

QIC 2011 Workshop, Track 1: Cellular Applications

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Heather Zecchini is deputy head of the Light Microscopy Facility at the Cancer Research UK Cambridge Research Institute. She received her doctorate from the University of Cambridge for studies of intestinal epithelial stem cells and progenitors using a novel in vivo clonal marking system. In the last five years she has worked with many of the researchers in the Institute on a range of cancer projects, to which she applies qualitative and quantitative imaging methods, particularly laser scanning cytometry, to study the processes involved in controlling the normal biological state of cells and tissues, the perturbation of these mechanisms leading to cancer and to aid in the development of cancer drug treatments.

Abstract

Laser Scanning Cytometry (LSC) is an exceptional tool for exploring many research areas, and it is particularly useful for addressing questions in cellular biology. At the core of many cellular applications is the ability to quantitate DNA content precisely and to determine the stages of the cell cycle. The ease of multiplexing additional markers with DNA content measurements allows correlation of drug treatments with their effects on cell cycle and marker expression. In this workshop we will demonstrate the basic concepts of cellular analysis using a study of the C4-2b prostate cancer cell line to explore the role of clathrin in cell cycle progression.

Clathrin is one of the best characterized endocytic coat components, with a fundamental role in vesicle formation and trafficking. During mitosis, clathrin associates with the mitotic spindle, and the depletion of its heavy chain prolongs mitosis and causes a series of mitotic defects, suggesting that clathrin heavy chain (CHC) is required for mitotic progression.

To further explore this in the context of prostate cancer, we reduced the expression of CHC in the prostate cancer cell-line C4-2b using a siRNA oligo knockdown approach. Cells were stained with a DNA marker and antibodies against CHC and phospho-histone H3 (PHH3; mitotic marker) followed by analysis using the iCys[®] Research Imaging Cytometer. In order to determine whether CHC depletion affects the kinetics of recovery from mitotic arrest, we treated control- and CHC-knockdown cells with monastrol (a drug which activates the mitotic checkpoint, thereby arresting cells in mitosis). Cells were scanned and analyzed during mitotic arrest and after recovery.

This workshop will include demonstration (instrument and software), discussion, and hands-on participation (software) to illustrate:

- Detailed sample preparation including plating, fixing, and staining
- Real-time scanning and data acquisition of stained cells
- Segmentation strategies to identify events
- Generating appropriate data analysis plots, gating of sub-populations, and image validation
- Statistical analysis and reports