



CPOS cry

# 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



**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

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Core

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Flow Cytometry  
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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](mailto:cryoem.cpos@hku.hk)

Tel: 3910-2938

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

CPOS

enquiry.cpos@hku.hk | 3910-6600

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

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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



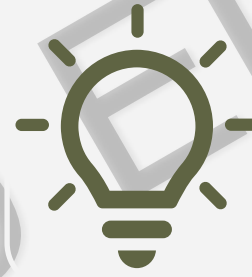
The Core is under surveillance **24/7**



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.



Sharps Disposal

Emergency Exit



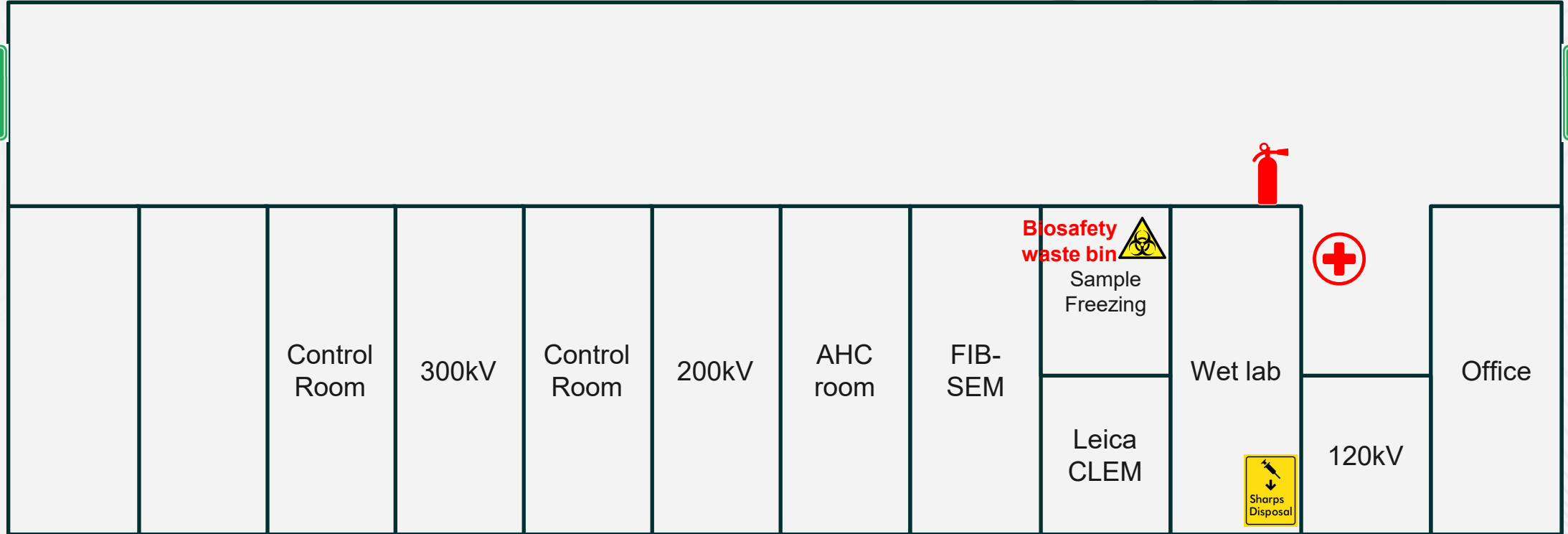
Biological waste bin



Sharp box



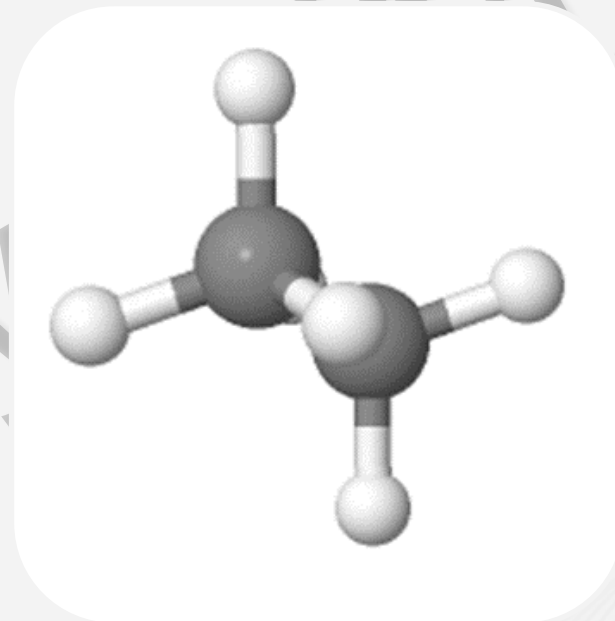
# Safety (Floor Plan)



## Safety (Gas)



Liquid Nitrogen



Ethane

# Containers for Liquid Nitrogen



Dewar

Always put on the floor



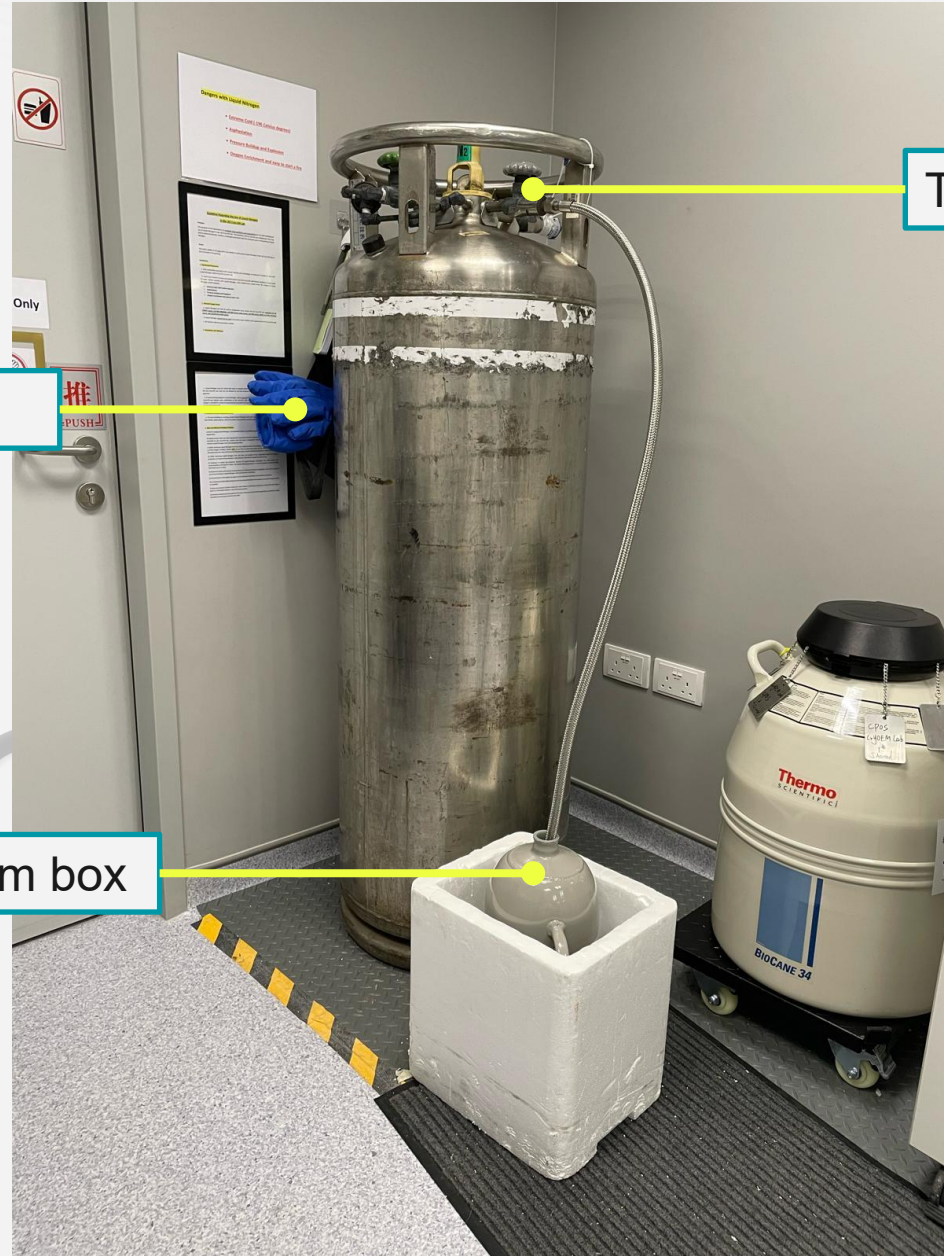
Stainless steel vacuum bottle (with loosen cover)



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

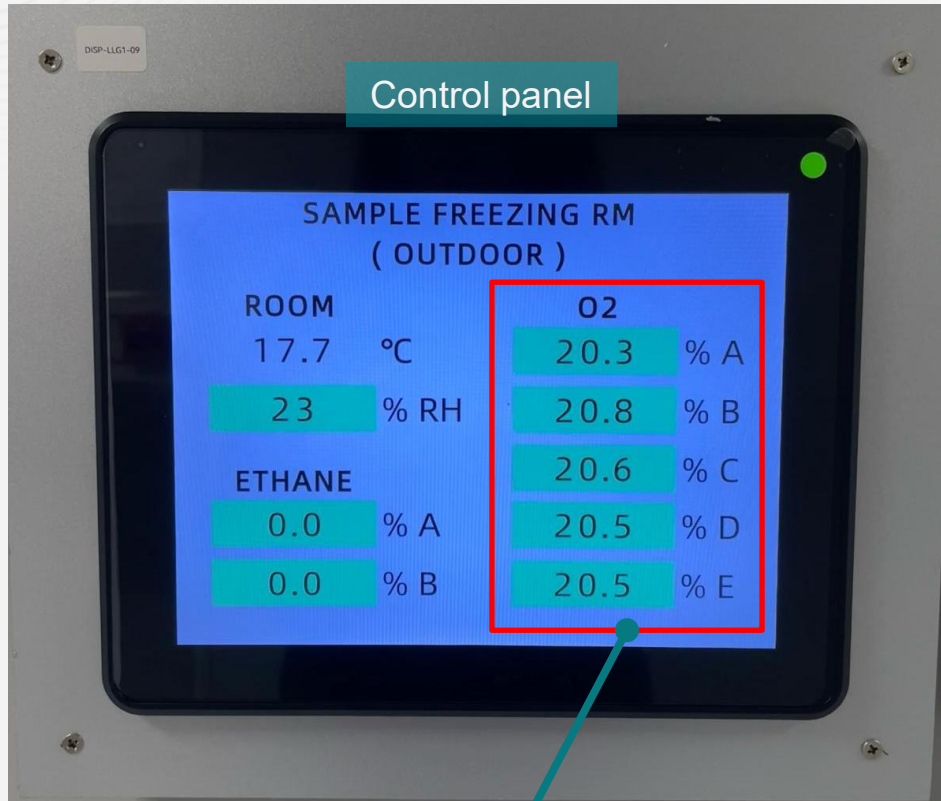
Turn on the switch

Put dewar in the big foam box

# Protection



# Oxygen Detector



If the O<sub>2</sub> 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 other

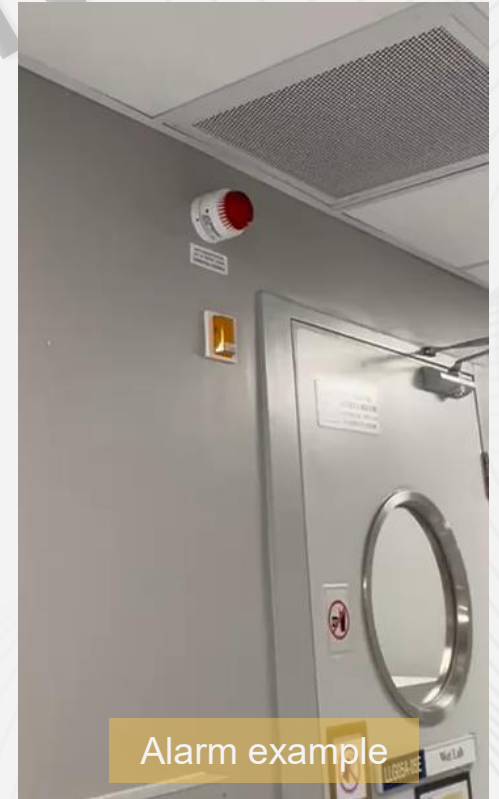
Access will be terminated if user violates this rule!

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.

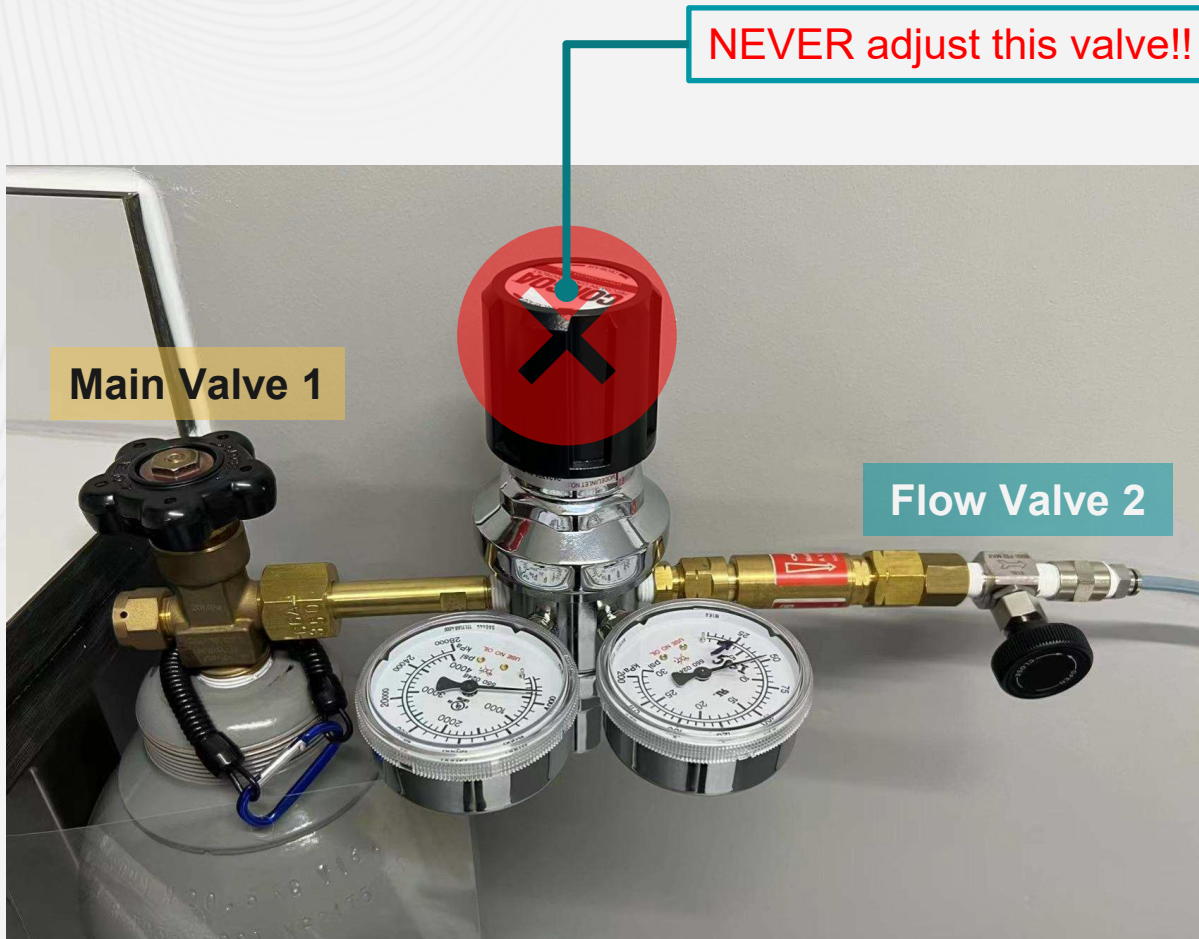
The ethane tank should be closed tightly and double-checked after usage.

If you see the light flash or hear an alarming sound, leave the room immediately.



Alarm example

# Open and Close Ethane Gas Flow



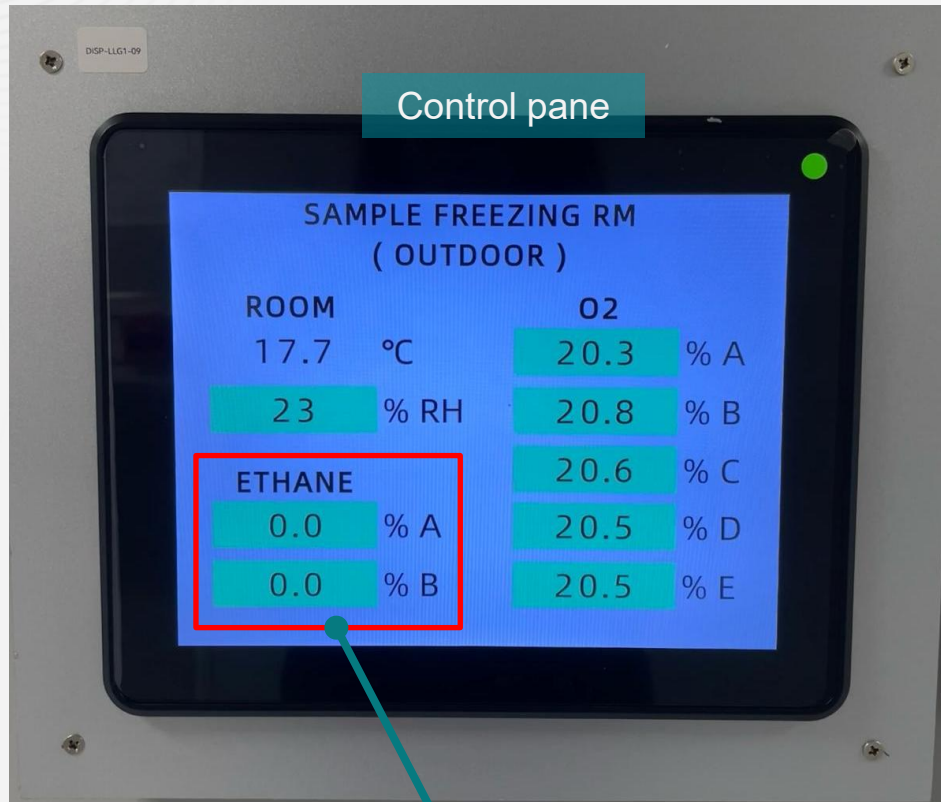
## Open the gas flow:

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](https://hku.au1.qualtrics.com/jfe/form/SV_4U6w9Xvill3F5gG)

b) iLab Registration Form to [enquiry.cpos@hku.hk](mailto:enquiry.cpos@hku.hk) and cc [cryoem.cpos@hku.hk](mailto:cryoem.cpos@hku.hk)

<https://info.cpos.hku.hk/wp-content/uploads/2025/07/iLab-User-Registration-Form-20250701.pdf>

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 1<sup>st</sup> booking**)

d) Email to [cryoem.cpos@hku.hk](mailto:cryoem.cpos@hku.hk) to confirm the availability

## 3. Training for 200kV (EPU operation)

a) HPC server account registration

b) Training (with sample prepared, submit Training Request)

c) Self-booking x 1



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](mailto:itsupport.cpos@hku.hk) and cc [cryoem.cpos@hku.hk](mailto: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](mailto:cpo@hku.hk) and cc [itsupport.cpos@hku.hk](mailto:itsupport.cpos@hku.hk) and [cryoem.cpos@hku.hk](mailto: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 <sup>st</sup> training	Free	\$500
Re-training	\$700	\$1000

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

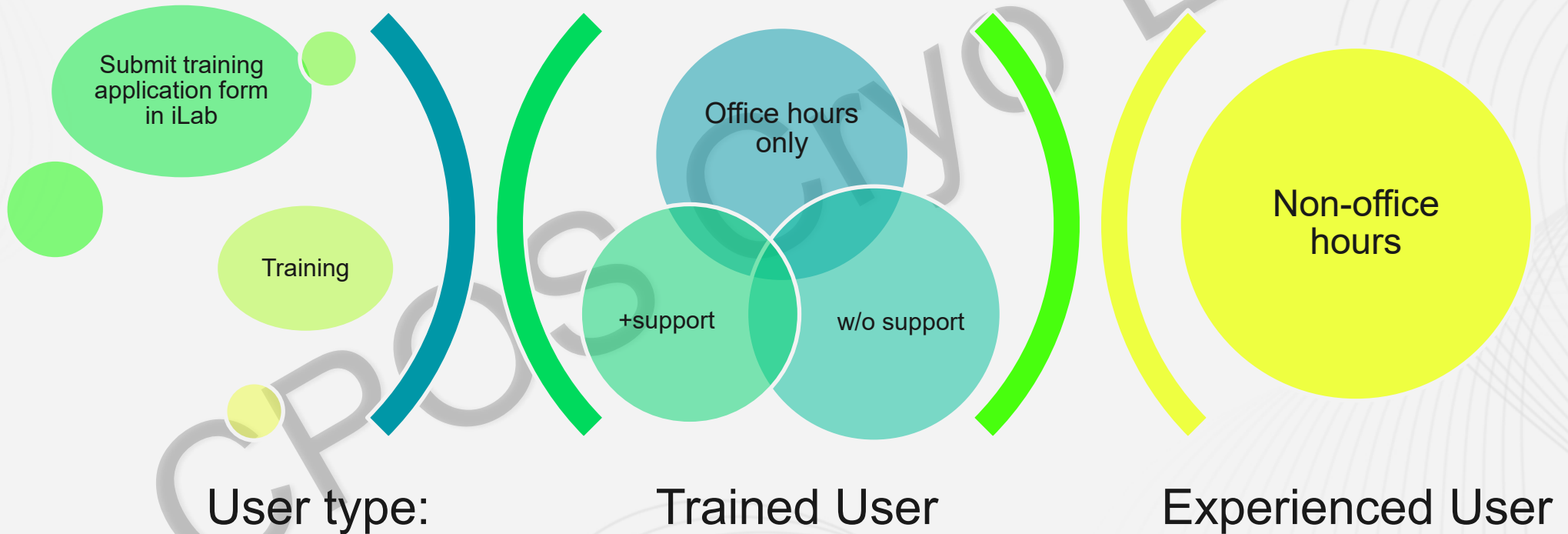
- If user has not booked within 2 months, **re-training** is required before using the instrument.

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**

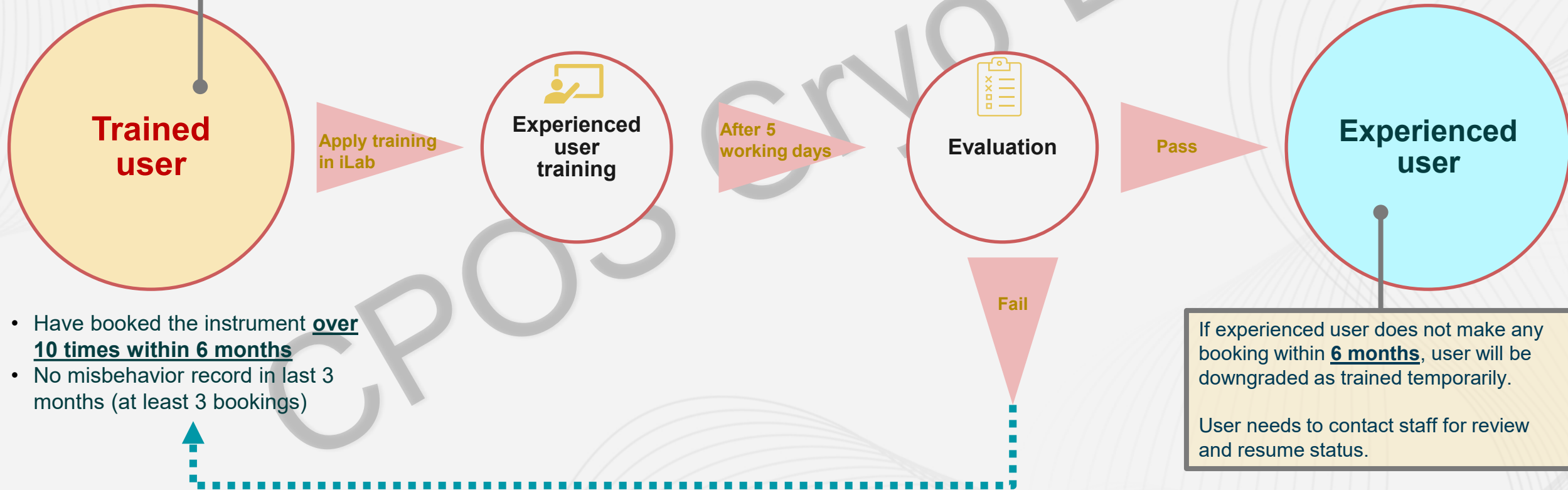
Fail

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.

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

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

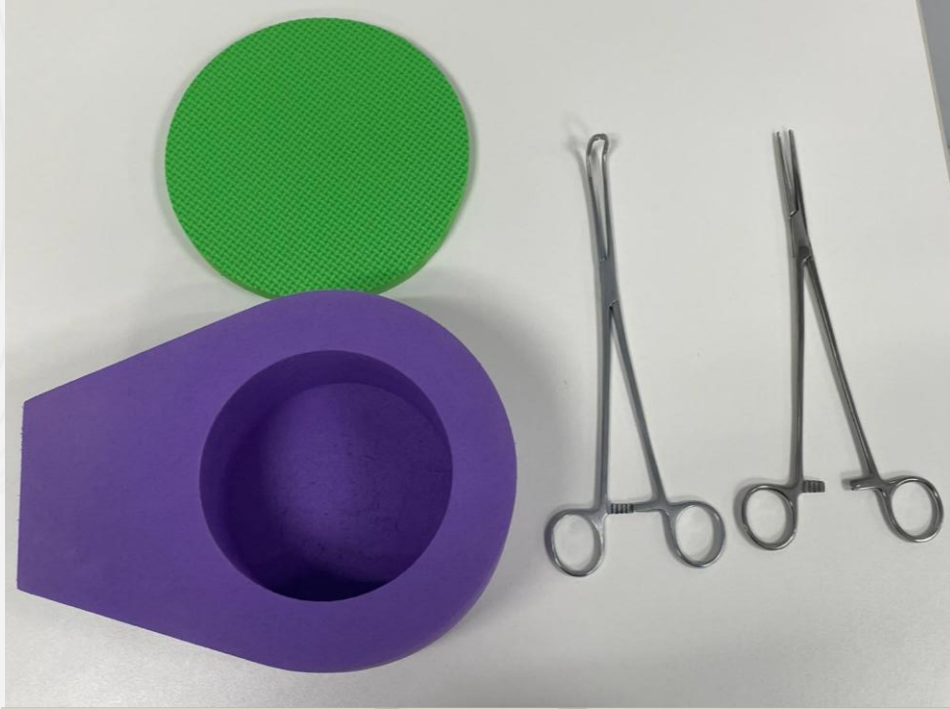




# Grid storage



# Grid storage

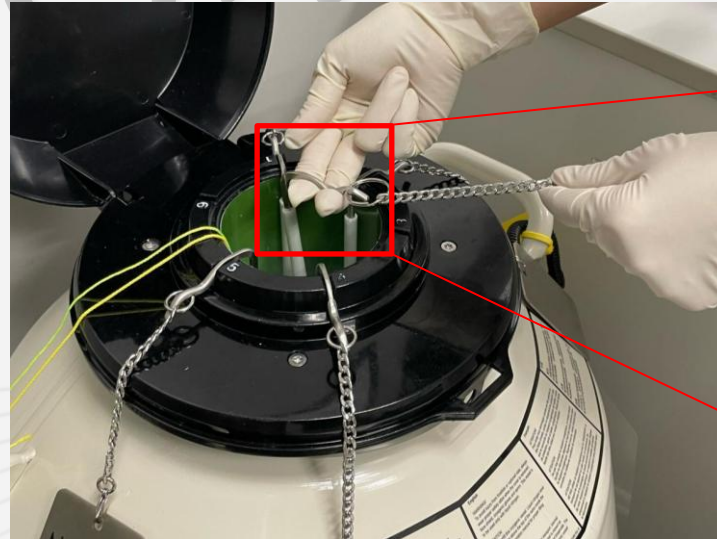
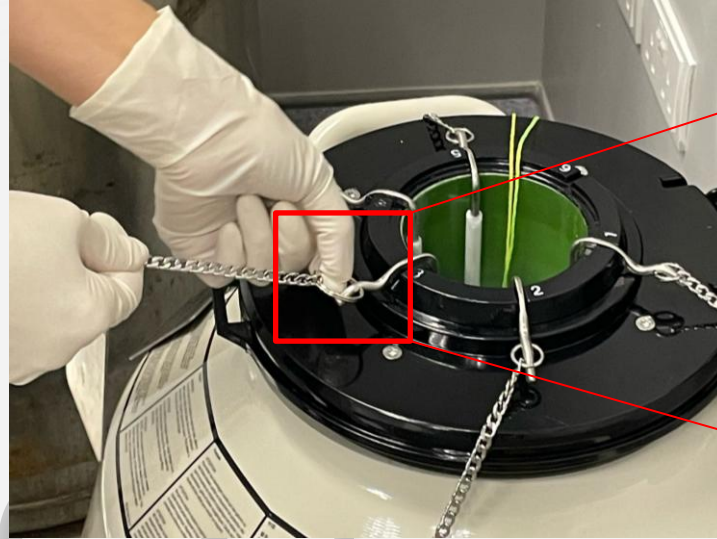


1

Tools

2

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



3

Put the puck system on the desk

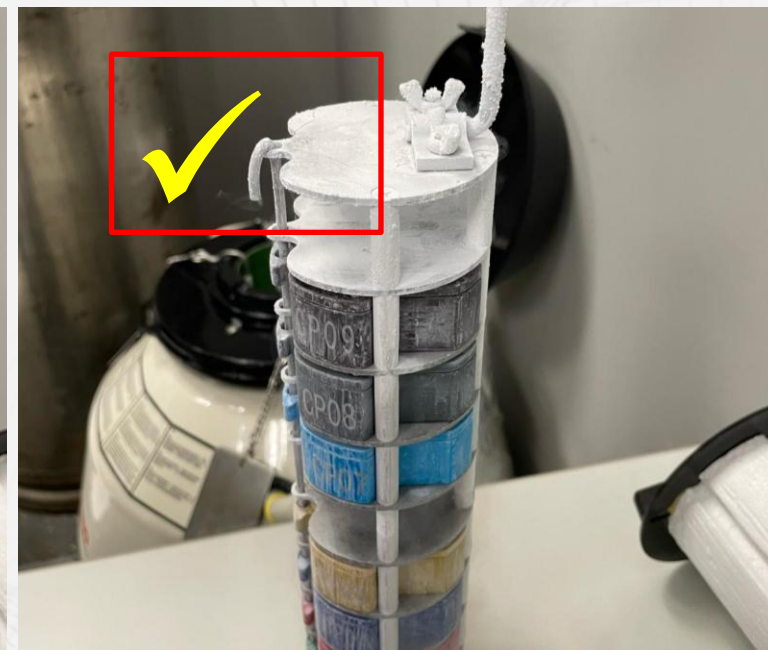
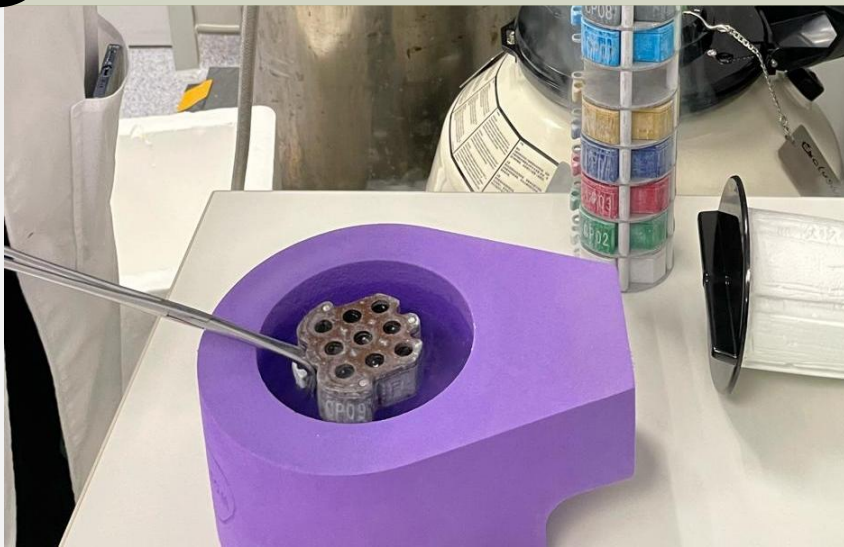


4

Take out the pin using tweezers

5

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



# Leica EM GP2

Vitrification

## Booking Sessions

### Office Hour

Session 1	10:00 am – 12:00 n
Session 2	2:00 pm – 4:00 pm

### Non-Office Hour (Experienced user only)

Session 3	6:00 pm – 8:00 pm
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# Booking Policy

Open for 28 days booking

Card access: User can access the instrument on the booking date

Drag to book the whole session (2 hours)

**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.  
1<sup>st</sup> Session: 10:00 - 12:00 (Mon - Fri)  
2<sup>nd</sup> Session: 13:30 - 15:30 (Mon - Fri)  
3<sup>rd</sup> Session: 15:30 - 17:30 (Mon - Fri)  
4<sup>th</sup> Session: 18:00 - 20:00 (only available on Mon-Thu for certified experienced users)  
5<sup>th</sup> 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] [charge and display on the invoice]

**Times**

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

Amount	HKU billing account number
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

List the users attending the session.

Cancellation

Billing Account

PI / other user's email

# Cancellation Policy

Before 24 hours → Free cancellation

Within 24 hours → 50% charge

Session starts → 100% charge

Payment 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

Invite additional people to this event by email

Please enter a comma separated list of valid email addresses

Payment 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

Invite additional people to this event by email

Please enter a comma separated list of valid email addresses



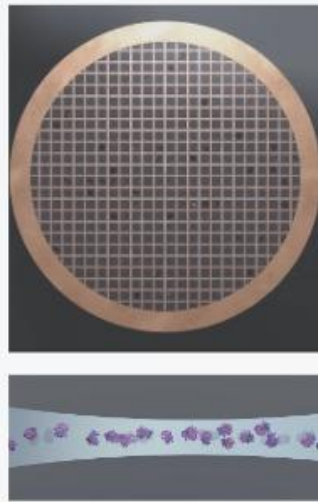
Cancel this event will result in a cancellation fee of \$150.00

01

AQUEOUS  
SPECIMEN

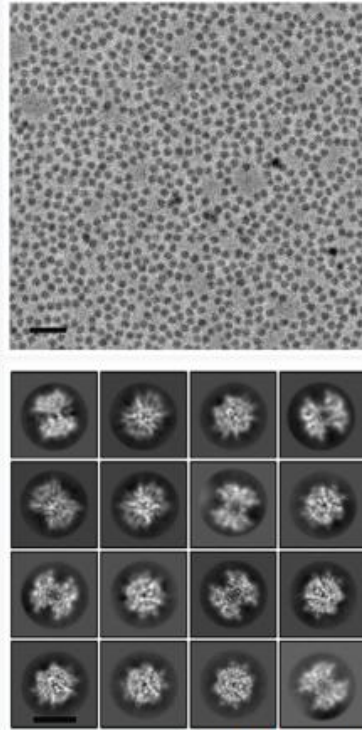
02

VITRIFICATION



03

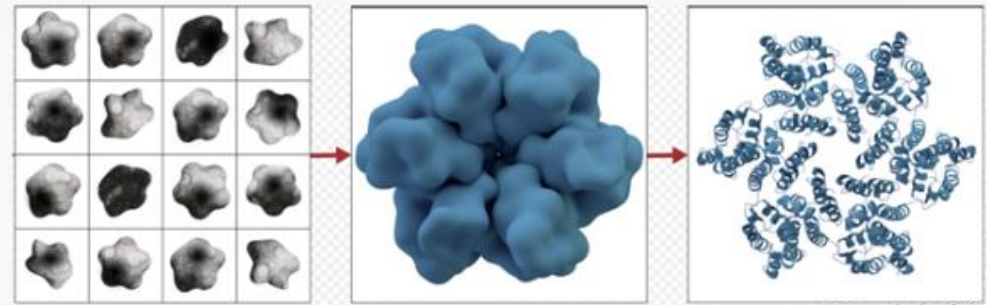
IMAGING



(X FAN et al., 2019)

04

SINGLE PARTICLE  
ANALYSIS / TOMOGRAPHY



(HZ Carreras, 2023)

05

3D MAPPING &  
RECONSTRUCTION

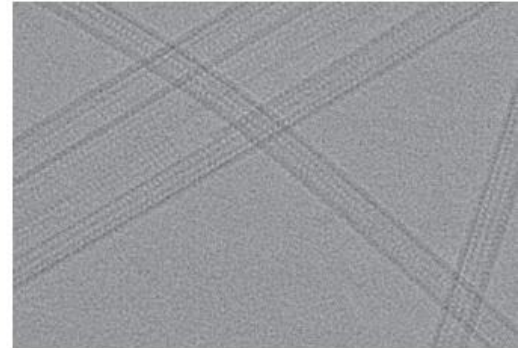
## Special Notice

Tip of the tweezer is very sharp.



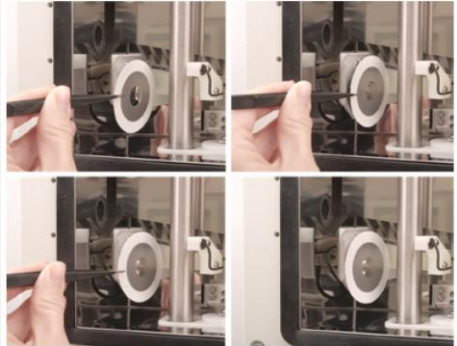
# Steps

1. Preparation of GP2
2. Preparation of Grid
3. Set-up of parameters and test
4. Condensation of Ethane
5. Sample Vitrification
6. Shut-down and Bake Out

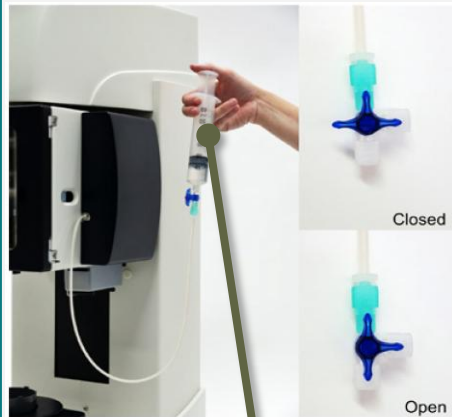


# Preparation of GP2

Place Filter Paper



Fill Humidifier with Water



1<sup>st</sup> fill: 90mL  
Refill: 30mL

Empty Drip Tray

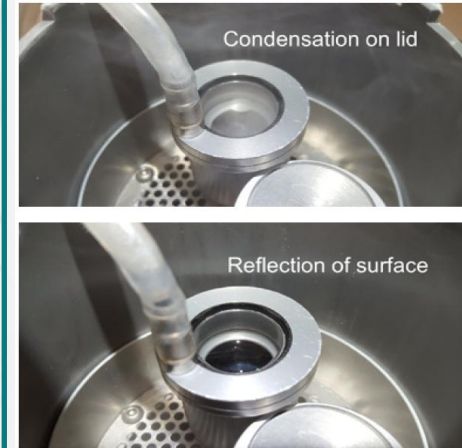


Freeze Chamber



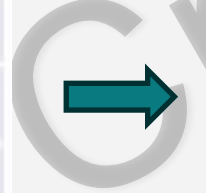
Cover the ethane container

Fill Ethane



# Set-up of parameters and test

The screenshot shows the main control interface of a Leica Microsystems system. At the top, there is a status bar with the Leica logo, a warning for a 'Humidifier empty' (refill 30ml), a program ID '37-95', and a red '5' indicating a program modification. Below this, the interface is divided into several sections. On the left, there are status indicators for 'Chamber' (Temperature: 38°C, Rel. humidity: 95%), 'Blots' (5), and 'Cryogen' (Temperature: -180°C, LN<sub>2</sub> level at 80%). In the center, there are large buttons for 'LOAD FORCECEPS', 'LOAD SPECIMEN', 'BLOT', 'PLUNGE', and 'TRANSFER'. On the right, there are smaller buttons for 'ROTATE BEFORE', 'ROTATE HOME', 'MULTIPLE BLOTS', 'HOME BLOTS', and 'ROTATED BLOTS'. At the bottom, there are navigation buttons for 'Settings', 'Programs', 'Rotation', and a red '3' indicating a rotation delay. The time is 13:47 on 2017.11.03.



The screenshot shows the 'Environment' settings screen for program '37-95'. The top bar includes the program ID, a date range ('Last loaded Nov 03, 2017 - Last modified Oct 24, 2017 18:08'), and an 'Edit' button. The main content is organized into sections: 'Environment', 'Load Specimen', 'Blot', and 'Plunge/Transfer'. The 'Environment' section is highlighted with a red box and contains the following parameters:

Parameter	Actual	Set	Control	Program
Chamber Temperature	28°C	37°C	Set (ON)	37°C
Chamber Rel. humidity	54 %	95 %	Set (OFF)	95 %
Chamber Window heater	-	55%	+ -	100 %

The 'Plunge/Transfer' section is highlighted with a blue box and contains:

Parameter	Actual	Set	Program	
Cryogen Temperature	-2°C	-180°C	Set	-180°C
Cryogen GN2 flow	-	100%	+ -	100 %

At the bottom, there are navigation buttons for 'Main', 'Programs', 'Save', and a red '3' indicating a rotation delay. The time is 13:21 on 2017.11.03.

# Set-up of parameters and test

## 1. Environment

The screenshot shows the 'Environment' settings page for program 37-95. The interface includes a sidebar with buttons for 'Environment', 'Load Specimen', 'Blot', and 'Plunge/Transfer'. The main area displays parameters for Chamber Temperature (28°C actual, 37°C set), Chamber Rel. humidity (54% actual, 95% set), Chamber Window heater (55%), and Cryogen Temperature (-2°C actual, -180°C set). A bottom navigation bar contains 'Main', 'Programs', 'Save', and a clock showing 13:21 on 2017-11-03.

## 2. Load Specimen

The screenshot shows the 'Load Specimen' settings page for program 4-95. The interface includes a sidebar with buttons for 'Environment', 'Load Specimen', 'Blot', and 'Plunge/Transfer'. The main area features a central image of the instrument and two checkboxes: 'Rotate before specimen application' and 'Rotate to home position after specimen application'. Below these is a 'Delay time before blotting' field set to 15.0 seconds. A bottom navigation bar contains 'Main', 'Programs', 'Save', and a clock showing 13:07 on 2017-11-03.

## 3. Blot

The screenshot shows the 'Blot' settings page for program BTV23-01. The interface includes a sidebar with buttons for 'Environment', 'Load Specimen', 'Blot', and 'Plunge/Transfer'. The main area displays settings for 'single blotting' (checked), 'Blot time [s]' (1.7), 'Sensor blotting' (checked), 'Blotting window for sensor blotting' (42.0 mm), 'additional move [mm]' (1.0), and 'Vertical blot position' (3.0 mm). A bottom navigation bar contains 'Main', 'Programs', 'Save', and a clock showing 14:40 on 2017-08-16.

## 4. Plunge / Transfer

The screenshot shows the 'Plunge / Transfer' settings page for program VLPfr 30 QF 1.2-1.3 Test. The interface includes a sidebar with buttons for 'Environment', 'Load Specimen', 'Blot', and 'Plunge/Transfer'. The main area displays settings for 'Automatically plunge after blotting' (checked), 'Post-blotting time [0 - 120 s]' (0.0), 'Skip transfer position' (unchecked), 'Automatically move to transfer position' (unchecked), and 'Transfer position above freezing position [mm]' (5.0). A bottom navigation bar contains 'Main', 'Programs', 'Save', and a clock showing 15:18 on 2017-10-07.

# Vitrification

Adjust the settings

Run the program



Load the  
Forceps

Load the  
specimen

Blot

Plunge

Transfer

## Shut down and Bake Out

Remove ethane container

Remove water from humidifier

Dispose filter paper and leave the chamber open

Bake out the system (~around 1-2 hours)

Put “HOT” sign in front of GP2

Switch off the system when the chamber reaches the lowest position