

## FACS data: Multiple Color Controls

by Brian Russell

A crucial part of any experiment is the controls. In FACS analysis, controls provide a basis for comparison when looking at the positive data, such that you will have an accurate measure of how successful the experiment was. Whether you are looking at a histogram or a dot plot, every cell line will exhibit some basal level of fluorescence that will show up in the control sample. When the samples are being collected, this basal level is set to the lower left corner of dot plots and against the left axis in histograms. The subsequent samples that are positively labeled will then shift to the right in histograms and both to the right and vertically on dot plots. The difference between the measured basal level and the shifted fluorescence level of the positive sample is determined during analysis by way of a bar region marker, or quadrant lines in the case of dot plots.

Many experiments in flow cytometry utilize multiple colors (FITC and PE are the most common) in order to test multiple characteristics in a single sample simultaneously. One very common use of multiple dye labeling is to discriminate dead

cells from live ones. This provides the opportunity to gate around only the live cells during acquisition, removing the dead cells from analysis and providing data that shows the full effectiveness of the experiment. Another frequent use for multiple dyes is for testing multiple receptors on the same cell type, such as CD4 and CD8 markers on T lymphocytes. Additional colors often necessitate the use of compensation between the fluorescent channels. Since fluorochromes emit light over a range of wavelengths, often when multiple dyes are used there is some overlap in the emission wavelength spectrum of the respective dyes. This is referred to as spectral overlap and can skew the data and provide inaccurate results. What is done in order to “compensate” for this effect is to subtract out the overlapping emission signals prior to acquisition. All of this adds up to the need for additional control samples that will make it possible to set up the acquisition phase of the experiment, such that the resulting data accurately represent the degree of success of the experiment.

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