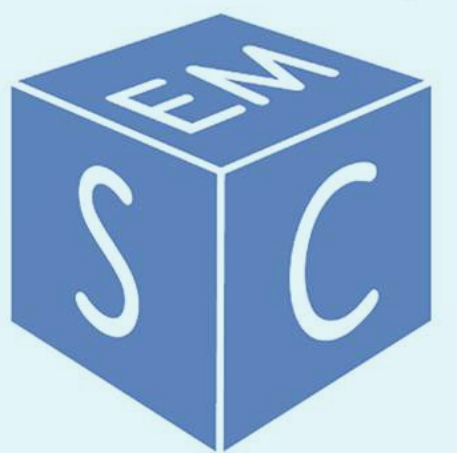


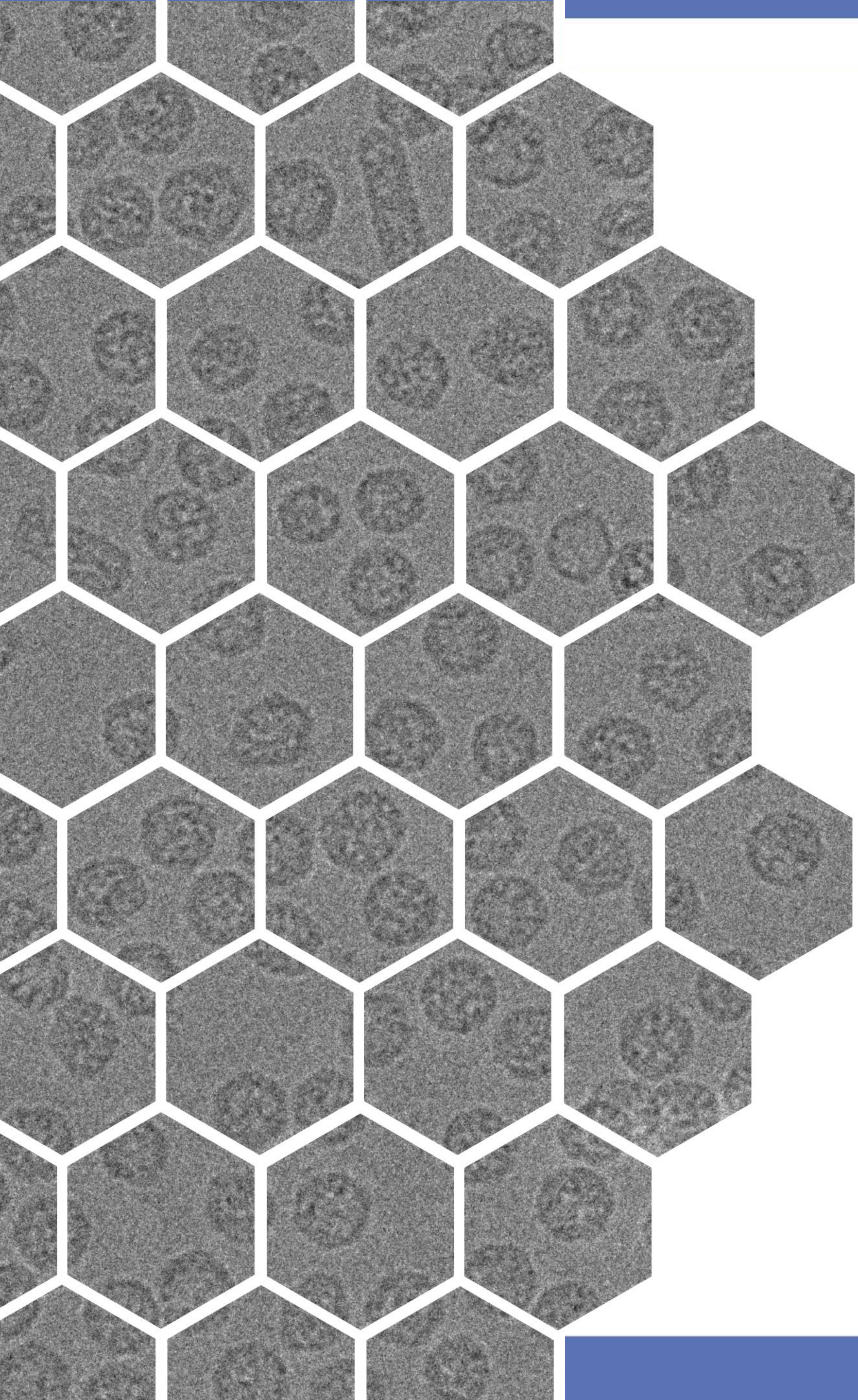
# 2026 cryoEM course

Considerations for biological cryoEM

February 2, 2026



**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

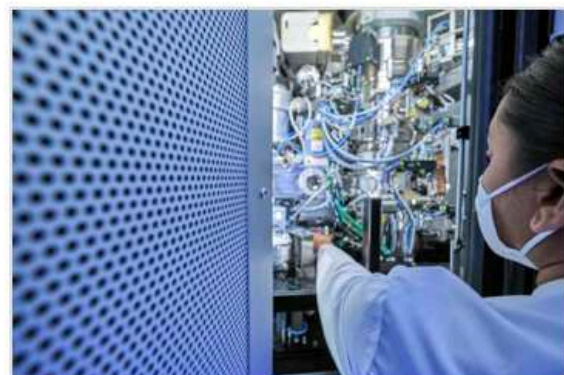
Section 3 : Tomography

Section 4 : Single Particle Analysis Short Course  
March 16-20

Section 5 : Future perspectives



# Course logistics: main topics



- 🕒 1 week.
- 👤 Morning lectures.
- 👤 Afternoon practicals.
- 📅 March 16-20, 2026.
- 💰 *students/postdocs \$375.*  
*staff/PIs/professionals \$450.*  
*industry \$625.*
- 🏠 Room/Accommodations NOT included.

[JOIN THE WAITLIST.](#)

Standard applications will close December 15, 2025. Late applications will be considered up to January 2, 2026.

Accepted applicants will be notified early January 2026.

For more information email [nccatuseroffice \[at\] nysbc.org](mailto:nccatuseroffice@nysbc.org)

## Single Particle Short Course March 16-20, 2026

NCCAT will offer a 1 week workshop focused on the theory and practice of single-particle analysis. The mornings are filled with lectures and stimulating round table discussions. The afternoons provide hands-on practicals to reinforce fundamental concepts and topics covered earlier in the day.

### Short-course Agenda

#### Monday, March 16, 2026

Session	Type	Lecturer	Topic
Morning	Lecture 1 – Keynote	Joachim Frank (Columbia University)	Intro and overview of SPA
Morning	Roundtable 1	students	Flashtalks from students
Afternoon	Lecture 2	SEMC staff	Sample preparation for biological samples
Afternoon	Practical 1	SEMC staff	cryoEM sample prep and grid prep stations

#### Tuesday, March 17, 2026

Session	Type	Lecturer	Topic
Morning	Lecture 3a	SEMC staff	Microscopes and tools of the trade
Morning	Lecture 3b	SEMC staff	Maintenance of cryoEM facilities



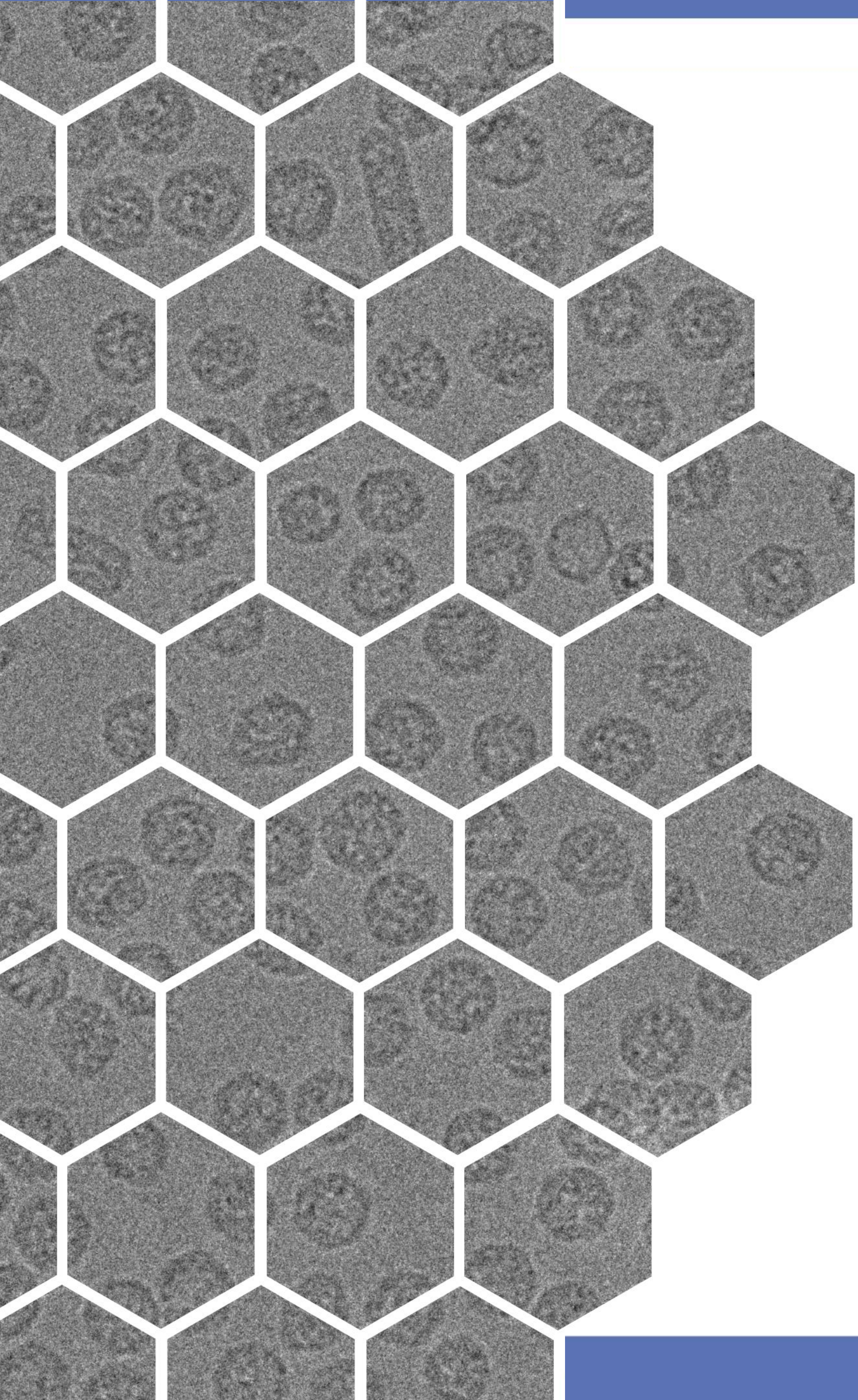
# Course logistics: Wednesday practical

**February 4, 2025**

Sample preparation practical

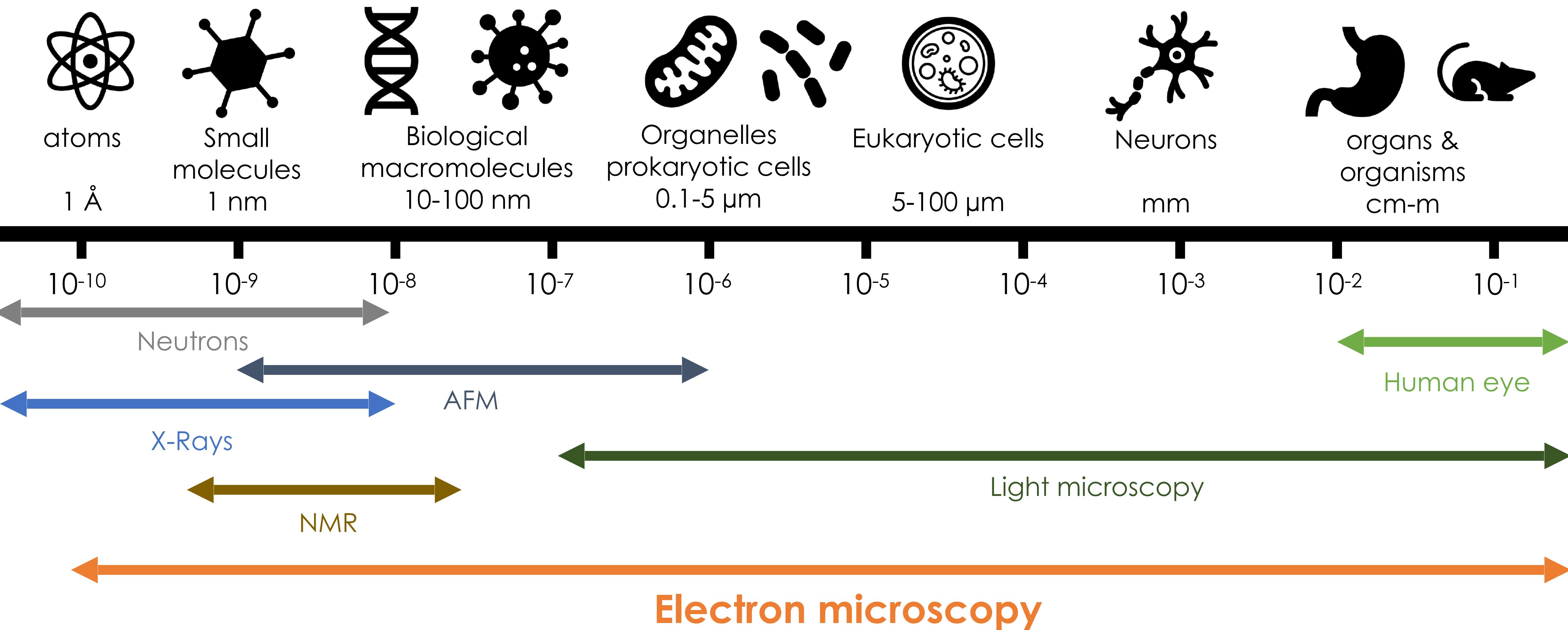
- one session or
- two sessions?





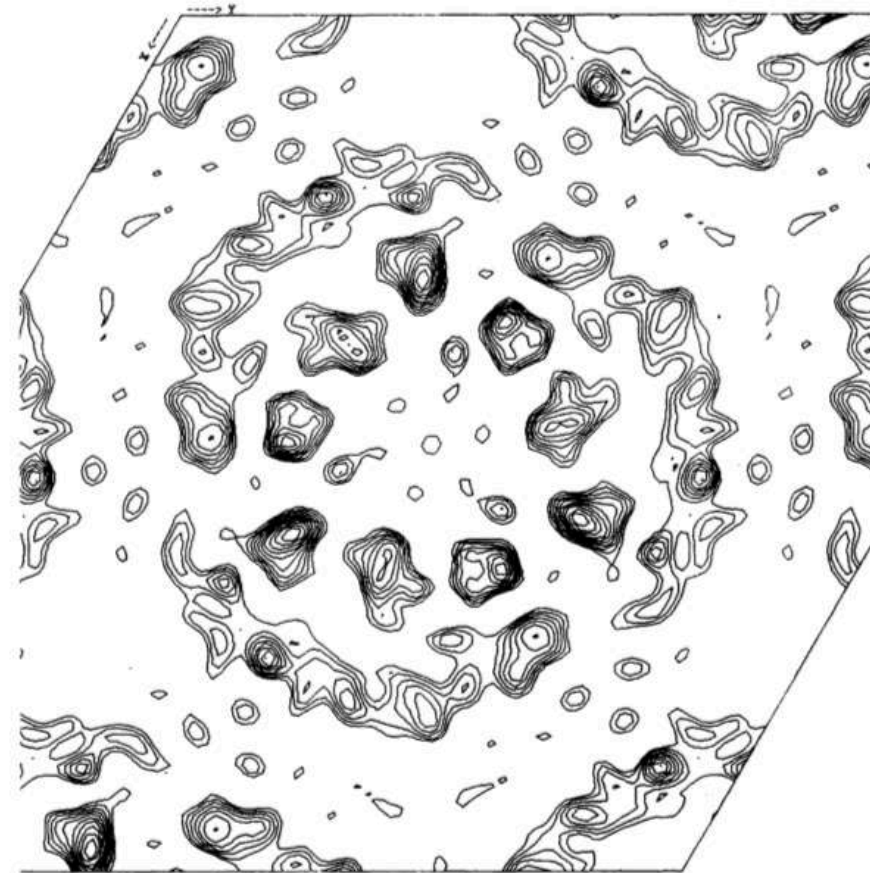
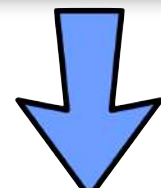
- ◆ Journal club and practical recap
- ◆ Considerations for biological cryoEM
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  - ◆ Grids
  - ◆ What happens to a sample
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# Scale of biology



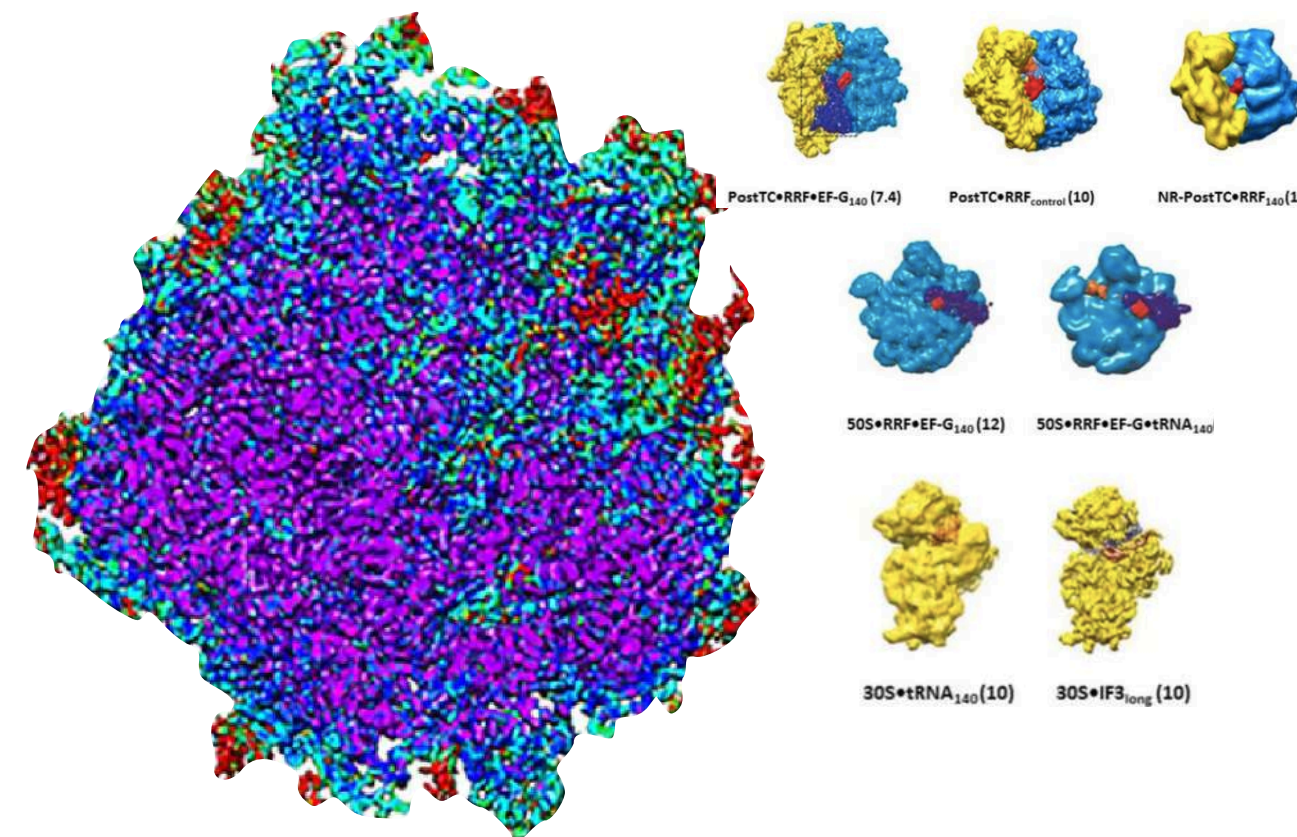
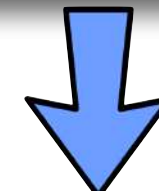
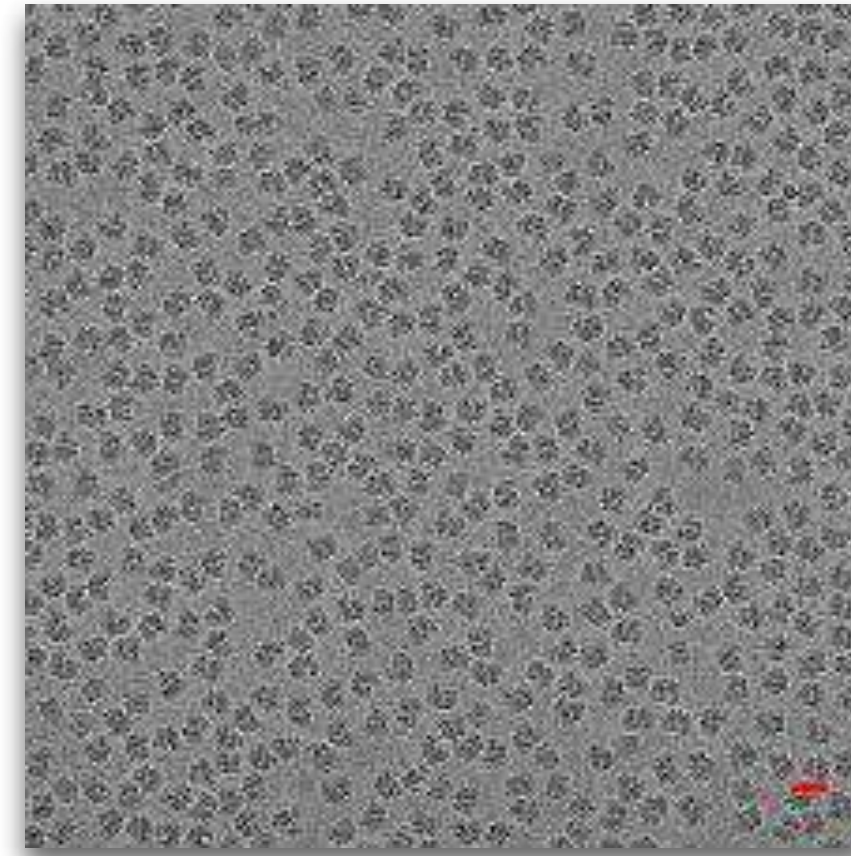
# cryoEM: technology on the rise

1986



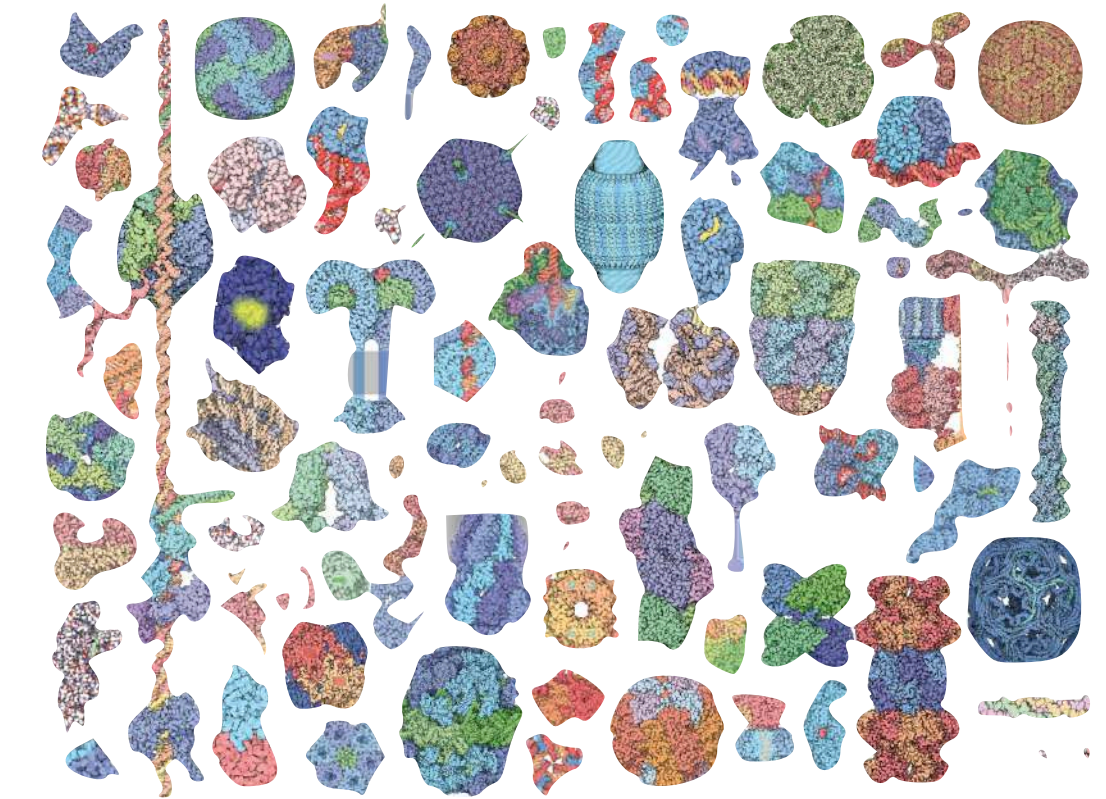
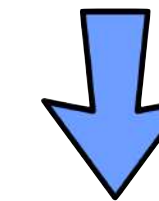
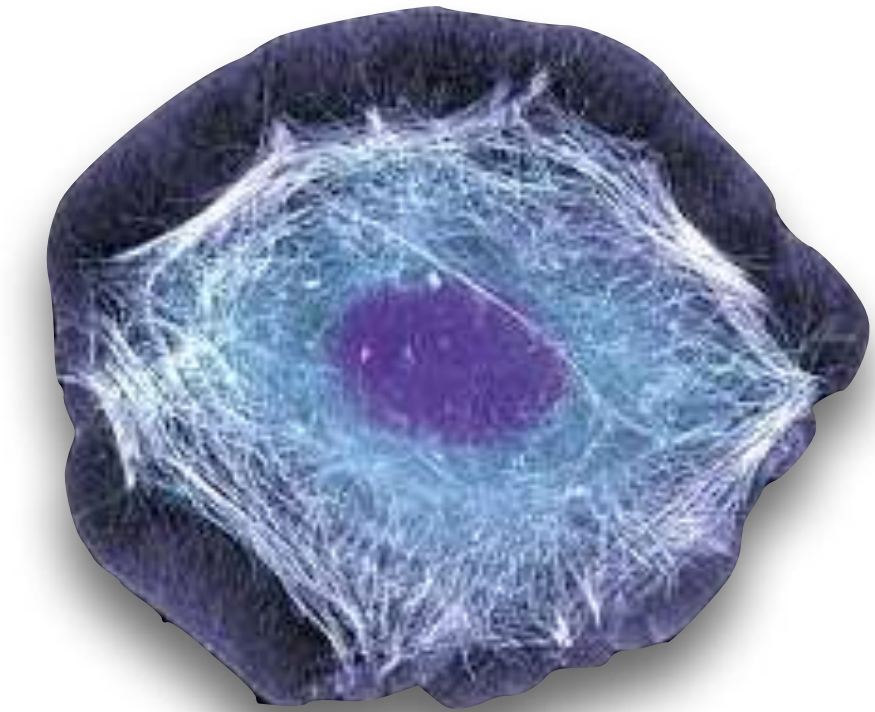
Henderson et al. (1986)

2017



Frank et al. (2017)

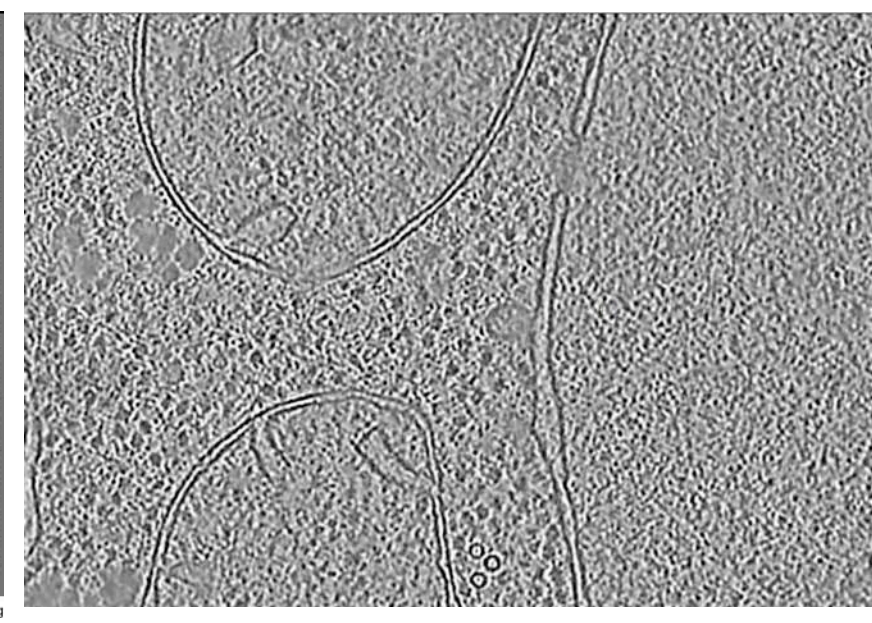
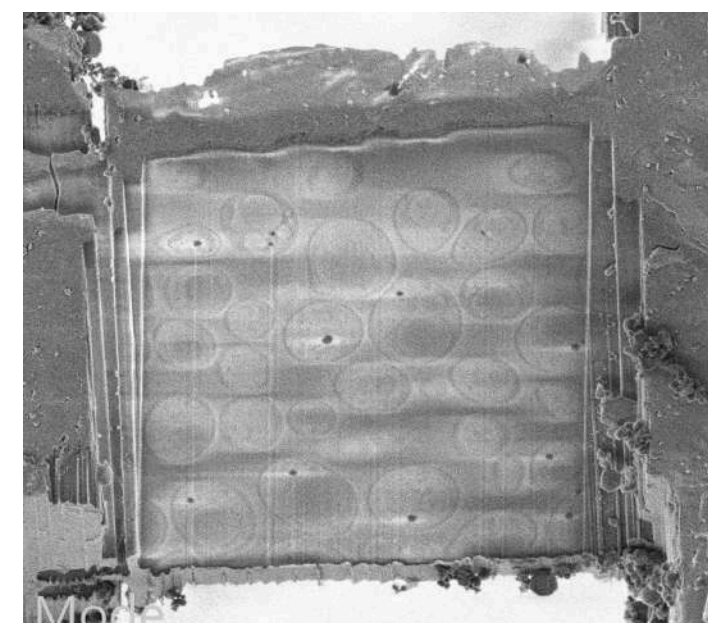
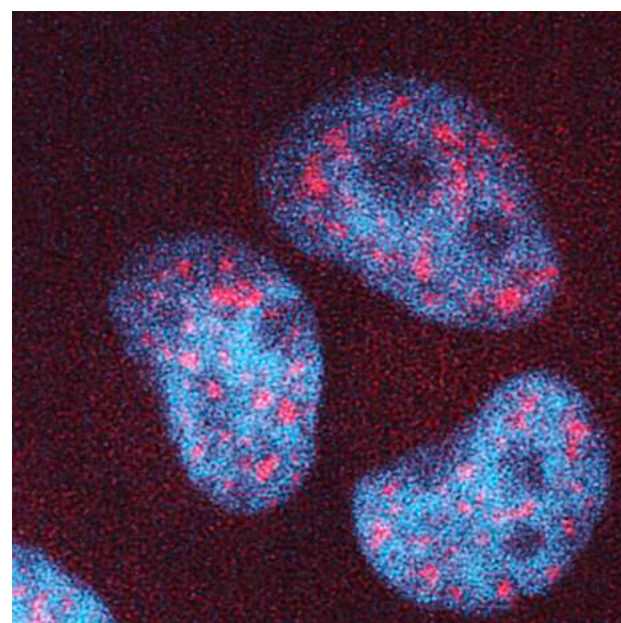
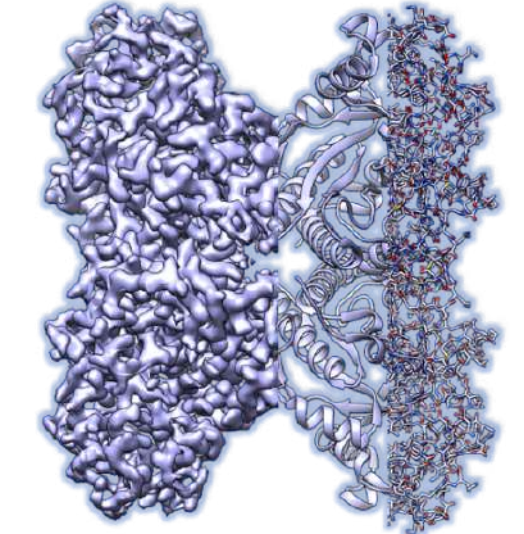
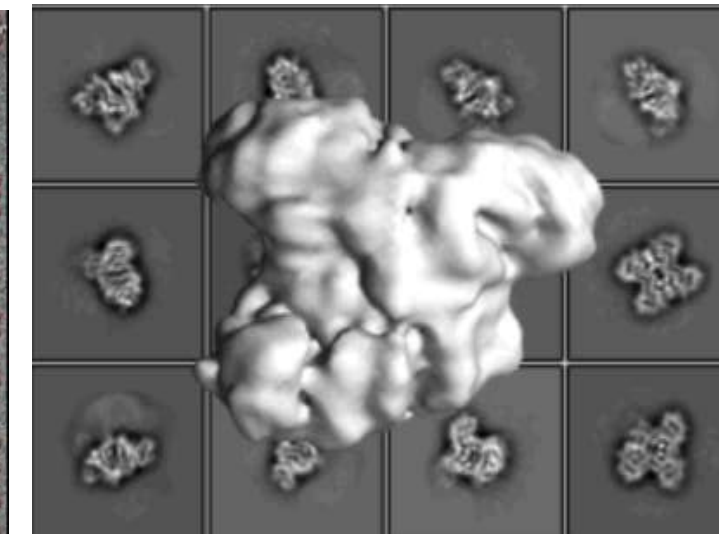
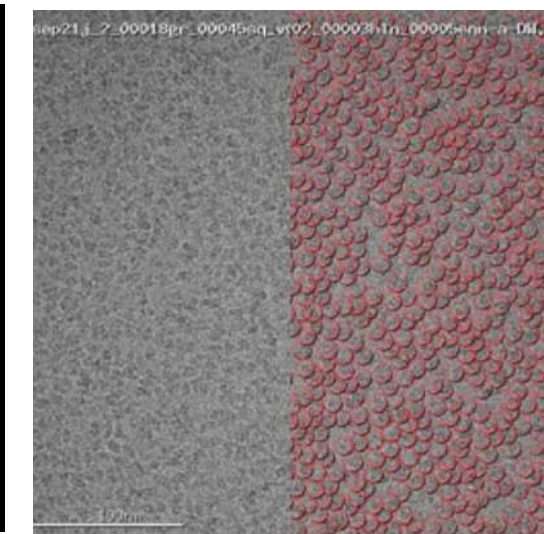
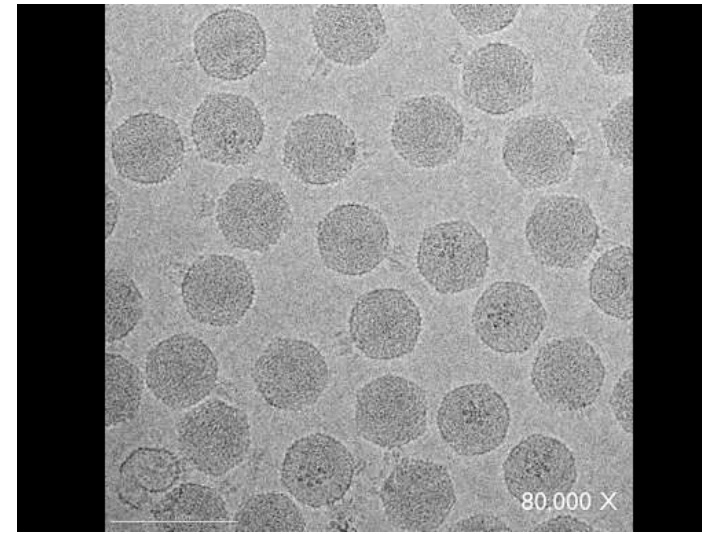
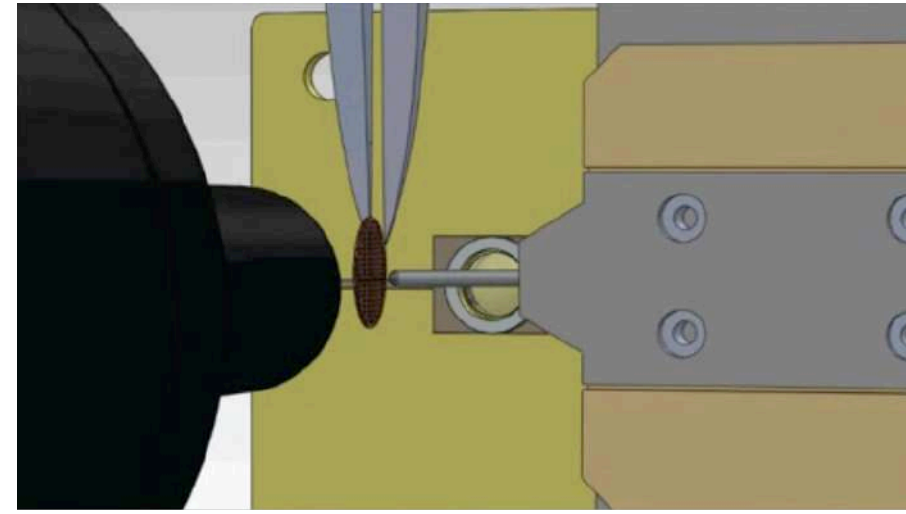
*in progress*



*the next chapter*

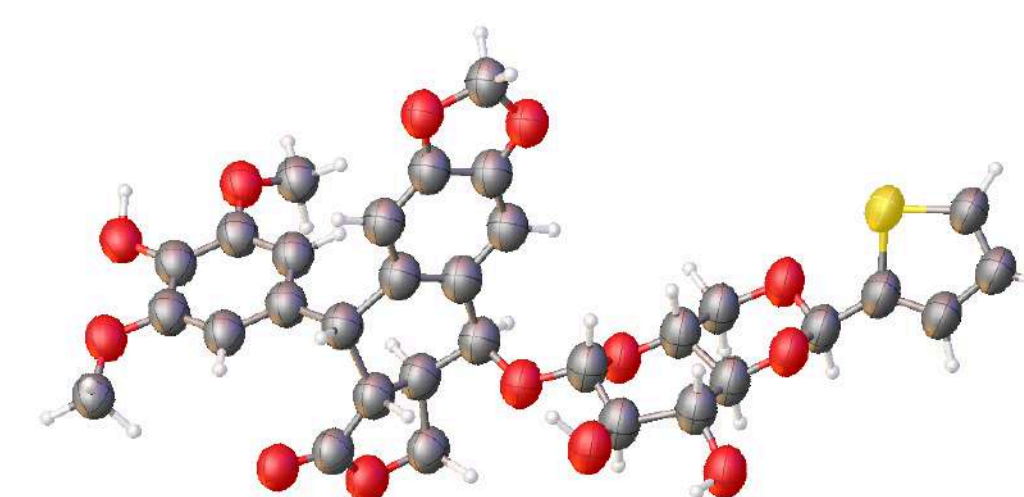
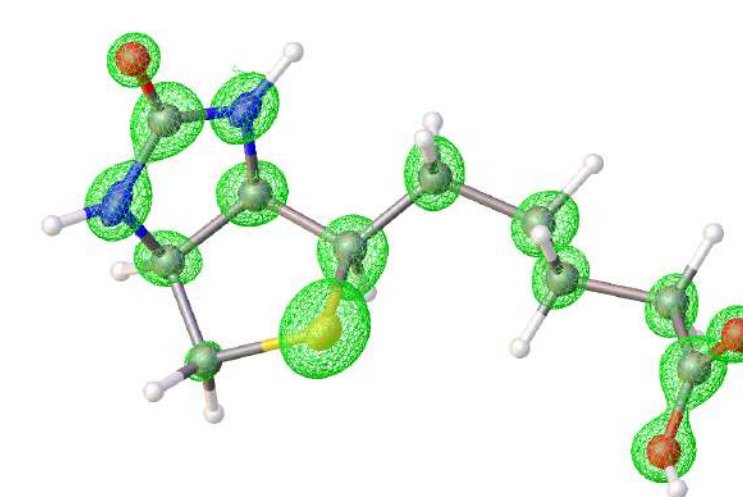
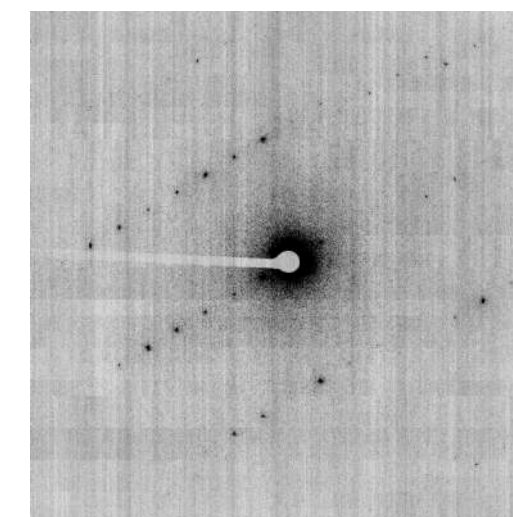
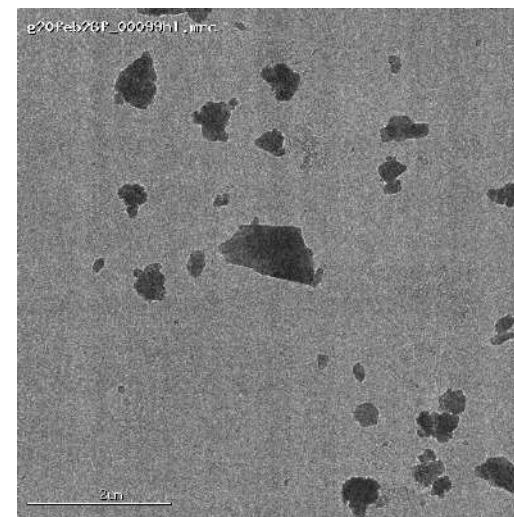
# cryoEM: a technology on the rise

Single particle cryoEM

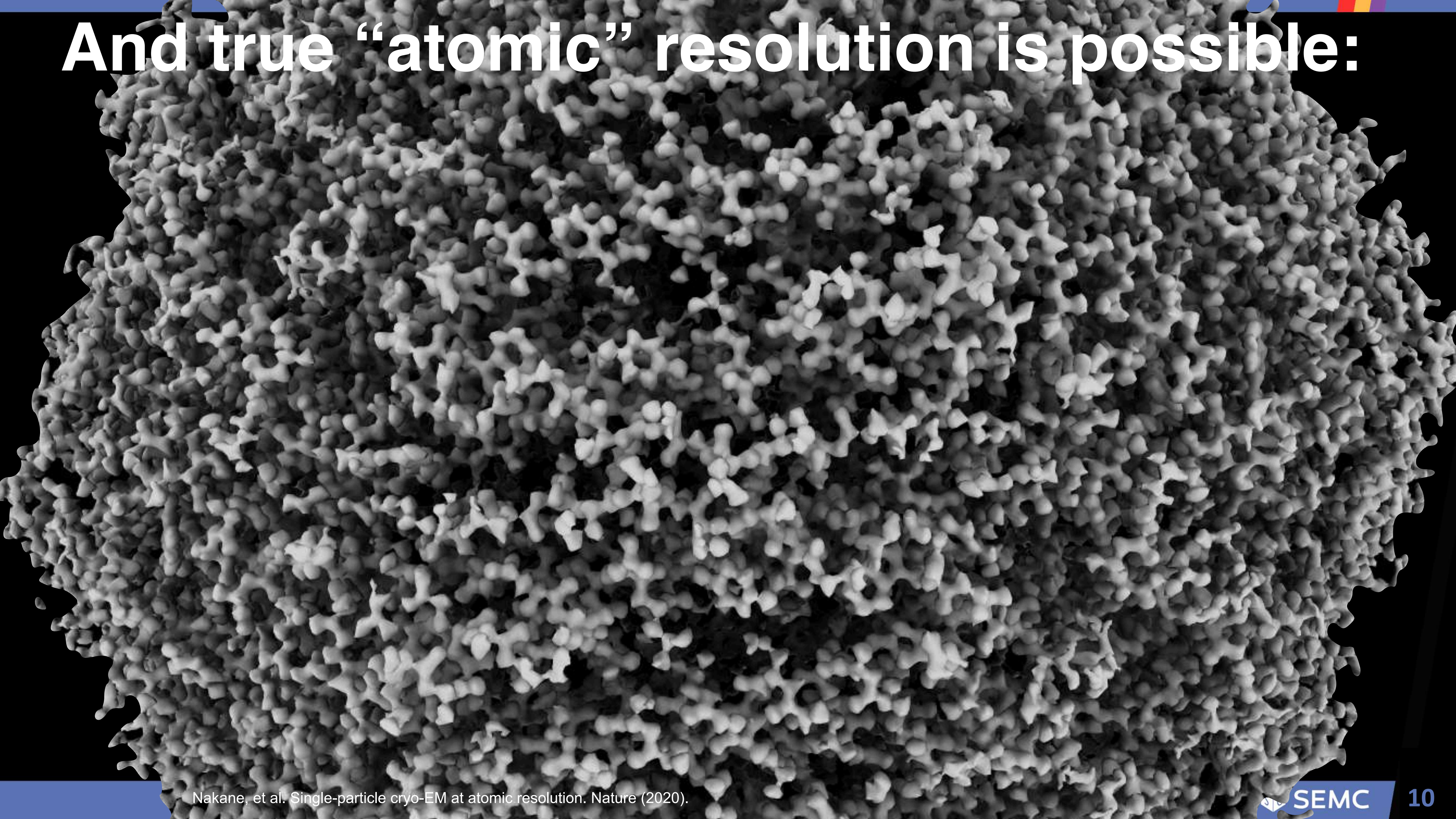


Cryo Electron Tomography (cryoET)

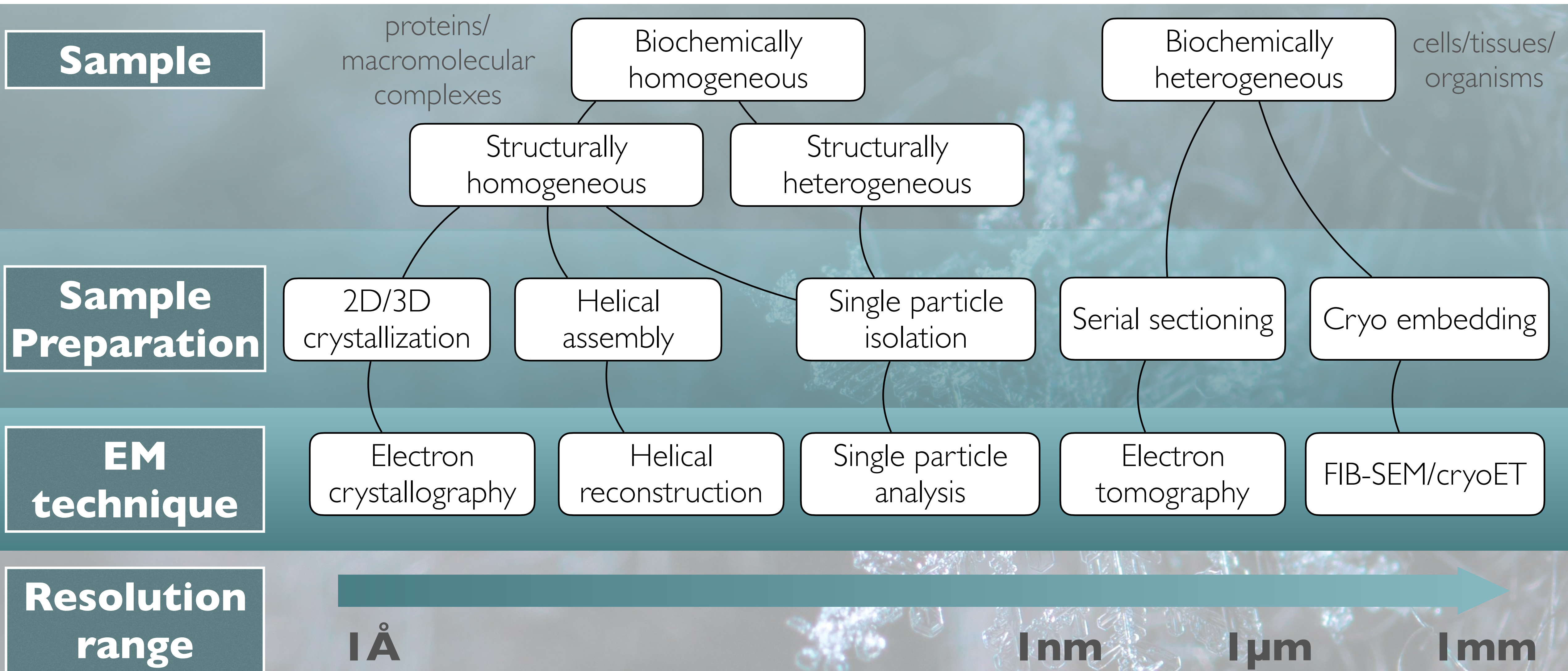
Micro crystal electron diffraction (microED)



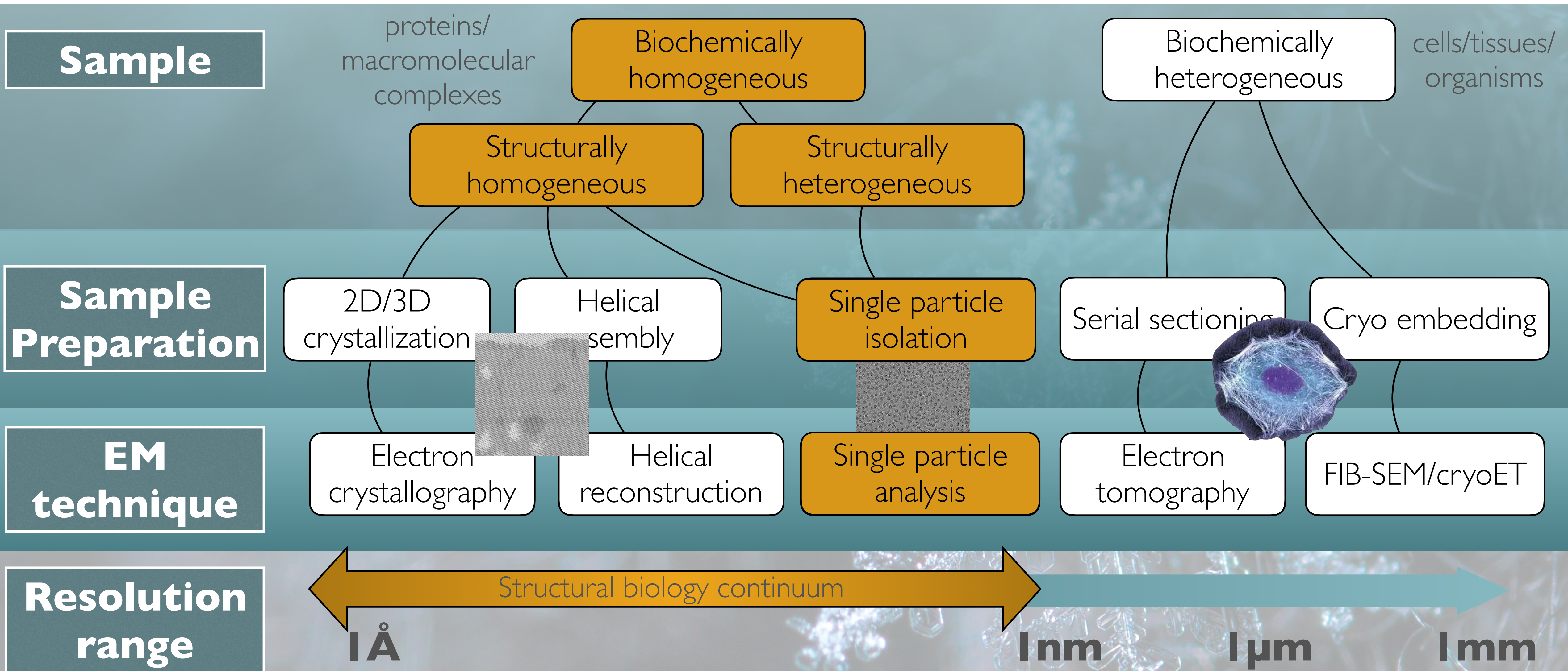
# And true “atomic” resolution is possible:



# How are samples prepared for cryoEM?

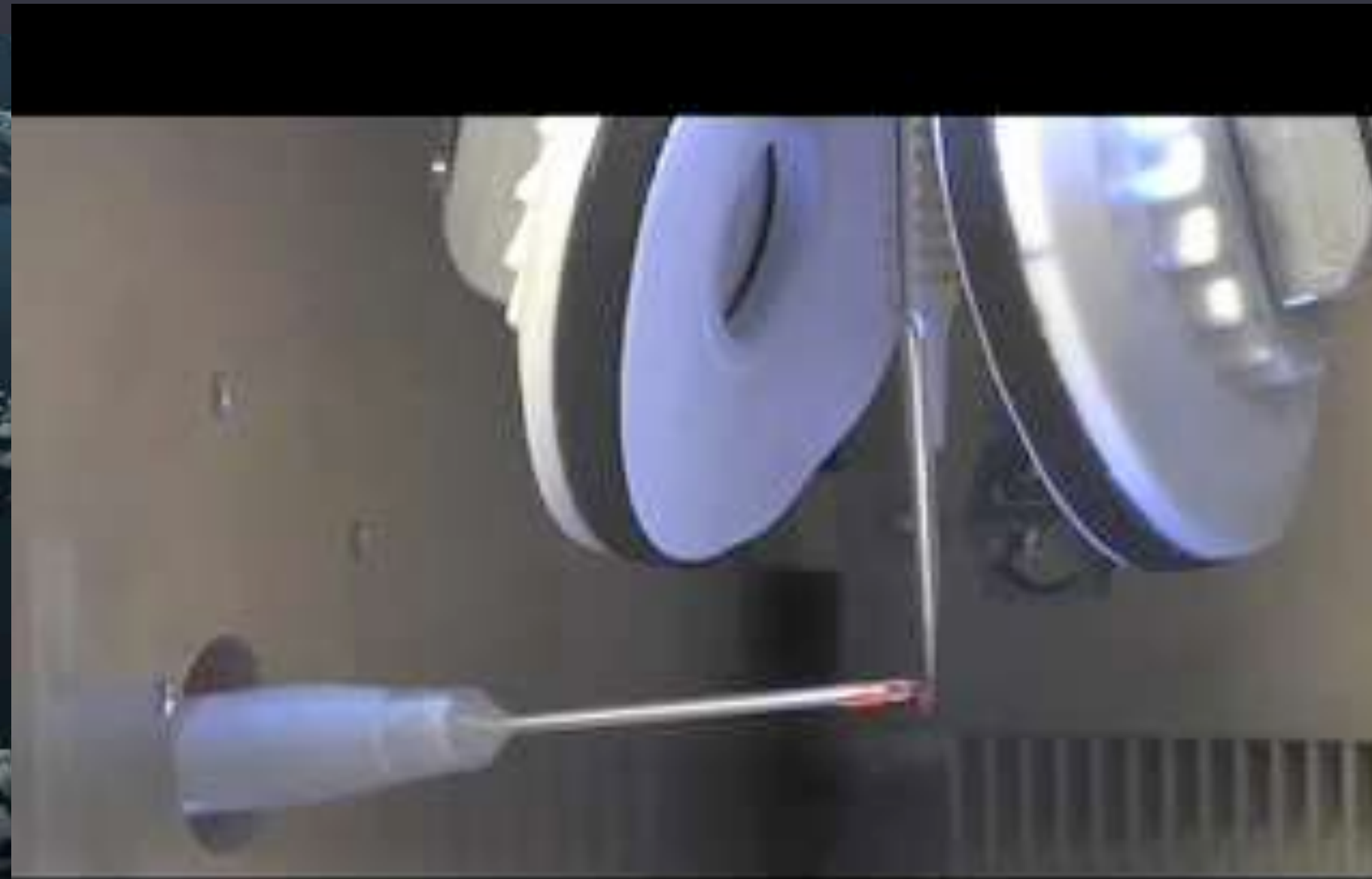
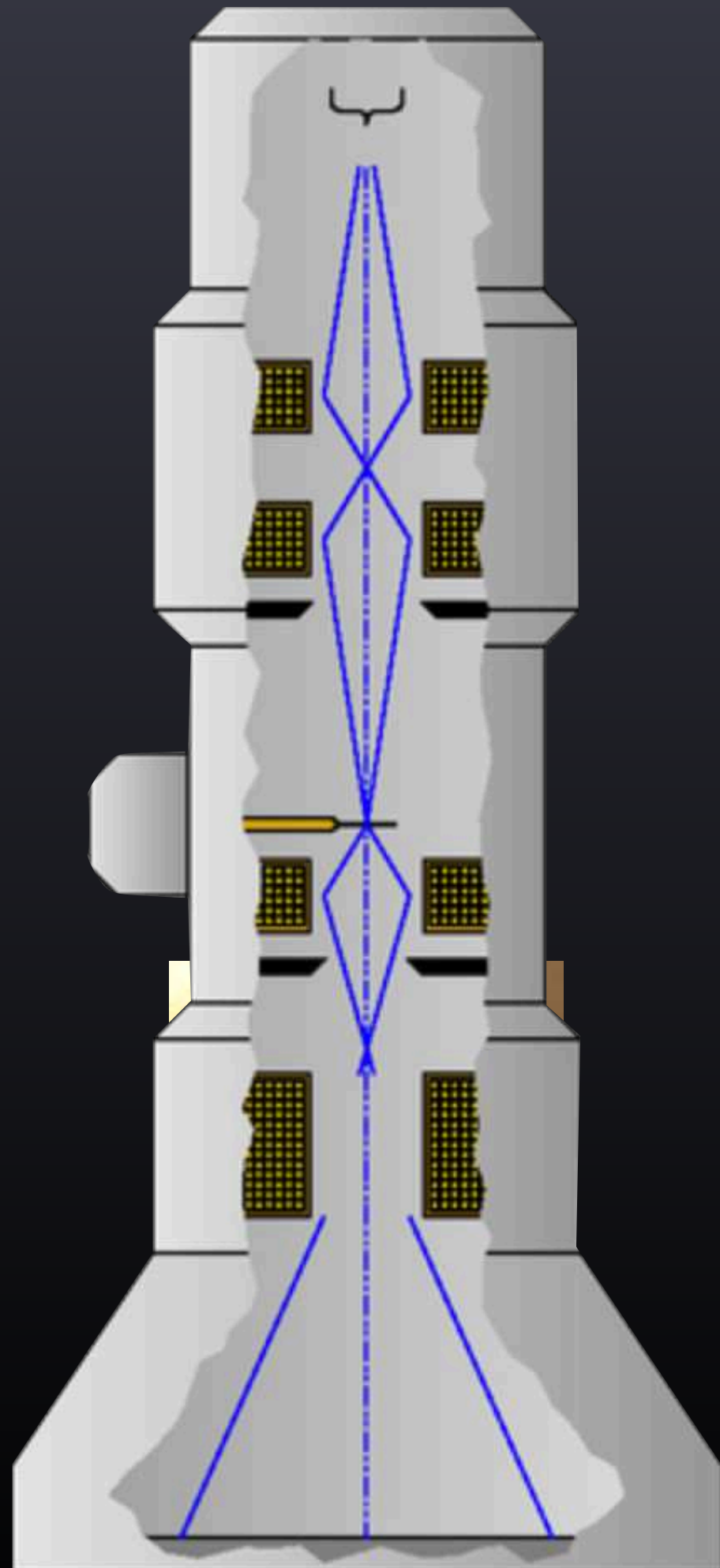


# How are samples prepared for cryoEM?



# How are samples prepared for cryoEM?

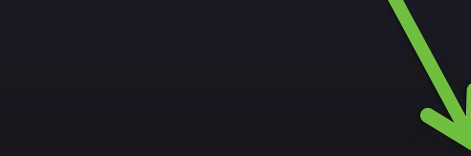
## Vitrifying a biological sample



>99.999%

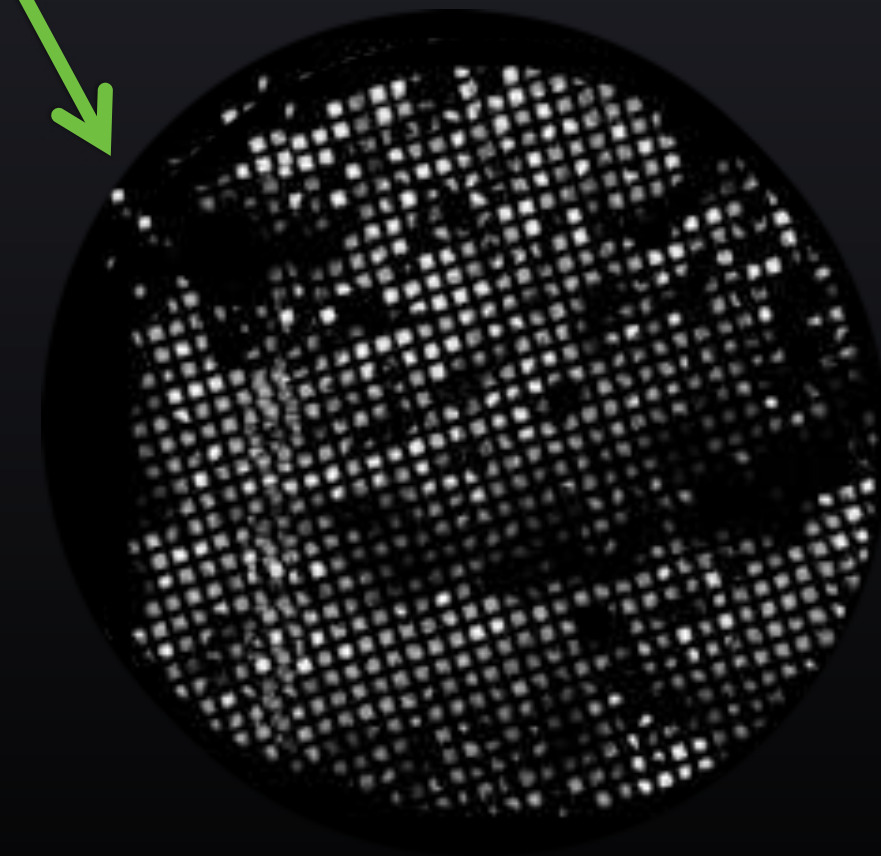


<0.001%



~3 $\mu$ l

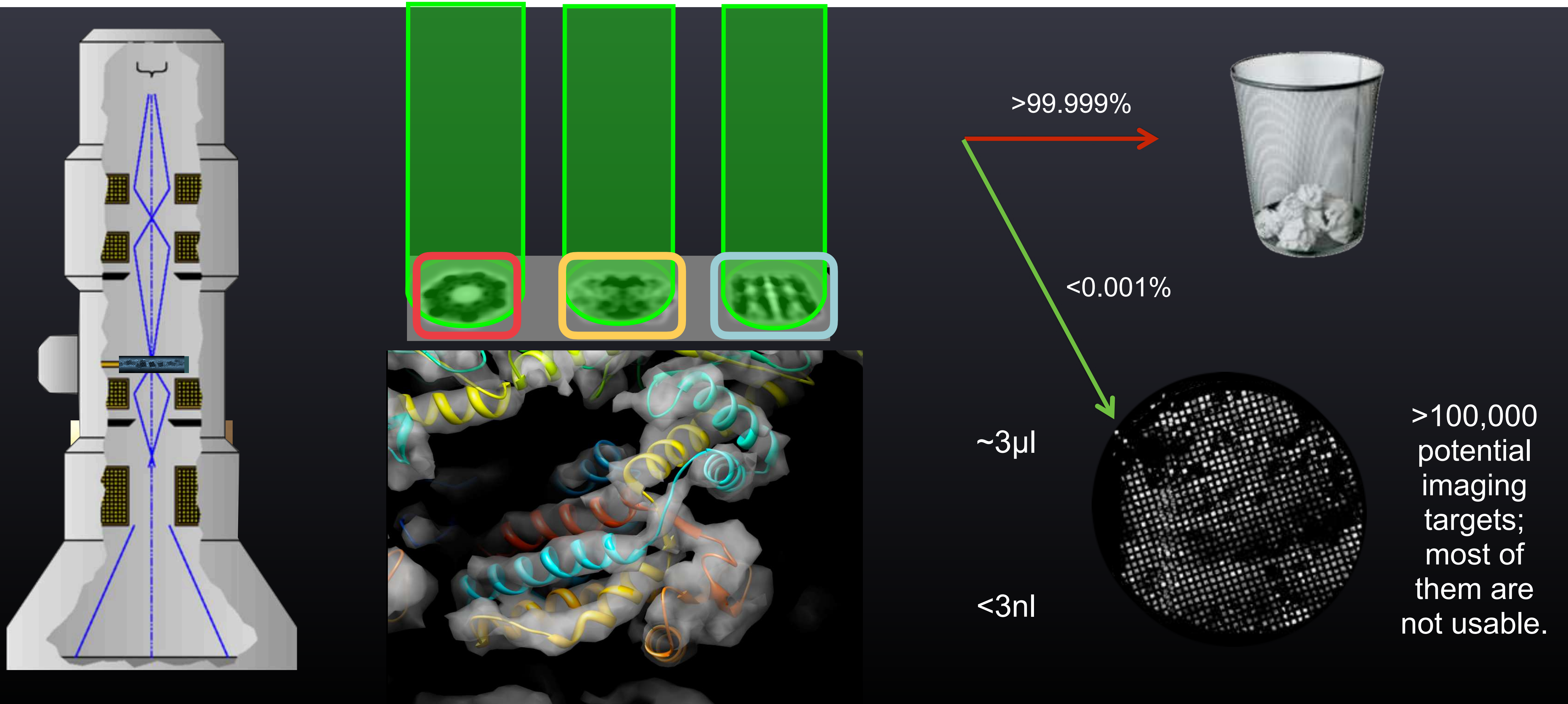
<3nl



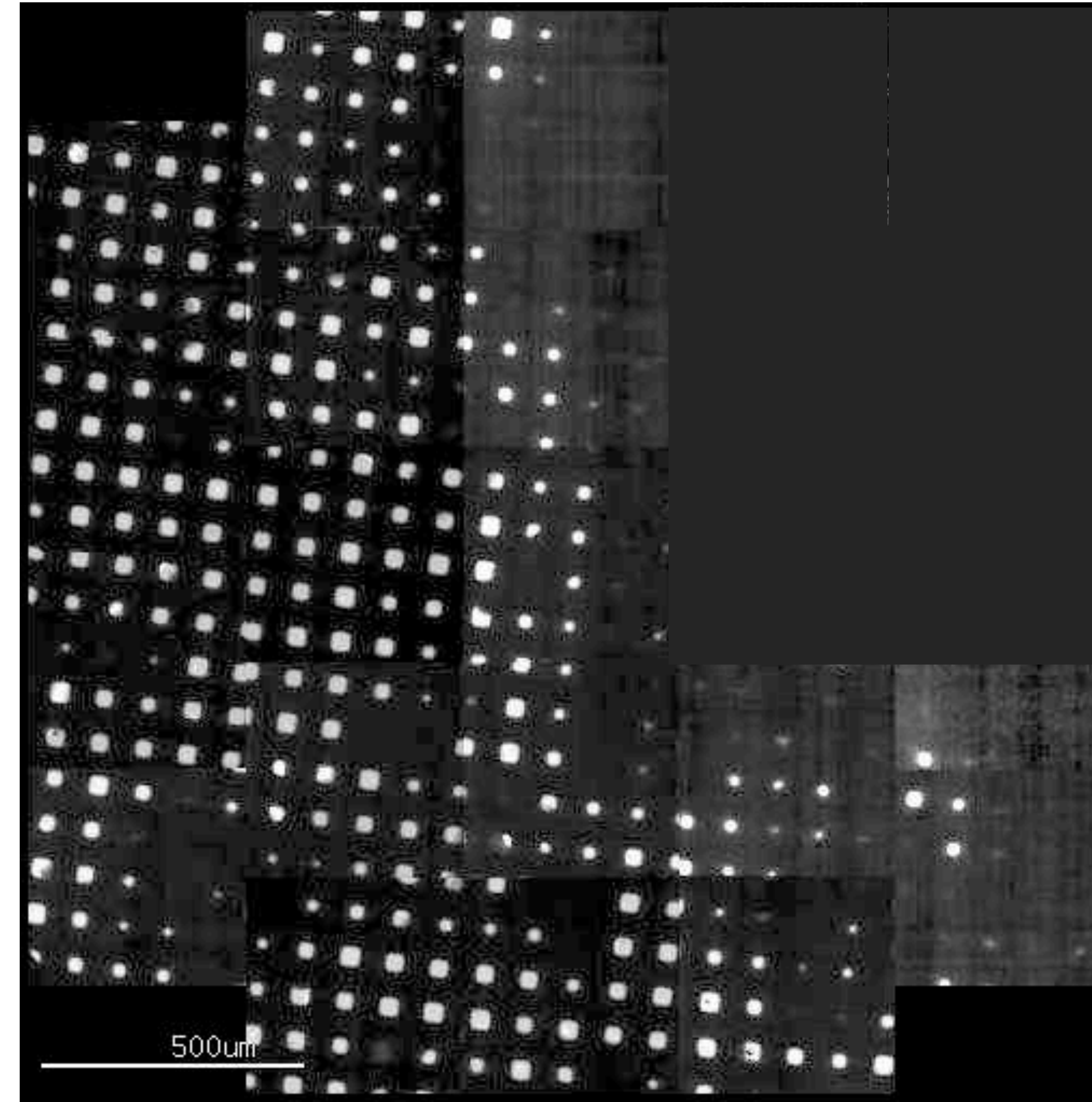
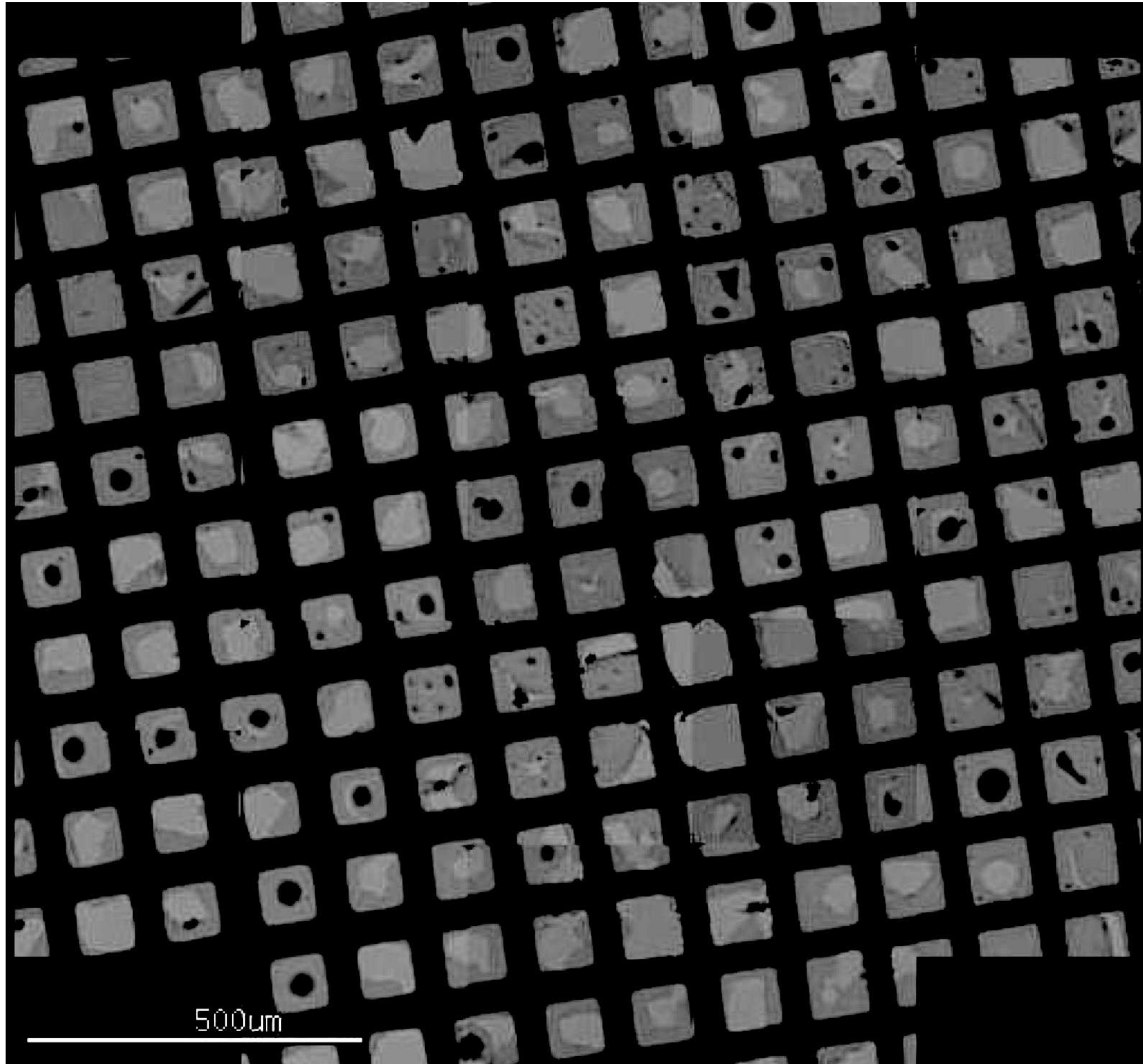
>100,000 potential imaging targets; most of them are not usable.

# How are samples prepared for cryoEM?

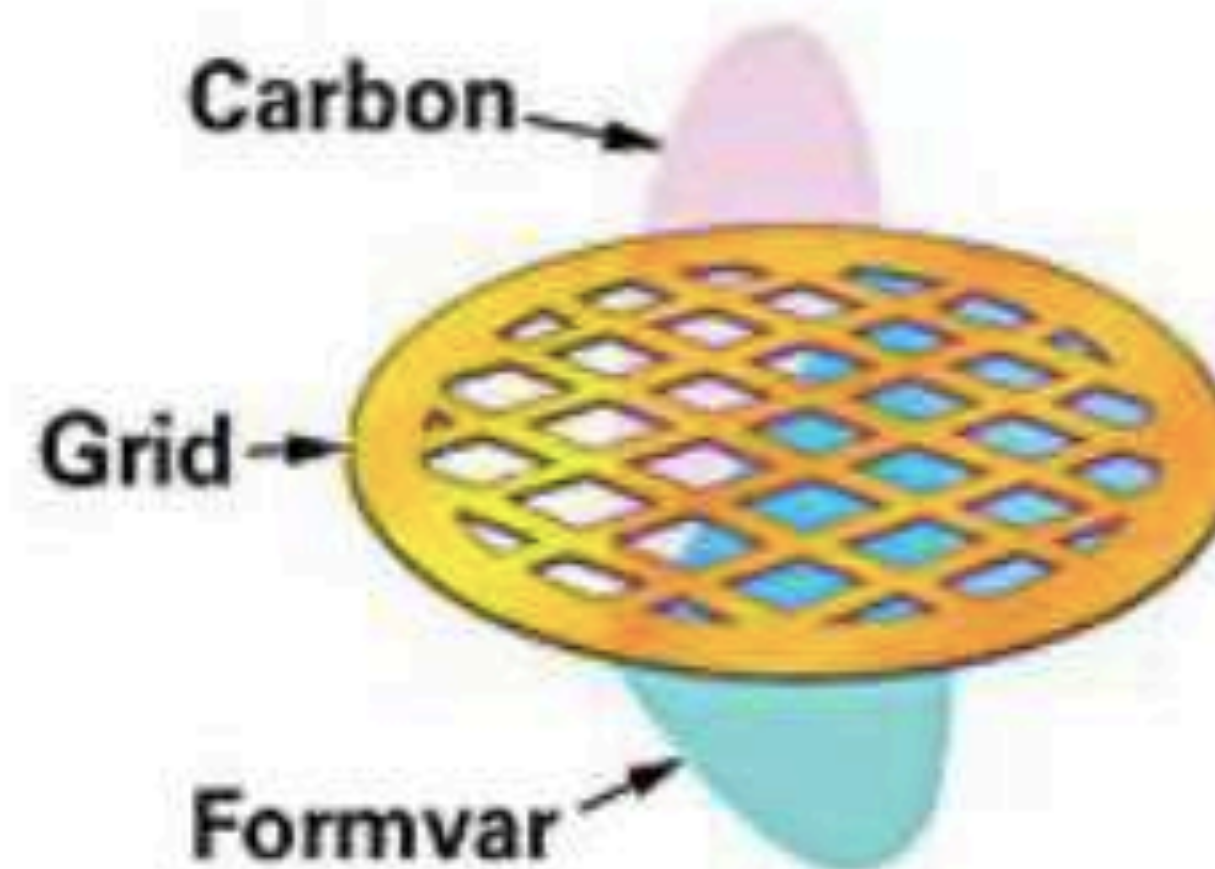
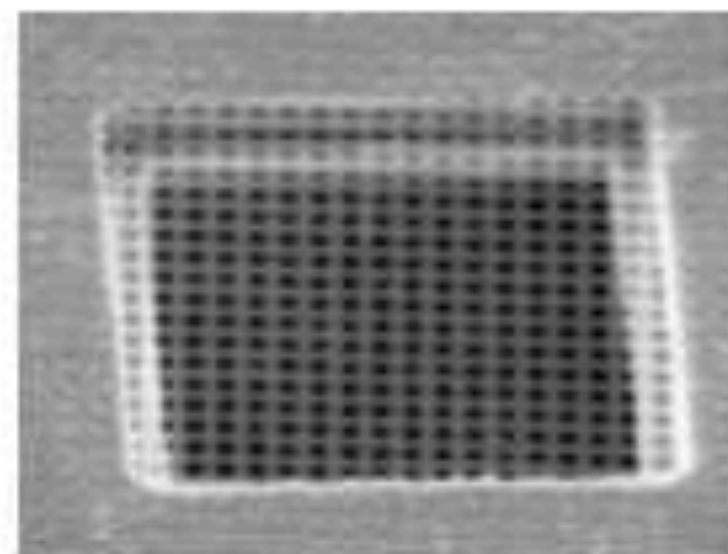
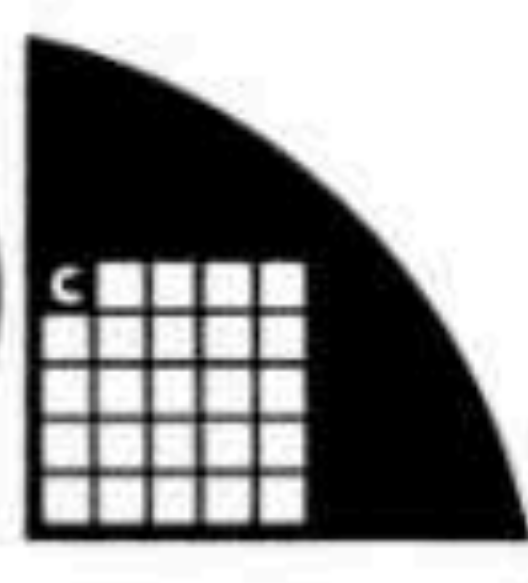
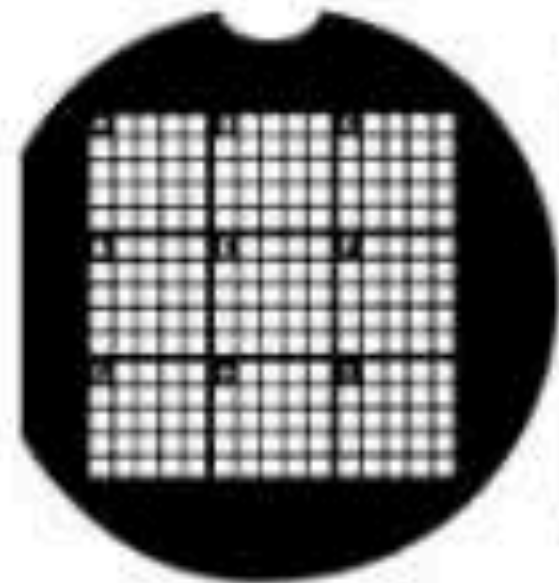
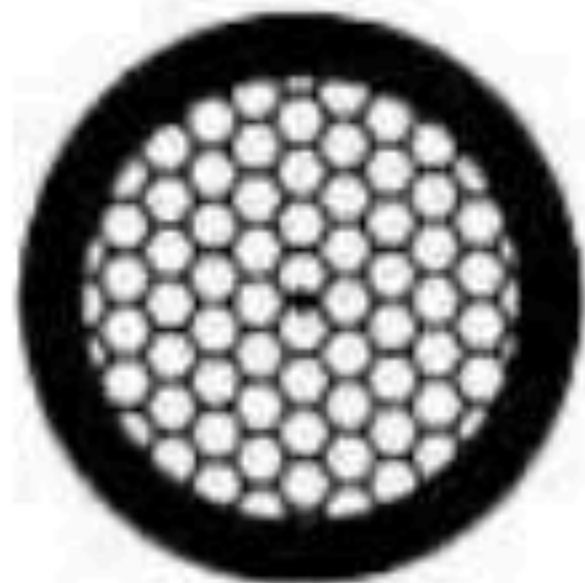
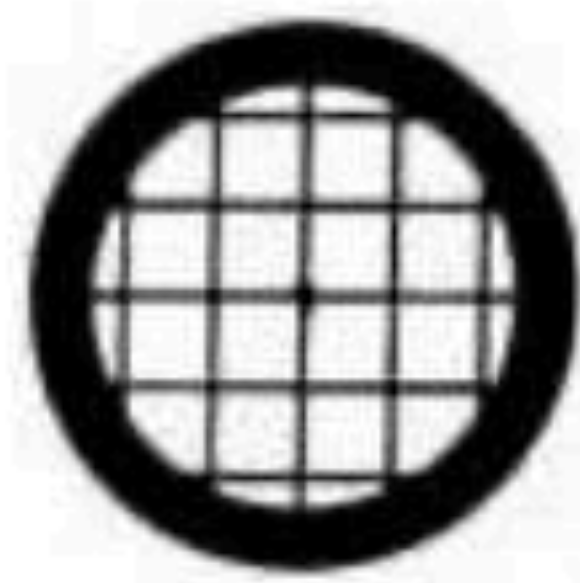
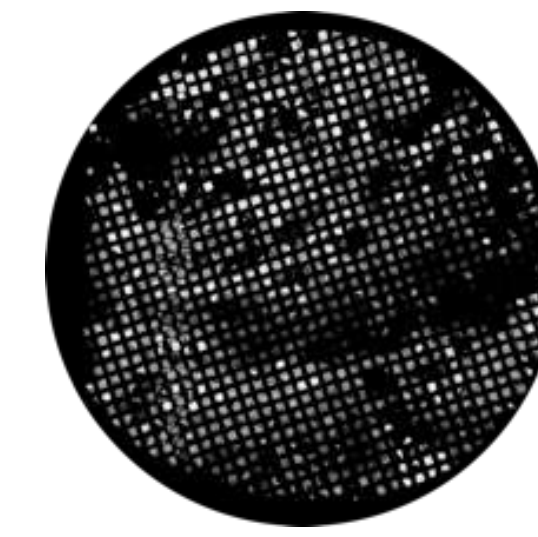
## Vitrifying a biological sample



# What do EM grids look like?



# What do EM grids look like?



## Common Materials

Copper

Nickel

Gold

Aluminum

Molybdenum

Titanium

Stainless Steel

[https://www.tedpella.com/grids\\_html/](https://www.tedpella.com/grids_html/)

# What do EM grids look like?



## Rough grid parameters

Rim Width: 350-400 $\mu$ m.

Thickness: approximately 25 $\mu$ m thick.

Diameter: 3.0 to 3.05mm

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

Example 200 mesh pitch =  $25.4/200 = 127\mu$ m

## PELCO® Grid Size

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

# What do EM grids look like?

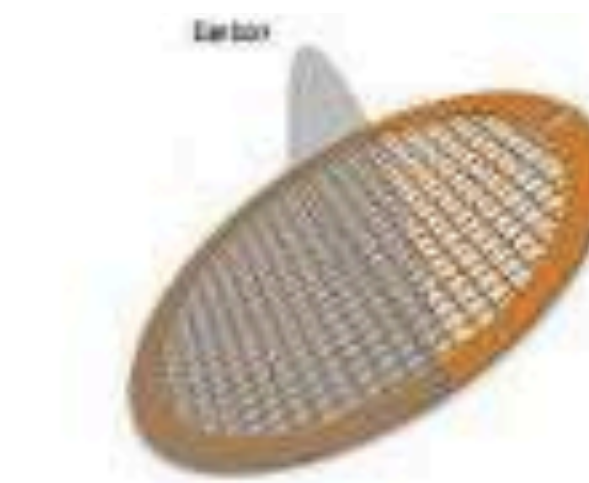
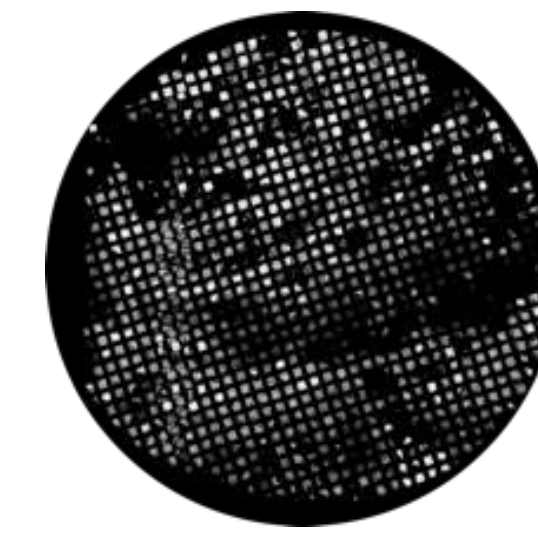
## TERMINOLOGY

Grid (Cu, Au, Mo, etc...)

- mesh

Foil (C, Au, etc...)

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



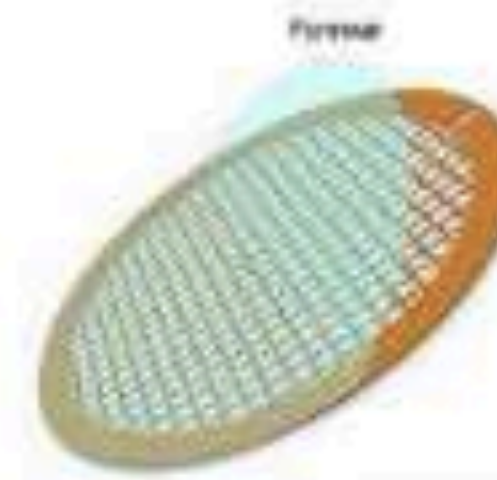
CARBON ONLY SUPPORT FILMS



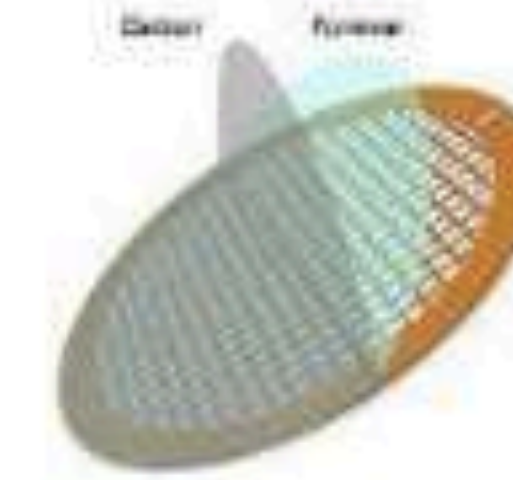
HOLEY CARBON SUPPORT FILMS



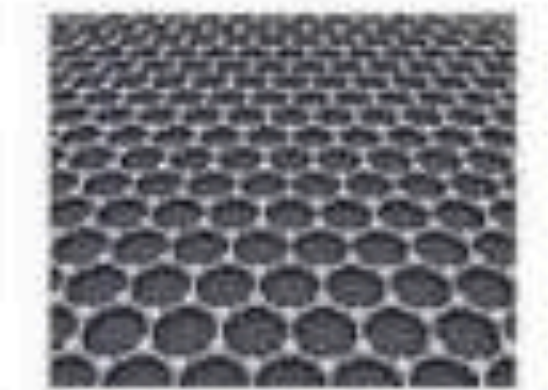
LACEY CARBON SUPPORT FILMS



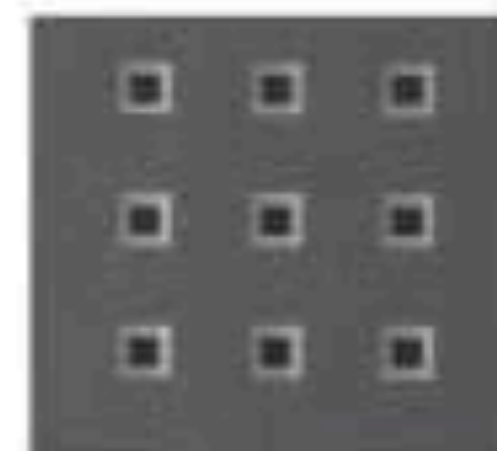
FORMVAR ONLY SUPPORT FILMS



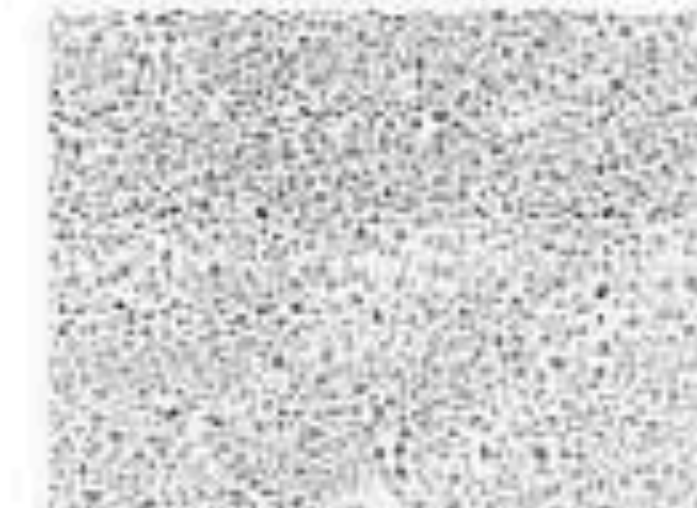
FORMVAR / CARBON SUPPORT FILMS



EM-TEC GRAPHENE SUPPORT FILMS



EM-TEC SILICON NITRIDE SUPPORT FILMS



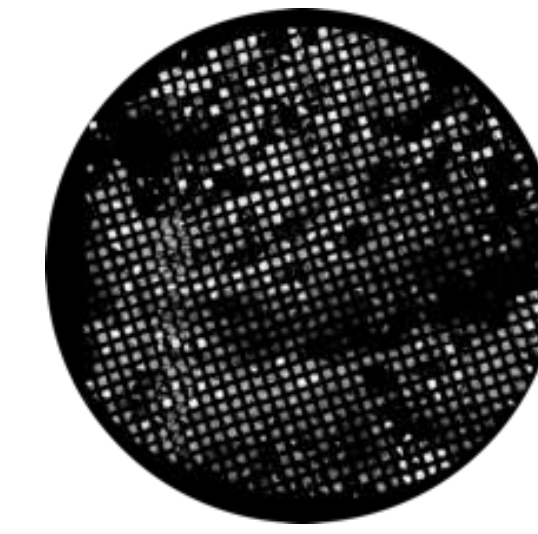
TEM CALIBRATION & TEST STANDARDS



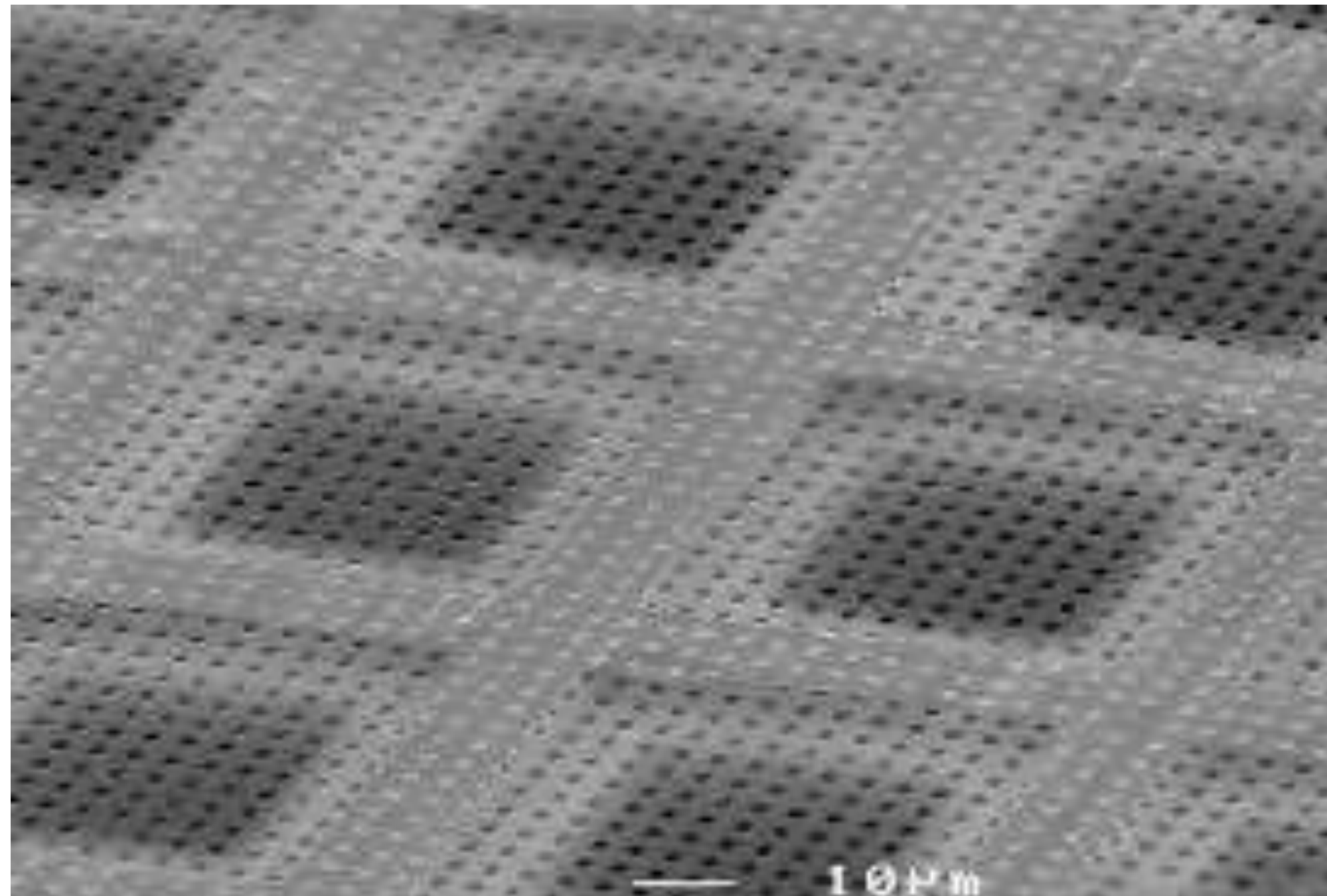
TEM GRID STORAGE BOXES

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

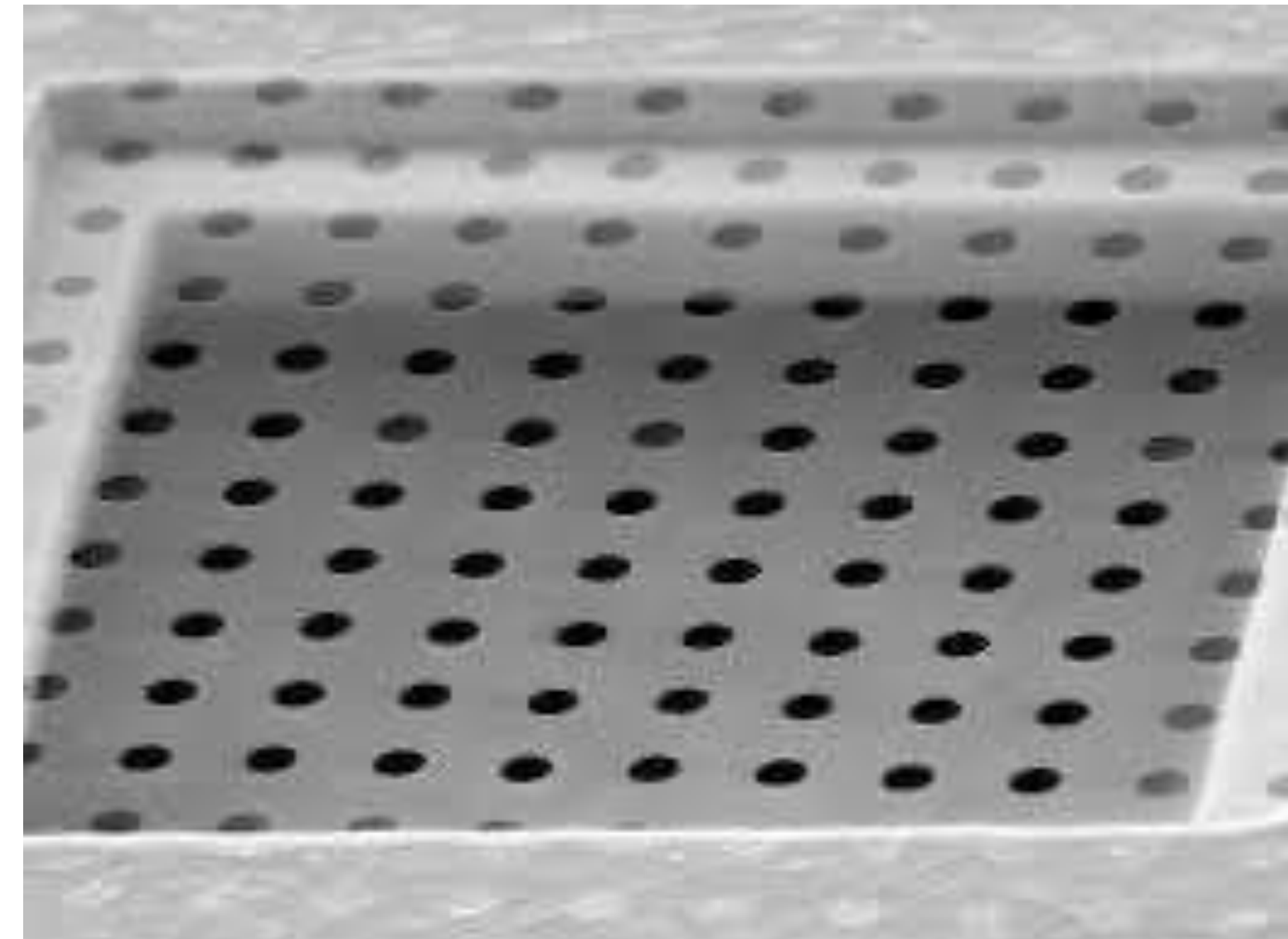
# What do EM grids look like?



## TERMINOLOGY

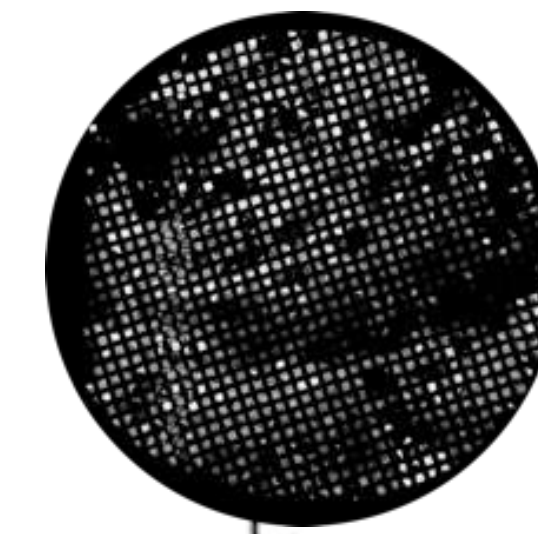


- [Protochips.com](https://www.protochips.com)



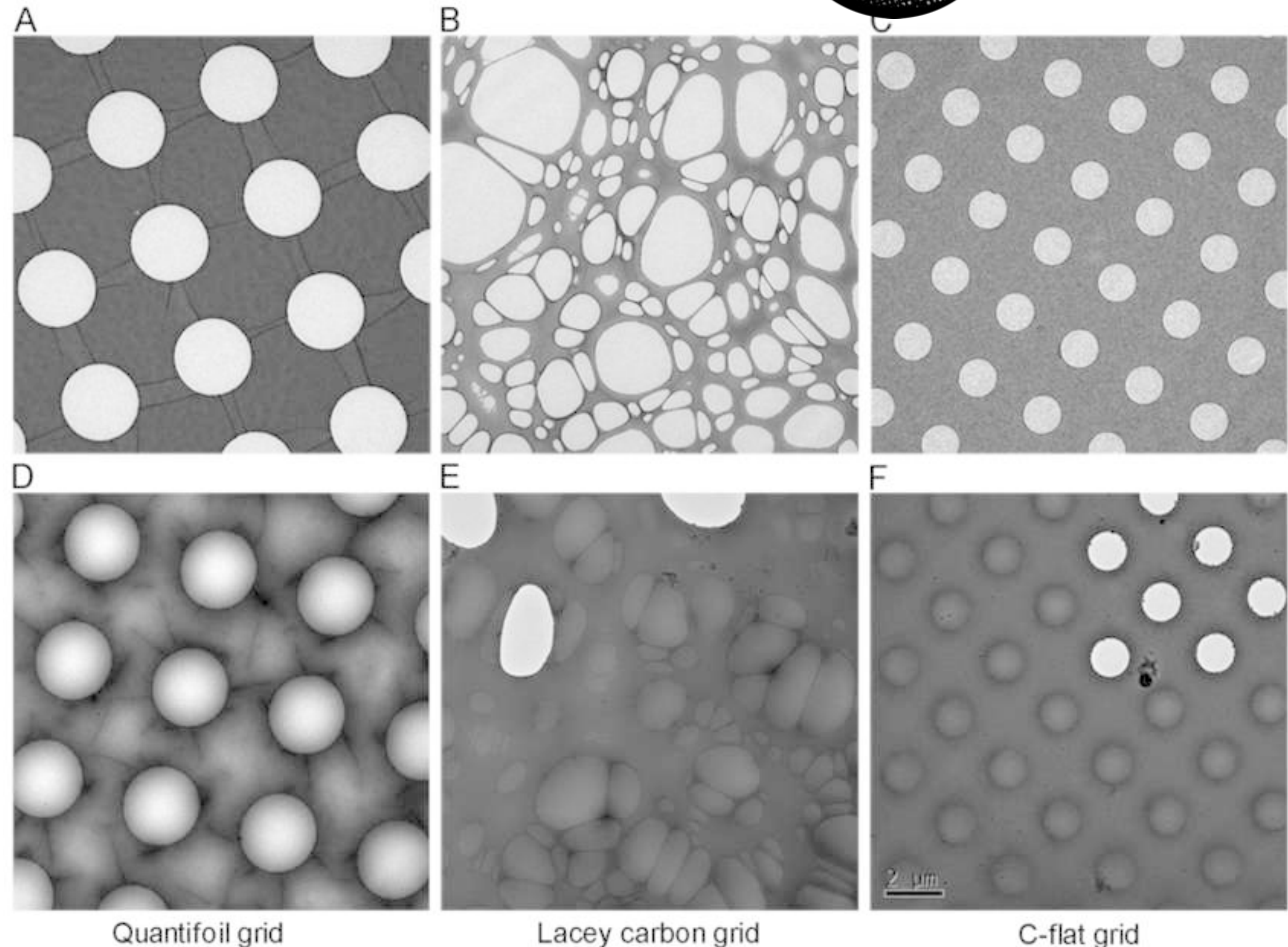
- [Quantifoil.com](https://www.quantifoil.com)

# What do EM grids look like?

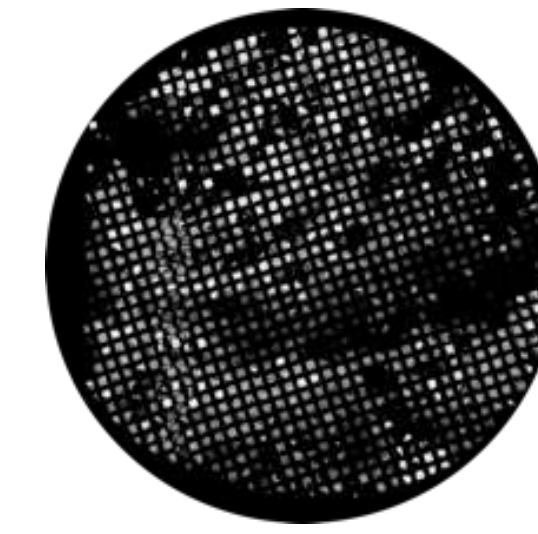


## TERMINOLOGY

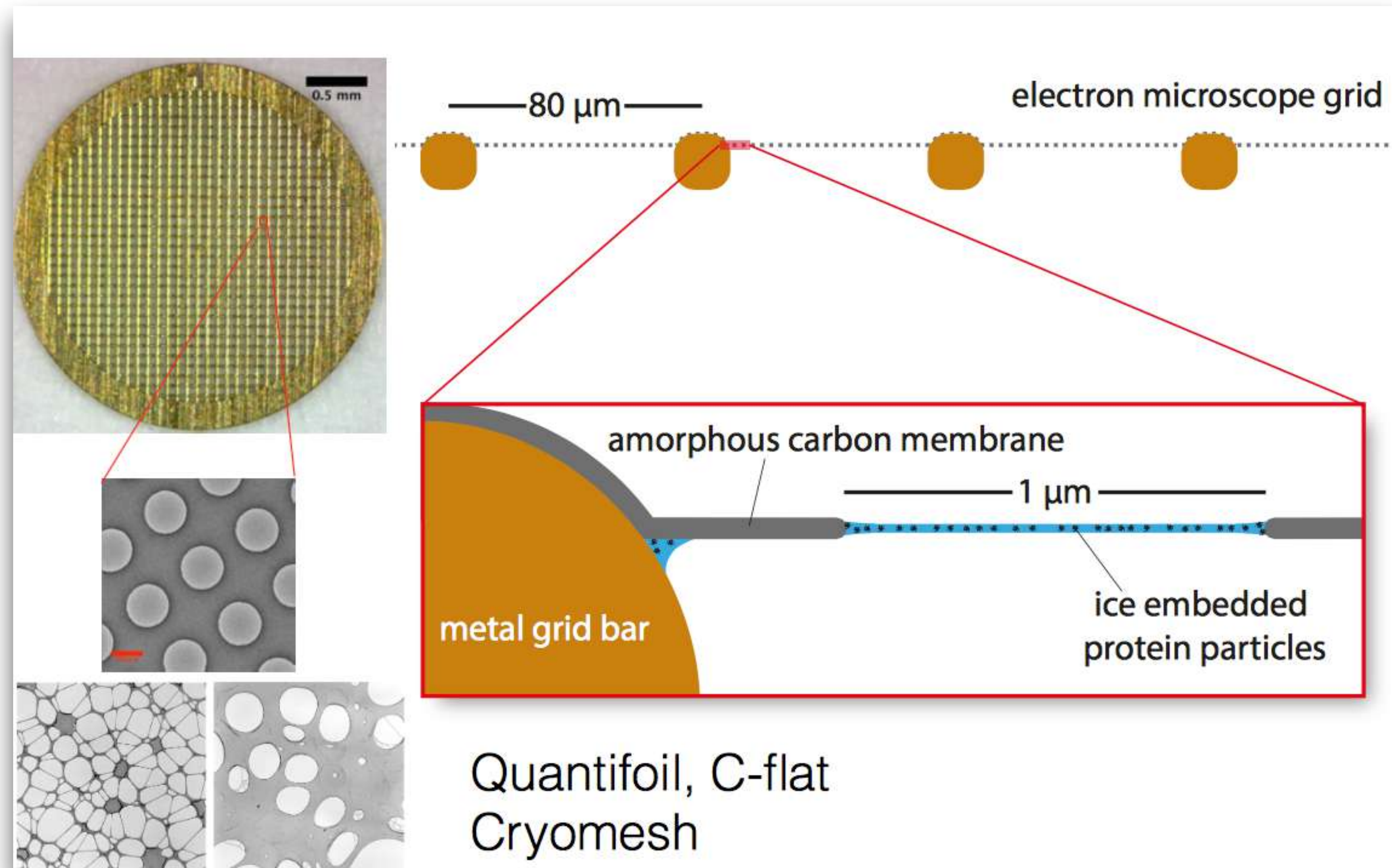
Cho, Hye-Jin & Hyun, Jae-Kyung & Kim, Jin-Gyu & Jeong, Hyeong & Park, Hyo & You, Dong-Ju & Jung, Hyun. (2013). Measurement of ice thickness on vitreous ice embedded cryo-EM grids: investigation of optimizing condition for visualizing macromolecules. *Journal of Analytical Science and Technology*. 4.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



# What do EM grids look like?



## TERMINOLOGY



# What do EM grids look like?

## TERMINOLOGY

- Holey gold foil on gold mesh grid

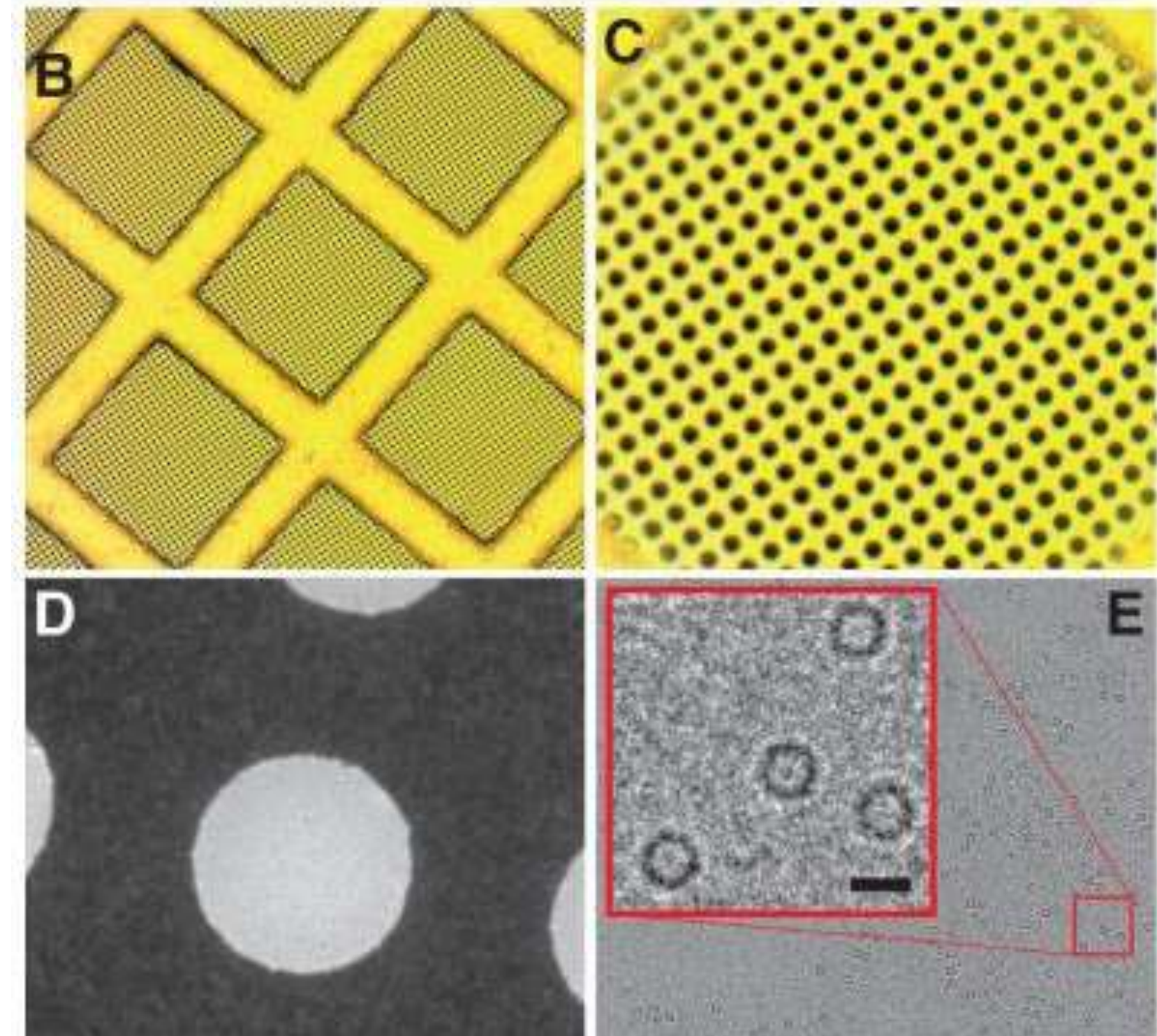
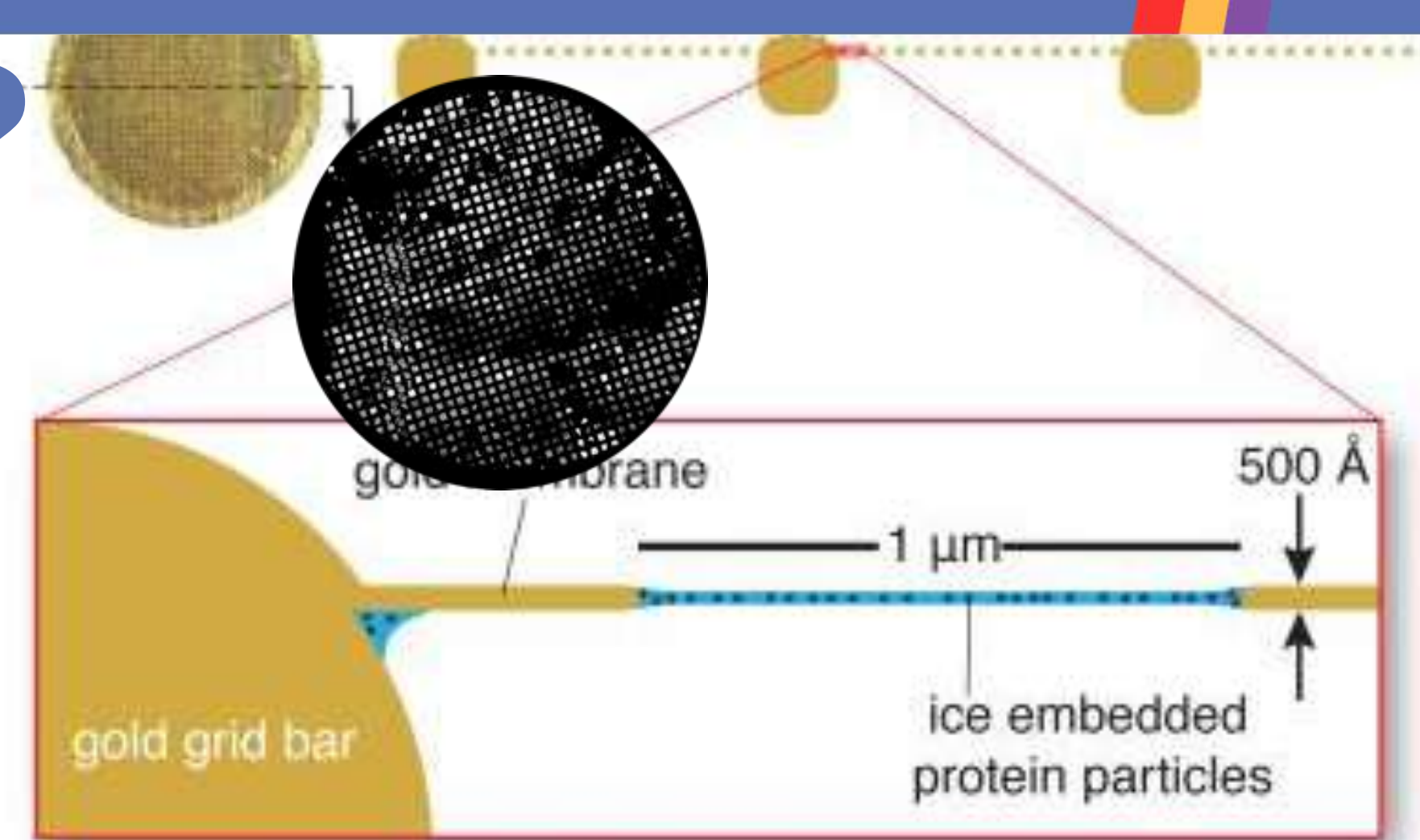
### Advantages:

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

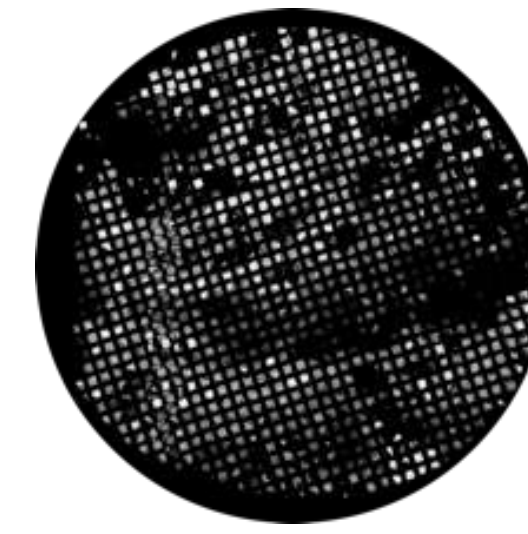
### Disadvantages:

- Difficult to find focus due to lack of amorphous substrate

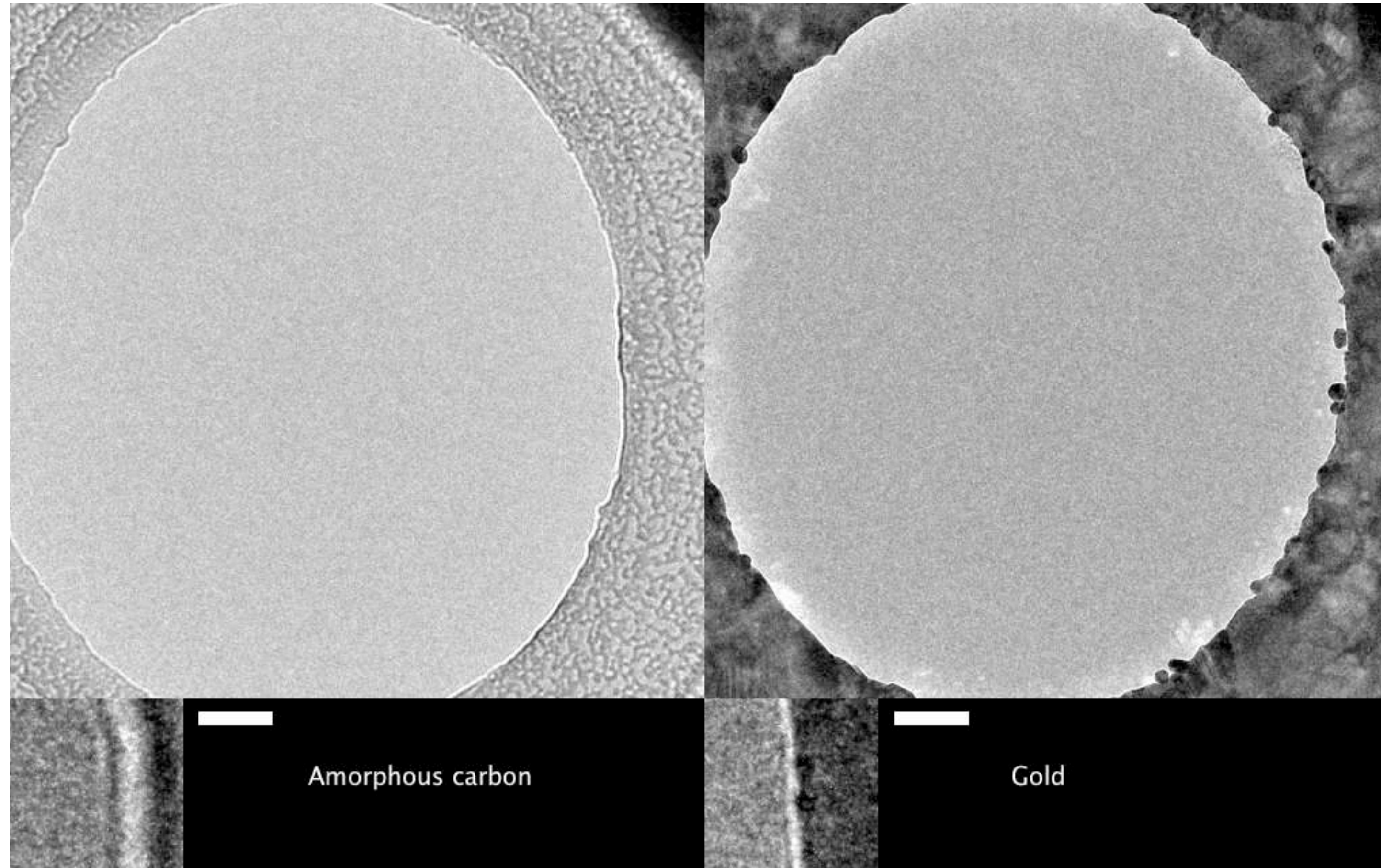
Russo & Passmore, 2015



# What do EM grids look like?



Gold grids

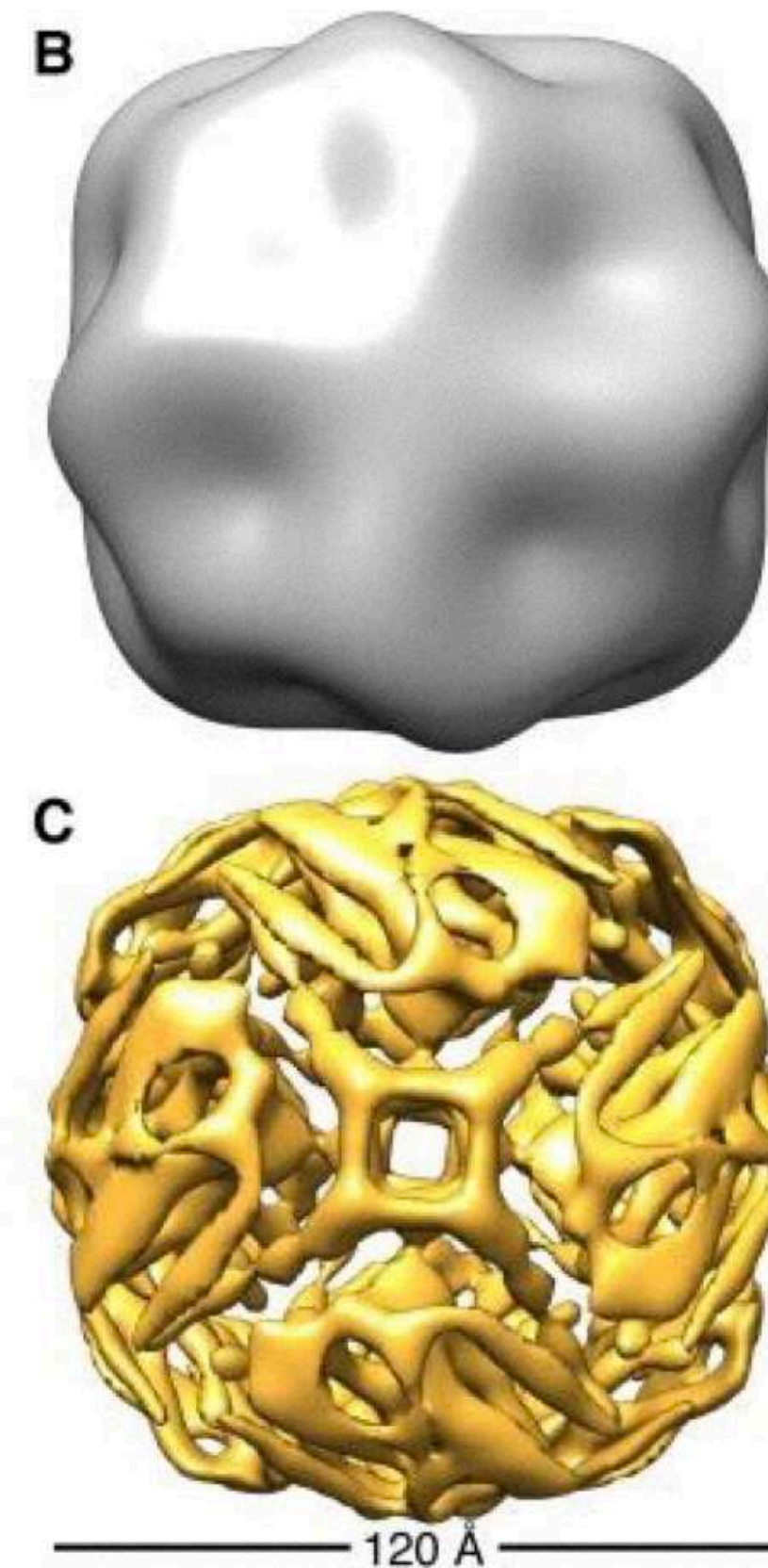
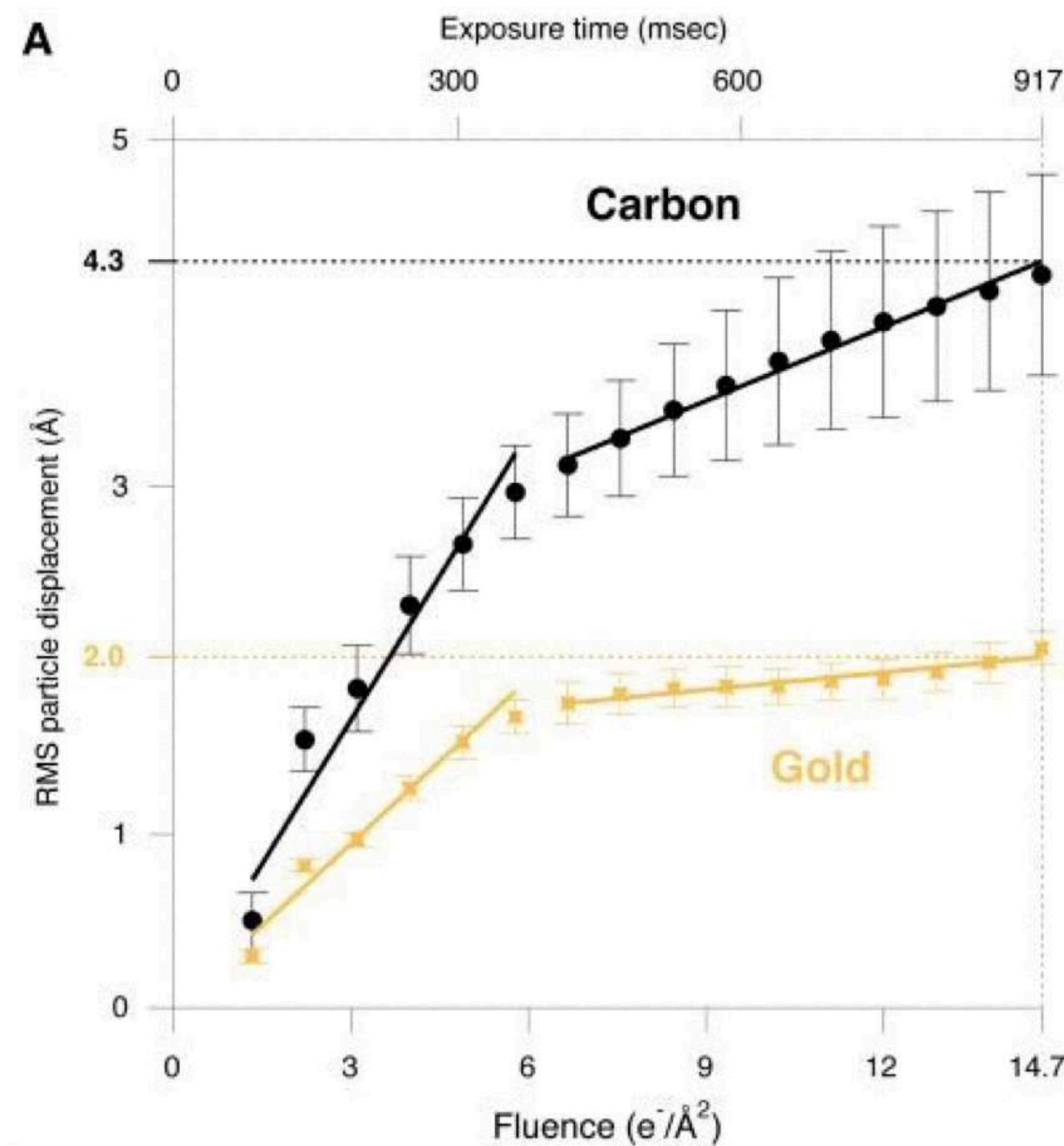


Russo & Passmore, 2015

# What do EM grids look like?



## Gold grids



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

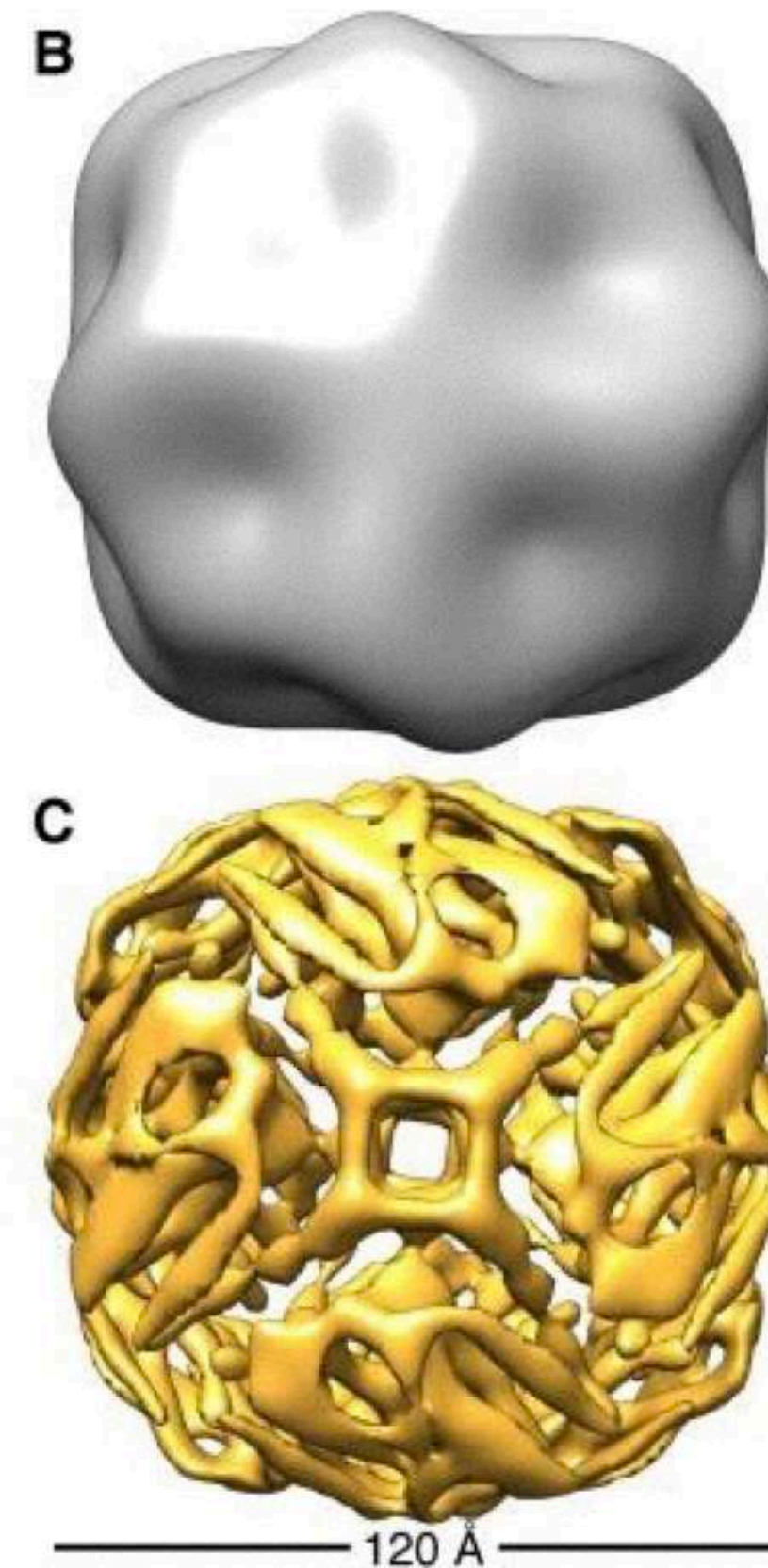
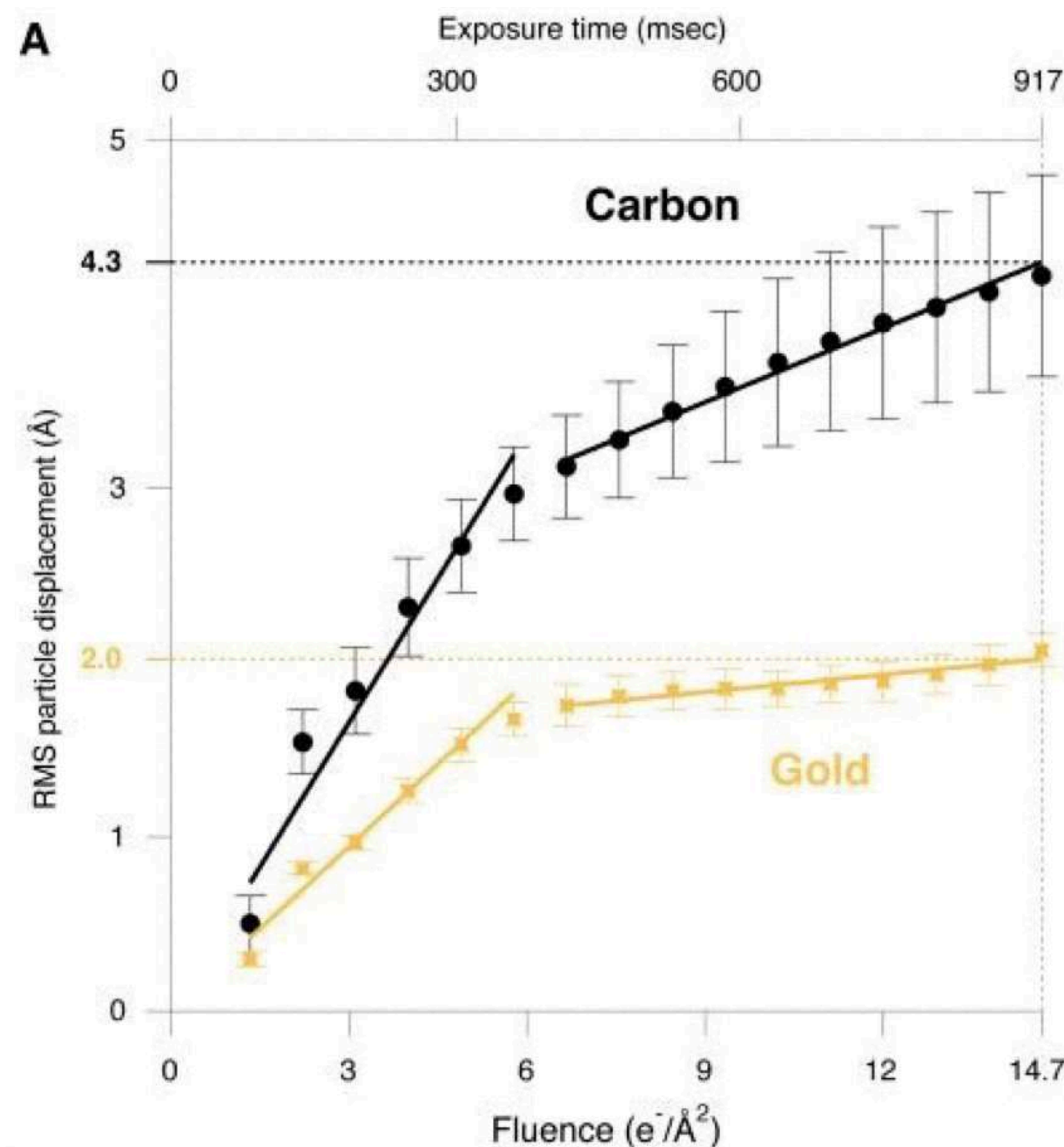
Apo ferritin density maps using same imaging conditions and identical processing for **B.** carbon and **C.** gold substrates. **B.** is at 25 Å and **C.** 8 Å resolution.

Russo & Passmore, 2015

# What do EM grids look like?



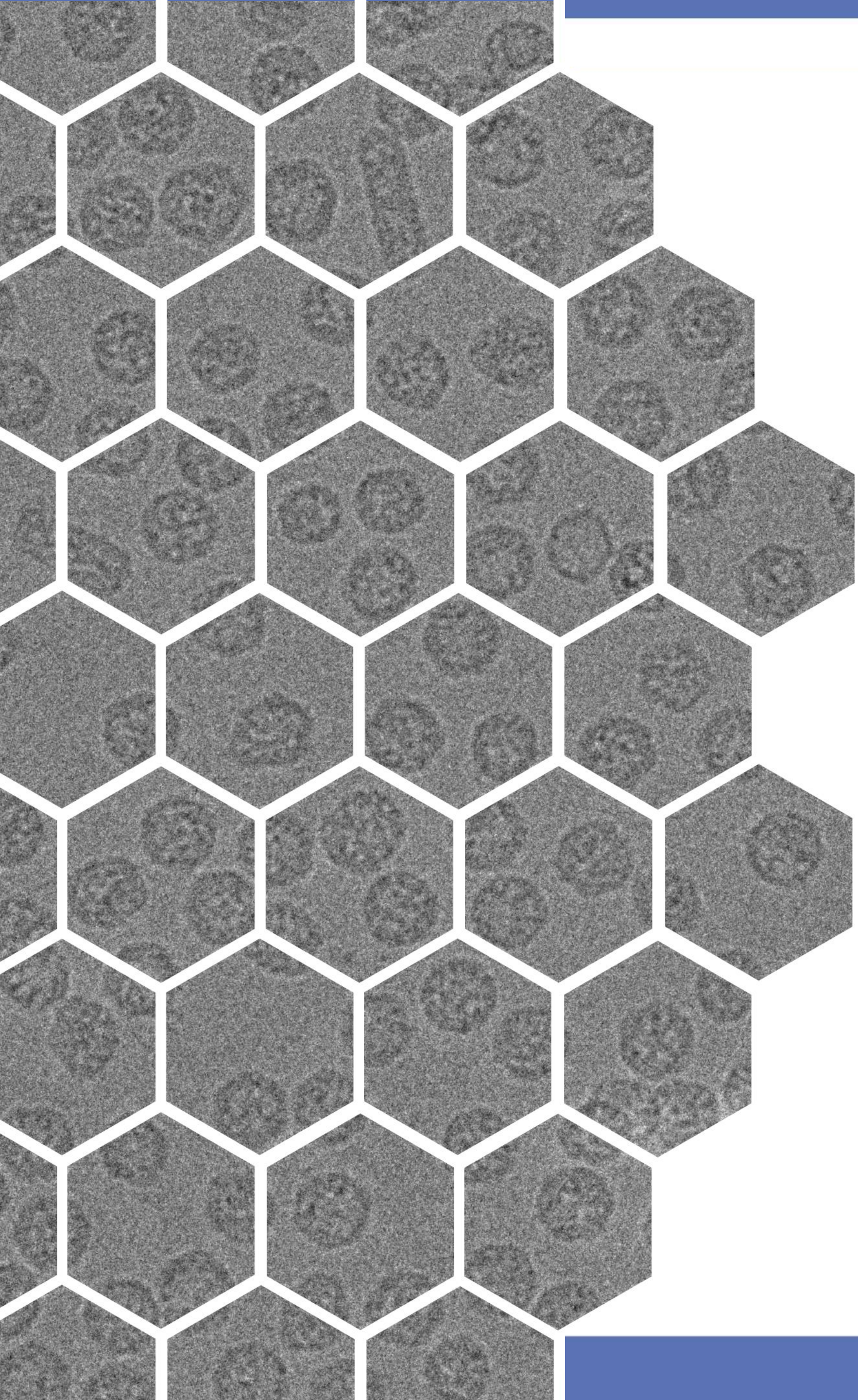
## Gold grids



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

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Russo & Passmore, 2015

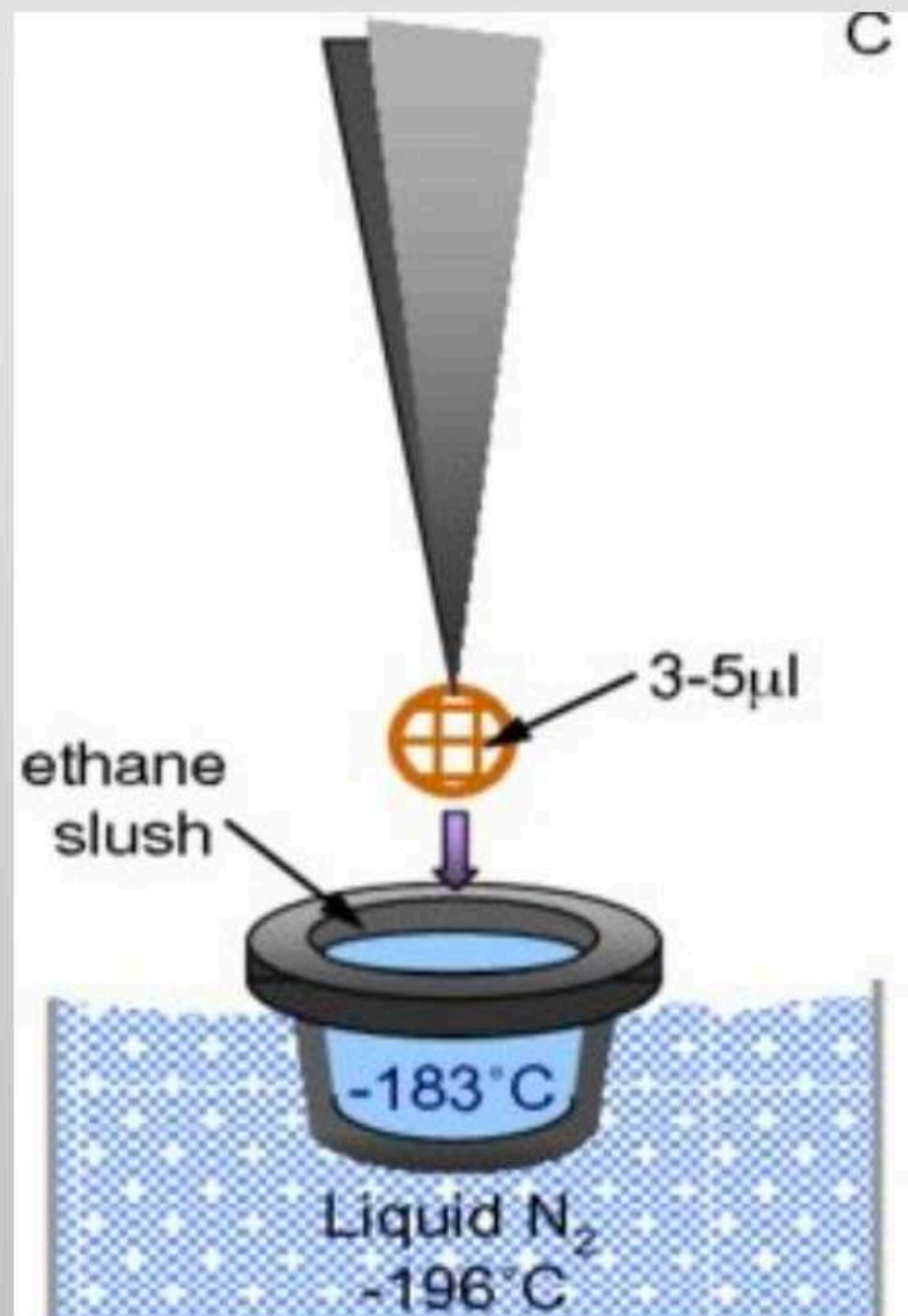


- ◆ Journal club and practical recap
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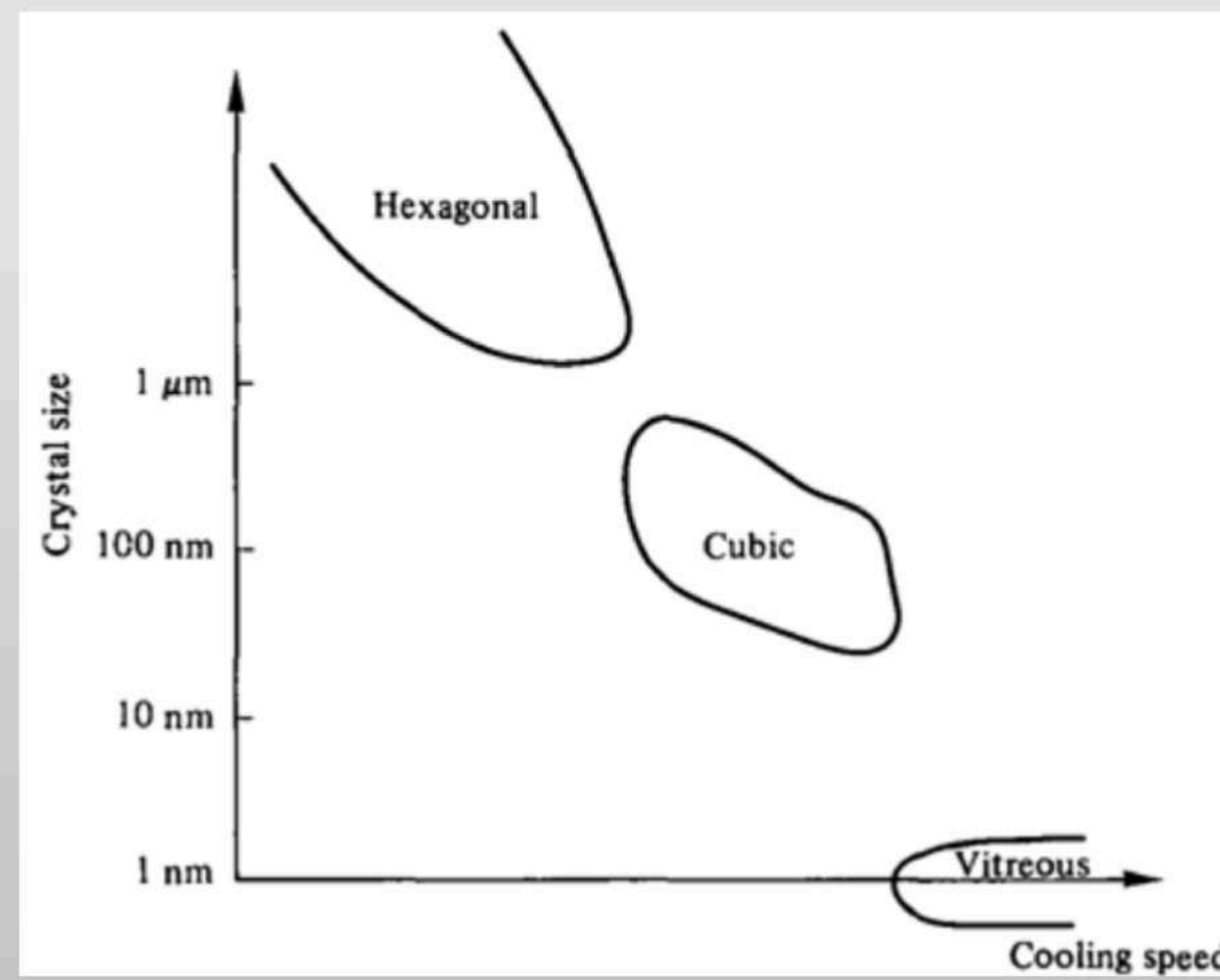
# How are samples prepared for cryoEM?

## Vitrification process

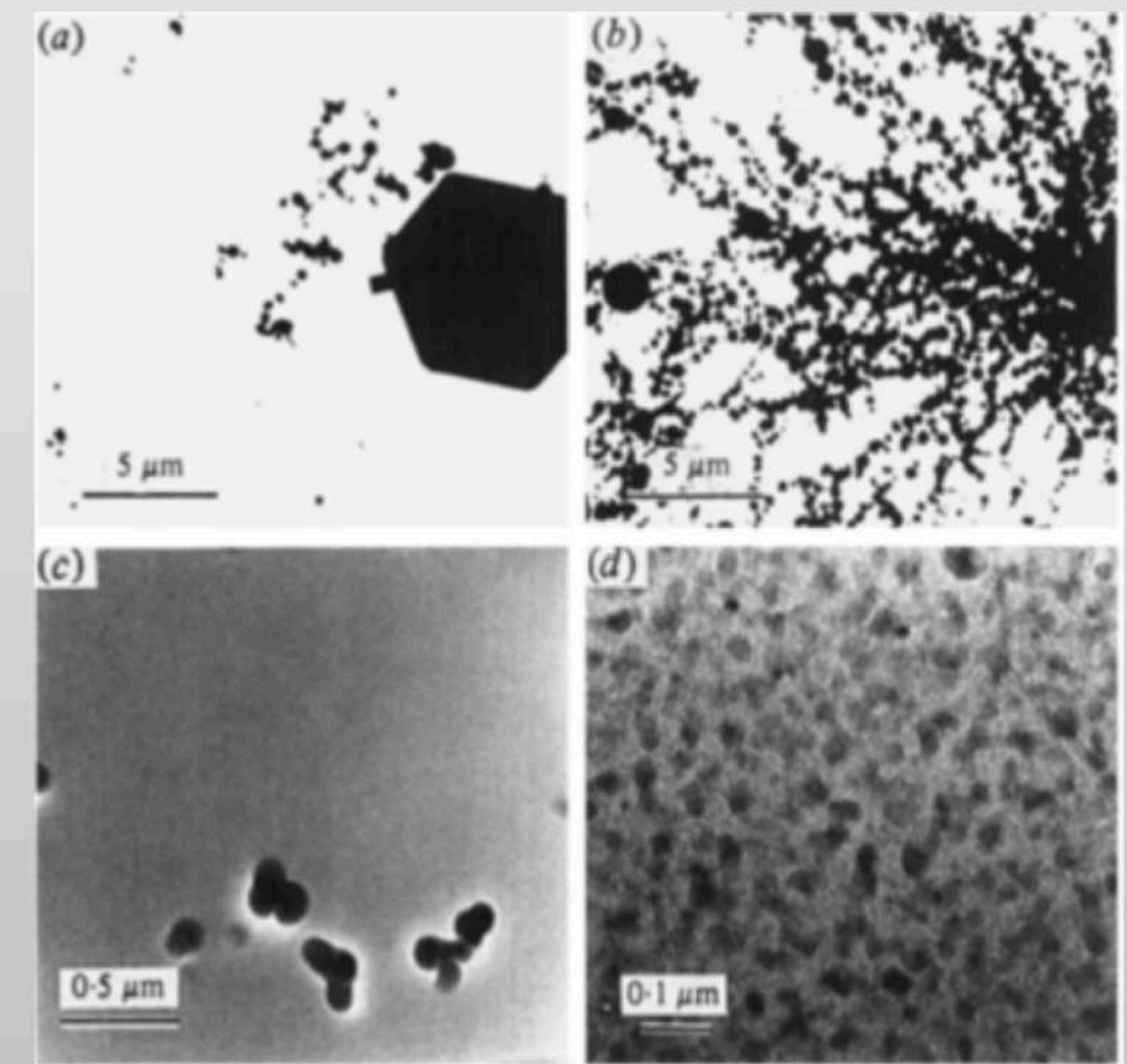
- Liquid ethane is a suitable coolant.
- Liquid nitrogen boils on contact, which makes it a poor coolant for cryo-EM.
- Cooling speed faster than  $10^5$ - $10^6$  K/s ensure the formation of vitrified ice.



Setup of liquid ethane  
(Image from Wen Jiang)



Cooling speed &  
forms of ice

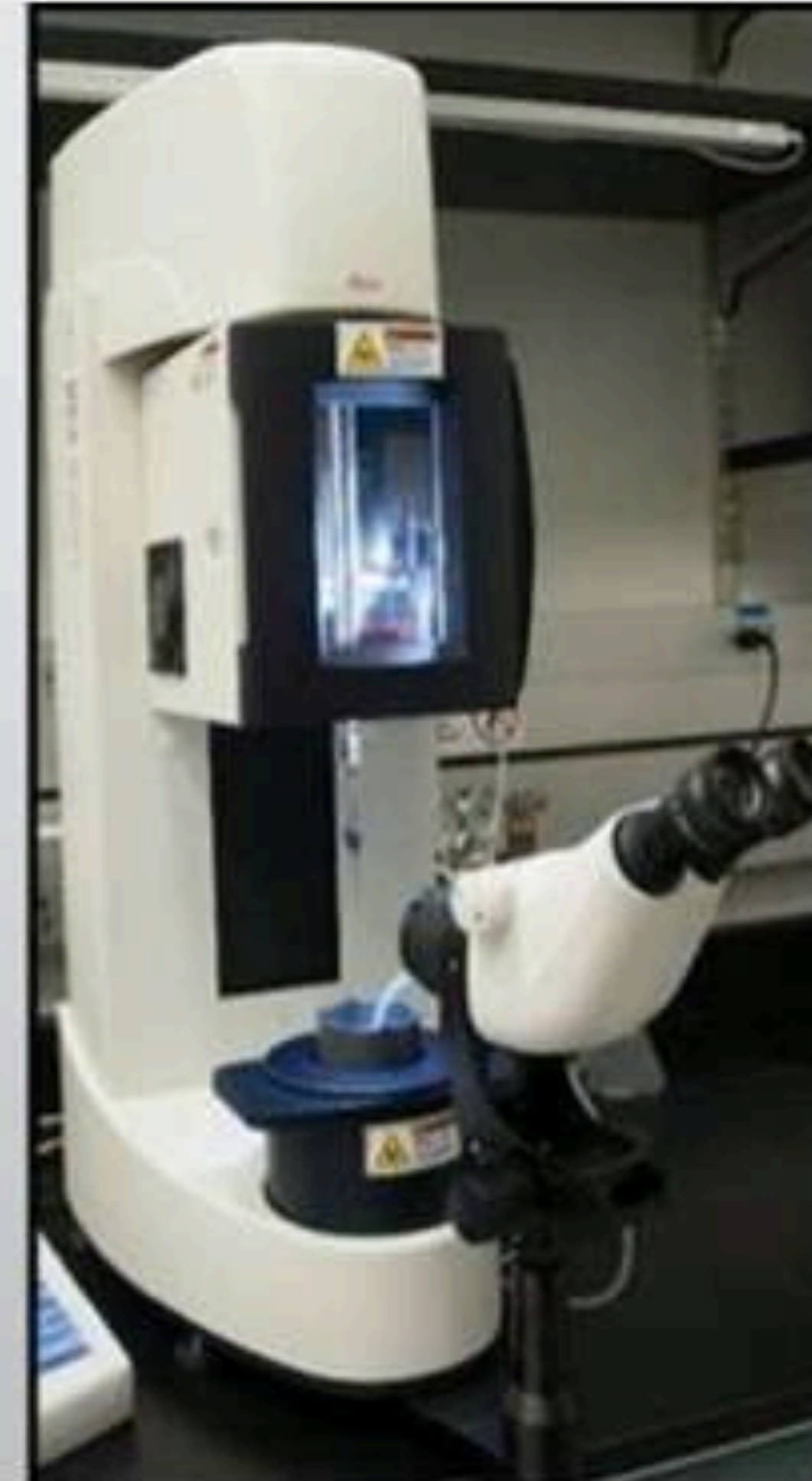


Different forms of ice contamination

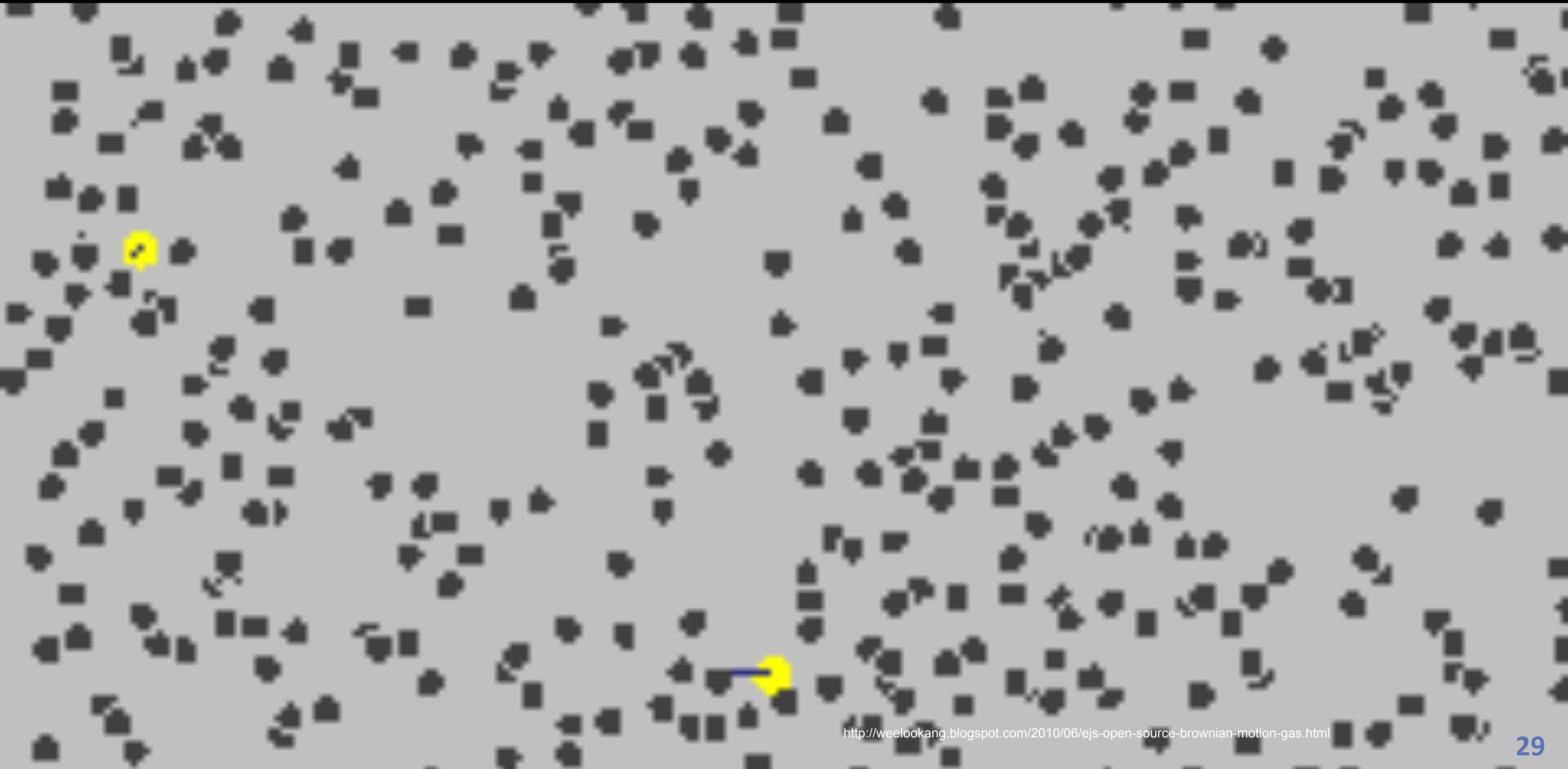
Jacques Dubochet et al., 1988

# How are samples prepared for cryoEM?

Vitrification process

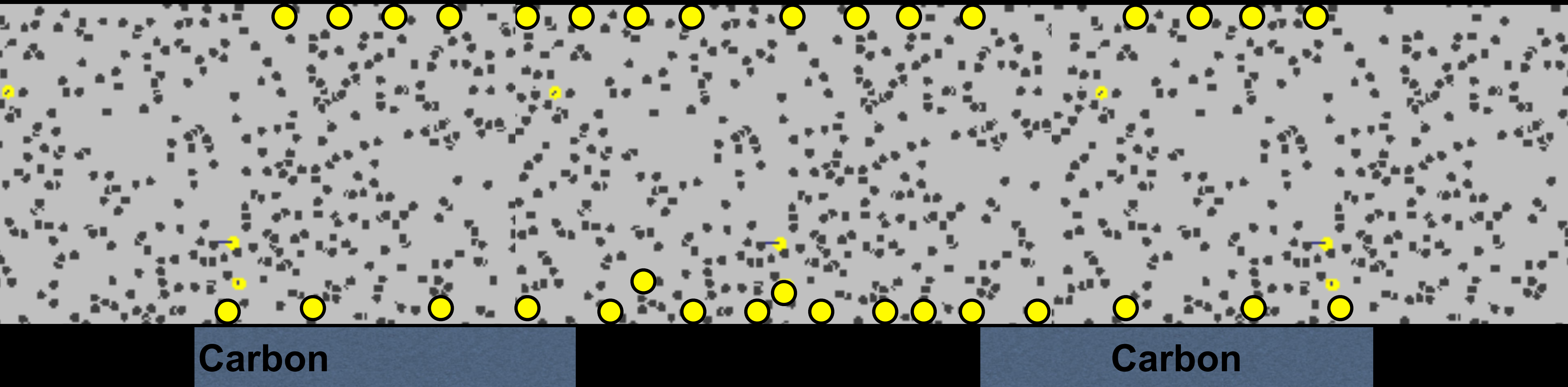


# What happens to samples during vitrification?



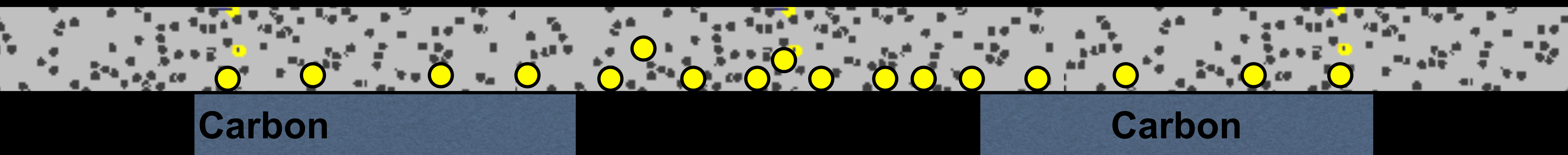
# What happens to samples during vitrification?

A hypothetical scenario during cryoEM grid preparation



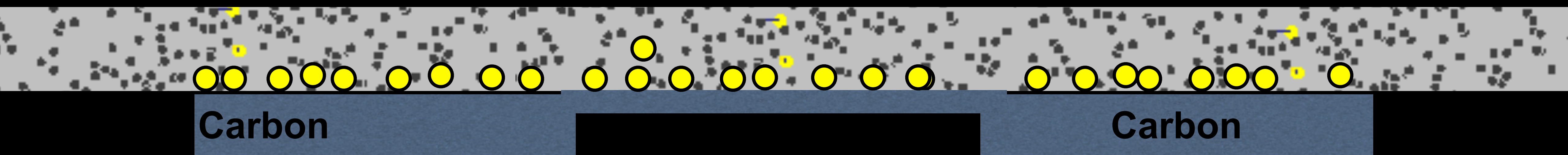
# What happens to samples during vitrification?

A hypothetical scenario during cryoEM grid preparation

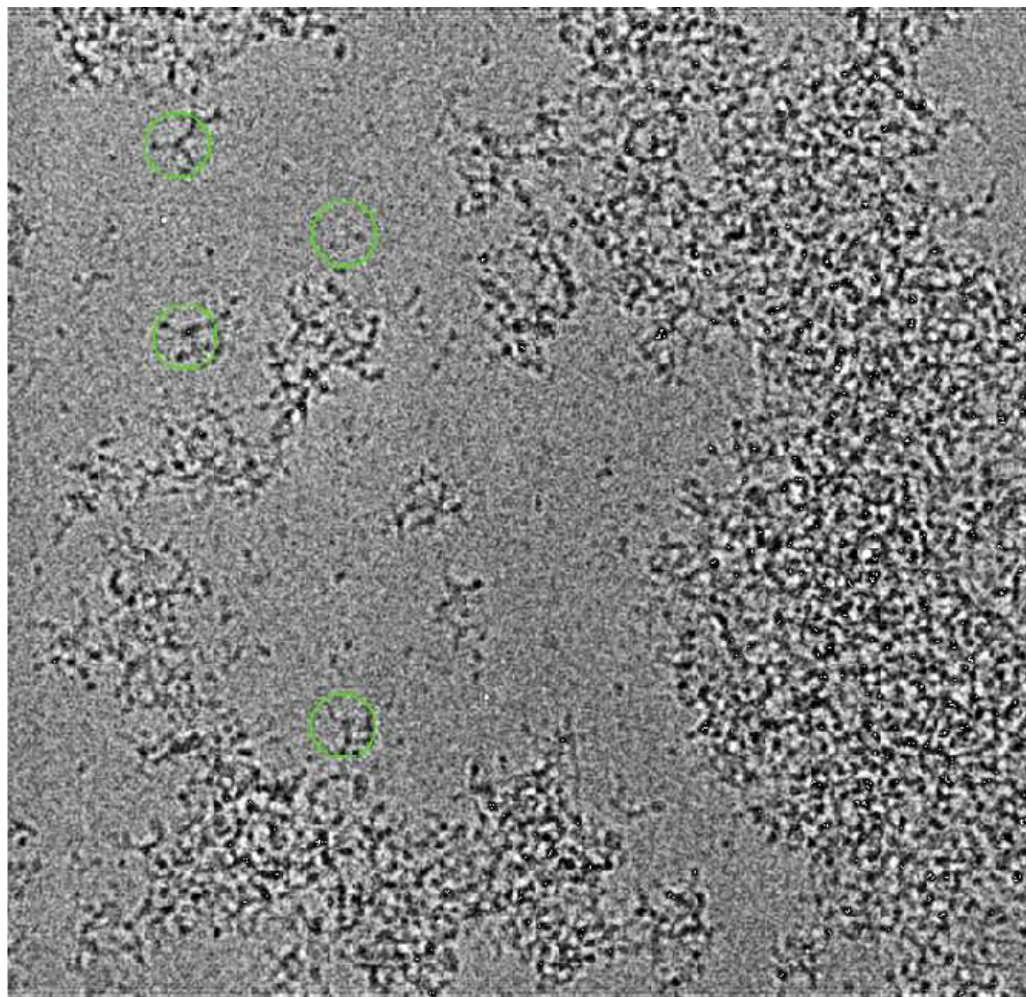


# What happens to samples during vitrification?

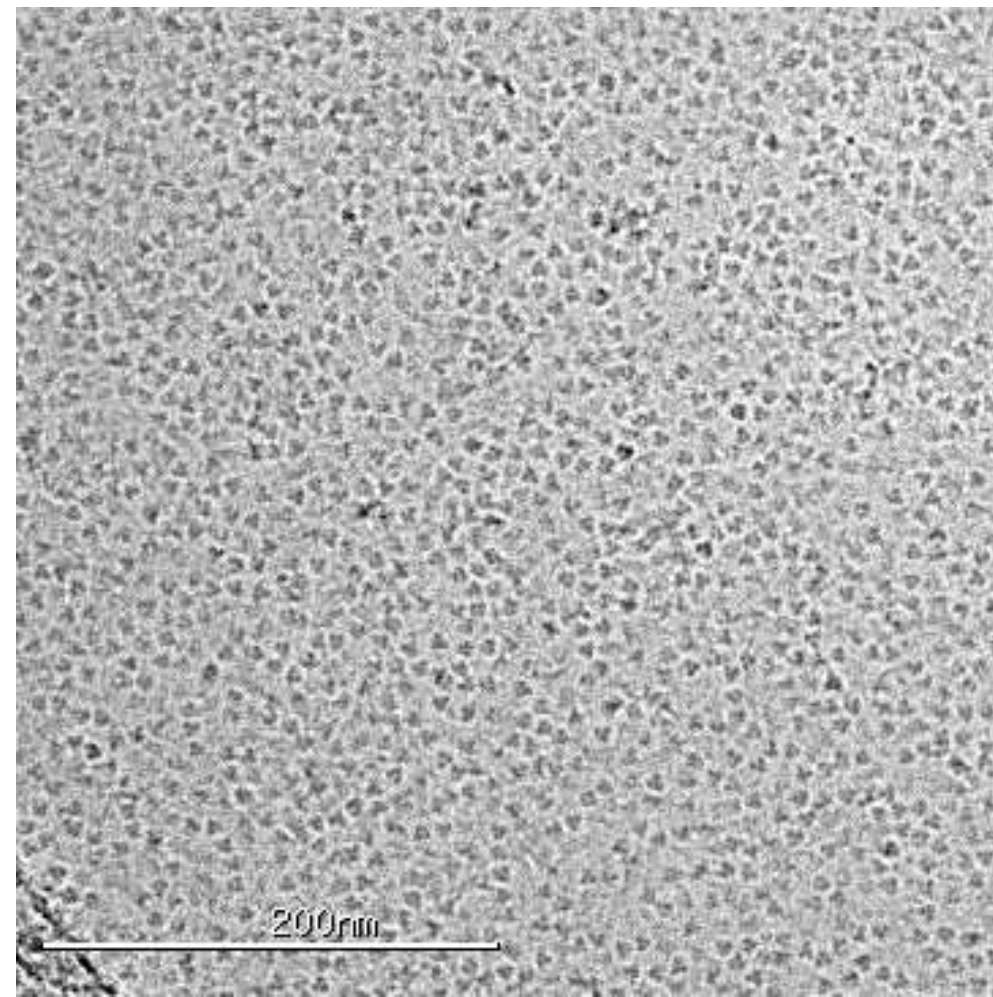
A hypothetical scenario during cryoEM grid preparation



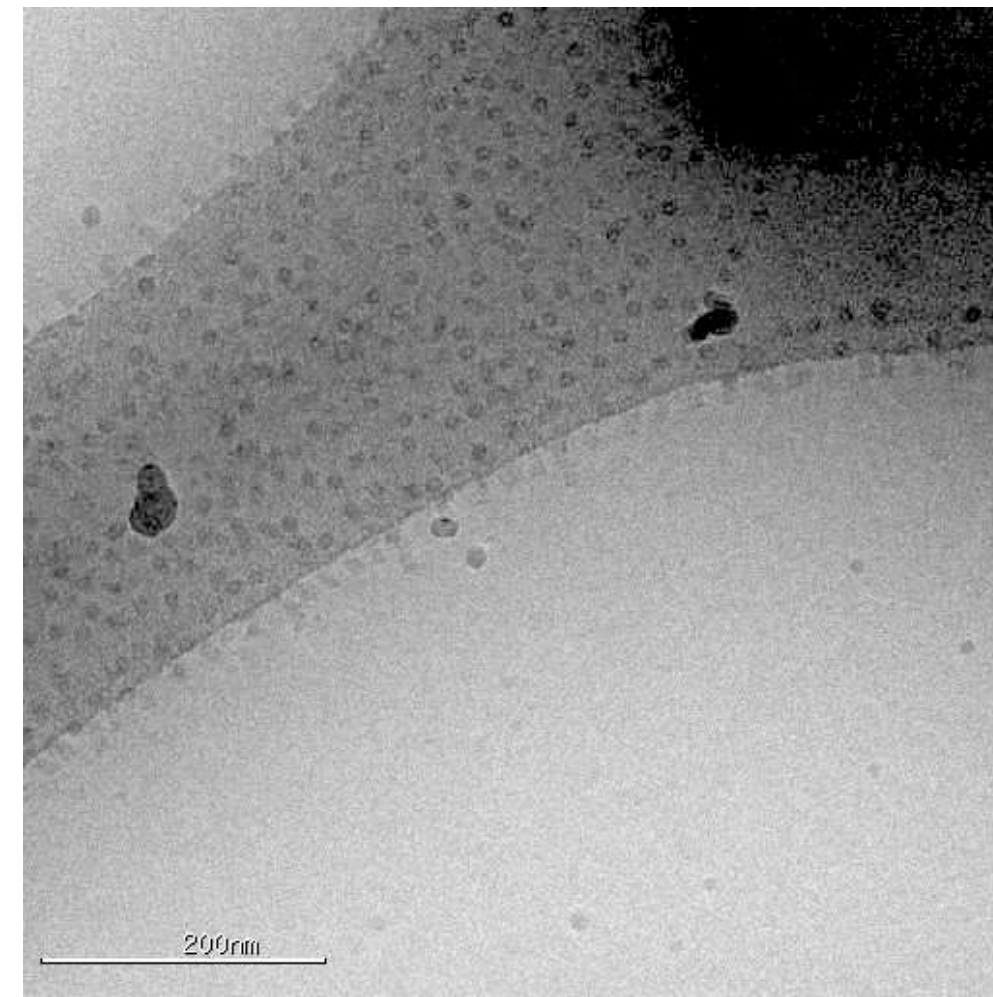
# What issues arise?



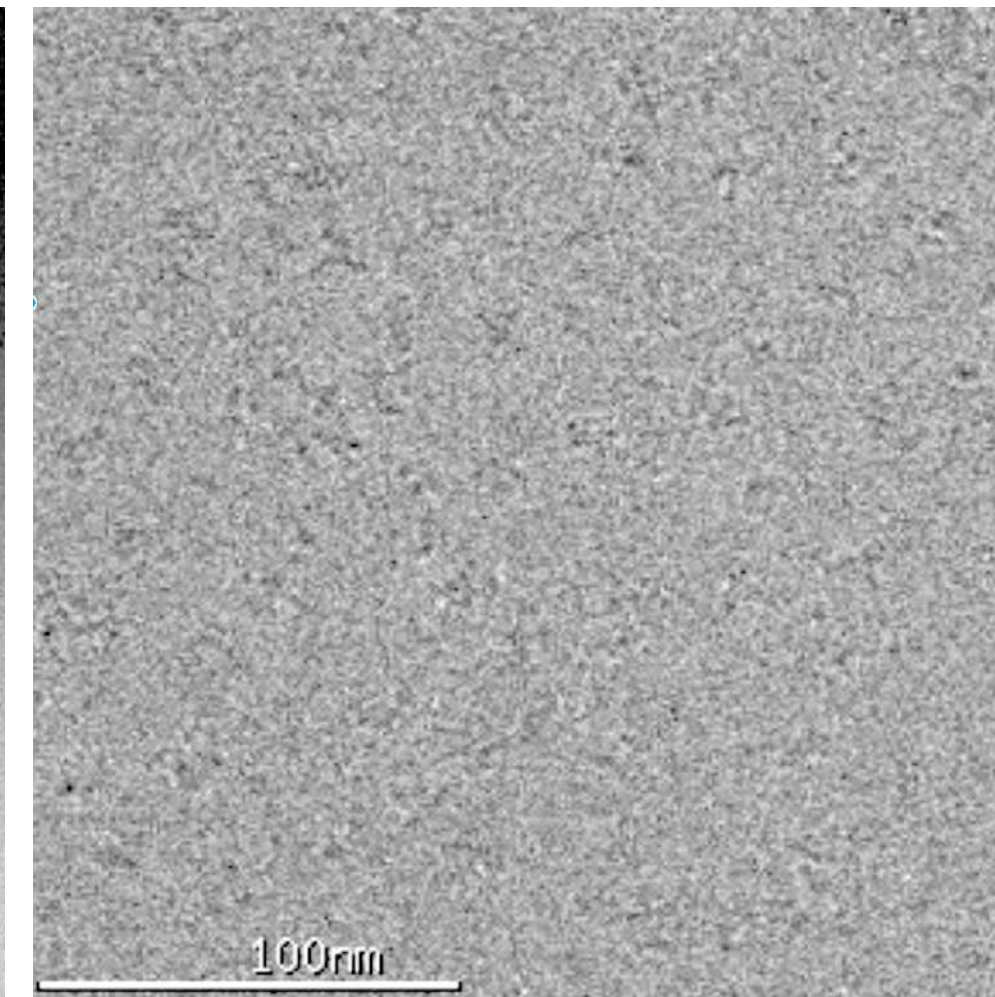
Aggregating in ice



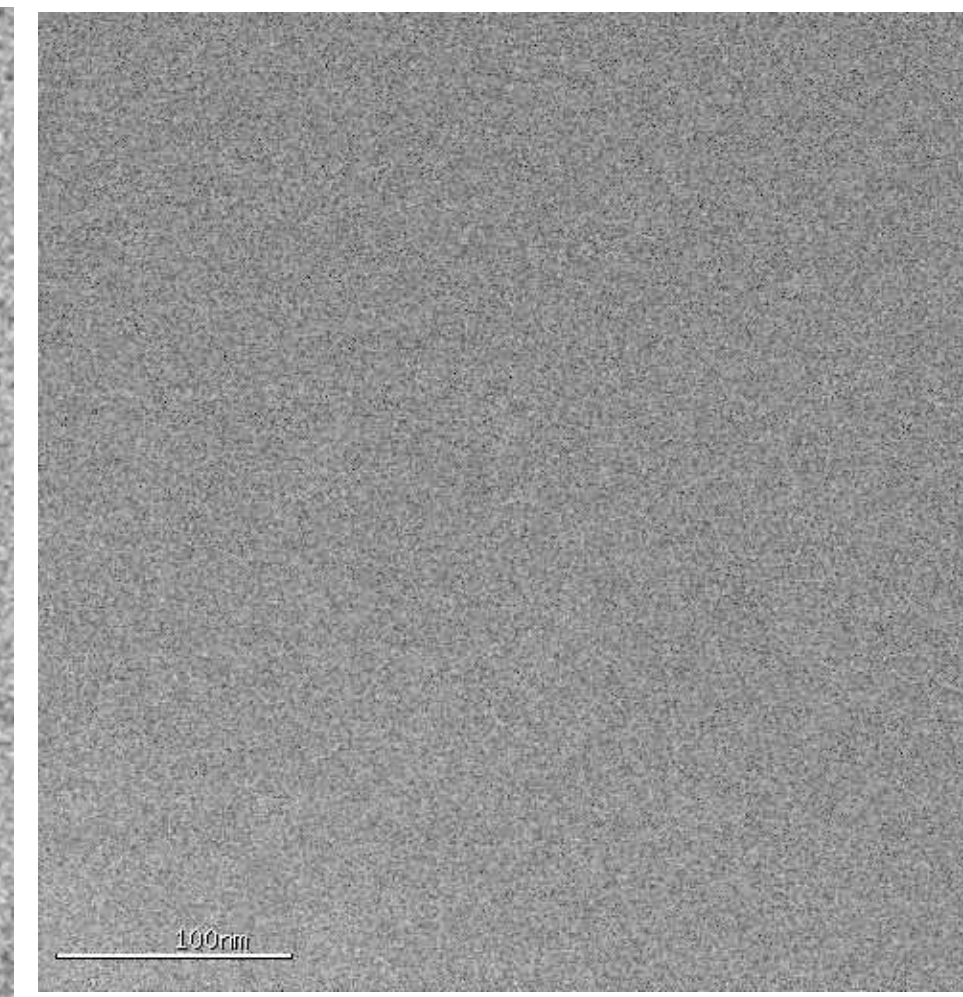
Preferred orientation



Particles not going into holes

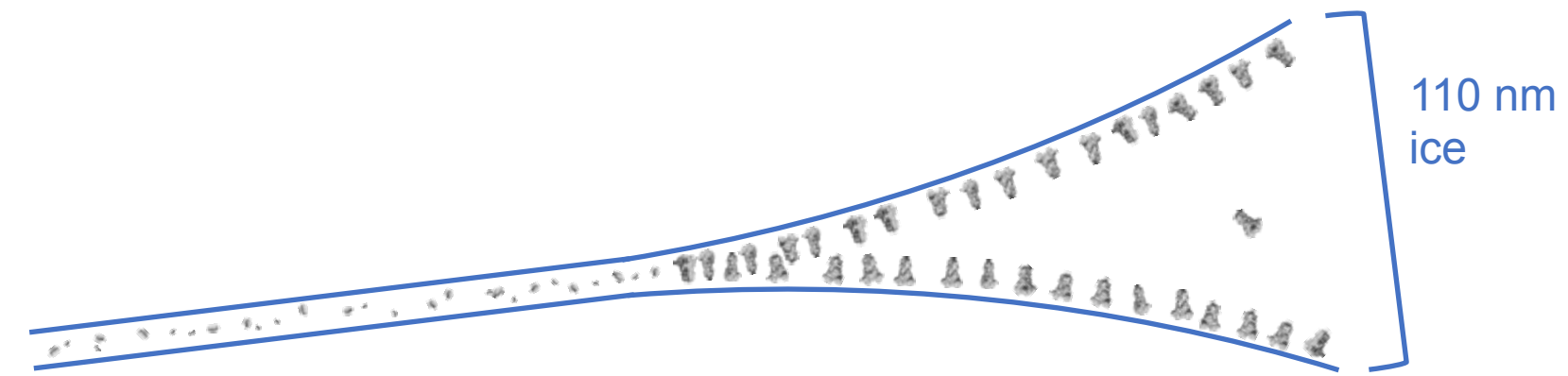


Rejecting 90% of particles

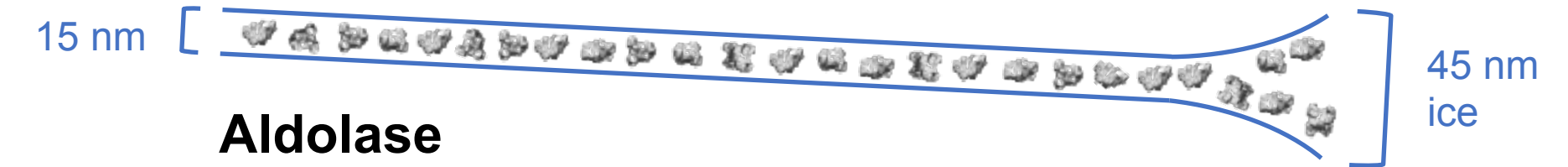


Particles disappearing in ice

# What issues arise?



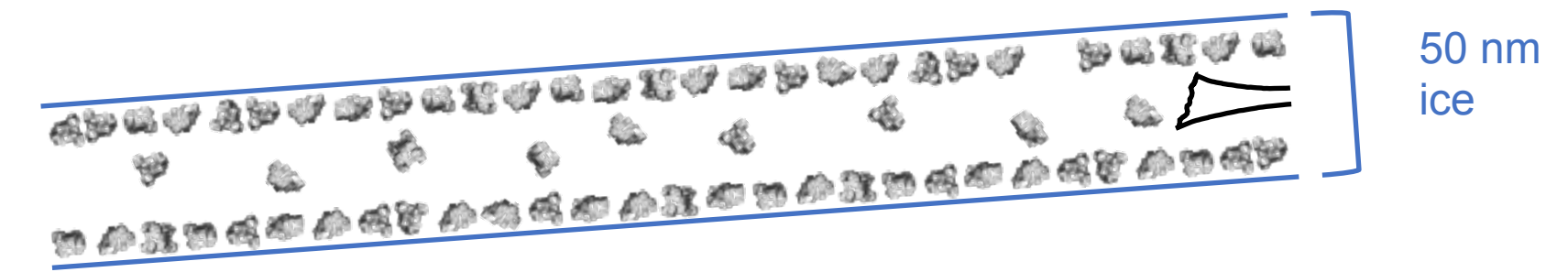
**Hemagglutinin**



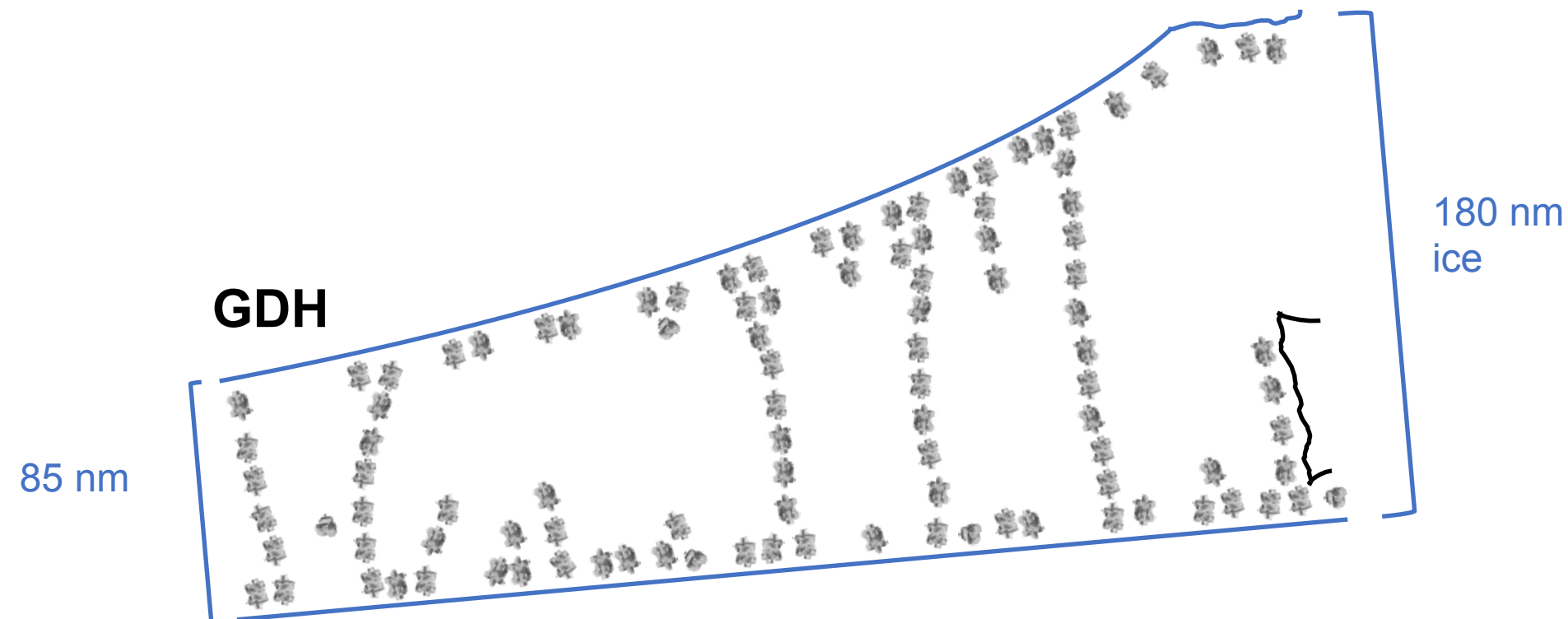
**Aldolase**



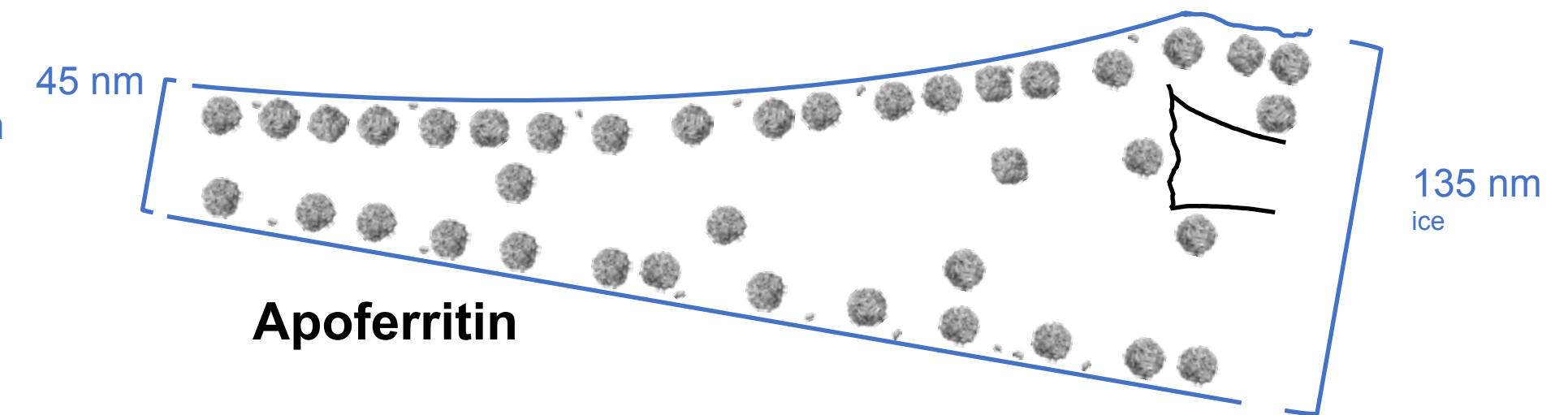
**Hemagglutinin**



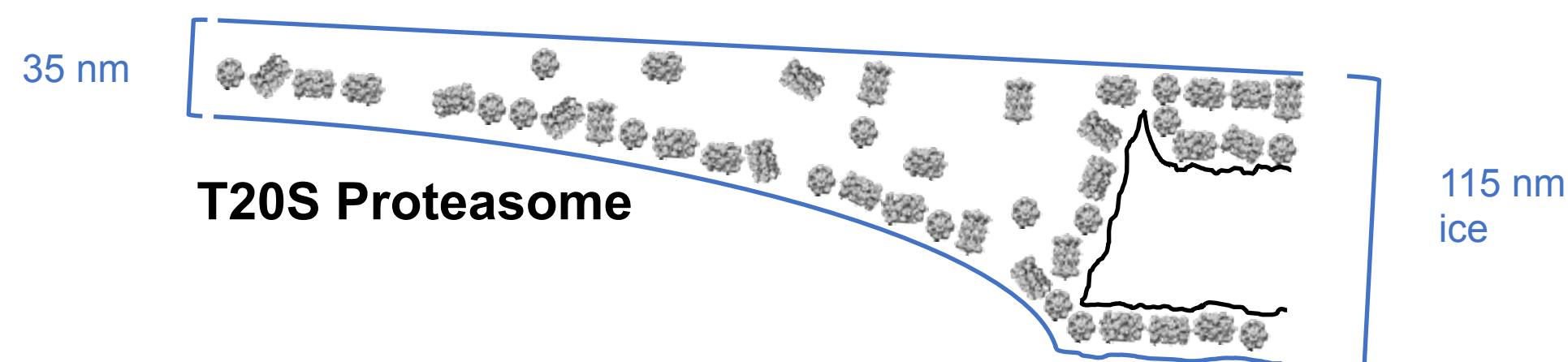
**Aldolase**



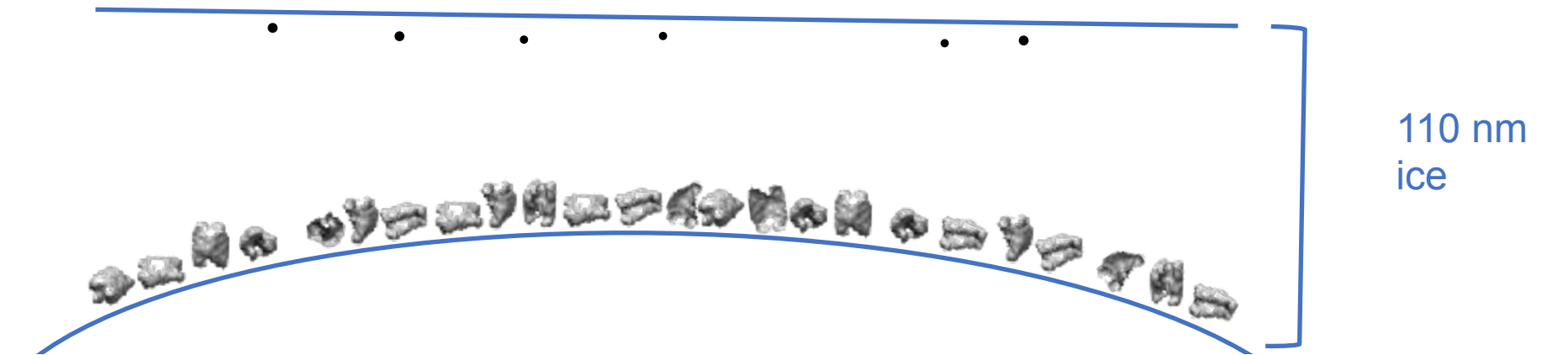
**GDH**



**Apoferritin**



**T20S Proteasome**



**DNAB Helices**

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

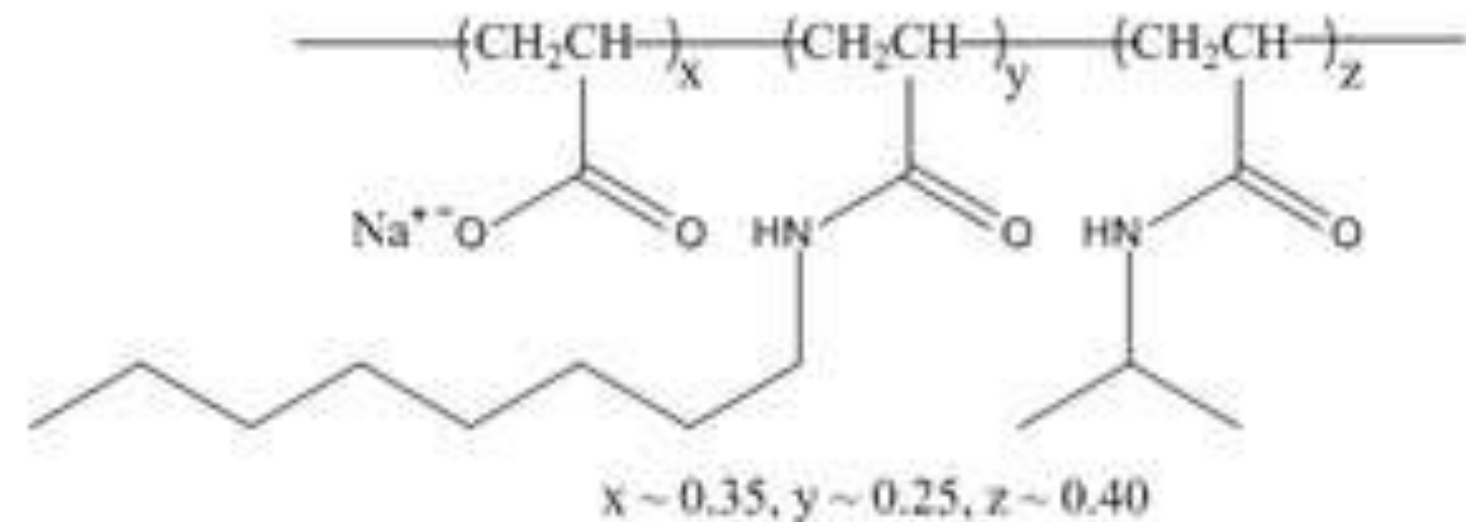
## Filamentous protein

- Segmented analysis

## Low concentration

- Multiple blots
- Affinity grids

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



Molecular Formula:

$(C_{6.2}H_{10.3}O_{1.35}N_{0.65}Na_{0.35})_{35}$

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 $\mu$ l	0.41% (w/v)	0.07% (w/v)	non-ionic detergent
Hexadecyl-trimethyl-ammonium Bromide (CTAB)	100 $\mu$ l	0.34% (w/v)	0.03% (w/v)	cationic detergent
n-Decyl- $\beta$ -D-Maltoside (DM)	100 $\mu$ l	0.87% (w/v)	0.09% (w/v)	non-ionic detergent
n-Decyl- $\alpha$ -D-Maltoside (DaM)	100 $\mu$ l	0.46% (w/v)	0.08% (w/v)	non-ionic detergent
n-Dodecyl- $\beta$ -D-Maltoside (DDM)	100 $\mu$ l	0.09% (w/v)	0.01% (w/v)	non-ionic detergent
Sodium Deoxycholate	100 $\mu$ l	1.66% (w/v)	0.17% (w/v)	anionic detergent
Triton X-100	100 $\mu$ l	0.15% (w/v)	0.01% (w/v)	non-ionic detergent
Tween 20	100 $\mu$ l	1% (w/v)	0.01% (w/v)	non-ionic detergent
CHAPSO	100 $\mu$ l	2.5% (w/v)	0.5% (w/v)	zwitterionic detergent
Amphipol A8-35	100 $\mu$ l	5% (w/v)		anionic surfactant
Glycerol	1 ml	30% (w/v)		cryoprotectant

- [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.

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

# 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

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

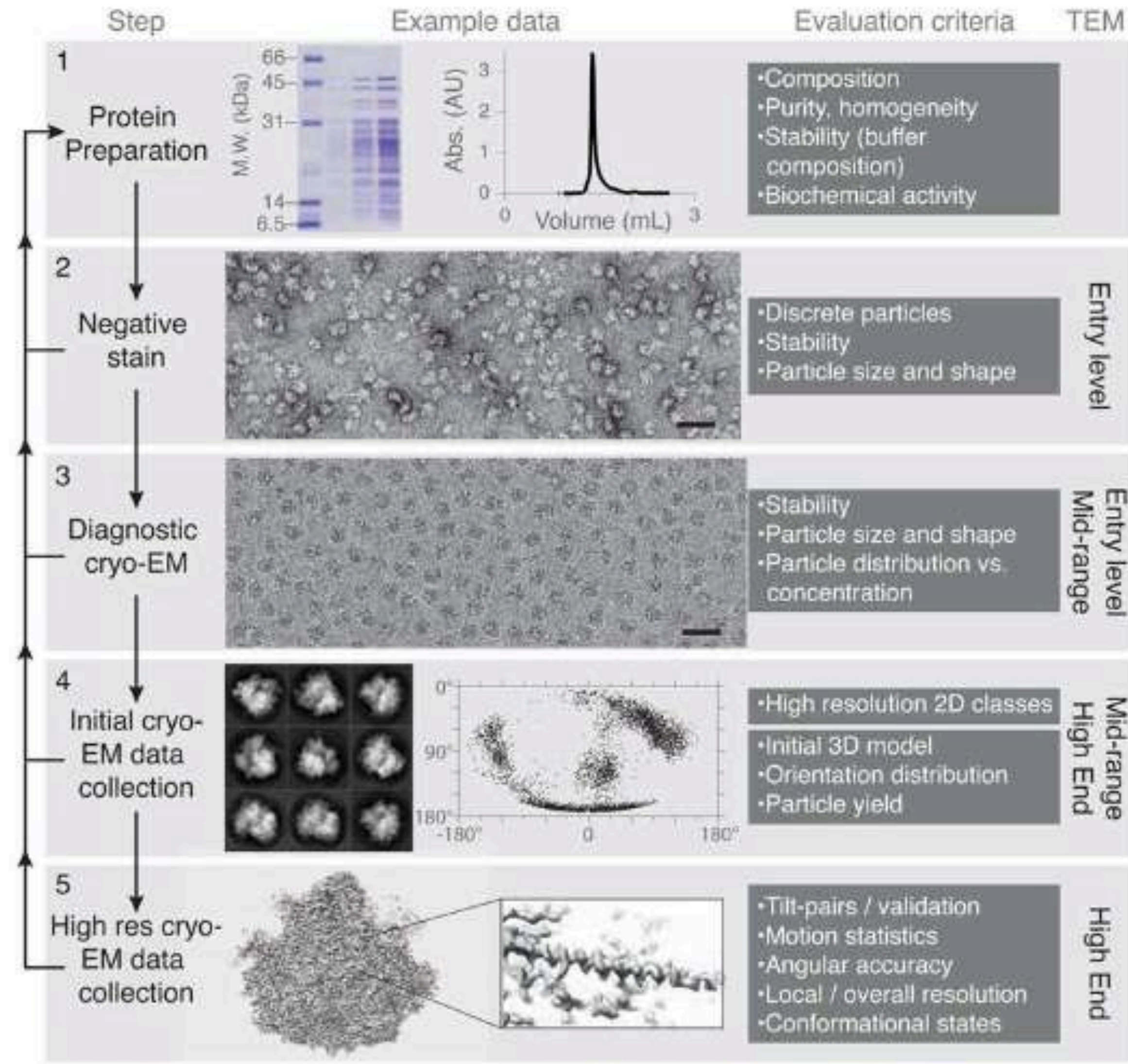
<https://www.anatrace.com/Landing/2020/Mar20-Newsletter>

# Preparing EM ready samples

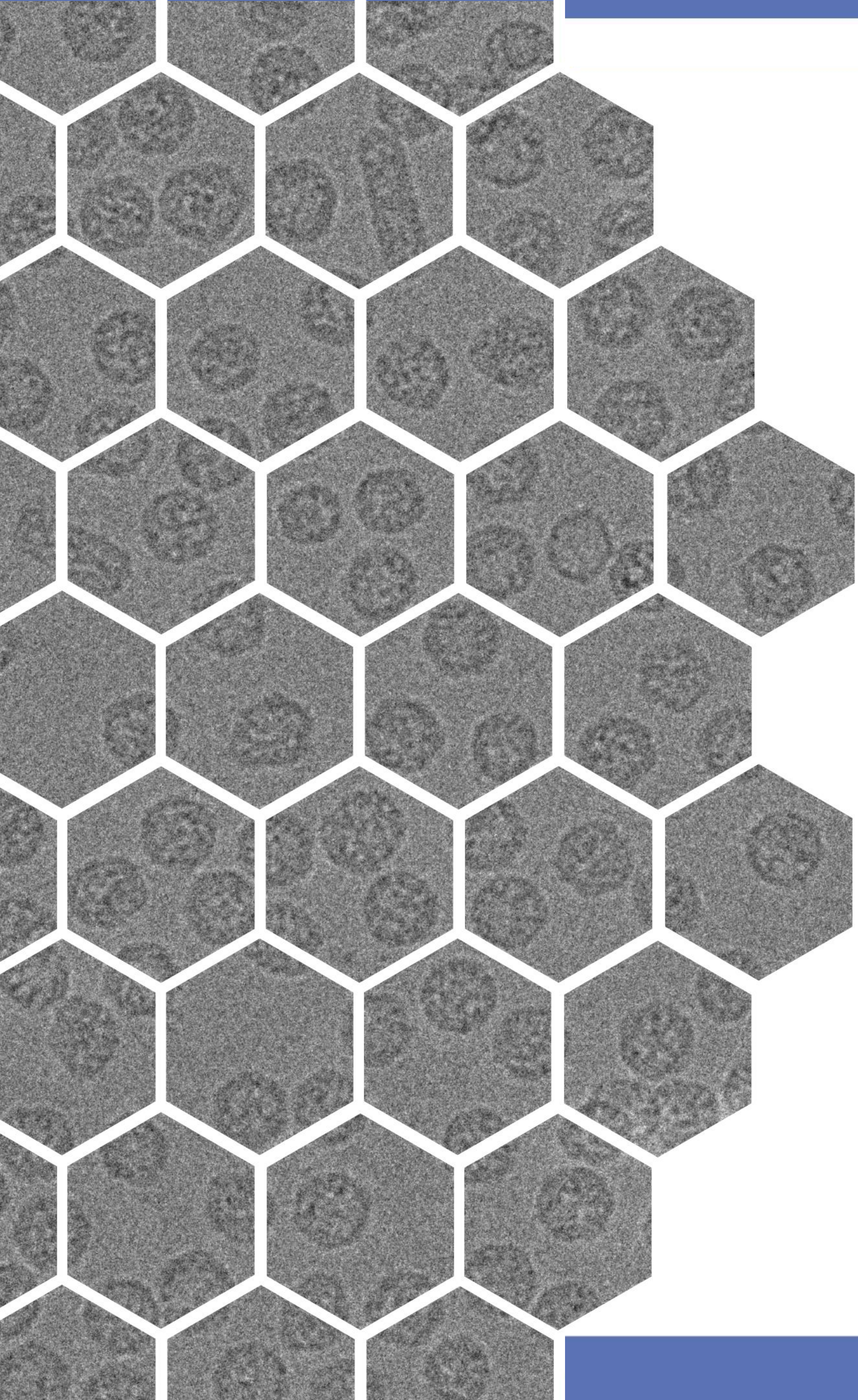
## THE OPTIMIZATION WORKFLOW

### 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/>



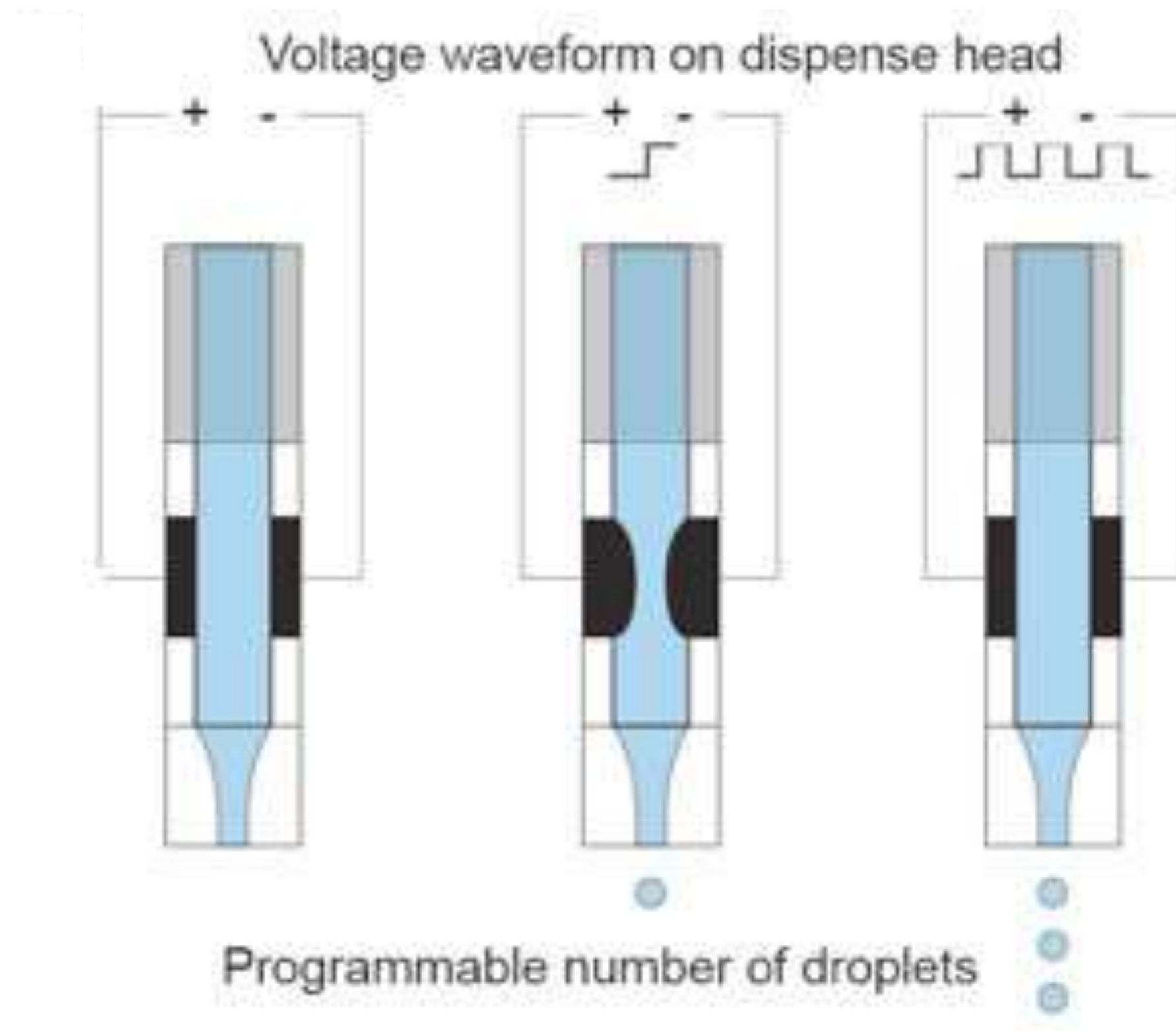
- ◆ Journal club and practical recap
- ◆ Considerations for biological cryoEM
  - ◆ Overview
  - ◆ Grids
  - ◆ What happens to a sample
  - ◆ Newer methods

# Other methods

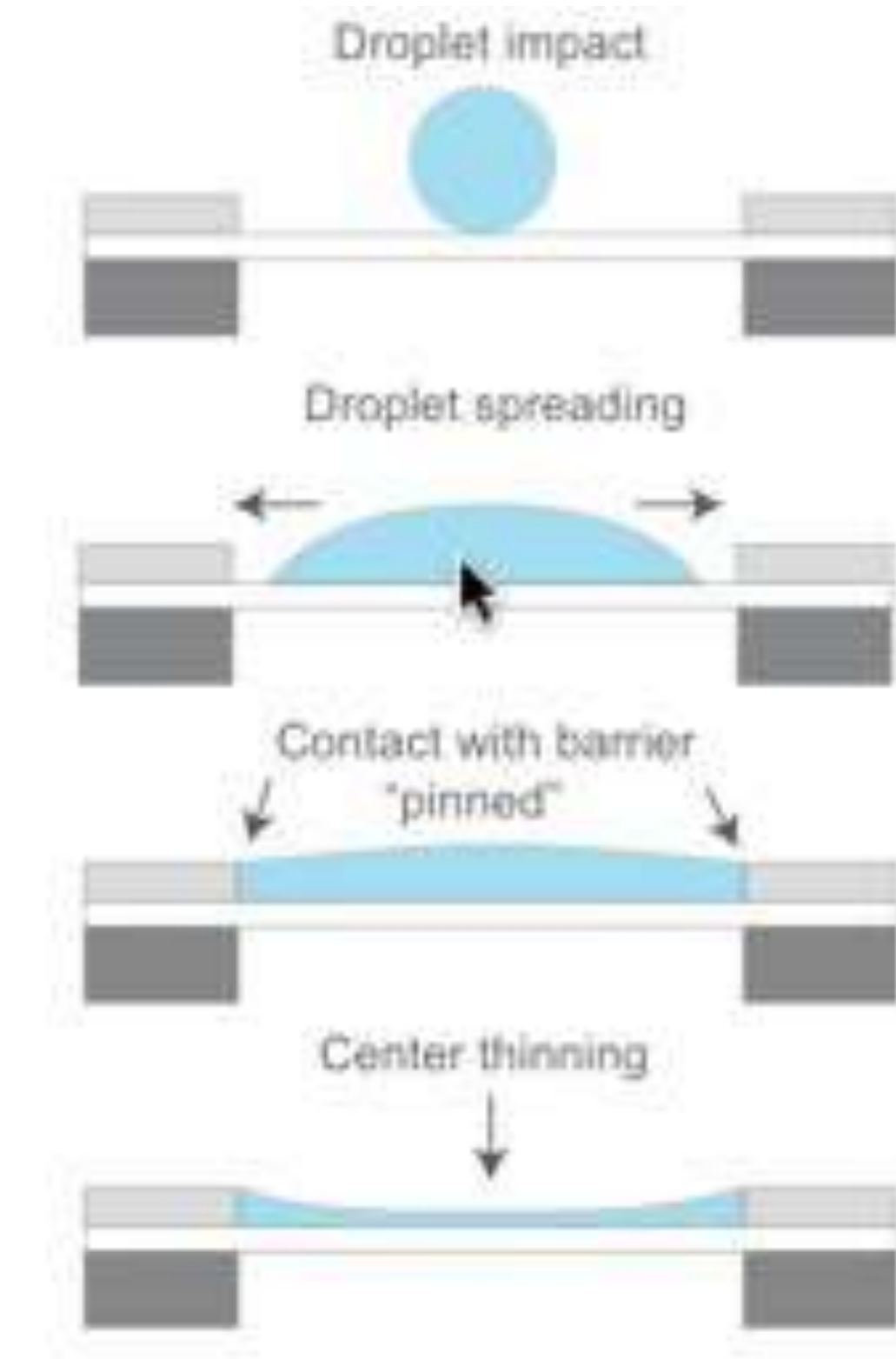


## Improving Current CryoTEM Grid Preparation Methods

### Accurate pL dispensing



### Thin films without blotting



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



## Improving Current CryoTEM Grid Preparation Methods

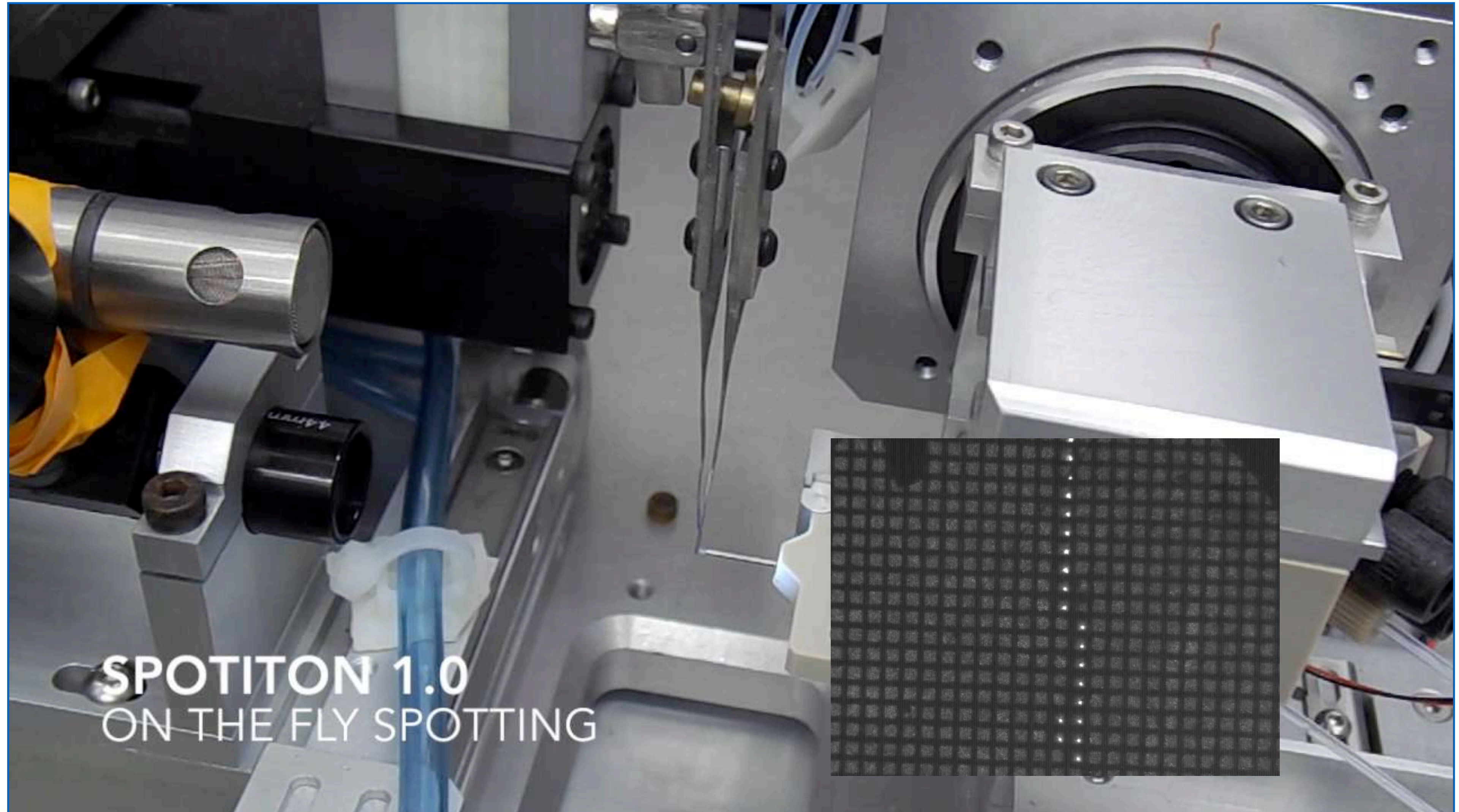
Dandey VP, Wei H,  
Zhang Z, Tan YZ,  
Acharya P, Eng ET,  
Rice WJ, Kahn PA,  
Potter CS, Carragher  
B. Spotiton: New  
features and  
applications. Journal  
of structural biology.  
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Venkat Dandey



Hui Wei



# Other methods



## Improving Current CryoTEM Grid Preparation Methods

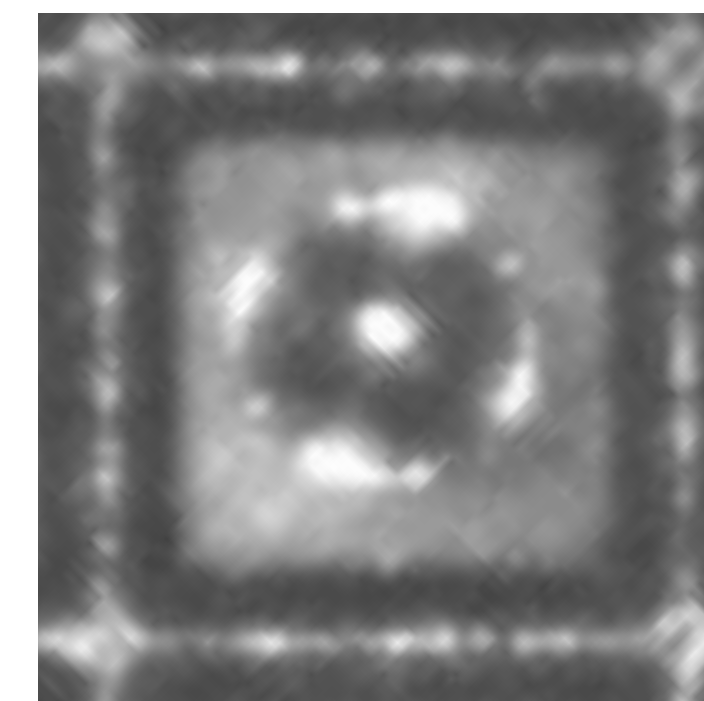
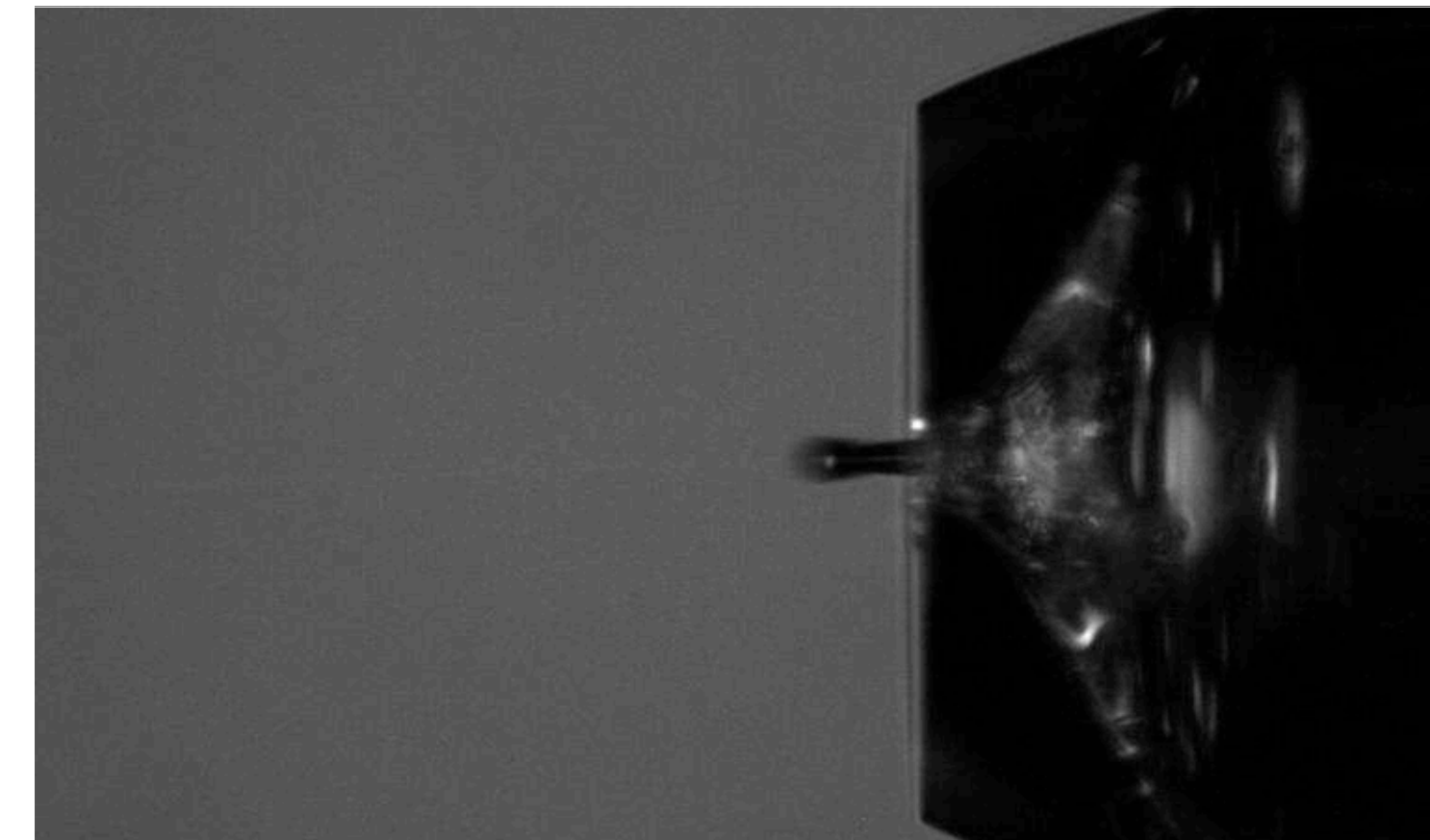
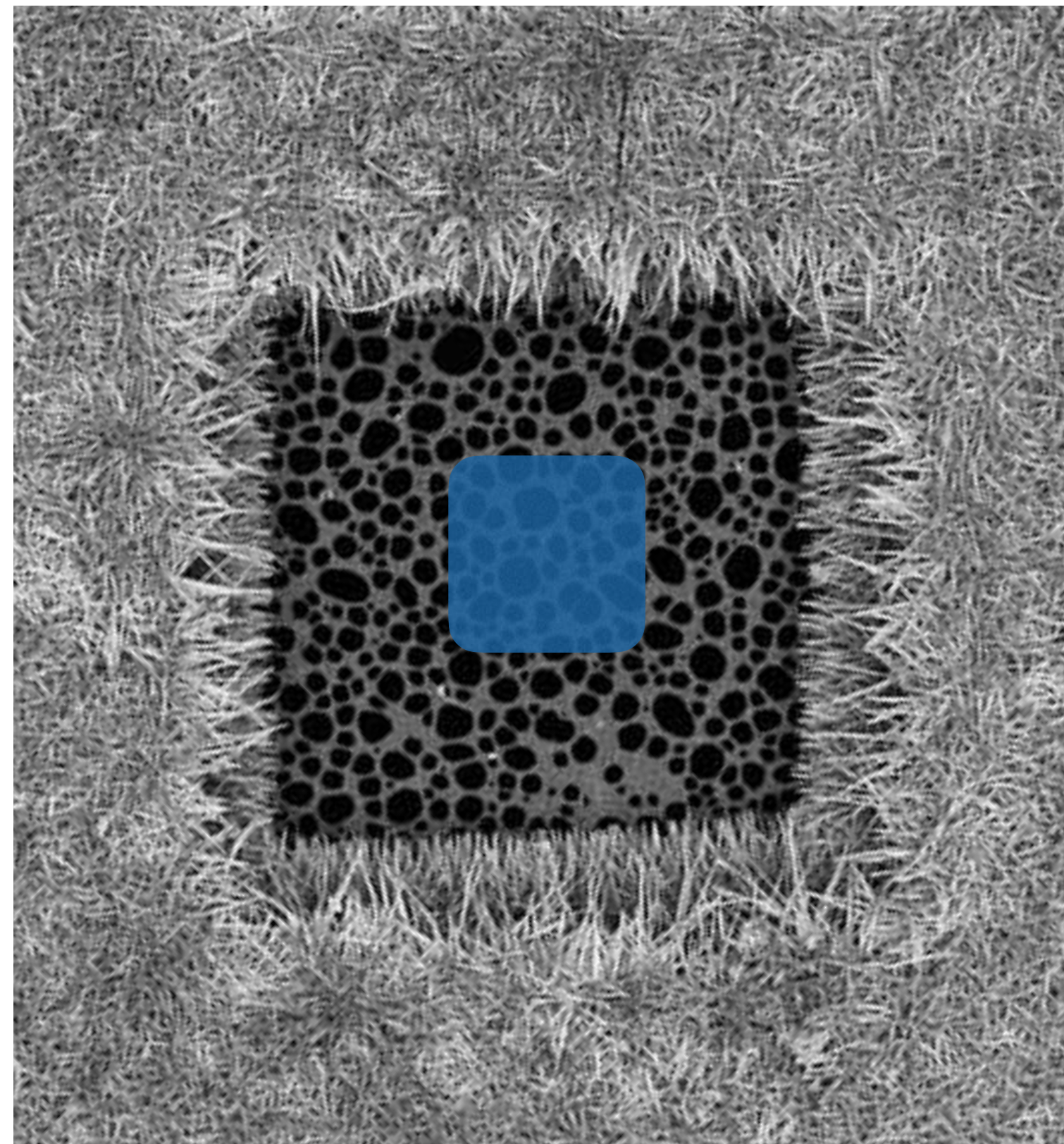
Wei H, Dandey VP,  
Zhang Z, Raczkowski  
A, Rice WJ,  
Carragher B, Potter  
CS. Optimizing "self-  
wicking" nanowire  
grids. J Struct Biol.  
2018;202(2):170-4.



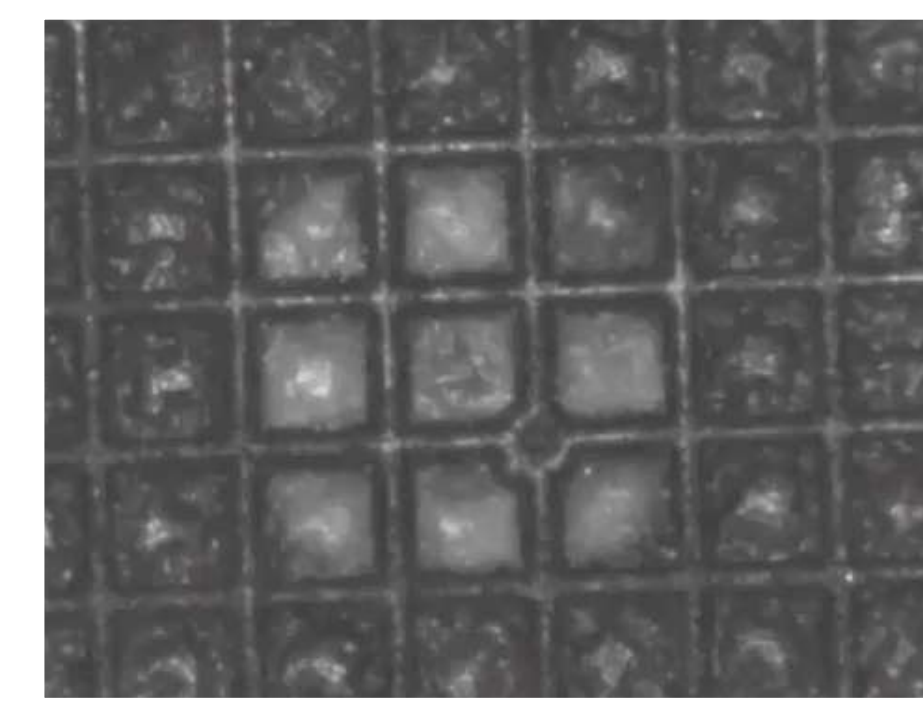
Venkat Dandey



Hui Wei



Single frame from loop



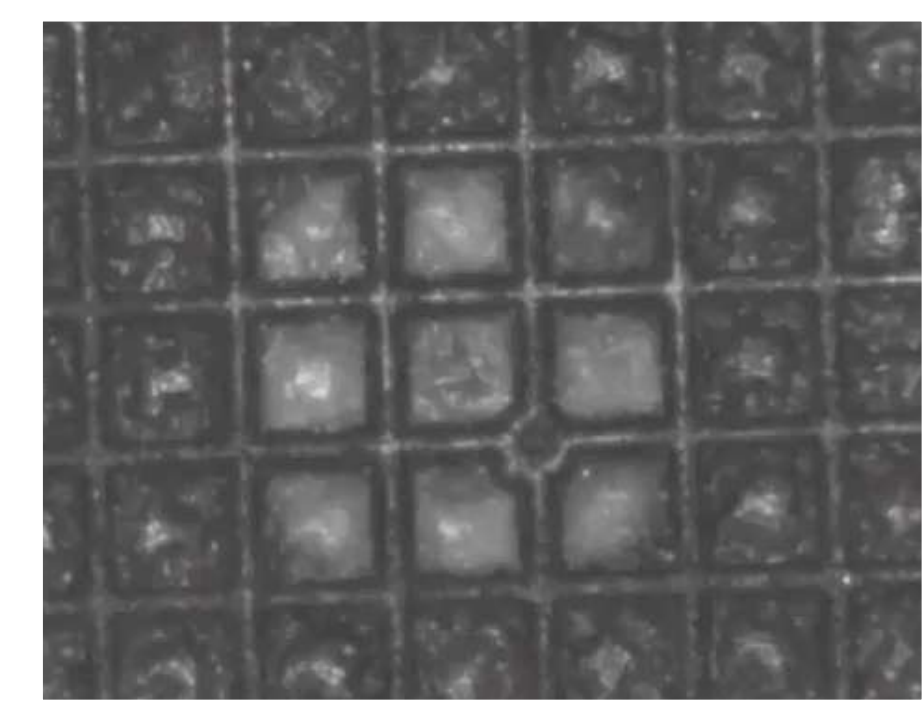
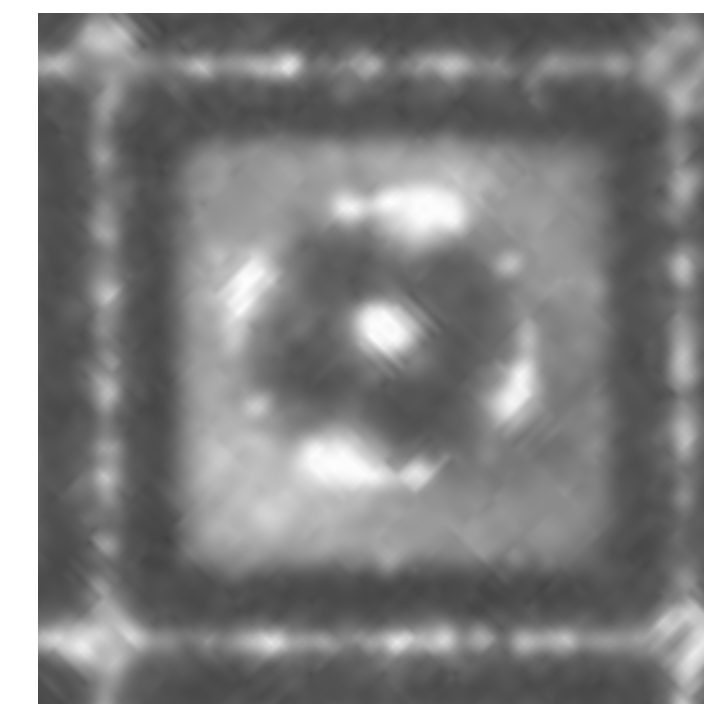
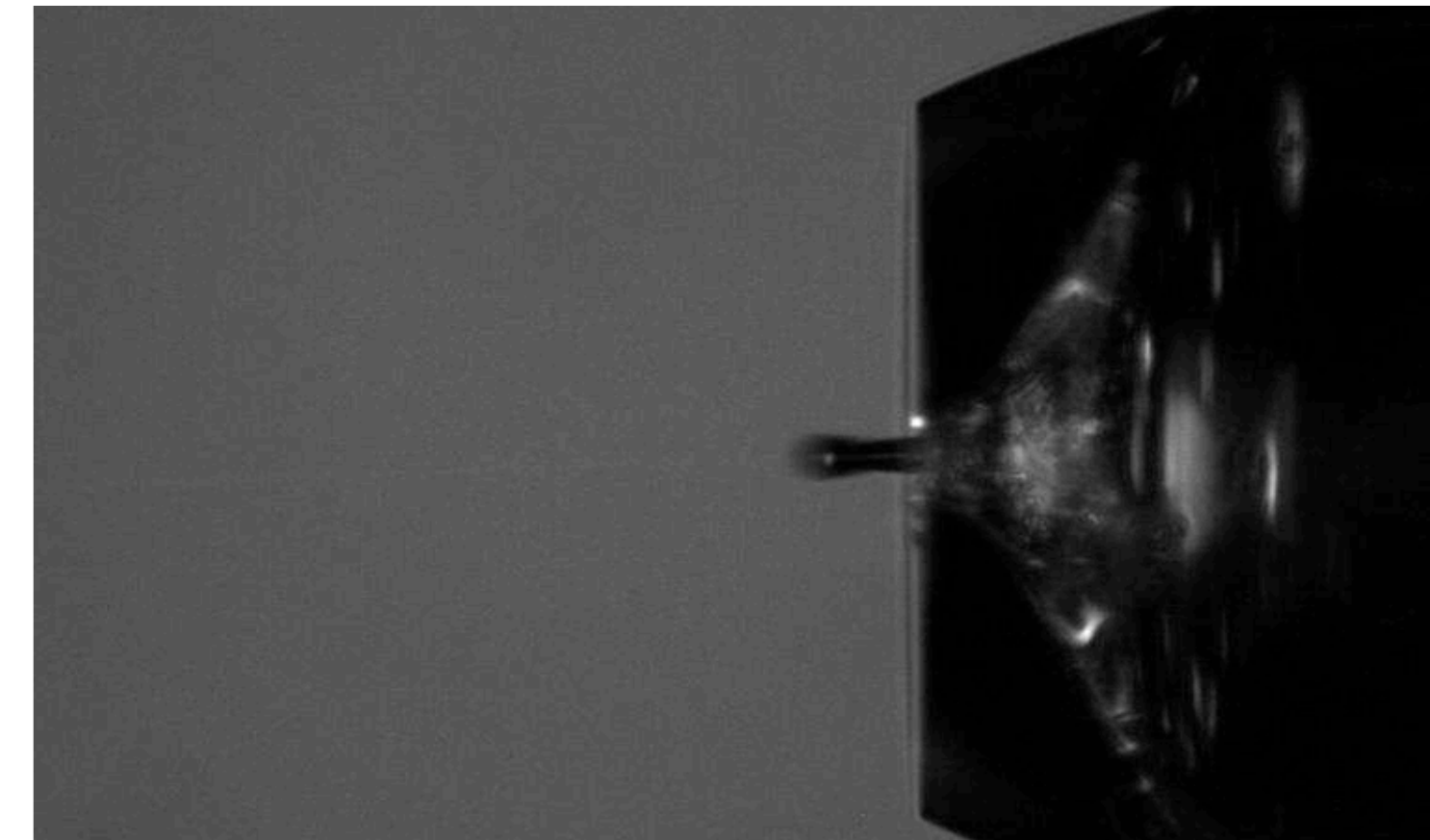
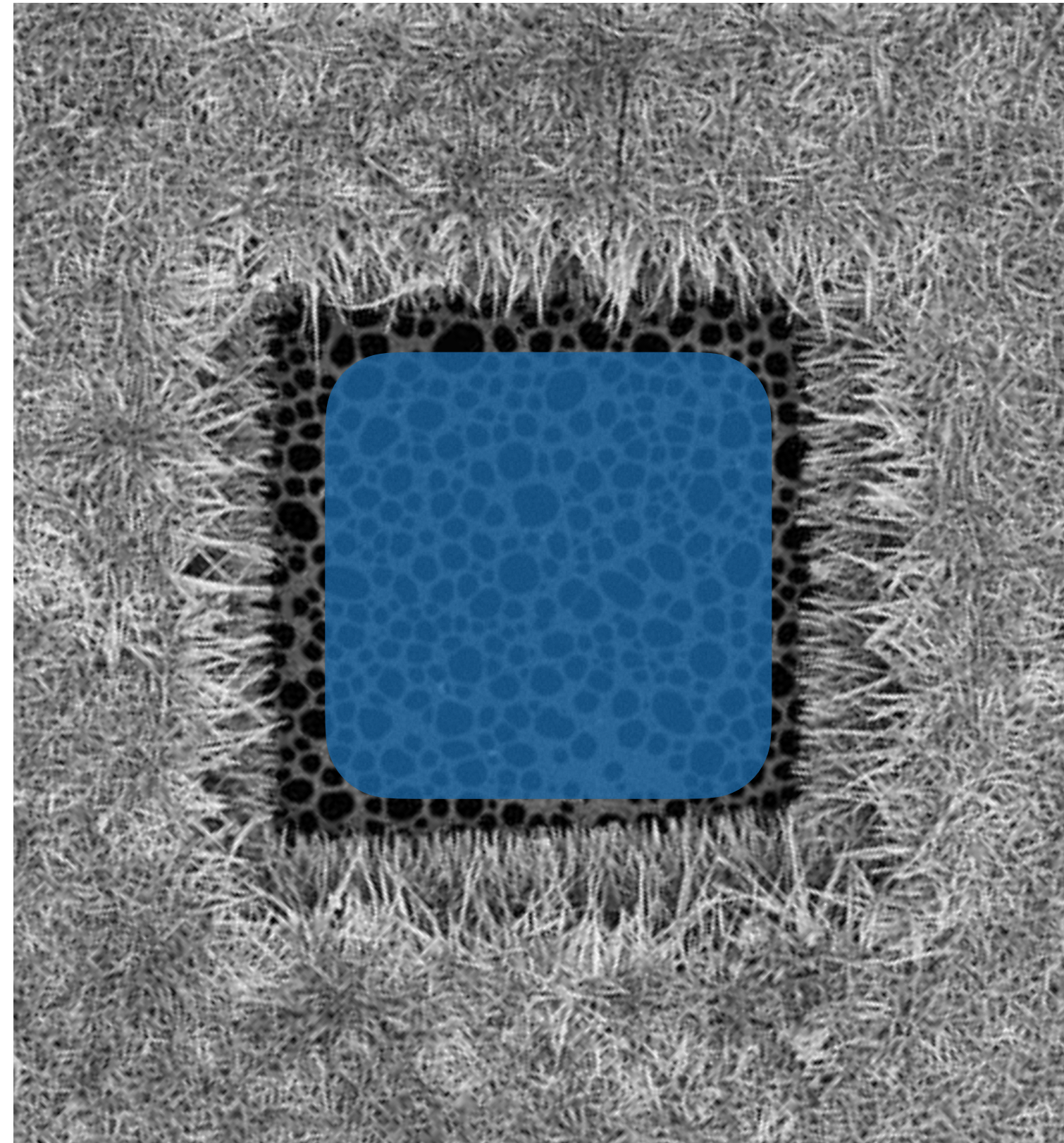
Video loop

# Other methods



## 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.



Single frame from loop

Video loop



Venkat Dandey



Hui Wei

# Other methods



## Improving Current CryoTEM Grid Preparation Methods

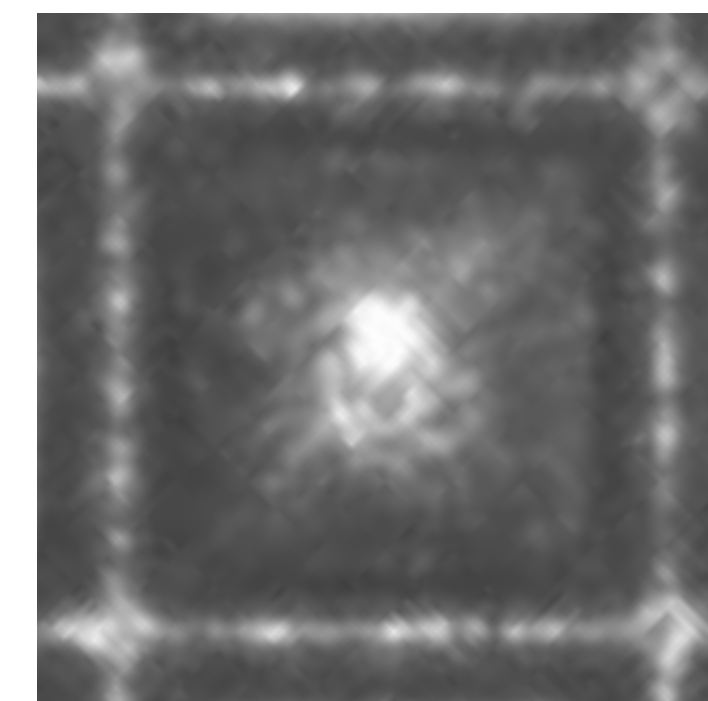
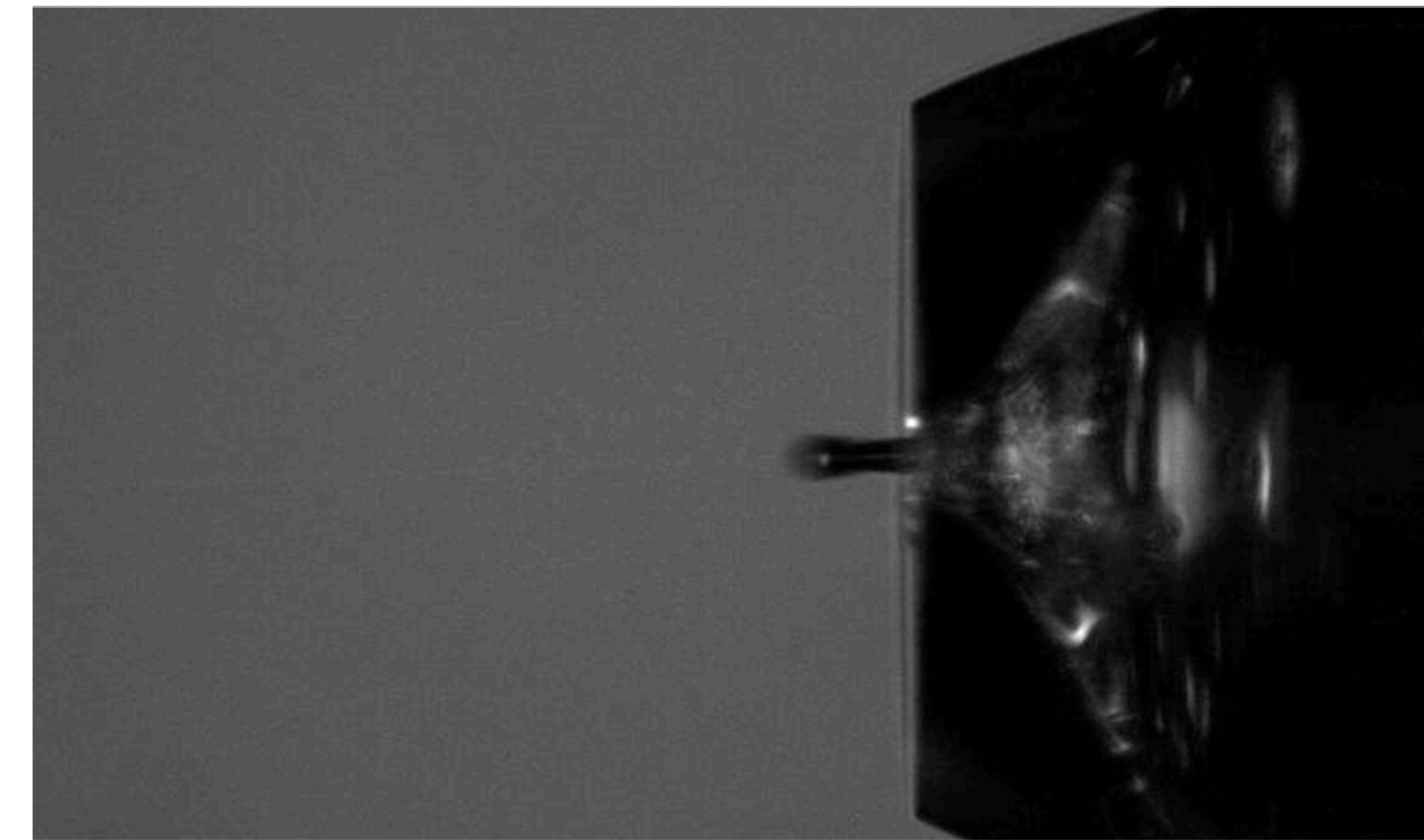
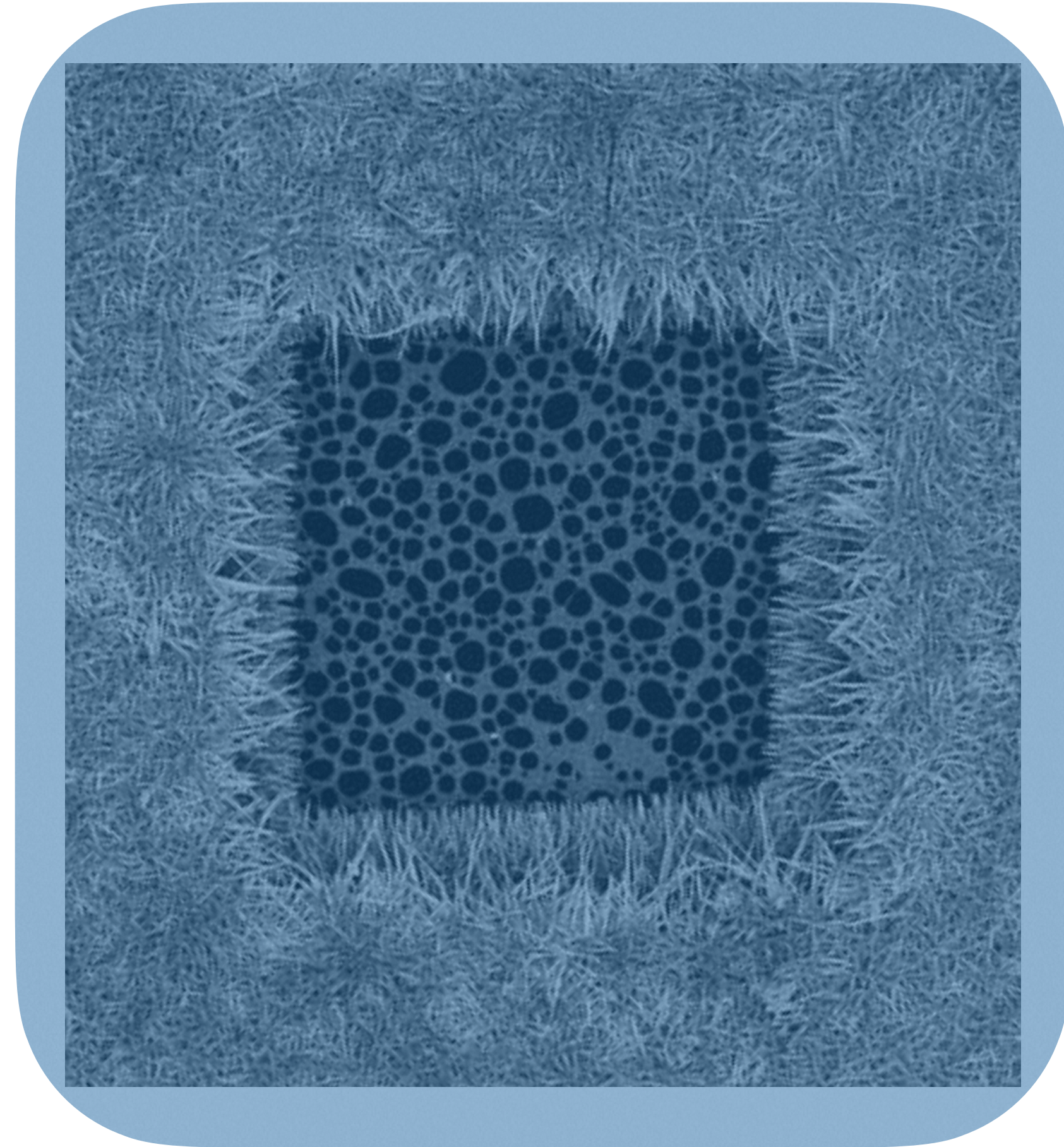
Wei H, Dandey VP,  
Zhang Z, Raczkowski  
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Carragher B, Potter  
CS. Optimizing "self-  
wicking" nanowire  
grids. J Struct Biol.  
2018;202(2):170-4.



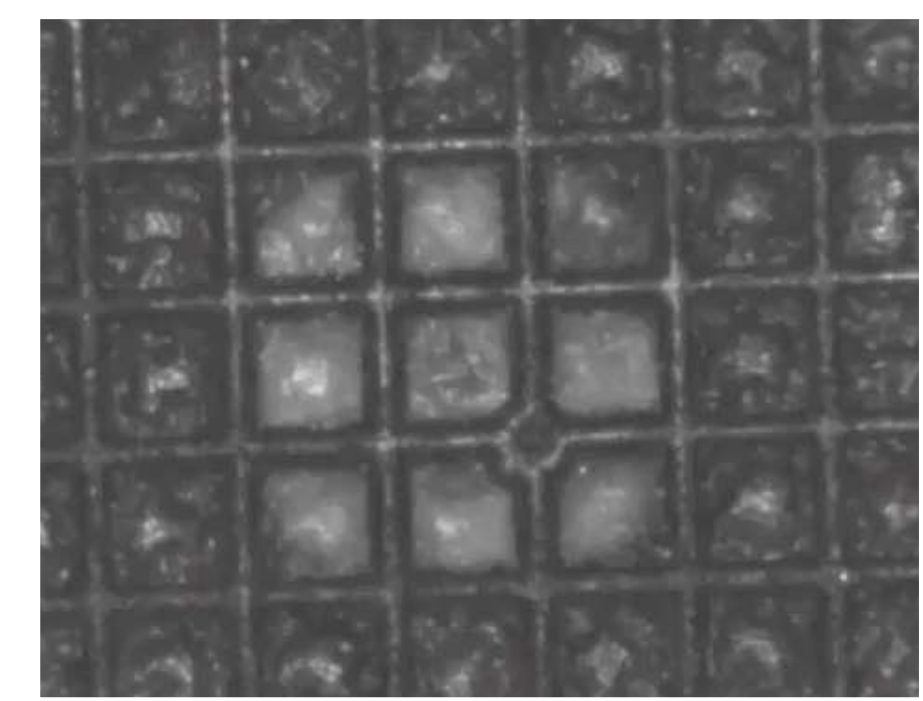
Venkat Dandey



Hui Wei



Single frame from loop



Video loop

# Other methods

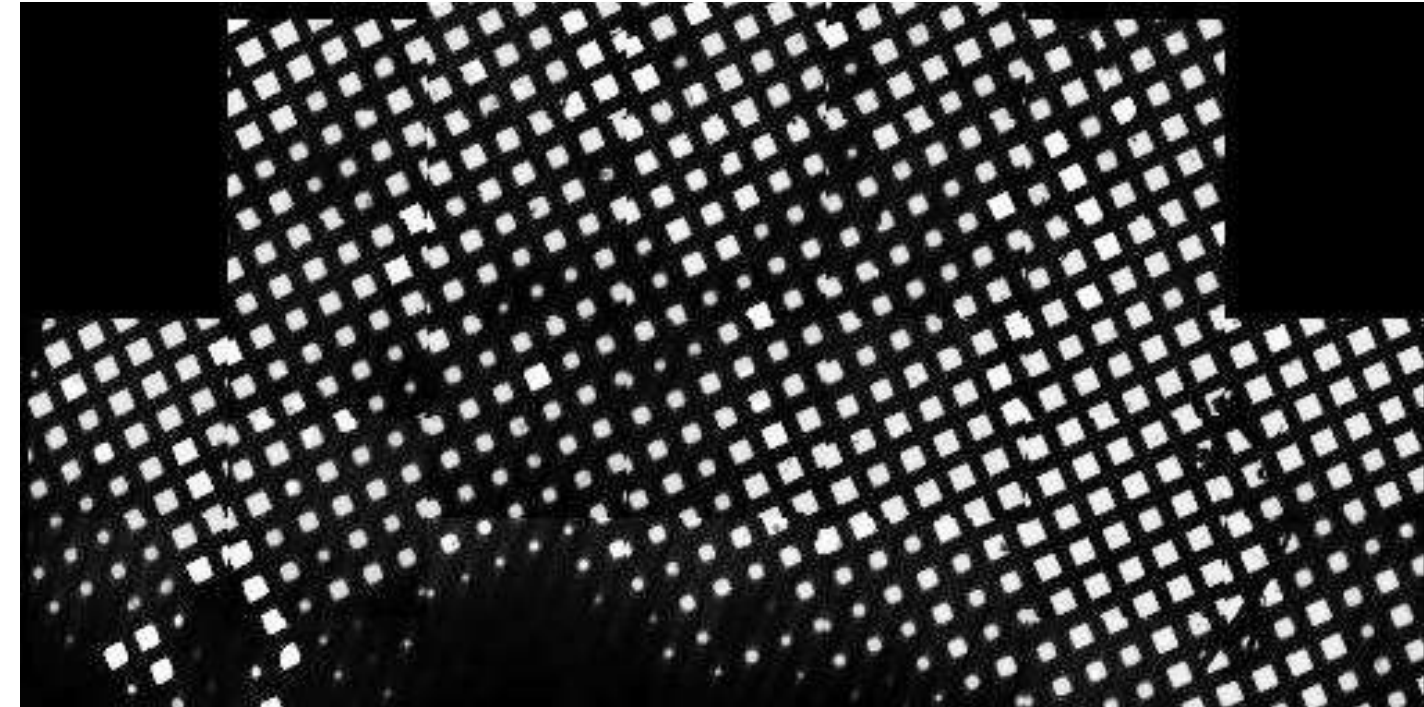


Spotiton

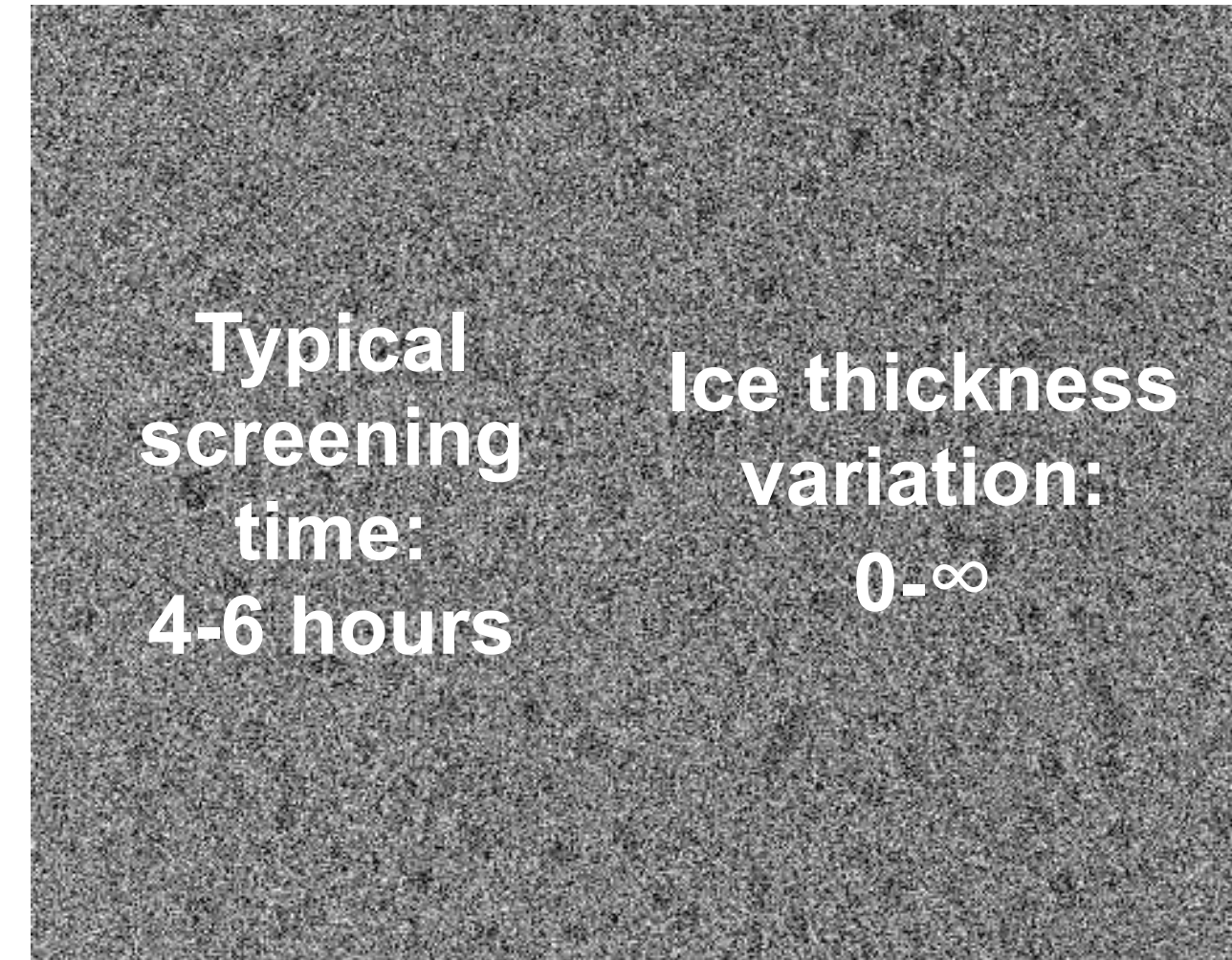
## Improving Current CryoTEM Grid Preparation Methods

Vitrobot

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

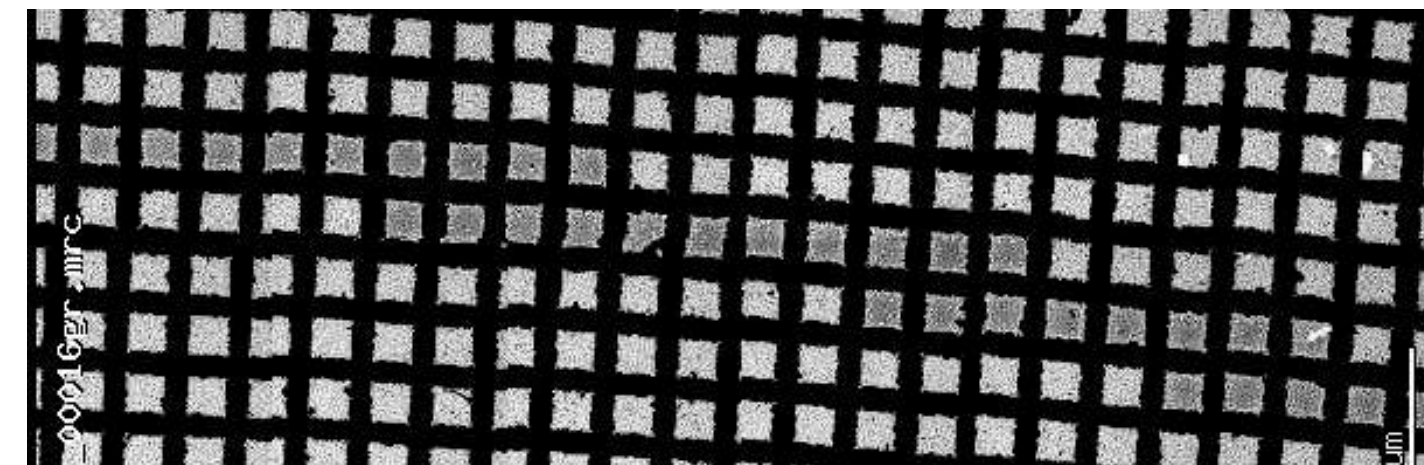


Usable area: ~0-10%

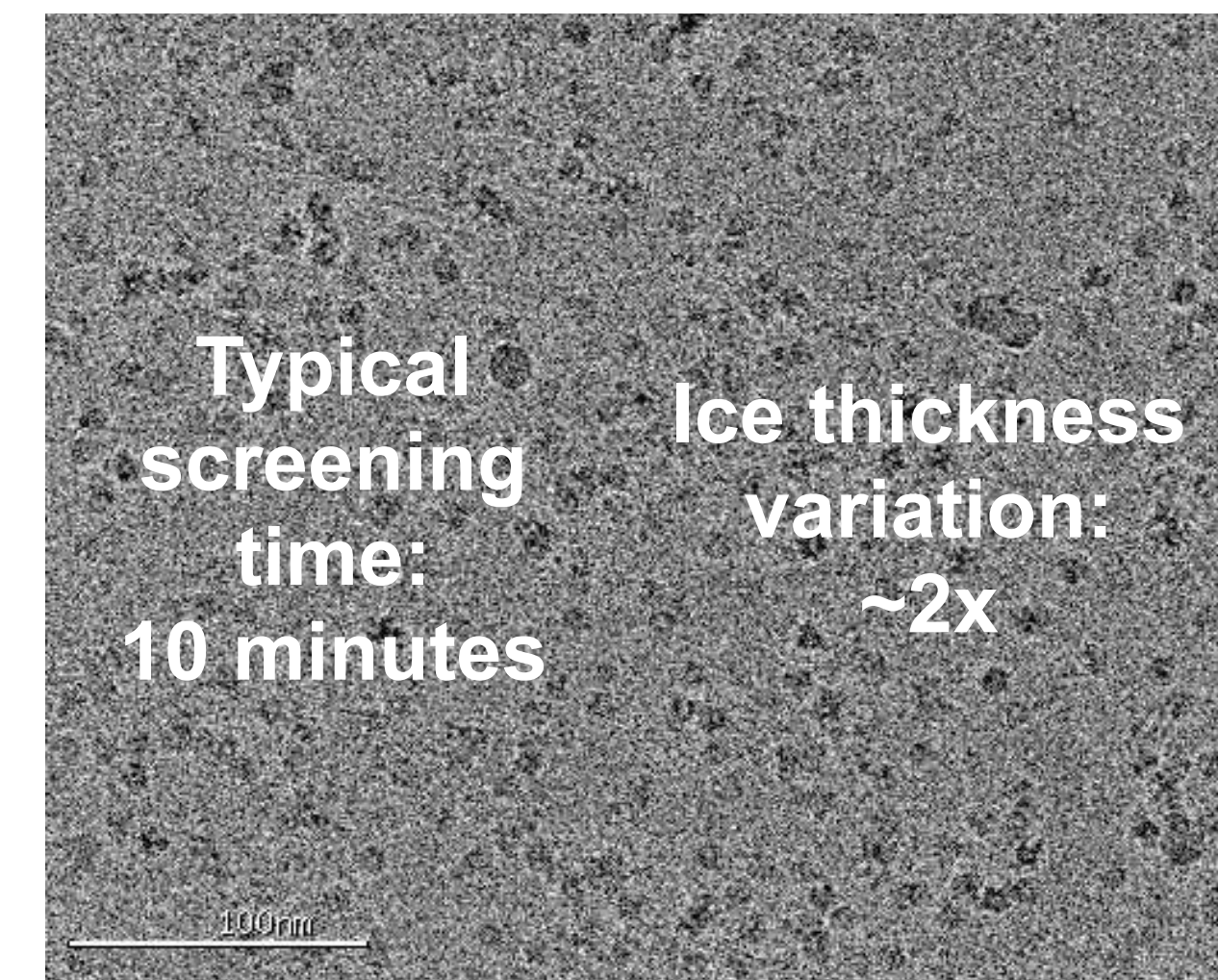


Spotiton

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



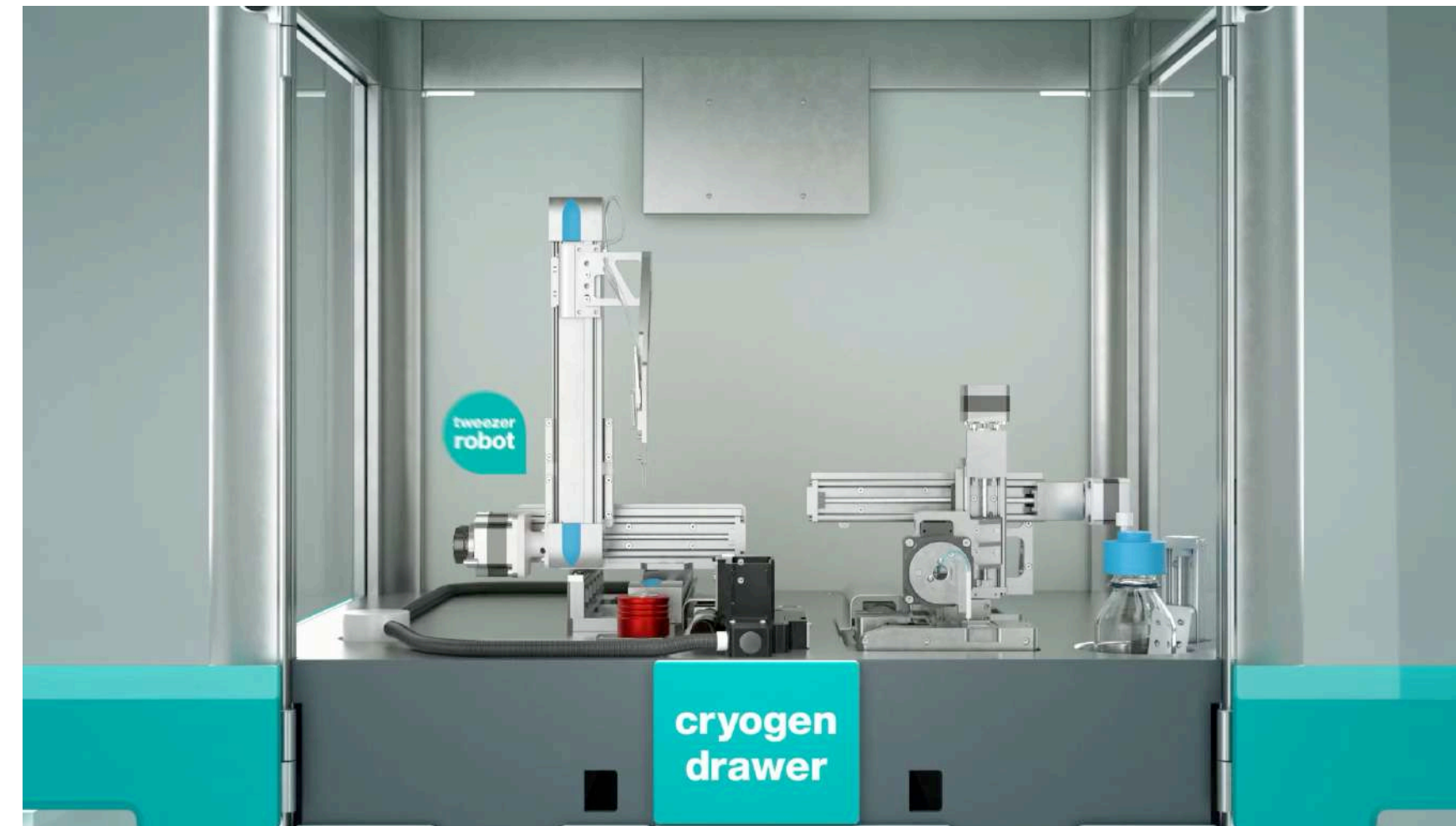
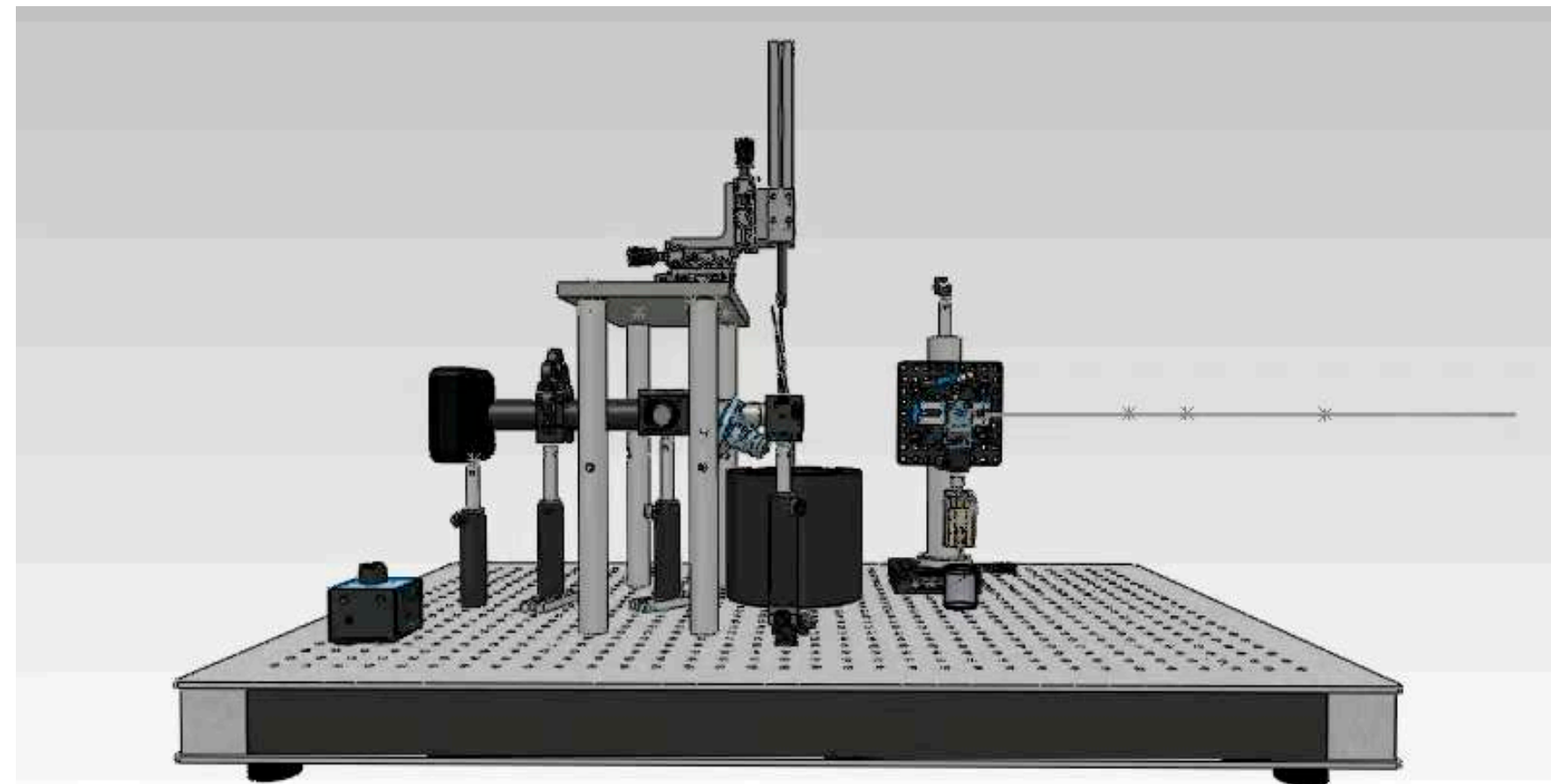
Hui Wei

# What is chameleon?

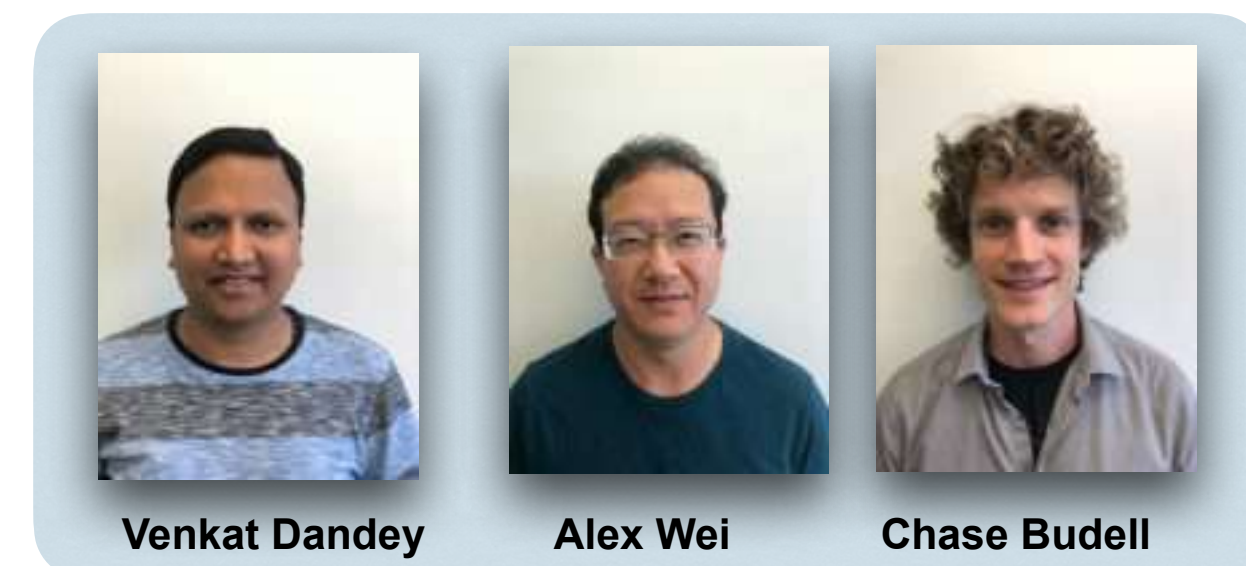
## The Spotiton Project: Commercialization

Spotiton concept: 2011

Chameleon: 2019



 **sptlabtech**



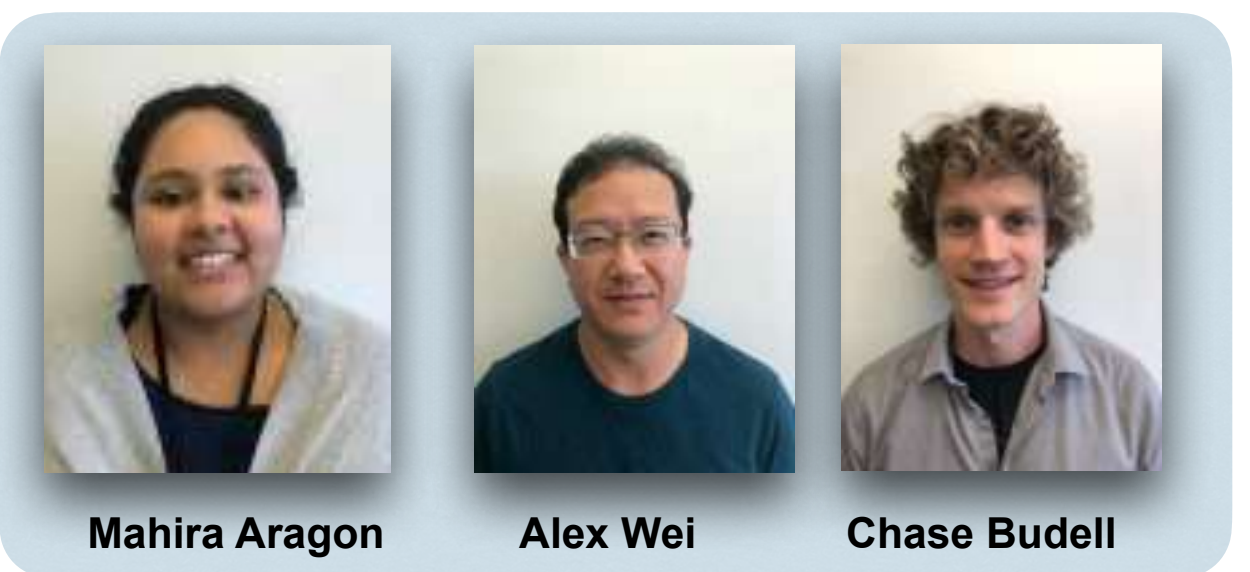
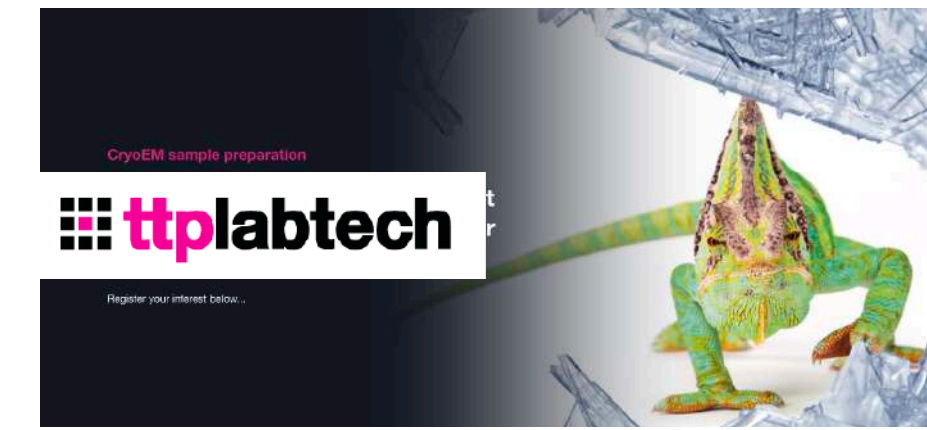
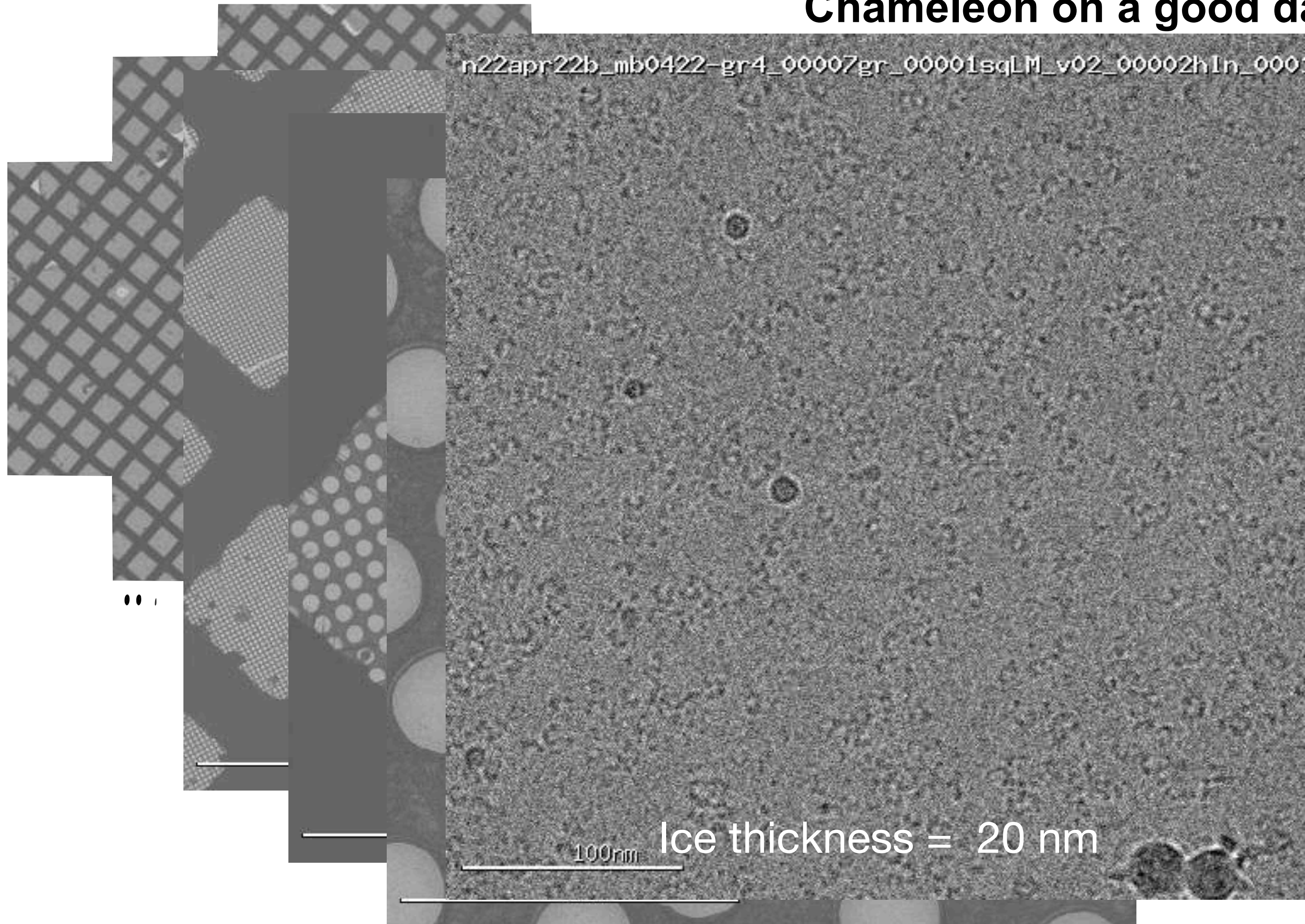
Venkat Dandey

Alex Wei

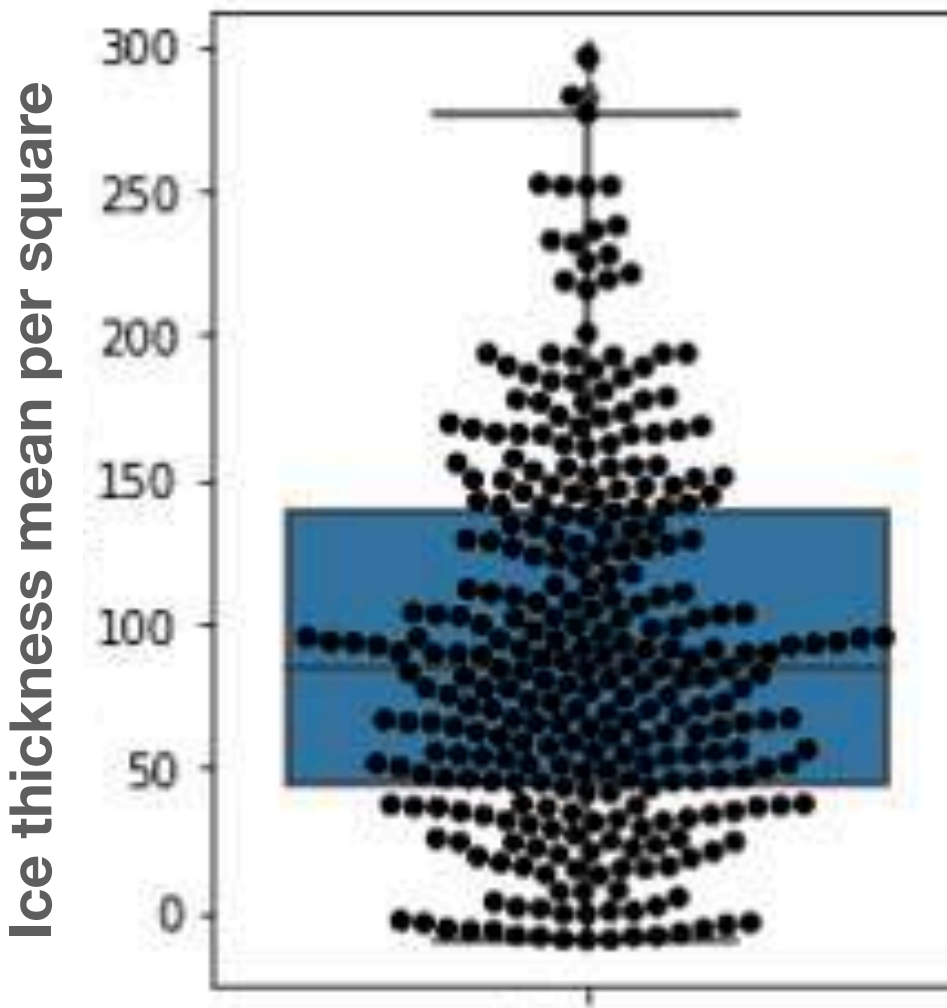
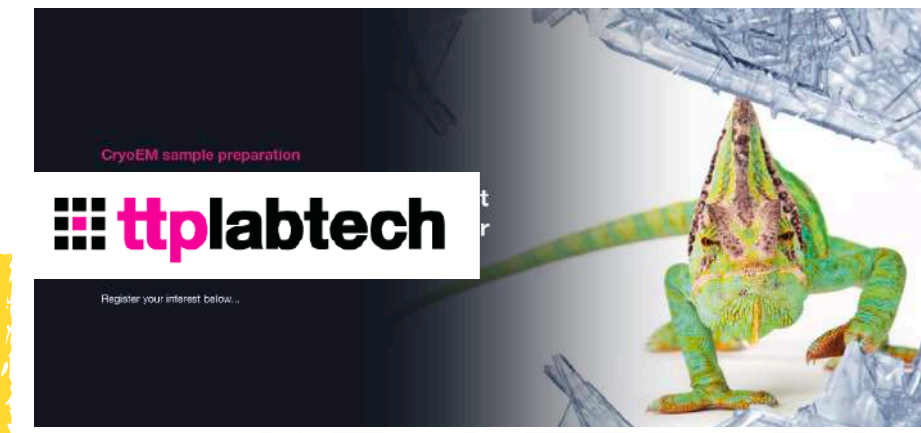
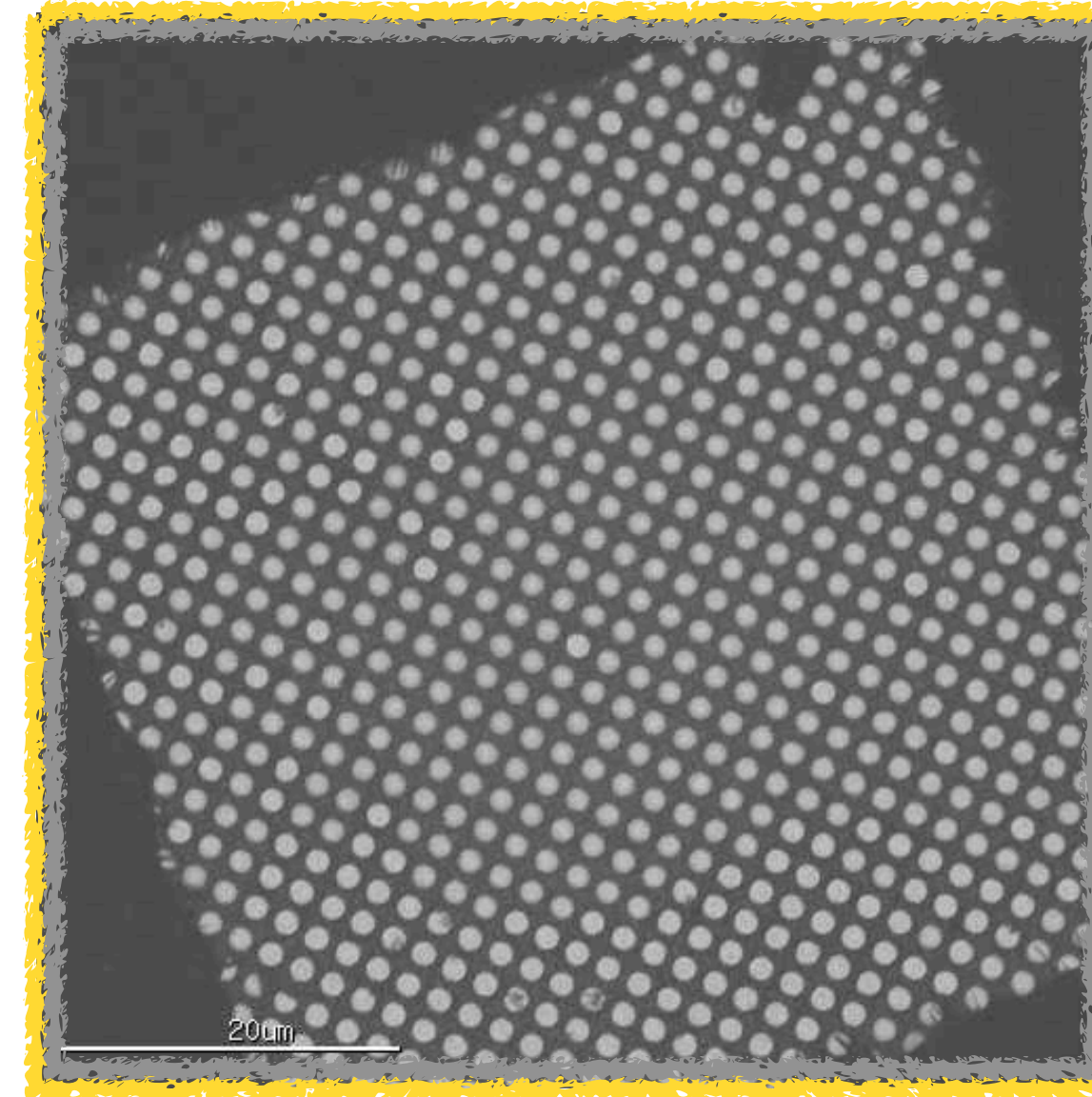
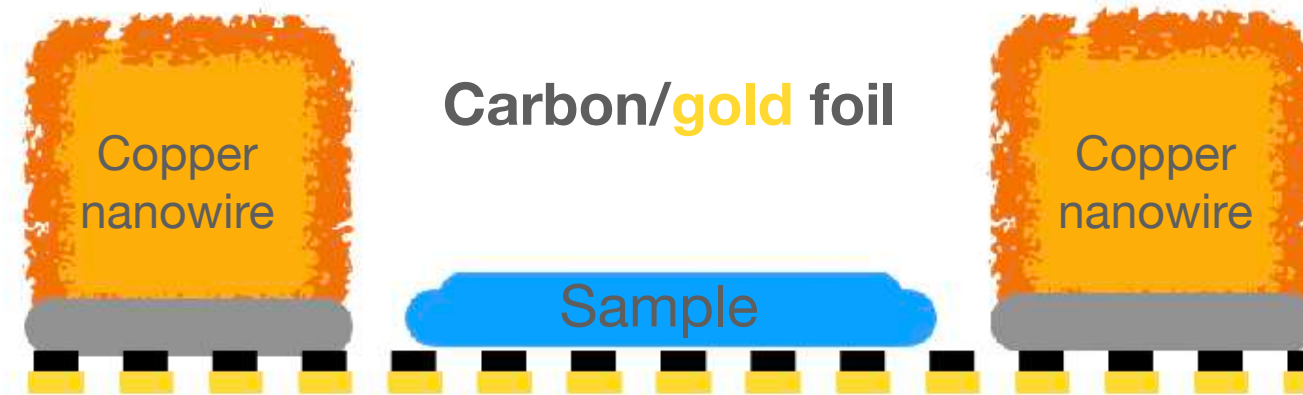
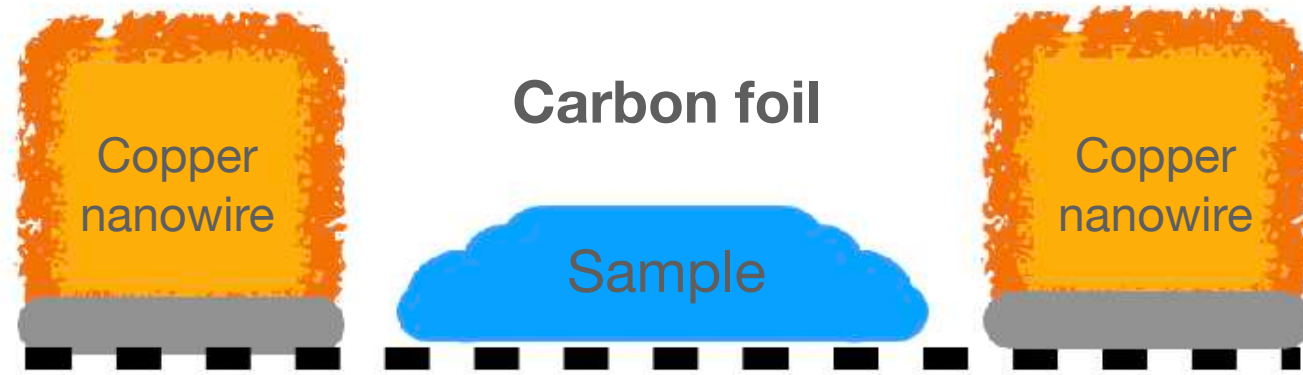
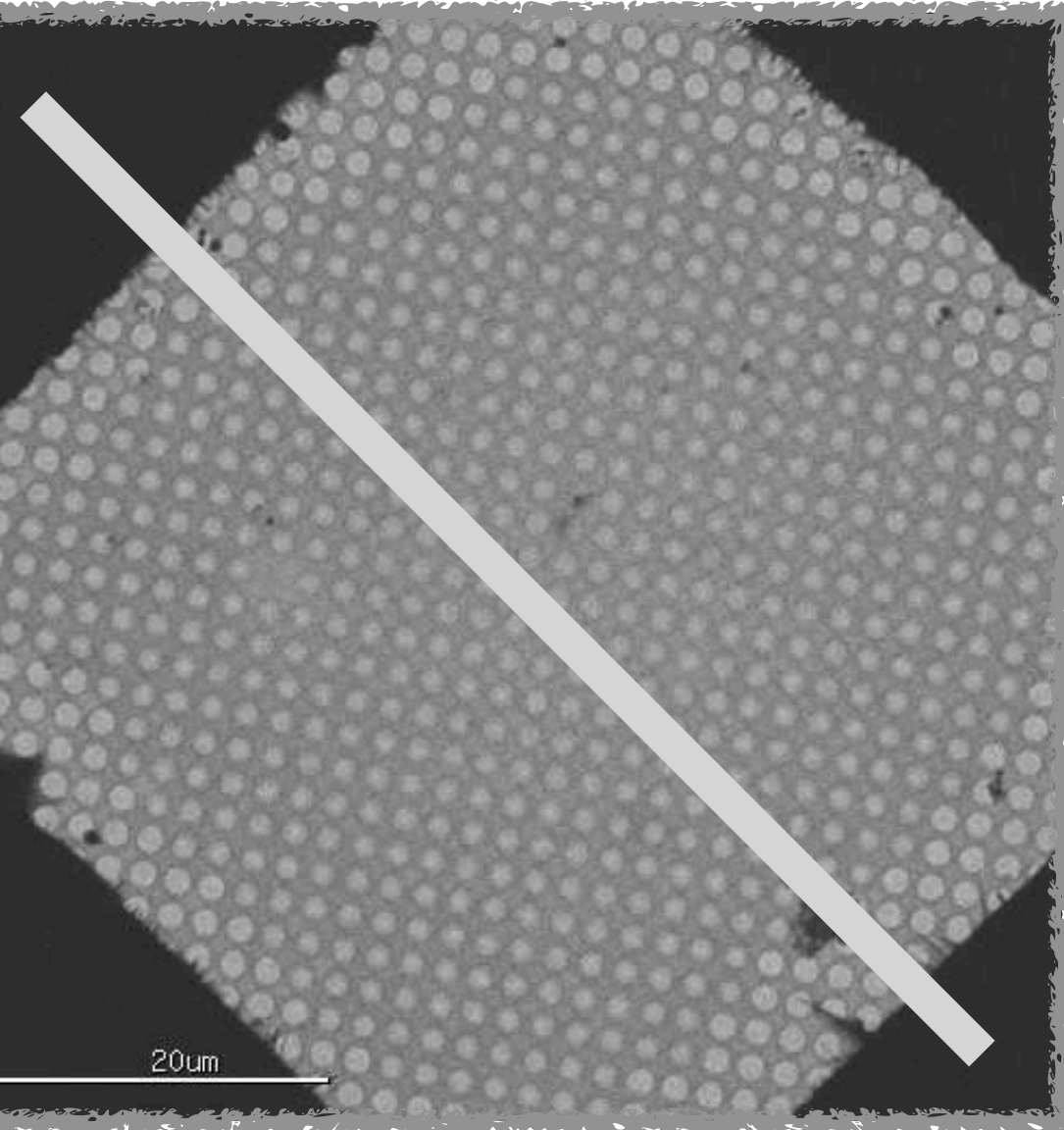
Chase Budell

# What is chameleon?

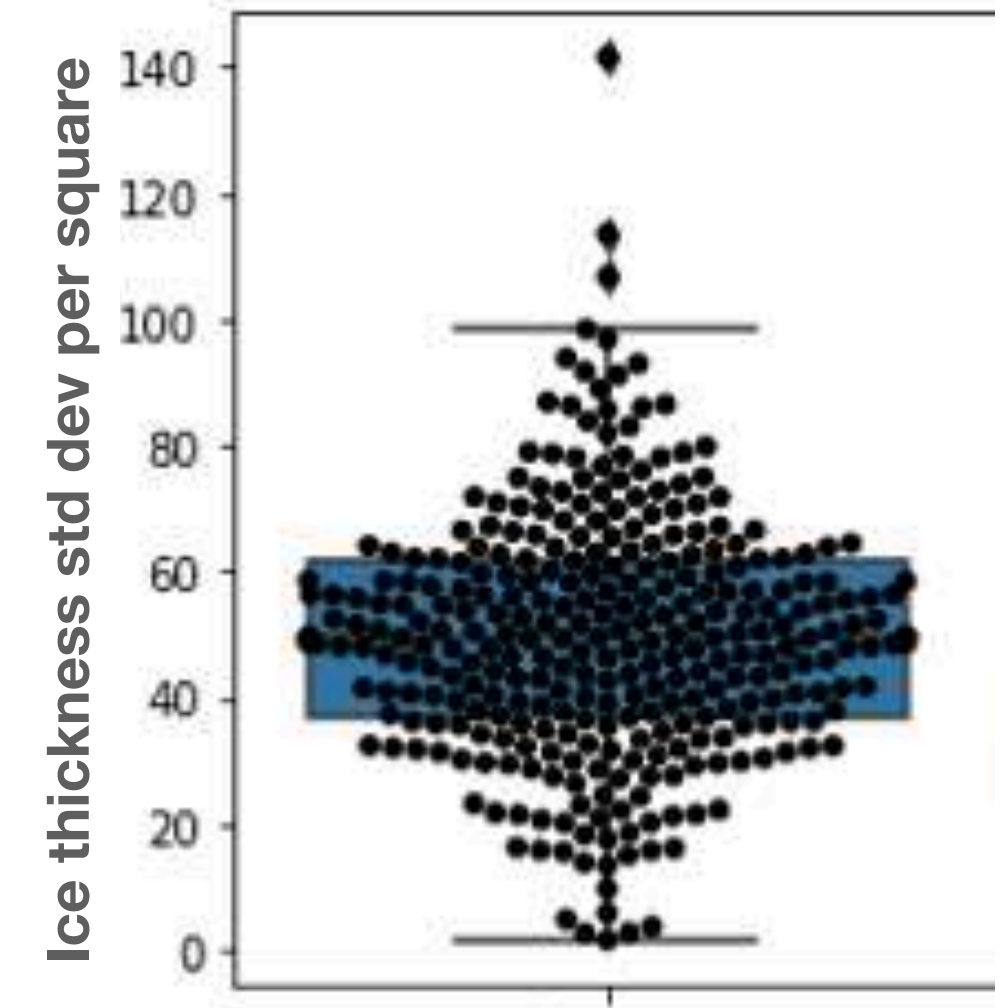
Chameleon on a good day



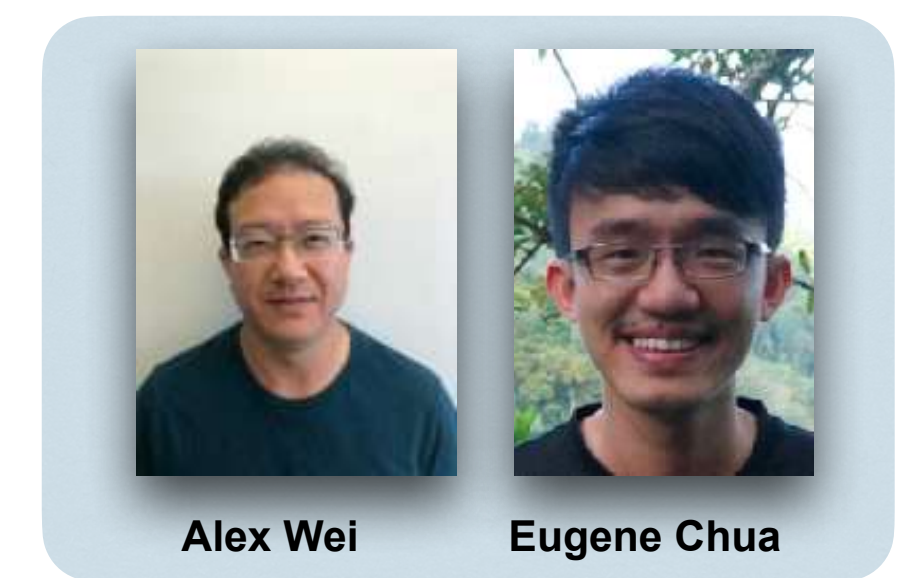
# What is chameleon?



Carbon foil



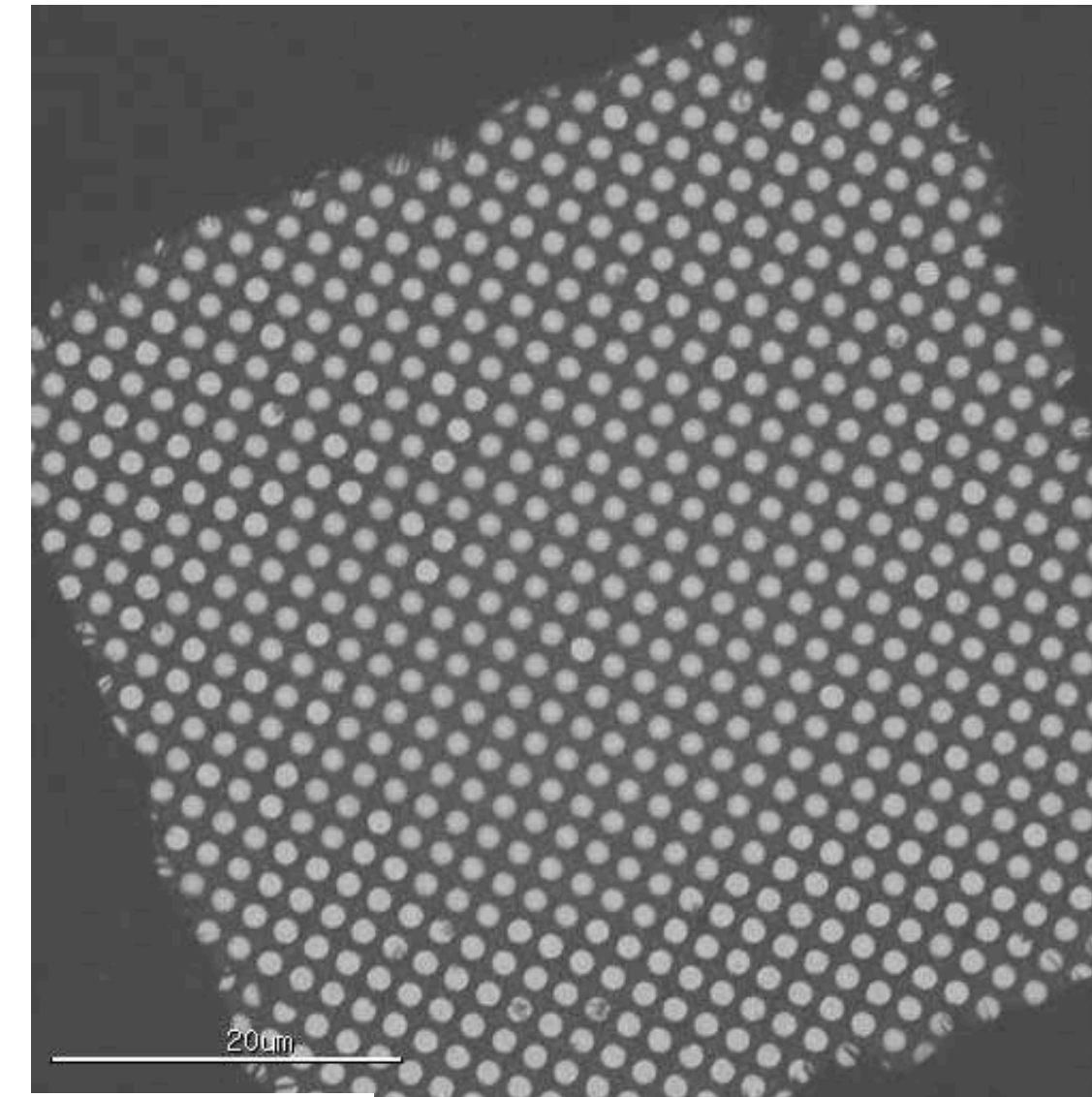
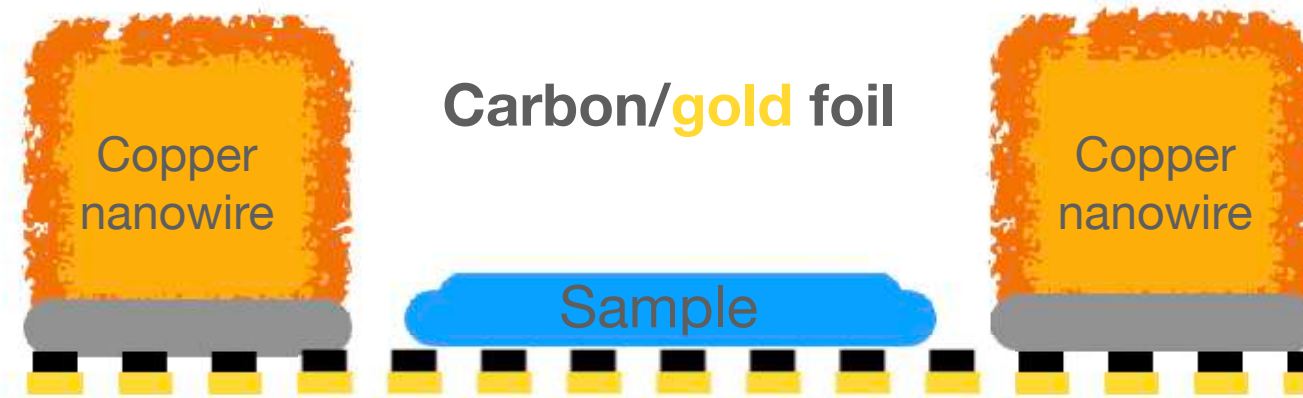
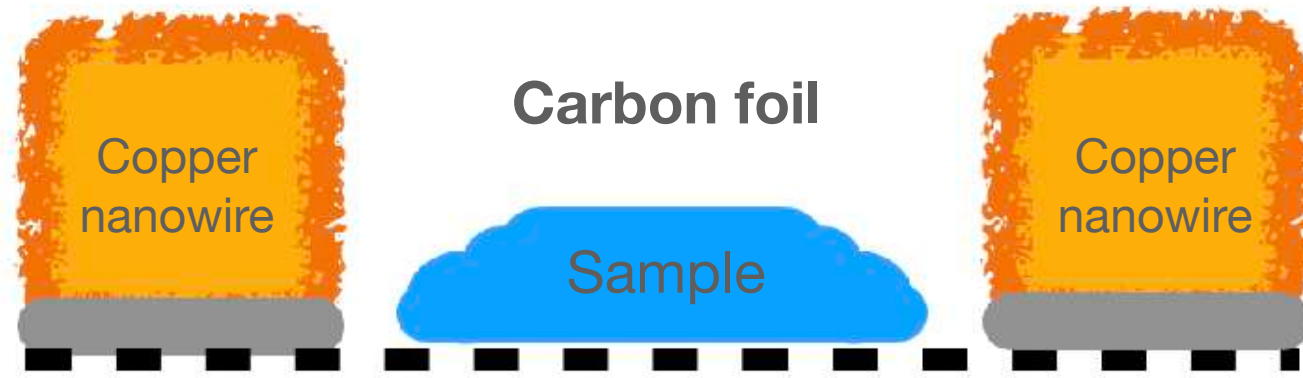
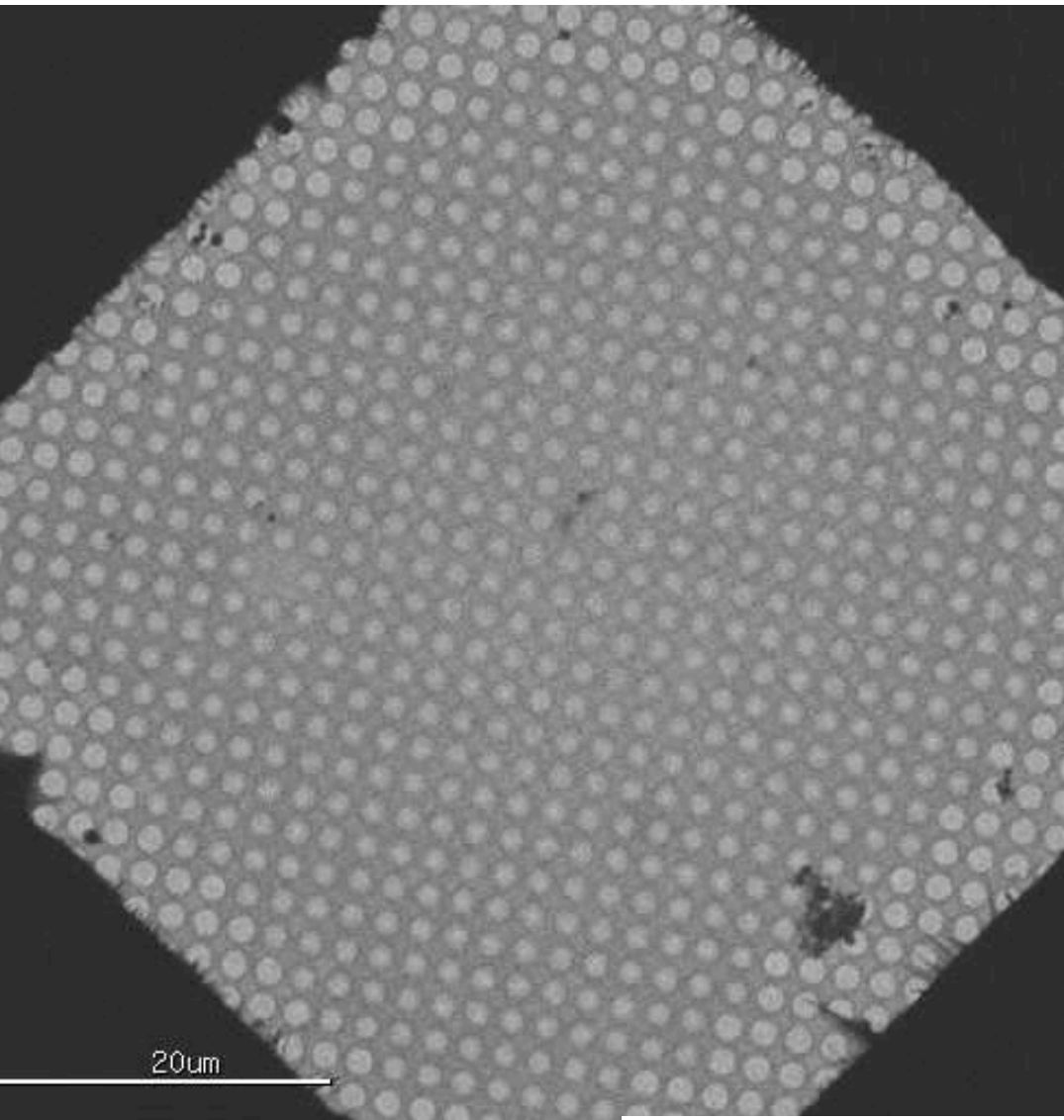
Carbon foil



Alex Wei

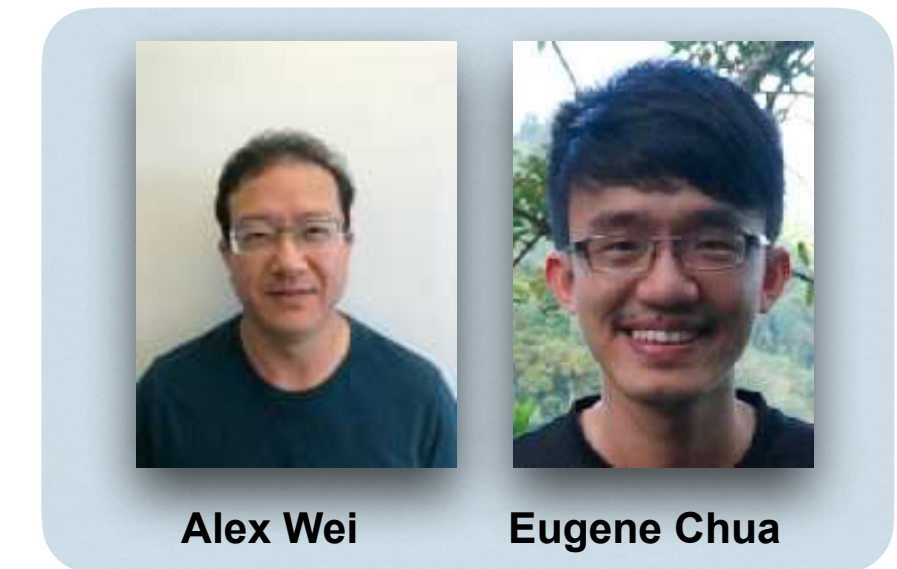
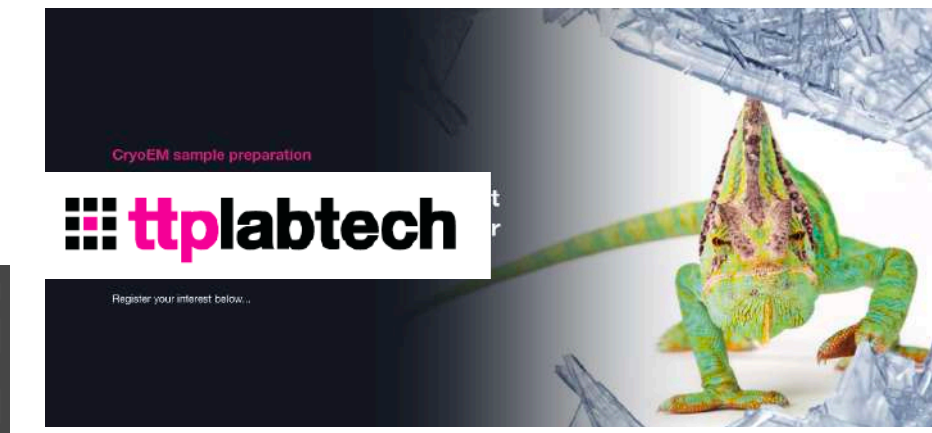
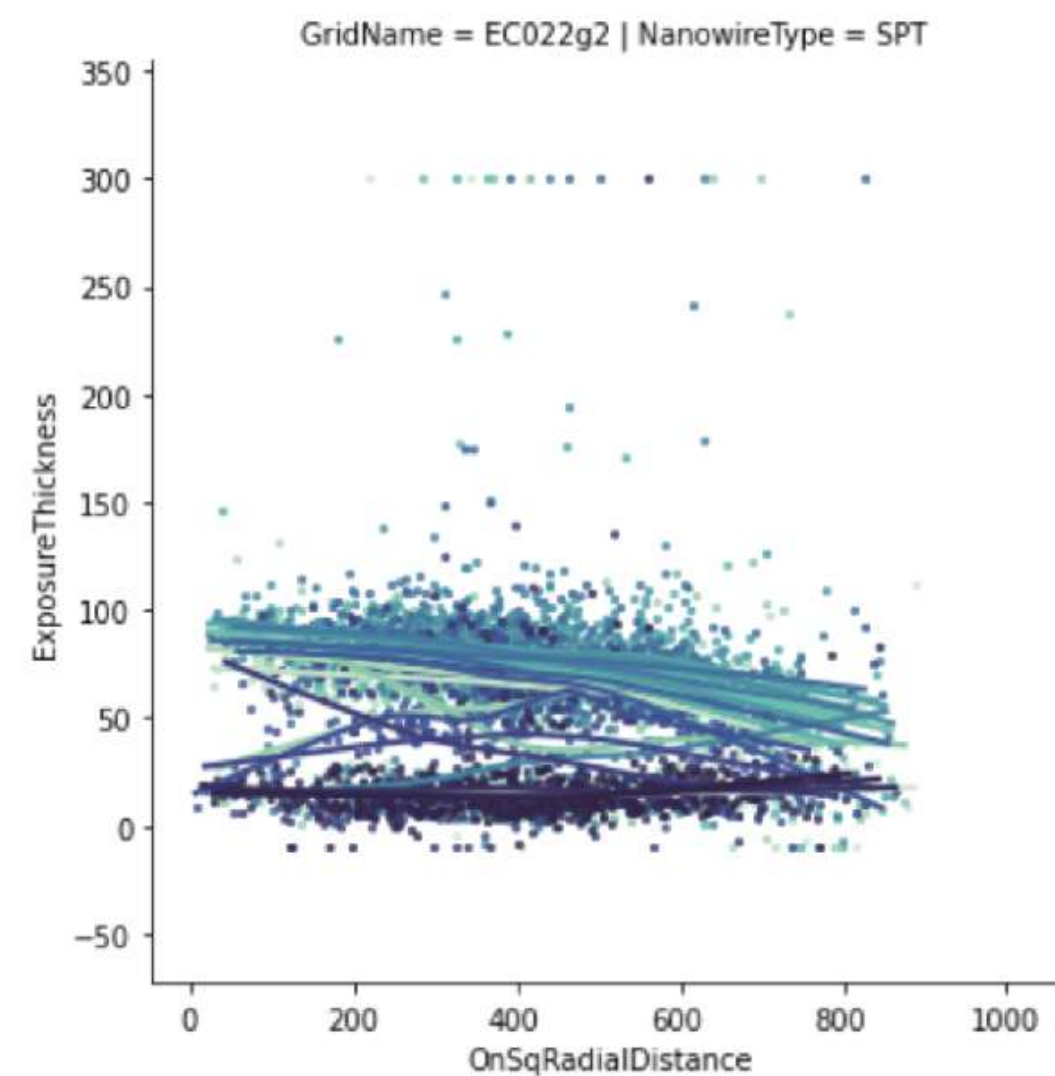
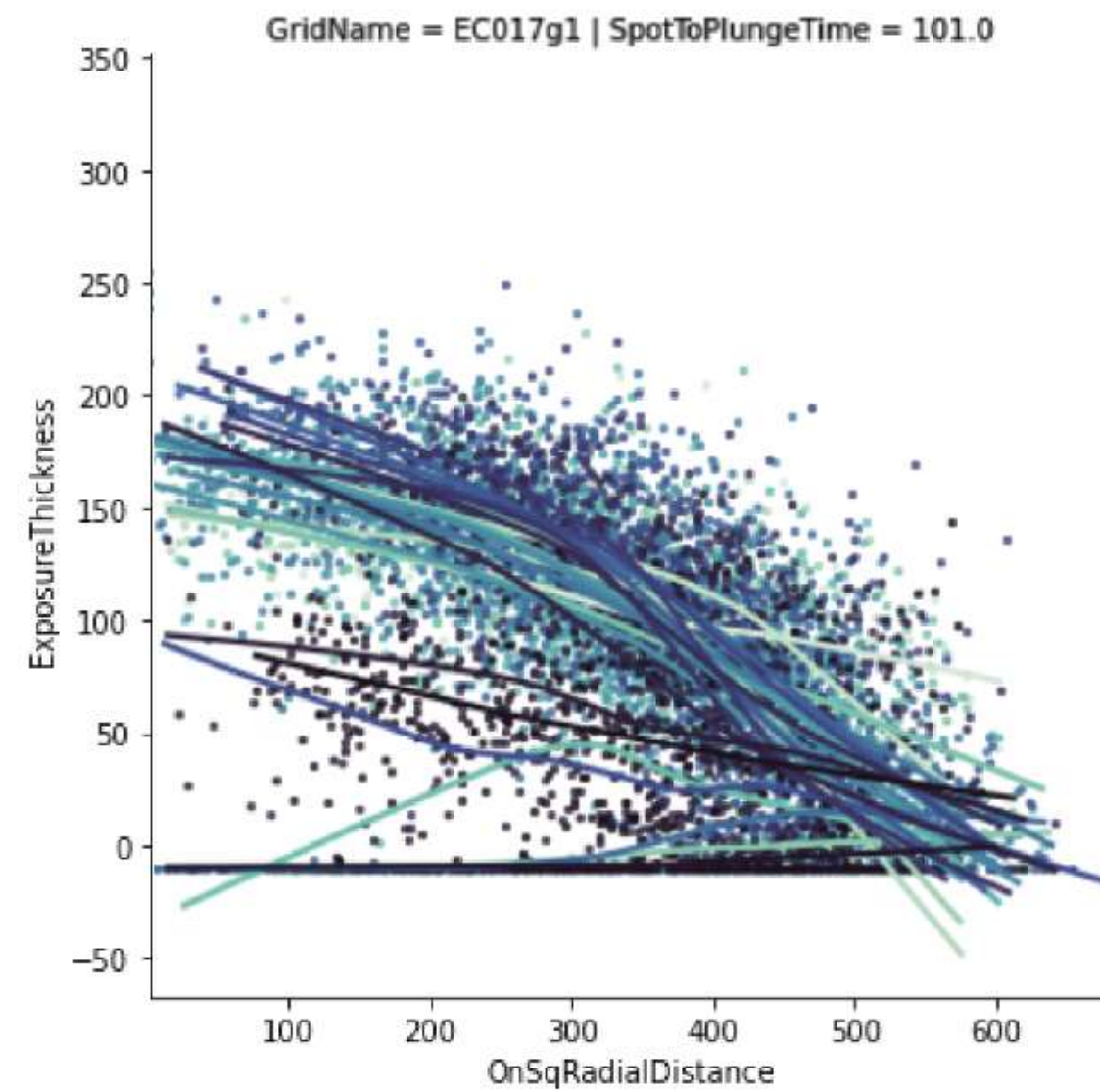
Eugene Chua

# What is chameleon?



Batch 413

Batch 392



Alex Wei

Eugene Chua

# What is chameleon?

Where is the ice relative to the grid?



Grid geometry

~1000 nm

~20 nm

Vitreous ice ideal -> typical thickness

~ 20 nm

.....

~ 100 nm

# What is chameleon?

Where is the ice relative to the grid?

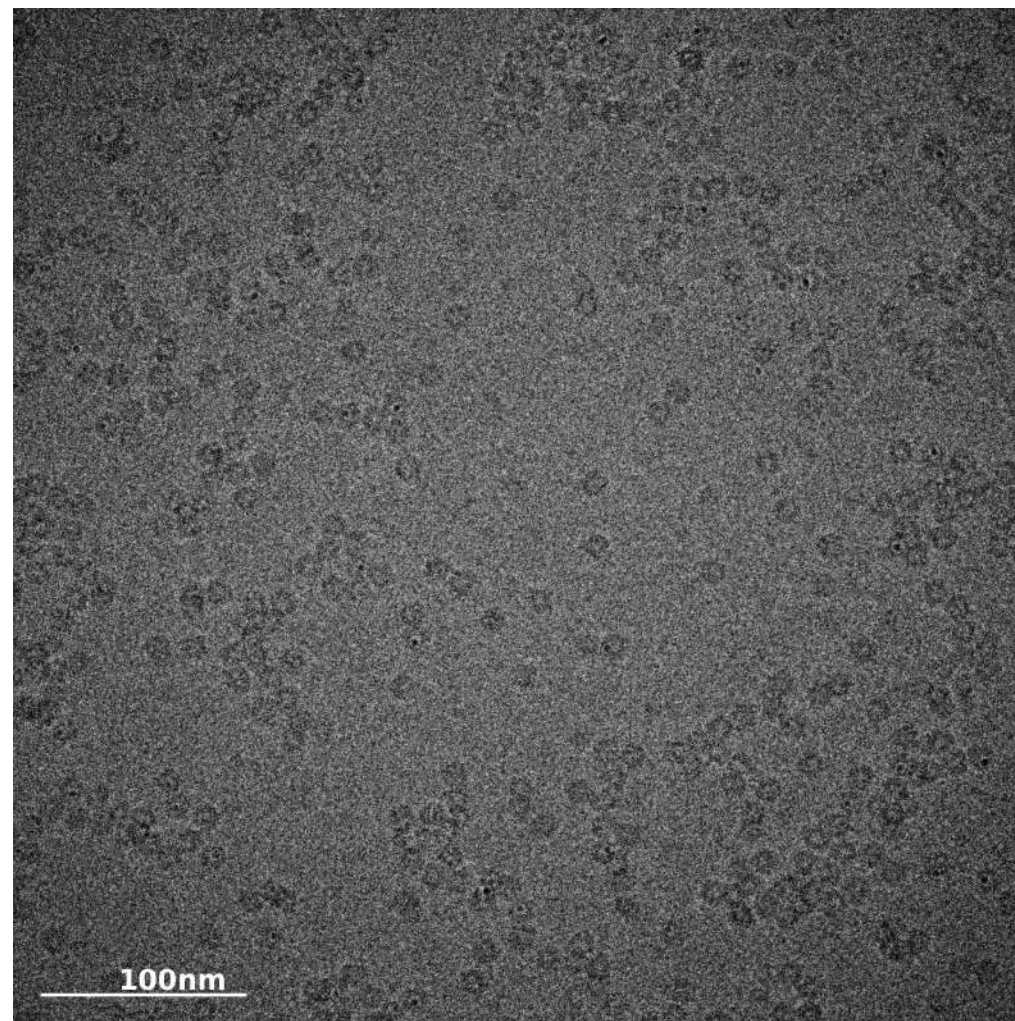


Does the substate determine this?

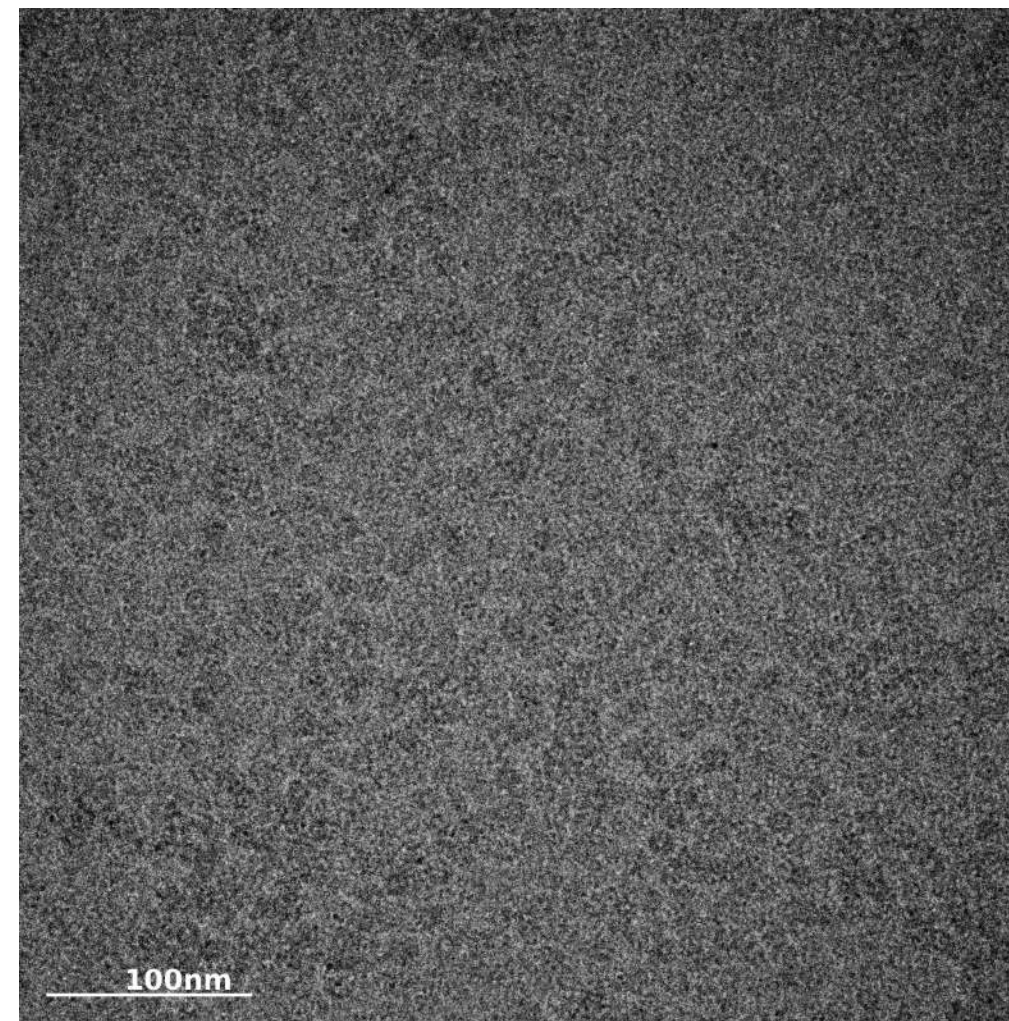
# What is chameleon? Where is the ice relative to the grid?



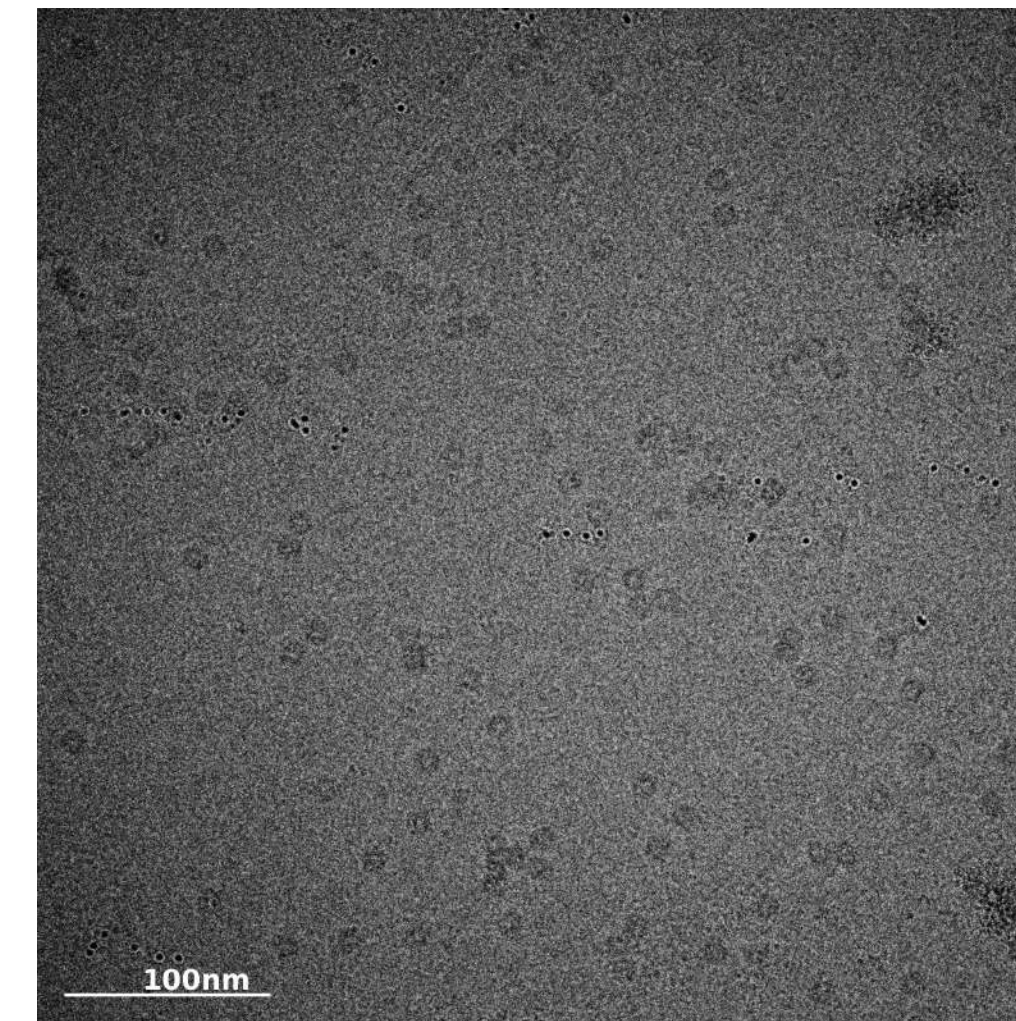
Add a graphene layer under the substrate



No graphene (8 mg/ml)



Graphene (8mg/ml)



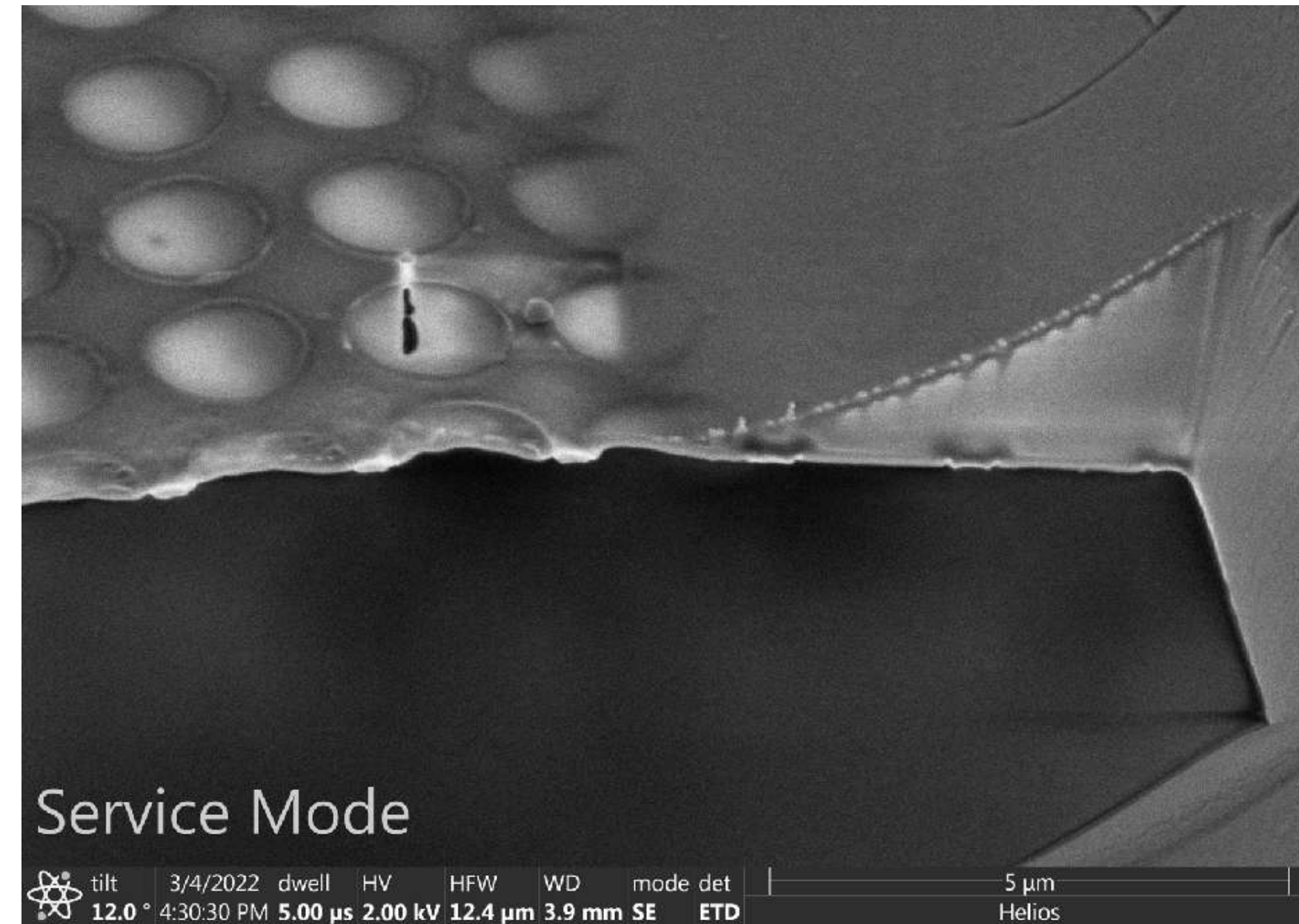
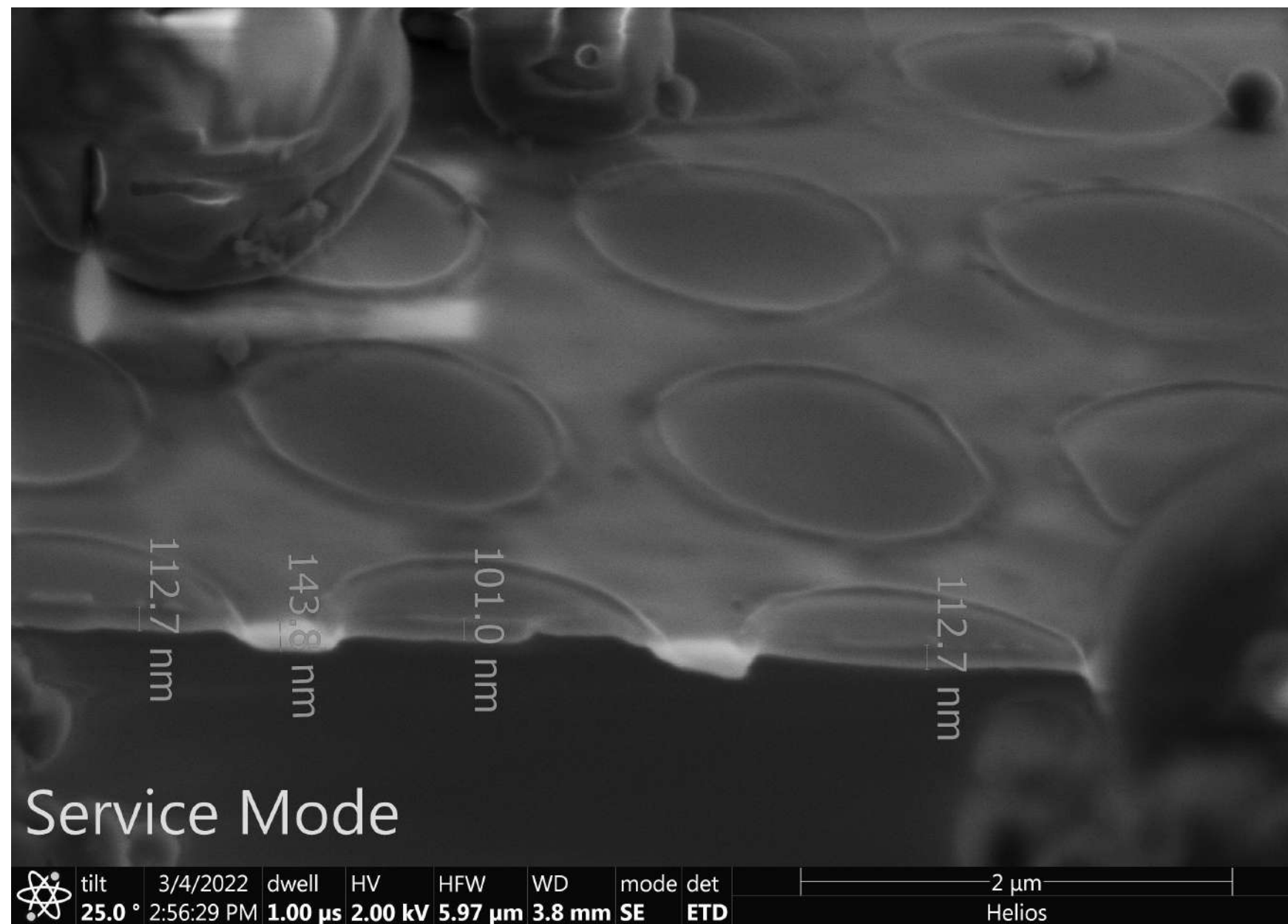
Graphene (0.8 mg/ml)

# What is chameleon?

Where is the ice relative to the grid?



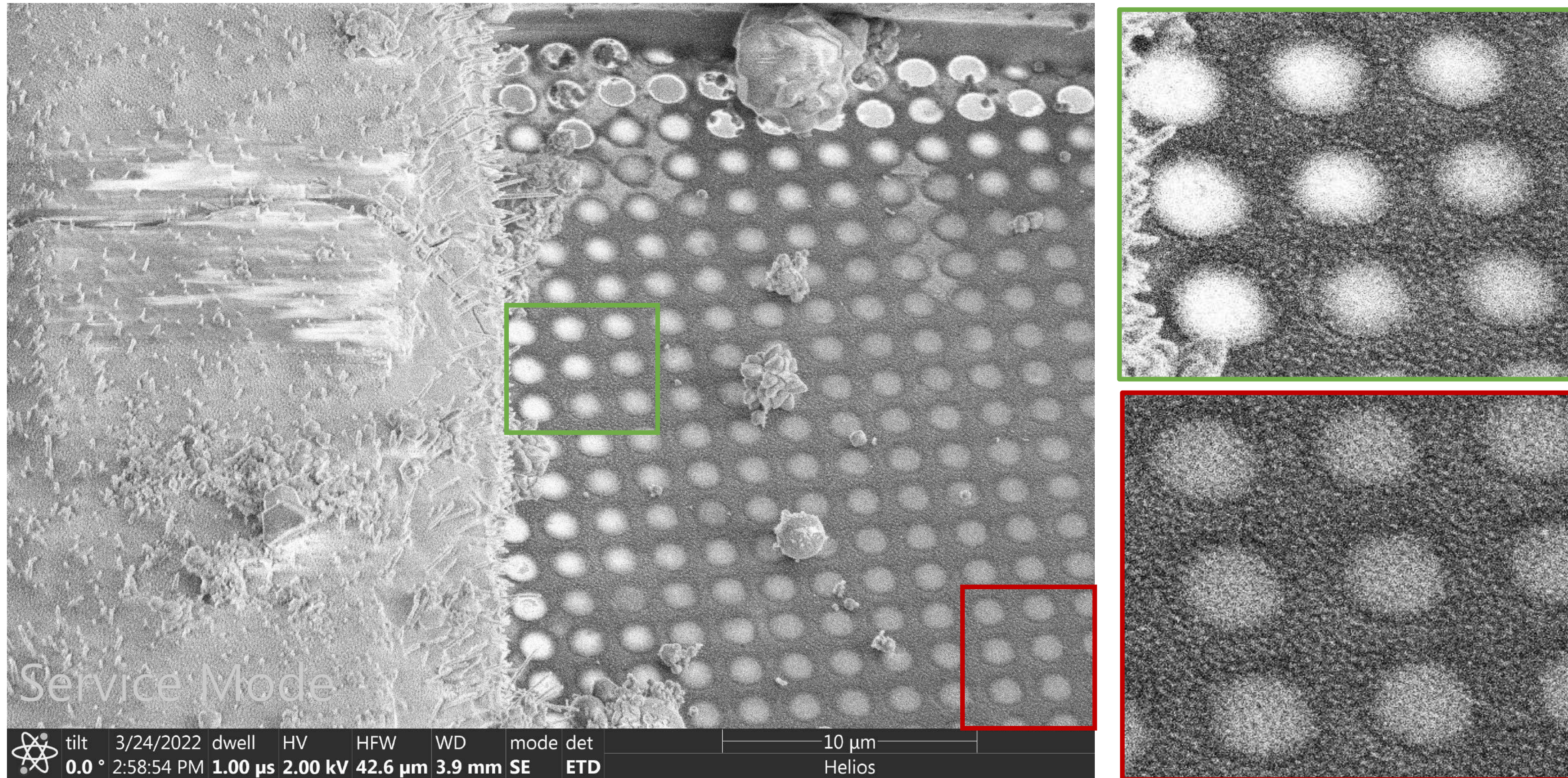
## Cryo FIB-SEM



# What is chameleon? Where is the ice relative to the grid?



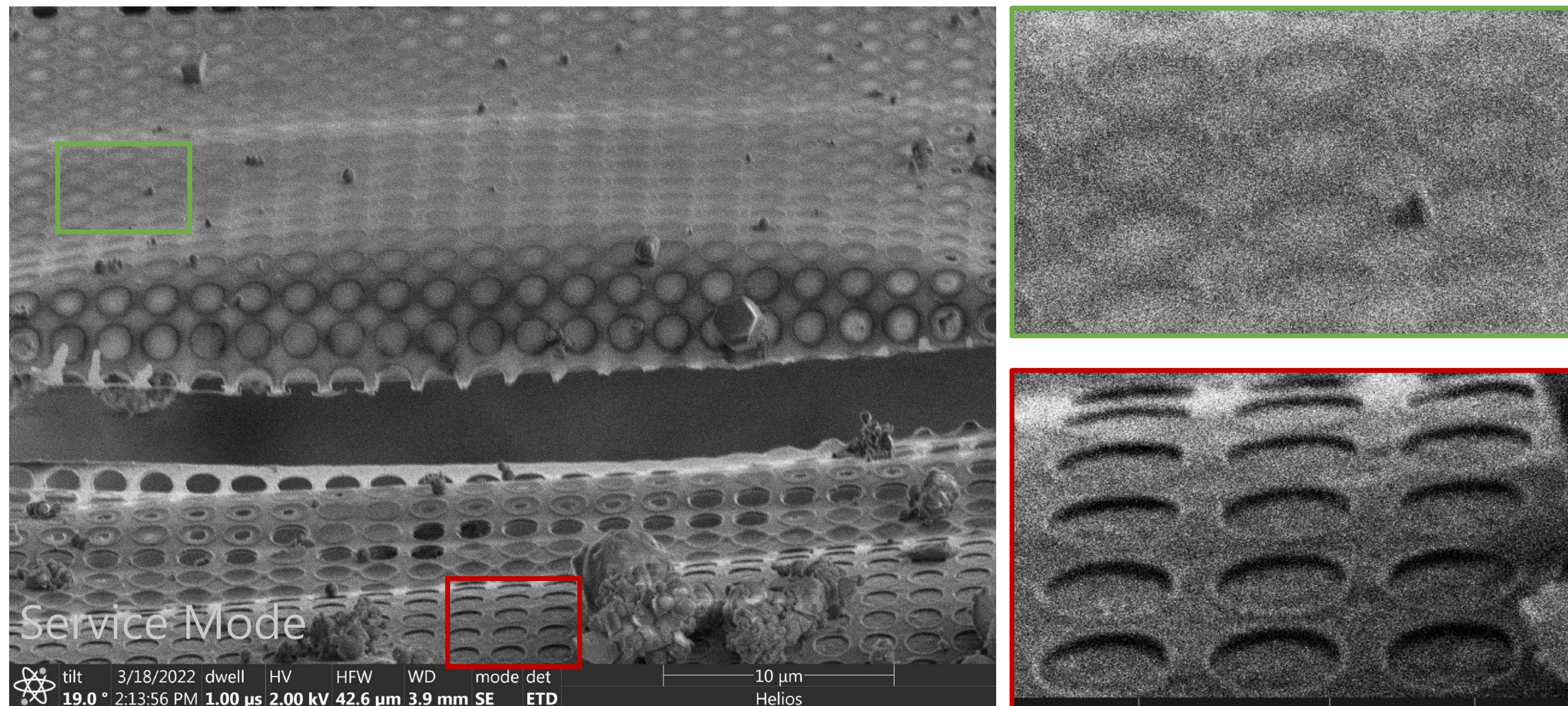
## Sample application side of grid



# What is chameleon? Where is the ice relative to the grid?



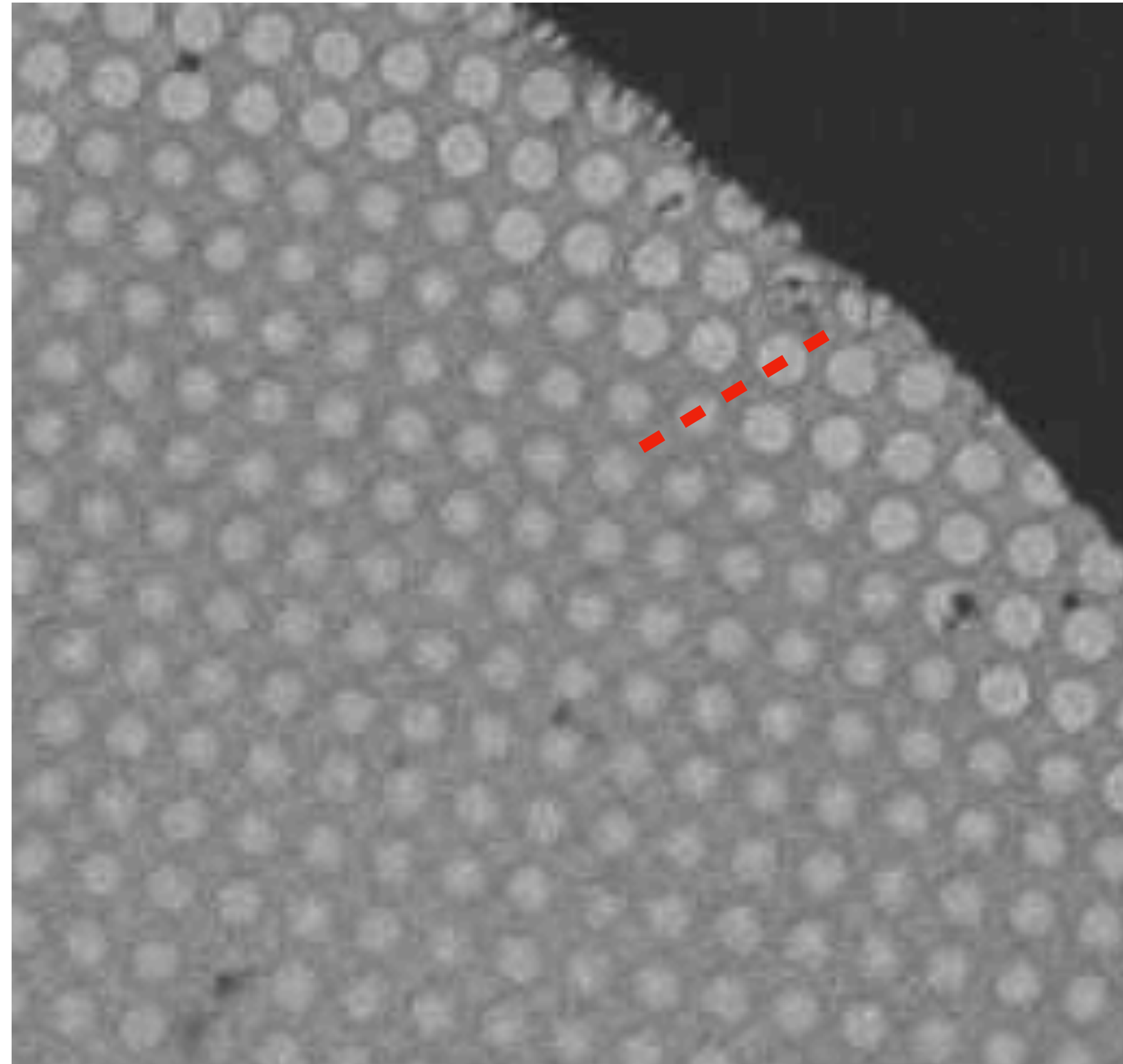
## Opposite side of sample application



# What is chameleon? Where is the ice relative to the grid?



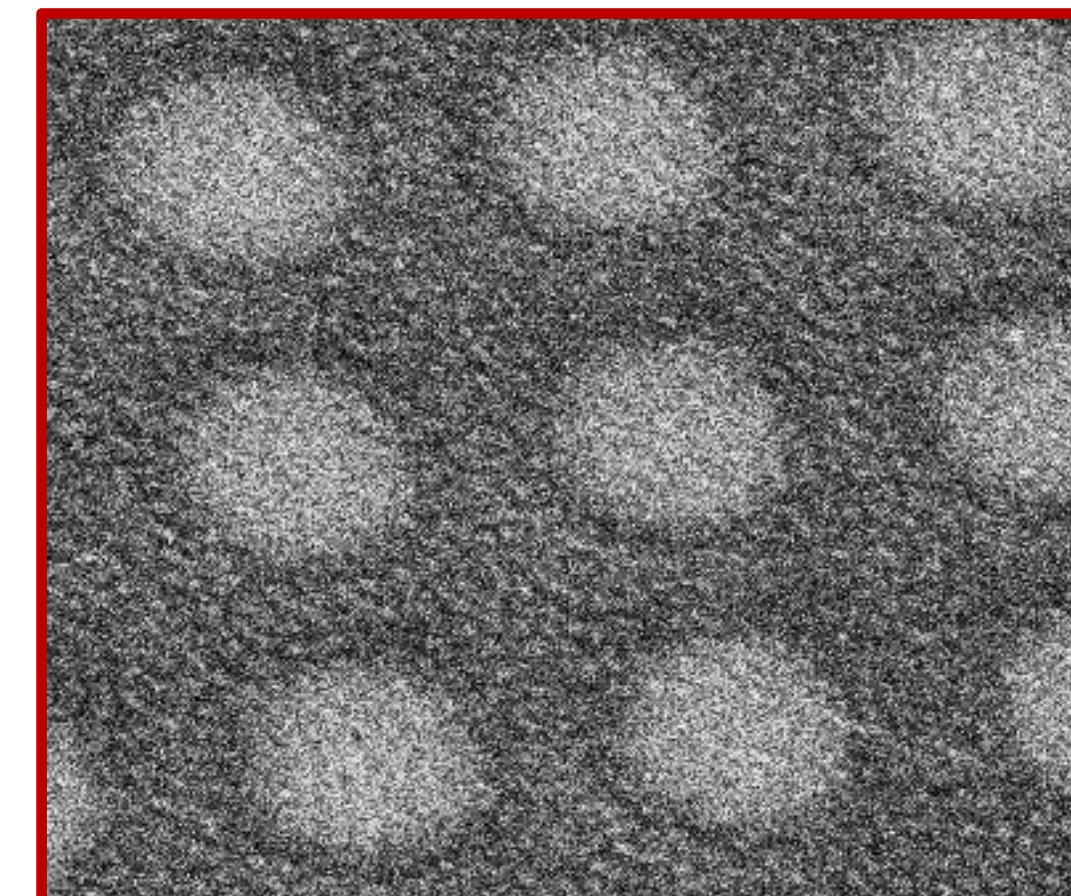
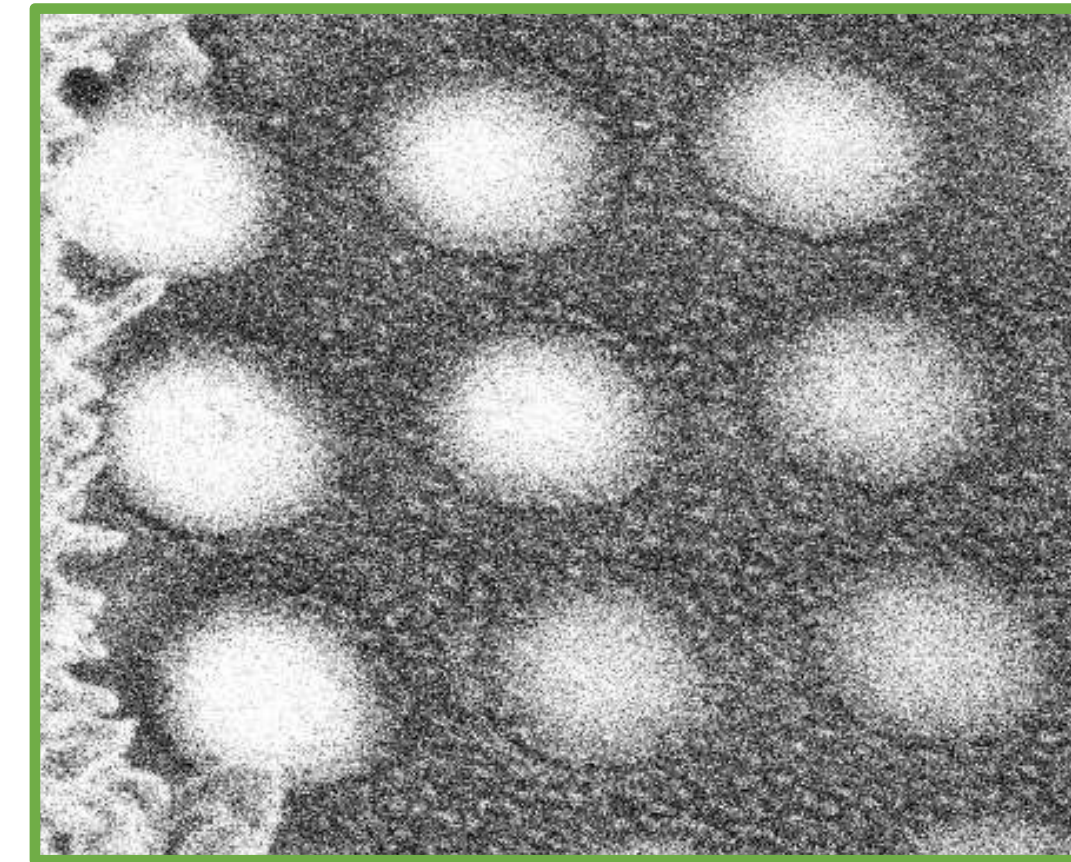
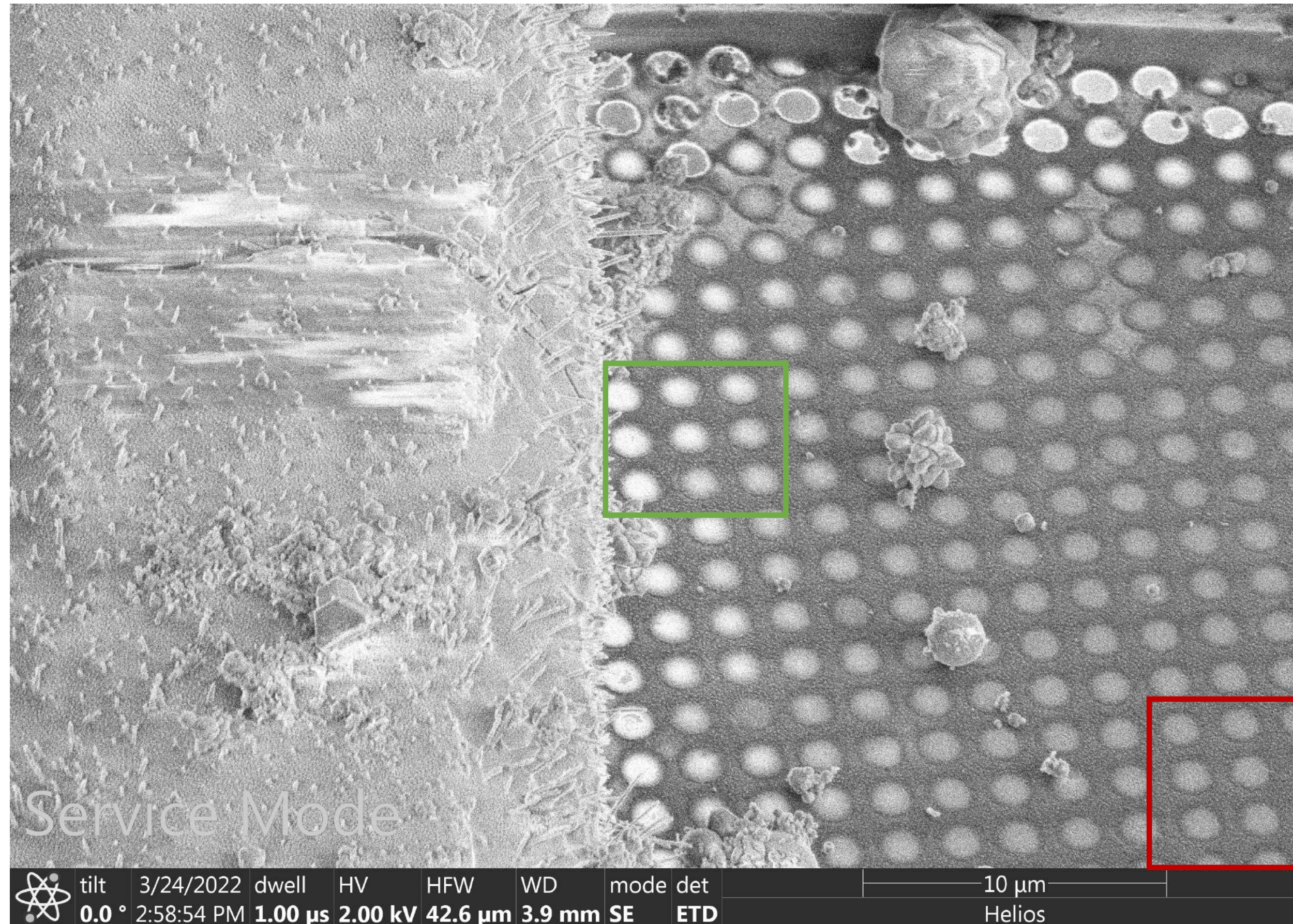
And why does this happen?

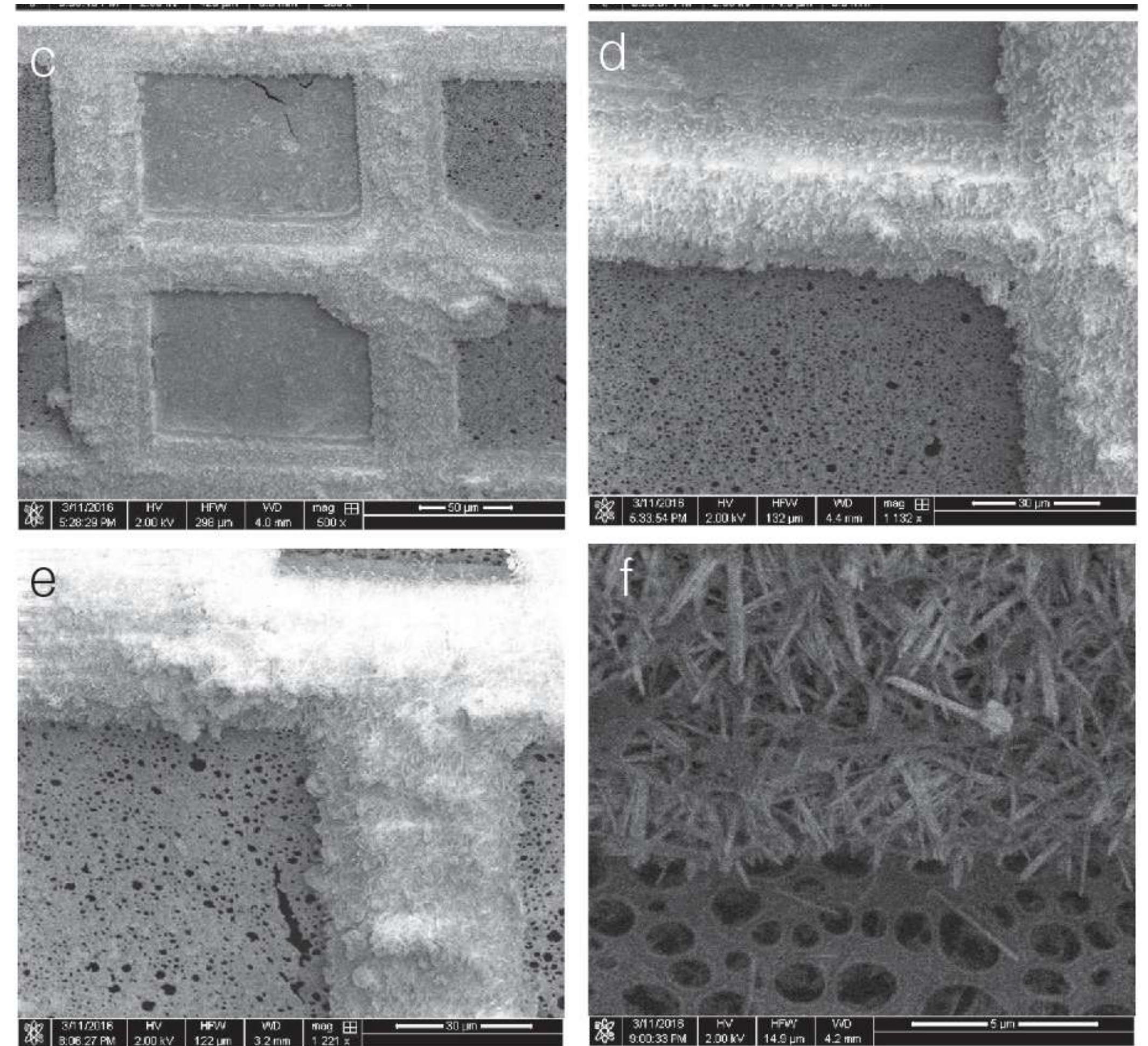
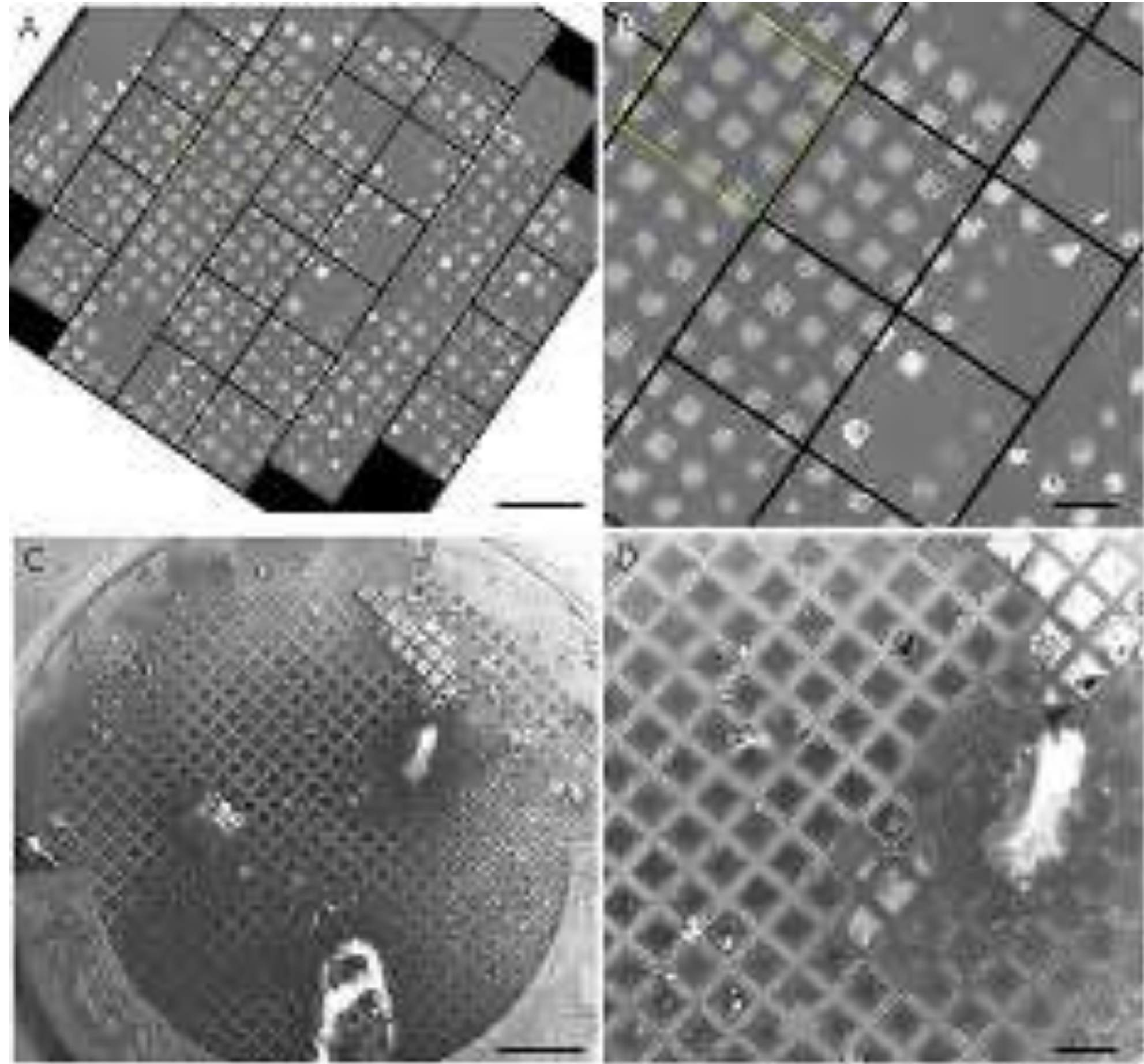


?



# WHAT DOES A GRID LOOK LIKE?

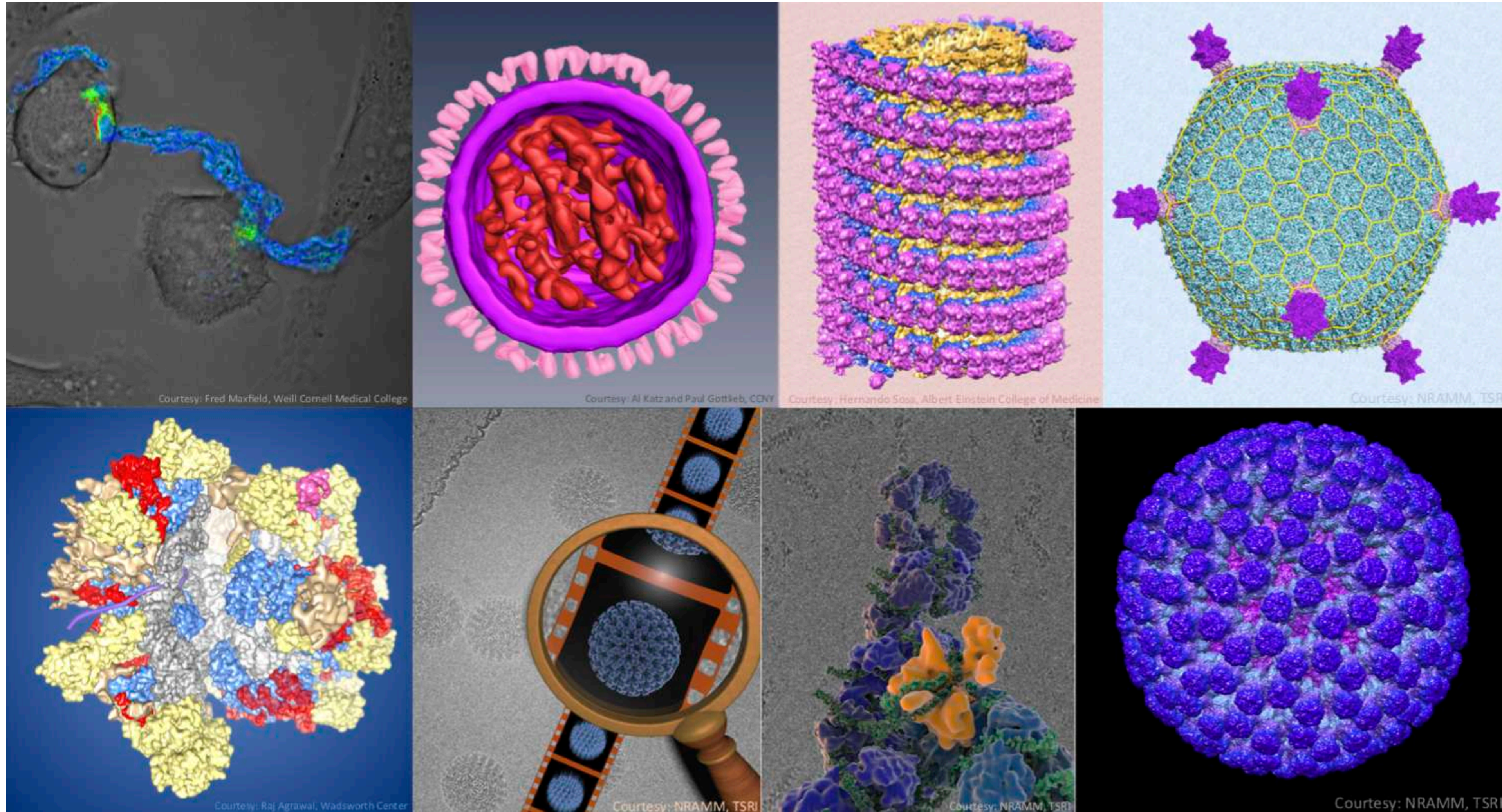




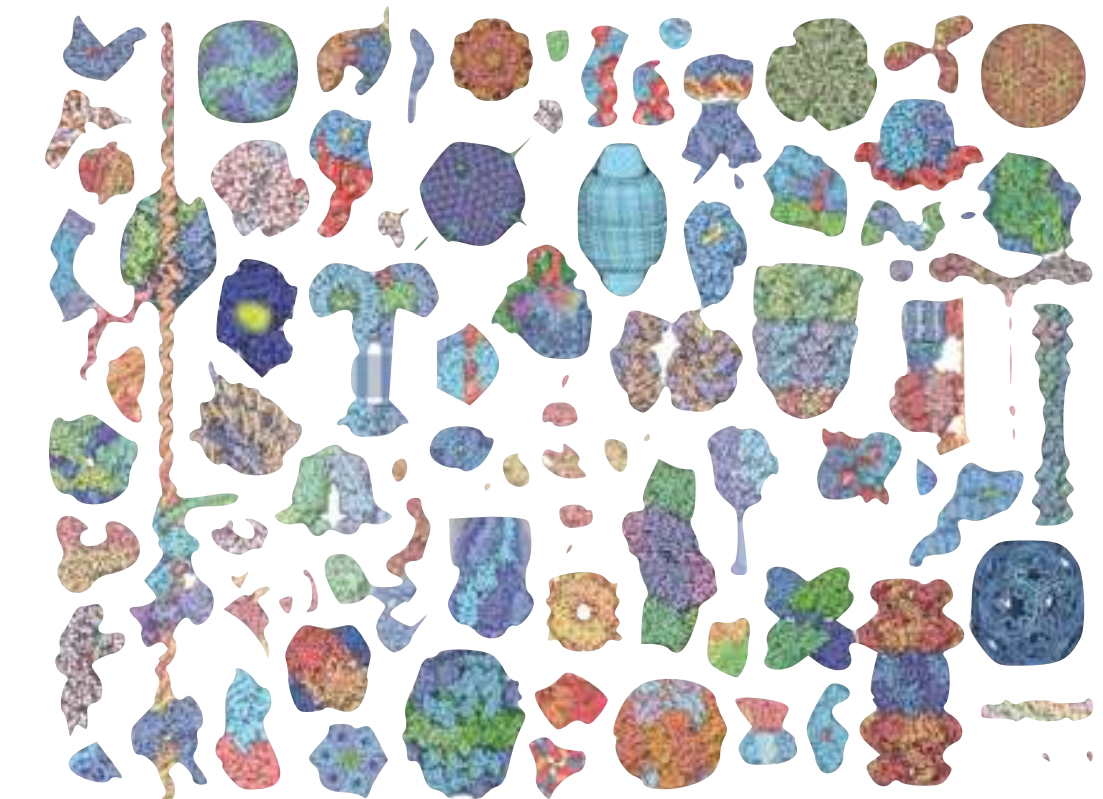
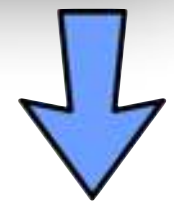
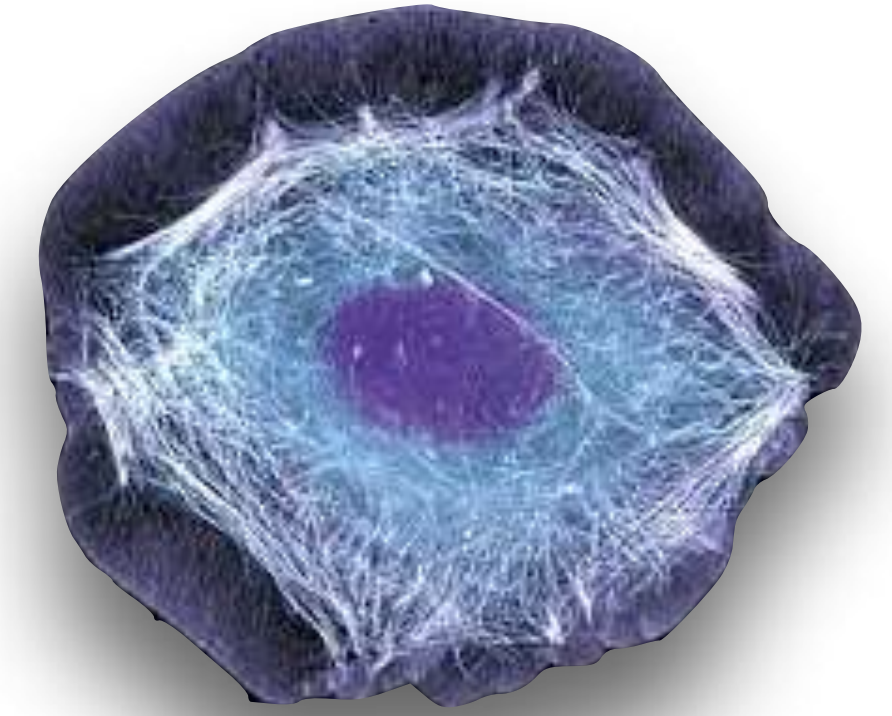
- Schmidli, Claudio & Rima, Luca & Arnold, Stefan & Stohler, Thomas & Syntychaki, Anastasia & Bieri, Andrej & Albiez, Stefan & Goldie, Kenneth & Chami, Mohamed & Stahlberg, Henning & Braun, Thomas. (2018). Miniaturized Sample Preparation for Transmission Electron Microscopy. *Journal of Visualized Experiments*.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



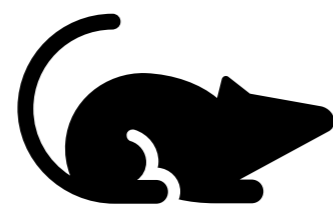
What about thicker samples?



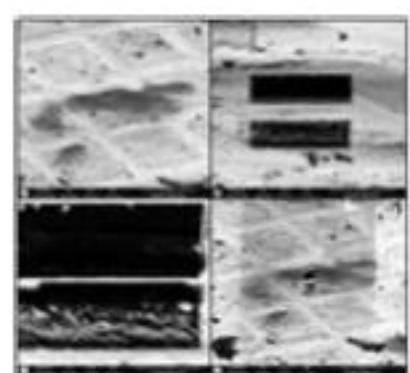
...

# How are samples prepared for cryoEM?

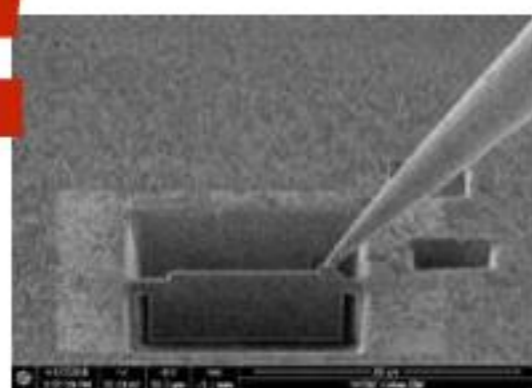
Towards Automation for  
In Situ CryoEM



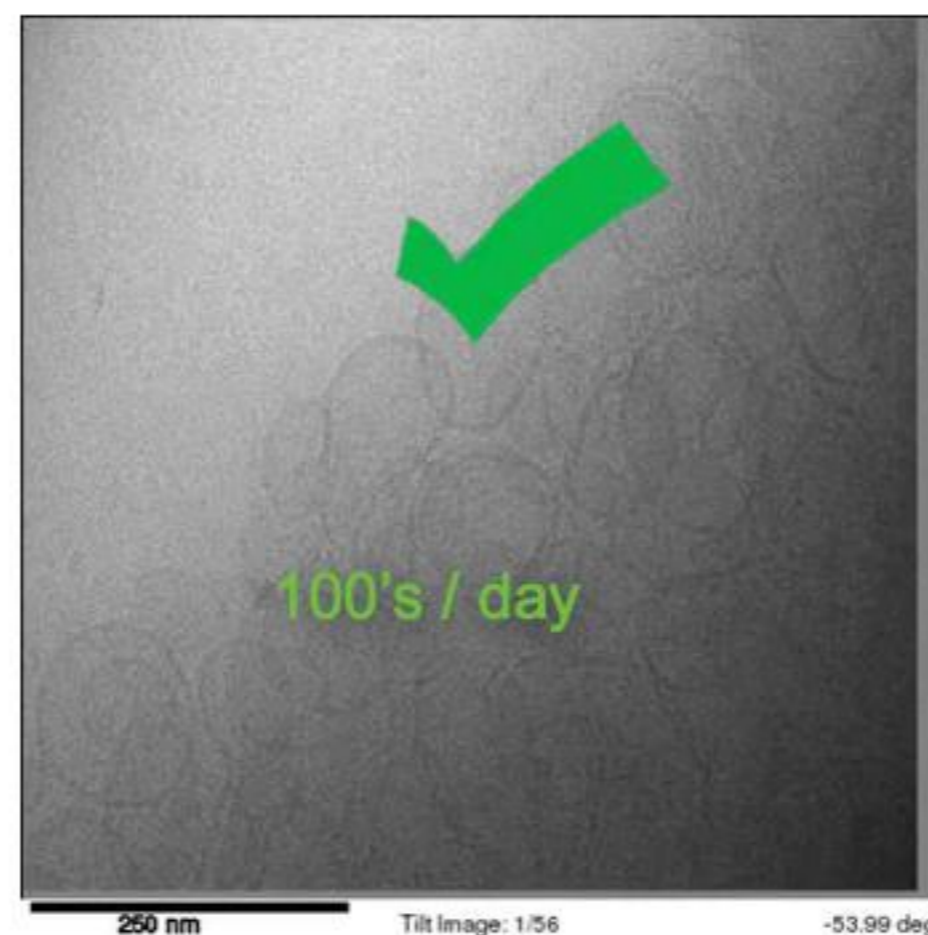
Sample



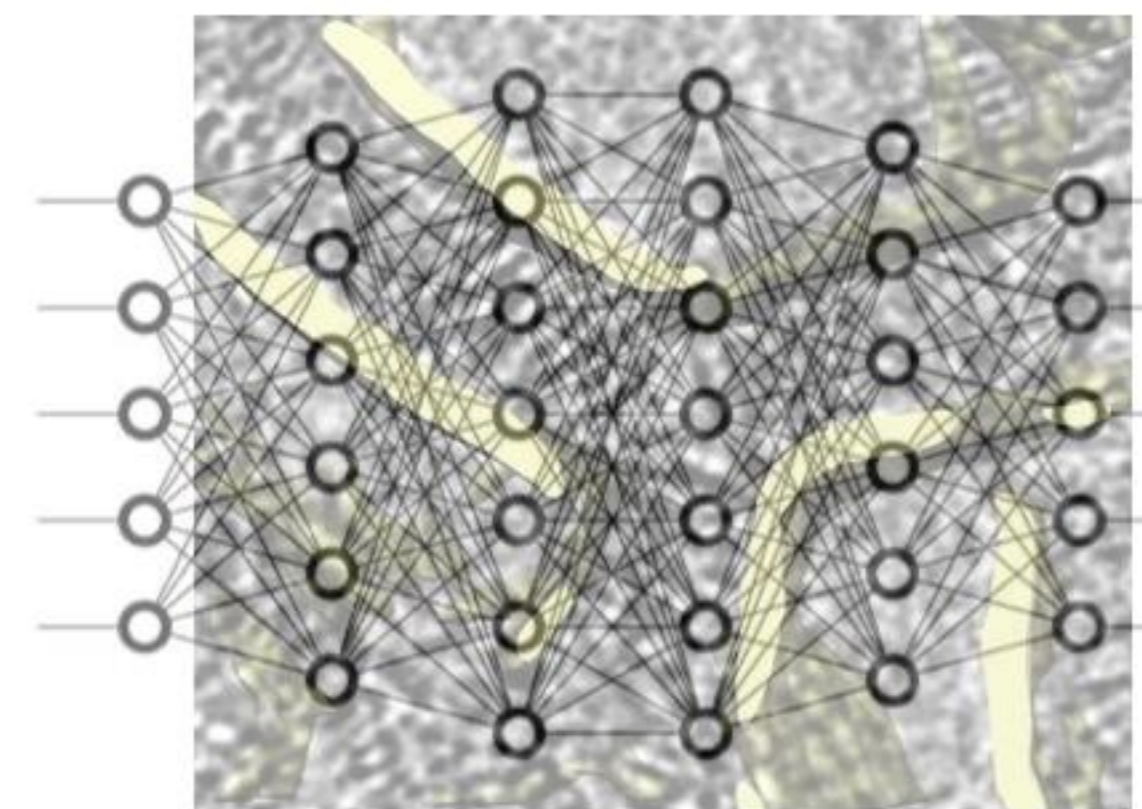
Milling  
Grid preparation



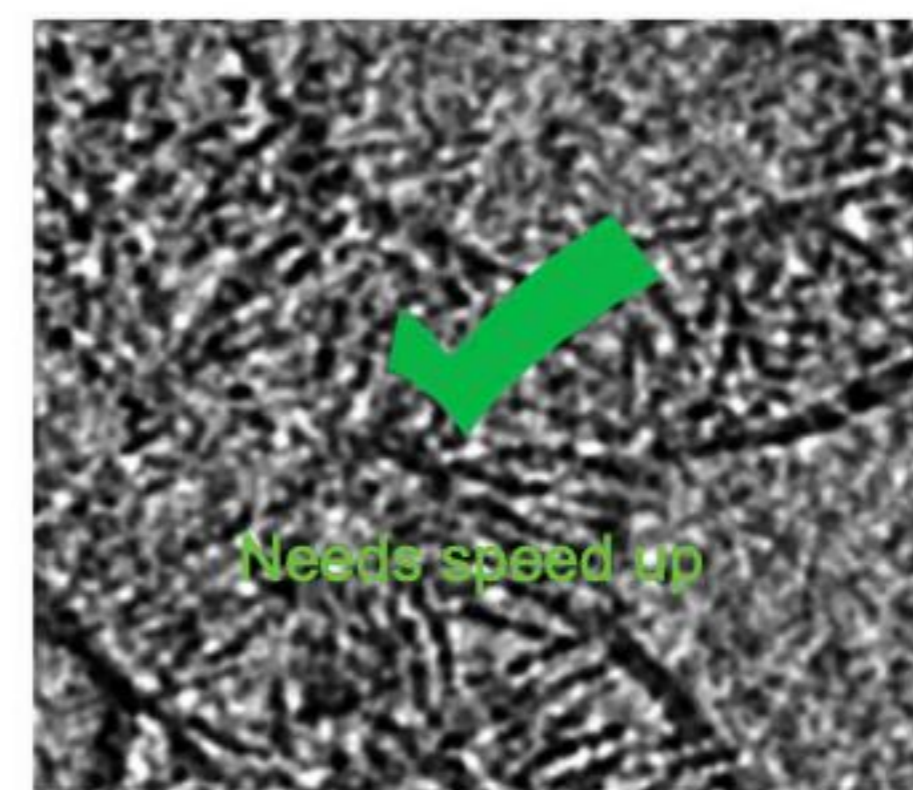
Lift out



Automated Data Collection  
(Leginon, etc.)



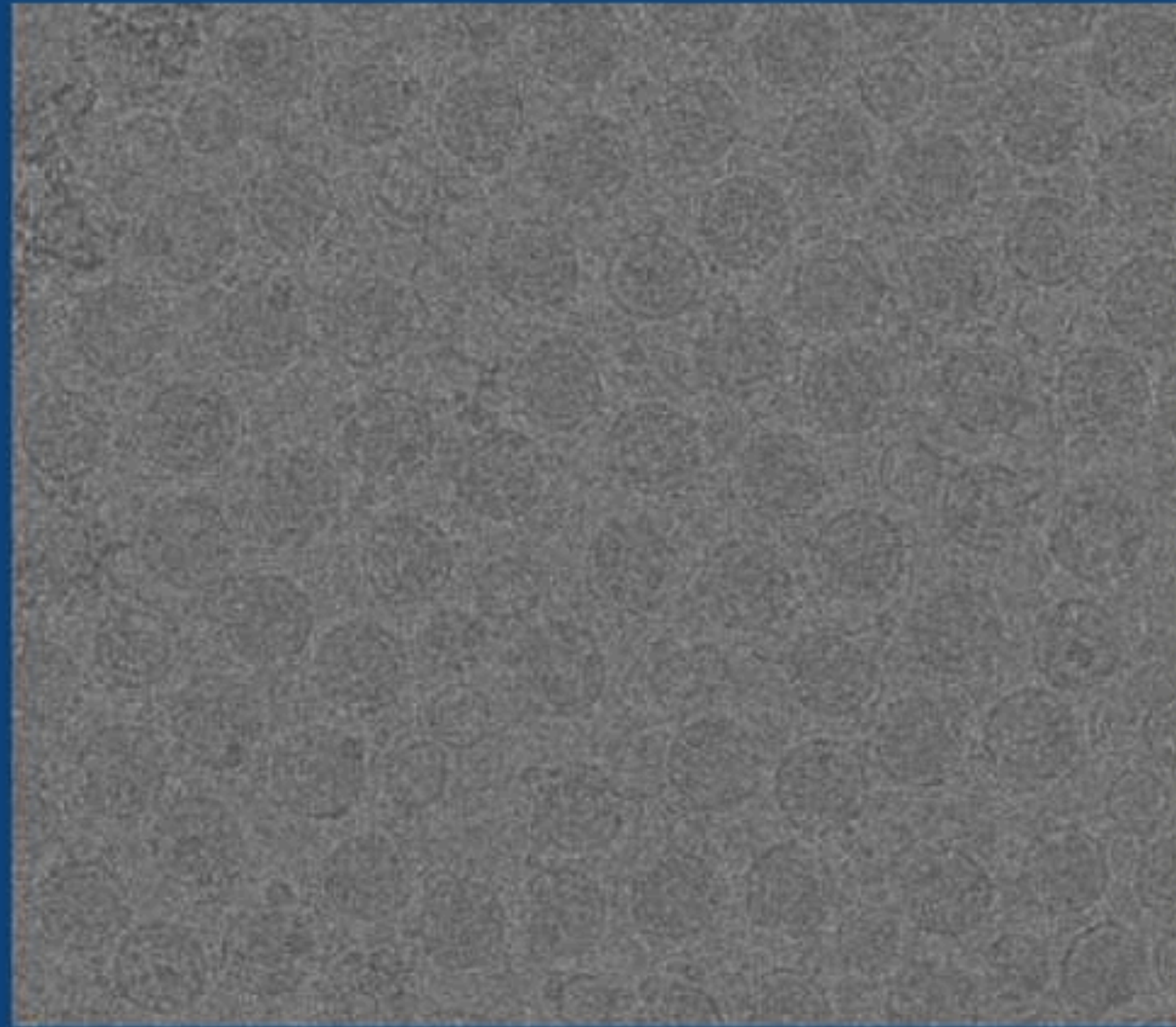
Deep learning?



Streamlined Processing  
(Appion Protomo)

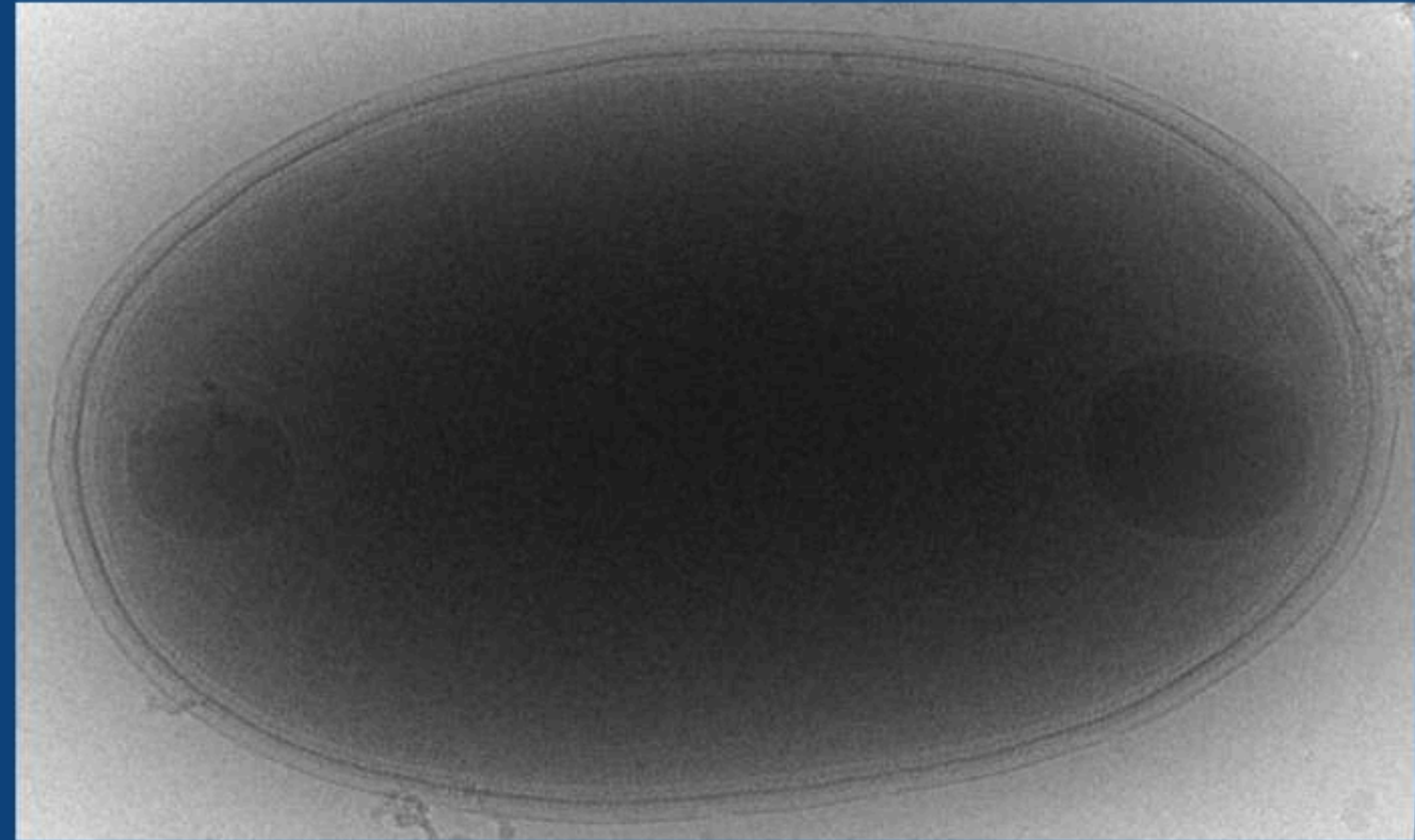
# How are samples prepared for cryoEM?

HOW THIN DOES THE SAMPLE NEED TO BE?



50 nm

Bacteriophage ( $\phi$ 12)

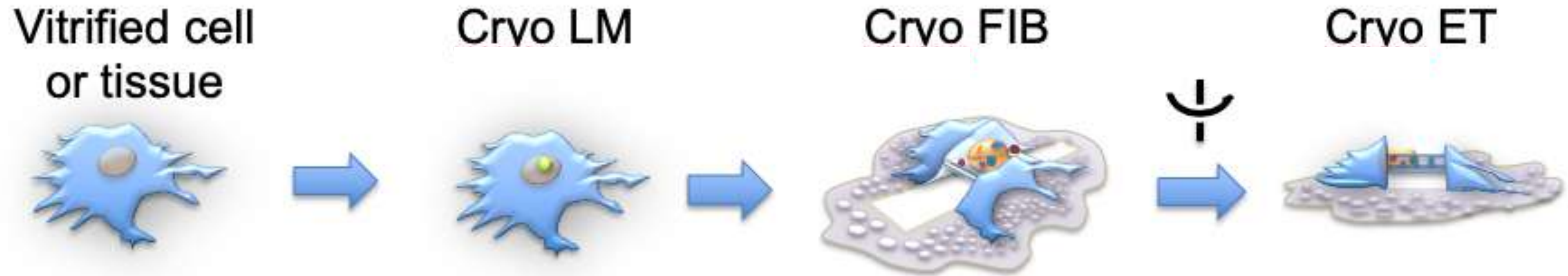


750 nm thick

E. coli, Salmonella, Cyanobacteria

# How are samples prepared for cryoEM?

## CLEM workflow



# How are samples prepared for cryoEM?

## STEP 1: Vitrify sample

### Plunge Freezing

- Rapid freezing in liquid nitrogen (LN2)-cooled liquid ethane



Leica Microsystems

### High Pressure Freezing (HPF)

- Rapid freezing at LN2 temps and high pressure



# How are samples prepared for cryoEM?

## STEP 1: Vitrify sample

**Sample:** cells (adherent, suspension)

**SEMC Equipment:** Leica EM-GP, Gatan CP3, manual plunger

**Bottlenecks:** sample concentration, sample buffer, preferred orientation of sample on grid, plunge freezing parameters, grid mesh, support film, warming up, ice thickness, vitrification

### Takeaways

- Vitrify up to ~10 um of sample
- One-sided blotting

# How are samples prepared for cryoEM?

## STEP 1: Vitrify sample

**Sample:** cells, tissue

**SEMC Equipment:** Wohlwend HPF Compact 01

**Bottlenecks:** sample amount, sample concentration, sample buffer, ice thickness, warming up, pressure, vitrification



# How are samples prepared for cryoEM?

## STEP 2: Cryo-LM

### Takeaways

- Do I have cells? Where?
- Is my target fluorescing?

**Sample:** vitrified cells on bare or clipped grid

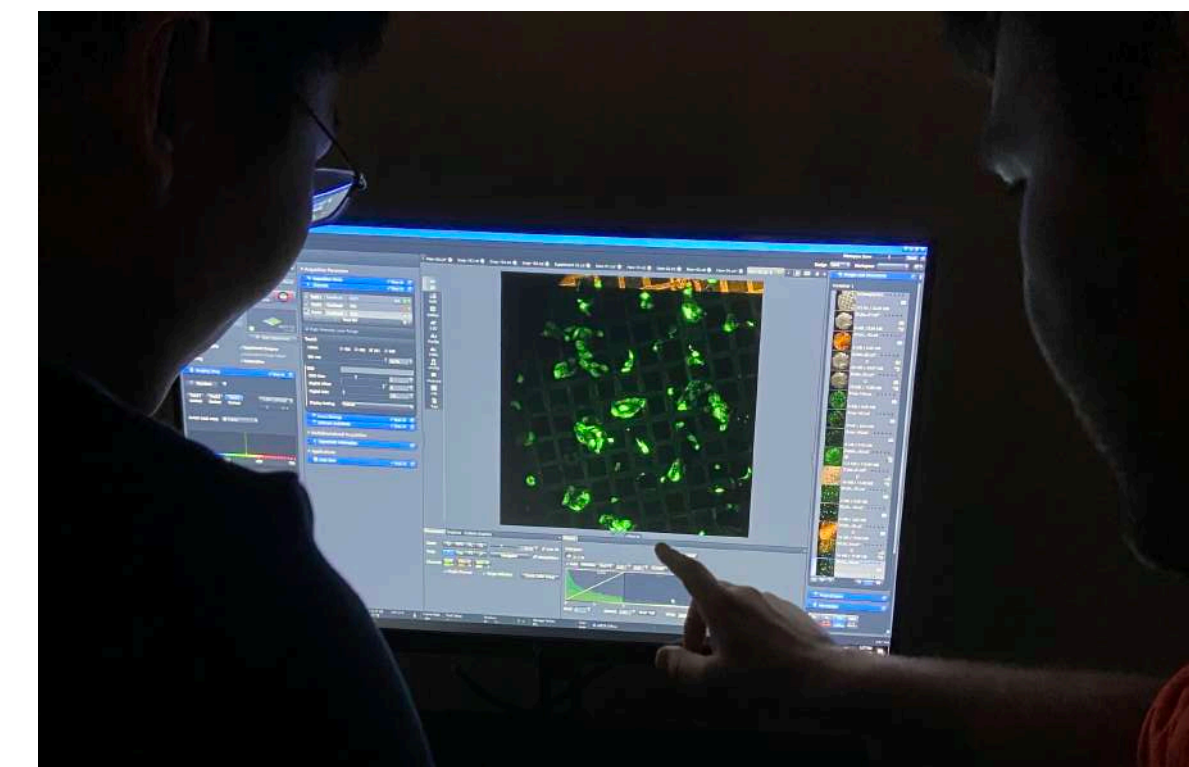
**SEMC Equipment:** Zeiss LSM 900 with Airyscan with Linkam Cryostage



Zeiss



**Bottlenecks:** sample concentration, sample location, support film, laser damage, ice thickness, autofluorescence, warming up



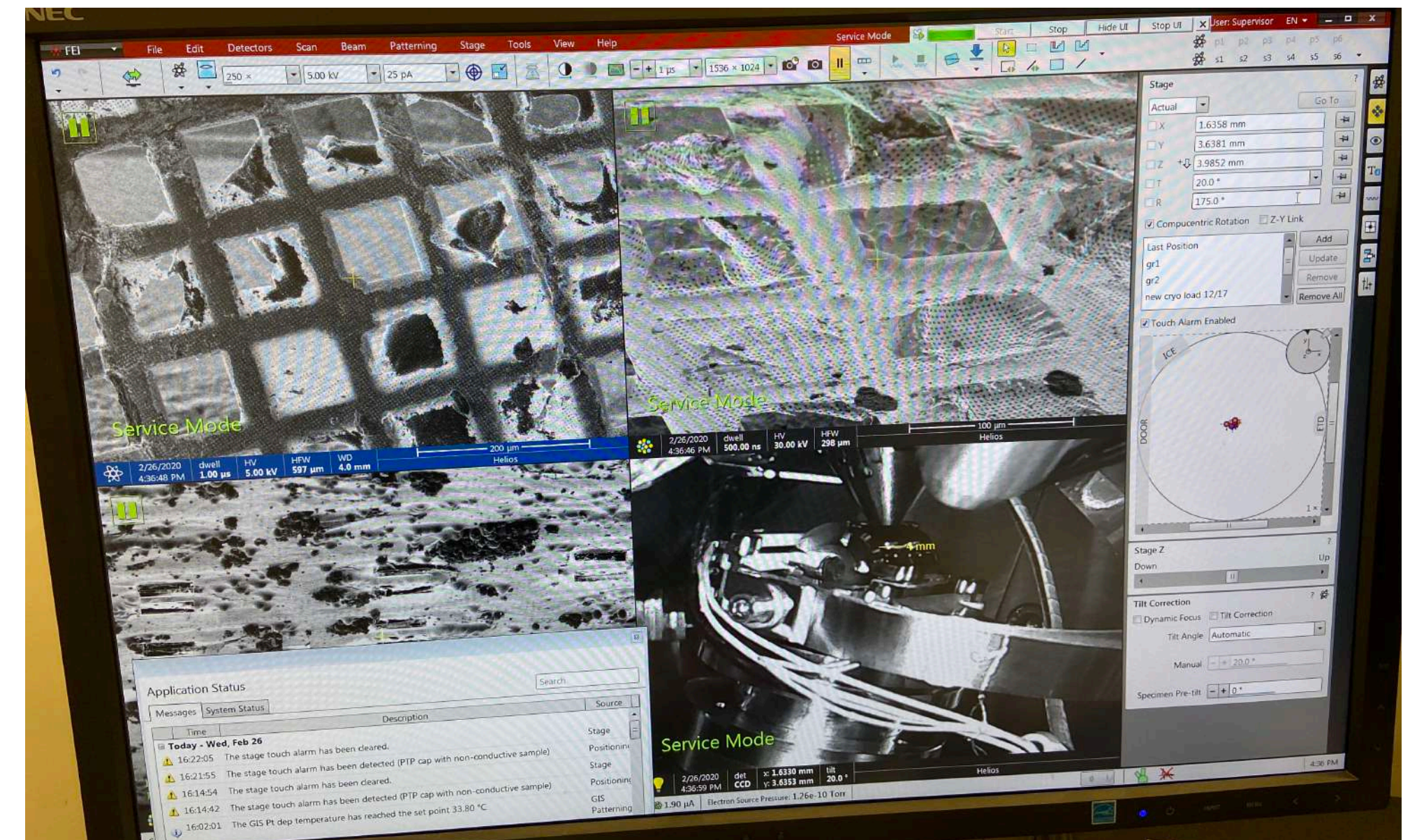
# How are samples prepared for cryoEM?

## STEP 3: Cryo-FIB milling

**Sample:** vitrified cells on a clipped grid

**SEMC Equipment:** Helios Nanolab G3 FIB-SEM, Leica VCT, VCM, & ACE

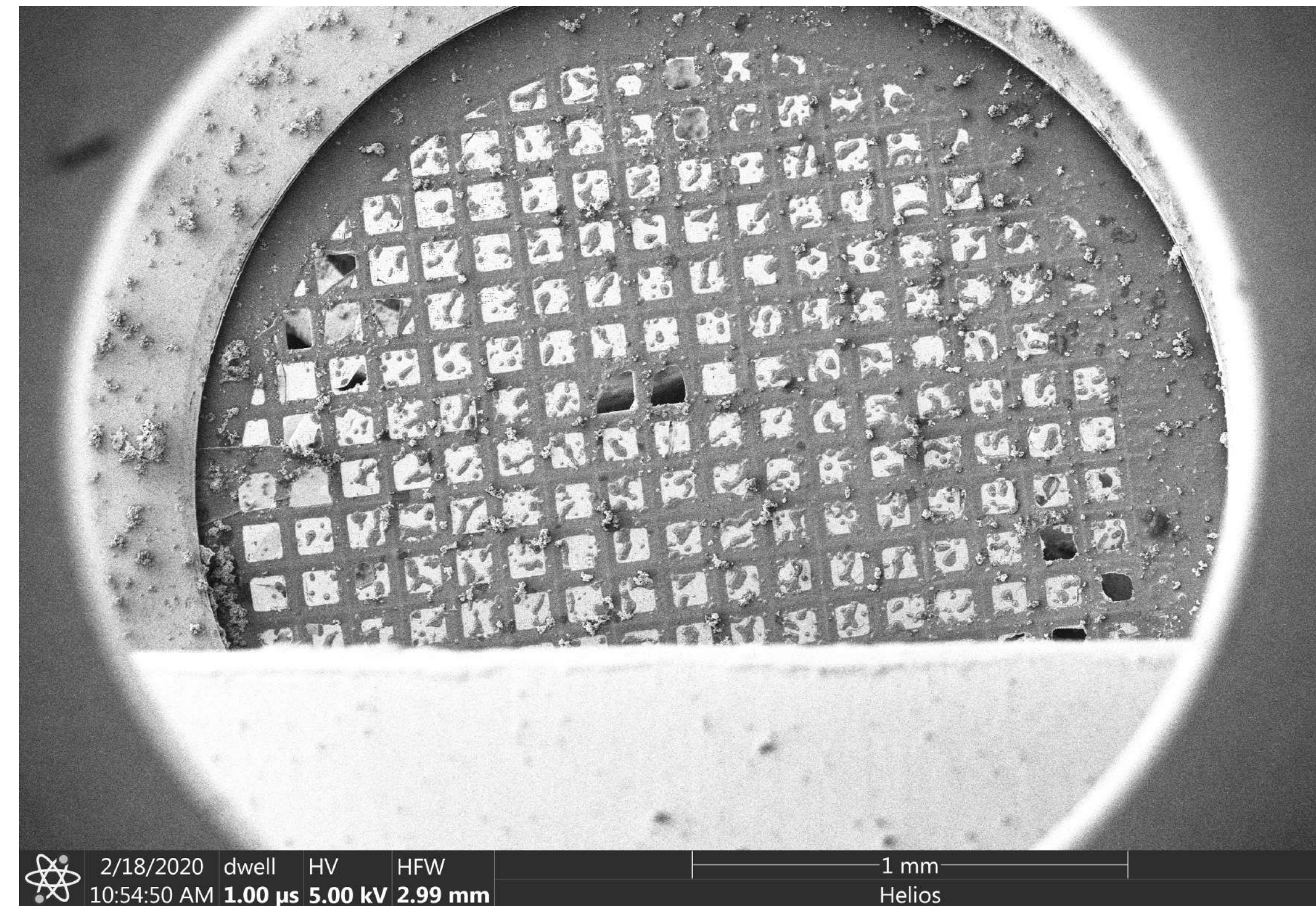
**Bottlenecks:** sample concentration, preferred orientation, imaging & milling parameters, grid mesh, support film, grid orientation, beam damage, charging, pole touches, dropping holder, image correlation, warming up, catching fire, ...



# How are samples prepared for cryoEM?

## STEP 3: Cryo-FIB milling

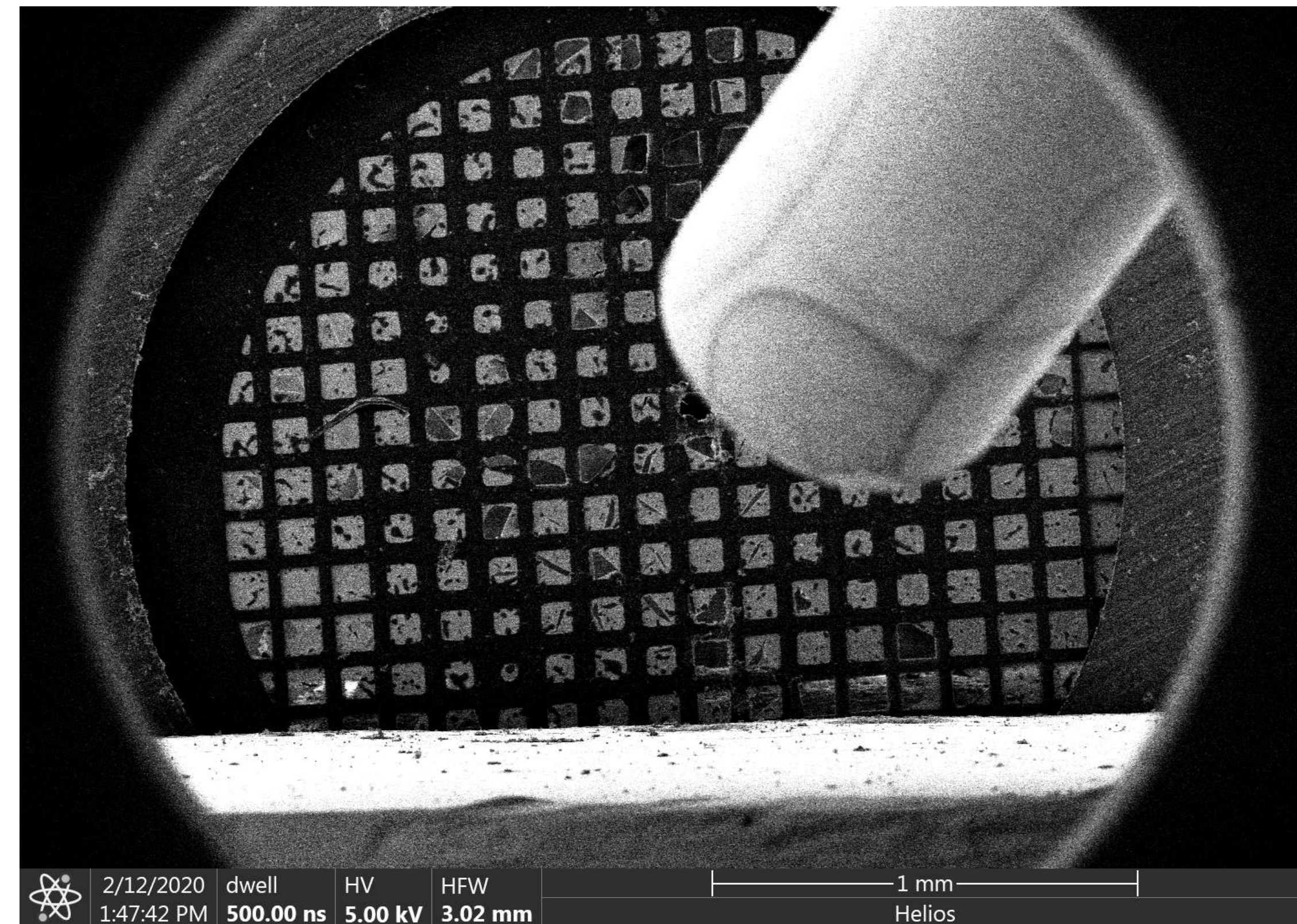
- 1) Grid overview/atlas images with electron and ion beam
- 2) GIS Platinum coat for 5 seconds
- 3) Mill large rectangular trenches on either side of each lamella site
- 4) Continue thinning lamella by milling on each side until  $<300$  nm thickness



# How are samples prepared for cryoEM?

## STEP 3: Cryo-FIB milling

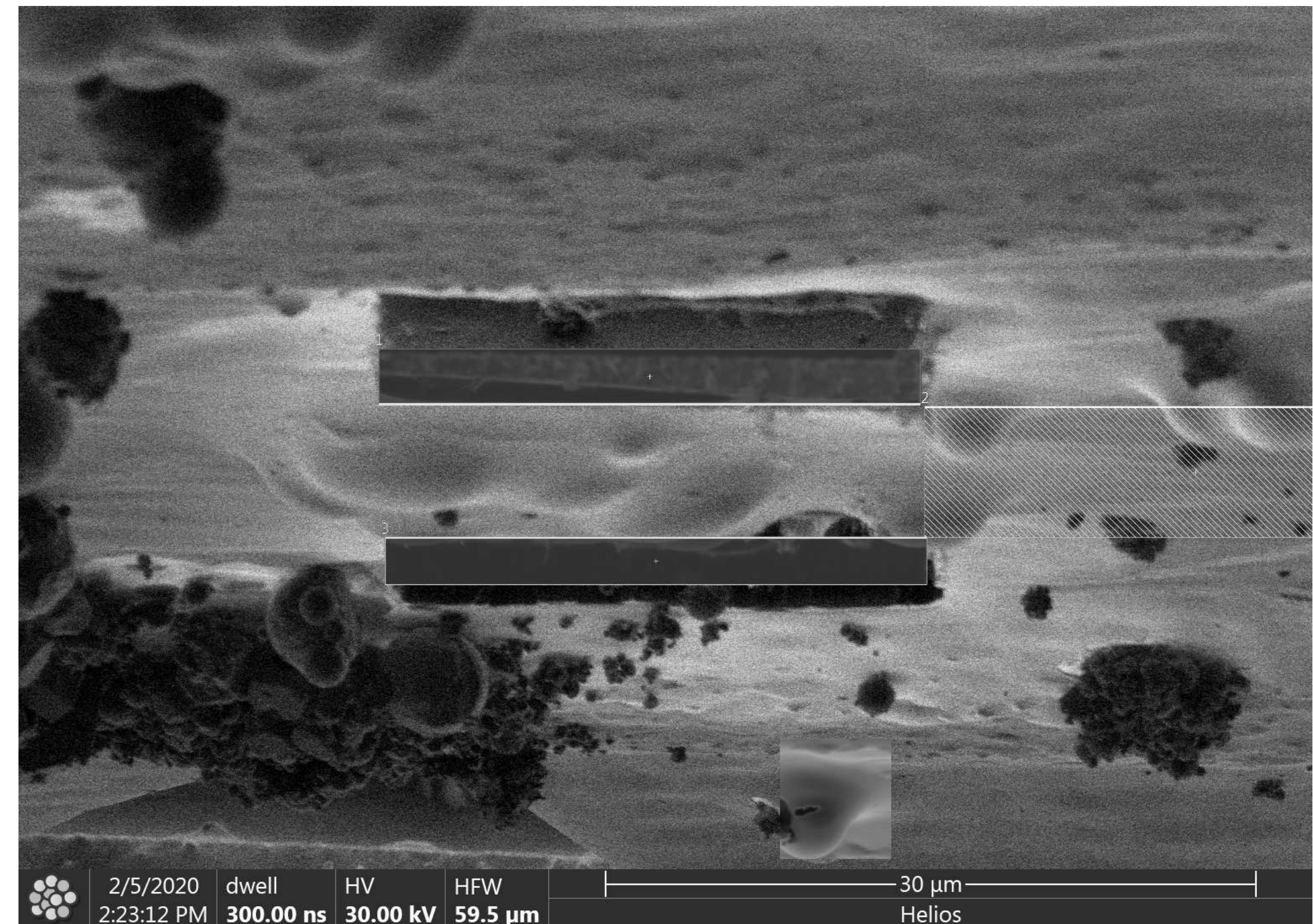
- 1) Grid overview/atlas images with electron and ion beam
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# How are samples prepared for cryoEM?

## STEP 3: Cryo-FIB milling

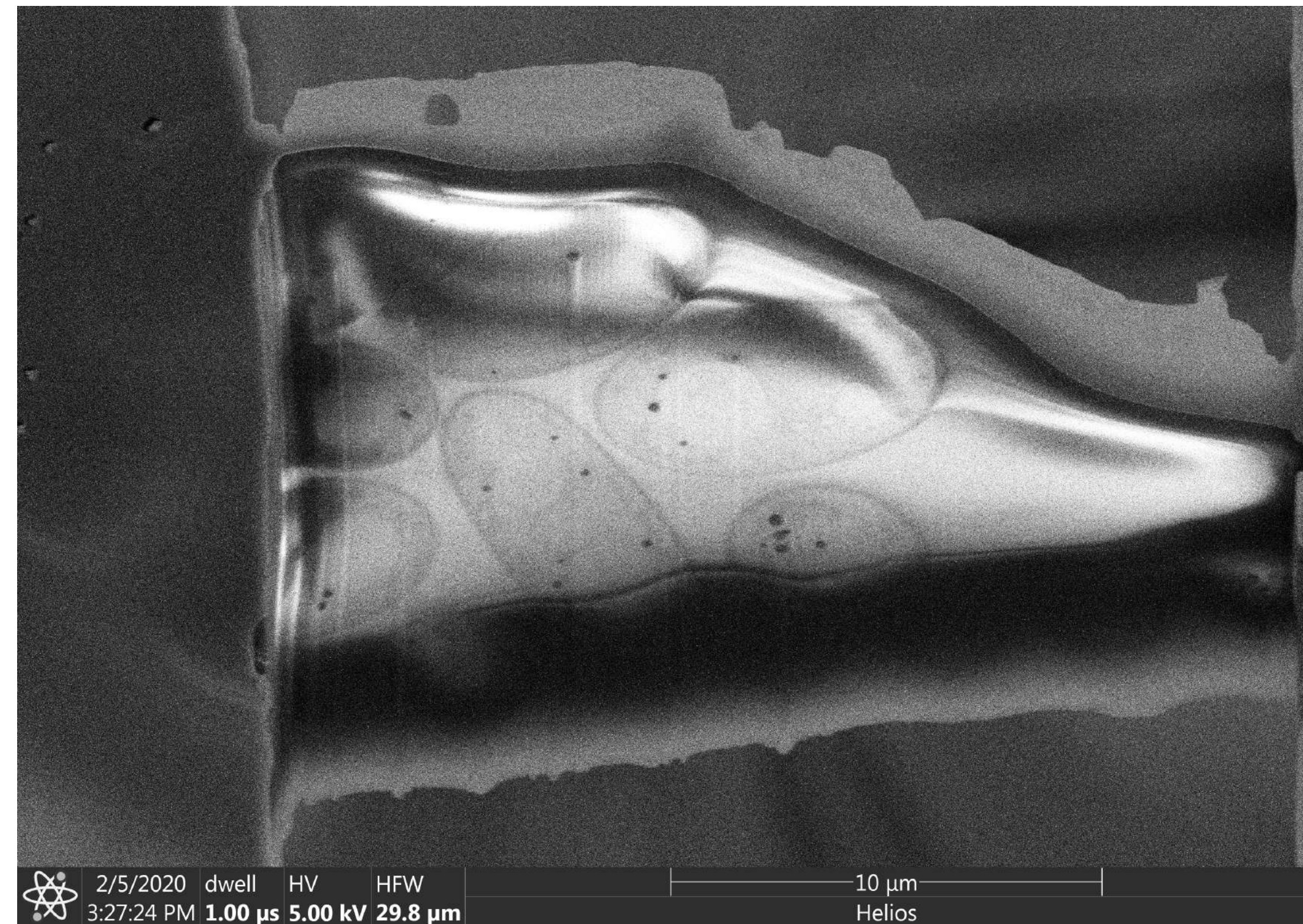
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# How are samples prepared for cryoEM?

## STEP 3: Cryo-FIB milling

- 1) Grid overview/atlas images with electron and ion beam
- 2) GIS Platinum coat for 5 seconds
- 3) Mill large rectangular trenches on either side of each lamella site
- 4) Continue thinning lamella by milling on each side until  $<300$  nm thickness

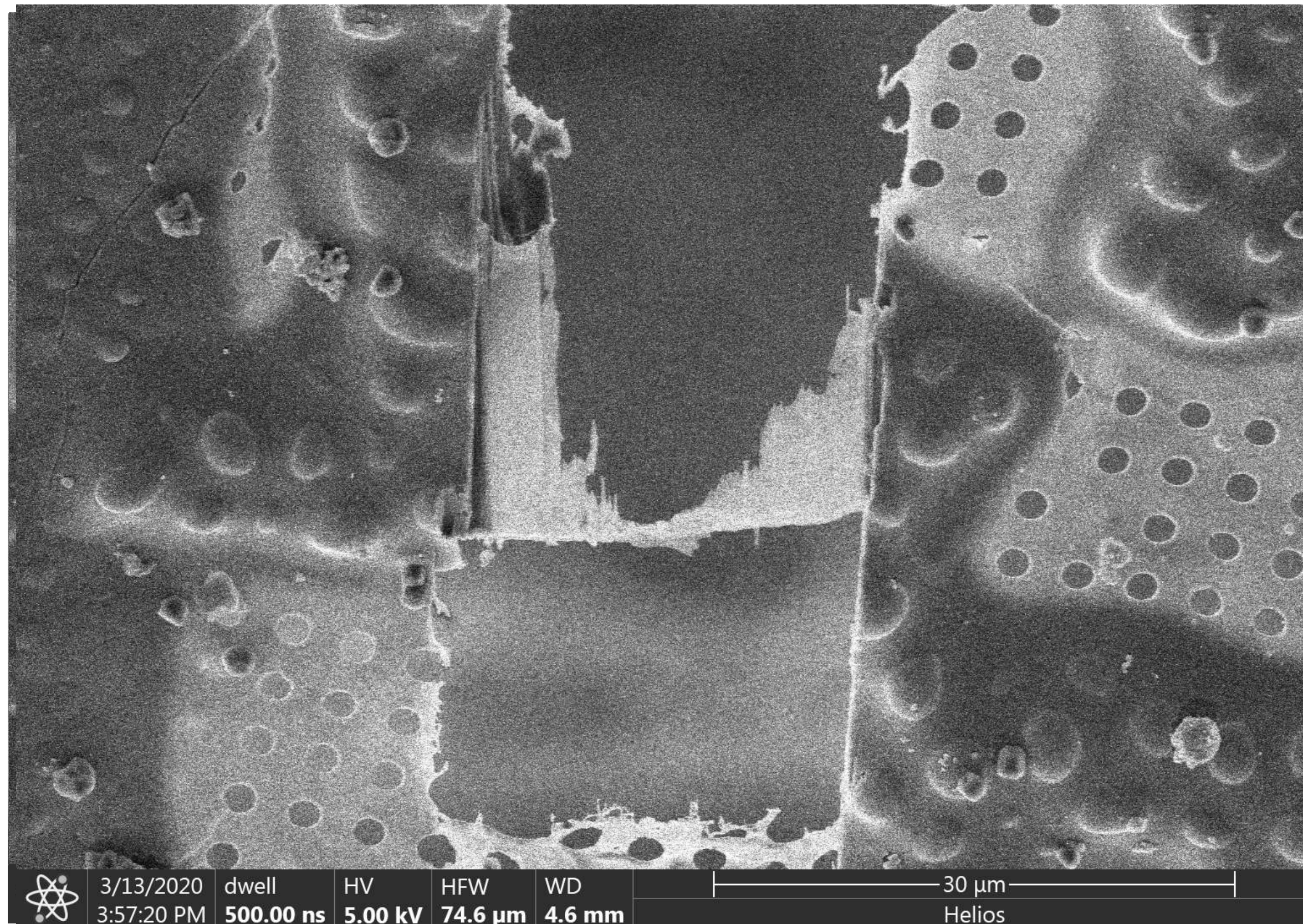


# How are samples prepared for cryoEM?

## STEP 3: Cryo-FIB milling

### Takeaways

- Large learning curve if done manually
- Many, many steps that all need to be completed successfully for experiment to work



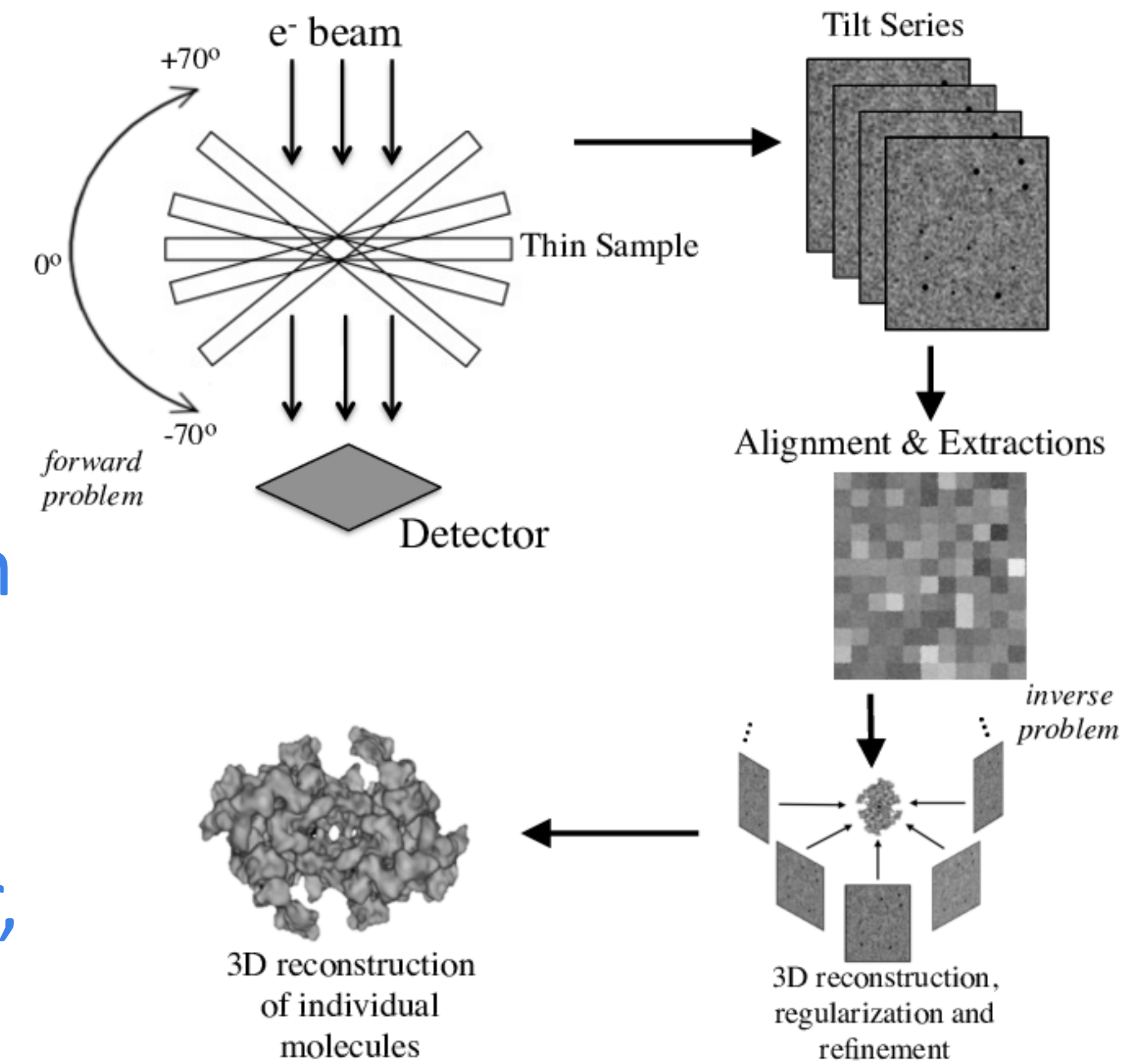
# How are samples prepared for cryoEM?

## STEP 4: Cryo-ET

**Sample:** whole cells or lamella on clipped grid

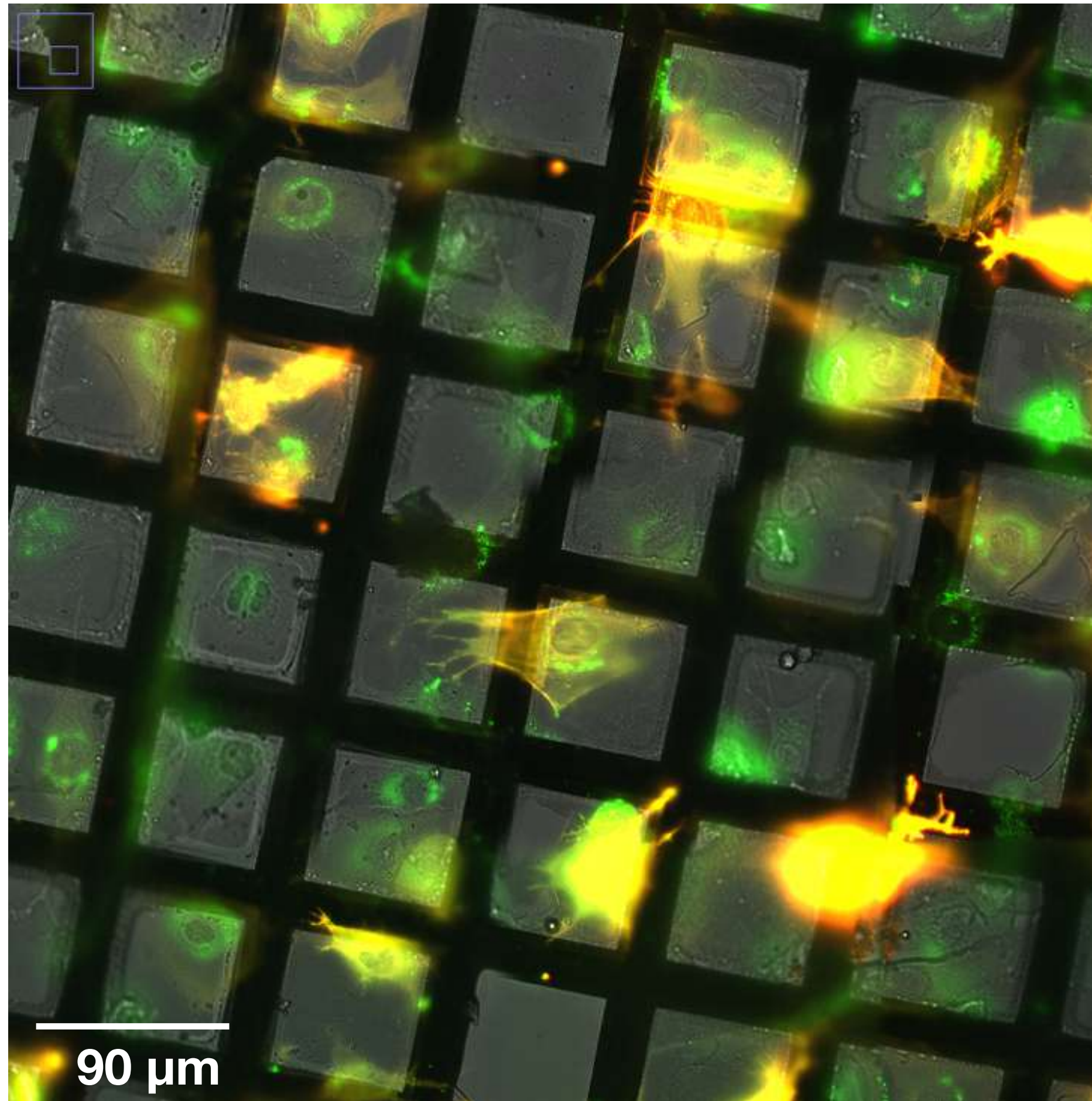
**SEMC Equipment:** TFS Titan Krios 300kV TEM with Energy filter + direct detector

**Bottlenecks:** sample concentration, sample buffer, preferred orientation, grid mesh, grid orientation, support film, warming up, ice thickness, vitrification, stage stability, beam damage, contamination, ...



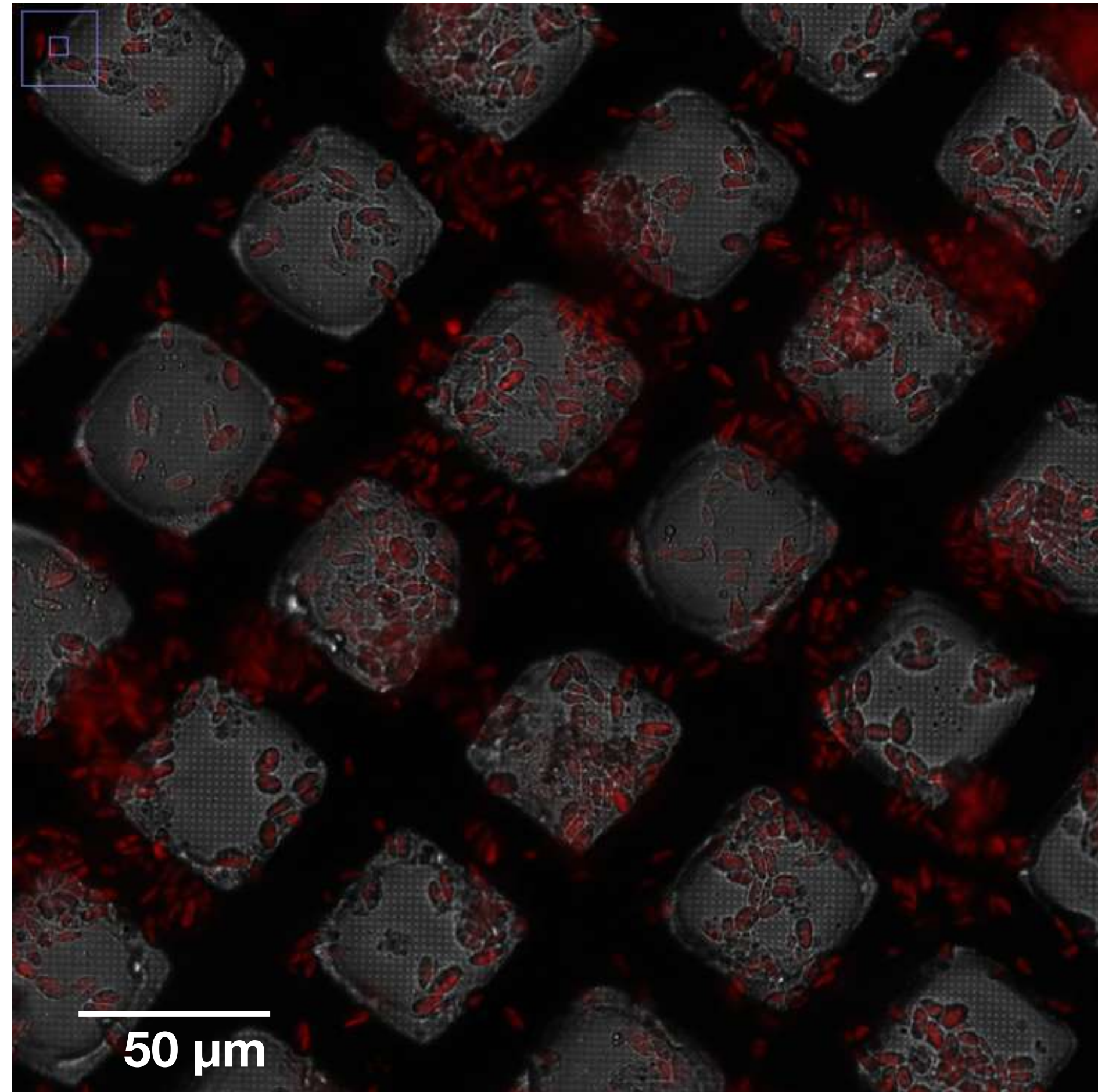
# How are samples prepared for cryoEM?

## Mouse fibroblasts



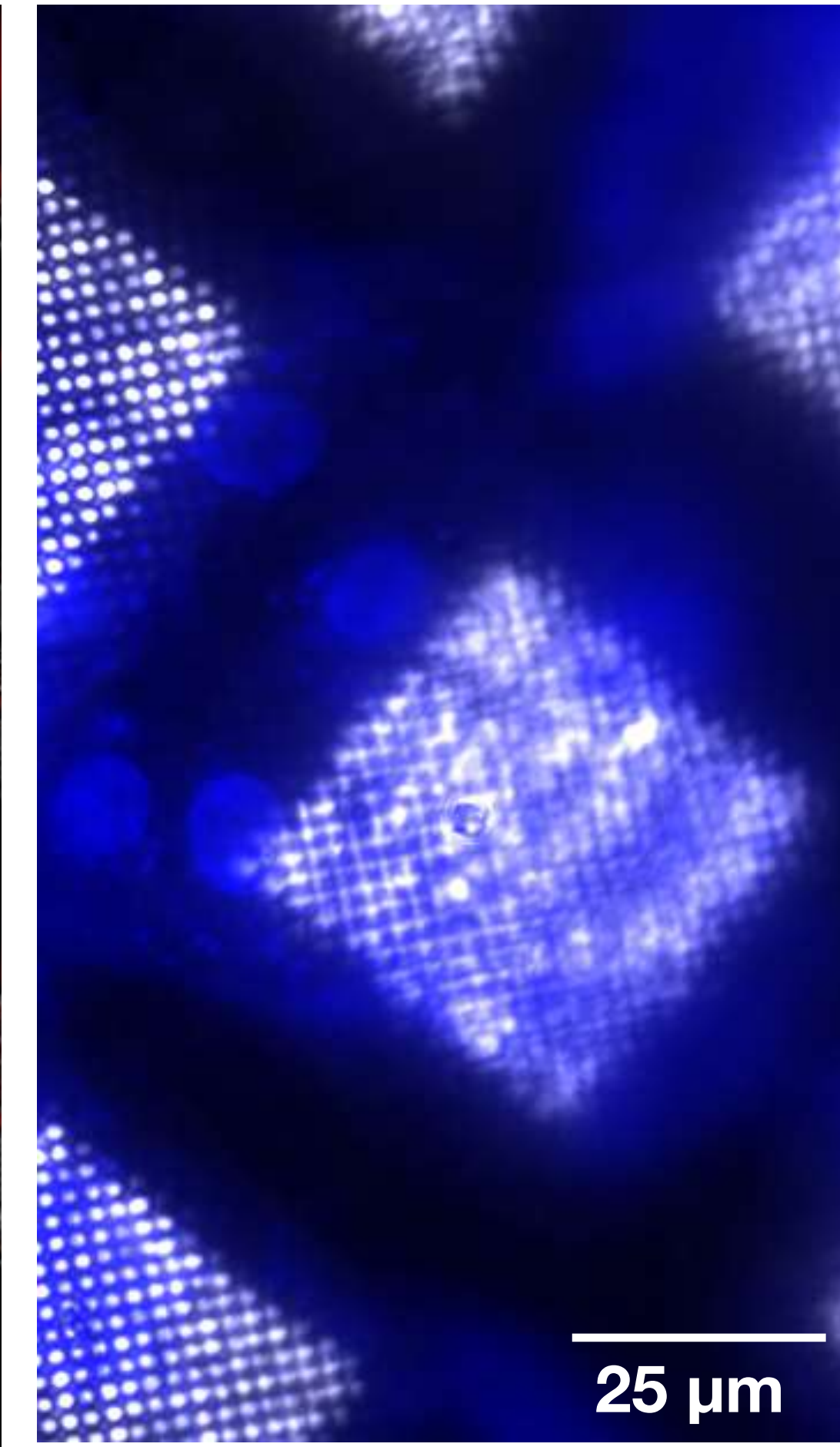
Transfected adhesion signaling protein tagged with GFP (green) and F-actin marker with mApple (red).  
Greg Alushin (RU)

## Diatoms



Auto-fluorescence.

## Microsporidia



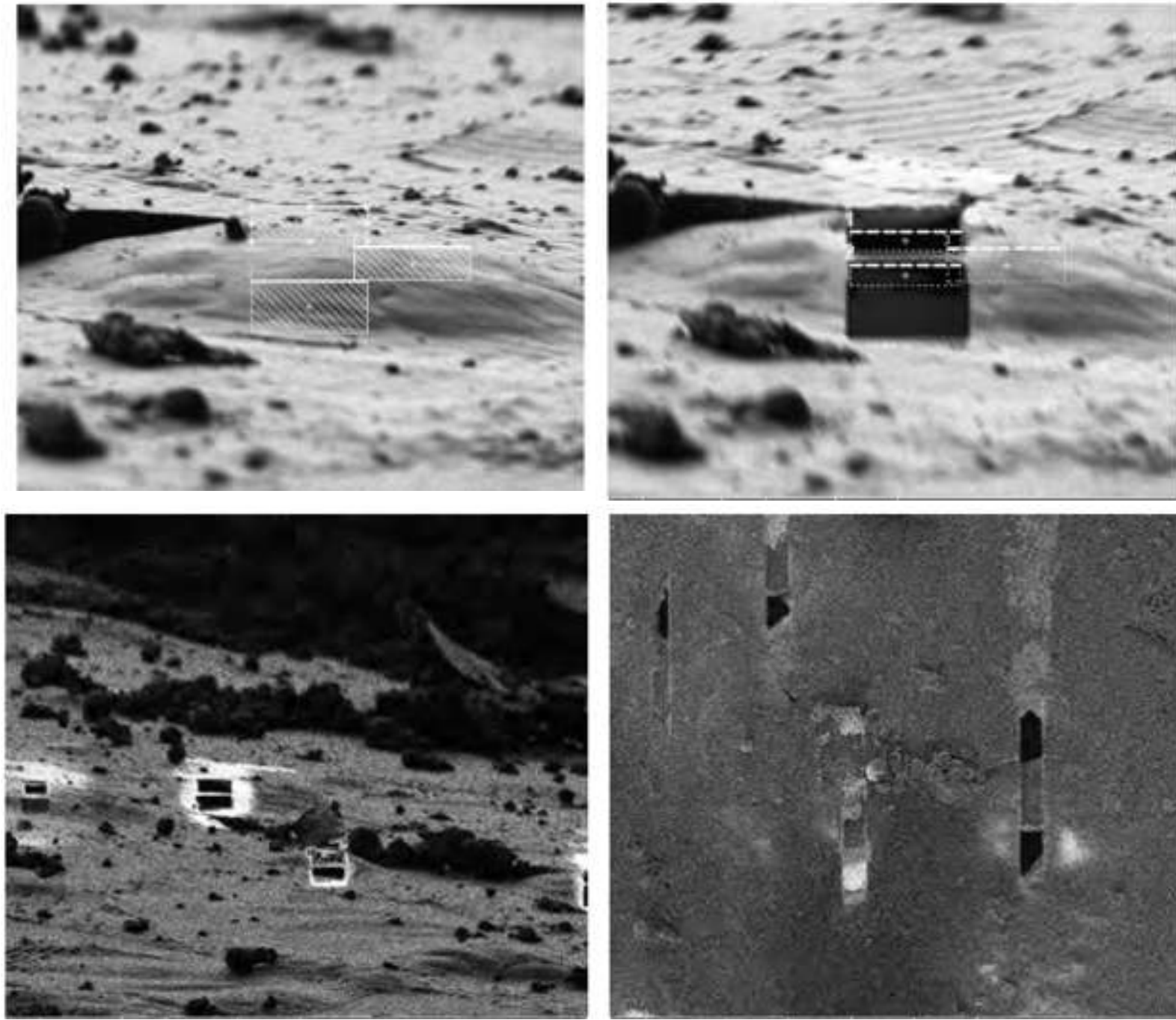
DAPI.

Wei Dai (Rutgers)

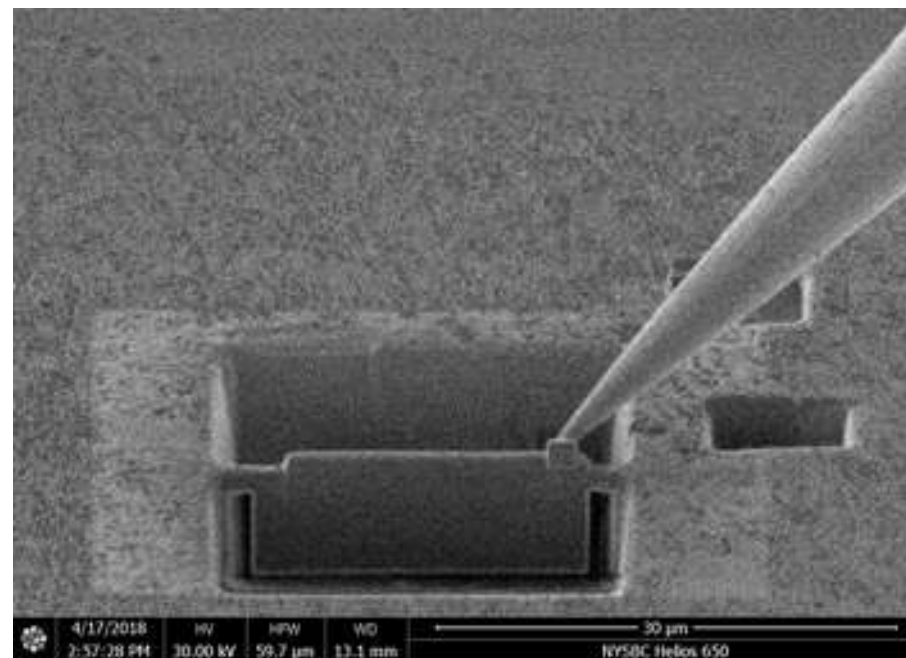
Gira Bhabha (NYU)

# How are samples prepared for cryoEM?

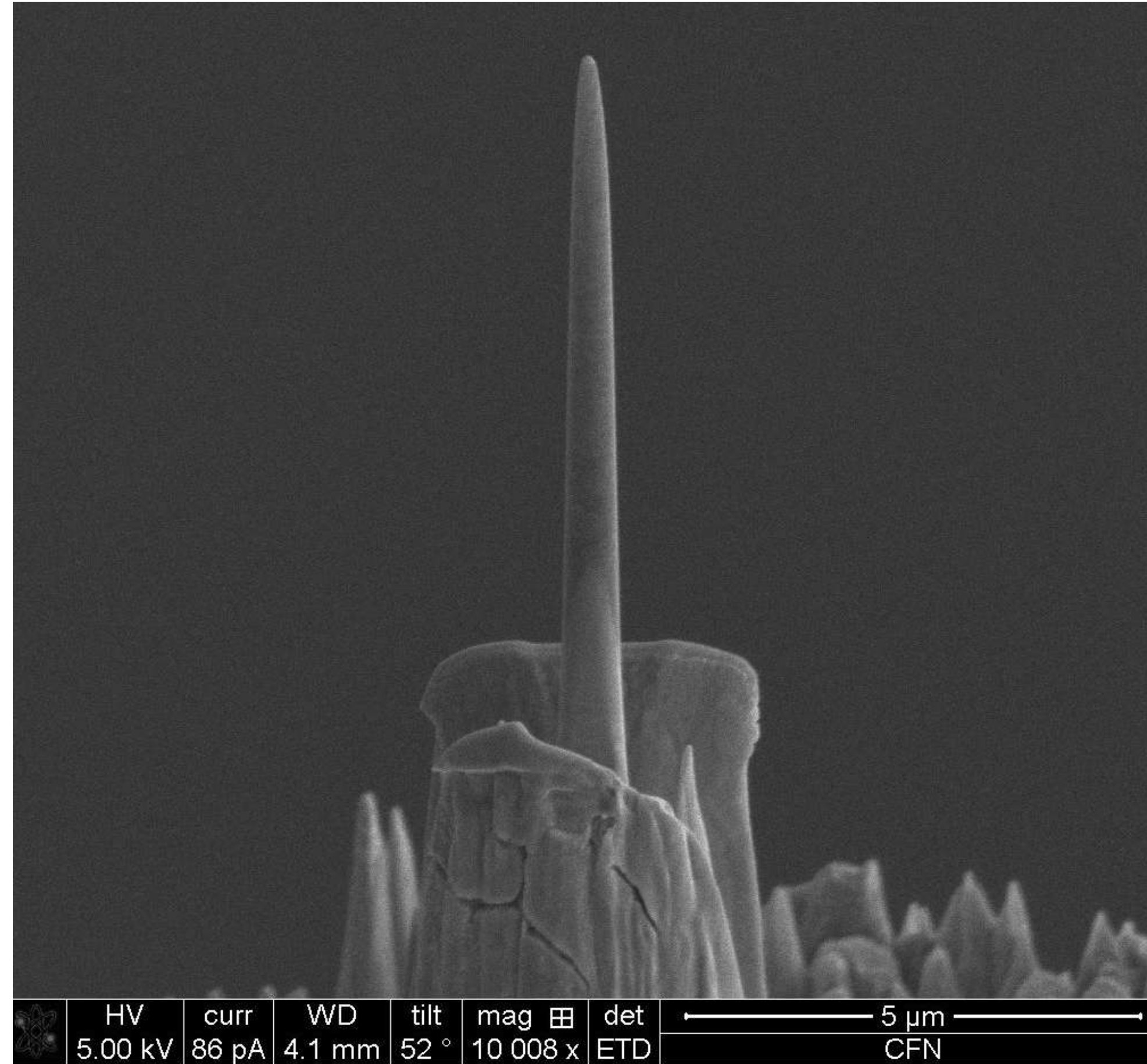
## Lamella



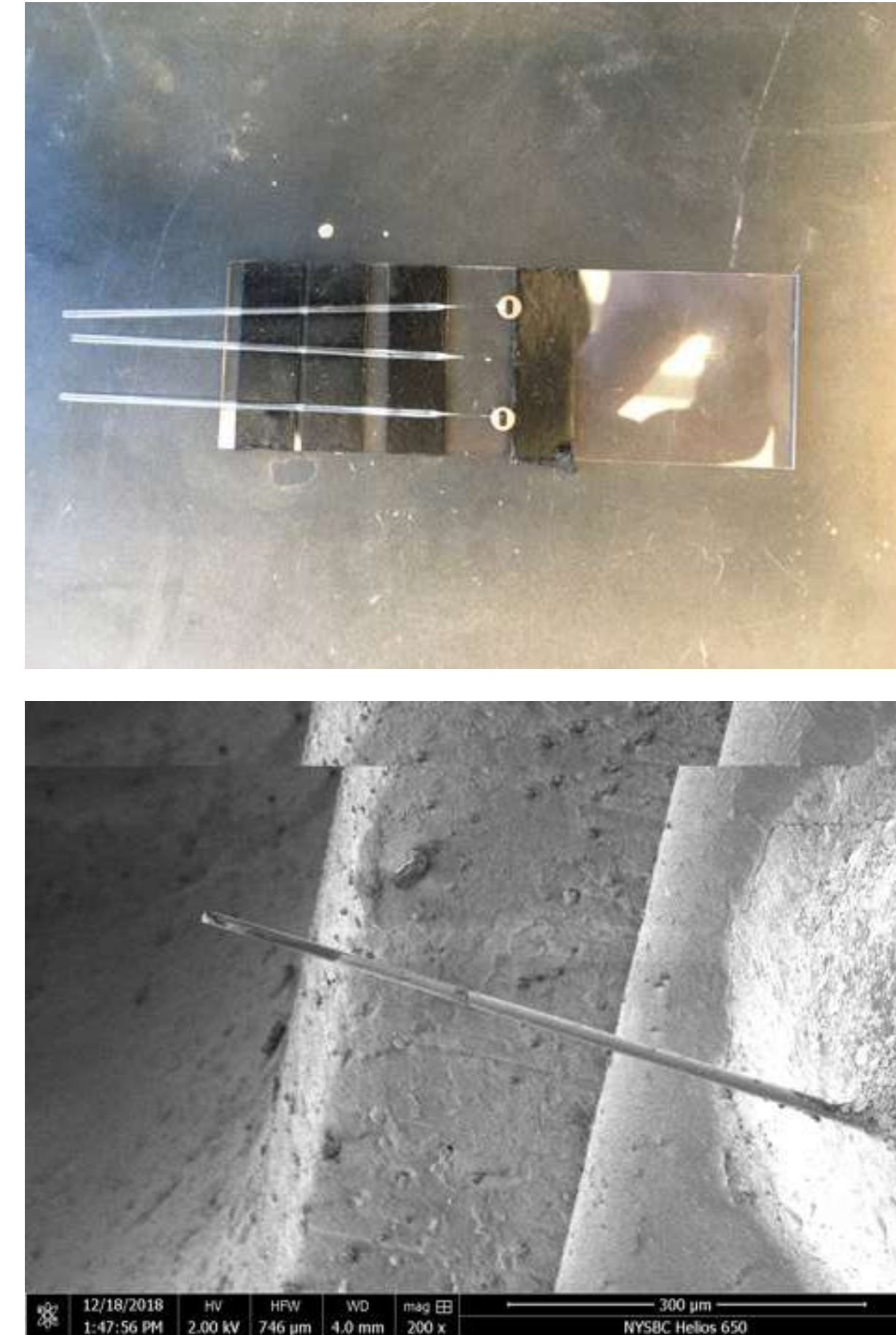
*with  
lift-out*



## Rods

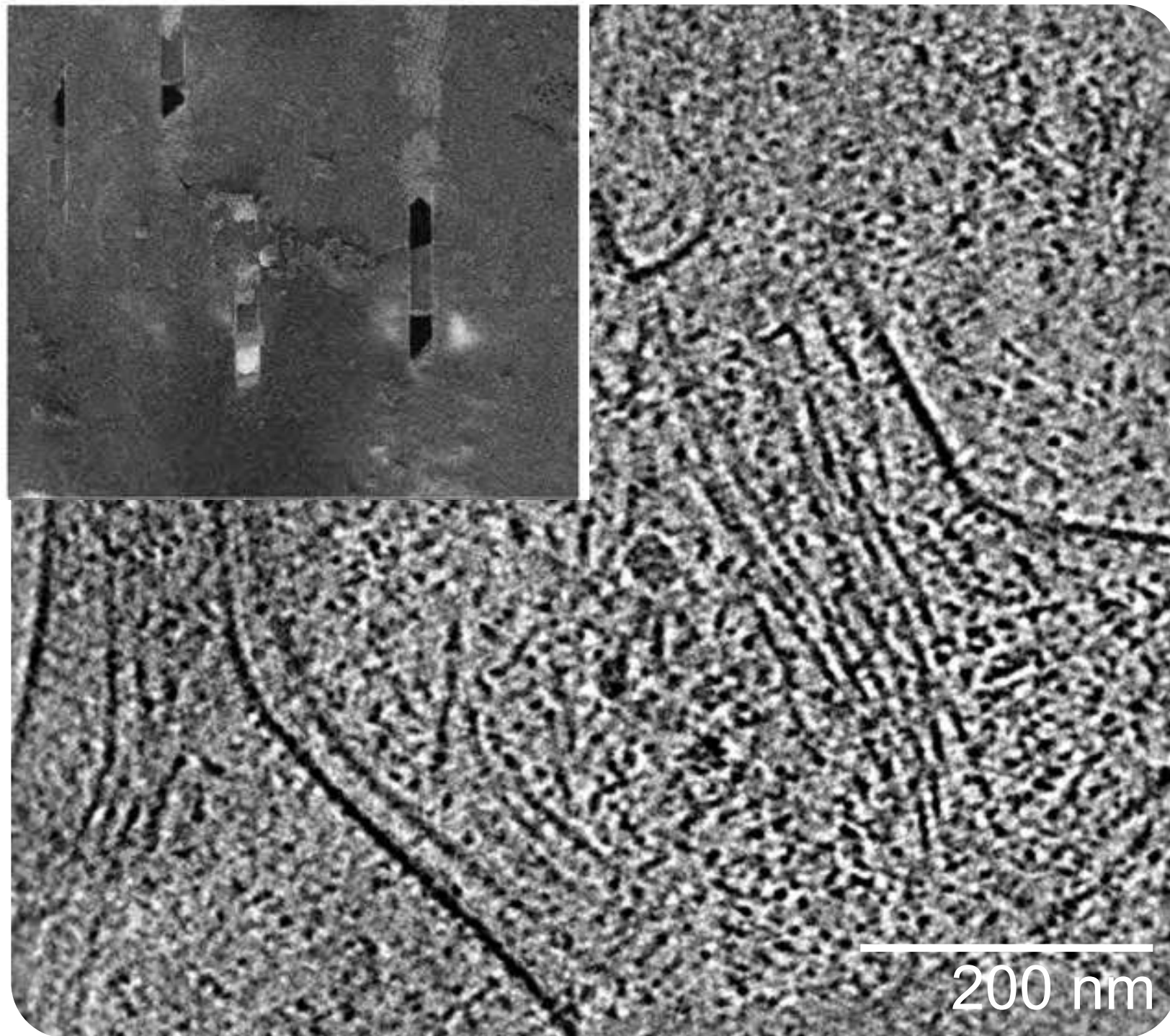


## Capillaries



# How are samples prepared for cryoEM?

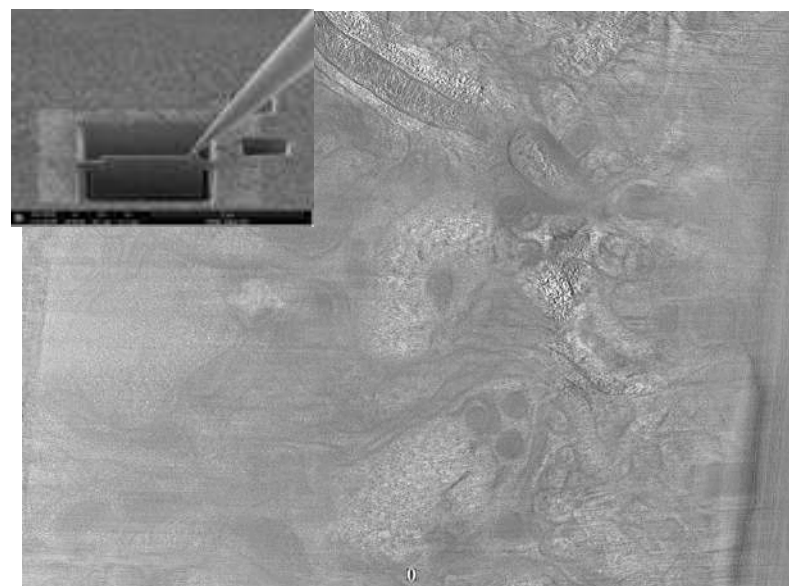
## Lamella



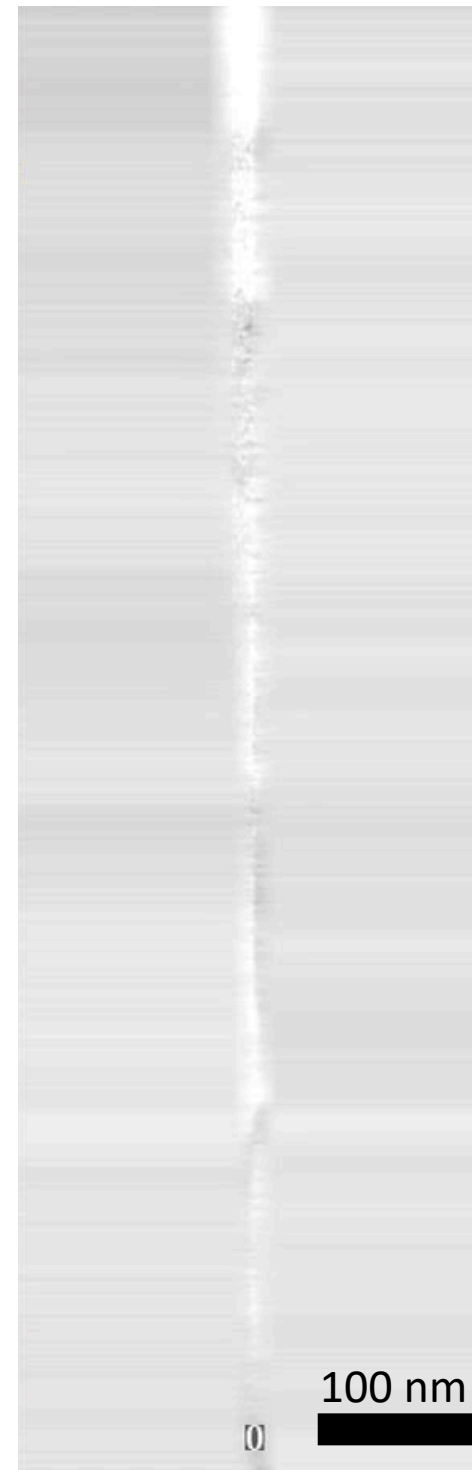
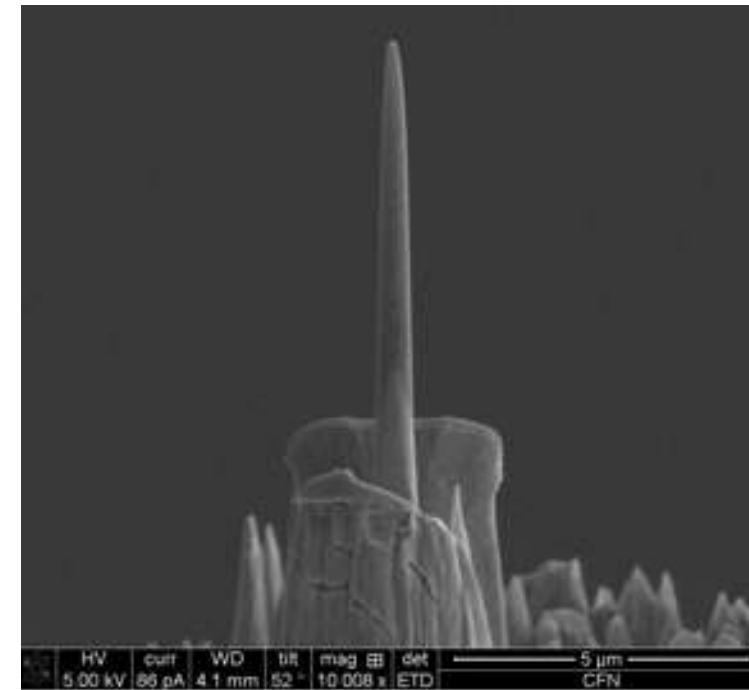
Zach Freyberg,  
(Univ. Pittsburg)

*with  
lift-out*

Kotaro  
Kelley  
(NRAMM)

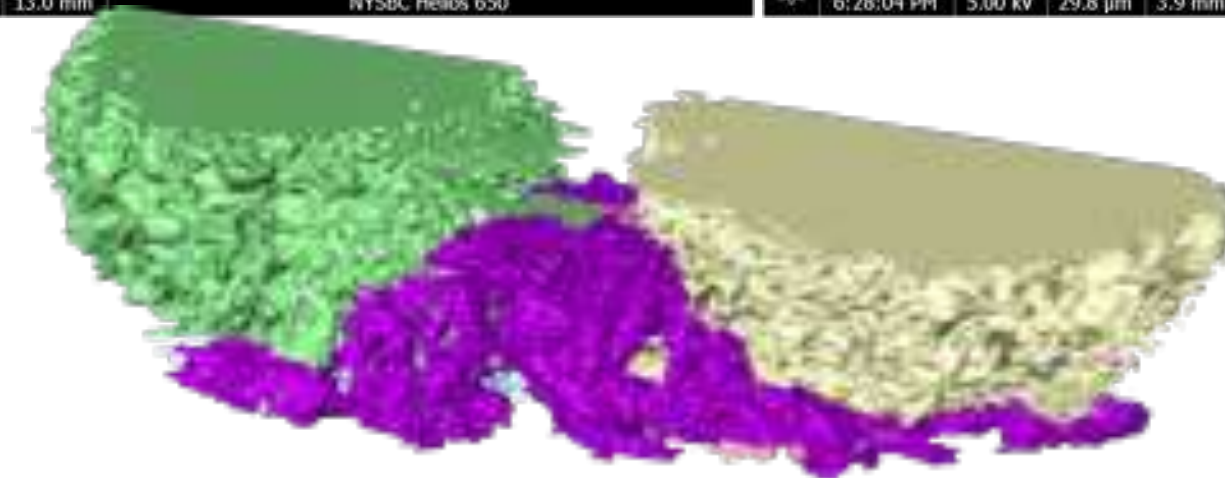
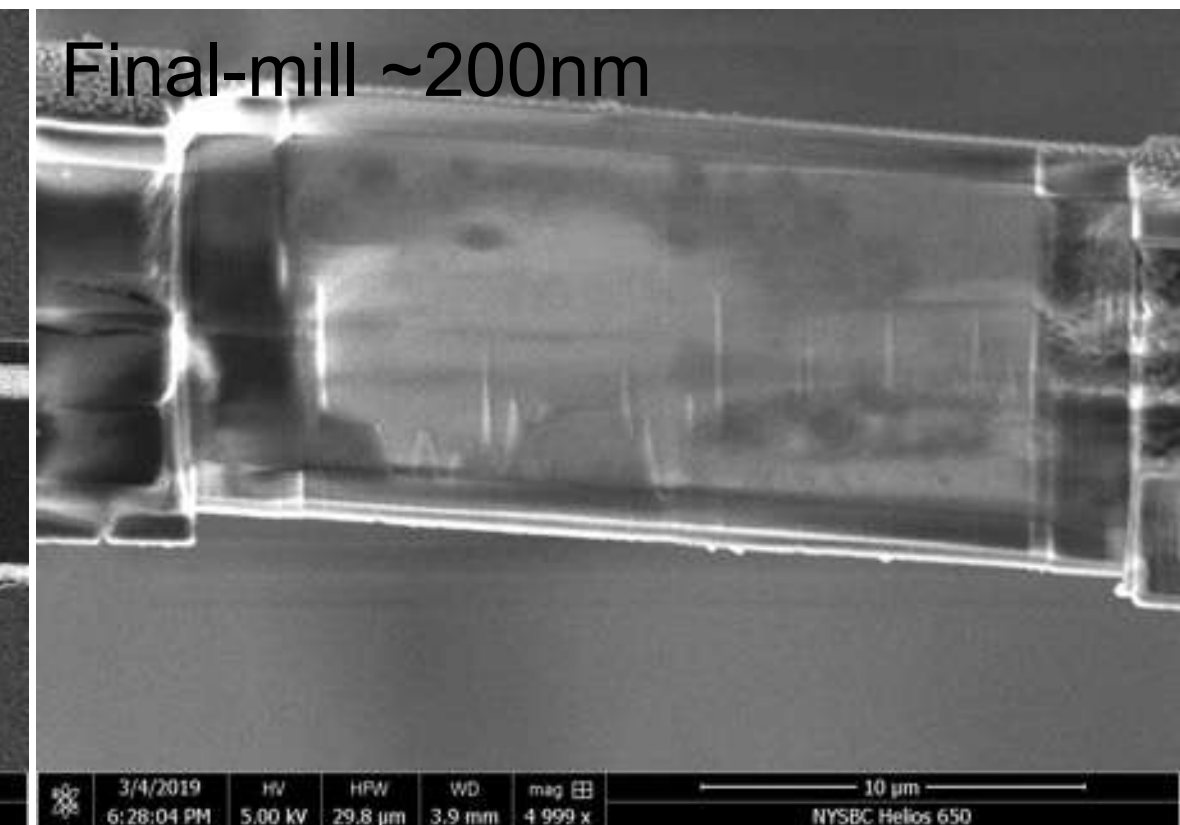
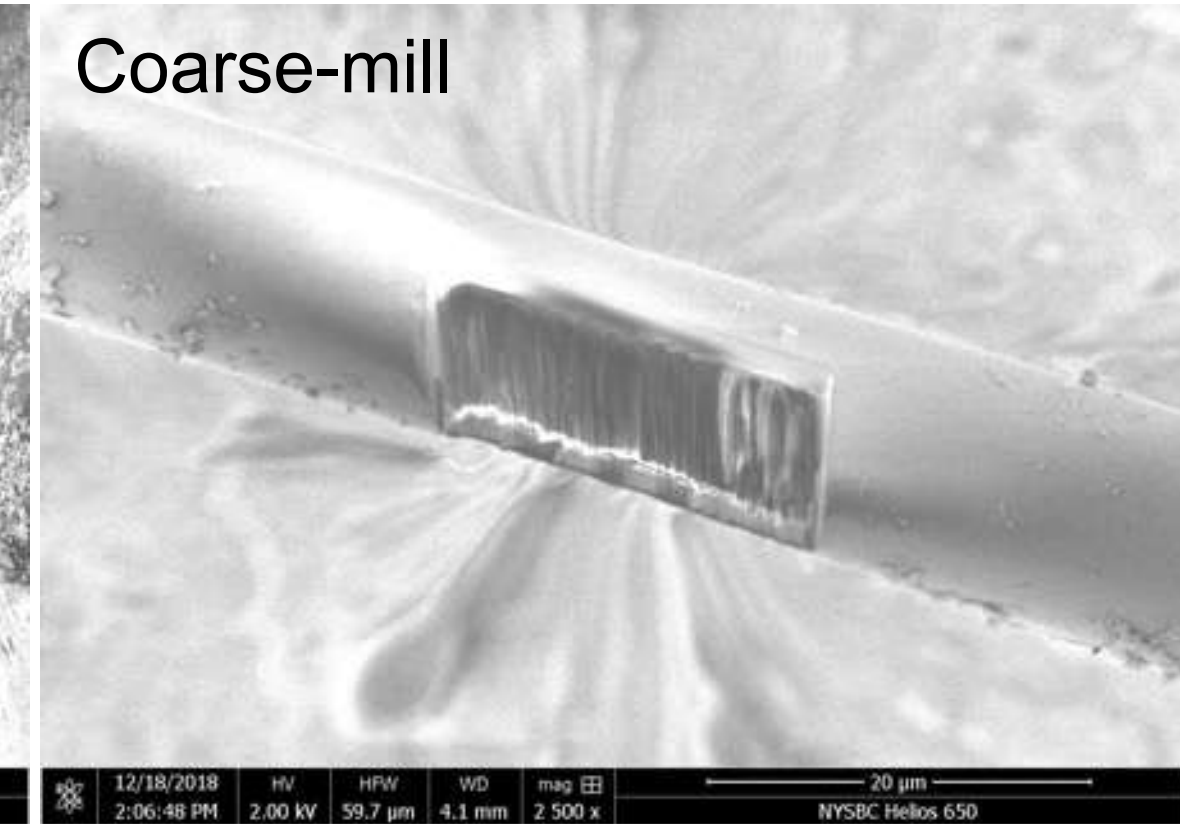
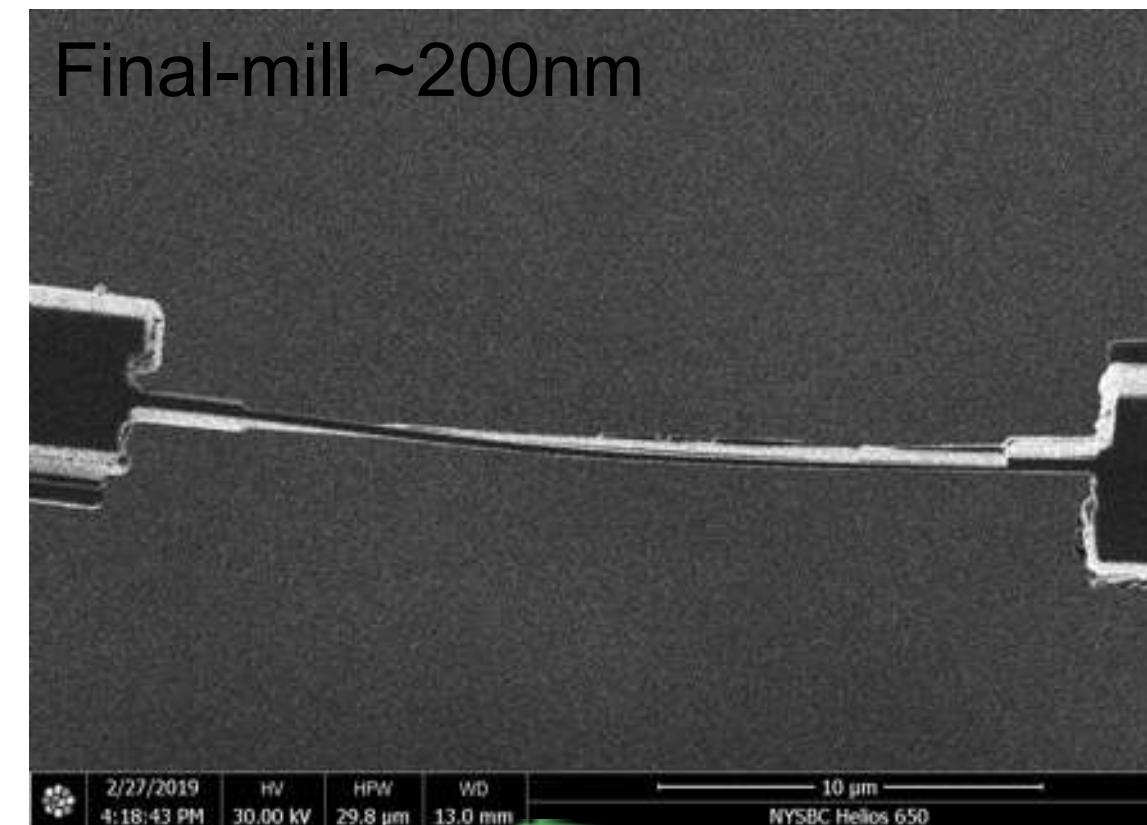
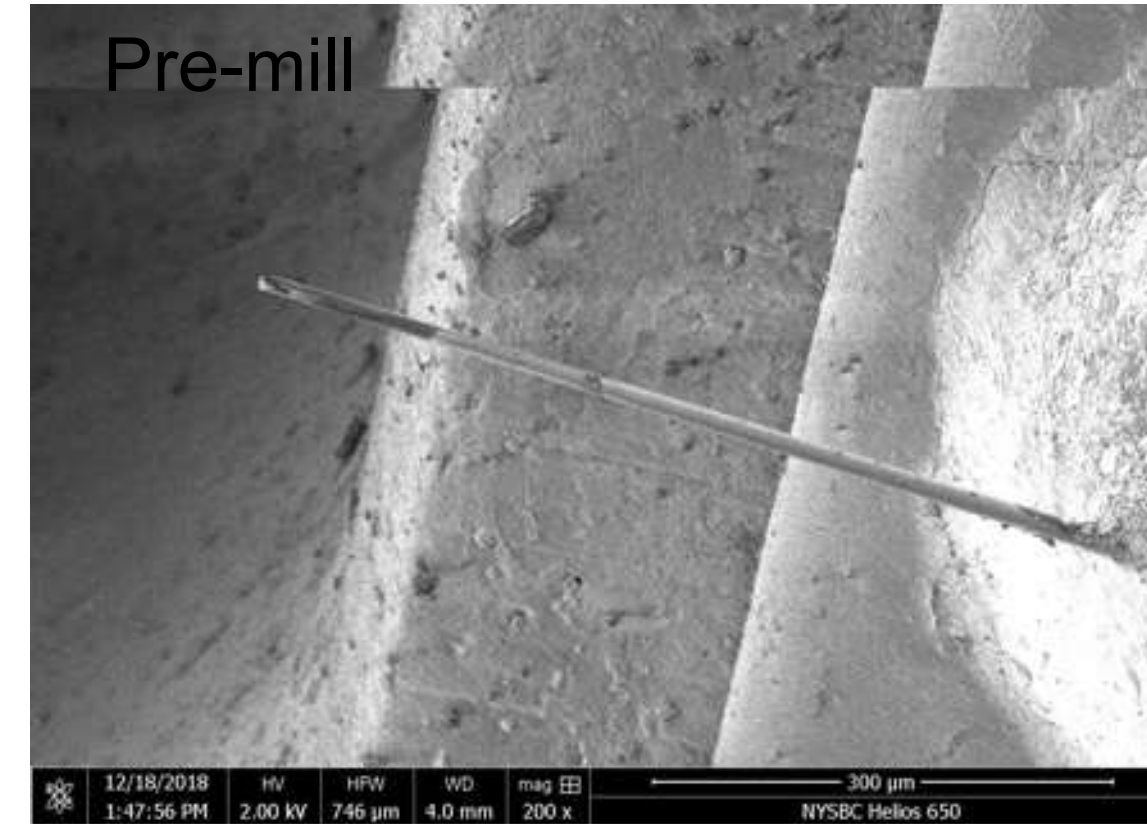


## Rods



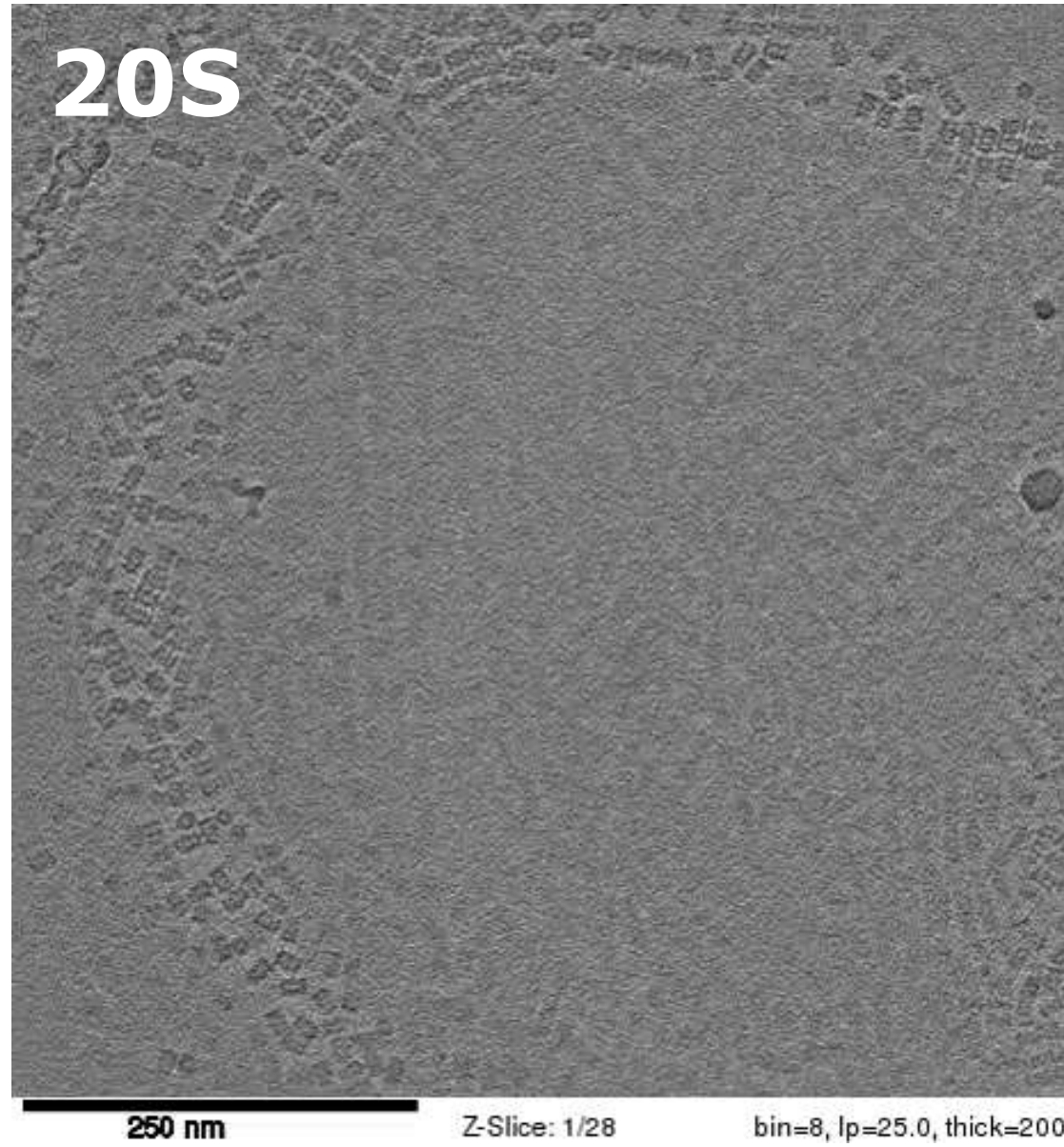
with Xin  
Group  
(BNL)

## Capillaries

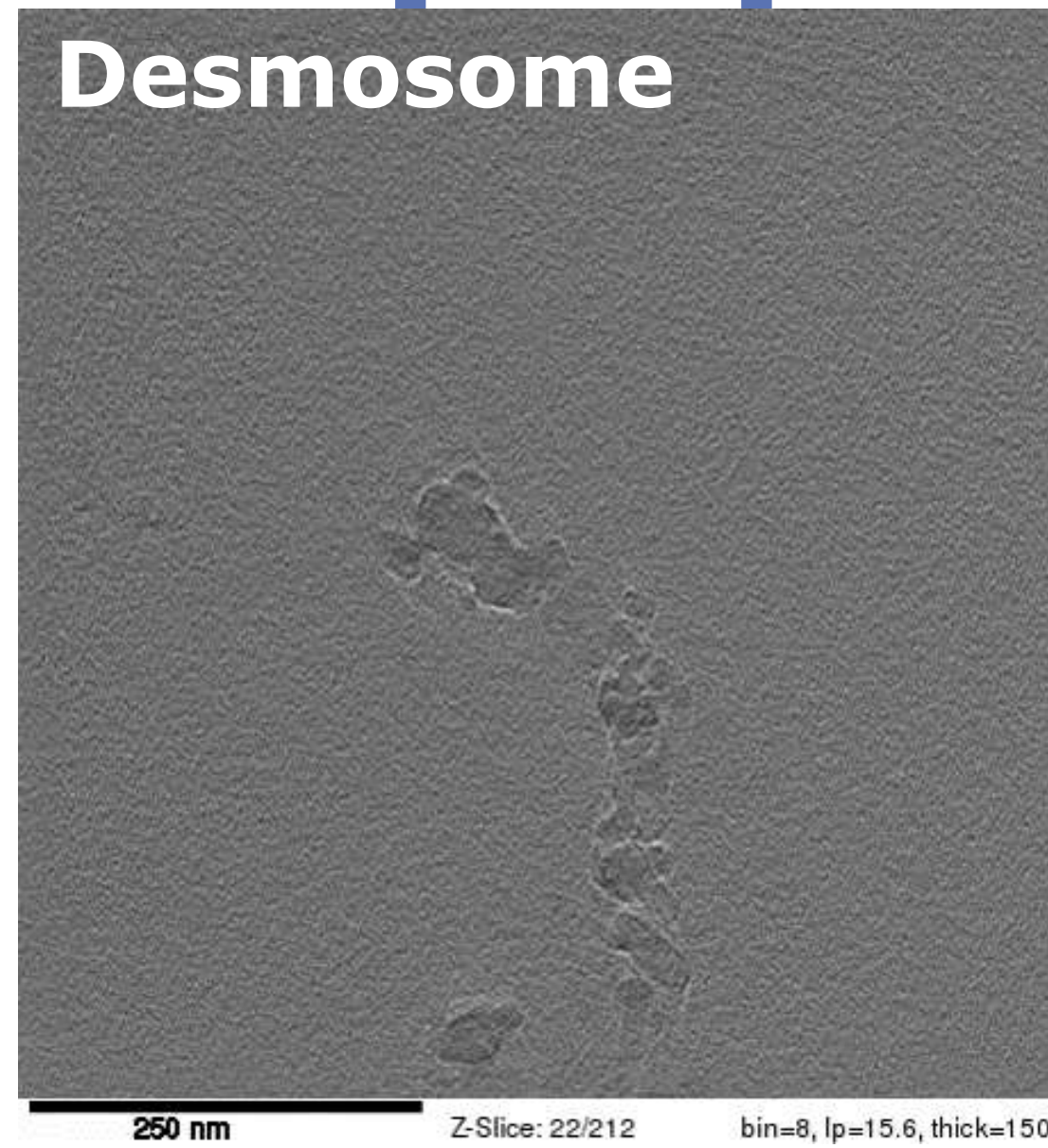


Kotaro  
Kelley  
(NRAMM)

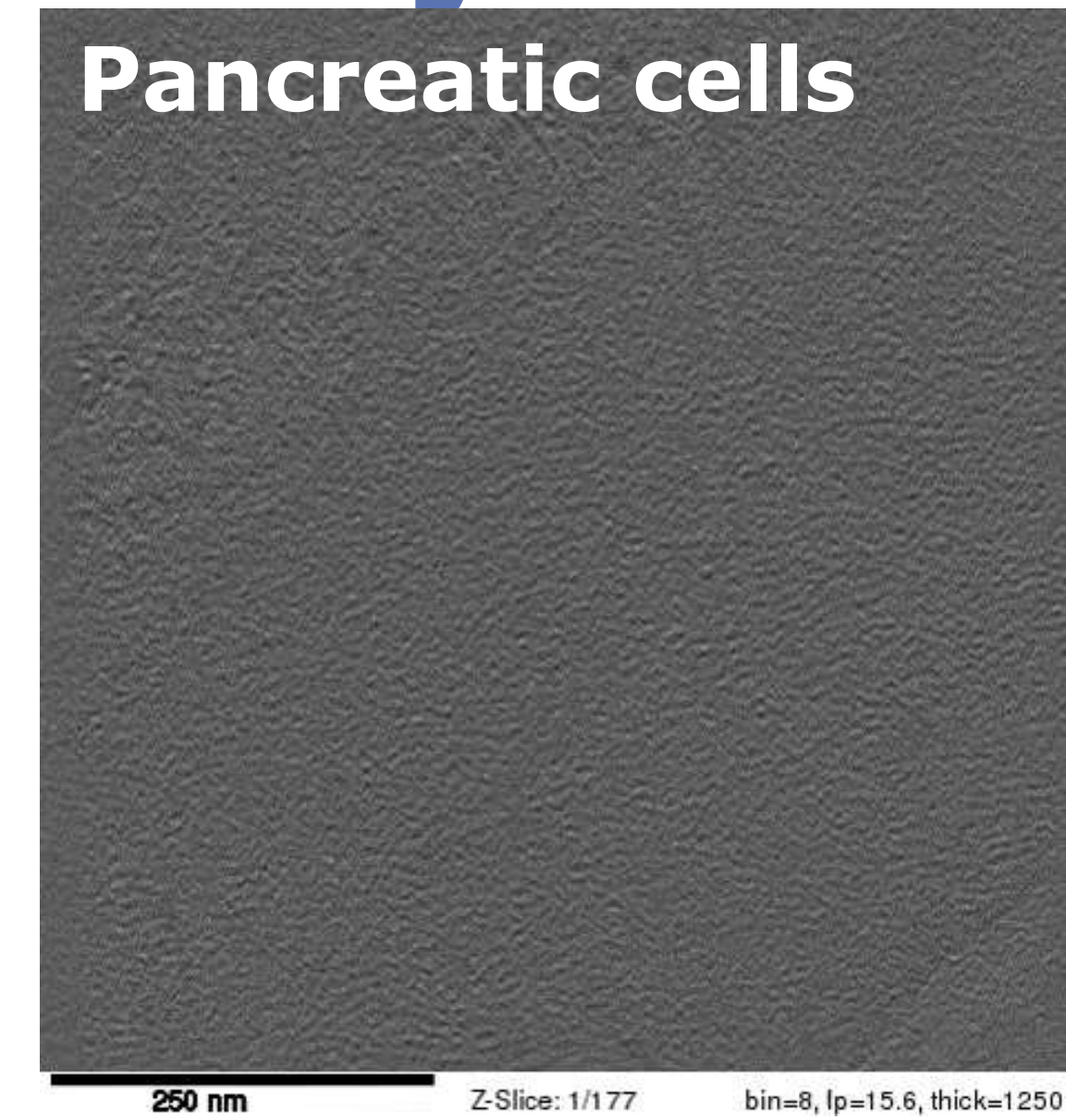
# How are samples prepared for cryoEM?



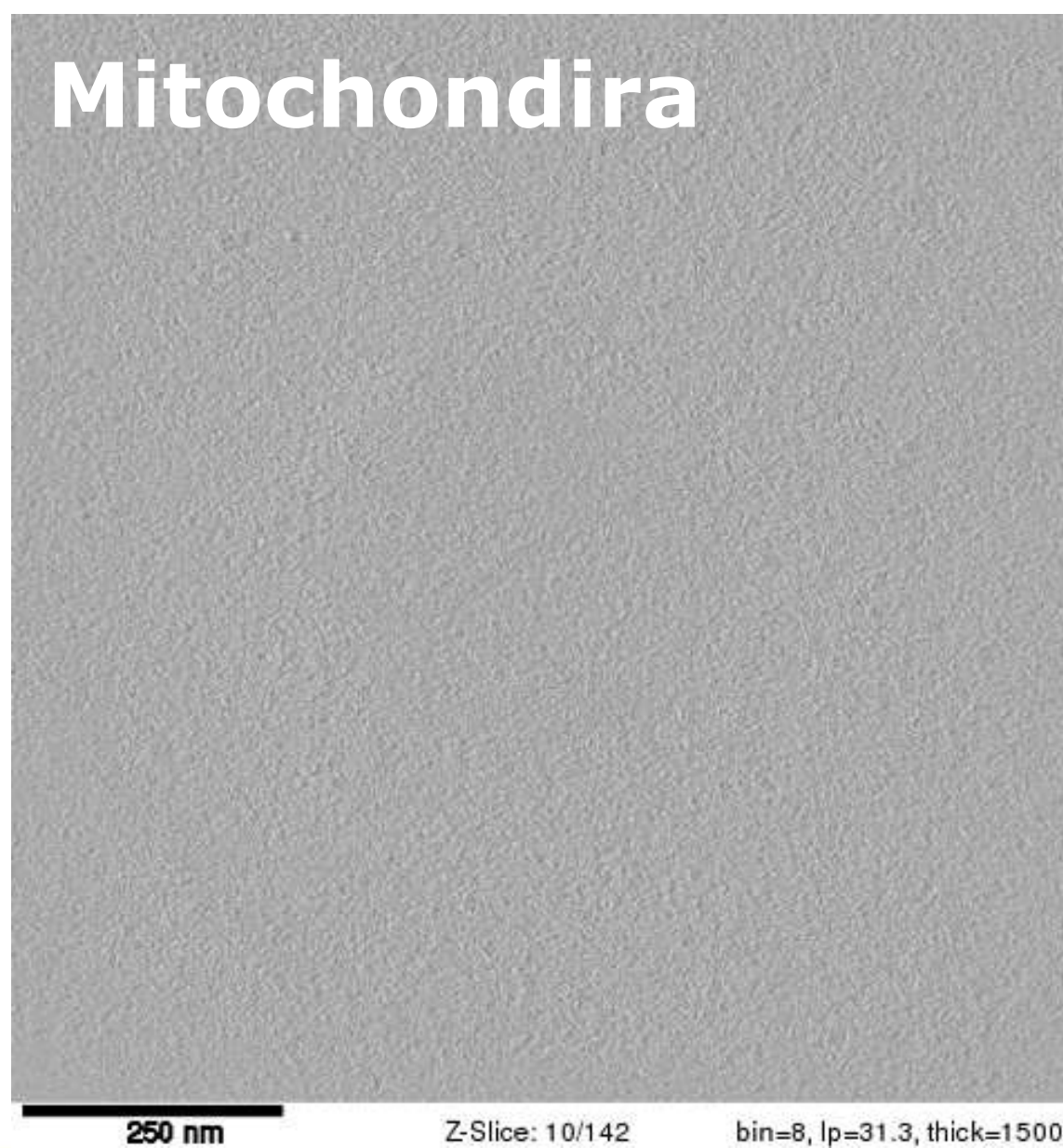
Alex Noble  
(NYSBC),  
Radostin Danev  
(MPI)



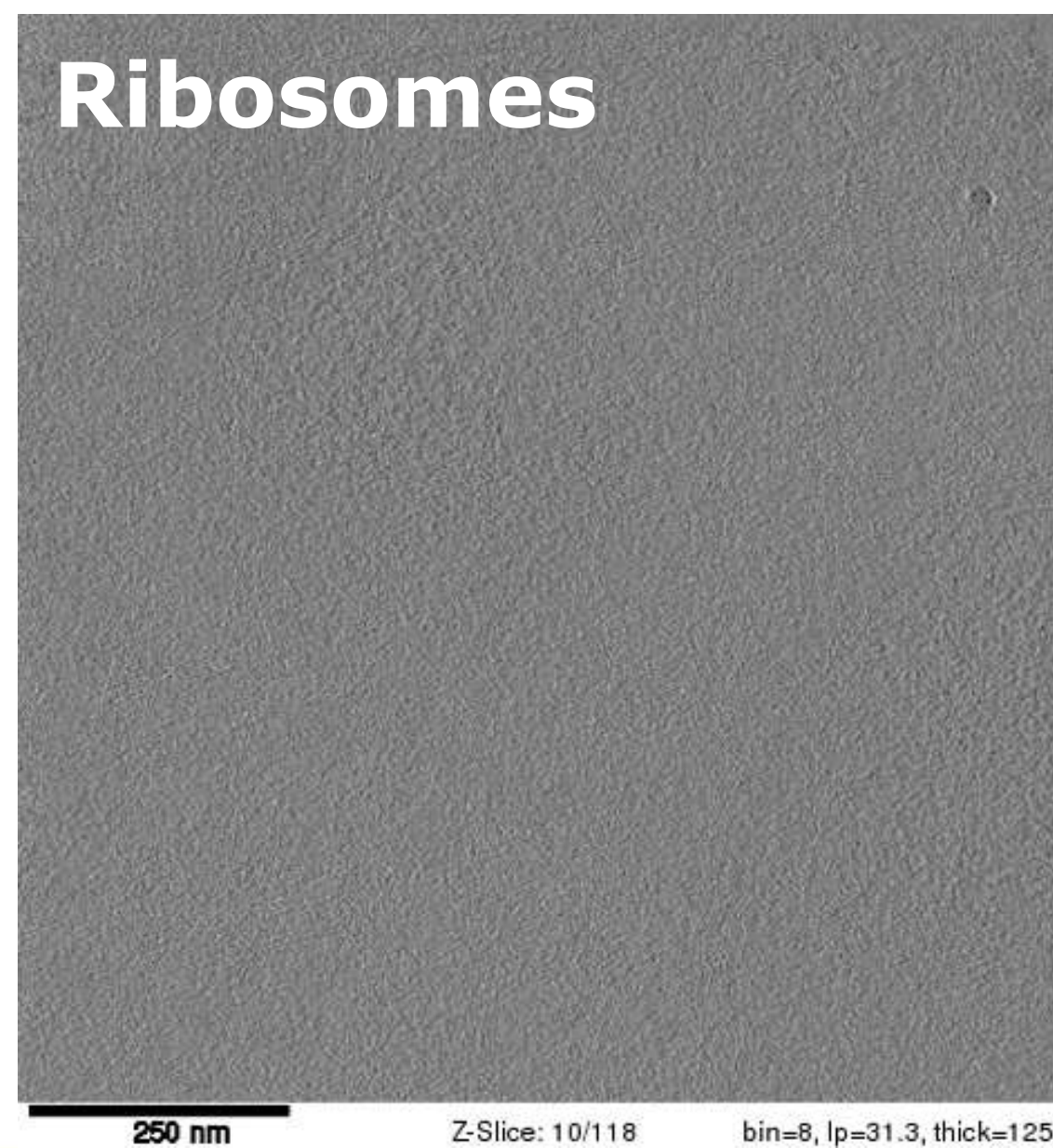
Julia Brasch  
(COLU)



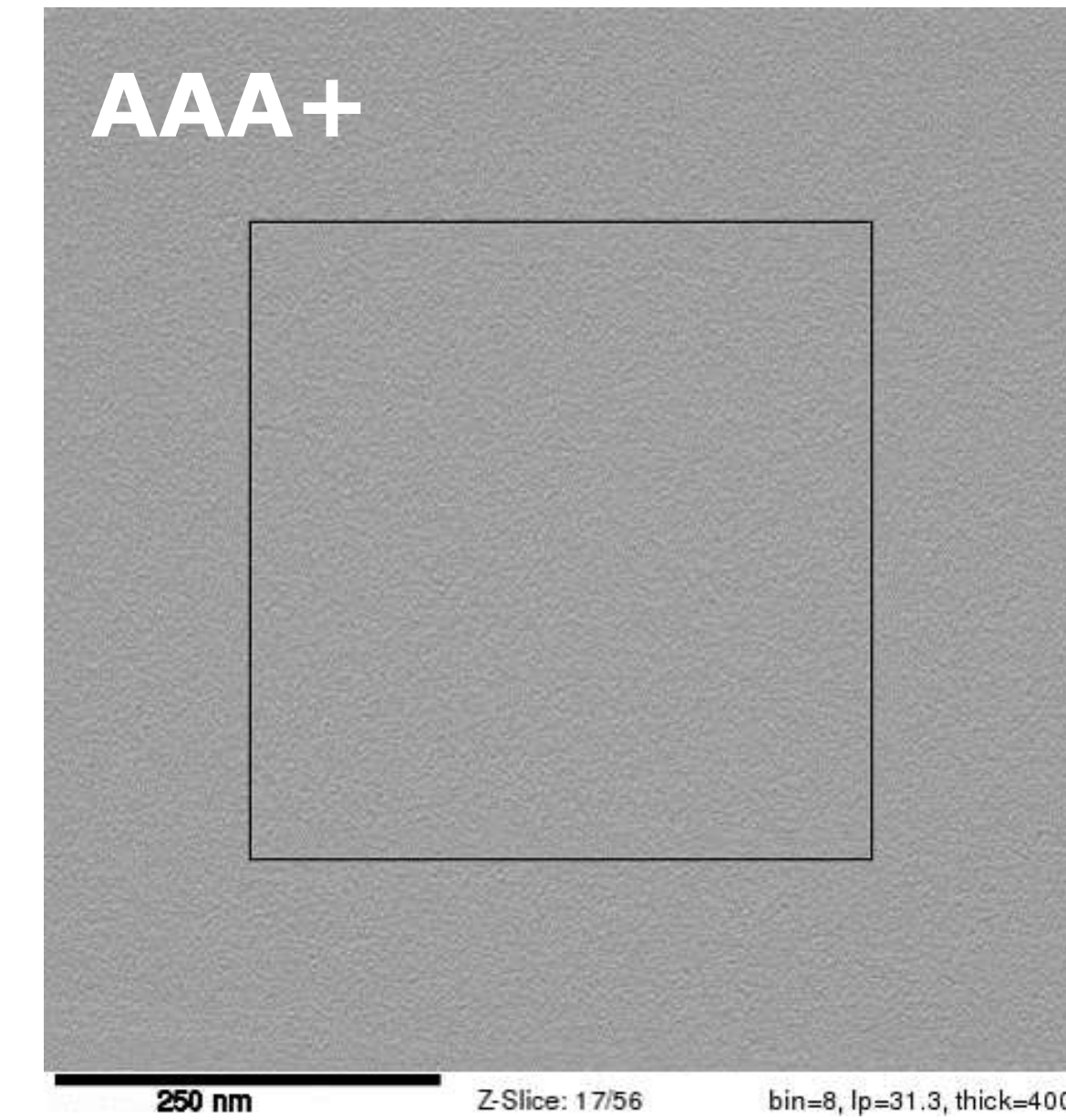
Zachary  
Freyberg  
(U. Pitt.)



Stephanie  
Siegmund  
(COLU)

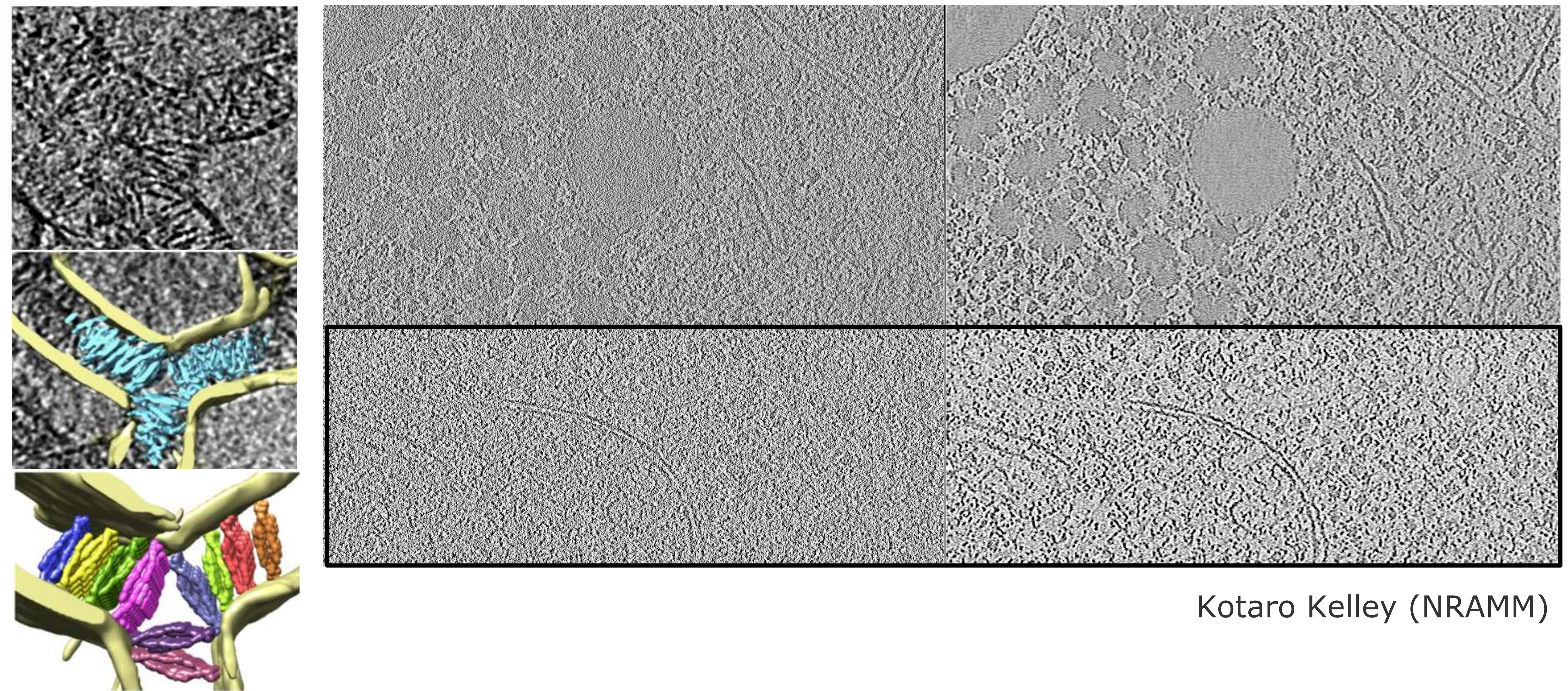


Stephanie  
Siegmund  
(COLU)



Jillian  
Chase  
(CUNY)

# How are samples prepared for cryoEM?

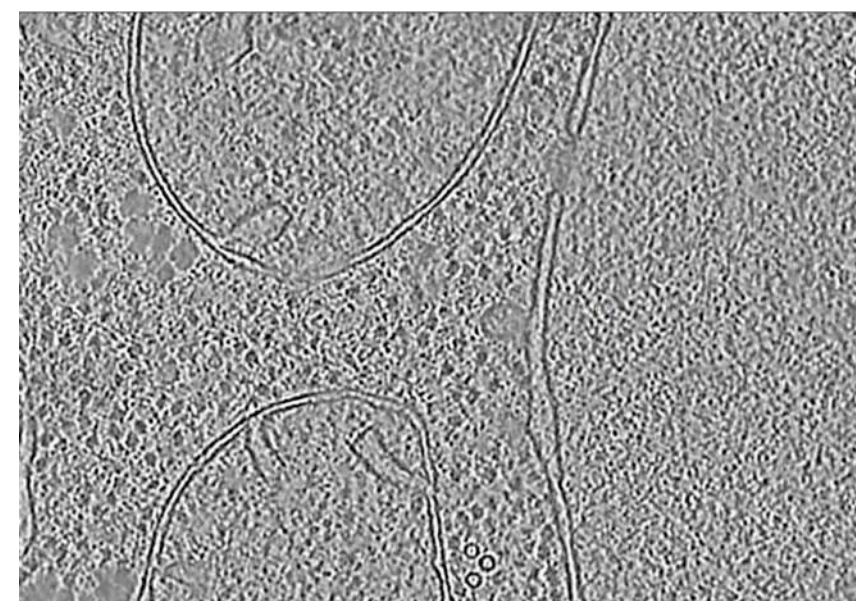
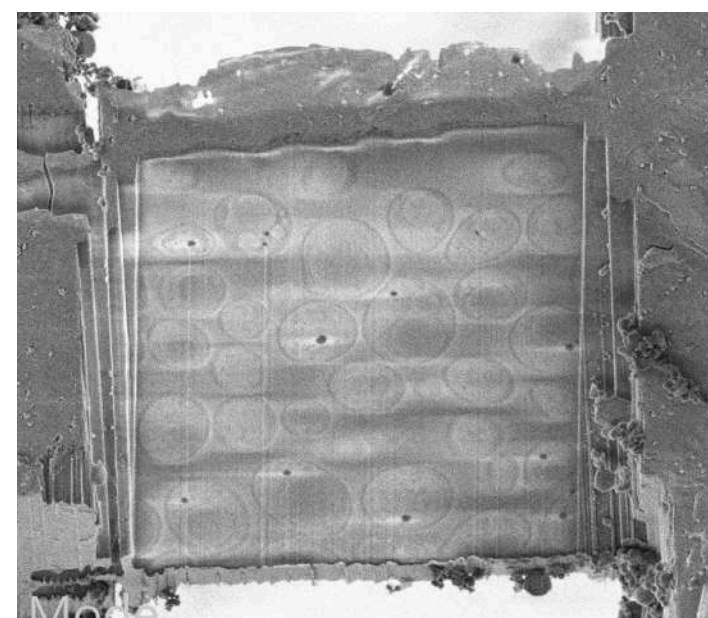
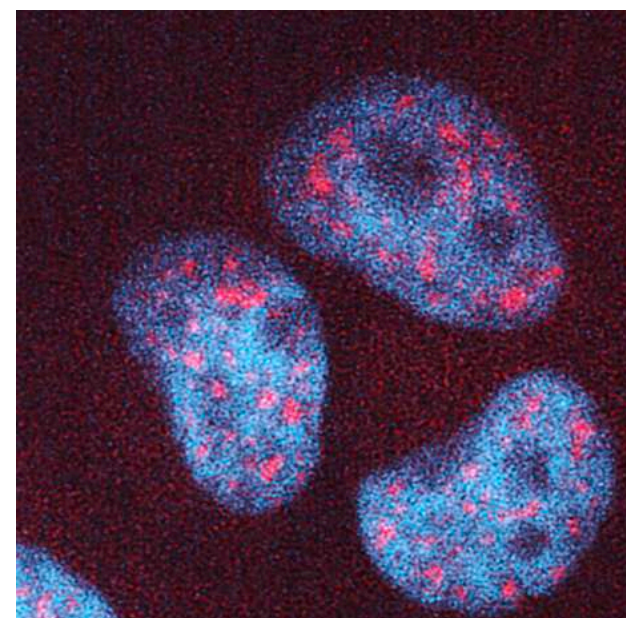
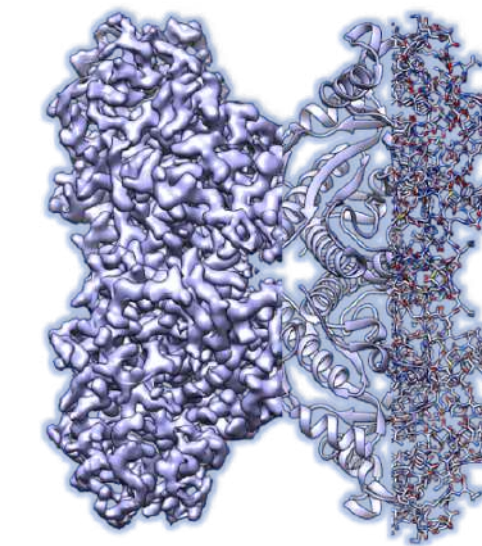
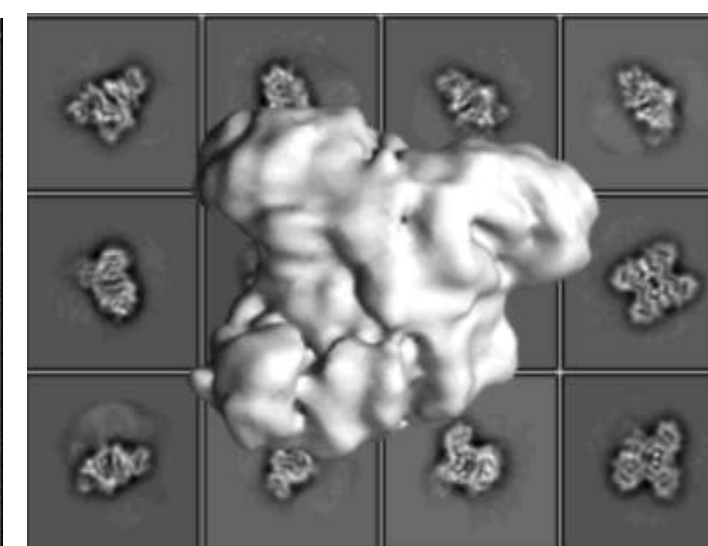
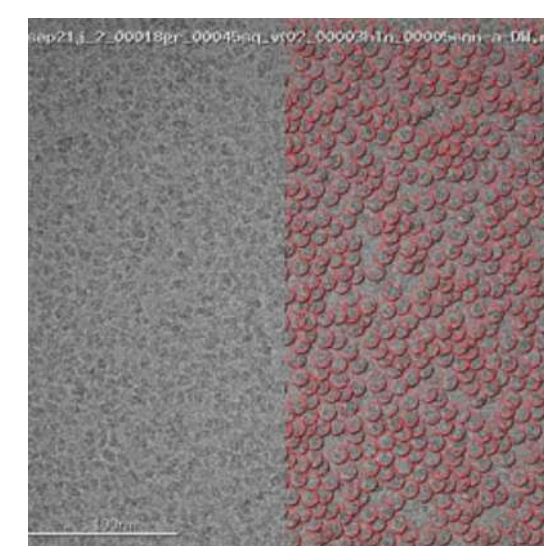
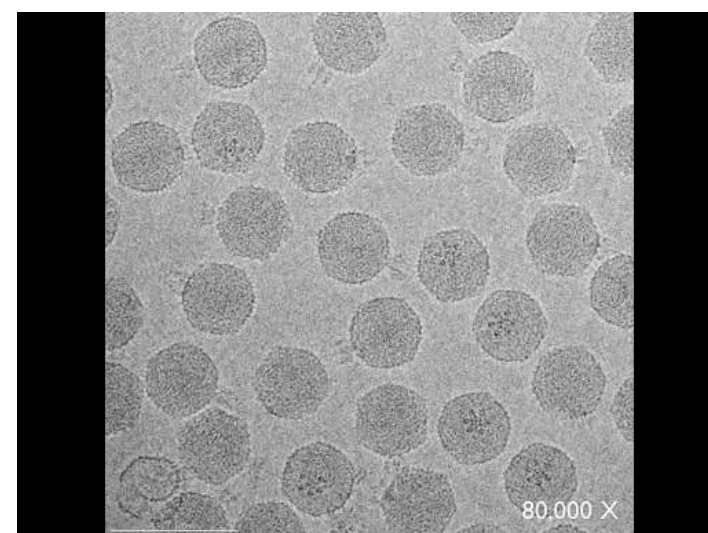
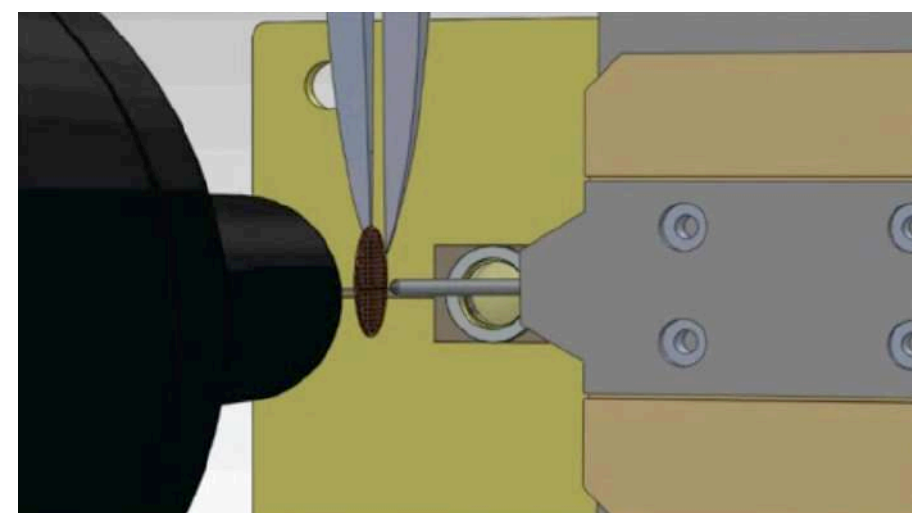


Kotaro Kelley (NRAMM)

Micah Rapp (COLU)

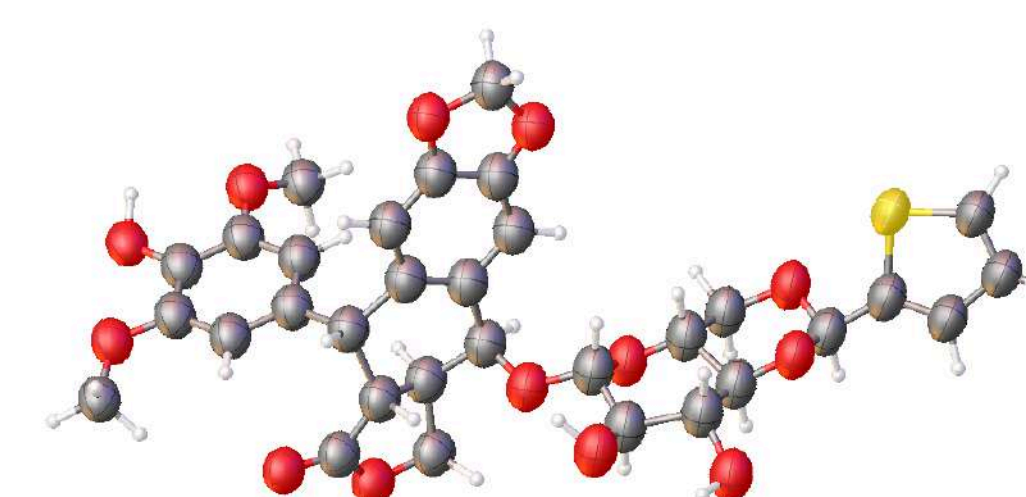
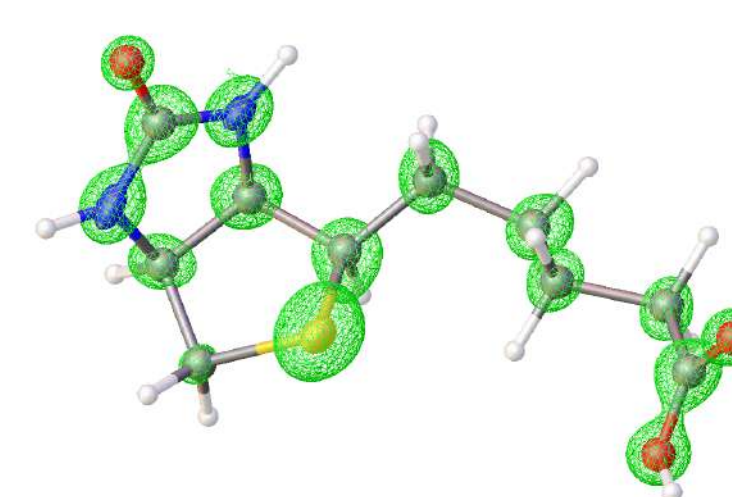
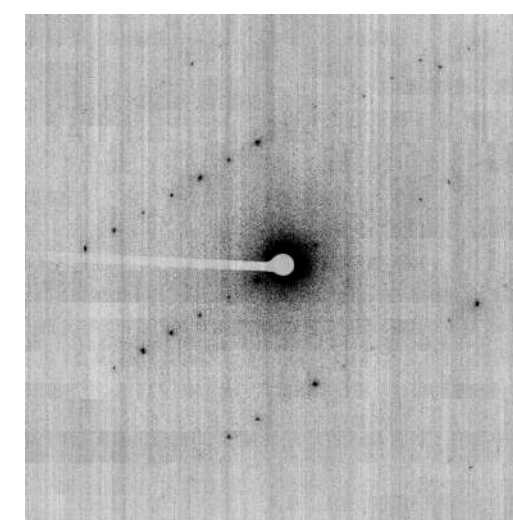
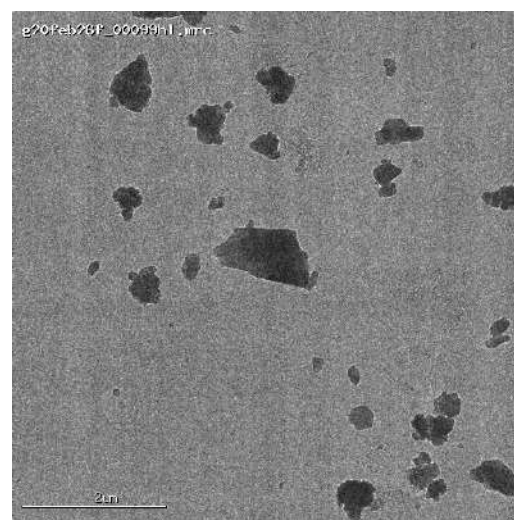
# cryoEM: technology on the rise

Single particle cryoEM



Cryo Electron Tomography (cryoET)

Micro crystal electron diffraction (microED)





TO BE CONTINUED

Questions?