

Monash Ramaciotti Centre for Cryo Electron Microscopy (CryoEM)

User Manual 2017



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INTRODUCTION

This Manual is a reference giving information about The Monash Ramaciotti centre (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, Dr. Georg Ramm (see contact details below).

ABOUT CryoEM

The Monash Ramaciotti centre (CryoEM) was established in 2015 as a dedicated EM facility for the life sciences housed in Building 75 on the Clayton Campus. Until 2015 the Bio EM capability was part of Monash Micro Imaging and housed in Building 13C.

CryoEM is a leading facility for life sciences electron microscopy. It houses Australia's first Titan Krios microscope, currently the most advanced microscope for biological EM. The Titan is equipped with two direct electron detectors: a Falcon II and a K2 Summit with an energy filter. The facility's expert team supports and collaborates on a large number of bio EM techniques ranging from standard SEM and TEM to immuno EM, correlative light and electron microscopy, cryo tomography and single particle analysis.

CryoEM Platform Vision

The Monash Ramaciotti Centre strives 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.

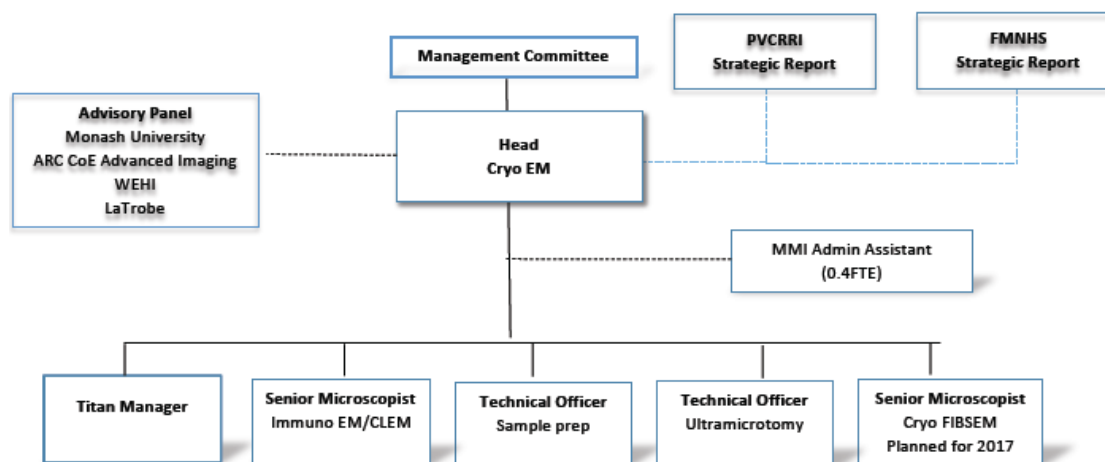
CryoEM Platform Mission

- 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

To learn more about CryoEM please visit our website:

<https://platforms.monash.edu/CryoEM/>

STAFF CONTACT DETAILS AND ROLES



Head

Dr. Georg Ramm

Head of Cryo EM Facility – Leadership and Management

Tel.: 03-9905 1280

Office: 15 Innovation Walk, Building 75, Rm G90

Email: georg.ramm@monash.edu

Cryo EM staff

Monash Ramaciotti staff work closely with the Head CryoEM to support the efficient operation of the laboratory. They assist with the development of training and operating procedures and with development of new microscope capabilities and techniques. Microscopists provide training and assistance to microscope Users and/or collaborate with Users on projects of mutual interest.

Name	Title	Responsibility
Viola Oorschot	Senior Research Officer	Immuno EM & CLEM
Hari Vanugopal	Senior Microscopist Titan	Structural cryo EM/ Titan
TBN	Senior Microscopist	Cryo FIBSEM / SEM
Joan Clark	Senior Technical Officer	Ultramicrotomy & SEM, TEM
Adam Costin	Senior Technical Officer	Sample preparation, (cryo)-TEM, Tomography

Web Page: <https://platforms.monash.edu/CryoEM/>

EM labs: 03-9905 4322

EM office: 03-9905 2746

LOCATION

The Monash Ramaciotti Cryo EM centre is located in Building 75 (STRIP1, 15 Innovation walk) with the general entrance in room G104 (see Figure 1).

For instructions on travelling to the Clayton campus, see <http://www.monash.edu/campuses/clayton.html>.

Information on Parking at Monash can be found at the following link:

<http://www.monash.edu/people/transport-parking>.

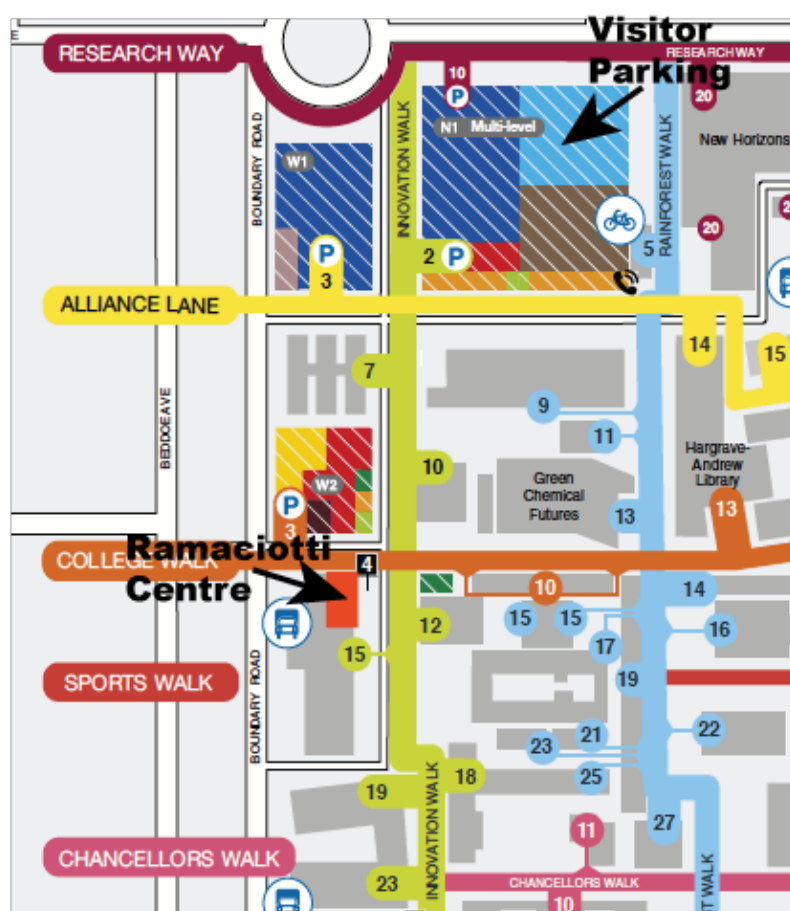


Figure 1. Location of the CryoEM Facility on the Monash University Clayton campus.

BUILDING DIRECTORY

Plans of the ground floor Figure 2. The Facility's room directory and telephone list is given in Table 1.

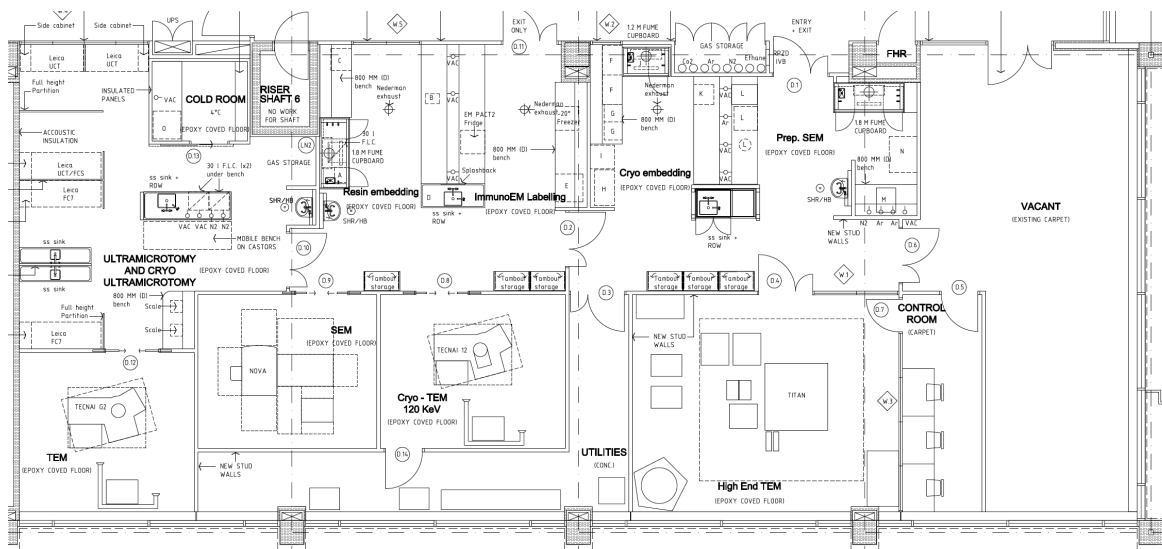


Figure 2. CryoEM, 15 Innovation Walk ground level.

Toilets

Male, Disabled and Female toilets are located on the Western side of the building foyer, near the lifts.

Smoke-free Campus

With effect from 1 January 2016, Monash is a smoke-free University.

Table 1. CryoEM Room Directory and Telephone List.

Room Number	Description	Telephone	Comments
G104	Monash Cryo EM		Main entrance
G90	Dr. Georg Ramm Cryo EM Head	03 9905 1280	Office
G103	Cryo EM Microscopists	03 9905 2746	Office
G103	Cryo EM General Office	03 9905 2746	CryoEM Staff Only
G104, G105,	Cryo EM Lab – SEM and cryo EM prep	03 9905 4322	CryoEM Staff and trained prep

G106			Users only
G107	Titan room		Cryo EM staff only, restricted area
G107A	Titan control room	03 9905 1262	CryoEM Staff and Titan users only
G108	Utility space		Cryo EM staff only, restricted area
G109, G110	Cryo EM Lab – immune EM and TEM sample prep	03 9905 4322	CryoEM Staff and trained Users only
G111	Tecnai Spirit TEM		CryoEM Staff and trained Users only
G112	Nova Nano SEM		CryoEM Staff and trained Users only
G113	Cryo EM Lab – Ultramicrotomy lab	03 9905 4322	CryoEM Staff and trained Users only
G114	Hitachi TEM		CryoEM Staff and trained Users only
G115	Cold room		CryoEM Staff and trained Users only

BUILDING ACCESS

Authorised access only. Authorised Facility Users can open the door to the laboratory area using their Monash ID card. Please contact the General Office if you change your ID card so that we can contact Security to activate your new card to allow access to the building. Visitors should ring the doorbell located next to the main entrance. Users will require local OHS induction/facility induction and LN2 training. Visitors have to be accompanied by a registered user or by a CryoEM staff member within facility.

Business Hours bookings on microscopes can be made between 9:00am and 5:00pm on normal university working days.

Monash staff and students must display their Monash ID card while in the Laboratory.

After-Hours Access

An After-Hours licence may be granted on the discretion of cryo EM staff once a microscope user has achieved a sufficient level of skill to be able to operate the microscope independently, but a minimum of 6 months incident free use of the facility after completion of training applies. Additional training is provided to allow the User to correctly rectify minor issues should these arise after hours when CryoEM staff are not available. After-Hours access is only available to Monash staff and students.

Users with an After-Hours licence have 24-hour/7-day access to the laboratories via the main entrance.

All people entering the building must ring Security (53059) on arrival and departure with the following exceptions:

- 1) between the hours of 7:00am to 8:00pm on normal University working days;
- 2) supervised official CryoEM activities such as Open Days, seminars and conducted tours.

Use of Monash ID cards

Monash Staff and students **must not** allow any other person to use their Monash University ID card. To do so is an extremely serious matter which could amount to conduct capable of disciplinary action. Staff and students who become aware of such misuse must immediately report the matter.

A CryoEM User is **not permitted** to let anyone else into the laboratory area except for visitors under their direct supervision. This includes another CryoEM User who didn't bring their ID card or someone who sneaks in behind while the door is open. Anyone without an ID card must contact a CryoEM staff member who will check that they are a current User before letting them enter the laboratory area. In some special cases such as when a card is lost or damaged a fob may be provided for temporary use.

The following advice on the use of Monash ID cards has been provided by the Monash Office of the General Counsel.

Staff

Staff **must not** allow any other person to use their Monash University ID card. To do so is an extremely serious matter which would be in breach of:

- the staff's employment contract. All staff employment contracts include a term requiring staff to comply with any obligations imposed by University policies and procedures. It is each staff member's responsibility to keep themselves informed of University policies and procedures at all times;
- the Access to Controlled Areas Policy (see <http://www.policy.monash.edu/policy-bank/management/facilities-services/access-to-controlled-areas-policy.html>). This policy applies to all staff and students of Monash. All persons to whom an access control card has been issued must only use the card to enter areas of campus for which they are currently authorised. Access cards must be used only by the person to whom they have been issued. They must not be lent, given to, or used by any other person to enter a controlled area for which they have no authorised right of entry. Anyone possessing or using an access control card to enter University premises without authorisation will be subject to disciplinary actions from the University or criminal charges where appropriate; and
- the terms upon which access was issued. CryoEM licensees are required to sign the Safety Inductions Checklist and User Agreement attached to the CryoEM User Manual, where licensees agree (amongst other things) that they have read and understood the Manual, to follow the CryoEM rules and procedures at all times and

that only authorised personnel (Licensed Users) may enter a laboratory unsupervised.

The above mentioned breaches are extremely serious matters and could amount to conduct capable of disciplinary action or dismissal under the Monash University Enterprise Agreement.

Students

Students **must not** allow any other person to use their Monash University ID card. To do so is an extremely serious matter which would be in breach of:

- the *Monash University (Council) Regulations*. Students are bound by University statute as a matter of law. When students enrol on the Web Enrolment System they also acknowledge and agree that they are bound by the statutes, regulations, policies and procedures of the University as amended from time to time. Regulation 30(2) states that a student who commits, or attempts to commit, or assists or encourages another student to commit, an act of general misconduct, academic misconduct or research misconduct commits a misconduct offence. General misconduct is conduct that is contrary to accepted standards of behaviour and includes conduct by which a student knowingly or recklessly breaches a University statute or a University regulation or a published policy or procedure of the University. A member of staff of the University who has reasonable grounds to believe that a student has committed an act of misconduct must report the matter to the responsible officer. General misconduct by a student is a serious matter which can result in disciplinary action and penalties, including exclusion from the University.
- the Access to Controlled Areas Policy (see <http://www.policy.monash.edu/policy-bank/management/facilities-services/access-to-controlled-areas-policy.html>). This policy applies to all staff and students of Monash. All persons to whom an access control card has been issued must only use the card to enter areas of campus for which they are currently authorised. Access cards must be used only by the person to whom they have been issued. They must not be lent, given to, or used by any other person to enter a controlled area for which they have no authorised right of entry. Anyone possessing or using an access control card to enter University premises without authorisation will be subject to disciplinary actions from the University or criminal charges where appropriate; and
- the terms upon which access was issued. CryoEM licensees are required to sign the Safety Inductions Checklist and User Agreement attached to the CryoEM User Manual, where licensees agree (amongst other things) that they have read and understood the Manual, to follow the CryoEM rules and procedures at all times and that only authorised personnel (Licensed Users) may enter a laboratory unsupervised.

The above mentioned breaches are extremely serious matters and could amount to conduct capable of disciplinary action or exclusion from the University under the *Monash University (Council) Regulations*.

Visitors and Tours

Visitors to the main Laboratory Area must be under the supervision of a CryoEM staff member or an authorised User at all times.

Guest wireless

Monash has three wireless networks available.

- Eduroam – for Monash staff and students and visitors from other eduroam institutions.
- Guest-wireless – for registered Monash visitors who have a temporary Monash account.
- Monash Free WiFi – open access for the general public on all Australian campuses. This network is not encrypted.

For further information see <http://www.monash.edu/wireless>.

Accommodation

For longer bookings and interstate / international visitors overnight accommodation if required is available nearby at the Australian synchrotron (<http://www.synchrotron.org.au/index.php/features/australian-synchrotron-guesthouse>).

INSTRUMENTATION AND SUPPORT FACILITIES

Electron Microscopes

FEI Titan Krios

Falcon 2 direct electron detector including dose fractionation

Gatan K2 Summit and energy filter

Ceta camera optimised for micro electron diffraction

Phase plate

Cryo functionality

- Cryo Autoloader
- Krios loading station
- 4x Krios Cassette, 2x NanoCab
- Krios Nitrogen Stage

Software:

- TEM scripting
- EPU (automatic image acquisition single particle EM)
- Tomo 4 package
- STEM package
- Serial EM
- Gatan Digital Micrograph 3.0

Tecnai G2 Spirit TWIN, 120kV

Eagle 4k HS camera

- LaB6 emitter
- Cryo Box

Holders:

- Compustage single-tilt holder
- 2 x 70 degree cryo transfer holder (Gatan 626)
- 655 Dry pumping station for cryo holders
- Tomography holder (high field of view)

Software:

- AutoAdjust and AutoGun
- TEM scripting



- Low dose exposure technique
- TIA Offline Additional Software
- Photomontage
- Tomography data acquisition software

FEI Nova Nano SEM 450

Column/Chamber

- High-resolution FEG column with immersion lens
- High-resolution multi-stub holder

Detection

- CCD IR inspection camera
- Helix detector
- Through-lens SED (TLD)
- Everhart-Thornley SED
- Low vacuum SED
- Through-lens BSED (TLD-B)
- Retractable DBS detector
- Retractable STEM3 Detector
- Beam deceleration
- 6-channel detector amplifier
- Nav-Cam+

Computation/Control

- MAPS Tiling & Stitching (integrated and offline license)
- Correlative Workflow (integrated and offline license)

Miscellaneous:

- Load base
- Quick loader STEM holder
- SEM sample holder kit
- Integrated Plasma Cleaner
- CryoCleaner EC + Spare Vessel
- Cryo Interlock Kit

Hitachi H-7500 TEM, 120 kV

1k digital camera (Gatan Multiscan 791)



Sample preparation suite

Cryo fixation:

- Vitrobot Mark IV
- High pressure freezer Leica EMPact2 + RTS (rapid transfer system)
- Leica CMC (metal mirror slam freezing and plunger freezing unit)

Sample preparation and processing:

- Automatic freeze substitution Leica AFS2 + FSP (freeze substitution processor)
- 2x Leica AFS
- Tissue Processor Leica EM TP
- Peelco Bio Wave Pro

Microtomy:

- 2x cryo ultramicrotome Leica UC7/FC7 with Leica EM CRION (electrostatic charge control) and micromanipulator for frozen hydrated sectioning
- 2x ultramicrotome Leica UCT (charge control and FCS cryochamber attachments)
- ultramicrotome Leica UCS
- ultramicrotome Reichert ultracut E (on loan to histology platform)

Microscopes (optical):

- Fluorescence Stereomicroscope Leica MZ10F with Fluo III
- Fluorescence Microscope Olympus BX60
- Stereomicroscope Leica M125 and others

Coaters and other ancillary equipment:

- glow discharge Peelco Easyglow
- Carbon Thread Coater Leica ACE200
- 4x knifemaker LKB7800 / Reichert-Jung
- gold sputter coater Baltec SCD005
- critical point dryer Baltec CPD030

Computer Laboratory – CryoEM supports and has licenses for ImageJ (FIJI), AMIRA, MAPS, Tomo4 package, eTomo, on dedicated computers.

Table 2. CryoEM software and computers.

Software	Computer platform
Amira 6.1	PC office
MAPS, Tomo4 package, eTomo, FIJI	PC office

EQUIPMENT MANAGERS, ENGINEERS AND TRAINERS

Equipment within CryoEM is supported by Equipment Managers and Microscopists (see Table 3) with the following responsibilities.

The **Equipment Manager** is the staff member who is most familiar with the detailed operation of the equipment.

A **Microscopist** provides training in use of the equipment.

Table 3. CryoEM Staff responsible for specific equipment.

Category	Equipment	Room	Manager	Trainers
Electron Microscopes	Titan Krios	G107	HV & GR (interim AC)	NA
	Tecnai Spirit	G111	AC	HV, VO, AC, GR
	Nova Nano SEM	G112	VO	AC, VO, JC, GR
	Hitachi TEM	G114	JC	AC, VO, JC, GR
Sample preparation	Baltec CPD	G105	JC	AC, JC
	Peelco glow discharge	G105	HV	AC, HV
	Leica Carbon coater	G104	AC	AC, VO, JC
	Baltec Gold coater	G104	JC	JC, AC
	Leica HPF EM Pact 2	G106	AC	AC, VO, GR
	Leica AFS2	G106	AC	AC, VO, GR
	Vitrobot Mark IV	G106	HV	HV, AC, GR
	Gatan pumping station	G106	HV	HV, AC, GR
	Peelco BioWave	G110	AC	AC
Optical Microscope	Olympus	G113	JC	VO, AC, JC
Microtomes	UCT	G113	JC	NA
	FC7/UC7	G113	VO	NA
	UCT	G113	AC	AC, JC, VO
	UCS	G113	JC	AC, JC, VO
	FC7/UC7	G113	AC	AC, VO, GR

USING CryoEM

Include access schedule

Enquiries regarding use of CryoEM facilities can be directed to the Head, Dr Georg Ramm.

Registration

The condition of usage form and the access can be filled out and send to the Facility Head and Margaret Rzeszutek (margaret.rzeszutek@monash.edu).

Initial User meeting

An initial user meeting involving the User and a supervisor is a prerequisite to using the facility. The meeting will include the Head of the facility and appropriate staff meeting.

Registration on ARIN to book microscopes

Prospective users can register with CryoEM via ARIN, which is available from the CryoEM website. From the ARIN menu request "Submit a new Job". This will open a new window, the Type will be "Register as a new user". Complete the details as required.

CryoEM will send through confirmation of your registration along with the CryoEM Conditions of Use form.

A separate ARIN request must be made for microscope training.

Costs

The CryoEM facility and staff are supported by Monash University, the Faculty of Medicine, Nursing and Health Sciences, and the School of Biomedical Science. Cost for use of the Cryo EM facility are charged at an hourly rate and capped for Monash and affiliated institute users. Access rates are shown below.

Safety Induction

You must complete an Induction before you can work unsupervised in the building.

Prior to attending the Induction you must download and read the CryoEM User Manual (this document). The Manual (PDF file) can be downloaded from the CryoEM web site (<http://www.CryoEM.monash.edu/>) or a printed copy can be obtained from the General Office. You must bring this Manual with you to the Induction session.

After completing the Induction you must complete and sign the *Safety Induction Checklist And User Agreement* on the last page of the Manual.

Note: The *User* is the person who has the microscope licence. The *Supervisor* is responsible for authorising the work and for payment of any costs incurred such as for training, consumables, equipment use or the cost of repairing equipment damaged as a result of incorrect operation by the *User*. The *User* and *Supervisor* can be the same person.

Supervisor's Responsibility

The Supervisor of a Project is responsible for authorising the work and for payment of any costs incurred such as for training, consumables, equipment use or the cost of repairing equipment damaged as a result of incorrect operation by a User on this Project.

TRAINING

Your training needs will be identified during your initial new meeting. CryoEM instruments can only be used by trained Users.

Training sessions will use standard samples and are not intended to yield scientific results.

By completing training to business hours level you will attain a standard of skill necessary to undertake basic microscope operations safely and effectively during normal business hours when assistance is available from CryoEM staff. After you have been using the microscope for some time, typically three months, you may request training for an After Hours licence.

Induction to Facility

To use systems at CryoEM researchers must attend the induction session. The one hour induction covers general information about the facility, the local OHS induction, and a tour to get familiar with the facility.

Practical Microscopy Training

CryoEM has a wide range of equipment, and you will discuss your requirements with CryoEM staff during the initial user meeting. CryoEM will determine the best microscope & sample prep techniques to train the user to achieve the desired outcomes of the project. Practical training on the microscope will take a minimum of 2 x 2 hours. For cryo TEM, prior training of operation of the Tecnai Spirit at room temperature is required. Initial TEM & SEM training consists of two 2-hour sessions. All training is conducted on user samples. After the initial training, the sStaff is available for further guidance

Additional Training

After you have completed your initial training and as your work progresses, you may require additional training. This could be in the application of more advanced techniques or in the operation of other microscopes. To arrange additional training, please contact the relevant Instrument Manager, see Table 3.

Refresher Training

If you do not make regular use of equipment you may require refresher training. The purpose of the refresher training is both to ensure that you know how to correctly operate the equipment and also to inform you of any recent changes in operating procedures.

Responsibilities

In order for microscope training to be as effective as possible, the following outlines the responsibilities of the Trainer and the Trainee.

Trainer's Responsibilities

To teach the Trainee how to operate the microscope safely, effectively and without damage.



To instruct the Trainee in the basic principles of operation of the microscope and to teach the Trainee how to perform basic adjustment of the microscope and accessories in order to achieve reasonable performance.

To teach the basic microscope techniques relevant to the Trainee.

To provide advice to the Trainee on optimisation of the microscope's performance.

Trainers will provide advice and assistance with imaging experiments however no responsibility is taken for data storage and analysis.

Trainee's Responsibilities

To comply with CryoEM and University OHS policies and procedures.

To always seek help if you are unsure of the correct operating procedure or if the equipment is not working properly.

To always follow the Trainer's instructions on the operation of the microscope.

To take detailed notes.

To learn the theory of microscopy and related techniques by consulting books and the scientific literature. Facility staff can suggest suitable references.

To become proficient in operating the microscope.

If you are conducting live cell imaging experiments –it is the users responsibility to check before conducting their imaging that environmental conditions are as required. This includes CO2 levels and incubator temperature. There are devices available on request to monitor these conditions.

BOOKING INFORMATION AND RULES (Except Titan, see below)

Microscopes can be booked through the ARIN web page, see <https://cfms.arin.org.au/cfms/Default.aspx>. You must be a licensed User to access the booking system. Users with a Client licence, Training licence or Supervised Use licence can check on bookings but cannot change them.

Bookings can only be made a limited time in advance, typically two weeks, and there will be a limit on the number of sessions or hours that can be booked within this period. Booking limits will vary from time to time depending on instrument load. Please contact the Instrument Manager if extra sessions are required urgently.

Activities such as repairs and maintenance, undergraduate classes and training of new users are usually undertaken during normal business hours and occasionally this will significantly limit the time available for regular bookings.

Please note that you are charged for the greater of either the booked time or the logged time.

From time to time you must check 'My Details' to ensure that your user details are correct. In particular, it is essential that your email address is correct. If email sent to you bounces then it will be assumed that you have left Monash University and your licences, projects and access will be cancelled.

While checking your details on the 'My Details' page you may wish to elect to receive an email alert when a microscope becomes available through a cancellation. This gives all people who urgently need microscope time an equal opportunity to take advantage of a cancellation. Please note that the normal booking rules still apply.

At the bottom of the list of your details is a series of checkboxes, one for each microscope that you are licensed to operate.

If you tick a box you will automatically be sent an email whenever a booking on that microscope is cancelled.

Booking Rules

The equipment in the Facility is in heavy demand. Please observe these simple rules so that we can optimize access for everyone.

You must attend, ON TIME, every session you are booked for, unless there are exceptional circumstances beyond your control (such as illness).

If you are unavoidably delayed in starting a session, you **MUST** contact the Instrument Manager or one of the Microscopists. Users who fail to notify the Facility of a delay within 30 minutes of their scheduled start time may have their session reassigned to another User. Persistent lateness may result in a reduction or suspension of booking entitlements.

A microscope can only be used by the person who booked it. Bookings are **NOT** transferable.

You must finish your session on time so that the next user is not inconvenienced.

If instrument time is available you can extend your microscope session outside the booked time (subject to your access time).

How To Make An Online Instrument Booking

Log in to the booking webpage at <https://cfms.arin.org.au/cfms/Default.aspx>. If you have forgotten your password, select 'Forgotten your password' and follow the prompts. A new password will be automatically emailed to you.

After login, click on the instrument that you wish to book. This will take you to the current week's table of bookings for that instrument.

Click on the desired day/date of your booking.

To complete the booking, press the 'Make Reservation' button. Your new booking should then appear on the re-displayed day bookings table. An email confirmation of the booking will also be sent to you.

Log out when you have completed your booking.

How To Cancel An Online Instrument Booking

After login, select 'Current' bookings and click on the 'View Bookings' button. This will display a list of your current (future) bookings for all instruments.

Click in the 'Action/Cancel' column for the booking to be deleted.

Select the number of hours that you wish to cancel.

To complete the cancellation, press the 'Delete Booking' button.

Your cancelled booking should then be erased from the re-displayed day bookings table. An email confirmation of the cancellation will also be sent to you.

You can cancel a booking at any time up to one day prior to the start of the session. To cancel a booking within one day of the session you will need to contact the Instrument Manager.

Please note that we expect that a late cancellation will be a very rare event, perhaps once or twice a year. People who make an excessive number of late cancellations may have their booking rights reduced. A log is kept of booking cancellations.

Internal and external Titan Krios access for structural cryo EM

The Monash Ramaciotti Centre for Cryo-EM welcomes new users and is committed to make high-resolution cryo-EM available as a technique to the broader research community in Australasia. For compliance reasons, best possible resource allocation and in the interest of bringing as many as possible cryo-EM projects to fruition, the following procedures apply:

Step 1 - Conception

A typical cryo-EM project is initiated by discussing the project proposal with Dr Georg Ramm

(Head facility) and Hari Vanugopal (Structural Cryo EM Specialist). The aim is to develop an assessment for the amenability of the system for high-resolution structure determination by cryo-EM. An important criteria is to identify the closest related successfully obtained structure in the literature and we recommend doing a search prior to approaching us. We highly encourage to bring any preliminary data & background info (e.g. biochemical data showing protein purification and homogeneity, prior negative staining and cryo-EM if available)

Step 2 - Compliance

Working at the Ramaciotti Centre for Cryo-EM involves dealing with delicate instrumentation and potential hazards. New users are required to sign up to the facility and complete an induction including all mandatory Health and Safety training. Outside users will get access via obtaining Monash Affiliation.

Step 3 - Sample optimisation

Sample preparation is the most critical step for Cryo-EM. Project owners will be trained in using all relevant instruments necessary for driving sample optimisation. Facility staff are available for training, advice and trouble shooting.

New projects typically start out by users first observing their specimen in negative stain and familiarise themselves with the use of TEMs such as the Tecnai 12 or Hitachi. Projects that appear suitable can proceed to cryo-EM, which requires additional training for using the Vitrobot and screening of vitrified specimen.

As is the case for protein crystallography, sample preparation for cryo EM is the most important and challenging part. Parameters that can affect achieving the right ice thickness and particle quality / distribution include buffer optimisation, choice of support film, protein concentration/quality, plunge freezing conditions and many other factors. In our experience, the individual owning the project is invariably the most knowledgeable about their sample. Accordingly, the central involvement of the project owner in preparing and imaging their own grids during sample optimisation is absolutely essential. Please note that for new projects optimisation of protein sample purification & preparation will most likely be required at this stage. For more mature projects, if prior cryo-EM data and optimisation has been done previously, this step will provide a quick check whether the samples are fit for Titan imaging.

Step 4 - Initial automated data collection

If warranted and after successful completion of Step 3, a protein sample will be ready for its first Titan run. The decision to go to Titan imaging will be made by facility staff together with the researcher who owns the project. Titan time will be allocated by facility staff to the next available time slot. Information on the current wait time will be provided by a queue advertised on the Titan noticeboard in room G107A and emailed to users and will be also given on the platform website (only project numbers will be visible).

Initially, an overnight run will be set up to collect enough images sufficient for structure-determination at sub-nanometer resolution.

Step 5 - Large-scale data collection

Titan resources for large-scale data collection will be allocated to projects that have high potential to achieve near-atomic resolution. The decision to allocate Titan time will be made

by facility staff in consultation with the researcher who owns the project. Titan time will be allocated by facility staff to the next available time slot.

Fee Structure Monash Ramaciotti Centre

Training & Registration fee: \$300 internal/\$500 external

Monash internal users & Partner Organisations

\$60/h capped at \$4,000 per person for Monash internal users (except Titan)

\$60/h for Partner Organisations (WEHI, LaTrobe, Baker, Burnet, MIMR) capped at \$4,000 per person.

\$100/h business hours (\$1,500 per day) Titan internal users & partner organisations capped at \$6,000.

External academic users

\$80/h uncapped for external academic users (except Tecnai/Titan)

\$100/h Tecnai uncapped outside academic

\$200/h business hours (\$2,500 per day) Titan uncapped for outside academic users.

Commercial users

\$120/h for Tecnai uncapped for commercial users

\$200/h additional charge for technician for commercial users

\$8,500/day Titan uncapped for commercial users (including technician time)

FMNHS & SBS users will receive up to \$20/h refund (capped at 2,000 per person) from a specific user subsidy contributed from a FMNHS & SOBS fund.

Exceptions:

- ½ price for unassisted use of ultramicrotomes at RT
- HPF (only assisted): a minimum of 2 h will be charged
- Vitrobot Mark IV: a minimum of 1 h will be charged
- AFS: a fee equivalent to 2 h will be charged for full run
- Carbon-coater & gold coater: a ¼ h fee will be charged per run
- Independent use of sample preparation lab and glow discharge unit is free of charge

Standard fees include all microscopes and assisted use of sample preparation equipment and assisted sample preparation.

PUBLICATIONS AND ACKNOWLEDGEMENTS

Your use of Facility facilities 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, the User, 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 your research work. It is a **condition of use** of the Facility that:

1. You provide 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.

Please acknowledge the contributions of the Facility in your publications simply by including:

Where Facility staff provided assistance (but not enough to justify co-authorship):

“The authors acknowledge use of the facilities and the assistance of NAME OF CryoEM STAFF MEMBER(S) at the Monash Ramaciotti Centre for Cryo Electron Microscopy.”

OR

Where you carried out all the work or one or more CryoEM staff members are co-authors:

“The authors acknowledge use of facilities within the Monash Ramaciotti Cryo EM platform.”

Publication references must be sent to Dr Georg Ramm. It would also be appreciated if a soft and/or a hard copy of the publication is provided, which we will place on a publication rack.

STORAGE OF MATERIALS AND DATA, COMPUTING

Facility Users are not permitted to store materials in the building. The Facility operates on a "Carry-in/Carry-out" basis. It is assumed that anything left behind is not needed and after a short grace period, these materials will be disposed of.

Samples and sample preparation materials labelled with the User's name, telephone number and date can be left in designated areas within the laboratories for short periods.

Computing

You **MUST NOT** install any software on Facility computers. If you need to use a program that is not currently installed please contact the Facility Head to discuss this. It may be possible to have the software installed.

Computer Room computers are provided to allow Users to process their data. They are not general use computers.

Access to some special-purpose Computer Room computers is restricted.

You are not permitted to use TeamViewer or any similar software running on a Facility computer to remotely access an external computer.

Laboratory computers are only to be used for instrument control, data acquisition, processing and transfer. Web browsing, Authcate login and use of TeamViewer or similar programs is not permitted.

USB Devices

Use of USB memory sticks, external USB drives or similar devices is **NOT** permitted on CryoEM computers as these are a source of virus infection. A number of computers are not protected by virus checkers as the manufacturer will not guarantee equipment performance if any additional software is installed.

Data storage and transfer

Storage and security of your data is your responsibility.

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

In general, you should not leave your data on Facility computers for prolonged periods of time (>1 month). The exception is images used to extract settings for future experiments, for this purpose there is a folder on every instrument computer called "User Settings", where you can have a folder of settings that you might need to recall in future experiments; ask an CryoEM staff member if you are unsure where this folder is. In the "User Settings" folder do not save large tiles, multi-position, z-stacks, or time experiments as this information is not required for future experiments and will make the file stored larger than is needed.

Every instrument PC has a "MyData" folder in which users must make a sub-folder using their Monash e-mail address. Under the email folder another sub-folder level must be created (it is recommended that a new folder, for example date is created every session) and the data collected for the session placed in that folder, which is then uploaded via the MyData application. The data are accessible online on . It is recommended to save your



image data to local storage, not directly to network storage during/after an experiment. Saving directly to network space can lead to file corruption. However, if you have network storage space (i.e. S:\“\\ad.monash.edu\shared”) you may wish to send a copy of your data to this space for quick access/analysis. This would be in addition to using the MyData setup, not instead of, and you must remember to disconnect from your network drive after transfer of your data is complete. To access any Monash network space you must go to the Map network drive tab, type in the address (i.e. <\\ad.monash.edu\shared>), select “connect using different credentials”, then enter in the user name “Monash\”your authcate” then your authcate password. For clarification of any of the above please see CryoEM staff.

SAFETY RULES AND LABORATORY PROCEDURES

The Safety Rules and Laboratory Procedures comprise part of the CryoEM Safety Induction that all Users are required to complete. Note that these are the general rules and procedures applying to all people using the Facility. You will be instructed in any additional rules and procedures that apply for specific items of equipment as part of your training.

For information regarding OHS Risk management at Monash University, see the Monash OHS Web Page: <http://www.monash.edu/ohs/index.html>

Where any hazard was identified with either your samples or the sample preparation methods to be used, a Risk Control Worksheet (RCW) must be prepared by the User and signed by the User, Supervisor and Instrument Manager (see Table 3).

If a hazard has been assessed, and, after taking into account all normal methods of risk minimization, the risk is still medium or high, then a Safe Work Instruction (SWI) must be prepared by the User if a suitable SWI has not already been prepared (Note: an SWI may also be called a Safe Working Procedure).

All RCWs and SWIs with risks that remain as medium or high must be checked and counter-signed by the Safety Officer or Deputy Safety Officer.

Materials Safety Data Sheets (MSDS) are downloadable using Chemwatch.

Failure to comply with CryoEM Rules and Procedures may result in a penalty being imposed. Penalties include warnings, reduction in licence level, temporary loss of licence, permanent loss of licence and permanent loss of access to CryoEM.

OHS Induction

Monash staff, students and long term visitors who need to carry out unsupervised work in laboratory areas (you will have a Monash ID card)

You must have already completed

- 1) **Online OHS induction** <https://my.monash.edu/services/training/ohs>)
- 2) your Department's or School's the **Local Area OHS induction**.
- 3) **LN2 online induction and local area induction**

In order to work in CryoEM laboratory areas without immediate supervision you must complete the CryoEM Safety Induction Checklist and User Agreement (last page of this manual).

In order to work in CryoEM computer room without supervision you must complete the following CryoEM Safety Induction Checklist and User Agreement (last page of this manual).

You are not permitted to use any equipment in CryoEM other than computers in the computer room and you must be under supervision while in laboratory areas.

Visitors or client users under supervision at all times

1. Short CryoEM Induction.

You are not permitted to operate any CryoEM equipment and you must be under supervision while in laboratory areas.

Contractors working under supervision at all times

1. Short CryoEM Induction.

Buildings and Properties staff and contractors

Buildings and Properties will arrange inductions for Buildings and Properties staff and contractors.

Visitors and contractors who need to carry out unsupervised work in laboratory areas (you will not have a Monash ID card)

Contractors must first complete the Monash Contractor Induction Program provided by the Buildings and Property Division (formerly Facilities & Services), see <http://www.monash.edu/contractors/contractor-induction>.

Computer room only users

In order to work in CryoEM computer room without supervision you must complete the following CryoEM Safety Induction Checklist and User Agreement (last page of this manual).

You are not permitted to use any equipment in CryoEM other than computers in the computer room and you must be under supervision while in laboratory areas.

Induction checklists and further information can be found at <http://www.monash.edu/ohs/ohs-training-and-induction/ohs-induction/local-area-ohs-induction>.

General Rules

If in doubt, always seek help!

You must comply with any temporary notices or tags that may be used from time to time in the Facility. For example, Do Not Operate notices and warnings of changed operating conditions due to equipment faults.

You may only operate equipment for which you have been trained.

Doors and emergency exits must be kept clear.

Do not tamper with fire extinguishers, first aid kits and other emergency equipment.

Children are not permitted in any laboratory, except under supervision on Open Day.

No running in the building.

The Facility is equipped with very sensitive smoke and fire detectors. Open flames are not allowed in the facility. Staff members must be notified before any work that may generate smoke or dust is carried out.

With effect from 1 January 2016, Monash is a smoke-free University.

After-Hours Operation

Facility business hours are 9am to 5pm Monday to Friday on normal University working days.

After hours access is only offered to experienced users (6 months incident free usage of the facility after completed training) and is at the discretion of facility staff.

The air-conditioning in the cryo preparation area G106 only allows for business hours use of cryo preparation equipment. Use of the cryo area is therefore restricted to business hours. In exceptional circumstances and if urgent after hours cryo work is required in G106, this needs to be discussed at least 2 days in advance with facility staff and at least two trained users need to be present during after hours cryo work.

After hours usage of equipment requires after-hours access. To apply for after-hours access, contact the head of the facility.

If the facility is used after 8pm, security must be informed on 53059 and called again when leaving the facility.

In the event of a fire alarm after hours, make the equipment safe and evacuate the building on the first fire alarm (Orange light, Beep-Beep alarm). Do not re-enter the building until you are advised that it is safe to do so by a University security staff member or Facility staff member.

Personal Protective Clothing and Equipment

Closed footwear must be worn in all laboratories.

Additional Personal Protective Equipment (e.g. laboratory coats, long trousers, protective aprons, safety glasses, full-face safety visor, gloves...) must be worn wherever indicated.

Personal protective clothing and equipment must not be worn in office areas or meals areas.

Personal protective equipment (PPE)

Lab coats must be worn within the PC2 facility.

Gloves and safety glasses must be worn when handling samples until they are placed in microscopes.

Handling and decanting liquid nitrogen requires additional safety equipment. We provide shield to worn in addition to safety glasses, apron, and thermal gloves in room G113.

Laboratory Use

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

You must follow the Operating Procedures provided with each piece of equipment and you must comply with all Laboratory Operating procedures.

Never attempt to repair any equipment. Any problems, faults, or unusual behaviour must be reported to a Facility staff member. Names of Facility staff members responsible for equipment can be found in Operating Procedures and in Table 3.

No items of equipment, tools or consumables can be removed from the room in which they belong without the permission of a Facility staff member.

Clear and clean your work area when finished. Glassware must be cleaned by the User immediately after use and must not be removed from the Facility.

Samples and sample preparation materials labelled with the User's name, telephone number, date and composition (where necessary) can be left in refrigerators for short periods.

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.

If equipment is not functioning as you expect, seek help!

Fume Cupboards

Work with the sash only open far enough to perform the procedure comfortably.

Do not leave any unnecessary glassware, chemicals, equipment in the fume cupboard.

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

For further information on use of fume cupboards see
<http://www.monash.edu/ohs/information-and-documents/all-information-sheets/use-of-local-exhaust-ventilation-systems-fume-cupboards>.

Samples and Chemicals

Materials Safety Data Sheets (MSDS) for each chemical stored or used in a laboratory are kept in the master MSDS folder located in room G55 or can be assessed online using ChemWatch. Ensure that you read the MSDS for all chemicals used by you and prepare Risk Control Worksheets and Safe Work Instructions as required. A glossary of terms used in the MSDS forms is located in each folder.

Only those chemicals listed for each laboratory in the front of the master MSDS folder may be stored or used in the Facility.

You must have carried out a Risk Assessment for your work (see www.monash.edu/ohs/forms/risk-management-program.pdf) and you must inform CryoEM of all Risk Phrases and/or Safety Phrases associated with each material that you intend to bring into CryoEM, whether the material is a sample or is used for sample preparation. Users **MUST NOT** bring any chemicals into the laboratory without permission. Permission must be sought from the Safety Officer, Adam Costin, before any new chemical may be introduced into a laboratory.

Store all chemicals in the appropriate cabinet.

Segregate different classes of chemicals (refer to MSDS for class).

Label all chemicals, reagents, samples and wash bottles. Labels on working solutions and preparations must include the chemical composition and product name, the preparation date, expiry date (if appropriate) and the owner's name and telephone number.

The following chemicals are strictly BANNED from the Facility.

- Hydrofluoric Acid
- Perchloric Acid/Acetic Anhydride mixes
- Nitric Acid/Ethanol mixes

Generator power back up

Red general power outlets (GPOs) in microscope rooms and in the General Office are connected to the building generator.

Oxygen sensors and alarms

Laboratory and Microscope areas are fitted with oxygen sensors and alarms that will operate if the oxygen level drops below 19.5% (normal reading ~20.9%). If a low oxygen level alarm sounds, leave the room and advise a Facility staff member of the alarm. If an Oxygen alarm is operating, you must not enter the laboratory. Oxygen levels can be monitored remotely on the control panel at the entrance to the facility (outside room G104).

Liquid Nitrogen

Liquid nitrogen (LN₂) is a cryogenic liquid and is the liquefied form of nitrogen gas. The major hazards from LN₂ as used in the Facility are cold burns (boiling point -196 °C), asphyxiation (gaseous nitrogen can displace oxygen from the air) and pressure build-up (liquid to gas expansion ratio 790 times). All facility users are must complete the LOCAL AREA OHS INDUCTION MODULE - CRYOGENIC FACILITY INDUCTION CHECKLIST plus online and

in-house training. For further information of handling and storage of liquid nitrogen see <http://www.monash.edu/ohs/information-and-documents/all-information-sheets/handling-and-storage-of-liquid-nitrogen>.

Liquid nitrogen is used in the labs G104, G113, and in all microscope rooms G107, G111, G112, G114.

Liquid nitrogen can cause severe burns and the eyes are particularly vulnerable.

Always wear the apron, full-face visor, insulating gloves and closed footwear when filling transfer containers from the liquid nitrogen storage vessels or when transferring and pouring liquid nitrogen from the transfer containers. You must wear closed shoes in the Cryo EM facility. Runners with mesh uppers and open shoes do not provide any protection from accidental liquid nitrogen spills.

Never handle liquid nitrogen whilst wearing disposable gloves.

Liquid nitrogen boil-off can displace oxygen so there is a risk of asphyxiation – minimize spillage and decant in well-ventilated areas. The 160ltr Dewar must not be stored or used anywhere other than the Gas Store (Room G38), which has direct external ventilation.

Liquid Ethane is used for plunge freezing. Additional care needs to be taken using liquid ethane.

Thermal shock can cause materials to fracture: only use the stainless steel or Nalgene Dewars provided, or Dewars provided by equipment manufacturers to handle liquid nitrogen.

As liquid nitrogen boils, there is a large volume increase – never store liquid nitrogen in sealed vessels.

Liquid nitrogen must not be removed from the Facility.

Be aware that liquid nitrogen will cause liquid oxygen to form on cold surfaces by condensation from the atmosphere.

Compressed Gases

The following compressed gases are used in the Facility:

CO₂, Ethane, Argon, N₂

All microscope rooms and most laboratories have N₂ reticulated.

The fume cupboard in the cryo preparation area has reticulated Ethane. Only when the fume cupboard is switched on the Ethane tap can be turned open. Users are also required to open and close the Ethane bottle in the gas storage cabinet.

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

Note:

Keep gas cylinders secured in their racks.

Do not move, connect or perform maintenance on gas cylinders, regulators or other gas fittings. This is done exclusively by Cryo EM staff.

Hazards And Incidents/Chemical Spills/Breakages

Any occurrence that leads to or *potentially* leads to injury or danger to health must be reported to the Safety Officer, Mr Stephen Firth. A Hazard and Incident Report must be submitted online to the Safety Analysis and Risk Analysis Hub (SARAH), see <http://www.monash.edu/ohs>.

Chemical spill kits are located in Corridor G60.

Chemical spills requiring clean-up are classified as Incidents and must be reported immediately to the Safety Officer.

Broken glass must be disposed of in the broken glass containers.

EMERGENCY EVACUATION

Normal Business Hours Evacuation

On hearing the ALERT tone (Beep Beep ...) or seeing the flashing orange ALERT light:

Open office or laboratory door and listen to instructions over the Public Address System;

Prepare to evacuate (put equipment into a safe standby state, save files, secure personal items...).

On hearing the EVACUATION tone (Whoop Whoop) or seeing the flashing red EVACUATION light:

Close but do not lock office/laboratory doors;

Follow instructions given by Floor Wardens (Yellow Hats);

Leave the building by the nearest safe exit;

Proceed to the ASSEMBLY AREA. For Building 75 this is the grassed area to the East of Building 75 and adjacent to Building 53, see Figure 3;

Remain at the ASSEMBLY AREA until a Warden or their delegate gives the ALL CLEAR.

After Hours Evacuation

Evacuate on either of the ALERT or EVACUATION warnings;

Proceed to ASSEMBLY AREA until ALL CLEAR is given, see Figure 3.

Power Failure

Microscopes 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 (see Compressed Gases).

IN AN EMERGENCY

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 (Foyer and Specimen preparation Laboratory G35).

The decision of calling emergency services is at the discretion of the person confronted with the situation. State:

- Nature of Emergency
- Address 15 Innovation Walk, Building Number 75
- Nearest room number
- Your name

Once the required emergency service has been notified, Monash Security must be contacted on **333** for notification in order that the emergency services can be escorted to the scene by security staff in a timely manner.

Further information can be obtained from the Emergency Procedures booklets that have detailed information on emergency response procedures including injury protocols. You should make yourself aware of the processes in these booklets which are next to every internal phone. The Monash Security website <http://www.fsd.monash.edu/security> also is an excellent source of information

For less urgent matters you can ring the Gate House on **53059**.

Fire Extinguisher Locations

Room G104 (Entrance Area, Lab coat area)

Room G109 (TEM sample prep lab)

Room G113 (Microtome Room)

Emergency Eye Wash And Showers

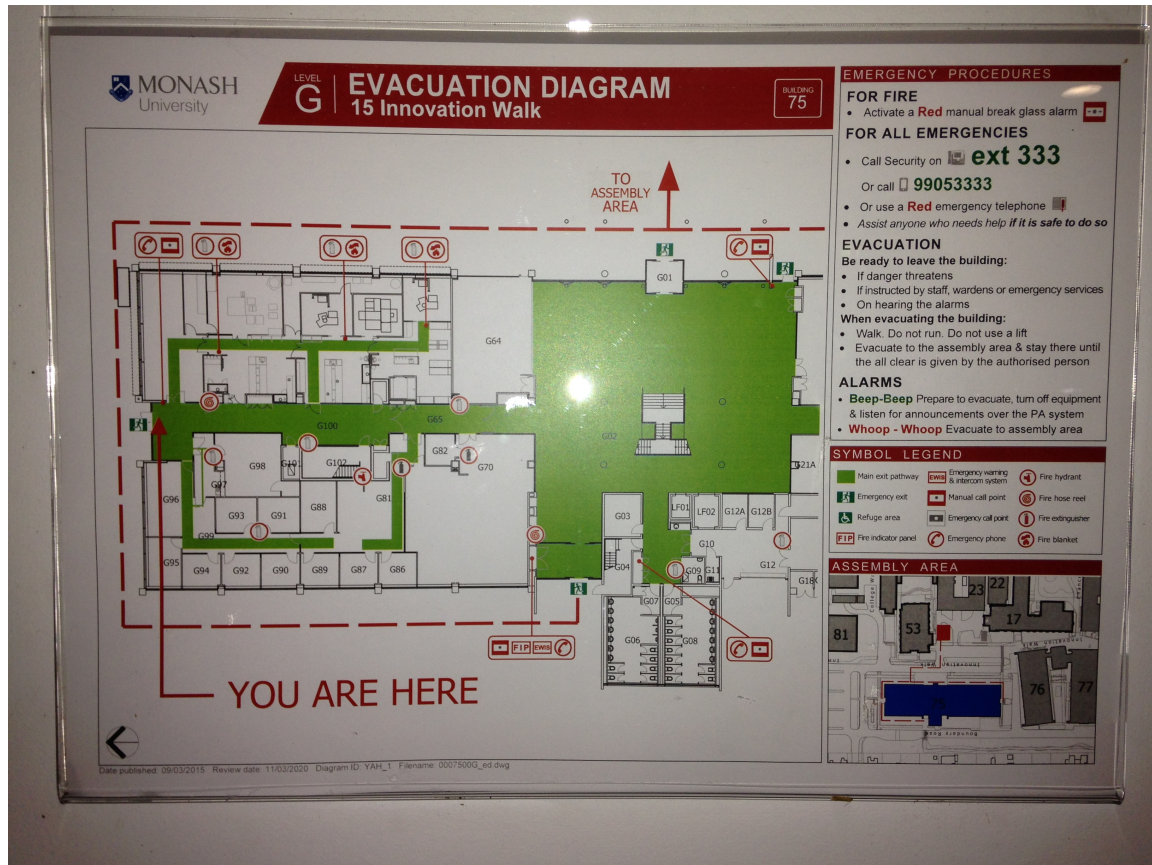
There are Eye Wash Stations and emergency showers in Rooms G104, G109, G113.

First Aid and Spill Kits

The first Aid kit is located in room G104 and Spill Kits are located in G109.

Emergency Evacuation Plan

Figure 3. Emergency evacuation plan.



Safety Officers

Adam Costin

Health and Safety Representative

Fire Wardens

Adam Costin

First Aid Officers

First Aid kit is located in Corridor G104.

The following CryoEM staff members are qualified as Monash Level 2 First Aiders:

Adam Costin

Nearest Medical Health service

The Monash University Medical Health Service clinic is located on the Clayton campus at 21 Chancellors Walk, Ground floor (see Campus Map Figure 1), tel. 990 53175.



MONASH University

Centre for Electron Microscopy

SAFETY INDUCTION CHECKLIST AND USER AGREEMENT

Name: _____ Monash ID: _____

Email: _____ Telephone: _____

Department: _____ Campus: _____

Supervisor: _____ Telephone: _____

I have read and understood the User Manual and I agree to follow the rules and procedures of Monash Micro Imaging as given in the current version of the User Manual (see CryoEM website), as instructed by CryoEM staff and as given in equipment operating procedures, other CryoEM documents and signs. Yes

I have received an introductory safety induction including instruction in the hazards in the Facility and on appropriate personal protective equipment. (Detailed instruction as required will be provided during training). Yes

I have been introduced to the Safety Officer. Yes

Where necessary, I have performed a Risk Assessment on my work and have informed the CryoEM Safety Officer and Manager of any associated hazards. Yes

I have been advised of the need to obtain a licence to use equipment and laboratories. Yes

I have been advised that only authorized personnel (Licensed Users) may enter a laboratory unsupervised. Yes

Signature of User: _____ Date: _____

Signature of CryoEM Head: _____ Date: _____

