BD Horizon Brilliant™ Ultraviolet Reagents

Enabling Deep Cell Analysis

Features

- UV (355 nm)–excitable dyes
- Obtain deep population analysis
- Low spillover into most detectors
- More choice and flexibility for multicolor panel design

In the recent past, deep immunophenotyping applications have been constrained by the limited selection of fluorochromes that offered spectral separation and that were excited by a variety of laser wavelengths. The establishment of a unique family of dyes excited by the previously underutilized ultraviolet laser (355 nm) dramatically expanded the number of markers that could be simultaneously investigated in a single tube assay. With the release of BD Horizon Brilliant™ Ultraviolet (BUV) Dyes, developed exclusively by BD Biosciences, researchers are able to expand their multicolor capabilities on flow cytometers equipped with a UV laser, by extending the usable emission spectrum. In addition to obtaining great population resolution of low expression markers, BUV dyes allow for the distribution of fluorochromes across more lasers and a wider spectrum, thereby, reducing spillover and facilitating panel design. BUV reagents also enable you to design lower parameter panels with lower spillover and spread, resulting in high-quality data.

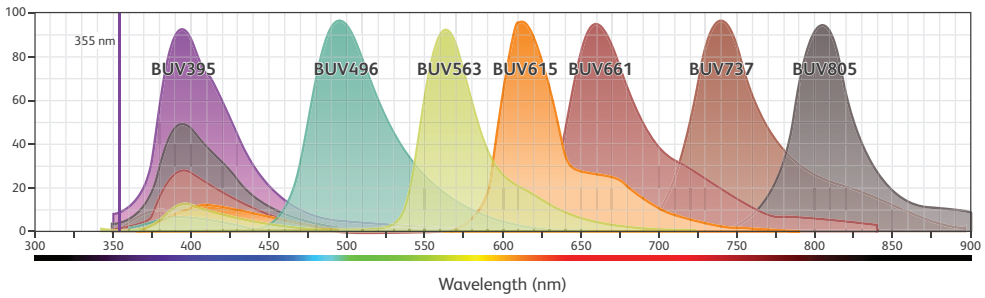

Figure 1. Emission profiles of BD Horizon™ BUV395, BUV496, BUV563, BUV615, BUV661, BUV737 and BUV805 Dyes

Family of BD Horizon Brilliant™ Ultraviolet Reagents

As the first UV reagent launched by BD, BD Horizon Brilliant™ Ultraviolet 395 (BUV395) quickly became the base polymer dye for a family of BD Horizon Brilliant™ Ultraviolet Tandem Reagents. Having virtually no spillover into any other detector, BUV395 was the ideal donor to aid in the expansion of fluorochromes throughout the UV spectrum, including BUV496, BUV563, BUV615, BUV661, BUV737 and BUV805 reagents. This same characteristic makes BUV395 the ideal fluorochrome to add to an existing panel, as it will minimally impact the resolution of the other markers in the panel. BUV395 is a moderately bright fluorochrome and can resolve a range of antigen expression levels (Table 1).

The line of BD Horizon Brilliant™ UV Reagents was specifically designed to expand the utility of the ultraviolet laser. BUV737, BUV563 and BUV661 are good choices for critical or low expression markers as these dyes provide bright resolution (Table 1). When coexpression is a factor, the low spillover profiles of BUV496 and BUV805 may provide optimal results due to their limited impact on the resolution of other fluorochromes.

With so many options for fluorochromes, managing spillover between reagents can be one of the more difficult elements of multicolor panel design. BUV reagents aid in distributing markers over multiple lasers, which can decrease spillover and spread. For example, by assigning one marker to the first fluorochrome excited by each laser, a 5-color panel with minimal spillover and resulting spread can be run on an instrument equipped with UV, violet, blue, yellow-green and red lasers.

Many BUV reagents are available through the line of BD OptiBuild™ Reagents, which offer researchers a vast selection of on-demand BUV-conjugated antibodies to meet their research needs.

BUV Reagents:

- Provide more options for markers of interest, thereby increasing panel design flexibility
- Simplify the design of high-quality, lower-parameter panels by reducing spread (Table 2 and Figure 3)
- Enable the detection of higher number of parameters in a single sample than previously possible (Table 3 and Figure 4)

Table 1

Fluorochrome	Filter	Relative resolution
BUV395 (donor dye)	379/28	
BUV496 (tandem dye)	515/30, 450	
BUV563 (tandem dye)	585/15, 535	
BUV615 (tandem dye)	610/20	
BUV661 (tandem dye)	670/25, 630	
BUV737 (tandem dye)	740/35, 690	
BUV805 (tandem dye)	820/60, 770	

Relative resolution key: Dim, Moderate, Bright, Very Bright

Table 1. Relative resolution of BD Horizon Brilliant™ UV (BUV) Reagents
Human blood samples were stained with several specificities conjugated to all BUV reagents and acquired on different flow cytometers. Relative resolution was determined based on the average stain index measured for each dye across specificities and instruments. Please note, relative resolution may change depending on instrument and instrument configuration, including lasers, laser power and filters.

Figure 2

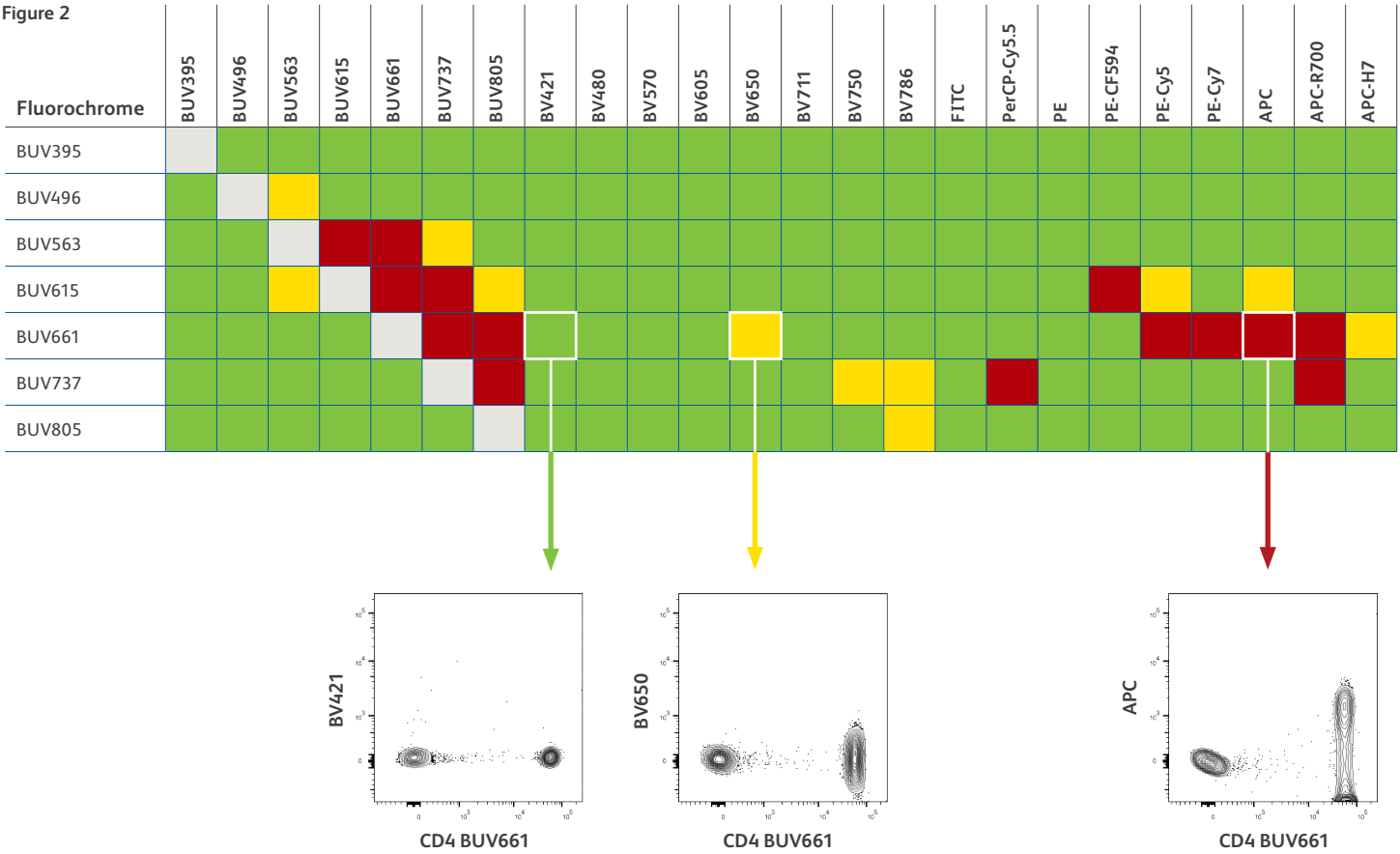

Figure 2. Representative summary of spread introduced by BD Horizon Brilliant™ UV Dyes into other detectors
A spread matrix was generated after staining human blood cells with CD4 conjugated with BD Horizon Brilliant™ UV Dyes (single stain). Samples were acquired on a BD FACSymphony™ A5 Cell Analyzer. Spread from an individual dye (row) into all the other detectors (columns) was visually assessed and color-coded as shown in the representative plots. Green=minimal spread; Yellow=medium spread; Red=high spread. The table shows how some BD Horizon Brilliant™ Dyes such as BUV395, BUV496 and BUV805 introduce very minimal to no spread into all the other detectors. Other BUV dyes have minimal impact on the majority of the other detectors. Please note that the amount of spread would vary depending on antigen density (lower spread for markers expressed lower than CD4 and higher spread for markers expressed higher) instrument configuration and set-up.

Table 2

Laser	Filter	Fluorochrome	Human T-cell panel	Human B-cell panel	Mouse B-cell panel
Ultraviolet 355 nm	379/28	BUV395	CD4	CD19	CD19
Violet 405 nm	450/40	BV421	CD3	CD27	CD23
Blue 488 nm	530/30	FITC	CD8	IgD	IgD
Yellow-Green 561 nm	610/20	PE	CD27	CD38	IgM
Red 640 nm	670/30	APC	CD45RA	IgM	CD21

Table 2. Example of instrument configuration and possible panels to achieve a 5-color panel with minimal spillover and resulting spread

Fluorochromes for a 10-color panel on a 5-laser instrument

To facilitate the development of a 10-color panel, we have assessed the spillover and resulting spread among fluorochromes compatible with a 5-laser configuration BD LSRFortessa™ X-20 Cell Analyzer. A strategic set of fluorochromes with minimal spillover, including three BUV reagents, was chosen (Figure 2).

This combination enables:

- Simplified panel design by reducing challenging fluorochrome combinations
- Generation of high-quality data through minimal introduction of spread

Foundation fluorochromes for a 10-color panel on a 5-laser instrument

Figure 3A

	BV421	PE-Cy7	PE	BUV615	BV480	BV750	BUV395	APC	BUV496	FITC	CD4 Stain Index
BV421											735
PE-Cy7											684
PE											433
BUV615											321
BV480											213
BV750											152
BUV395											150
APC											104
BUV496											103
FITC											49

Figure 3B

Fluorochrome	Marker	Clone	Volume Per Test	Cat. No.
BUV395	CD3	SK7	5 µL	564001
BUV496	CD4	SK3	5 µL	612936
BV750	CD8	RPA-T8	1.25 µL	747385
BV480	CD45RA	HI100	5 µL	566114
APC	CD197 (CCR7)	2-L1-A	5 µL	566762
PE	CD95	DX2	20 µL	555674
BUV615	CD27	M-T271	1.25 µL	751135
PE-Cy7	CD28	CD28.2	5 µL	560684
FITC	CD57	NK1	2.5 µL	555619
BV421	CD279 (PD-1)	EH12.1	5 µL	562516

Figure 3C

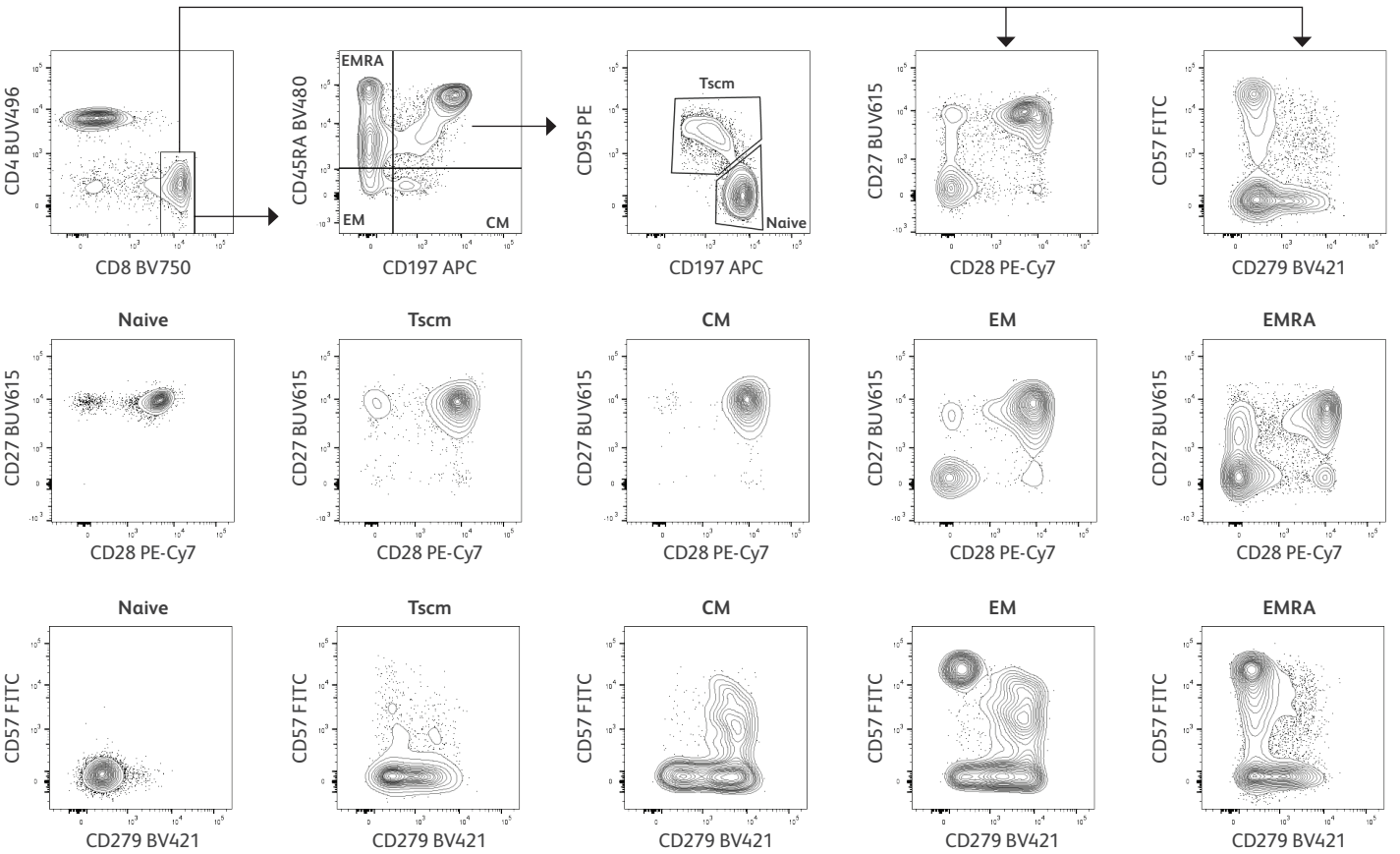

Figure 3. Example of a 10-fluorochrome combination with minimal spillover on a 5-laser (UV, violet, blue, yellow/green and red) BD LSRFortessa™ X-20 Cell Analyzer
A. Summary of spread across the selected fluorochromes generated using human peripheral blood mononuclear cells with mouse anti-human CD4, including stain index for CD4 calculated on the same instrument. The ability to distribute fluorochromes across more lasers enabled the expansion of the 5-color human T-cell panel (Table 2) to a 10-color panel with minimal increase in overall spillover and spread. Note that the amount of spread would vary depending on antigen density (lower spread for markers expressed lower than CD4 and higher spread for markers expressed higher). Similarly, the stain index values may change depending on instrument, instrument configuration and set-up. **B.** A 10-color panel was designed by taking into account fluorochrome resolution, antigen density and co-expression and potential impact from spread. **C.** Clear resolution of each population of interest was ensured by the overall minimal spillover and spread between fluorochromes in the panel.

28-color panel for analysis of immune cell composition

In this section, we describe a 28-color panel that was designed to identify and characterize various subpopulations of mouse leukocytes that play a role in both innate and adaptive immune responses (Table 3, Figure 4, Figure 5). The panel was developed so that it could be applied in cell analyses across varying lymphoid tissues and blood. Below are some of the markers used in the panel to identify major cell populations, including B-cells ($CD45R^+CD19^+$), T-cells ($CD3\varepsilon^+CD4^+$ or $CD8\alpha^+$), natural killer cells (NK, $NK1.1^+$) and dendritic cells (DCs, $CD11c^+$) as well as monocytes, macrophages and neutrophils. In addition to a total of 27 antibodies, the cells were stained with BD Pharmingen™ 7-AAD for exclusion of dead cells from the analyses (Table 3).

Table 3A

B-Cells $CD19^+$			
Immature/ Transitional	**Marginal Zone**	**Follicular**	**B-1a**
$B220^+$	$B220^+$	$B220^+$	$B220^{low}$
IgM^{high}	IgM^+	IgD^{high}	$CD43^+$
$CD93^+$	$CD21^{high}$	$CD23^+$	$CD5^+$

T-Cells CD3 ε^+			
CD4 Naïve	**CD8 Naïve**	**Central Memory**	**Effector Memory**
$CD4^+$	$CD8^+$	$CD62L^+$	$CD62L^{low}$
$CD62L^{high}$	$CD62L^{high}$	$CD44^{high}$	$CD44^{high}$

Dendritic Cells $CD11c^+$		
Myeloid	**Lymphoid**	**Plasmacytoid**
$I\text{-}A/I\text{-}E^{high}$	$I\text{-}A/I\text{-}E^{high}$	$I\text{-}A/I\text{-}E^{low}$
$CD11b^+$	$CD8a^+$	$B220^+$

Monocytes and Macrophages $CD11b^+$	
Macrophages	**Monocyte Subsets**
$Ly\text{-}6C^{low}$	$Ly\text{-}6C^{high}$
$F4/80^{high}$	$Ly\text{-}6C^{low}$

NK Cells $NK1.1^+$	Neutrophils $CD11b^+$
$CD27^{high/low}$	$Ly\text{-}6C^{low}$
$CD11b^{high/low}$	$Ly\text{-}6G^+$
$NKG2A^{+/-}$	

Table 3B

Laser	Fluorochrome*	Marker
Ultraviolet 355 nm 40 mW	BUV805	CD45R/B220
	BUV737	Ly-6C
	BUV661	CD21
	BUV615	CD314/NKG2D
	BUV563	F4/80
	BUV496	IgD
	BUV395	CD8a
Violet 405 nm 100 mW	BV786	IgM
	BV750	CD25
	BV711	CD27
	BV650	CD23
	BV605	CD192
	BV570	CD4
	BV480	I-A/I-E
	BV421	CD44
Blue 488 nm 100 mW	BB790	NK1.1
	BB755	Ly-6G
	7-AAD	live/dead
	BB660	CD127
	BB630	CD11c
	BB515	CD62L
	SSC	
Yellow-Green 561 nm 150 mW	PE-C7	CD43
	PE-C5	NKG2A/C/E
	PE-CF594	CD93
	PE	CD5
Red 628 nm 200 mW	APC-H7	CD19
	APC-R700	CD11b
	APC	CD3ε

Table 3. 28-color panel for analysis of immune cell composition in different mouse tissues
A. Description of the major leukocyte cell populations based on the expression of cell lineage and cell differentiation surface markers. **B.** BD FACSymphony™ A5 Flow Cytometer laser configuration and antibody conjugates used in the 28-color panel.

*BD Horizon Brilliant™ Blue (BB) Dyes, BD Horizon Brilliant Violet™ (BV) Dyes, BD Horizon Brilliant™ Ultraviolet (BUV) Dyes

Analysis of leukocyte populations

Bivariate plots were employed to assess the resolution of each population (Figure 4). The gating strategies illustrated in Figures 4 and 5 were used to identify several immune cell subsets.

Figure 4

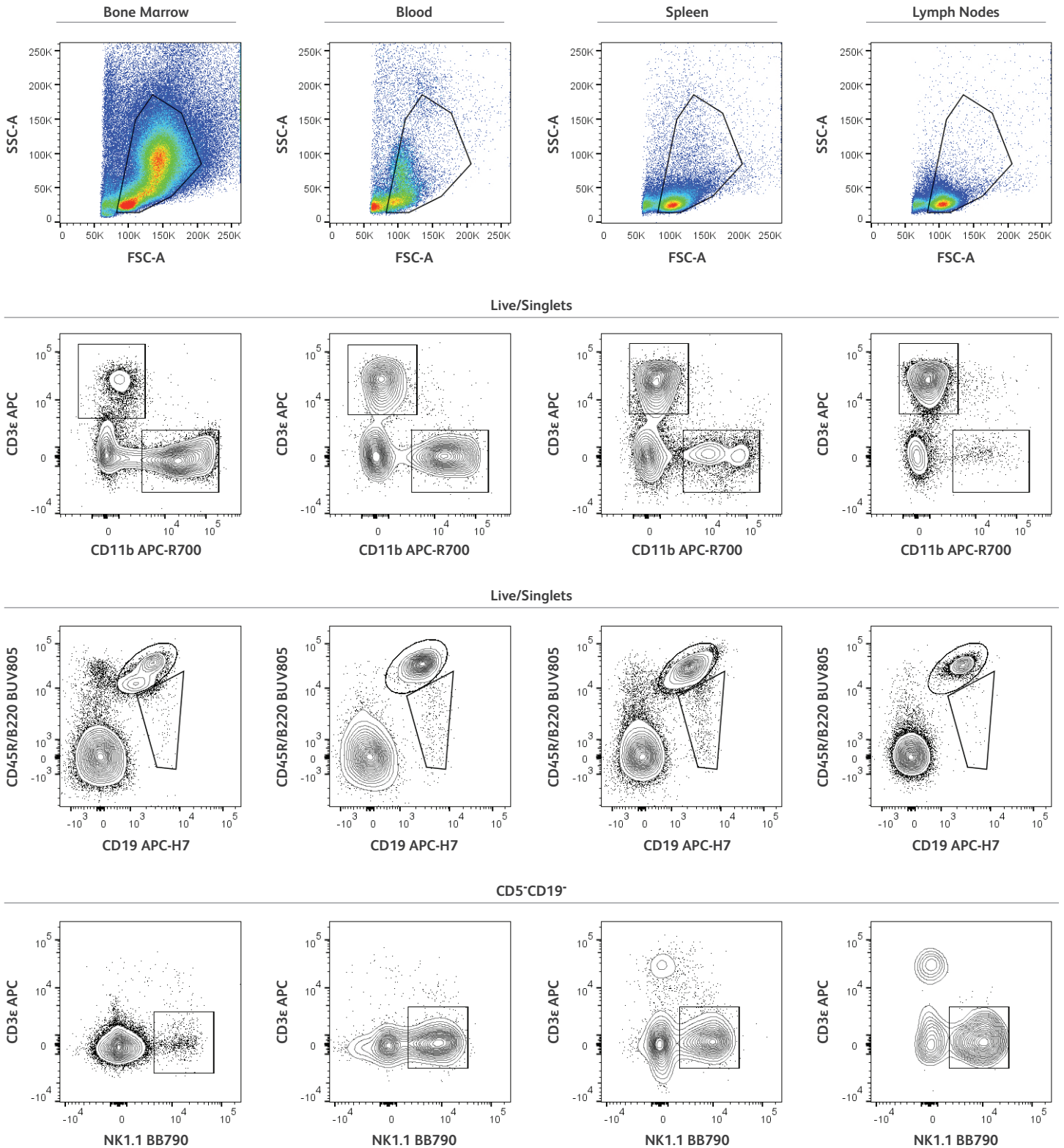

Figure 4. Supervised analysis of leukocyte populations across mouse tissues

The cell samples, stained as described in Table 3, were analyzed using a traditional manual gating strategy. The major immune cell populations of CD11b$^+$ myeloid cells, CD19$^+$ B cells , CD3ε$^+$ T cells and NK1.1$^+$ NK cells were gated as depicted in the plots. Similar to NK cells, DC populations were also analyzed within the CD5$^-$CD19$^-$ cells (not shown). Sequential gating for further analysis of cell subsets is illustrated in Figure 5.

Analysis of leukocyte subsets

Among the lymphoid populations, CD4$^+$ and CD8$^+$ T-cells were divided into memory and naïve subsets (Figure 5A), and B-cells were scrutinized into the various subsets of immature/transitional and mature cells. Analysis of IgD or CD21 expression in each B-cell subset further confirmed the phenotypes of these populations (Figure 5B). Within NK cells, CD11b^{high}CD27^{low} was the most abundant subset and a fraction of the total NK cells expressed high levels of NKG2A/C/E whereas the expression levels of NKG2D were overall very low (Figure 5C). The myeloid subsets of monocytes, macrophages, DCs and neutrophils were also investigated in the tissues (Figure 5D).

Figure 5

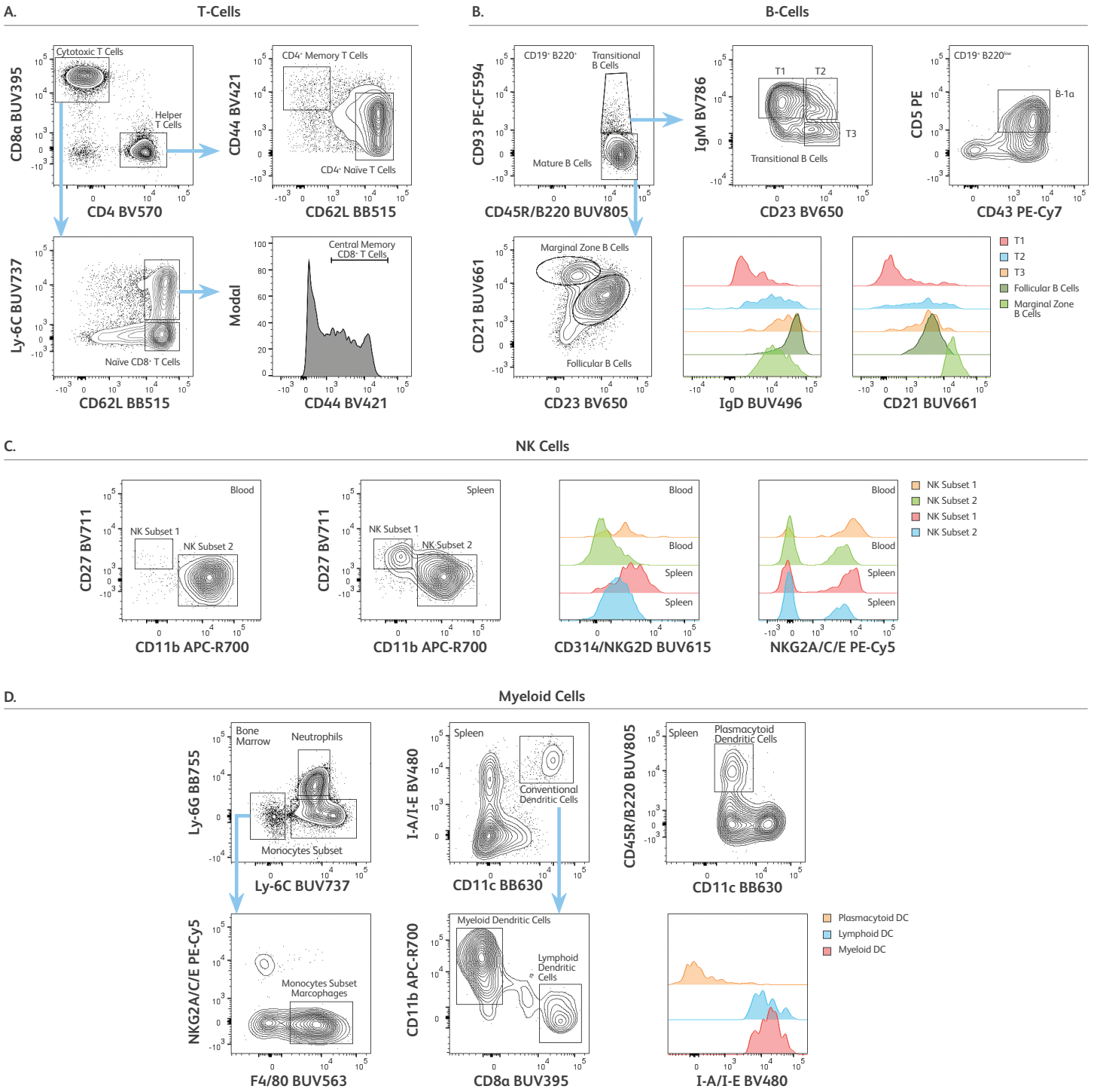

Figure 5. Representative analysis of leukocyte subsets in different mouse tissues

The major immune cell populations of CD11b$^+$ myeloid cells, CD19$^+$ B-cells, CD3$^+$ T-cells, NK1.1$^+$ NK cells and DCs were initially obtained as described in Figure 4. Then, further manual gating was used to define the indicated cell subsets in all four tissues: bone marrow, blood, spleen and lymph nodes. **A.** T-cell subsets in lymph nodes. **B.** B-cell subsets in spleen. **C.** NK-cell subsets in blood and spleen. **D.** Myeloid cell subsets in bone marrow and spleen. The plasmacytoid DC subset was analyzed within the gate of CD5$^-$CD19$^-$ CD11c$^+$ cells.

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