SYLVESTER COMPREHENSIVE CANCER CENTER
UNIVERSITY OF MIAMI HEALTH SYSTEM

Flow Cytometry Shared Resource

INTRODUCTION.

This document is for the attention of all Principal Investigators and Flow Cytometry Shared Resource (FCSR) users. The number of our users and the diversity of their applications continues to expand, as demand grows and the technology advances. Here we will review and update, on an annual basis, the guidelines which are in place for the use of this facility. Please read these guidelines, become familiar with them and share them with your staff.

The FCSR is an open facility, available to internal university users, as well as external academic and commercial institutions. The FCSR is open access, between normal business hours of 9:00am – 5:00pm (Eastern Time), Monday through Friday, with 24/7 access available to approved trained users (internal university users only).

The FCSR has the authority to establish these policies and has control of the associated Standard Operating Procedure (SOP) documents. The FCSR is responsible for the implementation of these policies, as well as for ensuring that facility staff and users understand, acknowledge and comply with these policies. Target users are: FCSR staff, internal (university members) and external (academic and commercial) users of the FCSR.

Use of the Flow Cytometry Shared Resource is for **RESEARCH PURPOSES ONLY**.

FACILITY ACCESS.

Prerequisites.

All users are required to register for an account in the iLab management system and must have active funding accounts assigned to them by their Principal Investigator. Each laboratory must submit all administrative requirements prior to receiving full access to the facility. These administrative items are in the form of the Cell Analysis Questionnaire and CyTOF Questionnaire.

Biosafety Questionnaire.

All laboratories must complete a Cell Analysis Questionnaire, regarding the types of samples which will be analyzed or sorted within the facility. This questionnaire must be completed within the iLab management system and is required prior to use of the facility and to remain compliant with the facility's policies and procedures. Access to the facility may be delayed or denied, if a completed questionnaire is not on file. An updated Cell Analysis Questionnaire, must be submitted to the facility for review and approval, if there are any changes to experimental plans, cell types, or any other information pertinent to the safe use of the facility.

CyTOF Questionnaire.

All laboratories that intend to use the CyTOF Mass Cytometer (Helios and Hyperion) must complete a CyTOF Questionnaire, regarding the types of samples which will be analyzed on the instrument. This questionnaire must be completed within the iLab management system and is required prior to use of the facility and to remain compliant with the facility's policies and procedures. Access to the instrument may be delayed or denied, if a completed questionnaire is not on file. An updated CyTOF Questionnaire, must be submitted to the facility for review and approval, if there are any changes to experimental plans, cell types, or any other information pertinent to the safe use of the instrument.

INSTRUMENT ACCESS.

Prerequisites.

Instrument access will be granted once all administrative and facility requirements have been completed. Users are encouraged to complete the *Basic Flow Cytometry Course*, however, it is not mandatory for access to the facility within normal business hours. The course is offered, free of charge.

After-hours instrument access.

It is required that all users attend the *Basic Flow Cytometry Course* prior to being granted after-hours instrument access, unless they are able to demonstrate appropriate previous experience, obtained elsewhere. The FCSR course is offered, free of charge. Users then must prove to facility staff that they understand and know all of the appropriate procedures to use each instrument. These include: proper startup and shutdown procedures, handling of necessary fluidics systems and correctly running samples. Users with after-hours access take full responsibility for the instrument(s) and any repairs needed after misuse of an instrument will be billed to their Principal Investigator. Trained and vetted internal university members may access the facility 24 hours a day, 7 days a week. This access is limited to the cell analyzers only. External users will not be granted after-hours access. Independent use of the facility requires that users be proficient in instrument operation and basic maintenance, including filling the sheath and emptying the waste reservoirs. The FCSR staff has final authorization and approval of individuals wanting to use the facility outside normal working hours.

Instrument reservations policy.

Reservations must be made within the iLab management system, through the user's personal iLab account, or by using the iLab Kiosk to make a walk-up reservation. Users should **NOT** make reservations for other members of their lab, or for anyone else. Users accept full responsibility for the instrument during their scheduled reservations and the reservation owner will be held accountable for any misuse of the instrument. Repairs needed because of instrument misuse will be billed to their Principal Investigator. Unregistered users are not permitted to operate the instruments, therefore, reservation owners must be present during the entire sample acquisition period. Reservations for the analyzers can be made up to 30 days in advance and walk-up reservations can be made on the spot, using the iLab Kiosk, provided there is at least 30 minutes available on the instrument calendar. Reservations for the cell sorters are subject to approval and must be made at least 12 hours in advance and up to 21 days in advance. This policy applies to the CyTOF Helios mass cytometer and Hyperion imager, as well. The facility reserves the right to cancel a reservation if the user/laboratory has not completed or submitted all the necessary documents which are required for continued access.

Missed reservations and late arrivals.

All missed reservations will be billed the standard instrument setup fee, for incurred costs. For late arrivals, extended time beyond their original scheduled reservations will not be grated, unless there is time available on the instrument.

Cancellation policy.

For the analyzers, users may cancel or edit their reservation, up to 30 minutes prior to their reservation start time. Cancellations cannot be made within the 30 minutes prior to a reservation start time and the reservation will be billed the standard instrument setup fee, for incurred costs. For the cell sorters and CyTOF instruments, reservations must be cancelled 24hrs in advance (at least the day before) and users must contact the facility staff to cancel their reservation. Same day cancellations are considered last minute cancellations and are subject to the appropriate standard instrument setup fees, for incurred costs. EXCEPTION: special consideration will be given to extreme circumstances, which could lead to a last minute cancellation and then billing of incurred costs may be waived.

SAFETY AND BIOSAFETY.

Regulations and Guidelines.

All cell analyzers, the FACS Aria-II (BSL-1) cell sorter and the CyTOF mass cytometer all operate under Biosafety Level 1 (BSL-1) conditions ONLY. If Biosafety Level 2 (BSL-2) samples need to be acquired on the cell analyzers, they must be fixed and rendered non-infectious using an appropriate fixative (4% paraformaldehyde, 4% formaldehyde, or 10% formalin; alcohols are not adequate in rendering BSL-2 cells to BSL-1). The FACS Aria-IIu (BSL-2) and FACS Aria Fusion (BSL-2) cell sorters can process Biosafety Level 2 cells.

Samples which require BSL-2 containment include all "normal" live human samples, human patient samples and cells transfected with retroviral/lentiviral vectors capable of infecting human cells. Biosafety Level 3 and Biosafety Level 4 cells **MUST NOT** be processed on any instrument within the facility. Clinical samples **MUST NOT** be processed in the FCSR. However, clinical samples designated "FOR RESEARCH ONLY" are acceptable and must be fixed, if using the analyzers, or processed on one of the two BSL-2 cell sorters.

Instrumentation.

Use of the FCSR instrumentation is restricted by Biosafety Level (BSL). All the analytical flow cytometers – FACS Canto-II, LSR-II, LSR-Fortessa-HTS and Cytek Aurora, as well as the FACS Aria-II cell sorter and the CyTOF mass cytometer, all are restricted to samples which are handled under Biosafety Level I (BSL-1) conditions only. The FCSR has a Baker BIOPROtect II cabinet, housing the FACS Aria-IIu and an integrated bio-safety cabinet, housing the FACS Aria Fusion cell sorters, which may analyze or sort samples under BSL-2 conditions.

Samples.

Samples which require special handling under BSL-3 or BSL-4 conditions are **NOT** permitted in the FCSR at all.

BSL-2 samples which have been fixed using the proper fixatives to render BSL-2 samples to BSL-1, are allowed to be analyzed on the BSL-1 flow cytometers. It is the responsibility of the Principal Investigator to ensure that all students, employees and trainees, are properly instructed in appropriate fixation protocols and that all personnel carry out these protocols correctly.

Users who plan to analyze human cells (live, or fixed), MUST designate that fact when reserving time on the pertinent cytometer in the iLab system. Similarly, genetically modified (virally transduced) cells also should be so identified. This signals to subsequent users and facility staff that these type of samples will be processed. Appropriate instrument decontamination procedures also must be followed after any such analysis.

Safety.

All users are instructed to apply Universal Safety Precautions and wear appropriate Personal Protective Equipment, when handling samples in the FCSR.

All users are expected to take the *Blood-Borne Pathogens Course,* administered through the Office of Environmental Health and Safety. Fulfillment of this requirement by all users is the responsibility of their Principal Investigator.

No "Sharps" are permitted in the FCSR.

INSTRUMENT MAINTENANCE.

Routine maintenance of the FCSR instrumentation is critical for optimal data collection. The FCSR performs daily Quality Control, through the use of Cytometer Setup and Tracking (CST) beads or SpectroFlo QC beads, and by performing routine maintenance on all instruments. It is not only important, but the responsibility of the FCSR users, to become familiar with and be able to perform correctly: startup, shutdown and cleaning procedures, on the instruments which they will be using, as well as refilling the sheath fluid and emptying the waste container.

FCSR users are expected to clean-up the work area which they have used, upon completion of their analysis, including refilling the instrument's sheath fluid reservoirs and emptying the waste containers, as necessary. IMPORTANT: bleach is to be added to emptied waste containers (>10% final volume), before reconnecting the container to the instrument. FACS Clean solution and then DI water are to be run through the flow cytometer, following completion of analysis, for two minutes each – NO EXCEPTIONS!

Spills or drips of any kind must be cleaned and disinfected immediately after occurrence. A disinfection/clean-up kit is located in the FCSR. Equipment, tips, sample tubes, tube racks, papers, etc. are to be removed, upon completion of analysis. Items left behind at the end of the day will be discarded. All bio-hazardous waste is to be placed in containers with red bio-hazard waste bags.

INSTRUMENT ISSUES AND PROBLEMS.

Always report any instrument faults, errors, problems, or issues, no matter how small or seemingly insignificant, to a FCSR staff member immediately. This is especially important if something occurs after hours, when a staff member is not immediately available, by telephone. Users are expected to understand the meaning of all instrument warning lights, alarms, etc. and to act accordingly when these may be activated.

DATA MANAGEMENT.

Policy.

The UM SCCC Flow Cytometry Shared Resource is not responsible for managing and storing acquisition data files. It is the user's responsibility for backing up and managing permanent storage solution of their data. Instrument work stations are purged frequently, in order to ensure reliable and efficient instrument operation, therefore, they are not considered to be a permanent storage method. Data management and storage is the personal responsibility of each respective user.

Data storage options.

Facility data server, SCCC FCCF (S:).
University cloud-based platform, BOX (box.miami.edu).
Other cloud based storage solutions (ex. Google Drive, etc…).
NOT ALLOWED are removable drives (ex. flash drive or external hard drives), because of the potential virus contamination risk through the USB port.

CITATIONS AND PUBLICATIONS.

Acknowledgments.

Principal Investigators/Laboratories agree to acknowledge the UM SCCC Flow Cytometry Shared Resource in all publications and grant applications, where data generated by use of the facility is presented. This includes: abstract and journal publications, poster presentations, seminar speeches and press documents and releases.
In addition, as required by the NCI and CCSG grant guidelines, the following statement must be included in all publications: "*Research reported in this publication was supported by the National Center Institute of the National Institutes of Health under Award Number P30CA240139. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health*." For publications that use CyTOF (Helios or Hyperion), please also add the following acknowledgement statement: *"The Mass Cytometry equipment was supported by a State of Florida Department of Health Bankhead-Coley Grant Program under award number 8BC09".*
Facility acknowledgment example: *"We would like to acknowledge the skilled assistance of the Flow Cytometry Shared Resource of the Sylvester Comprehensive Cancer Center at the University of Miami, Miller School of Medicine, for the provision of expert fluorescence analysis and cell sorting services".*

Co-Authorship.

Publication co-authorship with a Flow Cytometry Shared Resource staff member should be considered, where there was a significant contribution to the experimental design, or final outcome, of the study.

Notification.

The FCSR strives to maintain an up to date library of publications which have been made using our services. Please always send the FCSR an electronic copy of your newly accepted papers, if they contain data generated within the FCSR. Please also let the facility know when any grants, which contain FCSR-generated data, are awarded.