UCHC Guide to CellQuest Pro

FACS Calibur instruments

Startup:

Turn on Cytometer using green switch on the side of the cytometer
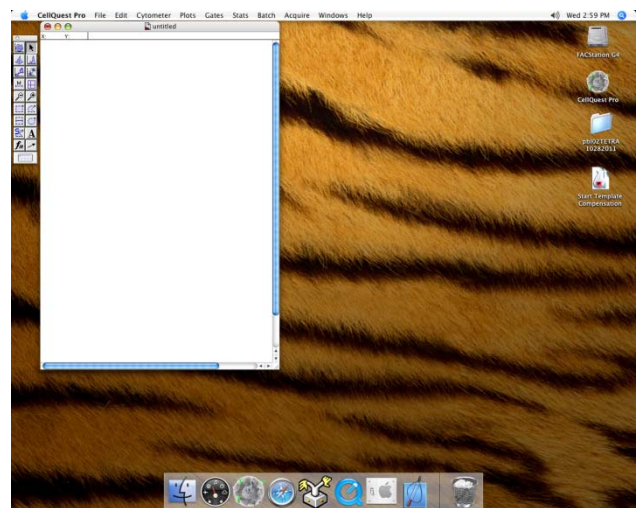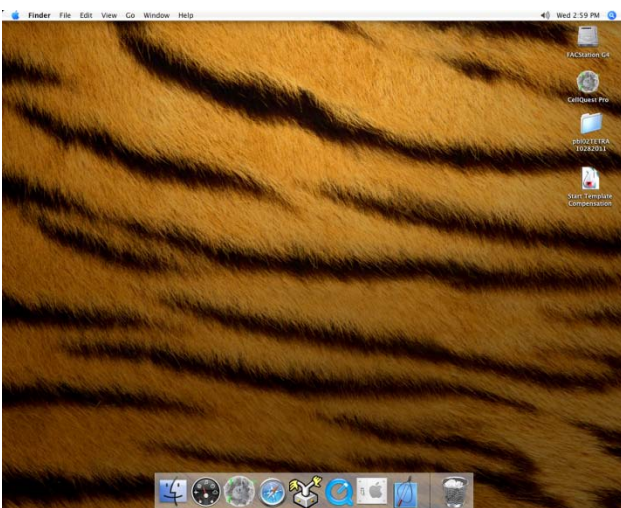
Cytometer Power Switch

Within a minute of turning on the cytometer; turn on the Apple Workstation. (Note that the most accessible power button may be located on the monitor or keyboard.)
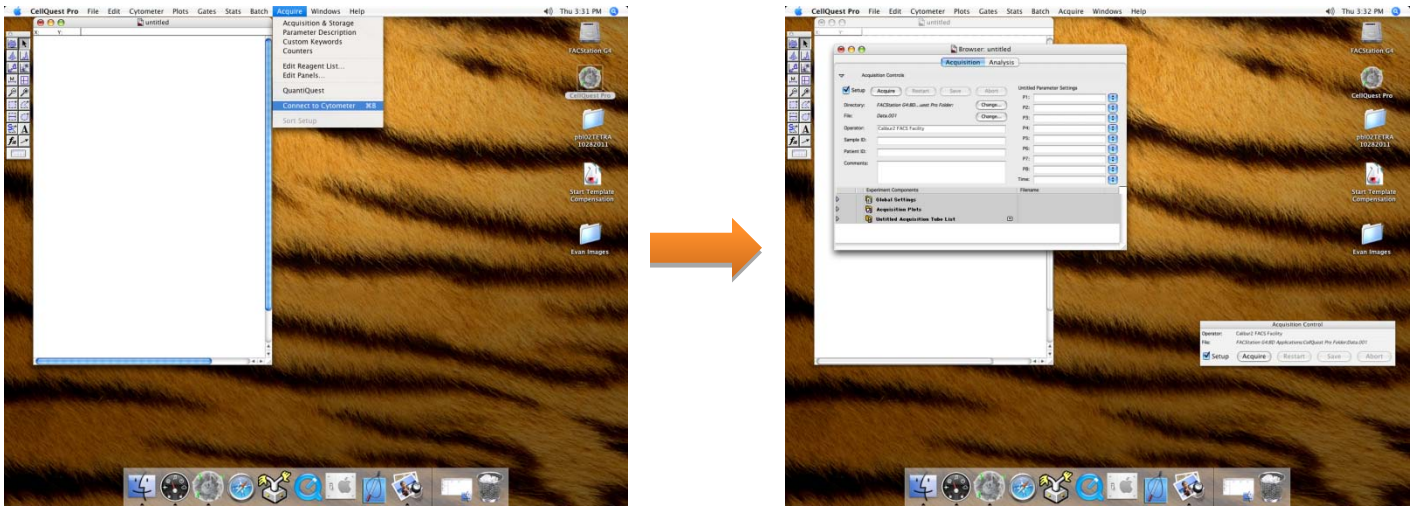
Additional power button locations
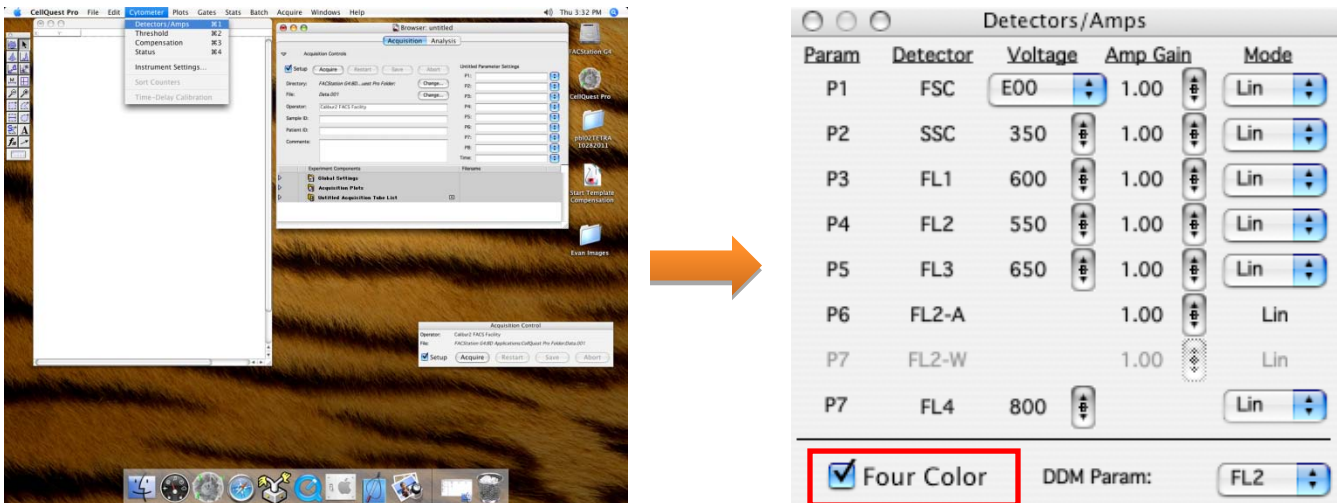
Open CellQuest Pro icon on desktop

Connect to cytometer by navigating to *Acquire > Connect to cytometer* (shortcut = ⌘B). A Browser window and Acquisition Control window will appear
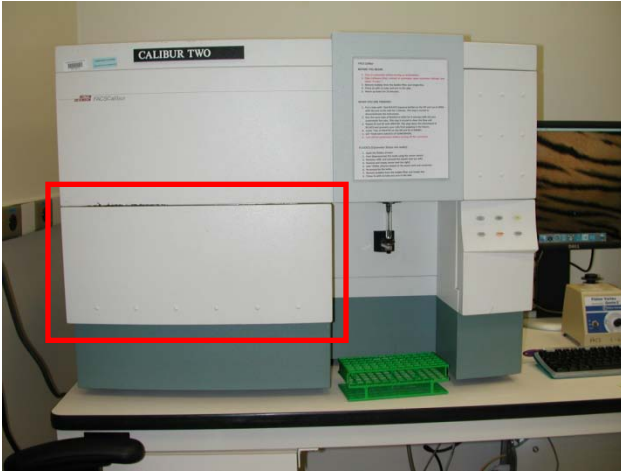

Navigate to *Cytometer > Detectors/Amps* (shortcut = ⌘1) to open the cytometer controls.

Turn on the 633nm laser by selecting "Four Color" in the Detectors/Amps window.
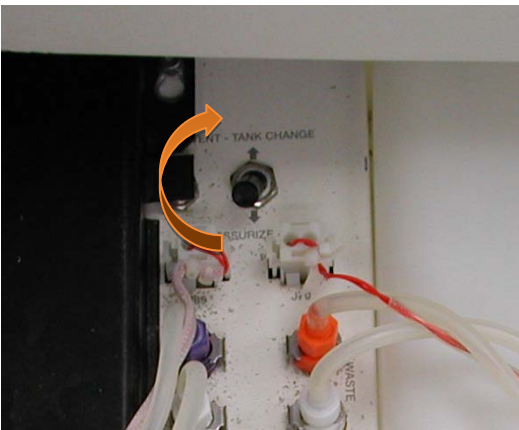
Fluidics:

Open the fluidics drawer and check that the sheath tank (Left) is full and that the waste tank (Right) is empty.

To change fluids, depressurize the fluidics system by flipping the black vent switch away from you.
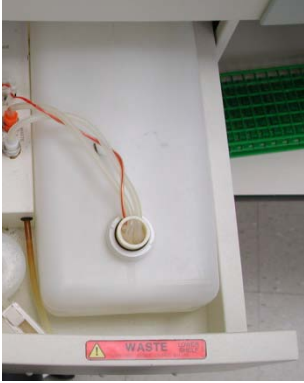

Remove the black metal sheath tank retention plate by sliding it away from you; then lift up.
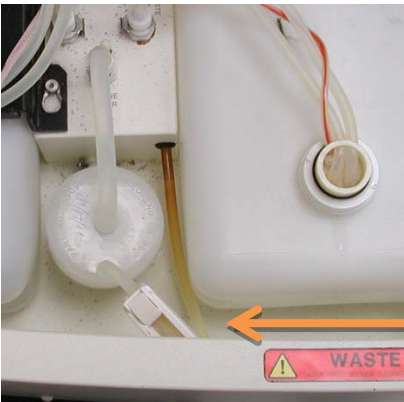
Unscrew the probe and intake lines and refill the tank. Reinstall intake lines and retention plate. Line up the four retention bolts and push down while sliding toward you.

Unscrew the probe and waste lines and carefully remove the waste tank. Empty the contents into the sink. Add ~250ml chlorine bleach to the waste tank and reconnect waste lines.

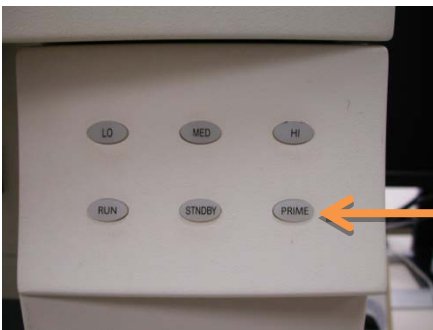Re-pressurize the system by flipping the vent switch towards you.

Remove bubbles from the bubble filter and intake line by releasing the roller-valve.

Roller-valve (drains to waste tank)

Once air is removed, close the roller-valve, and shut the fluidics drawer.

PRIME the cytometer twice with no tube and arm to the side.

Prime button

The instrument is now ready for use!

Cell Phenotyping Using Cell Quest Pro

Cytometer settings:

Open cytometer control panels: With the instrument connected (described above), open the cytometer controls by navigating to *Cytometer > Detectors/Amps, Cytometer > Threshold, Cytometer > Compensation,* and *Cytometer > Status*. Alternatively, use keyboard shortcuts ⌘+1, ⌘+2, ⌘+3, ⌘+4.

Move each window to a viewable location.

Detectors/Amps				
Param	Detector	Voltage	Amp Gain	Mode
P1	FSC	E00	1.00	Lin
P2	SSC	350	1.00	Lin
P3	FL1	600	1.00	Lin
P4	FL2	550	1.00	Lin
P5	FL3	650	1.00	Lin
P6	FL2-A		1.00	Lin
P7	FL2-W		1.00	Lin
P7	FL4	800		Lin

☑ Four Color   DDM Param: FL2

Threshold		
Value:	Primary Param:	Secondary Param:
52	⦿ FSC-H	
52	◯ SSC-H	◯ SSC-H
52	◯ FL1-H	◯ FL1-H
52	◯ FL2-H	◯ FL2-H
52	◯ FL3-H	◯ FL3-H
52		◯ FL4-H
		⦿ None

Compensation	
FL1 -	0 % FL2
FL2 -	0 % FL1
FL2 -	0 % FL3
FL3 -	0 % FL2
FL3 -	0 % FL4
FL4 -	0 % FL3

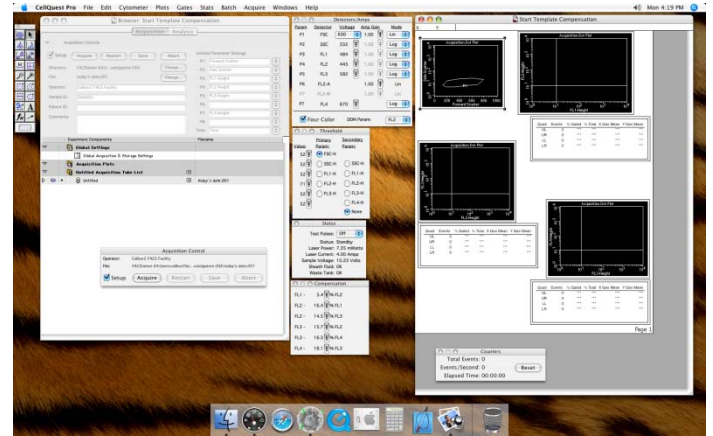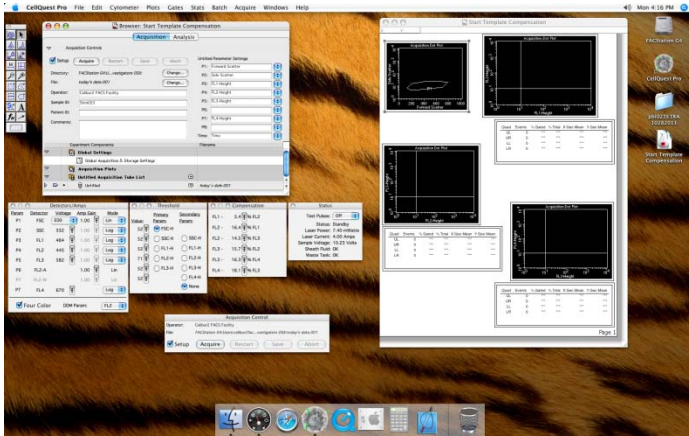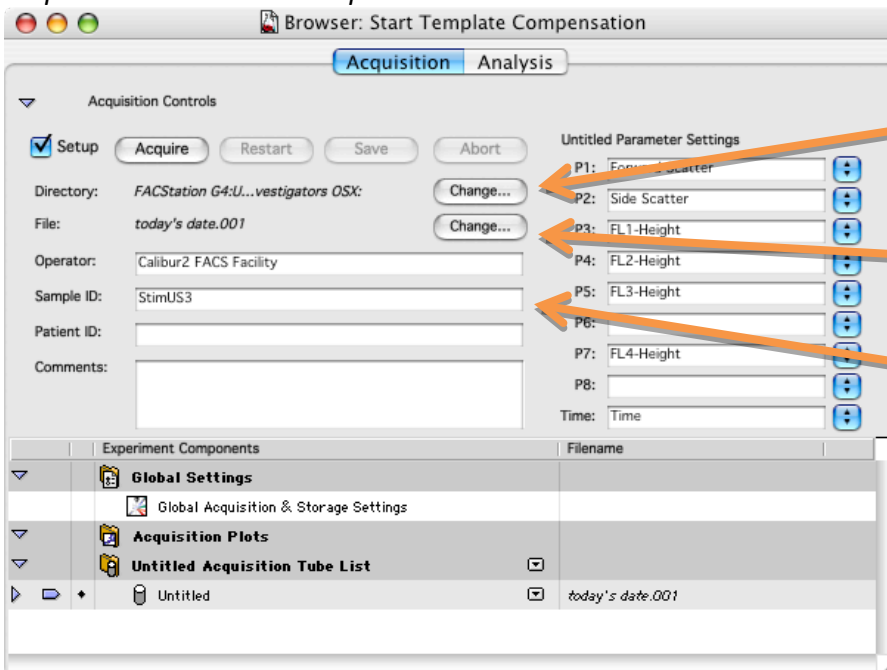| Status	
Test Pulses:	Off
Status:	Standby
Laser Power:	7.25 mWatts
Laser Current:	4.00 Amps
Sample Voltage:	10.23 Volts
Sheath Fluid:	OK
Waste Tank:	OK

You want to be able to see these controls, as well as the Browser window, Acquisition Control window and Worksheet window (and perhaps Counters window; *Acquire > Counters*)

Some CellQuest Pro desktop examples:

Select file save location and define reagents: If it isn't already, open the Browser window by navigating to *Acquire> Parameter Description*
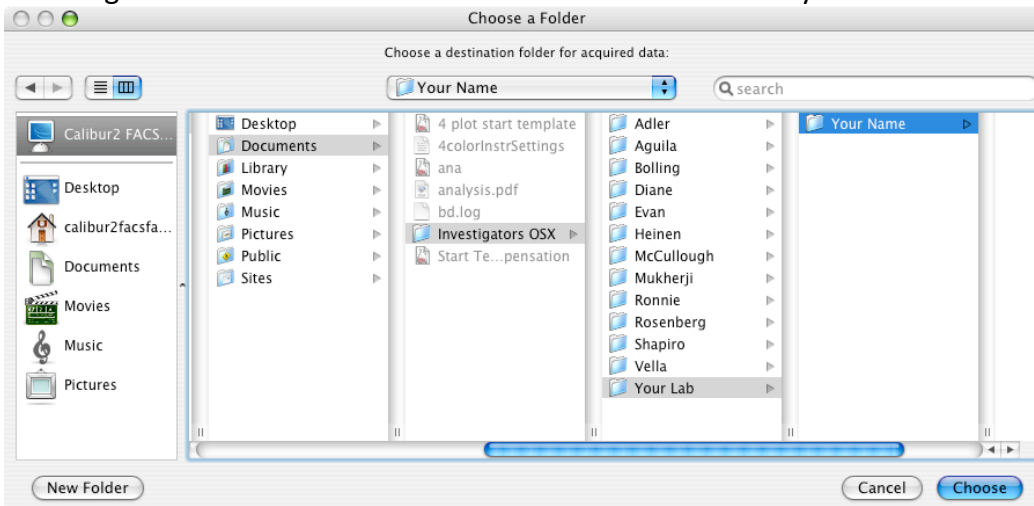

Directory: Choose the Folder where files will be saved
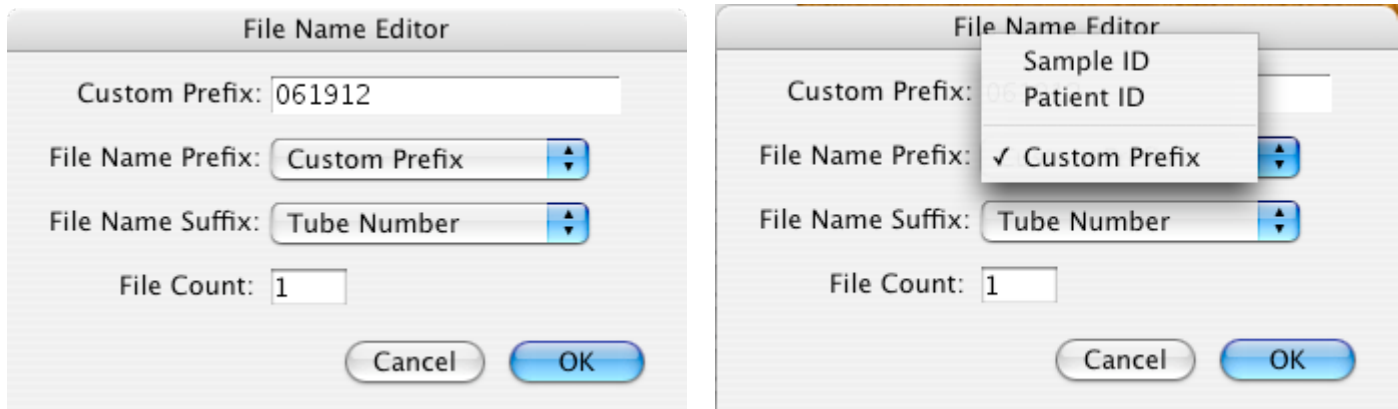
File: define name of files saved

Sample ID: create a description of the sample

In the Browser window, choose the folder where your files will be saved by selecting the "Change..." button next to "Directory:". In the next window, navigate to your lab's folder and create your own folder within it by selecting the New Folder button on the bottom left. Rename your folder and select Choose.

Define the name of each file by the selecting the "Change…" button next to "File:". In the next window you can choose a custom prefix for your file name or select one of the predefined prefixes such as Sample ID or Patient ID.
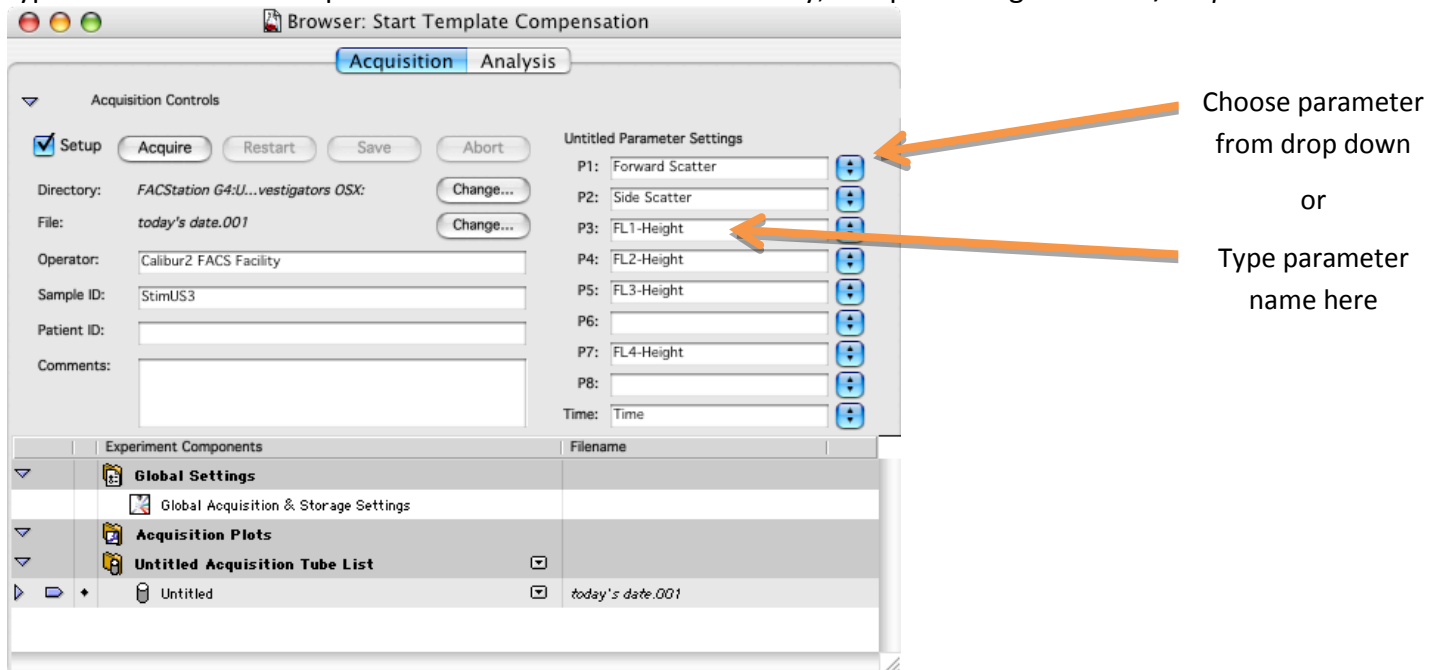

The File Name Suffix should be File increment unless you're using Tube Panels (**CellQuest Pro-advanced**).

In the Sample ID box, type in a name for your sample that describes it accurately. Some examples include: unstained control, Mouse 1, spleen 4, isotype control FITC, dox-treated 15, etc. These can be used in programs such as FlowJo to sort your samples during analysis.
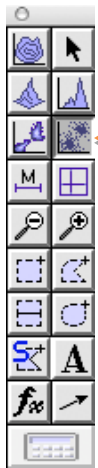
Note: you can save other Keywords that will be associated with your sample under "Patient ID:" and "comments:"

Select your parameter labels in the Browser window. Labels can be generic (e.g.: FL-1 Height) or specific (e.g.: CD3-PE). Some labels appear in the drop down menu beside each parameters, however, custom labels can be typed in the box. Match parameters and FL detectors carefully; compare using *Detectors/Amps* window.

Choose parameter from drop down

or

Type parameter name here

CellQuest Pro Advanced: Create and choose panels under *Acquire > Edit panels…*

Create Worksheet Elements: In the tool palette, select the dot plot tool.

 Dot Plot tool in the tool palette

Note: You can create a histogram, density plot, or contour plot by selecting those tools from the tool palette

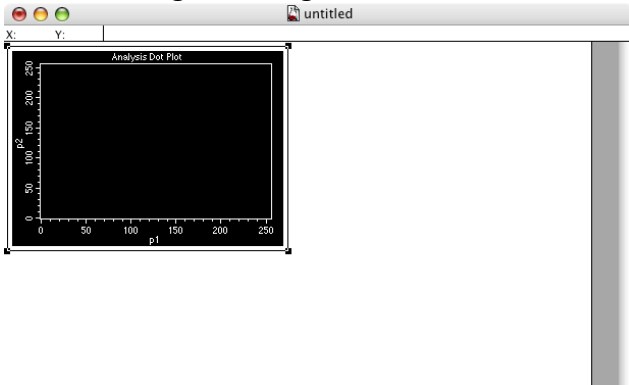Histogram tool   Density plot tool   Contour plot tool

Click and drag a rectangle in the worksheet to create a dot plot.

Select the white border of the dot plot, navigate to *Windows > Show inspector* (shortcut ⌘+ i)
In the inspector window, change the Plot Type to Acquisition or Acq->Analysis.
Change the X parameter to reflect your Forward Scatter Label and change the Y Parameter to reflect your Side Scatter label.
You may also adjust the number of events shown within the plot (lower numbers refresh faster) and the color of the dots within the plot.
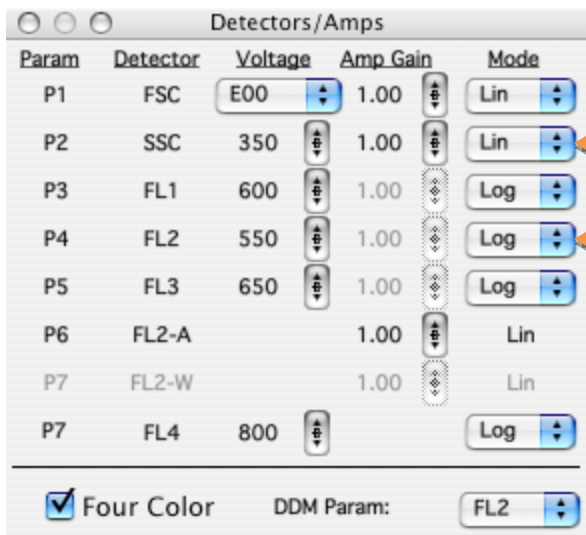
Dot color can be changed here

Create other plots on the worksheet and choose the fluorescence parameters that correspond to your experiment. You should try to observe all fluorescence parameters during collection.

Adjusting FSC and SSC Settings: You need to adjust the FSC voltage, FSC Amp Gain, and SSC voltages to appropriately display the scatter properties of your cells.

In the Detectors/Amps window, select linear Mode (Lin) for FSC. For SSC either Lin or log can be selected depending on your preference. Log should be selected for all fluorescence parameters **unless cell cycle analysis is to be performed** (See next section: addendum).
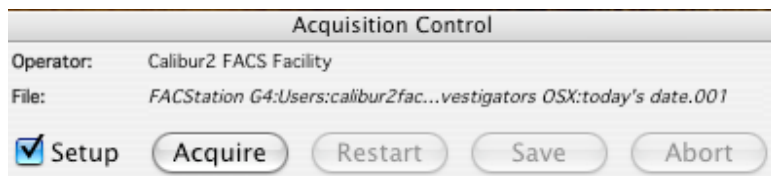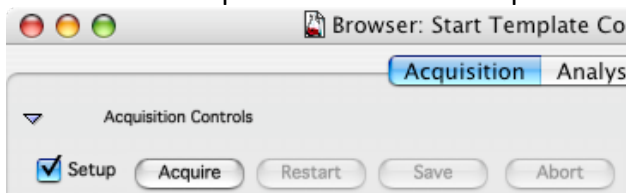
Lin or Log can be selected depending on your preference

Log should be chosen for all fluorescence parameters except when performing cell cycle analysis

Press RUN on the cytometer; then place an unstained control tube on the sample injection port (SIP).
TIP: Engage the arm underneath the tube quickly to avoid losing cells.

Ensure that Setup is checked in the Acquisition Control window or Browser window
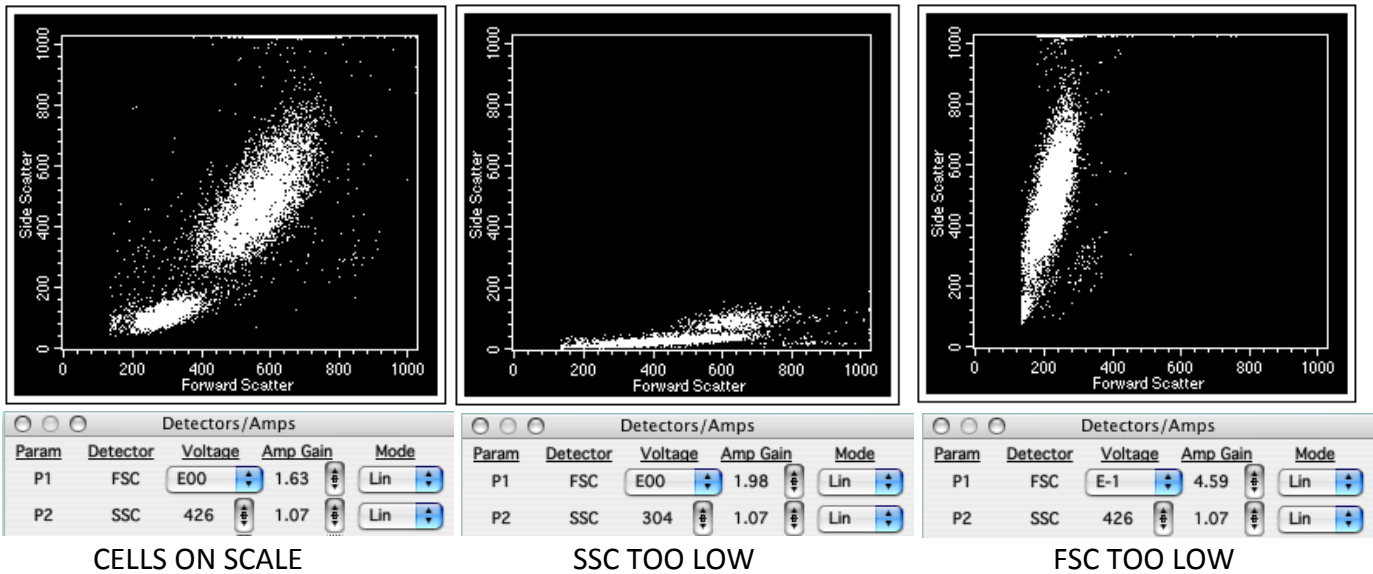

With setup checked, data **is not saved** to the computer's hard drive, it just appears on screen.

Acquire data. Note that Pause and Restart will refresh the data on the plots in the worksheet.
Adjust the FSC voltage multiplier from E00 to E-1, E01, or E02 as necessary to visualize your population of interest. Larger cells will need E-1 or E00; smaller cells may require E01 or E02. Next, adjust the FSC Amp Gain to place your population of interest on scale within the X axis of the FSC x SSC plot.
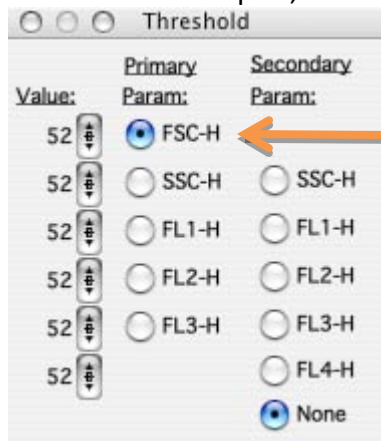

Adjust the FSC Amp Gain by clicking on the up or down arrow. Holding the option key while clicking adjusts the values by 10. You may also click the center of this button to reveal a slider that can be used to make large changes.

Adjust the SSC voltage to place your population of interest on scale within the Y axis of the FSC x SSC plot. Examples from lysed whole blood:
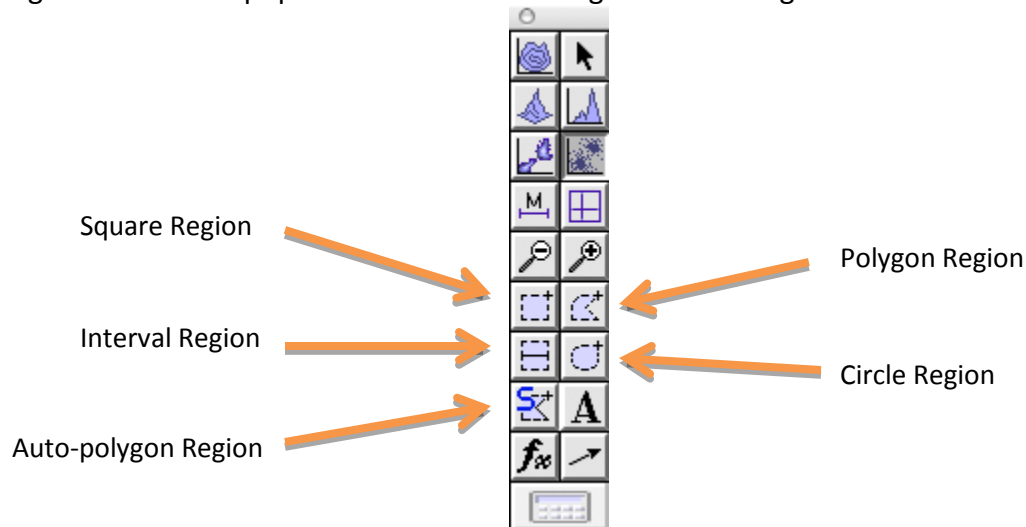

| | CELLS ON SCALE | SSC TOO LOW | FSC TOO LOW |

In the threshold window, adjust the FSC threshold value to remove debris from the dot plots.
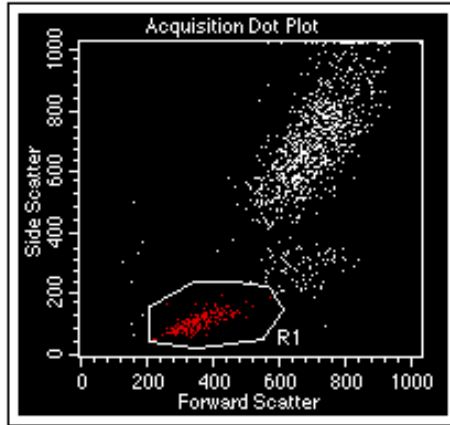In the above examples, the FSC threshold is set to 150.

Adjust the FSC-H threshold by clicking on the up or down arrow. Holding the option key while clicking adjusts the values by 10. You may also click the center of this button to reveal a slider that can be used to make large changes.

Draw a gate around the population of interest using one of the region tools in the tool palette.

Square Region
Interval Region
Auto-polygon Region
Polygon Region
Circle Region

Auto-polygon regions automatically adjust around the highest cell density. Use extreme caution when using these regions; make sure the automatic gate defines what you want it to define.

A regular polygon region is usually the best region to draw. For this tool, click to create a vertex move the mouse and click to create additional vertices, finish by double-clicking or click on the first vertex created. In the example below, the region is drawn around the lymphocytes from lysed whole blood.

Regions are not GATES! Regions can be turned into gates using the Gate definitions (⌘+G) or by navigating to *Gates>Gate List*.

Use your Region names to define Gates. Change the color of a gate and its dots by double clicking on the color next to the Gate in the Color column

CellQuest Pro uses logical operators for gating. For example, if you wanted to show a plot that shows events from two regions such as R1 and R2, then G1 would need to be defined as R1 AND R2. The other logical operators of gating are the words "OR", "NOT", & "AND NOT". By default R1=G1, R2=G2, and so on.
Logical command examples and their definitions:
"R1 AND R2" = everything in R1, then everything in R2 (event must appear in both R1 and R2)
"NOT R1" = everything outside of R1
"R1 OR R2" = everything inside R1 and R2
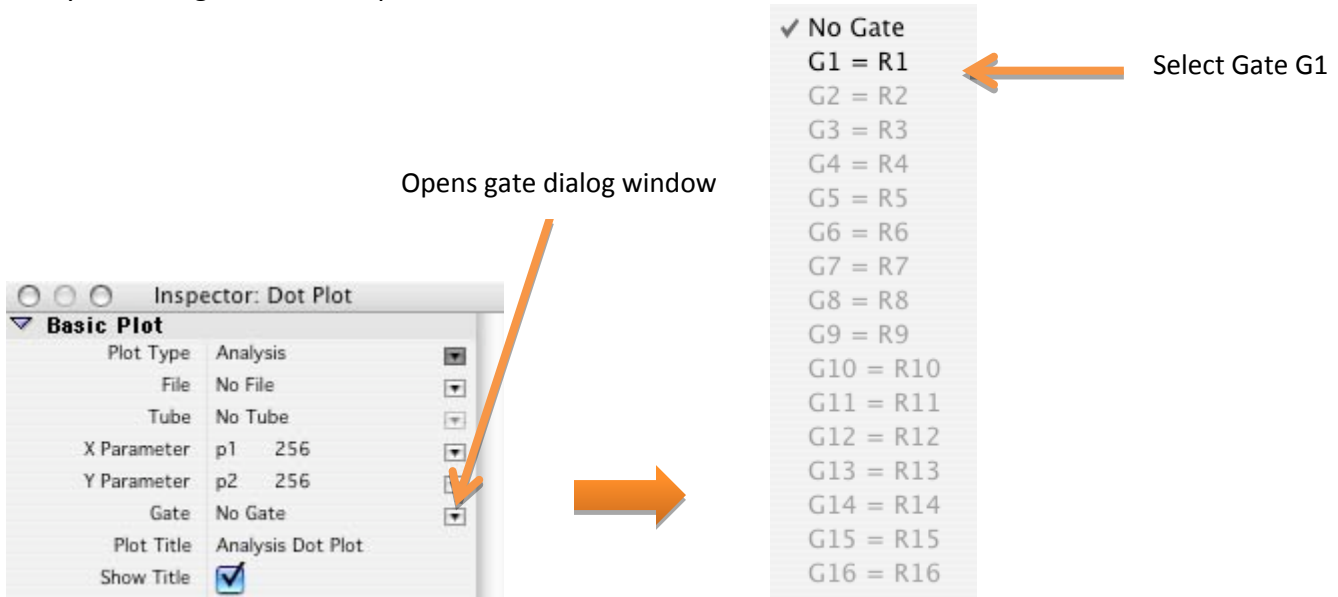"R1 AND NOT R2" = everything in R1 unless it appears in R2
"NOT R1 AND R2 = everything outside of R1 and R2
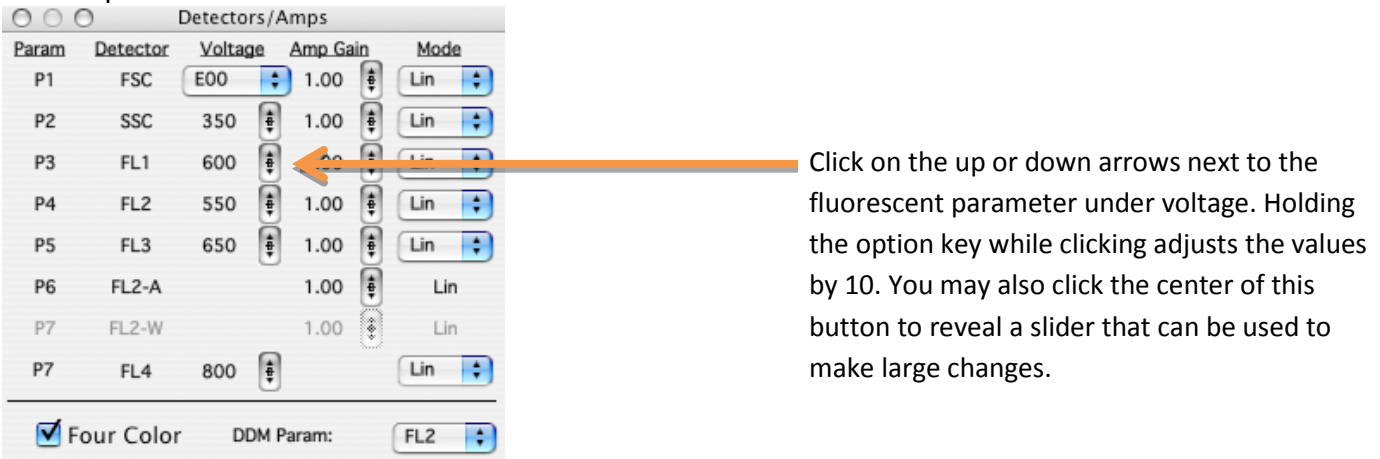The following command will not work: "R1 NOT R2"
Gates may also be used in logical operators e.g.: "G1 AND R2".
The more creative you get with these operators, the more likely you are to confuse yourself.
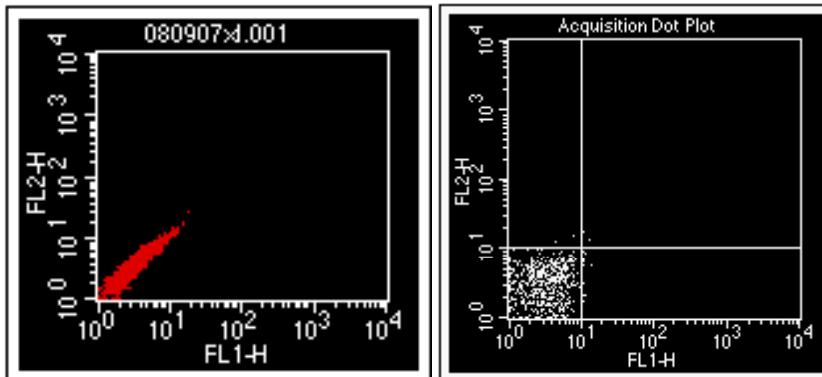
Apply the G1 gate to the fluorescent plots by selecting the plots (using the borders) and then choose your newly created gate in the Inspector window.

✓ No Gate
G1 = R1 ← Select Gate G1
G2 = R2
G3 = R3
G4 = R4
G5 = R5
G6 = R6
G7 = R7
G8 = R8
G9 = R9
G10 = R10
G11 = R11
G12 = R12
G13 = R13
G14 = R14
G15 = R15
G16 = R16

Opens gate dialog window

Inspector: Dot Plot
▽ **Basic Plot**
Plot Type	Analysis	▼
File	No File	▼
Tube	No Tube	▼
X Parameter	p1 256	▼
Y Parameter	p2 256	
Gate	No Gate	▼
Plot Title	Analysis Dot Plot	
Show Title	☑	

Now that the cells are on scale with regard to light scatter, begin to adjust the fluorescence detector voltages so that the unstained cells appear in the first log decade. Remember that Pause/Restart will quickly refresh the dot plots.

Detectors/Amps

Param	Detector	Voltage	Amp Gain	Mode
P1	FSC	E00	1.00	Lin
P2	SSC	350	1.00	Lin
P3	FL1	600		Lin
P4	FL2	550	1.00	Lin
P5	FL3	650	1.00	Lin
P6	FL2-A		1.00	Lin
P7	FL2-W		1.00	Lin
P7	FL4	800		Lin

☑ Four Color   DDM Param: FL2

Click on the up or down arrows next to the fluorescent parameter under voltage. Holding the option key while clicking adjusts the values by 10. You may also click the center of this button to reveal a slider that can be used to make large changes.

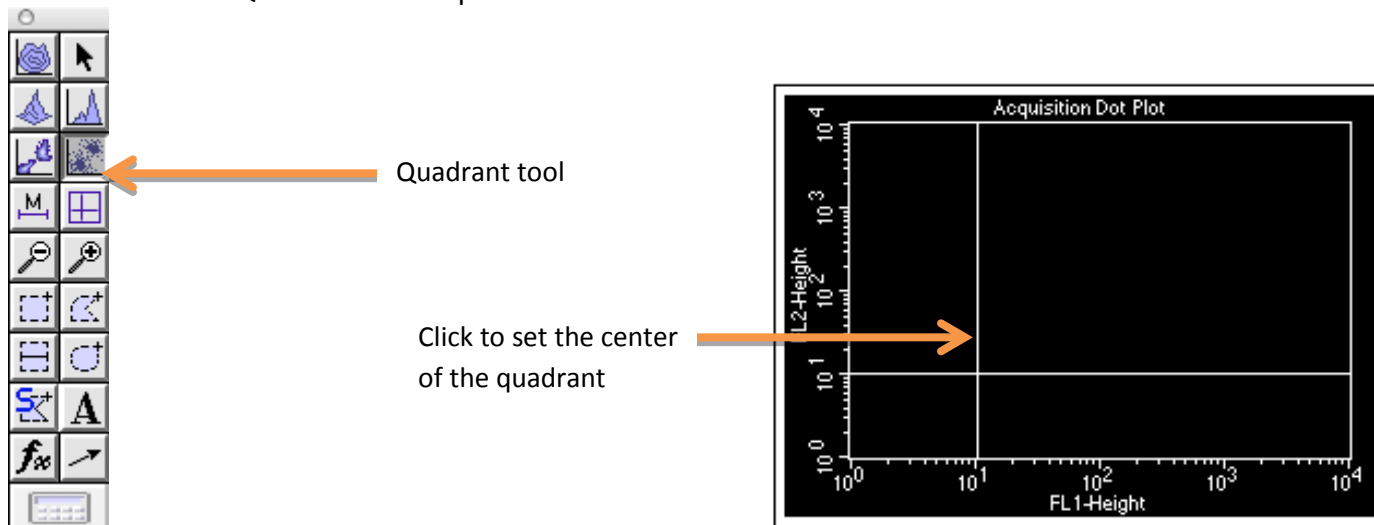Below: Examples of unstained cells with adjusted voltages from a cell line and lymphocytes, respectively.

Please note that you may need to reduce your voltages if positively stained cells are too bright and do not appear on scale.

Manual Compensation: Fluorescence spillover between detectors must be removed prior to acquisition of experimental samples.
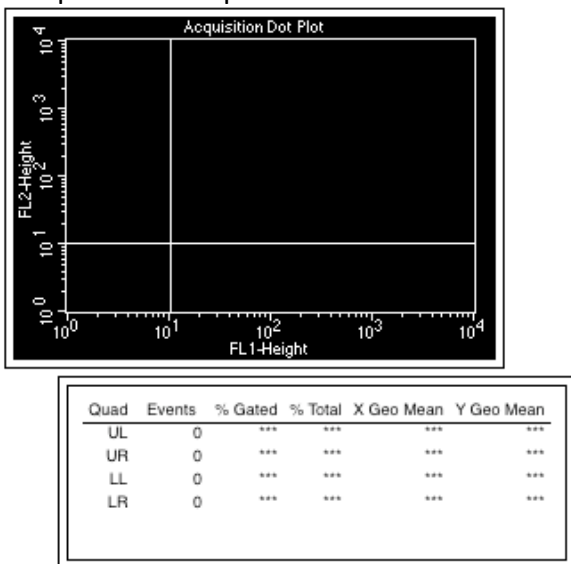
Single color controls are needed to adjust compensation values.

Add Quadrants to your fluorescence dot plots by selecting the Quadrant Tool, click within your plot, drag, and release when the Quadrants are in position.

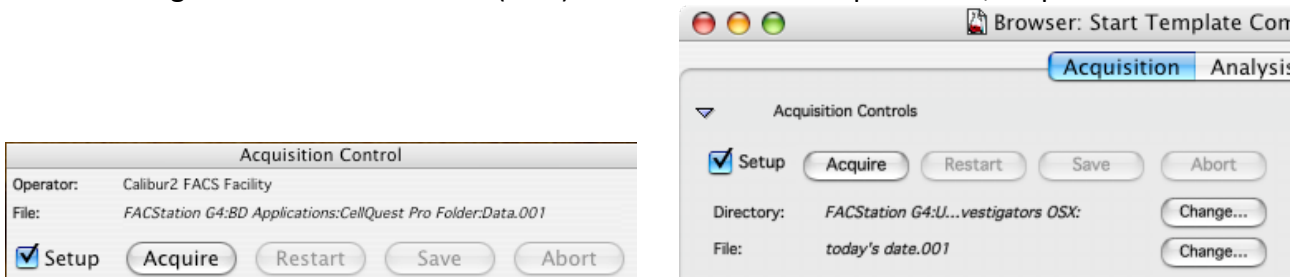
Quadrant tool

Click to set the center of the quadrant

Display Quadrant Statistics by selecting the Statistics menu and choosing Quadrants Stats.

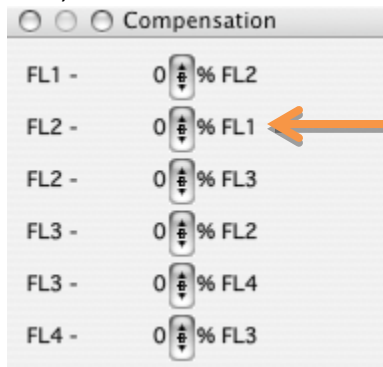Edit quadrant stats (select the border, then navigate to *Stat s> Edit Quadrant Stats*) to show the X Geo Mean and Y Geo Mean. Below is an example from the file "Start Template Compensation" which is located on the computer desktop.

Quad	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
UL	0	***	***	***	***
UR	0	***	***	***	***
LL	0	***	***	***	***
LR	0	***	***	***	***

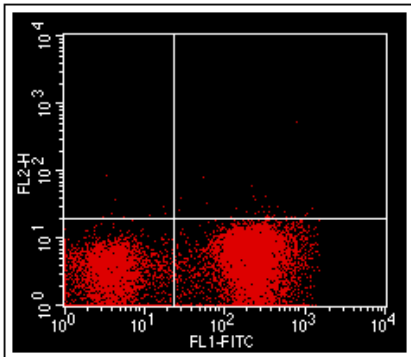Place the single color control for FL-1 (FITC) on the SIP. With Setup checked, Acquire data.

As the data populates your plots, adjust the values in the compensation window to remove FL1 spillover from FL-2, thus FL2-%FL1. Remember that Pause/Restart will quickly refresh the dot plots.
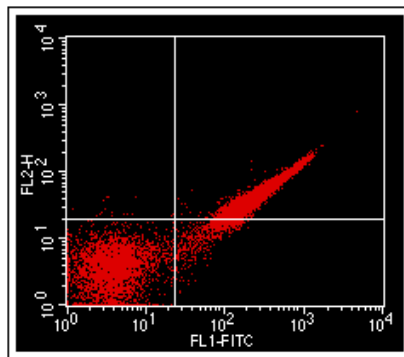

Click on the up or down arrows. Holding the option key while clicking adjusts the values by 10. You may also click the center of this button to reveal a slider that can be used to make large changes.
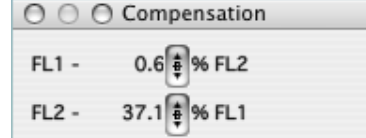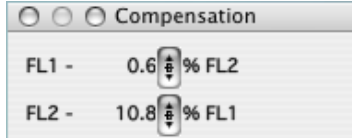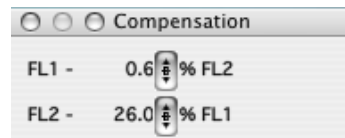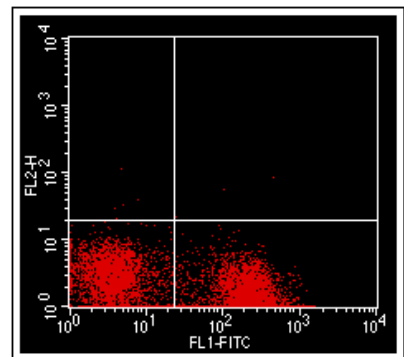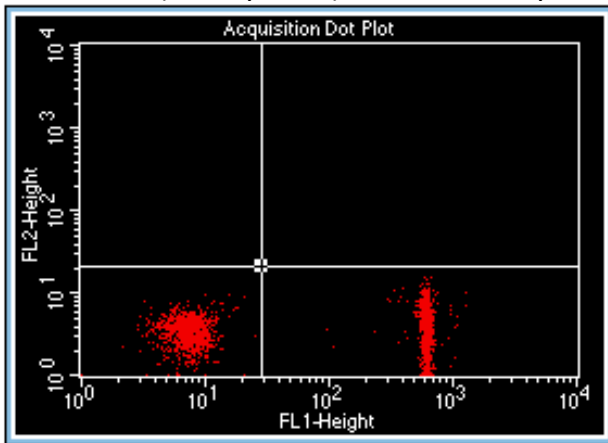
GOOD	TOO LITTLE	TOO MUCH

Use the Quadrant Stats to help adjust your compensation values. The Y Geo Mean (FL2) for LL and LR should be identical (or very close) NOTE: You may need to move the quadrants so all cells appear in one quadrant.

Quad	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
UL	0	0.00	***	***	***
UR	0	0.00	***	***	***
LL	21	52.50	***	7.29	3.50
LR	19	47.50	***	603.44	3.74

Continue compensation for all fluorescence parameters using single stained controls for FL2, FL3, and FL4.

When you are satisfied with your compensation, save your instrument settings by navigating to *Cytometer>Instrument Settings…* and select the Save… button. When prompted, select an appropriate file location and name the instrument settings file.

Save button- saves instrument settings file

Open button- open saved instrument settings file

Set button- applies instrument settings from opened file to the cytometer

Done- closes the window, <u>does not</u> set instrument settings
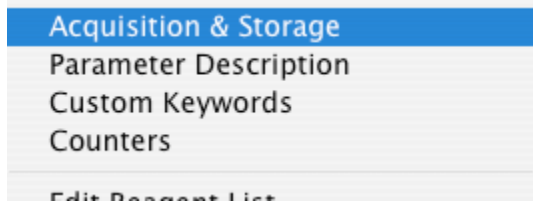
Rainbow Pinwheel (of death) - shhh… the MAC is thinking

You may use these settings in future experiments to save time during setup. To do so, navigate to *Cytometer>Instrument Settings…*, open your instrument settings file and select the Set button. Wait for the "Rainbow Pinwheel" to finish spinning and select the Done… button. **Always recheck voltages and compensation values for accuracy using single color controls.**
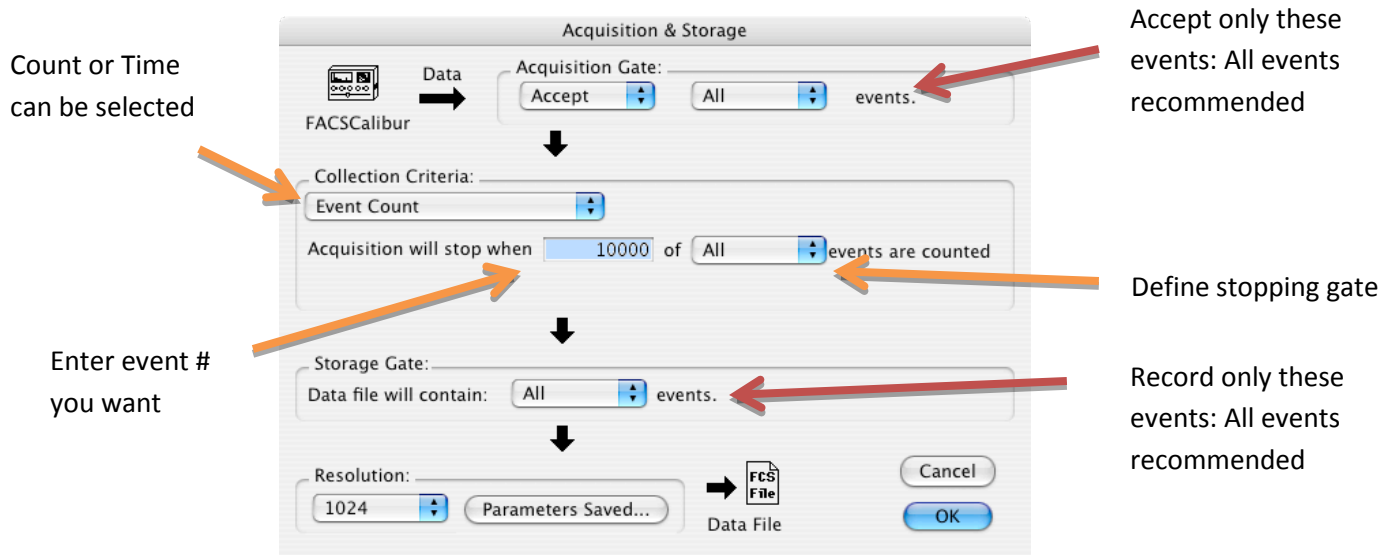
It is recommended that you acquire and save your unstained and single color controls as individual files for future reference before collecting your experimental samples. Remember to <u>uncheck Setup</u> from the Browser or Acquisition Control window when you are ready to collect experimental sample files.

Sample Collection:
Set the number of events you are interested in collecting by navigating to *Acquisition>Acquisition & Storage.*

In the Acquisition & Storage window, enter the appropriate information for your experiment.

Count or Time
can be selected

Accept only these
events: All events
recommended

Define stopping gate

Enter event #
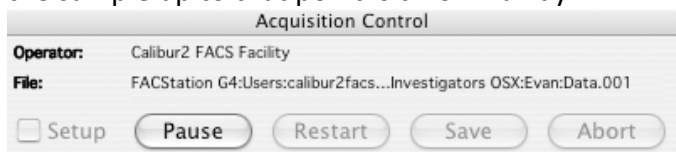you want

Record only these
events: All events
recommended

Select OK when finished.

Put your first experimental sample on the SIP.

In either the Browser or the Aquisition Control windows, select Acquire with Setup unchecked.

Data will begin to populate your plots.

You may elect to Pause & then Restart or Pause & then Abort if you want to re-record your sample; all data for the sample up to that point is thrown away.
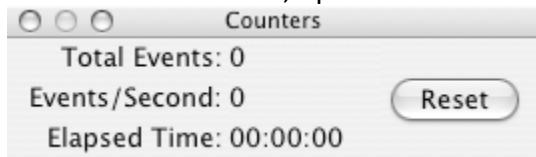

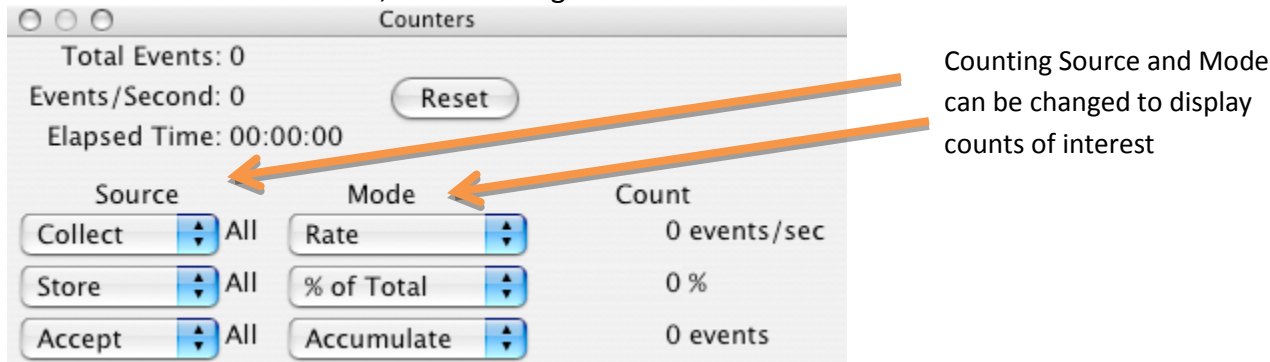If you believe you have finished collecting your sample or if your sample is gone, press Pause and then Save. DATA WILL NOT BE SAVED IF YOU PAUSE AND DO NOT HIT SAVE. However, if the event number set in Acquisition & Storage is fulfilled, the cytometer will automatically save the data file.

Keep the event rate lower than 3000 events/second during recording. Adjust the speed by pressing LOW, MED, or HIGH on the cytometer.

To see the event rate, open the counters window by navigating to *Acquire> Counters.*

For more advanced counters, click on the green "+" in the window bar.

Counting Source and Mode
can be changed to display
counts of interest

FCS2.0 files are saved in the location you defined during your initial setup. Back up these files and bring them with you for post-acquisition analysis.

Clean and Prepare for the next user:

Put a tube with ~3ml (~half tube) BLEACH (squeeze bottle) on the SIP and run in HIGH with the arm to the side for 1 minute. This step is crucial to decontaminate the instrument.

Run the same tube of BLEACH in HIGH for 3 minutes with the arm underneath the tube. This step is crucial to clean the flow cell.

Repeat #1 and #2 with dWATER. This step clears the instrument of BLEACH and prevents your cells from popping in the future.

Leave ~1mL of dWATER on the SIP and hit STANDBY.

Be Kind: Check sheath/waste and refill/empty as necessary.

If you finish after 6 PM on a normal weekday or any time on the weekend and you do not have visual or telephone confirmation from the next scheduled user, switch OFF the instrument.

Shutdown *(Last user of the day):*

Quit CellQuest pro.

Shut down the Macintosh workstation BEFORE turning off the cytometer.
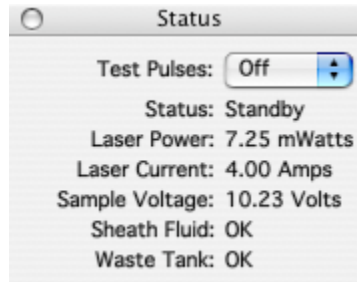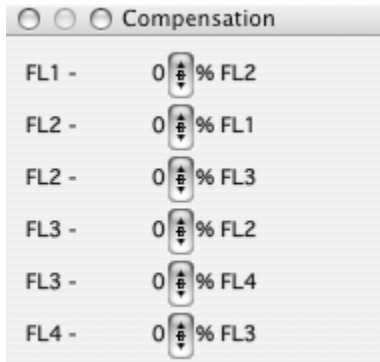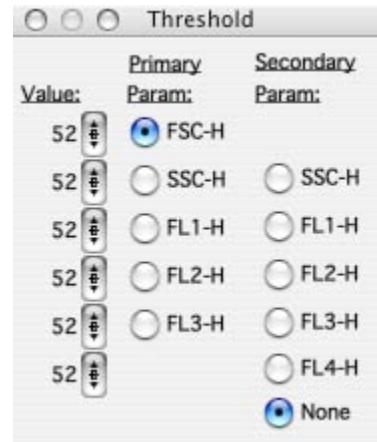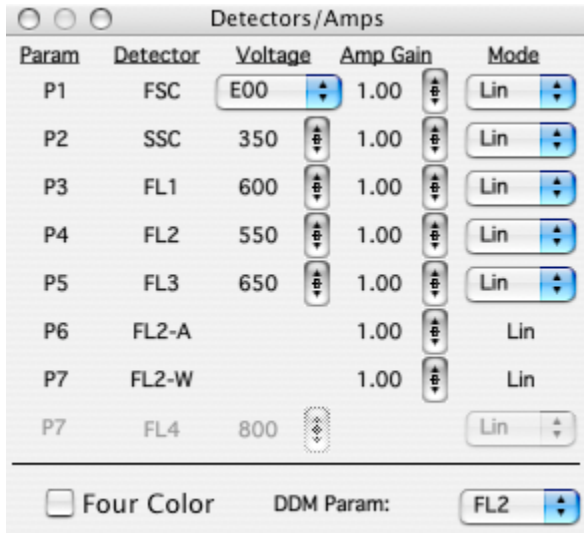
Addendum:

DNA / Cell Cycle Analysis Using CellQuest Pro

Cytometer settings:

Open cytometer control panels: With the instrument connected (described above), open the cytometer controls by navigating to *Cytometer > Detectors/Amps, Cytometer > Threshold, Cytometer > Compensation,* and *Cytometer > Status*. Alternatively, use keyboard shortcuts ⌘+1, ⌘+2, ⌘+3, ⌘+4.
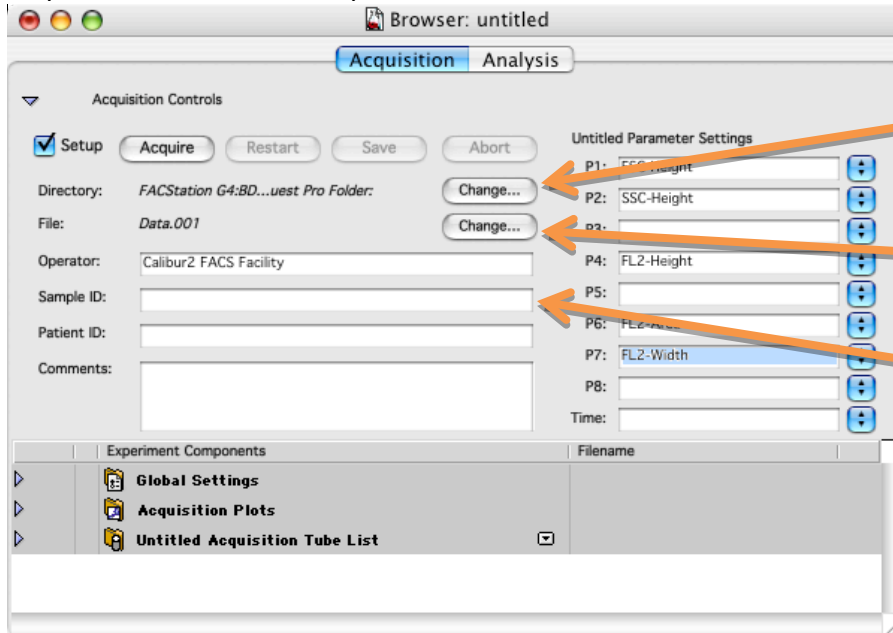
Move each window to a viewable location.

You want to be able to see these controls, as well as the Browser window, Acquisition Control window and Worksheet window (and perhaps Counters window; *Acquire > Counters*)

Be sure Four Color is <u>not</u> selected in the Detectors / Amps window.

Select file save location and define reagents: If it isn't already, open the Browser window by navigating to *Acquire> Parameter Description*
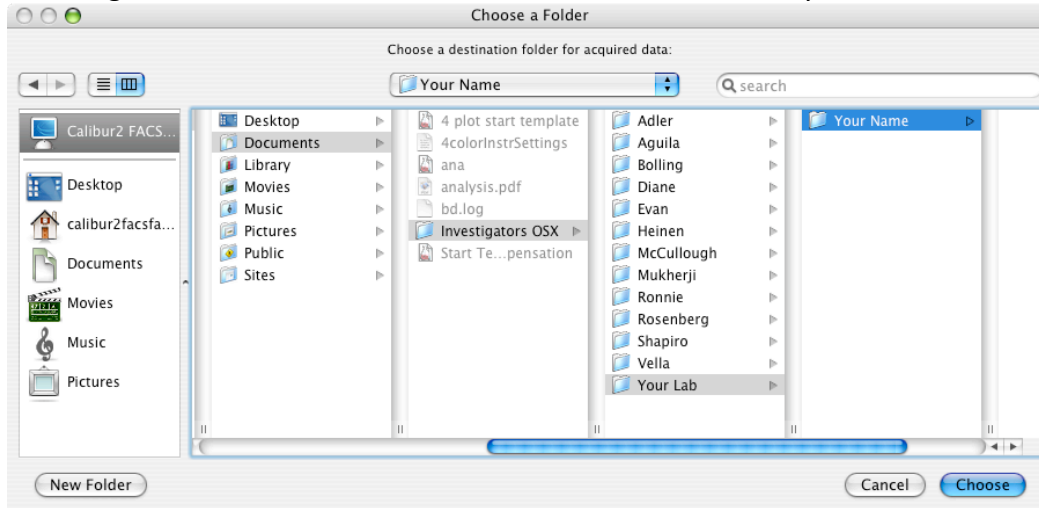

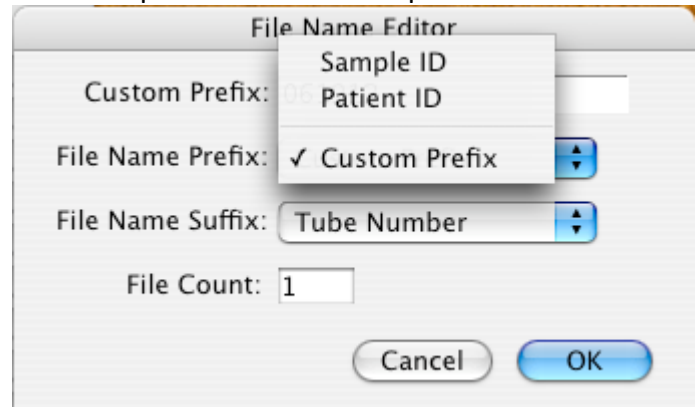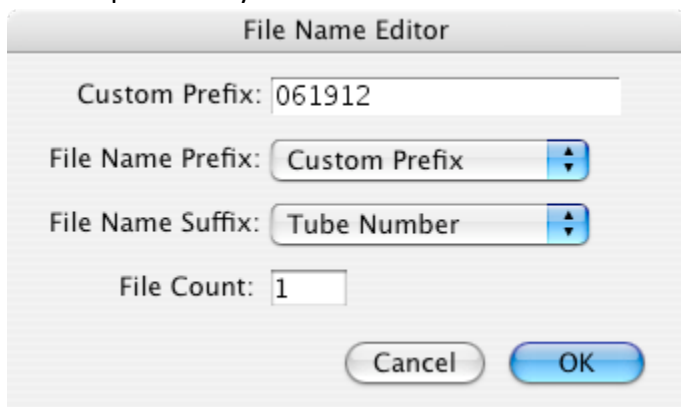Directory: Choose the Folder where files will be saved

File: define name of files saved

Sample ID: create a description of the sample

In the Browser window, choose the folder where your files will be saved by selecting the "Change…" button next to "Directory:". In the next window, navigate to your lab's folder and create your own folder within it by selecting the New Folder button on the bottom left. Rename your folder and select Choose.

Define the name of each file by the selecting the "Change…" button. In the next window you can choose a custom prefix for your file name or select one of the predefined prefixes such as Sample ID or Patient ID.
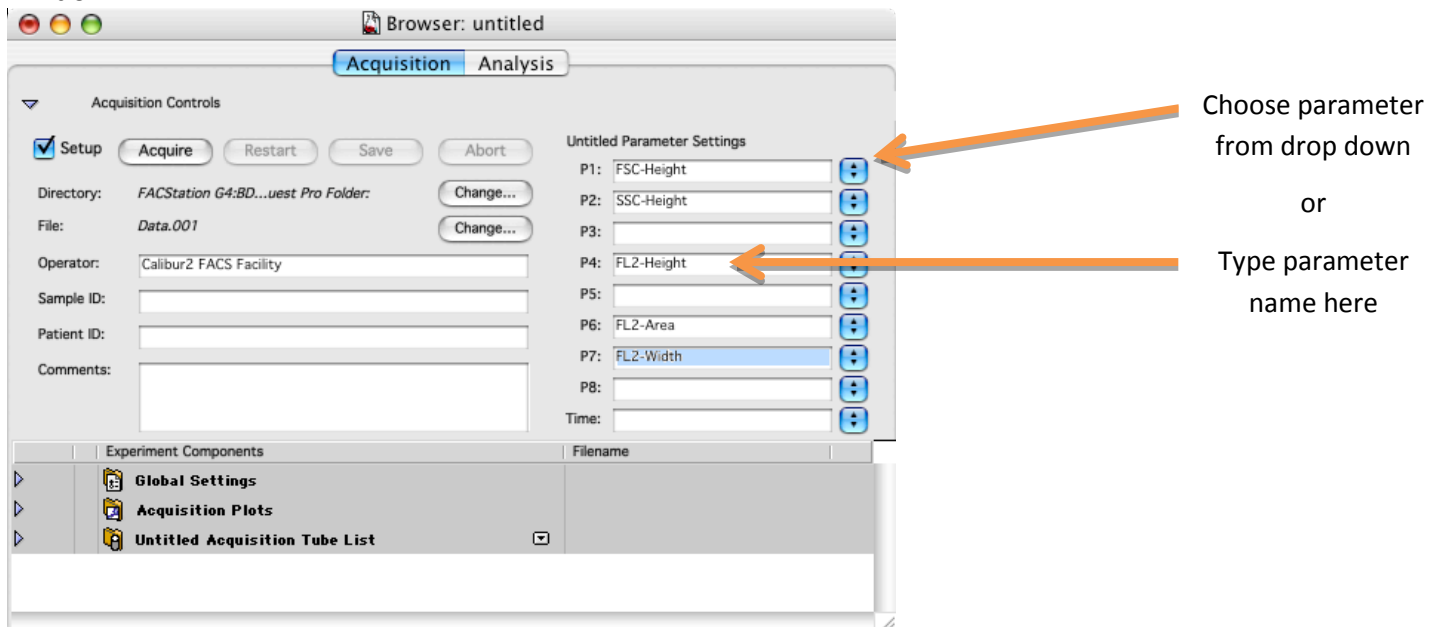
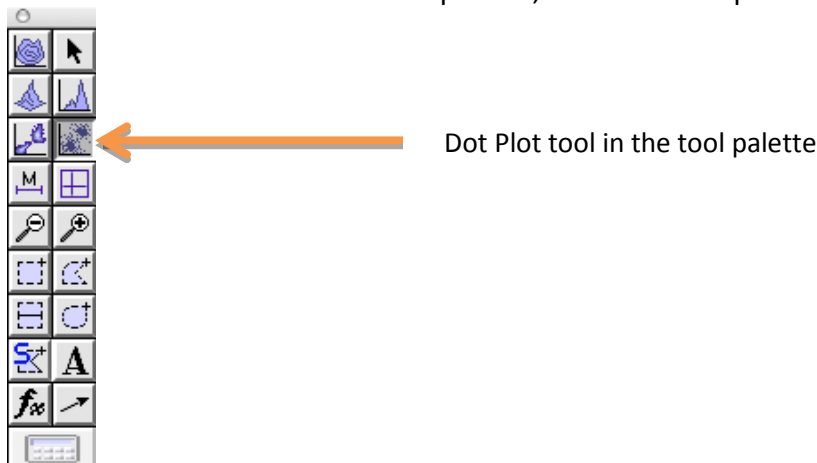The File Name Suffix should be File increment unless you're using Tube Panels (**CellQuest Pro-advanced**).

In the Sample ID box, type in a name for your sample that describes it accurately. Some examples include: unstained control, Mouse 1, spleen 4, isotype control FITC, dox-treated 15, etc. These can be used in programs such as FlowJo to sort your samples during analysis.

Note: you can save other Keywords that will be associated with your sample under "Patient ID:" and "comments:"

Select your parameter labels in the Browser window. Labels can be generic (e.g.: FL-1 Height) or specific (e.g.: Propidium Iodide). Some labels appear in the drop down menu beside each parameters, however, custom labels can be typed in the box. Match parameters and FL detectors carefully; compare using *Detectors/Amps* window.

Choose parameter from drop down

or

Type parameter name here

Create Worksheet Elements: In the tool palette, select the dot plot tool.

Dot Plot tool in the tool palette

Note: You can create a histogram, density plot, or contour plot by selecting those tools from the tool palette
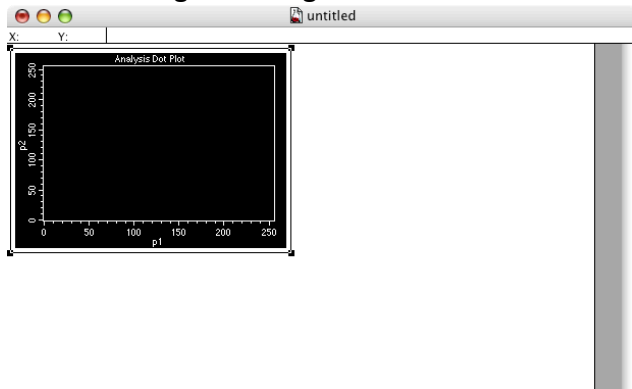
 Histogram tool   Density plot tool   Contour plot tool

Click and drag a rectangle in the worksheet to create a dot plot.

Select the white border of the dot plot, navigate to *Windows > Show inspector* (shortcut ⌘+I)

In the inspector window, change the Plot Type to Acquisition or Acq-> Analysis.

Change the X parameter to reflect your Forward Scatter Label and change the Y Parameter to reflect your Side Scatter label.

You may also adjust the number of events shown within the plot (lower numbers refresh faster) and the color of the dots within the plot.

Dot color can be changed here

Create other plots on the worksheet and choose the fluorescence parameters that correspond to your experiment. You should try to observe all fluorescence parameters during collection.

Below is an example of a PI/ Cell cycle analysis window:

This DNA cell cycle template file is saved on the desktop.

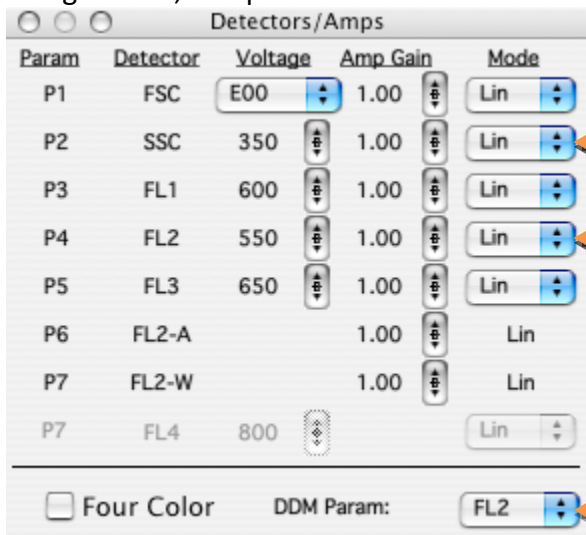**Adjusting FSC and SSC Settings:** You need to adjust the FSC voltage, FSC Amp Gain, and SSC voltages to appropriately display the scatter properties of your cells.

In the Detectors/Amps window, select linear Mode (Lin) for FSC. For SSC either Lin or log can be selected depending on your preference. Linear (Lin) should be selected for all DNA fluorescence parameters.

Doublet Discrimination: Select the doublet discrimination mode (DDM) that fits your experiment's DNA dye. Area (A) is the best parameter to use for detection of DNA content and is not available unless DDM is chosen correctly. You may choose to collect FL-1, FL-2, or FL-3 depending on your dye's fluorescence characteristics. PI is generally collected in FL-2, but FL-3 may also be used. If FL-1 fluorescence (GFP, etc.) is to be examined along with PI, compensation of FL1 and FL2 is possible, whereas compensation of FL1 and FL3 is not.

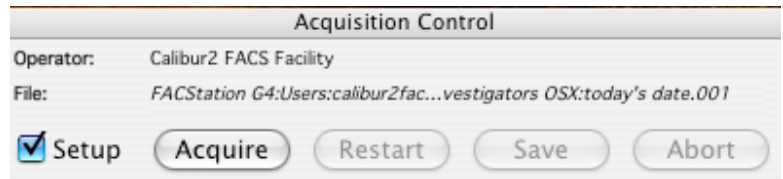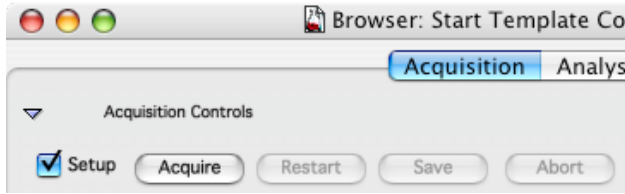Lin or Log can be selected depending on your preference

Lin should be chosen for all DNA fluorescence parameters

Choose DDM Parameter for DNA dye

Press RUN and LOW on the cytometer; then place an unstained control tube on the sample injection port (SIP).
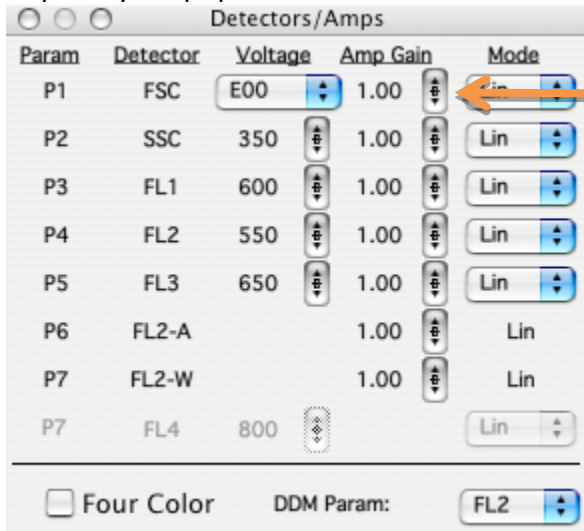**TIP:** Engage the arm underneath the tube quickly to avoid losing cells.

Ensure that Setup is checked in the Acquisition Control window or Browser window

With setup checked, data **is not saved** to the computer's hard drive, it just appears on screen.
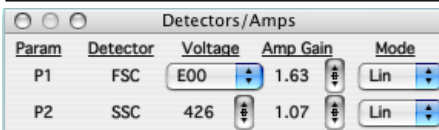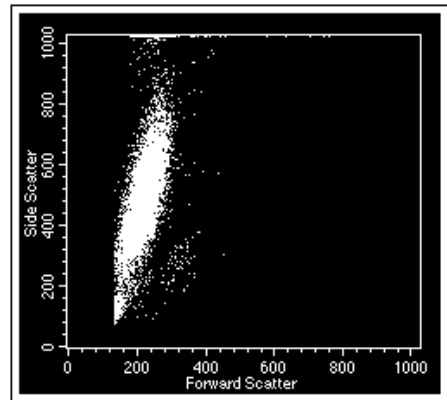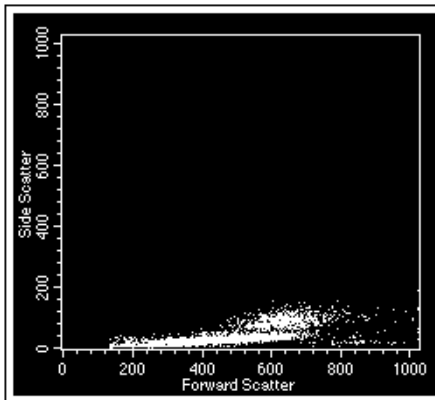
Acquire data. Note that Pause and Restart will refresh the data on the plots in the worksheet.
Adjust the FSC voltage multiplier from E00 to E-1, E01, or E02 as necessary to visualize your population of interest. Larger cells will need E-1 or E00; smaller cells may require E01 or E02. Next, adjust the FSC Amp Gain to place your population of interest on scale within the X axis of the FSC x SSC plot.

Adjust the FSC Amp Gain by clicking on the up or down arrow. Holding the option key while clicking adjusts the values by 10. You may also click the center of this button to reveal a slider that can be used to make large changes.

Adjust the SSC voltage to place your population of interest on scale within the Y axis of the FSC x SSC plot. Examples from lysed whole blood:

CELLS ON SCALE   SSC TOO LOW   FSC TOO LOW

In the threshold window, adjust the FSC threshold value to remove debris from the dot plots. In the above examples, the FSC threshold is set to 150.

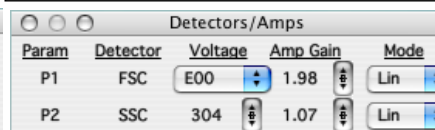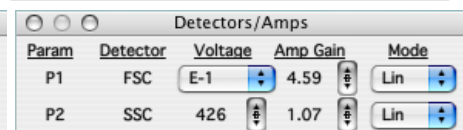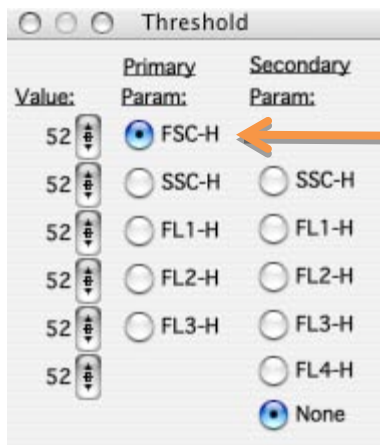Adjust the FSC-H threshold by clicking on the up or down arrow. Holding the option key while clicking adjusts the values by 10. You may also click the center of this button to reveal a slider that can be used to make large changes.

Draw a gate around the population of interest using one of the region tools in the tool palette.

Square Region

Interval Region

Auto-polygon Region

Polygon Region

Circle Region

Auto-polygon regions automatically adjust around the highest cell density. Use extreme caution when using these regions; make sure the automatic gate defines what you want it to define.

A regular polygon region is usually the best region to draw. For this tool, click to create a vertex, move the mouse and click to create additional vertices, finish by double-clicking or click on the first vertex created. In the example below, the region is drawn around the lymphocytes from lysed whole blood.

Regions are not GATES! Regions can be turned into gates using the Gate definitions (⌘+G)

Use your Region names to define Gates. Change the color of a gate and its dots by double clicking on the color next to the Gate in the Color column

CellQuest Pro uses logical operators for gating. For example, if you wanted to show a plot that shows events from two regions such as R1 and R2, then G1 would need to be defined as R1 AND R2. The other logical operators of gating are the words "OR", "NOT", & "AND NOT". By default R1=G1, R2=G2, and so on.
Logical command examples and their definitions:
"R1 AND R2" = everything in R1, then everything in R2 (event must appear in both R1 and R2)
"NOT R1" = everything outside of R1
"R1 OR R2" = everything inside R1 and R2
"R1 AND NOT R2" = everything in R1 unless it appears in R2
"NOT R1 AND R2 = everything outside of R1 and R2
The following command will not work: "R1 NOT R2"
Gates may also be used in logical operators e.g.: "G1 AND R2".
The more creative you get with these operators, the more likely you are to confuse yourself.

Fluorescence parameter adjustment:
Now that the cells are on scale with regard to light scatter, begin to adjust the fluorescence detector voltages so that G0/G1 cells appear directly over 200. Remember that Pause/Restart will quickly refresh the dot plots and histograms.

Click on the up or down arrows next to the fluorescent parameter under voltage. Holding the option key while clicking adjusts the values by 10. You may also click the center of this button to reveal a slider that can be used to make large changes.

Below is how the DNA histogram should look when the G0/G1 peak is properly adjusted:

Doublet discrimination: To eliminate doublets and debris from your analysis, make a doublet discrimination gate by displaying Width (W) versus Area (A) for your DNA dye. Gate on the events that have one width of DNA content. In the example below, the width parameter can be spread out by adjusting the FL2-W amp gain.

Cells with one width DNA content: single cells

Doublets & aggregates

Increase the spread of width data by adjusting Amp Gain

Histogram statistics: A good DNA / cell cycle analysis will center the G0/G1 peak over 200 and will also have a narrow (low CV) G0/G1 peak. To determine these values, create a marker over your G0/G1 peak by selecting the marker tool from the tool palette, then click and drag the mouse pointer over the G0/G1 peak.

 Marker tool

Show the histogram statistics for DNA histograms by selecting the border of the histogram, then navigate to *Stats>Histogram Stats.* When the histogram stats window appears, the inspector window (⌘ + i) will list the various information that can be displayed, be sure to include M1 statistics and display CV. Note the median of M1 in the sample below.

Histogram Statistics

File: bl032612.001   Sample ID: Hec59
Patient ID: Mock no treatment   Acquisition Date: 26-Mar-12
Gate: G5   Gated Events: 50411
Total Events: 56633

Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	0, 1023	50411	100.00	89.01	258.19	245.36	35.96	206.00	198
M1	162, 240	31391	62.27	55.43	200.10	199.76	5.91	199.00	198

When you are satisfied with your adjustments, save your instrument settings by navigating to *Cytometer>Instrument Settings…* and select the Save… button. When prompted, select an appropriate file location and name the instrument settings file.

Instrument Settings

Cytometer Type: FACSCalibur

Detectors/Amps:
Param	Detector	Voltage	AmpGain	Mode
P1	FSC	E00	2.50	Lin
P2	SSC	312	3.72	Lin
P3	FL1	655	1.00	Log
P4	FL2	555	1.00	Log
P5	FL3	752	1.00	Log
P6	FL1-A		1.00	Lin
P7	FL4	701		Log

Threshold:
Primary Parameter: FSC
Value: 52

Secondary Parameter: None

Compensation:
FL1 - 1.5 % FL2
FL2 - 14.7 % FL1
FL2 - 0.0 % FL3
FL3 - 19.4 % FL2
FL3 - 1.9 % FL4
FL4 - 2.1 % FL3

Displaying: Current Status   Revert

Print…   Save…   Open…   Set   Done

Save button- saves instrument settings file

Open button- open saved instrument settings file

Set button- applies instrument settings from opened file to the cytometer

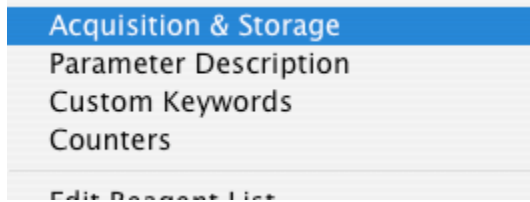**Done**- closes the window, <u>does not</u> set instrument settings

Rainbow Pinwheel (of death) - shhh… the MAC is thinking

You may use these settings in future experiments to save time during setup. To do so, navigate to *Cytometer>Instrument Settings…,* open your instrument settings file and select the Set button. Wait for the "Rainbow Pinwheel" to finish spinning and select the Done… button. **Always recheck Amp Gain and voltages for accuracy with each new experiment.**

Sample Collection:

Set the number of events you are interested in collecting by navigating to *Acquisition> Acquisition & Storage.*

In the Acquisition & Storage window, enter the appropriate information for your experiment.
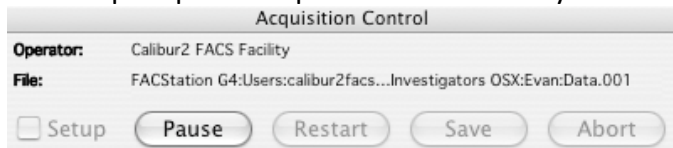

Select OK when finished.

Put your first experimental sample on the SIP.

In either the Browser or the Aquisition Control windows, select Acquire with Setup unchecked.

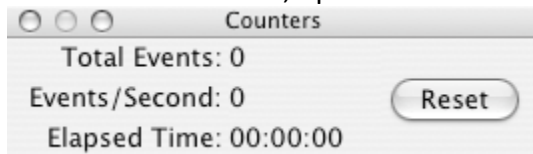Data will begin to populate your plots.

You may elect to Pause & then Restart or Pause & then Abort if you want to re-record your sample; all data for the sample up to that point is thrown away.
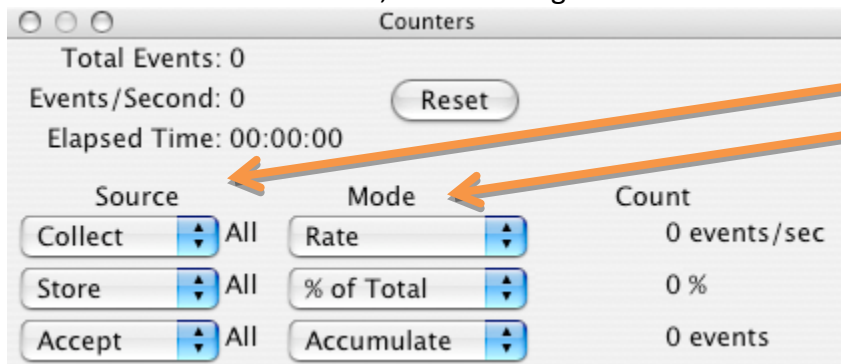

If you believe you have finished collecting your sample or if your sample is gone, press Pause and then Save. DATA WILL NOT BE SAVED IF YOU PAUSE AND DO NOT HIT SAVE. However, if the event number set in Acquisition & Storage is fulfilled, the cytometer will automatically save the data file.

Keep the event rate lower than 400 events/second during recording. Do not deviate from LOW. If your event rate is above 400 events/second, add more buffer with PI to your sample. Running in MED or HIGH will ruin the resolution of your experiment, increase your G0/G1 CV, and potentially prevent analysis of G0/G1 versus G2/M.

To see the event rate, open the counters window by navigating to *Acquire> Counters.*

For more advanced counters, click on the green "+" in the window bar.

Counting Source and Mode can be changed to display counts of interest

FCS2.0 files are saved in the location you defined during your initial setup. Back up these files and bring them with you for post-acquisition analysis.

Clean and Prepare for the next user:

Put a tube with ~3ml (~half tube) BLEACH (squeeze bottle) on the SIP and run in HIGH with the arm to the side for 1 minute. This step is crucial to decontaminate the instrument.

Run the same tube of BLEACH in HIGH for 3 minutes with the arm underneath the tube. This step is crucial to clean the flow cell.

Repeat #1 and #2 with dWATER. This step clears the instrument of BLEACH and prevents your cells from popping in the future.

Leave ~1mL of dWATER on the SIP and hit STANDBY.

Be Kind: Check sheath/waste and refill/empty as necessary.

If you finish after 6 PM on a normal weekday or any time on the weekend and you do not have visual or telephone confirmation from the next scheduled user, switch OFF the instrument.

Shutdown *(Last user of the day):*

Quit CellQuest pro.

Shut down the Macintosh workstation BEFORE turning off the cytometer.