A 61-parameter CyTOF panel for comprehensive profiling of human PBMC to characterize activation, differentiation, immune checkpoint and intracellular cytokines

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CyTOF™ XT PRO

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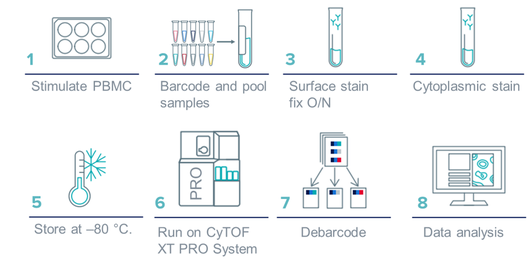
Introduction

CyTOF™ mass cytometry is a single-cell analysis platform that uses metal-tagged antibodies to resolve 50+ markers in a single tube. Notable benefits of CyTOF technology allow for antibody cocktails and stained samples to be frozen for later use and batch acquisition, which considerably minimizes technical variation and streamlines workflows. Samples can also be barcoded, allowing cells to be stained and acquired in a single tube. Unlike fluorescence-based cytometry, CyTOF systems have no autofluorescence and low signal spillover, and thus spectral compensation unmixing and single stain controls are not required. Therefore, it is uniquely possible with CyTOF to rapidly design high-parameter antibody panels, identify functional markers with low expression at high resolution, and use intracellular and functional markers to gain functional insights in rare cell populations.

High-parameter cytometric analysis is crucial in translational and clinical research to understand the functional phenotype of immune cells. It can allow accurate discoveries of mechanism of action, disease prognoses and potential therapeutic targets. A 61-parameter CyTOF panel was designed for in-depth functional immune profiling of human PBMC. The panel contains lineage markers for major immune cell subsets including B cells, T cells, myeloid cells, NK cells and a diverse array of phenotyping T cell targets focused on activation, differentiation, immune checkpoint and functional cytokines. The panel can be used to identify over 60 cell populations with a focus on cytokine-producing T cell subsets. Untreated and stimulated PBMC donor samples were barcoded, pooled together, labeled with viability and cell-identifying reagents, and stained with an antibody panel containing 36 surface and 14 cytokine targets. After cell staining, samples were frozen and acquired on a later day, using CyTOF XT PRO. Acquired data was debarcoded to analyze the immune responses of individual donors.

The data from this panel demonstrates how intracellular and functional markers can be used to identify rare cell populations and identify low-expressing targets at high resolution using CyTOF technology.

Methods and Materials



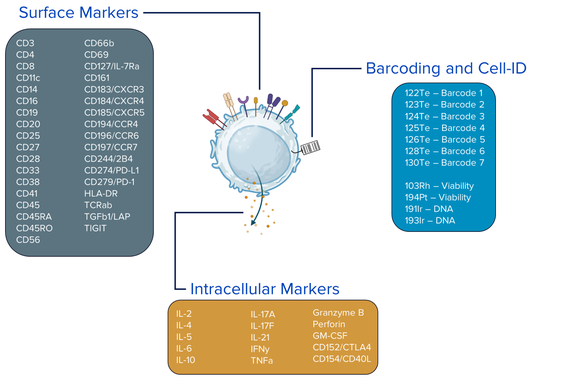


Figure 1. Experimental design. PBMC from three healthy donors were thawed and stimulated overnight with phytohaemagglutinin (PHA) and lipopolysaccharide (LPS), in triplicate. Eighteen hours post stimulation, cells were incubated with phorbol 12-myristate 13-acetate (PMA), ionomycin, brefeldin A and monensin for four hours. Live-cell universal barcoding using a tellurium-based 7-choose-3 strategy was carried out for batch staining. Pooled samples were stained with surface and cytoplasmic antibodies according to the Maxpar Cell Staining with Fresh Fix User Guide (FLDM-01319) and stored at –80 °C. The barcoded sample was thawed, washed, and acquired on a CyTOF XT PRO system with enhanced throughput (> 500 events/sec). Data normalization and debarcoding were performed using CyTOF Software (v9.2). Data analysis was carried out using CellEngine (CellCarta). The full panel list and purchase information can be downloaded using the QR code below.

Results

High-dimensional analysis of stimulated PBMC reveals deep immunophenotyping and exceptional resolution of cellular islands in t-SNE space

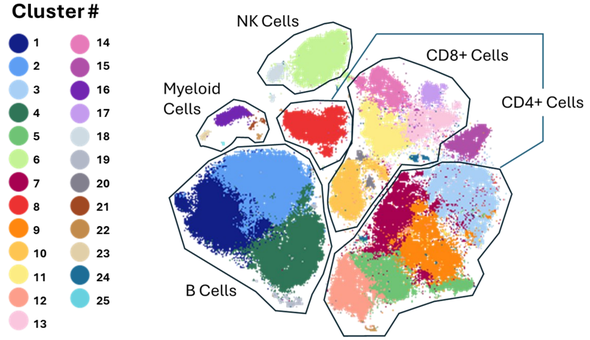


Figure 2. Major cell subsets present in PBMC samples.

An equal number of live singlet lymphocyte events from 3 stimulated donor PBMC samples were concatenated and subjected to t-SNE dimensionality reduction and PhenoGraph clustering algorithms. t-SNE map shows overlay of 25 clusters identified in the dataset based on differential expression of the surface and intracellular markers.

Excellent separation of major lineage populations including B cells, myeloid cells, NK cells and T cell subsets were observed.

Striking cross-lineage immuno-functional diversity captured via comprehensive immune checkpoint and cytokine profiling

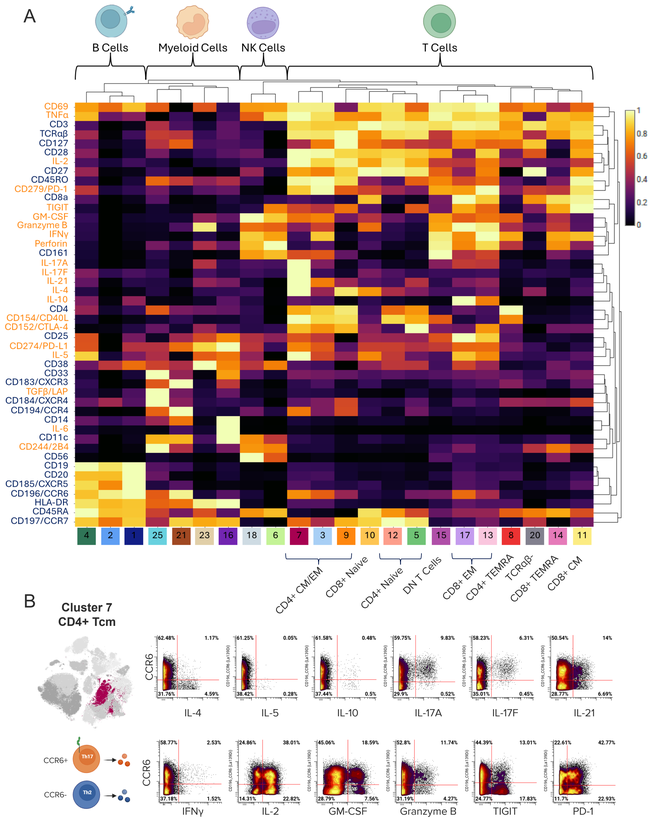


Figure 3. Deep insight into the complex nature of immune responses and cellular action unveiled.

A) Heatmap depicting hierarchal clustering of phenotypic and functional markers across the phonograph cellular clusters identified (color matched to the t-SNE map in figure 2 above). Clusters were efficiently organized into their respective lineage populations including B cells, myeloid cells, NK cells, T cells. A larger number of clusters were classified as T cell subsets based on the markers present in this panel (annotated along the X-axis). Effector, memory, cytotoxic, and regulatory cells were identified.

When deep phenotyping is paired with high-parameter functional analysis, a more in-depth view of poly-functionality and diversity at the single cell level is revealed. Expression beyond abundant and traditionally studied cytokines can be seen, confirming the increased need for multi-dimensional intracellular functional analysis.

B) Example of immuno-functional diversity observed: PhenoGraph cluster #7 is highlighted on the t-SNE map, which includes CD4+ T central memory populations (CD45RO+CD45RA-CD27+). Both Th2 and Th17 cells were identified within this cluster of memory cells (CCR4+CCR6- and CCR4+CCR6+ cells, respectively). Representative bivariate plots showing the expression of cytokine and immune checkpoint markers within the Th2 and Th17 cell populations.

Mapping the functional landscape of immune cell activation and cytokine expression of over 20 markers for unprecedented exploration of functional immunology

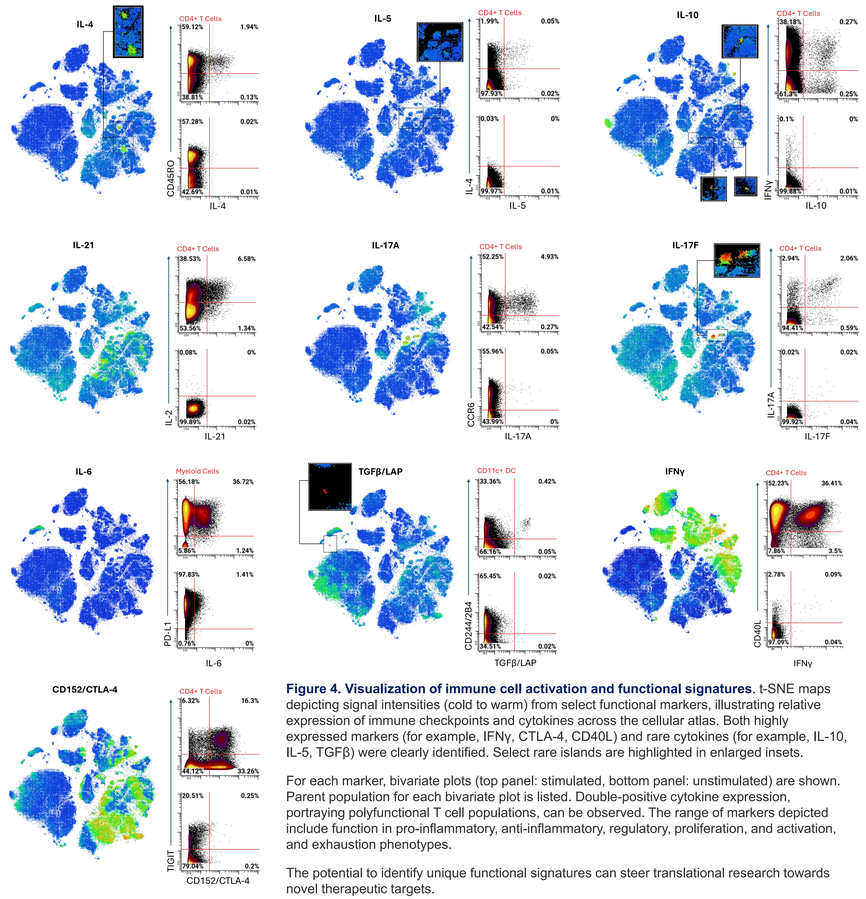


Figure 4. Visualization of immune cell activation and functional signatures. t-SNE maps depicting signal intensities (cold to warm) from select functional markers, illustrating relative expression of immune checkpoints and cytokines across the cellular atlas. Both highly expressed markers (for example, IFNγ, CTLA-4, CD40L) and rare cytokines (for example, IL-10, IL-5, TGFβ) were clearly identified. Select rare islands are highlighted in enlarged insets.

For each marker, bivariate plots (top panel: stimulated, bottom panel: unstimulated) are shown. Parent population for each bivariate plot is listed. Double-positive cytokine expression, portraying polyfunctional T cell populations, can be observed. The range of markers depicted include function in pro-inflammatory, anti-inflammatory, regulatory, proliferation, and activation, and exhaustion phenotypes.

The potential to identify unique functional signatures can steer translational research towards novel therapeutic targets.

Conclusions

CyTOF technology enables the highest number of simultaneous measurements of surface and intracellular targets in a single panel, allowing for wide immune coverage and exceptional resolution of intracellular targets to quantify rare cells and interrogate their functional potential.

CyTOF workflow advantages include sample barcoding and freezing stained samples for batch acquisition to reduce technical variation over clinical studies.

Striking functional diversity across the cellular immune landscape can be unveiled by combining deep immunophenotyping with multi-parametric intracellular and functional marker analysis.

Overall, studying functional immunology using CyTOF technology can elucidate the complex nature of immune responses to provide an understanding of how they relate to disease and treatment mechanisms.

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Supplemental Tables for Li et al

CyTOF XT PRO brochure

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