Ultra-high parameter, instrument-free, protein profiling by sequencing using TotalSeq™-A antibodies at scale.

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Background

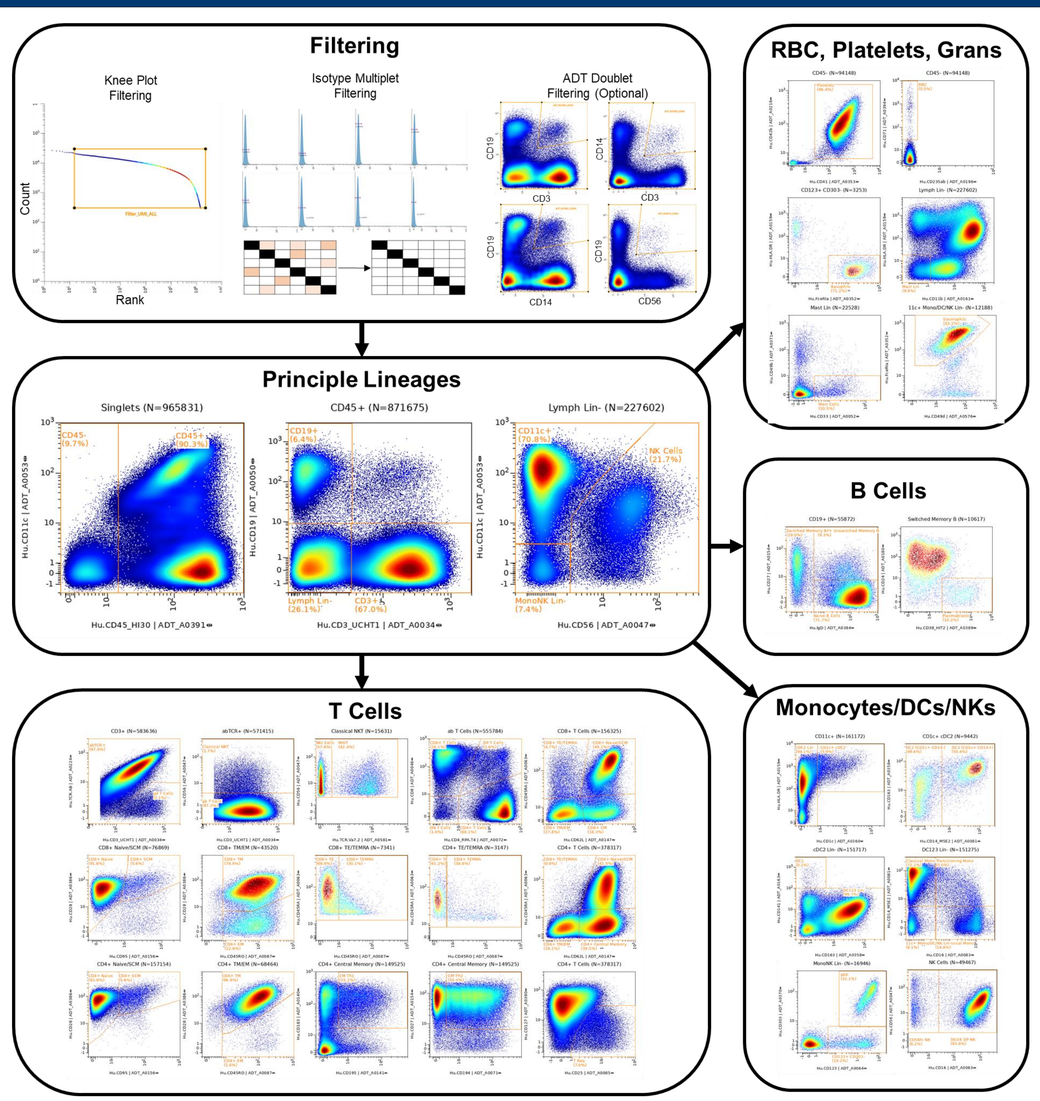
Problem
- Current protein profiling methods are limited, offering either high parameter analysis or high-throughput capability, but rarely both simultaneously.
- Achieving high-throughput analysis in conjunction with >100 protein parameters is particularly important in heterogeneous cell populations with rare cell types where there are still gaps in comprehensive cellular analysis.
- Analysis of many samples and parameters across millions of cells is costly, time-consuming, and currently necessitates complex workflows and dedicated instrumentation.

Solution
- Here we introduce a novel high-throughput, high-parameter protein profiling platform that employs oligo-tagged antibodies to profile >150 protein parameters in >1 million cells in a single experiment.
- ScaleBio's plate-based workflow allows processing of 1-96 samples in a single day from cells to sequencing library.

Experiment
- Our study utilized peripheral blood mononuclear cells (PBMCs) with a tailored plate-based protocol for high sensitivity and low background noise, profiling over 150 protein parameters on individual cells.

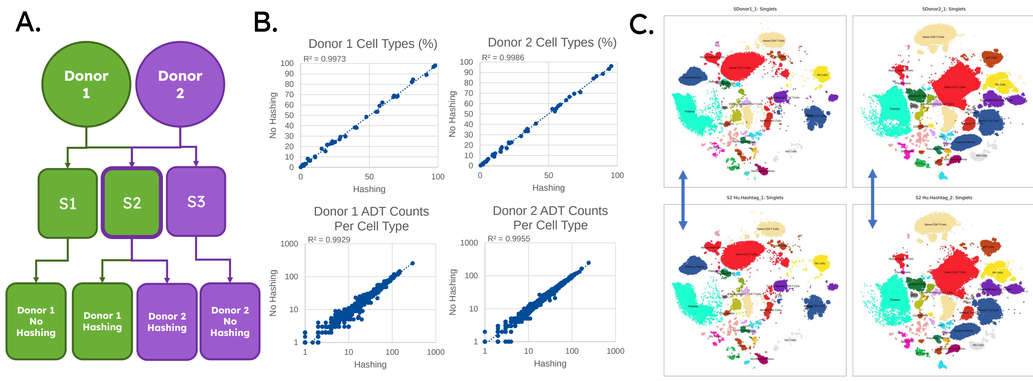
Results and Conclusions
- The platform successfully profiled over one million cells with high sensitivity across >150 proteins, and data quality meeting or exceeding current gold-standard technologies, a significant achievement in the field of cytometry and protein analysis.
- Bivariate gating analysis shows effective cell type identification with strong signal and low background that is able to accurately identify canonical cell populations originally defined by legacy cytometry technologies.
- We identified and characterized >60 different cell populations within the PBMC samples, showcasing the platform's ability to profile complex cellular diversity.
- This platform proved especially adept at detecting rare cell subsets, identifying populations with frequencies as low as 1 in 10,000 cells such as those in the dendritic cell niche.
- Across specific cell types biologically relevant expression patterns are able to accurately capture the diversity of protein expression in low frequency and phenotypically unique cell types.
- In summary, we provide a novel solution for an existing problem, enabling in-depth and scalable analysis of over 150 proteins at the single-cell level, expanding cell phenotyping beyond the most advanced multiparametric flow and mass cytometry.

Figure 1: High parameter & high-throughput single cell protein analysis overview



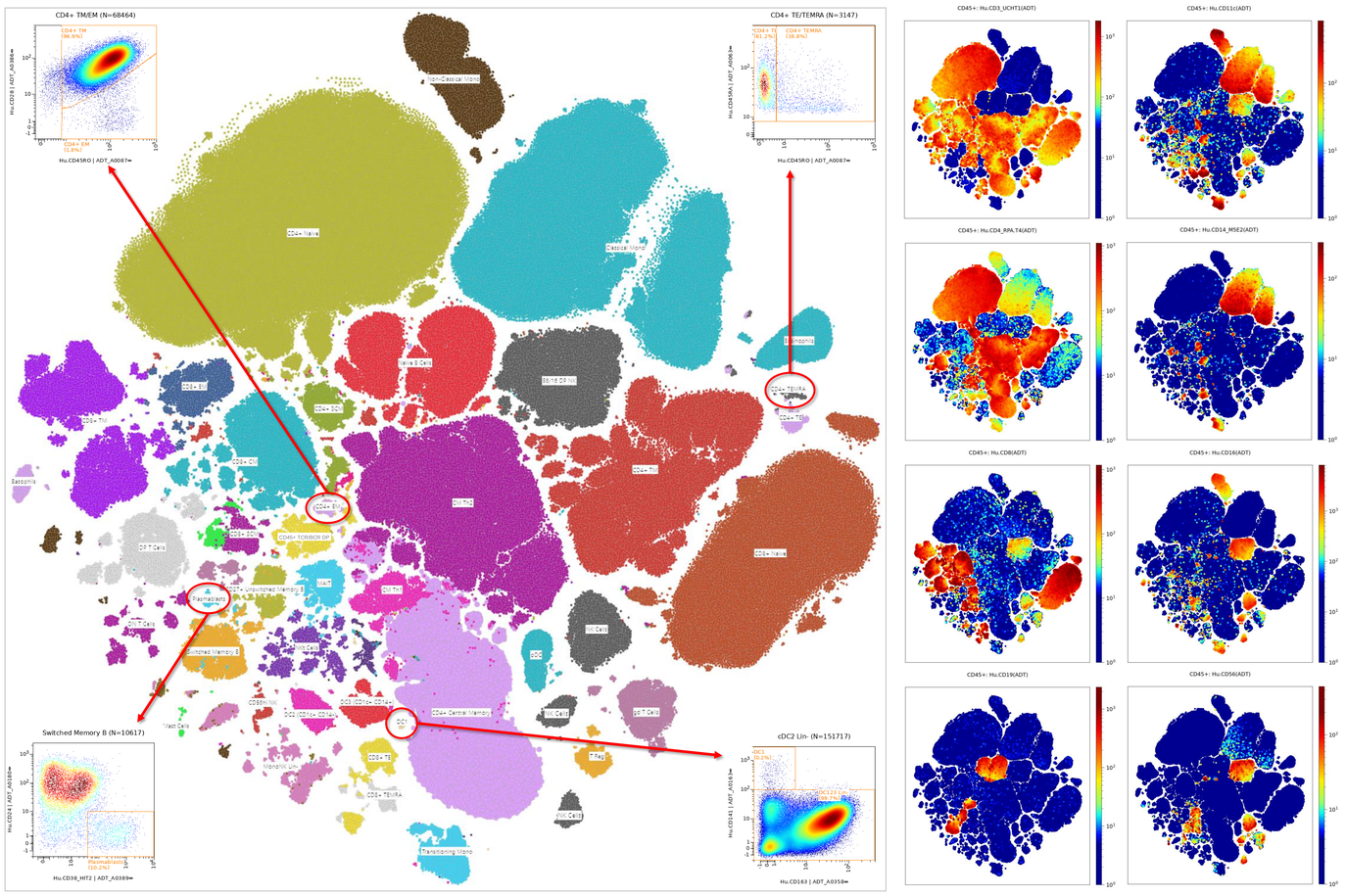
Resting PBMCs stained with 165-marker panel and run through the ScaleBio protein profiling workflow. After filtering approximately 1 million cells remain. Hierarchical bi-variate gating shown here was used to establish the cell-types supervising the tSNE seen in Figure 3. 42 parameters were used in this gating scheme, comparable to the highest number of dimensions used in flow and mass cytometry. Plots show clean data with strong ability to phenotype cell types using ADT data.

Figure 2: Hashtag sample multiplexing in conjunction with protein profiling does not impact performance



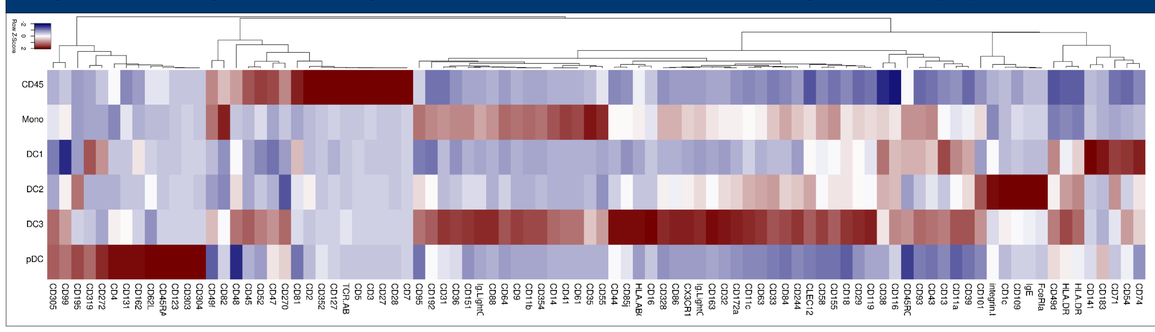
A. Fresh PBMCs were harvested from two donors, and stained with a panel of 165 antibodies in the presence or absence of hashing antibodies.
B. Top: Correlation of the percentage of cells found in 40 cell types with and without hashing (cell types were defined using gating scheme similar to that shown in Figure 1). Bottom: Correlation of median counts per ADT with and without hashing.
C. tSNEs generated on all samples show similar cell types and pattern with and without hashing.

Figure 3: Identification of >60 cell-types within PBMC population with highly specific protein expression



A. tSNE projection of all CD45+ cells. Approximately 1 million cells are shown with cell types labeled according to gating scheme shown in Figure 1. Plots in upper and lower corners show rare cell subsets (<0.01 to 0.01% of total cells).
B. Representative tSNEs shaded by expression of the indicated protein marker show clean signal and low background.

Figure 4: Biological diversity demonstrated within canonical myeloid cell populations



Expression matrix of 90 proteins across all CD45+ cells, classical monocytes, and the dendritic cell lineage. For representative cell-type gating reference "Monocytes/DCs/NKs" in Figure 1.