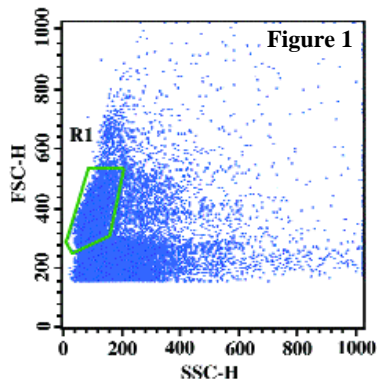


FACS data: The Dot Plot

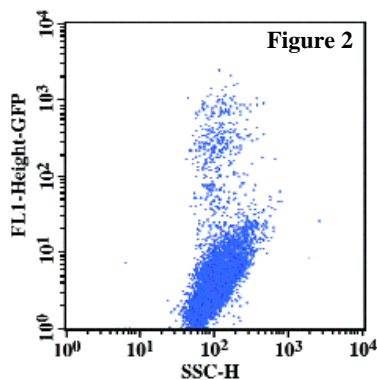
by Brian Russell

An important tool for evaluating any FACS data is the dot plot. As a cell intersects the laser beam, the instrument detects it as a point on an x-y graph. This form of data presentation looks at two parameters of the sample at the same time. The parameters can be any combination of scatter and fluorescence. Three common modes of use for the dot plot are the scatter plot: 1) forward scatter (FSC) vs. side scatter (SSC); 2) single color vs. side scatter; and 3) two-color fluorescence plot.

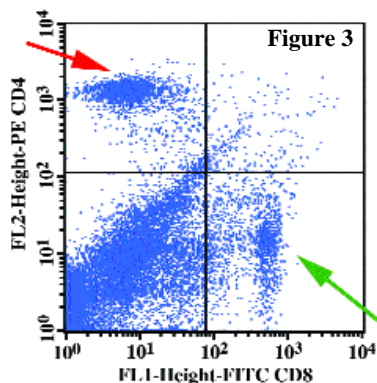
In the scatter plot of FSC vs. SSC, one is able to look at the distribution of cells based upon size. As the laser passes through the stream, light is deflected and refracted by the cells in the stream. This scattered light is collected by the FSC and SSC photodiodes. Collected light is interpreted by the FACS analysis machine and software to present a plot of points on a set of axes that correspond to the morphology of the cells in the stream.



The axes are set up such that SSC is most commonly on the x-axis, and FSC is on the y-axis. The distribution of the dots in the plot can distinguish one type of cell from another and allow one to gate around one particular population of cells for further analysis. Larger cells are represented as higher values along the y-axis, while cells that are more granular (more objects inside the cell to refract the laser) are represented as higher values along the x-axis. The example in **Figure 1** is of human blood cells. The lymphocytes (**gated**) form a tight population above the smaller red blood cells. The monocytes reside above the gated lymphocytes due to their larger size (measured along the FSC y-axis). The cells above and to the right of the gated region are granulocytes that are not only larger in size than the lymphocytes, but also are more granular and as such are expressed further to the right along the SSC x-axis.



When using only a single fluorescent dye (FITC, for example), you can use a dot plot vs. SSC to visualize the expression of the fluorescence of the cells (**Figure 2**). One advantage to looking at single color data with a dot plot as opposed to a histogram is that one can look at variances in expression as single events (dot plot), rather than as a collection of events shown as a vertical projection of counts at a particular fluorescence (histogram). Another advantage is that the events that demonstrate high levels of expression can be hard to visualize in the diminishing tail of a histogram fluorescent frequency diagram. With a dot plot on the other hand, because every event is expressed as a separate dot, the few events that may demonstrate high levels of fluorescence are also differentiated by granularity (how much stuff is inside the cell) with respect to SSC. Adding a second parameter gives the operator a clearer picture of the amount of expression in each sample.



The most common use for dot plots is to analyze multiple colors of fluorescence with respect to each other. This makes it possible to differentiate between those cells that express only one of the particular fluorescent markers, those that express neither, and those that express both. Frequently this method is used to discriminate dead cells from the live ones that are expressing the desired fluorescence. **Figure 3** shows a sample that has some single positives for FITC along the x-axis (**green arrow**) and some single positives for PE along the y-axis (**red arrow**). The upper right quadrant of the plot indicates cells positive for both fluorescent markers. One situation where a dot plot can be useful during the acquisition stage is to gate around just the double-positive cells and then collect only those cells for further analysis; this can allow you to narrow the analysis field to only those cells that express both of the fluorescent markers and from there, visualize the separation of events based on levels of fluorescence.

Next Issue:
Multiple Color Controls

Everything becomes clear when you know where all the dots fall!