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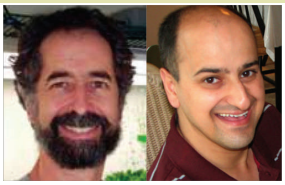
FLOW CYTOMETRY CORE FACILITY (FCCF)

IU Bloomington Flow Cytometry

Research Spotlight - The Innes Lab

By: Riyaz Bhat (Innes Lab); Edited: C. Hassel (FCCF)

Note: A color version of this PDF can be found at http://facs.bio.indiana.edu/newsletter.html



Roger Innes Riyaz Bhat

The Roger Innes lab is investigating the molecular mechanisms that mediate pathogen-triggered programmed cell death (PCD) in plants. They are combining flow cytometric assays with non-invasive confocal laser scanning microscopy (CLSM) to visualize and monitor changes in the mitochondrial membrane potential in intact plant protoplasts. Flow cytometry, being both qualitative and quantitative, allows screening of thousands of protoplasts to average mitochondrial fluorescence intensity.

The aim of one Innes lab project conducted by Riyaz Bhat is to understand the role of three genes, *EDR1, 2,* and *3* (*EDR* – enhanced disease resistance) that will help scientists understand how PCD is regulated at a molecular level during pathogen infection. The specific aims of the project are to assess 1) how *edr* mutations lead to activation of PCD and disease resistance and 2) what role mitochondria play in this process. In order to address these aims the lab has been assessing mitochondrial function in intact protoplasts isolated from wild type and *edr* mutant plants using novel fluorescent dyes that report on the mitochondrial membrane potential under different experimental conditions. These analyses will provide significant new insights into how pathogen-triggered PCD is regulated in plants. It will also shed light on mechanisms of pathogen defense and PCD in humans, as several of the proteins used in this study share significant similarity with human proteins, including some linked to PCD and mitochondrial function in human cells.

For specific mitochondrial staining and to measure mitochondrial membrane potential, a carbocyanine dye, 3,3' diheptyl-oxacarbocyanine iodide, $DiOC_7(3)$, is used. When protoplasts were treated with the commercially available protonophore uncoupling agent carbonyl cyanide m-chlorophenylhydrazone (50µM CCCP) in order to disrupt mitochondrial membrane potential, there was complete loss of fluorescence in protoplasts stained with 100 nM $DiOC_7(3)$. 30 – 50 µM CCCP leads to total loss of mitochondrial membrane potential in wild-type protoplasts (Fig. 1).

In further flow cytometry experiments, Calcein Violet (CV) was used to measure cell vitality. Since a change in mitochondrial membrane potential precedes other hallmarks of PCD, only protoplasts that are alive and undergoing mito-

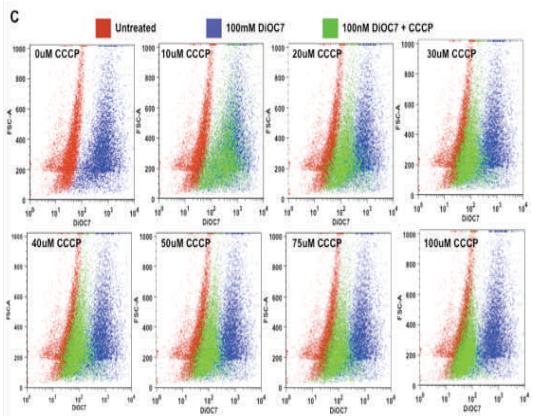


Figure 1. Flow cytometric analysis of DiOC7 stained protoplasts pretreated for 1 hr with different concentrations of CCCP.

Technology Spotlight - The ImageStream: *A Union of Flow Cytometry and Imaging*

By: Jeff Hudson (The Amnis Corporation)

Please note: we will be hosting a presentation by Amnis at our monthly flow cytometry roundtable on April 30; details on page 2

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

Image: http://www.amnis.com/

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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.

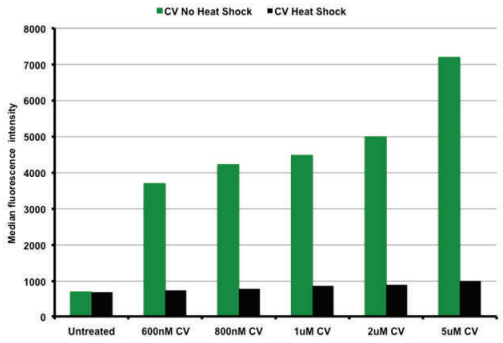

Figure 2. CV as a viability marker for plant protoplasts. Protoplasts were isolated from wild-type Arabidopsis Col-0 leaves. The CV signals were quantified as median fluorescence intensities. Note the loss of CV signal in heat shocked protoplasts

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

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