



CPOS Cryo

General Policy

Li Ka Shing Cryo-EM Laboratory

Cores in CPOS



**HKU
Med**

LKS Faculty of Medicine
Centre for PanorOmic Sciences
香港大學泛組學科研中心

Biobank Core

Genomics Core

Proteomics and
Metabolomics
Core

Bioinformatics
Core

Imaging and
Flow Cytometry
Core

Bioresearch
Support Core

Bioreagent Core

LKS Cryo-EM
Laboratory

HKUMed
Laboratory of
Cellular
Therapeutics

FMB Cores

Laboratory Block, 21 Sassoon Road

Online Platform



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LKS Cryo-EM
Laboratory

HKUMed
Laboratory of
Cellular
Therapeutics

iLab

PPMS

Bioreagent Core
Online Purchasing
System

iLab



Li Ka Shing Cryo-EM Laboratory

Email: cryoem.cpos@hku.hk

Tel: 3910-2938

Opening hours: 9:00 am to 5:30pm

HKU Med LKS Faculty of Medicine
Centre for PanorOmic Sciences
香港大學泛組學科研中心

enquiry.cpos@hku.hk | 3910-6600

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Li Ka Shing Cryo-EM Laboratory

Overview | Benefits | Applications | Process Steps | Imaging Equipment | Access Information During Soft Launch | Charges | Contact

Overview

Cryo-Electron Microscopy is the imaging of specimens frozen in vitreous ice and maintained at liquid nitrogen temperature using Electron Microscopes. In this method, specimens can be studied in their native state without dyes or fixatives, enabling the analysis of fine cellular structures, viruses, and proteins at molecular resolution. Despite being a decades-developed technique, Cryo-EM has been attracting interest since 2013 as a result of technological and algorithmic improvements that have driven a dramatic improvement in the resolution achievable using this technique (dubbed the 'resolution revolution'). In 2017, the technique won the Nobel Prize in Chemistry.

The Cryo-EM technique is becoming the first choice of many structural biologists when analyzing the protein structure experimentally. As a technique for determining the atomic structure of macromolecules that neither crystallize nor are difficult to crystallize under certain conditions, Cryo-EM has the same level of resolution as X-ray crystallography. Cryo-EM is the best way to study cell architecture, large proteins, membrane-bound receptors, or complexes of macromolecules.

General Rules and Security



The Core is under surveillance **24/7**



Do **NOT** lend account to other users



Only access booked equipment



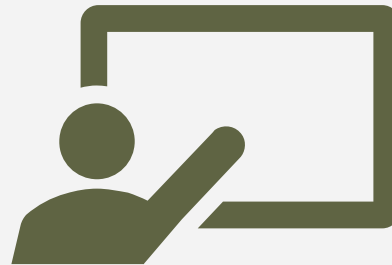
The last user of the day must turn off light and lock the doors



No Drinking And Eating



Turn off the machine right after use



Must attend training before using instrument



Always wear appropriate protective clothing and glasses when working in the laboratories.

User Responsibility



User should strictly follow the standard operation protocol (SOP)



Please operate the instruments carefully and gently



Keep workspace / sample preparation bench tidy and clean



Report any problem related to instruments



Write down experiment settings in logbook

If you are uncertain about performing a particular procedure, please contact Cryo-EM core staff.

General Safety



Staff has the right to query and, if necessary, stop any activity that is considered unsafe.



Avoid working alone during non-office hours in the laboratory.



Plan your work well before getting started.

General Safety



Fire Extinguisher

First Aid Box



Safety shower at corridor



Fire Escape Route and First Aid box.

No gloves on computer and areas accessible by others.



Dispose biological waste in designated bins.

Dispose sharps / glasses in sharp box.



Emergency Exit



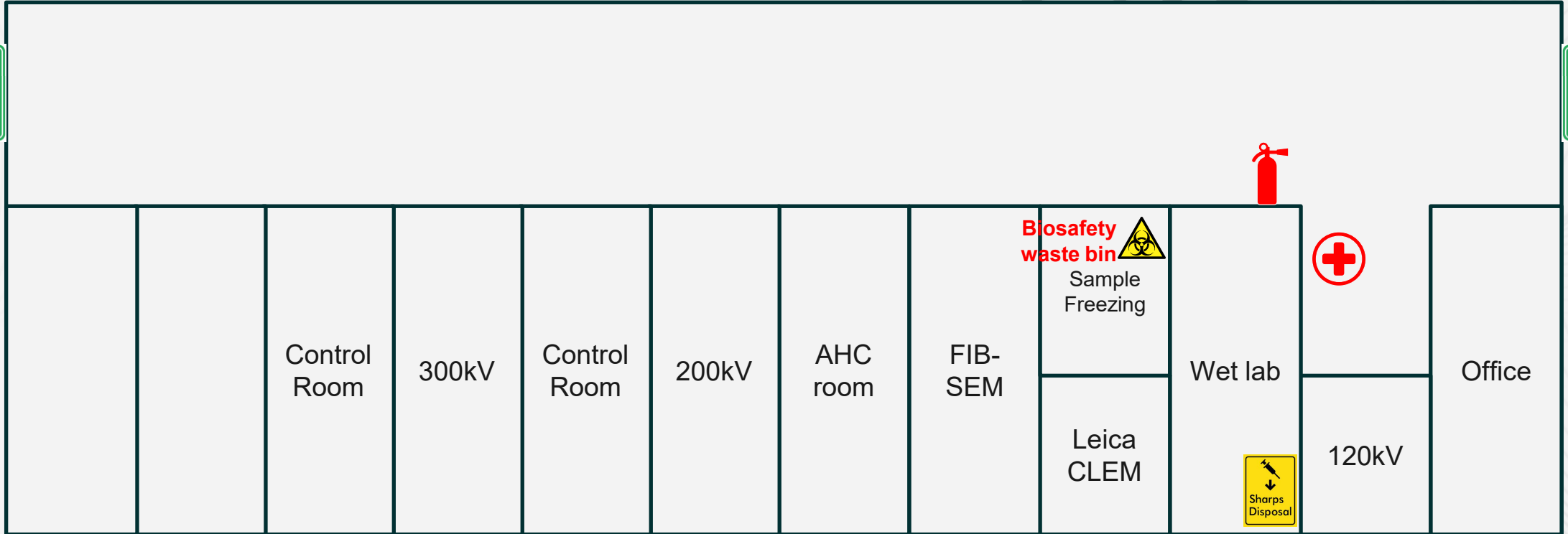
Biological waste bin



Sharp box



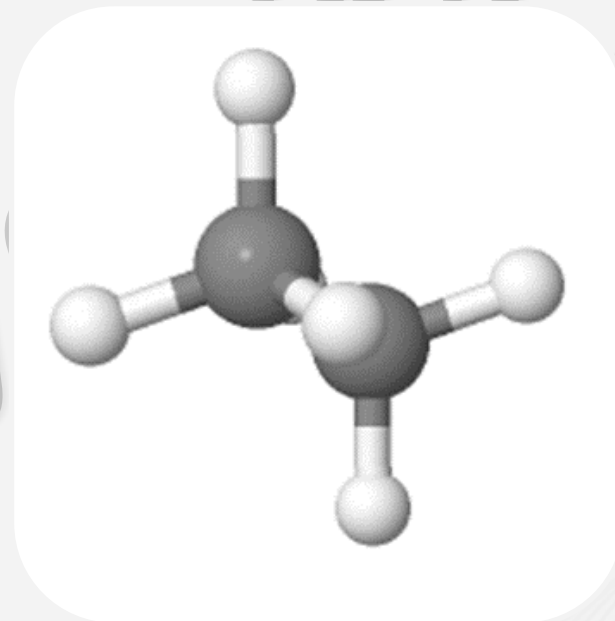
Safety (Floor Plan)



Safety (Gas)



Liquid Nitrogen



Ethane

Containers for Liquid Nitrogen



Dewar

Always put on the floor



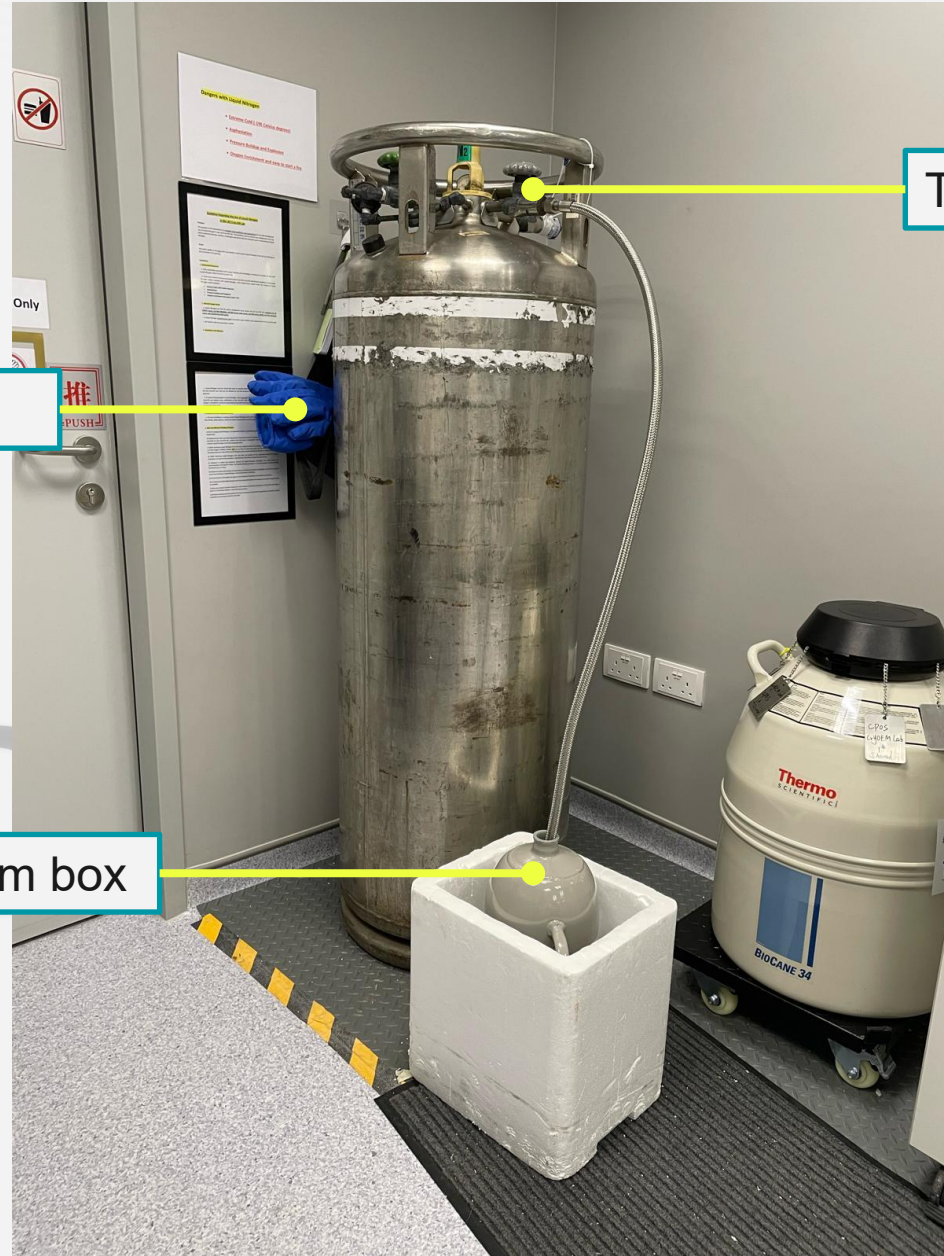
Stainless steel vacuum bottle (with loosen cover)



Foam Box

- **AM** Dewar: Available from 9 AM to 1 PM.
- **PM** Dewar: Available from 1:30 PM to 5:30 PM.
- **Night** Dewar: Available from 6 PM to 11 PM

Get Liquid Nitrogen



Use protective gloves

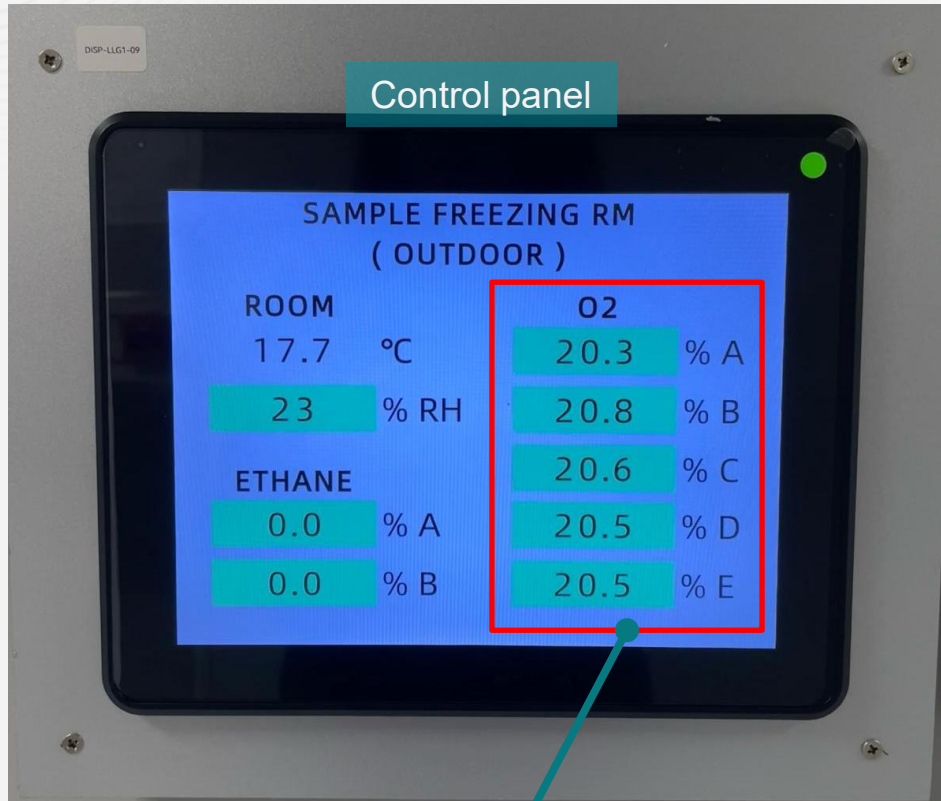
Turn on the switch

Put dewar in the big foam box

Protection



Oxygen Detector

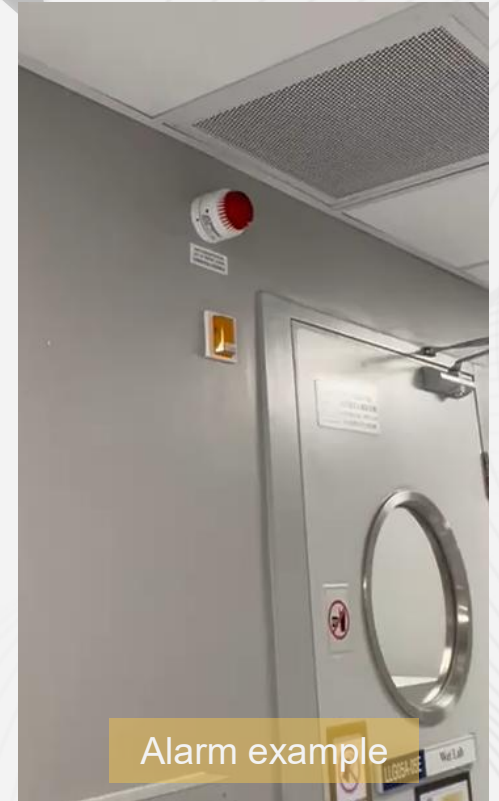


If the O₂ VALUE shows red or alarm in the room, user should leave the room immediately and report it to staff.

Ethane

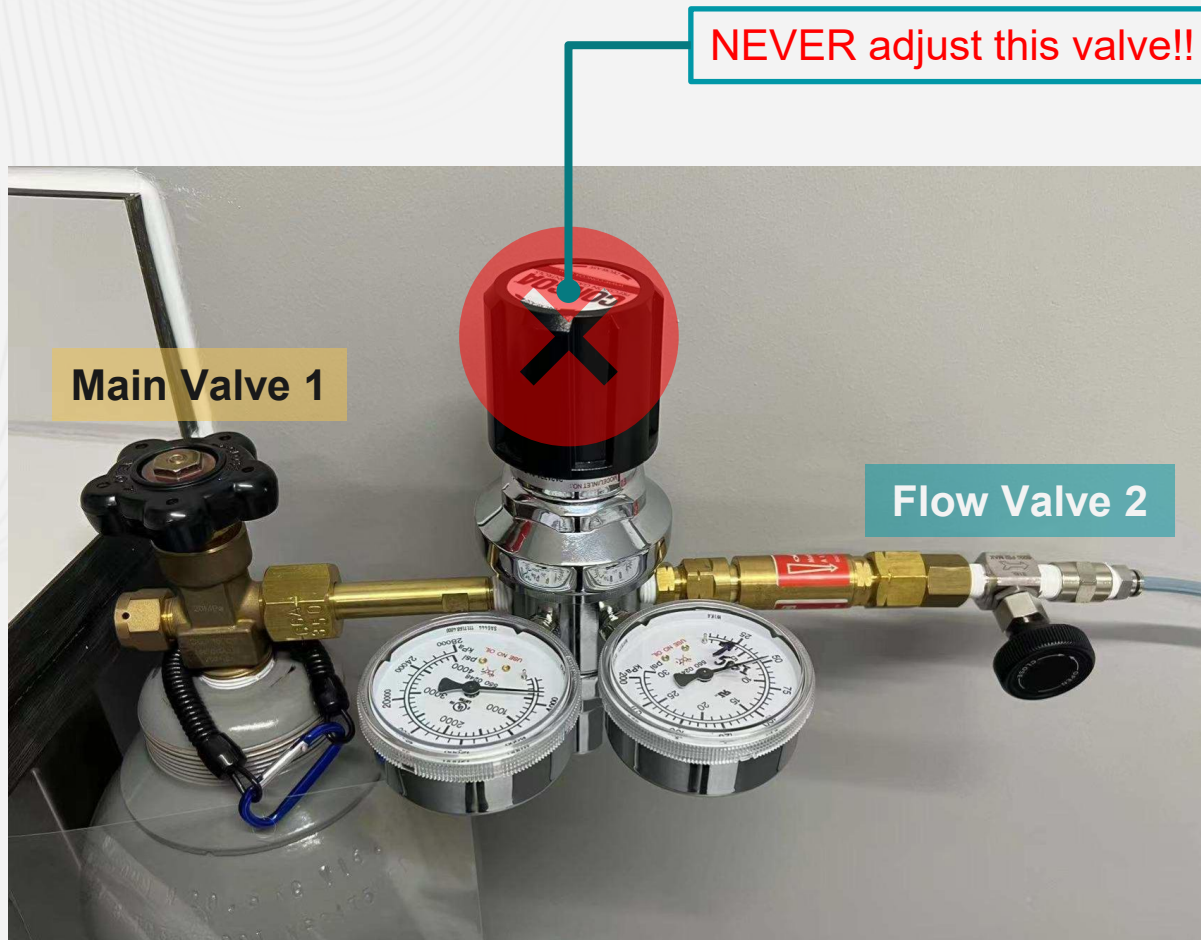
- Ethane is highly flammable and explosive.
- While working with an ethane cylinder, do not turn on/off any electric switch in the room.
- Wear safety goggles.
- **NEVER** point the ethane outlet tip toward yourself or others.
- Keep the ethane flow rate low to prevent splashing.
- If the ethane outlet tip is crowded by ethane ice or ethane liquid drop, close the valve, point the tip to a corner for 1-2 mins, and wait until the ice/liquid has fully disappeared in the air.
- The ethane tank should be closed tightly and double-checked after usage.
- If you see the light flash or hear an alarming sound, leave the room immediately.

Access will be terminated
if user violates this rule!



Alarm example

Open and Close Ethane Gas Flow



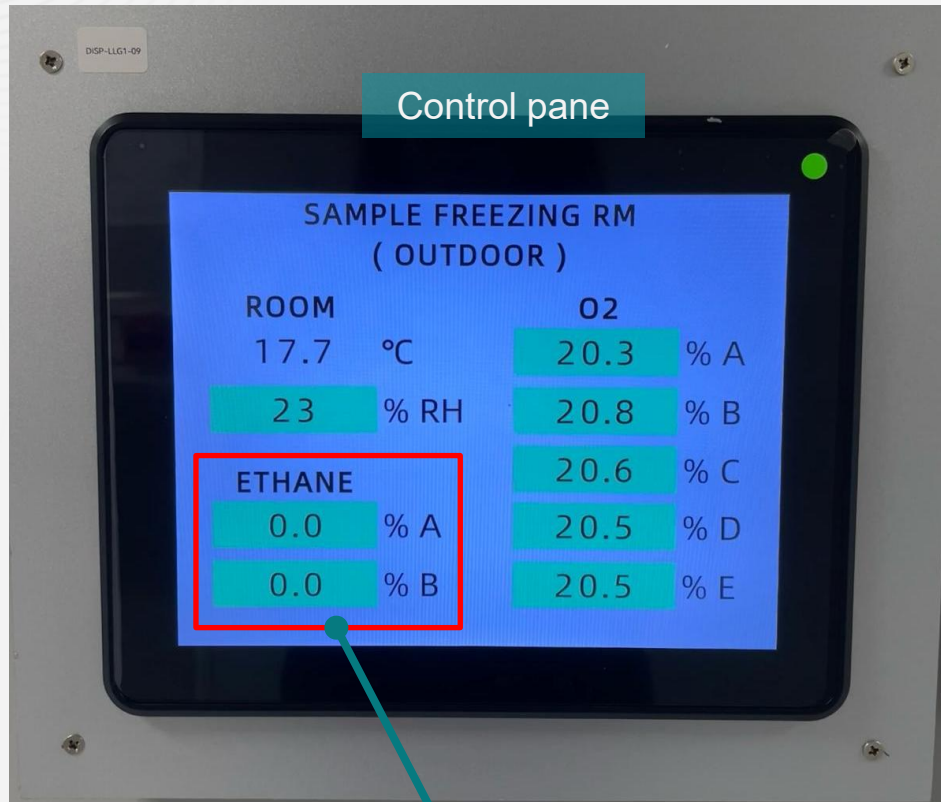
Open the gas flow:

1. Open **Main Valve 1**
2. Open **Flow Valve 2**
3. Adjust **Flow Valve 2** to control the flow rate

Close the gas flow:

1. Slow down the flow rate at **Flow Valve 2**
2. Close **Main Valve 1**
3. Drain the gas inside the tube
4. Close **Flow Valve 2**

Ethane Detector



If the ETHANE VALUE shows in red or alarm in the room, user should leave the room immediately and report it to core staff.

Getting Access to Cryo-EM Lab

1. Submit the following forms

a) Sample Safety Information e-Form (for new PI/ new project)

https://hku.au1.qualtrics.com/jfe/form/SV_4U6w9Xvill3F5gG

b) iLab Registration Form to enquiry.cpos@hku.hk and cc cryoem.cpos@hku.hk

<https://info.cpos.hku.hk/wp-content/uploads/2025/07/iLab-User-Registration-Form-202507.xlsx>

c) Training Application

Submit Training Request in iLab system

2. Training for Easi-glow, grid storage and GP2 / Vitrobot

a) Register your HKU card for access

b) Training

c) Self-booking (staff support is a must for 1st booking)

d) Email to cryoem.cpos@hku.hk to confirm the availability of staff

3. Training for 200kV (EPU operation)

a) HPC server account registration

b) Training (with sample prepared, submit Training Request in iLab system)

c) Self-booking x 1

4. 300kV self booking available

1. Contact staff for opening access of 300kV booking



Sample Information e-Form



iLab Registration Form

Each user should register one user account before processing to Imaging equipment training. If you are from cryoEM new PI group, please contact cryoEM team before submitting the following information to itsupport.cpos@hku.hk and cc cryoem.cpos@hku.hk for HPC account registration.

User Information

Preferred username*:

User full name:

User email:

Existing iLab user (Y/N)? :

If Yes, please provide your iLab login name (hku email address):

If No, please follow the below steps:

– Complete the [iLab user registration form](#)

– Submit the completed form to cpo@hku.hk and cc itsupport.cpos@hku.hk and cryoem.cpos@hku.hk

PI Information (For existing PI, please provide PI name only)

PI full name:

PI email:

Group Quota (20TB, 50TB, 100TB, 150TB):

CryoSparc admin account required (optional):

HKU billing account number:

* consists of letters and/or numbers (in 5-12 characters)

Charging

Training	HKU-Med	HKU
1 st training	Free	\$500
Re-training	\$700	\$1000

User should use the instrument **within 2 months** after training.

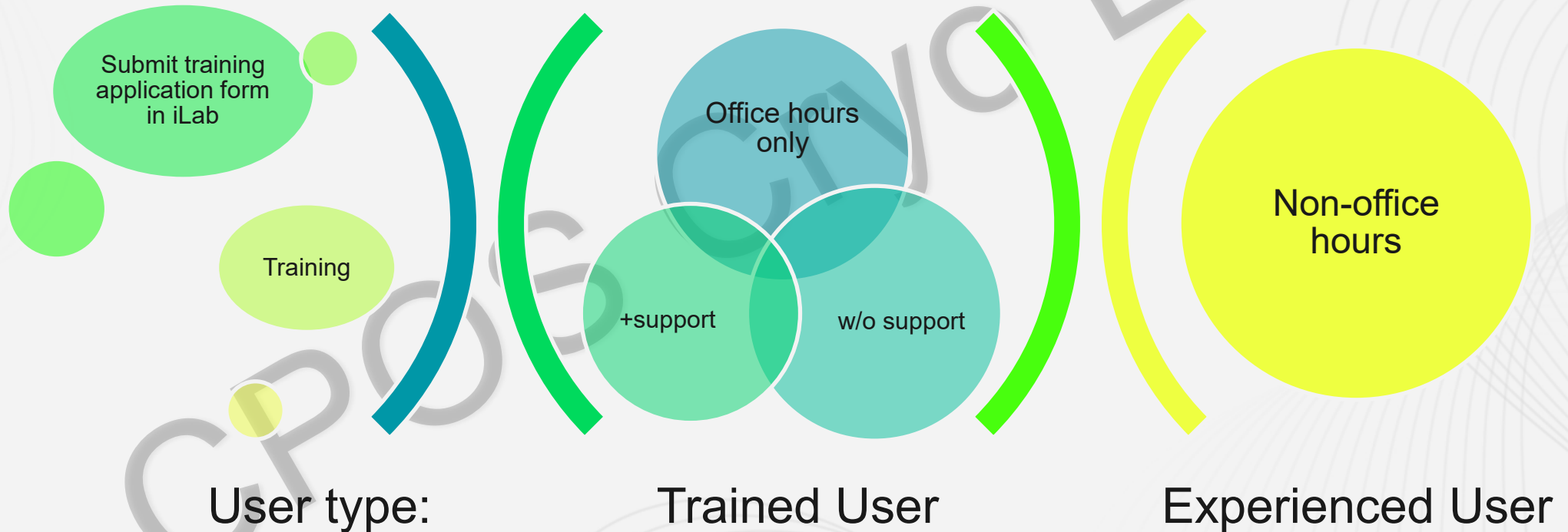
- If user has not booked within 2 months, **re-training** is required before using the instrument.

Usage	Min. usage / Session	HKU-Med	HKU
Sample Freezing with EasiGlow	2 hours	\$300 / 2 hours	\$330 / 2 hours

Charge will be based on **(1) booking** or **(2) usage** whichever longer. Same charge will be applied for no-show (not recommended).

User Type

- Do not transfer your booking session without notifying CPOS staff.
- Fill in correct information (e.g. usage time) in the logbook.



Upgrade from Novice to Experience

If trained user does not make any booking within **2 years**, the right will be removed.

User needs to contact staff for review and resume status.

Trained user

Apply training in iLab

Experienced user training

After 5 working days

Evaluation

Pass

Experienced user

Fail

- Have booked the instrument **over 10 times within 6 months**
- No misbehavior record in last 3 months (at least 3 bookings)

If experienced user does not make any booking within **6 months**, user will be downgraded as trained temporarily.

User needs to contact staff for review and resume status.

Apply again **after 3 months**





Easi-glow

Grid discharge treatment

LKS Cryo-EM Laboratory
(Wet Lab)

Imaging and Flow
Cytometry Core
(L601, 6/F)

Easi-Glow (In Cryo-EM Lab)

- Glow discharge treatment → mild plasma clean → turns the grids to be hydrophilic
- Discharge the grid just before vitrification
- The protocol should be optimized
- **NEVER** click "SAVE PROGRAM SETTINGS"
- Orientation of the grid: The side of carbon film should face upward

Standard Setting

Pressure	0.39mBar
SET	15mA
GLOW	00:01:00
HOLD	00:00:10
Polarity	Negative

User should record their own setting after optimization

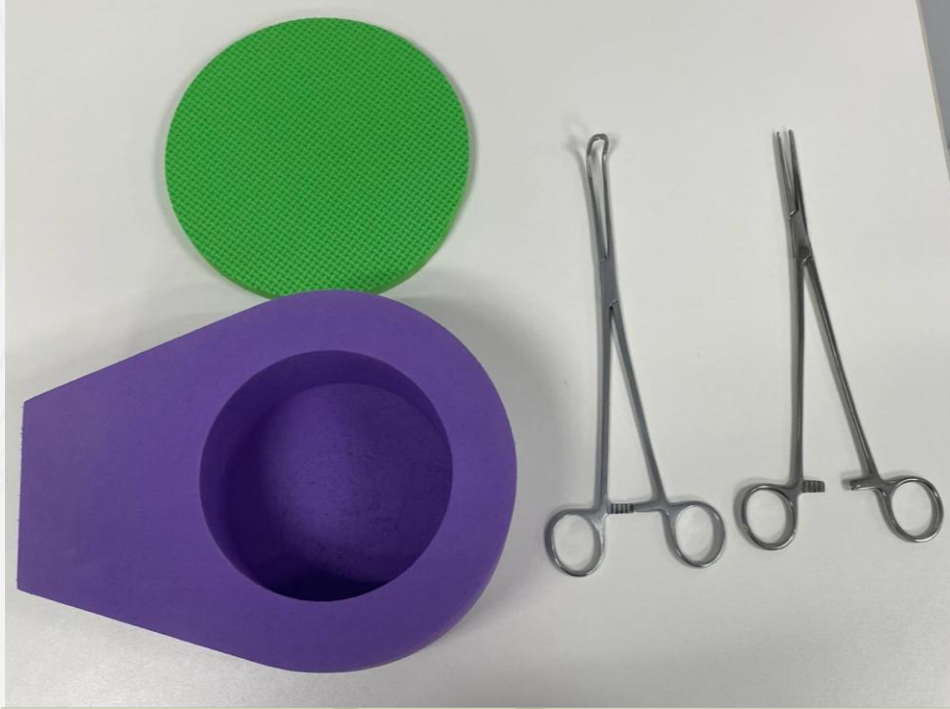




Grid storage



Grid storage

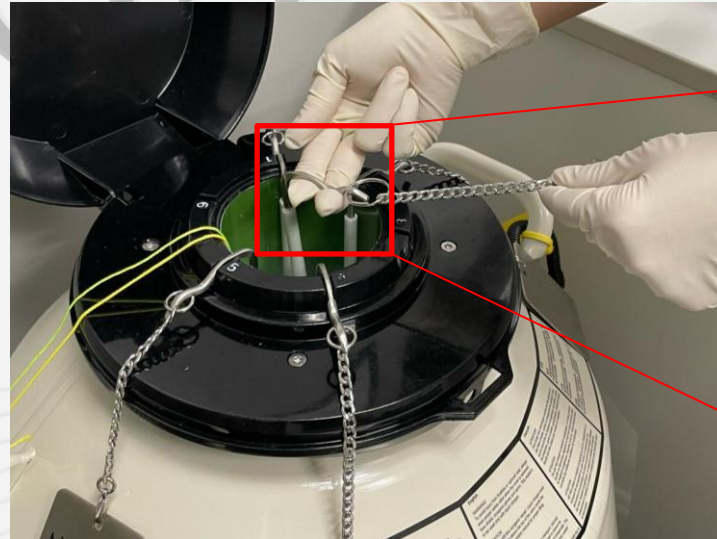
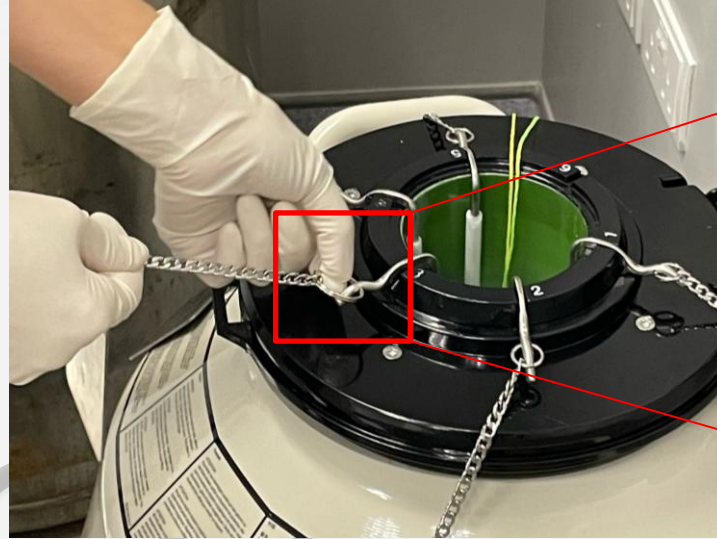


1

Tools

2

Do **NOT** take out the puck system using the o ring



3

Put the puck system on the desk

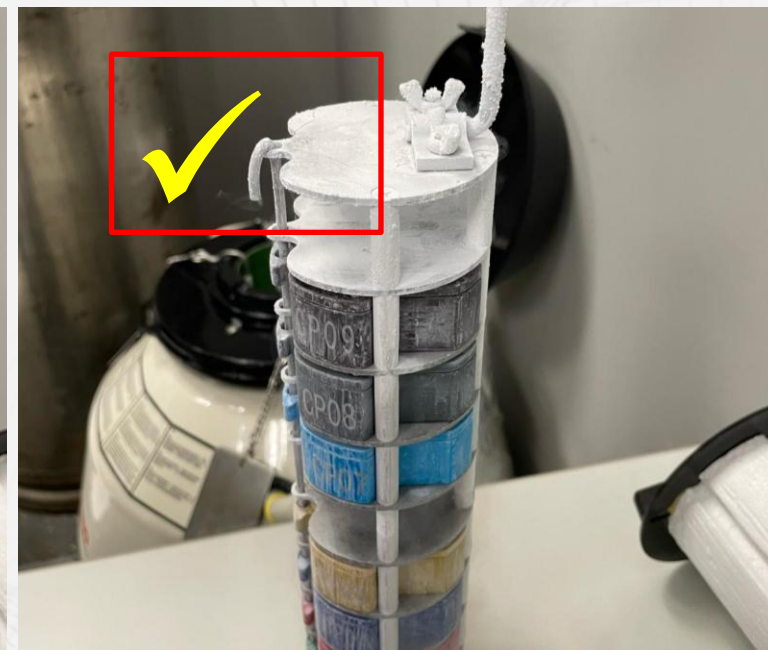
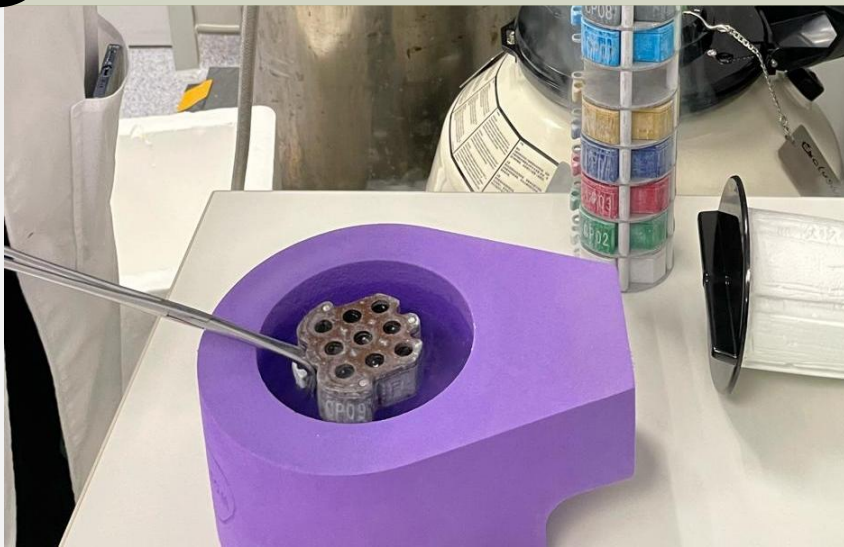


4

Take out the pin using tweezers

5

Use the holder to place the puck into liquid nitrogen foam box.





Vitrobot

Vitrification

Booking Sessions

Office Hour	
Session 1	10:00 am – 12:00 n
Session 2	1:30 pm – 3:30 pm
Session 3	3:30 pm – 5:30 pm

Non-Office Hour <small>(Experienced user only)</small>	
Session 4	6:00 pm – 8:00 pm
Session 5	8:00 pm – 10:00 pm

Booking Policy

- Open for 28 days booking
- Card access: User can access the instrument on the booking date

Agilent CrossLab | iLab Operations Software

Li Ka Shing Faculty of Medicine Centre for PanorOmic Sciences > View Schedule

Vitrobot 1 Receive cancellation notifications

Week (7 Days) Sun, 08 Sep - Sat, 14 Sep 2024 Hong Kong

	Sun, 08 Sep	Mon, 09 Sep	Tue, 10 Sep	Wed, 11 Sep	Thu, 12 Sep	Fri, 13 Sep	Sat, 14 Sep
10:00 AM		10:00 AM - 12:00 PM	Morning and Afternoon session	Morning and Afternoon session	Morning and Afternoon session	Morning and Afternoon session	
11:00 AM							
12:00 PM							
01:00 PM		Morning and Afternoon session	01:30 PM - 03:30 PM Kenneth Li	Morning and Afternoon session	01:30 PM - 03:30 PM Luo Jingyi	01:30 PM - 03:30 PM Pui Yi Trista KWAN Morning and Afternoon session / Price: \$150/hr (Experienced) test	
02:00 PM							
03:00 PM					03:30 PM - 05:30 PM Luo Jingyi		
04:00 PM							
05:00 PM							
06:00 PM		Morning and Afternoon session	Morning and Afternoon session	Morning and Afternoon session	Morning and Afternoon session		
07:00 PM							
08:00 PM							
09:00 PM							

General | Comments | Contacts

Reservation details | **Required forms**

For: Vitrobot 1 - Morning and Afternoon session \$150.00/hr (Experienced) - My Reservation
Lab: CHAN_Agnes (HKU) CPOS - Test
Created on: September 10, 2024 09:42

Minimum booking duration is 2 hours. For non-office hours bookings, please contact us at cryoem.cpos@hku.hk or 3910-2938 during office hours.

1st Session: 10:00 - 12:00 (Mon - Fri)
2nd Session: 13:30 - 15:30 (Mon - Fri)
3rd Session: 15:30 - 17:30 (Mon - Fri)
4th Session: 18:00 - 20:00 (only available on Mon-Thu for certified experienced users)
5th Session: 20:00 - 22:00 (only available on Mon-Thu for certified experienced users)

- Any reservation must be made 7 days in advance.
- Each user/PI group can only reserve up to 12 hours (or 6 sessions) at any given time.
- Cancellation policy: if cancellation is made within 24 hours of the reservation start time, there will be cancellation fee of 50% of the reservation total.
- Contact us for custom and urgent bookings.
- Total charge will appear after approval by Cryo-EM admin.

Event Notes: test note visible to anyone

Times

Scheduled	Start	End
Sep 13 2024	01:30 PM	03:30 PM

This event can be modified or deleted before 01:30 PM HKT on Sep 12, 2024

You are affected by the following capping rules

Scope	Booked amount	Capped amount
Vitrobot 1	2.0 hours	12.0 hours

Use and cost of reservation

Duration	Effective Rate	Amount	Use Type
2.0 hours	\$150.00	\$300.00	Morning and Afternoon session
2.0 hours			Basic Rate
Total Cost		\$300.00	HKUMed

Additional charges for this event

Please add other charges as needed
Final total charges will be adjusted according to actual usage.

Payment information

Please enter the HKU billing account number

%	HKU billing account number	Amount
100.0%	test	

100.0% Total Allocated

Use the same payment information for all add-on charges

Invite additional people to this event by email

Please enter a comma separated list of valid email addresses

PI / other user's email

Save Reservation | Cancel Changes | Delete Reservation

Cancellation Policy

- Before 24 hours → Free cancellation
- Within 24 hours → 50% charge
- Session starts → 100% charge

System Information

Please enter the HKU billing account number

%	HKU billing account number	Amount
1 100.0 %	test	

100.0% Total Allocated

Use the same payment information for all add-on charges

Before 24 hours

System Information

Please enter the HKU billing account number

%	HKU billing account number	Amount
1 100.0 %	test	

100.0% Total Allocated

Use the same payment information for all add-on charges

Within 24 hours
(Charge 50%)



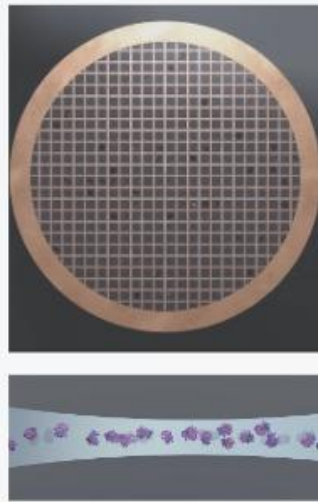
Canceling this event will result in a cancellation fee of \$150.00

01

AQUEOUS
SPECIMEN

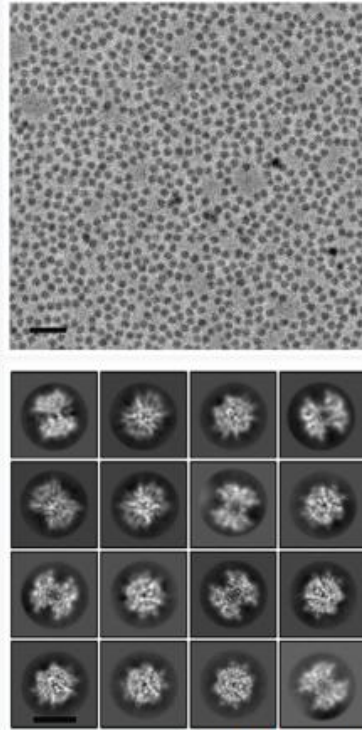
02

VITRIFICATION



03

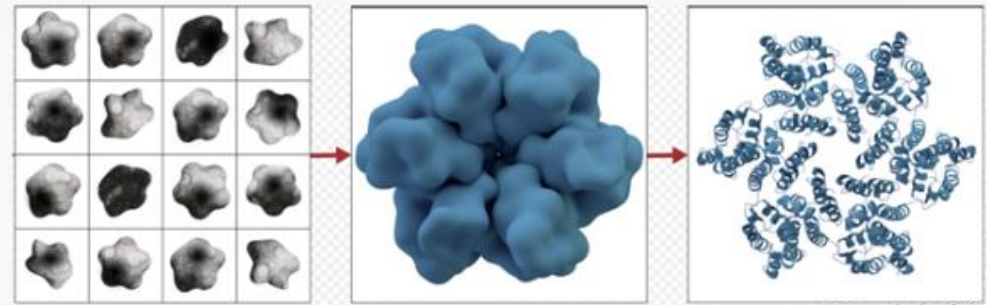
IMAGING



(X FAN et al., 2019)

04

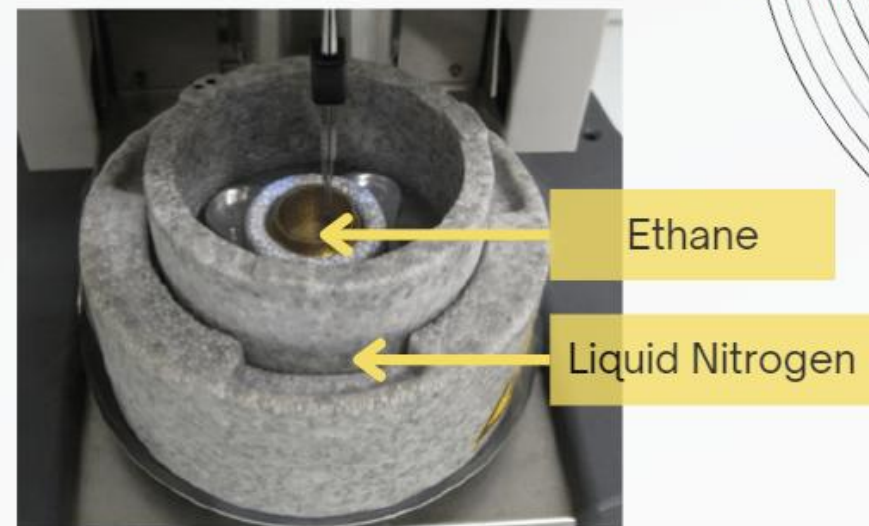
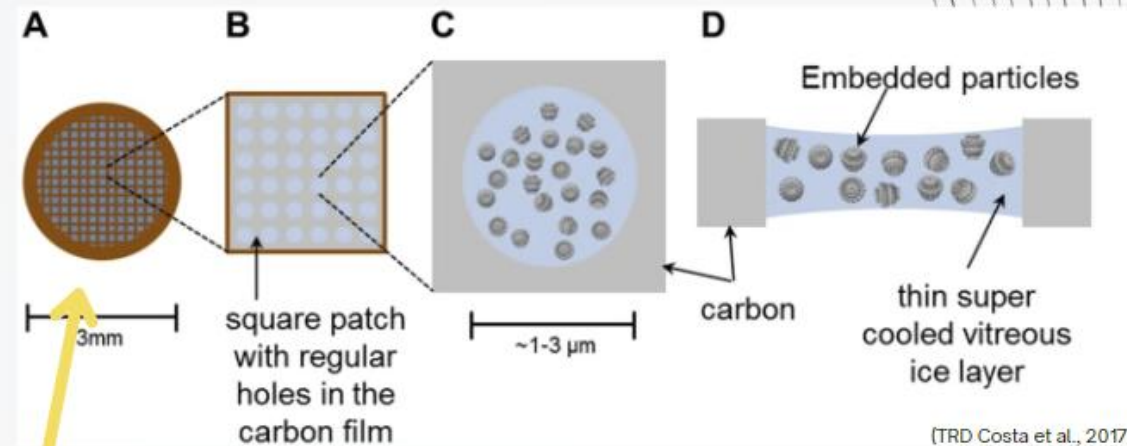
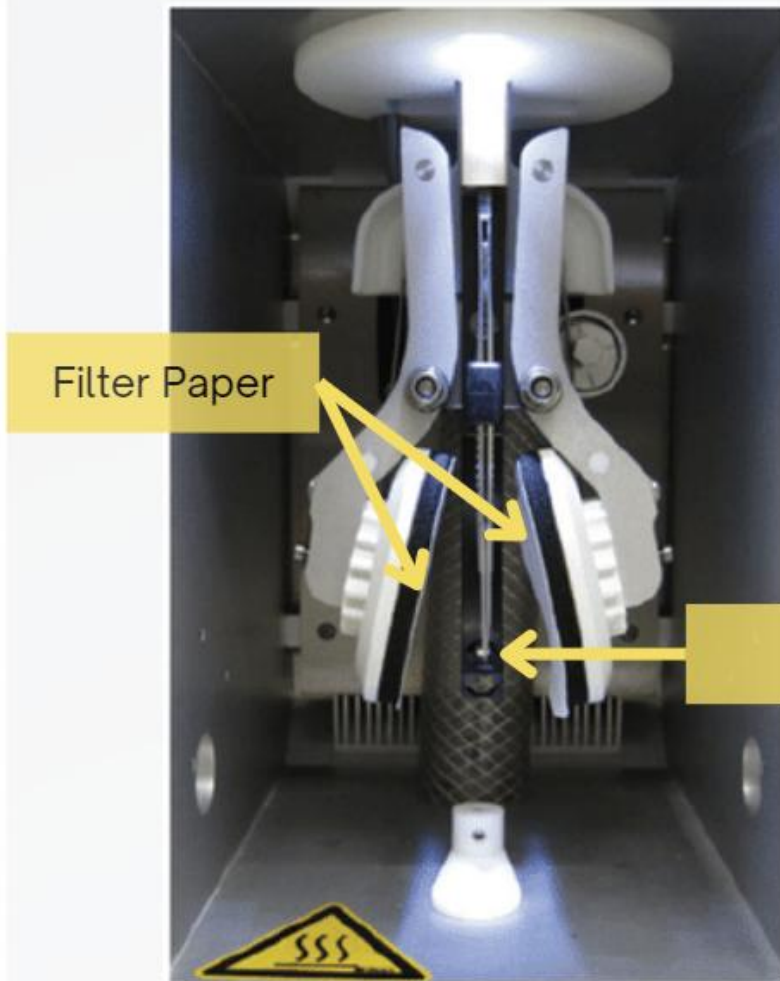
SINGLE PARTICLE
ANALYSIS / TOMOGRAPHY



(HZ Carreras, 2023)

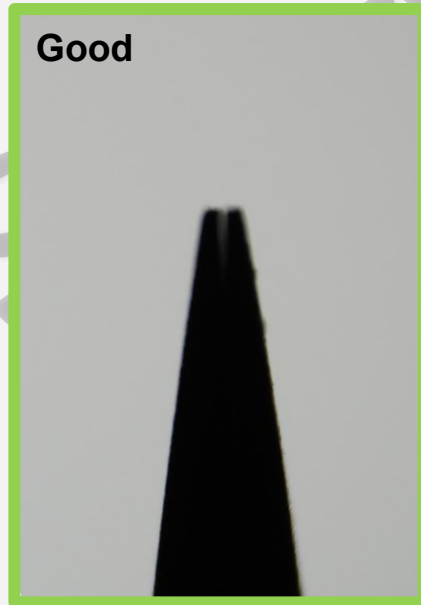
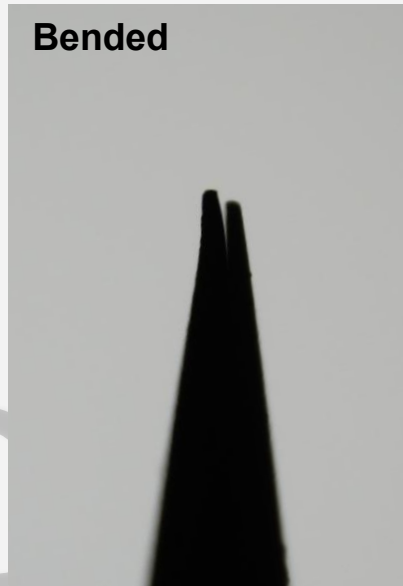
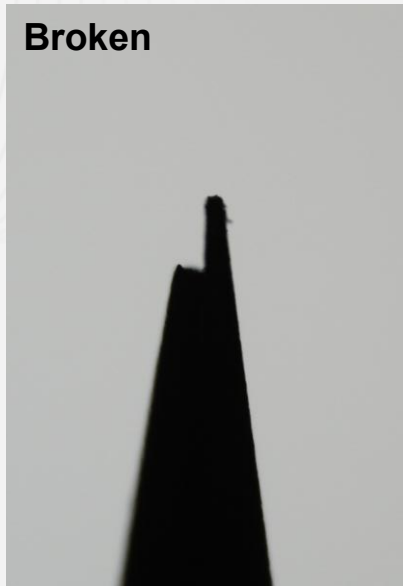
Vitrobot

VITRIFICATION



Special Notice

- Tip of the tweezers is very sharp.
- Only for grids.



Please handle the tweezers with extra caution.
It may be charged if they are broken.

Steps

1. Preparation of Vitrobot
2. Preparation of Grid
3. Condensation of Ethane
4. Sample Vitrification
5. System Shut-down

CPOS Cryo EM