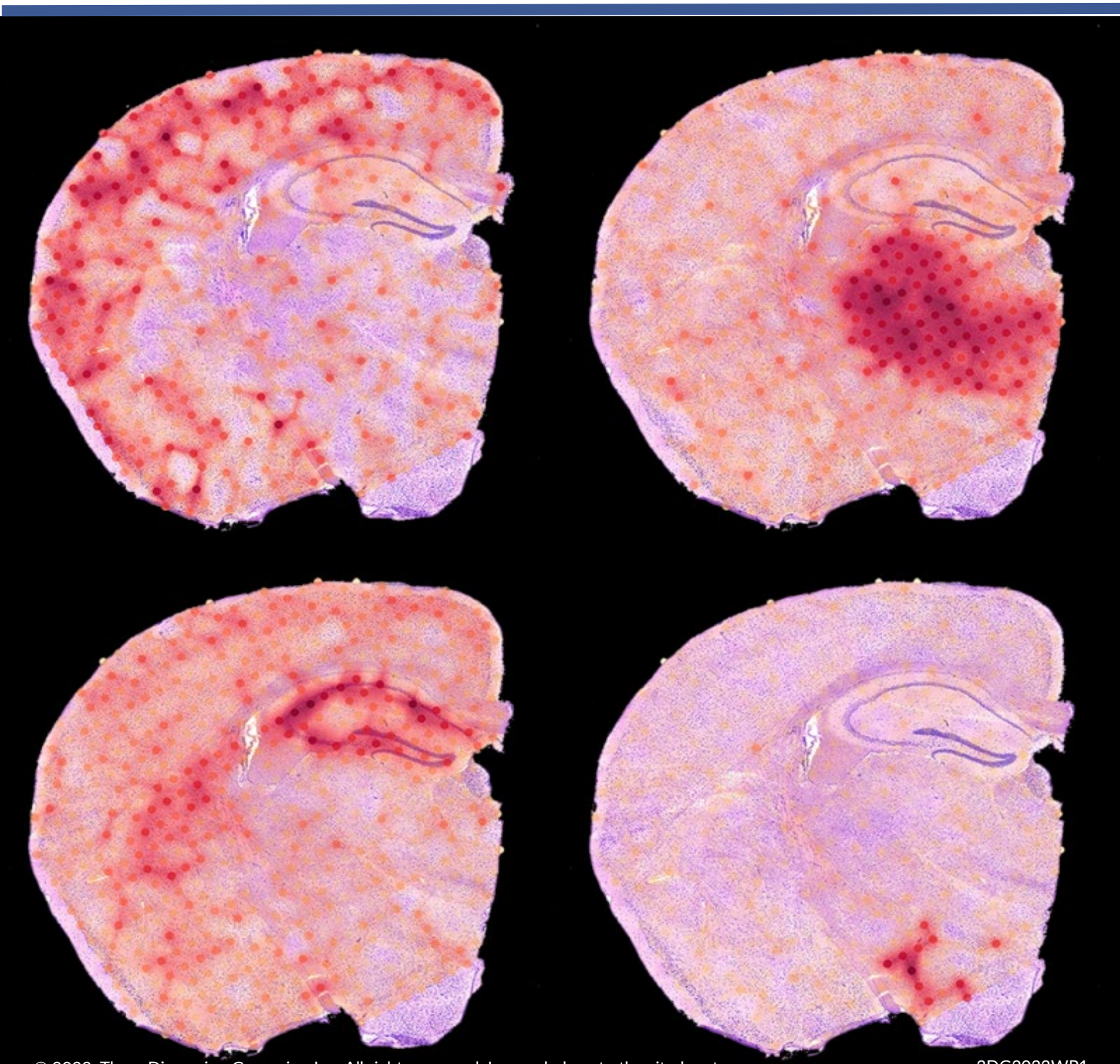
Understanding complex tissue functioning and its role in neurological disorders:

Applications of spatial transcriptomics and single-cell RNAseq



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Complex cell types make up the microenvironment surrounding tissues affected by neurological disease. Understanding how these cells function across the tissue can help provide insights into new therapeutic targets. This white paper is an overview of the challenges facing the biotech and pharmaceutical field in creating new therapeutics to combat neurological disorders, and the application of single-cell RNAseq and spatial transcriptomics to advance these efforts.

Introduction

A notable opportunity exists in discovering new pharmaceuticals for neurological disorders. Over the last 25 years big pharma has shifted focus away from neurological disorders to other fields such as heart disease and cancer. However, with new technologies like spatial transcriptomics and single-cell RNA-seq, new information and understanding of the mechanism and anatomical makeup of the microenvironments involved with these diseases are being discovered. This, along with the large unmet need of those afflicted by neurological disorders has motivated biotech and pharmaceutical companies to revisit drug discovery opportunities in neuroscience. In 2018, neuroscience received the second highest amount of investment dollars ($1.5 billion) just behind cancer research (2019 BIO Industry Analysis).

Brain tissue is highly complex in architecture with a diversity of cell types (133 cell types identified in Tasic et al., 2018) that are highly interconnected. When it comes to brain disorders, multiple cell types have been shown to be altered. These affected cell types can be further categorized into two classes, (1) primary and (2) secondary or “reactive” cell types. Primary cell types associated with primary disease pathology express gene variants/mutations most likely to develop the disease. Secondary or “reactive” cell types are cells that undergo change as a response to the primary cell type due to the interconnectedness of brain tissue. There is a lot of overlap in primary cell types as well as secondary cell types across diseases (Table 1).

TABLE 1 ORGANIZATION OF CELL TYPES AFFECTED FOR DIFFERENT NEUROLOGICAL DISEASES WITH RESOURCE INFORMATION.

Diseases	Cell types affected	Resources
Schizophrenia	pyramidal neurons; endothelial	Sabri et al., 1997; Ohnishi et al., 2000; Kindler et al., 2013
Autism	pyramidal neurons; endothelial	Skene and Grant 2016; Sabri et al., 1997; Ohnishi et al., 2000; Kindler et al., 2013
Alzheimer’s	microglia, astrocytes, and oligodendrocytes	Skene and Grant 2016; Mhatre et al.,2015
Multiple Sclerosis	Microglia, astrocytes, and oligodendrocytes	Skene and Grant 2016; Hemmer et al.,2015; Schirmer et al., 2019
Seizures	interneurons; astrocytes and oligodendrocytes	Skene and Grant 2016; Ogiwara et al.,2007; Hammad et al.,2014; Uhlmann et al.,2002
Parkinson's	cholinergic, monoaminergic, and enteric neurons; oligodendrocytes	Bryois et al., 2020

Understanding how these two classes of cell types interact and influence one another is important in understanding the mechanisms and the progression of the pathology. Key scientific challenges identified for the field of translational neurological medicine include mechanisms of disease, target identification and validation, predictive models, and biomarkers for patient stratification (Pankevich et al., 2015).

Identifying cell types

Single-cell RNA sequencing (scRNA-seq) allows gene expression profiling of individual cells. This technique has greatly advanced the ability to identify cell subpopulations within a given organ and reveal the heterogeneity of cell types in complex tissues like the brain. However, a necessary step to scRNA-seq is the dissociation of tissue to isolate individual cells. Tissue dissociation destroys the spatial/proximity information needed to understand cell interactions within a tissue, and is a key challenge in developing novel therapeutics for neurological disorders.

To overcome the spatial data limitations of scRNA-seq, spatial transcriptomics adds barcode sequences to mRNAs from small areas of tissue sections to map transcripts back to specific locations within the tissue. The use of spatial transcriptomics in neurological disorder studies has provided new insights addressing key challenges in the field including mechanisms of disease and identifying new therapeutic targets (Longo et al., 2021).

3D Genomics solutions

At Three Dimension Genomics, we use 10X Genomics' Visium platform for spatial transcriptomics (see **Visium technology**). This approach is at the cutting edge of ST technologies and has key advantages over earlier methods:

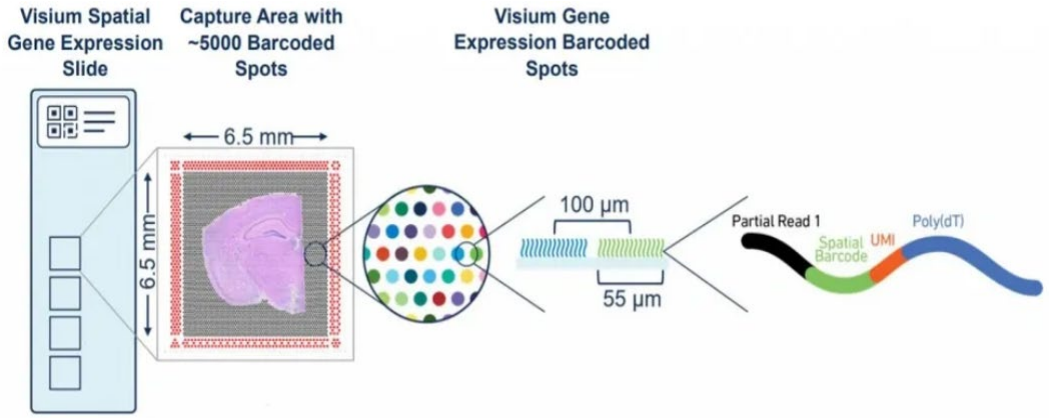
- **Tissue compatibility –** Visium is compatible with both fresh-frozen and formaldehyde-fixed, paraffin-embedded (FFPE) tissues
- **High resolution –** 55µm barcode spots enable 1-10 cell resolution depending on tissue type (HD Visium coming soon?)
- **Process entire sections –** while earlier microdissection techniques required the selection of regions of interest, 10X Visium enables profiling of entire sections

In dense tissues, **individual barcoded spots may contain multiple cells** and thus do not match the single-cell resolution of scRNA-seq approaches. To address this, signals from multiple cells within a spot can be deconvoluted into constituent cell type transcriptomes by combining with a single-cell or single-nuclei transcriptome dataset. This **multi-omic data integration** approach provides a powerful way to limit the weaknesses of any individual approach.

Visium technology

The **Visium platform** from 10X Genomics utilizes a spatial barcoding approach to map transcriptomic data to regions of tissue. A Visium Spatial Gene Expression slide has 4 captures areas that each span **6.5mm x 6.5 mm** and hold **~5,000 barcoded spots**. Since an individual spot is **55µm**, you can expect to capture between 1-10 cells per spot depending on the tissue type.

Tissue is mounted onto the capture area and imaged prior to mRNA extraction. Barcoded sequencing data can then be linked back to the specific region of tissue the mRNA originated from, providing a spatial map of gene expression across the full tissue section.



Tackling key scientific challenges in developing new therapeutics

As previously mentioned, several key scientific challenges exist in order to move the field forward and towards new therapeutics. At the core of these developments is a need for a better understanding of the disease itself or the mechanisms of disease. Having a full understanding of the mechanism and pathways by which the disease spreads allows for therapeutic targets to be identified. Although scRNA-seq had become a useful tool in discovering the individual cell types involved with each neurological disease, it wasn't until spatial transcriptomics was introduced that primary, secondary, and pathways of interactions between these cells could really be understood.

Case Studies

The following studies highlight some use cases in which scRNA-seq paired with ST has been used to address such key challenges. The first case study is focused on Alzheimer's disease studied in human brain tissue and the second case study is a great example of the power of using ST across time points in Amyotrophic lateral sclerosis (ALS) using spinal cord subsections in mice and humans.

Chen et al., 2021: Understanding Mechanisms of Disease in Alheimer's Disease

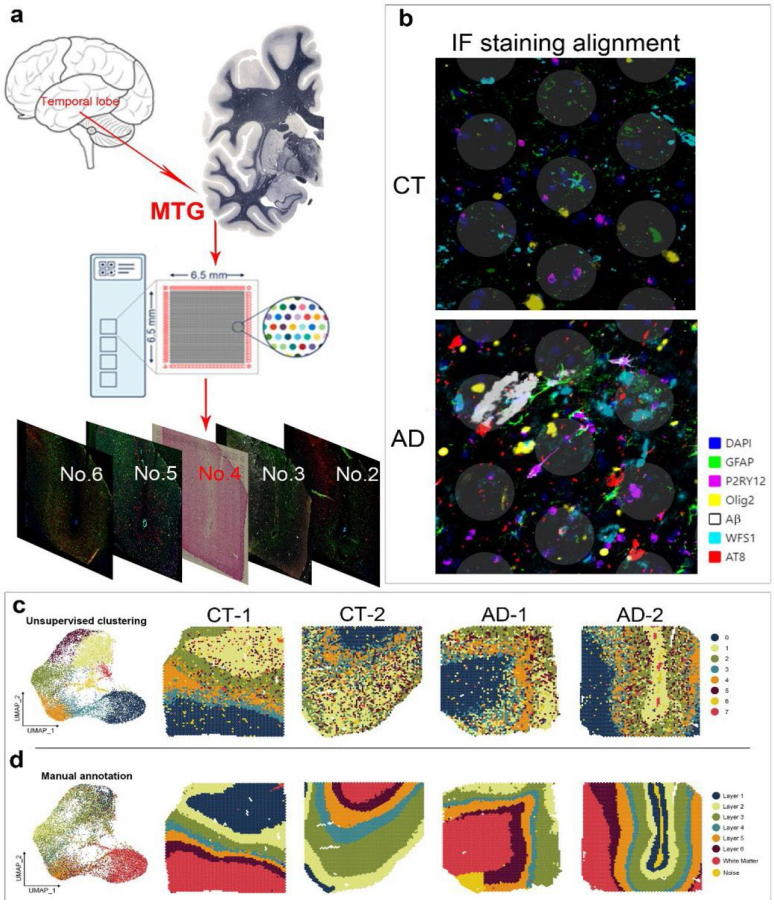


Figure 1 Source: Chen et al., 2021: Caption: Fig. 1. Spatial transcriptomics (ST) of the human middle temporal gyrus (MTG) (**a**) Sequential 10 µm sections of control (CT) and Alzheimer (AD) MTG brain regions were used for all experiments. The middle section (No.4) was used for ST on the Visium platform, and the four adjacent sections (No.2, 3, 5, 6) were used for immunofluorescence (IF) staining. (**b**) Alignment of ST with H&E staining, IF staining of nuclei (DAPI, blue) and cell-type specific markers (GFAP (green), P2RY12 (purple), Olig2 (yellow), WFS1 (teal)) and AD pathological hallmarks (Aβ plaques (white) and AT8+ pathological tau (red)). (**c**) Uniform manifold approximation and projection (UMAP) plots show eight clusters (0-7) were identified by the Seurat integration framework using ST spots from both control (CT-1, CT-2) and AD (AD-1, AD-2) human MTG. Spatial maps of the eight clusters for each individual sample from unsupervised clustering. (**d**) Manual annotation of six cortical layers and the adjacent white matter. The left panel figure shows the manually labeled spots (six cortical layers and the white matter) on UMAP space based on the Seurat integration framework. The right panel of the spatial maps show the localization of the manually labeled spots for each individual sample

In Chen et al. (2021), researchers used the 10X Genomics Visium platform in combination with co-immunofluorescence staining of disease associated markers to define the spatial gene expression of the middle temporal gyrus (MTG) of early Alzheimer's Disease (AD) and control groups.

▪ Through the application of Visium in human MTG for AD and control cases, the researchers were able to identify differentially expressed genes (DEGs) and gene clusters that corresponded to different anatomical layers of the human MTG (Figure 1). They identified novel marker genes for unique layers and the white matter which was validated by ISH data from the Allen Institute for Brain Science and by comparing their results to publicly available Visium data of the human frontal cortex. By finding these new layer-enriched marker genes, a better understanding of the anatomical structure of the

cortex is being developed and this also helps to determine layer-specific cellular vulnerability of AD pathology.

Cell types were identified using scRNA-seq and RNAscope smFISH in which DEGs specific to each cell type were identified. Co-expression patterns of neuron- and glia-related gene modules were found altered in AD pathology tissues which supports the hypothesis that interactions between neurons and glia are involved in AD pathogenesis. Overall, new gene signatures and pathways specific to AD pathology and the interactions between neurons and glia in early AD were brought to light in this study and suggest new targets for therapeutics.

Maniatis et al., 2019: Target identification and validation for new therapeutics

Amyotrophic lateral sclerosis (ALS) is a progressive disease whereby interactions between neurons and glia cell types contribute to motor neuron loss eventually leading to paralysis. Maniatis et al. (2019) employed the use of ST and scRNA-seq to investigate the spatiotemporal order of molecular events that contribute to the pathway of ALS and identify new therapeutic targets.

In this case study, mouse lumbar spinal cord tissue from ALS induced and control animals were sampled at 4 key stages of ALS: presymptomatic, onset, symptomatic, and end-stage time points. 80 lumbar sections from seven postmortem ALS human subjects were also analyzed. Initial data and findings suggested that microglial dysfunction was occurring well before symptom onset, preceded astroglial dysfunction, and was proximal to motor neurons.

In this study, the Visium analysis and immunofluorescence imaging (IF) showed that genes involved in autophagy were dysregulated in the spinal cord of ALS mice. The authors conducted an unbiased coexpression analysis of the mouse Visium data and found 31 major coexpression modules which they further grouped into submodules based on cell-type. They identified that the pathway activities encompassed by module 8 were responsible for the signing within and between cell types during early glial activation in intact tissue and these pathways were responsible for the maintenance and spread of the reactive phenotype in the ALS mouse model.

The authors then applied this same approach to cervical and lumbar tissue samples from seven postmortem human ALS patients. Four of these patients presented clinically with bulbar symptom onset and three with lower limb symptom onset. Here, they identified 28 human modules of gene pathways, some of which overlapped with the mouse model. The spatial mapping patterns revealed human expression in module 3 was attenuated across spinal cord sections at sites proximal to symptom onset (Figure 2 panel C) with the most pronounced expression occurring in the posterior white matter and anterior horns. KEGG analysis showed that human module 3 was enriched for several pathways including sphingolipid signaling. In previous studies, sphingolipid signaling has been proposed as a potential therapeutic for ALS by the improvement of disease in murine models of ALS (Dodge et al., 2015; Denic et al., 2015). By using Visium and scRNA-seq data in this study, the authors were able to detail the dynamics of sphingolipid signaling in multiple cell types, spinal cord regions, and disease stages, revealing a promising target for new therapeutics. Due to

the spatiotemporal nature of these techniques, new treatment strategies modulating the target activity of these pathways can be suggested.

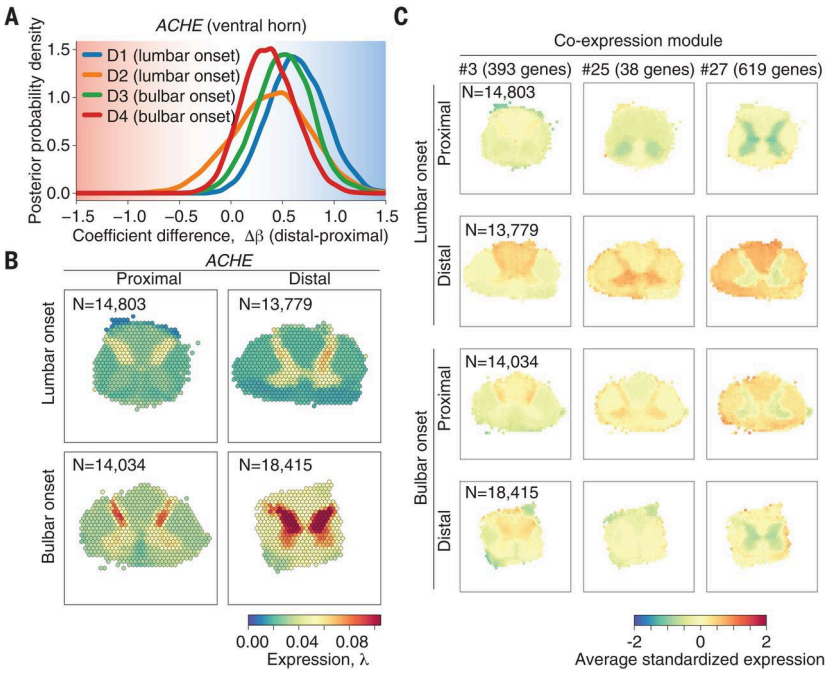


Figure 2 Source: Maniatis et al. 2019: Caption: Fig. 4 Spatiotemporal transcriptome of human postmortem spinal cord tissue from ALS patients. (**A**) The posterior difference distributions of the ventral horn coefficients for *ACHE* per patient (D1 to D4). Differences are calculated between distal and proximal regions with respect to the onset location. (**B**) Spatial mRNA expression of *ACHE* in human postmortem lumbar and cervical spinal cord. (**C**) Average spatiotemporal expression dynamics for human coexpression modules 3, 25, and 27 are visualized.

Summary

The central nervous system is complex and disorders involving this system have been found to have a lot of overlap in cell types. This creates a challenge in developing specific therapies for neurological disorders. Therefore both **cellular content** and **spatial organization** need to be studied to distinguish differences between different diseases. The use of scRNA-seq and ST show promise in helping scientists tackle the biggest challenges facing the field and development of new therapeutics.

About 3D Genomics

We are a group of Ph.D. scientists dedicated to providing the **highest quality research services** to help you in your biomedical and pharmaceutical research projects. We believe that every experiment is unique and deserves the **best custom research,** from design to execution to data analysis.

We specialize in providing **end-to-end services for single-cell multiomics**. We typically receive tissue samples for spatial projects and return transcriptome data with cluster analyses, H&E images, unstained frozen section slides, or images from fluorescent antibody imaging of client-specified antigens. We can isolate nuclei from tissues for single-cell RNAseq and integrate the transcriptomes with spatial transcriptomes from serial sections.

Areas of expertise

Single-cell multiomics technologies

- Spatial transcriptomics (10X Visium, typically integrated with 10X Genomics single nuclei RNAseq)
- Single-cell transcriptomics with large and small numbers of cells (10X Genomics and SmartSeq, respectively).
- Single-nuclei RNAseq from fresh-frozen and FFPE embedded tissues.
- IHC imaging (widefield/confocal, chromogenic/fluorescent)
- High Content Imaging (Cell Painting, HCS)
- Single-cell immune repertoire analysis, B-cell/T-cell clonotype studies, with cell type isolation by FACS and MACS.

Bulk cell technologies

- 3D organoid models for gene expression and drug response studies in multiple therapeutic areas using patient-derived and control cell lines
- Custom cell-based assay development and compound studies with multiple modality readouts.
- Capillary westerns (ProteinSimple)

Tissues for spatial transcriptomics

- Brain, kidney, lung, heart, etc.
- FFPE samples coming soon

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