The essential guide
Multiomic single cell immunology
# Table of contents

**Section 1**  
Introduction  

**Section 2**  
Explore what you can do with multiomic single cell immunology  
Tools for high resolution multiomic immunology  

**Section 3**  
Planning your experiment  
Getting started with data analysis  
Publications  
Resources from 10x Genomics
Section 1

Introduction

Immunologists have long relied on methods that provide analysis of individual immune cells to understand the immune system’s diversity and complexity. While technologies such as flow or mass cytometry have been pivotal for answering complex questions in immunology, continuing advances in the field will require the integration of multiomic single cell data.

Single cell multiomics for immunology provides access to multiple readouts at once for thousands to millions of single cells, giving you more from your single cell analyses. Imagine, instead of getting one measurement—protein expression—you could get everything at once: protein and gene expression, T- and B-cell receptor clonotypes, and antigen specificity; or coupled transcriptomic and epigenetic data. Instead of whittling down your markers of interest to a small panel that needs to be redesigned whenever new proteins are included, multiomic immunology lets you stain for all possible markers of interest at the same time, without the challenges of spectral overlap or compensation matrices. You can also combine immunophenotypic profiling and clonotype analysis with a readout of cell type and state for every cell. Single cell multiomic immunology means you can learn more from a single sample, without having to split it into parts, and improve reproducibility by profiling thousands of cells at a time and easily aggregating or comparing samples.
## Section 2

**Explore what you can do with multiomic single cell immunology**

Researchers have already started to answer a diverse set of immunological questions using single cell approaches. Here are some examples of immunology publications showing the unique ways these questions can be approached with single cell and spatial technology.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>From what progenitor are my cells derived?</td>
<td>Combine single cell gene expression analysis with lineage tracing</td>
<td>10, 36, 40</td>
</tr>
<tr>
<td>What cell types are responsible for disease?</td>
<td>Discover rare cell types, subtypes, and novel functions</td>
<td>24, 25, 27, 28, 81</td>
</tr>
<tr>
<td></td>
<td>Understand cellular interactions that contribute to disease and identify novel therapeutic targets</td>
<td>21, 60, 68, 75</td>
</tr>
<tr>
<td>What genes are important for immune activation?</td>
<td>Screen hundreds of genetic edits simultaneously with single cell CRISPR screens</td>
<td>14, 46, 47, 62</td>
</tr>
<tr>
<td>How rampant is an ongoing infection?</td>
<td>Capture host and pathogen RNA transcripts from the same cells</td>
<td>59, 60, 61, 66, 67</td>
</tr>
</tbody>
</table>
How do I understand the immune response to vaccination or infection in patients?

What dictates patient disease severity and response to therapy?

Measure vaccine response
Publications: 7, 64, 73, 74, 87, 89

Discover antibodies
Publications: 1, 4, 6, 8

Identify expanded T-cell or B-cell receptor clonotypes
Publications: 19, 22, 67

Identify immune factors that contribute to different disease response or prognosis
Publications: 71, 76, 77

Track patient responses to cell therapy
Publication: 53

Characterize immune cell activation state alongside clonotype and antigen binding specificity
Publications: 55, 63

Identify the gene regulatory networks that govern T-cell exhaustion
Publication: 45
Tools for high resolution multiomic immunology

Characterize complex cell populations by profiling hundreds of cell surface proteins along with gene expression and more, cell by cell, to gain an intricate picture of immunology and accelerate your discoveries with comprehensive solutions from 10x Genomics.

**Targeted Gene Expression**
Enrich sequencing libraries for your transcripts of interest using pre-designed or custom panels to focus on the genes that matter most.

**Single Cell Immune Profiling**
Get full-length, paired T- and B-cell receptor sequences, so you can match clonotype expansion to cell type and state, and generate functional receptors or antibodies for in vitro testing.

**Multiomic Cytometry**
Turn a next-generation sequencer into an ultra-high parameter cytometric detector to profile hundreds of protein markers at once, with optional transcriptome, T- and B-cell receptor sequences, or antigen specificity on top.

**Single Cell ATAC**
Survey the physical structure of the genome by identifying regions of open chromatin to reveal areas of active gene transcription, regulatory regions, and binding site motifs.

**Single Cell Multiome ATAC + Gene Expression**
Profile both the transcriptome and epigenome simultaneously in the same single cells, enabling deeper characterization of gene regulatory networks and cell state.

**Single Cell CRISPR Screening**
Directly assess CRISPR-driven gene edits or knockdowns and resulting gene expression phenotypes cell by cell to dissect molecular underpinnings of human immunity.

**Single Cell Gene Expression**
Obtain a digital readout of gene expression levels that lends insight into cellular heterogeneity, diversity, development, function, and response to external stimuli.

**Immune Receptor Mapping**
Characterize antigen specificity and phenotype with protein markers and/or gene expression to track vaccine response and accelerate antibody discovery.

**Spatial Transcriptomics and Proteomics**
Visium Spatial Gene Expression and Spatial Proteomics provide transcriptome and protein readouts from intact tissue sections to complement or extend your single cell analyses.

= mRNA  = CRISPR perturbation  = Chromatin accessibility
= T-cell receptor  = Cell surface protein
Depending on your biological question, different single cell approaches may be desired. If you want to study vaccine response or identify novel antibodies, Single Cell Immune Receptor Mapping can provide functional clonotype information and antigen binding specificity. For comprehensive phenotyping by gene expression and protein, Multiomic Cytometry may be your best choice. When you need to understand gene regulatory networks, Single Cell ATAC or Single Cell Multiome ATAC + Gene Expression can generate the data you require. Refer to the Tools for high resolution multiomic immunology section for a full list of single cell applications from 10x Genomics, or explore the product pages at 10xgenomics.com.

Sample type & preparation
It is critical that you obtain a clean single cell suspension free of cell debris, with minimal cell aggregates and high viability (>70%). It is also important to know the size range of the cells studied. The cell size is usually correlated with the number of transcripts expressed in the cell. A wide range of cell sizes (up to 30 μm) are compatible with Chromium Single Cell Next GEM Chips. In general, cell preparation protocols will vary depending on the tissue’s origin and the cell types studied. Each tissue type is unique, and thus, it is critical to optimize sample preparation before starting any single cell experiment. We recommend starting by reviewing our Cell Preparation Guide. Find more answers to common questions about sample prep on our Sample Prep FAQs page. We also have sample prep–focused webinars available for viewing in our Videos library.

Sample processing
The ability to process samples quickly after isolation or tissue dissociation is critical in maintaining cell integrity and preserving each cell’s transcriptome. Be aware that any sample manipulations may adversely affect gene expression profiles, cell states, or cell viability and introduce bias into the study (Van Den Brink et al., 2017).
Cell enrichment
When characterizing rare cell populations, for applications such as immune receptor mapping or antibody discovery, enriching for cells of interest prior to generating single cell partitions can help ensure adequate numbers of your cells of interest. The Chromium system is compatible with FACS and bead- or column-based enrichment methods.

Cells versus nuclei
While some applications, such as Multiomic Cytometry or Immune Receptor Mapping, require a single cell suspension as input, others, including Single Cell ATAC and Single Cell Multiome ATAC + Gene Expression, require nuclei isolation. Cells are recommended for Single Cell Immune Profiling, but either cells or nuclei can be used for gene expression analysis with Single Cell Gene Expression.

Available sample types can also dictate whether cells or nuclei must be used. For frozen tissue or archival samples, nuclei must be isolated directly. Alternatively, tissue can be dissociated and cryopreserved immediately after it is received, enabling cells to be stored long term.

Species compatibility
Single cell gene expression products from 10x Genomics have been used successfully on a wide range of organisms. The capability to extend single cell gene expression analysis to other species may depend on the quality of the available reference annotation. Similarly, measuring cell surface proteins in other species requires effective antibody reagents with oligonucleotide conjugations. Targeted Gene Expression pre-designed or custom panels are provided only for human genes, although a small number of exogenous sequences, such as reporter genes, can be added. Single Cell Immune Profiling provides ready-made primers for amplification of human or mouse T- or B-cell receptor transcripts, but experimental design is flexible and other species have successfully been clonotyped using custom primers (L Goldstein et al., 2019).

Number of cells
Deciding on the number of cells required depends on the expected cellular heterogeneity in the sample, the number of cells available, the minimum frequency expected of desired subpopulations, and the minimum number of cells of each cell type desired for data analysis (see online tool: satijalab.org/howmanycells). If the sample diversity is not known, a high number of cells at low sequencing depth may be the most flexible option to obtain a representative proportion of the cell population and meaningful biological information. Often, greater cell number, rather than sequencing depth, improves cell classification ability (J Ding et al., 2020). The Chromium system can recover up to ~65% of the cells loaded with a low doublet rate (0.9% per 1000 cells). For highly heterogeneous samples, thousands of cells may be required to fully resolve each subpopulation. However, the high cell recovery rate also makes Chromium suitable for samples with limited cell numbers.

Number of replicates
Determining the number of replicates depends on the research project, the type of sample, and the number of cells required in the study. The matter of biological replicates is still an open question in the field. In some studies, one sample alone can be seen as sufficient—with each cell representing a biological replicate, and different samples from different individuals accounting for the variability of a particular biological process. In other studies, to mitigate biological variability occurring in small cell populations across time, it can be beneficial to computationally aggregate cells from different samples to cover all aspects of the cell population being studied. Other cases may require the use of multiple replicates derived from a single sample to increase the total number of cells in the study.
Batch effects
Batch effects can be introduced at any stage of the workflow and are primarily due to logistical constraints that result in different preparation times, operators, and handling protocols. The 10x Genomics Chromium system demonstrates minimal technical variability across a variety of technical replicates. When combining data from multiple libraries, we recommend equalizing the sequencing read depth (depth normalization) between libraries before computationally merging to reduce batch effects introduced by sequencing. In addition, a number of computational tools including Seurat, scran, and scrone can correct batch effects. Cell Ranger can also perform batch correction for gene expression libraries.

Sequencing depth
The sequencing depth per experiment for gene expression libraries is dependent on total mRNA content in individual cells, and the diversity of mRNA species. In general, at the same transcript diversity, cells expressing a low amount of mRNA will require less sequencing depth than cells expressing a large amount of mRNA. When sequencing cost or capacity is limiting, there is often a trade-off between sequencing a higher number of cells versus sequencing a lower number of cells with more reads, or breadth versus depth. Single cell libraries for T- or B-cell receptor sequence, antigen specificity, protein markers, and CRISPR guides require less sequencing depth (minimum 5,000 read pairs per cell) than single cell ATAC (minimum 25,000 read pairs per cell) or single cell gene expression libraries (minimum 20,000 read pairs per cell).

10x Genomics single cell libraries are compatible with short-read sequencers and available in a dual indexing configuration. Our single cell gene expression workflows use unique molecular identifiers (UMIs) to barcode each transcript molecule before amplification takes place, resulting in a digital gene expression profile while accounting for PCR amplification bias.
Getting started with data analysis

Multiomic immunology solutions from 10x Genomics come with intuitive software for data analysis and visualization.

Simultaneously measure single cell modalities

<table>
<thead>
<tr>
<th>Gene expression</th>
<th>Full-length, paired V(D)J sequence</th>
<th>Chromatin accessibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell surface protein</td>
<td>Antigen specificity</td>
<td>CRISPR perturbation</td>
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</tbody>
</table>

Go from DNA sequence to immunophenotype with a single software package

Cell Ranger is our suite of analysis pipelines that turn your raw sequencing data into results.

Cell Ranger provides a count matrix consisting of columns for every cell barcode and rows for all measured features, including genes for transcriptome analysis, protein markers, and bound antigens or pMHC multimers.

When applicable, Cell Ranger will also perform assembly and annotation of supported immune repertoire clonotypes, including full-length, paired V, D, and J sequences.

Interactively explore your multiomic data

Loupe Browser lets you visualize and explore your results with point-and-click accessibility to:
- Enable manual annotation of cell clusters
- Import and overlay clonotype information
- Re-cluster cells based on alternative features such as protein markers

Take your analysis further with third-party tools

<table>
<thead>
<tr>
<th>Gene and protein expression data</th>
<th>Immune repertoire data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seurat, Scanpy, Bioconductor</td>
<td>Immunarch, VDJ tools, scRepertoire</td>
</tr>
</tbody>
</table>

Working with a bioinformatician:

For some questions, it may be helpful to work with a bioinformatician specializing in single cell sequencing data. Sharing some biological context can help them zero in on data most relevant to your research question. For example, how many different cell types are expected? Were cells sorted before sequencing, so that one cell type should dominate the population? How many cells were targeted, and at what sequencing depth? Of course, specific data analysis questions can also be directed to the expert 10x Genomics Software Support team at support@10xgenomics.com.
Publications

From immune cell discovery to therapeutic development, there are already a wealth of publications highlighting the power of single cell immunology that can help guide your own investigations.

**Antibody discovery**


**Assay method**

Autoimmunity


Cell atlas


Immunobiology


42. G Mollaoglu et al., The Lineage-Defining Transcription Factors SOX2 and NKX2-1 Determine Lung Cancer Cell Fate and Shape the Tumor Immune Microenvironment. *Immunity.* 49, 764–779.e9 (2018).


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**Immunotherapies**


61. V Cortez et al., Astrovirus infects actively secreting goblet cells and alters the gut mucus barrier. Nat Commun. 11, 2097 (2020).


66. R León-Rivera et al., Interactions of Monocytes, HIV, and ART Identified by an Innovative scRNAseq Pipeline: Pathways to Reservoirs and HIV-Associated Comorbidities. mBio. 11, e01037-20 (2020).


68. E Park et al., Toxoplasma gondii infection drives conversion of NK cells into ILC1-like cells. eLife. 8, e47605 (2019).


74. AT Waickman et al., Transcriptional and clonal characterization of B cell plasmablast diversity following primary and secondary natural DENV infection. EBioMedicine 54, 102733 (2020).

75. W Wen et al., Immune cell profiling of COVID-19 patients in the recovery stage by single-cell sequencing. Cell Discov. 6, 31 (2020).


**Inflammation and allergies**


**Transplant**


**Vaccine development**

87. PS Arunachalam et al., T cell-inducing vaccine durably prevents mucosal SHIV infection even with lower neutralizing antibody titers. *Nat Med*. 26, 932–940 (2020).


Resources from 10x Genomics

Application note
Discover how 10x Genomics technology is “Redefining Cellular Phenotyping” with this in-depth study of clonal expansion and antigen binding after viral infection.
Learn more →

Research snapshots
Explore how immunologists are leveraging single cell technologies to answer diverse questions in immunology.
Learn more →

Support
Visit the support site for documentation, software, and datasets that will help you get the most out of your 10x Genomics products.
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10x Genomics compatible products
Access our list of key partner products that have been certified compatible to work with our various solutions.
Learn more →

Solutions and products
Along with our suite of complete solutions, we offer an ever-growing catalogue of services to help you find the answers to your research questions.
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10x Blog
Keep up to date with the 10x Genomics Blog, where you’ll find everything from tips and tricks to the latest 10x news.
Learn more →