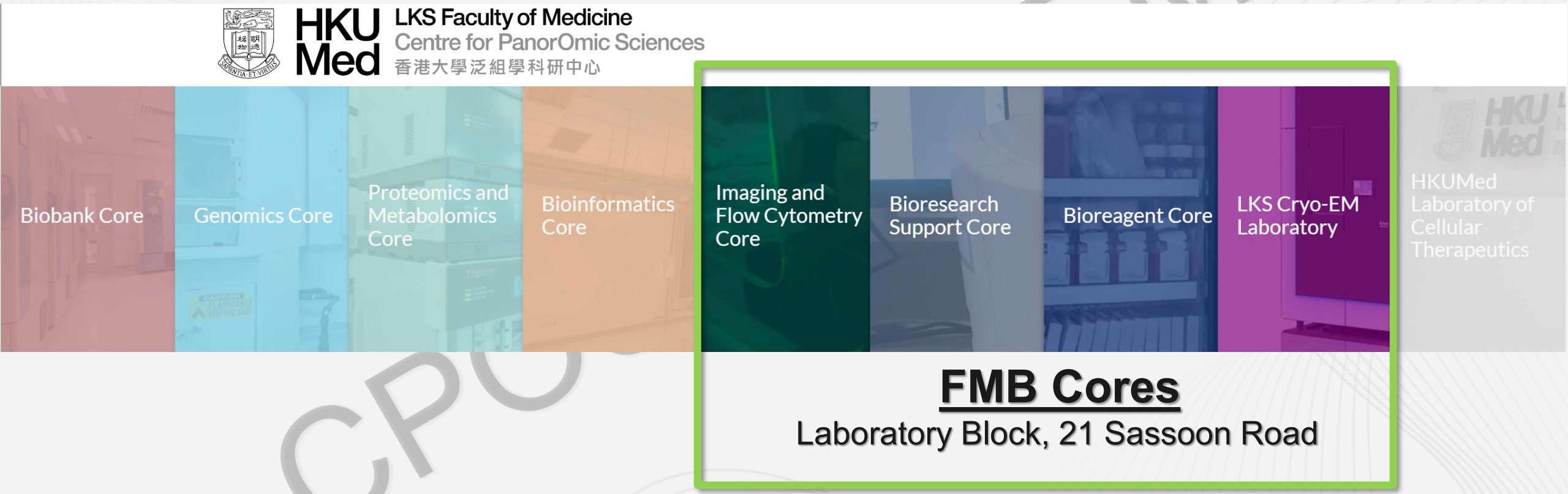
General Policy

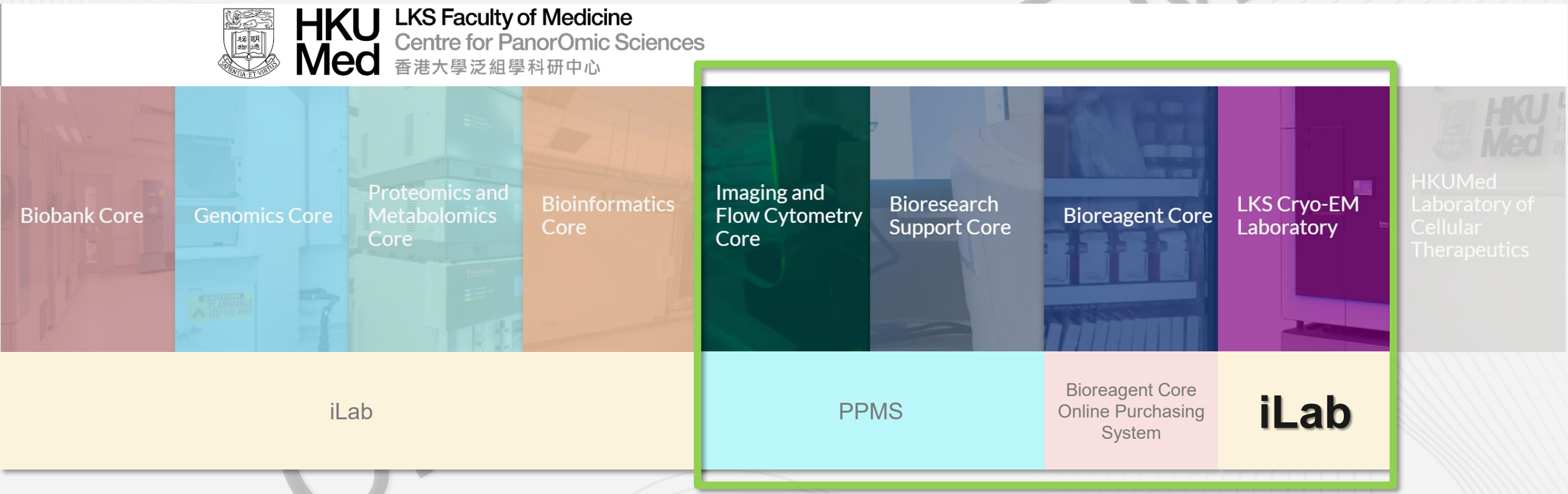
Li Ka Shing Cryo-EM Laboratory

CPOS Cry

Cores in CPOS



Online Platform

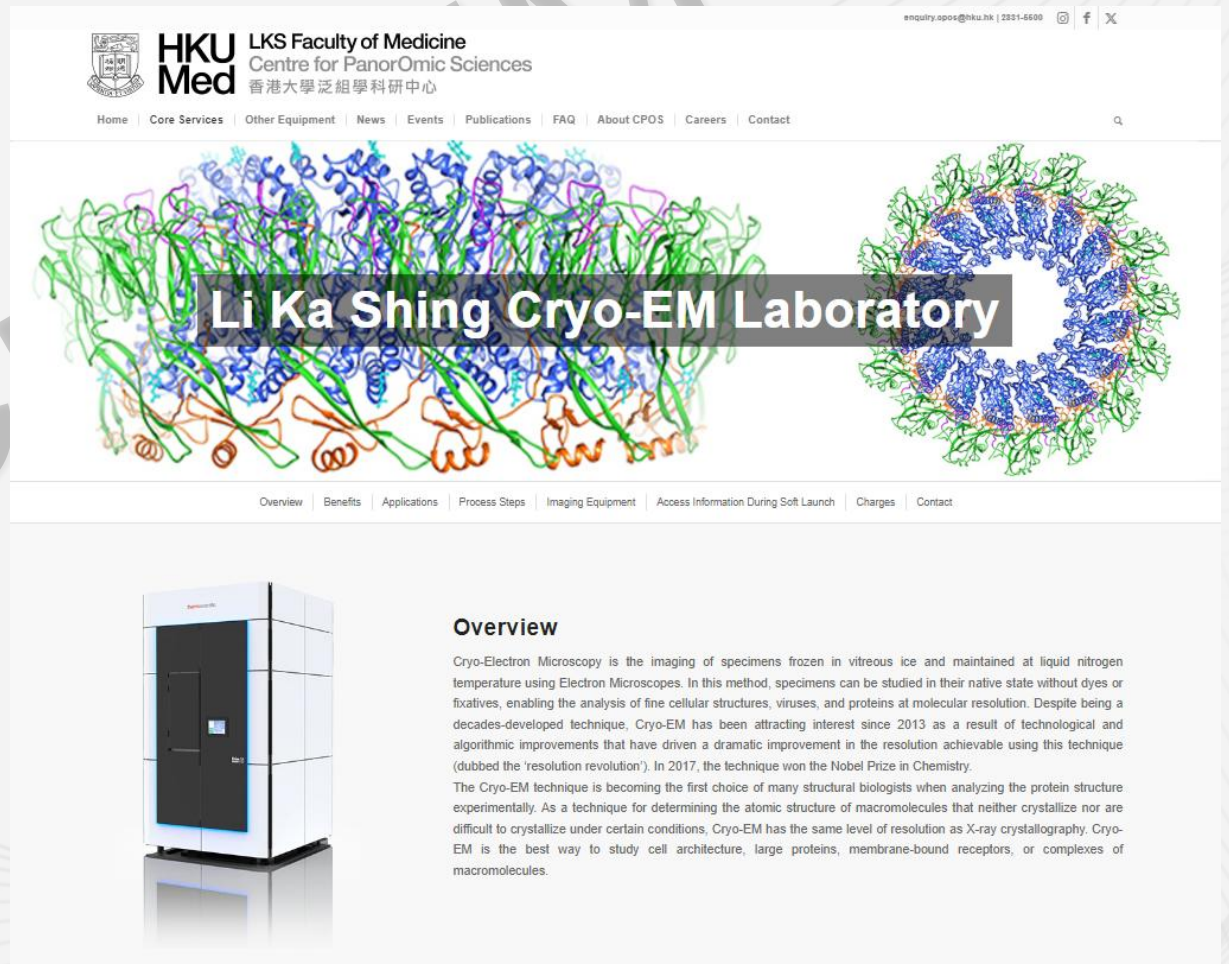


Li Ka Shing Cryo-EM Laboratory

Email: cryoem.cpos@hku.hk

Tel: 3910-2938

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



CPOS C

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.

CPOS Cryo-EM

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.

CPOS Cryo EM

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.

CROSS to EM

General Safety

Fire Extinguisher

First Aid Box

Safety shower at corridor

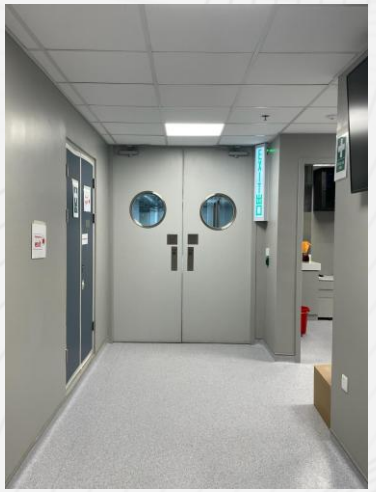
Fire Escape Route and First Aid box.

No gloves on computer and areas accessible by others.

Dispose biological waste in designated bins.

Dispose sharps / glasses in sharp box.

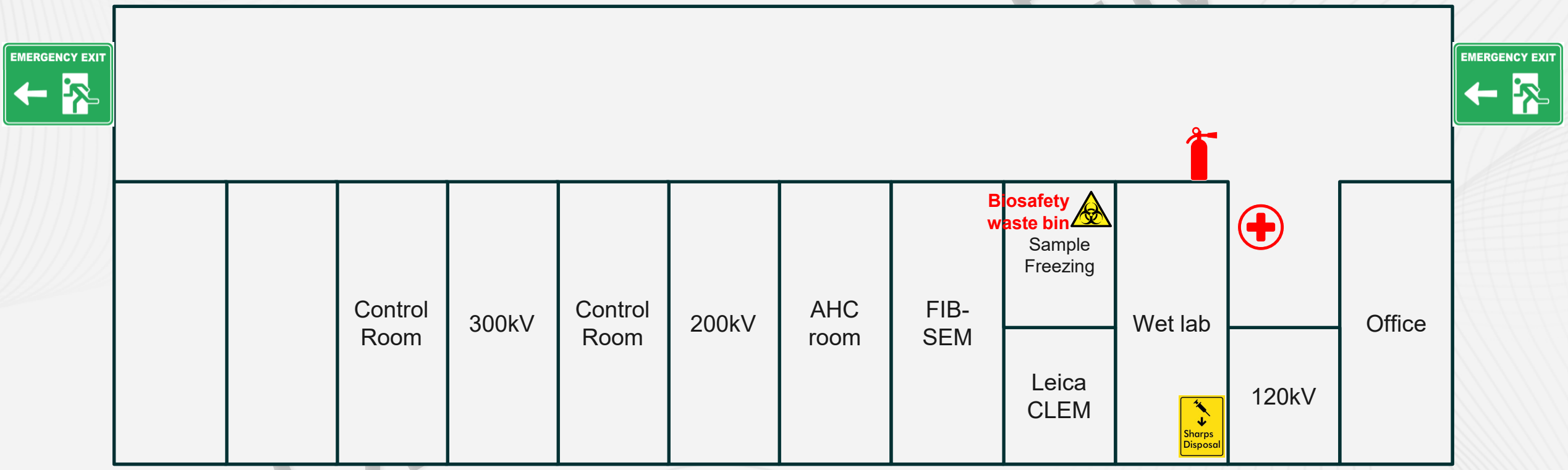
Emergency Exit



Biological waste bin

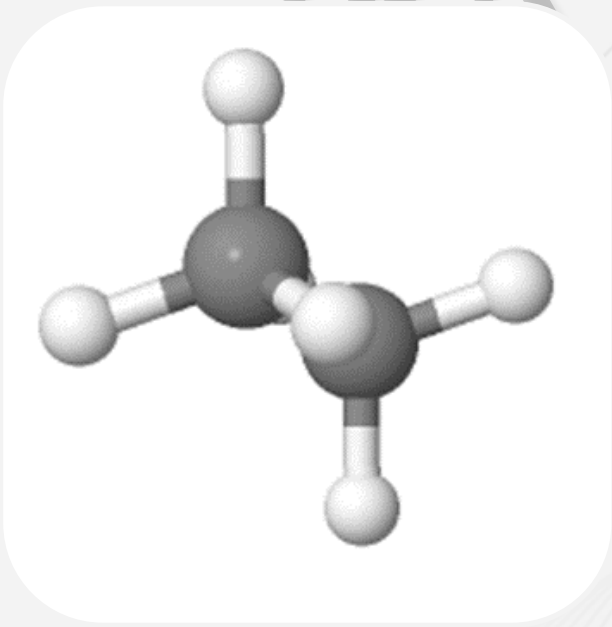
Sharp box

Safety (Floor Plan)



Safety (Gas)

Liquid Nitrogen



Ethane

CPCC Cryo

Containers for Liquid Nitrogen

Dewar

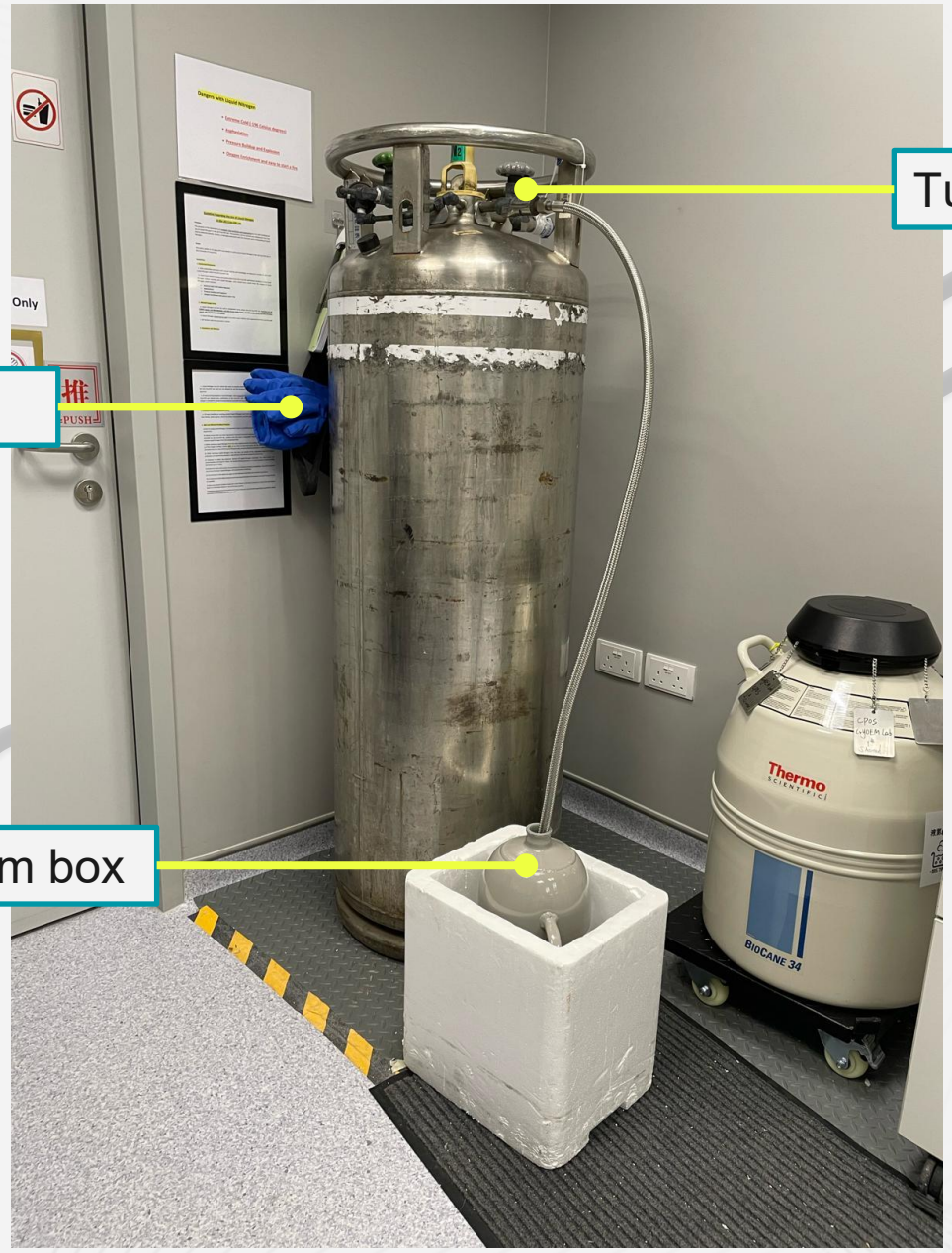
Always put on the floor

Stainless steel vacuum bottle (with loosen cover)

Foam Box

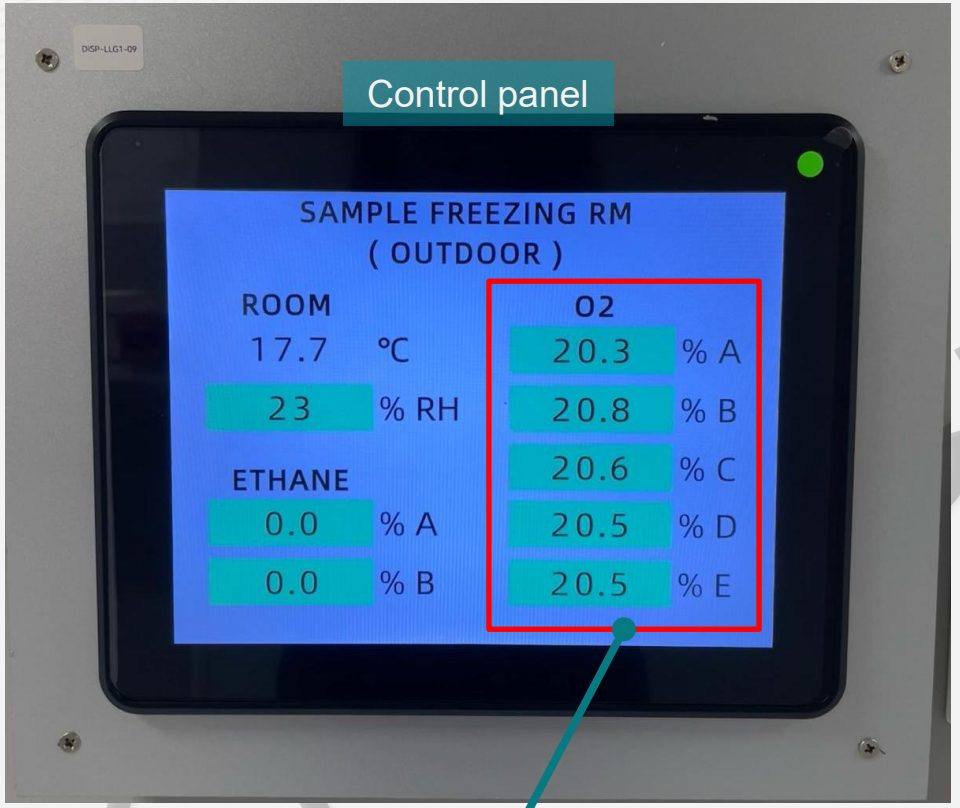
- **AM** Dewar: Available from 9 AM to 1 PM.
- **PM** Dewar: Available from 1:30 PM to 5:30 PM.
- **Night** Dewar: Available from 6 PM to 11 PM

Get Liquid Nitrogen



Protection

Oxygen Detector

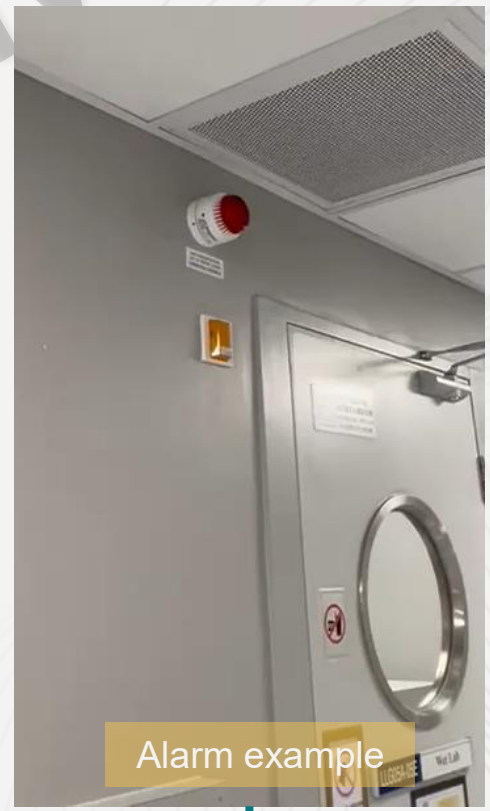


If the O_2 VALUE shows red or alarm in the room, user should leave the room immediately and report it to staff.

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
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.

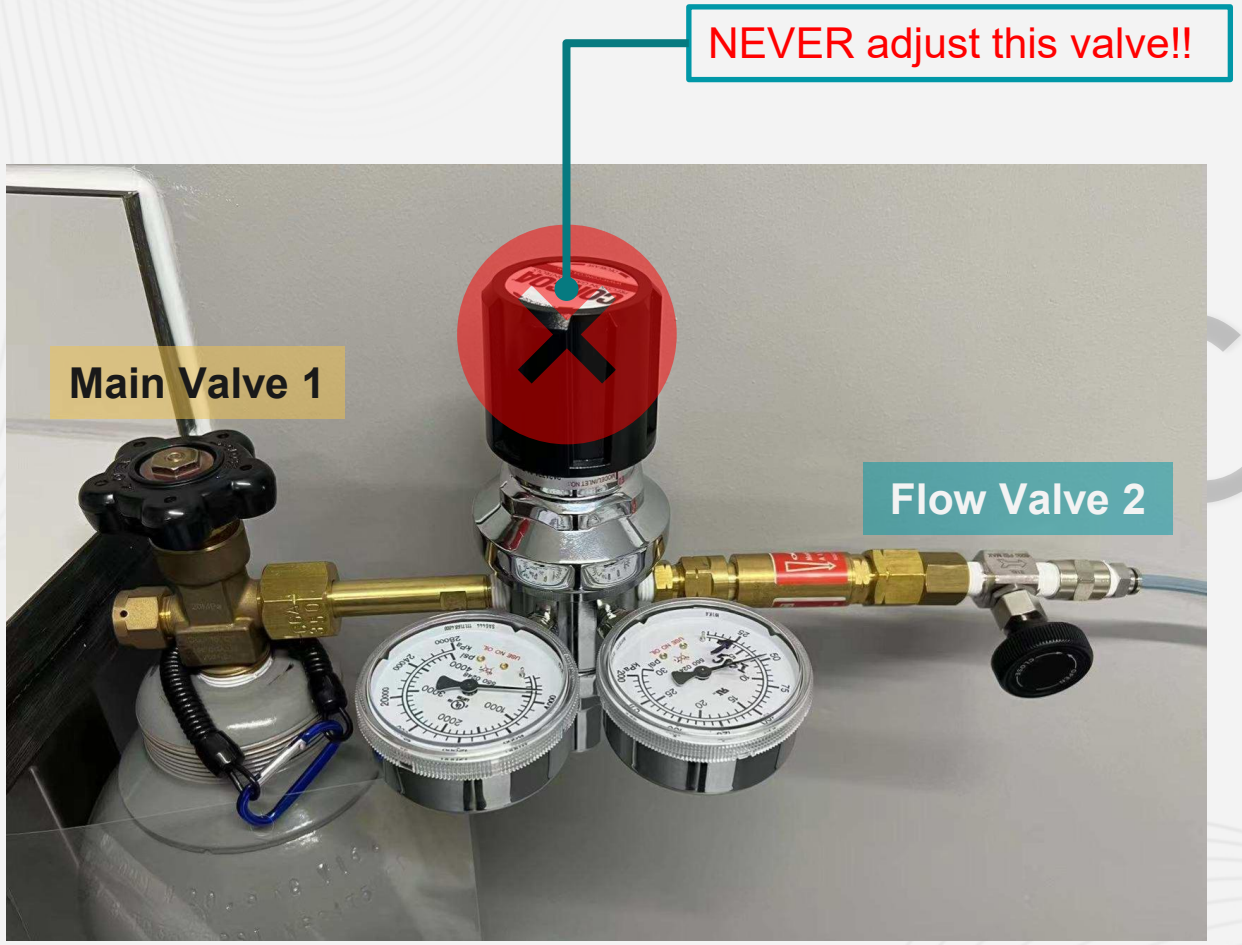
Access will be terminated if user violates this rule!



Alarm example

CPOS Cryo EM

Open and Close Ethane Gas Flow



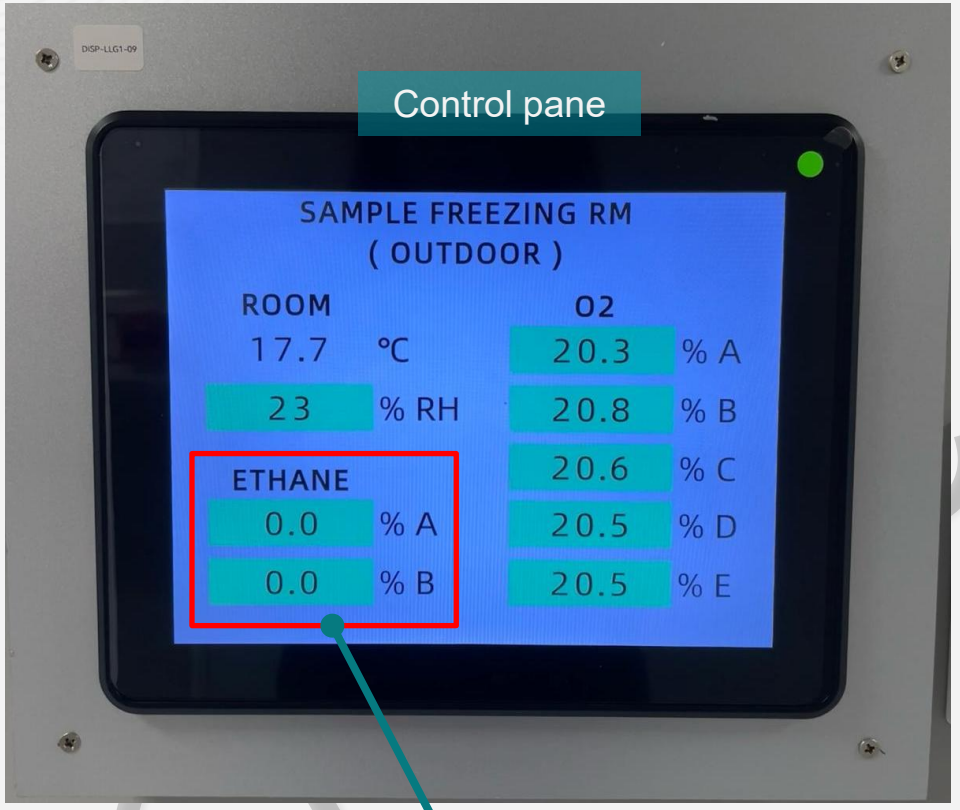
Open the gas flow:

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_4U6w9XvilI3F5gG

Sample Information e-Form

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-2025(

iLab Registration Form

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

3.Training for 200kV (EPU operation)

a)HPC server account registration

b)Training (with sample prepared, submit Training Request

c)Self- booking x 1

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 cpos@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)

CPOS Cryo EM

Charging

Training	HKU-Med	HKU
1st 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.

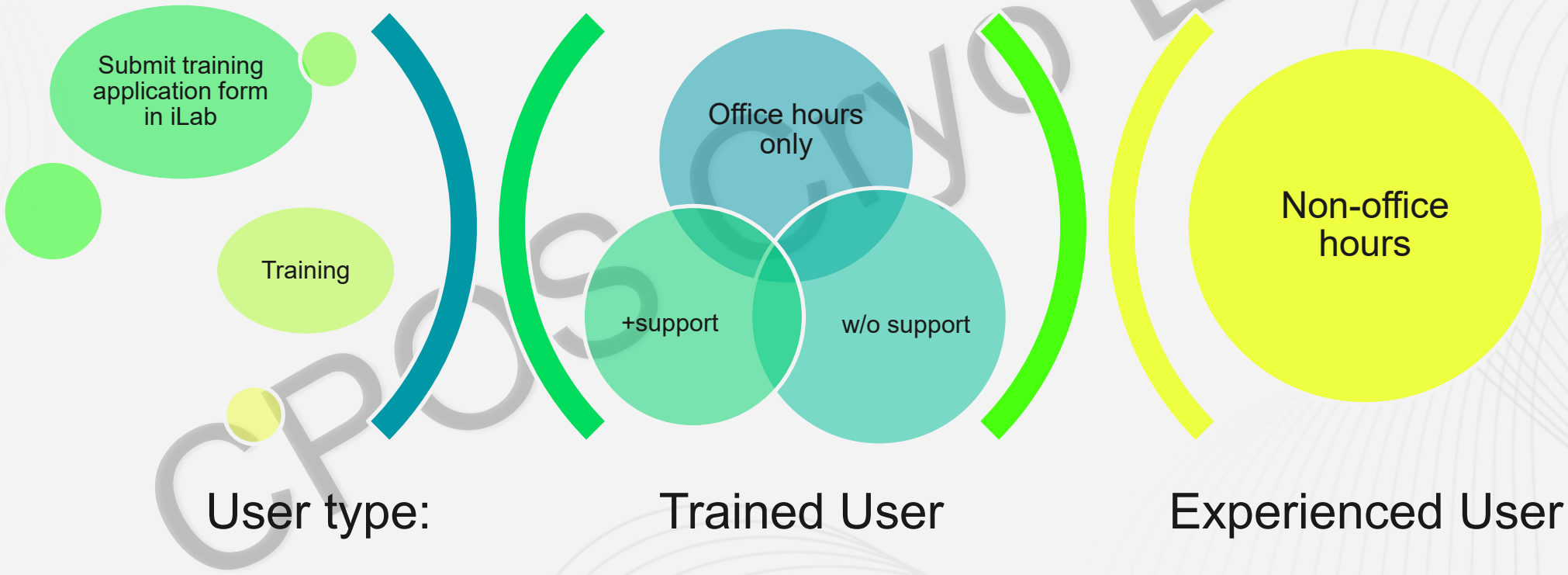
Usage	Min. usage / Session	HKU-Med	HKU
Sample Freezing with EasiGlow	2 hours	$300 / 2 hours	$330 / 2 hours

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

Cross Cryo

User Type

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

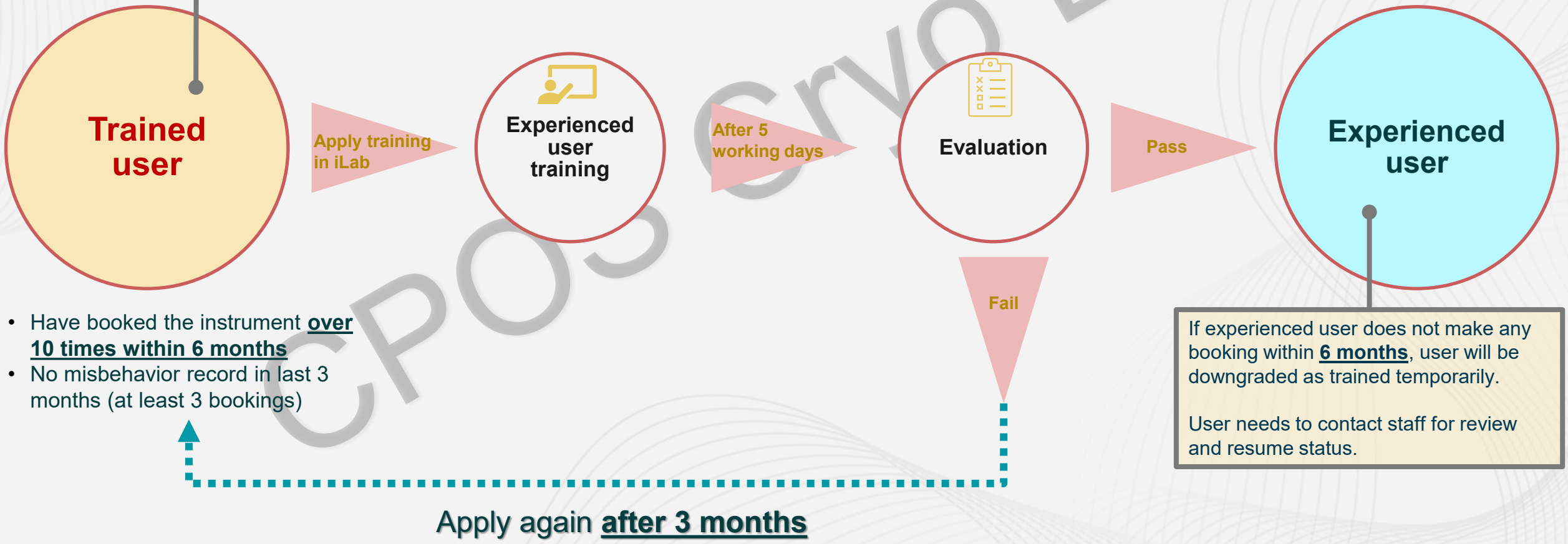


CPOS.cryo EM

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.



- 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**

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.

CPOS Cryo EM

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

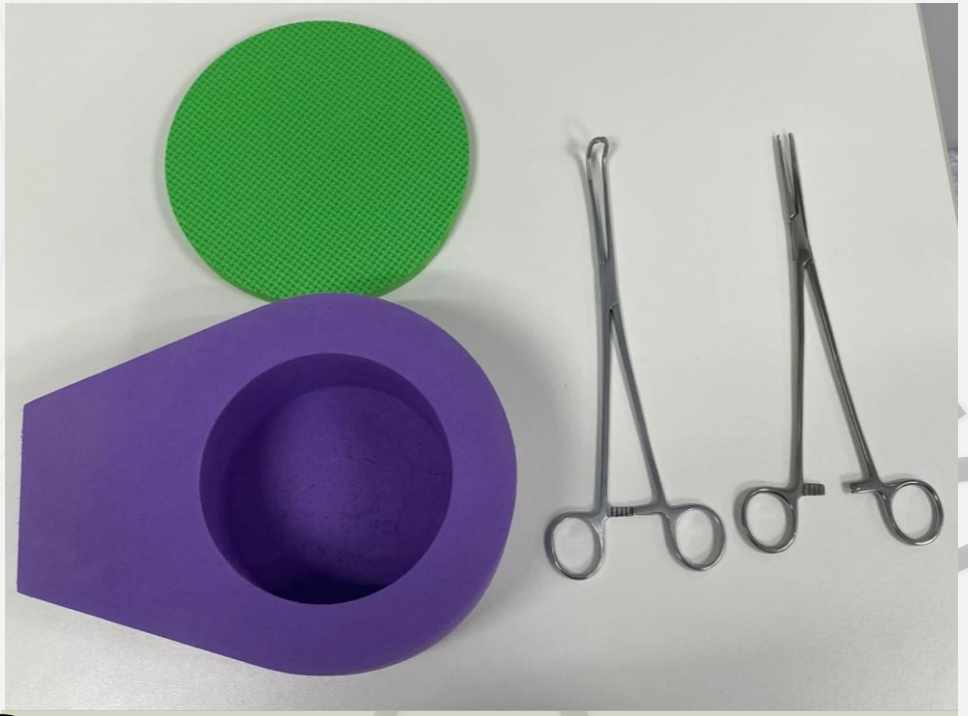
Pressure	0.39mBar
SET	15mA
GLOW	00:01:00
HOLD	00:00:10
Polarity	Negative

User should record their own setting after optimization

CPOS Cryo EM

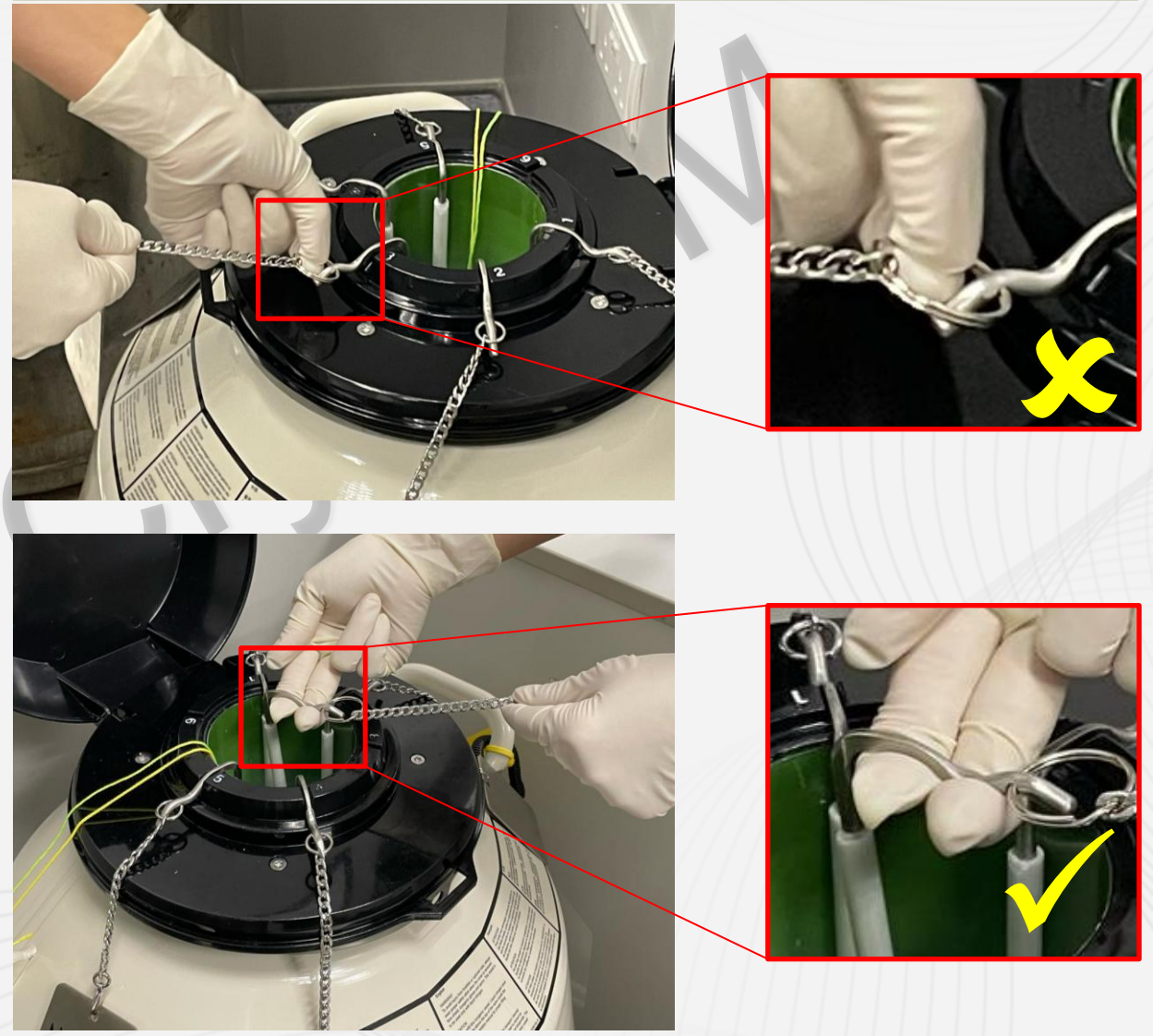
Grid storage

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 tweezer

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

Leica EM GP2

Vitrification

Booking Sessions

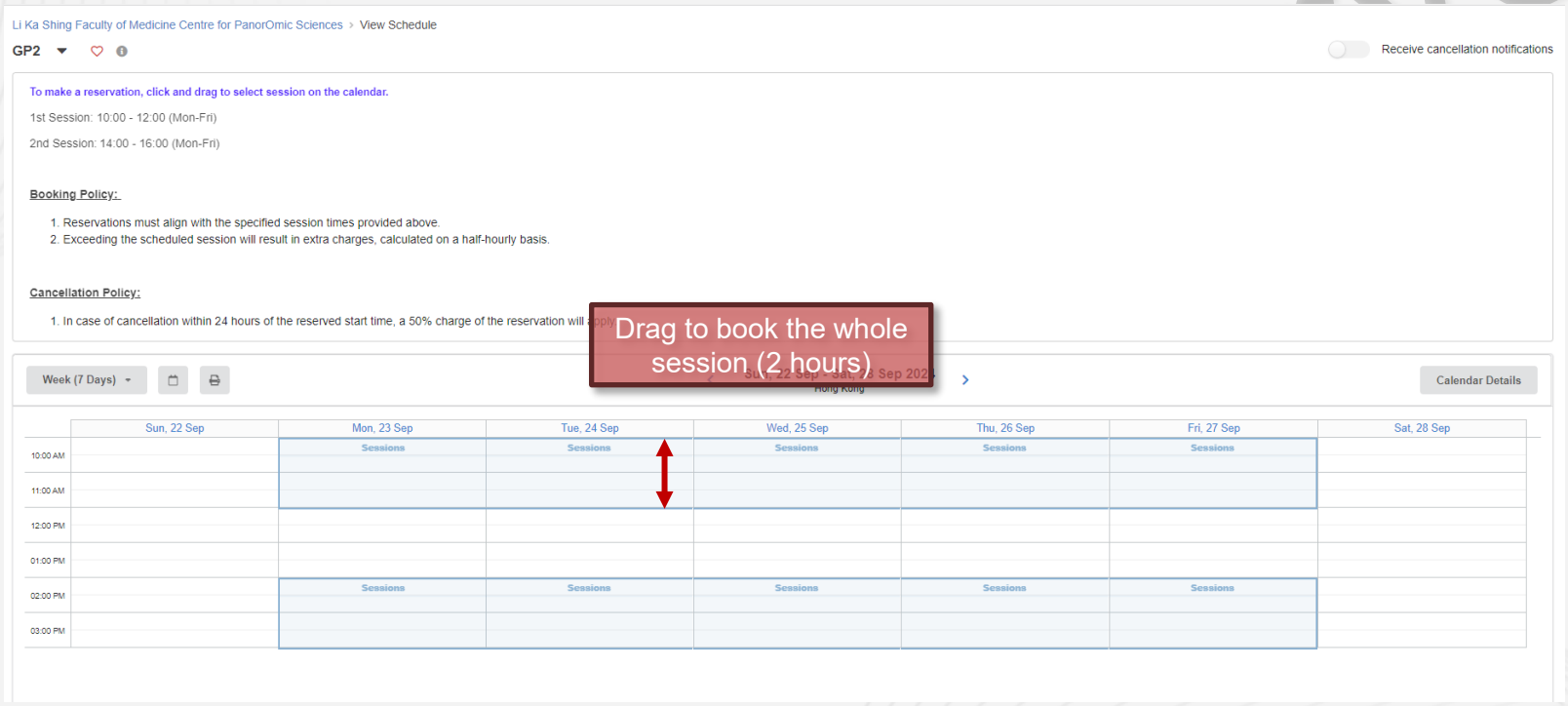
Office Hour	
Session 1	10:00 am – 12:00 n
Session 2	2:00 pm – 4:00 pm

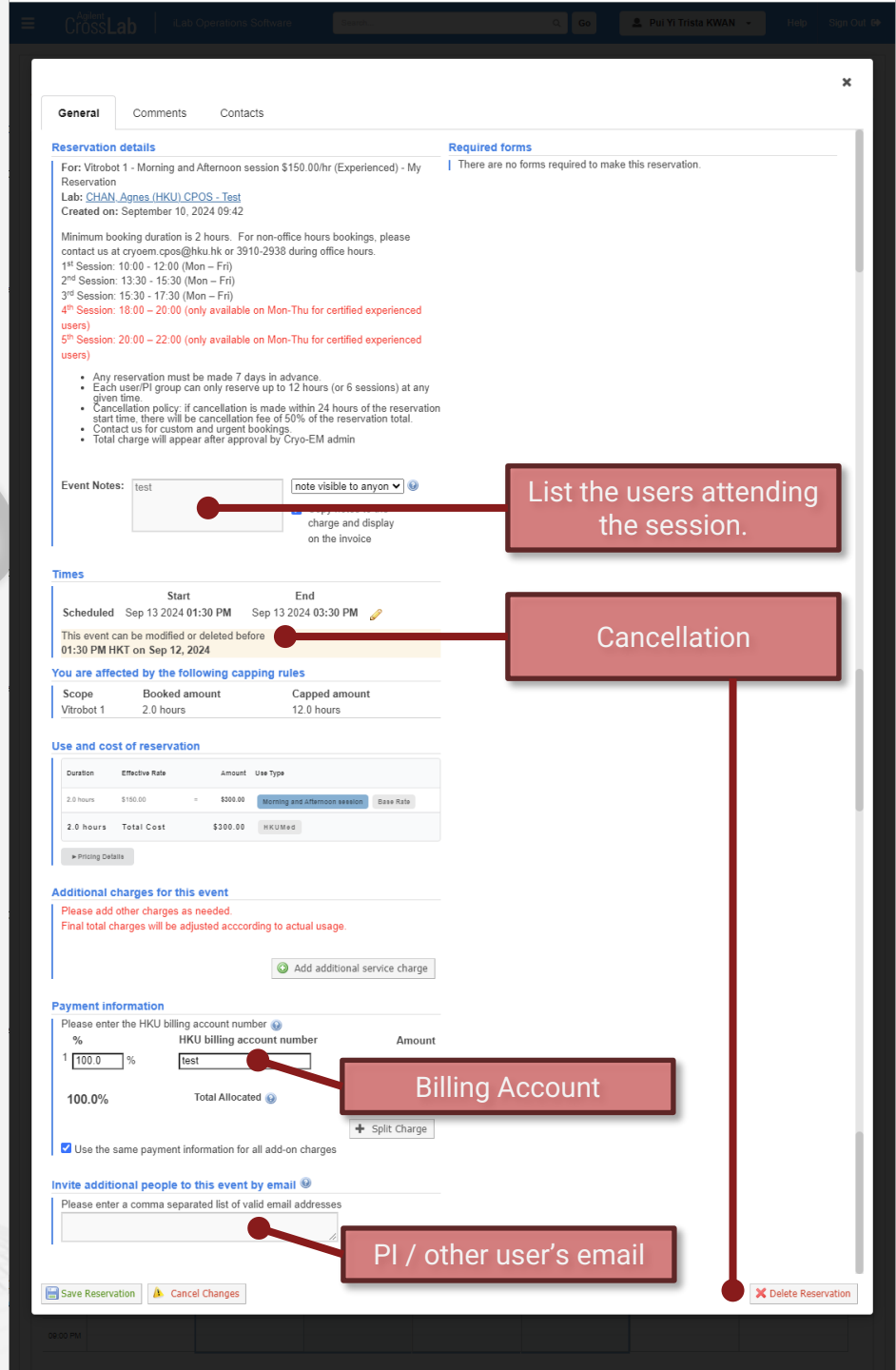
Non-Office Hour (Experienced user only)	
Session 3	6:00 pm – 8:00 pm

Booking Policy

Open for 28 days booking

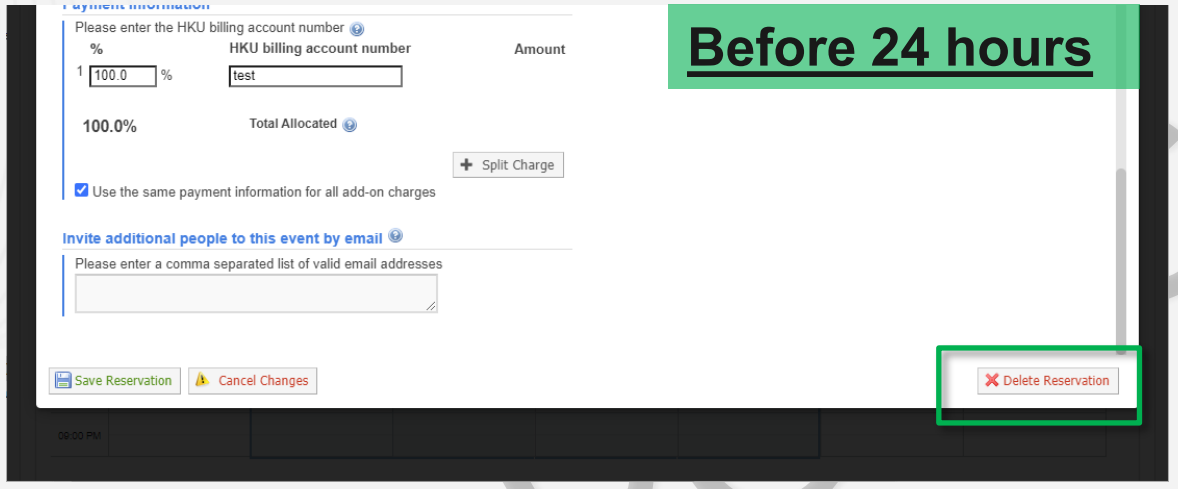
Card access: User can access the instrument on the booking date



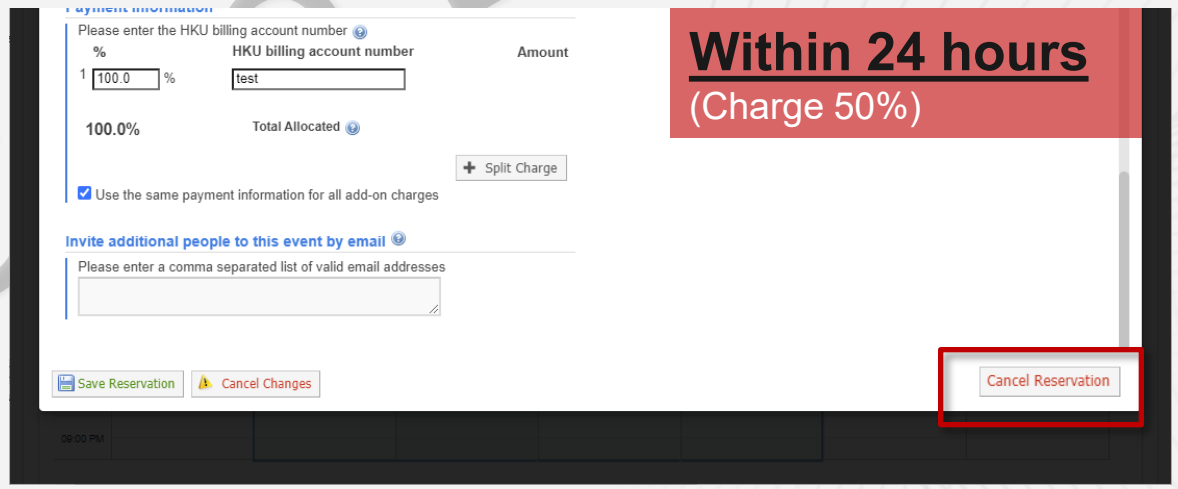


Cancellation Policy

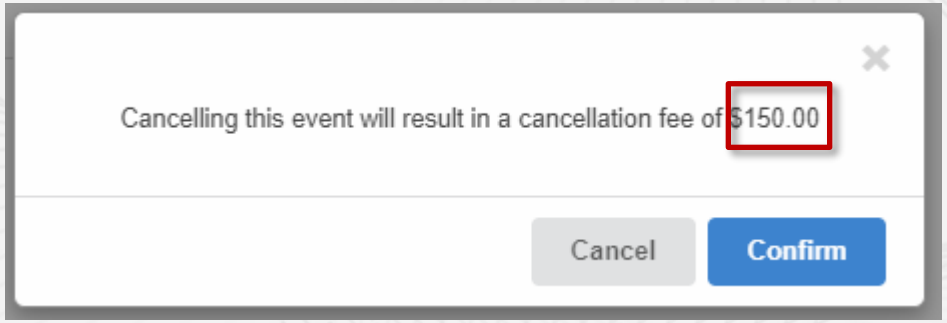
Before 24 hours → Free cancellation
Within 24 hours → 50% charge
Session starts → 100% charge



Before 24 hours



Within 24 hours
(Charge 50%)

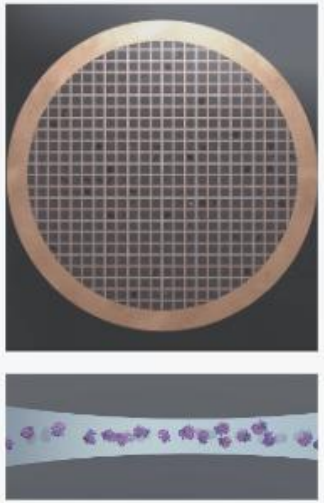


01

AQUEOUS SPECIMEN

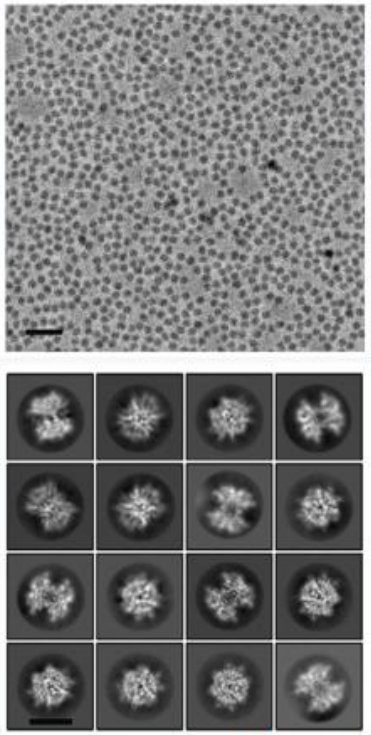
02

VITRIFICATION



03

IMAGING



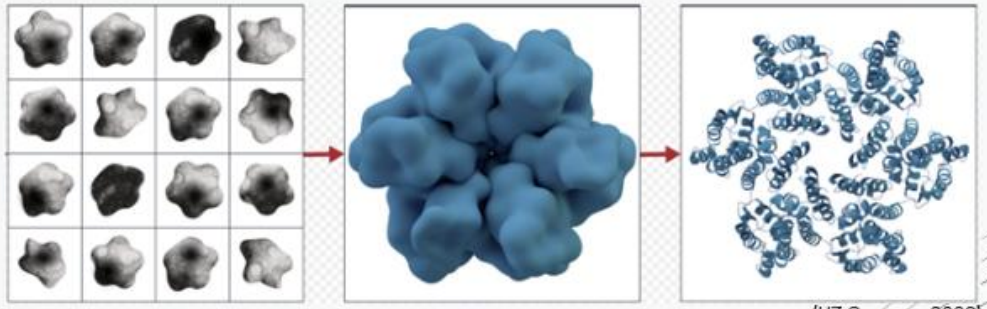
(X FAN et al., 2019)

04

SINGLE PARTICLE ANALYSIS / TOMOGRAPHY

05

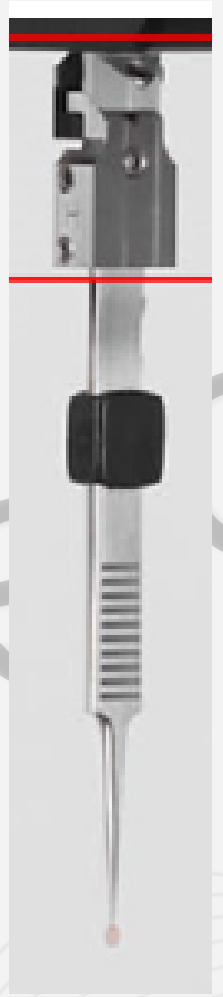
3D MAPPING & RECONTRUCTION



(HZ Carreras, 2023)

Special Notice

Tip of the tweezer is very sharp.



CPOSCHEM

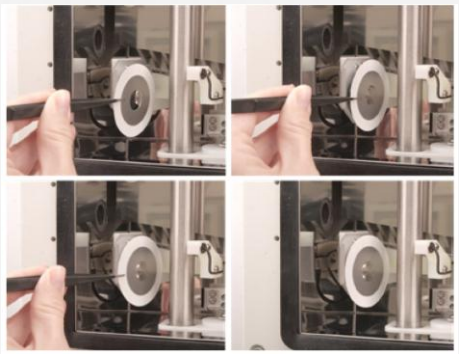
Steps

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

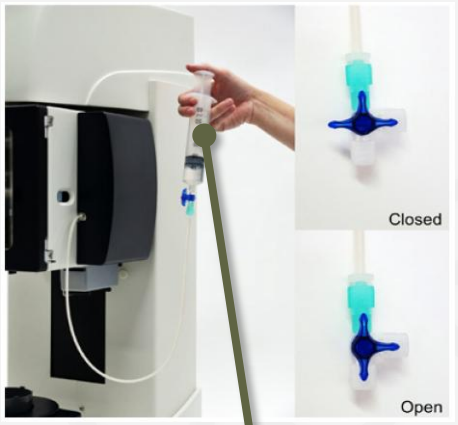
CPOS

Preparation of GP2

Place Filter Paper



Fill Humidifier with Water



1st fill: 90mL
Refill: 30mL

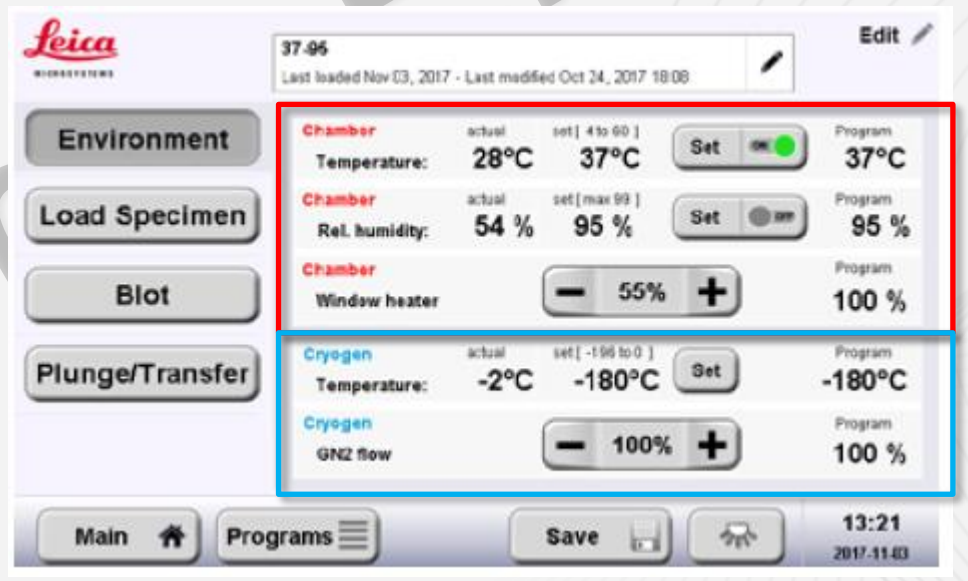
Empty Drip Tray

Freeze Chamber

Cover the ethane container

Fill Ethane

Set-up of parameters and test

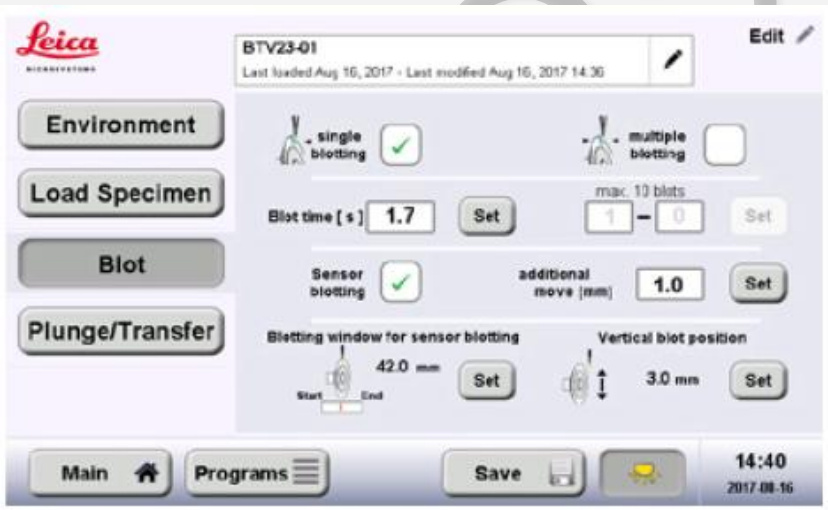


Set-up of parameters and test

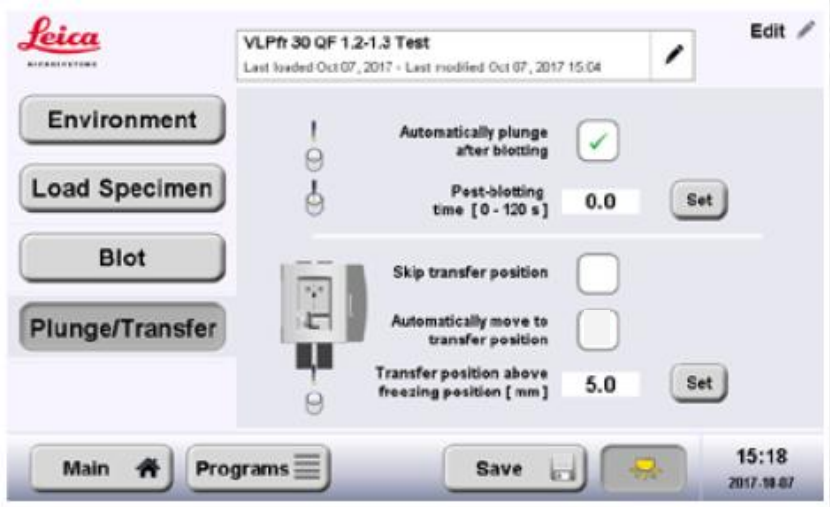
1. Environment

2. Load Specimen

3. Blot



4. Plunge / Transfer



Vitrification

Adjust the settings

Run the program

1. Load the Forceps
2. Load the specimen
3. Blot
4. Plunge
5. 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

CPOS Cryo EM