



*Quantitative Imaging Cytometry Centers of Excellence
Quantitative Imaging Cytometry Symposium
Boston, Massachusetts
September 27–29, 2011*

Track 2 / Workshop 2: “Hunting” for Rare Events

Wednesday, September 28, 1:00 – 5:00 pm

Enders Building, 8th Floor Conference Room

Facilitator: [Stephan Ruetz](#), Novartis Institute for Biomedical Research, Basel, Switzerland.

Panelists: [James Jacobberger](#), Case Western Reserve University, Cleveland, OH.
[Zbigniew Darzynkiewicz](#), Brander Cancer Research Institute, Valhalla, NY

One of the most striking advantages of Quantitative Imaging Cytometry using the iGeneration LSC technology platform is the ability to study individual cellular events with different or altered features within a large population of cells. Using cell-by-cell analysis, sub-populations of cells can be identified and classified using either fluorescent signals or morphometric markers, or both of these features in combination. In addition, LSC technology offers the unique ability to visualize rare events, not only helping to confirm the existence of different cells but also providing the opportunity to systematically hunt for altered or abnormal cellular events.

Sub-populations of cells with distinct features or properties are actually present in every cellular assay system. For instance, in any proliferating cell population only a few cells are in a particular phase of mitosis at any given time. Although their numbers may be very small, they differ from the other cells in their morphology, DNA content, composition and expression of mitosis-specific proteins, and thus can be clearly classified based on these properties. In contrast to these expected differences, there are also instances where rare events might not be expected or are not as obvious. Such situations are often seen in cell populations undergoing apoptosis. It is well established that programmed cell death occurs in individual cells in a highly structured and time-dependent manner. However, individual cells in a population do not enter apoptosis simultaneously; at any given time a variety of apoptosis markers are present. As a result, cells in a specific apoptotic stage are typically very rare and therefore often challenging to identify.

This workshop will address the challenges in analyzing rare events, as well as the opportunities. Both aspects--the identification as well as the “hunting” approach--will be covered. Based on their own data, the three speakers will present their strategies using iGeneration LSC technology, focusing on data analysis and interpretation of the results.

Panel discussion:

[Dr. Ruetz](#)

Dr. Ruetz will present different strategies for studying apoptotic processes using iGeneration LSC technology. The discussion will focus on suitable cellular systems and apoptosis markers, with an emphasis on the importance of appropriate controls. Starting from simple assay designs, the presentation will move to more advanced protocols including demonstration of an engineered cellular



model to study apoptosis. There will also be a demonstration of how different displays can be linked providing true high-content information. Finally, two case studies will show where an analysis failed to identify rare apoptotic events and discuss potential causes of limitations.

[Dr. Jacobberger](#)

Dr. Jacobberger will demonstrate the use of laser scanning cytometry for extracting quantitative cell cycle-related expression information and transitions through binary-state classification endpoints. As an example, he will show the extraction of the expression profiles for cyclin B1 and phospho-S10-histone h3, both of which oscillate in a cell cycle-dependent manner, and the occurrence of centrosome separation, cyclin B1 entry into the nucleus, nuclear membrane breakdown, and the stages of mitosis as a function of cell cycle time. Each of the latter events (centrosome separation, cyclin B1 entry, nuclear membrane breakdown, and mitotic stages) will be quantified as binary event states (either a cell belongs to the class or it does not). All parameters will be integrated into a single analysis. Topics for discussion include (1) the form and validity of rules for extracting expression profiles; (2) the value of extracting expression profiles; (3) improvements to image evaluation; (4) the pros and cons of binary classification versus feature identification and parameter quantification; and (5) automating the process.

[Dr. Darzynkiewicz](#)

Dr. Darzynkiewicz will discuss the detection of DNA replication by “click chemistry.” The “click chemistry” approach is based on the use of 5-ethynyl-2’deoxyuridine (EdU) as a DNA precursor which can be detected with fluorochrome-tagged azides by means of a copper (I) catalyzed [3+2] cyclo-addition reaction. The small size of the fluorescent azides offers an advantage because their accessibility to the incorporated EdU is greater than in the alternative cytometric assay of DNA replication based on incorporation of BrdU, to the BrdU-Ab. The greatest advantage of this approach stems from the fact that no DNA denaturation is required. It offers new opportunities for analyzing a correlation between DNA replication and other attributes of the cell detected immunocytochemically. Correlation between DNA replication and DNA damage response signaling (detected by histone H2AX phosphorylation and ATM activation) in cells treated with UV light, reactive oxidants, and DNA topoisomerase inhibitors camptothecin, mitoxantrone and etoposide, reactive oxidants and UV, assessed by laser scanning cytometry, will be presented.

Contributions by workshop participants are encouraged. If you plan to make a brief presentation of your work, please [submit your abstract](#).