

# Clinical Flow Cytometry Course XXI

## HANDS-ON LABORATORY-BASED COURSE

- Understanding your Flow Cytometer
- Multiparameter Flow Cytometry, Sorting, and Imaging Cytometry
- Cytokine Analysis
- Clinical Analysis Computer Laboratories I and II
- Immune Dysfunction
- DNA Analysis
- Clinical CD34 Counting / Rare Event Detection
- Bring your own Data (BYOD) Analysis Clinic

## COURSE DIRECTORS

- Albert D. Donnenberg
- Fiona Craig

## FACULTY

- Vera S. Donnenberg
- Joanne Lannigan
- Ray Lannigan
- Holden Maeker
- E. Michael Meyer
- William Morice
- Maurice O'Gorman
- Paul Wallace

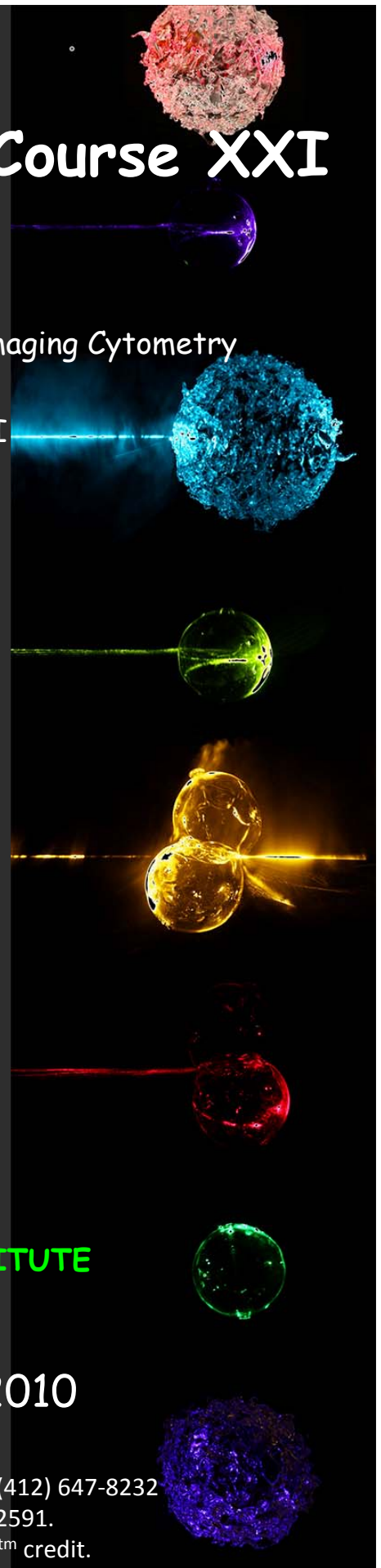
UNIVERSITY of PITTSBURGH CANCER INSTITUTE  
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Center for Continuing Education in the Health Sciences (412) 647-8232

<https://ccehs.upmc.com/formalCourses.jsp#2591>.

This course is approved for AMA PRA Category 1<sup>tm</sup> credit.



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### Lectures:

1. Introduction to flow cytometry. Fiona Craig.
2. Optimizing multicolor flow cytometry. Holden Maecker.
3. Quality control and performance monitoring. Joanne Lannigan.
4. An overview of the primary immunodeficiency diseases and the role of flow cytometry. Maurice (Mo) O’Gorman
5. Large granular lymphocyte disorders: pathobiology & diagnosis. William (Bill) Morice.
6. High sensitivity detection of paroxysmal nocturnal hemoglobinuria. Paul Wallace
7. Designing a flow cytometry experiment. Albert Donnenberg
8. Key note address. Alan Waggoner

### Laboratories:

1. Understanding your Flow Cytometer - Joanne and Ray Lannigan

2. Assays for the assessment of immune deficiency. Maurice (Mo) O’Gorman

We will perform 3 different procedures in this laboratory. The first will involve “Routine Immunophenotyping”. This is a very powerful screening assessment of the relative and absolute numbers of the major lymphocyte subsets that comprise a normal immune system in the peripheral circulation. A whole blood six color, two tube test will be performed to measure the relative percentages and absolute numbers of specific lymphocyte subsets. The second procedure is an assessment of the ability of granulocytes to elaborate an oxidative burst, essential for the killing of internalized pathogens. This is a whole blood oxidative burst assay which has become the gold standard for the screening diagnosis of chronic granulomatous disease. The last and most complex assay is a functional evaluation of the ability of CD4+ T cells to up-regulate the CD40 ligand (CD154) in response to in vitro stimuli. This test is valuable for the screening diagnosis of X-linked Hyper IgM syndrome or CD40-ligand deficiency as it was recently re-named. The applications of each of these assays in the diagnosis and assessment of primary immunodeficiency disease will be reviewed with additional focus on the technology and quality controls included in the design and execution of these tests.

3. Clinical Analysis Computer Laboratory I. William (Bill) Morice

This laboratory will address flow cytometric analysis for lymphoid neoplasms. After introductory presentations, participants will perform analysis of list mode data from illustrative cases. The laboratory will be divided into four portions addressing the following topics: identification of normal cell types and variations on normal, B-cell lymphoid neoplasms, plasma cell neoplasms and T- and NK-cell neoplasms.

4. Clinical Analysis Computer Laboratory II. Fiona Craig

This laboratory will begin with a discussion of flow cytometric analysis strategies used to identify populations of cells normally present in bone marrow. Participant will then apply these strategies to analyze list mode data from example clinical cases for the identification and characterization of abnormal cells. The following disease entities will be covered: acute lymphoblastic leukemia, including minimal residual disease detection, acute myeloid leukemia, myelodysplastic syndromes, myeloproliferative neoplasms, mast cell disease, and paroxysmal nocturnal hemoglobinuria.

5. Multiparameter Flow Cytometry, Sorting, and Imaging Cytometry – Albert Donnenberg

In this demonstration laboratory, peripheral blood mononuclear cells will be stained for 10-color analytical flow cytometry of lymphocyte subsets and myeloid cells, 8-color 4-way cell sorting and 4-color imaging flow cytometry.

Learning Objectives

- Review principles of cell staining and conserving expensive reagents
- Review offline (analytical) and run-time (sorting) spectral compensation
- Compare results generated by a flow-cell based analytical cytometer, a stream-in-air high speed sorter, and an imaging cytometer

6. Cytokine Analysis – Holden Maecker

In this laboratory, we will acquire and analyze a 10-color intracellular cytokine staining (ICS) experiment using pre-stained lyophilized cells. We will address instrument setup and optimization, and approaches for analyzing ICS data. We will also discuss key variables in setting up and running ICS assays, from sample handling and stimulation, to acquisition, gating, and analysis. The lab will also serve as an introduction to the use of lyophilized reagents for flow cytometry.

7. A Proliferation of Probes to Measure Ploidy and DNA Cell Cycle. Paul Wallace

In flow cytometry, analysis of DNA ploidy (DNA index or DI) and the fraction of cells in G<sub>0</sub>/G<sub>1</sub> versus S versus G<sub>2</sub>/M phases of the cell cycle is generally done by measuring cellular DNA content alone. Univariate DNA content analysis using propidium iodide (PI) with either ethanol fixed or detergent permeabilized cells are well-established clinical assay particularly for analysis of breast and colon tumor samples because they provides clinically relevant information regarding the tumors DNA content and kinetics. A multivariate approach using DRAQ5 is often used on hematological samples because it can be combined with surface phenotyping. We will start by demonstrating these univariate and multivariate ways that cells can be stained for DNA content; we will then look at the how the flow cytometer itself can affect the data derived from those stained cells; and finally we will discuss the ways to properly analyze and interpret DNA data.

8. Hematopoietic, Mesenchymal and Endothelial Progenitor Cell Enumeration. Vera Donnenberg

Cellular products for clinical use must meet stringent release criteria documenting purity, potency and safety, including an adequate progenitor cell dose, high viability and absence of bacterial or fungal contamination. A single platform, bead-calibrated assay will be used to assess CD34 purity, absolute CD34 content and CD34 viability, using the landmark ISAGE gating strategy. A modification of this strategy will also be presented for identification and enumeration of non-hematopoietic endothelial and mesenchymal progenitors in a paired specimen.

Learning Objectives

- To understand and perform daily instrument setup and quality assessment
- To understand the theoretical basis for single-platform flow cytometric determination of absolute counts (bead calibration, lyse no-wash)
- To understand the “ISHAGE” gating strategy for detection of CD34 cells, and the exclusion of non-viable cells by dye uptake
- To stain, acquire and interpret a “process control” sample
- To stain, acquire and interpret an unknown sample
- To determine absolute numbers of rare endothelial and mesenchymal cells in bone marrow and peripheral blood by relating CD34 proportion determined in a conventional assay to the absolute CD34 counted determined in an independent single platform assay.