Vol. 1 Issue 3

FLOW CYTOMETRY CORE FACILITY (FCCF)

IU Bloomington Flow Cytometry

Teaming up with IU Bloomington's Light Microscopy Imaging Center


Jim Powers

Microscope facilities are an integral part of science research, allowing researchers to identify minute details of organisms down to the cellular level, either by light or fluorescence microscopy. Previously part of the Multidisciplinary Microscopy Facility, Indiana University has established a Light Microscopy Imaging Center, located in Myers Hall 059. The imaging center is complementary to the flow cytometry facility, and vice versa.

The IU Bloomington Light Microscopy Imaging Center, managed by Jim Powers, contains several light and fluorescence microscopes. Microscopes include the following: a Leica Scanning Confocol, Spinning Disk Confocal, BioRad Scanning Confocal, Deltavision, and Nikon E800. Also part of the center, but not currently located in MY 059, are a Veritas Microdissection System (MY040) and Zeiss Axioplan (JH347). The center also offers Image Processing and analysis workstations

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New at the FCCF: LSRII moves; Z2 Coulter Counter & FACSAria II upgrades arrive

On July 8, 2008, the LSRII flow cytometer analyzer moved to its permanent location in the new BSL3 facility. While the LSRII is no longer available for general use, we have available for use the FACSAria sorter/analyzer as well as the FACSCalibur analyzer. The Aria is a technician-only instrument, however, training is provided for anyone who would like to self-run the Calibur analyzer. More information about each instrument can be found at http://facs.bio.indiana.edu/

We are pleased to announce that the facility has received FRSP funding, for which we applied earlier this year. With this funding the facility has purchased a Z2 Coulter Counter, as well as an upgrade for the current FACSAria. The Z2 instrument is a cell and particle counter that reports both cell counts and concentration of a cell population, as well as size distribution of that cell population. More information about the Z2 Coulter Counter can be found at http://www.beckman.com/products/instrument/partChar/pc_z2.asp


http://www.beckmancoulter.com/products/instrument/partChar/pc_z2.asp

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Spotlight - Fluorochromes for Flow and Imaging Cytometry

There are many fluorescent dyes, proteins and antibodies available to the research world. Many of these can be readily used for both flow and imaging cytometry assays. While the complete list of fluorophores is extensive, some of the more commonly used fluorophores include DAPI, GFP, FITC, R-PE, and APC, just to name a few. Experiments can be developed for both quantitative and qualitative analysis, especially with those resources we have available here at IU Bloomington. We have the ability to quantify, by fluorescence intensity, the average amount of fluorophore that a cell with a certain marker or DNA contains. From there, with the Aria cells sorter, cells, or in the case of the COPAS instrument, embryos and other large particles, can be sorted and recovered for culturing and further analysis, or for extraction purposes. The facility has the ability for slide based sorting, which then allows a user to take the cells or organisms of interest, and check for localized fluorescence.

A newer fluorescent technology aiding both flow cytometry and fluorescent microscopy are Quantum dots. Quantum dots are nanocrystals with a core of semiconductor material and are approximately 10-20nm in size. They are highly photostable and emit across the spectrum of light. Their excitation and emission spectrum separation is maximized compared to common fluorophores, decreasing spillover into other fluorescence channel. Quantum dots can all be excited by one excitation source, typically a 405nm Violet laser. With the correct emission filters, they can be utilized in a variety of assays either, complimenting commonly used fluorescent tags.

For more information about quantum dots, and specificially Qdots® by Invitrogen, see http://www.invitrogen.com/site/us/en/home/brands/Product-Brand/Qdot.reg.us.html

Fluorescence Microscopy (cont)

and software, a wet bench for sample preparation, and has 24 hour access available for trained researchers.

Researchers can receive qualitative data analysis of their cells through imaging, and high throughput quantitative data through flow cytometry. Researchers can also sort cells onto slides using flow cytometry instrumentation at the Flow Facility, and then take these slides over to the Imaging Center for analysis. For example, for researchers sorting mixed population GFP positive and negative cells, GFP positive-only cells can be isolated by flow sorting, then GFP localization analyzed by imaging.

To learn more about the LMIC and its services, see http://www.indiana.edu/~lmic/ or contact Jim Powers at japowers@indiana.edu, 856-1734.

New at the FCCF (cont)

The Aria upgrade is an equipment and software upgrade that will update our current Aria to the more advanced Aria II system. Included in the upgrade is a complete fluidics system replacement which will allow for an even more aseptic sort environment, and also includes a nozzle upgrade - 70, 85, 100 and 130um nozzle sizes - that will allow us to sort cells of a larger size than we are currently able to sort. More information about the FACSAria II can be found at http://www.bdbiosciences.com/facsaria_ii/

www.bdbiosciences.com

The Z2 Coulter counter has arrived and was recently installed. It will be available for use in the near future. The Aria II upgrade will occur in segments, in late-August or early September. The facility will send progress reports for each.

Upcoming events:

Wednesday August 27, 2008; 5pm, room JH009 - Flow Cytometry and Imaging Seminar, Graduate student orientation week
- Jim Powers (Light Microscopy Imaging Center) and Christiane Hassel (Flow Cytometry Core Facility)
- informal session open to all **new incoming Biology graduate students**

September 19-21, 2008 - Great Lakes International Imaging and Flow Cytometry Association (GLIIFCA) 17
- Midwest flow and imaging cytometry meeting in Milwaukee, WI - more information at www.gliifca.org

Beginning in September we will be offering a collaborative Flow Cytometry and Imaging roundtable open to all researchers at IU Bloomington - dates and times TBA

Oversight Committee

Roger Innes, Ph.D. (Biology)
Kris Klueg, Ph.D. (CGB)
Melanie Marketon, Ph.D. (Biology)
Thom Kaufman, Ph.D. (Biology)
Robert "Tank" Eisman, Ph.D (Biology)
Rich Hardy, Ph.D. (Biology)
Ken Nephew, Ph.D (Med Sci)
Curt Balch, Ph.D. (Med Sci)
Christiane Hassel, B.S. (Biology)
Kah Tan-Allen, Ph.D. (Optometry)

Contact Information

For more information about the facility contact:

Roger Innes - innes@indiana.edu
Kris Klueg - kklueg@cgb.indiana.edu
Christiane Hassel (manager/operator) - chassel@indiana.edu

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

FLOW CYTOMETRY
CORE FACILITY (FCCF)

1001 E 3RD STREET
JORDAN HALL 029
DEPARTMENT OF BIOLOGY

P.812-855-7101
F. 812-855-6705
http://facs.bio.indiana.edu/