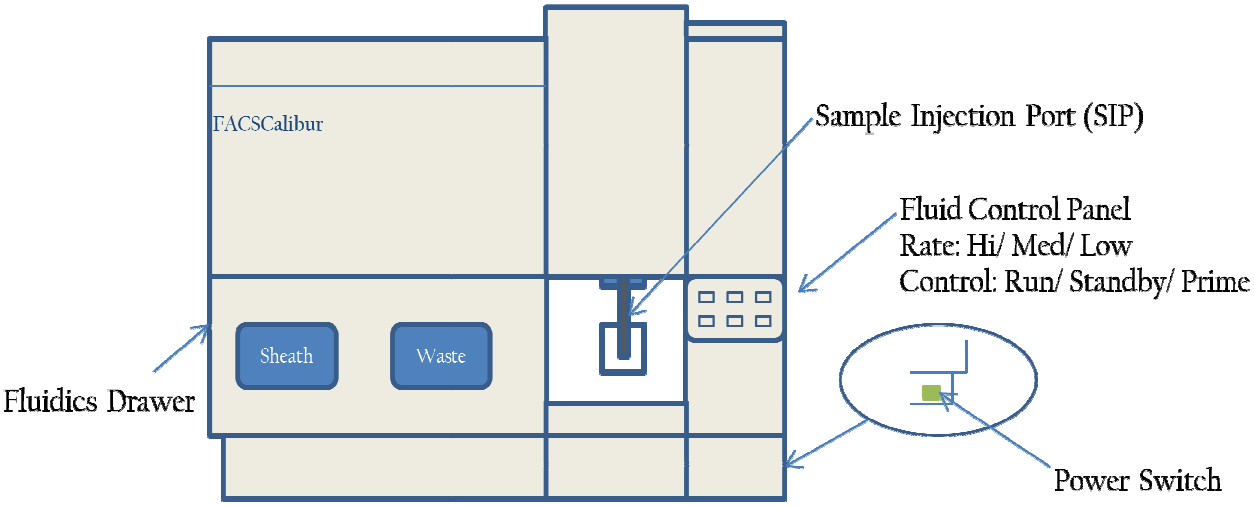
FACCalibur Users' Guide



FACSCalibur Start Up Procedure

If the Instrument is OFF:

1. **Check the sheath and waste tanks.**
   Open the fluidics drawer (front of instrument) and check the levels of sheath fluid and waste in reservoirs. Sheath fluid should be filled to 3/4 capacity (marked line). Waste should be empty except for ~10ml of bleach. **Fill and empty as necessary**.

2. **Pressurize the sheath tank.**
   Flip the switch between the two fluidic reservoirs towards you (Pressurize).

3. **Turn on cytometer.**
   The FACSCalibur must be turned ON **before** the computer. If you have a problem connecting to the cytometer, then the cytometer was not turned on in time to be recognized by the computer.

4. **Make sure Cytometer is in STANDBY mode.**
   Make sure the tube of water is on the SIP, the STANDBY button is lit, and the speed is set to LO.
   - If you are using the Red Laser (APC channel), then you must give the instrument 20 minutes to warm up

If the Instrument is ON already:

1. **Make sure the Cytometer is in STANDBY mode.**
   Make sure the tube of water is on the SIP, the STANDBY button is lit, and the speed is set to LO.

2. **DePressurize the Sheath Tank**
   Flip the switch between the two fluidic reservoirs away from you (depressurize-vent)

3. **Fill and Empty the Sheath and Waste Tanks as needed**
   Sheath Fluid should be filled to ¾ capacity (marked line). Waste should be empty except for ~10ml of bleach.

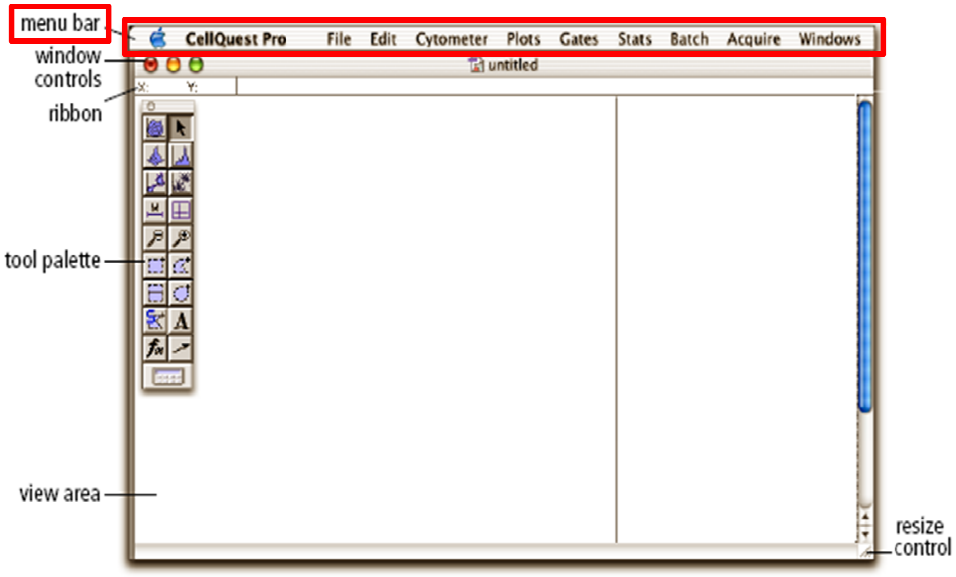
4. **Repressurize the Sheath Tank**
   Flip the switch between the two fluidic reservoirs toward you (pressurize)

CELLQuest Pro Software Start Up & Sample Acquisition

1. **Turn on computer, log in and open CELLQuest**

Click on the CellQuest Pro Icon in your menu bar.

The Following window will pop up:



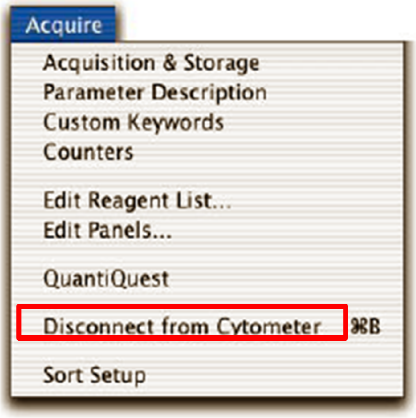
2. Connect to the Cytometer.

In the Menu Bar, Choose **Acquire: Connect to Cytometer**.

If you get an error message at this time, it means the computer was turned on BEFORE the instrument. You must restart the computer in order for the software to connect to the instrument.

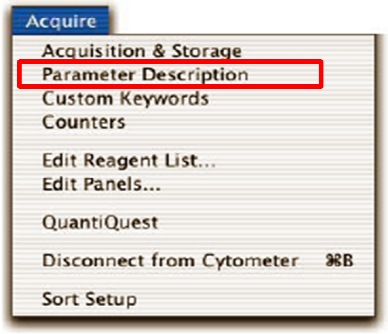
If the Following window (Acquisition Control) doesn't pop up when you connect to the cytometer:

You can try disconnecting:



…and reconnecting to the software.

You can also control acquisition in the Parameter Description window. It can be found in the Menu Bar under: **Acquire: Parameter Description**. This is also where you will choose where you save your experiment, name your samples, and label your axes.



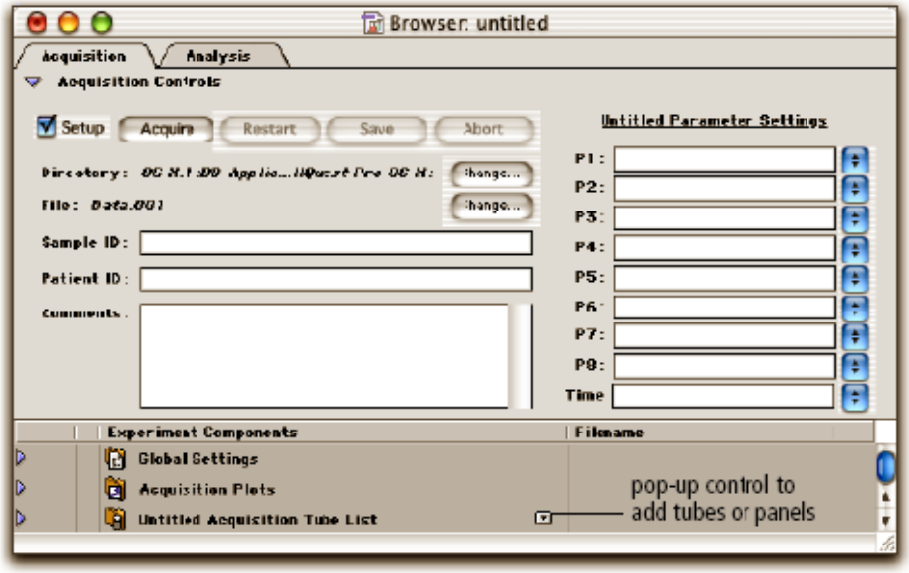


Figure 5-1 Acquisition Browser

3. Open your Acquisition Template.

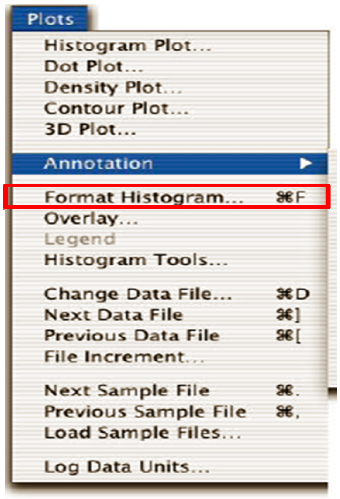
In the Main Menu Bar, Choose **File: Open Document...** If you have a template saved, look in the **DATA** drive, under:

- **Data: Your PI: Templates/Instrument Settings Folder**

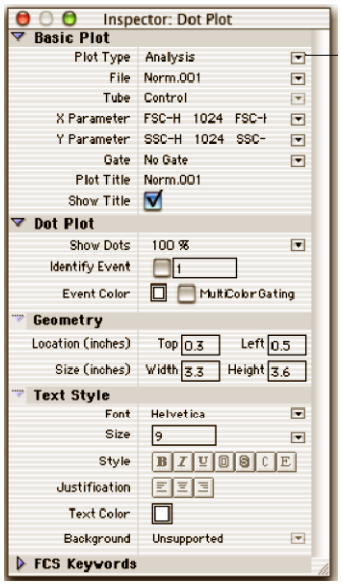
If you are making a new Template, use the toolbar on the left side of the screen to draw histograms and dot plots on the blank template (See New Template Protocol). Be sure to **SAVE the Template in the **Template/Instrument Settings folder** for your lab!!!

If you try to run and don't see any events, or your axes are labeled P1, P2, etc. make sure your **PLOT TYPE is **ACQUISITION** (or ACQUISITION>ANALYSIS). To check this, open:

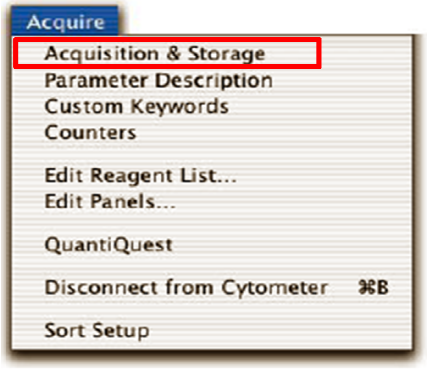
- **Plots: Format Histogram** (or Dot Plot, depending on the type of plot you've constructed)



- The following window will pop up. This allows you to change Plot Type from **'Analysis'** to **'Acquistion'**. You can also change things like your X parameter (histogram) or X and Y parameters (dot plot), how the plot is gated, etc.



4. **Set up your Acquisition and Storage** (the number of events to collect etc).
   Go to **Acquire: Acquisition and Storage**. (see explanation, next page)



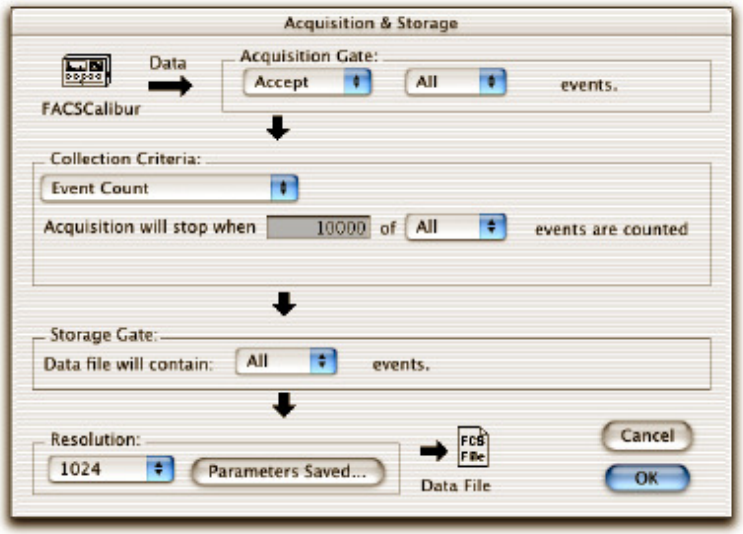


Figure 5-3 Acquisition & Storage settings dialog

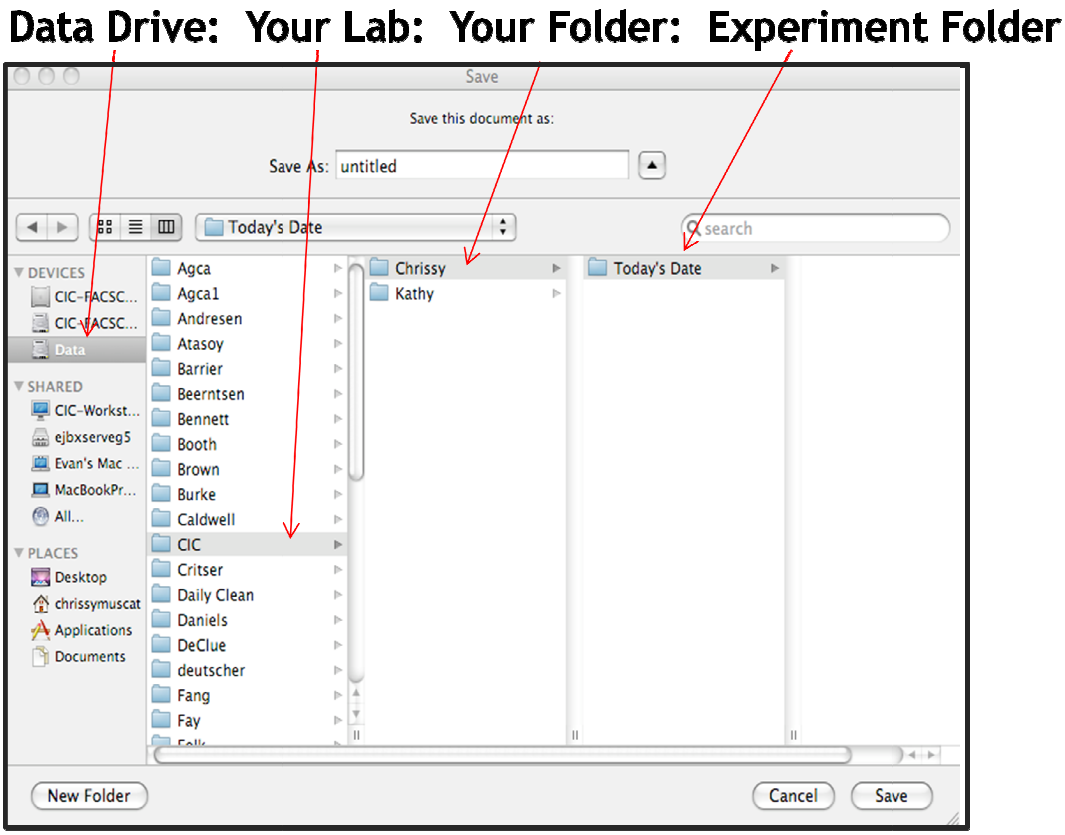
a. Make sure your **Acquisition Gate** is set to "Accept" "All" events.
b. Make sure your **Collection Criteria** is set properly. For example, if you would like to save 10,000 events in your 'live gate', you want to set collection criteria to "event count" and acquisition will stop when "10,000" of "G1=R1" (your primary population gate) events are counted.
c. Make sure your **Storage Gate** data file is set to contain "All" events and the file **Resolution** is 1024.
d. Click on **"Parameters Saved"** and the window below on the left will pop up: Be sure you have ALL the proper parameters checked!!!

e. Click on OK to exit Acquisition and Storage.

5. Create a folder for your data.

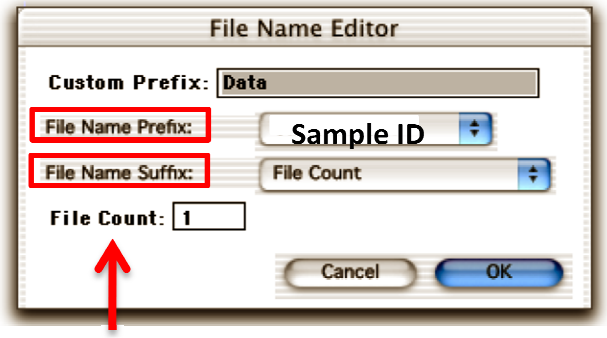
a. Open the **Parameter Description** Window (**Acquire: Parameter Description**)
b. In the Parameter Description window click on the '**Change…**' button next to the Directory and choose a location in which to save your data files.
c. Create a new folder for each experiment by clicking on 'Data' then choosing your lab's folder and your specific folder, once you are there, in the bottom left corner of the window choose '**Create New Folder**'.
d. Name the folder and Click on "Select [your chosen folder name]" to select that folder and exit Folder Information. (see next page)

e. Double check in the **'Parameter Description'** Window, that your **'Directory'** (or folder where your samples are being saved) is the proper folder.



6. Change the filename.

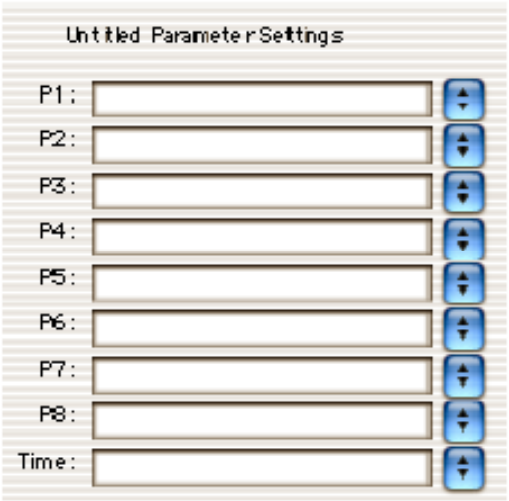
a. In the Parameter Description window click on the '**Change…**' button next to the File and choose a name for your data files
b. Set the **File Name Prefix** to "**Sample ID**".
c. Set the **File Name Suffix** to "**File Count**" and start file count from 001.
d. Click on OK to exit File Information.



7. Enter the names of your parameters

(e.g. Antibody conjugates, fluorescent dyes).

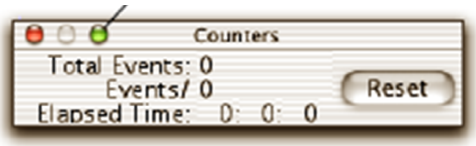
1. Type the name directly into the box for each parameter.
    - P1: FSC (Forward Scatter)
    - P2: SSC (Side Scatter)
    - P3: FL1 (FITC, GFP, Alexa 488)
    - P4: FL2 (PE, Cy3)
    - P5: FL3 (PI, PE-Cy5, PE-Cy7, 7AAD)
    - P6: FL4 (APC)



8. Open the Counters window.

Go to **Acquire: Counters**.

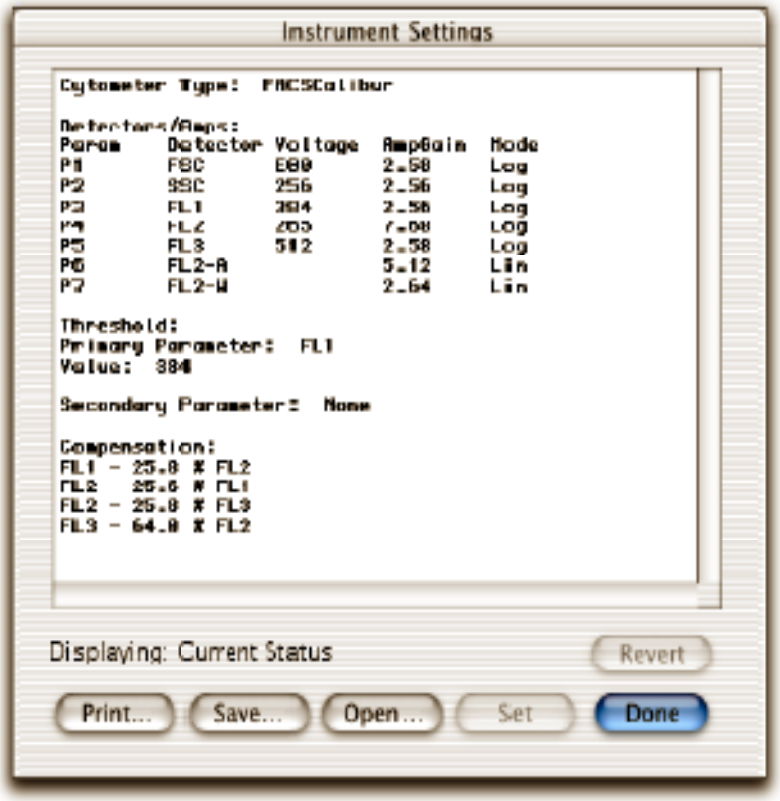
Please note: The Counters gives you a TOTAL event count ONLY (not cell count through gates).



9. Load Instrument Settings.

If you would like to use the same Detectors/Amps, Compensation and Threshold settings as in a previous experiment, you can open them by doing the following:

Go to **Cytometer: Instrument settings**. (picture, next page)



10. Open AND SET your Instrument Settings

Click the **OPEN** button. You can choose a specific Instrument Settings file ***or*** a previously recorded data file from your folder (choosing to use a previously recorded data file will copy the instrument settings from when that file was collected).
Remember that when you **SET** the instrument settings the color wheel will spin, and it might take a few seconds to set! Click on **DONE** to exit Instrument Settings.

11. Check sample settings before recording a file.

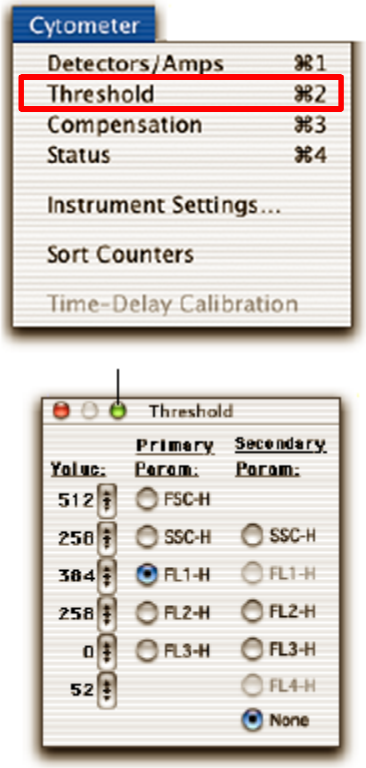
Tick the '**setup**' box in the Acquisition Control (or Parameter Description) window. Press 'Acquire' to start viewing sample data. ***you cannot record sample data file in setup mode**.*

Generally, the following steps (12-14) are done in **SETUP** mode. This way, you can change settings to get optimal signals before running your experiment. Steps 12 and 13 are usually set using your Unstained Sample, and Step 14 is done using your single stain compensation samples.

12. Check Threshold settings.

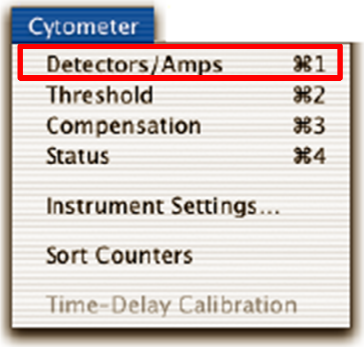
Go to **Cytometer: Threshold** and the window below will appear. Threshold is used to exclude data below a certain channel (or cells/debris below a certain size). Most of the time, threshold will remain the same. However, if you have very small cells, or a lot of debris you don't want counted, it helps to lower or raise the threshold respectively.

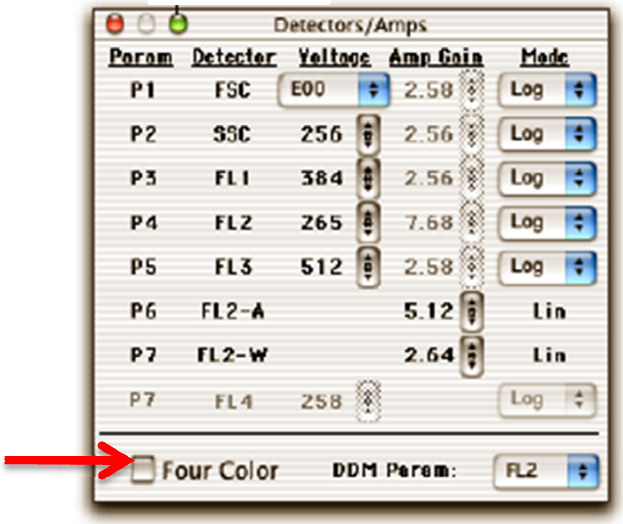
Normal settings: Primary Parameter: FSC-H value 52
Secondary Parameter: None



13. Check Detectors/Amps settings.

Go to **Cytometer: Detectors/Amps** the window on the next page will appear. Adjust each parameter as required. (see next page for guided instructions)





In set up mode (see step 11) run a sample of UNSTAINED cells and adjust the following:

MODE:

- **Log amplification** is often used to analyze samples with a large dynamic range of fluorescence signals. The log scale has four decades of range. **Set all fluorescent channels to LOG (unless doing cell cycle analysis)**
- **Linear amplification** is usually used for all light scatter parameters. It is also useful for fluorescent parameters used in DNA quantitation experiments. **Set FSC and SSC to LIN**
- **Linear amplifier** gain can be adjusted from 1.00–9.99. The chosen value multiplies the signal by a factor of 1.00–9.99.

VOLTAGE:

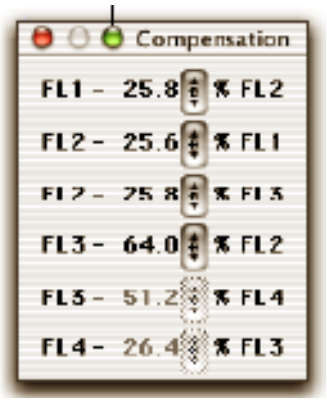
- As the voltage is increased, the detector sensitivity increases, resulting in increased signal.
- As the voltage is decreased, the detector sensitivity decreases, resulting in decreased signal.
- PMT voltages are adjusted using the Voltage sliders

FL-4 (P7):

- In order to Acquire the FL-4 channel (most commonly APC) you must check the **Four Color Box** at the bottom of the Detector/Amps Window.

14. Check Compensation settings.

Go to **Cytometer: Compensation** and the window on the next page will appear. (see Compensation tutorial for setting compensation on the FACSCalibur). In set up mode (see step 11) run each of your single stained samples, and adjust compensation so that there is minimal "spillover" of each signal into neighboring channels. (i.e. a FITC only sample doesn't appear to be FITC and PE positive)



***If you would like to save the changes your instrument settings in a separate file (instrument settings are the Threshold, Detectors/Amps and Compensation settings) go to **Cytometer: Instrument Settings** and in the window, click SAVE. Save the file in your Template/Instrument settings folder, and use it in future experiments for **STEP 9** (Load Instrument Settings.)*

15. Record a data file.

a. Enter your '**Sample ID**' in the Parameter Description window.
b. Uncheck the **Setup** box on the Acquisition Control window. Your first file (name.001) is ready for recording.
c. Vortex your sample, double check that there are no aggregates in your sample, and place on SIP.
d. **Run** cytometer on **Low** and click '**Acquire**' on the Acquisition Control window.
e. The number of events per second (Counter panel) should not exceed 2000. If your cells/events are running faster then remove from the SIP and dilute. If they are running slower then increase you sample speed to Medium or High (buttons above the RUN/STANDBY buttons).
f. If you do not reach the required number of events before the sample runs out, click '**Pause**' and then '**Save**'.
g. Repeat steps **a-f** for each sample.

16. Backup your data at the end of each run.

Either Bring a USB drive, or see the 'Data Transfer' Guide for transferring your data to another computer
The CIC facility is not equipped for data storage. Data is erased from all CIC computers (including workstations) on a monthly basis.

17. Please see the Data Analysis Protocols for analysis options.

FACSCalibur Shut Down Procedure

1. **Disconnect the software from the cytometer.**
   - After running the last sample, set the cytometer to STANDBY, make all necessary saves in CELLQuest Pro and choose **Disconnect from Cytometer** in the Acquire menu.
2. **Clean flow cell with 10% Contrad for 3 minutes.**
   - Place a tube with 3 ml of 10% Contrad on the SIP with the arm in the open (back) position and aspirate for 30 seconds. Then close the arm (forward) and RUN the tube on HIGH for 3 minutes.
3. **Rinse flow cell with water for 3 minutes.**
   - Place a tube with 3 ml of water on the SIP and aspirate for 30 seconds with the arm in the open position. Then close the arm and RUN on HIGH for another 3 minutes.
4. **Put the instrument in STANDBY** (turn the instrument off if necessary, see guidelines below) and depressurize the Sheath Tank by flipping the switch in the Fluidics Drawer to **"Vent- Change Tank".**

   - *If someone **is not** on the instrument within 2 hours after your appointment*, shut down the instrument
   - *If someone **is** on the instrument within 2 hours after your appointment*, keep the instrument turned on *(and in STANDBY mode)*
   - *You can check the reservation schedule by double clicking on the reservation schedule shortcut on your desktop or going to http://biotech.missouri.edu/cgi-bin/ureserve.pl*