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Commentary

Auto Fluorescent Cells, Including Mesenchymal Stem Cells, are Indeed Amenable to Flow Cytometric Analyses

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Abstract

Flow cytometric analysis is a valuable technique for identification and characterization of cells. However, flow cytometric analysis of auto fluorescent cells requires careful consideration. In particular, the outcome of fluorescence compensation in the presence of significant autofluorescence poses a challenge to the interpretation of multicolor flow cytometric data. In this paper, we explain the mathematical basis of fluorescence compensation and the effects of autofluorescence, both in theory and applied to mesenchymal stem cells, which are notoriously auto fluorescent. We illustrate how failure to understand the consequences of compensation can easily lead to critical errors in data interpretation, particularly when autofluorescence is involved. In the process, the common pitfalls of flow cytometric analysis of mesenchymal stem cells are presented, as are the simple measures necessary to avoid them. Specifically, the counterintuitive concept of "negative fluorescence values" is explained and exemplified, and the phenomenon of "population broadening" is addressed. Researchers must be acutely aware of the effects of compensation on the positioning of cells. We recommend always displaying data in biexponential or logical transformations and advice to include fluorescence-minus-one controls to establish thresholds of positivity.

Keywords: mesenchymal stromal cells; flow cytometry; cultured cells; cellular structures.

Commentary

The consequence of autofluorescence in compensation

Negative fluorescence values after compensation is a fairly common occurrence in flow cytometric analysis of cell types with high levels of auto fluorescence (e.g., mesenchymal stem cells). At first glance, the concept of negative fluorescence is counterintuitive, and researchers may instinctively begin to doubt the validity of the compensation, the flow cytometer, or suspect a "bug" in the flow cytometric software. Worse, the researcher may be sufficiently perplexed to conclude that flow cytometry of such cells "doesn't work" and abandon flow cytometric analyses altogether. However, negative fluorescence values do make sense when one considers what compensation does.

The classical approach to compensation involves recording the signals of single-stained compensation controls to determine the degree of spectral overlap between fluorochromes and detectors. Compensation is typically done by adjusting the values of the spectral overlap matrix elements (algorithmically by iteration or in rare cases manually by "visual inspection"), until the compensated median fluorescence intensity (MFI) of labelled cells (or beads) matches the compensated MFI of unlabeled cells in all detectors except the one assigned to the fluorochrome of the particular single-stained population.

The importance of fluorescence-minus-one controls

In a perfect flow cytometer, there would be no error in fluorescence measurements, and one could safely assume that the measurements were "true" indications of the actual fluorescences on each cell. The "width" (or spread, or variation) of a population of cells on any given parameter would then reflect the actual, physical variation of that parameter within the population, and nothing else. In this case, compensation would not affect the "width" or shape of the population (other than the purely visual changes introduced by redefining the basis of the coordinate system by shearing transformation) - and the "width" of the stained cell population would closely resemble that of the unstained cells, both before and after compensation (insofar as a "perfect" compensation truly eliminates the contribution of irrelevant fluorochromes on each individual cell, leaving only the natural variation of the background/auto fluorescence).

Conclusion

Auto fluorescent cells, including mesenchymal stem cells, are indeed amenable to flow cytometric analyses, in spite of high levels of auto fluorescence. However, researchers must be acutely aware of the effects of compensation on the positioning of cells (and thus always display data in biexponential or logical transformations). Compensation controls can be based on beads (recommended) or cells, or a combination thereof, with no theoretical implications for the compensation matrix, insofar as the matrix is solely based on slopes. Compensations must, however, compare stained and unstained populations with identical autofluorescences. Finally, researchers are advised to include fluorescence-minus-one controls to establish thresholds of positivity.

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