Solutions for high-dimensional flow cytometry

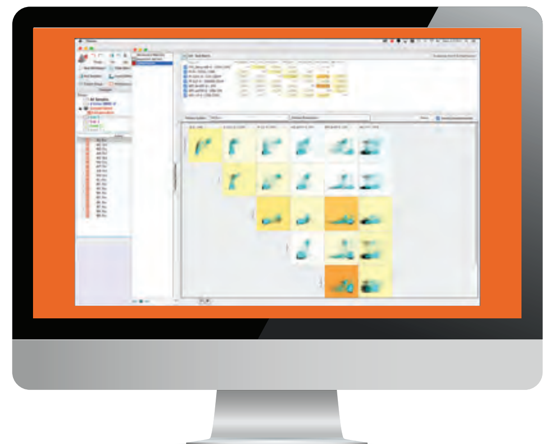
Deep exploration tools from BD Biosciences

BD presents the high-parameter solution

Figure 1: BD FACSymphony™ flow cytometer

Figure 2: BD Horizon™ Brilliant dyes (Brilliant Violet, Brilliant Ultraviolet and Brilliant Blue)

Figure 3: FlowJo™ version 10.5 advanced data analysis software



We support you with everything, from experimental design to analysis, to allow you to push your science to the limits.

High-dimensional flow cytometry

Bring state-of-the-art technology into your science

More colours

Allow a better understanding of the complex functions of the immune system.

Broad lineage panels

Allow better insights into the cellular composition of organs or peripheral blood in a single tube for applications where the target population is not known before the experiment.

Deep phenotyping panels

Allow the analysis of multiple expression patterns on a defined cell subset to learn more about the biology of immune functions

Optimising existing panels

With high-dimensional flow cytometry, existing panels can be optimised with better dyes to minimise compensation and data spreading. This will improve resolution and provide clearer information on receptor expression patterns.

Our new broad phenotyping panel

A large number of cell lineages in peripheral blood can now be screened with our new broad phenotyping panel.

Read on to discover the potential of the BD FACSymphony™ and its associated new dyes.

List of monoclonal antibodies and dyes for the 27-colour broad phenotyping panel

Antibody	Clone	Label	P/N
CD8	RPA-T8	BUV395	563796
CD4	SK3	BUV496	564651
CD19	SJ25C1	BUV563	565698
CD16	3G8	BUV615-P	*
CD38	HIT2	BUV661	565070
CD27	L128	BUV737	564302
CD45	HI30	BUV805	564915
CCR4	1g1	BV421	562579
IgD	IA6-2	BV480	566187
CD11c	B-ly6	BV570-P	*
CD20	2H7	BV605	563783
CD56	NCAM16.2	BV650	564057
CCR6	11A9	BV711	563923
CCR10	1B5	BV750-P	*
CD127	HIL-7R-M21	BV786	563324
CD45RA	HI100	BB515	564552
CD32	FLI8.26	BB630-P	*
CD64	10.1	BB660-P	*
PD1	EH12.1	BB700	566461
CD14	MφP9	BB790-P	*
CD57	NK-1	PE	560844
CD25	M-A251	PE-CF594	562403
CXCR3	1C6/CXCR3	PE-Cy5	561731
CD123	7G3	PE-Cy7	560826
CCR7	150503	Alexa647	560816
HLA-DR	G46-6	APC-R700	565128
CD3	SK7	APC-H7	560275

Table 1: List of monoclonal antibodies and dyes for the 27-colour broad phenotyping panel.
P stands for 'prototype dye'. The colours represent the excitation laser wavelengths

355 nm UV laser; 405 nm violet laser; 488 nm blue laser; 561 nm yellow-green laser; 637 nm red laser.

* Custom conjugates available upon request

The spillover spread matrix (SSM)

Buffy coats were obtained from the German red cross, from voluntary healthy donors. Samples were stained with mAbs in PBS with ETDA/FCS plus Brilliant stain buffer. mAbs were titrated for optimal stain concentration and minimal background. For the fully stained sample, a total of 900,000 events were recorded for analysis

For more information about the dyes, visit the BD Biosciences website at
www.bdbiosciences.com/us/applications/research/multicolor-flow/m/745795/overview

List of monoclonal antibodies and dyes for the 27-colour broad phenotyping panel

Dyes were matched to the markers using the spillover spread matrix (SSM) for the instrument generated from single colour stains in FlowJo™. The spillover spread matrix shows the impact of a dye in all the other channels and helps in choosing the appropriate dye for any marker and lineage.

Note that spreading is an issue only in strongly expressed markers. A medium or low expression will not have spreading issues.

	BB515	BB630-P	BB660-P	BB700	BB790-P	Alexa647	Alexa700	APC-Cy7	BUV395	BUV496	BUV563	BUV615-P	BUV661	BUV737	BUV805	BV421	BV480	BV570-P	BV605	BV650	BV711	BV750	BV786	PE	PE-CF594	PE-Cy5	PE-Cy7
BB515																											
BB630-P																											
BB660-P																											
BB700																											
BB790-P																											
Alexa647																											
Alexa700																											
APC-Cy7																											
BUV395																											
BUV496																											
BUV563																											
BUV615-P																											
BUV661																											
BUV737																											
BUV805																											
BV421																											
BV480																											
BV570-P																											
BV605																											
BV650																											
BV711																											
BV750																											
BV786																											
PE																											
PE-CF594																											
PE-Cy5																											
PE-Cy7																											

Table 2: The colour-coded SSM for the list of monoclonal antibodies. Green indicates no significant data spreading (<3), yellow a moderate effect (<6), and red indicates a strong data spreading (>6) into the affected channel.

For more information about the dyes, visit the BD Biosciences website at www.bdbiosciences.com/us/applications/research/multicolor-flow/m/745795/overview

Experimental results

Lymphocyte populations

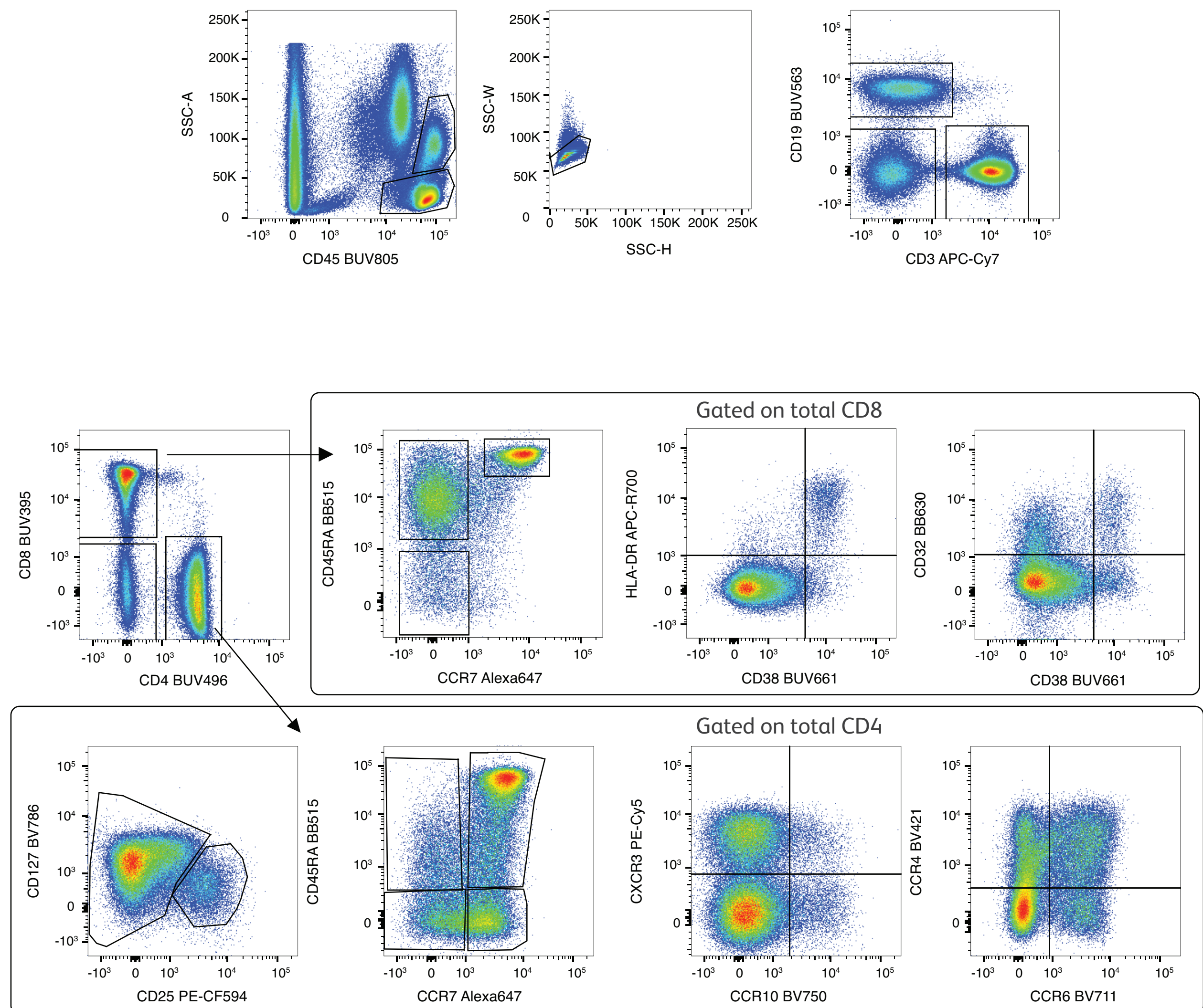


Figure 4:

Upper row: Lymphocytes were gated based on CD45 versus Side Scatter and singlets were gated based on Side Scatter-Width. CD3-positive T cells can be identified.

Middle row: Identification and analysis of CD4/CD8 positive T cells. The CD8-positive cells are then separated into naïve/memory state by CCR7/CD45RA staining. In the CD8 T cells, a CD38-positive subset can also be identified, and some of those cells express HLA-DR or CD32.

Lower row: Analysis of the CD4-positive T cells. Regulatory T-cells can be identified in a staining with CD127 and CD25. Also, in the CD4 subset, naïve/memory cells can be separated using CCR7/CD45RA staining. The last two plots show the expression of four chemokine receptors, CXCR3, CCR10, CCR4, and CCR6 for the CD4 T cells. With these parameters, the different T helper cell subsets can be identified.

Experimental results

Monocyte populations

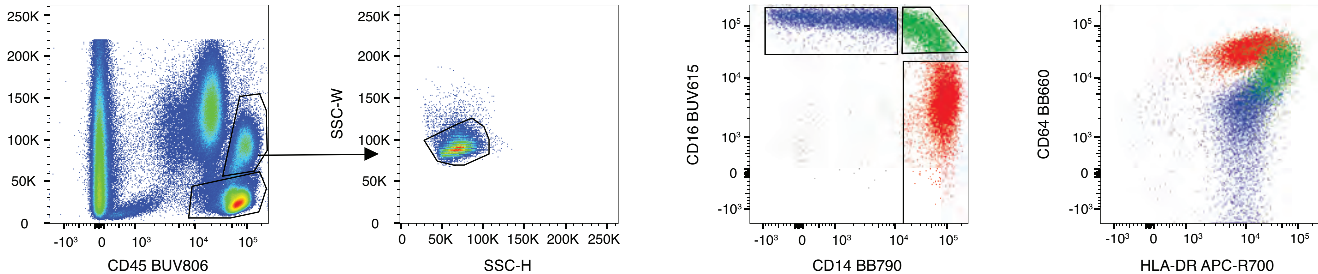


Figure 5: Monocytes were gated based on CD45 versus Side Scatter, and singlets were gated based on Side Scatter-Width. Classic monocytes are CD14-positive, but we can also identify a CD16-high intermediate population, and a CD14-neg/CD16-high non-classical monocyte population. The three populations also differ in the expression levels of HLA-DR and CD64, which is depicted in the last plot.

B-cell populations

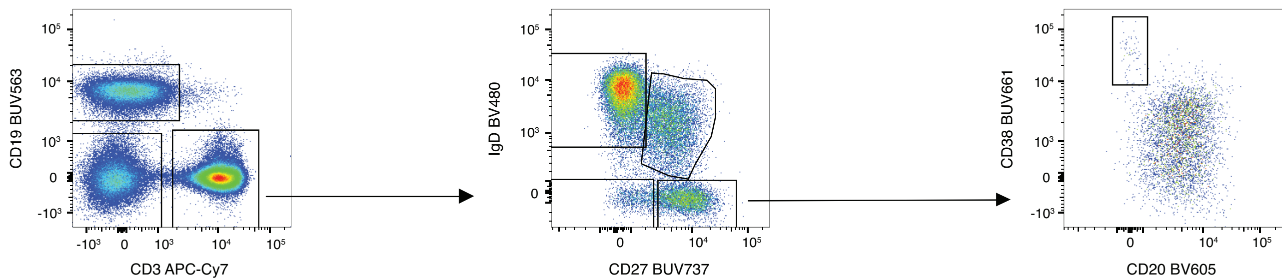


Figure 6: B-cells were identified as CD19-positive cells from the lymphocyte gate. They can be separated into naive/memory state by staining with anti-IgD surface immunoglobulin expression and CD27. The class-switched memory B-cells have lost all IgD expression and are CD25-high. These cells also contain the actively antibody secreting cells (plasmablasts), which can be identified in the last plot as CD38-high/CD20-low cells.

Dentritic cell populations

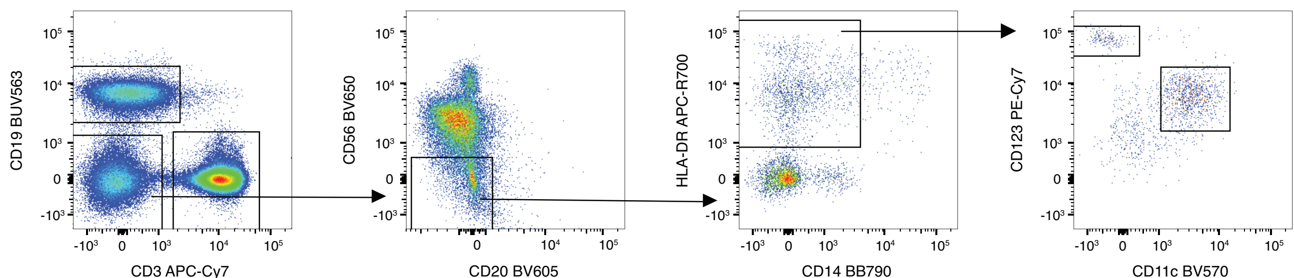


Figure 7: Dendritic cells (DC) can be identified by first gating on CD3/CD19/CD56 triple negative cells in the lymphocyte gate. In the next step, the gating is done on HLA-DR positive events and excludes any CD14-positive cells (contaminating monocytes in the lymphocyte gate). The last plot shows those cells stained with CD123 and CD11c to identify plasmacytoid DC (CD 123-positive) and myeloid DC (CD11c-positive).

Experimental results

NK cell populations

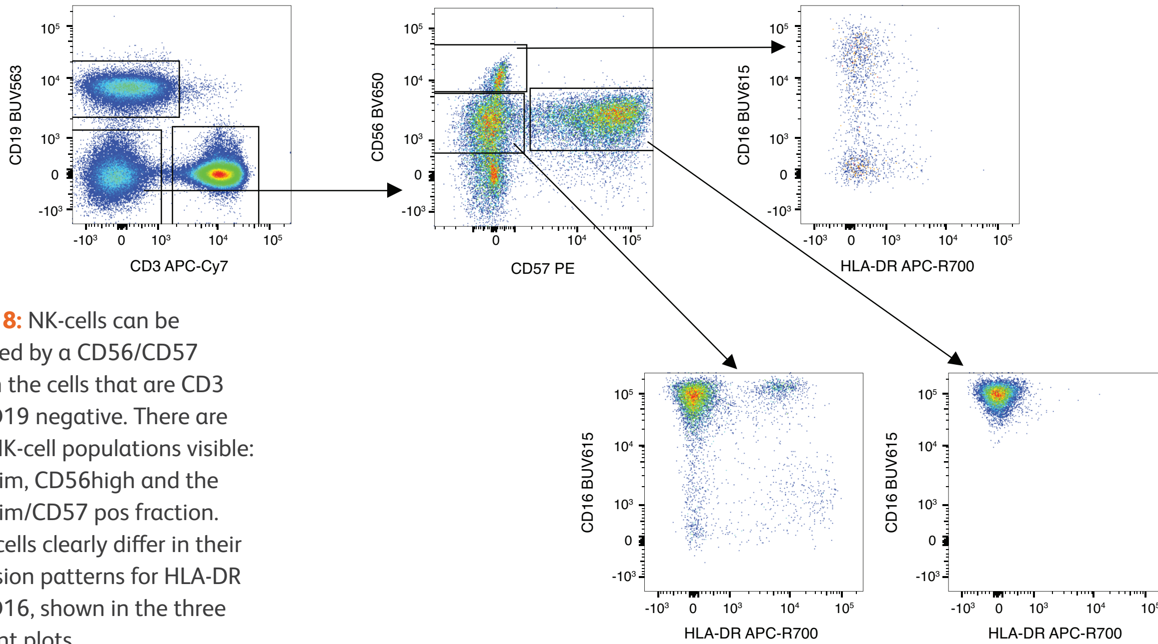


Figure 8: NK-cells can be identified by a CD56/CD57 stain in the cells that are CD3 and CD19 negative. There are three NK-cell populations visible: CD56dim, CD56high and the CD56dim/CD57 pos fraction. Those cells clearly differ in their expression patterns for HLA-DR and CD16, shown in the three adjacent plots.

Regulatory T-cell populations

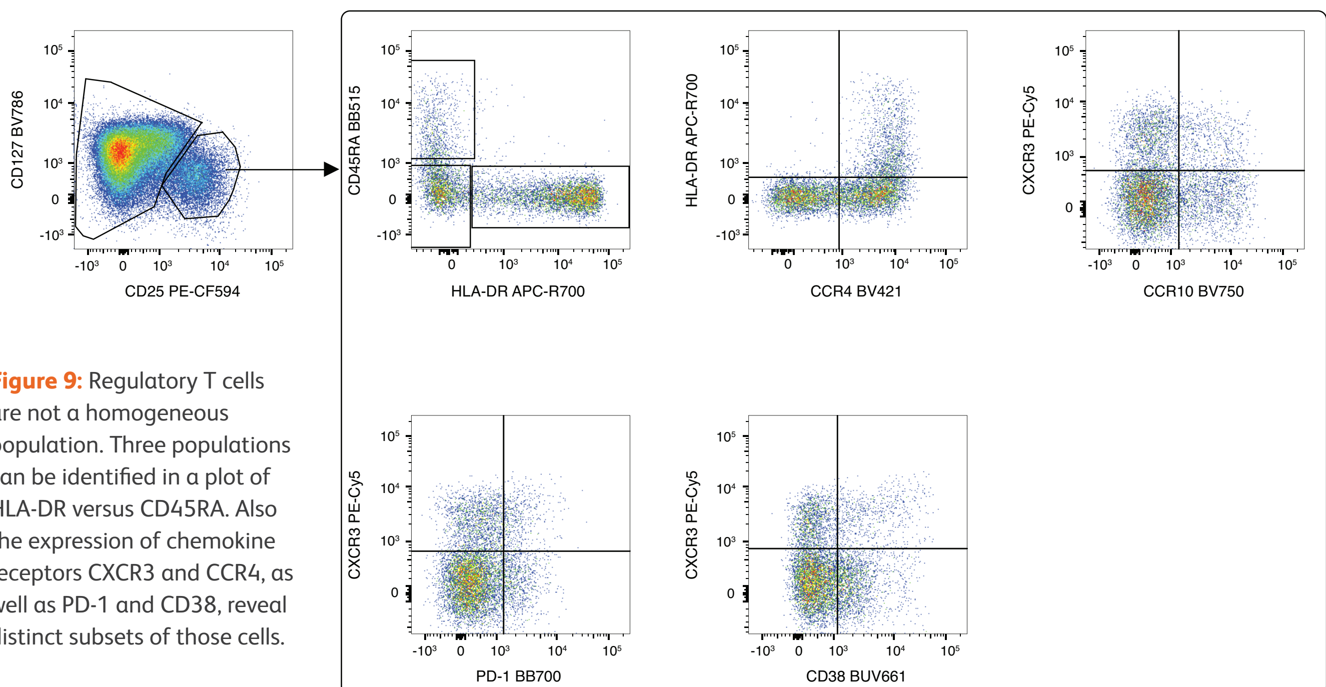


Figure 9: Regulatory T cells are not a homogeneous population. Three populations can be identified in a plot of HLA-DR versus CD45RA. Also the expression of chemokine receptors CXCR3 and CCR4, as well as PD-1 and CD38, reveal distinct subsets of those cells.

Experimental results

t-SNE plots

In high-dimensional flow cytometry, two-dimensional gating in dotplots to spot any difference in the sample sets is very time-consuming. Additionally, populations may be hidden in complex datasets that get lost by two-dimensional gating. With data reduction algorithms it is possible to visualise the expression patterns in two dimensions (t-distributed stochastical neighbor embedding, tSNE). This technology, available also in FlowJo™, allows the data pattern to be visualised in two-dimensional plots and enables an easier spotting of differences between different sample sets. Furthermore, this technology also eliminates the user bias in gating different subsets and the possibility of losing important cells that were not inside the parent gate for further analysis.

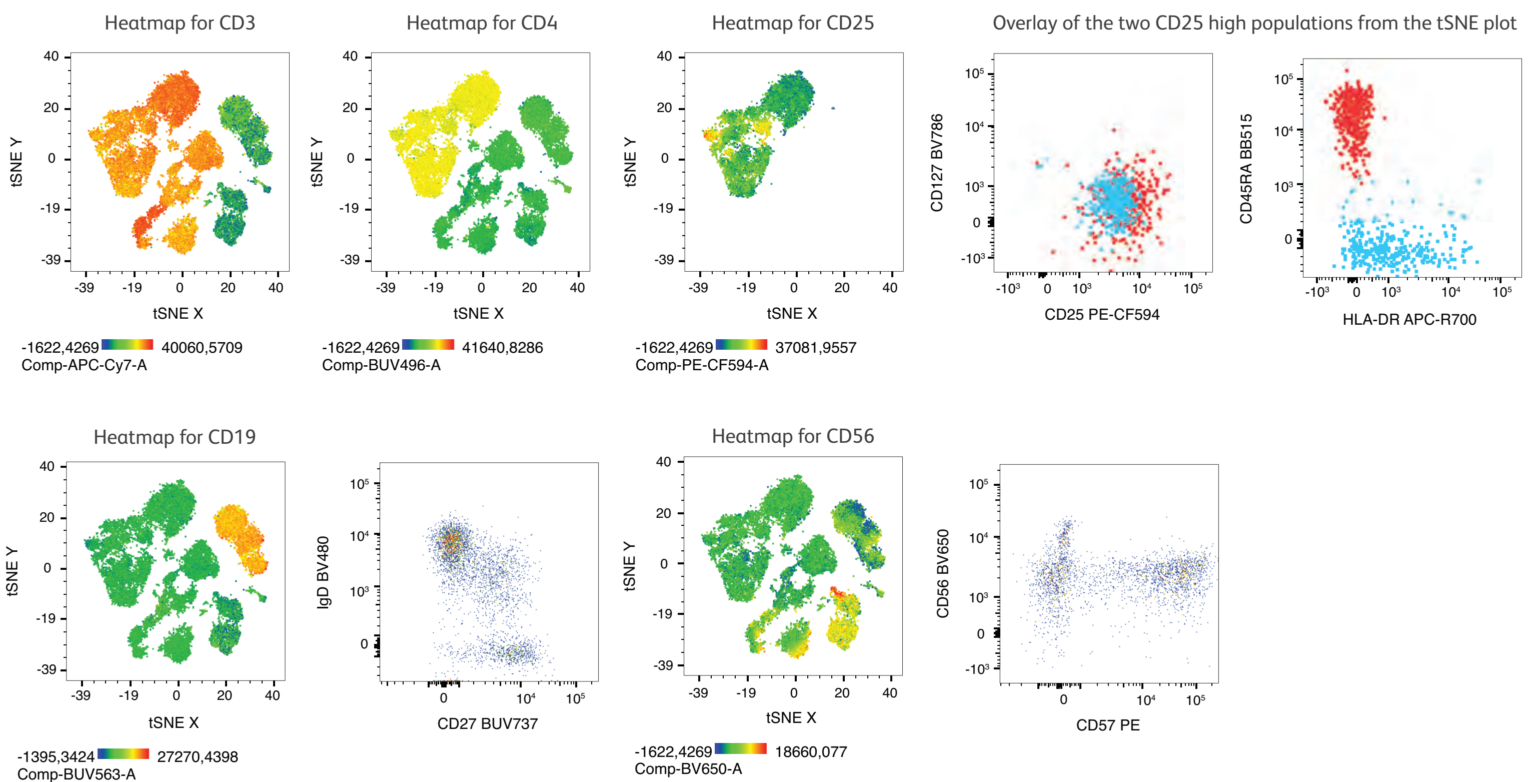


Figure 10:
The lymphocyte singlet gate was downsampled to 25000 events. tSNE calculation was performed in FlowJo™ 10.5 with 1000 iterations and a perplexity of 50.

Upper row: The upper row shows colour coded heatmaps for CD3, CD4, and CD25. The two CD25 high populations from the tSNE plots were gated and are shown as overlay dotplots. While both populations are clearly CD25 high and CD127 negative, there is a striking difference in CD45RA and HLA-DR expression. Due to low event numbers the size of the dots has been enlarged for better visibility.

Lower row: The lower row shows on the left the colour coded heatmaps for CD19 and a gated plot with CD27 and IgD. The three tSNE populations can be clearly seen in the dotplot and identified as naïve, non-switched memory and class switched memory B cells. On the right we have the colour coded heatmap for CD56, and the dotplot CD56 versus CD57 reveals the three distinct NK cell populations.

These examples show the potential of unsupervised analyses for high-parameter flow cytometry data.

Instrument configuration

Starting configuration for a BD FACSymphony™ A5

This configuration allows you to start your work immediately. Existing panels from a BD LSRFortessa™ or BD FACSAria™ can be transferred without any change, and you can start to add markers or build panels from scratch using the spillover spread matrix.

Laser	Power	Detector	Parameter	Bandpass	Dichroic
488	**200mW**	**7 Colours**			
		A	BB790-P	810/40	770LP
		B	BB755-P	750/30	735LP
		C	BB700	710/50	685LP
		D	BB660-P	670/30	635LP
		E	BB630-P	610/20	600LP
		F	BB515	530/30	505LP
		G	SSC	488/10	n.a.
405	**200mW**	**8 Colours**			
		A	BV786	810/40	770LP
		B	BV750	750/30	735LP
		C	BV711	710/50	685LP
		D	BV650	677/20	635LP
		E	BV605	605/40	595LP
		F	BV570-P	586/15	550LP
		G	BV510/BV480	525/50	505LP
		H	BV421	431/28	410LP
355	**100mW**	**7 Colours**			
		A	BUV805	810/40	770LP
		B	BUV737	735/30	690LP
		C	BUV661	670/25	630LP
		D	BUV615-P	605/20	595LP
		E	BUV563	580/20	550LP
		F	BUV496	515/30	450LP
		G	BUV395	379/28	370LP
637	**140mW**	**3 Colours**			
		A	APC-H7	780/60	750LP
		B	APC-R700	730/45	690LP
		C	APC	670/30	655LP
561	**200mW**	**5 Colours**			
		A	PE-Cy7	780/60	750LP
		B	PE-Cy5.5	710/50	685LP
		C	PE-Cy5	670/30	635LP
		D	PE-CF594	610/20	600LP
		E	PE	586/15	570LP

Table 3:
P stands for "prototype dye". These dyes are not listed in the web catalogue. BD will supply FACSymphony users with mAbs labelled with them on request. Our dedicated high-parameter reagent team can couple those prototype dyes to any of the mAbs from our portfolio of clones. Depending on the choice, up to a maximum of 10 lasers of various wavelengths and with multiple power ratings can be selected. BD is also working on expanding the selection of dyes. The configuration above is only an example and can be adapted to your specific requirements.

355 nm	**488 nm**	592 nm	785 nm
375 nm	505 nm	628 nm	
405 nm	514 nm	637 nm	
420 nm	**532 nm**	**640 nm**	
445 nm	552 nm	647 nm	
458 nm	**561 nm**	660 nm	
460 nm	568 nm	685 nm	
473 nm	588 nm	730 nm	

Table 4:
The 25 available lasers for the BD FACSymphony™ A5. Each laser has multiple power ratings that can be adjusted and stored. Depending on the choice, up to ten lasers can be selected and configured. Common laser choices are highlighted.

Other custom products and services

Delivering custom solutions

The BD Custom Technology Team (CTT) is a specialised contract research group with core competencies in flow cytometry and multiplexed protein assay development. Solutions include custom products, contract manufacturing, assay development and assay services that enable global biotechnology, pharmaceutical and contract research organizations to respond efficiently to the rapidly changing landscape of research and development.

These solutions expand beyond typical offerings to essential resources such as protocol development, flexible delivery options and quality documentation.

Typical assay services

BD has conducted cell and protein analysis studies for many major biotechnology and pharmaceutical companies. Examples of assay services include panel design* and manufacturing based on customer specifications. Thousands of samples have been processed in various studies for cancer, autoimmunity, infectious disease and HIV therapeutics and vaccines. Expertise in the typical study processes, combined with expertise in cell and protein analysis, can translate into efficient study completion for clients.

Solutions include:

- Proposal development that outlines the assays to be used
- Study protocol and SOP development that optimises collection and transport conditions in coordination with research and logistical teams
- Feasibility studies using known positive and negative controls
- Qualification studies to assess sample-to-sample variation, transport stability, intra-assay variability, inter-technician variability and normal range expectations
- Using established SOPs to process and analyse study samples
- Following assay protocols to process and analyse study samples
- Providing a cumulative data file and comprehensive bioanalytical study report with all information necessary to properly interpret study results

Reliable and flexible service and support

- Building on BD's immense experience with flow cytometers, reagents and data processing software
- Evaluation processes that meet your needs using small-scale (pilot) evaluation lots
- Panel development assistance for research use only (RUO) applications
- A wide variety of reagents and dyes for cocktailing, including BD Horizon Brilliant dye reagents*
- Options to add custom packaging, labeling and order management solutions, as well as accessory reagents, including counting beads

***Contact the BD custom team at BDB_Custom_Orders@bd.com for multicolour cocktails containing >1 BD Horizon Brilliant dye as well as lot-matched compensation controls for tandem dyes.**

For more information, please visit:
bdbiosciences.com or contact the BD custom team at
BDB_Custom_Orders@bd.com

Class I Laser Product. For Research Use Only. Not for use in diagnostic or therapeutic procedures.

BD - Europe, Terre-Bonne Park - A4, Route de Crassier -17, 1262 Eysins, Switzerland

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