BD FACSymphony™ A5 SE Cell Analyzer

Unlock the full potential of the BD FACSymphony™ platform with the flexibility of spectral unmixing or compensation workflows

Five lasers and 48 detectors for maximum coverage of the fluorochrome emission spectrum

Access spectral abilities on your BD FACSymphony™ A5 System with a BD FACSymphony™ A5 SE System upgrade kit

Choose between live spectral or compensation-based workflows in **BD FACSDiva™ Software**

Resolve critical cell populations with high autofluorescence using autofluorescence unmixing
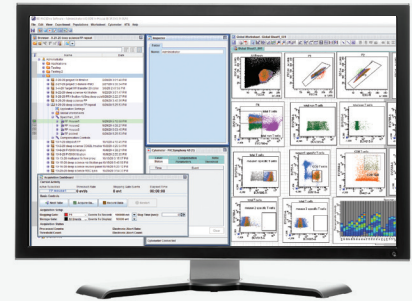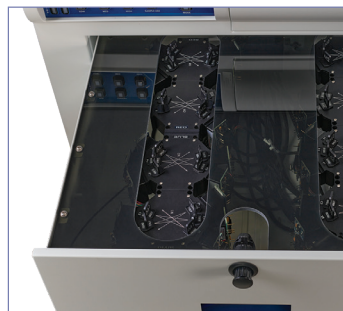
Perform spectral unmixing live on BD FACSDiva™ Software or export the data for analysis in FlowJo™ Software (v10.6 or later)
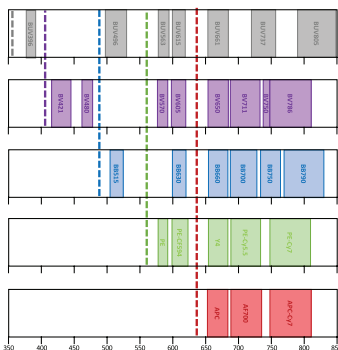
The BD FACSymphony™ A5 SE Cell Analyzer enables both spectral unmixing and compensation workflows

- Algorithmically optimized filter set collects the full spectrum of emitted light

- Algorithm trained on available fluorochromes and scalable detector array technology allows optimized placement of 48 detectors across five on-board lasers

- High-performance PMTs for enhanced resolution

- Using the familiar BD FACSDiva™ Software workflow, assign a fluorochrome to a detector with the option to analyze using compensation or spectral unmixing live during acquisition

- Visualize fluorochrome profiles with spectral plots

- Extract autofluorescence from highly fluorescent cells to potentially improve panel resolution

BD FACSymphony™ A5 Cell Analyzer

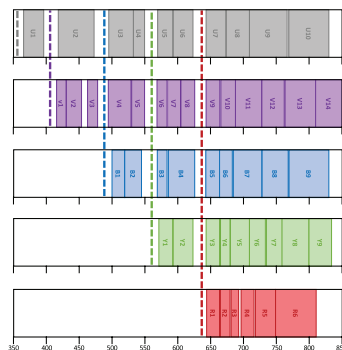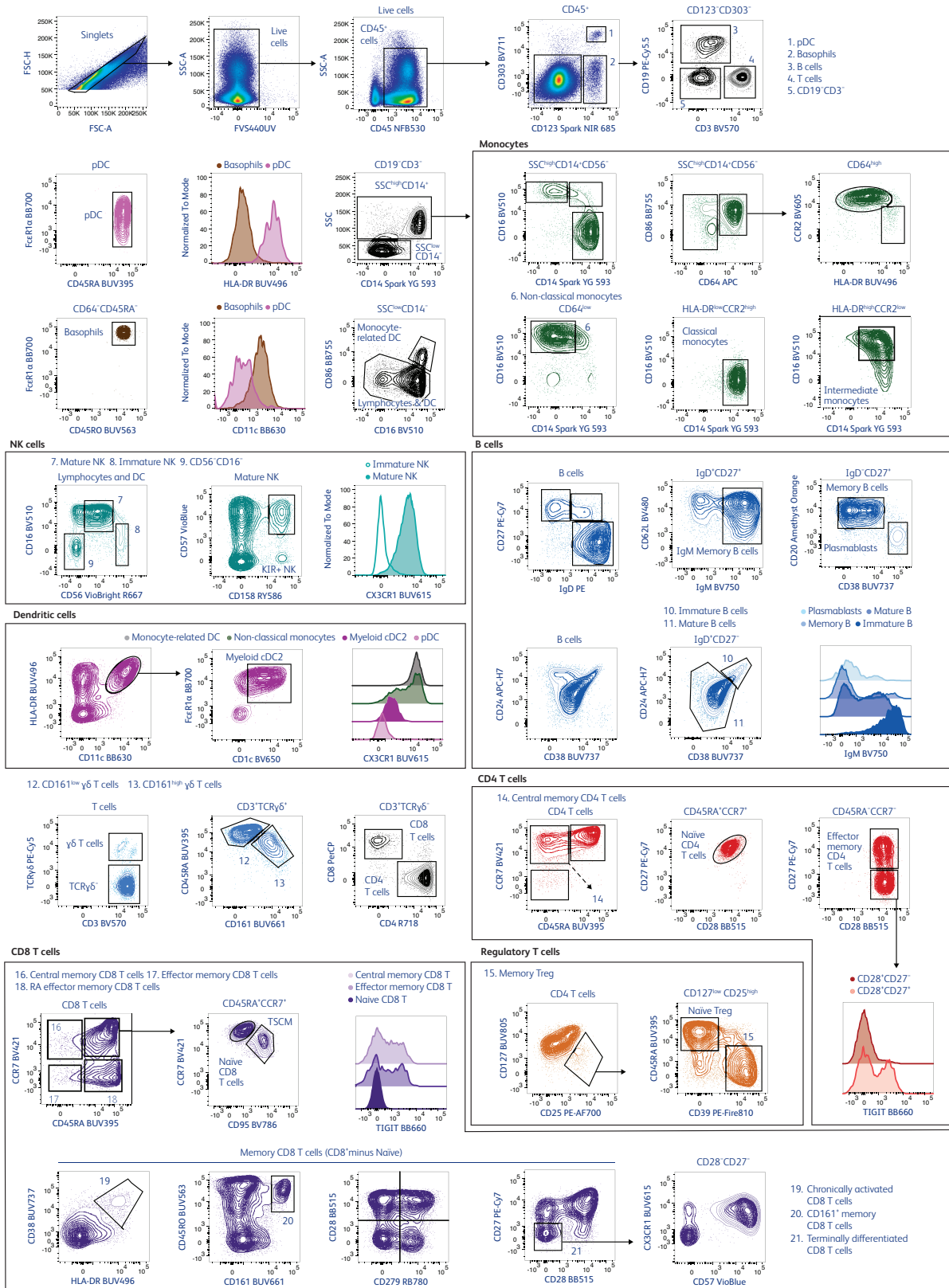**BD FACSymphony™ A5 SE Cell Analyzer**

Figure 1. Comparison of common BD FACSymphony™ A5 and A5 SE Cell Analyzer filter configurations

Distinguish between spectrally similar fluorochromes

The BD FACSymphony™ A5 SE Cell Analyzer features an optimized filter set to collect the full spectrum of emitted light, increasing flexibility in fluorochrome choices and enabling simultaneous analysis of fluorochromes with similar spectral signatures. The following panel contains a total of 40 fluorescence reagents. It includes the new spectrally optimized fluorochromes, BD Horizon RealYellow™ and RealBlue™ Reagents and was used to characterize a variety of immune cell populations in human peripheral blood.

Top gating row: Singlets (FSC-A / FSC-H) → Live cells (FVS440UV / SSC-A) → CD45⁺ cells (CD45 NFB530 / SSC-A) → CD45⁺ (CD123 Spark NIR 685 / CD303 BV711) → CD123⁻CD303⁻ (CD3 BV570 / CD19 PE-Cy5.5)
1. pDC
2. Basophils
3. B cells
4. T cells
5. CD19⁻CD3⁻

Monocytes — pDC (CD45RA BUV395 / FcεR1α BB700); Basophils • pDC (HLA-DR BUV496); CD19⁻CD3⁻ (CD14 Spark YG 593 / SSC); SSChighCD14⁺CD56⁻ (CD14 Spark YG 593 / CD16 BV510); SSChighCD14⁺CD56⁻ (CD64 APC / CD86 BB755); CD64high (HLA-DR BUV496 / CCR2 BV605). Basophils (CD45RO BUV563 / FcεR1α BB700); Basophils • pDC (CD11c BB630); SSClowCD14⁻ Monocyte-related DC, Lymphocytes & DC (CD16 BV510 / CD86 BB755). 6. Non-classical monocytes CD64low (CD14 Spark YG 593 / CD16 BV510); HLA-DRlowCCR2high Classical monocytes (CD14 Spark YG 593 / CD16 BV510); HLA-DRhighCCR2low Intermediate monocytes (CD14 Spark YG 593 / CD16 BV510).

NK cells — 7. Mature NK 8. Immature NK 9. CD56⁻CD16⁻; Lymphocytes and DC (CD56 VioBright R667 / CD16 BV510); Mature NK (CD158 RY586 / CD57 VioBlue); Immature NK • Mature NK (CX3CR1 BUV615); KIR+ NK.

B cells — B cells (IgD PE / CD27 PE-Cy7); IgD⁺CD27⁺ IgM Memory B cells (IgM BV750 / CD62L BV480); IgD⁻CD27⁺ Memory B cells, Plasmablasts (CD38 BUV737 / CD20 Amethyst Orange). 10. Immature B cells 11. Mature B cells; B cells (CD38 BUV737 / CD24 APC-H7); IgD⁺CD27⁻ (CD38 BUV737 / CD24 APC-H7); Plasmablasts • Mature B • Memory B • Immature B (IgM BV750).

Dendritic cells — Monocyte-related DC • Non-classical monocytes • Myeloid cDC2 • pDC; (CD11c BB630 / HLA-DR BUV496); Myeloid cDC2 (CD1c BV650 / FcεR1α BB700); (CX3CR1 BUV615).

CD4 T cells — 12. CD161low γδ T cells 13. CD161high γδ T cells; T cells (CD3 BV570 / TCRγδ PE-Cy5); γδ T cells, TCRγδ⁻; CD3⁺TCRγδ⁺ (CD161 BUV661 / CD45RA BUV395); CD3⁺TCRγδ⁻ CD8 T cells, CD4⁺ T cells (CD4 R718 / CD8 PerCP). 14. Central memory CD4 T cells; CD4 T cells (CD45RA BUV395 / CCR7 BV421); CD45RA⁺CCR7⁺ Naïve CD4 T cells (CD28 BB515 / CD27 PE-Cy7); CD45RA⁻CCR7⁻ Effector memory CD4 T cells (CD28 BB515 / CD27 PE-Cy7); CD28⁺CD27⁻ • CD28⁺CD27⁺ (TIGIT BB660).

CD8 T cells — 16. Central memory CD8 T cells 17. Effector memory CD8 T cells 18. RA effector memory CD8 T cells; CD8 T cells (CD45RA BUV395 / CCR7 BV421); CD45RA⁺CCR7⁺ TSCM, Naïve CD8 T cells (CD95 BV786 / CCR7 BV421); Central memory CD8 T • Effector memory CD8 T • Naïve CD8 T (TIGIT BB660).

Regulatory T cells — 15. Memory Treg; CD4 T cells (CD25 PE-AF700 / CD127 BUV805); CD127lowCD25high Naïve Treg (CD39 PE-Fire810 / CD45RA BUV395).

Memory CD8 T cells (CD8⁺ minus Naïve) — 19 (HLA-DR BUV496 / CD38 BUV737); 20 (CD161 BUV661 / CD45RO BUV563); (CD279 RB780 / CD28 BB515); 21 (CD28 BB515 / CD27 PE-Cy7); CD28⁻CD27⁻ (CD57 VioBlue / CX3CR1 BUV615).
19. Chronically activated CD8 T cells
20. CD161⁺ memory CD8 T cells
21. Terminally differentiated CD8 T cells

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Gating strategy for identification of immune cell subsets in human peripheral blood using a 40-color panel

Cell populations			Surface phenotype
Live leukocytes			Singlets+FVS440UV-CD45+
Basophils			Singlets+FVS440UV-CD45+CD123+CD303-CD64-CD45RA-FcɛR1α+CD45RO+
Plasmacytoid dendritic cells (pDC)			Singlets+FVS440UV-CD45+CD123+CD303+CD45RA+FcɛR1α^{low}
Monocytes			Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{high}CD14$^{high/low}$CD56-
Monocyte subsets	Classical monocytes		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{high}CD14$^{high/low}$CD56-CD86$^{high/low}$CD64^{high}CCR2^{high}HLA-DR^{low}CD14^{high}CD16^{low}
	Intermediate monocytes		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{high}CD14$^{high/low}$CD86$^{high/low}$CD64$^{high/low}$CCR2^{low}HLA-DR^{low}CD14^{high}CD16+
	Nonclassical monocytes		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{high}CD14$^{high/low}$CD86$^{high/low}$CD64^{low}CD14^{low}CD16^{high}
Dendritic cells	Monocyte-related DC		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{low}CD14-CD86+CD16+
	Myeloid dendritic cells (cDC2)		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{low}CD14-CD86-CD16-CD56-HLA-DR+CD11c+CD1c+FcɛR1α+CX3CR1^{low}
NK cells			Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{low}CD14-CD86-CD16+CD56$^{high/low}$
NK cell subsets	Immature NK cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{low}CD14-CD86-CD16-CD56^{high}CX3CR1-
	Mature NK cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{low}CD14-CD86-CD16-CD56^{low}CX3CR1+
	KIR⁺CD57⁺ NK cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19-SSC^{low}CD14-CD86-CD16-CD56^{low}CD158+CD57+
B cells			Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19+
B cell subsets	Immature B cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19+CD27-IgD+CD24^{high}CD38^{high}IgM^{high}
	Mature B cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19+CD27-IgD+CD24+CD38+IgM^{low}
	IgM memory B cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19+CD27+IgD+CD62L+IgM^{high}
	Memory B cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19+CD27+IgD-IgM-
	Plasmablasts		Singlets+FVS440UV-CD45+CD123-CD303-CD3-CD19+CD27+IgD-CD20-CD38^{high}IgM-
T cells			Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-
T cell subsets	γδ T cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ+
	γδ T cell subsets	CD161^{high} γδ T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ+CD45RA+CD161^{high}
		CD161^{low} γδ T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ+CD45RA+CD161^{low}
	CD4 T cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8-CD4+
	CD4 T cell subsets	Naïve CD4 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8-CD4+CD45RA+CCR7+
		Central memory CD4 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8-CD4+CD45RA-CCR7+
		Effector memory CD4 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8-CD4+CD45RA-CCR7-CD28+CD27+TIGIT+/-
		Effector Th1 CD4 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8-CD4+CD45RA-CCR7-CD28+CD27-TIGIT-
		Naïve regulatory T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8-CD4+CD127^{low}CD25^{high}CD39-CD45RA+
		Memory regulatory T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8-CD4+CD127^{low}CD25^{high}CD39+CD45RA-
	CD8 T cells		Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-
	CD8 T cell subsets	Naïve CD8 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-CD45RA+CCR7+CD95-
		T stem cell memory cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-CD45RA+CCR7+CD95+
		Central memory CD8 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-CD45RA-CCR7+TIGIT+/-
		Effector memory CD8 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-CD45RA-CCR7-
		RA effector memory CD8 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-CD45RA+CCR7-TIGIT+/-
		Terminally differentiated CD8 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-[CD45RA+CCR7+]-CD28-CD27-CX3CR1+CD57+
		Chronically activated CD8 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-[CD45RA+CCR7+]-HLA-DR+CD38+
		CD161⁺ memory CD8 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-[CD45RA+CCR7+]-CD161+
		CD279-expressing CD8 T cells	Singlets+FVS440UV-CD45+CD123-CD303-CD3+CD19-TCRγδ-CD8+CD4-[CD45RA+CCR7+]-CD279+

This gating strategy allows identification of a selected number of cell populations after staining of human peripheral blood mononuclear cells (PBMCs) with a cocktail of 39 antibodies and a viability dye.

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