CRYOEM 001 : EM SAMPLE PREPARATION — VITROBOT DEMO

NCCAT Embedded Training — Master Class series

October 28, 2020

New York Structural Biology Center



SIMONS ELECTRON MICROSCOPY CENTER



NATIONAL CENTER FOR CRYOEM ACCESS & TRAINING

CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA Building a cryoEM toolkit EM compatible samples EM support films and grids Sample preparation Tools of the trade: microscopes and detectors

Microscope operations Data collection strategies Data assessment & QC Data processing: cryoEM IT infrastructure On-the-fly feedback **3D** Reconstruction Visualization and validation



CRYOEM TOOLS & STORAGE

Fitting everything in 1 room

GRID BOXES AND PUCKS FOR STORAGE





HOW ARE SAMPLES PREPARED?



GRID PREPARATION IS A CHALLENGE



Graphics courtesy Gabe Lander

>100,000 potential imaging targets; most of them are not usable.

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 10⁵-10⁶ K/s ensure the formation of vitrified ice.









PLUNGE FREEZING

GRIDS & GLOW DISCHARGING — RECORD WHAT You do so you can repeat it next time



VITROBOT DEMO





Recording

01:56:13



3D PRINTED CLIPPING DEMO



https://mobile.twitter.com/carusl/status/1106443058189484033?lang=en

WHAT NEXT?

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cryoEM 001 : Single Particle Masterclass

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- 2. EM compatible samples
- 3. EM support films and grids
- 4. Sample preparation
- 5. Tools of the trade: microscopes and detectors
- 6. Microscope operations
- 7. Data collection strategies
- 8. Data assessment & QC
- 9. Data processing:
 - cryoEM IT infrastructure
 - On-the-fly feedback
 - 3D Reconstruction
- 10. Visualization and validation