

SCCC Flow Cytometry Shared Resource

New Standard Operating Procedures

6/17/20

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1. Introduction

This document provides a summary of new standard operating procedures (SOP's) that are required to be followed for use of the Sylvester Comprehensive Cancer Center (SCCC) Flow Cytometry Shared Resource (FCSR). These SOP's are aligned with university onsite research ramp up guidelines. Please note that due to physical distancing requirements and other safety measures, FCSR instruments and onsite services may not be available at full capacity. The university guidelines require all SR staff and users to adhere to new universal guidelines for all SR's (sent out separately) and to adhere to the following new FCSR specific onsite standard operating procedures.

2. Instrument use reservations and training calendars

- All of the FCSR's web based instrument calendars will remain off-line. Users are required to submit reservation requests in advance by emailing the facility staff. The Aria-II (BSL-1) sorter will be available only if the Aria-IIu (BSL-2) and Fusion (BSL-2) sorters are fully booked, and then only when appropriate physical distancing can be maintained between the Aria-II sorter and the Canto-II and Fortessa analyzers.
- Walk-up reservations for the cell analyzers will not be permitted for now, to facilitate compliance with new guidelines regarding room capacities and occupancy.
- Until further notice, the Flow Cytometry Training calendar will remain off-line and all hands-on onsite training will continue to be suspended. The FCSR will set up remote Zoom training sessions for the theory portion of the class workshop. The onsite practical portion will eventually be scheduled, with appropriate safety precautions in place. Users will continue to be informed about and encouraged to take advantage of the many available on-line webinars for analysis software training (e.g., DiVa, FCS Express, FlowJo, Astrolabe, Cytobank).

3. Access to the facility

- The door to the facility will remain locked at all times. Only users with pre-booked reservations may enter the facility, and they need to ring the doorbell to announce their presence and to be admitted by the facility's staff.
- Up to a maximum of four people (total number of staff and users) are allowed to be in the laboratory/instrument space of the facility at any one time, plus a maximum of two staff members in the office or conference space. Users are not allowed in the FCSR's staff office or conference room.
- It is the responsibility of users to bring all PPE required for their use of the facility. Users must wear appropriate PPE when entering the facility and also during their entire stay in the facility. This entails wearing a face mask when entering the facility and for the entire time while within the facility, having extra gloves, and possibly wearing a clean disposable gown/lab coat. Standard cloth lab coats are not permitted within the facility and disposable gowns/lab coats are highly recommended. Users without appropriate PPE will not be permitted access to the facility.

- Upon entry to the facility, users must remove their gloves (if they are wearing any) and wash their hands. Users need to bring a set of new gloves with them, and to don these gloves before logging-in to the kiosk to start their pre-scheduled reservations. Clean gloves must be worn while using any of the instruments in the FCSR. Users are required to remove their gloves and to wash their hands before exiting the facility.
- The availability of the instruments in the FCSR will continue to be restricted to comply with guidelines for physical distancing. Infection prevention measures, including cleaning and disinfection standard operating procedures, will be posted by each instrument.
- Consultations on project and reagent panel design, data collection, and data analysis will be performed via e-mail, phone call, texting, or Zoom.

4. Use of instruments (sorters and analyzers)

- Only instruments which have at least 6 feet of distance between their workstations will be available without scheduling restrictions (all iLab reservation calendars will remain off-line). This mainly affects the Aria-II (BSL-1) sorter, because there is only 5 feet between it and the Canto-II analyzer. Use of the Aria-II (BSL-1) will be restricted and will only be available when the Canto-II is not booked.
- Only one user will be allowed per instrument at any one time. Other user lab members and/or observers are generally not allowed at the same time at any instrument in use. However, if room occupancy and physical distancing allows, brief visits by others (e.g., to discuss progress) may be allowed by requesting permission from FCSR staff. Screen sharing software (e.g., Zoom) also may be used, for users to share their instruments' computer screens with colleagues.
- All human samples, except for cell lines established before January 2020 and designated as BSL-1, must be fixed with 4% paraformaldehyde, 4% formaldehyde, or 10% formalin, prior to being run on any of the cell analyzers. Ethanol and methanol fixation do not render cells BSL-1, so human samples fixed with these protocols must be analyzed one of the two BSL-2 sorters. Users must clearly state in their instrument reservation, if live human samples are to be acquired or sorted on any of the BSL-2 sorters, to allow the facility staff to take the appropriate precautions for handling.
- Users are required to perform basic cleaning procedures, in order to protect against potential instrument contamination. These procedures always must be completed upon completion of sample acquisition, at a minimum by running FACSClean, or 10% bleach, through the instrument for 2 minutes, followed by 3 minutes of DI water. It is highly recommended that users also complete this cleaning procedure prior to acquisition of their samples. The facility will provide the necessary cleaning solutions and users must ensure they have sufficient time to complete the appropriate instrument cleaning protocol, each time.
- For reservations requiring staff assistance (cell analyzers and cell sorters), users must provide written experimental details and/or gating schemes, in order for the FCSR staff to provide assistance safely. The written experimental details should include antibody markers and

fluorochromes, list of samples (including single color controls, positive and negative control, FMO's etc.), gating schemes and in case of cell sorting, desired populations to be sorted. Zoom (or other screen sharing software) may be employed to share the setting-up screen for sorting, where the user would drop off their samples and their precise experimental set-up details. The facility staff would then contact the user, to confirm sorting gate(s), etc., using screen sharing. These experimental details should be submitted by the user in advance, to enable staff to set up the experimental template prior to the scheduled reservation. These new standard operating protocols will allow the facility staff to set up experiments in compliance with physical distancing and other safety guidelines.

- Users must clean and disinfect work areas, including desk and bench surfaces and computer workstation keyboard/mouse and instrument high-touch areas, before and after use of each instrument. Spray bottles containing 70% EtOH and paper towels will be available at all stations for users to disinfect work areas.
- Users will not be allowed to remain in the facility while their samples are running on the sorters. However, if room occupancy and physical distancing allows, brief visits by users (e.g., to discuss progress) may be allowed by requesting permission from FCSR staff. Screen sharing software (e.g., Zoom) also may be used, for FCSR staff to share an instrument's computer screen with users for feedback and discussion.

5. Use of instruments (CyTOF - Helios and Hyperion)

- To comply with physical distancing guidelines, users are not allowed to enter the CyTOF room. Only one staff member is allowed in the CyTOF room at any one time. Cell suspension samples for the Helios and solid tissue slides for the Hyperion may be left with a FCSR staff member, or in the CyTOF Lab, for processing, by arrangement. Users will be contacted when the data collection for their sample(s) is complete. Results will be accessible through Box or similar tools or on the facility's data server.
- Experimental details for Helios cell suspensions must be provided in advance by users and should include a list of all metals/antibodies in the panel, the number of samples to be acquired, sample names, and the number of events to be collected.
- Properly labelled final resuspension media, consisting of CAS and beads, as well as filter tubes, must be provided by users in sufficient volume and quantity to accommodate all submitted samples.
- Experimental details for Hyperion tissue imaging must be provided in advance by users and should include a list of all metals/antibodies in the panel, the number of slides to be scanned, the type of tissue, and sample names. Clearly labeled slides (experimental and H&E, if available) should be provided for scanning, at least one day before the scheduled reservation. Zoom sessions will be scheduled to enable users to work remotely with facility's staff in real time, to determine placement and size of ROIs within each tissue section.

6. Compliance

SR users who do not comply with these FCSR standard operating procedures, and with all university physical distancing and other safety guidelines, will be asked to leave the facility and their access to the facility will be suspended until reviewed with their supervisor, the SR Director, and the SCCC Associate Director of Shared Resources. Any deviation thereafter may lead to a possible, temporary loss of on-campus privileges for that faculty member and their team.

7. Contact information

For information about the SCCC Flow Cytometry Shared Resource's instruments and services, or to discuss new or ongoing projects, or for questions on the FCSR specific SOP's described here, please contact Huw (Zip) Kruger Gray, Ph.D., FCSR Director, at hkgray@miami.edu.

For general information about the SCCC Shared Resources, or for questions about the onsite research ramp up universal guidelines for all SCCC Shared Resources, please contact George Grills, Associate Director of SCCC Shared Resources, at gxg766@med.miami.edu.