



COLD SPRING HARBOR LABORATORY QUANTITATIVE IMAGING CYTOMETRY (QIC) CENTER



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Center Objectives

Cold Spring Harbor Laboratory and CompuCyte Corporation have begun a new forum for quantitative imaging cytometry, with the objective of providing educational and training resources for the utilization of the emerging technology that combines quantitative measurement of cellular constituents (adherent cells, tissues and tissue microarrays) with comprehensive imaging at various degrees of resolution. Under the agreement, CSHL and CompuCyte will sponsor an annual symposium and several training programs throughout the year, focusing on new QIC techniques for researchers using LSC systems in cell and tissue-based research.

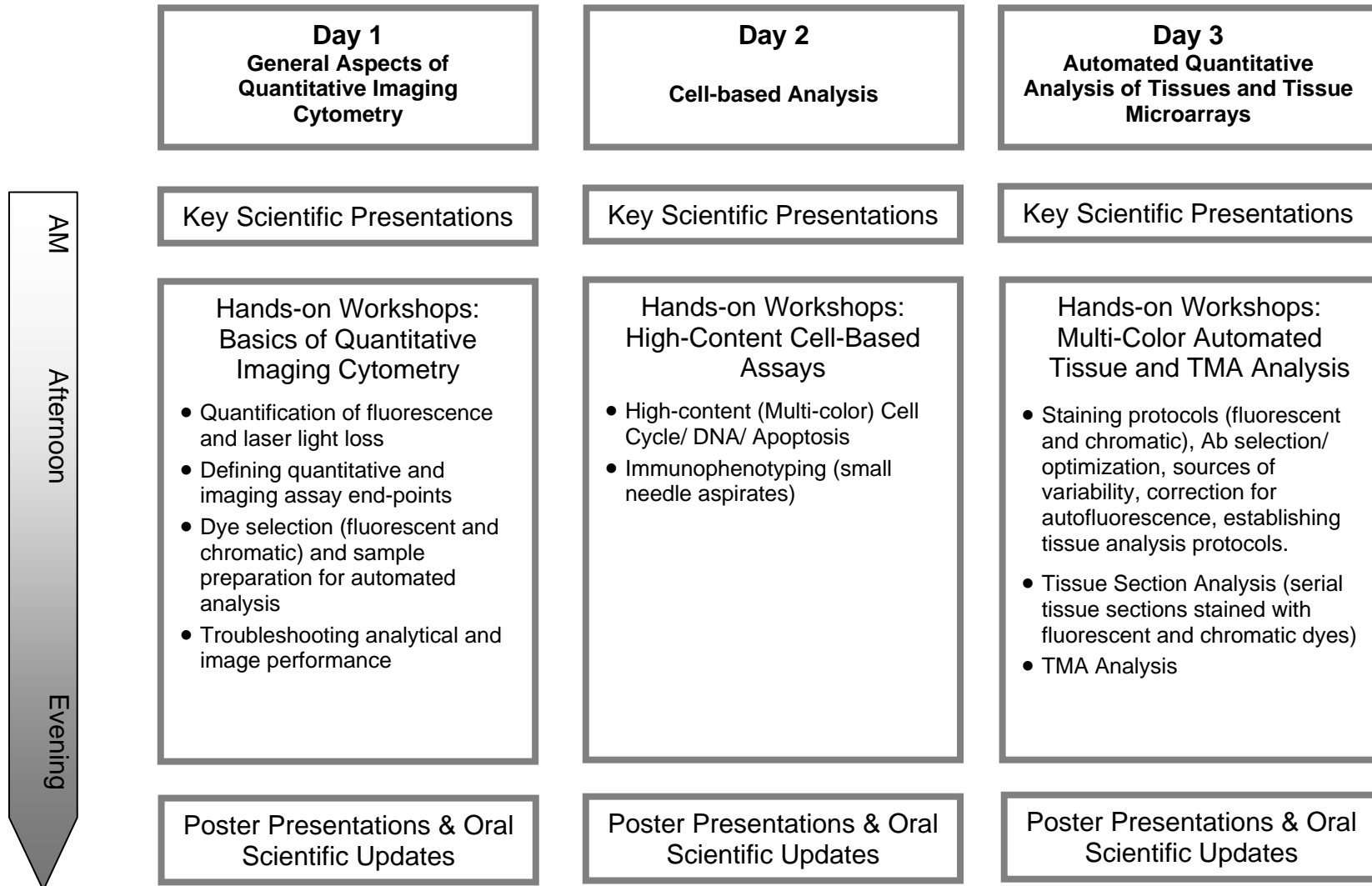
Advisory Committee:

The Center is guided by an advisory committee of renowned cytometry experts:

- **Zbigniew Darzynkiewicz**, M.D., Ph.D., Director, Brander Cancer Research Institute, New York Medical College, Valhalla, NY
- **William Geddie**, M.D., Department of Pathology, University Health Network and Assistant Professor, University of Toronto
- **James Jacobberger**, Ph.D., Professor of Oncology, Associate Director for Shared Resources, Director: Cell Analysis Core, Case Comprehensive Cancer Center, Cleveland, OH
- **Gloria Juan**, Ph.D., Sr. Scientist, Amgen, Thousand Oaks, CA
- **William Telford**, Ph.D., Director, Core Flow Cytometry Resource, National Cancer Institute of the National Institutes of Health, Bethesda, MD



Annual Quantitative Imaging Cytometry Course Structure



2008 QIC Course

The inaugural Quantitative Imaging Cytometry Course was held from January 30 to February 1, 2008 at the Cold Spring Harbor Laboratory QIC Center of Excellence. The 2008 symposium consisted of a number of educational activities, including morning presentations by distinguished scientists well known in the fields of cytometry and imaging cytometry, as well as afternoon practical hands-on sessions focused on solid-phase imaging cytometry techniques.

Morning sessions:

- ***Time, Biochemistry, and Cell States: The Role of Cytometry in Systems Biology*** - James Jacobberger, Professor of Oncology, Case Western Reserve University, and Director of the Cell Analysis Core, Case Comprehensive Cancer Center.
Dr. Jacobberger discussed multi-variate analysis of the discrete cellular “states.” A multi-variate analysis of cyclin expression in the mammalian cell cycle and FLT3 signaling in AML blasts was used to illustrate these cytometric properties along three different time scales and their value to dynamic mathematic modeling of cell processes (as a subset of Systems Biology).
- ***Laser Scanning Cytometry: Where It Fits into Modern Biomedical Analysis*** - William Telford, Flow Cytometry Core Laboratory, National Cancer Institute, National Institutes of Health, Bethesda, MD
Dr. Telford highlighted various imaging cytometry technology options available to scientists and the importance of critical assessment of choices based on specific application needs. He focused on the role of Laser Scanning Cytometry in characterizing individual cells in the process of analyzing biological and biomedical systems, providing an in-depth review of the technology itself and highlighting representative applications where LSC is particularly powerful.
- ***Unique Analytical Capabilities of Quantitative Image Cytometry and Their Applications in Cell and Molecular Biology*** - Zbigniew Darzynkiewicz, Professor of Pathology, Brander Cancer Research Institute, New York Medical College, Valhalla, NY
Dr. Darzynkiewicz reviewed the diverse analytical capabilities of quantitative imaging cytometry that allow its application to problems otherwise not permissible by flow cytometry. The nature of flow cytometry, with its suspension of cells in a stream of liquid that is often discarded following the measurement, has limitations: an inability to characterize time-resolved events, localize sub-cellular fluorochromes or analyze solid tissue, among others. Applications of QIC to the identification of cells that differ in degree of chromatin condensation, the detection of translocation between cytoplasm vs. nucleus, the detection of translocation of Bax to mitochondria and release of cytochrome C from mitochondria were highlighted.

Morning sessions (continued):

- ***Cell Fate Signaling in Tumor Cells*** - Shazib Pervaiz, Professor, National University of Singapore
Dr. Pervaiz presented data on his recent work exploiting simultaneous analysis of multiple parameters in tumor cells triggered to undergo drug-induced apoptosis. Additionally, he discussed recent unpublished data on the use of QIC for monitoring intracellular redox status and cell cycle, in an effort to underscore the utility of QIC technology for multi-parameter analysis of cellular events.
- ***Potential Mechanisms for the Generation of Chromosome Aneuploidy in Human Cancer*** - John M. Lehman, Professor of Pathology, Brody School of Medicine, East Carolina University, Greenville, NC
Dr. Lehman discussed his investigation of SV40-infected and uninfected cell populations using multi-parameter laser scanning cytometry to make simultaneous measurements of the cellular DNA content and MCM proteins (MCM2 and MCM3), as part of ongoing studies to understand the mechanism of SV40-induced re-replication of host-cell DNA, as this may contribute to the knowledge of DNA replication in human tumors and the generation of aneuploidy.
- ***The Impact of Systemic Chemotherapy on Circulating Epithelial Tumor Cells (CETCs) in Breast Cancer*** - Katharina Pachmann, Professor, Klinik für Innere Medizin II, Friedrich-Schiller Universität, Jena, Germany
Dr. Pachmann reported on her work in monitoring the number of CETCs in peripheral blood of cancer patients and the implications of those measurements. The number of CETCs can be influenced by natural shedding of cells from the tumor, mobilization of tumor cells by diagnostic and therapeutic manipulations (mammography, surgery) as well as apoptosis and cell death by systemic chemotherapy. She presented results indicating that monitoring CETCs may be the earliest and most reliable indicator of successful neoadjuvant treatment which is highly predictive for relapse-free survival.
- ***Applying Quantitative Imaging Cytometry to Diagnostic Cytopathology and Histopathology*** - William Geddie, Staff Pathologist, Cytopathology, Department of Laboratory Medicine and Pathobiology, Toronto General Hospital, University Health Network, Toronto, ON, Canada
Dr. Geddie reviewed the shortcomings of traditional morphologic classification and grading systems in diagnostic cytopathology and histopathology. He presented how QIC has been integrated into one high-volume diagnostic cytology laboratory, both for initial specimen triage and cell surface-marker immunophenotyping in fine needle aspirates, and provided data validating QIC analysis in comparison to flow cytometry. A significant portion of Dr. Geddie's presentation was focused on the potential of QIC for "making a little tissue do more" through multiplexed analysis, by combining multiple traditional stains on one sample—a significant feature in diagnostic pathology and tissue-based biomarker discovery.

Morning sessions (continued):

- ***QIC Applications in Pre-Clinical Drug Development*** - David L. Krull, Molecular and Ultrastructural Pathology Group, Safety Assessment, GlaxoSmithKline, Research Triangle Park, NC
Mr. Krull discussed several automated methods he has developed using imaging cytometry to quantify protein expression. He reviewed methods for protein labeling involving the use of immunohistochemical, immunofluorescent or histochemical stains and dyes, focusing on the evaluation of chromagens, Alexa Fluors®, Qdot nanocrystals and hematoxylin and eosin dyes.
- ***Quantitation of Caspase3 Activation as a Pharmacodynamic Endpoint in Fine Needle Aspirate (FNA) Biopsies*** - Gloria Juan, Manager, LSC Technology Group, Amgen Inc., Thousand Oaks, CA
Dr. Juan described her group's work on quantification of the activation of caspases 3 and 8 *in situ*, as a measure of the mechanism-specific biological activity of the anti-Trail Receptor immunoglobulin therapy AMG655, currently in clinical development. She presented data confirming that caspase activation is an early event that precedes tumor regression. Automated analysis by quantitative imaging cytometry correlated well with independent pathologist scoring. Biomarker validation studies in tumor biopsies and fine needle aspirates are progressing
- ***Quantitative Imaging of Biomarkers in Whole Sections and Tissue Microarrays Using Laser Scanning Cytometry*** - Vijayalakshmi Ananthanarayanan, Research Assistant Professor, Department of Pathology, University of Illinois at Chicago, Chicago, IL
Dr. Ananthanarayanan reviewed her work in investigating molecular markers in normal or pre-neoplastic lesions that predict progression to cancer, as potential endpoints for phase 2 chemoprevention trials. She described two approaches, using standard brightfield digital microscopy and laser scanning cytometry (LSC) for automated analysis of tissue microarrays. LSC analysis exhibited enhanced sensitivity and robust biological validity, allowing discrimination of recurrent from non-recurrent prostate cancer cases, based on p27 levels. It was suggested that automated image analysis systems could provide more precise and reproducible measurements for evaluation of tissue-based biomarkers and could greatly assist in characterization of early-morphological abnormalities associated with cancer (i.e., field effects).

Afternoon sessions:

Afternoons were devoted to practical instruction in various applications of quantitative imaging cytometry. The CSHL core facility is equipped with an iCys® Research Imaging Cytometer, representing state-of-the-art quantitative imaging cytometry technology. This location was used for more complex application development based on individual research.



The majority of attendees were involved in practical discussions related to sample preparation for automated analysis, the application protocol “decision tree,” selecting appropriate dyes, data acquisition and analysis of cellular and tissue specimens, cell surface immunophenotyping, extracting features for analysis, selecting and applying appropriate segmentation techniques, quality control, and many other subjects.

Instructors for the sessions were scientists from well-known pharmaceutical and research organizations:

- **William Telford**, National Institutes of Health
- **Jeffrey Smith, Steven Zoog** and **Gloria Juan**, all from Amgen
- **John Mahoney**, Dana-Farber Cancer Institute, Harvard Medical School
- **David Krull**, GlaxoSmithKline
- **Andrea Holme**, National University of Singapore

The sessions were equipped with individual data analysis stations and quantitative imaging analysis instrumentation utilizing principles of laser scanning cytometry.

Two evening receptions provided an opportunity for viewing posters presented at the symposium and discussions with authors. The Cold Spring Harbor Laboratory setting, as well as the on-campus accommodations, made for a collegial atmosphere of learning, networking and reflection.

