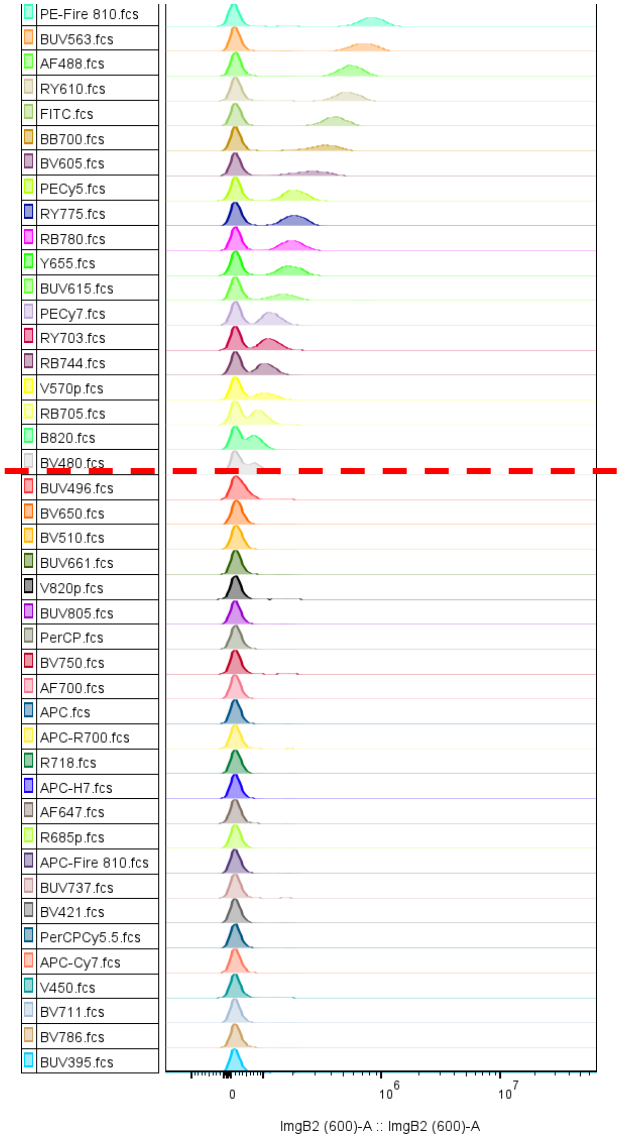
# Imaging Channel Spillover Assessment

on the BD FACSDiscover™ S8 Cell Sorter

BD

# Spillover assessment

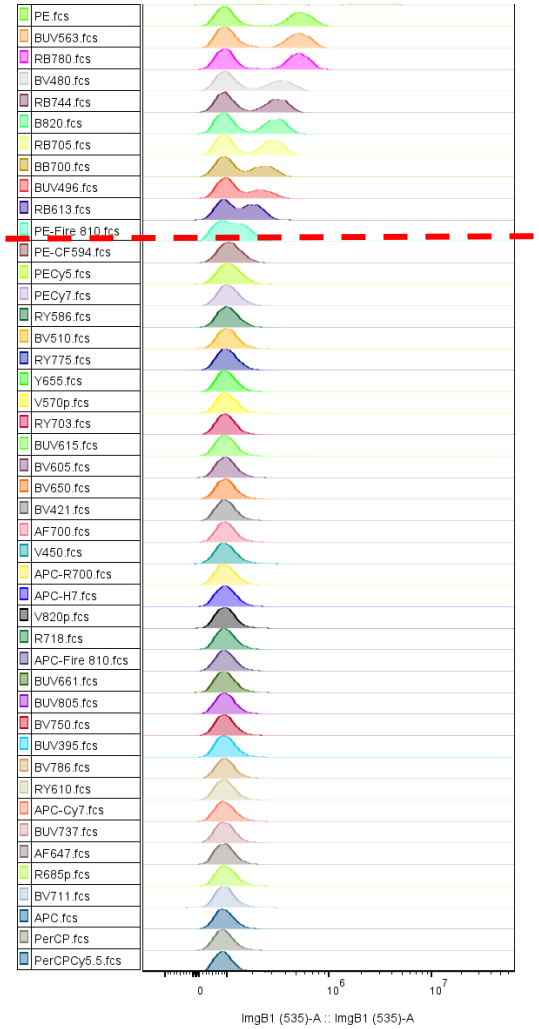



## What does this mean?

- Signal from these fluorochromes used for spectral or imaging is also detected in Imaging Channel 2
- Without compensation, this spillover can impact resolution and create artifacts
- Panel design can be used to mitigate this risk
- Safer and easier not to use these fluorochromes in combination with a fluorochrome detected in Imaging Channel 2

- Signal from these fluorochromes is not detected in Imaging Channel 2
- These fluorochromes can be safely used in combination with a fluorochrome detected in Imaging Channel 2

# Spillover into imaging channel 1

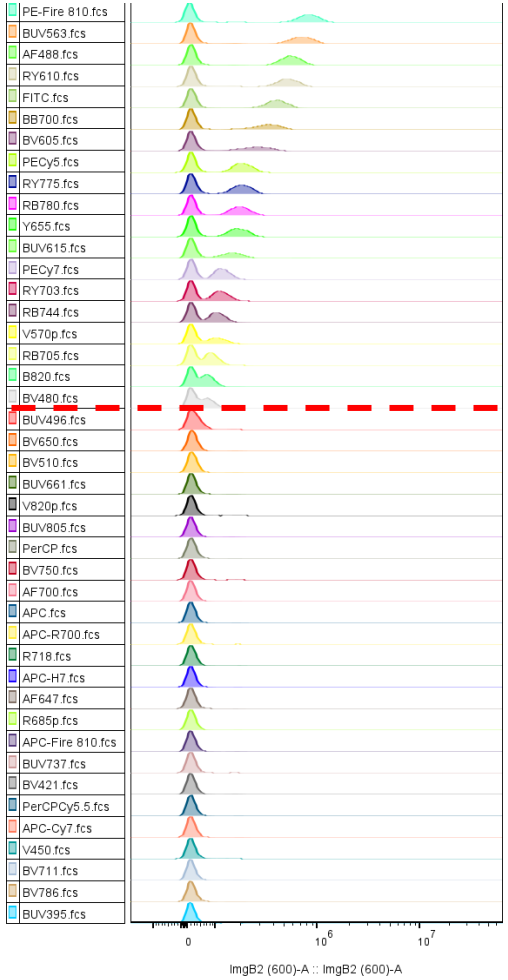



Use only if no other option is available
Use with diluted primary markers
Use with antigens expressed at low levels
Make sure the population of interest is brighter than any spillover background

Safe to use with antigens express as abundantly as CD4 or less

- Based on high expression of CD4
- The spillover will decrease as antibody dilution or use of antigens with lower density are used

# Spillover into imaging channel 2

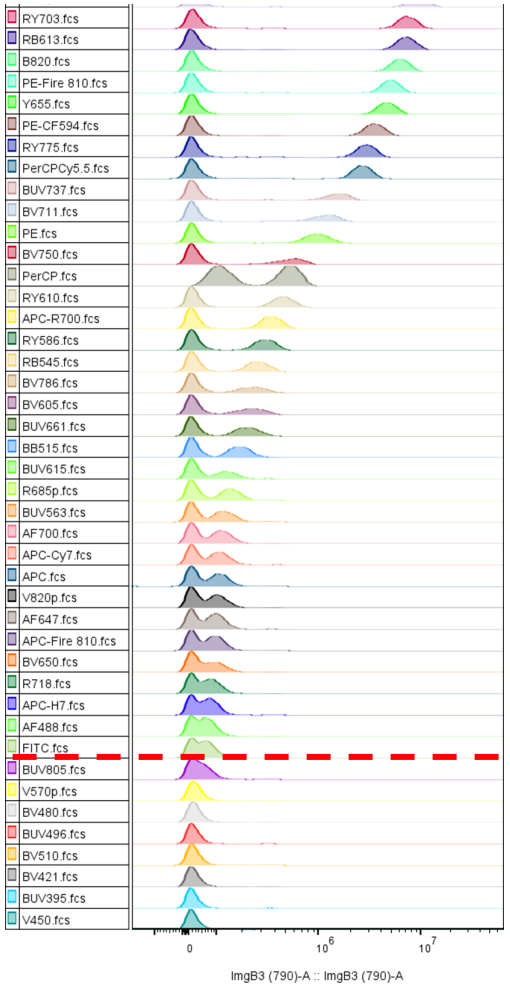



Use only if no other option is available
Use with diluted primary markers
Use with antigens expressed at low levels
Make sure the population of interest is brighter than any spillover background

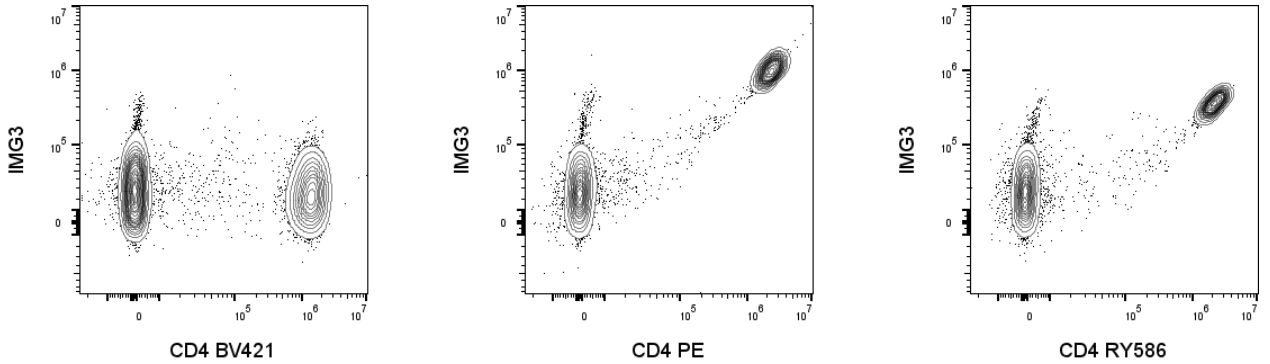
Safe to use with antigens express as abundantly as CD4 or less

- Based on high expression of CD4
- The spillover will decrease as antibody dilution or use of antigens with lower density are used

# Spillover into imaging channel 3




Use only if no other option is available
Use with diluted primary markers
Use with antigens expressed at low levels
Make sure the population of interest is brighter than any spillover background




Safe to use with antigens express as abundantly as CD4 or less

- Based on high expression of CD4
- The spillover will decrease as antibody dilution or use of antigens with lower density are used

# Spillover reference

| Primary fluorochrome | IMG 1 | IMG 2 | IMG3 |
|---|---|---|---|
| FITC | | | |
| AF488 | | | |
| BB515 | | | |
| RB545 | | | |
| PE | | | |
| RB613 | | | |
| PE-CF594 | | | |
| PerCPCy5.5 | | | |
| BB700 | | | |
| RB705 | | | |
| RB744 | | | |
| RB780 | | | |
| PECy7 | | | |
| BUV395 | | | |
| BUV496 | | | |
| BUV563 | | | |
| BUV615 | | | |
| BUV661 | | | |
| BUV737 | | | |
| BUV805 | | | |
| BV421 | | | |
| V450 | | | |
| BV480 | | | |
| BV510 | | | |
| BV570 prototype | | | |
| BV605 | | | |
| BV650 | | | |
| BV750 | | | |
| BV711 | | | |
| BV786 | | | |
| RY586 | | | |
| RY610 | | | |
| PECy5 | | | |
| RY703 | | | |
| RY775 | | | |
| PE-Fire 810 | | | |
| AF647 | | | |
| APC | | | |
| AF700 | | | |
| APC-R700 | | | |
| R718 | | | |
| APC-H7 | | | |
| APC-Fire 810 | | | |

Spillover 0-10%

Spillover 10-50%

Spillover >50%

## Key takeaways

- If using only one imaging detector, fluorochromes with no spillover into the imaging detector of choice are safe and should be prioritized
- Fluorochromes with spillover may be used if no safe options are available, if panel design rules and strict controls are used
- In the absence of compensation, IMG1 and IMG3 are the recommended detectors to be used together

Note: This table can be used as a universal reference based on CD4 stain, when default settings are used

# Proposed recommendations

- Use default settings
  - Assumption that all instrument defaults settings are close to optimal and are consistent over time
- Do not change settings unless:
  - Signal is saturated and reagent titration is not possible (GFP+)
- Do not use the wall to assess spillover
  - Bivariate plots, histograms and/or correlation are recommended
- Reagent titration and proper panel design (density vs brightness) are the best practices to manage signal resolution and spillover
- We can now provide consistent information on imaging fluorochrome SI and spillover into imaging channels

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