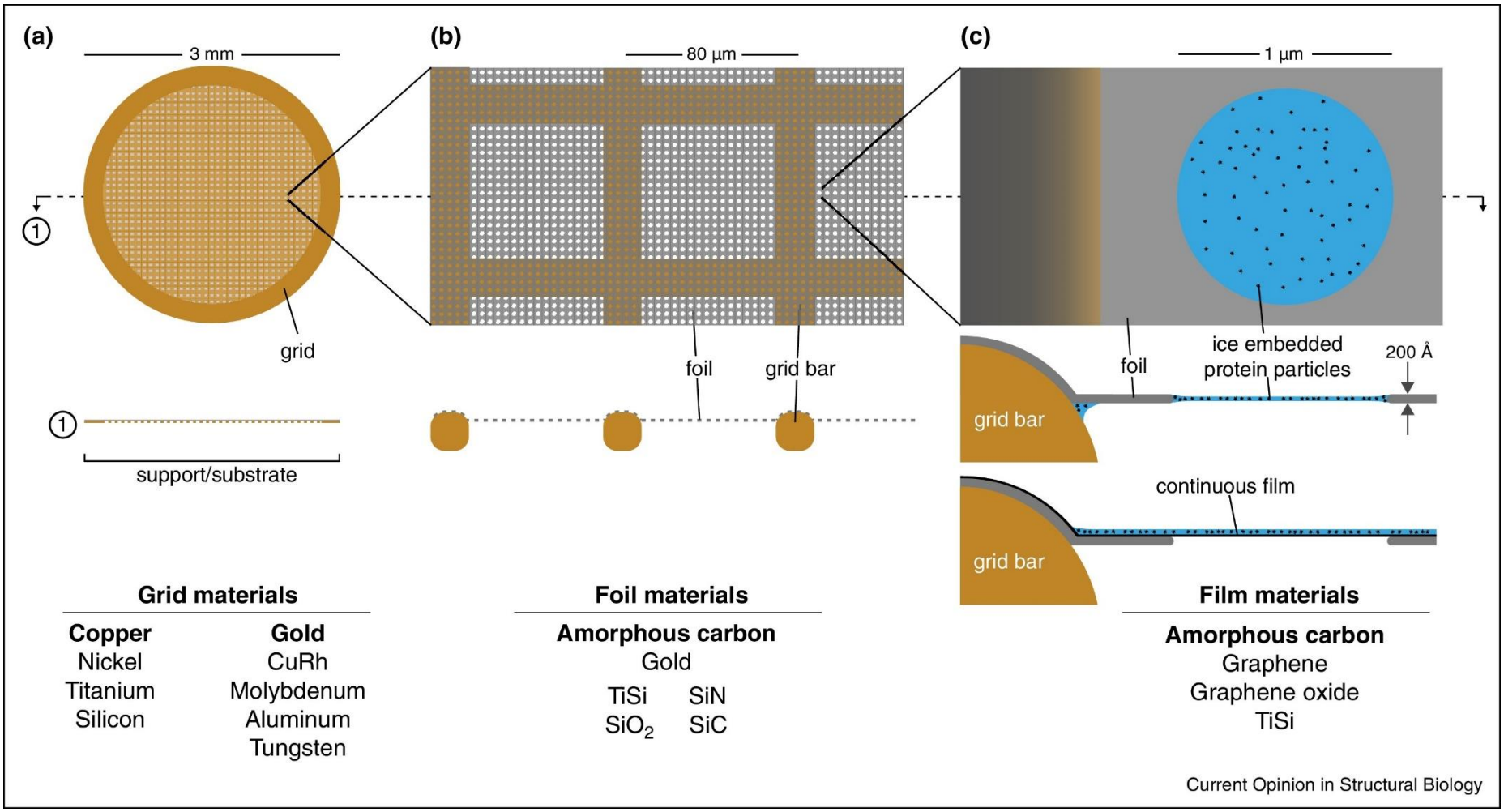
Selected Slides from SBWIP

James Chen (SBL)

Anatomy of a Grid



Quantifoil 300 mesh Cu R1.2/1.3

DUBOCHET'S VITRIFICATION METHOD

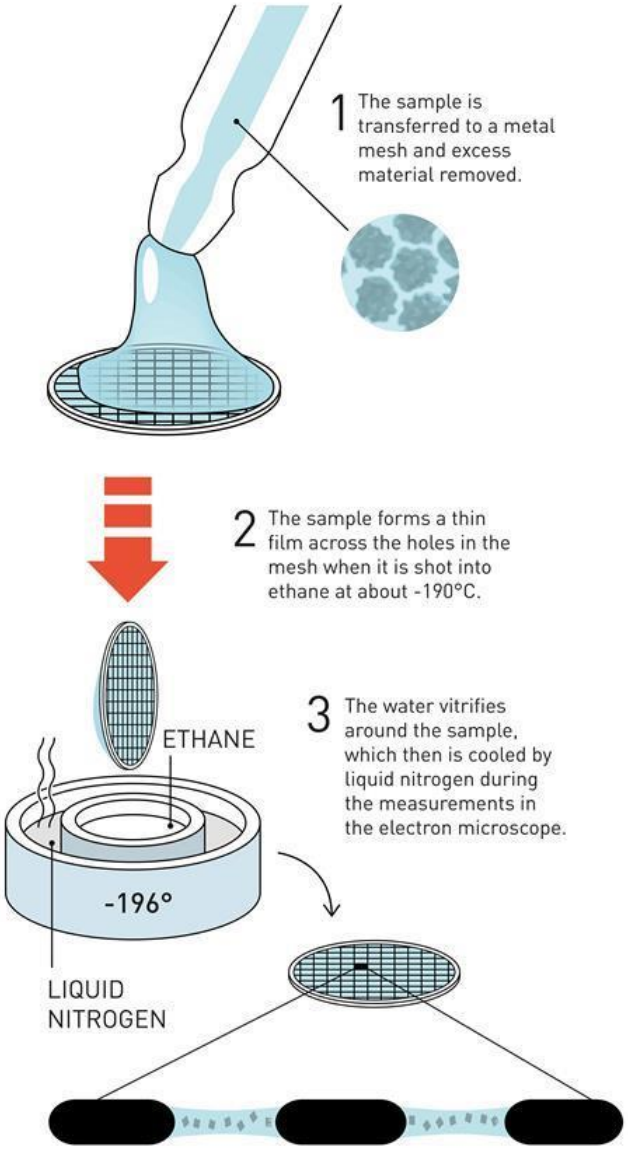
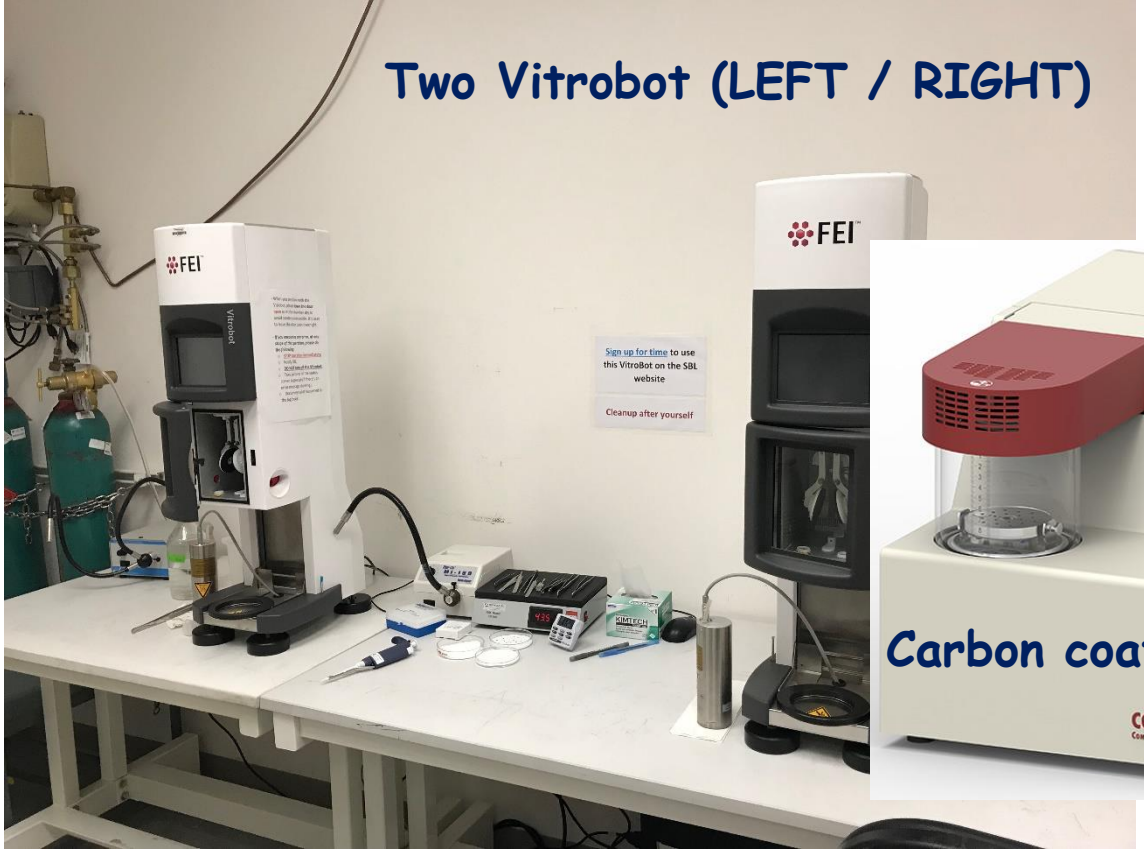
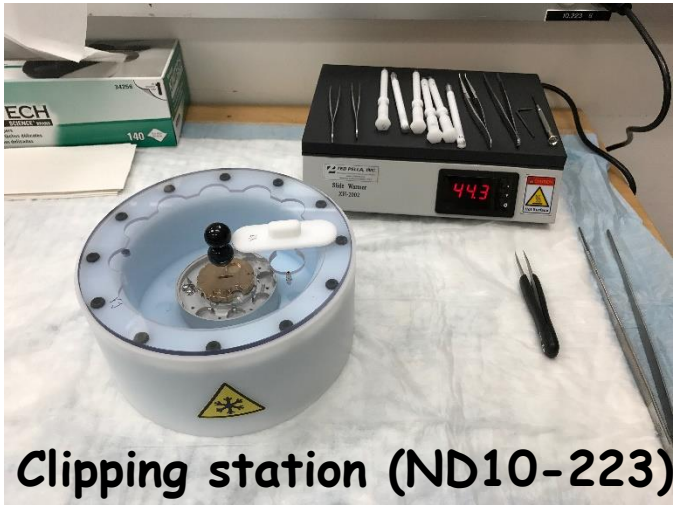


Illustration: ©Johan Jarnestad/The Royal Swedish Academy of Sciences


Two Vitrobot (LEFT / RIGHT)
FEI
FEI
Vitrobot
Sign up for time to use this VitroBot on the SBL website
Cleanup after yourself


safematic
Carbon coater
CCU-010
COMPACT COATING UNIT

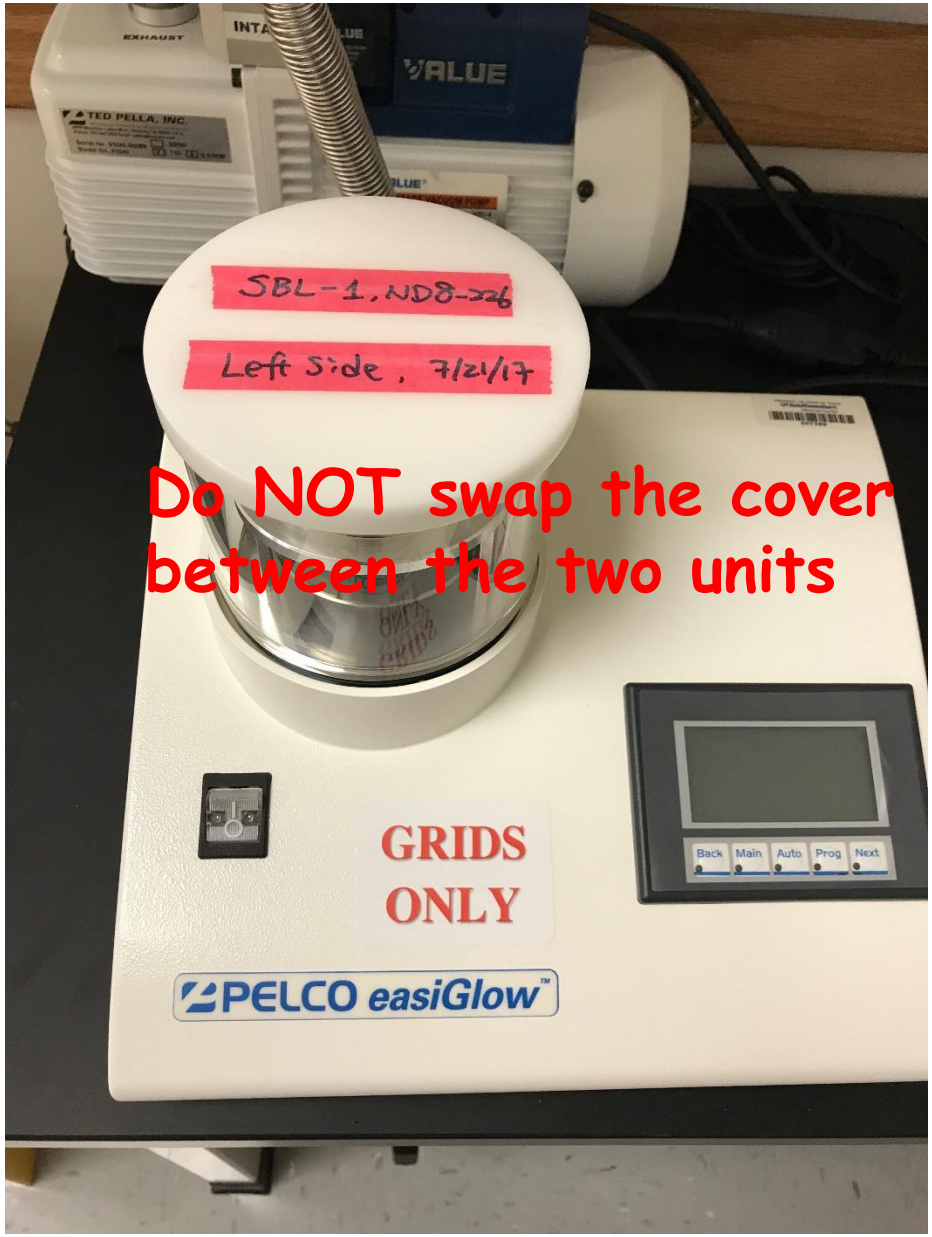

Glow discharger
SBL-1, ND8-226
Left Side, 7/21/17
GRIDS
ONLY
PELCO easiGlow


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).


SBL-1, ND8-226
Left Side, 7/21/17
Do NOT swap the cover between the two units
GRIDS ONLY
Back
Main
Auto
Prog
Next
PELCO easiGlow™


Handle the glass cover with care!

Glow Discharge

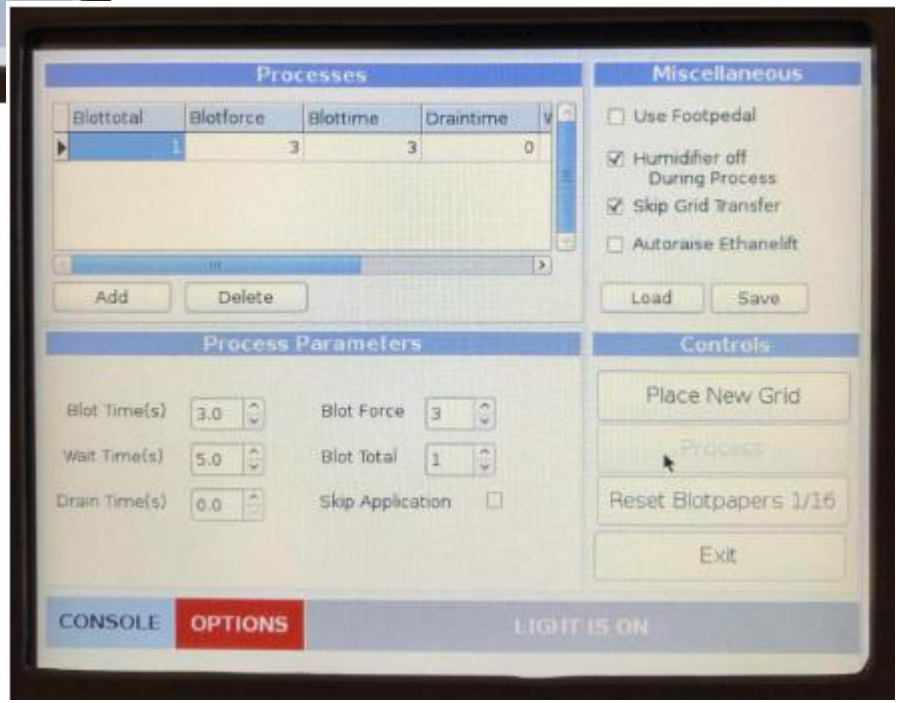
"The surface properties of QUANTIFOIL holey carbon support film, especially the wetting properties, may have to be adapted according to one's particular requirements. Untreated aging QUANTIFOIL tends to be hydrophobic. Hydrophillicity of the foil can be achieved by glow discharging in residual air or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




Humidifier Off during Process
Skip Grid Transfer
Autoraise Ethanelift
Add
Delete
Load
Save
Process Parameters
Controls
Blot Time(s) 3.0
Blot Force 0
Wait Time(s) 0.0
Blot Total 5
Drain Time(s) 0.0
Skip Application
Place New Grid
Busy ....
Exit
Console
Options
Manual
TH Log
Inifile
Light Is On
Vitrobot in action

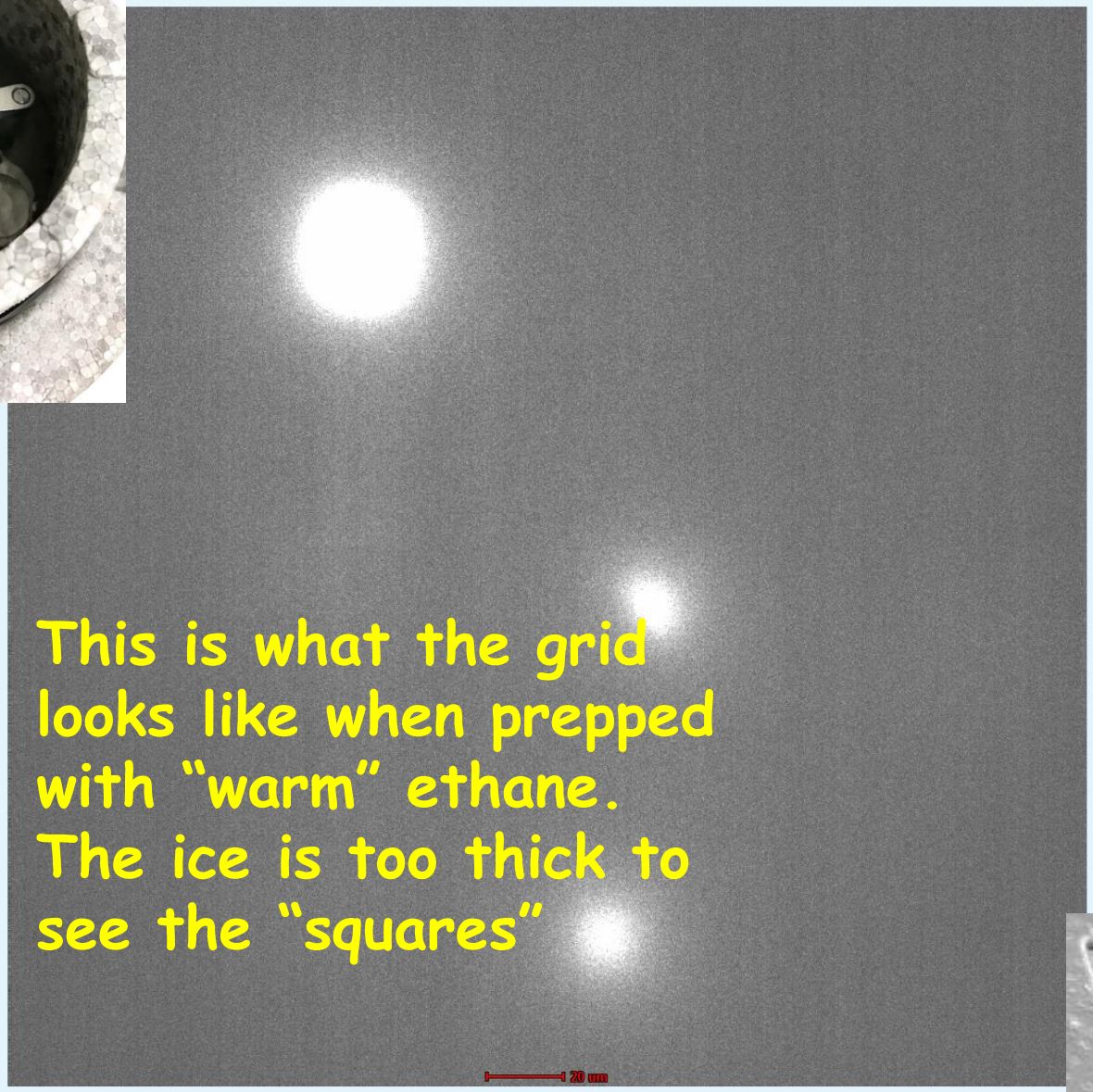
This is too warm

This is about right!

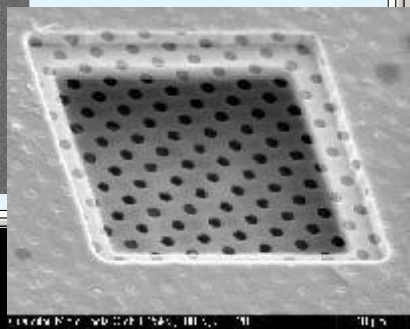
Use the long tweezers to lift the "spider"

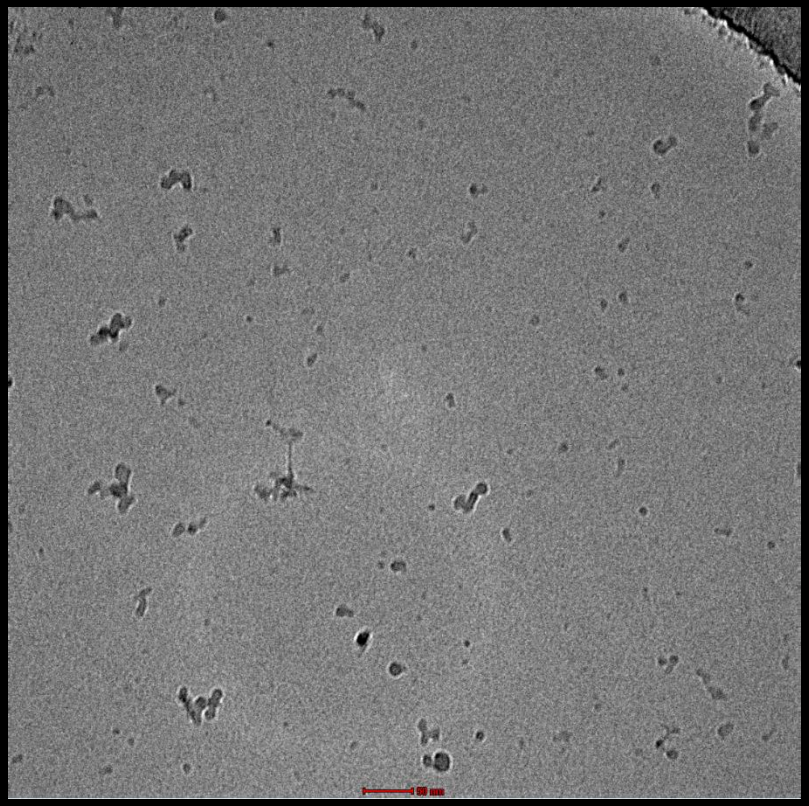
This is too warm

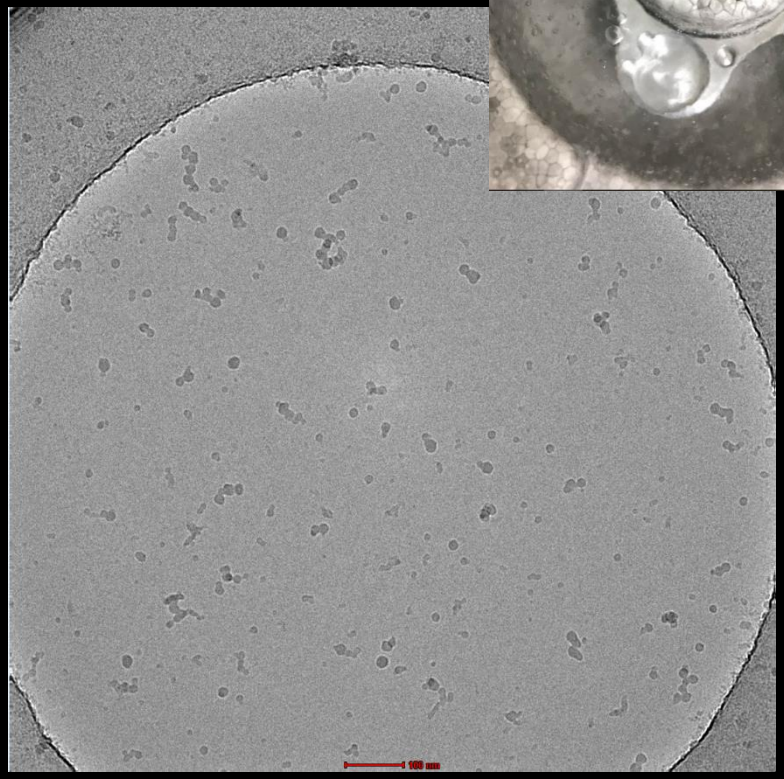
This is good, maybe just a bit too much solid ethane



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 !

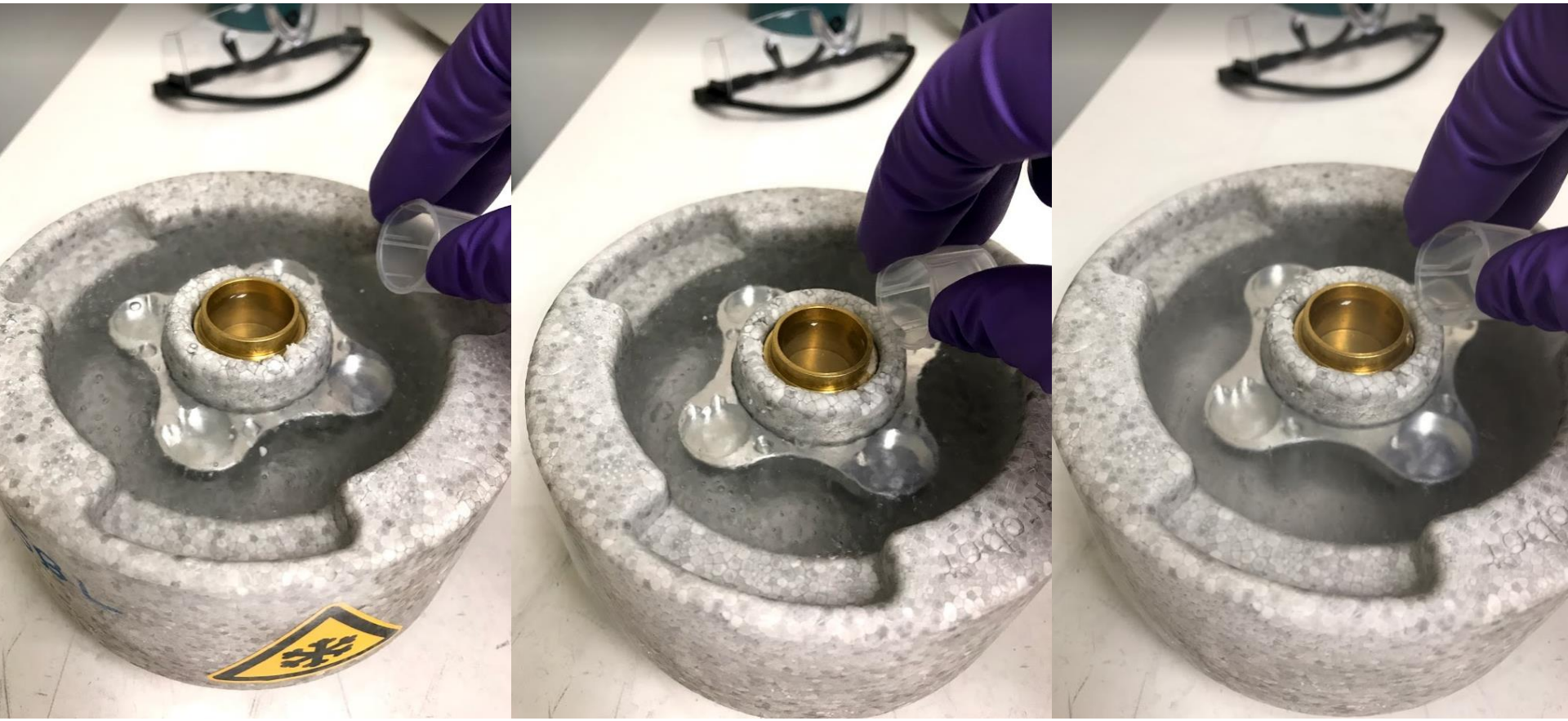
too warm

- 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).

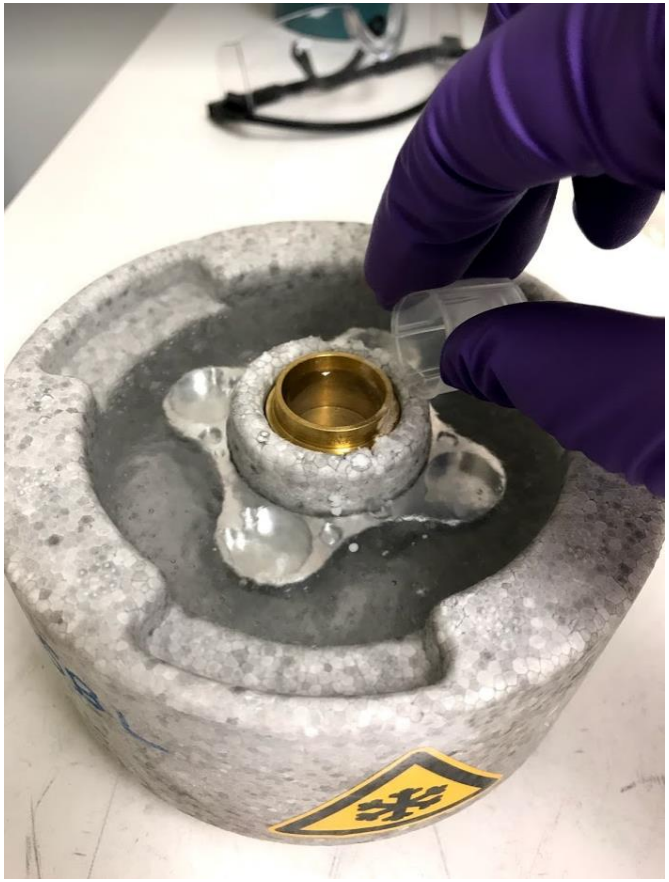
What to do when the ethane “warms up”?

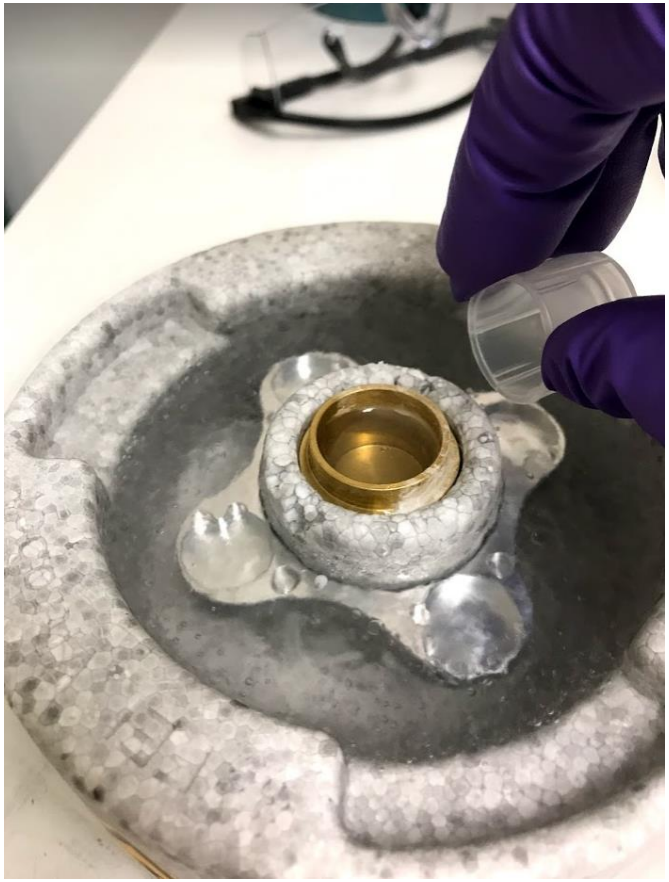
Option #1: Re-assemble/Repeat

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



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



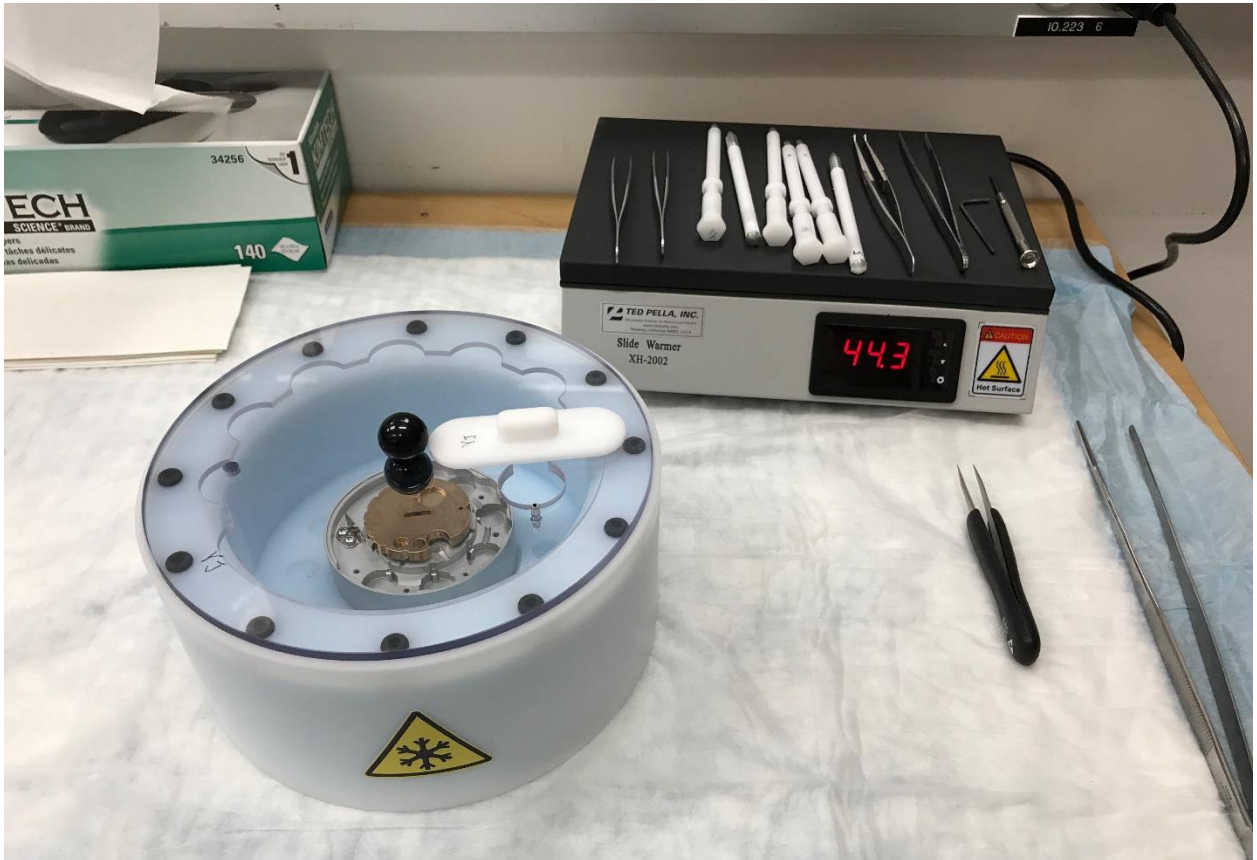


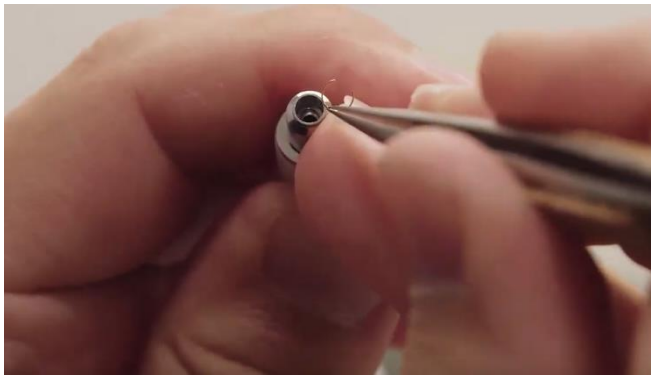
Advantages of using the “floater”

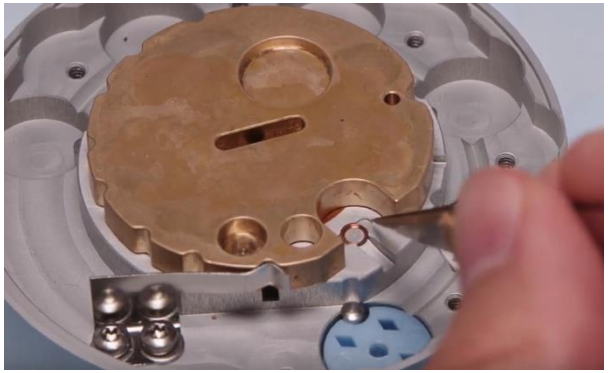
Good starting point

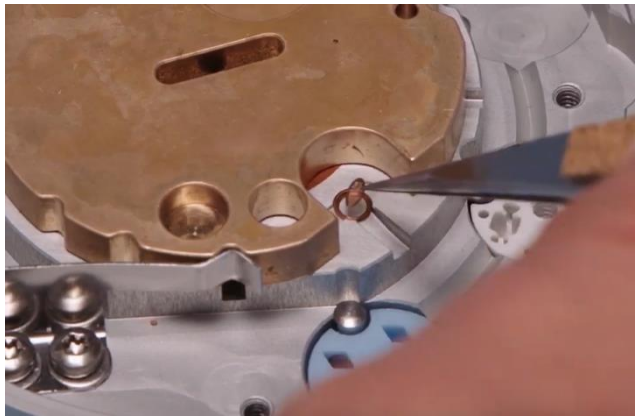
Check quality of “clipping”

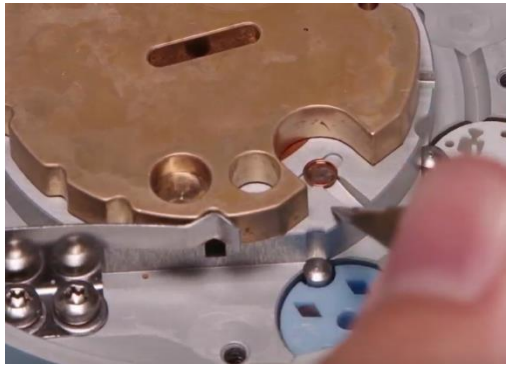
- Improperly assembled cassette may come apart on the microscope.
- Make sure that there’s not too much LN2 on top of the grid before “clipping’.

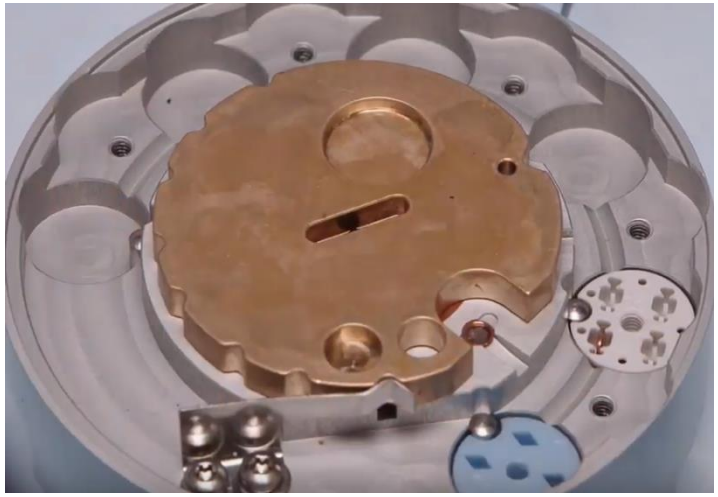




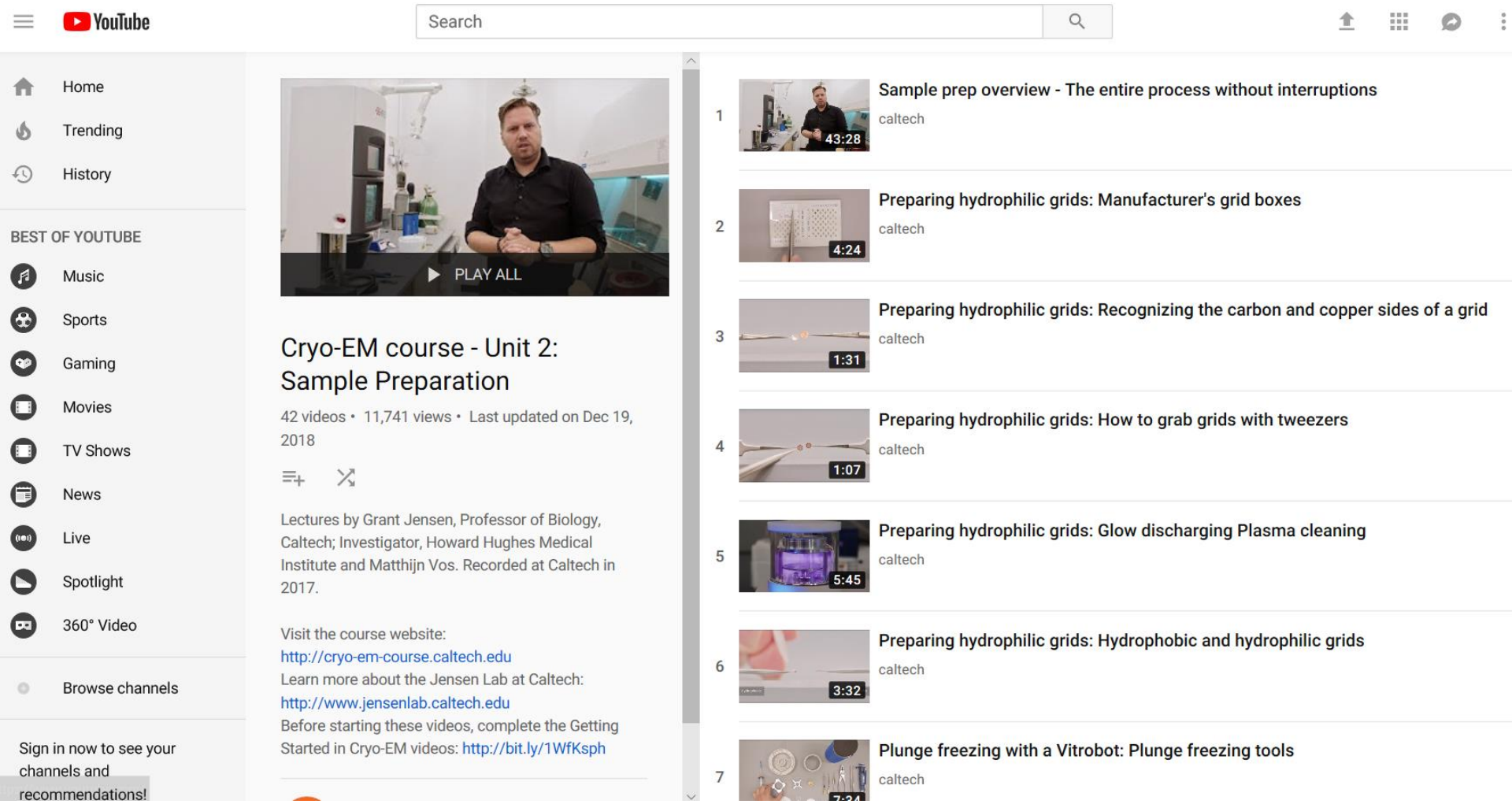




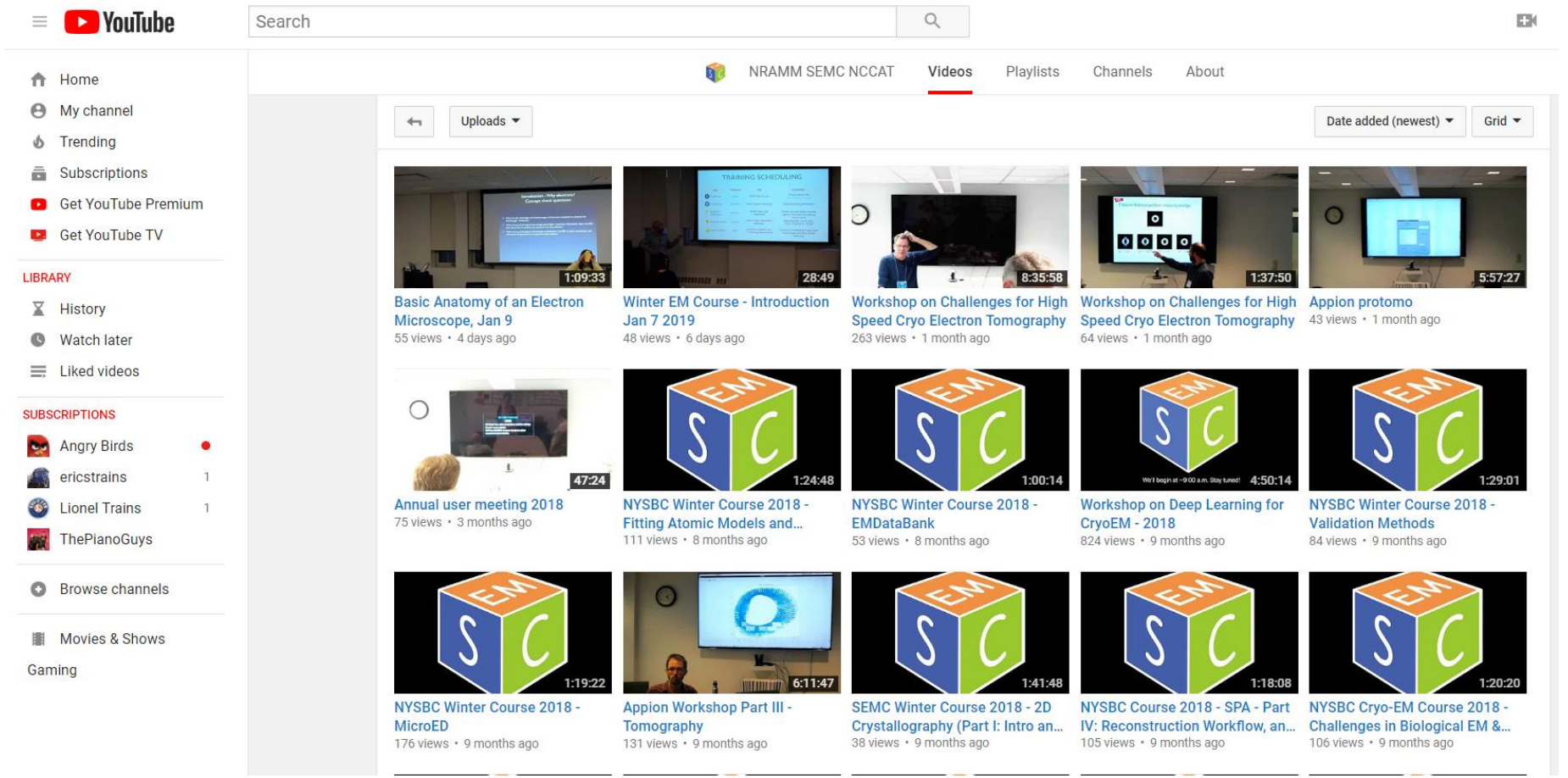




http://cryo-em-course.caltech.edu/



NRAMM / SEMC YouTube Channel

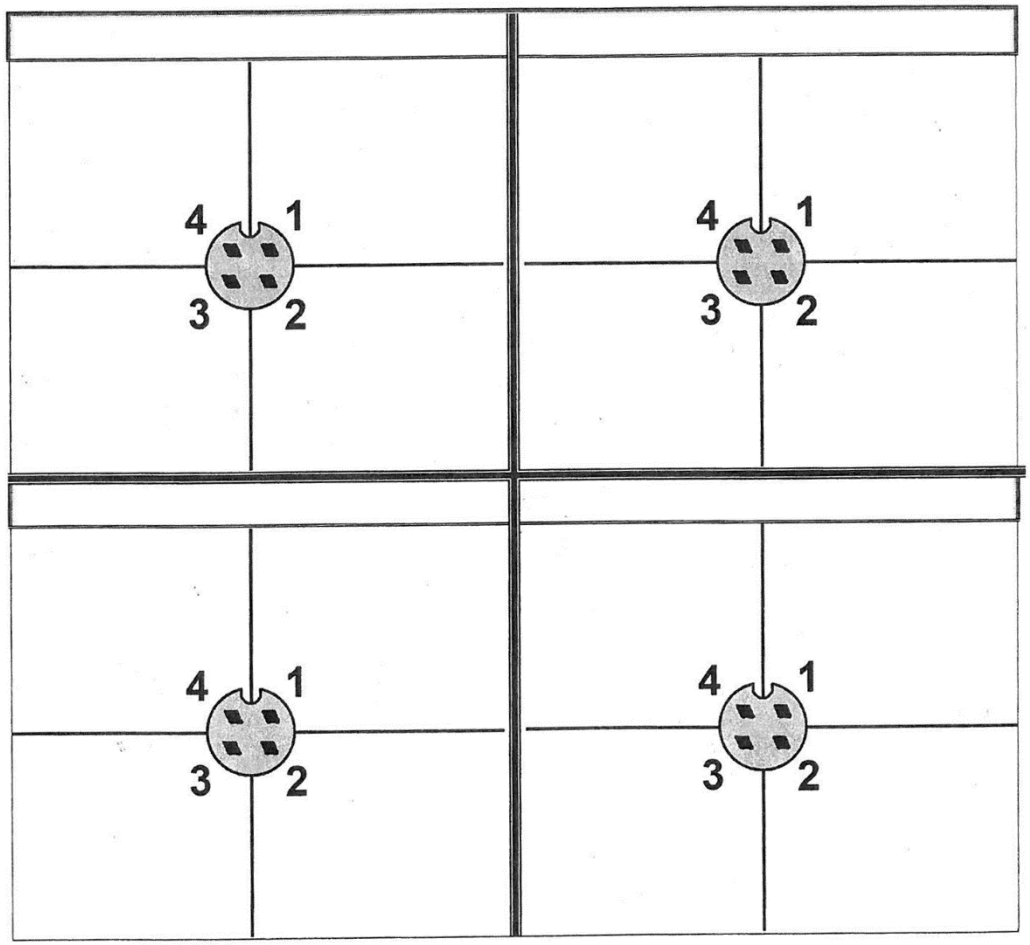


Plunge-FREEZING LOG

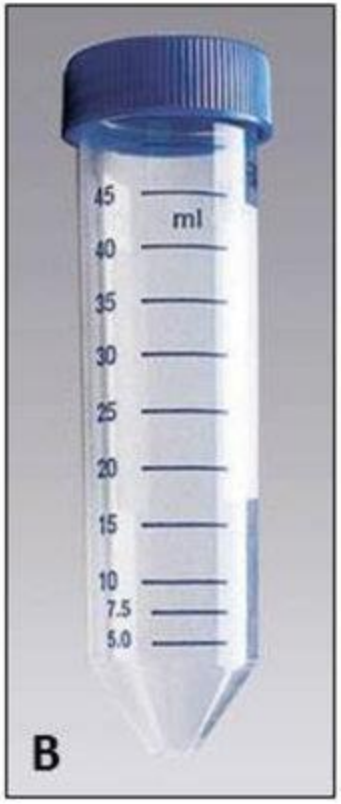
User:		Date:		Project:	
Sample:					

General conditions:	grid type	glow dis.	dry gold	wet gold	sample vol.	remarks

Grid box(es) stored in:



Notes:



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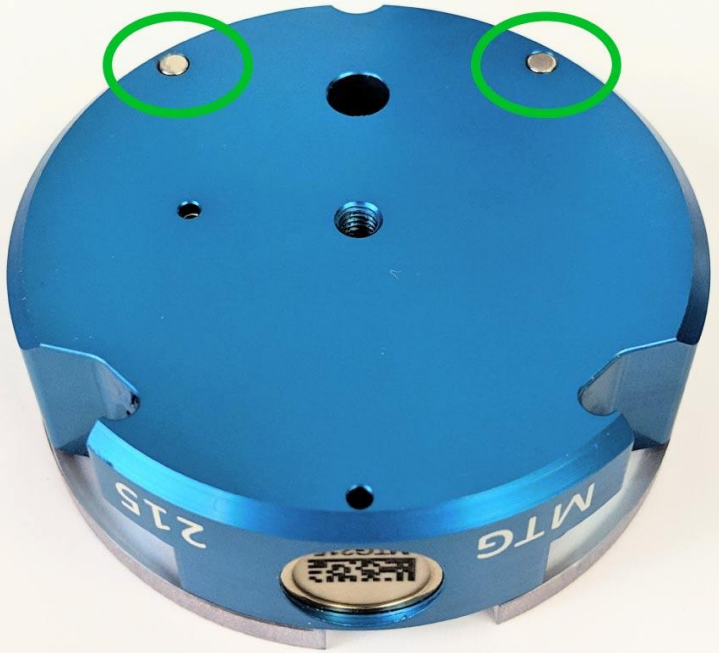
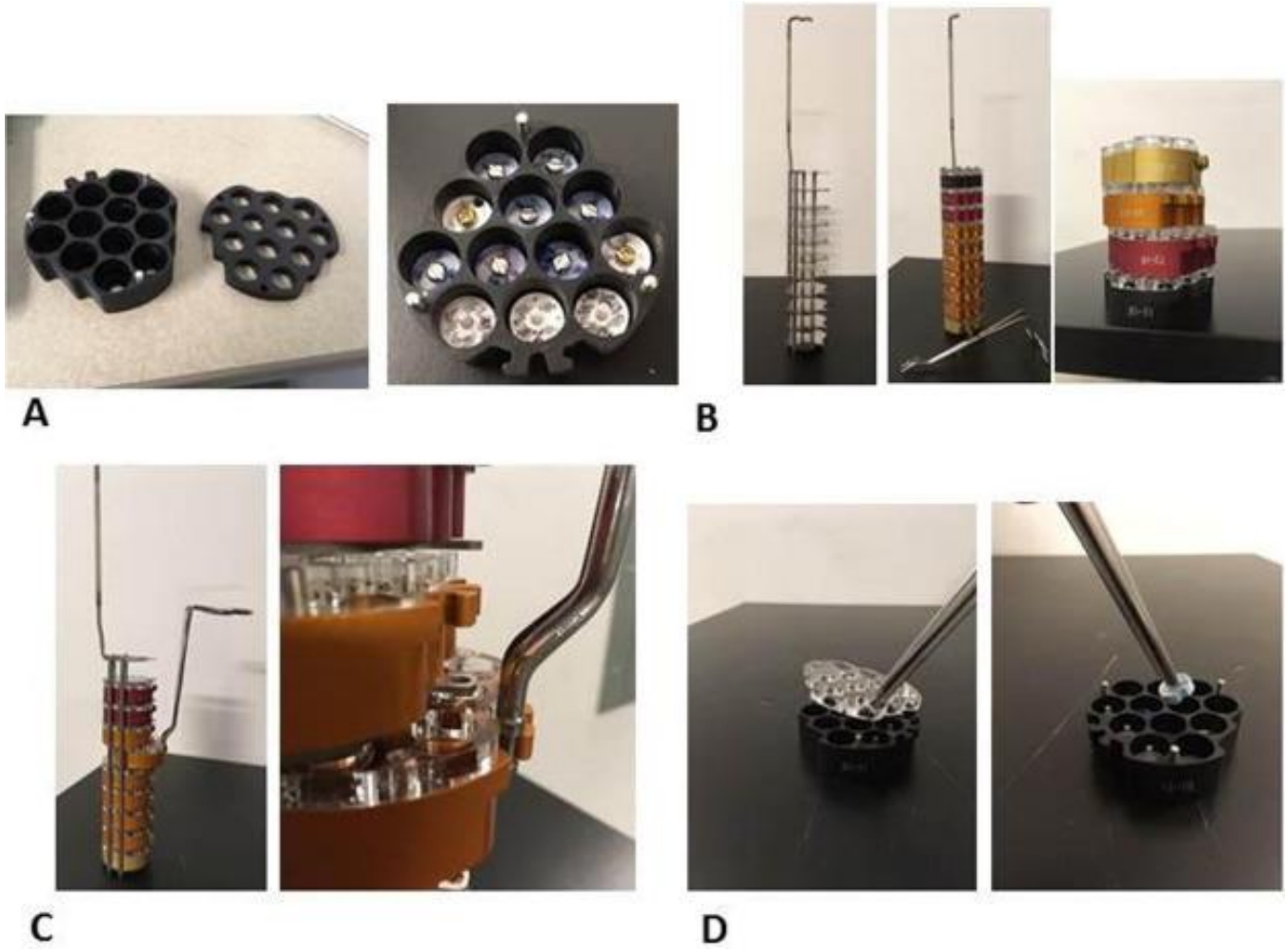
Bottom Magnets for Secure Retention in Storage and Shipping Canes





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