

CRYO-EM LAB GUIDELINES & RULES

Cryo-EM Laboratory Guideline for New Users

1. An initial meeting involving the users and Dr. Andrew Wong is prerequisite to using the lab. The objective is to identify suitable techniques, equipment and training required for the research project. The meeting will be scheduled by Dr Andrew Wong.
2. Before the initial meeting, users are required to upload the completed [Sample Declaration Form](#) via **FORM 1**. Thereafter, the meeting will be scheduled.
3. After the initial meeting, users are required to upload the below completed forms/documents via **FORM2**:
 - a. [Equipment Request Form](#)
 - b. [Acknowledgement Form](#)
 - c. Risk Assessment (only required if work is not covered in [Cryo-EM lab's RA](#))
 - d. Summary of NTU online safety course
 - e. [SBS Security Access Form](#) (only required for non-SBS users).
4. Users working with biological agents must submit an Online BPN Application for a separate review by the Institutional Biosafety Committee (IBC).
5. The EM lab currently **does not allow any live/active virus and pathogens** which are **Risk Group 2 and above**. Please kindly contact Dr Andrew Wong if you have any related questions.
6. Training session will be scheduled once all documents are reviewed and evaluated. Users will be granted access to the equipment after training and completion of quizzes (Refer to page 7).

Cryo-EM Laboratory General Guideline & Rules

7. Users must meet all the requirement stated above, "Cryo-EM Laboratory Guideline for New Users", before they are allowed to access the lab and its equipment. Any unauthorized access is forbidden.
8. The Cryo-EM lab is under 24-hour CCTV surveillance.
9. Personal access cards are not transferrable under any circumstances.
10. The access door separating the research lab (SBS-02n-01) from the EM Sample Preparation Room (SBS-02n-01e) has restricted access and can only be used by EM staff performing service work, when moving large equipment



which requires access to the red ramp, or by users during an emergency. This is because relative humidity (RH) in the Sample Preparation Room is kept at 30% (to minimize ice contamination in cryo-grids) whilst the research lab is kept in a much higher RH (~60 to 80%). Opening this lab door would cause the RH in the Sample Preparation Room to increase. Hence, users are not allowed to use it to access the Cryo-EM lab or vice versa, under normal circumstances.

11. Users are not allowed to conduct equipment training. Training can only be conducted by EM staff.
12. Users are not allowed to move or tamper with any EM tools and devices in the lab.
13. Users are reminded to handle all equipment and tools (e.g. tweezers, nanocabs, etc) with extra care.
14. Users who mishandle equipment:
 - a. are required to undergo refresher training before lab or equipment access can be granted again.
 - b. users could be asked to pay for the damages (depending on the situations).
15. Users must record the usage of c-clip rings and c-clips via **FORM3** as they are very expensive and proper usage record is necessary.
16. Users who uses the grid storage tank in SBS-02n-39R are responsible for its maintenance by refiling it with liquid nitrogen (at least once a week). Please refer to the duty roster or check with the Dr Andrew Wong.
17. Users must refill the LN2 dewars (LD4) immediately after use. Please dry out the dewars when there is a lot of ice contamination in the dewars. The refilled, and dried dewars (total of 4 dewars) must be kept at SBS-2n-39R when not in use. At any point in time, we should only have two filled, and two dry dewars.
18. Users are reminded to complete their work on time and should set aside time for data conversion, transfer and back up. No extra time will be given for data transfer and back up.
19. Users are reminded to back up their data and delete the data from the main PC, support PC and/or storage servers (offloaded). Data more than 7 days old will be deleted without prior notice given.

The Cryo-EM lab will not be held responsible for any loss of data.



20. Users must not restart or shutdown the microscope PCs (main or support) unless under direct supervision of Dr Andrew Wong.
21. Problems involving any of the equipment must be reported to EM staff immediately. Do not attempt to repair any equipment.

Specific Rules for T12

22. The last T12 user of the day must start the cryocycle. If a cancellation is made, which happens to be for the last session for the day, it is the sole responsibility of the user who cancelled the last session to ensure that the system is placed on cryocycle and standby.

Specific Rules for Arctica & Krios Usage

23. The Arctica can be used for screening and data collection.
24. The Krios is restricted to data collection, unless when the Arctica is down for an extended period of time (longer than 7 working days) and when there is no queue on the Krios for data collection. Data collection has priority.
25. Arctica and Krios users should screen the grids carefully and extensively, and only select the best grid for data collection.
26. Users are strongly **recommended to set up EPU for a short session (at least 1 hour) and to assess the quality of the data by on-the-fly processing.**
27. Users should proceed to **collect a large dataset collection only if the outcome of on-the-fly processing is promising. The Cryo-EM lab will charge users for the time utilised and will not waive any usage charges because the outcome of image processing of the entire dataset is disappointing.**
28. It is the user's responsibility to monitor the data collection, and to intervene when necessary, such intervention includes, but not limited to these:
 - Beam centring
 - monitoring the liquid nitrogen levels in tanks
 - Nitrogen gas pressure on liquid nitrogen tanks
 - changing the liquid nitrogen tanks
 - software issues, etc.
29. When the equipment is "down" due to technical issues, all sessions will be scheduled to a later date, following the same order as before. However, if the

users are not available on these dates, they can change their sessions to a later date, or swap their sessions with other users.

30. For efficient usage of the resource, cancelling a session halfway through is not encouraged, as it forces the facility to accommodate other users on very short notice.

Specific Rules for Titan Krios Usage

31. Users applying for time on Titan Krios are required to upload the completed [Krios Application Form](#) via **FORM4**.
32. Applications are to be evaluated by an evaluating committee and the given time based on scientific merit. **NOTES:** Users need to show in the proposal that suitable Cryo-EM samples/grids have been produced for data collection by including 2D classes.
33. Once users have been awarded time, users may email the EM staff (emicroscope@ntu.edu.sg) to schedule time on Titan Krios. As of now, the EM staff is helping to set up the automated data collection on the Titan Krios.
34. Data collection on the Krios will be scheduled in 24-hour increments. With the exception of data collected using the phase plate, which could require less data (at least 24 hours), users are recommended to use 48 hours per session. Longer sessions could be granted on a case-by-case basis. The Krios will have to undergo a cryo-cycle every 2 - 3 weeks, or when it is deemed to be necessary.
35. Krios users have to complete the back up of all data latest at 3pm on the last day of data collection. Users must start the [script to transfer and delete data remotely](#) at the beginning of the session, and not wait until the end of the session to do so.

Cryo-EM Laboratory Equipment Booking Rules

36. Only qualified users can book equipment via **FOM**. Before the user has completed training, equipment booking is made on behalf of the user.
37. Only experienced users are allowed to use the equipment independently. New users will operate the equipment under the supervision of the EM staff until he/she becomes an experienced user.
38. New users will not be allowed to use the equipment after office hours, (from 6pm to 9am on weekdays, during the weekends, and on public holidays, during which no EM staff is present).



39. As a general guideline, a minimum of 5 consecutive sessions of incident-free usage, excluding training session is required before users can be allowed to use the equipment after office hours. The Cryo-EM lab has the discretion to increase the minimum sessions required, depending on the situation.
40. Each user can only book one session per equipment in advance, and the next session can only be booked upon completion of the previous session.
41. **Equipment sessions are not transferrable, and equipment can only be used by the person who booked it.** If a user booked an equipment for another user, both users would have violated this rule, and user privileges could be suspended for a period of time.
42. Special experiments on the T12 which may require long acquisition times (more than 6 hours) could be granted on a case-by-case basis. Such requests should be sent to emicroscope@ntu.edu.sg in advance.
43. The user must utilize every booked session, unless there are exceptional circumstances beyond their control (such as illness). Cancellation is only allowed 24 hours before the start of the session. Charges will still apply if the user cancel the session less than 24 hours before the start of the session or did not turn up for session without a valid reason.
44. Users must not book the equipment unless they are sure that their sample will be ready so as to avoid last minute cancellation.
45. Activities such as repairs and maintenance, upgrades, workshops, undergraduate courses and training of new users are usually undertaken during normal business hours and occasionally this will significantly limit the time available for regular sessions.
46. Users could in very special circumstance opt for usage with assistance by EM staff, such as when equipment usage is going to be one-off, irregular, or if there just a few samples. There will be additional charges (minimum charge = 1 hour) for technical assistance.
47. **Users who repeatedly violate any of the above rules (1 to 46) could have their lab access suspended for a period of time.**
48. To determine downtime and computation of charges, users must keep an accurate record of microscope issues encountered during data collection. Users should inform EM staff immediately about such problems by providing snapshots of error messages displayed on the software, date and time of occurrence, duration of occurrence, and fill up the [Equipment Usage Record](#). The completed form can be uploaded via **FORM5**. The Cryo-EM lab will assess



the case, and the user could in certain circumstance, be given extra equipment time as a compensation for their lost time.

49. Users can use **FORM6** to let us know what you feel is working well and what concerns you would like addressed. The more specific your comments, the better we will be able to address your concerns.

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Application For Access to Cryo-EM Lab

