NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE | NTU Institute of Structural Biology

Guideline and Rules for NISB Cryo-Electron Microscopy Facilities
(NTU/NISB/SOP/17)

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Access Category: General

The NISB Cryo-EM facility is split into 2 different locations, School of Biological Sciences (SBS) and Facility for Analysis, Characterisation, Testing and Simulation (FACTS). The guidelines and rules stated in this document applies to both locations. Everyone plays a part in taking care of the facilities to ensure their longevity and continued operations. Safety of the users is also a top priority. Therefore, these guidelines are meant to protect both the user and the facility. Please note that the rules will be reviewed and are subjected to change.

1: Facility utilization

1.1 Pre-requisites for user access

i. To access the facilities, all users must follow the approval flow found in **Appendix A**.
ii. To identify suitable techniques and equipment required for their projects, potential users have to schedule a meeting with the facility staff.
iii. Before the facilities can be accessed by users, they must submit a platform application form via the submission portal, and complete all the requirements listed in the form.
iv. Users working with biological agents **MUST** submit an Online BPN Application for a separate review by the Institutional Biosafety Committee (IBC).
v. Users will only be allowed to utilize the facility equipment after training and being deemed independent by the facility staff. **Users are not allowed to conduct equipment training. Training can only be conducted by EM staff.**
vi. 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 safety courses.
vii. NISB must be acknowledged in publications. This is important for NISB to justify future funding which will be used to improve and procure new instruments.

"The authors acknowledge the use of (X-ray/cryo-EM/Biophysics and Biochemistry) platform at the NTU Institute of Structural Biology."

1.2 Security Access

i. Users must meet all the requirements stated in **section 1.1**, before they are allowed to access the facilities and its equipment.
ii. Users are not allowed to loan their access cards to unauthorized users to access NISB cryo-EM facilities.

1.3 Use of Shared Equipment and User Responsibilities

i. NISB provides shared instruments and tools to the research community. Users must treat the instruments with care and are not authorized to remove any instruments/tools/reagents at any time.
ii. The **Principle Investigator (PI)** is responsible for the action of the user(s) from their group, i.e. the PI must ensure that the user follows all Policies & Rules.
iii. If users abuse facility policies and/or act with a lack of respect toward other users, their access to the facility will be revoked.
iv. Users **MUST** clean up the shared equipment after use. **Spills and stains should be cleaned up immediately**.
v. All tools and accessories **MUST** be returned to their proper storage locations. Tools and accessories include, but are not limited to:
    a. Tweezers / forceps
    b. Blue grid boxes
    c. Micropipettes

    Some of the provided tools (such as the autogrid tweezers and Vitrobot precision tweezers) are precision machined and are extremely expensive. Any damage rendered to them due to user negligence will result in the user paying for the repair/replacement.
vi. Users are **NOT ALLOWED** to install any programs/software onto shared computers without permission.
vii. All data/files found to be saved outside proper folders will be removed without prejudice. Thus, users are encouraged to backup their results after the experiment.
viii. Disk space in computers / processing clusters provided by NISB are **NOT** meant for data archival. Their purpose is to serve as intermediaries for data transfer or to park data during active data processing (Refer to **Section 7** on utilization of NISB processing cluster).

Users **MUST** report any errors or issues to the facility staff as soon as possible. No issue is a non-issue. Report everything.

1.4 Equipment Bookings

i. Users must meet all the requirements stated in **Section 1.1 and 1.2**, and be trained before they are allowed to book equipment. Before the user has completed training, equipment booking is made on behalf of the user.
ii. New users are to contact the facility staff for training. Please take note that new users will work under supervision until they are deemed to be independent.
iii. Users **MUST** book the equipment before use. If any samples are found in the equipment without any bookings, they will be removed without prejudice.
iv. If users have previously booked a slot for the equipment but have no plans to utilize the slot, cancel the booking immediately to free it up for other users. Otherwise, charges will be imposed for unused slot.
v. At the end of each session, users should log off their booking to reflect the true utilization time. Otherwise, users will be billed based on the full booked slot.

vi. Equipment sessions are **NOT TRANSFERRABLE**, and equipment can only be used by the person who booked it. Booking equipment for others to use is **NOT ALLOWED** and user privileges will be suspended if caught.

1.5 Billing and charges

i. Billings for the current month (usage month) will be tabulated and sent out to the respective PIs by the first week of the following month (billing month).
ii. All billings should be approved by the end of the billing month.
iii. For any billing disputes, please contact Dr. Liew Chong Wai (cwliew@ntu.edu.sg) or Ms Lynette Liew (lynetteliew@ntu.edu.sg). Note that billing disputes are approved on a case-by-case basis.
iv. For the latest charges, please refer to our webpage **(LINK).**

2: Safety and Operations

2.1 General Safety

i. The attire in the laboratory space should be long pants (reaching down to at least the ankles), and covered shoes. Users with long hair should be tied back.
ii. Eating and drinking are not allowed in **ALL** EM facilities
iii. Donning of personal protective equipment (PPE) is a **MUST** while conducting experiments in the laboratory. The kind of PPE used should be relevant to the activities or experiments being conducted. PPE that is required is listed in the table below:

PPE Required	Activity		
	General wet laboratory experiments	Handling cryogenics	Handling UA
Labcoat	✓	✓	✓
Gloves (Nitrile or latex)	✓	✓	✓
Cryogloves		✓	
Face shield / eye googles	**	✓	

✓ - Mandatory PPE
** - Optional PPE

For more details on NISB PPE requirements, refer to NISB Laboratory Guidelines and Rules **NTU/NISB/SOP/02, section 2.2.**

2.2 Cryogenics Safety

i. Users **MUST** wear the appropriate PPE when handling liquid nitrogen (refer to **Section 2.1** for the appropriate PPE).
ii. This section is abbreviated to suit the scope of this SOP. For full details on cryogenic handling, please refer to **Cryogenics Handling for NISB (NTU/NISB/SOP/16)**.
iii. When dispensing cryogenics, ensure that the room is well-ventilated.

iv. Cryogenics that are no longer needed should be left in designated containers to allow to evaporate fully. **THEY SHOULD NEVER BE POURED DOWN THE SINK OR ON THE FLOOR.**

v. Users **MUST** refill the liquid nitrogen dewars after use. If the dewars are found to have ice contamination, users are to dry them out and inform the facility staff.

vi. Users who use the grid storage tank in **SBS-02n-39R** are responsible for maintaining the liquid nitrogen levels inside the storage dewar. Please refer to the duty roster or check with facility staff.

2.3 Uranyl Acetate Work

i. Users should wear proper PPE when performing radioactive work (refer to **Section 2.1** for the appropriate PPE).

ii. Users handling Uranyl Acetate (UA) should obtain a R1 radiation worker license from NEA before any work involving UA can be started.

iii. Valid R1 licenses should be uploaded via its submission portal **(LINK)**.

iv. Thermo-Luminescence Dosimeter (TLD) should be placed in the TLD holder and be worn at the chest level while working with UA.
Please refer to the **NEA radiation safety page** for more information on the use of TLD badges.

v. UA is given out on request to users. If additional UA is required, please contact facility staff at least **24 hrs in advance**.
   a. Issued UA should **ONLY** be used for the expressed purpose of sample staining and subsequent imaging on NISB microscopes. Users who only wish to utilize UA for their own experiments will **NOT** be issued any UA.

vi. Users should store their aliquots of UA in the provided cabinet drawer. **UA should never be brought out of the sample preparation room.**

vii. UA storage cabinet should always be locked when not in use.

viii. Users should record down percentage and amount of UA used after each experiment in the UA usage logbook, located at the staining station.

3: Sample Preparation Room

3.1 Pre-requisites for User Access

i. The sample preparation room is located at SBS-02n-39r.

ii. **Sections 1 to 3** are applicable to users who wish to utilize this facility.

iii. First time users, regardless new or experienced, **MUST** be briefed and trained by the facility staff.

iv. This room and its facilities are used to prepare cryo-grids for cryogenic electron microscopy and negative staining for room temperature microscopy.

3.2 General Operations

i. The access door separating the research lab (SBS-02n-01) from the Sample Preparation Room (SBS-02n-01e) has restricted access and can only be used during an emergency. This is to preserve the relative humidity (RH) of the Sample Preparation Room at 30%.

ii. Liquid nitrogen dewars are kept at **SBS-2n-39R.** Dewars should only be retrieved when needed, only one dewar should be retrieved. Dewars should be returned to **SBS-2n-39R** when not in use (refer to **Section 2.2** on liquid nitrogen handling and responsibilities).

3.3 Waste disposal

i. **Any biological or chemical waste** generated by users cannot be disposed in the Sample Preparation Room. Users should bring their own waste containers to contain their waste and dispose it properly in their own laboratory.

ii. **Solid radioactive waste** should be disposed of in the **red waste bag** contained in the provided Perspex box.
   a. Solid radioactive waste should be wrapped tightly in the provided parafilm before disposing into the Perspex box.

iii. **Liquid radioactive waste** should be adsorbed with absorbent material (*e.g.* vermiculite), and disposed as solid radioactive waste.

iv. The lid of the Perspex box should be closed **AT ALL TIMES**, except when disposing of radioactive waste.

4: T12

4.1 Pre-requisites for User Access

i. The T12 is located at SBS-02n-02.

ii. **Sections 1 and 2** are applicable to users who wish to utilize this facility.

iii. First time users, regardless new or experienced, **MUST** be briefed and trained by the facility staff.

4.2 Safety and operations

i. Users should wear proper PPE when handling grids / tweezers that have come into contact with UA (refer to **Section 2.1** for the appropriate PPE).

ii. The last T12 user of the work day (working hours are defined as weekdays, 0830 - 1730) must put the microscope into cryo-cycle. If the last user cancels their booking slot, it is their sole responsibility to ensure that the system is placed on cryo-cycle.

iii. Users are reminded to back up their data and delete the data from the T12 transfer PC. Data more than 7 days old will be deleted without prior notice.

NISB will not be held responsible for any loss of data.

5: Arctica

5.1 Pre-requisites for User Access

i. The Arctica is located at ABN B4b-71.

ii. **Sections 1 and 2** are applicable to users who wish to utilize this facility.

iii. First time users, regardless new or experienced, **MUST** be briefed and trained by the facility staff.

5.2 Safety and operations

i. Besides operating the microscope, the users are also responsible for monitoring and intervening in other aspects of the microscope operations during their session. These include but are not limited to:
   a. Monitoring the liquid nitrogen levels in tanks.
   b. Nitrogen gas pressure on liquid nitrogen tanks.
   c. Changing the liquid nitrogen tanks.
   d. Software issues.

ii. Errors encountered during data collection should be noted down and facility staff informed. Users should provide snapshots of error messages displayed on the software, date and time of occurrence, and duration of occurrence. Equipment time may be compensated on a case-by-case basis.

iii. Grids left in the microscope will be disposed of by default. If users wish to save their grids, they should inform the next user or the facility staff.

iv. Any liquid nitrogen used from the provided dewars should be refilled (refer to **Section 2.2** on user responsibilities with regards to liquid nitrogen use).

6: Krios

6.1 Pre-requisites for User Access

i. The Krios is located at ABN B4b-68.

ii. **Sections 1 and 2** are applicable to users who wish to utilize this facility.

iii. First time users, regardless new or experienced, **MUST** be briefed and trained by the facility staff.

6.2 Safety and operations

i. The Krios is restricted to data collection, unless when the Arctica is down for an extended period of time (longer than 7 working days) or when there is no queue on the Krios for data collection. Data collection has priority.

ii. Users are strongly recommended to set up EPU for a short session (at least 1 hour) and assess the quality of the data using on-the-fly processing.

iii. Users should proceed to collect a large dataset collection only if the outcome of on-the-fly processing is promising. **NISB will charge users for the time utilised and will not waive any usage charges based on poor image processing results of collected datasets.**

iv. Upon starting automated data-collection, users should start the data transfer script to automatically transfer data from the PC into the processing cluster (Refer to Data transfer and processing on local workstation (NTU/NISB/SOP/20) **[LINK]** for instructions).

v. Errors encountered during data collection should be noted down and facility staff informed. Users should provide snapshots of error messages displayed on the software, date and time of occurrence, duration of occurrence. Equipment time may be compensated on a case-by-case basis.

vi. Grids left in the microscope will be disposed by default. If users wish to save their grids, they should inform the next user or the facility staff.

vii. Any liquid nitrogen used from the provided dewars should be refilled (refer to **Section 2.2** on user responsibilities with regards to liquid nitrogen use).

7: Processing Cluster

NOTE: Storage space in the processing cluster is meant serve as an intermediary for data transfer or to park data during **active data processing**. Disk space in computers / processing clusters provided by NISB are **NOT** meant for data archival. **Data management is the responsibility of the user.**

7.1 Operations

i. To obtain access to the processing cluster, please contact Dr. Saw Wuan Geok (wgsaw@ntu.edu.sg).

ii. The processing cluster is mostly used to run GPU jobs. Other packages that rely heavily on multi-CPU processing (eg. cisTEM and EMAN).
   a. Packages that rely on CPU processing should only be used for simple image processing jobs such as denoising, filtering, visualisation and file conversion.

iii. To maximise the use of resources, each job should not exceed the following:
   a. 2 GPUs
   b. 10 CPUs
   c. 100 GB RAM

   Jobs that exceed any of the above can be stopped by facility staff at any time without prior warning.

iv. To maintain optimal operations of the processing cluster, the storage capacity will be kept **below 75% of the total storage capacity**.
   a. When the storage capacity has hit the 75% threshold, users will be given a dateline to transfer data out. Thereafter, data will be purged starting from the oldest dataset, until the occupied storage capacity falls below 75%.

v. For more details regarding the processing cluster operations, please refer to (Refer to NISB Server Usage Guidelines and Rules (NTU/NISB/SOP/21).

12: Revision History

This Table below reflects the summary of changes made to the document.

Version	Approved By	Approval Date	Effective Date	Sections Modified	Details of Change
0	Prof. Julien Lescar	25th Mar 2023	25th Mar 2023	N.A	Initial release

Appendix A: Flow of Approval for Platform Access