



# SKCCC Flow Cytometry Core

## Overview of Services

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The Flow Cytometry Core facility strives to provide instrumentation and technical knowledge to support various levels of research on a broad range of applications and topics. Recommendations are provided in selecting reagents, instrumentation and gating strategies, enabling researchers to answer pertinent questions related to cancer research, the immune system and basic biological sciences. Services include cell sorting, basic training in Flow Cytometry principles and software usage, panel design, acquisition and analysis of data files.

## Staff Contacts

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## Location and Hours of Operation

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Location: CRB1, Room 435

Hours: 9:30am – 6:00pm, Monday-Friday

## Instrumentation – Analyzers

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### FACSCalibur

We have three FACSCalibur flow cytometers, located in CRBI rooms 435 and 231, and in CRBII room 571. They are available 24/7 to users who have completed training at our facility. Each instrument is configured with 2 excitation sources: a 488nm (blue) laser and a 633nm (red) light emitting diode laser. A total of 4 fluorescent parameters as well as forward scatter and side scatter are available. A Mac computer with CellQuest Pro software is interfaced with each cytometer for acquisition and analysis.

### LSRII

This higher speed scanning cytometer is located in the core facility and is available to users who have completed a training course at our facility. The instrument is configured with four high powered solid state lasers which include wavelengths at 407nm (violet), 488nm (blue), 532 (green) and 639nm (red). In addition to forward scatter and side scatter parameters, a total of 14 fluorescent color parameters are available: 4 colors off of the violet laser, 2 colors off of the blue laser, 5 colors off of the green laser and 3 colors off of the red laser. Emission filters and mirrors can be interchanged to accommodate an ever expanding list of fluorophores. Acquisition and analysis of data files is performed on FACSDiva software.

### LSRFortessa

The LSRFortessa high-speed scanning cytometer is available to users who have completed the necessary training course at our facility. The instrument is configured with five high powered solid state lasers, which include wavelengths at 385nm (UV), 407nm (violet), 488nm (blue), 532 (green) and 640nm (red). In addition to forward scatter and side scatter parameters, a total of 20 fluorescent color parameters are available: 4 colors off the UV laser, 6 colors off of the violet laser, 3 colors off of the blue laser, 4 colors off of the green laser and 3 colors off of the red laser. Emission filters and mirrors can be interchanged to accommodate an ever expanding list of fluorophores. Acquisition and analysis of data files is performed on FACSDiva software.

## Instrumentation – Cell Sorters

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### FACSAria II

The Core operates and maintains two FACSAria II sorters. Both are operated by Core staff and can be scheduled by appointment Monday-Friday from 9:30am-6:00pm. Cells can be sorted into tubes, plates or slides

- BSL-2 sorter
  - can be used for animal cells that are designated for BSL-2
  - 13 fluorescent color parameters are available across 3 solid state lasers: 4 color parameters off of the 407nm (violet) laser, 6 colors off of the 488nm (blue) laser and 3 color parameters off of the 635nm (red) laser
- BSL-2 sorter
  - Used for human and animal cells designated for BSL-2
  - 11 fluorescent color parameters are available across 3 solid state lasers: 3 colors off of the 407nm (violet) laser, 5 colors off of the 488nm (blue) laser and 3 colors off of the 635nm (red) laser

Contact facility staff for additional information prior to sorting.

### Influx

The Influx high speed cell sorter is maintained and operated by a dedicated operator of the facility. The instrument is housed inside a biological safety cabinet, whereby human or animal cells classified as BSL-2 or lower, can be safely sorted. 13 fluorescent color parameters are available across 4 solid state lasers: 4 colors off of the 407nm (violet) laser, 2 colors off of the 488nm (blue) laser, 4 colors off of the 532nm (green) laser and 3 colors off of the 640nm (red) laser. This instrument enables up to 6-way population sorting into tubes, index sorting of single cells into 96 well plates and should provide exceptional excitation of mCherry protein through the use of the 532 green laser. The Sortware software is used for acquisition, analysis and sorting.

## Training

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Training on a flow cytometry instrument consists of 1-2 sessions, depending upon the user's previous flow cytometry experience and comfort in operating the instrument independently. Session 1 will consist of a short presentation on flow cytometry basics, demonstration of cytometer operation and information about Core policies and procedures. During session 2 the user provides practice samples and will acquire and analyze samples under the supervision of Core staff. Each session on the FACSCalibur will take ~1-1.5 hours, while LSRII/LSRFortessa sessions take ~1.5-2 hours.

**To schedule FACSCalibur training:** contact Amanda Bowers at [abowers1@jhmi.edu](mailto:abowers1@jhmi.edu)

**To schedule LSRII/LSRFortessa training:** contact Jessica Gucwa at [jgucwa1@jhmi.edu](mailto:jgucwa1@jhmi.edu)