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
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?
  • will be in practical

Baker, 2007

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

Protochips.com

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

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
NEXT GENERATION METHODS FOR SAMPLE PREPARATION

Spotiton
support film practical