The Winter-Spring 2022 EM Course

January 19, 2022

https://www.dailycal.org/2020/04/28/virtual-learning-standards-must-be-maintained/

Should we shift to remote except for on-site practicals?

Journal club schedule

1. Freezing, 1/26
   Dabbu Jaijyan, Agata Jacewicz
2. Voltage, 2/2
   Emmanuel Afriyie, Wang Zheng
3. Revolution, 2/9
   Bright Shi, Emmanuel Afriyie
4. Noise, 2/23
   Iden Sapse, Dabbu Jaijyan
5. Validation, 3/2
   Agata Jacewicz, Wang Zheng
6. New papers 3/7 and/or 3/9

Kiyano Madoo, Wang Zheng, Iden Sapse, others?

https://icelab.se/4754-2/

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.

What are SEMC's standard data collection parameters

- Titan Krios
- Gatan GIF – BioQuantum
- Gatan K3 camera
- E- flux: ~30 eps (15eps, 20eps also used) [for ref. K2 8 eps @200ms]
- Exp time: 2-2.5 sec
- Frame rate: 40-**50ms**
- **A/pix: 1.3Å/px ~1.1Å/px. | 0.83Å | 0.65Å. | 0.5/0.4Å**
- **Total dose: 50-65 e/Å2**

Re: [ccpem] Binning factor in Motioncor

DA

Collaborative Computational Project in Electron cryo-Microscopy
on behalf of Daniel Asarnow
Wed 12/23/2020 2:19 PM
To: CCPEM@JISCMAIL.AC.UK

Apologies, sent a draft.

"With an ideal single-particle sample at least 150% physical Nyquist is possible [3]."

-da

On Wed, Dec 23, 2020 at 11:18 AM Daniel Asarnow <asarnow@msg.ucsf.edu> wrote:

> There's no problem except for the intrinsic one that the DQE at a given resolution is worse than it would be at the same resolution with a higher magnification. In other words, SNR at some frequency will be higher in images with 0.25 A/px in superres with -FtBin 2 (effective pixel size of 0.5 A/px) than in images with 0.5 A/px in superres and -FtBin 1. Imagine a plot like Figure 1A from [1], but put the X-axis in 1/A instead of fraction Nyquist, and draw curves for two different magnifications.
>
> One must consider the pixel size, dose rate, number of particles per image, sample heterogeneity, and expected final resolution in order to choose an "ideal" imaging condition. Most of the time though we just choose one or two "standard" magnifications for our microscopes, so if you end up with Nyquist-limited resolution after -FtBin 2 at a lower magnification, you can try -FtBin 1 or 1.5. With an ideal single-particle sample at least 150% physical Nyq
>
> [1] https://www.nature.com/articles/nmeth.2472
> [2] https://www.sciencedirect.com/science/article/pii/S1047847713002815
> [3] https://www.biorxiv.org/content/10.1101/2020.11.08.372763v1
>
> Best,
> -da

Gatan K3

- Super res
- 149% Nyquist

Practical considerations for using K3 cameras in CDS mode for high-resolution and high-throughput single particle cryo-EM

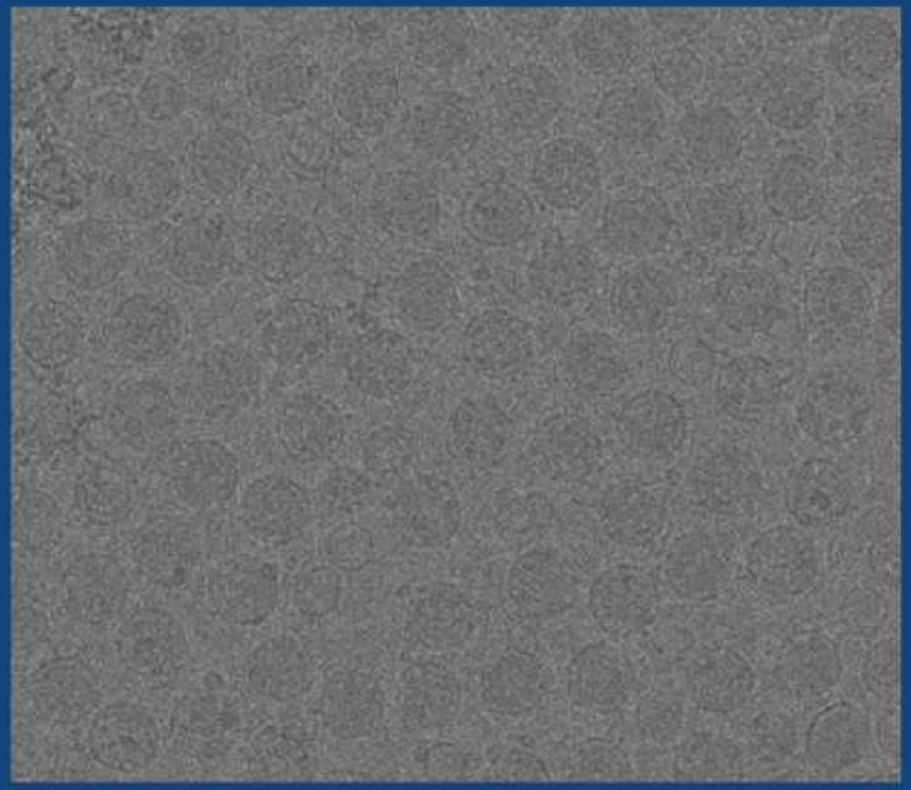
Ming Sun[*,a], Caleigh Azumaya[*,a], Eric Tse[*,b], Adam Frost[a,c], Daniel Southworth[b], Kliment A. Verba[c,d], Yifan Cheng[a,e] and David A. Agard[a,]**

[a] Department of Biochemistry & Biophysics, University of California, San Francisco, San Francisco, CA, 94143, United States
[b] Institute for Neurodegenerative Diseases, University of California, San Francisco, San Francisco, CA, 94143, United States
[c] Quantitative Biosciences Institute (QBI), University of California, San Francisco, San Francisco, CA, 94143, United States
[d] Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA, 94143, United States
[e] Howard Hughes Medical Institute, University of California, San Francisco, San Francisco, CA, 94143, United States
[*] Authors contributed equally.
[**] Correspondence: agard@msg.ucsf.edu, University of California, San Francisco, San Francisco, CA, 94143, United States

Abstract

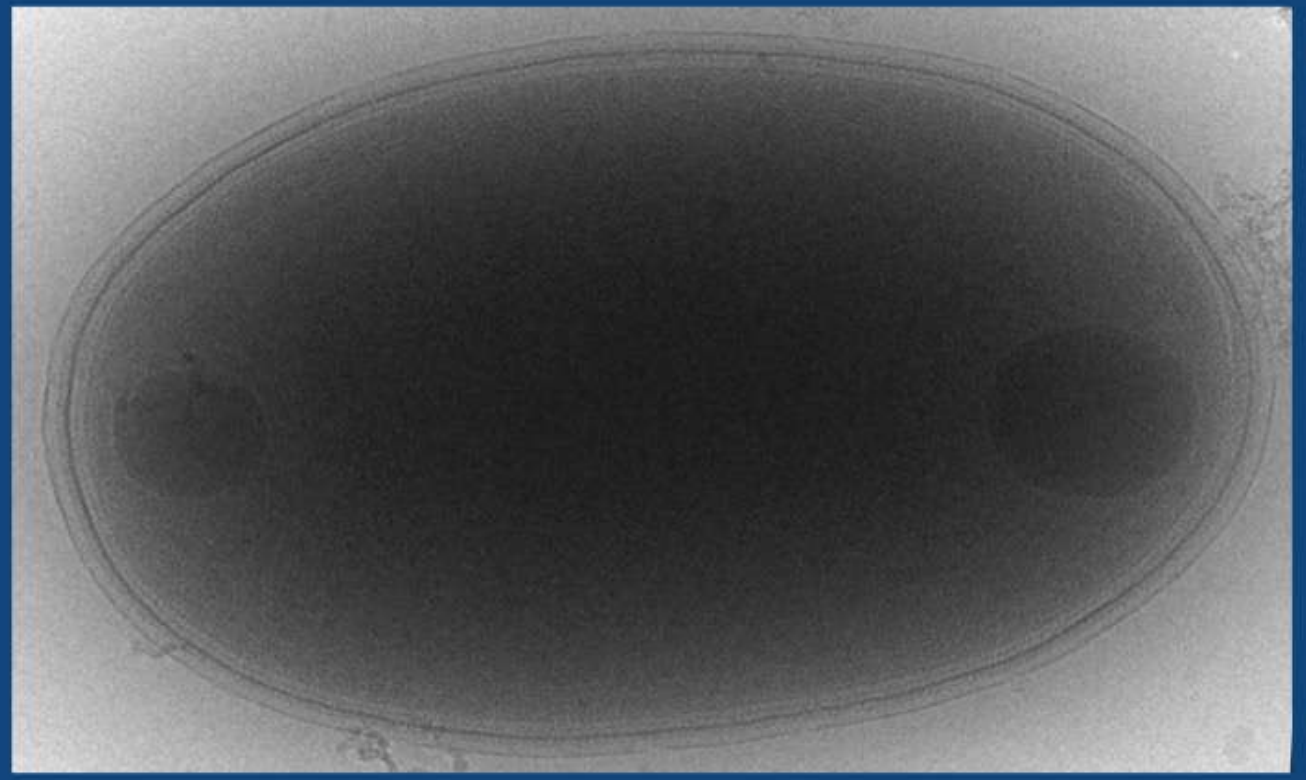
Detector technology plays a pivotal role in high-resolution and high-throughput cryo-EM structure determination. Compared with the first-generation, single-electron counting direct detection camera (Gatan K2), the latest K3 camera is faster, larger, and now offers a correlated-double sampling mode (CDS). Importantly this results in a higher DQE and improved throughput compared to its predecessor. In this study, we focused on optimizing camera data collection parameters for daily use within a cryo-EM facility and explored the balance between throughput and resolution. In total, eight data sets of murine heavy-chain apoferritin were collected at different dose rates and magnifications, using 9-hole image shift data collection strategies. The performance of the camera was characterized by the quality of the resultant 3D reconstructions. Our results demonstrated that the Gatan K3 operating in CDS mode outperformed nonCDS mode in terms of reconstruction resolution in all tested conditions with 8 electrons per pixel per second being the optimal dose rate. At low magnification (64kx) we were able to achieve reconstruction resolutions of 149% of the physical Nyquist limit (1.8 Å with a 1.346 Å physical pixel). Low magnification allows more particles to be collected per image, aiding analysis of heterogeneous samples requiring large data sets. At moderate magnification (105kx, 0.834Å physical pixel size) we achieved a resolution of 1.65 Å within 9 hours of data collection, a condition optimal for achieving high-resolution on well behaved samples. Our results also show that for an optimal sample like apoferritin, one can achieve better than 2.5 Å resolution with 5 minutes of data collection. Together, our studies validate the most efficient ways of imaging protein complexes using the K3 direct detector and will greatly benefit the cryo-EM community.

How thin do I need my sample?



50 nm

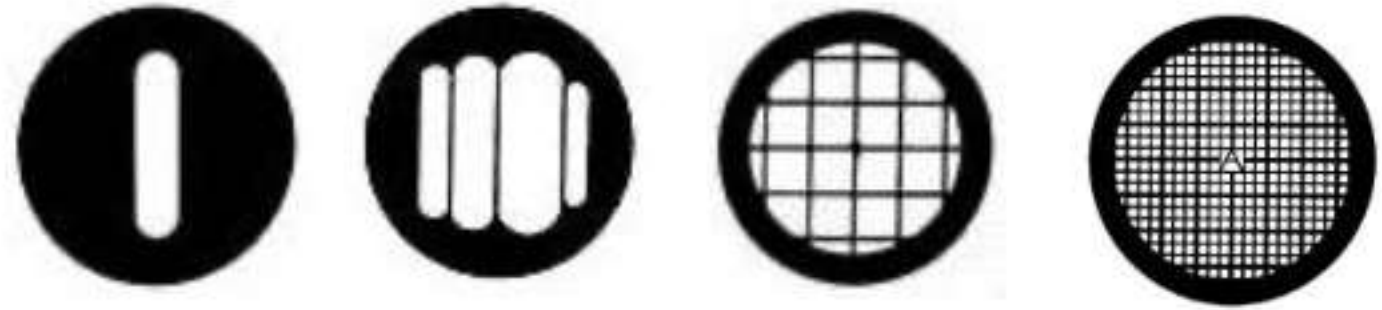
Bacteriophage (ϕ12)

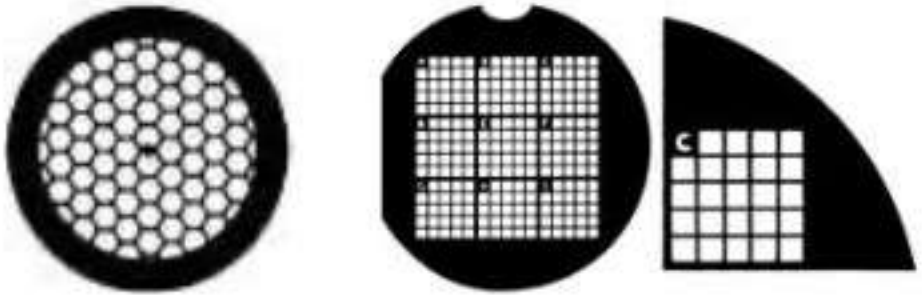


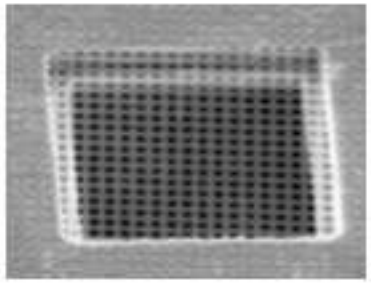
750 nm thick

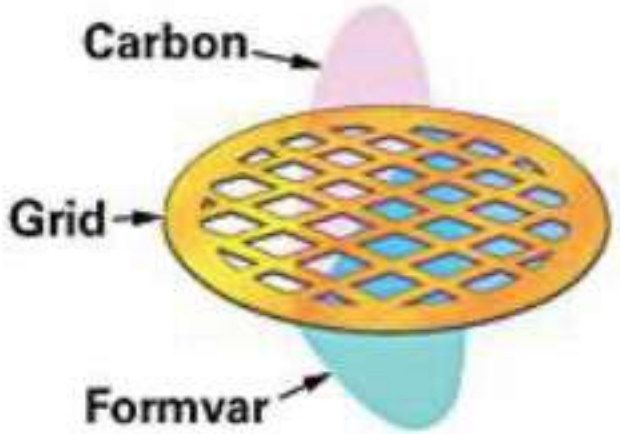
E. coli, Salmonella, Cyanobacteria

What are grids?









Common Materials

Copper

Nickel

Gold

Aluminum

Molybdenum

Titanium

Stainless Steel

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

Grids: Stats

Rough grid parameters

Rim Width: 350-400µm.

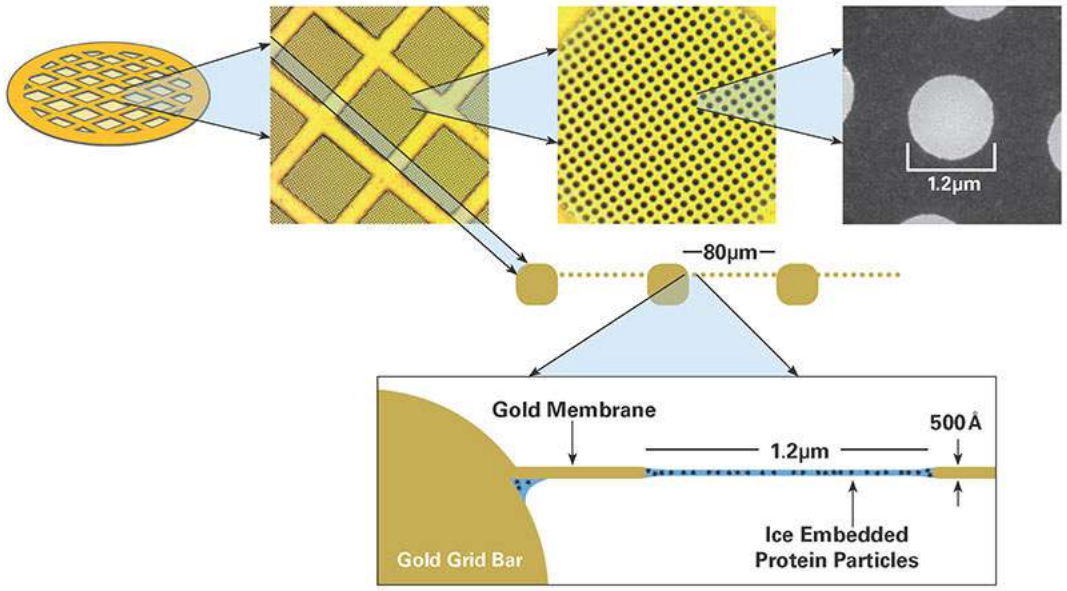
Thickness: PELCO® Grids are approximately 25µm thick.

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

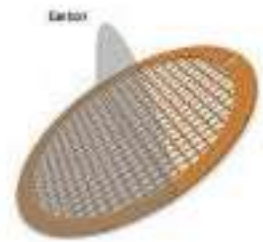
TERMINOLOGY

Grid (Cu, Au, Mo, etc…)

- mesh

Foil (C, Au, etc…)

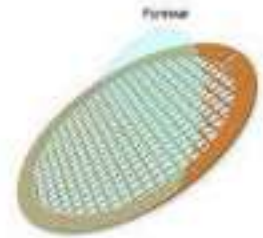
- Continuous
- lacy
- holey (hole size and spacing)



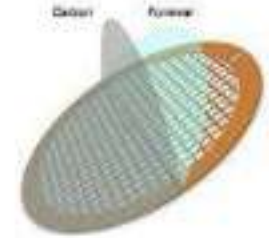
CARBON ONLY SUPPORT FILMS

HOLEY CARBON SUPPORT FILMS

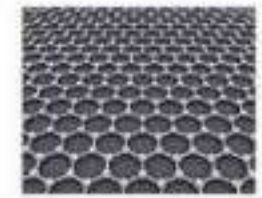
LACEY CARBON SUPPORT FILMS



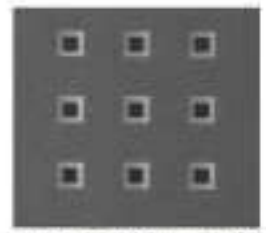
FORMVAR ONLY SUPPORT FILMS



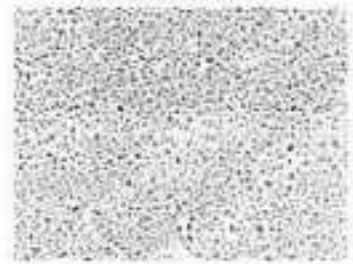
FORMVAR / CARBON SUPPORT FILMS



EM-TEC GRAPHENE SUPPORT FILMS



EM-TEC SILICON NITRIDE SUPPORT FILMS



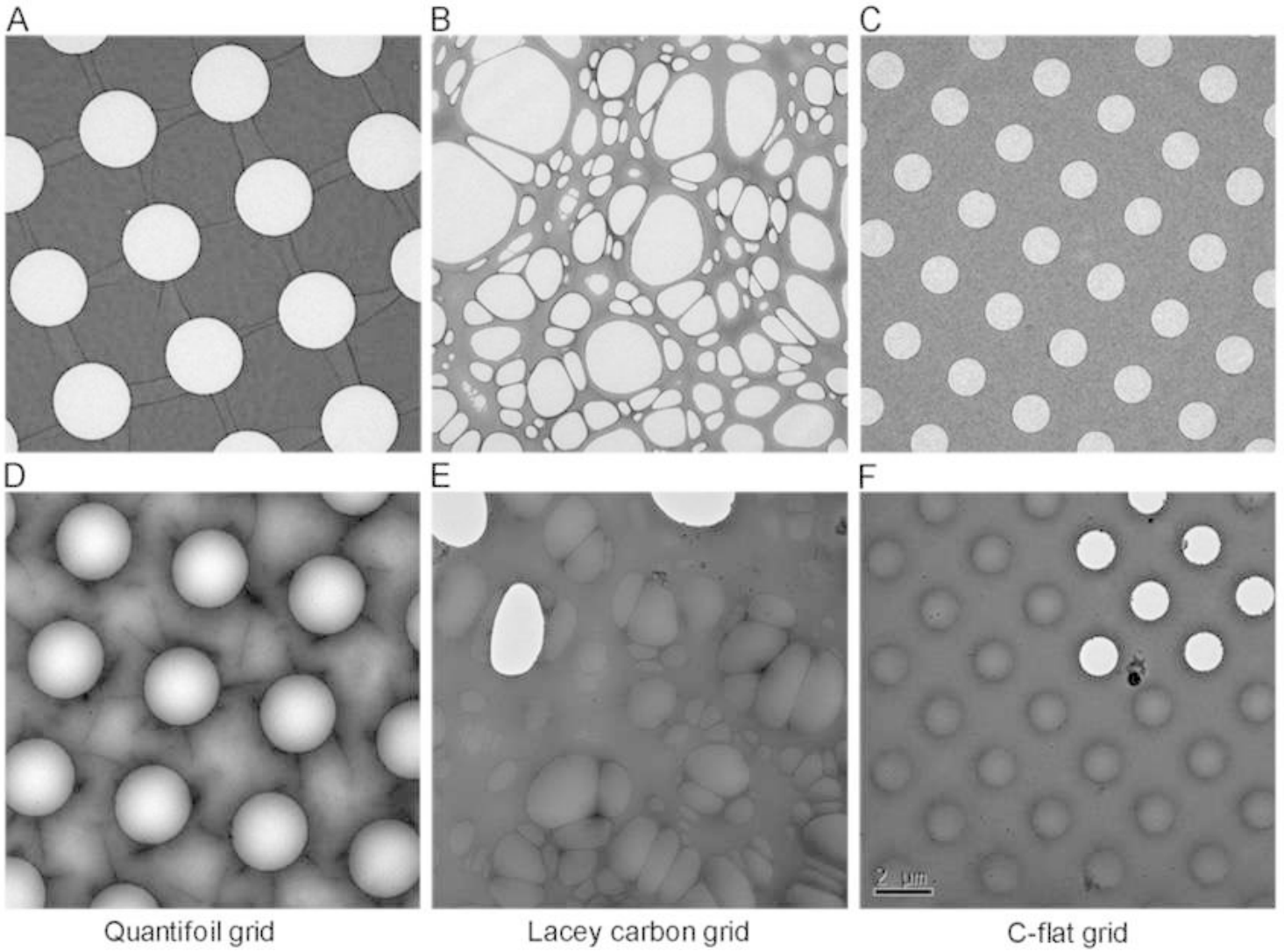
TEM CALIBRATION & TEST STANDARDS

TEM GRID STORAGE BOXES

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

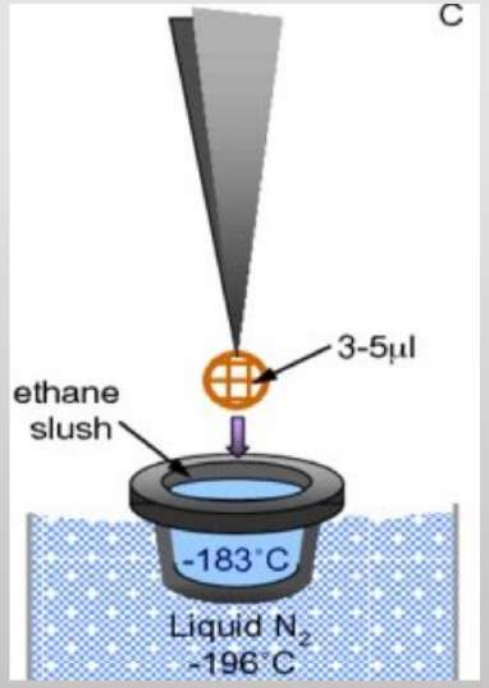
Support films

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.

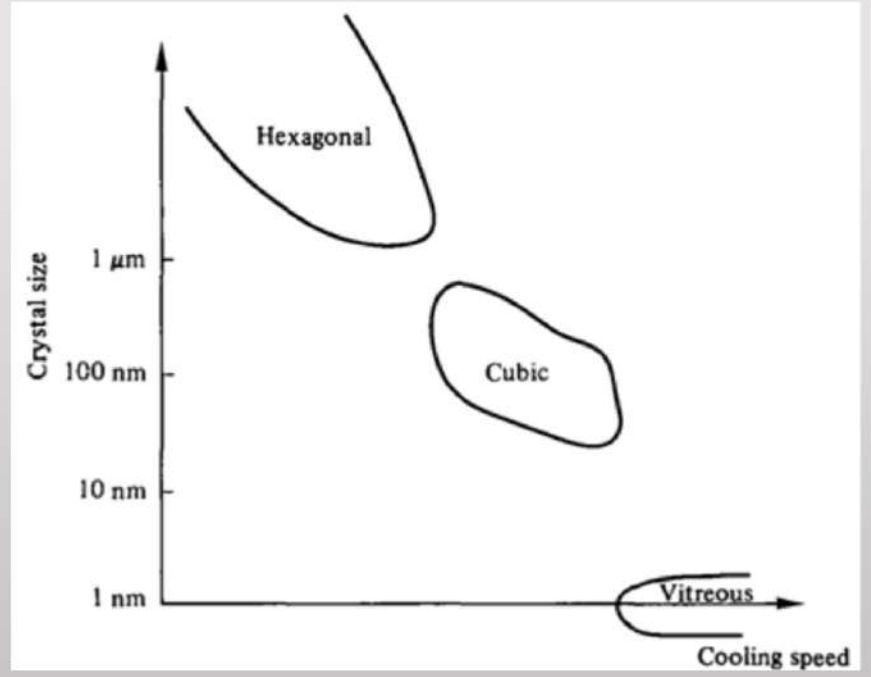


Plunge freezing

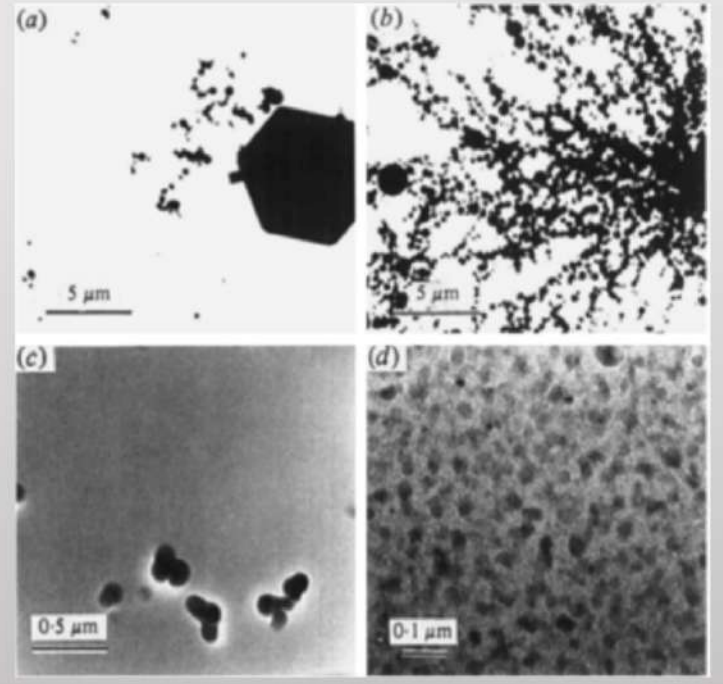
- Liquid ethane is a suitable coolant.
- Liquid nitrogen boils on contact, which makes it a poor coolant for cryo-EM.
- Cooling speed faster than 10^5-10^6 K/s ensure the formation of vitrified ice.



Setup of liquid ethane
(Image from Wen Jiang)



Cooling speed &
forms of ice



Different forms of ice contamination

Jacques Dubochet et al., 1988

Practical questions on Vitrobot / Leica

cryoEM merit badges

CryoEM
TRANSFORMATIVE HIGH RESOLUTION CRYO-ELECTRON MICROSCOPY

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.

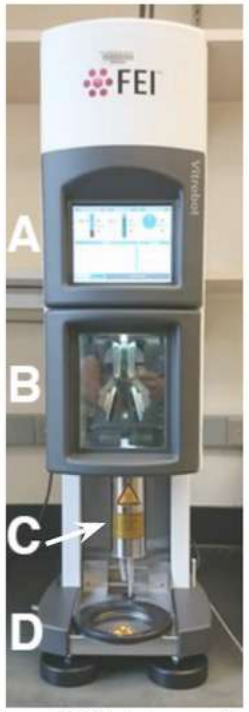


Figure 1. Vitrobot assembled and turned on. **A)** Screen. **B)** Environmental chamber with blotting pads. **C)** Humidifier. **D)** ethane lift.

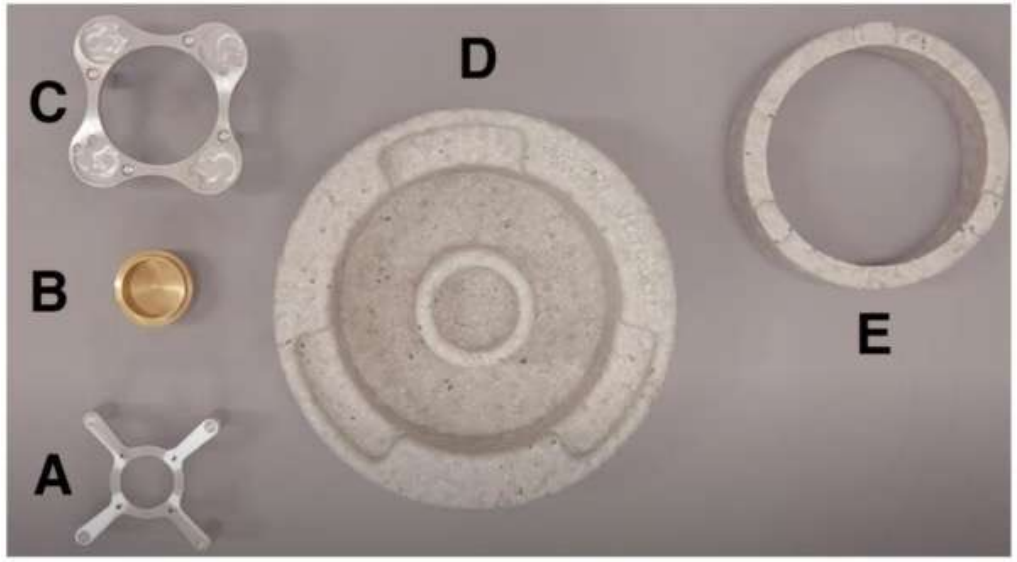
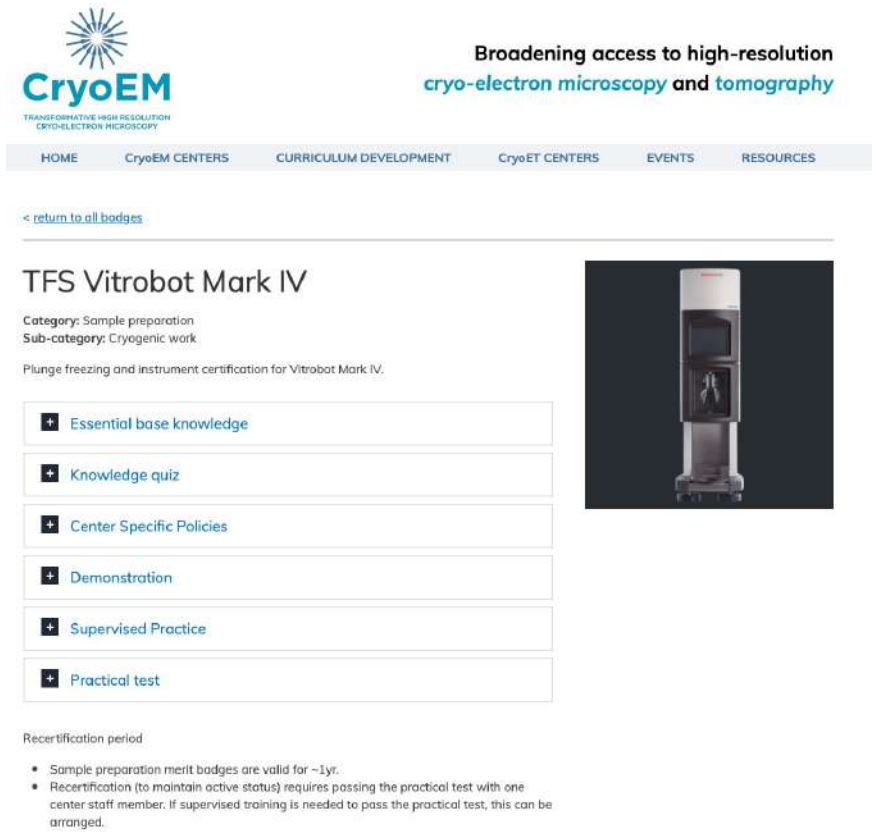
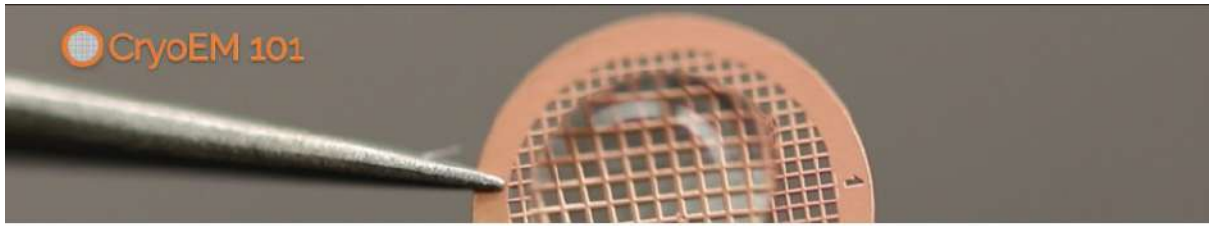


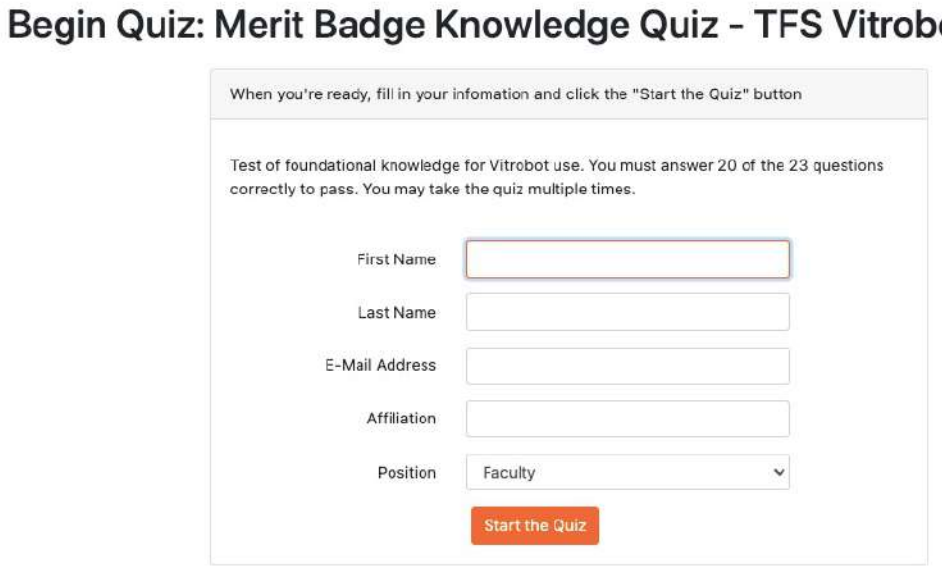
Figure 2. Ethane Holder. **A)** Spider. **B)** Brass Ethane Cup. **C)** Gridbox Holder. **D)** Base / Liquid Nitrogen Container. **E)** Anti-contamination Ring.

cryoEM merit badges

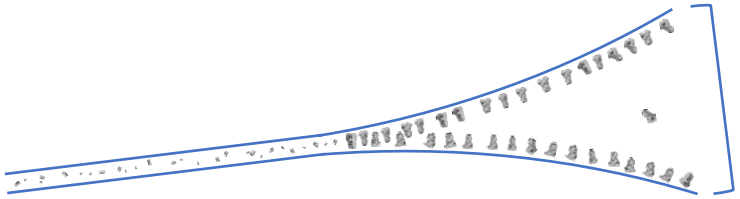
https://cryoem101.org/selftest/?test=19

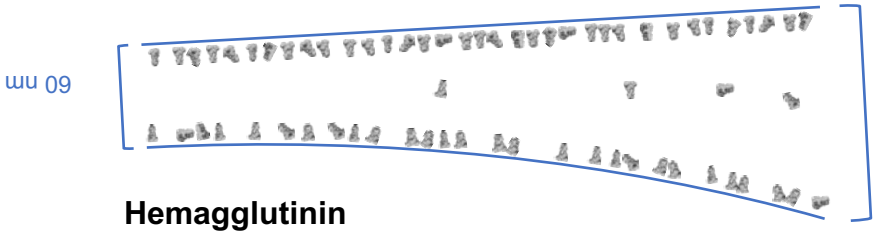


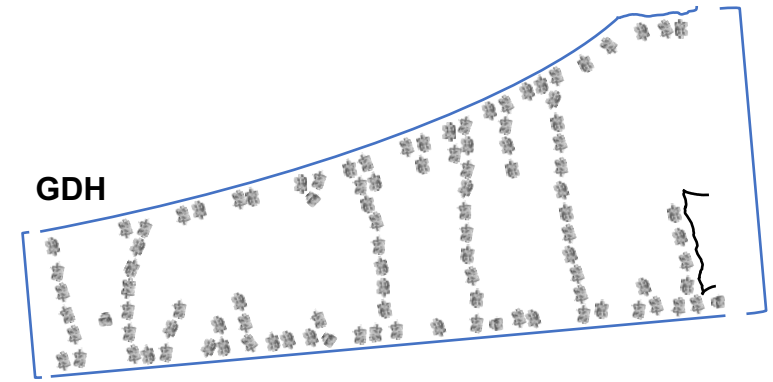


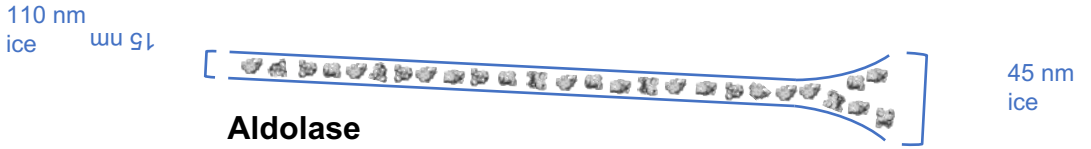


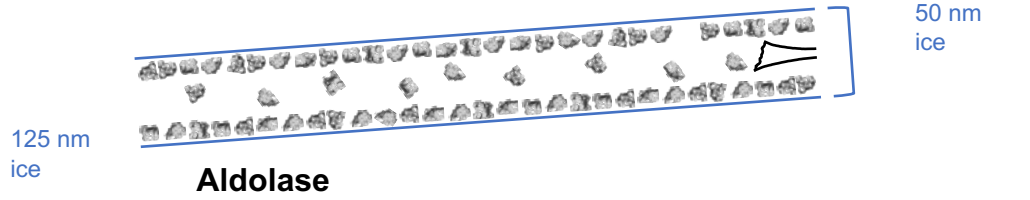
What issues arise?

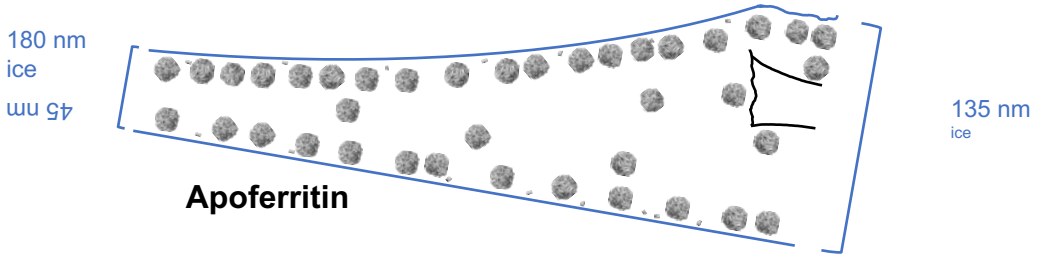


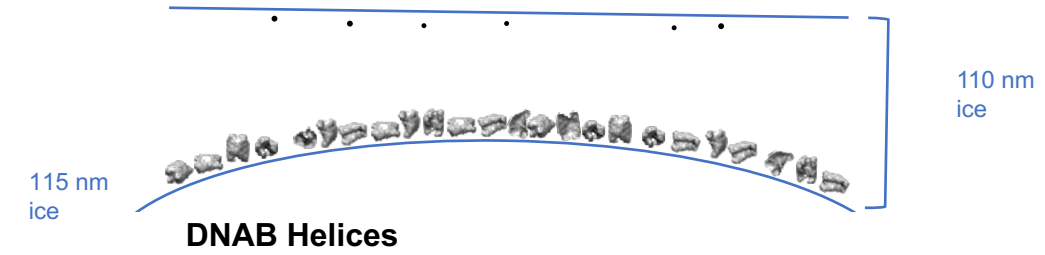








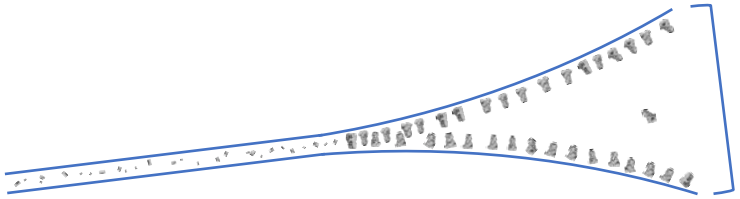


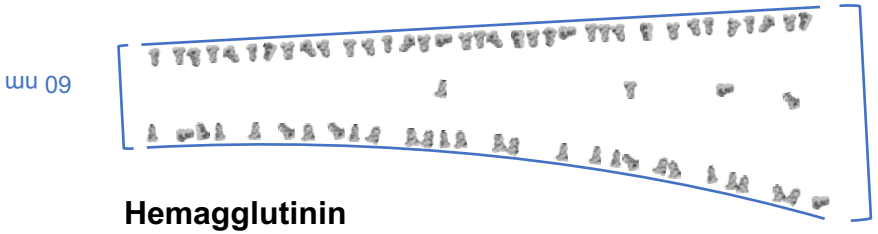


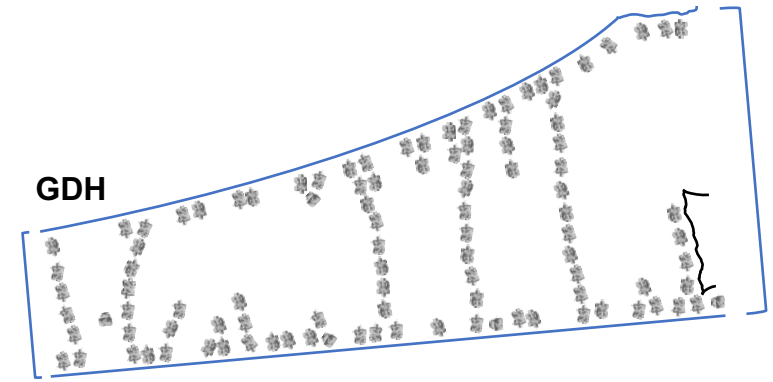
Noble AJ, et al. Routine single particle CryoEM sample and grid characterization by tomography. Elife. 2018;7.

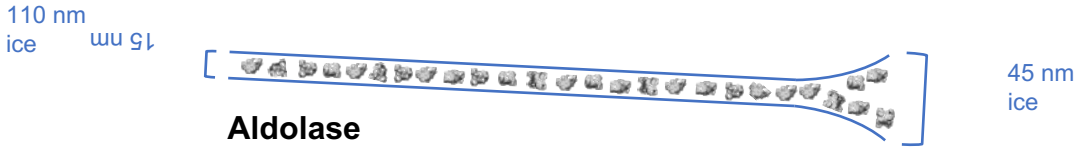
Alex Noble

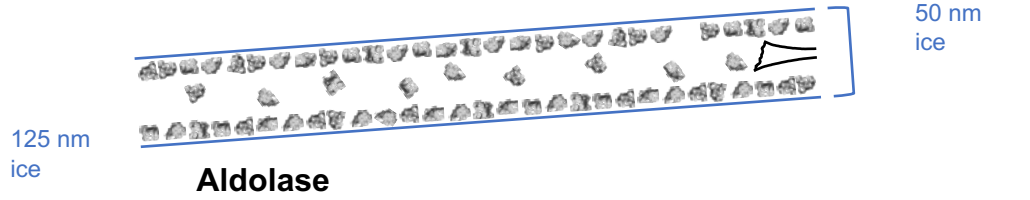
What issues arise?

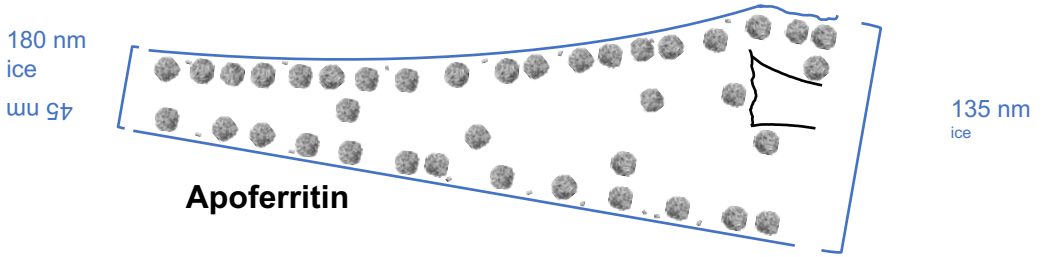


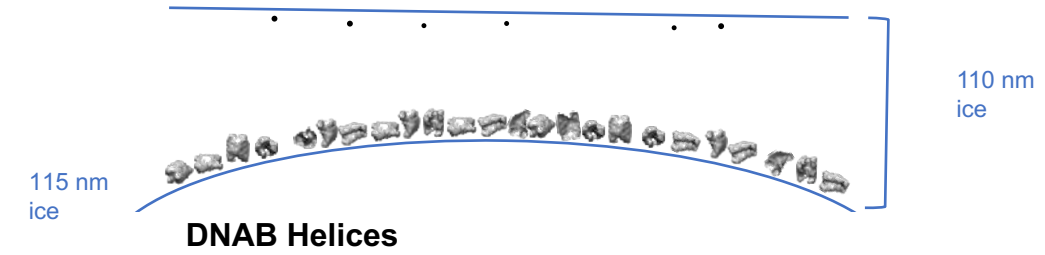








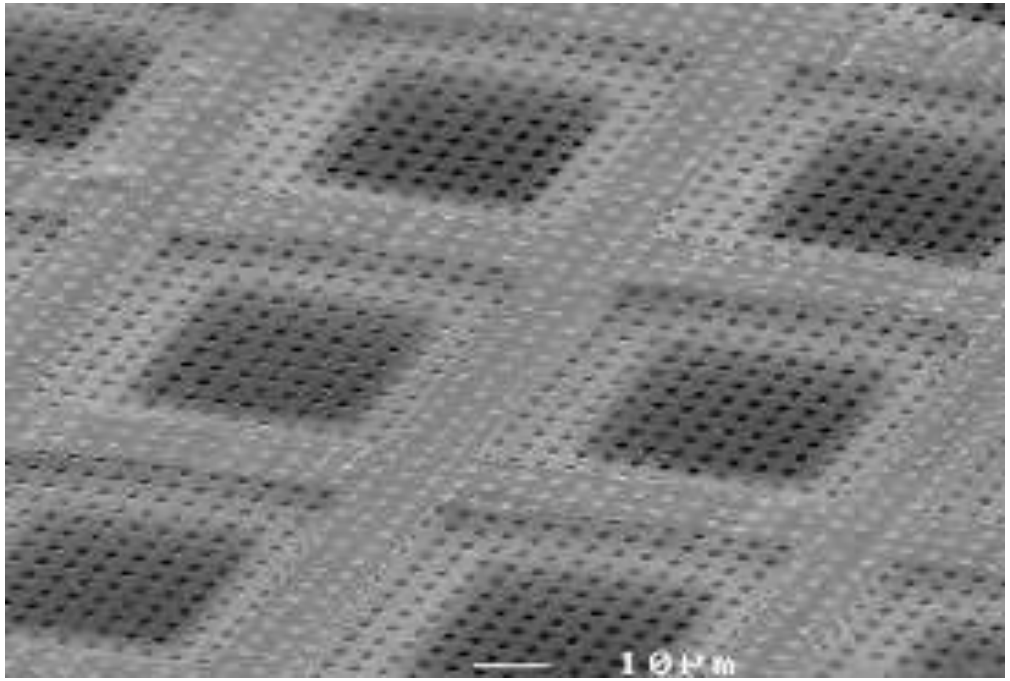




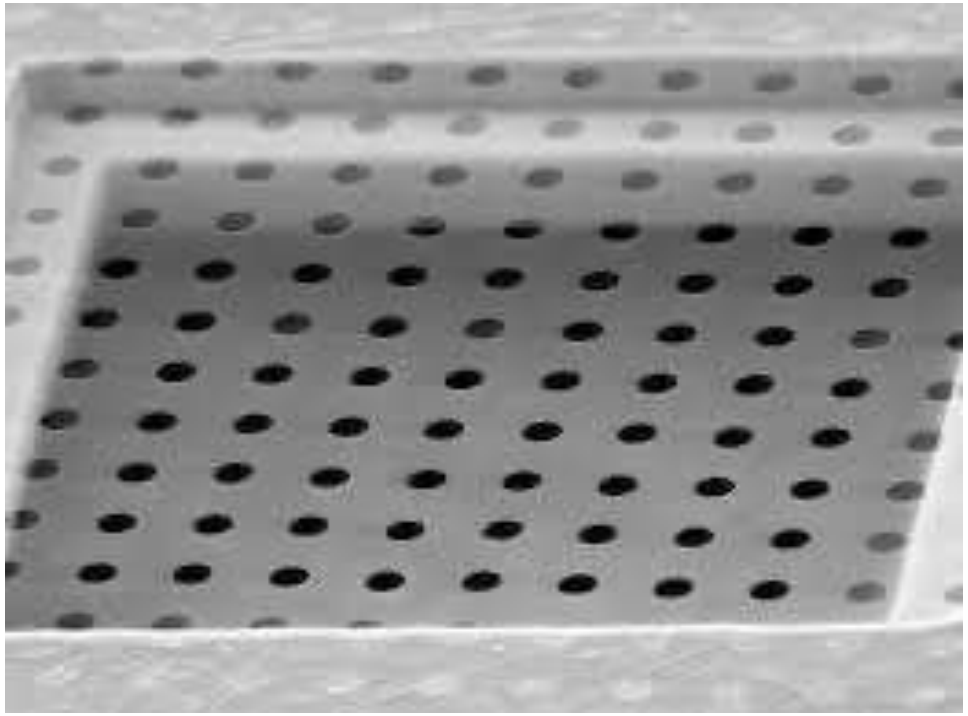
Noble AJ, et al. Routine single particle CryoEM sample and grid characterization by tomography. Elife. 2018;7.

Alex Noble

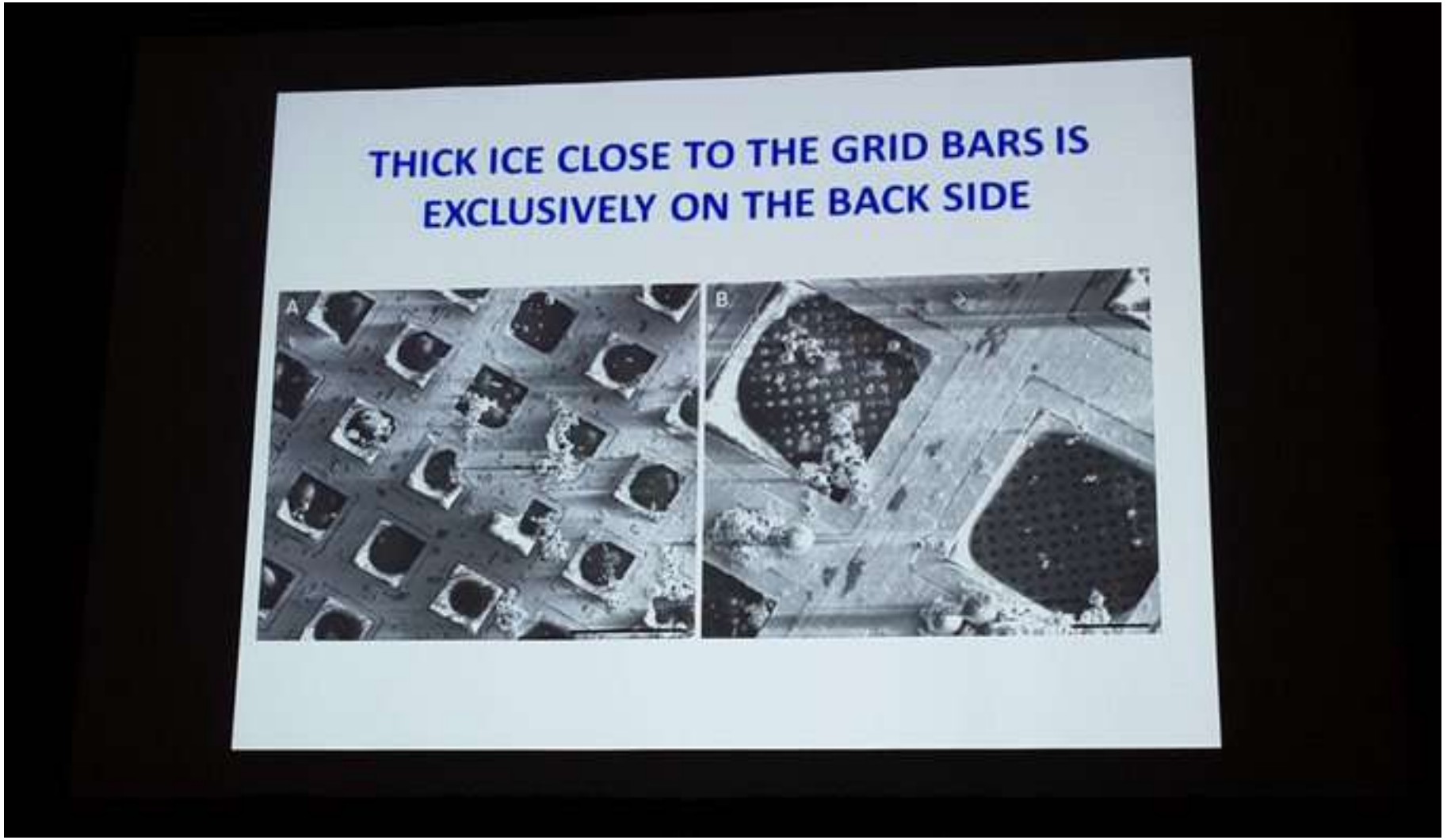
What does a holey carbon grid look like?



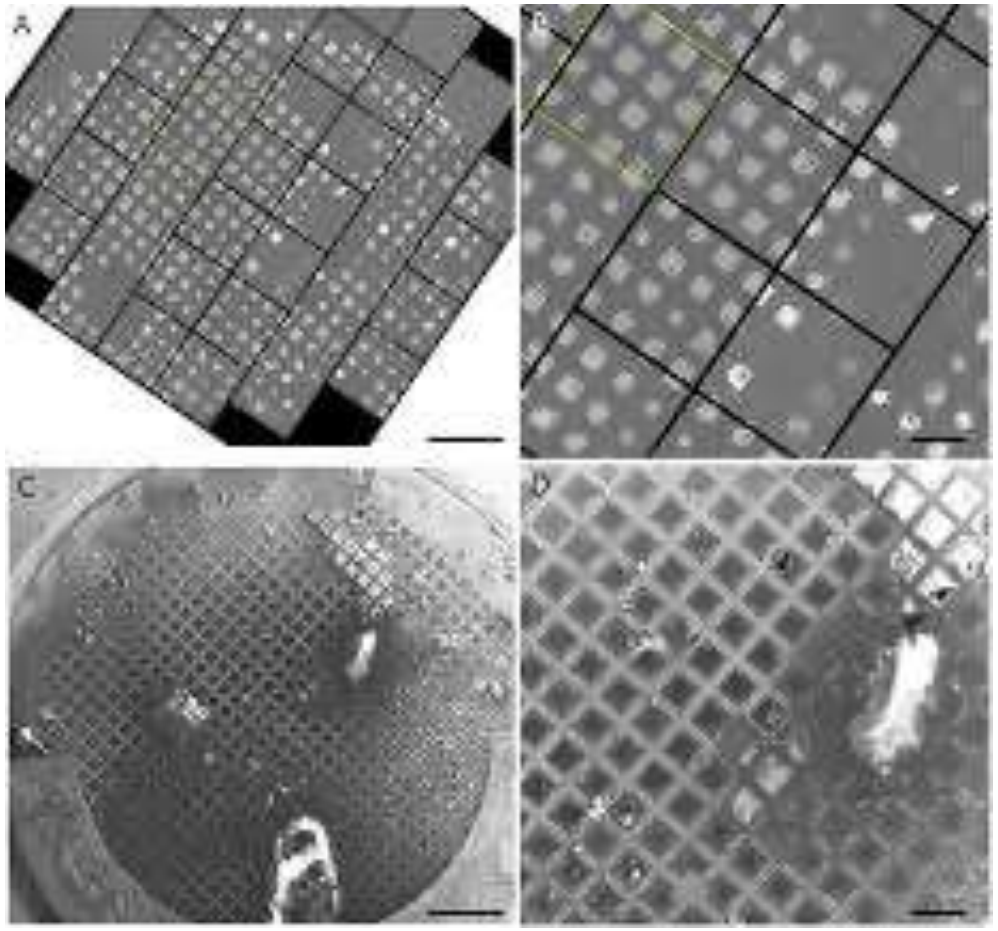
- Protochips.com

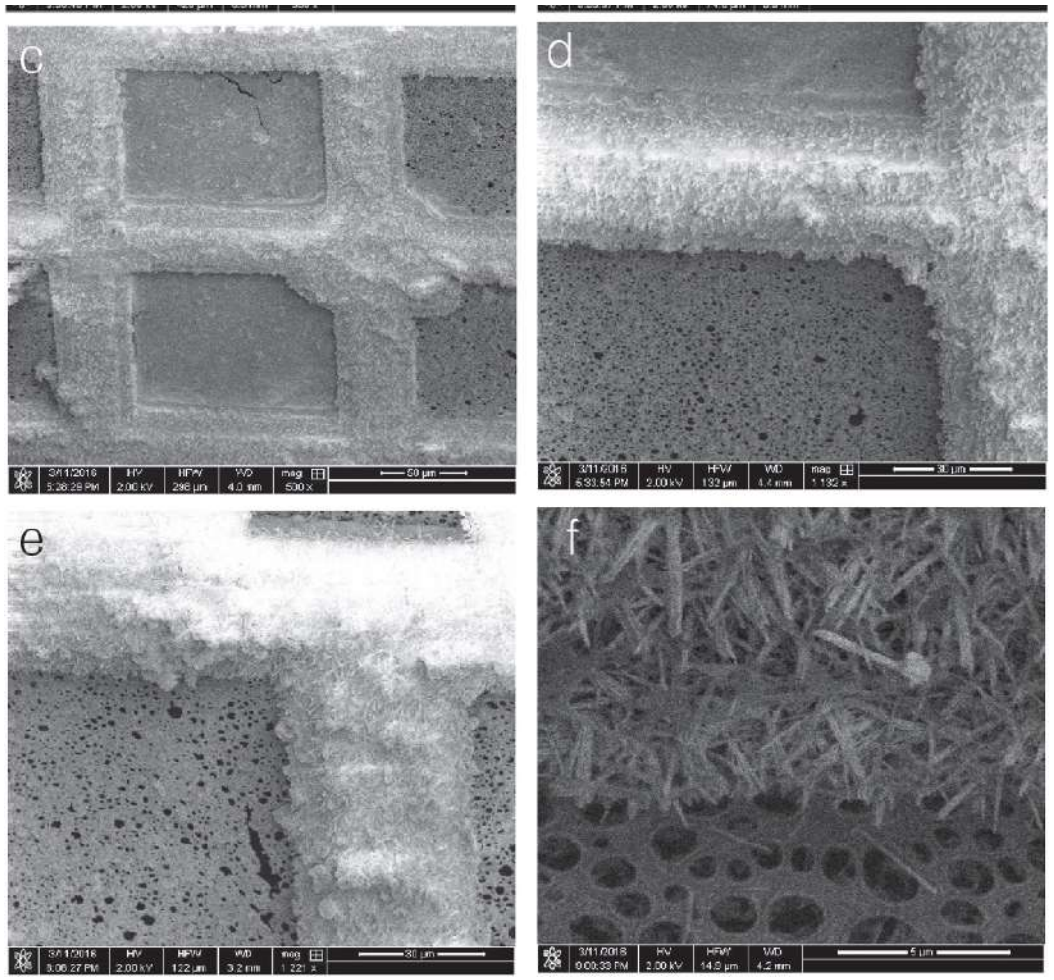


Quantifoil.com



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



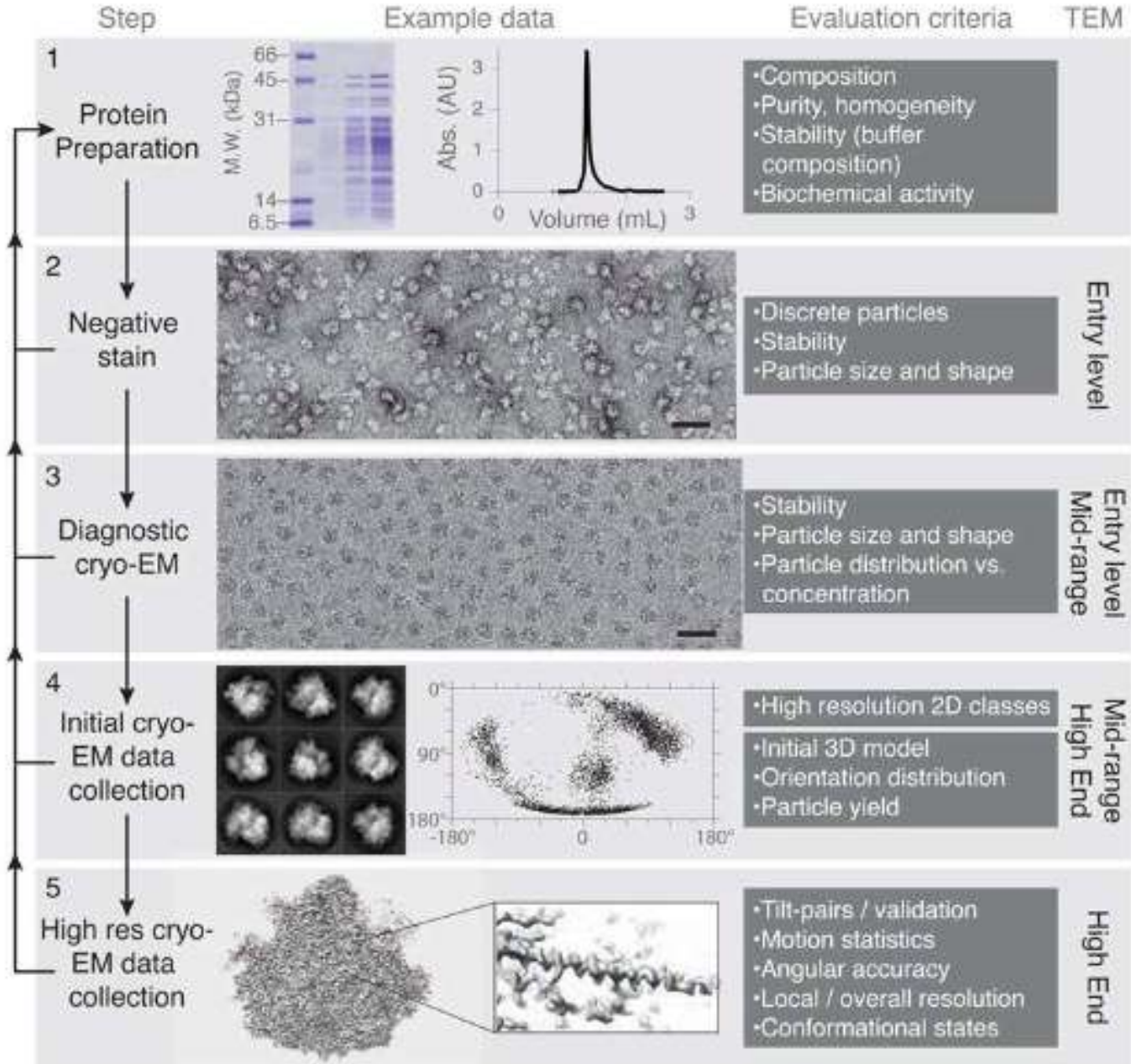


- Schmidli, Claudio & Rima, Luca & Arnold, Stefan & Stohler, Thomas & Syntychaki, Anastasia & Bieri, Andrej & Albiez, Stefan & Goldie, Kenneth & Chami, Mohamed & Stahlberg, Henning & Braun, Thomas. (2018). Miniaturized Sample Preparation for Transmission Electron Microscopy. Journal of Visualized Experiments.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

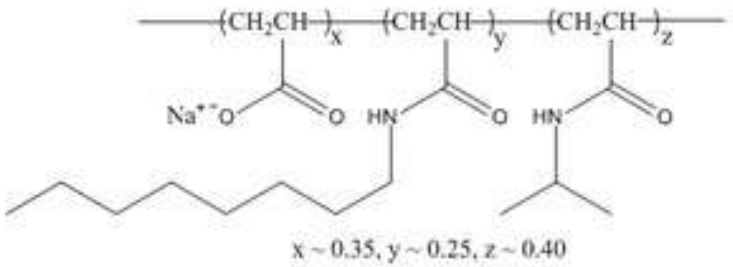
THE OPTIMIZATION WORKFLOW

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/

Reagents for improving vitrification of Cryo-EM grids used in single particle analysis.



Molecular Formula:
(C6.2H10.3O1.35N0.65Na0.35)35

Molecular Weight:
approx. 8 kDa

CAS#: 1423685-21-5

- Amphipol A8-35
- A short amphipathic polymer that is specifically designed for membrane protein stabilization. The surfactant possesses a very high affinity for the transmembrane surfaces and allows to solubilize membrane proteins in a detergent-free aqueous solution

Reagents for improving vitrification of Cryo-EM grids used in single particle analysis.

Surfactants and Cryoprotectants	Amount	Conc.	CMC	Class
Fluorinated Octyl Maltoside (FOM)	100 µl	0.41% (w/v)	0.07% (w/v)	non-ionic detergent
Hexadecyl-trimethyl-ammonium Bromide (CTAB)	100 µl	0.34% (w/v)	0.03% (w/v)	cationic detergent
n-Decyl-ß-D-Maltoside (DM)	100 µl	0.87% (w/v)	0.09% (w/v)	non-ionic detergent
n-Decyl-α-D-Maltoside (DαM)	100 µl	0.46% (w/v)	0.08% (w/v)	non-ionic detergent
n-Dodecyl-ß-D-Maltoside (DDM)	100 µl	0.09% (w/v)	0.01% (w/v)	non-ionic detergent
Sodium Deoxycholate	100 µl	1.66% (w/v)	0.17% (w/v)	anionic detergent
Triton X-100	100 µl	0.15% (w/v)	0.01% (w/v)	non-ionic detergent
Tween 20	100 µl	1% (w/v)	0.01% (w/v)	non-ionic detergent
CHAPSO	100 µl	2.5% (w/v)	0.5% (w/v)	zwitterionic detergent
Amphipol A8-35	100 µl	5% (w/v)		anionic surfactant
Glycerol	1 ml	30% (w/v)		cryoprotectant

https://www.mitegen.com/product/cryo-em-vitrification-starter-kit/

- [1] Noble *et al.* (2018) Routine Single Particle CryoEM Sample and Grid Characterization by Tomography. DOI: 10.7554/eLife.34257.
[2] Thonghin *et al.* (2018) Cryo-electron microscopy of membrane proteins. *Methods* **147**:176.
[3] Drulyte *et al.* (2018) Approaches to altering particle distributions in cryo-electron microscopy sample preparation. *Acta Cryst. D* **74**:560.
[4] Glaeser *et al.* (2017) Opinion: hazards faced by macromolecules when confined to thin aqueous films. *Biophys Rep* **3**:1.
[5] Gatsogiannis *et al.* (2016). Membrane insertion of a Tc toxin in near-atomic detail. *Nat. Struct. Mol. Biol.* **23**:884.
[6] Efremov *et al.* (2015) Architecture and conformational switch mechanism of the ryanodine receptor. *Nature* **517**:39.

Small protein

- VPP
- Thinner ice

Protein denaturation/Dissociation of protein complex

- Continuous carbon film
- Graphene oxide
- Cross-linking (GraFix)

Preferred orientation

- Tilt stage
- Cross-linking
- Detergent
- Glow-discharging conditions
- Support film (Graphene oxide)
- Image analysis (3D classification)

Flexibility

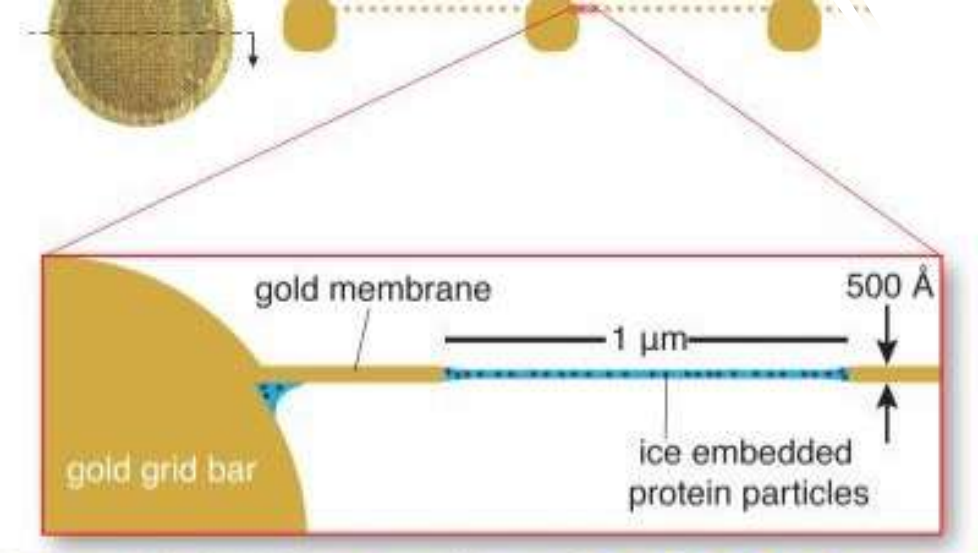
- Focused classification (subtraction)
- Multibody refinement

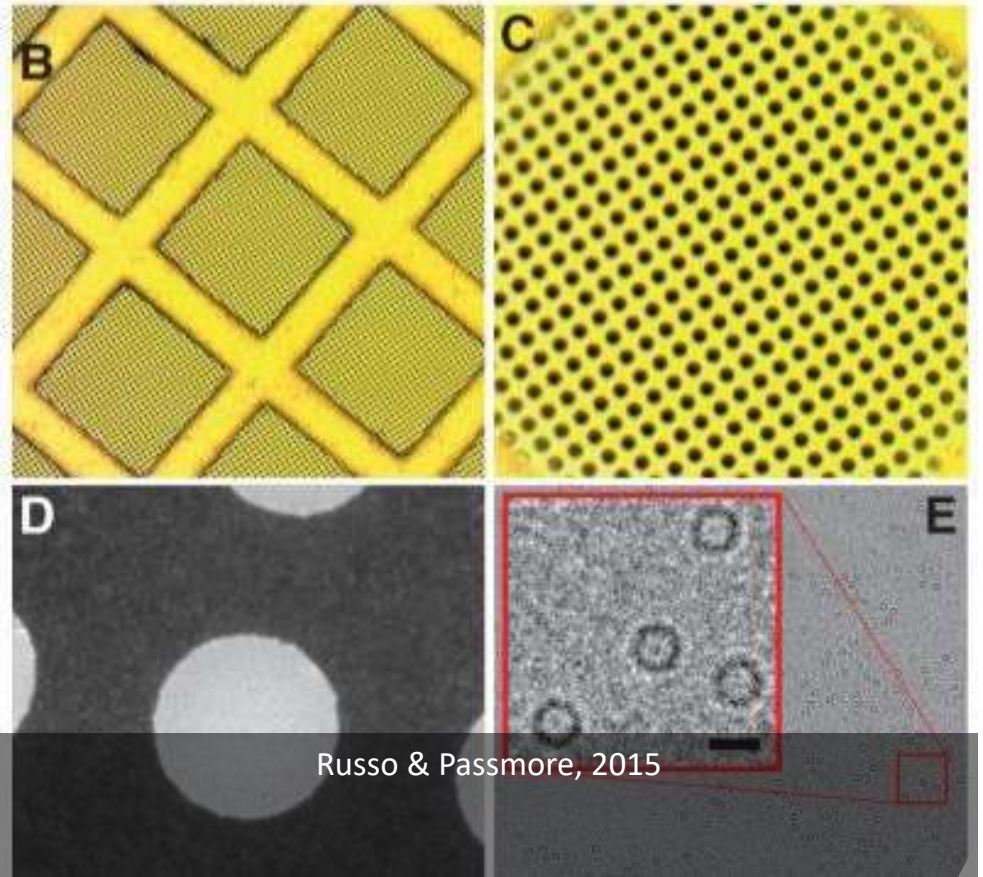
Filamentous protein

- Segmented analysis

Low concentration

- Multiple blots
- Affinity grids





Russo & Passmore, 2015

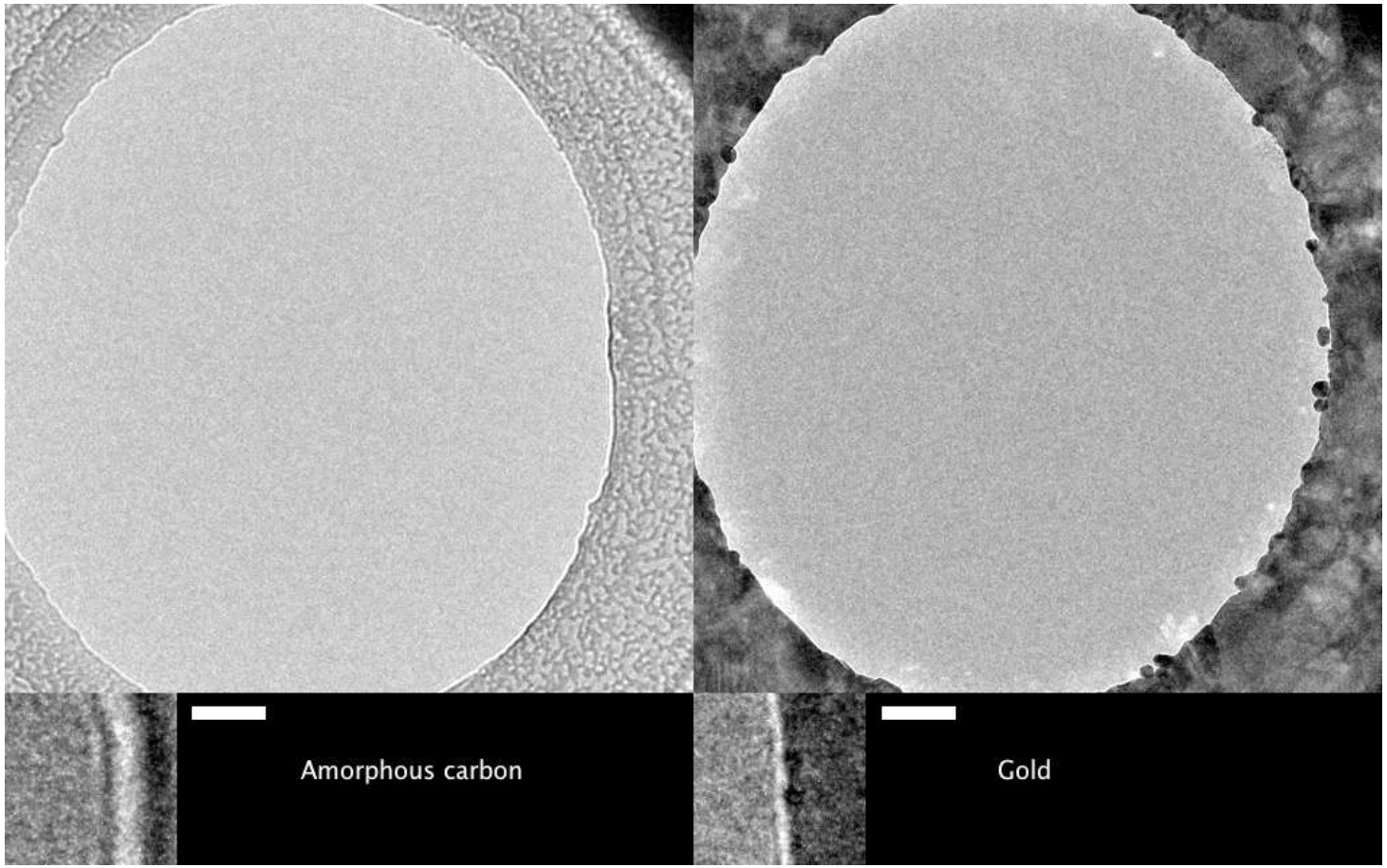
Gold grids

- 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

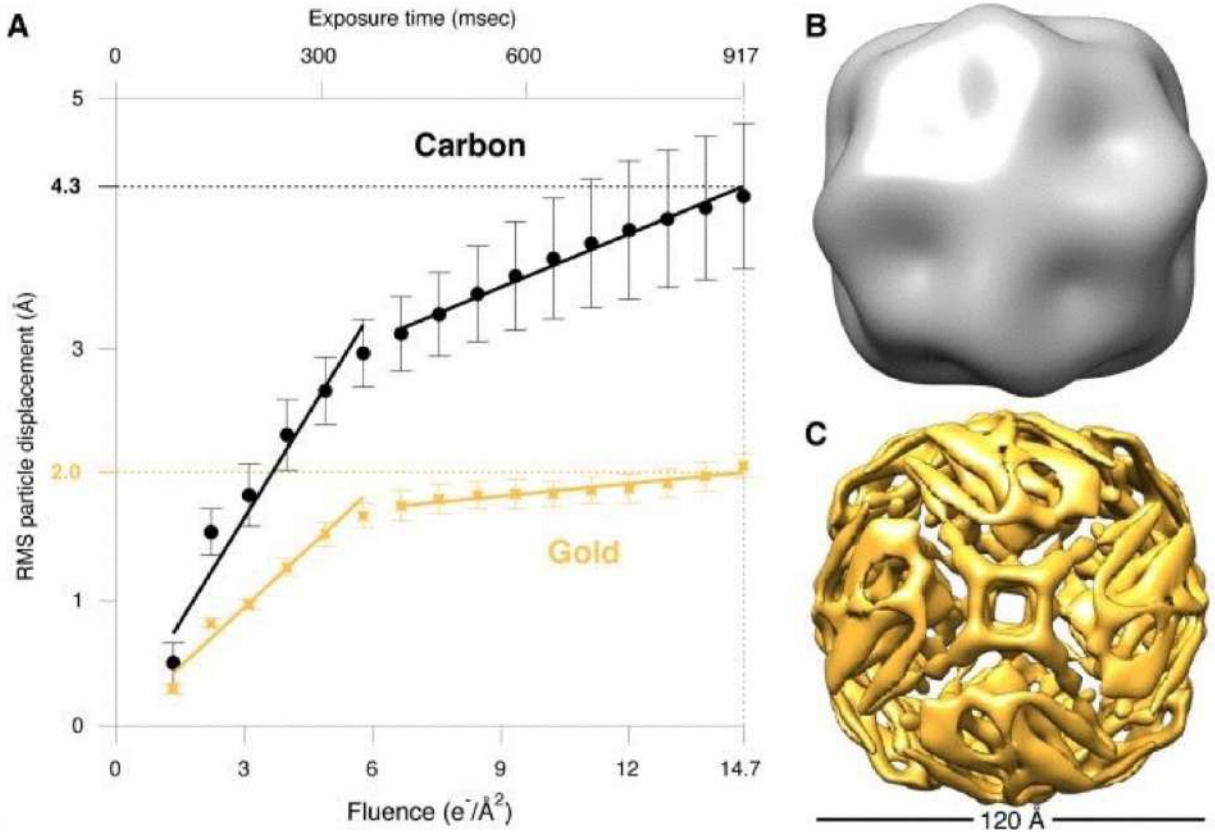
- Disadvantages:
  - Difficult to find focus due to lack of amorphous substrate

Gold grids



Russo & Passmore, 2015

Gold grids: how much better?



- **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.

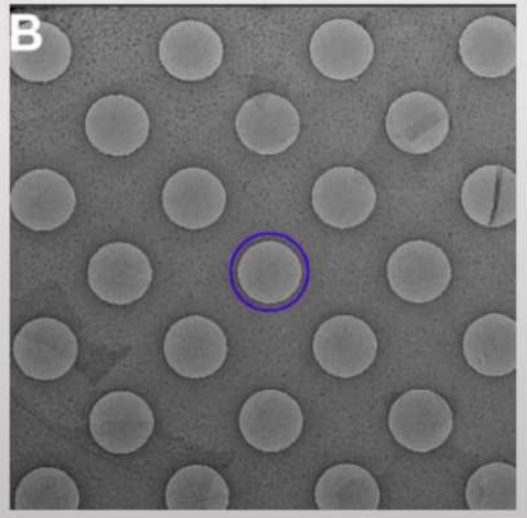
Russo & Passmore, 2015

Additional support film Topics

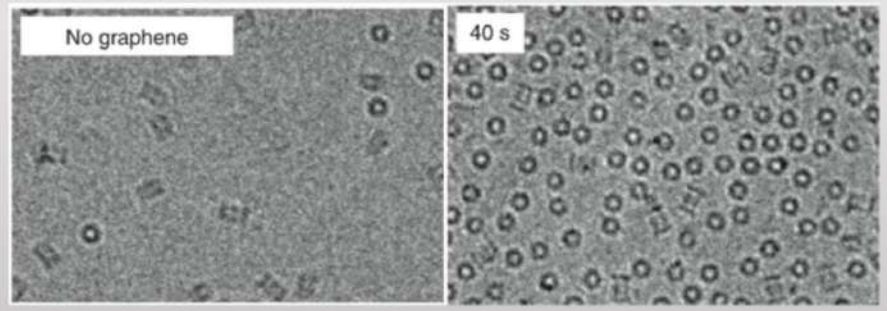
- Graphene Oxide
- Thin Continuous carbon
- Affinity grids

- Grid treatments
- Glow discharging
- Poly-lysine
- PEG
- ECM proteins

- Protect the protein from denaturation by blocking air-water interface
- Alleviate preferred orientation problem.
- Very thin (one layer of atoms).



Graphene oxide covered grids



Without Graphene With Graphene hydrogenation

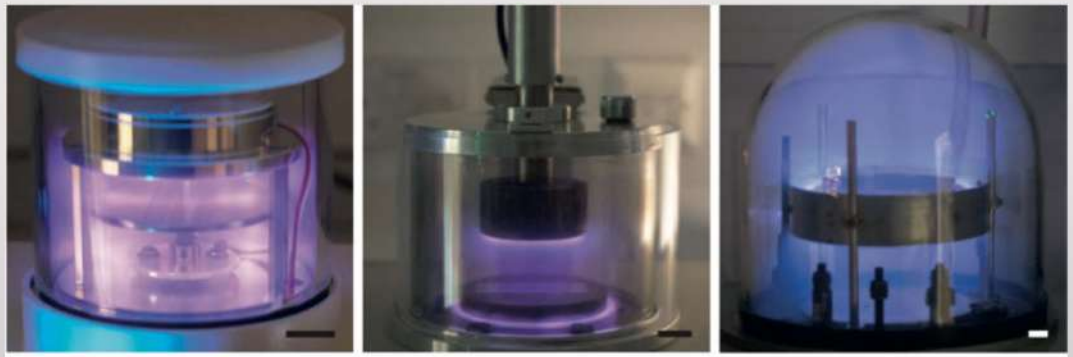
Eugene Palovcak *et al.*, 2018
Christopher J Russo *et al.*, 2014

Additional support film Topics

- Graphene Oxide
- Thin Continuous carbon
- Affinity grids

- Grid treatments
- Glow discharging
- Poly-lysine
- PEG
- ECM proteins

- Plasma is created by ionization
- Ions interact with grid surface to remove organic contamination and make the it hydrophilic



Ted Pella easyGlow (c. 2015) Edwards S150B (c. 1995) Edwards 12E6 (c. 1962)

L.A. Passmore *et al.*, 2016

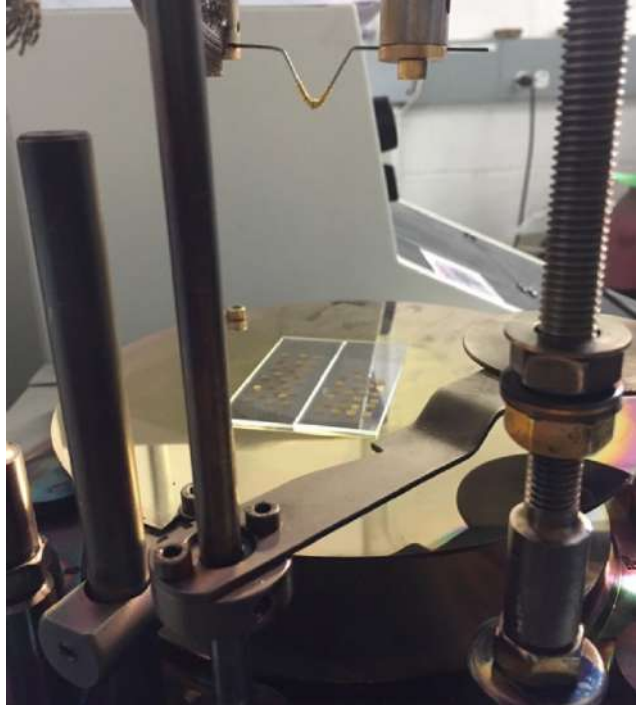

HOMEMADE
GOLD GRIDS

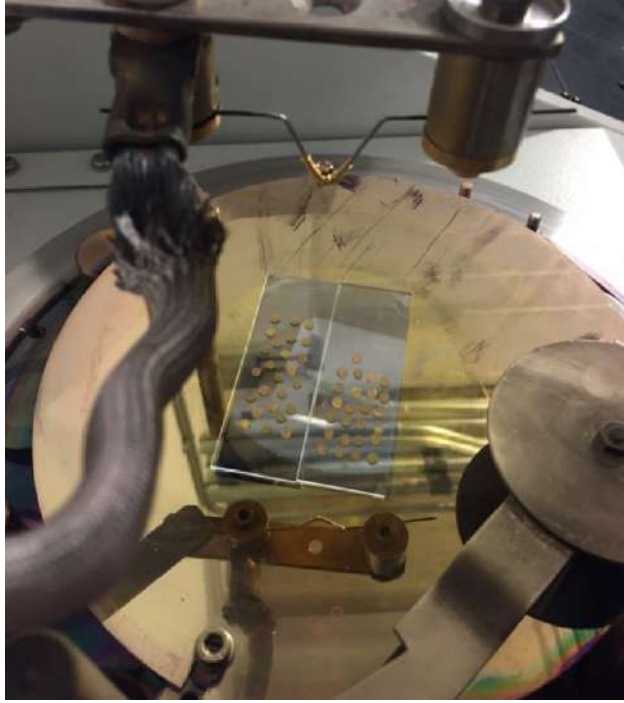
How to make your own Gold grids

1. Buy gold grids with holey carbon on them
2. Evaporate gold on the grids
3. Remove carbon

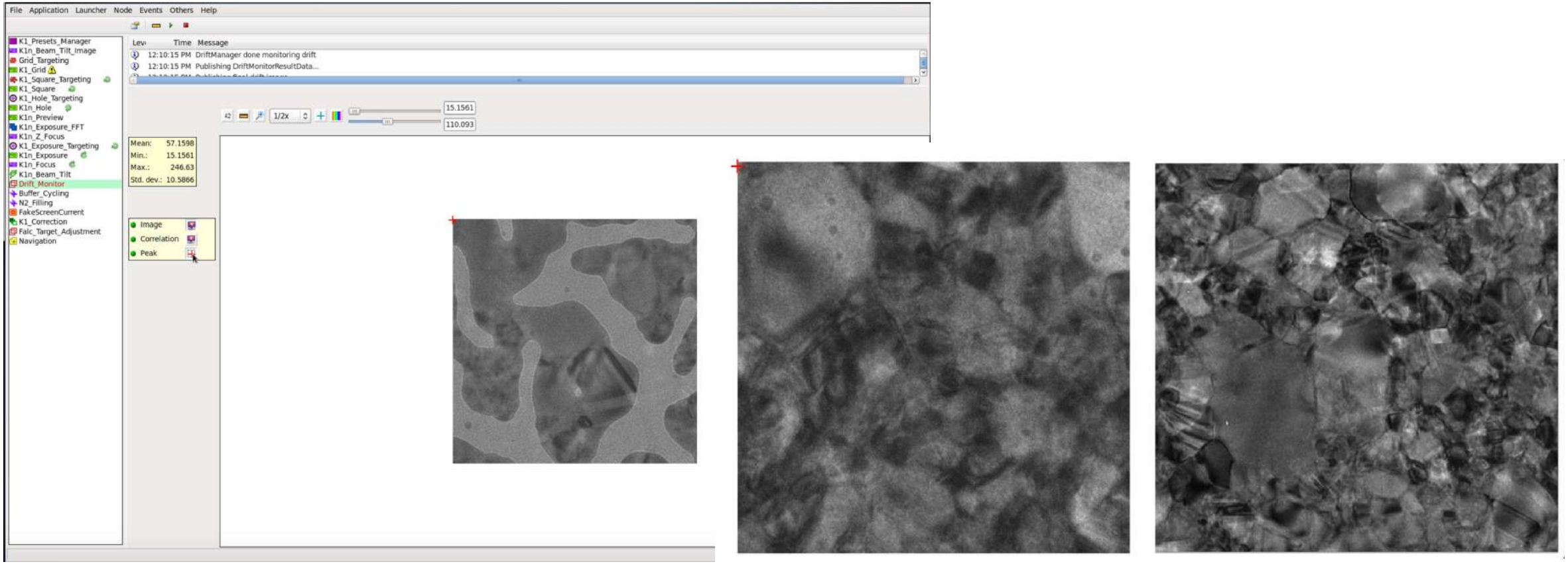
How to make your own Gold grids

Edwards Auto306

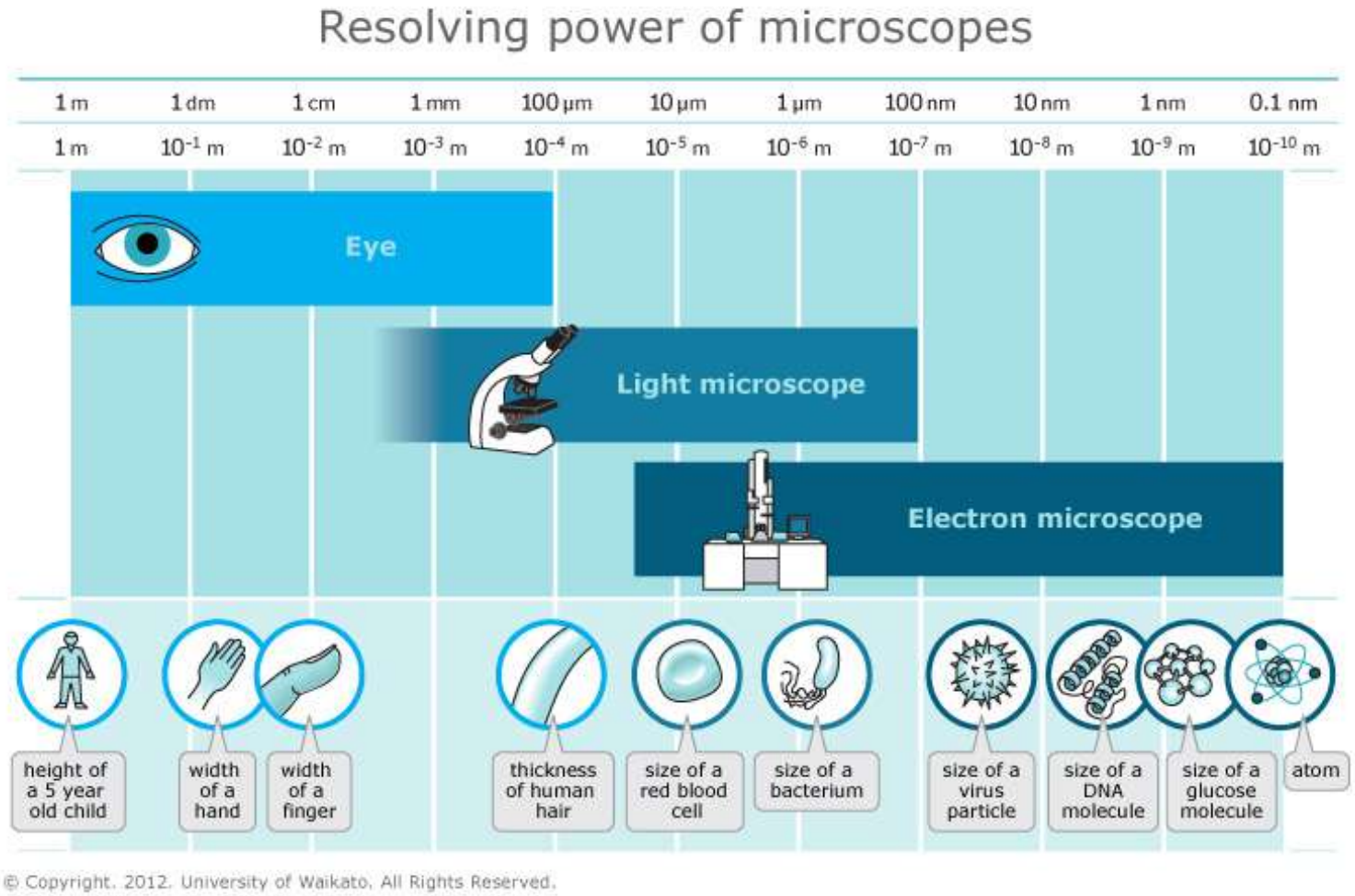




Why not just buy gold grids?



Support films and Grids



- http://www.boruhealthmachine.org/what-is-meant-by-the-resolving-power-of-a-microscope.html