

2012 PRECONGRESS PREPARATION
Descriptions for Intro Flow Modules

EXPERIMENTAL DESIGN AND THE APPLICABILITY OF FLOW AND/OR IMAGE CYTOMETRY:

This course will provide an introduction to experimental design of flow and image cytometry with case type scenarios that can carry through.

FLUORESCENCE AND FLUOROCHROMES:

An introduction to fluorescent dyes and proteins, Q-dots will be provided in this module. In addition, a discussion on Stoke shift, spectral overlap and color compensation will take place. The lab portion will include multicolor beads and a demonstration of the excitation, emission, compensation of fluorochromes and the optical elements used to collect the spectrum.

BASICS OF FLOW CYTOMETRY:

This module will be conducted in front of an instrument in which the faculty will cover excitation, emission, optics, fluidics and data collection and display. The log vs Lin, discriminators and gates would be explained followed by an actual looking at parts of a flow cytometer and running some beads to understand the principles. Again it would be essential to use an open access flow analyzer for this demo. This module will not be essential for participants who are familiar with flow Cytometry and only beginners should attend this.

CELL CYCLE AND PROLIFERATION:

This introductory lecture will include a wet lab demonstrating principles of DNA content measurement, CV and flow rate, doublet discrimination, cell cycle analysis and bivariate analysis of DNA content vs. Proliferation or some other marker (e.g., protein, RNA, ER, or Ber-Ep4). Covered will be fixed cell lines, tumor specimens, formalin-fixed-paraffin embedded tumors.

APOPTOSIS

A short lecture will review the biology behind all the major assays available. Demonstration may include using a cell line exposed to camptothecin and treated with annexin or caspase antibodies.

PHENOTYPE ANALYSIS

Phenotype analysis often requires specific staining protocols depending upon the type of cells and markers to be analyzed. In this wet lab, we will demonstrate and discuss

sample processing protocols used in context of phenotypic analysis of hematological malignancies. We will also review principles of antibody titration and discuss the relevance of different experimental controls (eg. isotype, compensation, FMO etc.) in multicolor phenotype analysis.

STEM CELL ANALYSIS

This course will cover two aspects of stem cell analysis: Aefluor, and a description of pro's and con'; and two Side population, the various methods of ensuring the jet in air and sense in quartz systems are up to the job. Rare event analysis statistics, and the confirmation of rare events being real will form part of the lecture, as well as the demonstration.

FUNCTIONAL ASSAYS

This will focus on calcium flux monitoring, membrane fluidity, cell surface charge and free radical generation. Also discussed will be LMD files to demonstrate these techniques after a brief lecture discussing the principles of the assays.

STANDARDS AND CONTROLS

Flow Cytometers have multiple subsystems that must perform optimally in order to generate good quality data. The fluidic, optical and electronic subsystems are all critical to achieving accurate and reproducible data. The use of the fluidics system for delivery of sample to the sensing area requires the fluid velocity to remain constant while particles must be focused to the point of the laser intercept and well aligned with the detection system in order to achieve maximum detection in a reproducible manner. Laser alignment, filter quality, and detection system optimization will significantly impact on the intensity and accuracy of signals to be processed. Accurate measurements of these signals require the electrical amplitude output signal be linearly proportional to the optical input signal. Due to inherent offsets introduced in the signal processing in the instrument electronics errors in linearity may occur. The ability to resolve dim populations from negative populations is a function of an instrument's photon detection efficiency (Q) and level of background noise (b) In this module we will discuss the methods used for characterizing an instrument's performance as well as the subsequent monitoring of performance over time. In addition, we will discuss important considerations in data acquisition (MIFlowCyt Standards), data analysis and data presentation.