Using a fluorescence or laser scanning microscope Safe Work Procedure

DETAILS			
Task / Activity:	Using a fluorescence or laser scanning microscope and other imaging equipment. This SWP does not include sample preparation or waste disposal.		
Centre / Department(s):	WIMR laboratories and Scientific Platforms: Cell Imaging		
SWP Reference:	WIMR-SWP-WHS-GEN-16.03	Prepared by:	Sarah Johnston & Hong Yu
Approval date:	2nd June 2020	Next review date:	2nd June 2022
Who was consulted?	Laurence Cantrill, Hong Yu, Virginia James, Susan Wan		

IDENTIFY TYPE OF HAZARD			
☐ Slip, trip or fall	☐ Animal bite/scratch	☒ Sharp object	☐ Heat / cold
☐ Corrosive chemical	☒ Toxic / harmful chemical	☒ Fire / explosion	☐ Violence / aggression
☒ Biological exposure	☐ Noise	☒ Manual handling / ergo	☒ Spill / release
☐ Ionising radiation	☐ Electrical	☐ Fall from height	☐ Psychological
☒ Non-ionising radiation	☐ Struck by or hit object	☐ Motor vehicle / plant	☒ Other (specify below):

RISK ASSESSMENT

Use the risk matrix (see below) to determine the risk rating using existing controls. Risk controls should be in accordance with the hierarchy of controls with preference for elimination, substitution or engineering controls that do not rely on human behaviour or PPE. Consult the safety data sheet and/or product safety information and applicable standards to complete this section.

Hazard / Risk	Risk rating	Risk controls
Serious damage to eyes or skin from exposure to laser radiation from Class 3B laser scanning microscopes (e.g. Leica TPS SC5 confocal microscope) and/or UV from fluorescence microscopes 	Medium	• Any activities that permit access to hazardous laser radiation or degrade the integrity of the protective housing shall only be completed by an external, qualified, manufacturer-authorised service engineer • Equipment has protective housing and shielding to contain laser and/or UV radiation, as far as possible • Equipment must be regularly inspected • Register of laser equipment kept by Laser Safety Officer • Warning signage on equipment indicating laser or UV hazard • Warning indicator light comes on when laser is emitting • Avoid exposing eyes or skin to direct or indirect radiation
Inhalation of mercury vapour or skin absorption resulting from a broken/exploded mercury lamp, may cause serious health effects or damage to the unborn child. 	Medium	• Lamp replacement by trained facility staff only • Lamps are replaced after they reach their recommended lifetime. Usage is monitored • Users are required to notify staff immediately of dim or flickering lamps • Users are trained not to turn on/off mercury lamps frequently. Mercury lamps must be allowed to cool before turning back on. After being turned on, mercury lamps must be left on for a minimum of 15 min to stabilise before being turned off

Hazard / Risk	Risk rating	Risk controls
		• Immediate evacuation of the area is required in the event of a lamp explosion, followed by shutting the room door • Only trained staff and/or the facility Spill Team may handle broken lamps or clean up cold mercury spills using the spill kit and personal protective equipment (PPE) provided • For serious mercury contamination i.e. mercury vapour from hot lamp explosion, call the fire brigade
Fire/explosion risk due to leakage of carbogen gas (95% v/v oxygen) near ignition sources 	Low	• Only authorised personnel may install cylinders and regulators • Gas cylinders are installed away from ignition or heat sources • Users trained to open the gas valve / regulator slowly to avoid sudden flow pressure blowing off
Asphyxiation by displacement of oxygen due to accidental release or leakage of CO_2 from equipment with environmental CO_2 chambers or liquid nitrogen 	Low	**Refer to SWP - Storage and handling of gas cylinders** **Refer to SWP – Liquid nitrogen and cryogenic liquids** • Set the gas flow pressure correctly • Oxygen deficiency monitoring and alarms are installed for all areas where carbogen or liquid nitrogen is used. Once the alarm goes on, users are to leave the lab immediately and press the red a/c purge button if possible
Pinch injury from motorised apparatus	Low	• Equipment guarding must be in place • Users trained to keep fingers away and not to open sample chamber when motor is active
Cuts from broken glass slides/coverslips	Medium	• Slides/coverslips are carried in suitable containers • Dispose of any broken slides and coverslips immediately in the provided sharps containers
Splash with liquid nitrogen (LN_2) while handling	Low	**Refer to SWP – Liquid nitrogen and cryogenic liquids** • Liquid nitrogen is contained within equipment so exposure is unlikely during normal operation • Filling of liquid nitrogen containers is done by facility staff
Skin or eye exposure to hazardous chemicals e.g. immersion oil 	High	• Avoid contact with oil and wash hands after use • Wear PPE appropriate to the substance used • Clean up any spills immediately with the provided cleaning materials (100% ethanol, cotton swabs, paper tissue etc.) • Refer to Safety Data Sheet / Chemalert database

Hazard / Risk	Risk rating	Risk controls
Working alone in a dark microscopy lab	Low	• Users are to notify a second person if they need to work along in a dark lab for over 3h or after hours
Eye fatigue / headaches or back, neck and shoulder discomfort associated with posture and prolonged working at the microscope	Medium	• Use a digital display if possible, rather than using the eyepiece/s for prolonged use • High-quality lens systems and eyepieces that reduce fatigue and long-term eye strain are desirable • Adjust oculars for comfort of user • Matching room lighting to the microscope field brightness to reduce eye strain • Chairs should have adjustable height, back rest and lumbar support • Maintain good posture at all times whilst working at the microscopes and computers • Take frequent breaks away from the microscope with physical stretches to relieve static sitting postures
Manual Handling: lifting microscopes	Low	• Users are not to lift or move microscopes unless trained and authorised by facility staff • Only small microscopes (e.g. stereo dissecting microscopes) designed to be portable may be moved • Small microscopes should be lifted with two hands, one hand on the arm, the other hand supporting the base • Store microscopes at bench level
Exposure to biological materials due to contamination or spills	High	**Refer to SWP - Biological Spills** • Acquisition of samples at the Cell Imaging Facility must only occur after the approval of an associated project in PPMS. Any projects to be run on the microscopes involving hazardous chemicals must have appropriate approval. OGTR requirements for safe work in a PC2 laboratory apply • Handle live cell cultures with care to avoid spilling and use standard precautions • Transport samples in secondary containment • Live samples must be sealed with permeable sealing film before being transported to the microscope room (at the host lab or in the biosafety hood in the Shared Lab on Level 2) • Disinfect outside of containers before transferring to live imaging equipment • Disinfect equipment with 70% alcohol after use • Any spills must be cleaned immediately with proper agents • Samples must be taken back to the host lab for disposal

Hazard / Risk	Risk rating	Risk controls
		• Gloves must be worn at all times when handling live cell cultures and/or hazardous materials, operating the microscope, and using the computer keyboard
Electrical shock due to faulty equipment	Low	• Regular electrical safety checks. Maintenance is performed by authorised electricians annually
Burns	Low	• Lamp replacement is only carried out by facility staff • Lamps are replaced in the morning before the system has been switched on to ensure that the housing and the old lamp are cool enough to touch

BACKGROUND INFORMATION

Standard microscopes

Upright microscopes are used for routine applications and diagnostics with thin specimens, usually on a glass slide. A compound microscope uses several lenses for higher magnification. Many different upright microscopes are used at WIMR including the Olympus BX53/DP80, Nuance/Leica DM 2500, two slide scanners (Olympus VS 120 and Nanozoomer) etc.

An inverted microscope allows samples to be studied from below, which is useful for cell cultures in liquid such as the Deltavision Elite microscope, two confocals (Olympus FV1000 and Leica SP5), Nanoimager, Zeiss live cell imaging system etc.

Stereo microscopes are generally used for low magnification observation of a sample, typically with light reflected from the surface of an object rather than transmitted through it. They generally use intense light sources such as halogen lamps or high power LEDs. They are often used for tissue dissection or small animal surgery. The stereo microscope uses two separate optical paths so each has a slightly different viewing angle, which provides depth of field.

The microscopes may have a single or double eyepiece, however, both see the same image. Digital cameras and displays may be integrated into the microscope.

Upright microscope

Stereo microscope

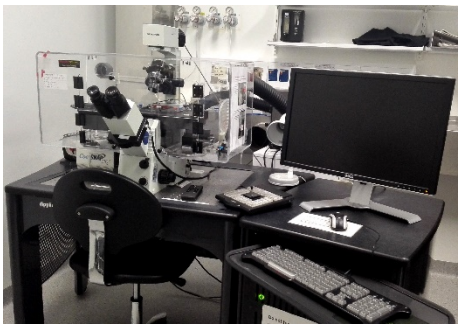
Inverted microscope

Fluorescence Microscopy

In traditional **widefield epifluorescence microscopy**, the entire specimen is subjected to intense illumination with light of a specific wavelength from a mercury arc lamp, mercury-containing metal halide lamp or LED illuminator. This results in emission of a longer wavelength that can then be viewed directly in the eyepieces or monitor. The **slide scanners (Olympus VS 120 and Nanozoomer), Zeiss live cell imaging system, Olympus BX53**, **Olympus CKX41, DeltaVision Elite, Nuance spectral imaging system** and the **Nanoimager** microscopes with widefield epifluorescence microscopes capabilities are located at WIMR.

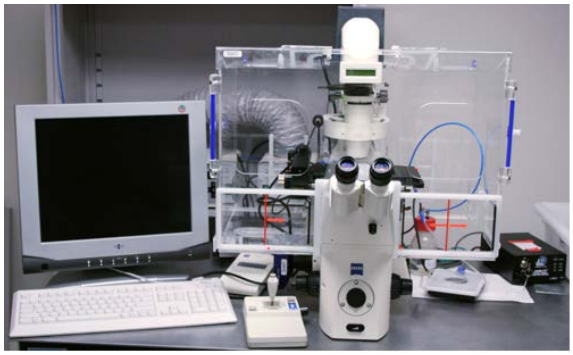
Laser confocal scanning microscopy is an optical imaging technique commonly employed to increase the optical resolution and contrast of an image. A routine problem encountered in imaging three dimensional biological samples with compound light or epifluorescence microscopes is that light is captured in multiple focal planes to create a single two-dimensional image. This can result in image distortion and loss of resolution when the sample is sufficiently thick. By using a spatial pinhole to block out-of-focus light in image formation, confocal microscopy focuses light into a single point within a defined plane, eliminating light noise from other planes; greatly reducing distortion and increasing resolution. The **Olympus FV1000** and **Leica TCS SP5** are laser scanning confocal microscopes used at WIMR.

Laser scanning confocal microscopes are examples of **Class 3B laser products**.



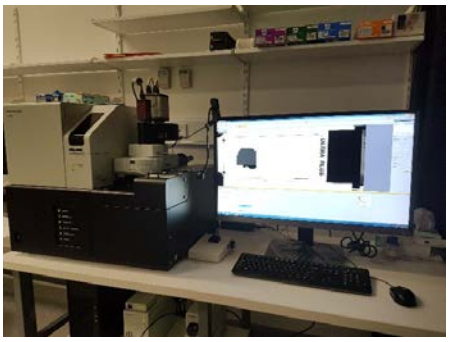
Live cell imaging systems

Live cell imaging systems are often integrated into microscopes, including the **Olympus FV 1000, Leica TCS SP5, Deltavision Zeiss Axiovert 200M, and JuliBr** microscopes. Live cell imaging requires a temperature controlled environmental chamber, usually with final 5% v/v CO_2 and so requires the instrument to be connected to a gas supply.



Slide scanner

Slide scanners are used to scan glass slides to convert them to digital microscopic images. The Olympus VS 120 and NanoZoomer slide scanners are used at WIMR.



GENERAL SAFETY PRECAUTIONS

e.g. housekeeping, standard precautions, PPE, PC2 or PC3 laboratory, induction, competencies, health counselling

Only authorised users may use the fluorescence or laser scanning microscopes or other cell imaging equipment. Booking is required.

All microscope users must be onboarded to WIMR or be under direct supervision.

Users must have completed the WIMR laboratory safety training module, signed the behavioural requirements on the PC2 laboratory form, and attended an induction for the relevant facility.

Reading and understanding this SWP and relevant user manuals is required for use of fluorescence/laser microscopes, live imaging equipment or slide scanners.

Researchers will only be given room access and access to the instrument booking calendar once they have shown competency in using the instrument.

Acquisition of samples at the Cell Imaging Facility must only occur after the approval of an associated project in PPMS. Any projects to be run on the microscopes involving hazardous chemicals must have appropriate approval. OGTR requirements for safe work in a PC2 laboratory apply.

Users must also refer to the following safe work procedures, as applicable:

- Use of gas cylinders and compressed gases
- Use of liquid nitrogen and cryogenic fluids
- Biological spills

Appropriate PPE and clothing must be worn when working with hazardous chemicals or biological material. Consult the Safety Data Sheet (SDS).

STEP-BY-STEP PROCEDURES

Include preparation of work area, equipment checks, area monitoring, signage & labelling, calibration, selection and donning of PPE, hand hygiene, how to perform the task safely, clean up & decontamination, waste disposal, record keeping

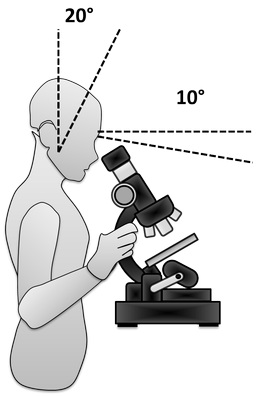
CAUTION:

- Always read and follow the user instructions for the instrument.
- Acquisition of samples at the Cell Imaging Facility must only occur after the approval of an associated project in PPMS. Any projects to be run on the microscopes involving hazardous chemicals must have appropriate approval. OGTR requirements for safe work in a PC2 laboratory apply.
- Do not look directly at laser/fluorescence illumination.
- Do not frequently switch on and off the fluorescence illumination and laser.
- Avoid contact with immersion oil, which irritates the skin or eyes.
- Seal the live sample containers with proper permeable sealing tape and wipe them with 70% ethanol before transferring to the live cell imaging system.
- Users are not permitted to interfere in any way with the microscope housing or beam delivery system, as this may render the system unsafe and cause the microscope to go out of alignment.

Set up your microscope ergonomically:

If you intend to spend a lot of time at the microscope it is important to set it up correctly so that you are sitting comfortably to avoid discomfort and potential injury and to minimise eye strain. Follow the steps below:

- Use a chair that provides back support.
- Ensure you legs go underneath the bench when sitting on a chair.
- Sit with your back against the backrest and your feet supported on the floor, chair ring or a footrest.
- Keep shoulders relaxed with elbows by your side. Use armrests if available.
- The chair and viewing height and angle of the microscope should be adjusted to the user. Ensure you are sitting upright and avoid leaning forward, looking straight down or raising the chin.
- Avoid leaning on hard edges.
- Close your eyes then focus on a distant object every 20 minutes to reduce eye strain.
- Take breaks. Every 30-60 minutes get up to stretch and move.
- Request an ergonomic assessment if you have ongoing discomfort from frequent or prolonged microscope use. Contact the WHS & Compliance Manager if you require an assessment.



Using microscopes with a mercury arc or mercury-containing metal halide lamp (fluorescence)

Mercury and metal halide lamps are used in many of the fluorescence microscopes. The UV light produced is dangerous to the eyes and skin, however, the microscopes have safety mechanisms to prevent exposure in normal use. There is a risk of exposure to toxic mercury vapour in the unlikely event that a mercury lamp explodes.

- Avoid frequent turning on and off of lamps. Leave the lamp on if another user is booked after you within half an hour as there is a minimum cool off time before the lamp can be turned back on – this time differs for individual lamps (minimum 15 minutes). Turn off the lamp if there is more than half an hour before the next booking and if you are the last user for the day.
- If you find that a lamp has been left on overnight, report this to the facility staff.
- Report issues such as dim or flickering lamps or lamps that do not turn on immediately to facility staff.
- Leave the room immediately if an explosion occurs and call the fire brigade.
- Do not look directly at the fluorescent light.
- Open shutters only after filters are engaged.
- Lamps get very hot – do not touch.
- Only facility staff are permitted to change lamps or troubleshoot any problems with the lamp housing or its interface with the microscope.

Summary of the lamp types on the fluorescent microscopes at the Cell Imaging Facility:

Microscope	Fluorescence Lamp Type	Lab
Olympus FV 1000 confocal	Traditional mercury arc lamp (100W)	J2.11
Deltavision	LED	J2.12A
Zeiss Live Cell Imaging	Traditional mercury arc lamp (100W)	J2.12C
Olympus BX53	Metal halide containing mercury (120W)	J2.08
Olympus VS 120 slide scanner	Metal halide containing mercury (130W)	J2.08
Nanozoomer slide scanner	Traditional mercury arc lamp (100W)	W1.02
Nuance (Leica DM 2500)	Metal halide containing mercury (100W)	J2.08
Leica SP5 confocal	Metal halide containing mercury (120W)	J2.10
Zeiss-PALM LCM	Traditional mercury arc lamp (100W)	J2.08

Using a laser-based microscope

Laser-based microscopes such as the scanning confocal microscopes (Olympus FV1000 and Leica SP5), the laser capture microdissection microscope (Zeiss-PALM LCM) and the Nanoimager are examples of **Class 3B laser products**, which means that there is a level of accessible emission in the specimen area, which can be harmful to the eyes and may also be harmful to skin. It is important to prevent eye exposure to the beam and guard against unintentional beam reflections. If the laser microscopes are used as prescribed there are no dangers to the user and laser safety glasses are not required. The laser is emitted from the objective tip.

- Turn on the equipment power and turn the key switch to on according to the instruction manual.
- Turn on the computer / software and set up.
- Lasers will only operate if the safety interlocks are on.

- Do not switch the lasers on if any part of the instrument housing is open.
- Never deactivate or disconnect the laser protection devices.
- Never look directly into a laser beam or reflection of the laser beam.
- Do not look into the eyepieces during the scan process or when switching the beam path.
- Do not introduce any reflective objects or your hand or fingers into the laser beam path.
- Do not set or remove a sample or alter the objectives or beam during scanning.
- All unoccupied positions in the objective turret must be closed using the supplied caps.
- Avoid frequent turning on and off of lasers. Leave the lasers on if another user is booked after you within two hours. Turn off the lasers if there are more than two hours before the next booking and if you are the last user for the day.
- Switch off the system according to the instruction manual.

Using a live imaging system

Some microscopes are connected to a CO_2 chamber to enable live cell imaging in chambered slides, petri dishes, microtitre plates, culture flasks etc. There are additional risks associated with live cell and tissue cultures and with connection to a gas supply. **Refer to SWP Using gas cylinders and compressed gases.**

- Samples must be transported to and from the facility in secondary containment with sufficient absorbent material to contain any spills.
- Samples must be labelled. GMO samples must be labelled as “GMO”.
- Live sample containers must be sealed with permeable sealing tape before being transported to the microscope room (at host lab or in the biosafety hood in the Shared Lab on Level 2)
- The outside of any containers must be decontaminated using an appropriate disinfectant before transfer to the chamber.
- Any spills must be cleaned up immediately with an appropriate disinfectant and absorbent material. Use a biological spill kit if the spill is significant.
- Uncontained bio-aerosols must not be produced.
- Gloves and gowns must be worn at all times except where designated.
- Connect the equipment to the CO_2 and compressed air gas regulators as applicable.
- Check that the correct gas has been connected and that all connections are tight.
- Check that the regulator is turned off. Then open the valve (clockwise).
- Open the regulator slowly and check that the pressure is correct for the application.
- When finished, close the regulator and valve and disconnect the apparatus.
- Apply standard precautions when working with live cells and tissue.
- If the low oxygen alarm activates, see below.

After Use:

- Always clean off the objectives and microscope stage after use with tissue soaked in 70% ethanol (or another disinfectant that is effective against the agent but will not damage the instrument).
- Do not leave any samples or used tissues on or near the microscopes.
- Biological waste must be disposed as clinical waste. Users must take any samples or waste back to their laboratory for disposal, unless otherwise agreed with the facility staff.

- Turn off microscopes and mercury lamps and replace dust covers if you are the last user for the day.
- Check that gas regulators and valves are off (if used).
- Leave the space clean and tidy for the next user.

To replace mercury lamps:

- Only trained facility staff are permitted to change mercury lamps.
- Wear PPE including gloves, lab coat and safety glasses.
- Ensure that the lamp is cool.
- Disconnect the lamp from the power by unplugging it from the wall outlet.
- Access the lamp according to the relevant microscope instructions.
- Remove the old lamp and store in the packaging it came in.
- Install the new lamp, close the housing, reconnect the lamp to the electricity supply and turn it on for half an hour to warm up. Align the lamp if required according to the microscope's instructions.
- Mercury waste is to be collected by a licensed hazardous waste collector or recycled by the supplier if possible. Contact Research Support services.

SPILL / INCIDENT / EXPOSURE RESPONSE

Describe how to deal with biological exposure/splash/needlestick, chemical spill/exposure (major and minor), equipment failure or emergency shutdown and other emergencies, as appropriate.

If anything seems to be out of order with the microscopes, STOP - do not proceed, record the situation carefully and contact the cell imaging specialist, technician, or research coordinator for the floor.

In the event of a mercury lamp breakage or explosion (rare)

Follow the Emergency Procedures – Chemical Spills. In summary:

- Evacuate the laboratory immediately.
- Once outside, shut the door and inform the Research Coordinator / Imaging Specialist. In the event of a mercury lamp explosion, call the fire brigade first.
- Contact an Emergency Warden / Spill Team.
- The room must be quarantined for at least half an hour before re-entering.
- Only trained personnel may clean up mercury spills. Note: a mercury spill kit with instructions is kept for this purpose in the Level 2 shared laboratory. PPE must be worn by all personnel involved in the clean up. Only the fire brigade can handle mercury vapour.
- Do not re-enter the laboratory until the spill team has finished.
- Submit an incident report form.

If exposed to mercury vapour from an explosion or if mercury contacts the skin:

- Remove the victim to fresh air and seek immediate medical advice.
- If breathing difficulties or bluish colouration of the skin develops, call an ambulance and apply artificial respiration. Oxygen is recommended if available and a trained person is present. Inform emergency services of mercury poisoning as an antidote is available (dimercaprol).

- Wash skin with soap and water and remove contaminated clothing. Seek medical advice if irritation occurs.

If the low oxygen alarm activates:

Follow the Emergency Procedures – Liquid nitrogen spill or low oxygen alarm. In summary:

- If trained to do so, turn off the CO2 gas supply
- Evacuate the area and notify laboratory supervisor or Facilities.
- Do not re-entre the room until the oxygen level is above 19.5%.

If immersion oil or liquid is spilled:

- If any liquid is spilled on or around the instrument, clean up the spill immediately. Unplug the equipment first if the spill is not contained or there is any possibility of electrical contact.
- Avoid contact with immersion oil as it is irritating to skin. Wash with soap and water if exposed.

Equipment maintenance:

- Only qualified service personnel may assemble or set up cell imaging equipment.
- Facility staff should monitor hours of use (metered as well as log book) to ensure that equipment is serviced as required.

REFERENCES

List relevant legislation, code of practice, standard, safety data sheet, manufacturer's instructions, research paper etc.

WIMR-SWP-WHS-GEN-01.02 Biological spills
WIMR-SWP-WHS-GEN-15.01 Liquid nitrogen and cryogenic fluids
WIMR-SWP-WHS-GEN-17.01 Using gas cylinders and compressed gases
Safety Data Sheet – BOC – Carbogen
Safety Data Sheet – Immersion Oil – GE Healthcare
Olympus FV1000 Confocal laser scanning biological microscope User Manual
Axiovert 200 M Operating Manual
AS/NZS 2243. Safety in laboratories
Emergency Procedures – Chemical Spills
Emergency Procedures – Liquid nitrogen spill or low oxygen alarm

RISK MATRIX		LIKELIHOOD				
		Almost Certain i.e. exposed to hazard continuously	**Likely** i.e. exposed to hazard regularly	**Possible** could happen i.e. exposed to hazard occasionally	**Unlikely** could happen but probably won't i.e. infrequently	**Rare** could happen but only in exceptional circumstance
CONSEQUENCES	**Catastrophic** Multiple death or permanent disability; huge financial /business impact	Very High	Very High	Very High	High	High
	Major Death or life threatening injury or illness; high financial/ business impact	Very High	Very High	High	High	Medium
	Moderate Serious injury / illness; significant financial / business impact	High	High	Medium	Medium	Medium
	Minor First aid or medical treatment; medium/low financial / business impact	Medium	Medium	Medium	Low	Low
	Minimal No or minor injury or illness; minor financial /business impact	Medium	Medium	Low	Low	Low