Secreted cytokine profiling with mRNA and surface proteins of peripheral blood immune cells using an integrated single-cell multiomics workflow

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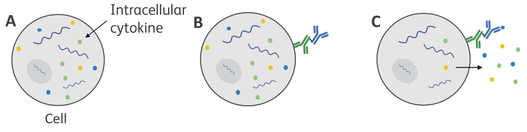
Abstract

Cytokines act as important messengers in immune cell signaling pathways. Current methods of analysis for cytokine measurement rely on blocking secretion followed by intracellular fixation/permeabilization staining protocols or solid phase capture of secreted proteins from serum, plasma or supernatant. Intracellular measurements offer a sensitive and high-throughput approach in single cells but may not accurately reflect the functional secretory response. Additionally, approaches that measure secreted cytokines after capture on adjacent solid surfaces have limitations in surface marker detection, ability to link the secretions to the originating cell or compatibility with further downstream analysis. Cytokine secretion assays (CSA) using bifunctional antibodies to directly capture secreted cytokines offer an alternative approach. Bifunctional antibody capture technology uses an antibody specific to a cell surface protein as an anchor conjugated directly to a secreted target-specific antibody. The surface protein-specific antibody acts as a tether to bind the bifunctional unit in place on the cell surface. As cytokines are secreted, they are captured by the target-specific antibody and are thereby bound to the surface of the cell.
Integrating the bifunctional capture method with a scCITE-seq approach to simultaneously measure protein and mRNA provides an opportunity to directly study secreted cytokine expression with surface protein and transcriptional profiling on a single-cell level. Here, we generated a protocol to incorporate these elements in an end-to-end workflow using stimulated PBMCs. We simultaneously profiled IFN-γ secreting CD4 T cells for 30 surface protein markers and whole transcriptome analysis. We were able to apply typical gating strategies used in CSA flow cytometry analysis to the combined data set to recapitulate flow staining results and link cytokine secretion directly with transcriptome analysis for secreting cells. Together these preliminary results suggest that analysis of secreted cytokines combined with transcriptomic and proteomic analyses on a single-cell level may be used to overcome current technical limitations and enable a deeper understanding of cytokine signaling and secretory processes.

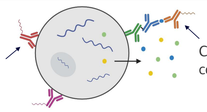
Methods

Cytokine Secretion Assay



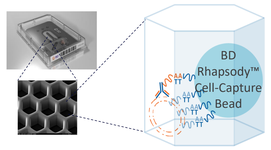
A) Cell stimulation induces the release of cytokines from the cell over time. **B)** Staining with a bifunctional antibody tethers a cytokine capture antibody to the surface of the cell. **C)** Cytokines are secreted from the cell and captured on the capture antibody.

BD® AbSeq Antibody and cytokine detection AbOligo staining



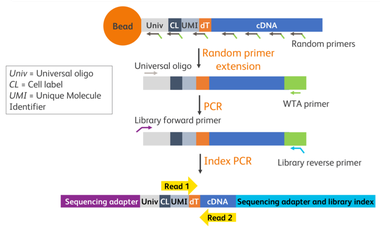
Surface protein staining with BD® AbSeq Immune Discovery Panel (IDP)

BD Rhapsody™ System workflow



mRNA and oligos from both BD® AbSeq Antibody and the cytokine of interest are captured on BD Rhapsody™ Cell Capture Beads.

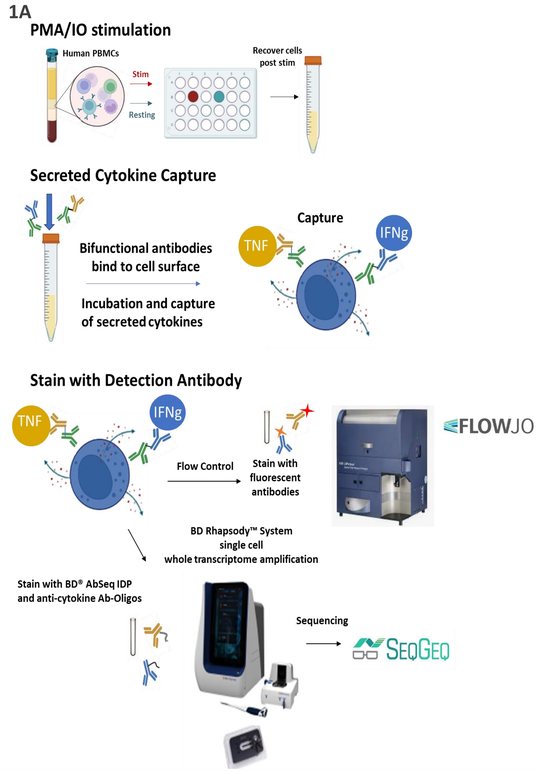
Library generation, sequencing, and analysis



Captured mRNA, BD® AbSeq Antibody oligos, and cytokine detection oligos are now associated with a cell label and unique molecular index (UMI). The sequences are amplified by the BD Rhapsody™ Whole Transcriptome Analysis (WTA) or AbSeq library preparation protocols.

Results

Workflow integration for bifunctional secreted cytokine capture with single-cell whole transcriptome and surface protein analysis



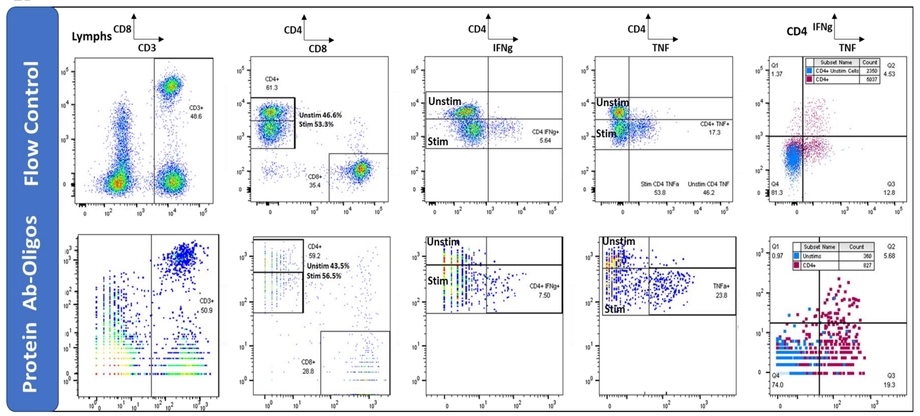
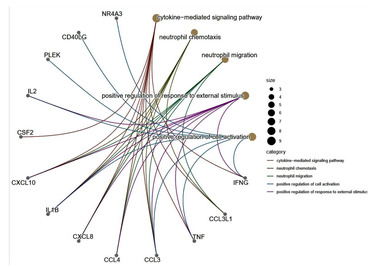


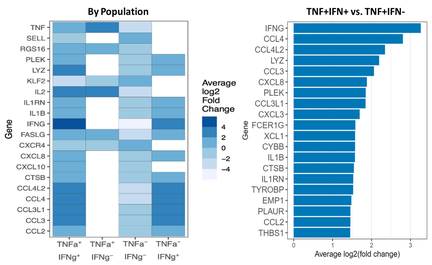
Figure 1. Workflow and data analysis integrating secreted cytokine capture with single-cell WTA and surface protein measurement. (A) Cell stimulation and staining workflow for bifunctional capture with readout by flow cytometry using fluorescent antibodies or single-cell labeling using oligo-conjugated antibodies with Rhapsody™ System workflow. (B) Demonstration of conventional surface protein staining compared with single-cell data obtained from ab-oligo labeling. (C) Differential expression analysis of mRNA transcript data can be analyzed after gating using surface protein and cytokine secretion.
Protocol was optimized over 20 development experiments. Data shown here is from N=1 representative experiment.

Methods and Workflow 1A figures created with Biorender.com.
Ashburner M, et al. Gene ontology: tool for the unification of biology. *Nat Genet.* 2000;25(1):25-29.
Gene Ontology Consortium. The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Res.* 2021;49(D1):D325-D334.

1C GO Analysis for cytokine secreting cells

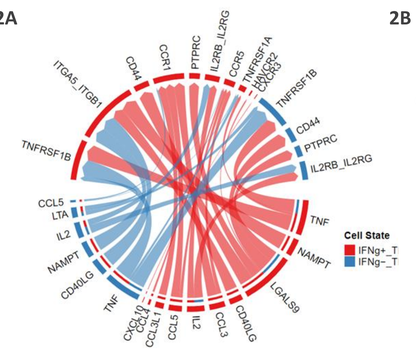


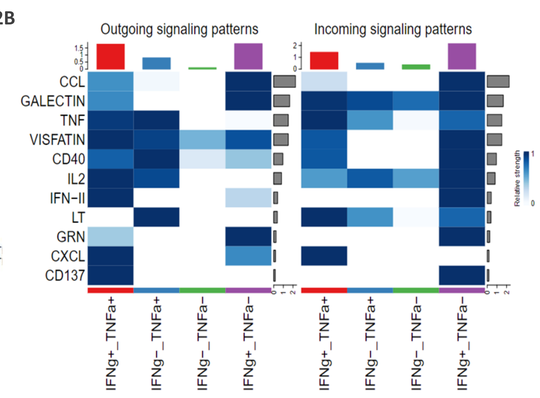
Gene expression fold change



Results

Cell-to-cell communication pathways





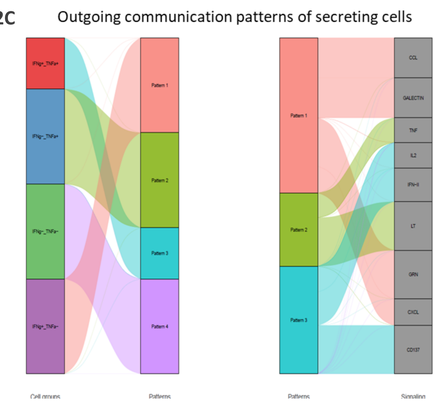


Figure 2. Inference of cell-cell communication for cell groups and cytokine secretion state using CellChat. (A) Chord diagram showing ligand-receptor interactions for stimulated T cell subpopulations with differing cytokine secretion patterns. (B) Heatmap identifying predominant outgoing and incoming signaling patterns from gene expression data for all cell groups in the sample. (C) River plot visualization of the association between communication patterns, signaling pathways and gene expression using cell groups.

Jin S. _CellChat: Inference and analysis of cell-cell communication from single-cell and spatial transcriptomics data_. R package version 1.6.1. 2023.
Hao Y, et al. Integrated analysis of multimodal single-cell data. *Cell.* 2021; 184 (13):3573-3587.e29.

Conclusions

- We have developed a protocol enabling the simultaneous analysis of mRNA, surface proteins and secreted cytokines from single cells. This protocol is used to directly assay cytokine secretions from live cells without fixation, permeabilization or transport inhibitors.
- Sequencing data for IFNg and TNFa expression detected with oligo-conjugated antibodies (Ab-oligos) showed high concordance with the flow cytometry control using conventional reagents.
- Ab-oligo labeling for surface protein and secreted cytokines facilitates the use of classical gating strategies with single-cell transcriptome data.
- Multiplexing of two secreted cytokine analytes provides the potential for deeper understanding of cellular states and allows analyses of signaling patterns between cells.