Enhancing the Performance of BD Horizon™ Brilliant Violet™ Reagents

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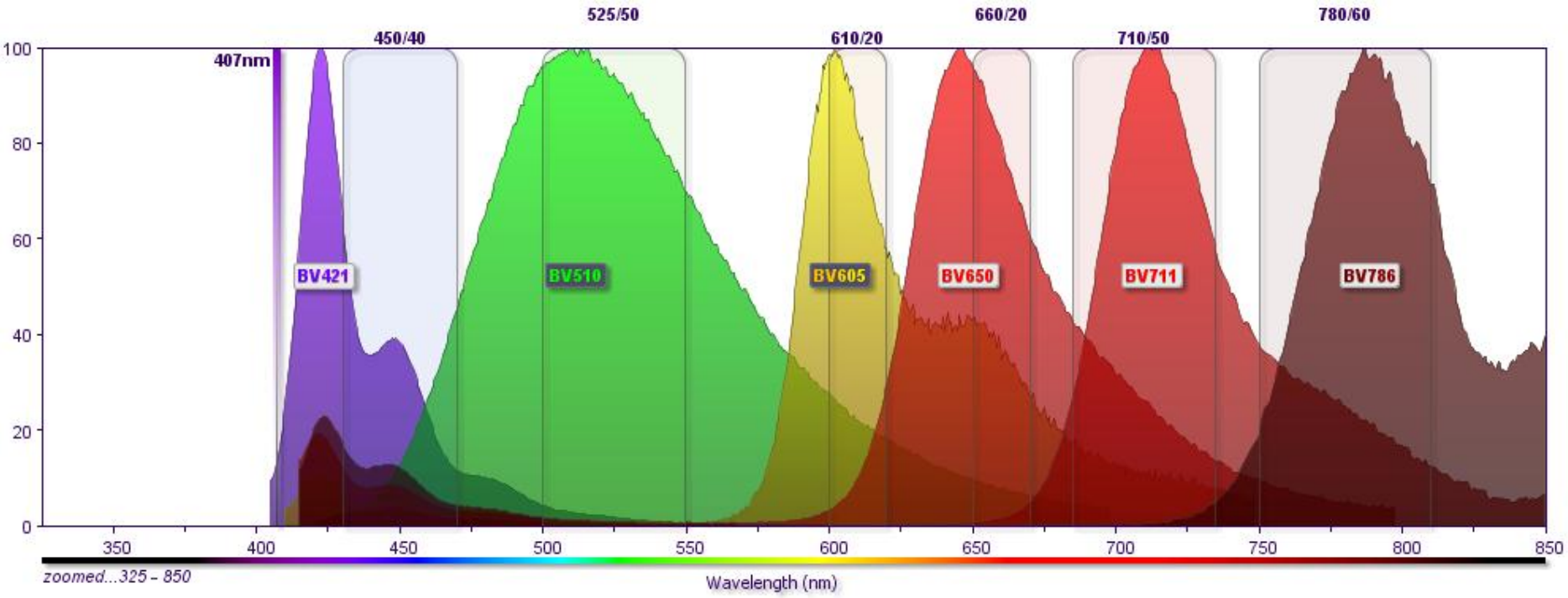
Outline

- BD Horizon™ Brilliant Violet™ overview
- Observations when combining BD Horizon Brilliant Violet (BV) reagents
  - Population shifts
  - “Budding” of the negative population
- Using BD Horizon™ Brilliant Stain Buffer to enhance performance
- Using Brilliant Stain Buffer in multicolor cocktails

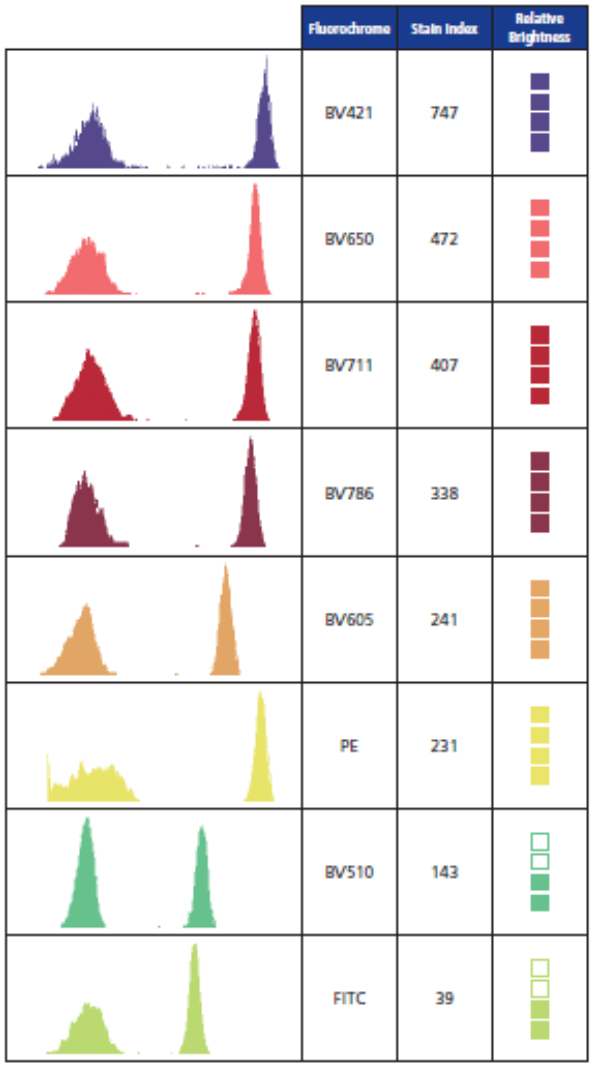
BD

BV Fluorochromes



BV421 BV510 BV605 BV650 BV711 BV786

Overview of BV Reagents



- Six bright dyes for the violet laser
  - Brightness comparable to PE for most dyes
- Enables 14-color panels
- Provides additional choices for multicolor panel design

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Fourteen-Color Panel: TBMNK

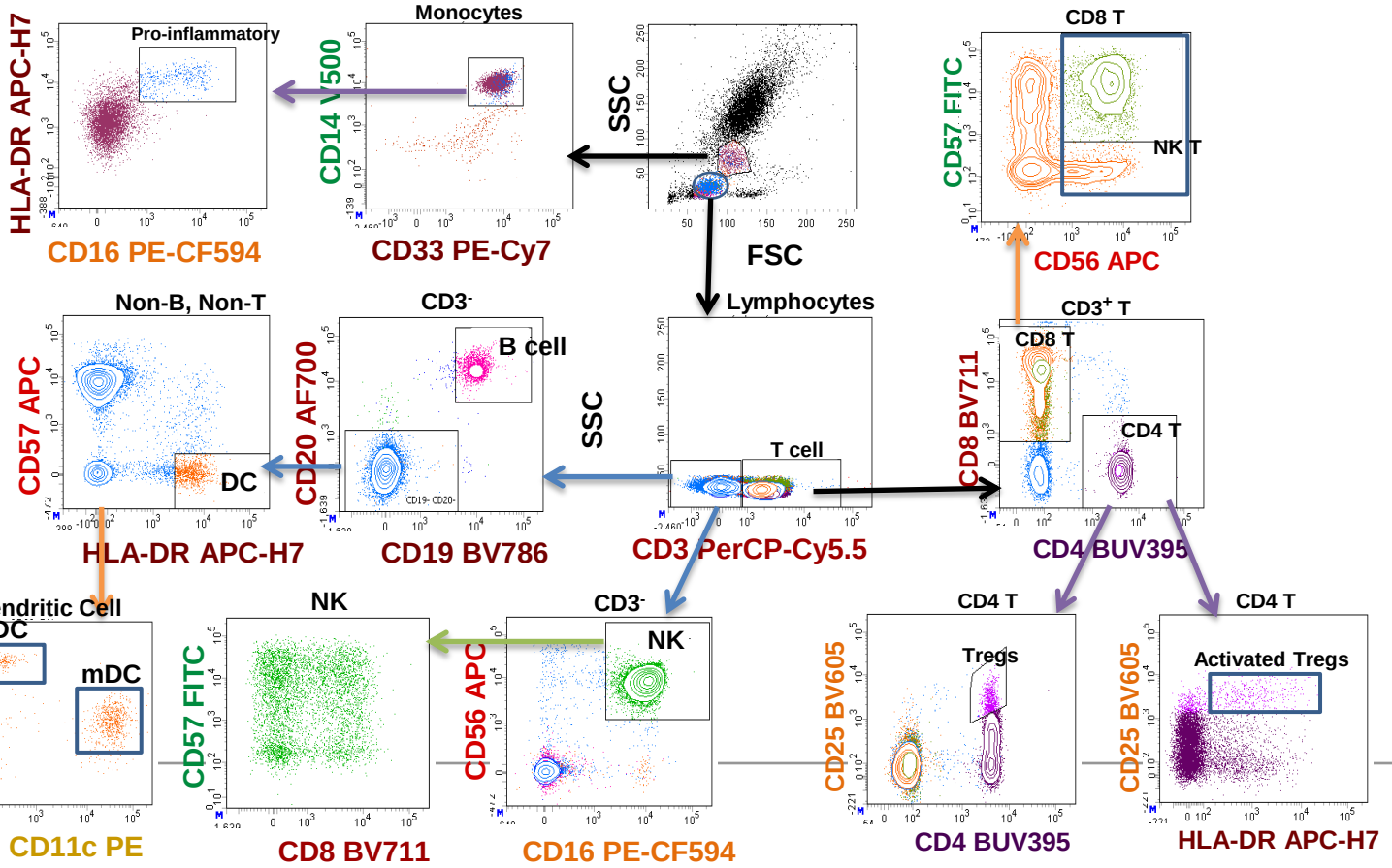
T, B, NK, NK-T, Mono, Dendritic cell subsets

14- Color Panel

UV Laser (355 nm)		**Violet Laser (405 nm)**	
BUV395	CD4	BV421	CD123
Blue Laser (488 nm)		V500	CD14
FITC	CD57	BV605	CD25
PerCP-Cy™5.5	CD3	BV711	CD8
		BV786	CD19

Yellow-Green Laser (561 nm)	
PE	CD11c
PE-CF594	CD16
PE-Cy™7	CD33

Red Laser (647 nm)	
APC	CD56
Alexa Fluor® 700	CD20
APC-H7	HLA-DR



Working with BD Horizon™ Brilliant Violet™ (BV) Reagents in Multicolor Panels

A New Class of Reagents

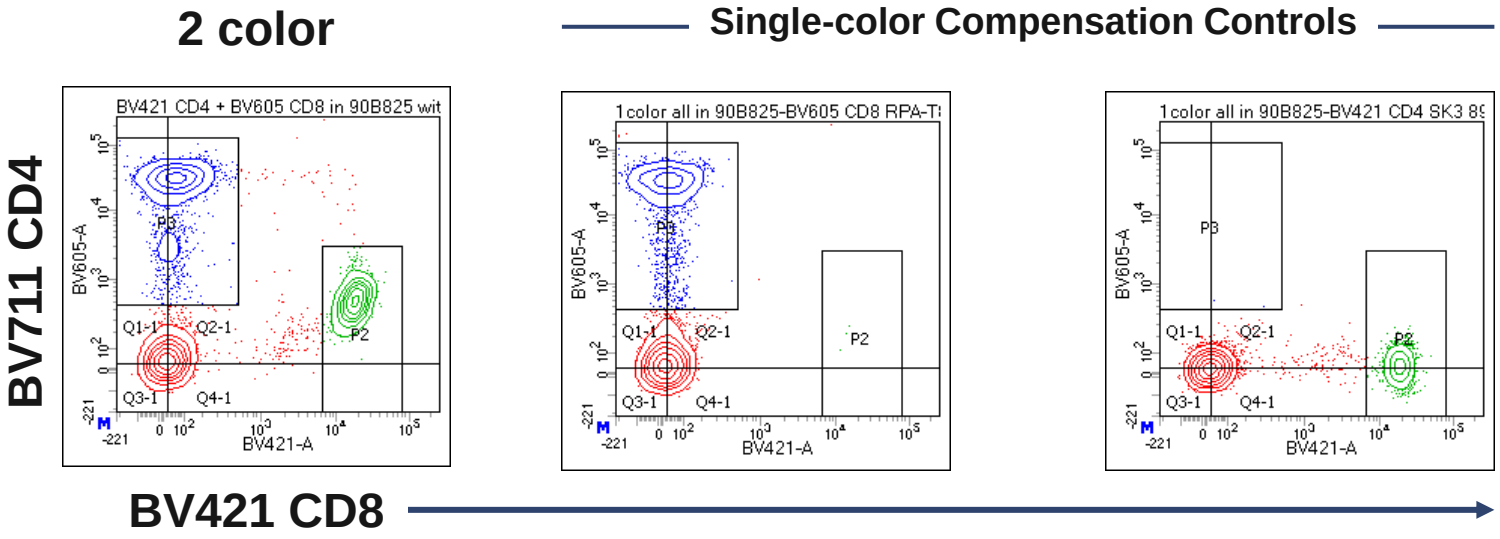
- Based on technology developed by Sirigen, BV polymer dyes represent a whole new class of fluorochrome reagents distinct from small organic molecules (FITC, Alexa Fluor® 700), fluorescent proteins (PE, APC), and their tandem conjugates.

- As seen with many new fluorochrome technologies, there might be special care required to achieve optimal results.
  - For example, setting up compensation for tandem fluorochromes.

- During the development and release of the various BV reagents, BD scientists noted that there could be anomalous staining patterns when multiple BV reagents were used in multicolor panels.

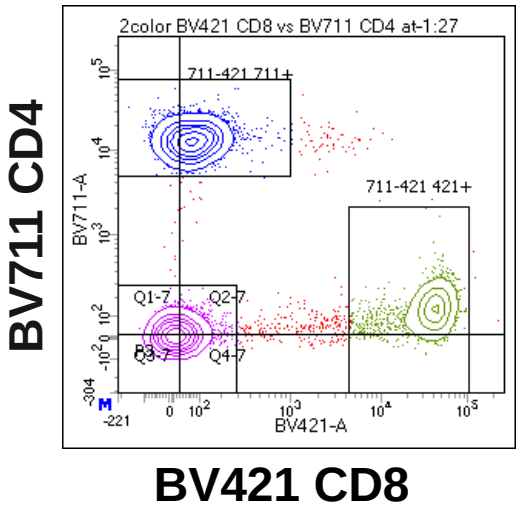
Interactions Between BV Reagents

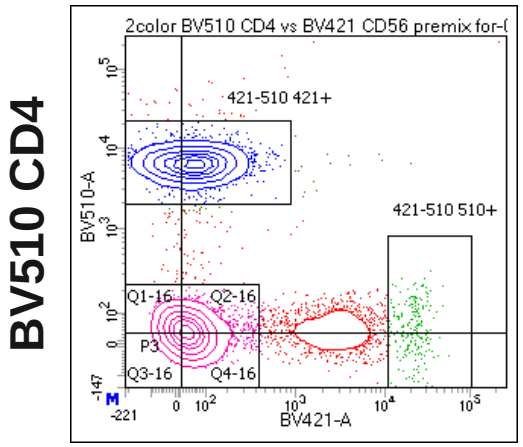
- Under some staining conditions, populations that should be negative for a given marker would show low to moderate levels of nonspecific background staining.
  - Cell populations appeared to be undercompensated.
- This is most easily observed on very bright orthogonal populations such as CD4 vs CD8.

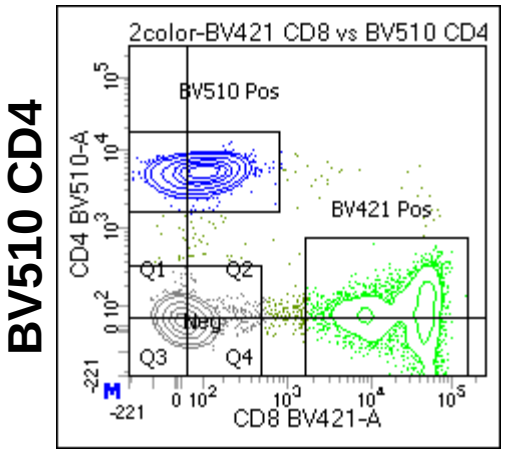


Interactions Between BV Reagents

- This interaction is not seen with all combinations of BV reagents.
- The impact is less apparent in multicolor cocktails.







In many cases, the interaction might not be observable in the data.

Development of Brilliant Stain Buffer

BD scientists have spent over a year studying when and how these reagent interactions occur and how they can be prevented.

- Our internal experiments purposely focused on reagent combinations that showed the worst interactions and backgrounds.
- Our studies showed that:
  - The interaction (background) was due to BV dyes on some antibodies interacting with each other.
  - The level of interaction is a function of:
    - Which reagents are used
    - The concentration of the reagents
    - The number of reagents in the cocktail
  - The interaction was increased with pre-mixing.

BD Horizon Brilliant Stain Buffer

The result of these studies is the development of **BD Horizon™ Brilliant Stain Buffer,** which dramatically improves the performance of all BV reagents in multicolor experiments.

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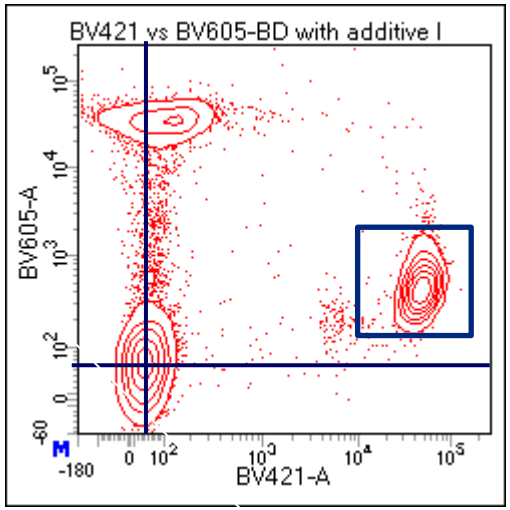
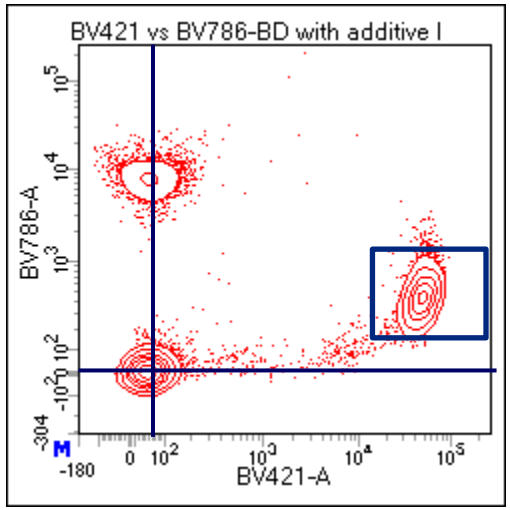
Brilliant Stain Buffer Blocks BV Reagent Interactions

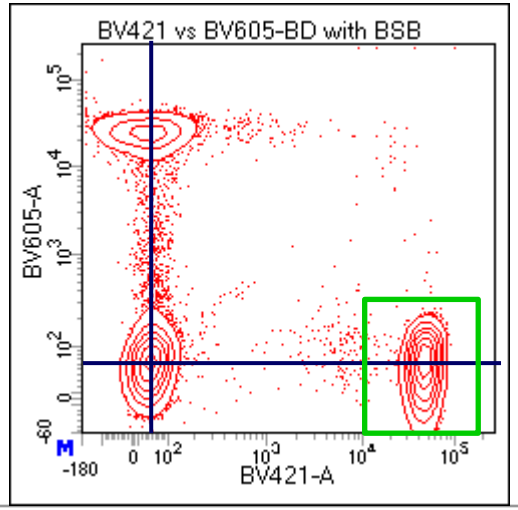
BD Horizon™ Brilliant Stain Buffer

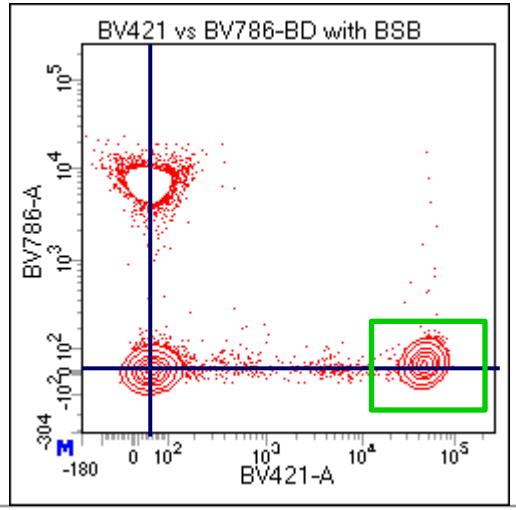
Without





Samples stained in the presence of Brilliant Stain Buffer are at their expected locations





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How to Use Brilliant Stain Buffer

Multicolor Staining of Human Cell Samples in Tubes or 96-well Plates Using Individual Staining Reagents

1. Add 50 µL of BD Horizon Brilliant Stain Buffer to all tubes or wells for the experiment.
2. Add each fluorescent reagent at the recommended volume per test (for example, 5 µL or 20 µL).

   Note: The 50-µL amount of Brilliant Stain Buffer per tube or per well does not depend on the final staining volume or amount of cells used per tube or the number of BD fluorescent antibodies used in staining.
3. Perform the standard staining protocol.

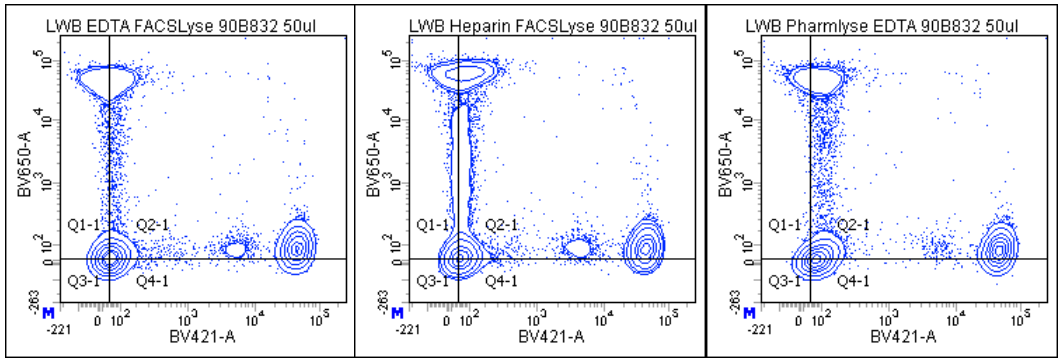
BD

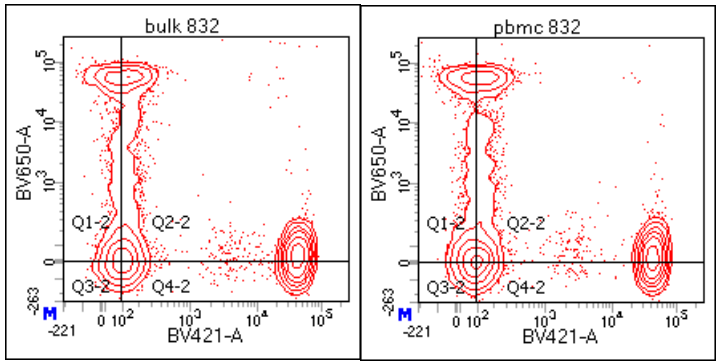
Brilliant Stain Buffer works with Standard Cell Preparation and Staining Protocols

BD FACS™ Lysing Solution | **BD Pharm Lyse™ buffer** | **Bulk Lyse** | **PBMCs**

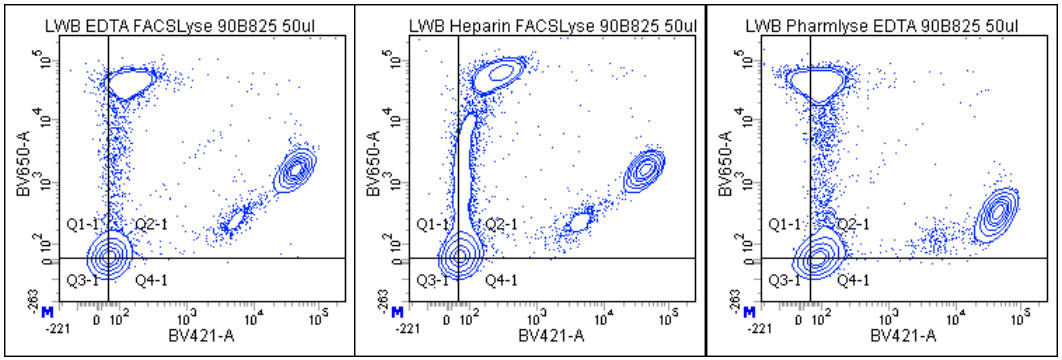
EDTA | **Heparin** | **EDTA**

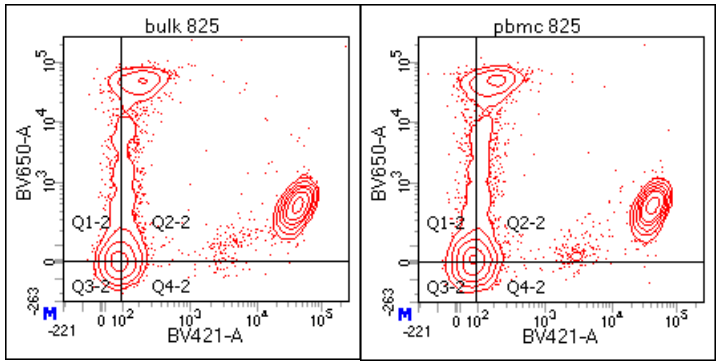
With Brilliant Stain Buffer





Without Brilliant Stain Buffer

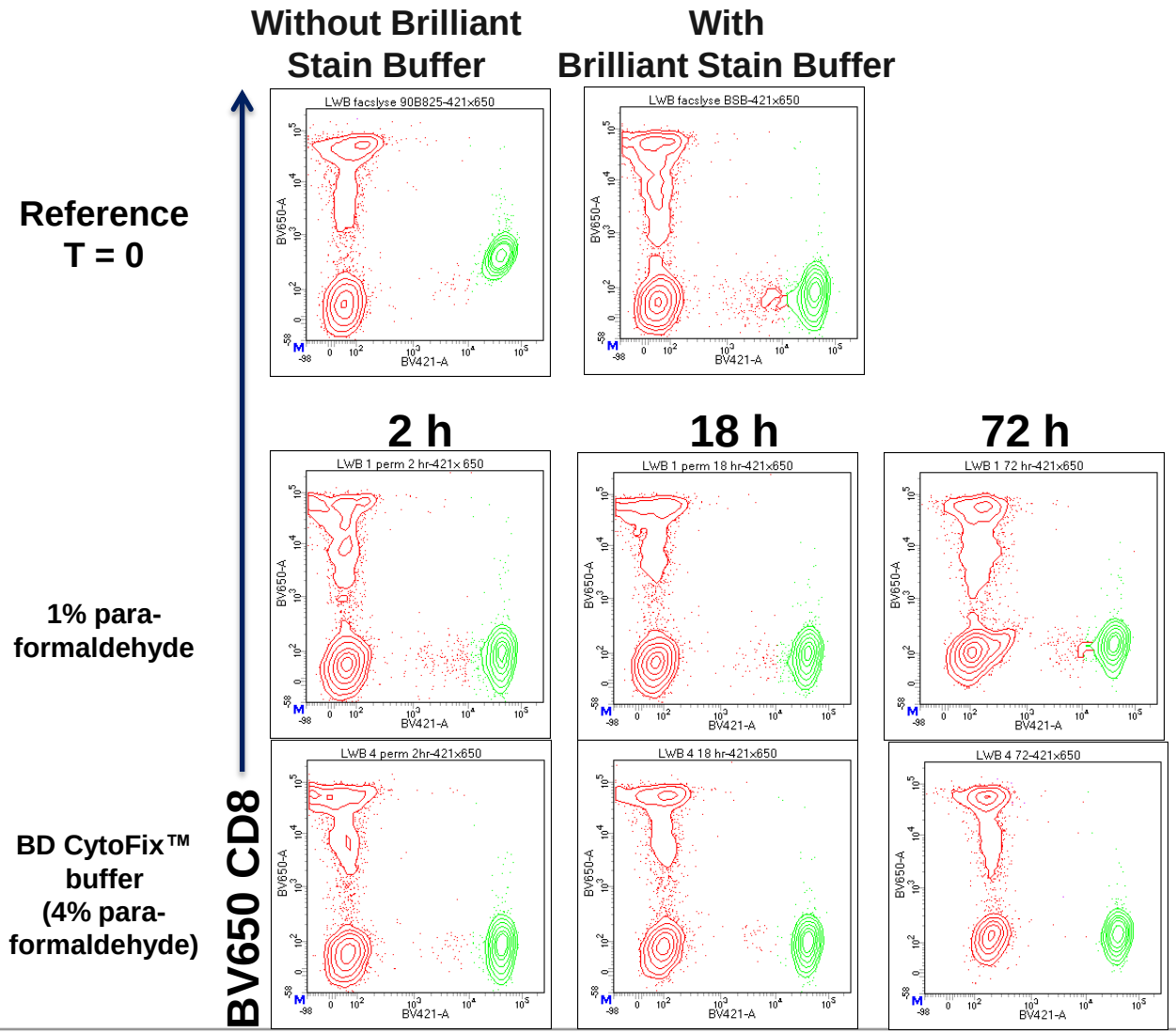




CD8 BV650

CD4 BV421

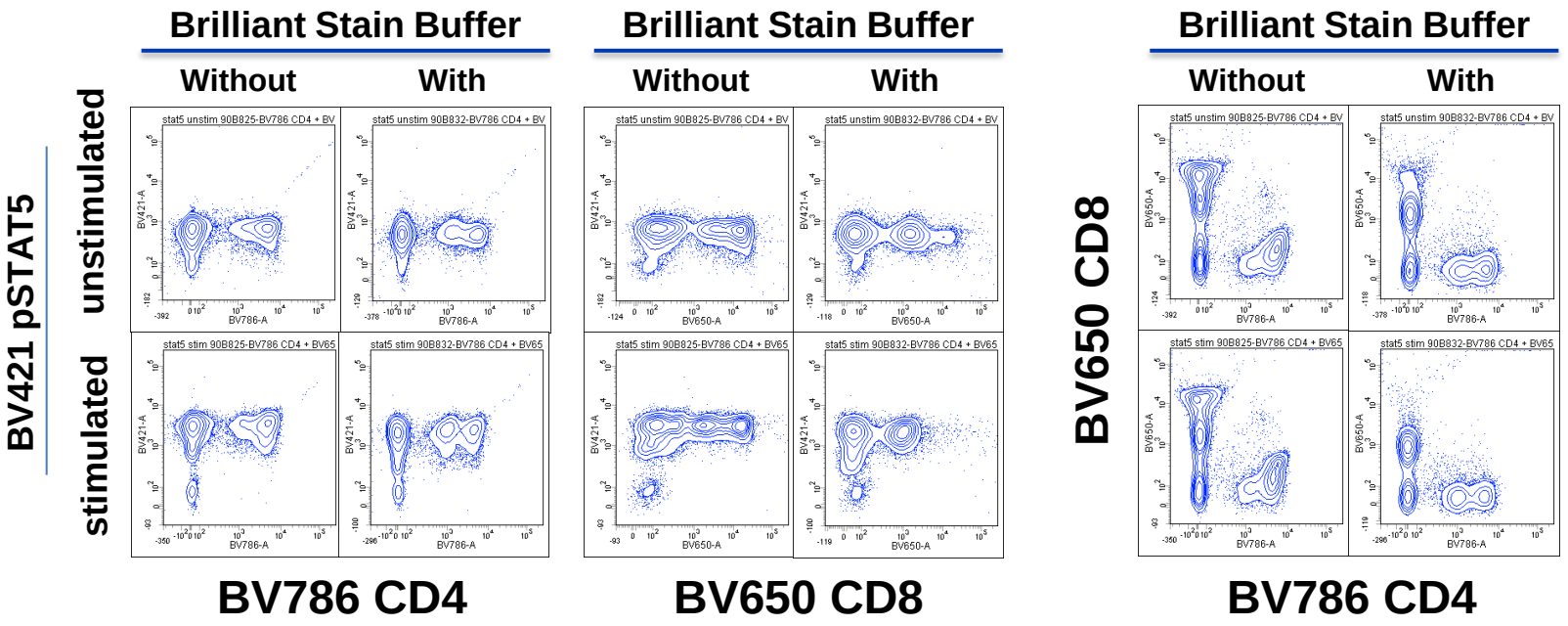
Brilliant Stain Buffer Provides Equivalent Performance after Fixation



- Overall background increases over time as a function of the fixation.
- Staining patterns remain constant.

Brilliant Stain Buffer is Compatible with Most Intracellular Staining Buffers

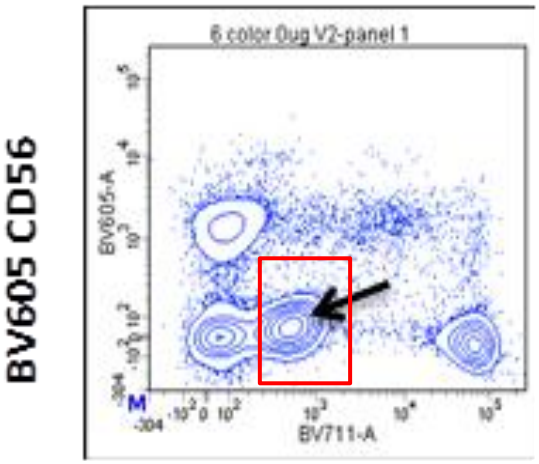
Three-color staining: BV786 CD4 + BV650 CD8 + BV421 pSTAT5
BD Phosflow™ Perm Buffer III



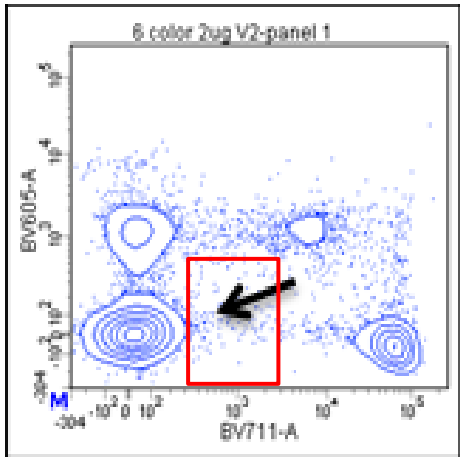
Multicolor Cocktails

Double-Negative Populations Might Appear

Without Brilliant Stain Buffer



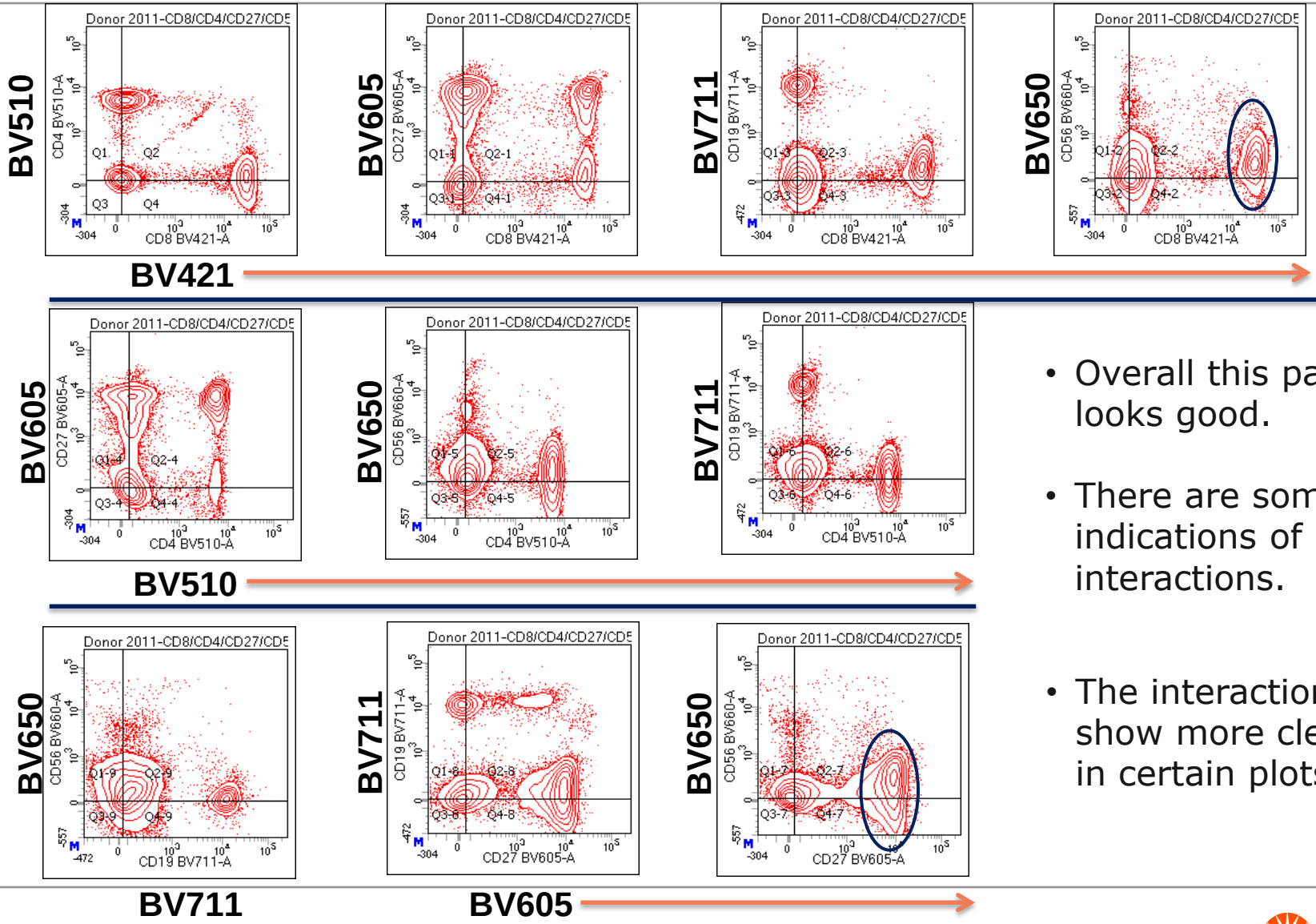
With Brilliant Stain Buffer



- In a multicolor experiment in which there are many double- and triple-positive populations, often the easiest way to detect aberrant staining is to look at the negative population.
- In some instances there is a “budding” of the negative population that should not be there.

Five-Color Analysis

BV421 CD8, BV510 CD4, BV605 CD27 BV650 CD56*, BV711 CD19



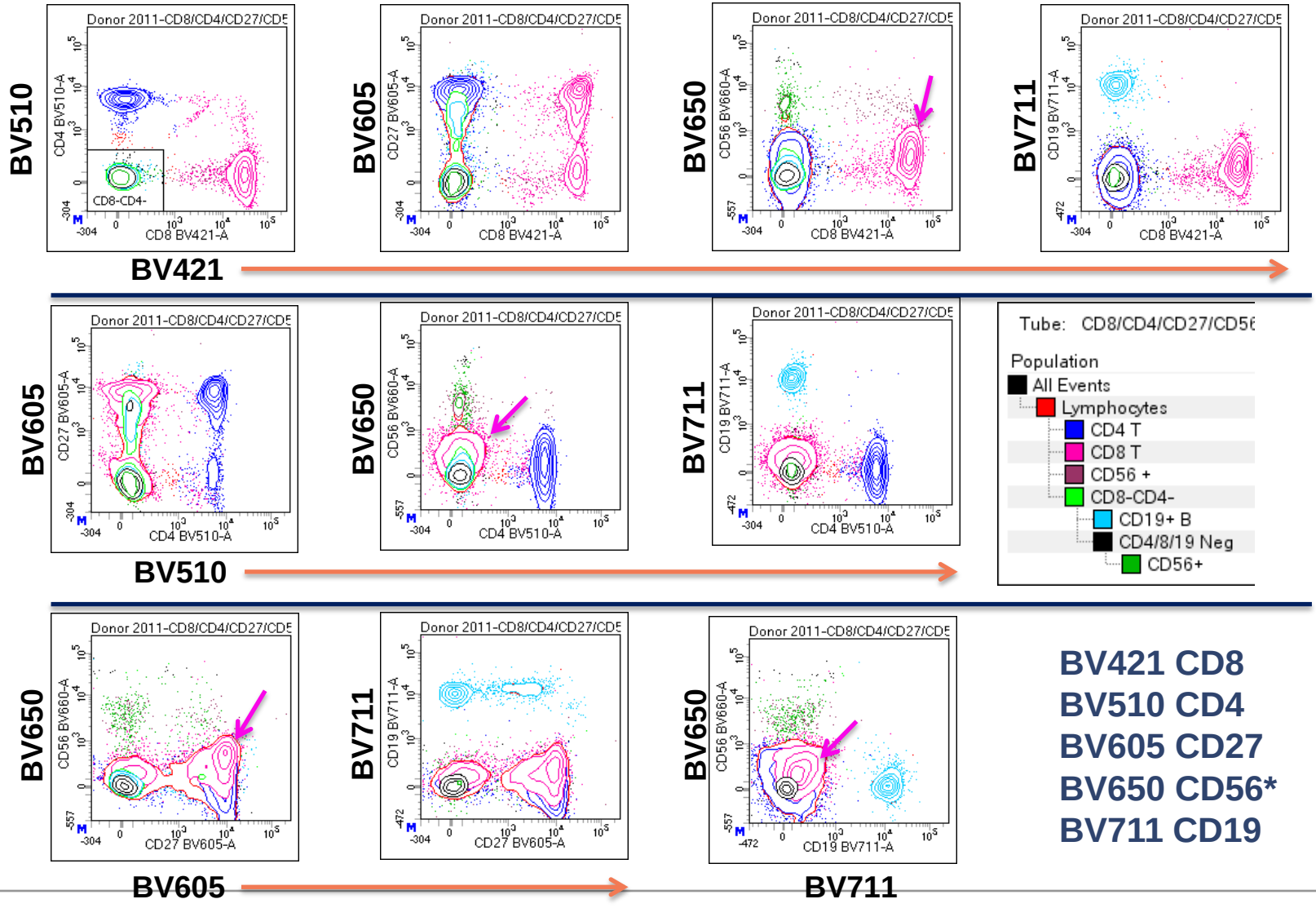
- Overall this panel looks good.
- There are some indications of interactions.
- The interactions show more clearly in certain plots.

* Product under development. Not for sale or use.

BD

Five-Color Analysis

BV421 CD8, BV510 CD4, BV605 CD27 BV650 CD56*, BV711 CD19



BV421 CD8
BV510 CD4
BV605 CD27
BV650 CD56*
BV711 CD19

* Product under development. Not for sale or use.

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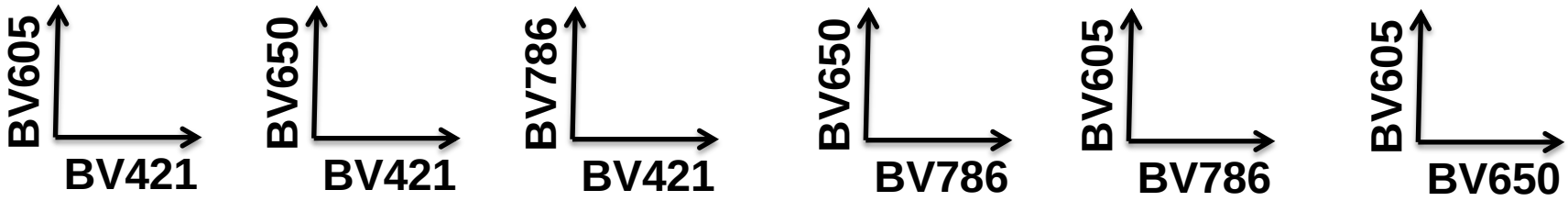
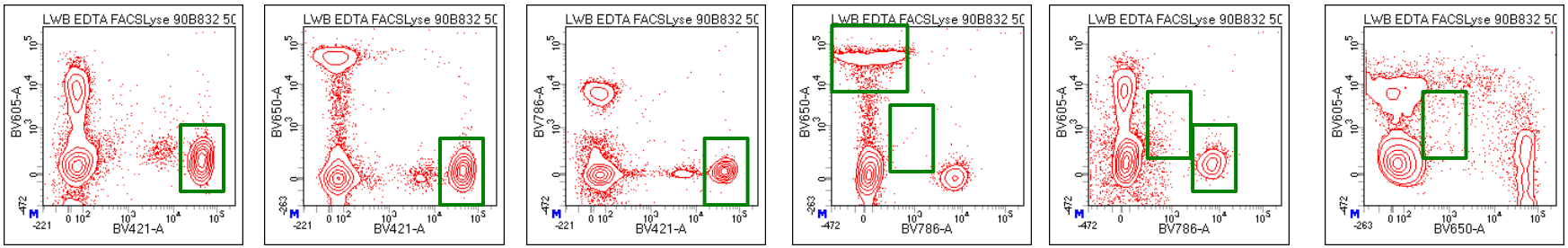
Four-color Cocktail with and without Brilliant Stain Buffer

CD4 BV421; CD56 BV605; CD8 BV650; CD19 BV786

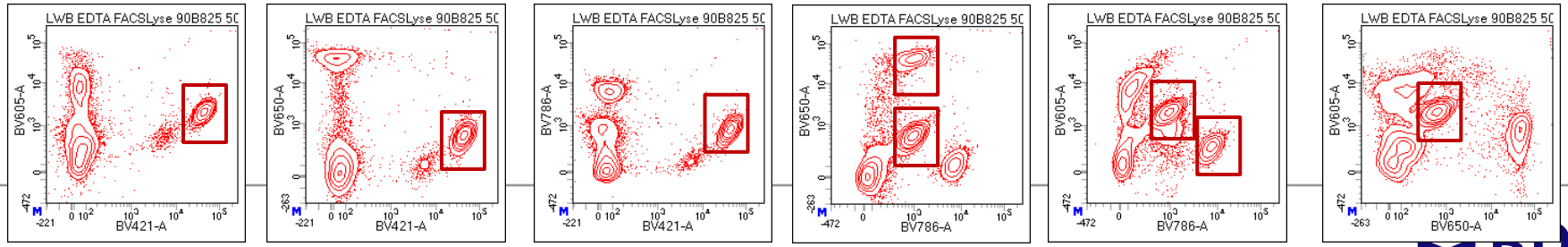
LWB – BD FACS Lysing Solution – EDTA



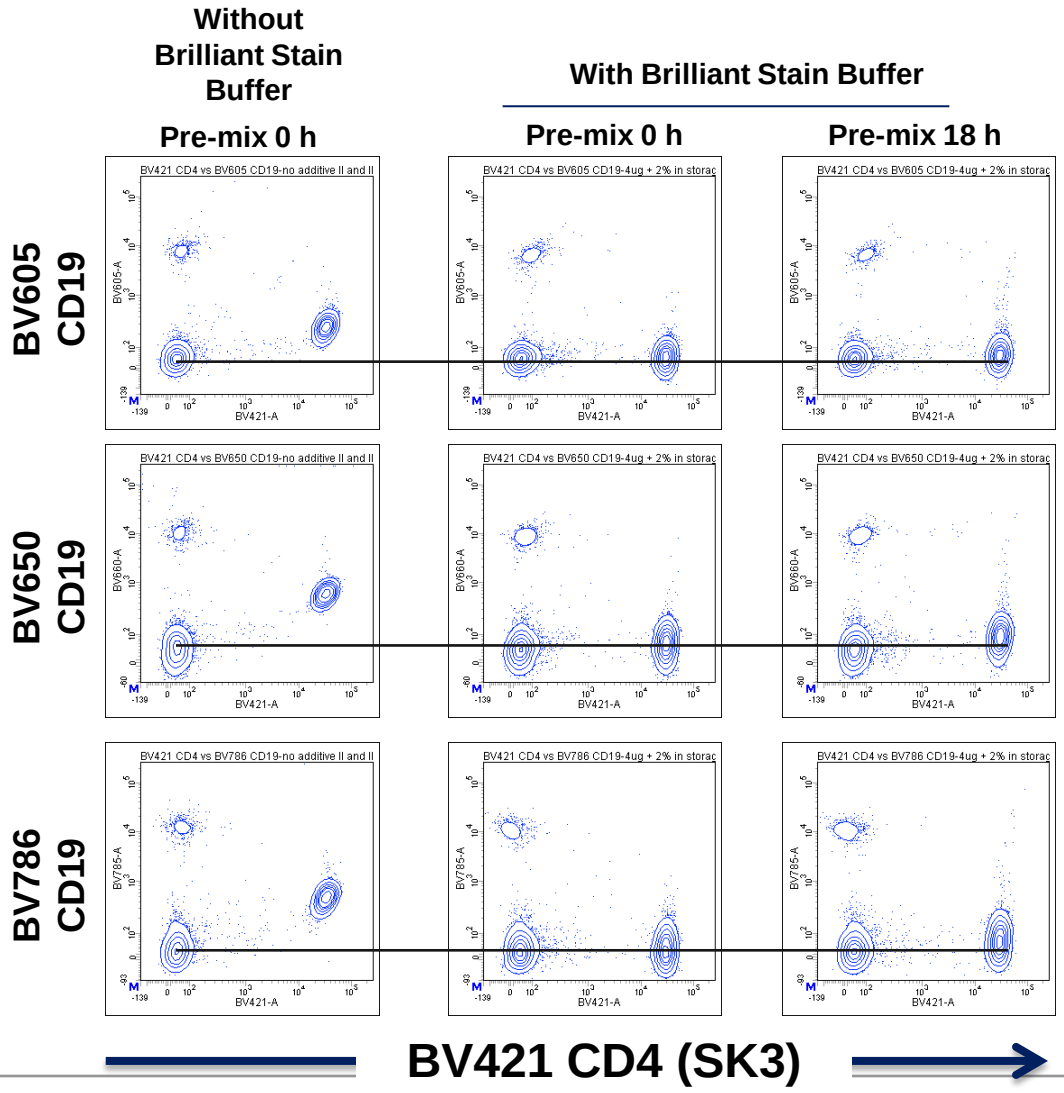
With Brilliant Stain Buffer



Without Brilliant Stain Buffer

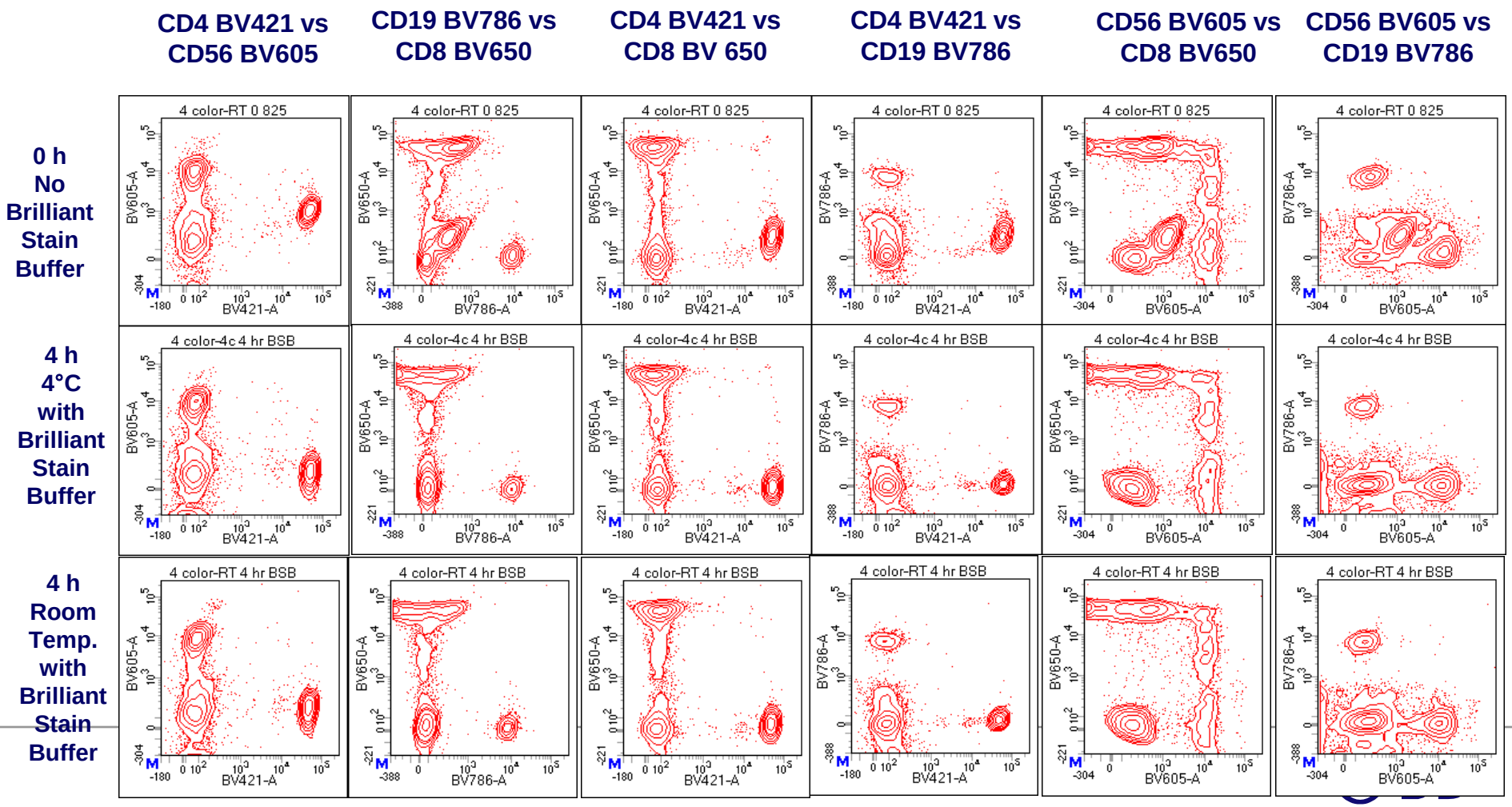


Brilliant Stain Buffer Improves Cocktail Pre-mixing



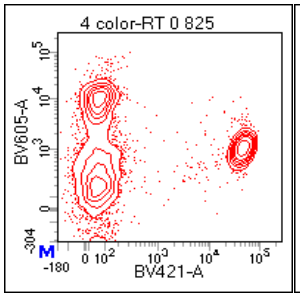
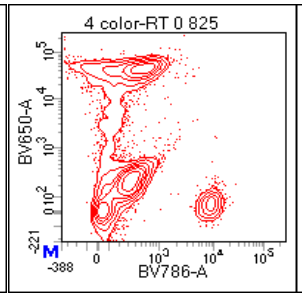
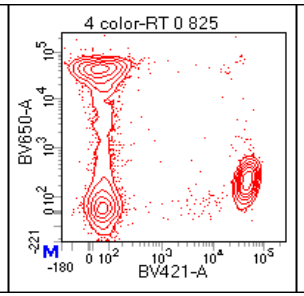
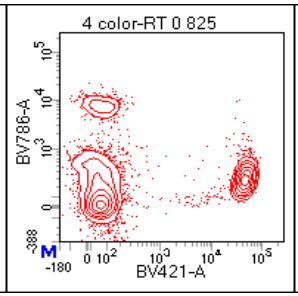
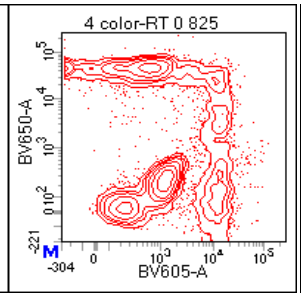
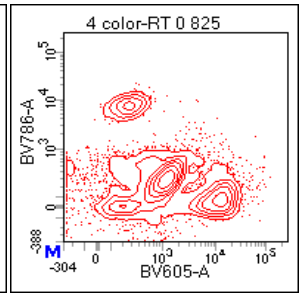
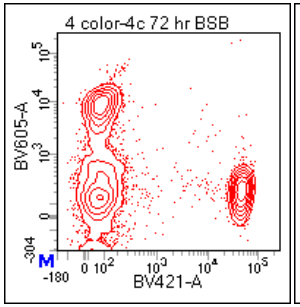
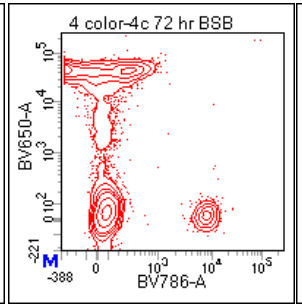
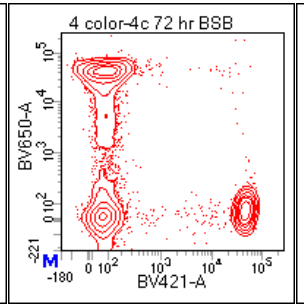
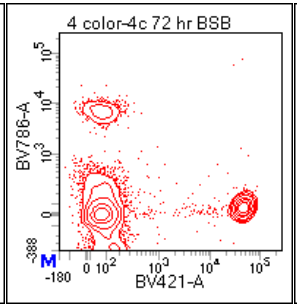
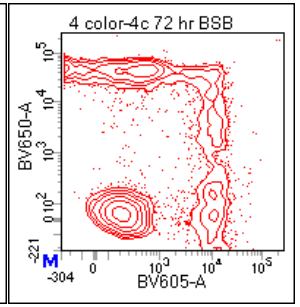
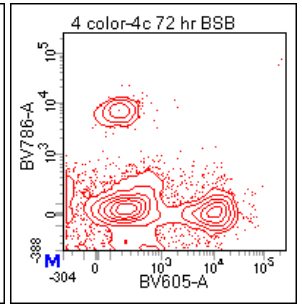
Four-color Cocktail, Pre-mixed

BV421 CD4, BV605 CD56, BV650 CD8, BV786 CD19
Time 0 without Brilliant Stain Buffer vs 4 h @ 4°C and 4 h @ RT with Brilliant Stain Buffer



Four-color Cocktail, Pre-mixed

BV421 CD4, BV605 CD56, BV650 CD8, BV786 CD19
Time 0 without Brilliant Stain Buffer vs 72 h @ 4°C

	CD4 BV421 vs CD56 BV605	CD19 BV786 vs CD8 BV650	CD4 BV421 vs CD8 BV650	CD4 BV421 vs CD19 BV786	CD56 BV605 vs CD8 650	CD56 BV605 vs CD19 BV786
0 h No Brilliant Stain Buffer						
72 h 4°C with Brilliant Stain Buffer						

How to Use Brilliant Stain Buffer

Pre-mixed Fluorescent Reagent Cocktails

Option A: Add all reagents at the same time

1. For each multicolor test of cocktailed fluorescent reagents:
   i) Add 50 µL of BD Horizon Brilliant Stain Buffer per test.
   ii) Add each fluorescent reagent at the recommended volume per test (5 µL or 20 µL).
   iii) Mix reagents (especially after adding BV reagents).
   iv) Store the cocktail at 4°C protected from light if it is to be used later.
   Note: Fluorescent reagent cocktails are best used within 24 hours after preparation.

   Example of creating a five-color fluorescent antibody cocktail containing two different BV conjugates

Reagent	Volume/Test (µL)	Total Number of Tests			
		1	3	5	10
Brilliant Stain Buffer	50	50	150	250	500
Reagent 1	5	5	15	25	50
Reagent 2	5	5	15	25	50
Reagent 3	20	20	60	100	200
Reagent 4 (BV)	5	5	15	25	50
Reagent 5 (BV)	5	5	15	25	50
Total Volume	**90**	**90**	**270**	**450**	**900**

Add the desired volume of reagent cocktail (90 µL in this five-color example) to all tubes using standard protocols for staining human cells.

How to Use Brilliant Stain Buffer

Pre-mixed Fluorescent Reagent Cocktails

Option B: Pre-make non-BV cocktails

1. Pre-make the cocktail of all non-BV reagents (for example, reagents 1, 2 and 3).
   (This cocktail can be stored for 1–3 weeks.)
2. For the full cocktail for each multicolor test of cocktailed fluorescent reagents:
   i) Add 50 µL of BD Horizon Brilliant Stain Buffer per test.
   ii) Add the pre-mixed non-BV cocktail (30 µL in this example).
   iii) Add the BV reagents.
   iv) Mix reagents (especially after adding BV reagents).
   v) Store the cocktail at 4°C protected from light if it is to be used later.
   Note: Fluorescent reagent cocktails are best used within 24 hours after preparation.

   Example of creating a five-color fluorescent antibody cocktail containing two different BV conjugates

Reagent	Volume/Test (µL)	Total Number of Tests			
		1	3	5	10
Brilliant Stain Buffer	50	50	150	250	500
Reagent 1+2+3	30	30	90	150	300
Reagent 4 (BV)	5	5	15	25	50
Reagent 5 (BV)	5	5	15	25	50
Total Volume	**90**	**90**	**270**	**450**	**900**

FAQs (1)

- Can I use BV reagents without Brilliant Stain Buffer?
  - *Yes. Depending upon the reagents used, the data might look perfectly fine.*
- How can I get Brilliant Stain Buffer?
  - *This product is shipped free of charge with all BV reagents.*
  - *You can request a free sample from the BD Biosciences website or your representative.*
- Are there any conditions in which Brilliant Stain Buffer will not work?
  - *To date we have not identified any conditions in which Brilliant Stain Buffer does not work.*
  - *Brilliant Stain Buffer does not impact staining with any non-BV reagents.*

FAQs (2)

- Is the order of Brilliant Stain Buffer addition important?
  - *Yes. Brilliant Stain Buffer should be added before any BV reagents are added to the staining tube or a cocktail.*
- Is the order of addition of cells and reagents important?
  - *No. No difference is seen when cells are added first and then reagents (with Brilliant Stain Buffer) second or when the reagents (with Brilliant Stain Buffer) are added first and cells second.*
- Does Brilliant Stain Buffer work with all BV reagents?
  - *Yes. Brilliant Stain Buffer does reduce reagent interaction between all BV reagents. However, Brilliant Stain Buffer and BD's BV reagents have been quality controlled and formulated to provide optimized results.*

FAQs (3)

- Does Brilliant Stain Buffer work with the newly released BD Horizon™ Brilliant Ultraviolet™ 395 (BUV395) reagents?
  - *Yes. Brilliant Stain Buffer blocks interactions between BUV395 and other BV reagents.*

Summary

- BV reagents offer a wide array of options for successful multicolor flow cytometry.
- Brightness is a key feature of BV dyes, which enhances resolution.
- As part of BD Biosciences ongoing commitment to quality, BV reagents have been optimized with:
  - A formulation that improves performance with samples containing serum.
  - A new Brilliant Stain Buffer that further enhances the performance of BV reagents.
    - For more information visit bdbiosciences.com/go/brilliant.

For more information…

If you have further questions:

Contact Technical Support (US) at:

877-232-8995, Prompt 3, 2

or email: ResearchApplications@bd.com

Please visit our multicolor flow cytometry resources site at: bdbiosciences.com/colors.

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