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365 5th Ave, New York, NY 10016

2017 MetroFlow Annual Meeting
Expanding the Spectral Palate in Multicolor Flow

Speaker Abstracts:

David Novo
Visualization of High Dimensional Flow Cytometry Data

The recent explosion in the number of parameters acquired per cell has increasingly challenged the standard approaches (gating and visualization on 1D and 2D plots) of flow cytometry data analysis. A number of high dimensional analysis tools imported and modified from other fields has been applied to flow cytometry data. Instead of alleviating data analysis challenges, this has primarily served to introduce an alphabet soup of algorithmic acronyms to the scientist, each algorithm claiming to be superior to previous methods. Choosing the appropriate tool has never been more difficult. This talk will review the major classes of algorithms in a manner accessible to the non-mathematician , emphasizing the advantages and disadvantages of each approach.

Pratip K. Chattopadhyay
Precision Immunology Through Deeper Single Cell Profiling

Three trends have dominated biomedical research over the last decade. The first, the NIH Roadmap's Single Cell Analysis Program, was founded on the principle that cells are extremely heterogenous, and that this heterogeneity is important in health and disease. For this reason, cells must be characterized individually, rather than by insensitive and misleading analysis of bulk cell populations. This trend renewed appreciation for cellular heterogeneity, and incited a revolution of new technologies that could comprehensively analyze single cells (the second trend, deep profiling). Finally, a third biomedical research trend was sparked by President Obama's Precision Medicine Initiative, which aims to define genomic and proteomic differences between patient groups, and use this information to inform treatment decisions. In this talk, I will discuss my work at the intersection of these three trends, and demonstrate the value of new technologies for comprehensive and complete cellular analysis. I will provide examples of how deep knowledge about immune responses can be attained, using examples drawn from our recent work in immunotherapy and fundamental immunology. This talk will highlight our work developing 30 parameter flow cytometry, single cell RNA sequencing, CITE-Seq (for simultaneous measurement of protein and transcripts), and new bioinformatic tools.

Katherine Drake
Defining a High Dimensional Analysis Workflow in Cytobank to Replace Biaxial Gating

Cytobank's cloud-based informatics platform allows scientists to make powerful discoveries by applying state of the art machine learning pipelines and creating visualizations that characterize changes in either single cell data or sample heterogeneity with more depth than traditional approaches. We'll discuss examples where Cytobank's tools were applied to find and compare rare and novel cell populations across samples, automate single-cell biomarker discovery, and identify similar samples from their biomarker signatures across hundreds of combined markers.

Amir Horowitz
Harnessing NK Cell Functions in Settings of Infections and Disease

Dr. Amir Horowitz's research interest is to study how immunogenetic variation of HLA, KIR and CD94:NKG2A genes governs the education of human NK cells and their ability to function within dynamic environments. The goal of Dr. Horowitz's research is to develop strategies for harnessing NK cell effector and helper functions for treatments of cancers and infectious diseases as well as for preventing autoimmunity. Dr. Horowitz's work has contributed to developing an understanding of human NK cells in peripheral blood and in peripheral tissues and their roles in microbial infections, cancers and following vaccination and hematopoietic cell transplantation (HCT). Dr. Horowitz pioneered the first studies of human NK cells by mass cytometry (CyTOF) and demonstrated an enormous breadth of phenotypic diversity and functions associated with specific HLA class I and KIR backgrounds. This research has led to the identification and characterization of numerous NK cell subset populations with unique activity and immunotherapeutic potential. For his presentation, Dr. Horowitz will present data focused on utilizing CyTOF to profile human NK cells at unprecedented resolution from individuals with known HLA and KIR genetic backgrounds. He will then demonstrate how these data are redefining previously established associations between polymorphisms in HLA class I and NK cell functions as profound determinants in control of HIV-1 infection.

James Wood
Optimizing Instrument Performance Using a Pulsed LED Light Source

The pulsed LED tool (e.g. quantiFlash™) has proven itself to be an indispensable tool to measure the photonic response and characteristics of a flow cytometer. The pulsed LED has advantages over the traditional multilevel bead sets including high precision (<0.2% CV) pulse amplitudes available over a wide dynamic range. However, disadvantages include the need to design custom device kits to introduce the LED light pulses into the flow cytometer light collection system.

For the manufacturers, the pulsed LED can be used to determine the integrity of the light collection system including optics, fiber cables, filters, and photo detectors (photomultiplier tubes (PMTs), and various photodiodes). For users, it can be used to setup a universal photonic scale in statistical photoelectrons (Spes), and to characterize the background noise (B) and detection efficiency (Q) of individual PMTs for improved experimental panel design. It can also be used to setup a PMT to have a specific dynamic measurement range that complements the required experimental application measurement range.

The background (B) electronic noise and optical laser noise associated with each PMT can be measured directly by triggering the flow cytometer either through the FSC or SSC detectors, or any fluorescent PMT. This is called the trigger-B method. It is measured with the lasers turned off and then with lasers turned on at several PMT high voltage settings. The squared standard deviation, SD2, of the noise peak is used to measure the noise intensity since it is proportional to the intensity of the optical noise. It is also independent of the influence of the base line restorers used in the flow cytometer signal processing. A high SD2 indicates an inherent high electronic detector noise, or a large level of optical noise from the lasers because of optical filter flaws and/or design, as well as

Raman scatter and fluorescence from optical components. The PMTs and other electronics causing high noise levels can be identified and repaired or replaced if appropriate. The voltage ranging helps to identify detectors that generate excessive noise at increasing high voltage levels.

Alternatively B values can be calculated by fitting a quadratic curve to the data, SD2 versus intensity (histogram channel or equivalent reference fluorophore, ERF), collected at several levels of LED light pulse intensities, i.e. pulse intensity ranging. Since the intrinsic coefficient of variation, CVintrinsic, of the LED pulse intensities is very low, it possible to ignore the quadratic term for all but the brightest light pulses, and use a linear fit for signal levels generating 10,000 Spes or less. B Values measured in this way are referred to as “calculated B values”.

The detection efficiency and resolution (Q) can be calculated from the fitted data set mentioned above. Q is 1/slope (or linear term) or the Spes/channel (or ERF). If a calibrated marker bead is available for a target dye, then the channel values can be converted to ERFs and the Q value will be referenced to the target dye.

To facilitate the calculation of Q and B, an Excel spreadsheet has been developed that uses multiple algorithms to calculate Q and B in channels or ERFs, and to correlate the calculated B to the directly measured trigger-B from above.

Krista D. Buono

Neural Flow Cytometry: Unraveling the Black Box of the Brain

“Since the mid 1970’s, by defining cell populations via intrinsic scatter properties, cell surface antigen expression and other fluorescence parameters, the use of fluorescence flow cytometry in establishing a hematopoietic cell lineage has grown exponentially. More recently, increased awareness of the quantitative potential of flow cytometry has inspired neuroscientists to utilize this powerful tool not only to identify the neural stem cell (NSC), but also to commence constructing a neural cell lineage. However, the application of flow cytometry to specifically analyze and isolate neural populations is challenging. In contrast to hematopoietic cells where light scatter properties and a single antibody can distinguish distinct populations of cell types, the neural cell scatter is undefined. No single known antigen can identify a distinct neural cell population. With such ambiguity in the definition of antigens for the specific identification of neural cells, it stands to reason that the next best solution is to use different surface cell antigens in combination to achieve greater specificity. Thus, we designed and executed a 5-color flow cytometric panel to unveil a neural cell lineage. The panel was comprised of monoclonal antibodies CD133: prominin-1; CD15: Lewis-X (LeX/SSEA1); CD24: heat stable antigen; NG2: neural/glial antigen 2, CD140a: platelet-derived growth factor receptor alpha (PDGFRα) and a viability dye. This simplistic four-antigen panel yielded eight phenotypically distinct neural cell types: a neural stem cell as well as four classes of multipotential progenitors and three classes of bipotential progenitors, several of which have not been described previously. This flow cytometric work has provided a novel selection method to isolate NSC, as well as specific subsets of their progeny, for experiments that previously were not possible. The findings also revealed that neural cells are far more heterogeneous than previously suspected. As more fluorescent monoclonal antibodies slowly become identified and added to this panel, the neural lineage may expand and l become more defined. These cell discoveries will not only be informative for the understanding of neural development and basic biology, but will also have tremendous impact on clinical applications. These include novel drug development in nervous system regeneration and repair and neuro-oncology. Thus, with continued diligence, scientists will be able to show that a vision of brain regeneration offered by a pioneering neuroscientist long ago is not a dream, but a reality. Although microscopy is the cornerstone tool of neurobiology, neuroscientist should be encouraged to add flow cytometry as a part of their repertoire.”

Evgenia Verovskaya

Multicolor Flow Cytometry as an Essential Tool in Studies of Hematopoietic Stem Cells and their Niches

The lifelong blood production is supported by functioning of hematopoietic stem cells (HSCs) that reside in the bone marrow. HSCs are unique in their ability to self-renew and differentiate into all mature blood and immune cells. Moreover, they have a remarkable regenerative capacity, and a single HSC can replenish blood system of an irradiated mouse. In order to perform their function, HSCs rely on cues coming from their microenvironment, also known as “HSC niches”. In particular, mesenchymal stromal cells (MSCs), their osteoblastic progenitors (OBCs) and endothelial cells (ECs) are essential for HSC maintenance by providing instructive signals via cell-cell interaction and secretory factors. Our laboratory at the Columbia Stem Cell Initiative is interested in understanding the crosstalk between HSCs and their niches in the normal and pathological conditions, such as cancer development and aging.

HSCs are extremely rare, constituting <0.01% of all bone marrow cells, and are morphologically indistinguishable from more differentiated progenitor populations. This makes multicolor flow cytometry an indispensable tool for isolation and functional characterization of hematopoietic system. We have developed a flow cytometry-based workflow for analysis of HSCs, their downstream progeny and the niche populations, and applied this method to characterize age-related changes in mouse bone marrow.

By combining flow cytometry-based cell isolation and in vitro and in vivo functional assays, we demonstrated that aging leads to remodeling of both hematopoietic microenvironment and the blood system. Aging resulted in changes in spatial distribution of the HSC niches in the bone marrow cavity, and development of pro-inflammatory milieu. Inflammation, in turn resulted in functional deterioration and changes in lineage commitment in old hematopoietic stem and progenitor pool. Genome-wide microarray and targeted Fluidigm gene expression analyses of purified populations highlighted activation of pro-inflammatory programs. Finally, single-cell RNA-Seq analyses of MSCs, OBCs, and ECs demonstrated changes in production of HSC-supporting factors from the niche populations, suggesting that pharmacological targeting of pro-inflammatory bone marrow microenvironment can be instrumental in reverting blood aging.

In conclusion, we demonstrate that flow cytometry is a powerful tool to characterize the hematopoietic system and the bone marrow microenvironment.