

# Imaging Channel Spillover Assessment

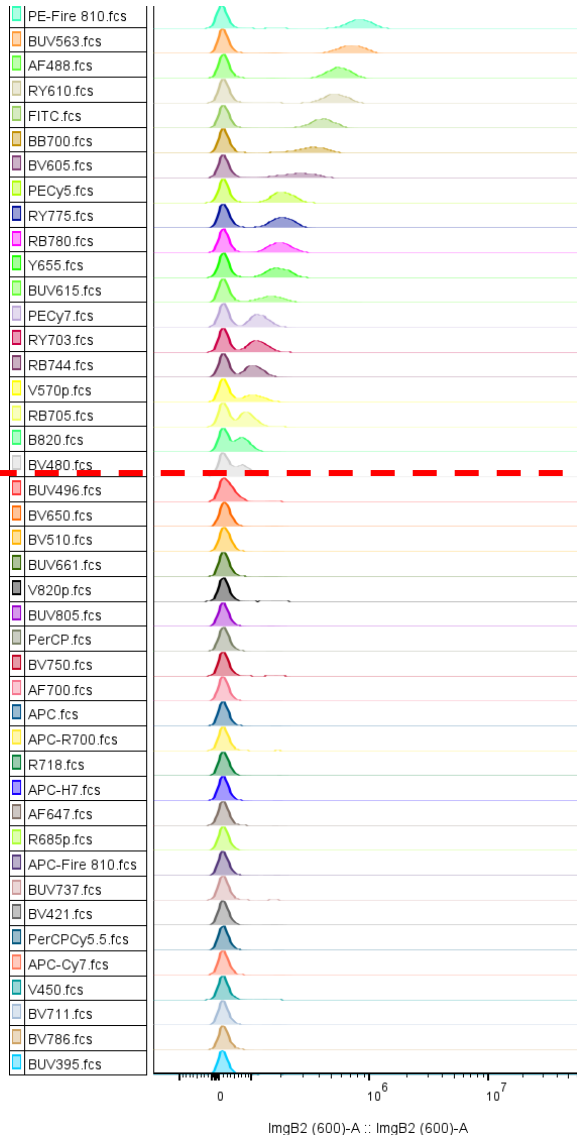
on the BD FACSDiscover™ S8 Cell Sorter



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# Spillover assessment

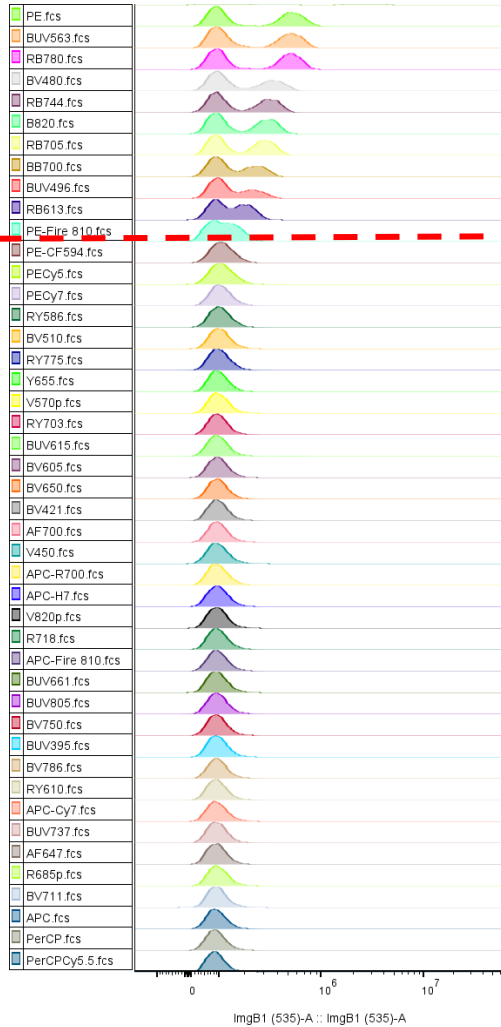
## What does this mean?



- 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

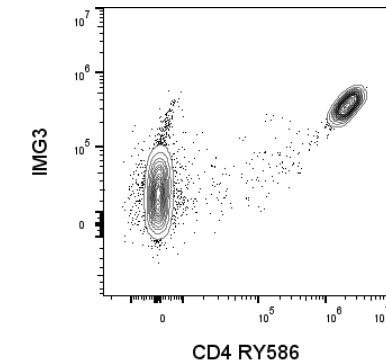
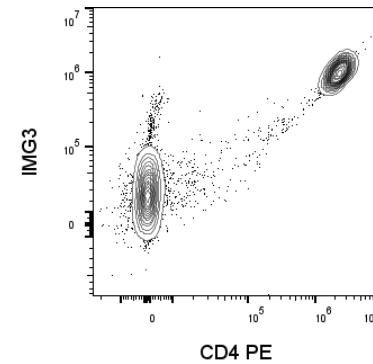
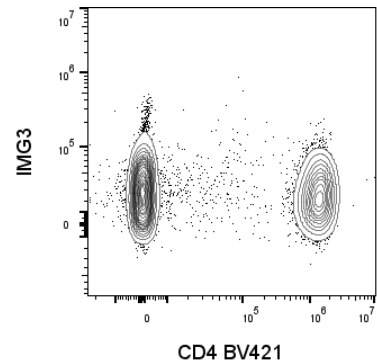
Safe to use with antigens express as abundantly as CD4 or less

- 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

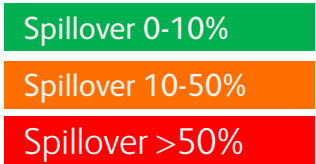


Safe to use with antigens express as abundantly as CD4 or less

- 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	Black	Red	Orange
AF488	Black	Red	Orange
BB515	Black	Red	Orange
RB545	Black	Red	Orange
PE	Orange	Black	Red
RB613	Green	Black	Red
PE-CF594	Green	Black	Red
PerCPy5.5	Green	Black	Black
BB700	Orange	Red	Black
RB705	Orange	Green	Black
RB744	Orange	Green	Black
RB780	Red	Orange	Black
PECy7	Green	Green	Black
BUV395	Green	Green	Green
BUV496	Orange	Green	Green
BUV563	Red	Red	Orange
BUV615	Green	Orange	Orange
BUV661	Green	Green	Orange
BUV737	Green	Green	Red
BUV805	Green	Green	Green
BV421	Green	Green	Green
V450	Green	Green	Green
BV480	Orange	Green	Green
BV510	Green	Green	Green
BV570 prototype	Green	Orange	Green
BV605	Green	Red	Red
BV650	Green	Green	Orange
BV750	Green	Green	Red
BV711	Green	Green	Red
BV786	Green	Green	Red
RY586	Green	Red	Orange
RY610	Green	Red	Red
PECy5	Green	Green	Red
RY703	Green	Green	Red
RY775	Green	Green	Red
PE-Fire 810	Green	Orange	Red
AF647	Green	Green	Orange
APC	Green	Green	Orange
AF700	Green	Green	Orange
APC-R700	Green	Green	Orange
R718	Green	Green	Orange
APC-H7	Green	Green	Orange
APC-Fire 810	Green	Green	Orange



## 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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