Title: STANDARD OPERATING PROCEDURE (SOP) FOR USE OF CONFOCAL MICROSCOPE			
Microscope Name: Upright Confocal Microscope – LSM800			
Laboratory (Location): Experimental Medicine Building (EMB), Zebra Fish Facility, Level 4, #04-22H-01, 59 Nanyang Drive, Nanyang Technological University, Singapore 636 921.			
N2 licence #: N2-04966-0066, item 23		**Machine Serial #: 2634000165**	
N3-licence waiver date:19 March 2020		**SOP effective date:30 June 2022**	
SOP No: IF/N3W/SOP/02		**Revision No:**	**03**
Prepared by:		**Balakrishnan Kannan Assistant Director, RASS**	
Approved by:		**Safety Committee, LKCMedicine**	
Validity:		**24 months**	

1.0 PURPOSE

To provide guidance on safe use of confocal microscope. The SOP is aims to standardize operation of the confocal microscope and to protect the user from potential hazards such as eye injury, mercury contamination. This procedure is to be followed by all users while using the Upright Confocal Microscope – LSM800.

2.0 RESPONSIBLE PERSONNEL

2.1 Principal Investigator (PI)/Mentor:

The PI/Mentor is to ensure that:

2.1.1 risk assessment is done for imaging projects using confocal microscope.

2.1.2 personnel have adequate knowledge about the reagents used in the experiment and the safety precautions that need to be observed.

2.1.3 all incidents or lapses are reported to the Safety Office (Dr Sini Mathew (sinimathew@ntu.edu.sg) and/or Mr Sowpati Jayaker (jayaker@ntu.edu.sg).

2.1.4 make sure precautions are taken if embarking on a biophysics/bioengineering project where the specimen or sample carrier is reflective/highly scattering (e.g., metal-coated surface in sample carrier, metal particles).

2.1.5 RASS Staff (Dr Balakrishnan Kannan, kbala@ntu.edu.sg) is aware of projects as in 2.1.4 above, prior to start of the imaging project in confocal microscope.

2.2 Laboratory Personnel:

The user is responsible for:

2.2.1 the safety precautions that need to be observed while using the microscope.

2.2.2 safe handling of the equipment and complying with guidelines as provided in this SOP or the latest version available at the in the staff intranet (Z:\Safety\School SOPs) or available with the lab safety representative.

2.2.3 report to the PI/RASS staff (Dr Balakrishnan Kannan kbala@ntu.edu.sg) of any lapses in the protocol.

3.0 PROCEDURES

3.1 BEFORE USE:

3.1.1 Users must hold valid N3 license or

3.1.2 Users must have completed the on-line course listed below from NTU and a copy of the transcript is sent to Imaging Facility staff and Safety Office:

3.1.2.1 OHS2NIR01: Working with Non-Ionising Radiation

3.1.3 Users must receive authorized training from Imaging facility staff and the training is documented. The training records to be maintained in the individual PI group Safety Management System too.

3.1.4 Users must have read and understood this SOP or the latest version available at the in the staff intranet (Z:\Safety\School SOPs) or available with the lab safety representative, the associated risk assessment(s) and the user notes for this equipment

3.1.5 Samples brought into the Imaging Room must be clean, dry, and sealed in case of fixed cells/tissue-sections. Slides must be free from

excess mounting media and the nail varnish/sealant must have had at least 60 minutes to cure.

3.1.6 Live cells, embryo, imaging medium must be transported with secondary containers in place.

3.2 TO USE:

3.2.1 User must comply with the **"before use"** criteria in the previous section.

3.2.2 User must follow instructions below for a) booking the equipment, b) operating the metal-halide (HXP) lamp, c) operating the microscope-frame, d) operating the lasers and e) additional SOP requirements as described below.

3.2.3 User should not attempt to modify/repair any part of the equipment. Maintenance/repair of the equipment is to be performed by authorized personnel (Imaging Facility staff, Vendor appointed Service Engineer and Applications Specialist) only.

4.0 BOOKINGS AND CANCELLATIONS:

4.1 User must a have a booking in the central equipment booking system the Pasteur Platform Management System (PPMS) (https://ppms.asia/singascope/login/?pf=8) to use the confocal microscope.

4.2 The user should use the equipment in his/ her own booked slot because of the presence of potential safety hazards while using the confocal microscope.

4.3 Allowing another user to use the confocal microscope during one's booking **will be treated as safety violation** and reported to Safety Committee for further action.

4.4 User must follow the Imaging Facility rules for booking/cancelling of the confocal microscope to ensure correct use of the metal-halide (HXP) lamp and the lasers.

5.0 USING METAL-HALIDE (HXP) LAMP:

5.1 The Metal-Halide lamp (HXP) has a bulb that contains Mercury and hence presents mercury contamination hazard.

5.2 If the lamp is turned ON and OFF too frequently, especially when it is hot, there is a possibility of explosion or implosion of the bulb leading to release of mercury (liquid or vapour) in the Imaging Room.

5.3 To avoid mercury contamination hazard, the HXP lamp must be operated strictly as below.

5.4 Before turning on, check that the lamp has been off for at least 30 minutes (according to the Confocal Microscope Logbook).

5.5 If it has been turned off within the last 30 minutes user must wait until 30 minutes has elapsed before turning on.

5.6 Before turning off the lamp, check the booking calendar in -PPMS to see if someone is using the microscope after you. If someone is using it within 1.5 hours of your session ending, leave it on.

5.7 Otherwise, turn it off so long as it has been ON for at least 30 minutes (see below).

5.8 Do not turn the lamp off within 30 minutes of being switched on. If you finish your work within 30 minutes (and no-one has booked to use it in the next 1.5 hours), you must wait until 30 minutes has elapsed.

5.9 Upon turning the lamp off (and the entire microscope) record the time to the nearest 5 minutes in the Confocal Microscope Logbook.

6.0 HXP LAMP ACCIDENT RESPONSE PROCEDURES:

6.1 If in the unlikely event of lamp bulb explosion (you will hear a loud “bang”) or implosion (glass crumbling sound), evacuate the room immediately, closing the door behind you.

6.2 User should not go back to the Imaging Room to pick-up personal effects or to turn off the microscope system.

6.3 If an accident happens,

6.3.1 the user might be affected by mercury vapour or panicked and hence might not be able to do the correct thing. User can contact RASS staff (65141256) / Safety officer (65923209, 65923913) / colleague and seek help to call the emergency line (NTU Fault Reporting Hotline: 6790 4777 (24-hour); LKCMedicine Emergency Helpline Number: 6592 3839 (24 hours); NTU Emergency and Security Hotline: 6790 5200 (24-hour)) or call Ambulance (995). The numbers are kept in a folder in the Imaging Room/cubicle.

6.3.2 If working after office hours, user should strictly follow “SOP for Lone Working”, call the emergency line (6.3.1).

6.3.3 Place “No Entry” sign across the door and wait for help. No Entry Signage is kept in the folder (6.3.1).

6.3.4 Report the incident to the RASS staff (kbala@ntu.edu.sg) / Safety Office (sinimathew@ntu.edu.sg; jayaker@ntu.edu.sg).

6.3.5 Any incident should be reported online on the Incident Investigation Reporting Form at the OHSE website within 24 hours.

7.0 UV ILLUMINATION FROM HXP LAMP:

7.1 While looking at nuclear counterstain such as DAPI, Hoechst etc. in the specimen through binocular, the specimen is illuminated with ultraviolet (UV) light. UV light may damage cornea, eye lens and the macula.

7.2 User should not look directly at the UV illumination.

7.3 The image seen through the binocular is in the visible range and hence safe to view.

8.0 LASER SAFETY AND POTENTIAL EYE INJURY HAZARD:

LASER stands for Light Amplification by Stimulated Emission of Radiation. All the light waves in a laser beam travel in the same direction forming a straight, intense, and nearly parallel beam of light, even over long distances. Laser can cause eye injury, skin burns, skin cancer (in the ultra-violet range) and fire hazard. However, the power level of Class 3b laser used in this microscope presents potential eye injury hazard. Not only the direct beam of laser light but also a specularly reflected laser light can cause eye injury.

The Laser Module (LM) in this confocal microscope delivers laser light at four discrete wavelengths (405 nm, 488 nm, 516 nm and 640 nm). The laser light output from the LM is coupled into the microscope through the scan-head using optical fibre.

The microscope is equipped with three laser-safety interlocks.

Interlock 1: When the user is looking through the binocular while setting up the imaging experiment, the laser light cannot illuminate the specimen. User can only employ either the HXP lamp or the Transmitted light to locate specimen.

Interlock 2: When the laser is scanning the specimen, the user can look at the image only on the computer screen. User cannot see anything through the binocular as the light path to the binocular is blocked.

The only location where the user can see the laser is at the sample illumination site. To guard against user staring at the laser light at the sample illumination site, an opaque barrier is provided to use when the laser is scanning the specimen.

9.0 USE OF THE MICROSCOPE:

[Note: This document does not constitute a manual or training guide. Users of this equipment must receive authorized training from Imaging Facility staff and use this Standard Operating Procedure in conjunction with the user notes (written during training) for this equipment and the risk assessment for the imaging project].

9.1 Follow the confocal microscope user notes for operation of the computer, microscope, scan-head, HXP Lamp and lasers.

9.2 In the confocal microscope, check that the lowest magnification objective (such as 5x, 10x) is in the imaging position before launching the control software (Zen).

9.3 Make sure that correct stage adapter that can hold the sample carrier is on the microscope stage.

9.4 With NO illumination (laser and HXP lamp shutters closed), lower the stage to “Load Position” and load your sample carrier on to the microscope stage.

9.5 Add a small drop of the immersion fluid on to the slide if the lens requires immersion fluid.

9.6 If using immersion fluid (for use with oil or water objective lenses only) make sure that you are familiar with the MSDS for the immersion fluid. A copy of MSDS is available at the microscope table.

9.7 Wash your hands if you touched the immersion oil inadvertently.

9.8 Bring back the stage to “Work Position”.
With NO illumination (laser and HXP lamp shutters closed), swing in the objective lens for use while imaging.

9.9 Sit comfortably on a lab chair and adjust the distance between the binocular tubes to match your inter-pupillary distance under brightfield illumination.

9.10 With the aid of short-cut buttons in the “Locate” tab in Zen software use either brightfield channel or any one of the fluorescence channels and adjust the stage height (Z-knob) to bring the specimen into focus, while viewing through the eyepieces.

9.11 Take particular care not to raise the stage too high beyond the focal point.

9.12 Raising too high/too fast may lead to the objective front lens crashing against the sample carrier.

9.13 Place the opaque barrier on the microscope stage.

9.14 Acquire images after optimizing the acquisition parameters in ZEN.

9.15 Save all the useful images in appropriate file format, in the data saving location advised by the Imaging Facility. Back-up data periodically.

9.16 Never look directly into the laser light scanning the specimen.

9.17 DO NOT manipulate or take a closer look at the specimen/sample carrier on the stage when the laser is scanning the specimen.

9.18 If required to manipulate the sample carrier with hands or required to take a closer look directly at the specimen/sample carrier on the stage, stop the laser scan by clicking stop button in the “Acquisition” tab in Zen.

9.19 Then proceed to manipulate or take a closer look.

9.20 If required to look through the binocular, go back to Locate tab and use the short-cut buttons for either brightfield channel or any one of the fluorescence channels.

9.21 To change/remove sample carrier, remove the opaque barrier from the microscope stage and keep it aside.

9.22 With NO illumination (laser and HXP lamp shutters closed), bring the stage to “Load Position” and remove your sample carrier from the microscope stage.

9.23 Load new sample carrier, if required, and bring back the stage to work position.

9.24 Start from 9.5 to set the imaging experiment, manipulate sample carrier, acquire images.

9.25 When imaging is completed, repeat **9.21-9.23,** and proceed to shut down the complete system.

10.0 LASER ACCIDENT RESPONSE PROCEDURES:

10.1 If the user accidentally looked at the laser beam or felt a flash in the eye(s), the eyes wink due to reflex action.

10.2 User should not try to look at the laser light again.

10.3 User should close the eyes, turn away from the microscope frame, open the eyes and walk out of the Imaging Room/cubicle.

10.4 User should not go back to the Imaging Room/cubicle to pick-up personal effects or to turn off the microscope system.

10.5 If an accident happens, the vision might be affected, or the user might be panicked and hence might not be able to do the correct thing. User can contact RASS staff (65141256) / Safety officer (65923209, 65923913) / colleague and seek help to call the emergency line (NTU Fault Reporting Hotline: 6790 4777 (24-hour); LKCMedicine Emergency Helpline Number: 6592 3839 (24 hours); NTU Emergency and Security Hotline: 6790 5200 (24-hour)) or call Ambulance (995). The numbers are kept in a folder in the Imaging Room/cubicle.

10.6 If working after office hours, user should strictly follow “SOP for Lone Working”, call the emergency line (10.5).

10.7 Place “No Entry” sign across the door and wait for help. No Entry Signage is kept in the folder (10.5)

10.8 Report the incident to the RASS staff (kbala@ntu.edu.sg)/ Safety Office (sinimathew@ntu.edu.sg; jayaker@ntu.edu.sg).

Any incident should be reported online on the Incident Investigation Reporting Form at the OHSE website within 24 hours.

11.0 CLEANING OBJECTIVE LENS/TIDYING THE WORK AREA:

11.1 Users must clean the oil/water immersion objective with lens cleaning paper only.

11.2 Use dry lens paper followed by lens paper sprayed with alcohol (Analytical-grade) provided near the microscope-frame.

11.3 DO NOT use swab-grade or 70% ethanol on Optics.

11.4 Clean immersion fluid, if spilled on the microscope stage, stage adapter, -microscope-frame, computer table, keyboard, mouse with Kim Wipes and Alcohol (swab-grade).

11.5 Dispose all soiled tissue in the waste bins.

12.0 ADDITIONAL SOP REQUIREMENTS FOR USE:

12.1 Maintain correct posture while working at the microscope and computer.

12.2 Do not work at the confocal microscope / computer for more than 2 hours without taking a break.

12.3 Make sure that the culture medium or the imaging buffer is not spilled on to the microscope/objective lens.

12.4 If there is a spill, clean immediately and report to Imaging Facility Staff and Safety Office.

12.5 Hazardous chemicals are not to be brought into the Imaging Room without prior consent from Imaging Facility Staff.

12.6 Microscope slides should be carried in an appropriate container. The broken slides/ coverslips should not be left at the imaging facility or on the microscope table and should be disposed off in sharp bins at the respective user's labs. Slides left in the Imaging Room shall be disposed without notice.

12.7 All the biological materials (specimen, buffer, drugs/reagents, syringes, needles, pipette tips) brought to the Imaging Room should be brought back to respective user's lab and disposed.

12.8 No biological material to be disposed in bins in the Imaging Room.

13.0 REFERENCES:

13.1 MSDS for the reagents in use

13.2 Lab risk assessment for imaging projects using confocal microscope

13.3 LKCMedicine Imaging Facility Confocal Microscope Training Records