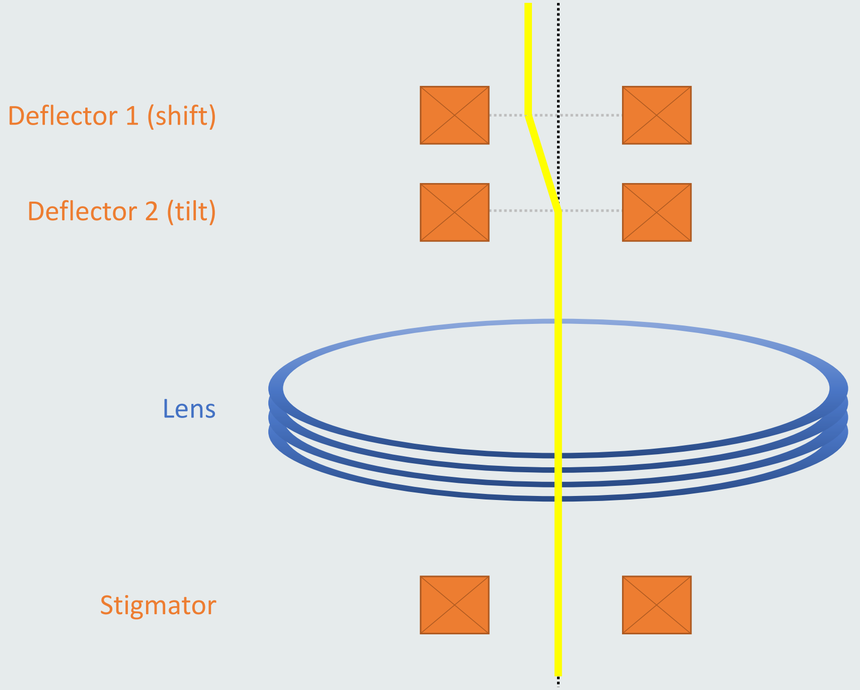
Lenses



What types of lenses do we have?

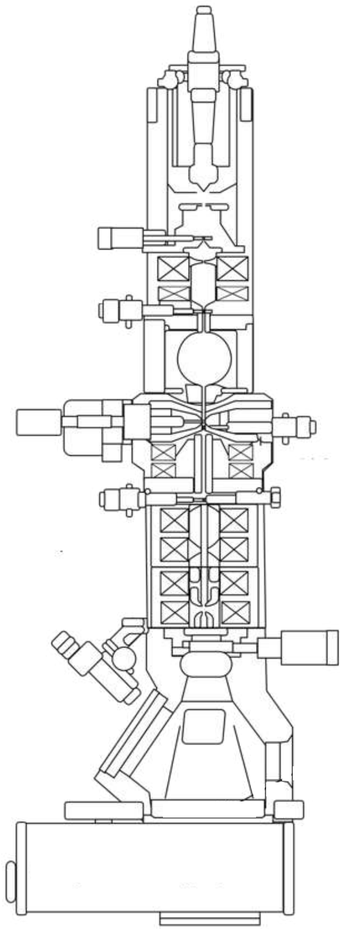


- Focus
- Magnify
- Rotate

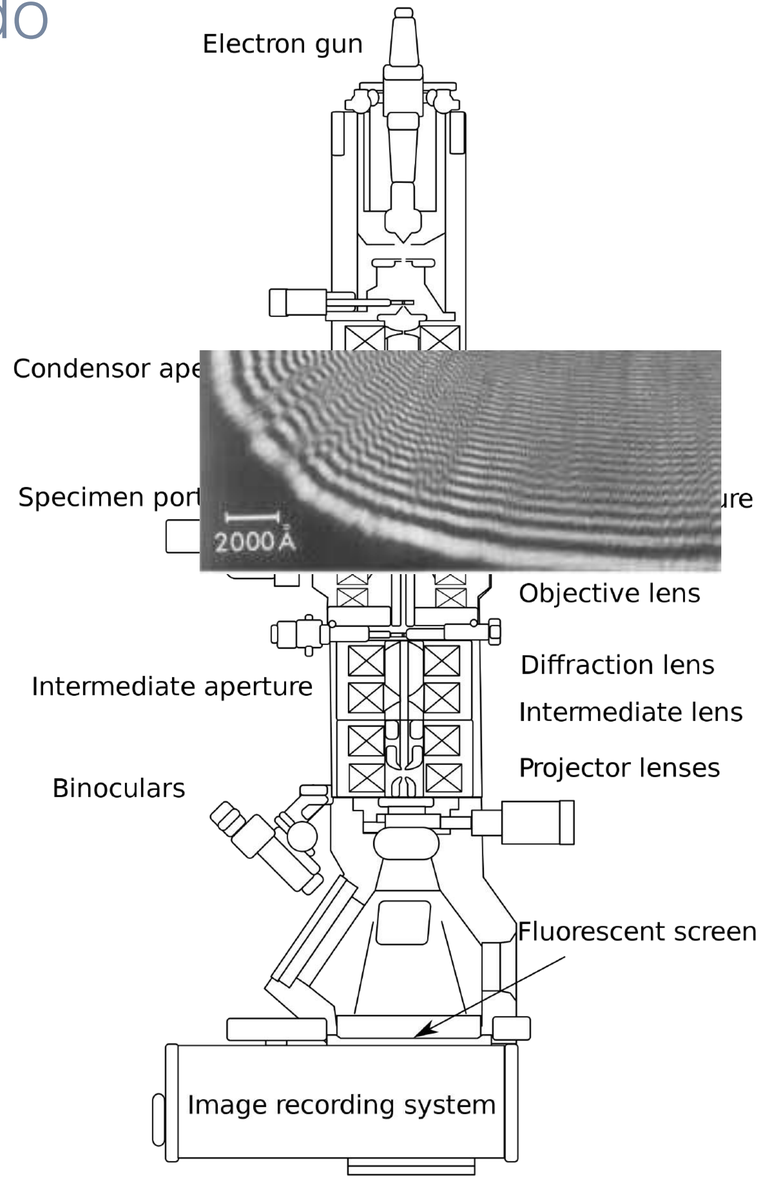


Lenses

Microscope Alignments
What to do & what not to do

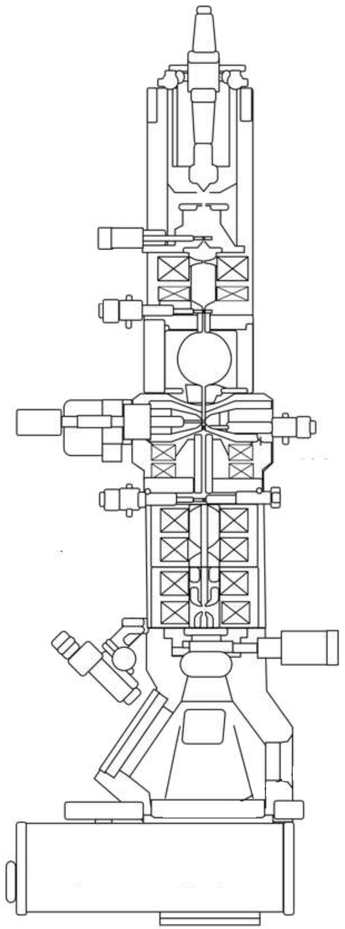


- Do:
  - Start at eucentric height and focus
  - Check if it is already good before attempt
  - Align from top to bottom
- Not to do:
  - ~~Align without a way to undo~~
  - ~~Align when TEM is not stable (i.e., temperature)~~

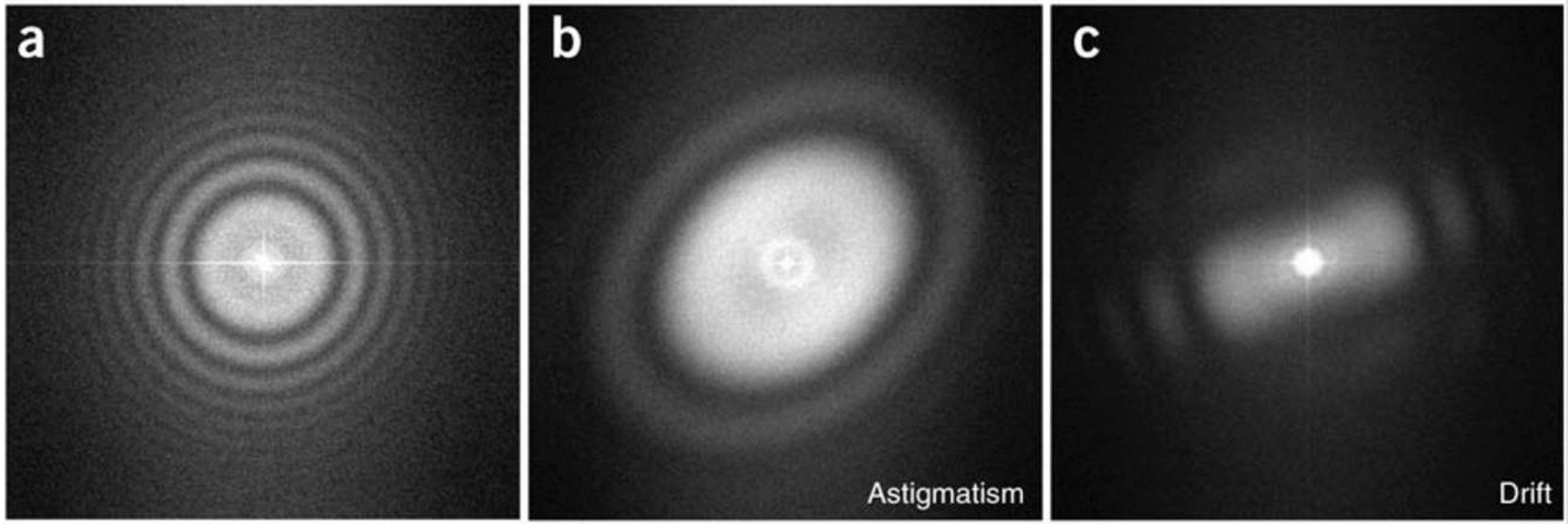


Lenses

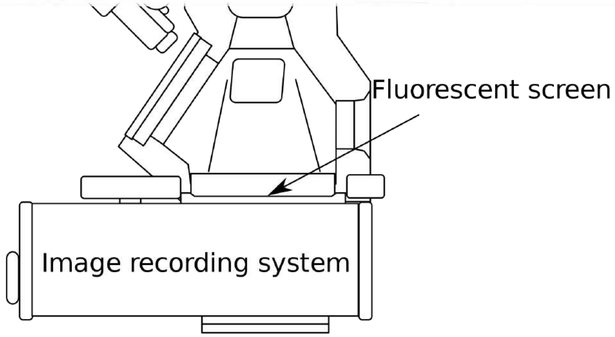
Microscope Alignments
What to do & what not to do



- Do:

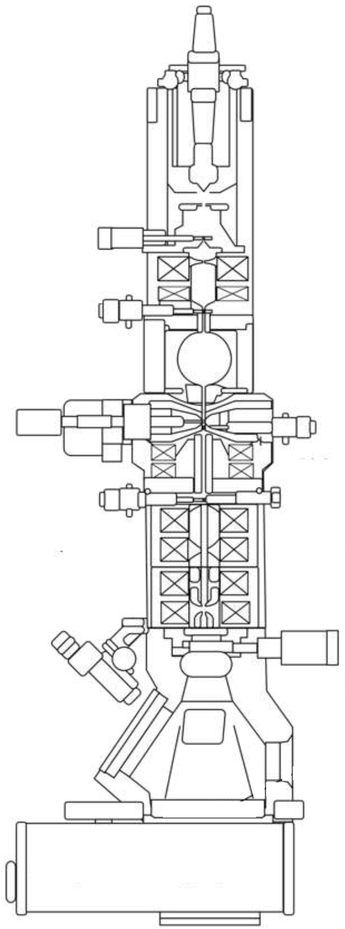


- ~~Align without a way to undo~~
- ~~Align when TEM is not stable (i.e., temperature)~~



Lenses

CTF



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

a

The phase shift (phase distortion function) due to the objective lens can be combined into a single phase factor χ, given by,

$$\chi(|g|)=\left(\frac{1}{2}\pi C_s\lambda^3|g|^4-\pi\Delta f\lambda|g|^2\right)$$ ---------- [4236a.a]

$$=\frac{2\pi}{\lambda}\left(\frac{1}{4}C_s\alpha^4-\frac{1}{2}\Delta f\,\alpha^2\right)$$ ---------- [4236a.b]

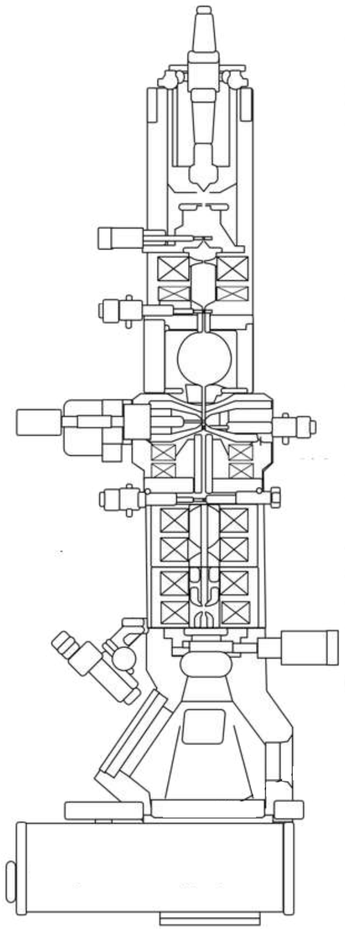
where,

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

Lenses

CTF



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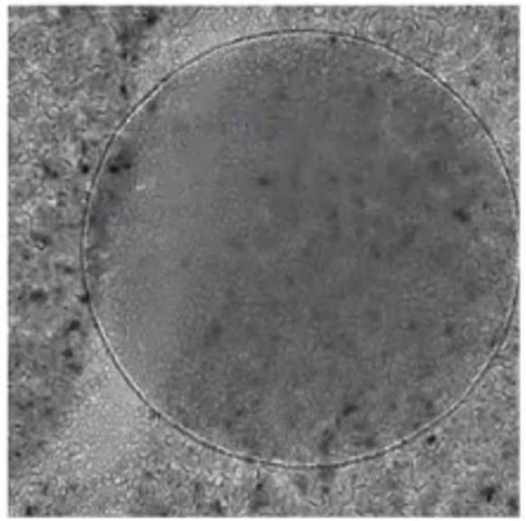
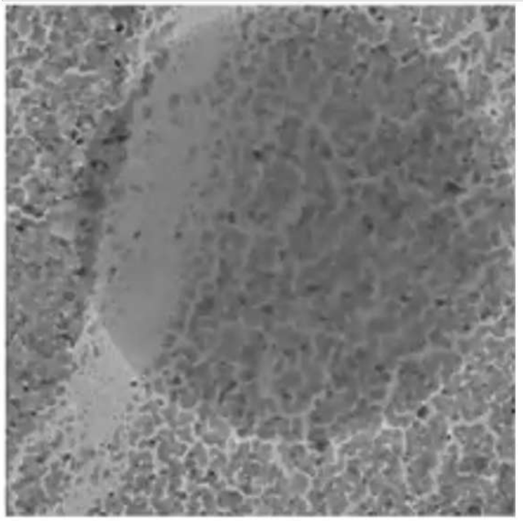
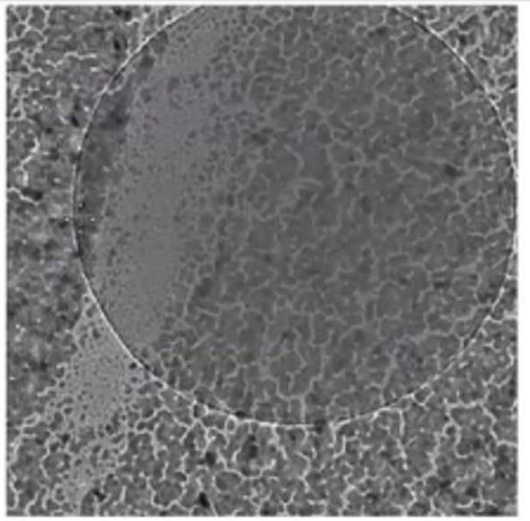
where







Lenses

How to increase efficiency?

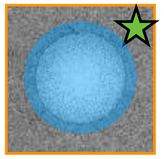
1 target/setup
80 s/image
~1000 images/day

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

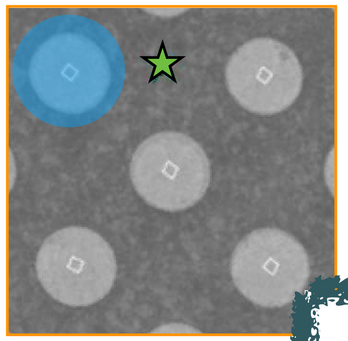
30 targets/setup
22 s/image
~3800 images/day

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

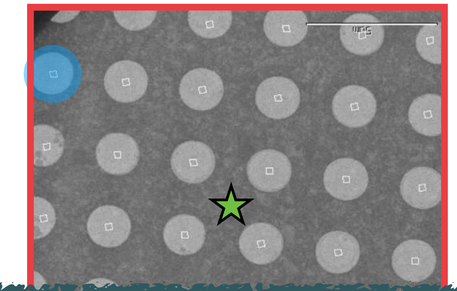
13.6MP

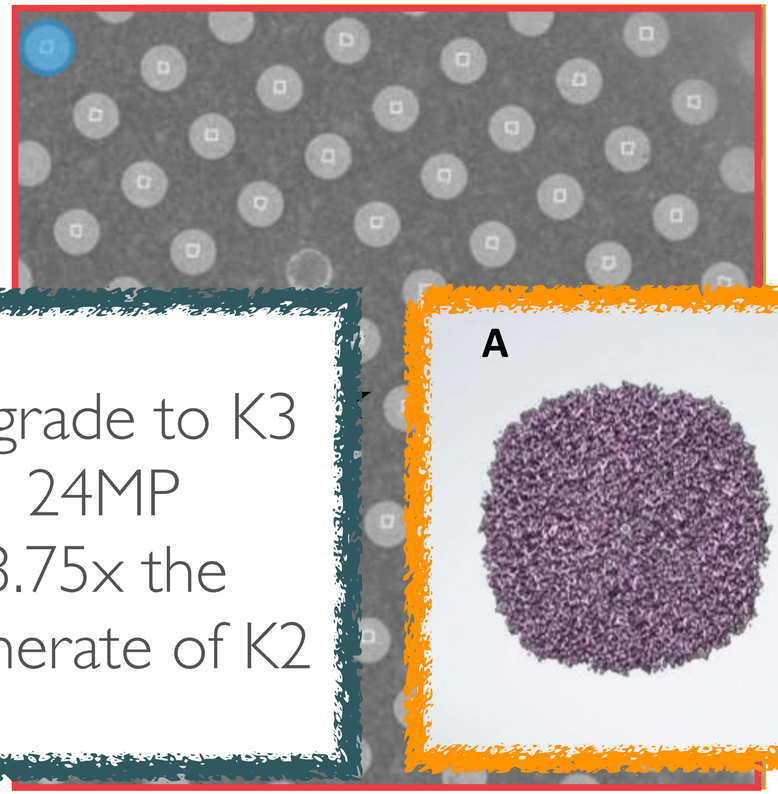


beam tilt
0 mrad



beam tilt
0.5 mrad





beam tilt
~3 mad

Upgrade to K3
24MP
3.75x the
framerate of K2

But… image shift ind

so… implement hardwar

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

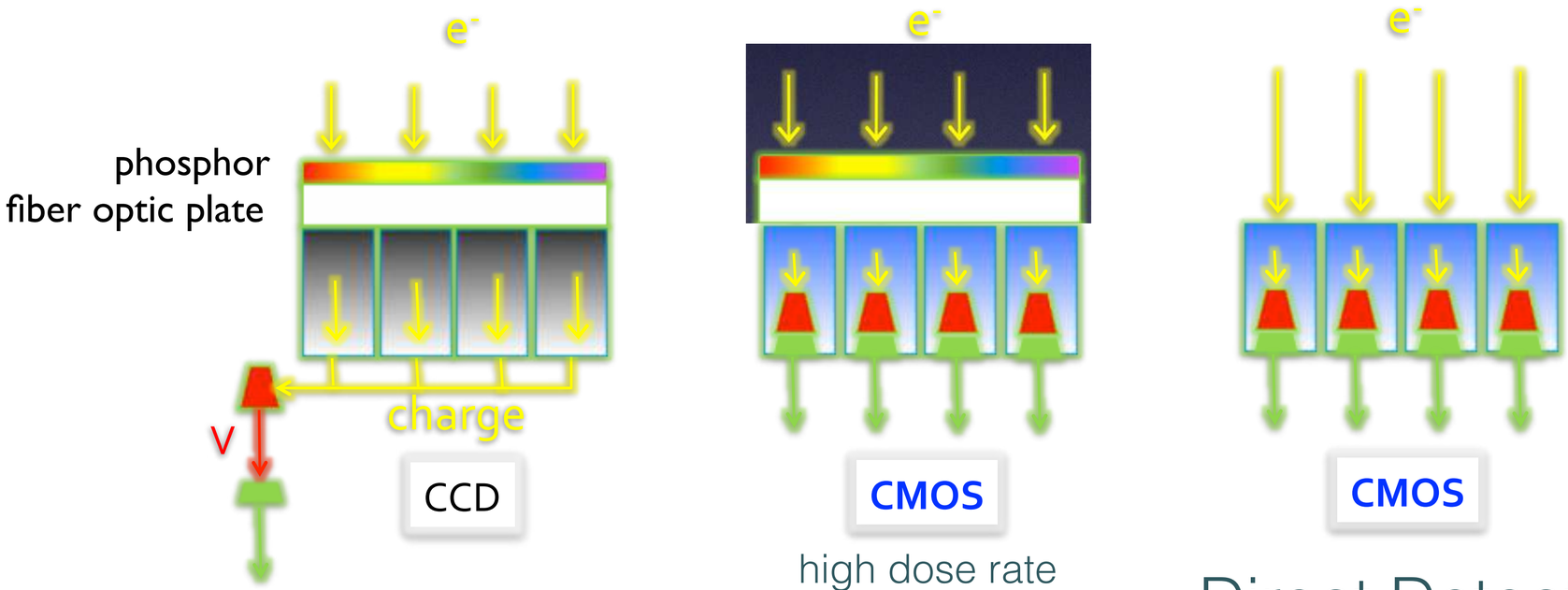
Detectors

Digital Cameras for TEM

- Photon converted
- Direct sensing

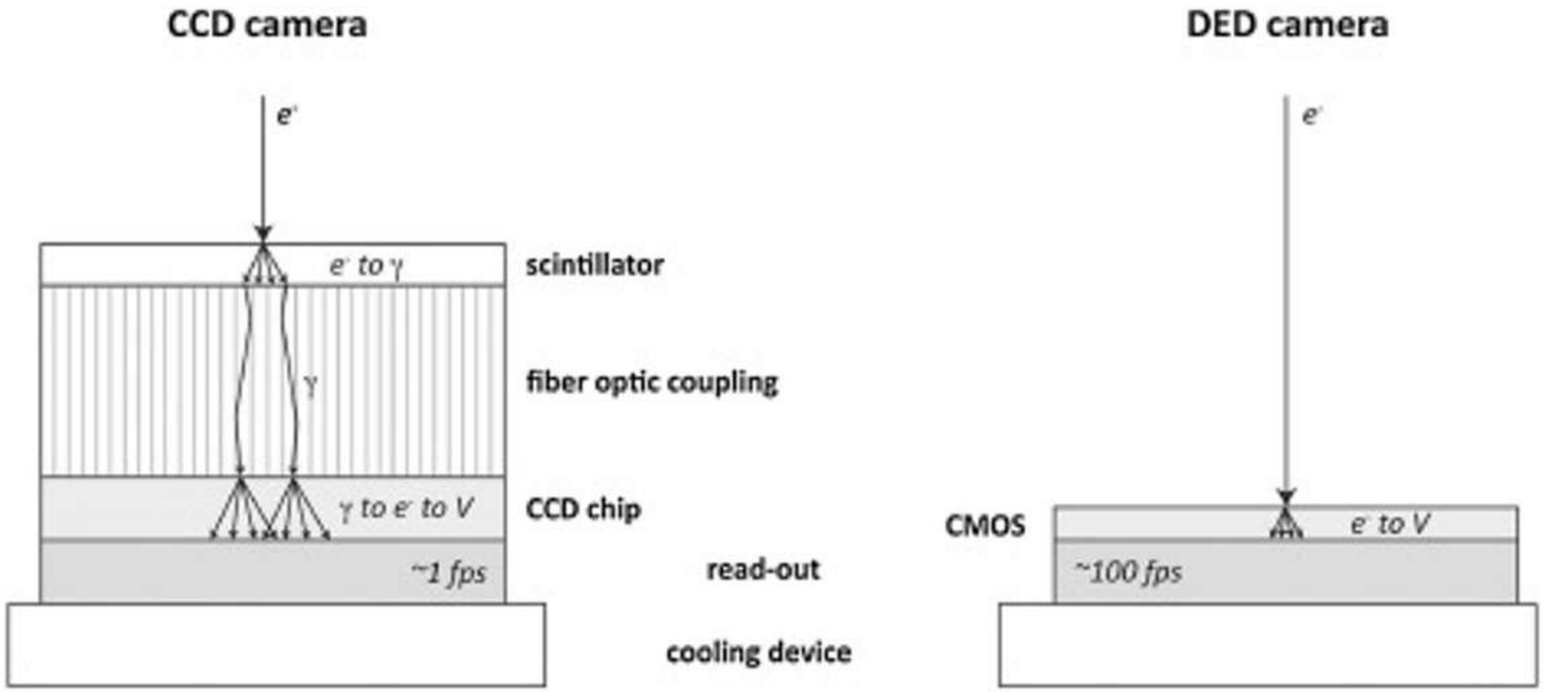
?

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



Detectors

Digital Cameras for TEM

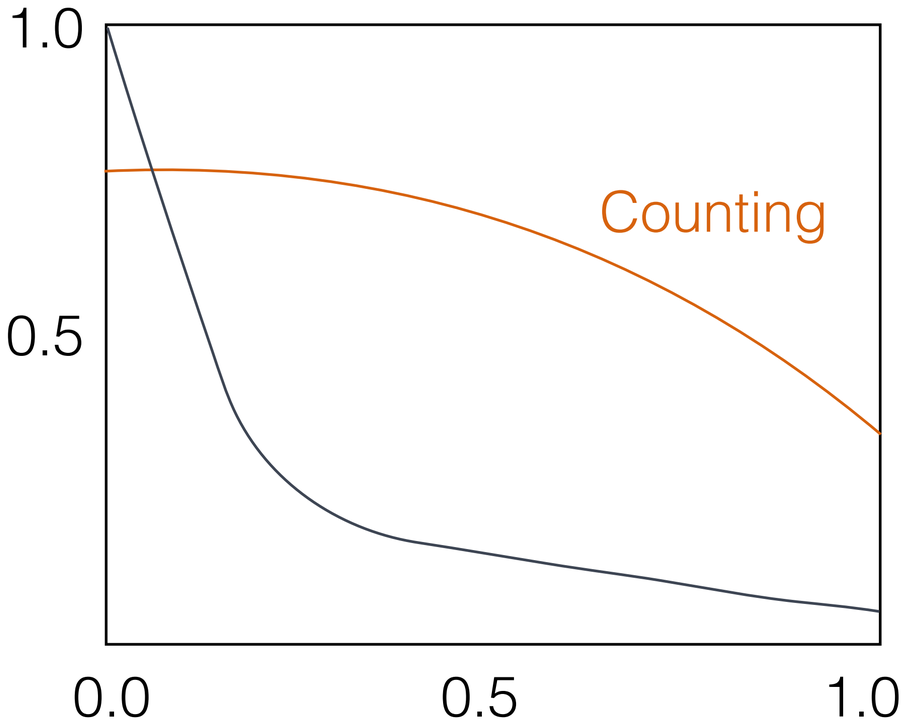


Koning et al. Ann. Anatomy 2018

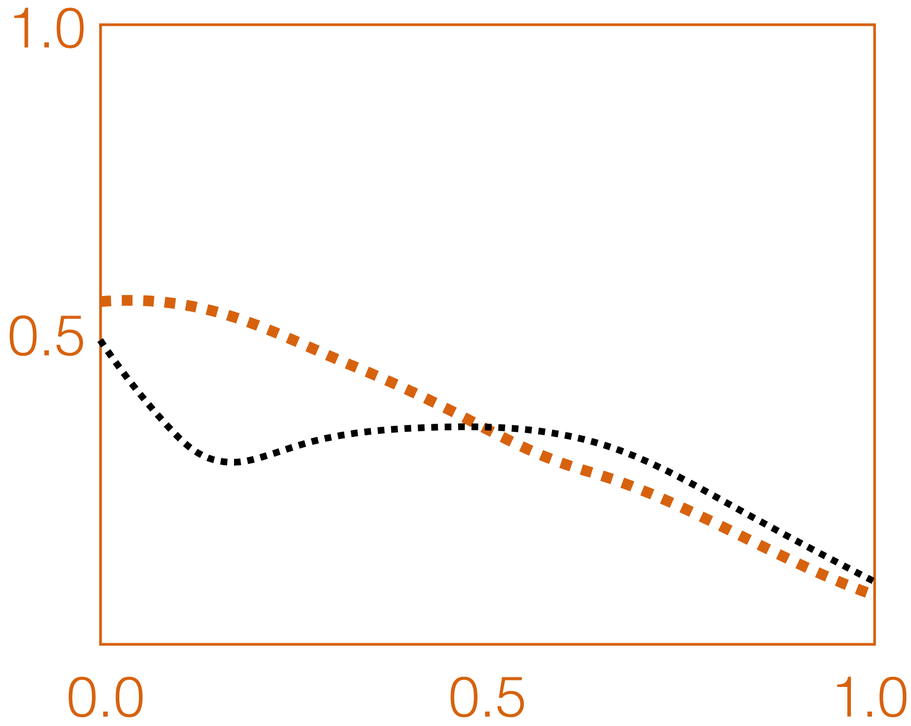
Detectors

Detector Performance Characterization

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



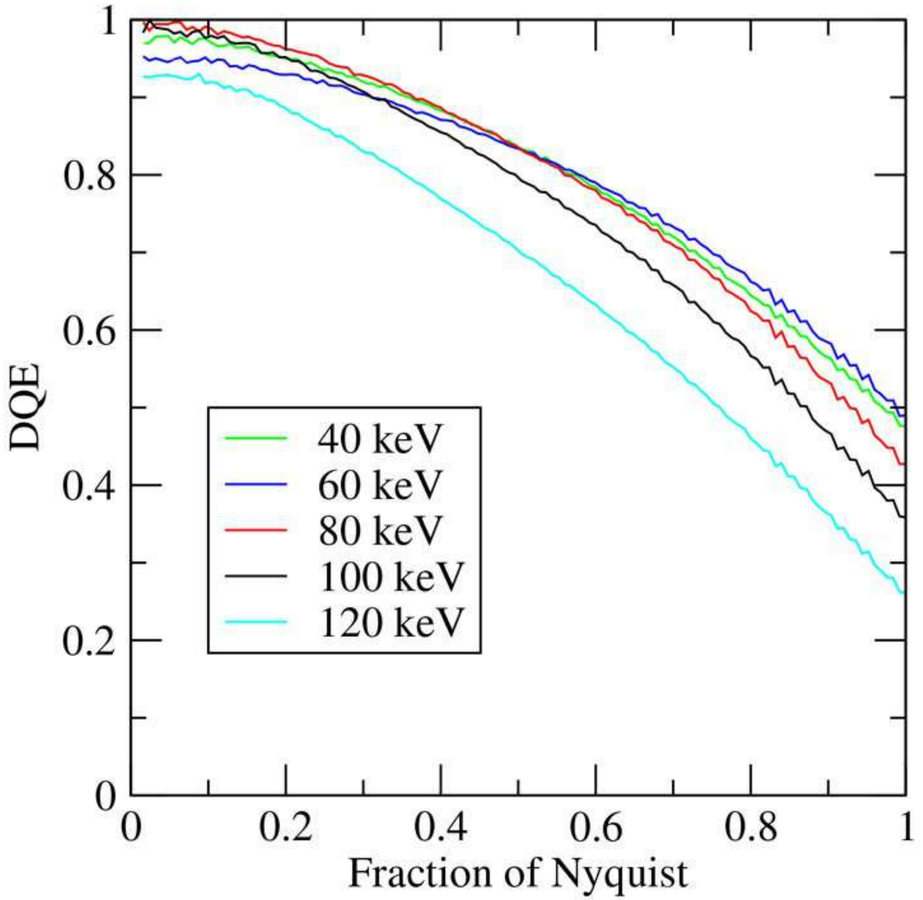
- DQE (Detector Quantum Efficiency)
  - S/N over spatial frequency range



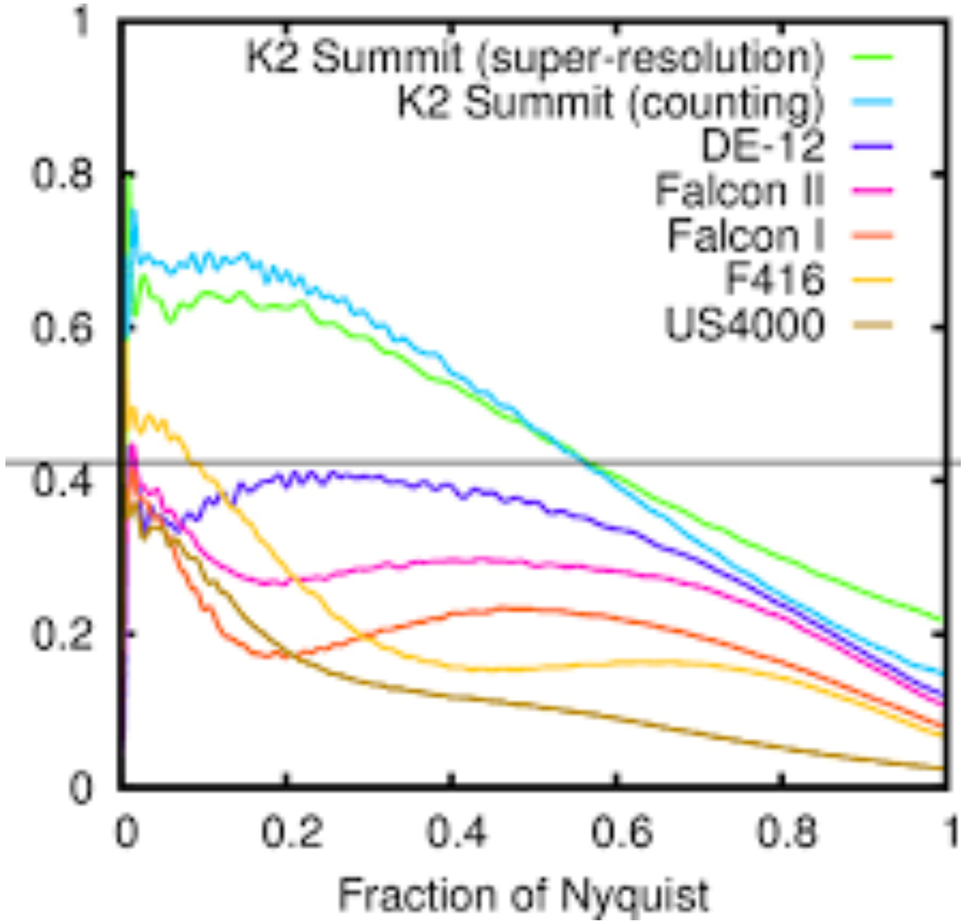
PSF: the point spread function describes the response of an imaging system to a point source or point object.
MTF: the modulation transfer function, is defined as the Fourier transform of the point spread function

Detectors

Detector Performance Characterization



dectris.com

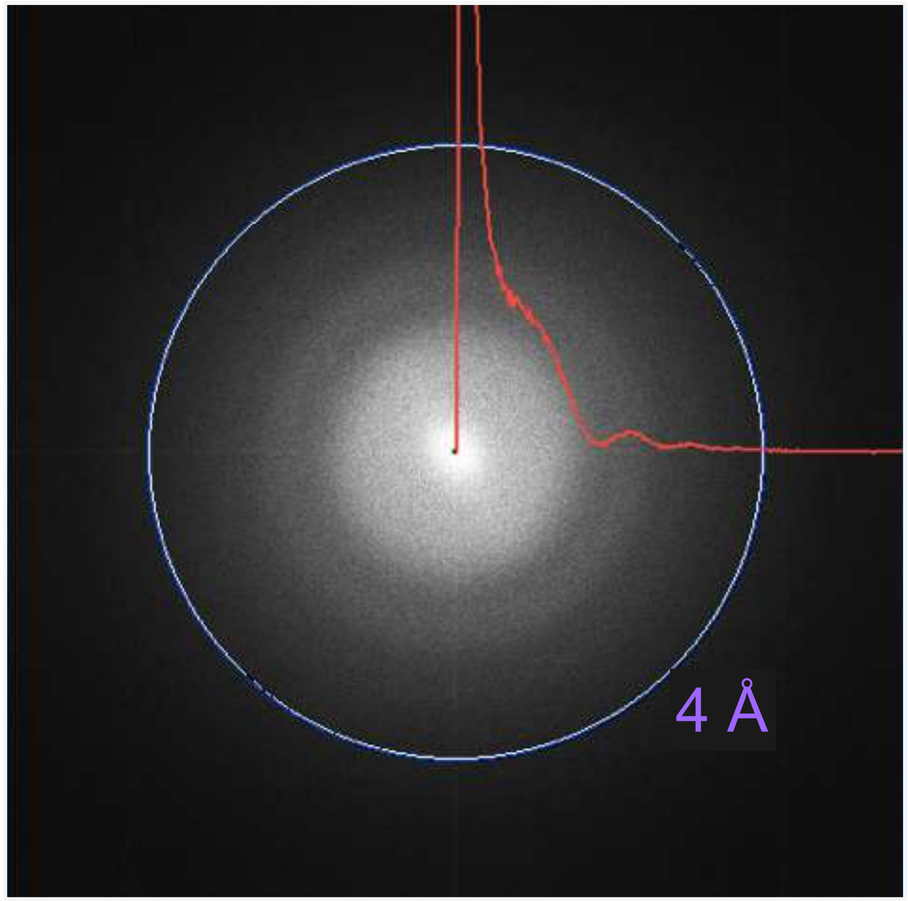


Ruskin, et al JSB

Detectors

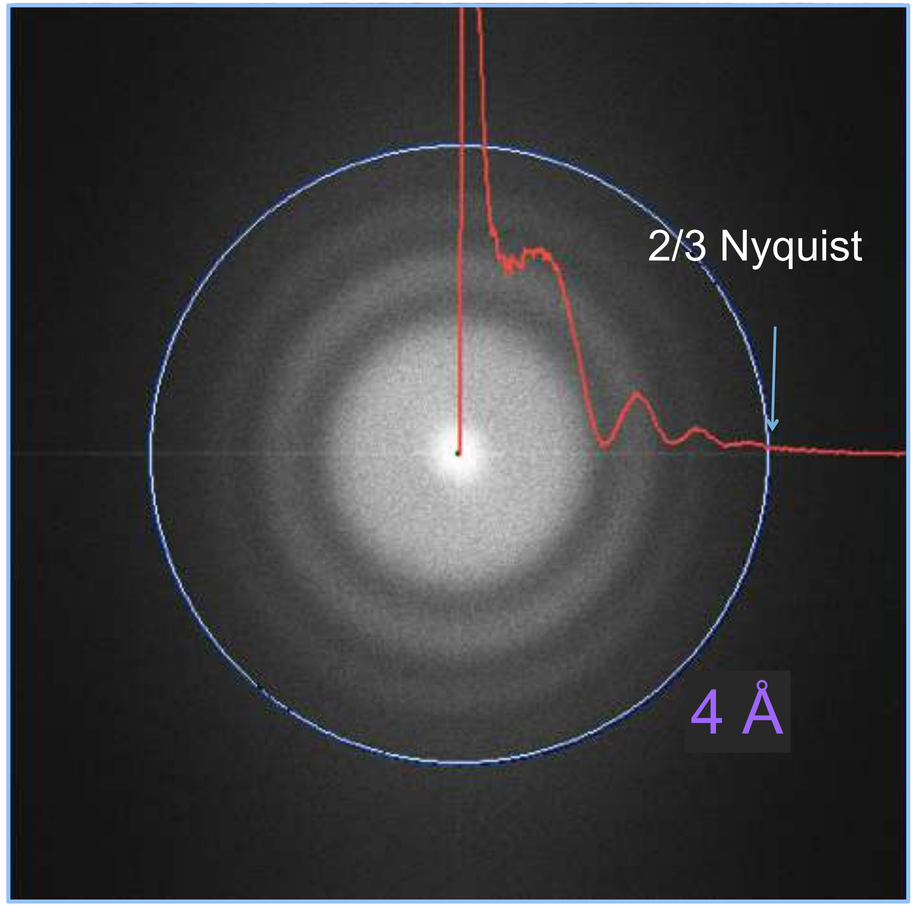
Improving the resolution:
Detecting electrons instead of photons

CCD



1.37 Å/pixel

DDD



1.38 Å/pixel

200KeV ; 20 $e^-/Å^2$; carbon film ;
3k x 3k image

Detectors

Improving the resolution:
Detecting electrons instead of photons

K3 specs

https://www.gatan.com/K3

Specifications

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

Specifications are subject to change without notice.

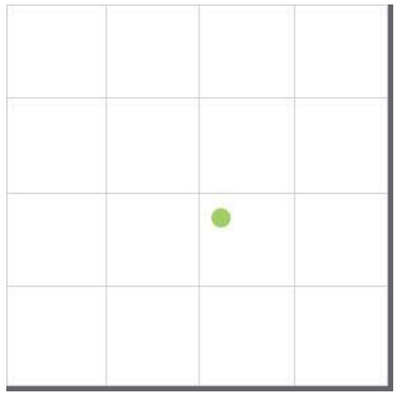
Detectors

Improving the resolution:
Detecting electrons instead of photons

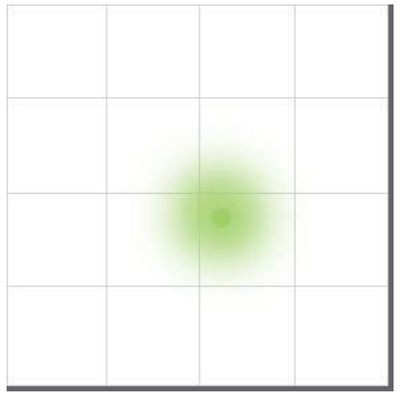
5,760 x 4,096 px

11,520 x 8,184 px

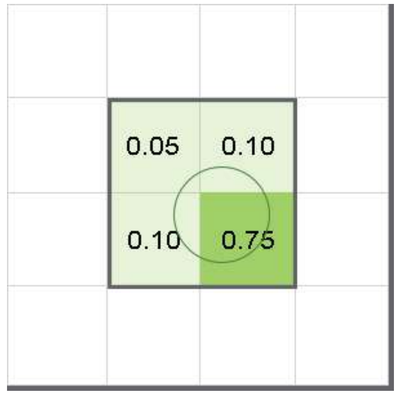
Counting mode



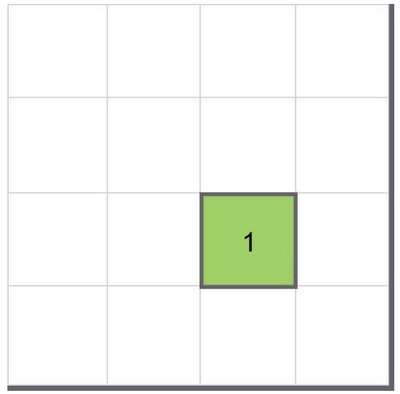
Electron enters detector.



Electron signal is scattered.



Charge collects in each pixel.



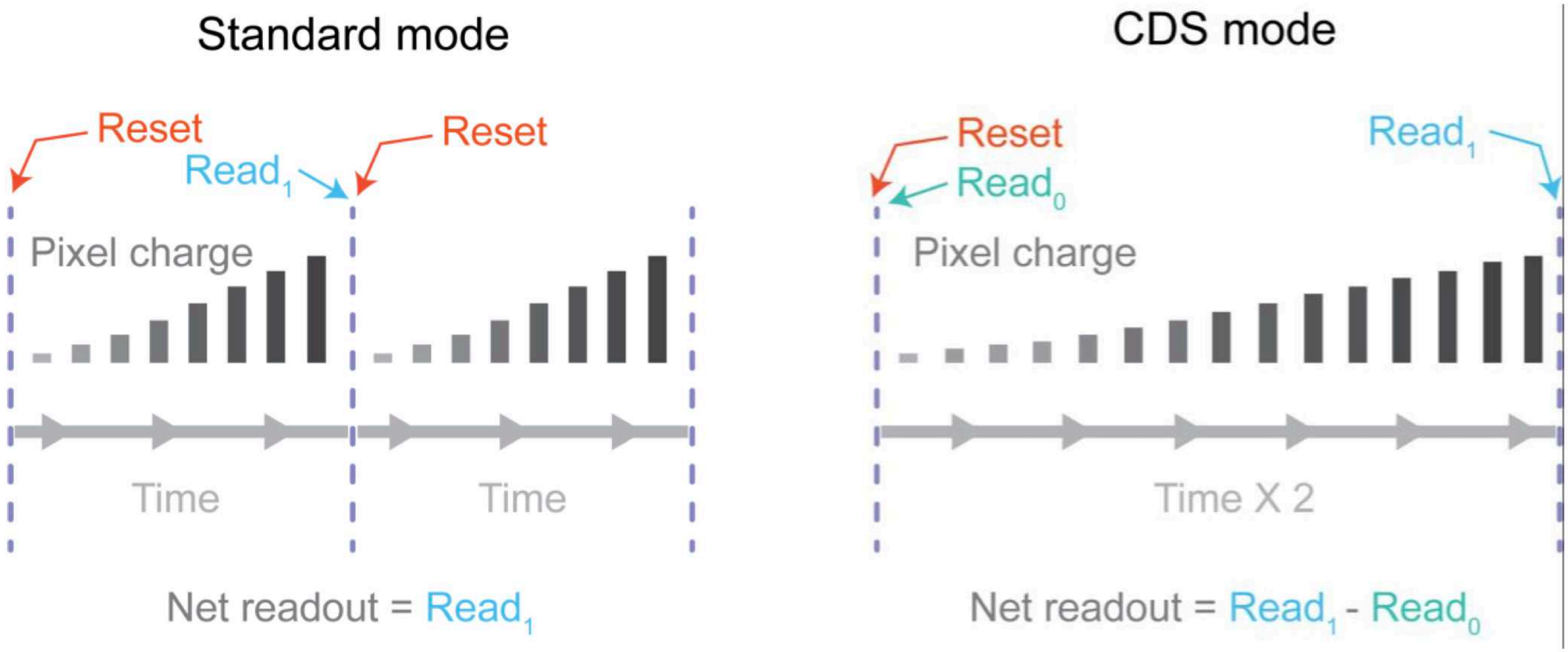
Events reduced to highest charge pixels.

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

Detectors

Improving the resolution:
Detecting electrons instead of photons

K3 lowers Read Noise with Correlated Double Sampling (CDS)

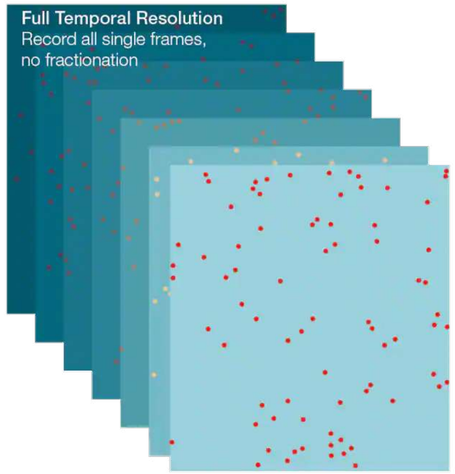


https://www.gatan.com/

Detectors

Improving the resolution:
Detecting electrons instead of photons

Falcon4 specs



Full Spatial Resolution
All localized events

Coordinates

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

Counted events of all raw frames with full temporal resolution (320 fps) and spatial resolution (events are localized to one-sixteenth of a pixel).

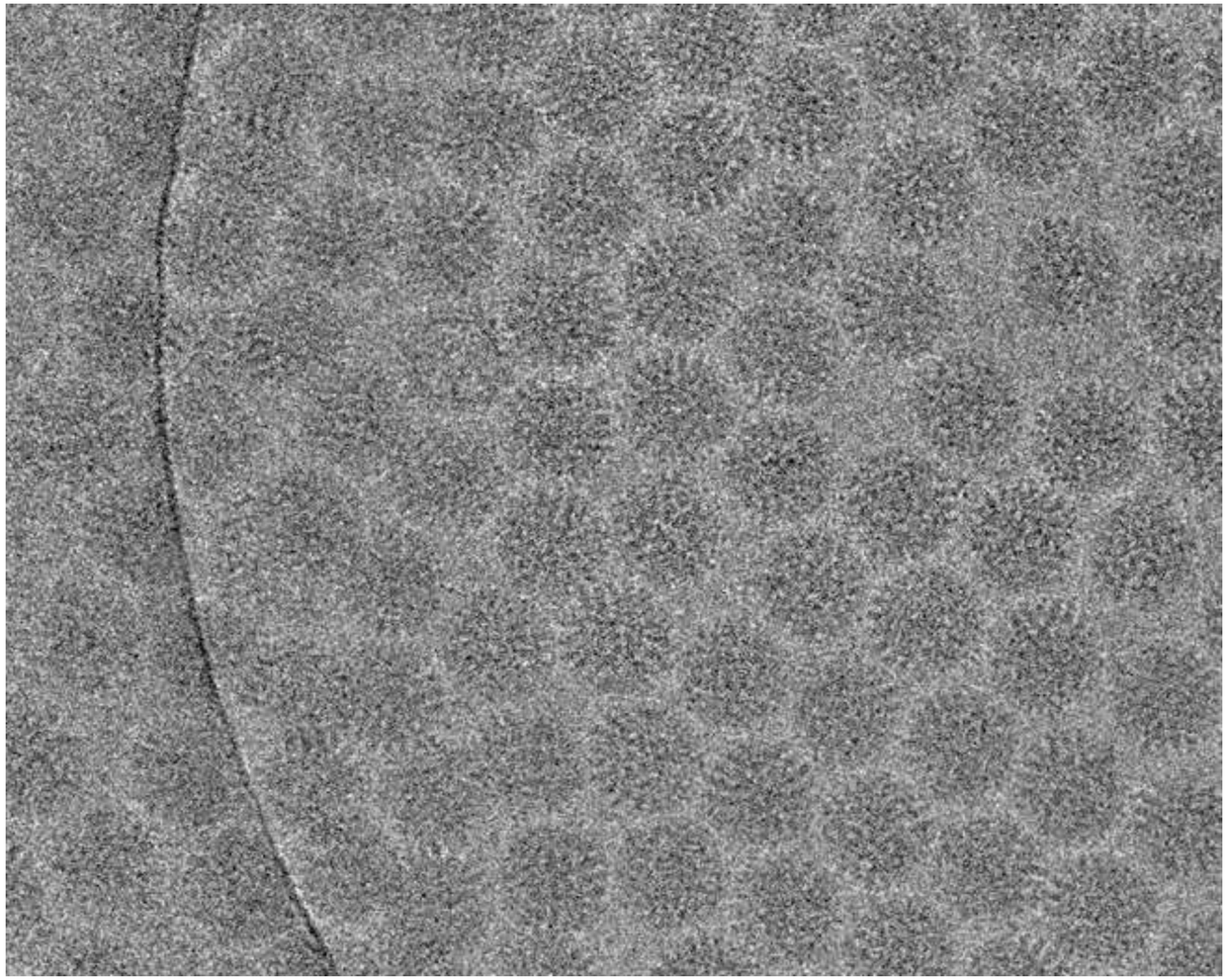
Camera architecture	Direct electron detection	
Sensor size	4,096 × 4,096 pixels, ~ 5.7 x 5.7 cm^2	
Pixel size	14 x 14 μm^2	
TEM Operating voltage	200 kV, 300 kV	
Internal frame rate	320 fps	
Frame rate to storage	320 fps (EER mode)	
Camera Overhead time	0.5 s per acquisition	
File formats	EER (native), MRC, TIFF, LZW TIFF	
Lifetime (<10% DQE degradation)	5 years in normal use (1.5Ge/px)	
Detection Modes	Electron counting mode Survey mode (fast linear mode)	
Imaging performance in EER mode (4k x 4k)	300 kV	200 kV
DQE (0)	0.92	0.91
DQE (½ Nq)	0.72	0.62
DQE (1 Nq)	0.50	0.33

Electron-event representation (EER)

https://www.thermofisher.com/us/en/home/electron-microscopy/products/accessories-em/falcon-detector.html

Detectors

Images are movies



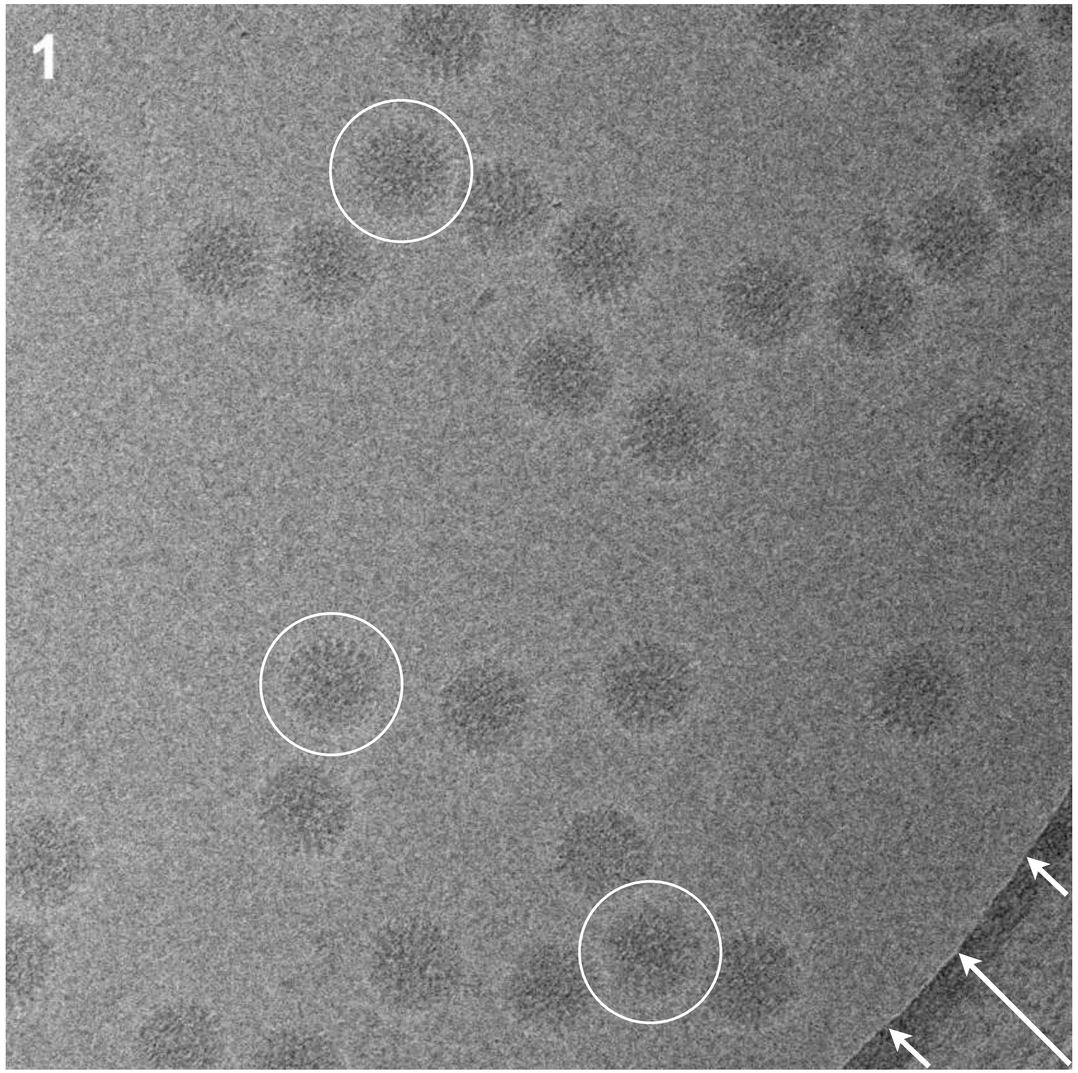
0.5 $e^-/Å^2$/frame

Image = Frame1 + Frame2 + Frame3 + Frame4 + Frame5

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

Detectors

Images are movies



10 frame averages

Each averaged frame corresponds to 0.25 s.

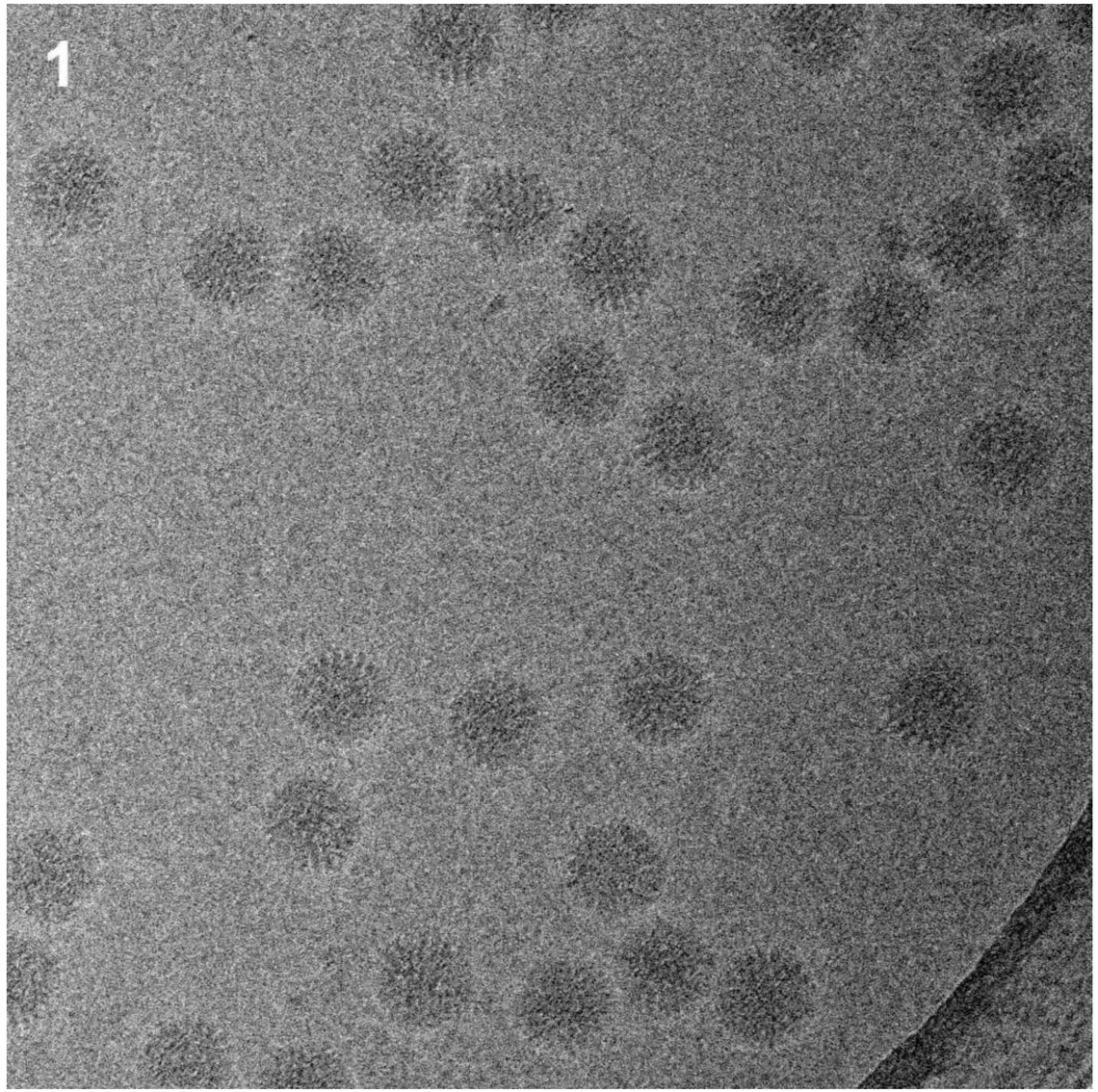
Dose/frame = 5 $e^-/Å^2$

A “movie” of rotavirus exposed to electron beam

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

Detectors

Images are movies



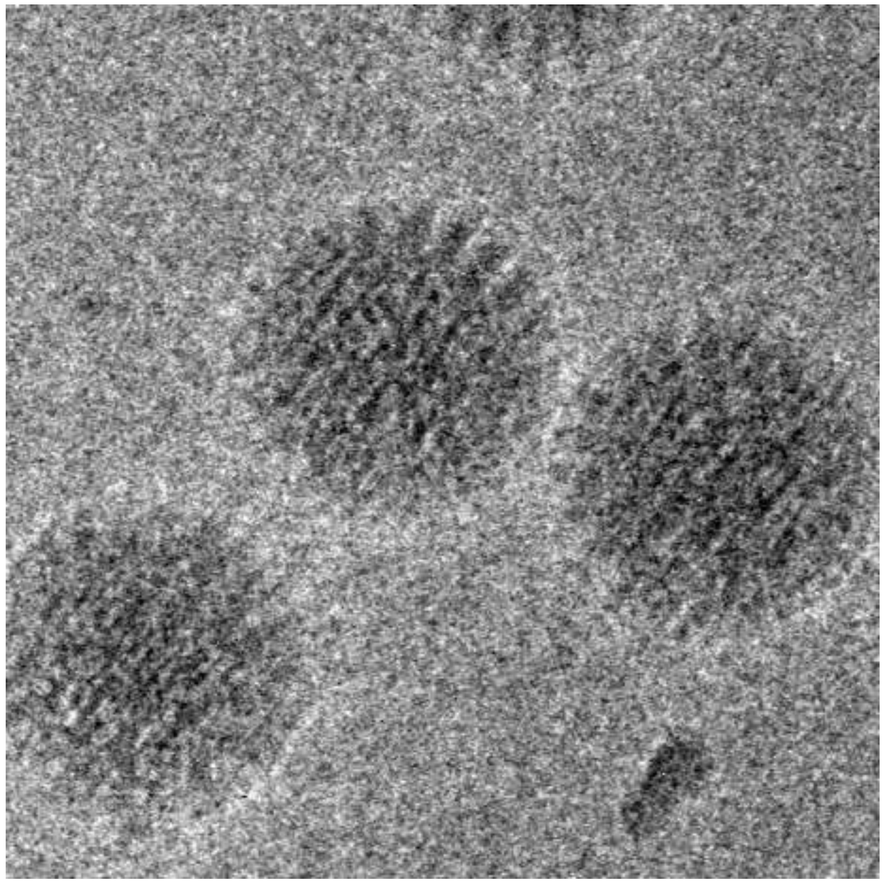
Translations over time

2.5nm

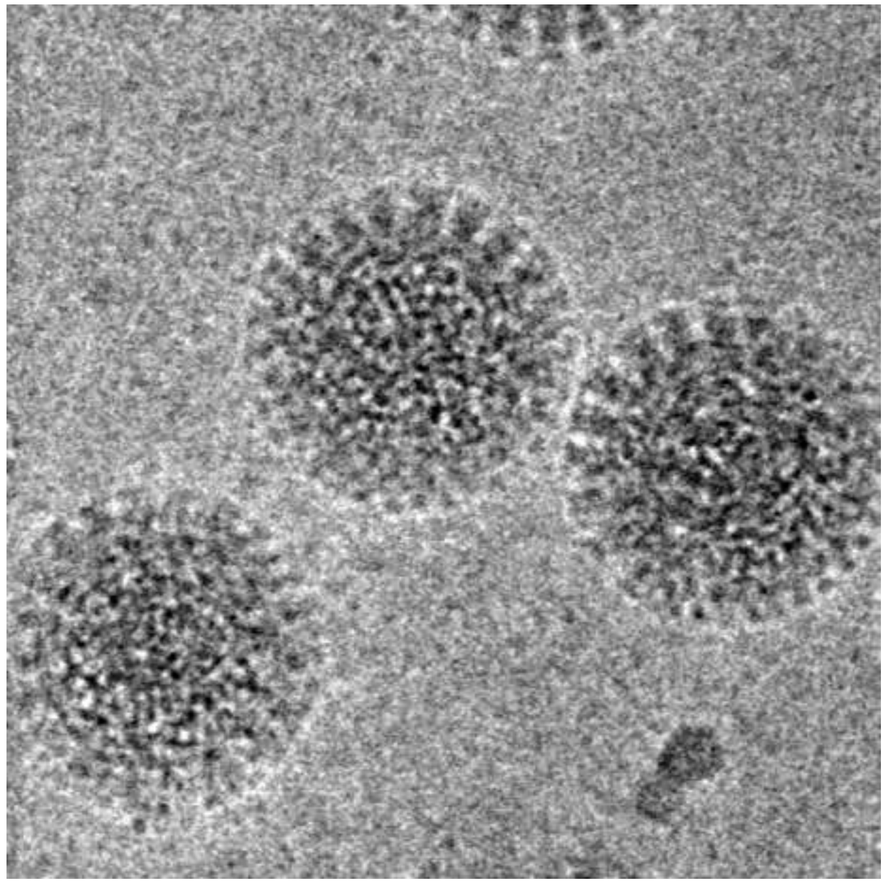
Detectors

Correcting for movement

60-frame average
(no alignment)



60-frame average
(translational alignment)



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

What brought about the resolution revolution?

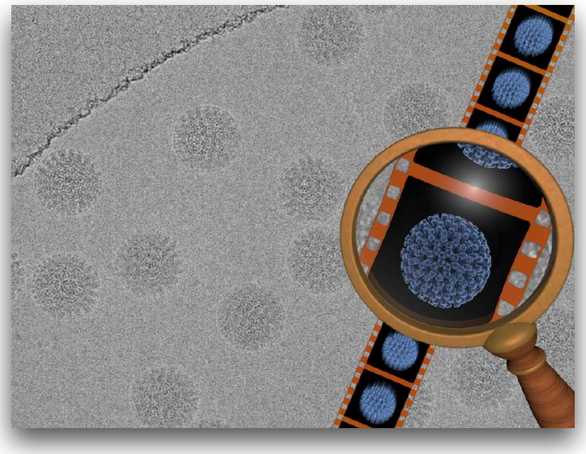
(~2012-2014)

Hardware

Software

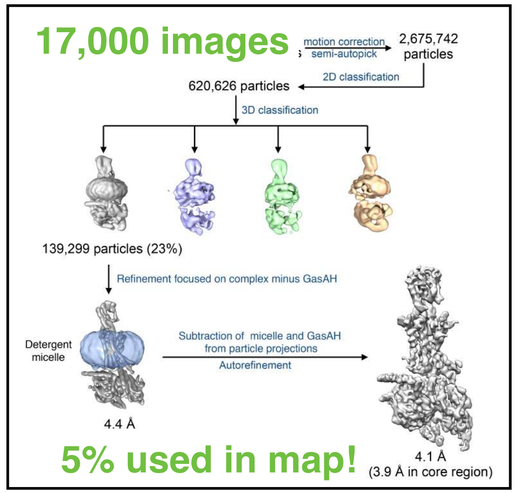
Microscopes

Direct Detectors



Computers

2012->2017
Cost reduced by 100x

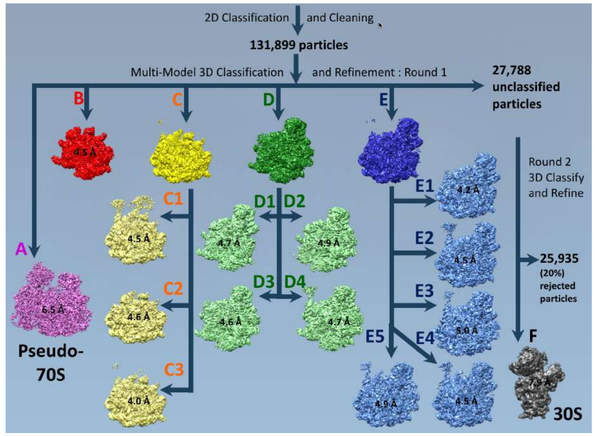


Leginon / SerialEM / EPU, …

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

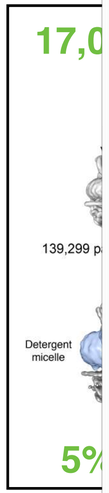
Denise Wootten, PhD

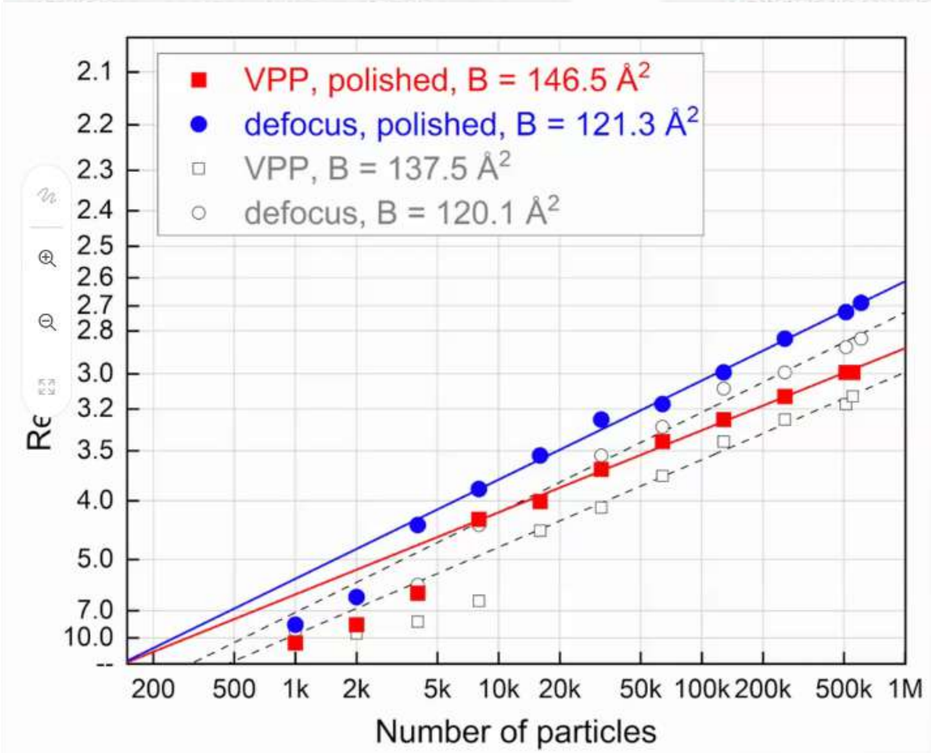
Monash Institute of Pharmaceutical Sciences, Monash University

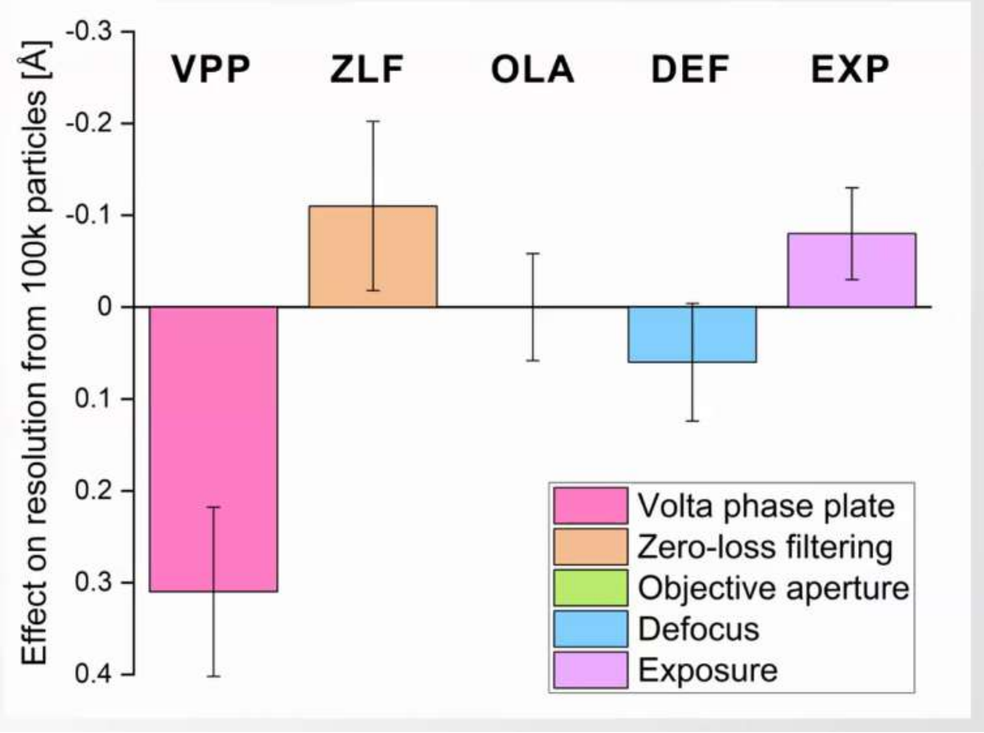
Radostin Danev, PhD

Graduate School of Medicine, The University of Tokyo

Software

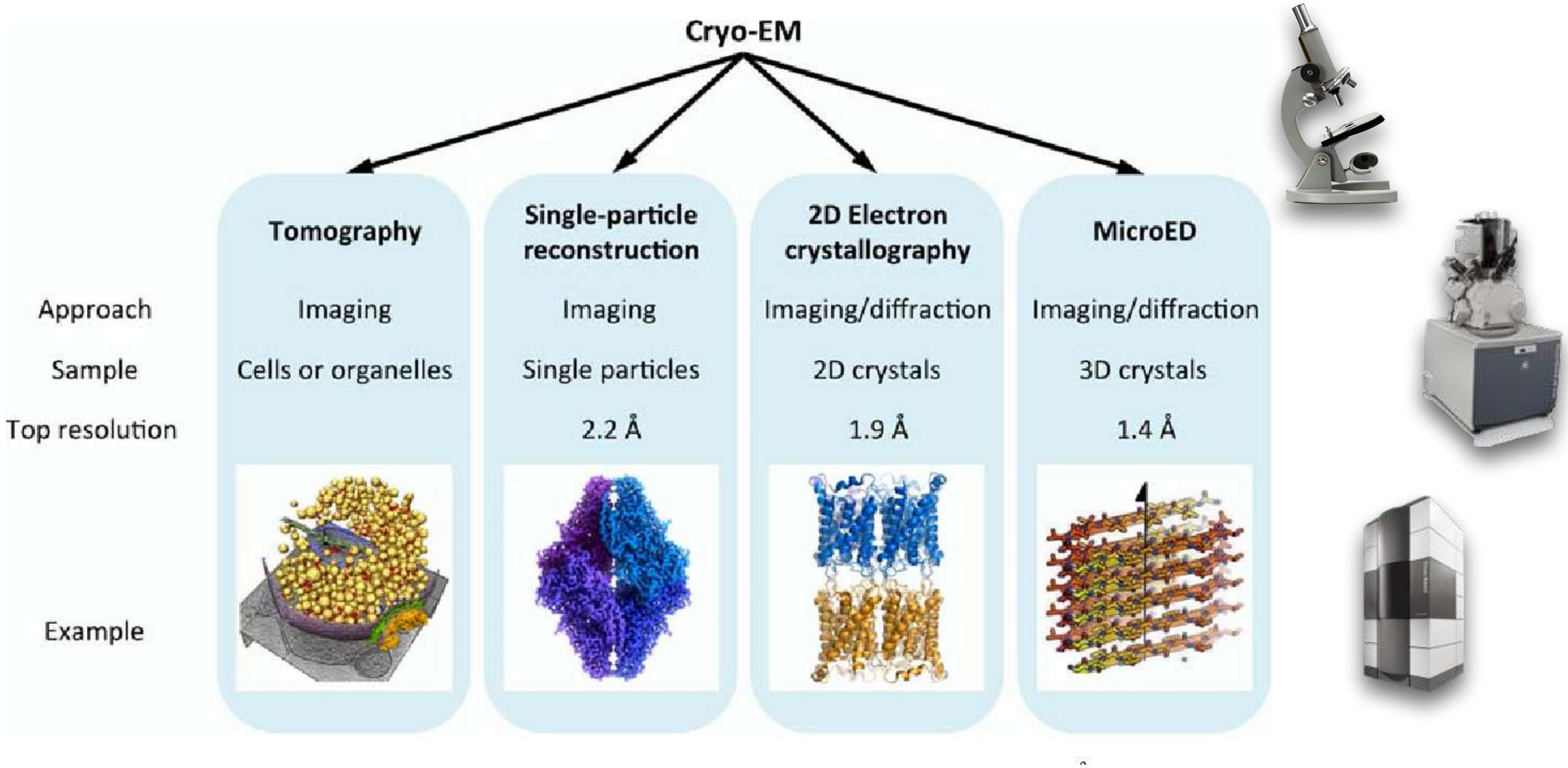






Nakane, et al. Single-particle cryo-EM at atomic resolution. Nature (2020).

Cryoem modalities and tools

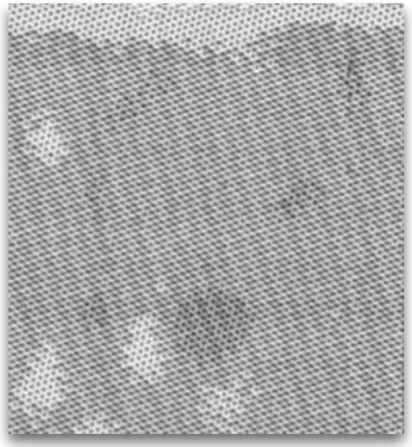


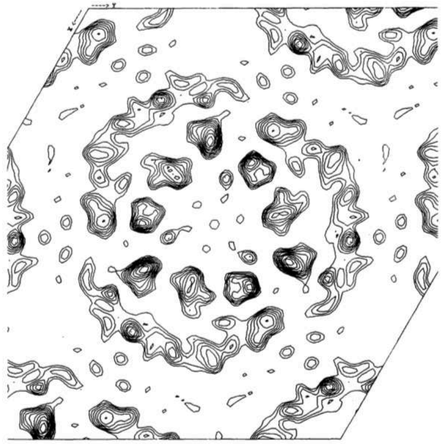
https://doi.org/10.1002/pro.2989

~3.4Å ~1.2Å ~1.9Å ~0.7Å

cryoEM: technology on the rise

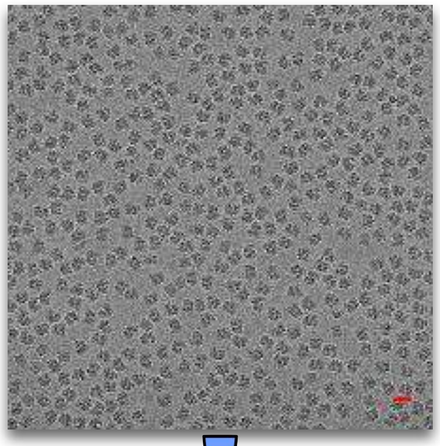
1986

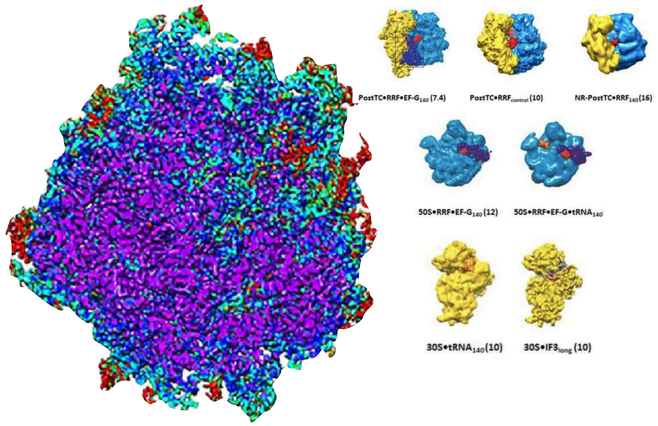




Henderson et al. (1986)

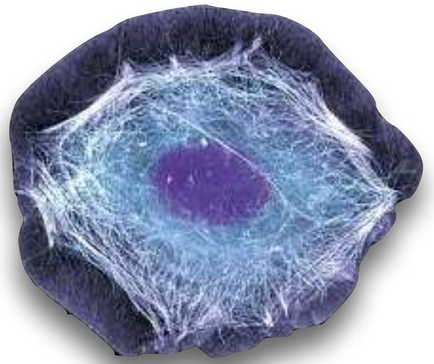
2017

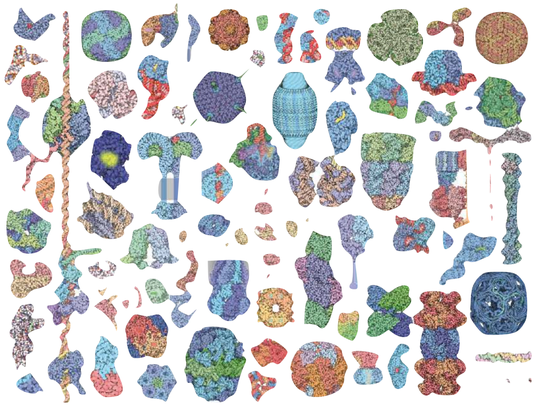




Frank et al. (2017)

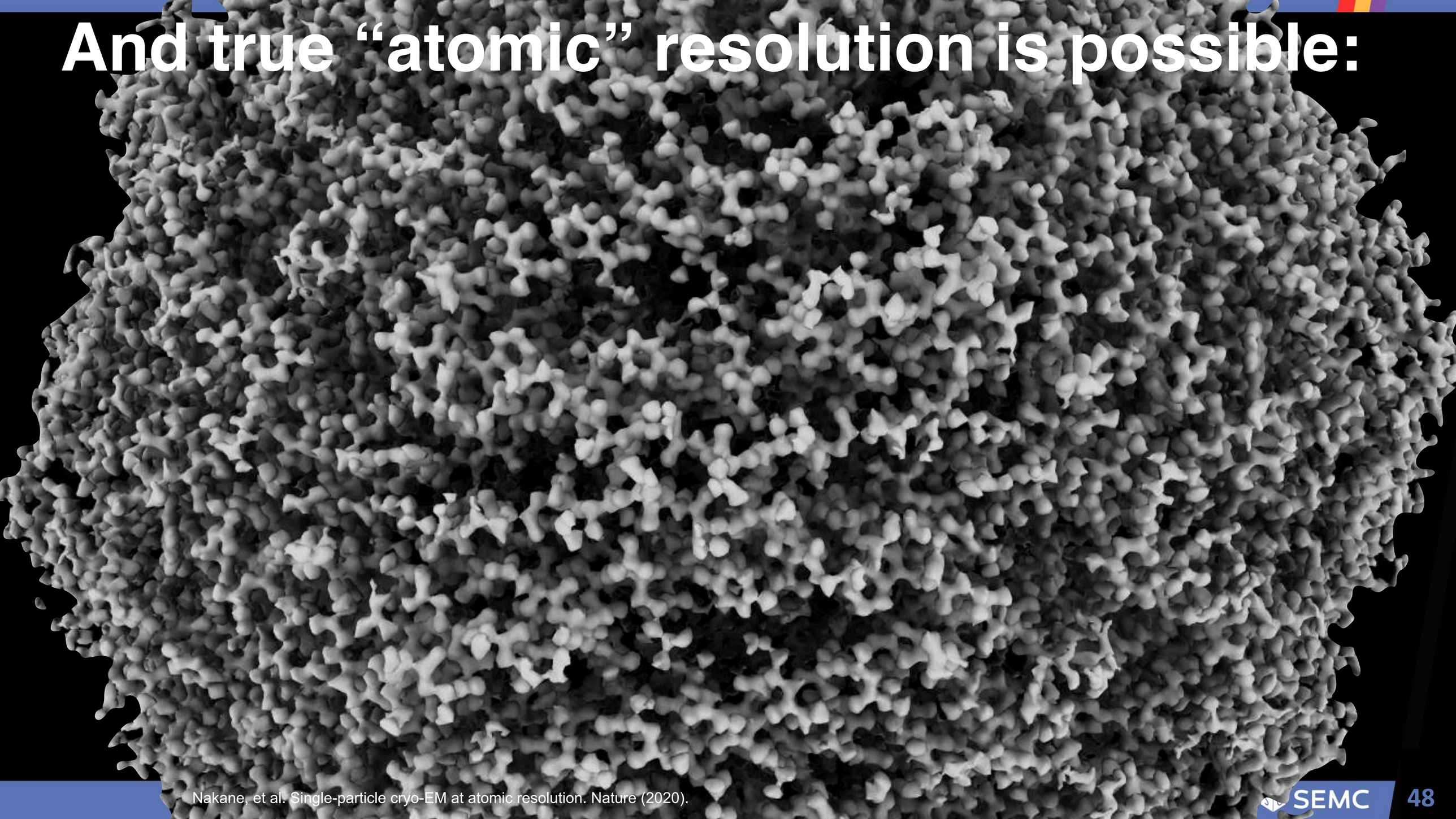
in progress





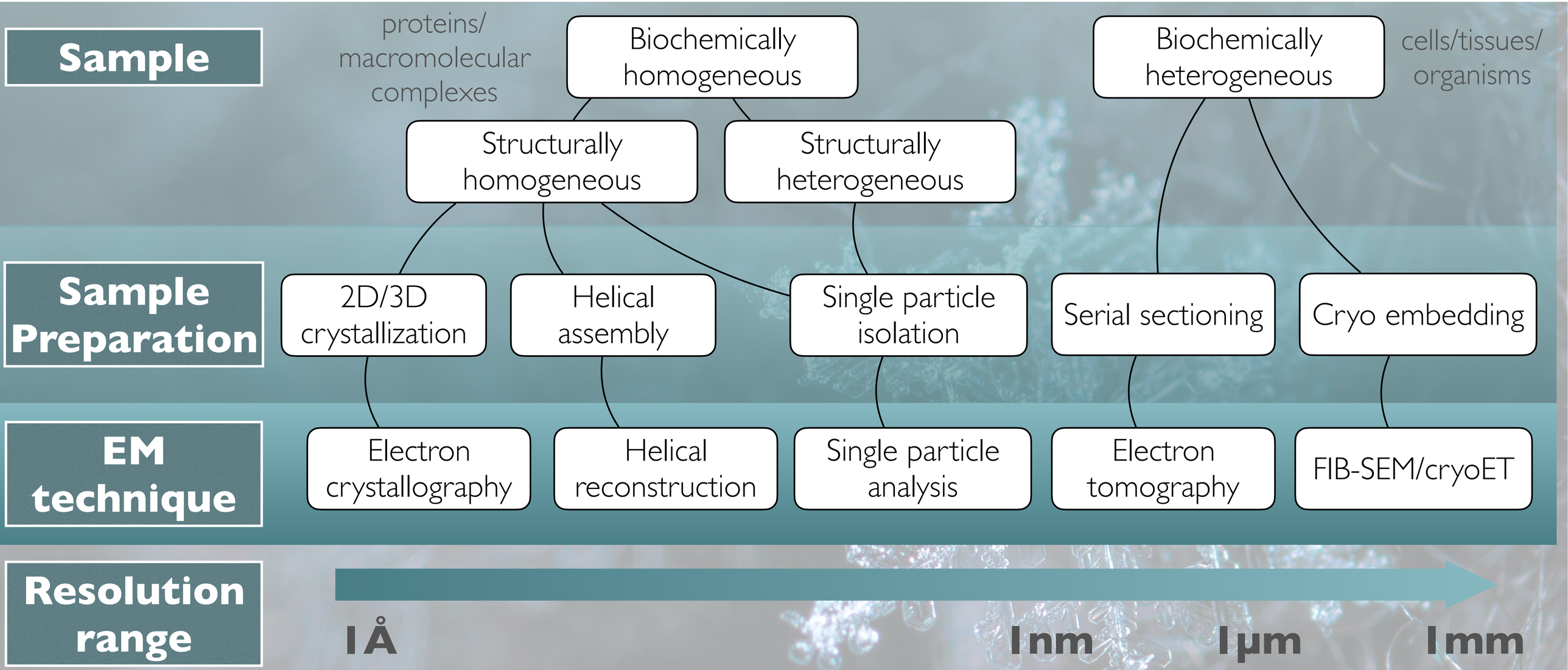
the next chapter

And true “atomic” resolution is possible:



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

How are samples prepared for cryoEM?



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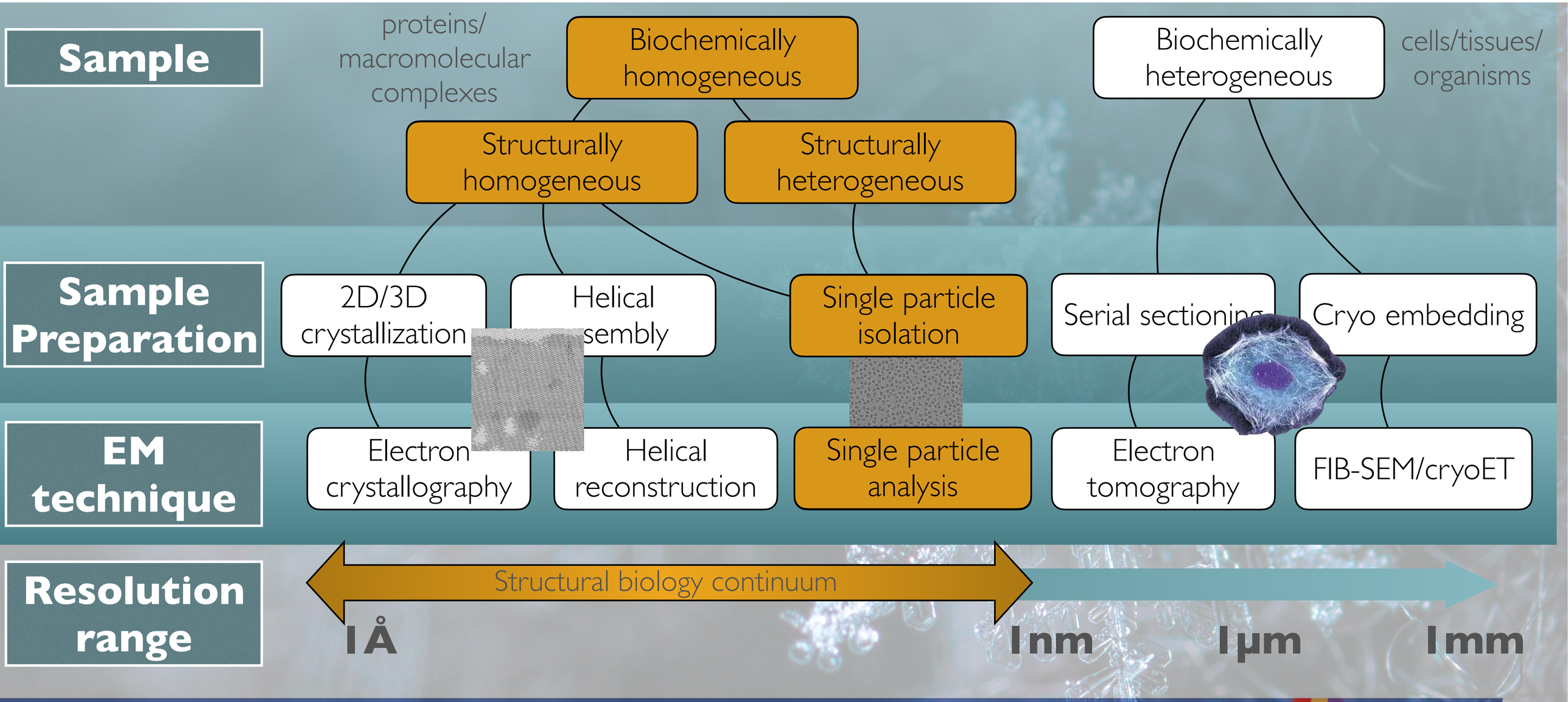
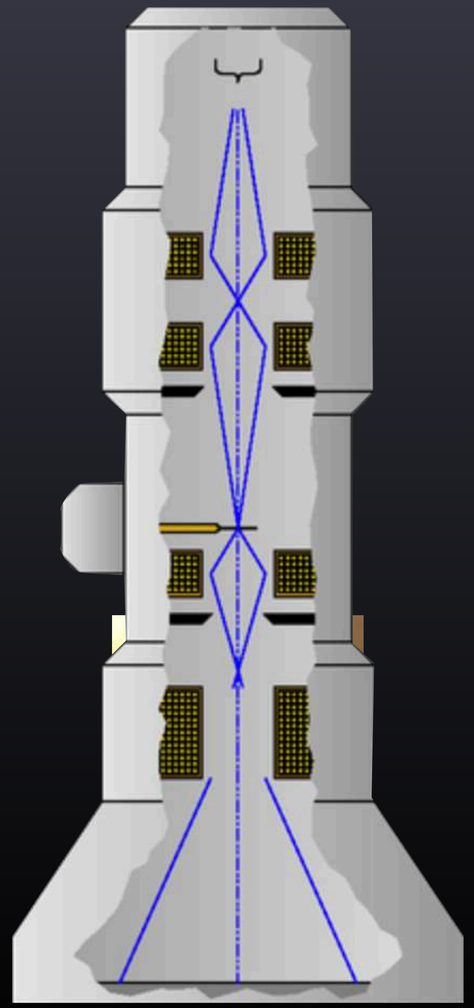
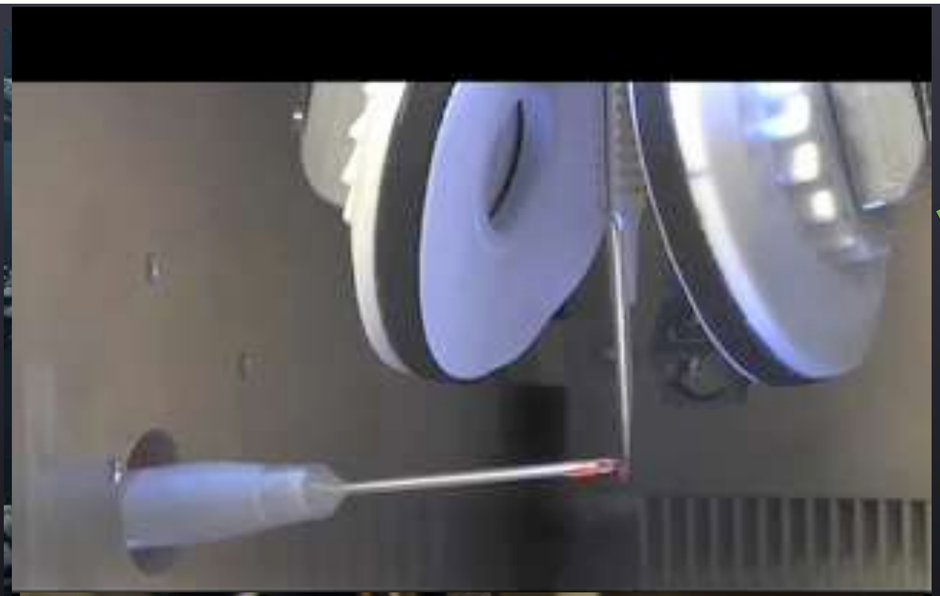


How are samples prepared for cryoEM?

Vitrifying a biological sample



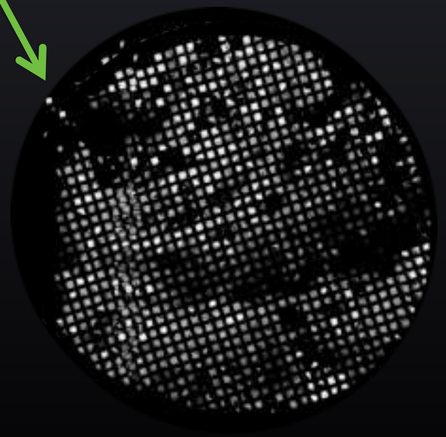


>99.999%

<0.001%

~3µl

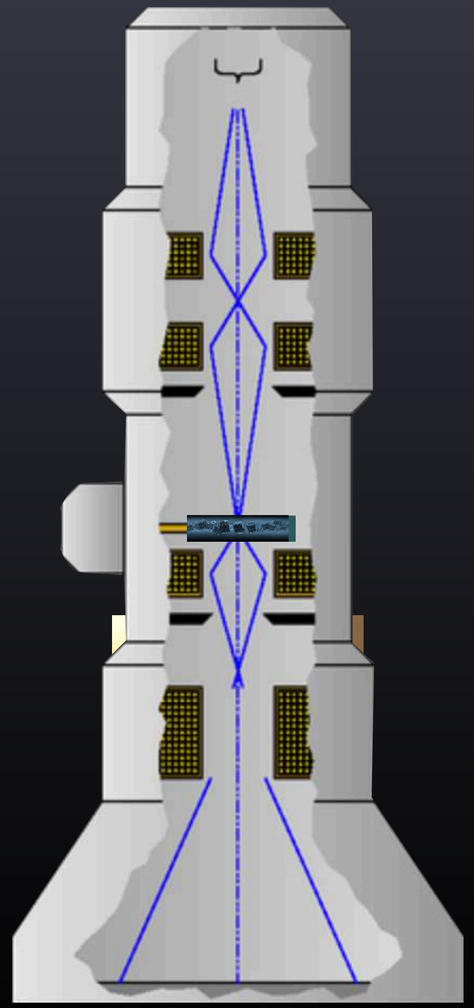
<3nl

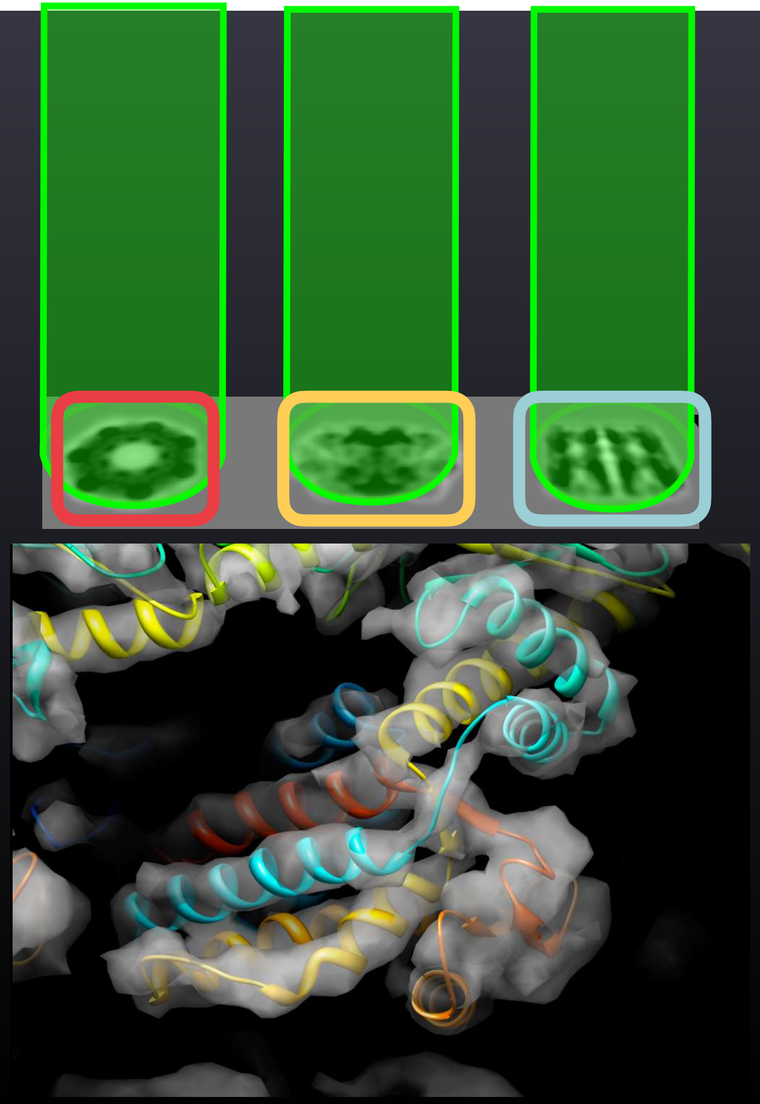


>100,000 potential imaging targets; most of them are not usable.

How are samples prepared for cryoEM?

Vitrifying a biological sample

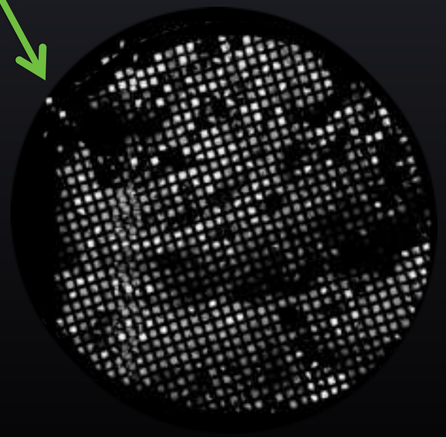




>99.999%

<0.001%

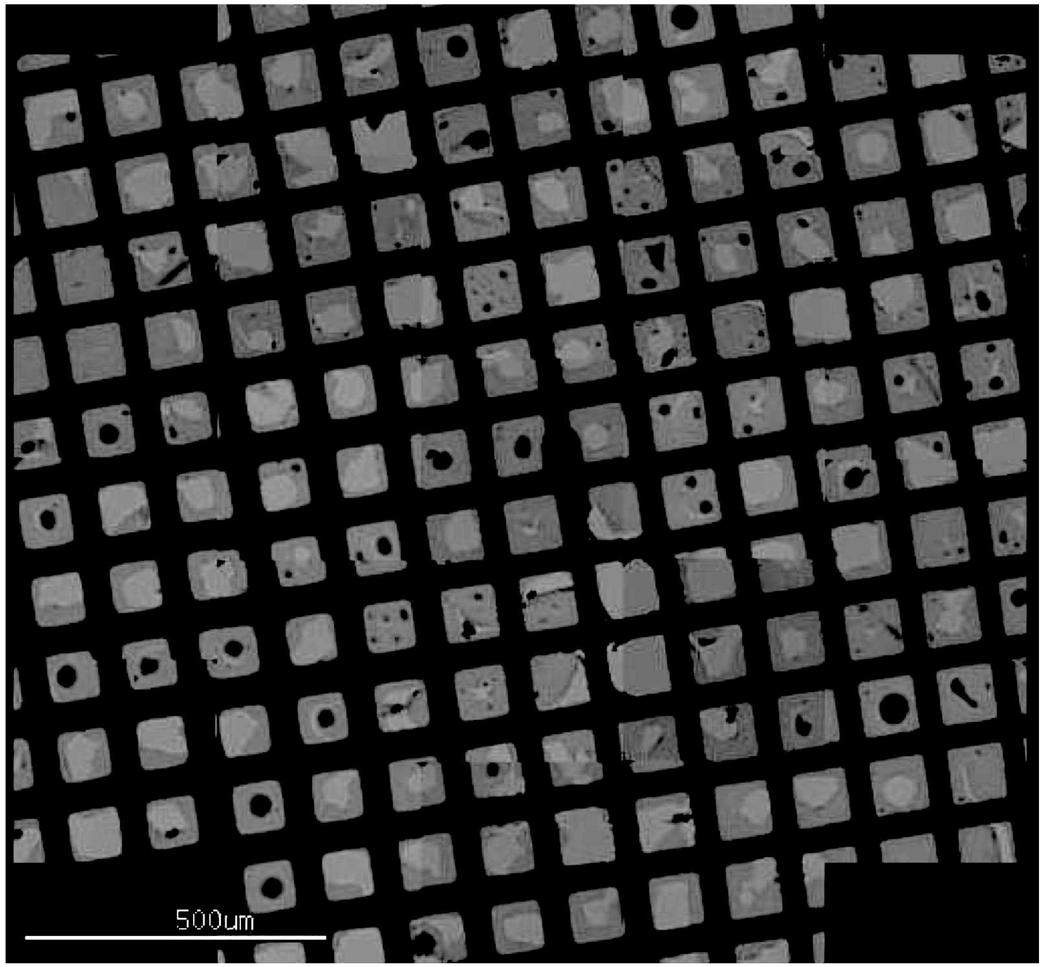
~3µl

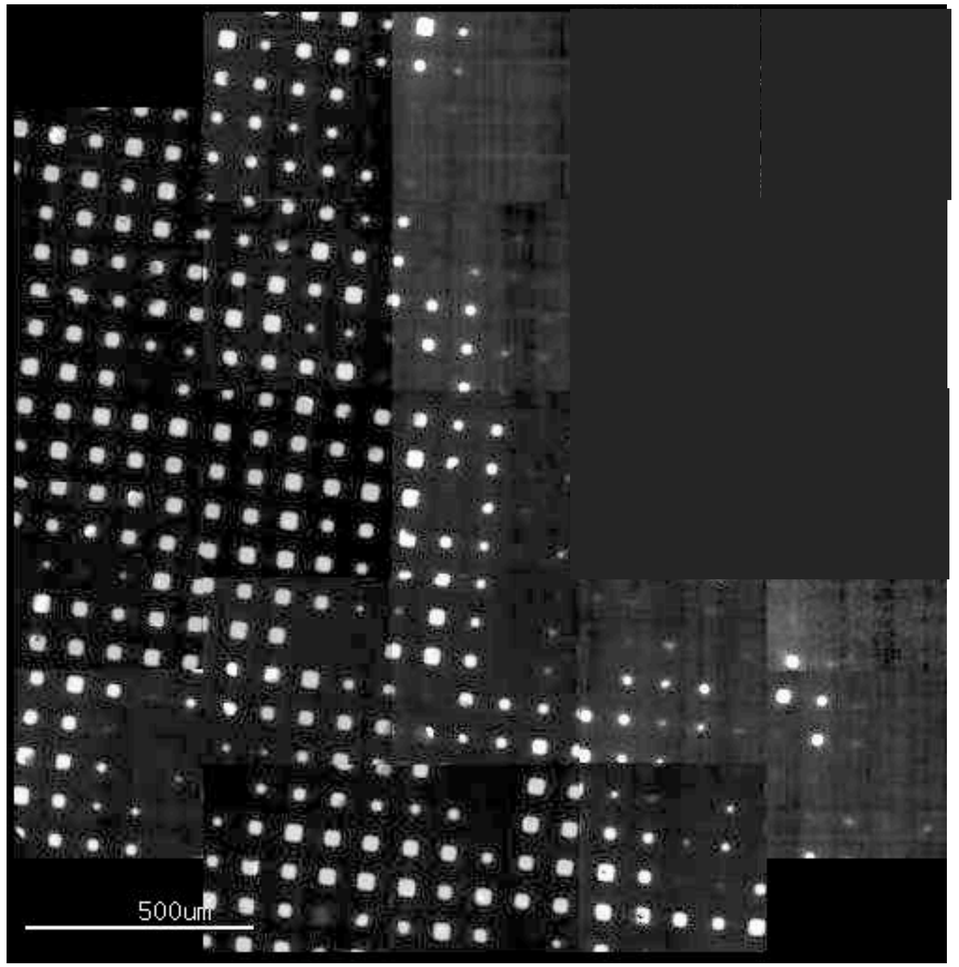


<3nl

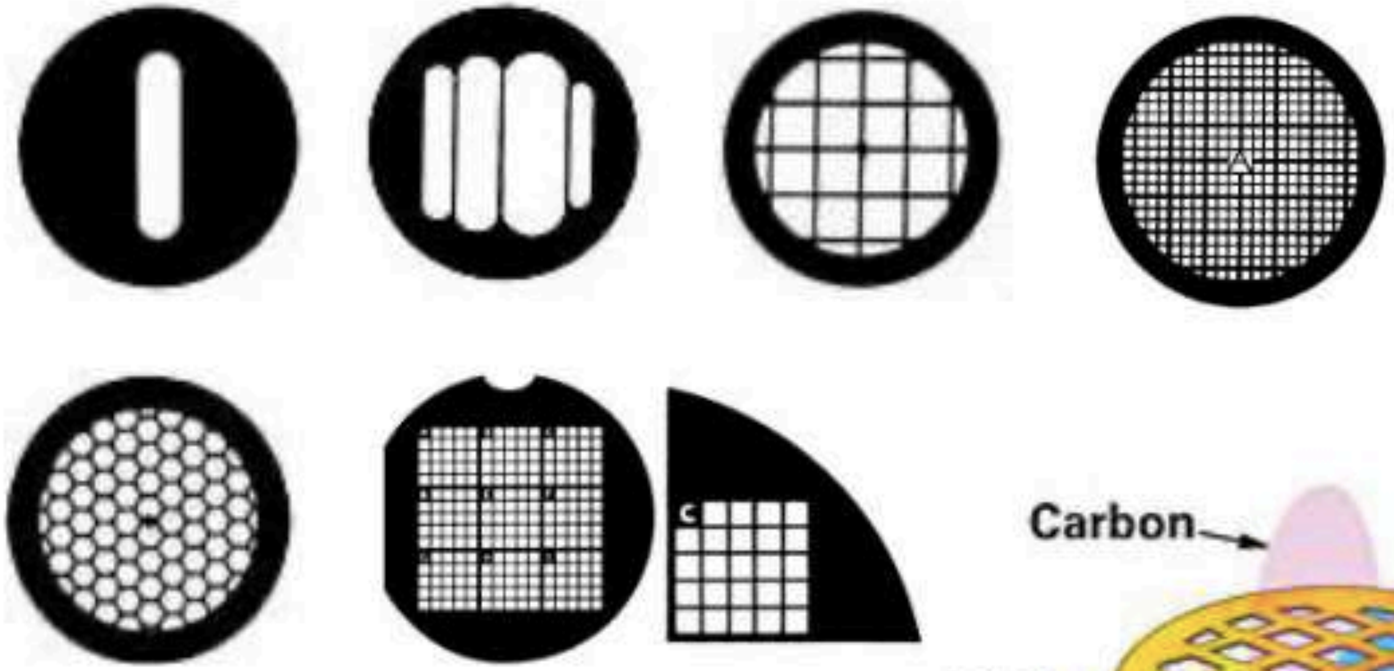
>100,000 potential imaging targets; most of them are not usable.

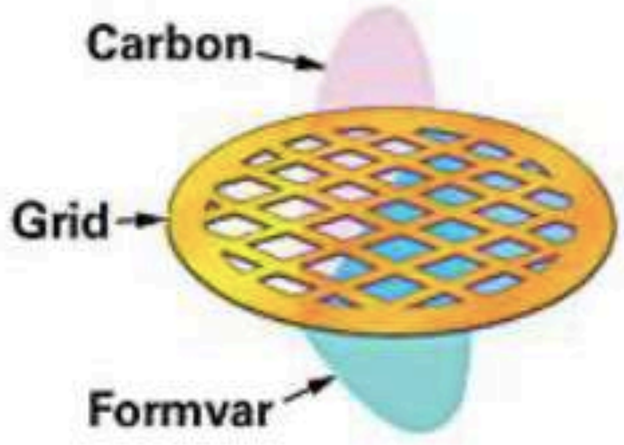
What do EM grids look like?





What do EM grids look like?





Common Materials

Copper

Nickel

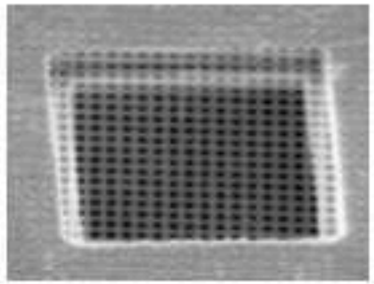
Gold

Aluminum

Molybdenum

Titanium

Stainless Steel



https://www.tedpella.com/grids_html/

What do EM grids look like?

Rough grid parameters

Rim Width: 350-400µm.

Thickness: approximately 25µm thick.

Diameter: 3.0 to 3.05mm

Pitch: Is 1"/mesh or 25.4mm/mesh

Example 200 mesh pitch = 25.4/200 = 127µm

PELCO® Grid Size

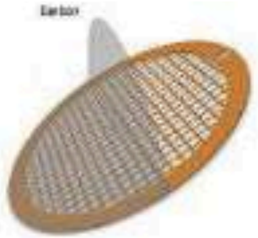
Square Mesh	Pitch µm	Hole µm	Bar µm	% Trans-mission
50	508	425	83	70
75	339	284	55	70
100	254	204	50	65
150	169	125	44	60
200	127	90	37	50
300	85	54	31	40
400	64	38	26	35
500	51	28	23	30

What do EM grids look like?

TERMINOLOGY

Grid (Cu, Au, Mo, etc…)
- mesh

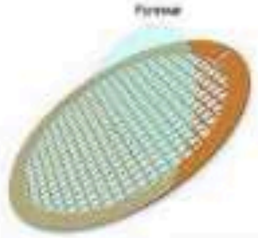
Foil (C, Au, etc…)
- Continuous
- lacy
- holey (hole size and spacing)



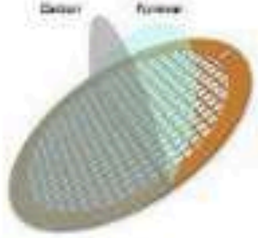
CARBON ONLY SUPPORT FILMS

HOLEY CARBON SUPPORT FILMS

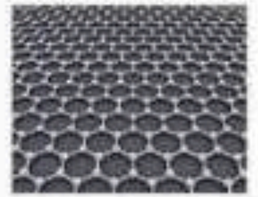
LACEY CARBON SUPPORT FILMS



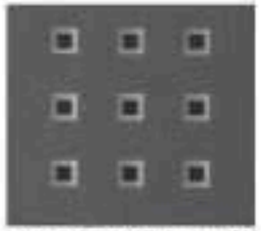
FORMVAR ONLY SUPPORT FILMS



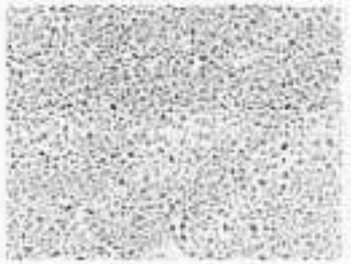
FORMVAR / CARBON SUPPORT FILMS



EM-TEC GRAPHENE SUPPORT FILMS



EM-TEC SILICON NITRIDE SUPPORT FILMS



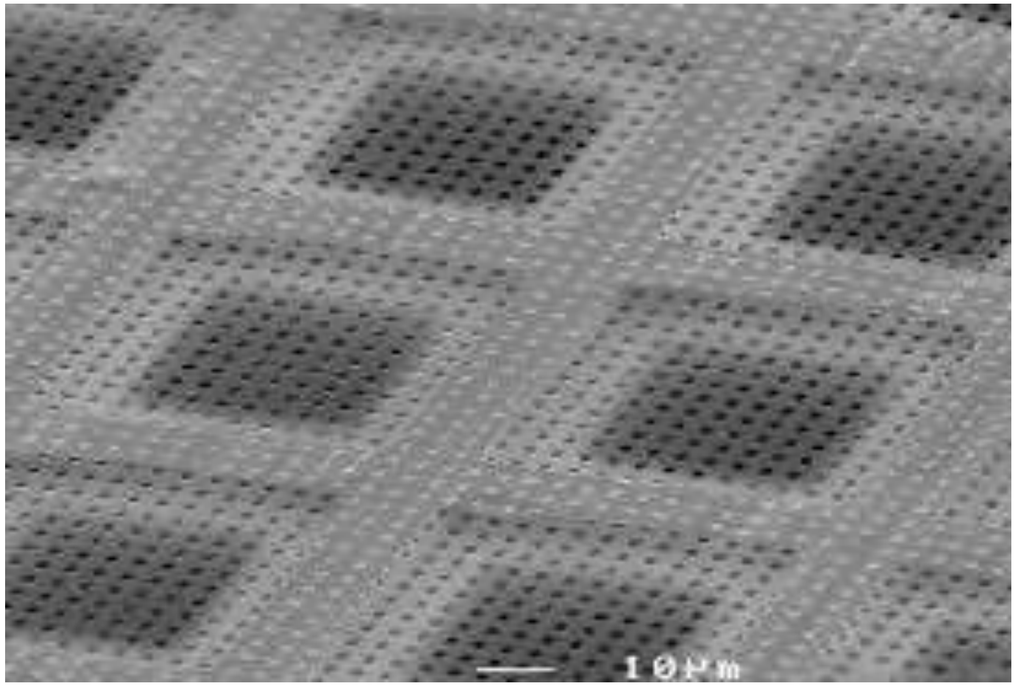
TEM CALIBRATION & TEST STANDARDS

TEM GRID STORAGE BOXES

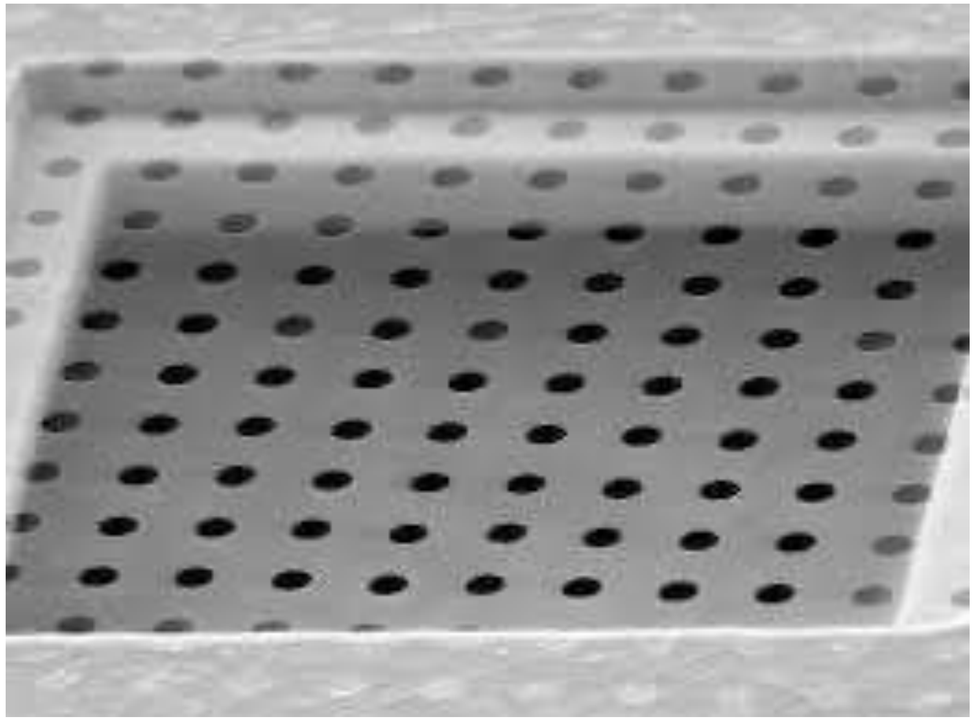
https://edgescientific.com/product-category/tem-supplies/tem-support-films/

What do EM grids look like?

TERMINOLOGY



- Protochips.com

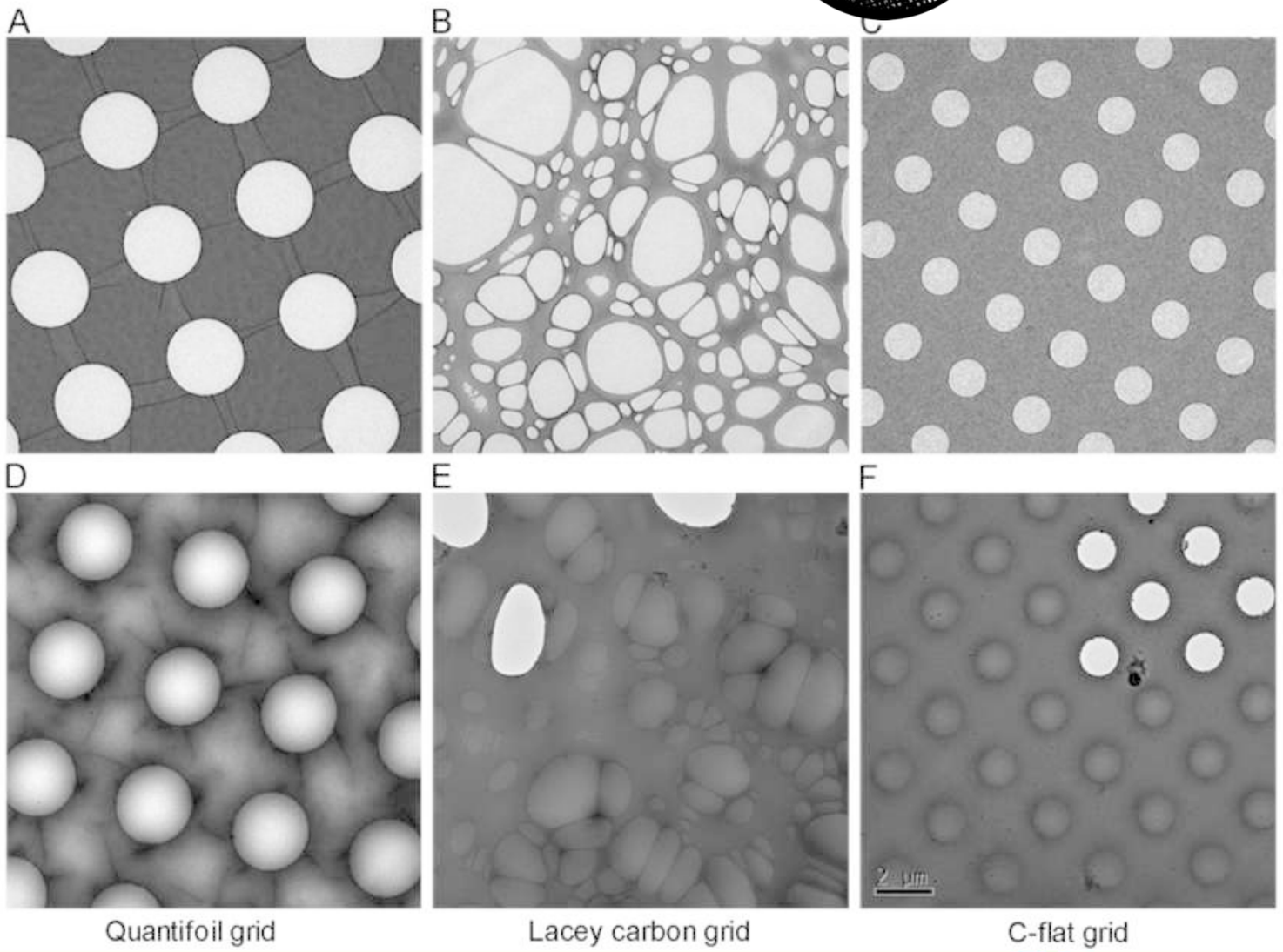


Quantifoil.com

What do EM grids look like?

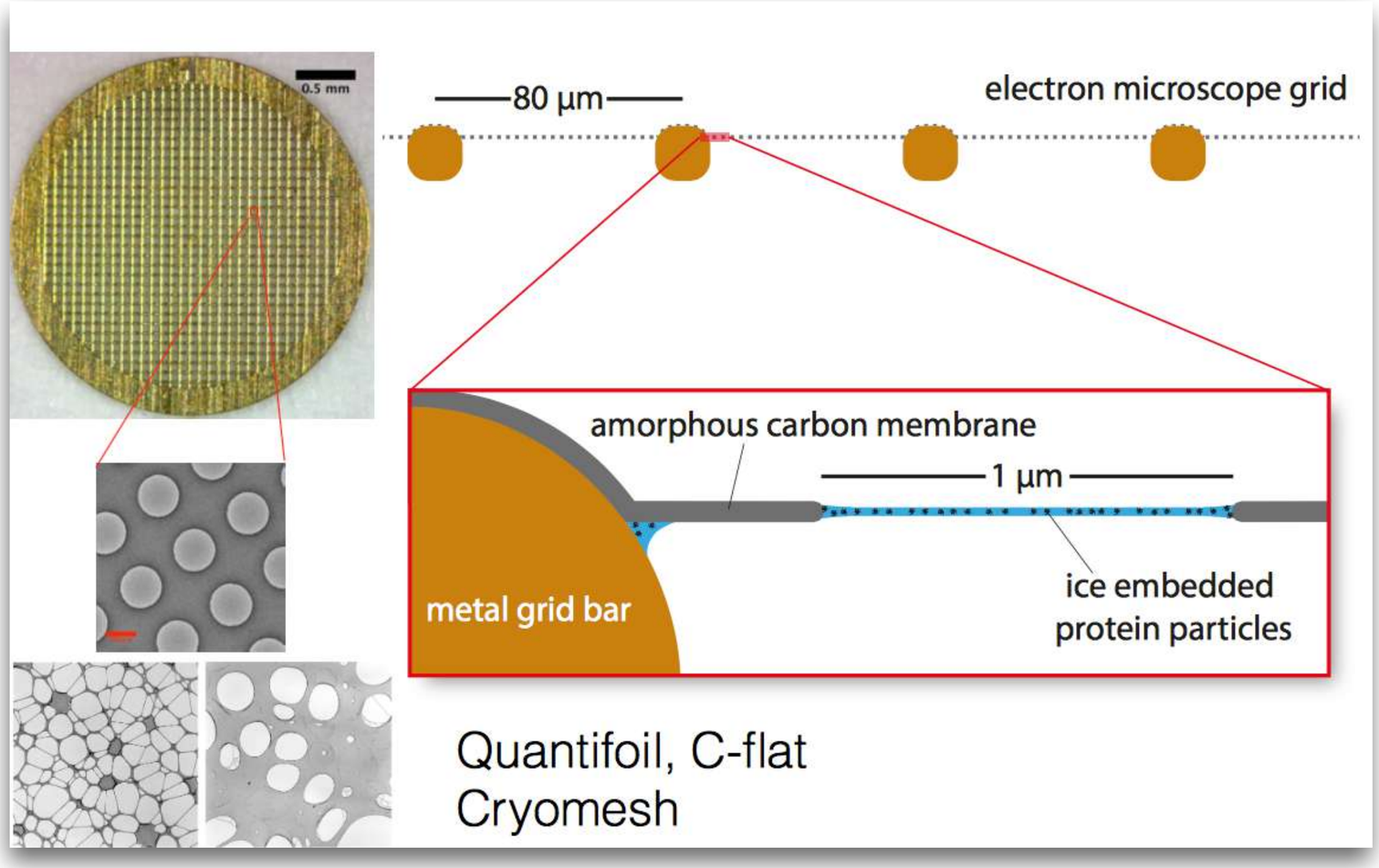
TERMINOLOGY

Cho, Hye-Jin & Hyun, Jae-Kyung & Kim, Jin-Gyu & Jeong, Hyeong & Park, Hyo & You, Dong-Ju & Jung, Hyun. (2013). Measurement of ice thickness on vitreous ice embedded cryo-EM grids: investigation of optimizing condition for visualizing macromolecules. Journal of Analytical Science and Technology. 4. 10.1186/2093-3371-4-7.



What do EM grids look like?

TERMINOLOGY



Russo & Passmore, 2015

What do EM grids look like?

TERMINOLOGY

- Holey gold foil on gold mesh grid

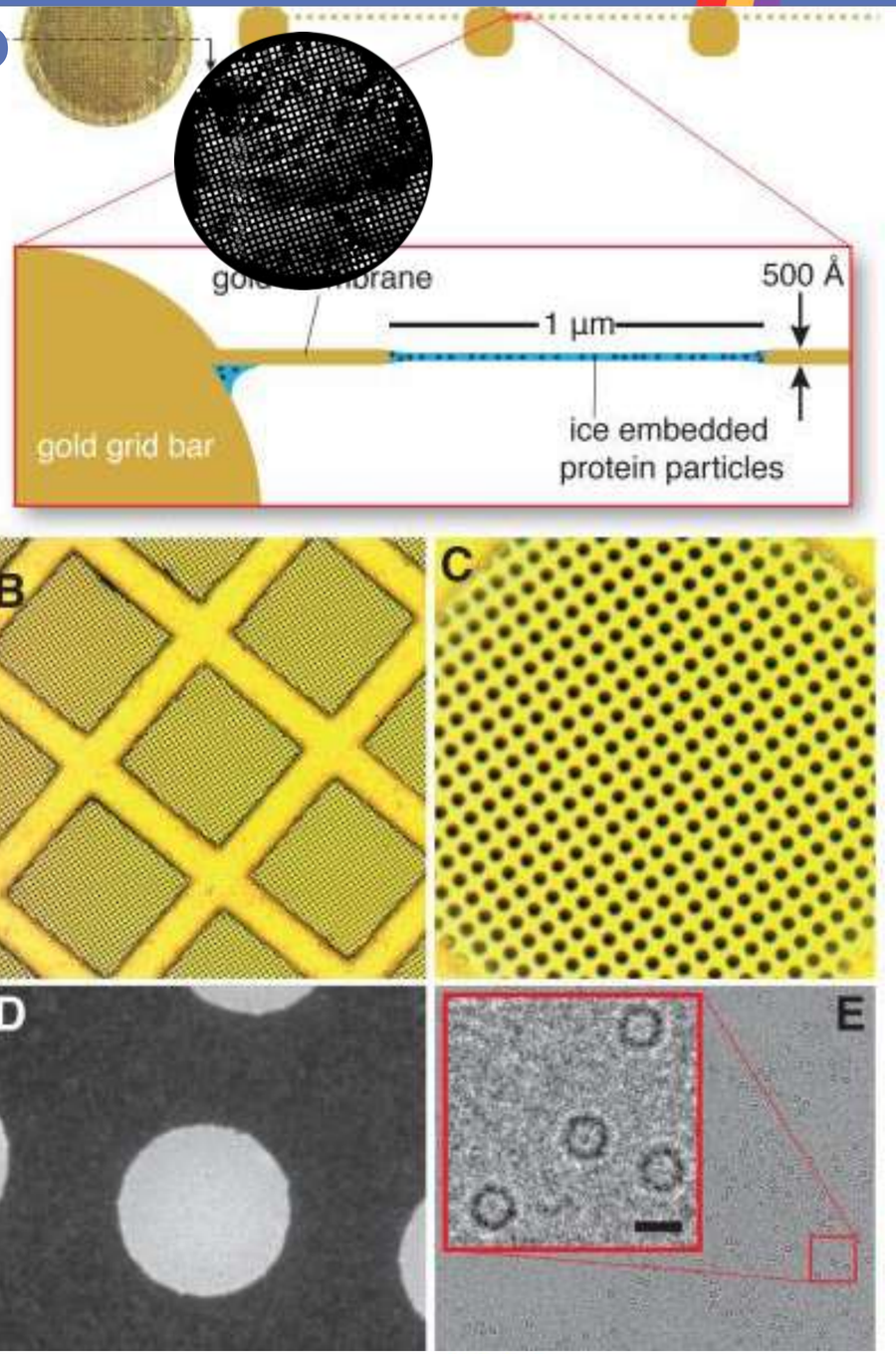
Advantages:

- Prevents differential thermal contraction when freezing
- Reduces beam-induced specimen movement
- Combined with direct detector technology allows for near atomic resolution

Disadvantages:

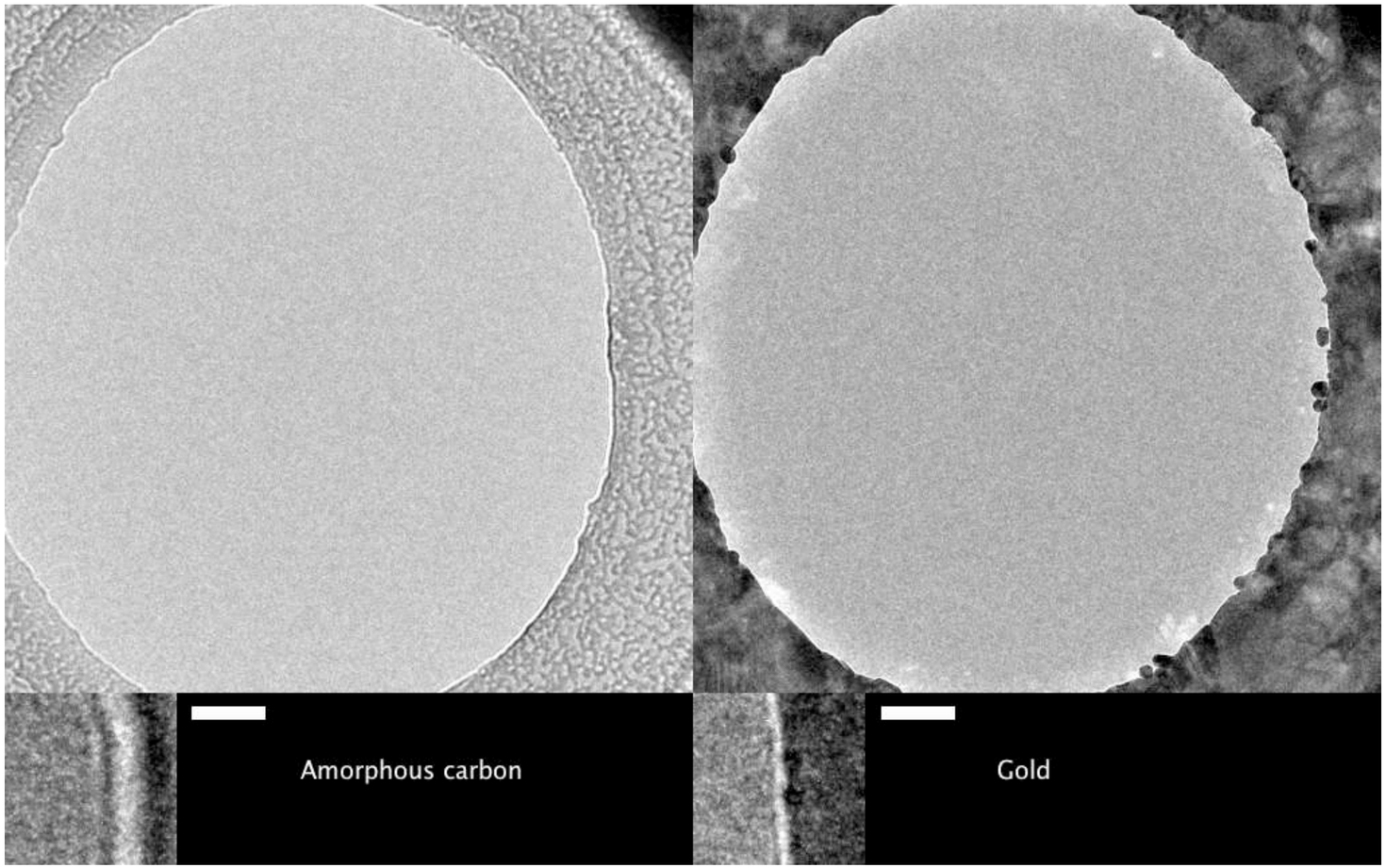
- Difficult to find focus due to lack of amorphous substrate

Russo & Passmore, 2015



What do EM grids look like?

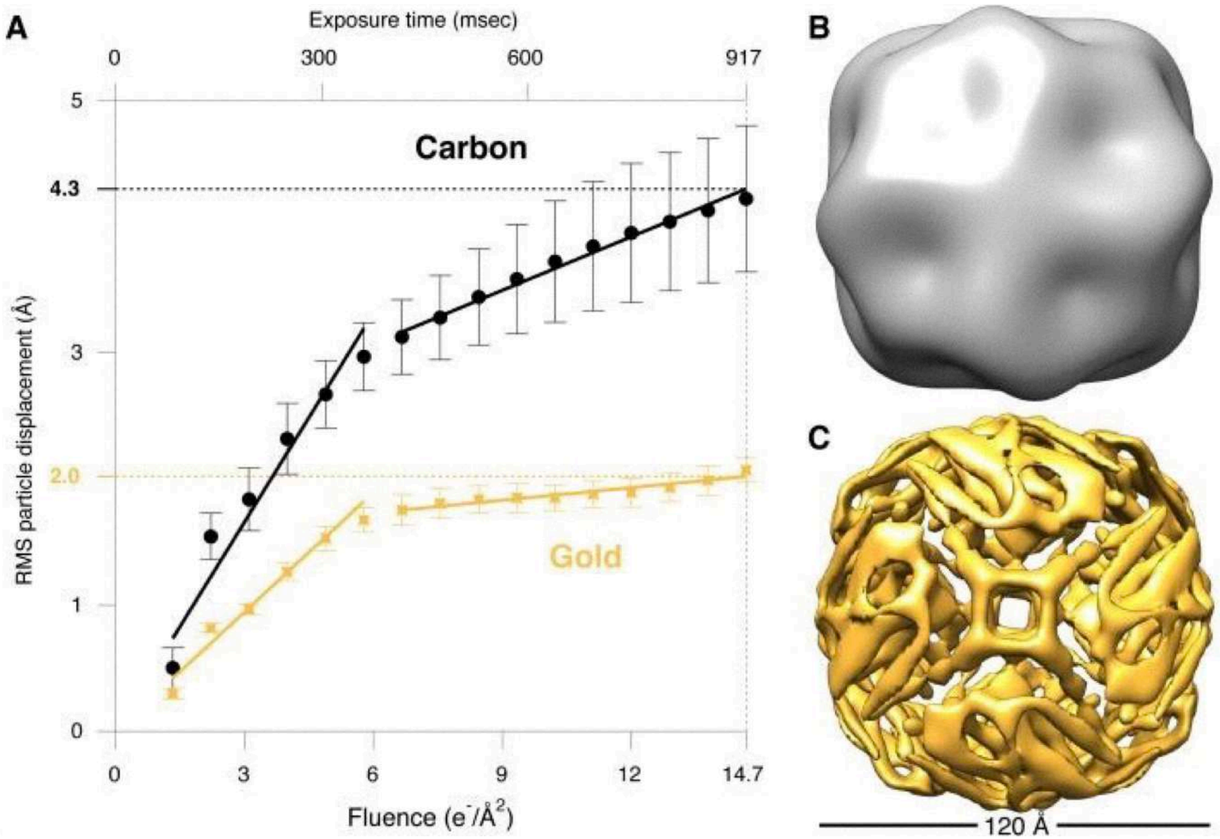
Gold grids



Russo & Passmore, 2015

What do EM grids look like?

Gold grids



A. 80S ribosome movement during irradiation supported by amorphous carbon and gold using same imaging conditions.

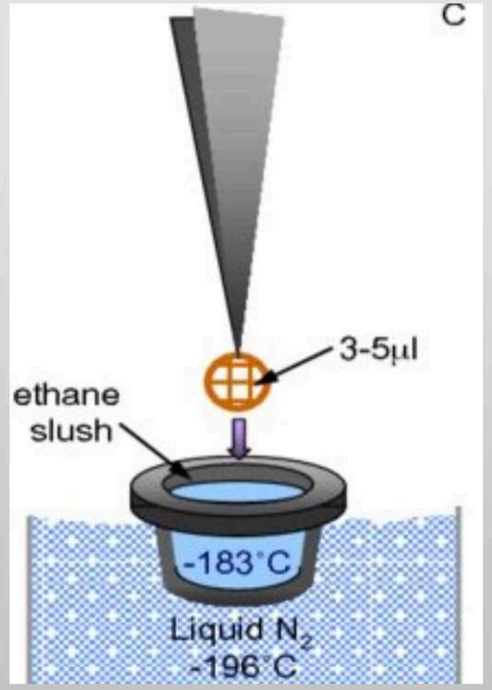
Apoferritin density maps using same imaging conditions and identical processing for **B.** carbon and **C.** gold substrates. **B.** is at 25 Å and **C.** 8 Å resolution.

Russo & Passmore, 2015

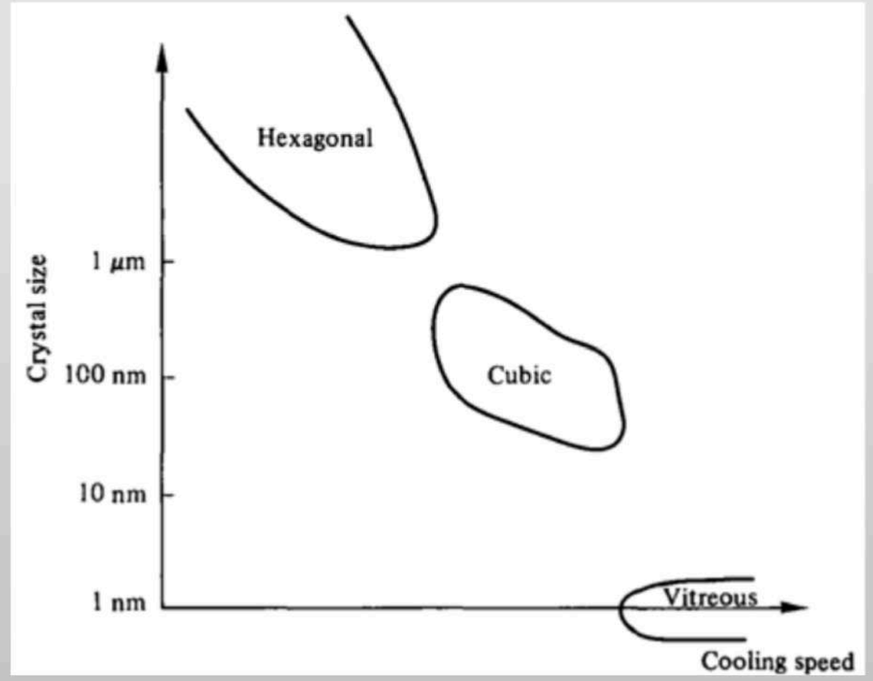
How are samples prepared for cryoEM?

Vitrification process

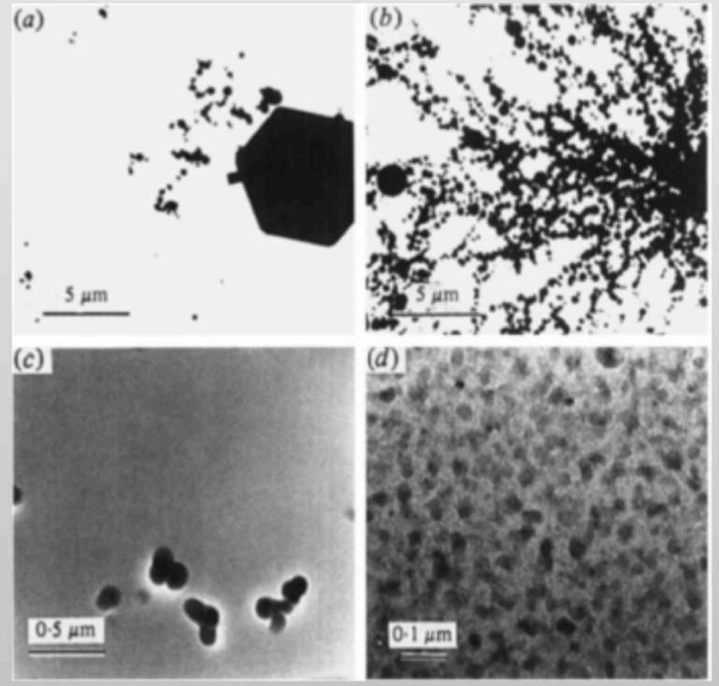
- Liquid ethane is a suitable coolant.
- Liquid nitrogen boils on contact, which makes it a poor coolant for cryo-EM.
- Cooling speed faster than 10^5-10^6 K/s ensure the formation of vitrified ice.



Setup of liquid ethane
(Image from Wen Jiang)



Cooling speed &
forms of ice



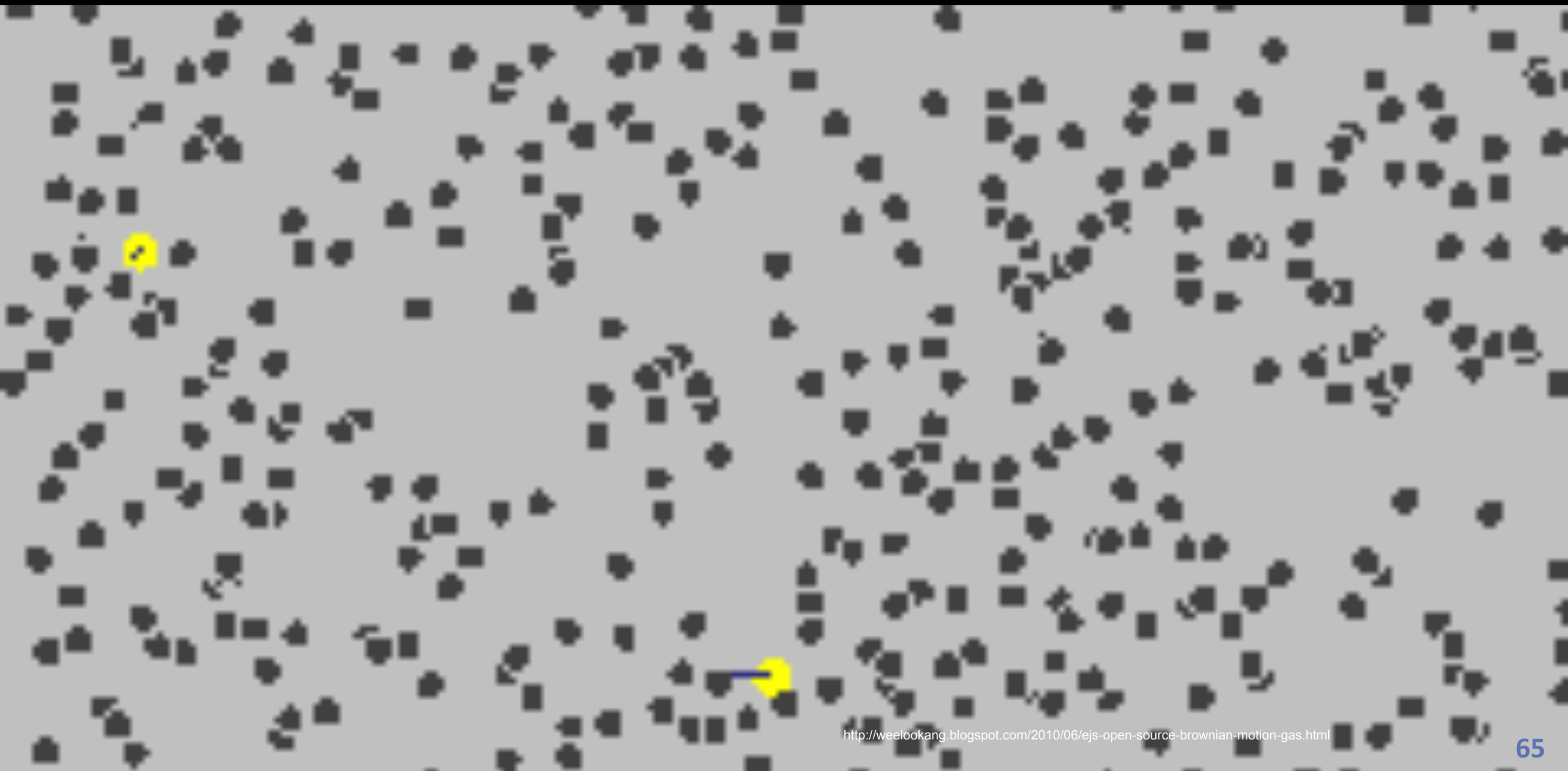
Different forms of ice contamination

Jacques Dubochet et al., 1988

How are samples prepared for cryoEM?

Vitrification process

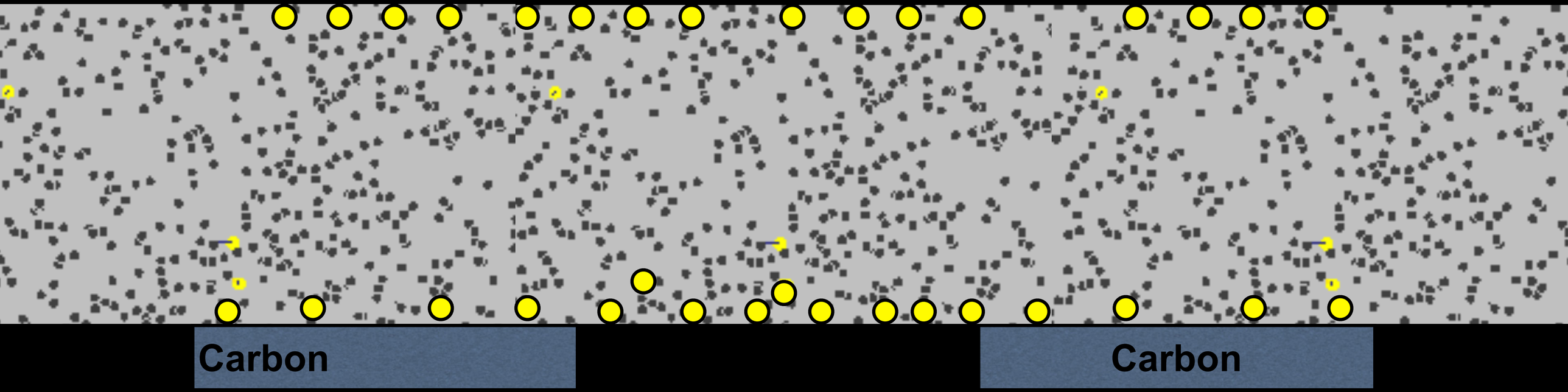
What happens to samples during vitrification?



http://weelookang.blogspot.com/2010/06/ejs-open-source-brownian-motion-gas.html

What happens to samples during vitrification?

A hypothetical scenario during cryoEM grid preparation



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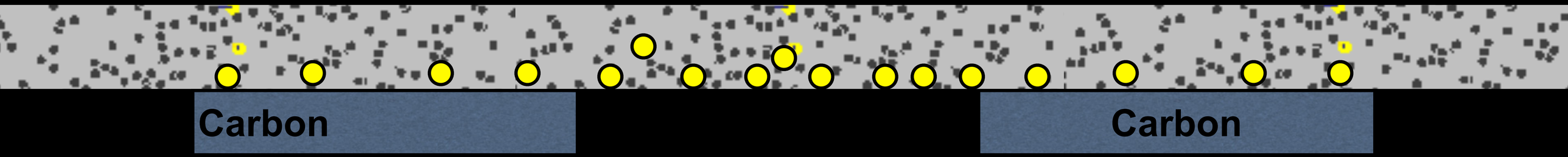

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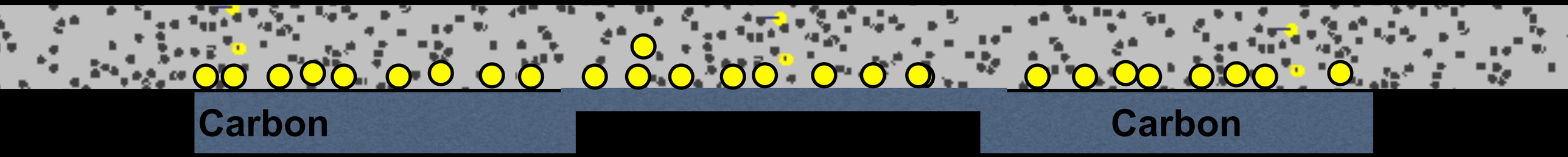
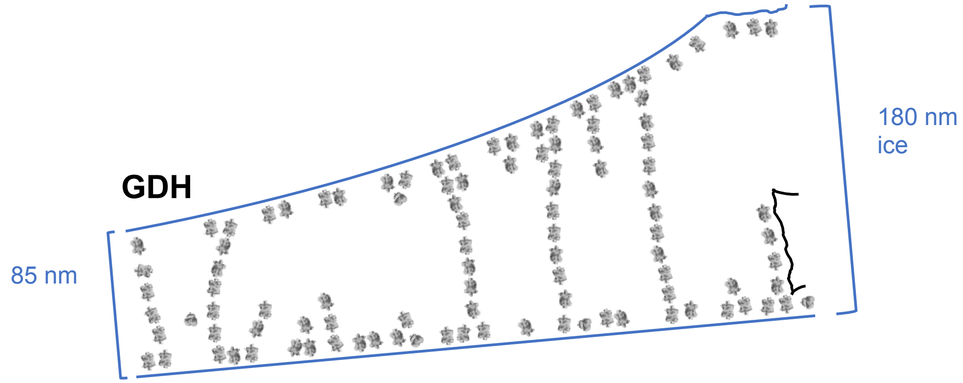


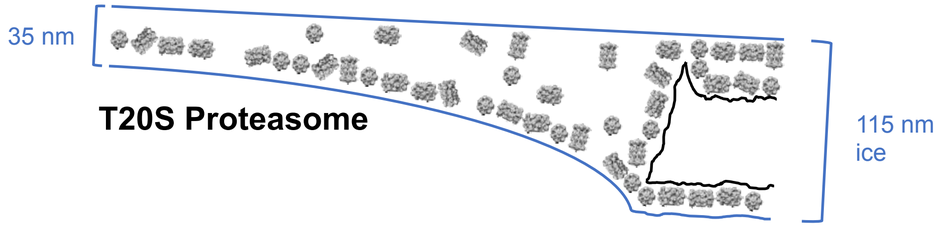
http://weelookang.blogspot.com/2010/06/ejs-open-source-brownian-motion-gas.html

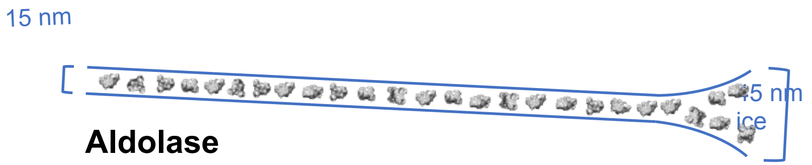
What issues arise?

Hemagglutinin

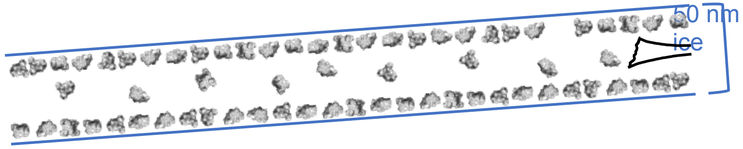
Hemagglutinin



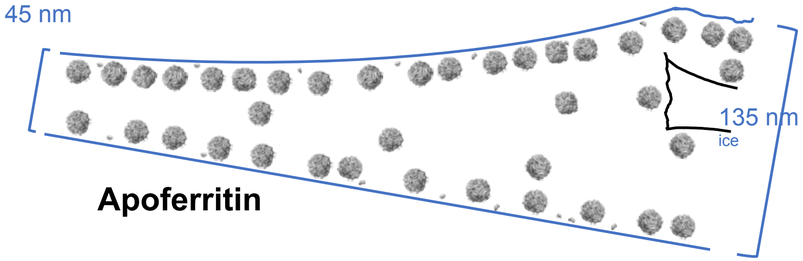




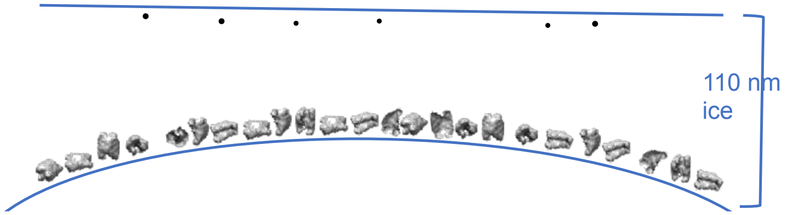
Aldolase



Aldolase



Apoferritin



DNAB Helices

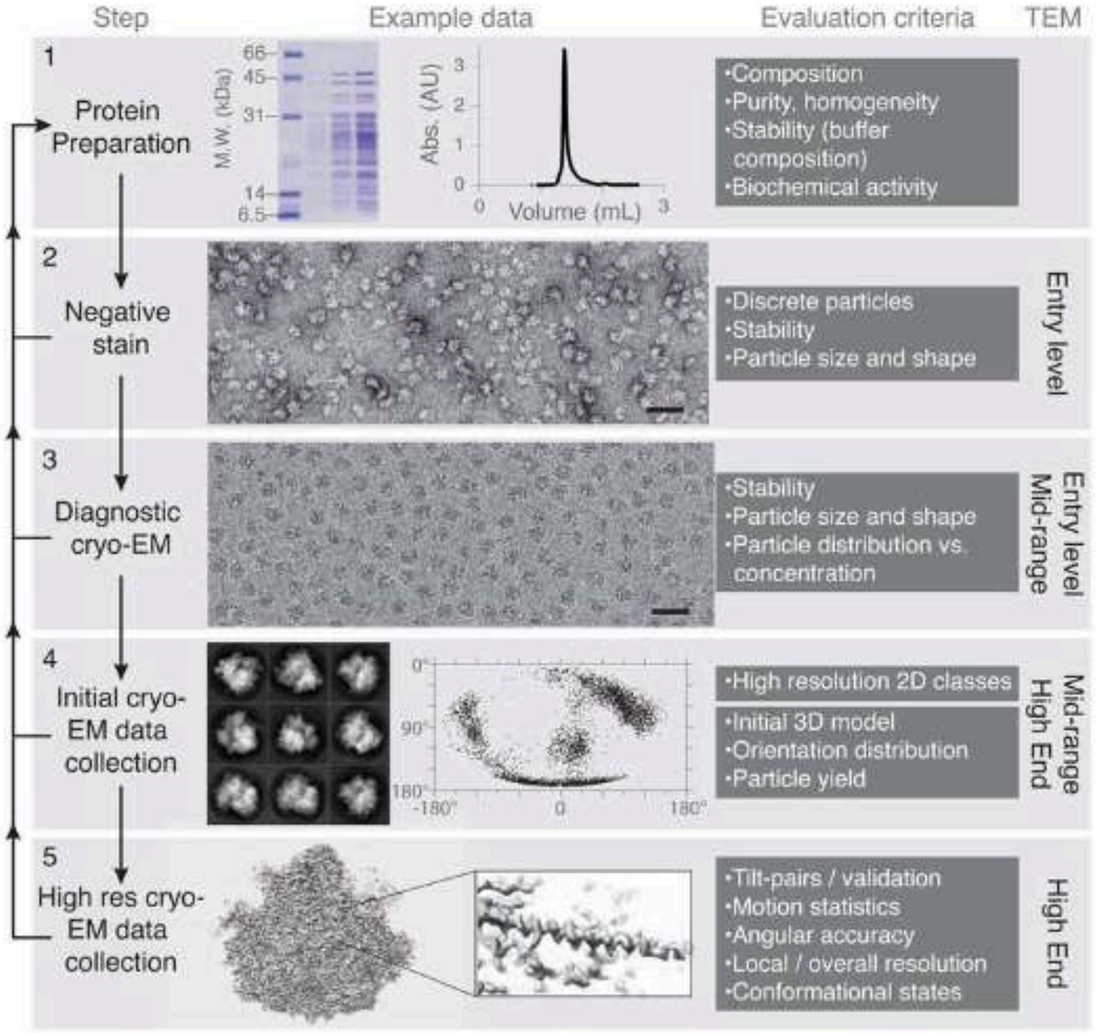
Noble AJ, et al. Routine single particle CryoEM sample and grid characterization by tomography. Elife. 2018;7.

Alex Noble

How are samples prepared for cryoEM?

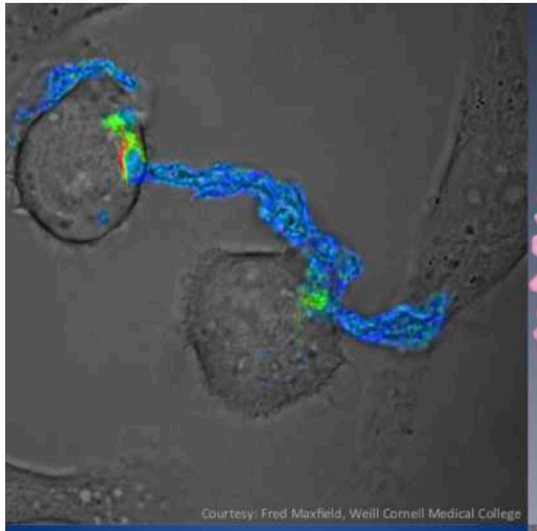
THE OPTIMIZATION WORKFLOW

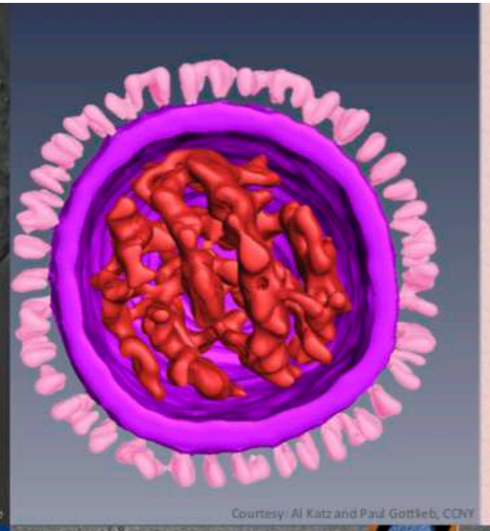
Structure determination by cryo-EM. A systematic approach to 3D structure determination is shown. In the left column, the major steps are listed. Each step should be performed successively and only after one has been completed successfully should the scientist move onto the next step. In the second column, example data are shown for ribosomes (details in text). Scale bars on the micrographs are 500 Å. Each step should be evaluated with the criteria listed in the third column, returning to earlier steps for troubleshooting.

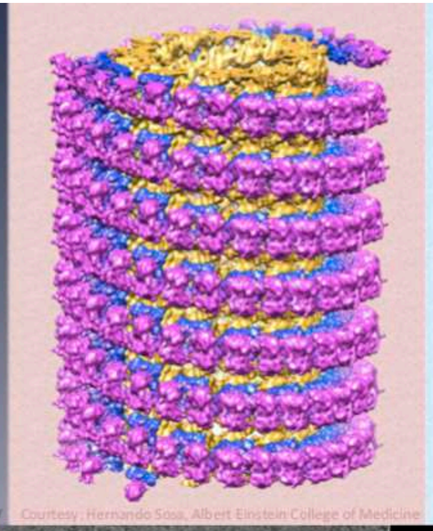


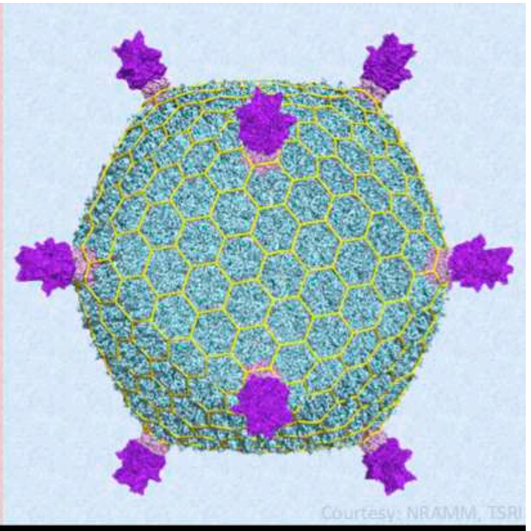
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5140023/

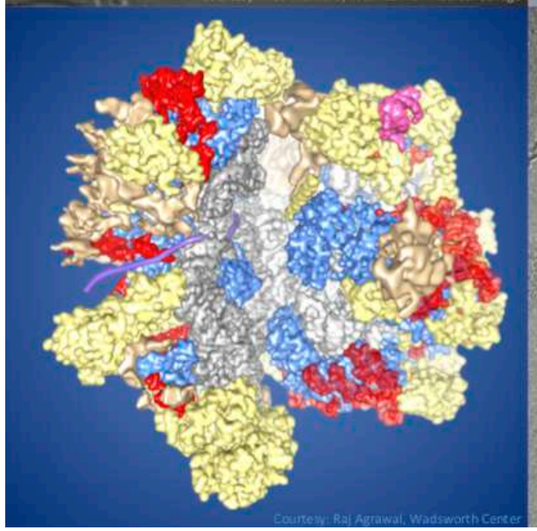
How are samples prepared for cryoEM?

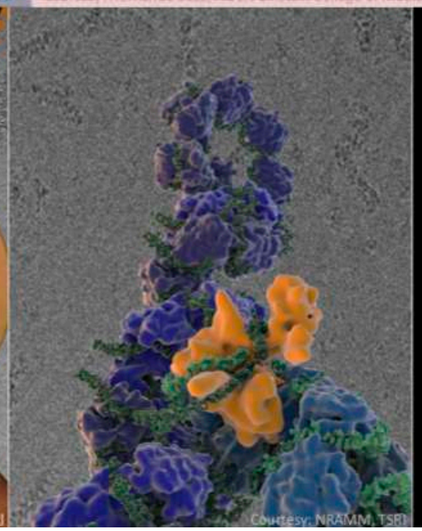


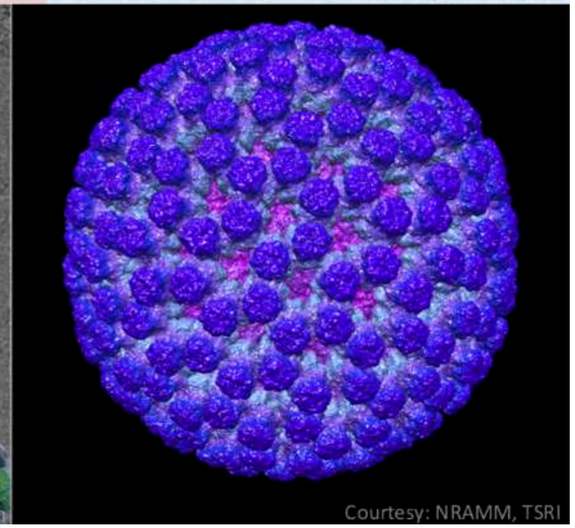












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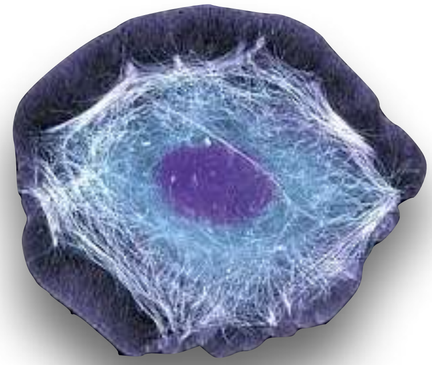
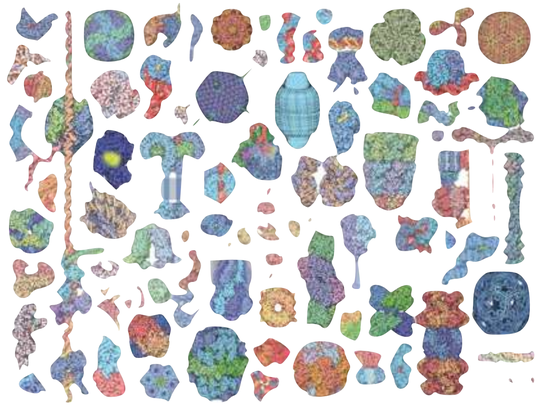


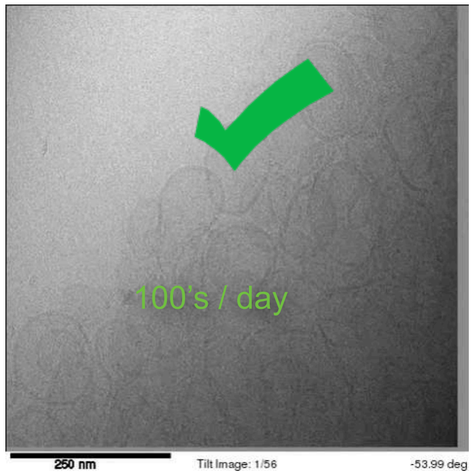


TBD (20??)

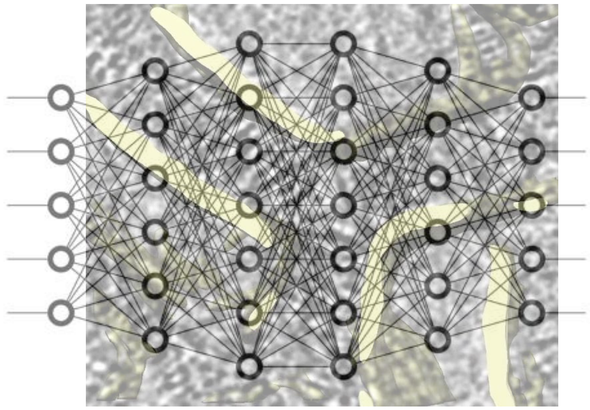
How are samples prepared for cryoEM?

Towards Automation for
In Situ CryoEM

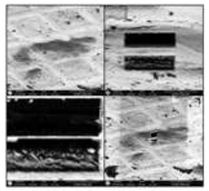
Sample

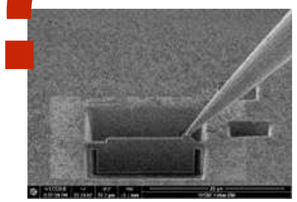


Automated Data Collection
(Leginon, etc.)



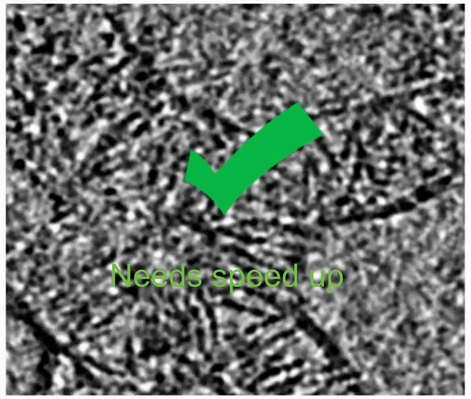
Deep learning?





Milling
Grid preparation

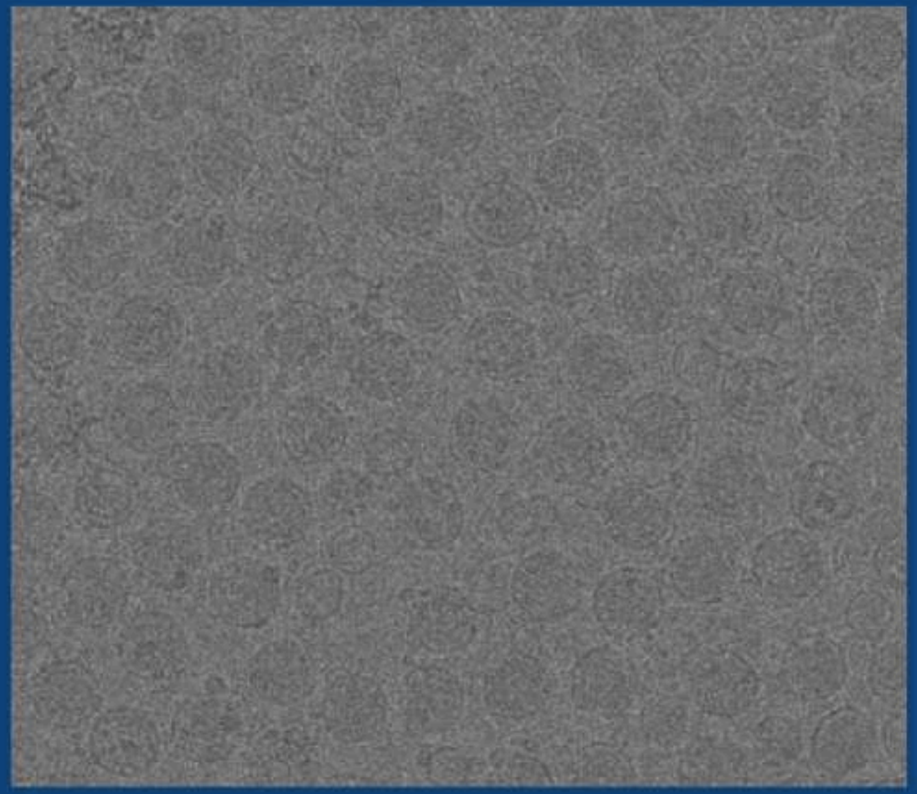
Lift out



Streamlined Processing
(Appion Protomo)

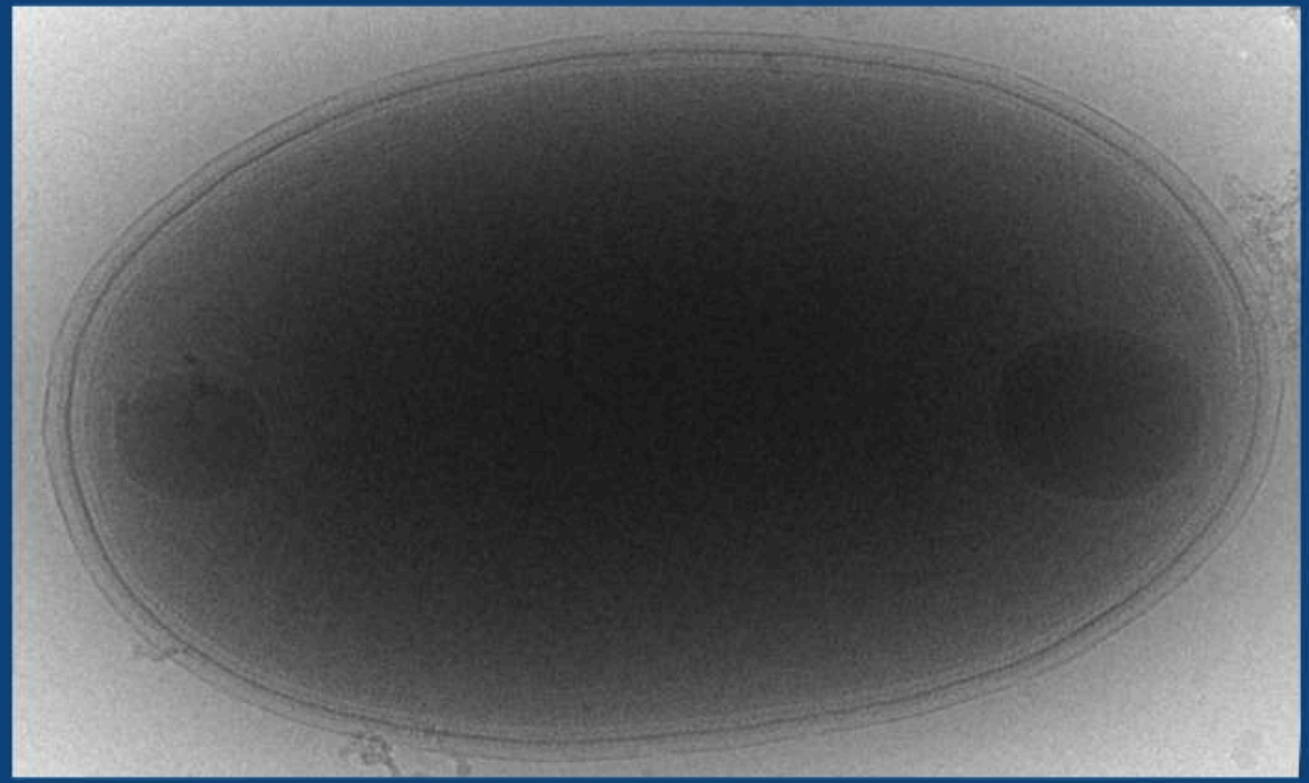
How are samples prepared for cryoEM?

HOW THIN DOES THE SAMPLE NEED TO BE?



50 nm

Bacteriophage (ϕ12)



E. coli, Salmonella, Cyanobacteria

How are samples prepared for cryoEM?

CLEM workflow