Instruments and equipment

SR-FCM core maintains the following instruments for users:

FCM analyzer:

Our Name Code	Description
Accuri-Open	Two-laser, 4-color, BD Accuri C6 compact benchtop analyzer (installed in open space)
Accuri-BSC	Two-laser, 4-color, BD Accuri C6 compact benchtop analyzer (installed in biosafety cabinet)
Guava	Two-laser, 6-color, Millipore Guava EasyCyte 8HT compact benchtop analyzer
Fortessa	Five-laser, 21-color, BD FACS LSRFortessa (SORP) high-performance analyzer

FCM sorter:

Jazz	Three-laser, 6-color, BD FACSJazz cell sorter (installed in biosafety cabinet under BSL2)

Other Equipment:

MACS	Miltenyi Biotec MACS, manual magnetic antibody bead separator and related consumables
Dissociator	GentleMACS™ Dissociator, 2-port type automated processor for tissue dissociation to single cell suspension
Others	Cell Culture Resources in FACS room (for cells that will be sorted or have been sorted) – a ClassA2 biosafety cabinet, CO2 incubator, Evosfl fluorescence microscope, refrigerator, low-speed centrifuge

Workstations:

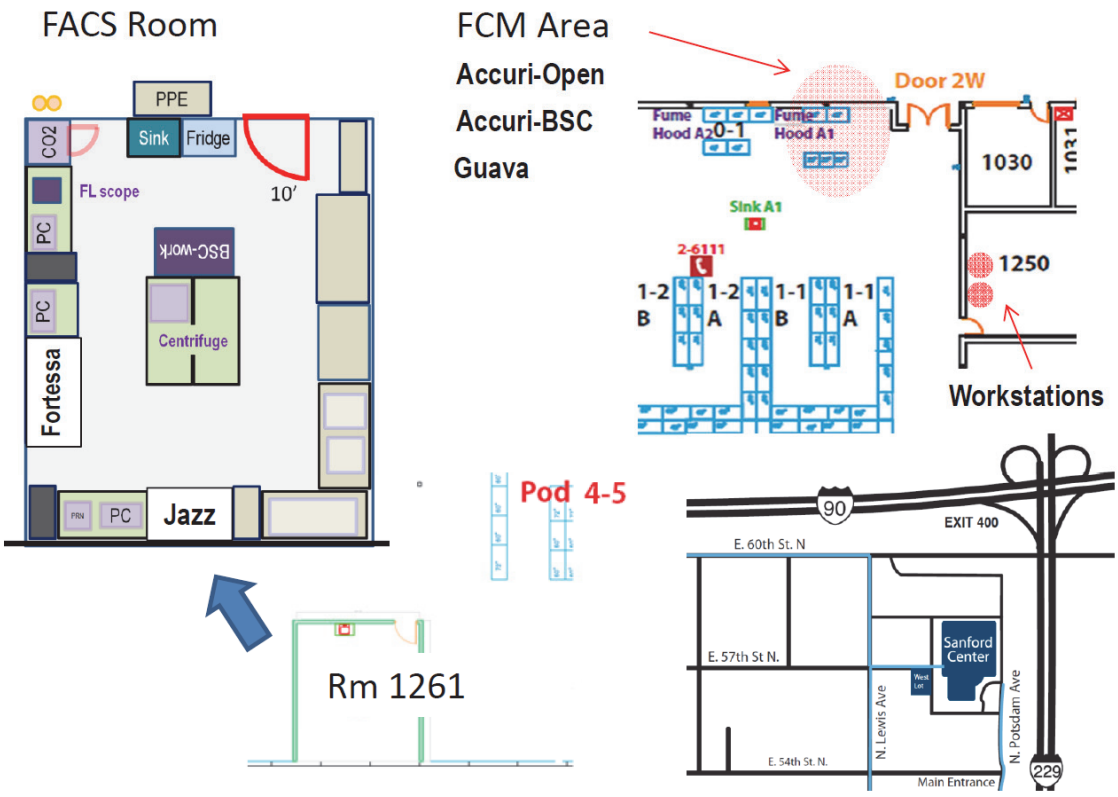
- **2 PCs** in data analysis room are dedicated for FCM core use and are available for all users.
- Both of the workstations are accessible with or without connection to SH network.

Software:

- **FlowJo**, **FCSExpress**, **CFlow**, and **MS Office** are available in both of the workstations.

- **Guava Soft** and **Modfit LT** are available in the right workstation.
- *<only for heavy users>* FCM core could provide internet dongle access to FCSExpress software (accessible from any PCs) upon the request.

Facility Oversight and Contact Information



SR-FCM core is located at:
Sanford Research Center
2301 E. 60th Street-North, Sioux Falls, SD 57104-0589
flowcytometrycore@sanfordhealth.org

Satoshi Nagata, PhD, FCM Core director
Tomoko Ise, MD, PhD, Staff Scientist

Samples that can be analyzed and sorted in FCM core

- Mammalian cells are anticipated samples. If you need to run other samples (microorganism or particles), please discuss with FCMC director in advance.
- Whenever possible, cell samples should be fixed (biologically inactivated, typically with paraformaldehyde or alcohol) before running on instruments.
- Live cell analysis and sorting under BSL2 conditions are allowed depending on the instruments. For the detail, please consult “Biohazard” section below. Note that projects

involving in known or potential biohazard must have been listed in institutional biosafety committee (IBC) research registration.

- Samples requiring BSL3 or BSL4 conditions cannot be handled in this facility.
- On an annual basis, all principal investigators are required to submit a FCM sample biohazard questionnaire form. The form will be reviewed in institutional biosafety committee.

Hours of Operation

- Accuri-Open, Accuri-BSC, and Guava are available 24/7 for self-service to the internal users (in Sanford Center) and Monday - Friday, 10:00 - 20:00 for self-service to the external users.
- Fortessa is available 24/7 for self-service to the qualified internal users and Monday - Friday, 10:00 - 20:00 for self-service to the qualified external users. After user registration, 2-4 training sessions are provided by FCM core. The trainings will be customized to user's application. FCM core qualify the user for self operations at some point.
- For cell sorting by Jazz, 4 sessions per week (Monday, Tuesday, Thursday, Friday) are available for users' semi self-operation (up to ~5 hrs). Please consult your experiment design and schedule with FCM core at least 10 days in advance. Start-up (~ 1hr) will be conducted by core staff. The setting will be adjusted to the users' application. Users will conduct sample application and shut-down (according to the SOP we provide). The core will provide the assistance and training up to 3-times for the sample application part.

  A set-up includes, starting the fluidics, laser warm up, nozzle setup, remove bubbles, aligning lasers, tuning the laser delay, QC of analysis part, making the droplets, adjust the drive frequency and amplitude, adjust the side stream positions, adjust the drop delay, stabilizing droplets formation, create and open the template for user's specific application.

User Registration (prerequisites):

Registration per instrument (required training is different for each model of instruments). Please identify the instrument that you want to use based on the following information. If needed, consult FCM core.

Instrument	Configu-ration	Pre-Requirement for use	Typical applications	Comments by FCM core
Accuri-Open Accuri-BSC Model- BD Accuri C6 analyzer	2 lasers 4 color	A training session with an experienced user	Apoptosis assay Cell Growth Assay (Count, Viability, CFSE dilution) Cell Cycle assay 1-4 color staining Measure GFP expression	Easy to use (user-friendly software) Recommended to a beginner Samples with potential biohazard (BSL2) can be run in Accuri-BSC unit installed in a Biosafety Cabinet (class II, type A2) – please see the "Biohazard" Section

Guava Model- Guava EasyCyte 8HT	2 lasers 6 color	A training session with an experienced user	Apoptosis assay Cell Growth Assay (Count, Viability, CFSE dilution) Cell Cycle assay 1-6 color staining Measure GFP expression	Recommended to an experienced user of Accuri C6 Individual samples are mixed by an integrated devise Only "height" parameters available for some parameters
Fortessa FACS LSRFortessa analyzer	5 lasers 18 color	Previous experience with Accuri C6 or other flow cytometers A few training sessions (~2-4) with FCM core staff	Virtually any analytical applications of flow cytometry can be conducted Suitable for >4 multicolor analysis FRET Please contact FCM core for more detail	state-of-the-art instrument Required to understand: (1) filter configuration (2) adjusting sensitivity of detectors (3) compensation Installed in BSL2 facility for samples with potential biohazard (BSL2) – please see the "Biohazard" Section
Jazz Model- FACSJazz cell sorter	3 laser 6 color	Previous experience with Accuri C6 or other flow cytometers Semi self-operated sorting: application-specific training sessions will be required with FCM core staff (up to 3 times)	Sorting of GFP-positive cells Sorting of subpopulation of lymphocytes	Every day-startup and set-up (and QC) will be performed by FCM-core staff Sort setting will be performed by core staff depending on your application Required to understand: (1) filter configuration (2) adjusting sensitivity of detectors (3) compensation Installed in Biosafety Cabinet. BSL2 facility for samples with potential biohazard (BSL2) – please see the "Biohazard" Section

To initiate the **registration**, please send a request by an e-mail to **"flowcytometrycore@sanfordhealth.org"**.

The core director will reply the e-mail with instruction on how to proceed. The appropriate training session(s) will be arranged.

Applications that can be performed in each instrument (examples for users)

If you are still not sure what experiment can be done with each instrument, please refer to the following general guide.

Cell cycle analysis (1D or 2D) – Accuri-Open

Assessment of apoptosis with various probes – Accuri-Open, Accuri-BSC

Cell number and viability assessment, dye dilution proliferation assays - Accuri-Open, Accuri-BSC, Guava

Screening (>200 samples) – Guava, Fortessa

Multiparameter immunophenotyping – Accuri-Open (<4 colors), Accuri-BSC (<4 colors), Guava (<6 colors), Fortessa (many colors)

Applications requiring UV-laser – Fortessa

Fluorescence resonance energy transfer (FRET) – Fortessa

Sorting experiments – Jazz

Important – We usually request new sorter users to perform a pilot test with analyzers before the cell sorting (analyzed cells cannot be harvested in the pilot test). The first step in sorting is effective cell analysis. The analytical part of the experiment must be pre-optimized to obtain the best sorting result.

Acknowledgements:

Please help us to develop and maintain the core. If your research supported by our FCM core results in publication, please include FCM core in acknowledgements section in your publication:

"This project used Sanford Research Flow Cytometry Core Facility that is supported in part by a COBRE grant from the National Institutes of Health (P20 GM103548)"

Please send copies of the publications to the FCM core director.

Biohazard (and other hazard):

-Five things you need to know-

1. Bench top cytometers are fully enclosed. The biological hazards associated with them relate to sample preparation rather than the instrument itself.

2. In contrast, the cell sorter generates droplets and aerosols during their normal operation, thereby increasing the biohazard potential of the experiments. Aerosol production can increase substantially during instrument failures, e.g., a partial clog in the nozzle tip.

3. Other sorter-specific risks are the high voltage applied to the deflection plates during sorting process and the laser beam possibly exposed when alignment.

4. Biohazard risk can be minimized by fixing samples before analysis (paraformaldehyde or alcohol treatment). Fixation of samples is a common practice of FCM.

5. Shut-down protocols provided in SOPs for each instrument contain decontamination routine by running a disinfectant (diluted bleach) regardless of the samples. The waste tanks contain bleach in sufficient quantity to achieve a final concentration of 2.5% when the tank is full.

-The most important thing you need to take great care-

Securely place sample trays (96 well plate) or tubes into the plate holder or the sample introduction port (to avoid splash). Don't drop your samples.

-Practices and examples-

The FCM Core is a biohazard safety level 2 (BSL-2) facility.

The core can sort NIH/CDC designated biohazard 2 agents which include primary human cells and cell lines, transfected cell lines, and live cells containing known level-2 pathogens.

However, you CANNOT analyze or sort any samples that are strongly suspected to be infected with a high level of HIV, HCV, HBV or other pathogens requiring higher than standard BSL-2 level containment or other special precautions. When a BSL-2 virus is being produced in high concentrations, additional risk assessment is required.

Please consult with FCM core director if you have questions.

- A guide -

1. Fixed cells
   – can be run in: All instruments

2. Live mouse primary cells, human cell lines without possibility of virus infection
   – can be run in: All instruments

3. Human primary cells, any cells infected with viruses or virus vectors
   – can be run in: Accuri-BSC, Fortessa with HTS, Jazz cell sorter

4. Human patients' cells without high potential of infection of transmissible clinically relevant viruses (such as HIV, HBV or HCV)
   – can be run in: Accuri-BSC, Fortessa with HTS, Jazz cell sorter

5. Samples requiring BSL3 or BSL4 conditions cannot be analyzed or sorted in this facility.

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<For Sanford network users> Please refer to the following SharePoint site for more information:
http://intrashare1:52001/dept/research/equpiment/Flow%20Cytometry%20Core/Forms/AllItems.aspx