Research Flow Cytometry Facility

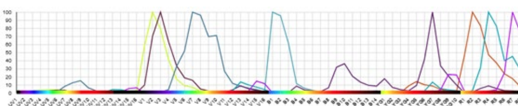
Quarterly Newsletter

Information Provided by your CCHMC RFCF

Why Spectral Flow Cytometry?

Spectral Flow Cytometry allows researchers to maximize the information a single sample can provide and allows the evaluation of more parameters simultaneously.

The primary difference between conventional and spectral flow cytometry is the amount of the emitted spectrum that is captured by the detectors. In conventional, only a portion of the fluorochrome's emission is collected by one detector. In spectral the entire spectrum is collected by multiple detectors. The collection of more data points with spectral flow cytometer allows more dissection of the signals which improves resolution between fluorochromes resulting in better resolved populations. Spectral flow cytometry allows cellular autofluorescence to be included as a parameter which often makes data easier to interpret. Spectral also provides greater flexibility in panel design.



Conventional flow cytometry uses a process known as compensation to subtract background signals from overlapping spectra due to multiple fluorochromes in a detector. Spectral flow cytometry uses spectral unmixing algorithms to distinguish the spectral profile of individual fluorochromes allowing individual spectral signatures of highly overlapping fluorochromes to be resolved. This increases the number of fluorochromes that can be used simultaneously in spectral versus conventional flow cytometry. The ability of spectral flow cytometry to differentiate a vast combination of fluorophores increases flexibility in application design and enables the development of complex multicolor panels for comprehensive immunophenotypic analysis.

If you would like to try Spectral Flow Cytometry, consider using the A5 SE, Auroras, or Northern Lights analyzers. For sorting with spectral consider using the S6 SE or Bigfoot. Please contact RFCF staff for information.

New autoMacs Neo

The RFCF has a new autoMACS® NEO Separator, an automated magnetic cell instrument. The fast and gentle isolation of any cell type makes it ideal for downstream applications, such as flow cytometry, functional assays, or omics studies. Proven in thousands of peer-reviewed publications. Please contact RFCF staff for information.

Transporting Samples

Best practice for sample transport within the CCHMC research facility requires 3 elements.

1. **Sealed capped primary tube(s)**.
2. A **sealed LEAK PROOF** secondary container with a biohazard symbol on it if it is BSL2 or above. (Examples below)
3. Absorbent between primary and secondary containers to absorb the entire contents.

Thank you to users already following these guidelines set by the CCHMC Biosafety Committee.

Biomarker Detection (MSD)

Meso Scale Discovery (MSD) enables researchers to measure very low levels of secreted and intracellular biomarkers. If you are utilizing ELISA, Western Blots, or Bead-based immune assays consider using the MSD that is in the RFCF. Join us Wednesday, April 17th, 12-1, T3-121 for a lunch and learn. RSVP to Brian Gribble at bgribble@meso-scale.com

Sample Concentration

The recommended sample concentration of 1-5x10^6 cells/mL is advised for the flow analyzers. Bring sample buffer with you to the facility to dilute your sample in case it is too concentrated.

A cloudy sample is too concentrated and will clog the instrument costing you instrument time and inconveniencing everyone else who needs to use the instrument.

Cellometer Cell Counter

The Nexcelom Cellometer Vision Trio cell counter is setup with a new software. This automated cell counter measures brightfield, and fluorescence in up to 2 channels (510-570 and 570-650nm). Applications include: cell count and size, cell viability (with dyes e.g. PI, Trypan Blue, Acridine Orange), cell apoptosis (by Annexin-V detection), fluorescent protein expression quantification (e.g. GFP, YFP).

Dates to Note

April 17, 12-1pm, T3.121: MSD lunch and learn.
April 19, 9-10am, S6.125: High Parameter (HP) meeting. Tamara Tilburgs & Zachary Koenig: Computational analysis workflow of clinical pregnancy samples using high dimensional flow cytometry data.
April 24, 1-2pm, S6.125: ORVCA meeting. Staining protocol for flow cytometry by Rebecca Bulterma from Nodexus.
May 22, 1-2pm, S6.125: ORVCA meeting. Conjugation kits for antibodies by Austin Marshall from Proteintech and Cassandra Clark from ThermoFisher.
June 26, 1-2pm, S6.125: ORVCA meeting. Panel design by Mary Mullen and Krystal Seger from RFCF.

Sealed LEAK PROOF Containers

The secondary container must be able to contain the sample(s) in case the primary container breaks.

These containers are not LEAK PROOF

Unacceptable secondary containers

include sample racks, ice buckets, aluminum foil wrappers, unsealed toolboxes, unsealed plastic boxes and any container that is not leak proof or has a cracked lid.

Acceptable secondary containers

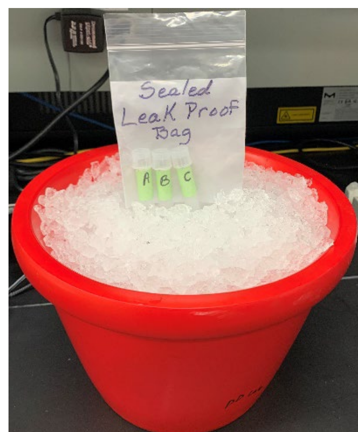
include plastic buckets or containers with a screw cap, plastic container with **LEAK PROOF** lid, plastic bag with leak proof seal, Playmate chests with lockable lids, Rubbermaid, or Ziploc containers.

Storage Box with Gasket Staples, Home Depot, Grainger, Big Lots

Sample/Specimen Transport Box $136

Examples:

Sealed LEAK PROOF secondary container with absorbent can be placed in ice.



Sealed samples can be placed in a container with ice which is then placed in sealed LEAK PROOF secondary container with absorbent.