



**Northwest Cytometry User's Group and  
Cascade Cytometry User's Group present the  
2007 Northwest Regional Cytometry Meeting**

Thanks to Nolwenn Le Meur and FHCRC for providing photos.

**Presentations include:**

**Garry Nolan, "Single cell analysis of phosphorylation: inference engines to derive signaling map topologies from clinical samples"**

**Frank Traganos, "Phosphorylated forms of ATM and histone H2AX provide for cytometric detection of DNA damage"**

**Ger van den Engh, "Flow cytometry of marine microorganisms: dealing with extremely complex samples"**

**John Nolan, "Sweating the small stuff: flow cytometry for the quantitative analysis of natural and synthetic nanoparticles"**

**Mike Loken, "Defining 'normal' in 6 dimensional space: a statistical basis for detecting residual disease"**

**James Leary, "Finding rare 'needles' in a cytometry haystack: applications in cancer, stem cells, drug discovery, and nanomedicine"**

**David Basiji, "Practical considerations of managing and analyzing large files of flow image data"**

**Steve DeRosa, "Standardized and semi-automated analysis of 8 color flow cytometric data for evaluation of vaccine-induced T cell responses"**

## **Order in cytometric complexity: quantitation, inference, and data management**

Cytometry, already providing plenty of data, is straining to accommodate cytomics, a more systems biology approach aimed at understanding the molecular architecture of the cell, as it comes of age. Inevitably the rate of data production will go up. With this cornucopia of data, or burden, comes the question of how best to visualize and analyze it – while data management is obviously going to be key, it's also likely other hardware, reagent, and software innovations that facilitate the automation of analyses will come to play a larger role. Discussion of how to better utilize data, how to turn the abundance of it into information and knowledge, framed in the context of biological and medical topics such as mapping phosphorylation networks and nanomedicine, is the substance of what this meeting is about – please join us.

**Place: Fred Hutchinson Cancer Research Center, Seattle**

**Date: March 22, 23 – 24, 2007**

### *Registration, hotel, and other information*

*Registration for the 2007 Northwest Regional Cytometry meeting is free thanks to the generous support of our sponsors listed on page four. Register by sending a note to [nwrcytometry@gmail.com](mailto:nwrcytometry@gmail.com), including name, email address, affiliation, and mailing address, and this will be replied to with confirmation of registration. Auditorium seating is limited, and early registration is advised. FHCRC is located at 1100 Fairview Ave. N, telephone (206) 667-5000. Hotels close to FHCRC include the Marriott Residence Inn 206-624-6000 (walking distance), Silver Cloud Inn 206-447-9500 (walking distance), and Marriott Courtyard 800-321-2211 (free shuttle). Both Silver Cloud and Marriott Residence Inn have a preferred FHCRC rate, obtained by mentioning it. Parking information will be sent out later with the more detailed program. For other information, contact either Julie Hill at [hilljul@gmail.com](mailto:hilljul@gmail.com) or Allan Kachelmeier at [kachelme@ohsu.edu](mailto:kachelme@ohsu.edu).*





# Order in cytometric complexity: quantitation, inference, and data management

## Friday, March 23

### *Inference, statistics, and data mining*

Garry Nolan, Professor, Stanford Department of Microbiology and Immunology, "Single cell analysis of phosphorylation: inference engines to derive signaling map topologies from clinical samples"

James Leary, SVM Endowed Professor of Nanomedicine, and Director, Molecular Cytometry, Purdue University, "Data mining across cytomic and cytometric platforms"

Mike Loken, PhD, President, HematoLogics, Inc., "Defining 'normal' in 6 dimensional space: a statistical basis for detecting residual disease"

### *Methods and heuristics*

Frank Traganos, PhD, Associate Director, Brander Cancer Research Institute, "Phosphorylated forms of ATM and histone H2AX provide for cytometric detection of DNA damage"

Ger van den Engh, Research Professor, UW School of Oceanography, and founder of Cytopeia, Inc., "Flow cytometry of marine microorganisms: dealing with extremely complex samples"

### *New technology*

John Nolan, Professor, La Jolla Bioengineering Institute, "Sweating the small stuff: flow cytometry for quantitative analysis of natural and synthetic nanoparticles"

James Leary, "Finding 'rare' needles in a cytometry haystack: applications in cancer, stem cells, drug discovery, and nanomedicine"

## Saturday, March 24

### *Standards and data management*

David Basiji, PhD, founder and CEO of Amnis Corporation, "Practical considerations of managing and analyzing large files of flow image data"

Steve DeRosa, MD, Fred Hutchinson Cancer Research Center, "Standardized and semi-automated analysis of 8 color flow cytometric data for evaluation of vaccine-induced T cell responses"

David Novo, PhD, President, DeNovo Software, "Data display transformations in flow cytometry"

Ryan Brinkman, Senior Scientist, British Columbia Cancer Research Centre, "Gates to the road forward: data standards for high throughput flow cytometry"

Listed on the right are the major talks Friday and Saturday. Other events, such as vendor talks and roundtable discussions, are not listed. A full detailed schedule of events will be emailed to meeting registrants later, closer to the date. The meeting runs from 9:30am to 7pm on Friday, and from 9am to 12 noon on Saturday. Lunch and refreshments will be provided.

## Thursday, March 22

Registrants might elect to come a day early and take advantage of one of the following two additional opportunities.

The Flow Informatics and Computational Cytometry Society (FICCS; <http://ficc.org>) meeting. Members of FICCS share an interest in new software tools, methods, and standards for flow cytometry, and include bioinformaticists, computational statisticians, software and hardware developers and clinician scientists, from both academia and industry (details of the FICCS meeting on page 5). Contact Ryan Brinkman at [info@ficc.org](mailto:info@ficc.org) for more information.

Advanced users course, "DNA and Cell Cycle Analysis", taught by Dr. Frank Traganos. The course covers both basic and multiparameter DNA cell cycle analysis techniques, including proliferation assays and measurement of DNA damage (syllabus on page 5). Contact Sue DeMaggio at [flocyte@flocyte.com](mailto:flocyte@flocyte.com) for more information and enrollment.



# Order in cytometric complexity: quantitation, inference, and data management



**Michael Loken**, PhD, President, HematoLogics, Inc. began his work in flow cytometry in 1973 on the first fluorescence activated cell sorter. The techniques he developed to simultaneously quantify multiple antibodies have been universally adopted. He was also the first to show neoplastic cells exhibit aberrant antigen expression, and these results have been applied to diagnosis of hematological abnormalities. Michael will discuss a 6 dimensional concept of normality as it relates to detection of residual disease.

**Garry Nolan**, Professor, Stanford Department of Microbiology and Immunology. Garry received his PhD under the mentorship of Leonard and Leonore Herzenberg, and later did postdoctoral work under David Baltimore at MIT and Rockefeller University, work in which he cloned and characterized the p65 subunit of NF- $\kappa$ B. Recent work has focused on analysis of biological events at the single cell level using genetic and FACS-based approaches, particularly analysis of phosphoproteins, with clinical development of diagnostics one of the goals.



**John Nolan**, Professor, La Jolla Bioengineering Institute. Dr. Nolan received his BS in Biology and Chemistry from the University of Illinois and PhD in Biochemistry from Penn State. From 1993-2004 he was at Los Alamos National Laboratory as a Post-Doc, Technical Staff Member, and, most recently, Director and PI of the National Flow Cytometry Resource. His research interests are in the area of quantitative molecular and cell analysis. John will discuss the roles of small cell-derived nanoparticles in physiology, the opportunities nanoparticles offer for diagnostics and therapeutics, and the challenges for the quantitative analysis of natural and synthetic nanoparticles.



**James Leary**, SVM Endowed Professor of Nanomedicine and Director of the Molecular Cytometry Facility, Purdue University. In a career spanning more than 30 years, Dr. Leary has pioneered rare-event analysis and other applications in cytometry and in the new field of cytomics. In his afternoon talk he will discuss high-speed approaches to finding rare cancer and stem cells, selection of aptamer sequences in combinatorial chemistry libraries for drug discovery, and targeting of nanoparticles to cells in vivo. In a separate talk Jim will discuss cytometric data mining, and approaches which enable mining of mixed data types.



**David Basiji**, PhD, is a founder of Amnis Corporation and currently serves as the company's CEO. He is a co-inventor of the ImageStream platform and various related technologies. Dr. Basiji earned his Ph.D. in Bioengineering from the University of Washington in 1997 for the development of an ultra-sensitive DNA and protein analysis platform. He currently holds 25 U.S. Patents. David will discuss the practical aspects of handling and manipulating gigabyte-sized flow image files. The talk will cover some of the issues associated with transforming such data sets into meaningful information, as well as some of the practical matters of managing the terabytes of data that accumulate over time.



**Steve DeRosa**, MD, Assistant Professor, Department of Laboratory Medicine, University of Washington. After studying bioengineering at Columbia, and training in pediatrics at Stanford, Dr. DeRosa began full-time research with Len and Lee Herzenberg. Working with Mario Roederer, he helped extend flow cytometry to 10 colors, and later at NIH developed 14-color staining panels. He now works with the HIV Vaccine Trials Network. He will discuss the 8-color intracellular cytokine staining they have developed, which, with protocol, set-up, and data analysis standardized, enables quality high-throughput analysis. A web-based system they developed automatically computes compensation matrices and applies standard gates.



**Frank Traganos**, Associate Director of the Brander Institute, New York Medical College at Valhalla, began his career in flow cytometry with Dr. Myron Melamed at Sloan-Kettering, who at that time was collaborating with Dr. Lou Kamentsky on development of a commercial flow cytometer. He later joined Dr. Zbigniew Darzynkiewicz in founding the Flow Cytometry Core Facility at Sloan-Kettering. He has been at Valhalla since 1990, where he is Professor of Medicine, Experimental Pathology and Immunology and Microbiology, as well as Associate Director. The vast majority of his research has been in study of cell proliferation and cell death with the tools of cytometry.



**David Novo**, PhD, President of De Novo Software. David learned flow cytometry from Howard Shapiro, and after working with Howard for a year, wrote the first version of his flow cytometry data analysis software, FCS Express. Currently, FCS Express is used by hundreds of researchers worldwide. David will discuss some of the issues relating to presentation of flow cytometry data on non-linear scales and, in particular, how the scaling can sometimes give a distorted view of the underlying data.



**Ger van den Eng**, PhD, Research Professor, School of Oceanography, University of Washington, and founder of Cytopeia, Inc. Widely known for having developed the first commercial high-speed sorter, Ger's dominant interest of late is isolation and characterization of microorganisms from ocean samples. Ger will discuss the problem of how to deal with complex samples in which each particle is unique.



**Ryan Brinkman**, Senior Scientist, British Columbia Cancer Research Centre and Assistant Professor, Medical Genetics, University of British Columbia. Dr. Brinkman has been leading an effort to develop data standards for high throughput flow cytometry, with associated research efforts in automated gating and flow cytometry based patient classification. Ryan will discuss the proposal for FCS4 and associated standards (e.g., gating) and what these will mean for flow cytometry data management in the future.

Special thanks to all our sponsors who made this meeting possible.



## Flow Informatics and Computational Cytometry Society meeting at Fred Hutchinson Cancer Research Center

FICCS ([ficc.org](http://ficc.org)) will hold its third meeting of groups interested in the collaborative development of software tools to facilitate high throughput flow cytometry on March 22. The meeting will be held at the Fred Hutchinson Cancer Research Center in Seattle.

FICCS 3 will include a morning session of presentations, followed by an afternoon group workshop focused on the development of an open source database infrastructure to support high throughput flow cytometry experiments.

Morning:

Byron Ellis (Nolan Lab, Stanford University), “flowCore: An open source software package in R for the analysis of flow cytometry data”

Nolwenn LeMeur/ Deepayan Sarkar (Fred Hutchinson Cancer Research Center), “flowViz/flowUtils: Open source software packages for the management and display of flow cytometry data in R”

Adam Treister (Tree Star Inc.), “FlowJo's integration with R”

Nikesh Kotecha (Nolan Lab, Stanford University), “CytoBank”

Afternoon:

Several members of the FICCS community have expressed an interest in co-developing software infrastructure for data management, with support for linkage to R. The afternoon session will focus on organizing this effort. Any group interested in participating is invited to provide a 3-5 minute presentation outlining their use, requirements, and required / available resources. The afternoon session will also include a session on data standards ([flowcyt.sourceforge.net](http://flowcyt.sourceforge.net)).

Contact Ryan Brinkman at [info@ficc.org](mailto:info@ficc.org) for further information.

## FloCyt course “DNA and Cell Cycle Analysis” – taught by Dr. Frank Traganos, software assistance from Mark Munson (Verity) and new DNA dye information from Jolene Bradford (Invitrogen)

The course begins with a brief introduction to the DNA cell cycle and associated protein production at different stages of the cycle, and then progresses into the main topic, incorporation of cell cycle analysis in multiparameter cytometry. Students will have ample opportunity to ask questions relevant to their own research. A sketch of the syllabus is below. For more information contact Sue DeMaggio at [flocyte@flocyte.com](mailto:flocyte@flocyte.com).

Topics covered

Basics of cell cycle analysis, A very brief history of cell cycle, Going from cell cycle to single parameter DNA histogram

What do DNA histograms tell us – what other information is required for interpretation?

Brief discussion of DNA dyes – do's and don'ts

Examples of perturbations in DNA histograms

Sub-G1 cells – how to identify apoptotic cells from debris

Multiparameter cell cycle

BRDU to more precisely determine S phase, Traditional method, New SBIP method

Other assays for proliferation – Ki-67, cyclins

Early two parameter approaches, DNA versus protein, DNA versus RNA

Native versus Denatured DNA for identification of mitosis

Use of cyclins to study cell cycle

Background on role of cyclins, cdk's, and ckis

Discussion of each of the useful cyclins: D, E, A, and B

Use of cyclins to study: Refinement of cell cycle compartments, Drug treatment, Unbalanced growth, Drug point of action – stathmokinosis, Difference between cancer and normal cells

Three parameter approaches using cyclins and DNA

Multiparameter approaches using modification of chromatin proteins

Phosphorylation of histone H3 to identify mitotic cells

Phosphorylation of histone H2AX to identify DNA double-strand breaks, Sensitivity of the assay, Detection of DNA damage by a variety of clastogens

Measuring DNA damage using ATM

Other multiparameter approaches, Inactivated versus total retinoblastoma protein

Mitochondrial membrane potential