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Complementarity of Flow Cytometry and Fluorescence Microscopy

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Flow cytometry and fluorescence microscopy both provide single-cell analysis using different but complementary sets of data, essentially population-based target intensities versus target morphology in relatively small sample sizes. Both approaches generally employ optical filters to analyze fluorescence emissions, and have to overcome some of the same physical limitations, including spectral overlap of dyes and the dynamic range limits of measuring systems. Some of the technical challenges differ: dye photostability is more critical to microscopy; creating suspensions from adherent cells can impact flow cytometric analysis. With extensive image acquisition and processing, the microscopist may arrive at quantitative data. However, the cooperative use both flow cytometry and microscopy can provide more robust interrogation of biological phenomena.

A number of examples will be presented to illustrate both the complementarity that microscopy and flow cytometry perspectives bring to new applications and ultimately, biological questions. They also illustrate the constraints to migrating assays between platforms. Viability assays based on membrane integrity or esterase activity generally provide bright, well-resolved signals easily read by microscopy or flow cytometry (Figures 1-2). Microscopy has helped verify mitochondrial specificity and responsiveness with mitochondrial function assays that can be quantified by flow cytometry. However, assays that depend on translocation rather than overall intensity change, such as loss of cytochrome c from mitochondria, are better performed by microscopy. Flow cytometry can readily quantify live cells in various stages of cell cycle using a number of cell-permeant DNA dyes; microscopy confirms nuclear labeling and also indicates that some of these dyes may have adverse morphological affects. Kinetic studies, such as calcium response, can be accomplished on individual cells by microscopy, and easily quantified on populations by flow cytometry. Morphological information, as with organelle structure, is readily available through microscopy, but flow cytometry may provide little useful information about morphology. One example of complementarity between microscopy and flow cytometry is provided by analysis of mitochondrial DNA (mtDNA) depletion. Microscopy has demonstrated the decrease of mtDNA-encoded COXI, including mosaicism, in individual fibroblasts following progressive exposure to anti-retroviral drugs. Flow cytometry has provided rapid population statistics to support the visual results.[1,2]

Fluorescence microscopy is well suited to the resolution of cell and tissue architecture, and to following kinetic and trophic responses in single cells. Flow cytometry rapidly quantifies small differences between cell populations using statistically-significant numbers of events. Flow cytometry can represent a “black box” when looking at the magnitude of a population response; fluorescence microscopy can help verify that measured results represent meaningful biological effects.

References

[1] M.S. Janes et al., *J Histochem Cytochem* 52 (2004) 1011
[2] R.A. Capaldi et al., *Mitochondrion* 4 (2004) 417

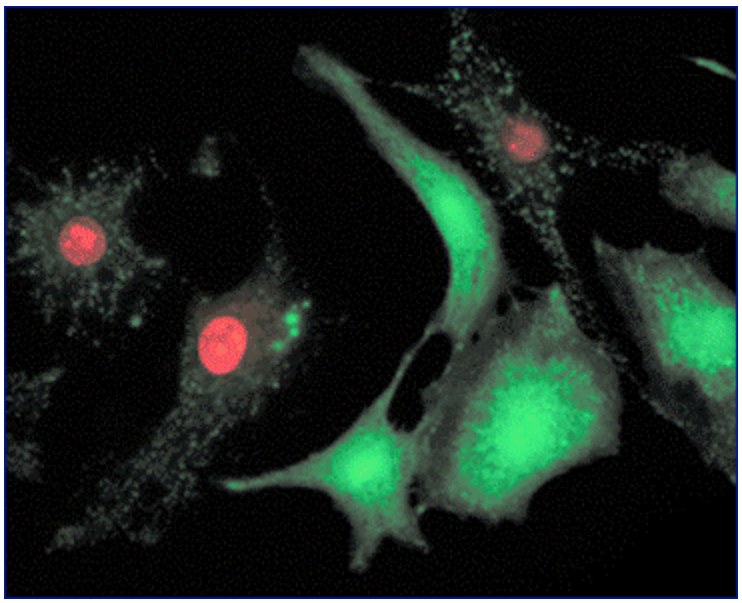

FIG. 1. An older culture of bovine pulmonary aortic endothelial cells was stained with the calcein AM and ethidium homodimer-1: live cells show green calcein fluorescence throughout their cytoplasm; dead cells show red nuclear fluorescence.

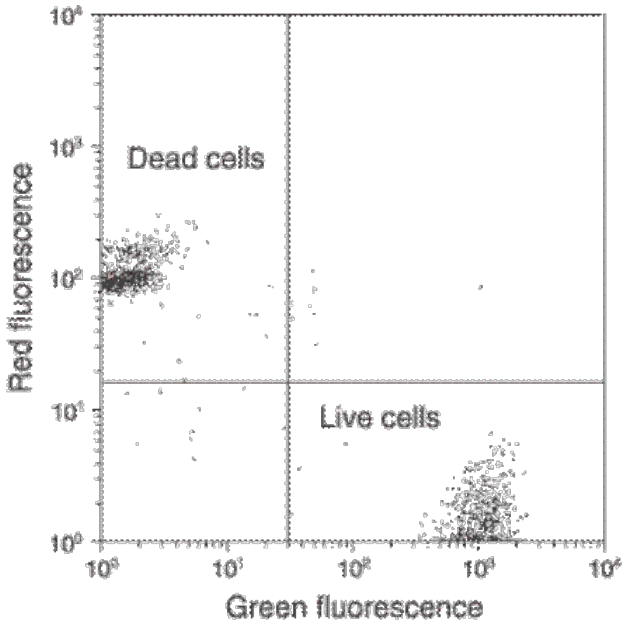

FIG. 2. An equal mixture of live and ethanol-fixed human B cells was stained with calcein AM and ethidium homodimer-1. Flow cytometric analysis was carried out after 5 minutes with 488 nm excitation. The dot plot shows the clear separation of the green-fluorescent (530 nm bandpass) live-cell population from the red-fluorescent (585 nm bandpass) dead-cell population.

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