LAB 1
Build a Flow Cytometer
Instructors: Martin, Wilder, Jett
This lab is designed to give the students a better appreciation of the inner workings of a flow cytometer, taking away some of the mysteries of what is hidden inside the cabinet. In this lab session you will assemble a small flow cytometer and use it to measure a sample of fluorescent microspheres. You will learn some of the important steps used in setting up a flow cytometer, some diagnostic clues that are useful for evaluating a flow cytometer's performance and an appreciation for what is involved in constructing flow cytometers. This compact flow cytometer is assembled using modular parts according to a detailed protocol. It includes the following components: laser, laser beam shutter, laser power attenuator, beam block, CCD camera and video monitor (used for viewing the laser beam - sample stream intersection region), flow chamber, fluorescence collection optics, fluorescence detector, electronics, oscilloscope and a computer. Because you will be installing and aligning optical components along a laser beam line good laser safety practices will be discussed and stressed. By the end of the lab session you will have assembled a working cytometer and will be analyzing the microsphere sample and optimizing the final adjustments to obtain the best CV. In recent years, CVs below 2% have been achieved.

LAB 2
Basic Data Acquisition and Analysis
Instructors: Naivar, Freyer
The laboratory will focus on the key concepts of flow cytometric data acquisition and how acquisition and display parameters affect downstream data analysis. Students will acquire data from a simulated flow cytometer and explore the effects of changing acquisition settings on the instrument. Emphasis will be placed on the basic concepts of sample delivery, signal intensity, dynamic range, thresholding and coincidence. Basic data analysis concepts will be reviewed, including data scaling, display parameters, gating and simple statistical analyses. Students will “acquire” data under various acquisition settings and explore the impact these parameters have on subsequent data analysis and interpretation. We will also take advantage of the fact that we are using a simulator, which allows students to see the effects of changing several “magical” acquisition settings that do not exist on current instruments but may be available in the not-so-distant future.

LAB 3
Automate Cytometry Applications and Visualize High-dimensional Data
Instructor: Bagwell
This will be a brand new laboratory with a focus on automating cytometry applications and visualizing high-dimensional data, arguably two of the most important topics in modern cytometry. Cytometry is evolving away from manual and subjective gate-based analysis systems to objective and automated systems. As modern cytometers evolve, the number of correlated measurements is steadily increasing, making it impractical to examine dot-plots. This laboratory presents one exciting solution to both problems, probability state modeling. The laboratory is entirely hands on where you will not only get to automate the selection of specific cell types in bone marrow and peripheral blood, but also will visualize and analyze very high-dimensional data. After finishing this laboratory, you will never look at cytometry data in the same way.

LAB 4
A Typical Cytometry Day
Instructors: Pletcher, Wilshire
You walk into your laboratory in the morning with plenty of samples to run. What do you do to ensure your experiment will work at each step from sample prep -> acquisition -> sorting your cells? We will take a "Forensic Cytometry" approach to your flow experiment by reviewing what can go wrong... and show you how to prevent it by following the 9 Practical Rules of Flow Cytometry.
We'll review how to prevent bad data during sample prep by titering your antibodies, Fc blocking, adding a viability dye, and doublet removal. On the machine we'll go over QC and how to set voltages. We'll take apart the analyzer so that you can see which components are being adjusted and not view the machine as a "mysterious black box". We'll go over the do's and don'ts for compensation as well as FMO controls for gating. We'll then move to the sorter where we will guide you through alignment of lasers, stream and pinholes - we guarantee that anyone can do it!

LAB 5
Advanced Sorting
Instructor: Trotter
This practical laboratory session will focus on several areas of interest in cell sorting that apply to particle sorting in general. We will cover instrument setup based on the task at hand. In other words, how to realistically approach optimizing nozzle size, stream stability, deflection envelope, break off, drop rate and sample rate for any given experiment. The lab will try to provide the attendee with tools for use in their own facility in problem solving a wide variety of sorting experiments, regardless of the cytometer they use, including suggestions on advising facility users on sample preparation. Among the topics to be covered will be standard sorting, enrichment sorting, and high speed sorting of various sample types such as, dendritic cells, microglial cells, adherent cells expressing GFP, activated cells, and normal lymphoid cells.

LAB 6

Imaging Flow Cytometry: Combining Morphology and Classic Flow Cytometry

Instructor: McGrath

Imaging flow cytometry resembles classic flow cytometry in that fluorescent data are collected on single cells in flow. However instead of total cellular intensity values, this technology collects pictures of the cell where each pixel has an independently compensated value for brightfield and multiple fluorescent channels using the ImageStream or FlowSight (Amnis Corp.). The images can then be analyzed for levels of intensity as well a number of morphological aspects of the intensity (shape, size, texture) as well as comparing locations of different stains. In the lab we will learn tips on sample preparation and data collection and get a tour of the machine. But the real fun and challenge is in the analysis, and that is where we will spend the most time. There are a series of labs available that range from absolute beginner to black belt masking. You will have the opportunity to learn how to quantify nuclear localization, apoptosis as well as a wide array of morphological and fluorescent features. We will also have some group fun trying to "name that feature" to help us brainstorm on what feature is the best to discriminate and match our wits with the computer "Wizard" feature-finder program. Ultimately the goal of the course is to enable the participants to learn if you can see it, you can gate it and that integrating looking at cells adds incredible power to flow cytometric analysis.

LAB 7

Monitoring Cell Function and Proliferation

Instructors: Tario, Wallace, Muirhead

This lab module will consider different methods of measuring immune cell function by flow cytometry including:

- Multimer staining and phenotypic profiling of antigen specific T cells
- Proliferation monitoring using dye dilution

The focus of the laboratory will be critical issues for cell function assays using commercially available probes. Students will be divided into small groups for "hands on" experience with:

- Staining and analysis of multimer binding lymphocytes.
- Staining and proliferation analysis using protein-reactive or membrane intercalating dyes (CFSE, PKH26 and newer analogs of each).

We will also cover several instrument setup and data collection issues likely to be of interest even if your laboratory is not already doing functional assays. These will include:

- Probe selection and analysis of multicolor data correlating cytokine expression, multimer binding and differential lymphocyte subset proliferation.
- Color compensation – recognizing problems, optimizing probe combinations to minimize them
- Collection and analysis of data on low frequency subpopulations – are these events real or are they junk?

LAB 8

Fluorescent Protein Analysis – Conventional, Spectral, and Fluorescence Lifetime Measurements

Instructors: Hawley, Galbraith, Houston

Fluorescent Proteins (FPs) comprise a family of related reporter molecules that can be conveniently expressed and detected within living cells and organisms. Currently, the color palette of useful FPs spans the ultraviolet, visible, and near-infrared spectra. The ability to detect multiple FPs simultaneously by flow cytometry provides the opportunity to non-invasively differentiate various populations, assess gene functions, and/or monitor protein-protein interactions in individual cells. Examples of applications will be discussed. Due to broad excitation and emission spectra, FPs often exhibit significant spectral overlap when used in combination. In this hands-on laboratory, using mammalian cells expressing various combinations of cyan, green, yellow, and red FPs, we will show that careful selection of excitation wavelengths and detection optics can result in successful resolution of five FPs on a conventional flow cytometer by performing spectral compensation. In addition, we will explore two methodologies that have been recently implemented in flow cytometry, spectral flow cytometry and fluorescence lifetime measurements. The former resolves multiple FPs by spectral unmixing and the latter resolves them using fluorescence decay kinetics. Upon completion, participants should come away with the knowledge to devise feasible detection strategies and the skill to analyze FP data.

LAB 9

Intracellular Cytometry: Signaling and Cell Cycle Regulation

Instructors: Chow, Hedley, Shankey

The large majority of proteins involved in the regulation of cell signaling, survival, and growth regulation are intracellular. This lab covers the technical aspects of intracellular antigen staining for flow cytometry. It includes approaches to cell fixation and permeabilization that optimize intracellular labeling while preserving light scatter and phenotypic markers. Although more demanding than cell surface staining, mastery of intracellular cytometry enables the study of fundamental regulatory mechanisms of normal and abnormal cell biology, many of which remain under-explored. In the second half of the lab we will illustrate this with (1) the activation of signal transduction pathways that regulate the acute inflammatory response via the NFkB transcription factor, and (2) molecular mechanisms that regulate movement through the cell division cycle.

LAB 10
Apoptosis and Autophagy
Instructors: Telford, Tamul, Bradford
This laboratory will focus upon different methods for the measurement of apoptosis by flow cytometry, with an emphasis on combining methods into more informative multiparametric assays. This is a hands-on laboratory where participants will get to perform several apoptosis assays as well as participate in the data analysis and group discussion of all the results. Participants will use live mouse tumor cells that have been induced to undergo apoptosis, followed by labeling with one of several fluorogenic caspase substrates, annexin V and a DNA binding dye. Participants will then acquire their samples on one of several flow cytometers under the supervision of the laboratory faculty, and will analyze their data. Additional assays, including tests for mitochondrial membrane potential, DNA damage via TUNEL assay, immunolabeling for active caspases and autophagy assays may also be available as time and materials permit. When completed, the participant should have an enhanced understanding of analyzing this important cellular phenomenon by flow cytometry.

LAB 11
Multicolor Immunophenotyping
Instructors: Preffer, Kelliher
Flow cytometry is a method for analyzing cells for multiple surface and intracellular proteins utilizing excitation lasers and monoclonal antibodies conjugated to unique fluorescent tags. Additionally, simultaneous light scatter measurements that impart cell size (forward light scatter) and complexity (90^{o} light scatter) are coupled with this information to identify and describe individual leukocyte cell populations. In our laboratory, we will acquire data on a 10 color BD flow cytometer. There will be a concentrated discussion about the antibody/antigen reaction, antibody kinetics, antibody titering, conjugated versus unconjugated antibodies, the importance of choosing the correct monoclonal antibody clone as well as choosing the correct antibody/fluorochrome combination. We will go through the steps of successful panel design utilizing common T-cell lineage and activation markers. We will perform an antibody titration and calculate the antibody's signal-to-noise ratio which will suggest the best titration to utilize. We will stain different combinations of reagents utilizing the results of the titration to determine both the optimal antibody clone and its preferred fluorochrome combination. We will discuss the mechanics of staining, surface versus intracellular as well as all the reagents necessary to properly develop successful staining.

LAB 12
Standardization and Optimal Panel Design
Instructors: Inokuma, Hingorani
This laboratory will focus on the principles involved in building a multicolor flow panel. We will discuss the key factors to building a robust multicolor panel, such as the wide arrays of fluorochromes available; understanding fluorochrome characteristics, understanding antibody specificity and spillover issues. The lab will provide tools to understand spillover, techniques to determine the brightness of antibody signal, and techniques to quality check your panel. Additionally, this lab will cover the principles involved in standardization across cytometers. We will discuss how to determine the dynamic range of the cytometer, determining a set of MFI target values, generating applications settings and verifying standardization accuracy using BD FACSDiva software, and BD FACSDIVA CS&T system.

LAB 13
Measuring Extracellular Vesicles
Instructors: Lannigan, Solga
This laboratory will focus on the important considerations necessary when measuring Extracellular Vesicles (EVs) by flow cytometry. Most flow cytometers were designed to measure cells ranging from 2-70 μm in size and with fluorescence ranging in the tens to several hundreds of thousands of fluorescent molecules. EVs on the other hand are generally 50 nm-1 μm in size and contain only hundreds to a few thousand molecules of fluorescence associated with them, making their detection, quantitation, and characterization challenging using conventional flow cytometers. This laboratory will present some valid approaches for: 1) assessing the limits of detection for an instrument; 2) the validation of single particle detection; 3) quantifying fluorescence expression in MESF units; and 4) calculation of particle concentrations. This laboratory will be conducted using a conventional, as well as an imaging flow cytometer.

LAB 14
Microfluidic Cell Sorting: So Not a Droplet Sorter
Instructors: Grummitt, Foster
This lab will cover many interesting aspects of cell sorting with Microfluidic Chips and no sheath fluid, as a sharp comparison to droplet sorting. This will include sorting without any cell centering, and a lab comparison of sorting with hydrodynamic focusing. The lab will also allow students to explore the flexibility allowed by Microfluidic Chips and perform Sequential Sorting in which a previously sorted population can be sorted again, or the non-sorted fraction can be sorted. Further, the students will be able to ascertain purity and yield in their experiments in a way not possible with conventional droplet sorting. And lastly, the students will be able to perform a clinically-relevant therapeutic sorting exercise in which all the elements of clinical cell sorting will be in effect, including sterility, ease-of-use, cell viability and disposability.

LAB 15
Cytometric Analysis and Sorting of (i) Large Particles and Cells and (ii) Complex Tissues from Animals and Plants
Instructors: Galbraith, Alexander, McGuirk, Aman

Flow cytometry is ideally suited for measurement of single cell suspensions, and the standard designs of flow cytometers and cell sorters are based on the typical size ranges of blood cells, i.e. diameters <20 μm. However, this situation is not always that simple. Many eukaryotic cells are larger than this, sometimes much larger. Furthermore, eukaryotic organisms are infrequently found in the form of natural single cell suspensions, instead forming tissues and organs -- complex three-dimensional interspersions of different cell types. Converting organs and tissues to single cell suspensions is not necessarily a simple task, since it requires dissolution of the extracellular matrices that interlink the cells.

This laboratory provides practical strategies for handling unusual samples in flow cytometry and cell sorting, including (i) dealing with large cells and other large biological particles, and (ii) dealing with complex organs containing many different cell types. The lab demonstrates how simple homogenization techniques can be used to release suspensions of nuclei for flow analysis and sorting, using both plants and animals as sources of organs and tissues. It also demonstrates how single cells and nuclei can be readily encapsulated and processed within a microfluidic device (the Chromium™ System) for convenient, highly parallel analysis of transcription via RNA-Seq.

All flow operators should benefit from the materials covered in this lab, since inevitably they will be confronted by users that wish to analyze and sort these unusual samples.