Humans can rapidly differentiate cells with very subtle differences in their imagery. However, manual classification suffers from a lack of objectivity, repeatability, throughput, and statistical significance, and such per-cell subjectivity makes it difficult to identify changes in large populations of cells using traditional microscopic techniques. Amnis Corporation has developed the ImageStream high speed multispectral imaging flow cytometer that addresses these traditional limitations. This instrument automatically acquires up to six different spatially registered images (brightfield, darkfield, and four fluorescent) per cell in flow at very high rates using a digital CCD camera. The digital imagery obtained is analyzed using the IDEAS statistical image analysis program which provides tools for the objective numerical scoring and discrimination of cells based on the characteristics of their imagery, including size, shape, signal strength, texture, and location/co-localization based metrics. Thus, the ImageStream combines the quantitative power of large sample sizes common to flow cytometry with the high information content,
Spotlight (cont)

-Chondrial permeability transition were included in analyses. Cell vitality is measured by intracellular esterase activity as a recognized parameter of cell death. CV, a 405nm violet laser excitable dye, reports on the esterase activity within living cells, and only viable protoplasts retain CV signal (figure 2).

When protoplasts prepared from edr2 and edr3 mutant plants were used for analysis, the uptake of DiOC7 was consistently lower in the mutant protoplasts than the protoplasts prepared from wild-type Col-0 leaves. Protoplasts prepared from acd2.2 (accelerated cell death 2.2) gave comparable results. These observations would suggest that mitochondria in edr2 and edr3 cells may be predisposed to undergo permeability transition and thus leak apoptotic effectors into the cytoplasm. An alternative explanation would be that edr2 and edr3 plants contain less mitochondria per cell than the wild type plants, thus resulting in lower overall signal from these mutants.

Of particular relevance to the Innes lab research is that some agents known to trigger apoptosis in animal cells also trigger PCD in plant cells. Regulation of PCD via mitochondria may be a central point of integration for various cell death triggers in both plants and animals.

*To learn more about this project, please contact Riyaz Bhat (Innes lab post-doc) - rbhat@indiana.edu
**For more information about the flow cytometry facility and flow cytometry applications contact Christiane Hassel (FCCF manager/operator) - chassel@indiana.edu

Amnis (cont)

Present in microscopic images, enabling objective image-based cytometry for large populations of cells. Here we demonstrate application of ImageStream cytometry to several biological systems, including the analysis of nuclear translocation, internalization and sub-cellular organelle trafficking, localization to the immune synapse, cell cycle, and apoptosis. Data from cell lines as well as rare primary cells will be presented. More information about the ImageStream can be found at www.amnis.com

We will be hosting a presentation by Amnis of the ImageStream Technology at our monthly flow cytometry roundtable - Thursday, April 30, 2009, 10:15am in the Lieber Room (JH123). Please e-mail the facility at chassel@indiana.edu if you there are specific topics in which you are interested in hearing and to schedule individual meeting times with the presenter, Jeff Hudson.

Contact Information

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Facility Hours

Monday - Friday, 9am-5pm: Closed on major holidays; other closings will be announced through the flow cytometry listserv; special hours available upon request and operator availability. Please see the facility calendar for up-to-date schedule availability - http://facs.bio.indiana.edu/calendar.html

Oversight Committee

Roger Innes, Ph.D. (Biology) Kris Klueg, Ph.D. (CGB) Melanie Marketon, Ph.D. (Biology)
Thom Kaufman, Ph.D. (Biology) Rich Hardy, Ph.D. (Biology) Robert "Tank" Eisman, Ph.D (Biology)

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