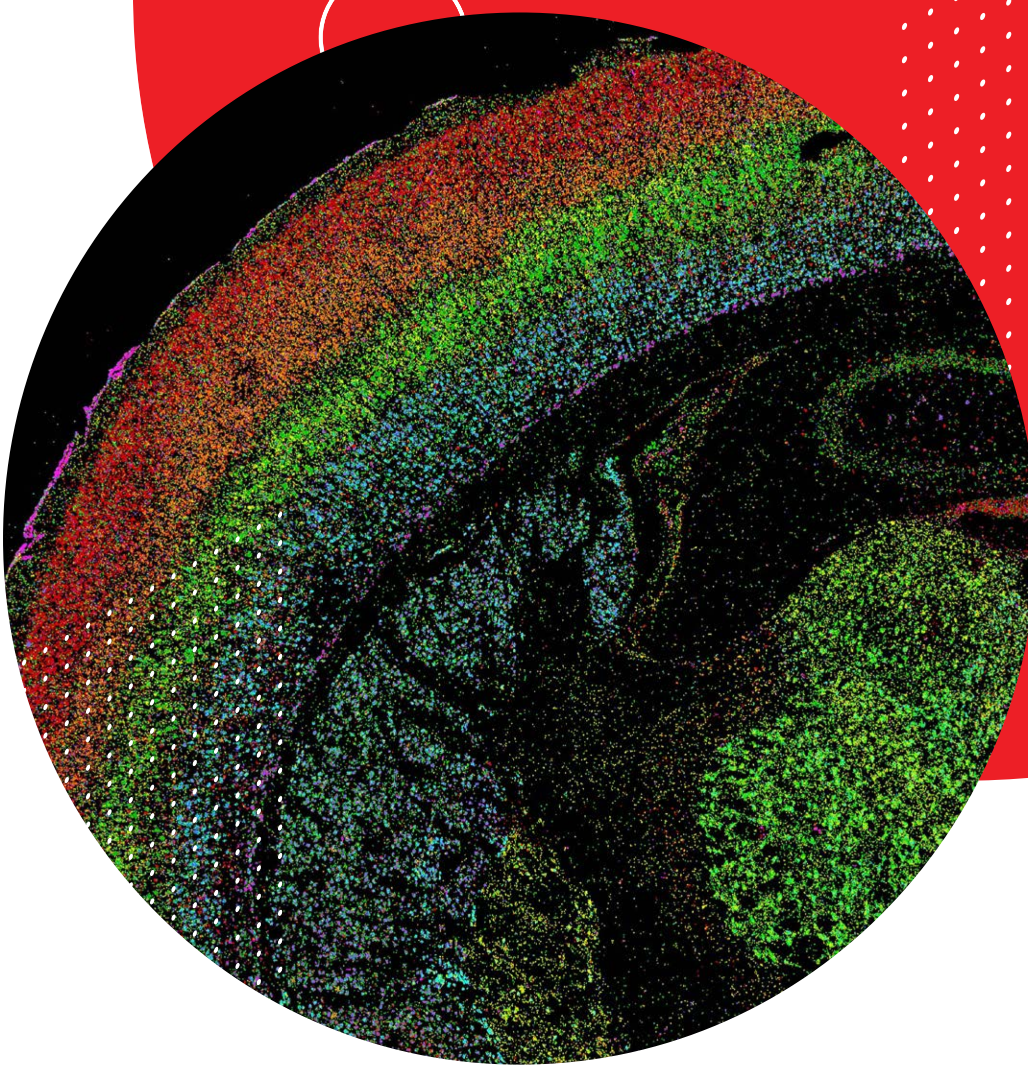
UNRAVELING THE CELLULAR AND SUBCELLULAR LANDSCAPE USING SPATIAL BIOLOGY



Sponsored By

resolve biosciences


Location Matters
Spatial biology allows you to visualize specific genes and cell types involved with disease progression.

UNDERSTANDING SPATIAL BIOLOGY

How genes control the generation, shape, position, and function of individual cells and facilitate cell interactions is a perennial question in biology. Constructing atlases of the location, timing, and level of gene expression in specific cell types unveils biological pathways in development and disease. Single-cell transcriptomics provides a quantitative evaluation of thousands of transcripts expressed in a cell, but omits their spatial context. Researchers have now developed numerous techniques to incorporate spatial information in gene expression profiles. These spatial transcriptomics techniques facilitate the visualization of gene expression patterns *in situ*.

> Spatial transcriptomics approaches that resolve gene expression patterns *in situ* are classified into microdissection, *in situ* hybridization, *in situ* sequencing, and *in situ* capturing methods.

Approaches in Spatial Transcriptomics

Spatial transcriptomics is a rapidly growing field that combines cutting-edge methods in tissue segmentation, imaging, and single-cell RNA sequencing (scRNAseq) with powerful computational algorithms. Together, these techniques generate comprehensive, spatial gene expression profiles in a range of tissue samples. Methods that allow the assessment of gene-expression profiles at known tissue locations can be classified into four approaches: microdissection, *in situ* hybridization, *in situ* sequencing, and *in situ* capturing.[1] These methods allow scientists to discover previously unknown genes in biological processes and reveal new cellular identities; however, each has unique advantages and shortcomings.

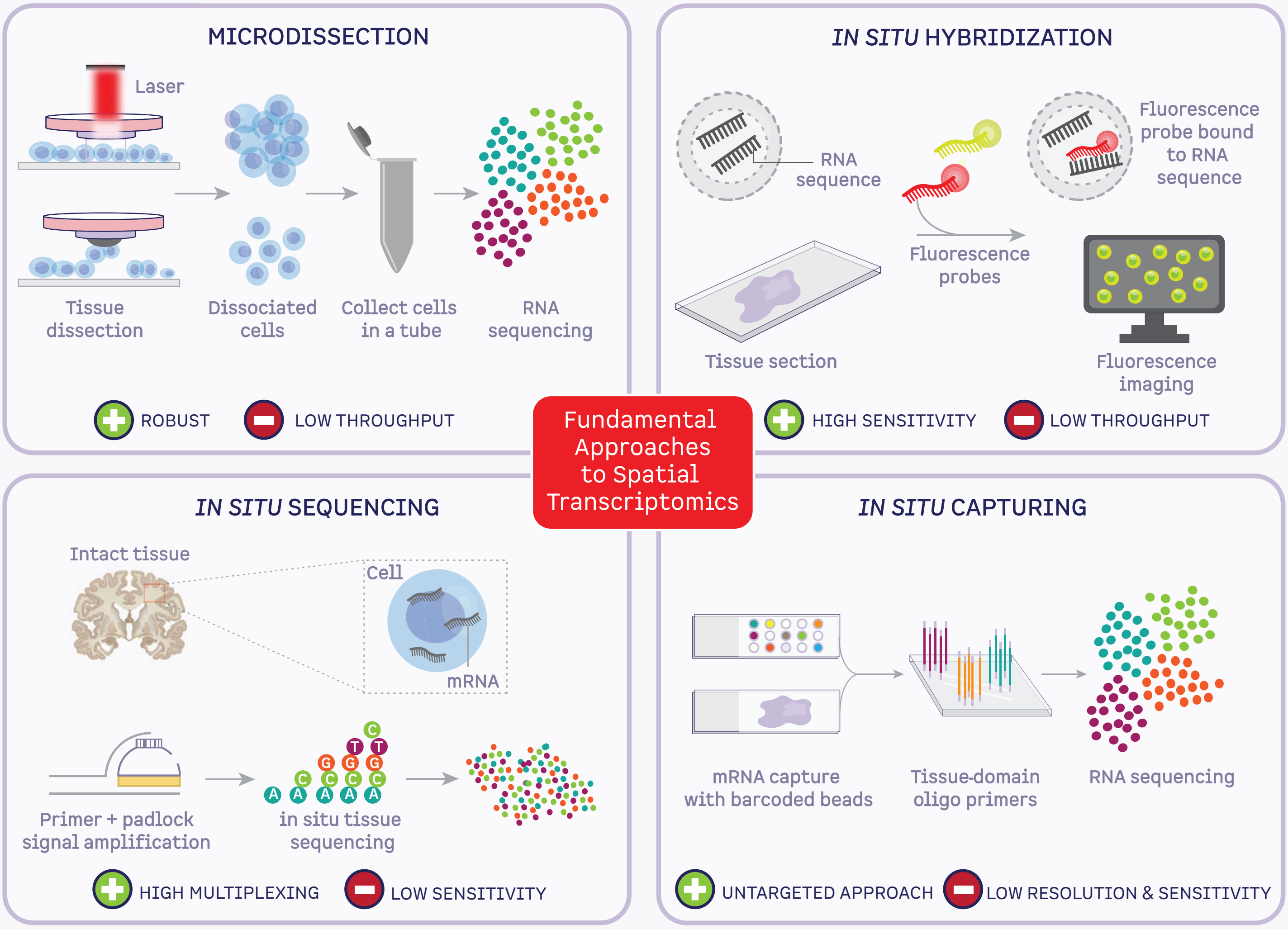
To determine the molecular profiles in a given tissue space, researchers employ laser-capture tissue microdissection to ablate a small group of cells from specific locations.[2] For increased resolution, methods such as Geo-seq employ laser microdissection to obtain cell clusters made of as few as 10 cells.[3] The tissue samples are then analyzed through bulk or single-cell sequencing. However, microdissection-based methods are low throughput and cumbersome because they only cover small tissue sections at a time.

Traditional *in situ* hybridization (ISH) is a common method to visualize transcripts of interest with spatial precision. Instead of removing cells and extracting mRNA, researchers performing ISH introduce specialized probes against target sequences directly into tissue and visualize them using fluorescence or light microscopy. While ISH has good sensitivity and offers subcellular spatial resolution, it has several limitations.[1,2] First, a major drawback is that only a limited number of predetermined transcripts are visualized at a time. In addition, probe-binding efficiency varies from sample to sample, which drastically reduces the reproducibility for quantitative applications. To circumvent some of these challenges, methods such as seqFISH, MERFISH, and RNAscope provide multiplexing solutions by employing more fluorescent probes, rapid probe stripping protocols, and a combinatorial barcoding approach to enhance the number of target genes. However, these enhancements make the techniques expensive, time consuming, and often destroy tissue integrity, making DNA and protein detection impossible. Molecular Cartography, a novel high-throughput, quantitative method, uses non-destructive technology, allowing researchers to perform follow-up experiments.

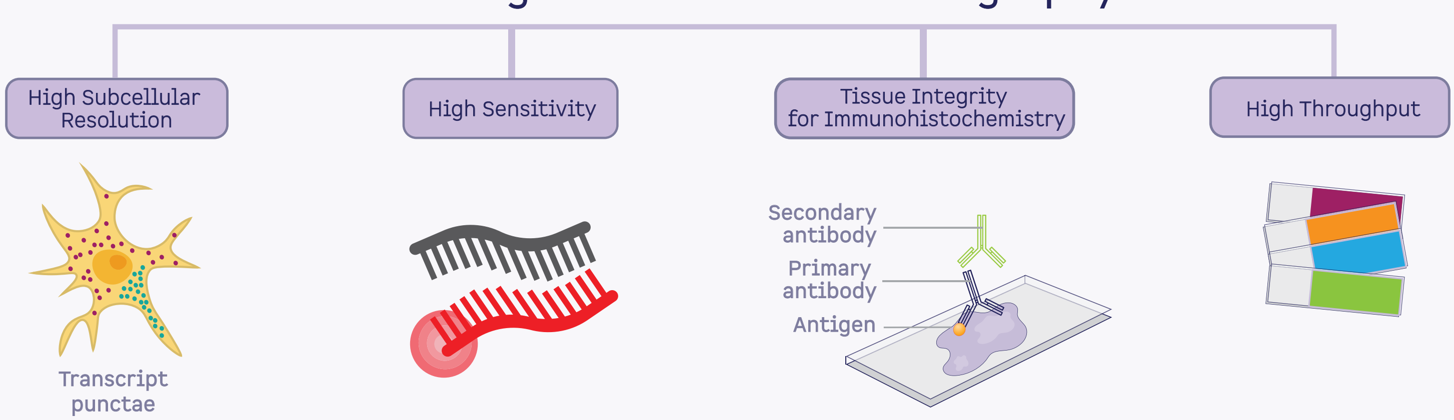
In situ sequencing approaches circumvent some of the traditional ISH limitations by utilizing probe designs that directly capture and amplify RNA from the intact tissue space. While *in situ* sequencing methods such as STARmap and CARTANA facilitate visualization of up to 1000 different transcripts within a tissue space, these methods are insensitive, detect only predetermined targets, and cover a limited field of view.[4,5,6] Unbiased *in situ* capturing methods solve the underlying limitations of *in situ* sequencing by detecting unknown sequences. In this technique, specialized barcoded beads hybridize and capture all transcripts within a cell and mark the cell's location.[1,2] The barcoded beads provide sequencing libraries for unbiased transcriptome datasets and spatial information. However, efficiently capturing RNA probes for sequencing is difficult and creates a bottleneck that reduces the accuracy of transcript expression quantifications.

While all spatial transcriptomics approaches have their strengths and weaknesses, emerging technologies are driving new discoveries in neuroscience, oncology, toxicology, and beyond.

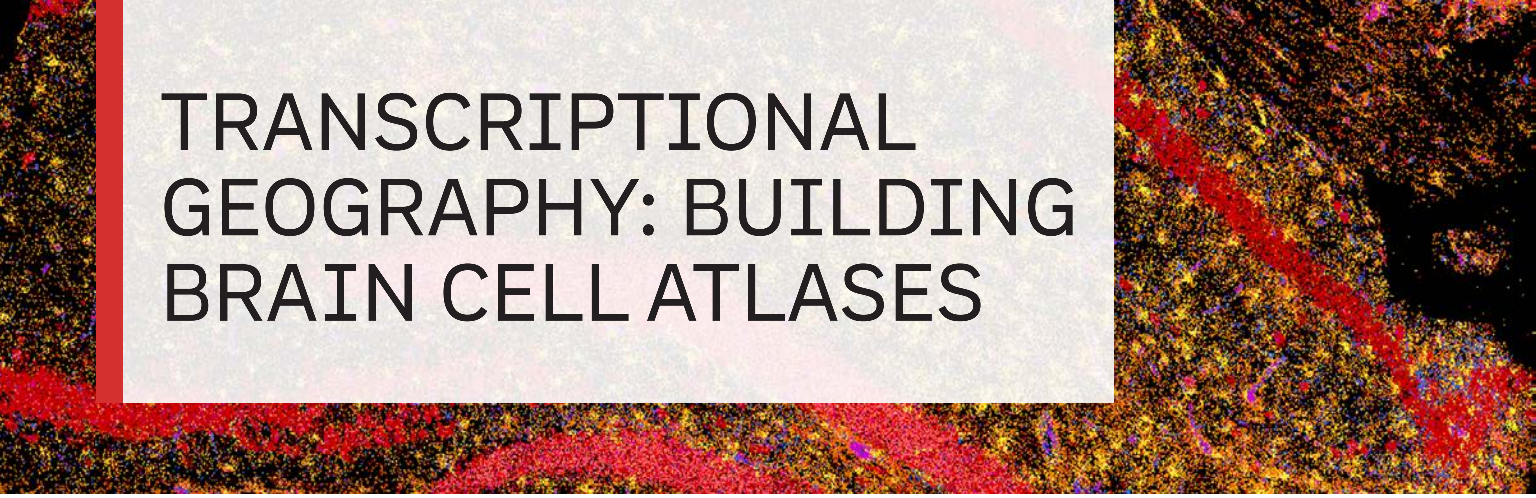
Molecular Cartography: Resolving Subcellular Activity with Spatial Transcriptomics



Advantages of Molecular Cartography



TRANSCRIPTIONAL GEOGRAPHY: BUILDING BRAIN CELL ATLASES



The brain is organized into distinct regions and anatomical circuits that execute specialized functions. Brain cells, such as neurons and glia, display astonishing diversity in morphology, physiology, and connectivity. Coincidently, distinct cellular subtypes assume stereotypical topographical positions in the brain, orchestrating structural motifs that are important for function. Therefore, to understand behavioral and disease mechanisms, researchers must also determine the overall composition and organization of the distinct brain regions. Advancements in spatial transcriptomics provide an opportunity to map the spatial distribution of brain cell types within a region, visualize the transcripts in subcellular compartments, and pinpoint pathways that are altered during development, adulthood, and disease states in a cell-type and circuit-specific manner.[1]

Building Brain Cell Atlases

Throughout brain development, distinct spatial domains with unique transcription factors set up lineages that give rise to region-specific cell types. Many of these cells migrate great distances and differentiate along the way before integrating into a circuit. A range of local cues, gradients of secreted signaling molecules, and other cell-cell communication mechanisms influence cell birth, migration, differentiation, and maturation steps. Using spatial transcriptomics, researchers develop brain cell atlases that harbor positional and molecular information of the developing and adult brain, which they can use to address questions in brain development and function.[2]

Recent work from Sten Linnarsson and his colleagues at the Karolinska Institute uncovered the transcriptional diversity of the mouse brain from gastrulation to birth at spatial and temporal resolution.[3] *In situ* sequencing of 119 genes important for embryonic brain development identified region- and lineage-specific gene expression patterns. The authors reported that radial glia, stem cells in the brain that give rise to neurons and glia, are both transcriptionally and spatially diverse. These cells were shown to secrete a greater variety of morphogens than previously thought, which provide cues for neuronal differentiation. This prenatal mouse brain atlas provides a wealth of information on time- and space-restricted gene expression patterns. Researchers can utilize this information to develop genetic targeting tools for neurodevelopmental disorders and brain cancers.

Visualizing Plaque-Induced Genes

Spatial gene expression analysis also provides deeper insight into neurological diseases such as Alzheimer's disease (AD). AD pathology is defined by the presence of amyloid ß (Aß) plaques. Historically, single cell RNA sequencing (scRNAseq) studies showed gene expression changes in normal versus AD brains and revealed the cell types that become activated in response to Aß pathology. However, the impact of Aß plaques on neurodegeneration is not well defined. Therefore, researchers employed *in situ* sequencing in mouse brains to determine the interaction of Aß plaques with the nearby tissue environment.[4] Transcriptomics of cells surrounding the plaque revealed previously undefined plaque-induced genes (PIGs) in microglia and astrocytes. The PIGs in the AD brains reveal altered crosstalk between glial cells due to disruptions in the endosomal and lysosomal pathways. The subcellular distribution of PIGs provides new insights into how plaques develop in time and space.

Molecular Cartography

Molecular Cartography™ is a novel highly quantitative spatial transcriptomics technique available to the neuroscientific community that allows researchers to identify cellular heterogeneity with spatial context. Molecular Cartography is based on combinatorial single-molecule fluorescent *in situ* hybridization (smFISH). By hybridizing transcript-specific probes per target RNA, researchers can generate molecular maps of a mouse brain to analyze expression patterns of up to 100 unique RNA per sample. Through a proprietary colorizing and de-colorizing chemistry during imaging, this technology accurately identifies individual transcripts with unprecedented specificity and sensitivity, allowing for quantitative analysis with one spot corresponding to one transcript. An automated workflow offers transcript visualization with high-throughput processing. Additionally, researchers can identify individual transcripts, analyze their subcellular distribution in 3-D within a 10 µm thick tissue section, and reuse tissue for downstream immunohistochemistry applications.

Taken together, spatial transcriptomics is overlaying molecular and morphological changes in neurological disease pathologies for the first time, allowing researchers to follow clinically relevant circuits and pathways.

HOT OR COLD: DEFINING THE TUMOR MICROENVIRONMENT

Tumor mass is made up of heterogeneous cells creating a microenvironment that varies from patient to patient. The complexity of the mass and the hierarchical organization of cell types is crucial to understand a tumor's characteristics. Cancer cells in the tumor microenvironment are under evolutionary pressure to mutate and evade immune surveillance. To adapt to the local environment, tumors accumulate dynamic changes that generate spatial and temporal heterogeneity in gene expression. As a result, patients show variable responses to therapies, so there is an urgent need to design personalized therapies based on tumor molecular and cellular profiles.[1,2]

There are two main tumor types, where the presence of infiltrating immune cells defines their characteristics and predicts therapeutic success. Hot tumors contain immune cells such as cytotoxic T cells within their microenvironment, whereas cold tumors do not have immune cell infiltration.[3] Within each tumor type, the cellular arrangement and cell-cell interactions underlie key features of the tumor microenvironment, such as changes in extracellular matrix proteins and cytokine release.

The tumor microenvironment remains largely uncharacterized and holds a wealth of information about molecular and biochemical anomalies that could help guide new therapeutic design. Traditionally, researchers employed basic histology methods that explore tumor characteristics by assessing one highly-expressed protein marker at a time. Newer imaging techniques that use multiple markers reveal tumor heterogeneity to only a certain extent. However, a comprehensive analysis of RNA, protein, and biochemical activities of individual cells in the tumor microenvironment with spatial and temporal context is critical to better characterize heterogeneous cancers and move toward precision medicine. Recent advances in the simultaneous detection of hundreds of tumor biomarkers using single-cell omics methodologies with spatial context have been a game changer for cancer researchers.[1,2]

Classifying Tumors

A spatial transcriptomics study from Joakim Lundeberg and his colleagues at the Royal Institute of Technology in Sweden classified the prostate cancer microenvironment for the first time.[4] A tissue and transcriptome-wide analysis of the prostate tumor mass revealed unique tissue organization involving cancer, normal, and immune cells. The researchers reported differences in gene expression patterns between normal prostate epithelium and cancer cells within the tumor. Further, they identified gene expression differences between the cancer core and periphery thanks to their spatial information. This type of transcriptomics data could help determine whether the periphery promotes tumor initiation and progression, and may provide new tools for early cancer detection.

Glioblastomas are aggressive brain and spinal cord cancers displaying massive intra- and inter-tumor heterogeneity. Using spatial transcriptomics, Dieter Heiland and his colleagues at the University of Freiburg in Germany discovered that glioblastoma cells assume developmental and inflammatory trajectories that increase heterogeneity in the tumor microenvironment.[5] They also found that aging accelerates metabolic modifications in the tumor microenvironment that drive inflammation changes. This work shows how brain tumors adapt to metabolic changes in an age-dependent manner to increase inflammation.

Tumor Heterogeneity and Patient Survival

Recent work from Zohar Yakhini and his colleagues at the Technion - Israel Institute of Technology showed how tumor heterogeneity predicts survival in breast and lung cancer.[6] Using machine learning tools, the researchers "spatially resolved" bulk mRNA and miRNA expression in patient tissue samples. They uncovered high levels of oxidative stress within the reactive stroma in the tumor. This suggested that the stroma releases energy to sustain the growth and survival of cancer cells. Further, the spatial analysis described reactive stroma as an emerging hallmark of cancer initiation and progression. Finally, using a tumor heterogeneity index based on the spatial mapping, researchers showed a significant link between heterogeneity and patient survival, where survival decreases as tumor heterogeneity increases in both tumor types.

Overall, cancer researchers leverage new spatial transcriptomics methodologies to detect cancer biomarkers within their native environment. Such analyses provide a comprehensive understanding of cell-to-cell variation within and between individual tumors, facilitating a leap towards personalized cancer medicine.

References

Article 1: Understanding Spatial Biology

1) M. Asp et al., "Spatially resolved transcriptomes—next generation tools for tissue exploration," *BioEssays*, 42:e1900221, 2020.
2) L. Waylen et al., "From whole-mount to single-cell spatial assessment of gene expression in 3D," *Commun Biol*, 3:602, 2020.
3) J. Chen et al., "Spatial transcriptomic analysis of cryosectioned tissue samples with Geo-seq," *Nat Protoc*, 12(3):566-80, 2017.
4) J. Lee et al., "Highly multiplexed subcellular RNA sequencing in situ," *Science*, 343:1360-3, 2014.
5) X. Wang et al., "Three-dimensional intact-tissue sequencing of single-cell transcriptional states," *Science*, 361:6400, 2018.
6) I. Hernández et al., "Application note: Mapping brain cell types with CARTANA in situ sequencing on the Nikon Ti2-E microscope," *Nat Methods*, 2019.

Article 2: Transcriptional Geography: Building Brain Cell Atlases

1) E. Lein et al., "The promise of spatial transcriptomics for neuroscience in the era of molecular cell typing," *Science*, 358:64-9, 2017.
2) S.M. Sunkin et al, "Allen brain atlas: an integrated spatio-temporal portal for exploring the central nervous system," *Nucleic Acids Res*, 41: D996-D1008, 2013.
3) G. La Manno et al., "Molecular architecture of the developing mouse brain," *Nature*, 596, 92-96, 2021.
4) W. Chen et al., "Spatial transcriptomics and in situ sequencing to study Alzheimer's disease," *Cell*, 182:976-91, 2020.
5) B. Nilges et al., "Every cell matters: Molecular Cartography™ of the mammalian brain," *Virtual Neuroscience*, Society for Neuroscience, 2021.

Article 3: Hot or Cold: Defining the Tumor Microenvironment

1) M. Allam et al, "Multiplex bioimaging of single-cell spatial profiles for precision cancer diagnostics and therapeutics," *npj Precis Onc*, 4:11, 2020.
2) S.M. Lewis et al, "Spatial omics and multiplexed imaging to explore cancer biology," *Nat Methods*, 18:997-1012, 2021.
3) J. Galon, D. Bruni, "Approaches to treat immune hot, altered and cold tumours with combination immunotherapies," *Nat Rev Drug Discov*, 18:197–218, 2019.
4) E. Berglund et al, "Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity," *Nat Commun*, 9:2419, 2018.
5) V.M. Ravi et al, "Spatiotemporal heterogeneity of glioblastoma is dictated by microenvironmental interference," *bioRxiv*, 2021.
6) A. Levy-Jurgenson et al, "Spatial transcriptomics inferred from pathology whole-slide images links tumor heterogeneity to survival in breast and lung cancer," *Sci Rep*, 10:18802, 2020.