# CRYOEM 001 :
# CRYO SAMPLE PREPARATION

NCCAT Embedded Training – Master Class series

June 16-17, 2021

NATIONAL CENTER FOR
CRYOEM ACCESS & TRAINING

New York Structural
Biology Center

SIMONS ELECTRON
MICROSCOPY CENTER

# WEEK 2
# JUNE 6/16-17

Wed 6/16 3-5pm

Overview of cryo sample prep

Introduction to merit badges

Practical: Vitrobot Mark IV and Glacios

Thur 6/17 2-5pm

Screening microscope (Glacios) in detail

Screening with Leginon

Core knowledge

Sample preparation

Microscope operations & Data collection

Processing & Data analysis

# SCHEDULE

I. Sample purification and grid preparation
   a) cryoEM merit badges
   b) Chameleon demo
overview of blot free vitrification vs plunge freezing methods

II. Grid screening & evaluation
   a) Sample holders
   -Side entry systems: Gatan 626/Elsa holder and loading
   -Autoloader systems: autogrid clipping and loading
   b) F20 setup and demo of screening with Leginon

III. Cryo-EM data collection
   a) Glacios setup and advanced sample screening/preliminary data collection with Leginon
   b) Krios high res data collection with Leginon

IV. Image (pre)-processing
   a) On the fly feedback cryoSPARC live
   b) Working with your own data

# NCCAT CROSS-TRAINING RESOURCES

**Supplemental cryoEM masterclass materials for cohort3**

- Intro to sample preparation – negative stain and screening on an F20
- Negative stain and F20 Manuals and Checklists:
  - Solarus plasma cleaner manual
  - Negative-stain manual
  - Negative-Stain Independence Checklist
  - TF20 user manual
  - T12-F20 Checklist


# CRYOEM MERIT BADGES

CryoEM
TRANSFORMATIVE HIGH RESOLUTION CRYO-ELECTRON MICROSCOPY

Broadening access to high-resolution
cryo-electron microscopy and tomography

HOME | CryoEM CENTERS | CURRICULUM DEVELOPMENT | CryoET CENTERS | EVENTS | RESOURCES

< return to all badges

## TFS Vitrobot Mark IV

**Category:** Sample preparation
**Sub-category:** Cryogenic work

Plunge freezing and instrument certification for Vitrobot Mark IV.

- + Essential base knowledge
- + Knowledge quiz
- + Center Specific Policies
- + Demonstration
- + Supervised Practice
- + Practical test

Recertification period

- Sample preparation merit badges are valid for ~1yr.
- Recertification (to maintain active status) requires passing the practical test with one center staff member. If supervised training is needed to pass the practical test, this can be arranged.

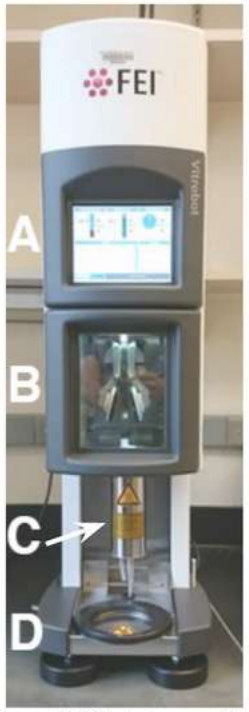



**Figure 1.** Vitrobot assembled and turned on. **A)** Screen. **B)** Environmental chamber with blotting pads. **C)** Humidifier. **D)** ethane lift.

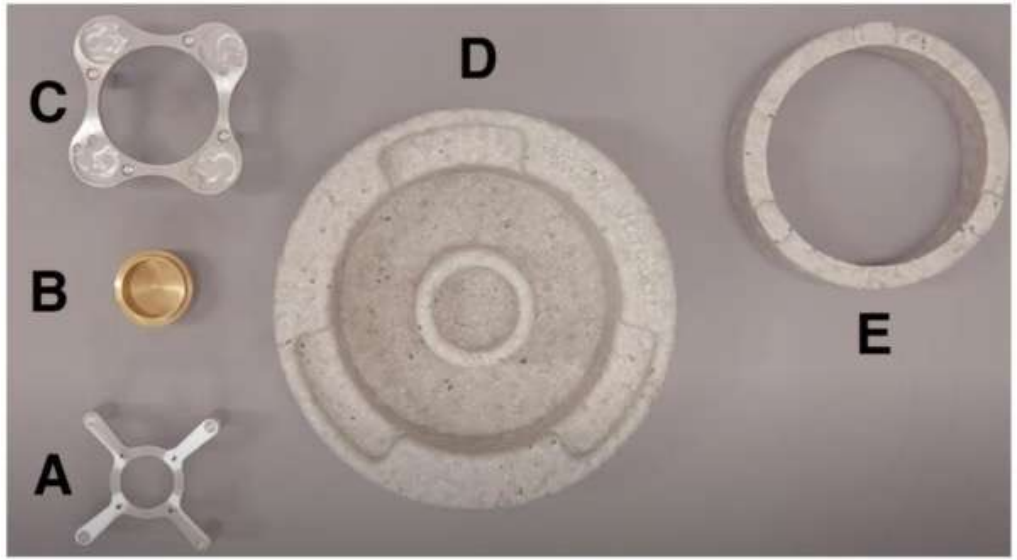
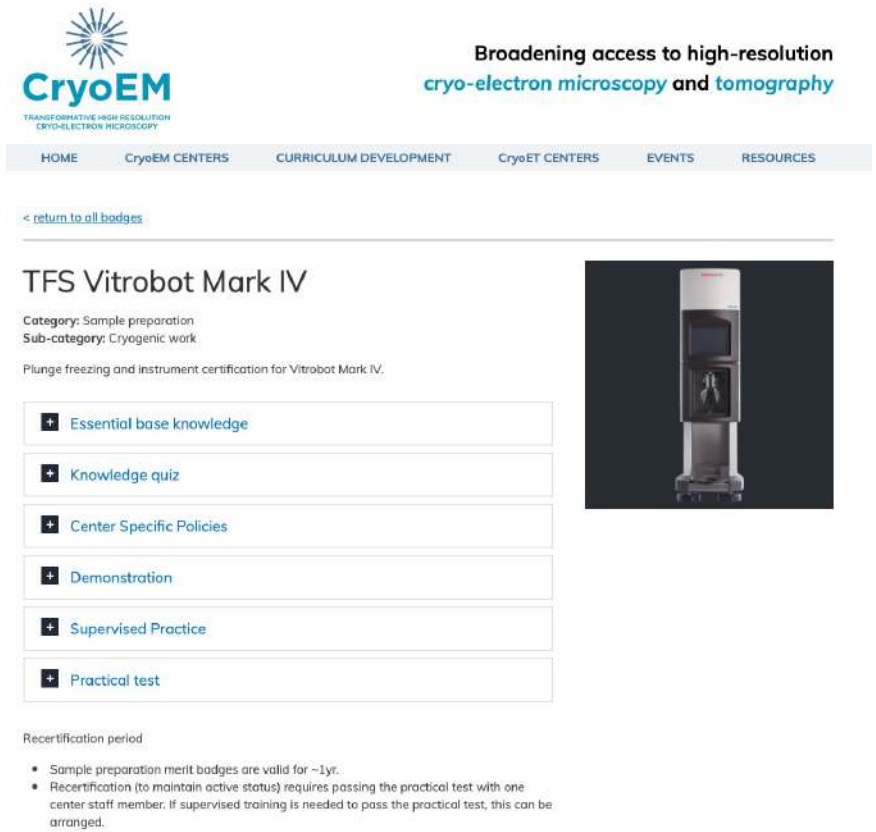
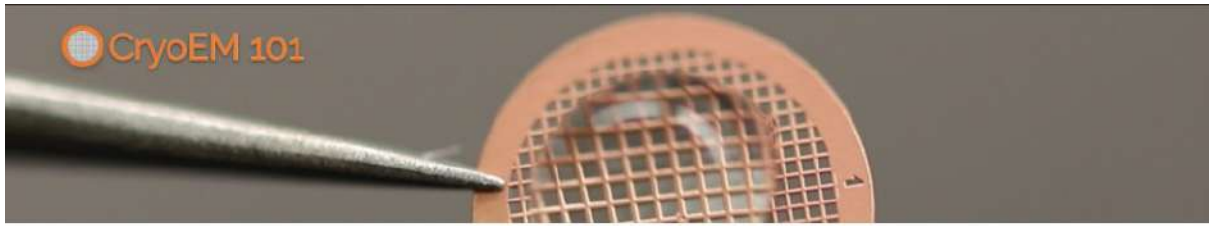


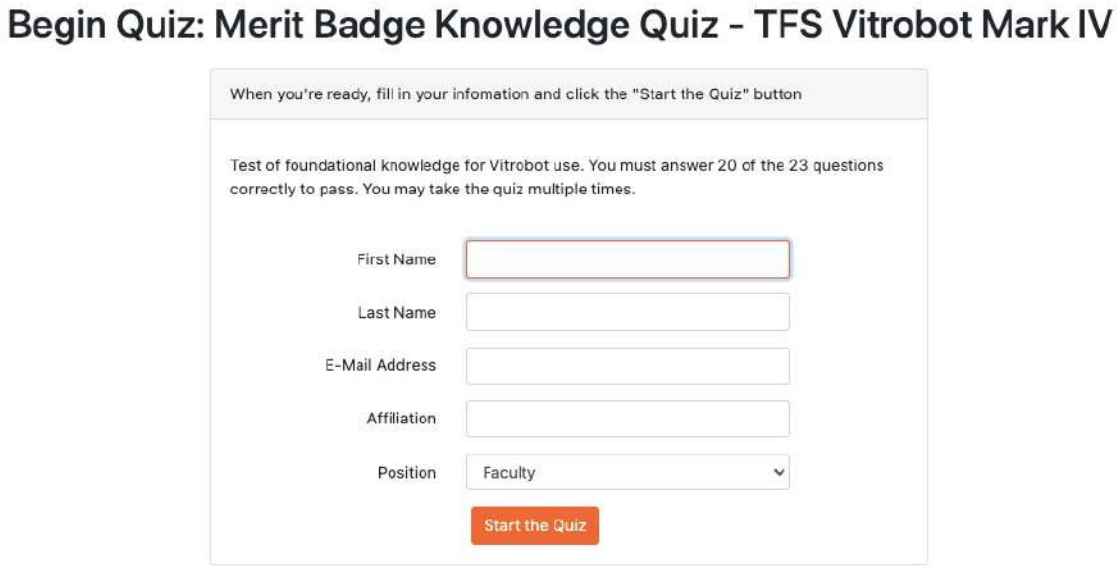

**Figure 2.** Ethane Holder. **A)** Spider. **B)** Brass Ethane Cup. **C)** Gridbox Holder. **D)** Base / Liquid Nitrogen Container. **E)** Anti-contamination Ring.

# CRYOEM MERIT BADGES

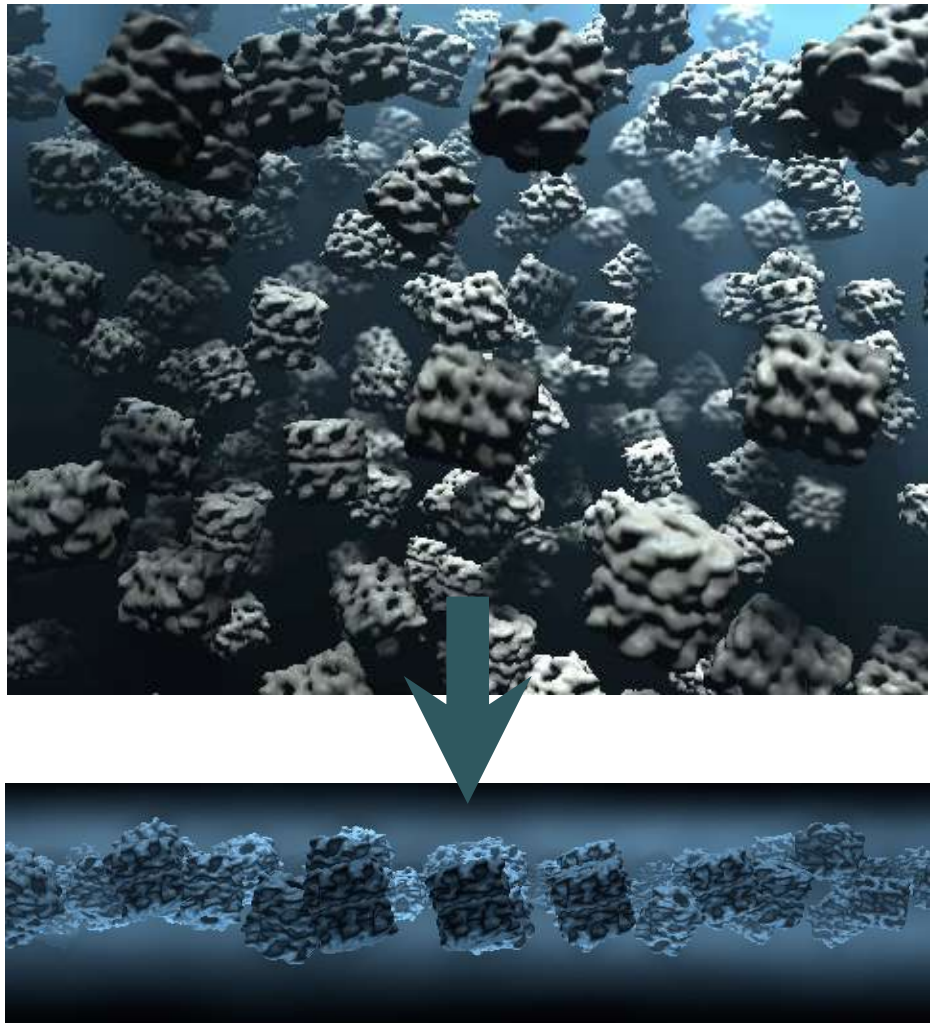
https://cryoem101.org/selftest/?test=19

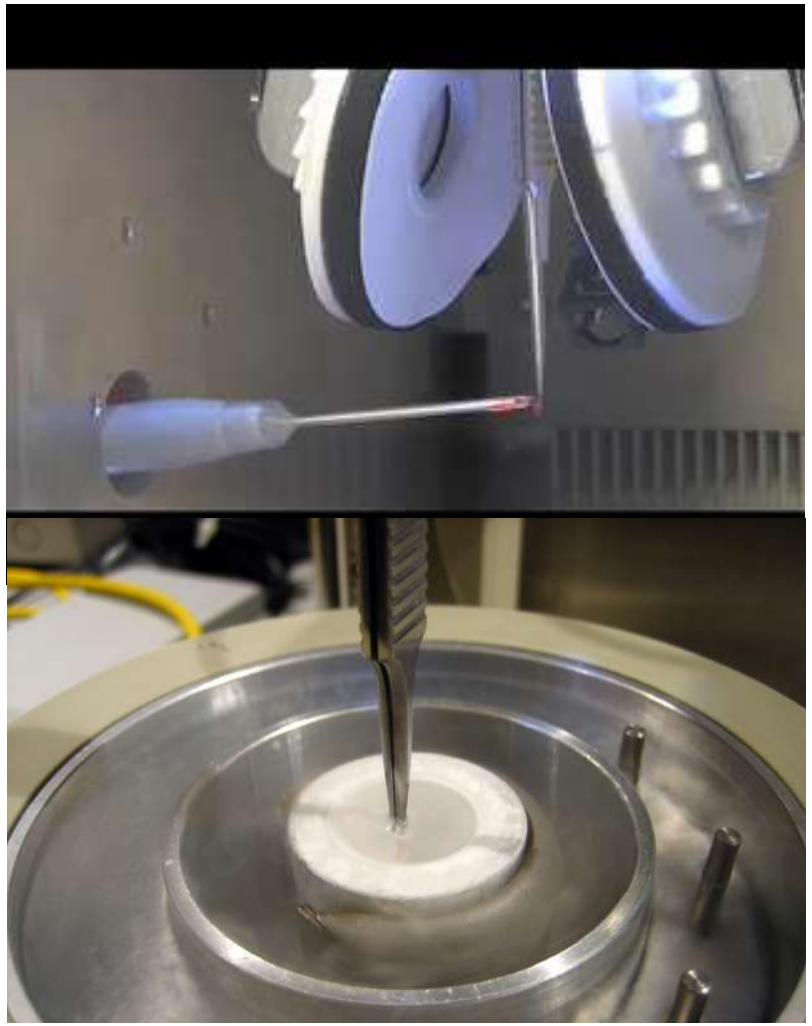


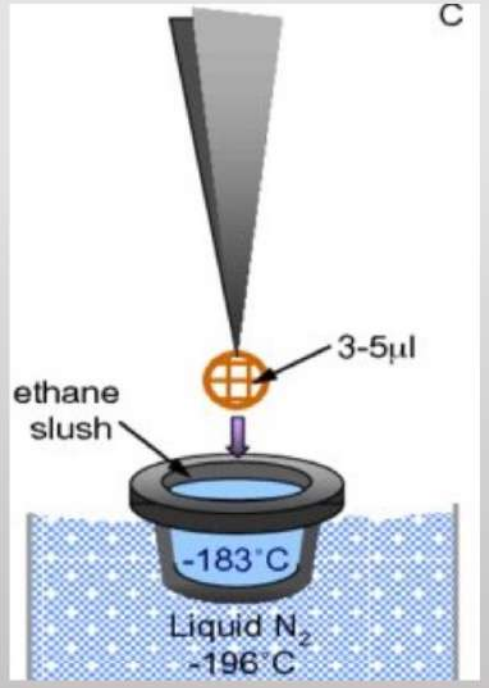
# PLUNGE FREEZING



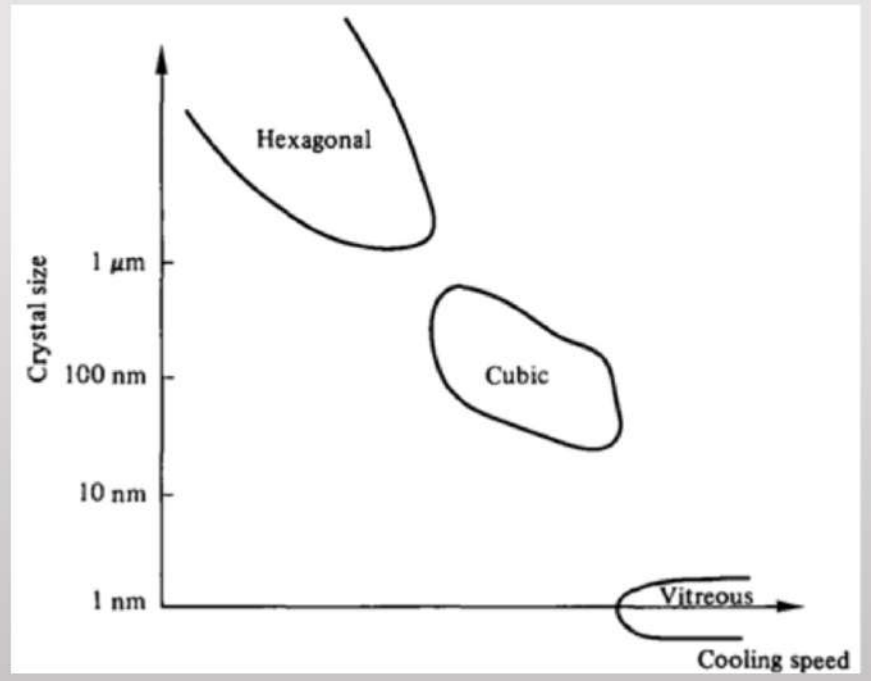


# PLUNGE FREEZING

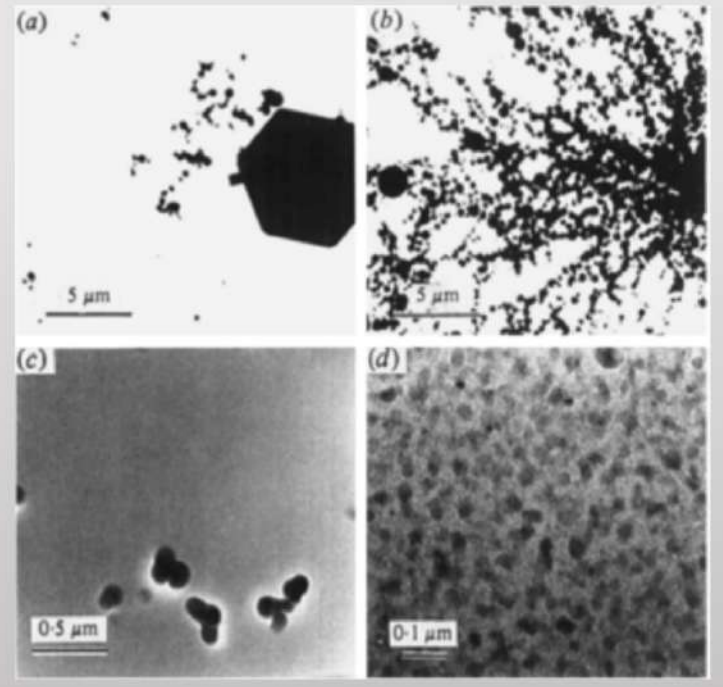
- Liquid ethane is a suitable coolant.
- Liquid nitrogen boils on contact, which makes it a poor coolant for cryo-EM.
- Cooling speed faster than $10^5$-$10^6$ K/s ensure the formation of vitrified ice.




Setup of liquid ethane
(Image from Wen Jiang)



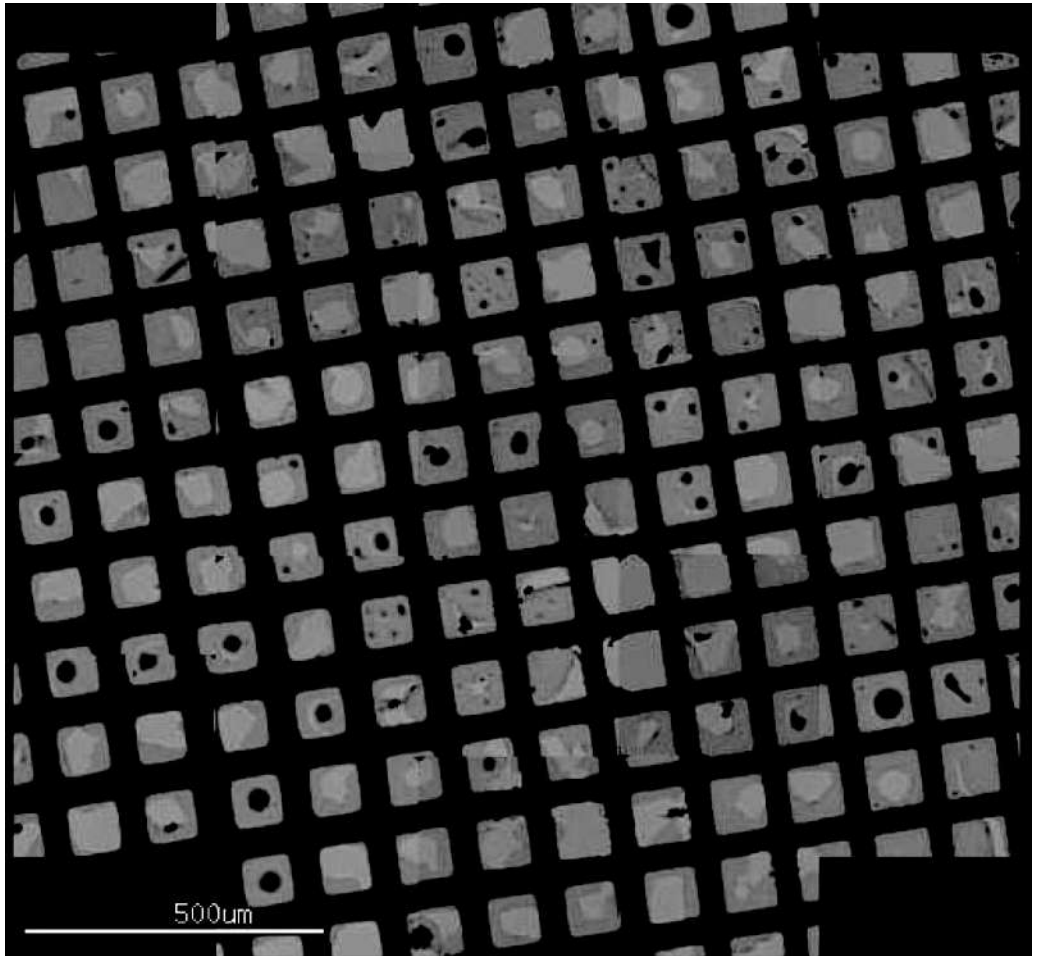

Cooling speed &
forms of ice

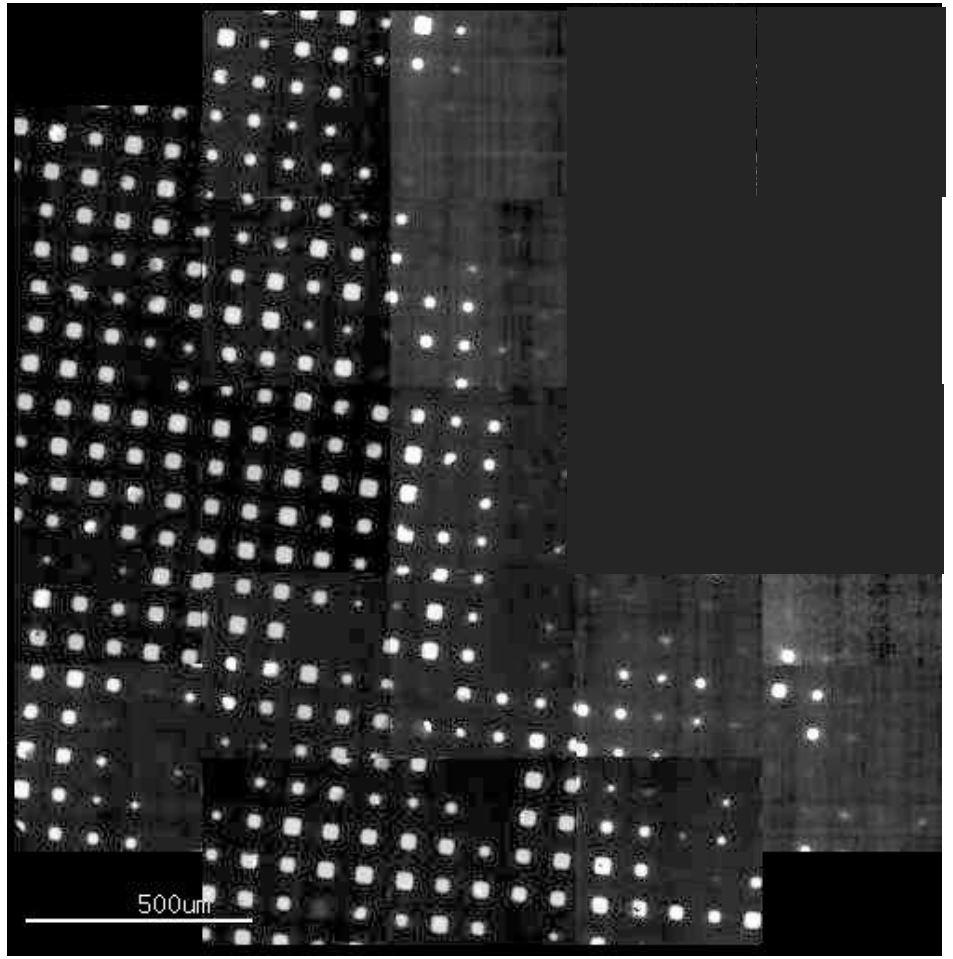



Different forms of ice contamination

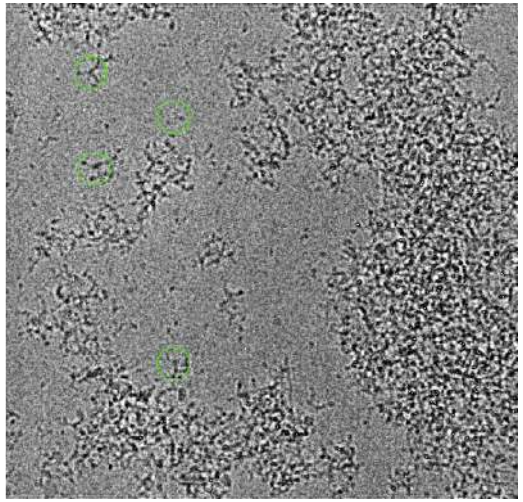
Jacques Dubochet et al., 1988

# WHAT DO GRIDS LOOK LIKE?

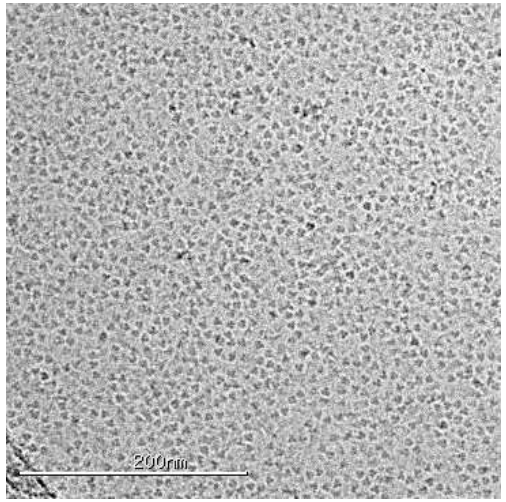


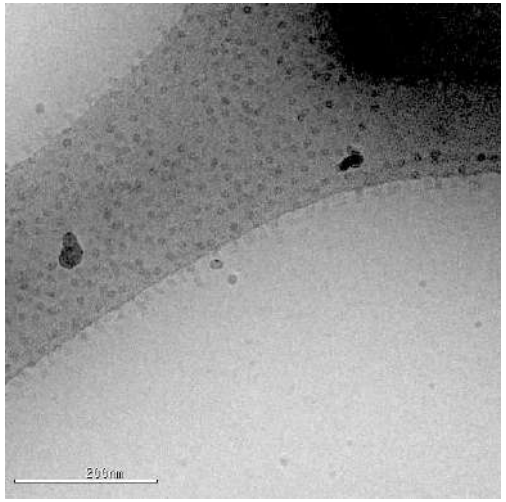
# WHAT ISSUES ARISE?



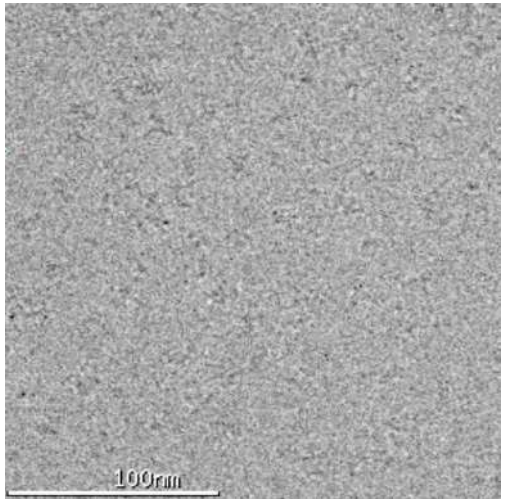
Aggregating in ice



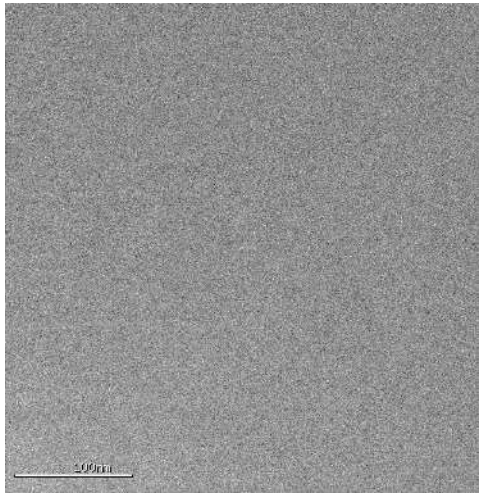

Preferred orientation



Particles not going into holes



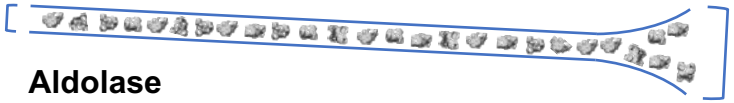

Rejecting 90% of particles



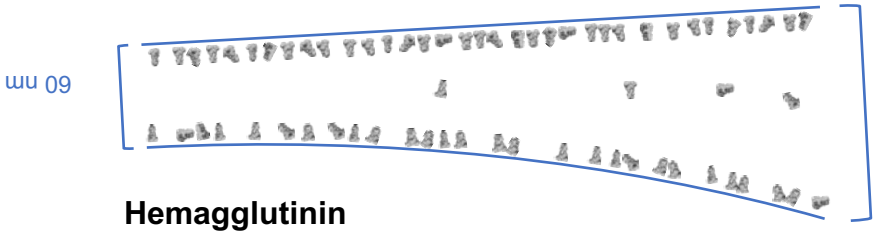
Particles disappearing in ice

# WHAT ISSUES ARISE?

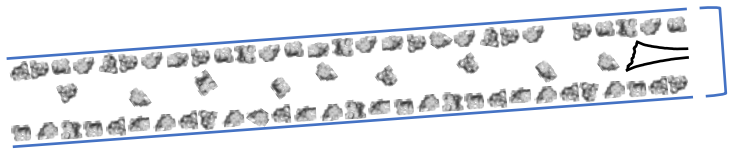

Hemagglutinin



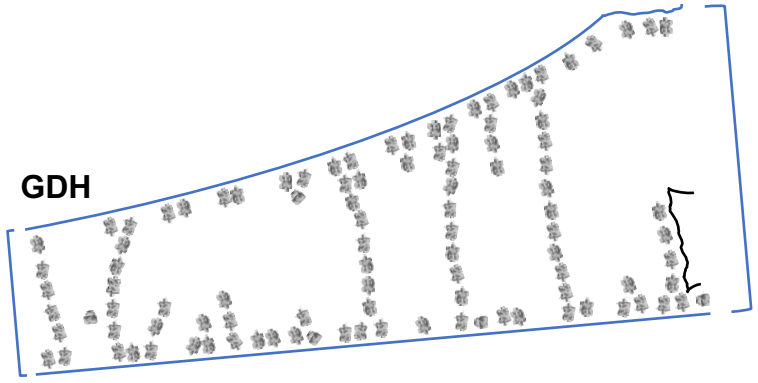

Aldolase

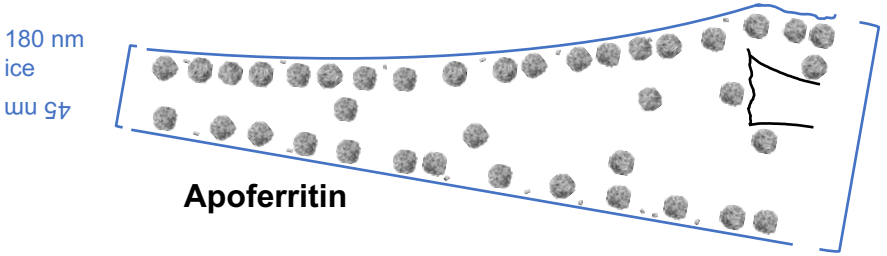



Hemagglutinin

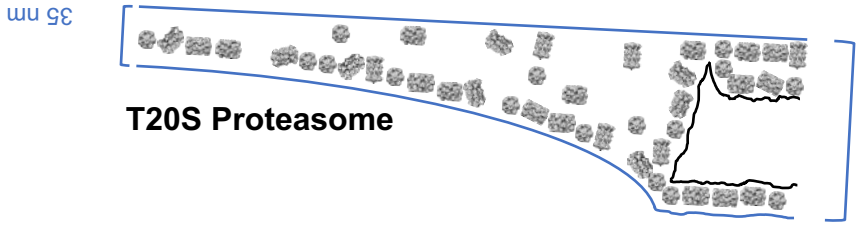



Aldolase

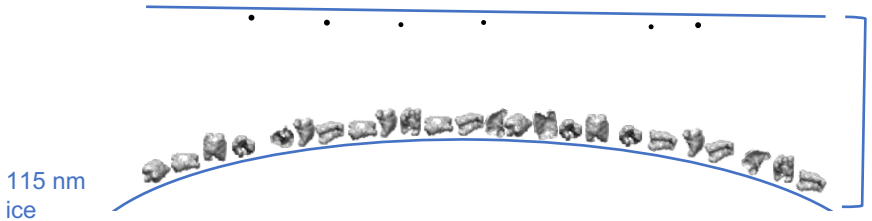



Apoferritin




T20S Proteasome




DNAB Helices

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

Alex Noble

# REAGENTS FOR IMPROVING VITRIFICATION OF CRYO-EM GRIDS USED IN SINGLE PARTICLE ANALYSIS.

| Surfactants and Cryoprotectants | Amount | Conc. | CMC | Class |
|---|---|---|---|---|
| Fluorinated Octyl Maltoside (FOM) | 100 µl | 0.41% (w/v) | 0.07% (w/v) | non-ionic detergent |
| Hexadecyl-trimethyl-ammonium Bromide (CTAB) | 100 µl | 0.34% (w/v) | 0.03% (w/v) | cationic detergent |
| n-Decyl-ß-D-Maltoside (DM) | 100 µl | 0.87% (w/v) | 0.09% (w/v) | non-ionic detergent |
| n-Decyl-α-D-Maltoside (DαM) | 100 µl | 0.46% (w/v) | 0.08% (w/v) | non-ionic detergent |
| n-Dodecyl-ß-D-Maltoside (DDM) | 100 µl | 0.09% (w/v) | 0.01% (w/v) | non-ionic detergent |
| Sodium Deoxycholate | 100 µl | 1.66% (w/v) | 0.17% (w/v) | anionic detergent |
| Triton X-100 | 100 µl | 0.15% (w/v) | 0.01% (w/v) | non-ionic detergent |
| Tween 20 | 100 µl | 1% (w/v) | 0.01% (w/v) | non-ionic detergent |
| CHAPSO | 100 µl | 2.5% (w/v) | 0.5% (w/v) | zwitterionic detergent |
| Amphipol A8-35 | 100 µl | 5% (w/v) | | anionic surfactant |
| Glycerol | 1 ml | 30% (w/v) | | cryoprotectant |

https://www.mitegen.com/product/cryo-em-vitrification-starter-kit/


[1] Noble *et al.* (2018) Routine Single Particle CryoEM Sample and Grid Characterization by Tomography. DOI: 10.7554/eLife.34257.
[2] Thonghin *et al.* (2018) Cryo-electron microscopy of membrane proteins. *Methods* **147**:176.
[3] Drulyte *et al.* (2018) Approaches to altering particle distributions in cryo-electron microscopy sample preparation. *Acta Cryst. D* **74**:560.
[4] Glaeser *et al.* (2017) Opinion: hazards faced by macromolecules when confined to thin aqueous films. *Biophys Rep* **3**:1.
[5] Gatsogiannis *et al.* (2016). Membrane insertion of a Tc toxin in near-atomic detail. *Nat. Struct. Mol. Biol.* **23**:884.
[6] Efremov *et al.* (2015) Architecture and conformational switch mechanism of the ryanodine receptor. *Nature* **517**:39.

# REAGENTS FOR IMPROVING VITRIFICATION OF CRYO-EM GRIDS USED IN SINGLE PARTICLE ANALYSIS.

| PDB Release Date | PDB | Protein | Additive |
|---|---|---|---|
| 2020-01-08 | 6PWN | MscS mechanosensitive channel | 0.01% f-OM |
| 2019-09-04 | 6KG7 | Piezo2 mechanosensitive channel | 0.65 mM f-FC8 |
| 2019-08-28 | 6QTI | Nicotinamide nucleotide proton channel | 0.05% CHAPS |
| 2019-08-07 | 6R7L | SecYEG translocon | 0.2% f-OM |
| 2019-02-06 | 6E0H | TMEM16 scramblase | 3 mM f-FC8 |
| 2018-12-19 | 6N3Q | Sec protein-translocation channel complex | 3 mM f-FC8 |
| 2018-11-07 | 6H3I | Type 9 secretion system translocon | 1.5 mM f-FC8 or 0.7 mM f-OM |
| 2018-10-24 | 6DMR | TRPV5 ion channel | 3 mM f-FC8 |
| 2018-10-17 | 6D3R | CFTR | 3 mM f-FC8 |
| 2018-09-26 | 6HJR | Influenza Hemagglutinin | 2% Octyl Glucoside |
| 2018-08-08 | 6FOO | Ryanodine receptor 1 | 0.2% f-OM |
| 2018-08-01 | 6CJQ | SthK CNG Potassium channel | 3 mM f-FC8 |
| 2018-05-23 | 5YX9 | TRPC6 ion channel | 0.5 mM f-OM |
| 2018-01-31 | 6C0V | P-Glycoprotein transporter ABCB1 | 3 mM f-FC8 |
| 2017-12-27 | 6B5V | TRPV5 ion channel | 3 mM f-FC8 |
| 2017-12-13 | 6BPQ | TRPM8 channel | 2% DMSO |

https://www.anatrace.com/Landing/2020/Mar20-Newsletter

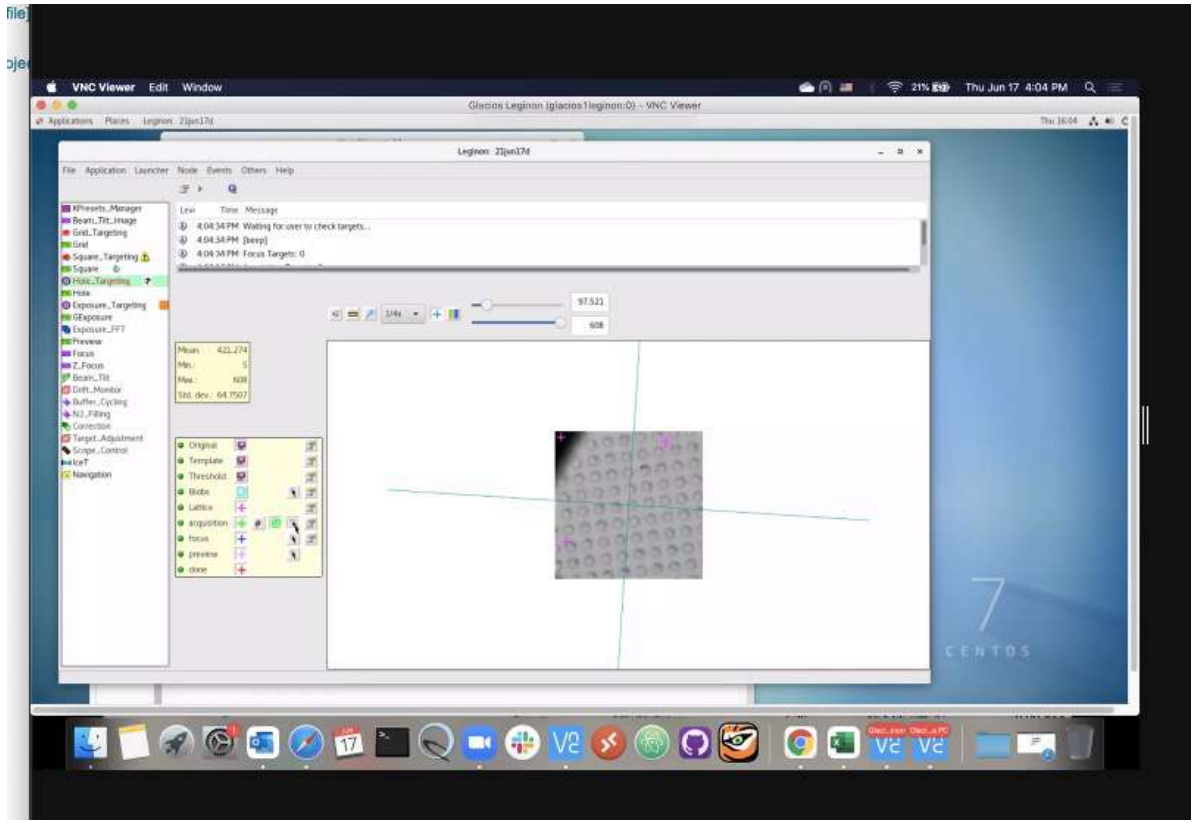

Glaeser, RM, et al. (2017) Biophys Rep 3(1), 1-7.

Noble, AJ, et al. (2018) Nat Methods 15(10), 793-795.

Drulyte, I et al. (2018) Acta Crystallogr D Struct Biol 74(Pt 6), 560-571.

Chen, J, et al. (2019) J Struct Biol X Volume 1. DOI: 10.1016/j.yjsbx.2019.100005

# DIFFICULT SPECIMENS




**Small protein**

- VPP
- Thinner ice

**Protein denaturation/Dissociation of protein complex**

**Continuous carbon film**

**Graphene oxide**

**Cross-linking (GraFix)**

**Preferred orientation**

**Tilt stage**

**Cross-linking**

**Detergent**

**Glow-discharging conditions**

**Support film (Graphene oxide)**

**Image analysis (3D classification)**

**Flexibility**

**Focused classification (subtraction)**

**Multibody refinement**

**Filamentous protein**

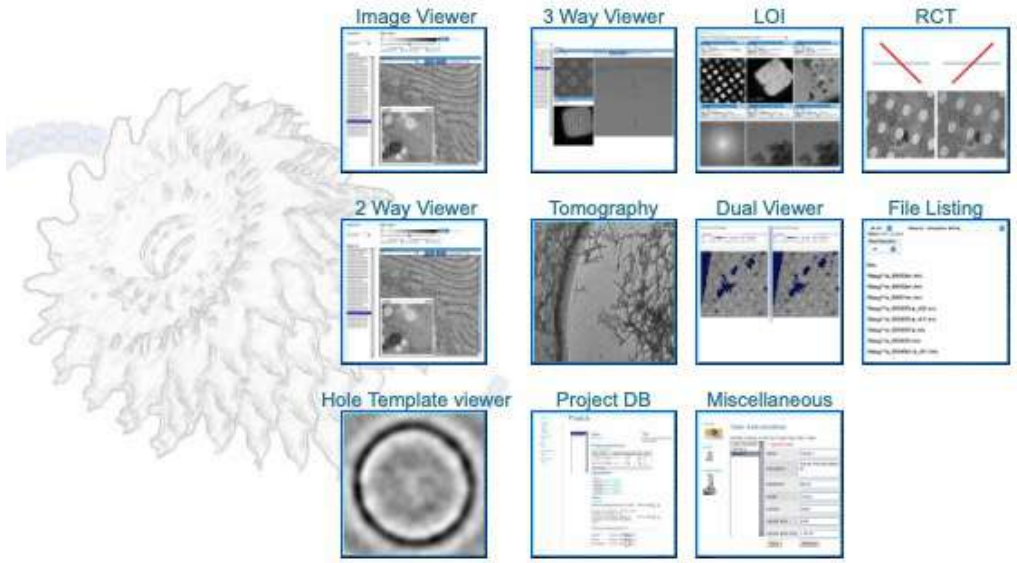
- Segmented analysis

**VI. Low concentration**

Multiple blots

Affinity grids

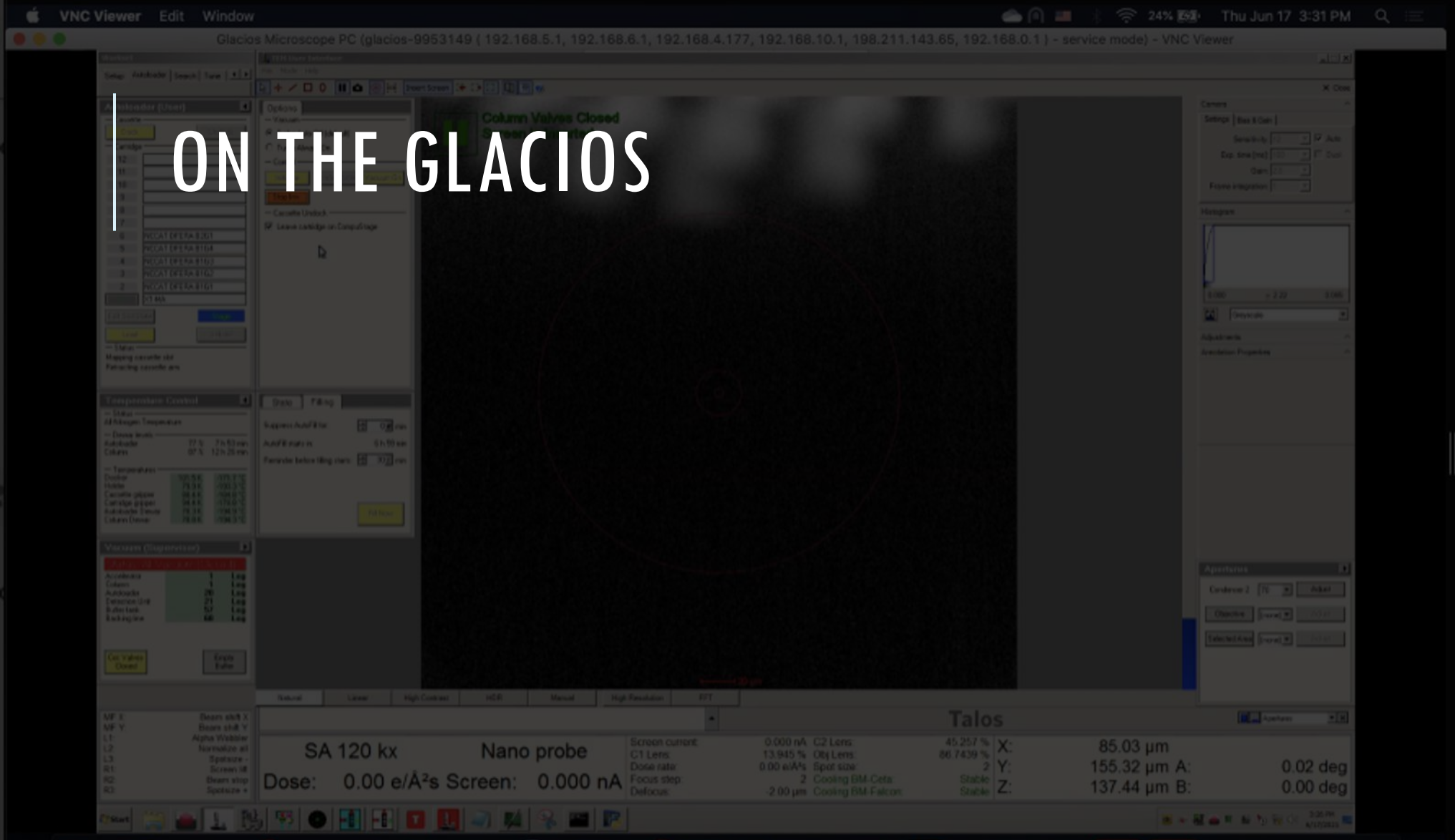
## EMGWEB.NYSBC.ORG Appion and Leginon Tools

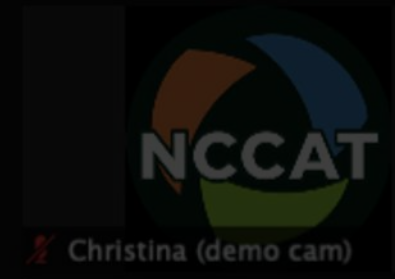



# EMGWEB.NYSBC.ORG

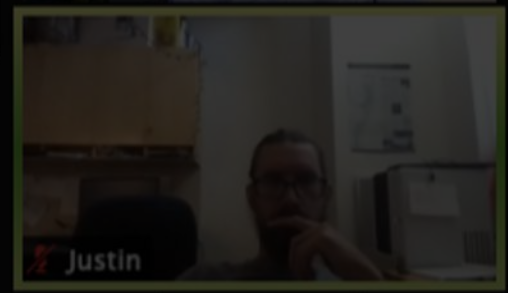
ninfo / nccat032020

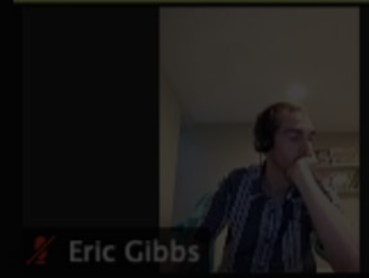
# ON THE GLACIOS








# CRYOEM ~~101~~ 002

WEEK 1
JUNE 6/09-10

WEEK 2
JUNE 6/16-17

WEEK 3
WK OF JUNE 21+

Practical: High end data collection

Data collection on Krios

Leginon automation

Pre-processing / cryoSPARC LIVE