Flow Cytometry Orientation

http://web.mit.edu/flowcytometry/www/

Glenn Paradis
Director of the Koch Institute Flow Cytometry Core Facility
Sorting Facility    76-279
Analyzer Facility    76-273
Spectra of Fluorophores
Quartz Cuvette-1
Quartz Cuvette-2

Waste/bleach

635 nm laser

488 nm laser

Green Ready/Run light = tube is pressurized

Air pressure in tube
Hi = 60ul/min.
Med = 35ul/min.
Lo = 12ul/min.

H2O

Laminar flow
Do not run test tubes dry!!

Air disrupts laminar flow

H2O Laminar flow

Air pressure in tube
Hi = 60ul/min.
Med = 35ul/min.
Lo = 12ul/min.

635 nm laser
488 nm laser

Waste/bleach
Monitor the Ready/Run Light

Waste/bleach

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H2O

Waste/bleach

635 nm laser

488 nm laser

Glenn Paradis KI Flow Cytometry Core
Facility at MIT 2012
Pulse Height

- Signal Strength (channel)
  - Linear: 256
  - Log: 260,000
- Time (microseconds)
  - 500
- Height

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Pulse Area

Signal Strength (channel)

Area

Time

500
Pulse Width

Signal Strength (channel) vs. Time

Width
Your Cell is represented by a tick mark

FL1-H, FL2-H subset
MLN stain 3
Event Count: 1

Data from one cell

Channel 500

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Facility at MIT 2012
Data Presentation Formats

Contour Plot

Density Plot

Histogram Plot

Dot Plot

Glenn Paradis KI Flow Cytometry Core Facility at MIT 2012
## Detector Measurements
### Scatter Parameters

<table>
<thead>
<tr>
<th>Detector</th>
<th>Wavelength</th>
<th>Measurement</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSC-Forward Scatter</td>
<td>488nm</td>
<td>Refraction/Diffraction</td>
<td>not size</td>
</tr>
<tr>
<td>SSC-Side Scatter</td>
<td>488nm</td>
<td>Reflection @ 90° angle</td>
<td>internal complexity</td>
</tr>
</tbody>
</table>

![Cell Cluster Diagram]

- **Granulocytes**
- **Monocytes**
- **Lymphocytes**
## Detector Measurements

### Scatter Parameters

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![Image of scatter parameters](image)

- **Granulocytes**
- **Monocytes**
- **Lymphocytes**

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How Are FSC Measurements Made?

Air pressure in tube
Hi = 60ul/min.
Med = 45ul/min.
Lo = 30 ul/min.

Glen Paradis KI Flow Cytometry Core Facility at MIT 2012
How Are FSC Measurements Made?

- 488 nm laser
- FSC photodiode
- Waste/bleach
- Air pressure in tube:
  - Hi = 60 ul/min.
  - Med = 45 ul/min.
  - Lo = 30 ul/min.

Glenn Paradis KI Flow Cytometry Core Facility at MIT 2012
Detector Arrays

Trigon and octagon detector arrays
Detector Configurations

<table>
<thead>
<tr>
<th>LSR Fortessa</th>
<th>355</th>
<th>405</th>
<th>488</th>
<th>561</th>
<th>640</th>
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<tbody>
<tr>
<td></td>
<td>379/28</td>
<td>450/50</td>
<td>530/30</td>
<td>610/20</td>
<td>670/30</td>
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<td></td>
<td>BUV 396</td>
<td>BV 421, Pacific Blue, DAPI</td>
<td>FITC, GFP, Alexa 488</td>
<td>PE, dTomato</td>
<td>APC</td>
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<td>BUV 737</td>
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<td>PE-Texas Red, mCherry</td>
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<td></td>
<td></td>
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<td>PE-Cy5.5</td>
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<td></td>
<td></td>
<td>BV 711</td>
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<td>PE-Cy5.5</td>
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<td>BV 786</td>
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<td>PE-Cy7</td>
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<td></td>
<td>BUV 737</td>
<td>BV 605</td>
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Optical Layout

Air-cooled, compact, long-life laser

Sheath and waste containers with level sensors

Beam shaping prismatic expander for high intensity illumination

Flowcell optically coupled to condenser lens for high collection efficiency

Three high performance photomultipliers and filter sets for multicolor fluorescence analysis

Sample and sheath pressure pump and regulators maintain steady flow

Low-angle forward scatter detection for small particle resolution

High efficiency Brewster beam splitter for side scatter detection

The droplet containment system prevents droplet formation, and controls aerosols and splashes between samples
Data Management

• Store data only in the currently monthly folder.
• Back up your data to your personal Dropbox account.
• I will delete old data from the local HD with no warnings when hard drive fills up.
Flow Cytometry Core Facility Analyzer Policy

1. **Appointment wait period:** If wait periods for any instrument become greater than two weeks, labs with KI or Whitehead Institute affiliation or with NCI funding will be given preference for booking appointments. Any lab without such affiliation/funding may only book appointments within two weeks from the day of booking.

2. **Schedule changes:**
   a. Cancellations must be made with 24 hours advance notice; otherwise the entire time scheduled will be billed.
   b. You are billed on the greater of the time you reserve or the time you use on the flow cytometer. Instrument use time is calculated from the beginning of your scheduled time to your log out time.
   c. We reserve the right to restrict your access to the facility in the event of frequent last minute cancellations, late arrivals or not showing up for your appointments at all.

3. **Rate changes:** Periodically check our web page for updates on the rates charged for our services. Our web site rates will be updated immediately if there is a change.

4. **Overbooking:** No one lab may book more than 50% of the weekday hours between 10am-6pm in any given week on a particular instrument.

5. **Instrument malfunction:** We may have to cancel your appointment if the flow cytometer breaks down. Make sure to get trained on a backup analyzer.

6. **Fire alarms:** The analyzer rooms and building must be evacuated in the event of a fire alarm. There are no exceptions to this MIT policy. Delays caused by fire alarms will reduce the length of your appointment.

7. **Computer management**
   a. Data backups are the investigator’s responsibility.
   b. Data may be deleted at anytime.
   c. There is no web site browsing/reading emails or any other internet activity on our data collection computers. Bring a laptop if you must.

8. **Restricted access to the facility will be enforced if** any 3 combinations of the following activities occur within 1 year. This means we will log you in and out and you will lose 24/7 facility access.
   a. Training fellow investigators on how to use our equipment. **Training must be done by our staff.**
   b. Sharing your computer account password. Neither you nor your fellow investigator will have access to the facility.
   c. You must clean the instrument with 5 minutes 10% bleach, followed by 5 minutes DI H₂O.
   d. You must put the cytometer in Standby mode.
   e. You must turn off the instrument after 5pm M-F and always on weekends + holidays.
FACS Diva Training Video

http://web.mit.edu/ist-train/Koch/story.html

FACS Canto II HTS-1

FACS LSR II HTS-1

FACS LSR II HTS-2

FACS Fortessa HTS-1
Book Up Cytometer and Staff Using iLab

https://mit.ilabsolutions.com/account/login

**Flow Cytometer Names**
- FACS Calibur-1
- FACS Calibur HTS-2
- FACS Canto II HTS-1
- FACS LSR II HTS-1
- FACS LSR II HTS-2
- FACS LSR Fortessa HTS-1

**Facility Staff**
- FACS Training-Help Analyzers
  - Mike Monday and Thursday
  - Michele Tuesday
  - Glenn Wednesday and Friday