CAF Fluorescence Microscopy Unit

Laboratory Standard Operating Procedures

BIOSAFETY GUIDELINES FOR BSL-2 CONTAINMENT: BD FACSMELODY CLEANING PROCEDURES			
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Content:

A. Purpose

This document serves as part of the Biosafety Manual and Standard Operating Procedures for the Central Analytical Facility (CAF) Fluorescence Microscopy Unit at the University of Stellenbosch, Room 2022-2025, Mike de Vries Building. It has been developed from earlier model Manuals and Standard Operating Procedures (SOPs) currently in place in the laboratory as well as Exposure Control SOPs, Safety Manuals, guidelines of the World Health Organisation and guidance of The Division of Occupational Health and Safety Office of Research Services, NIH.

All users of the CAF Fluorescence Microscopy 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, serious injuries or harm.

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.

This SOP have to be considered together with all other SOPs of the unit.

The purpose of this SOP is to describe the cleaning procedures to be followed for the daily and weekly cleaning, as well as cleaning the instrument prior to aseptic sorting.

B. Basic cleaning after each experiment.

1. After the last sample of an experiment has been acquired, the following basic cleaning procedure applies:

    a. Run a 5 ml FACS tube about ¾ filled with 70% ethanol for 5 minutes without recording.

    b. Run a 5 ml FACS tube about ¾ filled with freshly prepared 10% bleach for 5 minutes without recording.

    c. Run a 5 ml tube about ¾ filled with dH_2O for 5 minutes without recording.

2. When the cleaning procedure has been completed, open the Stream window in the software and stop the stream so to initiate standby mode.

3. If there are users that have booked the instrument for use later the day (refer to the online booking system), the current user may leave the instrument in the standby mode.

4. If the current user is the last person to use the instrument for the day, but the instrument is booked for sample analyses the following day (please refer to the online booking system), the user should perform a Daily Shutdown procedure.

    a. Go to the *Cytometer* tab and select *Daily Shutdown*.

    b. Follow the prompts and on the last step load a 5 ml tube about ¾ filled with dH_2O

    c. Switch off the instrument when prompted to do so.

5. Wipe all work surfaces, including keyboard and mouse with 70% ethanol and discard any biohazard waste in the red biohazard bins.

C. Weekly cleaning procedures

1. Upon the acquisition of the last sample for the day at the end of the week, the user should first follow the procedure for basic cleaning of the instrument (Refer to section B), followed by cleaning the flow cell before performing the long term shutdown procedure.

2. Following basic cleaning of the instrument, stop the stream

3. Clean the Flow Cell repeatedly as per the following procedure:

    a. Go to the *Cytometer* tab and select *Flow Cell Clean*.

    b. Follow the prompts and when requested load the appropriate tubes:

        - 10% bleach (repeat 3x)
        - 70% ethanol (repeat 3x)
        - dH_2O (repeat 3x)

4. Perform the *Long Term Shutdown* as per the following procedure:

    a. Fill the extra stainless steel ethanol tank with 70% ethanol, **ONLY** up to the weld line.

    b. Go to the *Cytometer* tab and select *Long Term Shutdown*.

    c. Follow the prompts and where the instructions indicate to exchange the sheath with ethanol, disconnect the blue line from the sheath tank's bubble filter and connect it to the ethanol tank's bubble filter.

d. Disconnect the clear air line from the sheath tank and connect it to the ethanol tank.

e. Continue following the prompts (this step should continue for approximately 5 minutes)

f. On the last step, load a 5 ml tube filled with dH_2O

g. Switch off the instrument when prompted to do so.

D. Exchanging the flow cytometer waste tank

5. The levels of the waste tank should be manually checked prior to the start of sample analysis or sorting on the flow cytometer, but also throughout sample acquisition by checking the green indicator in the bottom left corner in the software.

6. If the tank is filled at approx. 80% of its volume or more, the waste tank should be exchanged.

7. Stop the stream

8. Prepare the empty waste tank by adding two sachets of Medisure Chlorine (2x 6g) to the tank.

9. Disconnect the full waste tank, but keep a paper towel beneath the entry pipe to collect any dripping waste. Put the entry pipe into the empty tank and close securely.

10. Close the lid of the full waste tank tightly (users might detect a faint chlorine smell)

11. The deactivated waste can be discarded in the laboratory sink (room 2026).

12. Both the full waste tank and exchange waste tank should be labelled with the biosafety hazard labels.

E. Cleaning for aseptic sorting

1. The BD FACSMelody is equipped with an extra stainless steel Sheath tank. This empty tank should be autoclaved prior to cleaning for aseptic sorting.

2. The BDFACSMelody is also equipped with an additional stainless steel tank which will be used for running bleach and dH2O in succession.

   a. First fill this tank to the weld line with freshly prepared 10% bleach solution

b. Ensure that the bubble filter line dedicated for bleach is attached to the tank

c. Close the lid tightly.

3. Prior to cleaning for aseptic sorting, the flow cell clean procedure should be followed (Refer to C.3)

4. After the flow cell cleaning, find the *Cytometer* tab and select *Prepare for aseptic sorting*

5. When prompted to run bleach through the system, do the following:

   a. Disconnect the blue line from the bubble filter of the sheath tank and connect it to the bubble filter of the tank filled with bleach.

   b. Exchange the clear air line as well from the sheath tank to the tank filled with bleach.

   c. Click *Continue*.

6. When prompted to run dH_2O do the following:

   a. Disconnect the tank filled with bleach and pour the bleach into an empty glass container for use in other cleaning applications.

   b. Fill the tank with dH_2O up to the weld line

   c. Exchange the bubble filter for the one dedicated for dH_2O

   d. Connect all tubes again and close the lid tightly

   e. Click *Continue*

7. In the meantime, fill the stainless steel ethanol tank with 70% ethanol up to the weld line and close the lid tightly.

8. When prompted to run ethanol through the system, do the following:

   a. Disconnect all tubing from the tank filled with dH_2O and connect to the filled ethanol tank.

   b. Click *Continue*.

9. In the meantime, fill the autoclaved sheath tank with sterile Sheath up to the weld line.

10. When prompted to run detergent, only load a FACS tube filled with dH_2O into the cytometer.

11. Disconnect all the tubing from the ethanol tank and connect them correctly to the Sheath tank.

12. Find the *Cytometer* tab and select *Startup procedure*

13. Follow the startup procedure as per normal.

14. Ensure the workspace (desk space, mouse, keyboard) is cleaned with 70% ethanol to limit contamination of the sort samples.