Flow Post-its Kathy Daniels

Flow Cytometry

Core Facility

Impacts of Spread

When designing and executing multiparameter flow cytometry experiments, instrument **configuration** and **panel design** both play a very important role. Just because a cytometer can detect fluorochromes does not mean that it will work with your experiment. Care must be taken when designing your panel to **avoid** fluorochrome combinations that introduce **high spread** with **coexpressing markers**.





Experiment Comparison

Original: Double positive population of BV711 and BUV737 needed to be isolated for sorting. A gate was set on what was believed to be this population. Post sort purity revealed **purity of 66.9%**. During troubleshooting, samples were visualized in biexponential display and it was identified that there was **high spread** of BV711 into BUV737, resulting in no clear BUV737+ population. Sorter proved to be operational as purity of 98.5% was observed when gating on BV711+ population.

Optimized: Panel was adjusted with to minimize impacts of spread. New combination of BUV₇₃₇ and APC-Cy₇ was used. Double positive population resolved well and post sort check showed **purity of 98.3%**



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