Expanding the Maxpar Direct Immune Profiling Assay to Enable Comprehensive Antigen-Specific Immune Analysis

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Introduction

The complex nature of the immune system requires deep interrogation at the single-cell level. Mass cytometry, which uses CyTOF® technology, utilizes antibodies tagged with monoisotopic metals, resulting in discrete signals that enable highly multiparametric characterization on a single-cell level. CyTOF can currently enable cellular phenotyping of over 50 single-cell parameters simultaneously, including phenotypic and functional markers, thereby significantly increasing the ability to comprehensively evaluate immune responses.[1]

The **Maxpar® Direct™ Immune Profiling Assay™** is a pre-titrated, dried-down, 30-marker antibody cocktail for immune profiling of human whole blood and peripheral blood mononuclear cells (PBMC) by CyTOF (Figure 1). Using **Maxpar Pathsetter™** software, stained samples are automatically resolved into **37 immune populations**. This assay was designed with customization in mind, with 18-plus open channels available for panel expansion.

This study highlights the use of the **Maxpar Direct T Cell Activation Expansion Panel** and **live-cell barcoding** to add 15 parameters to the backbone Maxpar Direct panel. The array of key surface and intracellular functional parameters in this expanded panel enables comprehensive analysis of **immune cell activation and antigen-specific recall responses**. Such phenomena are hallmarks for research on infection, vaccine development, and immunotherapy.

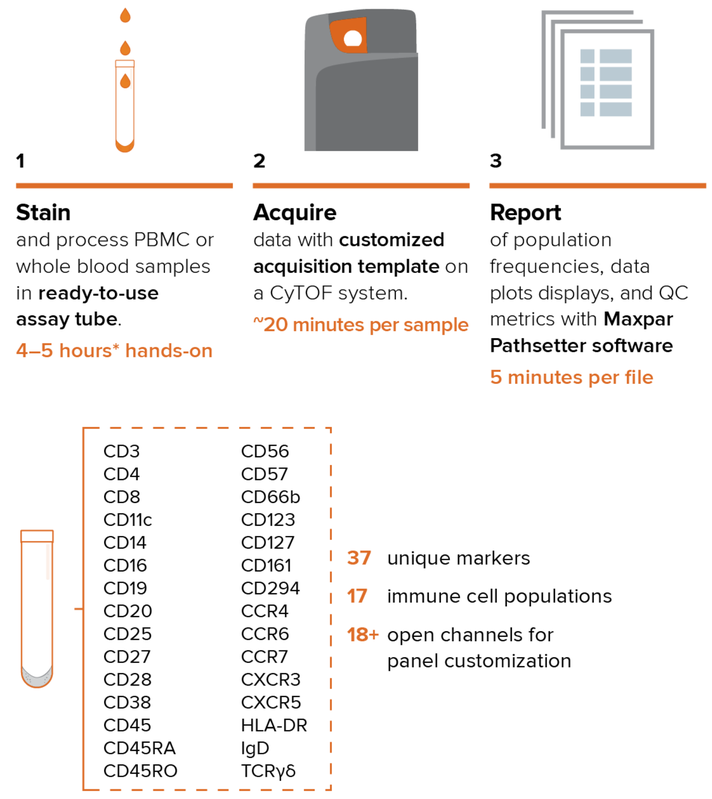
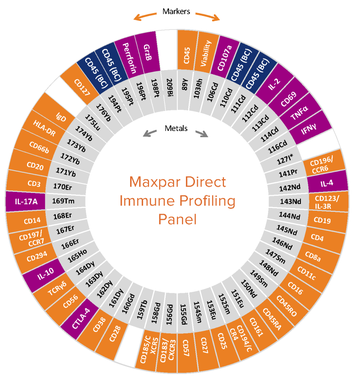


Figure 1. The Maxpar Direct Immune Profiling Assay Workflow (top) and list of markers in the panel (bottom)

Materials and Methods

Building a 47-Parameter Panel for Functional Profiling of Activated Immune Cells



Target (clone)	Isotope	Staining
CD107a (H4A3)	106Cd	Surface
CD45 (HI30)	110Cd	Surface (barcode)
CD45 (HI30)	111Cd	Surface (barcode)
IL-2 (MQ1-17H12)	112Cd	Intracellular
CD69 (FN50)	113Cd	Surface
TNFα (Mab11)	114Cd	Intracellular
IFNγ (B27)	116Cd	Intracellular
IL-4 (MP4-25D2)	142Nd	Intracellular
CTLA-4 (14D3)	162Dy	Intracellular
IL-10 (JES3-9D7)	165Ho	Intracellular
IL-17A (BL-168)	169Tm	Intracellular
CD45 (HI30)	194Pt	Surface (barcode)
CD45 (HI30)	195Pt	Surface (barcode)
Perforin (B-D48)	196Pt	Intracellular
Granzyme B (Gb11)	198Pt	Intracellular

Figure 2. This 47-parameter panel includes 41 phenotypic and functional markers, 4 barcode tags, and 2 Cell-ID® reagents. (Left) Panel wheel illustrating cellular markers and isotope channels used in this study, which combines the base Maxpar Direct panel (Cat. No. 201334, orange boxes), the Maxpar Direct T Cell Activation Expansion Panel (Cat. No. 201409, purple boxes), and anti-CD45 antibodies for live-cell barcoding (Cat. Nos. 3110001B, 3111001B, 3194001B, 3195001B, green boxes). Empty boxes indicate open channels. (Right) Table detailing expansion panel antibody clones, barcoding reagents, isotope tags, and staining method required.

Study Design: Sample Preparation, Staining, and Analysis

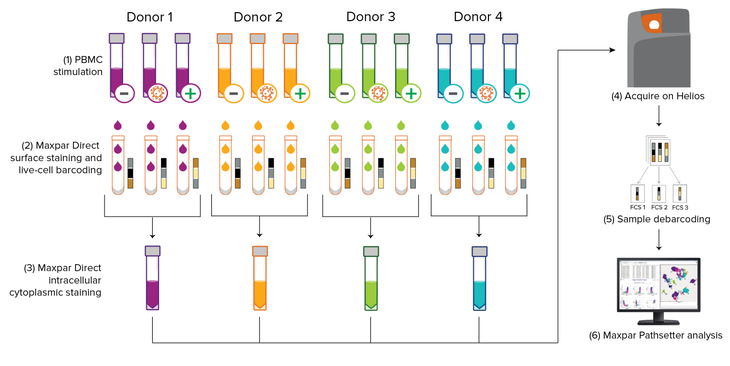


Figure 3. Experimental design

Materials and Methods

- Human PBMC from four different donors were rested overnight and either left untreated (➖) or stimulated with PMA/ionomycin (➕) or a mix of immunodominant microbial peptides (🦠). Brefeldin A and monensin were added to all samples 1 hr post-stimulation and incubated for an additional 4 hr.
- After stimulation, cells were added to the dried-down Maxpar Direct antibody pellet and concurrently stained with additional surface markers according to the Maxpar Direct User Guide (PN 400286).
- A unique 4-choose-3 anti-CD45 barcode mix for each stimulation condition was added to corresponding tubes (▮▮▮).
- Multiplexed samples from each PBMC donor were combined into a single tube, fixed, permeabilized, and stained with the intracellular antibodies.
- After acquisition on a Helios™ instrument*, sample debarcoding[2] was carried out and individual FCS files were uploaded into Maxpar Pathsetter software. For automated analysis of the 11 additional markers introduced in the Maxpar Direct T Cell Activation Expansion Panel, a customized model was created following the Method Develop: Customize the Maxpar Direct Immune Profiling Assay User Guide (FLDM-00151).

* This assay can also be acquired on a CyTOF XT™ system

Results

Optimizing Workflow and Data Quality with Sample Multiplexing and Automatic Analysis

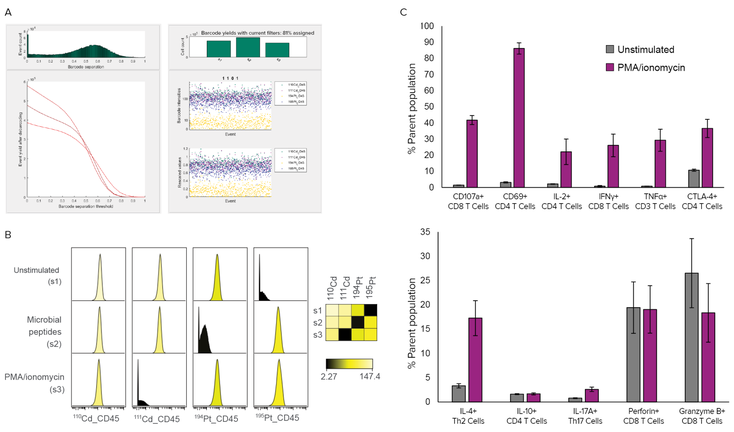


Figure 4. A) Representative sample debarcoding analysis from PBMC Donor 3 illustrates efficient sample separation between the positive and negative barcode channels used, which resulted in a barcode yield of 81%. **B)** After sample deconvolution, FCS files were further evaluated for data quality and consistency. All debarcoded files exhibited a positive signal for the expected barcode channels (yellow histograms) and had minimal signal overlap into negative barcode channels (black histograms). This was also visualized in a heat map depicting median signal intensities for each barcode channel. **C)** The Maxpar T Cell Activation Pathsetter Model incorporates new functional information based on the activation markers and cytokines in this panel and results in >100 additional measurements within specified immune cell subsets. Bar graphs depict % CD4 or CD8 T cell populations expressing the functional markers from this panel (mean ± SEM from all 4 PBMC donors) after PMA/ionomycin stimulation compared to unstimulated controls.

Mapping the Functional Landscape of Activated Immune Cells

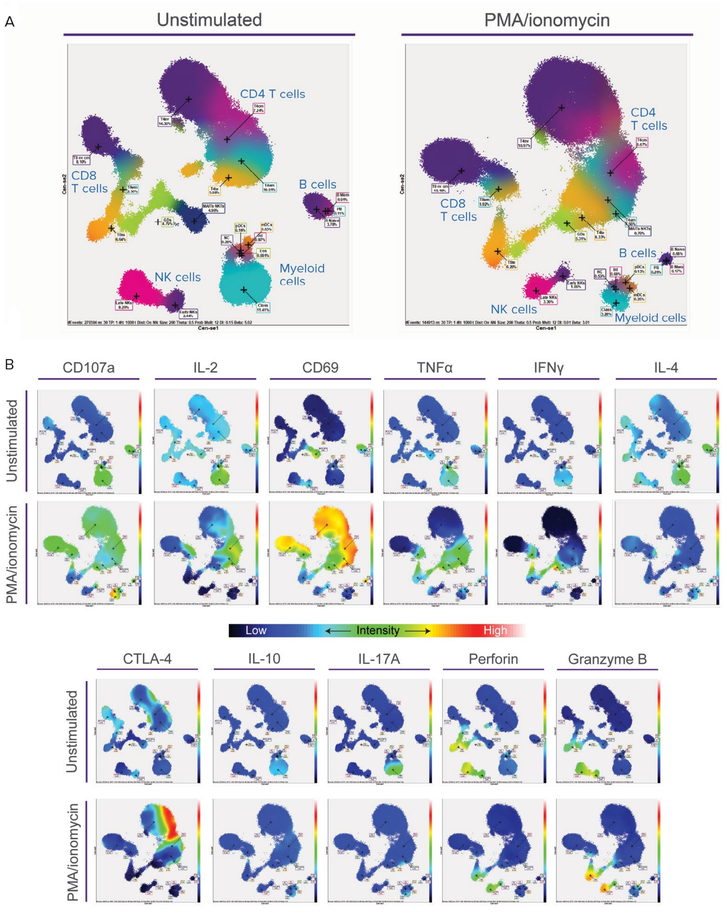


Figure 5. Visualization of immune cell activation via high-dimensional Cen-se′[3] analysis. Maxpar Pathsetter generated Cen-se′ maps of unstimulated and PMA/ionomycin-stimulated samples from PBMC Donor 2 are shown. **A)** Clusters are colored by cell types and subsets. CD8 T cells, CD4 T cells, B cells, NK cells, and myeloid cells are labeled. Indeed, this high-dimensional modelling illustrated expansions or reductions in specific cellular islands when stimulated by PMA/ionomycin. **B)** Heat maps of signal intensities illustrating relative expression across immune cell populations for each of the 11 functional markers from the Maxpar Direct T Cell Activation Expansion Panel are shown for both unstimulated and PMA/ionomycin stimulated samples. These heat maps revealed more intricacies of the cellular response to stimulation in the Cen-se′ plot such as ubiquitously high expression of CD69 across the T cell islands, exhausted CTLA-4+ CD4 T cells, and increased production of cytokines such as IFNγ in specific T cell clusters.

Results

Cytokine Profiling of T Helper Cell Subsets

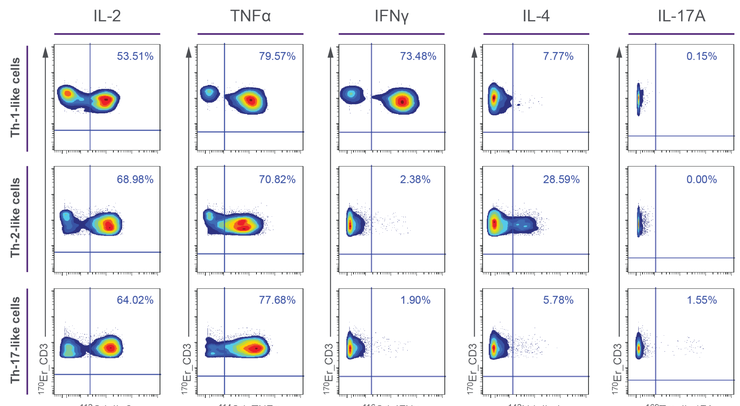


Figure 6. The addition of select activation and intracellular markers can also complement the phenotyping carried out by the base Maxpar Direct panel. For example, the T helper (Th) cell subsets Th1, Th2, and Th17 are defined by expression of specific chemokine receptors in the base Maxpar Direct assay. Investigation of cytokine profiles in this work confirmed the definition of these subsets. CD3 vs. IL-2, TNFα, IFNγ, IL-4, or IL-17A bivariate plots are displayed for Th1-like, Th2-like, and Th17-like populations from PMA/ionomycin-stimulated Donor 3 as a representative sample. Gate labels display percent of cells positive for the corresponding cytokine expression. As expected, PMA/ionomycin-stimulated samples revealed that while IL-2 and TNFα were ubiquitously expressed across all three 3 Th subsets, IFNγ, IL-4, and IL-17A were predominantly expressed in Th1, Th2, and Th17 populations, respectively.

Exploring T Cell-Mediated Host Immune Responses to Foreign Antigens

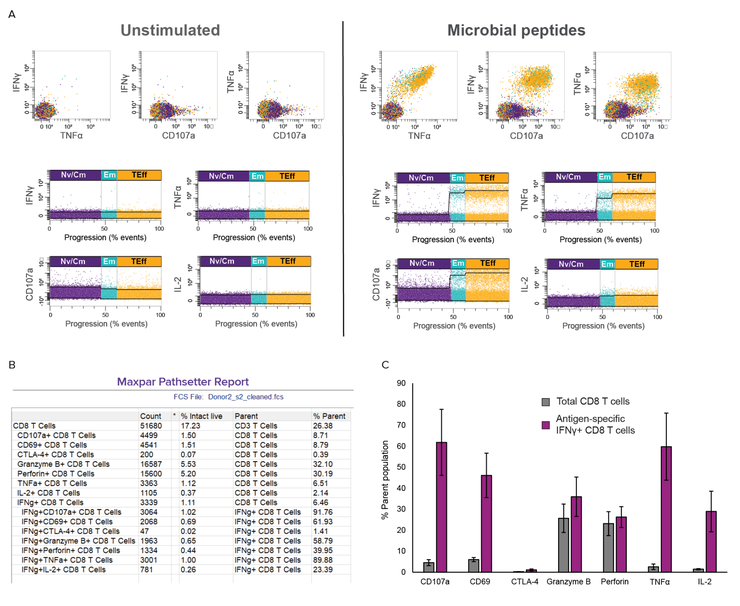


Figure 7. Antigen-specific CD8 T cells identified using Maxpar Pathsetter. **A)** Bivariate plots from CD8 T cells showing IFNγ vs. TNFα, IFNγ vs. CD107a, and TNFα vs. CD107a for unstimulated and peptide stimulated samples from PBMC Donor 2 (top panel). Upregulation and positive correlation of functional markers highlight antigen-specific T cells identified in microbial peptide-stimulated samples. Expression profile plots of IFNγ, TNFα, CD107a, and IL-2 across naïve/central memory (Nv/Cm), effector memory (Em), and terminal effector (TEff) CD8 T cell memory subsets (bottom panel). All plots are color-coded by CD8 T cell memory subsets. **B)** Screen capture of the updated Maxpar Pathsetter report page for peptide-stimulated CD8 T cells from PBMC Donor 2. Frequency of CD8 T cells expressing CD107a, CD69, CTLA-4, granzyme B, perforin, TNFα, and IFNγ is shown. Additional breakdown of these frequencies on antigen-specific CD8 T cells, defined as IFNγ+, is reported. **C)** Greater frequencies of activation markers and cytokine production can be observed in antigen-specific CD8 T cells when compared to global CD8 T cell populations across all PBMC donors tested (bar graphs depicting mean % parent population frequency ± SEM). These antigen-specific T cells were polyfunctional, as assessed by co-expression of numerous activation and cytolytic markers, which is a correlate of T cell efficacy.[4]

Conclusions

- A **47-parameter** CyTOF panel was successfully developed by combining the base Maxpar Direct Panel, the Maxpar Direct T Cell Activation Expansion Panel, and Maxpar anti-CD45 LCB reagents.
- The preconfigured expansion panel included 9 antibodies targeting cytoplasmic markers, thereby introducing a novel workflow of **intracellular staining** to the Maxpar Direct Immune Profiling Assay.
- The existing Pathsetter model was adapted to incorporate new functional immune markers resulting in **>100 additional measurements** on various cell subsets.
- **Automated analysis** successfully classified new populations such as **antigen-specific T cells** in response to microbial peptide pools.
- Similar experimental workflows and antibody panels to the one described herein can easily be applied to other infectious disease models such as SARS-CoV-2 infection[5] or monitoring the response to vaccination[6].

References

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