# Gates, Plots and Regions

## To help analyze your flow cytometry

Flow cytometry data analysis is built upon the principle of gating. Gates and regions are placed around populations of cells with common characteristics, usually forward scatter, side scatter and marker expression, to investigate and quantify these populations of interest. Here we will show what the common flow cytometry graph outputs look like and how in a few simple steps you can identify different cell populations that have been stained with antibodies conjugated to fluorophores.

1

### Forward and side scatter

The first step in gating is often distinguishing populations of cells based on their forward and side scatter properties. Forward and side scatter can give an estimation of the size and granularity of the cells respectively, although this can depend on several factors such as the sample, the wavelength of the laser, the collection angle and refractive index of the sample and the sheath fluid. The red/yellow/green/blue hot spots indicate increasing numbers of events resulting from discrete populations of cells. Debris often has a lower level of forward scatter and is found at the bottom left corner of the density plot. The forward scatter threshold can be increased to avoid collecting these events, or they can be removed by gating on the populations of interest, shown by the red gates in figure 1b and 1c.

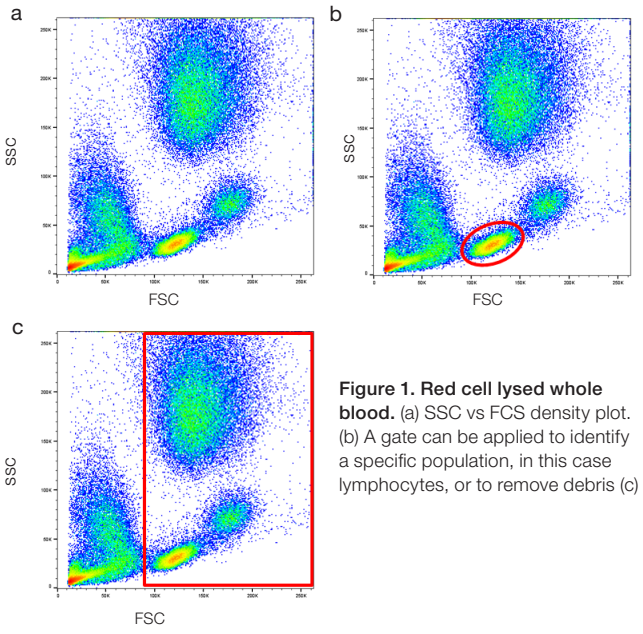



**Figure 1. Red cell lysed whole blood.** (a) SSC vs FCS density plot. (b) A gate can be applied to identify a specific population, in this case lymphocytes, or to remove debris (c)

2

### Single parameter histograms

The events or cells within a gate can be further analyzed for expression of markers by fluorescence and the data expressed in a histogram. In order to accurately identify the positive dataset flow cytometry should be repeated in the presence of appropriate controls. This is particularly necessary if a single distinct peak is observed, however often in flow cytometry multiple peaks are observed due to mixed populations as can be seen in figure 2 where CD3 expression in analyzed.

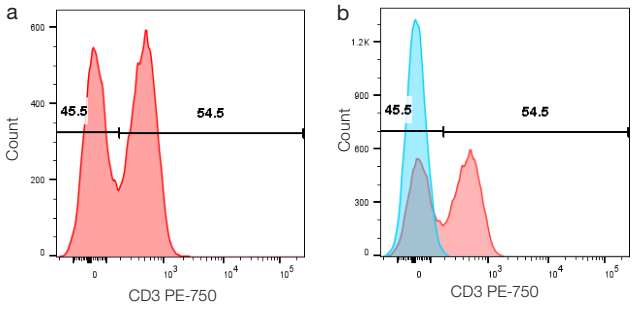



**Figure 2. Single parameter histograms.** (a) Cells within the lymphocyte gate defined in figure 1(b) are represented in a histogram to evaluate the relative expression of CD3. (b) Overlay of a negative population (blue) onto the stained population allows easy identification of the positive cells.

## 3 Two-parameter density plots

These graphs display two measurement parameters, one on the x-axis and one of the y-axis and the events as a density (or dot) plot. In figure 3, the lymphocytes determined by forward and side scatter (a) were stained with CD3 and CD19 to identify the T and B cell populations. This data can be visualized where the density plot is split into four quadrants allowing you to determine the cells single positive for each marker and both double negative and double positive (b).

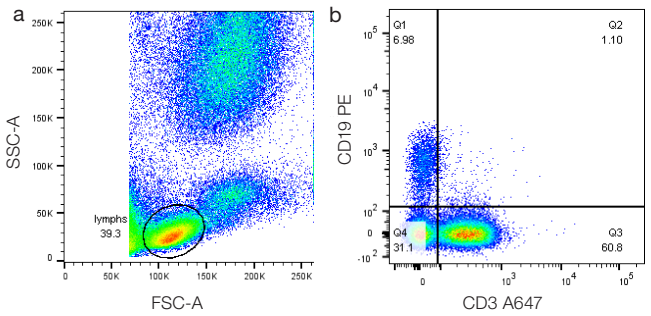



**Figure 3. Two-parameter (dual color fluorescence) density plot.** Red cell lysed whole blood was stained with CD3 and CD19. The lymphocytes were determined by the forward and side scatter profile (a) and the B and T cell percentages determined (b).

## 4 Backgating

Backgating is a useful method of identification of cells to confirm a staining pattern or gating method. It allows you to analyze cells identified in a gate on dot plots with different parameters. This can be of use if you are unsure of your gates, the expression levels, non-specific binding or the presence of dead cells and need additional information to identify your cells.

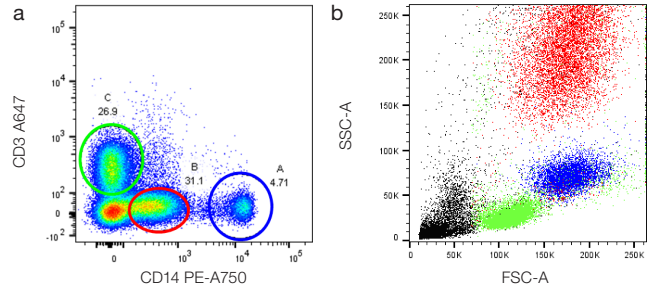
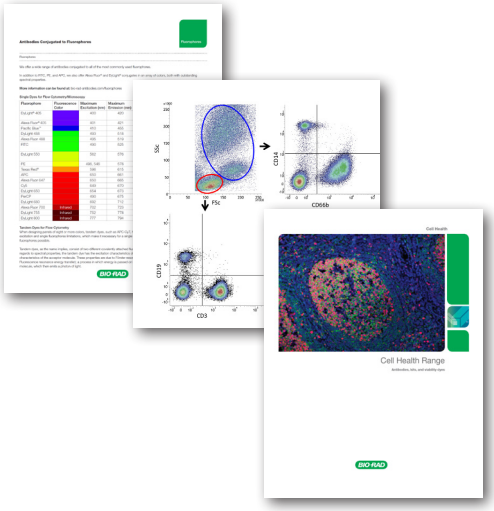



**Figure 4. Backgating to identify leukocyte subsets.** (a) red cell lysed whole blood was stained with CD3 and CD14. (b) cells in gates a, b and c were backgated onto FSC vs SSC (c) to identify specific leukocyte populations.

To get more detailed information practical advice and to see further examples of sequential gating strategies, see our dedicated gating strategies webpage at **bio-rad-antibodies.com/gatingstrategies**

Interested to learn more about flow cytometry? We offer a range of application guides, protocols, controls and hands-on tips and tricks. Visit **bio-rad-antibodies/flow-resources** to find out more.





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