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# Automated analysis of 16-color polychromatic flow cytometry data maps immune cell populations and reveals a distinct inhibitory receptor signature in systemic sclerosis

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# **Automated Analysis of 16-Color Polychromatic Flow Cytometry Data Maps Immune Cell Populations and Reveals a Distinct Inhibitory Receptor Signature in Systemic Sclerosis**

## **Abstract**

**Background.** The phenotypic profiles of both peripheral blood and tissue-resident immune cells have been linked to the health status of individuals with infectious and autoimmune diseases, as well as cancer. In light of the promising clinical trial results of agents that block the Inhibitory Receptor (IR) Programmed Death 1 (PD-1) axis, novel flow cytometric panels that simultaneously measure multiple IRs on several immune cell subsets could provide the distinct IR signatures to target in combinational therapies for many disease states. Also, due to the paucity of human samples, larger (14+ color) '1-tube' panels for immune cell characterization ex vivo are of a high value in translational studies. Development of fluorescent-based panels offer several advantages as compared with analogous mass cytometric methods, including the ability to sort multiple populations of interest from the sample for further study. However, automated platforms of multi-dimensional single cell analysis that allow objective and comprehensive population characterization are severely underutilized on data generated from large polychromatic panels. **Methods.** A 16-color flow cytometry (FCM) panel was developed and optimized for the simultaneous characterization and purification of multiple human immune cell populations on a 4-laser BD FACSARIA II cell sorter. FCM data of samples obtained from healthy subjects and individuals with systemic sclerosis (SSc) were loaded into Cytobank cloud, then compensated and analyzed with SPADE clustering algorithm. The viSNE algorithm was also employed to compress the data into a 2D map of phenotypic space that was subsequently clustered using SPADE. For comparison, the FCM data were also analyzed manually using FlowJo software. **Results.** Our novel 16-color panel recognizes CD3, CD4, CD8, CD45RO, CD25, CD127, CD16, CD56,  $\gamma\delta$ TCR,  $\nu\alpha$ 24, PD-1, LAG-3, CTLA-4, and TIM-3; it also contains a CD1d-tetramer and a live-dead dye (with CD19 and CD14 included as a combined dump channel). This panel allows combinational IR signatures to be determined from CD4<sup>+</sup> T, CD8<sup>+</sup> T, Natural Killer (NK), invariant Natural Killer (iNKT), and gamma delta ( $\gamma\delta$ ) immune cell subsets within one sample. We have successfully identified all subsets of interest using automatic SPADE and viSNE algorithms integrated into Cytobank services, and demonstrated a distinctive phenotype of IR distribution on healthy versus systemic sclerosis subject groups. **Conclusions.** Methods of automatic analysis that were originally developed for processing multi-dimensional mass cytometry can be applied to polychromatic FCM datasets and provide robust results, including subset identification and distinct IR signatures in healthy compared to diseased subject groups.