Flow Cytometry Technologies

From discovery to sorting: Integrated spectral analyzer and sorter solutions optimized by built-in tSNE/UMAP tools

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Abstract

Spectral cytometry has expanded high-parameter multicolor flow cytometry, enabling increasingly complex panels and deeper exploration of novel and rare immune cell populations. However, these advances place greater demands on instrument speed, acquisition efficiency, sorting performance, and data analysis. To address these challenges, we developed the Invitrogen™ Attune™ Xenith™ Flow Cytometer, which integrates spectral detection with proven acoustic focusing technology to deliver efficient, high-parameter sample acquisition at rates up to 1000 µL/min while maintaining the resolution required to detect rare populations. Complementing this system, the Invitrogen™ Bigfoot™ Spectral Cell Sorter features a shared optical design and collection deck architecture to streamline panel transfer between platforms and incorporates innovative hardware to enable efficient up to six-way spectral sorting with high purity, efficiency, recovery, and throughput - providing a cohesive workflow solution from acquisition through sorting.

Using high-parameter immunophenotyping panels in mouse tissues and human peripheral blood mononuclear cells (PBMCs), we acquired data on the Attune Xenith Flow Cytometer and performed dimensionality reduction using tSNE or UMAP within Invitrogen™ Flowscape™ software. Flowscape software utilizes automated identification and gating of populations of interest directly within UMAP space, facilitating rapid, data-driven population discovery. These analyses inform and optimize downstream sorting strategies on the Invitrogen™ Bigfoot™ Spectral Cell Sorter. Together, the Attune Xenith Flow Cytometer and Bigfoot Spectral Cell Sorter provide users with rapid detection, analysis, and high-purity isolation of rare immune cell subsets, supporting streamlined, high-dimensional flow cytometry workflows.

Introduction

With the development of new expansive panels, spectral cytometry is continuing to push the limits of information that users can garner from individual samples. Larger panels allow immunologists to dive deep into cellular populations and discover new subsets of cells that were previously unknown. To help facilitate expression of antigens in diverse cellular populations, dimensionality reduction tools have been utilized, such as t-distributed stochastic neighbor embedding (t-SNE) and uniform manifold approximation and projection (UMAP). These tools can be used to visualize population differences and expression patterns across various cellular populations, making them helpful tools to analyze large data sets and antigens that may have an array of expression across cellular subsets.

To address the need of researchers to use these dimensionality reduction tools we implemented UMAP and t-SNE capabilities in Flowscape™ software. Users are able to generate dimensionality reduction maps, analyze results via marker expression, and back-gate onto desired populations of interest. Here, the Invitrogen Attune Xenith Flow Cytometer was used to collect samples at speeds ranging from 200-1000µl/min in high-parameter spectral panels to generate both UMAP and t-SNE plots using both human PBMCs as well as mouse tissue samples. Back-gating on desired UMAP populations provided markers to understand what type of cells were in different clusters and allowed for down-stream sorting on the Invitrogen Bigfoot Spectral Cell Sorter.
Using these features, users can quickly evaluate their high-parameter panels using dimensionality reduction features and evaluate changes in populations for further studies.

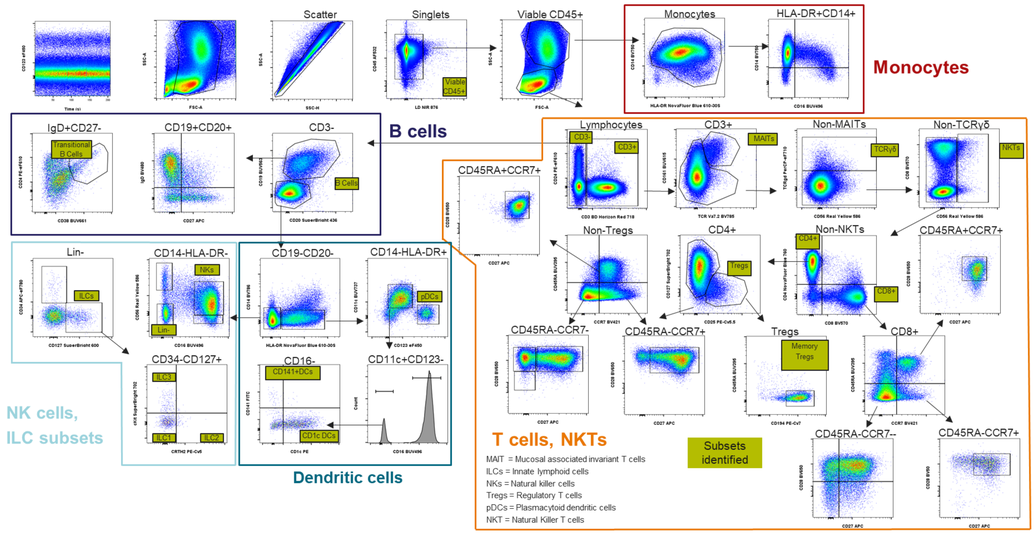
Finding and sorting unique populations using this method is a powerful tool for exploratory research, and with the capability to directly sort UMAP and t-SNE populations from the Bigfoot Spectral Cell Sorter, users can isolate these populations within the same protocol. Future studies will aim to sort different populations within the same UMAP or t-SNE cluster to identify variances within the same cluster, as well as demonstrate other downstream applications of UMAP or t-SNE sorted populations, such as RNA-sequencing.

Materials and methods

List of fluorophores used in the PBMC panel: CD45RA BUV395 (Thermo Fisher), CD16 BUV496 (Waters), CD19 BUV563 (Thermo Fisher), CD161 BUV615 (Waters), CD38 BUV661 (Waters), CD11c BUV737 (Thermo Fisher), PD-1 BUV805 (Thermo Fisher), CD197 BV421 (Thermo Fisher), CD20 SB436 (Thermo Fisher), CD123 eF450 (Thermo Fisher), IgD BV480 (Thermo Fisher), CD57 BV510 (Biolegend), CD8 BV570 (Biolegend), CD127 SB600 (Thermo Fisher), CD28 BV650 (Thermo Fisher), cKit SB702 (Thermo Fisher), CD14 BV750 (Biolegend), TCR Vα7.2 BV785 (Biolegend), CD141 FITC (Thermo Fisher), HLA-DR NFB610-30S (Thermo Fisher), TCRγδ PerCP-eF710 (Thermo Fisher), CD4 NFB760 (Thermo Fisher), CD1c PE (Thermo Fisher), CD56 RY586 (Waters), CD24 PE-eF610 (Thermo Fisher), CD294 PE-Vio670 (Miltenyi), CD25 PE-Cy5.5 (Thermo Fisher), CD194 PE-Cy7 (Biolegend), CD27 APC (Thermo Fisher), CD3 R718 (Waters), CD34 APC-eF780 (Themro Fisher), LD NIR876 (Thermo Fisher)

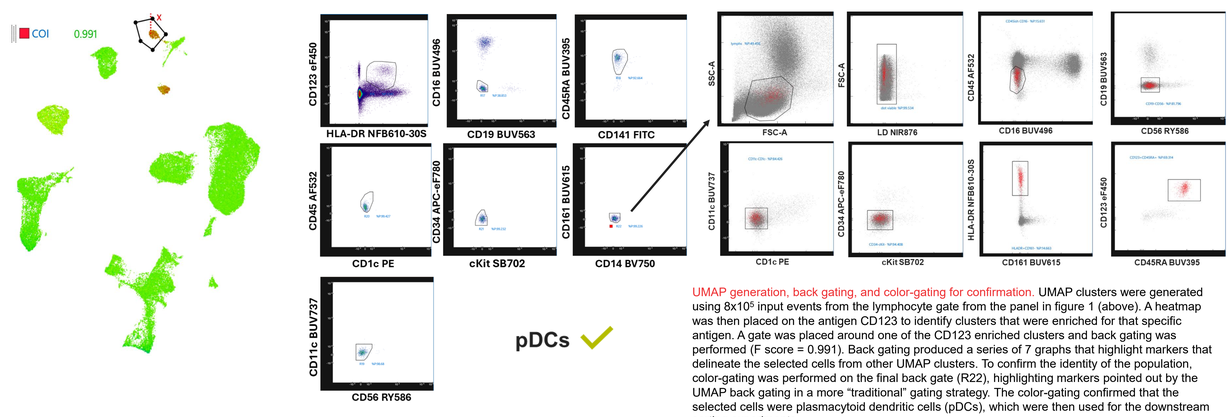
Results

Figure 1. Human PBMC immunophenotyping panel



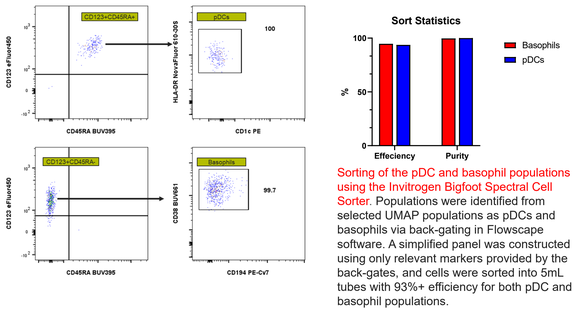
Human PBMC immunophenotyping panel. 1x10^6 human PBMCs were collected using the Attune Xenith Flow Cytometer at 200µl/min. Immunophenotyping panel was designed to investigate several different immune cell subsets including T cells, monocytes, B cells, dendritic cells and ILCs. This 33-color panel was used as the backbone to generate our UMAP data shown in the next figure. Data was Subsets identified using the panel are shown here in green.

Figure 2. UMAP generation from PBMC immunophenotyping data



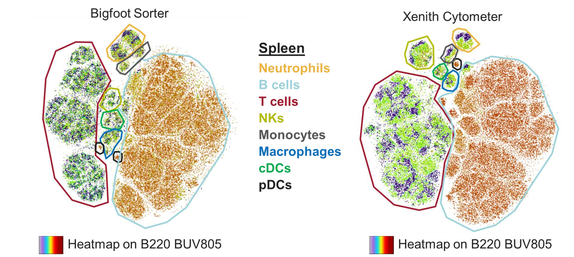
UMAP generation, back gating, and color-gating for confirmation. UMAP clusters were generated using 8x10^5 input events from the lymphocyte gate from the panel in figure 1 (above). A heatmap was then placed on the antigen CD123 to identify clusters that were enriched for that specific antigen. A gate was placed around one of the CD123 enriched clusters and back gating was performed (F score = 0.991). Back gating produced a series of 7 graphs that highlight markers that delineate the selected cells from other UMAP clusters. To confirm the identity of the population, color-gating was performed on the final back gate (R22), highlighting markers pointed out by the UMAP back gating in a more “traditional” gating strategy. The color-gating confirmed that the selected cells were plasmacytoid dendritic cells (pDCs), which were then used for the downstream sorting experiments.

Figure 3. Sorting of identified UMAP populations



Sorting of the pDC and basophil populations using the Invitrogen Bigfoot Spectral Cell Sorter. Populations were identified from selected UMAP populations as pDCs and basophils via back-gating in Flowscape software. A simplified panel was constructed using only relevant markers provided by the back-gates, and cells were sorted into 5mL tubes with 93%+ efficiency for both pDC and basophil populations.

Figure 4. Comparison of t-SNE data from both platforms



Platform parity between both the Attune Xenith Flow Cytometer and Bigfoot Spectral Cell Sorter. Data collected from a 37-color mouse spleen immunophenotyping panel was run on both the Attune Xenith Flow Cytometer and Bigfoot Spectral Cell Sorter platforms. t-SNE maps were generated on both platforms showing the uniformity of dimensionality reduction plot generation between both the Bigfoot Spectral Cell Sorter and Attune Xenith Flow Cytometer.

Summary

Utilizing dimensionality reduction analysis methods for large panels can be a powerful tool for researchers to allow for visualization of unique cellular populations between different collected samples/groups. Using the dimensionality reduction plot building and back-gating tools available in Flowscape software, we show here how to identify, confirm, and sort clusters of interest from dimensionality reduction plots collected on the Attune Xenith Flow Cytometer and Bigfoot Spectral Cell Sorter. This method allows users to rapidly collect cell clusters of interest for downstream experimentation.

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