Flow Cytometry Technologies

Fast, efficient analysis of challenging and clog-prone tissue types using the Attune Xenith Flow Cytometer

Susan O'Meara, Venetia Morris, Brandon Trent, Mitch Klomp, Dan Troast, Nate Trujillo. Thermo Fisher Scientific,145 E. Mountain Ave, Fort Collins, CO, 80524

Abstract

Mouse small intestine is a challenging sample for flow cytometry due to fibrous material, cellular debris, extracellular matrix components, and frequent clogging. We evaluated the Invitrogen™ Attune™ Xenith™ Flow Cytometer using a 12-color murine intestinal immunophenotyping panel focused on lineage analysis across the CD8 T cell pathway. The panel included markers for T cell identification, CD8 lineage resolution, T cell subset characterization, and viability-based exclusion.

The Attune Xenith Flow Cytometer enabled uninterrupted acquisition of digested intestinal tissue preparations containing substantial cellular and extracellular debris at high flow rates, including 1,000 µL/min and up to 33,000 events/second, while maintaining clear resolution of CD8-associated populations. The system's acoustic focusing with hydrodynamic technology and clog-resistant fluidics reduces acquisition interruptions and minimizes user intervention during analysis of digested mouse small intestine tissue.

These findings support the Attune Xenith Flow Cytometer as a robust, high-throughput platform for focused multicolor analysis of challenging digested tissue samples, including mouse small intestine workflows centered CD8 T cell pathway characterization.

Why acoustic focusing?

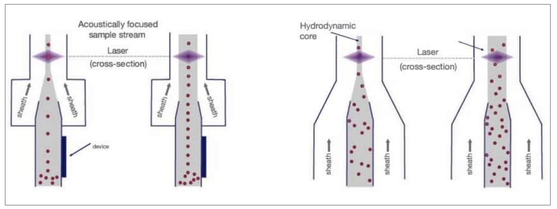

Figure 1. Acoustic focusing with a syringe-driven system. Invitrogen™ Attune™ Flow Cytometers use acoustic focusing with a syringe-driven fluidic system to gently position cells in space, reduce clogging, support higher sample injection rates, and enable absolute counting capabilities.

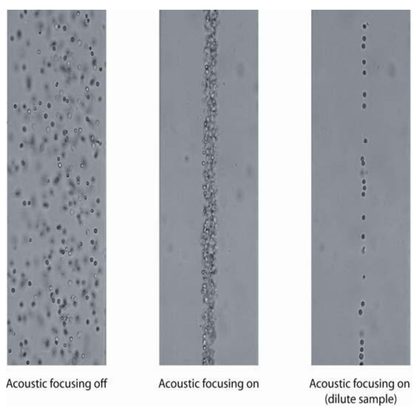

Figure 2. Attune acoustic-assisted hydrodynamic focusing combines acoustic focusing with dilution-based hydrodynamic focusing to narrow and align the sample stream. Dilution increases particle spacing, helping reduce coincident events and support cleaner, more reliable data acquisition at higher sample input rates.

Clog resistance

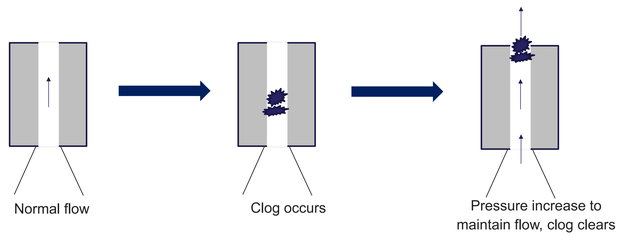

Figure 3. Attune Xenith Flow Cytometer is driven by a volumetric flow and can cause pressure changes to help remove a clog.

Time to results

Instrument and flow rate	Time to 5.8 Million Cells	Relative rate
60 µL/min (industry standard)	67 minutes	-
Acoustic focusing, 200 µL/min	20 minutes	~5x faster
Acoustic focusing at 1000 µL/min*	4 minutes	~16.7x faster

Figure 4. Faster acquisition with acoustic focusing. Acoustic focusing enabled higher flow-rate acquisition and reduced the time required to collect 5.8 Million cells with the sample concentration approximately 1.5 Million Cells/mL. Compared with a calculated flow rate of 60 µL/min, which would require approximately 67 minutes to collect the same number of cells, acoustic focusing reduced acquisition time to 20 minutes at 200 µL/min and 4 minutes at 1000 µL/min. This represents an approximately 16.7-fold improvement in time to results at the highest acoustic focusing flow rate.

Materials and methods

Test method

Testing was performed on an Attune Xenith Flow Cytometer run at a flow rate of 200 µL/min and 1000 µL/min.

Data analysis

All of the data analysis was performed in FlowJo™ Software.

Sample preparation

Small intestine tissue from BALB/c mouse was flushed with cold HBSS, cut into small fragments, and incubated in EDTA to remove epithelial cells. Released cells were collected, pelleted, and washed. The remaining tissue was digested with Gibco™ TrypLE™ Express, filtered through a 70 µm mesh strainer, washed, and centrifuged. The final cell pellet was used for staining and then fixed prior to acquiring.

Panel

Marker	Fluor / color
CD3	PE-Cyanine5
CD4	APC
CD8α	eFluor™ 450
CD11b	BUV496
CD19	PE-Cyanine7
CD44	BUV563
CD45	Alexa Fluor™ 700
CD62L	BV421
CD127	PE
Live/Dead	Near-IR 876
CD25	BUV395
TCRγδ	BV750

Figure 5. Immunophenotyping panel used for small intestine T-cell subset analysis.

Gating strategy

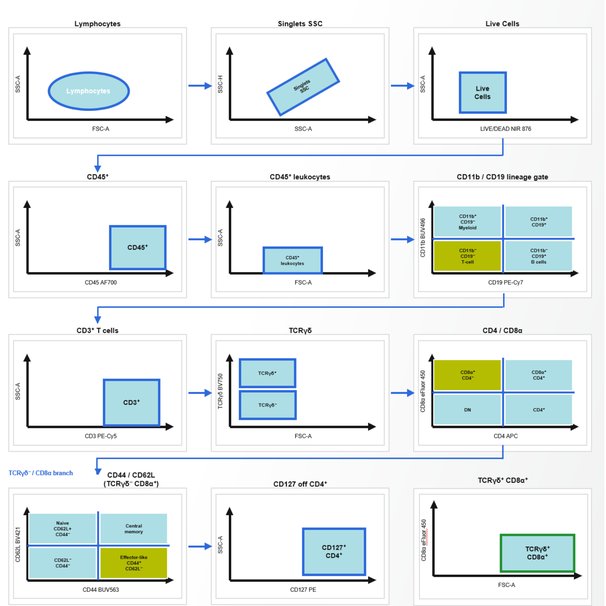

Figure 6. Small intestine T-cell gating strategy. Lymphocytes were gated through singlets, live cells, CD45+ leukocytes, CD11b-CD19- T cells, and CD3+ T cells. TCRγδ- and TCRγδ+ branches were further analyzed for CD4/CD8α, CD44, and CD62L expression.

Time plots

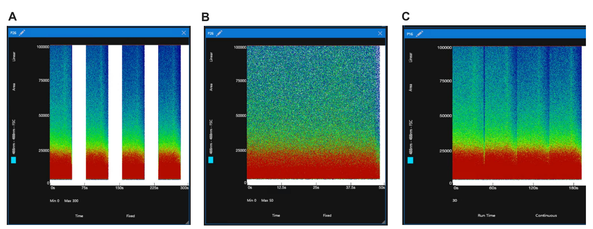

Figure 7. Continuous acquisition of a 4 mL sample on the Attune Xenith Flow Cytometer.
A. Time plot collected during the first sample draw at 1000 µL/min with sample concentration approximately 1.5 Million cells/mL.
B. Expanded time plot of the first draw, showing stable event collection during high-flow acquisition.
C. Full time plot showing four sequential sample draws across the 4 mL sample volume. Vertical transitions indicate movement between draws and demonstrate sustained high-flow acquisition with consistent event recovery. Across four draws, the mean event count was 1.44 × 10^6 cells per draw, with SD = 7.81 × 10^4 cells.

Results

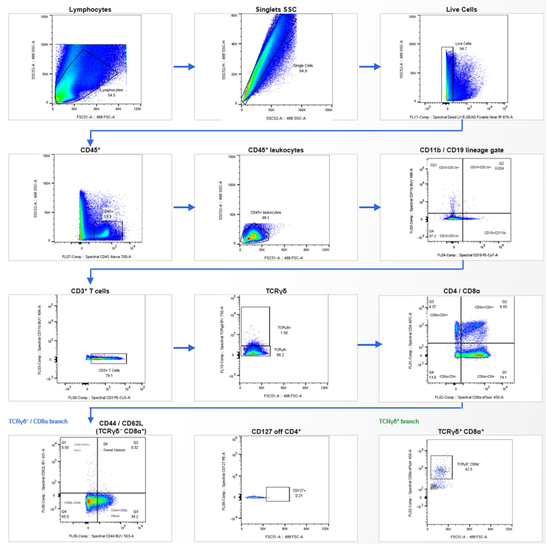

Figure 8. Representative small intestine T-cell gating strategy. Cells were gated as lymphocytes by FSC-A/SSC-A (37.8%), followed by singlets (91.4%), live cells (93.8%), CD45+ leukocytes (19.7%), CD11b-CD19- cells (97.2%), and CD3+ T cells (74.6%). CD3+ cells were separated into TCRγδ- (99.4%) and TCRγδ+ (0.55%) populations. TCRγδ- cells were further analyzed for CD4/CD8α, CD44+ activated/memory phenotype, and CD62L- effector-like phenotype; TCRγδ+ cells were assessed for CD8α+ expression (45.9%).

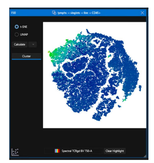

Figure 9. t-SNE visualization of small intestine CD45+ leukocytes**.** t-SNE was used as a dimensionality-reduction tool to visualize phenotypic relationships among gated live CD45+ cells. Events with similar marker-expression profiles cluster together, enabling visualization of population structure and identification of rare or distinct subsets. The highlighted region represents the TCRγδ+ population within the broader intestinal leukocyte landscape.

Conclusions

- Acoustic focusing enabled faster acquisition of challenging small intestine samples, reducing the time to collect 5.8 Million cells from an estimated 67 minutes at 60 µL/min to 4 min at 1000 µL/min.
- Anti-clogging fluidics supported continuous acquisition of debris-rich intestinal samples and helped minimized workflow disruption during high-flow analysis.
- The 12-color panel resolved key intestinal immune populations, including live CD45+ leukocytes, CD3+ T-cells, TCRγδ+/TCRγδ- subsets, CD4/CD8α populations, and CD44/CD62L activation-memory phenotypes.
- Together, these results support the Attune Xenith Flow Cytometer as an efficient platform for high-throughput analysis of challenging, clog-prone tissue-derived samples.

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