

IU Bloomington Flow Cytometry

Instrument Overview - BD FACSAria

The BD FACSAria is a high-speed cell sorter with the capability of sorting up to 70,000 events per second. It has four lasers and a forward scatter (FSC) PMT for detecting light scatter and fluorescent signals. The laser excitation sources include a Blue 488nm, Red 633nm, Violet 405nm and UV 355nm source. In addition to normal light scatter profiles, the added FSC-PMT allows smaller cells or organisms to be detected more accurately, and to be differentiated from debris and instrument noise.

The Aria can sort cells or single-celled organisms from approximately 0.2-30 μ m in diameter. The Aria has the ability to perform up to 4-way sorting; sending up to four populations of interest into their own collection device. For example, in doing a two color experiment, a researcher may choose to sort two single-positive populations, a double negative population, as well as a double positive population. All of these populations may be sorted at one time.

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Flow Cytometry Research at IU Bloomington - Diversity

Research taking place in the IU Bloomington flow facility since its establishment has been extremely diverse. The facility serves the entire university, aiding in the research of members of the Biology, Bio-complexity (Physics), Center for Genomics and Bioinformatics, Chemistry, Medical Sciences and Optometry departments. Past and present research has included analysis of human, mouse, hamster, plant, bacteria, *Drosophila*, snail, bovine, and more. Analyses that have been performed on the flow cytometry instrumentation include cell cycle analysis, GFP detection, apoptosis and mitochondrial membrane potential studies, cell proliferation, antibody staining, and more. Future research includes lymphocyte cell subset detection in mouse cells, virus sorting, *Drosophila* embryo sorting, and other fluorescence detection assays.

The facility is more than happy to demonstrate sorting and analysis capabilities. For a tour of the facility, to speak with facility personnel about your research interests, and/or to set up a demonstration of one or more of our instruments, please call, e-mail or stop by the facility in Jordan Hall 029.

Product Spotlight - AmpliGrid by Advalytix (text and images courtesy of Advalytix)

Molecular analysis of FACS sorted single cells made easy with the Advalytix AmpliGrid platform

Advalytix' single cell molecular analysis platform consists of the FACS-compatible AmpliGrid slide and AmpliSpeed slide cyler. The platform enables highly sensitive amplification of DNA, mRNA, or mtDNA from single cells in a simple workflow. Cells are deposited onto the AmpliGrid slide via FACS (or alternatively via laser capture microscopy or micromanipulator). A custom slide adaptor for the BD FACSAria and quick start guides for BD's FACSAria and Vantage, and Beckman Coulter's MoFlo enable highly precise sorts (placement accuracy > 95%) and quick implementation. Simple visual inspection via fluorescent microscope verifies correct placement of single cells after sorting - no more "black box" single cell sorts into MTPS (8-methoxypyrene-1,3,6-trisulfonic acid, trisodium salt).

After adding a 1 microliter assay - either manually with a pipette or with an available automated liquid handler solution - AmpliGrid slides are thermocycled on the world's smallest thermocycler - the AmpliSpeed. Post PCR, amplicons are retrieved from the slide and analyzed via gel, capillary electrophoresis or sequencing. Popular applications include immunology, infectious disease, oncology, and stem cells where single cell molecular analysis drives the next level of biological insight.

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Instrument Overview - BD FACSAria (cont)

An automated cell deposition unit (ACDU) allows researchers to perform single- or multi-cell sorting of populations onto slides or multi-well plates. This is especially useful for researchers doing cloning experiments, or who will be doing further analysis by fluorescence microscopy.

In addition to its cell sorting abilities, the Aria is also equipped with an aerosol containment system for anyone working with BSL2 agents. This system increases the safety of both technicians and researchers in the facility, and minimizes the chances for cross-contamination of other samples due to aerosol release. For anyone needing a temperature controlled environment for cell collection, a re-circulating water bath is an additional feature of the Aria. It comes with attachments for both tube holders and the ACDU, therefore keeping slides, multi-well plates, and tubes at a controlled temperature while sorting.

The facility has recently applied for FRSP funding to upgrade our current Aria to the Aria II. If this Aria II upgrade comes to fruition, the sorter will have even more capabilities, including the ability to sort larger cells sizes (up to approximately 40µm in diameter) and improved aerosol containment function. This will allow the facility to stay on the cutting edge of analysis and sorting technologies.

*For more information about the Aria or other facility instrumentation, please contact the flow facility.

Product Spotlight: Advalytix (cont)

In addition, Advalytix just introduced the CellEvator, a sample mixer for BD's FACS ARIA. It uses surface acoustic waves (SAW) to mix the sample in the Aria's intake tube to prevent clogging during long sorts.

- Please visit the Advalytix website (www.advalytix.com)

or contact Grant Hill (grant.hill@olympus.com) for more information.

**The AmpliGrid technology is not currently available on the IU Bloomington campus, however, we will be hosting a demo in May 2008 (see "Upcoming Events" for more information) to ascertain interest in this technology. We are looking for a volunteer for the AmpliGrid demo, especially anyone looking at gene expression in single cells. Please contact the facility if you are interested.



Upcoming events:

Wednesday May 28, 2008 - Advalytix demo - AmpliGrid single cell RT-PCR; starts at 9am in the flow facility, JH029
- more info about the AmpliGrid system at www.advalytix.com - **Please RSVP to chassel@indiana.edu**

Wednesday May 28th, 2008 - Indianapolis Flow Users Group meeting at **IU Bloomington** - JH009
- 5:30-6pm, light dinner; 6-7pm; presentation by Natasha Crosby (Daleke lab) - **Please RSVP to chassel@indiana.edu**

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. Candidate (Optometry)

Contact Information

For more information about the facility contact:

Roger Innes - rinnes@indiana.edu

Kris Klueg - kklueg@cgb.indiana.edu

Christiane Hassel (manager/operator) - chassel@indiana.edu

Facility Hours

Monday - Friday, 9a-5p: Closed on major holidays; other closings will be announced through the flow cytometry listserv; special hours available upon request and operator availability

- A facility calendar is available on our website

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