Title: Massively parallel single cell-omic approaches reveal high parameter correlation of protein and mRNA expression in individual immune cells

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Abstract: Since the efforts associated with the sequencing of the human genome, biomedical research Massively parallel single cell-omic approaches reveal high parameter correlation of protein and mRNA expression in individual immune cells of complex biological systems and networks. Tools for molecular and cell analysis have continued to evolve to address these new challenges and opportunities in many different biological fields. Flow cytometry, the tool of the trade of today's immunologists, is a highly multi-parametric platform, capable of high speed quantitative assessment of cells and other particles, at the single cell level. Today, as we continue to innovate on our flow cytometry platforms, which are capable of reaching up to 50 parameters, flow cytometry is opening a range of new applications stemming from opportunities presented by the advancements of genomics, proteomics, systems immunology and biology. The inevitable impact of these efforts, are in turn impacting decisions in clinical diagnosis and advancements in a deeper understanding of cancer biology, vaccine development and drug discovery.Today, with the introduction of new high parameter flow cytometry platforms (BD FACS Symphony A3, A5 and S6), the development of a large array of new Sirigen polymer fluorochromes and the completion of 2 studies for both mouse and human receptor density and expression, this year saw the demonstration of highly sensitive 25 to 28-color flow cytometry panels for analytical and sorting applications directly linked to single cell multi-omic applications.

With the advancements of High Parameter Flow Cytometry and Single-Cell mRNA sequencing we have enhanced our knowledge of the cell populations found in individual tissues or disease states. However, low correlation between mRNA and protein expression levels (not exceeding 30 color HP Flow Cytometry) hinders our understanding of biology and disease. Traditional single cell protein assays, such as flow cytometry are limited in the number of parameters that can be achieved within given experiment. Herein we use oligo-conjugated antibodies (Ab-seq) in combination with massively parallel single cell mRNA sequencing on the BD Rhapsody™ platform to simultaneously measure the protein and mRNA content of individual human blood cells (PBMCs) and mouse splenocytes. To accomplish this, we will use several Ab-seq panels against immune relevant cell surface markers (for analyzing both T regulatory cells, human ILC1, 2 and 3 cell populations and a broad mouse phenotyping panel) paired with the BD Rhapsody™ Immune Response panel (a targeted gene expression panel consisting of 399 targets). The Multi-omics data from this single workflow experiment provides a measurement of both gene expression and protein expression. The digital measurements are rendered free of PCR bias through the use of the unique molecular indices (UMIs). In addition, we will demonstrate the utility of this data to develop more comprehensive HP flow panels that can be used to functional panels using high parameter sorting technology. Our results show that protein expression detected with Ab-seq is highly sensitive and specific. Protein expression patterns correlate well with results from flow cytometry data on the same samples. Ab-seq allowed the robust detection of expressed genes even when their cognate mRNA transcripts have low abundance. Our study shows successful analysis of protein and mRNA expression data within a single workflow, and should enable the further elucidation single cells within different tissues, developmental time points, and disease states.