




**CAF Fluorescence Microscopy Unit
 Laboratory Standard Operating Procedures**


BIOSAFETY GUIDELINES FOR BSL-2 CONTAINMENT: GENERAL PROCEDURES FOR ALL ACTIVITIES			
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A. Purpose

This document serves as the Biosafety Manual and Standard Operating Procedure (SOP) for the Central Analytical Facility (CAF) Fluorescence Microscopy (FM) Unit at the University of Stellenbosch, Room 2022-2025, Mike de Vries Building. It has been developed from previous Manuals and SOPs as well as Exposure Control SOPs, Safety Manuals and SOPs developed at other Departments of Stellenbosch University, from the University of Cape Town and guidelines of the World Health Organisation.

All users of the FM unit are required to fully understand the potential hazards involved in using these facilities and to follow safety practices at all times. Failure to do so can result in costly instrument damage and can also harm the user or cause serious injuries.


Use of the equipment is a privilege and not a right. No individual shall enter the facility or use any equipment without the approval of a CAF staff member. Training can be provided, however, it remains the discretion of CAF staff to allow independent use of any equipment.

The SOP described in this manual apply to all research staff, postgraduate students, visitors and guests, volunteers, building staff and service staff who enter the laboratory. This manual will be reviewed annually by the Unit Manager to ensure that it is accurate and up to date.

Research involving recombinant/synthetic nucleic acids, infectious agents, creation of genetically modified cell lines, acute biological toxins, unfixed non-human primate materials or human blood, cells or unfixed tissues must first submit an application for research registration through the REC:BEE before commencing. This work may require prior review and approval by the REC:BEE, and must comply with their Guidelines.

The facility will be a registered Biosafety Level 2 facility, hence no samples of risk level 3 or higher may be submitted to the unit. All samples have to be pro-actively assessed for appropriate risk level and only BSL2 or lower risk samples may be submitted to the laboratory according to the correct procedures (SOP 4 and 5).

When performing a risk assessment of laboratory procedures, all potential routes of exposure should be evaluated. Most laboratory-acquired infections have resulted from cuts with sharps, inhalation of aerosols, splashes or sprays, hence, it is good practice to look for potential exposures via routes of entry such as ingestion, inoculation, inhalation, and contamination of skin and mucous membranes. If a high risk for exposure is defined during the assessment, safer alternative procedure needs to be identified or other risk mitigation strategies applied.

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B. Background

CAF houses high-end equipment which are made available for the sample analyses of researchers and post-graduate students from Stellenbosch University, other research institutions as well as clients from industry. The different subunits of CAF are managed by experienced staff and provides in addition to analytical services also training of postgraduate students.

The FM unit of CAF houses equipment to analyse fluorescence signal in stained samples and includes fluorescence microscopes and flow cytometers. One branch of the unit is located in Stellenbosch (Mike de Vries building, room 2022-2025) and the other branch is located at the Tygerberg Medical Campus (room 3046 of the Clinical building and room 5033 of the Education building).

The FM unit in Stellenbosch houses the following equipment:

1. Zeiss LSM780 ELYRA super-resolution microscope
2. BD FACSMelody Flow cytometer and Cell Sorter
3. Leica CM1860 cryostat with UV
4. Vivid Air Fume Extraction Cabinet
5. NuAire Level II Biological Safety Cabinet (BSCII)
6. Olympus IX81 fluorescence microscope


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C. General Procedures of the FM Unit applicable to all users

C.1. Administrative procedures

Potential users have to consult with staff of the FM unit prior to the start of a study to determine whether the instrument(s) of the unit are suited to perform the intended analyses.

1. A new user is required to :
 - a. Register for a CAF ID. This unique user ID is obtained by completing the CAF client registration form uploaded at www.sun.ac.za/CAF. Choose the option "Client ID Number".
 - b. Register for the online CAF booking system which will enable the user to view the booking schedule of the instruments of the FM unit and to book a suitable day and time for sample analyses.
 - The URL to make bookings to use CAF equipment is: [www.supersaas.com/schedule/CAF Booking systems](http://www.supersaas.com/schedule/CAF_Booking_systems)
 - Select the option "STB Fluorescence Microscopy"
 - First time users need to select the option "Sign in" and complete the information requested when choosing the option "Create new user account".
2. The FM unit maintains a 24 hour booking cancellation policy. Should users need to cancel within this window period, they need to notify the CAF staff in writing and provide the reason for the cancellation. It remains the discretion of CAF staff to decide whether the cost for this booking should be waved or not.
3. A non-trained user is required to, in addition to booking the instrument, also book for assistance online, 24h before the date of proposed use of equipment and to supply CAF staff with information on the study, planned experiment and samples to be analysed.
4. Complete the Sample Submission Form of the FM Unit to determine the risk level of the samples to be analysed.
 - a. A risk assessment of samples should be conducted and both the intrinsic hazards of the samples as well as considerations of individuals handling the samples, addressing the potential for emergent hazards should be taken into account.

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- b. Any genetic modifications to samples need to be declared before sample submission and the required form for GMO samples need to be completed. Also, documentation needs to be provided that the laboratory where the GMOs were produced is registered or in the process of registration as a GMO facility.
5. All users need to sign the General Safety Agreement of the FM unit (Appendix 1). Independent users also need to sign the declaration document attached to the SOP of the instrument they were trained to use.
6. After use of the instrument, the user should log the time spent analysing the samples, adding notes of any technical issues experienced to the instrument logbook. Also, inform the unit staff member.

C.2. Laboratory security and access management

1. Security measures of the FM unit include:
 - a. an alarm system, that is armed at the end of each day
 - b. a card reader for controlled access to the unit after hours (17:00-8:00). Each door with card access has a emergency door release (green) unit next to the card reader for emergencies. Breaking the glass of this unit will allow opening the door manually.
 - c. The front door has a code lock installed.
 - d. There are three panic buttons in the unit
 - One positioned at the door to the confocal microscope room (room 2024);
 - One positioned in the Unit office below the desks, against the wall;
 - One positioned on the alarm system panel at the door to room 2025.
2. Users of the unit will only be given access via their student/staff cards after training and upon the discretion of the CAF staff that the user is able to work independently.
3. An untrained person accompanying a trained person should not be left alone in the unit
4. No person is allowed to use another user's card to gain access to the facilities.

5. When working in the laboratory, a user must keep a charged mobile phone with them to use in the case of an emergency. Use of a mobile phone in the laboratory for personal use or other purposes are not allowed. No landline is available in the unit.
6. When working independently the doors to a laboratory must be kept closed.
7. It is the responsibility of the user to ensure the door of the unit is locked and the alarm is set when they leave the facility.

C.3. Data storage and backups

1. Data safety cannot be guaranteed, therefore, it is expected from all users to copy their data immediately after the last sample was analysed. CAF also urges users to make sufficient backups of their data. It should be noted that CAF reserves the right to remove any data of a user from any equipment or analyses computer on day 7 post the date when data was generated.
2. No USB flash drives or external hard drives may be used to transfer data directly from the microscope computers. All microscopes have direct links to an analysis station to which data can be transferred before copying to the user's own storage device.
3. Only dedicated, *formatted* flash drives provided by the unit may be used to copy data from equipment which are not linked to analyses computers. Users should bring their own flash drive or external drive to copy the data to from the CAF flash drive.
4. All user data on equipment computers and analyses stations should be saved in the following prescribed manner:
 - a. Each user will have a folder labelled with their name and department
 - b. Subfolders should be created with the date set as follows "year month day" (e.g., 2020 01 20),
 - c. all data acquired on that day needs to be stored in the accordingly labelled folder.

C.4. General Laboratory Safety Rules

It is the responsibility of each user to be attentive to every action taken in the FM unit, to consider the potential impact on possible exposure or contamination and to follow established SOPs and protocols diligently and without variance.

All users of the unit, whether trained or untrained, must read the SOPs that is applicable to the analysis they will be performing. It is also required that the user sign the safety agreement of the 1) unit and 2) equipment they will be using.

The general rules and regulations of the unit includes the following:

1. Upon entering the lab, wash hands thoroughly and don Personal Protective Equipment (PPE).
2. Standard PPE that is required to be worn in the lab and especially when handling samples include gloves, a lab coat and closed shoes. Personal clothing that would still expose a greater area of skin after donning a lab coat should not be worn (e.g., skirt or short pants). Depending on the hazard level of the samples, also eye protection or face masks should be worn.
3. No eating or drinking is allowed in the laboratory.
4. All potentially hazardous samples submitted to the unit should be sealed in airtight containers or sample holders.
5. No user is allowed to perform any action they were not trained for, i.e., removal of any part or to open any section of equipment where the electronics and lasers are located. Services will only be performed by a designated engineer and certain maintenance tasks will be executed by CAF staff.
6. All workspaces need to be wiped with 70% ethanol after use and the laboratory should be left neat and clean.
7. All waste should be discarded into the correct waste containers provided in the laboratory. No regular waste (packaging material, paper notes, etc.) should be placed in the biohazard waste bins.
8. No one shall abuse any of the emergency safety systems in place when there is no emergency.
9. When leaving the unit, all PPE should be removed and hands washed thoroughly

C.5. Laboratory Signage

The biohazard symbol and BSL2 designation will be posted at the entrance to rooms where Risk Group 2 agents are analysed and discarded.

BSL 2 labs will have the following permanently affixed decals:

1. Biohazard symbol with "BSL-2" designation
2. Exit Requirements: reminders to remove PPE and wash hands prior to exiting.



D. Training activities in the CAF Fluorescence Microscopy Unit

Staff of the FM unit either analyse samples on behalf of a user, assist a user with the analyses of their samples or train users upon request to be signed off as an independent user of specific equipment in the unit. Training to use a specific instrument is either in the format of a one to one setting or a more structured one to two day course.


D.1. One-to-one training

When a trainee delivers their samples to the laboratory, a FM unit staff member will train them on the use of the equipment so to acquire data or images of the samples.

The training will include:

- a. The procedure to securely switch the instruments on and off
- b. Proper cleaning of the instruments
- c. The software specific setup of the experiment conducted at the time.
- d. Risks involved with the use of the equipment
- e. Safe handling and disposal of the users' samples
- f. General rules and regulations for use of the unit

This format of training is a prerequisite for all users who wishes to use the equipment independently in the unit. A user may only work independently on equipment and perform the specific techniques practised during the one-on-one training session. If the user wishes to use a different technique on the same equipment, or other equipment, they would need to

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receive the necessary training. **Training may only be provided by CAF unit staff and no other student or researcher.**

D.2. Training Courses

One- or two-day training courses are presented in the unit to teach participants the principles of the analytical techniques applied in the unit. The courses include theoretical presentations in the CAF House meeting room (16 De Beers Street, Stellenbosch) and hands-on training of the equipment in the laboratory. The hands-on training includes software setup of different experiments on the equipment and the acquisition of fixed pre-prepared samples.

This training is recommended for all users of the unit but it is not a prerequisite for independent use of the equipment.

D.3. Biosafety Training

Although the one-on-one training in the unit will include an overview of all the rules and regulations of the FM unit, which includes those of a BSL2 facility, a separate basic biosafety training session need to be attended by all users. This will be in the format of an online webinar (, with an evaluation form to be completed.

When a user brings samples to the unit that can be classified as BSL2, they need to provide confirmation that they have received the required training by their department where the samples were prepared. The CAF unit staff will however monitor their skill to ensure that safe techniques are applied as required for BSL2 facilities.

E. Emergency Procedures

E.1. Fire escape plan

1. The fire escape plan is put up against the walls along the corridor to the unit.
2. Directions to the exit are indicated along the corridors of the building. The fire evacuation plan is practised twice a year and the fire alarm is tested regularly.
3. When the fire alarm is activated, a designated team of staff in the building are to investigate the incidence, immediately. If the thread is eminent, staff members will sound manual horns to warn all and to set the evacuation plan in motion.
4. Location and operation of the fire extinguisher:

- a. The fire extinguisher is located just outside the front door of the FM unit.
 - b. Fire extinguishers should be used only if the fire is small and confined to one small area! USE JUDGEMENT IN THIS! DO NOT CREATE A LIFE-THREATENING SITUATION WHILE TRYING TO EXTINGUISH A FIRE! (Lab users are not obligated to use the fire extinguishers but should make alarm and evacuate immediately).
 - c. To operate, pull the pin to release the handle.
 - d. Position yourself at a safe distance from the fire (as directed on the fire extinguisher).
 - e. Aim the nozzle at the base of the fire, squeeze the handle to discharge the agent and sweep from the left and right for a few seconds, cease the action when the fire has been extinguished
5. Fire extinguishers are inspected annually by the contracted company. Check the gauge periodically to ensure operational status.
 6. Members of the Building Health and Safety Committee dedicated as fire marshals should receive training according to the required schedule, every 2 years.

E.2. Medical emergencies

1. An emergency shower and eye wash station are located just outside the front door of the FM unit.
2. A first aid kit is available in the Unit office as well as in the corridor towards the main staircase.
3. There are dedicated first aiders identified in the building and their contact details are displayed in the unit, behind the front door.
4. For any serious medical incidence, Campus Health or ER24 will be contacted immediately, and the person taken to medical facilities or an ambulance will be summoned to collect the person.
 - a. The incident should be reported immediately to the Campus Health or Facilities management. The medical costs resulting from an injury in the workplace are covered by the workmen's compensation commissioner, if a claim is submitted in

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good time, paperwork completed correctly, and the claim accepted. If a staff member is injured while performing his/her duties in the workplace, the medical practitioner treating the injured person must be informed that the case is an injury on duty.

- b. The Campus Health Services must be informed of the injury during working hours, while injuries after hours must be reported to Facilities Management. In emergencies, staff may be treated at the emergency unit of the local Medi-Clinic, bearing in mind that the injury must be treated as an injury on duty.

- c. See SOP2 for more information and the incident report template. More information is also available at <http://www.sun.ac.za/english/CampusHealth/medical-services/occupational-health#injury>

5. Emergency Phone Numbers

Campus Security	0218082333 0218084202
Campus Security Whatsapp	082 808 2333
Campus Health Occupational Medicine: Sr Anneke van Heerden	021 808 3494/6
Laboratory Manager: Ms Lize Engelbrecht	079 699 4131
Fire Department / HAZMAT	021 808 8888
Ambulance	084 124 (ER24) 082 911 (Netcare)
Medi-Clinic 24h Emergency	021 861 2000
CSCD 24-Hour Crisis Service	082 557 0880

E.3. Electrical shock

1. Immediately switch off the power supply. Do not touch or move the person. Call the nearest trained first aider to apply first aid.
2. Report the incident to Facilities Management.

CAF Fluorescence Microscopy Unit
 Room 2022, Mike de Vries Bldg., Private Bag X1, Matieland, 7602, South Africa
 Tel: +27 21 808 9327/4030; lizeb@sun.ac.za

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Commented [DSDD]3: Waar is die local medi-clinic in Stellenbosch

CAF Fluorescence Microscopy Unit Usage and Safety agreement

I, _____, undertake to

- Familiarise myself with the risks involved with the use of the equipment prior to the start of analysis;
- Timely inform a CAF staff member on the proposed biohazardous sample to be analysed, the safety risks involved as well as the procedure to follow upon possible exposure;
- Read all documentation with regard to safety, instruments and reagents (e.g. MSDS) used by the Unit;
- Use the equipment I have been trained to use in the appropriate way, else book the assistance of a CAF staff member;
- Immediately notify other individuals working close by the laboratories and a member of the CAF staff of any dangerous situation.
- Adhere to all rules and policies of the CAF Fluorescence Microscopy Unit, including that of the specific laboratories within the unit.

Name of user: _____ SU Number (if applicable): _____

Signature: _____ Date: _____

Name of Supervisor: _____

Supervisor E-mail: _____