CRYOEM 001 :
EM SUPPORT FILMS AND GRIDS

NCCAT Embedded Training – Master Class series

September 16, 2020

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING

New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER

CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA

Building a cryoEM toolkit

EM compatible samples

EM support films and grids

Sample preparation

Tools of the trade:

- microscopes and detectors

Microscope operations

Data collection strategies

Data assessment & QC

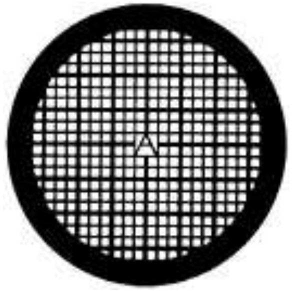
Data processing:

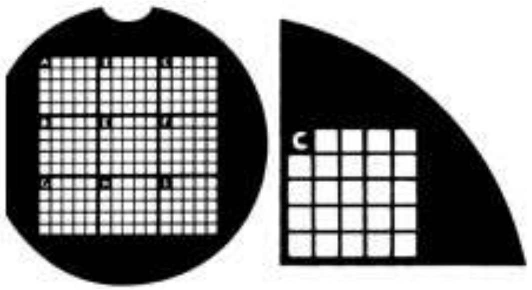
- cryoEM IT infrastructure
- On-the-fly feedback
- 3D Reconstruction

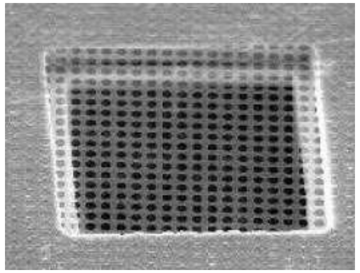
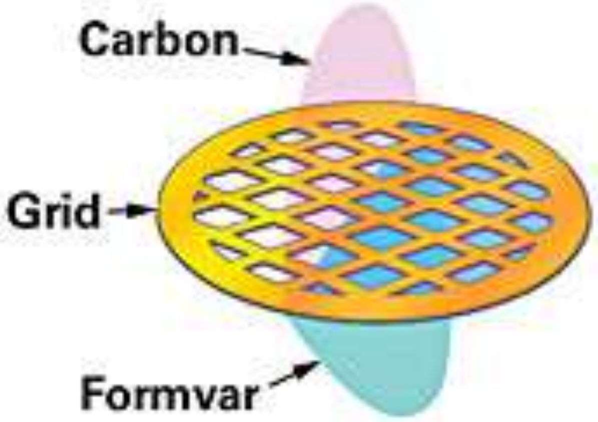
Visualization and validation

GRIDS





Common Materials

Copper

Nickel

Gold

Aluminum

Molybdenum

Titanium

Stainless Steel

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

GRIDS: STATS

Rough grid parameters

Rim Width: 350-400µm.

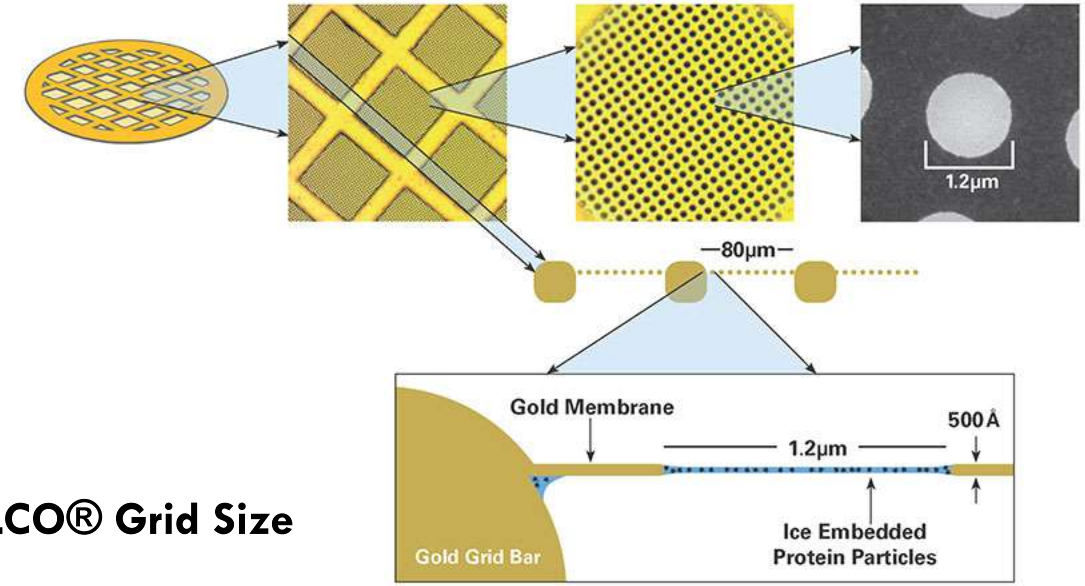
Thickness: PELCO® Grids are approximately 25µm thick.

Diameter: 3.0 to 3.05mm

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

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

Example 200 mesh pitch = 25.4/200 = 127µm



PELCO® Grid Size

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

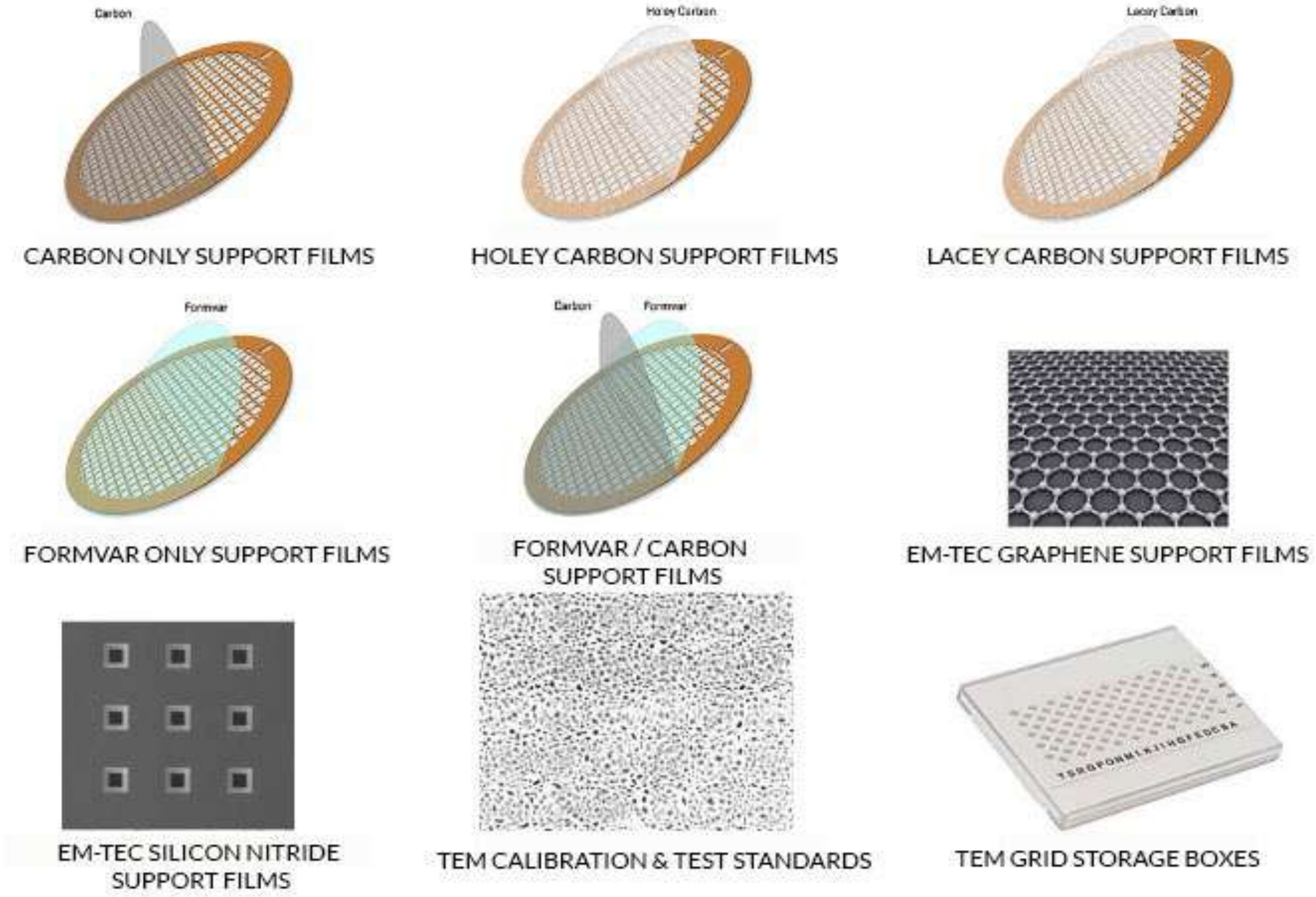
TERMINOLOGY

Grid (Cu, Au, Mo, etc…)

- mesh

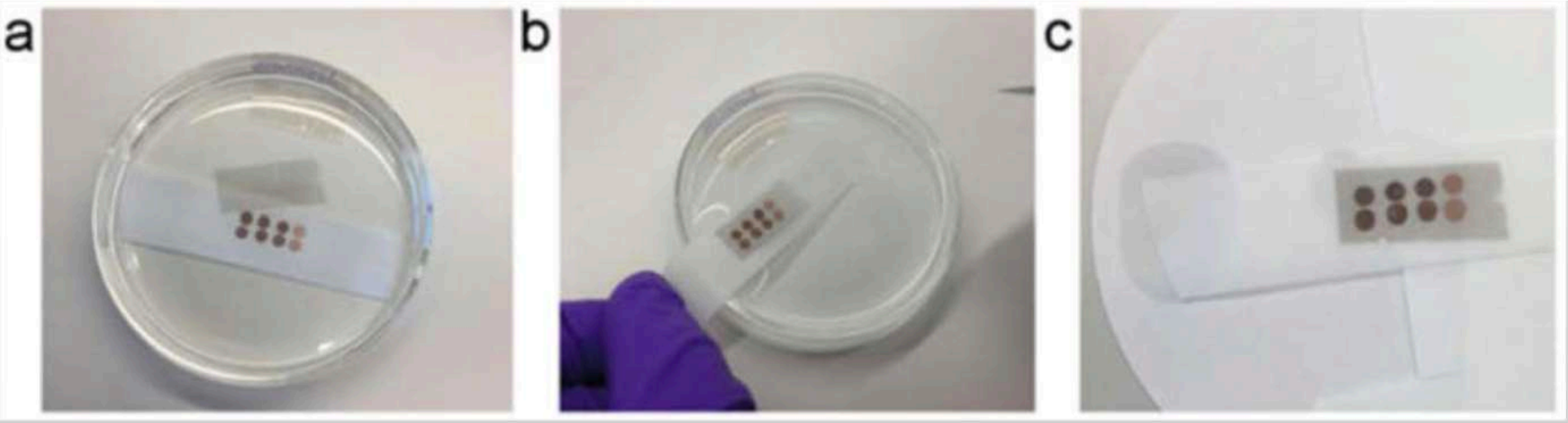
Foil (C, Au, etc…)

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



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

FLOATING SUPPORT FILMS

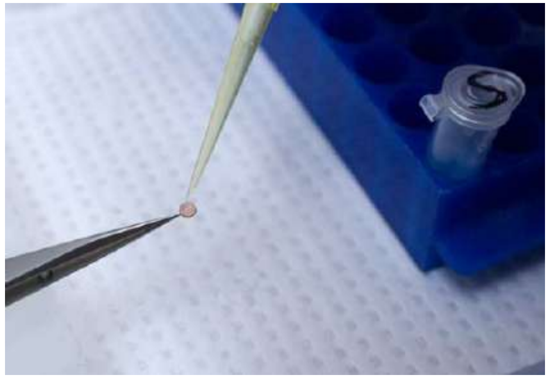


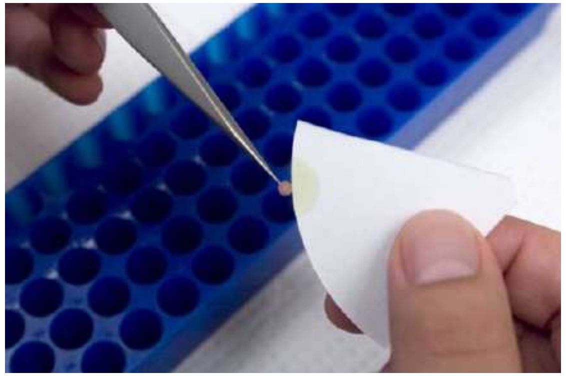
Procedure for applying support films

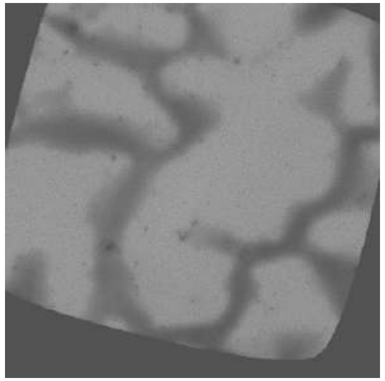
Chang and Barford, 2018

SUPPORT FILMS

Support films used in negative stain







Baker, 2007

www.mcb.ucdavis.edu/cryoem/microscopy101.html

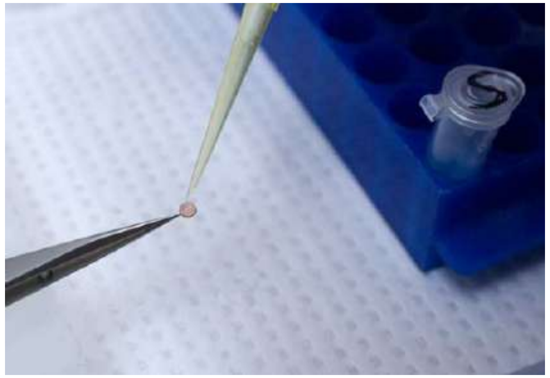
NEGATIVE STAINING

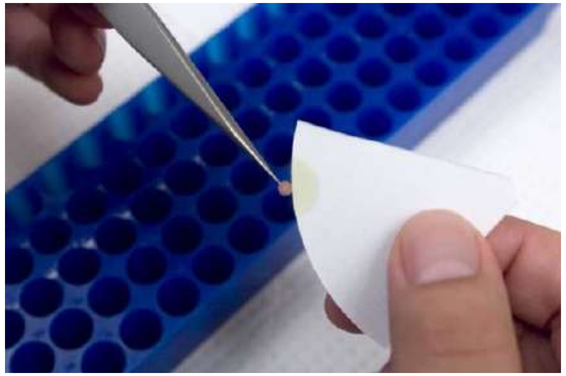
Heavy metal salt solution surrounds sample

- Continuous carbon support film
- Protocol: glow discharge, sample, wash, stain
- NCCAT: UA/UF, PTA, ammonium molybdate

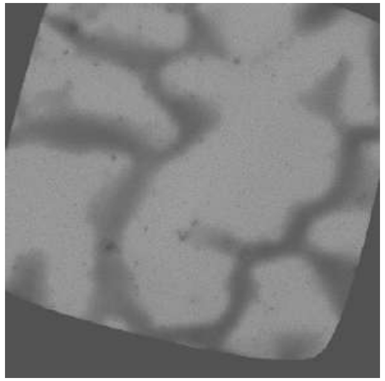
Advantages: high contrast, easy to learn, high SNR, radiation resistant, 3D reconstruction possible

Disadvantages: structural collapse & flattening artifacts, non-native environment, ~20 Å max resolution





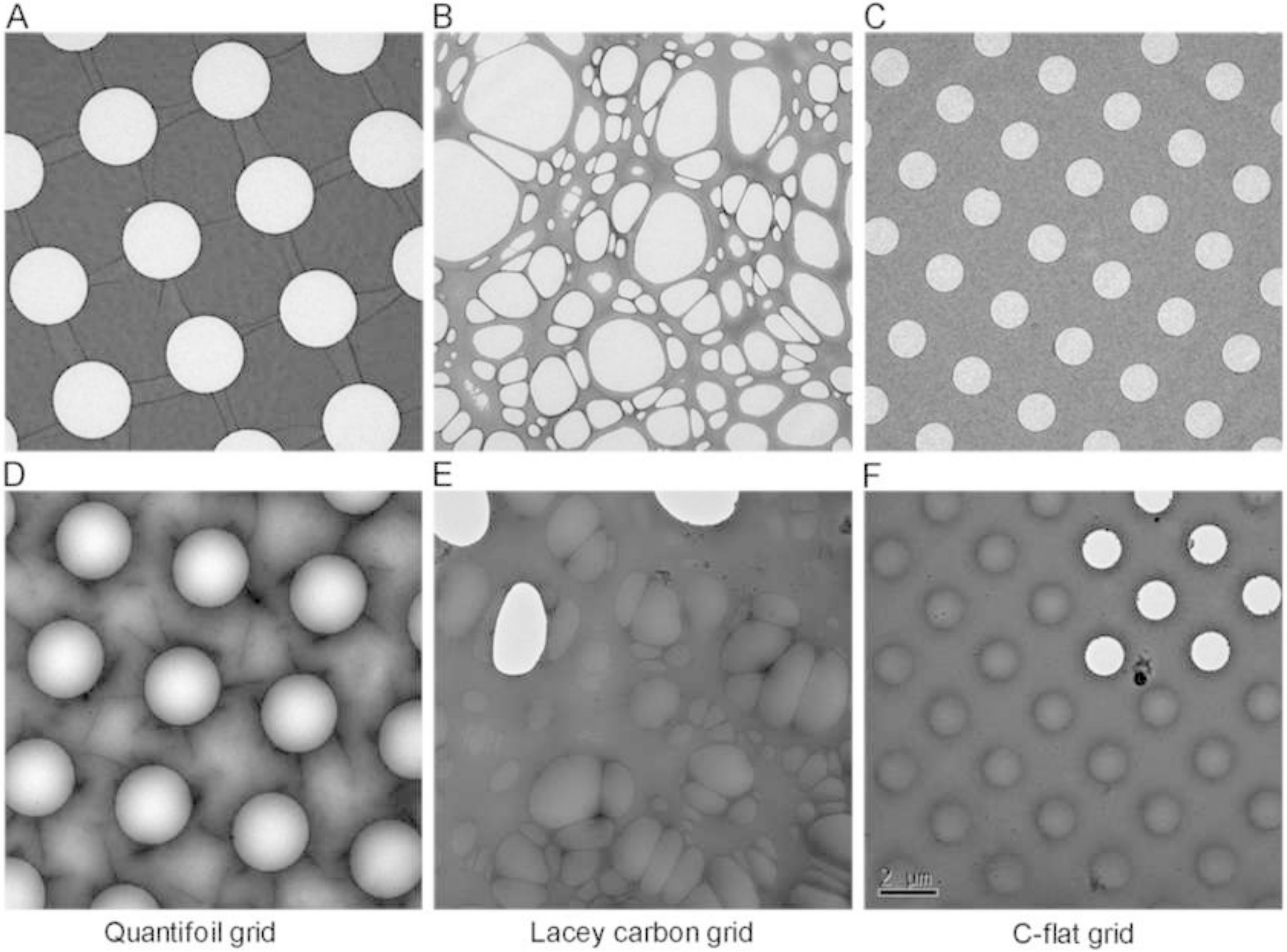
Baker, 2007



www.mcb.ucdavis.edu/cryoem/microscopy101.html

SUPPORT FILMS

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



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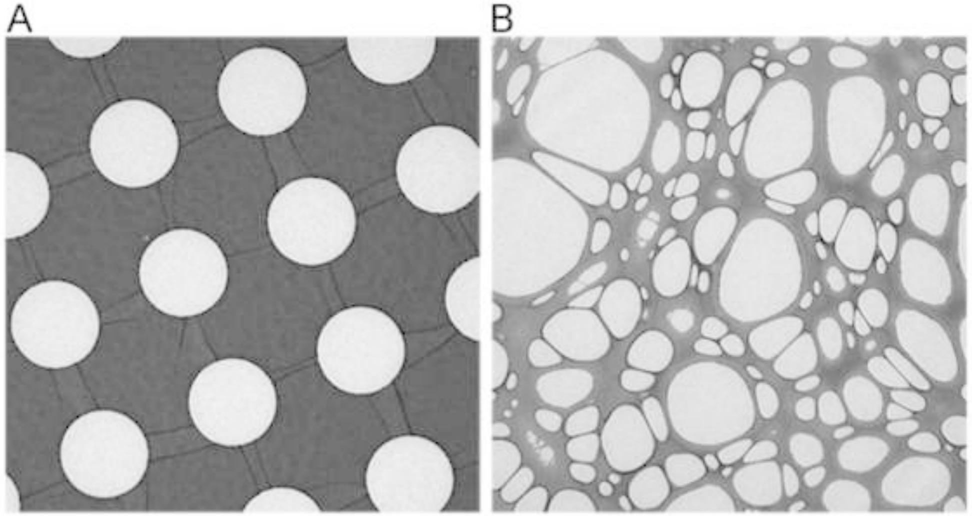

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Making your own holey carbon:
http://dx.doi.org/10.1016/j.jsb.2013.11.002

Making your own lacey carbon:
https://www.2spi.com/making-lacey-carbon/

HOMEMADE HOLEY CARBON

Journal of Structural Biology 185 (2014) 42–47

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journal homepage: www.elsevier.com/locate/yjsbi

Fabrication of carbon films with ~500 nm holes for cryo-EM with a direct detector device

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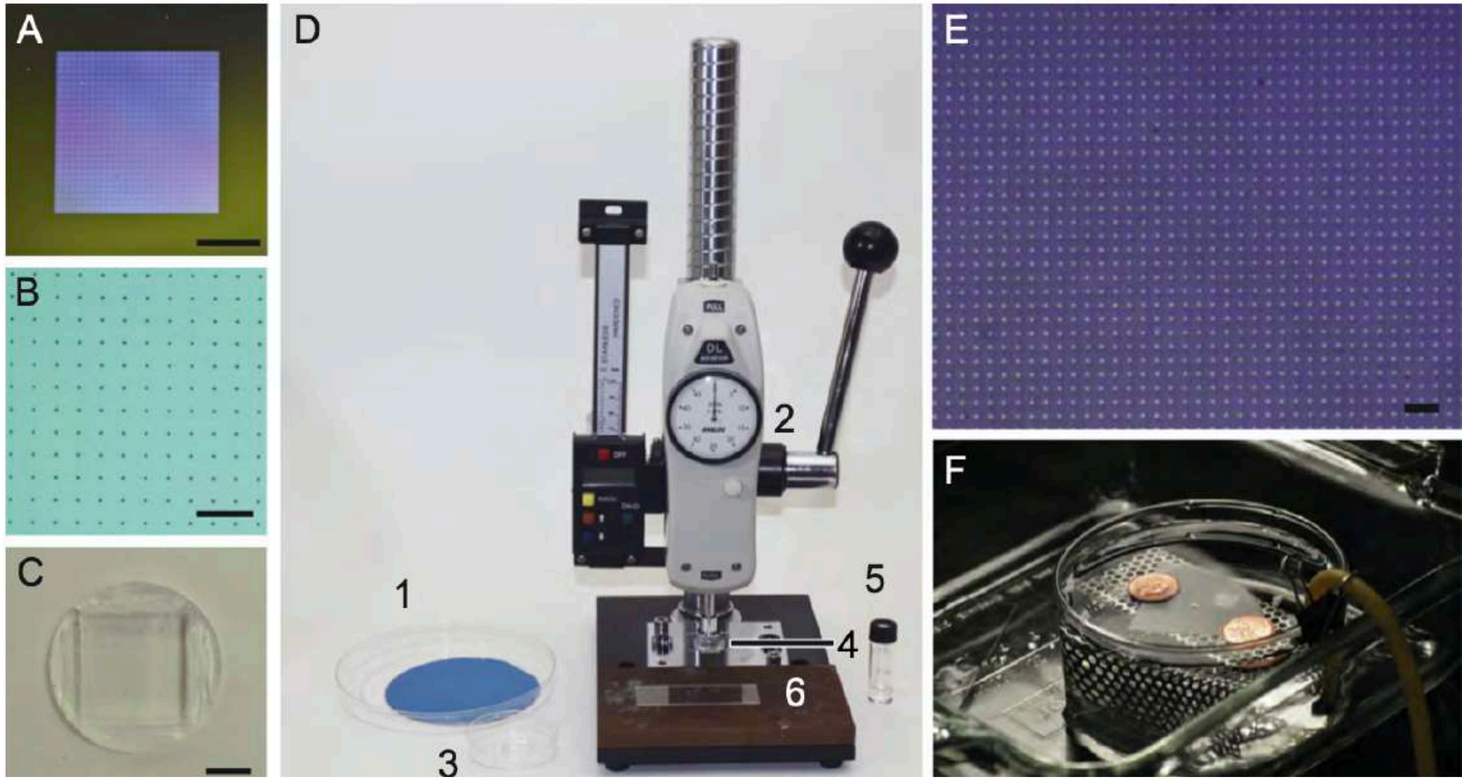
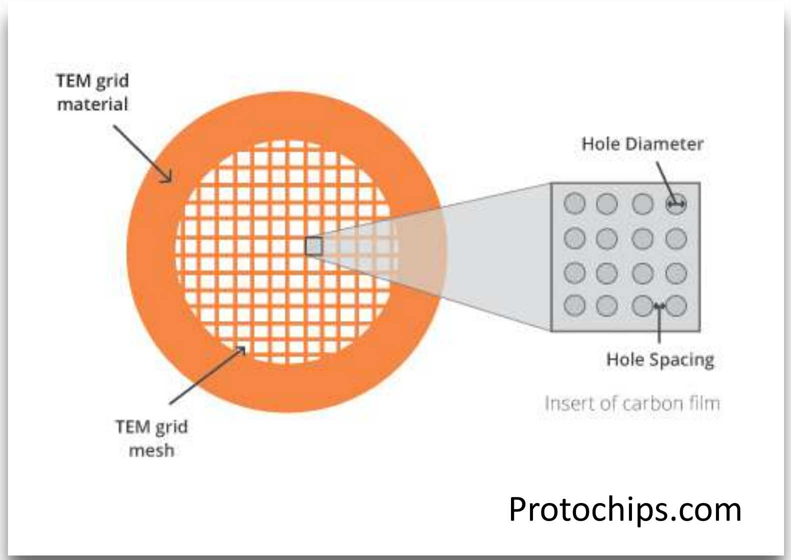
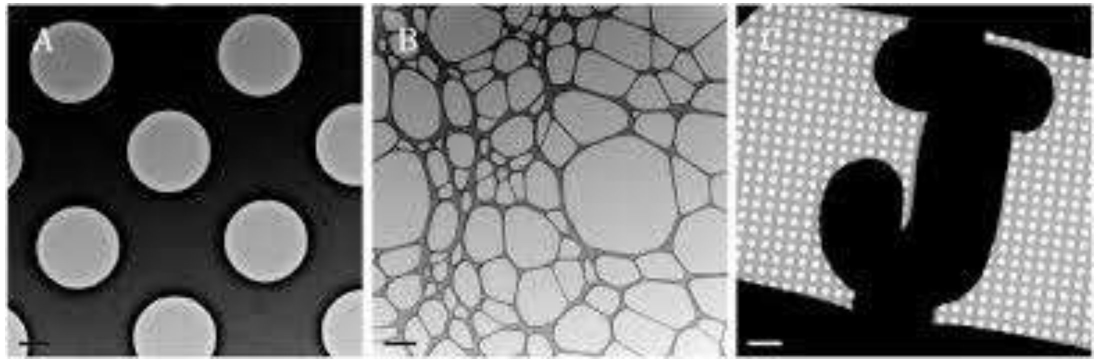
ABSTRACT

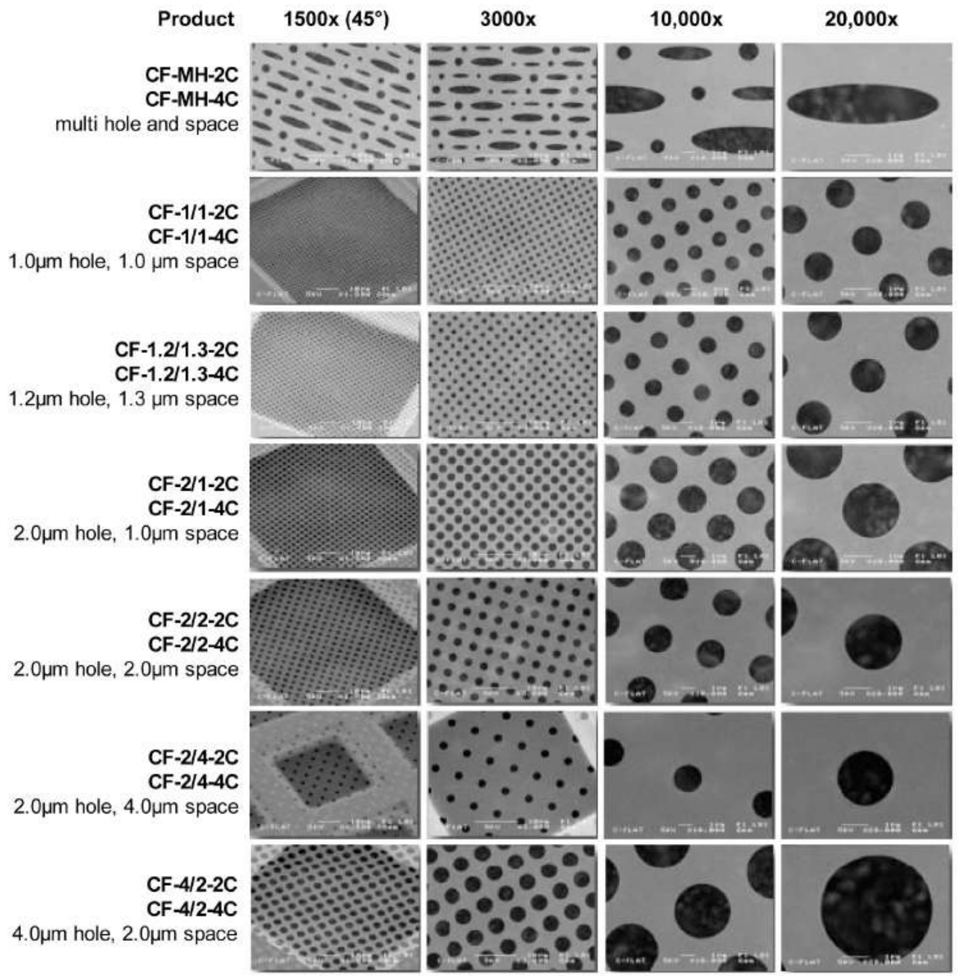
Single particle electron cryomicroscopy (cryo-EM) is often performed using EM grids coated with a perforated or holey layer of amorphous carbon. Regular arrays of holes enable efficient cryo-EM data collection and several methods for the production of micropatterned holey-carbon film coated grids have been described. However, a new generation of direct detector device (DDD) electron microscope cameras can benefit from hole diameters that are smaller than currently available. Here we extend a previously proposed method involving soft lithography with a poly(dimethylsiloxane) (PDMS) stamp for the production of holey-carbon film coated EM grids. By incorporating electron-beam (e-beam) lithography and modifying the procedure, we are able to produce low-cost high-quality holey-carbon film coated EM grids with ~500 nm holes spaced 4 μm apart centre-to-centre. We demonstrate that these grids can be used for cryo-EM. Furthermore, we show that by applying image shifts to obtain movies of the carbon regions beside the holes after imaging the holes, the contrast transfer function (CTF) parameters needed for calculation of high-resolution cryo-EM maps with a DDD can be obtained efficiently.



PLUNGE FREEZING







https://scienceservices.de/de/verbrauchsmaterial-werkzeuge/tem-grids/info-tem-grids/coatings-and-coating-materials

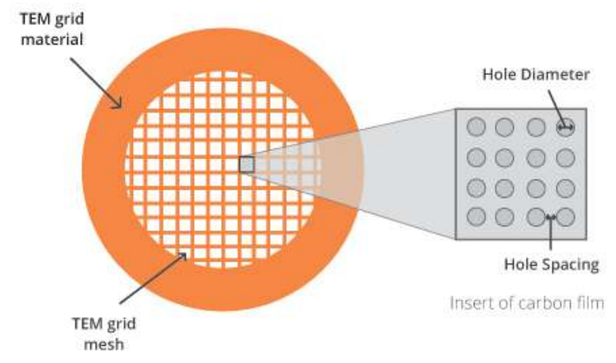
PLUNGE FREEZING

Sample suspended in physiological buffer

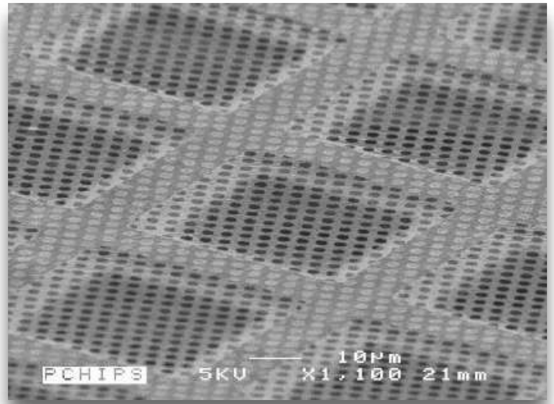
- Holey carbon support film: C-flats, Quantifoil
- Protocol: glow discharge, sample, blot, plunge freeze
- NCCAT: TFS Vitrobot, Leica EM GP, Gatan CryoPlunge Freezer 3, manual plunge freezer

Advantages: no fixation/dehydration/staining artifacts, learning curve, random orientation, higher resolution than stain

Disadvantages: low contrast, low SNR, radiation sensitive, difficult to visualize <100 kD, freezing artifacts

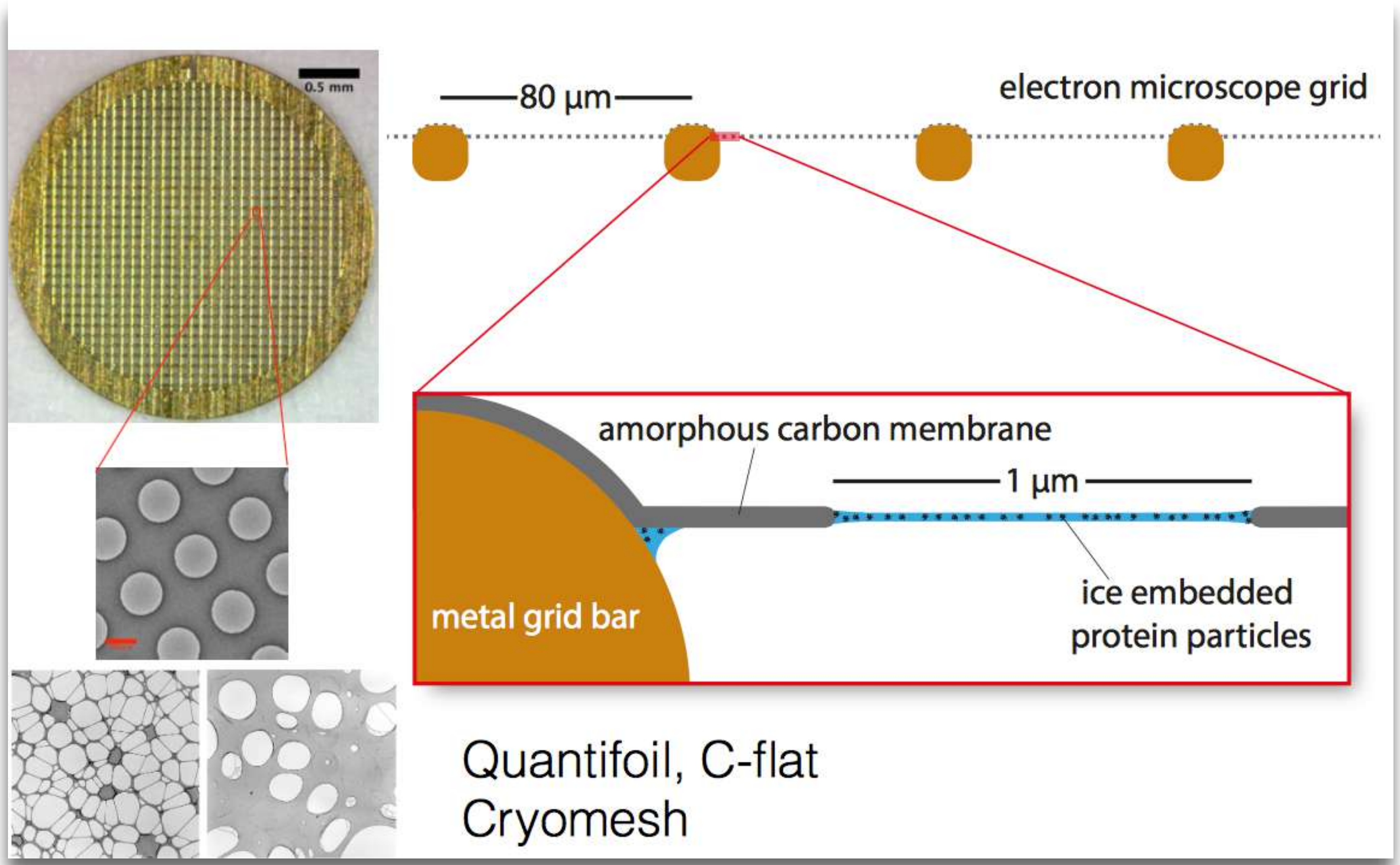


Protochips.com



www.mcb.ucdavis.edu/cryoem/microscopy101.html
www.mcb.ucdavis.edu/cryoem/microscopy101.html

TRADITIONAL SUBSTRATES FOR CRYOEM



Russo & Passmore, 2015

CHALLENGES

Proteins interact with surfaces present during the blotting process

Denaturation of proteins, preferential orientations

Electron radiation induces motion of the particles and substrates

Image blurring

Additional layer of carbon reduces signal to noise per particle

alignment more difficult

Overall lack of reproducibility from grid to grid

GOLD GRIDS

- Holey gold foil on gold mesh grid

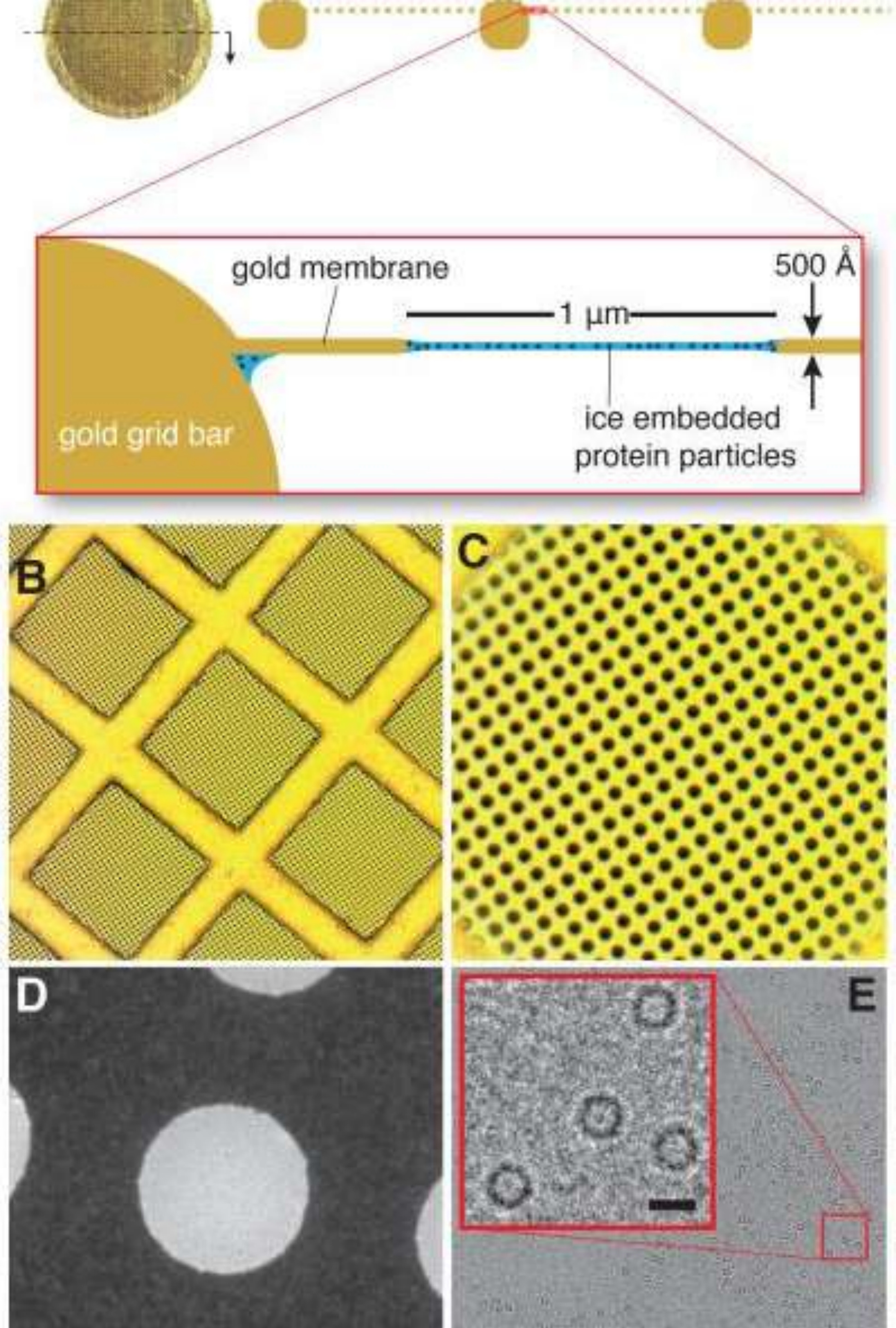
Advantages:

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

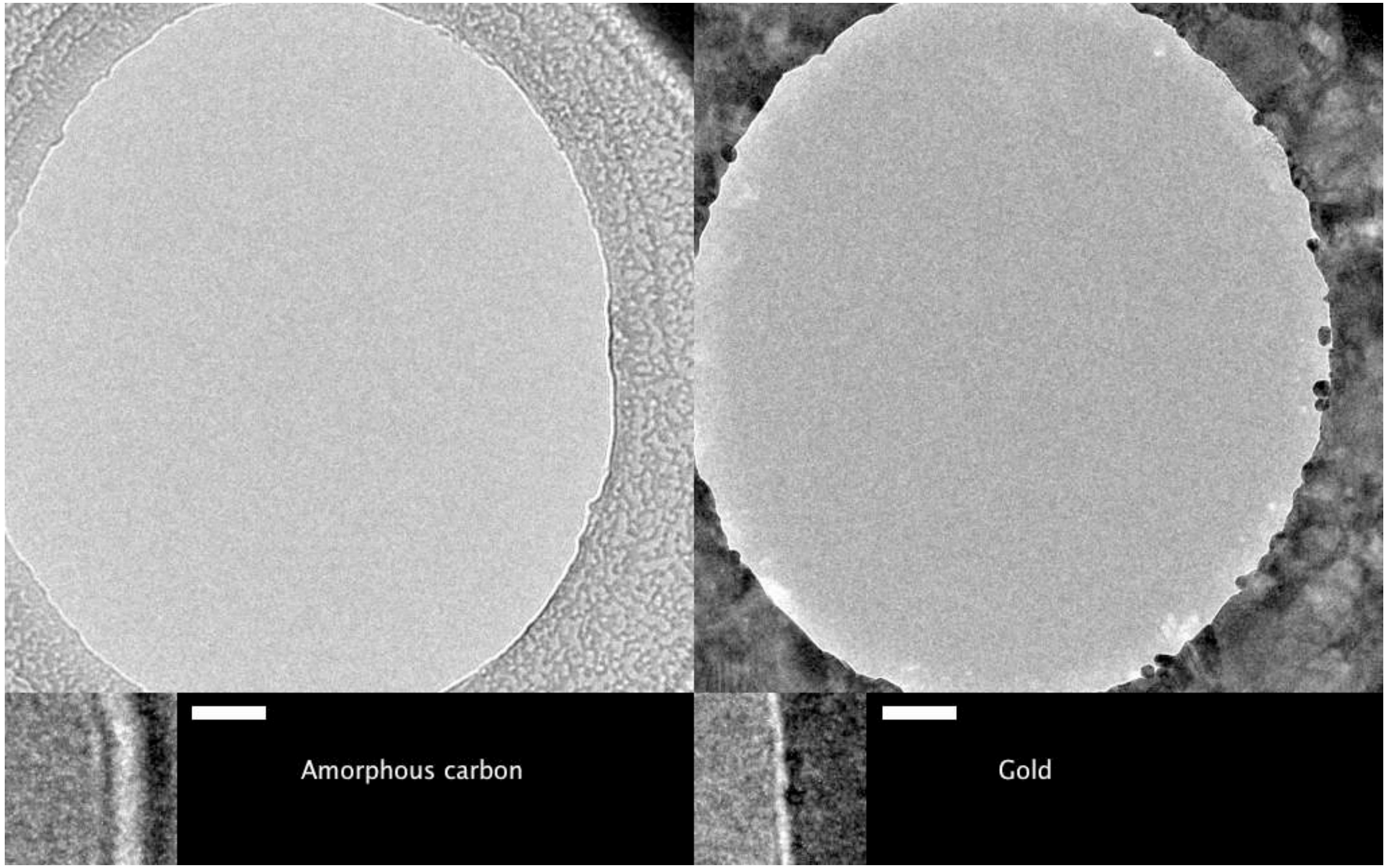
Disadvantages:

- Difficult to find focus due to lack of amorphous substrate

Russo & Passmore, 2015

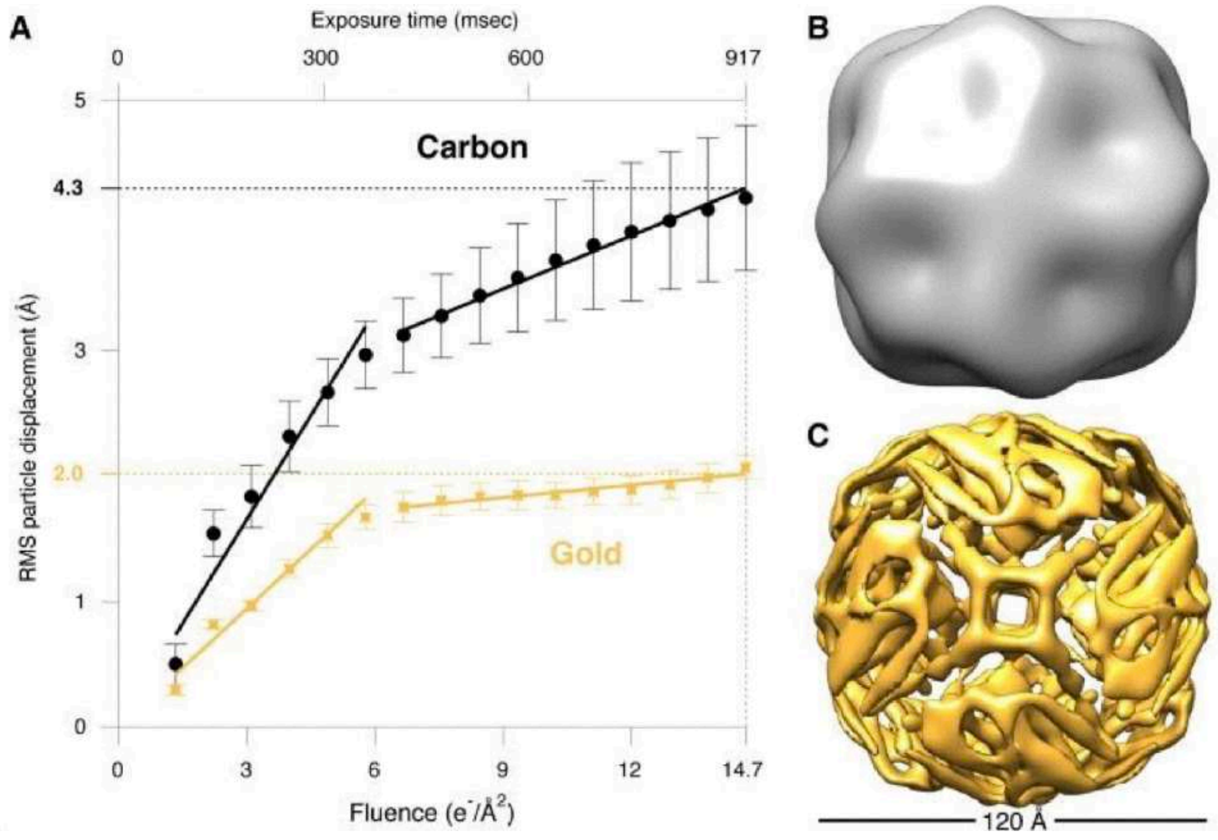


GOLD GRIDS



Russo & Passmore, 2015

GOLD GRIDS: HOW MUCH BETTER?



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

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

Russo & Passmore, 2015

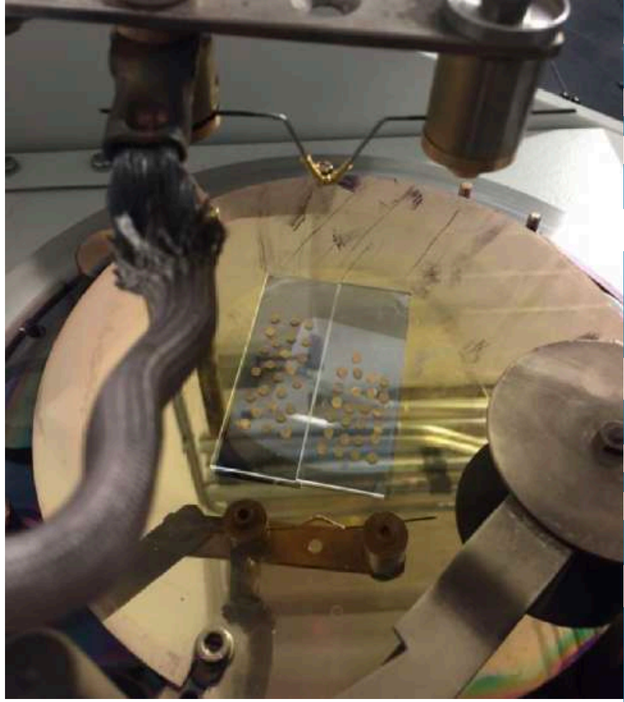
HOMEMADE GOLD GRIDS

HOW TO MAKE YOUR OWN GOLD GRIDS

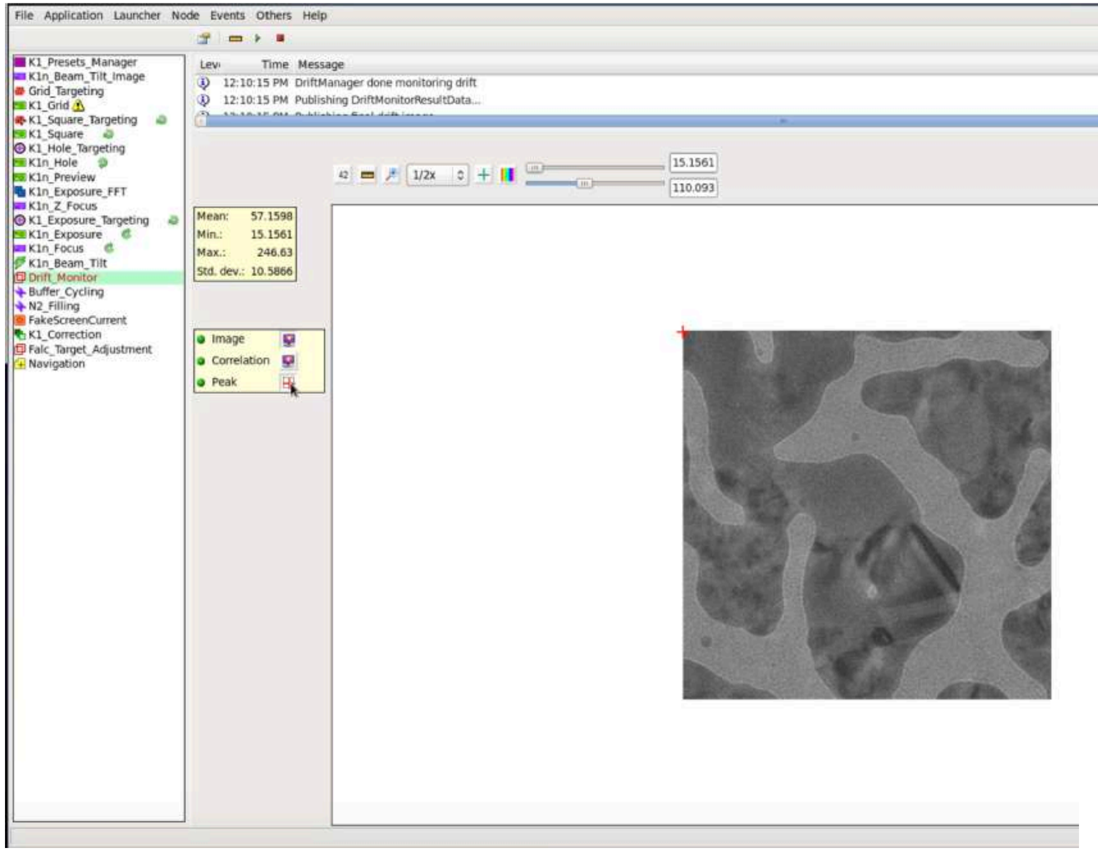
1. Buy gold grids with holey carbon on them
2. Evaporate gold on the grids
3. Remove carbon

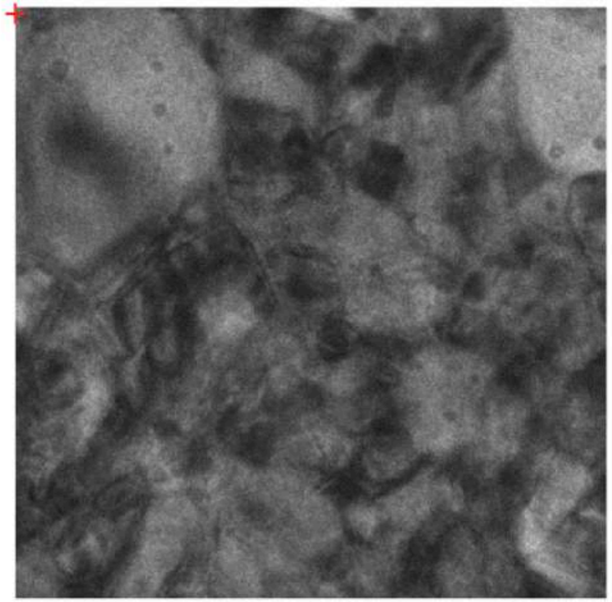
HOW TO MAKE YOUR OWN GOLD GRIDS

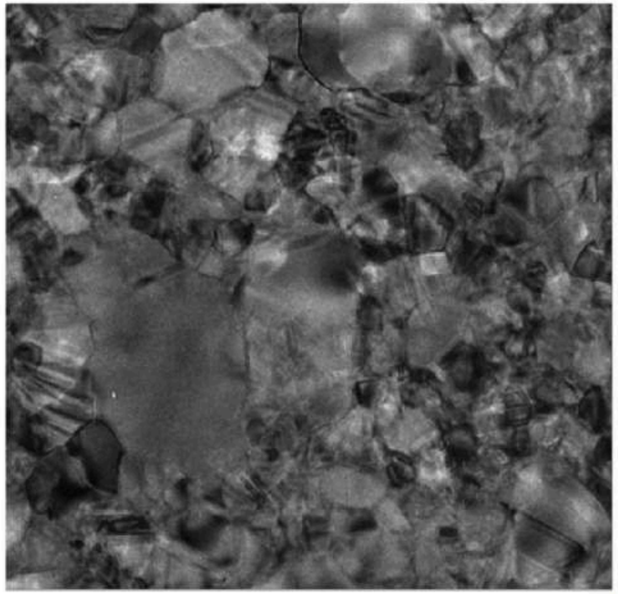
Edwards Auto306



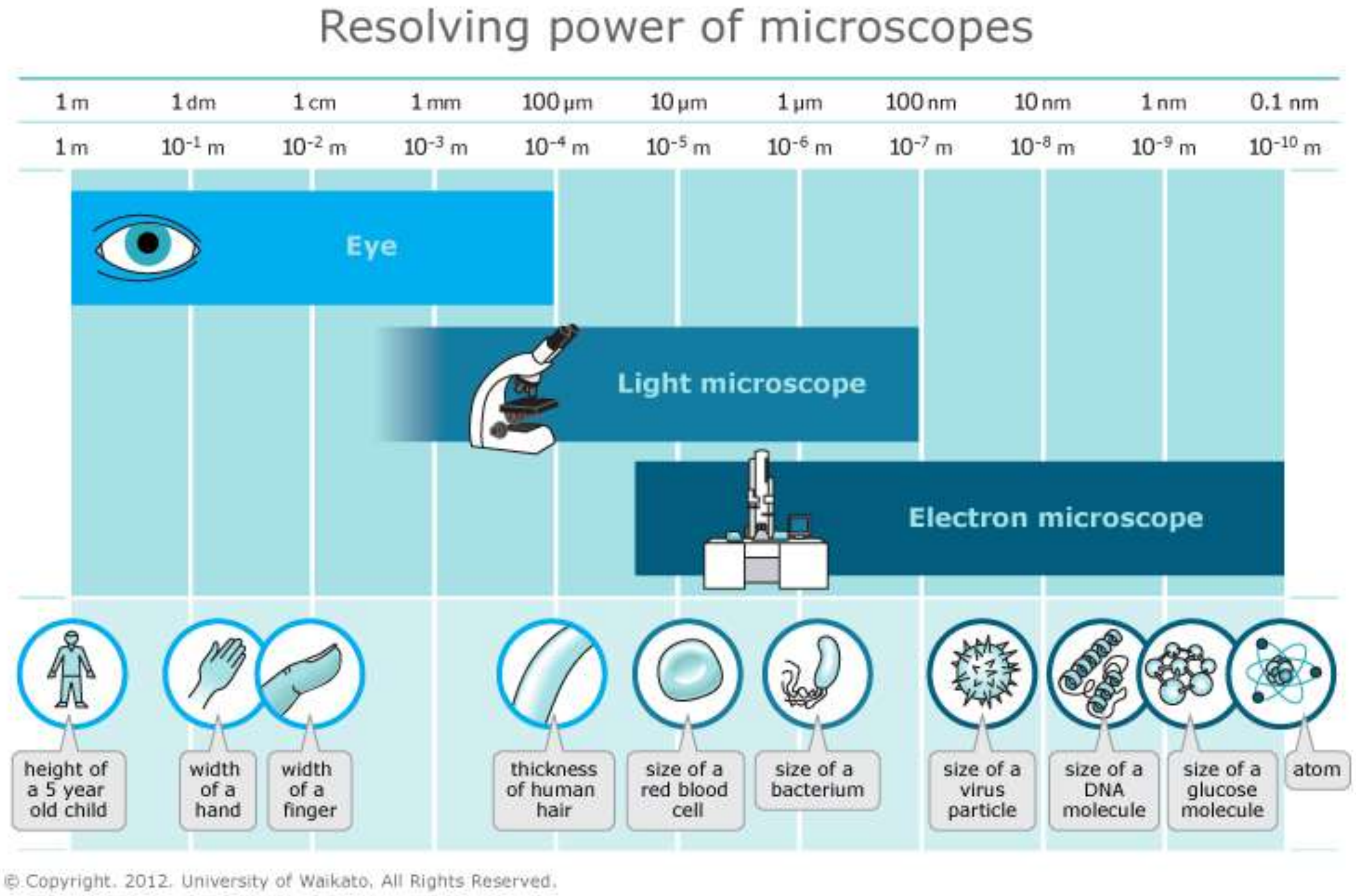
WHY NOT JUST BUY GOLD GRIDS?







SUPPORT FILMS AND GRIDS



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

ADDITIONAL SUPPORT FILM TOPICS

Graphene Oxide

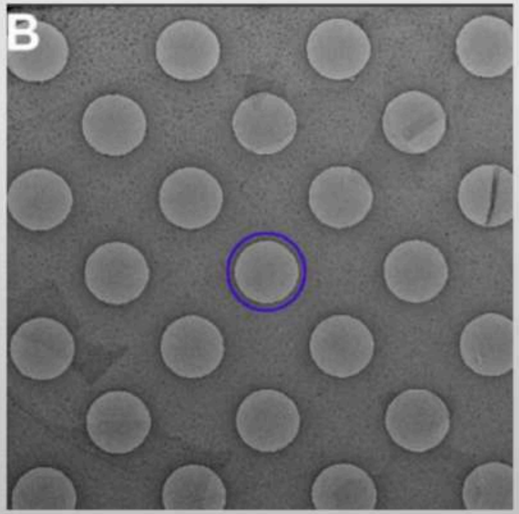
Thin Continuous carbon

Affinity grids

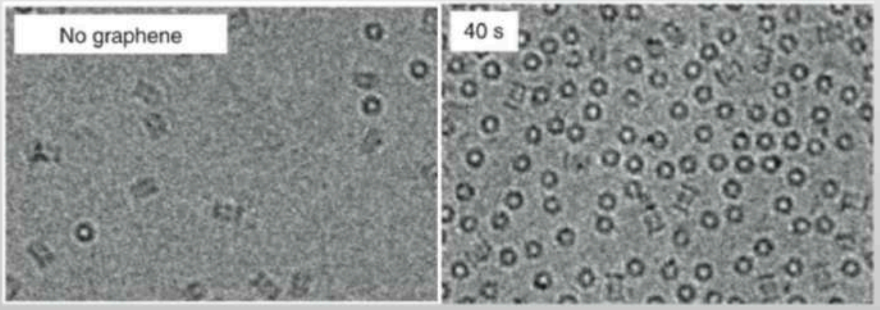
Grid treatments

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

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



Graphene oxide covered grids



Without Graphene With Graphene hydrogenation

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

ADDITIONAL SUPPORT FILM TOPICS

Graphene Oxide

Thin Continuous carbon

Affinity grids

Grid treatments

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

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

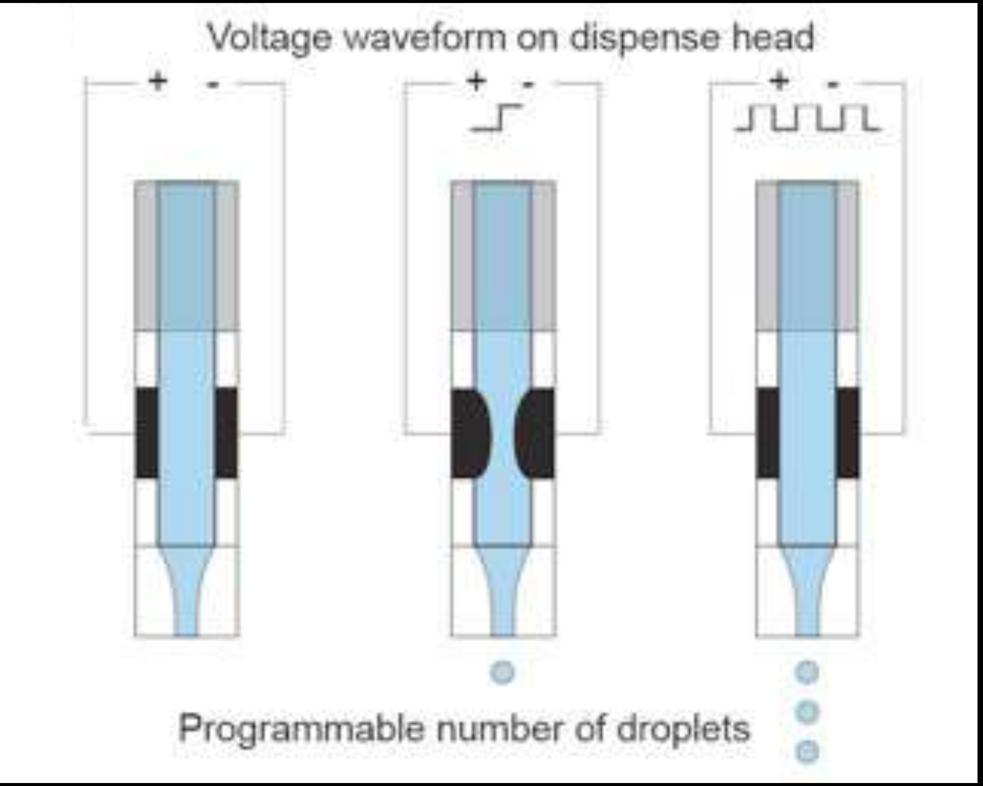
Ted Pella easyGlow (c. 2015) | Edwards S150B (c. 1995) | Edwards 12E6 (c. 1962)

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

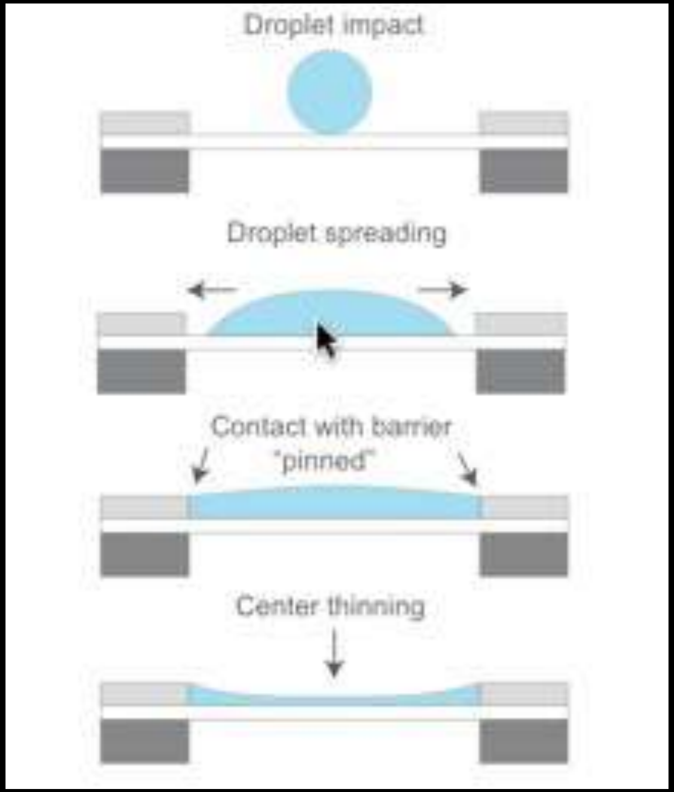
The Spotiton Project: Improving cryoTEM grid preparation

New Technologies

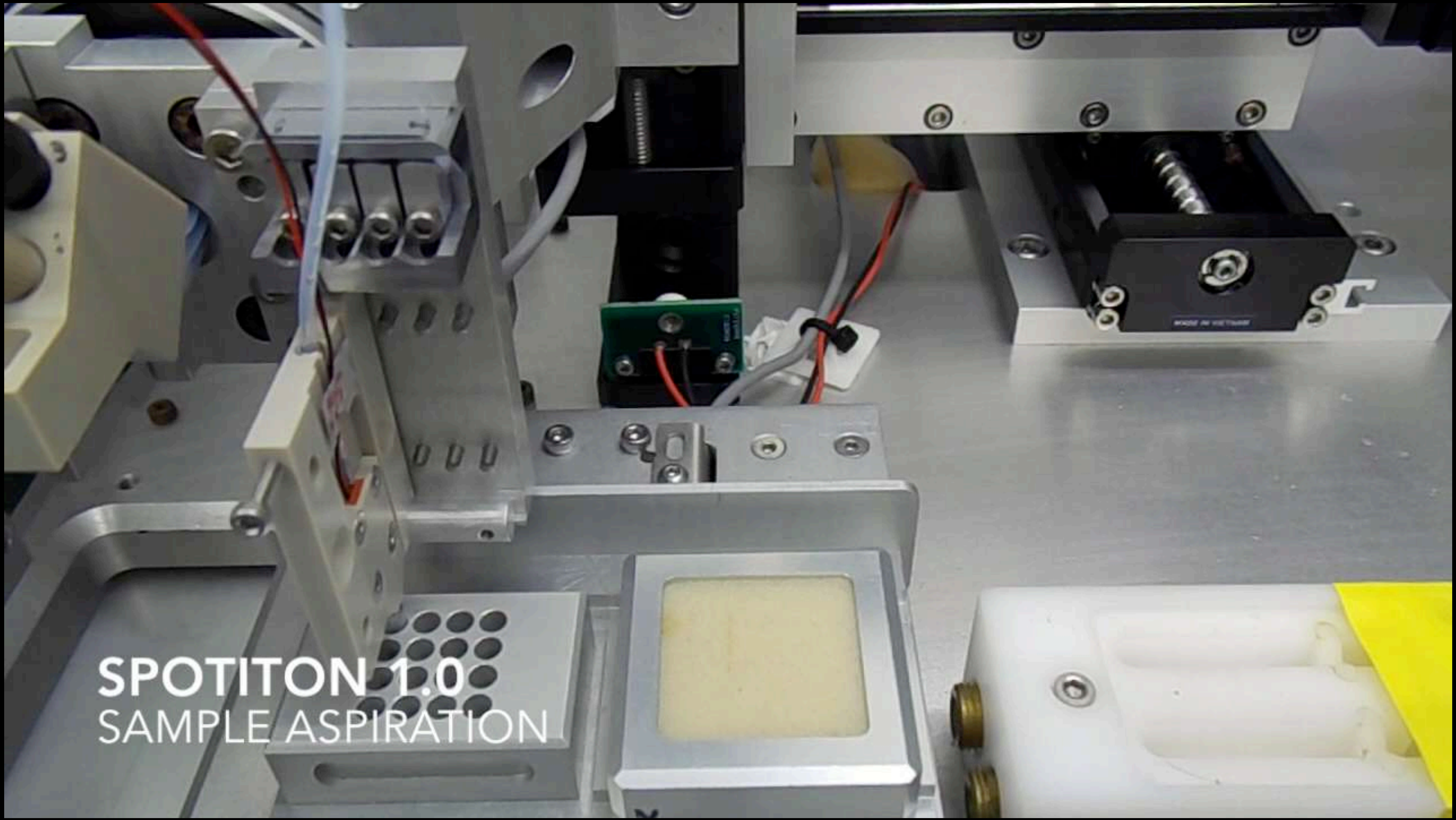
Accurate pL dispensing



Thin films without blotting



The Spotiton Project: Improving cryoTEM grid preparation



Venkat Dandey

Hui Wei

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 biolog
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.

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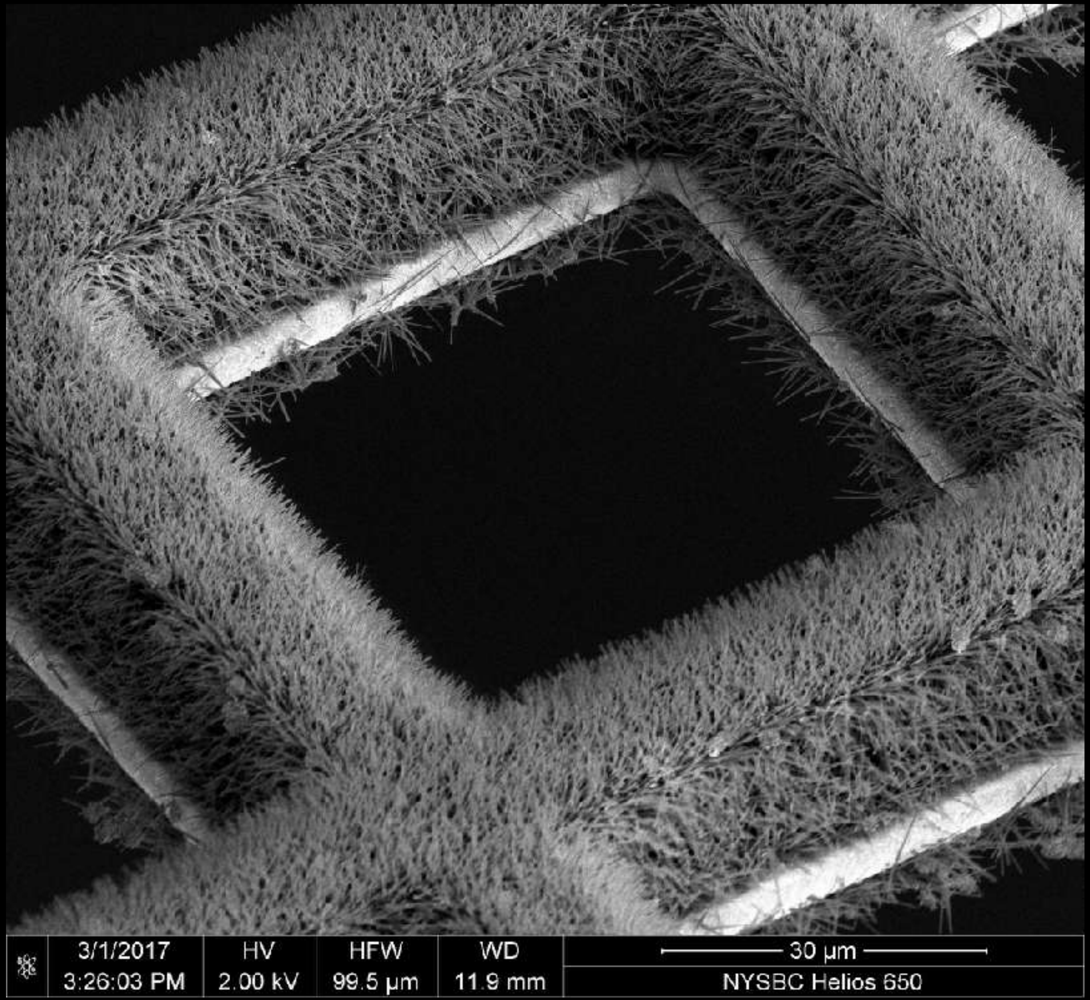
The Spotiton Project: Improving cryoTEM grid preparation

"Self-blotting" nanowire grids

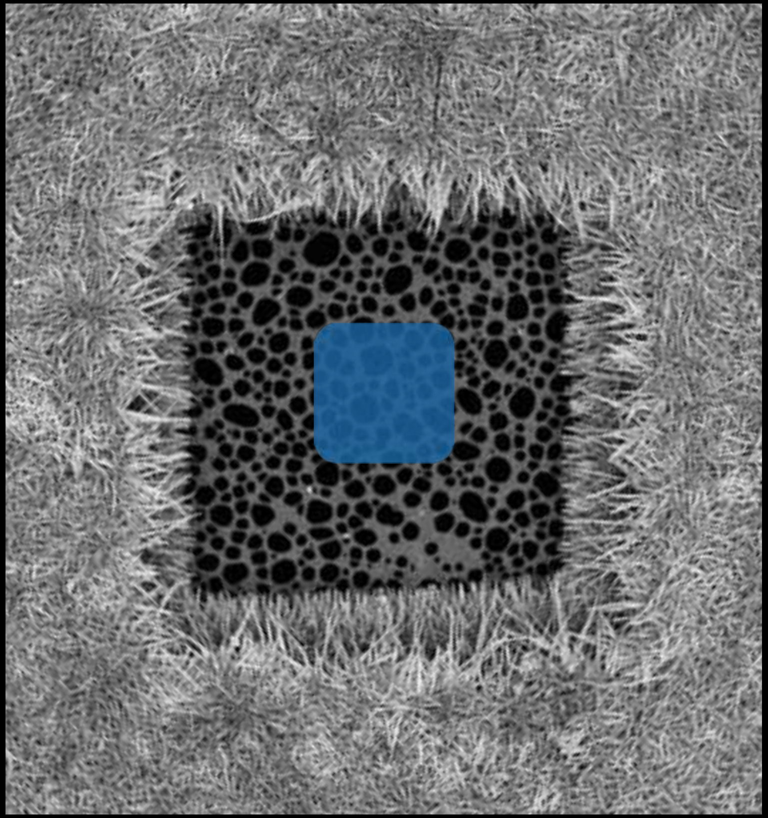


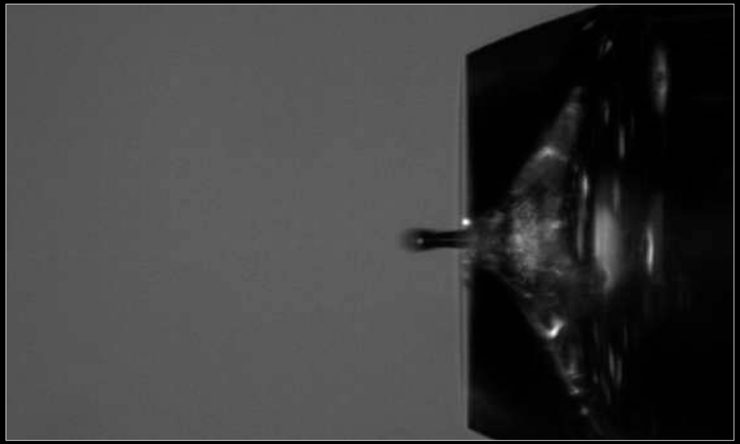
Hui Wei

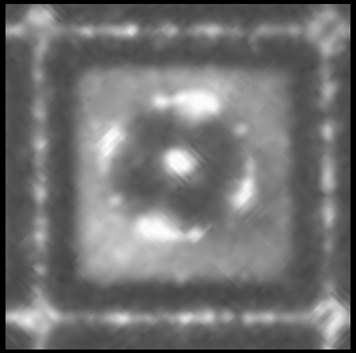
Nanowires can be grown on copper grids using a simple chemical treatment

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.

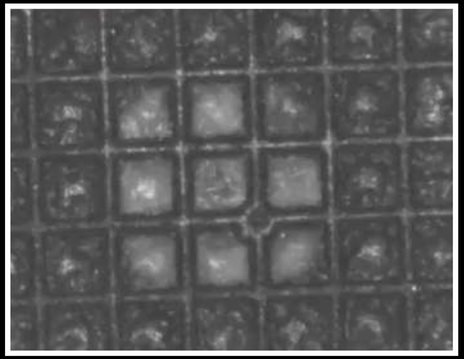
The Spotiton Project: Improving cryoTEM grid preparation





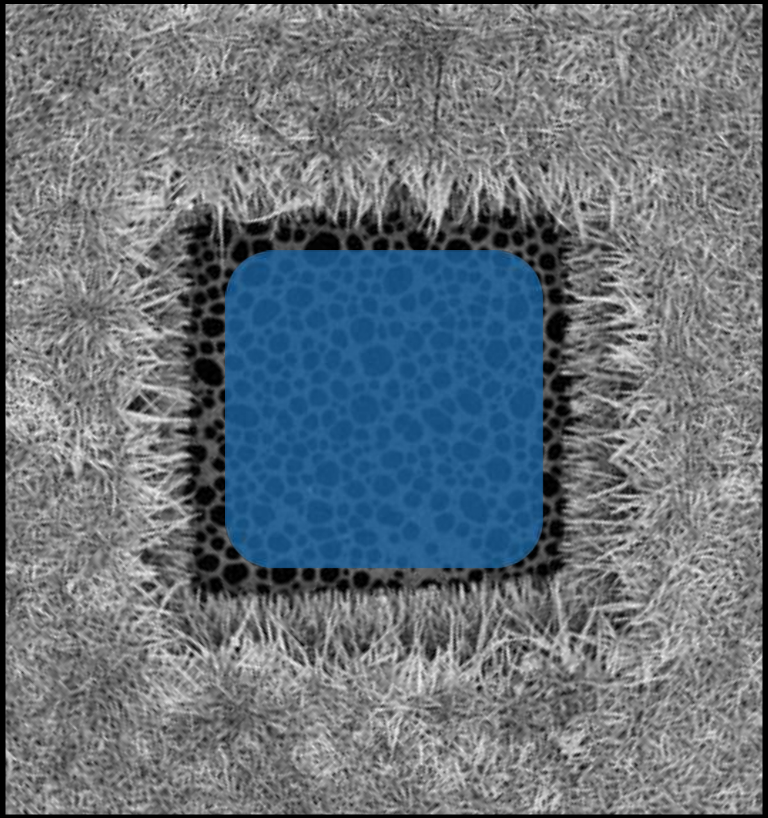


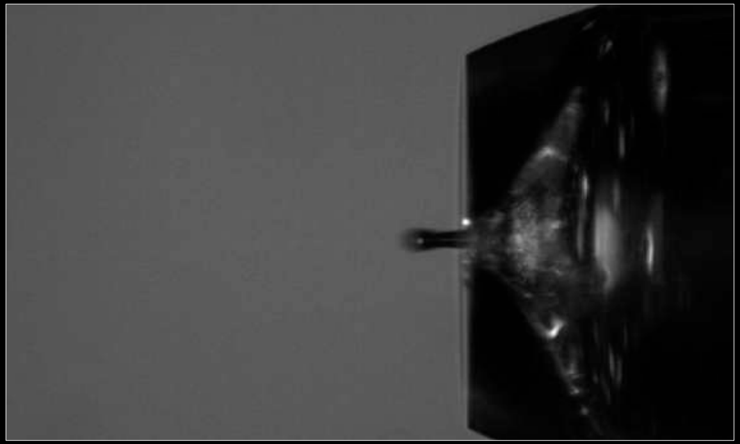
Single frame from loop

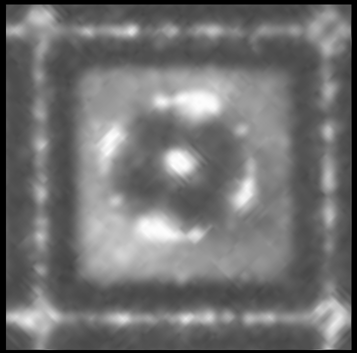


Video loop

The Spotiton Project: Improving cryoTEM grid preparation







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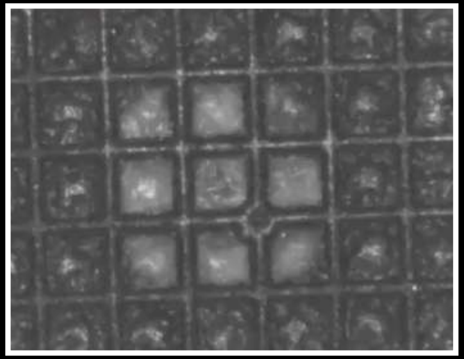
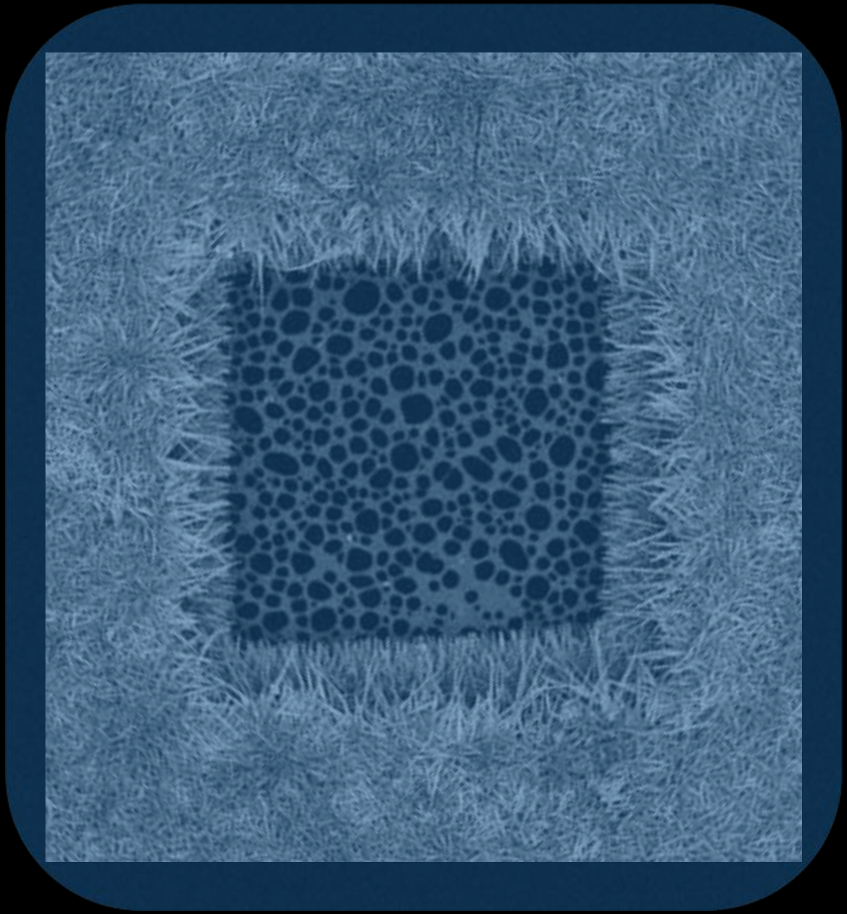
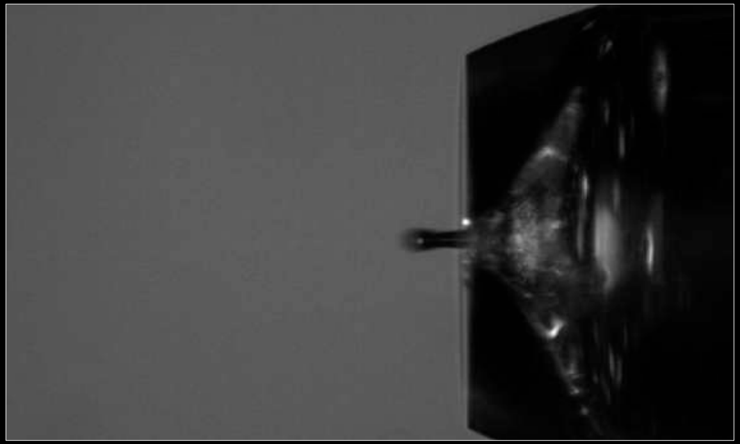
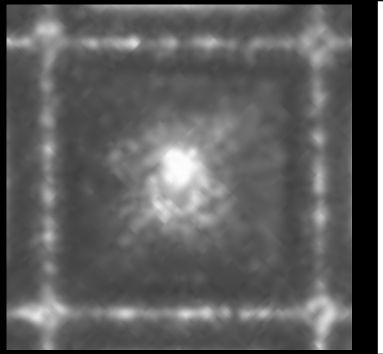


Video loop

The Spotiton Project: Improving cryoTEM grid preparation







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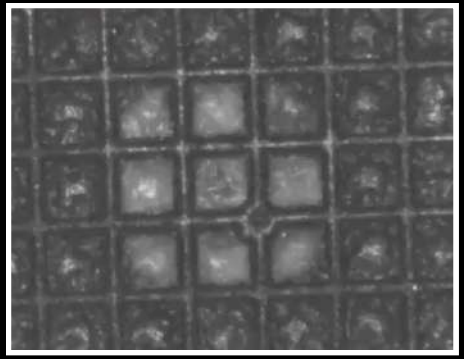
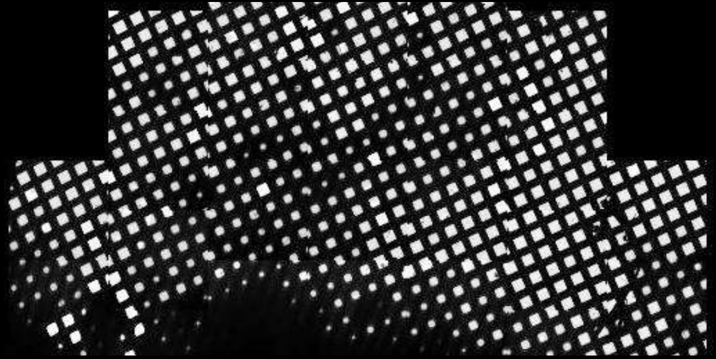


Video loop

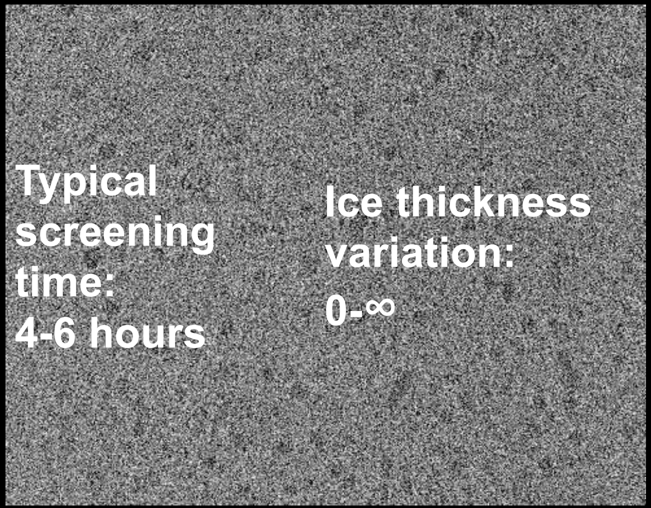
The Spotiton Project: Improving cryoTEM grid preparation

Vitrobot

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



Usable area: ~0-10%

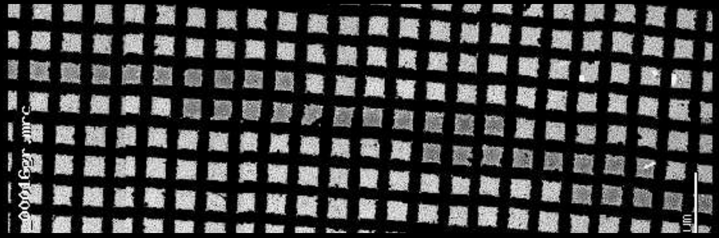


Typical screening time: 4-6 hours

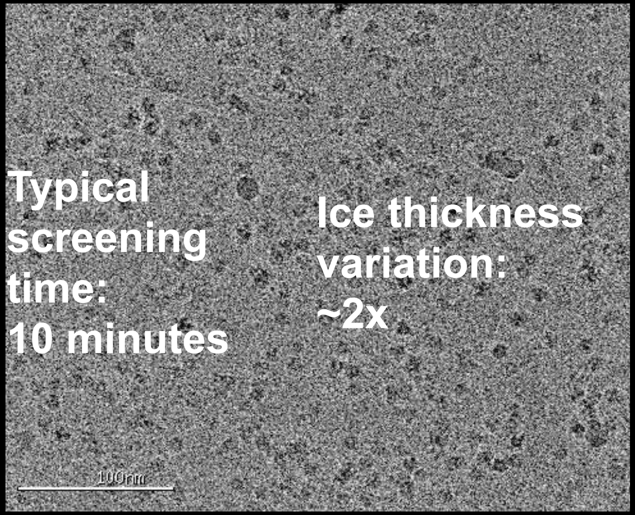
Ice thickness variation: 0-∞

Spotiton

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



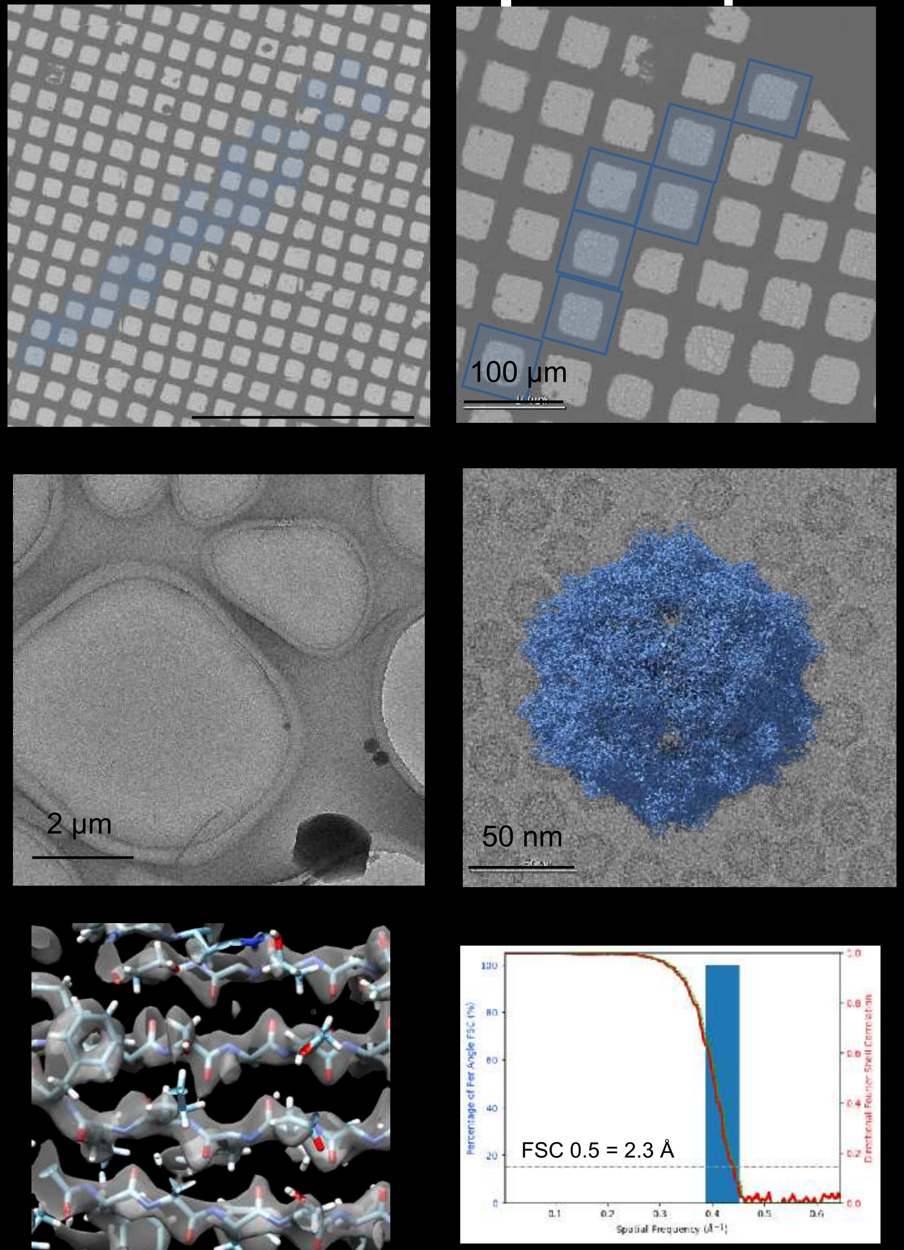
Usable area: ~100%

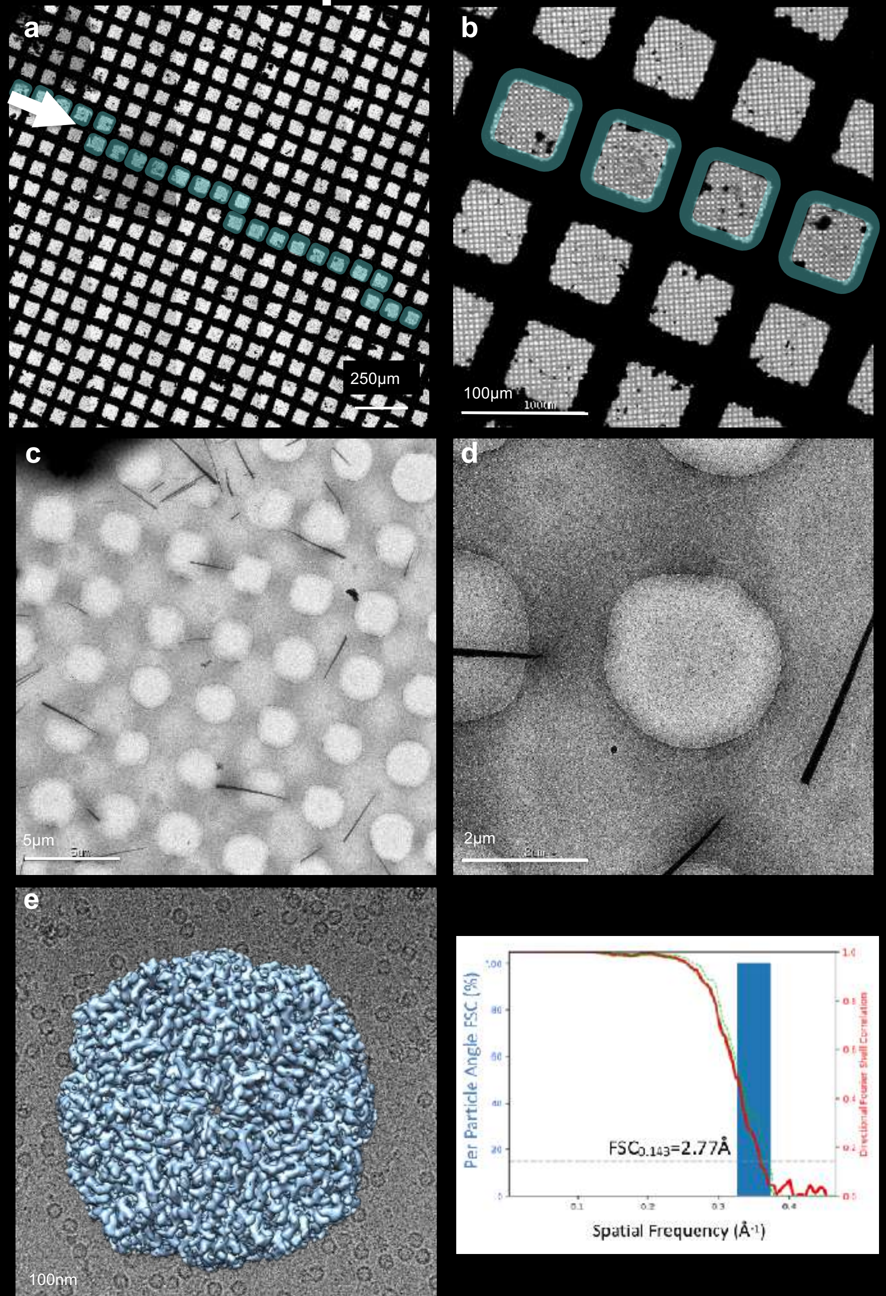


Typical screening time: 10 minutes

Ice thickness variation: ~2x

The Spotiton Project: proof of concept





SUPPORT FILMS AND GRIDS

Questions?

WHAT NEXT?

cryoEM 001 : Single Particle Masterclass

1. Building a cryoEM toolkit
2. EM compatible samples
3. EM support films and grids
4. Sample preparation
5. Tools of the trade: microscopes and detectors
6. Microscope operations
7. Data collection strategies
8. Data assessment & QC
9. Data processing:
   - cryoEM IT infrastructure
   - On-the-fly feedback
   - 3D Reconstruction
10. Visualization and validation