Flow Post-its
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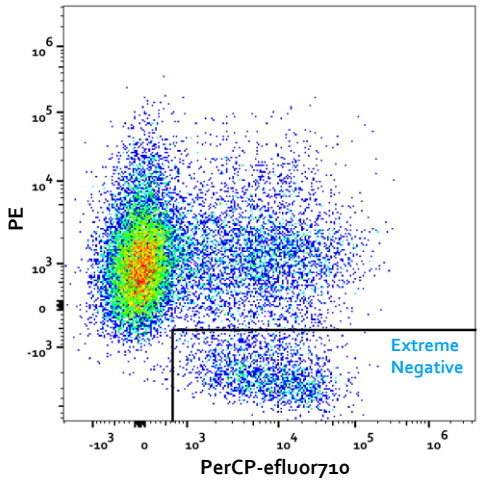
Extreme Negatives and Proper Controls

January 2021

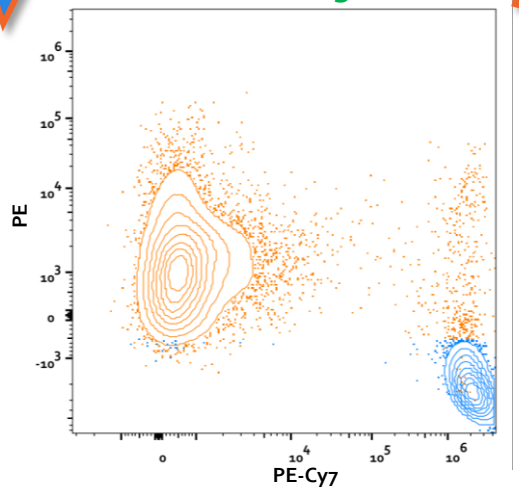
When analyzing multiparameter flow cytometry data where **spillover correction** has been carried out, a quality check should always be done to determine the accuracy of results. Visualizing NxN plots with the appropriate scaling to view all events is necessary when doing these quality assessments. **Extreme negatives** are typically indicative of **overcompensation** or **overestimation of unmixing.**

Extreme Negative Identified



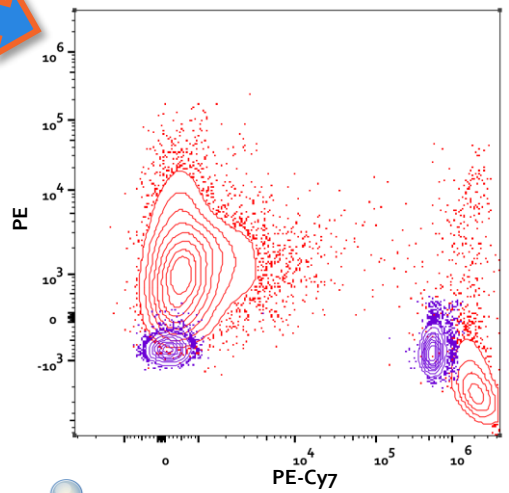
During analysis, a population of cells is seen that is below the autofluorescence of the unstained population for PE. The spillover correction via unmixing for PE and PerCP-efluor710 seems to be correct. Further investigation of all flurochromes via an NxN plot is warranted to determine what caused this extreme negative.

"Over-Unmixing" Seen



Using the extreme negative population from the previous plot, a Boolean gating strategy was created for ***extreme negative*** and ***"NOT"***. When looking at the NXN plots, it was identified that unmixing underestimated the amount of PE in the PE-Cy7 single positive population.

Root Cause Found



Rules of Compensation & Unmixing

- ☑ **Rule 1:** Autofluorescence of the positive and negative need to match.
- ☑ **Rule 2:** Single color and experimental fluorochromes must be the same.
- ☒ **Rule 3:** The **single color controls** need to be as bright or brighter than **experimental samples.**

Every flow cytometry experiment should be checked to ensure that the results are accurate and as expected. **Extreme negatives indicate that something needs to be corrected**. In the example outlined above, it was found that a single color control was not as bright as an experimental sample. *By re-running a single color that is as bright as the sample, unmixing will be more accurate and the data will be improved.*