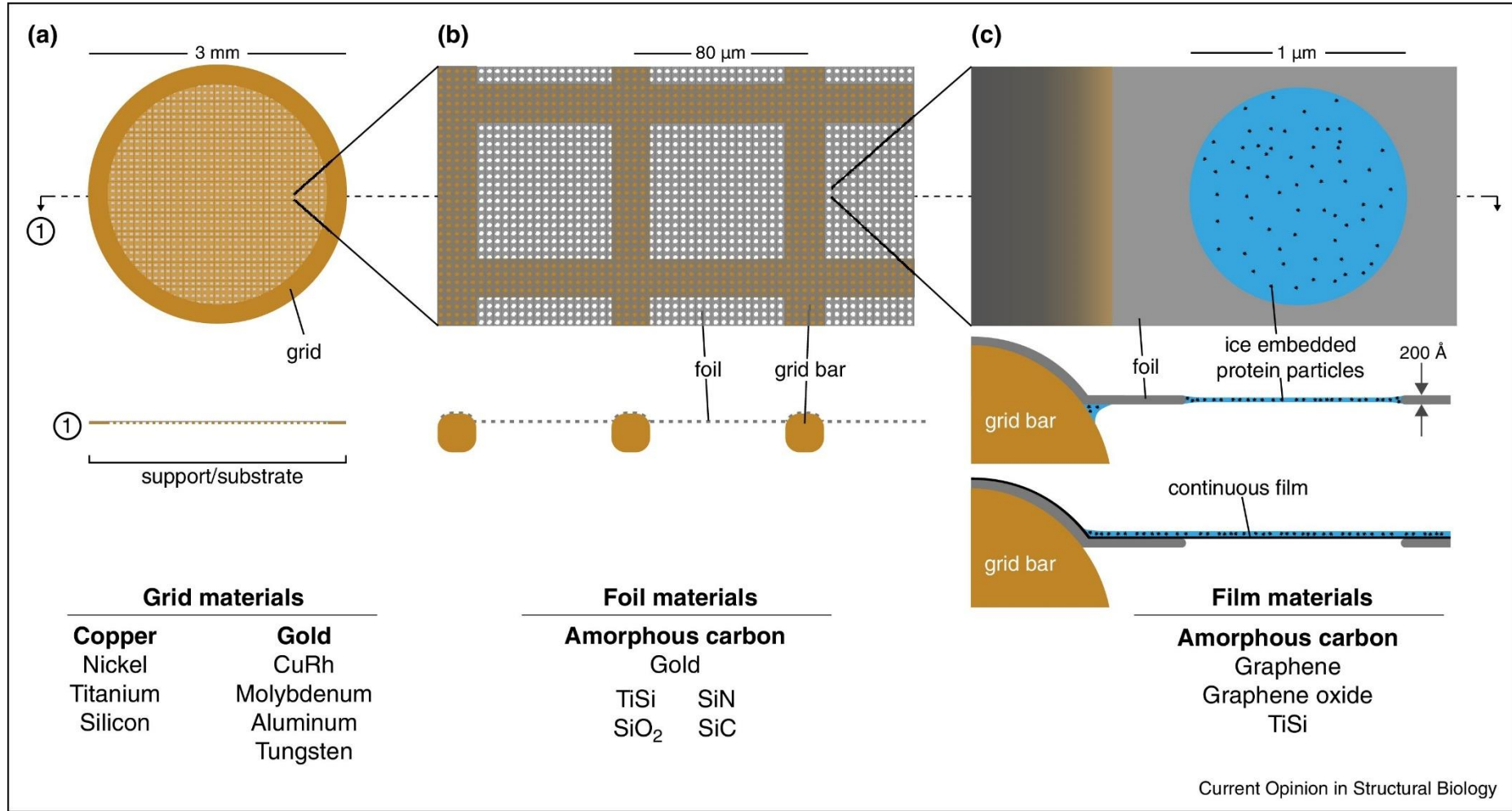


Selected Slides from SBWIP

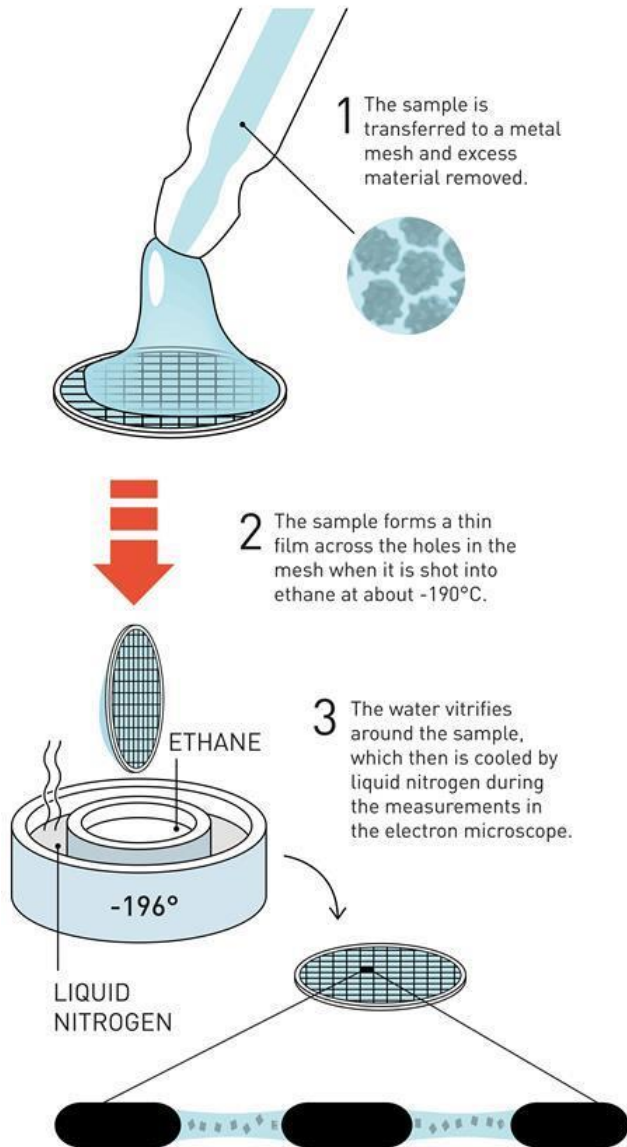
James Chen (SBL)

Anatomy of a Grid

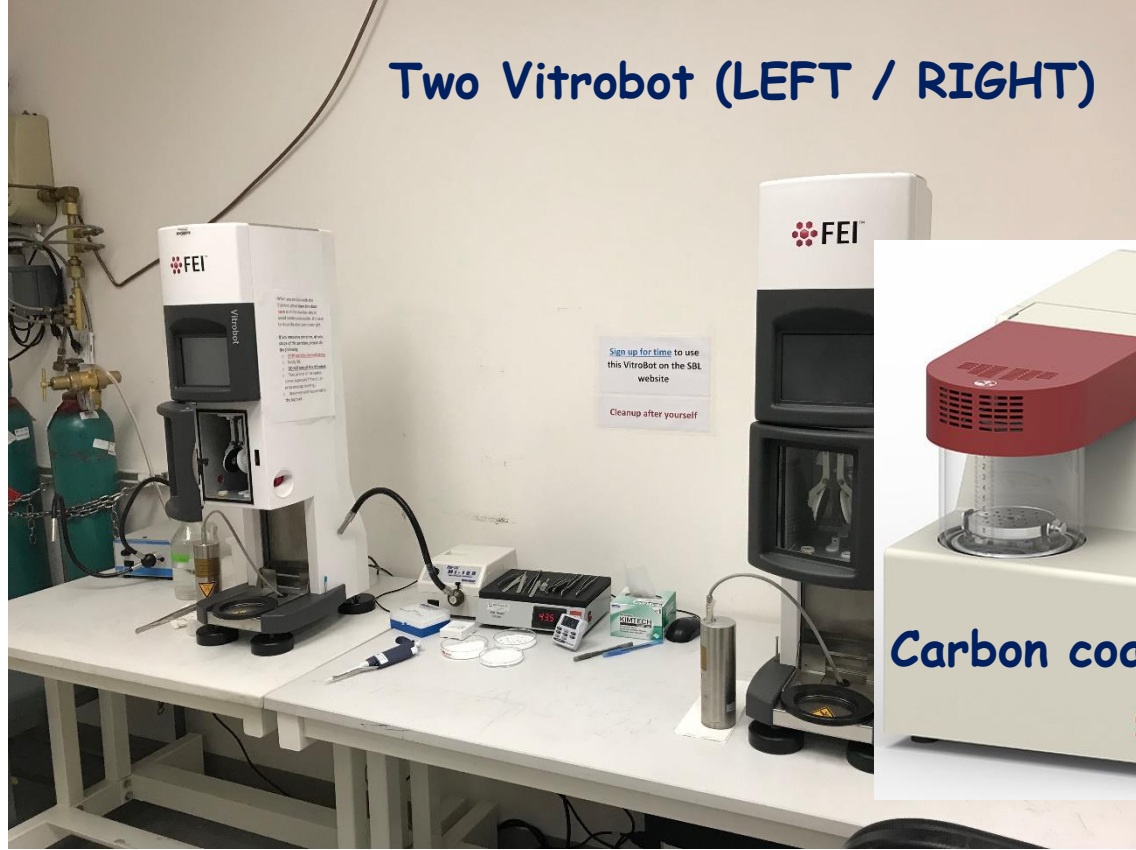


Quantifoil 300 mesh Cu R1.2/1.3

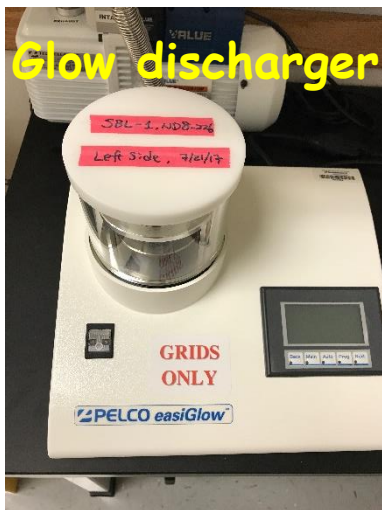
DUBOCHET'S VITRIFICATION METHOD



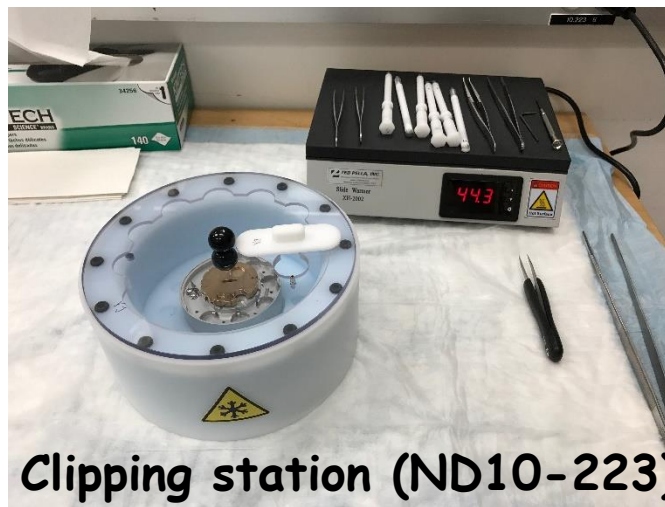
Two Vitrobot (LEFT / RIGHT)



Carbon coater



Glow discharger



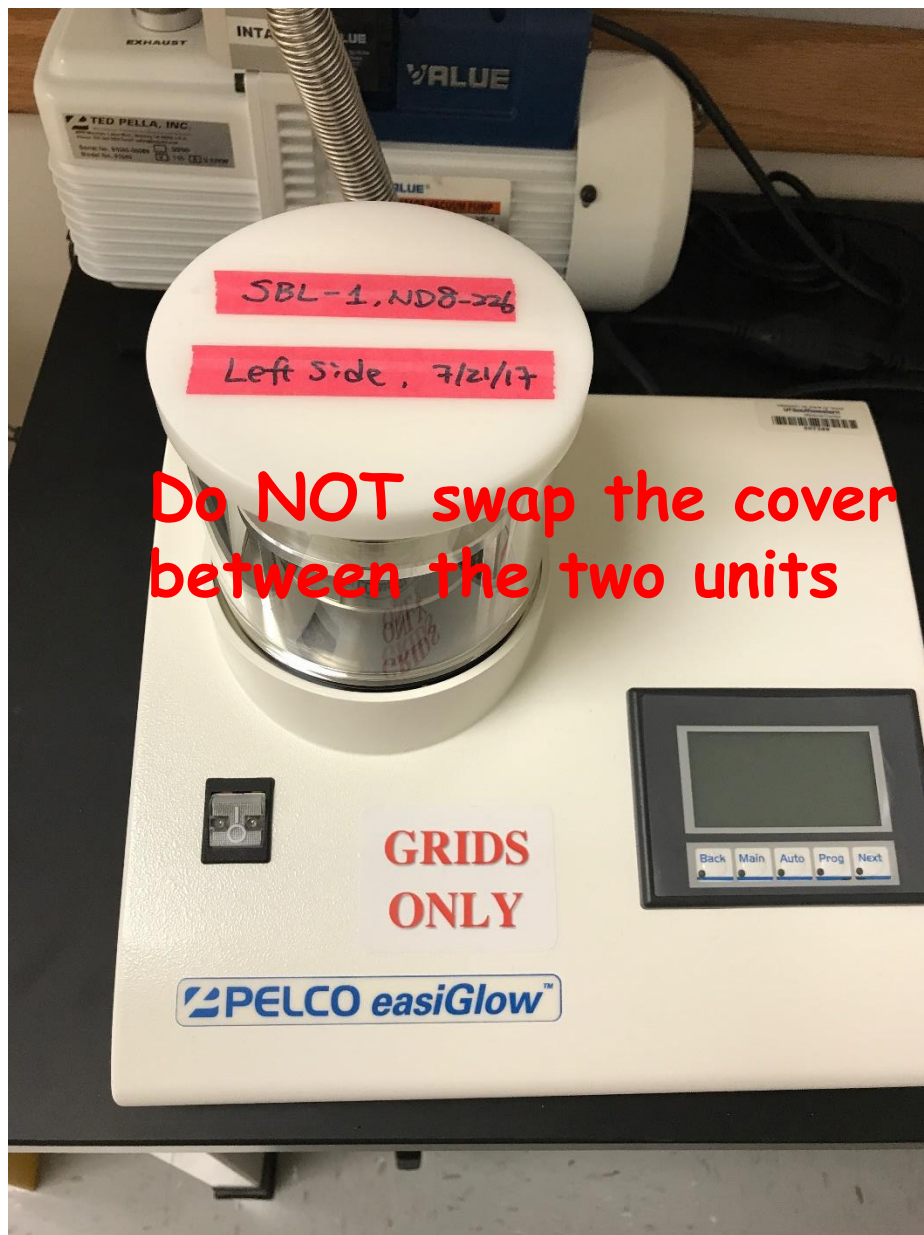
Clipping station (ND10-223)



Clipping station

Common Issues

- Using the facility without signing up (especially with the clipping station in the X-ray lab);
- Mistakenly opened the ethane tank valve all the way up;
- Misplacing items (especially transport dewars);
- Long term storage of grids in short term storage dewars;
- Informing SBL about items/supplies running out (gloves, pipet tips, blotting papers, etc).



Glow Discharge



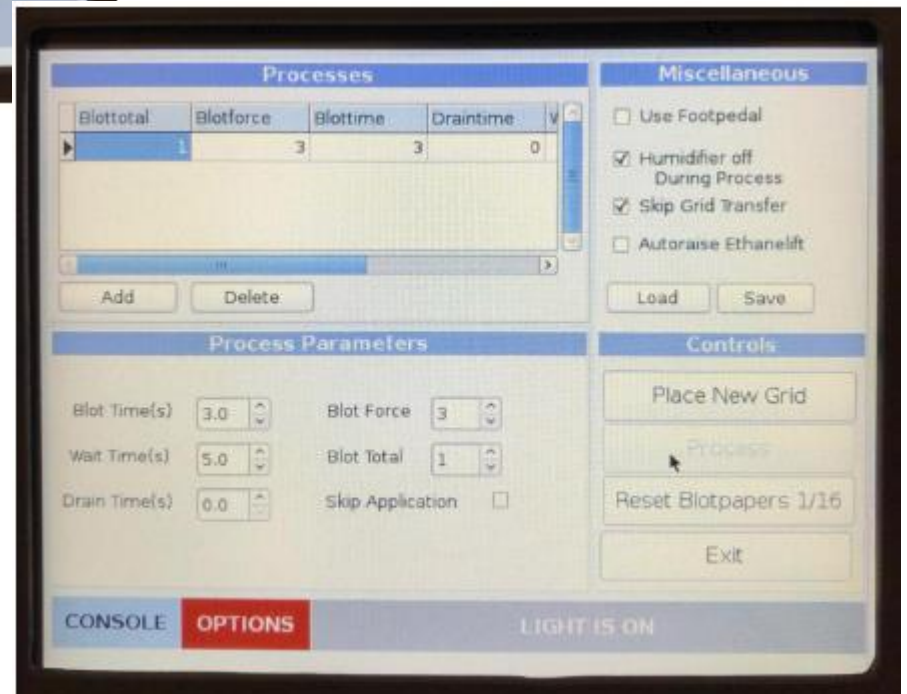
“The surface properties of QUANTIFOIL holey carbon support film, especially the wetting properties, may have to be adapted according to one's particular requirements. Untreated aging QUANTIFOIL tends to be hydrophobic. Hydrophilicity of the foil can be achieved by glow discharging in residual air or by metal coating.”

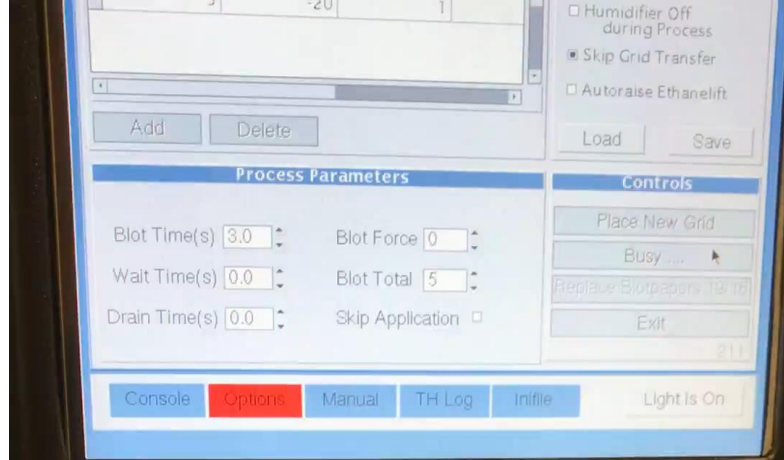
- 30mA, 80 seconds (default)
- 30mA, 30 seconds for continuous carbon grids
- PNCC: 25mA, 30 seconds
- Weiwei: 30mA, 30 seconds



The cable connector at the bottom of the water reservoir is very stiff, and easily breakable. Please handle it with both hands gently.

Vitrobot User Interface





Vitrobot in action



This is too warm



This is about right!

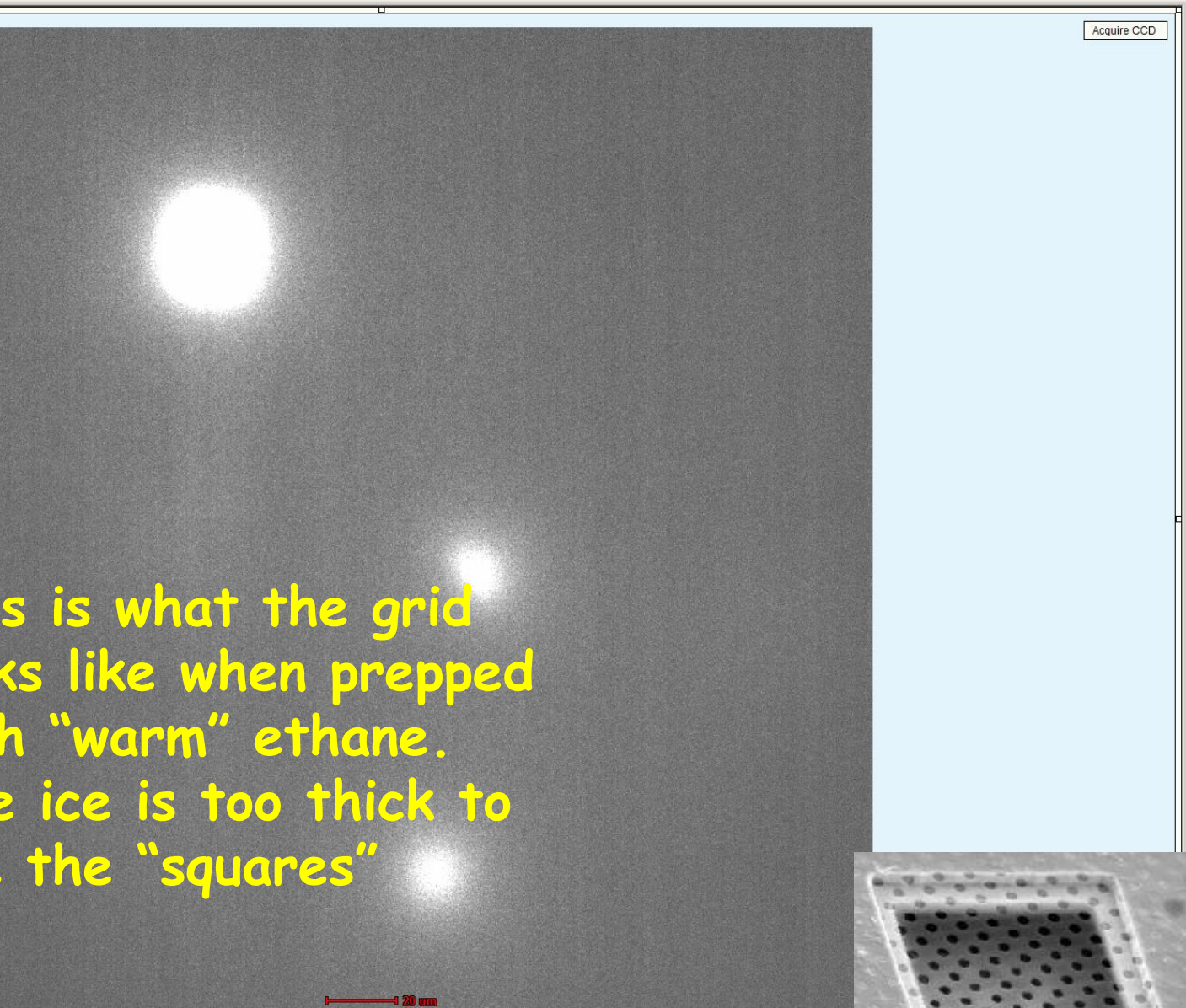
Use the long tweezers to lift the "spider"



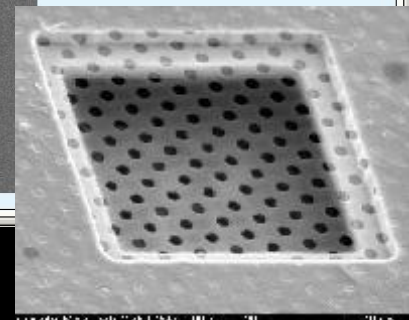
This is too warm

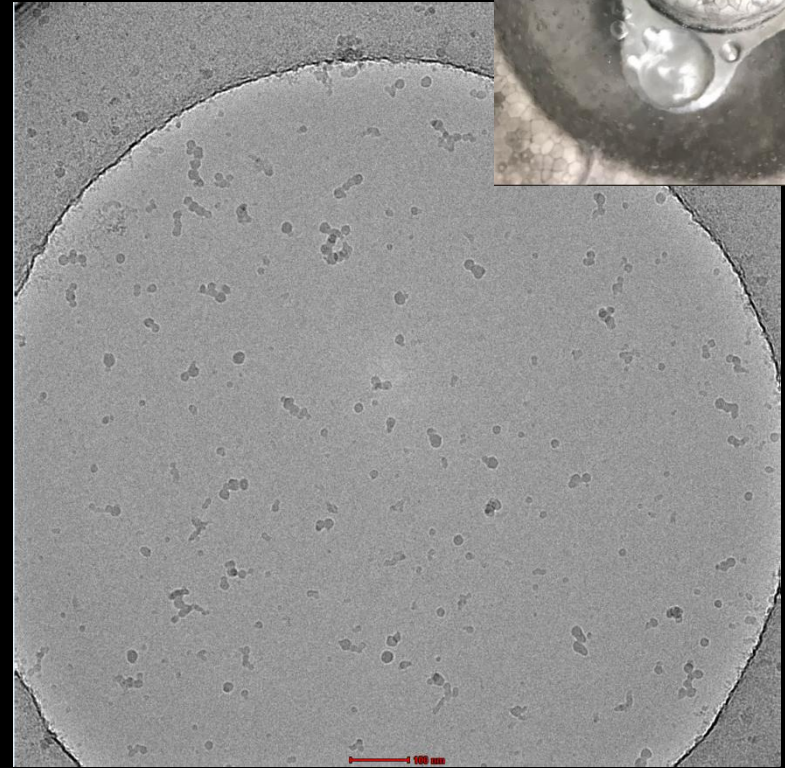
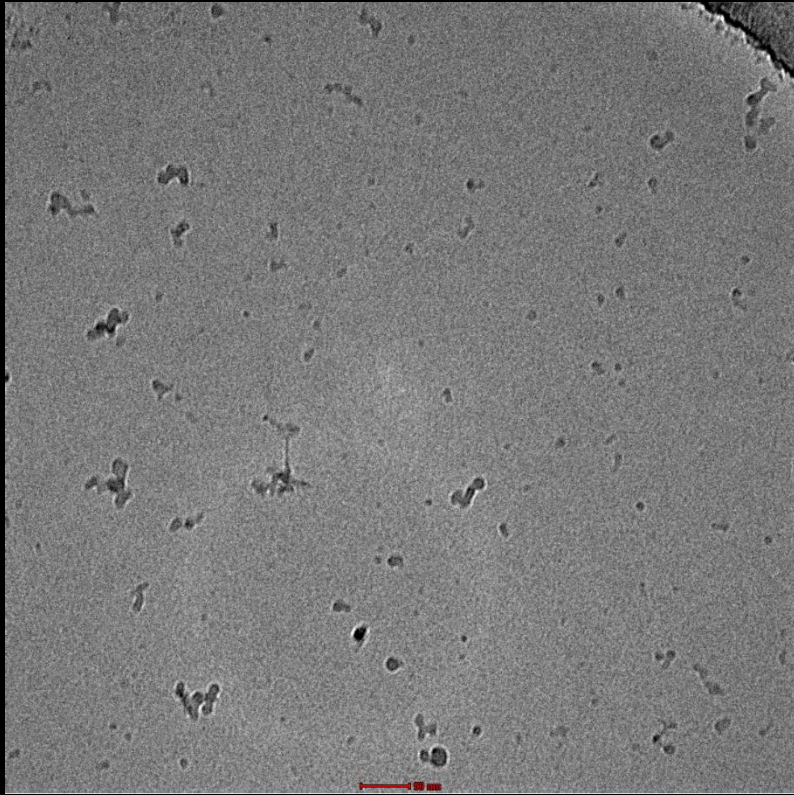


This is good, maybe just a bit too much solid ethane



This is what the grid looks like when prepped with "warm" ethane. The ice is too thick to see the "squares"





This is what the grid looks like when prepped with too much solid ethane. The dark/black spots (irregular shape) inside the holes are ethane "contaminants".



These are all good !



too warm

- There's no perfect answer for "**how much solid ethane should one have**".
- It boils down to how long it take from the point one "readies" the ethane to the point when the grid gets plunged into it.
- Normally this will take 1-2 minutes.
- Also consider that the tweezers (tip) and grids will be "warmer" (than ethane) when plunged.
- Sometimes one may end up setting up a reaction on the fly, which will take longer (more solid ethane needed).

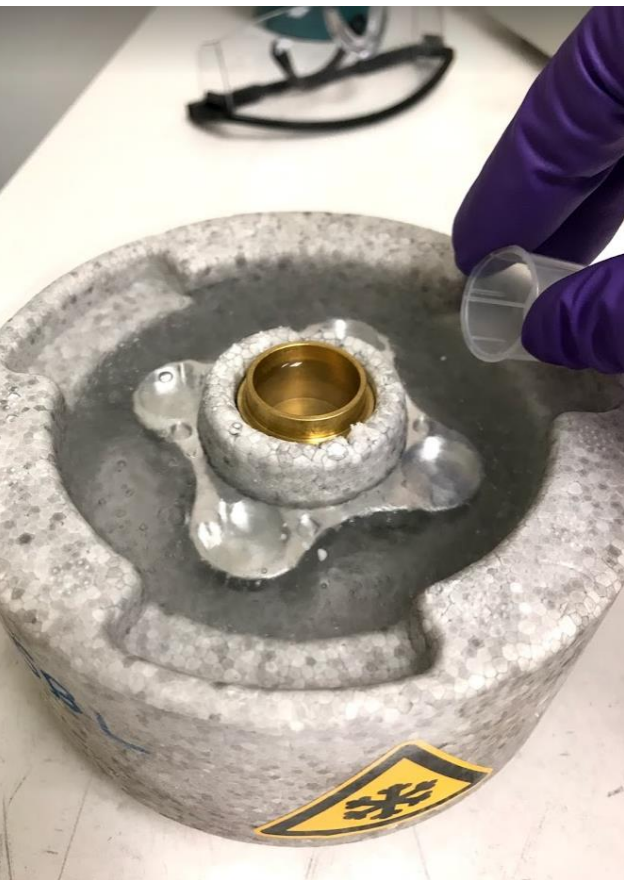
What to do when the ethane
“warms up”?



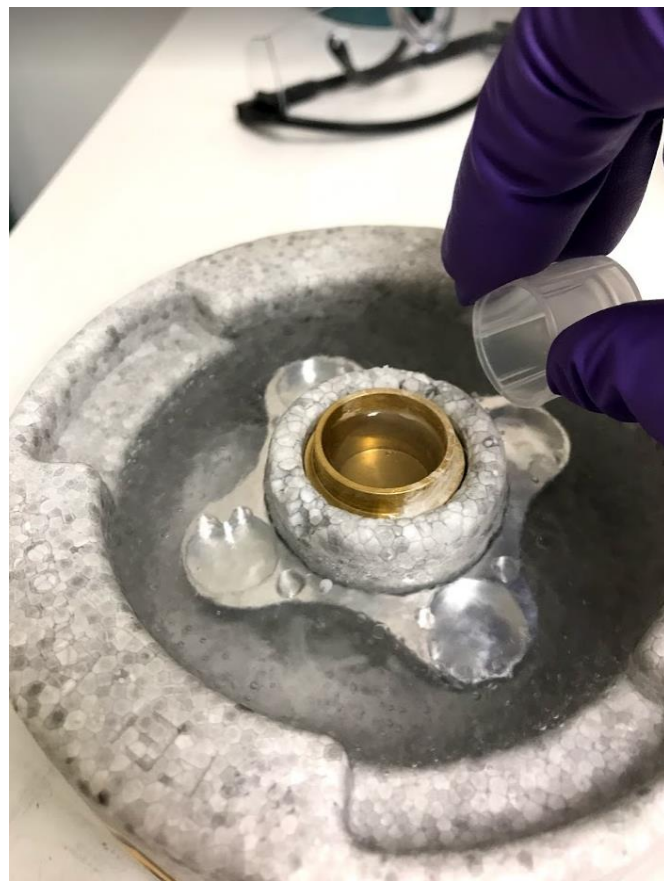
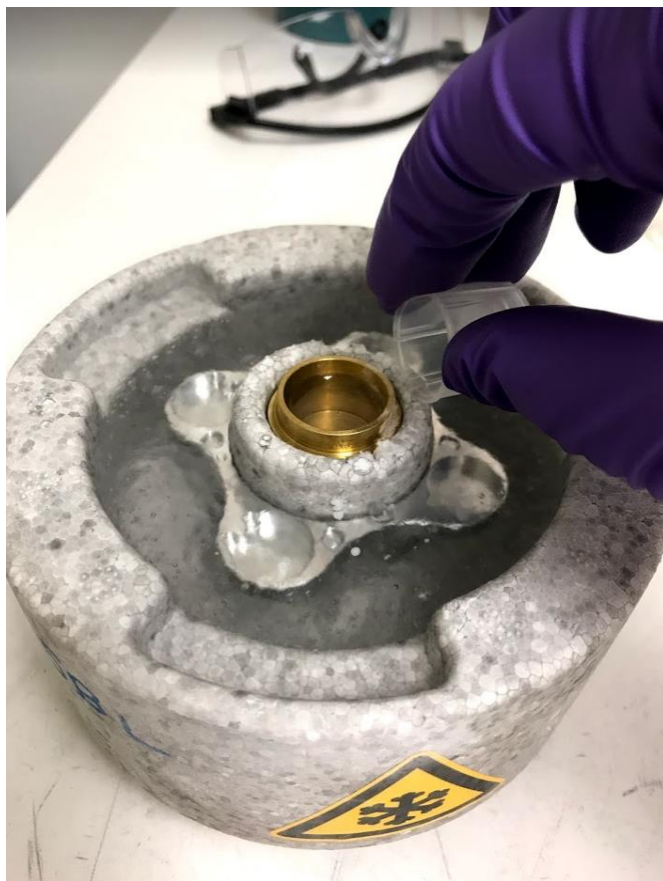
Option #1: Re-assemble/Repeat



Option #2: The “cap trick” (courtesy of Yang Li)



Option #2: The “cap trick” (courtesy of Yang Li)



Advantages of using the “floater”

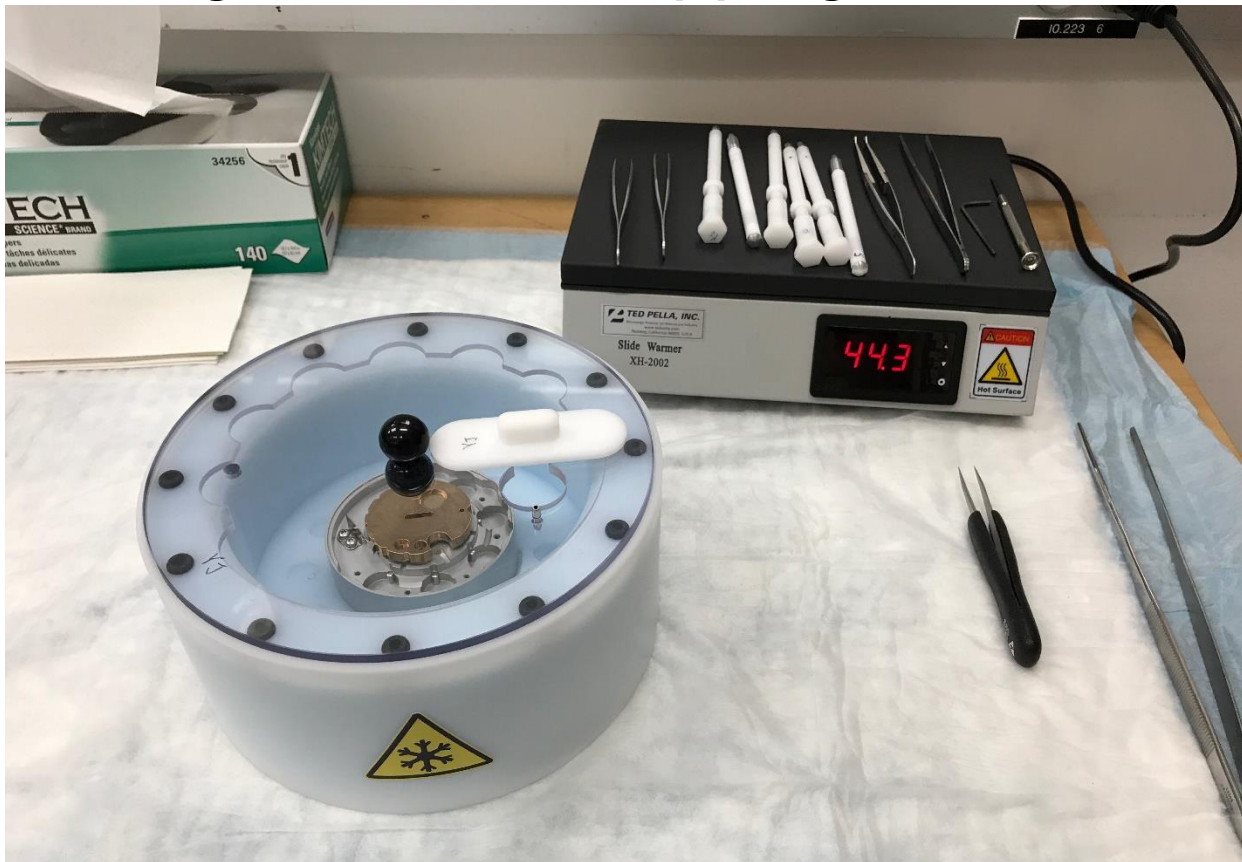


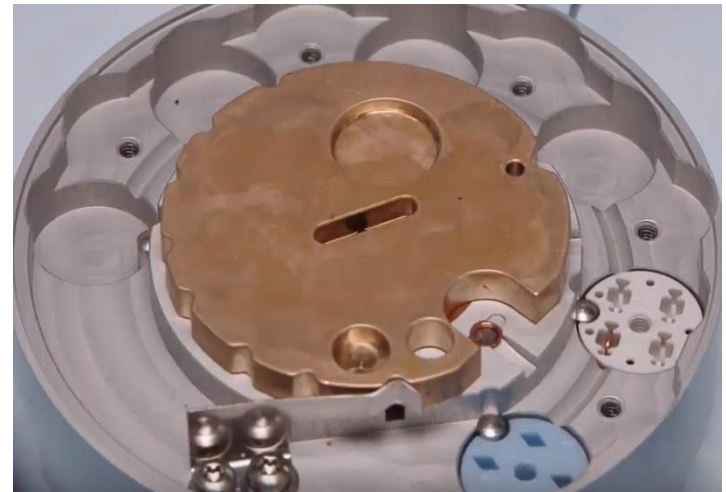
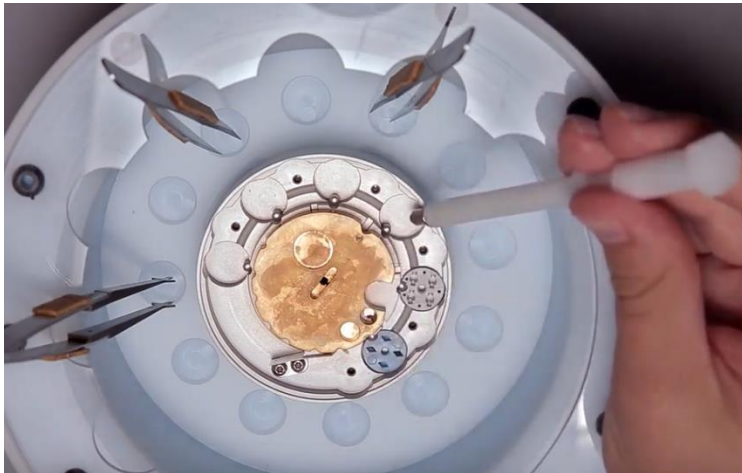
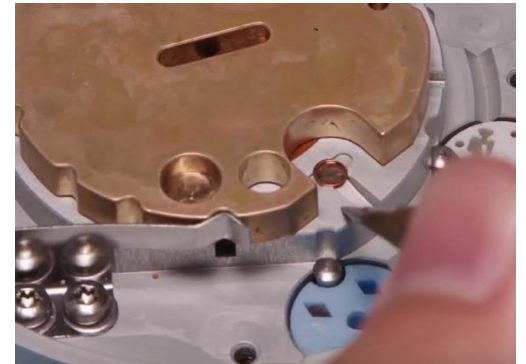
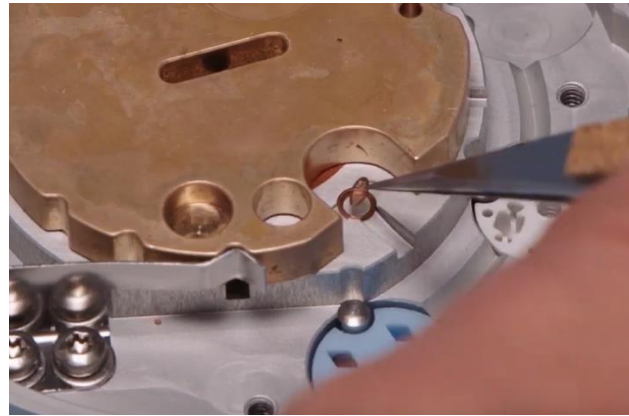
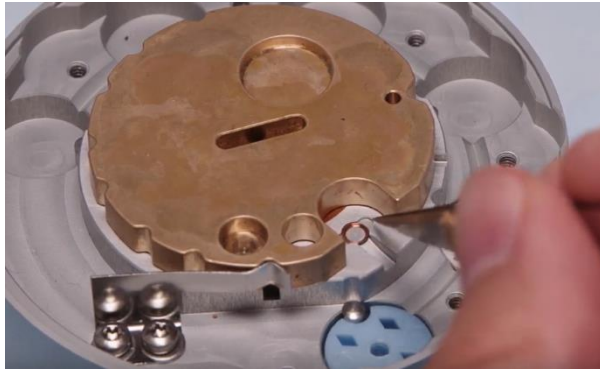
Good starting point



Check quality of “clipping”

- Improperly assembled cassette may come apart on the microscope.
- Make sure that there’s not too much LN2 on top of the grid before “clipping”.





<http://cryo-em-course.caltech.edu/>

YouTube Search

Home Trending History

BEST OF YOUTUBE

- Music
- Sports
- Gaming
- Movies
- TV Shows
- News
- Live
- Spotlight
- 360° Video

Browse channels

Sign in now to see your channels and recommendations!

Cryo-EM course - Unit 2: Sample Preparation

42 videos • 11,741 views • Last updated on Dec 19, 2018

Lectures by Grant Jensen, Professor of Biology, Caltech; Investigator, Howard Hughes Medical Institute and Matthijn Vos. Recorded at Caltech in 2017.

Visit the course website: <http://cryo-em-course.caltech.edu>
Learn more about the Jensen Lab at Caltech: <http://www.jensenlab.caltech.edu>
Before starting these videos, complete the Getting Started in Cryo-EM videos: <http://bit.ly/1WfKsph>

1 **Sample prep overview - The entire process without interruptions**
caltech 43:28

2 **Preparing hydrophilic grids: Manufacturer's grid boxes**
caltech 4:24

3 **Preparing hydrophilic grids: Recognizing the carbon and copper sides of a grid**
caltech 1:31

4 **Preparing hydrophilic grids: How to grab grids with tweezers**
caltech 1:07

5 **Preparing hydrophilic grids: Glow discharging Plasma cleaning**
caltech 5:45

6 **Preparing hydrophilic grids: Hydrophobic and hydrophilic grids**
caltech 3:32

7 **Plunge freezing with a Vitrobot: Plunge freezing tools**
caltech 7:21

NRAMM / SEMC YouTube Channel



Search



NRAMM SEMC NCCAT

Videos

Playlists

Channels

About

- Home
- My channel
- Trending
- Subscriptions
- Get YouTube Premium
- Get YouTube TV

LIBRARY

- History
- Watch later
- Liked videos

SUBSCRIPTIONS

- Angry Birds
- ericstrains 1
- Lionel Trains 1
- ThePianoGuys

Browse channels

Movies & Shows

Gaming

Uploads

Date added (newest)

Grid



Basic Anatomy of an Electron Microscope, Jan 9
55 views • 4 days ago



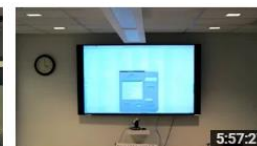
Winter EM Course - Introduction Jan 7 2019
48 views • 6 days ago



Workshop on Challenges for High Speed Cryo Electron Tomography
263 views • 1 month ago



Workshop on Challenges for High Speed Cryo Electron Tomography
64 views • 1 month ago



Appion promo
43 views • 1 month ago



Annual user meeting 2018
75 views • 3 months ago



NYSBC Winter Course 2018 - Fitting Atomic Models and...
111 views • 8 months ago



NYSBC Winter Course 2018 - EMDataBank
53 views • 8 months ago



Workshop on Deep Learning for CryoEM - 2018
824 views • 9 months ago



NYSBC Winter Course 2018 - Validation Methods
84 views • 9 months ago



NYSBC Winter Course 2018 - MicroED
176 views • 9 months ago



Appion Workshop Part III - Tomography
131 views • 9 months ago



SEMC Winter Course 2018 - 2D Crystallography (Part I: Intro an...
38 views • 9 months ago



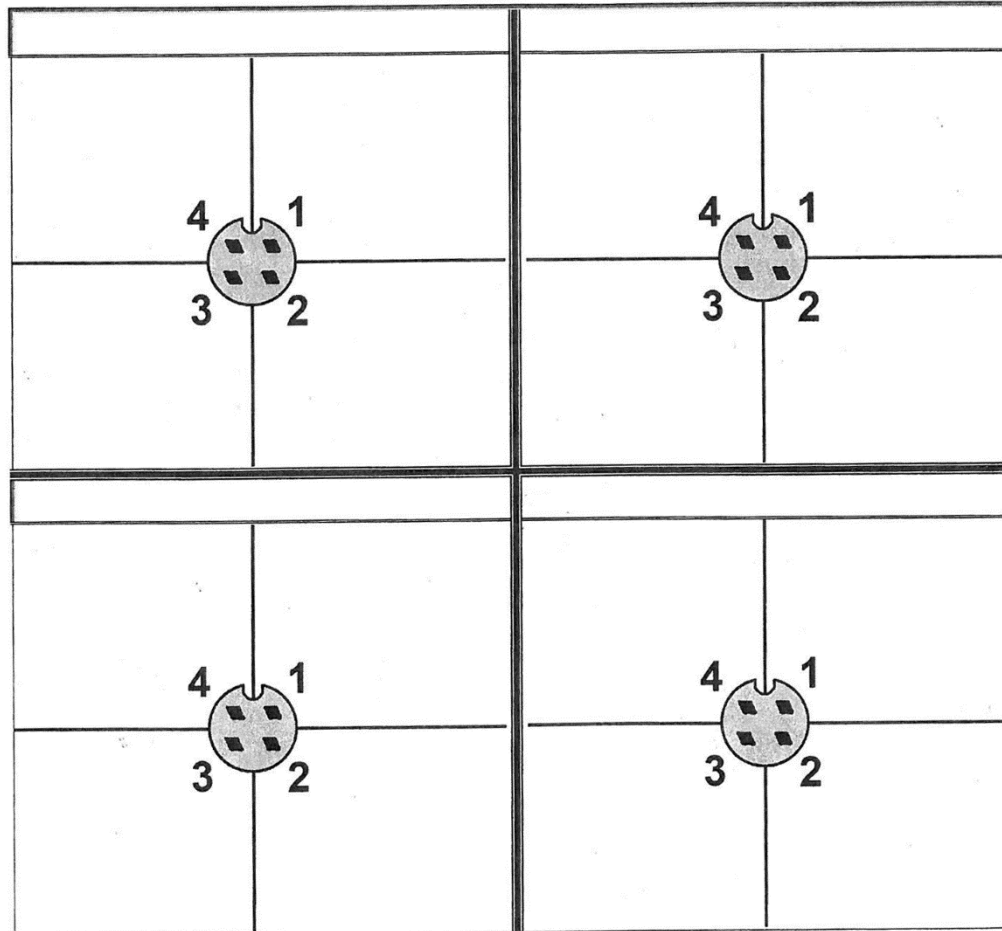
NYSBC Course 2018 - SPA - Part IV: Reconstruction Workflow, an...
105 views • 9 months ago



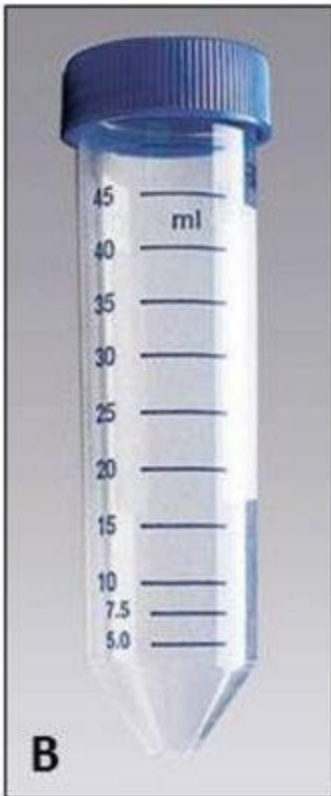
NYSBC Cryo-EM Course 2018 - Challenges in Biological EM &...
106 views • 9 months ago

Plunge-FREEZING LOG

User:		Date:		Project:		
Sample:						
General conditions:	grid type	glow dis.	dry gold	wet gold	sample vol.	remarks
Grid box(es) stored in:						



Notes:





**Bottom Magnets for Secure Retention
in Storage and Shipping Cans**





A



B



C



D