Title: STANDARD OPERATING PROCEDURE (SOP) FOR USE OF CONFOCAL MICROSCOPE WITH RESONANT SCANNER		
Microscope Name: Live Cell Confocal Microscope with resonant scanner AXR		
Laboratory (Location): Clinical Science Building (CSB), Support Room 10, Level 11, 11 Mandalay Road, Nanyang Technological University, Singapore 308 232.		
N2 licence #: N2/2025/00119, item 4	**Machine Serial #: 551725**	
N3-licence waiver date: 22 May 2026	**SOP effective date: 22 May 2026**	
SOP No: IF/N3W/SOP/04	**Revision No:**	**0**
Prepared by:	**Esther Koh Facility Manager, NOBIC**	
Approved by:	**Safety Committee, LKCMedicine**	
Validity:	**24 months**	

1.0 PURPOSE

To provide guidance on safe use of the confocal microscope. The SOP aims to standardise the operation of the confocal microscope and to protect the user from potential hazards such as eye injury. This procedure is to be followed by all users while using the live cell confocal microscope with resonant scanner - AXR.

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 the confocal microscope.

2.1.2 personnel have adequate knowledge about the reagents and other materials 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 - Mr Sowpati Jayaker (jayaker@ntu.edu.sg).

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

2.1.5 Imaging Facility staff (Dr. Esther Koh, nobic.facilities @e.ntu.edu.sg) is aware of projects as in 2.1.4 above, prior to start of the imaging project using the 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 confocal 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 staff intranet (Z:\Safety\School SOPs) or available with the lab safety representative.

2.2.3 report to the PI/Imaging Facility staff (Dr. Esther Koh, nobic.facilities @e.ntu.edu.sg) of any lapses in the protocol.

3.0 PROCEDURES

3.1 BEFORE USE:

3.1.1 Users must hold valid N3 licenses or

3.1.2 Users must have completed 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 authorised 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 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 be completely hardened.

3.1.6 Live cells, imaging medium must be transported with secondary containers that are spill-proof.

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 microscope-frame, c) operating the lasers and d) 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 authorised personnel (Imaging Facility staff, Vendor appointed Service Engineer and Applications Specialist) only.

4.0 BOOKINGS AND CANCELLATIONS:

4.1 User must have a booking, in the Pasteur Platform Management System (PPMS) (https://ppms.asia/singascope/login/?pf=8) or the booking system currently in use, 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 an unauthorised 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 lasers.

5.0 UV ILLUMINATION FOR EYEPIECE VIEWING:

5.1 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.

5.2 User should not look directly at the UV illumination.

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

6.0 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 six discrete wavelengths (405 nm, 445nm, 488 nm, 561 nm, 640nm and 730 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 laser-safety interlocks in a fully enclosed system. When any of the doors of the enclosure is open, the laser/s stop firing.

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 LED lamp or the Transmitted light to locate specimen.

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.

If the transmitted light arm is pushed up regardless of whether the laser is scanning or not, the laser output will be blocked.

There is no way that the user can see the laser beam when it is scanning the specimen.

7.0 USE OF THE MICROSCOPE:

[Note: This document does not constitute a manual or training guide. Users of this equipment must receive authorised 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].

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

7.2 In the confocal microscope, check that the lowest magnification objective (2X) is in the imaging position before launching the control software (NIS Elements).

7.3 With NO illumination (laser and LED shutters closed), select the objective lens required for imaging.

7.4 Push up the transmitted light arm.

7.5 Add a small drop of the immersion fluid if the lens requires immersion fluid.

7.6 If using immersion fluid (for use with oil, silicone or water objective lenses only) make sure that you are familiar with the MSDS for the immersion fluid.

7.7 Wash your hands if you touched the immersion oil or silicone inadvertently.

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

7.9 With NO illumination (laser and LED lamp shutters closed), lower the objective lens to "Load Position" and load your sample carrier on to the microscope stage.

7.10 Return the transmitted light arm to the vertical position.

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

7.12 Set the microscope either to brightfield or widefield epifluorescence illumination.

7.13 Look at the objective while you use the focusing wheel of the microscope to move the objective close to the sample. If immersion fluid is used, the objective must contact the immersion fluid. Care must be taken not crush the objective against the sample.

7.14 While looking at the sample through the binoculars, use the focusing wheel to slowly move the objective away from the sample until the sample is sharply focused.

7.15 If you have reached the lowest point of the travel range for the objective without seeing the sample in sharp focus, close the LED shutter (if applicable) and repeat steps **7.13 and 7.14**.

7.16 Take particular care not to raise the objective lens too high beyond the focal point.

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

7.18 Once the sample is in sharp focus, ensure that all the doors of the black enclosure are closed before proceeding to the laser scanning mode.

7.19 Acquire images after optimising the acquisition parameters in NIS Elements.

7.20 The laser power selected for imaging must be kept at no more than 50%.

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

7.22 If you need to check the location of your sample in relation to the objective, or change the sample, always stop the laser scanning mode before you open any of the enclosure doors.

7.23 To remove/change sample, open the enclosure's top doors before pushing up the transmitted light arm.

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

7.25 Load/remove new sample carrier if required.

7.26 Pull down the transmitted light arm.

7.27 Start from **7.12** to get your new sample into focus and acquire images.

7.28 When imaging is completed, repeat **7.21 to 7.24** and proceed to shut down the complete system.

8.0 LASER ACCIDENT RESPONSE PROCEDURES:

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

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

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

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

8.5 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 NOBIC staff (6904 7023, 8131 7460) / Safety officer (65923913, 65923209) / 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.

8.6 If working after office hours, user should strictly follow "SOP for Lone Working", call the emergency line (8.5).

8.7 Place "No Entry" sign across the door and wait for help. No Entry Signage is kept in the folder (8.5)

8.8 Report the incident to the NOBIC staff (esther.koh@ntu.edu.sg)/ Safety Office (jayaker@ntu.edu.sg, chitra@ntu.edu.sg).

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

9.0 CLEANING OBJECTIVE LENS/TIDYING THE WORK AREA:

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

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

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

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

9.5 Dispose all soiled tissue in the waste bins.

10.0 ADDITIONAL SOP REQUIREMENTS FOR USE:

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

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

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

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

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

10.6 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.

10.7 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.

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

11.0 REFERENCES:

11.1 MSDS for the reagents in use

11.2 Lab risk assessment for imaging projects using confocal microscope

11.3 LKCMedicine Imaging Facility Confocal Microscope Training Records