SEMC workshop series

Gold Grid Making Workshop



SIMONS ELECTRON MICROSCOPY CENTER February 14, 2014

New York Structural Biology Center





WORKSHOP

Schedule February 14, 2018 2:30 pm-3:30 pm

- 1. Workshop intro
- Why gold?
- 2. Photo [Crystal]
- 3. Demo of gold grid making
- 4. Wrap up



[Ed]

[Ed]





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







Current cryo-EM sample prep





TRADITIONAL SUBSTRATES FOR CRYO-EM





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



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

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

А

RMS particle displacement (Å)

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.



Carbon-Gold VS Gold-Gold Grids



Amorphous carbon





- them

• Buy gold grids with holey carbon on

• Evaporate gold on the grids Remove carbon















and the second second





























WORKSHOP

Schedule February 14, 2018 2:30 pm-3:30 pm

- 1. Workshop intro
- Why gold?
- 2. Photo [Crystal]
- 3. Demo of gold grid making
- 4. Wrap up

[Ed]

[Ed]

