

# **C** Simons Electron Microscopy Center

#### Challenges in biological EM & Sample Prep

#### **2019 Winter EM Course**



NATIONAL CENTER FOR **CRYOEM ACCESS & TRAINING** 

**25 Min lecture + 30 min practical** 14 Jan 2019





# **CLASS ORGANIZATION**

Section I: EM fundamentals section Section 2: Tomography section Section 3: Single particle section Section 4:2D crystallography section Section 5: EM challenges and new frontiers







#### **Electron Microscopy**

Σ ш S



Naked eye

#### **Light microscopy**



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# **OBTAINING A 3D OBJECT FROM A 2D** PROJECTION









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



#### **OBTAINING A 3D OBJECT FROM A 2D** PROJECTION





# **RT & CRYO SAMPLE PREP METHODS**



perature EW

Cryo-EW





resolution range

0.7Å

# 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





sh, stain date



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

#### 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





#### **CURRENT CRYO-TEM SPECIMEN** PREPARATION









#### Challenges of CryoEM: Radiation damage



#### "Low-dose" imaging





# PLUNGE FREZING

- Sample suspended in physiological buffer
- Holey carbon support film: C-flats, Quantifoil
- Protocol: glow discharge, sample, blot, plunge freeze
- •
- random orientation, higher resolution than stain
- visualize <100 kD, freezing artifacts



SEMC: Gatan CryoPlunge Freezer 3, FEI Vitrobot, manual plunge freezer

**Advantages:** no fixation/dehydration/staining artifacts, learning curve, **Disadvantages:** low contrast, low SNR, radiation sensitive, difficult to



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### CRYO FIB MILLING







# CRYO FIB MILLING





#### **SPOTITON / CHAMELEON**





#### **SPOTITON EP2** AUTOMATIC GRID PICKUP





#### Sample preparation practical