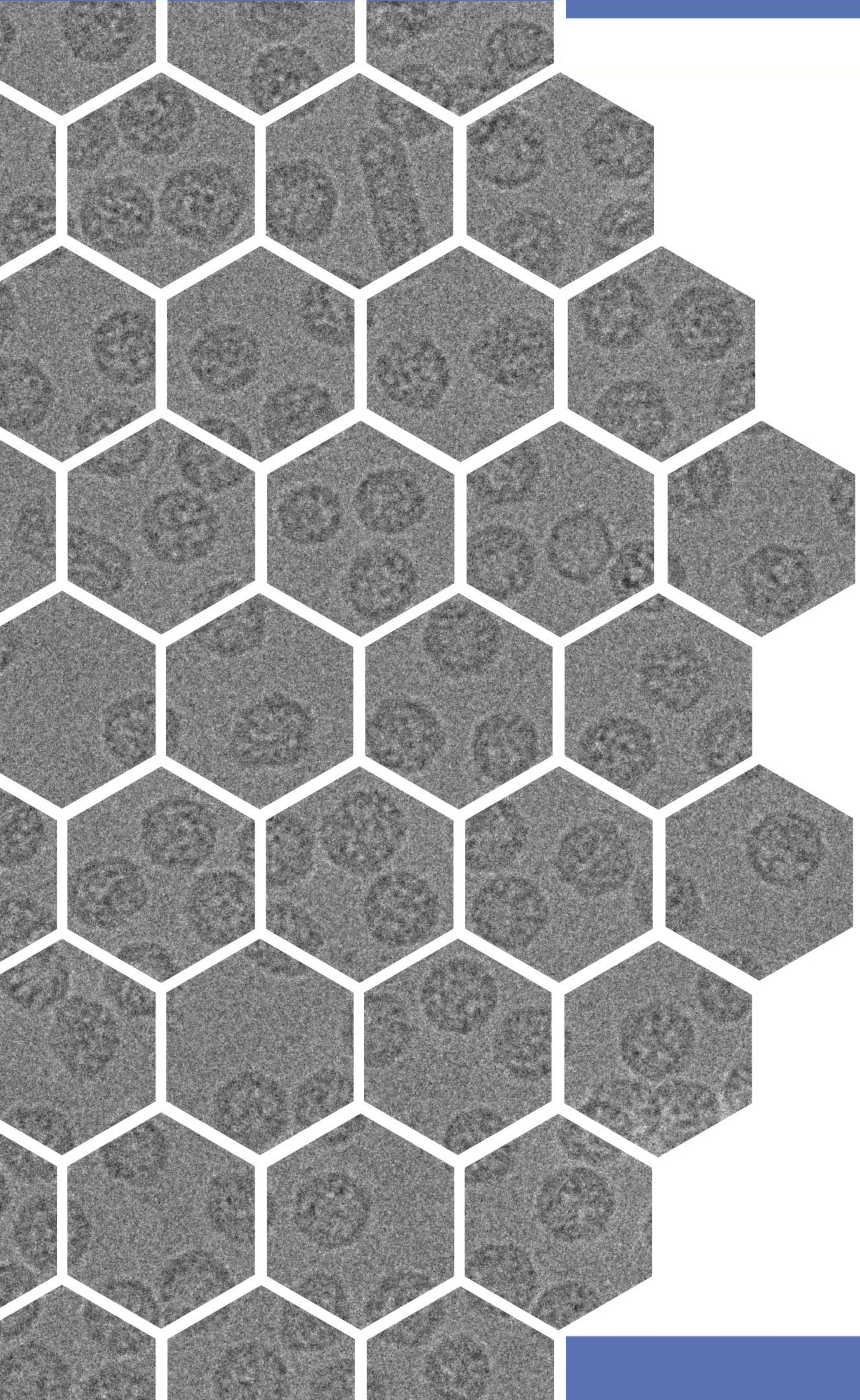
2025 Winter cryoEM course

Considerations for biological cryoEM

February 3, 2025

NYSBC SEMC



- Journal club and practical recap
- Considerations for biological cryoEM
  - Overview
  - Grids
  - What happens to a sample
  - Newer methods

Course logistics: main topics

Section 1 : EM fundamentals

Section 2 : EM crystallography

Section 3 : Single Particle Analysis

Section 4 : Tomography Short Course
March 31-April 4

Section 5 : Future perspectives

Course logistics: main topics

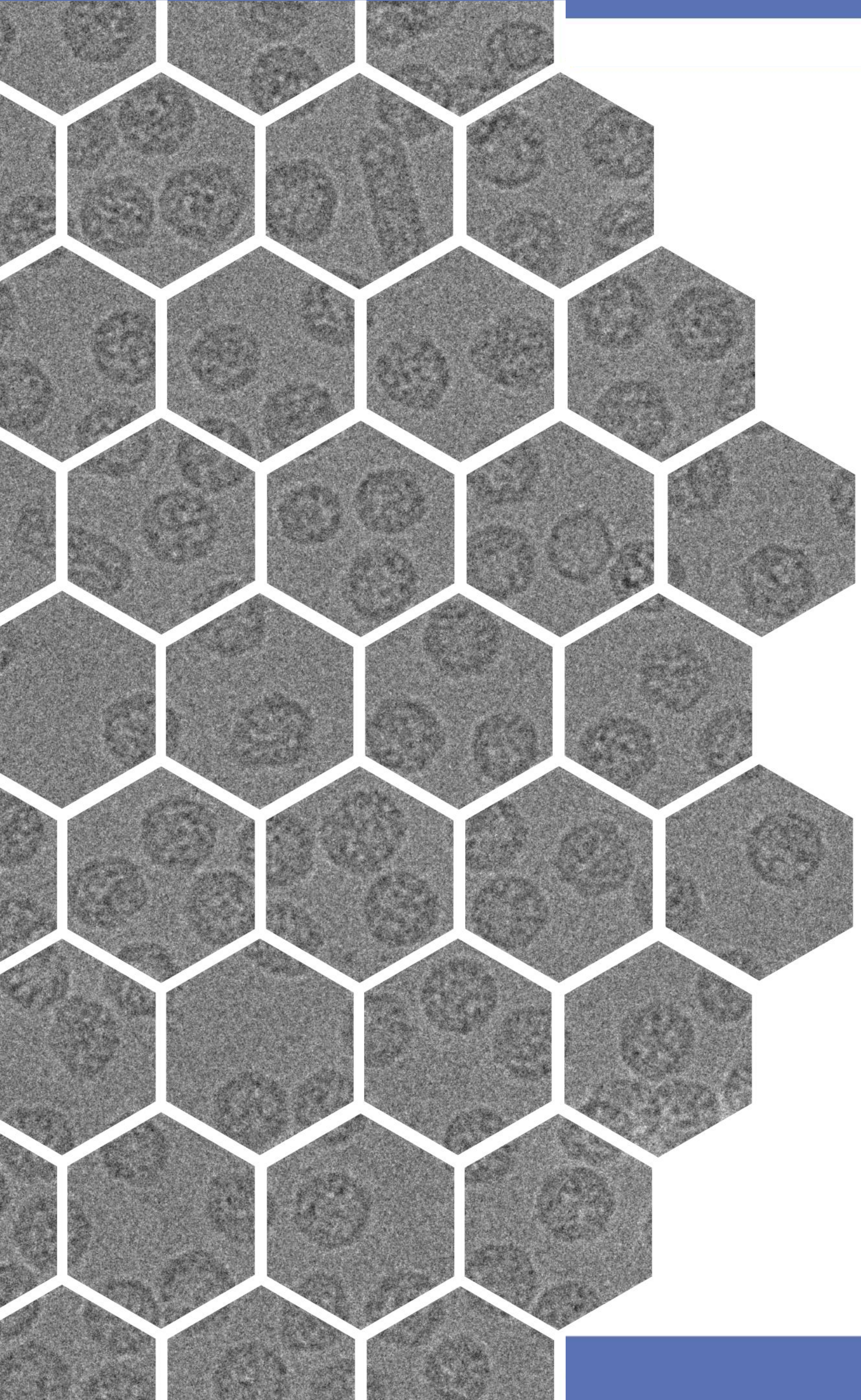
Course logistics: Wednesday practical

February 5, 2025

Sample preparation practical

-one session or

-two sessions?



- Journal club and practical recap
- Considerations for biological cryoEM
  - Overview
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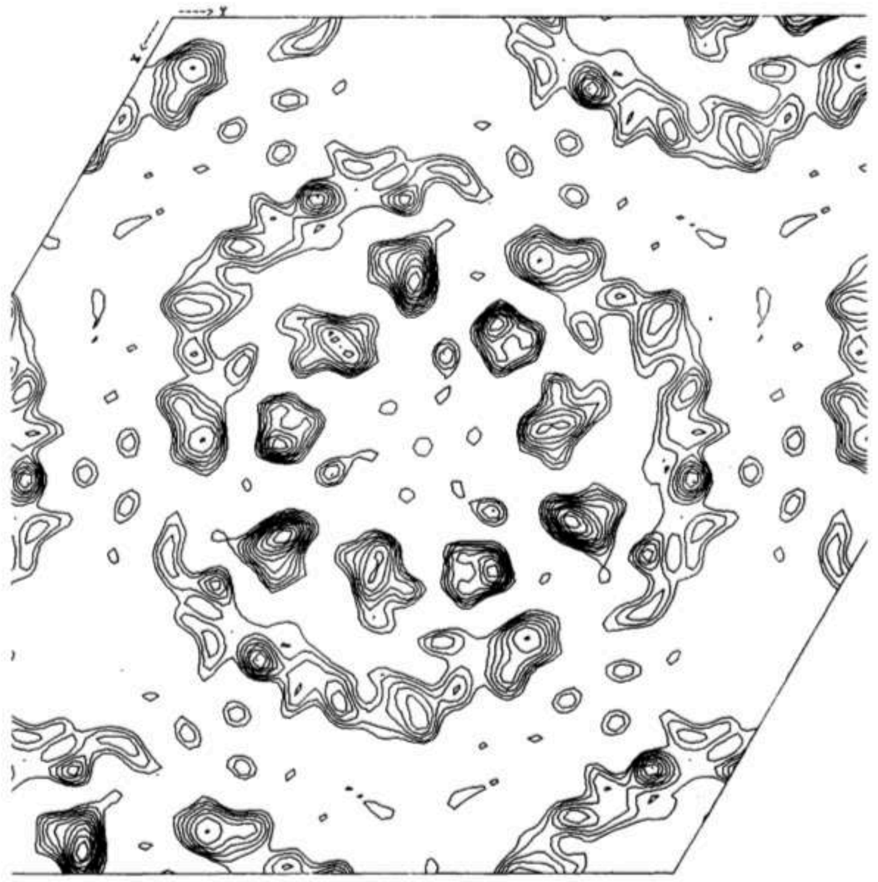
  - Newer methods

Scale of biology

doi: 10.2210/rcsb_pdb/goodsell-gallery-014

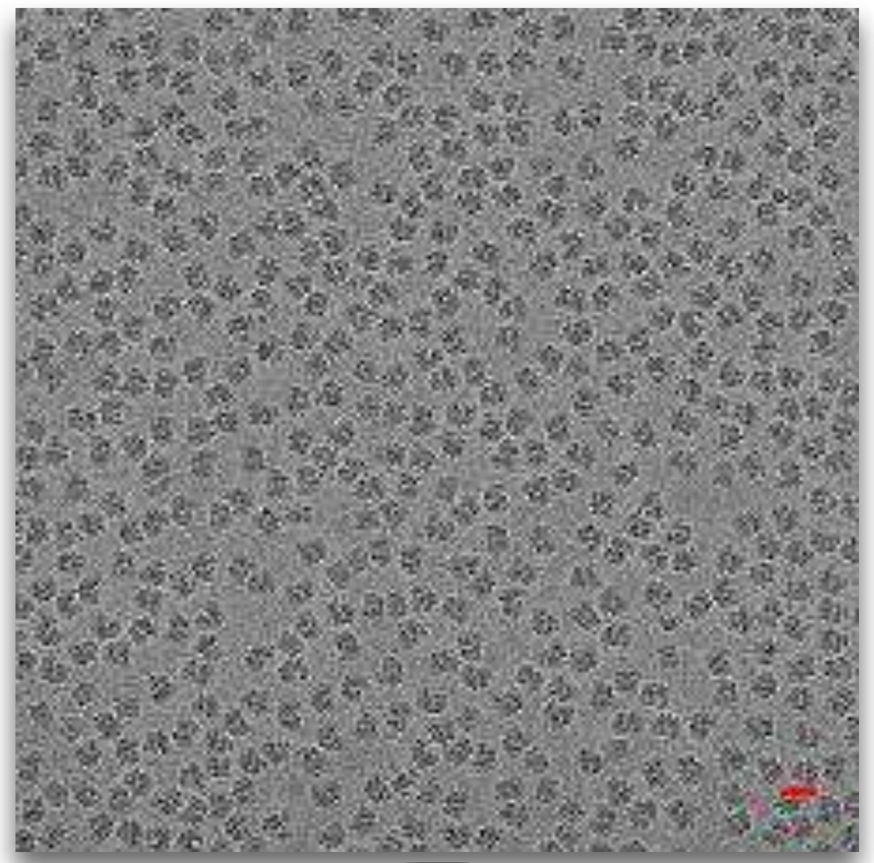
cryoEM: technology on the rise

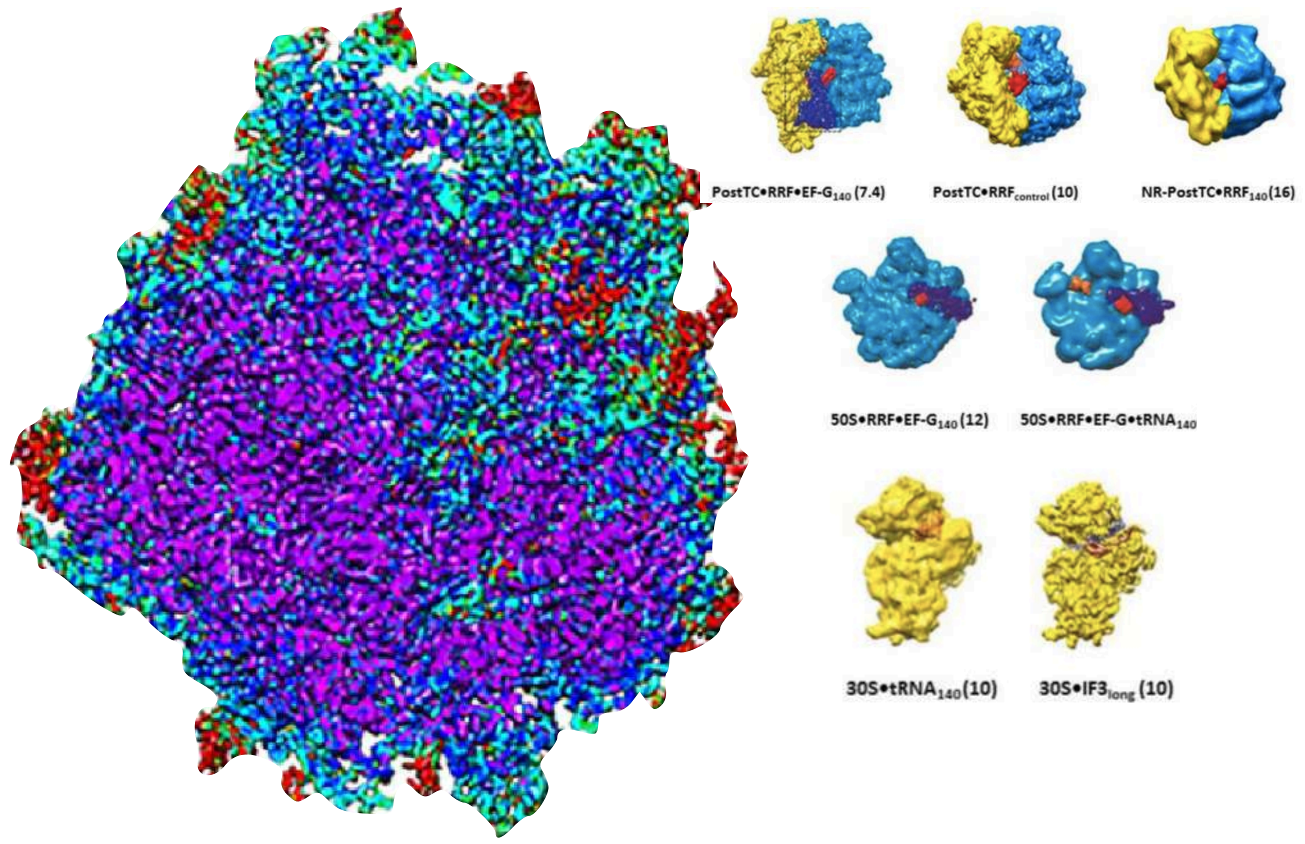
1986



Henderson et al. (1986)

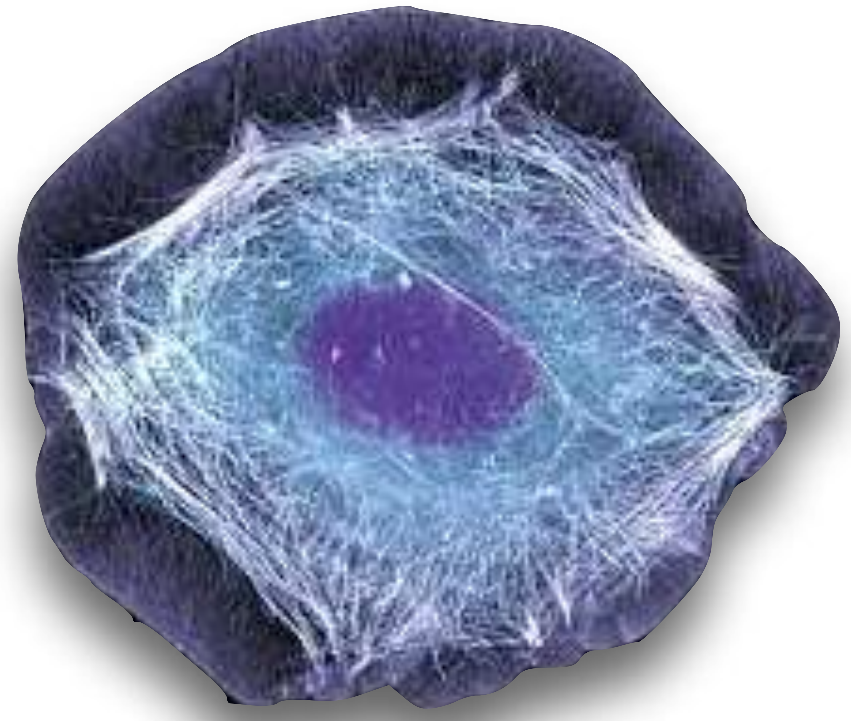
2017

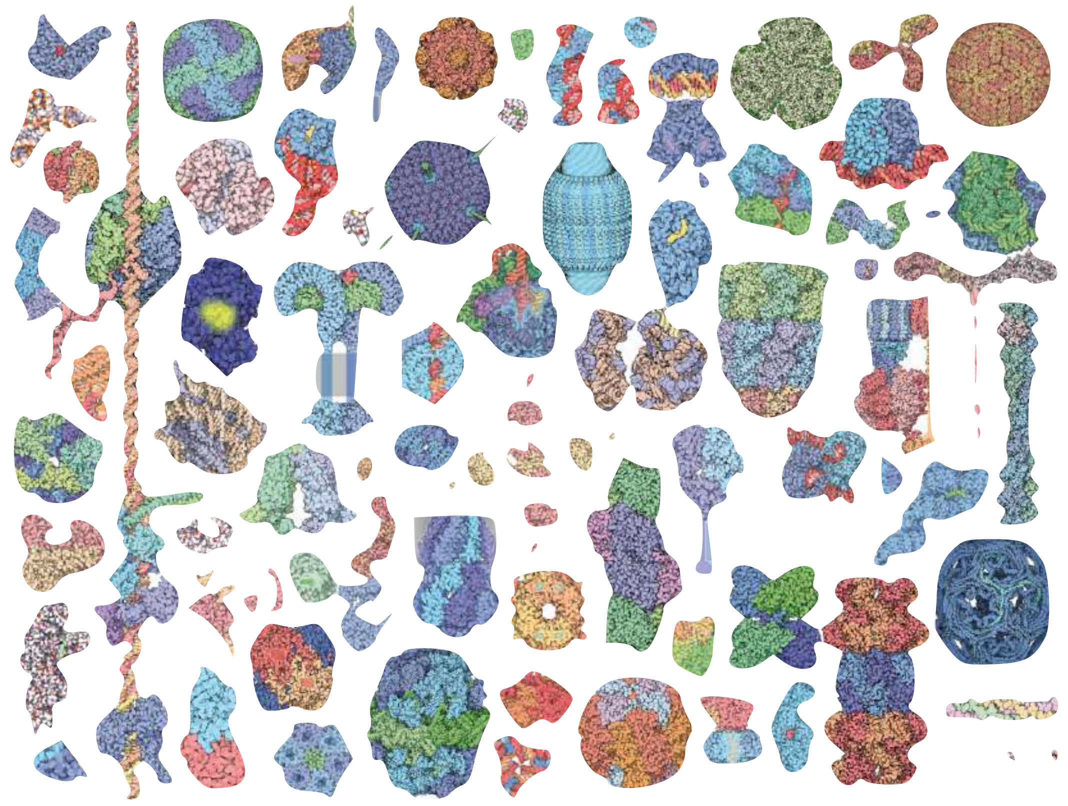




Frank et al. (2017)

in progress

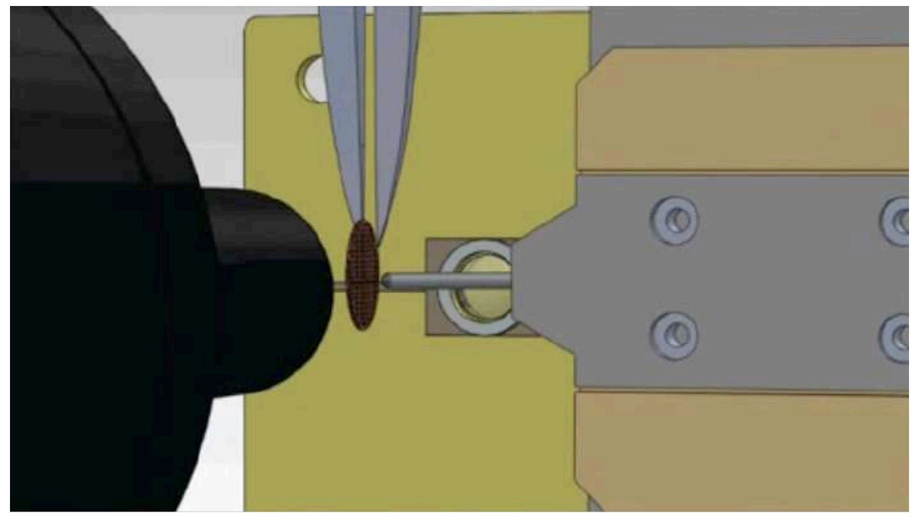
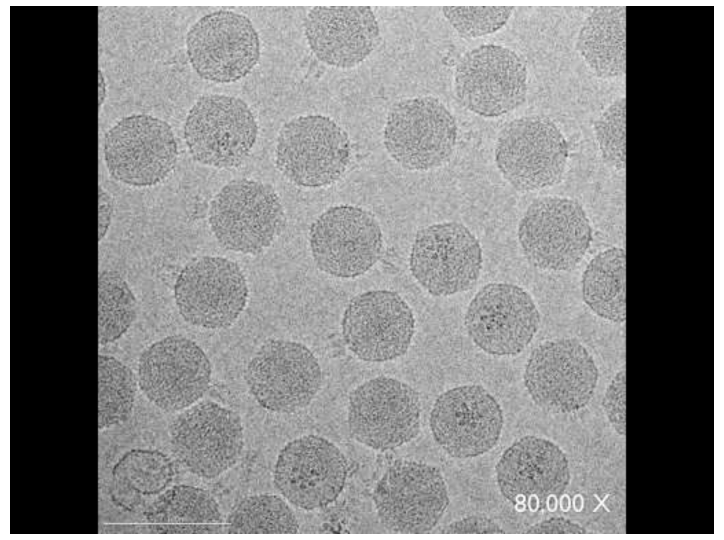


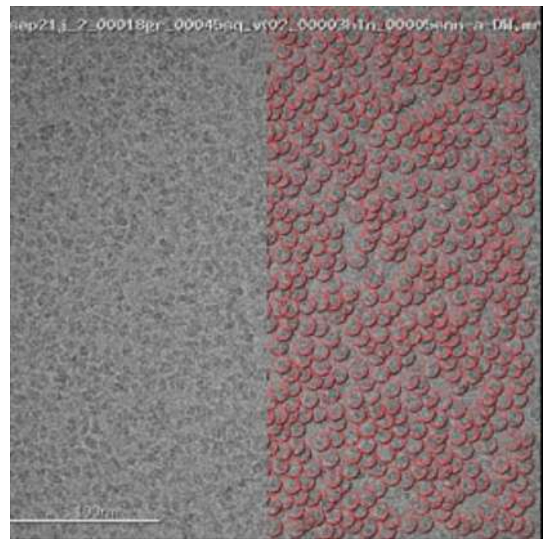
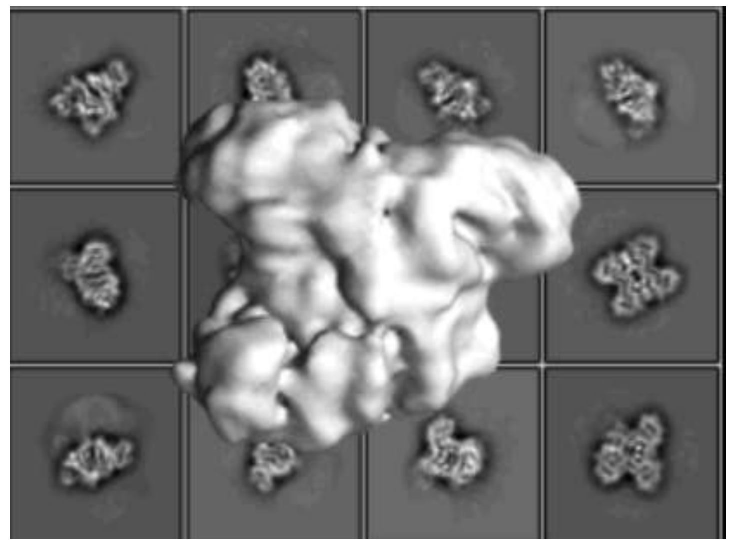
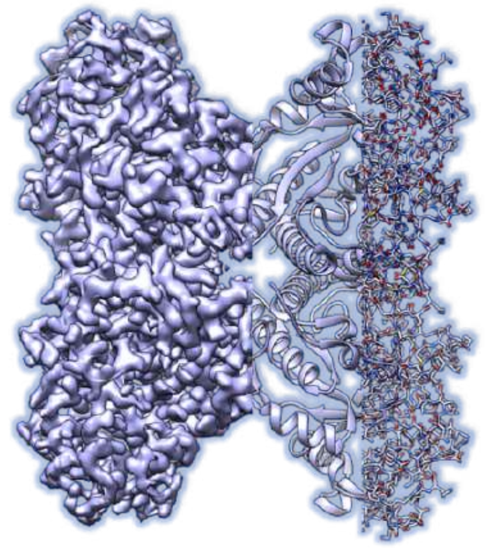


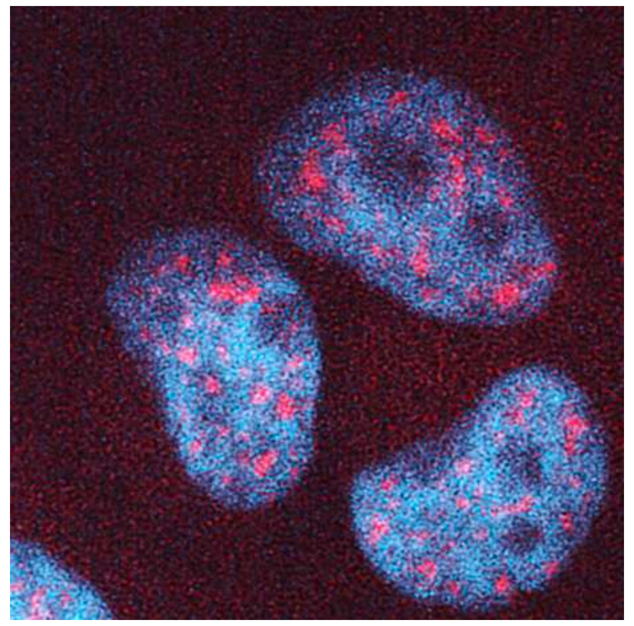
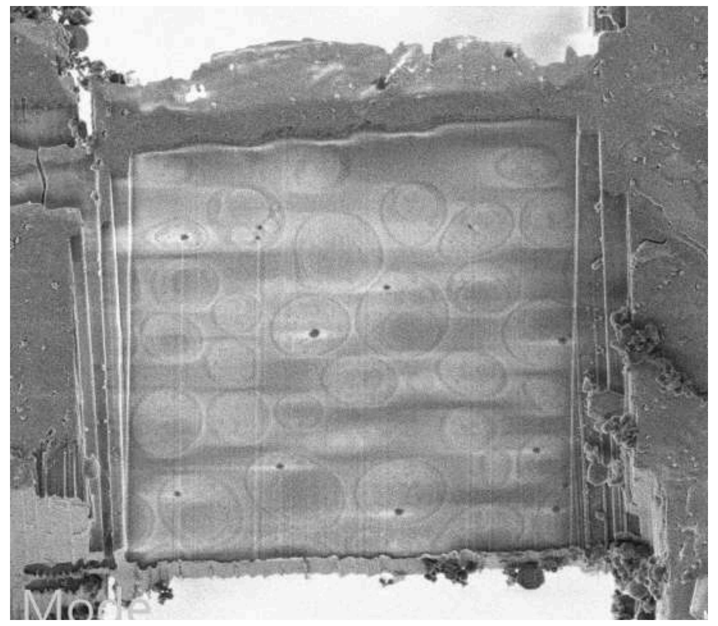
the next chapter

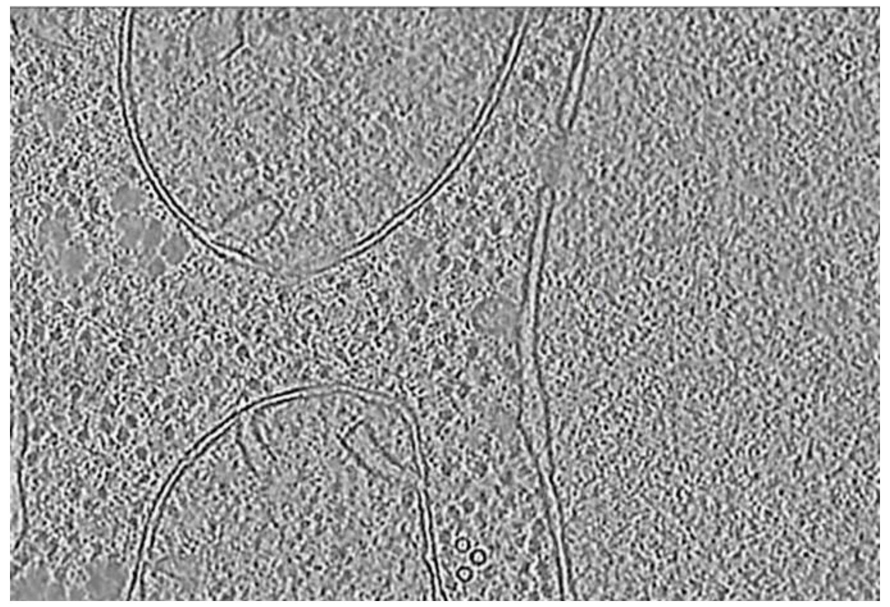
cryoEM: a technology on the rise

Single particle cryoEM

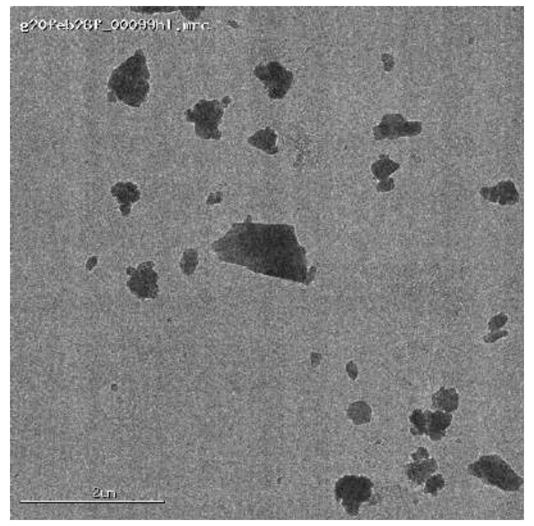
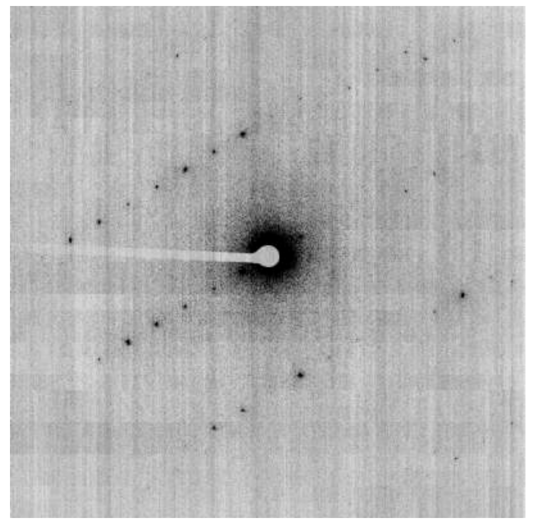
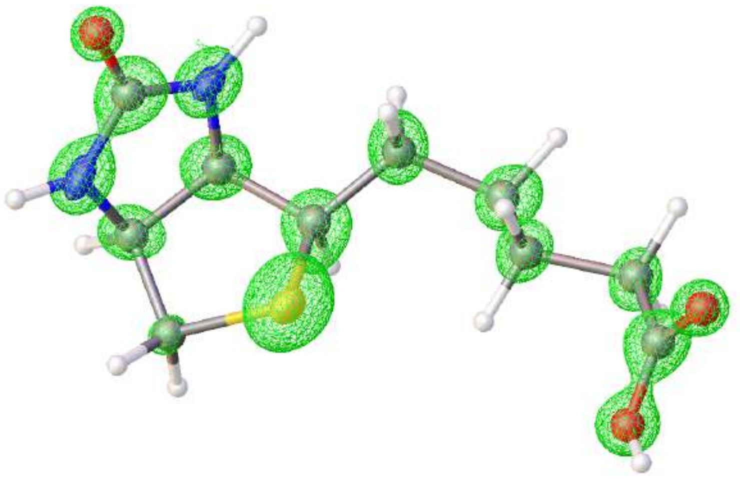
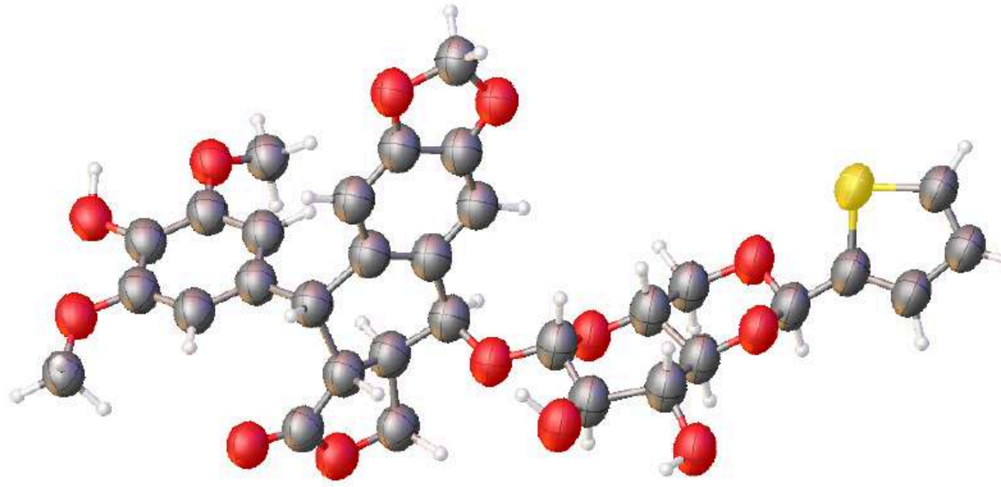




Cryo Electron Tomography (cryoET)

Micro crystal electron diffraction (microED)

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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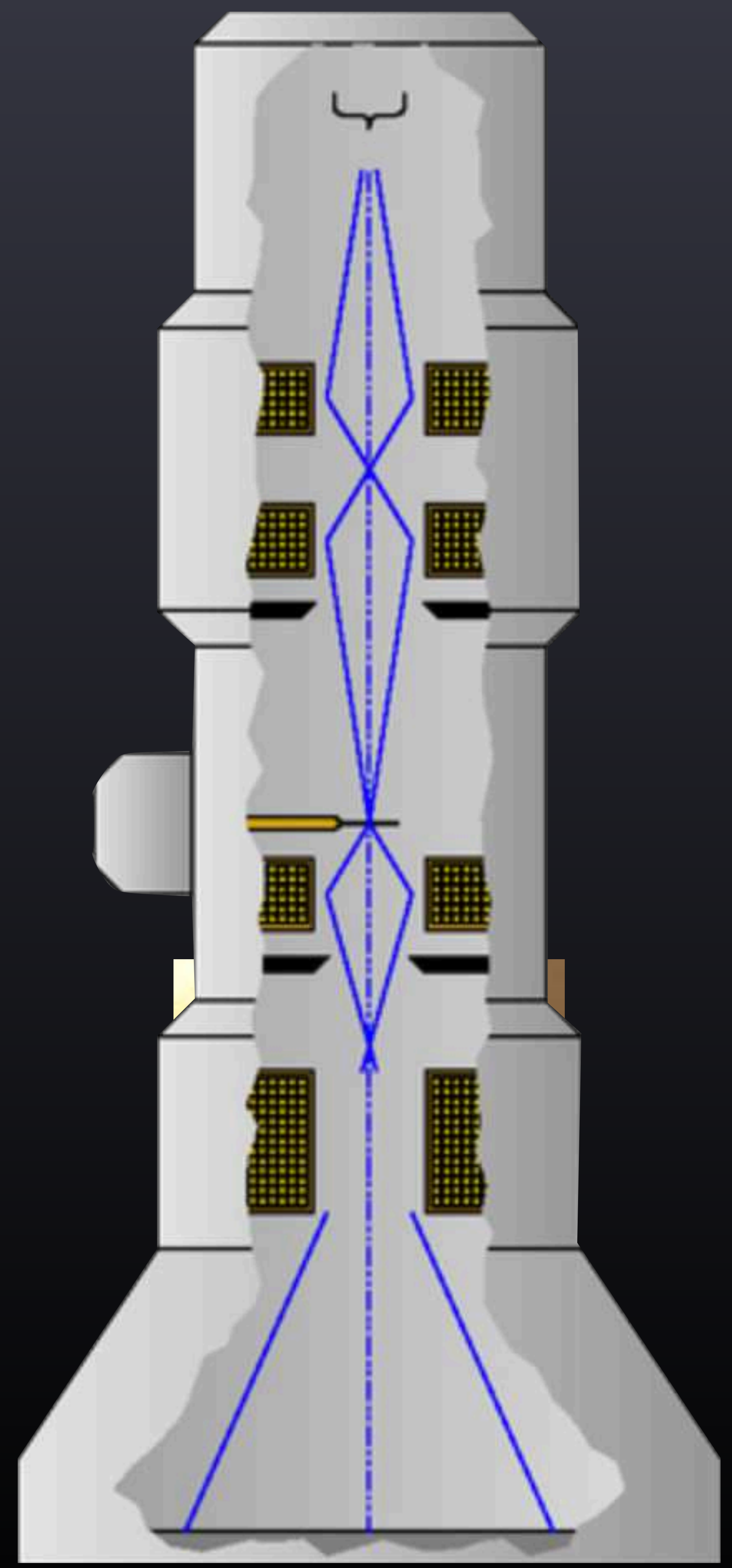
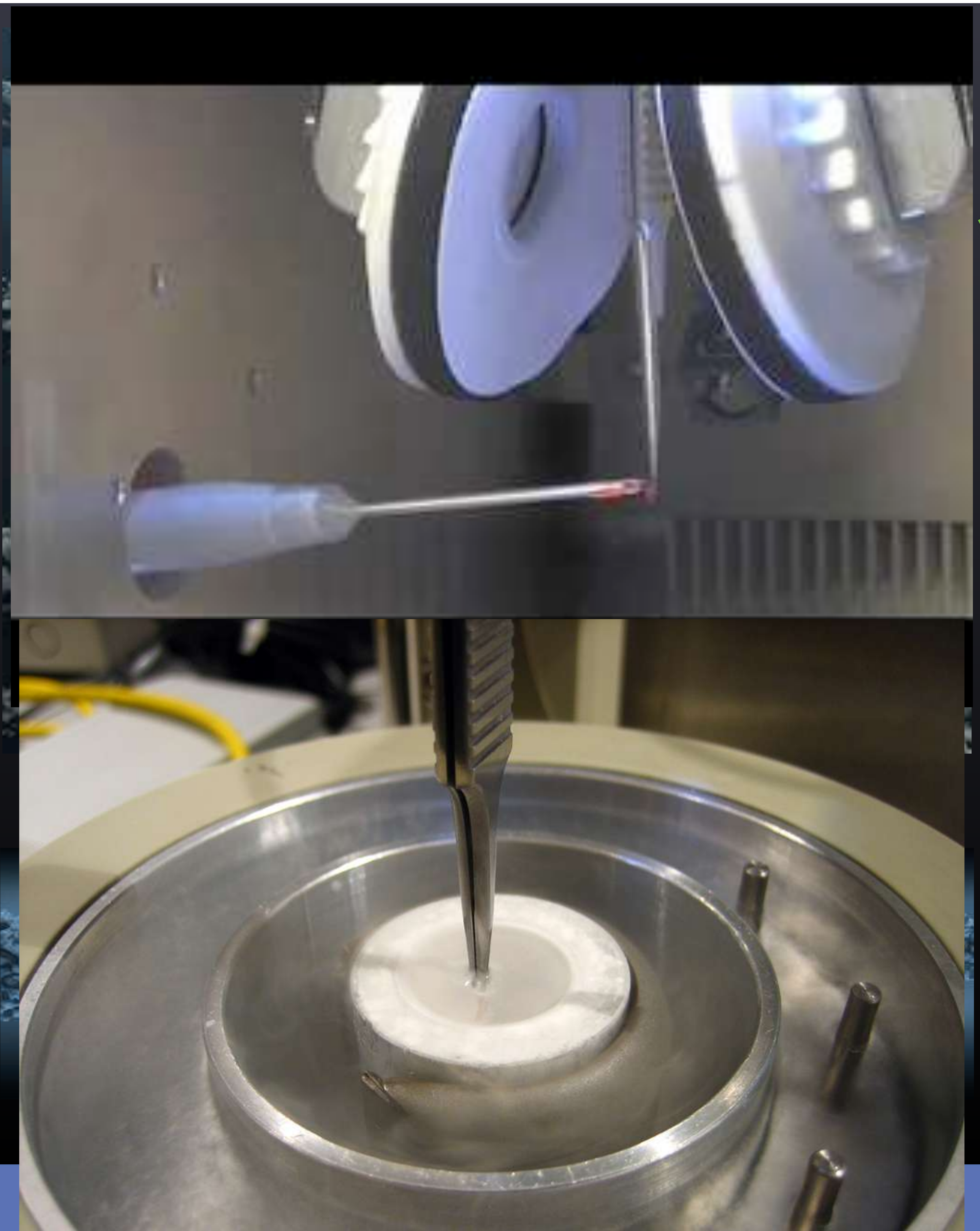
How are samples prepared for cryoEM?

Vitrifying a biological sample



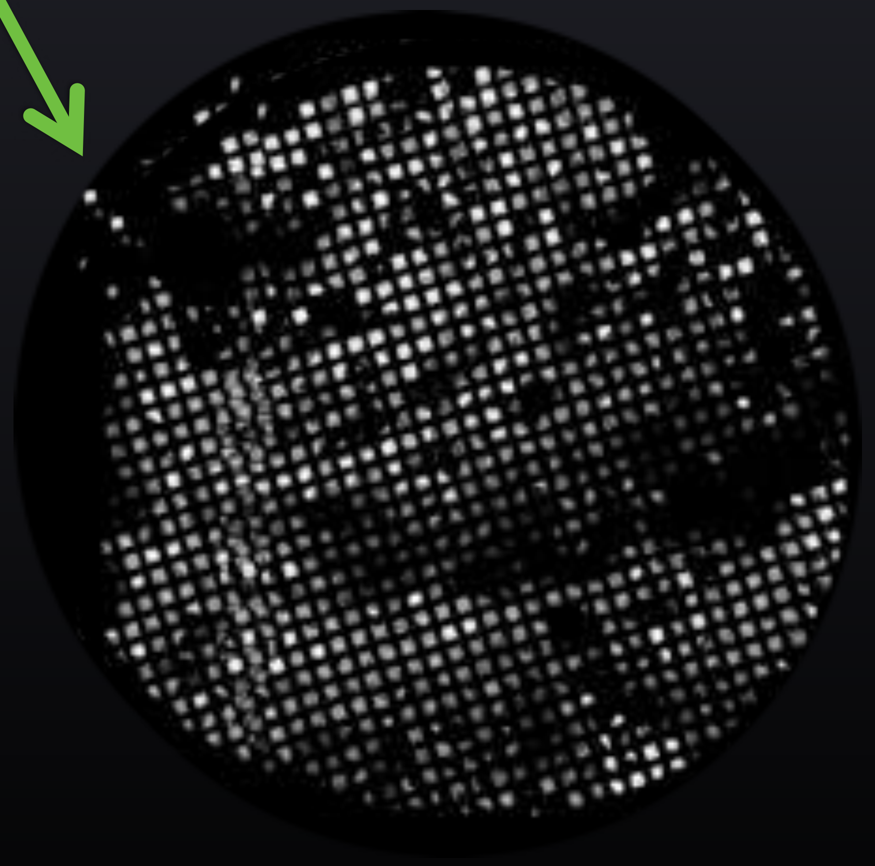


~3μl

<3nl

>99.999%

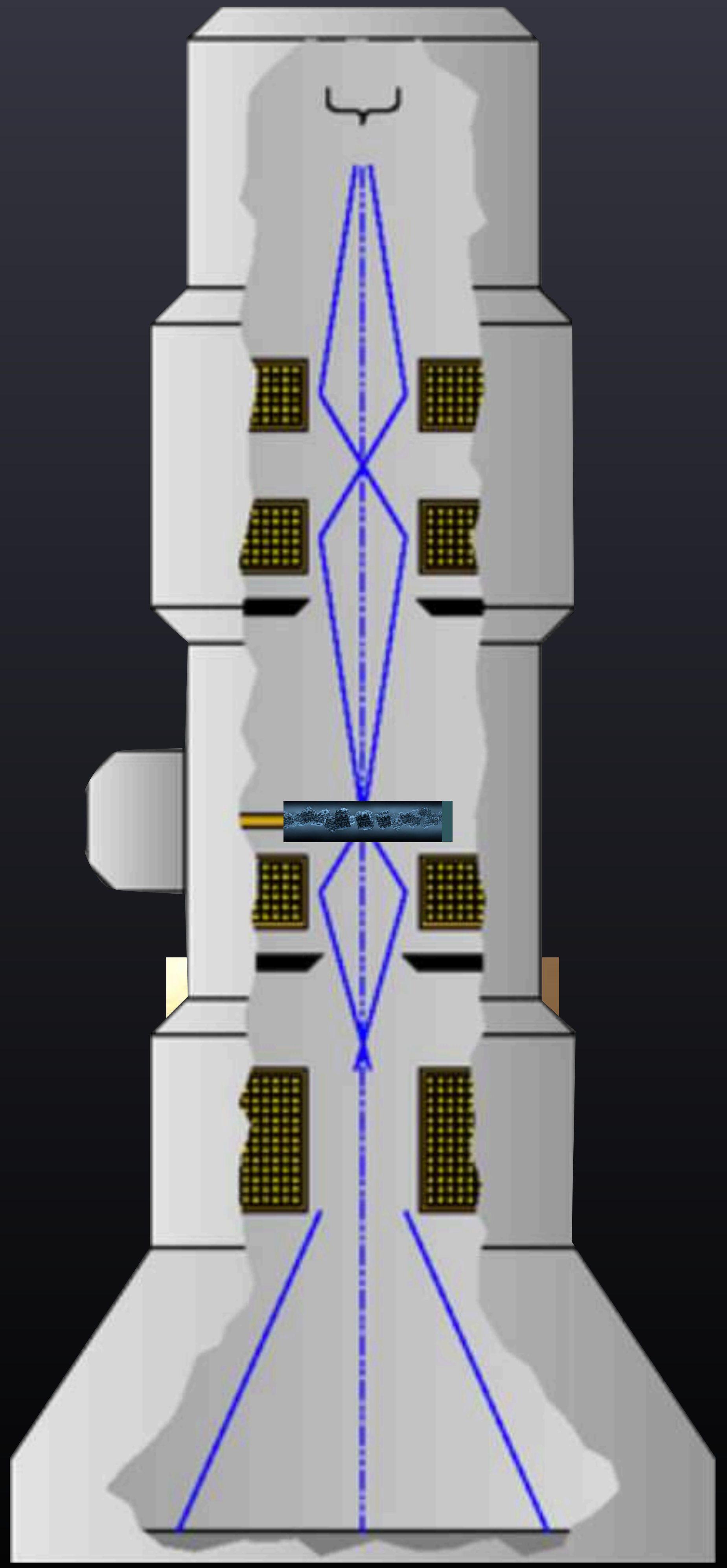
<0.001%

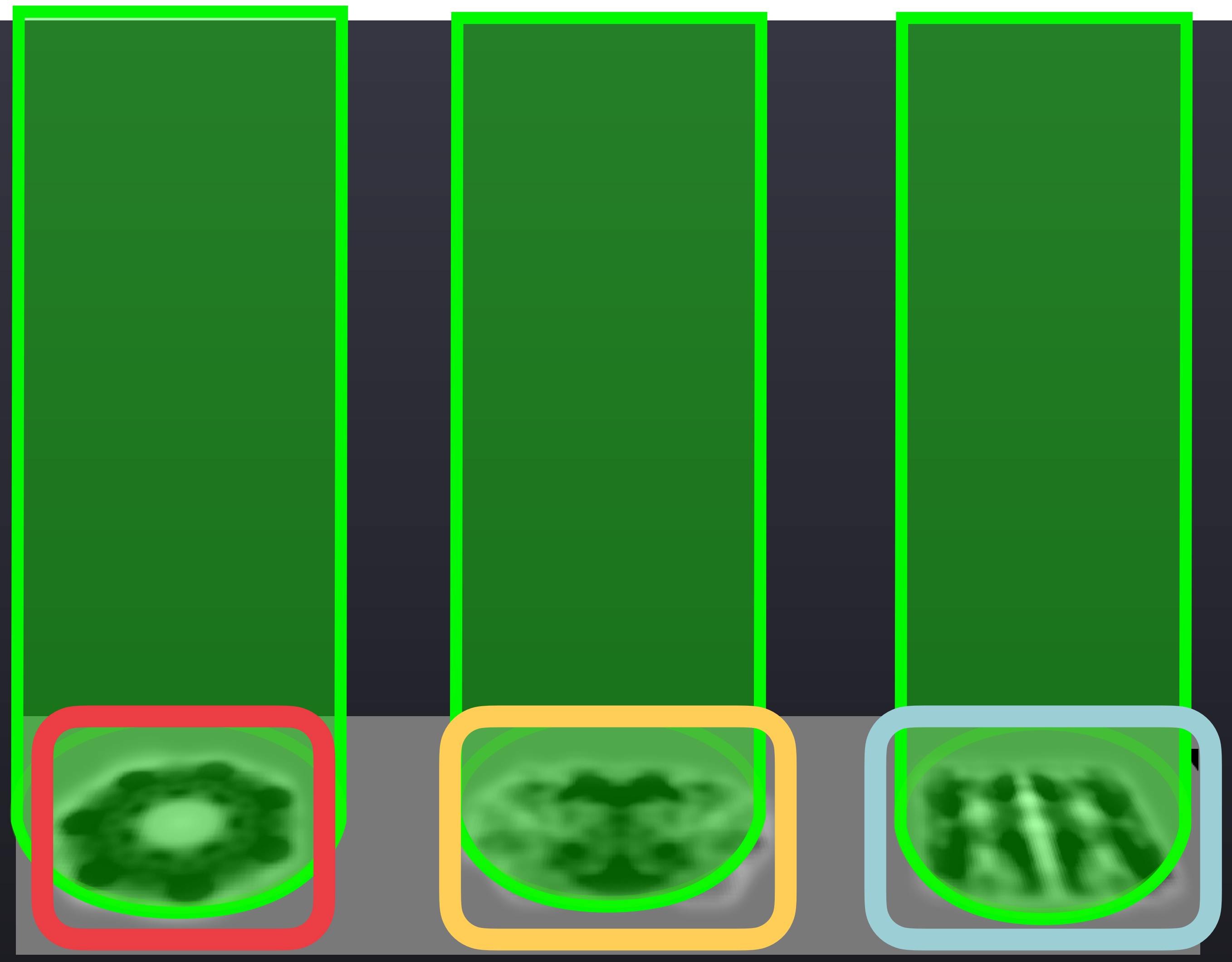


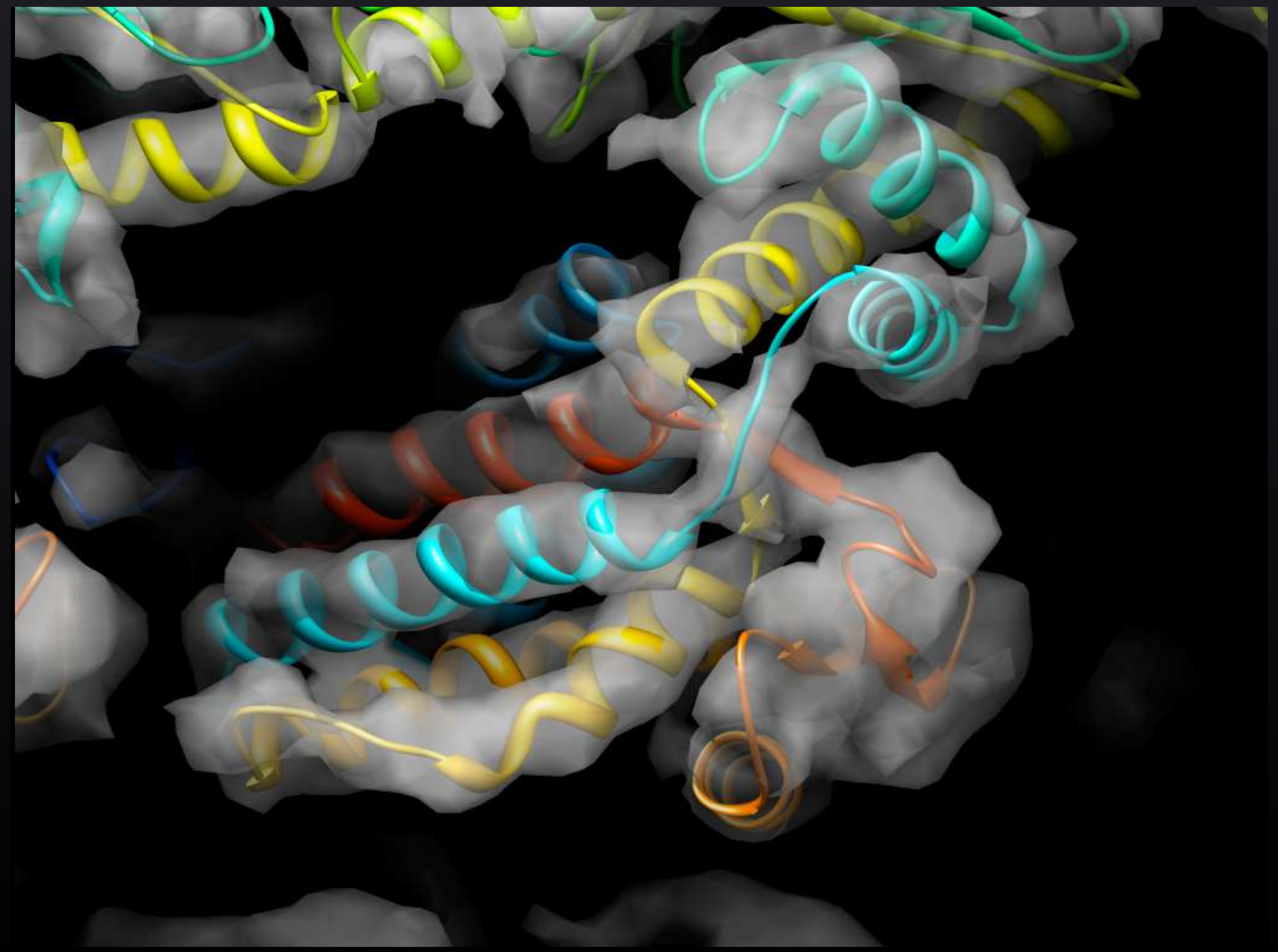
>100,000 potential imaging targets; most of them are not usable.

How are samples prepared for cryoEM?

Vitrifying a biological sample







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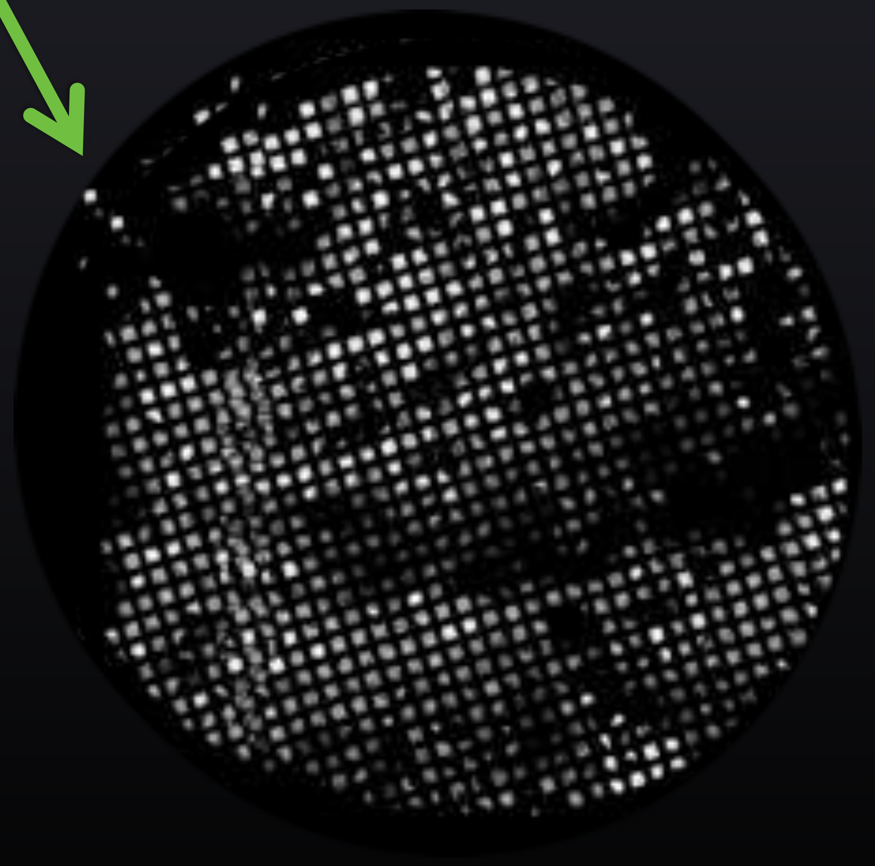
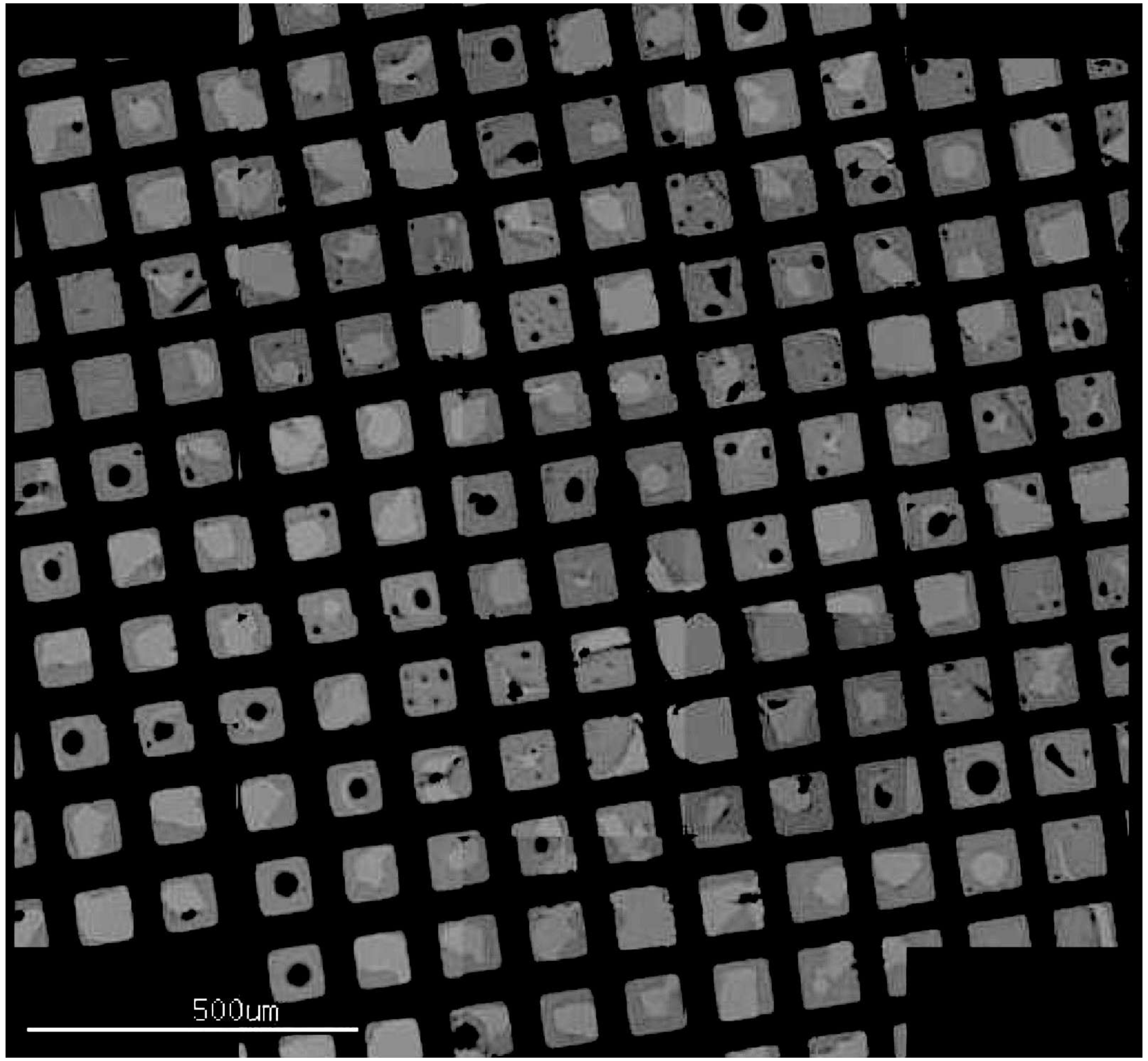
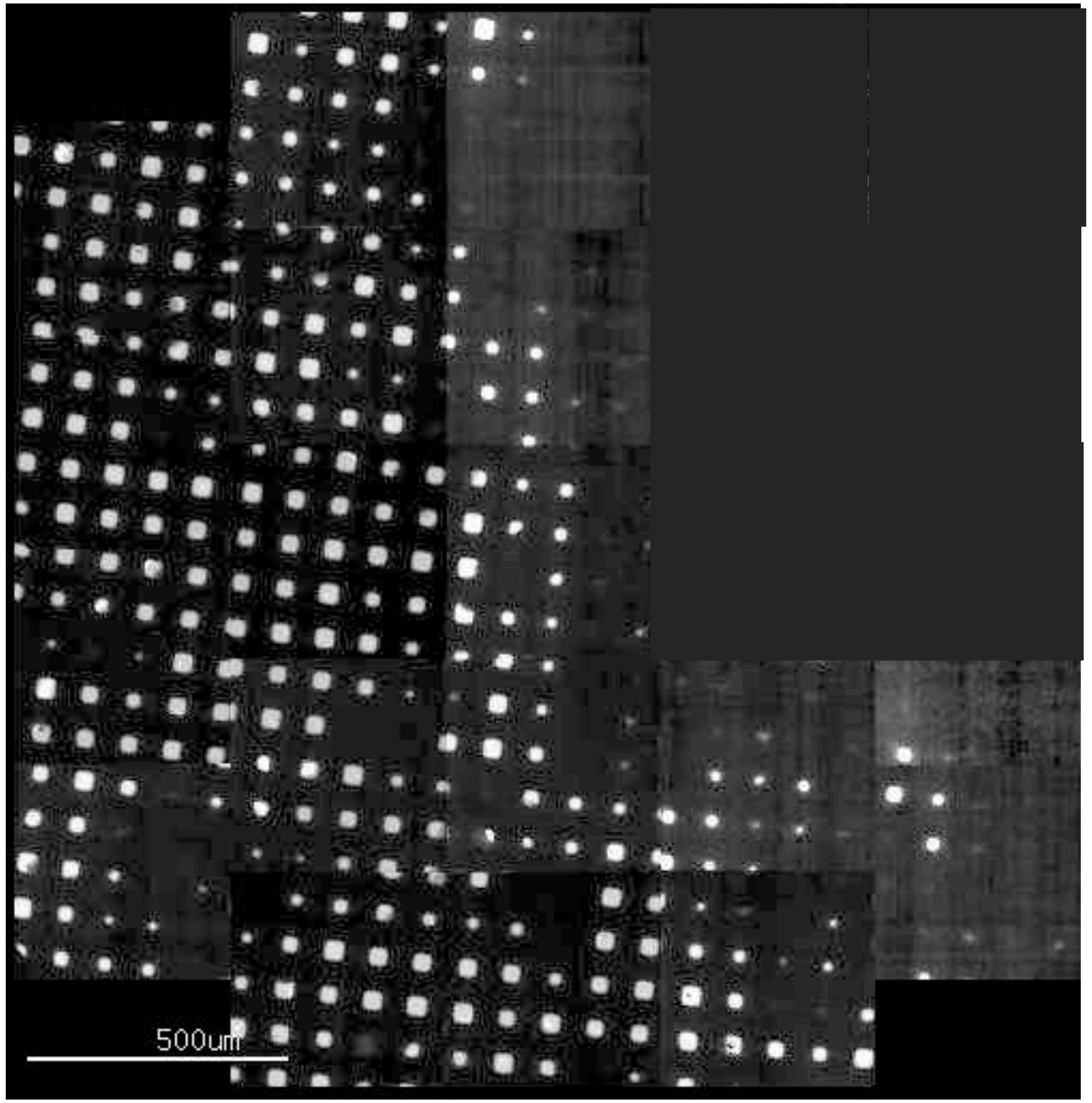


>100,000 potential imaging targets; most of them are not usable.

What do EM grids look like?





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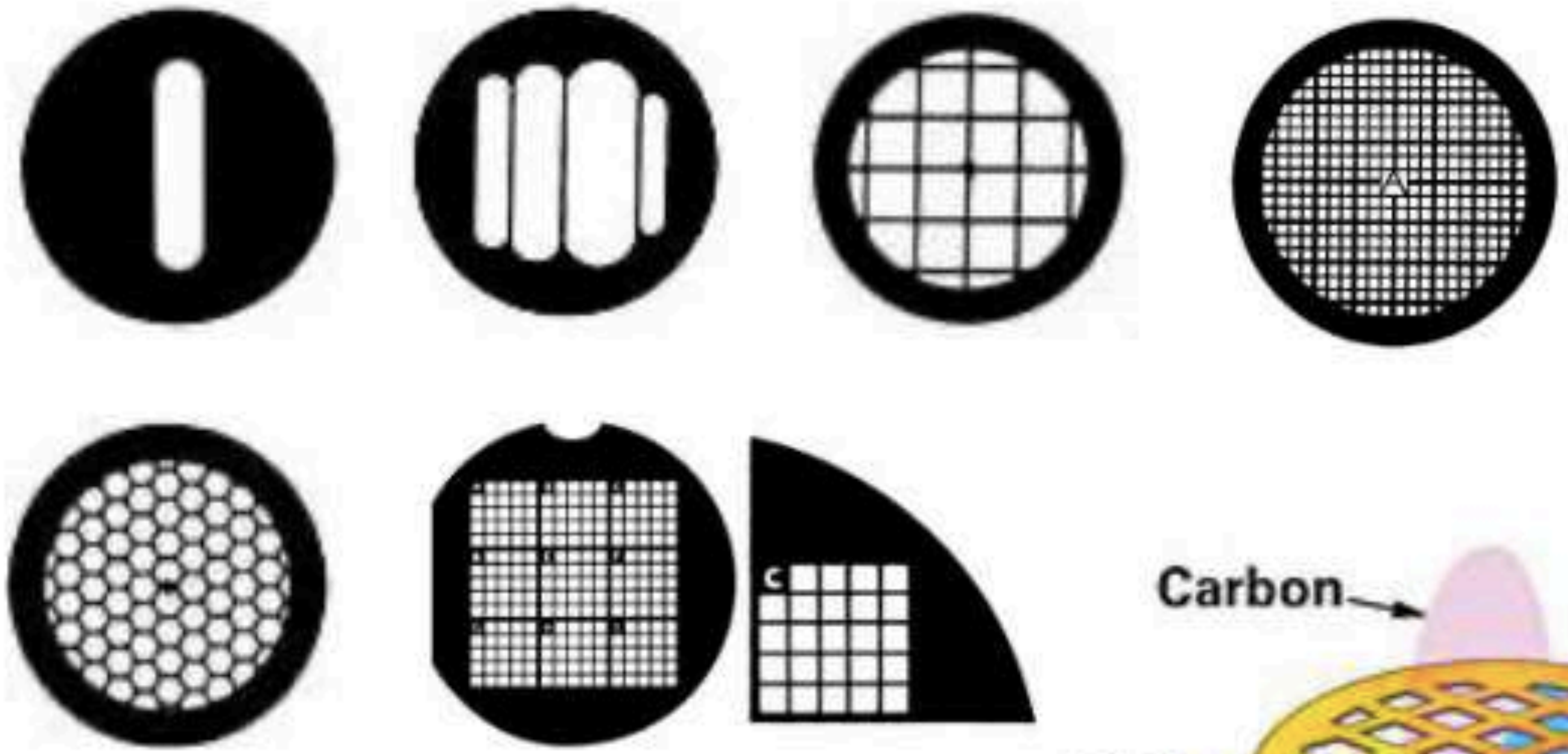
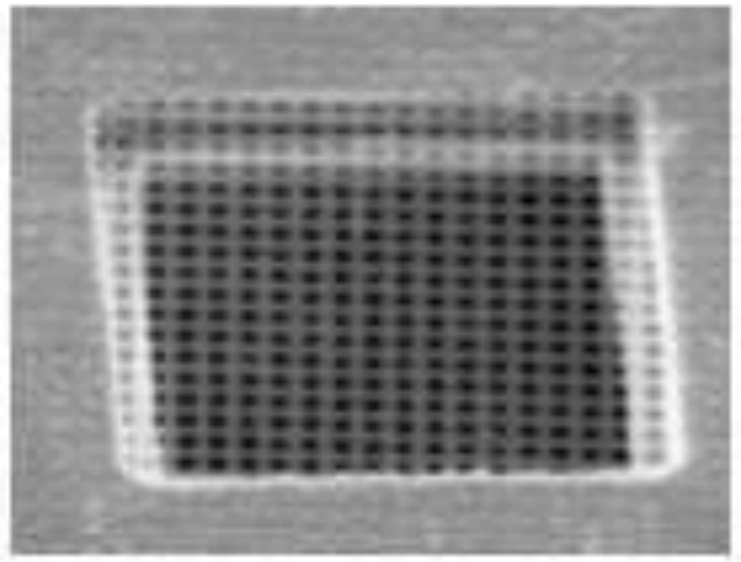
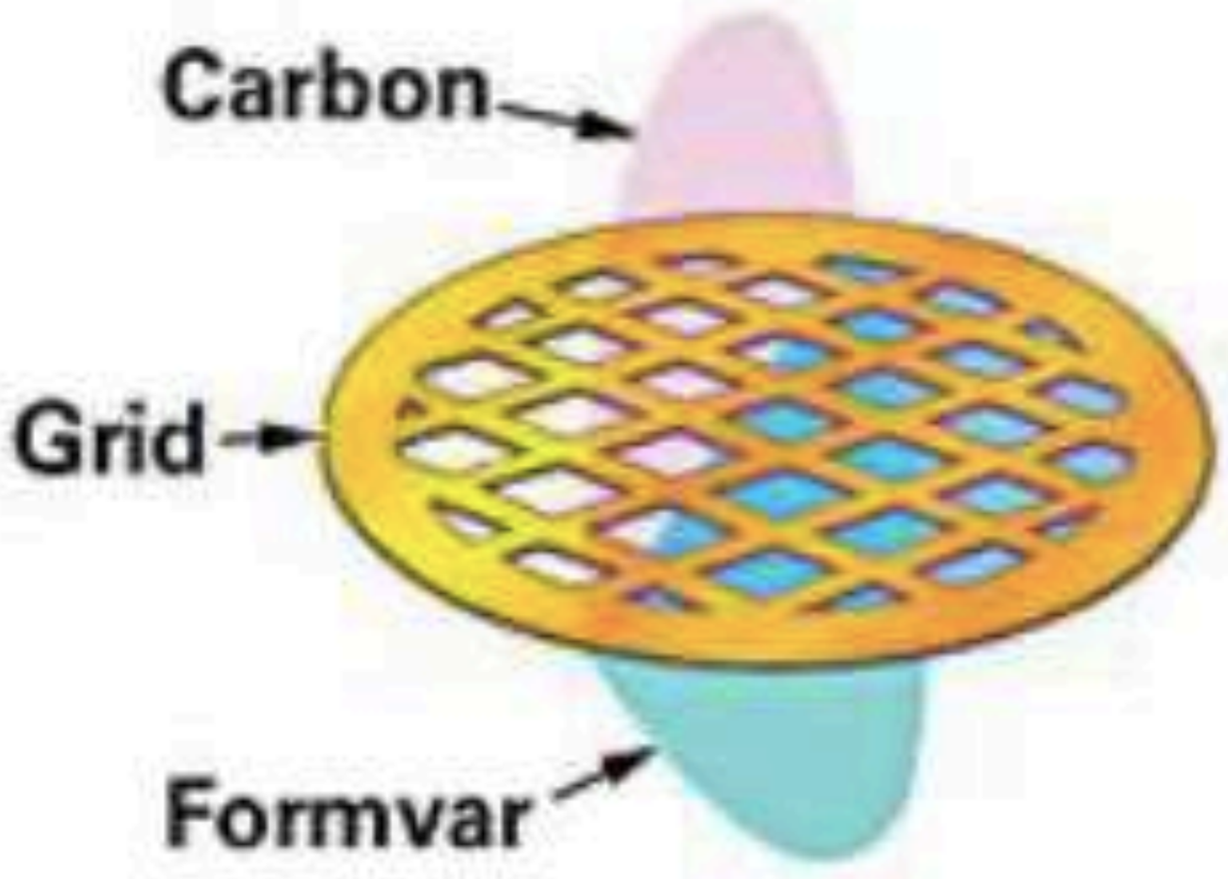






Common Materials

Copper

Nickel

Gold

Aluminum

Molybdenum

Titanium

Stainless Steel

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

What do 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

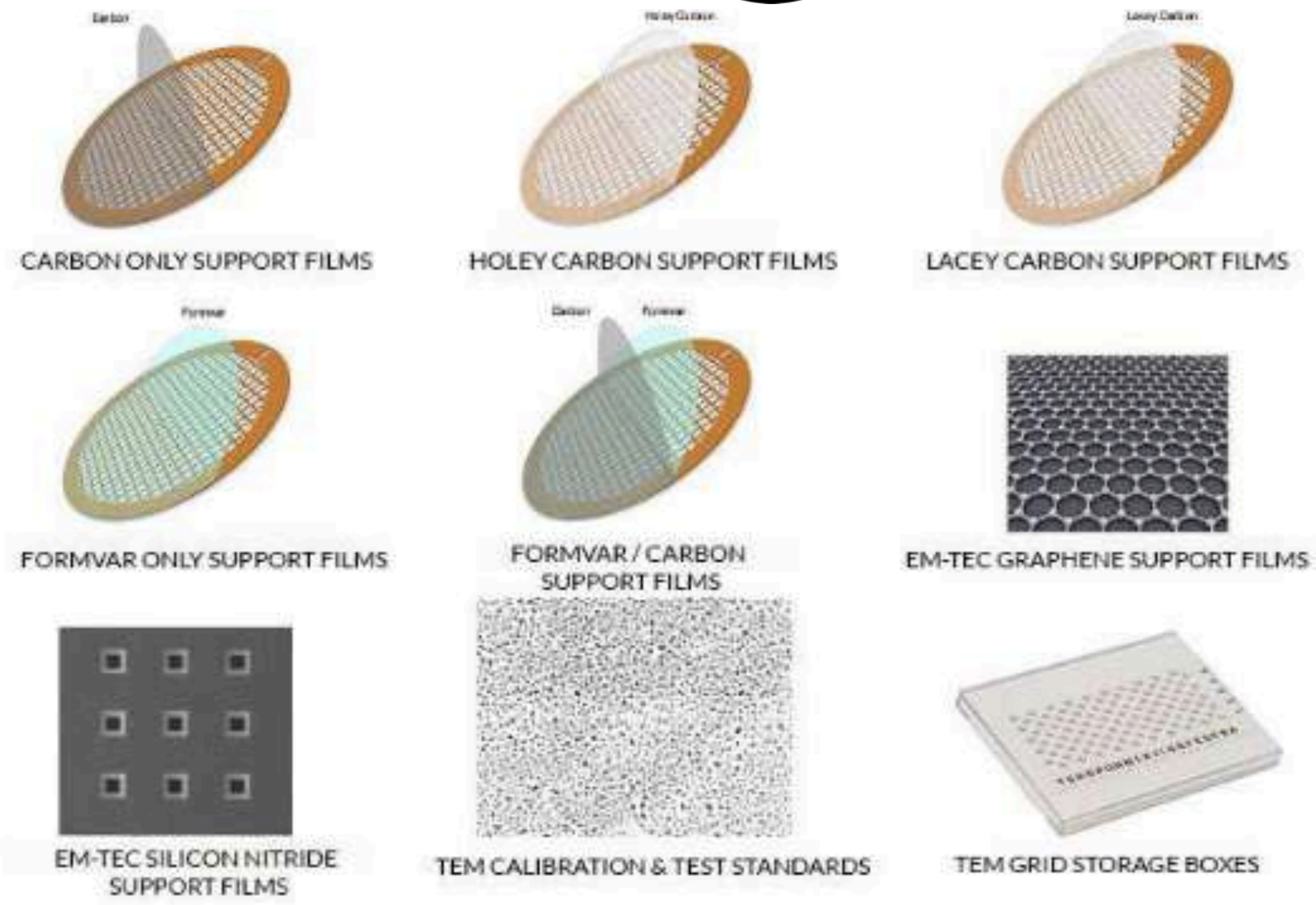
Square Mesh	Pitch µm	Hole µm	Bar µm	% Trans-mission
50	508	425	83	70
75	339	284	55	70
100	254	204	50	65
150	169	125	44	60
200	127	90	37	50
300	85	54	31	40
400	64	38	26	35
500	51	28	23	30

What do EM grids look like?

TERMINOLOGY

Grid (Cu, Au, Mo, etc…)
- mesh

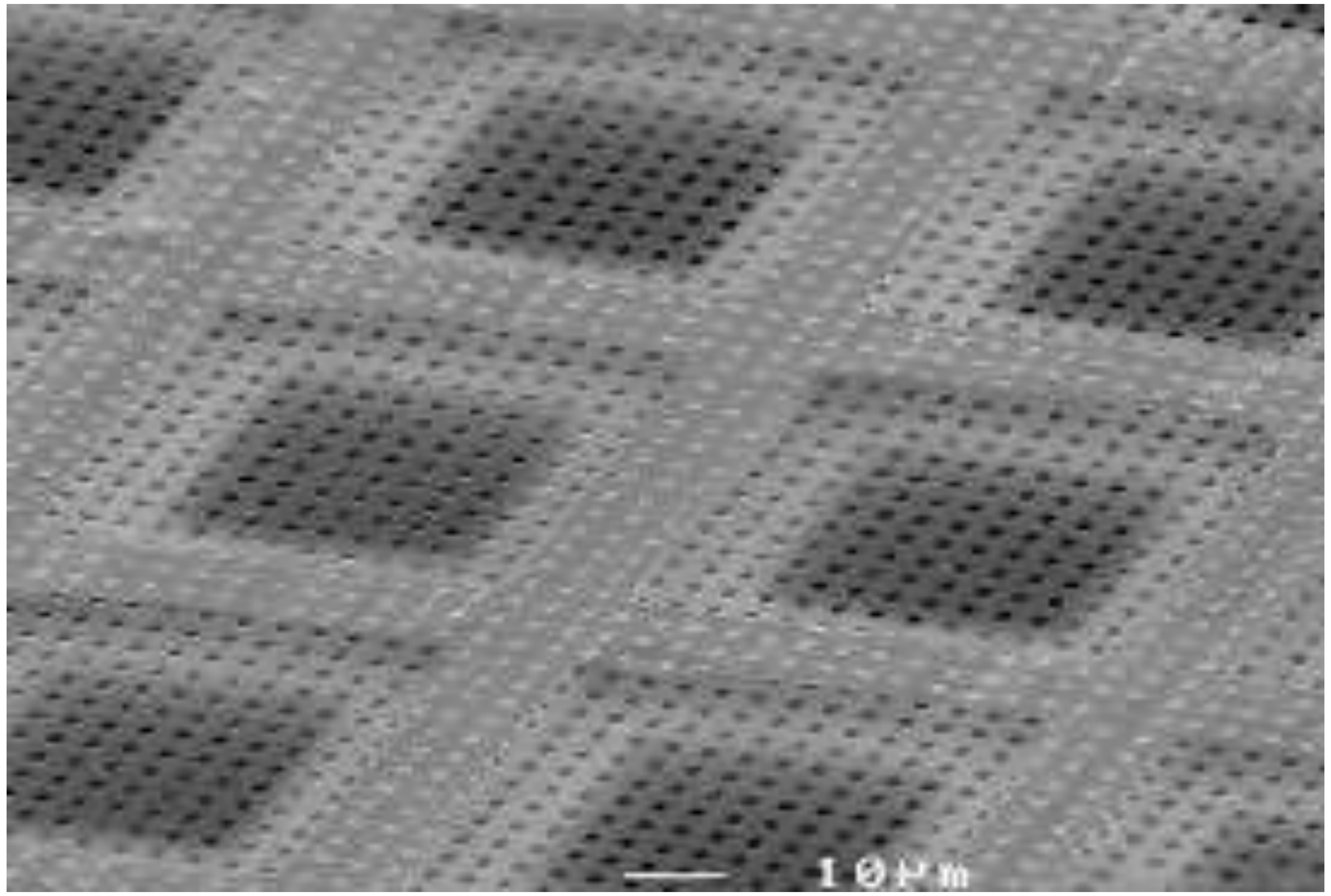
Foil (C, Au, etc…)
- Continuous
- lacy
- holey (hole size and spacing)

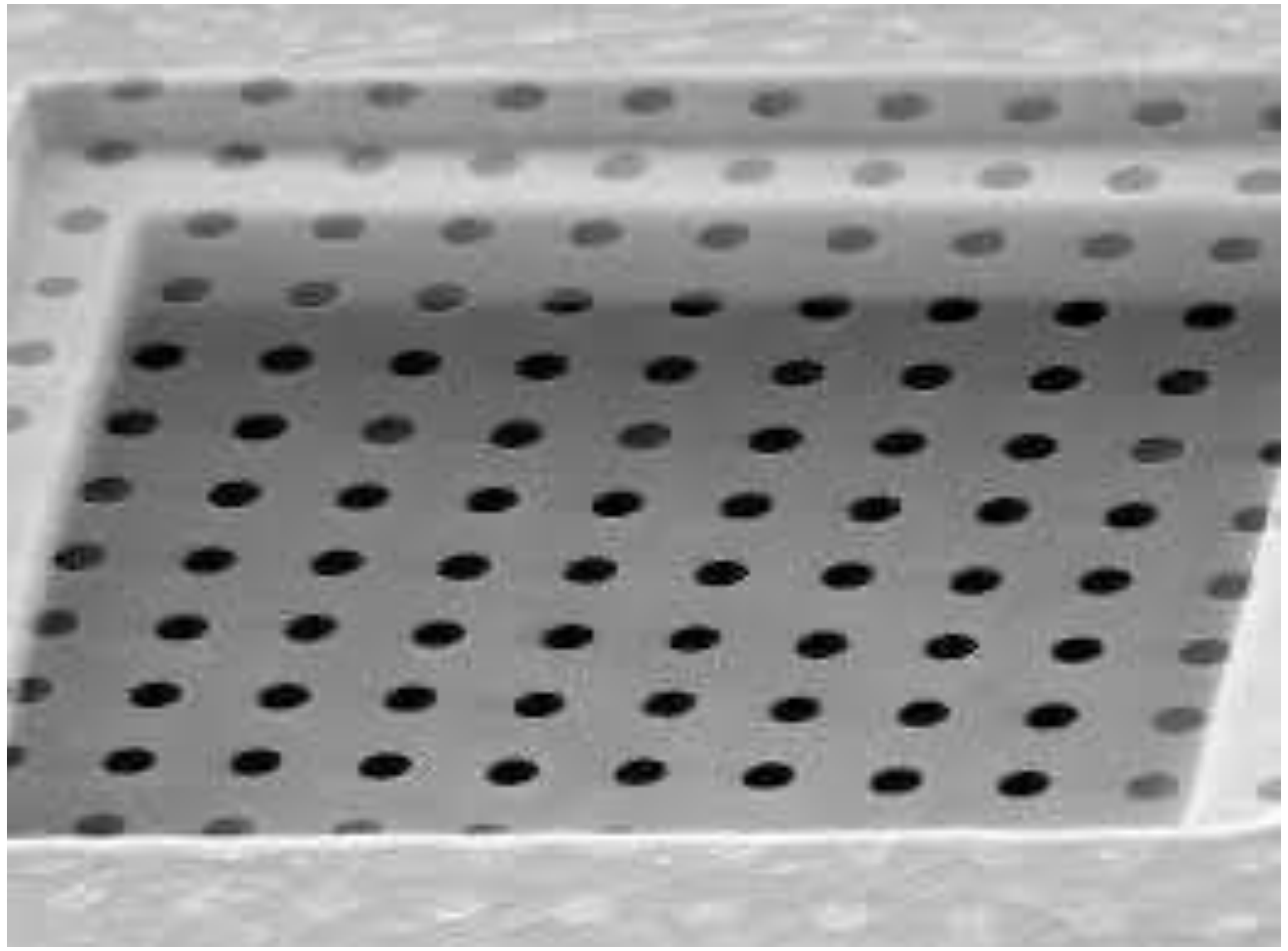


https://edgescientific.com/product-category/tem-supplies/tem-support-films/

What do EM grids look like?

TERMINOLOGY



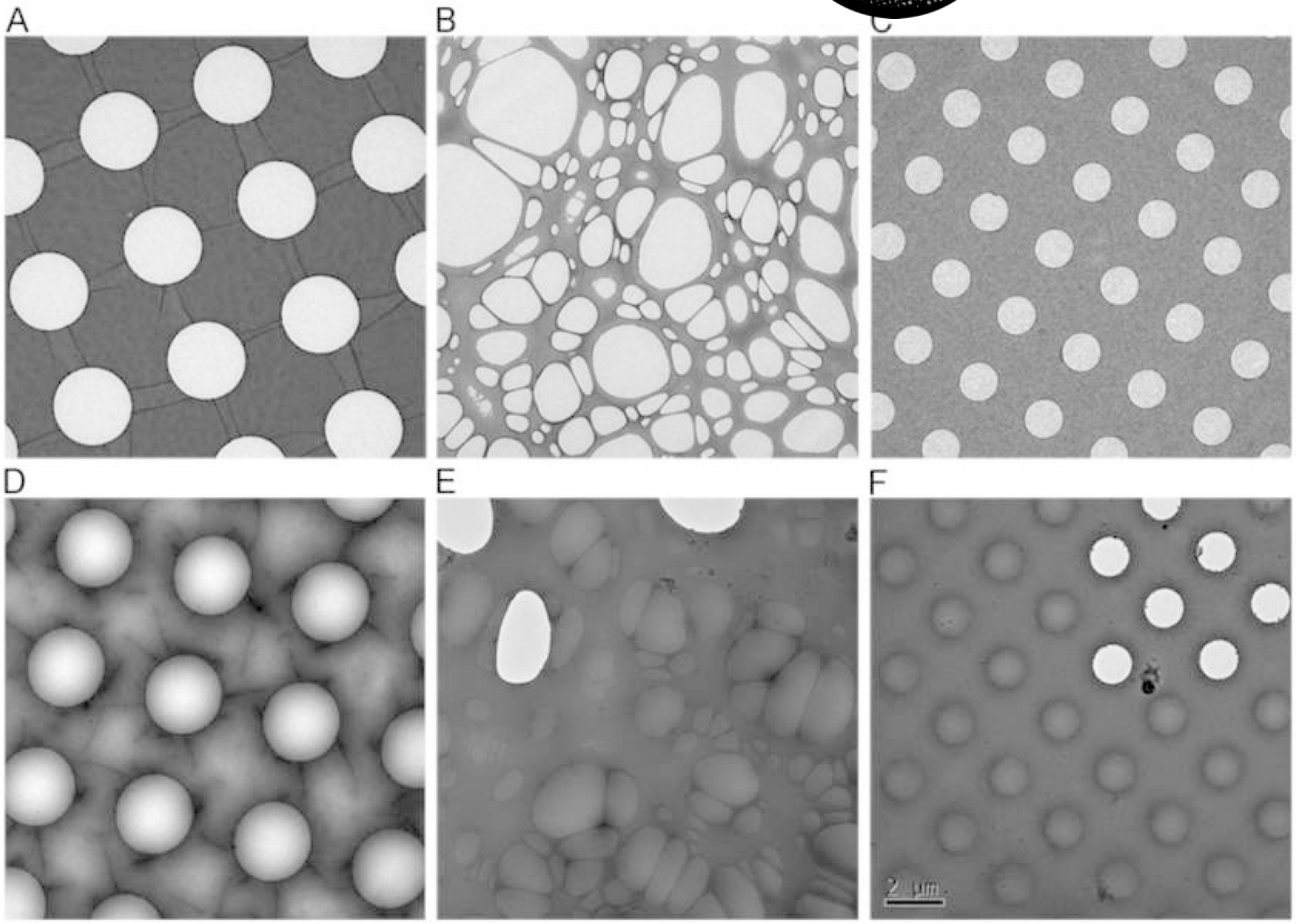


- Protochips.com

Quantifoil.com

What do EM grids look like?

TERMINOLOGY



Quantifoil grid   Lacey carbon grid   C-flat grid

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?

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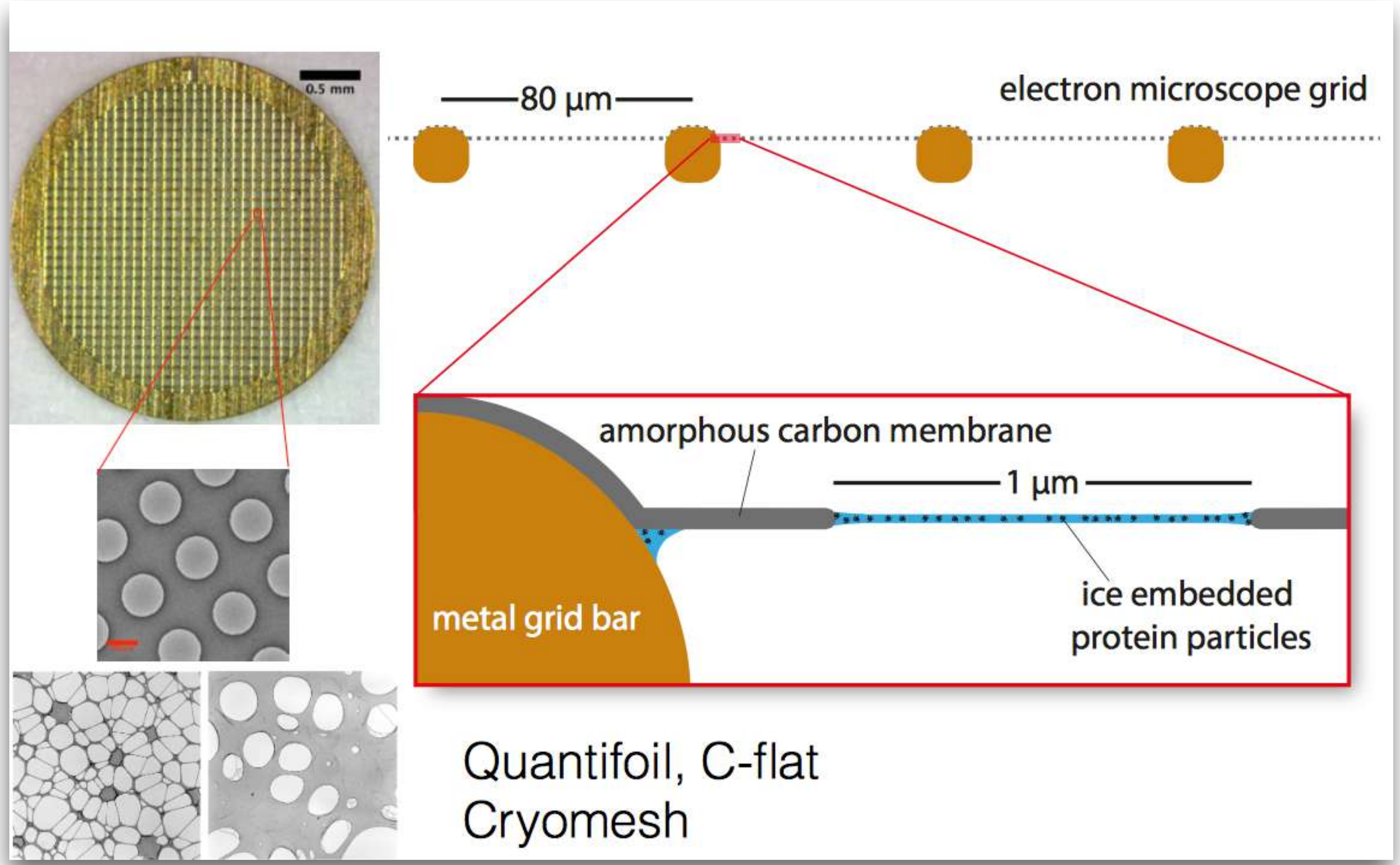


Russo & Passmore, 2015

What do EM grids look like?

TERMINOLOGY

- Holey gold foil on gold mesh grid

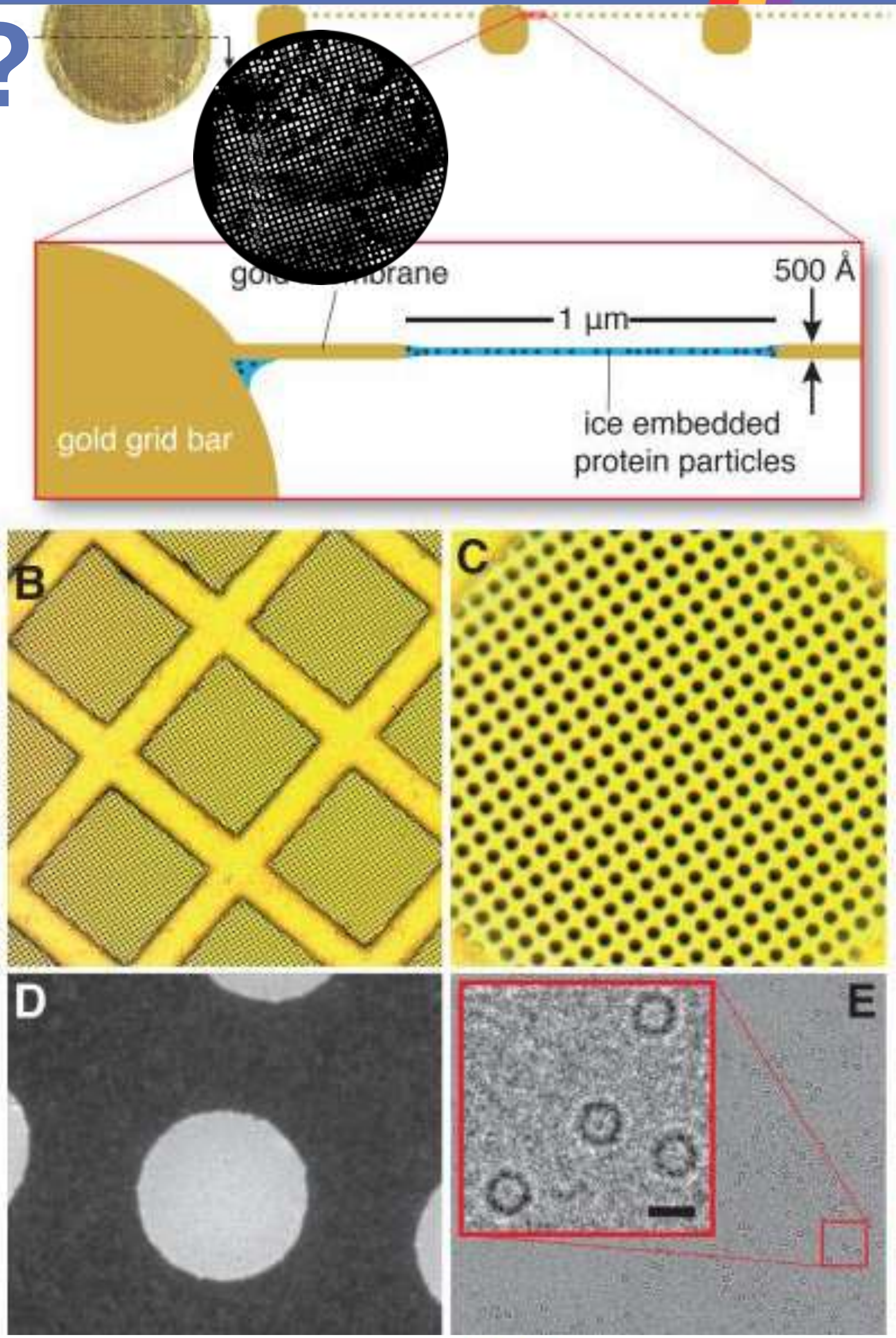
Advantages:

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

Disadvantages:

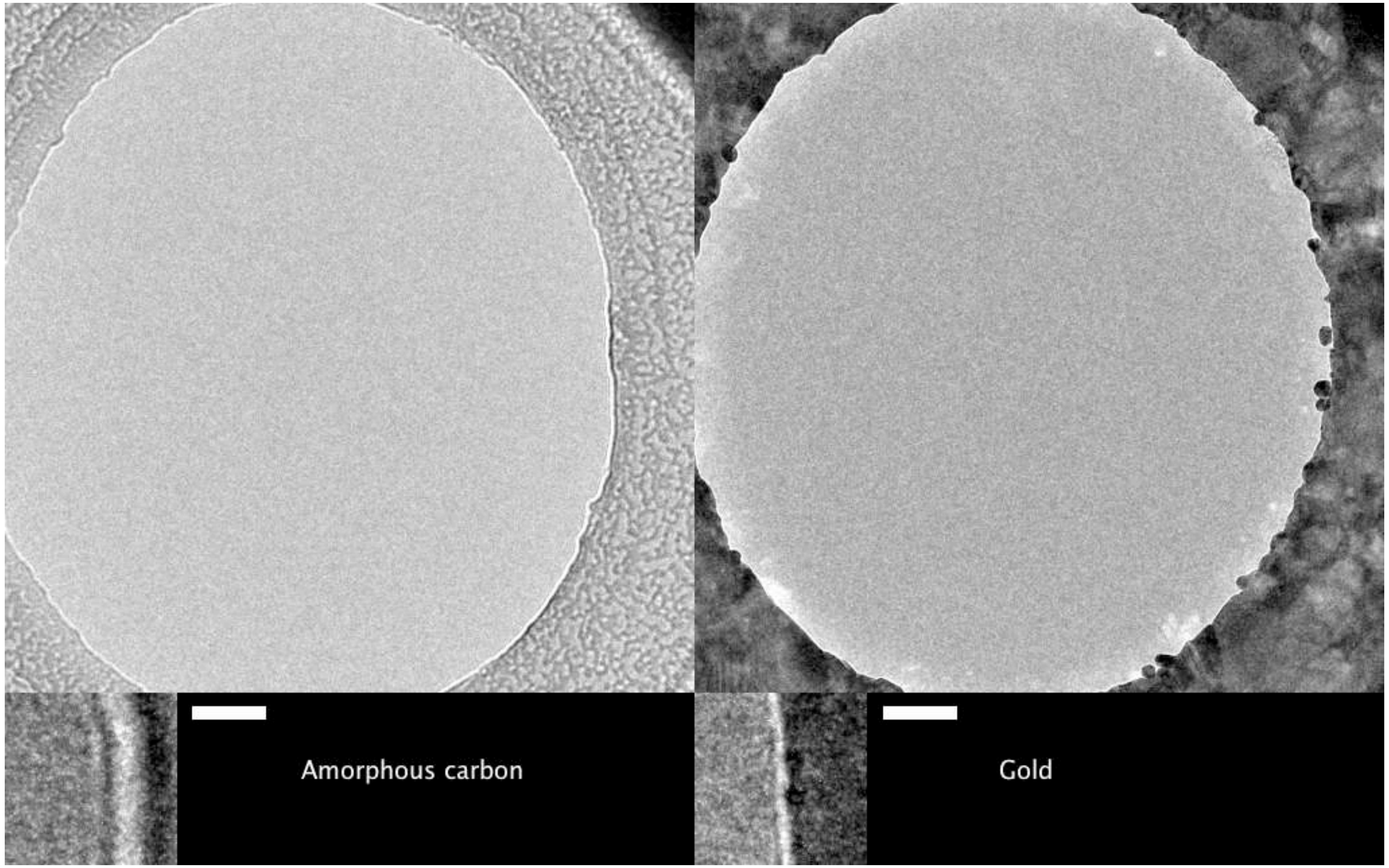
- Difficult to find focus due to lack of amorphous substrate

Russo & Passmore, 2015



What do EM grids look like?

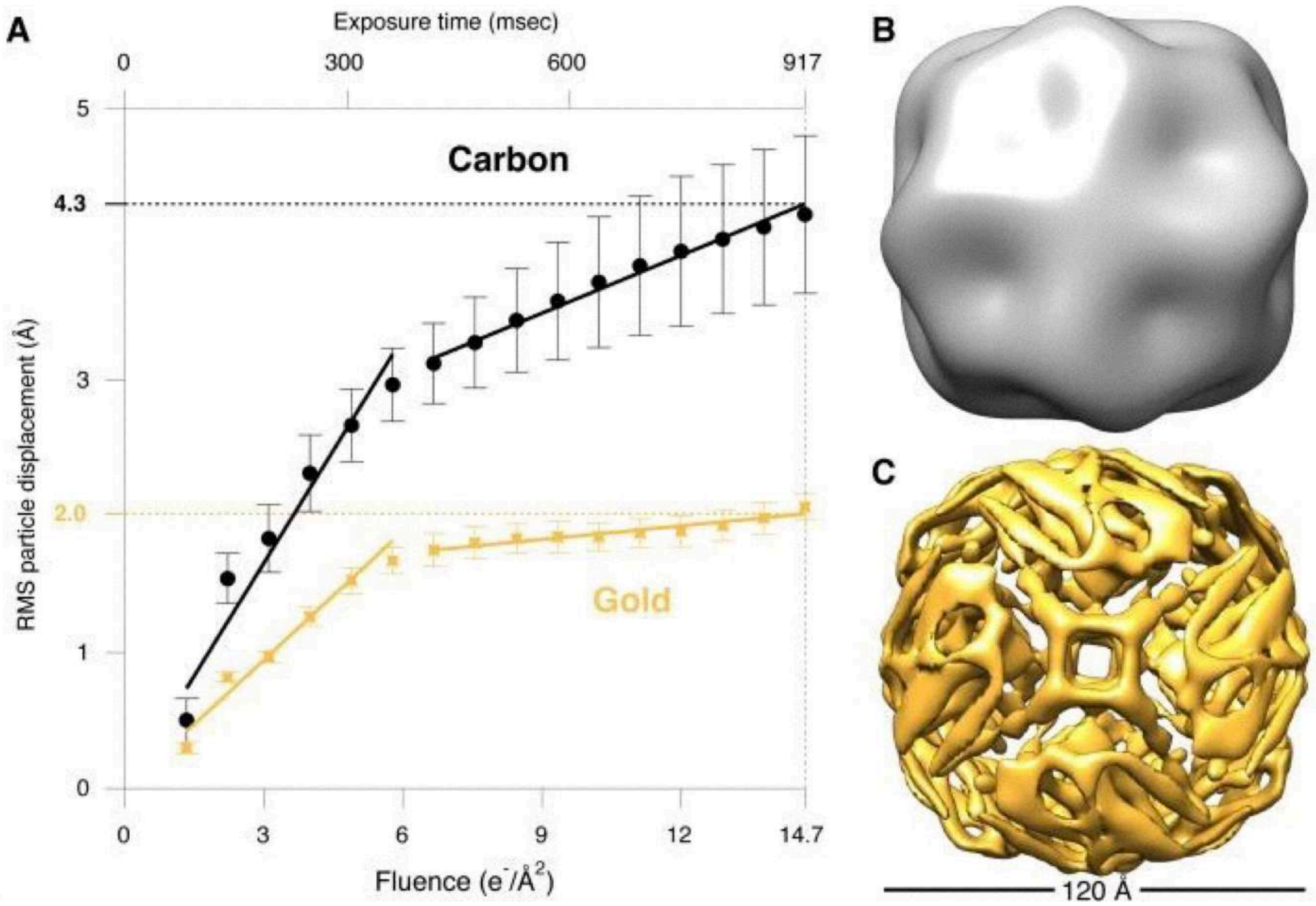
Gold grids



Russo & Passmore, 2015

What do EM grids look like?

Gold grids



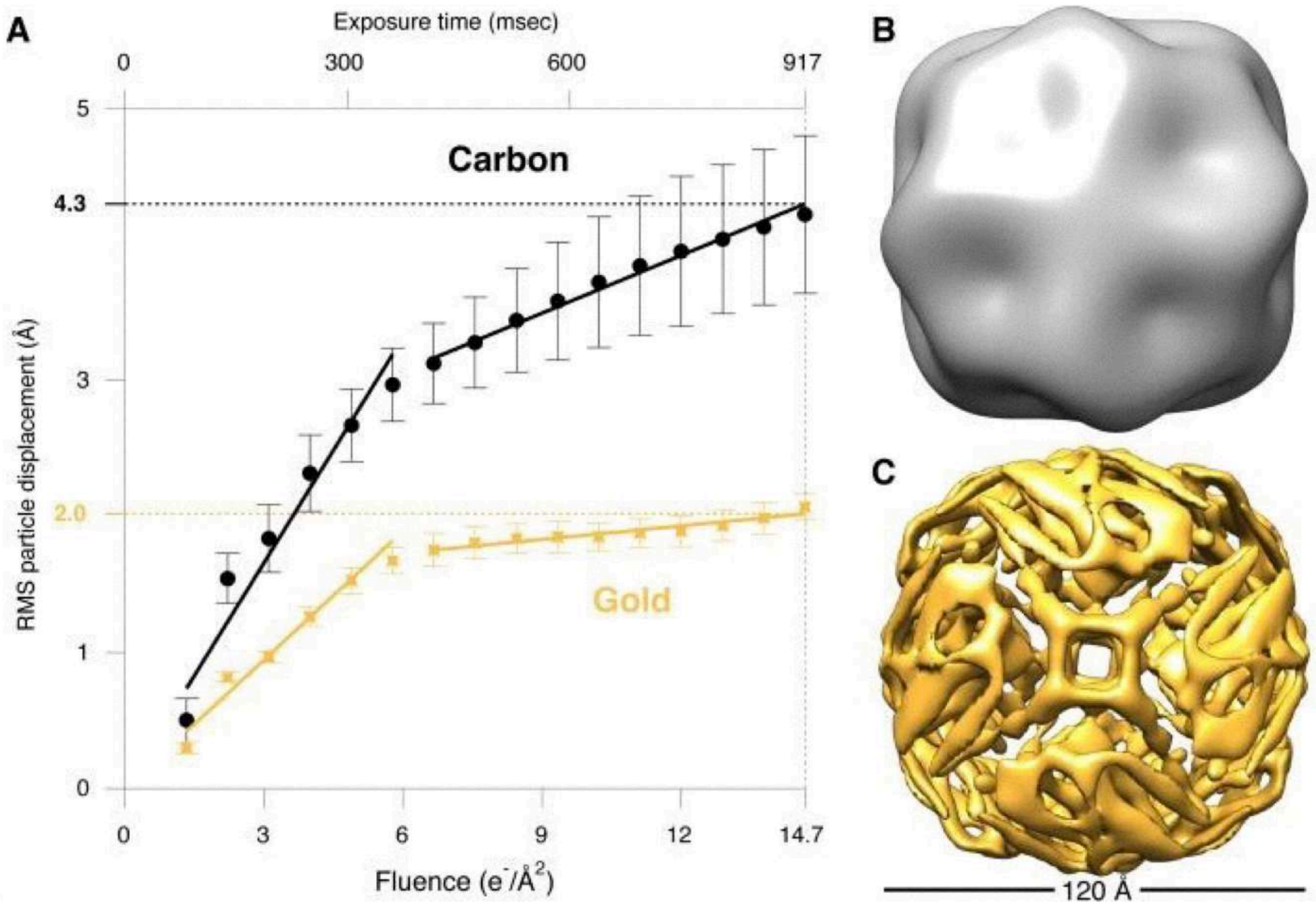
A. 80S ribosome movement during irradiation supported by amorphous carbon and gold using same imaging conditions.

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

What do EM grids look like?

Gold grids



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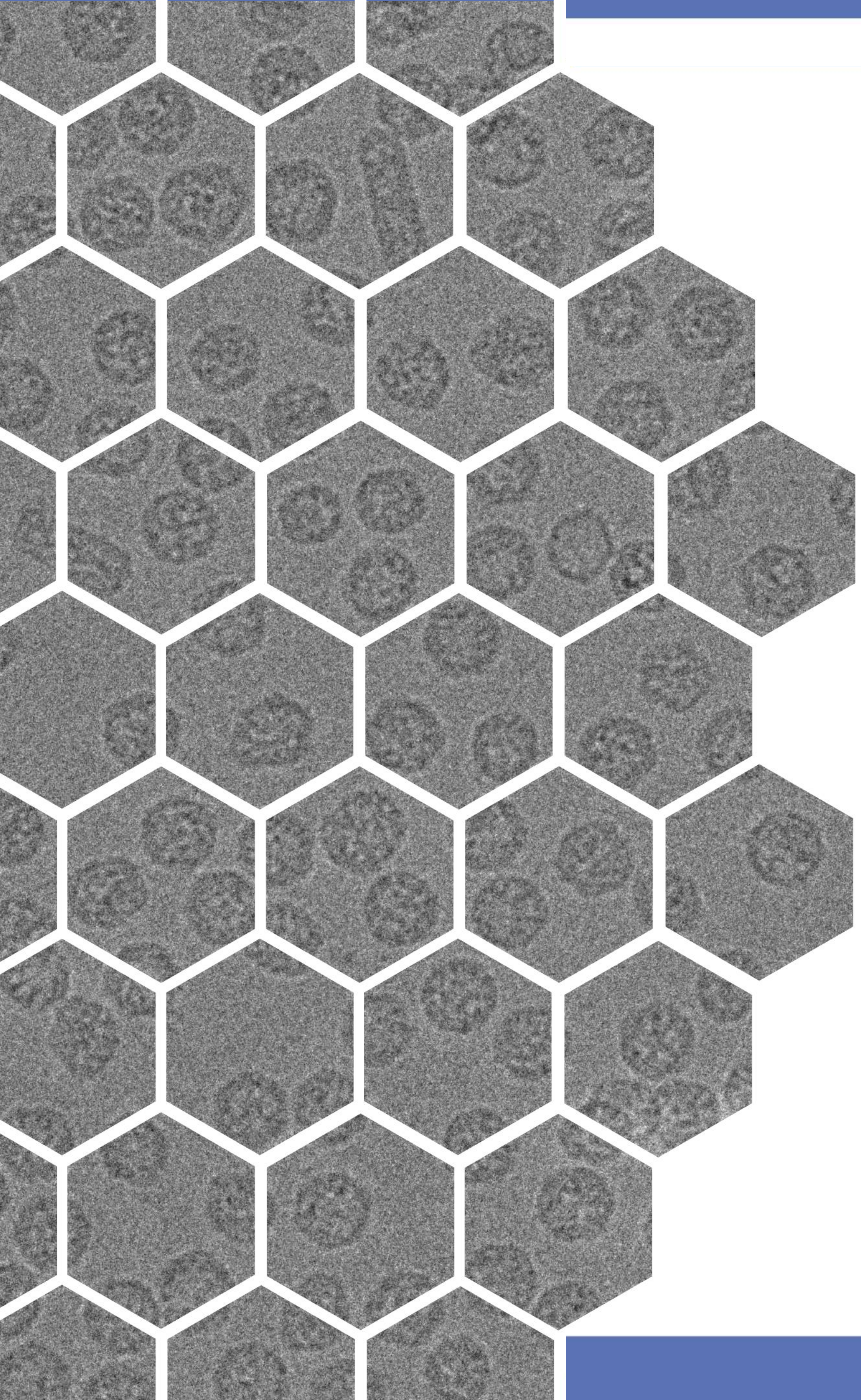

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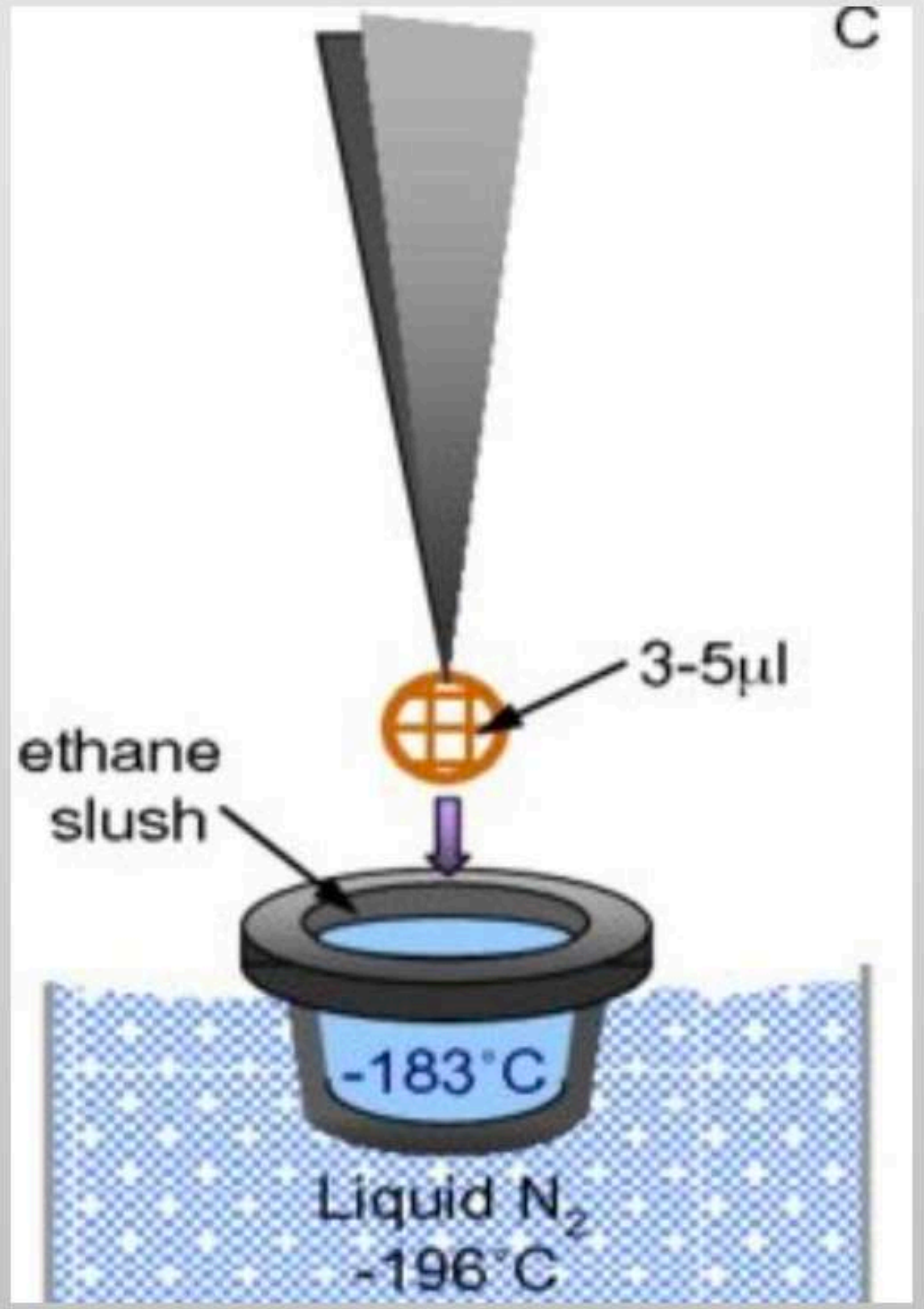
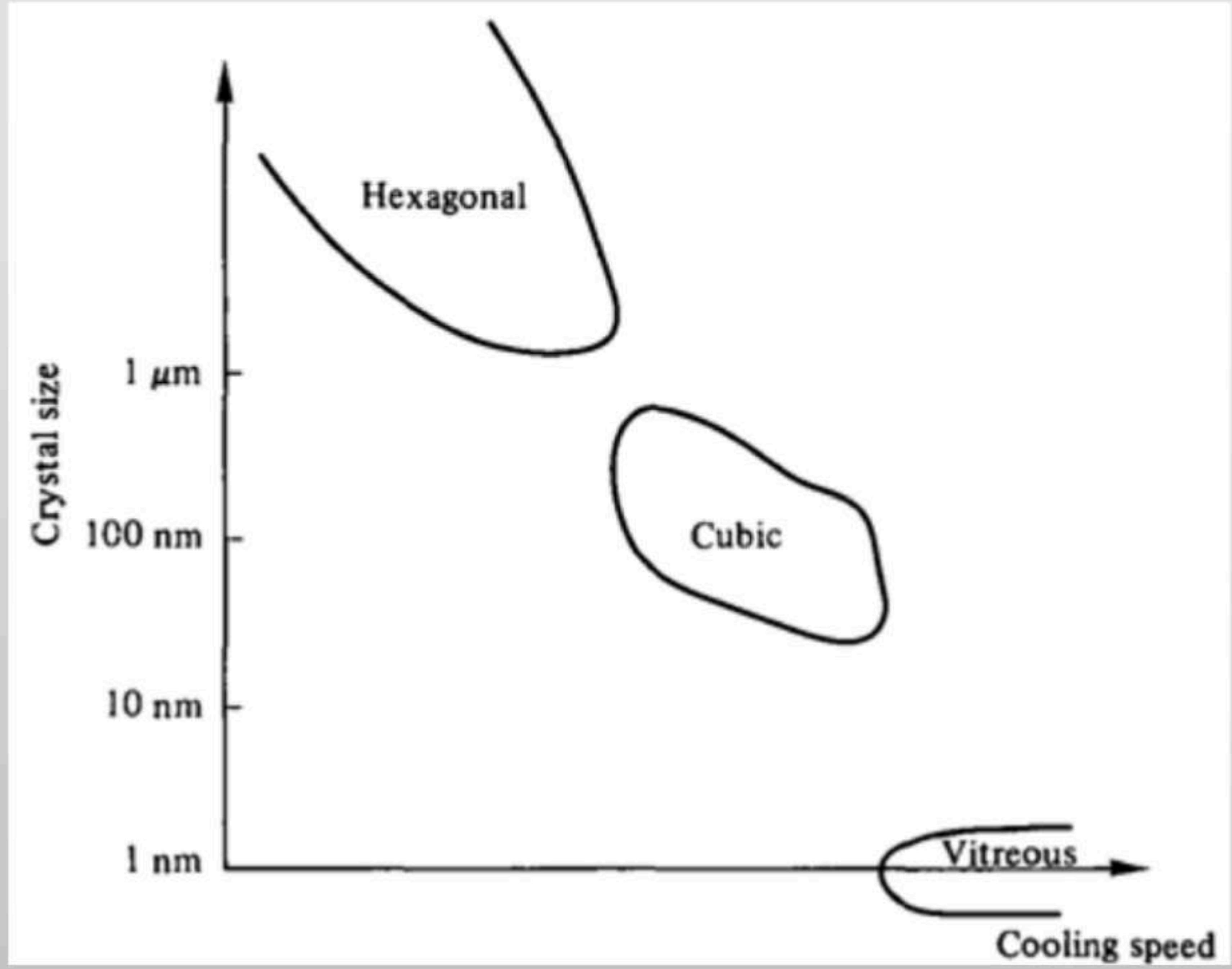
How are samples prepared for cryoEM?

Vitrification process

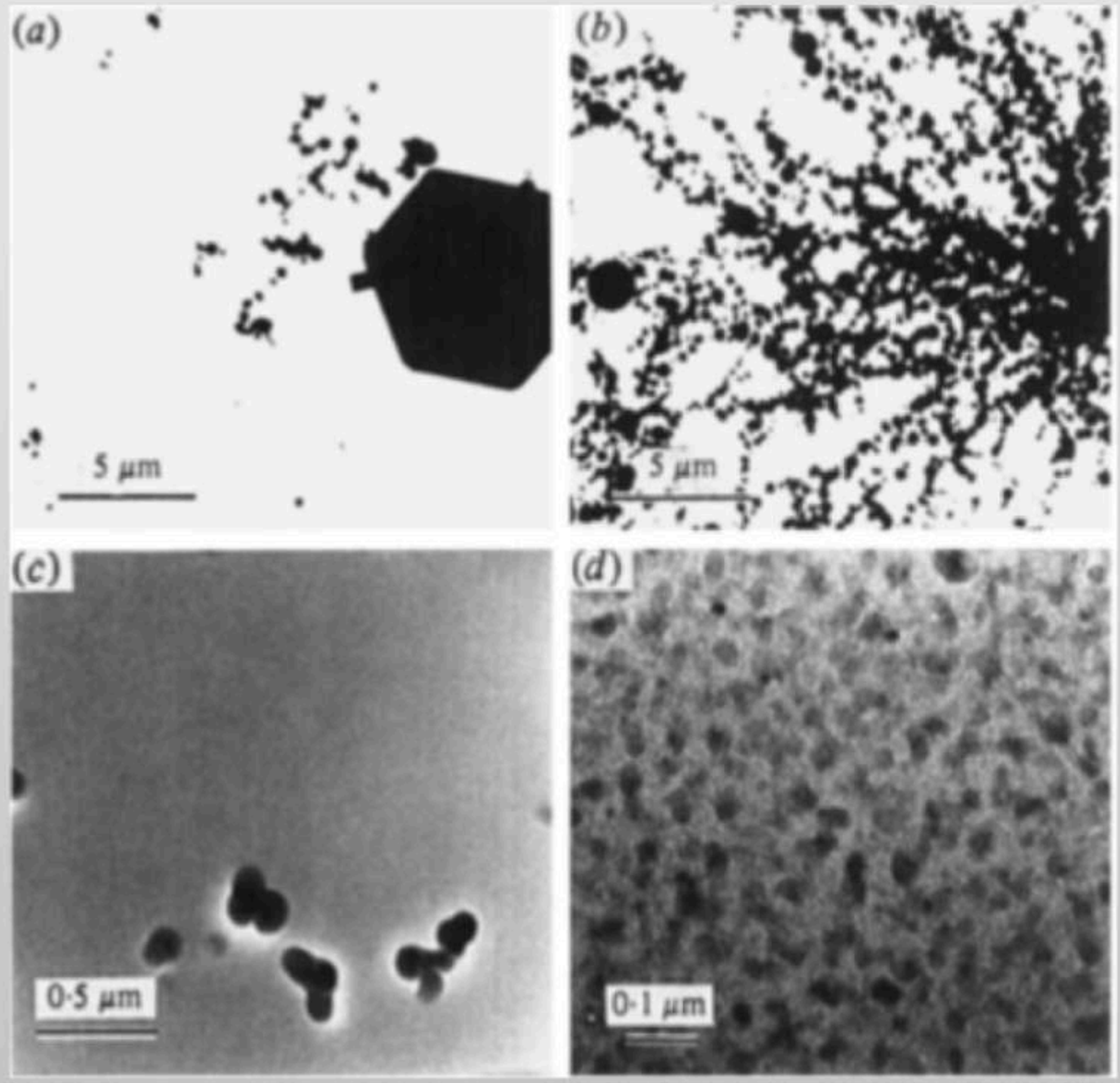
- Liquid ethane is a suitable coolant.
- Liquid nitrogen boils on contact, which makes it a poor coolant for cryo-EM.
- Cooling speed faster than 10^5-10^6 K/s ensure the formation of vitrified ice.



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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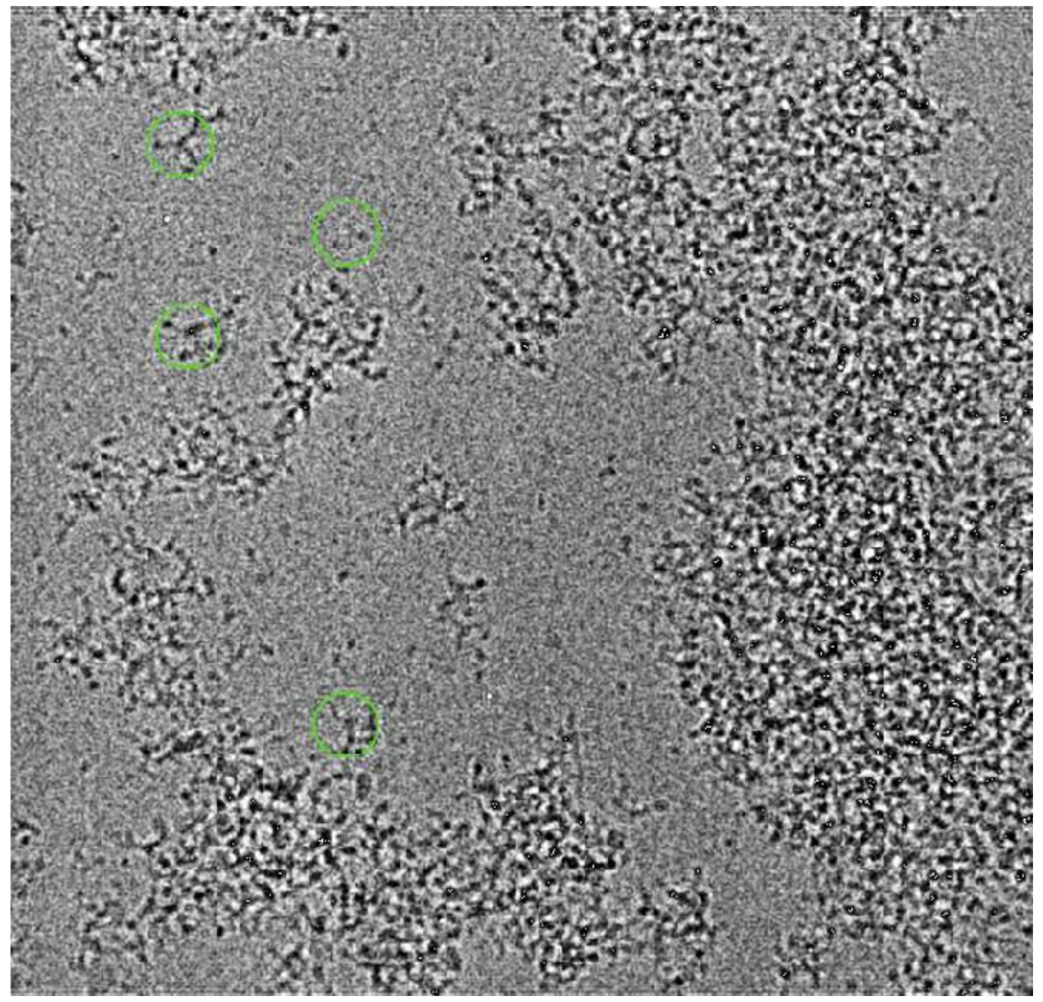
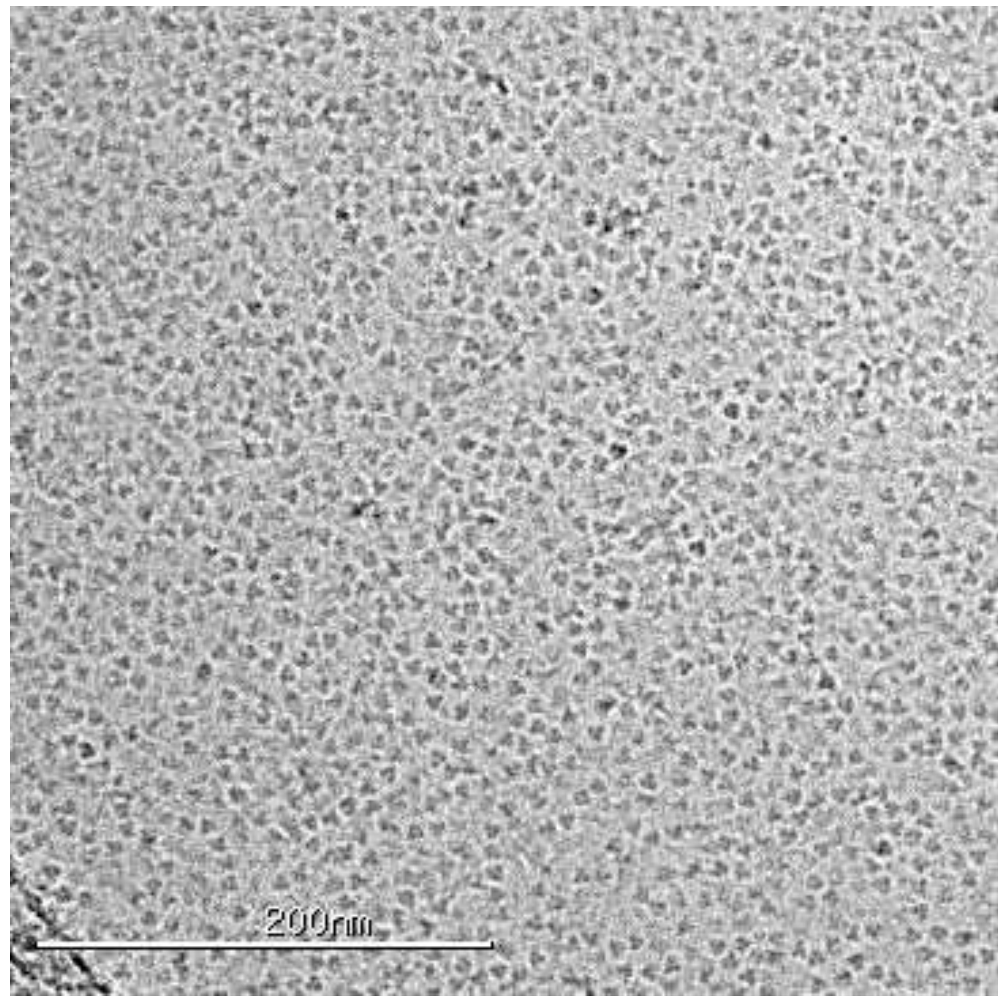
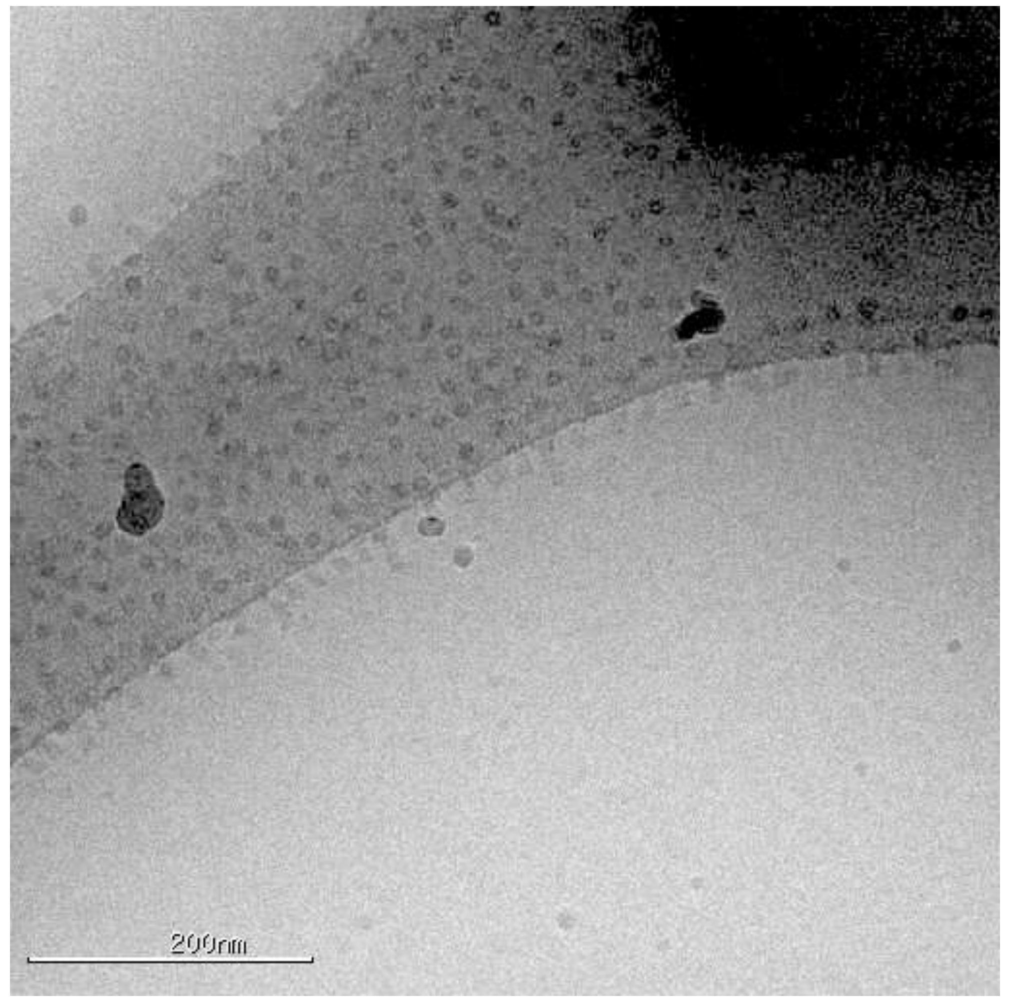
What issues arise?



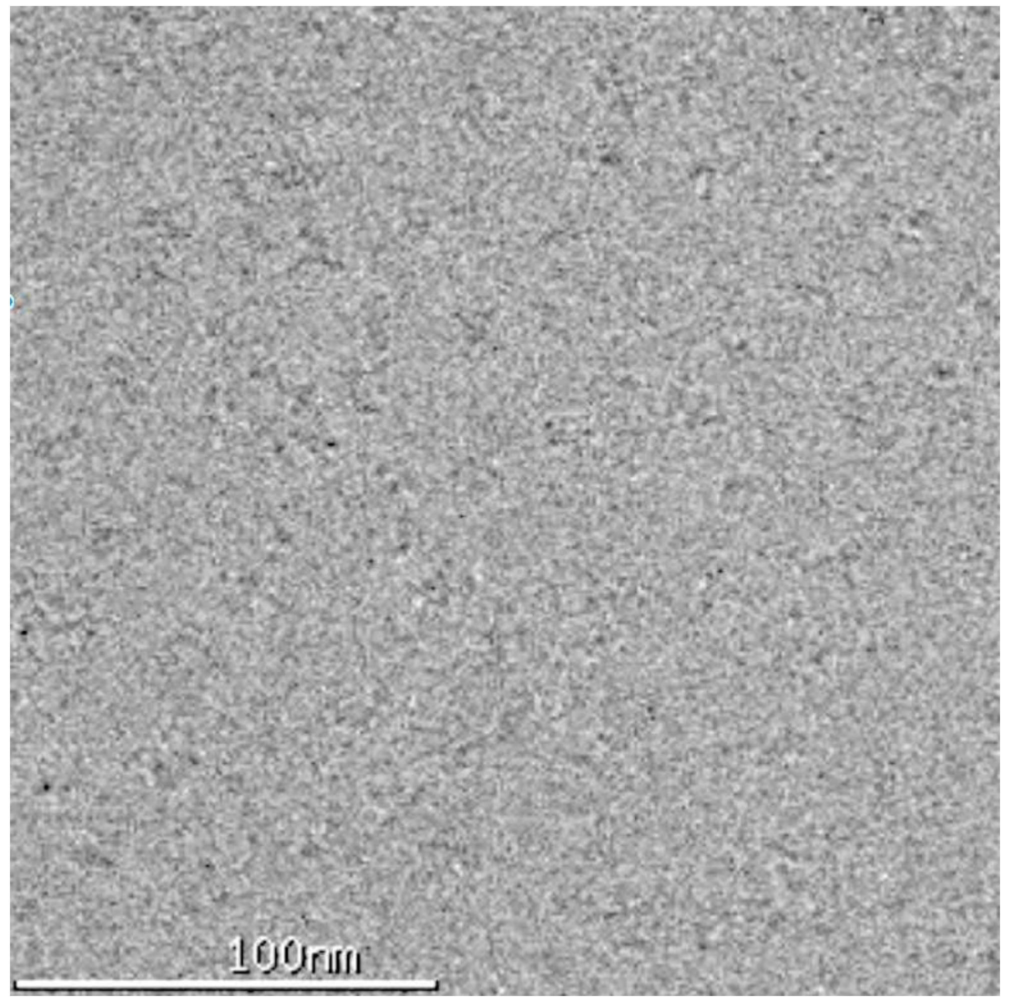
Aggregating in ice



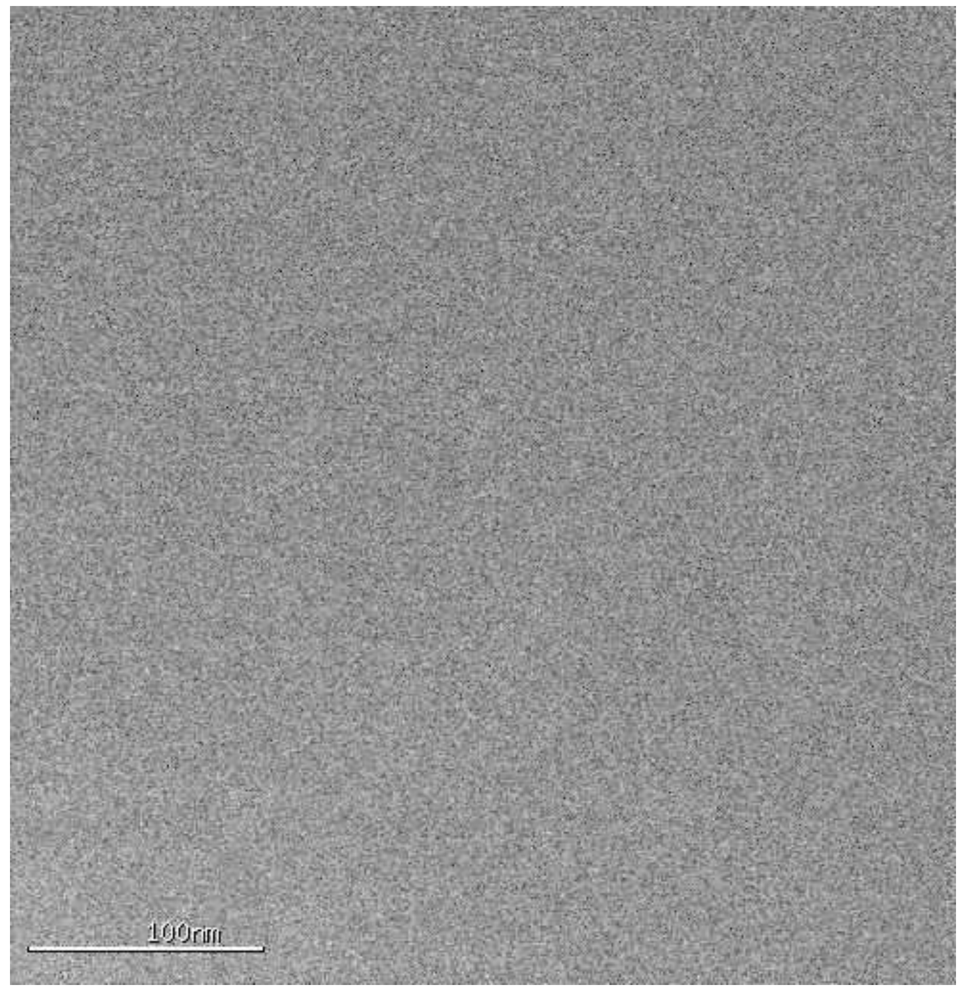
Preferred orientation



Particles not going into holes

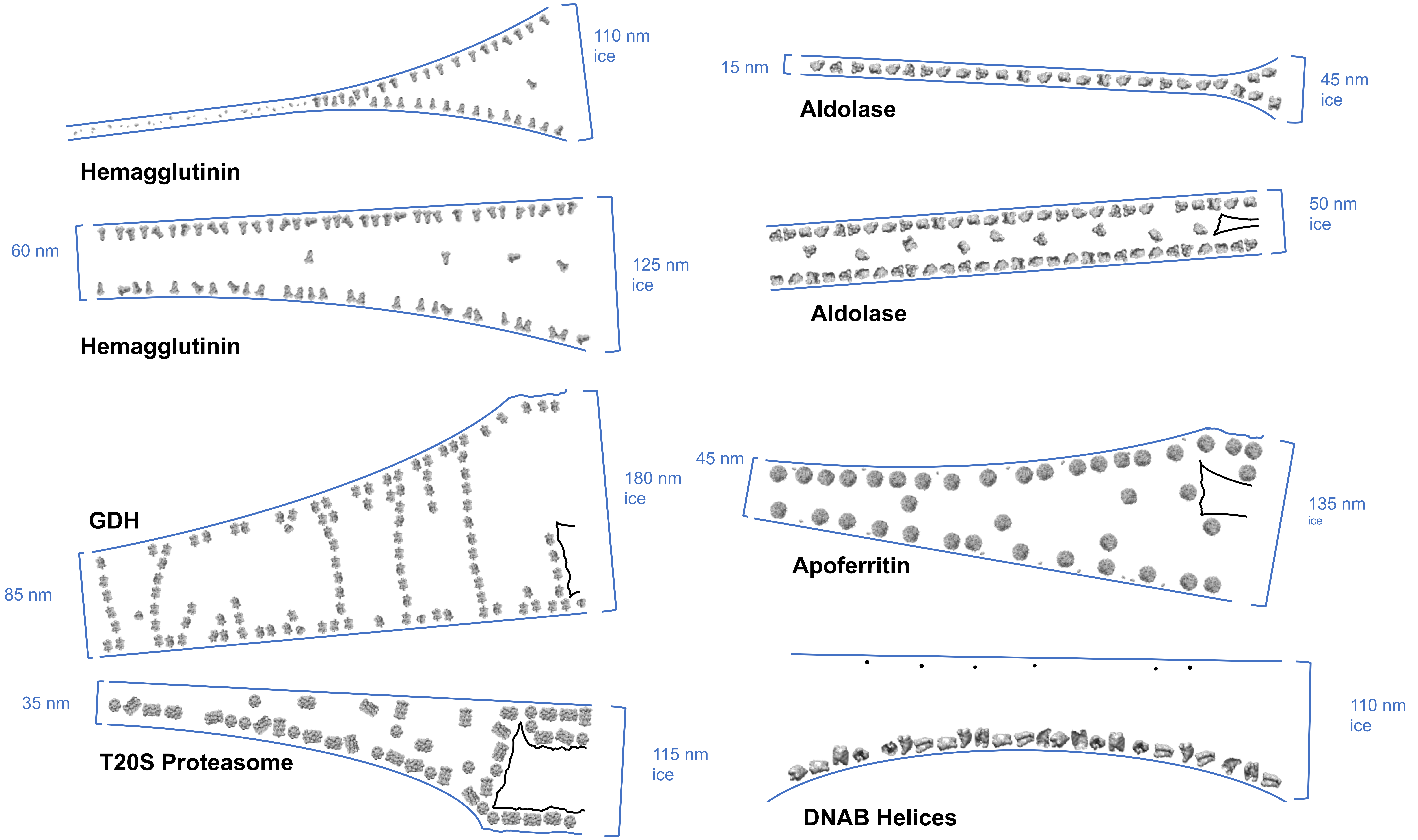


Rejecting 90% of particles



Particles disappearing in ice

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?

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

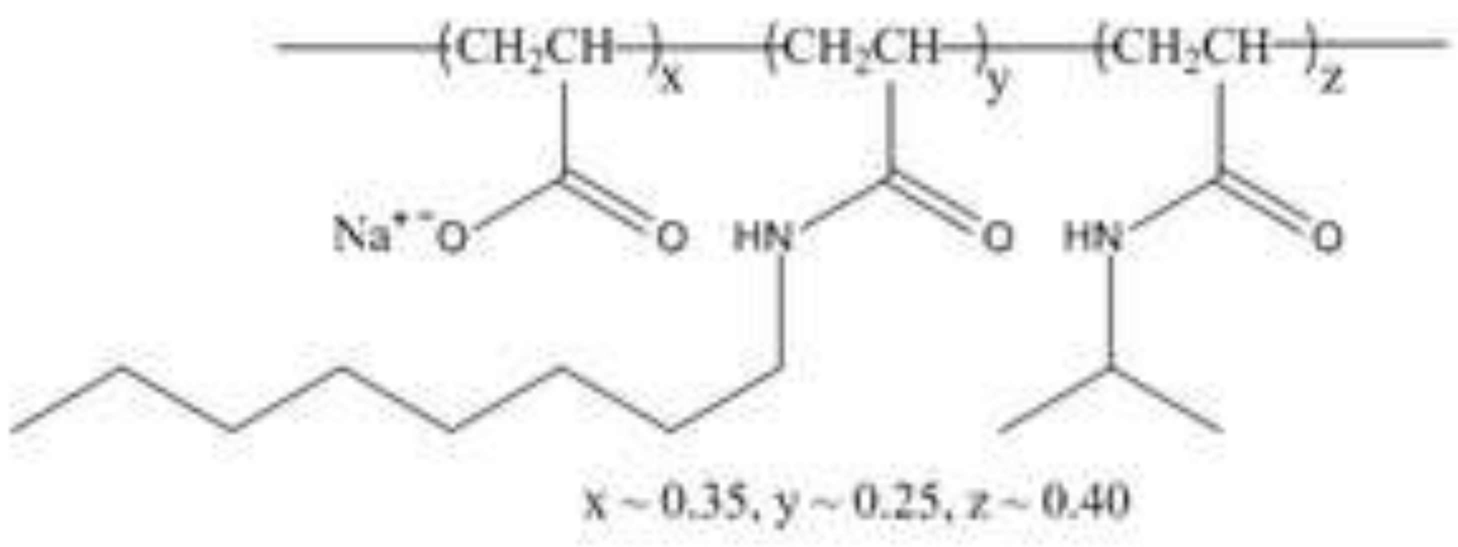
Filamentous protein

- Segmented analysis

Low concentration

- Multiple blots
- Affinity grids

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.

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

PDB Release Date	PDB	Protein	Additive
2020-01-08	6PWN	MscS mechanosensitive channel	0.01% f-OM
2019-09-04	6KG7	Piezo2 mechanosensitive channel	0.65 mM f-FC8
2019-08-28	6QTI	Nicotinamide nucleotide proton channel	0.05% CHAPS
2019-08-07	6R7L	SecYEG translocon	0.2% f-OM
2019-02-06	6E0H	TMEM16 scramblase	3 mM f-FC8
2018-12-19	6N3Q	Sec protein-translocation channel complex	3 mM f-FC8
2018-11-07	6H3I	Type 9 secretion system translocon	1.5 mM f-FC8 or 0.7 mM f-OM
2018-10-24	6DMR	TRPV5 ion channel	3 mM f-FC8
2018-10-17	6D3R	CFTR	3 mM f-FC8
2018-09-26	6HJR	Influenza Hemagglutinin	2% Octyl Glucoside
2018-08-08	6FOO	Ryanodine receptor 1	0.2% f-OM
2018-08-01	6CJQ	SthK CNG Potassium channel	3 mM f-FC8
2018-05-23	5YX9	TRPC6 ion channel	0.5 mM f-OM
2018-01-31	6C0V	P-Glycoprotein transporter ABCB1	3 mM f-FC8
2017-12-27	6B5V	TRPV5 ion channel	3 mM f-FC8
2017-12-13	6BPQ	TRPM8 channel	2% DMSO

https://www.anatrace.com/Landing/2020/Mar20-Newsletter

Glaeser, RM, et al. (2017) Biophys Rep 3(1), 1-7.

Noble, AJ, et al. (2018) Nat Methods 15(10), 793-795.

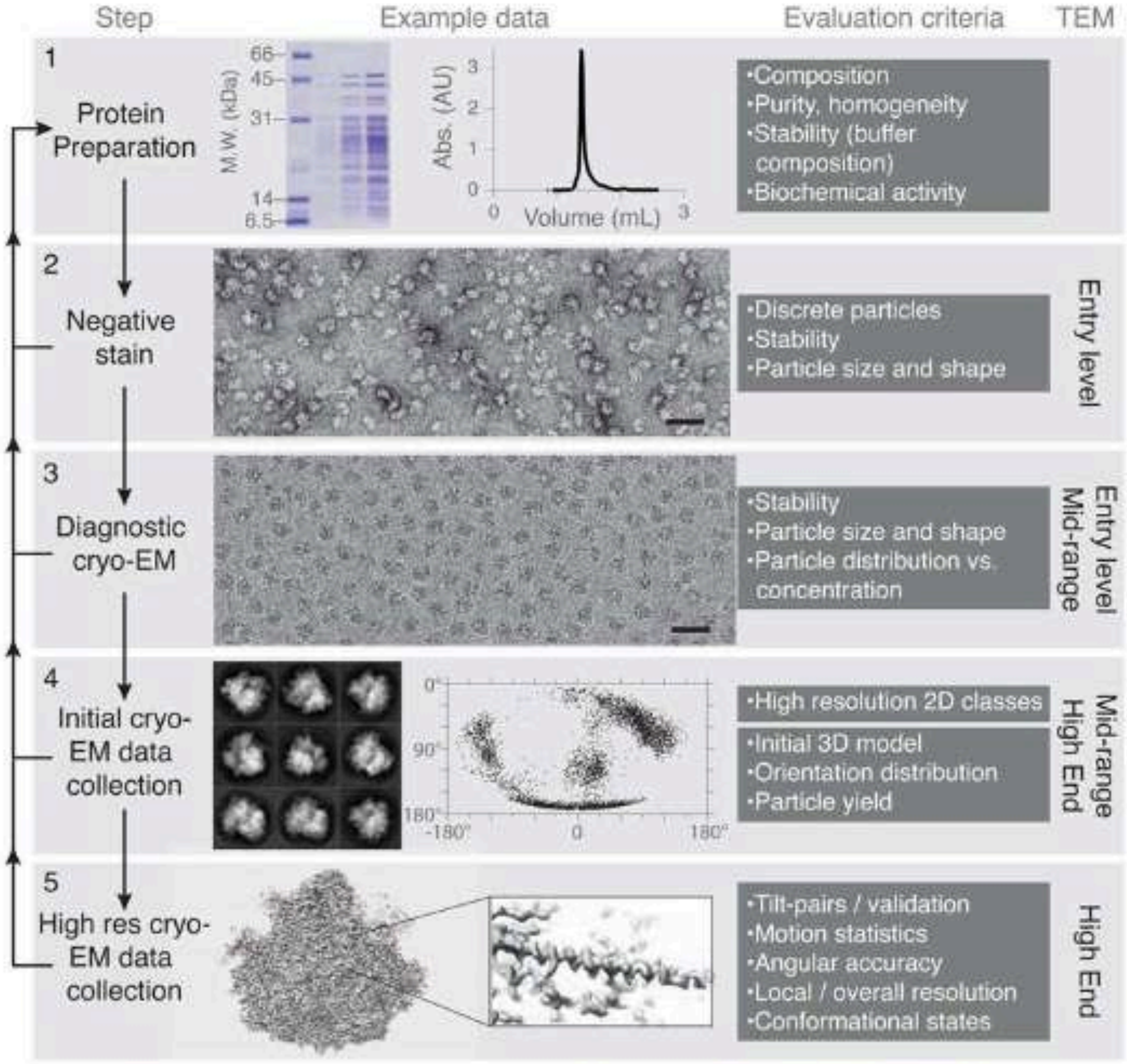
Drulyte, I et al. (2018) Acta Crystallogr D Struct Biol 74(Pt 6), 560-571.

Chen, J, et al. (2019) J Struct Biol X Volume 1. DOI: 10.1016/j.yjsbx.2019.100005

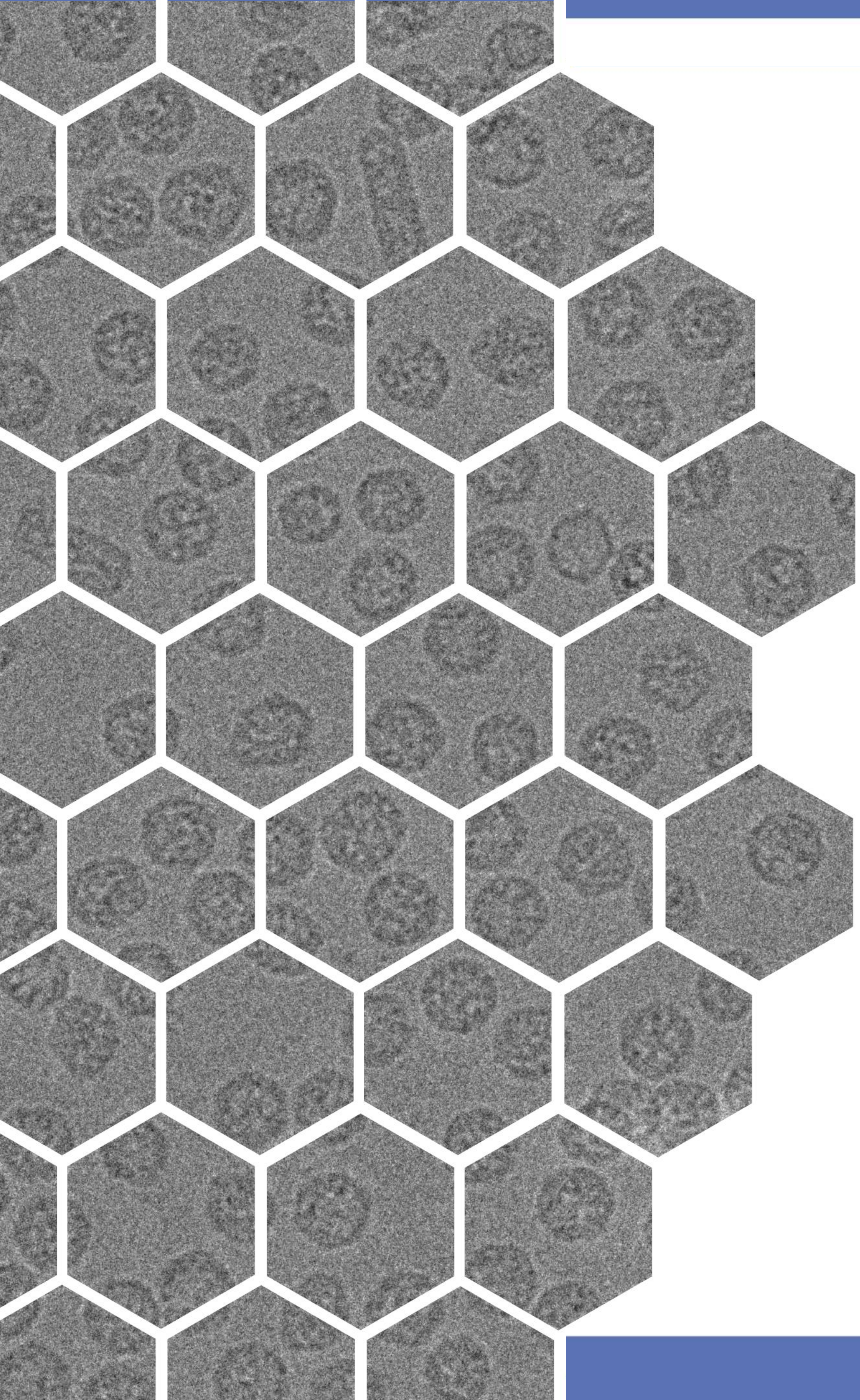
Preparing EM ready samples

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/



- Journal club and practical recap
- Considerations for biological cryoEM
  - Overview
  - Grids
  - What happens to a sample
  - Newer methods

Other methods

Spotiton

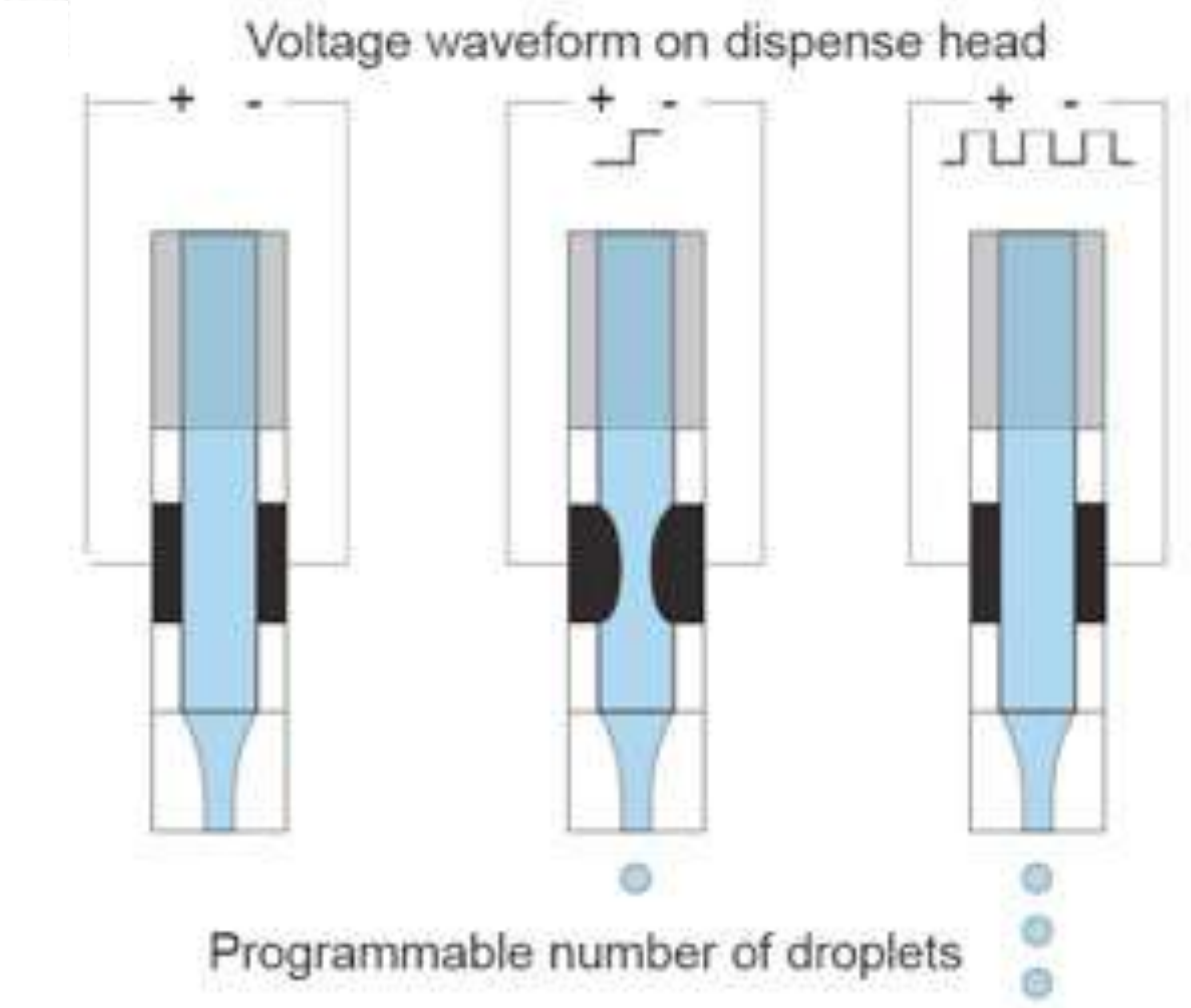
Improving Current CryoTEM Grid Preparation Methods

Dandey VP, Wei H, Zhang Z, Tan YZ, Acharya P, Eng ET, Rice WJ, Kahn PA, Potter CS, Carragher B. Spotiton: New features and applications. Journal of structural biology. 2018;202(2):161-9

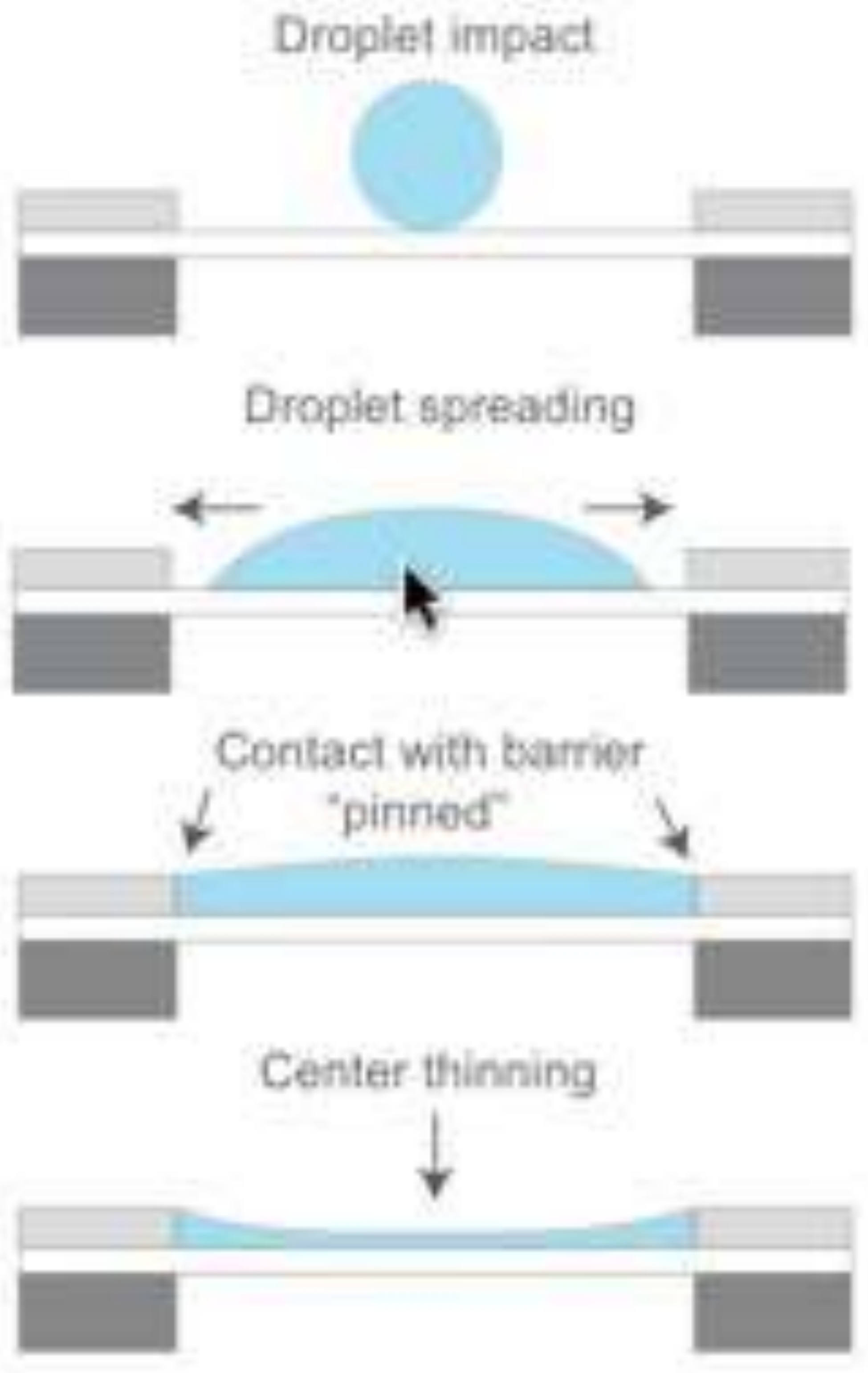
Venkat Dandey

Hui Wei

Accurate pL dispensing



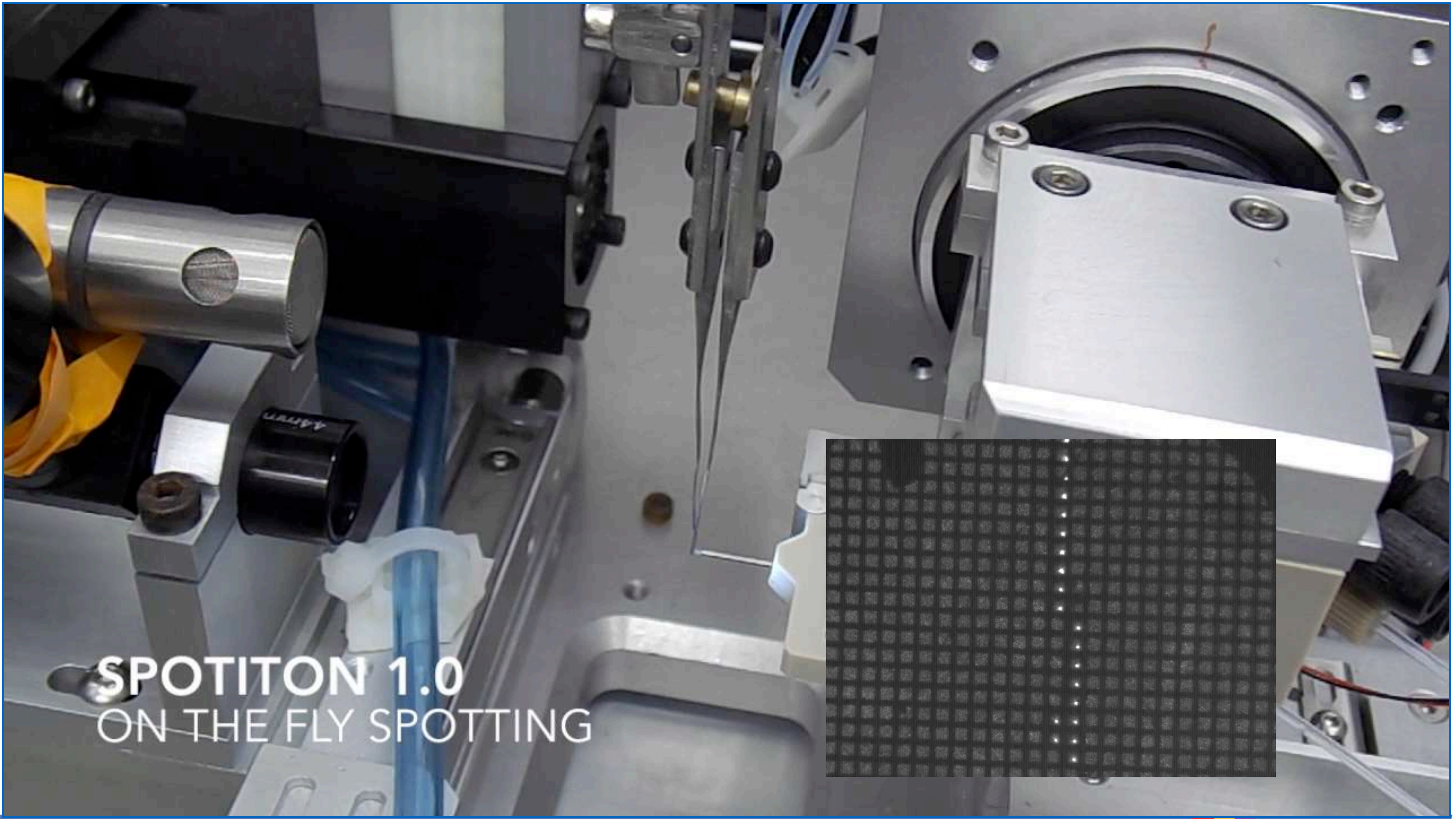
Thin films without blotting



Other methods

Spotiton

Improving Current CryoTEM Grid Preparation Methods



Dandey VP, Wei H, Zhang Z, Tan YZ, Acharya P, Eng ET, Rice WJ, Kahn PA, Potter CS, Carragher B. Spotiton: New features and applications. Journal of structural biology. 2018;202(2):161-9

Venkat Dandey

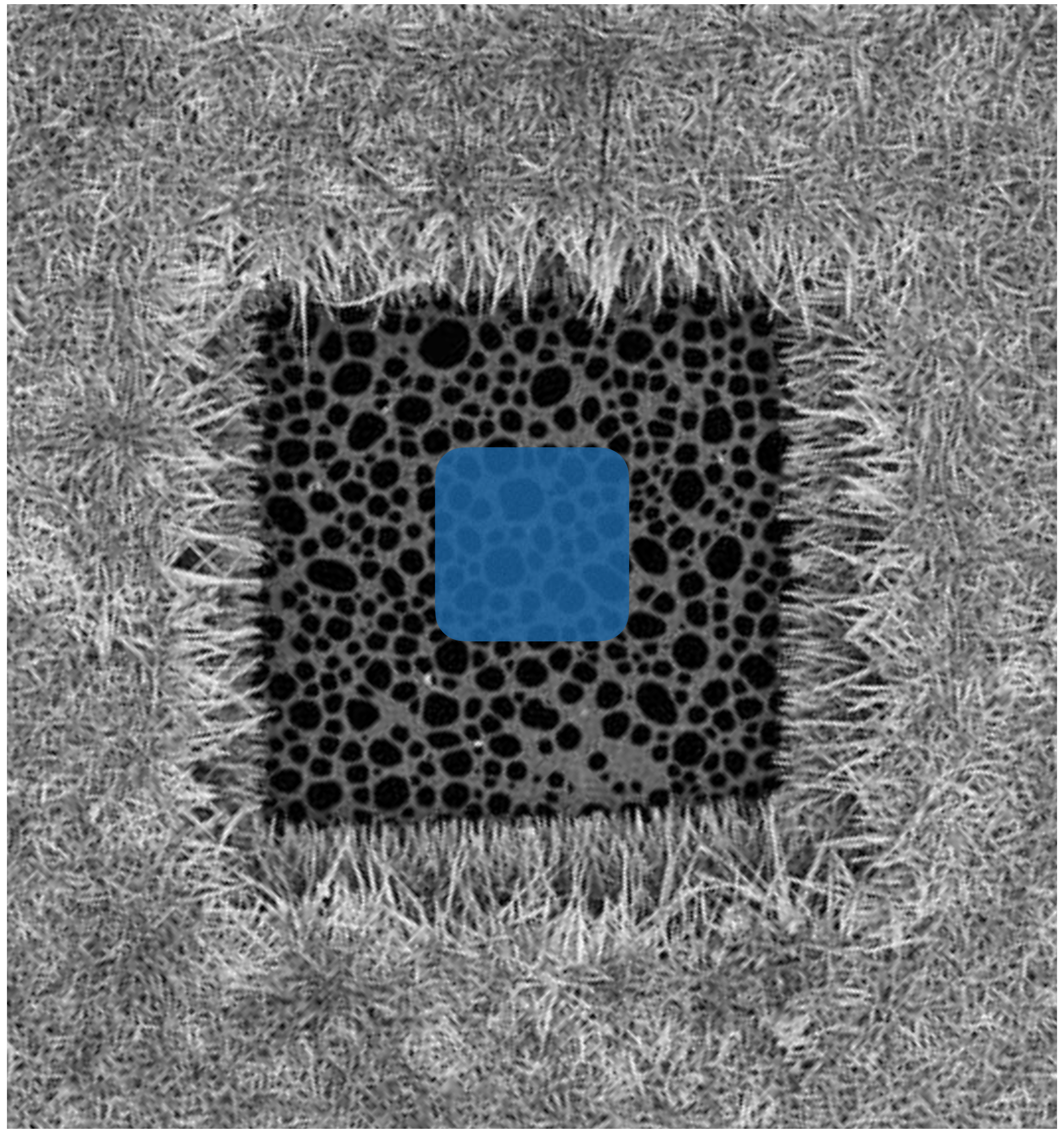
Hui Wei

Other methods

Spotiton

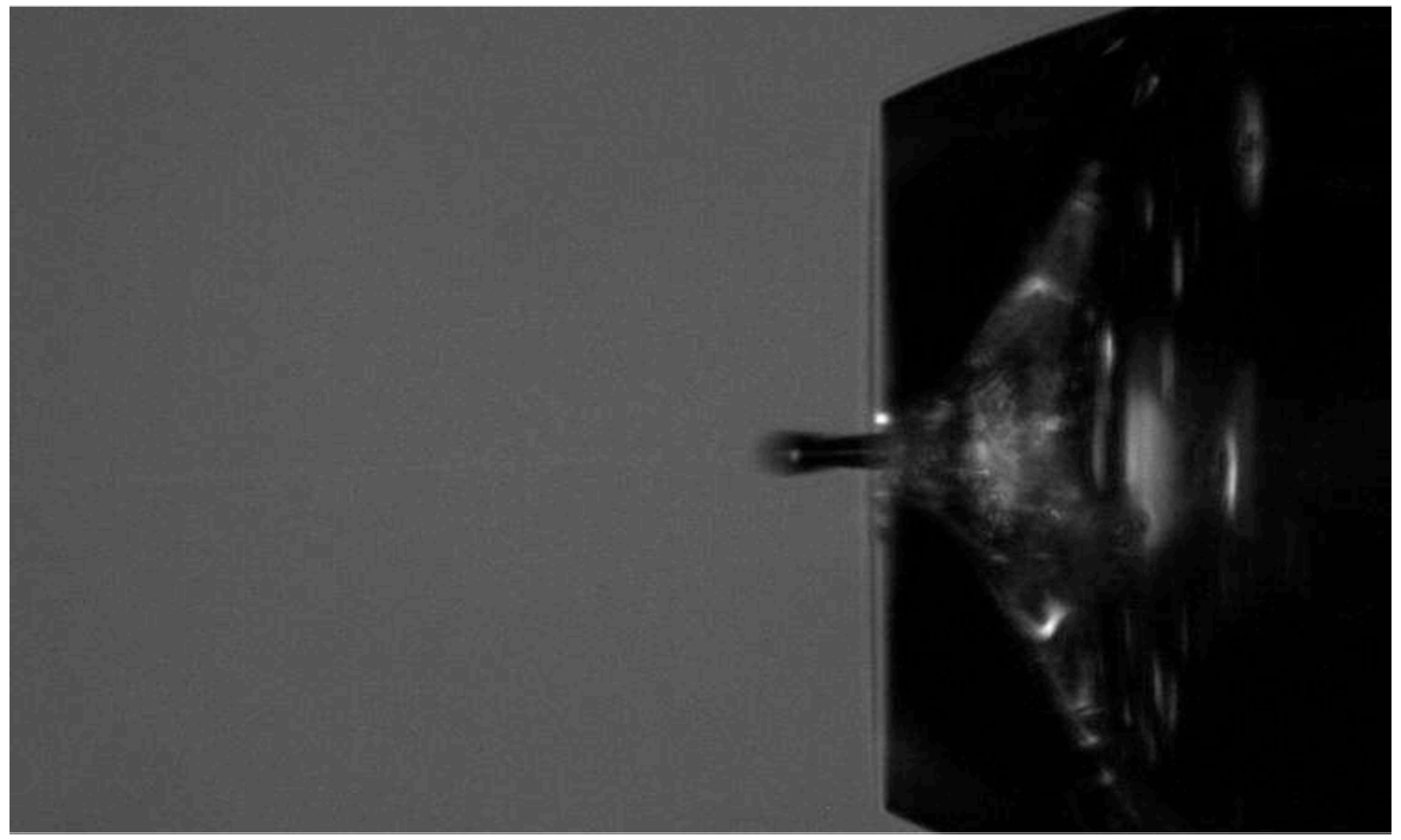
Improving Current CryoTEM Grid Preparation Methods

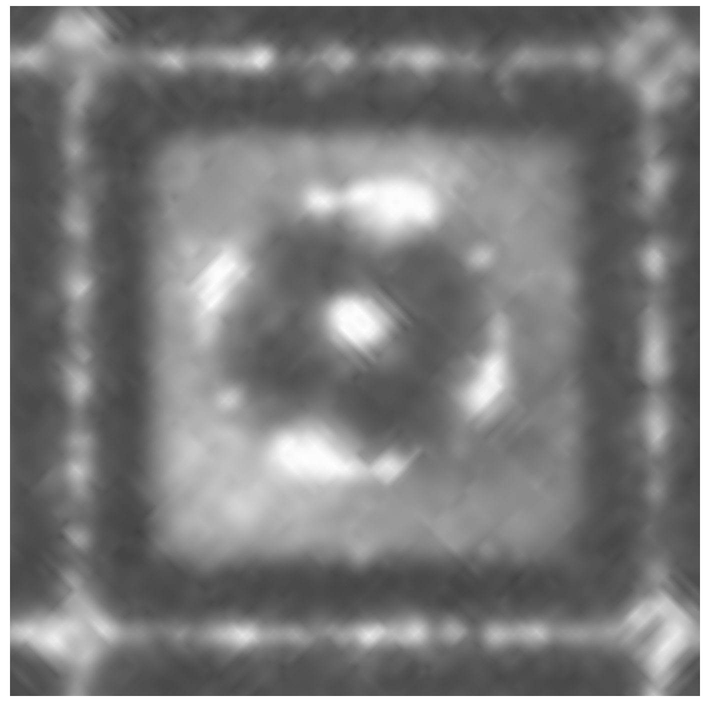
Wei H, Dandey VP, Zhang Z, Raczkowski A, Rice WJ, Carragher B, Potter CS. Optimizing "self-wicking" nanowire grids. J Struct Biol. 2018;202(2):170-4.



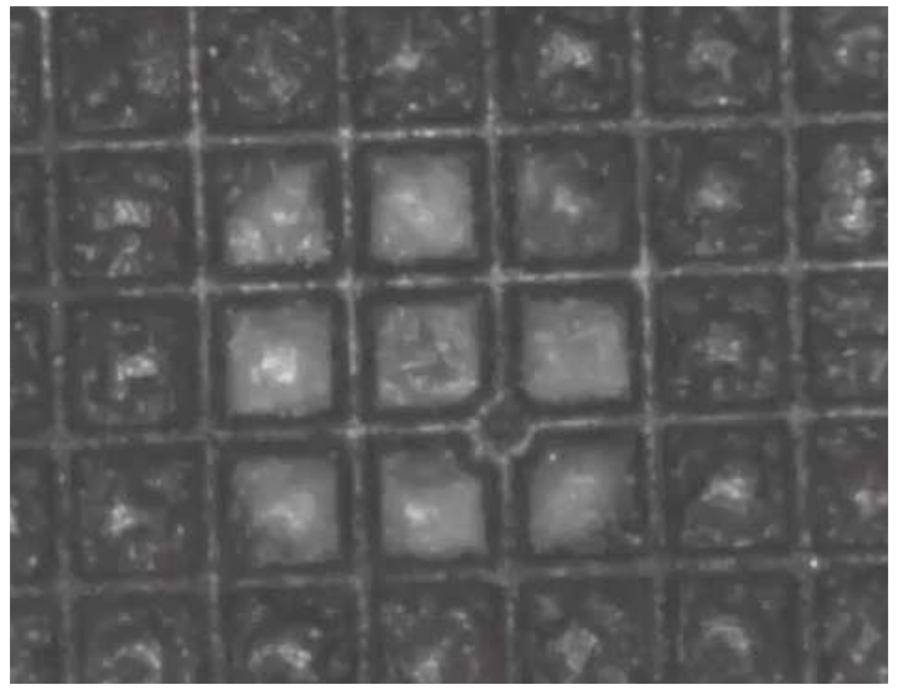
Venkat Dandey

Hui Wei





Single frame from loop



Video loop

Other methods

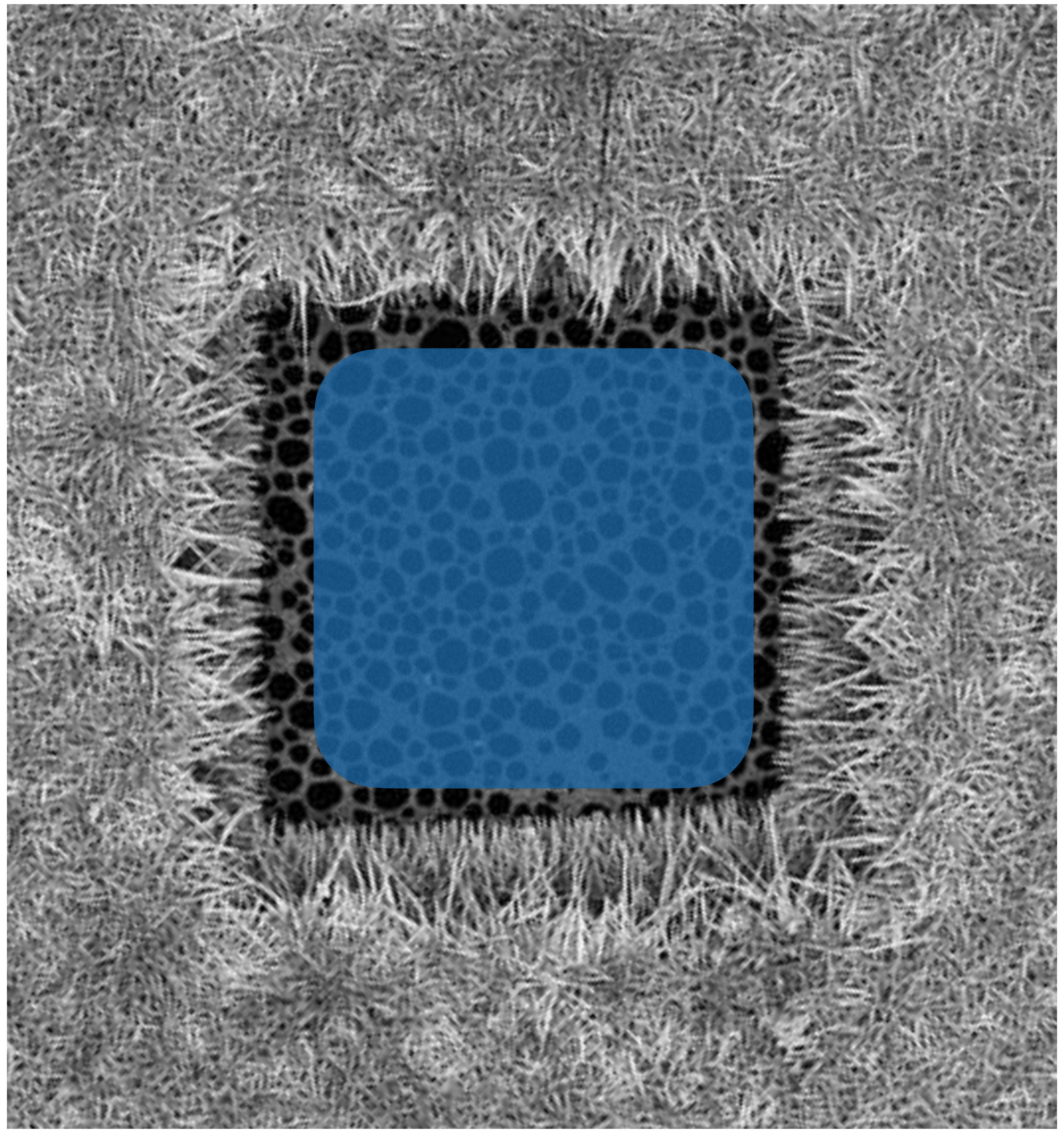
Spotiton

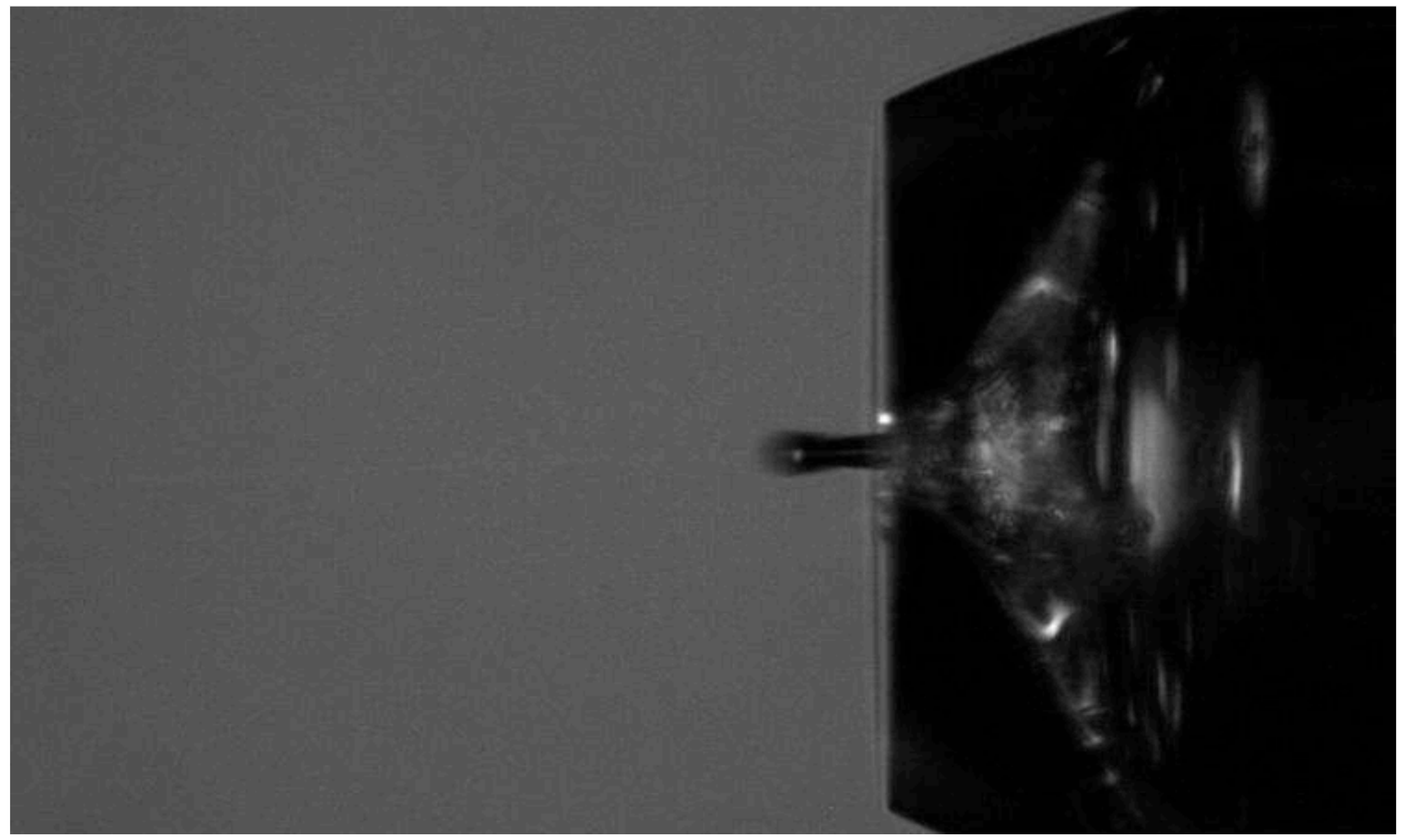
Improving Current CryoTEM Grid Preparation Methods

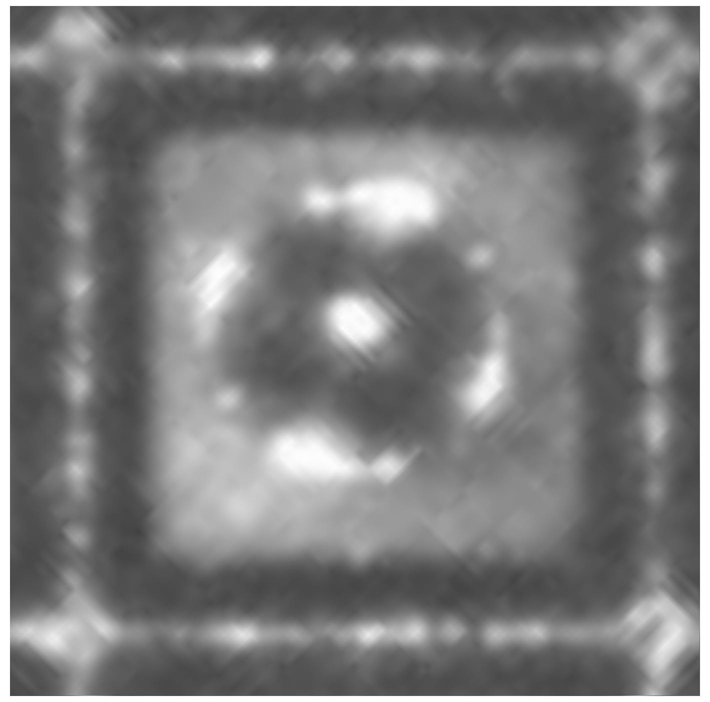
Wei H, Dandey VP, Zhang Z, Raczkowski A, Rice WJ, Carragher B, Potter CS. Optimizing "self-wicking" nanowire grids. J Struct Biol. 2018;202(2):170-4.

Venkat Dandey

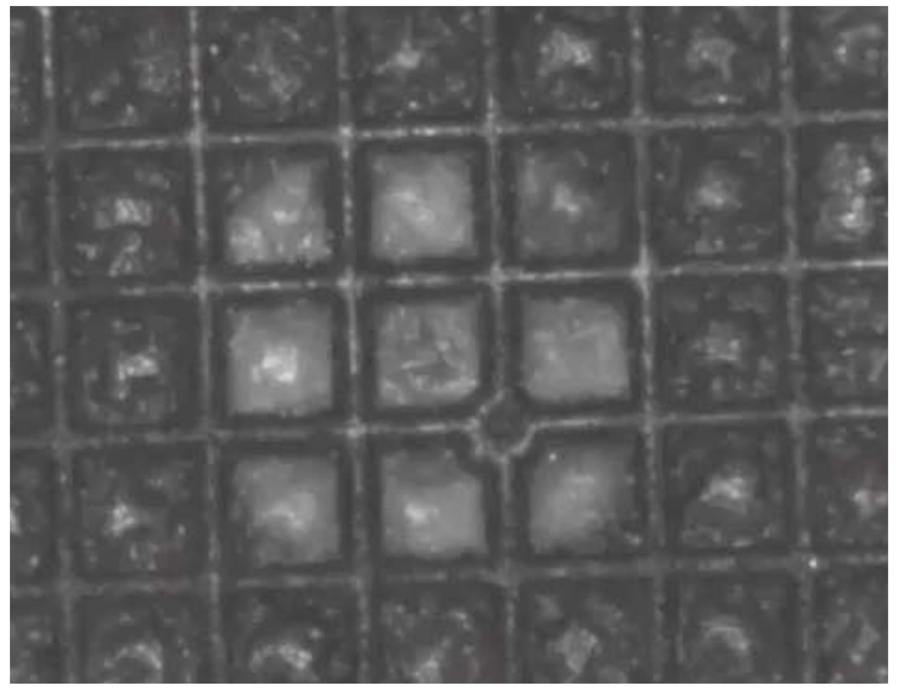
Hui Wei







Single frame from loop



Video loop

Other methods

Spotiton

Improving Current CryoTEM Grid Preparation Methods

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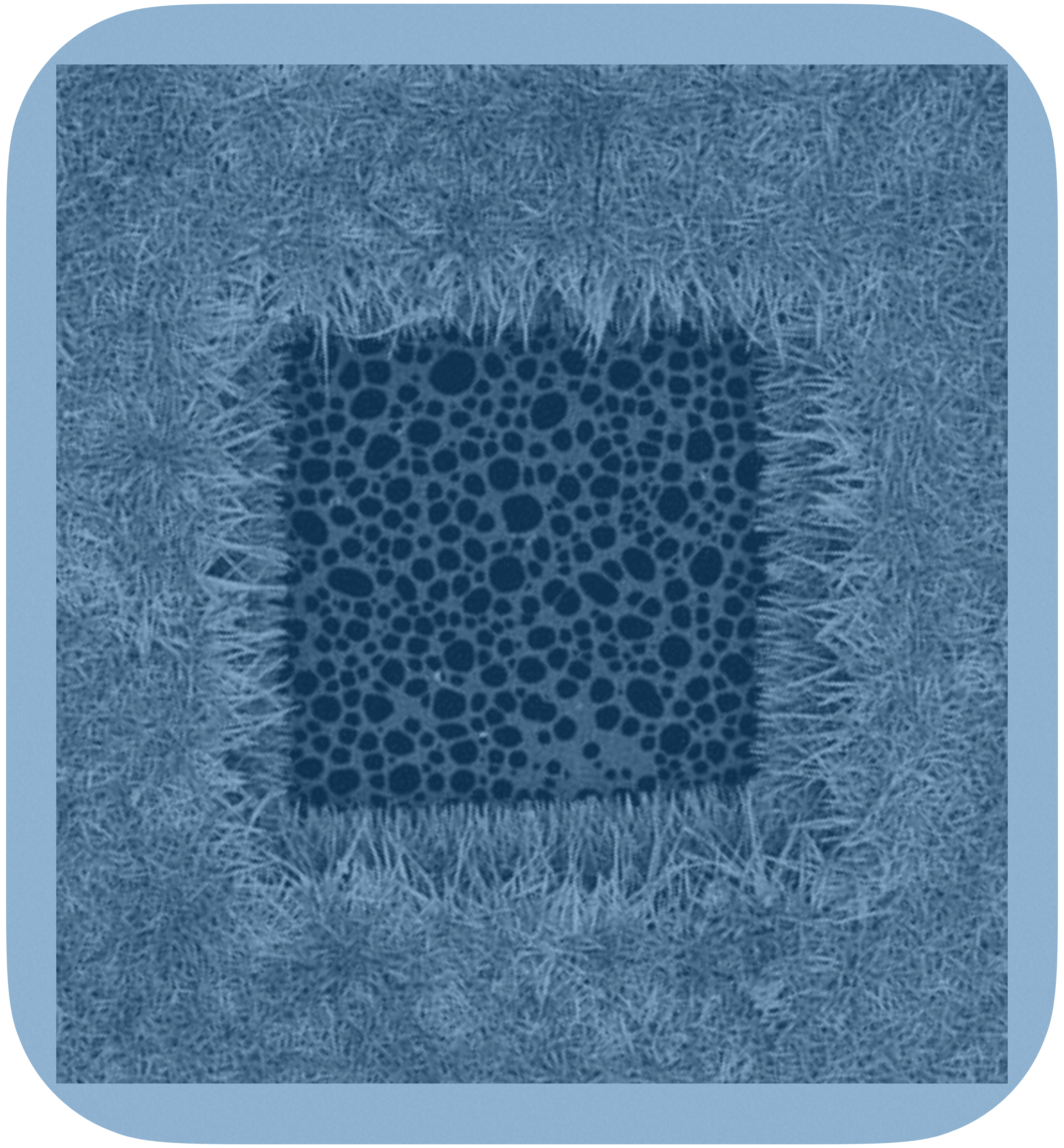
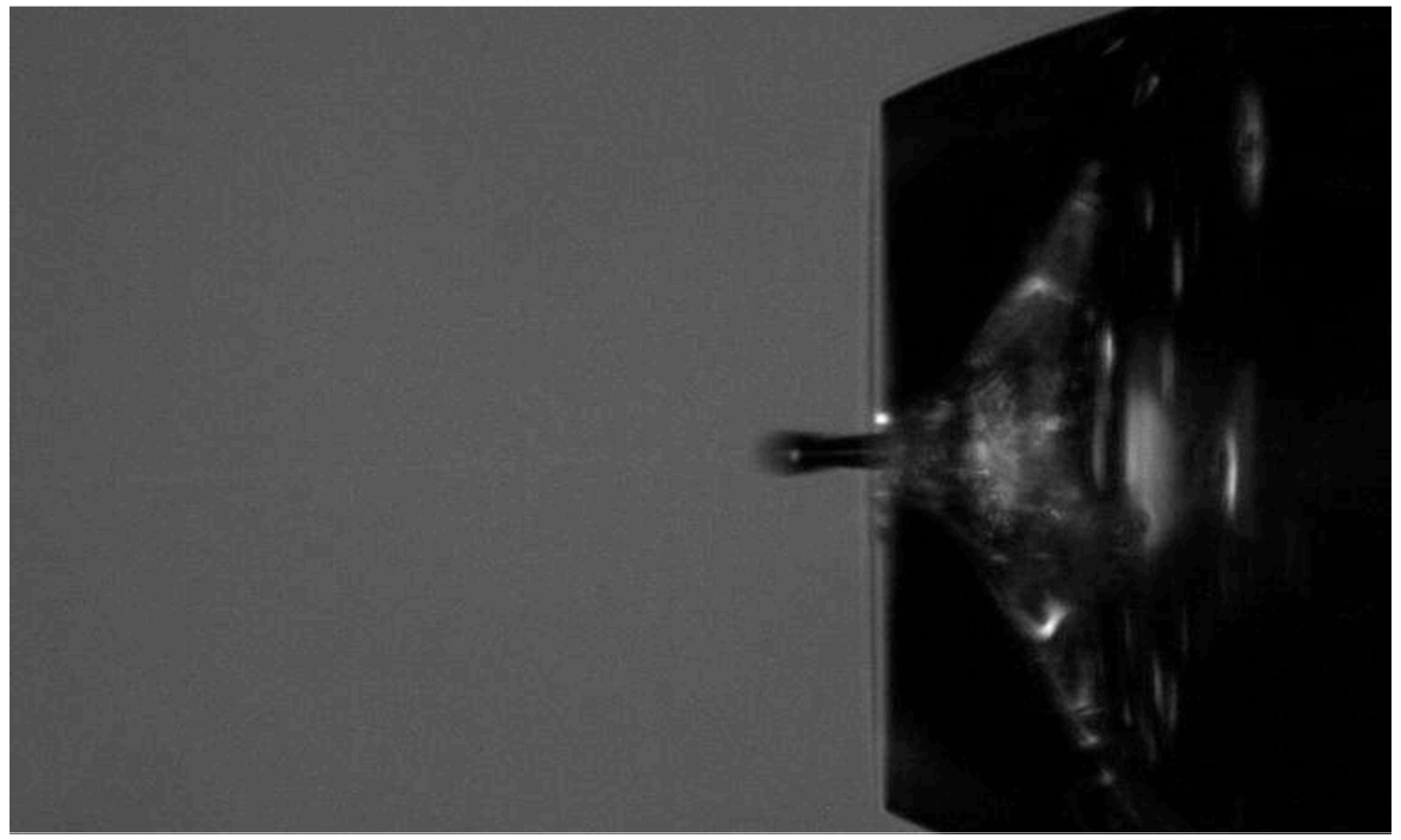
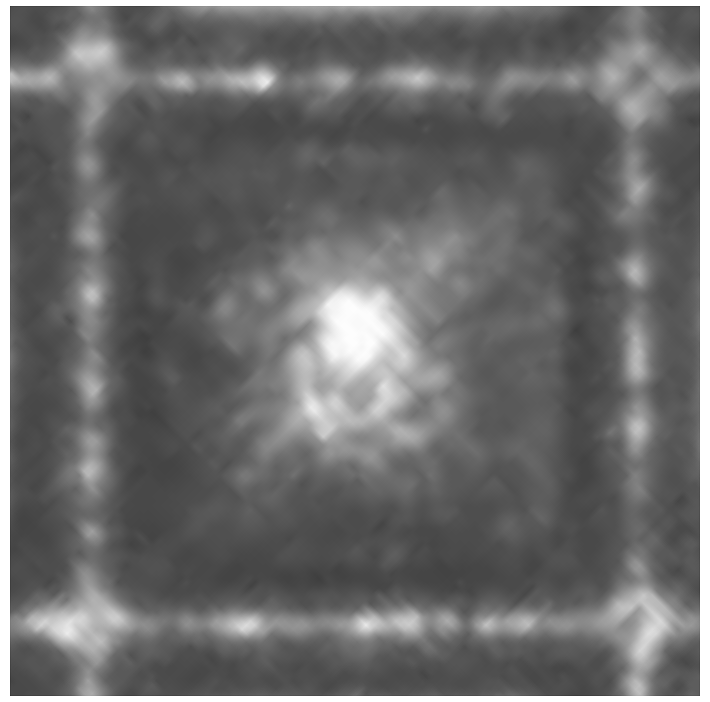
Venkat Dandey

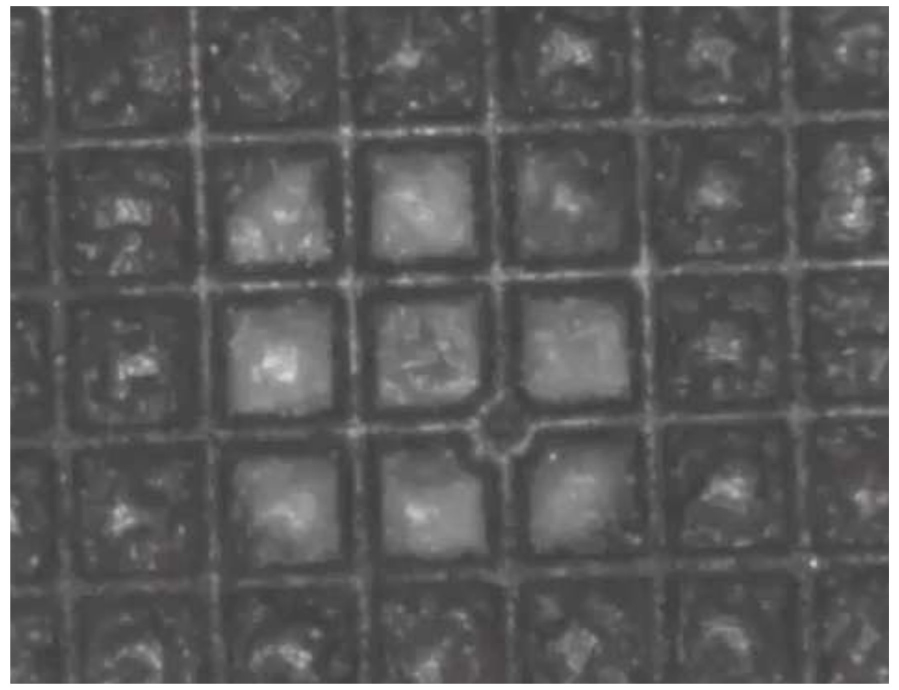
Hui Wei







Single frame from loop



Video loop

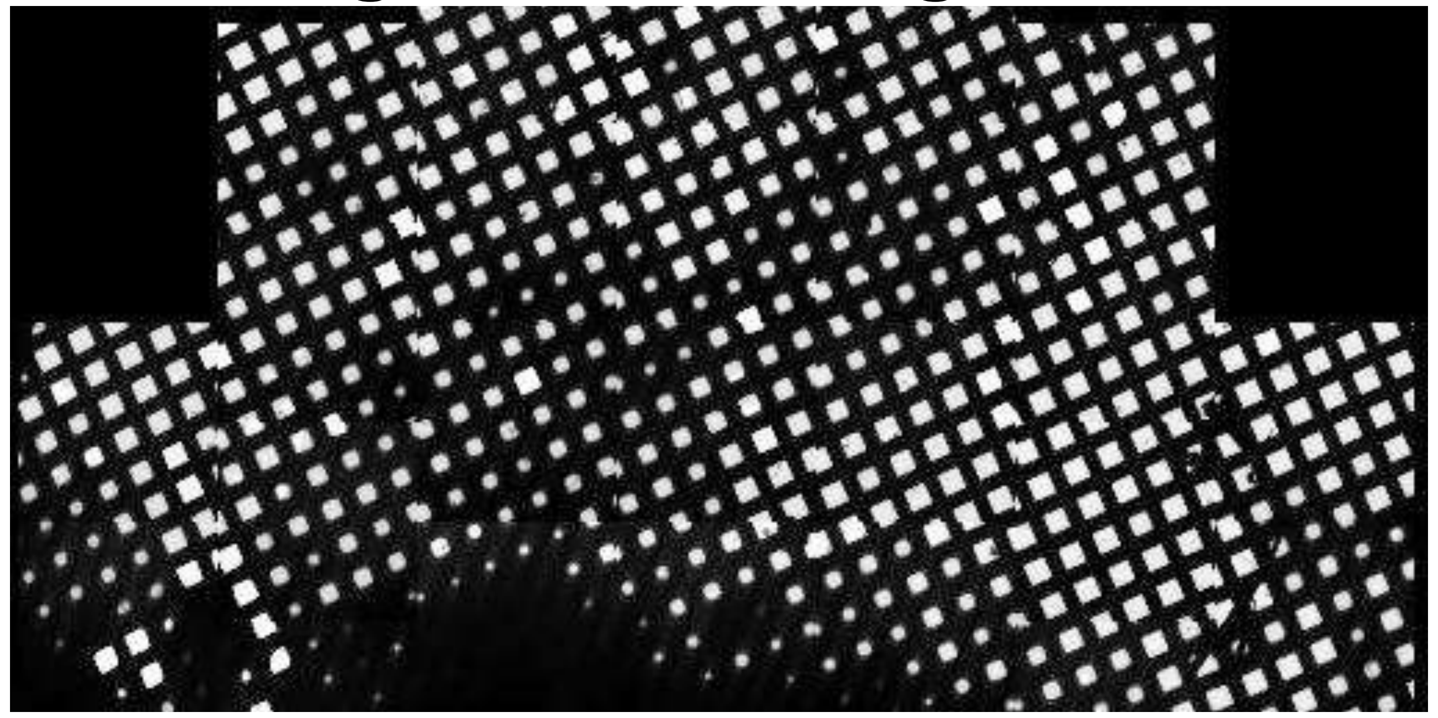
Other methods

Spotiton

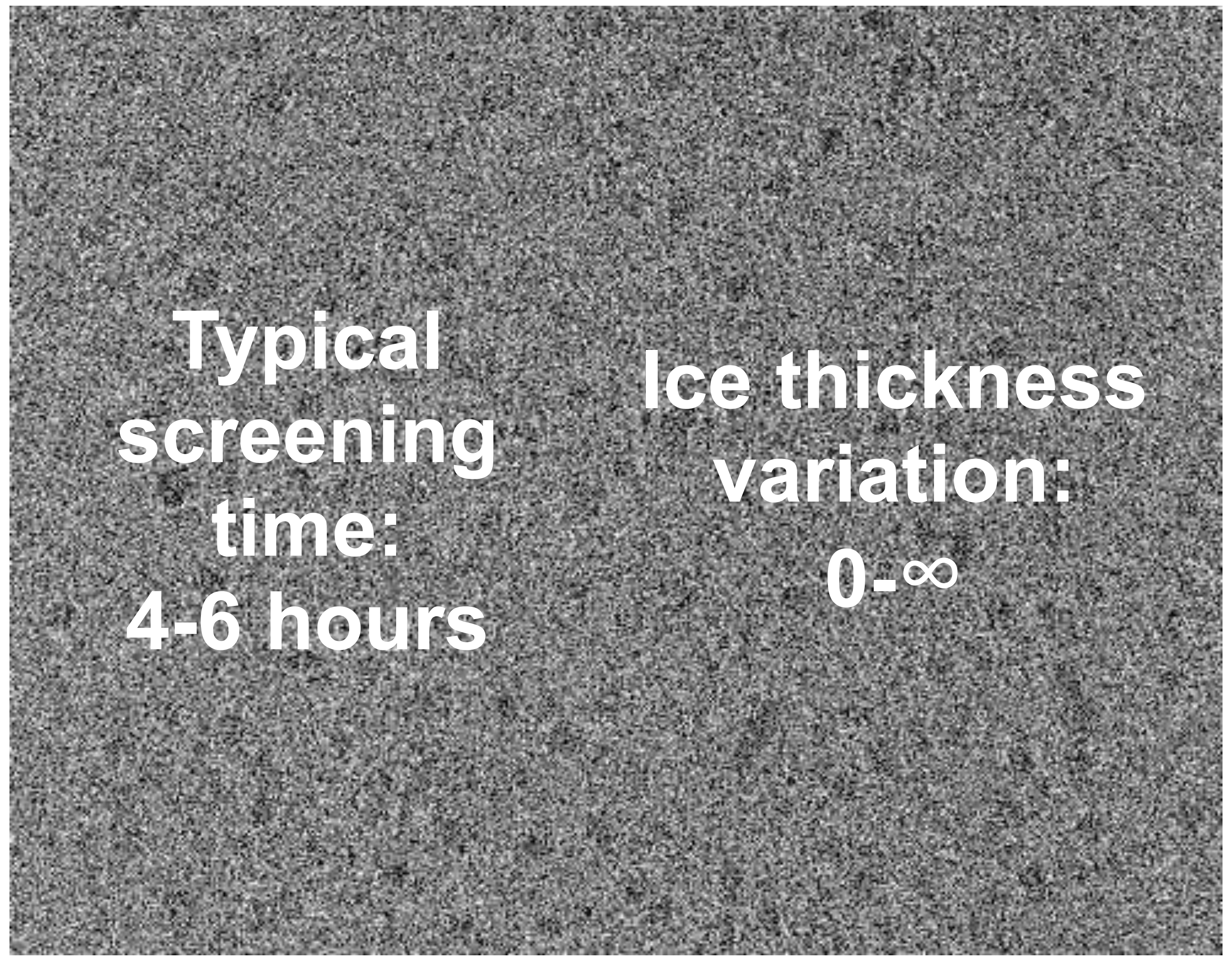
Improving Current CryoTEM Grid Preparation Methods

Vitrobot

3 uL of sample required for each grid; ~2nL on grid

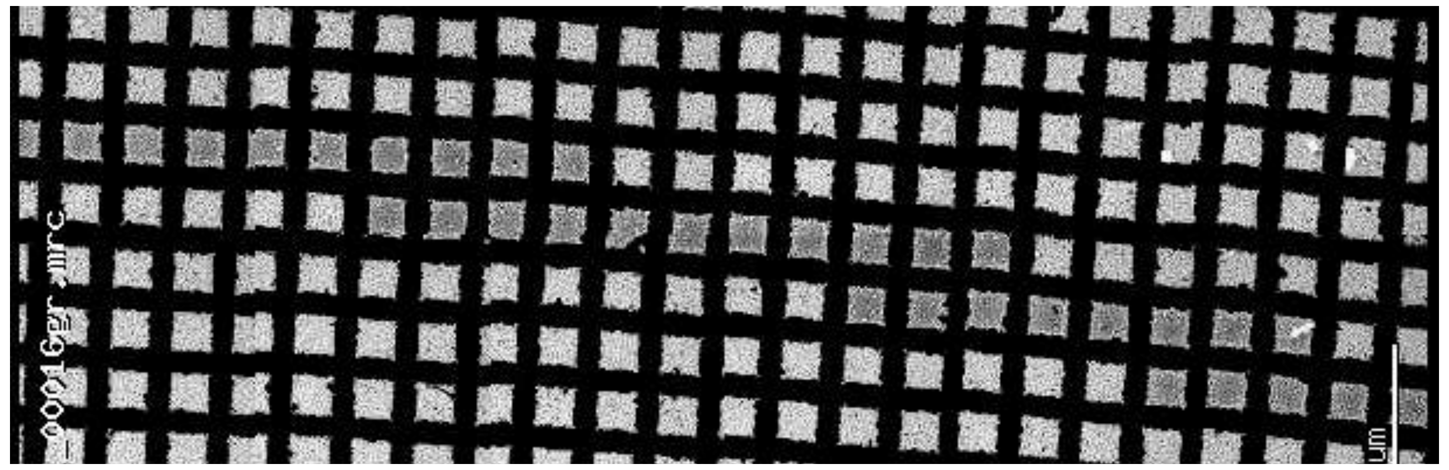


Usable area: ~0-10%

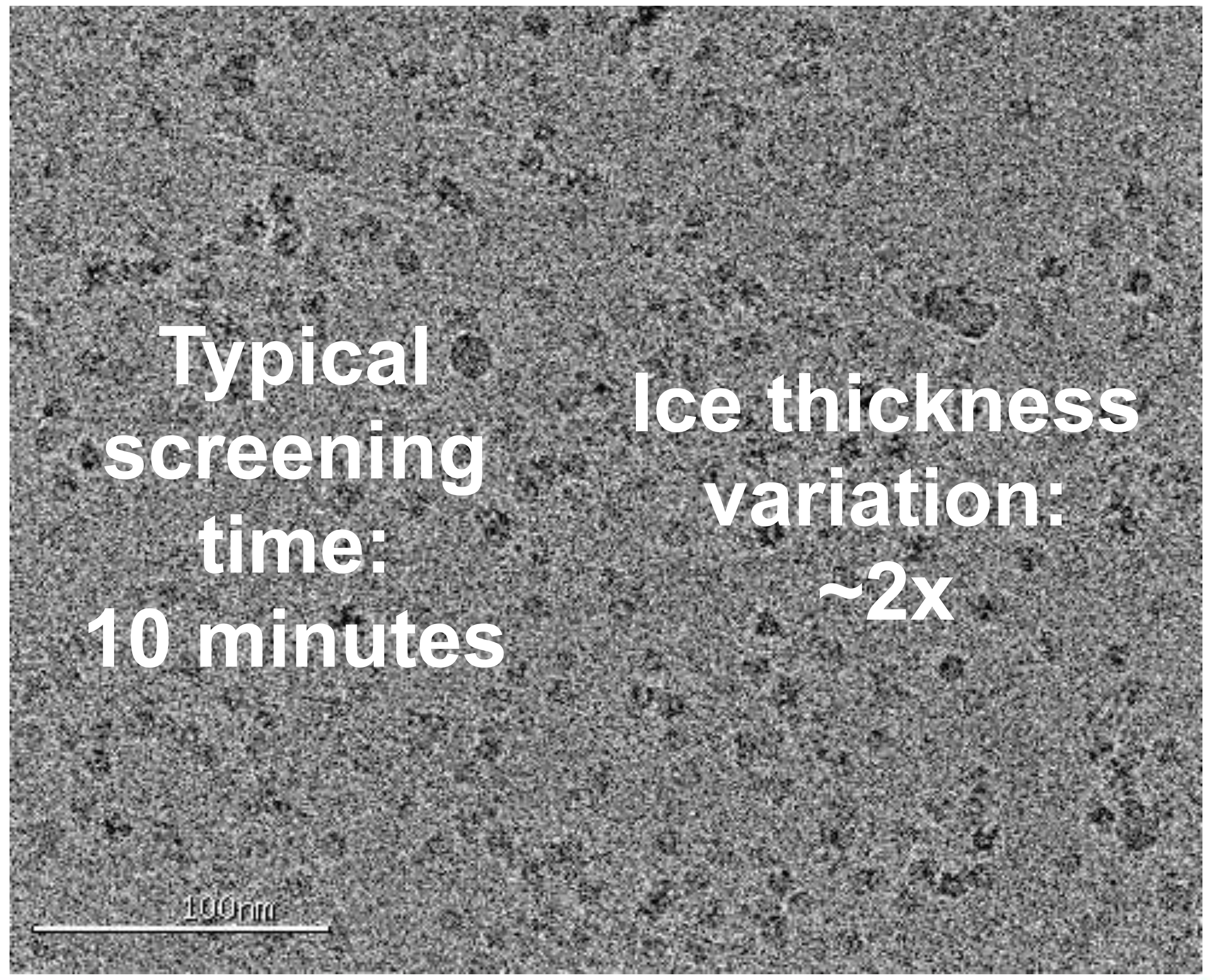


Spotiton

3 uL of sample enough for >100 grids; ~500pL on grid



Usable area: ~100%



Wei H, Dandey VP, Zhang Z, Raczkowski A, Rice WJ, Carragher B, Potter CS. Optimizing "self-wicking" nanowire grids. J Struct Biol. 2018;202(2):170-4.

Venkat Dandey

Hui Wei

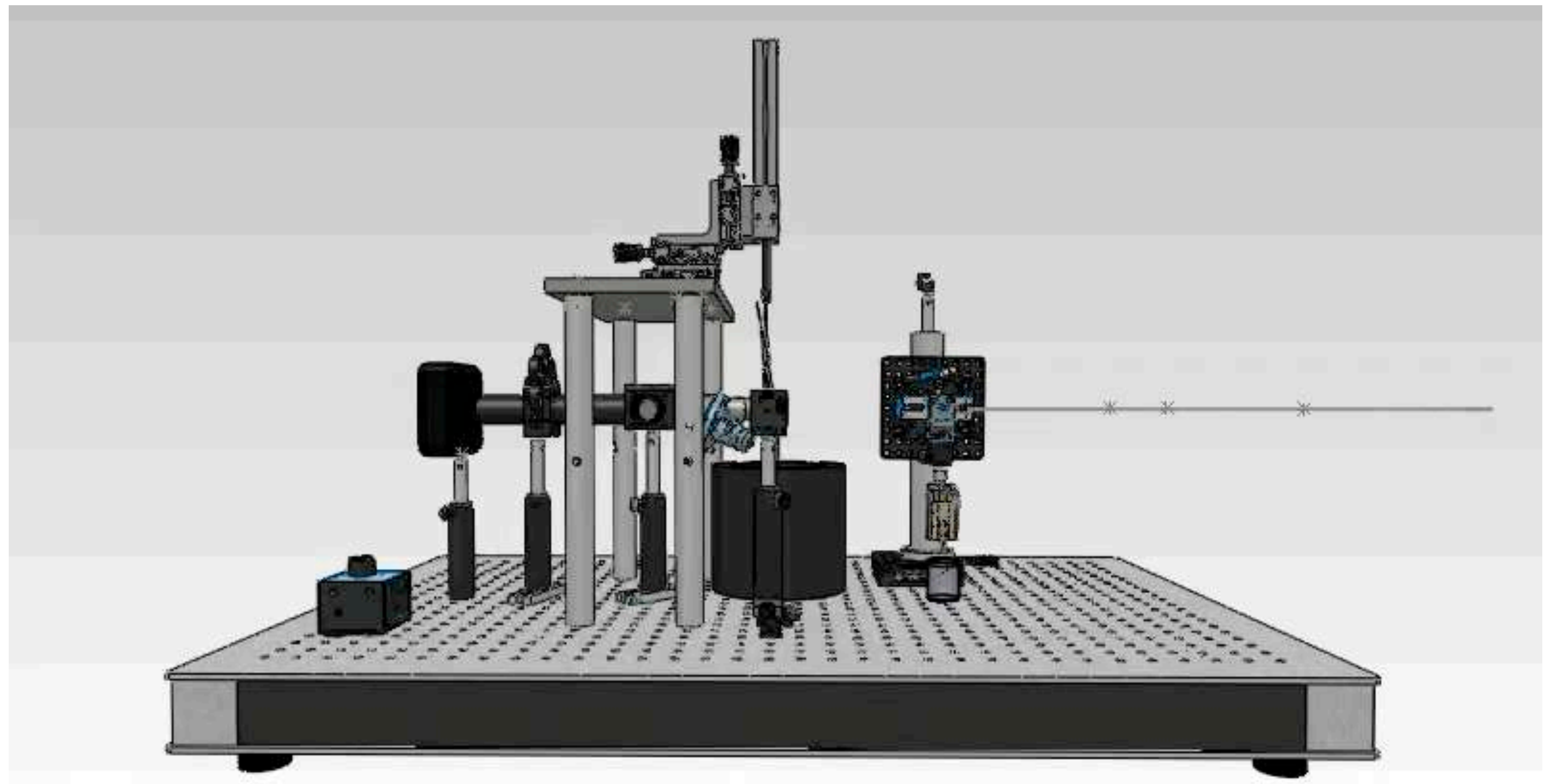
What is chameleon?

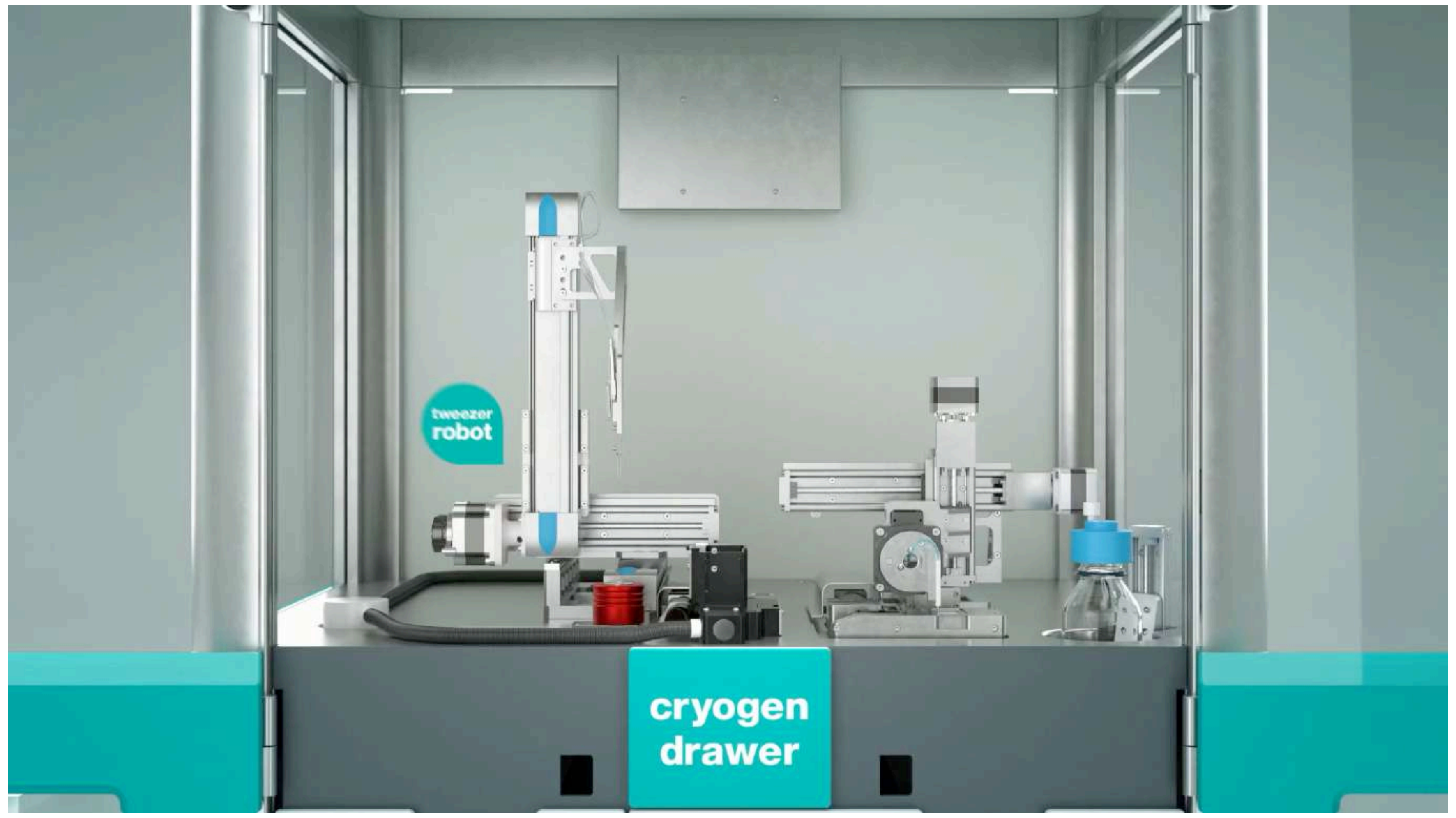
Spotiton

The Spotiton Project: Commercialization

Spotiton concept: 2011

Chameleon: 2019



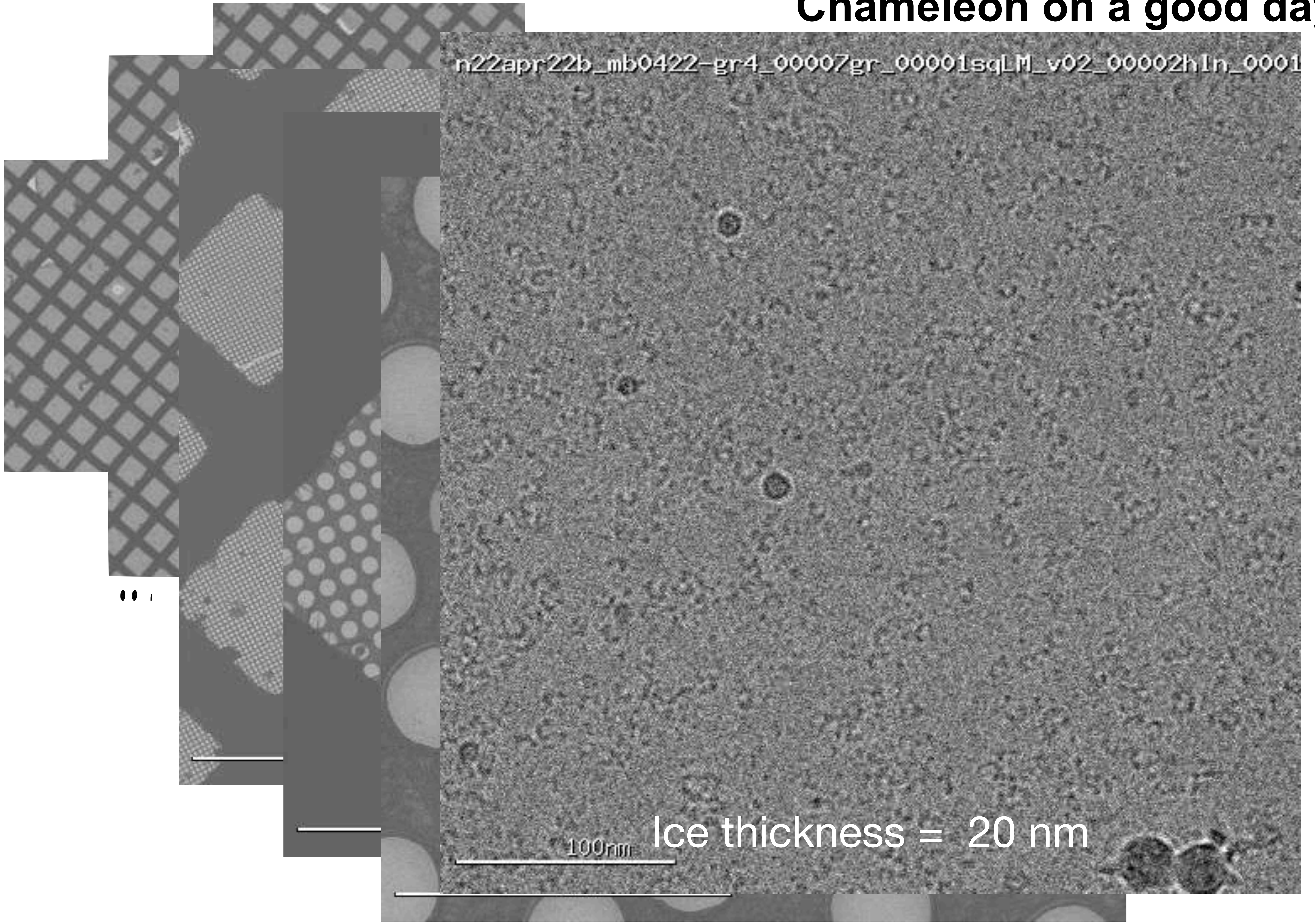


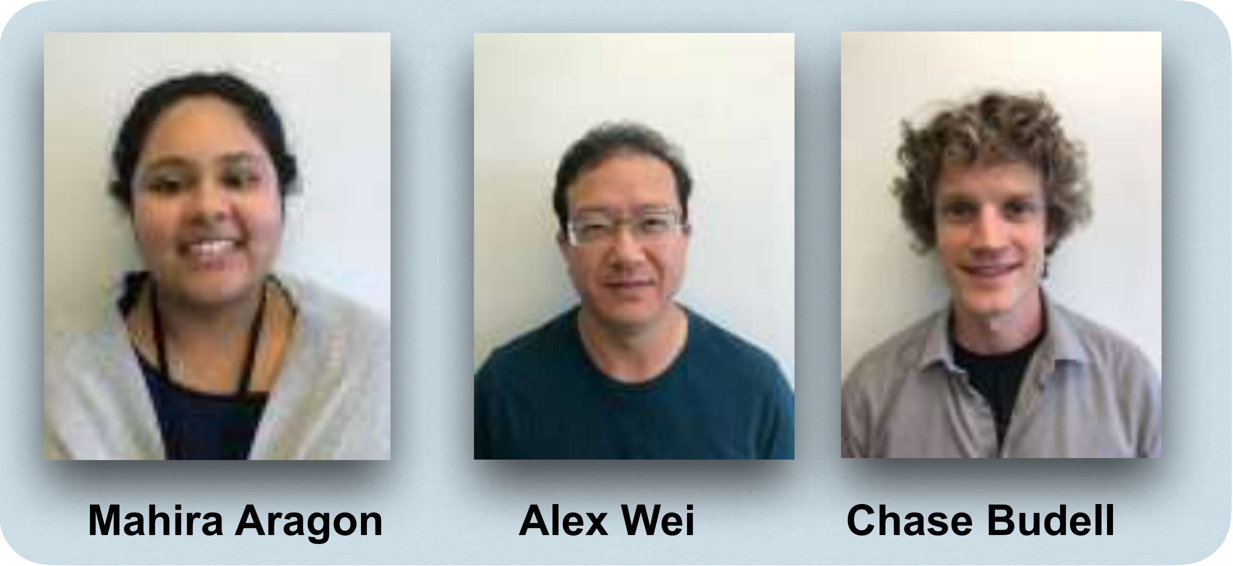
sptlabtech

Venkat Dandey | Alex Wei | Chase Budell

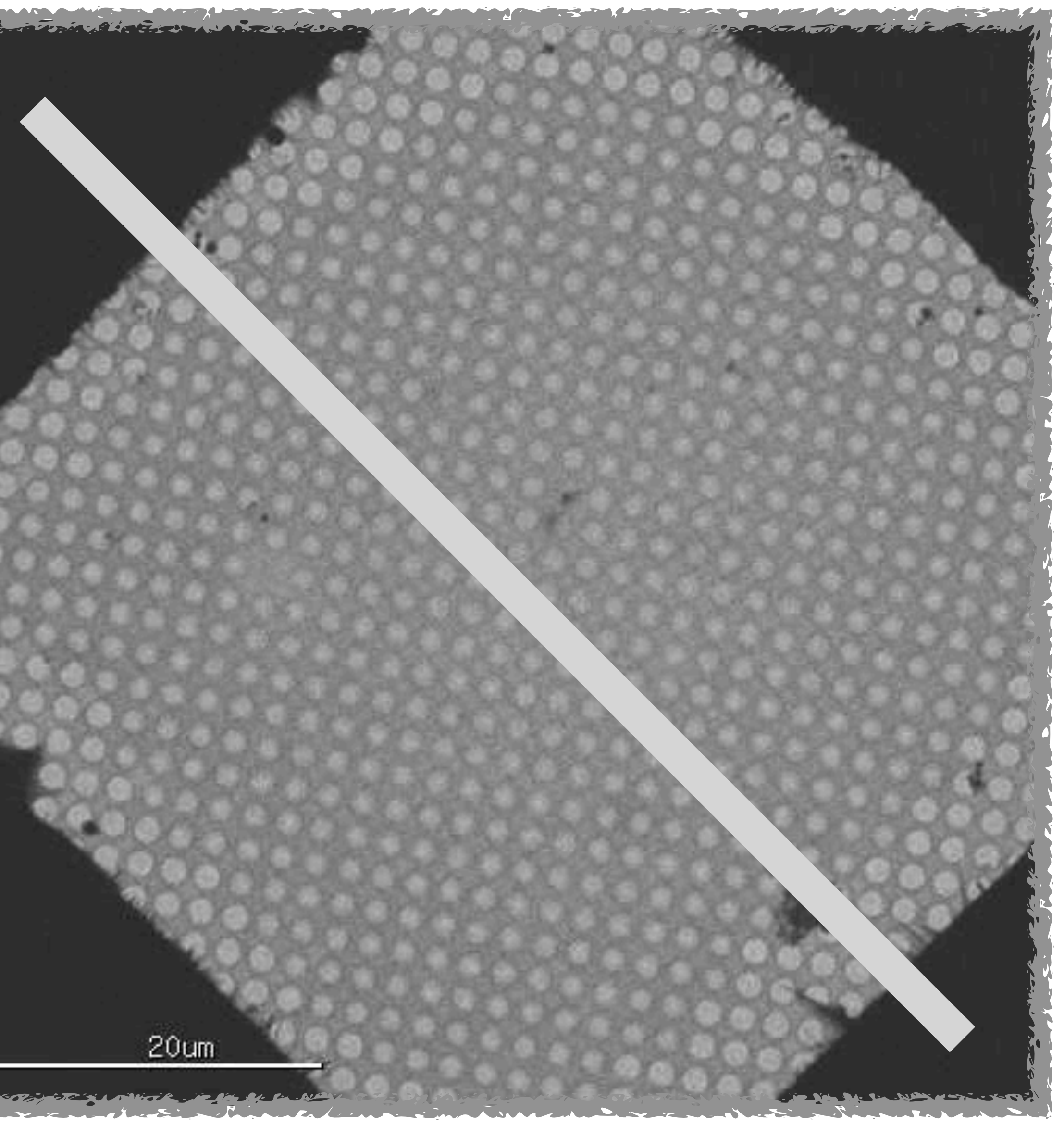
What is chameleon?

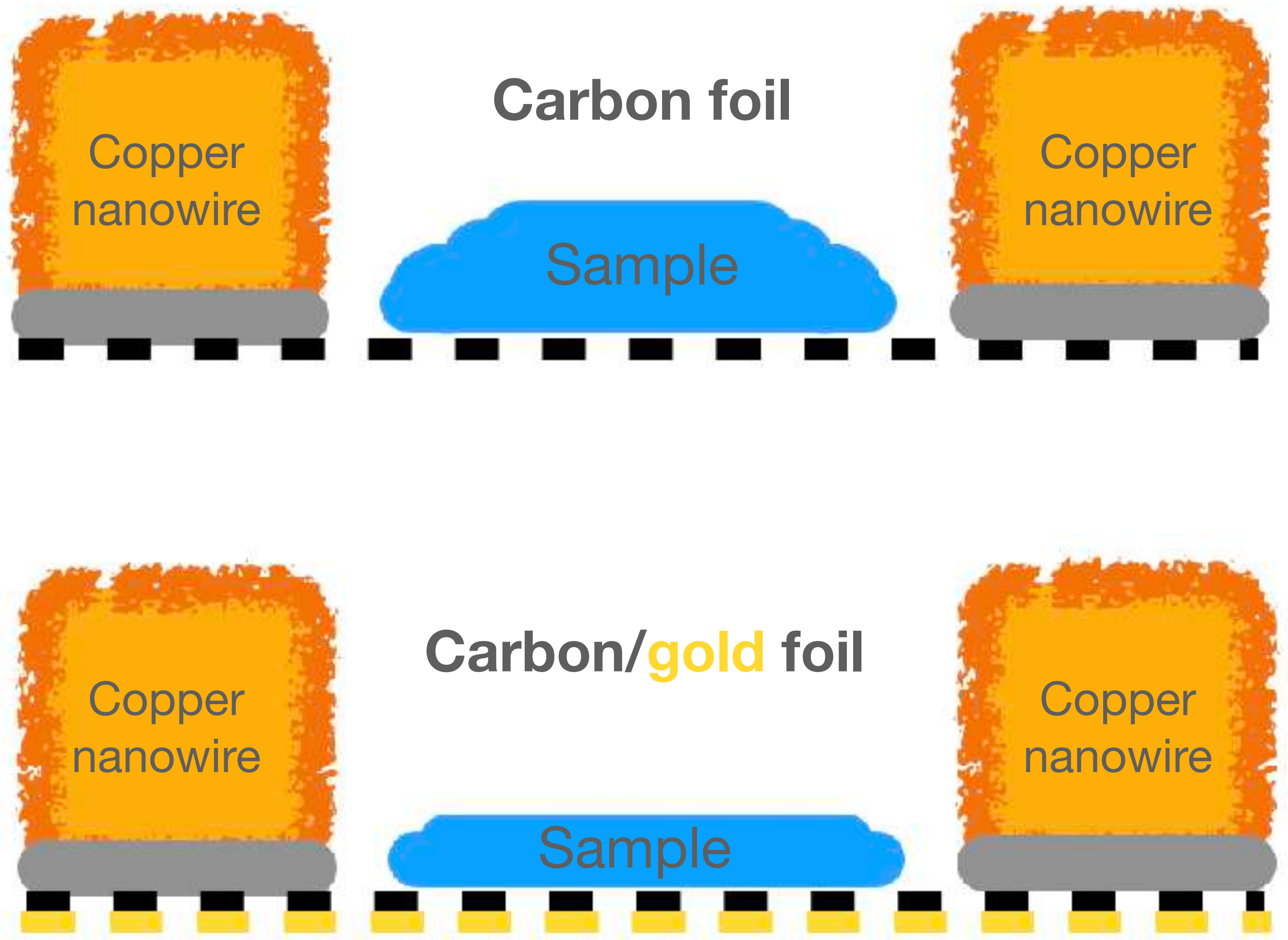
Chameleon on a good day

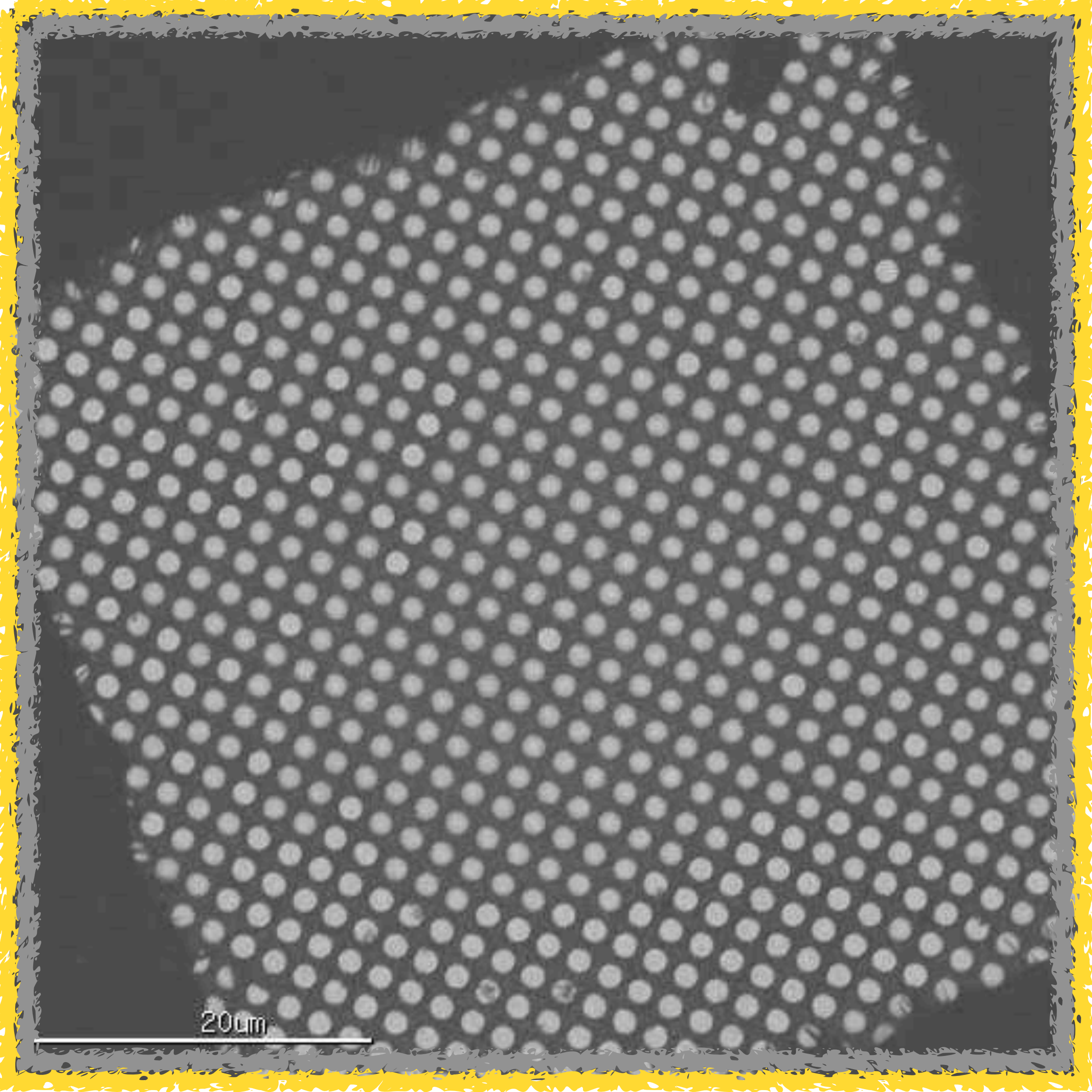




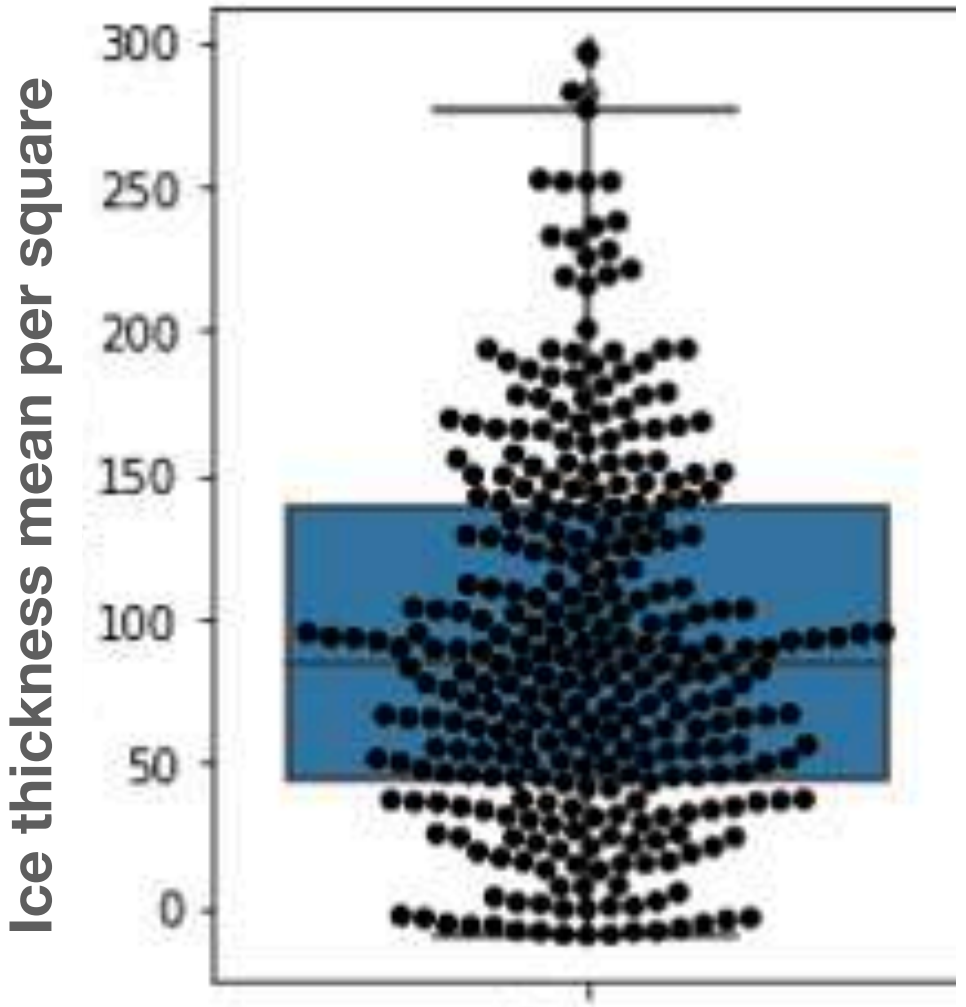
What is chameleon?



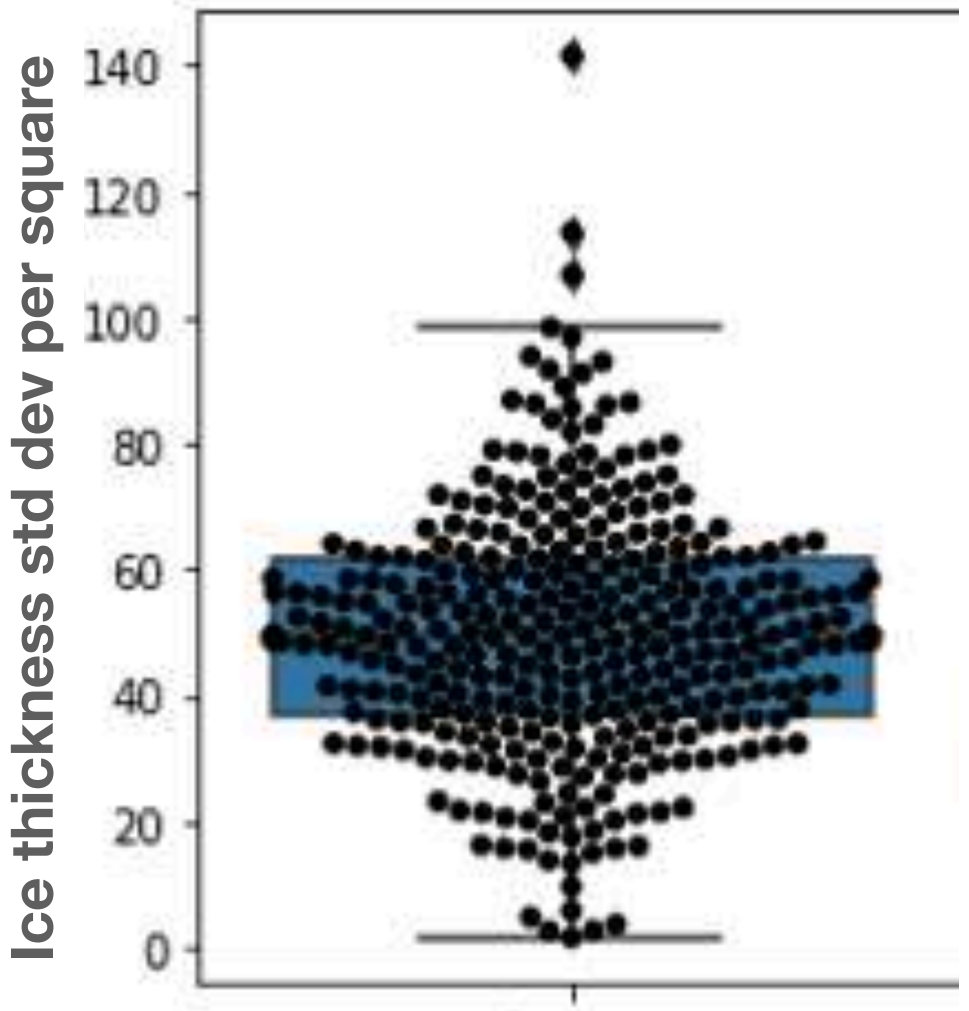




Spotiton



Carbon foil



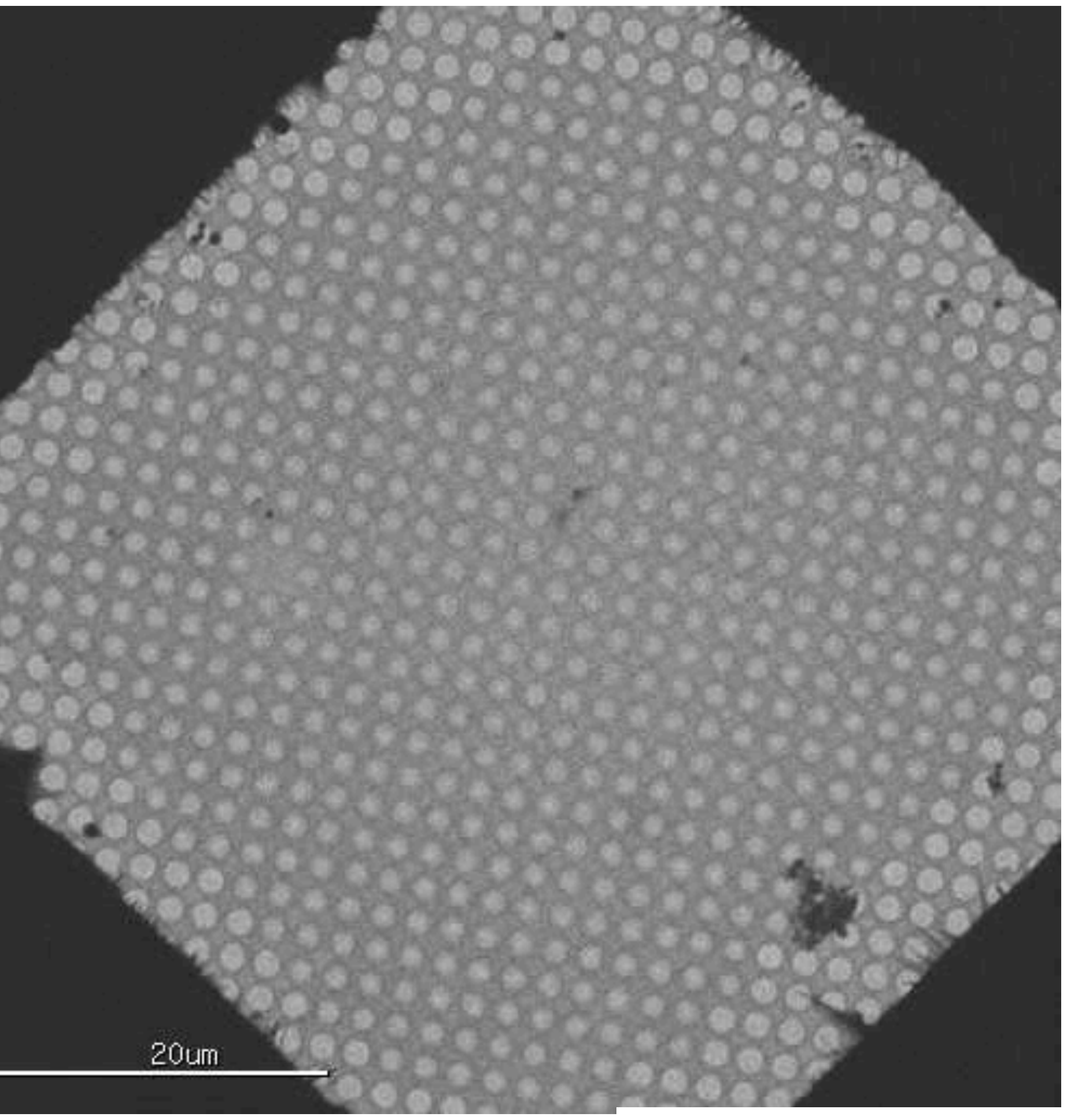
Carbon foil

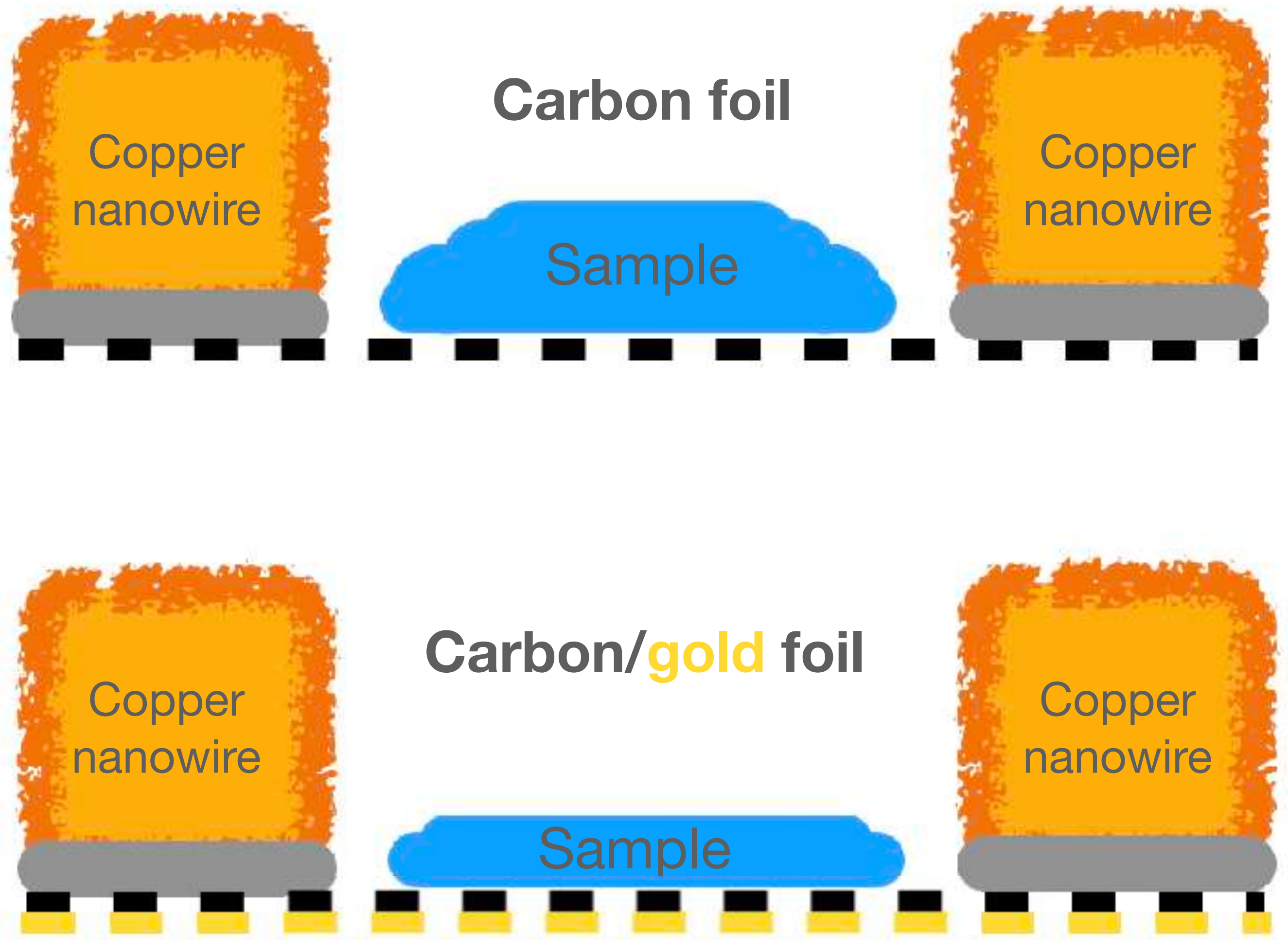
Alex Wei

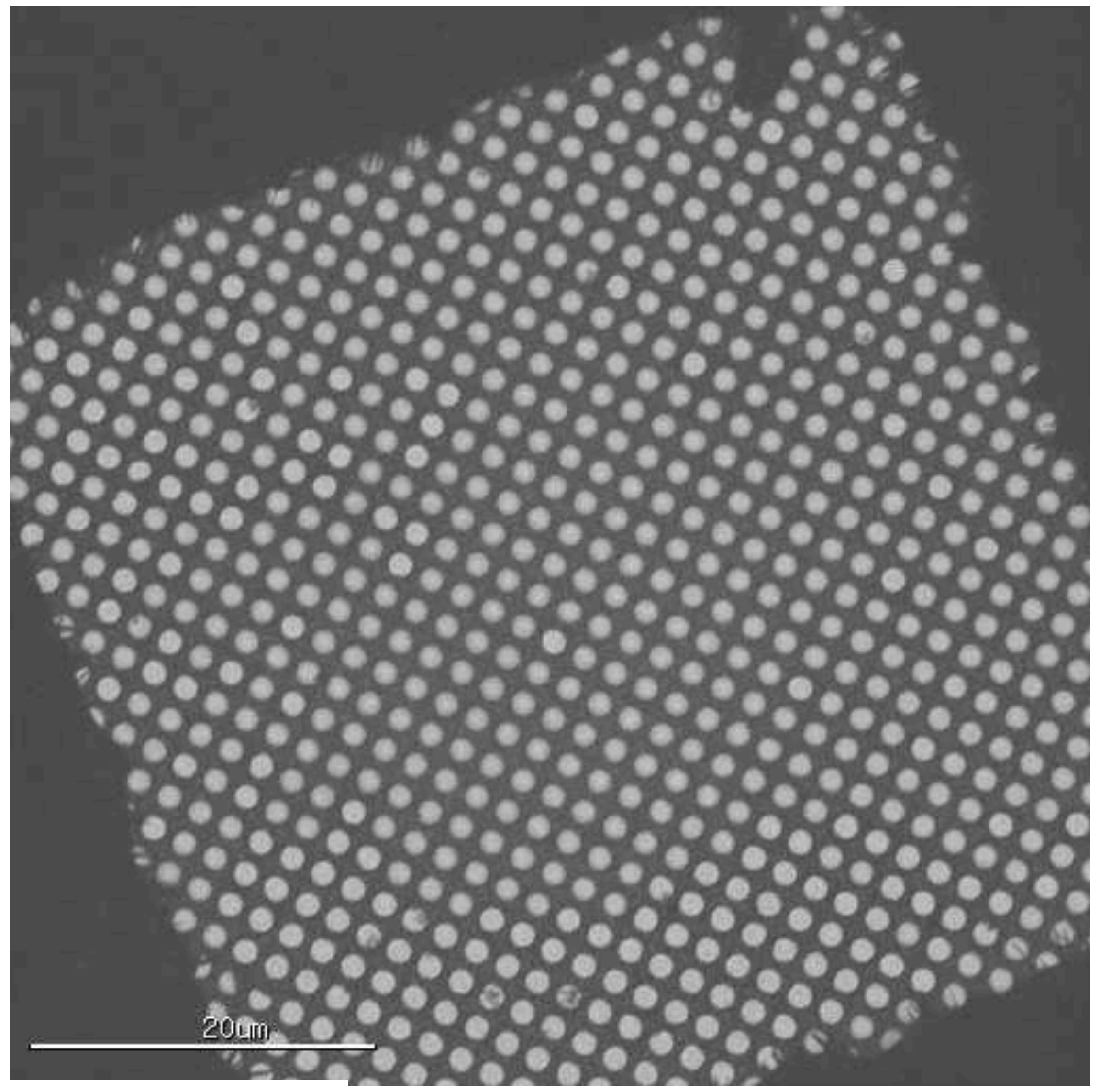
Eugene Chua

What is chameleon?

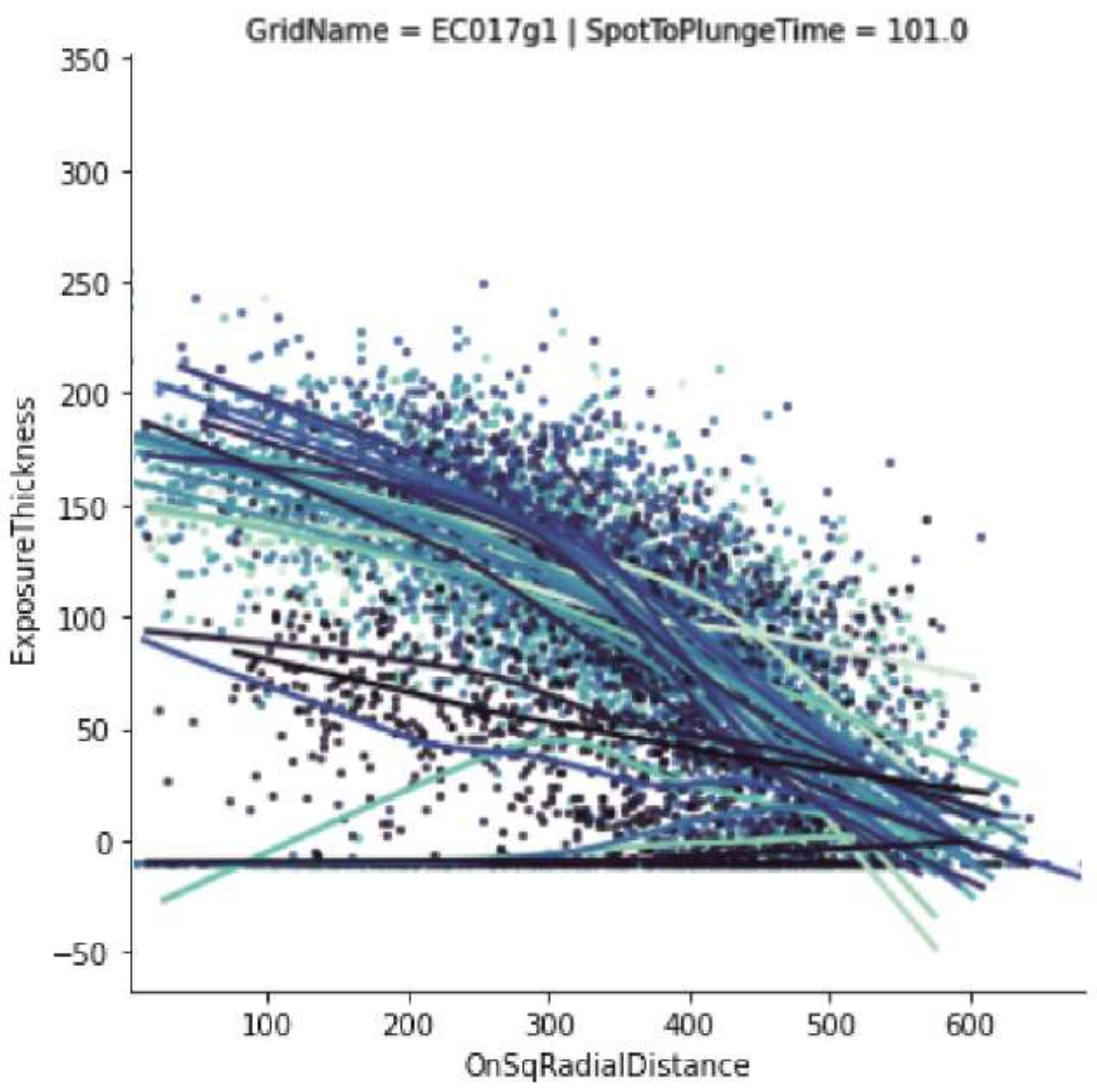
Spotiton



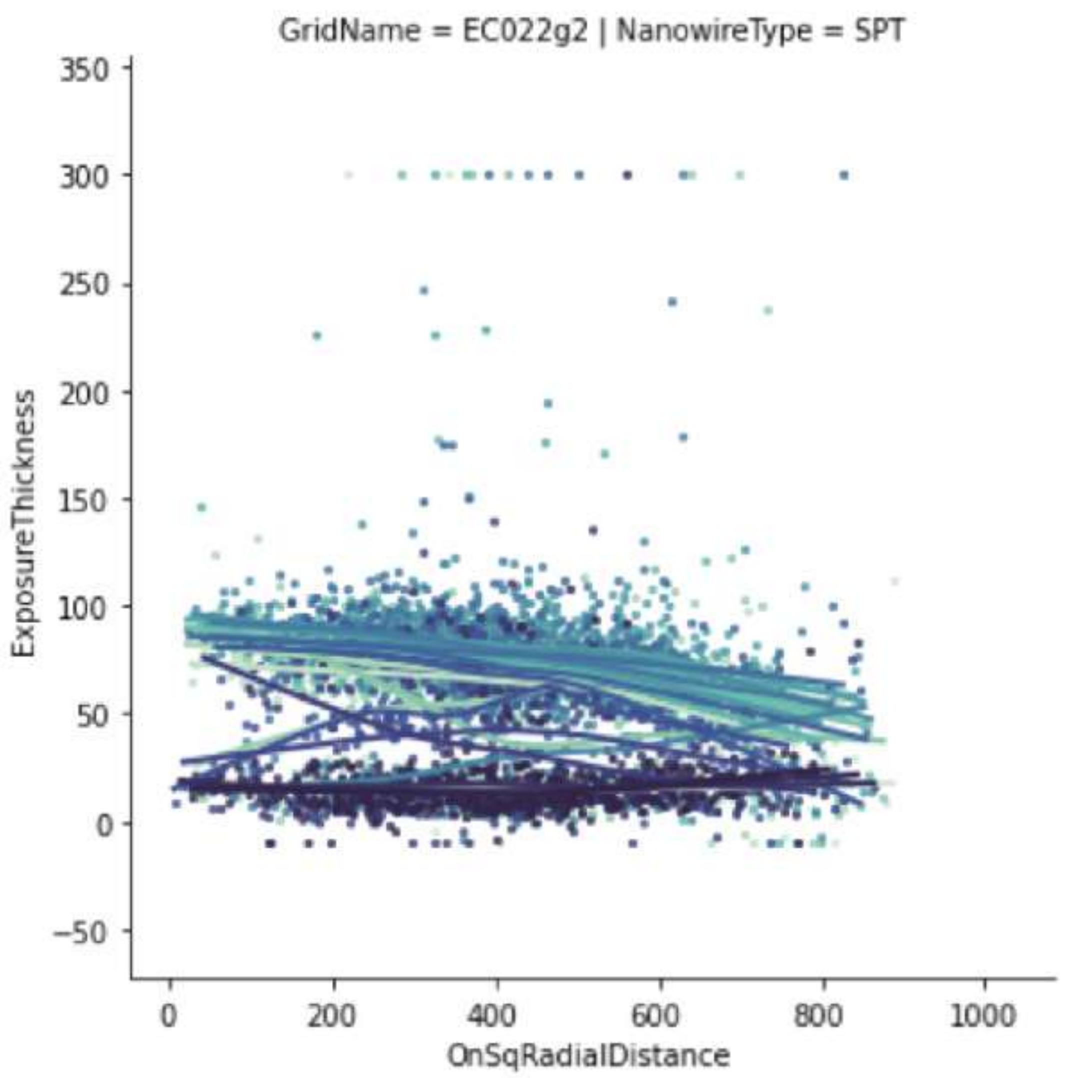




Batch 413



Batch 392



Alex Wei Eugene Chua

What is chameleon?

Where is the ice relative to the grid?

Spotiton

Grid geometry

Vitreous ice ideal -> typical thickness

What is chameleon?

Where is the ice relative to the grid?

Spotiton

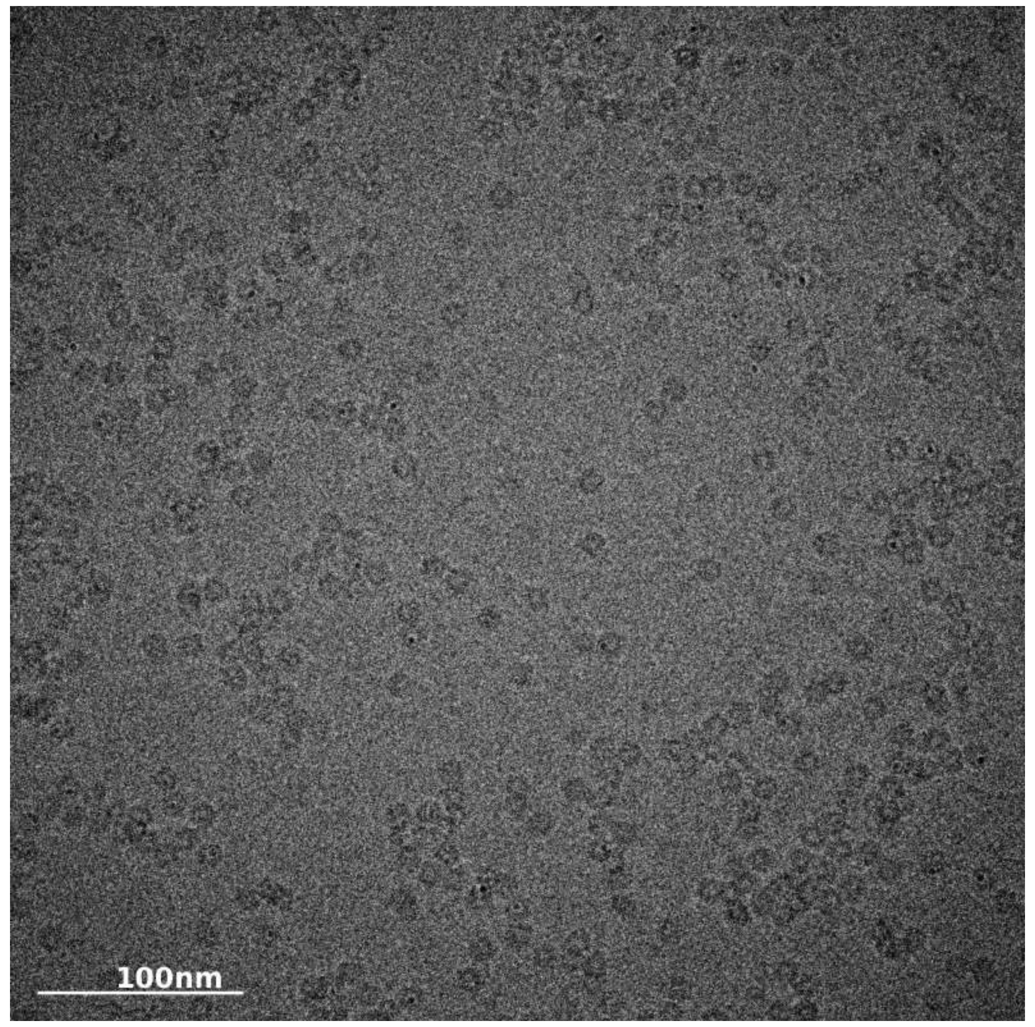
Does the substate determine this?

What is chameleon?

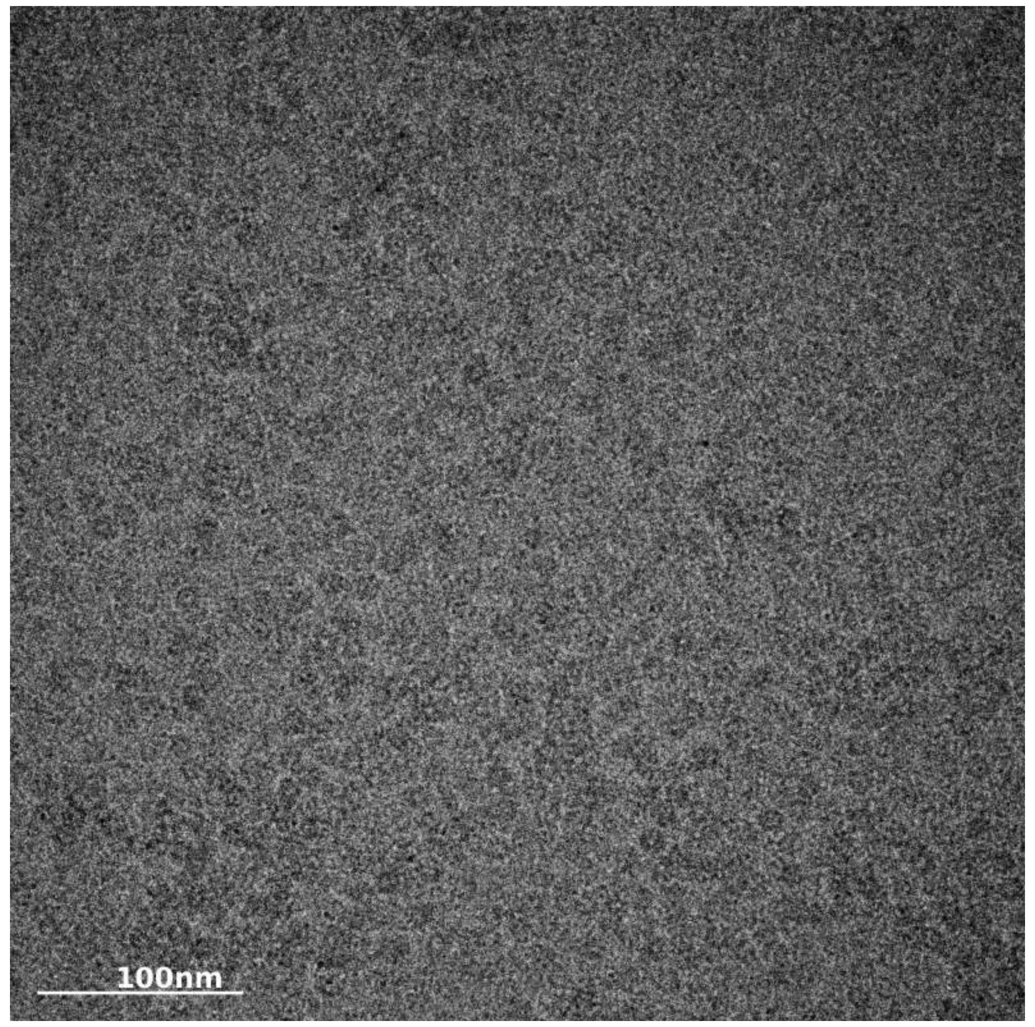
Where is the ice relative to the grid?

Spotiton

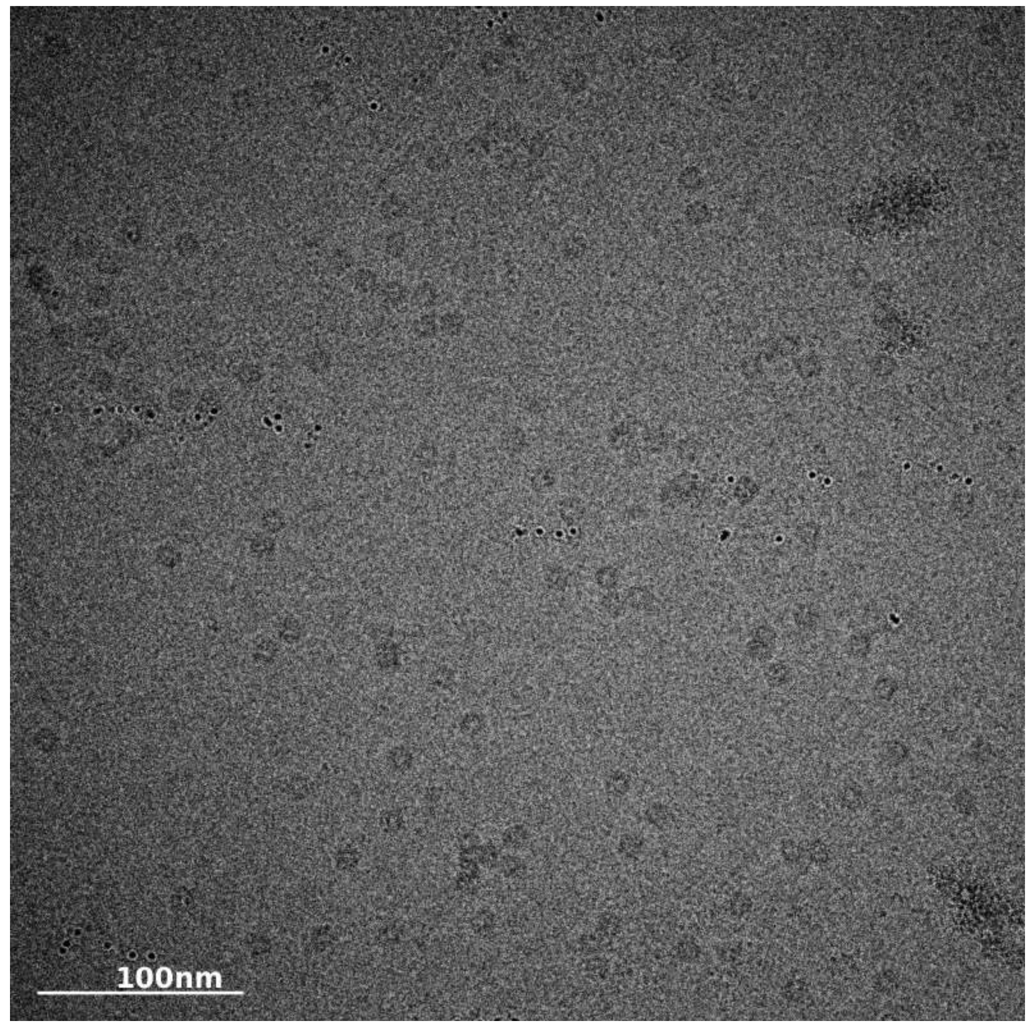
Add a graphene layer under the substrate



No graphene (8 mg/ml)



Graphene (8mg/ml)



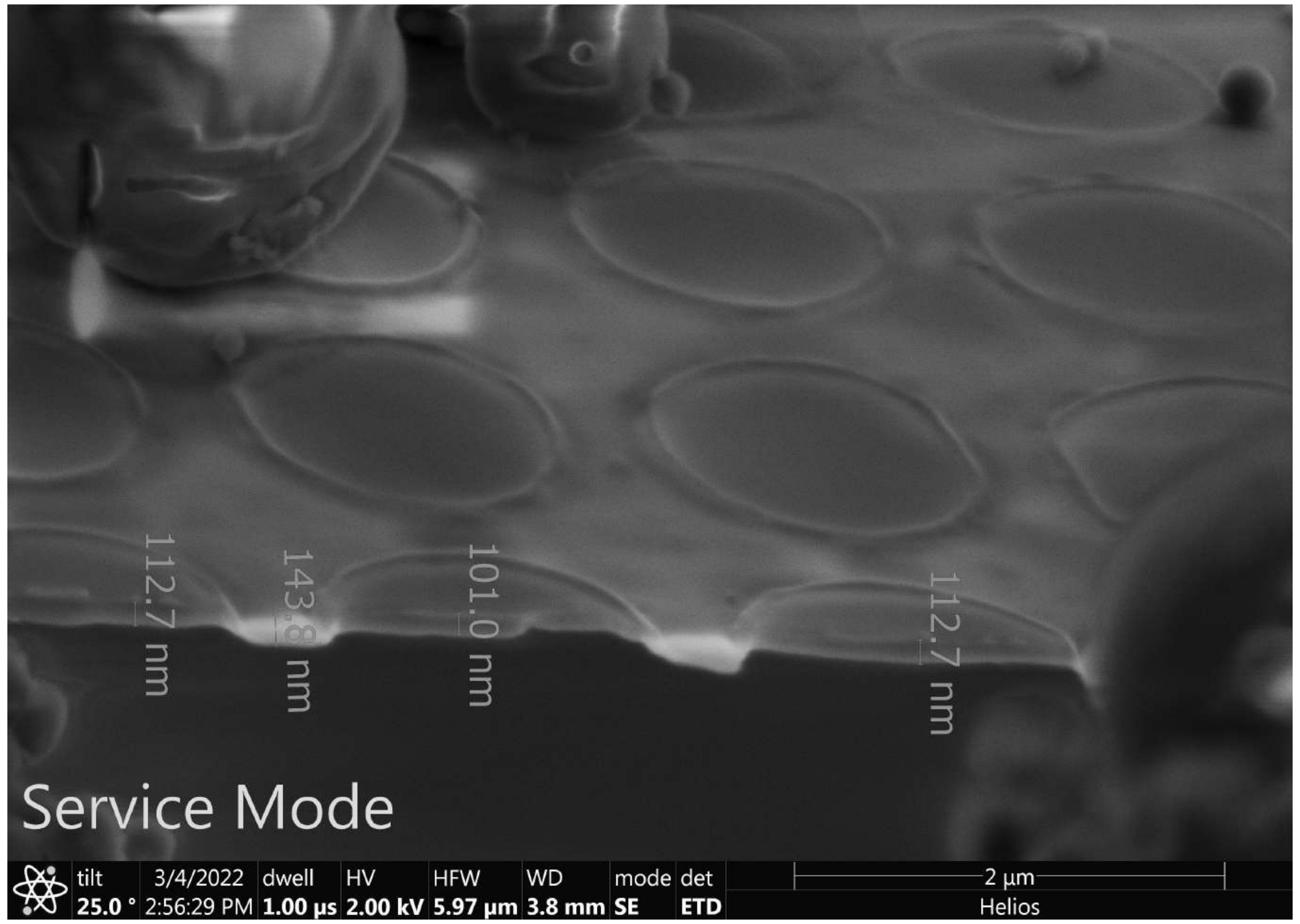
Graphene (0.8 mg/ml)

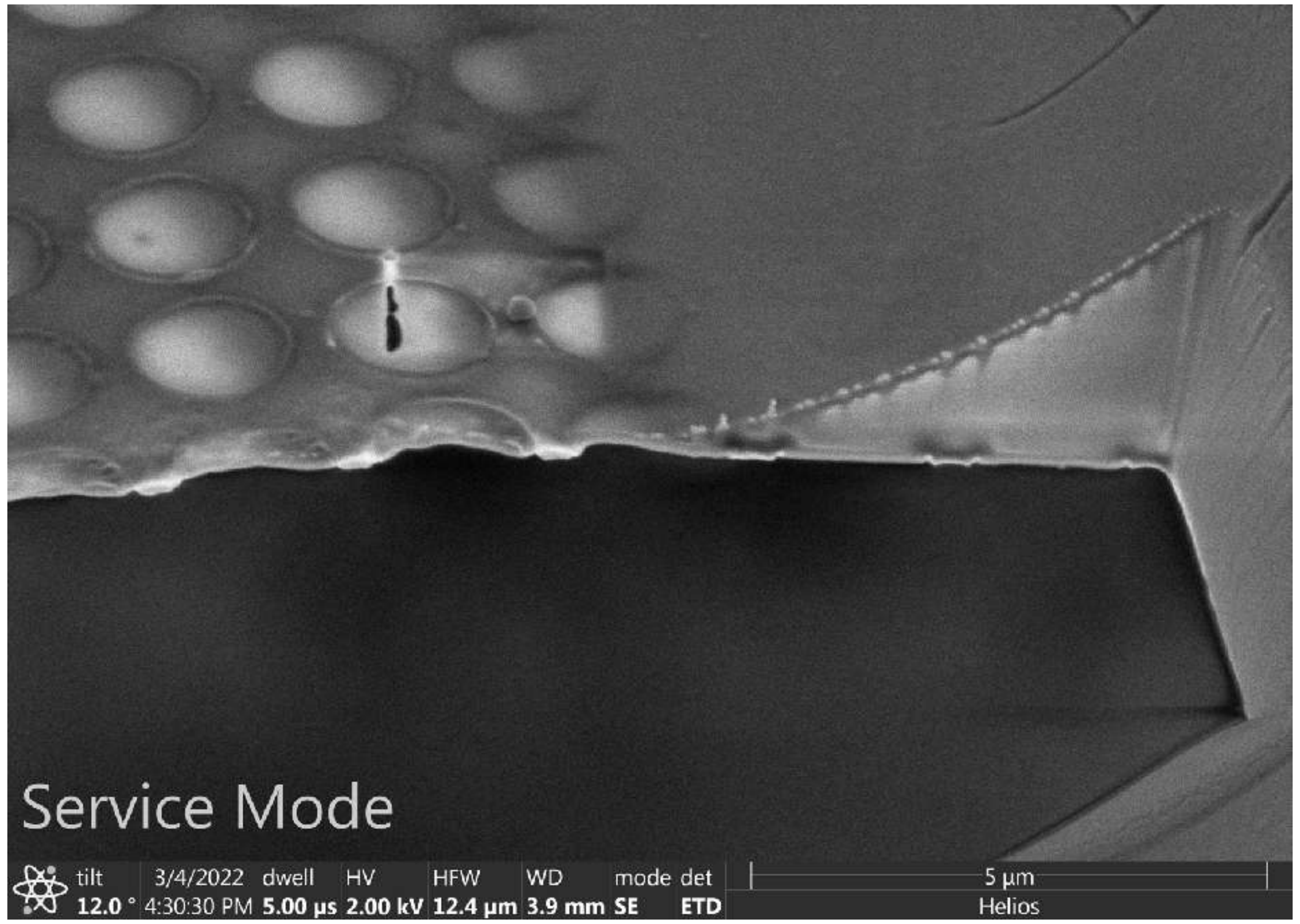
What is chameleon?

Where is the ice relative to the grid?

Spotiton

Cryo FIB-SEM



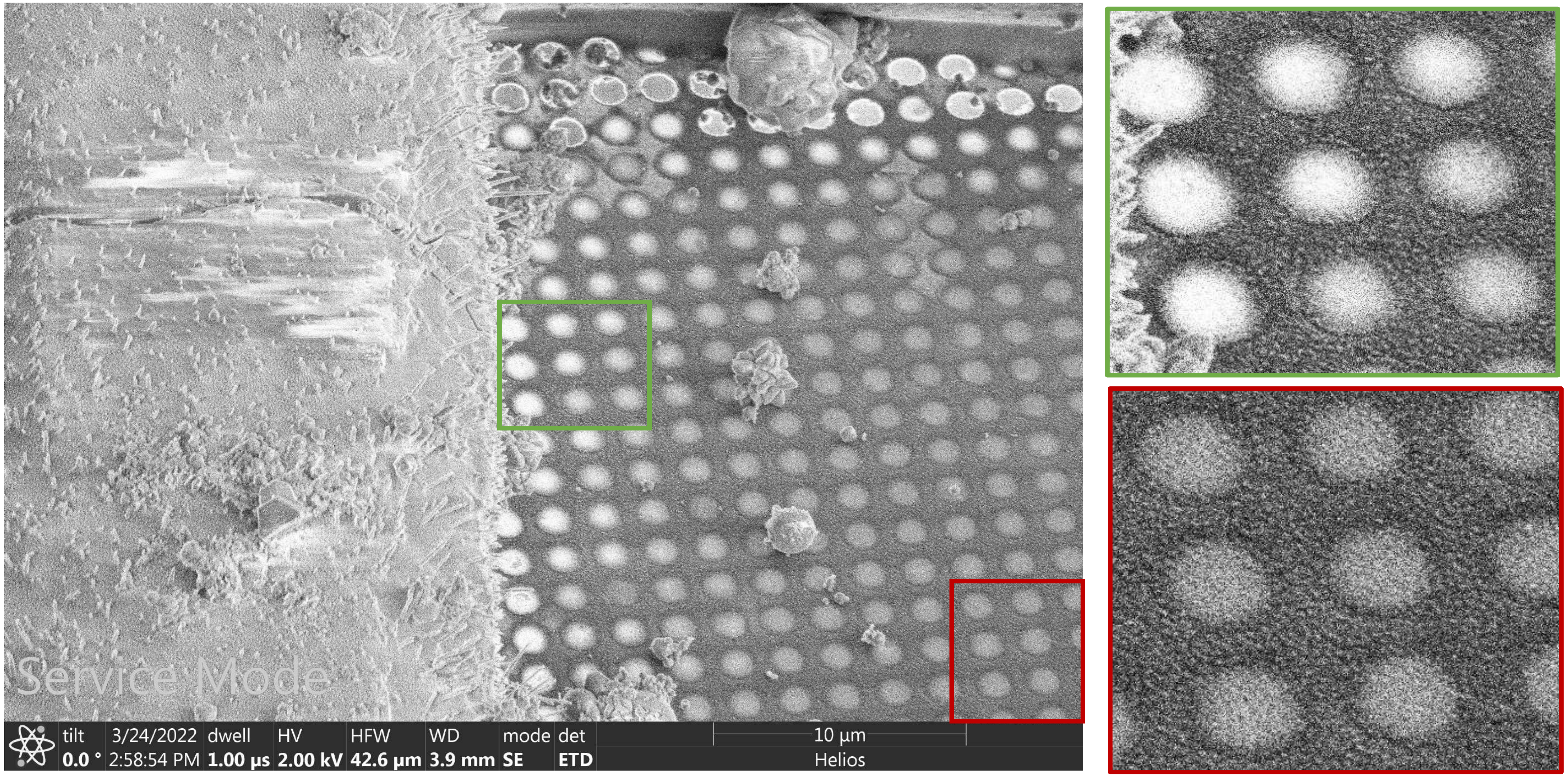


What is chameleon?

Where is the ice relative to the grid?

Spotiton

Sample application side of grid

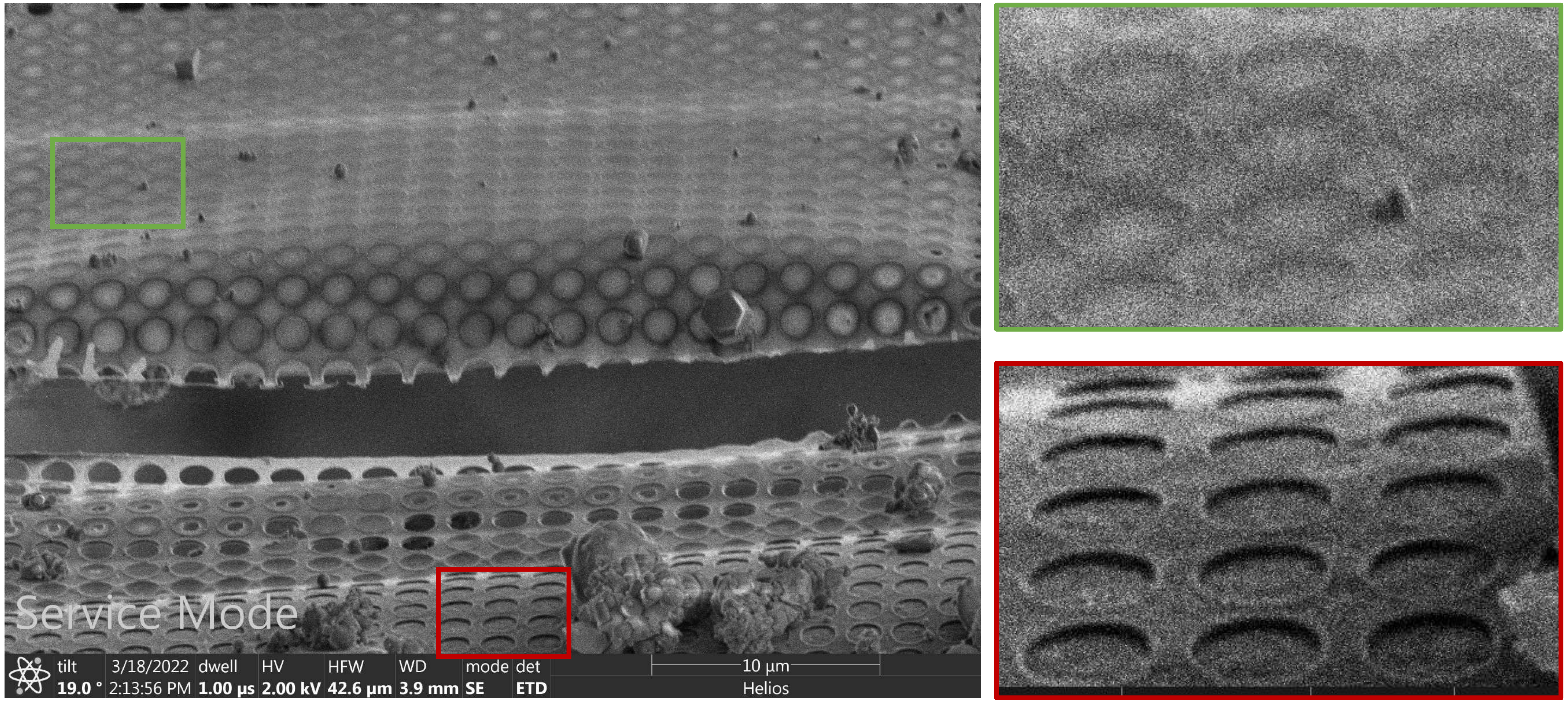


What is chameleon?

Where is the ice relative to the grid?

Spotiton

Opposite side of sample application

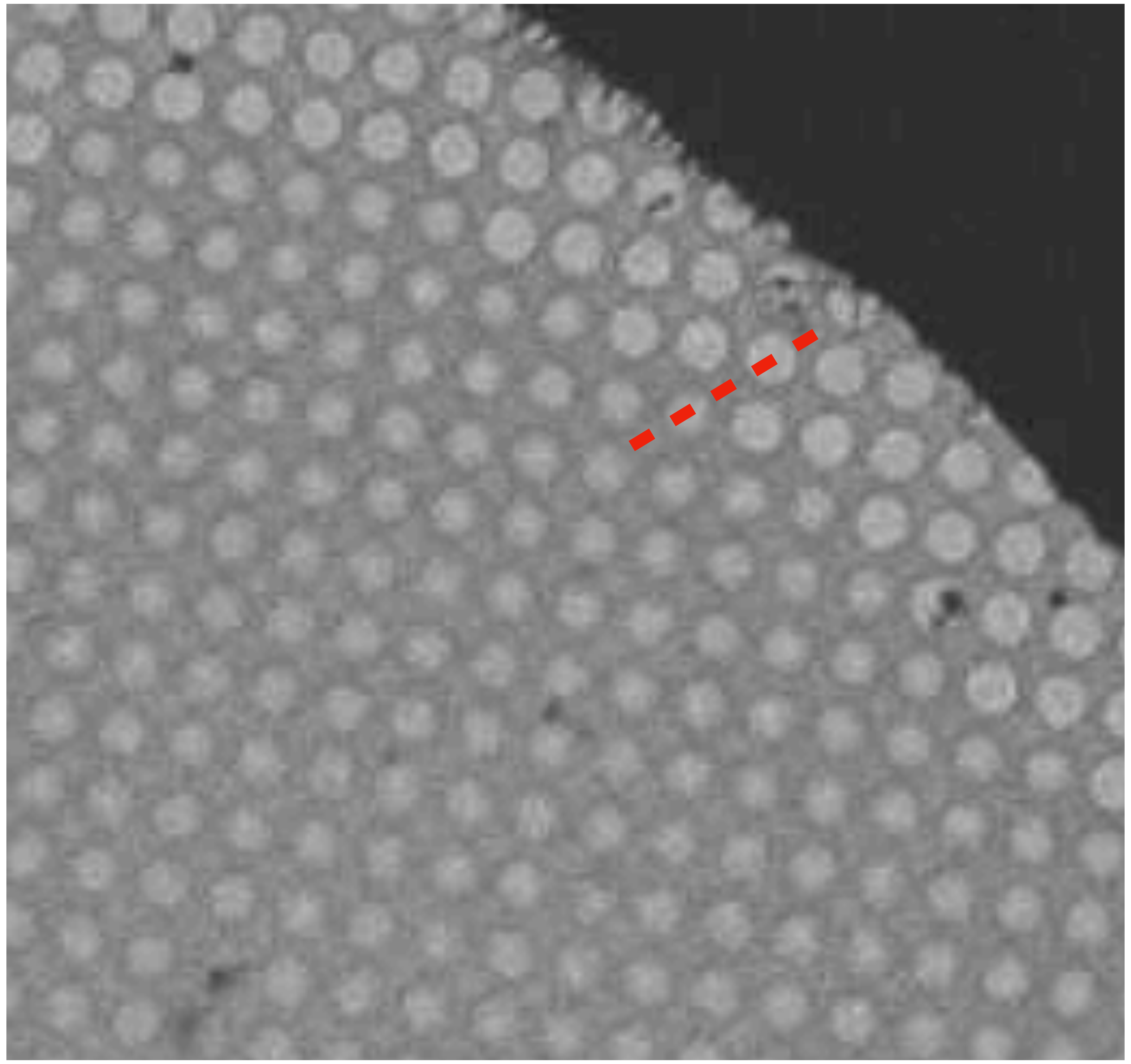


What is chameleon?

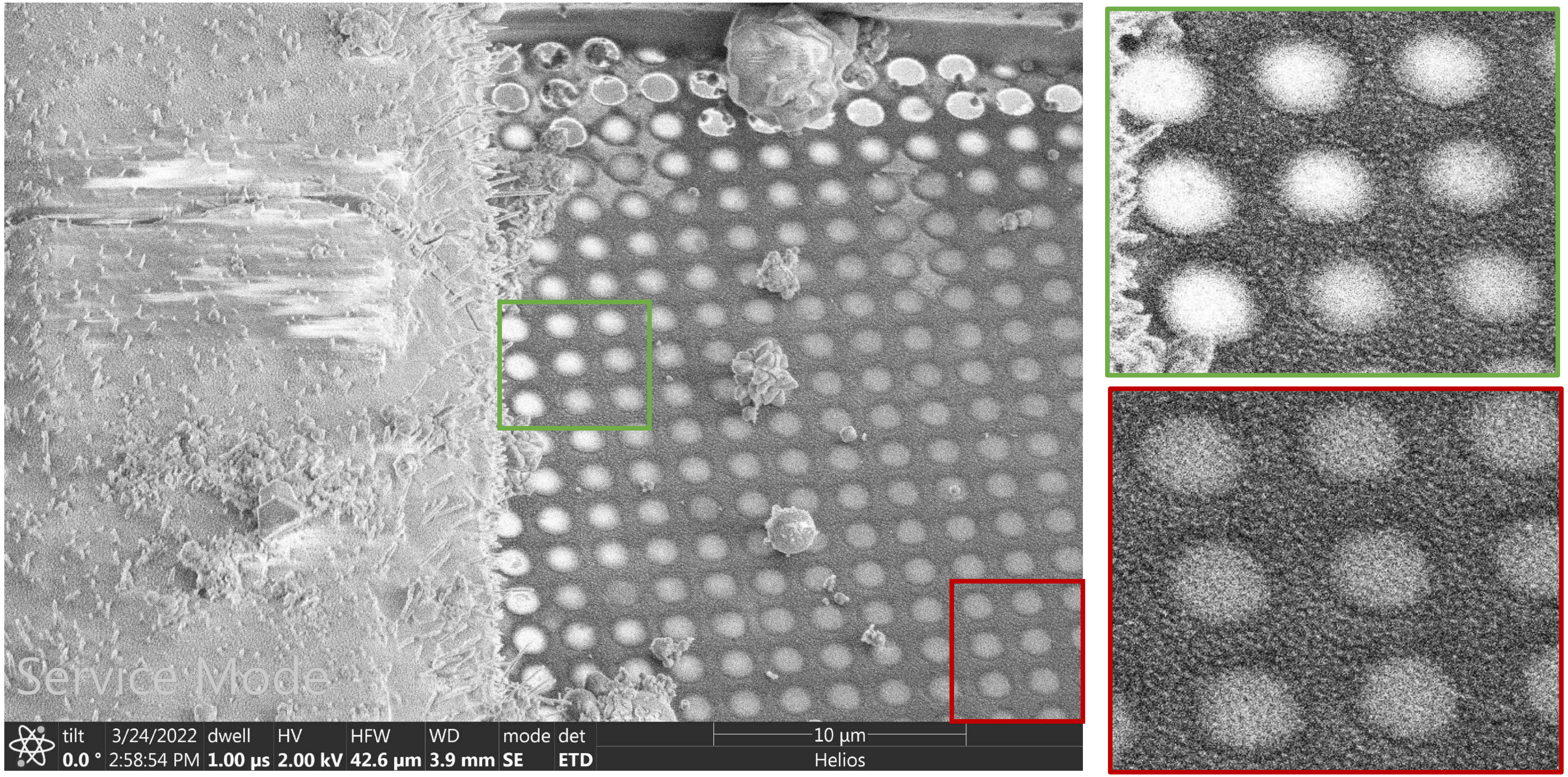
Where is the ice relative to the grid?

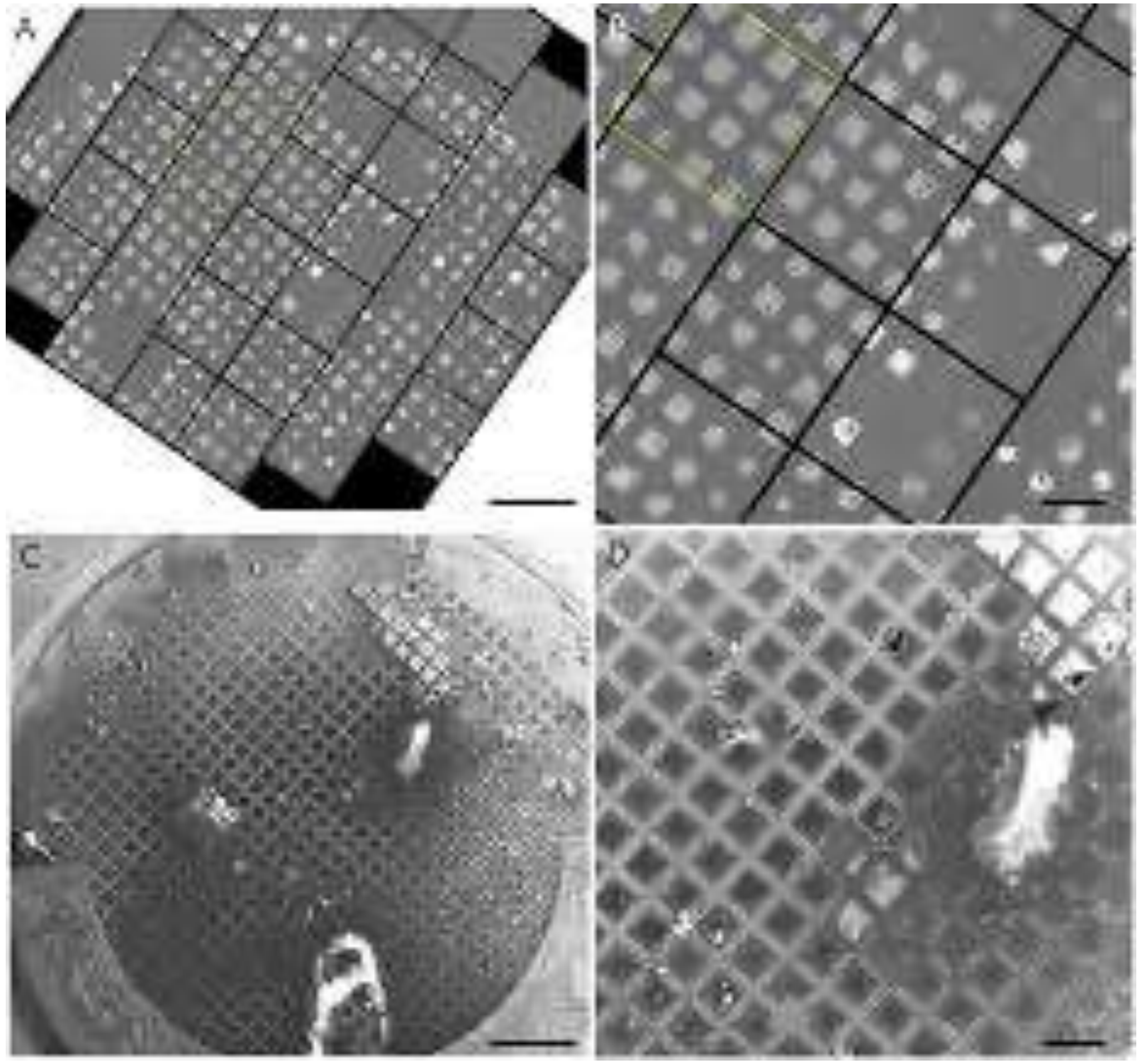
Spotiton

And why does this happen?

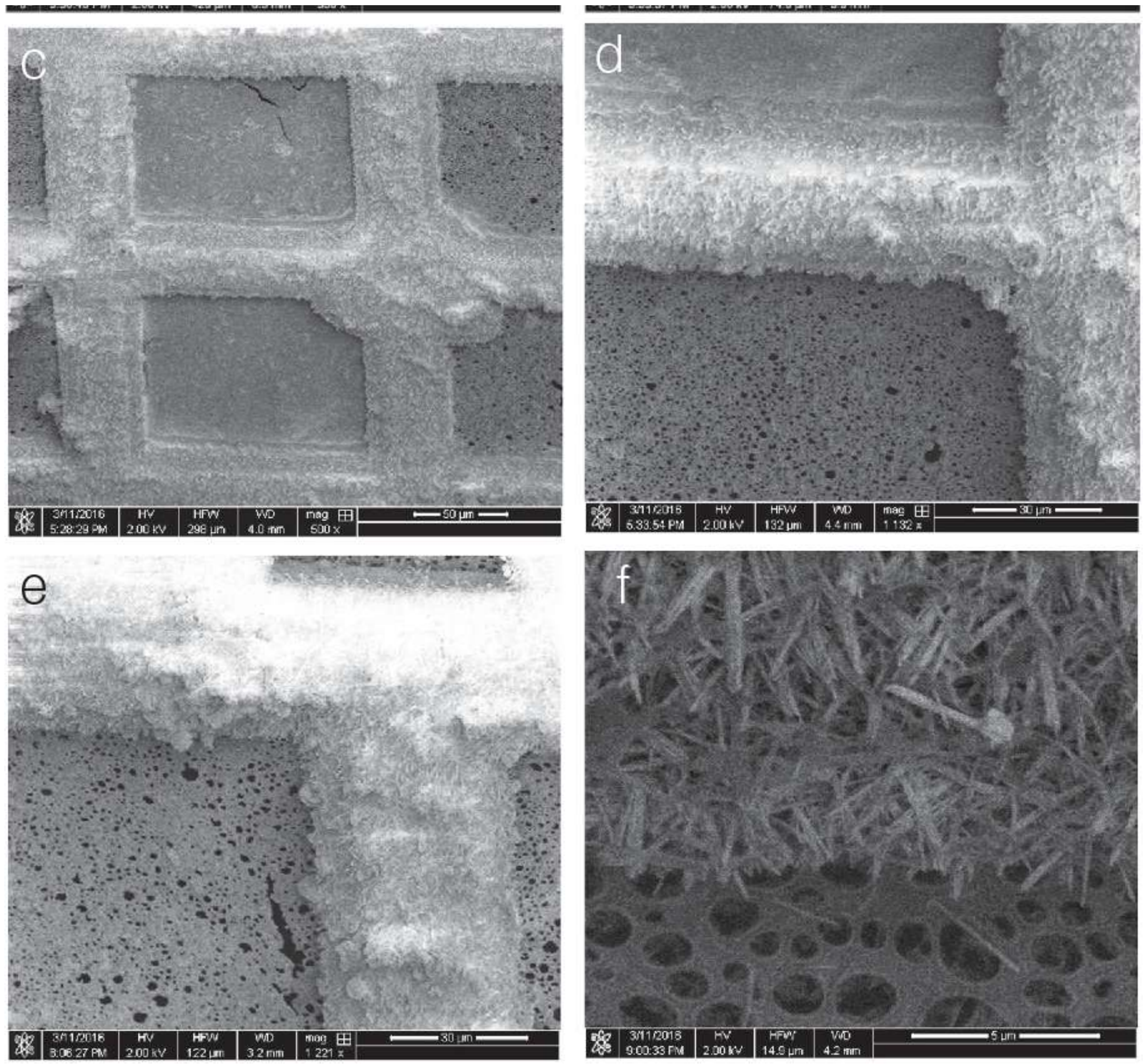


WHAT DOES A GRID LOOK LIKE?



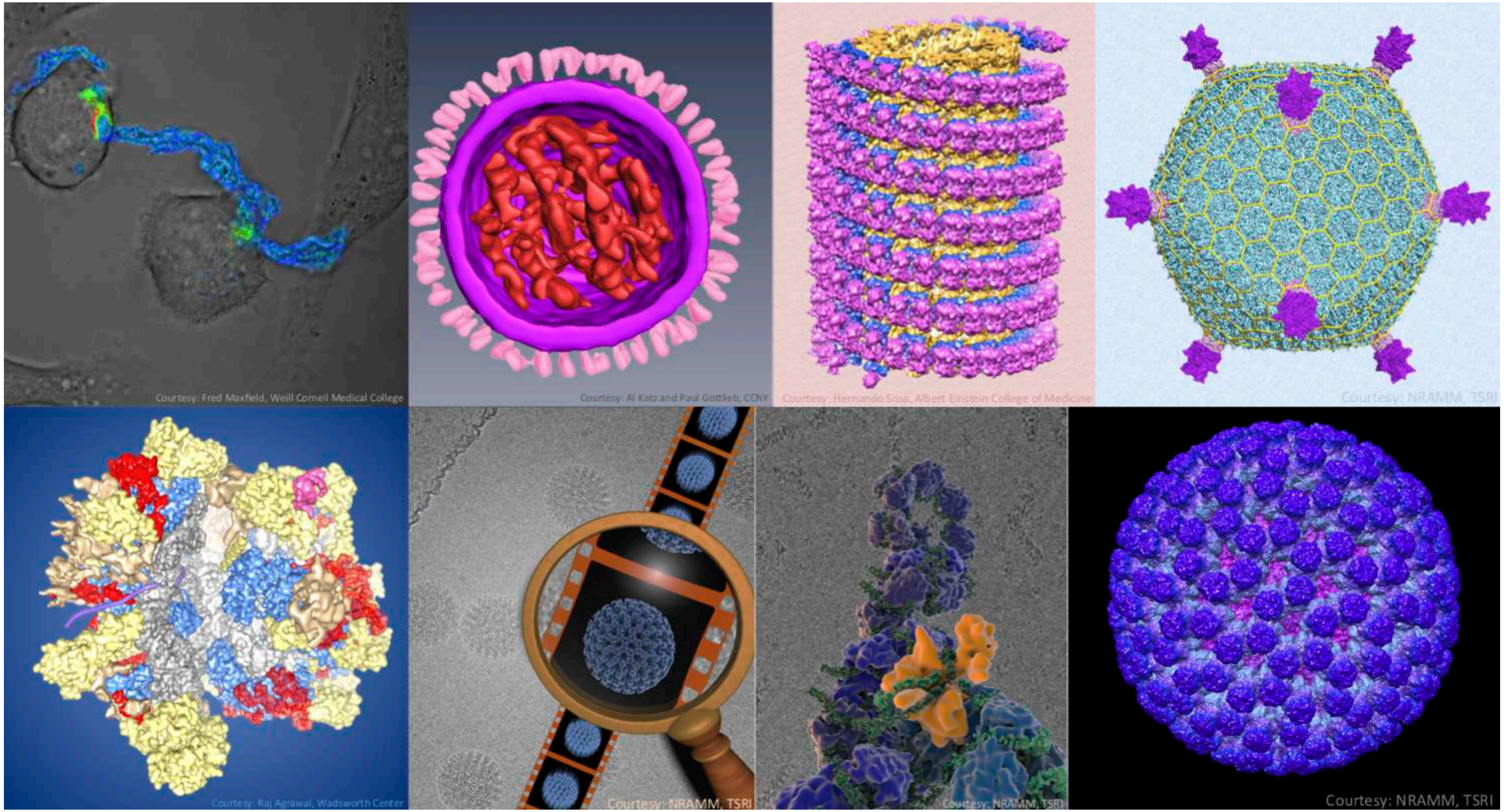


- Schmidli, Claudio & Rima, Luca & Arnold, Stefan & Stohler, Thomas & Syntychaki, Anastasia & Bieri, Andrej & Albiez, Stefan & Goldie, Kenneth & Chami, Mohamed & Stahlberg, Henning & Braun, Thomas. (2018). Miniaturized Sample Preparation for Transmission Electron Microscopy. Journal of Visualized Experiments.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

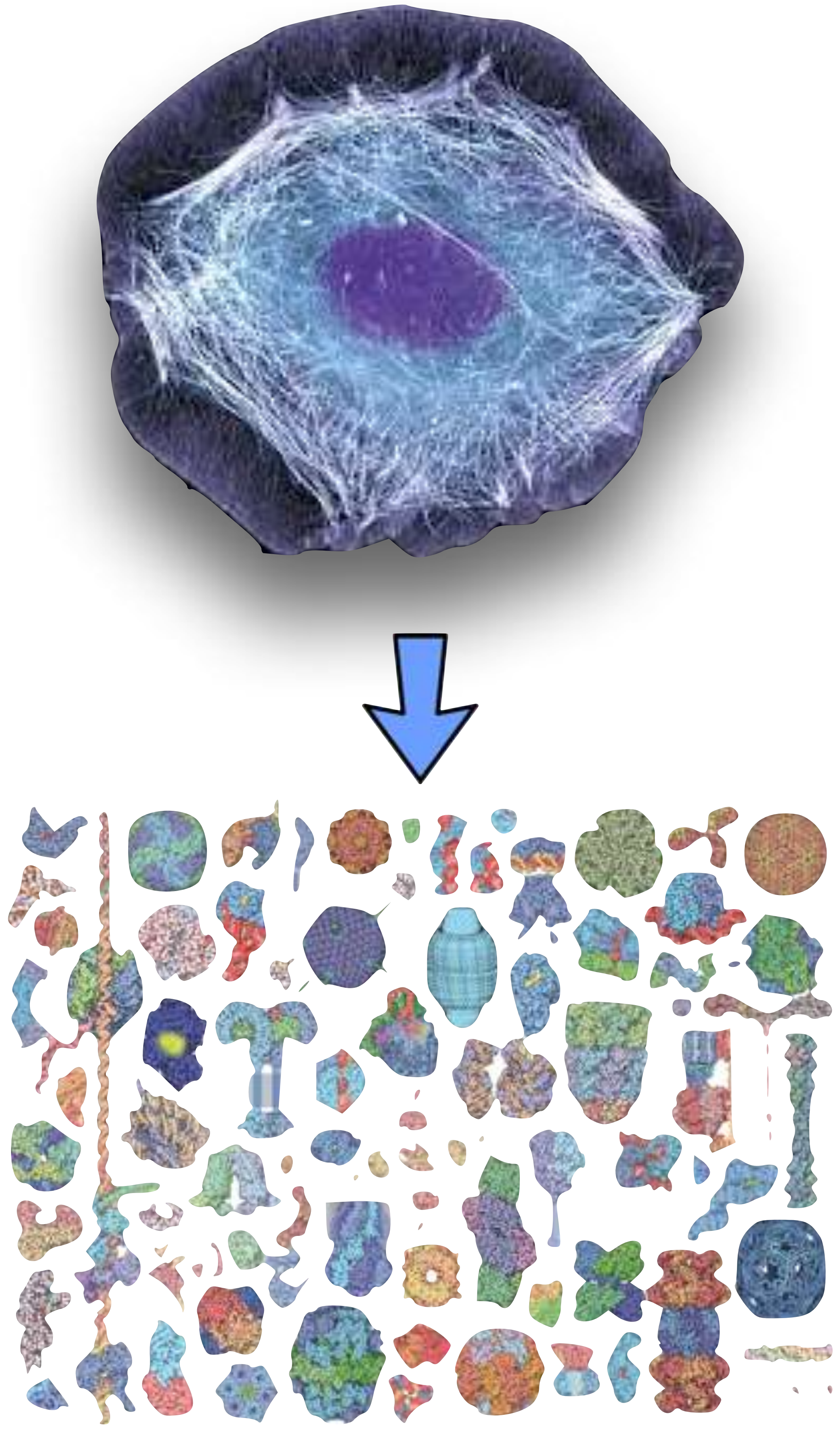


- Razinkov, I., Venkata P. Dandey, Hui Wei, Z. Zhang, D. Melnekoff, W. Rice, Christoph Wigge, C. S. Potter and B. Carragher. “A new method for vitrifying samples for cryoEM.” *Journal of structural biology* 195 2 (2016): 190-198 .

How are samples prepared for cryoEM?



What about thicker samples?

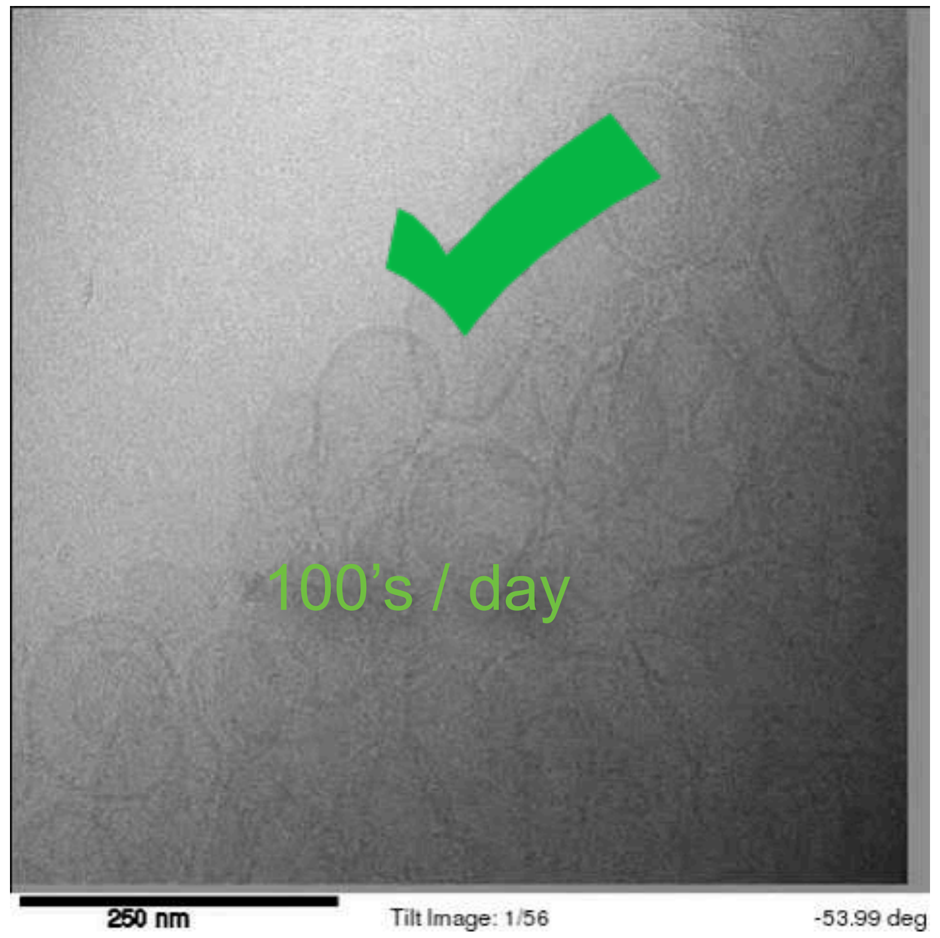


...

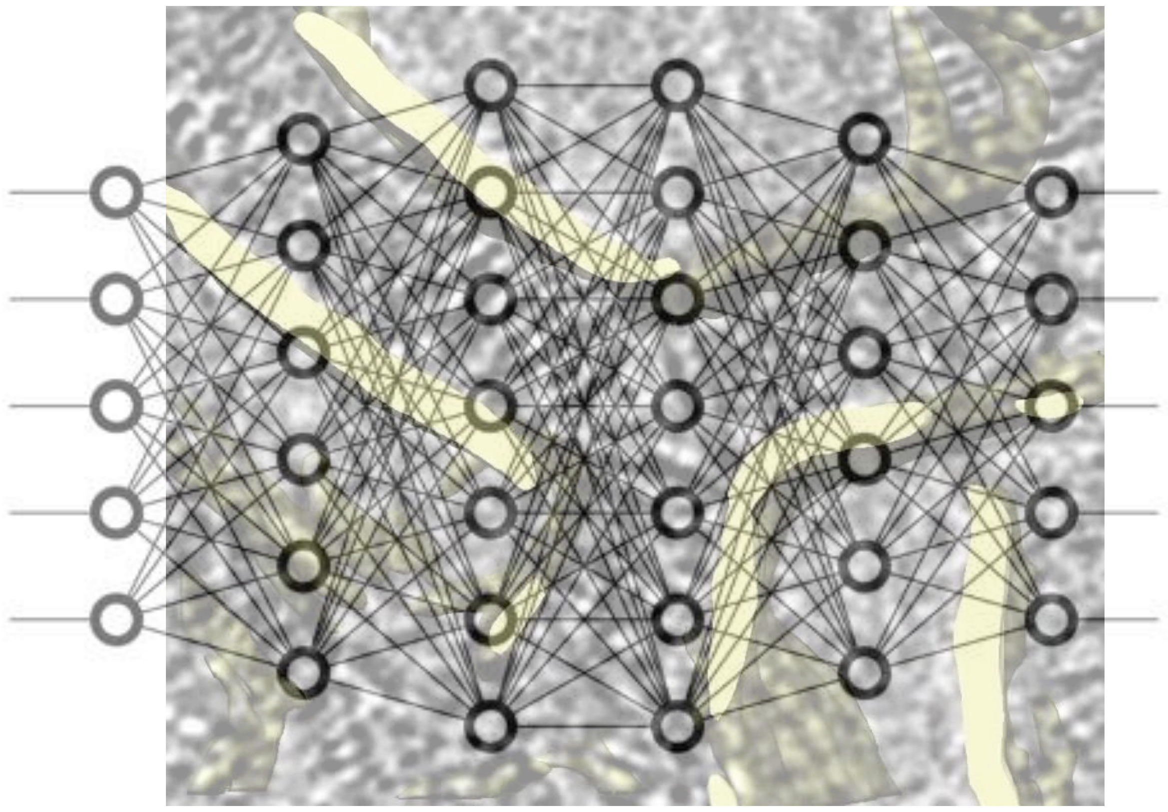
How are samples prepared for cryoEM?

Towards Automation for
In Situ CryoEM

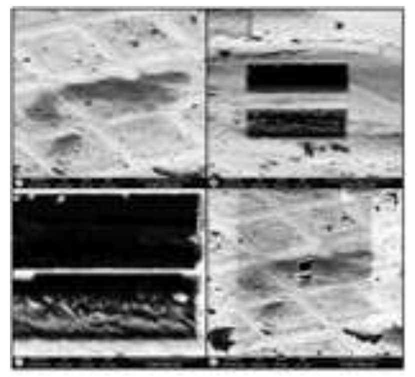
Sample

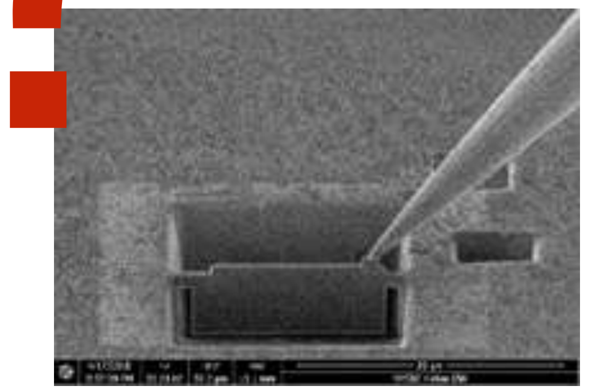


Automated Data Collection
(Leginon, etc.)



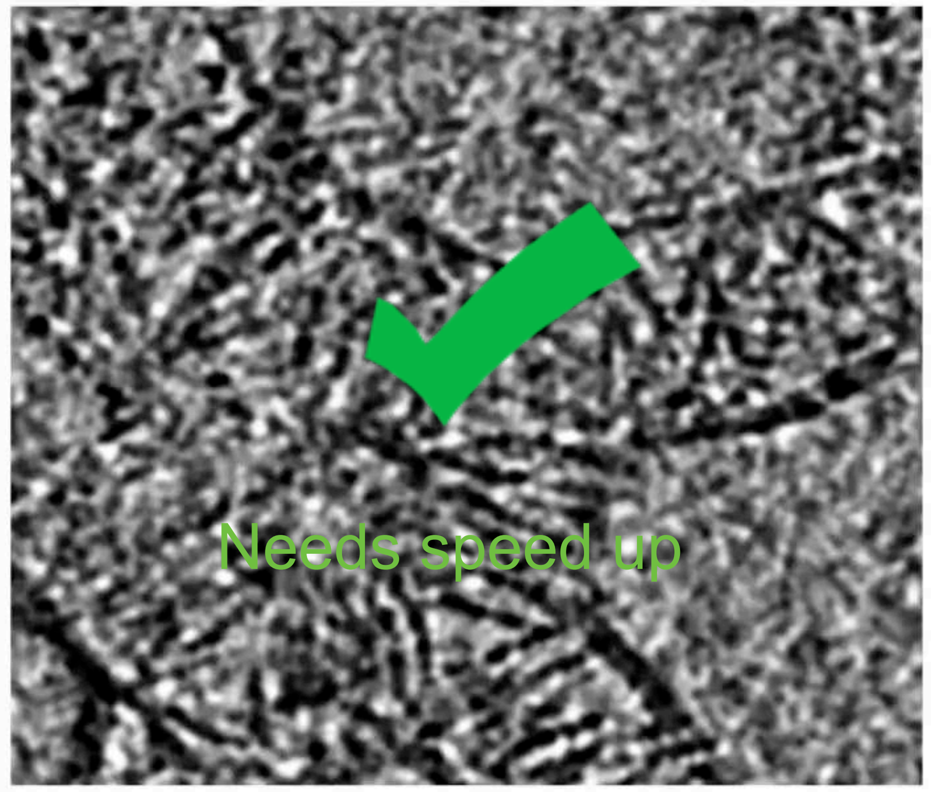
Deep learning?





Milling
Grid preparation

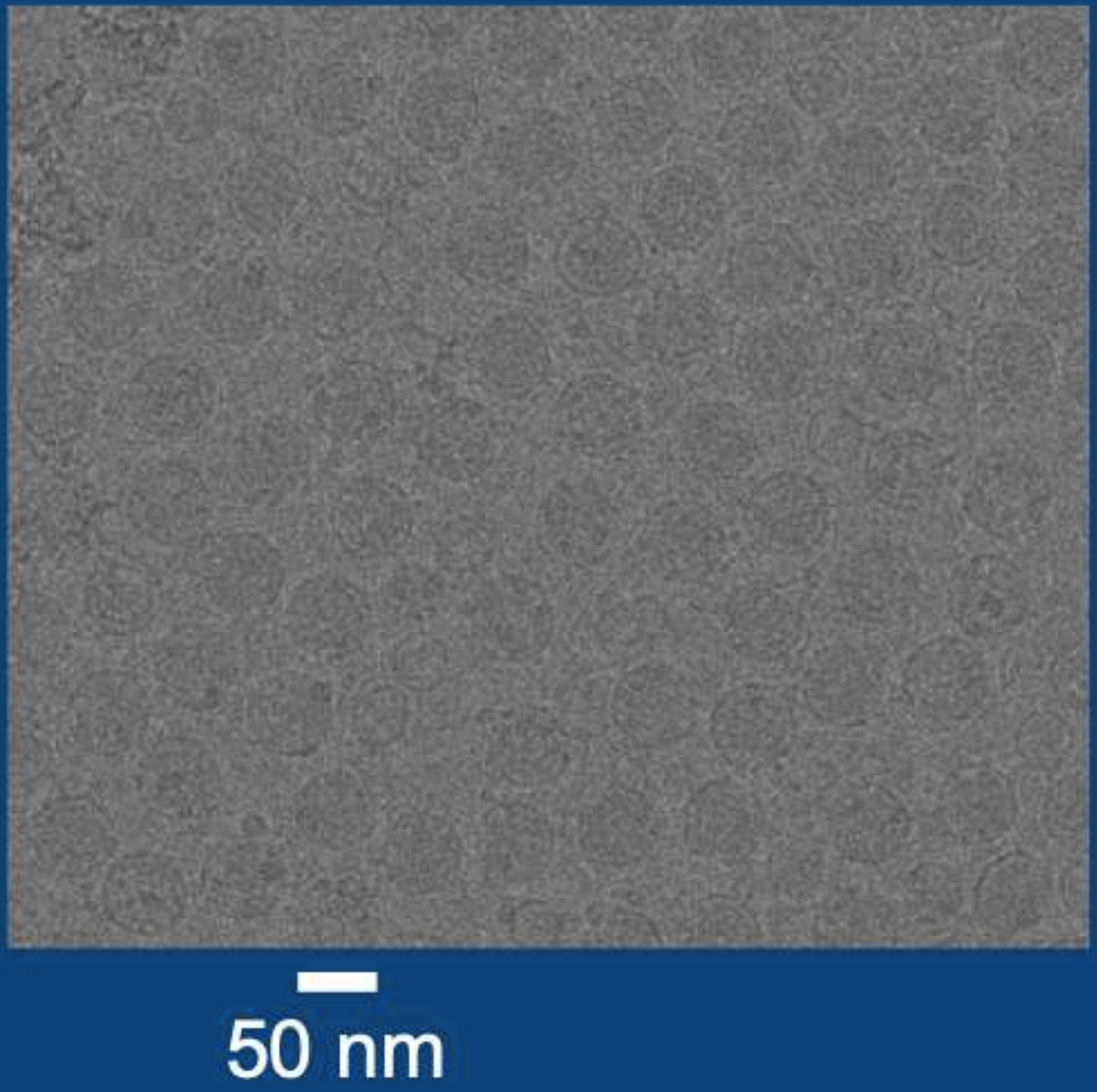
Lift out



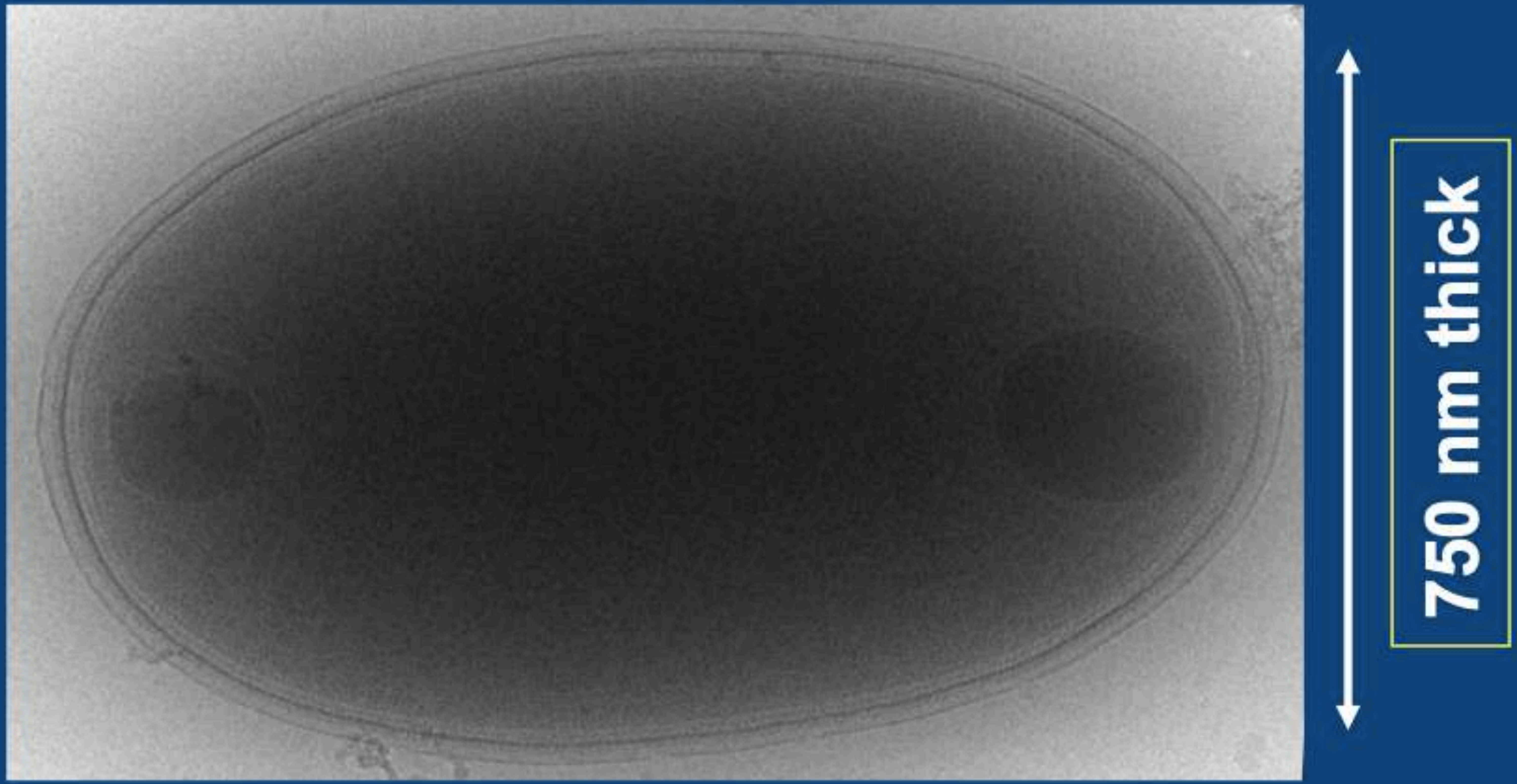
Streamlined Processing
(Appion Protomo)

How are samples prepared for cryoEM?

HOW THIN DOES THE SAMPLE NEED TO BE?



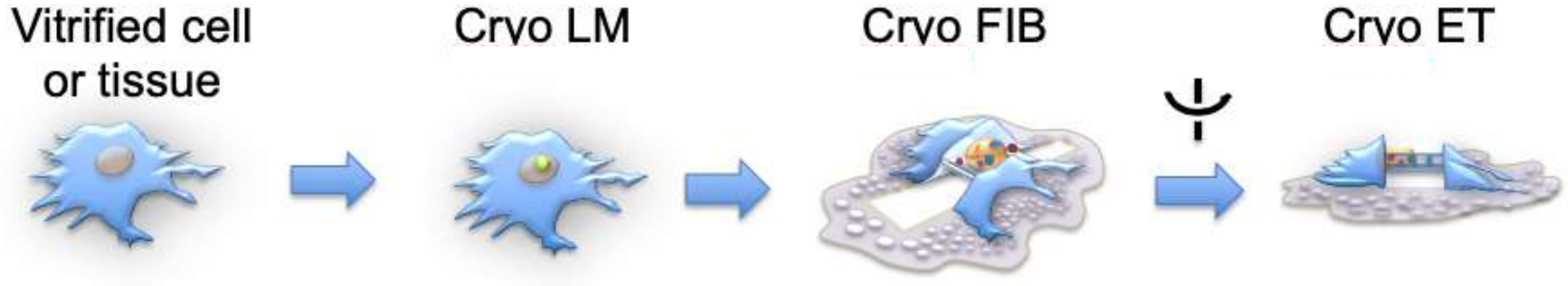
Bacteriophage (ϕ12)



E. coli, Salmonella, Cyanobacteria

How are samples prepared for cryoEM?

CLEM workflow



TO BE CONTINUED

Questions?