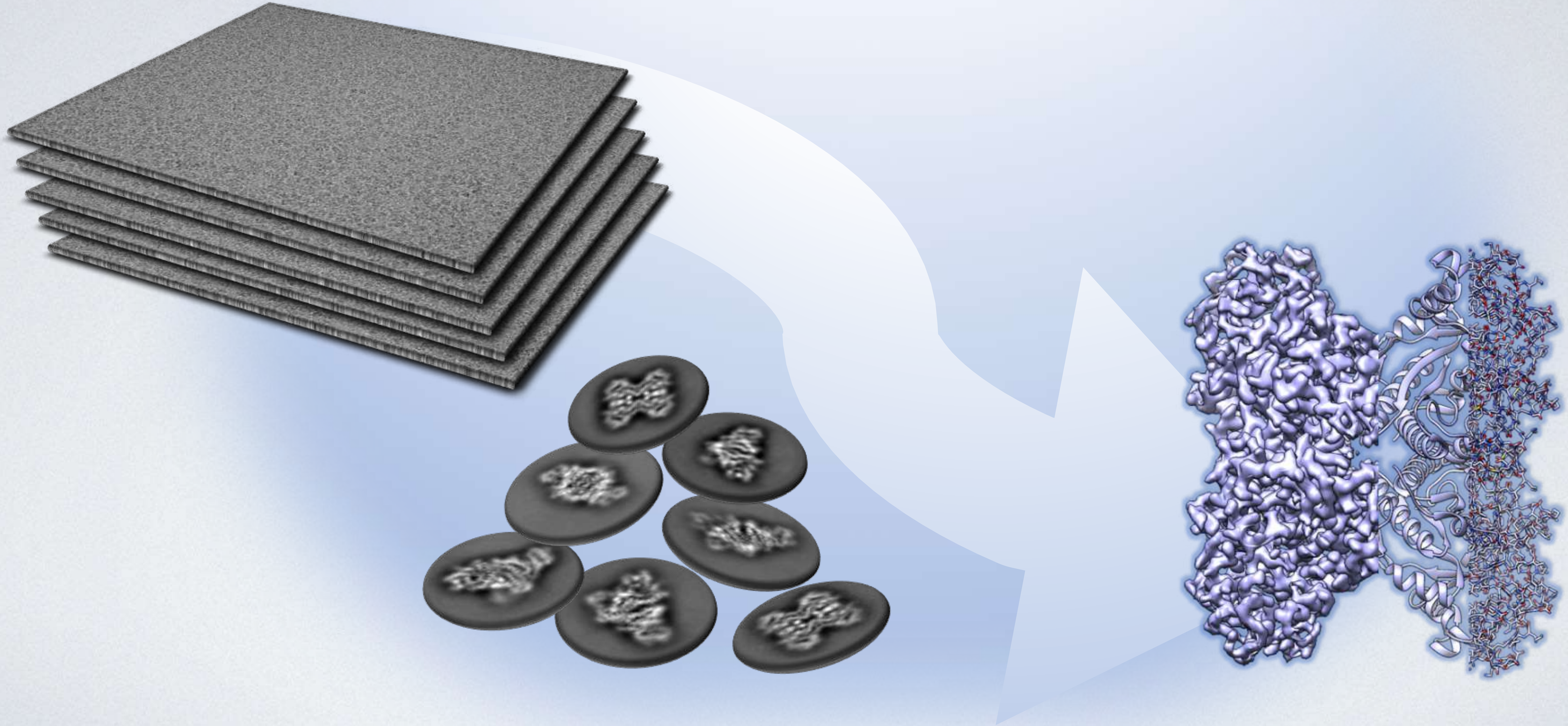
New York Structural Biology Center
SIMONS ELECTRON MICROSCOPY CENTER

Tools of the trade: Microscopes, grids and more

NCCAT SPA Short-course

Edward T Eng
March 15, 2022

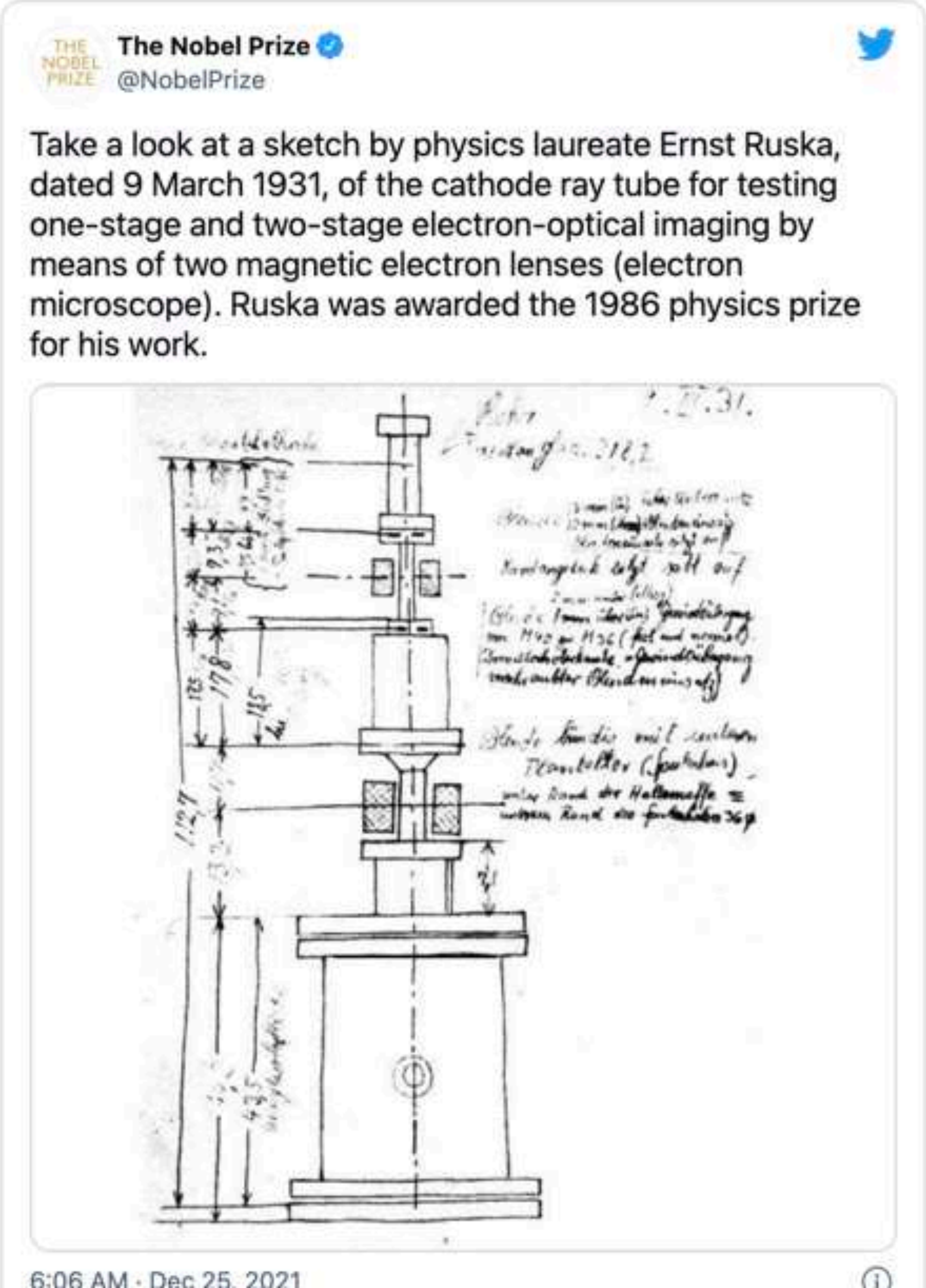
WHAT IS POSSIBLE TODAY?

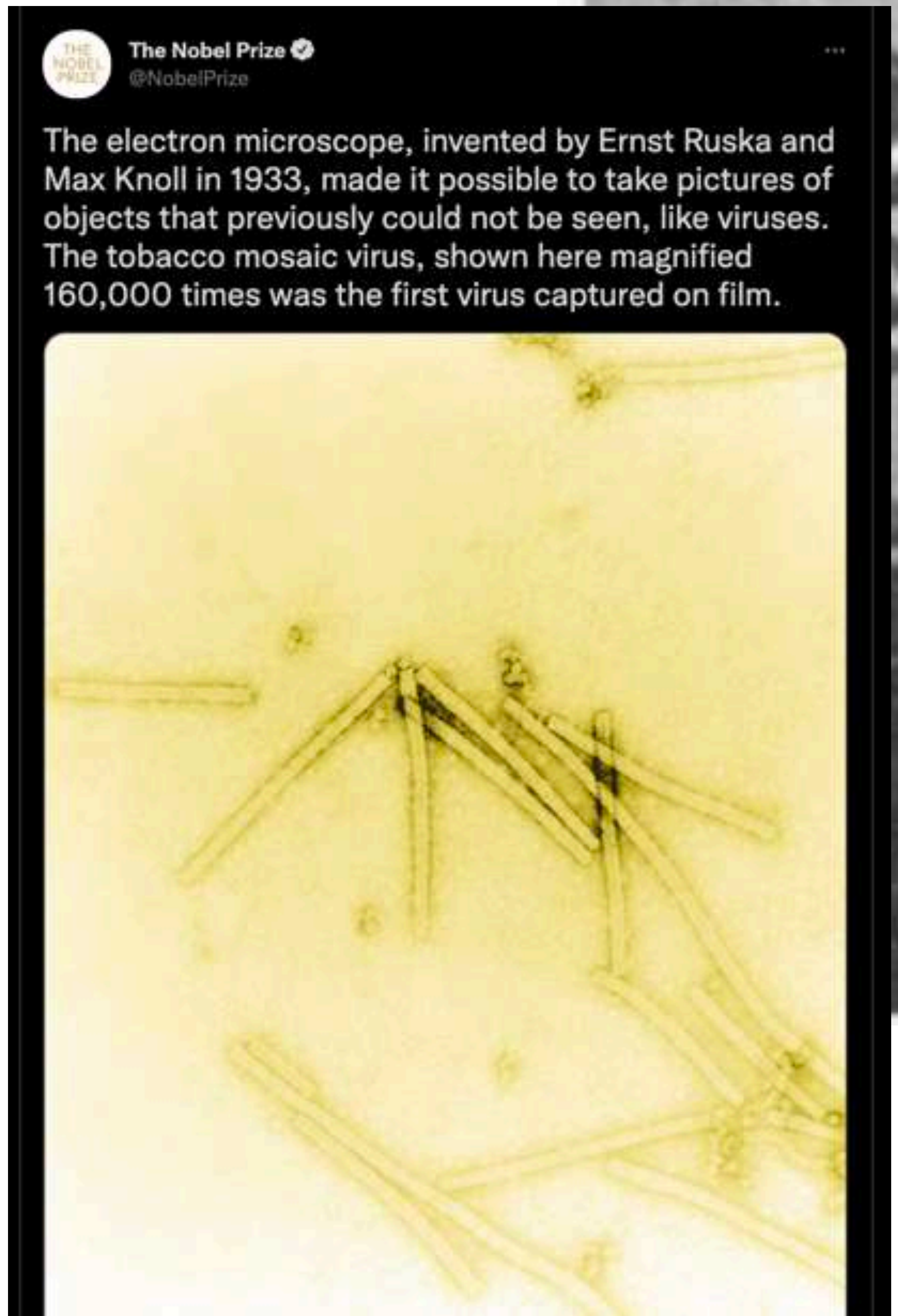


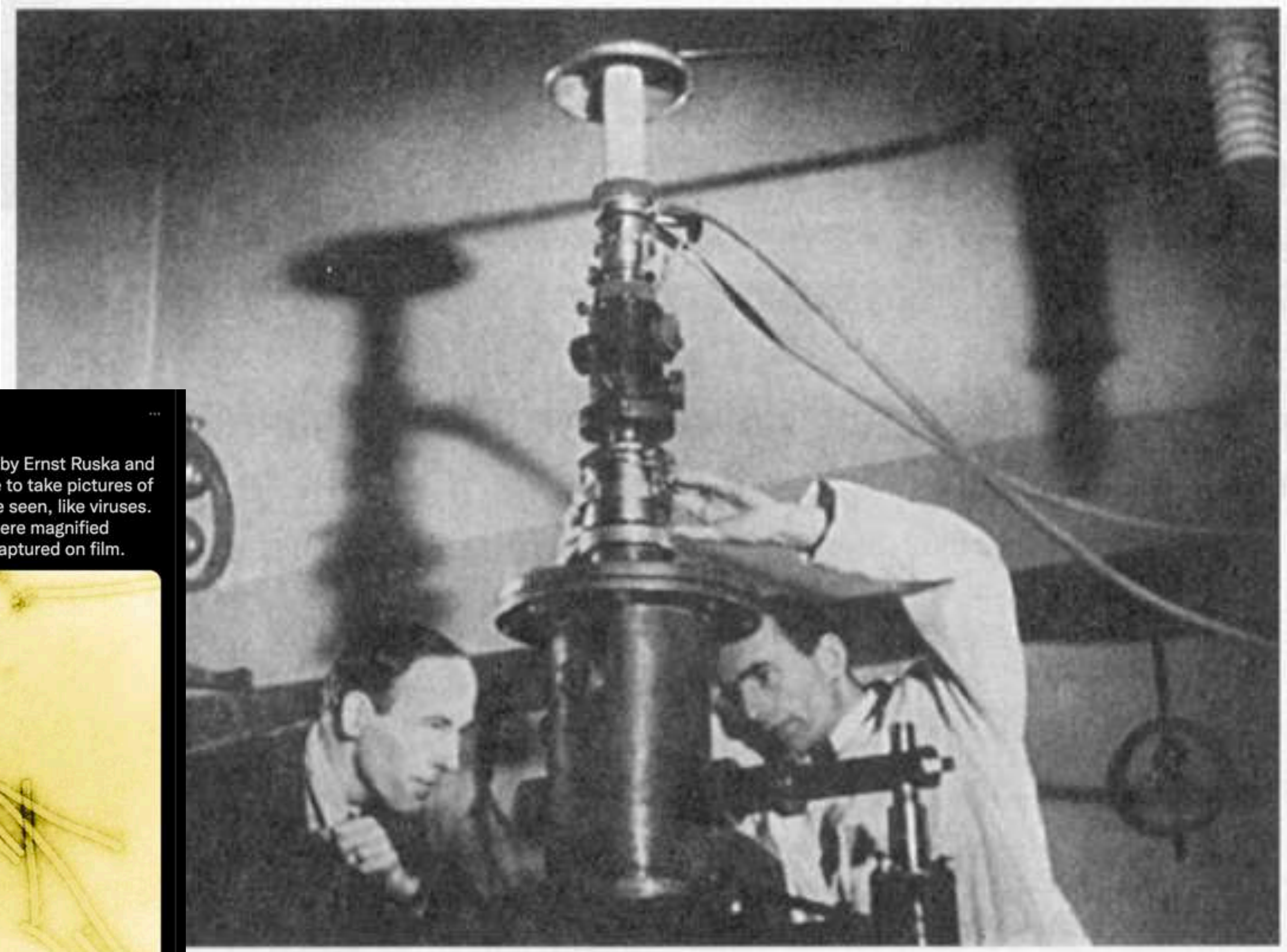
THE ELECTRON MICROSCOPE

Ruska and Knoll in Berlin in the early 1930s

-Wikipedia





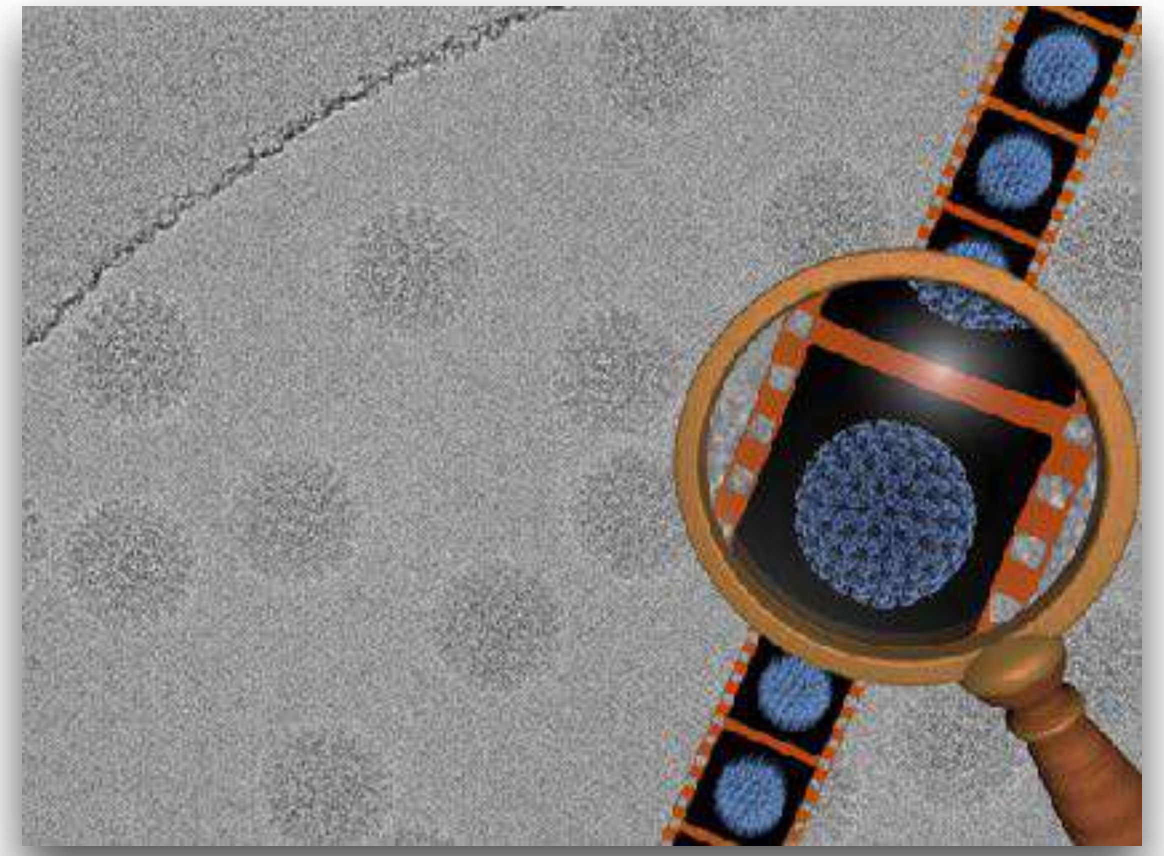


WHAT BROUGHT ABOUT THE RESOLUTION REVOLUTION? (~2012-2014)

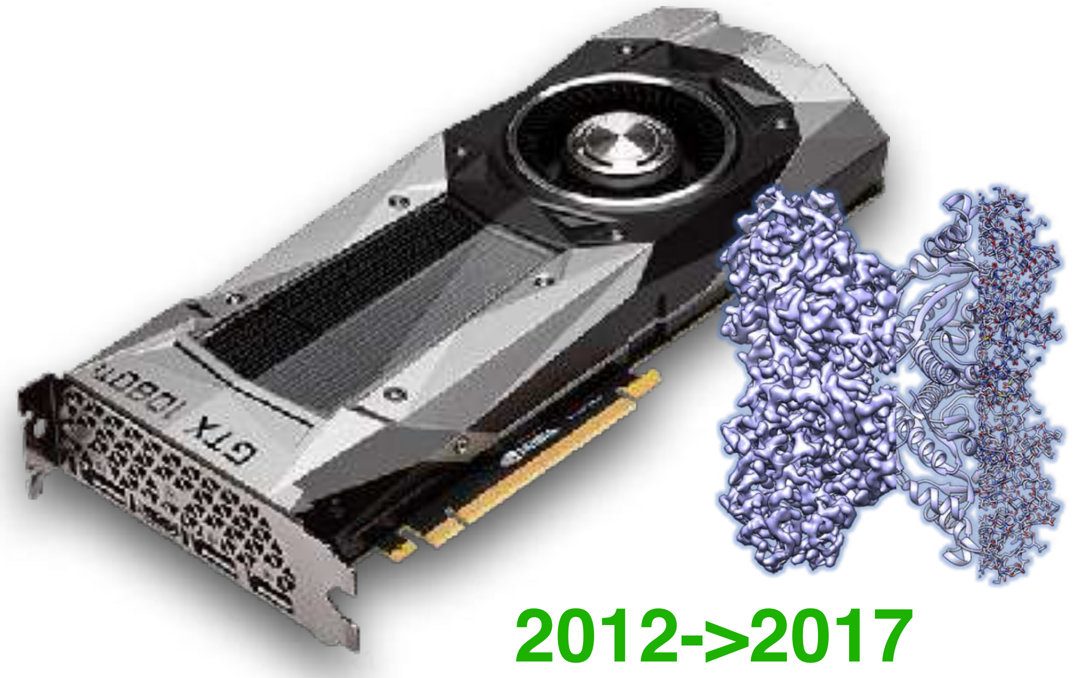
Hardware

Microscopes

Direct Detectors

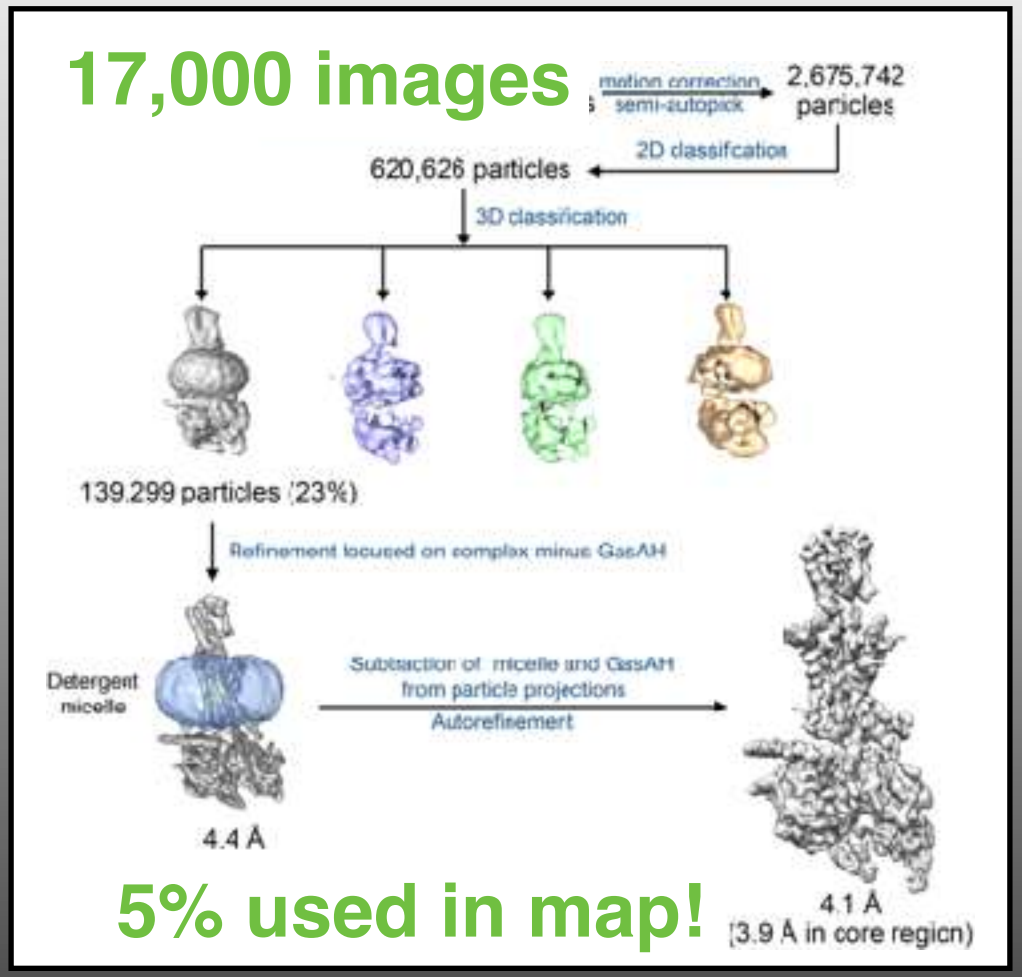


Computers



2012->2017
Cost reduced by 100x

Software

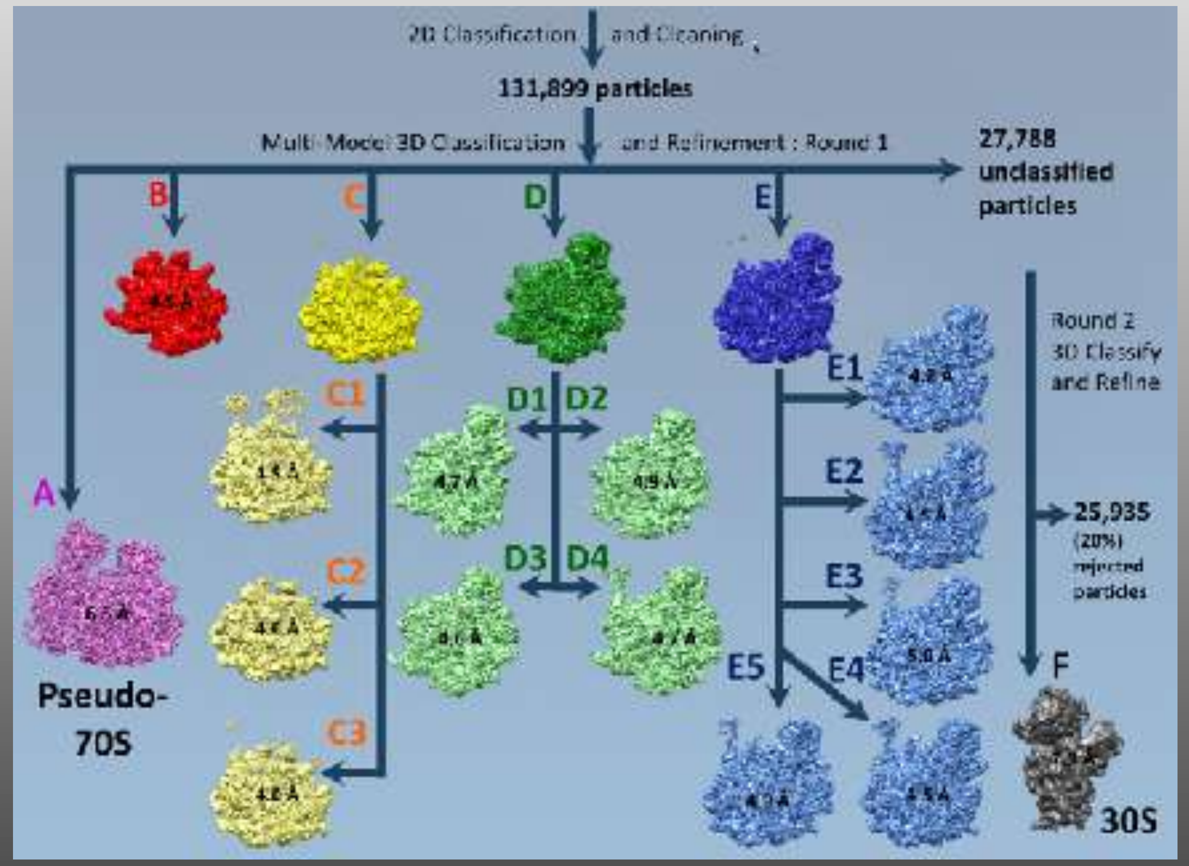


Leginon / SerialEM / EPU, …

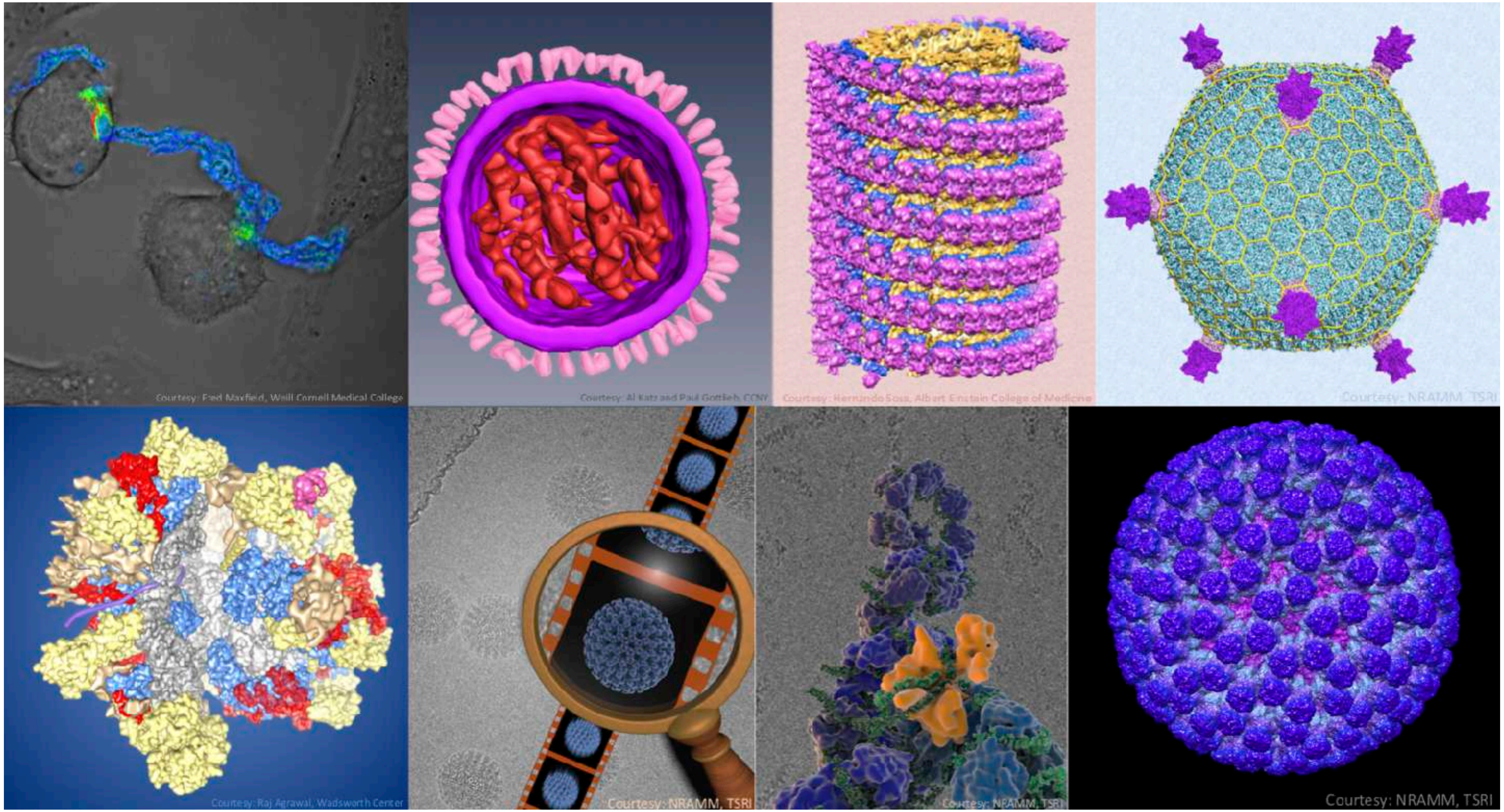
MotionCorr2, Unblur, …

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

14 independent structures



CRYOEM: TECHNOLOGY ON THE RISE



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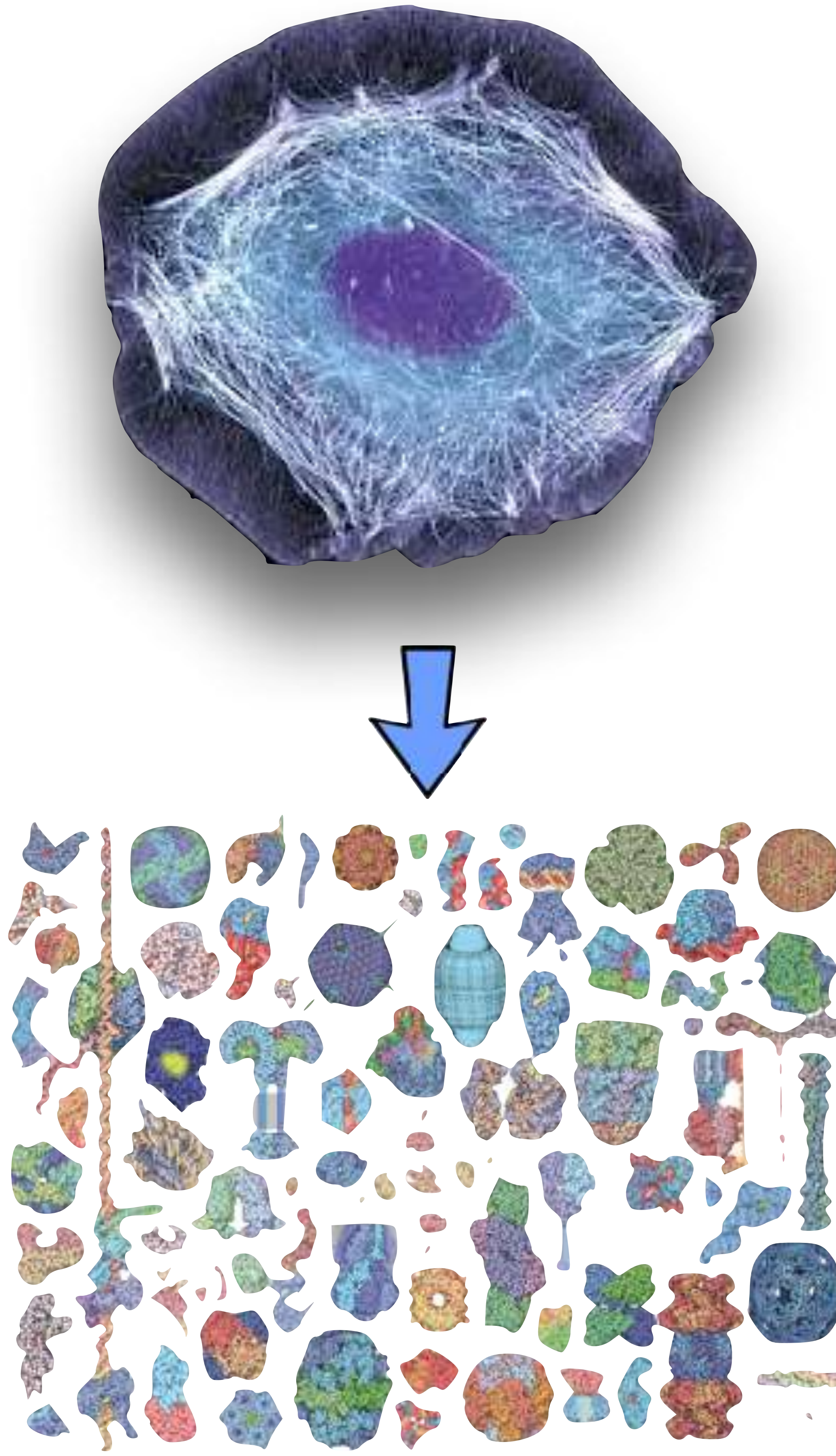
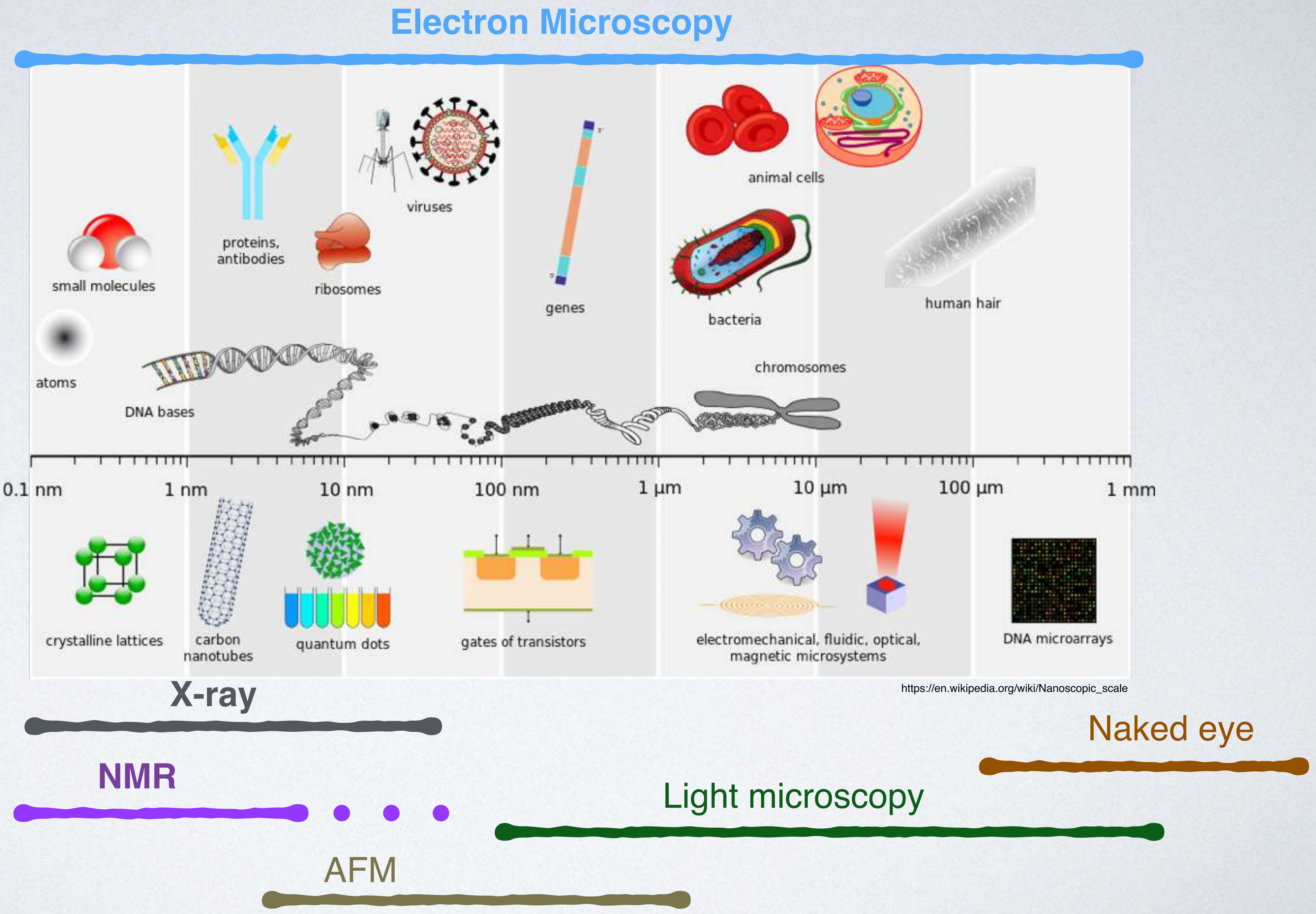


TBD (20??)

CRYOEM: SCALE WITHIN BIOLOGY



WHY ELECTRONS

CRYOEM: WHY ELECTRONS?

Pros

Small wavelength

Can be focused

Cons

Damages sample

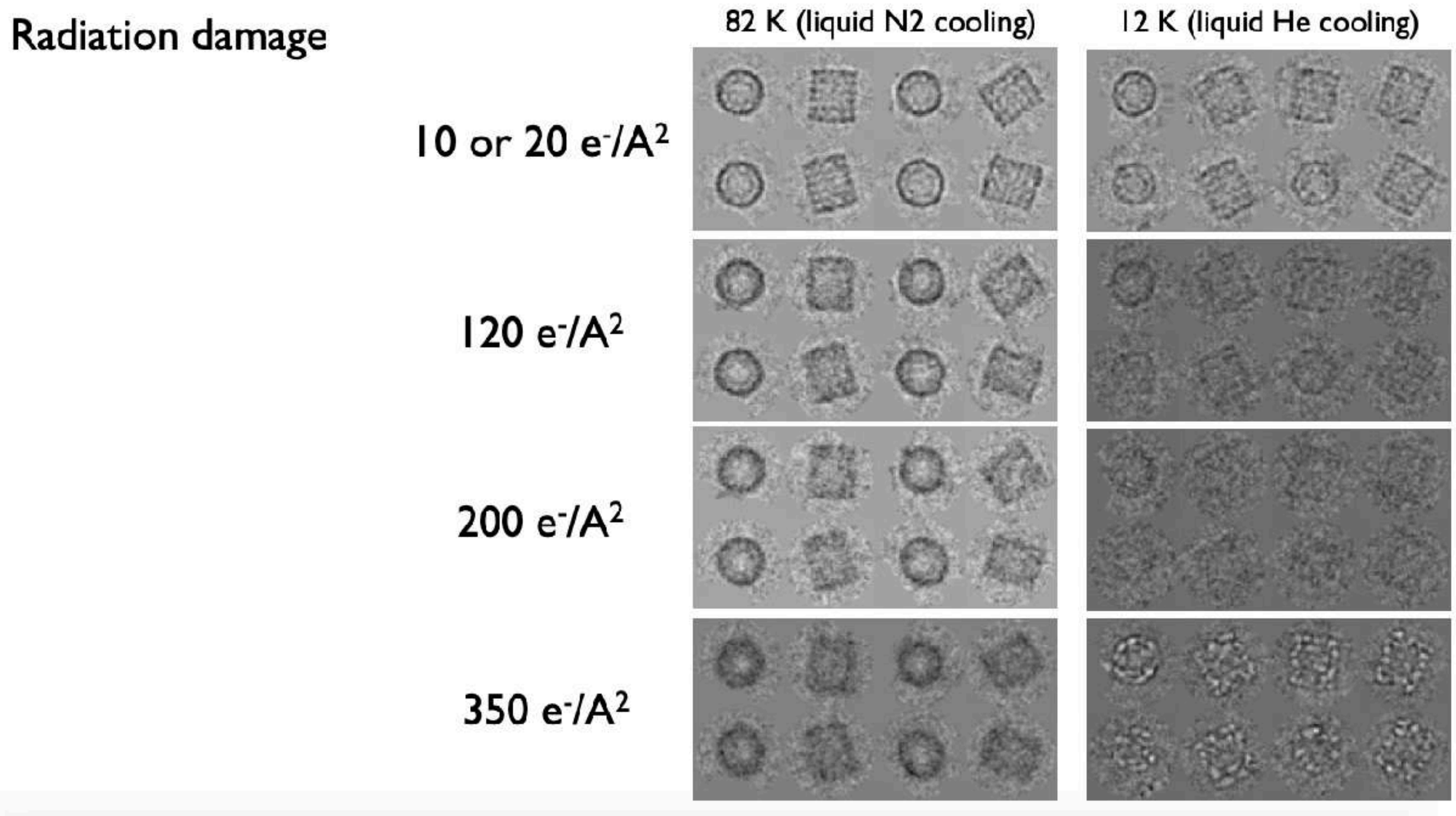
worse with faster electrons

Poor penetration

better with faster electrons

DETECTORS

Ideal dose for cryoEM?



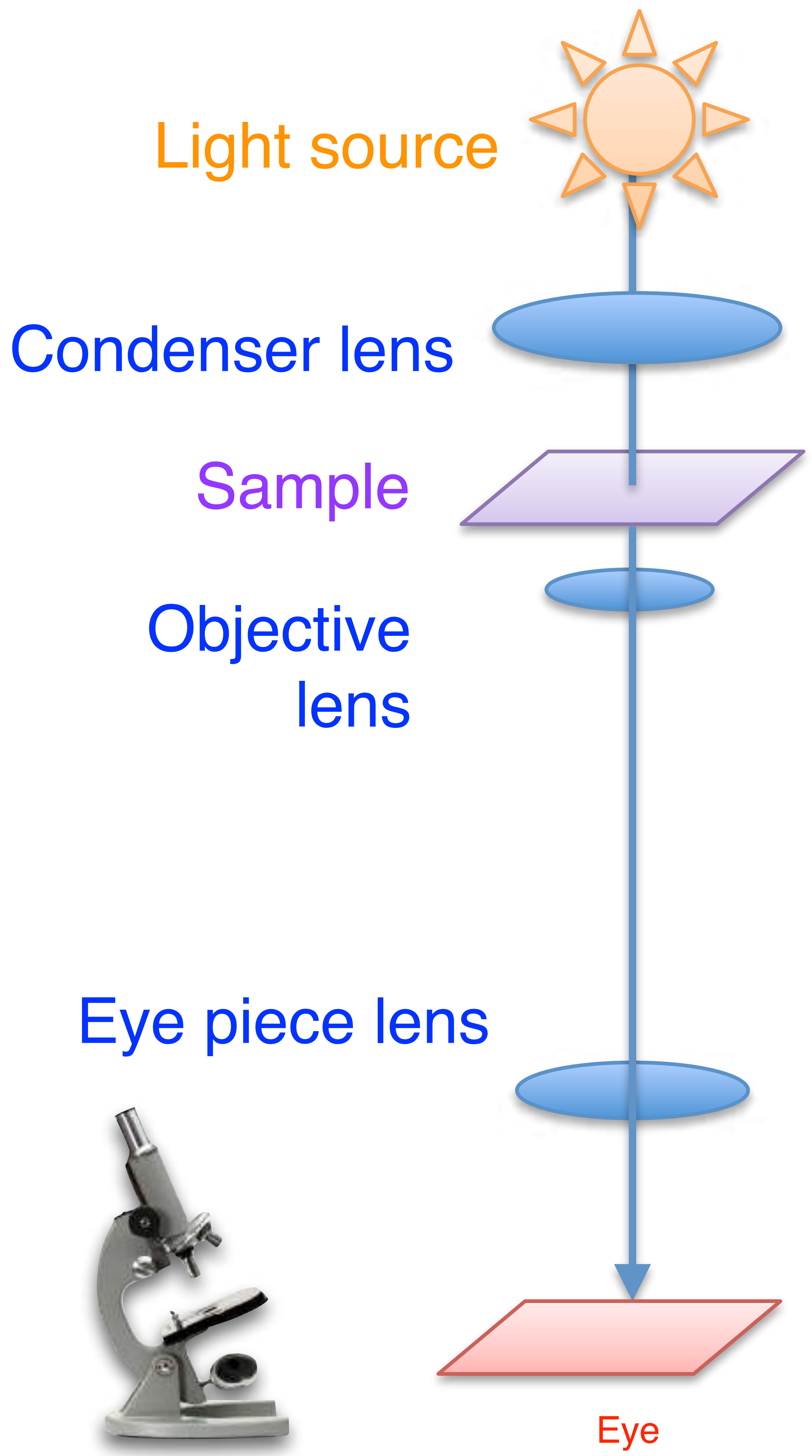
https://cryo-em-course.caltech.edu/

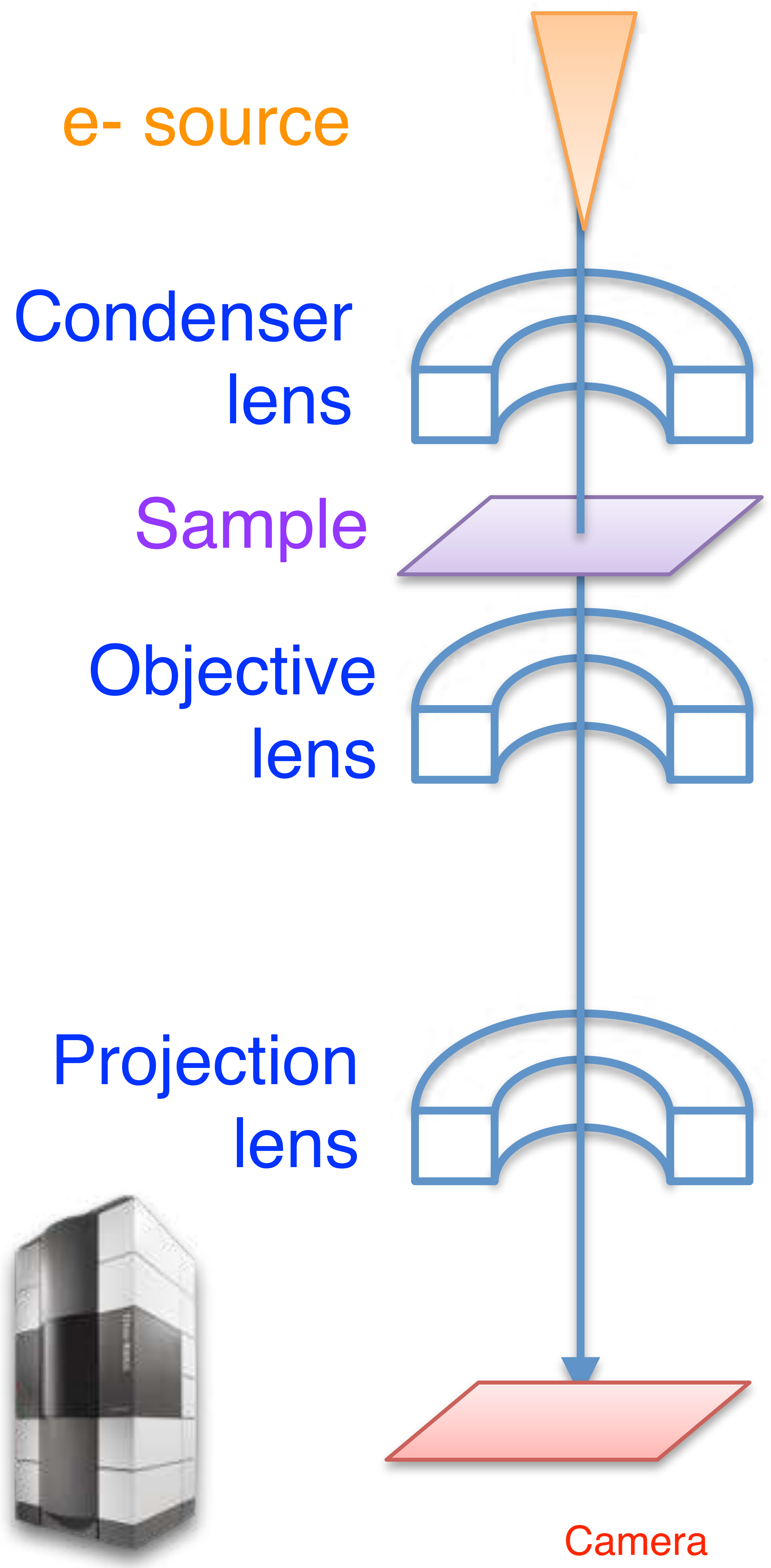
Specimen Behavior in the Electron Beam

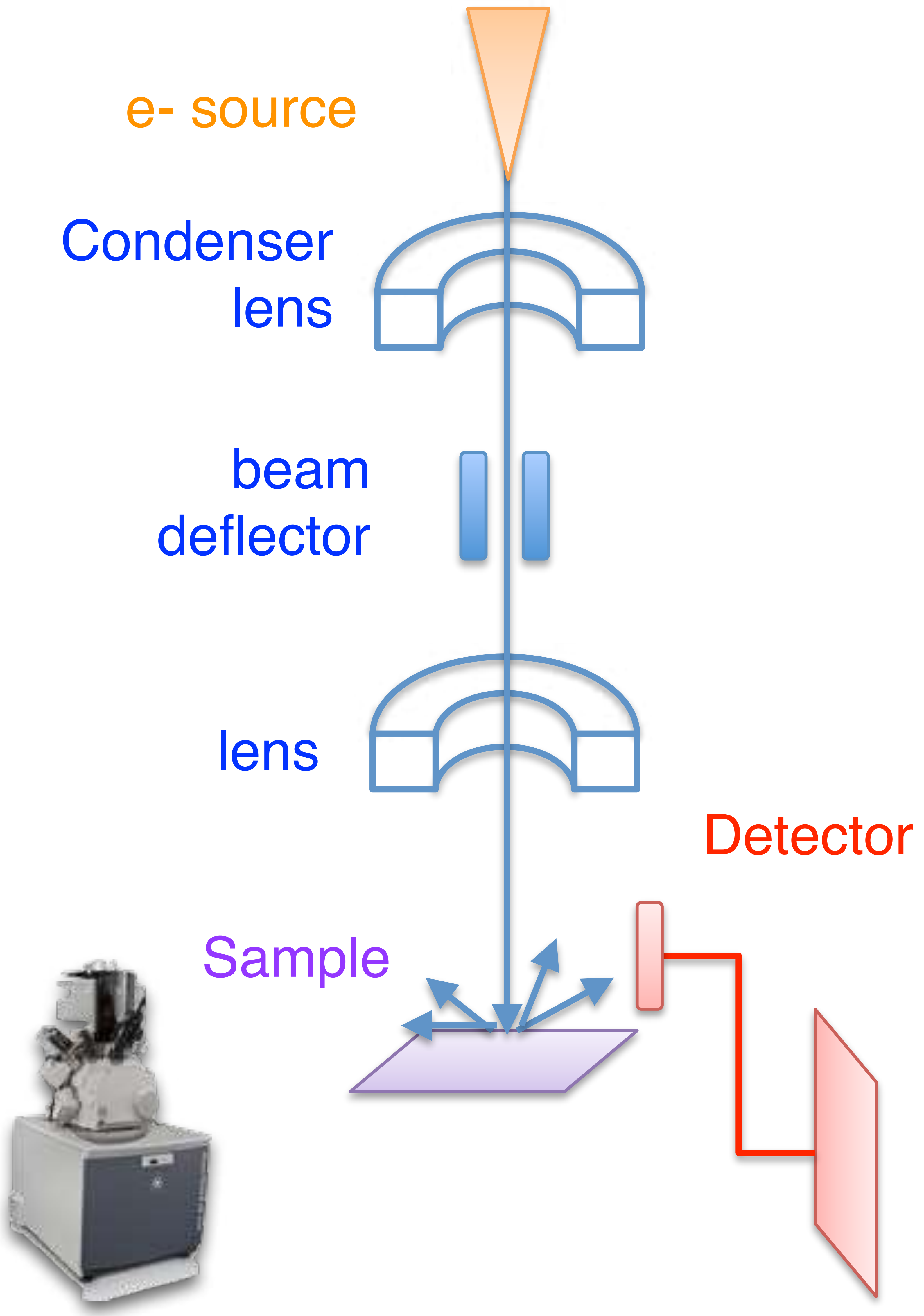
R.M. Glaeser[1]
Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, United States
[1]Corresponding author: e-mail address: rmglaeser@lbl.gov

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

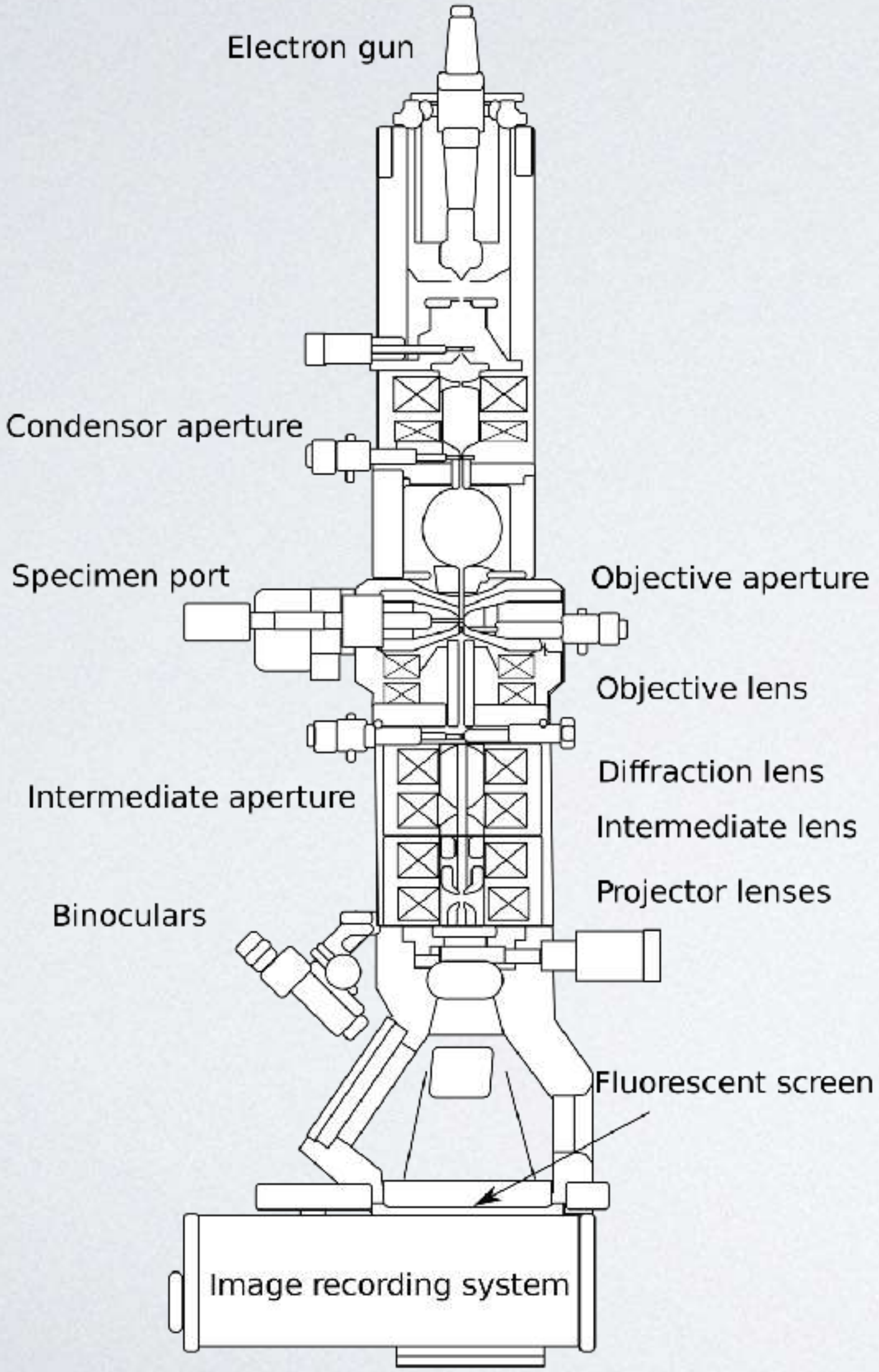
CRYOEM: TOOLS







MAIN PARTS OF AN EM



Electron sources

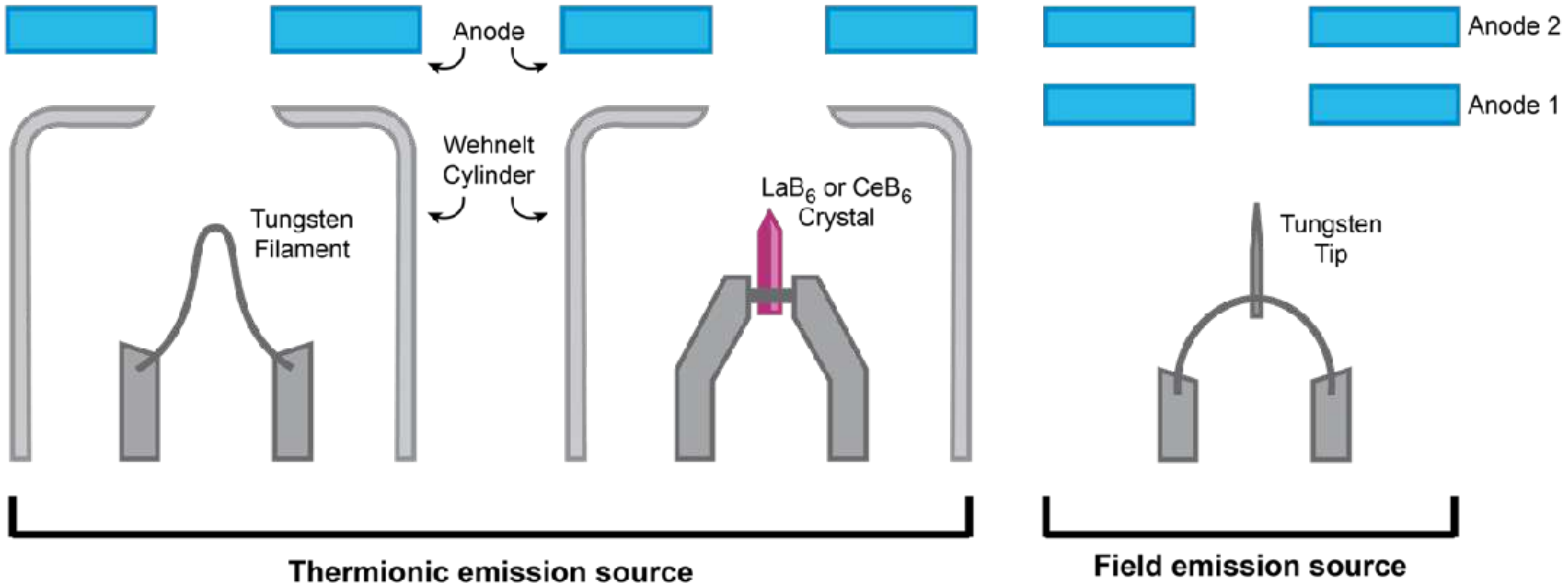
Vacuum systems

Lenses

Detectors

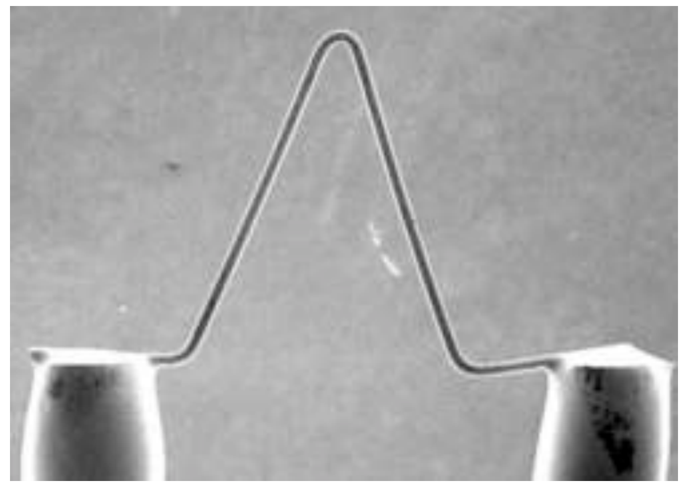
ELECTRON SOURCES

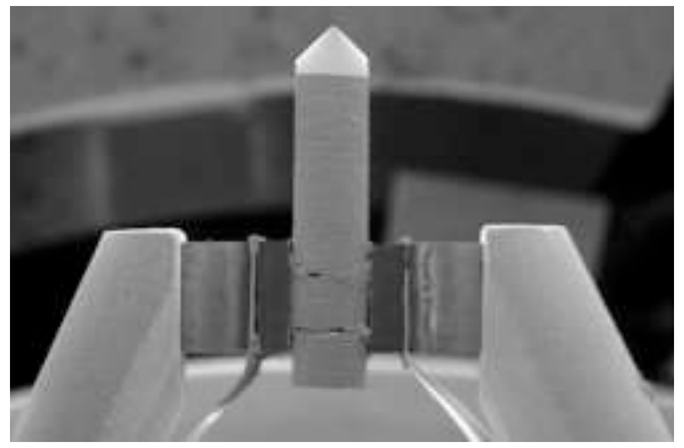
What are the 3 main kinds of electron sources?

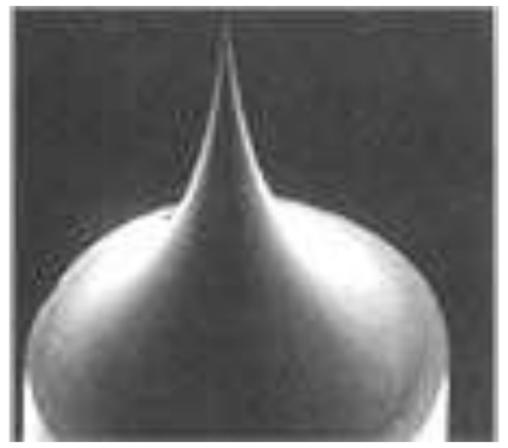


www.thermofisher.com

nanoscience.com

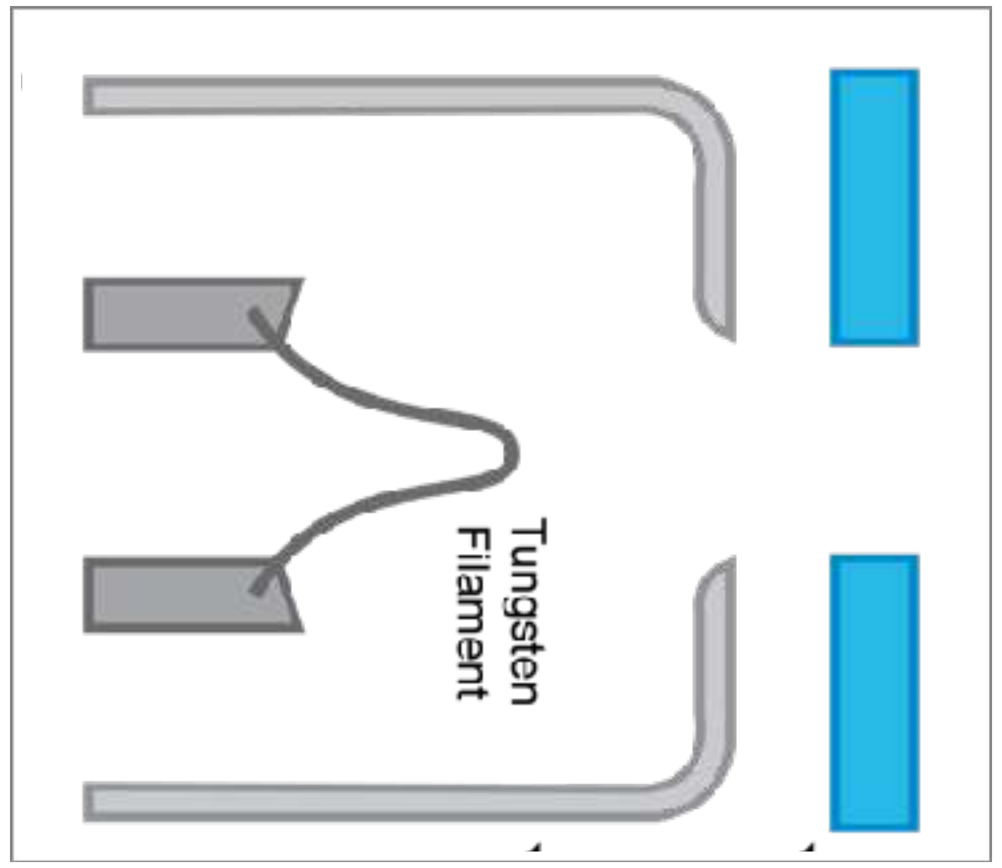


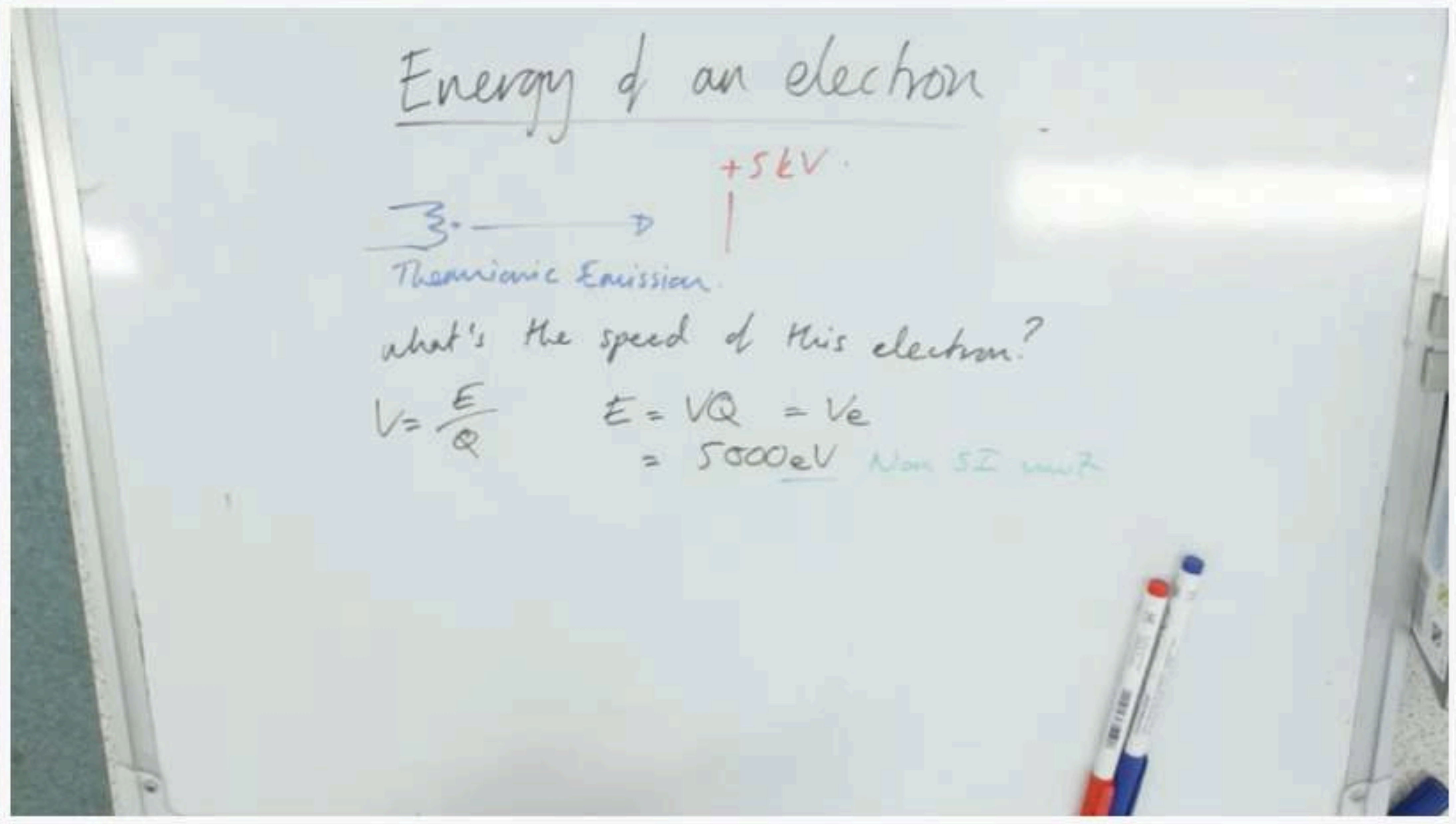




ELECTRON SOURCES

How fast are the electrons moving?





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

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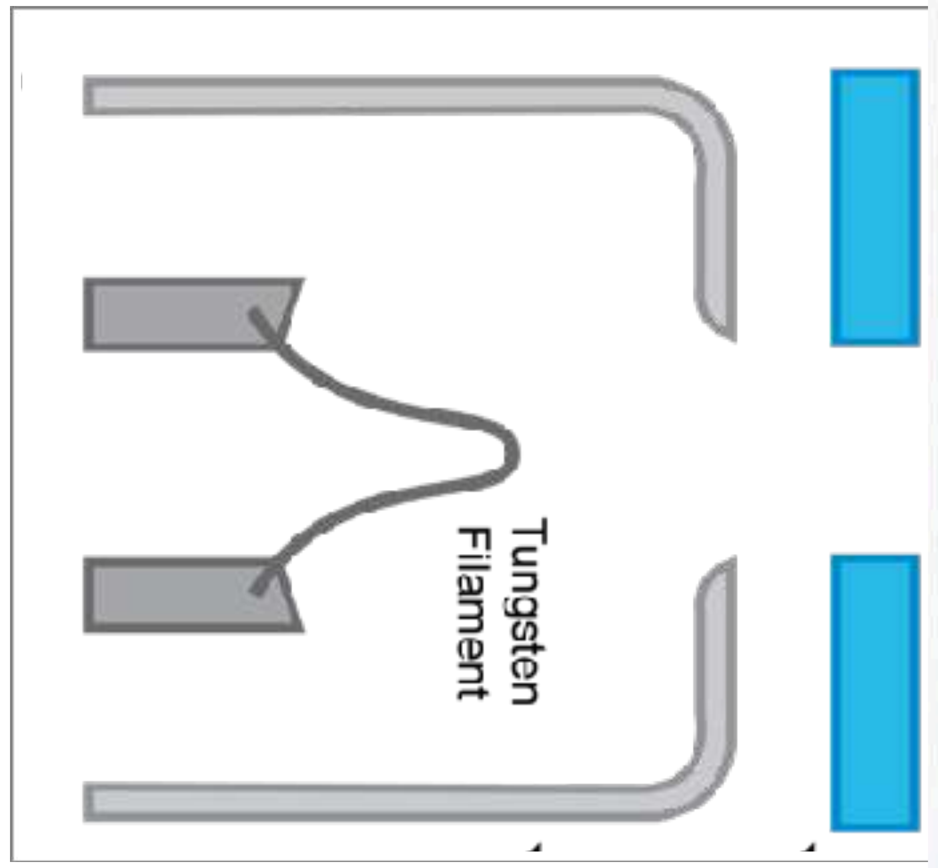

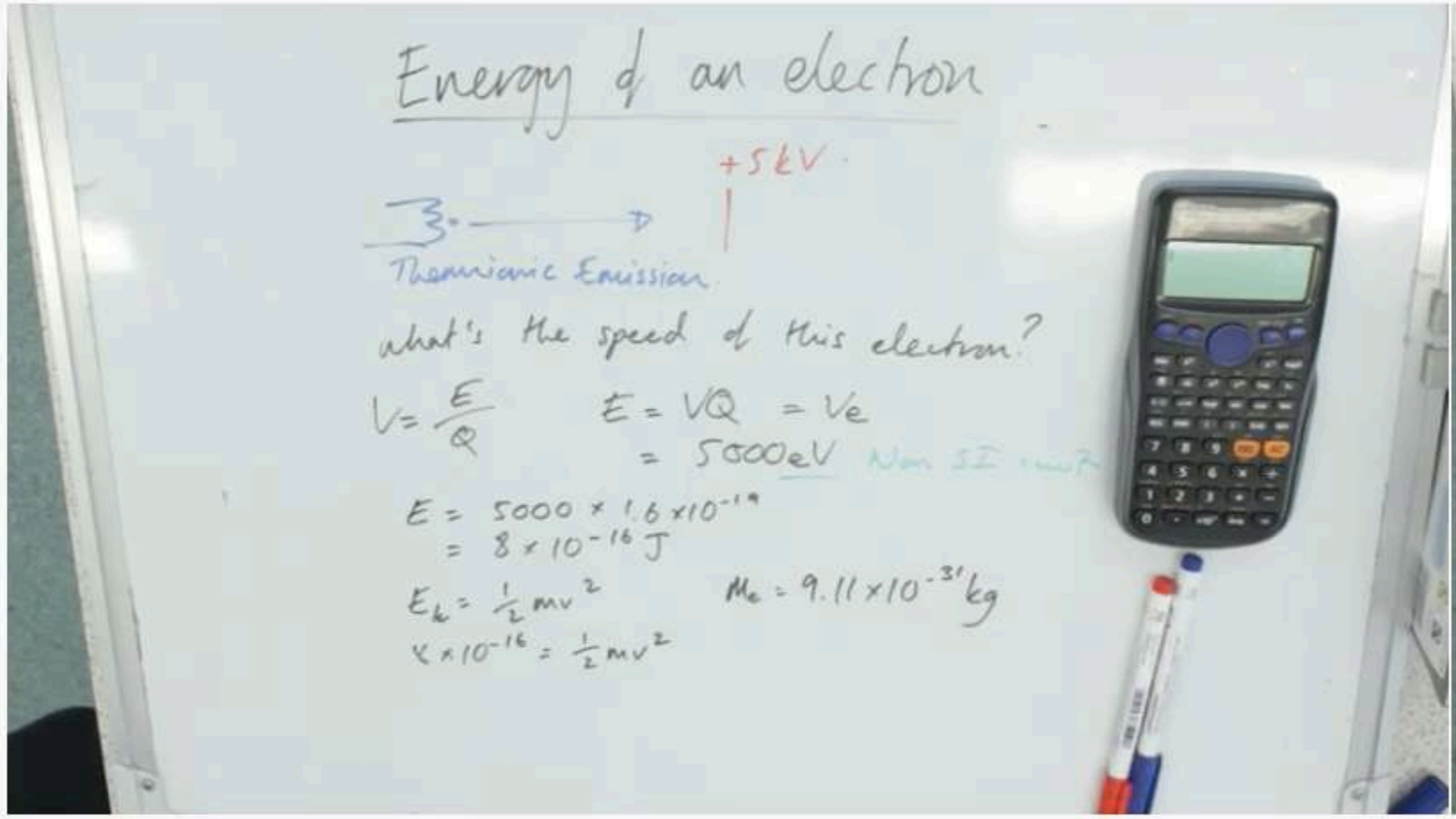

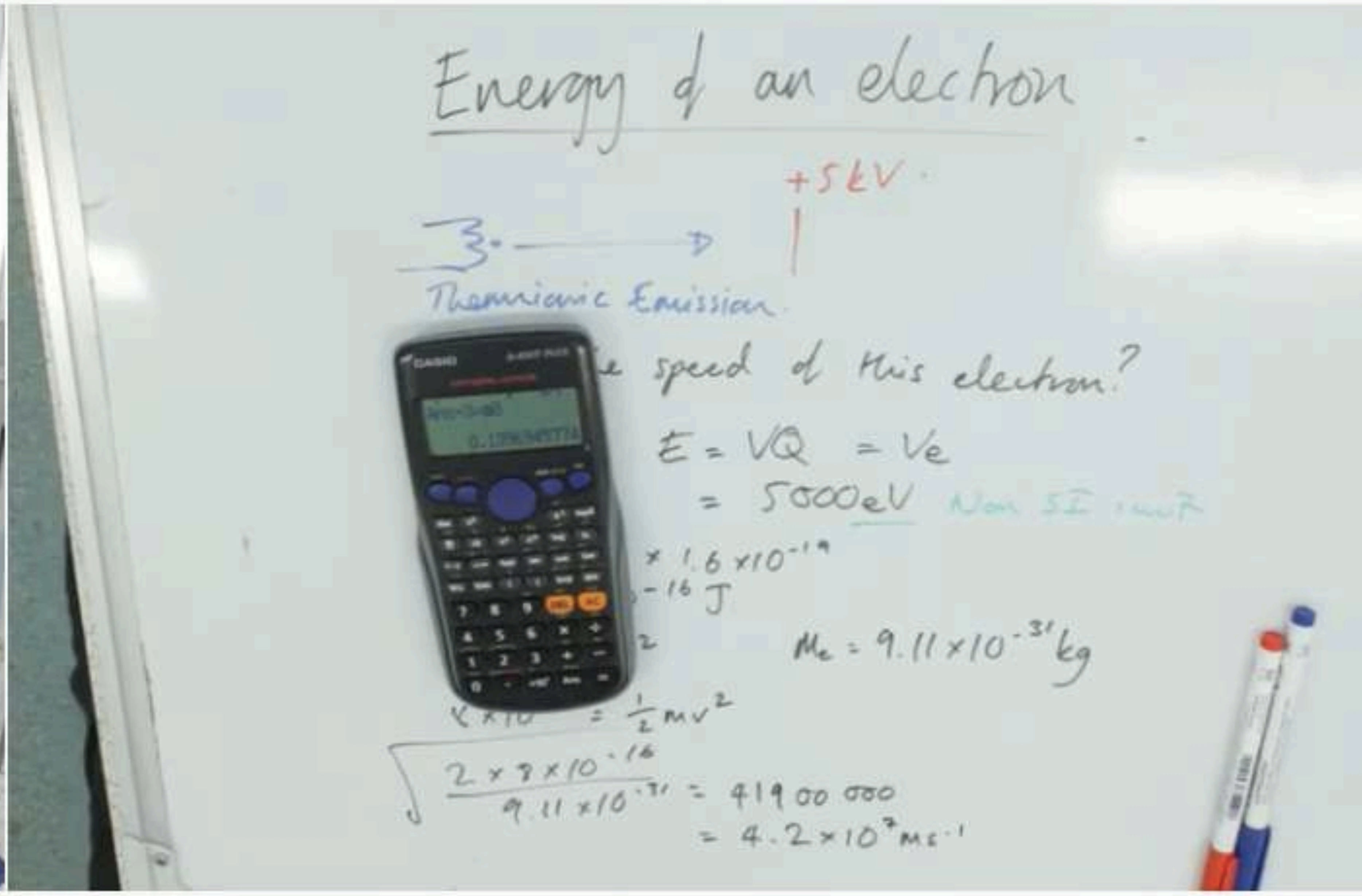

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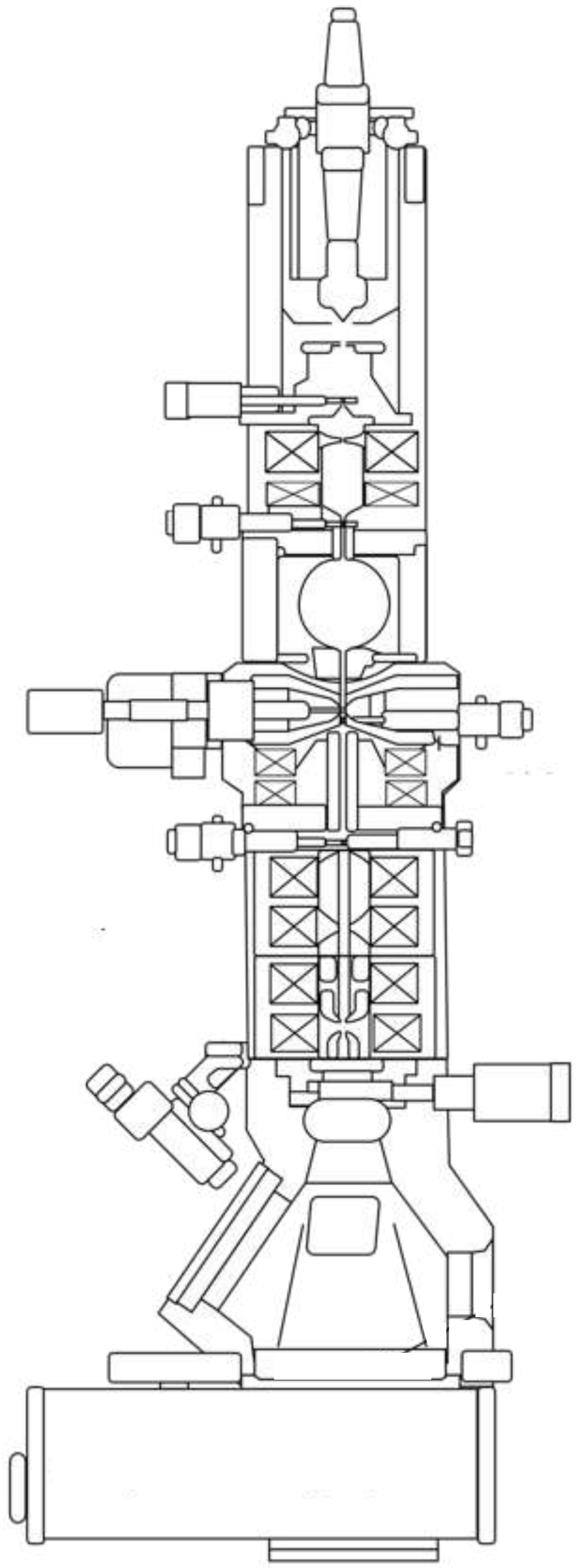
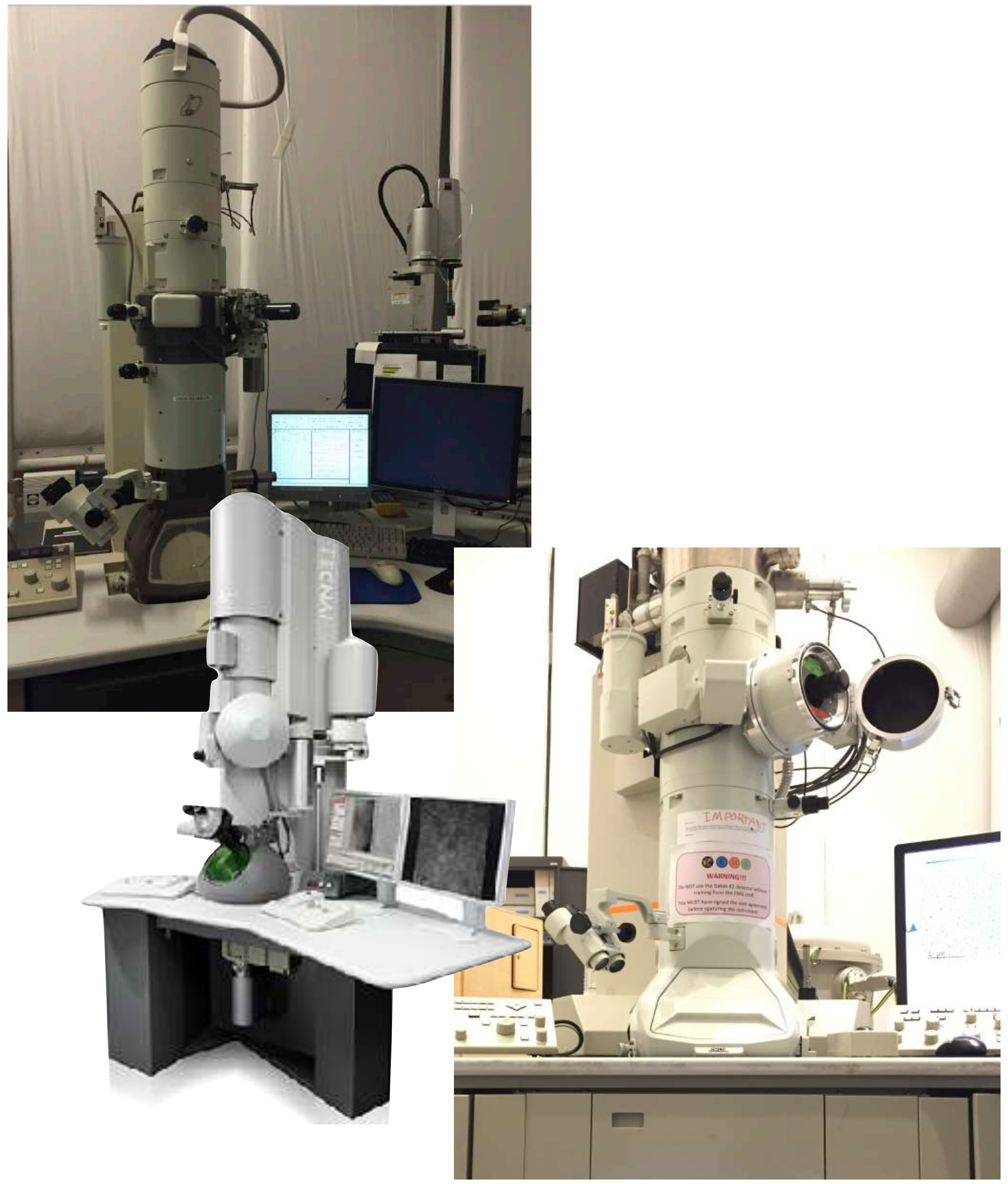
ELECTRON SOURCES & TYPES OF CRYOEM



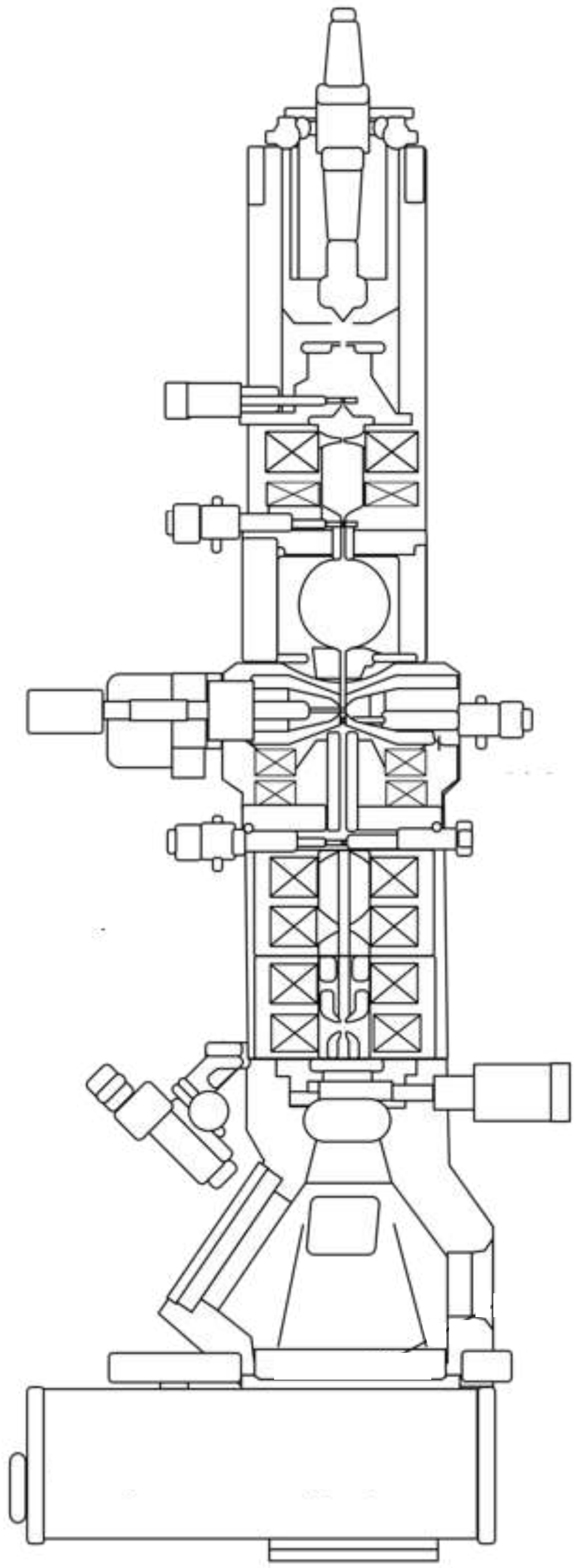
80-120 kV: JEM 1230; Tecnai T12
W or LaB6
High contrast & robust
sub-nm resolution

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

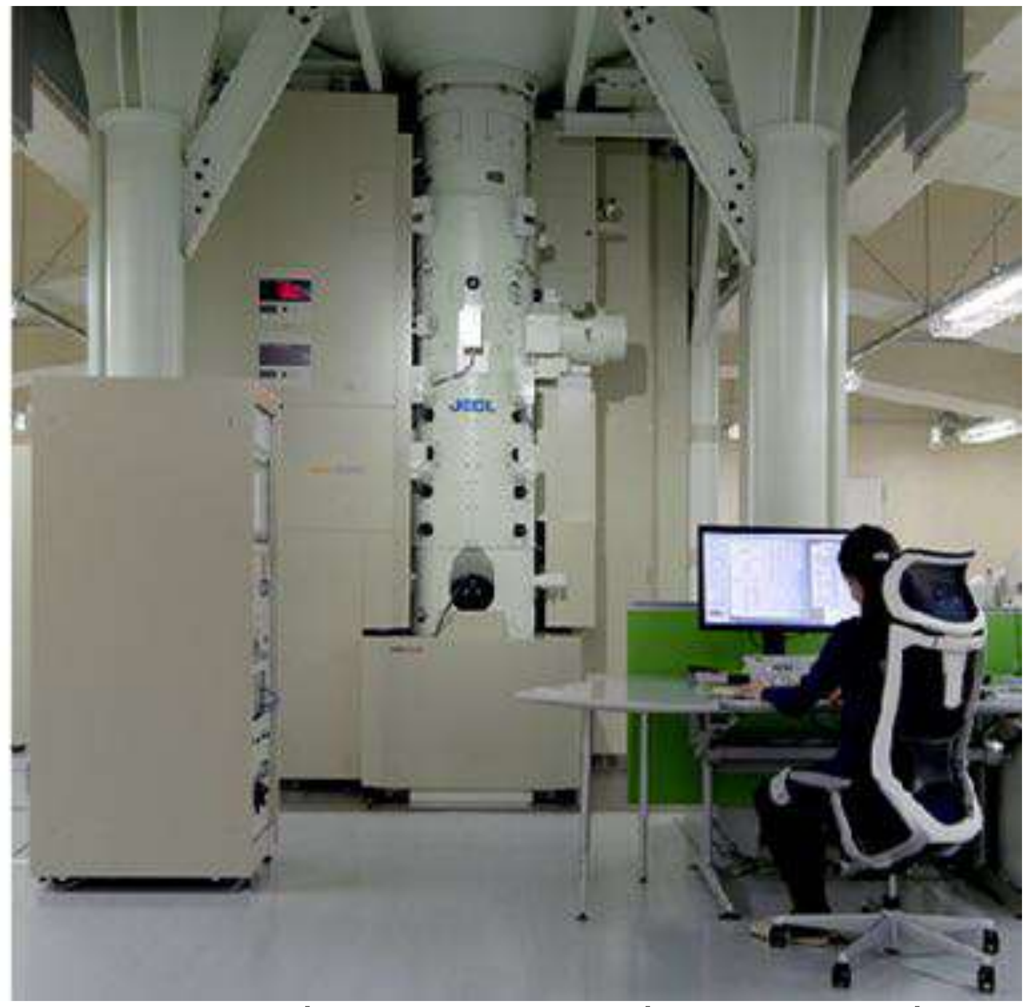
300 kV: JEM 3200FSC, cryo-ARM, Krios, Polara
FEG
Smaller effect on unwanted lens aberration
1.5-3 Å resolution



ELECTRON SOURCES & TYPES OF CRYOEM

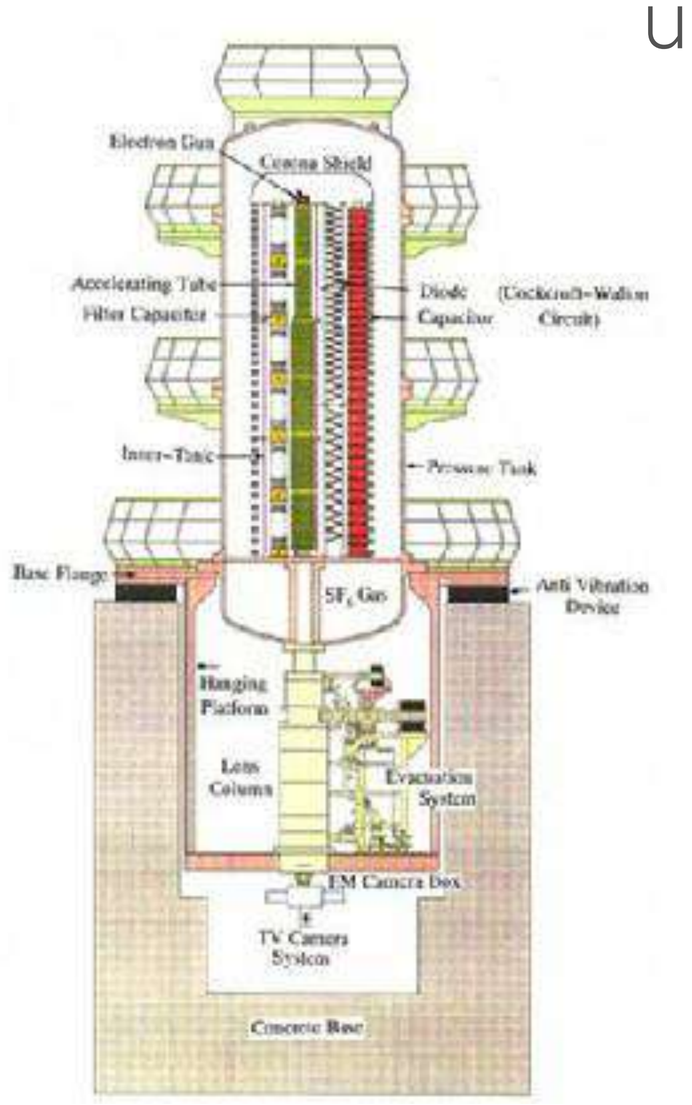


1-1.2 MV: Hitachi, JEOL
LaB6



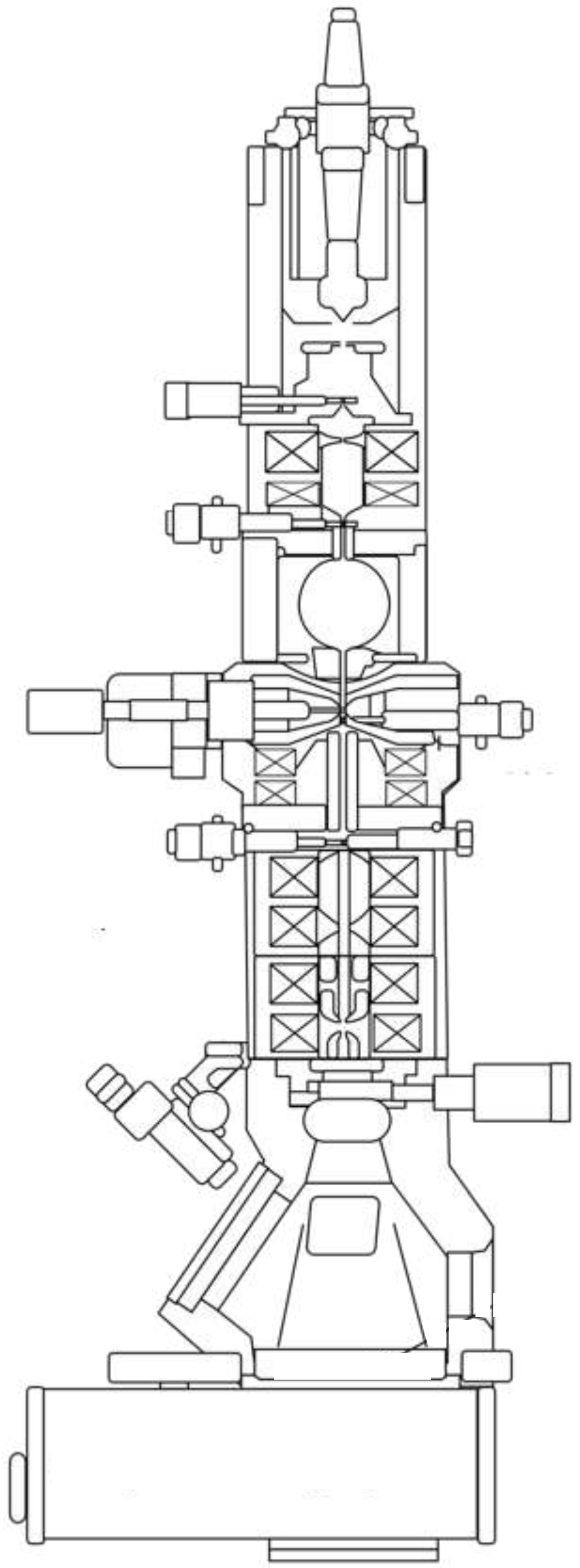
uhvem.osaka-u.ac.jp

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

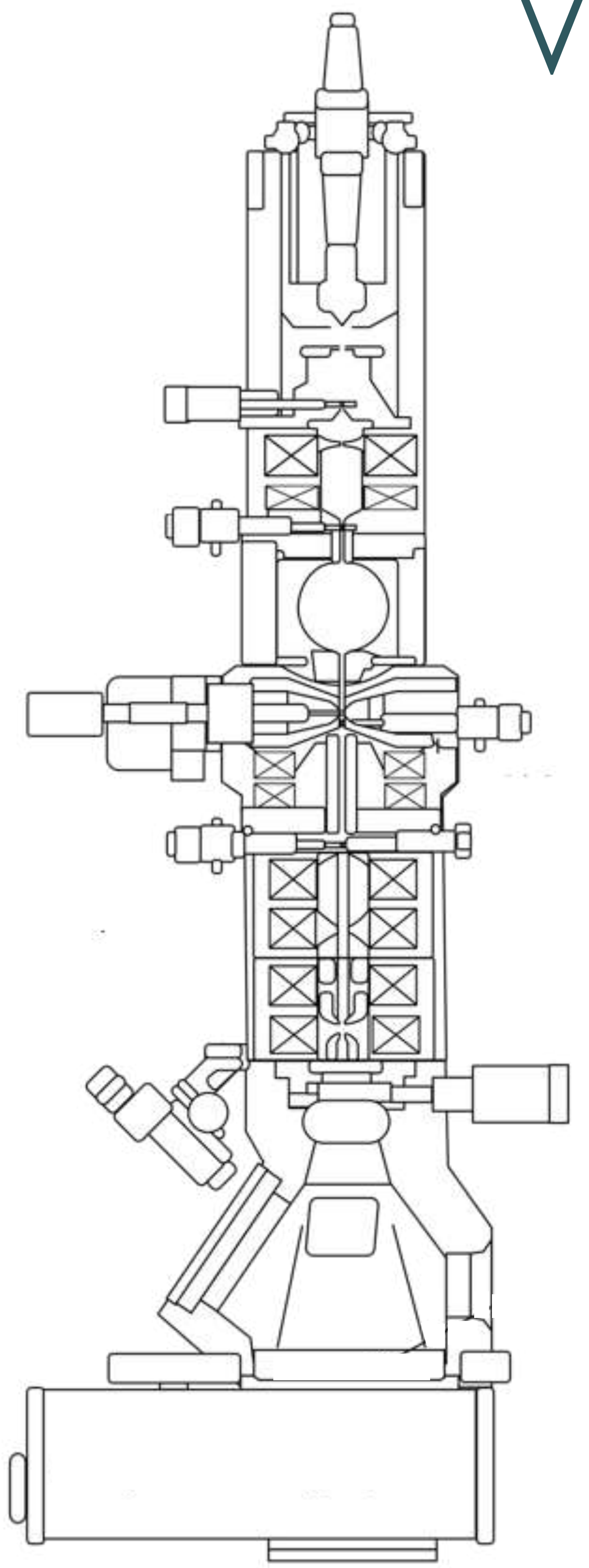
Filament - O2 will burn out source

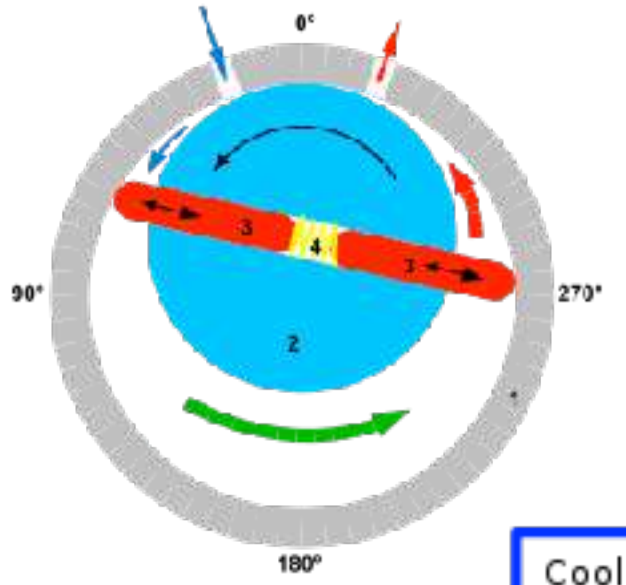
Contamination - reduce interaction gas, e-beam and sample

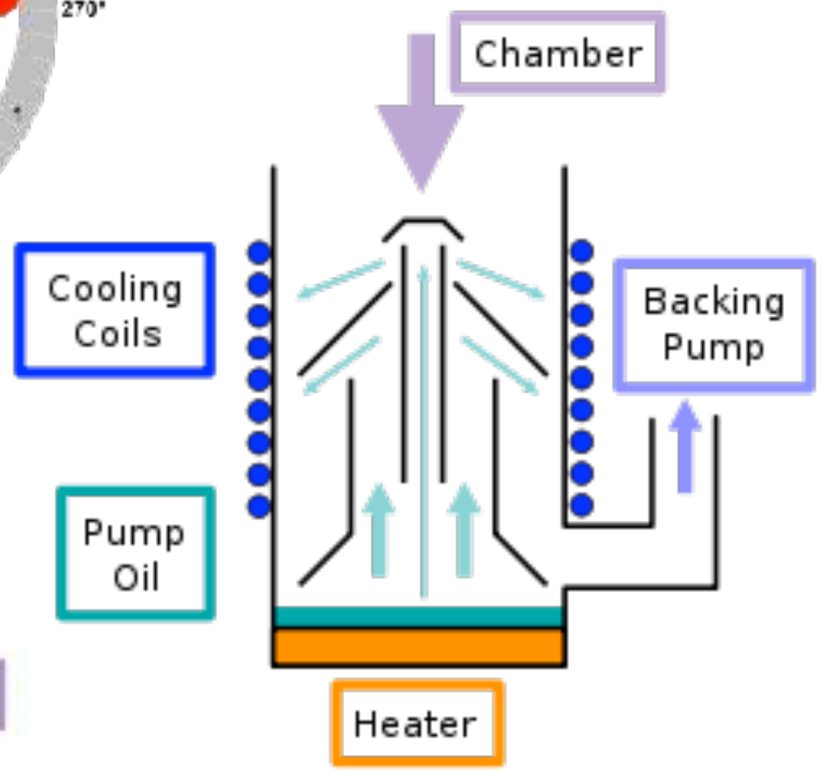
VACUUM SYSTEMS

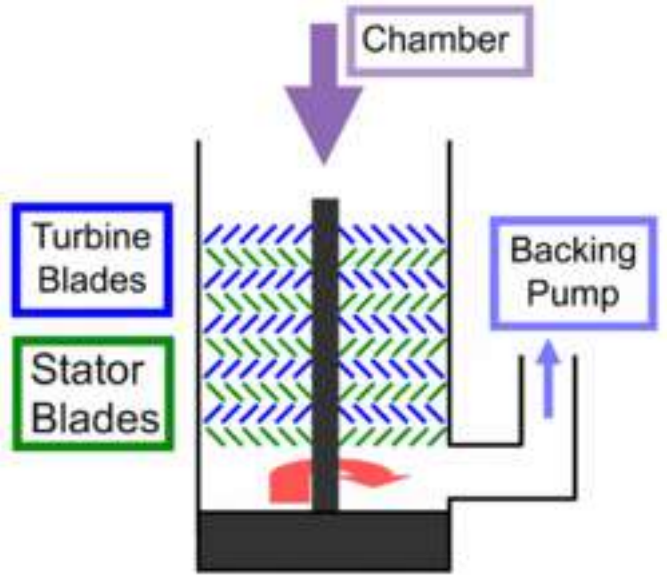
What types of pumps do we have?

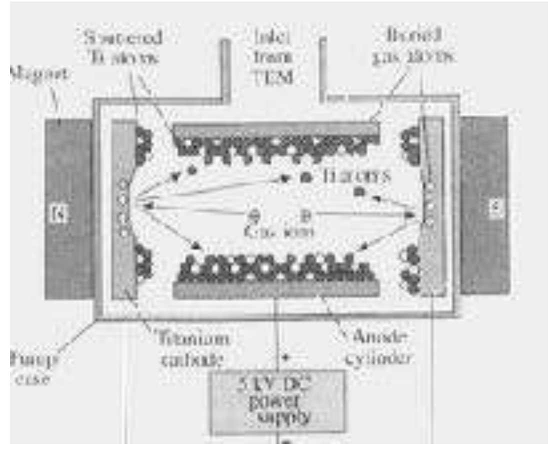
1 mm Hg = 1 Torr = 10^2 Pa
1 atm = 760 Torr = 7.5×10^4 Pa







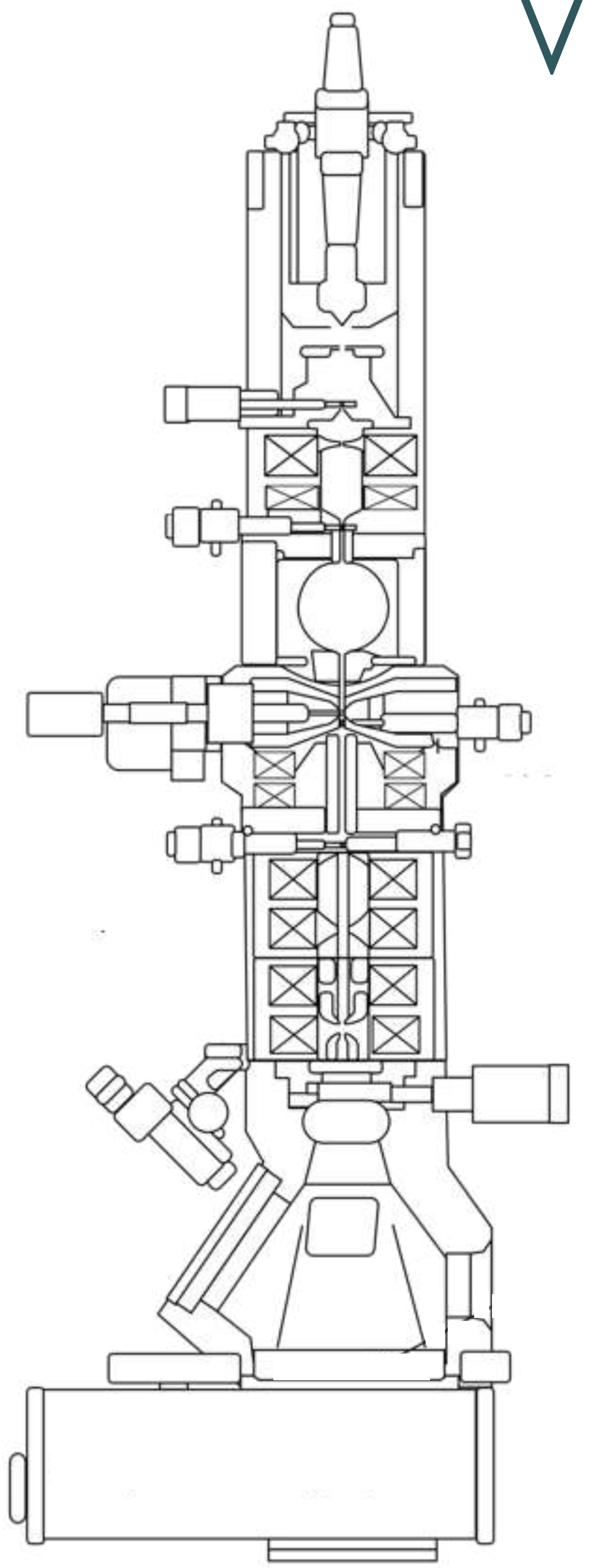




PVP / Rotary	$1-10^{-3}$ Torr	>0.1 Pa
Diffusion	$10^{-3}-10^{-6}$ Torr	$0.1-10^{-4}$ Pa
Turbo	$10^{-6}-10^{-9}$ Torr	$10^{-4}-10^{-7}$ Pa
IGP	$10^{-9}-10^{-12}$ Torr	$10^{-7}-10^{-9}$ Pa

wikipedia.com

VACUUM SYSTEMS

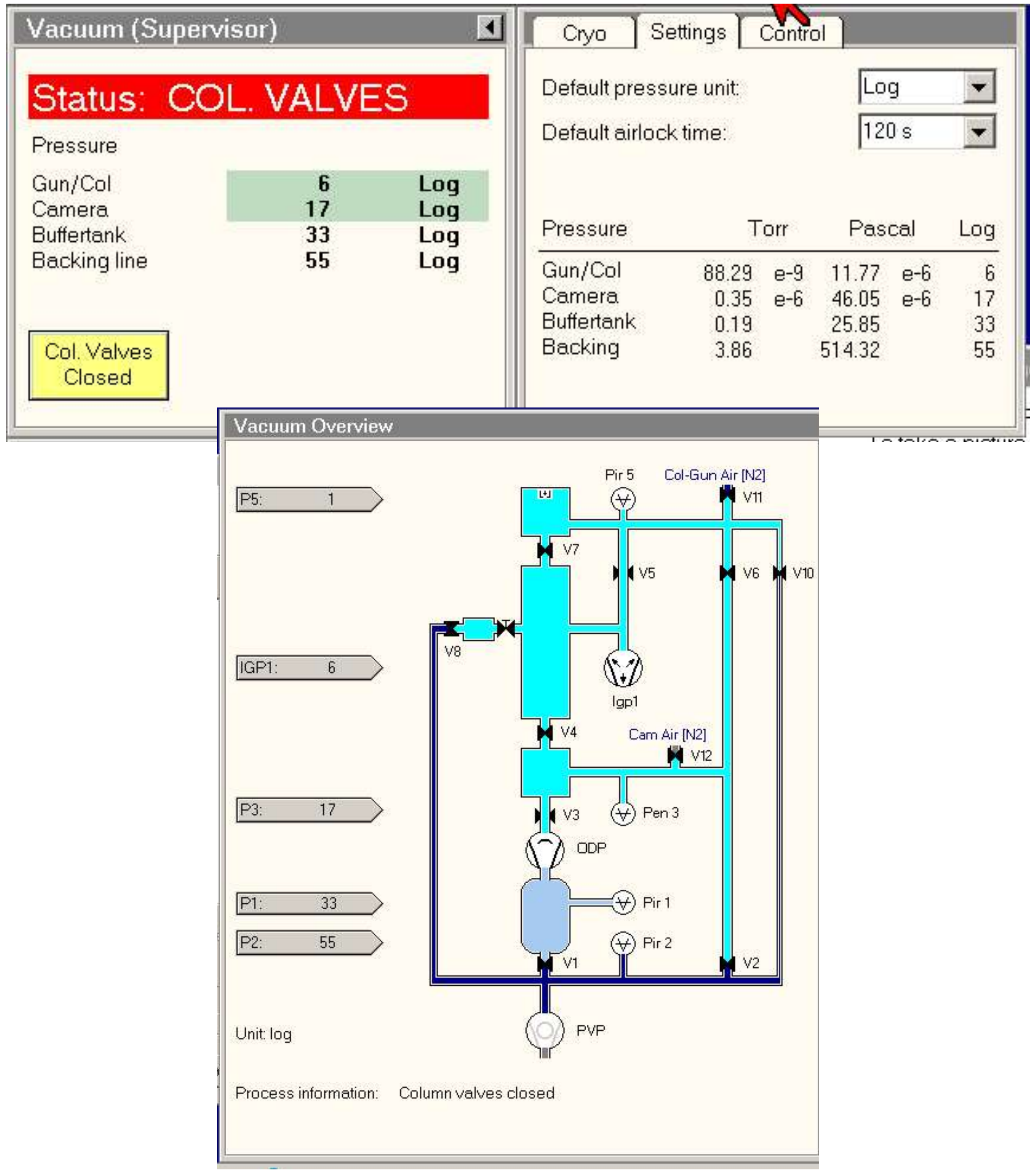


What types of pumps do we have?

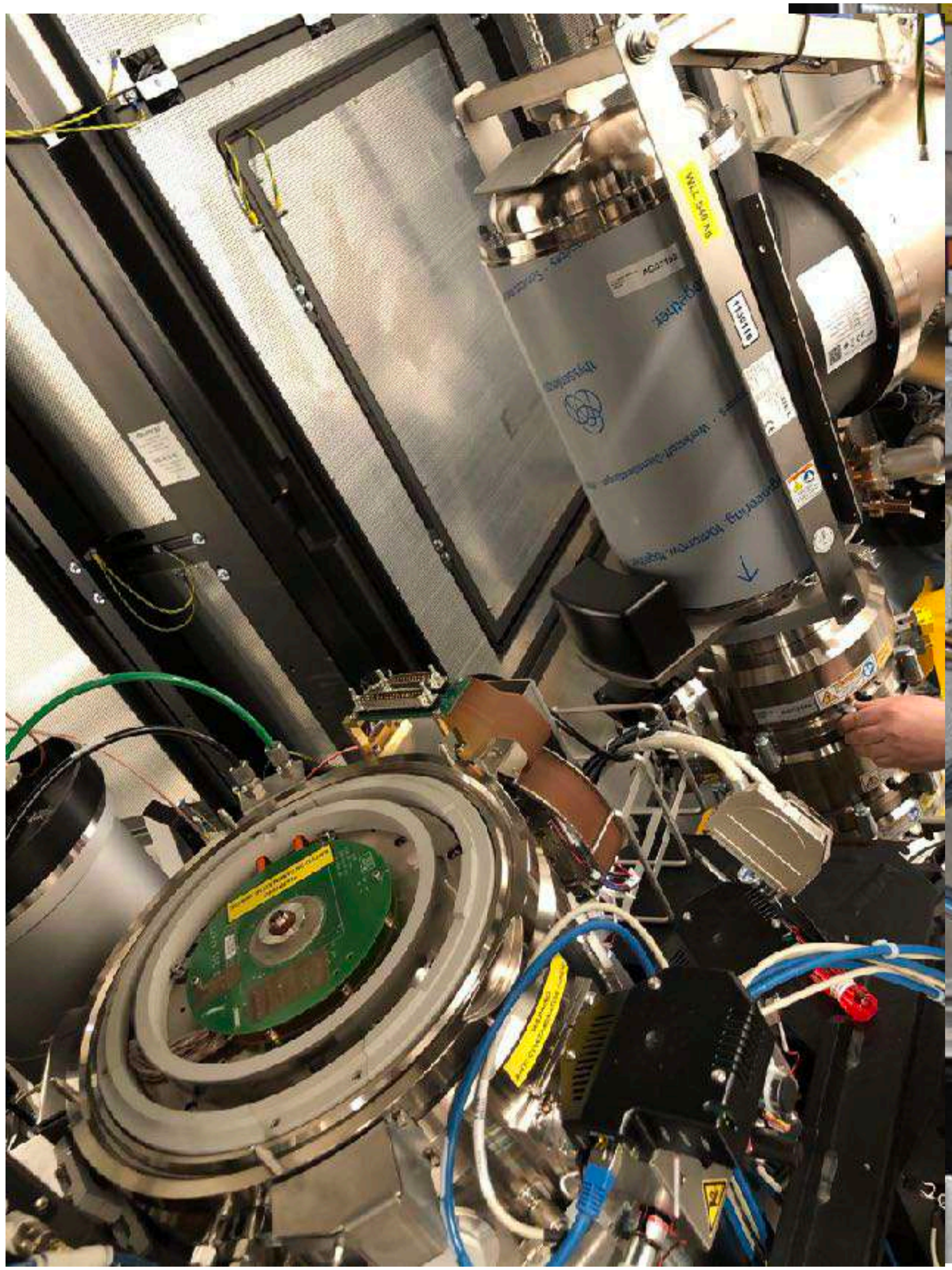
$1 \text{ mm Hg} = 1 \text{ Torr} = 10^2 \text{ Pa}$

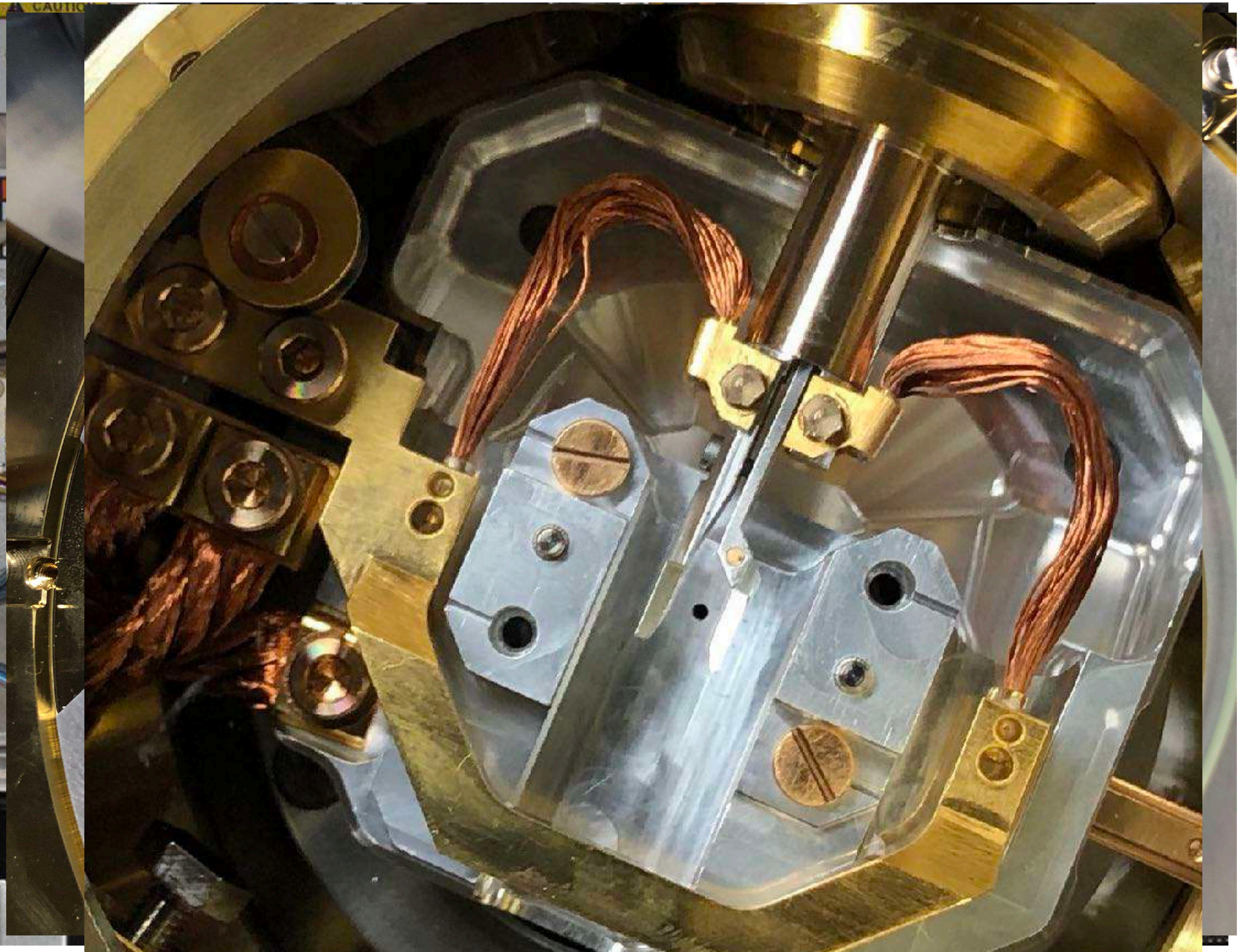
$1 \text{ atm} = 760 \text{ Torr} = 7.5\times10^4 \text{ Pa}$

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



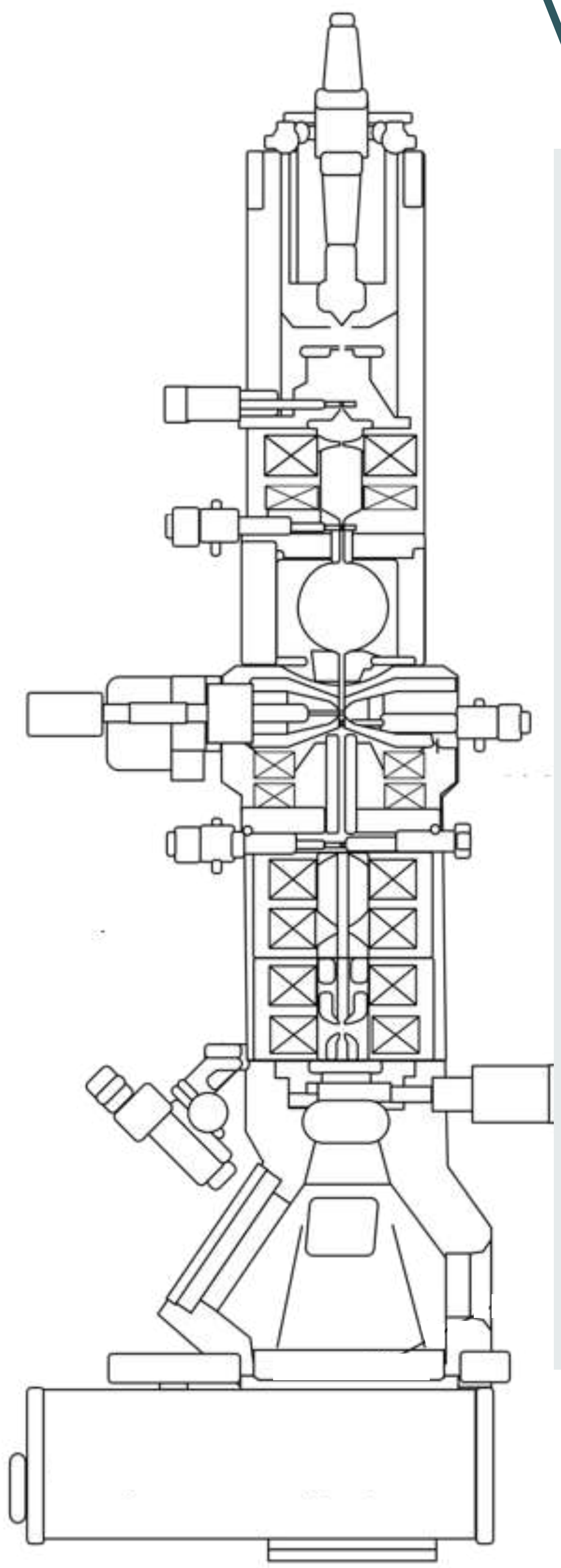
VACUUM SYSTEMS

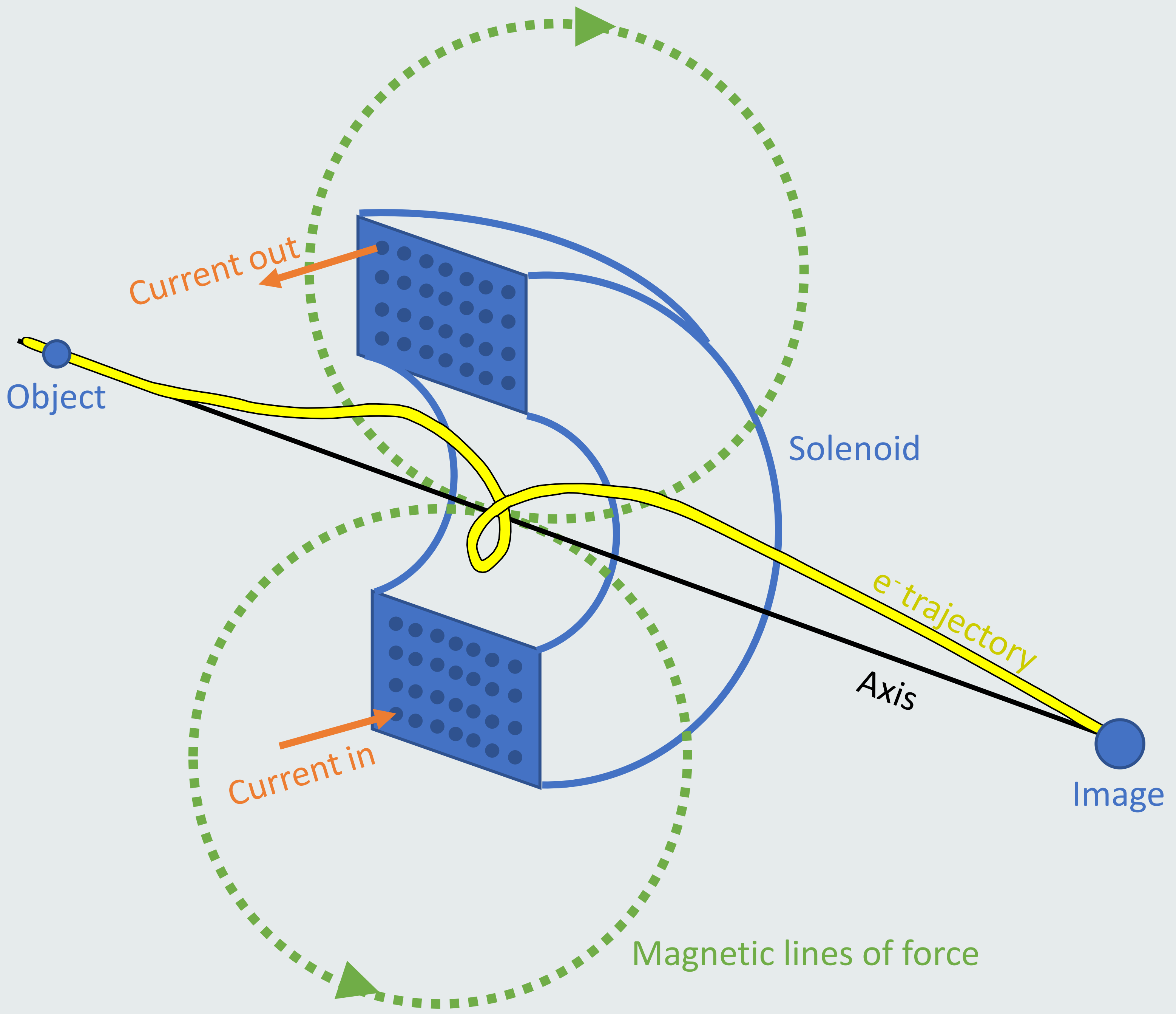




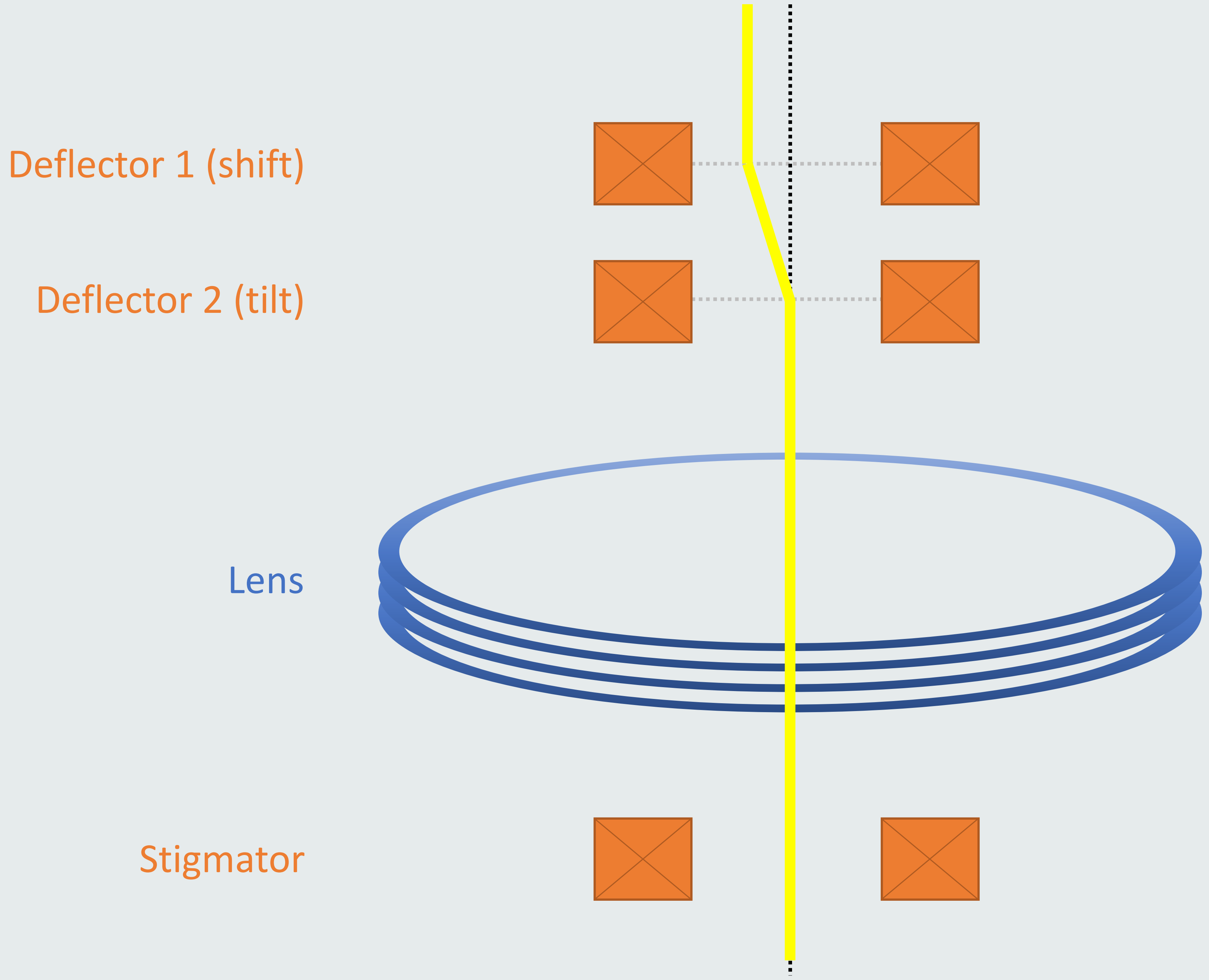
LENSES

What types of lenses do we have?





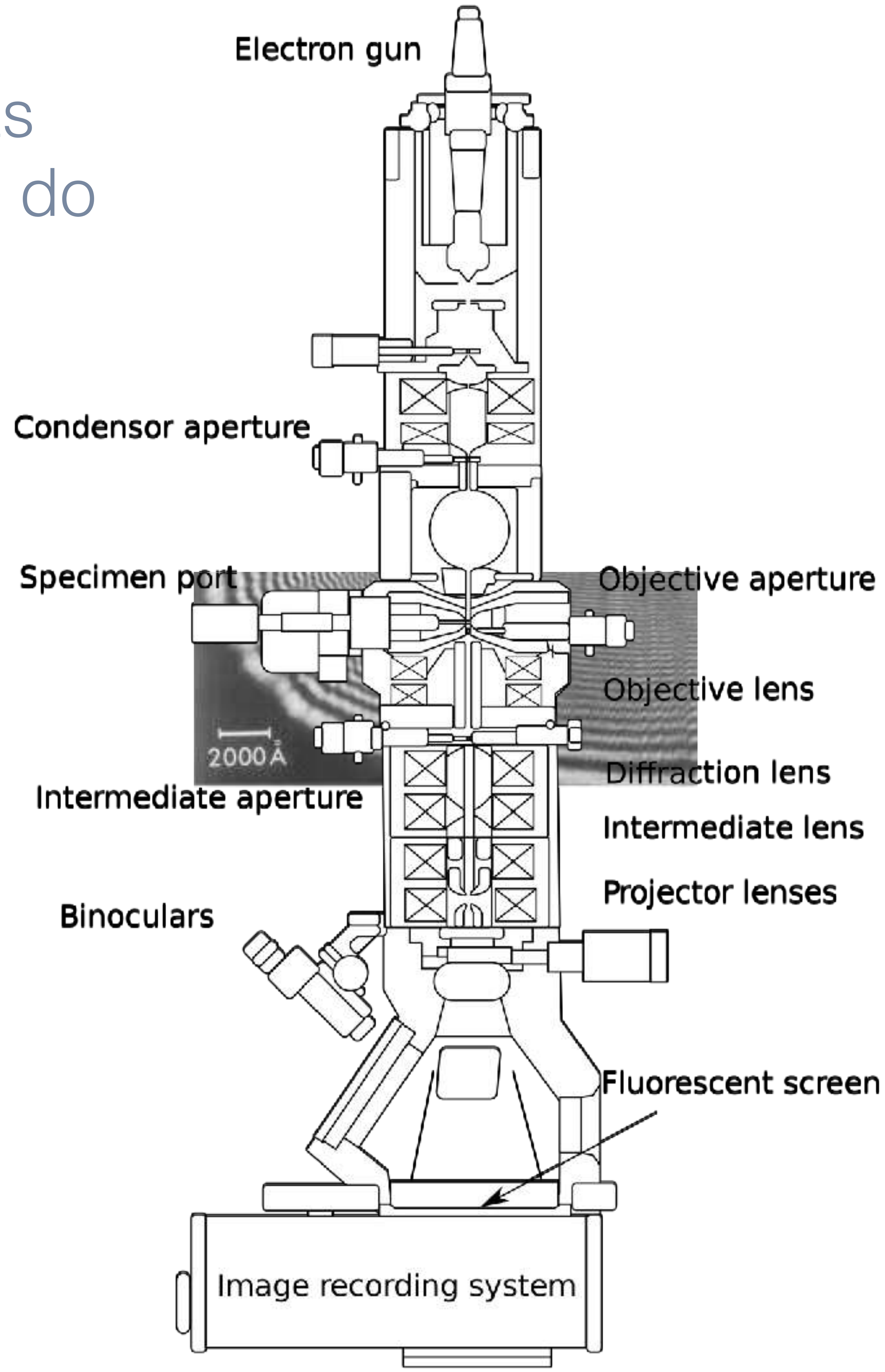
- Focus
- Magnify
- Rotate



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)~~

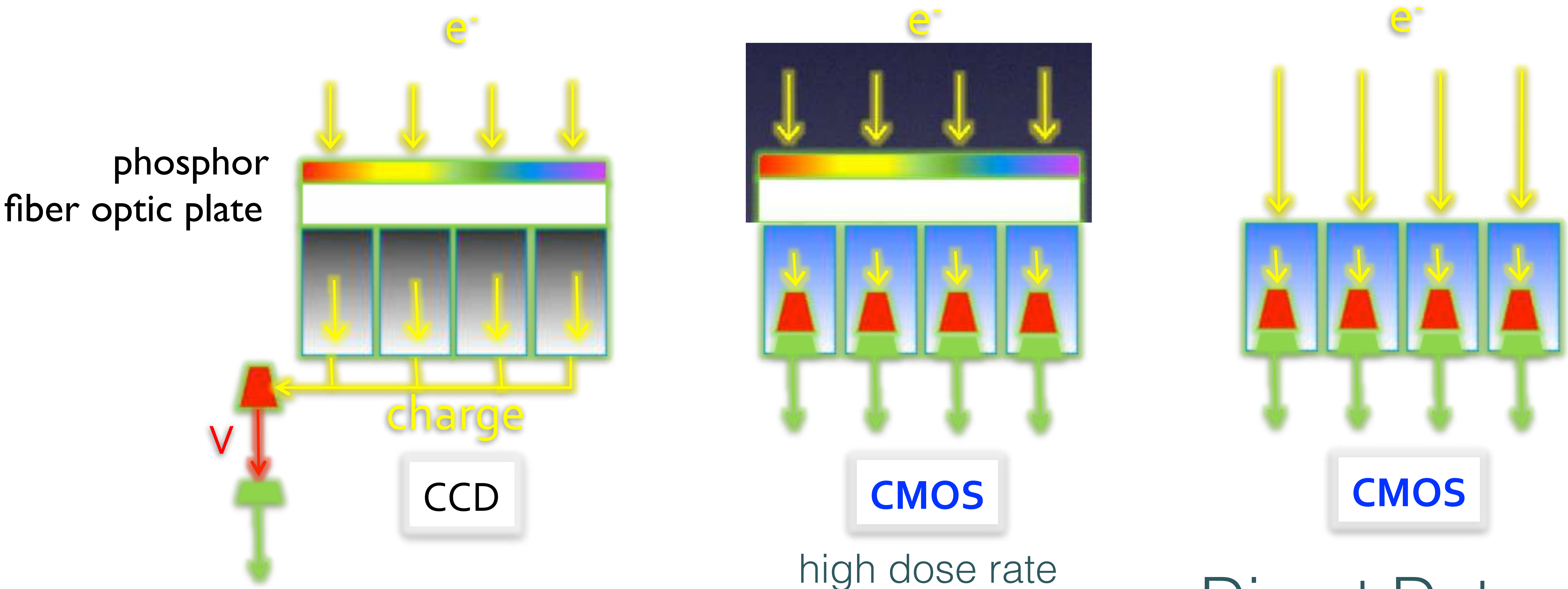


DETECTORS Digital Cameras for TEM

- Photon converted
- Direct sensing

?

- CCD Charge Coupled Device
- CMOS Complementary Metal Oxide Semiconductor

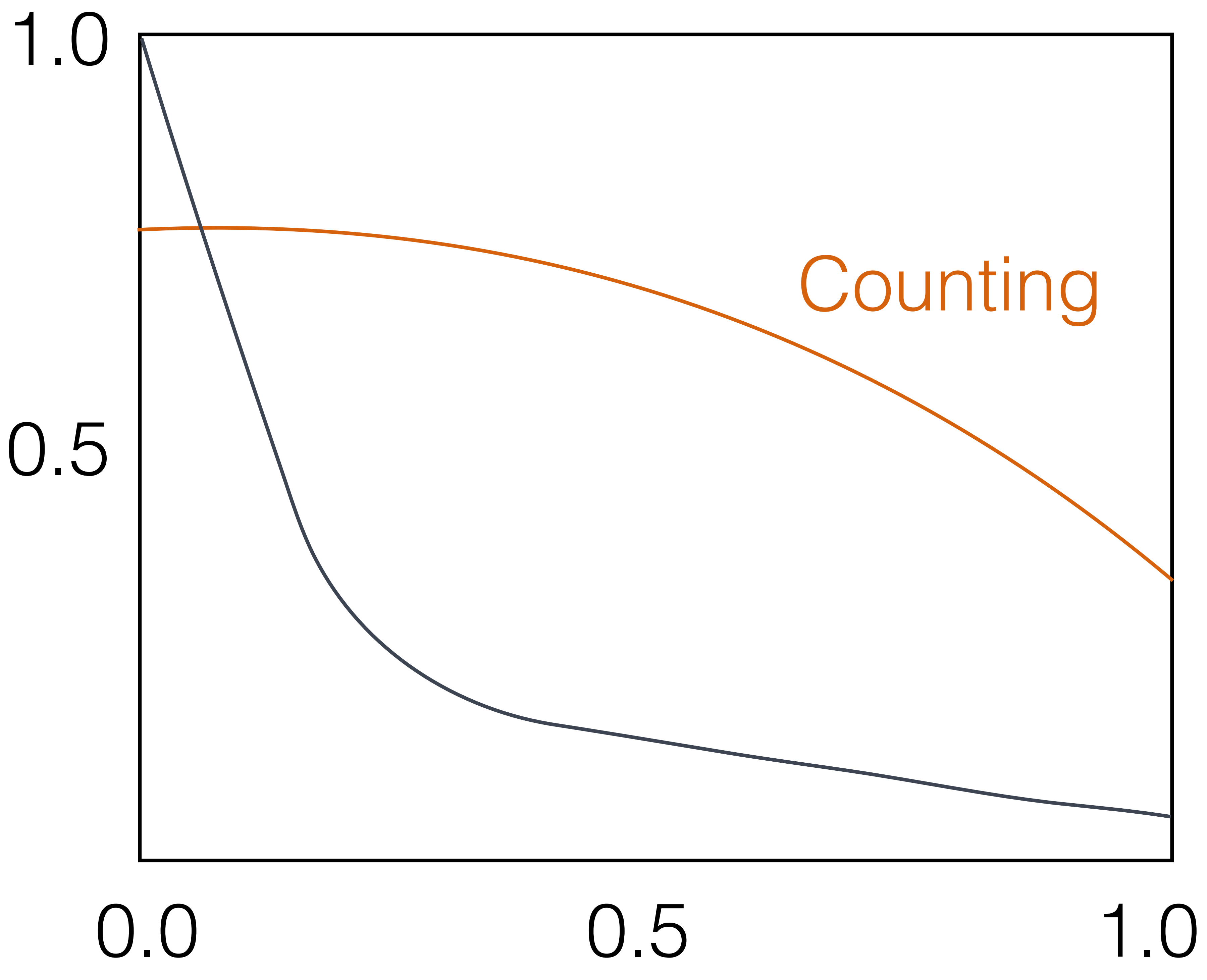


high dose rate

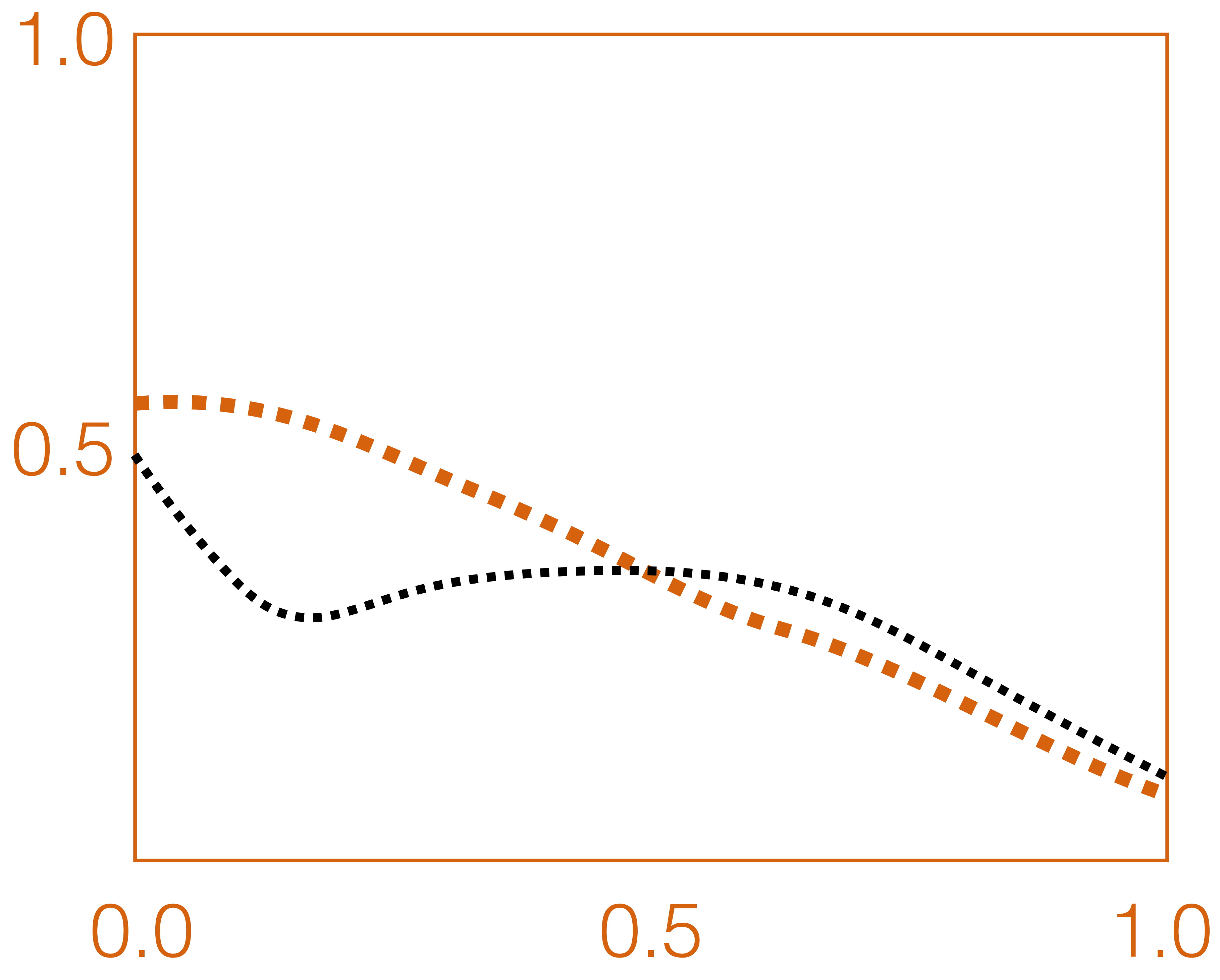
Direct Detectors

DETECTORS Detector Performance Characterization

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



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



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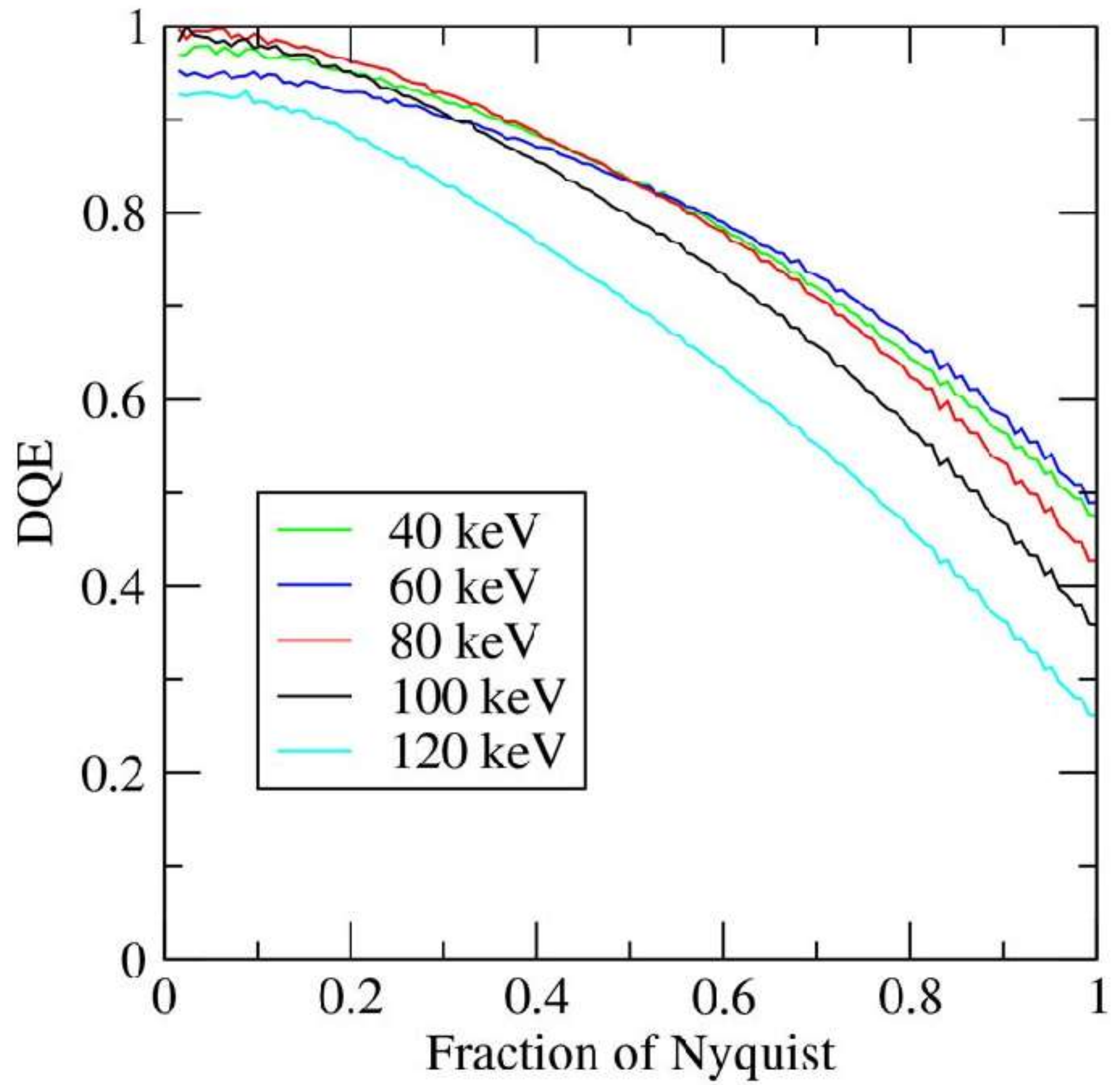

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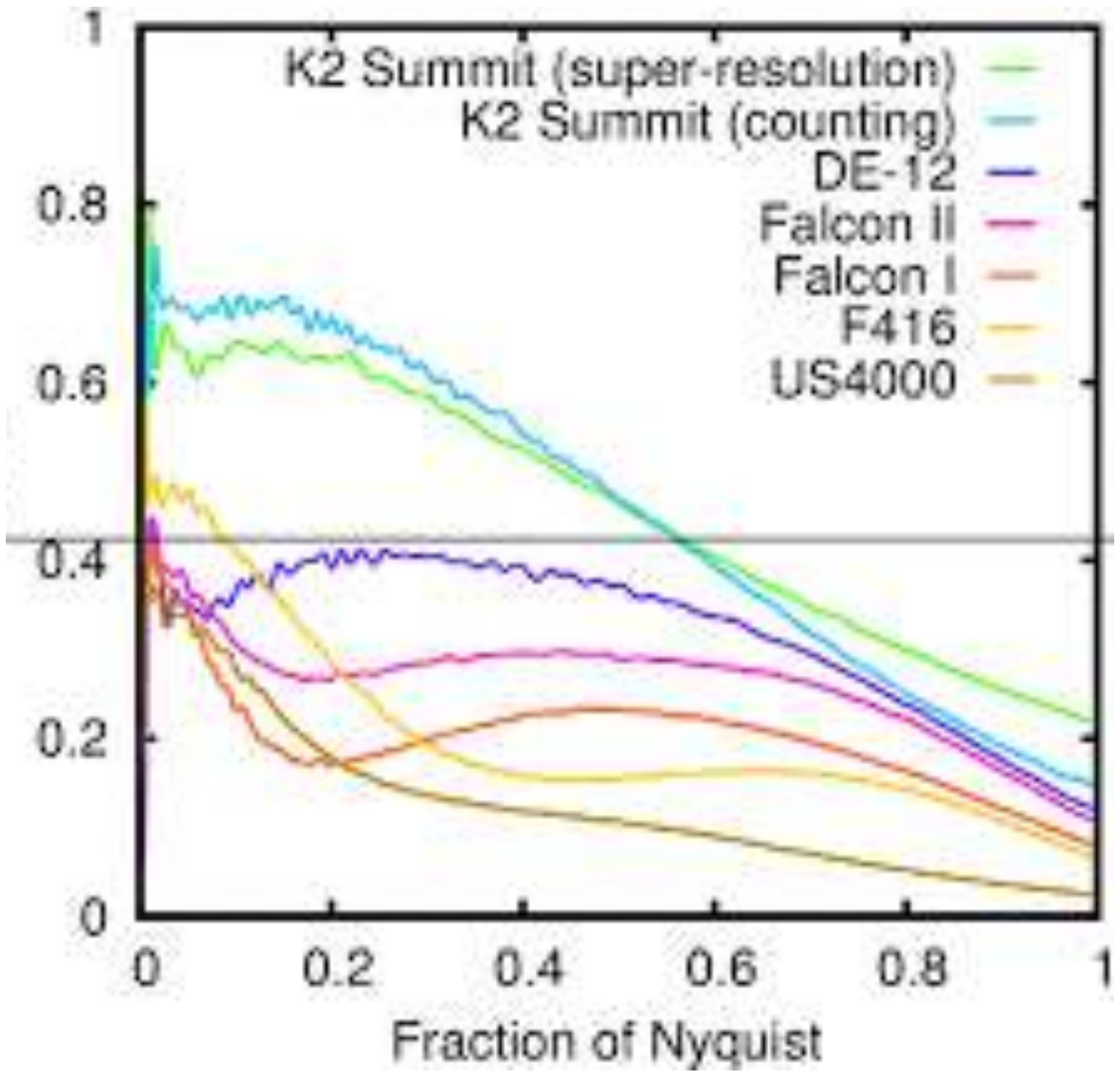

Ruskin, et al JSB

HOW ARE SAMPLES PREPARED?

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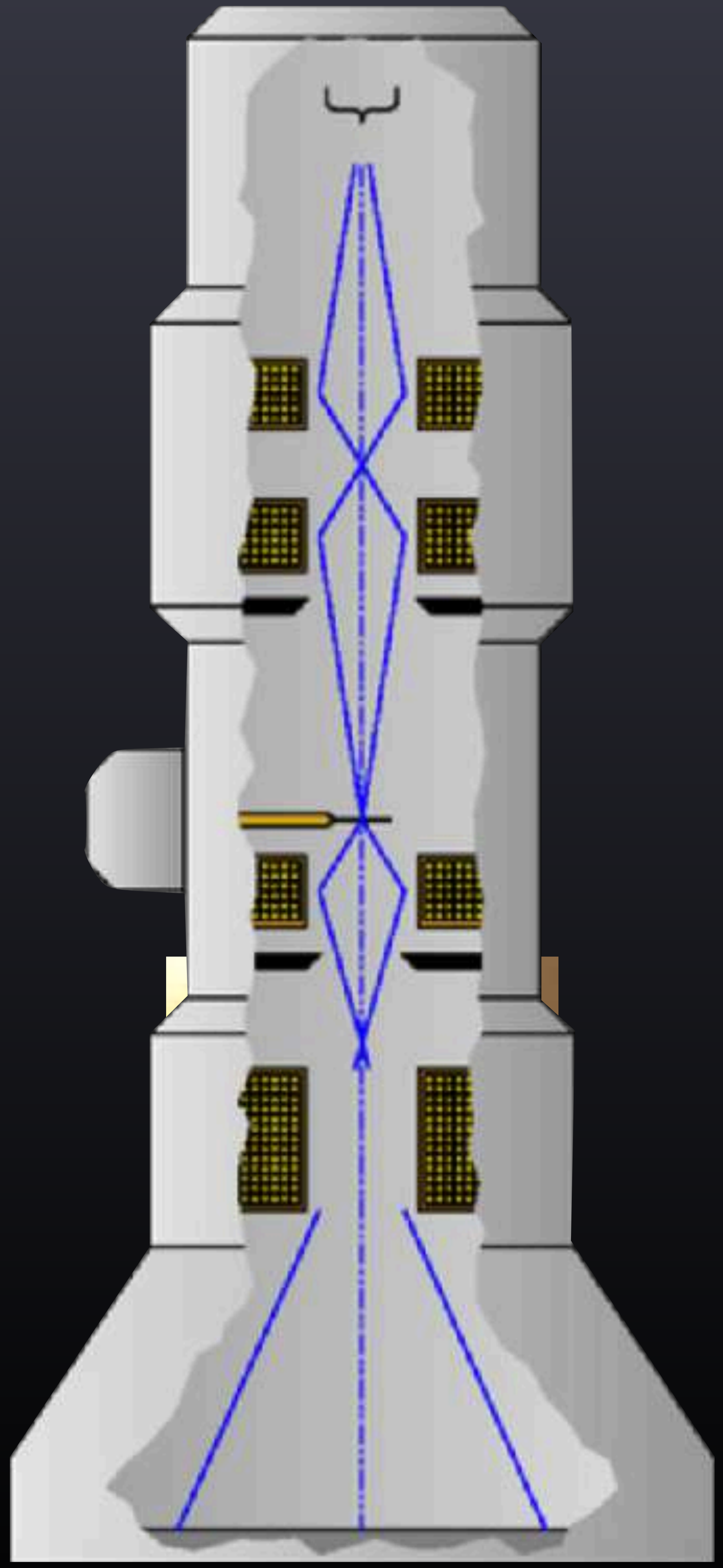
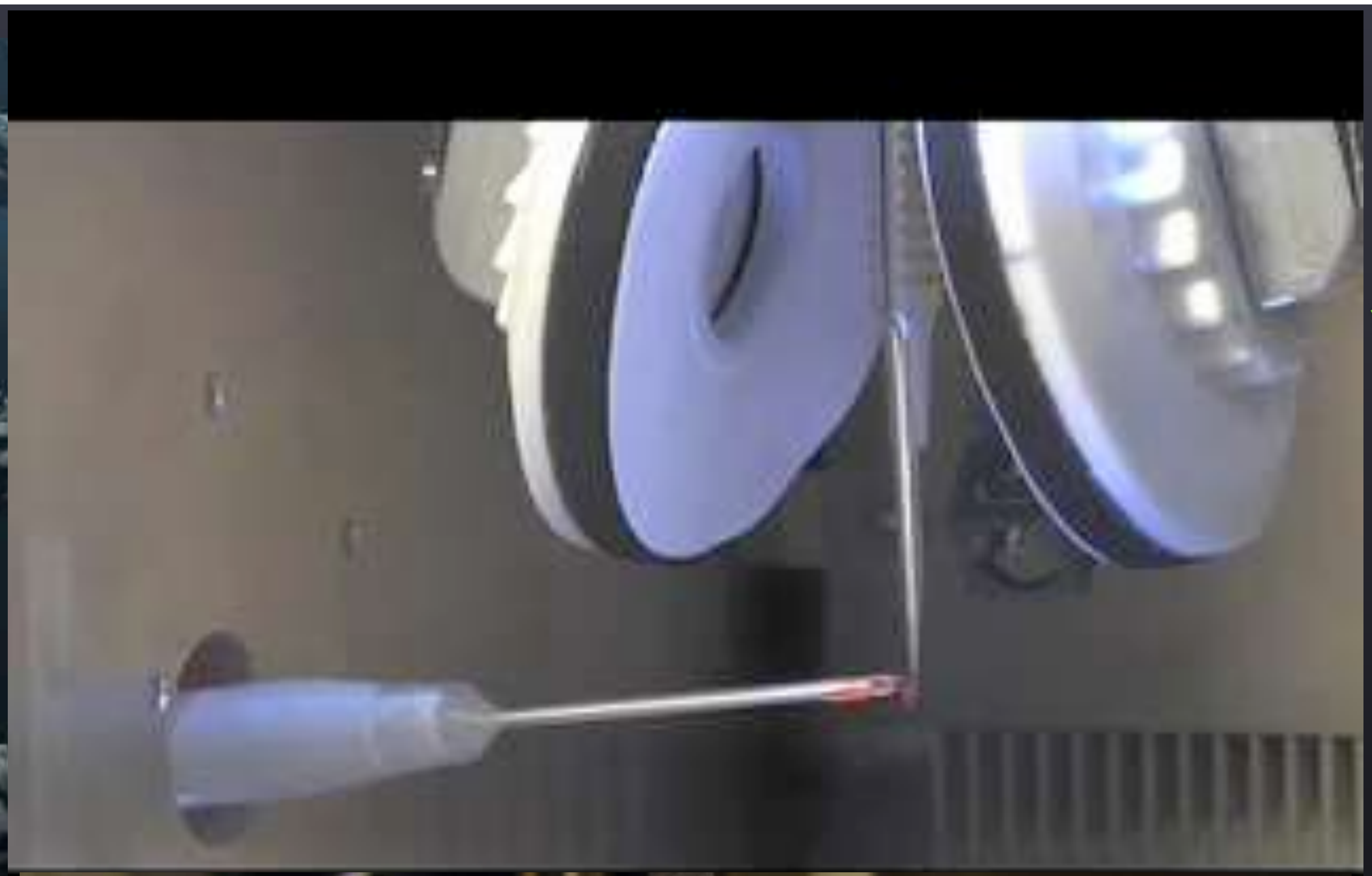
HOW ARE SAMPLES PREPARED?

Vitrifying a biological sample



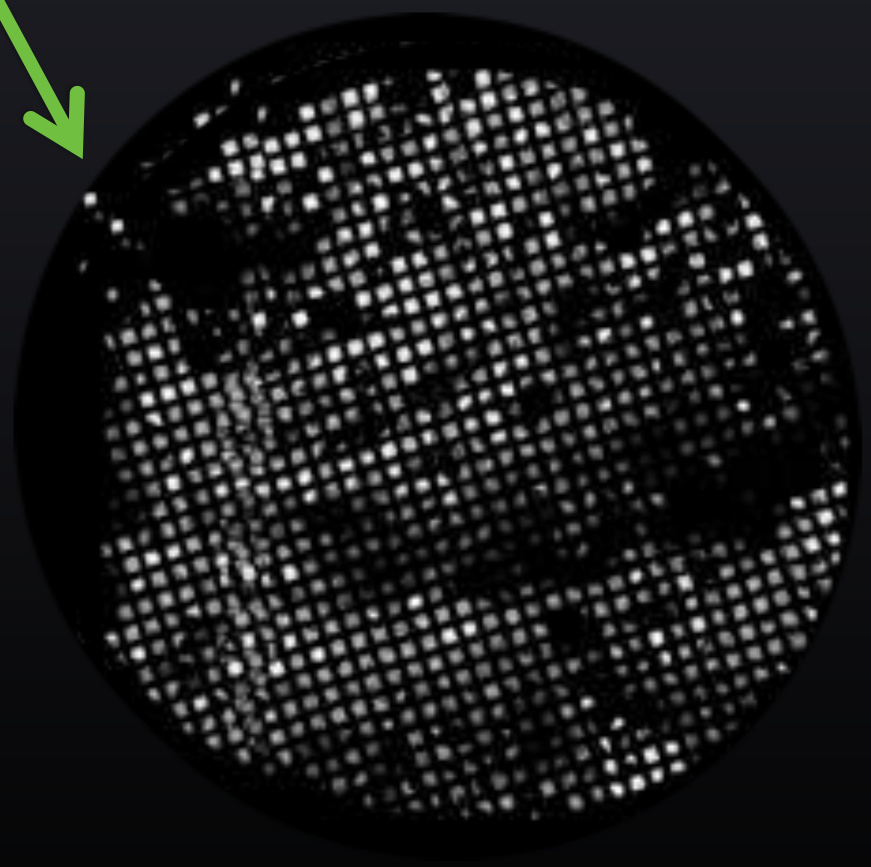


~3µl

<3nl

>99.999%

<0.001%



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

HOW ARE SAMPLES PREPARED?

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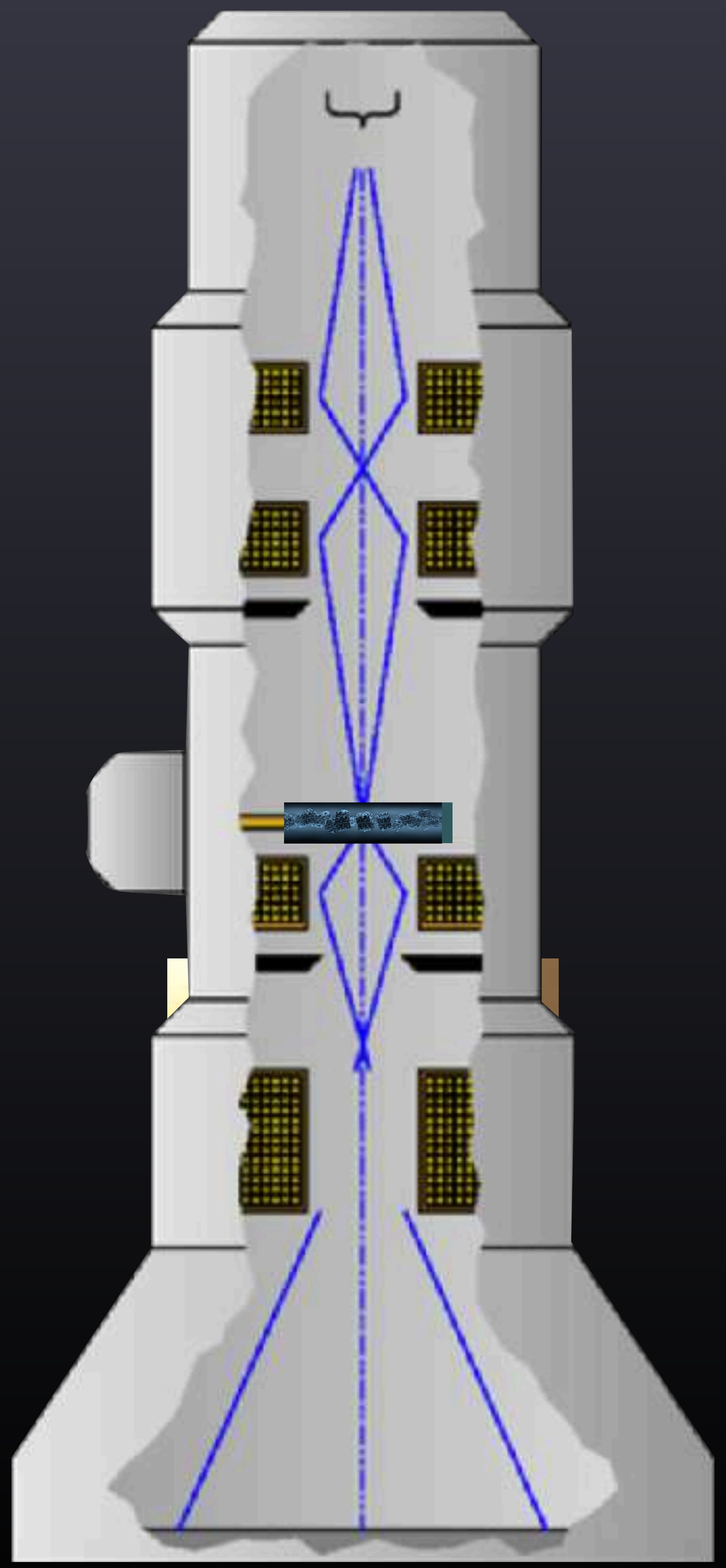
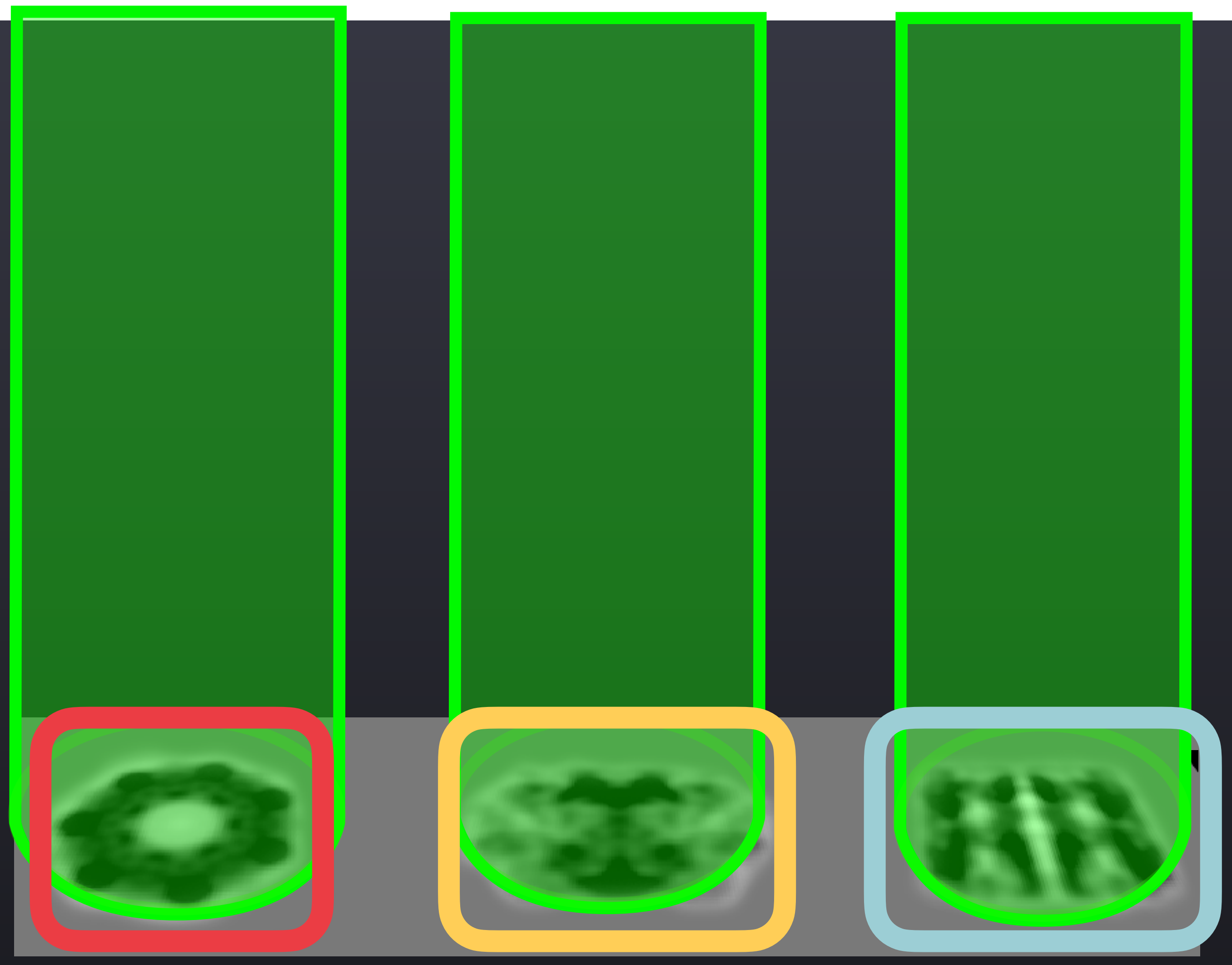
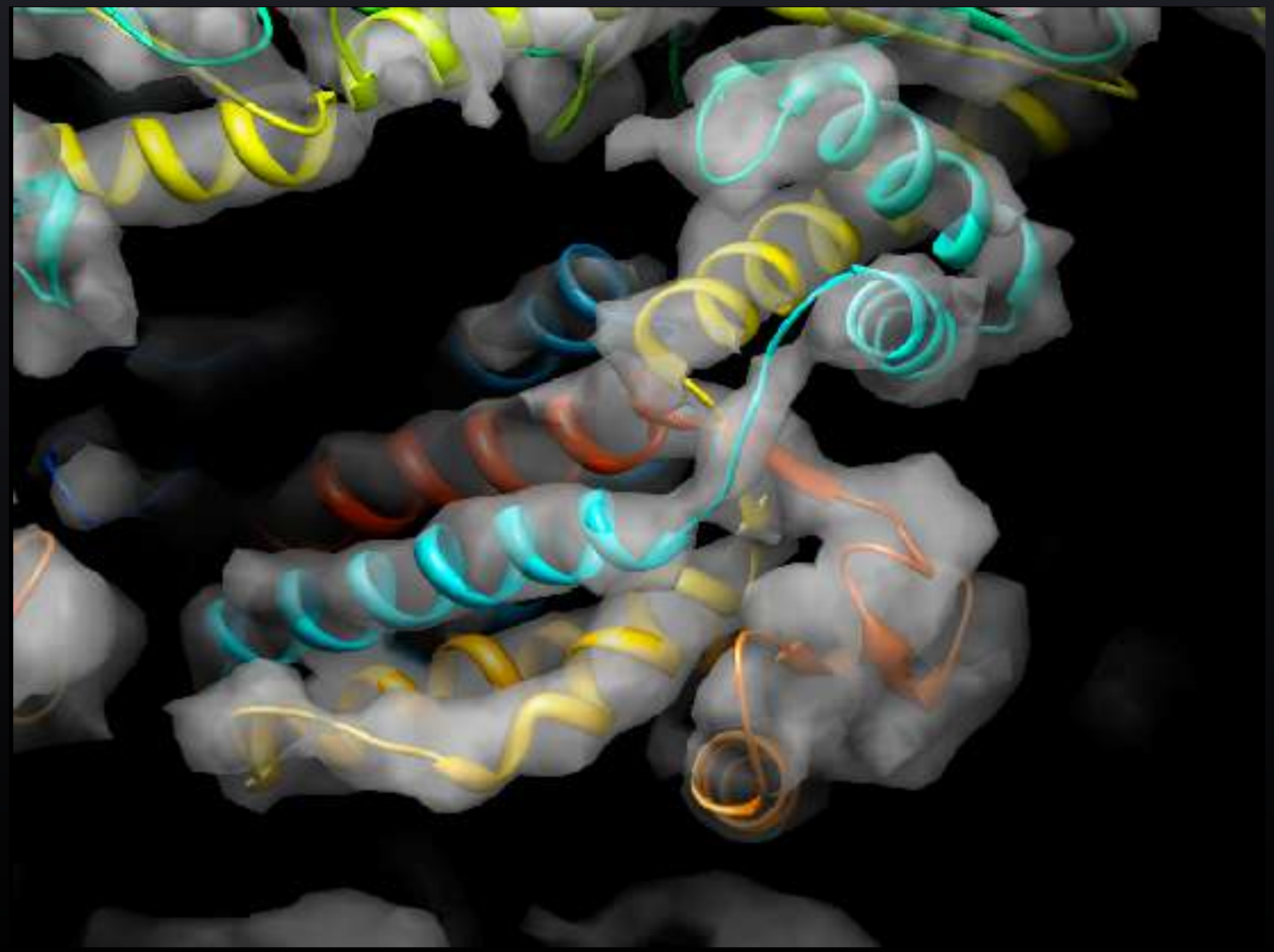




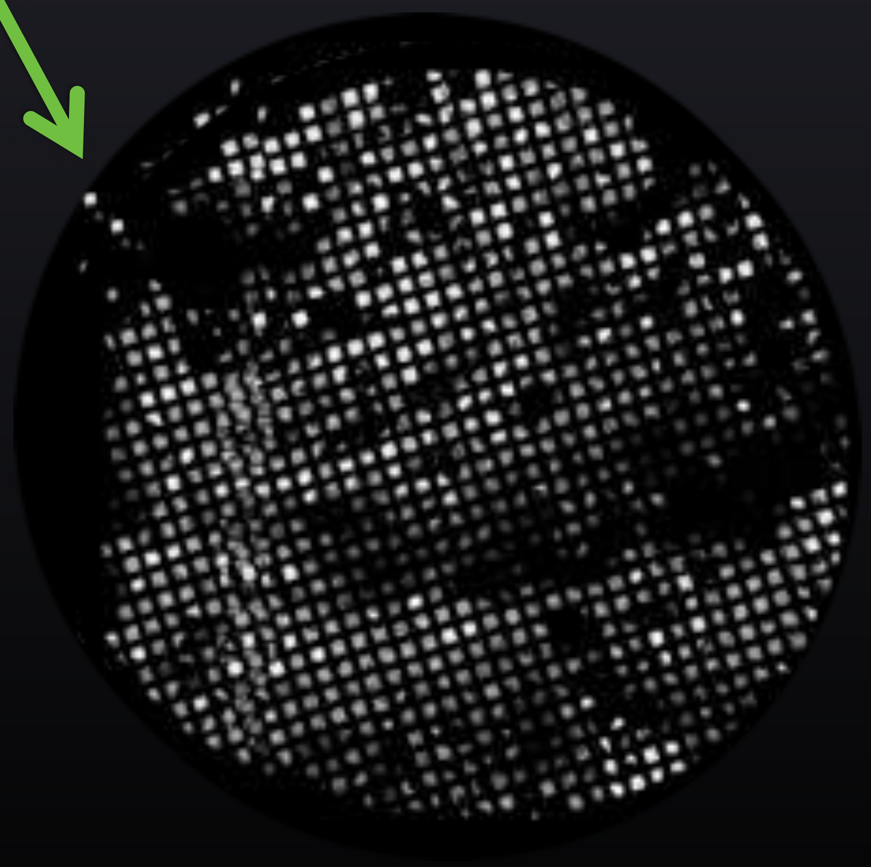


>99.999%

<0.001%

~3µl

<3nl



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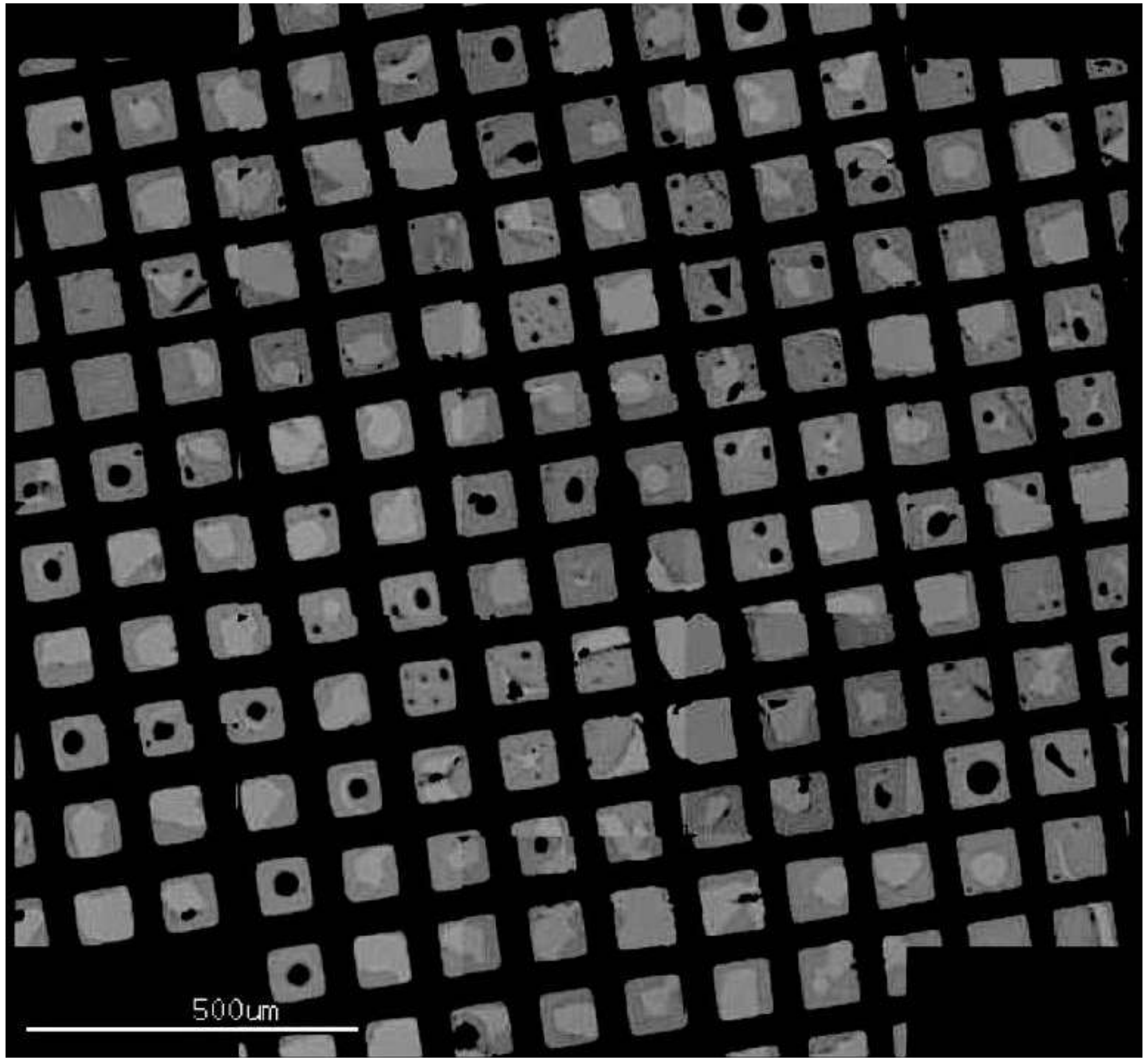
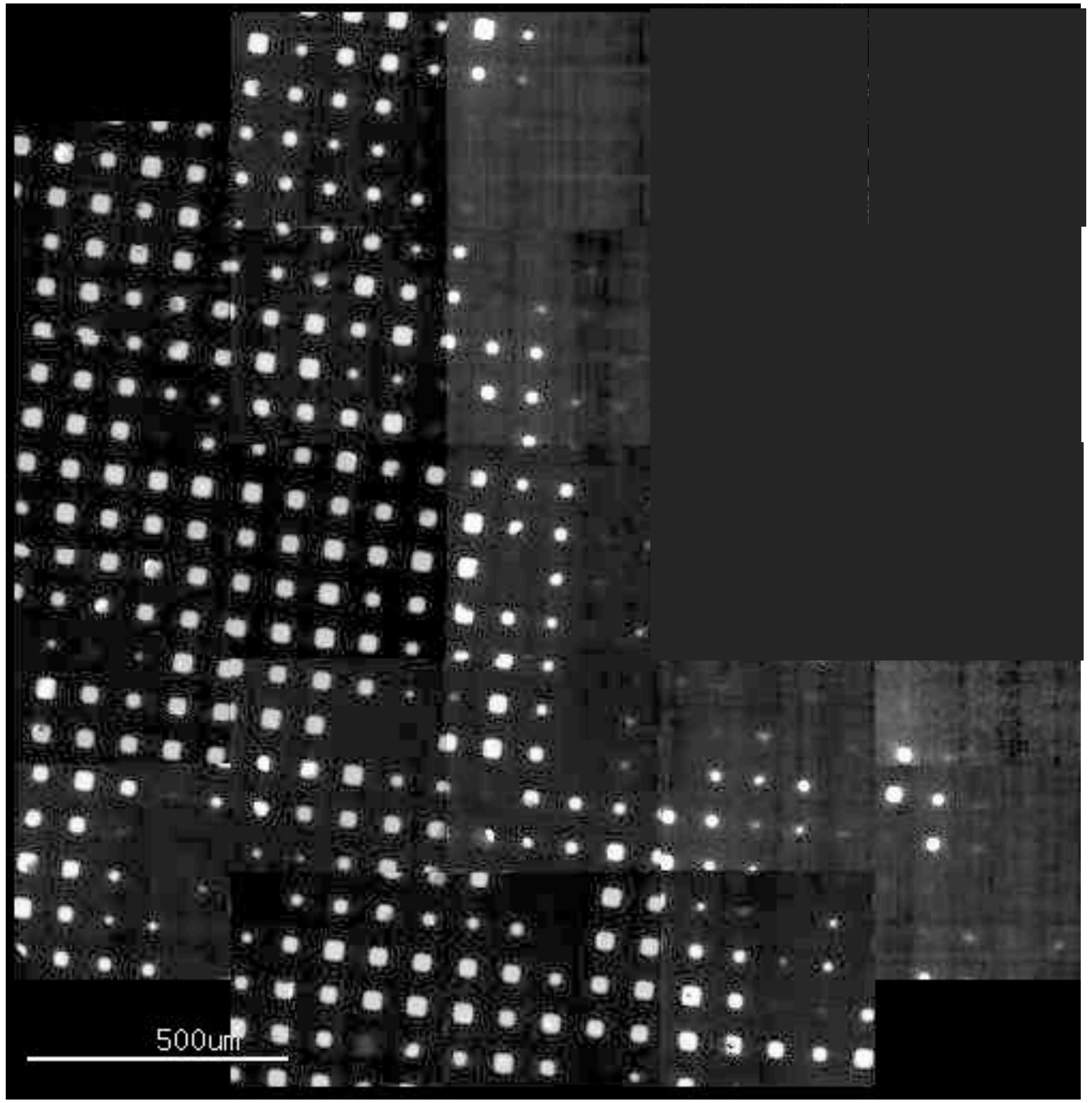
WHAT DO GRIDS LOOK LIKE?





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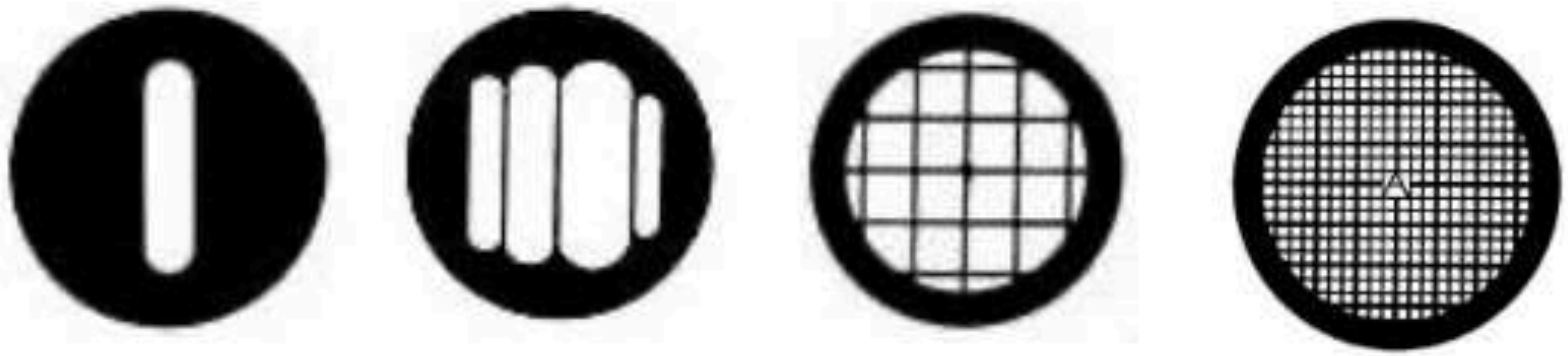
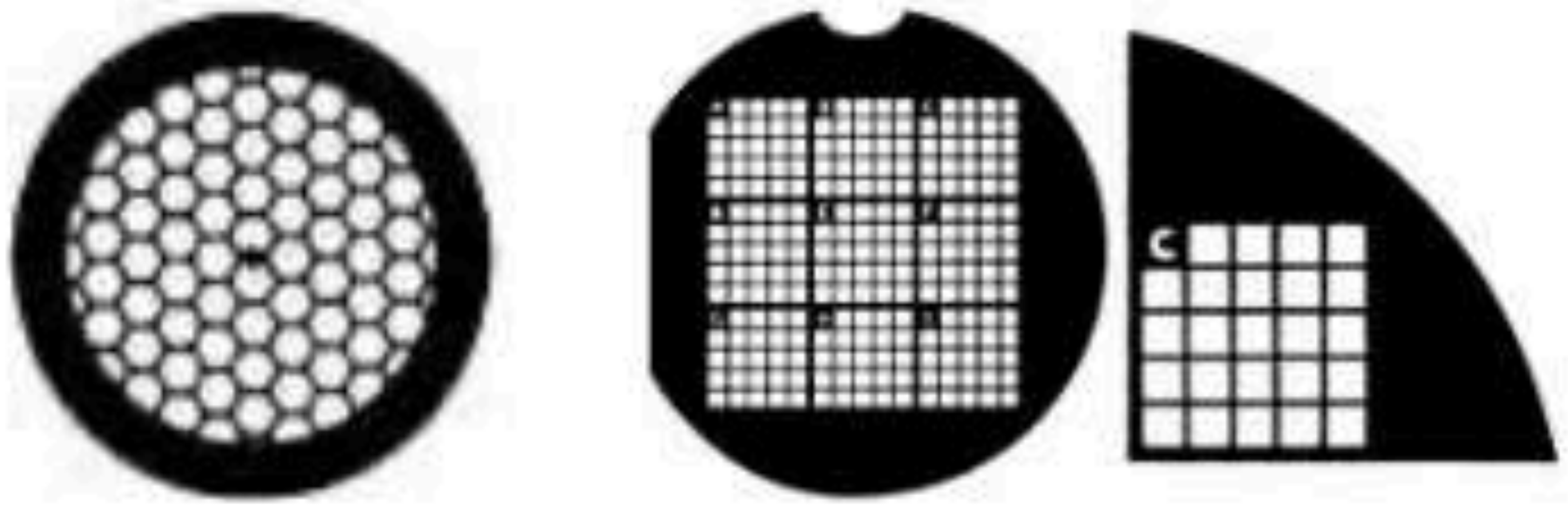
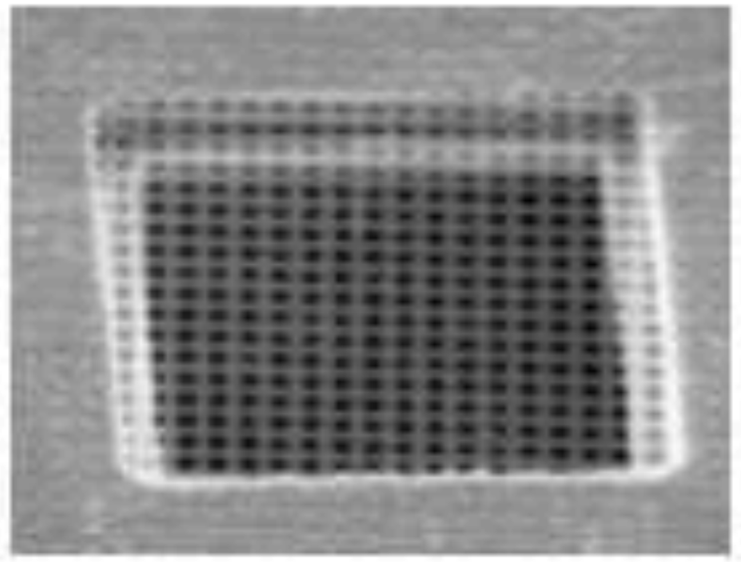
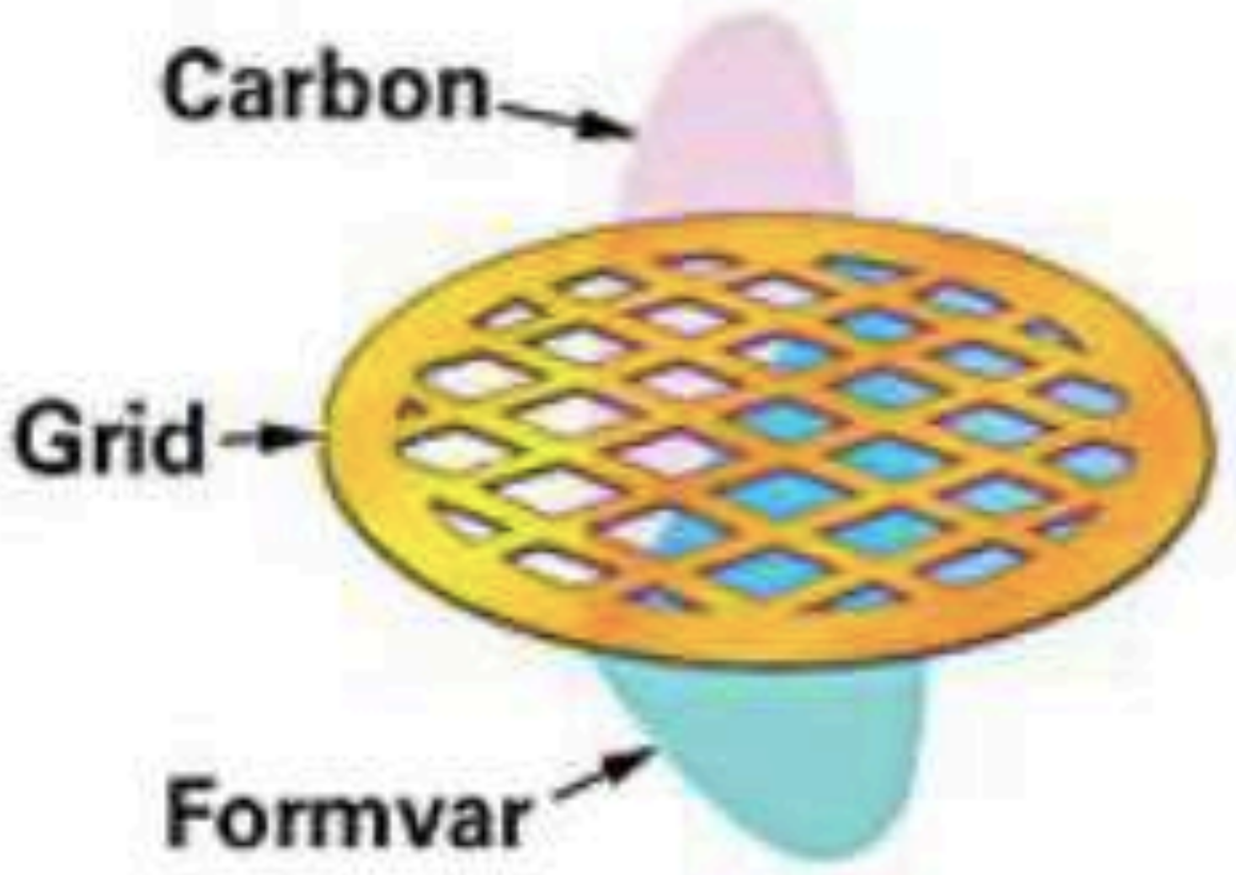








Common Materials

Copper

Nickel

Gold

Aluminum

Molybdenum

Titanium

Stainless Steel

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

WHAT DO GRIDS LOOK LIKE?

Rough grid parameters

Rim Width: 350-400µm.

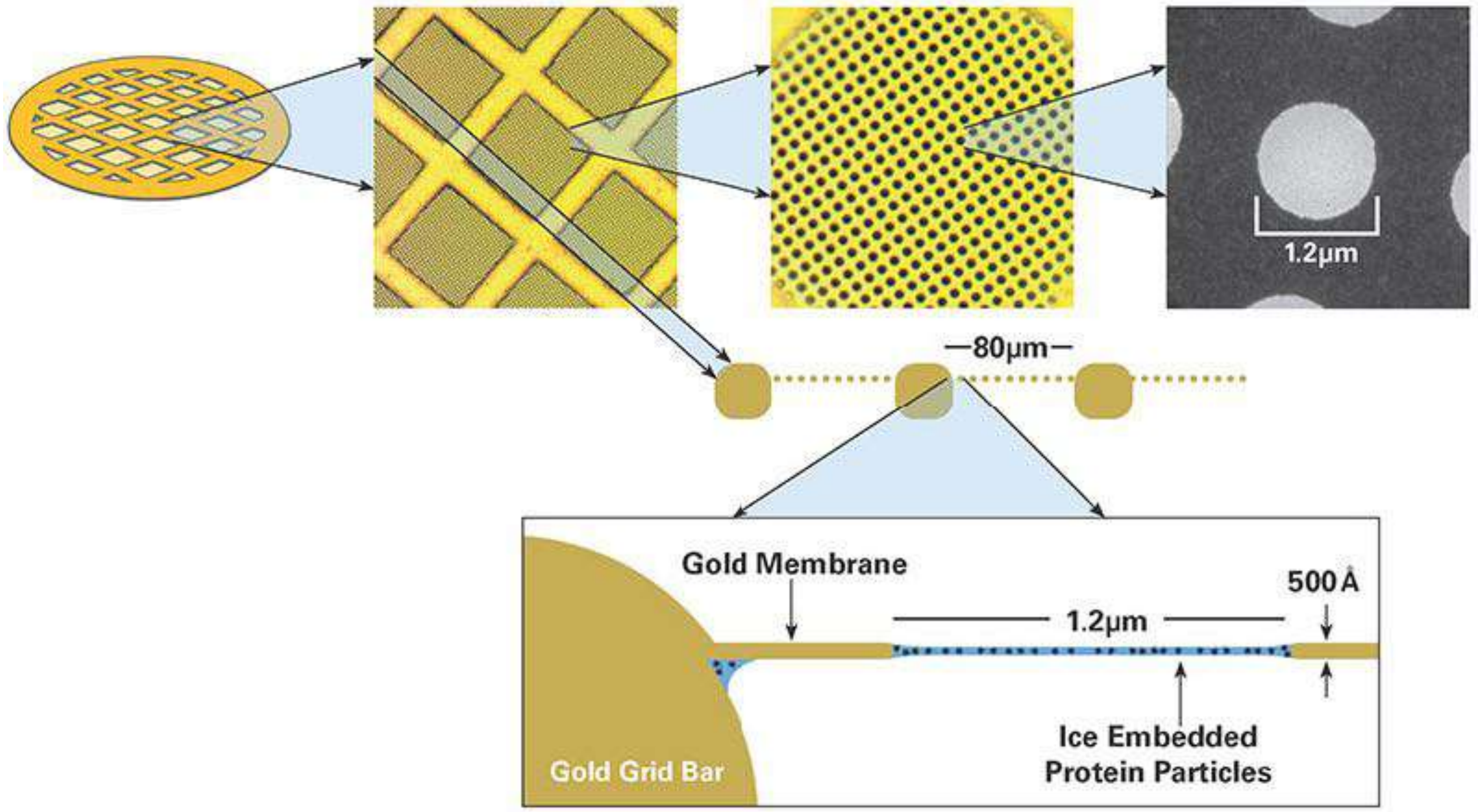
Thickness: PELCO® Grids are approximately 25µm thick.

Diameter: 3.0 to 3.05mm

Finish: Copper, Nickel and Gold grids have a matte finish on one side and a shiny finish on the other side.

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

Example 200 mesh pitch = 25.4/200 = 127µm



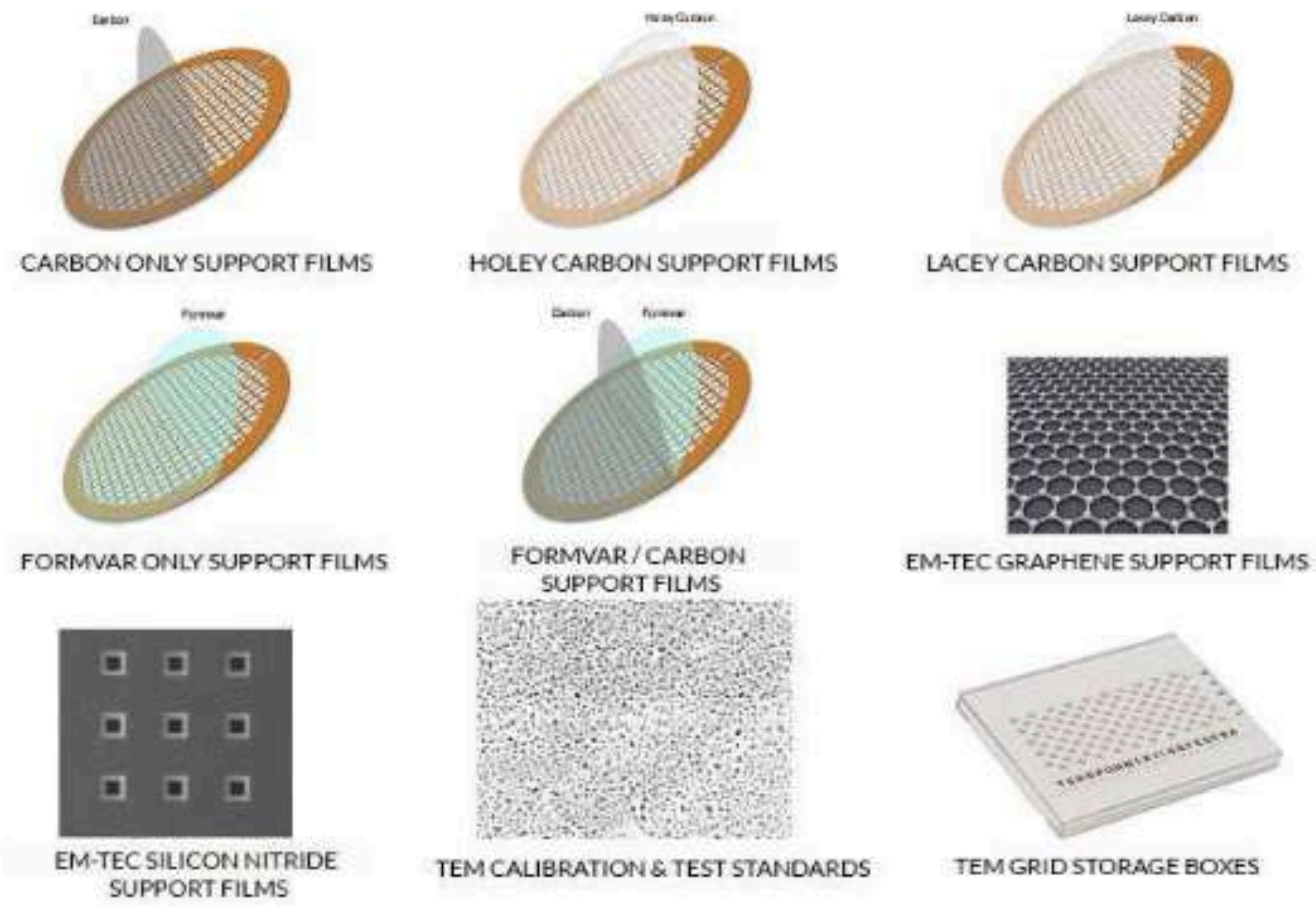
PELCO® Grid Size

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

TERMINOLOGY

Grid (Cu, Au, Mo, etc…)
- mesh

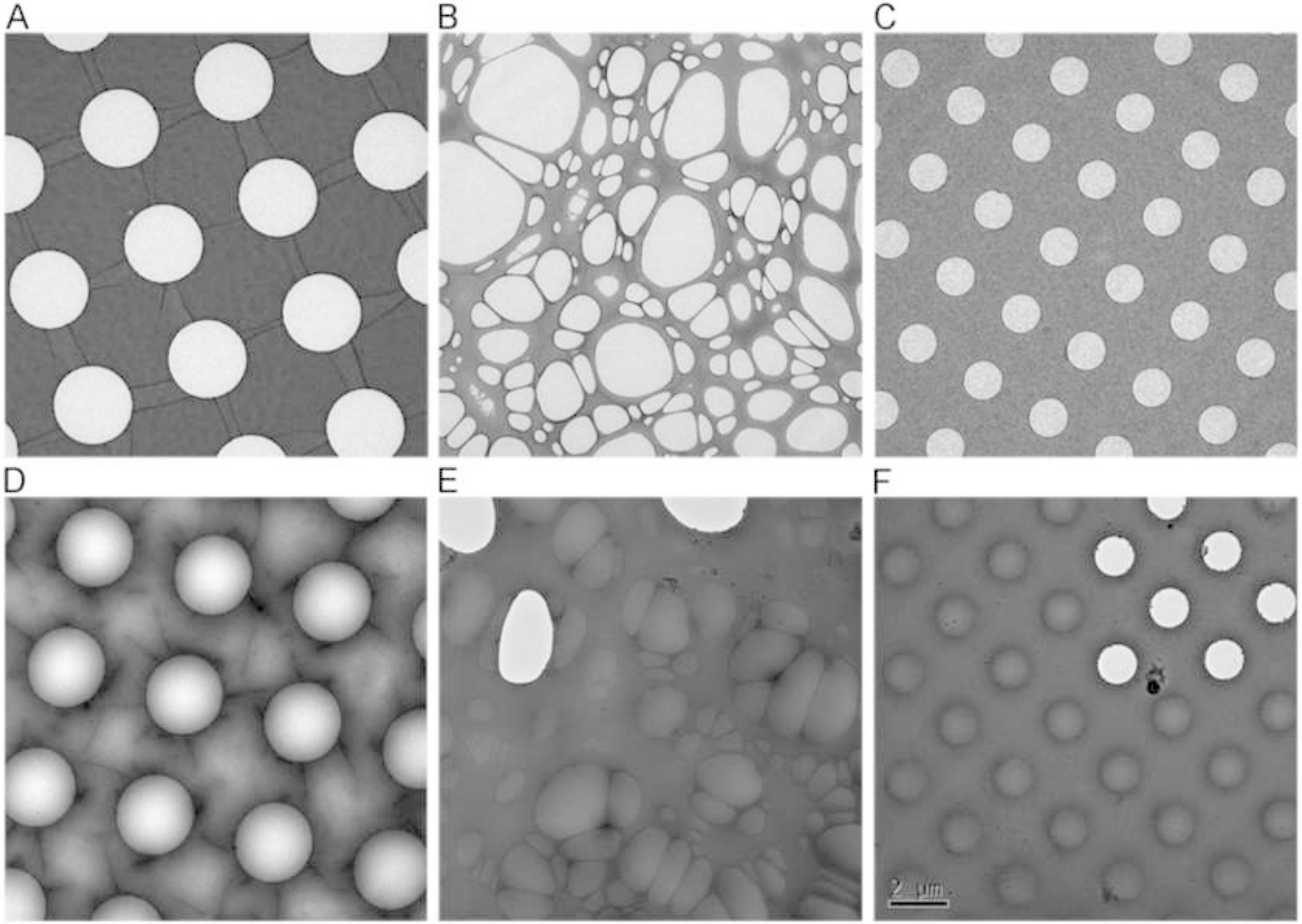
Foil (C, Au, etc…)
- Continuous
- lacy
- holey (hole size and spacing)



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

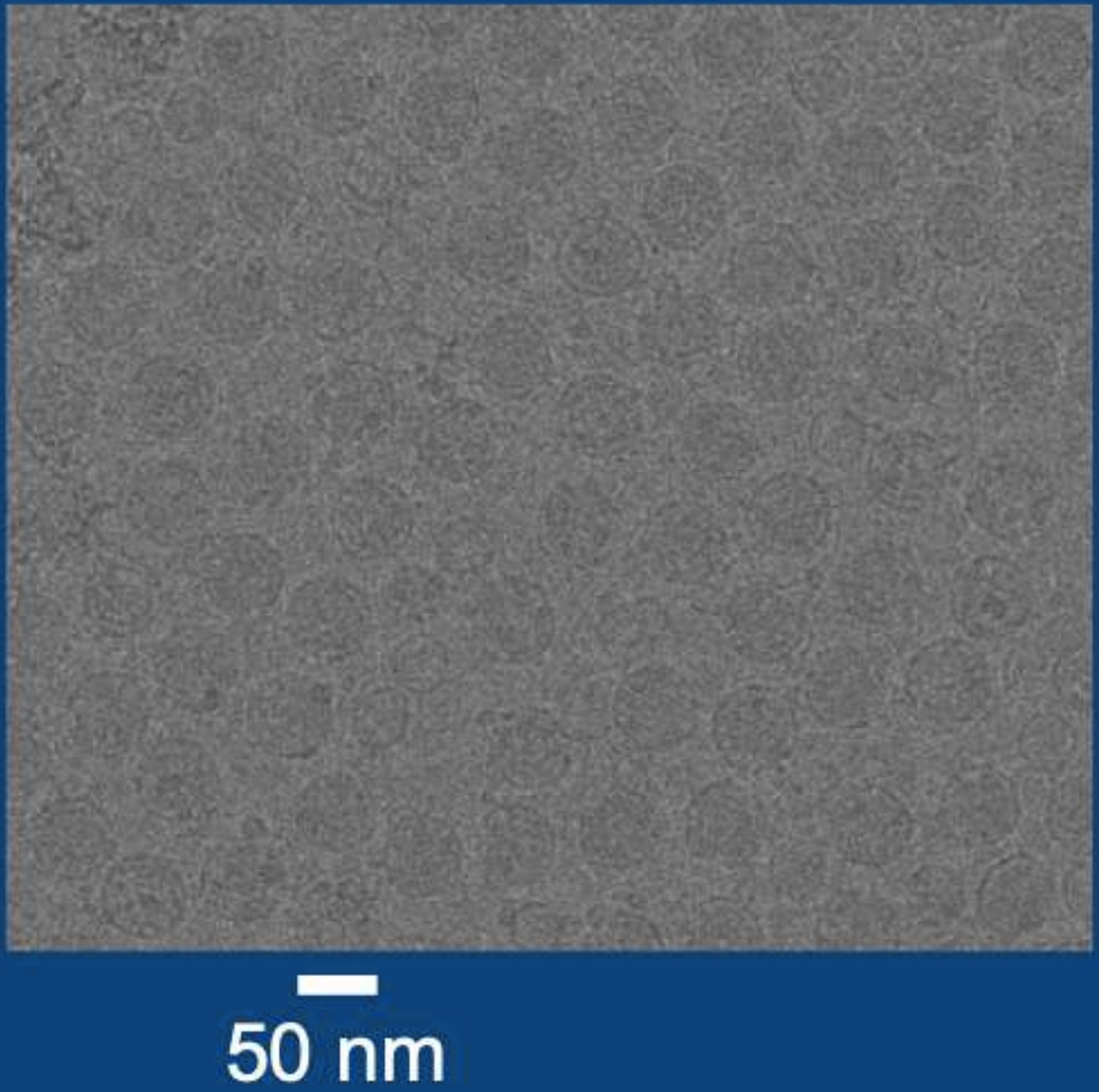
VARIATIONS ON THE FOIL OF GRIDS

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.

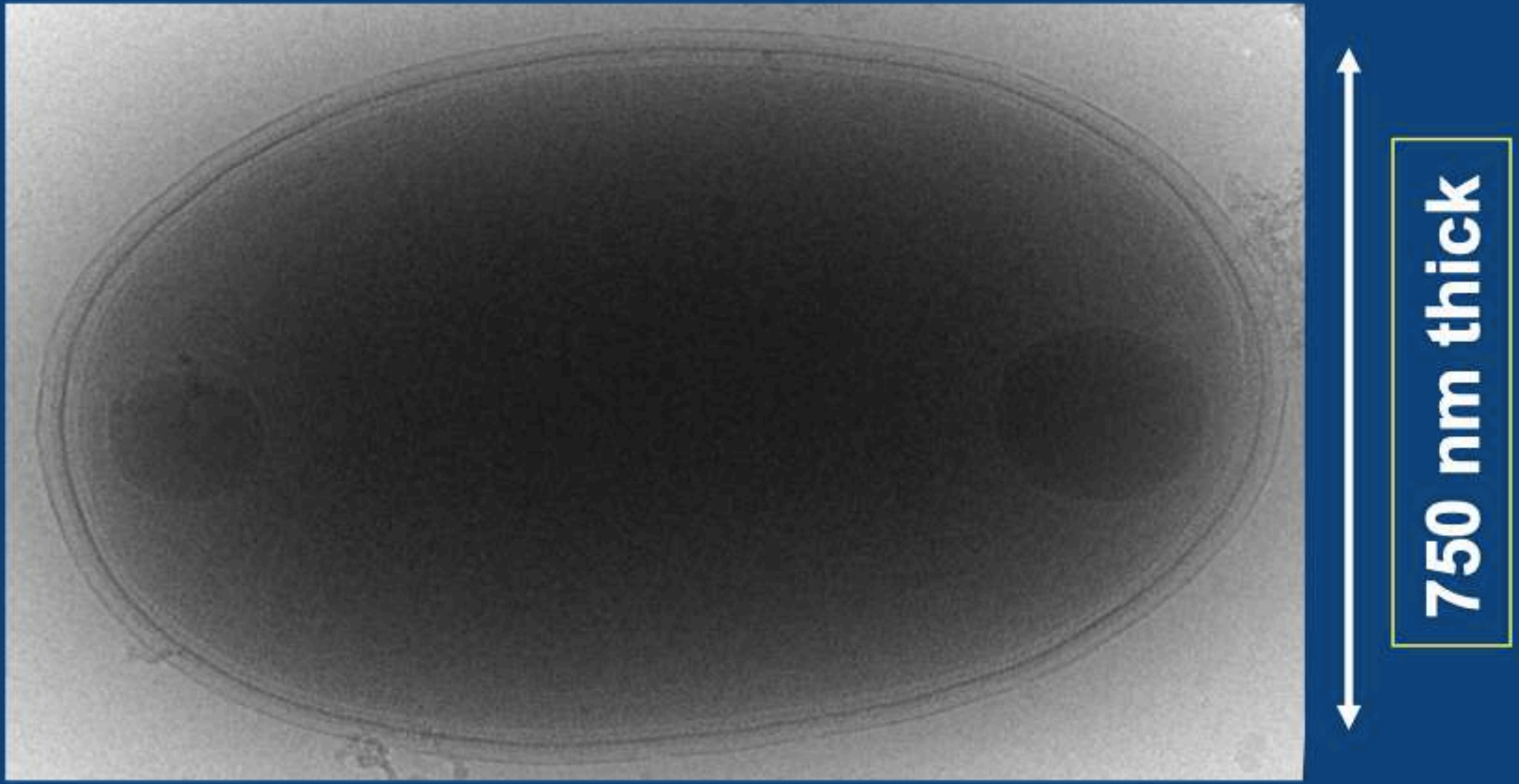


Quantifoil grid   Lacey carbon grid   C-flat grid

HOW THIN DOES THE SAMPLE NEED TO BE?



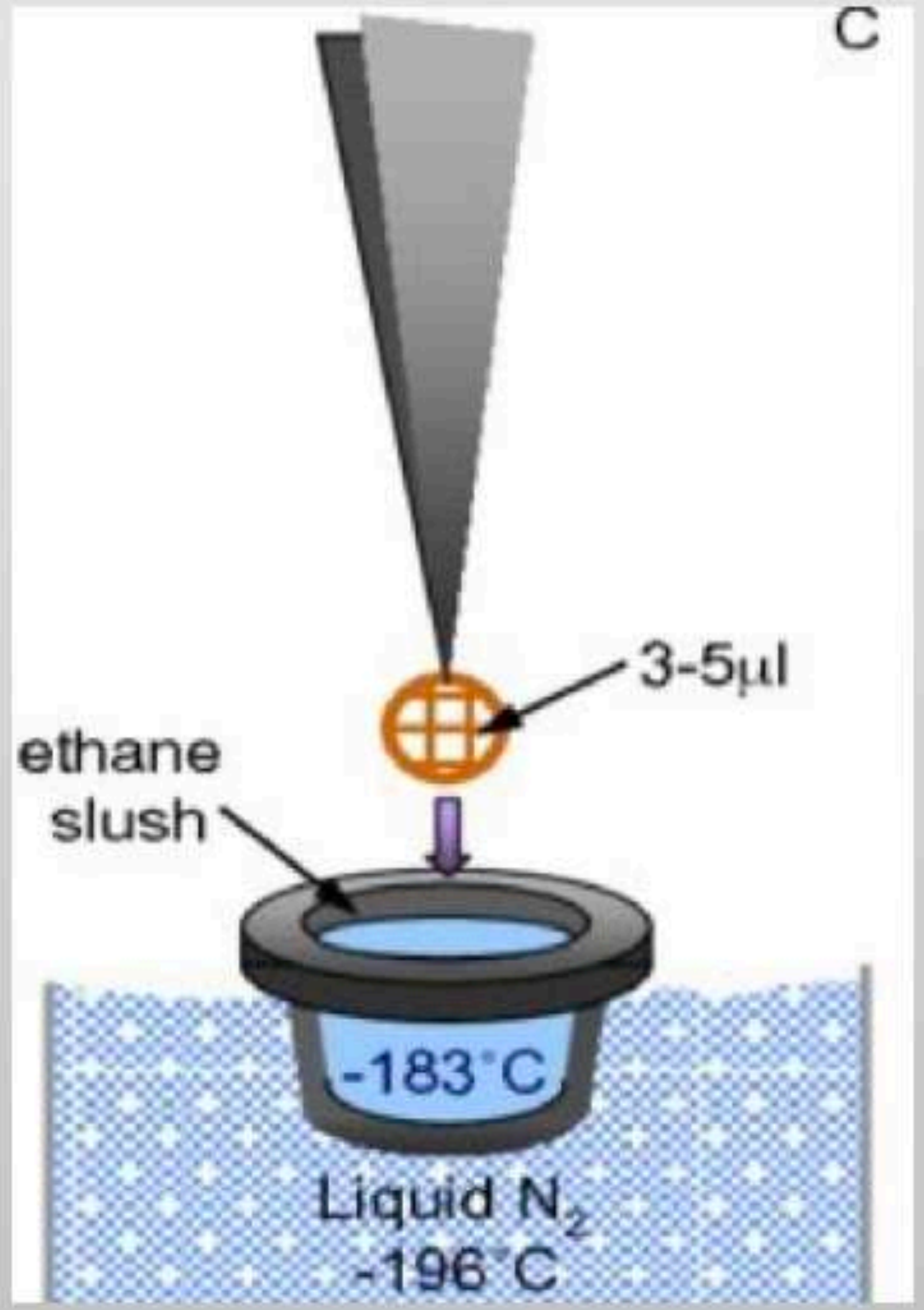
Bacteriophage (ϕ12)



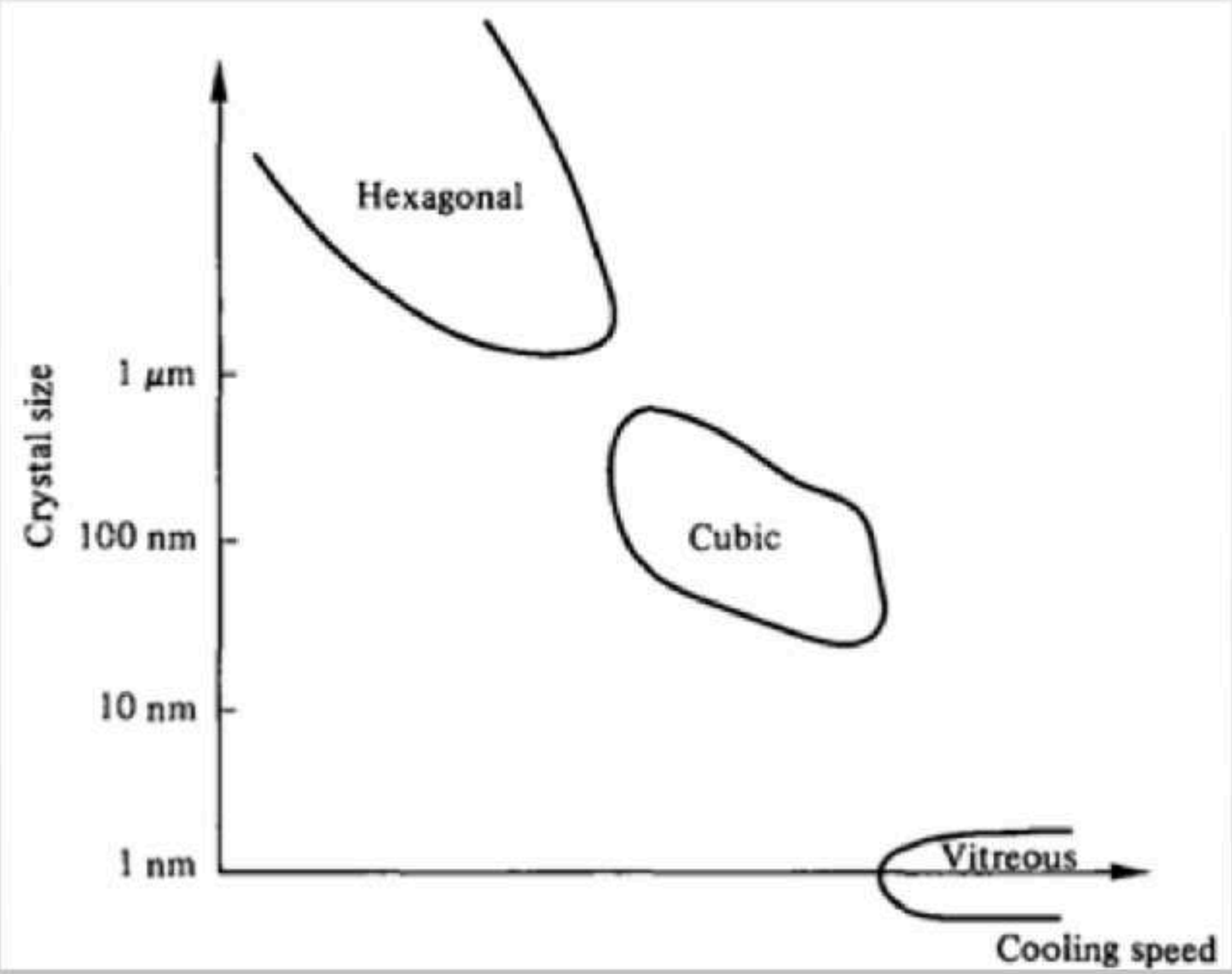
E. coli, Salmonella, Cyanobacteria

VITRIFICATION PROCESS

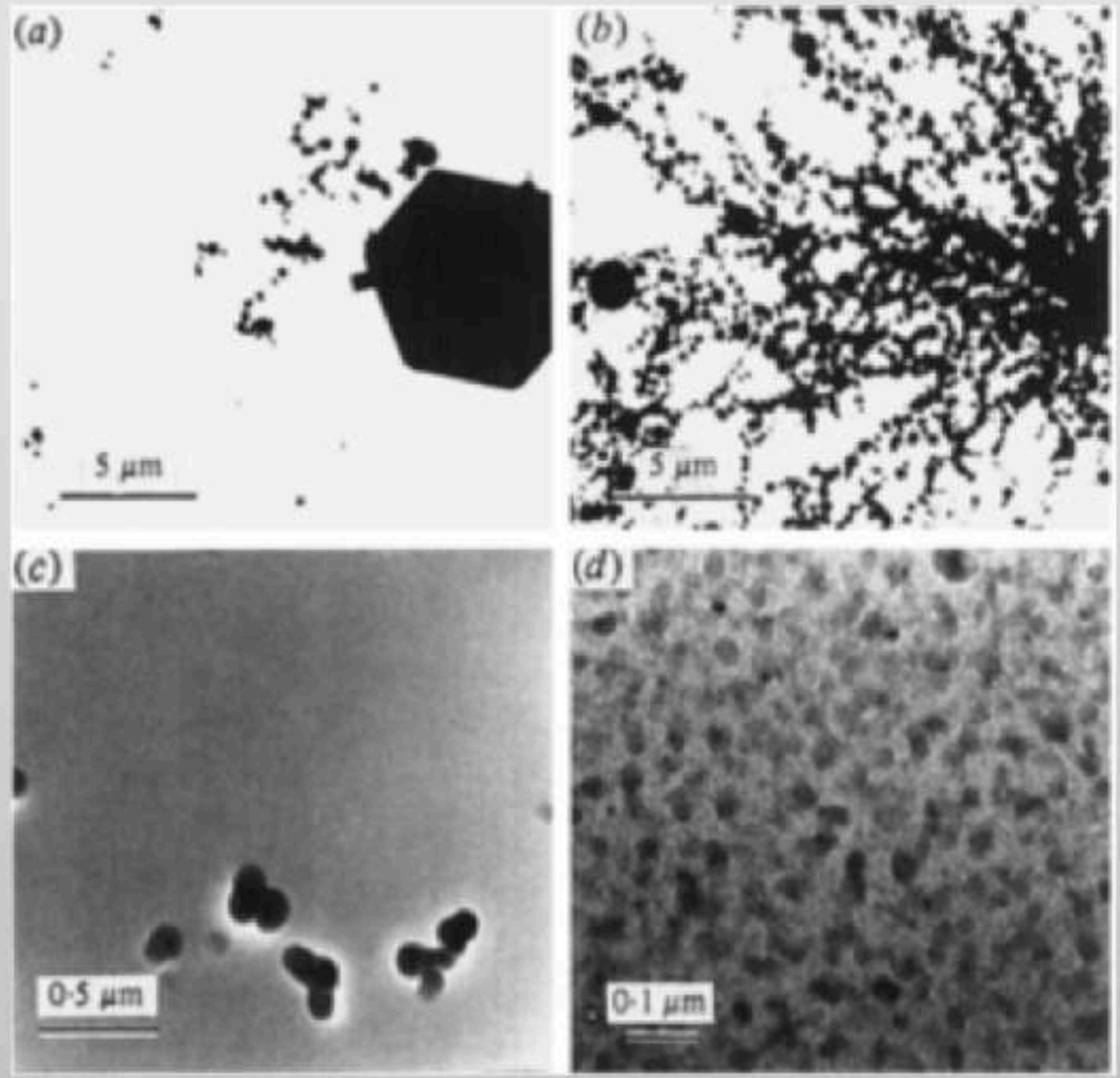
- Liquid ethane is a suitable coolant.
- Liquid nitrogen boils on contact, which makes it a poor coolant for cryo-EM.
- Cooling speed faster than 10^5-10^6 K/s ensure the formation of vitrified ice.



Setup of liquid ethane
(Image from Wen Jiang)



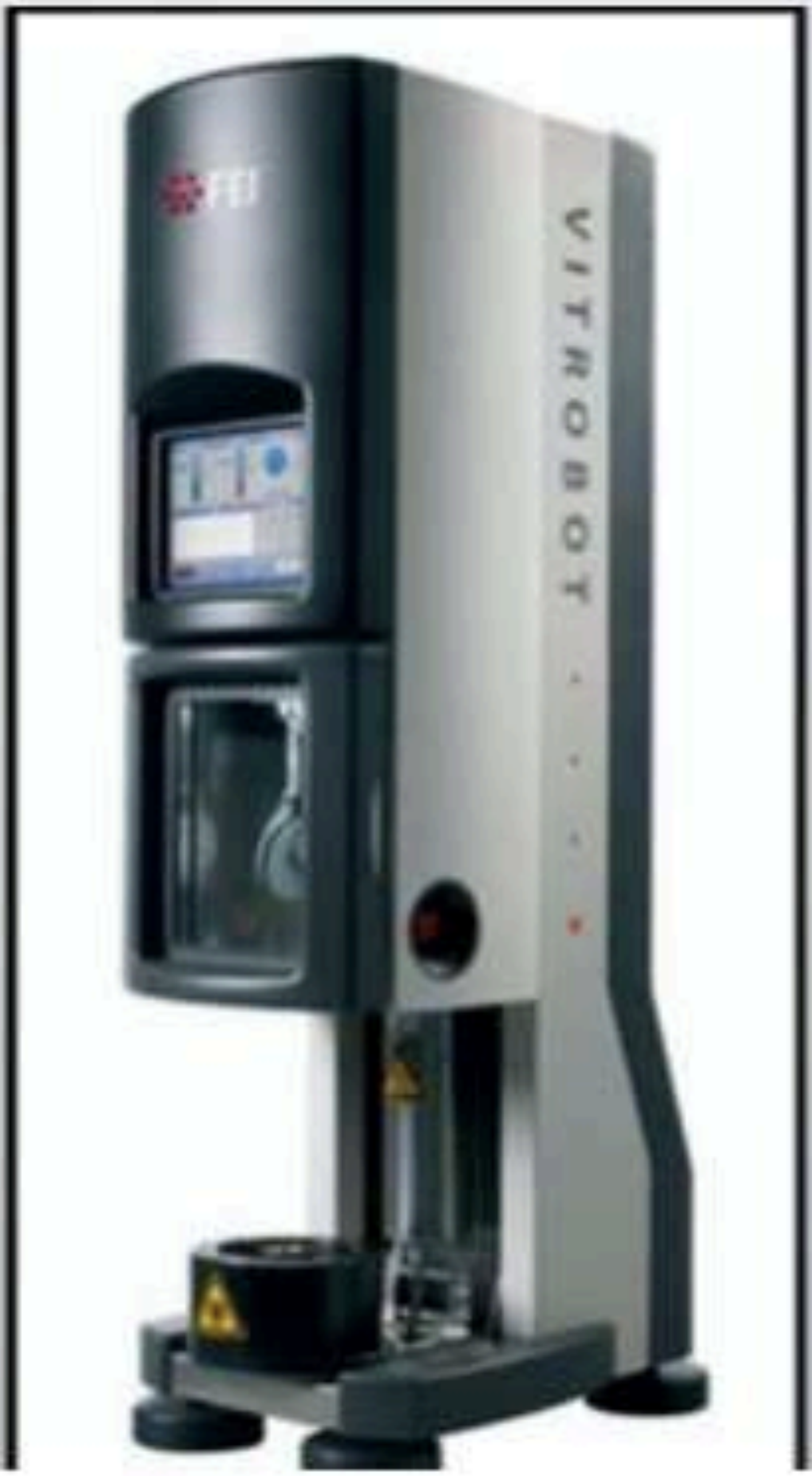
Cooling speed &
forms of ice



Different forms of ice contamination

Jacques Dubochet et al., 1988

VITRIFICATION PROCESS

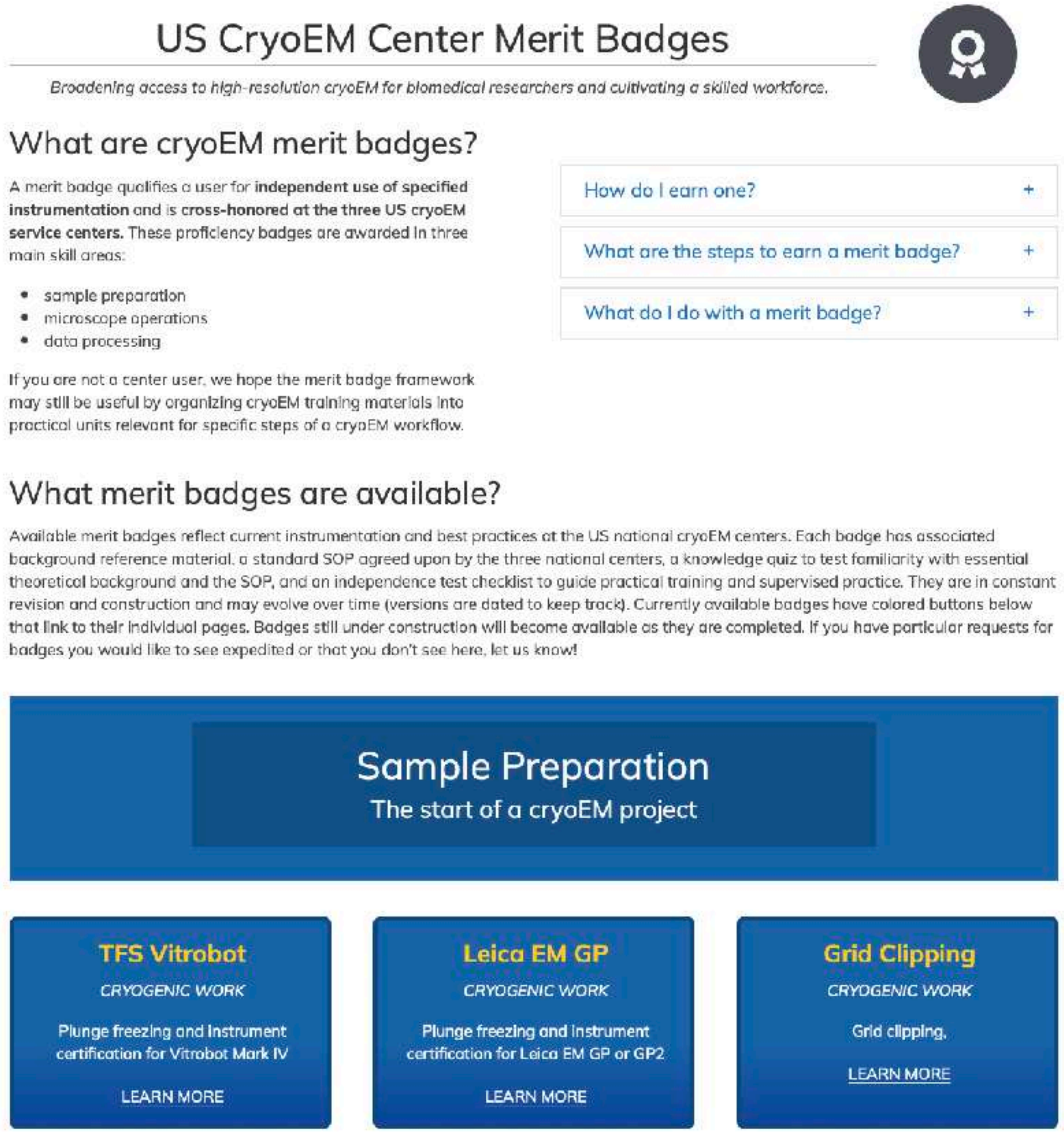


CRYOEM MERIT BADGES

Standards

Best practices

Demonstration of proficiency



US CryoEM Center Merit Badges

Broadening access to high-resolution cryoEM for biomedical researchers and cultivating a skilled workforce.

What are cryoEM merit badges?

A merit badge qualifies a user for **independent use of specified instrumentation** and is **cross-honored at the three US cryoEM service centers.** These proficiency badges are awarded in three main skill areas:

- sample preparation
- microscope operations
- data processing

If you are not a center user, we hope the merit badge framework may still be useful by organizing cryoEM training materials into practical units relevant for specific steps of a cryoEM workflow.

How do I earn one? +

What are the steps to earn a merit badge? +

What do I do with a merit badge? +

What merit badges are available?

Available merit badges reflect current instrumentation and best practices at the US national cryoEM centers. Each badge has associated background reference material, a standard SOP agreed upon by the three national centers, a knowledge quiz to test familiarity with essential theoretical background and the SOP, and an independence test checklist to guide practical training and supervised practice. They are in constant revision and construction and may evolve over time (versions are dated to keep track). Currently available badges have colored buttons below that link to their individual pages. Badges still under construction will become available as they are completed. If you have particular requests for badges you would like to see expedited or that you don't see here, let us know!

Sample Preparation

The start of a cryoEM project

TFS Vitrobot

CRYOGENIC WORK

Plunge freezing and instrument certification for Vitrobot Mark IV

LEARN MORE

Leica EM GP

CRYOGENIC WORK

Plunge freezing and instrument certification for Leica EM GP or GP2

LEARN MORE

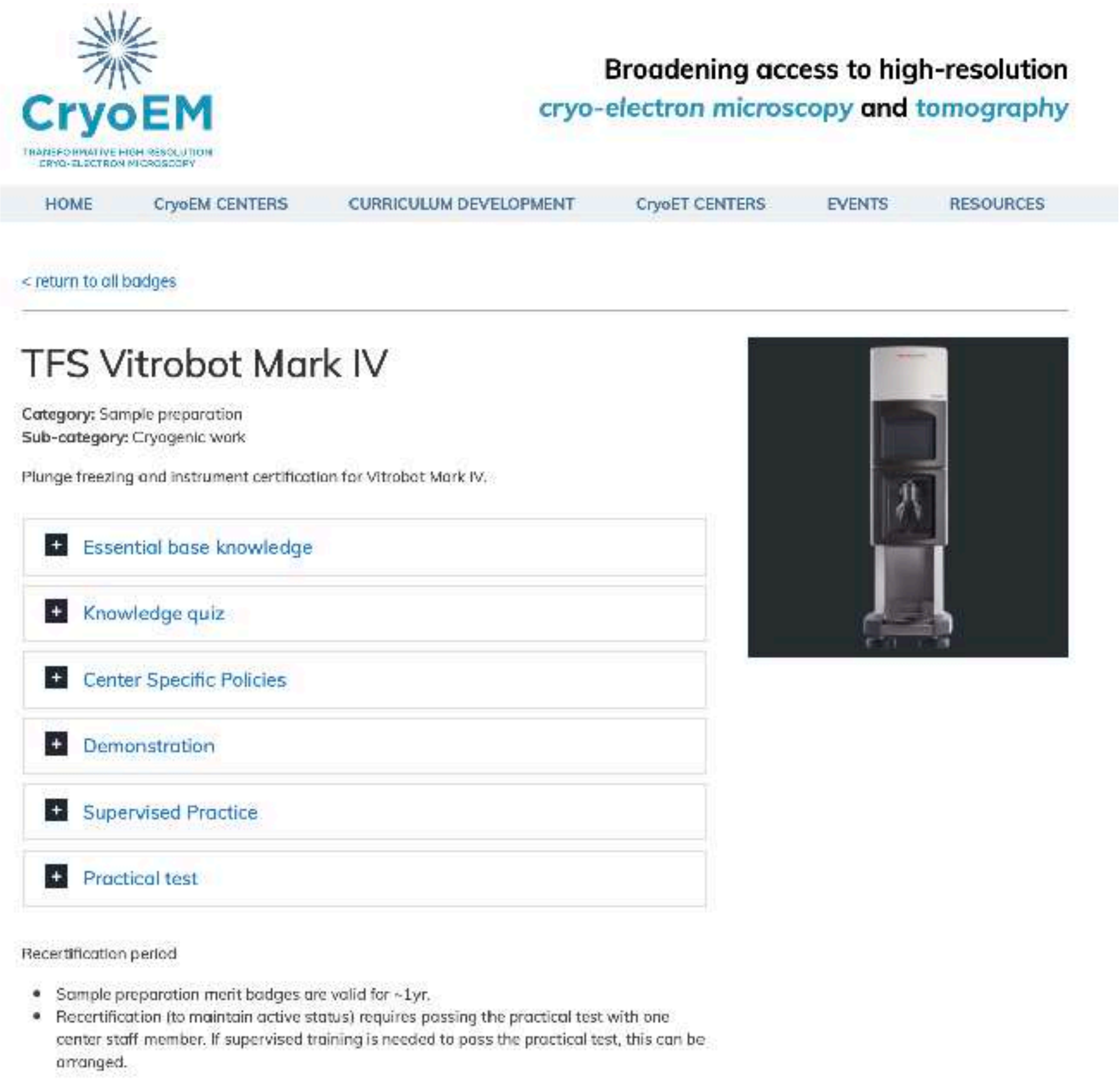
Grid Clipping

CRYOGENIC WORK

Grid clipping.

LEARN MORE

CRYOEM MERIT BADGES



CryoEM

Broadening access to high-resolution
cryo-electron microscopy and tomography

HOME | CryoEM CENTERS | CURRICULUM DEVELOPMENT | CryoET CENTERS | EVENTS | RESOURCES

< return to all badges

TFS Vitrobot Mark IV

Category: Sample preparation
Sub-category: Cryogenic work

Plunge freezing and instrument certification for Vitrobot Mark IV.

- Essential base knowledge
- Knowledge quiz
- Center Specific Policies
- Demonstration
- Supervised Practice
- Practical test

Recertification period

- Sample preparation merit badges are valid for ~1yr.
- Recertification (to maintain active status) requires passing the practical test with one center staff member. If supervised training is needed to pass the practical test, this can be arranged.

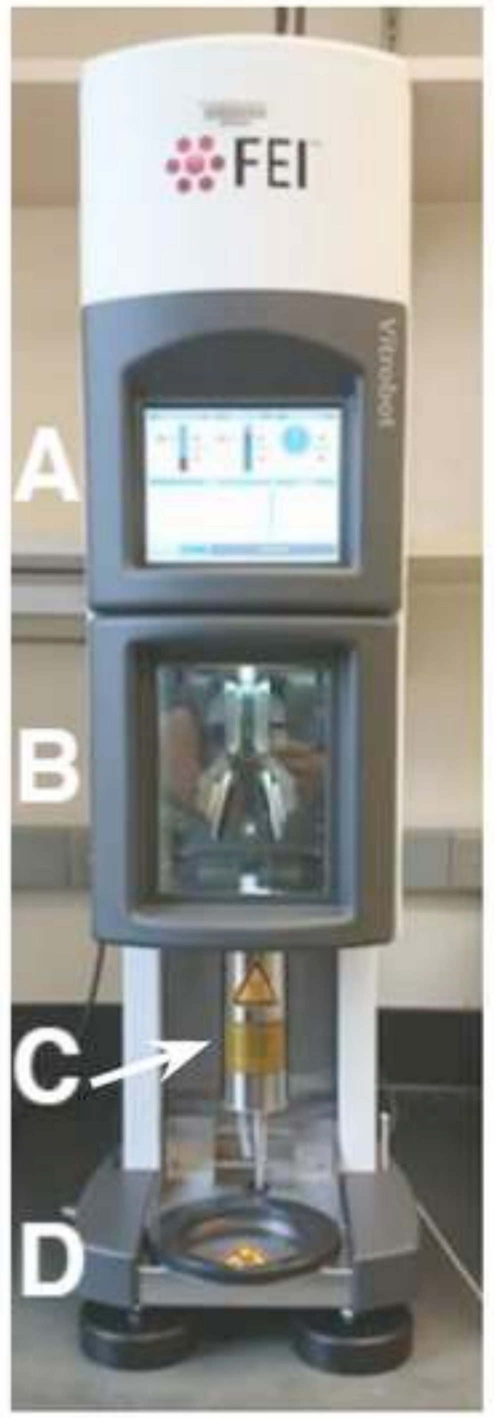


Figure 1. Vitrobot assembled and turned on. **A)** Screen. **B)** Environmental chamber with blotting pads. **C)** Humidifier. **D)** ethane lift.

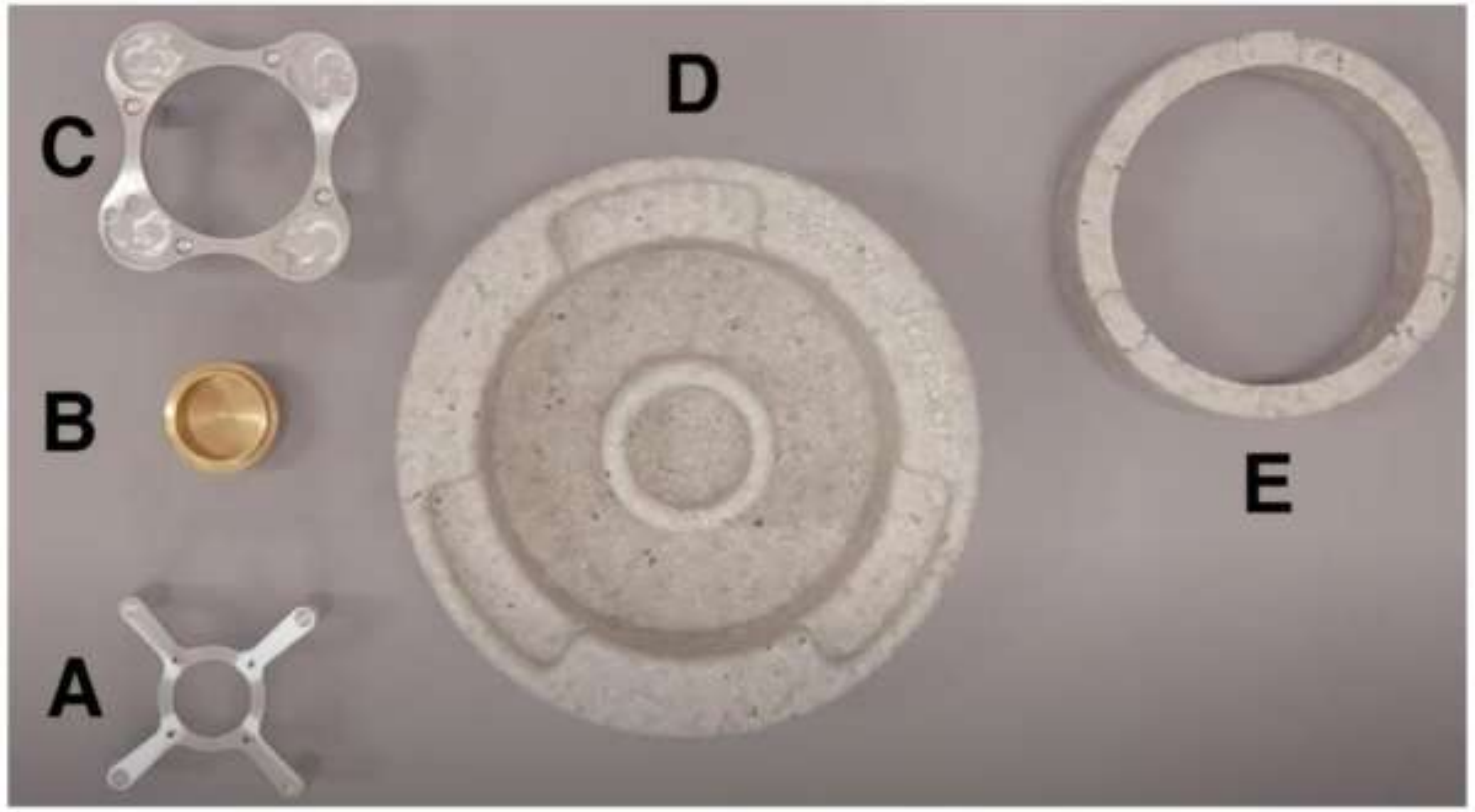
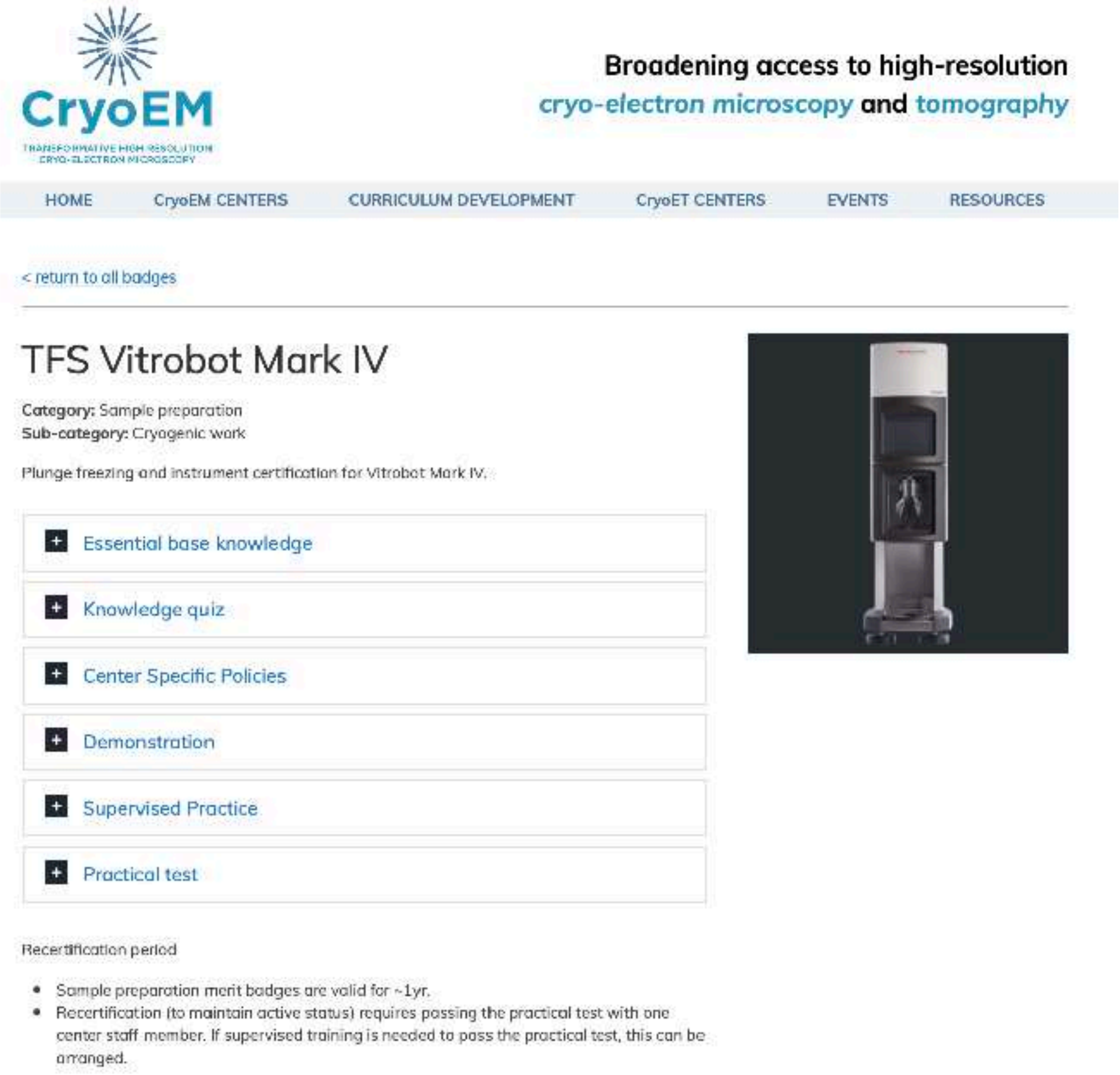
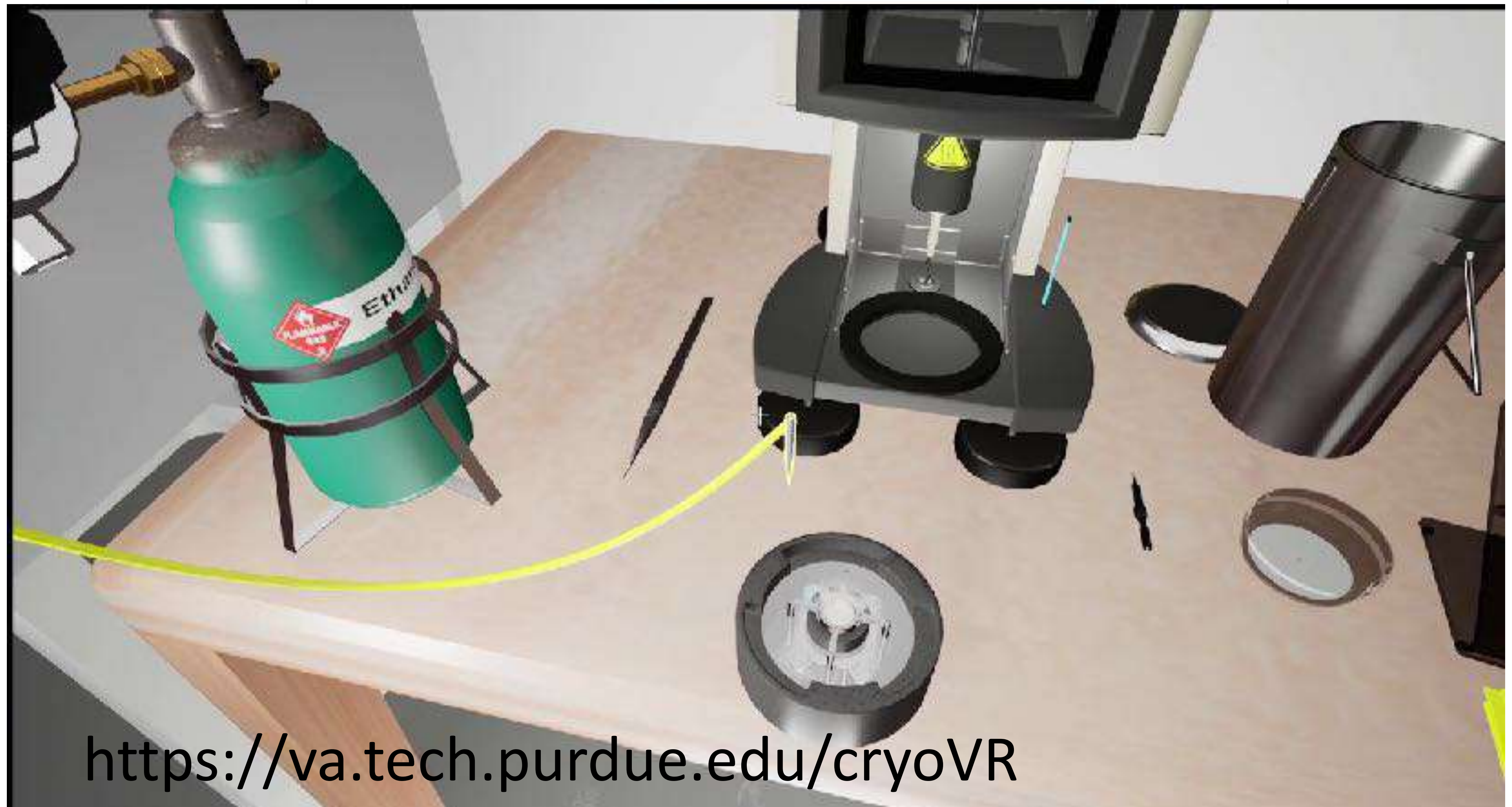


Figure 2. Ethane Holder. **A)** Spider. **B)** Brass Ethane Cup. **C)** Gridbox Holder. **D)** Base / Liquid Nitrogen Container. **E)** Anti-contamination Ring.

CRYOEM MERIT BADGES

https://cryoem101.org/selftest/?test=19





https://va.tech.purdue.edu/cryoVR

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

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

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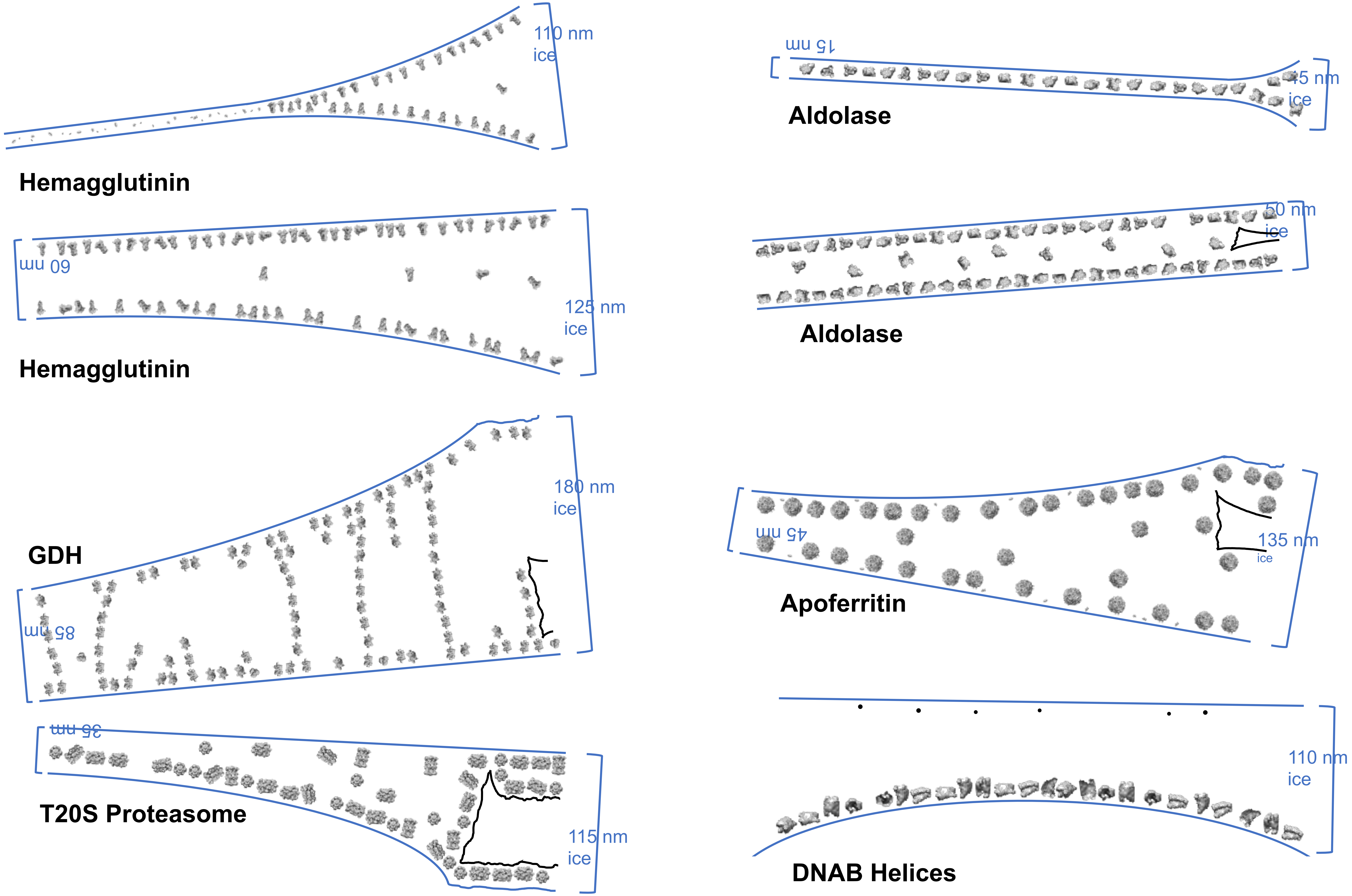
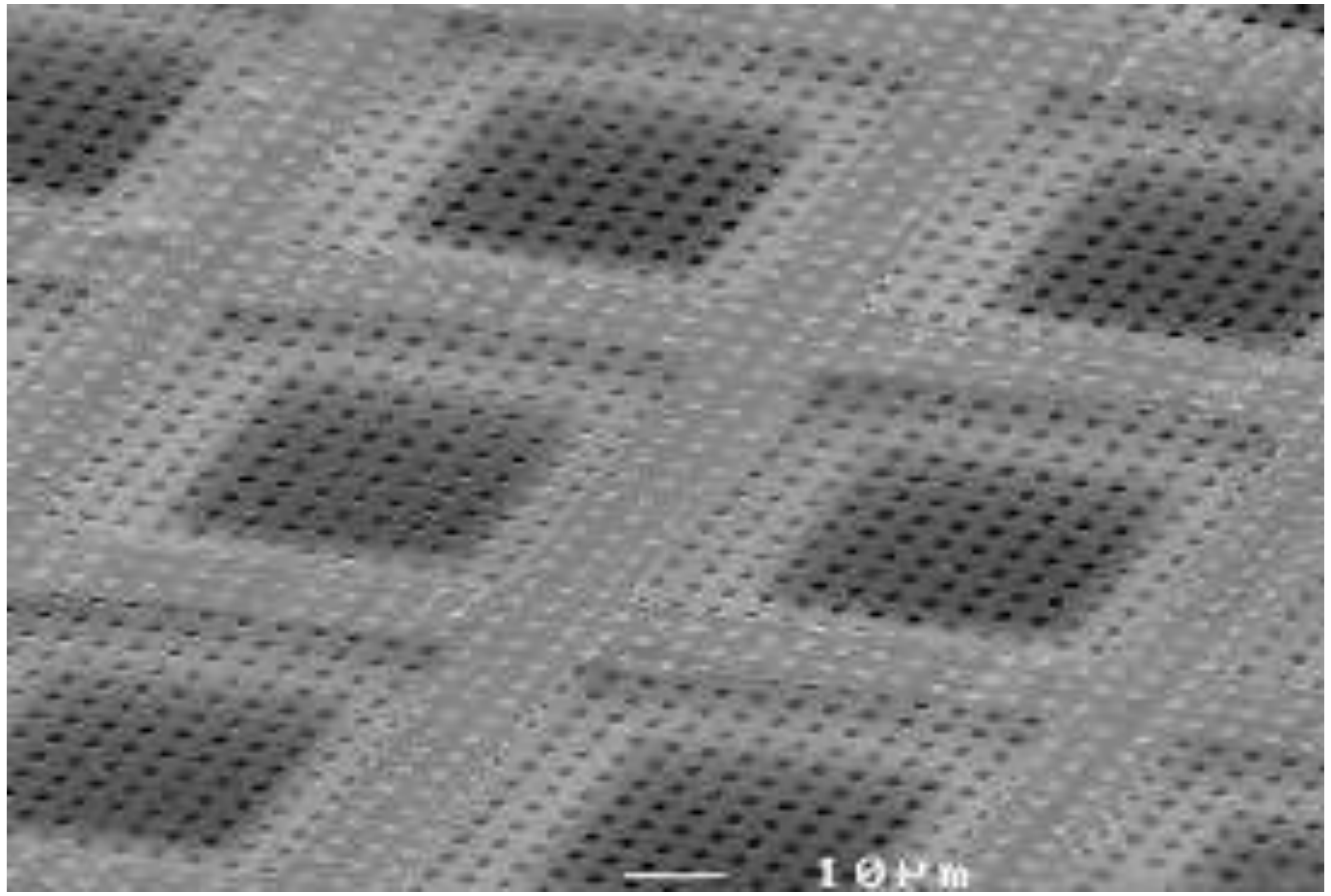
What issues arise?

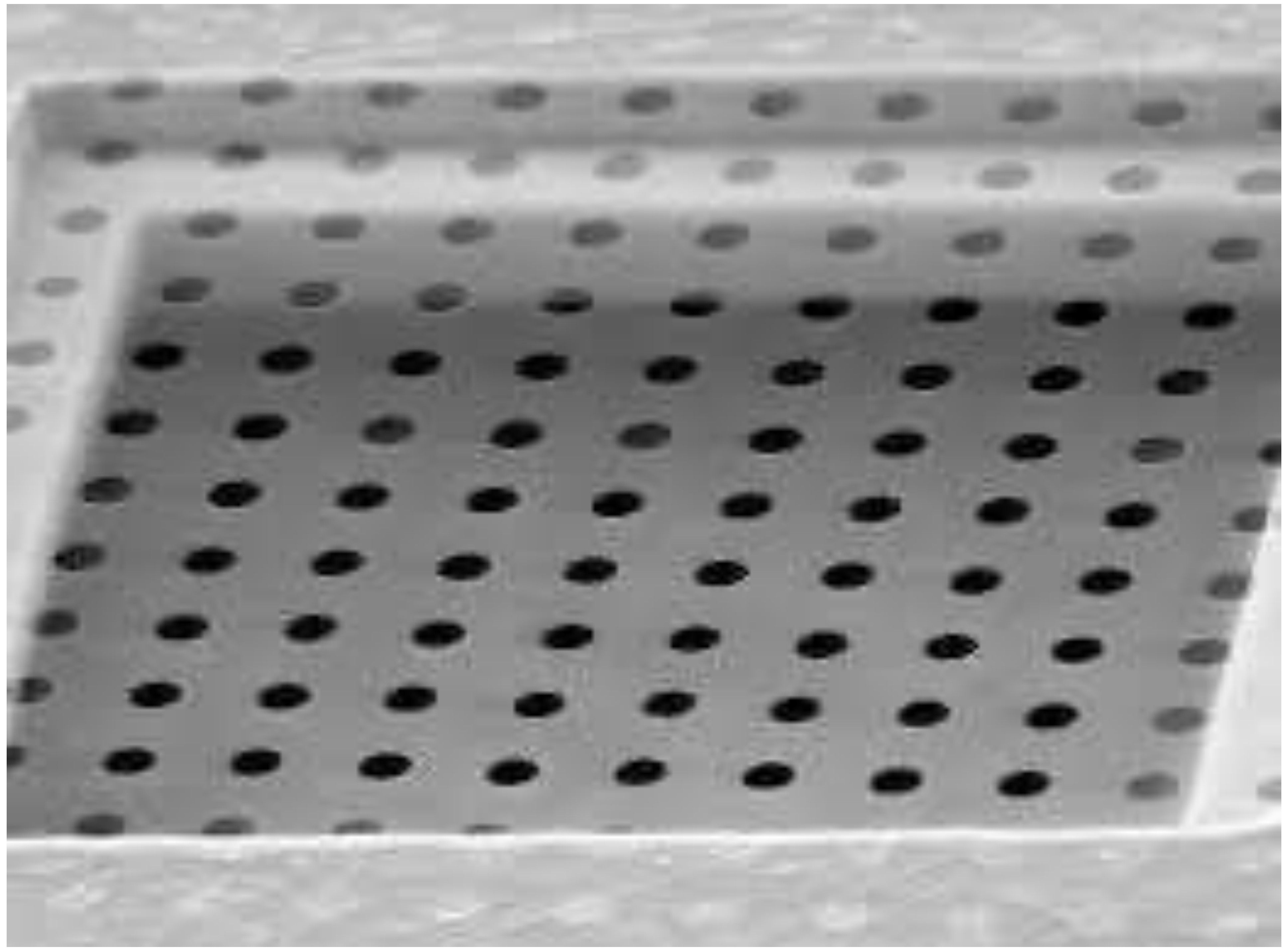


Noble AJ, et al. Routine single particle CryoEM sample and grid characterization by tomography. Elife. 2018;7.

Alex Noble

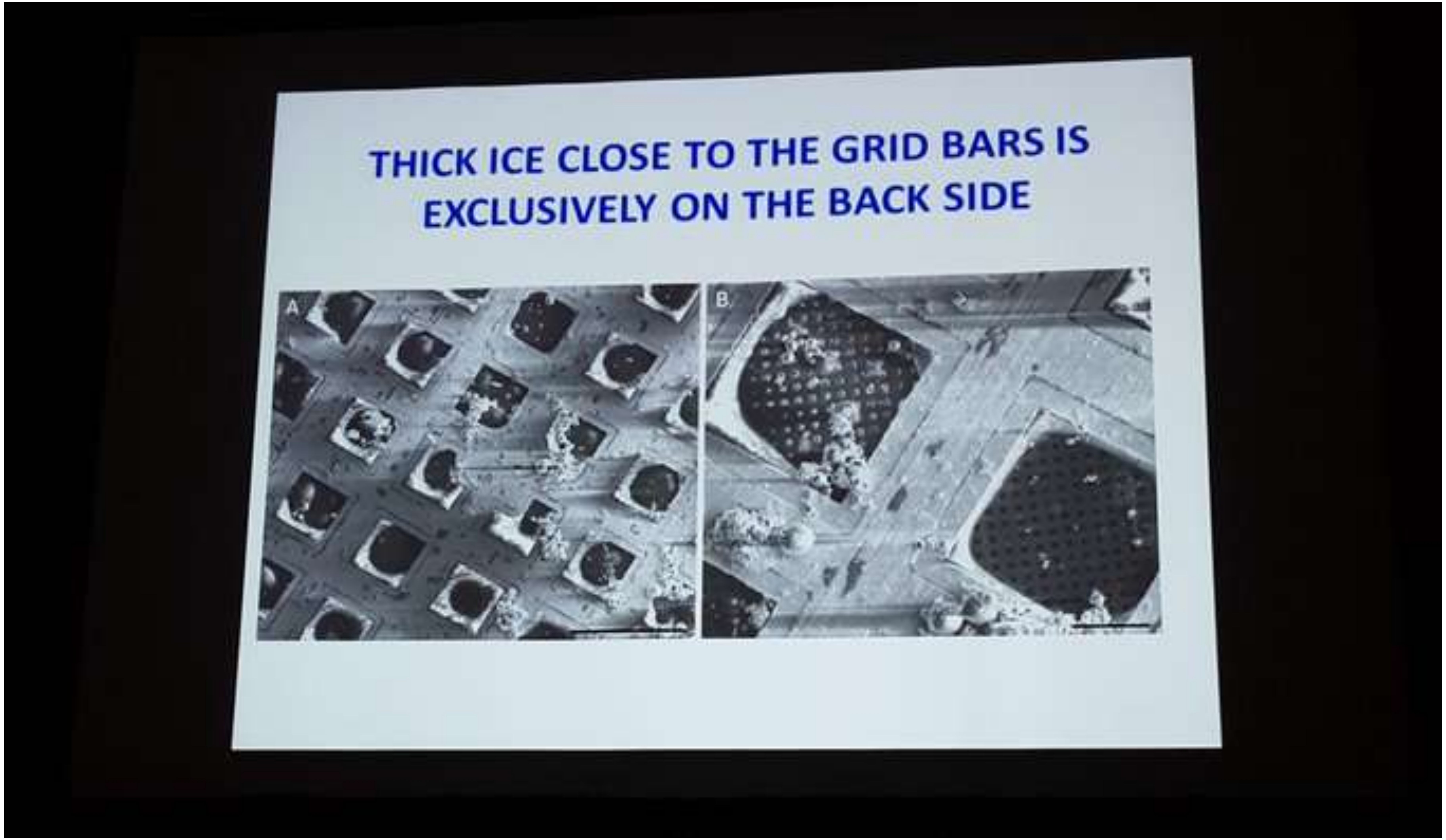
What does a holey carbon grid look like?



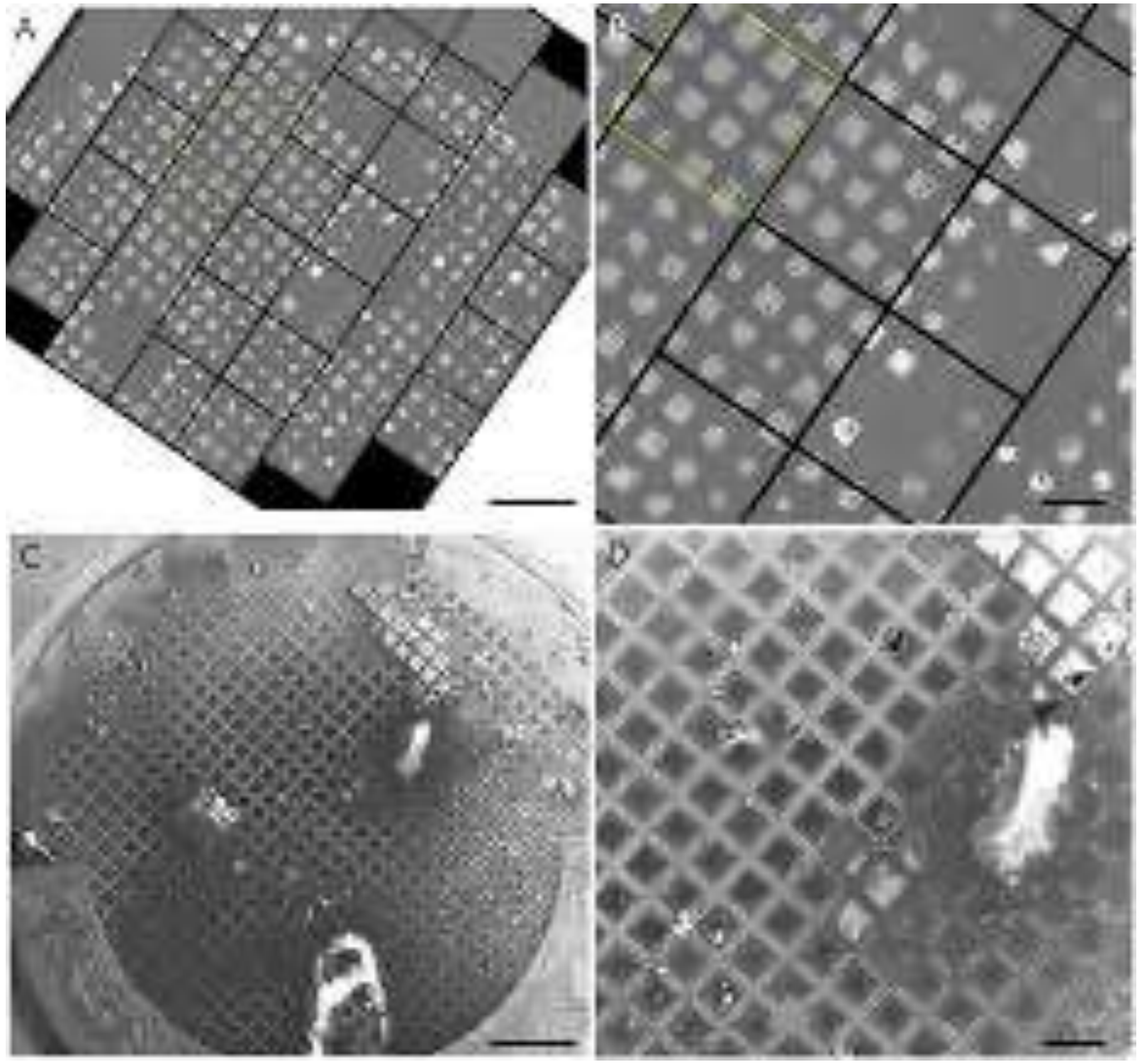


- Protochips.com

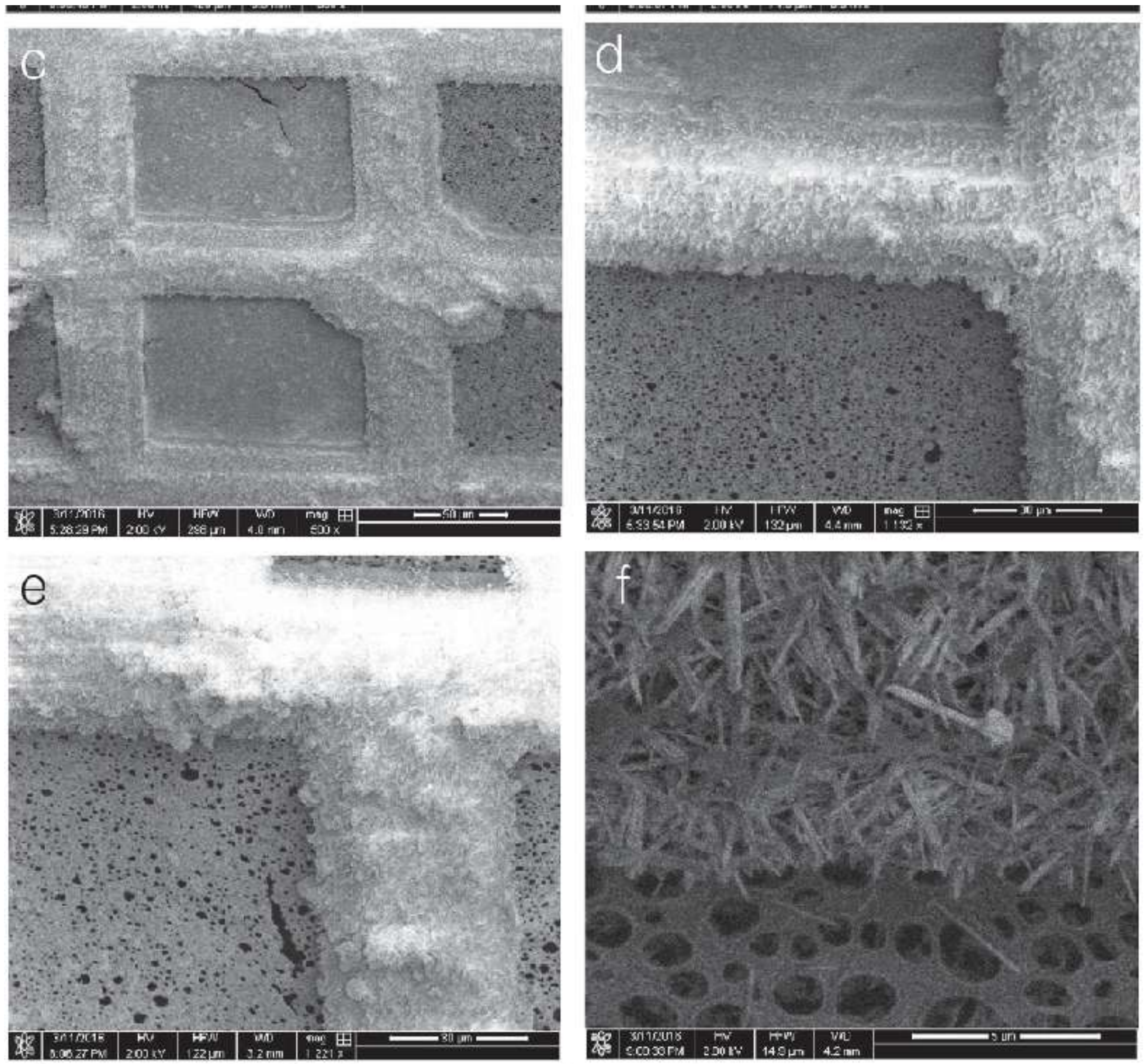
Quantifoil.com



- Bob Glaeser shows an SEM of a [#cryoEM]() grid https://twitter.com/annotated_sci/status/1158810644600119297?s=20



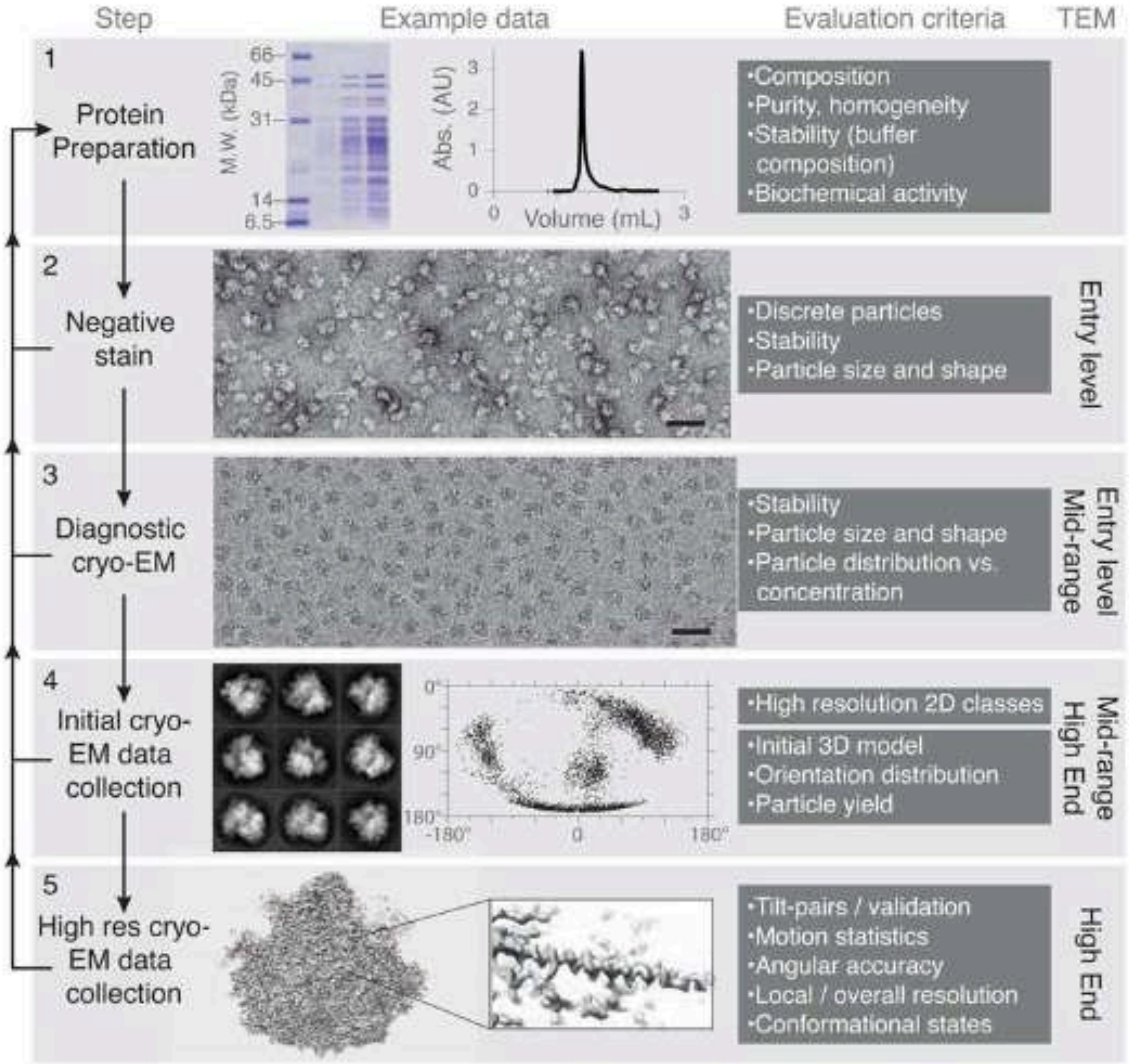
- Schmidli, Claudio & Rima, Luca & Arnold, Stefan & Stohler, Thomas & Syntychaki, Anastasia & Bieri, Andrej & Albiez, Stefan & Goldie, Kenneth & Chami, Mohamed & Stahlberg, Henning & Braun, Thomas. (2018). Miniaturized Sample Preparation for Transmission Electron Microscopy. Journal of Visualized Experiments. 2018. 10.3791/57310.



- Razinkov, I., Venkata P. Dandey, Hui Wei, Z. Zhang, D. Melnekoff, W. Rice, Christoph Wigge, C. S. Potter and B. Carragher. “A new method for vitrifying samples for cryoEM.” *Journal of structural biology* 195 2 (2016): 190-198 .

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/

TOOLS OF THE TRADE: MICROSCOPES, GRIDS AND MORE

Questions?