P095

Concurrent protein profiling of intracellular cytokines and phosphoproteins integrated into a high-parameter surface protein assay at the single-cell level.

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Poster# P095

Abstract

BACKGROUND: Characterizing a cell's protein composition provides essential information about its phenotype, state, and functionality. Both cell surface and intracellular proteins are crucial for comprehensive cell understanding. Intracellular markers, especially those involved in signaling pathways like the STAT family, are particularly important but challenging to profile due to their transient phosphorylation states.

CHALLENGES:

Current technologies (flow cytometry and CyTOF) have significant limitations:

- Restricted number of markers per experiment and High instrument costs
- Complicated panel design requirements
- Difficulty simultaneously profiling cytokines and phosphoproteins due to incompatible buffer requirements

SOLUTION

- A protein-only single-cell workflow that enables simultaneous profiling of both cell surface and intracellular proteins
- A universal fixation/permeabilization buffer system compatible with antibody DNA oligo tags
- A bioinformatic pipeline that annotates cells using sequencing reads from ADT and detects protein expression without requiring mRNA data

RESULTS AND CONCLUSIONS

•Successfully profiled around 80 protein markers simultaneously (including 30 intracellular proteins)

•Captured key signaling proteins (STAT1, STAT3, STAT4, STAT5, STAT6), transcription factors (FoxP3), and cytokines (IL-2, IFN-γ, TNF-α)

•Demonstrated the ability to conduct protein-only focused studies without significant limitations on the number of proteins characterized

•Expanded multiplexing capabilities for measuring both intracellular and surface protein expression profiles at the single-cell level

Methods

1A. BD® OmicsOne Immune Profiler Protein panel (30 Plex), 20 Drop-ins, and 29 Intracellular markers

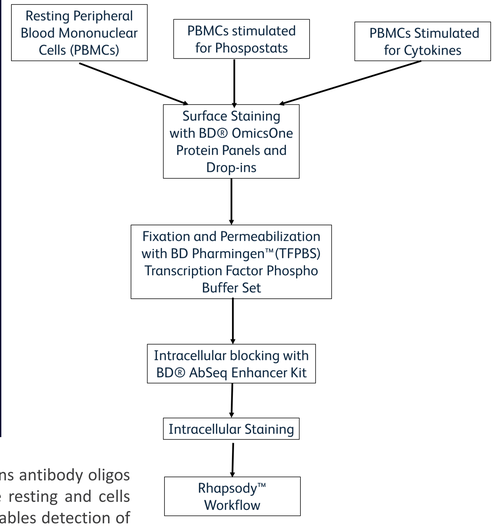
Surface Markers

BD® OmicsOne Immune Profiler panel		Drop-ins
CD3	CD137	CD154
CD4	CD161	CD69
CD8	CD183 (CXCR3)	CD95
CD11c	CD185 (CXCR5)	CD137
CD14	CD186 (CXCR6)	CD194
CD16	CD196 (CCR6)	CD184
CD19	CD197 (CCR7)	CD103
CD25	CD272	CD20
CD27	CD278	CD138
CD28	CD279	CD10
CD45RA	CD357 (GITR)	CD21
CD56	CD366 (Tim3)	CD223-LAG-3
CD62L	HLA-DR	CD275
CD127	IgM	CCR7
CD134	IgD	CD152
		CD73
		TCR-γδ
		CD360-IL-21R
		CD34
		CD274

Intracellular Markers

Intracellular Proteins and Isotype Control	Phosphoprotein Markers	Cytokines
Helios	Stat4	TGF-β1
FoxP3	Stat6	IL-21
T-bet	Stat5	IL-17A
Eomes	Stat3	IL-6
Granzyme B	Stat1	IL-12
GATA -3	p38 MAPK	IL-4
BCL-6		IL-10
cPARP		IL-13
H2AX		IFN-γ
Caspase 3		TNF-α
Ki-67		IL-2
MsIgGK		

1B. Experimental Workflow



1C. BD Rhapsody™ Single Cell System

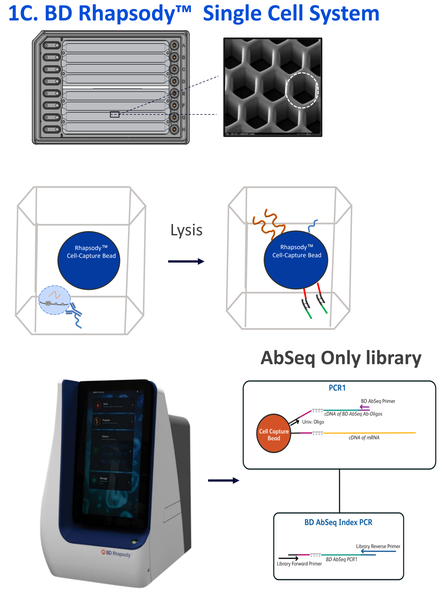


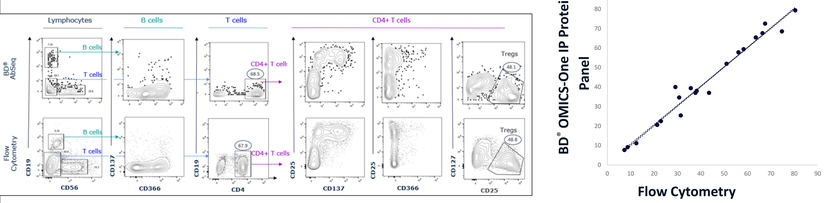
Figure 1. (A). BD® OMICS-One Immune Profiler Protein Panel, BD® AbSeq Drop-ins antibody oligos and Custom antibody oligos to detect Intra Cellular markers used to stain the resting and cells stimulated for phosphoproteins and Cytokines. (B). Staining workflow which enables detection of phosphorylated markers and cytokines using a universal buffer (BD Pharmingen™ Transcription Factor Phospho Buffer Set) is shown. (C). Infographic showing the BD Rhapsody™ system which enables single cell capture followed by library prep.

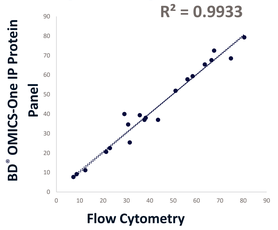
Conclusions

- **High-plex protein profiling**: Detected 78 surface and intracellular proteins simultaneously
- **Sample Tag Compatible**: Multiple samples were stained together with BD® AbSeq SMK
- **Flexible panel design**: Used BD® OMICS-One Protein Panels along with dropped-in BD® AbSeq reagents for specific study objectives and needs.
- **Straightforward workflow:** A universal fixation/perm buffer was used for simultaneous surface, cytoplasmatic and nuclear protein staining. After fixation, samples can be stored at -20°C (for up to a week) for workflow adjustments.
- **Simplified library prep:** Library prep has a quick turnaround on protein-only workflows (without RNA-seq).
- **Reliable data output**: High correlation(<.99) between BD® OMICS-One Protein Panel and 30 antibodies for flow cytometry was demonstrated.
- **Data analysis and visualization:** A cost- and programming-free data display and interpretation was obtained from using BD Cellismo™ Data Visualization Tool.

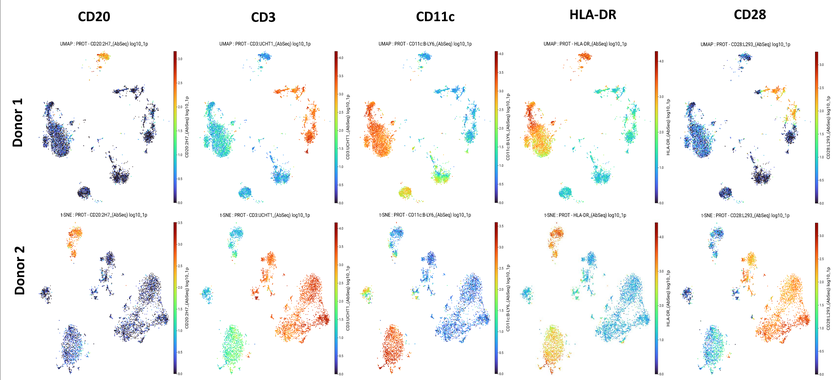
Results (1)

2A. BD® AbSeq protein profiling has high correlation to flow cytometry

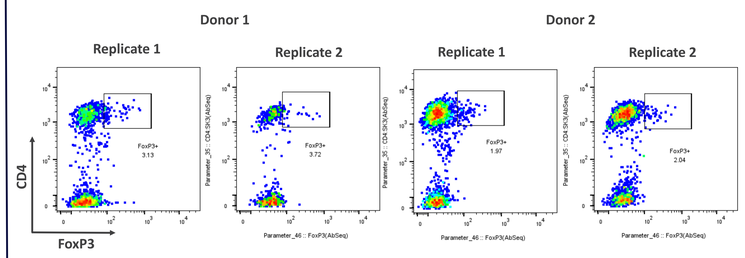




2B. High specificity of key Surface primary markers in resting PBMCs



2C. FOXP3 detection in resting PBMCs



2D. Phosphorylated marker detection in resting and stimulated cells

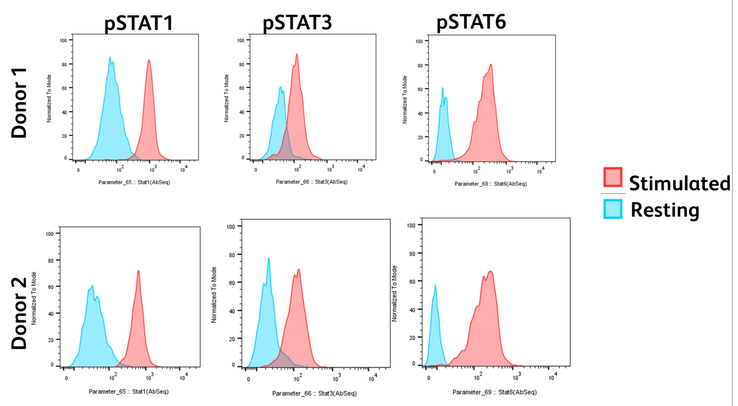
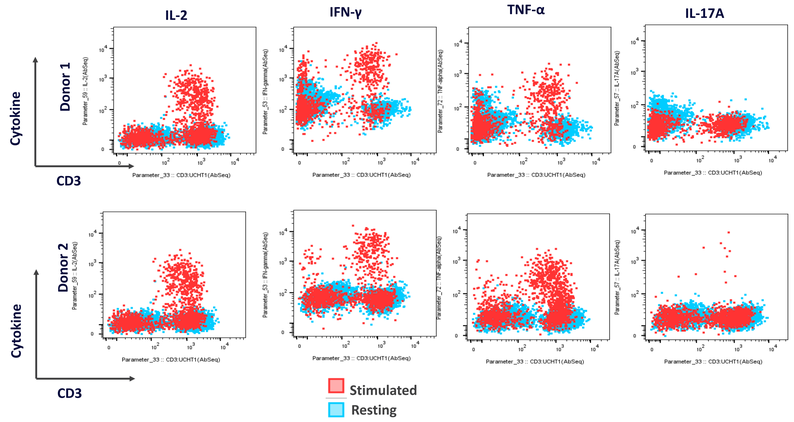


Figure 2. (A). Same sample was stained with BD® OMICS-One Immune Profiler Protein Panel and corresponding flow antibodies for primary oligo-based and it shows high concordance with flow antibodies. The correlation between flow cytometry and Antibody oligo-based approach is >.99. (B). Key primary surface markers present in the BD® OMICS-One Immune Profiler Protein Panel allow for cell calling and clustering using the bioinformatics pipeline/BD Cellismo™ Data Visualization Tool and the markers show high specificity (C). FoxP3 was included in the panel and identified the small Treg population. (D). PBMCs were stimulated with cytokines prior to staining and fixation to elicit phosphorylation in key phosphoproteins and the stimulation status is detected in the stimulated population when compared to the corresponding resting cells.

Results(2)

3A. Cytokine detection in PMA/Ionomycin stimulated cells



3B. Utility of Isotype marker for noise removal

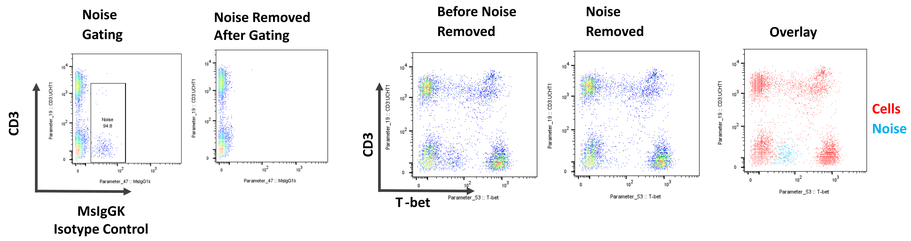


Figure 3. (A). Cells were stimulated with PMA and Ionomycin for 12 hours (Red) with BD GolgiStop™ to prevent cytokines from being secreted or rested (Blue) prior to fixation with TFPBS and then stained with custom made intracellular markers to detect cytokines such as IL-2, IFN-γ, TNF-α. (B). An Isotype marker was also included in the Intracellular panel which can assist with noise removal in problematic samples.

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