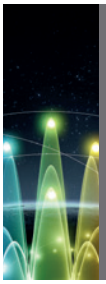

Gates, Plots, and Regions

To Help You Analyze Your Flow Cytometry

Flow cytometry data analysis is built upon the principle of gating. Gates and regions are placed around cell populations with common characteristics — usually forward scatter (FSC), side scatter (SSC), and marker expression — to investigate and quantify the populations of interest. Here we 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 (Figure 1A). FSC and SSC 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, the refractive index of the sample, and the sheath fluid. The colored hot spots indicate increasing numbers of events resulting from discrete populations of cells. Debris often has a lower level of FSC and is found at the bottom left corner of the density plot. The FSC threshold can be increased to avoid collecting these events, or they can be removed by gating the populations of interest, as shown by the red gates in Figures 1B and 1C.

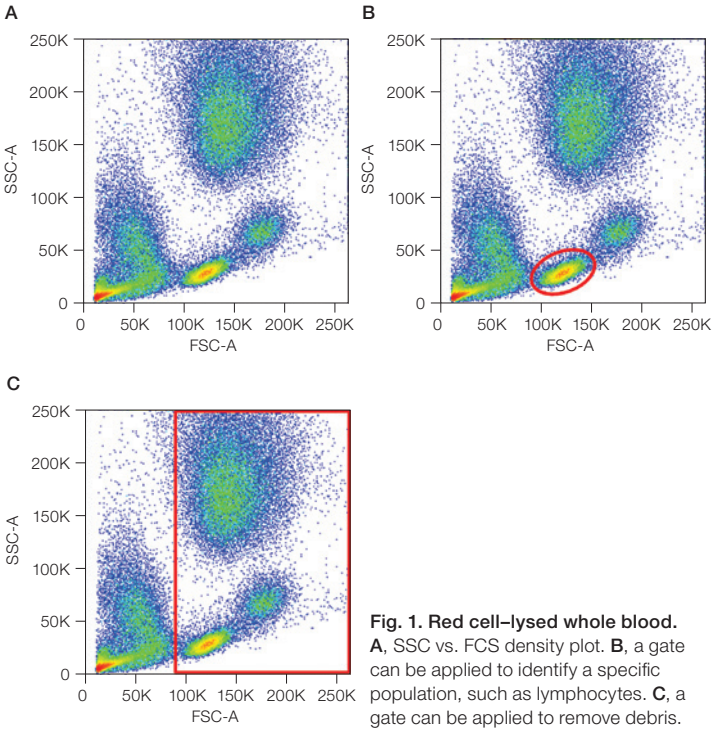

Fig. 1. Red cell–lysed whole blood. **A**, SSC vs. FCS density plot. **B**, a gate can be applied to identify a specific population, such as lymphocytes. **C**, a gate can be applied to remove debris.

2 Single-Parameter Histograms

The events or cells within a gate can be further analyzed for marker expression by fluorescence, and the data can be expressed in a histogram. To accurately identify the positive population, appropriate controls should be used during flow cytometry. This is particularly necessary if a single distinct peak is observed but also when multiple peaks are observed due to mixed populations, as is often the case (Figure 2).

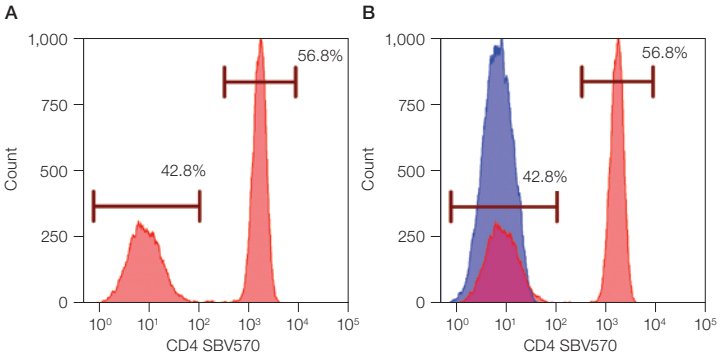

Fig. 2. Single-parameter histograms. **A**, cells within the lymphocyte gate defined in Figure 1B (red) are represented in a histogram to evaluate the relative expression of CD4 SBV570 (MCA1267SBV570). **B**, overlay of a negative population (blue) onto the stained population allows easy identification of the positive cells. SBV, StarBright™ Violet.

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3 Two-Parameter Density Plots and Sequential Gating

These graphs display two measurement parameters, one on the x-axis and one on the y-axis, and the events as a density (or dot) plot. An example is provided in Figure 3. Lymphocytes were identified by FSC and SSC and then were stained with CD3 and CD19 to identify the T- and B-cell populations with gates placed around each single-stained population. The T-cell population can then be further subdivided into CD4-positive and CD8-positive populations to identify T-helper and T-cytotoxic cells, respectively, and the CD4-positive population can be further gated to identify the CD127^{lo} CD25^{hi} regulatory T cells. This principle can be continued with more markers, but the number of cells will diminish with each gate.

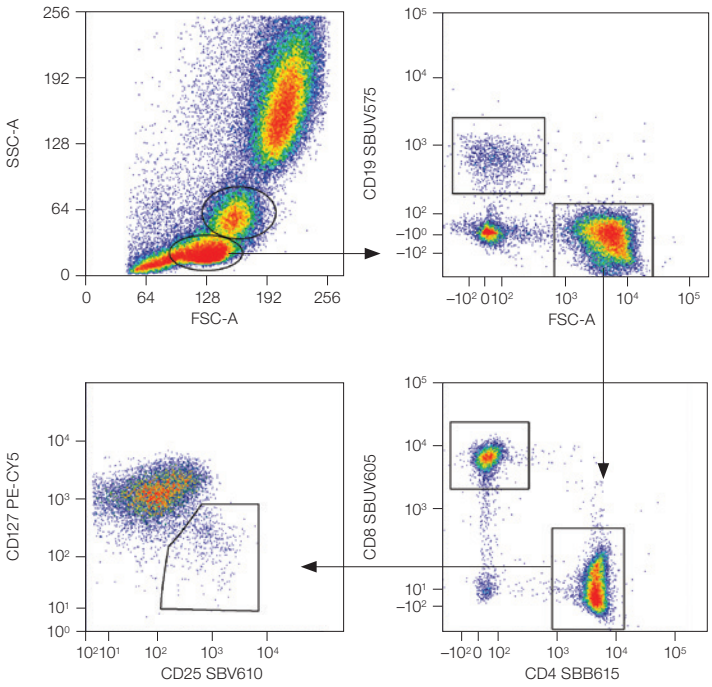

Fig. 3. Two-parameter density plots with sequential gating to identify specific peripheral blood subsets. Red cell–lysed whole blood was stained with CD19 (MCA1940SBUV575), CD3 (MCA463SBUV795), CD4 (MCA1267SBB615), CD8 (MCA1226SBUV605), CD25 (MCA2127SBV610) and CD127. The gating strategy is shown by the arrows. Cy, cyanine; PE, phycoerythrin; SBB, StarBright Blue; SBUV, StarBright UltraViolet; SBV, StarBright Violet.

This simple principle of gating can be applied to subsequent populations again and again to further determine the expression patterns on cell types. This is particularly useful as the number of markers and fluorophores in a single experiment increases.

4 Backgating

Backgating is a useful method for confirming that a staining pattern or gating method identifies a particular cell population. 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 about your gates, marker expression levels, nonspecific binding, or the presence of dead cells and need additional information to identify your cells.

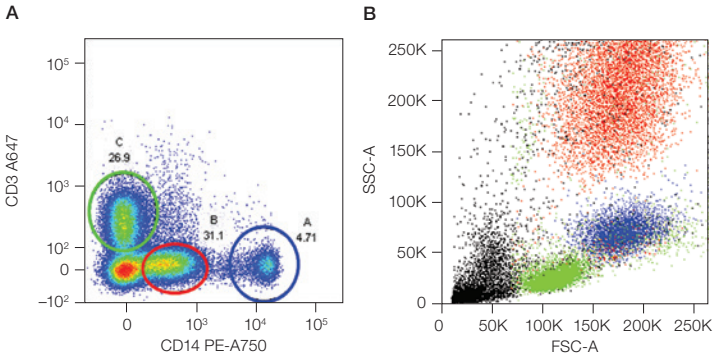

Fig. 4. Backgating to identify leukocyte subsets. A, gates are drawn on red cell–lysed whole blood stained with CD3 (MCA463A647) and CD14 (MCA1568P750). **B**, cells in gates A, B, and C were backgated onto FSC vs. SSC to identify specific leukocyte populations. Axxx, Alexa Fluor; PE, phycoerythrin.

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

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.com/flow-resources to find out more.

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