Flow Cytometry and Imaging Technologies

Accelerating high parameter flow cytometry: Enhancing analysis time with rapid data acquisition

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Abstract

The advent of spectral data unmixing has significantly enhanced the capability for high parameter multicolor analysis in flow cytometry. However, this advancement has also led to increased acquisition and analysis times for high-dimensional data. To address these challenges, the Invitrogen™ Attune™ Xenith™ Flow Cytometer has been developed, incorporating proven acoustic focusing core technology to deliver efficient, rapid, and accurate full-spectrum data acquisition.

In this study, we utilized a 37-color mouse panel to perform comprehensive immunophenotyping of tissue digests. Our results demonstrate that the Attune Xenith Flow Cytometer can acquire data at reduced instrument run times without excessive sample dilution, even with clog-prone samples. At acquisition rates of 1000 µL/minute, data resolution was maintained, and we identified distinct subpopulations, including rare subsets, from spleen and bone marrow.

The high sample throughput capability of the Attune Xenith Flow Cytometer significantly reduces time to results while maintaining uncompromised spectral resolution, even with highly complex panels. This efficiency facilitates the identification of rare cell subsets in mouse tissues and enhances overall laboratory productivity. Here, we illustrate the capability of the Attune Xenith Flow Cytometer to resolve complex high parameter data accurately and rapidly, thereby minimizing instrument run time and optimizing workflow efficiency.

Materials and methods

Bone marrow (BM) and spleen (SP) cells from BALB/c were isolated by standard methods. Cells were stained in Invitrogen™ eBioscience™ Flow Cytometry Staining Buffer, Invitrogen™ Super Bright™ Complete Staining Buffer and Invitrogen™ CellBlox™ Plus Blocking Buffer were used to prevent non-specific polymer interactions and non-specific binding, respectively. Invitrogen™ LIVE/DEAD™ Fixable Near IR 876 viability dye was used to clearly distinguish live from dead/dying cells. Following incubation with the viability dye cells were fixed in 2% formaldehyde. The antibodies used in this panel are listed in the table below. Samples were acquired on a Attune™ Xenith™ Flow Cytometer.

For spectral unmixing controls, Invitrogen™ Ultracomp eBeads™ Plus Compensation Beads were used for most markers. The exceptions for which either bone marrow cells or spleen cells were used are highlighted in yellow on the table below.

Data analysis was performed both with FlowJo™ v10.10.0 software (BD Biosciences) and Invitrogen™ Flowscape™ Software.

The aim with this experiment was to display the Attune Xenith fast acquisition and event rate speeds in a high parameter panel without compromising the resolution of identified populations. We demonstrate precise rare subpopulation identification that's virtually unchanged between 200 and 1000 µL/min, including concentrated samples that were recorded at around 50000 events per second.

Table 1. 37-color mouse immunophenotyping panel showing antigens/antibody clone/dye pairings and what was used as single-color controls for unmixing our spectral experiment.

Laser and channel	Filter	Fluorophore	Antigen	Clone	Unmixing control	Fluorescence minus-one (FMO) controls
349 UV-1	387/11	Brilliant Ultraviolet 395	CD5	53-7.3	Beads	Yes
349 UV-6	507/19	Brilliant Ultraviolet 496	CD19	eBio1D3	Mouse cells	
349 UV-8	575/15	Brilliant Ultraviolet 563	CD44	IM7	Mouse cells	
349 UV-9	615/24	Brilliant Ultraviolet 615	CD11c	N418	Beads	
349 UV-10	670/30	Brilliant Ultraviolet 661	CD93	AA4.1	Beads	Yes
349 UV-11	728/40	Brilliant Ultraviolet 737	TCRgd	GL3	Beads	Yes
349 UV-12	750/LP	Brilliant Ultraviolet 805	B220	RA3-6B2	Mouse cells	
405 V-1	420/10	Brilliant Violet 421	Siglec H	551	Beads	
405 V-2	434/17	Super Bright 436	F4/80	BM8	Beads	
405 V-3	455/14	eFluor 450	CD21	eBio4E3	Beads	
405 V-4	473/15	Brilliant Violet 480	CD62L	MEL-14	Beads	
405 V-5	507/19	Brilliant Violet 510	CCR2	475301	Beads	
405 V-7	575/15	Brilliant Violet 570	Ly6G	1A8	Mouse cells	
405 V-8	615/24	Super Bright 600	I-A/I-E	M5/114.15.2	Mouse cells	
405 V-9	661/20	Super Bright 645	CD25	PC61.5	Beads	Yes
405 V-10	710/20	Super Bright 702	IgD	11-26c	Beads	
405 V-11	747/33	Brilliant Violet 750	CD4	GK1.5	Beads	
405 V-12	770/LP	Super Bright 780	CD11b	M1/70	Mouse cells	Yes
488 B-1	520/40	FITC	CD38	90	Beads	
488 B-2	549/15	RB545	TCRb	H57-597	Beads	Yes
488 B-4	615/24	NovaFluor Blue 610-70S	CD8	53-6.7	Beads	
488 B-5	670/30	NovaFluor Blue 660-120S	CD205	205yekta	Beads	Yes
488 B-6	720/24	PerCP-eFluor 710	CD1d	1B1	Beads	Yes
488 B-7	750/LP	Real Blue 780	CD24	M1/69	Mouse cells	
561 Y-1	575/15	PE	CD127	A7R34	Beads	
561 Y-2	589/15	RY586	CD27	LG.3A10	Beads	Yes
561 Y-3	605/15	PE-eFluor 610	IgM	II/41	Mouse cells	
561 Y-5	661/20	PE-Cy5	CD3	17A2	Beads	
561 Y-7	700/13	PE-Cy5.5	CD49b	DX5	Beads	Yes
561 Y-9	760/60	PE-Cy7	CD23	B3B4	Beads	
561 Y-10	810/25	PEFire810	CX3CR1	SA011F11	Beads	Yes
637 R-1	661/20	APC	CD335	29A1.4	Beads	Yes
637 R-2	700/13	NovaFluor Red 700	CD274	MIH5	Beads	Yes
637 R-3	720/24	Alexa Fluor 700	CD45	30-F11	Mouse cells	
637 R-5	785/LP	APC-eFluor 780	Ly6C	HK1.4	Mouse cells	
781 IR-1	810/25	APC-Fire 810	TER-119	TER-119	Beads	
781 IR-3	860/LP	LIVE/DEAD Fixable NIR876	Dead cells		Mouse cells	

Results

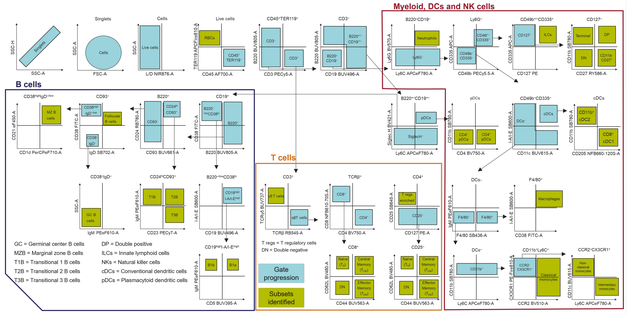


Figure 1. 37-color gating strategy overview. Sequential gate progression starts at the top from left to right and is depicted in blue. When more than one population is in the same plot, arrows are used to delineate gate progression. Green shapes represent the final gating strategy for the subpopulations identified.

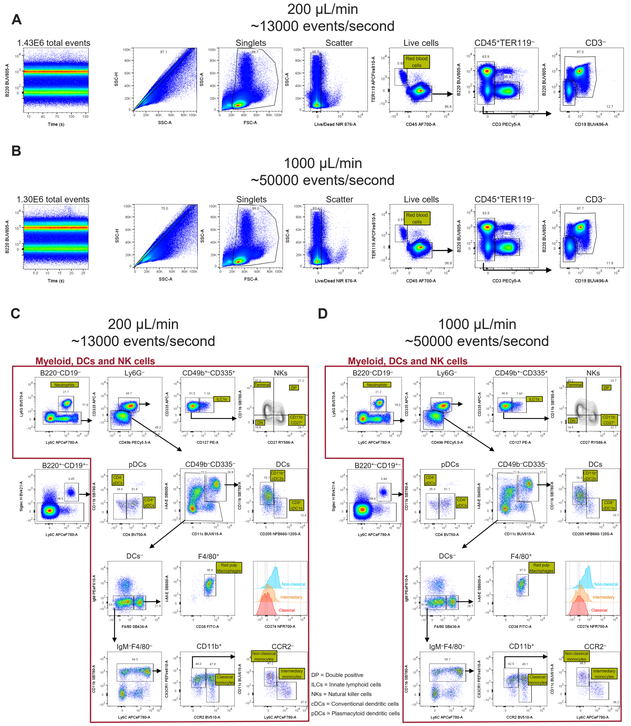
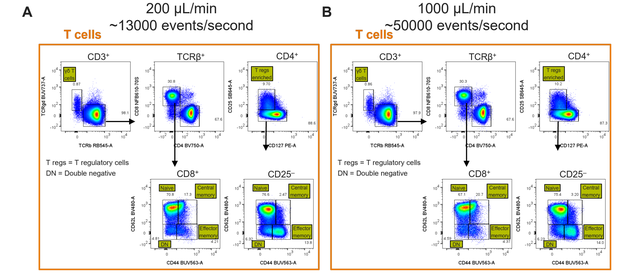


Figure 2. 37-color immunophenotyping panel analysis of the spleen: Comparison of flow rates and event rates. Population resolution is maintained across flowrates, including at up to 50000 events/second. A) and B) Time plots showing run stability, initial gate clean up and progression. C) and D) Myeloid, dendritic cells and natural killer cells resolution comparison at different flowrates and event rates.

Results (continued)



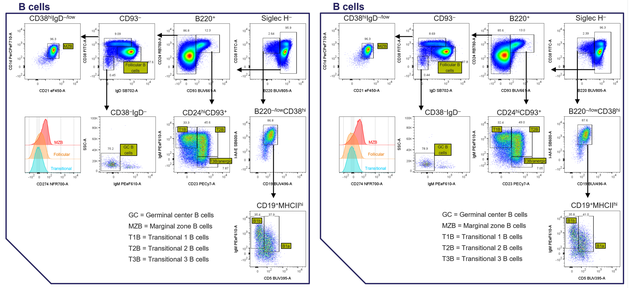


Figure 3. 37-color immunophenotyping panel analysis of the spleen: Comparison of flow rates and event rates. Population resolution is maintained across flowrates, including at up to 50000 events/second. A) and B) showing T cell subsets identified. C) and D) B cell subsets identified.

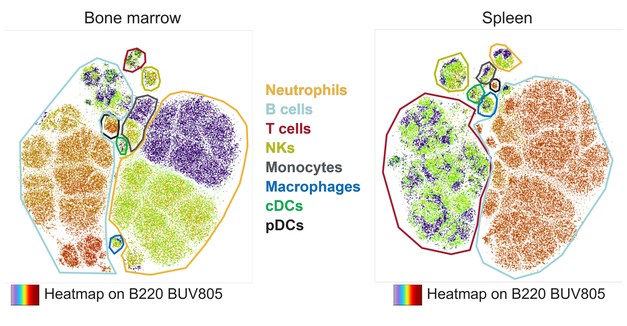


Figure 4. Flowscape Software dimensionality reduction algorithms. Population resolution is maintained across flowrates, including at up to 50000 events/second. A) and B) Time plots showing run stability, initial gate clean up and progression. C) and D) Myeloid, dendritic cells and natural killer cells resolution comparison at different flowrates and event rates.

Results (continued)

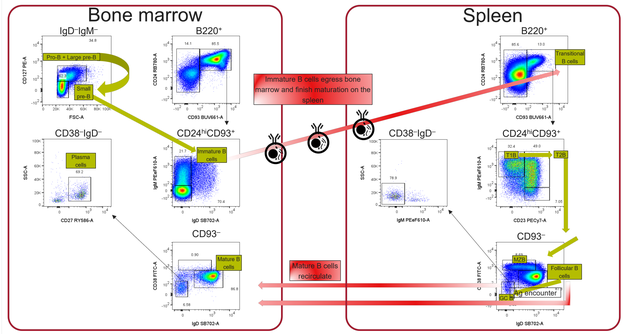


Figure 5. A glance into B cell development. Using the same 37-color immunophenotyping panel on primary and secondary lymphoid organs allow us to visualize B cell development from progenitors to plasma cells differentiation.

Conclusions

- Attune Xenith Flow Cytometer: A rapid path to discovery
  - Robust handling of tissue digests = no clogs
  - 37-colors in-depth immunophenotyping with acoustic-focusing:
    - 36 distinct subpopulations identified across 2 lymphoid organs
    - No compromise on resolution up to 50000 events/second
    - Diluting samples: reduces aggregates and accelerates acquisition
  - Reduced experiment time = more time for analysis
    - Flowscape Software: Dimensionality reduction analysis

Acknowledgements

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