Using Digital Light Sheet on Stellaris 5 Confocal DLS Microscope Safe Work Procedure

DETAILS			
Description of task / activity:	Using Digital Light Sheet (DLS) on Stellaris 5 Confocal DLS Microscope. Note this SWP is to be used in conjunction with SWP – Using a fluorescence or laser scanning microscope.		
Centre / Department(s):	WIMR laboratories and Scientific Platforms: Cell Imaging		
SWP Reference:	WIMR-SWP-WHS-GEN-63.01	Document Author/s:	Hui Zhang
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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 Stellaris 5 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 are only to be completed by a qualified service engineer • Equipment has protective housing and shielding to contain laser and/or UV radiation into appropriate location • Equipment is regularly inspected and maintained • Instrument is included on register of laser equipment • Warning signage indicating laser or UV hazard • Warning indicator light lit when laser is emitting • User training covers laser safety
Asphyxiation by displacement of oxygen due to accidental release or leakage of CO2 from equipment with environmental CO2 chambers or liquid nitrogen 	Medium	• System is under electronic feedback control • Room has active air circulation • CO2 feed is regulated • Oxygen deficiency / CO2 monitoring and alarms. Ensure users are aware of systems in place. Once the alarm goes on, users are to leave the lab immediately, press the red a/c purge button if possible, and notify staff
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

Hazard / Risk	Risk rating	Risk controls
Cuts from broken glass slides/petri dish/coverslips with subsequent biological exposure	Medium	• Slides/petri dish/coverslips are carried in suitable containers • Instrument Z stage travel limits • User training • Gloves • Disposal of any broken slides and coverslips immediately in the provided sharps containers
Eye fatigue / headaches or back, neck and shoulder discomfort associated with posture and prolonged working at the microscope	Medium	• When using the microscope stretching breaks should be taken. It is recommended to spend at least approximately 1 minute stretching every 30 minutes on the microscope. • Use a digital display if possible, rather than using the eyepiece/s for prolonged use • Adjust oculars for comfort of user • Match room lighting to the appropriate brightness to reduce eye strain when viewing dim signals • Chairs have adjustable height, back rest and lumbar support, ensure adjusted appropriately for use • 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
Musculoskeletal injury when changing the condenser in the microscope due to poor manual handling techniques	Low	• Users are to be trained for changing condenser by facility staff • Do not overstretch while installing and keep ergonomic posture. Users should request assistance if required. • Condenser is less than <5kg
Exposure to biological materials during DLS imaging, or due to contamination or spills	Medium	**Also 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/unfixed samples must be sealed with permeable sealing film (unless sealed otherwise) before being transported to the microscope room (at the host lab or in the biosafety hood in the Shared Lab on Level 2) for confocal imaging only. This does not apply for samples to undergo lightsheet microscopy as sample immersion will need to occur • Additional PPE including P2/N95 mask and safety glasses MUST be worn when handling live /unfixed samples, uncontained, in the incubation chamber on the microscope

Hazard / Risk	Risk rating	Risk controls
		• Disinfect outside of containers before transferring to live imaging equipment • Disinfect equipment with 70% alcohol after use • Biological and potentially infectious material is used on the scopes such as unscreened human tissue or samples that have been infected with viral vectors. All scope work should be performed following PC2 work guidelines and any biological material spilt must be cleaned up with 70% ethanol. Samples must be taken back to the host lab for disposal, or disposed of with appropriate inactivation in the biological safety cabinet • 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	• Maintenance is performed by authorised electricians annually
Burn injury: use of hot glue gun, handling hot agarose (65°)	Low	• Do not overheat agarose • Unplug heat gun when finished and ensure cooling period is performed in a safe manner, with labelling if necessary • Clean up any spills immediately • Refer to Safety Data Sheet / Chemalert database for SDS

BACKGROUND INFORMATION

e.g. legal framework, supporting information

The Leica Stellaris 5 Confocal DLS microscope combines the latest technologies of confocal microscopy, digital light sheet (DLS) and live cell imaging. Light sheet is ideal for fast, gentle, and volumetric imaging of whole living model organisms, cleared tissues, and cells as they develop over a period of time.

The operation of DLS may require biological samples to be uncontained so there is a risk of exposure in the event of a spill. Appropriate PPE must be worn if unfixed or live biological samples are uncontained in case of a spill or splash.

TRAINING & INDUCTION

e.g., induction, competencies, pre-requisites, assessment

Training in this SWP is required. Training is to be provided by Cell Imaging Facility Staff

All researchers must have completed a laboratory safety induction consisting of:

- WIMR online Laboratory Safety Training module
- PC2 laboratory induction
- Signed the behavioural requirements on the PC2 laboratory form

All users of the DLS Stellaris confocal microscope must also have completed training in SWP – Using a fluorescence or laser scanning microscope which covers the risks associated with using confocal microscopes.

Additional training / information is required:

- SWP – Essential Laboratory Safety Rules
- SWP – Using a fluorescence or laser scanning microscope
- SOP – Operation on Stellaris 5 for confocal imaging
- SOP – Operation on Stellaris 5 for DLS imaging

Instrument training competency is assessed via demonstration of independent instrument operation, in conjunction with verbal explanation of all aspects of operation and troubleshooting common faults. After training is carried out, a training competency quiz is to be completed and passed before instrument access is authorised by Imaging staff.

GENERAL SAFETY PRECAUTIONS

e.g. standard precautions, authorisations, health counselling, vaccination, compliance requirements

Acquisition of samples at the Cell Imaging Facility requires approval of the research project in PPMS. In addition, prior to the commencement of the project, researchers are to discuss their project with imaging staff.

Researchers must provide evidence of IBC project approval for any work involving GMOs.

Equipment booking is required in PPMS. Only authorised users who have completed the training may use Stellaris 5 Confocal DLS microscope.

Primary and any secondary containers used to transport any organisms out of a PC2 facility must be free of contamination with GMOs or infectious agents prior to being transported out of the facility.

Users must be onboarded to WIMR or be under direct supervision.

All users must follow the WIMR Essential Laboratory Safety Rules.

PERSONAL PROTECTIVE EQUIPMENT & CLOTHING

e.g. mandatory PPE, PPE for specific circumstance

The following PPE is required:

- Long-sleeve lab gown
- Enclosed non-slip and water-resistant shoes
- Disposable gloves when handling hazardous materials or operating the instrument (including keyboard)

Additional PPE is required for use of the DLS with live/unfixed samples that are uncontained (i.e. unsealed chamber)

- P2/N95 face mask and safety glasses to be worn when loading or unloading samples and while the samples are uncontained during operation and when decontaminating the instrument including the mirror/lens.

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 SOP and SWP for the instrument.
- Do not look directly at laser/fluorescence illumination.
- Do not frequently switch on and off the fluorescence illumination and laser.
- Avoid contact live/unfixed samples by wearing appropriate PPE.
- Care needs to be taken not to generate aerosol or splash.
- 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.

Refer to SWP- Using a fluorescence or laser scanning microscope for ergonomic set up of microscopes, laser safety and use of the live imaging system.

Refer to Appendix and SOP - Operation Stellaris 5 for DLS imaging for detailed operating procedures.

Imaging of live or unfixed samples

Due to the potential risk of exposure to biohazardous materials while loading and unloading samples, additional requirements apply for unfixed or live samples that are risk group 2 (i.e. human or animal cells or tissue):

- No risk group 3 or higher unfixed samples are allowed to be imaged.
- For long term live sample imaging, live/unfixed samples are loaded and contained in a sealed chamber filled with 5% CO_2. Refer to SWP - Using a fluorescence or laser scanning microscope.
- Specific approval is required for imaging of unfixed or live samples that are either (i) GMO or (ii) infectious via the respiratory route (e.g., samples infected with SARS-C0V-2). In this case, a P2/N95 mask and safety glasses must be worn by all room users and appropriate signage placed on the door before sample containers are opened within the incubation chamber at all times while the chamber is open.
- During operation, care needs to be taken not to generate aerosols or splashes by loading/unloading the sample/mirror slowly, no quicker than 1 turn every 30 seconds (or whatever is appropriate).
- Proper instrument decontamination is required at the end of the experiment.

Operation:

- Place prepared sample on the stage adaptor and focus the microscope
- For live imaging, use CO_2. Turn on the CO_2 supply using the valve on the wall and replace cover to create a sealed chamber filled with 5% CO_2.
- Start acquisition.
- Complete acquisition.
- Clean the objectives and microscope stage with 100% ethanol or other suitable disinfectant that will not damage the microscope (check with the imaging staff).
- Turn off CO_2 supply valve.

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.

Biological spill:

Any spills must be cleaned immediately with proper agents - refer to SWP – Biological Spills.

Standard precautions apply when cleaning up spills – wear gloves, gown and other PPE as appropriate.

Small spills can easily be managed by wiping the area immediately with paper towelling, tissue or cotton wool, then clean the area with water and detergent followed by a hospital grade disinfectant or 70% ethanol.

For objectives and microscope stage, clean up with lens tissue or paper tissue soaked in 100% ethanol (or another disinfectant that is effective against the agent but will not damage the instrument).

For larger spills use a biological spill kit. This involves use of an absorbent pad to soak up the spill then use an appropriate disinfectant (Virkon, bleach or 70% ethanol) to clean up. Wear safety glasses and avoid generating aerosols. Unplug the equipment first if there is any possibility of electrical contact.

Dispose of waste in a clinical waste bin.

In the event of an exposure or sharps injury

Follow the Biological Exposure Action Plan.

Notify your supervisor and submit an incident report for any significant spill or biological exposure.

If the low oxygen alarm or CO_2 alarm activates:

Follow the Emergency Procedures – Liquid nitrogen spill or low oxygen alarm or Emergency Procedure – CO2 alarm, as appropriate. In summary:

If trained to do so, turn off the gas supply

Evacuate the area and notify laboratory supervisor or Facilities. Allow time for ventilation to replace air. Use the A/C purge button to speed up ventilation if installed.

Do not re-entre the room until the oxygen level is above 19.5% and CO2 alarm has stopped.

In the event of instrument failure or electrical or laser hazard:

Do not attempt to fix the instrument.

If there is unexpected power interruption the UPS will provide power to the instrument for several minutes to retrieve the biological samples from the imaging chamber and place back to its container if it is safe to do so.

Emergency evacuation

In the event of immediate emergency evacuation (e.g., fire, bomb threat), abort the imaging, retrieve the biological samples from the imaging chamber and place back to its container if it is safe to do so, and leave the facility safely.

Reporting:

Report all incidents and instrument failures or major spills to westmead.cytometry@sydney.edu.au.

A WIMR incident report must be completed within 24 hours for any exposure or major spill or near miss.

Contact the WHS & Compliance Manager and/or Chief Warden immediately if a serious incident occurs or emergency services were called. Notification to a regulatory authority may be necessary.

REFERENCES

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

- SOP – Operation on Stellaris 5 for confocal imaging
- SOP – Operation on Stellaris 5 for DLS imaging
- WIMR-SWP-WHS-GEN-16.03 – Using a fluorescence or laser scanning microscope
- SWP – Essential Laboratory Safety Rules
- SWP – Working with carcinogens and highly toxic substances
- SWP - Biological spills
- Emergency Procedures – Chemical Spills
- Emergency Procedures – Liquid nitrogen spill or low oxygen alarm

RISK MATRIX		LIKELIHOOD				
		Almost Certain i.e. exposed to hazard daily	**Likely** i.e. exposed to hazard regularly	**Possible** could happen / exposed occasionally /monthly	**Unlikely** could happen but probably won't i.e. infrequently	**Rare** could happen in exceptional circumstance
CONSEQUENCES	**Catastrophic** Multiple death or permanent disability; huge financial /business impact	**Extreme**	**Extreme**	**Very High**	**Very High**	**High**
	Major Death or life threatening injury or illness; high financial/ business impact	**Extreme**	**Very High**	**Very High**	**High**	**Medium**
	Moderate Serious injury / illness; > 4 days lost time; notifiable; significant business impact	**Very High**	**Very High**	**High**	**Medium**	**Low**
	Minor First aid or medical treatment; medium/low financial / business impact	**Very High**	**High**	**Medium**	**Medium**	**Low**
	Minimal No or minor injury or illness; minor financial /business impact	**Medium**	**Medium**	**Low**	**Low**	**Low**

Appendix: Detailed Operating Procedures

Hardware preparation

- switch off the system
- hardware prep/installation
    1. turn on the camera
    2. change the BF knob to DLS position
    3. swing out TLD and insert DLS magnetic insert
    4. replace the condenser with the DLS one and bring up the condenser to a safe level
    5. screw in the right detection lens capped with the right mirrors
    6. tilt the transmitted light arm down
- switch on the system

Open LASX software and log into PPMS

Preparation on the microscope

- place the sample into the stage adaptor in the right orientation, such as a FEP tube or U -shape capillary, filled with your samples in either water or agarose, sits in the middle cross the bottom of a 35mm petri dish
- focus on your sample with fluorescence illumination by adjusting Z-wide
- fill the petri dish with distilled water/medium
- carefully lower the DLS condenser by adjusting the silver knob till the mirror cap immerses in the water/medium (DO NOT go too fast to avoid mirrors breaking the petri dish bottom!)
- slowly move the DLS condenser further down and check in the eyepieces till you see sharp sample reflections in both mirrors

Using a live imaging system for long term imaging

- Turn on the Okolab temperature and CO_2 control screen
- Turn on the valve of CO_2 supply on the wall
- Place the cover on top of the stage adaptor to create a sealed chamber filled with 5% CO2.

Calibration

- Step 1: detection to bring your sample into focal plane of the detection objective
- Step 2: illumination to measure the Z position of the detection objective to be able to ultimately place the illumination objective at the optimal position at later steps
- Step 3: optimisation to focus the “beam waist” of the light sheet in the focal plane

DLS acquisition

- set up fluorescence channels (adding new channels for multi-color imaging, laser and camera settings etc)
- calibrate the mirror positions for all channels by defining the “mirror position”
- set up Z stack

- Start acquisition

System shut-down

- lower the illumination objectives to the lowest position from microscope touch screen
- exit LASX software
- turn off the system hardware
- log out from PPMS
- Leave the computer on all the time to allow daily data sync

Clean-up

- Always clean off the objectives and microscope stage after use with tissue soaked in 100% 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.
- Check that Okolab control screen and valves of CO_2 supply are off (if used).
- Leave the space clean and tidy for the next user.