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Single-cell and spatial multi-omics for mapping the brain across molecular layers

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Abstract

Understanding how molecular mechanisms occur and shape brain function and dysfunction remains a central challenge in neuroscience. Although bulk omics methods have contributed significantly to the field, they fail to address the cellular and spatial heterogeneity of the brain. Single-cell and spatial multi-omics approaches emerged to address these limitations by enabling integrated, high-resolution profiling of molecular layers while preserving cellular and tissue context. However, despite their impact on basic neuroscience, the clinical translation of these methods remains limited by cost, technical complexity, and analytical challenges. In this review, we summarize recent advances in single-cell and spatial multi-omics applied to brain research, critically evaluating their technological capabilities, translational potential, and current limitations. We further highlight emerging directions, including spatiotemporal integration, morphomics, improved reproducibility, and the expansion of multi-omics research to biologically and environmentally diverse populations.

Keywords: Brain, psychiatry, multi-omics, single-cell, translational omics

1. Introduction

Over the past five decades, molecular biology has been transformed by breakthrough technologies enabling large-scale characterization of nucleic acids, proteins, and metabolites, laying the foundation for the modern omic sciences[1]. Since then, omics approaches have advanced our understanding of biological systems by enabling the identification and quantification of broad sets of molecules across samples[2]. This systems-level perspective is especially valuable for biologically complex tissues with limited sample accessibility, such as the human brain.

The major omics-genomics, transcriptomics, proteomics, and metabolomics- have made extensive contributions to neuroscience using bulk tissue profiling, though they are beyond the scope of this review (for detailed discussions, see Konopka and Bhaduri[3], Restrepo-Lozano *et al.*[4], Lein *et al.*[5], Coppola *et al.*[6], and Fingleton *et al.*[7]). These bulk approaches average signals across heterogeneous cell populations and anatomical regions, consequently masking cell type-specific mechanisms and spatial patterns that are fundamental to brain physiology and pathology.

Single-cell omics methods have emerged to overcome these limitations by enabling high-resolution characterization of cell types, subtypes, and states. Despite their growing coverage and accessibility, most single-cell workflows require sample dissociation. As such, subpopulation data often comes at the price of the loss of spatial context, tissue architecture, and microenvironmental information. Since brain function is strongly linked to precise spatial organization and connectivity, this represents a critical limitation for an integrative biological understanding[8]. To address this loss of information, spatial omics methods have been developed to profile molecular features while preserving the spatial context.

In addition to cellular and spatial complexity, biological systems are driven by interactions across multiple molecular layers. However, most developed omics methods are restricted to a single molecular layer, which is insufficient to capture the full complexity of the brain[9]. Therefore, integrating epigenomic, transcriptomic, proteomic, and metabolic data at single-cell and spatial resolutions is necessary for a more comprehensive understanding of the molecular mechanisms of the brain in both health and disease.

2. New Technologies and Modern Multi-Omics

Recent advances in single-cell and spatial technologies have expanded the study of brain biology beyond conventional bulk measurements. By profiling multiple molecular layers at cellular or spatial resolution, single-cell and spatial methods can reveal cell-type-specific regulatory programs, molecular interactions, and regionally organized biological processes. This section summarizes the major single-cell and spatial multi-omics strategies currently applied in neuroscience, together with representative applications and emerging technological directions. Figure 1 provides an overview of how these approaches relate to different experimental objectives.

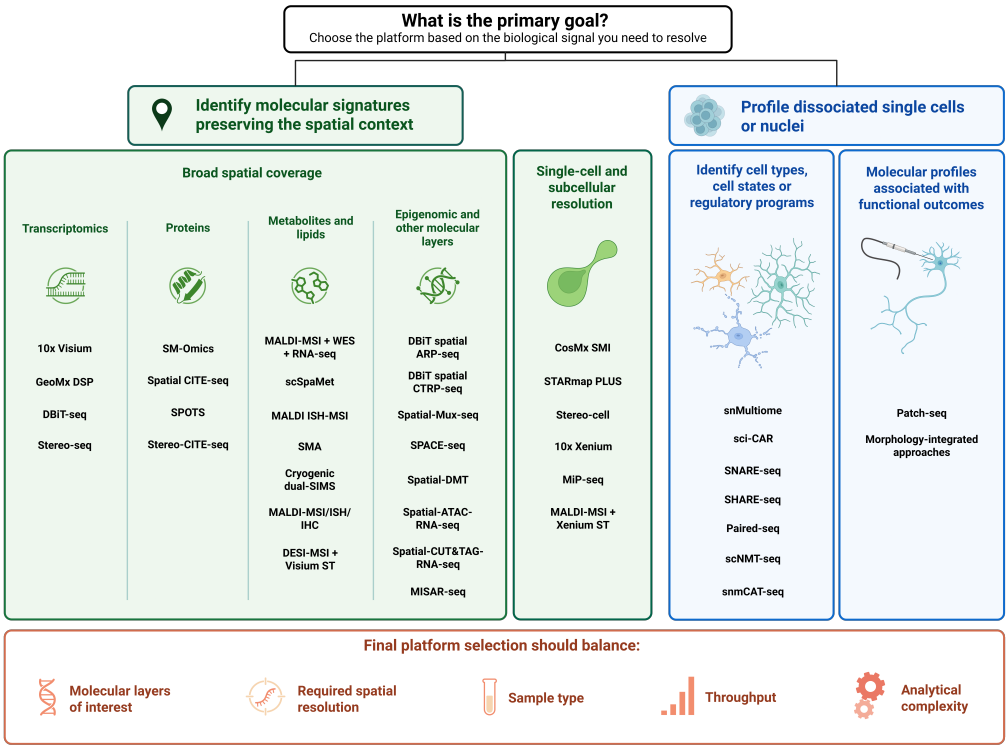

Figure 1. Application-driven framework for selecting single-cell and spatial multi-omics methods in neuroscience. Representative methods are organized according to the primary biological objective, the need to preserve spatial context, the molecular layers of interest, and the required spatial resolution. Methods are positioned according to typical use cases; several approaches support multiple modalities, resolutions, and biological goals. Final platform selection should also consider sample type and preservation, throughput and scalability, analytical complexity, and the maturity of validation in neuroscience. Created in BioRender. Ikeda, V. (2026) https://BioRender.com/t9fwjv5. DSP: digital spatial profiling; DBiT-seq: deterministic barcoding in tissue for spatial omics sequencing; Stereo-seq: spatial enhanced resolution omics-sequencing; CITE-seq: cellular indexing of transcriptomes and epitopes by sequencing; SPOTS: Spatial PrOtein and Transcriptome Sequencing; MALDI-MSI: matrix-assisted laser desorption/ionization mass spectrometry imaging; WES: whole-exome sequencing; scSpaMet: single cell spatially resolved metabolic; SMA: spatial multimodal analysis; SIMS: secondary ion mass spectrometry; ISH: in situ hybridization; IHC: immunohistochemistry; DESI: desorption electrospray ionization; ATAC: assay for transposase-accessible chromatin; DBiT ARP-seq: spatial ATAC-RNA-Protein-seq; SPACE-seq: SPatial Assay for Accessible chromatin, Cell lineages, and gene Expression with sequencing; Spatial-DMT: spatial joint profiling of the DNA methylome and transcriptome; CUT&Tag: cleavage under targets and tagmentation; MISAR-seq: microfluidic indexing-based spatial assay for transposase-accessible chromatin and RNA-sequencing; SMI: spatial molecular imager; STARmap: spatially-resolved transcript amplicon readout mapping; MiP-seq: multi-omics in situ pairwise sequencing; snMultiome: single-nucleus multiome; SNARE-seq: single-nucleus chromatin

accessibility and mRNA expression sequencing; SHARE-seq: simultaneous high-throughput ATAC and RNA expression with sequencing; scNMT-seq: single-cell nucleosome, methylation and transcription sequencing; snmCAT-seq: single-nucleus methylcytosine, chromatin accessibility, and transcriptome sequencing; ST: spatial transcriptomics.

2.1 Multi-omics profiling of individual cells and nuclei

Single-cell transcriptomics has changed neuroscience by revealing a highly complex landscape of diverse cell types and states[10]. However, transcriptomics alone provides an incomplete view of cellular identity and function. Cellular phenotypes emerge from coordinated cross-talk between molecular layers, such as epigenetic modifications, transcript and protein abundance and stability, and metabolic state, not all of which are necessarily directly correlated[11]. Therefore, integrating multiple omics modalities at single-cell resolution has increasingly been applied in neuroscience to elucidate the regulatory mechanisms underlying brain development, function, and pathologies.

Among all single-cell multi-omics methods, the simultaneous profiling of epigenomic features and gene expression has been the most widely adopted. Early proof-of-concept studies, such as single-cell genome-wide methylome and transcriptome sequencing (scM&T-seq)[12], single-cell chromatin accessibility and transcriptome sequencing (scCAT-seq)[13], combinatorial indexing-based coassay that jointly profiles chromatin accessibility and mRNA (sci-CAR)[14], and single-nucleus chromatin accessibility and mRNA expression sequencing (SNARE-seq)[15], demonstrated the feasibility of capturing both layers from individual cells and nuclei. These approaches laid the foundation for other methods, including parallel analysis of individual cells for RNA expression and DNA accessibility by sequencing (Paired-seq)[16] and simultaneous high-throughput ATAC and RNA expression with sequencing (SHARE-seq)[17], which improved scalability by introducing combinatorial barcoding. These led to the development of standardized commercial platforms, such as 10x Genomics single-nucleus multiome (snMultiome), whose improved reproducibility and standardized workflow have facilitated its wide adoption. Detailed comparisons of these methods in neuroscience have previously been published[18].

Recently, snMultiome has also become widely used, mainly due to its standardized workflow and high reproducibility. This approach has already been extensively applied in the study of neurodegenerative disorders. In Alzheimer's disease (AD), snMultiome analyses across multiple brain regions have revealed impairments in transcriptional and epigenomic regulation[19-23]. Liu *et al*. analyzed the single-cell epigenomes and transcriptomes of 3.5 million cells from 384 postmortem brain samples, demonstrating a widespread erosion of epigenomic identity, characterized by altered chromatin compartmentalization[24]. Preserved epigenomic stability was associated with cognitive resilience, whereas epigenomic disorganization correlated with neuronal vulnerability and disease progression, especially in excitatory neuronal populations[24].

Use of single-cell multi-omics in Parkinson's disease (PD) remains comparatively limited but has provided relevant insights. Across studies, glial cells, particularly oligodendrocytes[25] and microglia[26,27], exhibited transcriptional and epigenomic alterations linked to oxidative stress, protein misfolding, immune activation, and metabolic dysregulation. While many of these findings have already been observed using monoculture *in vitro* methods, single-nucleus omics studies have confirmed them with a more integrated approach, considering the interplay among cell types[27]. Furthermore, 13 disease-associated microglia (DAM) subpopulations were identified, revealing region-specific dysregulation between pro-inflammatory and stress-responsive states[28].

Single-cell multi-omics approaches have also been applied to psychiatric disorders. Integrative analysis of multiple PsychENCODE datasets revealed extensive remodeling of cell-type-specific regulatory mechanisms across aging and neuropsychiatric conditions[29]. Rather than focusing only on dysregulated genes and pathways, this study provided what they considered "*an extensive collection of inferences and predictions for neuroscientists to verify in new cohorts, populations, assays, and experimental conditions*", thus paving the way to precision medicine[29]. Moreover, snMultiome analysis of postmortem samples from schizophrenia (SCZ) and mood disorder patients showed that most differentially regulated genes were found in excitatory neurons, whereas glial cells showed the strongest associations with genetic risk variants[30].

Beyond pathological conditions, single-cell multi-omics have also been applied to investigate neurodevelopmental and evolutionary contexts. During human brain development, joint profiling of gene expression and chromatin accessibility across multiple regions and developmental states identified age as the primary driver of molecular variability, followed by brain region[31]. Pathways associated with neurogenesis, synaptic maturation, and circuit formation were found to be dynamically regulated over time[31].

Similar findings were observed during human cortical development, where distinct transcription factors (TFs) responsible for neurogenesis and gliogenesis were identified, and lineage-determining TFs acquire an active chromatin state early in differentiation, becoming "ready to activate" before full lineage commitment[32]. Complementing these findings, during cortical interneuron development, RNA expression can provide a measure of a cell's instantaneous developmental state, while chromatin accessibility is associated with both the history of the developmental process and the prediction of its future identity[33]. Notably, throughout development, RNA-seq and ATAC-seq data remain different until interneurons commit to the terminal differentiation, when interneurons reach their settling position and begin to integrate circuits in the cortex[33]. These findings highlight that coordinated, yet asynchronous, regulation across molecular layers is important for cell fate determination[33].

Extending these approaches across species has further revealed that although cortical cell types are broadly conserved, corticogenesis diverges through a combination of compositional shifts in progenitor subpopulations and extensive cis-regulatory changes within conserved cell types, with human-gained accessible chromatin regions linked to cognitive traits and psychiatric risk[34].

Several alternative single-cell multi-omics methods have been developed but remain less widely used in neuroscience. Single-cell nucleosome, methylation and transcription sequencing (scNMT-seq) enables simultaneous profiling of chromatin accessibility, DNA methylation, and the transcriptome, with limited applications demonstrating dynamic epigenetic regulation during neural differentiation and the resetting of epigenetic fate restriction in injury responses[35,36].

Similarly, single-nucleus methylcytosine, chromatin accessibility, and transcriptome sequencing (snmCAT-seq) profiles the same three layers using nucleosome occupancy and methylome sequencing (NOMe)-based accessibility labeling in nuclei rather than whole cells, making it applicable to frozen and postmortem material, contributing to a foundational regulatory atlas of the human brain[37]. To the best of our knowledge, applications beyond the original study in the neuroscience field are currently limited. Furthermore, cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq)[38] has revealed dynamic immune remodeling across aging, central nervous system (CNS)-border immunity, brain tumors, and epilepsy by linking transcriptional states to surface protein phenotypes in microglia and macrophage populations[39-42].

Beyond molecular layers, Patch-seq extends single-cell profiling to a multimodal framework by integrating electrophysiological, transcriptomic, and morphological data from individual neurons[43,44]. This method has provided new insights into neuronal diversity[45-49], functional specialization[50-54], and disease-associated dysfunction[55-57]. Collectively, these studies demonstrate how Patch-seq multimodal single-cell data can connect transcriptomic, electrophysiological, and morphological data at single-cell resolution, offering insights into new mechanisms across several disorders. This approach provides an integrative framework for linking molecular profiles to functional outcomes.

It is important to note that although previous reviews have extensively discussed other single-cell multi-omics methods[58-62], most of these methods have not yet been applied in neuroscience and are therefore outside the scope of this article. Furthermore, we mainly focused on paired multi-omic methods (Table 1), since they provide a direct link between omics within individual cells, enabling mechanistic inference that is difficult to achieve with unpaired approaches.

Table 1. Comparative overview of single-cell multi-omics methods relevant to neuroscience. The table summarizes molecular modalities profiled, major strengths, limitations, and tissue compatibility for each multi-omic method discussed in this review.

Method	First reported	Modalities profiled	Biological input	Strengths	Limitations	Tissue compatibility
scM&T-seq[12]	2016	Transcriptome and genome-wide DNA methylation	Whole cells	Jointly profiles gene expression and DNA methylation in the same cell	Low-throughput; technically demanding	Fresh/viable single cells suspensions
Patch-seq[43,44]	2016	Transcriptome, morphology, and electrophysiology	Live neurons	Links gene expression with electrophysiology and morphology	Low-throughput; technically demanding; mostly limited to viable neurons in acute slices or cultures	Live neurons in brain slices or cultured neurons
CITE-seq[38]	2017	Transcriptome and cell surface proteins	Whole cells	Scalable and widely used; simultaneous RNA and surface-protein profiling; compatible with droplet-based workflow	Limited to antibody-detectable surface proteins; isolating single cells from tissue can cleave or alter surface proteins; no epigenetic layer	Fresh/viable single-cell suspensions; commonly applied to immune cells
scNMT-seq[35]	2018	Transcriptome, chromatin accessibility, and DNA methylation	Whole cells	Captures transcriptome, chromatin accessibility, and DNA methylation in the same cell	Low-throughput and technically complex; limited scalability compared with droplet	Fresh isolated single cells; low-throughput sorted cells

					or combinatorial-indexing methods	
sci-CAR[14]	2018	Transcriptome and chromatin accessibility	Whole cells or nuclei	Scalable combinatorial-indexing strategy; jointly profiles chromatin accessibility and gene expression in thousands of single cells	Per-cell coverage can be sparse; workflow is more complex than unimodal scRNA-seq or scATAC-seq; no proteomics layer	Fixed or permeabilized cells/nuclei from cell lines and tissues
scCAT-seq[13]	2019	Transcriptome and chromatin accessibility	Whole cells	Simultaneously profiles full-length gene expression and chromatin accessibility	Low-throughput, plate-based workflow; technically complex nucleus-cytoplasm separation	Fresh/viable single cells suspensions
SNARE-seq[15]	2019	Transcriptome and chromatin accessibility	Nuclei	Droplet-based joint profiling of nuclear transcriptome and chromatin accessibility; suitable for nuclei and therefore useful for frozen or difficult-to-dissociate tissues	Nuclear RNA has lower transcript complexity than whole-cell RNA; ATAC and RNA signals may be sparse; no proteomics layer	Isolated nuclei; suitable for frozen tissue and difficult-to-dissociate tissues
Paired-seq[16]	2019	Transcriptome and chromatin accessibility	Whole cells or nuclei	Ultra-high-throughput joint profiling of transcriptome and accessible chromatin; suitable for atlas-scale studies and large cell numbers	Combinatorial-indexing workflow is technically demanding; sparse per-cell coverage; no proteomics layer	Fixed/permeabilized cells or nuclei
SHARE-seq[17]	2020	Transcriptome and chromatin accessibility	Whole cells or nuclei	Highly scalable; links chromatin accessibility with gene expression in the same cell	Computationally demanding; chromatin and RNA coverage can be sparse; no proteomics layer	Fixed cells/nuclei
10x Chromium Multiome ATAC + Gene Expression (snMultiome)	2020	Transcriptome and chromatin accessibility	Nuclei	Commercially standardized; scalable; widely adopted for paired nuclear RNA and ATAC profiling	No proteomics layer; nuclear RNA has lower transcript complexity than whole-cell RNA	Nuclei isolated from fresh or frozen tissue
snmCAT-seq[37]	2022	Transcriptome, chromatin accessibility, and DNA methylation	Nuclei	Measures nuclear transcriptome, chromatin accessibility, and DNA methylation in the same nucleus	Technically complex; lower throughput than RNA+ATAC-only methods; computational integration is demanding	Nuclei isolated from frozen or postmortem tissue

scM&T-seq: single-cell genome-wide methylome and transcriptome sequencing; CITE-seq: cellular indexing of transcriptomes and epitopes by sequencing; scNMT-seq: single-cell nucleosome, methylation and transcription sequencing; sci-CAR: combinatorial indexing-based coassay that jointly profiles chromatin accessibility and mRNA; scRNA-seq: single-cell RNA sequencing; scATAC-seq: single-cell assay for transposase-accessible chromatin using sequencing; scCAT-seq: single-cell chromatin accessibility and transcriptome sequencing; SNARE-seq: single-nucleus chromatin accessibility and mRNA expression sequencing; SHARE-seq: simultaneous high-throughput ATAC and RNA expression with sequencing; snMultiome: single-nucleus multiome; snmCAT-seq: single-nucleus methylcytosine, chromatin accessibility, and transcriptome sequencing.

2.2 Spatially resolved multi-omics

Despite the advances generated by single-cell multi-omics methods, the spatial context, a major dimension of brain biology, is being lost. Spatial context plays a key role in cell identity, function, and interactions within a tissue. In the brain, for example, morphogen gradients such as Sonic Hedgehog (Shh) specify dorsoventral neuronal fates through concentration-dependent signaling, thereby directing cell identity[63,64]. Recognizing the importance of the spatial dimension to biological function motivated the development of spatial omics technologies that preserve positional information while capturing molecular complexity.

Spatial transcriptomics and related single-omics methods were initially developed to measure molecular profiles in their spatial localization. Broadly, these approaches can be divided into two complementary categories. Sequencing-based approaches use spatial barcoding or in situ labeling to generate high-plex spatial data, often at whole-transcriptome scale, typically trading off cellular resolution. Imaging-based approaches achieve true single-cell and even subcellular resolution by visualizing transcripts or proteins in situ through iterative cycles of hybridization and imaging. However, the multiplexing capacity is usually lower than that of sequencing-based approaches[65]. Beyond imaging- and sequencing-based approaches, mass spectrometry imaging (MSI) provides complementary data for spatially resolved analysis. By detecting the mass-to-charge ratio of molecules, MSI enables label-free mapping of metabolites, lipids, and peptides, capturing additional dimensions of cellular dynamics.

Foundational spatial methods (such as single molecule fluorescence in situ hybridization (smFISH)[66,67], multiplexed error-robust FISH (MERFISH)[68], sequential FISH (seqFISH)[69], Spatial Transcriptomics[70], co-detection by indexing (CODEX)[71], and spatial enhanced resolution omics-sequencing (Stereo-seq)[72]) have already contributed to new insights into molecular organization in the brain. Building on these foundations, spatial multi-omics approaches integrate multiple molecular layers within a shared coordinate system, combining the strengths of spatial biology and multimodal profiling. This integration provided a better understanding of how the epigenome, transcriptome, and proteome are organized across brain regions and how these relationships change in disease[73]. Table 2 compares the platforms discussed across these categories.

Table 2. Comparative overview of spatial omics and spatial multi-omic methods relevant to neuroscience. The table summarizes molecular layers, spatial resolution, strengths, limitations, and tissue compatibility across sequencing-based, imaging-based, mass-spectrometry-based, and emerging spatial multi-omic platforms.

Method	Modalities profiled	Spatial unit/Resolution*	Strengths	Limitations	Tissue compatibility
Sequencing-based					
10x Visium[70]	Whole transcriptome + histology image	55 µm spots; 100 µm center-to-center	Widely validated commercial workflow; broad ecosystem; transcriptome-wide profiling mapped to tissue morphology	Not single-cell resolution; each spot captures mixed cell types	Fresh-frozen and FFPE (assay-version dependent)
DBiT-seq[74]	Whole transcriptome + targeted protein markers	10-50 µm pixels (channel-width dependent)	RNA and protein co-measured at high resolution from the same section; no specialized imaging required	Targeted protein panel; microfluidic workflow and alignment requirements; pixel-level mixing possible in dense tissue	Formaldehyde/PFA-fixed tissue sections; fixed-frozen and FFPE adaptations reported
GeoMx DSP[75]	Whole transcriptome and/or targeted protein markers	User-defined ROIs or segmented masks; molecular output is aggregate ROI/segment-level	Enables targeted profiling of rare populations or specific morphological regions guided by histology; compatible with archival tissue; flexible RNA/protein panel	Bulk ROI/segment-level profiling, not cell-by-cell mapping; sensitivity depends on ROI design and sufficient material; panel/probe-dependent	FFPE and fresh-frozen tissue sections

			selection		
Stereo-seq[72]	Whole transcriptome	~ 0.22 μm DNB features; 0.5 μm center-to-center	High-density capture with large field of view; scalable to whole-organ or whole-embryo profiling	Specialized DNB-array workflow; library preparation and data handling are complex; submicron capture grid is not the same as direct single-molecule imaging	Fresh-frozen, fixed-frozen and FFPE
SM-Omics[76]	Whole transcriptome + targeted protein markers	55 μm spots; 100 μm center-to-center (Visium-based)	Automated high-throughput workflow for combined spatial transcriptomics and antibody-based protein detection	Not single-cell resolution; protein readout is antibody/protocol-dependent	Fresh-frozen tissue sections
SPACE-seq[77]	Chromatin accessibility + whole transcriptome + mtDNA variants	55 μm spots; 100 μm center-to-center (Visium-based)	Co-profiles chromatin accessibility and gene expression on a standard Visium/CytAssist platform; can infer mtDNA variants and extrachromosomal DNA copy-number features	Spot-level rather than single-cell; ATAC and mtDNA signals can be sparse	Fresh-frozen tissue sections
Spatial-CUT & Tag-RNA-seq[78]	Whole transcriptome + selected histone modification (e.g., H3K27me3, H3K27ac, or H3K4me3)	20-50 μm pixels	Links selected chromatin-state marks directly with gene expression in the same section	One selected histone mark per experiment; not a genome-wide catalogue of all epigenetic features; microfluidic workflow; pixel-level mixing possible	Frozen tissue sections
SPOTS[79]	Whole transcriptome + targeted protein markers	55 μm spots; 100 μm center-to-center (Visium-based)	Adds protein-marker detection to commercial Visium spatial transcriptomics	Not single-cell resolution; protein detection is panel-dependent; lower protein multiplexing than high-plex imaging/ADT methods	Fresh-frozen tissue sections
Spatial-ATAC-RNA-seq[78]	Whole transcriptome + chromatin accessibility	20-50 μm pixels	Co-maps genome-wide chromatin accessibility and gene expression in the same section	Pixel-based rather than segmented single-cell; microfluidic workflow; chromatin-accessibility signal can be sparse	Frozen tissue sections
DBiT spatial ARP-seq[80]	Whole transcriptome + chromatin accessibility + targeted protein markers	25-50 μm pixels	Simultaneous spatial epigenome, transcriptome and proteome profiling from the same section	Microfluidic workflow; protein detection is panel-dependent; pixel-based rather than true segmented single-cell	Fresh-frozen and fixed-frozen tissue sections

DBiT spatial CTRP-seq[80]	Whole transcriptome + histone modifications + targeted protein markers	25-50 μm pixels	Simultaneous spatial chromatin-state, transcriptome and proteome profiling from the same section	Microfluidic workflow; protein detection is panel-dependent; pixel-based rather than true segmented single-cell; one histone mark per experiment	Fresh-frozen and fixed-frozen tissue sections
Stereo-cell[81]	Whole transcriptome + cell morphology + targeted protein markers	~ 0.22 μm DNB spots; 0.5 μm center-to-center	Droplet-free high-throughput single-cell profiling that preserves cell morphology and spatial coordinates on-chip; useful for large/fragile cells and extracellular vesicles	Not a native tissue-section spatial omics method; spatial context is reconstructed on-chip rather than preserved in tissue; specialized DNB-array workflow; high sequencing depth	Intact cells or cell suspensions on DNB-array chips
Spatial CITE-seq[82]	Whole transcriptome + high-plex cell-surface proteins	25 μm pixels	Co-maps whole transcriptome with high-plex protein panels from the same section	Protein detection is panel-dependent and restricted to cell-surface markers; lacks subcellular resolution	PFA-fixed tissue sections
MISAR-seq[83]	Whole transcriptome + chromatin accessibility	50 μm pixels	Co-maps chromatin accessibility and gene expression in the same spatial tissue section	Not single-cell resolution; microfluidic workflow; chromatin-accessibility signal can be sparse	Frozen tissue sections
Spatial-Mux-seq[84]	Two histone modifications + chromatin accessibility + whole transcriptome + targeted protein markers	20-50 μm pixels	Simultaneously profiles up to five molecular modalities in the same section	Technically complex microfluidic/in situ barcoding workflow; protein detection is panel-dependent; limited to two histone marks per experiment; pixel-based rather than segmented single-cell	Fixed-frozen tissue sections
Spatial-DMT[85]	Whole-genome DNA methylome + whole transcriptome	10-50 μm pixels	Jointly maps DNA methylation and gene expression from the same tissue section; early demonstration of spatial whole-genome methylome profiling	Lower-resolution pixels may contain mixed-cell signals; microfluidic/bisulfite workflow complexity	Fixed-frozen tissue sections
Imaging-based					
CosMx SMI[86]	Targeted RNA panels or whole-transcriptome assay + targeted protein markers	Single-cell segmentation with subcellular transcript localization; imaging-based cyclic ISH	High-plex RNA and protein imaging in intact tissue; subcellular transcript localization	Panel-dependent; cyclic imaging is time-consuming and expensive; segmentation quality affects cell-level results	FFPE and fresh-frozen tissue

10x Xenium[87]	Targeted RNA panels + optional targeted protein subpanels	Single-cell segmentation with subcellular transcript localization; imaging-based in situ assay	High-plex targeted spatial RNA detection with subcellular localization; non-destructive workflow compatible with downstream histology	Panel-dependent and not whole-transcriptome; protein capability is panel/workflow-dependent; segmentation affects results	Fresh-frozen and FFPE tissue sections
MiP-seq[88]	Targeted DNA, RNA and proteins, including mutations, allele-specific expression and RNA modifications	Subcellular resolution; imaging-based in situ sequencing	Multiplexed spatial multi-omics in intact samples; can be combined with calcium or Raman imaging for functional integration	Probe-based rather than unbiased whole-transcriptome; complex imaging and in situ sequencing workflow	Fixed cells and tissue sections
STARmap PLUS[89]	Targeted RNA panel + targeted protein markers	Single-cell/subcellular 3D imaging; voxel size ~ 200 nm lateral x ~ 350 nm axial (experiment-dependent)	RNA and protein co-measured at subcellular resolution; applicable to three-dimensional intact tissues	Panel-dependent; complex multiplexed FISH, hydrogel/tissue-processing and imaging workflow	Intact mouse brain tissue
Mass spectrometry-based					
MALDI-MSI + WES + RNA-seq[90]	Spatial metabolome (MALDI-MSI) + bulk WES + bulk RNA-seq	MALDI-MSI: instrument-dependent, typically ~ 10-100 μm; WES and RNA-seq are non-spatial	Integrated metabolic, genomic, and transcriptomic profiling from the same FFPE section; enables retrospective use of archival samples	Genomic and transcriptomic layers lack spatial resolution; metabolite annotation can be ambiguous	FFPE tissue sections
MALDI ISH-MSI[91]	Spatial metabolomics (MALDI-MSI) + targeted RNA transcripts (MALDI-ISH)	~ 20 μm pixels	Co-detects metabolite signals and targeted gene-expression patterns from the same section using a MALDI-based readout	Targeted RNA only; metabolite annotation can be challenging	Fresh-frozen tissue sections
Cryogenic dual-SIMS[92]	Spatial metabolome + targeted protein markers	~ 3 μm for metabolite SIMS; ~ 1 μm for protein SIMS	Preserves near-native metabolic and lipid states via cryogenic imaging; integrates metabolites, lipids, and protein-defined cell types at single-cell level	Requires specialized cryogenic SIMS instrumentation and complex sample handling; protein detection is panel-dependent; metabolite annotation can be challenging	Cryogenic tissue sections
SMA[93]	Whole transcriptome + spatial metabolome	Transcriptomics: 55 μm Visium spots, 100 μm center-to-center; MALDI-MSI:	Enables same-section spatial transcriptome and metabolite co-profiling; compatible	Not single-cell resolution; metabolite annotation can be challenging	Fresh-frozen tissue sections

		instrument-dependent	with commercial Visium slides		
MALDI-MSI + 10x Xenium[94]	Spatial metabolomics (MALDI-MSI) + targeted transcriptomics (10x Xenium)	MALDI-MSI: ~ 5 μm pixels in reported workflow; Xenium: single-cell segmentation with subcellular transcript localization	Same-section integration of metabolic features with single-cell spatial gene expression, reducing misalignment versus adjacent sections	In reported validation, MALDI-MSI before Xenium reduced transcripts per cell; metabolite annotation can be challenging; Xenium remains panel-based; technically complex	Fresh-frozen tissue sections
DESI-MSI + 10x Visium[95]	Untargeted spatial metabolomics (DESI-MSI) + whole transcriptome (10x Visium) + histology	DESI-MSI: ~ 100 μm; Visium: 55 μm spots, 100 μm center-to-center	Enables metabolite-transcript correlation from the same section; DESI-MSI can be compatible with downstream RNA profiling	Not single-cell resolution; metabolite annotation can be challenging; transcriptomic layer remains Visium spot-level; compatibility is workflow- and tissue-dependent	Fresh-frozen tissue sections
MALDI-MSI/ ISH/IHC[96]	Label-free metabolites/lipids (MALDI-MSI) + targeted RNA transcripts (MALDI-ISH) + targeted proteins (MALDI-IHC)	~ 5 μm for MALDI-IHC; ~ 20 μm for combined MALDI-MSI/ISH workflows (instrument-dependent)	Enables spatial detection of metabolites, targeted transcripts and targeted proteins from the same section using MALDI-based readouts	RNA and protein detection are panel-dependent; metabolite annotation can be challenging; technically complex workflow	Fresh-frozen tissue; MALDI-IHC also compatible with FFPE
scSpaMet[97]	Spatial metabolome by TOF-SIMS + targeted protein markers	< 1 μm per pixel for metabolite imaging (TOF-SIMS)	Links metabolic features to protein-defined cell types in the same section, enabling cell-type-associated spatial metabolomics	Metabolite annotation can be challenging; protein detection is panel-dependent; requires specialized SIMS instrumentation	FFPE and frozen tissue sections

*Values report each platform's nominal capture-unit (spot, pixel, or DNB feature size and center-to-center pitch), not necessarily demonstrating single-cell or single-molecule resolution. The gap between nominal and effective resolution reflects capture efficiency of the underlying chemistry, lateral diffusion of the captured analyte prior to barcoding, and downstream computational assignment of signal to cells via image-guided segmentation. This is most consequential for sub-cellular-scale pixel/DNB grids (Stereo-seq, DBiT-seq and its extensions), where the nominal feature size can be mistaken for achieved resolution. FFPE: formalin-fixed paraffin-embedded; DBiT-seq: deterministic barcoding in tissue for spatial omics sequencing; PFA: paraformaldehyde; DSP: digital spatial profiling; ROIs: regions of interest; Stereo-seq: spatial enhanced resolution omics-sequencing; DNB: DNA nanoball; SPACE-seq: SPatial assay for Accessible chromatin, Cell lineages, and gene Expression with sequencing; mtDNA: mitochondrial DNA; ATAC: assay for transposase-accessible chromatin; CUT&Tag: cleavage under targets and tagmentation; SPOTS: Spatial PrOtein and Transcriptome Sequencing; ADT: antibody-derived tag; DBiT spatial ARP-seq: spatial ATAC-RNA-protein-seq; CTRP-seq: CUT&Tag-RNA-protein-seq; Stereo-cell: spatial enhanced-resolution single-cell sequencing; CITE-seq: cellular indexing of transcriptomes and epitopes by sequencing; MISAR-seq: microfluidic indexing-based spatial assay for transposase-accessible chromatin and RNA-sequencing; Spatial-DMT: spatial joint profiling of the DNA methylome and transcriptome; SMI: spatial molecular imager; ISH: in situ hybridization; MiP-seq: multi-omics in situ pairwise sequencing; STARmap: spatially-resolved transcript amplicon readout mapping; FISH: fluorescence in situ hybridization; MALDI-MSI: matrix-assisted laser desorption/ionization mass spectrometry imaging; WES: whole-exome sequencing; SMA: spatial multimodal analysis; DESI: desorption electrospray ionization; IHC: immunohistochemistry; scSpaMet: single cell spatially resolved metabolic; TOF-SIMS: time-of-flight secondary ion mass spectrometry.

2.2.1 Sequencing-based approaches

2.2.1.1 GeoMx digital spatial profiling

GeoMx digital spatial profiling (DSP) is a commercial platform capable of high-plex spatial profiling of RNA and/or proteins within user-defined regions of interest (ROIs)[75]. It is non-destructive and compatible with fresh tissue, wet-stored samples, and formalin-fixed paraffin-embedded (FFPE) slides preserved for extended periods[98]. DSP uses oligonucleotide tags conjugated to antibodies or RNA probes via photocleavable linkers, which, upon ultraviolet (UV) illumination of selected ROIs, are released for sequencing-based or optical counting readouts.

To our knowledge, few studies have applied DSP in a true multi-omic configuration to study the brain. One example is the spatial proteogenomics (SPG) assay, which was validated in glioblastoma multiforme (GBM) samples, enabling whole-transcriptome profiling alongside > 100-plex protein quantification[99]. This approach identified spatial differences in transcript and protein levels across pathological regions, including immune infiltration patterns and tumor-associated protein signatures.

Although DSP can quantify RNA and protein simultaneously, most published neuroscience studies to date have employed it in a single-omic mode. In high-grade glioma, DSP resolved tumor spatial domains with distinct immunological landscapes[100] and linked oxygenation levels to modulation of immune-related proteins, highlighting metabolic-immune crosstalk in glioma pathology[101]. Comparative spatial analysis of paired primary and recurrent glioblastomas revealed highly heterogeneous distributions of immune markers with no consistent direction of change between stages, suggesting complex molecular remodeling[102]. Furthermore, perivascular stromal cells, beyond their structural functions, were identified as active contributors to tumor progression and immune evasion through crosstalk with immunosuppressive myeloid populations[103]. DSP has also been used in therapeutic contexts to evaluate biomarkers associated with spatial signatures, including the relationship between O^6-methylguanine-DNA methyltransferase (MGMT) methylation state and immuno-oncology profiles[104], and molecular responses to programmed cell death protein 1 (PD-1) blockade in recurrent GBM[105].

In neurodegenerative diseases, DSP has been mostly applied to AD. Preliminary spatial proteomics of the suprachiasmatic nucleus from postmortem AD brain revealed a loss of arginine vasopressin (AVP) and vasoactive intestinal peptide (VIP)-expressing neurons, linking circadian rhythm dysfunction to AD progression[106]. In the prefrontal cortex, it identified differentially expressed proteins in neurons, including neprilysin, an amyloid beta-degrading enzyme, and, controversially, microglial activation markers[107]. Spatial proteomics profiling across hippocampal subregions identified molecular changes distinguishing AD, primary age-related tauopathy (PART), and chronic traumatic encephalopathy (CTE), highlighting spatially specific amyloid beta and/or pTau accumulation[108,109].

Cerebral amyloid angiopathy (CAA), a common AD pathology, was also explored by DSP. It was used to identify immune responses linked to vascular damage[110], capturing transient and chronic inflammatory changes over progression in APPSw mice[111], and identifying spatially localized immune deficits in triggering receptor expressed on myeloid cells 2 (TREM2)-risk variant carriers[112]. Further DSP studies compared immune activation patterns across AD-related pathologies and Lewy body disease[113], showing region-specific immune activation in AD, while pure Lewy body disease presented subtle but global immune responses. Neurofibrillary tangle (NFT)-bearing neurons from cognitively resilient individuals were associated with microenvironments marked by lower oxidative and energetic stress[114]. Another study analyzed retinal tau isoforms as potential predictors of brain tauopathy and cognitive decline[115].

Beyond AD, DSP has also been applied to other neurological diseases. In PD, substantia nigra profiling indicated overlapping but distinct molecular profiles between PD and the Parkinsonian subtype of multiple system atrophy (MSA-P)[116]. Gut-brain axis studies reported alpha-synuclein accumulation in the myenteric plexus, associated with intestinal inflammation and active neuronal regeneration signatures in PD patients[117]. In frontotemporal dementia, AD, and C9orf72-related disorder, the DSP protein panel revealed selective localization of classical pathological markers in subregions of the cortex, contrasting with the widespread localization of other disease-associated proteins[118]. In epilepsy, DSP revealed that memory impairment is associated with specific molecular profiles in hippocampal and temporal neocortex subregions, independent of seizure frequency or severity[119]. Similarly, in the ischemic stroke mouse model, mechanisms associated with stroke, such as apoptosis and inflammation, appear to be spatially compartmentalized, with peri-infarct regions showing higher astrocytic reactivity and neurodegenerative markers[120].

2.2.1.2 10x Genomics Visium

10x Genomics Visium, a commercially available platform derived from the original spatial transcriptomics method by Ståhl *et al.*[70], is a widely used sequencing-based platform for spatial transcriptomics that also spatially resolves immunofluorescence-based protein readouts. Similarly to GeoMx DSP, applications of 10x Genomics Visium in multi-omic settings (e.g., 10x Visium proteogenomics assay) in the brain remain limited but illustrative.

10x Visium-SPG integrated with single-nucleus RNA sequencing (snRNA-seq) enabled the generation of a dorsolateral prefrontal cortex (DLPFC) multi-omic atlas, with refined cortical sublayer definition and cell-type localization, and linked layer-specific ephrin A5 (EFNA5)-EPH receptor A5 (EPHA5) signaling to SCZ risk genes[121]. In the inferior temporal cortex from late-stage AD, Visium-SPG identified a transcriptional profile associated with proximity to amyloid beta plaque, involving dysregulation in protein degradation, inflammation, and synaptic functions, which were further validated at higher resolution using RNAscope smFISH[122], highlighting a powerful workflow in which sequencing-based methods provide discovery and targeted imaging approaches provide validation. Although the name was not defined as Visium-SPG, Visium transcriptomics, combined with quantitative immunohistochemistry (IHC), has also been used to study molecular responses to brain electrical stimulation[123].

Even when used only for transcriptomics, 10x Visium has generated new insights in neuroscience. In AD, Visium mapped plaque-associated dysregulated genes and layer-specific markers in the human middle temporal gyrus[124]. In mouse AD models, it has also been used to evaluate immunomodulatory treatments, identifying cell types and region-specific transcriptomic changes

associated with improvements in behavioral tests[125]. In CTE, spatial profiling of lesions revealed an astrocyte-enriched signature associated with neuroinflammation, extracellular matrix remodeling, and blood-brain barrier dysfunction[126]. In Multiple Sclerosis (MS), integrated 10x Visium and snRNA-seq of non-lesional brains revealed that even normal-appearing brain tissue can show glial and neuronal signatures that are similar but attenuated to those observed in active lesions[127]. Similarly, in Huntington's disease (HD) models, integration of 10x Visium with matched snRNA-seq across stages identified early mitochondrial deficits and Tcf4 dysregulation, followed by time-dependent changes in neuropeptide signaling[128].

In neuro-oncology, 10x Visium has been used to resolve spatial heterogeneity and assess treatment responses, including the identification of treatment-responsive regions in the Shh-medulloblastoma model[129] and the spatial segregation of GBM tumor cells and microenvironments[130,131]. Beyond oncology, 10x Visium also supported SCZ findings by corroborating upper-layer neuron vulnerability[132] and enabled the identification of new genes associated with alcohol dependence[133]. Additional work mapped layer-specific expression in postmortem DLPFC and associated neuropsychiatric risk genes to specific cortical layers[134].

Beyond pathology, Visium has been applied to resolve spatiotemporal patterns in transcriptional programs during nervous system development and maturation. For example, integrating TF-seqFISH with Visium and snRNA-seq enabled analysis of spatial gene regulation in human spinal cord development[135]. Furthermore, spatial atlases of aging and senescence in non-pathological DLPFC have suggested that aging-associated programs are distributed across microglia, endothelial cells, and astrocytes[136].

2.2.2 Imaging-based approaches

2.2.2.1 CosMx spatial molecular imager

CosMx spatial molecular imager (SMI) is an imaging-based platform capable of spatially profiling RNAs and proteins at true single-cell and subcellular resolution[86]. Compared with ROI-based approaches such as DSP, CosMx prioritizes spatial precision, making it particularly suited for resolving cellular neighborhoods and subcellular localization patterns, though it usually offers lower multiplexing capacity than sequencing-based whole-transcriptome methods.

Despite its advantages, its application in neuroscience remains limited, with only a few studies employing the platform to date. In PD, SMI was used to compare cortical neurons with and without alpha-synuclein pathology, identifying an excitatory neuron subtype associated with Lewy pathology, leading the authors to describe a disease-linked transcriptional signature termed Lewy-associated molecular dysfunction from aggregates (LAMDA)[137]. In AD, SMI corroborated previous findings by identifying microglial accumulation near amyloid plaques and linking this to impaired astrocyte signaling[138]. SMI was also used to explore the impact of radiotherapy on the brain, where it found prominent morphological changes in glial cells but no increase in cellular senescence markers[139].

2.2.2.2 10x Genomics Xenium

Xenium is the imaging-based platform from 10x Genomics that enables in situ profiling of RNA together with targeted protein panels at subcellular resolution[87]. Current applications largely rely on predefined transcriptomic gene panels, with ongoing expansion of multi-omic configurations.

To date, a notable multimodal combination of 10x Xenium with hyperplexed immunofluorescence imaging (HIFI) has identified that fibrotic areas triggered by several GBM therapies are pro-tumor-survival regions by encapsulating surviving glioma cells. Inhibition of treatment-associated fibrosis significantly improved survival[140]. Most neuroscience applications have used 10x Xenium mainly for spatial transcriptomics. In AD, Xenium identified a terminally inflammatory microglial (TIM) state in mouse models and localized analogous TIM-like cells near amyloid beta plaques in the human AD brain[141]. In MS, 10x Xenium identified centrifugal propagation of lesions and disease-associated glia, which emerged not only in lesion cores but also in perilesional and surrounding tissue[142]. In neuro-oncology, Xenium has been used in engineered GBM organoid models[143] and in diffuse intrinsic pontine glioma (DIPG)[144] to link tumor transcriptional changes with remodeling of surrounding glial and immune microenvironments. 10x Xenium has also been used to validate post-traumatic stress disorder (PTSD)-associated gene changes spatially[145], and to generate high-resolution spatial atlases in epilepsy models[146]. Furthermore, Xenium, combined with single-cell RNA sequencing (scRNA-seq), has helped map oligodendrocyte precursor cell (OPC) maturation trajectories[147] and identified distinct neural stem cell (NSC) niches and injury-responsive programs in the ventricular-subventricular zones[148]. Atlas-scale applications have also been reported[149,150].

It is important to note that most studies using 10x Genomics Xenium are still preprints[151-154]. Therefore, future reviews about the use of multi-omic approaches in neuroscience should address these new applications.

2.2.3 Mass spectrometry imaging

MSI, despite a long methodological history, has only recently been integrated into spatial multi-omic workflows. Most MSI-based spatial multi-omics frameworks combine spatial metabolomics with spatial transcriptomics to generate matched biochemical and gene expression profiles. Examples include integration of desorption electrospray ionization (DESI)-MSI with 10x Visium[95], and matrix-assisted laser desorption/ionization (MALDI)-MSI with Visium (spatial multimodal analysis, (SMA))[93] to align metabolite distributions with spatial transcriptional regions. Efforts toward higher spatial resolution include MALDI-MSI integration with 10x

Xenium to achieve high-resolution metabolomics and transcriptomics[94]. Likewise, the MALDI in situ hybridization (ISH) MSI framework enables in situ profiling of transcriptomic and metabolomic data from a single tissue section[91].

Beyond the integration of transcriptomics and metabolomics, recent methods have also expanded to include a proteomic layer. Single cell spatially resolved metabolic (scSpaMet) is a workflow for untargeted spatial metabolomics combined with targeted multiplexed protein imaging[97]. Complementarily, Kreutzer developed a framework that profiles whole-exome sequencing (WES), gene expression, and metabolites from the same fixed tissue, identifying high levels of correlation[90].

Further extending the molecular layers, cryogenic dual-secondary ion mass spectrometry (SIMS) imaging is a framework that, using water-gas cluster ion beam secondary ion MSI, allows integrated profiling of metabolites, lipids, and proteins at the single-cell level[92]. Finally, Bell *et al*. developed a spatial triomic workflow that integrates MALDI-MSI with MALDI-ISH and MALDI-IHC, allowing the profiling of label-free metabolites, targeted transcripts, and targeted proteins within the same tissue[96].

2.2.4 Frontiers in spatial multi-omics methods

The methods previously discussed were grouped by their primary detection modality. However, a complementary way to organize the field is by the gaps that current spatial multi-omics methods are aiming to address. Some of these approaches were developed in parallel with the platforms described earlier, but their application in neuroscience remains relatively limited, often restricted to proof-of-concept studies or a small number of disease-focused applications. Therefore, they should be considered technological frontiers that highlight where spatial multi-omics is likely to expand next, as the field moves toward higher molecular coverage, improved spatial resolution, cross-modal integration, and broader use in complex brain tissues.

2.2.4.1 Extending spatially resolved proteomics

One frontier is the incorporation of spatial proteomics into spatial workflows. This is important because RNA levels are not necessarily correlated with protein abundance, especially for secreted factors, cell-surface receptors and post-translationally regulated proteins[155]. Therefore, several methods have addressed this by extending spatially resolved transcriptomics to profile the proteome within the same sample, though they differ in strategy and trade-offs. Antibody-capture approaches, including spatial protein and transcriptome sequencing (SPOTS)[79], Spatial CITE-seq[82], and Stereo-CITE-seq[156], pair oligonucleotide-tagged antibodies with spatially barcoded capture arrays, enabling quantification of proteins alongside the transcriptome. In situ imaging approaches, such as spatially resolved transcript amplicon readout mapping with protein localization and unlimited sequencing (STARmap) PLUS[89], usually preserve subcellular localization of both RNA and protein signal at the cost of lower multiplexing capacity. SM-Omics[76] prioritizes throughput, integrating spatial transcriptomics with multiplexed immunofluorescence in an automated workflow suited for larger sample cohorts. Collectively, these approaches address the discordance between transcript and protein levels and enable more direct validation of cell states and microenvironmental phenotypes within spatial domains.

2.2.4.2 Spatial epigenomics: localization of regulatory programs

Transcriptional patterns observed across spatial regions can reflect either local regulatory remodeling (changes in chromatin accessibility, histone modification state, or TF binding) or simply the redistribution of different cell types between regions. Platforms that profile only RNA cannot discriminate between these biologically distinct scenarios. Microfluidic indexing-based spatial assay for transposase-accessible chromatin and RNA-sequencing (MISAR-seq) addresses this by enabling simultaneous spatial profiling of chromatin accessibility and transcription. When applied to the developing mouse brain, it revealed spatially organized regulatory programs governing fate determination that were not apparent from transcriptional data alone[83]. Spatial-ATAC-RNA-seq and spatial CUT&Tag–RNA-seq further preserve tissue integrity while jointly capturing open chromatin or histone modification profiles alongside the transcriptome[78].

An important contribution to this frontier is the deterministic barcoding in tissue for spatial omics sequencing (DBiT-seq) family, which uses deterministic barcoding via orthogonal microfluidic channels to co-capture transcriptomes and proteins with high spatial precision[74]. Extensions DBiT spatial ATAC-RNA-protein sequencing (ARP-seq) and DBiT spatial CUT&Tag-RNA-protein-seq (CTRP-seq) add chromatin accessibility and histone modification readouts alongside proteins and the transcriptome from a single tissue section[80]. Applied to postnatal cortical maturation and a lysolecithin-induced demyelination model, these methods revealed that cortical layer identity can be maintained at the chromatin level even when transcriptional output declines and identified chromatin priming at myelination-associated loci preceding transcriptional activation, establishing the regulatory sequence of remyelination programs[80]. A pathology-compatible extension, Patho-DBiT, adapts this framework for FFPE tissue and, as far as we know, is currently the only demonstrated spatial platform capable of profiling non-polyadenylated small RNAs, including tRNAs, accessing a class of molecular alterations that all poly(A)-based spatial methods systematically miss[157].

2.2.4.3 Higher-dimensional spatial multi-omics: From 3 to 5 layers

A third frontier extends beyond two-layer co-profiling toward three to five molecular modalities within a unified spatial coordinate system. This is motivated by the recognition that chromatin accessibility, histone modifications, transcription, and protein levels

each reflect distinct and partially independent aspects of cellular state. Spatial assay for accessible chromatin, cell lineages, and gene expression with sequencing (SPACE-seq)[77] extends the 10x Visium workflow to jointly capture chromatin accessibility, whole transcriptome, and mitochondrial DNA (mtDNA) variants, co-registering regulatory programs with somatic mtDNA variations. Multi-omics in situ pairwise sequencing (MiP-seq) enables subcellular-resolution profiling of DNA, RNA, and proteins via padlock-probe ligation and can be co-registered with functional imaging modalities to link molecular states directly to physiological readouts at the level of individual cells[88]. Spatial-Mux-seq represents the current dimensionality frontier, simultaneously profiling five layers in space: two histone modifications, chromatin accessibility, the whole transcriptome, and a targeted protein panel; and it was capable of identifying spatially dynamic epigenetic remodeling within radial glia niches during neuronal differentiation[84].

2.2.4.4 Stable regulatory layers and higher spatial resolution

A fourth frontier targets molecular layers that are more stable than chromatin accessibility, while simultaneously increasing spatial resolution toward subcellular scales. Unlike chromatin accessibility, which often reflects dynamic regulatory activity and can change rapidly in response to developmental cues or environmental stimuli, DNA methylation provides a comparatively stable epigenetic mark of cell identity, lineage history, and developmental state[158,159]. Spatial joint profiling of the DNA methylome and transcriptome (Spatial-DMT) explores this stability by joint profiling the DNA methylome and transcriptome in tissue sections, revealing region- and cell-type-specific coordination between methylation states and spatial gene expression in the mouse brain[85]. In the brain, where neurons, glia, and vascular cells maintain distinct and stable epigenetic identities, methylation spatial profiling offers a robust signal for cell-type mapping and developmental lineage tracing that transcriptomic approaches alone cannot address[160].

Increasing the resolution represents another important frontier. Earlier sequencing-based spatial platforms, such as 10x Visium, profile at spot-level resolution of 55 μm, capturing signals from multiple cells simultaneously without resolving subcellular compartments. High-density DNA nanoball-patterned arrays reduce the capture pitch to approximately 0.22 μm. Stereo-seq applies these arrays to intact tissue sections, capturing the whole transcriptome while preserving architecture, although cell assignment remains computational and the capture grid does not correspond directly to effective molecular resolution[72]. Spatial enhanced-resolution single-cell sequencing (Stereo-cell) instead captures intact cells on-chip and integrates morphological imaging with transcriptomic and antibody-based protein readouts, extending unbiased profiling to large or fragile cells and to extracellular vesicles, at the cost of native tissue context[81]. In principle, this transition brings the capture grid below the scale of neuronal processes, opening the possibility of resolving transcripts localized to dendrites and axons of the same neuron and of delineating molecular interfaces at specialized structures such as the glia limitans and the blood-brain barrier. Whether this potential is realized in practice depends on capture efficiency, lateral diffusion, and molecule-to-cell assignment rather than on feature pitch alone. Together, Spatial-DMT, Stereo-seq, and Stereo-cell illustrate how expanding the spatial multi-omics methods reveals complementary dimensions of brain physiology in health and disease (Table 2).

3. Translational Relevance of Single-Cell and Spatial Multi-Omics

The translational relevance of single-cell and spatial multi-omics relies on their capability to change how disease mechanisms are mapped, compared, and prioritized. A recent study outside neuroscience illustrates this trajectory. Using deep visual proteomics on archived FFPE biopsies from toxic epidermal necrolysis, Nordmann *et al.* identified cell-type-resolved interferon and Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway activation, functionally validated JAK inhibition in experimental models, and reported rapid clinical recovery in treated patients[161]. This study demonstrates that spatially resolved omics can connect tissues from diseases to a druggable mechanism and, in selected contexts, guide therapeutic intervention.

In neuroscience, comparable clinical translation remains less mature, but the conceptual path is similar. Current studies show that single-cell and spatial multi-omics can localize disease-associated signals to specific cell populations, anatomical structures, and molecular regulatory programs. This is particularly important in brain disorders, where similar clinical or histopathological phenotypes may arise from different combinations of neuronal vulnerability, glial reactivity, immune infiltration, or regional tissue damage[23]. By linking these changes to chromatin accessibility, transcriptional state, protein abundance, and spatial organization, these methods can generate more precise hypotheses for biomarker discovery, patient stratification, therapeutic target selection, and evaluation of treatment response.

Table 3 summarizes representative disease-associated studies discussed in this review. Organized by disease area rather than platform, the table includes paired and integrated multi-omic approaches, multimodal methods, and representative single-omic single-cell and spatial studies.

4. Pitfalls and Current Limitations in Single-Cell and Spatial Multi-Omic Methods

Single-cell and spatial multi-omics have expanded the molecular resolution of neuroscience. However, these methods also introduce technical, analytical, and tissue-specific biases that can affect cell-state definition, spatial assignment, cross-study reproducibility, and translational interpretation (Figure 2). These limitations should therefore be carefully considered when interpreting results.

Table 3. Representative disease-focused applications of single-cell, single-nucleus, spatial, and multi-omic approaches in neuroscience. The table includes paired and integrated multi-omic studies, multimodal approaches, and representative single-omic studies.

Key finding	Methods	Modalities profiled in the study**	Tissue/model	Reference
Alzheimer's disease, AD-related pathology, and tauopathies				
Identified AD-associated cis-regulatory elements and candidate regulators, including ZEB1 and MAFB	snMultiome	RNA + chromatin accessibility	Human postmortem brain	Anderson *et al*., 2023[19]
Identified two AD phases via pseudoprogression modeling (early microglial/astrocyte activation with SST+ interneuron loss, later excitatory neuron and PVALB+ /VIP+ loss) spatially confirmed with MERFISH	snRNA-seq + snATAC-seq + snMultiome + MERFISH	RNA + chromatin accessibility	Human postmortem brain	Gabitto *et al*., 2024[20]
Defined cell-subtype-specific cis-regulatory elements and TFs (e.g., ELK1 in excitatory neurons, APOE in microglia), yielding 53 new candidate LOAD genes	snRNA-seq + snATAC-seq	RNA + chromatin accessibility	Human postmortem brain	Gamache *et al*., 2023[21]
Identified oligodendrocyte-associated regulatory module linking APOE and CLU, and highlighted SREBF1 as a disease-relevant TF	snRNA-seq + snATAC-seq	RNA + chromatin accessibility	Human postmortem brain	Morabito *et al*., 2021[22]
Identified 32 shared and 14 disease-specific cell states across AD, FTD, and PSP, with selective vulnerability of layer 5 IT neurons in AD, layer 2/3 IT neurons in FTD, and layer 5/6 neurons in PSP	snRNA-seq + snATAC-seq	RNA + chromatin accessibility	Human postmortem brain	Rexach *et al*., 2024[23]
Epigenomic erosion in entorhinal cortex/hippocampus excitatory neurons is associated with AD progression, while preserved epigenomic stability was linked to cognitive resilience	snMultiome	RNA + chromatin accessibility	Human postmortem brain	Liu *et al*., 2025[24]
Preliminary data suggest a selective vulnerability of the suprachiasmatic nucleus to tau pathology and immune dysregulation in early AD, with reduced AVP+ and VIP+ neuron counts relative to neighboring hypothalamic nuclei	GeoMx DSP	Proteins	Human postmortem brain	Son *et al*., 2023[106]
Identified 18 differentially expressed proteins in AD neurons, including elevated neprilysin (also elevated in microglia) and the neuroinflammatory markers CD11b, CD11c, and CD163	GeoMx DSP	Proteins	Human postmortem brain	Gholampour *et al*., 2025[107]
AD, PART, and CTE can be differentiated	GeoMx DSP	Proteins	Human	Richardson *et al*., 2025[108]

based on the proteomic composition of NFT- and non-NFT-bearing neurons, which are largely correlated with the presence or absence of amyloid beta			postmortem brain	
Found that synaptic health is inversely correlated with local pTau burden, and that neurons in possible PART cases are proteomically more similar to AD than to "real" PART cases	GeoMx DSP	Proteins	Human postmortem brain	Walker *et al*., 2024[109]
Found that amyloid beta-immunotherapy-induced smooth muscle cell loss is linked to peripheral T-cell infiltration rather than resident perivascular macrophages, with both tissue-resident and monocyte-derived Trem2+ macrophages accumulating around vascular amyloid deposits	GeoMx DSP + IF	RNA + proteins	PDAPP and hTau APP KI mice	Taylor *et al*., 2024[110]
CAA accumulation is associated with brain-wide vascular and inflammatory changes, including distinct protein profiles between CAA-positive and CAA-negative vessels, with progression differing by sex	GeoMx DSP + IHC	RNA + proteins	APPSw mouse model	Krick *et al*., 2025[111]
TREM2 risk-variant carriers show reduced plaque-associated microglia and increased dystrophic neurites and tau pathology compared to non-carriers, with brain-region-dependent differences in immune response	GeoMx DSP + IHC/IF	RNA + proteins	Human postmortem brain	Prokop *et al*., 2019[112]
Found strong local immune activation (DAM markers MERTK, CLEC7A, GPNMB) around amyloid beta plaques in AD/MIX cases, whereas pure Lewy body disease showed a more attenuated, globally distributed immune signature	GeoMx DSP + IHC	RNA + proteins	Human postmortem brain	Bathe *et al*., 2024[113]
Found that NFT-bearing neurons and their microenvironments in cognitively resilient individuals show lower neuroinflammation (CD68, GFAP) and reduced oxidative/energetic stress (PINK1, IDH1), alongside markers of better-preserved synapses	GeoMx DSP	Proteins	Human postmortem brain	Walker *et al*., 2022[114]
Found that retinal Oligo-tau and PHF-tau levels correlate strongly with brain Braak stage, NFT burden, and cognitive scores, with GeoMx confirming elevated retinal pTau in MCI patients	GeoMx DSP + IHC/IF	Protein	Human retina	Shi *et al*., 2024[115]

Identified amyloid beta-proximal depletion of autophagy, ubiquitination and synaptic genes (UCHL1, ATG5, SHANK3) alongside enrichment of the complement gene C3, validated at cellular resolution using RNAscope smFISH	Visium-SPG	RNA + protein	Human postmortem brain	Kwon *et al*., 2023[122]
Identified three novel amyloid beta/tau-associated genes (KIF5A, PAQR6, SLC1A3) alongside known DEGs, validated at single-cell resolution via RNAscope	10x Visium	RNA	Human postmortem brain	Chen *et al*., 2022[124]
Anti-CD4 antibody and NK cell supplement treatment improved behavior impairment in 5xFAD mice, and identified the specific brain cell types and regions whose transcriptomic changes tracked with this improvement	10x Visium	RNA	5xFAD mouse model	Lee *et al*., 2024[125]
Increasing microglial density around plaques could drive astrocytes toward a more neurotoxic phenotype, disrupting neuronal synaptic balance via increased GABAergic and decreased glutamatergic signaling	CosMx SMI + Stereo-seq	RNA	AD mouse model	Mallach *et al*., 2024[138]
Identified TIMs enriched with age and APOE4 genotype, functionally impaired in amyloid beta clearance, and modulated by aducanumab treatment, and localized analogous cells near amyloid beta plaques in human AD cortex	snMultiome + 10x Xenium	RNA + chromatin accessibility	AD mouse model and human postmortem brain	Millet *et al*., 2024[141]
VTA dopamine neurons in 3xTg-AD mice are hyperexcitable due to reduced SK channel activity driven by upregulated CK2, with pharmacological CK2 inhibition restoring normal firing pattern	Patch-seq + IHC	RNA + electrophysiology + proteins	AD mouse model	Blankenship *et al*., 2024[55]
Microglia near amyloid plaques undergo glycolytic and lipid-metabolism reprogramming (Hif1a, Apoe, Lpl) at the expense of homeostatic markers, with disease-reactive subtypes preferentially localized near VIP+/PVALB+ interneurons	10x Xenium	RNA	AD mouse model and human postmortem brain	Saito *et al*., 2025[162]
Identified a 21-gene CTE lesion signature, with GFAP, APLNR, AQP1, and TNC upregulated, implicating astrocytic activation, neuroinflammation, blood-brain barrier dysfunction, and extracellular matrix remodeling	10x Visium + IHC	RNA + proteins	Human postmortem brain	Suter *et al*., 2025[126]
Frontotemporal dementia, C9orf72-related disease, and amyotrophic lateral sclerosis				

Found reduced STMN2 and endolysosomal trafficking proteins (retromer/GARP complexes) and elevated CNTNAP2 in ALS motor neurons, with similar changes present in neurons both with and without detectable TDP-43 cytoplasmic inclusions, indicating early, pathology-independent protein dysregulation	Single-cell mass spectrometry proteomics	Proteins	Human postmortem spinal motor neurons	Guise *et al*., 2024[163]
Each of the five types of dementia (AD, C9orf72, MAPT, FTLD-TDP, GRN) displayed a largely distinct cortical-layer protein signature, with P2ry12 downregulation the only marker shared across all five	GeoMx DSP	Proteins	Human postmortem brain	Bolen *et al*., 2025[118]
Parkinson's disease and synucleinopathies				
Microglia and oligodendrocytes are further altered in PD beyond normal aging, identifying a disease-associated oligodendrocyte subtype linked to loss of CARNS1 and to 89 PD-associated SNP loci	snMultiome	RNA + chromatin accessibility	Human postmortem brain	Adams *et al*., 2024[25]
Identified 13 DAM subpopulations and found that a CD83+/HIF1A+ subpopulation enriched for antigen-presentation and heat-shock genes is selectively depleted in the substantia nigra in PD, while distinct pro-inflammatory subpopulations are enriched there	snMultiome	RNA + chromatin accessibility	Human postmortem brain	Chatila *et al*., 2023[28]
Profiled 113,207 substantia nigra nuclei to annotate 128,724 cis-regulatory elements and build 3D chromatin contact maps, identifying 656 target genes of dysregulated regulatory elements, uncovering potential and known PD risk genes	snRNA-seq + snATAC-seq + ChIP-seq + Hi-C	RNA + chromatin accessibility + 3D chromatin architecture + histone modifications	Human postmortem brain	Lee *et al*., 2023[26]
Identified a subpopulation of cortical glutamatergic excitatory neurons with robust SNCA overexpression and altered activity of the TFs YY1, SP3, and KLF16, driving PD-associated gene dysregulation	snRNA-seq + snATAC-seq	RNA + chromatin accessibility	Human postmortem brain	Shwab *et al*., 2024[27]
APP was the top network hub gene, downregulated in both PD and MSA-P substantia nigra, with MSA-P showing more pronounced immune, mitochondrial, and protein-synthesis pathway downregulation than PD	GeoMx DSP	RNA	Human postmortem brain	Shin *et al*., 2025[116]
Found alpha-synuclein accumulation in the myenteric plexus of all PD patients,	GeoMx DSP + IHC	RNA + proteins	Human colon and myenteric plexus	Shin *et al*., 2025[117]

associated with upregulated neuronal regeneration genes (TUBB2A, S100B), and upregulated interferon/lymphocyte-activation genes in the colon intestinal epithelium				
Found that Lewy pathology selectively affects layer 5 IT and layer 6b excitatory neurons, and defined an associated "LAMDA" transcriptional signature (downregulated synaptic, mitochondrial, and proteostasis genes; upregulated DNA-repair and complement genes) shared between human PD cortex and a mouse alpha-synuclein fibril model	GeoMx DSP + CosMx SMI	RNA	PD mouse model and human postmortem brain	Goralski *et al*., 2024[137]
Hippocampal CA1 neurons were most vulnerable to alpha-synuclein pathology, followed by CA2/3, while DG neurons were nearly resistant, with Plk2 expression partially explaining this vulnerability gradient	10x Xenium + IF	RNA + proteins	PD mouse model	Horan-Portelance *et al*., 2026[164]
Psychiatric and substance-use-related disorders				
Profiled 2.8 million nuclei from 388 human prefrontal cortices to identify over 550,000 cell-type-specific regulatory elements and 1.4 million single-cell eQTLs across 28 cell types	snMultiome + snRNA-seq + snATAC-seq	RNA + chromatin accessibility	Human postmortem brain	Emani *et al*., 2024[29]
Diagnosis-related dysregulation concentrated in excitatory neurons while genetic risk predominantly affected glial and endothelial cells, with INO80E and HCN2 dysregulated in layer 2/3 excitatory neurons, influenced by SCZ polygenic risk	snRNA-seq + snATAC-seq	RNA + chromatin accessibility	Human postmortem brain	Gerstner *et al*., 2025[30]
Identified EFNA5-EPHA5 as a consensus SCZ-risk ligand-receptor pair via both data-driven cell-cell communication and clinical risk-gene analyses, with co-expression concentrated in layers 5/6 excitatory neurons	snRNA-seq + 10x Visium (SPG)	RNA + protein	Human postmortem brain	Huuki-Myers *et al*., 2024[121]
Reduced GABAergic and increased principal neuron abundance in upper cortical layers, with the most extensive transcriptomic changes in upper-layer GABAergic neurons (downregulated energy metabolism, upregulated neurotransmission genes)	snRNA-seq + 10x Visium + IHC	RNA + protein	Human postmortem brain	Batiuk *et al*., 2022[132]
Mapped layer-enriched gene expression	10x Visium	RNA	Human	Maynard *et al*., 2021[134]

across the six DLPFC cortical layers and validated novel markers via smFISH, showing differential layer-specific expression of genes associated with SCZ and autism spectrum disorder			postmortem brain	
Profiled over 2 million nuclei from 111 postmortem brains and found PTSD-associated gene alterations concentrated in inhibitory neurons, endothelial cells, and microglia, implicating glucocorticoid signaling, GABAergic transmission, and neuroinflammation, with Xenium confirming a subset of these genes at single-cell spatial resolution	snATAC-seq + snRNA-seq + snMultiome + 10x Xenium	RNA + chromatin accessibility	Human postmortem brain	Hwang *et al*., 2025[145]
Identified Cpa6 as a hub gene of downregulated co-expression modules in the alcohol-sensitive inhibitory neuron subtype and in oligodendrocytes, and Gpc5 as a hub gene of an upregulated astrocyte module that was cross-validated in human postmortem data	snRNA-seq + 10x Visium	RNA	Alcohol dependence mouse model	Salem *et al*., 2024[133]
Neuro-oncology				
Developed the DSP SPG assay, and applied it to show giant cell GBM has higher CD3+/CD8+ T-cell infiltration and distinct protein signatures (S100B, CD44, MBP) compared to standard GBM	GeoMx DSP (SPG)	RNA + proteins	Human GBM	Bonnett *et al*., 2023[99]
Cellular tumor regions were actively cycling (G1/G2M phase) while necrotic/perinecrotic regions were non-cycling. IDH-wildtype tumors showed increased mesenchymal and progenitor-like states, and immune cell infiltration varied between spatially proximal but transcriptionally distinct tumor domains	GeoMx DSP + CosMx SMI + 10x Visium + 10x Xenium	RNA	Human GBM	Moffet *et al*., 2023[100]
Hypoxic GBM regions showed upregulated CD44, beta-catenin, and B7-H3 alongside downregulated VISTA, CD56, KI-67, CD68, and CD11c, while PD-L1/PD-1 were unaffected by oxygenation status	GeoMx DSP	Proteins	Human GBM	Petterson *et al*., 2023[101]
Immune-marker heterogeneity was primarily between patients, with each primary/recurrence pair following a different trajectory in both tumor core and periphery. HLA-DR and B7-H3 were differentially localized and proposed as candidate targets	GeoMx DSP	Proteins	Human GBM	Loussouarn *et al*., 2023[102]

Identified PDGFRβ as a driver gene for mesenchymal stem cell differentiation into perivascular stromal cells that promote microvascular proliferation, which was associated with increased immunosuppression and poor outcome	scRNA-seq + GeoMx DSP + IF	RNA + proteins	Human GBM	Poon *et al*., 2025[103]
10 of 27 immuno-oncology proteins - including CD4, CD8A, B7-H3, PD-L1, FOXP3, and CD44, were significantly increased in the tumor core of MGMT-methylated versus unmethylated IDH-wildtype GBM	GeoMx DSP	Proteins	Human GBM	Barber *et al*., 2021[104]
PD-1 blockade (nivolumab) produced no measurable spatial transcriptomic changes in tumor cells or tumor-associated macrophages in recurrent GBM, consistent with the drug's lack of clinical benefit	GeoMx DSP	RNA	Human GBM	Artzi *et al*., 2025[105]
CDK4/6 inhibition (palbociclib) reduced tumor heterogeneity and induced neuronal differentiation throughout the tumor bulk, but a spatially distinct tumor-microenvironment interface enriched for astrocytes and microglia continued to proliferate despite treatment	10x Visium	RNA	Medulloblastoma mouse model	Vo *et al*., 2023[129]
Characterized the GL261-GSC immunocompetent mouse model at multiple disease stages and treatments, finding it recapitulates the human TME GBM subtype and identifying immunoevasion (Cd274/PD-L1) and immunosuppression (Csf1r, Arg1, Mrc1) targets	snRNA-seq + 10x Visium	RNA	GBM mouse model	García-Vicente *et al*., 2025[130]
OPC-like and NPC-like tumor cell states spatially segregate within GBM, with perinecrotic regions more immunosuppressive than the endogenous microenvironment and perivascular regions more pro-inflammatory	snRNA-seq + 10x Visium	RNA	Human GBM	Liu *et al*., 2024[131]
Anti-CSF-1R therapy triggers fibrotic pro-tumor-survival niches, mediated by perivascular fibroblast-like cells via TGF-beta signaling, that encapsulate dormant, immune-evading glioma cells, and that combined inhibition of fibrosis and CSF-1R significantly improved survival	scRNA-seq + 10x Xenium + HIFI	RNA + proteins	GBM mouse model and human GBM	Watson *et al*., 2024[140]
CRISPR-engineered brain organoids carrying Proneural (TERT + TP53) or	scRNA-seq + 10x Xenium	RNA	Engineered GBM brain organoids	Ishahak *et al*., 2025[143]

Mesenchymal (TERT + NF1 + PTEN) GBM mutations disrupt neurodevelopmental gene networks and, upon xenotransplantation, form tumors that recapitulate subtype-specific spatial organization matching human GBM				
Identified PIK3CA/MTOR as targetable dependencies in DIPG; phosphoproteomics revealed that PI3K inhibition (paxalisib) triggers compensatory PKC activation, and combining PKC inhibition (enzastaurin) with metformin and radiotherapy synergistically extended survival	10x Xenium + bulk RNA-seq + bulk ATAC-seq + Phosphoproteomics	RNA + chromatin accessibility + phosphoproteome	DIPG human cell and mouse models	Duchatel *et al*., 2024[144]
Mapped ECM genes across GBM and normal brain, identifying at least four GBM cell populations with distinct, spatially enriched ECM expression profiles, and elevated ECM proteins (IGFBP2, MGP, ANXA1, ANXA2) distinguishing GBM from lower-grade astrocytoma	10x Xenium + IHC/IF	RNA + proteins	Human GBM	De *et al*., 2025[151]
TIL-generating gliomas were marked by IL7R expression, structured perivascular immune clustering, and the metabolic gene ACSS3, while TIL-resistant tumors showed neuronal lineage signatures, immunosuppressive transcripts (TOX, FERMT1), and tumor-connected macrophages	scRNA-seq + 10x Xenium + CODEX	RNA + proteins	Human glioma	Hotchkiss *et al*., 2026[153]
Identified that, across tumor core/edge regions in WHO grade 2-4 diffuse gliomas, edge-enriched SOX4+ NPC-like cells and GPNMB+ MES-like cells mark distinct regional metabolic vulnerabilities in IDH-wildtype GBM	10x Xenium + imaging mass cytometry + MSI	RNA + proteins + metabolites	Human gliomas	Ma *et al*., 2025[154]
EGFR amplification via ecDNA, rather than linear chromosomal amplification, could drive a distinct tumor microenvironment enriched in MES-like and pericyte cells in close spatial proximity, with higher hypoxic/metabolic activity	10x Xenium + WGS + Hi-C + bulk RNA-seq	RNA + 3D chromatin architecture + genome	Human gliomas	Zhao *et al*., 2025[165]
A subset of cells in IDH-mutant gliomas are hybrid cells firing single action potentials with a novel GABAergic-neuron/OPC transcriptional signature (termed GABA-OPCs), and higher hybrid-cell firing activity	Patch-seq	RNA + electrophysiology + morphology	Human gliomas	Curry *et al*., 2024[56]

was associated with better patient outcomes				
Microglia- and monocyte-derived TAMs are self-renewing populations that compete for space and can be depleted via CSF1R blockade. Microglia-derived TAMs are predominant at diagnosis but outnumbered by monocyte-derived TAMs at recurrence, especially in hypoxic regions	scRNA-seq + CITE-seq	RNA + surface proteins	Human GBM and glioma mouse model	Pombo Antunes *et al*., 2021[41]
Cranial radiotherapy induces glial morphological changes but does not increase cellular senescence or SASP markers	CosMx SMI + IHC	RNA + proteins	Mouse model	Kuil *et al*., 2025[139]
Multiple sclerosis				
Non-lesional MS tissue shows oligodendrocytes with lower myelination gene expression and higher CRYAB+ oligodendrocytes across all three regions. Also presents disease-specific microglial subgroups (HIF1A+/SPP1+ specific to MS, SOCS6+/MYO1E+ specific to secondary demyelination) with NAWM changes similar but attenuated to those seen in active lesions	snRNA-seq + 10x Visium	RNA	Human postmortem brain	Lam *et al*., 2023[127]
Disease-associated glia arise independently of lesions and precede their formation, dynamically appearing and resolving over the disease course, while active lesions evolve via centrifugal propagation from the core outward	10x Xenium	RNA	Mouse EAE model and human MS spinal cord	Kukanja *et al*., 2024[142]
In a lysolecithin demyelination model, CD11c+ microglia/macrophages spread beyond the lesion core into distal white-matter tracts, while lesion-resident microglia resolve into neuron/myelin-supportive (Nrxn3, Cntn2) versus pro-inflammatory/phagocytic (Apoe, Abca1, Btk) subpopulations that partly recapitulate developmental programs	DBiT spatial ARP-seq + DBiT spatial CTRP-seq + CODEX	RNA + chromatin accessibility + histone modifications + proteins	Lysolecithin-induced demyelination mouse model	Zhang *et al*., 2025[80]
Huntington's disease				
Mutant Htt cell-autonomously decreased GABAergic synaptic output in striatal neurons and identified dysregulated genes correlating with these deficits, with HDAC1/3 inhibition (RGFP109) partially reversing the functional and transcriptional phenotypes	Patch-seq	RNA + electrophysiology + morphology	HD mouse model cells	Paraskevopoulou *et al*., 2021[57]

Mitochondrial deficits emerge earliest, followed by early Tcf4 dysregulation linked to cortical changes. Time-dependent dysregulation of neuropeptide Y/cAMP-PKA signaling implicated in differential Drd1 vs Drd2 neuron vulnerability	10x Visium + snRNA-seq	RNA	HD mouse model	Burns *et al*., 2025[128]
Epilepsy				
Identified physical interactions between microglia and T cells with mutually enhanced pro-inflammatory function, and integrin-collagen signaling as the main mechanism of immune cell infiltration	CITE-seq	RNA + surface proteins	Human epileptic lesions	Kumar *et al*., 2022[40]
Episodic memory impairment in TLE is associated with subregion-specific molecular signatures independent of hippocampal cell loss, demographic variables, and disease characteristics, with the strongest signal in CA3, where BDNF emerged as a central hub gene for memory-related networks	GeoMx DSP	RNA + proteins	Human temporal lobe epilepsy tissue	Busch *et al*., 2022[119]
Identified Spp1, Trem2, and Cd68 as consistently upregulated across glial cell types and spatial data, and Penk, Sorcs3, and Plekha2 as consistently upregulated across neuronal cells	scRNA-seq + snRNA-seq + 10x Xenium	RNA	Epilepsy mouse model	Liu *et al*., 2024[146]
Ischemia				
Ischemic core border showed decreased neuronal markers (Map2, NeuN) alongside increased immune infiltration (Iba1, CD45, CD11b), autophagy and neurodegenerative-related proteins (BAG3, CTSD, BACE1, APP, A β42, phospho-tau), while the peri-infarct region showed a milder profile dominated by astrocytic reactivity and phospho-tau, with peri-infarct normal tissue largely unaffected	GeoMx DSP	Proteins	Ischemic stroke mouse model	Noll *et al*., 2022[120]
Neural stem cells have a distinct DNA methylome despite near-identical transcriptomes to common astrocytes, and ischemia transiently reprograms striatal astrocytes into this stem-cell methylome via DNMT3A, whose conditional knockout abolished ischemia-induced neurogenesis	scNMT-seq	RNA + chromatin accessibility + DNA methylation	Ischemia mouse model	Kremer *et al*., 2024[36]

**Modalities include both omic-scale profiling and targeted validation (e.g., IHC, IF), therefore, the presence of multiple modalities does not necessarily indicate simultaneous or paired multi-omic profiling. AD: Alzheimer's disease; ZEB1: zinc finger E-box binding homeobox 1; MAFB: MAF bZIP transcription factor B; snMultiome: single-nucleus multiome; SST: somatostatin; PVALB: parvalbumin; VIP: vasoactive intestinal peptide; FISH: fluorescence in situ hybridization; MERFISH: multiplexed error-robust fluorescence

in situ hybridization; snRNA-seq: single-nucleus RNA sequencing; ATAC: assay for transposase-accessible chromatin; TFs: transcription factors; APOE: apolipoprotein E; LOAD: late-onset Alzheimer's disease; CLU: clusterin; SREBF1: sterol regulatory element binding transcription factor 1; FTD: frontotemporal dementia; PSP: progressive supranuclear palsy; IT: intratelencephalic; AVP: arginine vasopressin; DSP: digital spatial profiling; PART: primary age-related tauopathy; CTE: chronic traumatic encephalopathy; NFT: neurofibrillary tangle; CAA: cerebral amyloid angiopathy; IHC: immunohistochemistry; TREM2: triggering receptor expressed on myeloid cells 2; DAM: disease-associated microglia; PINK1: PTEN induced kinase 1; PD: Parkinson's disease; MSA-P: multiple system atrophy, parkinsonian type; HD: Huntington's disease; SCZ: schizophrenia; PTSD: post-traumatic stress disorder; GBM: glioblastoma multiforme; DIPG: diffuse intrinsic pontine glioma; scRNA-seq: single-cell RNA sequencing; ATAC-seq: assay for transposase-accessible chromatin using sequencing; smFISH: single-molecule fluorescence in situ hybridization; SPG: spatial proteogenomics; CITE-seq: cellular indexing of transcriptomes and epitopes by sequencing; CODEX: co-detection by indexing; MSI: mass spectrometry imaging; scNMT-seq: single-cell nucleosome, methylation and transcription sequencing; HIFI: hyperplexed immunofluorescence imaging; DLPFC: dorsolateral prefrontal cortex; OPC: oligodendrocyte precursor cell; PD-1: programmed cell death protein 1; LAMDA: Lewy-associated molecular dysfunction from aggregates; FTLD-TDP: frontotemporal lobar degeneration with TDP-43 pathology; ALS: amyotrophic lateral sclerosis; MCI: mild cognitive impairment; pTau: phosphorylated tau; PHF-tau: paired helical filament tau; MS: multiple sclerosis; TLE: temporal lobe epilepsy; snATAC-seq: single-nucleus assay for transposase-accessible chromatin sequencing; ChIP-seq: chromatin immunoprecipitation sequencing; Hi-C: high-throughput chromosome conformation capture; IF: immunofluorescence; WGS: whole-genome sequencing; CRISPR: clustered regularly interspaced short palindromic repeats; VTA: ventral tegmental area; NAWM: normal-appearing white matter; TIM: terminally inflammatory microglia; TAM: tumor-associated macrophage; TME: tumor microenvironment; TIL: tumor-infiltrating lymphocyte; NPC: neural progenitor cell; MES: mesenchymal; GABA: gamma-aminobutyric acid; NK cell: natural killer cell; EAE: experimental autoimmune encephalomyelitis; ECM: extracellular matrix; SASP: senescence-associated secretory phenotype; HLA-DR: human leukocyte antigen-DR; CSF-1R: colony-stimulating factor 1 receptor; TGF-β: transforming growth factor beta; PI3K: phosphoinositide 3-kinase; PKC: protein kinase C; PKA: protein kinase A; CK2: casein kinase 2; CDK4/6: cyclin-dependent kinases 4 and 6; HDAC1/3: histone deacetylases 1 and 3.

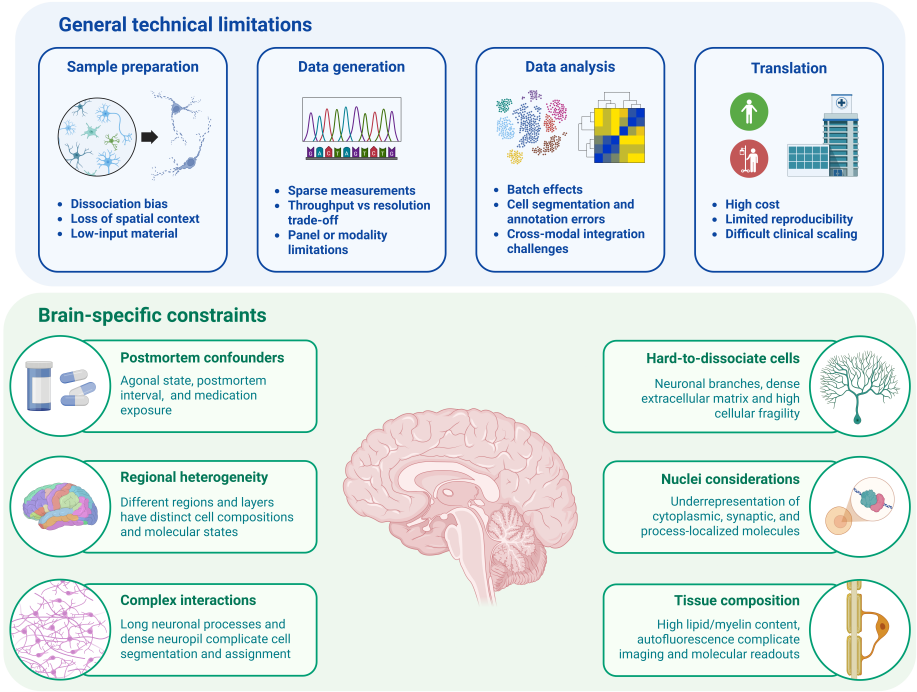

Figure 2. General and brain-specific limitations of single-cell and spatial multi-omics in neuroscience. General limitations include dissociation bias, data sparsity, analytical variability, high costs, and limited clinical scalability. Brain-specific constraints include postmortem confounders, regional heterogeneity, complex cytoarchitecture, difficult cell dissociation, incomplete nuclear profiles, and interference from lipid-rich tissue and autofluorescence. Created in BioRender. Ikeda, V. (2026) https://biorender.com/m7msl51.

4.1 Limitations of single-cell methods

A major limitation of single-cell methods is data sparsity. Single-cell datasets typically contain large entries of zeros that can be due either to methodological limitations or to biologically non-expressed values for the gene[166], often making it impossible to distinguish between the two. Although several imputation methods were developed to overcome this limitation, it remains a challenge to handle appropriately, as these methods rely on their own data (circularity), which can artificially amplify the signal, leading to inflated correlations[166].

Another limitation is cell-type annotation, which often relies on unsupervised clustering followed by manual curation, introducing subjectivity and limiting reproducibility across studies[166]. This limitation becomes more complex in multi-omic datasets, because different molecular layers may capture overlapping but non-identical aspects of cellular identity and state, meaning that transcriptomic, epigenomic, proteomic, or metabolomic profiles do not always classify cells in the same way[167]. In addition, partial or context-dependent correlations between chromatin accessibility and gene expression further reflect the complexity of gene regulation, which can be influenced by additional regulatory layers such as DNA methylation, histone modification, and trans-acting factors that are frequently not captured in current approaches[168].

4.2 Limitations of spatial omics methods

Spatial multi-omics introduces additional challenges. Most platforms involve tradeoffs between multiplexing, resolution, and throughput. Sequencing-based approaches typically maximize multiplexing but may average signals across spots or ROIs, whereas imaging-based platforms maximize spatial resolution but often rely on targeted panels. Mass-spectrometry imaging profiles metabolites and small molecules, but annotation and spatial resolution depend strongly on the instrument, tissue preparation, and analytical workflow.

These differences directly affect biological interpretation. In spot- or ROI-based methods, aggregated measurements can hide cellular heterogeneity and confound cell-type attribution[169]. In imaging-based approaches, segmentation determines which transcripts or proteins are assigned to each cell, making cell boundaries, marker selection, and computational pipelines important analytical variables[170]. Similar issues apply to spatial multi-omic approaches based on adjacent tissue slides across modalities, where imperfect alignment can affect the interpretation of relationships between transcripts, proteins, metabolites, and histological structures[171].

A consequence of these platform-specific differences is limited cross-platform compatibility. Spatial datasets are not always interchangeable because different methods represent tissue through different units of analysis (e.g. spots, ROIs, or segmented cells). Therefore, even datasets generated from similar biological material, measuring the same molecular layer, may capture different aspects of tissue organization[172]. This is particularly relevant in the brain, where disease-associated changes may occur across multiple spatial scales.

For this reason, harmonization cannot rely only on batch correction[173]. Integration methods can reduce technical variation, but they cannot recover signals that were measured at a different spatial unit. This limits meta-analysis, cross-cohort validation, disease biomarker discovery, reference atlas construction, and the development of AI models trained on heterogeneous spatial multi-omic datasets[174].

Finally, spatial proximity cannot be extrapolated to interaction or causality. Co-localization between cell types, transcripts, proteins, or metabolites may suggest potential communication or shared microenvironmental regulation, but these associations require validation using orthogonal evidence, such as higher-resolution imaging, perturbation experiments, functional assays or independent protein readouts[175].

4.3 Brain-specific limitations

Brain tissue also presents specific challenges. Molecular profiles change substantially across anatomical regions, cortical layers, white and gray matter compartments, and disease-associated microenvironments[176,177]. Consequently, differences in dissection, section orientation, anatomical annotation, or sampling depth can introduce molecular variation that reflects regional anatomy rather than disease mechanisms.

These anatomical differences are compounded by the physical and biochemical properties of brain tissue. White matter and myelin-rich regions have high lipid content which can affect probe penetration, autofluorescence, and image interpretation[178,179]. Because spatial transcriptomic readouts are sensitive to tissue handling, permeabilization, RNA diffusion, and capture efficiency, these properties can influence molecular recovery[180]. These trade-offs are especially important when comparing tissue compartments with distinct composition and molecular accessibility.

Human brain studies are further shaped by sample-level covariates. Postmortem interval, agonal state, RNA integrity, fixation, freezing, storage time, tissue pH, antemortem medication exposure, and donor variability can influence molecular preservation and assay performance[181]. This creates a particular challenge for multi-omics, because a sample that is suitable for transcriptomic profiling may not preserve proteins, lipids, metabolites, chromatin accessibility, or DNA methylation with equivalent quality.

Cell morphology adds another brain-specific constraint. Neurons are highly polarized, with dendrites, axons, and synaptic compartments extending far from the soma, and many molecular species are distributed across these processes rather than confined to the cell body. Assigning spatial RNA, protein, or metabolite signals to a single cell can therefore be difficult when segmentation is based primarily on nuclei or soma boundaries[182]. This issue is especially relevant at synapses, perivascular regions, and glia-neuron interfaces, where closely packed structures may generate overlapping molecular signals[183].

Finally, intact brain cells, particularly mature neurons with long and fragile processes, are difficult to dissociate without cell loss, selective depletion, or stress-induced artifacts[184]. Single-nucleus sequencing partially mitigates these issues and is well suited for frozen or fragile brain tissue. However, it introduces a trade-off by profiling nuclear RNA rather than the full cellular transcriptome, thereby underrepresenting cytoplasmic, synaptic, and process-localized molecules that may be important for biological interpretation[185].

4.4 Reproducibility, scalability, and clinical translation

Beyond method-specific limitations, the translation of single-cell and spatial multi-omics depends on whether results can be

reproduced, scaled across cohorts, and interpreted within clinically feasible workflows. This remains challenging because variability can be introduced at nearly every step of the experiment, including tissue processing, nuclei or cell isolation, fixation, library preparation, staining, imaging conditions, segmentation, batch correction, and computational preprocessing, affecting downstream interpretation of tissue niches and disease-associated molecular signatures[186-190].

Scalability is also limiting. Each additional donor, brain region, tissue section, or molecular modality increases the burden of tissue-quality control, anatomical matching, sectioning, staining, and expert annotation[191,192]. High-resolution spatial methods are particularly difficult to scale because imaging-based platforms can involve long acquisition times and limited slides per run, whereas sequencing-based platforms may process more samples in parallel but still require careful ROI selection, and standardized tissue handling.

Current spatial multi-omics platforms illustrate these barriers. For example, the GeoMx DSP platform enables high-plex RNA and protein profiling from selected ROIs in FFPE or fresh-frozen tissue sections while maintaining the tissue integrity. Reported throughput varies by workflow and laboratory implementation, with one report describing up to 8 slides per week[193]. Still, it remains cost-intensive and ROI-based, limiting true single-cell interpretability and making segmentation and ROI definition critical determinants of biological interpretation[193]. As a result, DSP works as a complementary tool to image-based analyses, requiring the integration of multiple areas of expertise to interpret results and translate them into clinically meaningful outcomes for patients.

These limitations help explain why most translational multi-omics studies in neuroscience still rely on bulk omics. Although these approaches lack cellular and spatial resolution, they are easier to standardize, more compatible with large cohorts, and more feasible in clinical or resource-limited settings. For single-cell and spatial multi-omics to move toward routine clinical application, the field will need standardized quality-control metrics, reference datasets, harmonized analytical pipelines, reduced costs, and independent validation showing that high-resolution molecular information provides clinical value beyond established histopathological or imaging measurements.

5. Future Avenues in Single-Cell and Spatial Multi-Omics Methods

5.1 Artificial intelligence

Artificial intelligence is reshaping multiple fields of science, including single-cell and spatial multi-omics, driving a transition from data generation toward more effective integration, interpretation, and predictive modeling. Key advances are emerging across areas such as multi-omics integration, foundation models, perturbation prediction, and automated analysis.

5.1.1 Multi-omics integration and cross-modal alignment

A central challenge in multi-omics is aligning single-cell modalities with distinct feature spaces, noise structures, and measurement scales into a unified representation of cell state. A widely used framework for paired multi-omics data is the weighted nearest-neighbor (WNN) implemented in Seurat, which constructs a cell-specific weighted combination of omic-level neighbor graphs, learning per-cell contributions from each molecular layer to yield a consensus which can be clustered and visualized[167]. Complementing this, probabilistic deep generative models such as total variational inference (totalVI) and MultiVI represent paired omics data within a shared latent space while accounting for batch effects and modality-specific noise distributions[194,195]. For unpaired ("diagonal") integration, graph-linked approaches such as graph-linked unified embedding (GLUE) couple omic layers via gene regulatory networks, enabling cross-modal alignment and regulatory inference without requiring cellular correspondence[196]. For mosaic integration, where datasets only partially overlap in their measured modalities, methods such as stabilized mapping for mosaic single-cell data integration (StabMap) and Multigrate offer flexible frameworks that can be used across several modality combinations[197,198].

A comprehensive benchmarking evaluated 40 integration methods across seven analytical tasks, including dimension reduction, batch correction, clustering, classification, feature selection, imputation, and spatial registration, on 64 real and 22 simulated datasets[199]. Key findings revealed that method performance is both dataset-dependent and modality-dependent. For vertical integration of paired RNA and antibody-derived tag (ADT) data, Seurat WNN, Multigrate, and Matilda consistently performed well across datasets. For diagonal integration, scBridge ranked highest for dimension reduction and clustering. At the same time, GLUE performed best in batch correction, highlighting a recurrent trade-off between preserving biological signals and removing technical variation. For mosaic integration, StabMap demonstrated strong performance across dimensionality reduction, clustering, and classification tasks. For cross-integration across multiple batches of all modalities, Multigrate emerged as the most broadly applicable option.

Collectively, this landscape is converging toward flexible frameworks that can handle the full range of experimental designs, from fully paired to fully unpaired, within a coherent, shared representation space.

5.1.2 Single-cell and spatial foundation models

Transformer-based foundation models trained on large single-cell and spatial datasets are emerging as general-purpose backbones

for representation learning and transfer across tasks. scGPT, for example, uses generative pretraining on large cell atlases to learn context-aware gene-expression representations, enabling gene network inference and perturbation-response prediction[200]. An important frontier is extending such models from single-cell data to spatially informed representations. Nicheformer takes a step in this direction by modeling tissue-neighborhood biology using dissociated single-cell and targeted spatial transcriptomic data[201]. Future multimodal foundation models may provide unified embeddings that connect molecular state with spatial context, enabling consistent annotation, cross-dataset harmonization, and the identification of conserved versus context-specific cell states across tissues and conditions.

5.1.3 Predictive perturbation modeling

Emerging machine learning methods aim to predict how cells respond to genetic or pharmacological perturbations, learning mappings from baseline molecular states to post-perturbation transcriptomes. Early approaches established the conceptual framework. ScGen used variational autoencoders and latent-space vector arithmetic to model perturbation effects across unseen cell types[202], while the compositional perturbation autoencoder (CPA) extended this to combinatorial drug and genetic perturbations, learning representations of cell identity and perturbation to predict responses to unseen treatment combinations at single-cell resolution[203]. Graph-based approaches have since incorporated biological prior knowledge. Graph-enhanced gene activation and repression simulator (GEARS) uses gene-gene interaction graphs derived from biological databases to predict transcriptional responses to novel multigene perturbations, including emergent non-additive genetic interaction effects[204]. More recently, large perturbation models (LPMs) have sought to integrate heterogeneous perturbation experiments across chemical and genetic modalities, disentangling perturbation, readout, and biological context as independent dimensions to enable generalization across experimental settings[205].

Despite this progress, a benchmarking study found that none of the five foundation models, nor two other deep learning models, outperformed simple linear baselines for predicting transcriptome changes after single or double perturbations, challenging assumptions about the value added by model complexity[206]. Adding to this, the Systema benchmark demonstrated that current methods struggle to generalize beyond systematic variation (consistent transcriptional differences between perturbed and control cells arising from selection biases or confounders) and that common metrics are susceptible to these biases, leading to overestimated performance[207].

5.1.4 Analysis and benchmarking of real datasets

Large language model (LLM)-based artificial intelligence (AI) agents are increasingly used for end-to-end analysis of single-cell and spatial multi-omic datasets. Task-based benchmarks, such as scBench and SpatialBench, are early efforts to assess whether such systems can reproduce standard analyses, recover known biology, and generate reproducible outputs; however, performance to-date remains limited. In scBench, the best model reached 52.8% accuracy, whereas in SpatialBench, the best base-model accuracy was 38.4%, underscoring the need for continued development and validation before such systems can be reliably used[208,209].

5.1.5 AI limitations

A general limitation across all AI applications is that data scale alone does not guarantee generalization. Model performance depends on the diversity and representativeness of training data, and there is increasing concern that heavy reliance on synthetic or model-generated data can degrade learning dynamics. In particular, “model collapse” has been demonstrated when models are trained on recursively generated data[210], which could lead to a progressive loss of rare modes and distorted estimates of the true data distribution. Related distributional bias has been observed in single-cell data generation, where deep generative models trained on small datasets could amplify sampling artifacts, overrepresent abundant cell populations, and fail to reproduce less abundant cell types[211]. For single-cell and spatial multi-omics, this reinforces that the next gains in AI performance will likely depend less on incremental increases in dataset size and more on diverse training data spanning donors, brain regions, technical platforms, and environments, coupled with rigorous benchmarking, sample annotation, and interpretability.

5.2 Proteomics and metabolomics down to single-cell resolution

5.2.1 Single-cell and spatial proteomics

Single-cell proteomics has emerged as a natural complement to single-cell transcriptomics, as it may enable direct quantification of proteins, the molecular layer most closely linked to cellular phenotype and function. Major advances in the field have been driven by technologies such as Single Cell ProtEomics by Mass Spectrometry (SCoPE-MS), which introduced multiplexed workflows using tandem mass tags and carrier proteomes to improve peptide identification from individual cells[212]. Subsequent developments, including ultra-miniaturized sample preparation platforms such as nanodroplet processing in one pot for trace samples (nanoPOTS)[213] and nano-proteomic sample preparation (nPOP)[214], and multiplexed data-independent acquisition strategies such as plexDIA[215], have improved sensitivity, quantitative robustness, and throughput. These methods are moving single-cell proteomics from proof-of-concept toward biologically driven applications.

Applications in neuroscience remain limited but illustrate the promise of the field. Ultrasensitive proteomic workflows coupled to patch-clamp isolation have demonstrated the feasibility of profiling proteins from individual neurons while linking molecular states to electrophysiological phenotypes[216]. Likewise, single-cell proteomics of postmortem motor neurons with TAR DNA-binding protein 43 (TDP-43) pathology revealed disease-associated protein alterations not readily inferred from transcriptomic data alone[163], while recent studies in developing mouse brain uncovered cell-type-specific protein networks missed at the RNA level[217]. Together, these studies highlight the potential of single-cell proteomics to investigate neuronal heterogeneity and protein-level mechanisms in the nervous system.

Despite being promising, single-cell proteomics remains substantially less advanced than single-cell transcriptomics. Current transcriptomic platforms routinely profile thousands of genes across millions of cells using standardized and scalable workflows. In contrast, single-cell proteomics still faces limitations in proteome depth, throughput, reproducibility, and computational standardization. Thus, at present, single-cell proteomics should be viewed not as a mature counterpart to single-cell transcriptomics, but as an emerging complementary technology. Its current strength lies in addressing questions inaccessible to RNA-centered approaches. As sensitivity, scalability, and integration continue to improve, single-cell proteomics is likely to become an increasingly important layer in neuroscience multi-omics.

5.2.2 Single-cell and spatial metabolomics

Single-cell metabolomics emerges as a complementary frontier in multi-omics by enabling direct interrogation of metabolites, the molecular products most proximal to cellular physiology and phenotype. Unlike transcripts or proteins, metabolites provide a dynamic readout of pathway activity, energy metabolism, neurotransmission, and environmental interactions, features particularly relevant in neuroscience. Metabolites are especially relevant for neurodegeneration and neuroinflammation studies.

Recent advances in MS-based technologies, including capillary electrophoresis MS, live single-cell sampling, and SIMS, have enabled increasingly sensitive profiling of metabolites from individual cells[218,219]. These approaches revealed metabolic heterogeneity among neural cells and offered opportunities to study cell-specific physiological states inaccessible to transcriptomic approaches. However, similarly to single-cell proteomics, compared with other single-cell omics, applications in neuroscience still need to be more explored.

Spatial metabolomics currently represents a more mature frontier than single-cell metabolomics itself. MSI platforms such as MALDI-MSI, DESI-MSI, and related multimodal approaches now enable increasingly resolved mapping of metabolites in tissue and have become major contributors to spatial multi-omics, some of which we discussed above.

Single-cell metabolomics is strategically an emerging technology in terms of complementarity to other omics. As eventual barriers are overcome, single-cell metabolomics may become an increasingly important layer for understanding brain function and disease.

5.3 Less explored layers: Morphomics and temporal localization

Despite advances in molecular multi-omic methods, morphological information remains underexplored, even though it is biologically fundamental. In tissues, morphology is strongly linked to the function[220], a concept that can be extended to the cellular level. Morphology was one of the main criteria for cell type classification, and nowadays, it is well established that structural features reflect the physiological state or specializations of the cells. This morphofunctional relationship is relevant in neuroscience, as cell shape, size, and connectivity are associated with neural function in health and disease[221,222].

Outside neuroscience, some recent studies have already found relevant findings by associating morphological features with the prediction of cancer molecular subtypes[223,224] and patient survival[225]. Together, these findings highlight the potential of morphomics as an additional layer in multi-omic methods for studying brain processes.

Morphomics can be divided across three scales: macroscopic, or radiomics, which captures organ-level features; microscopic, or pathomics, which focuses on tissue- or cell-level features; and ultrastructural, which includes subcellular features[226]. Among these, pathomics (the study of the pathome, i.e., all the cells' morphological features[226]) has only been partially integrated into multi-omic analyses. While some methods, such as Patch-seq, briefly explored the morphology, the field still lacks standardized workflows and pipelines.

Some computational methods have been developed to fill this gap. MorphNet[227] pioneered the prediction of cell morphology from gene expression profiles. MorphDiff[228] extends this concept by modeling cell morphological changes under perturbations. Morphology-enhanced spatial transcriptome analysis integrator (METI)[229] uses spatial transcriptomics with morphological features to deeply profile tumor cells and their microenvironment. Similarly, framework for large-scale histomorphometry (FLASH)[230] provides a high-throughput quantitative morphometry of cells, and MorphLink[231] integrates morphological measurements with molecular profiles to identify relationships between structure and function.

Experimental methods have also been developed, mainly outside neuroscience. Single-cell biophysical fractometry[232] enables high-throughput quantification of cellular morphology based on fractal-related physical properties, distinguishing histological

subtypes and drug responses in lung cancer cells. Senescence subtype classifier based on observable unique phenotypes (SenSCOUT)[233] is another method that aims to bridge the gap between experimental and computational approaches by integrating imaging and machine learning to identify senescence subtypes, using cell and nuclear morphologies alongside biomarker expression profiles. Therefore, these emerging studies and technologies highlight that integrating morphomics into multi-omic frameworks could deepen understanding of cellular dynamics in the brain.

Another layer less explored is the temporal localization of molecular events. Static assays, by capturing just a snapshot, frequently lose relevant information. One example is the low correlation between transcripts and proteins, which is common in neuro-immune studies, where cell-surface markers can be detected by immunocytochemistry despite low or undetectable mRNA levels. Therefore, exploring differences across omic layers not only at a single time point but also over time could provide more information about brain dynamics.

A foundational study in the field was the RNA velocity framework[234]. By modeling the ratio of unspliced to spliced transcripts in single cells, RNA velocity estimates future transcriptional states, enabling the reconstruction of developmental trajectories from scRNA-seq datasets. Building on this rationale, new approaches aim to measure molecular mechanisms across time. Temporally resolved in situ sequencing and mapping (TEMPOmap)[235] spatially profiles RNA across time at a subcellular resolution, identifying RNA life cycles. Extending this framework, Ren *et al.* (2026) integrated STARmap PLUS, ribosome-bound mRNA mapping (RIBOmap)[236], and TEMPOmap data to achieve a spatially resolved mRNA life cycle, by capturing transcription, translation, and degradation[237]. Although complex, these frameworks are important steps toward spatiotemporal multi-omics, in which molecular mechanisms are resolved in both space and time.

5.4 Expanding global representation in single-cell and spatial multi-omics

Another important advance for the field is the global expansion of single-cell and spatial multi-omic studies. Despite rapid technological advances, the field still predominantly relies on data from individuals of European ancestry, leading to disease models and therapeutic targets that may not generalize across populations. This bias is particularly relevant for brain disorders, since genetic risk and environmental exposures vary substantially across populations[238].

These gaps are especially pronounced in underrepresented regions such as Latin America, Africa, and the Caribbean, where neuropsychiatric disorders are a growing public health burden. Sociocultural, environmental, and genetic factors are unique to these regions and may modulate disease risk and therapeutic response differently, yet remain largely unexplored in current multi-omic datasets[239].

Progress in these regions is constrained by significant challenges, including the high cost of reagents, limited expertise, and limited access to instruments[240]. However, progress is beginning to emerge. Initiatives such as LatinCells, Project JAGUAR, Immune Cell Atlas of Indigenous South American Populations, and African Caribbean Single-Cell Network, all supported by the Chan Zuckerberg Initiative, are pioneering this effort, demonstrating the feasibility of building new scientific communities in the field[240].

By broadening participation and addressing gaps in infrastructure, training, and resource access, the field can move toward a next generation of multi-omic tools that are truly translational across diverse populations.

6. Conclusion

The rapid evolution of single-cell, spatial, and multimodal omics has fundamentally reshaped the way we investigate the human brain. By integrating molecular layers with spatial context and cellular phenotypes, these technologies have revealed regulatory mechanisms, cellular ecosystems, and microenvironmental interactions that were previously inaccessible. Despite these advances, important challenges remain: limited reproducibility, high cost, uneven global representation, and the absence of standardized analytical frameworks continue to restrict scalability and clinical translation.

Addressing these barriers will require coordinated efforts across technology development, computational innovation, workforce training, and global infrastructure building. As analytical pipelines become more robust and accessible, and as more diverse populations are incorporated into large-scale datasets, multi-omics is poised to transition from a research-driven mapping tool to a transformative framework for understanding disease mechanisms, refining diagnostic categories, and guiding precision therapeutics in neurology and psychiatry.

Declarations

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Ito-Silva VI: Conceptualization, writing-original draft, writing-review & editing.
Silva-Costa LC: Writing-original draft, writing-review & editing.
Belangero SI, Smith BJ: Writing-review & editing.
Martins-de-Souza D: Conceptualization, writing-review & editing.

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The authors declare no conflicts of interest.

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