

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

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 | 2351-6600

Home | Core Services | Other Equipment | News | Events | Publications | FAQ | About CPOS | Careers | Contact

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



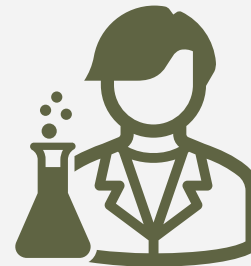
The last user of the day must turn off light and lock the doors



No drinking and eating at the computer area



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



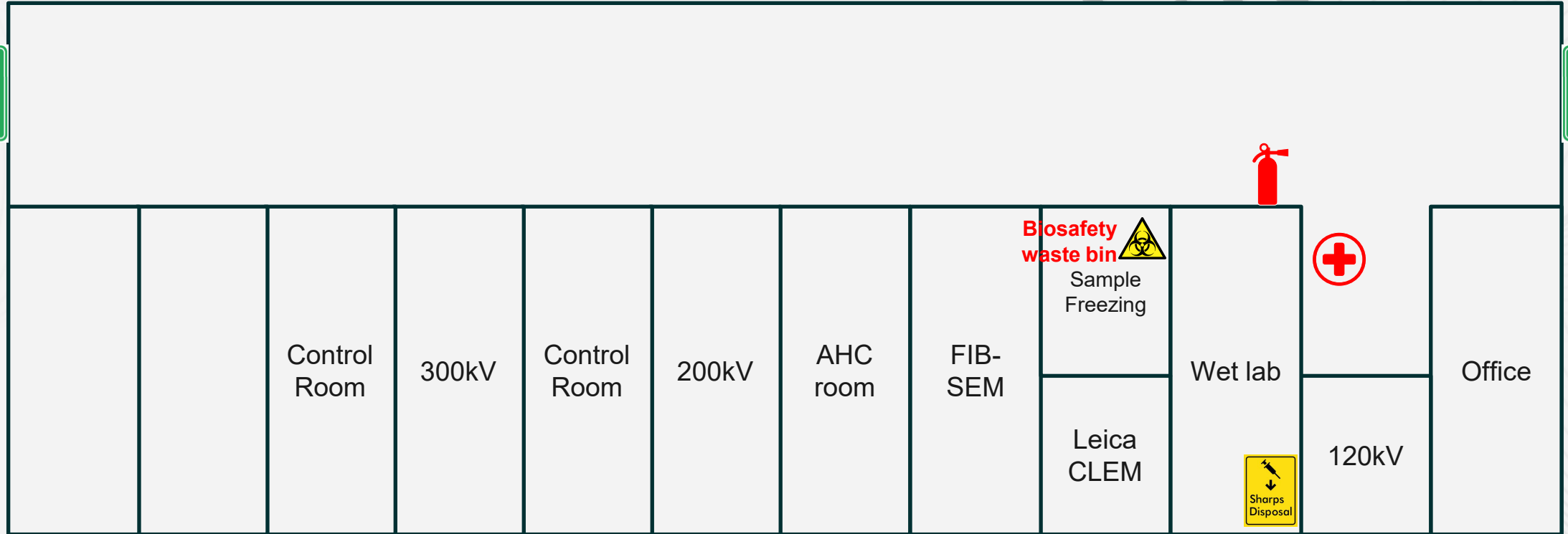
Biological waste bin



Sharp box



Safety (Floor Plan)



Safety (Gas)



Liquid Nitrogen

Containers for Liquid Nitrogen



● Dewar

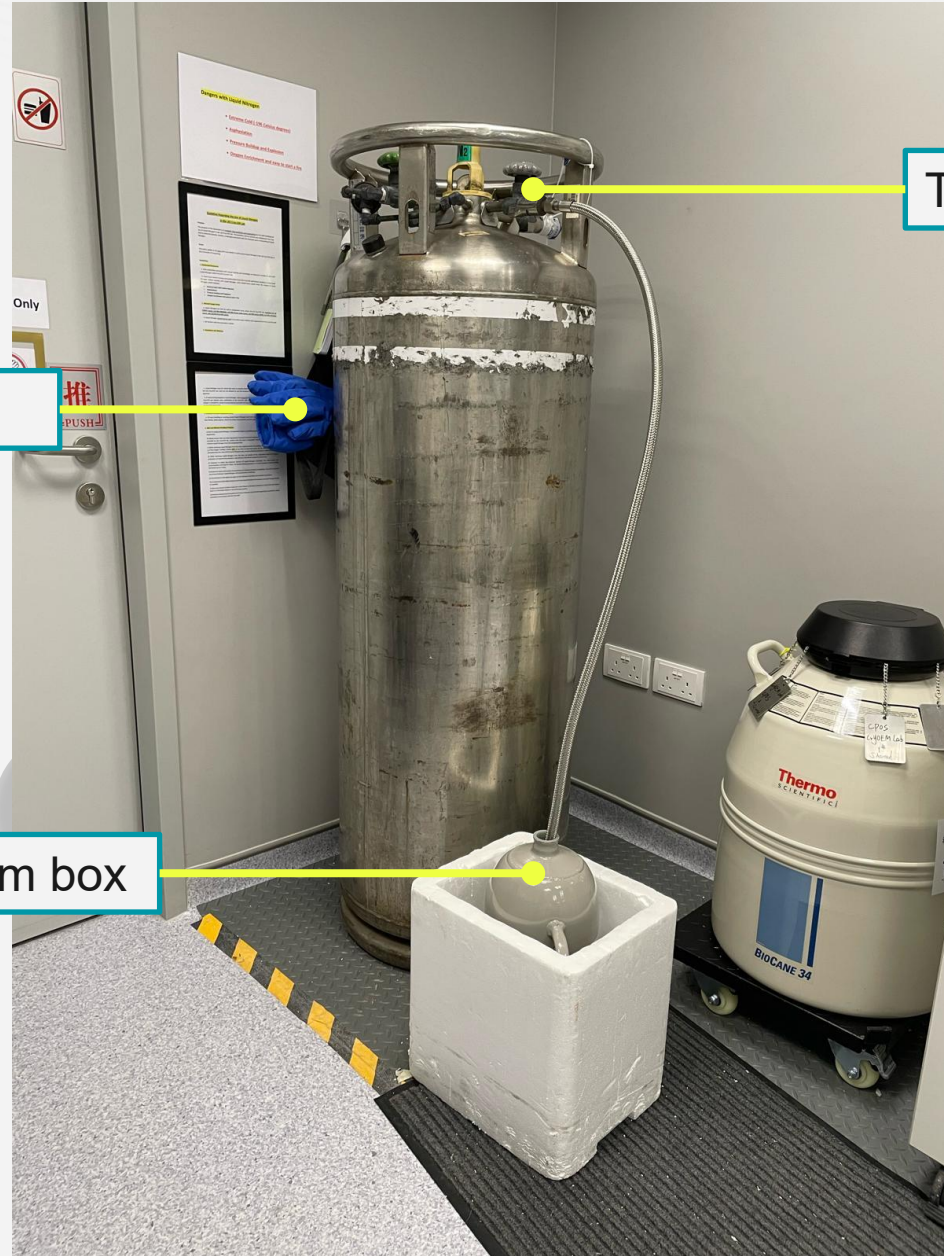
Always put on the floor

- **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



Stainless steel vacuum bottle (with loosen cover)

Get Liquid Nitrogen



Use protective gloves

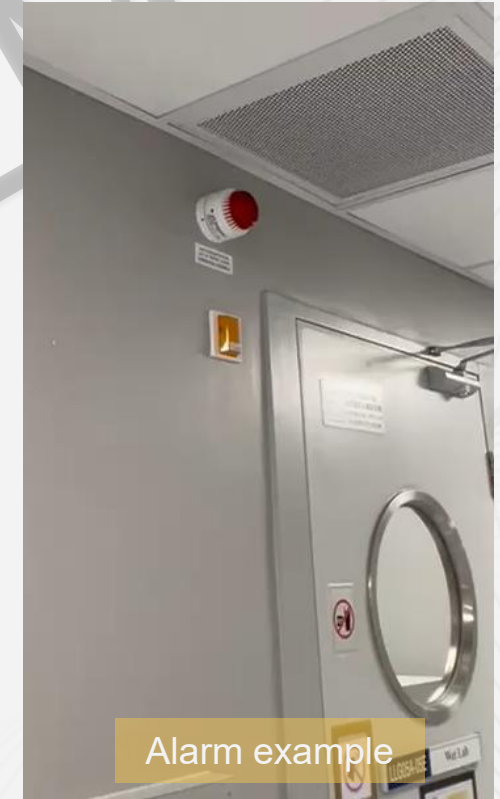
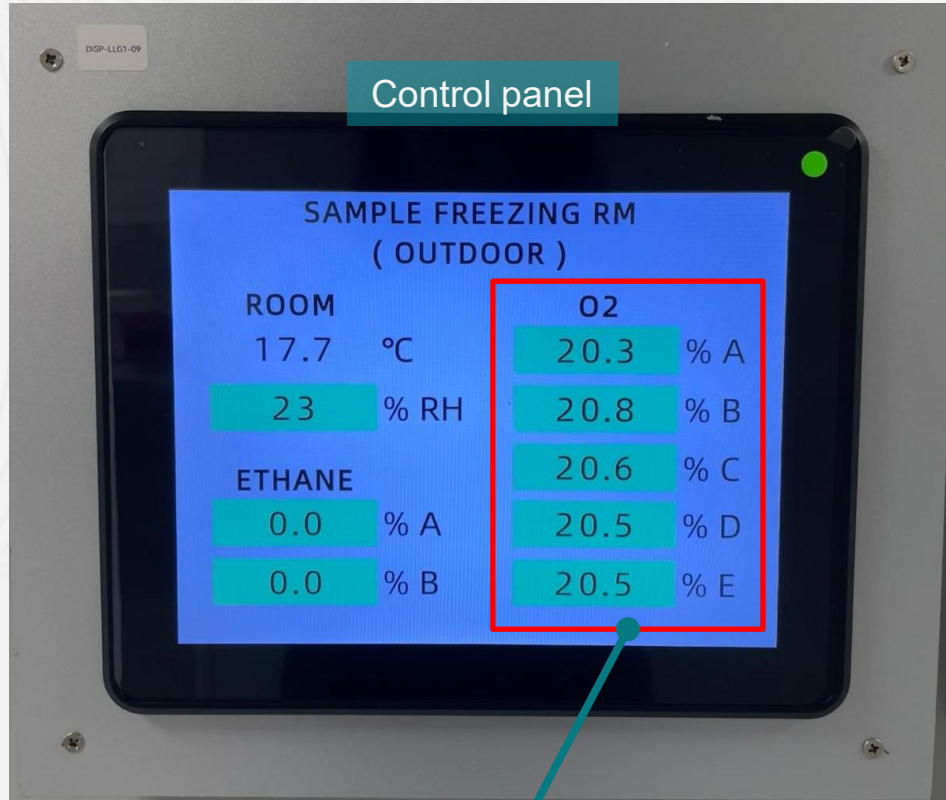
Turn on the switch

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

2. Training for 120kV

a) Submit training request in iLab

b) Training (with sample prepared, contact staff for booking)



Sample Information e-Form



iLab Registration Form

Charging

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

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

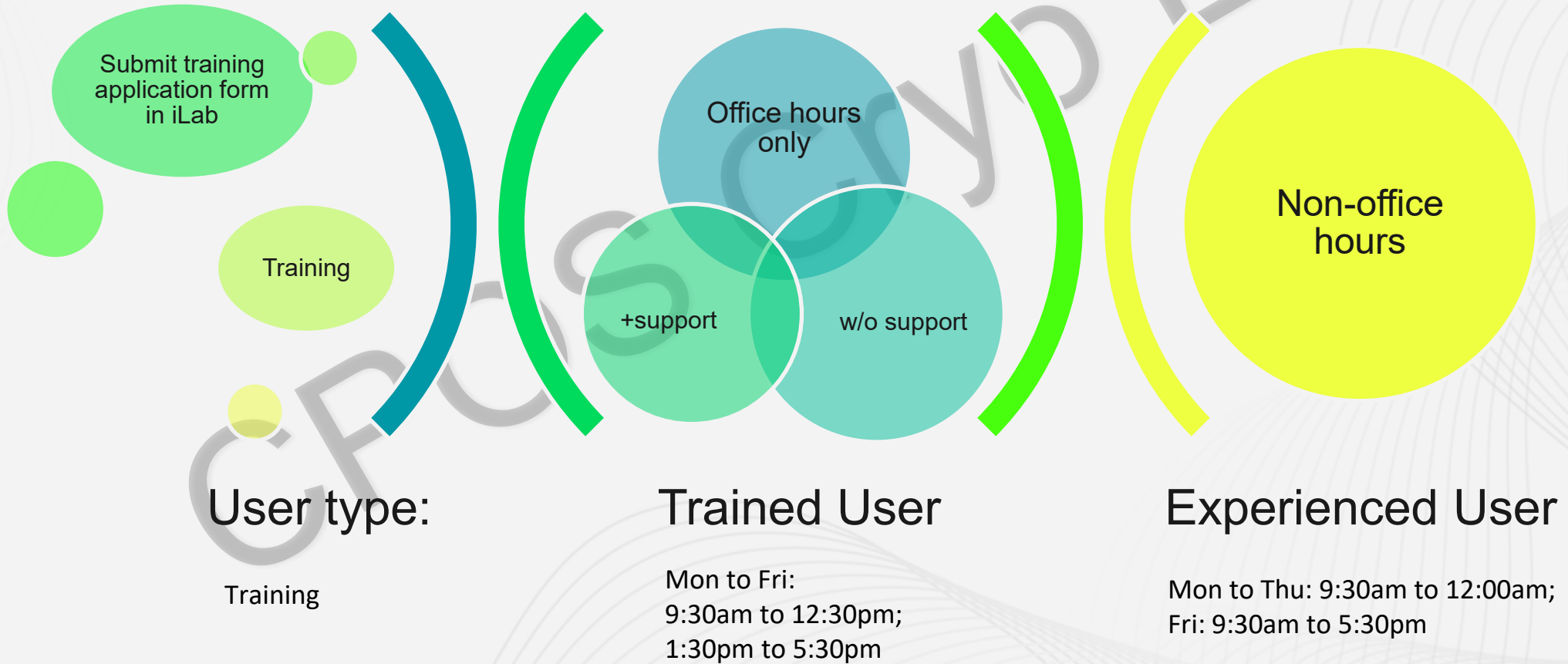
- If user has not booked within 2 months, **re-training** is required before using the instrument.

| Usage | Min. usage / Session | HKU-Med | HKU |
|-------|----------------------|--------------|--------------|
| 120kV | 1 hours | \$150 / hour | \$160 / 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.





Talos L120C TEM (120kV)

Booking Sessions

| Office Hour | |
|-------------------|--------------------|
| Morning Session | 9:30 am – 12:30 pm |
| Afternoon Session | 1:30 pm – 5:30 pm |

Minimum booking hours: 1 hour

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

Invite additional people to this event by email

Please enter a comma separated list of valid email addresses

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

Invite additional people to this event by email

Please enter a comma separated list of valid email addresses



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



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



Preparation

1: Check vacuum and stage is home

Workset

Setup Search Tune CCD camera

Vacuum (Supervisor)

Status: All Vacuum (Closed)

| | | |
|----------------|----|-----|
| Accelerator | 1 | Log |
| Column | 6 | Log |
| Detection Unit | 12 | Log |

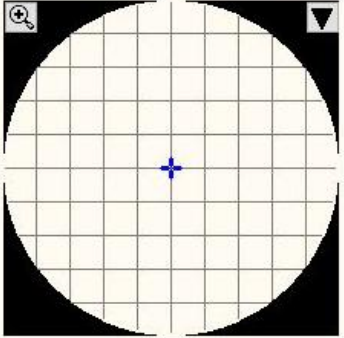
Nitrogen level **Low**
Turbo **Zero speed (0.0%)**

Col. Valve Closed Turbo pump

Stage²

Go Add Update Delete

Auto Euc Height Find Tracks



Control File S

Positions / tracks
Delete All Clear Tracks

Stage control
Power step (1/8 .. 8) 1/8
 XY separately above 500000 x

Reset
Holder XY A

Alpha wobbler Wobbler
0 5 10 15

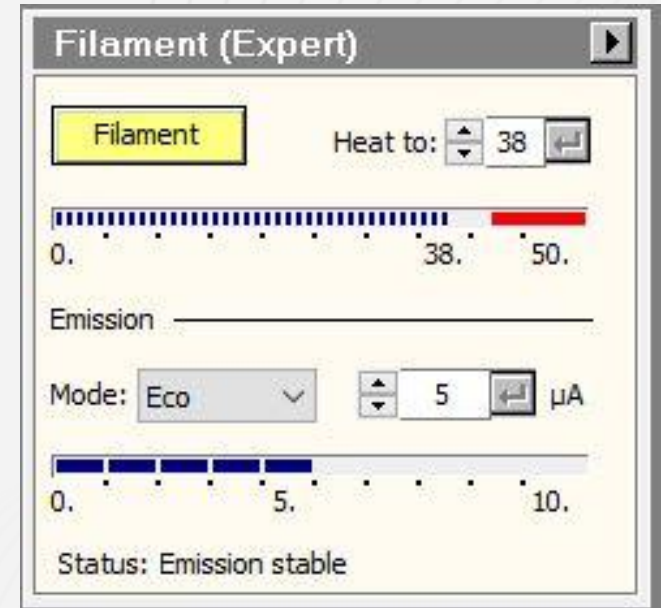
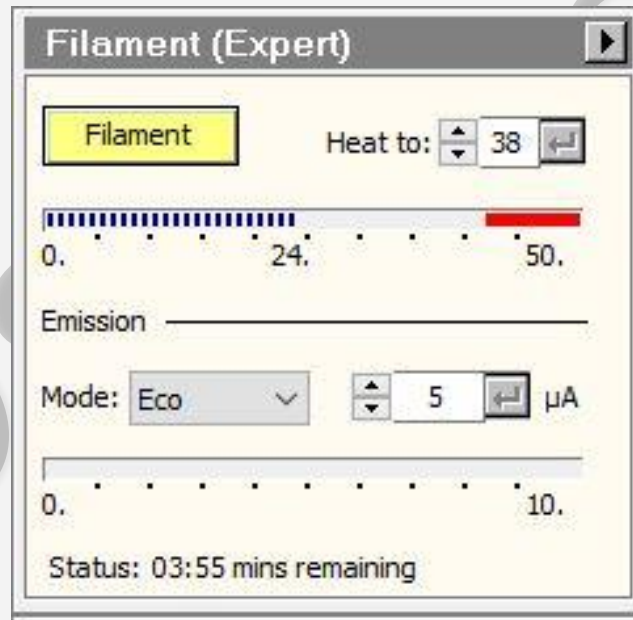
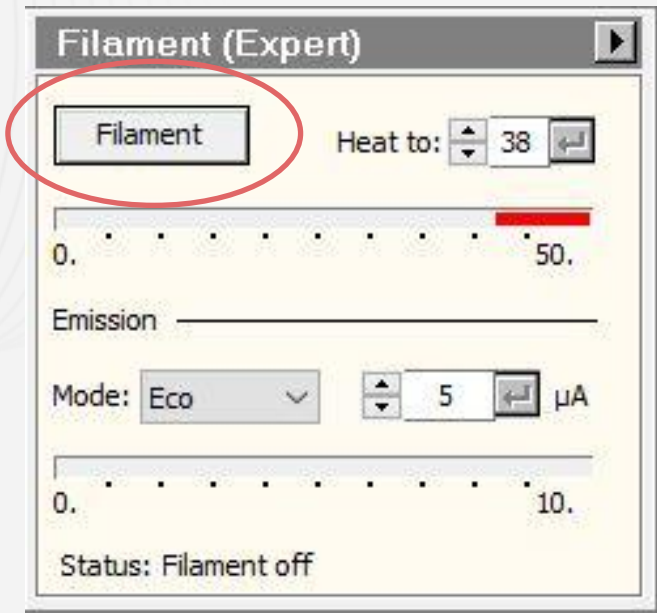
Alpha toggle
Set Alpha 15 °

S

| | | | |
|----|----------|-------------|-----------|
| X: | -0.00 μm | A: | -0.00 deg |
| Y: | 0.01 μm | | |
| Z: | 0.01 μm | Cool. BM-C: | Stable |

Preparation

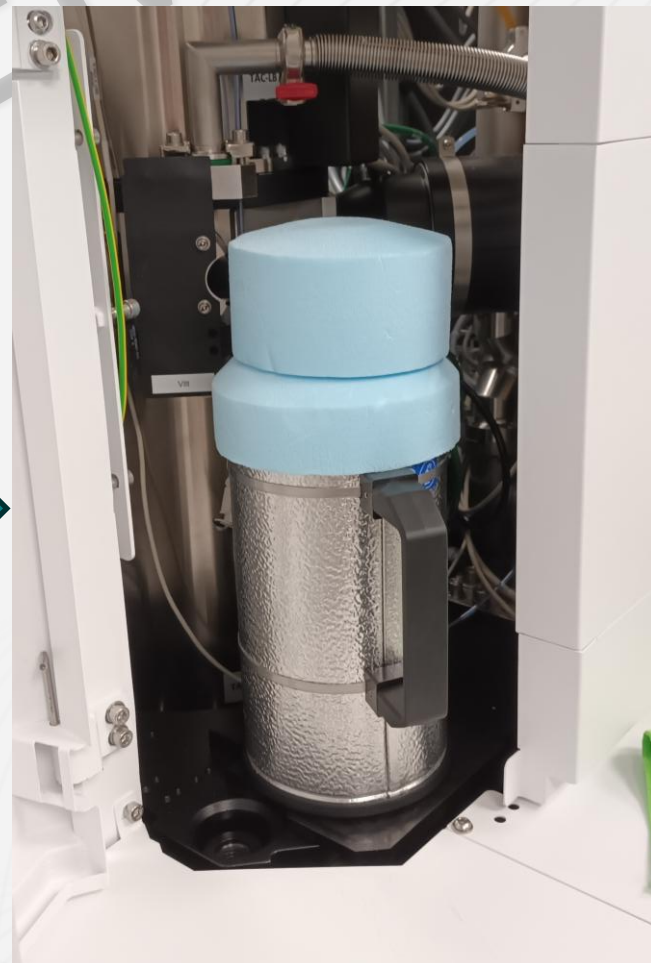
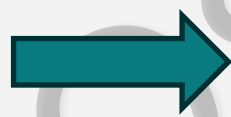
2: Filament On (10 minutes)



EM

Preparation

3: Filling LN2



Preparation

3: Filling LN2

When low level, refill LN2 (about 3hrs)

Workset

Setup Search Tune CCD camera

Vacuum (Supervisor)

Status: All Vacuum (Closed)

| | | |
|----------------|-------------------|-----|
| Accelerator | 1 | Log |
| Column | 6 | Log |
| Detection Unit | 12 | Log |
| Nitrogen level | Low | |
| Turbo | Zero speed (0.0%) | |

Col. Valve Closed Turbo pump



Workset

Setup Search Tune CCD camera

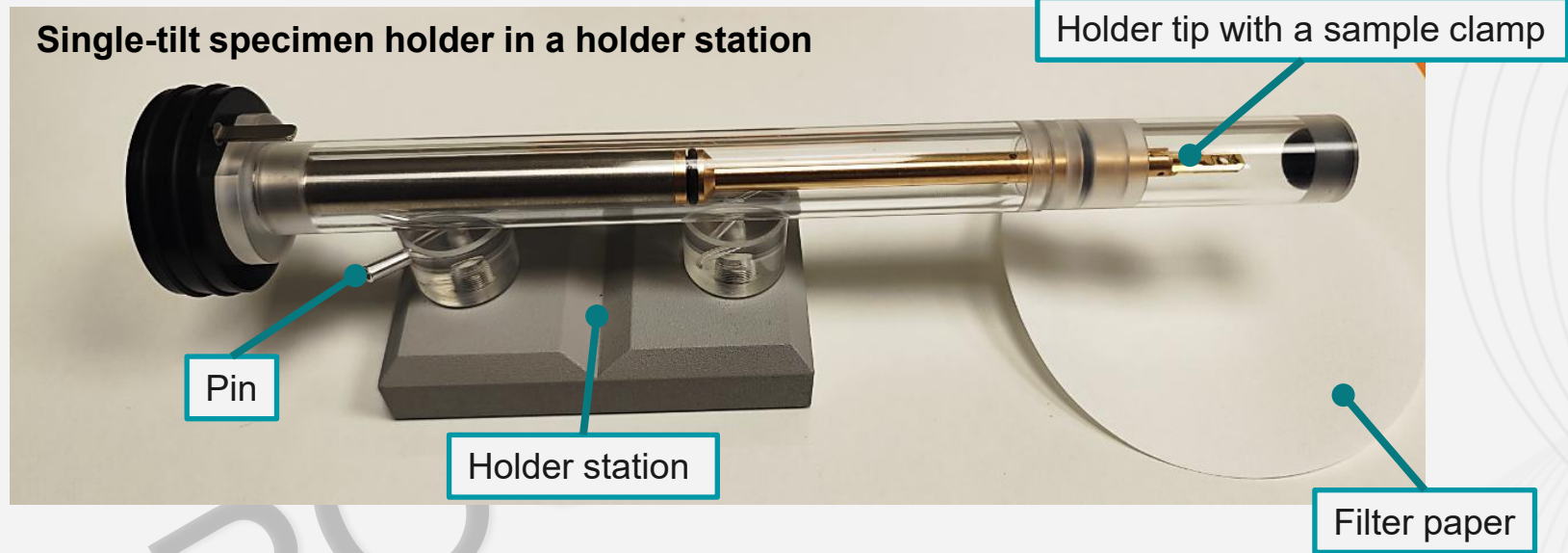
Vacuum (Supervisor)

Status: All Vacuum (Closed)

| | | |
|----------------|-------------------|-----|
| Accelerator | 1 | Log |
| Column | 6 | Log |
| Detection Unit | 12 | Log |
| Nitrogen level | OK | |
| Turbo | Zero speed (0.0%) | |

Col. Valve Closed Turbo pump

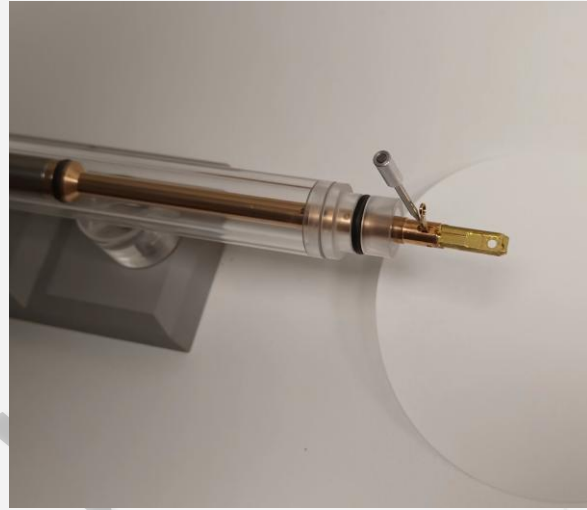
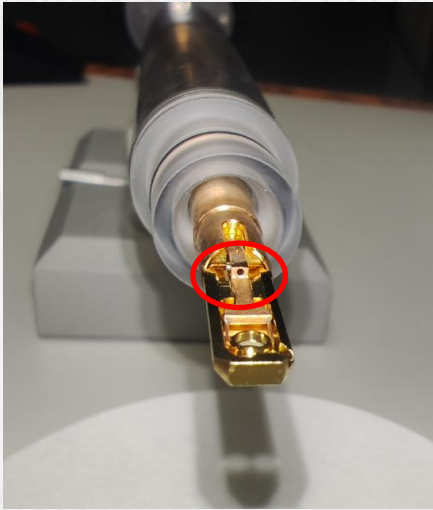
Loading Sample



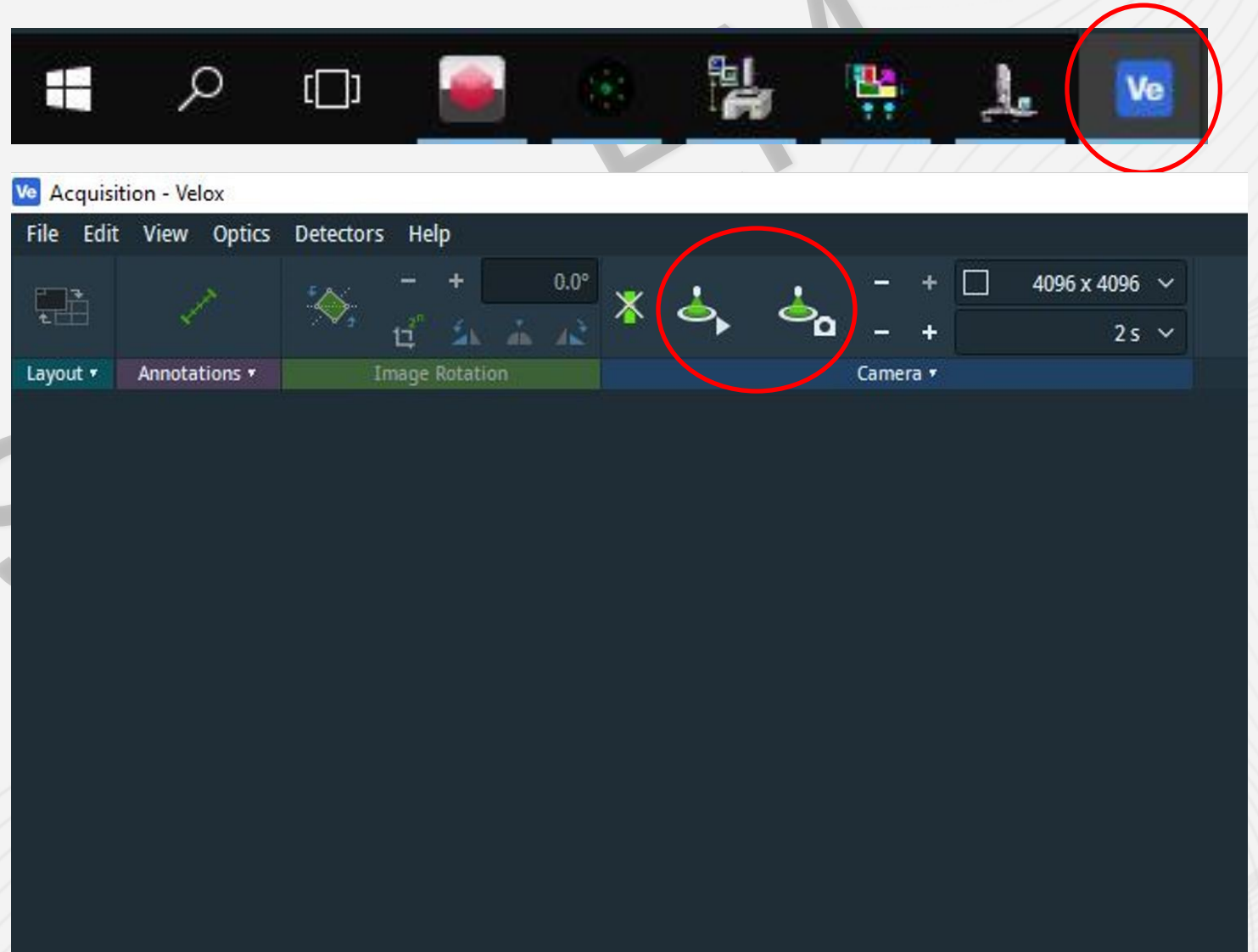
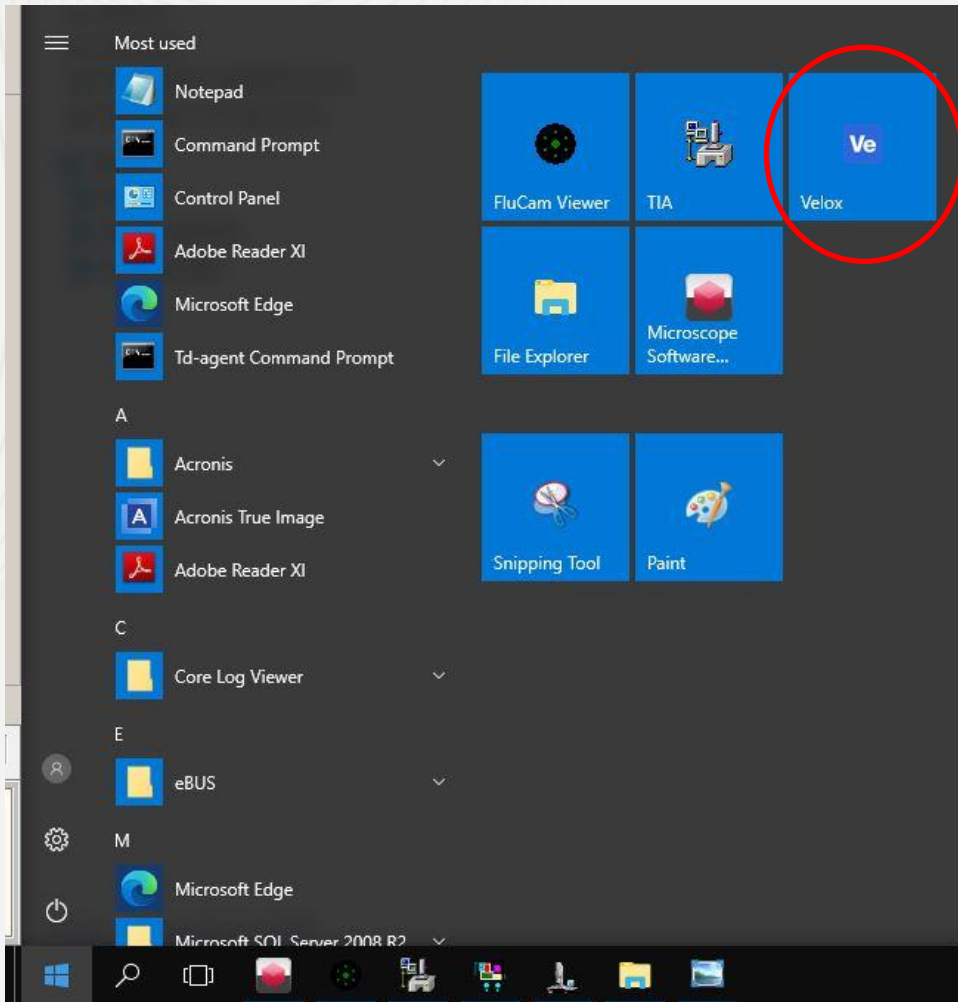
Loading Sample



Loading Sample

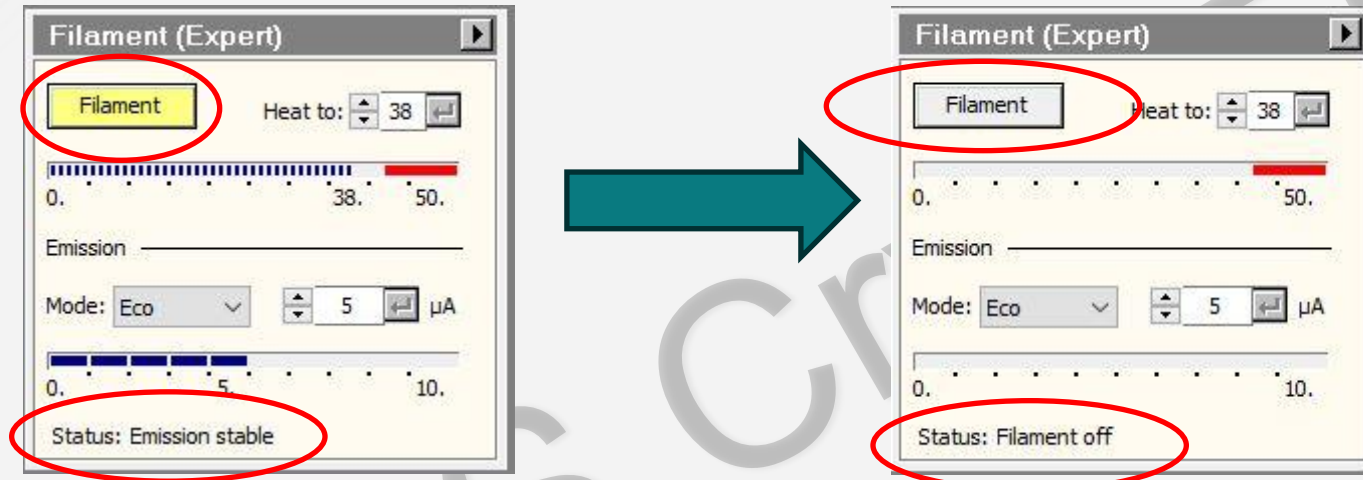


Beam Alignment and Image Acquisition



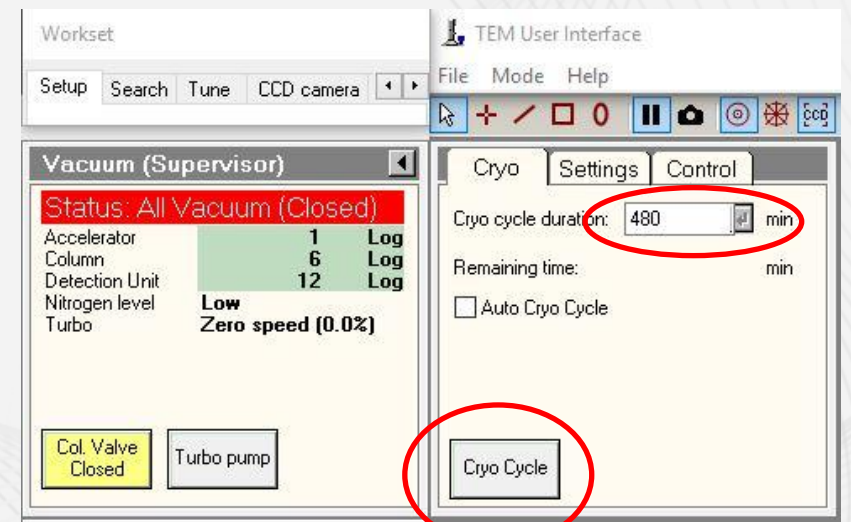
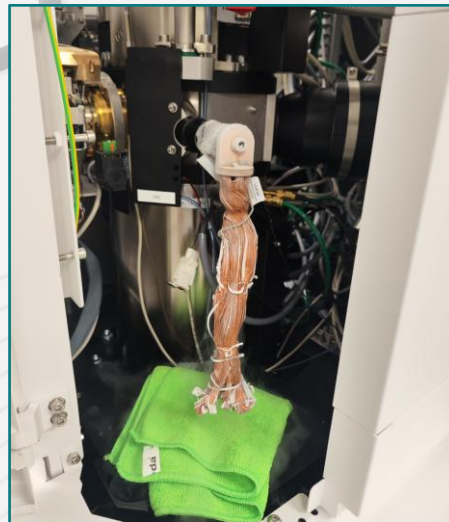
After Experiment

1: Filament off



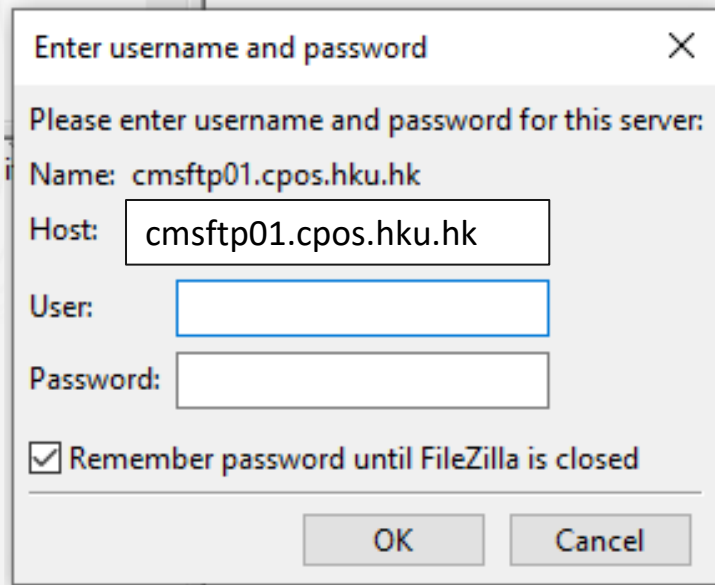
2: Cryo Cycle (for users finished by 17:00)

- (1) Unload sample (Remember: home stage first)
- (2) Take away LN2
- (3) Click cryo cycle.



Data Transfer

- Transfer files in the software FileZilla.



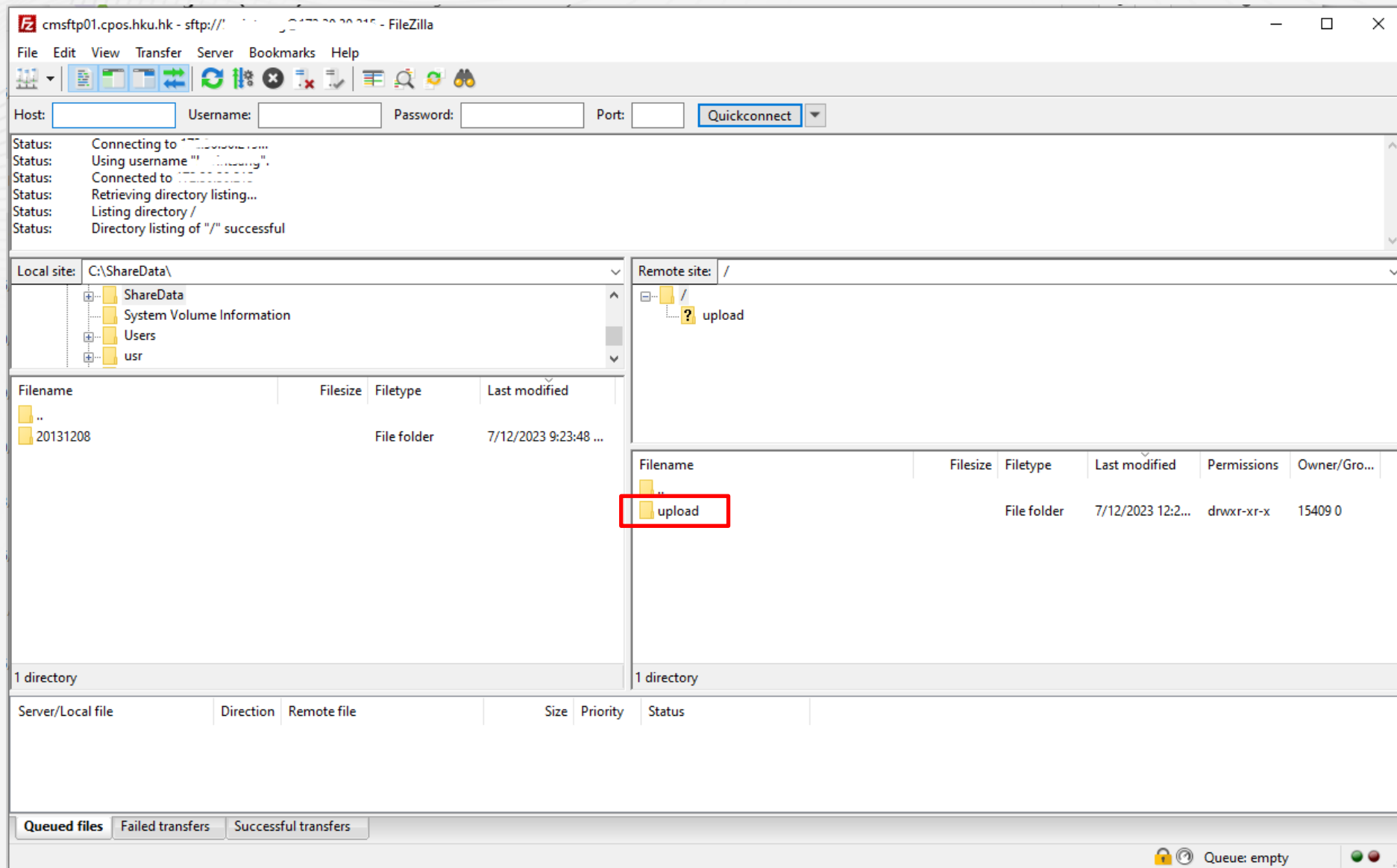
The image shows a screenshot of the FileZilla 'Enter username and password' dialog box. The dialog has a title bar with a close button (X). Below the title bar, it says 'Please enter username and password for this server:'. The 'Name:' field is filled with 'cmsftp01.cpos.hku.hk'. The 'Host:' field is also filled with 'cmsftp01.cpos.hku.hk'. The 'User:' field is empty and has a blue border. The 'Password:' field is empty. There is a checked checkbox labeled 'Remember password until FileZilla is closed'. At the bottom, there are 'OK' and 'Cancel' buttons.

For HPC user:

Please input your HPC ID and Password respectively.

For FTP user:

Please acquire the ID and Password from staff.



Your data in DMP server will only be kept for 7 days.
Expired data will be deleted without further notification.