



# Seven Tips for Achieving the Perfect Panel for Multicolor Flow Cytometry

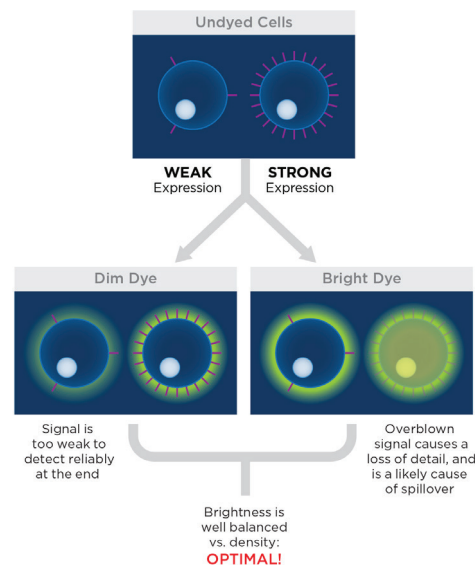
With well-designed panels and high-quality reagents, multicolor flow cytometry is a powerful tool for simultaneously detecting and monitoring multiple sample parameters. Proper panel design and well-tuned instrumentation is essential to achieving high-quality data. Together with the following tips, you can avoid common pitfalls and improve the quality of your data.

## 1. Fluorochrome Selection

Some dyes are extremely bright while others are dim by comparison. When it comes to choosing fluorochromes, the brightest isn't always the best option while a dimmer dye isn't always the most prudent.

Overall, it's advisable to choose a dye that is sufficiently bright to detect your antigen of interest while minimizing spillover (or bleed) into other detectors. Brighter emission means more potential for spillover and data spreading which can negatively impact resolution. Avoid this by choosing brighter dyes for weakly-expressed antigens and dimmer dyes for strongly-expressed antigens.

### TIP1



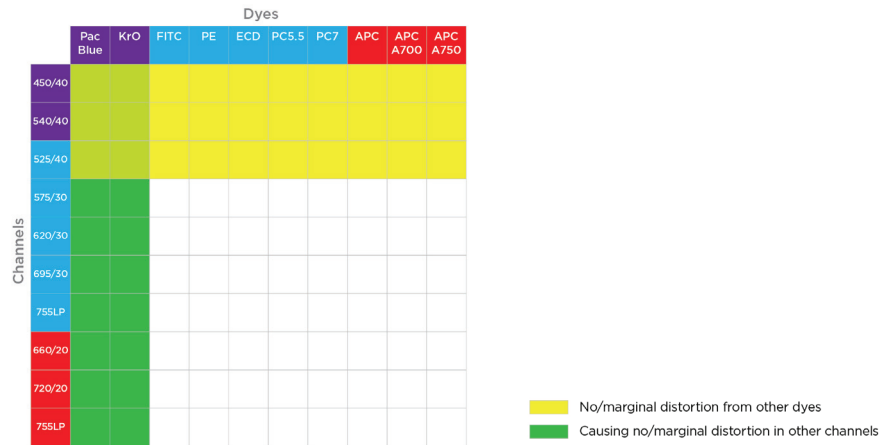
*Spillover happens when the emission spectrum of one fluorochrome is picked up by a detector set to measure a different fluorochrome, thereby contributing to unwanted signal in the channel of the second fluorochrome.*

## 2. Channel selection

Optimize your panel by choosing fluorochromes with 'less' influence on channels for strongly-expressed antigens (green columns) and weakly-expressed antigens for fluorochromes with a detection channel minimally impacted by spillover from other fluorochromes (yellow rows).

Whether a fluorochrome falls into one of these categories (or not) depends on your instrument configuration and choice of fluorochromes. Be sure to test fluorochrome and channel combinations to determine the best lineup for your panel. Completely eliminating spillover is challenging for multichannel assays. Using the guide shown below can help minimize the impact of spillover.

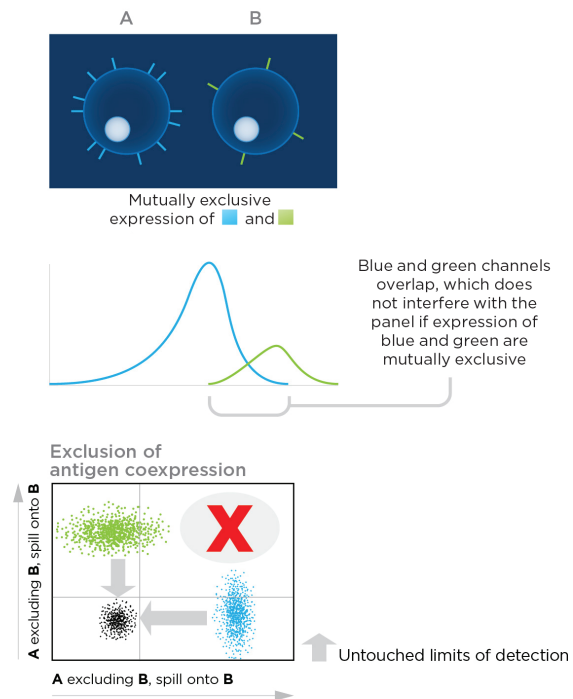
### TIP2



## 3. Antigen Exclusion

Spillover between the emission spectra of mutually exclusive antigens won't interfere with your panel as the antigens are not expressed together on the same cells in the cell gate of interest.

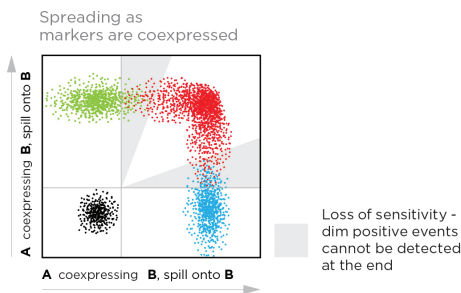
### TIP3



## 4. Antigen Coexpression

Spillover can cloud your data and mask potential modulations of antigen expression. Only spillover from non-coexpressed antigen fluorochrome labels won't impact sensitivity and resolution for modulated or weakly expressed antigens. If a marker has a modulated or weak expression density, the sensitivity impact from coexpressed antigen labels should be minimized through selection of fluorochromes with little or no spillover.

### TIP4

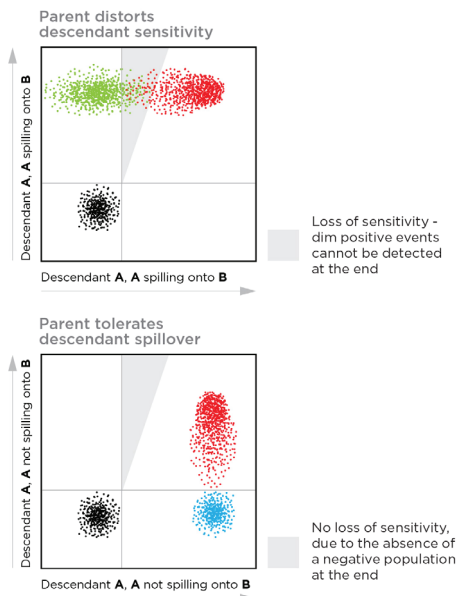


## 5. Parent Markers vs Descendants

It's acceptable for a subpopulation (descendant) marker to spill over (or cross-talk) to the gating marker (parent). The parent marker will tolerate this spillover because the descendant will always be positive. For example, if CD4 shows a strong spillover in the CD3 channel within a lymphocyte gate, this spillover will not cause background spreading in the CD3 channel as CD4+ T-cells are also CD3+.

Watch out for the reverse scenario where the parent marker cross-talks with the descendant marker because the parent signal's spillover will contaminate the background of the descendant signal.

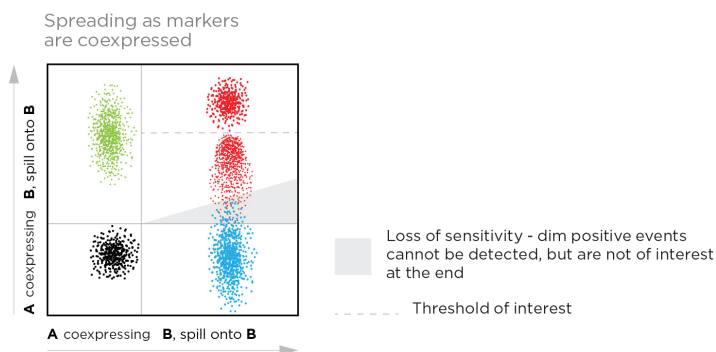
### TIP5



## 6. Positive Threshold

Spillover to a coexpressed antigen is allowable if you're looking to identify only brightly positive cells or discriminate between bright and medium-strong/dim expression. Spillover can't be tolerated when solely discriminating between dim and negative expression as this will cloud the data and conclusions leading to false positives.

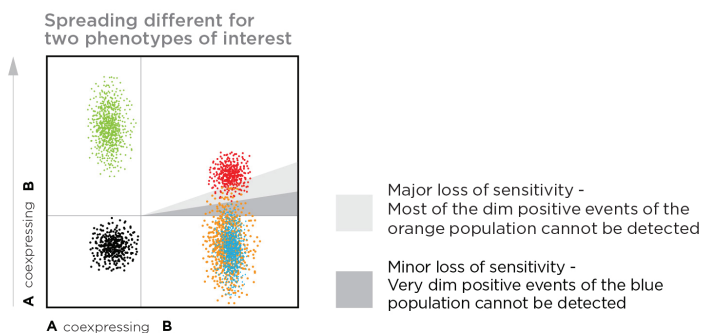
### TIP6



## 7. Spillover Complexity

Keeping spillover patterns simple and straightforward is a best practice for avoiding phenotype-dependent detection limits. It can be challenging however to control spillover and spread from fluorochromes not shown in your dot plot. 'Keeping it simple' requires sound knowledge of the fluorochrome spillover characteristics and the coexpression patterns of the labeled antigens.

### TIP7



For optimal signal detection and confidence in your results, plan your next multicolor flow cytometry assay with these tips in mind. To further ensure your success with multicolor flow cytometry, choose high-quality reagents manufactured according to "Current Good Manufacturing Practice (cGMP)"