NEW YORK STRUCTURAL BIOLOGY CENTER

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING

Simons Electron Microscopy Center

Challenges in biological EM
Support films & Sample prep

2020 Winter EM Course

25 Min lecture + 45 min practical
Jan 08, 2020

Course logistics: main website

semc.nysbc.org/the-winter-spring-2020-em-course/

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semc.nysbc.org/the-winter-spring-2020-em-course/

SIMONS ELECTRON MICROSCOPY CENTER

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EVENTS

Upcoming Events

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Workshops and Symposia at Outside Organizations

The Winter-Spring 2020 EM Course

General registration for the 2020 winter course is closed. If you registered for this course through your institution's registrar (with a cutoff in January), then please let us know as we have separate reserved spots for students taking the course for academic credit.

About the course

Electron microscopy in combination with image analysis is increasingly powerful in producing 3D structures of individual molecules and large macromolecular complexes that are unapproachable by other methods. This course is focused on the concepts and theories behind electron microscopy and will be taught in a reverse classroom format based on Grant Jensen's online course (Getting Started in Cryo-EM from Caltech). Students will be responsible for watching these online lectures prior to class. Each week guest lecturers and SEMC staff lead discussions on the practice of solving molecular structures by electron microscopy.

The course will be held at the New York Structural Biology Center at 89 Convent Ave (133rd St).

Course Schedule

Classes in SEMC or NYSBC seminar rooms (Winter course: Mondays 3:30-5pm and Wednesdays 3:30-5pm | Short course weeks: 9:30am-11am)

EM fundamentals section (month of January)

Jan 6 : Lecture – Introduction & SEMC tour (Ed Eng – NYSBC)
Jan 8 : *Practical* – Sample Preparation & Support films (Micah Rapp/Ed Eng – COLU/NYSBC)
Jan 13 : Lecture – Basic anatomy of the electron microscope (Micah Rapp – COLU)
Jan 15 : *Practical* – TEM use
Jan 20 : *MLK Jr holiday – No class*

Course Administrator:
Ed Eng (eeng@nysbc.org)

Teaching Assistant:

Micah Rapp
(mar2294@columbia.edu)

Course logistics

Mondays
3:30-5pm - A-11 seminar room / SEMC conference room

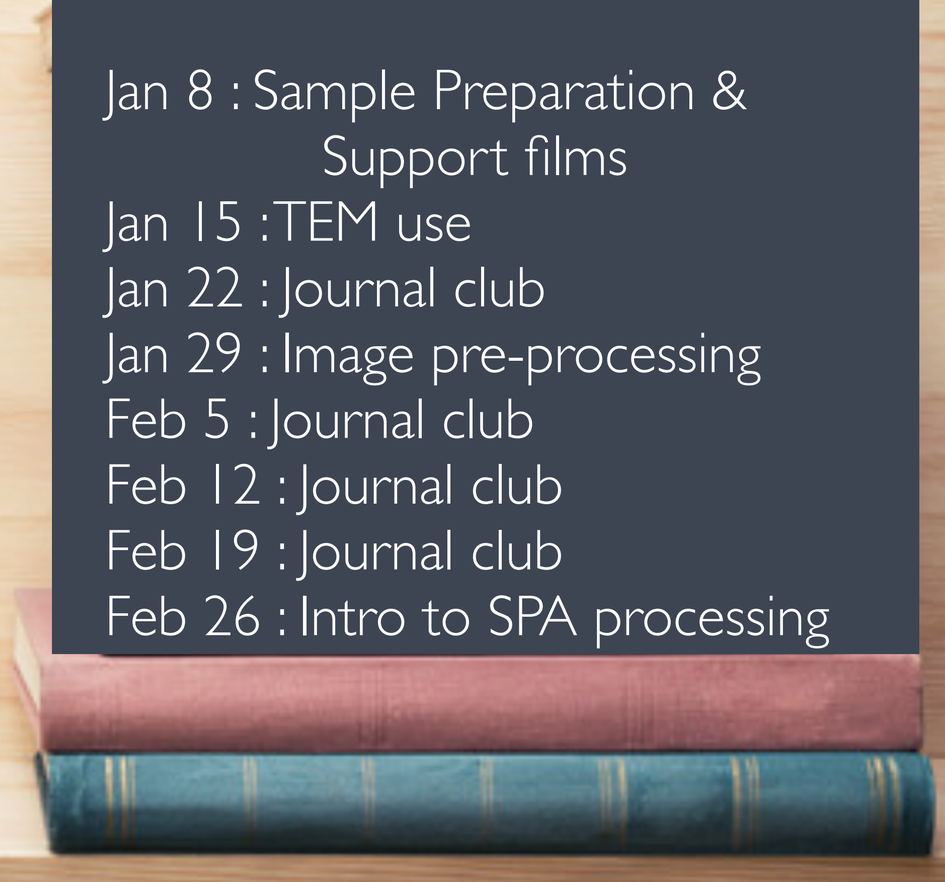
Wednesdays
Starts at 3:30 - SEMC conference room

Lecture schedule

Jan 6 : Introduction & SEMC tour
Jan 13 : Basic anatomy of the electron microscope
Jan 20 : *MLK Jr holiday – No class*
Jan 27 : Fourier transforms and Image Formation
Feb 3 : MicroED (Bill Rice – NYU)
Feb 10: Helical reconstruction (Hernando Sosa – Einstein)
Feb 17 : *President's day holiday – No class*
Feb 24 : Q&A – open forum & primer to SPA

Recitation schedule

Jan 8 : Sample Preparation & Support films
Jan 15 : TEM use
Jan 22 : Journal club
Jan 29 : Image pre-processing
Feb 5 : Journal club
Feb 12 : Journal club
Feb 19 : Journal club
Feb 26 : Intro to SPA processing



Appion part I
www.surveymonkey.com/r/BHVHYK3

Course logistics

Section 1a : EM fundamentals section
b : 2D EM section
c : *SEMC Appion workshops* - ***Jan 30***

https://www.surveymonkey.com/r/BHVHYK3

Section 2 : Single-particle short-course - March 2

1d : **Additional journal clubs**

Section 3 : Tomography short-course - April 13

1e : **Course wrap up** - TBD

Recitation schedule

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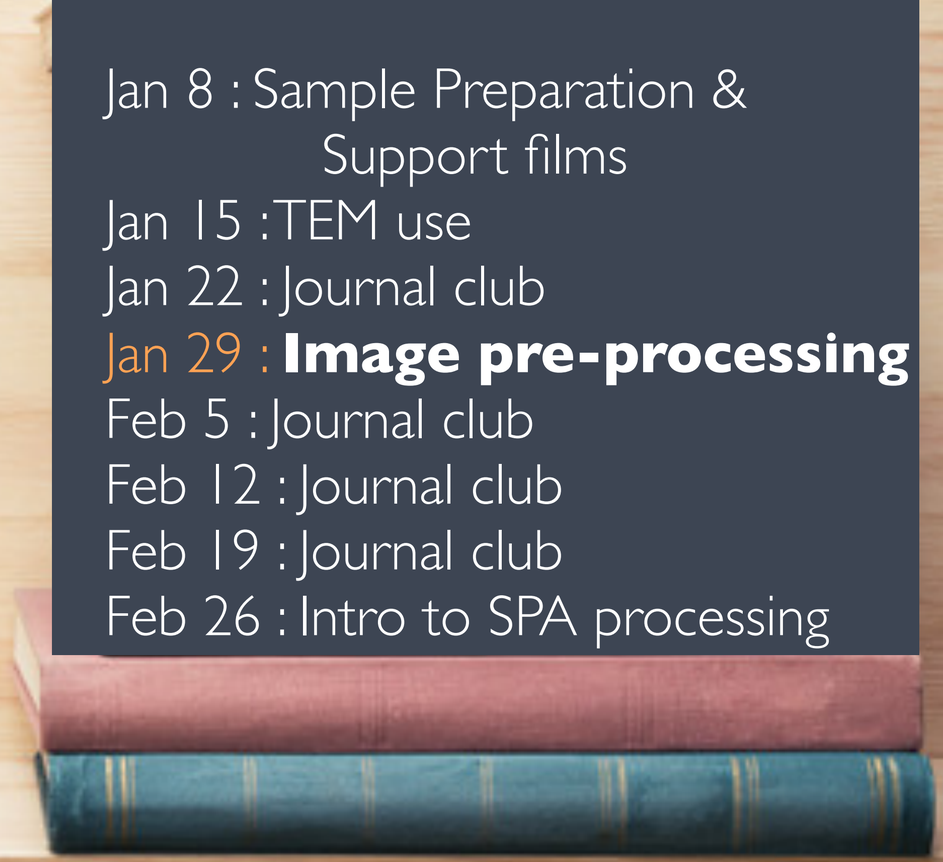


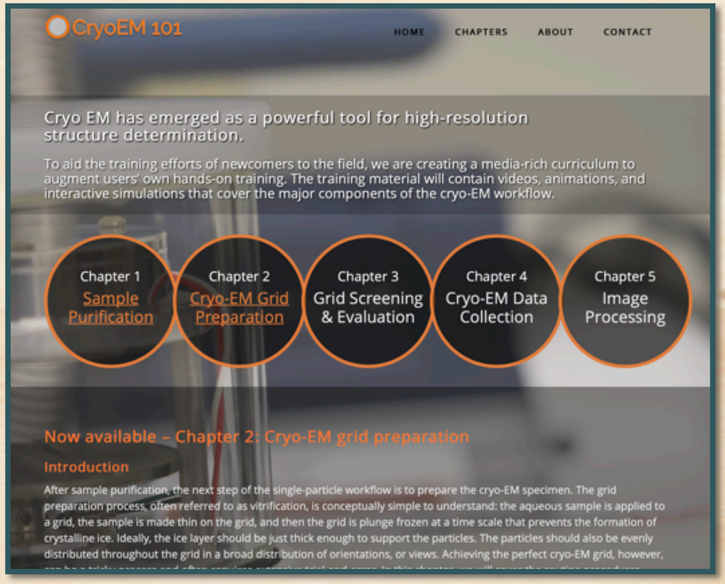
Course logistics: recitations

cryo-em-course.caltech.edu/videos

Part 4: Fundamental Challenges in Biological TEM & Sample Prep

Unit 2: Sample Preparation - youtube.com/playlist?list=PL8_xPU5epJdfd5fM2CjQItR-iRIIEIJk8

cryoem101.org



Chapter 1: Sample Purification

Chapter 2: Cryo-EM Grid Preparation

Wednesdays

Starts at 3:30 - SEMC conference room

Recitation schedule

Jan 8 : Sample Preparation & Support films

Jan 15 : TEM use

Jan 22 : Journal club

Jan 29 : Image pre-processing

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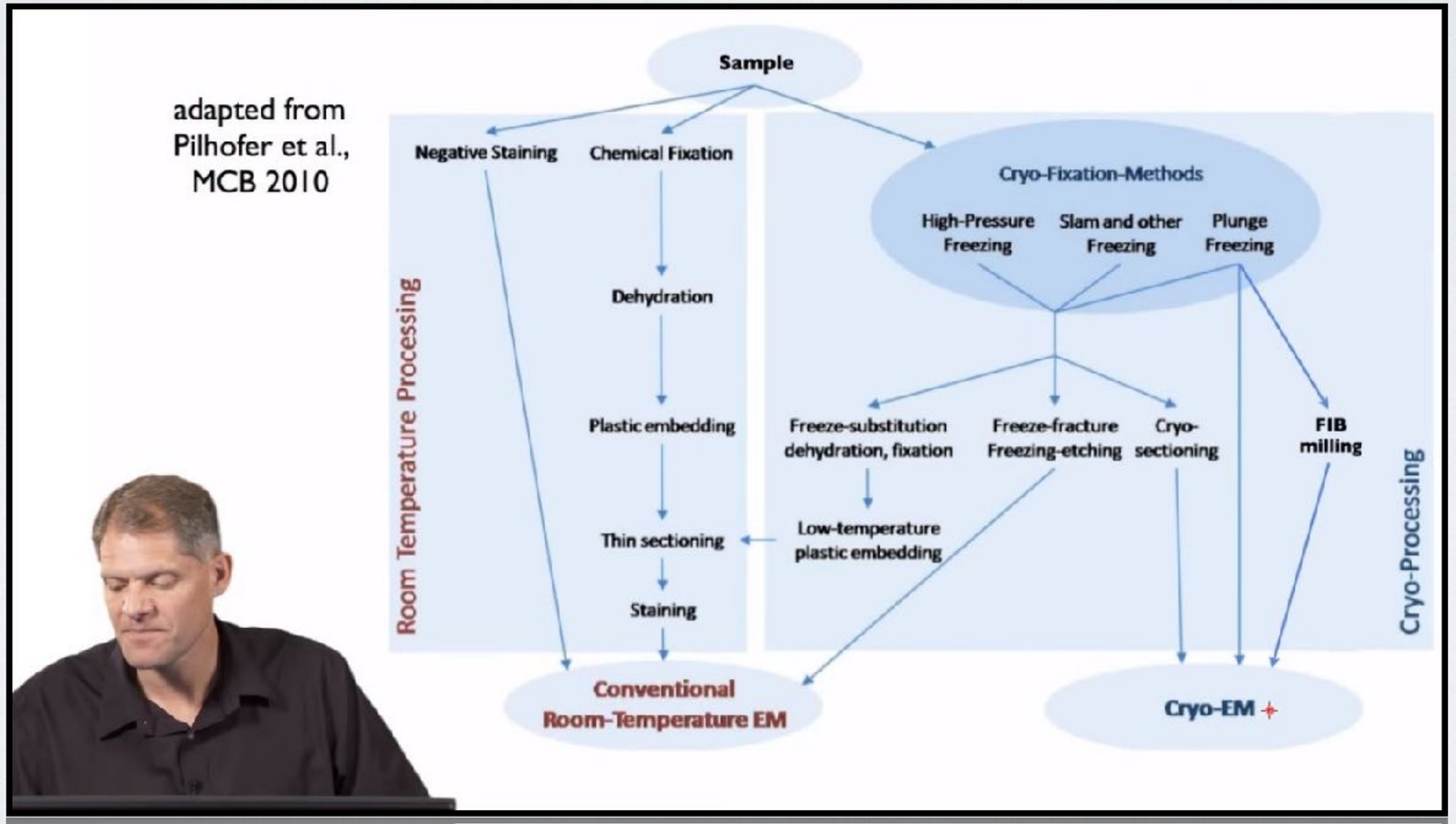
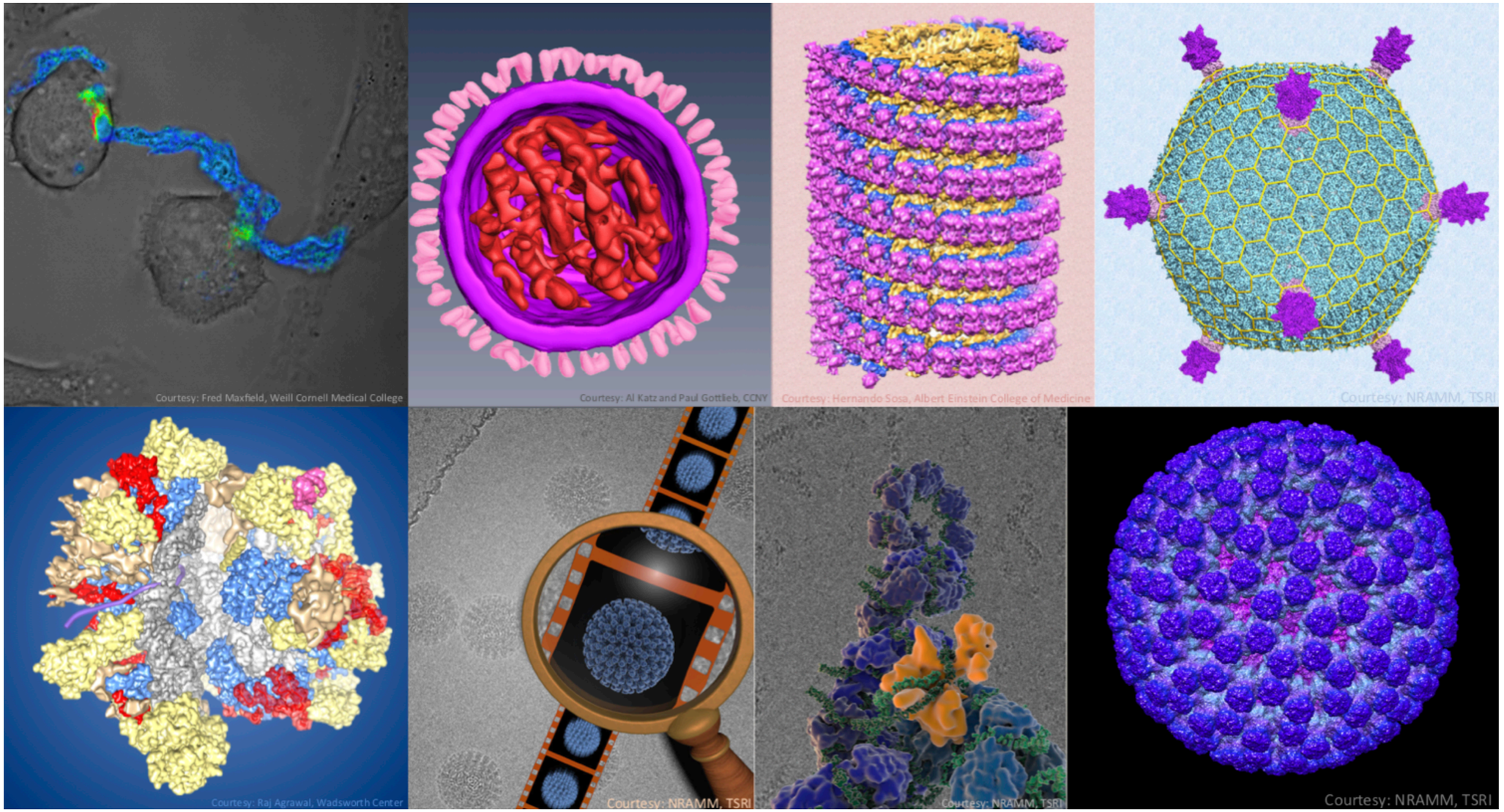
RT & CRYO SAMPLE PREP METHODS

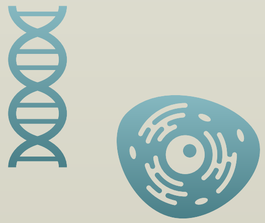


CRYOEM: TECHNOLOGY ON THE RISE



THAT'S GREAT…. HOW DO I START?

Core knowledge



Biochemistry & Sample preparation

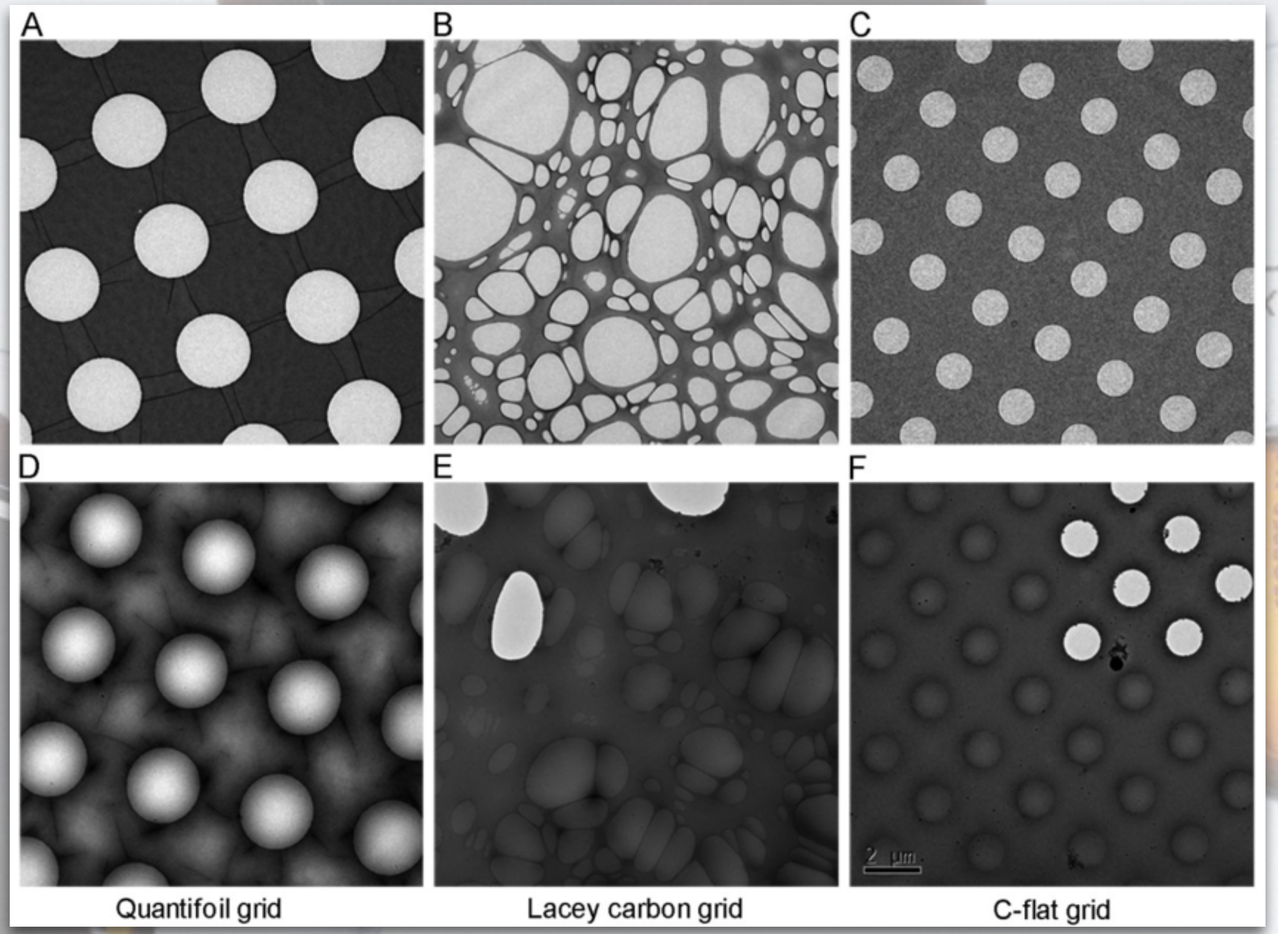
Data collection

Processing & Data analysis

FOCUS ON 4 AREAS

Challenges in biological EM
Support films

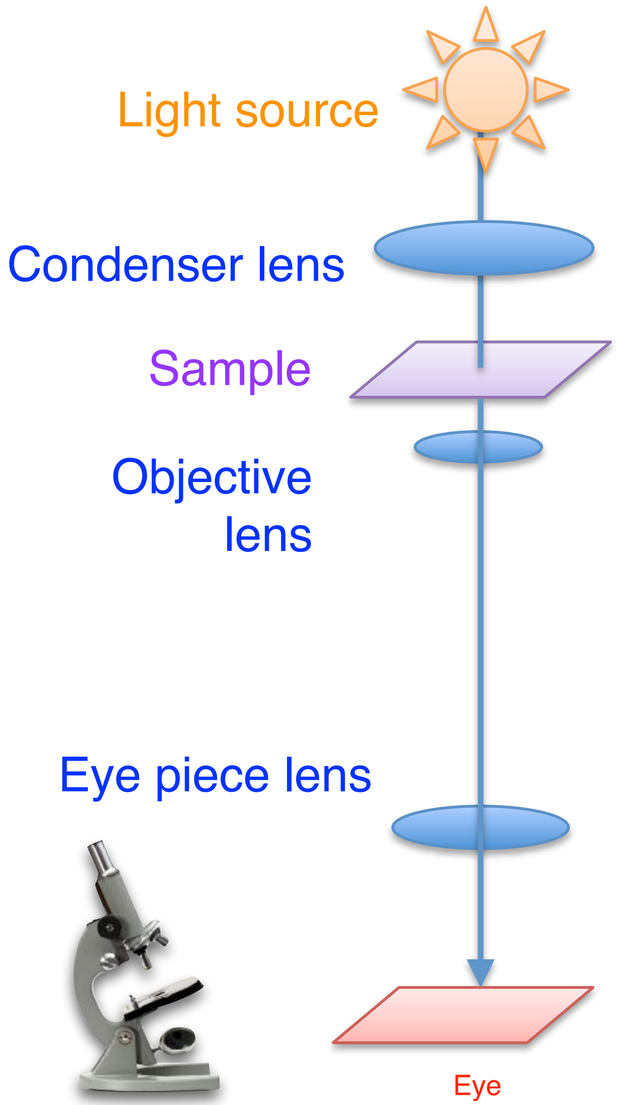
Cho, Hye-Jin & Hyun, Jae-Kyung & Kim, Jin-Gyu & Jeong, Hyeong & Park, Hyo & You, Dong-Ju & Jung, Hyun. (2013). Measurement of ice thickness on vitreous ice embedded cryo-EM grids: investigation of optimizing condition for visualizing macromolecules. Journal of Analytical Science and Technology. 4. 10.1186/2093-3371-4-7.

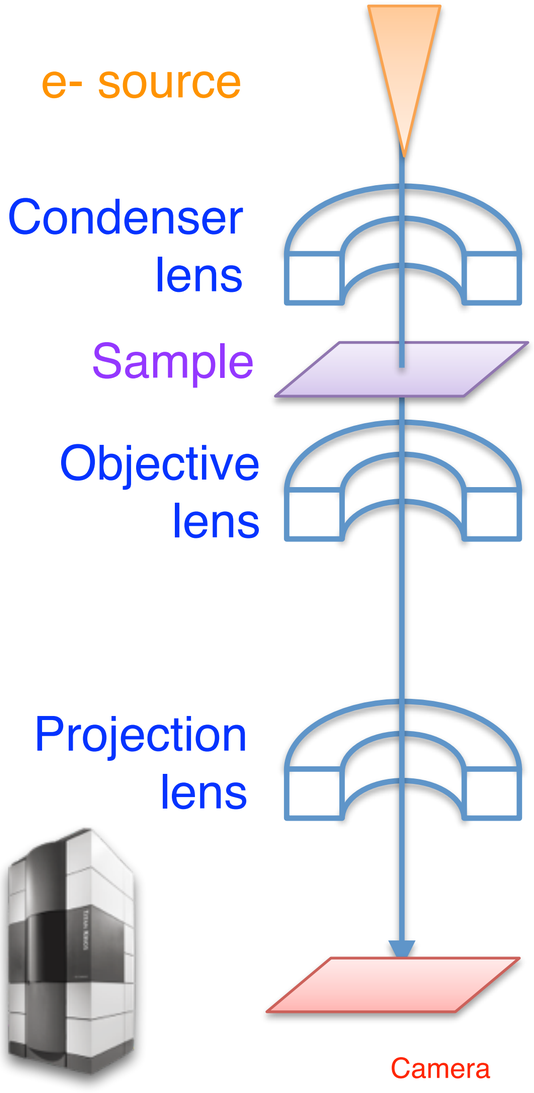


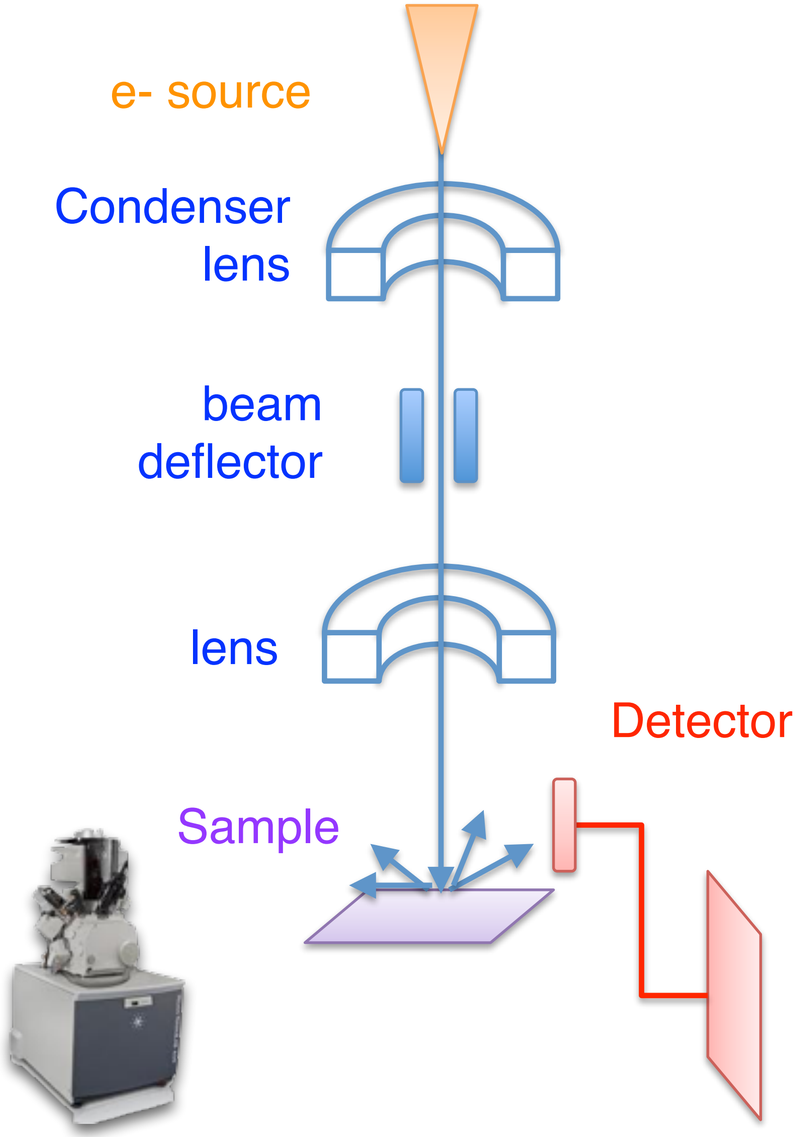
DOI: 10.1186/2093-3371-4-7

Sample Preparation

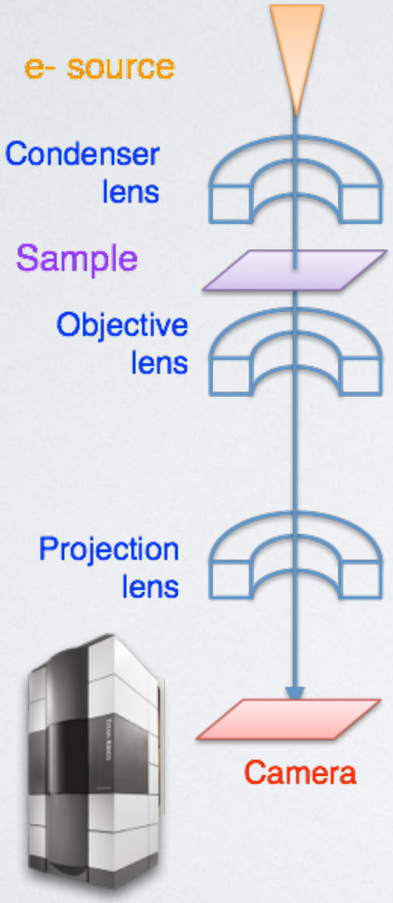
MICROSCOPES

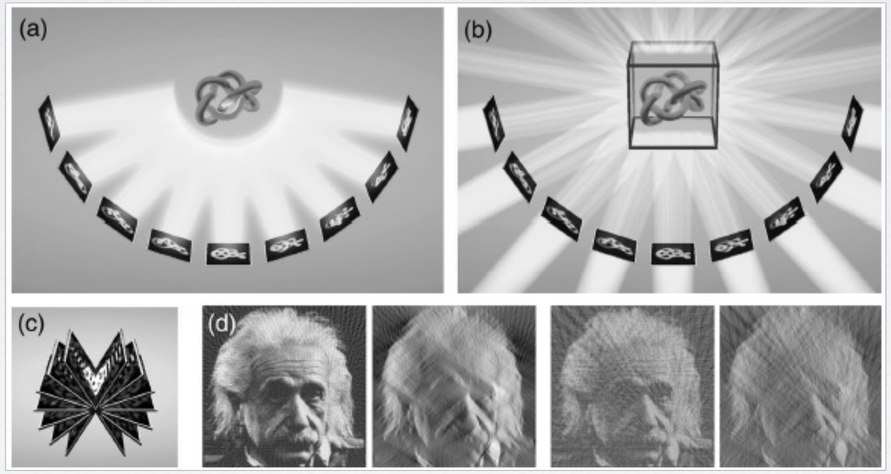




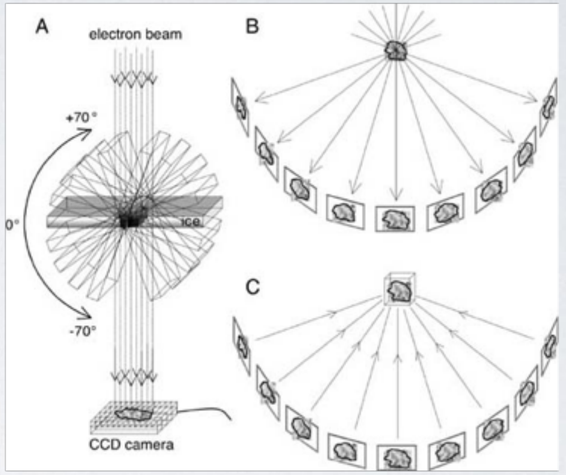


OBTAINING A 3D OBJECT FROM A 2D PROJECTION





From W. Baumeister et al. Trend in Cell Biology 9(1999)81



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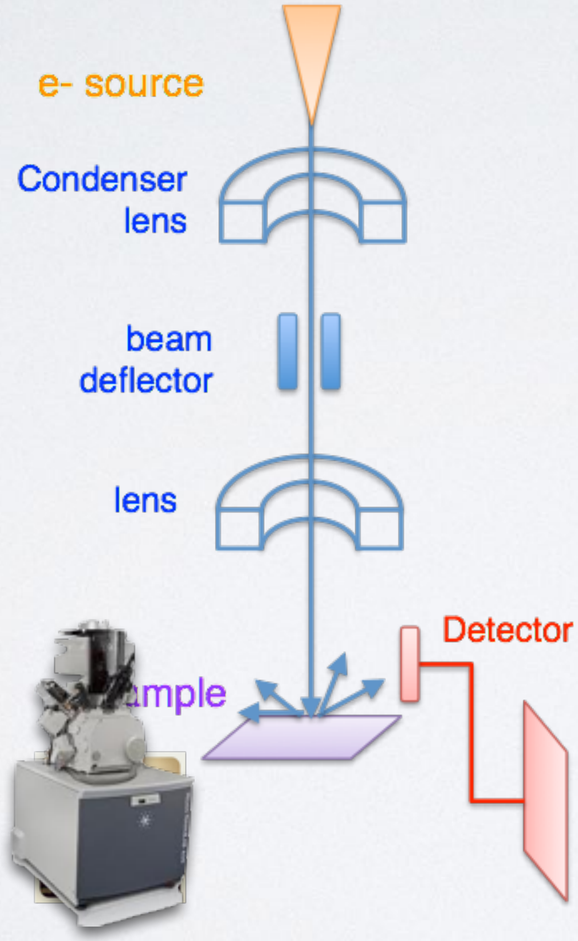
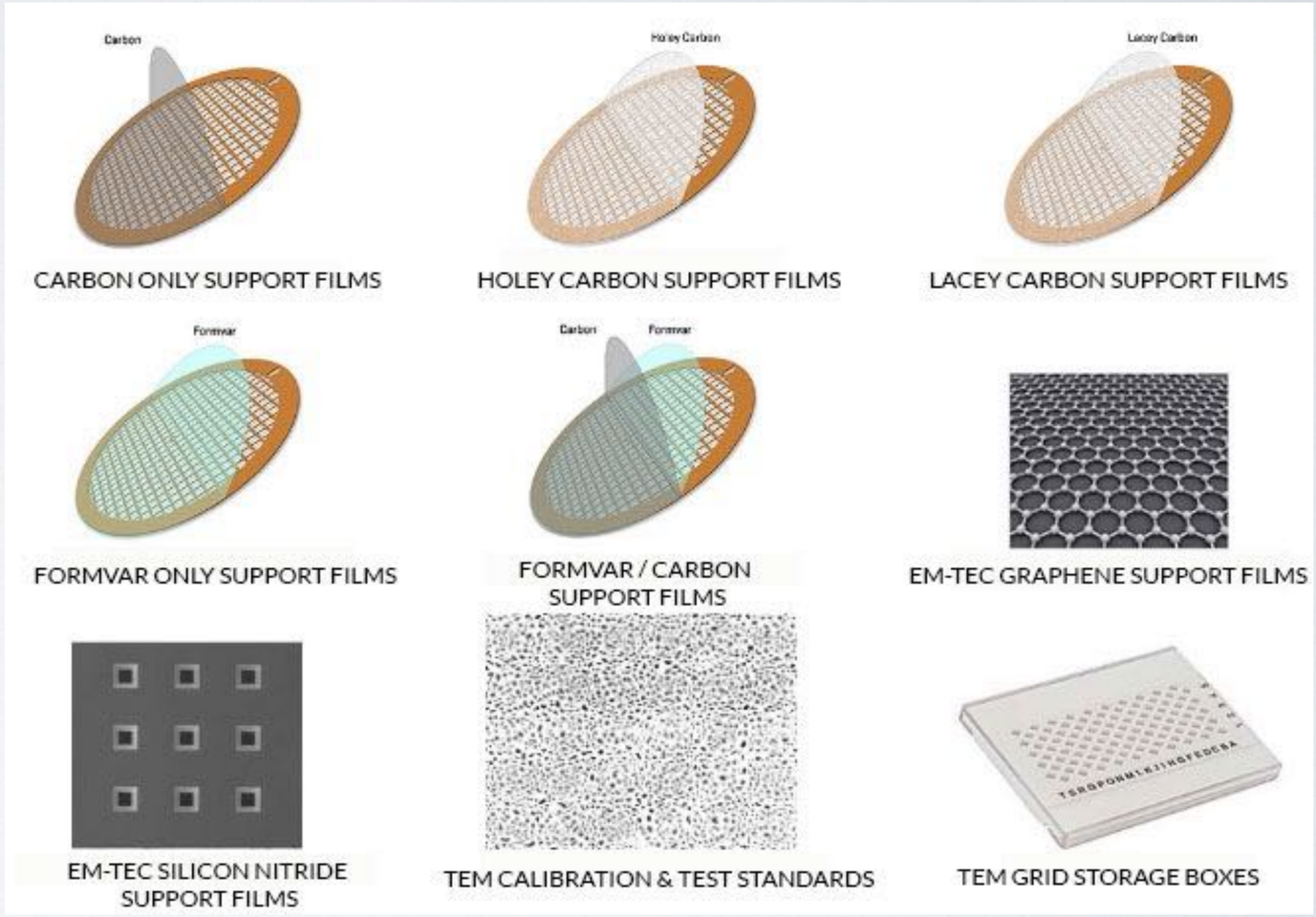


WHAT HOLDS OUR SAMPLE?

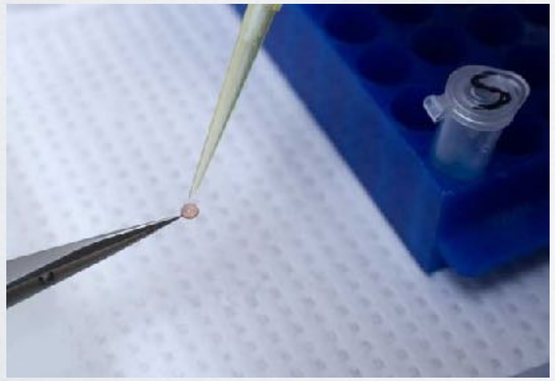
- Terms
  - Grid (Cu, Au, Mo, etc…)
    - mesh
  - Foil (C, Au, etc…)
    - Continuous
    - lacy
    - holey (hole size and spacing)



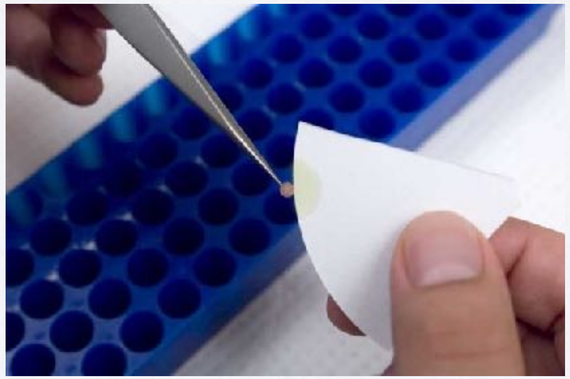
https://edgescientific.com/product-category/tem-supplies/tem-support-films/

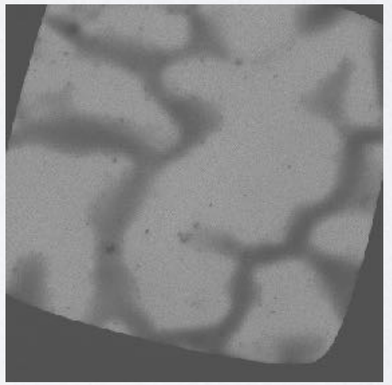
NEGATIVE STAINING

- What support films are used?



Baker, 2007



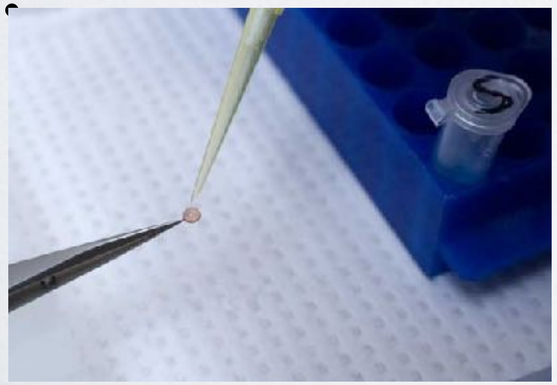


www.mcb.ucdavis.edu/cryoem/microscopy101.html

NEGATIVE STAINING

- Heavy metal salt solution surrounds sample
- Continuous carbon support film
- Protocol: glow discharge, sample, wash, stain
- SEMC: 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



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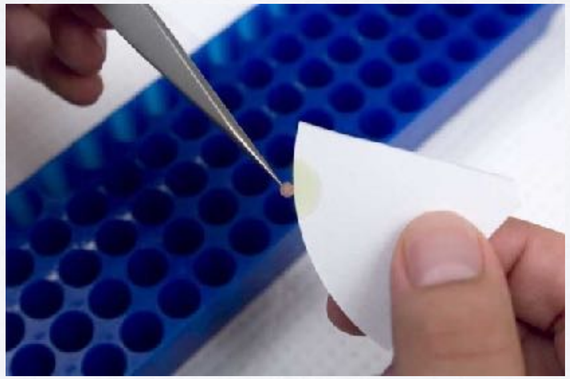
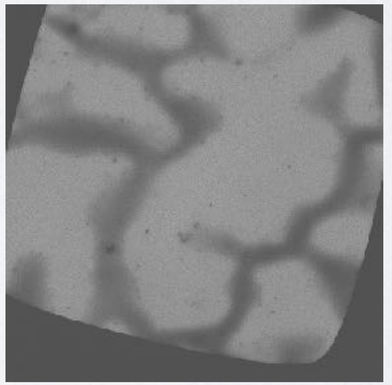


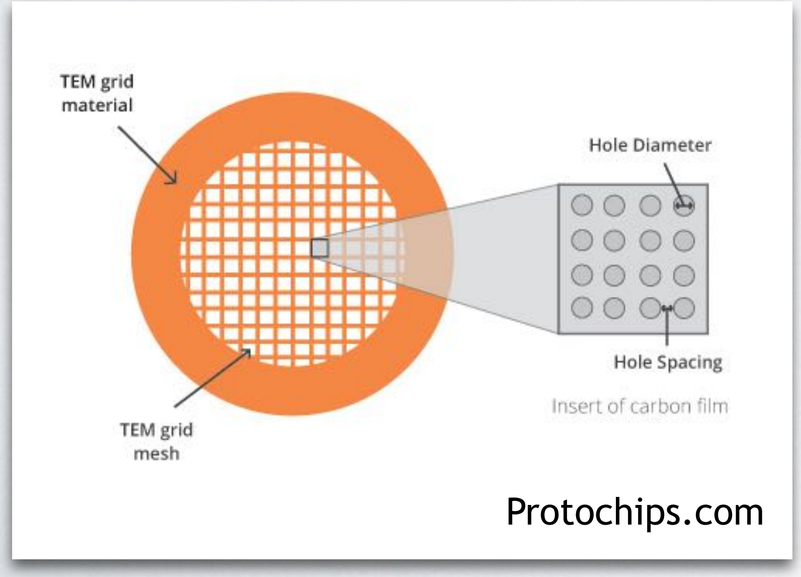


www.mcb.ucdavis.edu/cryoem/microscopy101.html

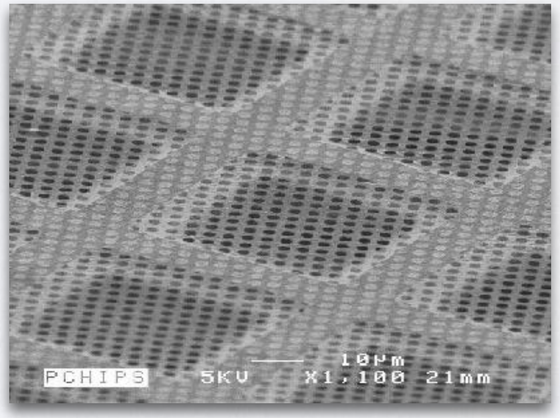
PLUNGE FREEZING

- Sample suspended in physiological buffer
- Holey carbon support film: C-flats, Quantifoil
- Protocol: glow discharge, sample, blot, plunge freeze
- SEMC: Gatan CryoPlunge Freezer 3, FEI Vitrobot, 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



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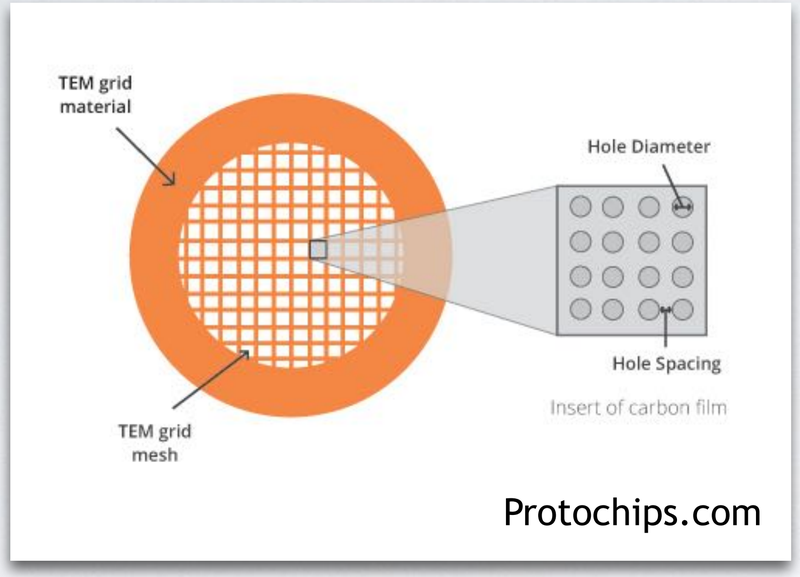
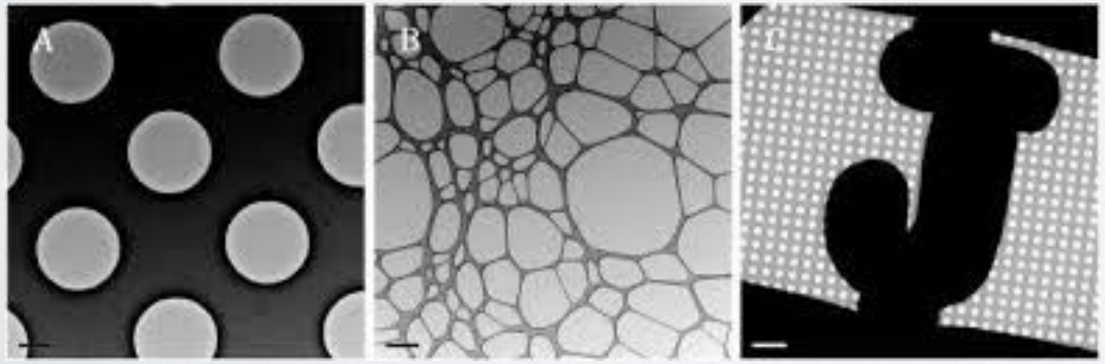
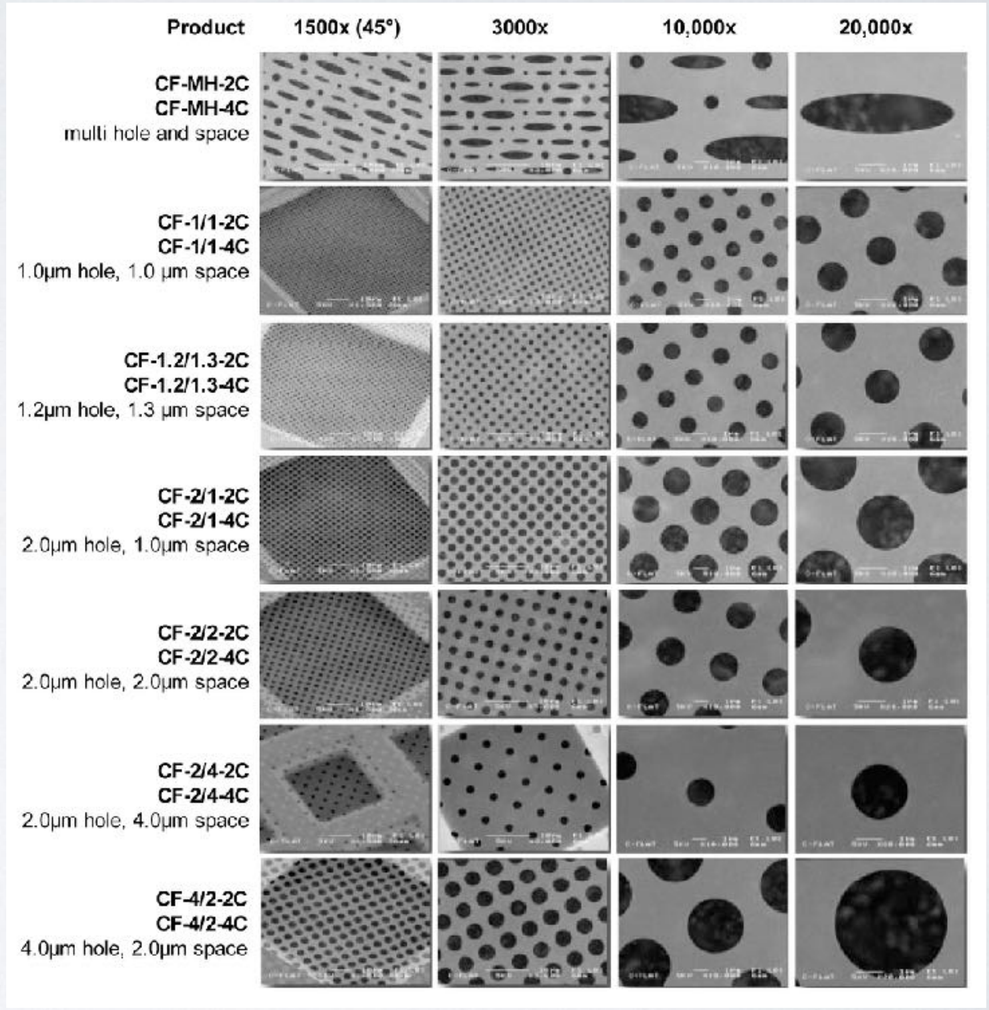
PLUNGE FREEZING

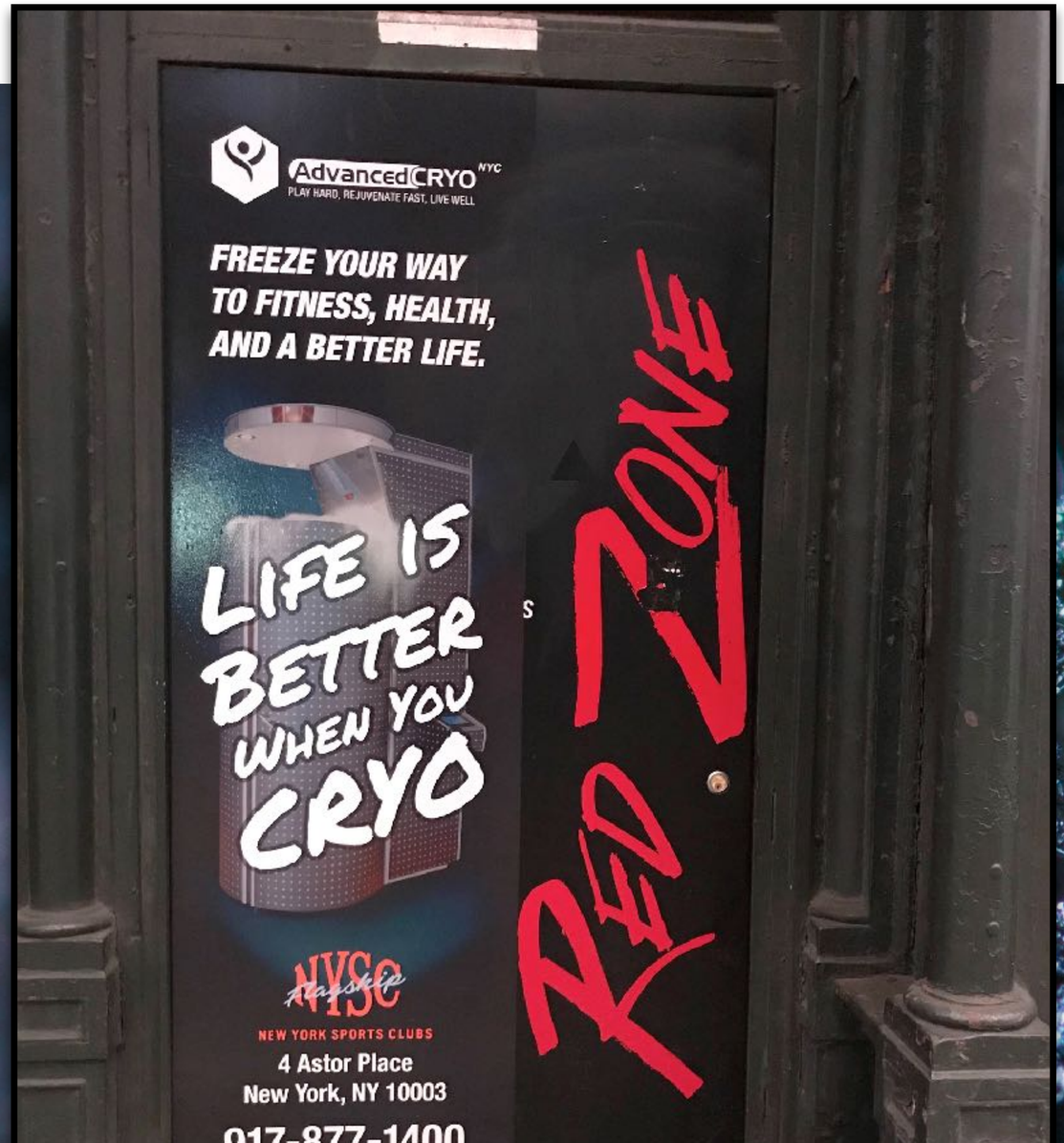






https://scienceservices.de/de/verbrauchsmaterial-werkzeuge/tem-grids/info-tem-grids/coatings-and-coating-materials

HOW SAMPLES ARE PREPARED?



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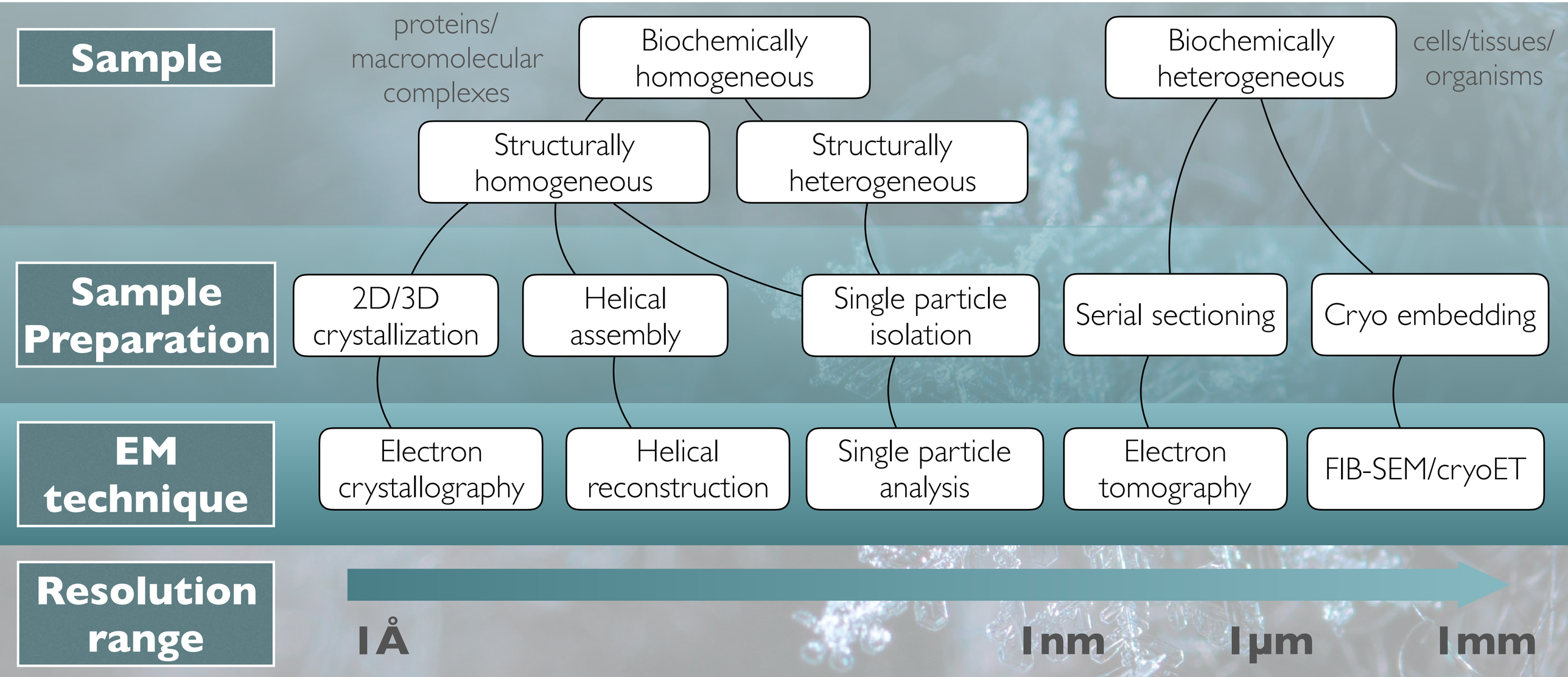

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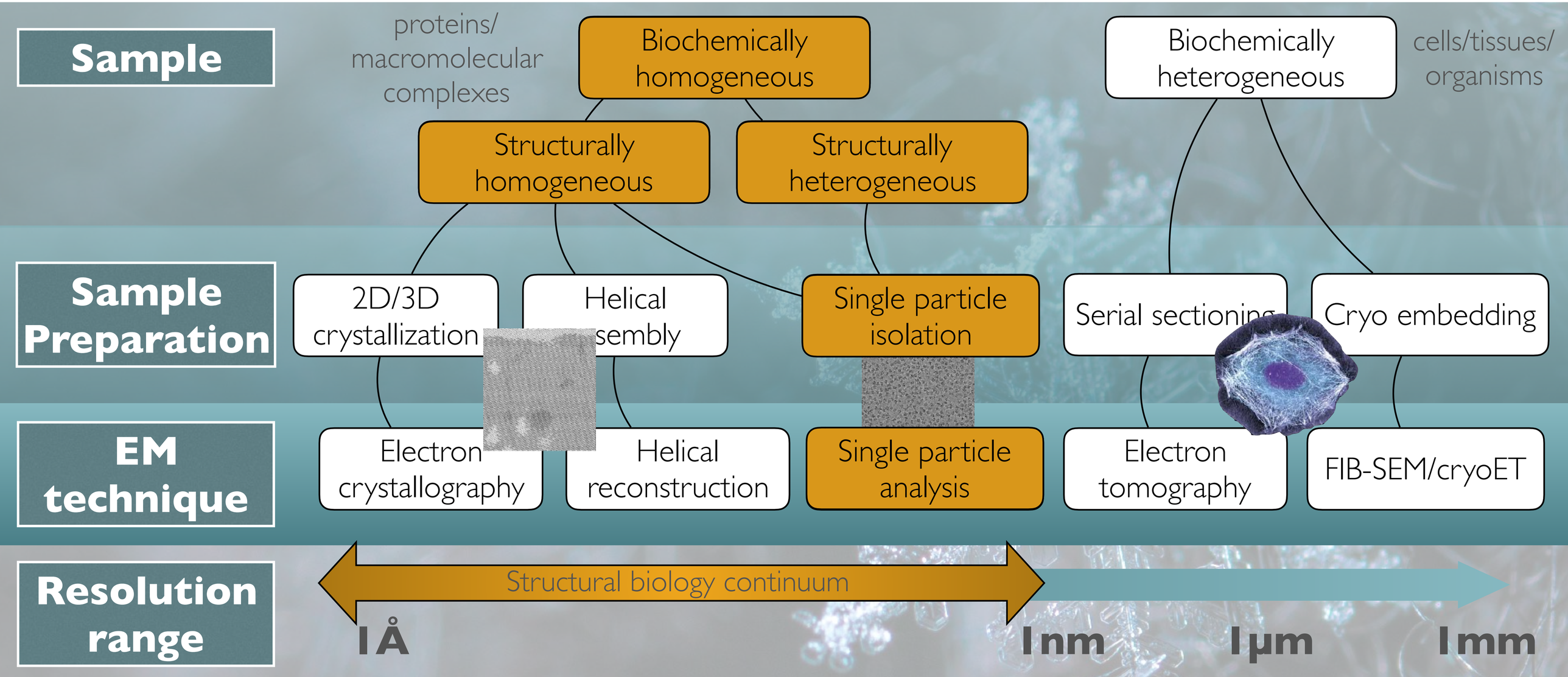
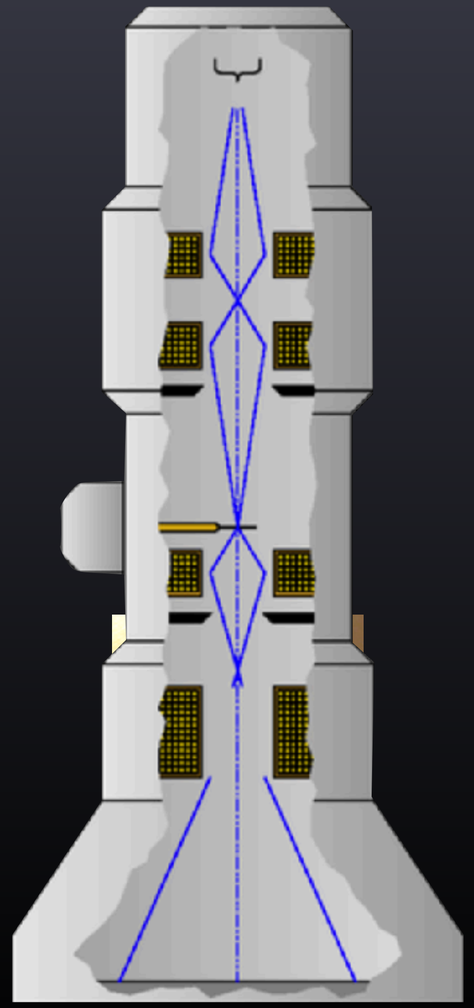
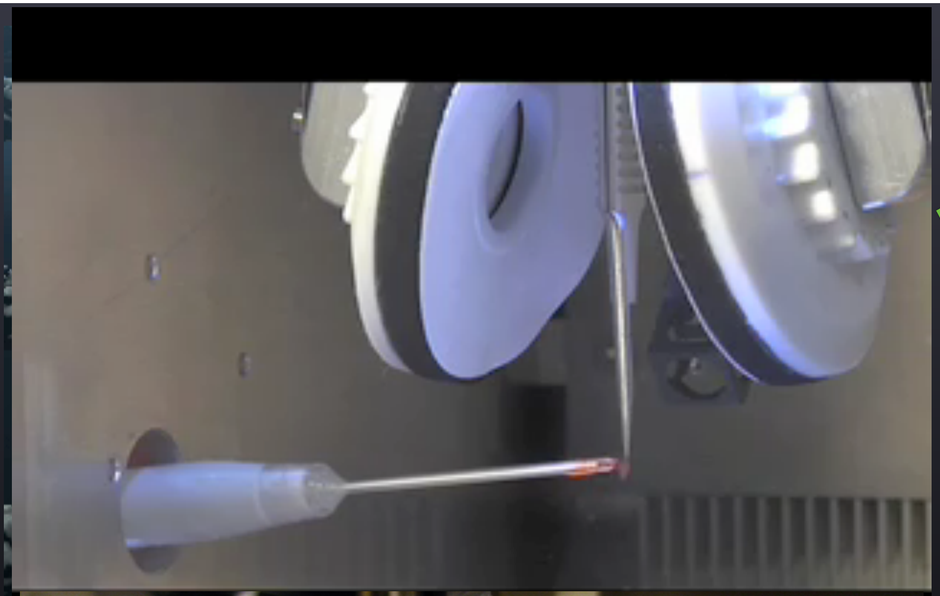


HOW SAMPLES ARE PREPARED?

Vitrifying a biological sample





>99.999%

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~3µl

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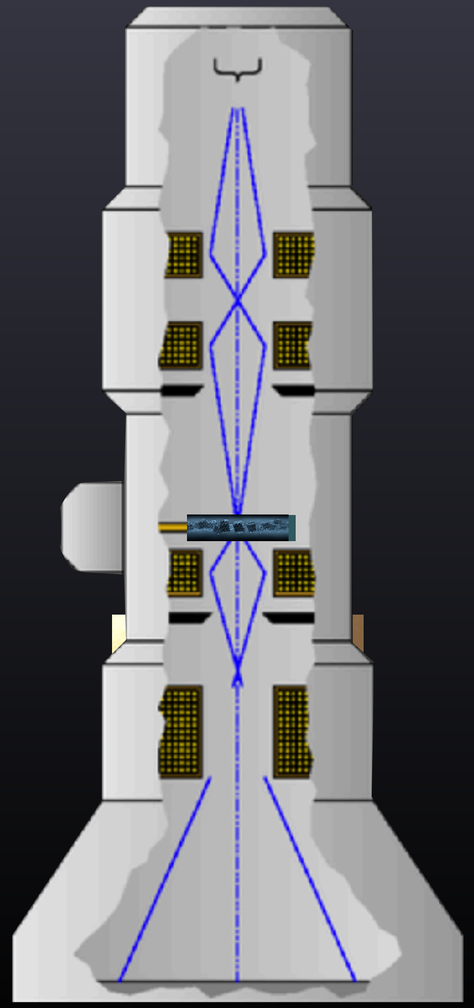
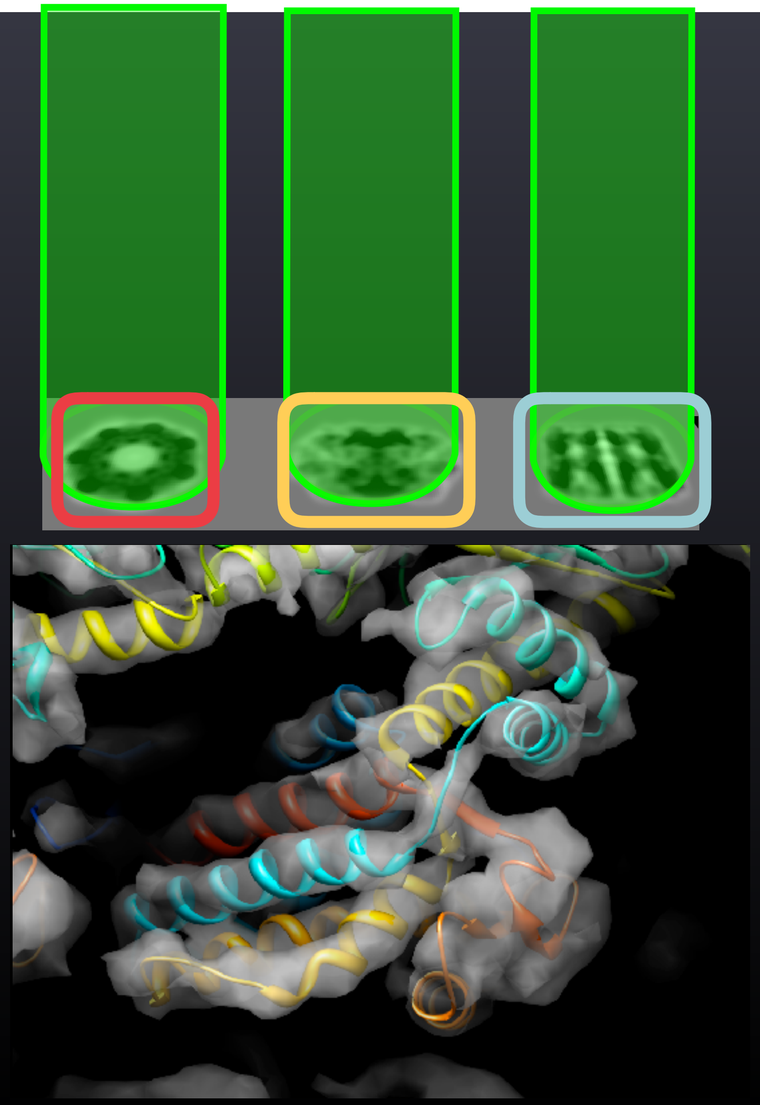
>100,000 potential imaging targets; most of them are not usable.

HOW SAMPLES ARE PREPARED?

Vitrifying a biological sample





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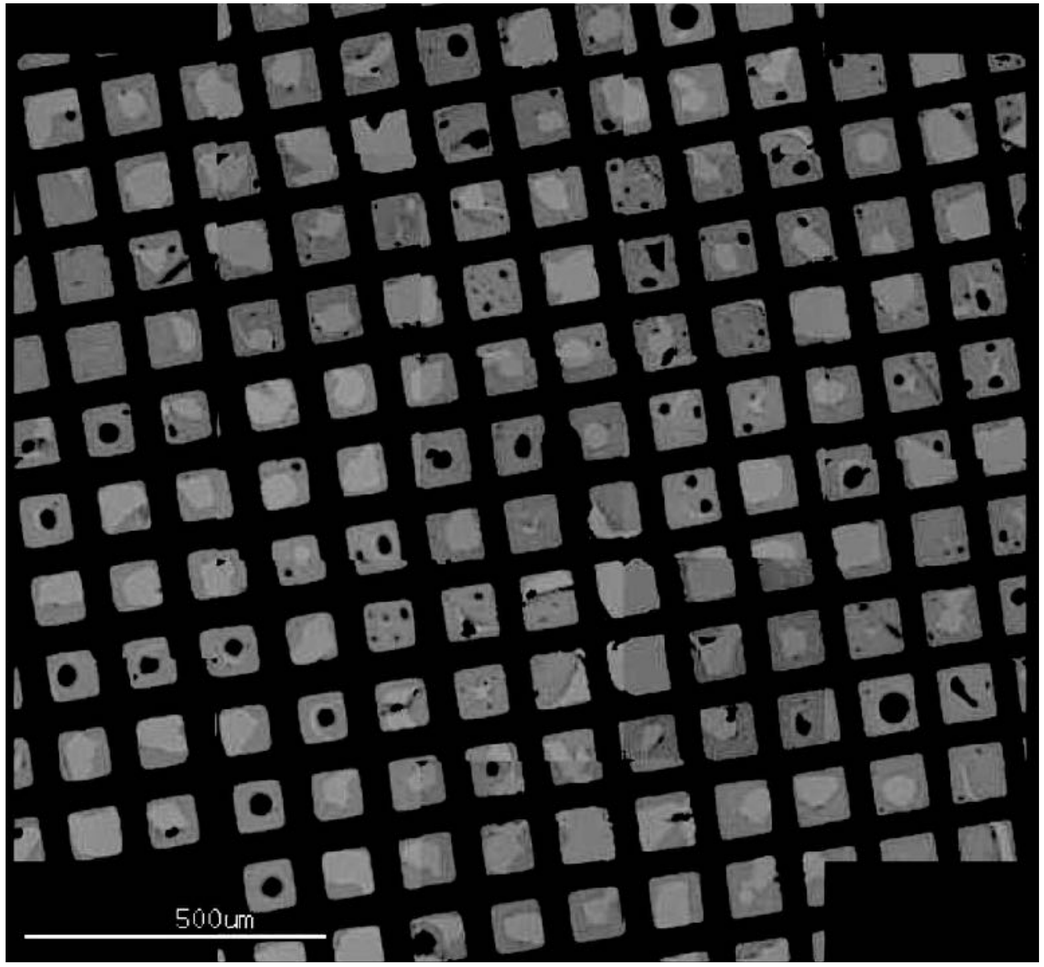
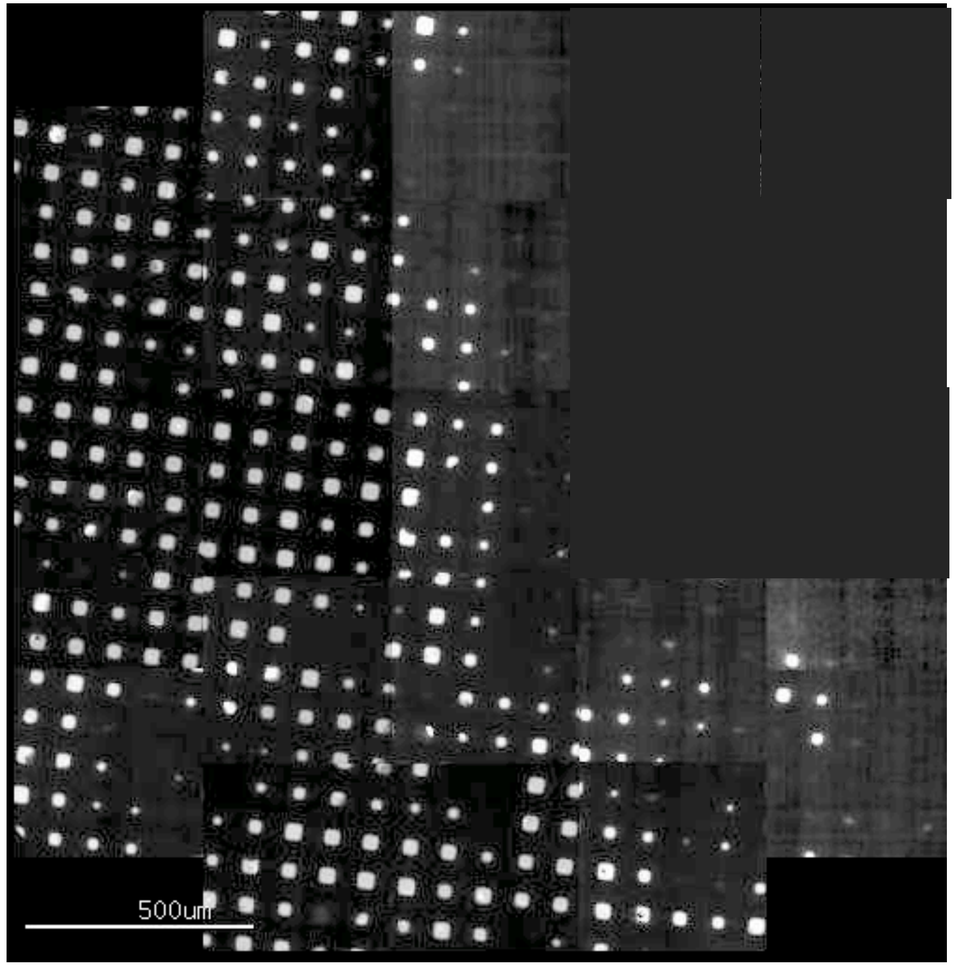
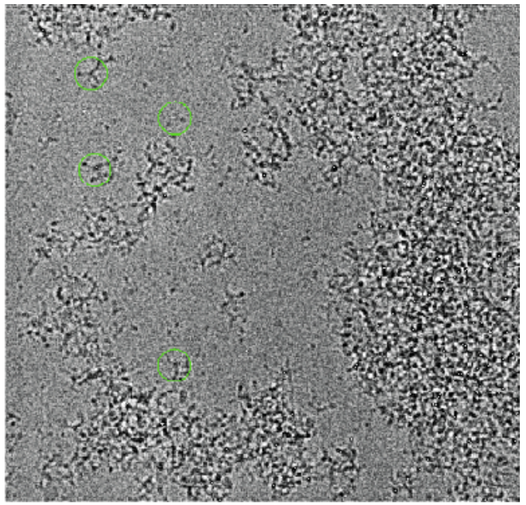
>100,000 potential imaging targets; most of them are not usable.

WHAT DO GRIDS LOOK LIKE?

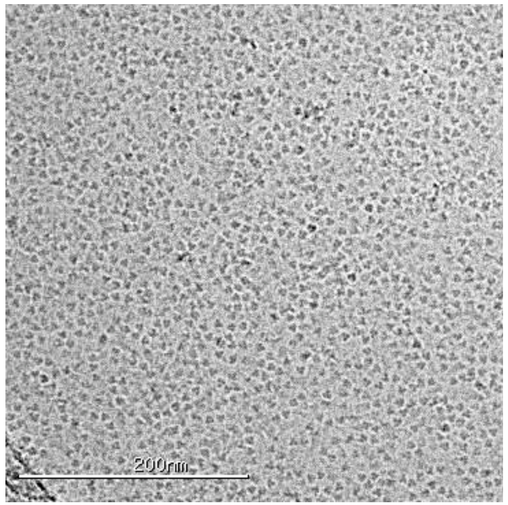




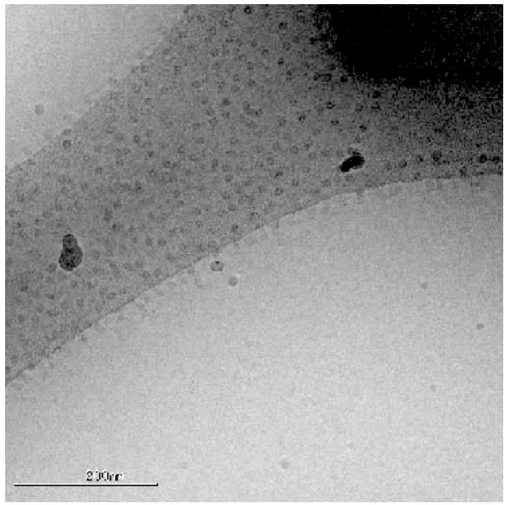
WHAT ISSUES ARISE?



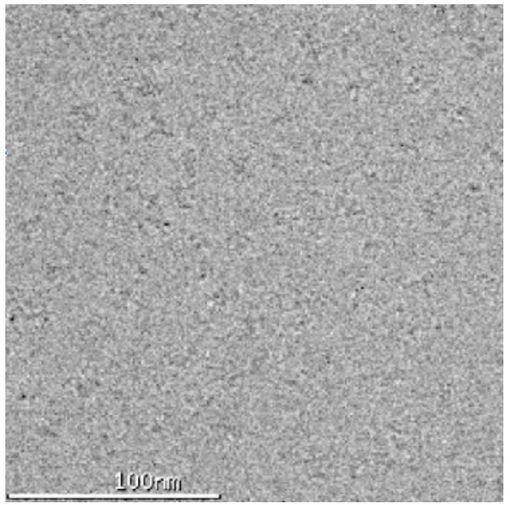
Aggregating in ice



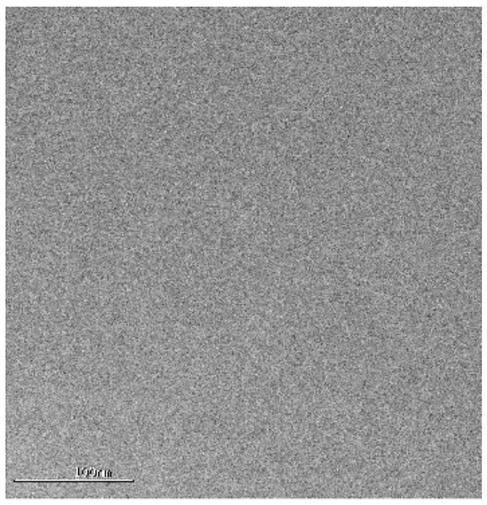
Preferred orientation



Particles not going into holes

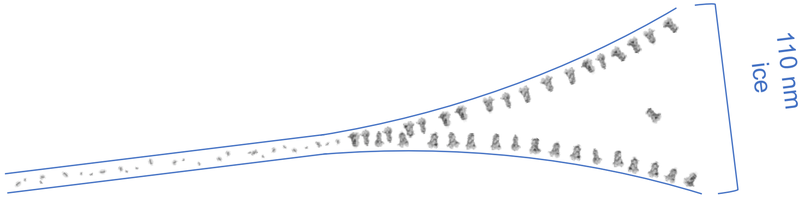


Rejecting 90% of particles

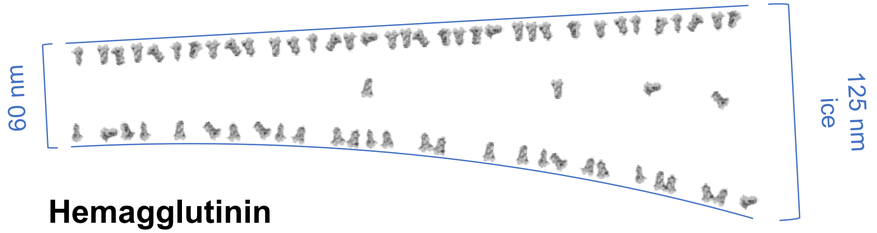


Particles disappearing in ice

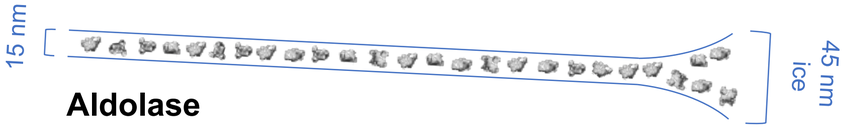
WHAT ISSUES ARISE?



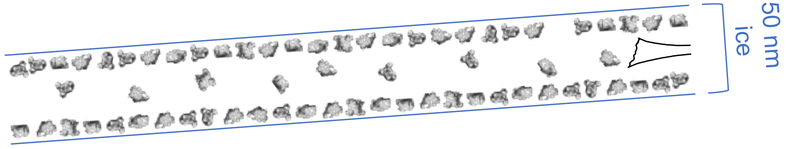
Hemagglutinin



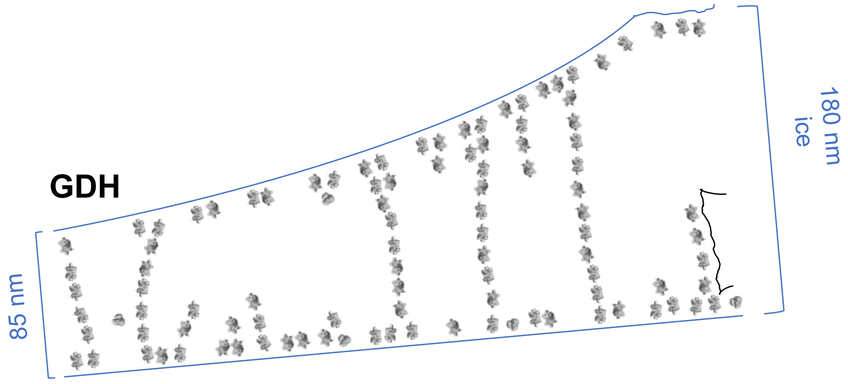
Hemagglutinin



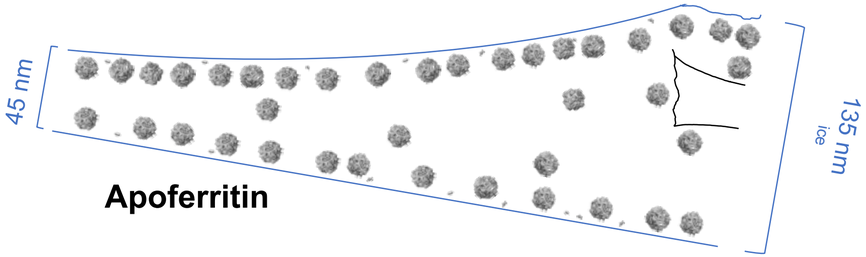
Aldolase



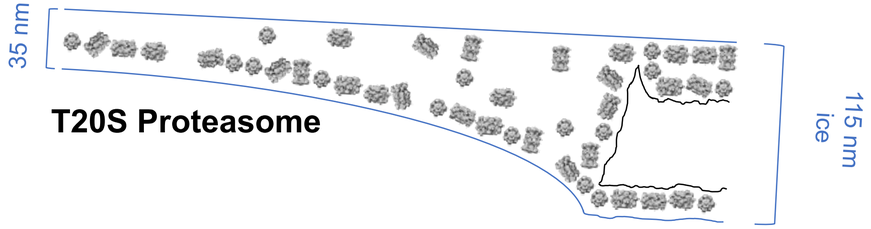
Aldolase



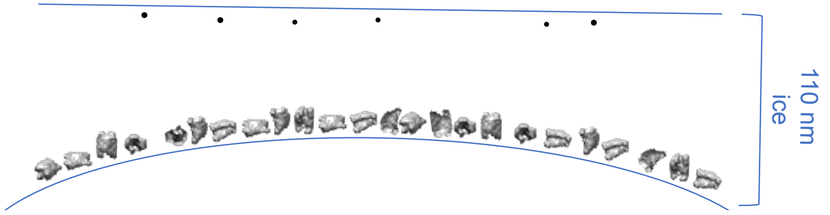
GDH



Apoferritin



T20S Proteasome

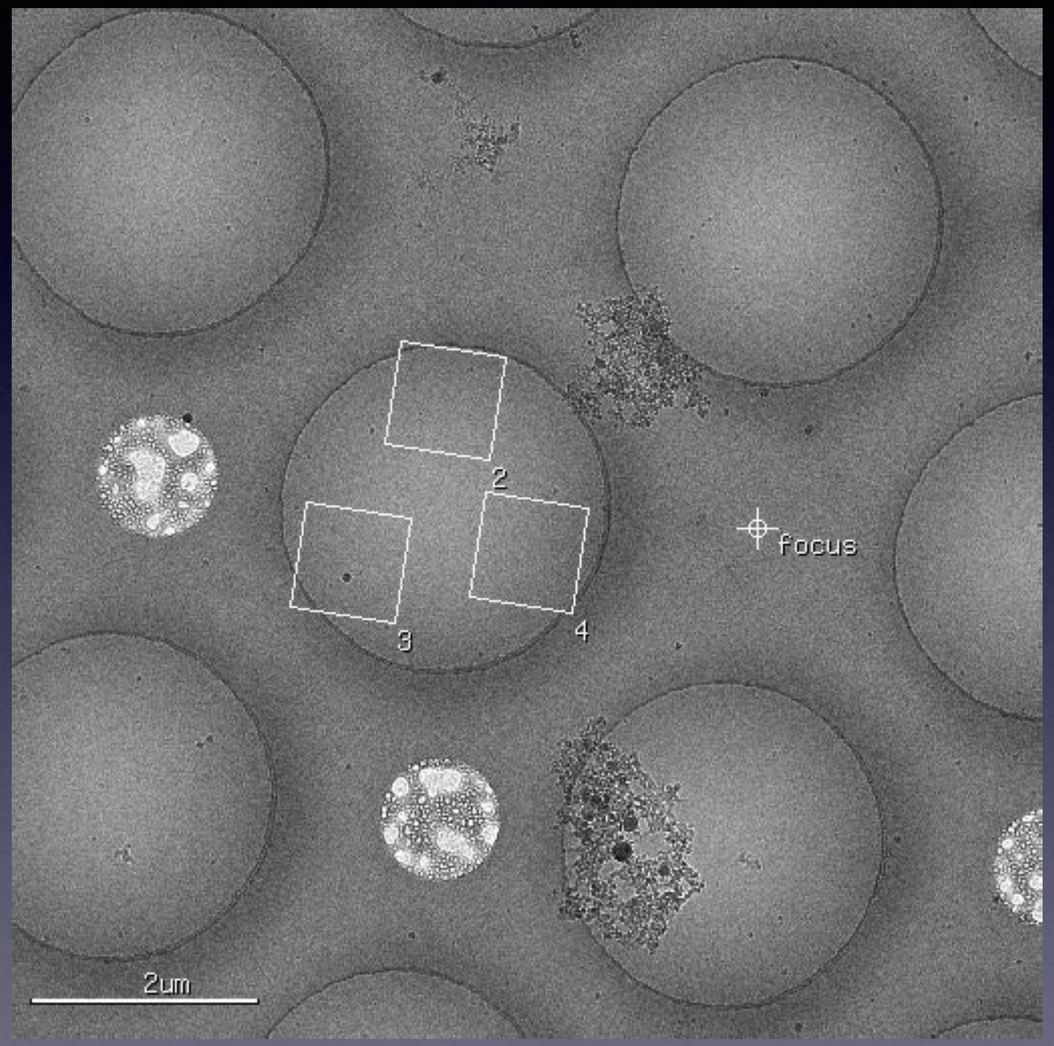


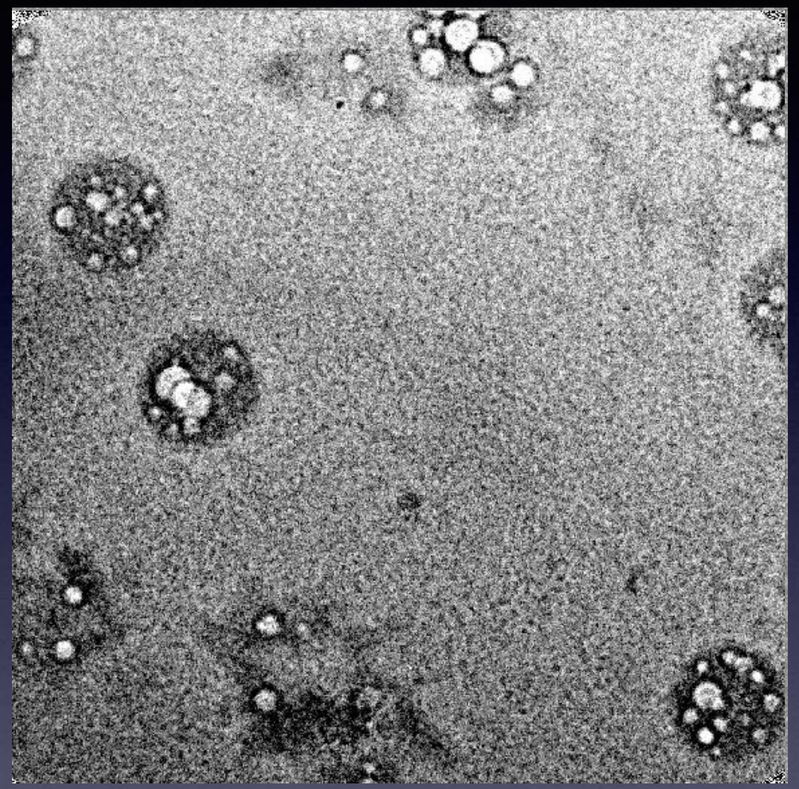
DNAB Helices

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

Alex Noble

Challenges of CryoEM: Radiation damage





“Low-dose” imaging

TRADITIONAL SUBSTRATES FOR CRYO-EM

- 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

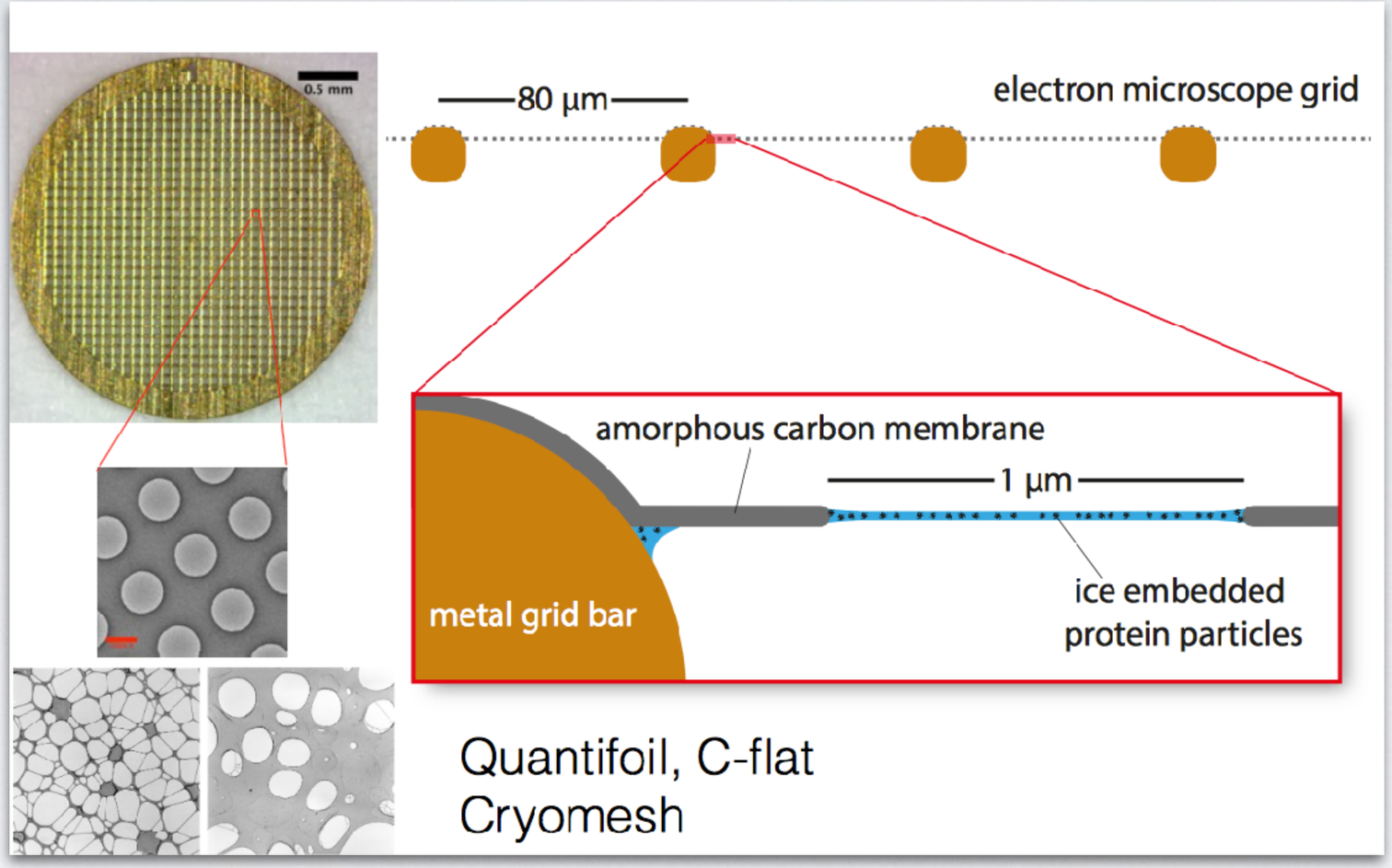
- Additional layer of carbon reduces signal to noise per particle

  ➡ alignment more difficult

- Overall lack of reproducibility from grid to grid

Russo & Passmore, 2015

TRADITIONAL SUBSTRATES FOR CRYO-EM



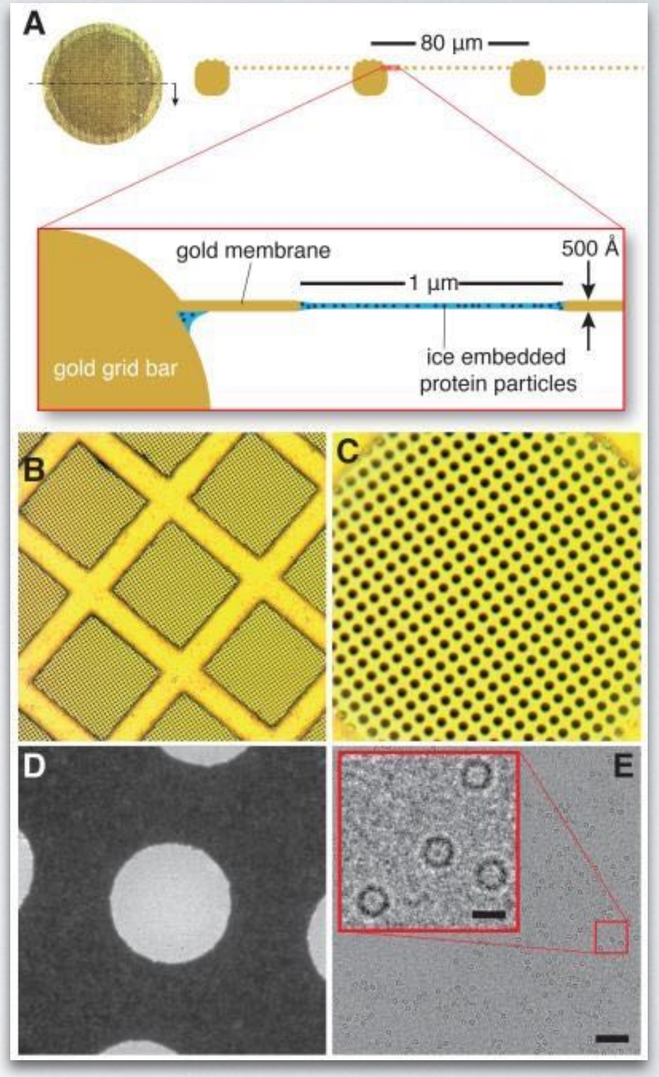
Russo & Passmore, 2015

GOLD GRIDS

- Holey gold foil on gold mesh grid

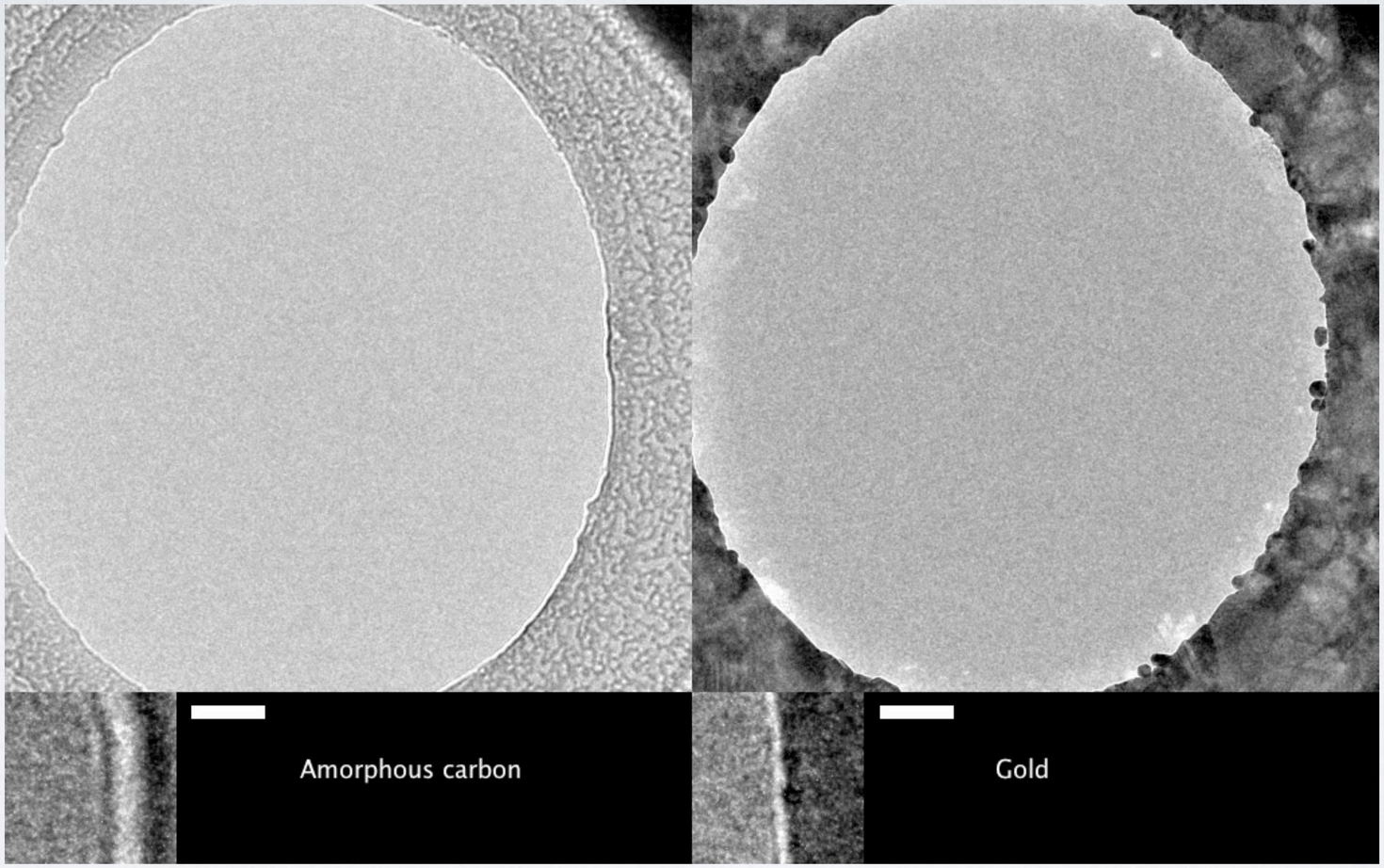
Advantages:
- Prevents differential thermal contraction when freezing
- Reduces beam-induced specimen movement
- Combined with direct detector technology allows for near atomic resolution

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



Russo & Passmore, 2015

GOLD GRIDS



Russo & Passmore, 2015

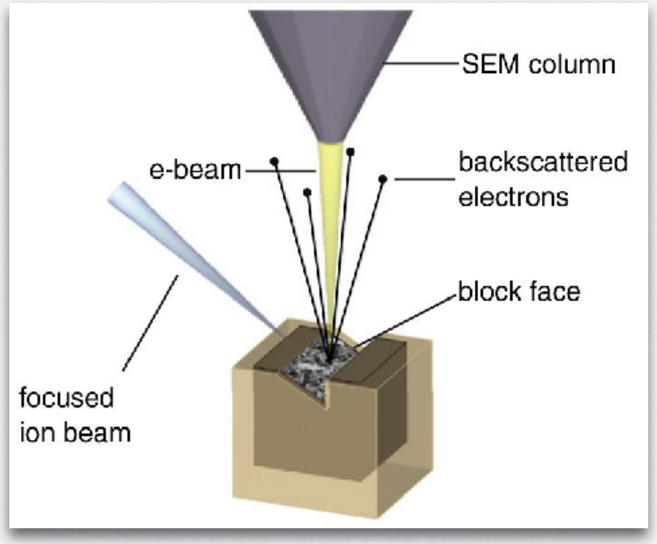
FIB/SEM VS THIN SECTION SAMPLE PREP

- Chemical fixation
- Staining
  - En bloc, enhanced contrast and electrical conductivity
- Dehydration
- Embedding

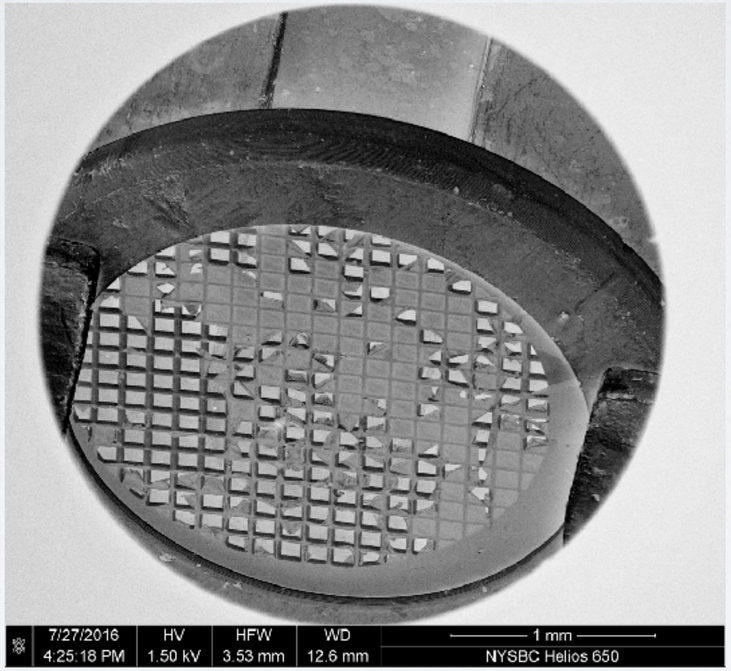
- Au/Pd coat
  - Conductivity

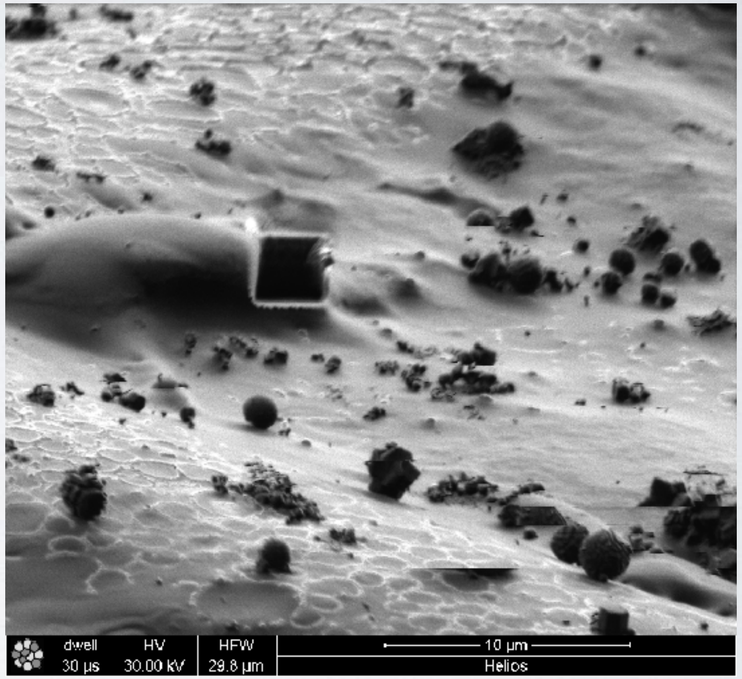
Cryofixation: High pressure freezing
Dehydration: Freeze substitution

- Chemical fixation
- Dehydration
- Embedding
- Sectioning
- Staining



CRYO FIB MILLING





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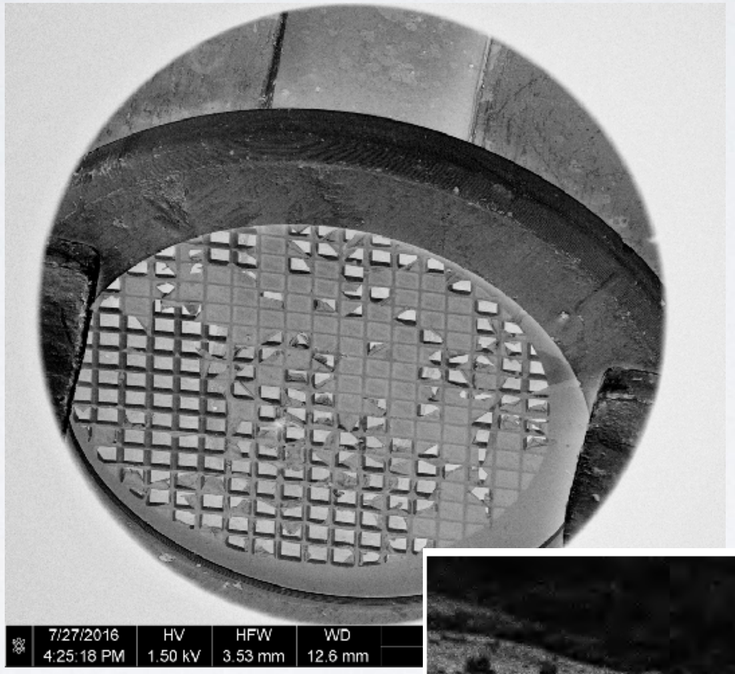

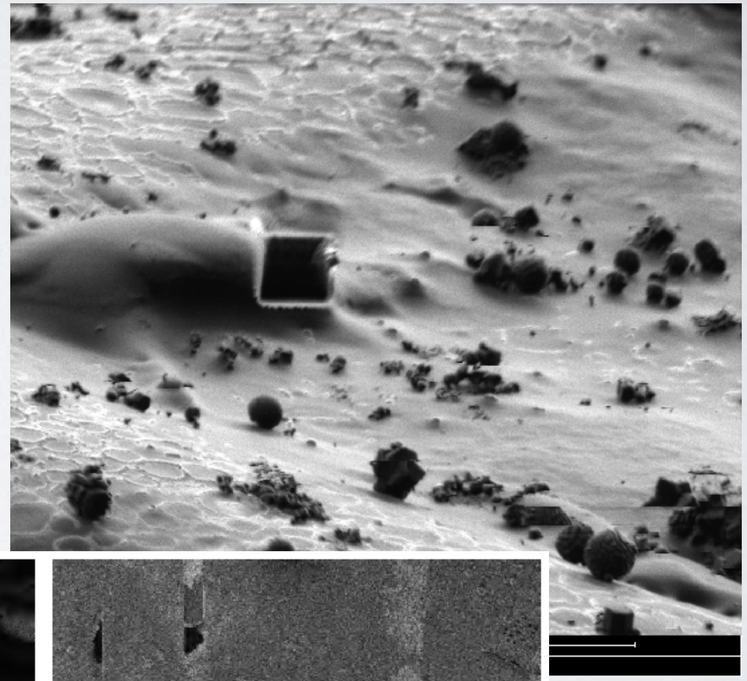

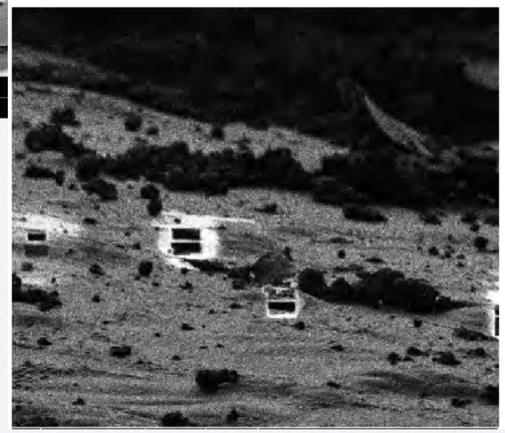

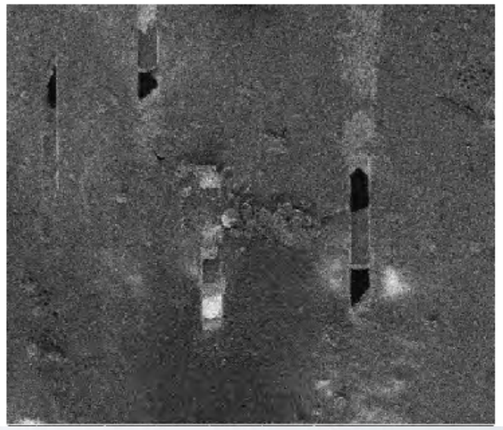

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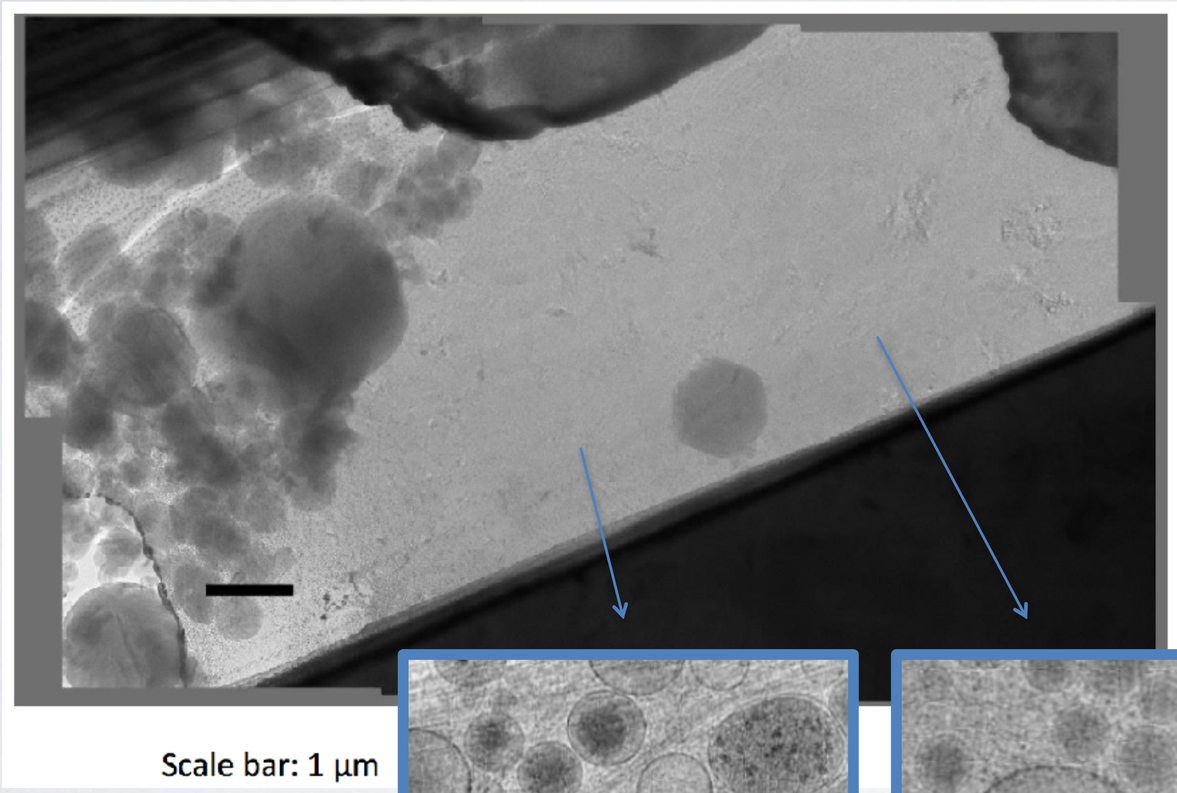
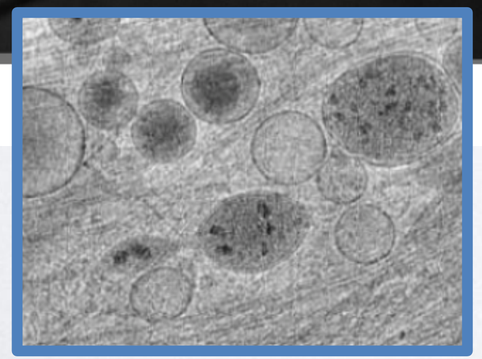
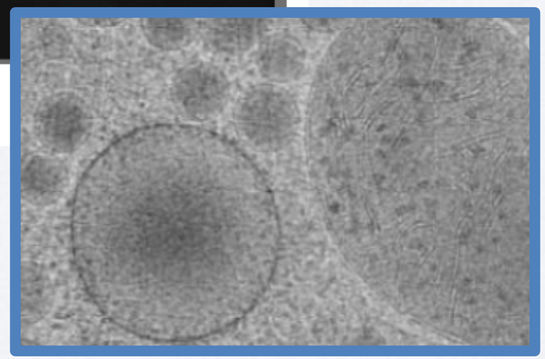
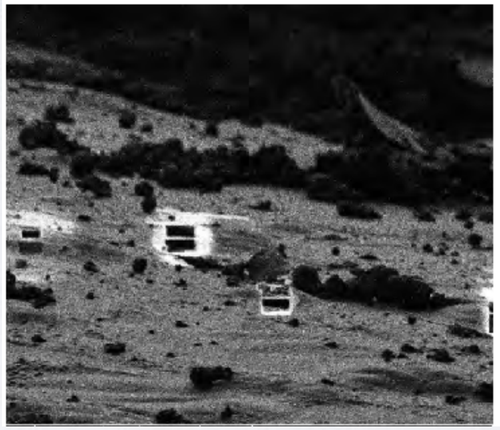
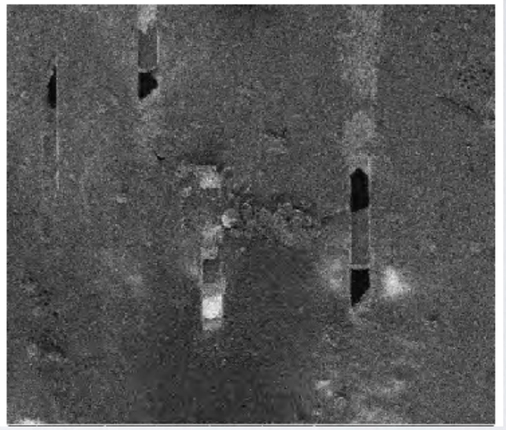


Scale bar: 1 µm

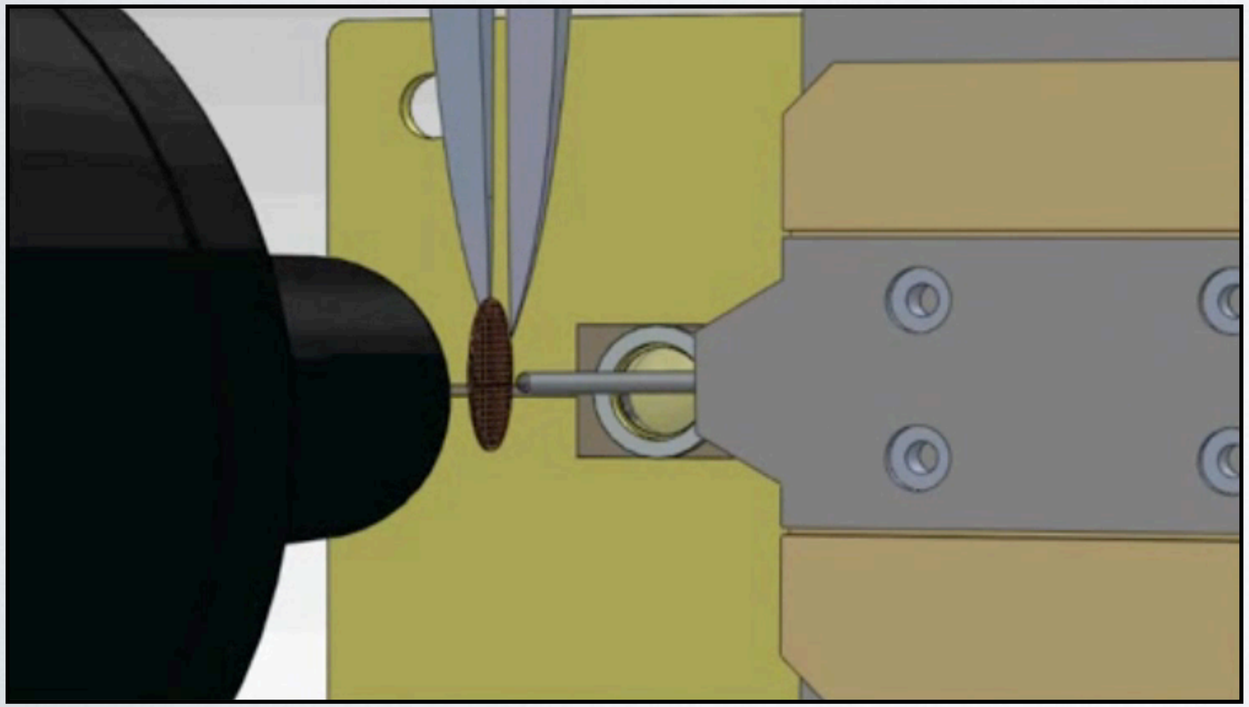


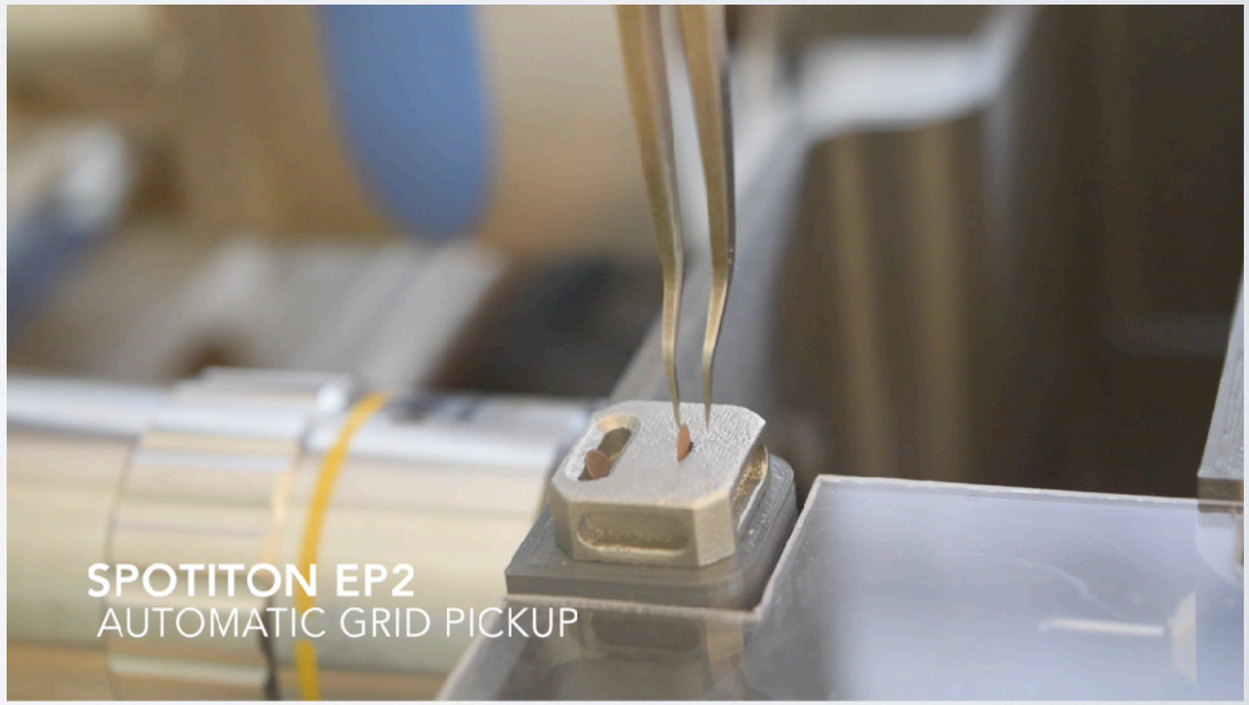






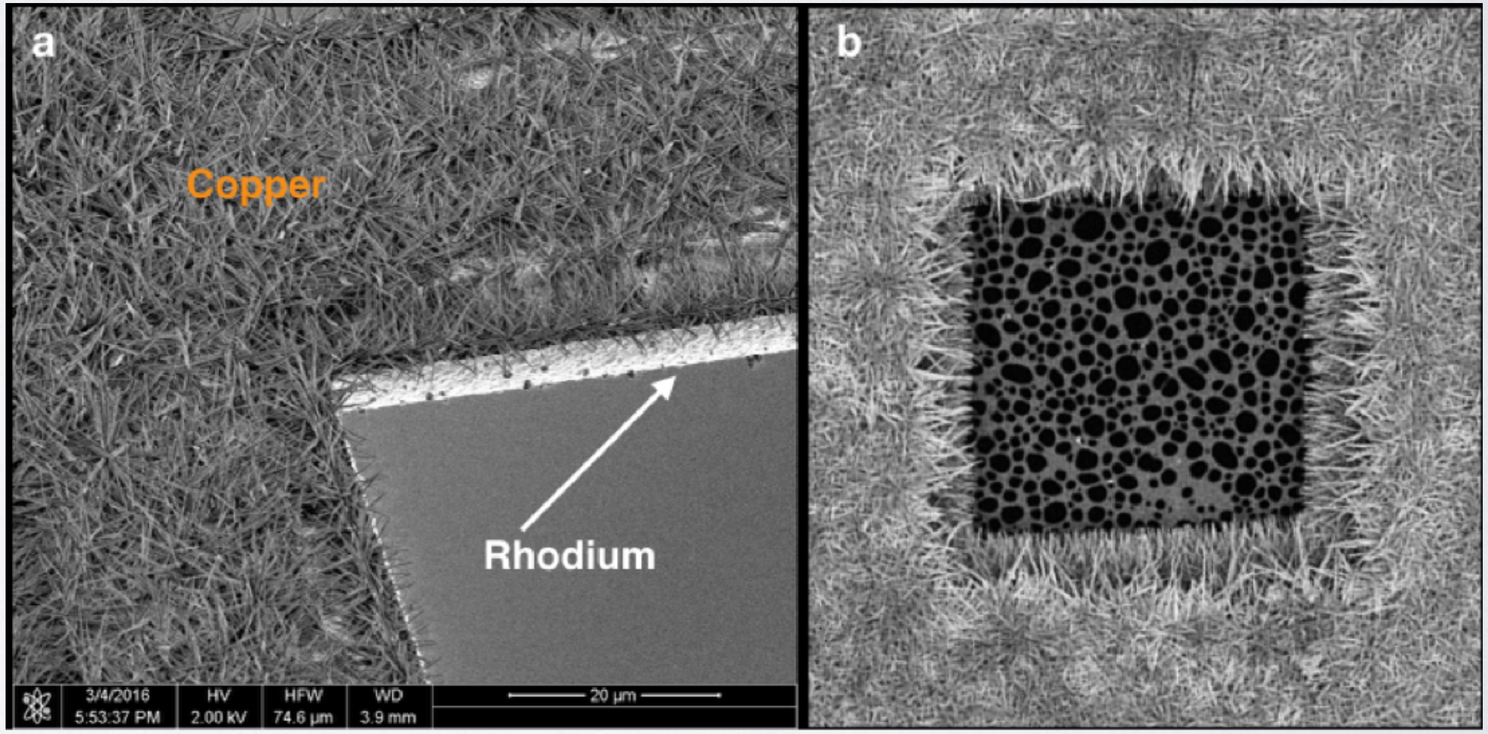
SPOTITON / CHAMELEON





NEXT GENERATION METHODS FOR SAMPLE PREPARATION

Spotiton



Sample preparation and support film practicals

FINISH

START