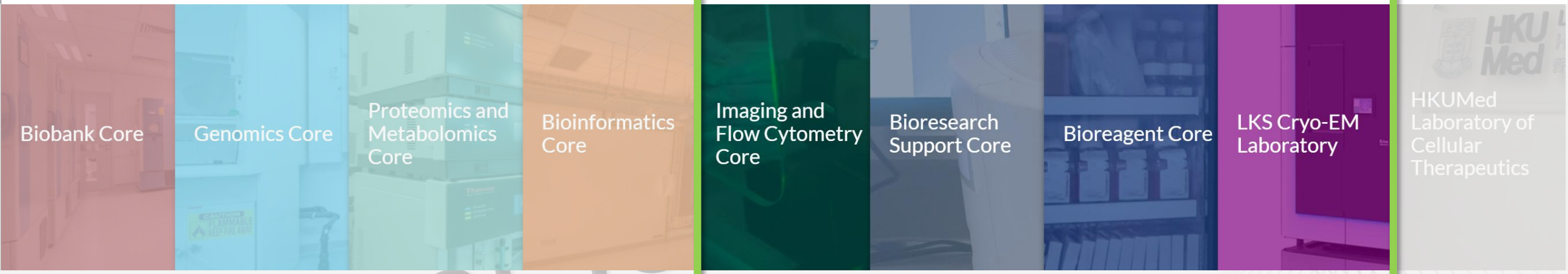
General Policy

Li Ka Shing Cryo-EM Laboratory

Cores in CPOS

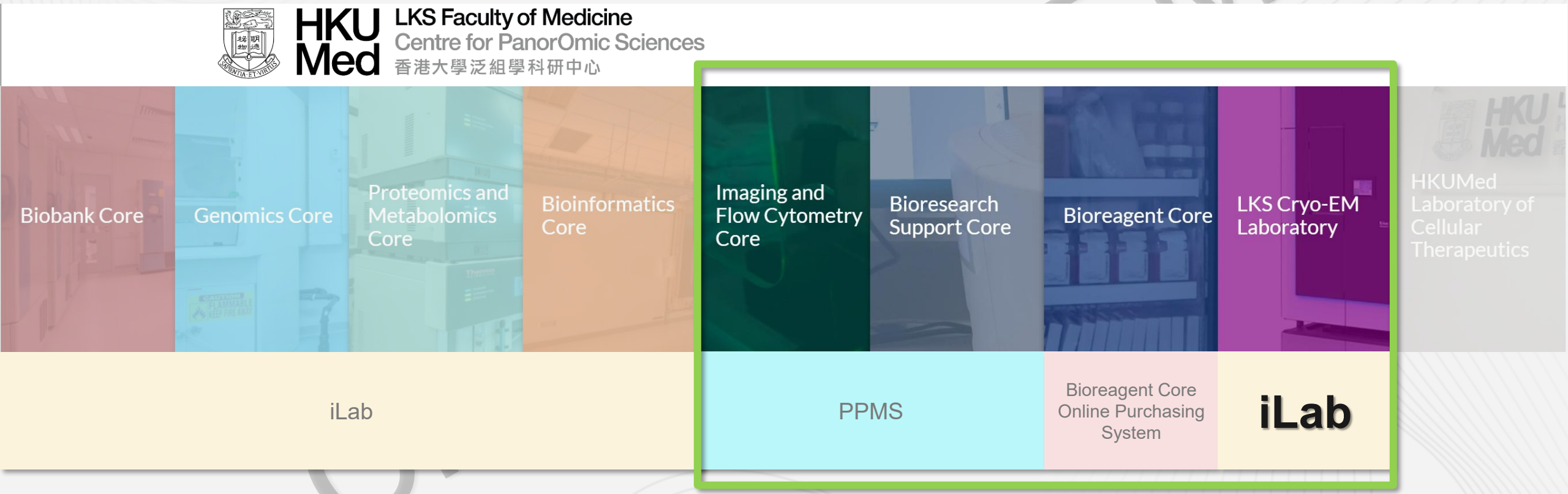
HKU Med LKS Faculty of Medicine
Centre for PanorOmic Sciences
香港大學泛組學科研中心



FMB Cores

Laboratory Block, 21 Sassoon Road

Online Platform

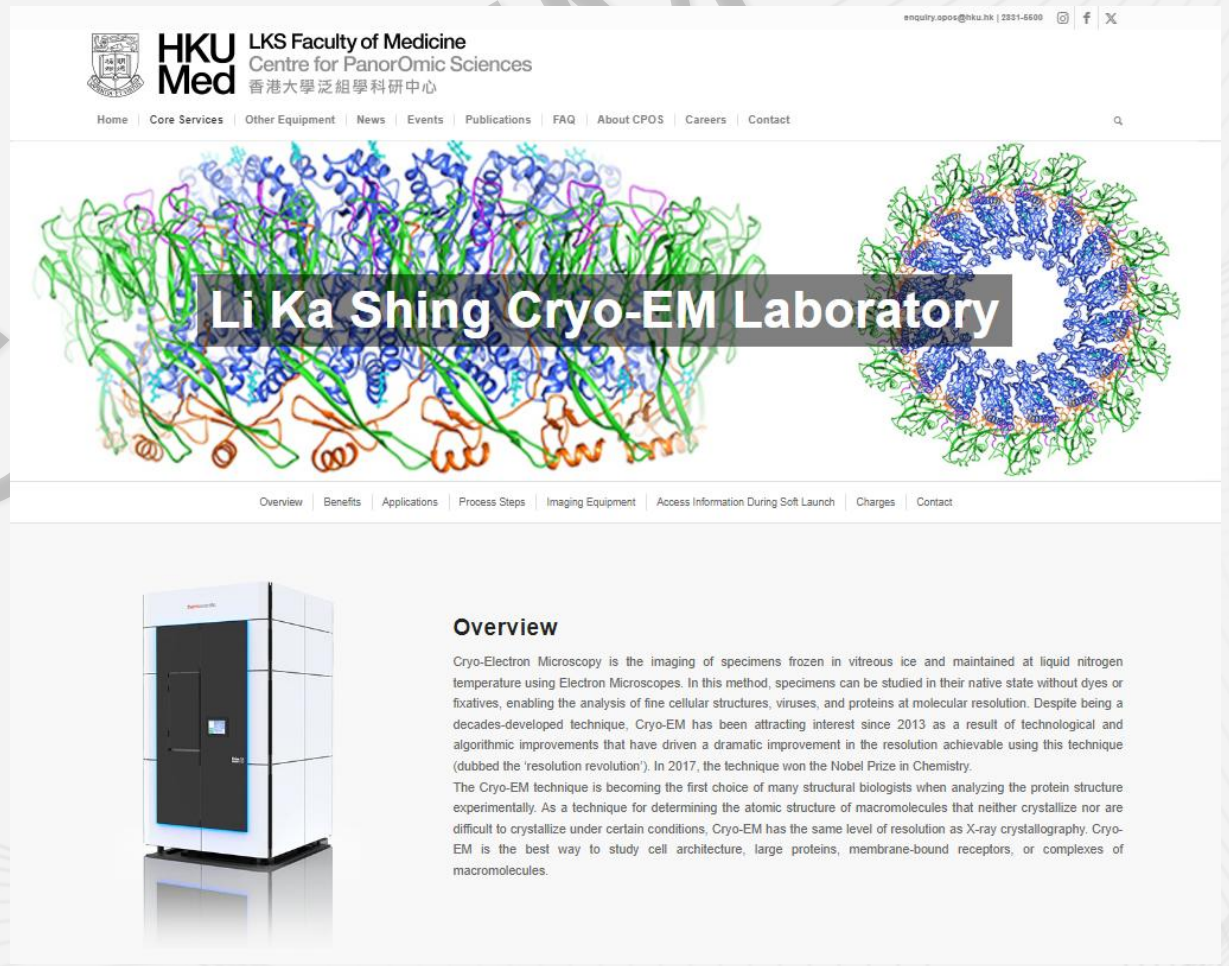


Li Ka Shing Cryo-EM Laboratory

Email: cryoem.cpos@hku.hk

Tel: 3910-2938

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



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

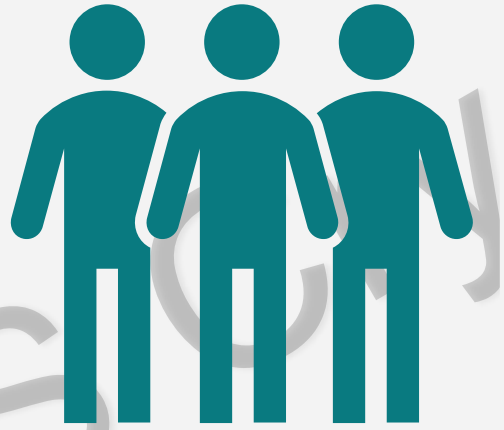
Report any problem related to instruments

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

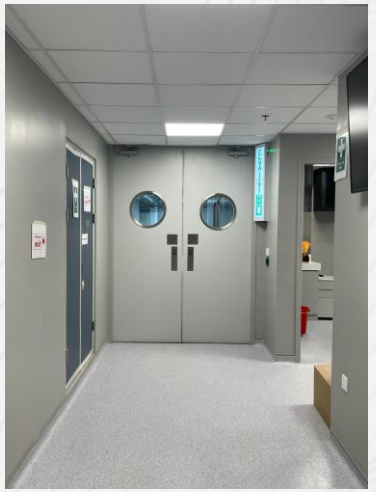
Fire Escape Route and First Aid box.

No gloves on computer and areas accessible by others.

Dispose biological waste in designated bins.

Dispose sharps / glasses in sharp box.

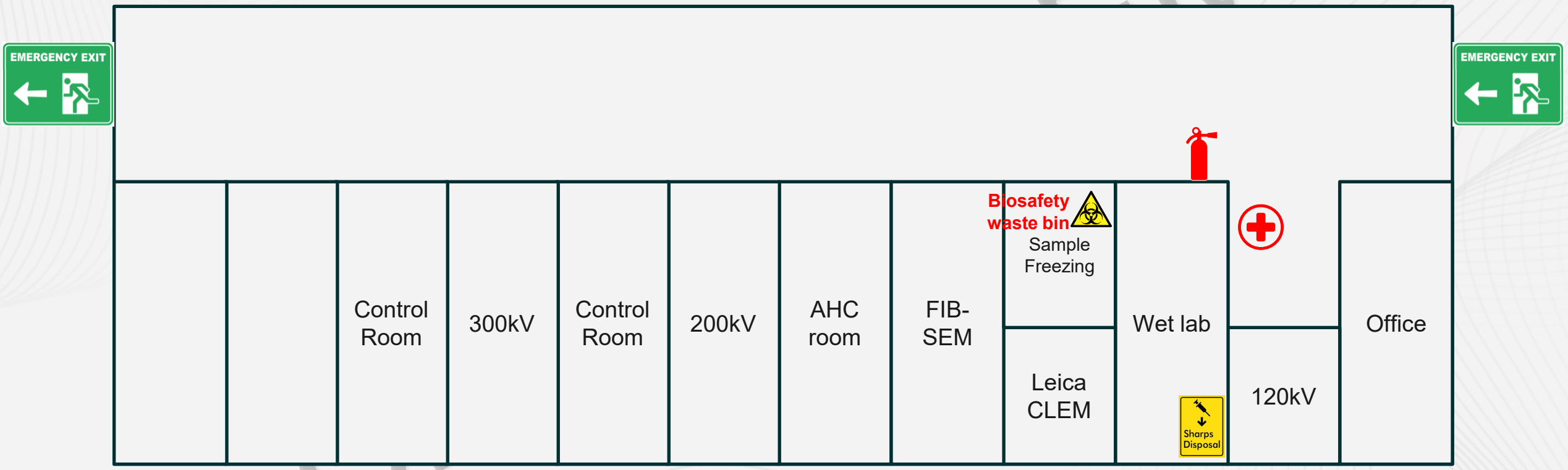
Emergency Exit



Biological waste bin

Sharp box

Safety (Floor Plan)



Safety (Gas)

Liquid Nitrogen

Containers for Liquid Nitrogen

Dewar

Always put on the floor

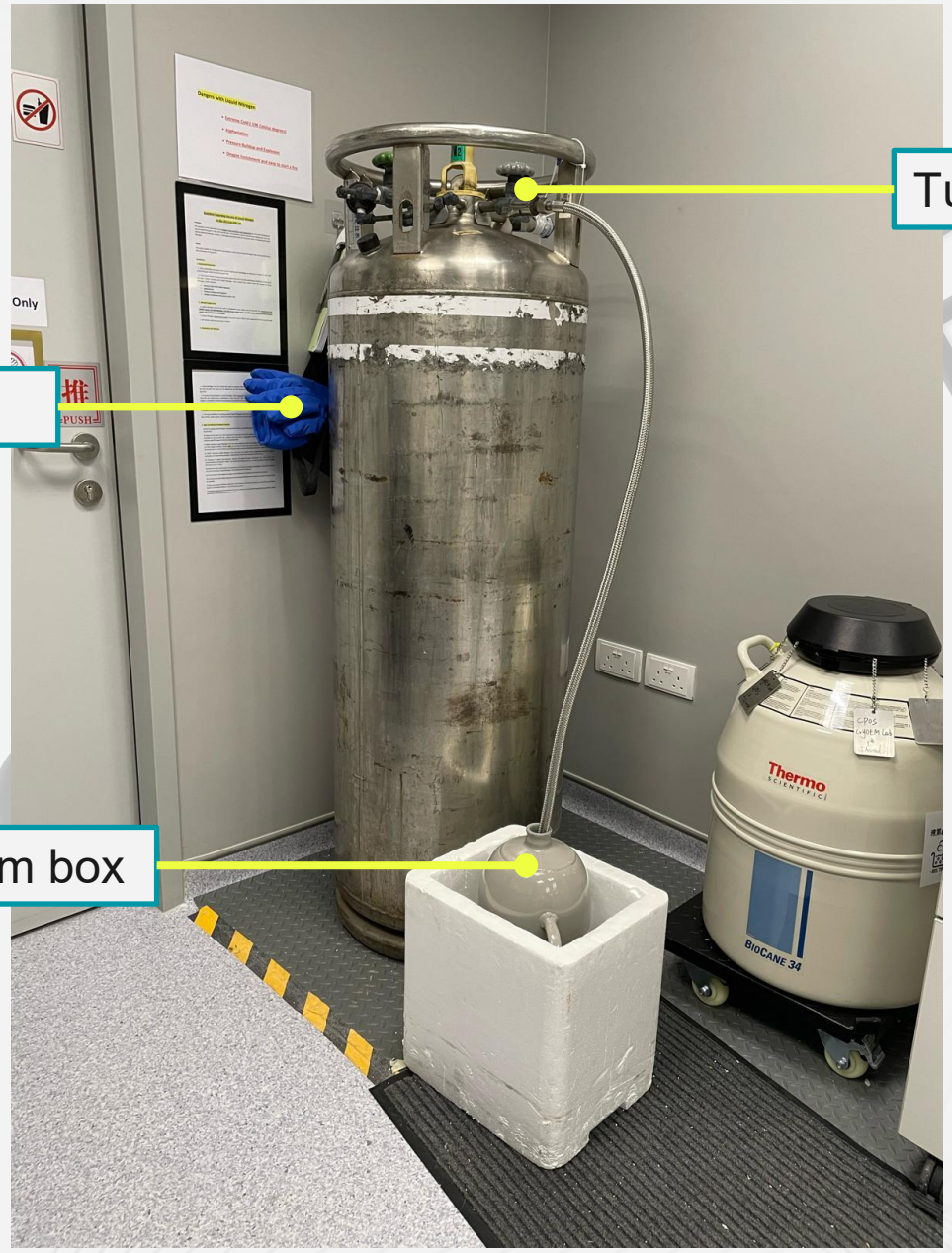
Stainless steel vacuum bottle (with loosen cover)

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

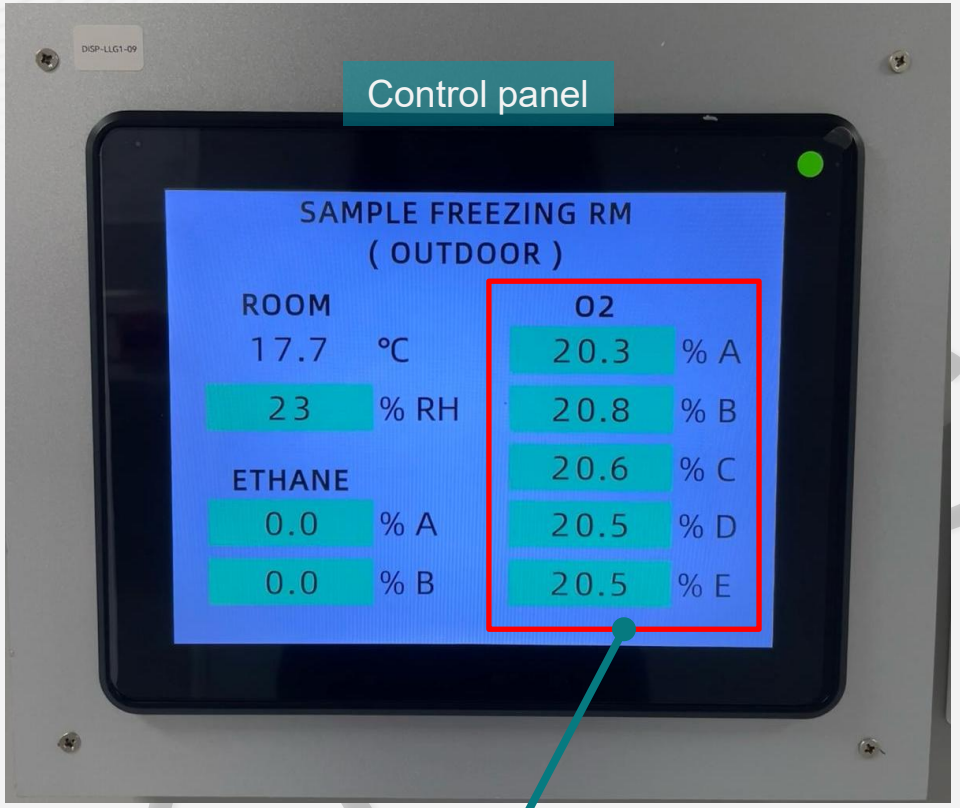
(For ICE user, you can also use FIB dewar if needed.)

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.

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

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

d) Register your HKU card for access

Sample Information e-Form

iLab Registration Form

2.Training for ICE

a)Training

3.Training for FIB

a)Training (with sample prepared)

4.Training for 300kV (Tomo5)

a)HPC server account registration

b)Training (with sample prepared)

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)

Charging

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

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

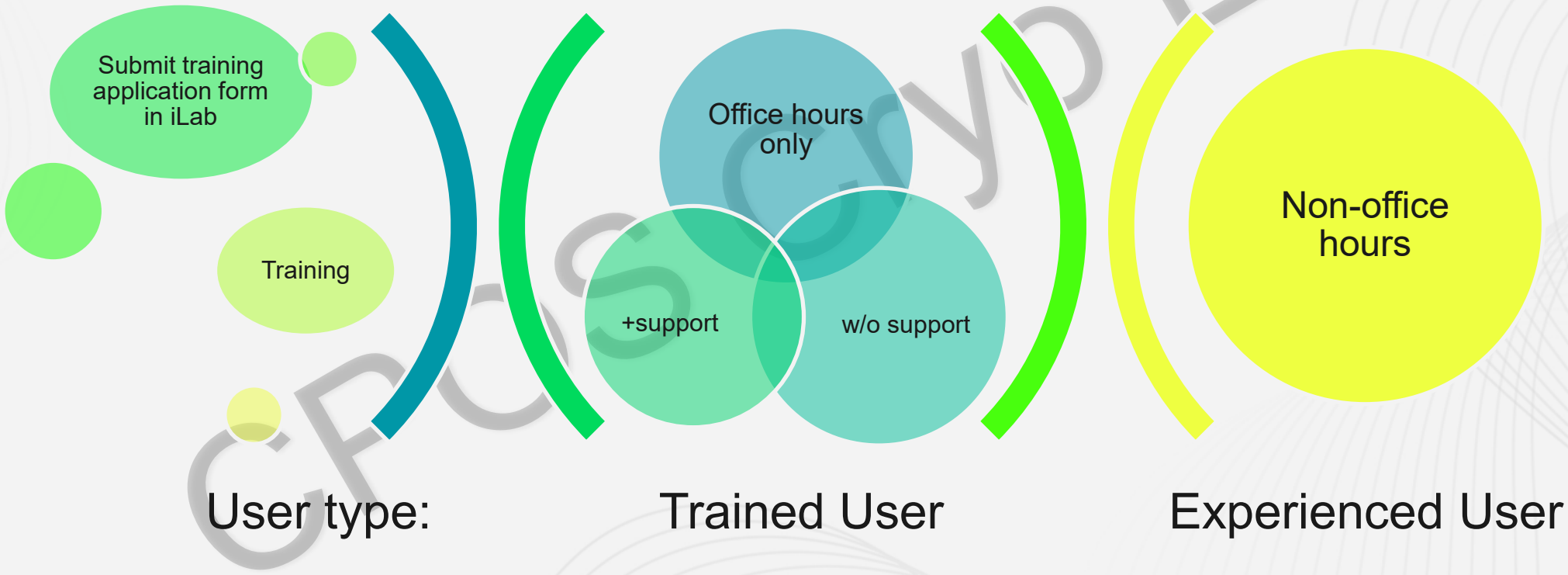
- If user has not booked within 2 months, **re-training** is required before using the instrument.

Usage	Min. usage / Session	HKU-Med	HKU
Leica EM ICE High Pressure Freezer	2 hours	$300 / hour	$330 / hour

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

User Type

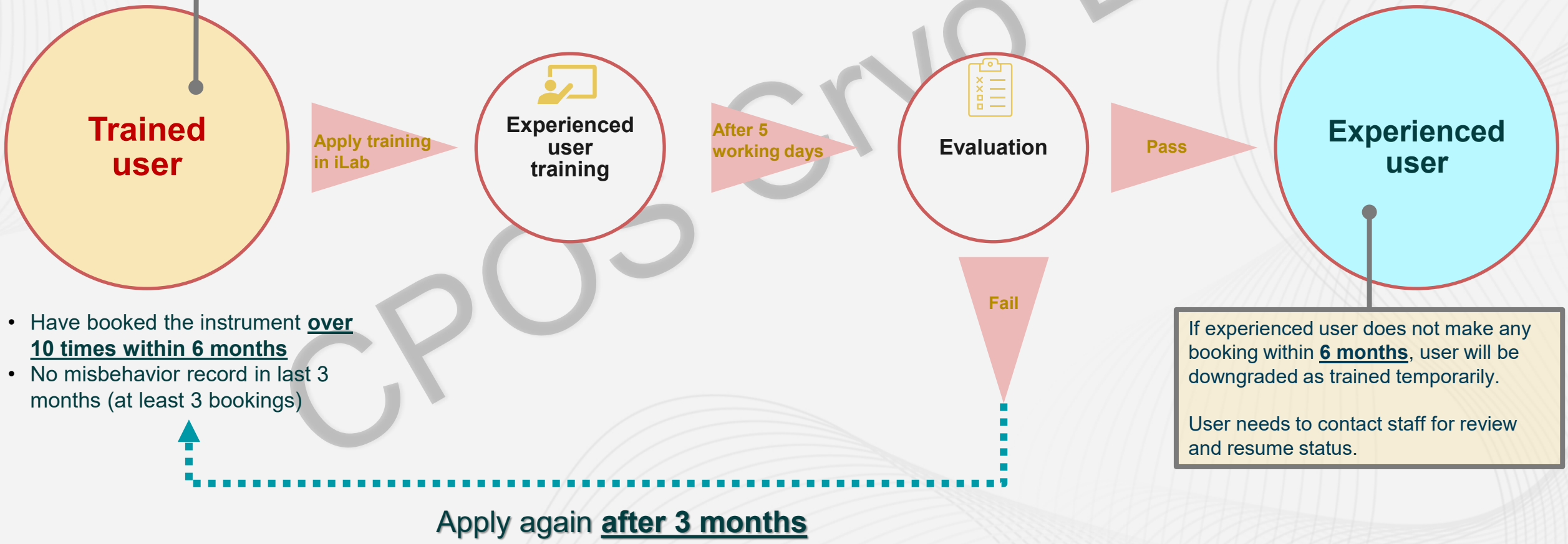
- Do not transfer your booking session without notifying CPOS staff.
- Fill in correct information (e.g. usage time) in the logbook.



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.



- 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.

Easi-glow

Grid discharge treatment

Cryo EM

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

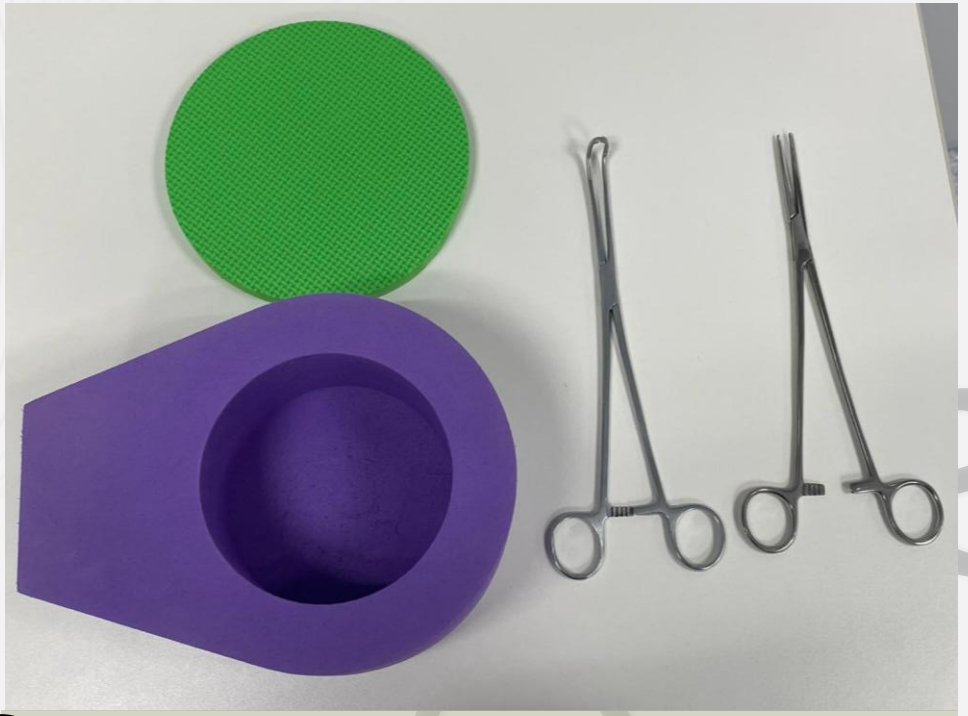
Standard Setting

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

User should record their own setting after optimization

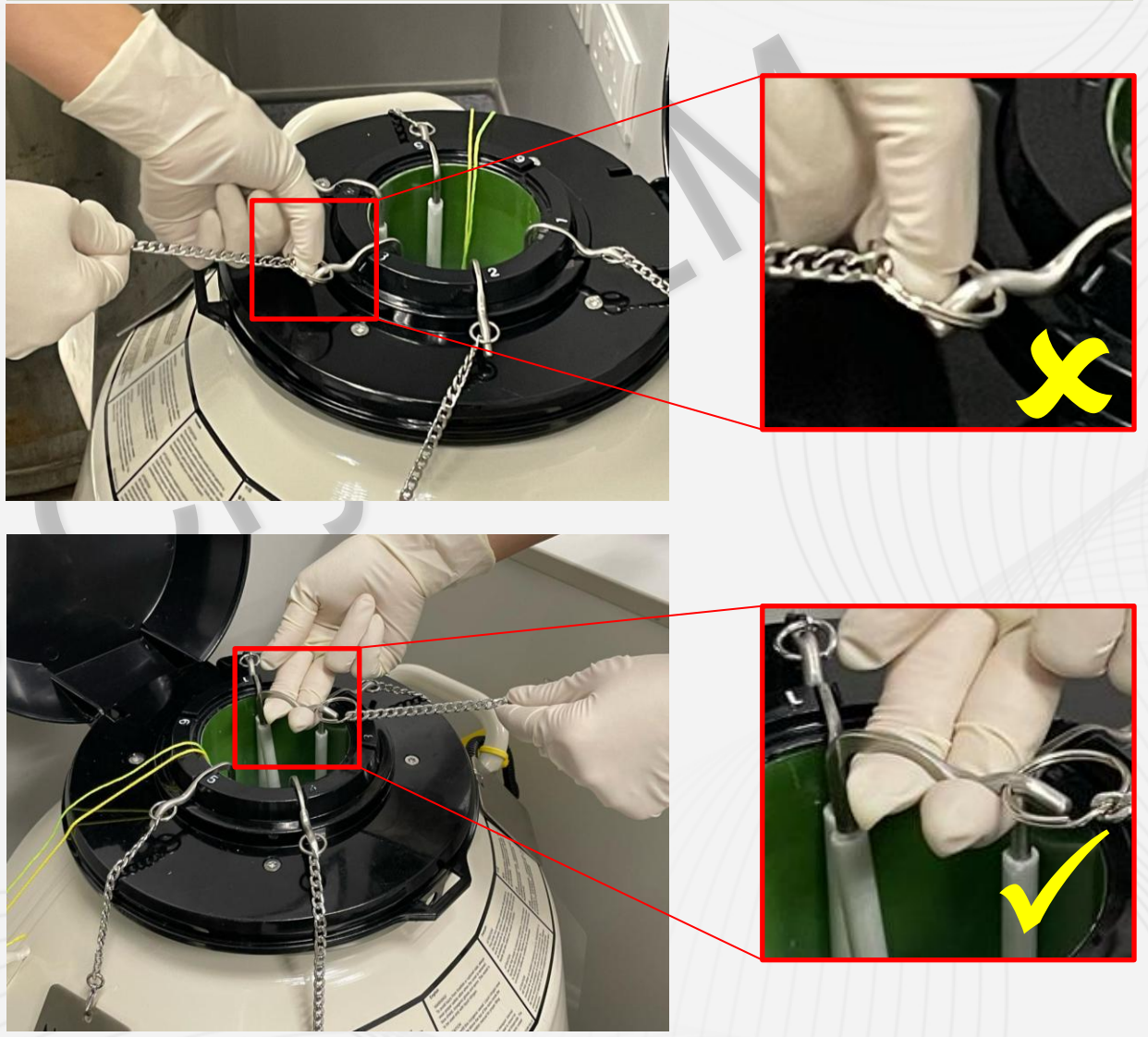
Grid storage

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 tweezer

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

Leica EM ICE High Pressure Freezer

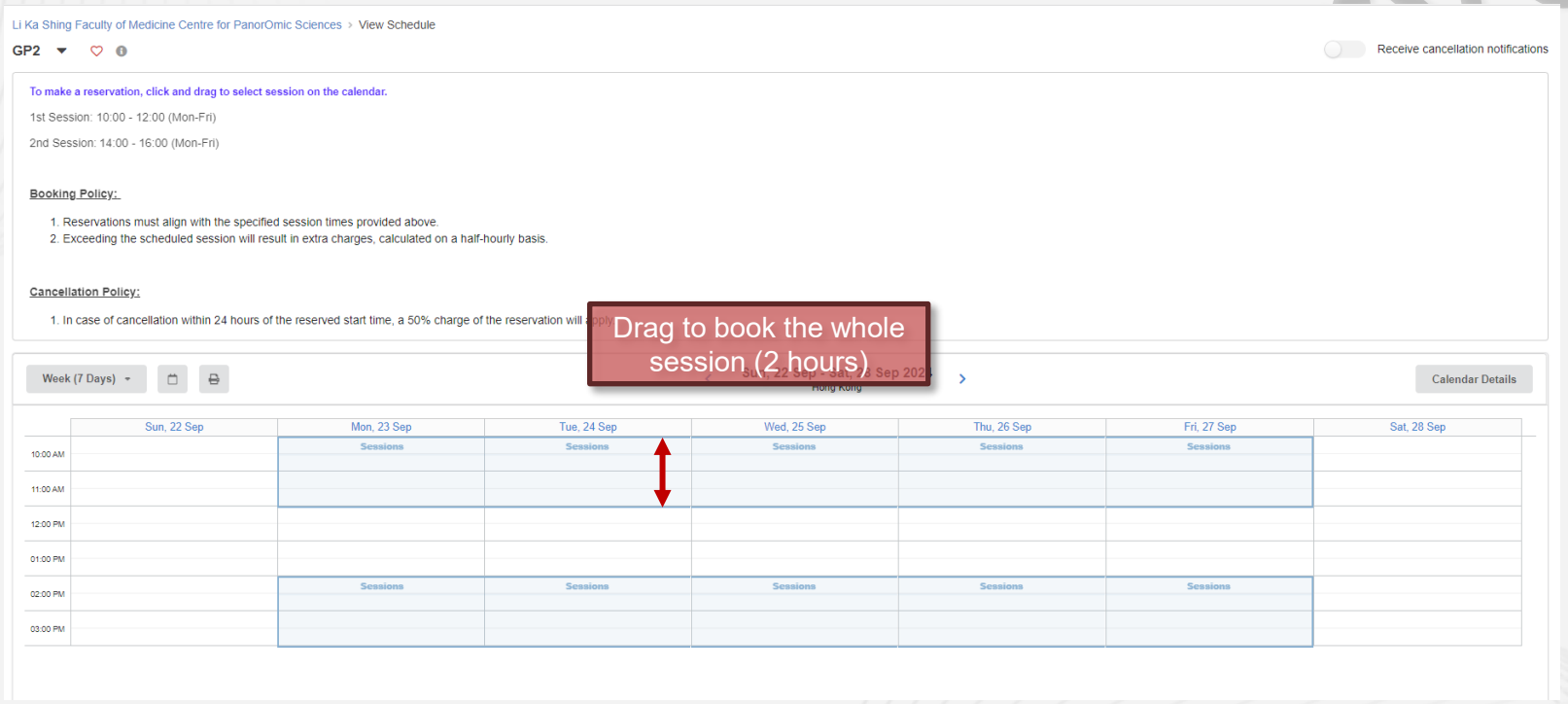
Vitrification

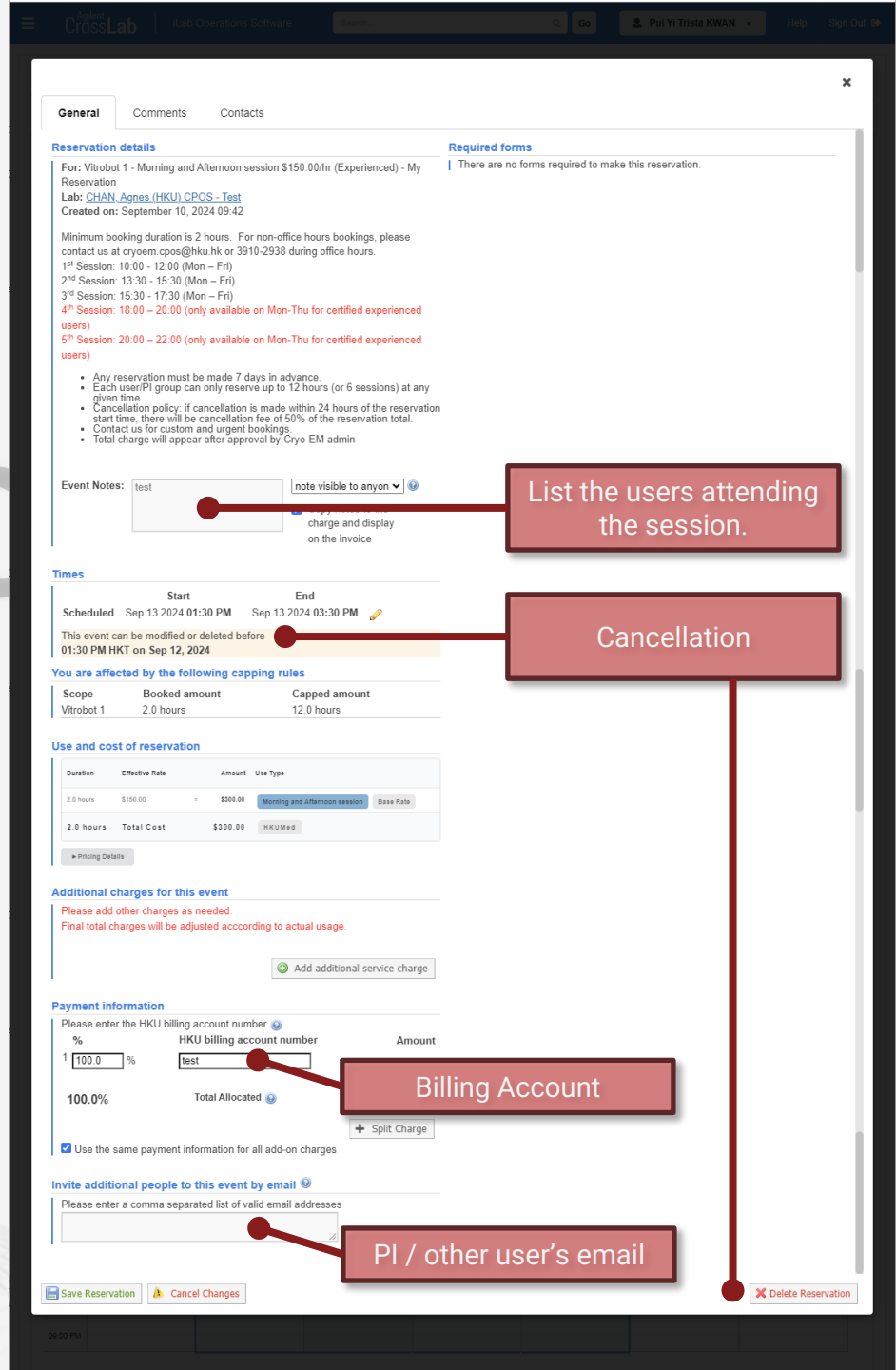
Booking Sessions

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

Booking Policy

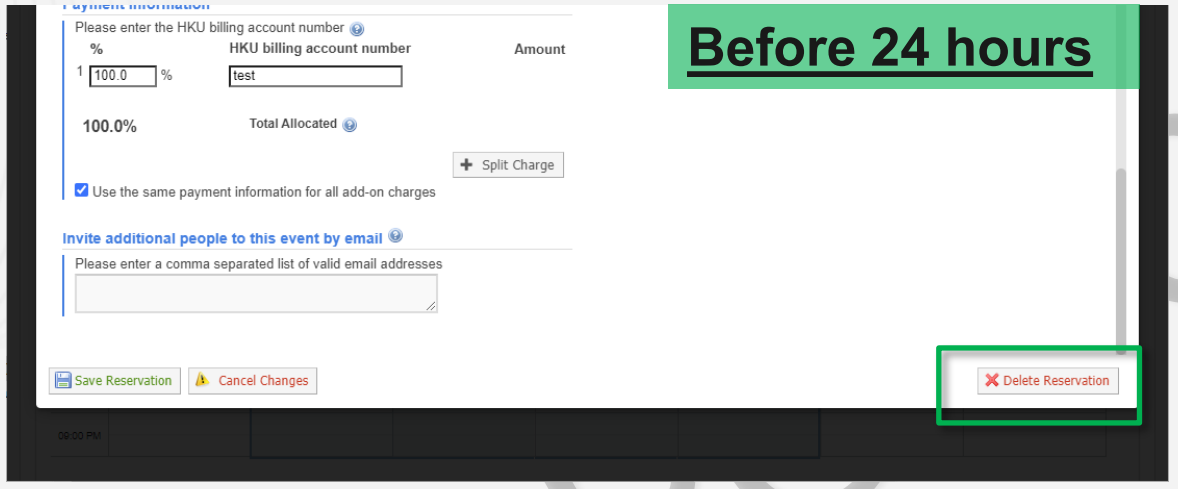
- Open for 28 days booking
- Card access: User can access the instrument on the booking date



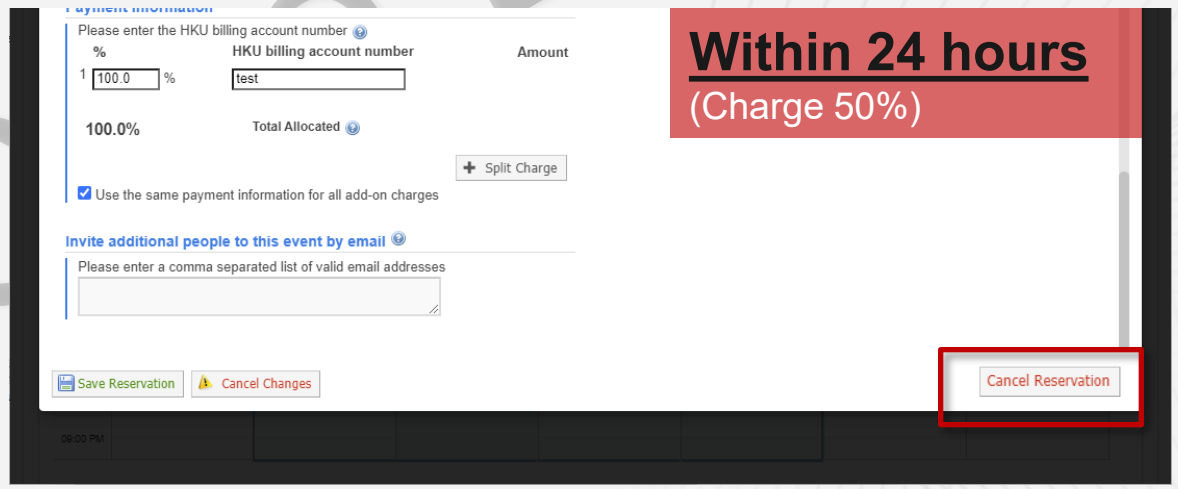


Cancellation Policy

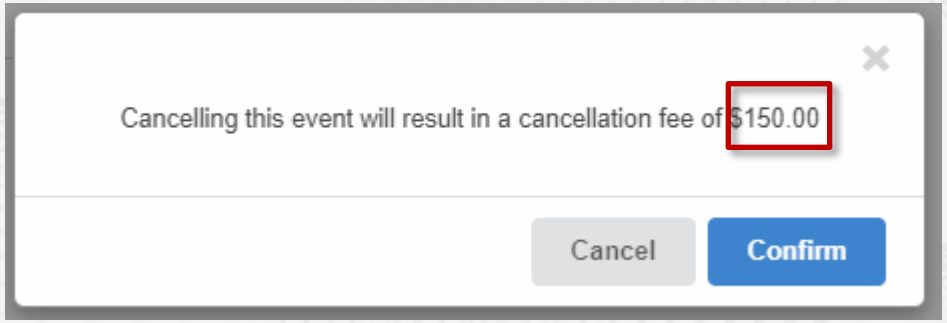
- Before 24 hours → Free cancellation
- Within 24 hours → 50% charge
- Session starts → 100% charge



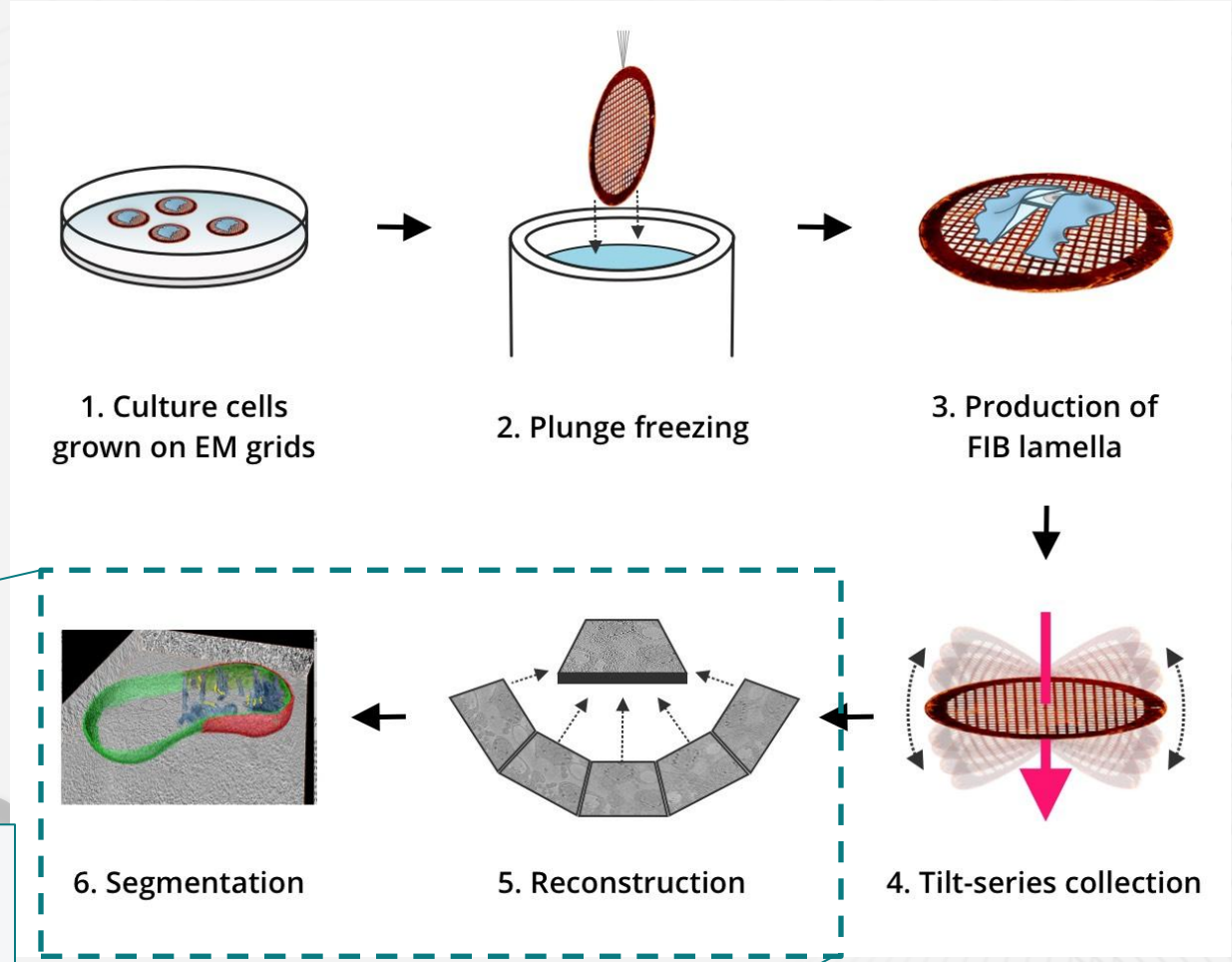
Before 24 hours

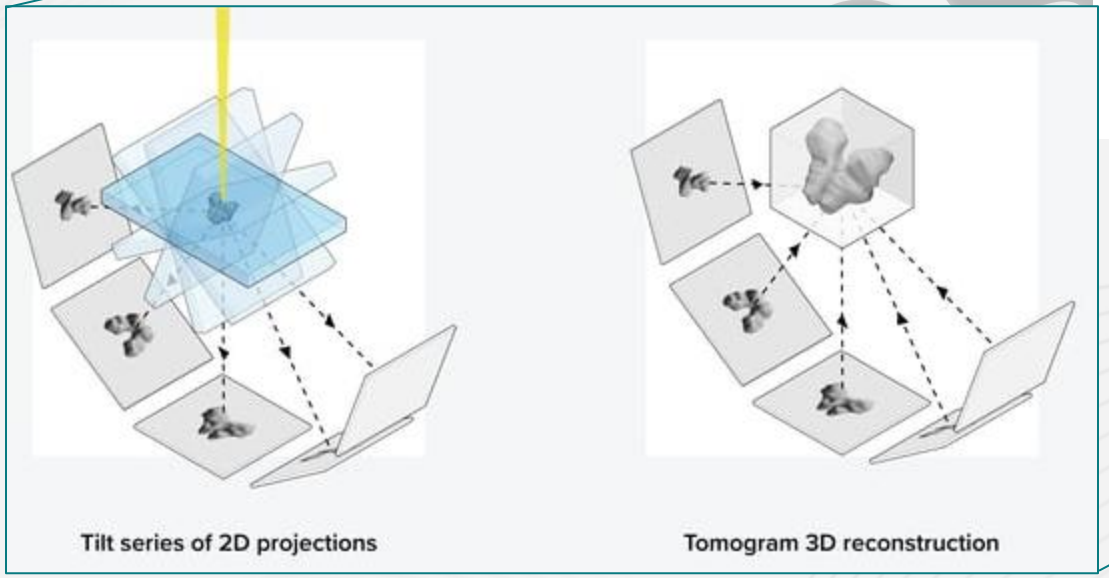


Within 24 hours
(Charge 50%)

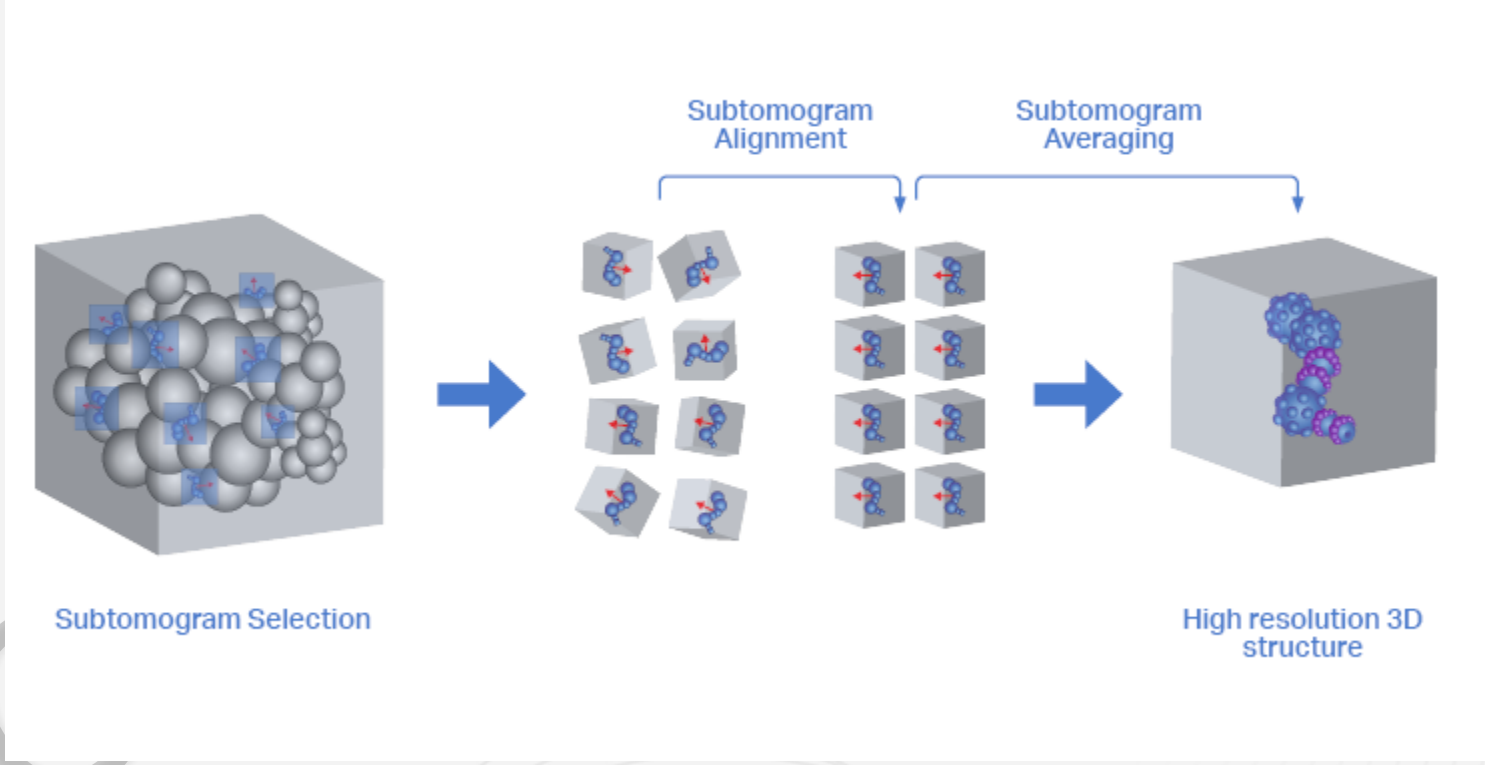


Cryo-ET (Tomography)





Sub-tomogram Averaging

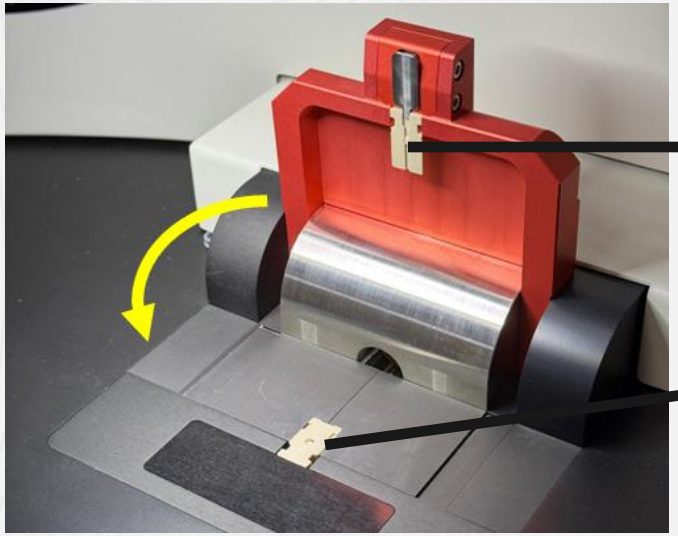


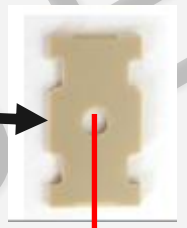
Sample Freezing

Thickness (less than 30um)	Thickness (30-200um)
GP2 	HPF 
Cell (monolayer) Protein sample	Cell Tissue

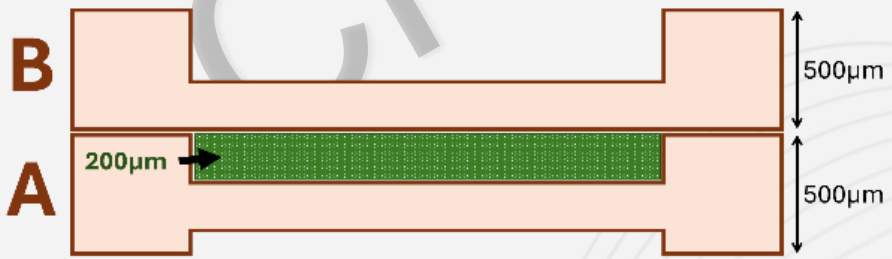
Tools

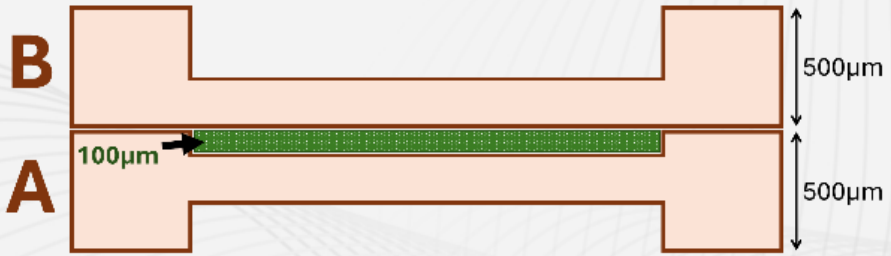
- Cartridge (upper / lower half cylinder and middle plate)
- Tissue punch
- Cryo-storage container
- Microscope stage insert (for sapphire system)
- Cryo-box
- Sample Carrier Release
- Trisection pod holder
- Cryo-storage holder container
- Cryo-protectant, e.g. Hexadecene, sucrose (prepared by user)



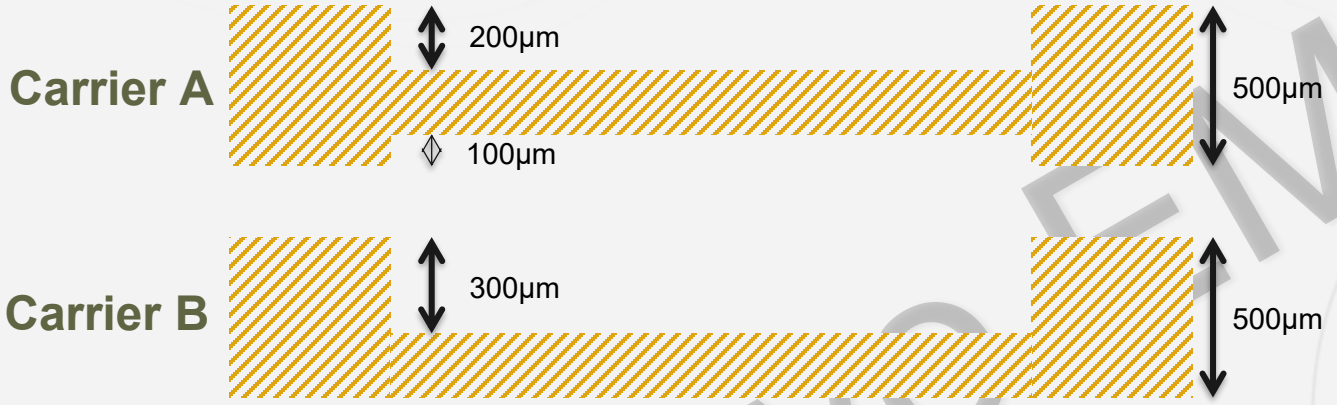


Set up

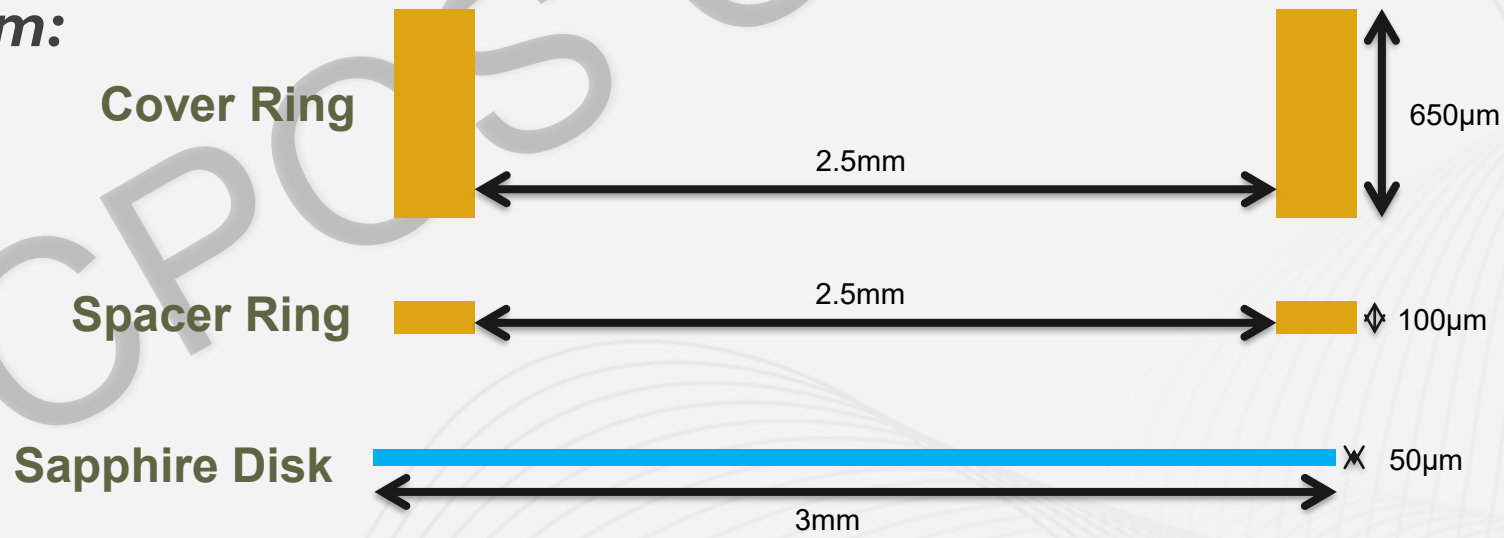




Specimen system:



Sapphire system:



Consumables

3mm Specimen system:

Sample holder middle plate	**1mm**	
	Thickness	Inner height
Carrier (A)	500um	100 / 200um
Carrier (B)	500um	300um

3mm Sapphire system

Sample holder middle plate	**850um**
	Thickness
Sapphire disc	50um
Cover ring	300um

6mm Specimen system:

Sample holder middle plate	**1mm**	
	Thickness	Inner height
Carrier (A)	500um	100 / 200um
Carrier (B)	500um	300um

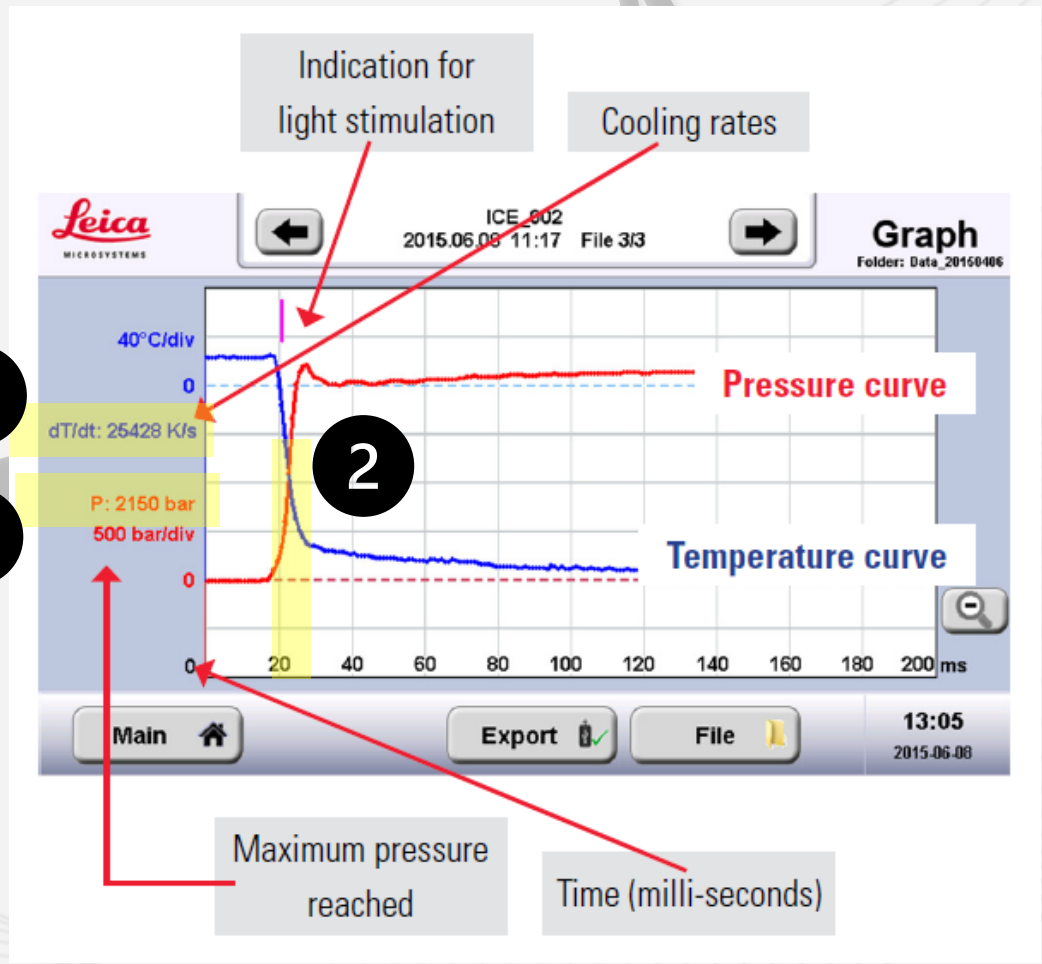
6mm Sapphire system

Sample holder middle plate	**820um**
	Thickness
Sapphire disc	120um
Spacer ring	200um

Consumables	Price
Carrier, Cu	$150
Spacer ring / Cover ring	$100
Sapphire discs, 3mm	$200
Sapphire discs, 6mm	$400

Blank Run Checking

dT/dt	larger than 16000 K/s
The interaction between T curve/ P curve	within 30ms
The Pressure	~ 2100 bar



Shut down and Bake Out

- Check with staff if there is next user on that day
- Drain LN2
- Bake out the system (5-6 hours)
- Put caution sign in front of ICE
- Switch off the system