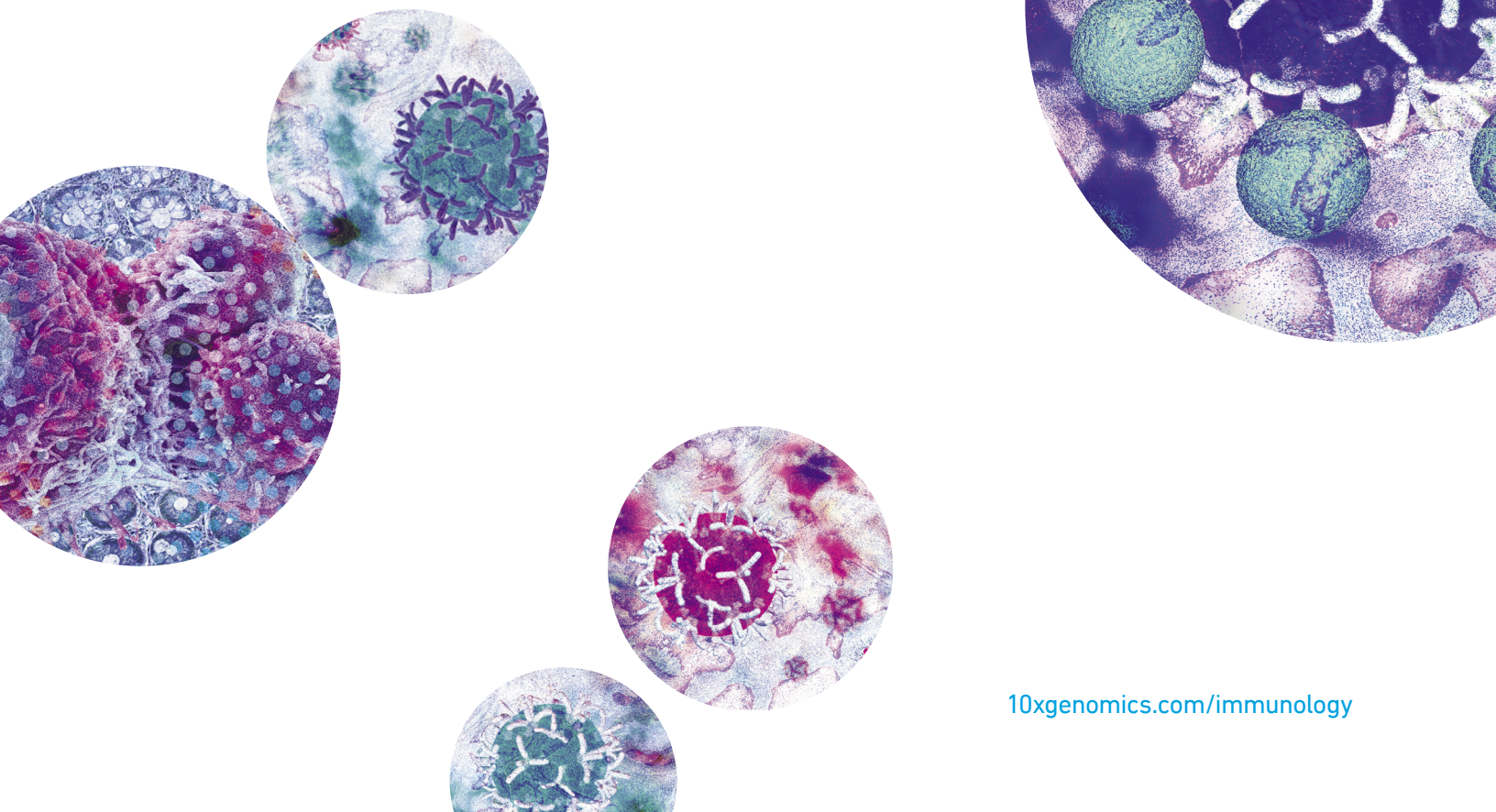
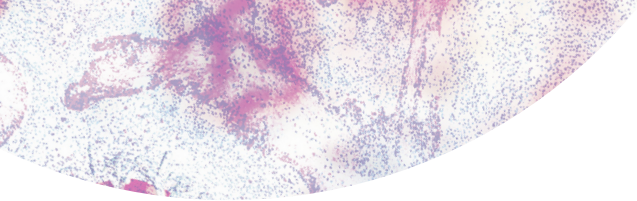


IMMUNOLOGY RESEARCH

Beyond the Surface

Uncovering the layers
of immune cell complexity





Gain a Holistic View of the Immune System

Immunology research has been at the forefront of many important scientific and medical breakthroughs in the past century, including vaccine development, tissue matching for safe organ transplantation, and immuno-oncology therapies. Today, immunology and infectious disease researchers continue to advance our understanding of significant health issues including cancer, autoimmune diseases, and emerging pathogens. However, their efforts face significant challenges due to the complex, dynamic, and heterogeneous nature of the immune system and the limitations of prevailing research tools.

In order to comprehensively monitor and understand an immune response, scientists need the ability to characterize cell types and functional states in individual cells at high throughput. To this end, techniques such as flow cytometry (Flow) and mass cytometry (cytometry by time of flight, or CyTOF) have enabled immunologists to sort and classify cells into distinct types and states based on cell surface proteins. However, relying on cell surface markers alone leaves most of a cell's molecular characteristics hidden and fails to further differentiate cells that express the same surface proteins or between different clonotypes of T or B cells.

10x Genomics offers a selection of solutions to meet the challenges of immunological studies while allowing the flexibility to incorporate these solutions with your current flow cytometry workflow.

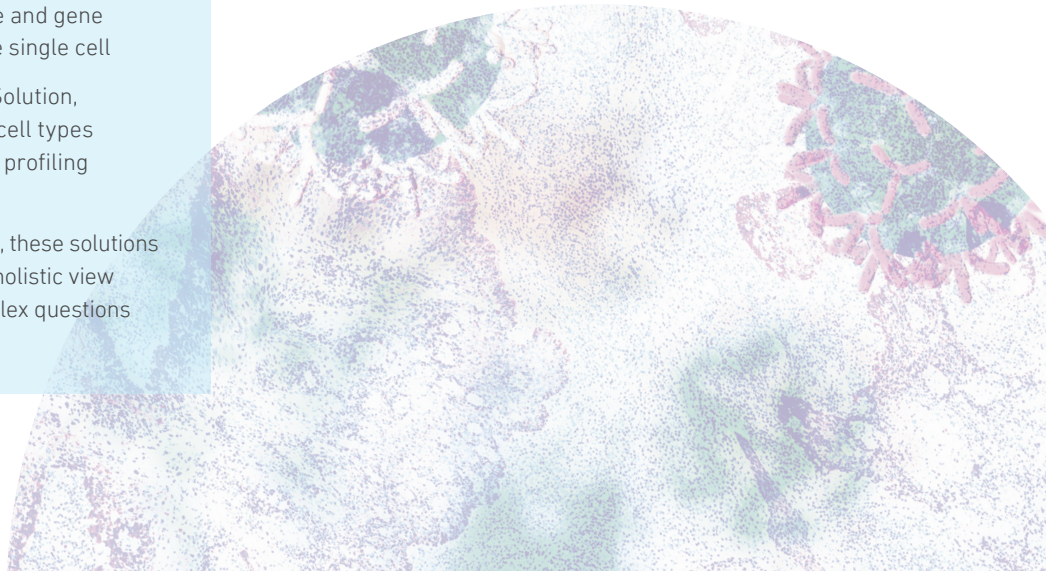
- Use our Chromium Single Cell Immune Profiling and Gene Expression Solutions with Feature Barcoding technology to perform multiomic phenotyping in thousands of single immune cells
- Multiomics allows you to characterize cell surface protein and antigen specificity and combine these outputs with immune repertoire and gene expression analysis all from the same single cell
- With the Chromium Single Cell ATAC Solution, you can deeply characterize immune cell types and dissect developmental lineage by profiling chromatin accessibility

Together, along with our turnkey software, these solutions are enabling researchers to gain a clear, holistic view of the immune system and address complex questions that have evaded previous technologies.

Combine Gene and Cell Surface Marker Measurements for Improved Subtype and Cell State Identification

High throughput single cell RNA sequencing technologies have enabled important advances in the understanding of innate and adaptive immunity. For example, using the Chromium Single Cell Gene Expression Solution, researchers have discovered evidence for the generation of memory-like CD4+ T cells in the human fetal intestine (1), charted the evolutionary architecture of the innate immune response (2), and unveiled previously unappreciated levels of heterogeneity in the bone marrow microenvironment (3). However, while gene expression profiling provides information about the type and quantity of mRNA transcripts produced, the abundance and isoforms of expressed proteins cannot always be inferred directly from mRNA readout alone (4). Likewise, relying on cell surface markers alone will leave much of the dynamic biology of immune cells undiscovered (5), and it is well understood that extensive heterogeneity exists even within immune cell populations classified as a single lineage based on surface markers (6).

Today, immunology and infectious disease researchers continue to advance our understanding of significant health issues including cancer, autoimmune diseases, and emerging pathogens.



To more accurately characterize cellular identity, state, and function, and to define novel cell subtypes in healthy or diseased models, it is important to measure multiple cellular readouts. The Chromium Single Cell Immune Profiling and Gene Expression Solutions with Feature Barcoding technology enable a multiomic approach by labeling cell surface proteins with DNA barcode conjugated antibodies, instead of traditional fluorophore conjugated antibodies, and integrating measurements of cell surface proteins and transcriptomes into a single readout.

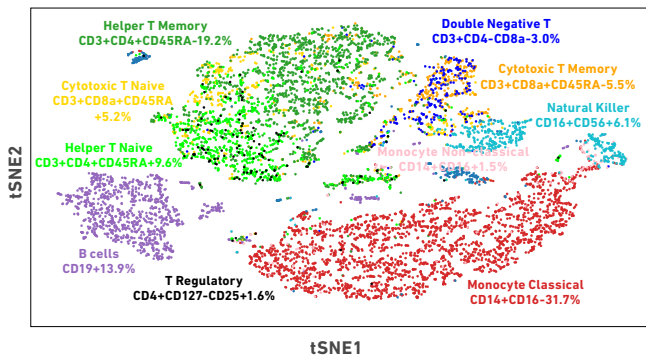
Using the Chromium Single Cell Immune Profiling Solution with Feature Barcoding technology, we profiled peripheral blood mononuclear cells (PBMCs) and clustered cells based on gene expression and cell surface protein expression (Figure 1 A, B).

Compared to gene expression data alone, the additional information from antibody Feature Barcode counts better resolved many canonical cell types, such as double negative and memory T cells, CD4+ and CD8+ T cells, and memory and naïve CD4+ or CD8+ T cells (Figure 1B, cell clusters marked with an asterisk). A quantitative comparison between Feature Barcoding technology and flow cytometry showed that the two approaches reveal similar cell populations (Figure 1C).

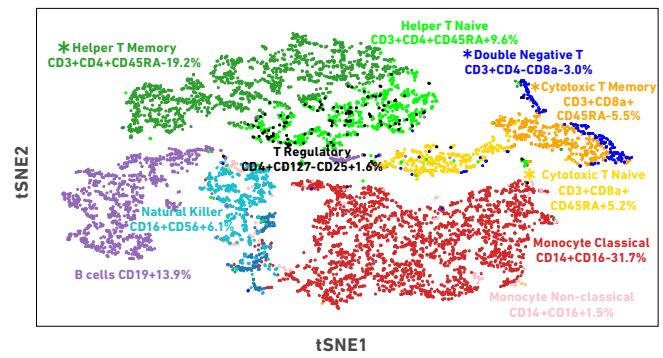
Together these results demonstrate that Feature Barcoding technology performs as well as flow cytometry to identify cell types and provides the added ability to combine protein and transcriptome measurements in the same cell. An additional advantage of Feature Barcoding technology is the massive diversity afforded by the DNA barcode conjugated antibodies.

Figure 1

A. Gene Expression



B. Cell Surface Protein



C. Quantitative comparison between Feature Barcoding technology and flow cytometry

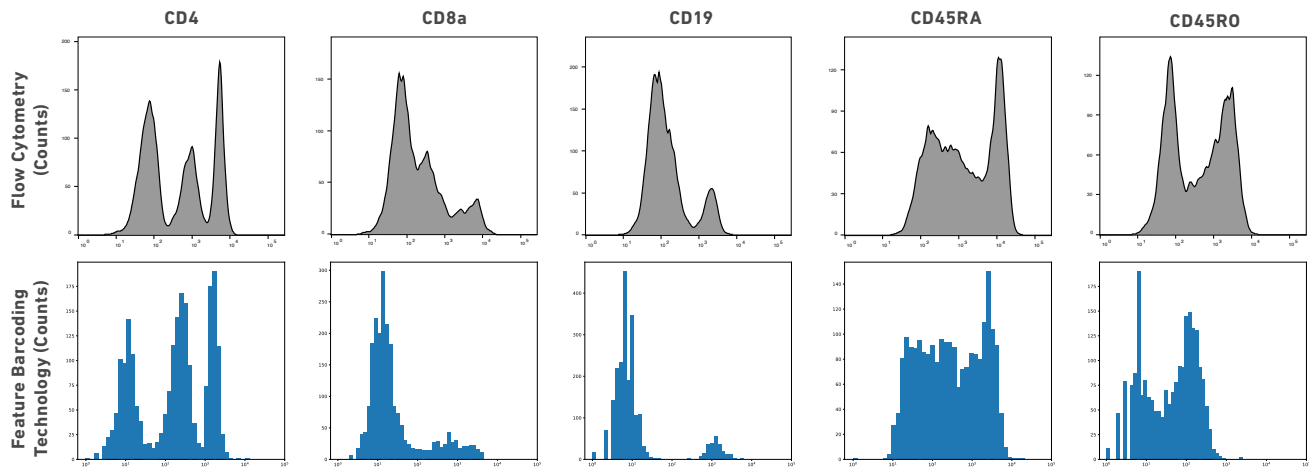


Figure 1. t-Distributed Stochastic Neighbor Embedding (tSNE) projections of ~8000 peripheral blood mononuclear cells (PBMCs), using the Single Cell Gene Expression Solution (A, B) and quantitative comparison between Feature Barcoding technology and flow cytometry, using the Single Cell Gene Expression Solution (C). For panels A and B, every dot is a single cell and cells are clustered together based on their gene expression and/or protein expression profiles. Cell annotation was performed manually by reviewing the highly expressed genes/proteins in each cluster and assigning a cell type based on the published literature. **A.** PBMCs are grouped together based on digital gene expression information. **B.** PBMCs were then grouped by cell surface protein expression profiles. The additional information from antibody unique molecular identifier (UMI) counts better resolved many canonical cell types compared to gene expression data alone (populations denoted with asterisk*). **C.** Flow cytometry and Feature Barcoding technology reveal similar cell populations when fluorescence intensity is compared with UMI counts per cell.

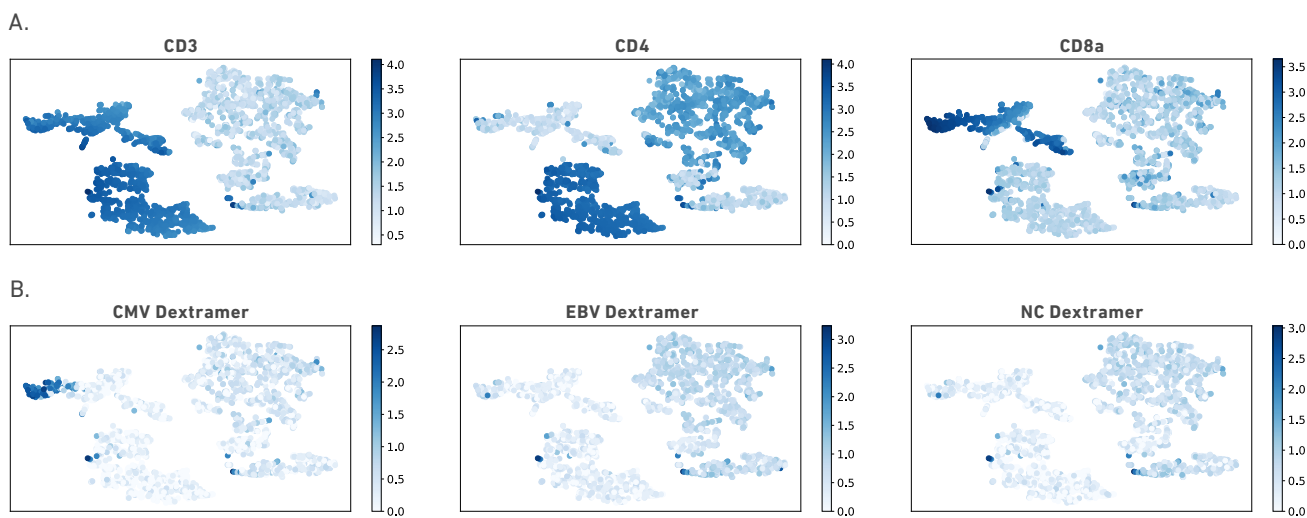
Determine Antigen Binding Specificities of T Cells Combined with TCR Sequences for a More Detailed Characterization of the Immune Response

The adaptive immune response is mediated by major histocompatibility complex (MHC) cell surface proteins presenting peptides to T cells. T cells are activated when a T-cell receptor (TCR) recognizes its target antigen, a peptide bound to a major histocompatibility complex (pMHC) on antigen-presenting cells, triggering multiple downstream activities that activate the T cell. In order to characterize adaptive immune responses to infections, autoimmunity, and cancer, it is necessary to measure multiple dimensions of T cell biology, including the transcriptional state, cell surface protein markers, and antigen specificity (TCR-pMHC interaction), and to link all of these phenotypes to the expressed TCR clonotype. Our Chromium Single Cell Immune Profiling Solution with Feature Barcoding technology for antigen specificity uses multiomics for a much higher throughput and higher resolution view of the behavior of immune cells than has previously been possible. This solution can be combined with existing flow cytometry workflows, enabling researchers to focus on the cells they are interested in and increasing the ability to detect rare clones.

Using the Chromium Single Cell Immune Profiling Solution with Feature Barcoding technology, we characterized a sample of PBMCs combined with cytomegalovirus positive (CMV+) T cells. Cells were labeled with a panel of DNA barcode conjugated cell surface protein antibodies along with dCODE™ Dextramers® displaying CMV, Epstein-Barr virus (EBV), and nonbinding control (NC) antigens. Cells were clustered using the cell surface protein expression dataset, revealing multiple distinct populations of T and B cells among other immune cell types (Figure 2A). Overlaying the antigen dataset on the clustered cells revealed a population of CD8+ T cells that bind specifically to Dextramers® displaying a CMV antigen (Figure 2B). We also assembled paired T-cell receptor clonotypes and identified an expanded clonotype among the CD8+ cells (Figure 2C).

Although for this example we show cell surface marker and antigen specificity information, it is important to note that we have retained gene expression and immune receptor data that can also be linked with these other cellular readouts (for more information about how this was done, including how flow cytometry was incorporated, read the [Redefining Cellular Phenotyping Application Note](#)). Thus, this solution provides an unparalleled approach for identifying the discrete cellular phenotypes that underlie immune receptor specificity and antigen binding capabilities, which is critical for developing a better understanding of the adaptive immune response and its relation to disease.

Figure 2



C.

	V	D	J	C
α	TRAV26-2	-	TRAJ43	TRAC
α	TRAV40	-	TRAJ53	TRAC
β	TRBV30	-	TRBJ2-4	TRBC2

Figure 2. CMV-negative PBMCs mixed with 2% CMV expanded T cells and labeled with a panel of cell surface protein-specific antibodies and dCODE™ Dextramers® displaying CMV, EBV, and nonbinding control (NC) antigens. Cell populations are clustered using tSNE projections based on surface protein expression. **A.** T cells are identified by CD3+, CD4+, and CD8+ expression. **B.** CD8+ T cells bind specifically to Dextramers® displaying a CMV antigen (left panel). The sample does not contain EBV-specific T cells, so the EBV Dextramer® staining is similar to that of the nonbinding Dextramer® control (middle and right panels). **C.** Paired T-cell receptor clonotypes are assembled for the T cells. The table outlines gene calls for the most prevalent TCR clonotype alpha and beta chains.

Unlock the True Diversity of the Immune Repertoire for the Identification of Functional Phenotype and Clonality

The specificity of adaptive immunity relies on a vast repertoire of T- and B-cell receptors generated through somatic recombination of multiple possible gene segments, which results in unique antigen receptor expression on a cell-by-cell basis. Our Chromium Single Cell Immune Profiling Solution can also be used to simultaneously profile gene expression and examine paired full-length receptor sequencing for unbiased clonotype analysis of T- and B-cell populations in individual cells. We used this solution to investigate the tumor microenvironment (TME) as well as the adaptive immune phenotype in tumor samples from a colorectal cancer (CRC) and a squamous cell non-small cell lung carcinoma (NSCLC).

In the first step of this analysis, we examined gene expression profiles in each sample to characterize the heterogeneous cell populations. Both tumors showed significant populations of immune cell types, such as tumor infiltrating lymphocytes (TILs), including T cells, B cells, and plasma cells (Figure 3). While the presence of these immune cell populations in the tumor could be indicative of an active immune response, without examining the lymphocyte receptor sequences it is difficult to obtain a true understanding of the adaptive immune response in these tumors.

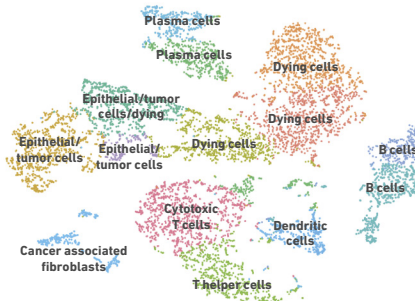
In the CRC tumor, the top T-cell clonotype displayed limited expansion (<1% of all T-cell clonotypes). However, when the Ig repertoire of the plasma cell cluster was examined, the dominant clonotype made up >4% of all B-cell clonotypes. Overlaying the clonotype Ig data on gene expression based cell cluster projections revealed that the dominant clonotype occurs within the plasma cell cluster (Figure 3C). This striking clonal expansion suggests that these plasma cells were generating tumor-specific antibodies.

In the NSCLC tumor, the most abundant Ig clonotype showed limited clonal expansion. The top paired clonotype contributed to just over 1% of all clonotypes and was not localized to a specific cluster. Examining the TCR sequencing data in conjunction with the gene expression data revealed that the top two clonotypes identified were cytotoxic T cells (CD8+), although limited expansion of these clones was seen (~1% of all clonotypes). Thus, we observed no evidence of a tumor-specific immune response in the lung cancer tumor.

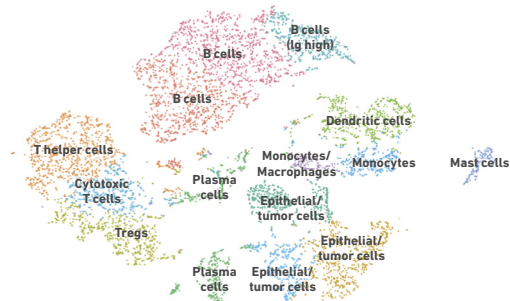
Using the Chromium Single Cell Immune Profiling Solution to combine gene expression and immune repertoire sequencing data for the same single cells, we were able to deduce both the functional phenotype of the immune cells that infiltrated the tumor and the clonality of these cells. While a large immune cell infiltrate was observed in gene expression data from both tumors, only by coupling this to immune cell clonotypes were we able to observe the extent of expansion and, by implication, the presence or absence of a tumor-specific immune response.

Figure 3

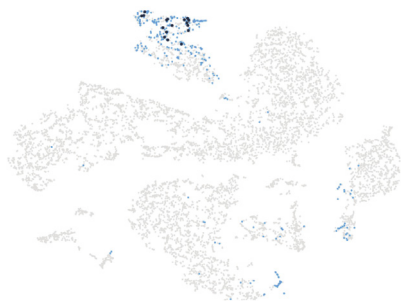
A. CRC Tumor



B. NSCLC Tumor



C.

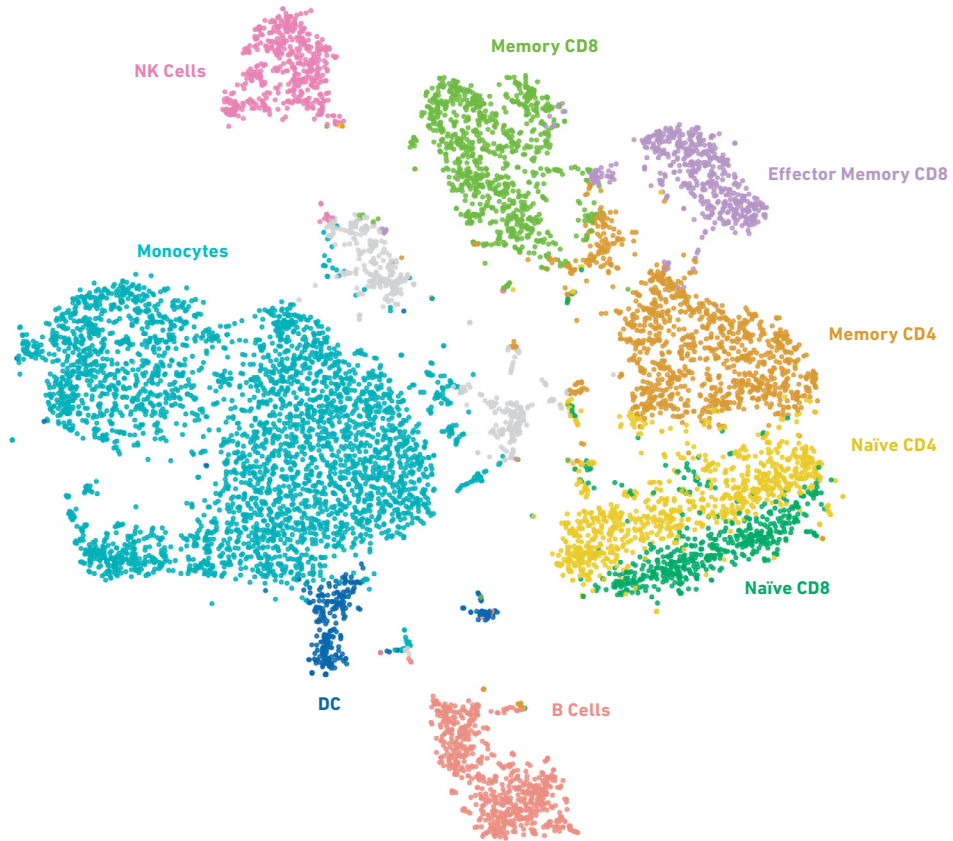


	V	D	J	C
H	IGHV1-3	IGH10R15-1A	IGHJ5	IGHA1
λ	IGLV2-14	-	IGLJ1	IGLC1

Figure 3. tSNE projections of cells output by Cell Ranger and visualized using the Loupe Cell Browser. Every dot is a single cell, and cells are clustered together based on their gene expression profiles. **A.** and **B.** Cell annotation was performed manually by reviewing the highly expressed genes in each cluster and assigning a cell type based on the published literature on cells from the CRC tumor (A) and NSCLC (B). **C.** (left) Overlay of gene expression and Ig clonotypes for the CRC sample. Light blue dots indicate an Ig clonotype call. Dark blue dots show the location of the most prevalent Ig clonotype in the plasma cell cluster. The table at the right outlines the gene calls for the heavy (H) and lambda light (λ) chain.

Figure 4

A.



B.

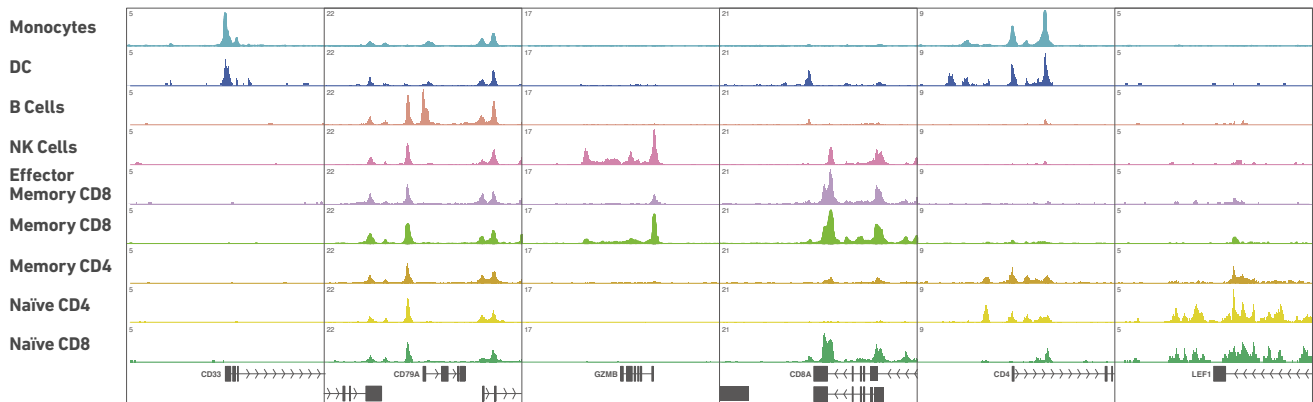


Figure 4. Heterogeneity in chromatin accessibility delineates cell types. A. Clustering of single nuclei accessibility profiles reveals nine cell groups in PBMCs. B. Plots show aggregate chromatin accessibility profiles for each cluster at several marker gene loci.

Understand the Impact of Epigenetics for Subtype Classification and Lineage Determination

While assaying RNA and protein expression at single cell resolution has improved the ability to classify cell types and measure cell-to-cell phenotypic variation, the underlying epigenetic changes that drive lineage commitment and regulate gene expression are still not well known. Understanding the epigenetic relationships between the various cell lineages that emerge during hematopoietic differentiation, as well as the molecular pathways that regulate gene expression during transitions between distinct lymphocyte activation states, is essential for understanding the immune response and developing new immune-therapeutic protocols (7). Recent innovations have enabled researchers to begin studying the epigenetic landscape at the single cell level by measuring cell-to-cell variations in the open chromatin landscape.

The Chromium Single Cell Assay for Transposase Accessible Chromatin (ATAC) Solution provides a robust approach to profile the chromatin landscape in hundreds to tens of thousands of cells in parallel. Using this solution, we profiled >9,000 nuclei from PBMCs and clustered the single nuclei accessibility profiles with Cell Ranger ATAC, our turnkey analysis software for analyzing single cell epigenetic data. This analysis identified nine distinct functional cell types and enabled clear distinction between effector, memory, and naïve T cells (Figure 4A).

Next, we examined chromatin accessibility at known marker loci by aggregating reads from all cells within a cluster and demonstrated that the accessibility of chromatin at each cell surface marker gene examined was associated with the cell type. For example, monocytes and dendritic cells showed open chromatin at the CD33 locus while all lymphoid lineage clusters shared a common pattern of open chromatin around the CD79A locus (Figure 4B). Importantly, chromatin accessibility at memory-associated loci, such as LEF1 (a lineage-determining transcription factor), and effector function loci, such as Gzmb (a serine protease), could be used to distinguish cell activation states within cell types. Thus, assaying chromatin accessibility at single cell resolution enabled us to dissect cellular heterogeneity and identify cell-type-specific gene regulatory patterns.

Although single cell epigenetic profiling techniques have only been available for a short time, they have already made a mark in immunology research. Using various single cell ATAC protocols, researchers have characterized leukemic and nonleukemic regulatory pathways in patient T cells, observed *cis* and *trans* regulators of naïve and memory T cell states, and identified epigenetic biomarkers for T cell exhaustion (8, 9). Most recently, researchers used the Chromium Single Cell ATAC Solution to profile chromatin in the development of human immune cells and T cell exhaustion in tumors (10).

Furthering the Study of Infectious Disease, Autoimmunity, and Cancer with Single Cell Sequencing Tools and Multiomics

Developing an extensive understanding of the immune system, both innate and adaptive, requires the proper tools to further dissect the complex orchestration of the immunological response to infections, autoimmunity, cancer, and other pathologies (11). Single cell sequencing technologies can be used to profile tissues and identify the molecular drivers of disease, study immune function, and identify developmental trajectories.

Methods such as the Single Cell Solutions from 10x Genomics are the only way to directly observe antigen binding to T cells while, at the same time, generating sequences of paired and recombined alpha and beta TCR genes, measuring gene and cell surface protein expression, and directly linking all of these outputs together. Single Cell ATAC will provide even more improvements to immune phenotyping and enable researchers to study the epigenetic pathways underlying allergies, autoimmune disorders, and immunotherapy resistance. The insights gained can be applied to identify functional states and discover neo-antigens, or, in therapeutic applications, to significantly impact the study of human health and disease.

RESOURCES

Chromium Single Cell Gene Expression
[10xgenomics.com/solutions/single-cell/](https://www.10xgenomics.com/solutions/single-cell/)

Chromium Single Cell Immune Profiling
[10xgenomics.com/solutions/vdj/](https://www.10xgenomics.com/solutions/vdj/)

Chromium Single Cell ATAC
[10xgenomics.com/solutions/single-cell-atac/](https://www.10xgenomics.com/solutions/single-cell-atac/)

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