

Cryomesh™: A new grid substrate for Cryo-EM



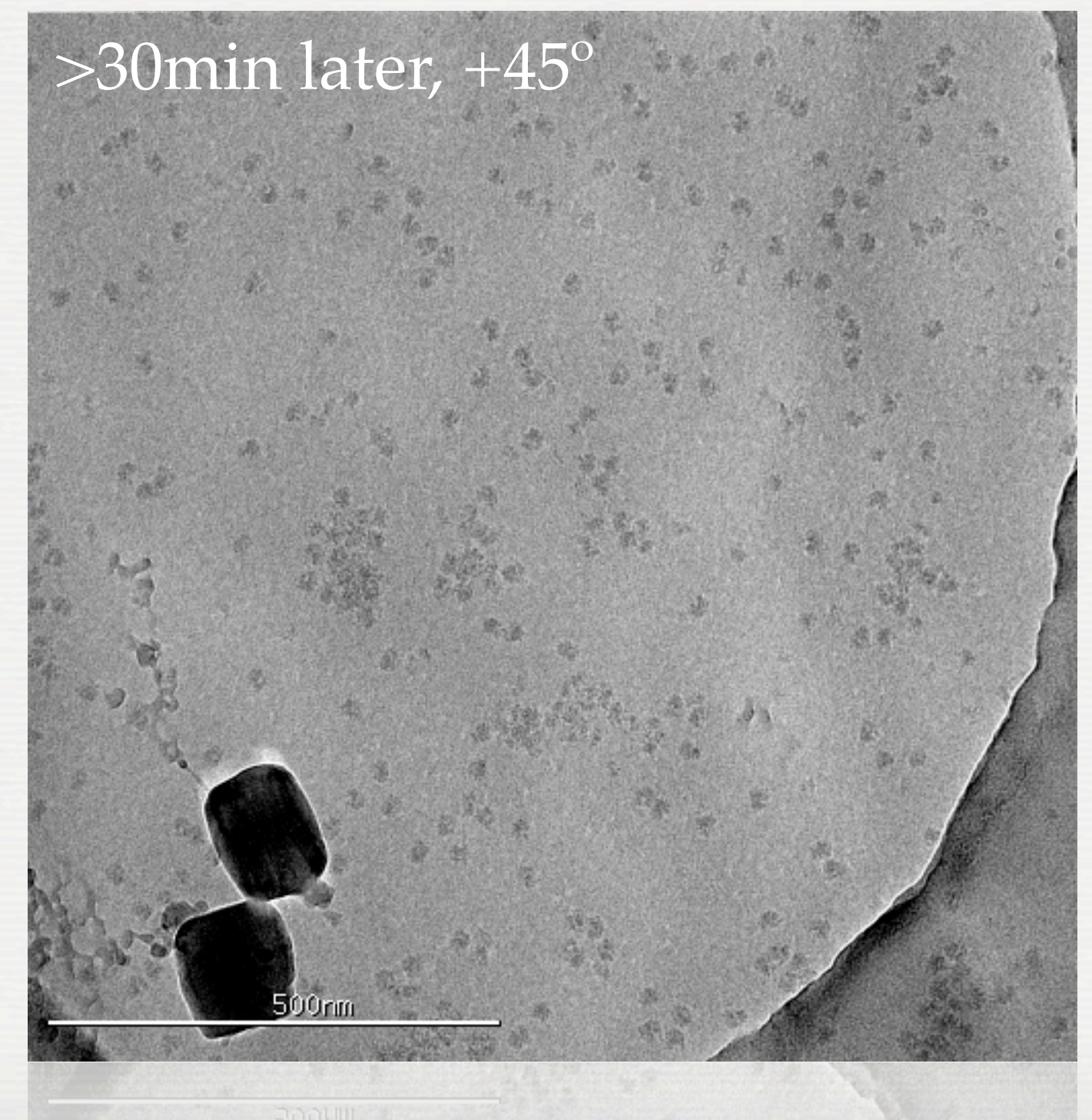
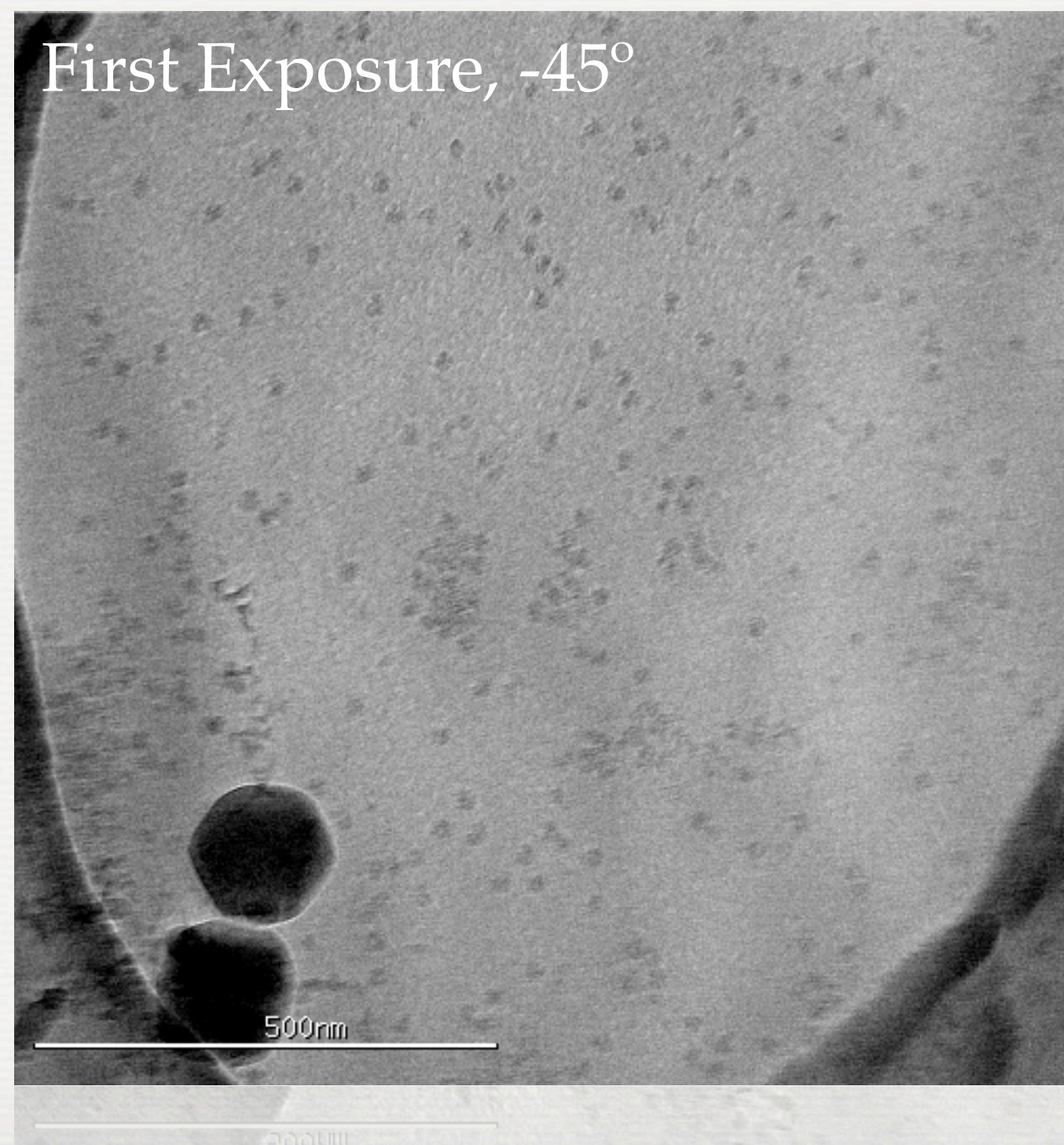
Craig Yoshioka
NRAMM Cryo Course '09

Motivation For New Grid Tech

- **Reduction in beam-induced motion (BIM) !!**
 - Tilted cryo data collection
 - Improve resolution?
- Improved Imaging Platform
 - Faster, more consistent imaging
 - Automated microscope calibration?

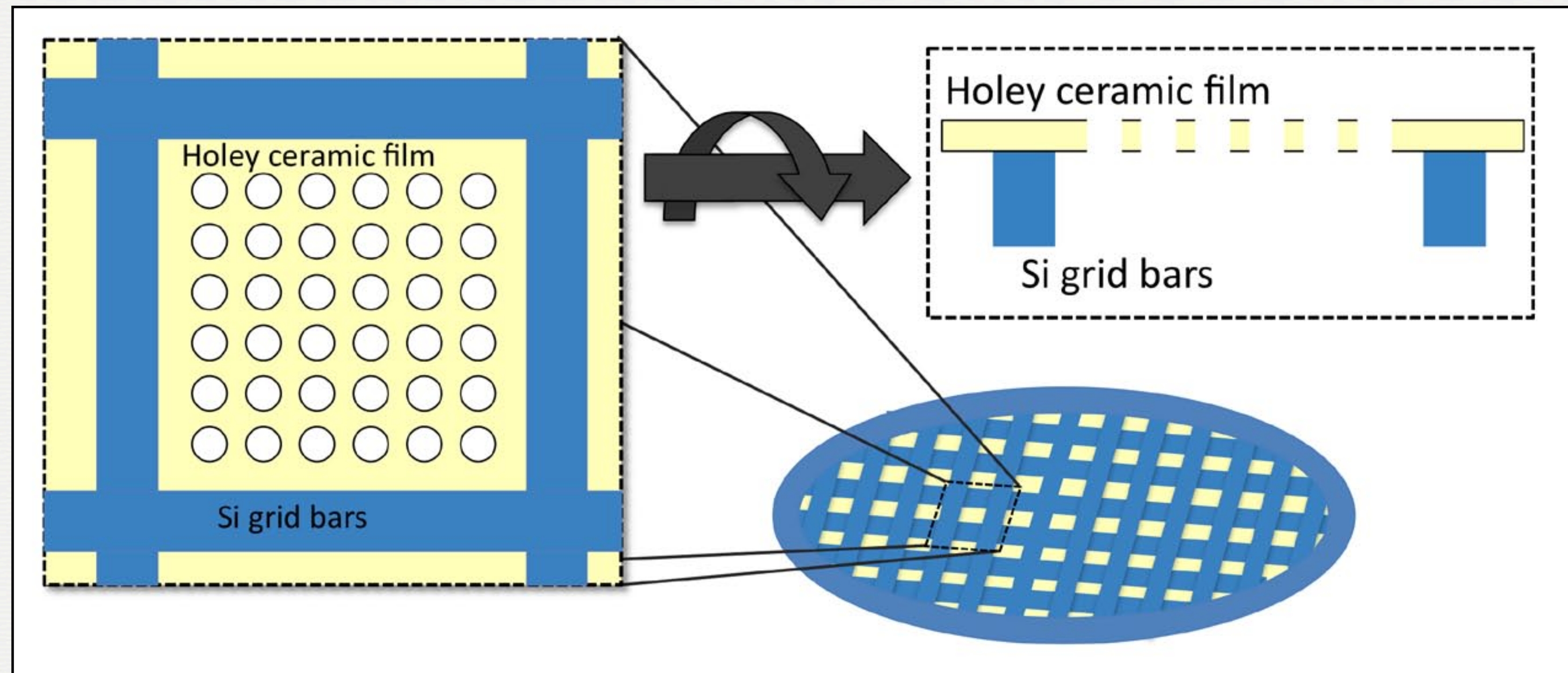
Beam-Induced Movement

- A phenomenon (commonly observed, not entirely understood)
 - Arises from charge build-up in specimen
 - Mechanical Movement, i.e. tension release?
 - Electrostatic Lensing Effect?
 - Both?

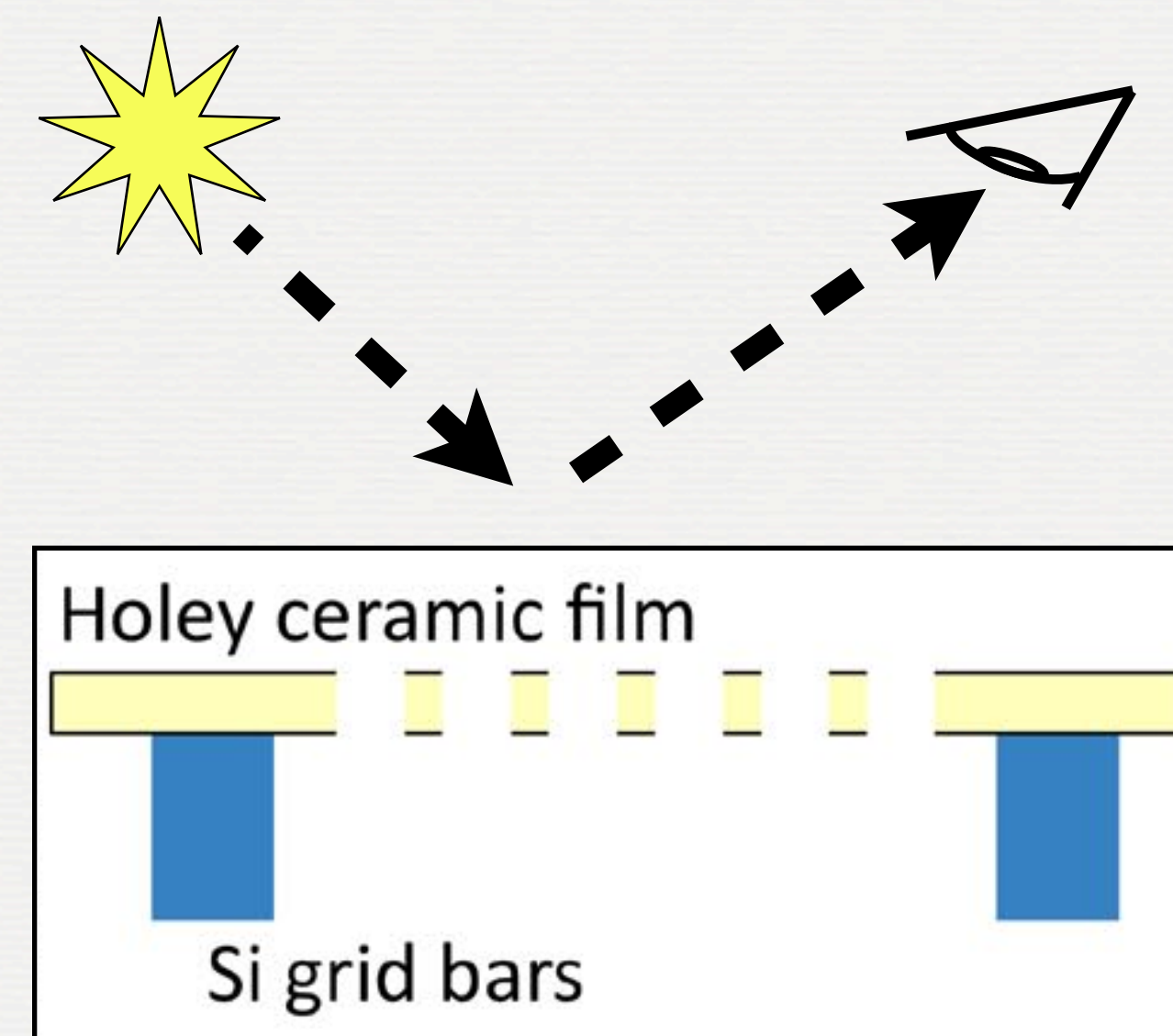
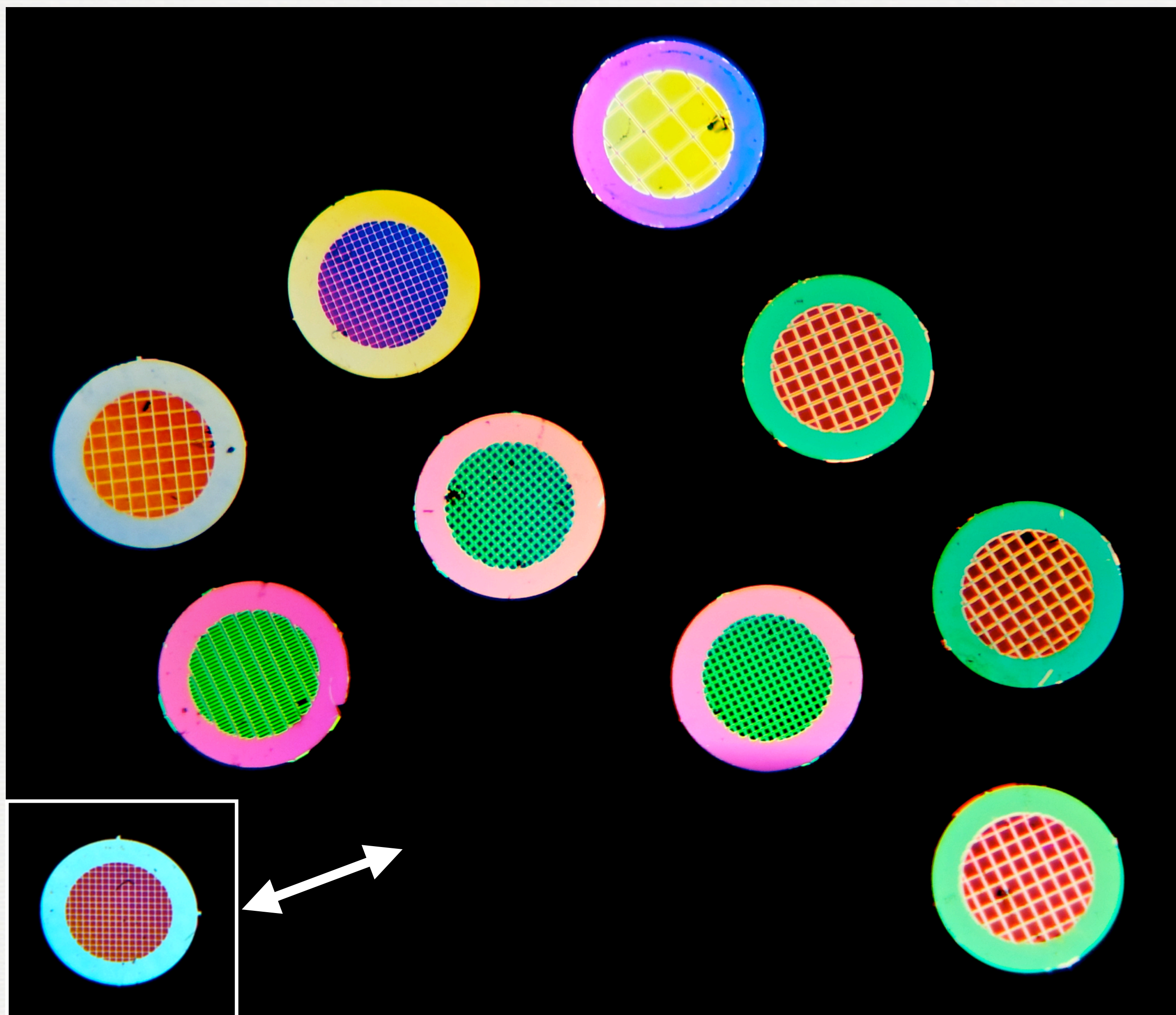


Hence: Cryomesh

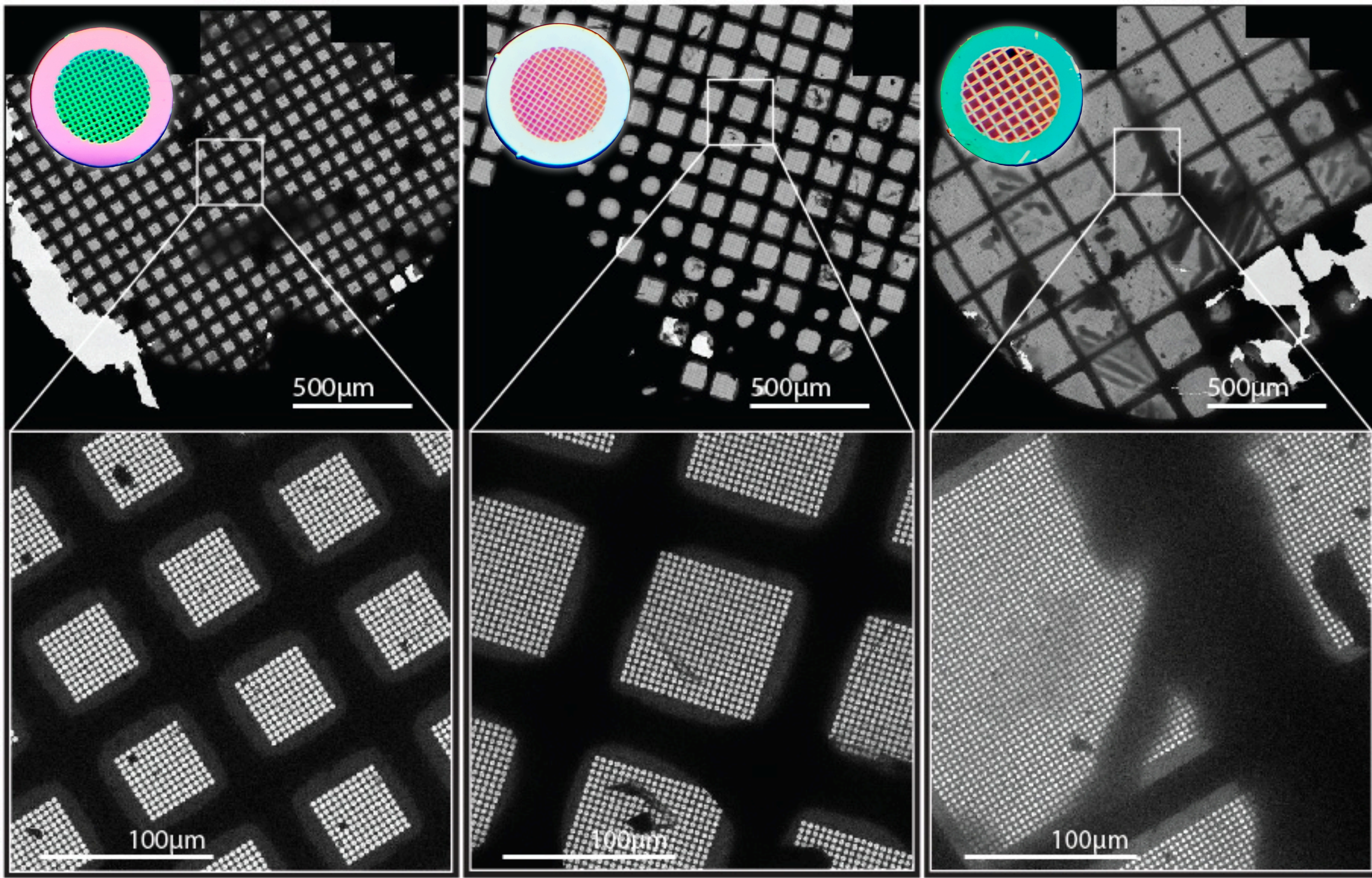
- More conductive at all temps than carbon
- Stronger and more rigid than carbon



Cryomesh Characteristics

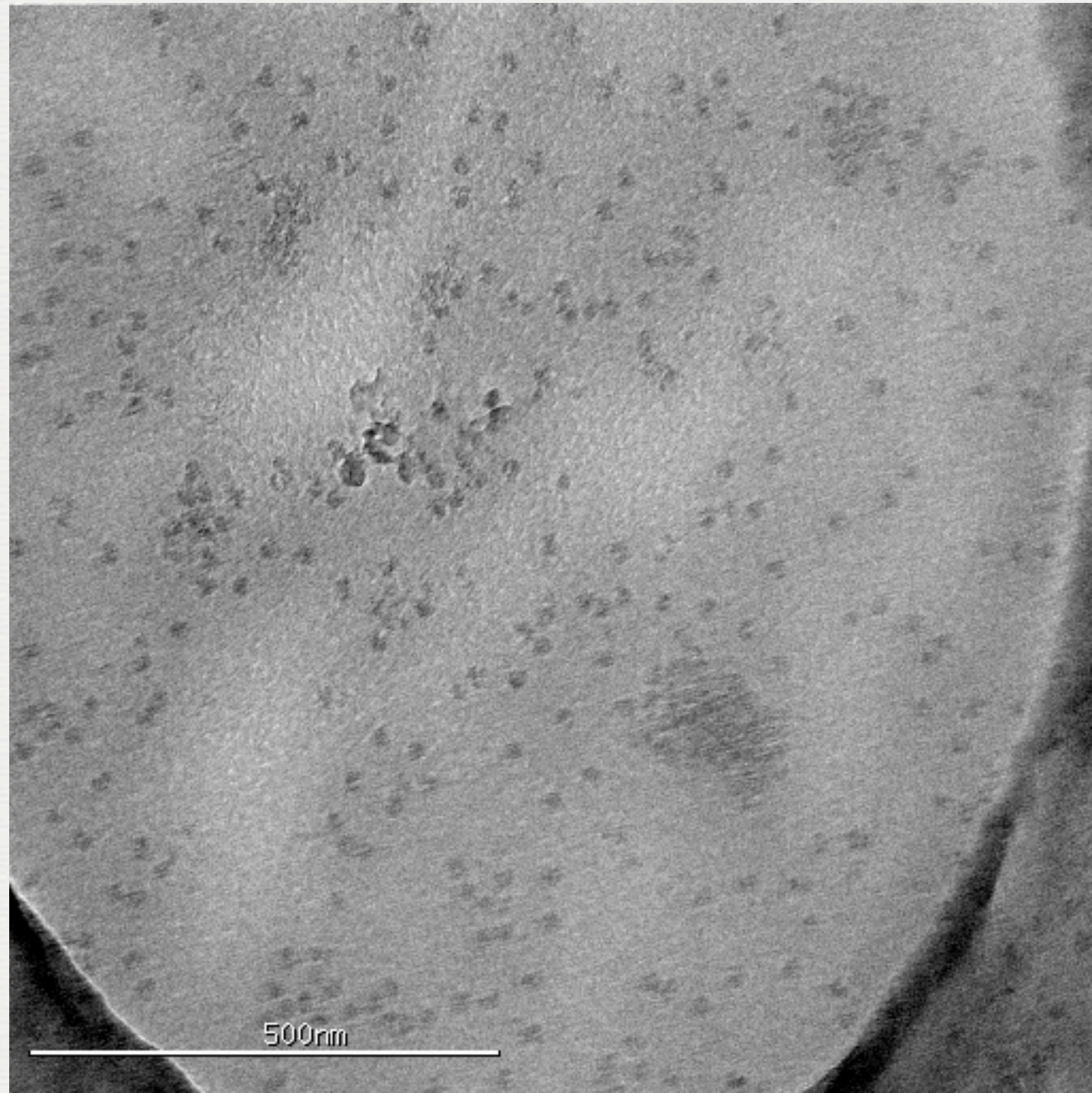


Cryomesh Characteristics

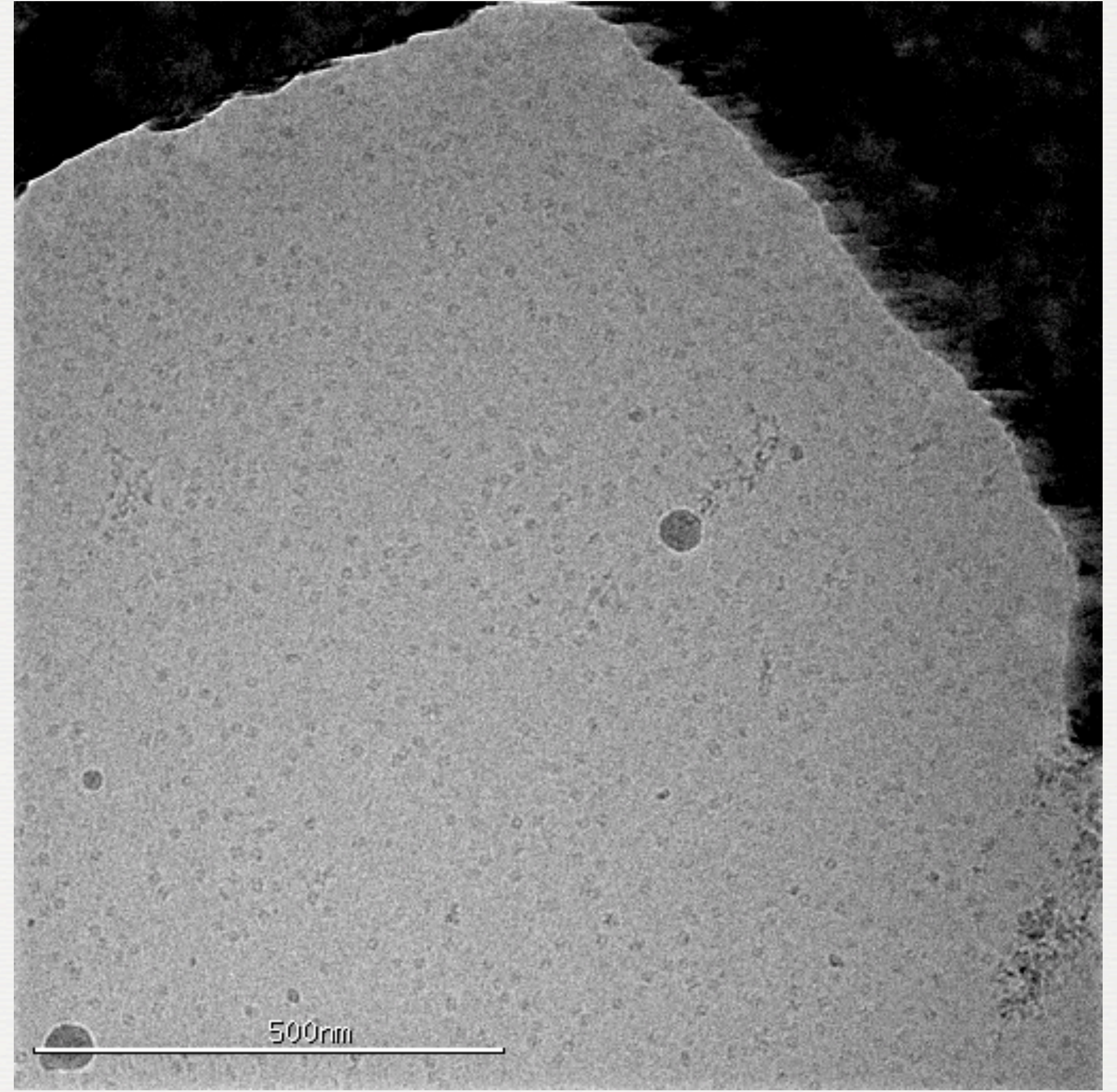


Imaging Cryo at Tilt

C-Flats @ 45-55°

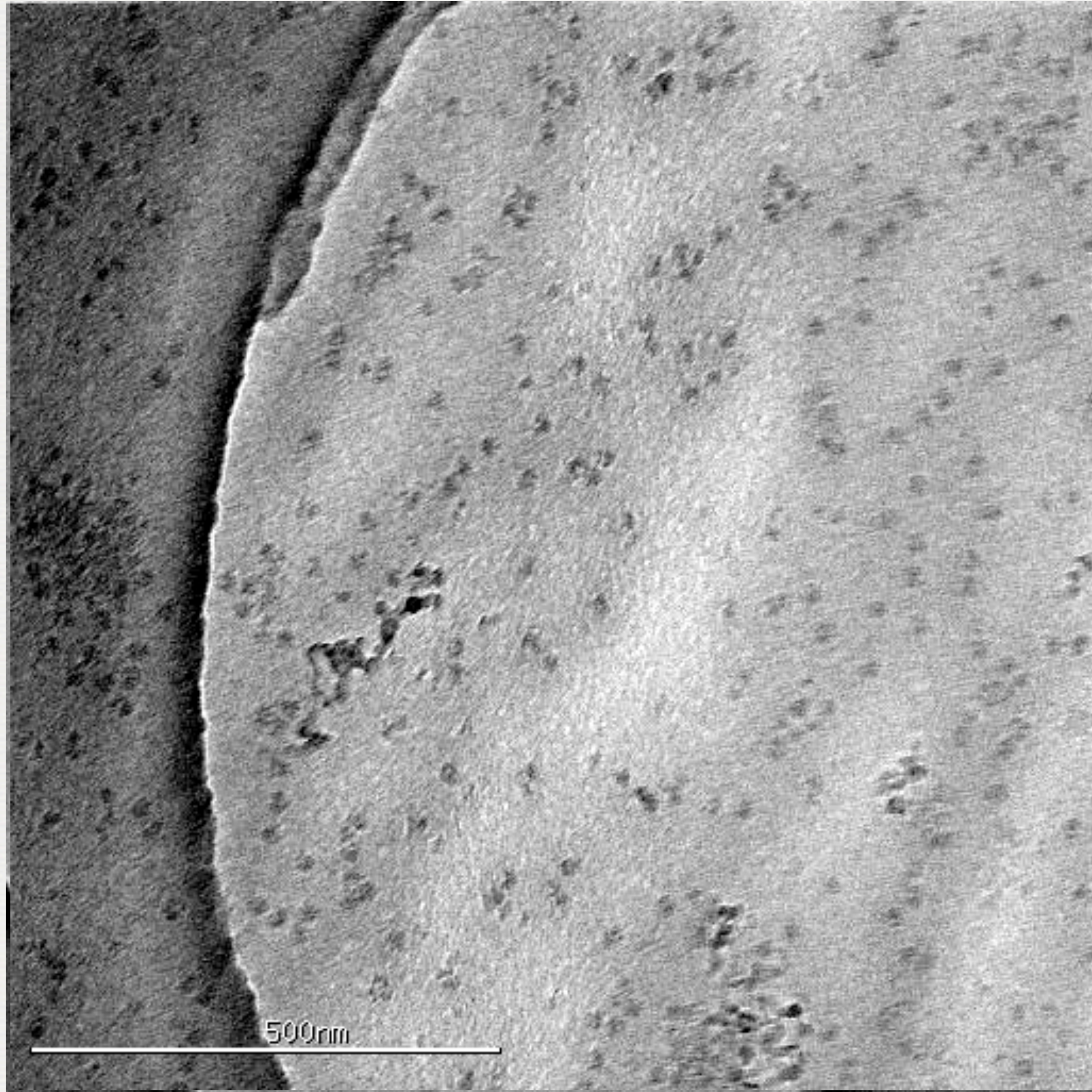


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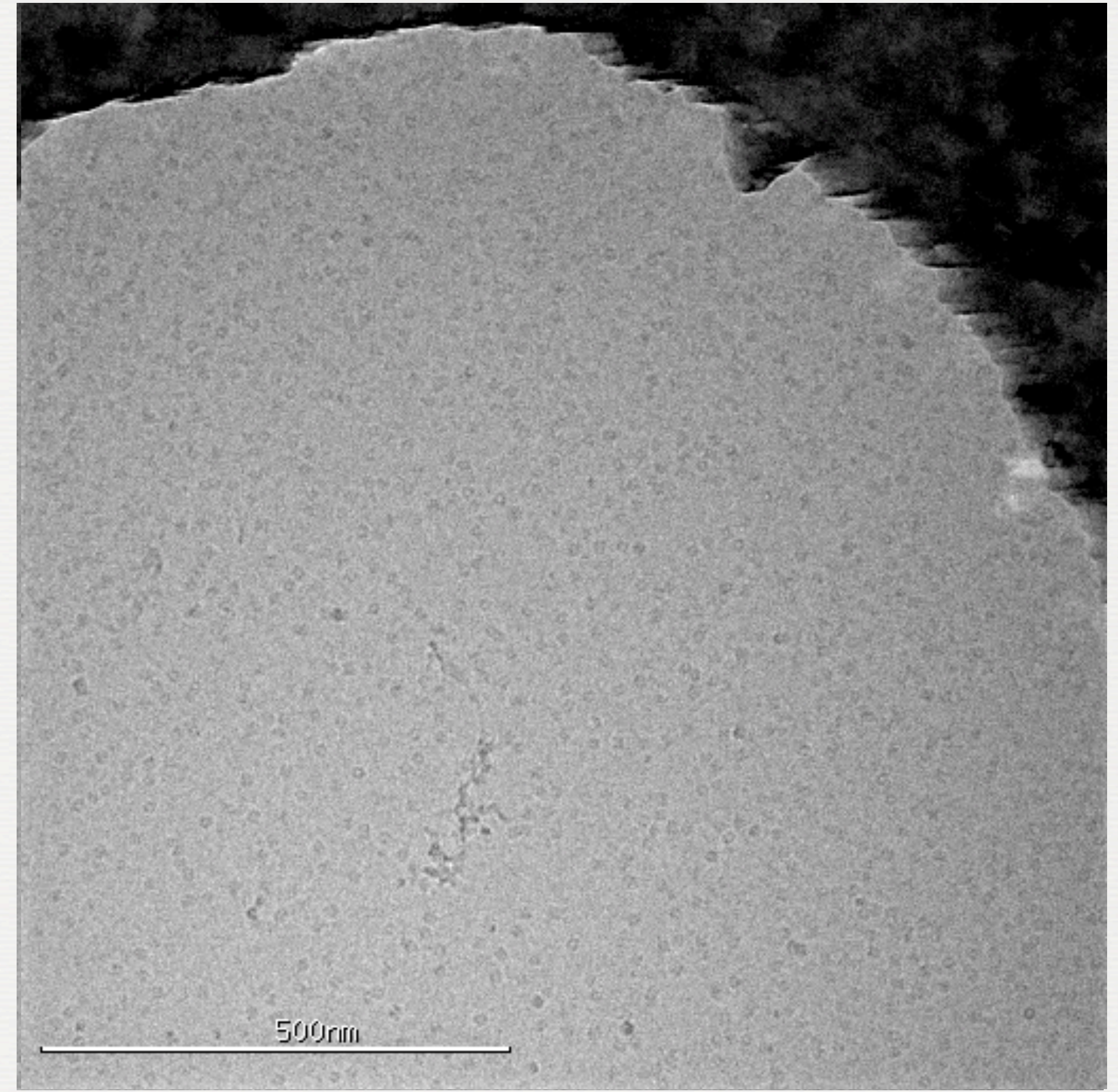


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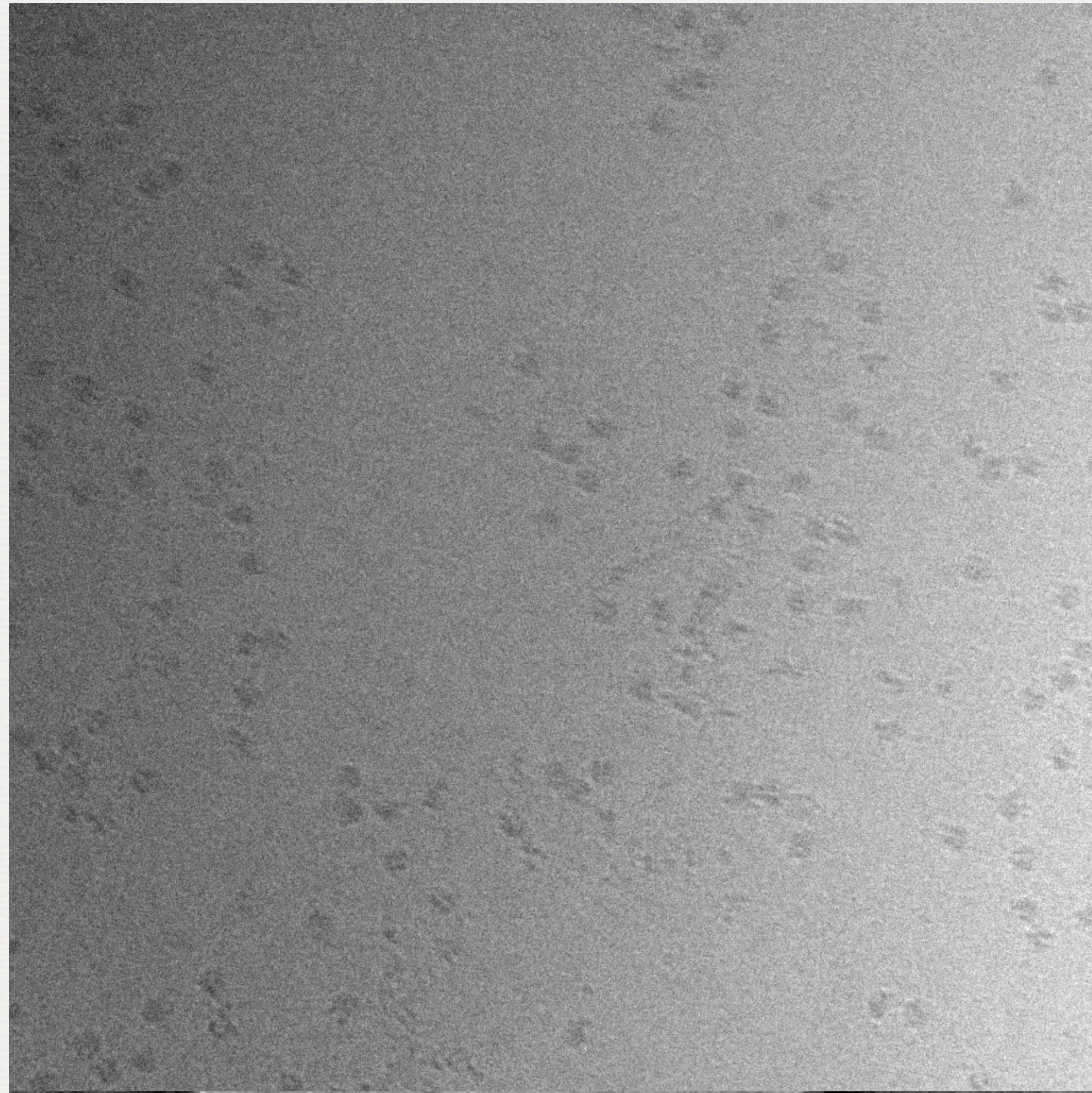


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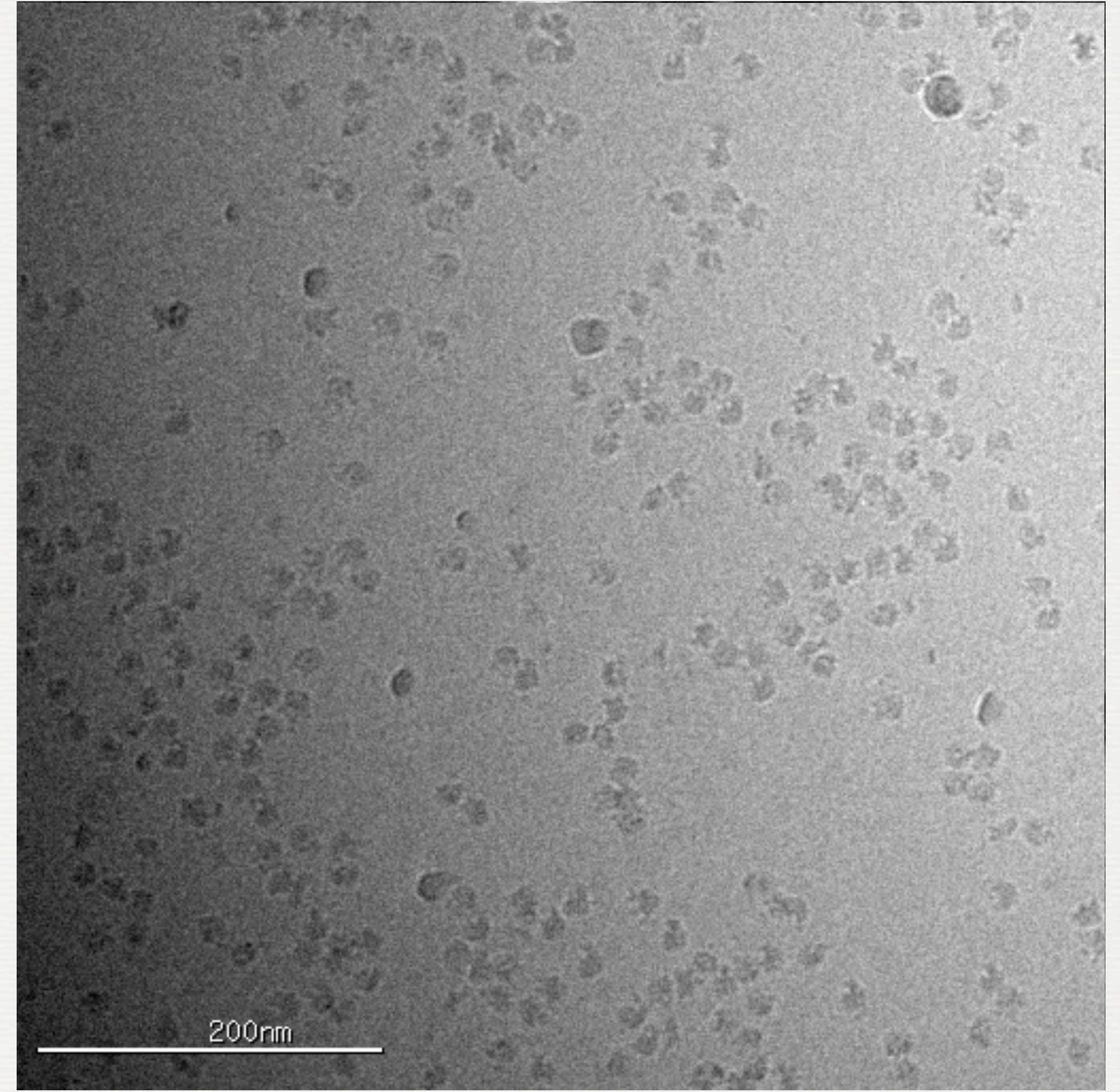


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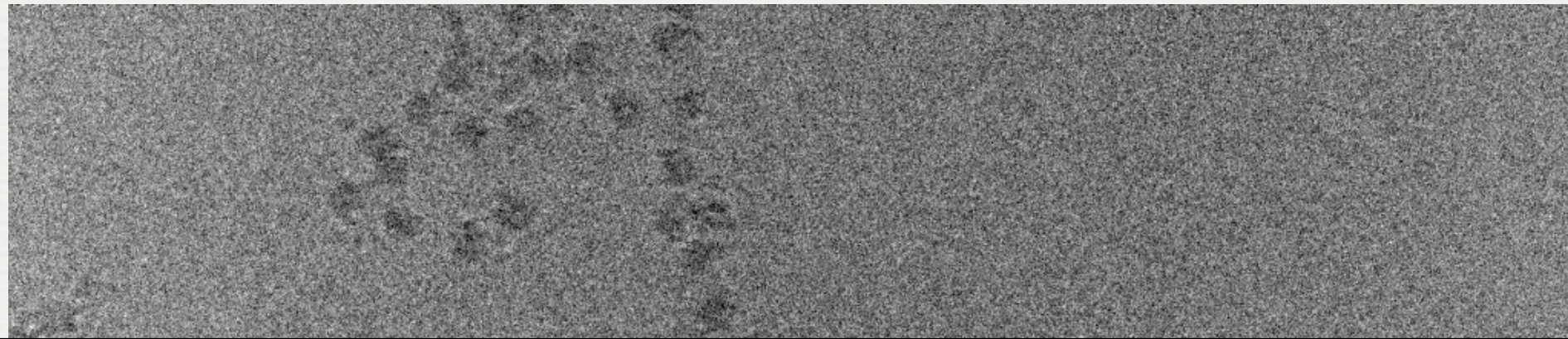


Cryomesh @ 45-55°

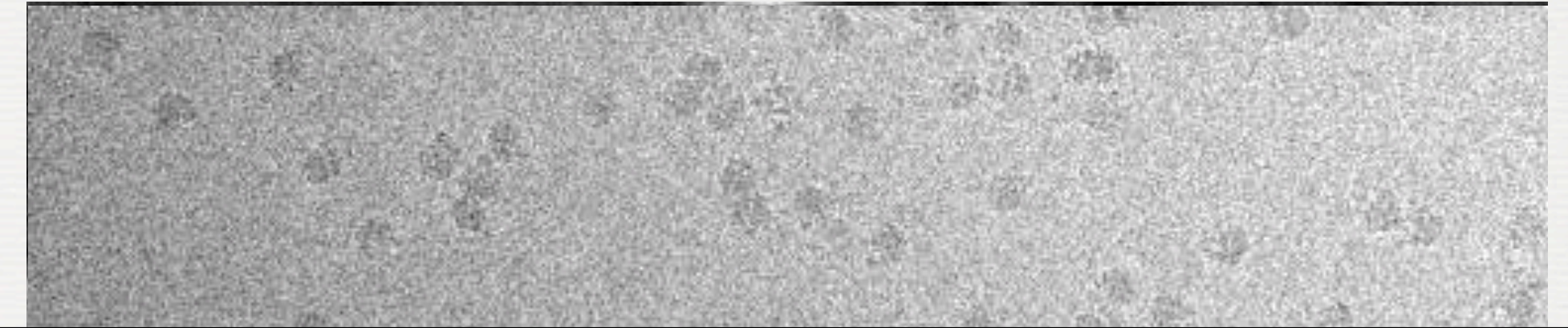


Imaging Cryo at Tilt

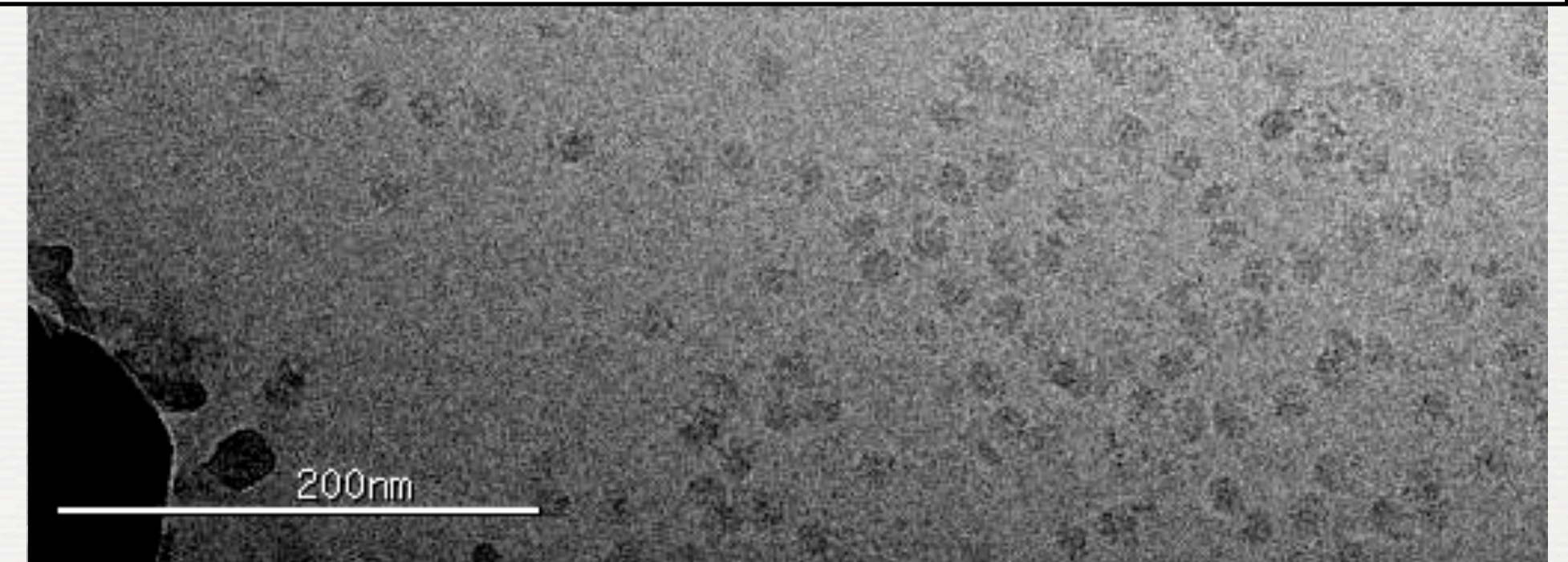
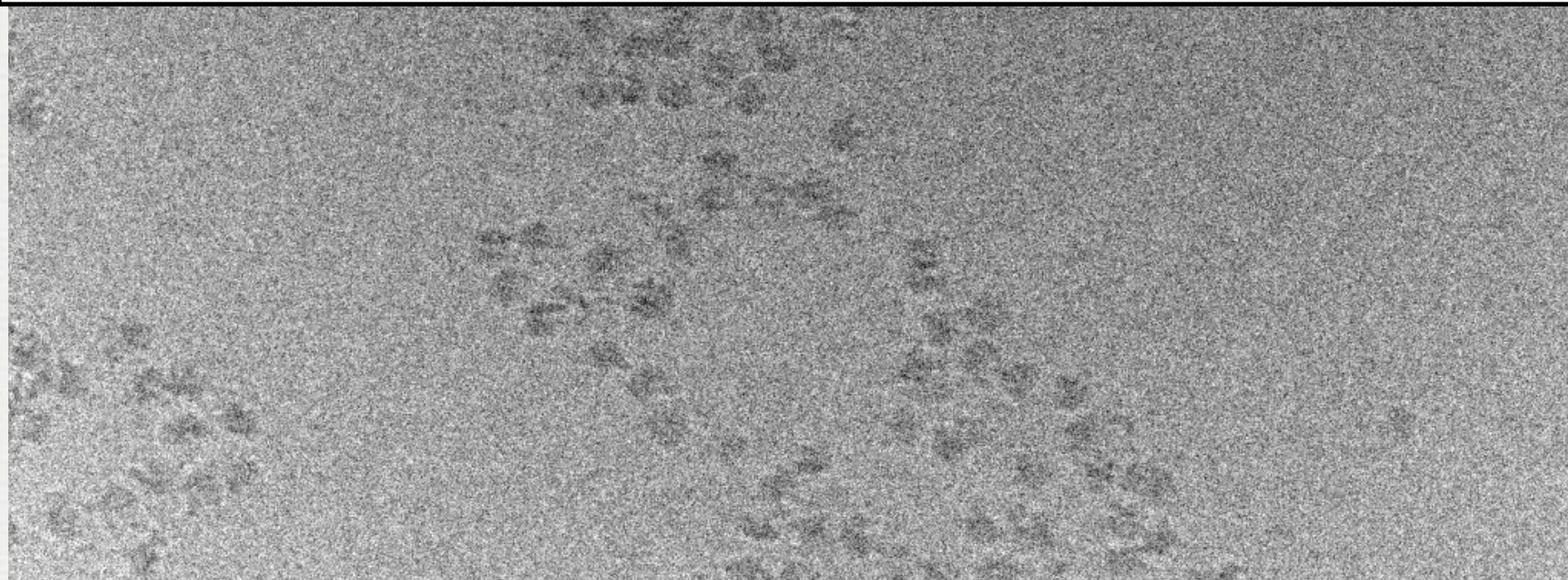
C-Flats @ 45-55°



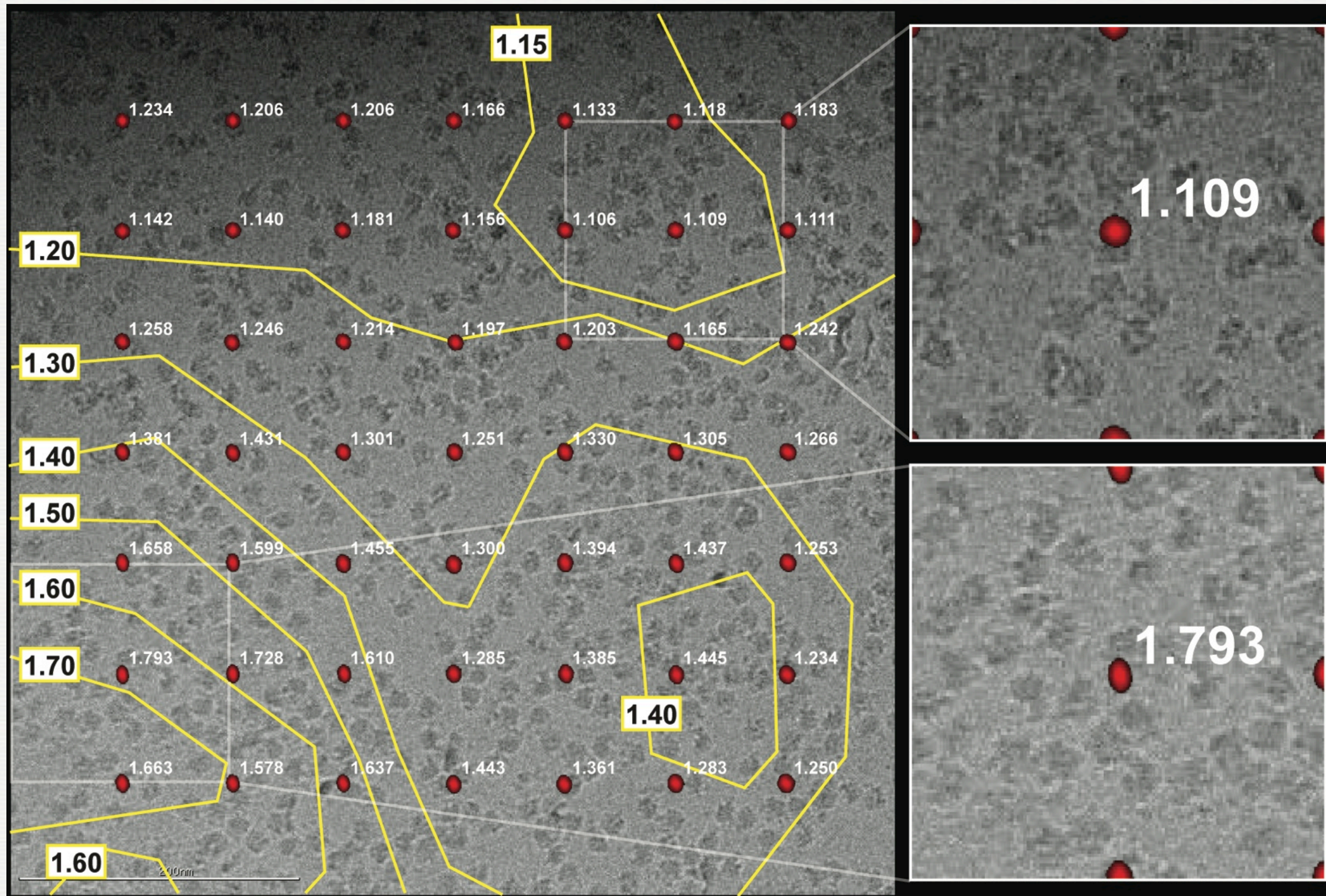
Cryomesh @ 45-55°



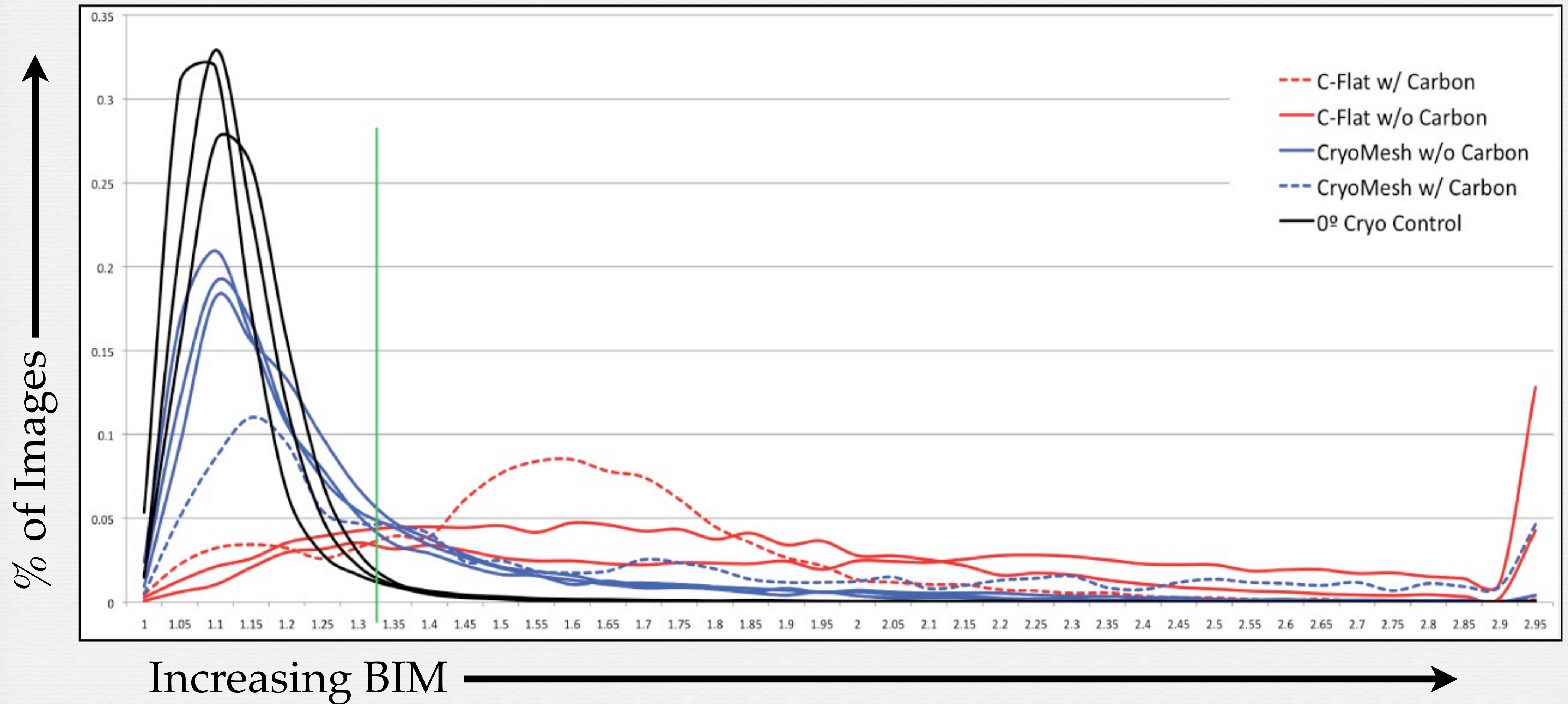
Experiment	Grid Type	Additional Carbon Layer ?	% BIM free	# of images
08feb04c	C-Flat	No	16% (9/57)	83
09jun04a	C-Flat	No	13.9% (10/72)	68
07nov27b	C-Flat	Yes	5% (4/81)	52
09jun11a	CryoMesh	No	73.4% (179/244)	138
09mar12c	CryoMesh	No	63.8% (74/116)	197
08feb05c	CryoMesh	Yes	48.2% (137/284)	32



Evaluating BIM Locality and Severity

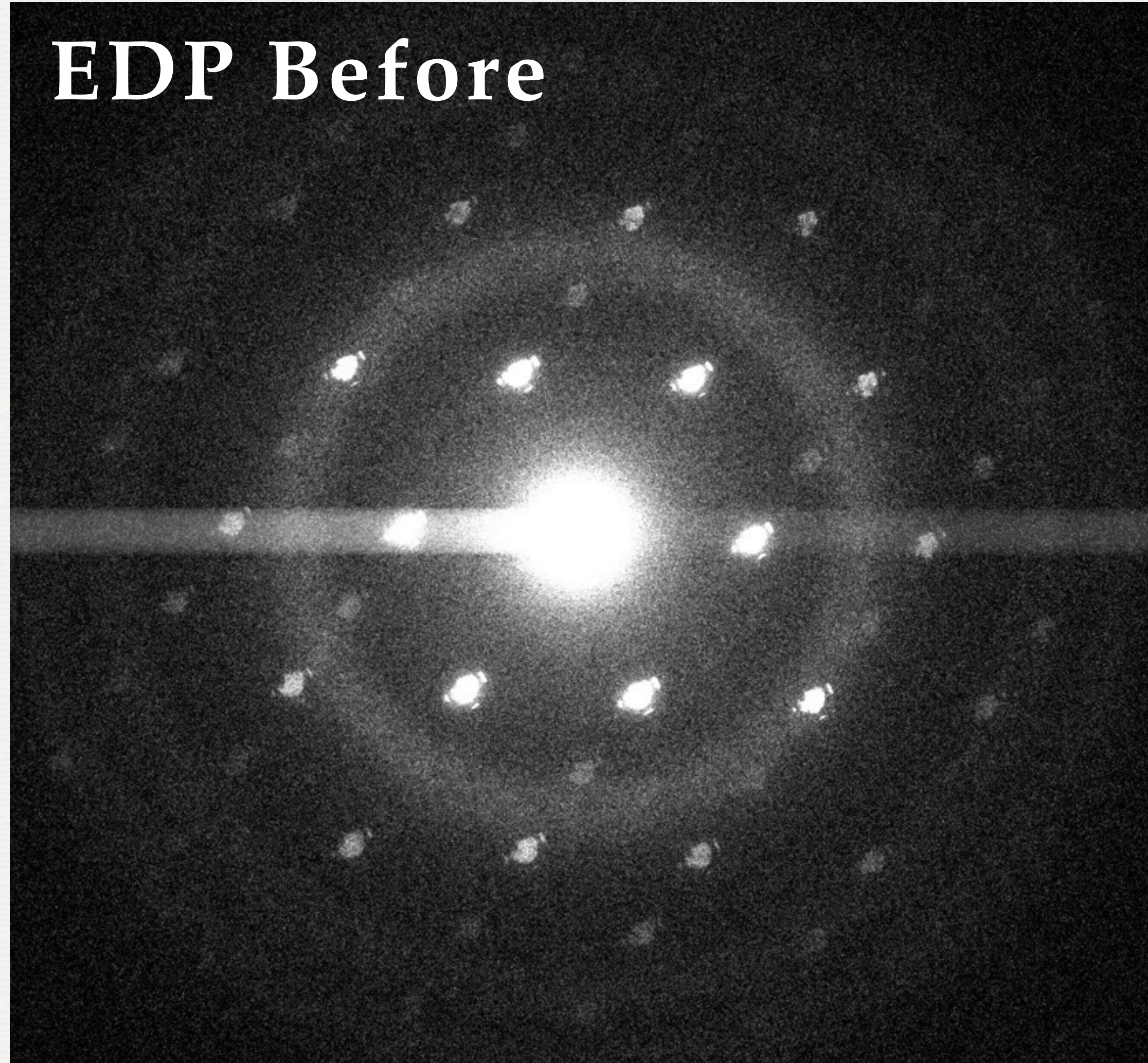


Evaluating BIM Locality and Severity



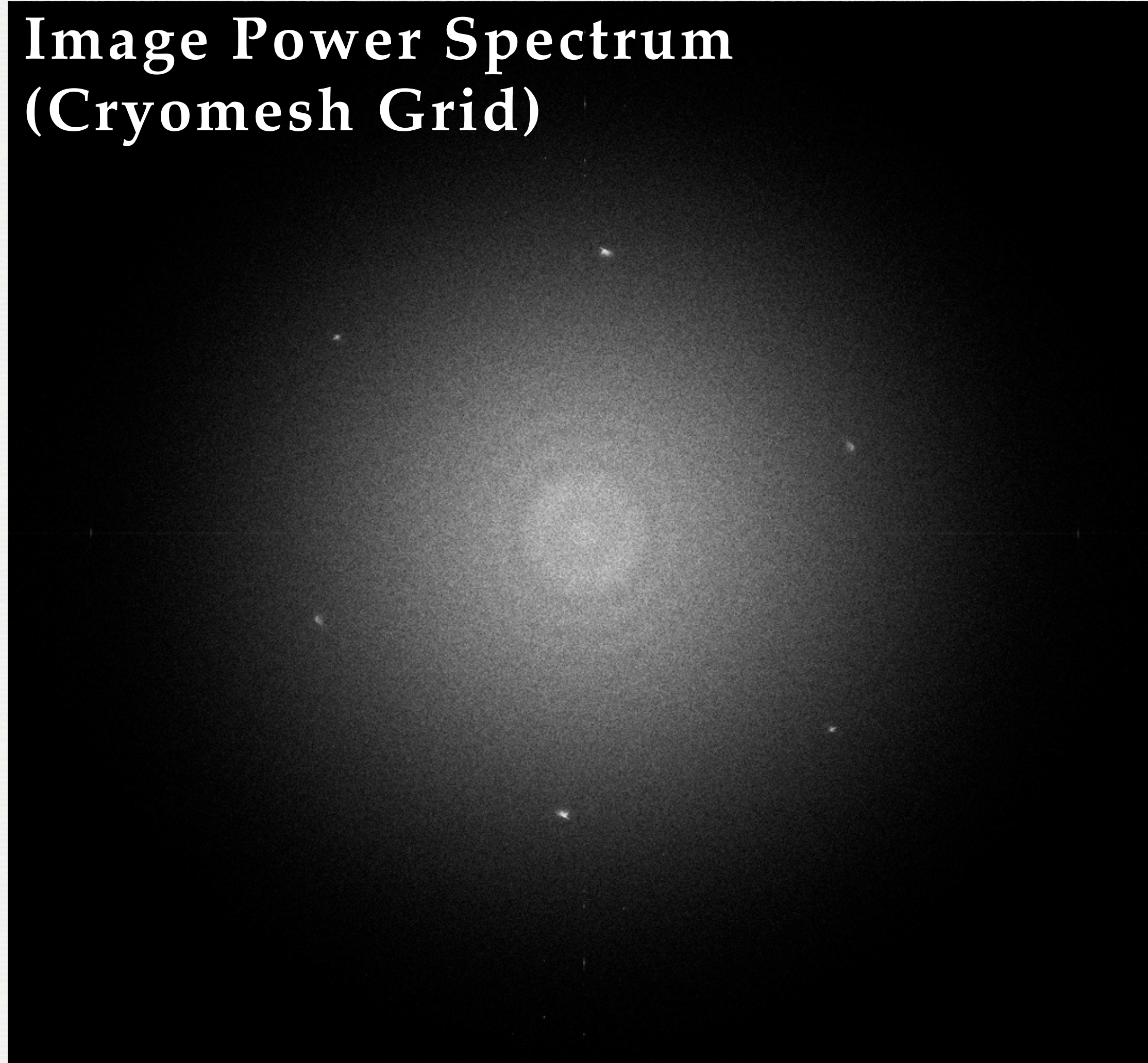
CryoMesh Paraffin Diffraction

EDP Before

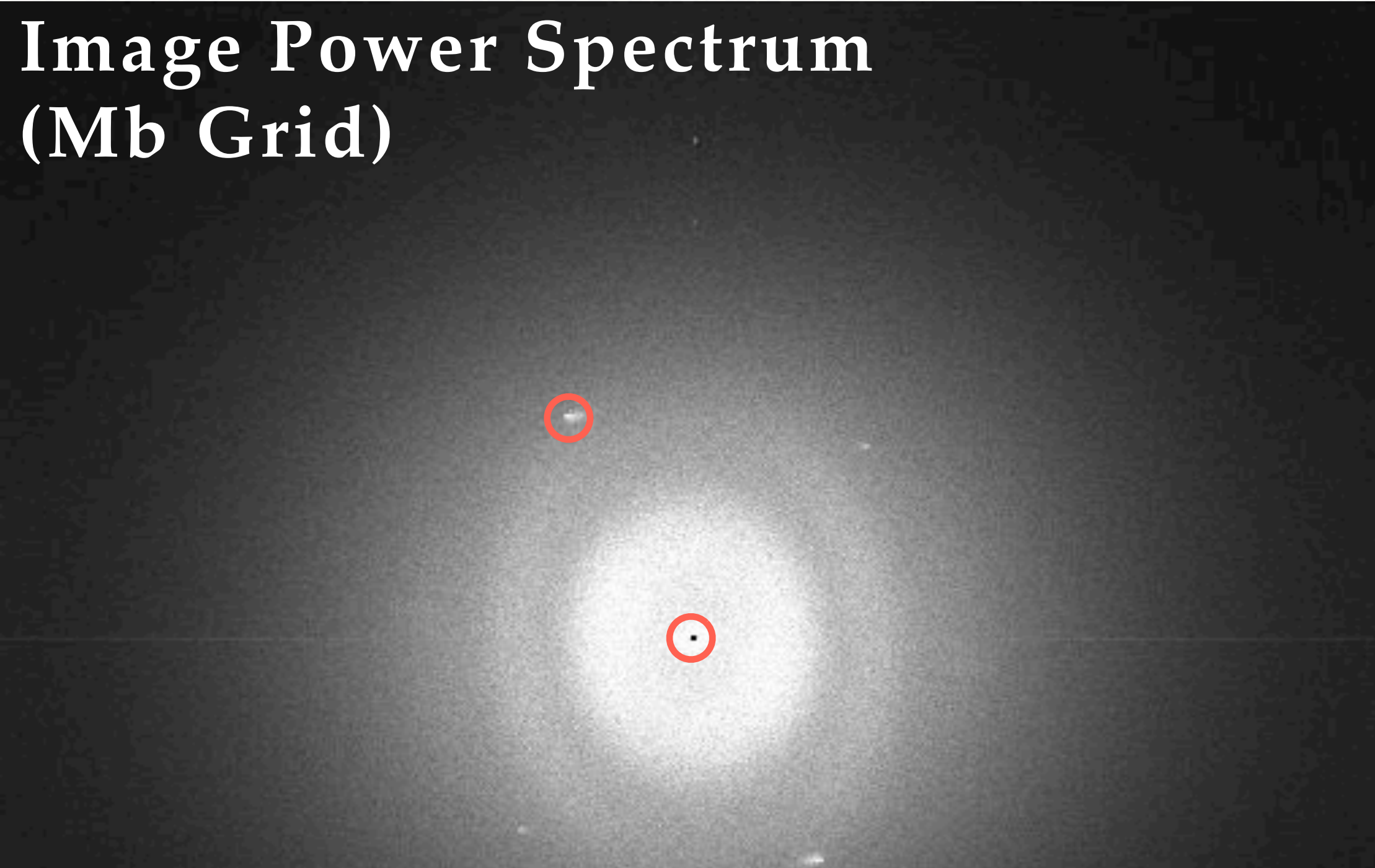


Cryomesh Paraffin Diffraction

**Image Power Spectrum
(Cryomesh Grid)**

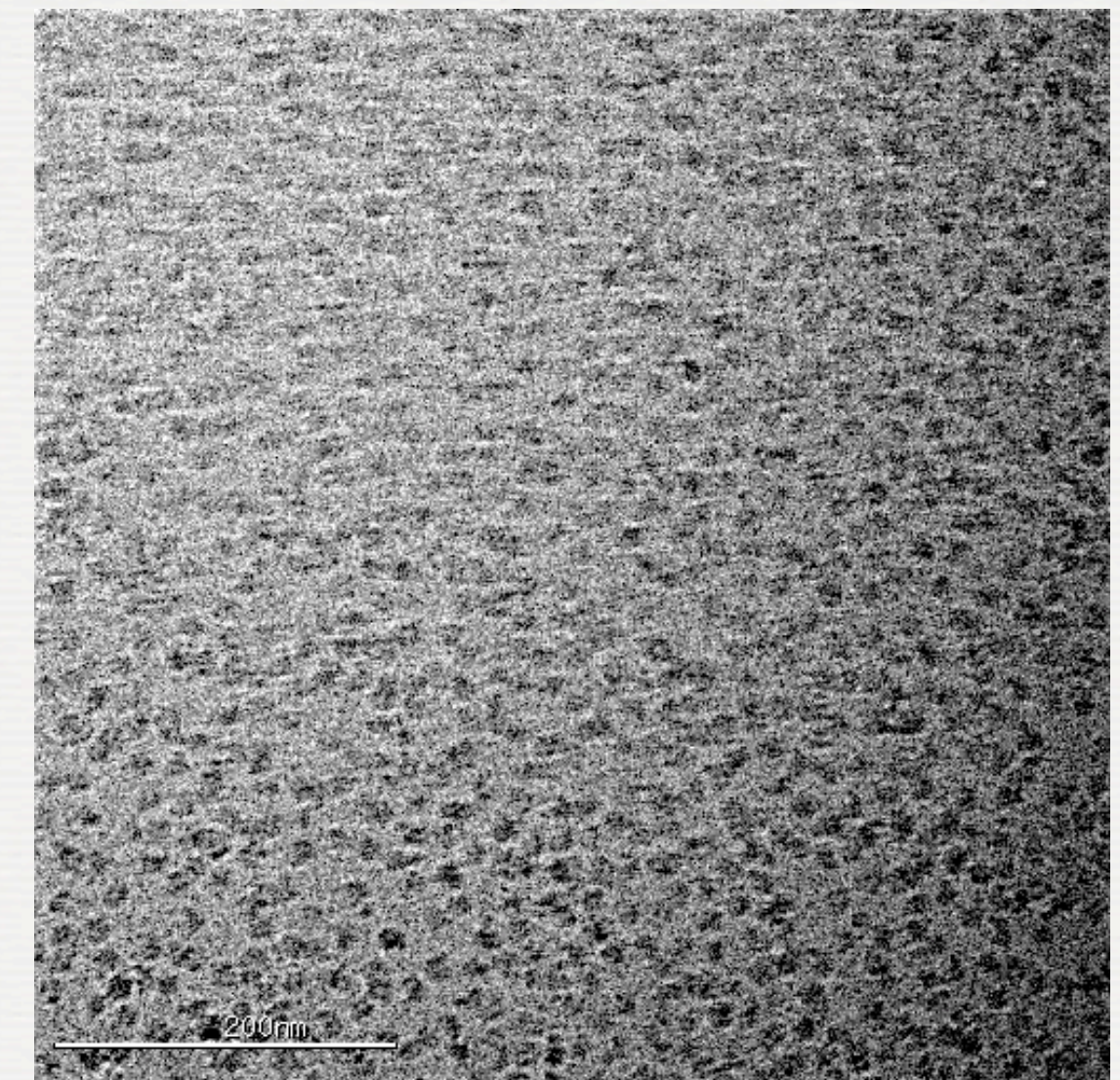
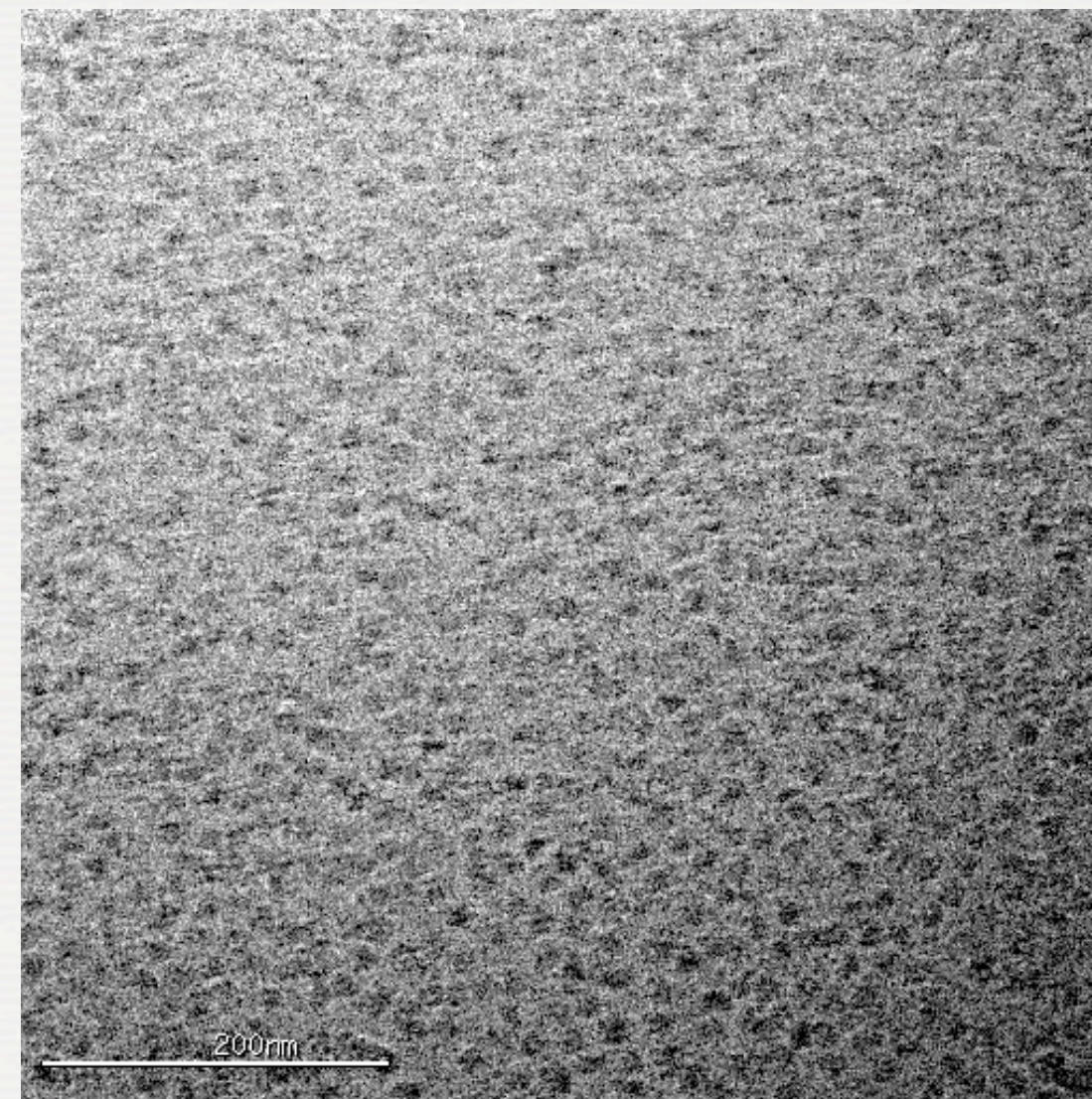
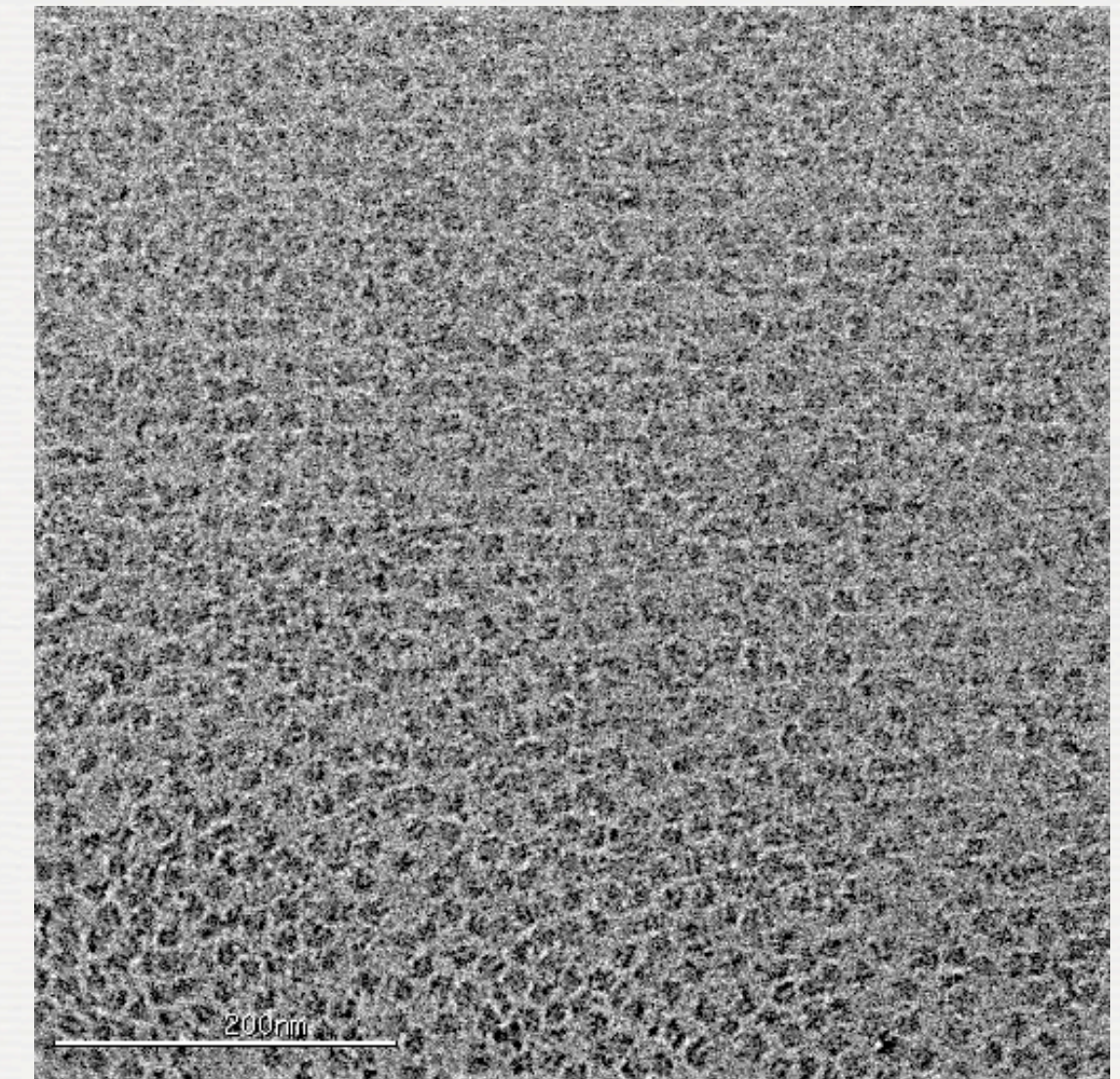
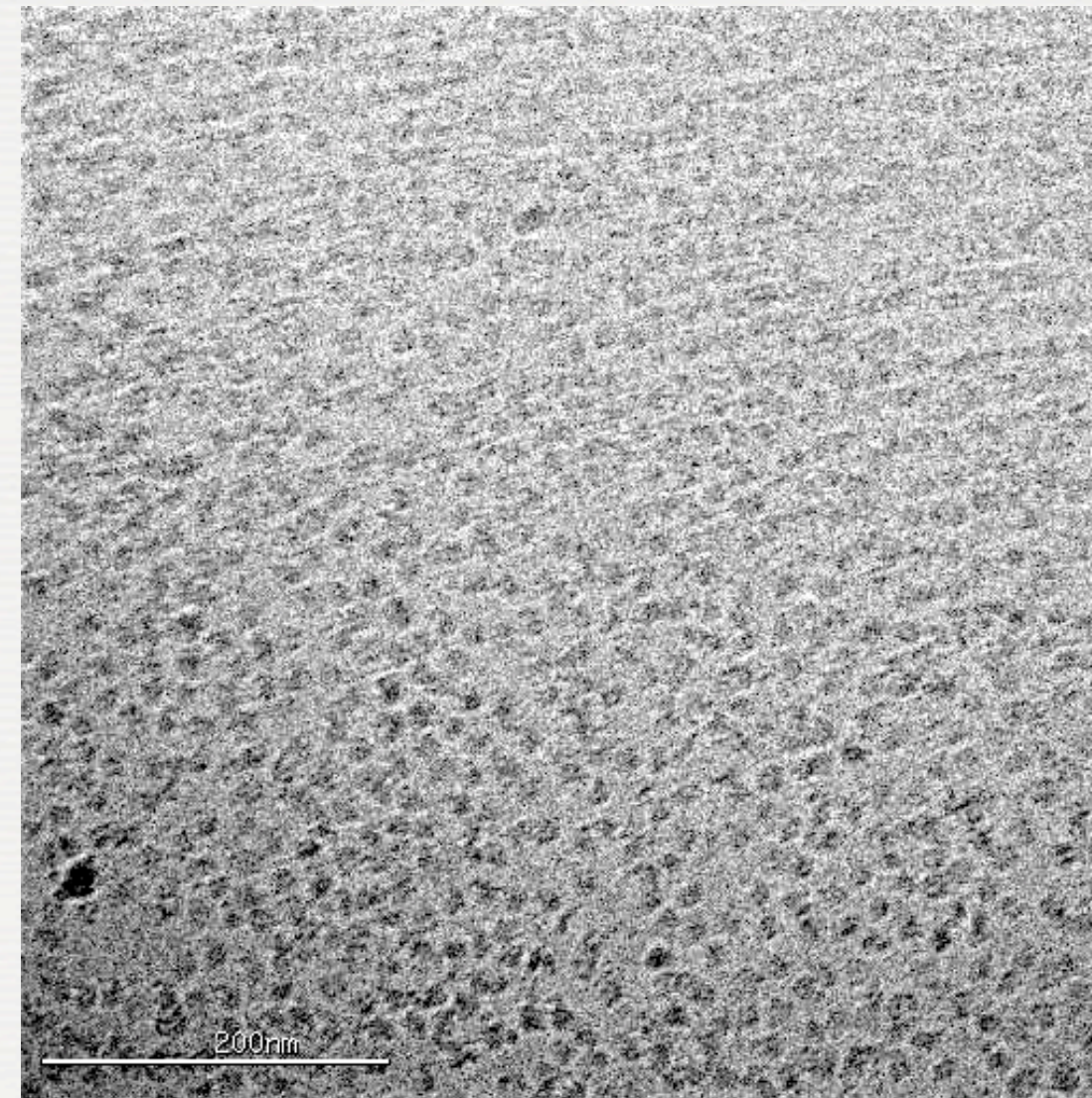
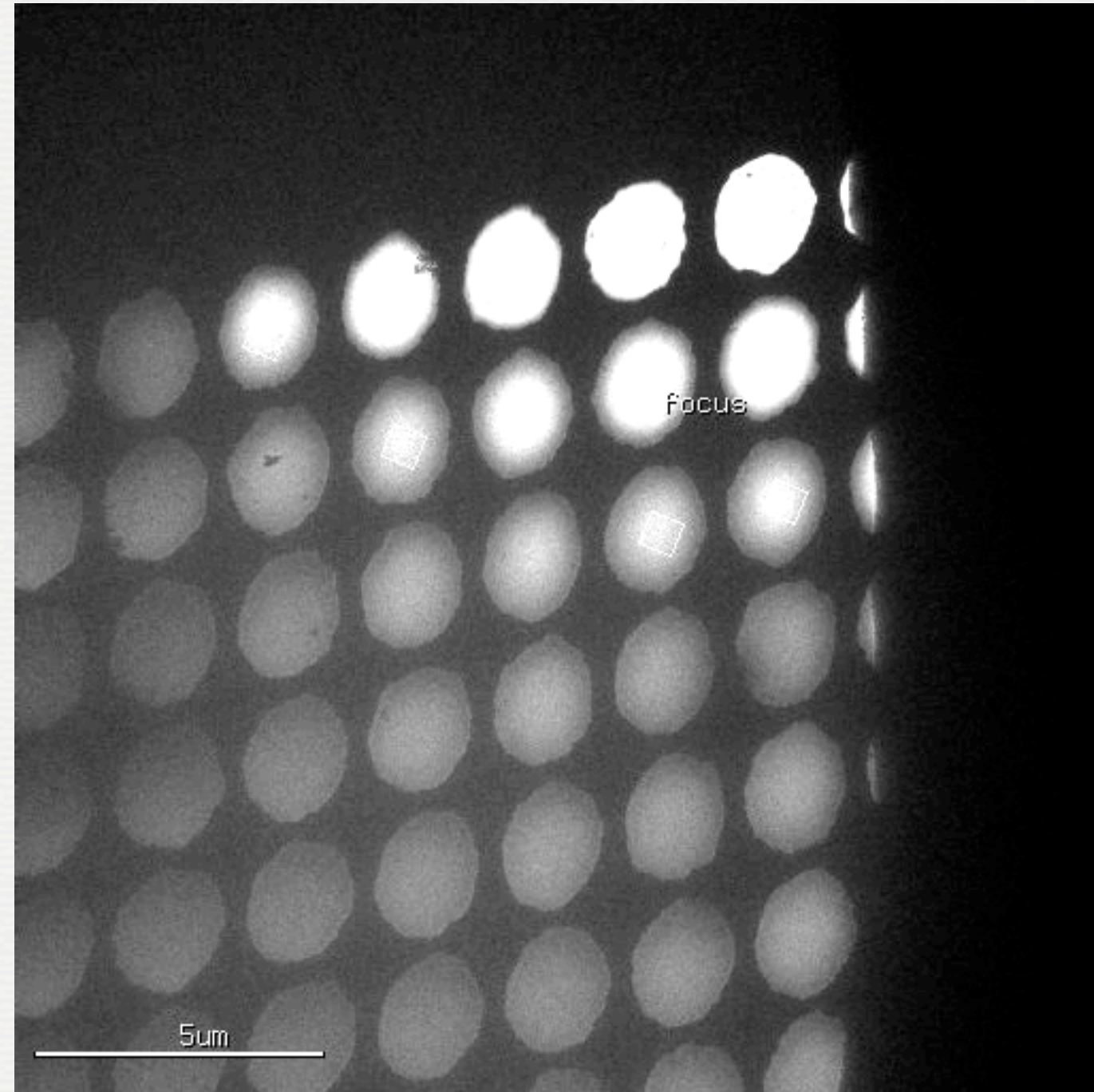


Cryomesh Paraffin Diffraction

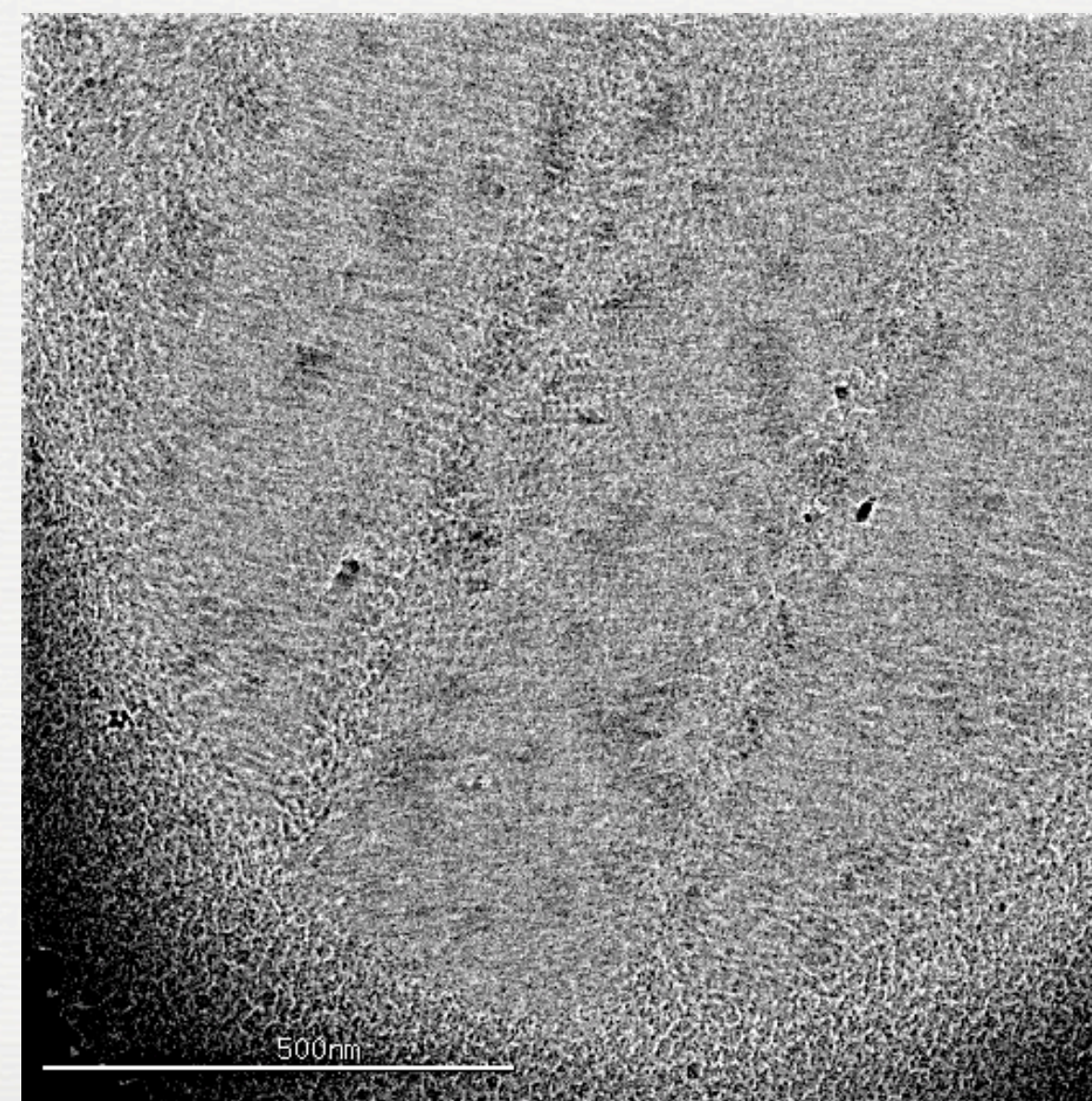
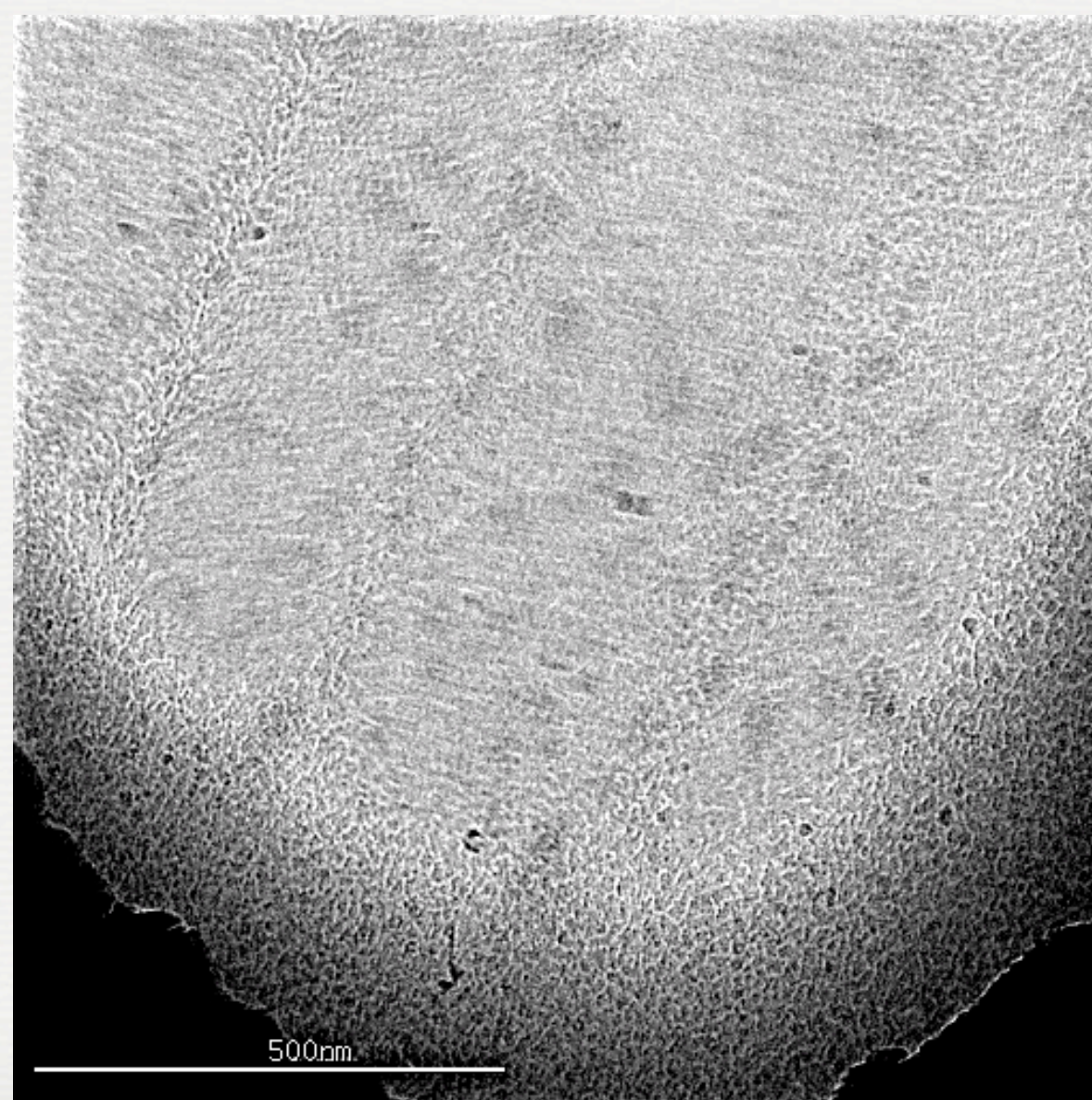
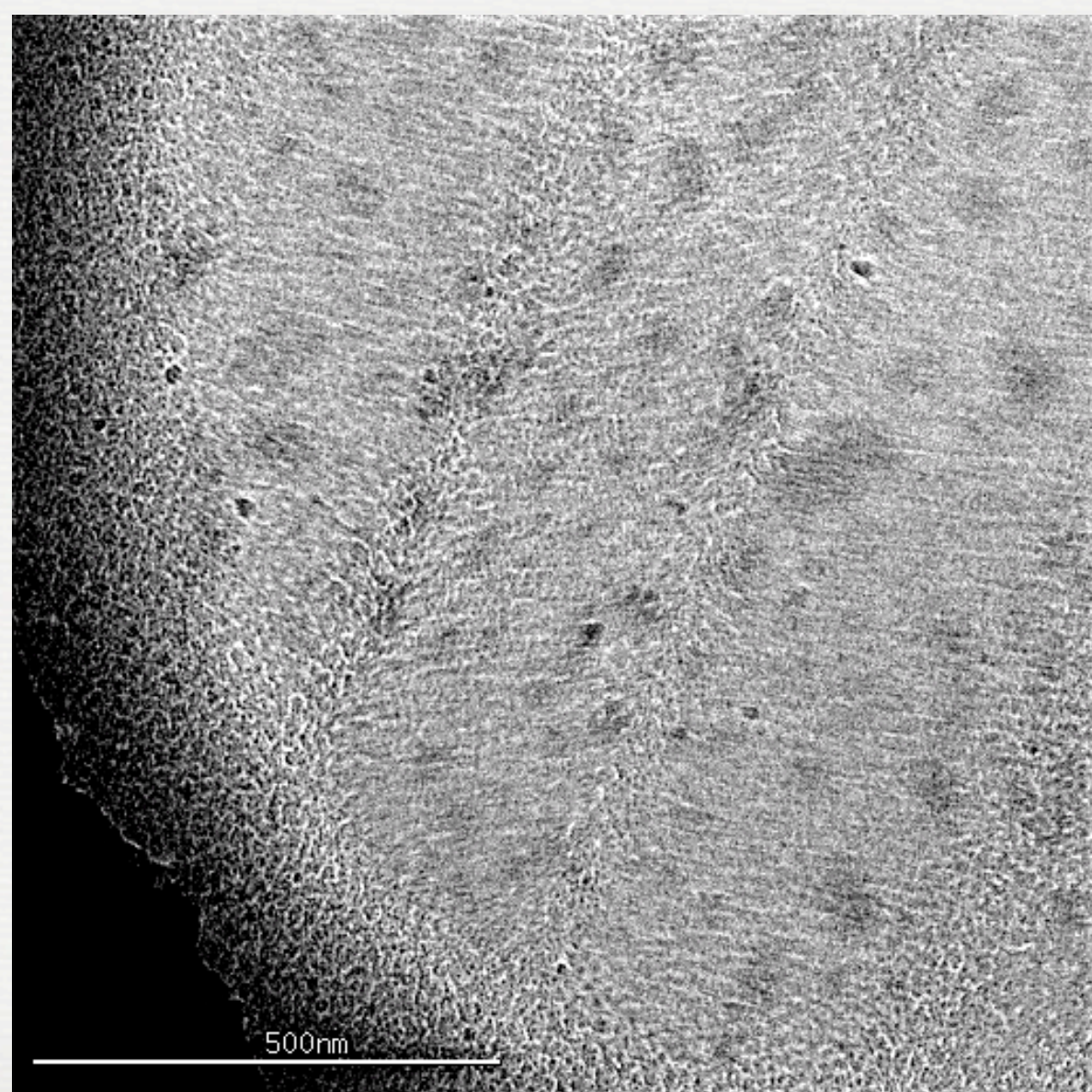
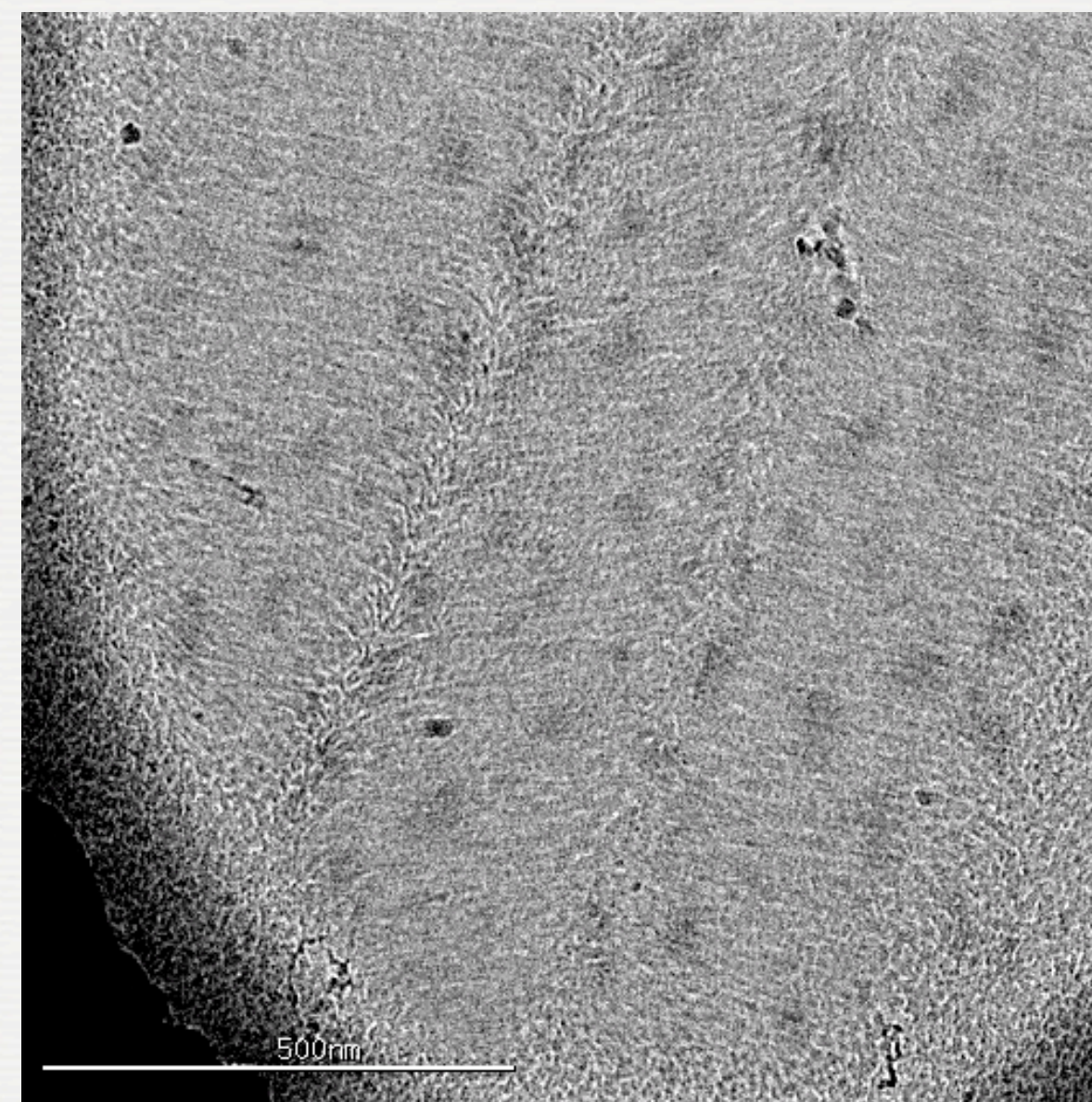
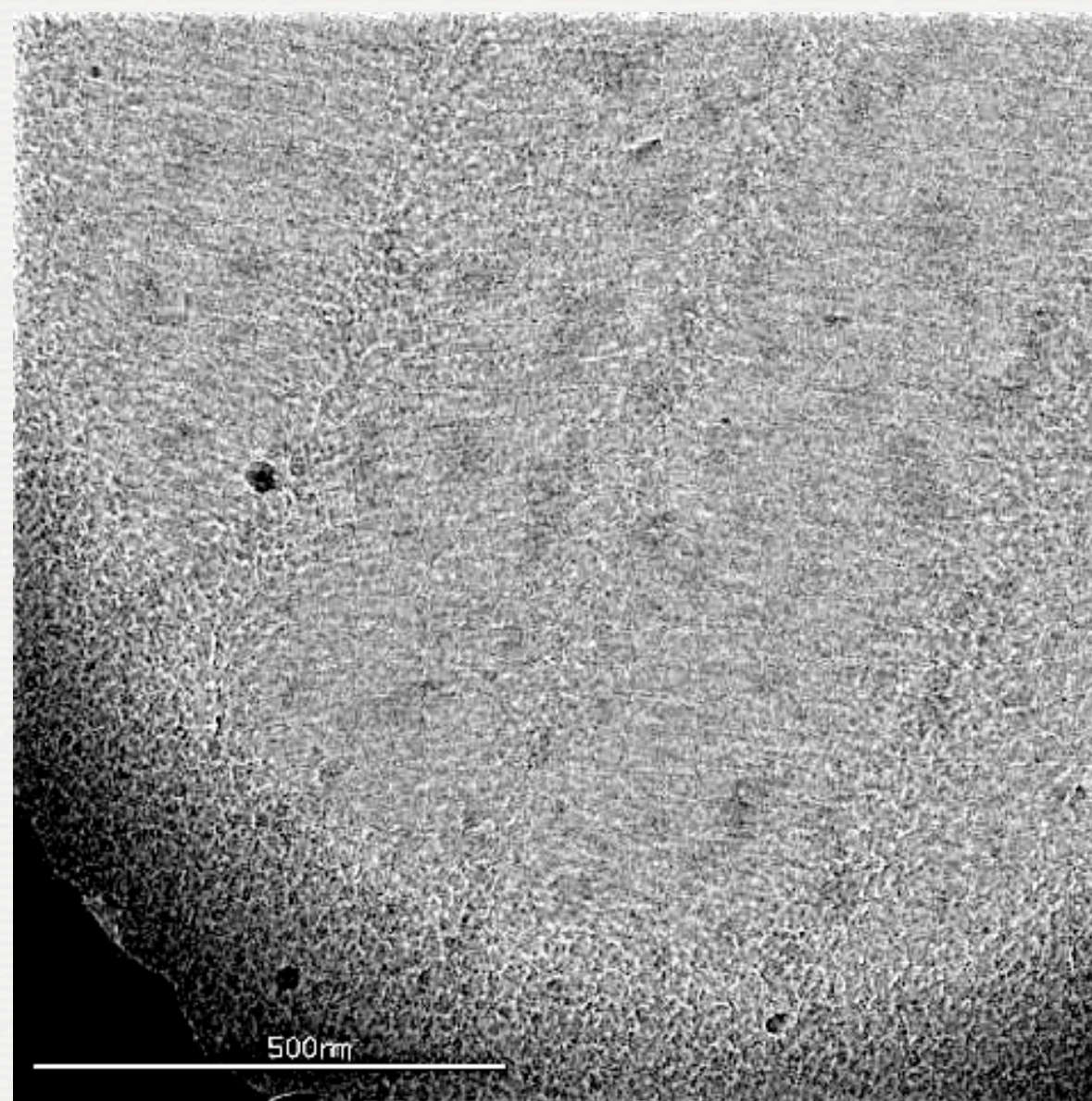
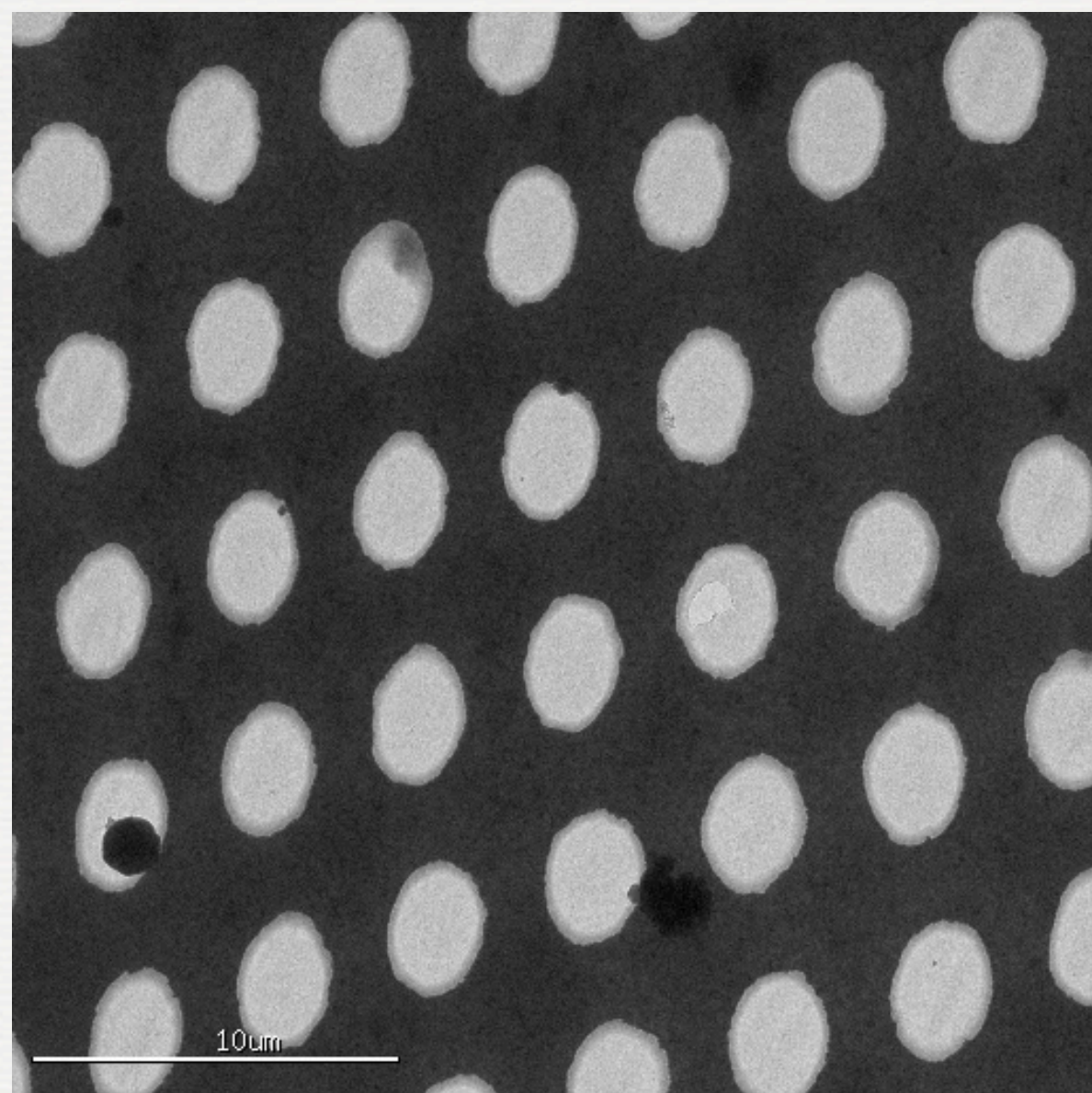


	Max I_g/I_o	Min I_g/I_o	Overall Avg
Cryomesh	0.0365	0.0138	0.0245
Mb Grids	0.00927	0.00358	0.00634

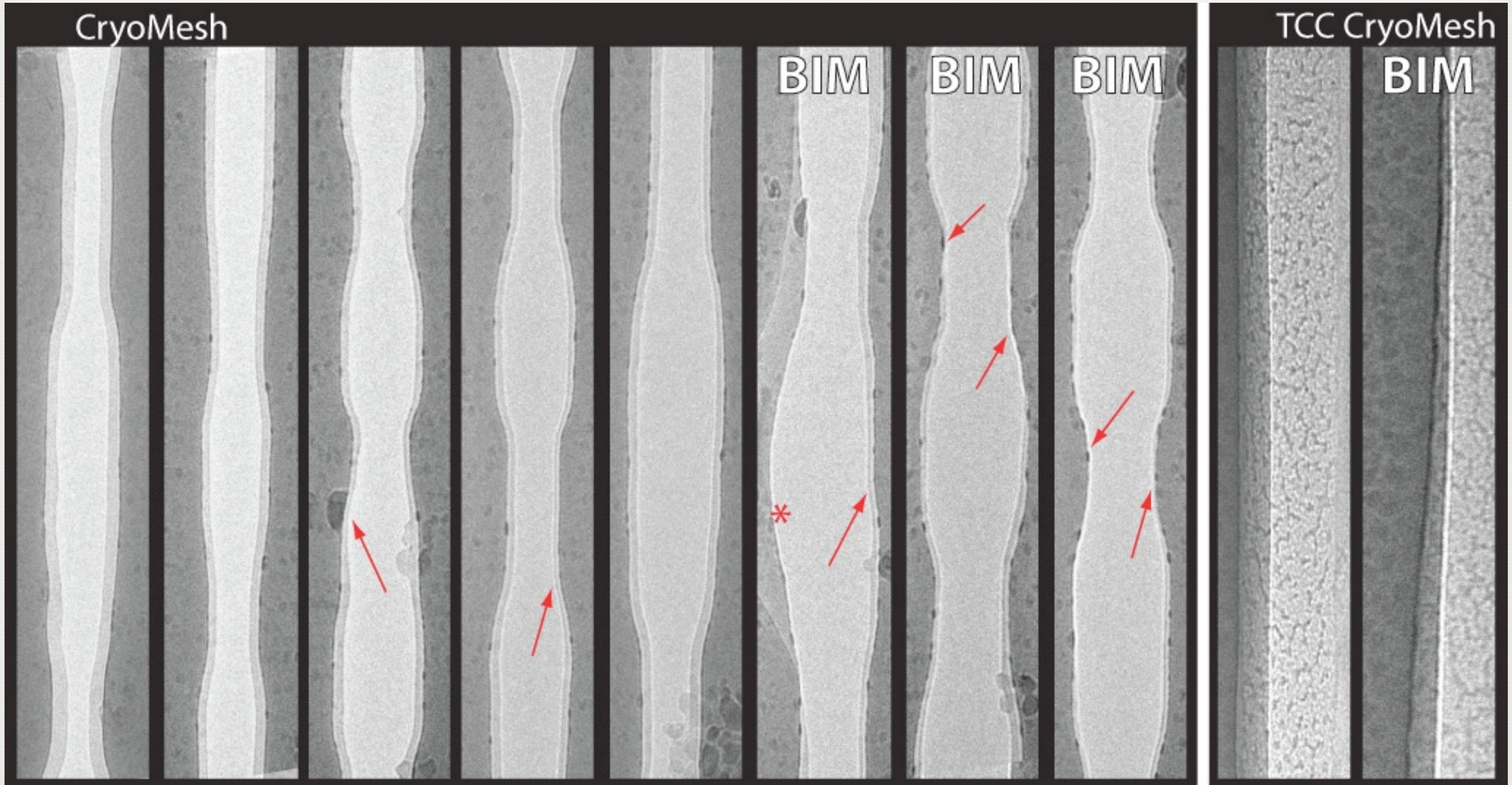
Uneven Ice On Cryomesh



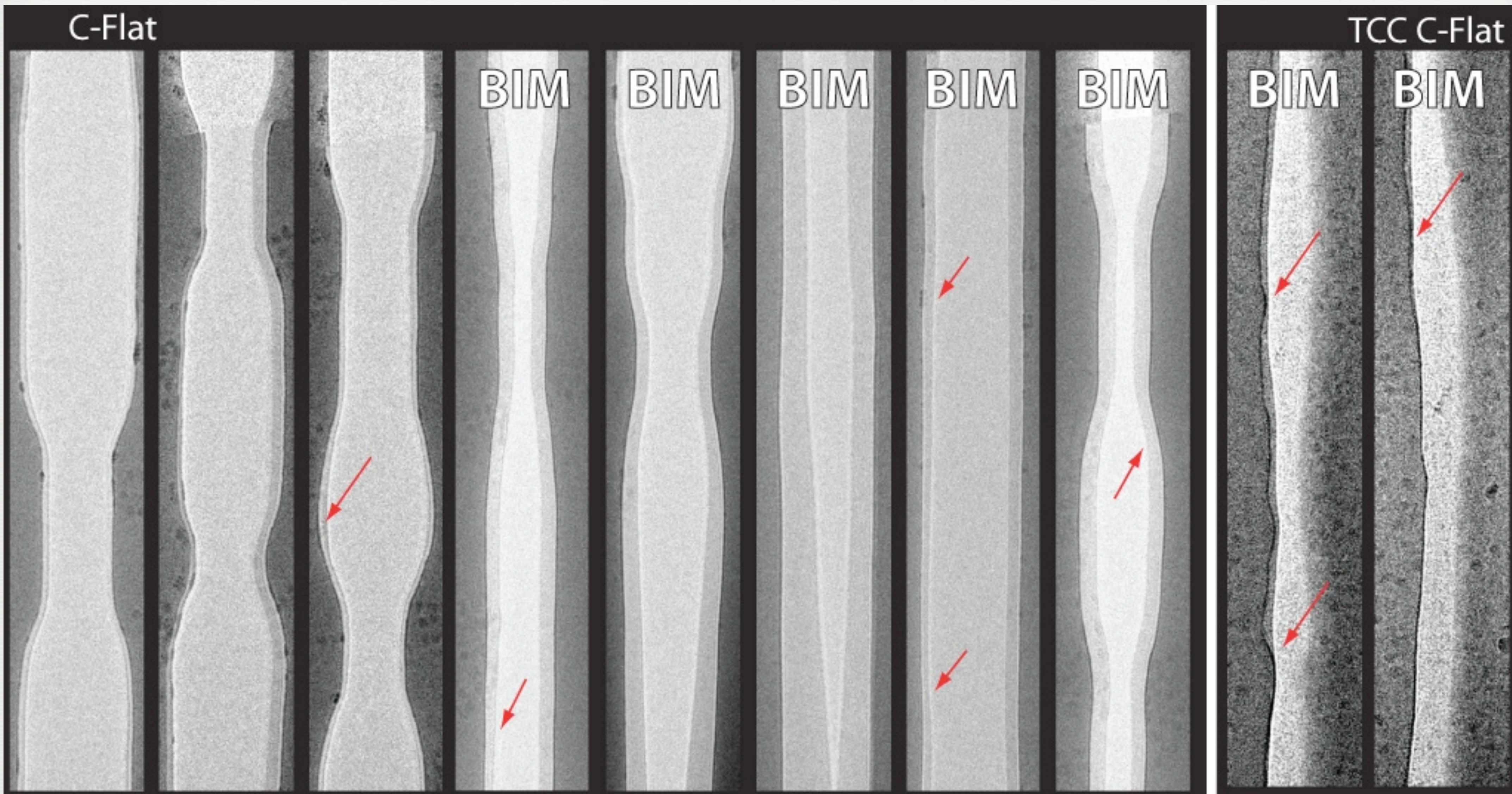
'Eutectic' Ice On Cryomesh



Ice Channels In Cryomesh



Ice Channels In C-Flats



Summary

- BIM Observations
 - Exact mechanism undetermined, but 'quality' of ice appears important
- CryoMesh Observations
 - Their flatness / rigidity appears to be an important characteristic
 - Brittle (hard to work with), tend toward thick ice, difficult to focus on
 - Could increase useable cryo images at tilt from ~10% to ~75% (or higher)

Acknowledgements



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Cryomesh Catalase Diffraction at Tilt

Cryomesh 0°

Cryomesh 45-50°

