

CROSS Cryo-EM

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

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.



Emergency Exit



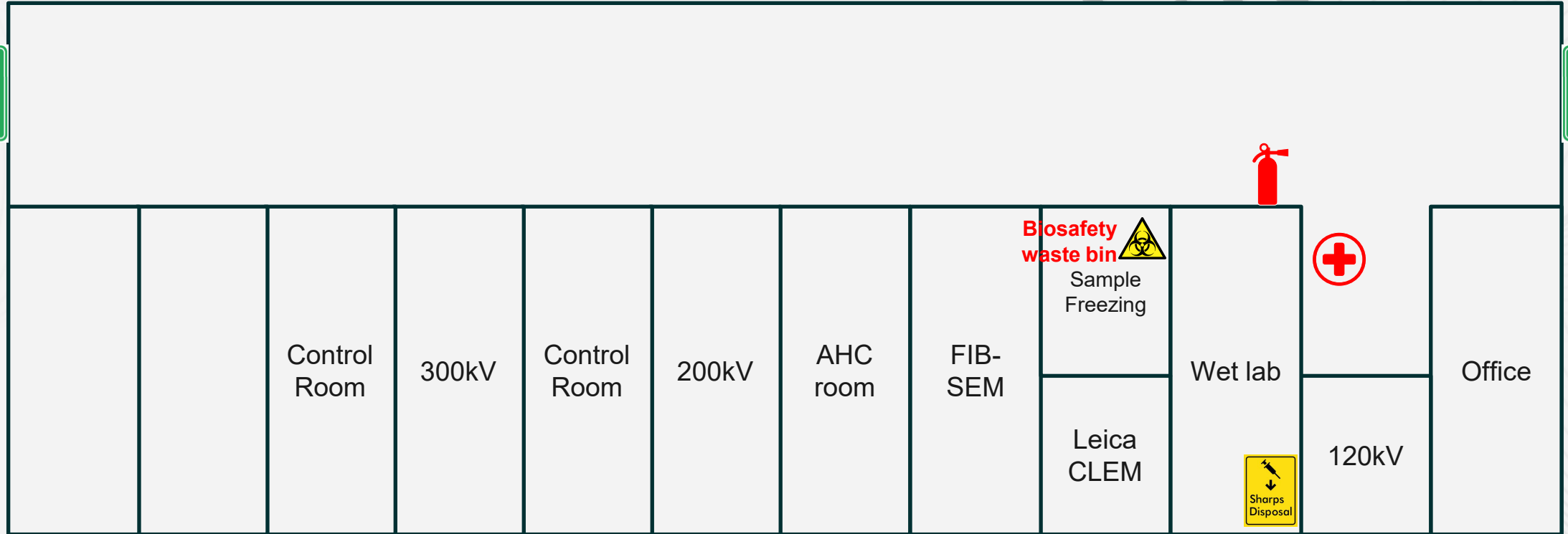
Biological waste bin



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)

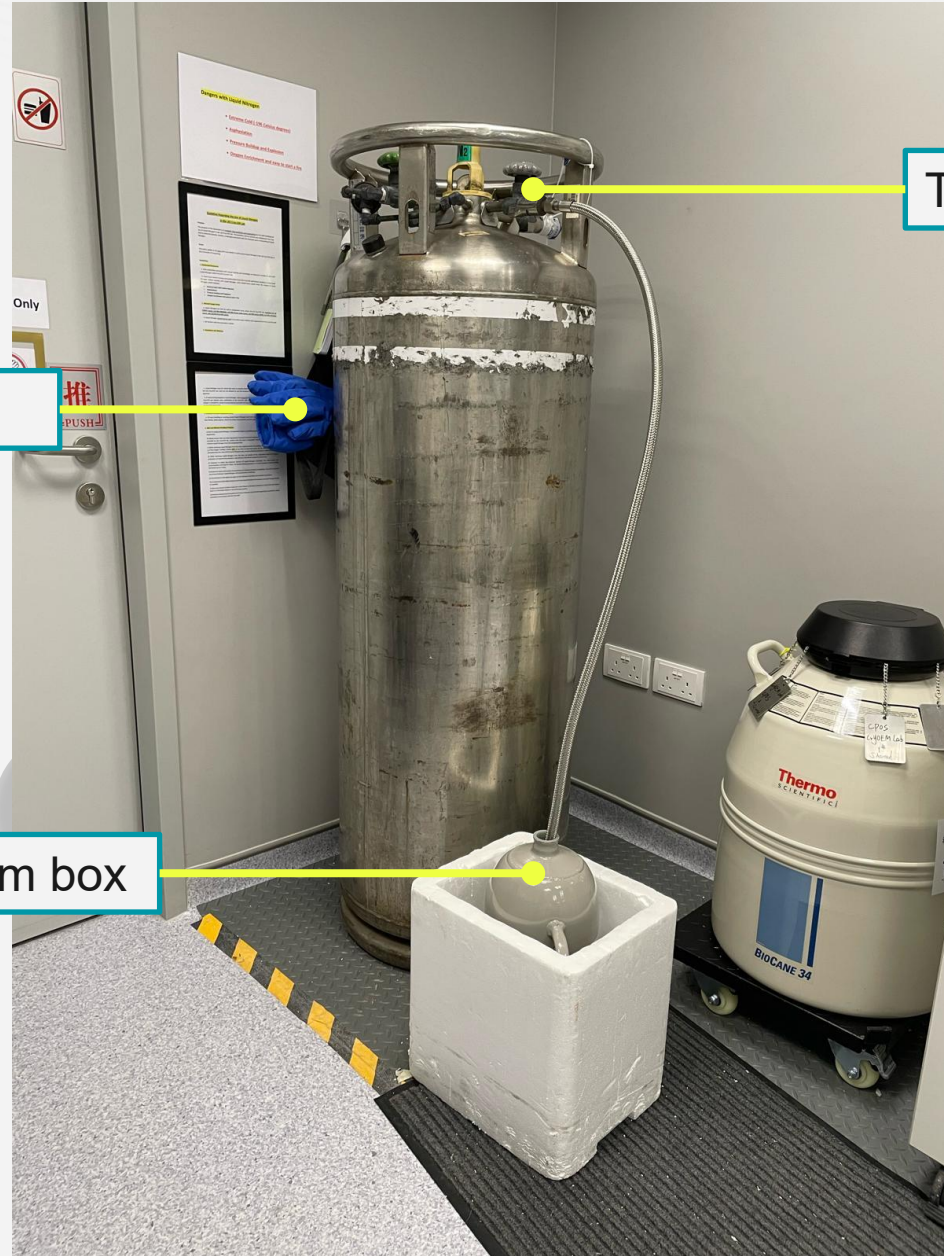


Foam Box

- **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



Use protective gloves

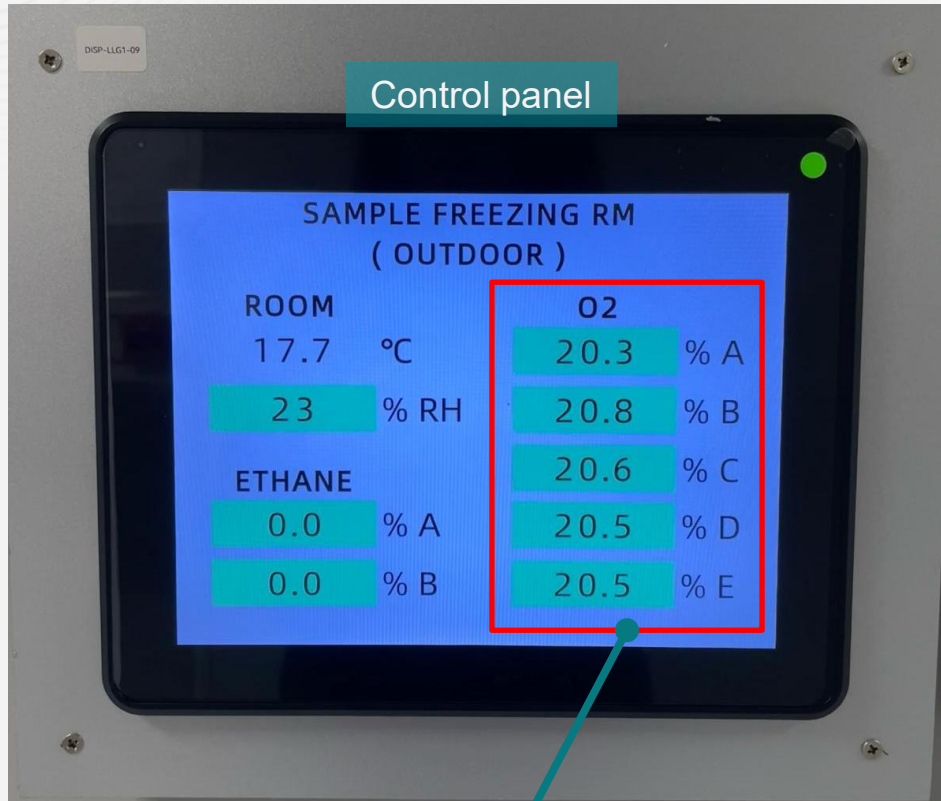
Turn on the switch

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.

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

d) Register your HKU card for access

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)



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 cp@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.

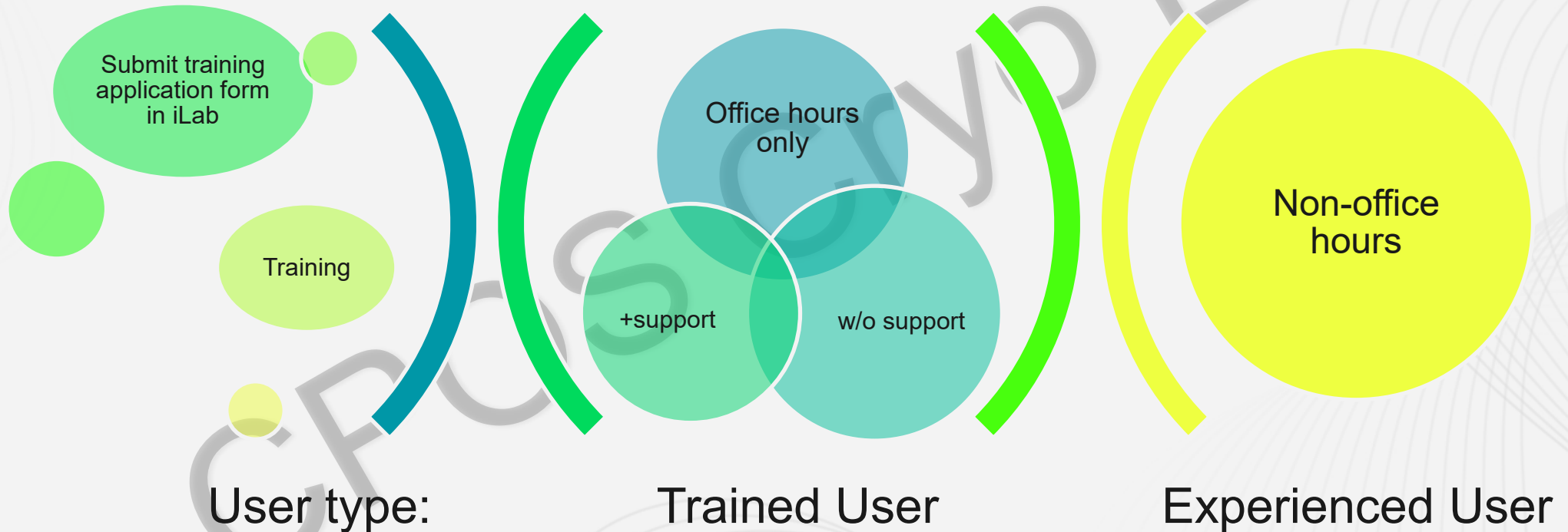
- If user has not booked within 2 months, **re-training** is required before using the instrument.

Usage	Min. usage / Session	HKU-Med	HKU
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.



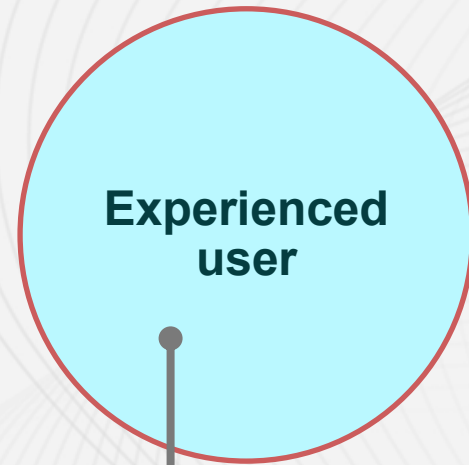
Apply training in iLab



After 5 working days



Pass



- 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

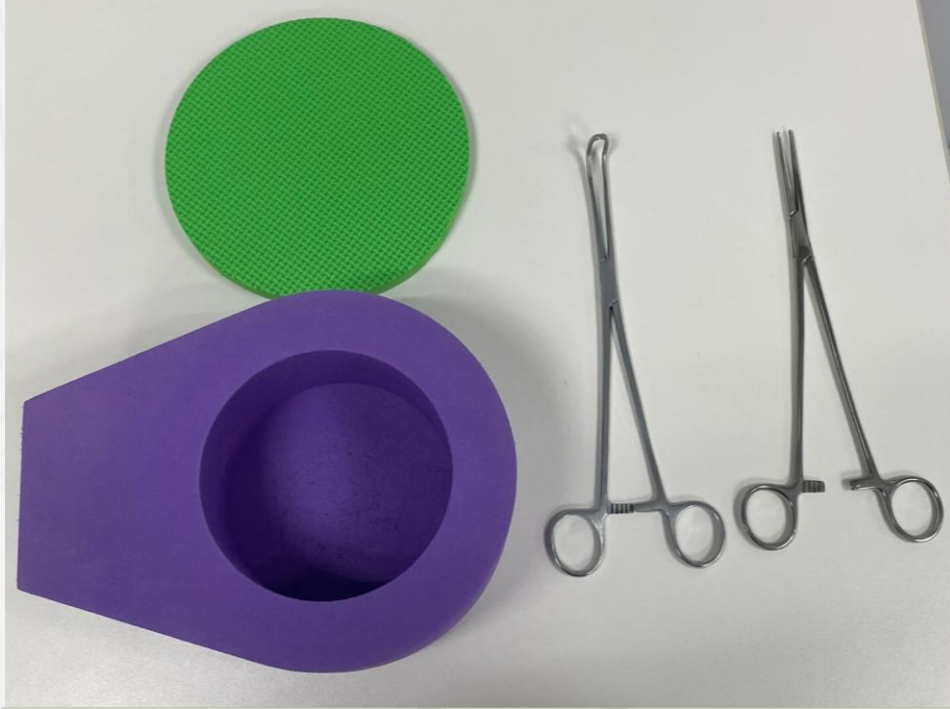




Grid storage



Grid storage

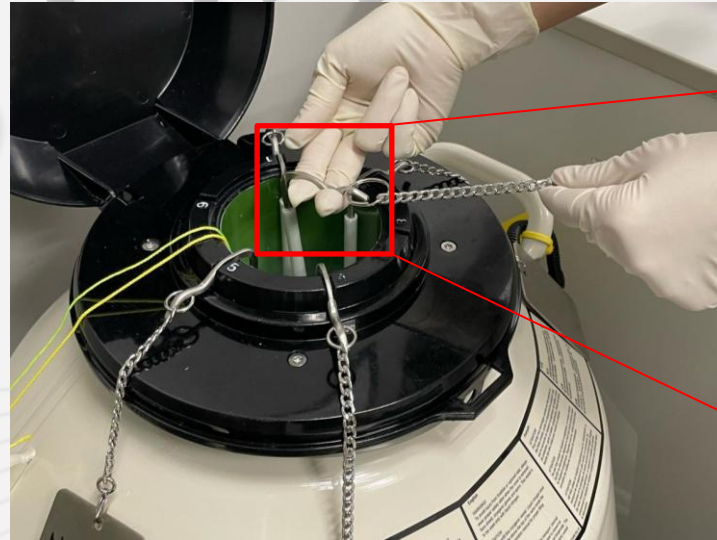
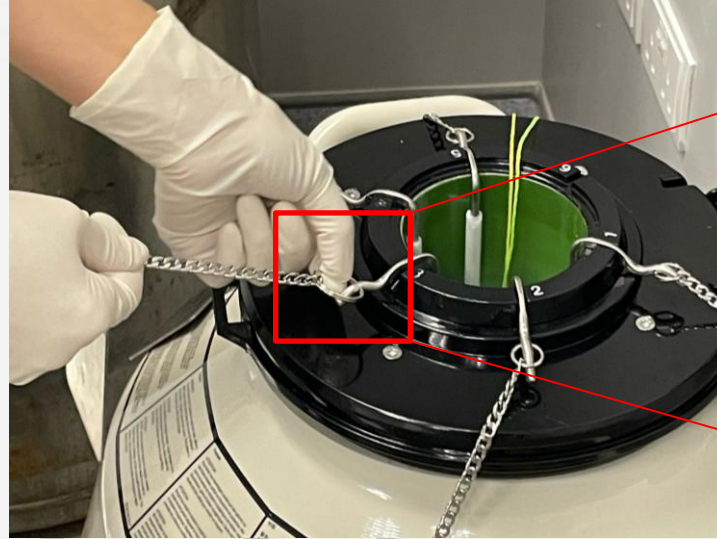


1

Tools

2

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



3

Put the puck system on the desk

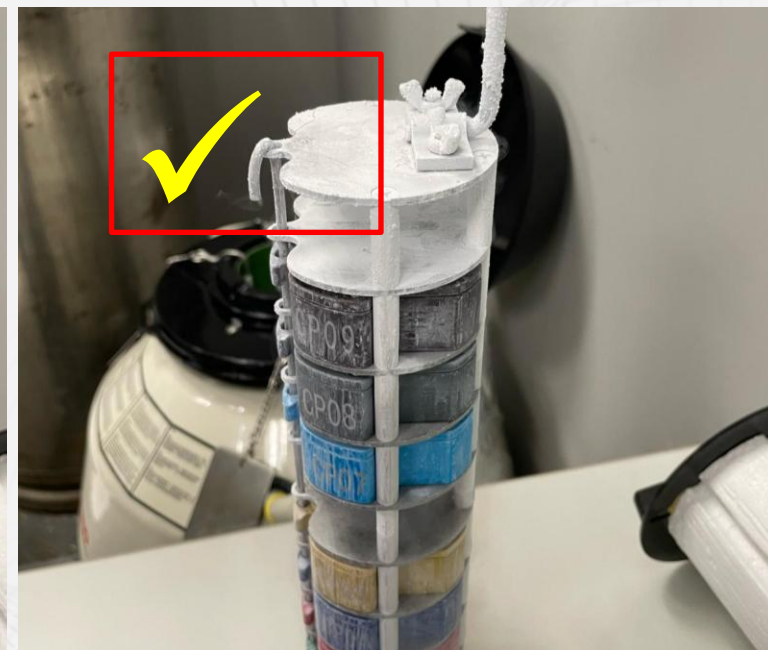
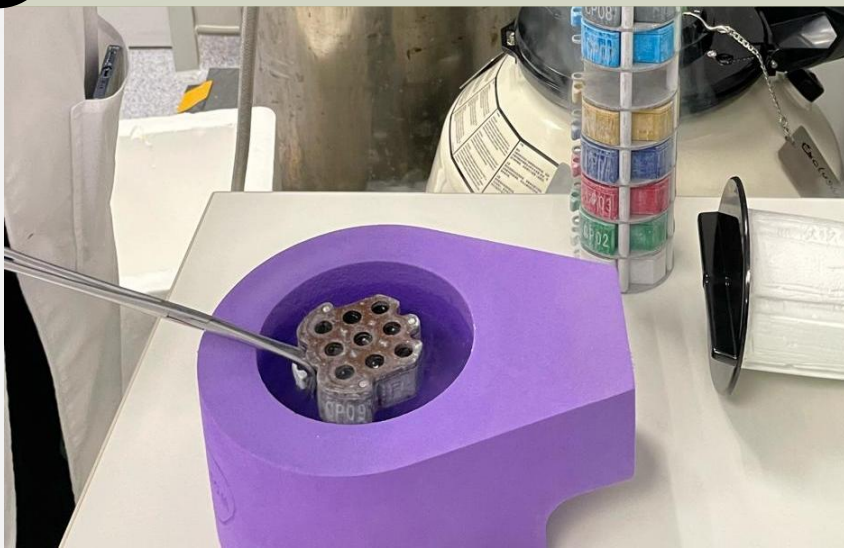


4

Take out the pin using tweezers

5

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



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

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.
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] [charge and display on the invoice]

Times

Scheduled	Start	End
	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

Save Reservation | Cancel Changes | Delete Reservation

Callouts:

- 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

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

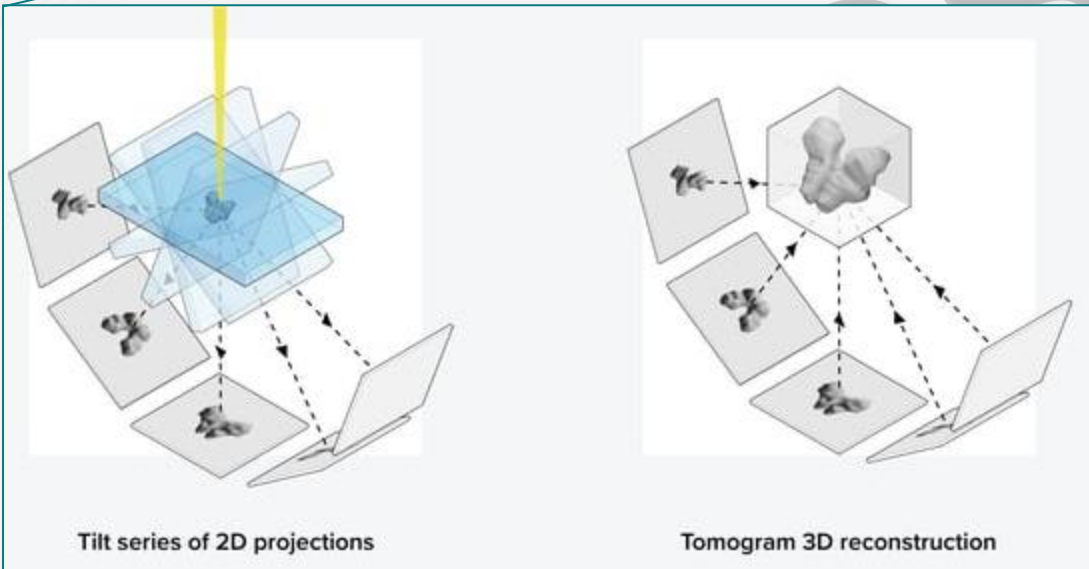
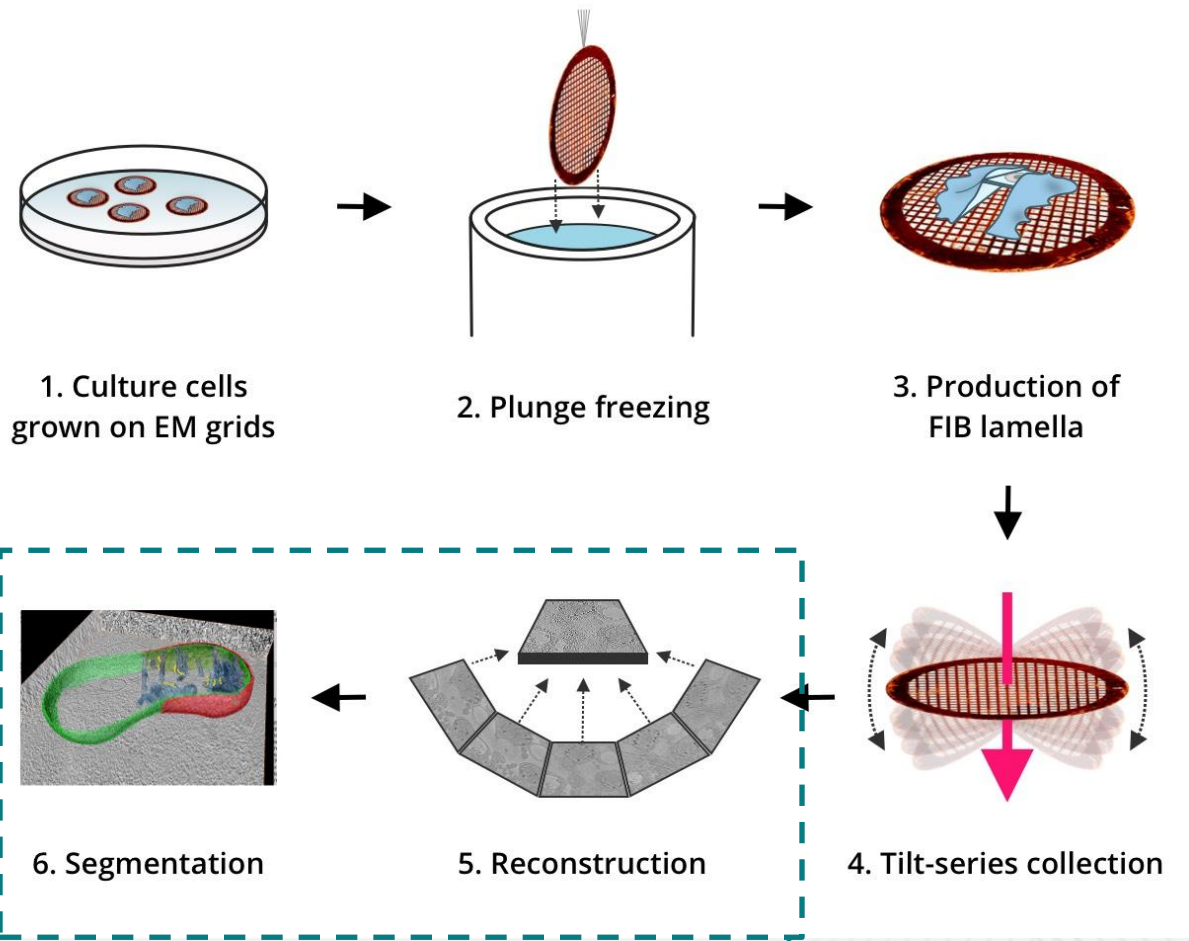
Within 24 hours
(Charge 50%)



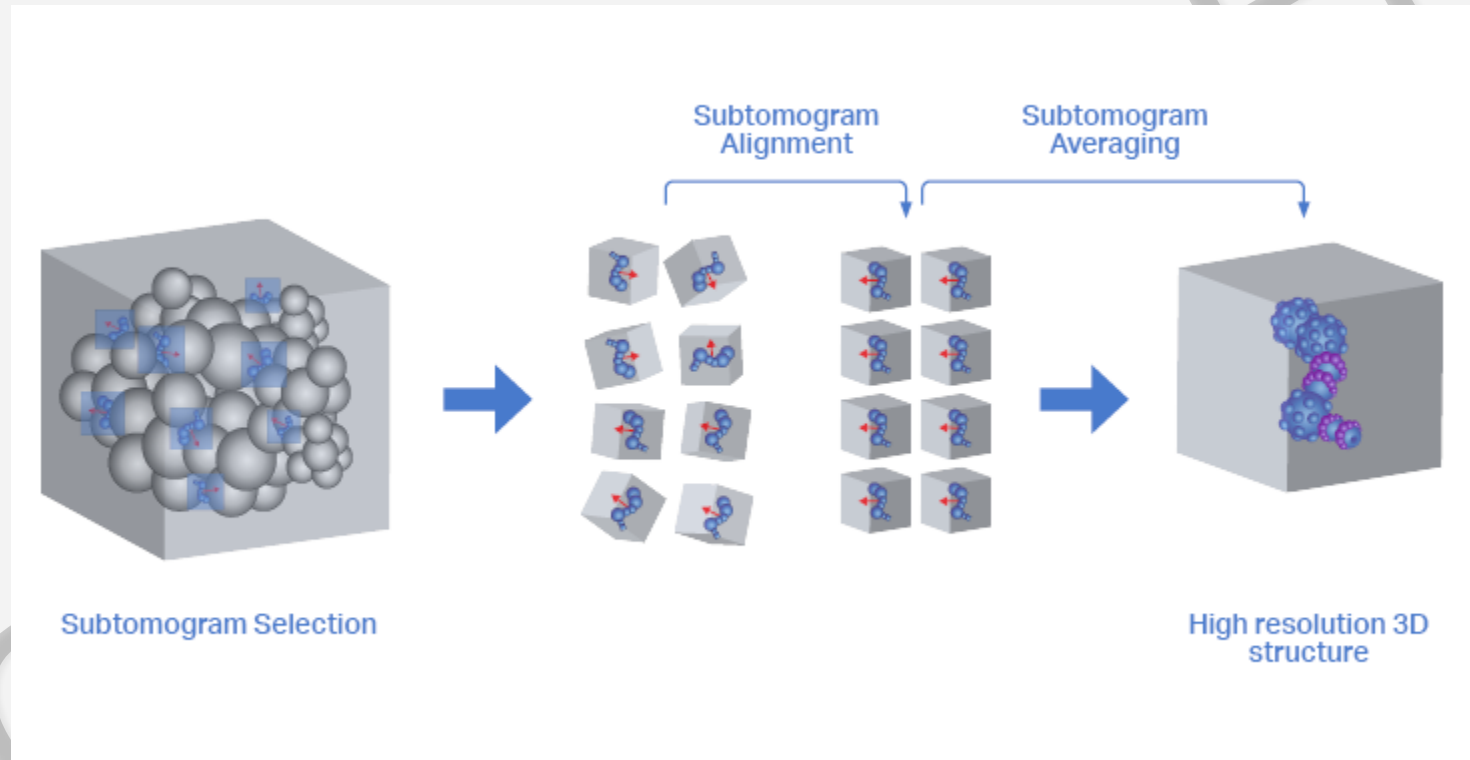
Cancel ×

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

Cryo-ET (Tomography)



Sub-tomogram Averaging



Sample Freezing

Thickness (less than 30um)

GP2



Cell (monolayer)
Protein sample

Thickness (30-200um)

HPF



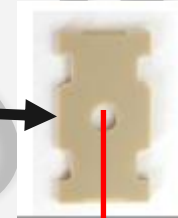
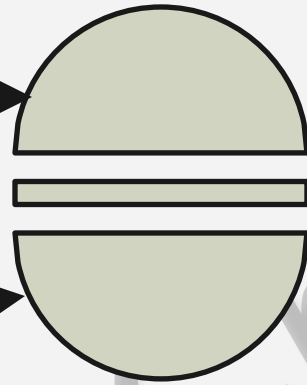
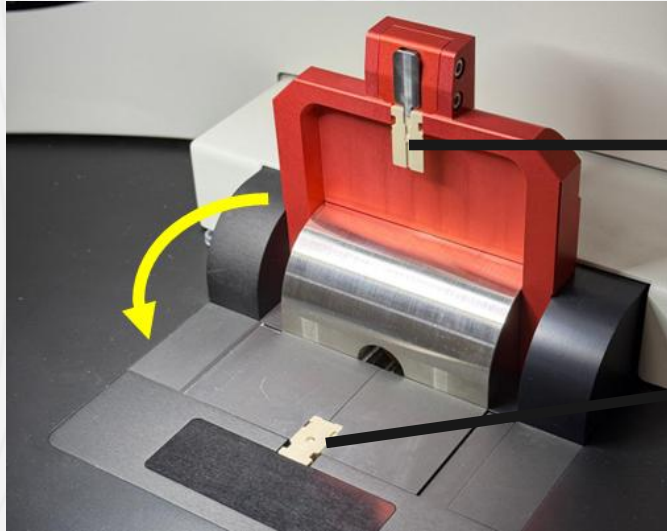
Cell
Tissue

Tools

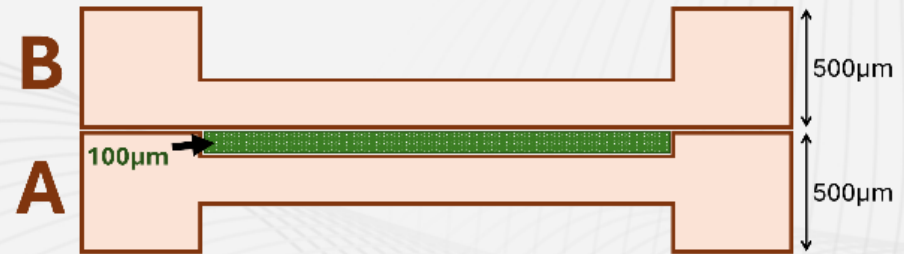
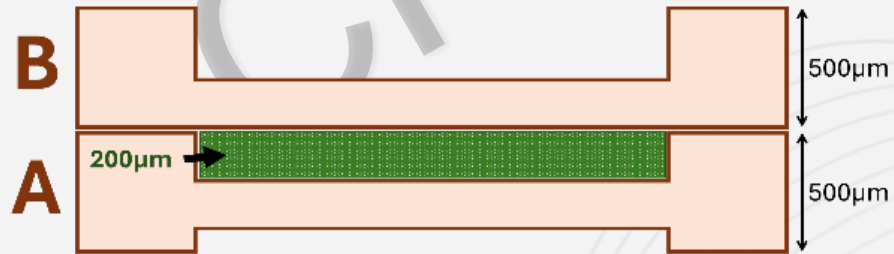
- Cartridge (upper / lower half cylinder and middle plate)
- Tissue punch
- Cryo-storage container
- Microscope stage insert (for sapphire system)
- Cryo-box
- Sample Carrier Release
- Trisection pod holder
- Cryo-storage holder container
- Cryo-protectant, e.g. Hexadecene, sucrose (prepared by user)



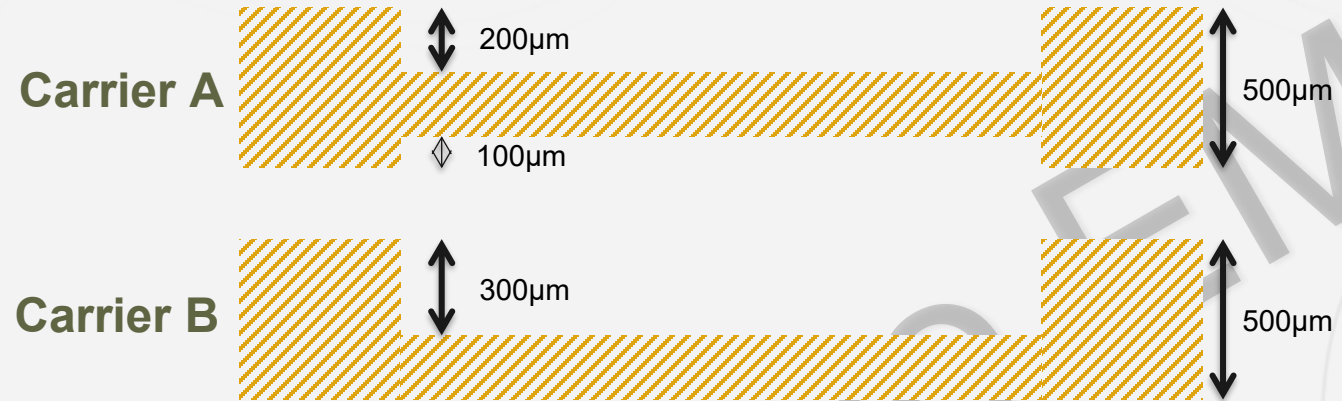
CPOS Cryo EM



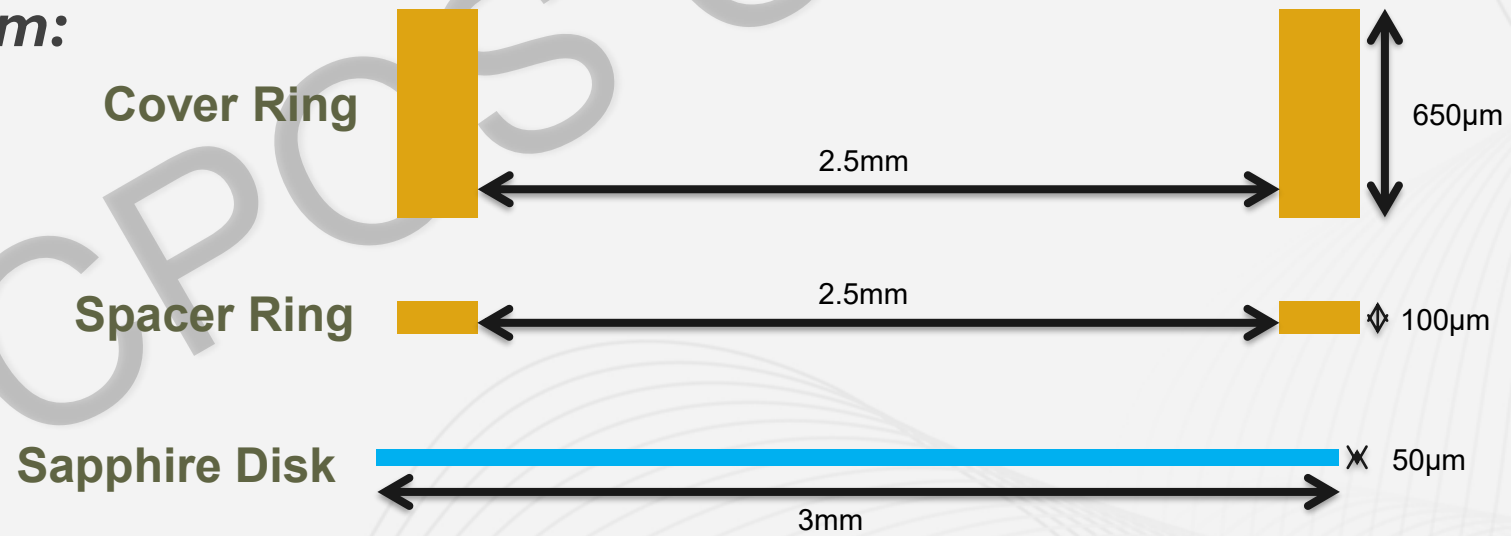
Set up



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

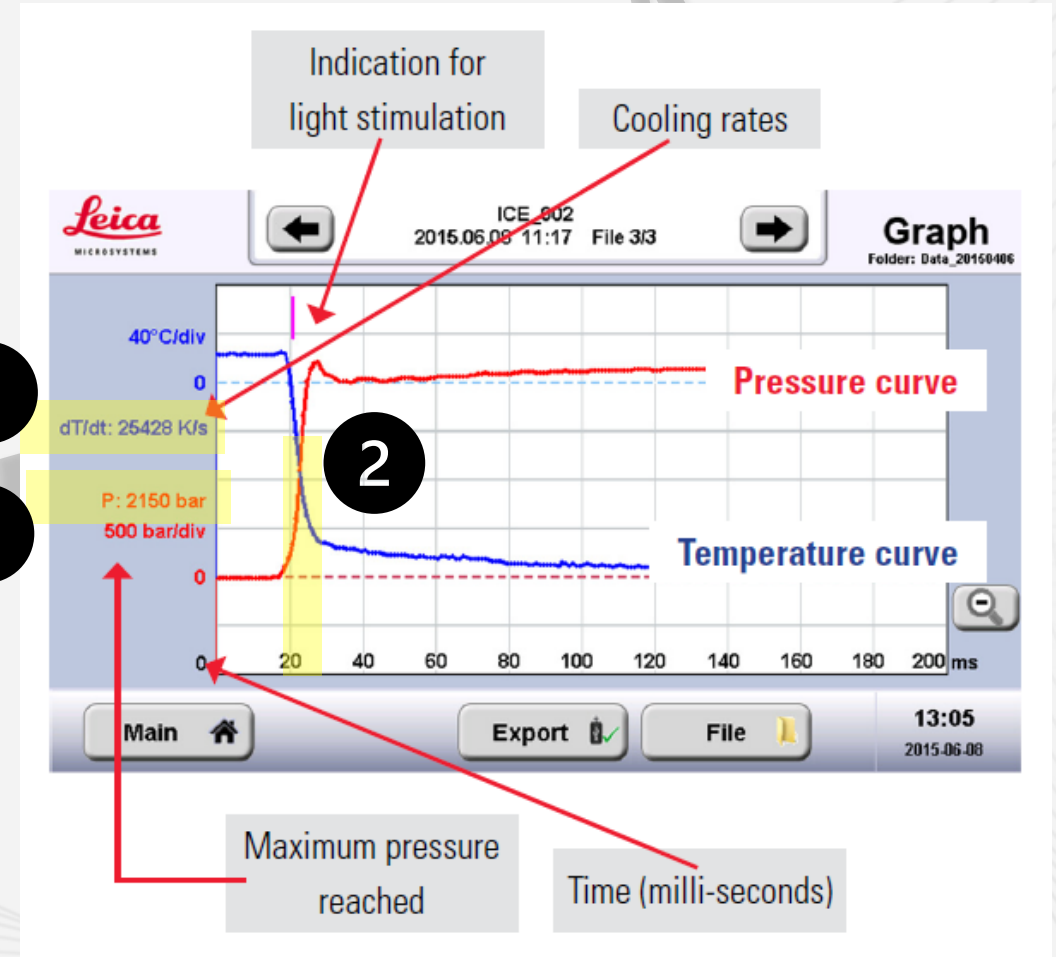
6mm Sapphire system

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

CPOS Cryo EM