

Centre for Advanced Histology and Microscopy (CAHM)



Equipment Usage Policy

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1) Preamble

The Centre for Advanced Histology and Microscopy (CAHM) encompasses four individual but overlapping platform technologies: histology, optical microscopy, electron microscopy and image analysis. Dedicated support is provided from experienced professionals. This document outlines the procedures involved in accessing the optical microscopy platform.

All microscopes and ancillary items within CAHM are available for use by Peter Mac's researchers. As we are an open access facility, researchers from external institutions are welcome to use the equipment/services within CAHM (see Section 3.0 for more information).

Access to the microscopes or histology equipment is granted following a tutorial (and in some cases a competency evaluation) performed by a CAHM staff member.

2) Acknowledgement/Authorship

Guidelines for Authorship on Manuscripts

The Association of Biomolecular Resource Facilities: (<http://www.abrf.org/index.cfm/page/resources/Authorship.htm>) provides an excellent guideline to follow when considering authorship by CAHM personnel. See also Angeletti *et al* (1999. *FASEB Journal*, 13:595).

Two excerpts from the website are presented below:

“Core facilities must charge for services rendered according to cost accounting practices set up at each institution. Charging for services does not preclude authorship on manuscripts provided the Core laboratory individual has contributed to the research in a substantial way”.

“Important reasons for acknowledging contributions from core facilities in publications, by co-authorship or by formal mention in the acknowledgments section, include:

1. Core facility personnel are scientists. When they make a substantial intellectual and/or experimental contribution to a publication they deserve to be acknowledged just as any other co-author.
2. The existence of core facilities depends in part on proper acknowledgment in publications. This is an important metric of the value of most core facilities.

Proper acknowledgment of core facilities enables them to obtain financial and other support so that they may continue to provide their essential services in the best ways possible. It also helps core personnel to advance in their careers, adding to the overall health of the core facility”.

All work performed within CAHM must be acknowledged in publications. As per NHMRC guidelines, intellectual inputs sought from a CAHM member to complete a body of work generally warrants authorship.

3) Gaining Usage Rights to the Equipment

All researchers wishing to use the optical microscopes must receive a tutorial and, for the confocal microscopes, an associated competency evaluation. With completion of the tutorial/evaluation, the researcher is designated a registered user of that specific microscope.

Researchers are only permitted to use microscopes they are registered for. Registered users are **not permitted** to show other non-registered users how to use any of the microscopes. Only CAHM staff members are authorised to train new researchers.

3.1 Tutorials and Tests

Prior to using a microscope for the first time, email the head of CAHM, A/Prof Sarah Ellis to arrange a tutorial. If you wish to use one of the high-end confocal microscopes and you are a student, please organise a meeting between your supervisor, yourself and Sarah Ellis to discuss your experimental design and suggested Tutorials range from 15 minutes to 2 hours depending on the complexity. One-on-one tutorials are preferred to accommodate individual learning preferences.

The following section details the process required to a registered user of a particular microscope within CAHM.

3.1.1 Widefield Microscopes

The widefield microscopes available for use are:

- **Olympus IX81 (live cell).**
- **Olympus IX83 (live cell).**
- **Olympus BX51.**
- **Olympus BX53.**
- **Olympus BX61.**
- **Olympus VS120 (slide scanner)**
- **PerkinElmer Vectra (mIHC slide scanner)**
- **Zeiss Axiovert.**
- **Leica DMIRB.**

The tutorial on the widefield microscopes involves the following:

- Overview of microscope components and their function.
- Alignment of light through the microscope (Köhler illumination).
- Use and alignment of DIC (Differential Interference Contrast) optics or phase contrast (if required).
- Use of fluorescent compensators (BX51) for use with dual or triple filter sets.
- Use of the correction collar on objectives (when available) to correct for the varying thickness of coverslips.

- Use of the SPOT camera and software to obtain optimal image quality:
 - Flatfield correction – for brightfield.
 - Background subtraction – for fluorescence.
 - White balance – for brightfield.
 - Adding and merging scale bars on the acquired images.
 - Tips on saving data (8bit or 16bit), image compression and further analysis.

3.1.2 Live Cell Imaging - Olympus IX81 and IX83

In addition to the above, the tutorial on live cell imaging (widefield) involves the following:

- Setting up the experiment using MetaMorph or CellSens software including discussing techniques to prolong cell life, such as minimising exposure time, binning, optimising light intensity, and objective choice.
- Setting up an overview scan of the well plates or a whole slide (IX83)
- Setting up multiple stage positions.
- Setting up z-series image acquisition.
- Setting up time-lapse experiments.
- Setting up gas supply for long term time-lapse experiments.
- Reviewing and exporting data sets once acquired.
- Image playback including addition of scale bars, time stamps, making movies, light equalisation and so forth. Image analysis is dealt with separately.

3.1.3 Slide Scanning - Olympus VS120 and BX61

The facility provides a slide scanning service for both brightfield and fluorescence slides. In addition to the above, the tutorial on slide scanning involves the following:

- Using the dedicated 100 slide capacity **Olympus VS120 Slide Scanner** for batch scanning of up to 100 slides.
- Using **Metamorph** software on the BX61 to automatically scan a section.

3.1.4 Automated Multispectral IHC Slide Scanner – PerkinElmer Vectra

The facility provides a 200 slide capacity automated multispectral slide scanning service for multiplex immunohistochemistry (mIHC). The tutorial on mIHC slide scanning involves the following:

- Marking the region of interest (ROI) on the slides for automated scanning.
- Setting up the exposure time for each wavelength for image acquisition.
- Setting up batch scanning of selected ROIs or whole slide scanning.

3.1.4 Confocal Microscopes

The confocal microscopes available for use are:

- **Zeiss 780 ELYRA (live cell).**
- **Leica SP5 (live cell).**
- **Andor Spinning Disc (live cell).**
- **Nikon C2.**
- **Olympus FV1000.**
- **Olympus FV3000 (live cell).**
- **BioRad MRC1024.**

To become a registered user of a confocal microscope the researcher must first have a tutorial on the **BioRad MRC1024** to cover basic confocal principles.

The tutorial on the **BioRad MRC1024** involves the following:

- Overview of confocal microscopy.
- Overview of the microscope and scan head components and their function.
- Procedure for turning on the system and shutting it down.
- Alignment and setting up of Köhler illumination and DIC.
- Correct objective settings.
- Adjusting settings for optimal image acquisition by avoiding oversaturated and under-saturated pixels.
- Optimising scanning speed and its effect on image quality.
- Kalman averaging to reduce noise.
- Sequential scanning to avoid bleed-through between adjacent fluorescence wavelengths.
- Choosing the correct pixel size and Z-slice to conform to Nyquist sampling theory.
- Choosing the correct objective according to their numerical aperture.
- Collecting a Z-series.
- Projecting a Z-series (maximum intensity projection).
- Adding scale bars.
- Saving the experiment.

Following the tutorial, researchers are expected to spend time (*approximately 5-10 hours*) working through a tutorial booklet with slides provided by the Core. When the researcher is confident, they will complete a competency evaluation with A/Prof Sarah Ellis. On some occasions, it may be recommended the researcher undertake further practise before moving on to the other confocal microscopes. If this is deemed necessary, the researcher may retake the evaluation after 24 hours (providing the head of CAHM is available). Researchers are required to undertake the test **within one month** of taking the tutorial. If one month is exceeded a new tutorial must be given.

Researchers who are deemed competent in the use of the **BioRad MRC1024** may receive a tutorial on any of the other confocal microscopes. The tutorial provides an overview of how these confocal microscopes differ from the **BioRad MRC1024**, and the various

software options available. The tutorial is given once and then the researcher is free to use the confocal at their leisure. A staff member from CAHM will sit with the researcher for their first few sessions if required to provide support and ensure correct capture of images. Assistance will be provided whenever needed between the hours of 9am to 5pm with other times by prior arrangement.

3.1.5 Multiphoton Microscope

The **Olympus FVMPE-RS Multiphoton** is available for live cell and intravital imaging. Anaesthetic apparatus is provided for mice. Use of this microscope is by arrangement only. Please see the head of CAHM for further details. We have an experienced microscopist to assist you with intravital experiments on live mice/*drosophila*/zebra fish.

3.1.6 Laser Capture Microscope

The tutorial on **Arcturus Veritas Laser Capture Microscope** involves the following:

- Overview of microscope components and their function.
- Acquiring overview image.
- Focusing the cutting laser.
- Setting the optimal capture laser power.
- Marking out the section for capture.

3.1.7 Image Analysis Software

The facility also provides access to and instruction on different image analysis software packages. The available image analysis software is:

- **Adobe Photoshop**: digital darkroom for image manipulation.
- **Metamorph**: 2D image analysis (e.g. cell counting analysis, intensity quantification, time lapse video, etc).
- **Imaris 8.2 CL and Imaris Suite 8.4**: 3D image visualisation and analysis, co-localisation analysis, iso-surface mode with depth effect, image plane at different angle, 3D cropped dataset, 3D video editing tool, etc.
(**Note**: only **Imaris 8.2 CL** has *ImarisXT module* which links Imaris to other image analysis or programming software including **Fiji/ImageJ** and **MatLab**)
- **Volocity**: 3D image analysis, 3D time-lapse image analysis, cell counting, volume measurement, powerful co-localisation analysis.
- **Fiji/ImageJ (free download)**: 2D image analysis and limited 3D image analysis.
- **Graphpad Prism (v7)**: statistical analysis.
- **Matlab**: Macro development.
- **FlowJo (v10)**: FACS analysis.
- **Tibco Spotfire**: multiplex immunohistochemistry (mIHC. Aka OPAL) staining image analysis.
- **Inform (PerkinElmer)**: specialised mIHC multispectral image (**MSI** file) analysis.

- **Arivis Vision4D:** specialised for large data image file stitching and analysis (up to TB data).

In addition to the above, microscope-associated viewer and analysis software is also available (**NOTE:** most of these viewers are also able to open **TIFF** and **JPG** image files):

- **LAS AF lite:** Leica confocal image file (**LIF**) viewer.
- **Zeiss Zen Software:**
 - **Zen Blue** edition: Zeiss confocal image file (**CZI** and **LSM**) viewer and analysis; batch processing image function.
 - **Zen Black** edition: Zen black has additional functions to the Zen blue edition, including spectral unmixing, 3D analysis, FCS, FRAP and FRET analysis.
- **FV10-ASW:** Olympus confocal files (**OIB**, **OIF**) viewer and analysis.
- **Olympus FV31S-DT & SW:** Olympus confocal files (**OIB**, **OIF** and **OIR**) viewer and analysis (e.g. deconvolution, spectral unmixing, cell counting and measurement, colocalization, etc).
- **NIS AR & Nikon Elements v4.0:** Nikon confocal files (**ND2**) viewer and analysis.
- **OlyVIA:** Olympus image file (**VSI**) viewer and analysis.
- **Olympus CellSens:** Olympus image files (**OIB**, **OIF** and **OIR**) viewer and analysis (e.g. cell counting and measurement, object tracking, image comparison, intensity analysis, etc).

Tutorials on software will be tailored to suit individual needs. Group tutorials on various aspects of image analysis (cell counting, size measurements, 3D modelling etc.) will be held on a regular basis for all research staff to attend.

3.2 Refresher Tutorials

Refresher tutorials are available on request for any of the equipment within CAHM. A refresher tutorial is recommended if you have not used a certain microscope for more than 6 months.

3.3 Access by External Researchers

As we are an open access facility, we welcome researchers from external Universities and MRIs to CAHM. All equipment bookings are through the iLab booking system (see below and require a *Purchase Order (PO) number* for billing purposes prior to booking. Please contact Cameron Skinner (cameron.skinner@petermac.org) and/or Alysha Dew (alysha.dew@petermac.org) to arrange access and support.

4) Booking of Equipment

All equipment must be booked using **iLab** booking system:

<https://petermac.corefacilities.org/landing/1607>

It is the responsibility of each researcher to make sure their usage is accurately portrayed in the online booking calendar including providing the correct cost centre and actual time used.

Any researcher that does not turn up for their booking within 30min (without prior notice to other users) will forfeit their booking. Their booking may be taken up by another researcher on a first come, first served basis. If you are the second last user on a microscope for the day, please ensure the last user turns up for their session.

Researchers are not allowed to edit their booking during/after their reservation time. In the event of booking time changes (e.g. extensions on the microscope usage or finishing earlier than booked time), please kindly notify the staff via email (cameron.skinner@petermac.org and/or alysha.dew@petermac.org) regarding any changes to the booking to be updated on the **iLab** booking system.

5) Charges for Using Equipment

Most of the microscopes and equipment within CAHM are charged on a per hour basis, which varies depending on the instrument. A full list of all charges can be obtained from the head of CAHM.

6) Data Storage

All data saved from experiments is the responsibility of the researcher. CAHM **DOES NOT** accept any responsibility for the security of data.

All researchers must save their data to their specified user folder. Any data that is found anywhere else on the computer will be deleted without consultation.

All data will be deleted at the end of each month. All data in individual researcher's folders will be assumed to be backed up unless prior arrangement has been made with a staff member from CAHM. The backed up data will be stored for **2 weeks** before deleted from CAHM back up hard drive.

Live cell microscope computers are not connected to the Peter MacCallum Network.

7) Enforcement of Policy

If a researcher is found to be in breach of this policy (for example; providing tutorials to unregistered users) they will be reminded of their obligation to adhere to CAHM policy. Consistently ignoring CAHM policy will result in suspension of access accounts for a given period.