



Simons Electron Microscopy Center

Challenges in biological EM & Sample Prep

2019 Winter EM Course

25 Min lecture + 40 min practical
14 Jan 2019



COURSE STRUCTURE

Monday and select Wednesdays
3:30-5pm - A-11 seminar room /
SEMC conference room

1.5 hr class

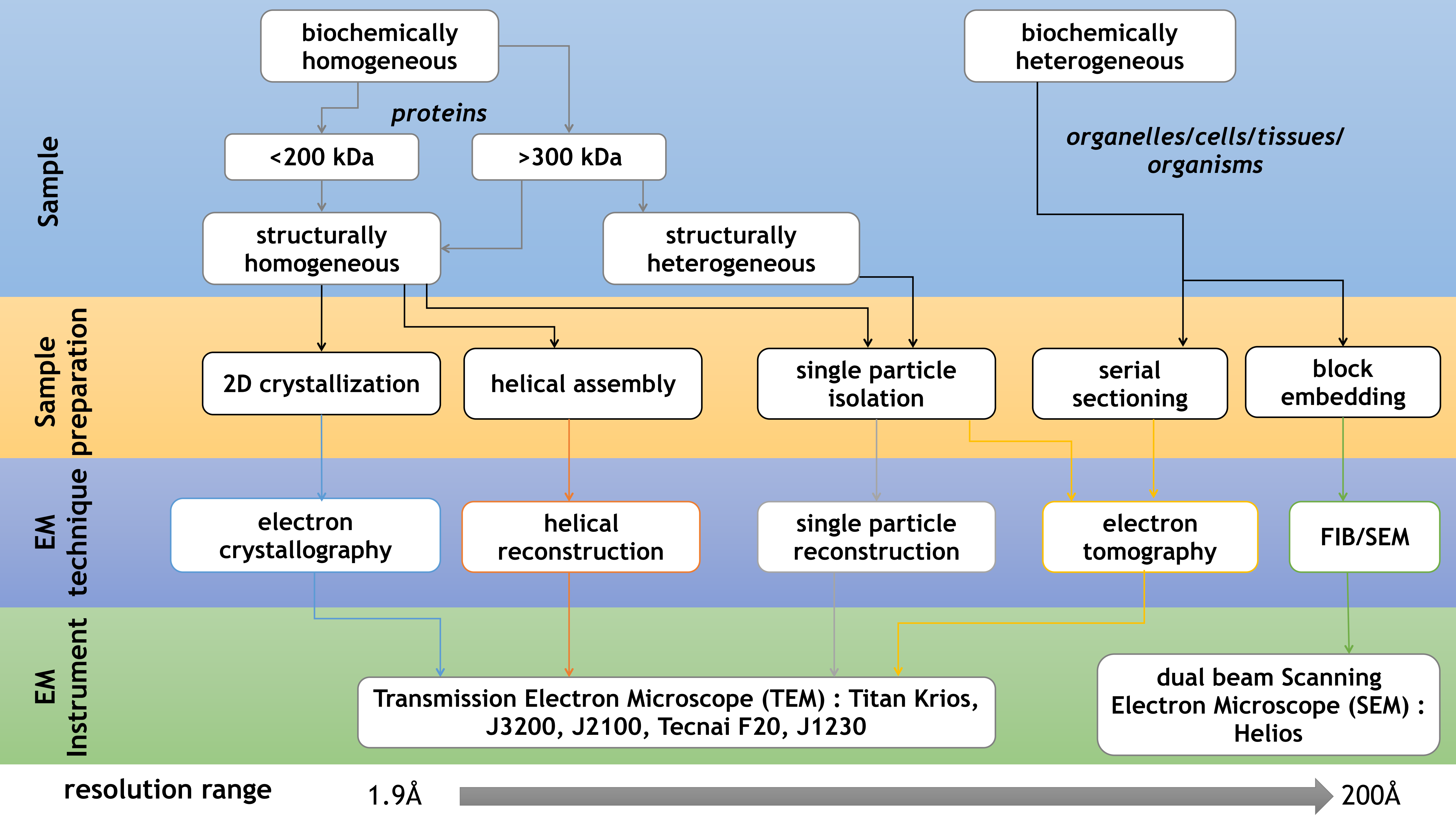
Class handbook

Wednesdays
Starts at 3:30 - SEMC conference room

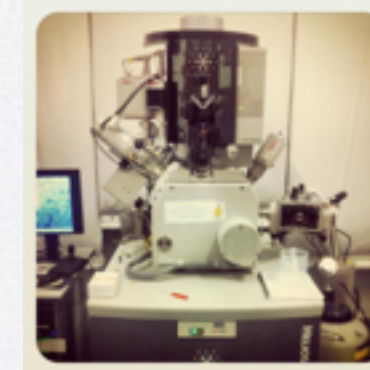
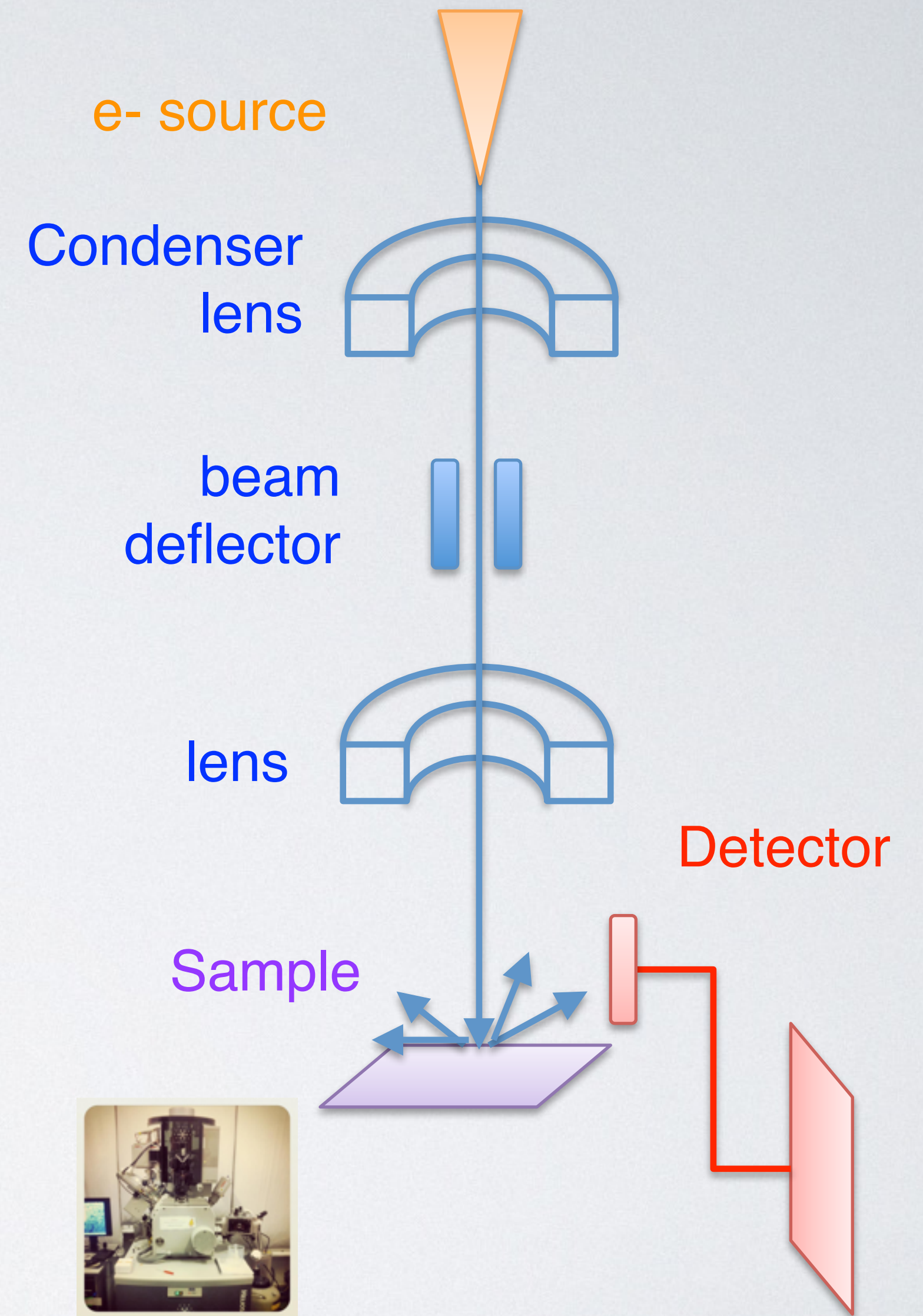
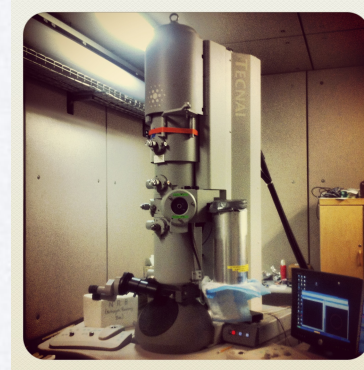
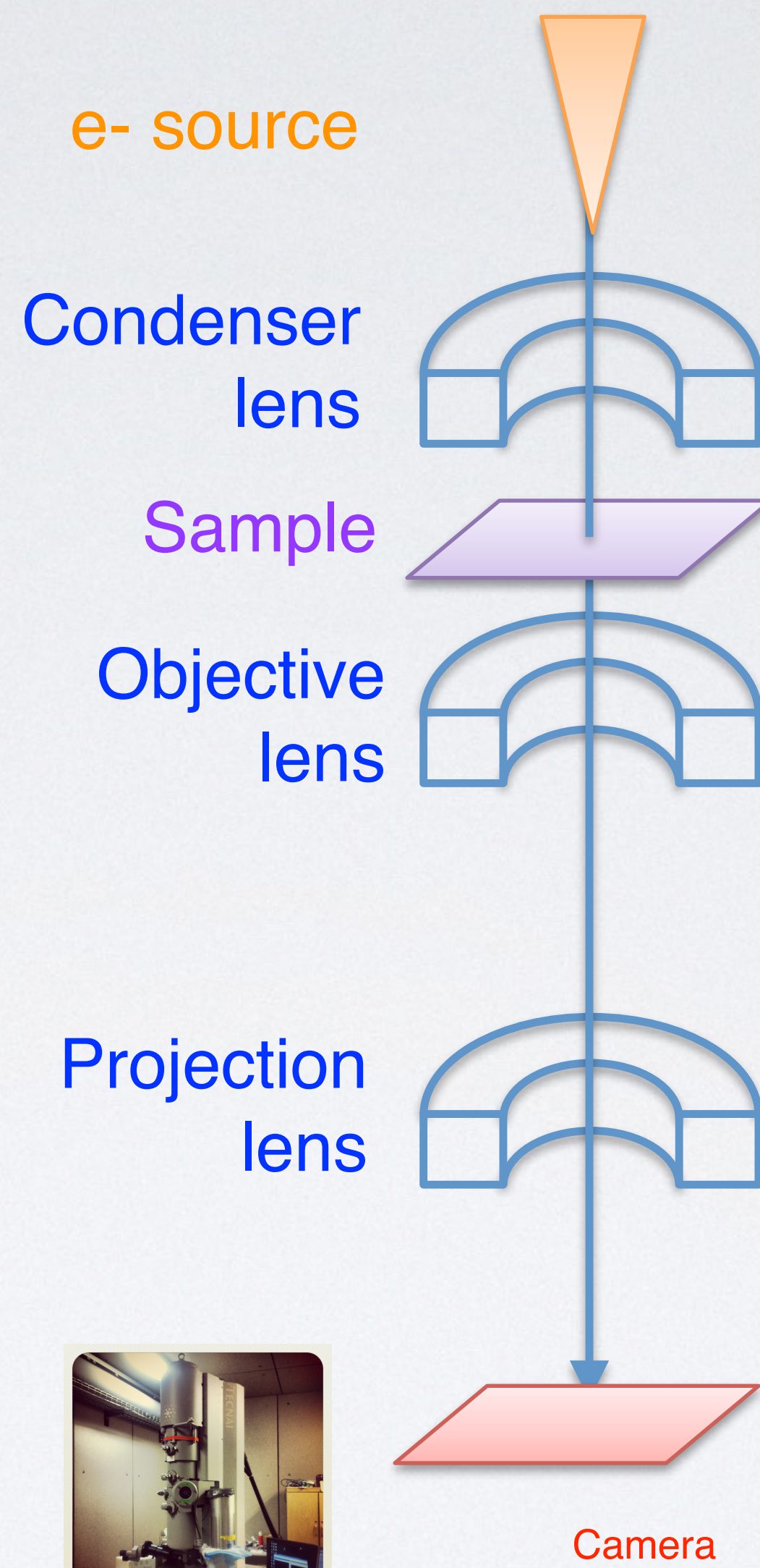
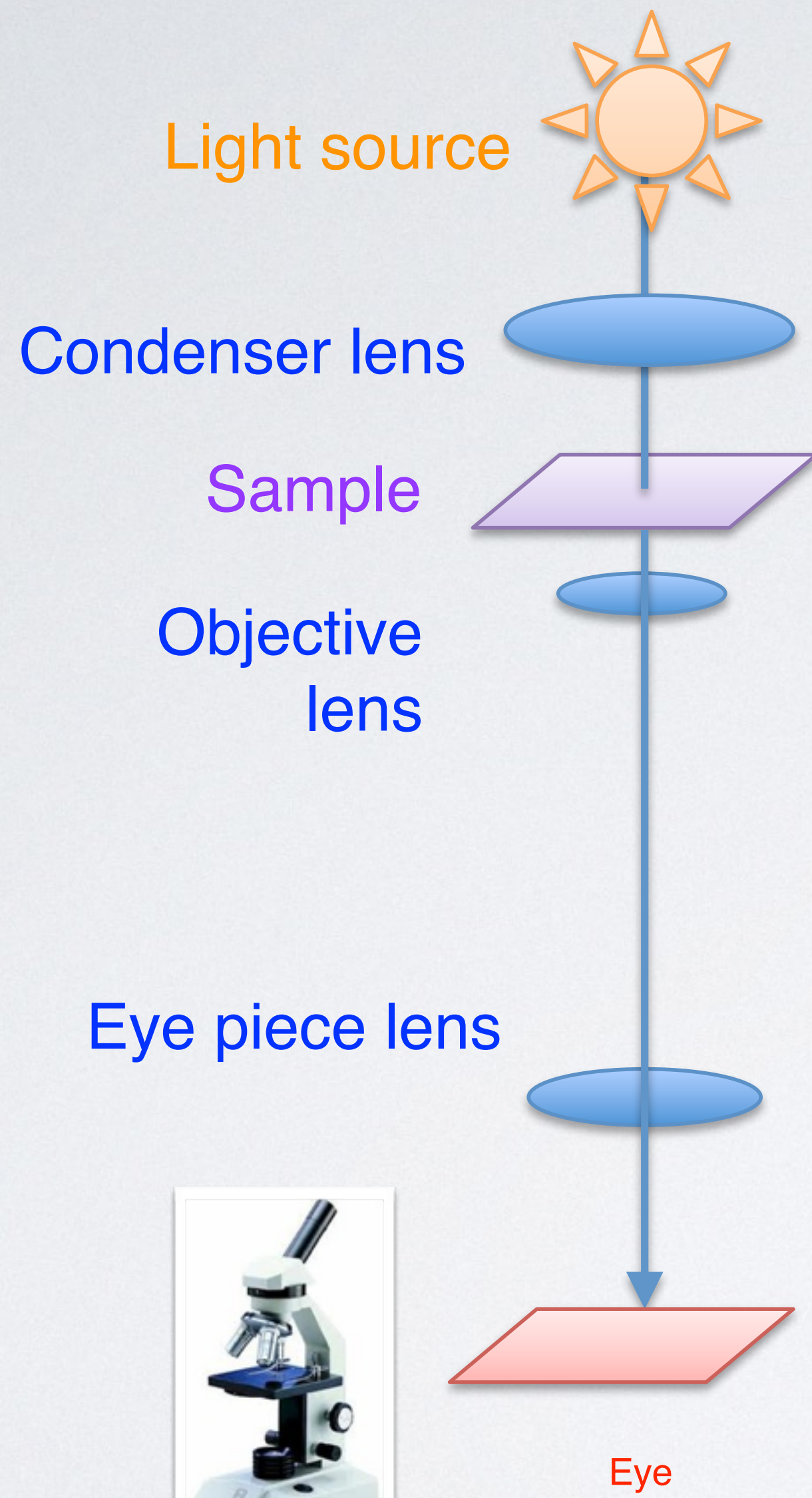
Recitation section
Journal club and practicals

Recitation schedule

Jan 30: Microscope Practical
Feb 6: Journal Club 1
Feb 13: Tomography Practical
Feb 20: Tomography Practical 2
Feb 27: Journal club 2
Mar 6: Single Particle Practical
Mar 13: Journal club 3
Mar 20: Journal club 4
Mar 27: Molecular fitting
Apr 3: Journal club 5
Apr 10: Journal club 6
Apr 17: Journal club 7
Apr 24: Journal club 8

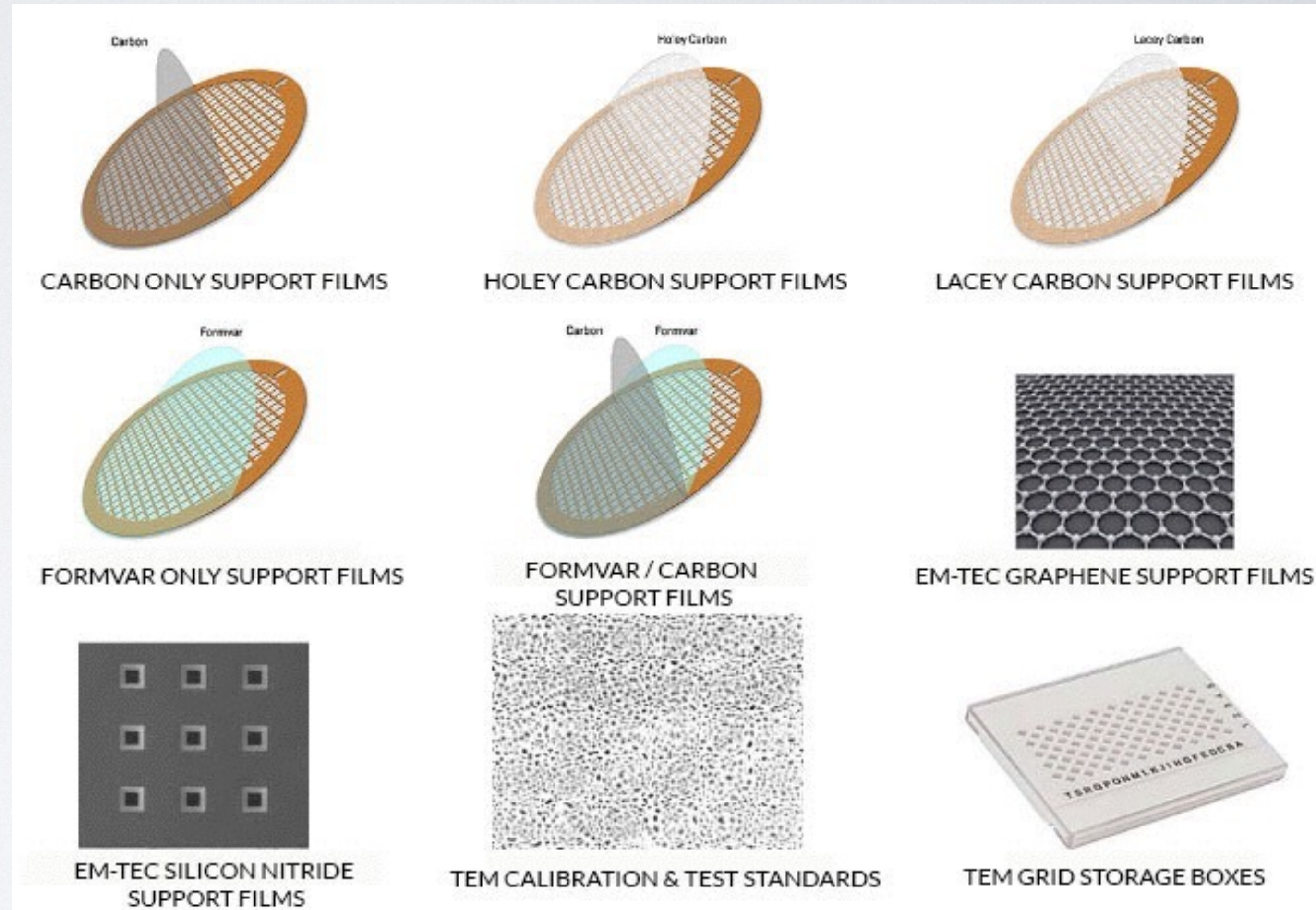


TOOLS OF THE TRADE



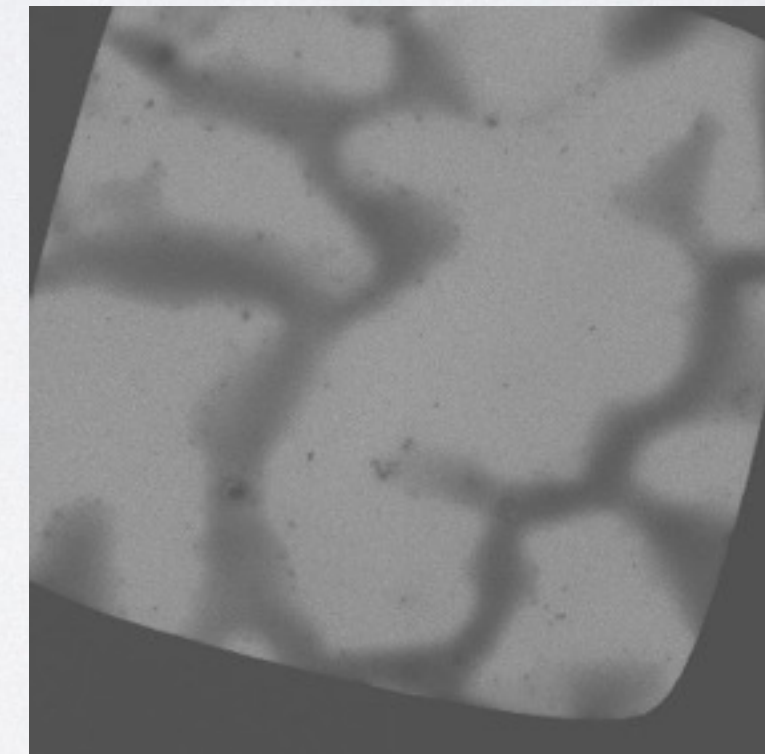
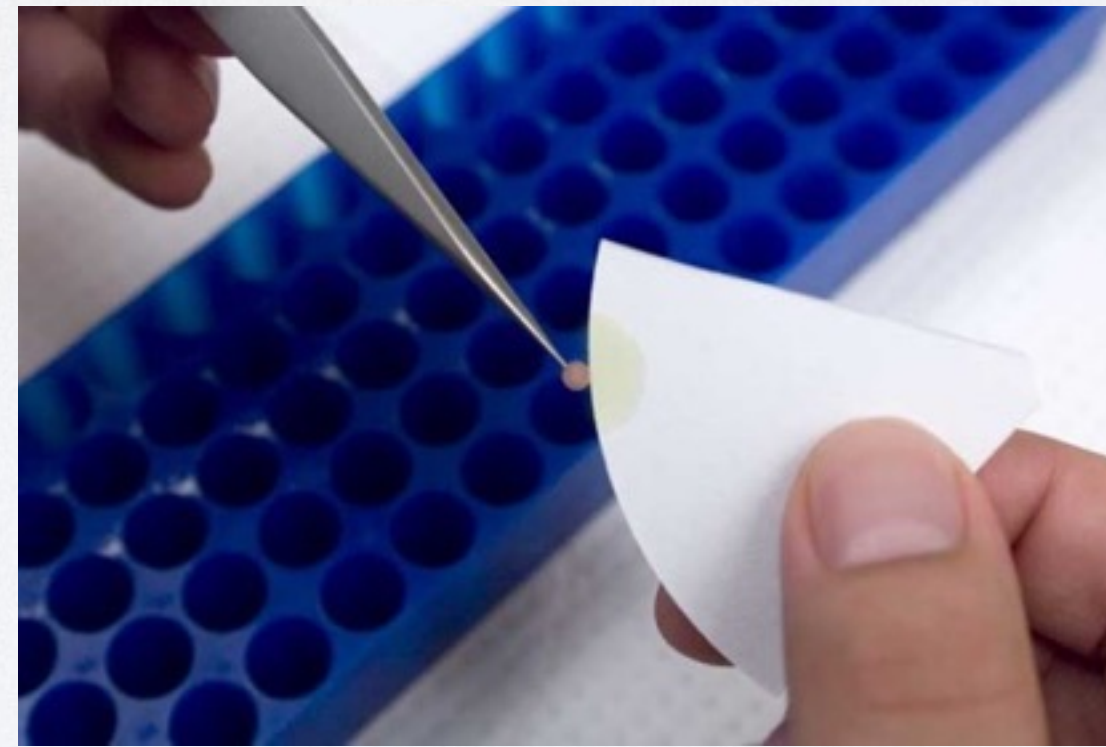
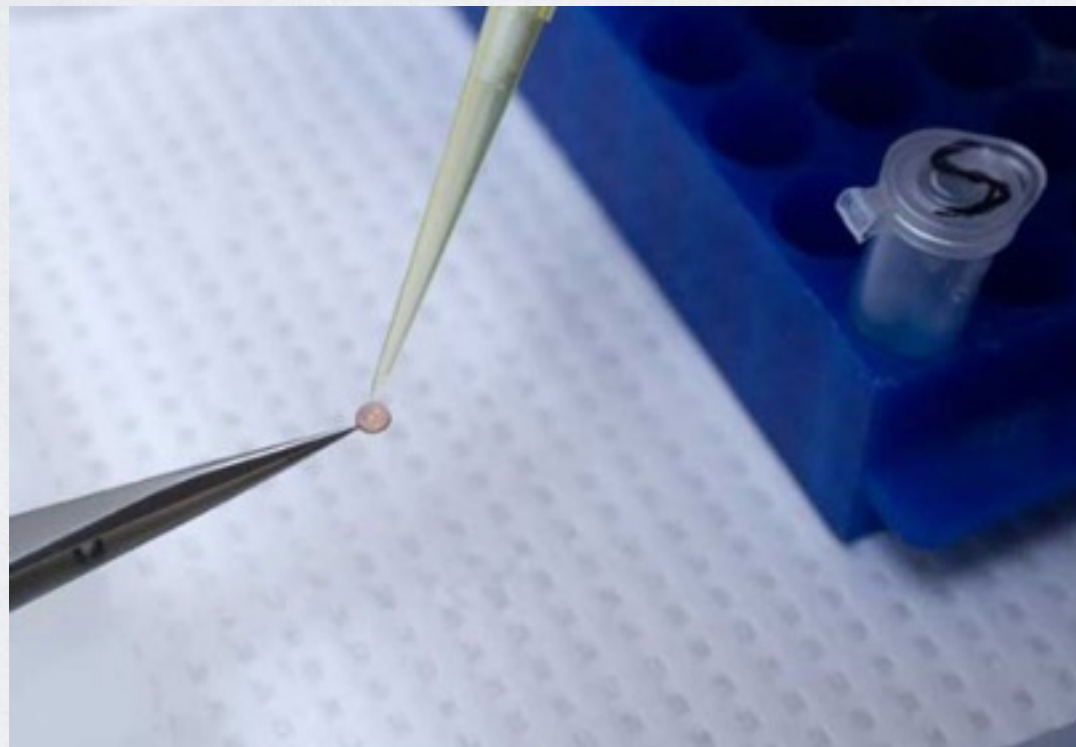
WHAT HOLDS OUR SAMPLE?

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



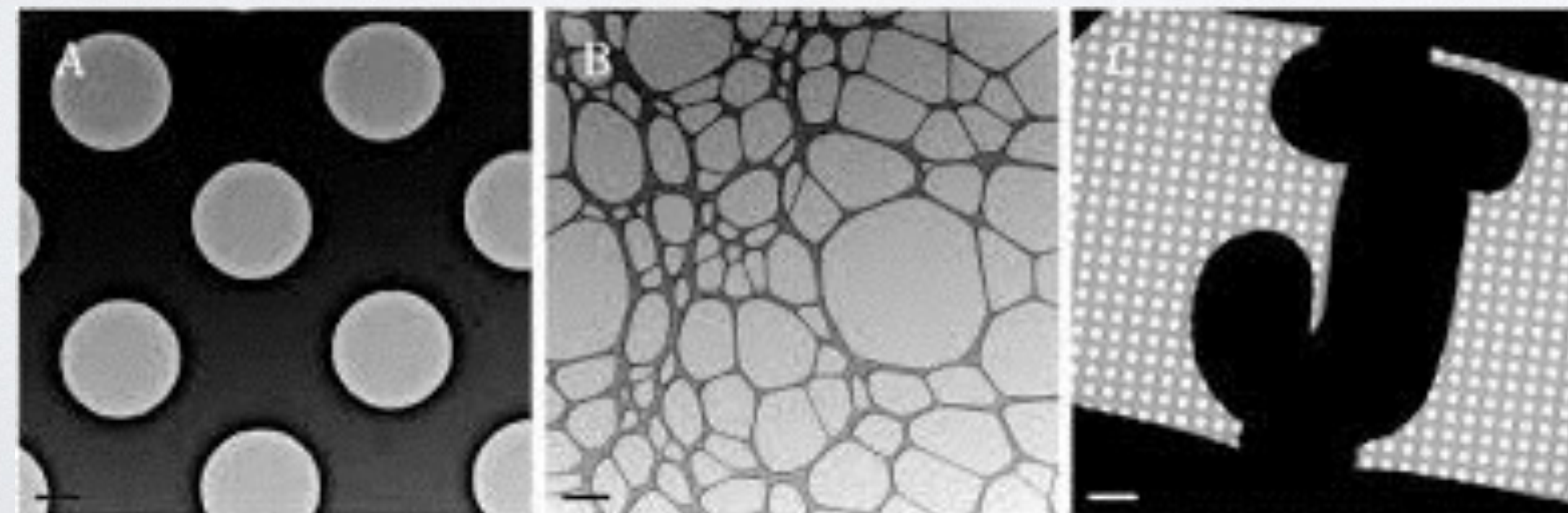
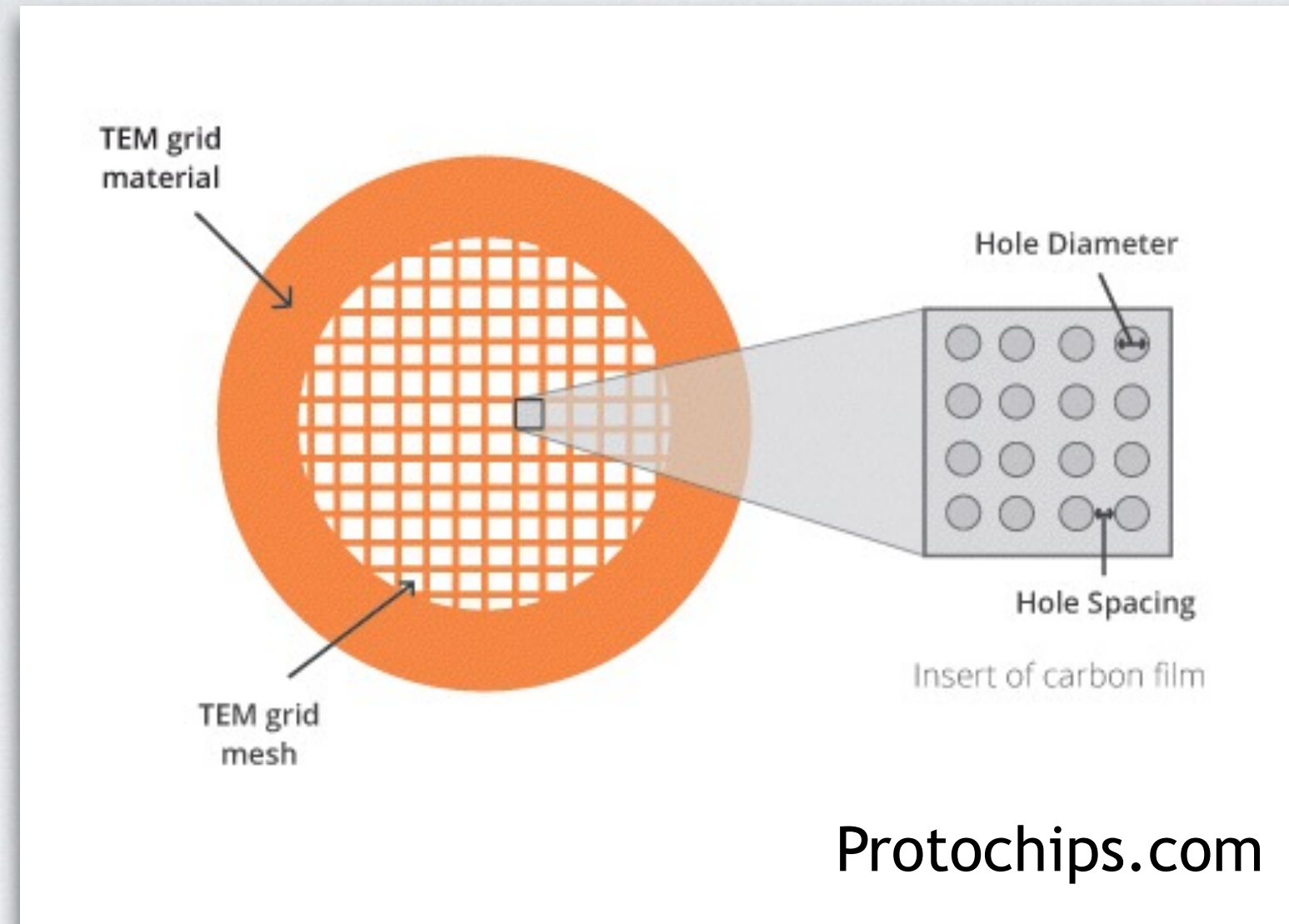
NEGATIVE STAINING

- What support films are used?
 - will be in practical



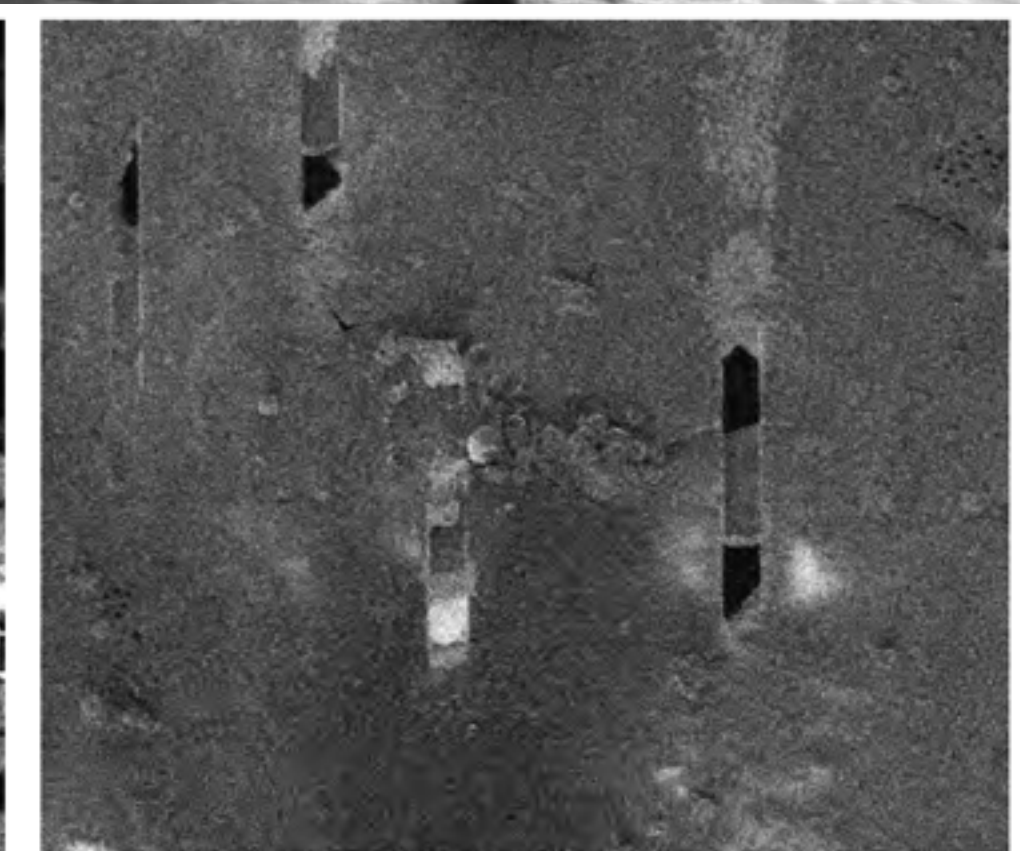
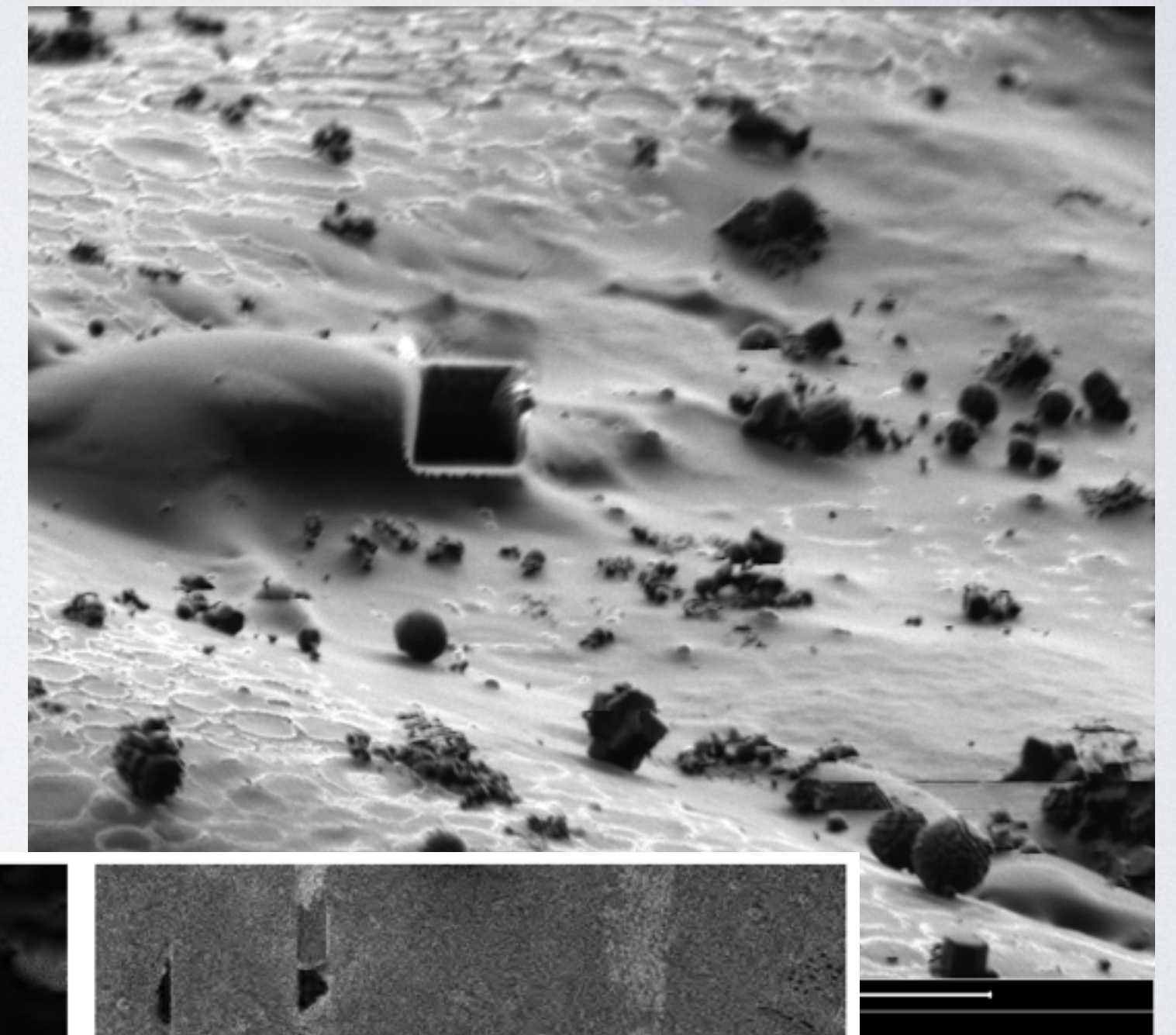
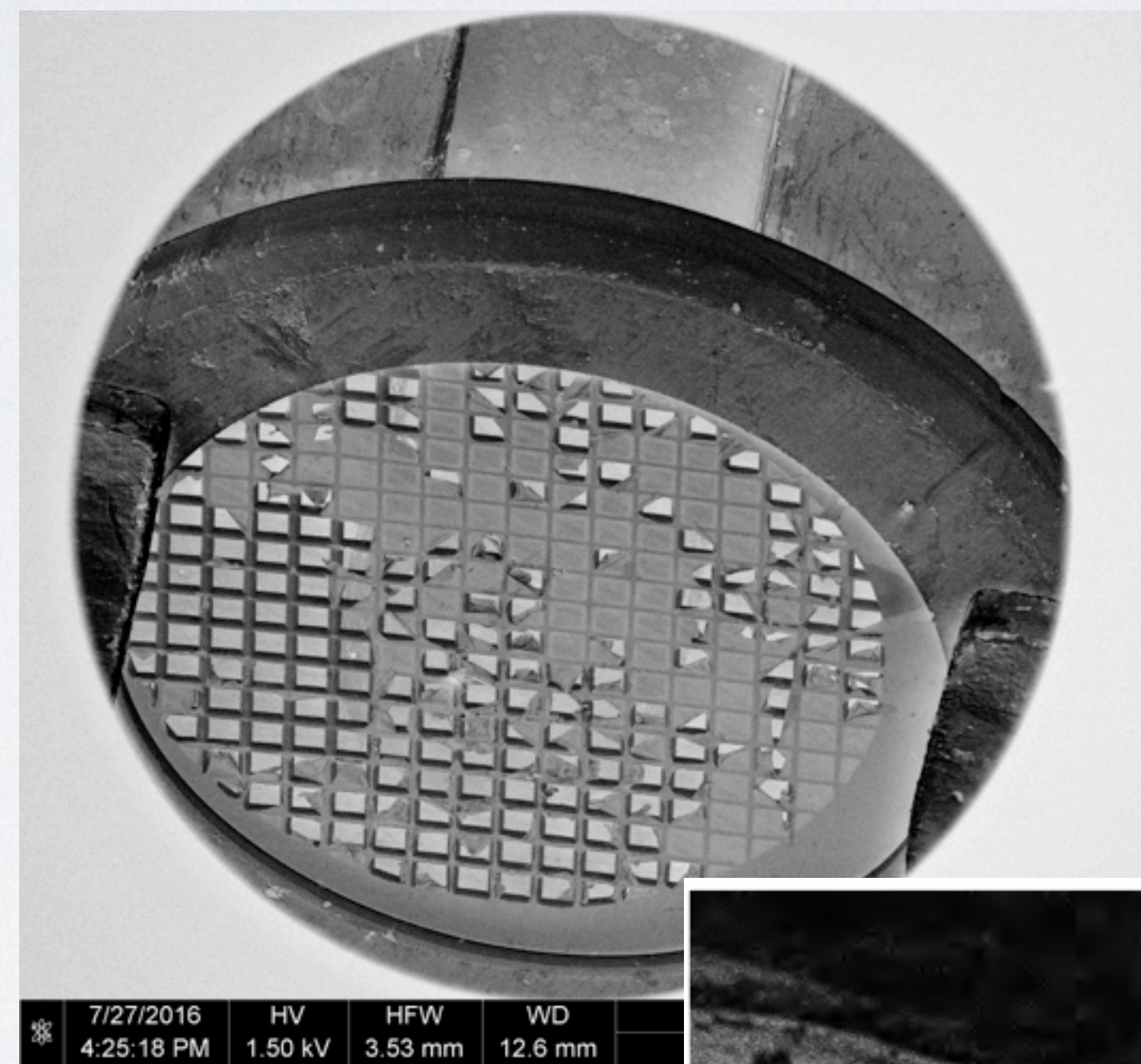
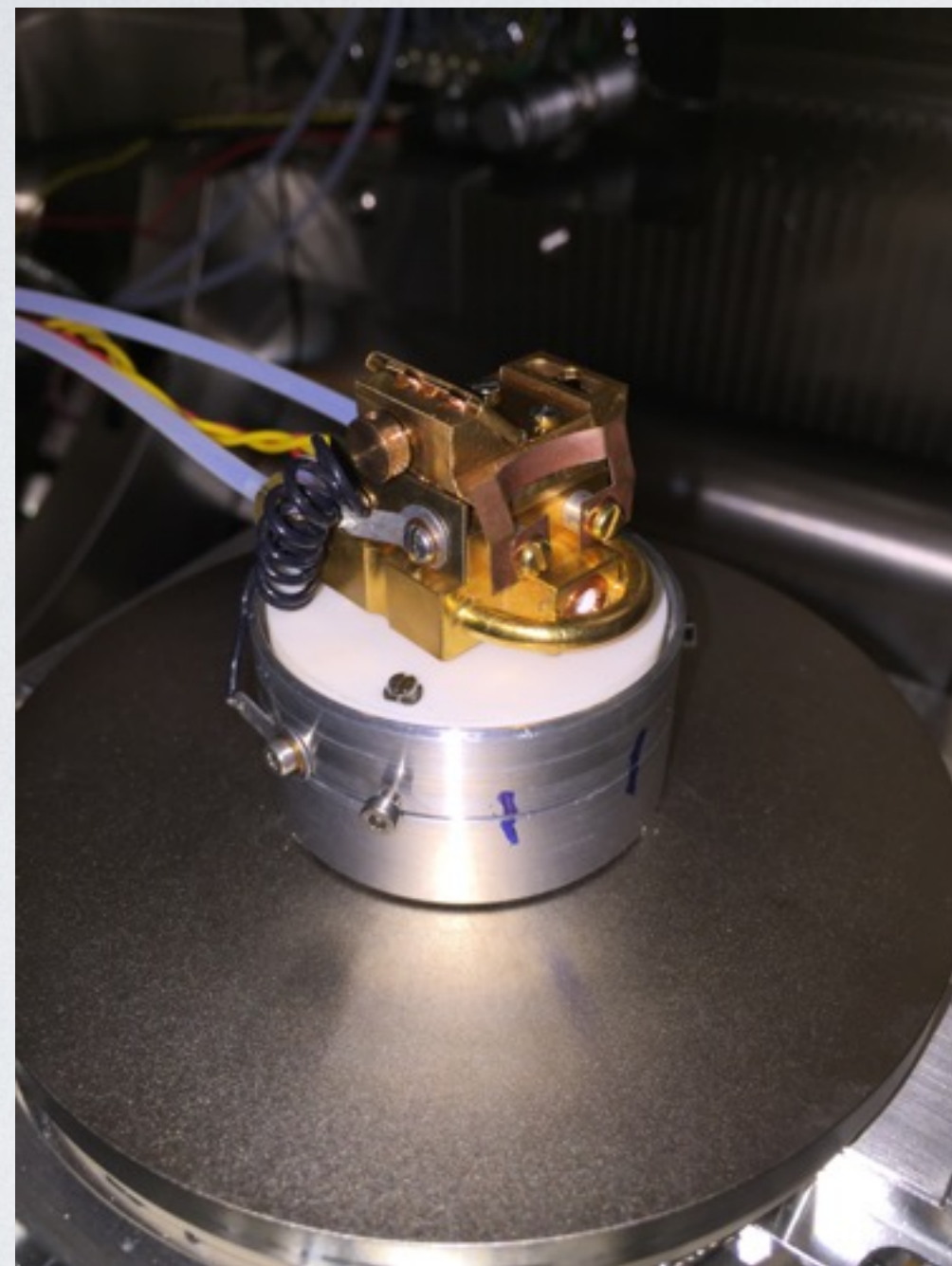
Baker, 2007

PLUNGE FREEZING



Product	1500x (45°)	3000x	10,000x	20,000x
CF-MH-2C CF-MH-4C multi hole and space				
CF-1/1-2C CF-1/1-4C 1.0µm hole, 1.0 µm space				
CF-1.2/1.3-2C CF-1.2/1.3-4C 1.2µm hole, 1.3 µm space				
CF-2/1-2C CF-2/1-4C 2.0µm hole, 1.0µm space				
CF-2/2-2C CF-2/2-4C 2.0µm hole, 2.0µm space				
CF-2/4-2C CF-2/4-4C 2.0µm hole, 4.0µm space				
CF-4/2-2C CF-4/2-4C 4.0µm hole, 2.0µm space				

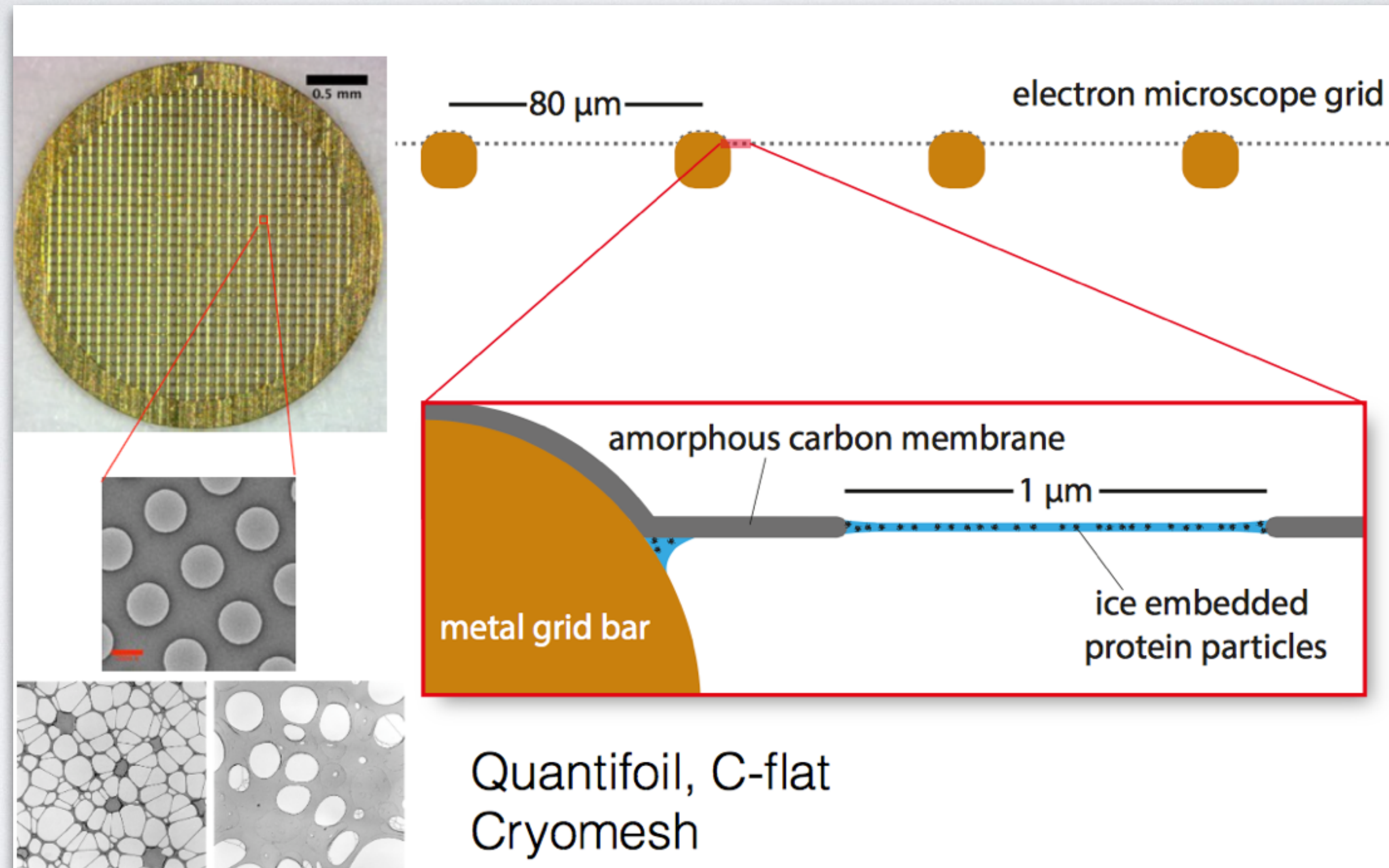
CRYO FIB MILLING



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

TRADITIONAL SUBSTRATES FOR CRYO-EM



GOLD GRIDS

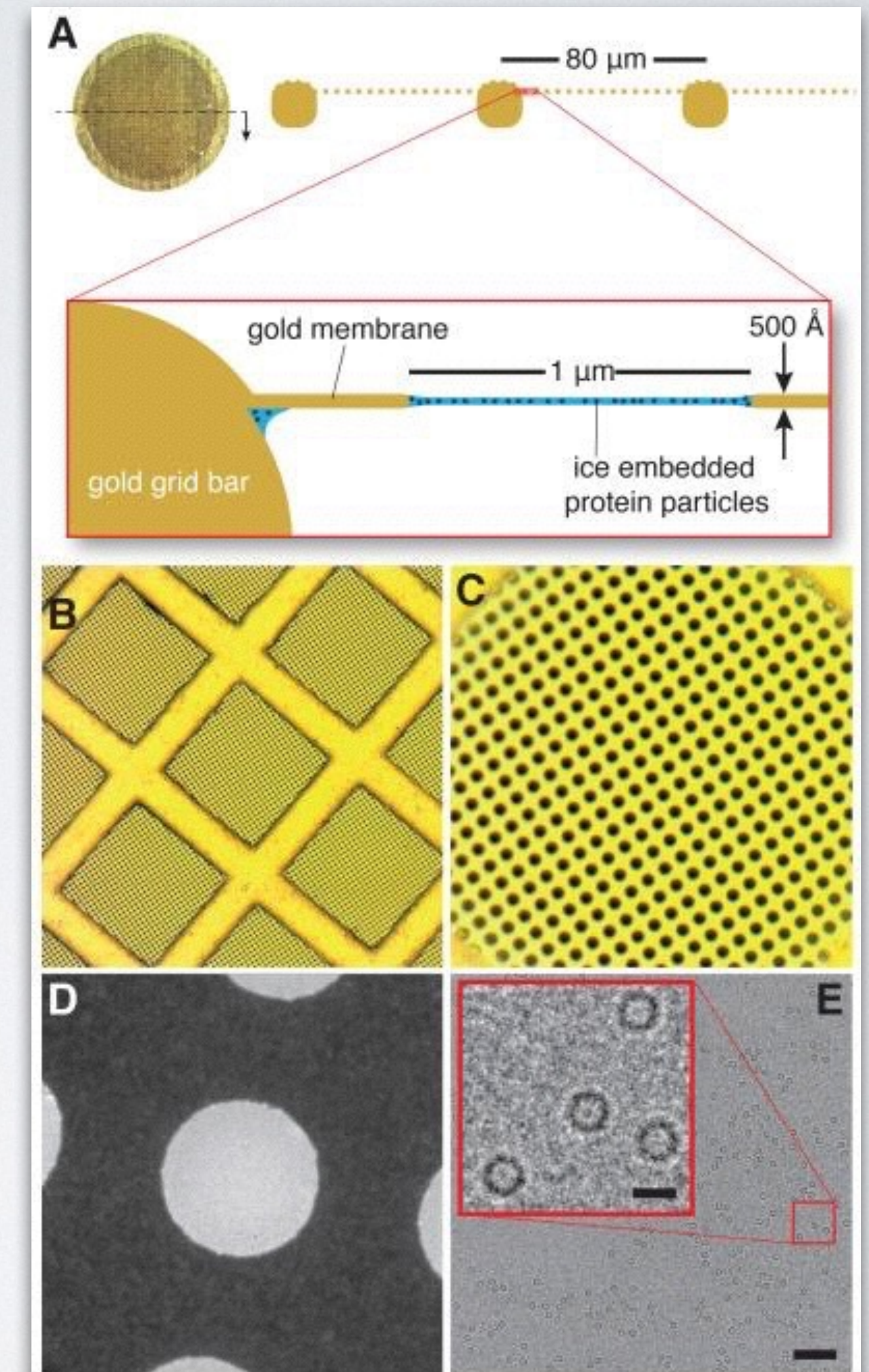
- Holey gold foil on gold mesh grid

Advantages:

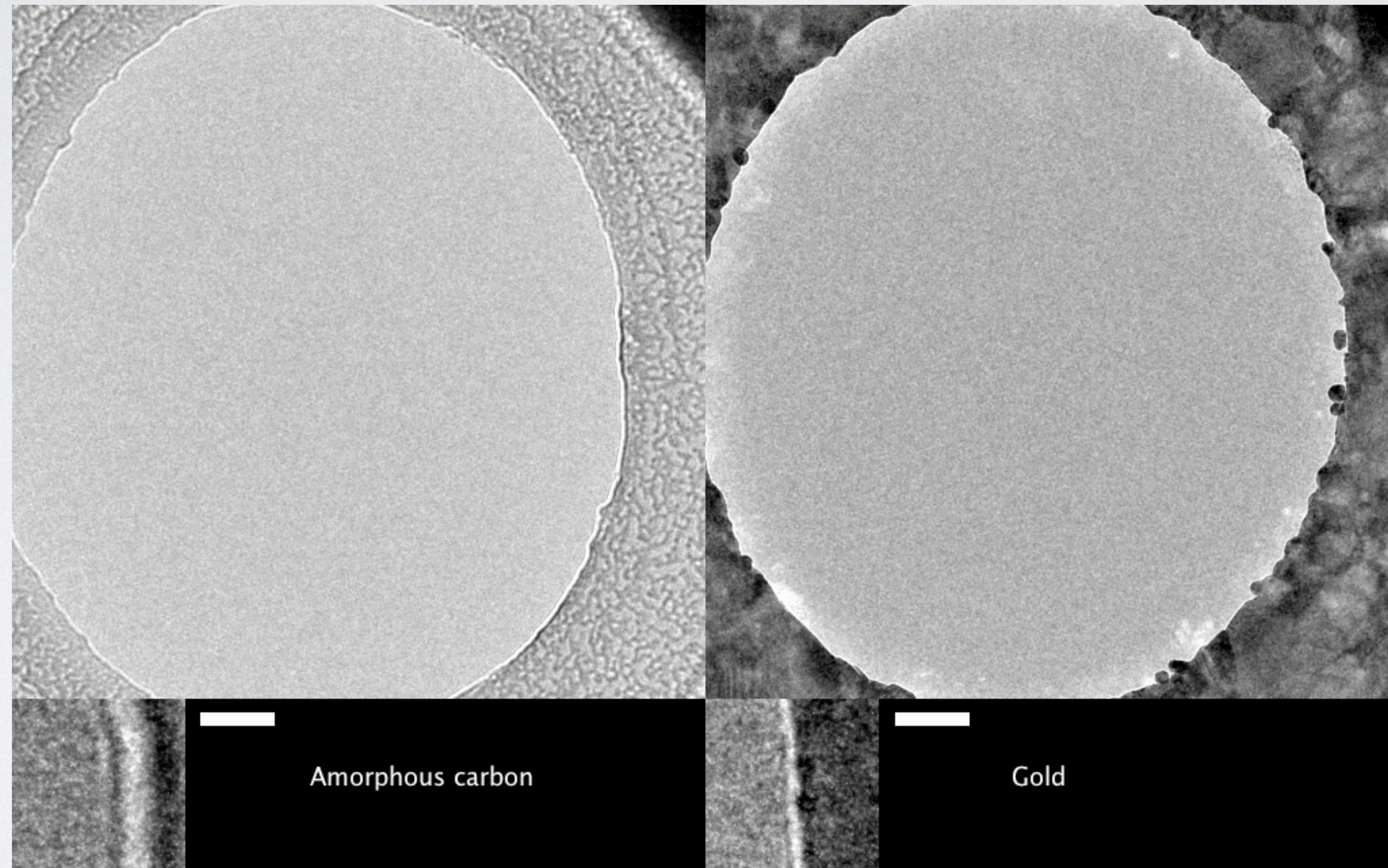
- Prevents differential thermal contraction when freezing
- Reduces beam-induced specimen movement
- Combined with direct detector technology allows for near atomic resolution

Disadvantages:

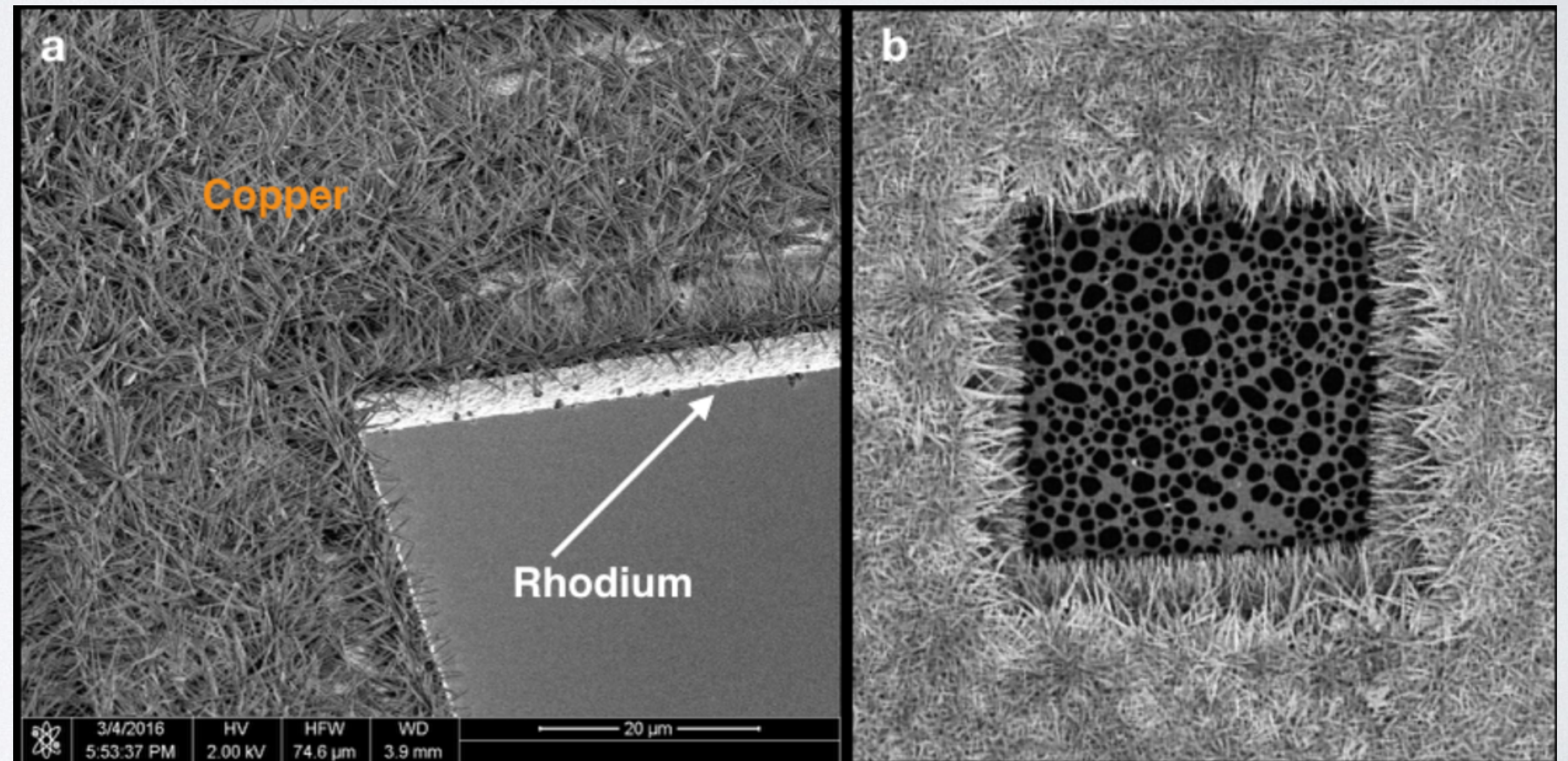
- Difficult to find focus due to lack of amorphous substrate



GOLD GRIDS



NEXT GENERATION METHODS FOR SAMPLE PREPARATION



support film practical