

Hollings Cancer Center Flow Cytometry and Cell Sorting Shared Resource Rules and Regulations

Objective:

The Flow Cytometry & Cell Sorting (FCCS) Shared Resource Facility is co-sponsored as a Hollings Cancer Center (HCC) Core Facility and a University Research Resources Facility. The goal of the FCCS Shared Resource is to provide consultation, comprehensive analytic flow cytometry and high speed cell sorting, and assay development to facilitate and expand the flow cytometry-based research of HCC investigators.

Services:

Analytical Use: LSRFortessa, LSRFortessa X-20, FACSVerse, CytoFLEX

The analytical cytometers are available for use by investigators or their staff. Facility staff will also run samples at your request at an assisted user fee rate. Independent operation is provided at a reduced rate but comes with the expectation that the users are properly trained. Facility staff provides individual and laboratory training free of charge. Please note, all new FCCS users must be trained through the facility regardless of prior experience before independently operating facility cytometers. Failure of any user to conform to FCCS regulations outlined below may result in suspension of facility privileges.

- **Reserved time:** Please be courteous to your fellow investigators. If you do not cancel your reservation for independent usage within 24 hours of your scheduled time, you will be charged for up to the first hour of time you booked. If you exceed your previously scheduled reservation it is up to the discretion of the following investigator to allow you extended time. Only book the amount of time you will need and during a time when you are sure samples will be ready. Overbooking precludes others from running their experiments in a timely fashion. Please be considerate.
- **Sample preparation:** All samples must be filtered prior to analysis.
- **Cleaning:** Following every use of the LSRFortessa or LSRFortessa X-20, investigators must clean the instrument by running a solution of 30% Contrad for 5 minutes followed by DI Water for 5 minutes on high speed. Note, it is the responsibility of the user to incorporate this time into their scheduled experiment. Users may log out of Infinity tracker screen to avoid charges during the mandatory cleaning process. When cleaning the FACSVerse, initiate the Daily Cleaning mode and follow instructions to run 30% Contrad then DI Water. When cleaning the CytoFLEX, run File/Daily Clean and follow instructions to run 30% Contrad then DI Water.
- **Fluidics:** Following every use of the cytometer, sheath and waste containers must be checked. If the sheath container is less than half full, investigators must replenish sheath fluid by filling the tank up to the top line. If the waste container is greater than half full, empty the waste container and add 200ml bleach when

finished, or roughly enough to cover the bottom of the tank. Please be courteous to your fellow investigators by maintaining cytometer fluidics.

- **Shut Down:** If you are the final user for a cytometer for the day, turn off cytometer by pressing the green buttons on the instrument and the fluidics cart.
- **Reagents:** The FCCS shared resource will provide sheath fluid, bleach, and all reagents necessary for the maintenance and operation of flow cytometers. All additional supplies are the responsibility of the investigator.

Cell Sorting: FACSria, MoFlo Astrios

- Sorters are only operated by Facility staff. For all sorts, we will conduct a post-sort analysis to verify the sort purity unless instructed otherwise by the investigator.
- Before a sort is started we will ask you to dictate gating strategies and verify appropriate sort logic.
- Samples must be filtered. Use 35-60um mesh from one of the following products: Falcon 2235 tubes with integrated filter cap (VWR or Fisher); CellTrics sterile filter caps from Partec; SpectraMesh nylon filters (VWR) is sold by sq. feet.
- Samples can be accepted in capped and labeled 1 ml (BioRad Microtiter), 5 ml (any 12x75mm), 15 ml (conical) tubes. Please prepare an appropriate collection tube or plate for each sample to be sorted.
- Make sure to include the proper controls to set up the instrument. You should include at least one negative (i.e. unstained) for each cell type and one compensation (i.e. single-stained) control for every fluorochrome or dye that you use in your staining procedure. Contact Facility staff if you require advice on planning/generating proper controls for your experiments.
- For a cell sorting experiment to be successful, you will need to know beforehand the scattering profile and fluorescent patterns of your target cells. Therefore, it might be necessary to perform flow cytometric analysis of your cells before the actual sort can be performed.

Imaging Analysis: Luminex ImageStream ISx

- The ImageStream is only operated by facility staff.
- Users should consult with facility staff prior to booking initial appointment.
- Samples must be filtered. Use 35-60um mesh from one of the following products: Falcon 2235 tubes with integrated filter cap (VWR or Fisher); CellTrics sterile filter caps from Partec; SpectraMesh nylon filters (VWR) is sold by sq. feet.
- Samples must be run in 1.5mL or 0.5mL microcentrifuge tubes.

- Make sure to include the proper controls to set up the instrument. You should include at least one negative (i.e. unstained) for each cell type and one compensation (i.e. single-stained) control for every fluorochoime or dye that you use in your staining procedure. Contact Facility staff if you require advice on planning/generating proper controls for your experiments.
- Each appointment comes with 4 hours of guided data analysis. Please consult with facility staff to schedule your analysis session.

Creating Reservations and Project Requests:

The FCCS uses the Infinity system allowing investigators to reserve time and request assisted-use time online at their convenience. Arranging for the FCCS facility to assist you through cell sorting or analytic cytometry can be initiated by providing experimental details and goals via the “Schedule Use” tab, then requesting a reservation to process your samples. Details and instructions to get started can be found at <http://www.hollingscancercenter.org/research/shared-resources/cell-evaluation-therapy/flow.html> under the “Schedule Use” tab.(Need to change all instructions) Please note that if a requested reservation cannot be confirmed due to conflicts, the FCCS staff will contact the investigator to reschedule the experiment at the soonest available time.

Training:

All users must be trained by HCC FCCS staff for unassisted use on any machine. The FCCS staff provides training for independent use of the LSRFortessa, LSRFortessa X-20, and FACSVerse cell analyzers free of charge. Training sessions are approximately 1 hour and can be repeated if necessary until the user is comfortable operating the cytometer. We recommend that the investigator brings practice cells to analyze to become acclimated to the settings they will use in future experiments. Note that it is unlikely that usable data will be collected during training. Please contact the FCCS staff for a consultation of your needs and to schedule training.

Service Fees:

- Charges are based on the time a user is logged in to Infinity tracking software. This represents time a user is occupying the equipment and thus, prohibiting others from utilizing the resource.
- Billable time accrues in 15 minute increments.
- If a user repeatedly reserves time in Infinity in excess (greater than one hour) of that necessary to complete their experiment, the user may be subject to billing for total time scheduled.
- There is a 15% hourly usage subsidy for HCC Research and Associate Research Members for all services provided.
- After hours and weekend access are possible after activation of the user’s ID badge.

- Revised service fees are listed at:
http://www.hollingscancercenter.org/research/shared-resources/cell-evaluation-therapy/fee_structure.pdf

Cancellation Policy:

- Unassisted cytometry: There is no penalty when a user cancels a reservation for independent operation of cytometers given they cancel their reservation in Infinity up to one hour beforehand allowing other investigators to utilize this time. If an investigator fails to cancel their reservation less than 1hr prior to its start time, the user will be charged for the full time reserved. (Can we figure out a way to do this?) The FCCS Staff may waive charges if user has a reasonable excuse (i.e., you are sick, kid is sick, etc.) but must be notified by either call to the FCCS Facility or email staff) If Infinity will not allow the user to cancel, they can contact the FCCS staff and they can adjust the appointment. (Add In?)
- Assisted Cytometry: There is no penalty when a user cancels a reservation for assisted cytometry, consisting of cell sorting and analytic cytometry requiring a FCCS staff member, if at least 24 hours' notice is provided. If an investigator cancels a reservation for assisted cell analysis less than 24 hours prior to the appointment, the investigator will be charged for the time reserved.
- Sorting: If an investigator cancels a reservation for cell sorting less than 24 hours prior to the appointment the investigator will be charged for only one hour regardless of the duration of their reservation. The sort setup fee will also be waived. Again, if we can use some or all of your previously reserved time for another investigator you will not be charged for the portion of your scheduled time that they use. (Don't really do this either)
- No Shows/Overbooking: Failure to keep an appointment without contacting the FCCS Staff will result in a charge for the full amount of time scheduled. If a user repeatedly makes appointments and does not use the time, the FCCS management will contact the PI of the laboratory to discuss billing issues.

Biosafety Regulations:

The use of analytic flow cytometers and, especially, aerosol-generating sorters with samples that contain viable infectious agents presents a serious risk to the FCCS Facility users and staff.

- **Analyzers:** *The use of the LSRFortessa, FortessaX20, and FACSVerser with un-fixed biohazardous samples is strictly prohibited.* All such samples must be sterilized with the use of an approved agent - usually 1-2% paraformaldehyde. Per MUSC biosafety regulations, materials that are not to be used in a viable state on these instruments include human cells of any type (includes human cell lines as well as primary cells) whether it is known or unknown if the cells contain infectious agents (HIV, Hepatitis, etc.) and cells with recombinant DNA vectors (e.g. lentivirus, adenovirus, plasmids). If you are in doubt of if your samples meet these requirements please discuss this with the Facility manager (B. Jacob Kendrick) at kendricb@musc.edu and or MUSC biosafety officer (Dr. Chris Voelkel-Johnson, johnsocv@musc.edu). If you would like to analyze un-fixed

biohazardous samples, a member of the FCCS staff will be required to analyze these samples for you using the biocontained FACSAria at standard assisted analysis charge.

- **Sorters:** All BSL-2 designated samples can be sorted on the FACSAria or MoFlo Astrios.

Data Management:

All user data will be deleted from cytometers within 30 days of collection. Storage of user data is solely the responsibility of the investigator. User generated data will be backed-up and then deleted from machines on the last day of every month. Facility data backup is provided as a courtesy, and will be overwritten each month due to space requirements.

Data Analysis:

A workstation with FlowJo v10.7 is available for remote access to those who have previously set up that option. Contact staff to establish access. In addition, users may sign out one of two FlowJo security keys (USB Dongle) for periods of 48 hours at a time (no fee associated). The key is located in a dropbox outside HO308 and can be reserved under the equipment section of Infinity.

Each ImageStream appointment comes with 4 free hours of guided analysis using Luminex's data analysis software IDEAS. Users must schedule guided analysis with facility staff.