

Multicolor Cytometry using the WOLF Sorter

Introduction: Multicolor flow cytometry is widely used to sort subpopulations of cells. Antibodies and/or viability markers are used to tag proteins as the basis for sorting. In multicolor flow cytometry, compensation is required because fluorescence from more than one fluorochrome may be detected due to the overlap between the emission spectra. Compensation is generally performed with single color compensation beads or cells. The WOLF Cell Sorter has two modes of compensation available to the user (automatic and manual). Here, we demonstrate the use of the WOLF Cell Sorter’s ability to compensate 3 colors using FITC, PE and PE-Cy5 labeled beads, and sort these beads after compensation.

Methods: Calibration beads from Spherotech were used for the compensation and bulk sorts. Beads were FITC, PE or PE-Cy5.5 positive. These beads were mixed to a total concentration of 100 beads per uL and approximately 1000 beads of each color were sorted into FACs tubes. The sorted beads were analyzed using the Accuri.

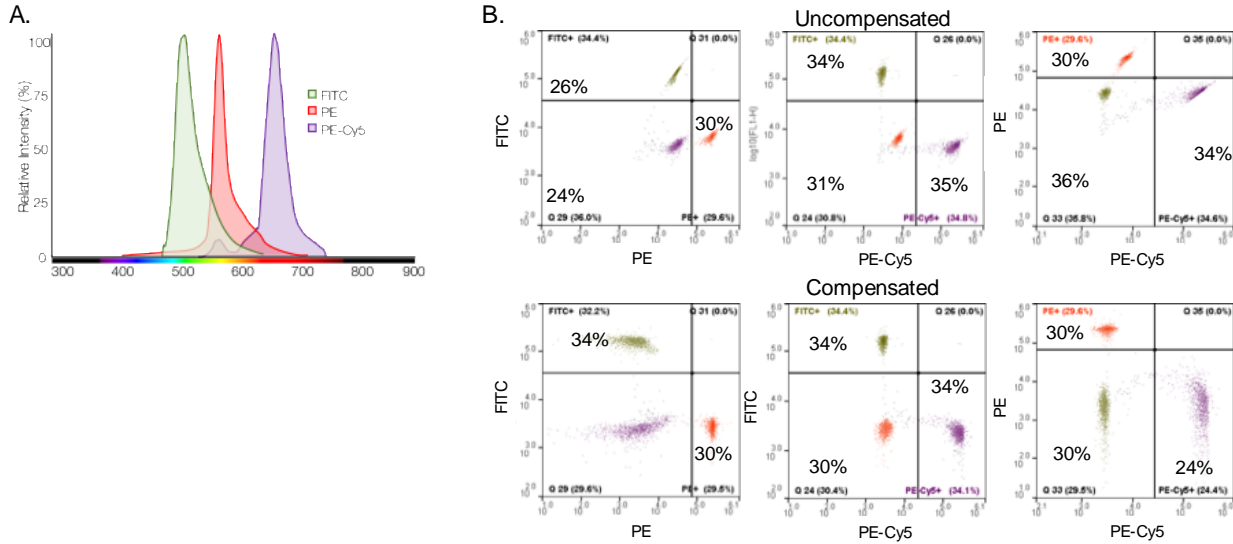


Figure 1. A) Graph of emission spectra of FITC, PE and PE-Cy5. The overlap between the emission spectra needs to be removed in order to decouple the real total intensity from each fluorophore. **B)** Before and after compensation comparison of the three different bead populations. Compensation was done automatically using the Wolf Viewer software.

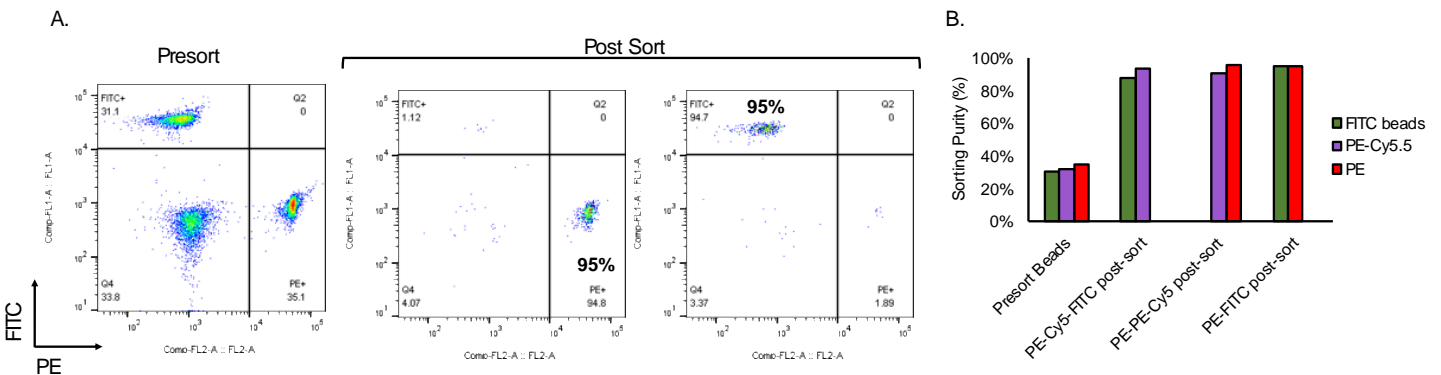


Figure 2. A) The Wolf Cell Sorter is able to perform a 2-way sort from each of the three fluorophores from a mixed bead population. **B)** The purity of the sorted population was 95% from a ~33% starting population.

Summary of Results: The WOLF is able to successfully isolate subpopulations of each type of bead from a mixed population after compensation. Compensation allows for spillover of signal into neighboring channels to be corrected and populations of particles to be properly gated. Here, we successfully sorted two compensated populations of beads at the same time (2-way sort). The purity of the sort is high and the isolation is successful regardless of the initial distribution of the beads.