Instrumentation

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Parameters</th>
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<tr>
<td>BDFACS Aria II</td>
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The UWCCC Flow Lab and the UW Biosafety Office have updated institutional guidelines for live cell sorting and flow cytometry to reflect recent changes in NIH/ISAC recommendations. See page 3 for details.

We have opened business on the new ARIA III cell sorter. This sorter is placed in a Level II biosafety cabinet providing sterile conditions for sorts and BSL2 containment for human samples. All human samples and virally transfected cells are sorted on this instrument.

FlowJo Site License

The University of Wisconsin has acquired an institutional, shared-cost software license. Register any computer on- or off-campus for a $240 annual fee. Turn in a dongle for a credit for 4 years of use. The license ensures immediate access to any new versions of the software on any Mac, PC or Linux system.
NIH/NIAID has constructed a website where users can import and deposit their raw flow files. The site offers other analysis tools as well for both flow cytometry (FLOCK) and genetic analysis (ie. MHC Analysis, tagSNP). There is a tentative plan that all journals will require deposition of raw flow files so that other investigators can perform secondary analyses after publication.

http://immport.niaid.nih.gov

**Flow Cytometry Clustering without K**

- computationally determines the number of unique populations in high dimensional flow data using a rapid binning approach
- maps populations across independent samples
- calculates summary statistics
- find the most informative parameters
- reduces subjective factors in manual gating
- can handle non-spherical hyper shapes

http://exon.niaid.nih.gov/spice
Biosafety Guidelines for Live Cell Sorting

Institutional protocols updated reflecting recent changes in NIH/ISAAC guidelines

What has not changed:

• all live cell sorting experiments have to be included in the biological safety protocol and pre-approved by the IBC prior to appointment requests

• this is a longstanding institutional regulation and is now strictly enforced to ensure safety of both core users and staff at our high-traffic BSL2 facility

What has changed:

Instrument assignment / containment:
• all live cells of human origin must be sorted in a biological safety cabinet (BSC)
• most BSL-2 samples will be sorted in the BSC including any virally transfected samples

Pre-screening requirements:
• all primary human samples including embryonic stem cell lines must be screened for infectious diseases to prevent aerosolization of BSL-3 agents (ie. HIV-1,2, HepB, HepC, HTLV-1,2)
• all virally transfected samples and infected material must be pre-tested to minimize risk of transmission

Improved communication:
• researcher is responsible for providing detailed information on any live samples delivered to the facility to enable proper risk-assessment and to ensure assignment of appropriate staff and containment equipment

Why Biosafety is so important in live cell sorting?
Live cell sorting involves the purposeful aerosolization of cell suspensions. The instrument generates a stream of single cell droplets in order to deflect single particles. The single-droplet stream typically contains ‘satellite’ droplets, which fall in the size experimentally proven to be the most successful in aerosol transmission of infectious particles to the lower airways. It is also not uncommon for aerosol incidents to occur during regular sorter operation as a result of clogging, air introduction and stream instability. Live cell sorting therefore is a serious health risk to the operator.

The availability of novel reagents to achieve efficient genetic engineering have greatly increased the number of sort samples associated with agents considered BSL2+ when in aerosol (lentiviral systems, CMV, EBV, HTLV, etc.). Stem cell technologies have introduced an increasing number of samples expressing oncogens and tumor-suppressor knock-down sequences. Human clinical trials in general are sampling a population typically at increased risk of contracting or originally carrying infectious diseases (patients affected by cancer, immunosuppression, etc.). For all these reasons, biosafety is one of the most important components for live cell sorting.
Editor: Erika Héninger, eheninger@wisc.edu

Core Services

Data Analysis & Consultation
Need help with data analysis or guidance to navigate the myriad of new options in flow cytometry & imaging? Schedule a consultation and we will be happy to assist you with both experimental design and interpretation of data. We also offer assistance with preparation of publication-ready figures and other flow cytometry data presentations.

Assay Services
Our facility offers standardized assay services including everything from sample processing to data interpretation. Besides the assured Quality Control and high-throughput options, this service has been providing great value to laboratories without equipment or trained staff to perform flow cytometry & imaging. Contact us to see what we can offer to develop customized protocols for your experimental models.

Training & Education
For new users we offer a monthly lecture series titled “Introduction into Flow Cytometry”. We provide one-on-one, hands-on training sessions on all of the instruments. Assisted services are also available for inexperienced users and experienced users with unfamiliar assays, on schedule or upon request. Training sessions are customized to our users' individual experimental needs.

“Introduction to Flow Cytometry”
Lecture Series
Spring/Summer schedule
May 20, 22, 24
June 24, 26, 28
July 22, 24, 26