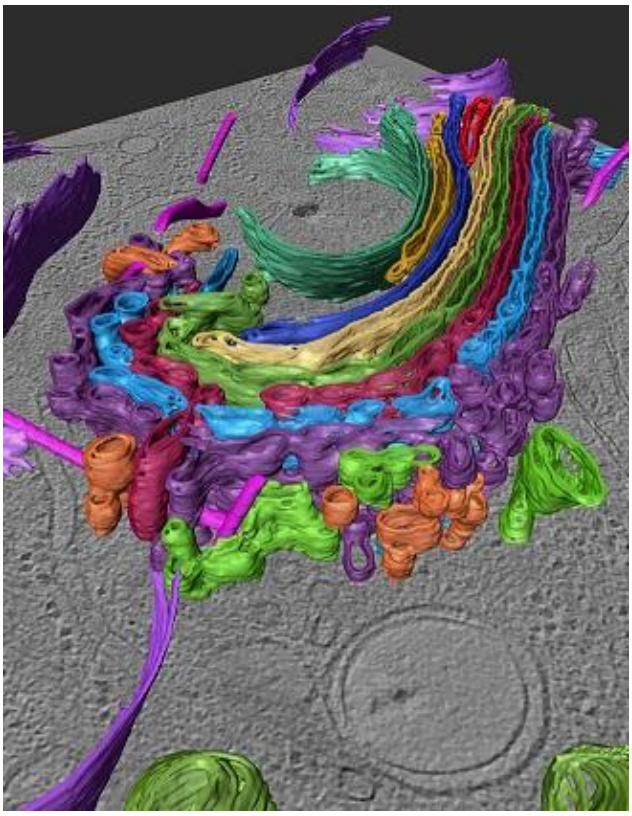

An Introduction to the Ramaciotti Centre for Cryo-Electron Microscopy

USER HANDBOOK

Contents

INTRODUCTION

This handbook contains information about The Monash Ramaciotti Centre for Cryo-Electron Microscopy (CryoEM), its staff and facilities, operating procedures and occupational health and safety matters.

If you have any questions or comments regarding the Manual or CryoEM, please contact the Head of the Facility, A/Prof. Georg Ramm (georg.ramm@monash.edu) or the Manager, Katie Levick (katie.levick@monash.edu).

ABOUT CRYOEM

We are a leading facility for life sciences electron microscopy. We provide expertise, training and research support in advanced biomedical electron microscopy to researchers from the University, government and industry sectors. The expert team in the facility supports and collaborates with researchers on a large number of bio-EM techniques ranging from standard SEM and TEM to advanced techniques, including Volume EM, immuno-electron microscopy, correlative light and electron microscopy, cryo-electron tomography, and protein structure determination using single particle analysis and sub tomography averaging. We maintain a suite of advanced electron microscopes (including two Titan Krios Cryo-TEMs) and the associated specimen preparation equipment.

The Monash Ramaciotti Centre for Cryo Electron Microscopy (CryoEM) was established in 2015 as a dedicated electron microscopy facility for the life sciences. The majority of the facility is housed in Building 75 on the Clayton Campus. This site houses 6 electron microscopes, and all of the associated specimen preparation equipment. In 2023, the facility was expanded to house the new Titan G4 cryo-TEM in building 13A. In 2024, we acquired the Helios Hydra G4 PFIB, which is housed in building 13C.

In 2019 CryoEM joined with the Monash Centre for Electron Microscopy to form the Victorian node of Microscopy Australia (https://micro.org.au). Microscopy Australia is a consortium of university-based microscopy facilities united by values of collaboration, accessibility, excellence and innovation, which aims to provide world-leading microscopes and expertise that keep Australia at the forefront of global research.

CryoEM Platform Vision

To maintain and further develop a cutting-edge life science EM facility that incorporates new approaches and developments in both structural and cellular electron microscopy.

CryoEM Platform Mission

– To provide state of the art instrumentation for life sciences electron microscopy
– To provide expertise in structural and cellular electron microscopy
– To provide excellence in research training in electron microscopy
– To collaborate in structural and cellular life science research and innovate new imaging approaches

CryoEM Web Page

https://www.monash.edu/researchinfrastructure/cryo-em

Location

The CryoEM facility is located on the Clayton campus of Monash University. The main lab is in Building 75 (STRIP1, 15 Innovation Walk). There are also Cryo-EM facilities in building 13A and 13C. A map of the Clayton campus can be found here: Clayton-campus-map.pdf (monash.edu).

For instructions on travelling to and parking on the Clayton campus see: Transport and parking - Monash University.

The facility is housed on the ground floor at the northern end of building 75 (at the corner of Boundary Road and College Walk). The staff offices are in rooms G89 to G98. The main entry to the laboratory is room G104. If you do not have card access, there is a doorbell at the entry of the office area and the lab.

Toilets are located on the Western side of the building foyer, near the lifts.

CRYOEM STAFF

PROF. GEORG RAMM

Head of Cryo-EM Facility – Leadership and Management
Chan Zuckerberg Initiative Imaging Scientist
T: 03 990 51280 M: 0417 341 774
Office: G90, 15 Innovation Walk
georg.ramm@monash.edu

KATIE LEVICK

Manager
T: 03 990 58905 M: 0405 540 943
Office: G89, 15 Innovation Walk
katie.levick@monash.edu

MARGARET RZESZUTEK

Administrative officer
T: 03 990 20878
Margaret.Rzeszutek@monash.edu

HARI VENUGOPAL

Manager – single particle Cryo EM
Office: G92, 15 Innovation Walk
Hari.Venugopal@monash.edu

DR SERGEY GORELICK

Manager – Focused Ion Beam Scanning Electron Microscopy
Office: G91, 15 Innovation Walk
Sergey.Gorelick@monash.edu

DR JILL DANNE

Manager – Immuno-EM
Office: G93, 15 Innovation Walk
Jill.Danne@monash.edu

JOAN CLARK

Microscopist – TEM sample preparation
Office: G96, 15 Innovation Walk
Joan.Clark@monash.edu

DR SIMON CRAWFORD

Microscopist – TEM and SEM training
Office: G94, 15 Innovation Walk
Simon.Crawford@monash.edu

DR RACHEL TEMPLIN

Manager – CLEM and advanced life science EM *(on leave until Jan 2026)*
Office: G93, 15 Innovation Walk
rachel.templin@monash.edu

DR SYLVAIN TREPOUT
Research Fellow – Cryo-ET specialist
Office: G92, 15 Innovation Walk
Sylvain.Trepout@monash.edu

DENIS KORNEEV
Research Fellow – Multimodal imaging
Office: G91, 15 Innovation Walk
denis.korneev@monash.edu

NATASHA KAPOOR-KAUSHIK
Microscopist – CLEM and advanced life science EM
G93, 15 Innovation Walk
natasha.kapoor-kaushik@monash.edu

INSTRUMENTS AND SUPPORT FACILITIES

Electron Microscopes

FEI Titan Krios Cryo-TEM

Manager: Hari Venugopal
Location: G107, 15 Innovation Walk (building 75)

Thermo Fisher Krios G4 Cryo-TEM

Manager: Dr Sylvain Trepout
Location: AG22, 37 Rainforest Walk (building 13a)

Thermo Fisher Talos Arctica Cryo-TEM

Manager: Hari Venugopal
Location: G103, 15 innovation Walk (building 75)

FEI Tecnai G2 Spirit TWIN TEM

Manager: Dr Simon Crawford
Trainer/s: Dr Simon Crawford, Dr Sylvain Trepout, Natasha Kapoor-Kaushik
Location: G111, 15 innovation Walk (building 75)

FEI Nova NanoSEM 450 FEGSEM

Manager: Dr Simon Crawford
Trainer/s: Dr Simon Crawford, Dr Rachel Templin, Natasha Kapoor-Kaushik
Location: G112, 15 Innovation Walk (building 75)

JEOL JEM-1400Flash TEM

Manager: Dr Jill Danne
Trainer/s: Dr Simon Crawford, Dr Jill Danne, Natasha Kapoor-Kaushik
Location: G114, 15 Innovation Walk (building 75)

FEI Helios 5 UX Cryo-FIBSEM

Manager: Dr Sergey Gorelick
Location: G103A, 15 Innovation Walk (building 75)

FEI Helios 5 Hydra DualBeam Cryo-PFIBSEM

Manager: Dr Sergey Gorelick
Location: CG36, 37 Rainforest Walk (building 13c)

Optical Microscope

Zeiss LSM900 Airyscan confocal microscope

Manager: Dr Jill Danne
Trainer/s: Dr Jill Danne
Location: G113, 15 Innovation Walk

Specimen preparation suite

The CryoEM facility's extensive specimen preparation suite includes equipment for standard and cryo-fixation and processing of a wide variety of samples.

This includes equipment for:

- Cryo-ultramicrotomy
- Ultramicrotomy
- Critical point drying
- Automated freeze substitution
- Plunge freezing
- High pressure freezing
- Specimen coating

Data analysis

The CryoEM facility has three dedicated GPU workstations which are used to analyse data. These are equipped with software including Dragonfly and ImageJ.

All users of Cryo-TEM must acquire access to high performance computing on MASSIVE M3 (MASSIVE third stage). MASSIVE is an ISO quality-accredited, high performance data processing facility that provides access to data processing capacity at the scale required to make impactful research discoveries. Monash users can apply for access via Monash eResearch. External users should speak to CryoEM staff about access.

ACCESSING THE CRYOEM FACILITY

Access and training requests, instrument bookings and consumable orders are all made via the iLab online booking system (Ramaciotti Centre for Cryo-Electron Microscopy).

Users of the facility may either submit samples for preparation and microscopy by facility staff or be trained to prepare samples and operate microscopes independently. In most cases, it is a mixture of the two, and some of the work is done by facility staff and some by the users. This requires significant communication and cooperation between facility staff and users.

Charges

The CryoEM facility and staff are supported by Monash University, the Faculty of Medicine, Nursing and Health Sciences, and Microscopy Australia. Usage of the facility is charged at an hourly or daily rate (depending on application). Total annual charges for instrument use are capped for internal Monash University users and affiliated Institute users. Current rates are shown later in this document (Appendix 1). Consumables charged to the user (e.g. TEM grids) are charged at cost.

Charges for instrument use applied to internal users represent a small fraction of the actual cost of maintaining and operating multi-million-dollar instruments, the cost of providing expert and experienced staff, and the on-going cost of replacement/upgraded equipment. The rates are very heavily subsidised by the University.

Registration

All users of the facility must register for access annually by completing the registration form on iLab. This registration is for a calendar year (Jan-Dec). The registration fee contributes to the cost of lab consumables, training and instrument maintenance.

Access to the labs is revoked at the end of each calendar year and will be renewed once the registration for the following year is received. (There is a grace period of 2 months at the beginning of each year, but users are encouraged to register early each year to avoid any interruption to their access).

New Project meeting

Prospective users of the facility must complete a New Project Discussion request on iLab. This form will ask questions about the proposed project, including the sample type, the project aims, the number of samples, and the timeframe involved. A meeting will then be arranged which will involve the project requester, their supervisor (mandatory if the requester is a student) and relevant cryo-EM facility staff. If the project is feasible, a plan will be determined including the CryoEM staff to be involved, training needs, specific safety considerations, sample preparation requirements and any other relevant information.

New group members who are joining existing projects must still complete a New Project Discussion request so that the specifics of their contribution to the project can be discussed (along with any training needs etc).

Users may request a meeting at any time if there are any concerns about progress or if there are changes to the project.

General facility Induction

A facility induction must be completed before you can work unsupervised in the laboratory. CryoEM facility inductions are held monthly for all new users. The session takes approximately 1 hour and consists of a presentation followed by a lab tour. This induction is general in nature and does not permit you to use any CryoEM equipment or processes independently.

Laboratory access

Independent access to CryoEM laboratories is available to authorised users only. Registered users will be granted independent access only if they have completed the following WHS online training modules, in addition to the general facility induction:

- Biosafety Basic Principles
- Biosafety: Gene Technology and Biosecurity Principles
- Dangerous Goods and Hazardous Substances
- Cryogenic Material Basic Safety Principles

(Non-Monash users must certify that they have completed equivalent training in their own institution before they can be given independent access to the laboratories.)

Monash users will be able to use their Monash ID card to swipe in to the laboratory. You may not allow anyone else to use your ID card, or to enter the laboratories.

The standard access level will allow users to access the CryoEM lab in building 75 between 9am and 5pm on working days, as well as the CryoEM staff office area. Access to the laboratories in building 13a and 13c is restricted to CryoEM staff.

Visitors must be supervised by CryoEM staff or by authorised users. Visitors may not use any equipment or chemicals in the lab. There is a doorbell outside the lab and the CryoEM office area.

After-hours access

Access to the CryoEM facility may be granted only to users who have demonstrated safe and competent use of the facility over an extended period of time. There are some activities which are not permitted after-hours due to their hazardous nature (e.g. decanting of liquid nitrogen from pressurised vessels).

To apply for after-hours access, please speak to the CryoEM staff member you have been working with. If they approve, you will be required to complete an after-hours induction and gain permission from your supervisor before access will be granted. Users with after-hours access will have 24/7 access to the laboratory.

Users who do not have permission to be in the facility after-hours must not enter before 9am or remain after 5pm.

Contractors

Contractors must complete the general facility induction before working in the lab, and should be supervised at all times.

Monash BPD staff, contractors and visitors who need to work in the facility unsupervised must complete the Monash Contractor induction: Contractor induction - Buildings and Property Contractors

PUBLICATIONS AND ACKNOWLEDGMENTS

Your use of the CryoEM facility is heavily subsidised by the University and by grants from external funding agencies. In order to comply with current funding obligations and to apply for new funding, the facility must report on the number and type of publications produced by users of the facility. It is essential that you provide us with this information, so that we can continue to provide the advanced instrumentation, staff, training and assistance that you and other researchers require to undertake research work. It is a condition of use of the CryoEM facility that:

1. You provide us with the reference for all publications and reports that contain results obtained using Facility facilities, including instrumentation, computer software and staff advice;
2. You acknowledge the Facility and any assistance provided by Facility staff in the publication or report;
3. Facility staff members who have acquired and/or interpreted data on your behalf should be invited to be co-authors on the publication, as is usual practice. (see the Monash University Research Authorship and Attribution Policy or the Royal Microscopy Society Imaging Facility Publication Guidelines (also appendix 2)).

Publication references may be sent to the CryoEM facility manager (katie.levick@monash.edu)

Acknowledgment templates:

The authors acknowledge the use of instruments and scientific and technical assistance at the Ramaciotti Centre for Cryo-Electron Microscopy at Monash University, a Microscopy Australia (ROR: 042mm0k03) facility enabled by NCRIS.

OR

(if the work was carried out with help from CryoEM staff but not enough to justify co-authorship)

The authors acknowledge the use of instruments and scientific and technical assistance of [NAME OF CRYOEM STAFF MEMBER(S)] at the Ramaciotti Centre for Cryo-Electron Microscopy at Monash University, a Microscopy Australia (ROR: 042mm0k03) facility enabled by NCRIS.

In addition, specific instruments must be acknowledged as follows:

This research used equipment funded by (select from list below):

Equipment:	**Funding body:**	**Grant number:**
Cryo-PFIBSEM (TBC)	ARC LIEF	LE250100012
Gatan K3 camera	ARC LIEF	LE200200045
Zeiss LSM 900 Airyscan 2 with Linkam cryo-stage	ARC LIEF	LE180100202
Wohlwend High Pressure Freezer	ARC LIEF	LE180100202
FEI Talos Arctica	ARC LIEF	LE 170100016
FEI Helios G5 Cryo-FIBSEM	ARC LIEF	LE150100132
FEI Titan Krios Cryo-TEM	ARC LIEF	LE120100090
Leica suite ((cryo-)ultramicrotomes, AFS2, EMPact2)	ARC LIEF	LE100100165
Gatan Alpine camera	Ian Potter Foundation	

TRAINING

Your training needs will be identified during the New Project meeting, and will be tailored to help you achieve the desired outcomes of your project.

- Training is conducted exclusively by CryoEM staff on a one-on-one basis, usually on your own samples.
- Each instrument and technique requires specific training and independent use of an instrument or technique is not permitted unless training has been successfully completed and you have been given permission by CryoEM staff.
- During your training your sessions will be booked by the staff member who is training you and you will be closely supervised.
- Once you have completed the training you will be permitted to book sessions on iLab independently during standard working hours (9am to 5pm weekdays).
- CryoEM staff are available during standard working hours to provide support and answer questions.

Additional training

After you have completed your initial training and as your work progresses (or if your project changes), you may require additional training. This could be in the application of more advanced techniques or in the operation of other techniques entirely. If this occurs, please contact CryoEM staff.

Refresher training

If you have not used an instrument or technique for some time (6 months), or you are unsure of how to operate, you must request refresher training before you make a new booking. This allows us to:

- ensure that you are still able to confidently do the task,
- communicate any changes in the operation of the task that we have made
- check that there have been no changes to your project that may require additional training.

Microscopy theory

All users of the CryoEM facility are encouraged to deepen their understanding of microscopy and how it applies to their projects by consulting the scientific literature and textbooks. Facility staff can suggest suitable references.

MyScope is an online microscopy training resource which has been developed by Microscopy Australia with contributions from microscopists around Australia (including from the CryoEM facility). This resource offers valuable background information about electron microscopy concepts and techniques and is specifically targeted towards beginners.

STORAGE OF SAMPLES AND MATERIALS

CryoEM users are not permitted to store samples or other materials in the labs. The only samples or materials that are permitted to remain in the lab are those that are in immediate use or where there is a specific plan for their examination. Unattended samples must be labelled with the user's name, contact number, date, and sample type. The CryoEM facility cannot guarantee the safety of user samples or materials that are left in the lab.

Samples which have been prepared for cryo-TEM must be removed from the short-term storage dewars in the lab as soon as they have been imaged. Samples in the temporary storage dewars must be labelled using the tags supplied.

Other samples (such as resin-embedded tissue) must be retrieved by users and stored in their own labs as soon as possible after the project is complete. Samples which have not been retrieved within 3 months of the completion of a project may be discarded by CryoEM staff.

COMPUTING

You must not install any software on CryoEM facility computers. If you need to use software that is not currently installed please contact CryoEM staff. Laboratory computers are to be used only for instrument control and data acquisition, processing, and transfer. There is a shared use computer at the rear of the lab that may be used for checking email etc.

DATA STORAGE AND TRANSFER

Storage and security of your data is your responsibility. Data can be lost at any time due to hardware or software faults, or user error.

USB devices (memory sticks, external drives or similar devices) are not permitted for use on any CryoEM computers (particularly microscope computers).

USING ILAB

Monash uses the Agilent iLab booking system to manage access requests, equipment and service bookings: https://monash.ilab.agilent.com/landing/63

How to register for iLab

Monash users: You can find instructions for how to register here (your supervisor will also need to register if they have not already done so).

External users: You can find instructions for how to register here. Or contact us.

How to submit a request on iLab

- Log in to iLab
- Select the *Ramaciotti Centre for Cryo-Electron Microscopy*
- Select the *New Users and Service Request* tab
- Select the *request service* button that applies and fill in the required information
- CryoEM staff will respond to your request

(If you are already a user of the facility and you wish to create a new project or discuss changes to your active project, please submit a New Project Discussion Request).

How to make an equipment booking on iLab

You will only be able to book equipment once you have completed the relevant training and your trainer has given you booking access to that equipment. If you are being trained or if staff are operating equipment on your behalf, staff will make bookings under your name.

- Log in to iLab
- Select the *Ramaciotti Centre for Cryo-Electron Microscopy*
- Select the *Schedule Equipment* tab
- Select the equipment calendar
- Booking rules or restrictions will be shown on the calendar
- Select the date required
- Click and drag on the calendar interface to select the time span for your booking
- In the pop-up menu select your lab
- Complete the details in the dialogue screen
    - assign a project to your booking
    - The booking time may be modified by clicking on the pencil icon
    - A note may be added if you wish
- Click *Save Reservation* to save the booking

How to cancel/change an equipment booking on iLab

In most cases, equipment bookings may not be cancelled on the day of the booking. If you need to cancel a booking at short notice and are unable to do so through the iLab calendar, please contact CryoEM staff.

- Log in to iLab
- Select the *Ramaciotti Centre for Cryo-Electron Microscopy*
- Select the *Schedule Equipment* tab
- Select the equipment calendar
- Click on the booking, and then the details icon to open the booking details
- To cancel the booking, click on *Delete Reservation.* This will delete the booking from the calendar.
- To change the booking
    - Change the time of the booking,
        - Click on the pencil icon under *Times*
        - Adjust the times as required
        - Click Save
    - Change other booking details as required
    - Click Save Reservation to save the booking

WORKING SAFELY IN CRYOEM LABS

CryoEM Safety Team

Safety Officer and Fire Warden

Dr Simon Crawford
Office G94

First Aid Officers

Dr Jill Danne
Office G93

Katie Levick
Office G89

First Aid kits are located in G104 in building 75, on the outer wall of the Krios G4 room in building 13A, and outside CG36 in building 13C.

The nearest Medical Health Service clinic is located on the ground floor of the Campus Centre on Clayton Campus (21 Chancellor's Walk). The phone number is 990 53175.

Safety rules and laboratory procedures

It is essential that you understand and follow CryoEM's safety rules and laboratory procedures, which comprise part of the CryoEM facility induction. These general rules and procedures apply to everyone working in CryoEM facilities. You will be instructed in any additional rules and safety procedures that apply for specific pieces of equipment or procedures as part of your training.

CryoEM facilities are PC2 certified and everyone working in the facility must follow PC2 principles.

Any occurrence that leads to (or *potentially* leads to) injury or danger to anyone working in the facility must be reported to the Safety Officer (Dr Simon Crawford). A Hazard and Incident Report must be submitted online to the Safety Analysis and Risk Analysis Hub (SARAH^{+}).

For information regarding OHS Risk Management at Monash University, see the Monash OHS web page: https://www.monash.edu/hsw

General rules

If in doubt, always seek help!

You may only use microscopes, equipment, or chemicals independently after you have received specific training and been given permission by CryoEM staff.

Only CryoEM staff are permitted to train users.

No food or drink may be brought, stored or ingested in any laboratory.

People working in the lab must wear a lab coat and safety glasses, as well as fully enclosed shoes. Gloves may be required depending on the activity.

You must wash your hands before you leave the lab.

Bags and coats should be stored outside the laboratory (there is a dedicated cupboard in the office area).

Headphones and earbuds must not be worn in the lab.

Portable devices such as mobile phones or laptops must not be touched with gloves or placed on lab surfaces other than areas signed as 'clean/no gloves' areas. Ideally, they should not be brought into the lab.

You must follow operating procedures provided for equipment or procedures.

Never attempt to repair any equipment. Any problems, faults, or unusual behaviour must be brought to the attention of CryoEM staff.

No items of equipment, tools or consumables may be removed from the room in which they belong without permission of CryoEM staff.

Clear and clean your work area when finished.

Samples and sample preparation materials must be labelled with the user's name, telephone number, date, and type if they are being left unattended for any period of time.

Samples may not be stored in the lab. Samples may only be left in the lab if they are part of a current experiment (e.g. cryo-TEM samples which are due to be examined in a booked microscope session).

It is assumed that anything left behind is not needed and, after a short grace period, these materials will be disposed of.

Keep benches, sinks, and fume hoods clean and clear of clutter.

Children are not permitted in the laboratory unless they are under the direct supervision of a parent or guardian if attending an approved teaching or outreach activity.

You must not enter or remain in any CryoEM laboratory while the air conditioning is inoperative (this is due to the presence of liquid nitrogen, reticulated gases and other chemicals present in the labs).

Risk Assessments

The risks and hazards associated with the standard operations of the laboratory are well understood and managed. CryoEM risk assessments are available on SARAH and safe work procedures are available either as hard copies in the laboratory or on request.

If a hazard is identified with either your samples or your proposed sample preparation method, you must prepare a risk assessment on SARAH (CryoEM staff can help with details) **before** the work proceeds. The SARAH reference number must be supplied to the Safety Officer and Lab Manager for review and approval.

If your work involves a hazard with a risk assessed as medium or higher, you must prepare a Safe Work Instruction and submit it to the Safety Officer and Lab Manager for approval before the work proceeds. All SWIs must be checked and countersigned by either the Safety Officer or the Lab Manager.

Dangerous goods and hazardous substances

Chemicals

You must not use any chemicals in the lab unless you have received training and permission from CryoEM staff. Safety Data Sheets for all chemicals used or stored in the lab can be accessed using ChemWatch. You must read the SDS for all chemicals you use and, if required, prepare Risk Assessments and Safe Work Instructions.

All chemicals, reagents, samples and wash bottles must be labelled appropriately. Labels on working solutions and preparations must include the chemical composition and product name, the preparation date and expiry date and the owners name and telephone number. All chemicals in the lab must be stored appropriately – please seek advice from CryoEM staff if you are unsure.

No chemicals may be disposed of down the sinks. Chemicals must be disposed of into designated, appropriately labelled waste containers. If you are unsure, please seek advice from CryoEM staff.

You must not bring any chemicals into the facility without permission from the Safety Officer. The facility stocks all of the chemicals that are required for most experiments. If your experiment requires new chemicals, you must seek permission from the Safety Officer well before your experiment is due to start. You will need to provide information about what the chemical is required for, how to use and store it safely, and how to dispose of waste.

The following chemicals are BANNED from the facility:

- Hydrofluoric acid
- Perchloric acid/acetic anhydride mixes
- Nitric acid/ethanol mixes

There is a chemical spill kit in room G109.

Liquid nitrogen

Liquid nitrogen is used throughout the CryoEM laboratories and everyone who works in the facility must be aware of the risks associated with its use. Liquid nitrogen (LN2) is a cryogenic liquid and is the liquefied from of nitrogen gas. The major hazards from LN2 as used in the facility are cold burns

(LN2 has a boiling point of -196°C), asphyxiation (gaseous nitrogen can displace oxygen from the air) and pressure build-up (liquid to gas expansion ratio = 790x).

Everyone who works in the CryoEM labs must confirm that they have successfully completed training in the safe handling of cryogenic materials. Monash staff and students must complete the online training module – Cryogenic Material Basic Safety Principles – which can be accessed from your MyDevelopment page. External users must certify that they have done equivalent training at their own institutions.

Anyone who needs to use LN2 in the facility must also complete the local area Cryogenics Induction.

Rules for using liquid nitrogen:

- Safety glasses and a lab coat must be worn.
- Fully enclosed footwear must be worn (eg. no mesh runners, sandals, or open toed footwear).
- Long trousers should be worn.
- When decanting from a pressurised vessel, a full-face shield, cryogenic apron and cryogenic gloves must be worn in addition to the other PPE.
- Never store LN2 in a sealed vessel – thermal expansion can cause explosive results.
- Thermal shock or embrittlement can cause materials to fracture. Only use equipment designed for use with cryogens and check regularly for signs of wear or cracking.
- Liquid oxygen may condense on exposed LN2, leading to a risk of explosion. Ensure that there are no spark sources or open flames nearby and that containers of LN2 are not left uncovered for long periods.
- If the O2 depletion alarm sounds, evacuate the area and do not re-enter until the O2 levels have returned to normal.
- Avoid spilling LN2 on the floor and splashing on surfaces. Over time, this leads to damage to floor coatings and surfaces.
- If you are pouring LN2 into a warm container ensure that you are pouring slowly and in a controlled manner so that you minimise splashing.
- To dispose of LN2, pour the excess into the polystyrene box provided in the fume hood in the cryo-prep area and allow it to evaporate.

Liquid Ethane

Liquid ethane is a cryogen that is used to plunge-freeze samples for Cryo-TEM. The major hazards associated with liquid ethane as it is used in the facility are that it is flammable and that it can cause cold burns. The liquid nitrogen supply is reticulated into the fume hood in the cryo-preparation area from a cylinder in the external gas cupboard. The ethane tap in the fume hood can only be opened if the fume hood is turned on. The ethane gas cylinder must be closed unless ethane is in use.

Rules for using liquid ethane

- You must wear the same PPE as for LN2.
- Ensure that you are ready to plunge-freeze your samples before you open the ethane cylinder, and that you have closed the cylinder at the end of your session.
- Ensure that you remove your PPE before you go out of the lab to open or close the ethane cylinder.
- Ensure that there are no spark sources or open flames nearby.

- To dispose of liquid ethane, pour the excess into the polystyrene box provided in the fume hood in the cryo-prep area and allow it to evaporate.

Compressed gases

In addition to ethane, the following compressed gases are used in the facility – nitrogen, argon, and carbon dioxide. Each of these gases can reduce the oxygen levels in the air if they are present in large quantities. O2 depletion monitors are installed throughout the labs.

Nitrogen gas is reticulated into all labs and microscope rooms. It is used to provide pressure for opening and closing microscope valves, venting microscope specimen chambers, and other functions in the lab. Nitrogen gas is non-toxic and non-flammable.

Argon gas is reticulated into room G105 from a cylinder stored in the external gas cupboard. It is used in the sputter coaters. Argon gas is non-toxic and non-flammable.

Carbon dioxide (CO2) is supplied from a cylinder that is secured in room G105. It is used for critical point drying of samples. The gas cylinder should only be open if the critical point drier is in operation.

Only CryoEM staff are permitted to move gas cylinders or adjust regulator settings or other fittings. Facility user interaction with gas cylinders is limited to opening or closing the cylinder while they are using equipment.

Biological samples

The CryoEM facility is rated PC2. Biological materials that are rated higher than PC2 must not be brought into the facility. If your sample is rated PC2 you must inform us before your project starts.

You must notify us and provide a Reference ID number if your sample involves any of the following GMO dealings:

- Dealing involving intentional release of a GMO (DIR)
- Dealing not involving intentional release of a GMO (DNIR)
- Notifiable low-risk dealing (NRLD)
- Exempt dealing (ED)

Samples may not be brought into the facility without prior approval by CryoEM staff. If you are a current user and you need to start working on a different type of sample you must request a New Project Meeting and obtain approval before you start working on the new sample in the CryoEM lab.

If you intend to use live animals or cells in the facility, you must provide details of any potential biological or other hazards and the controls. For work with live animals, you must demonstrate that you have completed the appropriate animal ethics approvals. If requested, you must provide us with a risk assessment and/or a safe work instruction.

There is a biological spill kit in room G109.

Fume Cupboards

Work with the sash only open far enough to perform the activity comfortable and safely. A fully open fume cupboard will not protect you and others. The sash should be fully closed if the fume cupboard is not in use.

Do not leave any unnecessary glassware, chemicals, or equipment in the fume cupboard. These can interfere with the air flow and reduce the effectiveness of the fume cupboard.

Never use a fume cupboard if the extraction fan or backwash are not working. Report any faults immediately to a CryoEM staff member.

EMERGENCY RESPONSE

In an emergency the most important things are to ensure your safety and the safety of others. Please report any emergency situations as quickly as possible.

Emergency evacuation

There is a two-stage alarm – alert and evacuation.

- ALERT tone (Beep, Beep…) or a flashing orange ALERT light
- EVACUATION tone (Whoop, Whoop…) or a flashing red EVACUATION light

Evacuate the building on hearing or seeing any evacuation alarm.

Standard Business Hours Evacuation

On hearing the ALERT tone (Beep, Beep…)

- Open the office/laboratory door and listen for instructions over the public address system
- Prepare to evacuate (put equipment, chemicals, samples, or data into a safe state).
- Seek help if you will require assistance to evacuate

On hearing the EVACUATION tone (Whoop, Whoop…)

- Leave the building by the nearest safe exit.
- Close, but do not lock office/laboratory doors as you leave.
- Follow instructions given by floor wardens
- Proceed to the Assembly area for the building you are working in (see below)
- Remain at the Assembly area until the floor warden or a Monash security staff member gives the ALL CLEAR

After-hours evacuation

Evacuate on either the ALERT or the EVACUATE warning, proceed to the Assembly area and remain there until the ALL CLEAR is given.

Contacting emergency services

If emergency services (Ambulance, Police, Fire Brigade) need to be contacted:

immediately dial 000 from your mobile telephone, or

dial 0000 from a university telephone with access to an outside line.

The decision to call emergency services is at your discretion. State:

- Nature of the emergency
- Address:

  - 15 Innovation Walk, Building number 75, or
  - 37 Rainforest Walk, Building 13A, or
  - 10 Chancellor's Walk, Building 13C
- Nearest room number
- Your name

Once the required emergency service has been contacted you must notify Monash Security on 9905 3333 (Telephone/Mobile) or 333 (Red Phone) in order that the emergency services can be escorted to the scene by security staff in a timely manner.
For less urgent matters you can ring the Security on 990 27777.

Power failure

All microscopes (except for the JEOL 1400) are powered by Uninterruptible Power Supplies (UPS) backed up by a generator and will continue to operate in the event of a power failure. However, all other services such as general power outlets, lighting and air conditioning will fail. Emergency lighting is provided in microscope rooms.

In the event of a power failure lasting more than a minute or so you should put the equipment into a safe standby state (see microscope Operating Procedures) and leave the laboratory area. You must remain outside the laboratory area until the situation is resolved. If the power failure lasts more than 30 minutes then you must not re-enter the laboratory area until a Facility staff member advises you that it is safe to do so.

YOU MUST NOT ENTER OR REMAIN IN A LABORATORY WHILE THE AIRCONDITIONING IS INOPERATIVE.

Gas alarm

Evacuate the lab as directed. Do not re-enter until the alarm is resolved.

- O2 depletion alarms will be triggered if the O2 level falls below 19.5%
- SF6 alarms will be triggered if a leak of SF6 is detected

Chemical spill

Clear the area and seek assistance from CryoEM staff.

- Spill kits are located G109 and?

Biological spill

Clear the area and seek assistance from CryoEM staff.

- Spill kit is located in G109

Liquid nitrogen spill or leak

Clear the area and wait until the liquid nitrogen has evaporated. If the O2 depletion alarm is triggered, evacuate the lab and do not re-enter until the alarm is resolved.

- If the spill is on a person, run cool water over the affected area for 15 – 20 minutes and seek first aid assistance

Injury or illness

Seek assistance from CryoEM first aid officers (Jill Danne and Katie Levick) or any staff member.

- In a life-threatening situation, contact
    - emergency services 000 (mob) or 0000 (landline)
    - and Monash security 9905 3333 (mob) or 333 (landline)

First aid kit

Main lab: room G104 (near lab entry)
13A: room AG15, on outside wall of microscope room
13C: in corridor outside microscope room

Defibrillator

Main lab: Ground floor, building 75, near elevators
13A: Ground floor, building 13C, near entry to Padua Theatre
13C: Ground floor, building 13C, near entry to Padua Theatre

Fire extinguisher locations

Main lab

- Room G104 (entrance area, lab coat area)
- Room G109 (TEM sample prep lab)
- Room G113 (microtome room)

Building 13A

- Room AGPS02, microscope room airlock

Building 13C

- in corridor outside microscope room

Safety shower and eyewash stations

Main lab

- Room G104
- Room G109

Building 13A

- As for 13C (also toilets opposite main entry to microscope space)

Building 13C

- In corridor outside microscope room (CG26)

Appendix 1: Ramaciotti Centre for CryoEM Charges

Annual registration fee: $300 for Monash users, $500 for external users (per calendar year)

Instrument	Internal*/partner**	External (academic)	Commercial
Microscope time			
JEOL 1400	$60 per hour capped at $4000 per person per year	$80 per hour	$120 per hour
Nova NanoSEM	$60 per hour capped at $4000 per person per year	$80 per hour	$120 per hour
Tecnai	$60 per hour capped at $4000 per person per year	$80 per hour	$120 per hour
Helios Dualbeam and Hydra PFIB	$100 per hour or $750 per day[a], capped at $6000 per year	$200 per hour or $1500 per day[a]	$4500 per day[a]
Talos	$750 per day[a], capped at $6000 per year	$1500 per day[a]	$4500 per day[a]
Titan G1 and Krios G4	$1500 per day[a], capped at $6000 per year	$2400 per day[a]	$8500 per day[a]
Zeiss Airyscan	$60 per hour capped at $4000 per person per year	$80 per hour	$120 per hour
Specimen preparation equipment (included in caps)			
Ultramicrotome (RT, unassisted)	$30 per hour	$40 per hour	$60 per hour
Cryo-ultramicrotome	$60 per hour	$80 per hour	$120 per hour
HPF (minimum of 4 hours)	$60 per hour	$80 per hour	$120 per hour
Grid plunger (Vitrobot or GP2) (minimum of 1 hour)	$60 per hour	$80 per hour	$120 per hour
AFS	$120 per run	$160 per run	$240 per run
Carbon/gold coaters	$15 per run	$20 per run	$30 per run

* FMNHS and SOBS users will receive a discount of $20 per hour for JEOL, Nova NanoSEM, Tecnai, Airyscan, HPF, and Vitrobot. Yearly fees including microscope, sample prep equipment and staff time are capped at $2000 per person without Helios/Talos/Titan and at $4000 with Helios/Talos/Titan.

**Hudson and WEHI users are charged at internal/partner rates, with a cap of $6000.

a. Per day rate = 24 hour run. Minimum run time for Talos and Titan microscopes is 1 day.

Appendix 2: RMS Acknowledgement Guidelines

Imaging Facility Guidelines for Acknowledgement

1. All publications resulting from the use of instruments within the facility should acknowledge the facility as a whole, e.g. 'the authors gratefully acknowledge the [core facility name] for their support & assistance in this work' and the facility should be informed of the publication.

2. Specific grants that have funded the facility instruments used for the work to be published must be acknowledged if the data was acquired during the active period of that grant. Facility staff will advise users of such grant codes.

3. Assistance above the technical or routine level, with any facility staff providing scientific input and expertise in experimental set-up, acquisition or analysis, should be recognised through co-authorship on resulting publications. Please discuss acknowledgements with facility staff prior to manuscript submission.

Example scenarios with baseline recommendations:

Sample Preparation	Fast, routine sample preparation with standard protocol.	Simple acknowledgement
	Development of new sample preparation protocols. Optimisation of existing protocols for specific samples.	Inclusion of specific facility member on author list
Image Acquisition	Training of users to acquire images themselves. Simple acquisition of raw data.	Simple acknowledgement
	Operational image acquisition with input and decisions dependent on expertise. Design or re-design of experimental conditions.	Inclusion of specific facility member on author list
Image Analysis	Recommendation of analysis software and tools. Basic data analysis help and advice.	Simple acknowledgement
	Constructive data analysis and interpretation. Creation of complex custom image analysis tools.	Inclusion of specific facility member on author list

Based on the publication policy compiled by Natasha Stephen, Plymouth Electron Microscopy Centre, after discussions with the RMS EM-UK community

RMS Royal Microscopical Society

BioImagingUK