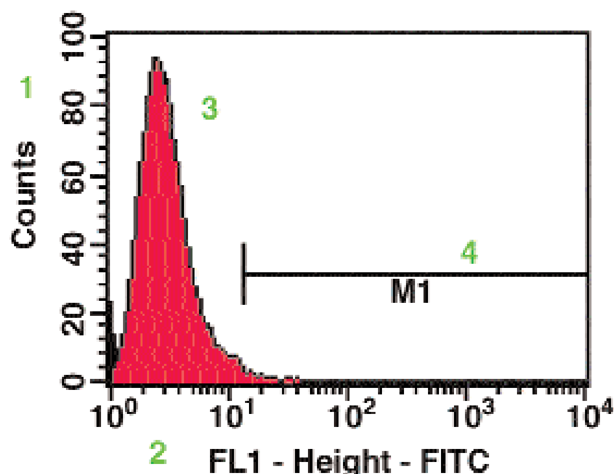
Understanding FACS

FACS data: The Controls

by Brian Russell

In every biological experiment, controls are a vital and necessary part. The same is true for flow cytometry: negative control samples establish a baseline fluorescence intensity to which positive samples can be compared. The figure shows a typical negative control. In this case, autofluorescence is minimal. Other controls that could overlap with this histogram are cells incubated with either primary or secondary antibody. Any shift in these controls would be due to non-specific binding, and may be considered better controls because they measure the non-specific binding that will need to be subtracted from the experimental sample. Many researchers also use positive controls; these will be discussed in the next issue of this newsletter.



1. The "counts" represent the number of cells or events, per sample, detected at a particular intensity of fluorescence. Generally 10,000 events are collected per sample, but a low cell number in an individual sample can result in fewer events collected, and will then express as a smaller peak.
2. The x-axis is usually labeled with the type of fluorescent dye used (in this case FITC), and describes a gradient of fluorescence intensity. An increase in fluorescence intensity (shifting of the peak to the right) is a representation of the increased ratio of moles of dye bound to the cell.
3. This histogram is typical of what a control sample should look like. The peak is very focused, narrow, and tall. This would indicate that any fluorescence that is being emitted is due to the inherent characteristics of the cell in question. The location of the peak along the x-axis is adjusted prior to running the samples so that the left edge of the control peak just touches the left hand corner.
4. The marker (M1) is placed in the histogram to identify a region; the left edge of that marker is a reference point to compare different samples in a particular group, relative to the control. Markers are set during the analysis of the samples and have no effect on the number or type of cells collected. The marker is set such that <1% of the control peak extends beyond the left edge of the marker into the M1 region. It is important to have a small portion of the control peak in the marker region so that an accurate comparison can be made to later samples. However, it is essential to have the marker set at <1% so that small shifts can also be detected. Any shift to the right in subsequent samples will result in more of the peak inside the marker region as compared to the control.

Gate: No Gate — Gated Events: 10000
Total Events: 10000 — X Parameter: FL1-H FL1-Height-FITC (Log)

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Marker	Left, Right	Events	% Gated	% Total	Mean	Geo Mean	CV	Median	Peak Ch
All	1, 9910	10000	100.00	100.00	3.33	2.89	71.87	2.69	2
M1	13, 9910	90	0.90	0.90	19.09	18.28	32.99	17.00	14

5. The table of statistics shows a number of key facts. The first column lists the names of the markers used in the histogram. The numbers to the right of the names indicate the position of the left and right edges of each marker in relation to the x-axis. The "Events" column lists how many cells were collected (All) and how many fell inside a particular marker's parameters (M1). The "% Gated" column indicates the percentage of the events collected that fall inside the marker (M1) region. The larger the "% Gated" number, the greater the number of events that express higher levels of fluorescence, and correspondingly fall inside the marker region. The other important column is the "Mean" column which conveys the average fluorescence intensity for the chosen parameter. Two samples can both demonstrate a 99% shift compared to the control, but the mean value can indicate which one has the higher fluorescence intensity.

Next Issue: A discussion of the various types and shapes of peaks that demonstrate positive expression.