





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-16.01	Prepared by:	Sarah Johnston & Hong Yu
Approval date:	27 th September 2019	Next review date:	27 th September 2022
Who was consulted?	Laurence Cantrill, Hong Yu, Virginia James, Susan Wan		




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

RISK ASSESSMENT
<i>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.</i>

Hazard / Risk	Risk rating	Risk controls
<p>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</p>  	Medium	<ul style="list-style-type: none"> Any activities that permit access to hazardous laser radiation or degrades the integrity of the protective housing shall only be completed by an external, qualified, manufacturer-authorized 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
<p>Inhalation of mercury vapour or skin absorption resulting from a broken/exposed mercury lamp, may cause serious health effects or damage to the unborn child.</p>  	Medium	<ul style="list-style-type: none"> Lamp replacement by trained facility staff only Lamps are replaced after they reach their recommended lifetime. Useage is monitored Users are required to notify staff immediately of dim or flickering lamps Immediate evacuation of the area is required in the event of a lamp explosion Only trained staff may handle broken lamps or clean up mercury spills using the spill kit and PPE provided

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		<ul style="list-style-type: none"> Mercury lamp must be allowed to cool before turning back on
Fire/explosion risk due to leakage of carbogen gas (95% v/v oxygen) near ignition sources 	Low	<ul style="list-style-type: none"> Only Facilities 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 for equipment with environmental CO ₂ chamber or liquid nitrogen 	Low	<p>Refer to SWP - Storage and handling of gas cylinders Refer to SWP – Liquid nitrogen and cryogenic liquids</p> <ul style="list-style-type: none"> 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.
Pinch injury from motorised apparatus	Low	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> 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	<p>Refer to SWP – Liquid nitrogen and cryogenic liquids</p> <ul style="list-style-type: none"> Liquid nitrogen is contained within equipment so exposure is unlikely during normal operation. Filling of liquid nitrogen container is done by facilities staff.
Skin or eye exposure to hazardous chemicals e.g. immersion oil 	High	<ul style="list-style-type: none"> Avoid contact with oil and wash hands after use Wear PPE appropriate to the substance used Clean up any spills immediately Refer to Safety Data Sheet / Chemalert database
Working alone in the dark microscopy lab	Low	<ul style="list-style-type: none"> Users are to let a second person know if they need to work alone 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	<ul style="list-style-type: none"> 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.

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		<ul style="list-style-type: none"> 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 should be performed
Manual Handling: lifting microscopes	Low	<ul style="list-style-type: none"> 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	<p>Refer to SWP - Biological Spills</p> <ul style="list-style-type: none"> Handle live cell cultures with care to avoid spilling and use standard precautions Transport samples in secondary containment No infectious agents allowed in cell imaging facility Disinfect outside of containers before transfer to live imaging equipment Wear gloves when handling live cell cultures Disinfect equipment with 70% alcohol after use Any spills must be cleaned immediately Samples must be taken back to the host lab for disposal. Gloves may be worn inside environment chambers when handling hazardous material, but no gloves may touch the microscope housing or the computer keyboard
Electrical shock due to faulty equipment	Low	<ul style="list-style-type: none"> Regular electrical safety checks. Maintenance is performed by authorised electricians annually
Burns	Low	<ul style="list-style-type: none"> 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 Leica DM 2500.

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.

An inverted microscope allows samples to be studied from below which is useful for cell cultures in liquid. The Zeiss AxioVert 200M fluorescence/live cell imaging system uses an inverted microscope.

The microscopes may have a single or double eye piece, 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 epi-fluorescence microscopy**, the entire specimen is subjected to intense illumination with light of a specific wavelength from a mercury or xenon arc-discharge lamp. This results in emission of a longer wavelength which can then be viewed directly in the eyepieces or monitor. The **Olympus BX53**, **Olympus CKX41**, **DeltaVision Elite**, **Nuance spectral imaging system** and the **Nanoimager** microscopes with widefield epi-fluorescence 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 epi-fluorescence 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 Axiovert 200M** and **Zeiss Axiovert 200M** microscopes. Live cell imaging requires a temperature controlled environmental chamber, usually with final 5% v/v CO₂ 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 and signed the behavioural requirements for PC2 laboratory form and have attended an induction for the relevant facility.

Training in this SWP and relevant user manuals by the Cell Imaging Specialist or a nominated Research Coordinator 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 on using the instrument.

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

Personal protective equipment and clothing must be worn.



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

Using the microscopes:

CAUTION:

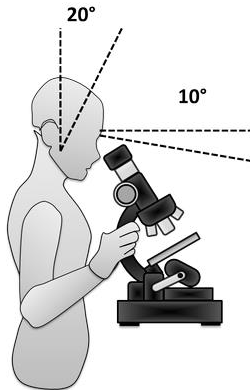
- Always read and follow the user instructions for the instrument.
- All projects must be approved. Infectious agents must not be brought into the cell imaging facility.
- Do not look directly at laser/fluorescence illumination.
- Avoid contact with immersion oil, which irritates the skin or eyes.
- 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 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 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. Maximum 5 hours in a day.

- 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 lamp (fluorescence)

Mercury 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 or a lamp does not turn on and raise the alarm.
- 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 trouble shoot any problems with the lamp housing or its interface with the microscope.

Using a laser scanning confocal microscope

Laser scanning confocal microscopes 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 scanning microscope is 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. An LED is lit while the laser beam is emitting (check instruction manual as this differs depending on the equipment).

- 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 is 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 CO₂ chamber to enable live cell imaging in 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”.
- 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.
- Wear gloves when handling biological samples, but remove gloves before touching the microscope housing or computer/keyboard.
- Connect the equipment to the CO₂ 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 using 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. External 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)

- Evacuate the laboratory immediately. Mercury vapour has an 8hr TWA of 0.003 ppm (0.025 mg/m³)
- Once outside, shut the door and inform the Research Coordinator / Imaging Specialist.
- Contact an Emergency Warden / Spill Team.
- The green a/c purge button should be activated to extract any airborne vapour. 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 cell imaging laboratory. A cartridge respirator must be worn by all personnel involved in the clean up.
- Do not re-enter the laboratory until the spill team has finished.
- Submit an incident report form.

If exposed to mercury vapour or if mercury contacts the skin:

- Remove 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 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

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

