Flow Cytometry Orientation

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

Glenn Paradis
Sorting Facility 76-279
Analyzer Facility 76-273

Know the Excitation and Emission Spectra of Your Fluorophores

Quartz Cuvette

Do Not Run Your Test Tube Dry

Do not run your test tube dry. Air disrupts laminar flow.

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

Waste/bleach
H₂O
Laminar flow

488 nm laser

Absorption Spectra

Emission Spectra

488 nm laser
H₂O Tries To Leave Cuvette By Way Of The Sample Injection Port

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

Green Ready/Run light = tube is pressurized

Make Sure Sample Test Tube Has Pressure In It Or Else Sample Tube Will Fill With H₂O

Make Sure Sample Test Tube Has Pressure In It Or Else Sample Tube Will Fill With H₂O

Pulse Height

Pulse Area
**Data Presentation Formats**

- Contour Plot
- Density Plot
- Histogram Plot
- Dot Plot

**Autofluorescence**

- Negative control
- Density Plot
- Mixture Histogram
- Mixture Dot Plot

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**Pulse Width**

- Log vs. Linear
- Signal vs. Time
- Width vs. Time

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**Your Pulse is represented by a tick mark**

- Time
- Width

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**Event Count**: 1

- FL1-H: FL2-H subset
- MLN stain
- # Cells: 10,000
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>488 nm</td>
<td>Refraction/Diffraction</td>
<td>not size</td>
</tr>
<tr>
<td>SSC-Side Scatter</td>
<td>488 nm</td>
<td>Reflection @ 90° angle</td>
<td>Internal Complexity</td>
</tr>
</tbody>
</table>

How Are FSC Measurements Made?

Green Ready/Run = tube is pressurized

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

#### Fluorescent Parameters

<table>
<thead>
<tr>
<th>Detector</th>
<th>Wavelength</th>
<th>Color</th>
<th>Fluorophore</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>530/30 nm</td>
<td>green</td>
<td>FITC, DP, Alexa 488</td>
</tr>
<tr>
<td>F2</td>
<td>585/42 nm</td>
<td>yellow</td>
<td>PE</td>
</tr>
<tr>
<td>F3</td>
<td>&gt;650 nm</td>
<td>red</td>
<td>PI, TandemR, Cy5, 5</td>
</tr>
<tr>
<td>F4</td>
<td>660/20 nm</td>
<td>red</td>
<td>APC, Cy5</td>
</tr>
</tbody>
</table>

### FACScan Optical Layout

### Data Management

- Store data only in the currently monthly folder.
- Back up your data. Use USB Memory/flash drives on Macs only. We also have a server.
- I will delete old data with no warnings when hard drive fills up.
Flow Cytometry 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 on TechTime with 24 hours advance notice, otherwise the entire time scheduled will be billed. For Monday cancellations, you must delete your TechTime appointment before 10AM.
   b. You are billed on the greater of the time you reserve in TechTime or the time you use on the flow cytometer.
   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.

3. **Rate changes**: Periodically check our web page for updates on the rates charged for our services.

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

5. **Instrument malfunction**: We may have to cancel your appointment if the flow cytometer breaks down.

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

7. **Restricted access to the facility** will be enforced if any 3 combinations of the following actions occur within 1 year.
   a. Training fellow investigators on how to use our equipment. Training must be done by our staff.
   b. Sharing your username and password. Neither you nor your fellow investigator will have access to the facility.
   c. Not following the shutdown procedure to completion (i.e. not leaving the cytometer in Standby mode or leaving the cytometer on all night).
   d. Throwing bio samples in the regular trash. We have a carry in carry out policy.

8. Users are responsible for providing an account number and updating it when it expires.

User Signature________________________________________  Date____________________

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**Flow Cytometer Names**
- FACScan Left
- FACScan Right
- FACScan Calibur Right
- FACScan LSR II HTS-1
- FACScan LSR II HTS-2
- FACScan LSR Fortessa-1
- FACScan Canto

**Facility Staff**
- FACS Training-Help
- Glenn Paradis -> Wednesday + Friday
- Michael Jennings -> Monday + Thursday
- Xindi Song -> Tuesday

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**Booking Up Cytometers and Staff Using Tech Time**

[http://calendar.mit.edu](http://calendar.mit.edu)