KOCH INSTITUTE
for Integrative Cancer Research at MIT

Flow Cytometry Orientation

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

Glenn Paradis
Director of the Koch Institute Flow Cytometry Core Facility

Sorting Facility 76-279

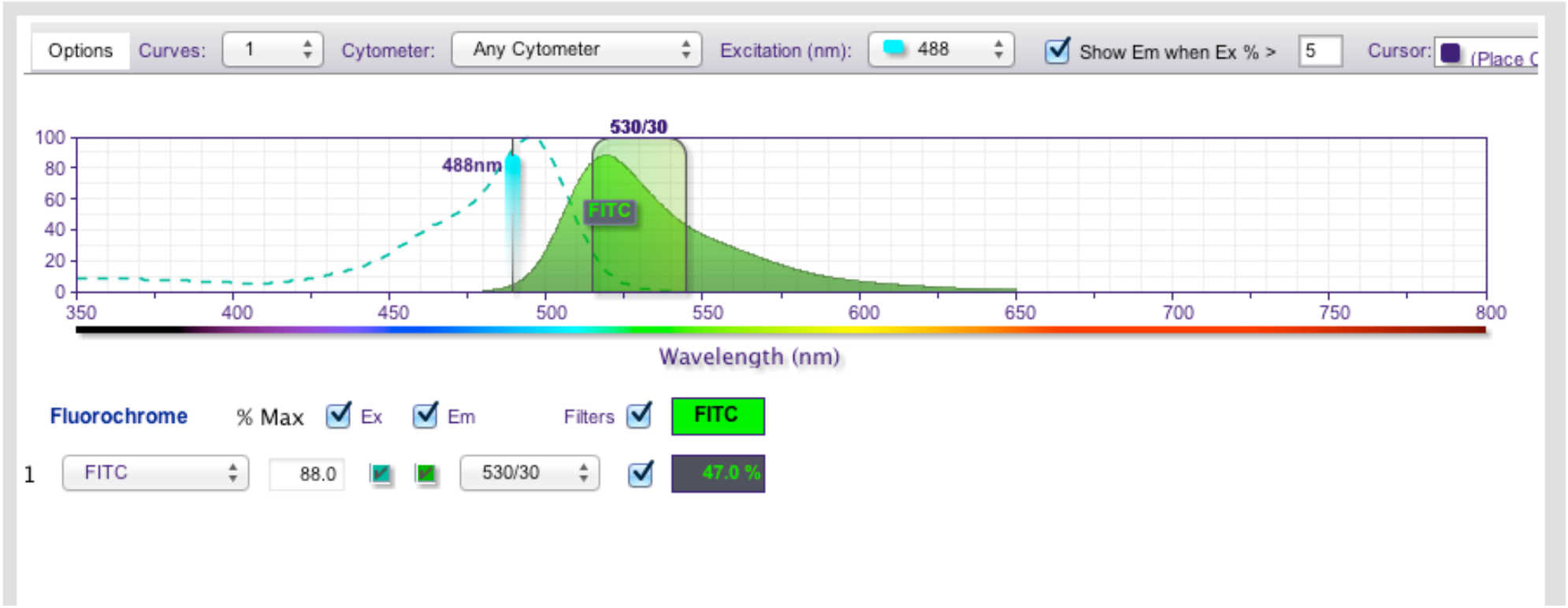
Analyzer Facility 76-273

Spectra of Fluorophores

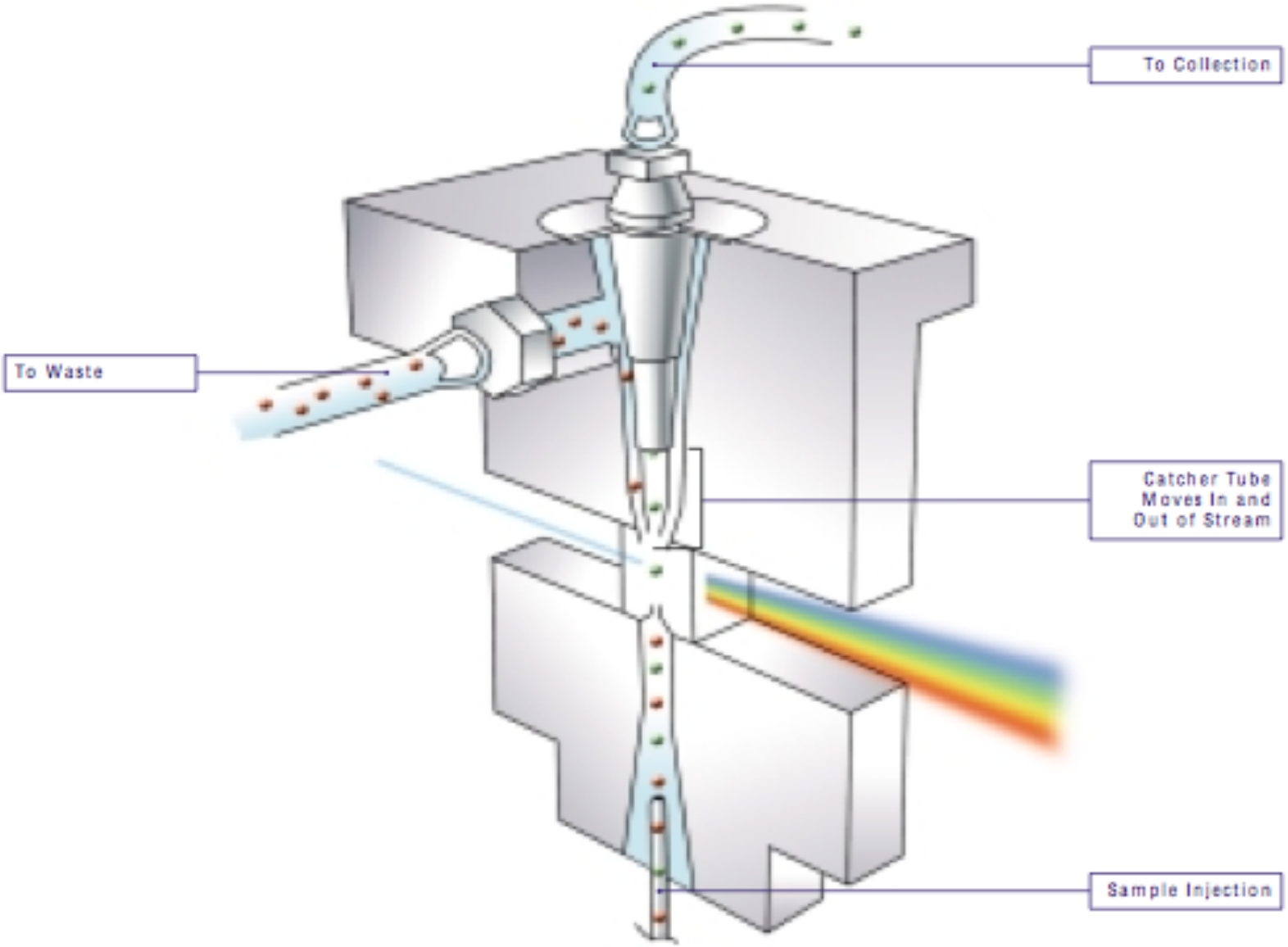
BD FLUORESCENCE SPECTRUM VIEWER A MULTICOLOR TOOL

Notice: A recent Java® security update has disabled the spectrum viewer for some users.

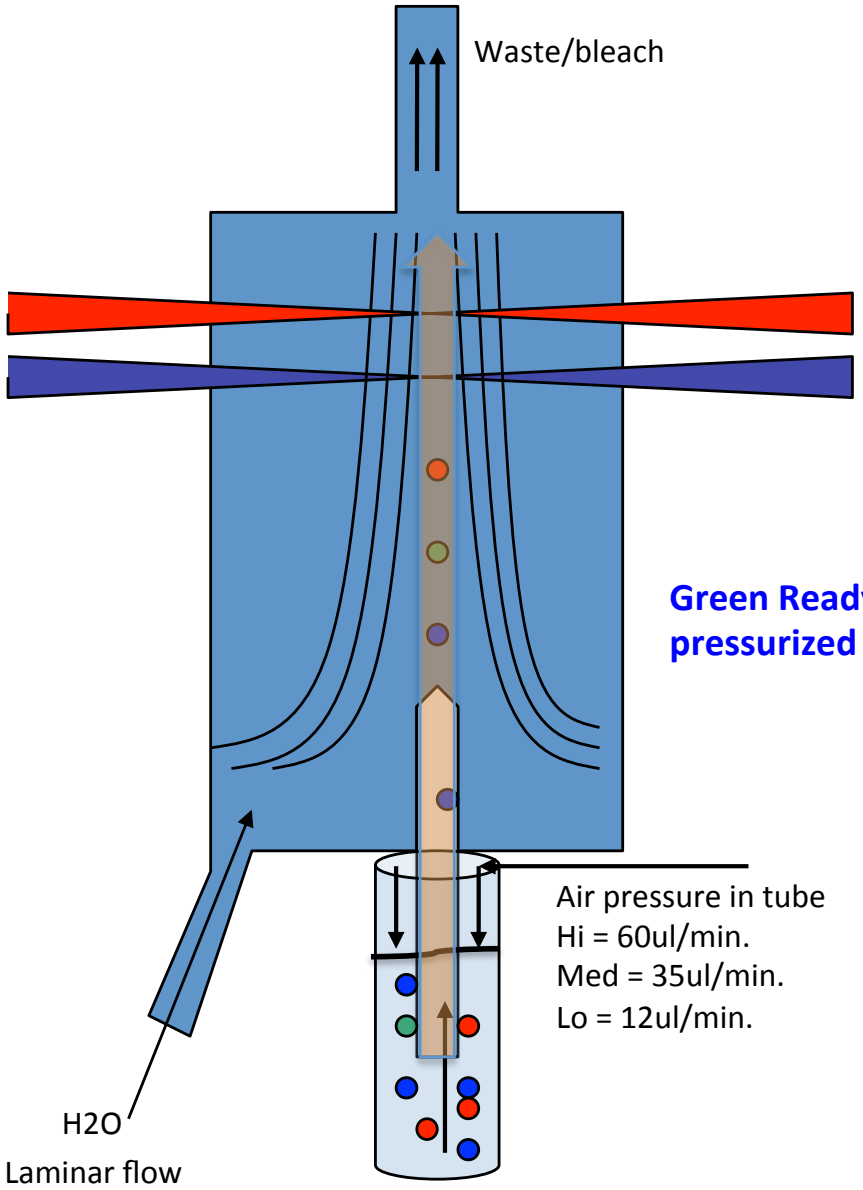
Please check this document for troubleshooting tips. If you are still unable to use the program, please contact us.



Quartz Cuvette-1

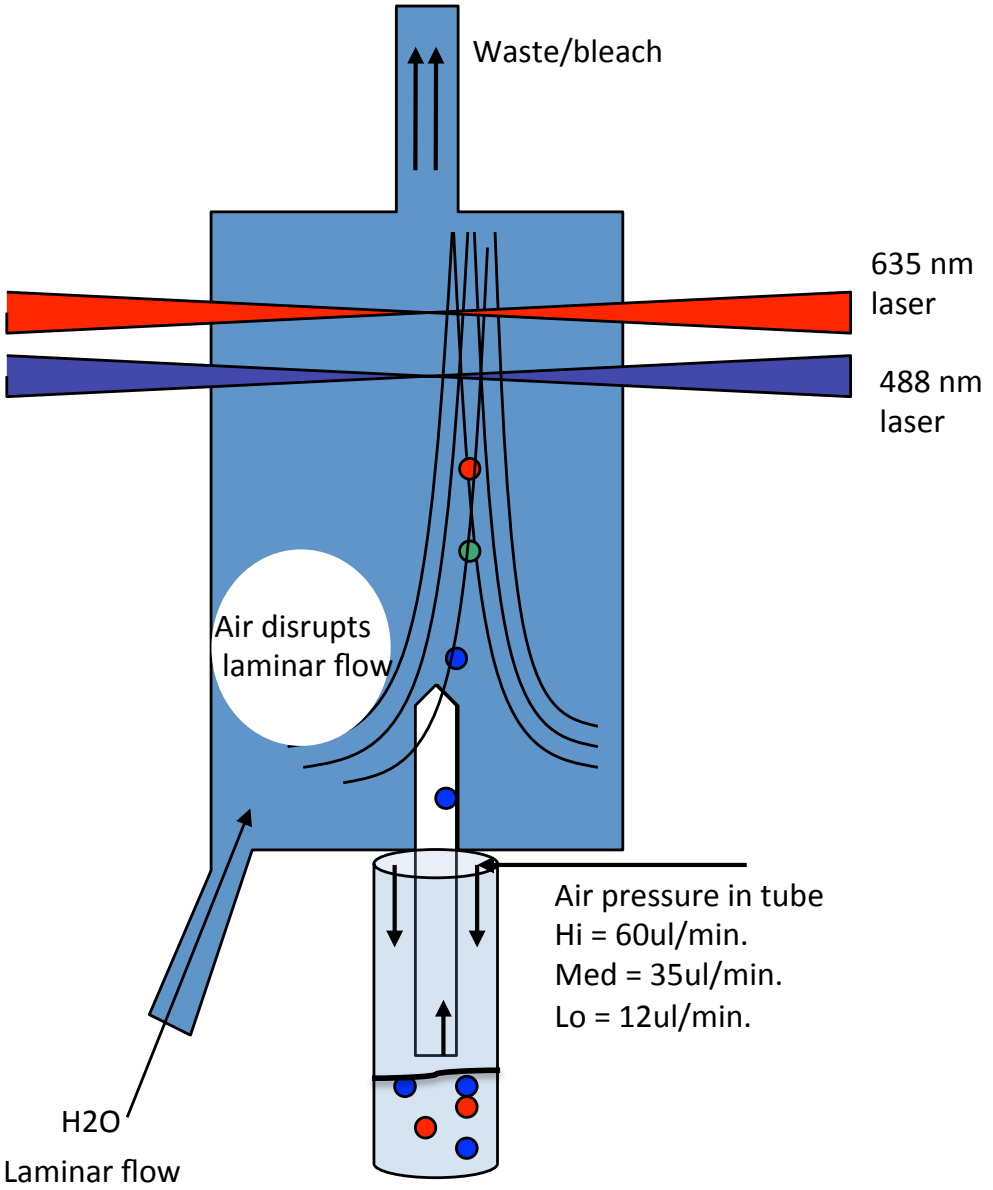


Quartz Cuvette-2

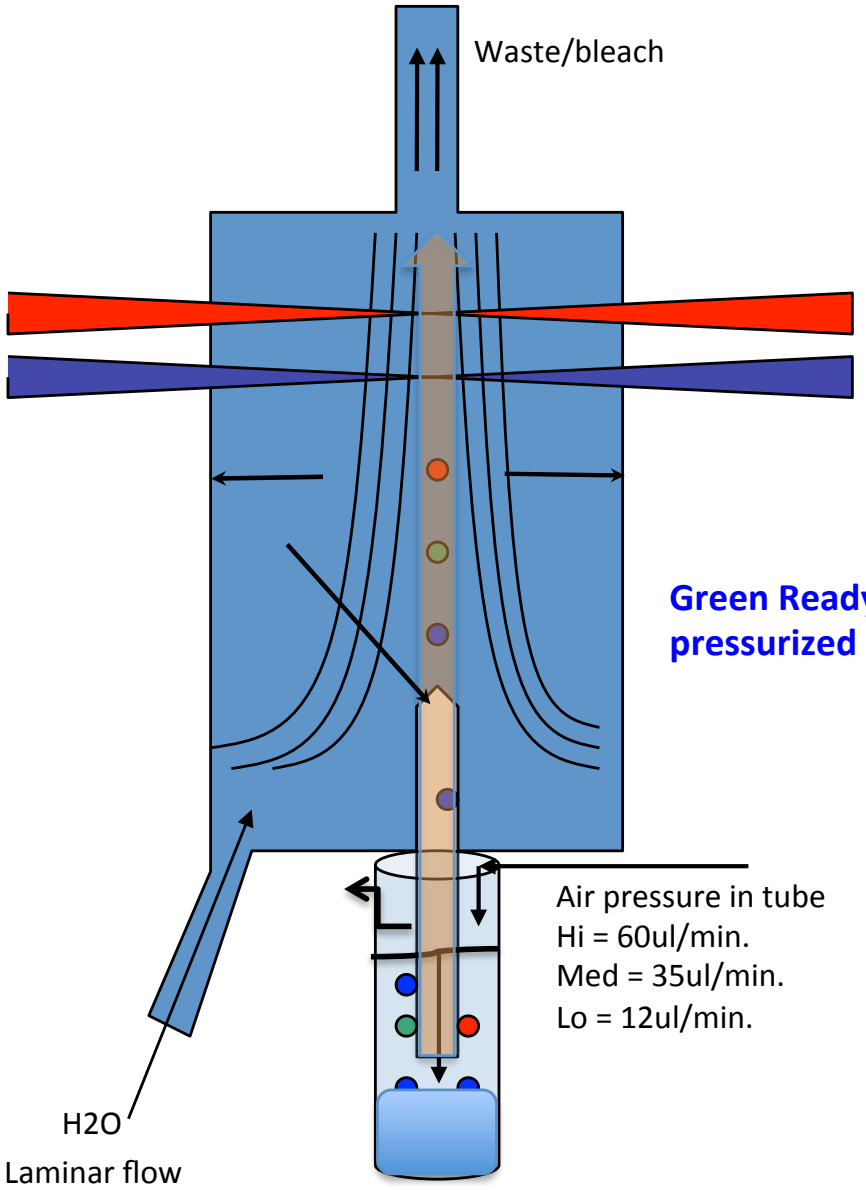


Green Ready/Run light=tube is pressurized

Do not run test tubes dry!!



Monitor the Ready/Run Light



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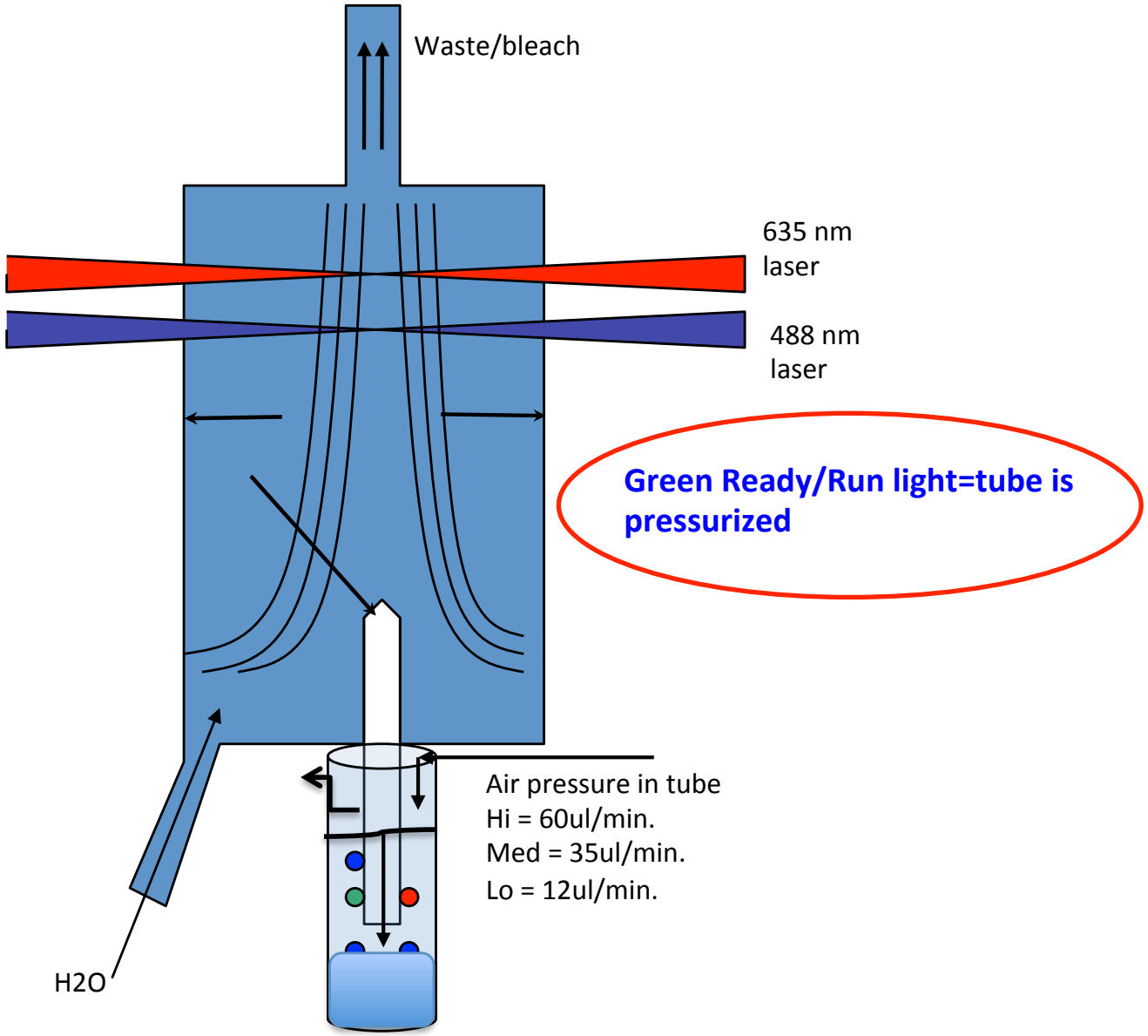
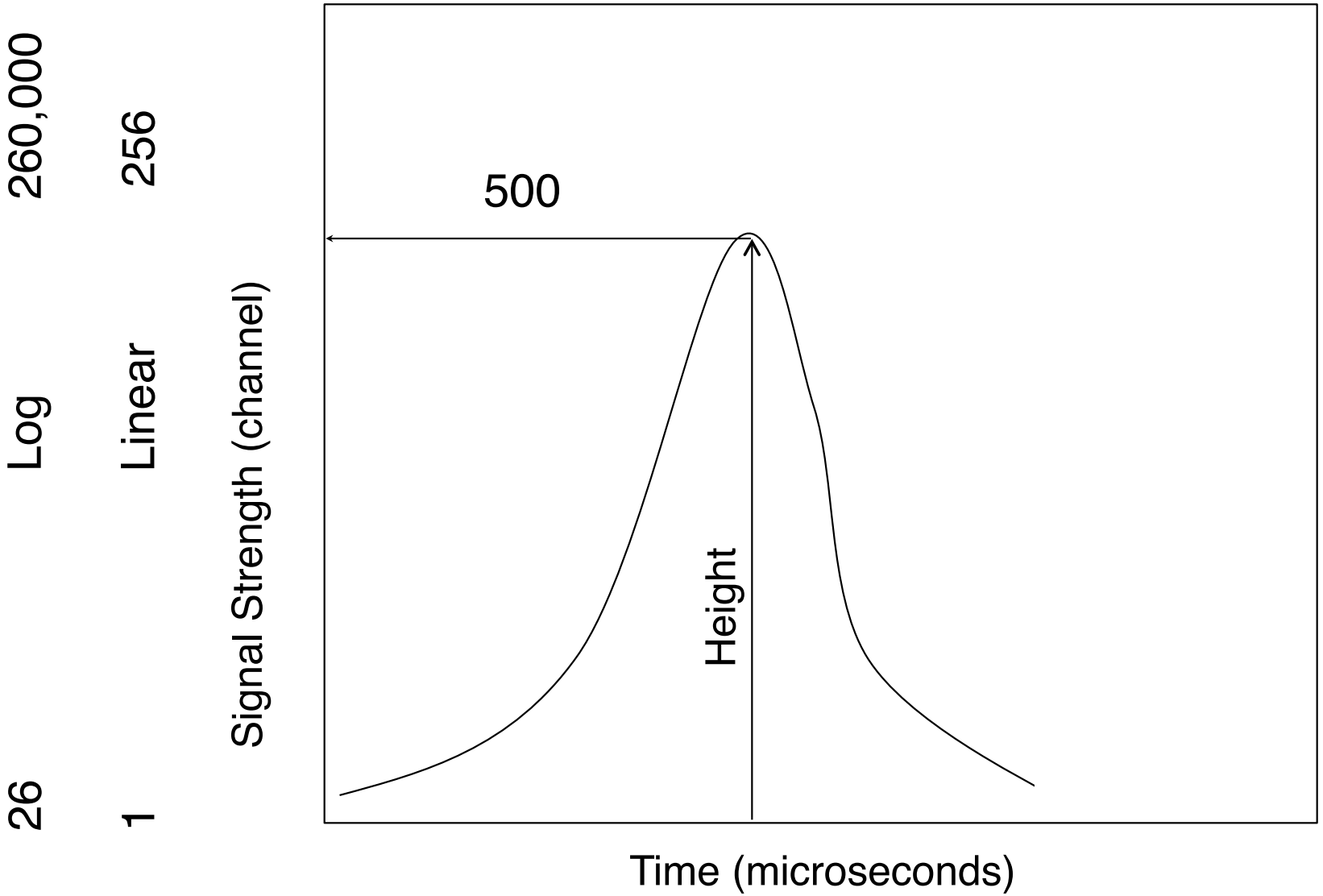
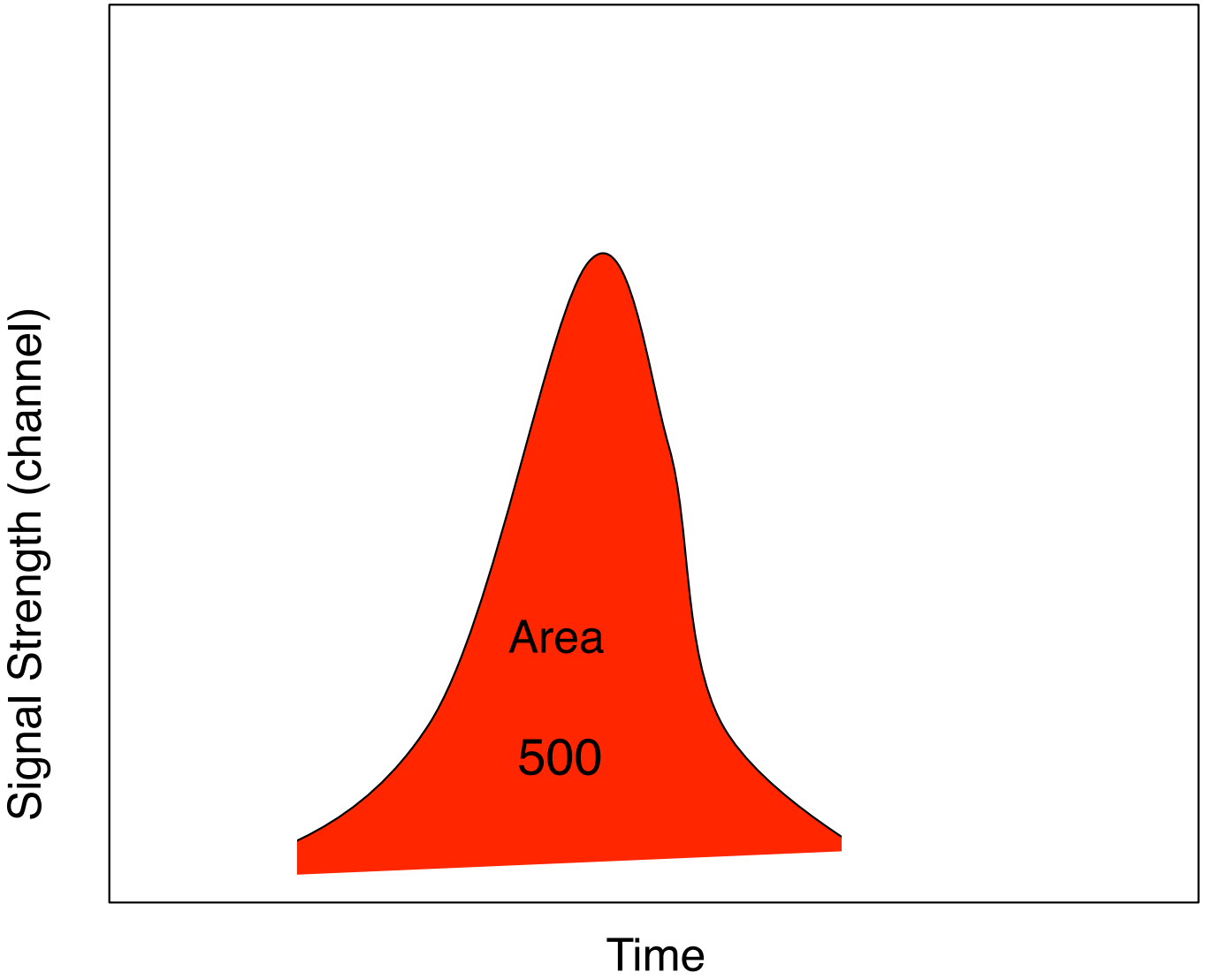


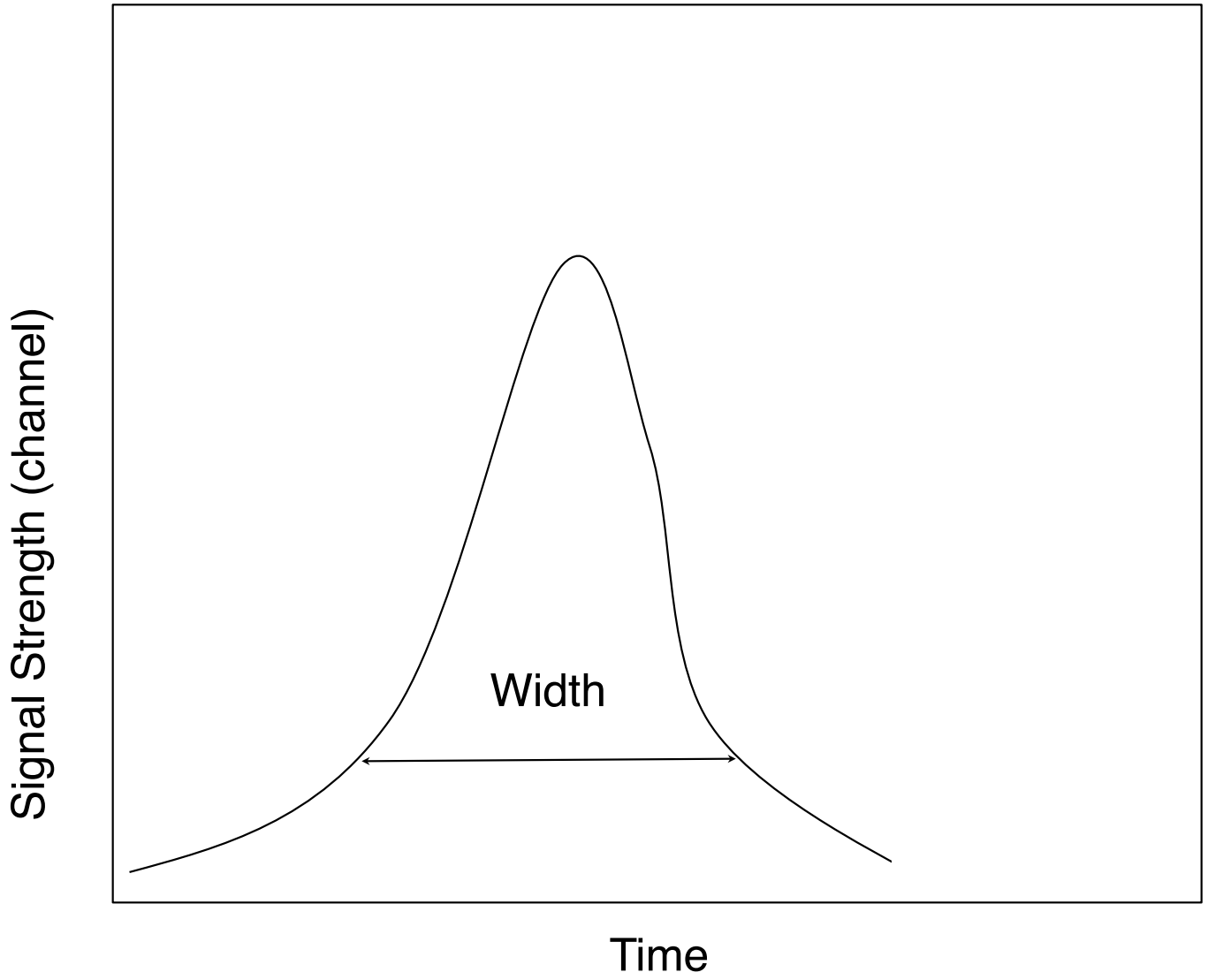
Pulse Height



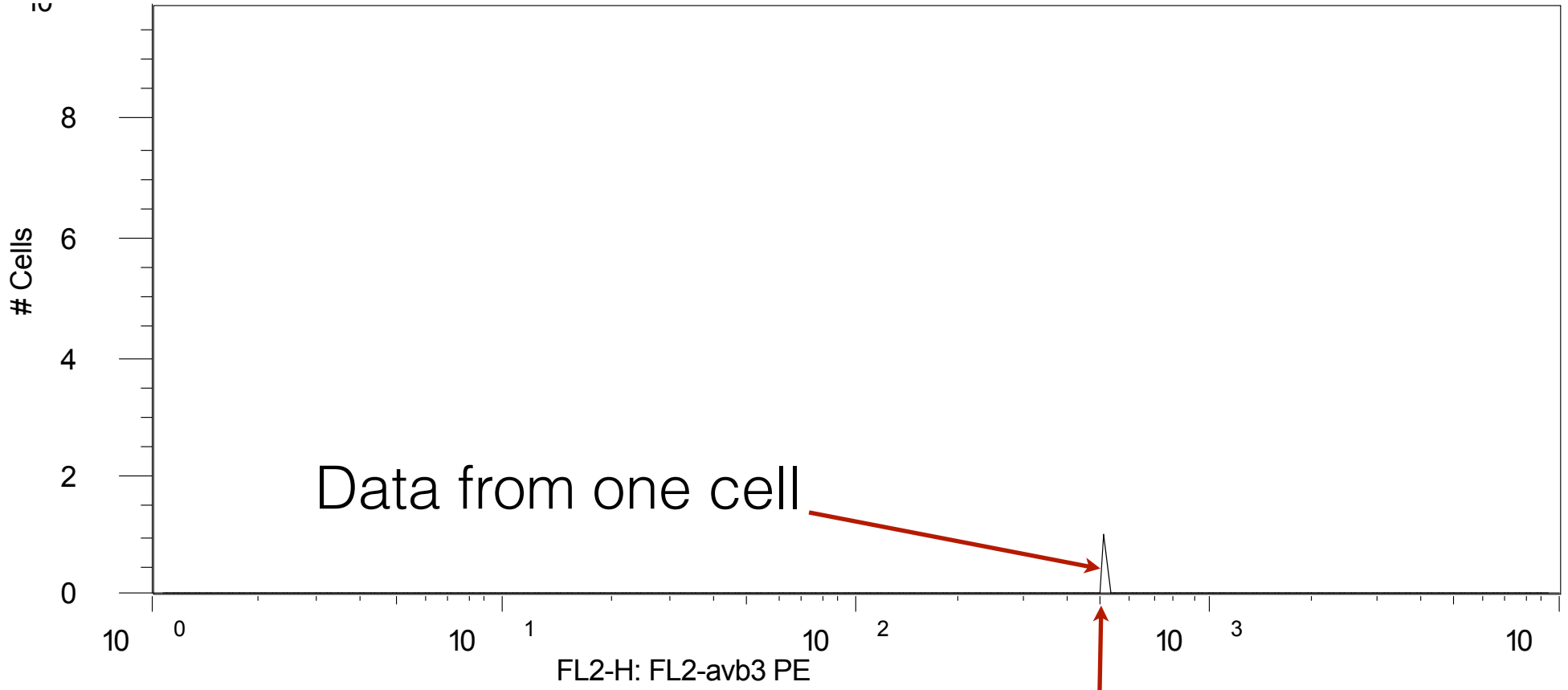
Pulse Area



Pulse Width



Your Cell is represented by a tick mark

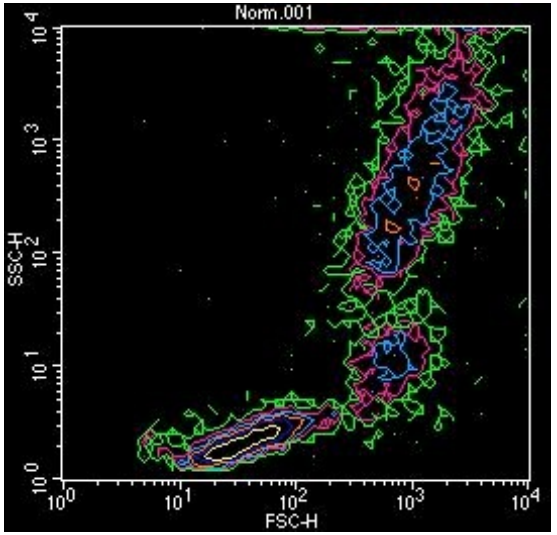


FL1-H, FL2-H subset
MLN stain 3
Event Count: 1

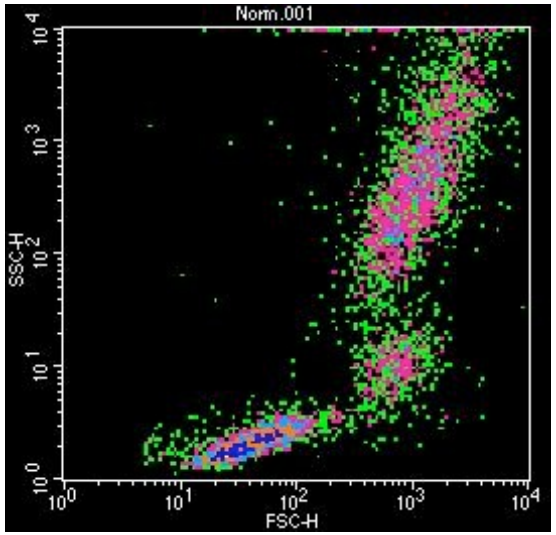
Channel 500

Data Presentation Formats

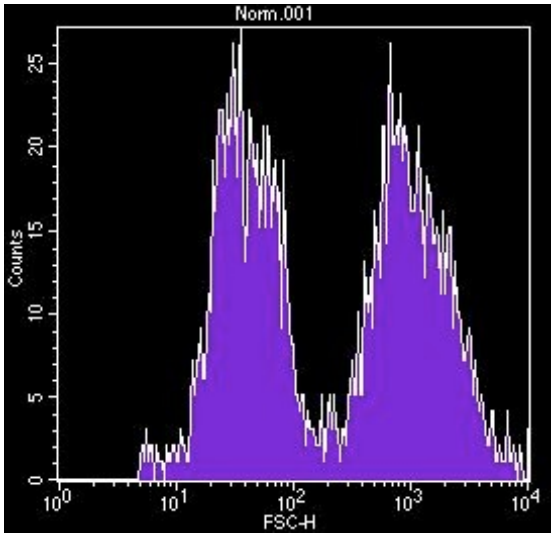
Contour Plot



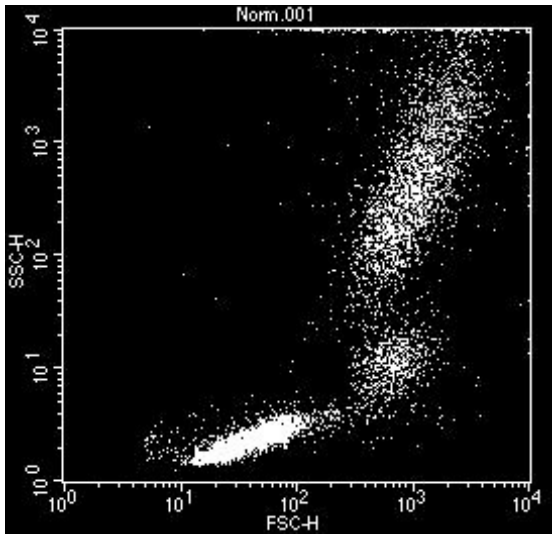
Density Plot



Histogram Plot

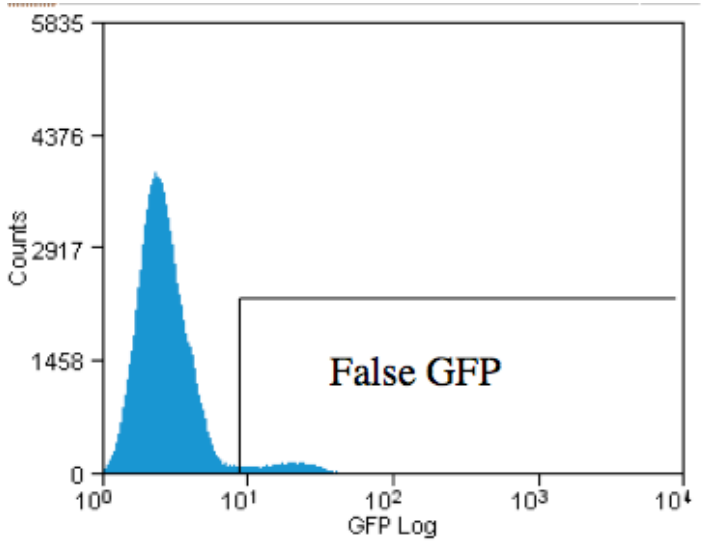


Dot Plot

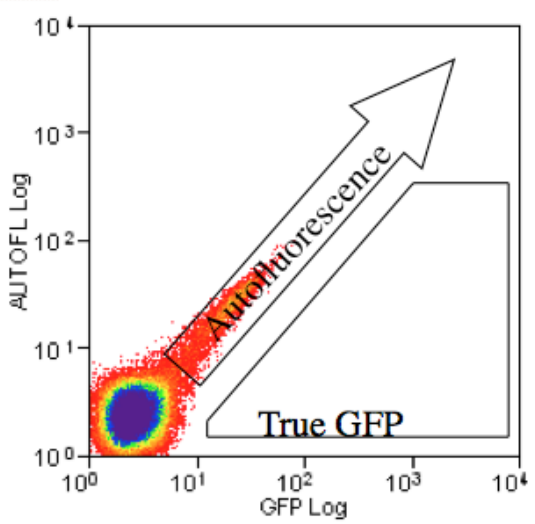


Autofluorescence

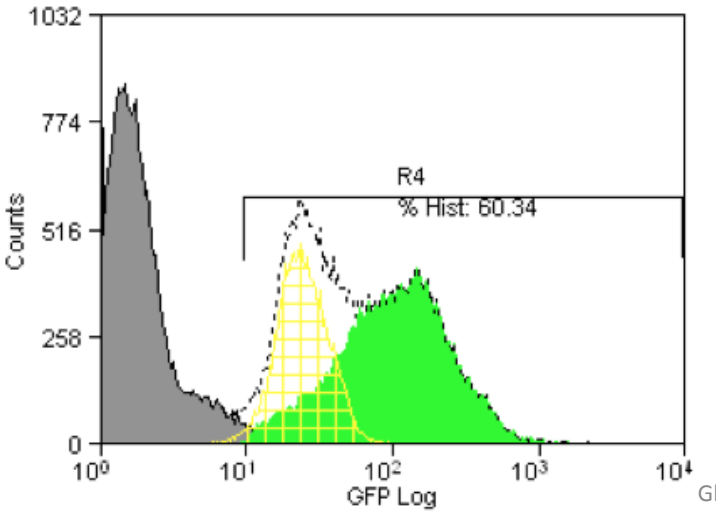
Negative control



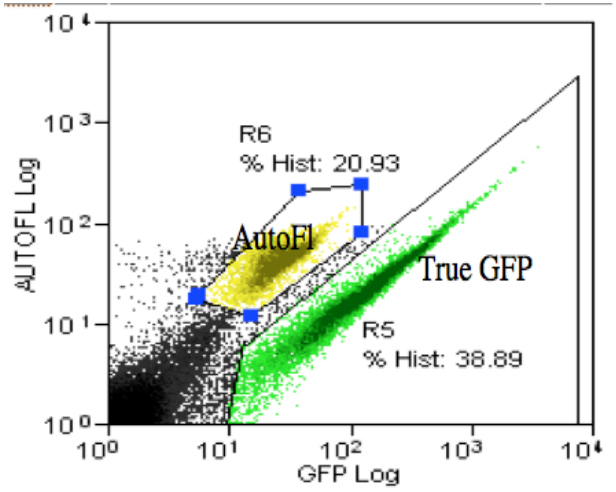
Density Plot



Mixture Histogram

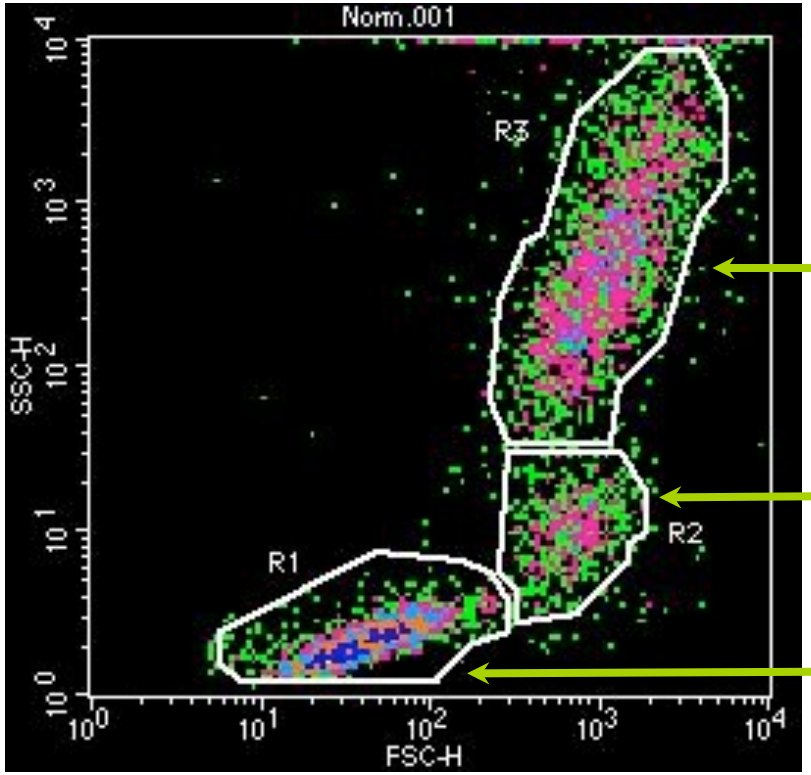


Mixture Dot Plot



Detector Measurements
Scatter Parameters

Detector	Wavelength	Measurement	Property
FSC-Forward Scatter	488nm	Refraction/Diffraction	not size
SSC-Side Scatter	488nm	Reflection @ 90^{o} angle	internal complexity



Granulocytes

Monocytes

Lymphocytes

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Scatter Parameters

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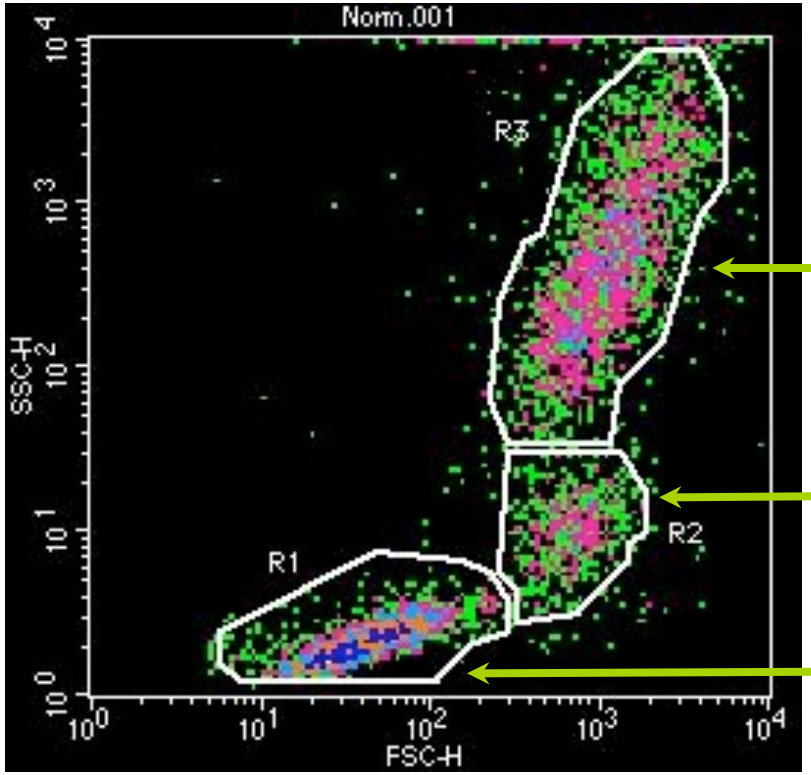

Granulocytes

Monocytes

Lymphocytes

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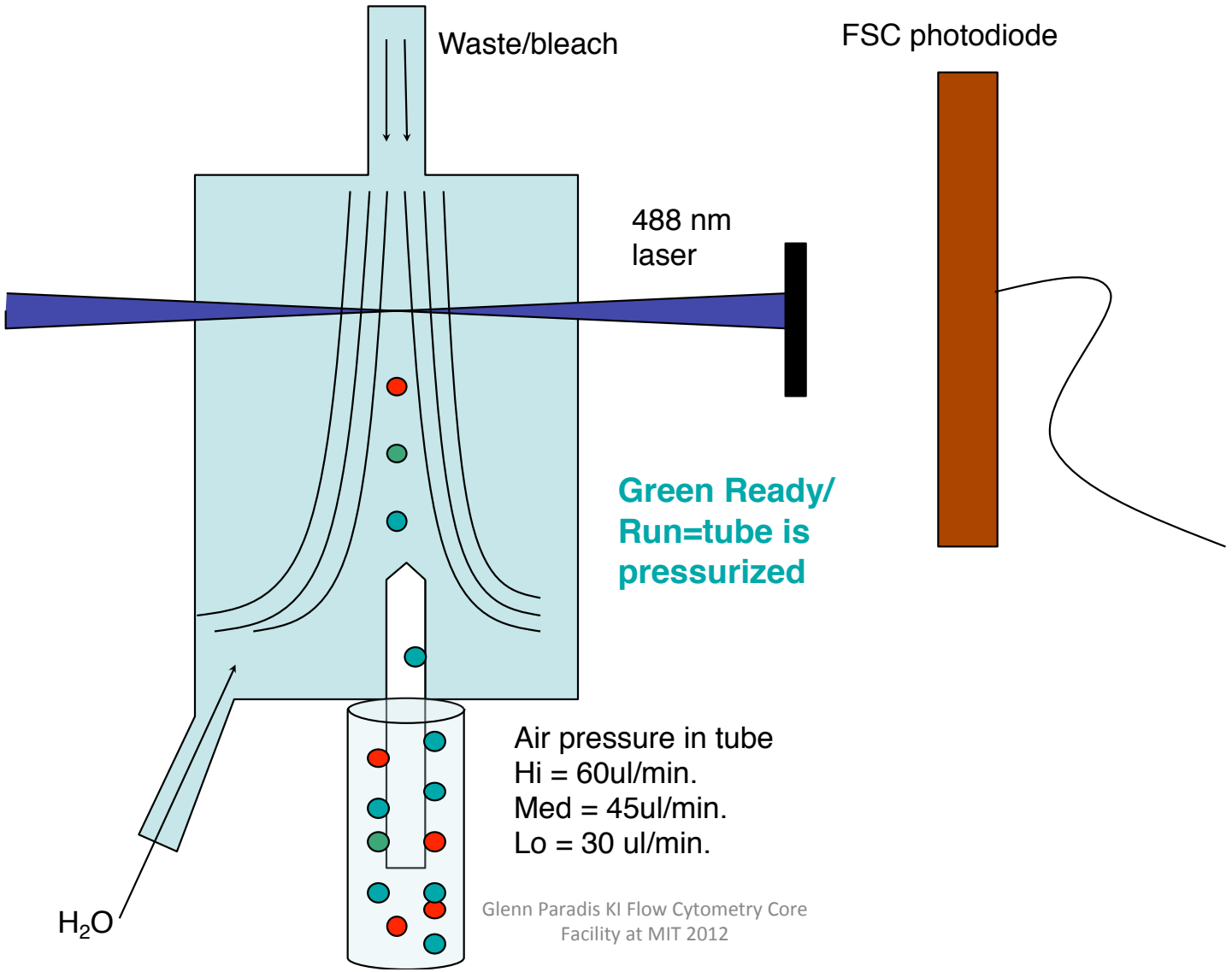
How Are FSC Measurements Made?



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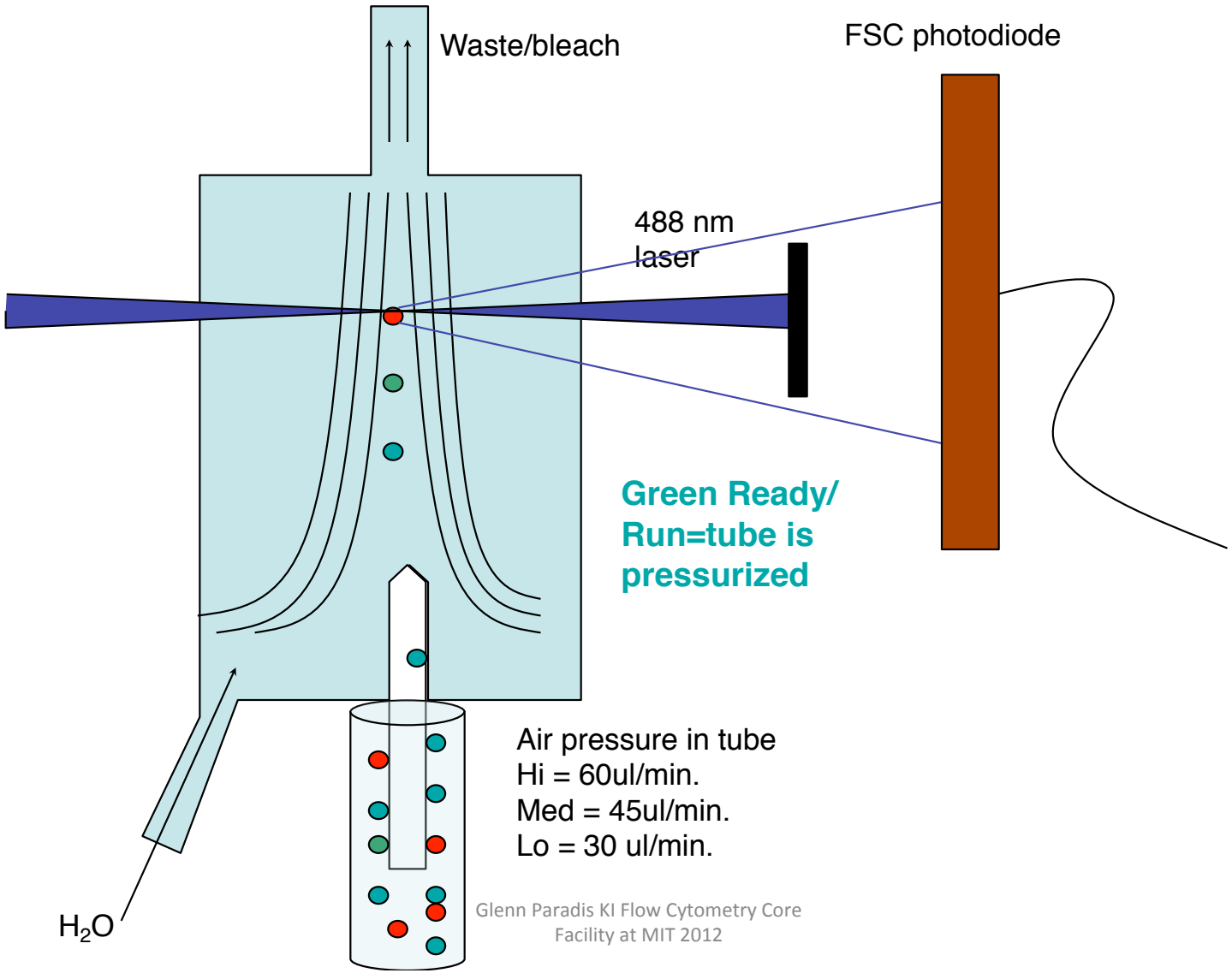

Detector Arrays

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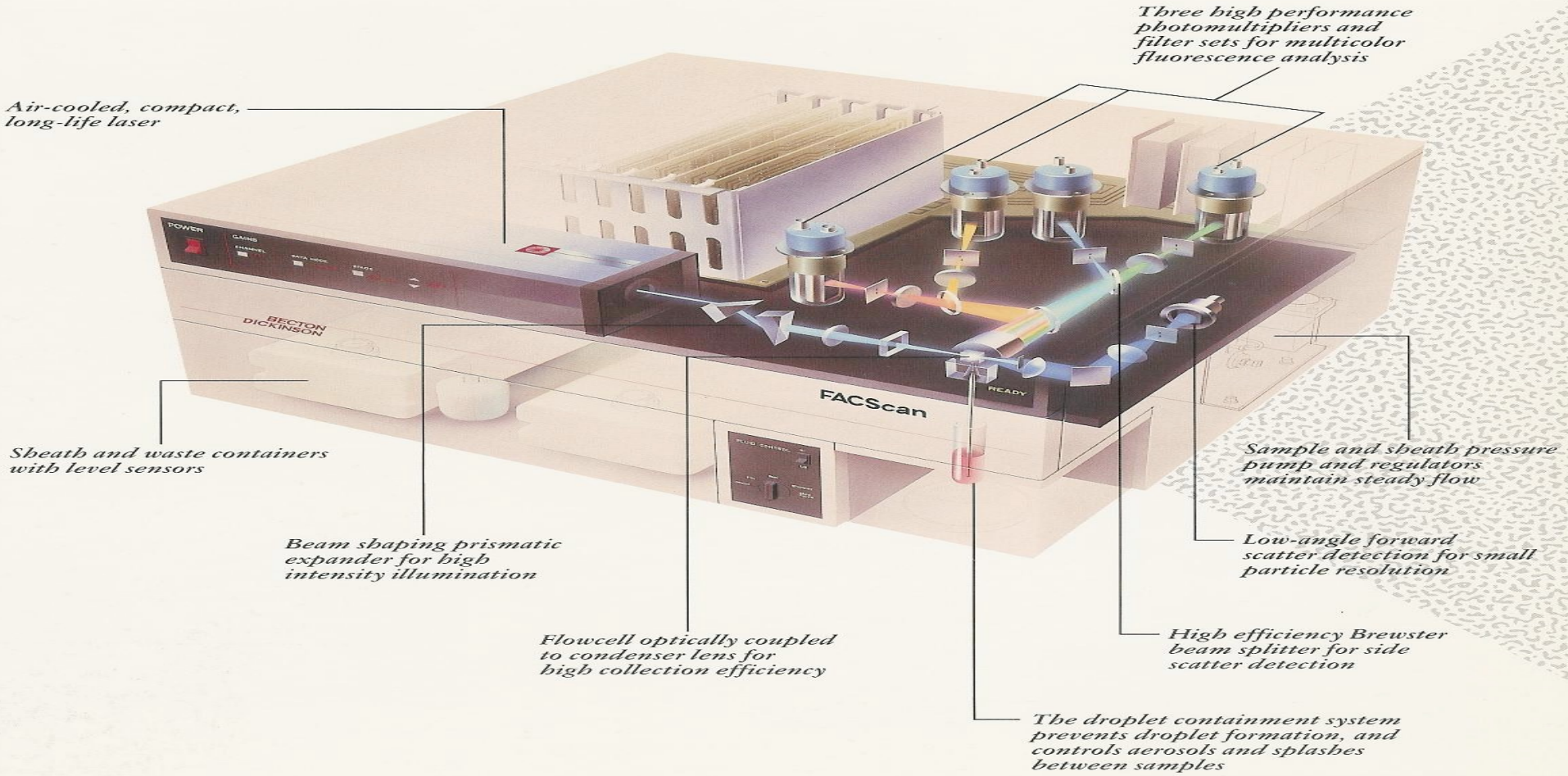
Detector Configurations

LSR Fortessa	355	379/28	BUV 396
		740/35	BUV 737
	405	450/50	BV 421, Pacific Blue, DAPI
		525/50	BV 525, AmCyan, Aqua
		605/12	BV 605
		710/50	BV 711
		800/30	BV 786
	488	530/30	FITC, GFP, Alexa 488
		695/40	PerCP-Cy5.5
	561	582/15	PE, dTomato
		610/20	PE-Texas Red, mCherry
		695/40	PE-Cy5.5
		780/60	PE-Cy7
	640	670/30	APC
		710/50	Alexa Fluor 700
		780/60	APC-Cy7

Optical Layout



KI Elevator Safety

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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

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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 H_2O.
   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