

REVIEW

Open Access



Spatial omics in 3D culture model systems: decoding cellular positioning mechanisms and microenvironmental dynamics

Liwei Du¹ and Huayu Yang^{1*} 

Abstract

Recent advances in spatial omics have revolutionized our ability to decode cell-positioning dynamics within three-dimensional (3D) culture models, such as organoids, tumor spheroids, and 3D bioprinting constructs, which faithfully mimic in vivo tissue architecture. By “spatially encoding” high-dimensional molecular information while preserving native microenvironmental context, spatial transcriptomics, proteomics, metabolomics, and epigenomics provide unprecedented maps of gene, protein, metabolite, and chromatin landscapes. When integrated with 3D culture systems, these approaches enable real-time visualization of how cells interact, self-organize, and respond to local biochemical and biophysical cues. Notably, spatial profiling of tumor spheroids has revealed discrete gene-expression gradients and region-specific metabolic heterogeneity, illuminating mechanisms by which the tumor microenvironment (TME) drives therapeutic resistance and immune evasion. Despite these insights, systematic integration of complex multimodal datasets and their translation into clinically actionable biomarkers remain formidable challenges. Emerging high-resolution spatial-omics platforms, which paired with precision-engineered 3D models such as bioprinted tissues and microfluidic organ-on-chip devices are beginning to bridge these gaps by enabling multiplexed, longitudinal analyses under physiologically relevant flow and mechanical stimulation. Looking ahead, ongoing improvements in imaging resolution, probe multiplexing, computational data-fusion, and standardized analytical pipelines are poised to deepen our understanding of tissue patterning, disease progression, and the spatial dynamics of therapeutic intervention. This review uniquely focuses on the seamless integration of cutting-edge spatial-omics technologies into 3D culture models, a synergy that provides unprecedented maps of gene, protein, metabolite, and chromatin landscapes while preserving native microenvironmental context, which will yield more predictive in vitro systems and accelerate the discovery of personalized treatment strategies.

Keywords Spatial omics, 3D model, Spatial positioning, Cellular interaction, Tumor microenvironment

*Correspondence:

Huayu Yang
dolphinyahy@hotmail.com

¹Department of Liver Surgery, Peking Union Medical College (PUMC) Hospital, Peking Union Medical College (PUMC), Chinese Academy of Medical Sciences (CAMS), Beijing 100730, China



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Introduction

The tumor microenvironment (TME) is highly dynamic and heterogeneous in both space and time, and this heterogeneity drives cancer evolution and therapy resistance [1, 2]. Genetic diversity within tumor cells and local factors (hypoxia, acidity, stromal context) create distinct micro-niches that select for resistant clones, undermining precision therapies [1]. Conventional single-cell genomics has catalogued the diverse cell types in the TME, but by dissociating tissues it loses critical spatial information about “location-dependent” functions. Indeed, as Hunter et al. note, dissociative RNA-seq requires tissue dissociation and thus obscures how neighboring cells interact in situ [3]. This gap has impeded our understanding of how cell positioning and cell–cell contacts (e.g., immune checkpoint ligand engagement, metabolic coupling, or stromal support) shape cell fate and drug response.

To address these limitations, spatial omics approaches (imaging-based and sequencing-based) have rapidly emerged to map molecular profiles in intact tissue context [3, 4]. For example, spatial transcriptomics (ST) methods preserve 2D tissue architecture while profiling gene expression [5]. Such methods are now decoding the TME as a network of spatially organized cell states rather than an unordered cell-type list. Recent studies have uncovered microniches of key interactions: for instance, spatial profiling of head-and-neck cancers shows PD-L1 + tumor-associated macrophages co-localizing with exhausted CD8 + T cells, which revealed that primary tumors generally contain more alpha SMA + stromal cells, and are surrounded by CD4 + T helper cells and B cells, with higher MHCII positivity, highlighting local immune-evasive niches [6]. Similarly, spatial sequencing of oral cancers revealed that hypoxic core regions (with anaerobic, glycolytic metabolism) are enriched in immunosuppressive fibroblasts and have high PD-1/PD-L1 signaling, whereas adjacent oxygenated regions favor oxidative metabolism and active immune infiltration [7]. In pancreatic and other cancers, spatial analyses show that cancer-associated fibroblasts (CAFs) cluster at invasive fronts with specific epithelial and immune cells, reconfiguring the extracellular matrix in a way that promotes invasion [8]. These studies redefine the “molecular geography” of TME and illustrate a new paradigm of “location equals fate” in tumor biology.

Despite exciting mechanistic insights, translating spatial omics to the clinic faces significant challenges. As experts have noted, integrating spatial assays into clinical workflows will require expensive prospective validation trials, robust bioinformatic pipelines to interpret high-dimensional spatial data, and more streamlined high-throughput platforms. Many precision diagnostics were established without spatial data, so it remains to be proven which novel biomarkers or therapeutic strategies

spatial analysis can uniquely deliver. Nevertheless, initial studies suggest spatial profiling can refine patient stratification: for example, spatial signatures of immune vs. stromal niches in pancreatic and lung tumors have been correlated with treatment response [8, 9]. Bridging this promise to practice will demand overcoming cost and complexity barriers, as well as standardization of spatial workflows.

Concurrently, 3D culture technologies are transforming in vitro cancer models. Organoids, spheroids, 3D bioprinting technologies and organ-on-chip systems now recapitulate many features of in vivo tissue architecture and microenvironments. Organoids, self-organizing 3D cultures derived from stem or tumor cells that generate miniaturized tissues with multiple differentiated cell types and organ-like structure [10, 11]. Spheroids capture simple 3D cell-cell and diffusion gradients, while microfluidic organ-on-chip platforms supply perfusion, mechanical forces and multi-cell complexity [12, 13]. 3D bioprinting further allows precise placement of cells, organoids and extracellular-matrix (ECM) hydrogels to engineer patient-specific tumor models with defined geometry [14]. This technology employs bioinks, mixtures of viable cells, and supportive hydrogels to achieve precise spatial deposition and assembly of cellular and biomaterial components within three-dimensional architectures, thereby constructing complex structures that recapitulate in vivo microenvironments [15]. Collectively, these 3D systems better mimic the cell-cell and cell-ECM interactions of real tumors than 2D cultures. For example, co-culturing tumor organoids with stromal and immune cells can reproduce spatial gradients of oxygen, nutrients and cytokines found in tumors. Such models have enabled more predictive drug screening and disease modelling, narrowing the gap between in vitro assays and patient tumors [16–19]. However, despite the morphological and functional proximity of 3D models to native tissues, their core scientific value lies in unraveling the fundamental mechanisms driving spatial cell positioning specifically, how cells perceive local cues, establish polarity, select niches, and ultimately assemble into functional architectures. This process entails the multiscale integration of gene expression regulation, protein interaction networks, metabolic adaptation, and mechanotransduction signaling dynamics that conventional methodologies often fail to capture holistically, yielding only fragmented insights.

Applying spatial omics to these 3D models is beginning to reveal how cell positioning and micro-niches emerge. A striking example is the recent Smart-seq3D method, which maps single-cell transcriptomes in tumor spheroids along their core–periphery axis. Using this approach on triple-negative breast cancer spheroids, Mahmoud et al. identified thousands of genes whose

expression varies continuously from the spheroid core to its edge [20]. These spatial gene expression patterns corresponded to regional biology: for instance, the hypoxic core upregulated glycolysis and survival pathways, while the outer rim showed stress-response and migratory signatures. Notably, 3D spheroids captured heterogeneity seen in vivo that is absent in 2D culture, including spatial determinants of drug resistance [20]. In this study, spatial profiling of spheroids exposed gradients of drug response and immune checkpoint markers that matched invasive tumor fronts, highlighting that 3D models in combination with spatial sequencing can reveal how cells sense local cues and organize in space. Recent reviews have highlighted the rapid advancement of spatial omics technologies and their applications in 3D breast cancer models in vitro [21, 22]. While these studies provide valuable overviews of technical progress and translational perspectives, our review aims to offer a broader and more integrated perspective. Specifically, we simultaneously cover all major categories of 3D culture systems, including spheroids, organoids, assembloids, 3D-bioprinted constructs, and organ-on-a-chip models, which systematically compare how spatial omics methodologies can be applied to each of them. Furthermore, we accentuate cross-technology comparisons and the integration of multi-omic layers (transcriptomics, proteomics, metabolomics, and epigenomics). Finally, we expand on the clinical and translational implications, illustrating potential applications across diverse fields of oncology. These features collectively distinguish our work and contribute a uniquely comprehensive resource to the field.

In total, these advances demonstrate that 3D culture combined with spatial multi-omics provides a unique window into the mechanisms of tissue patterning - from gene regulatory networks and metabolic interactions to mechanical signaling.

Methodology This narrative review was conducted based on a comprehensive literature search performed on PubMed, Web of Science, and Google Scholar databases. The search terms included combinations of “spatial omics”, “3D model”, “organoid”, “spheroid”, “assembloid”, “3D bioprinting”, “organ-on-a-chip”, “tumor microenvironment”, and “cancer”. The search was focused on articles published primarily between 2018 and 2025 to capture the most recent advancements. Both original research articles and review articles were considered. Selected studies were included based on their relevance to the application of spatial omics technologies in 3D model systems, with a particular emphasis on cancer research, technical innovation, and biological insight.

Analytical workflows in spatial omics

Analysis of spatial-omics data typically begins with pre-processing (image alignment, spot/gene calling or feature quantification) and then annotation of cell types or states. A common strategy is to use a matched single cell RNA-seq dataset as a reference: single-cell clusters or gene signatures are mapped or deconvolved onto the spatial spots [23, 24]. Spatial transcriptomics methods can be broadly categorized into image-based and capture-based approaches, each with trade-offs (Fig. 1). Image-based methods (FISH and in situ sequencing) use fluorescent probes and microscopy to record transcripts in place. For example, MERFISH and seqFISH use cycles of hybridization and imaging to build up multiplexed gene expression “barcodes” in tissue, achieving subcellular resolution.

Imaging-based techniques offer the highest spatial resolution, enabling the precise localization of individual RNA molecules within tissue sections [25]. Thin tissue slices are fixed, permeabilized, and subjected to iterative hybridization with fluorescent probes to capture targeted transcripts. Signals are acquired over multiple cycles using high-resolution fluorescence microscopy, and images are registered across rounds to allow quantitative analysis of RNA abundance and subcellular spatial distribution.

Single-molecule RNA FISH (smFISH), one of the earliest spatial transcriptomic methods, was limited in the number of genes that could be detected simultaneously [26, 27]. In contrast, MERFISH (Multiplexed Error-Robust FISH) employs multi-round hybridizations and microscopic imaging to simultaneously localize thousands of RNA species within individual cells [28]. Similarly, seqFISH (sequential FISH) uses iterative cycles of hybridization, imaging, and probe stripping to decode barcoded mRNAs, enabling the in situ detection of thousands of genes in tissue samples [29, 30].

The strengths of in situ hybridization-based imaging include high gene detection efficiency and single-molecule resolution. However, these methods also face challenges such as high computational costs, large image files and 3D stacks require high-performance computing resources for image alignment, segmentation, and data storage. Furthermore, techniques based on multi-round in situ hybridization often struggle to detect shorter transcripts and exhibit limited scalability for large tissue areas.

Another category of in situ sequencing (ISS) methods relies on rolling circle amplification (RCA) as a key step to generate cloned amplicons, known as RCA products (RCPs) that contain numerous copies of the target sequence. These RCPs are subsequently sequenced via ligation-based chemistry, enabling quantitative analysis of transcript expression. For example, fluorescence in situ sequencing (FISSEQ) sequences amplified cDNA clusters

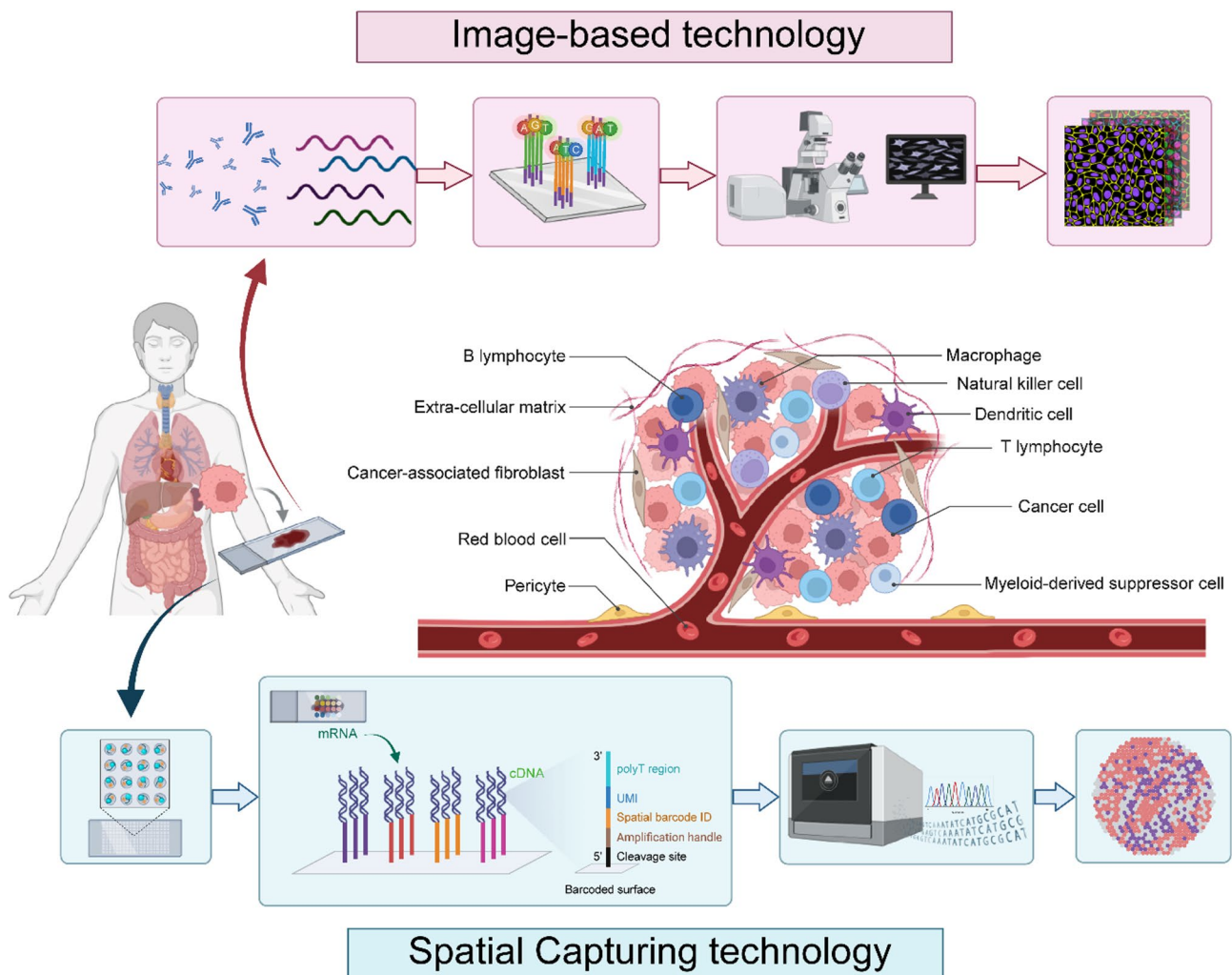


Fig. 1 Typical workflow for studying the tumor microenvironment via spatial omics. Tissue or 3D culture samples are sectioned (fresh-frozen or FFPE) and analyzed through either imaging-based assays or capture-based arrays. Imaging-based techniques (upper panel) rely on fluorescent probe hybridization or in situ sequencing to directly visualize transcripts within tissue sections. Typical steps include probe hybridization to target RNA, iterative imaging cycles using high-resolution microscopy, image alignment/registration, and computational spot detection coupled with cell segmentation to generate single-cell or subcellular expression profiles. Capture-based techniques (lower panel) employ spatially barcoded oligonucleotide arrays or beads to capture RNA molecules released from tissue sections. mRNA hybridizes to spatially barcoded primers containing unique molecular identifiers (UMIs), which are reverse-transcribed into cDNA and converted into sequencing libraries. Sequencing reads retain positional barcodes, enabling reconstruction of transcriptome-wide gene expression profiles at a resolution defined by spot or bead size. This figure was drawn using Biorender

within fixed tissues, increasing the detectable number of transcripts and achieving whole-transcriptome coverage [31]. Similarly, STARmap (Spatially Resolved Transcript Amplicon Readout Mapping) extends the capabilities of imaging-based spatial omics by integrating targeted in situ sequencing with hydrogel-tissue chemistry and three-dimensional optical sectioning. This integrated approach enables highly multiplexed RNA analysis within intact tissue specimens as well as in vitro 3D models, providing high-resolution transcriptomic profiling in structurally complex and physiologically relevant environments.

These imaging-based approaches provide exceptionally high spatial resolution, reaching single-cell or even

subcellular levels, along with high sensitivity for target RNA molecules. However, they suffer from limited throughput, typically only enabling the detection of dozens to hundreds of pre-selected genes, and require multiple rounds of imaging, resulting in extended experimental timelines [32].

Employing innovative strategies to capture transcripts in situ onto microscopically patterned arrays, sequencing-based spatial transcriptomics enables the simultaneous acquisition of spatial and molecular information at cellular resolution, with evolving capture methodologies progressively enhancing precision from tissue-level down to subcellular scales.

In capture-based spatial transcriptomic methods, fresh-frozen or FFPE tissue sections are placed on spatially barcoded oligonucleotide arrays. Following tissue permeabilization, released mRNAs bind to these barcoded spots. On-slide reverse transcription generates cDNA tagged with spatial barcodes and unique molecular identifiers (UMIs). After imaging, the cDNA is collected for library preparation and sequencing. The resulting reads, which include spatial barcodes, allow reconstruction of gene expression profiles for each spot or pixel. After alignment to a reference genome, transcripts are quantified based on these barcodes, enabling near-complete capture and quantification of the protein-coding transcriptome.

Prominent examples of such platforms include 10x Visium and Slide-seq: Visium employs arrays with spatially barcoded primers, each capture spot having a diameter of approximately 55 μm ; Slide-seq uses a monolayer of beads with known spatial barcode sequences to capture RNA, achieving a resolution of about 10 μm . While these methods support genome-wide transcriptome profiling, their spatial resolution remains relatively low, often necessitating the averaging of signals from multiple cells within a single capture spot.

More recently, higher-resolution capture-based technologies have emerged. For instance, High-Definition Spatial Transcriptomics (HDST), developed by Vickovic et al., achieves a resolution of around 2 μm using densely packed barcoded bead arrays [33]. Similarly, Seq-Scope, introduced by Cho et al., leverages the self-assembly of barcoded oligonucleotides on an Illumina sequencing flow cell to reach an impressive resolution of 0.5–0.8 μm [34]. Despite these advances, expression signals within individual capture points often still represent averaged profiles from multiple cells.

Following preprocessing, spatial omics data undergo normalization and downstream analysis. Key steps often include the identification of spatially variable genes, the clustering of spots or cells into distinct domains, and the inference of cell-cell interactions. Integration with histological images, such as H&E staining, and AI-powered computational approaches can further enhance interpretability. Overall, spatial analysis pipelines generate high-dimensional matrices linking molecular counts to tissue coordinates, yet they face challenges related to data volume and multimodal integration.

Thus, although capture-based methods offer high scalability and are suitable for large-scale studies, they generally exhibit limited sensitivity and fall short of achieving true single-cell resolution.

Emerging techniques also combine modalities: for example, digital spatial profiling (Nanostring GeoMx) uses UV-cleavable barcodes on antibodies or probes in selected regions-of-interest. Overall, imaging-based

strategies offer maximal spatial detail (often single-cell or subcellular) at the cost of gene panel size and throughput, whereas capture-based strategies offer transcriptome-wide depth but typically yield lower spatial resolution. Choice of method thus depends on the experimental priorities (resolution vs. throughput vs. target breadth).

Spatial omics to unravel the interactions of cellular components in the tumor microenvironment

Single-cell sequencing has not only revolutionized our understanding of tumor biology but also fundamentally reshaped the broader landscape of cancer research by enabling unprecedented resolution of the intricate multicellular ecosystems within tumors and their microenvironment. This transformative approach is essential for deconvoluting the complex mechanisms underlying tumor progression, immune evasion, and therapeutic resistance, delivering multidimensional insights unattainable through conventional methodologies. Furthermore, integration with complementary spatial omics technologies significantly enhances our capacity to dissect the dynamic crosstalk between malignant and stromal cell populations. State-of-the-art algorithms fall into two categories: “mapping” methods (e.g. Tangram, NovoSpaRc, CellTrek) align individual cells from scRNA-seq to spatial coordinates, while “deconvolution” methods (e.g. RCTD, cell2location, DestVI) infer proportions of reference cell types in each spatial spot. These pipelines produce spatial localization maps showing where each cell type or state resides in the tissue. Once cell types are localized, one can perform spatially resolved functional analyses. Differential expression testing can compare gene expression across different regions or niches in the tissue, revealing spatially variable genes or pathway activities. Gene-set and pathway enrichment analyses are often applied to cells or spots from specific domains to infer underlying biology. Spatial omics also enables inference of cell-cell interactions in situ: for example, co-localization of ligand and receptor transcripts (or proteins) can be assessed directly, or spatial graph methods (e.g. Giotto) can predict cell-cell signaling events. In practice, researchers often overlay immune markers or deconvolution scores to map immune infiltration. For example, the densities of T cells, macrophages or other immune subsets can be plotted across the section to identify “hot” and “cold” regions of the tumor microenvironment. This reveals insights into immune exclusion or zonation that are critical for understanding therapy response. In summary, analysis workflows combine cell-type annotation with spatial localization, followed by specialized tests such as spatial differential expression, pathway enrichment, and cell-cell interaction modeling to interpret the 3D biology.

It is crucial to acknowledge the limitations of these computational approaches. Deconvolution algorithms

heavily depend on the quality and comprehensiveness of the scRNA-seq reference and may struggle to accurately resolve rare or novel cell states absent from the reference. Inference of ligand-receptor interactions from spatial co-localization is suggestive but does not constitute functional validation, necessitating orthogonal experimental confirmation. Furthermore, each computational tool incorporates inherent assumptions and parameter dependencies that may influence outcomes and complicate direct comparison across studies. As the field continues to evolve rapidly, standardized benchmarks and best practices are still emerging.

Glioblastoma

Recent spatial omics studies have revealed a complex, layered architecture in glioblastoma, driven in part by hypoxia. Integrative spatial transcriptomics (10× Visium and Slide-seq) combined with single-cell and imaging proteomics shows that glioma ecosystems organize into “layers” of cellular states. For example, Greenwald et al. reported that gliomas comprise small local neighborhoods each dominated by one tumor-cell state, with stereotyped pairwise co-locations of states defining five global layers; hypoxic niches lie at the center of this tissue hierarchy [35]. Spatial profiling of clinical GBM specimens (using technologies such as NanoString CosMx and GeoMx on FFPE sections) showed that tumor core regions are enriched for hypoxic mesenchymal-like tumor cells and vascular proliferation, whereas invasive margin regions (residual tumor in healthy brain) are dominated by non-mesenchymal states with glial differentiation programs. Together, these data indicate that cell-cell interactions (e.g. spatial co-incidence of TAMs with hypoxic tumor niches) and long-range signals (hypoxia gradients) structure the GBM microenvironment. Functionally, spatial co-expression analyses uncovered candidate ligand-receptor axes and regulatory programs in situ. For instance, radial glial-like stem-state tumor cells are enriched at neuron-rich invasive fronts in glioma, and genes such as *FAM20C* were shown to drive their invasion in model systems [36]. Clinically, spatial architectures have prognostic significance: areas with dense astrocyte-like tumor clusters correlate with poorer survival [37], and a gene module associated with glial differentiation in infiltrative cells was linked to worse outcomes [38]. These findings highlight spatial heterogeneity and cell-cell crosstalk (notably hypoxia-driven niches and TAM associations) as key determinants of GBM progression and therapy resistance.

Hepatocellular carcinoma

In hepatocellular carcinoma (HCC), spatial transcriptomics has delineated a unique “invasive zone” at the tumor margin that fosters immunosuppression and

tumor spread. Using high-resolution Stereo-seq and 10× Visium on tumor-border sections, Liu et al. identified a 500 µm border region in HCC enriched for immunosuppressive macrophages, metabolic remodeling and hepatocyte injury [39]. Within this invasive zone, a subset of hepatocytes overexpressing serum amyloid A (SAAs) was detected immediately adjacent to tumor cells. Mechanistically, malignant cells secrete CXCL6, triggering JAK-STAT3 activation in neighboring hepatocytes and induction of SAA1/2 expression. The SAAs, in turn, recruit and polarize TAMs toward an M2-like phenotype, closing an immunosuppressive feedback loop [39]. Spatially resolved single-cell studies further showed that HCC exhibits inter-tumor heterogeneity: for example, HCC generally displays disordered metabolic programs and diverse T-cell infiltration, while intrahepatic cholangiocarcinoma (ICC) is a major source of cancer-associated fibroblasts [40]. These analyses also uncovered a specialized tumor-peritumor junctional niche rich in intermediate endothelial cells (marked by CPE) with mixed ‘normal’ and ‘tumor-associated’ signatures [40]. Researchers have identified triadic “immune niches” in tumors: intratumoral clusters of CXCL13⁺ CD4⁺ T helper cells, mature dendritic cells and CD8⁺ T cells that expand after PD-1 blockade [41]. Together, these findings show that spatial mapping in liver cancer reveals gradients of hypoxia and metabolism, cell-cell circuits, and stromal topologies. Clinically, such insights suggest that targeting components of the invasive-zone circuit or the DC-T-cell hubs could improve ICB response and mitigate immunosuppressive TME features.

Pancreatic cancer

Spatial profiling of pancreatic ductal adenocarcinoma (PDAC) has illuminated how tumor subtypes co-exist and resist therapy within complex niches. In treatment-naïve and chemoresistant PDAC samples, single-cell, proteomic and spatial-omics (e.g. 10X Visium) revealed layered tumor architecture. Spatial deconvolution identified transitioning epithelial cells (acinar-to-ductal metaplasia, PanIN lesions) and malignant subclones with KRAS-driven and epithelial-mesenchymal states [42]. Chemotherapy-resistant PDACs showed a marked increase in inflammatory CAFs (iCAFs) co-localized with tumor, upregulating stress-response genes [43]. More recent work has uncovered a fundamental interplay between tumor-intrinsic epigenetic programs and macrophage niches. Spatial transcriptomics and multi-omics found that basal-like (squamous) vs. classical tumor states are regulated by AP1/HDAC transcriptional circuits and amplified by TNF-α⁺ macrophages. These macrophages accumulate in basal regions and correlate with reduced CD8⁺ T cell infiltration. Critically, targeting TNF-α in mouse models reprogrammed the TME:

macrophages were depleted and CD8⁺ T cells infiltrated the tumor, improving survival. Overall, spatial omics in PDAC reveal that intratumoral heterogeneity and niche signaling drive subtype co-existence and may underlie refractory behavior.

Lung cancer

In non-small-cell lung cancer, spatial transcriptomics has mapped the divergent architectures of adenocarcinoma and squamous tumors. Integrative scRNA-seq and 10× Visium profiling of treatment-naïve non-small cell lung cancer (NSCLC) showed that overall cell-type composition is similar in lung adenocarcinoma (LUAD) versus lung squamous cell carcinoma (LUSC), but their spatial interaction networks differ markedly [9]. Key findings include an “inverse” spatial relationship between M2-like macrophages and cytotoxic NK/T cells – regions dense in anti-inflammatory TAMs exclude NK cell, and a transcriptional reprogramming of TAMs. Within tumors, macrophages adopt a developmental (fetal-like) metabolic program favoring cholesterol export and iron efflux. Spatial L-R inference confirmed extensive macrophage–tumor and macrophage–immune crosstalk, including checkpoint interactions. In early-stage lung adenocarcinoma, imaging-based spatial surveys (mass cytometry) have further shown that T-cell and B-cell neighborhoods predict patient relapse, but 10× Visium data specifically highlighted that LUAD cells often assemble an “immune barrier” of exhausted T cells at the invasive front. Spatial omics thus pinpoints where immune evasion occurs in lung tumors and suggests prognostic markers and strategies.

Colorectal cancer

Spatial studies in colorectal carcinoma (CRC) have leveraged cutting-edge platforms to dissect TME heterogeneity. For example, using 10× Visium HD (sub-spot resolution), Romero et al. profiled CRC surgical specimens and identified diverse macrophage subsets occupying distinct niches [44]. One subset co-localizes with tumor cells and exhausted CD8⁺ T cells, while another lies near tertiary lymphoid structures with effector T cells. Ligand-receptor analysis implicated these macrophages in shaping the immune milieu: for instance, pro-tumor macrophages in “hot” regions expressed LAG3-LGALS9 and other checkpoint axes co-localized with T cells. Notably, spatial clonality inference showed that locally expanded T-cell clones often reside adjacent to M1-like macrophages with anti-tumor features.

Prostate cancer

Spatial omics has begun to elucidate the immunologically “cold” nature of prostate tumors and the stromal niches that accompany therapy resistance. Integrated single-cell

and spatial maps of prostatectomy specimens ($n > 100$ patients) identified a distinct epithelial subset termed “club-like” cells that reside at the tumor-stroma interface [45]. These club-like cells display a senescence-associated secretory phenotype (SASP) with low androgen signaling and high luminal progenitor markers. Crucially, regions enriched for club-like cells coincided with dense infiltrates of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs). Spatial ligand-receptor analysis suggests that club cells secrete chemokines and cytokines (e.g. CCL2, CXCL8) that recruit and activate suppressive myeloid cells. Concurrent spatial Slide-seq profiling confirmed disrupted glandular architecture in tumors (loss of normal epithelial arrangement) and expansion of fibroblasts and cancer-associated fibroblasts, with markedly reduced T/B cell presence compared to normal prostate [46]. These data imply a functional circuit in which club-like tumor epithelia drive myeloid inflammation. Clinically, club cell abundance was linked to prior androgen-deprivation therapy and is hypothesized to contribute to castration resistance and immune evasion.

Oral squamous cell carcinoma (OSCC)

In head and neck carcinomas, spatial transcriptomics has defined core vs. invasive front programs and metabolic-immune niches. A Visium-based study of HPV-negative OSCC showed that tumor cores (TC) and leading edges (LE) have conserved yet distinct signatures: the LE expresses stress and invasion genes and is enriched for TGF- β signaling and myofibroblasts, whereas the TC harbors proliferative tumor cells and dense lymphocytes [5]. Strikingly, the LE gene signature (involving EMT and immune-regulation) predicted poor survival across multiple cancers, whereas the TC signature correlated with better prognosis. This suggests that spatial position within the lesion governs phenotype and outcome. Moreover, new multi-omics spatial work has linked metabolism to immunosuppression in OSCC. Mapping glycolytic activity at subregional resolution revealed “hypermetabolic” zones (high lactate) where fibroblasts convert to inflammatory CAFs (iCAFs) by taking up tumor-derived lactate. These iCAFs overexpress HIF1A and produce CXCL12, which recruits Tregs and induces local TGF- β expression, creating immune-suppressive niches [7]. Thus, lactate gradients drive a fibroblast–Treg axis in situ. In summary, spatial profiling of oral tumors uncovers that metabolic and cell composition gradient.

Spatial omics in 3D in vitro models

Spatial omics technologies have revolutionized the study of gene and protein expression in situ. In recent years, researchers have begun to apply these methods to complex 3D cell culture models - including multicellular spheroids, organoids, assembloids, and 3D-bioprinted

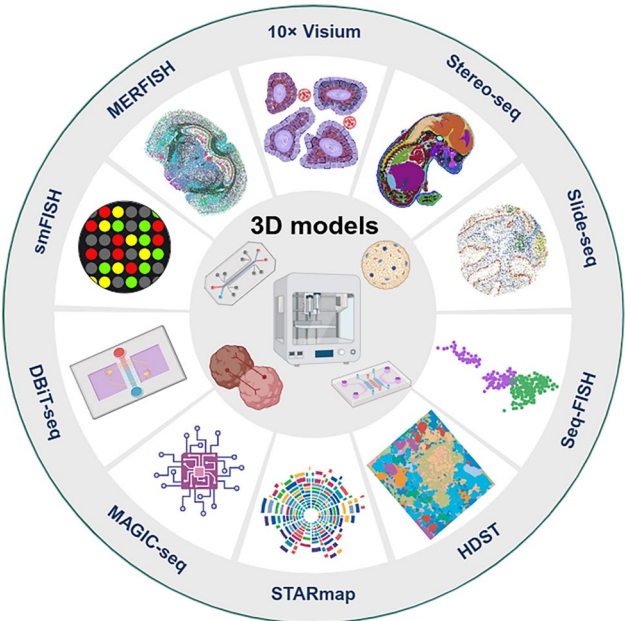


Fig. 2 Schematic diagram of the application of various 3D culture techniques in spatial omics. This figure was drawn using Biorender

tissues to better mimic the architecture of native tissues. These approaches enable mapping of transcriptomes, proteomes, metabolomes, and epigenomes within intact 3D cultures, revealing how cells interact and self-organize. Major platforms include sequencing-based methods

(e.g. 10× Visium, Stereo-seq) and imaging-based methods (e.g. MERFISH/seqFISH, NanoString GeoMx/CosMx, multiplex immunostaining) (Fig. 2; Table 1). Below, we review key advance in spatial transcriptomics, proteomics, metabolomics, epigenomics, and multi-omics integration across different 3D models, highlighting representative studies, findings, and technical adaptations.

Spatial omics in spheroids

Spheroids (e.g. tumor spheroids or co-cultured cell aggregates) are one of the earliest and most widely used 3D models. Spatial profiling of spheroids has provided insights into gradients of gene expression and cell-cell interactions in 3D tumor or disease niches that are not observable in traditional 2D cultures. For example, Koc-Günel et al. (2025) combined 10× Visium spatial transcriptomics with high-content imaging to study rare lung lymphangioleiomyomatosis (LAM). They embedded primary LAM fibroblasts (LAMFs) and lymphatic endothelial cells (LECs) into 3D spheroids and profiled lung tissue and spheroids. Spatial gene clusters revealed co-localized fibroblast-LEC niches enriched for VEGF-A and FGF signaling [51]. In a 3D spheroid co-culture, LAMF-LEC spheroids showed enhanced invasion that was potently blocked by sorafenib, pinpointing VEGF-A as a candidate therapeutic target.

Cougnoux et al. developed Smart-seq3D, a sequencing-based spatial transcriptomics approach for tumor

Table 1 Applications of Spatial omics in 3D in vitro models

Year	Technology	3D model	Key findings	Ref
2023	10× Vi-sium (spatial transcriptomics)	Embryonic HEMO organoid (human)	Integrated with scRNA-seq (SpatialScope) to map cell types; discovered yolk-sac erythro-megakaryopoietic niche and TB-like signaling in organoid.	[47]
2023	10× Visium on PET membrane	SHH-inducible neural organoids (hPSCs)	Adapted a PET insert culture onto Visium slide; captured localized SHH pathway activation; found peak SHH targets near induction site despite low UMI counts.	[48]
2024	BGI Stereo-seq (LOSRT lamination)	Primary lung & liver organoids (mouse)	Developed lamination device to flatten organoids; Stereo-seq identified diverse cell types (alveolar cells, macrophages, hepatocytes, etc.) in intact laminated organoids.	[49]
2023	4i multiplex IF (53-plex proteomics)	Retinal organoid sec-tions (human iPSC)	Generated a high-resolution protein atlas (41 sections, 6–39 weeks) with 53 antibodies; integrated with scRNA + scATAC to build a “digital organoid” revealing retinal cell layer organization and regulatory factors.	[50]
2025	10× Visium + imaging	LAM spheroid co-culture (human LAMF + LEC)	Spatial transcriptomics on LAM lung tissue and LAMF–LEC spheroids showed co-localized fibroblast and lymphatic endothelial signatures (VEGF-A, FGF); sorafenib reduced LAMF invasion.	[51]
2025	Smart-seq3D (scRNA + diffu-sion labeling)	TNBC tumor spheroids (3D culture)	Inferred radial positions of cells in spheroids via dye diffusion and Smart-seq3; identified thousands of spatially variable genes and continuous core–periphery expression gradients, capturing 3D-specific heterogeneity.	[20]
2024	HiFi-Slide spatial RNA sequencing	BBB assembloid (PSC-derived brain + vasculature)	Achieved 3D spatial transcriptomics on a PSC-derived brain–blood–vessel assembloid; iden-tified 12 spatial domains and colocalized cell types (e.g. ECs with GABAergic neurons, SMCs with glutamatergic neurons), recapitulating neurovascular arrangement.	[52]
2023	MALDI-MSI (metabolomics)	Cortical brain organ-oid (human iPSC)	Optimized mass spec imaging of organoid sections; detected ~ 260 lipids and mapped their localization. Found lipid species enriched in neurogenic rosettes, suggesting roles in progenitor maintenance vs. differentiation.	[53]
2021	MALDI-MSI (metabolomics)	Tumor spheroids & patient-derived organoids	Reviewed examples of MSI in 3D cultures: MALDI-MSI visualizes drug/metabolite distribu-tions in spheroids and organoids, enabling spatial pharmacology studies in cancer models.	[54]

spheroids (Fig. 3A) [20]. They perfused triple-negative breast cancer spheroids with a fluorescent dye, then dissociated and profiled single cells with Smart-seq3xpress (Fig. 3). A probabilistic “diffusion” model inferred each cell’s position along the spheroid radius. Smart-seq3D identified thousands of spatially varying genes and continuous core-periphery expression gradients in the tumor spheroid (e.g. hypoxia and proliferation signatures at the core) (Fig. 3B). This technique distinguished expression patterns present in 3D spheroids that are absent in 2D cultures, illustrating how spatial context shapes tumor heterogeneity and drug response (Fig. 3C). Spatial proteomics and metabolomics have also been applied to spheroids. Mass spectrometry imaging (MSI) is a powerful label-free tool, Hummon et al. reviewed multiple examples of MALDI-MSI mapping drug and metabolite distributions in tumor spheroids and patient-derived organoids [54]. MSI visualizes how drugs penetrate and are metabolized in 3D cultures, aiding personalized medicine. For instance, MALDI-MSI studies have spatially mapped chemotherapeutics and lipids in colon cancer spheroids, revealing heterogeneous drug uptake. Overall, spatial MS imaging in spheroids underscores drug metabolism niches inaccessible to bulk assays.

Spheroids are typically 200–500 μm in diameter, so one can section them thinly for array-based assays (Visium) or image them by confocal. Nevertheless, technical limitations remain. Large spheroids ($>300\text{ }\mu\text{m}$) are difficult to section without distortion, and physical sectioning often disrupts the continuous spatial context. Diffusion-based methods (like Smart-seq3D) overcome this by inference but require rigorous modeling. Imaging of intact spheroids is limited by light scattering, so most spatial methods rely on slicing. Photobleaching and antibody penetration can be issues in thick samples. Biological variability also poses a challenge, as spheroids may develop necrotic cores or exhibit structural heterogeneity depending on their size and culture conditions, leading to potential inconsistencies across experiments. In summary, while spatial omics has significantly advanced our understanding of spheroid organization, these technical and biological constraints should be carefully considered when interpreting experimental results. New methods (e.g. microtome sectioning of spheroid pellets, CLARITY clearing for imaging) are being explored to enhance resolution in true 3D spheroids.

Spatial omics in organoids

Organoids, self-organized 3D cultures derived from stem cells that mimic organ structures have seen extensive spatial omics. A major innovation is lamination of organoids to enable array capture. Ma et al. introduced LOSRT (Lamination-Based Organoid Spatially Resolved Transcriptomics) for primary tissue-derived organoids

(mouse lung and liver). They used a custom device to gently flatten (laminate) single organoids underweight compression, then placed them on BGI’s Stereo-seq chips [49]. Lamination preserved epithelial layers and most cells, making them amenable to high-resolution spatial RNA capture (Fig. 4A). Stereo-seq profiling resolved diverse cell types (e.g. alveolar type I/II, airway basal cells, macrophages, hepatocytes, stellate cells) and confirmed them by marker staining (Fig. 4B and C). This automated LOSRT pipeline overcame the issue that standard microtome sectioning can miss cells or distort organoids, enabling rapid spatial profiling of organoid heterogeneity.

Sequencing-based spatial transcriptomics (Visium, Slide-seq, Stereo-seq, etc.) has been adapted to organoids by various strategies. For example, Xiao et al. generated a human embryoid organoid (HEMO) capturing multiple germ layers (Fig. 4D). They applied 10 $\times$ Visium to organoid sections at day 15 (peak hematopoiesis) and integrated it with single-cell RNA-seq using the Spatial-Scope algorithm [47]. This yielded single-cell-resolution gene maps, revealing distinct niches such as a yolk-sac erythro-megakaryopoietic region (co-localized erythroid, megakaryocyte, and yolk sac endoderm cells). Notably, SpatialScope integration also uncovered variability between organoid replicates due to differences in cutting plane and polarization, highlighting a technical challenge in 3D profiling.

Cerda Jara et al. (2023) tackled spatial sequencing in neural organoids with optogenetic stimulation (Fig. 4E). They grew hiPSCs on thin polyester (PET) inserts and induced localized SHH expression. The PET membranes were cut and mounted on a 10 $\times$ Visium slide (after fixation) to capture spatial transcriptomes [48]. Because RNA capture was non-uniform across the membrane, they aggregated counts in concentric circles around the stimulation center. SmartVisium analysis showed that SHH target genes peaked near the ROI and that the SHH receptor PTCH1 was upregulated in adjacent regions. This study illustrates how Visium can be adapted to organoid-like cultures (via culturing on detachable membranes) and how spatial transcriptomics can validate engineered patterns in 3D.

Spatial proteomics has also been applied in organoids. Philipp Wahle et al. created a highly multiplexed protein atlas of human retinal organoids (and adult retina) using iterative indirect immunofluorescence imaging (4i) [50]. They stained 41 organoid Sects. (6–39 weeks) for 53 antibodies (photoreceptor, bipolar, glia, and signaling markers) in 21 cycles (Fig. 4F). This yielded ~ 400 million pixels of spatial proteome data across development. By integrating these 4i images with single-cell RNA-seq and ATAC-seq (multiome) data from matching timepoints, they constructed a “digital organoid” model of retinal development. This multi-omic atlas identified cell-type

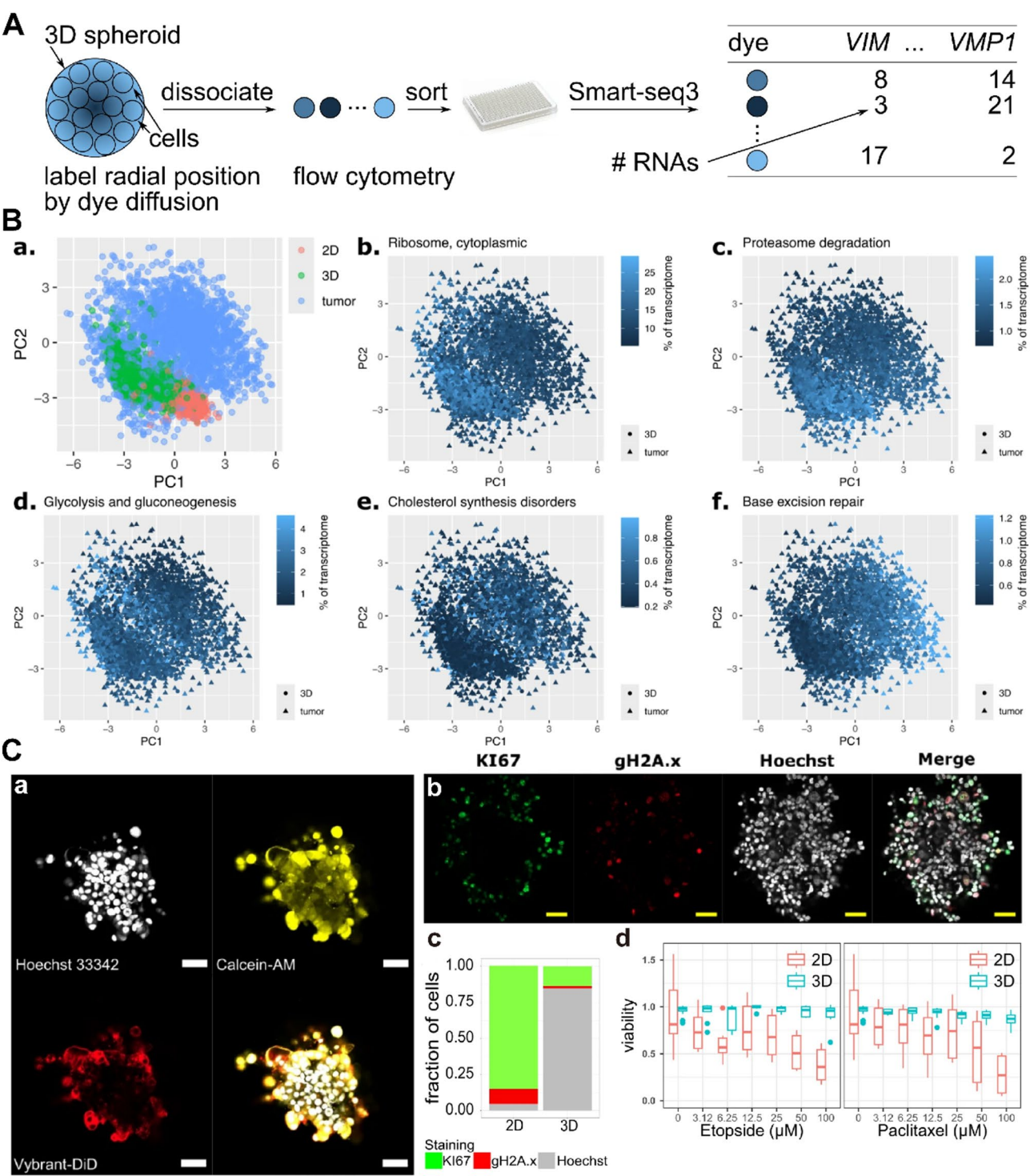


Fig. 3 Applications of spatial omics in 3D spheroids. **(A)** Workflow for applying Smart-seq3D to spatial and single-cell transcriptomics. **(B)** Smart-seq3D demonstrates that 3D spheroids more faithfully recapitulate the transcriptional heterogeneity observed in in vivo tumors. **(C)** Immunofluorescence (IF) staining was employed to validate the spatial features of 3D spheroids and confirm their concordance with Smart-seq3D results. These images are reproduced with the permission of Refs [20]

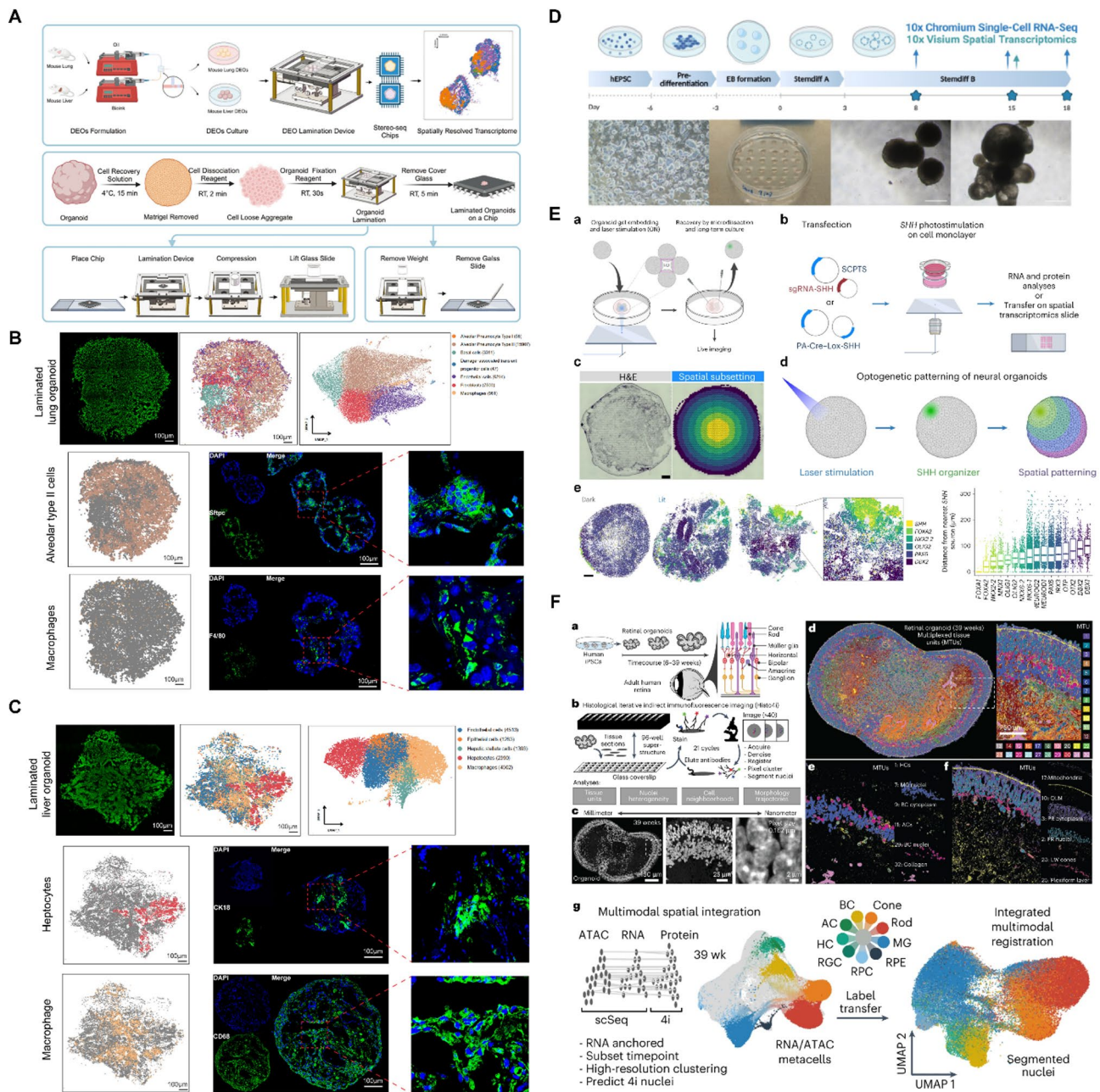


Fig. 4 Applications of spatial omics in organoids. **(A)** Workflow of the LOSRT technique, including organoid generation, lamination, fixation, and sequencing. **(B)** ssDNA imaging was performed on laminated lung organoids, followed by spatially resolved cell-type annotation and classification. The spatial distributions of alveolar type II cells (SFTPC-positive) and macrophages (F4/80-positive) were mapped and subsequently validated via immunofluorescence staining of cryosectioned organoid slices. **(C)** ssDNA imaging was performed on laminated liver organoids, followed by spatially resolved cell-type annotation and classification. The spatial distributions of hepatocytes (CK18-positive) and macrophages (CD68-positive) were mapped and subsequently validated via immunofluorescence staining of cryosectioned organoid slices. These images are reproduced with the permission of Refs [49]. **(D)** Differentiation workflow for human embryonic organoids (HEMOs). HEMOs were harvested at defined developmental stages for 10x Chromium single-cell RNA sequencing and 10x Visium spatial transcriptomics, enabling the identification of distinct cellular niches. These images are reproduced with the permission of Refs [47]. **(E)** Spatial omics analysis of neural organoids under optogenetic stimulation. (a, b) Schematic of the optogenetic stimulation protocol for SHH and its integration with spatial transcriptomic readouts. (c) H&E-stained image of hiPSC-derived neural organoid sections transferred onto a 10x Visium slide, with a spatial subset of Visium spots centered on the SHH-induced region. Scale bar = 500 μ m. (d) Optogenetic stimulation pattern applied to neural organoids. (e) Spatial distance distributions of Visium spots in control (dark) versus SHH-induced (light) organoids. Scale bar = 100 μ m. These images are reproduced with the permission of Refs [48]. **(F)** Spatial Proteomic Atlas of Human Retinal Organoids. (a, b) Schematic of the 4i workflow and downstream analysis, applied across the developmental time course of retinal organoid maturation. (c) Representative 4i dataset image showing Hoechst-stained nuclei in a section of a 39-week-old retinal organoid. (d-f) Example pixel-based clustering results for the same 39-week organoid section, illustrating distinct proteomic domains. (g) Overview schematic for the integration of spatial proteomic data with complementary transcriptomic datasets. These images are reproduced with the permission of Refs [50]

spatial arrangements (e.g. developing photoreceptor layers) and regulatory factors (e.g. OTX2) controlling retina neurogenesis. In other organoid systems, multiplexed imaging (e.g. CODEX, MIBI, or Imaging Mass Cytometry) could similarly map protein landscapes, though published examples in organoids are still emerging.

Spatial metabolomics has been pioneered in organoids by mass spectrometry imaging. Gerarda Cappucci et al. reported MALDI-MSI on human cortical brain organoids [53]. They optimized fixation, embedding, and matrix application to preserve morphology and measured lipid distributions at $\sim 50\ \mu\text{m}$ resolution. Over 260 lipid species were detected, including some that localized specifically to neuroepithelial rosettes (neural progenitor niches), for example, ceramide-phosphoethanolamine CerPE 36:1; O2 was enriched in rosettes, whereas PE 38:3 was excluded. This suggests certain lipids may regulate progenitor proliferation or differentiation. This proof-of-principle study established protocols for MSI in organoids and showed that spatial metabolite patterns can reveal developmental biology. Epigenomics in situ (e.g. spatial ATAC-seq or CUT&RUN) is still in early days for organoids. However, non-spatial multi-omic sequencing (scRNA + scATAC) has been performed on organoids and integrated with spatial maps. Epigenomics in situ (e.g. spatial ATAC-seq or CUT&RUN) is still in early days for organoids. However, non-spatial multi-omic sequencing (scRNA + scATAC) has been performed on organoids and integrated with spatial maps. For instance, the retinal organoid study above combined chromatin accessibility profiling with spatial proteomics. Emerging methods for co-profiling epigenome and transcriptome spatially (e.g. co-ATAC/RNA) may soon be applied to organoid slices [50].

Despite these advances, spatial profiling of organoids faces hurdles. Organoids vary greatly in size and shape. Embedding and sectioning can cause loss of 3D context and uneven cell recovery. Methods like lamination or culturing on removable membranes help, but introduce new technical steps [48, 49]. The thick Matrigel in organoids can hinder diffusion of probes or stains. Also, organoids lack a defined orientation: two siblings may be cut at different axes, complicating data alignment [47, 52]. Software tools (e.g. SpatialScope, SpaceFlow) have been developed to integrate multiple slices and enrich resolution [52]. In short, while initial studies demonstrate the feasibility of organoid spatial omics, current results should be viewed cautiously. Variations in slicing depth, orientation, and ECM composition can lead to differences in observed spatial patterns. Future protocols and 3D imaging (light-sheet, multiplexed 3D FISH) may help overcome these challenges. Careful slicing protocols, image registration, and computational deconvolution are often needed to maximize spatial signal in organoids.

Spatial omics in assembloids

An assembloid is a three-dimensional multicellular construct engineered by combining two or more organoids or organized cell aggregates, such as spheroids, stromal cells, immune cells, or endothelial cells in order to reconstruct complex tissue architectures and intercellular dynamics in vitro [55–58]. First popularized in neural studies to model circuitry (e.g., cortex-ganglionic eminence fusions), assembloids have since been adopted in cancer research to emulate tumor microenvironments by combining patient-derived tumor organoids with stromal or immune components like fibroblasts or T cells [59–64]. This approach facilitates high-resolution investigation into spatial organization and functional integration, capturing context-dependent interactions that simpler co-culture or organoid models cannot. Assembloids recapitulate the complexity of in vivo microenvironments by enabling cell-cell physical contacts, paracrine signaling, and ECM remodeling. This allows them to model spatially dependent biological processes, such as liver zonation, cancer stem cell niche formation, organ development, immune responses, and disease progression [65, 66]. Beyond serving as functional units, assembloids act as spatial frameworks for transcriptional gradients (e.g., Wnt/BMP signaling pathways), metabolic compartmentalization (e.g., hypoxic cores), and cell fate determination (e.g., asymmetric stem cell division) [39, 67]. They thus provide a foundational model for deciphering position-dependent gene expression. Compared to homogeneous 2D cultures or single-cell-type 3D spheroids, assembloids replicate the spatiotemporal coordination of multicellular interactions, offering precise experimental platforms to investigate cellular crosstalk across space and time.

Spatial omics can reveal how different compartments intermix. A landmark example is Dao et al., who created a human blood–brain barrier (BBB) assembloid by combining cerebral brain organoids and vascular organoids from PSCs (Fig. 5A). They applied a customized spatial RNA-tagging platform (HiFi-Slide, repurposed Illumina flow cell) to $10\ \mu\text{m}$ sections of a $3 \times 3\ \text{mm}$ assembloid. This yielded ~ 178 million reads, with ~ 227 genes per $10\ \mu\text{m}^2$ spot. Spatial clustering (SpaceFlow) partitioned the assembloid into 12 domains with distinct cell-type enrichments [52]. For example, one domain co-localized endothelial cells (ECs) with GABAergic neurons; another paired smooth muscle cells (SMCs) with glutamatergic neurons. These co-enrichments (validated by immunostaining) recapitulated neuro-vascular interactions in the BBB. Thus, spatial transcriptomics in the assembloid revealed how vascular and neural lineages arrange relative to each other, offering a more in vivo-like architecture. Assembloids can also be engineered by directed methods. For example, Roth et al. described a bioprinting-based platform (SPOT) that magnetically patterns

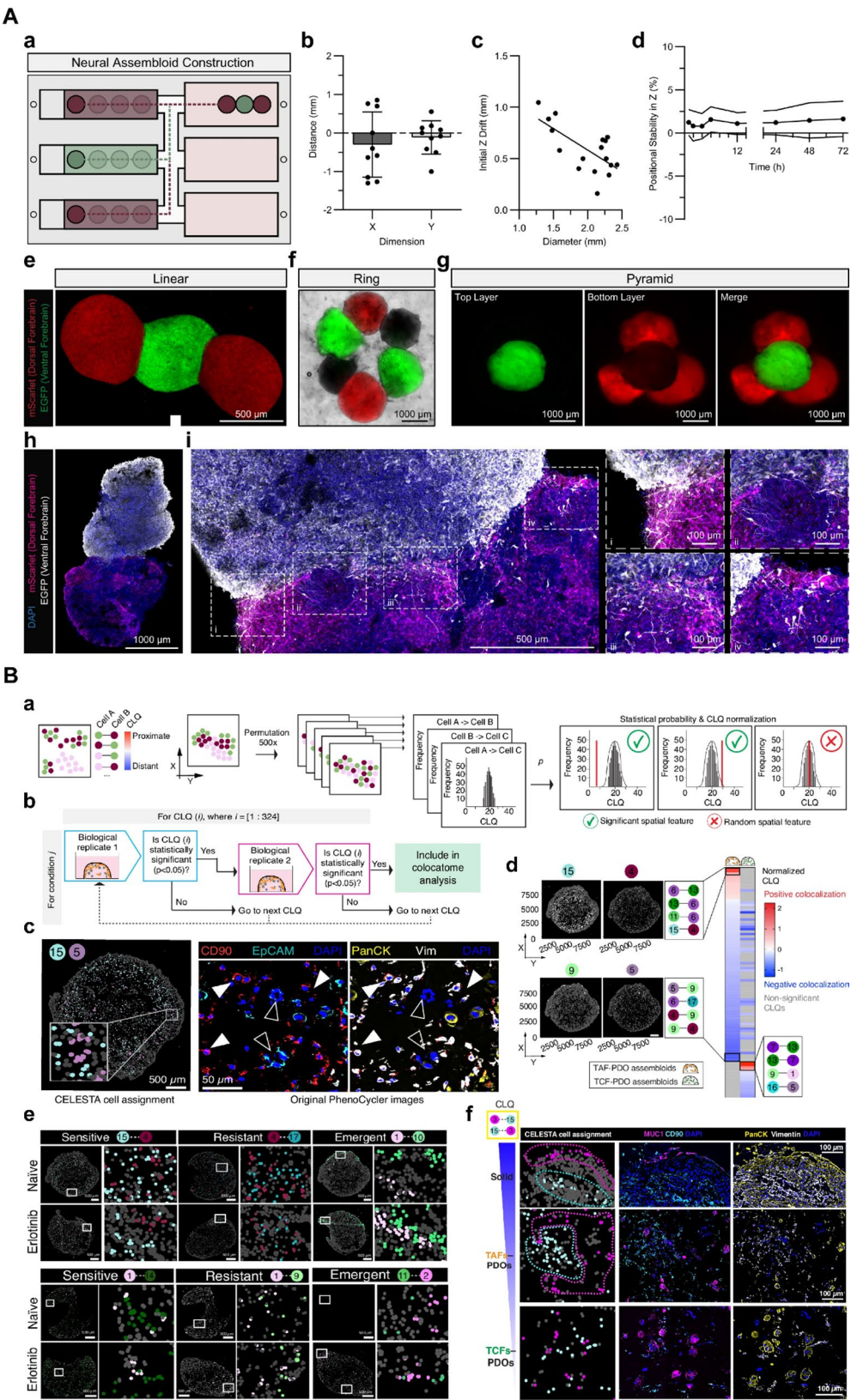


Fig. 5 (See legend on next page.)

(See figure on previous page.)

Fig. 5 Applications of spatial omics in assembloids and 3D bioprinting. **(A)** Construction of 3D-bioprinted neural assembloids using the SPOT method. (a) Schematic overview of the SPOT automated workflow. (b) Accuracy of alginate microgel transfer in the X and Y dimensions. (c) Drift measured along the Z axis. (d) Positional stability of alginate microgels over 72 h. (e–g). Representative fluorescence images showing linear, ring-shaped, and pyramidal fusion between eGFP-expressing ventral forebrain and mScarlet, expressing dorsal forebrain neural organoids. (h–i) Representative immunofluorescence images of integrated ventral and dorsal forebrain neural organoids. These images are reproduced with the permission of Refs [68]. **(B)** Quantitative spatial framework for analyzing intercellular positioning within assembloids. (a) Schematic representation of the colocalization quotient (CLQ) and the workflow for spatial cellular positioning analysis. (b) Strategy for selecting statistically significant pairwise spatial features within each assembloid. (c) Representative images validating significant colocalization; solid white arrows denote fibroblasts; transparent arrows denote tumor cells. (d) Significant heterotypic negative (blue) and positive (red) colocalizations in TAF-PDO and TCF-PDO assembloids. (e) Representative examples of erlotinib-sensitive, resistant, and emergent heterotypic colocalizations in TAF-PDO and TCF-PDO assembloids. Scale bar = 500 μm . (f) IF staining validation of negative colocalization between CD90⁺ fibroblasts and MUC1⁺ malignant cells in representative LUAD tumor regions. These images are reproduced with the permission of Refs [69]

organoids into complex assembloid architectures (Fig. 5B) [68]. Such techniques allow predefined spatial layouts of organoids. While spatial omics has yet to be reported on printed assembloids, these advances pave the way. Future integration of spatial profiling (e.g. multiplexed imaging) could assess how printed patterns affect tissue self-organization. Recent studies have highlighted the spatial heterogeneity of cancer-associated fibroblasts (CAFs) within the tumor microenvironment. Fibroblasts located at the tumor periphery (TAFs) exhibit pro-migratory and immunosuppressive profiles, while core-residing CAFs (TCFs) are more involved in matrix remodeling. This zonal specialization may contribute to differential therapeutic responses, though the spatial mechanisms underlying resistance remain unclear. Colocatome analysis is a novel quantitative framework introduced by Bouchard et al. to compare spatial colocalization patterns between in vitro tumor assembloids and patient-derived pathological samples [69]. Using LUAD as a model, the study demonstrated that cancer-associated fibroblasts (CAFs) from different tumor regions - tumor-adjacent (TAFs) versus tumor-core (TCFs) - exert distinct effects on tumor-stroma organization. By co-culturing LUAD patient-derived organoids with TAFs or TCFs and applying colocatome analysis, the researchers revealed that TAF - assembloids closely resembled the solid growth pattern of clinical LUAD tissues, while TCF-assembloids aligned with acinar morphologies. This approach highlights how regional CAF heterogeneity contributes to spatial architecture and underscores the value of assembloids as tractable models for studying spatial dynamics in the tumor microenvironment.

Assembloids combine multiple tissue types, making dissociation for single-cell data more difficult. Spatially, each organoid component may have different densities or sizes, complicating sectioning. Embedding large assembloids often requires larger arrays or tiled imaging. Sectioning artifacts can be more pronounced at fusion boundaries. Consequently, spatial resolution may be inconsistent, with certain regions potentially subject to undersampling. Furthermore, current assembloid studies often focus on a limited number of cell types or small-scale structures. Thus, although initial findings

are encouraging, they remain largely proof-of-concept. The generalizability of these spatial patterns across different assembloid systems has yet to be systematically established. In summary, while assembloids expand the experimental toolkit, technical challenges in 3D imaging and computational segmentation mean that conclusions should be drawn cautiously, with clear acknowledgment of current limitations. Ongoing advances in imaging (light-sheet microscopy, 3D FISH) and computational reconstruction (3D interpolation of serial sections) may help fully capture the 3D spatial biology of assembloids.

Spatial omics in 3D bioprinting

The biomedical research landscape is undergoing a transformative shift driven by the convergence of two revolutionary technologies: 3D bioprinting and spatial omics [70]. This synergy is redefining our understanding of biological complexity [71]. 3D bioprinting enables the precise deposition of living cells and biomaterials to engineer highly biomimetic tissue architectures, while spatial omics transcends the spatial limitations of conventional bulk-omics by resolving molecular profiles within their native tissue context. The integration of these cutting-edge approaches provides unprecedented multidimensional insights into tissue development, disease mechanisms, and drug responses. Conventional in vitro models often fail to recapitulate the spatial heterogeneity of native tissues. The core strength of 3D bioprinting lies in its ability to orchestrate spatial cell organization, constructing complex structures with vasculature, extracellular matrix (ECM), and multicellular interfaces that faithfully mimic the 3D structure and function of living tissues [72]. Concurrently, spatial omics technologies, including spatial transcriptomics and proteomics, which bridge the critical gap between molecular mechanisms and tissue architecture [73, 74]. They empower researchers to decipher gene expression heterogeneity, cell-cell communication, and microenvironmental signaling networks while preserving essential spatial information [21]. The convergence of 3D bioprinting and spatial omics is ushering in a new era of biomedical research [75]. On one hand, 3D bioprinting provides physiologically relevant, controllable tissue models for spatial omics studies.

On the other hand, spatial omics offers robust tools for validating the biological fidelity of 3D printed constructs [76]. This synergistic integration not only accelerates research in disease pathogenesis and drug discovery but also paves the way for innovative platforms in personalized medicine [77].

3D bioprinting uses scaffolds and bioinks to create tissue-like constructs with precise architecture. This field is newer in spatial omics, but some relevant work is emerging. For example, printing has been used to assemble pre-formed organoid clusters or cell aggregates into larger tissues [68]. These printed tissues can incorporate multiple cell types (e.g. neural and tumor organoids) or gradients of biomaterials. Spatial omics in 3D bioprinted models remains at an early stage. One can imagine combining printing with in situ assays: for instance, printing thin hydrogel layers that are easily sectioned, or embedding barcoded microarrays into printed constructs. Alternatively, thick constructs might be analyzed by 3D imaging approaches. High-fidelity spatial architecture provides critical guidance for constructing physiologically relevant 3D bioprinted models. Yuan et al. analyzed histological images of resected primary tumors from three breast cancer patients, identifying distinct spatial patterns based on vascular morphology and staining [71]. Using extrusion-based multi nozzle 3D bioprinting, they engineered a spatially heterogeneous TME model by patterning triple-negative MDA-MB-231 cancer cells, vascular endothelial cells (ECs), and human mammary carcinoma-associated fibroblasts (hMCAFs) within a biomimetic ECM bioink. This model recapitulated two key compartments: cancer cell-rich (CCR) regions and adjacent stroma-rich (SR) regions. Crucially, it demonstrated spatially heterogeneous resistance to angiogenesis and ECM stiffness remodeling in breast cancer.

Currently, most spatial work in bioprinting has focused on verifying printed pattern fidelity by imaging rather than global omics. However, as printing becomes more prevalent for organoid assembly, spatial omics methods will follow. Comprehensive analysis of 3D bioprinted structures remains challenging, as bioinks must be chemically compatible with standard fixation and staining protocols, and thicker constructs are difficult to section without structural deformation. Consequently, publicly available spatial transcriptomic or proteomic datasets derived from bioprinted tissues remain extremely limited. One proposed strategy involves embedding barcoded chips during printing or within sequentially laminated layers, though such techniques are still largely experimental. In summary, spatial omics applied to bioprinted models is still in its infancy. While initial studies have successfully confirmed structural integrity, high-resolution molecular spatial mapping remains underdeveloped. Further technical advances, such as the development of

novel bioinks and optimized sectioning methods, which are essential to unlock the full potential of this integrative approach. For example, after printing a cardiac or vascular network, one could perform CODEX/Imaging Mass Cytometry to map dozens of proteins in situ. Key challenges will include designing bioinks compatible with fixation and staining, and developing sectioning methods that preserve the printed architecture.

Spatial omics in microfluidic 3D models and organ-on-chip systems

Microfluidic platforms and organ-on-chip (OoC) devices have emerged as transformative in vitro models that recreate complex physiological conditions, such as fluid flow, shear stress, chemical gradients, and multicellular co-cultures, while maintaining precise architectural control over 3D environments [78–83]. Recent advances in spatial omics have enabled these platforms to incorporate high-resolution molecular mapping, thereby allowing the study of tissue-like behavior at a spatially resolved and multimodal level.

Developed in 2020, DBiT-seq (Deterministic Barcoding in Tissue sequencing) pioneered microfluidic barcode delivery directly onto tissue sections using orthogonal PDMS channels [84–86]. With spot sizes ranging from 10 to 50 μm , DBiT-seq enables near-single-cell resolution of both transcriptome and proteome, as antibody-derived tags (ADTs) are integrated into the same barcoding workflow [85]. While originally designed for tissue slides, its planar microfluidic design is easily transferable to flat-mounted organoids or OoC constructs. Subsequent extensions like spatial ATAC-seq and CUT&Tag seq incorporated epigenetic layers into the microfluidic platform [87–89]. As a result, DBiT-based microfluidics now support high-resolution, co-localized measurement of RNA, protein, and chromatin accessibility in engineered 3D models.

MAGIC-seq enhances spatial transcriptomics throughput and field of view via grid-pattern microfluidic barcoding [89–91]. By utilizing combinatorial barcoding across serpentine chips, MAGIC-seq can process tens of centimeters-square areas with $< \mu\text{m}$ level of sensitivity. It has been demonstrated on 93 serial sections of mouse brain development to construct a volumetric 3D transcriptome atlas. Although clinical OoC structures are thinner, the scalability and cost-effectiveness of MAGIC-seq make it well-suited to map full cross-sections of microfluidic chips containing co-cultures and gradient environments. Reviews on spatial omics instrumentation highlight the rise of various designs, such as HDST, Slide-seq, Stereo-seq, Pixel-seq, sci-Space, and Matrix-seq, that integrate microfluidic arrays for precise spatial barcoding. These systems share the design principle of delivering bar-coded oligos through microfluidic channels or patterned

arrays, enabling customizable spot sizes and throughput. Their modularity and flexibility lend themselves to OoC and spheroid-on-chip applications, supporting modular expansion to study organ-interaction models. However, the application of spatial omics to microfluidic cultures faces several technical challenges. Many current platforms utilize thin chambers or membrane-based designs, which require specific adaptations for the delivery of spatial barcodes. Moreover, data acquisition from organ-on-chip models often necessitates integration across multiple sections or microfluidic channels. As these systems become increasingly modular, standardized protocols for spatial molecular profiling on chips will be essential. Despite these complexities, early implementations demonstrate that organ-on-chip platforms can be successfully integrated with spatial omics approaches to investigate processes such as drug responses under flow conditions and inter-organ interactions in a spatially resolved manner.

Discussion

Core features of spatial omics technologies

Spatial omics broadly refers to methods that couple high-throughput molecular profiling with precise tissue localization, enabling multiplexed “omics” readouts in situ. In practice this encompasses spatial transcriptomics, proteomics, metabolomics, and epigenomics, each of which maps gene expression, protein abundance, metabolic states or chromatin marks onto the spatial coordinates of tissues or 3D models [92, 93]. These approaches integrate imaging or barcoding techniques with sequencing or mass-spectrometry to provide high-dimensional molecular maps that preserve native cellular context. By combining spatial resolution with whole-transcriptome or proteome scale data, spatial omics opens a window on tissue architecture and function that neither bulk sequencing nor dissociative single-cell methods can capture.

Spatial-omics platforms share three key attributes. First, they aim for high spatial resolution, often at or near single-cell scale. Imaging-based methods such as MERFISH, seqFISH or 10× Xenium use repeated rounds of fluorescence in situ hybridization or sequencing to detect RNA molecules at submicron accuracy. Second, they support multi-omics integration: for example, imaging mass spectrometry (e.g. MALDI or imaging mass cytometry) can map hundreds of proteins, metabolites or lipids in situ, while chromatin FISH and CUT&Tag-MERFISH approaches probe spatial epigenetic states. Third, spatial omics produces very high-dimensional data, often covering thousands of genes or analytes per location. This high dimensionality is both a strength and a challenge [92, 93].

However, most current platforms trade off breadth for resolution. In particular, sequencing-based arrays (e.g.

10× Visium, Slide-seq, Stereo-seq) capture whole-transcriptome data but have spots on the order of 10–100 μm , each encompassing multiple cells [23, 24]. Conversely, highly multiplexed FISH methods achieve true single-cell or subcellular mapping of transcript sets but are limited to hundreds or thousands of preselected targets. As a result, most spatial datasets are not automatically at single-cell resolution, and downstream analysis typically requires integration with single-cell RNA-seq or proteomic references to deconvolve and annotate cell types. For example, each Visium spot ($\approx 55 \mu\text{m}$) often contains transcripts from tens of cells, so computational deconvolution with scRNA-seq is used to infer the likely cell-type composition of each spot. Thus, there is a trade-off between resolution and breadth: current technologies either sacrifice some transcriptome coverage for single-cell accuracy, or capture many genes per location at lower spatial granularity.

Limitations in 3D models

A major limitation of current spatial omics is that they almost all operate on two-dimensional tissue slices. In complex 3D systems, such as tumors, organoids, spheroids, or engineered tissues, crucial information resides along the third dimension and the temporal axis. By analyzing only 2D sections, one may miss vertical heterogeneity. For example, in a tumor slice the invasive front and hypoxic core may be on different planes and partially lost in a single section. Important structures such as blood vessel networks or immune cell niches that span across layers cannot be fully captured. In organoid or spheroid models, phenotypic gradients (e.g. oxygen or drug penetration) form in 3D, so any single slice only provides a partial view. Thus, spatial 2D methods collapse a volume into a plane, potentially obscuring true 3D spatial relationships. This is particularly problematic for tumor models, where invasion and metastasis occur at 3D interfaces, and for organoids that aim to mimic whole-tissue physiology. Attempts to overcome this include serial sectioning and computational 3D reconstruction, but these add complexity and can introduce registration errors. As one recent review notes, most spatial omics approaches “remain limited to 2D sections” [67]. Fully volumetric spatial profiling methods are still in their infancy, and traditional 2D assays will inevitably lose information that is unique to 3D model.

Beyond the limitations of two-dimensional systems, several additional challenges hinder the application of spatial omics to three-dimensional models. Technical variability and reproducibility remain major concerns: differences in sample preparation, such as freezing protocols, section thickness, and fixation methods can markedly affect RNA integrity, antigenicity and overall data quality, complicating cross-study comparisons.

Cross-platform and cross-modal integration is also problematic, because each spatial technology carries distinct biases, sensitivities and spatial resolutions; for example, harmonizing whole-transcriptome, lower-resolution data from 10x Visium with targeted, high-resolution measurements from MERFISH requires sophisticated computational frameworks and rigorous normalization. Many high-plex spatial methods are costly and have limited throughput, which constrains their use in large-scale screening of 3D models. Computationally, the scale and complexity of spatial datasets place heavy demands on storage and processing resources, and the registration and three-dimensional reconstruction of serial sections introduce additional analytical challenges. The lack of standardized analysis pipelines further increases result variability, since different algorithms or parameter choices can yield divergent conclusions. Functional validation is essential, with spatially inferred interactions requiring confirmation in 3D models, commonly through genetic or pharmacological perturbations, though such experiments remain technically challenging. Addressing these limitations will require coordinated effort across biology, technology development, computational science and clinical expertise to establish best practices and robust workflows.

Emerging AI and deep learning solutions

To address the 2D limitation and other challenges, artificial intelligence and deep generative models are being developed. One strategy is to reconstruct 3D molecular maps from limited 2D data. These AI-driven methods promise dense, non-destructive 3D spatial profiling without the need for exhaustive sectioning.

AI is also applied to spatial inference and multimodal integration. Deep generative approaches can fuse scRNA-seq and spatial RNA data to infer missing genes or to enhance resolution. For instance, models like SpatialScope train on paired single-cell and spatial data to impute full transcriptomes at single-cell resolution [94]. Other tools integrate histology images (H&E) with spatial profiles: deep learning models can predict gene-expression patterns directly from pathology slides, effectively linking morphology to molecular state. AI methods are also used for batch correction (combining data across experiments), spatial gene expression prediction, and even biomarker discovery by mining multimodal data [67]. In sum, AI complements spatial omics by inferring hidden spatial patterns, correcting technical noise, and integrating histological context, pointing the way toward 3D and multimodal tissue atlases.

Future perspectives

In the coming years, we anticipate that spatial omics will increasingly merge with advanced 3D culture systems.

State-of-the-art organoids, spheroids and tumor-on-chip models can be profiled with multiplexed spatial methods to more faithfully recapitulate in vivo biology. Combining spatial transcriptomics/proteomics with live-cell imaging and lineage tracing will add dynamic and temporal dimensions. Crucially, integrating multimodal spatial data (e.g. jointly measuring RNA, protein and metabolism) can bridge molecular mechanisms and phenotypic outcomes.

Spatial omics holds exciting potential for clinical applications. It can reveal disease tissue architecture, guide biomarker discovery, and stratify patients based on microenvironment features. Notable examples include identifying prognostic spatial features in glioblastoma or predicting immunotherapy response based on spatial patterns [35, 95]. The ability to analyze patient-derived organoids (PDOs) using spatial omics offers a platform for personalized drug testing and outcome prediction, serving as “patient avatars.” In the long term, spatial omics may be integrated into digital pathology workflows, enriching traditional H&E morphology with molecular mapping to improve diagnostic accuracy and prognostic assessment.

However, clinical translation faces several barriers. Current platforms are expensive and complex; sequencing-based spatial assays require specialized instrumentation and costly reagents, hindering routine clinical adoption [96]. While innovation in multiplexing and computational efficiency may mitigate these issues, cost remains a challenge.

Standardization presents another major hurdle. Pre-analytical factors such as tissue fixation and processing time, along with operator variability, considerably impact data quality, yet standardized protocols are lacking [96]. To ensure reliability, inter-laboratory reproducibility and quality control benchmarks must be established. Analytical pipelines also require standardization: although numerous algorithms exist for cell typing and spatial analysis, consensus on best practices is still evolving.

Spatial datasets are often massive, ranging from gigabytes to terabytes per sample, and frequently multimodal. Their effective use requires advanced data management and sharing infrastructures, many of which remain in development. Regulatory and practical considerations further complicate adoption. Any clinical spatial assay must demonstrate added value over existing diagnostics. Experience from initiatives such as the Molecular Microscope Diagnostic System (MMDx) in kidney transplantation, a bulk transcriptomic platform, which illustrates that establishing comprehensive reference databases can take decades. Similarly, spatial profiling will require large, well-annotated cohorts to achieve statistical power.

In summary, while spatial omics shows great promise for advancing precision medicine, its routine clinical

implementation will require overcoming challenges related to cost, standardization, data infrastructure, and regulatory validation.

As one perspective notes, further technical development will “facilitate the integration of ST and SP with 3D tumor models (organoids, tissue scaffolds, and 3D bio-prints)” [97]. In the future, AI-driven tissue reconstruction, high-throughput spatial assays, and sophisticated 3D systems promise to connect mechanistic discovery directly to clinical translation. Together, these advances will help build comprehensive 4D atlases of disease, revealing how cells and molecules orchestrate complex phenotypes in space and time.

Abbreviations

3D	Three-dimensional
TME	Tumor microenvironment
ST	Spatial transcriptomics
CAFs	Cancer-associated fibroblasts
ECM	Extracellular-matrix
AI	Artificial intelligence
smFISH	Single-molecule RNA FISH
MERFISH	Multiplexed Error-Robust FISH
seqFISH	Sequential FISH
ISS	In situ sequencing
RCA	Rolling circle amplification
FISSEQ	Fluorescent in situ sequencing
STARmap	Spatially Resolved Transcript Amplicon Readout Mapping
UMIs	Unique molecular identifiers
HDST	High-Definition Spatial Transcriptomics
PDAC	Pancreatic ductal adenocarcinoma
NSCLC	Non-small cell lung cancer
LUAD	Lung adenocarcinoma
LUSC	lung squamous cell carcinoma
CRC	Colorectal carcinoma
SASP	Senescence-associated secretory phenotype
MSI	Mass spectrometry imaging
HEMO	Human embryoid organoid
BBB	Blood-brain barrier
ECs	Endothelial cells
SMCs	Smooth muscle cells
OoC	Organ-on-chip
DBIT seq	Deterministic Barcoding in Tissue sequencing
FFPE	Formalin-Fixed, Paraffin-Embedded
LCM	Laser Capture Microdissection
scRNA-seq	Single-Cell RNA Sequencing
NGS	Next-Generation Sequencing
ROI	Region of Interest
DSP	Digital Spatial Profiler

Acknowledgements

None.

Author contributions

Conceptualization: Liwei Du, Huayu Yang. Writing-original draft: Liwei Du. Writing-review & editing: Liwei Du. All authors approved the final manuscript for publication.

Funding

This work was supported by the National Natural Science Foundation of China (32271470).

Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

Received: 4 July 2025 / Accepted: 27 October 2025

Published online: 26 November 2025

References

- Ge R, Wang Z, Cheng L. Tumor microenvironment heterogeneity an important mediator of prostate cancer progression and therapeutic resistance. *NPJ Precision Oncol.* 2022;6:31.
- Zhang A, Miao K, Sun H, Deng C-X. Tumor heterogeneity reshapes the tumor microenvironment to influence drug resistance. *Int J Biol Sci.* 2022;18:3019.
- Hunter MV, Moncada R, Weiss JM, Yanai I, White RM. Spatially resolved transcriptomics reveals the architecture of the tumor-microenvironment interface. *Nat Commun.* 2021;12:6278.
- Choi S-W, Kim JH, Hong J, Kwon M. Mapping immunotherapy potential: Spatial transcriptomics in the unraveling of tumor-immune microenvironments in head and neck squamous cell carcinoma. *Front Immunol.* 2025;16:1568590.
- Arora R, Cao C, Kumar M, Sinha S, Chanda A, McNeil R, Samuel D, Arora RK, Matthews TW, Chandarana S. Spatial transcriptomics reveals distinct and conserved tumor core and edge architectures that predict survival and targeted therapy response. *Nat Commun.* 2023;14:5029.
- Blise KE, Sivagnanam S, Banik GL, Coussens LM, Goecks J. Single-cell Spatial architectures associated with clinical outcome in head and neck squamous cell carcinoma. *NPJ Precision Oncol.* 2022;6:10.
- Liu Z, Zhang Z, Zhang Y, Zhou W, Zhang X, Peng C, Ji T, Zou X, Zhang Z, Ren Z. Spatial transcriptomics reveals that metabolic characteristics define the tumor immunosuppression microenvironment via ICAF transformation in oral squamous cell carcinoma. *Int J Oral Sci.* 2024;16:9.
- Moncada R, Barkley D, Wagner F, Chiodin M, Devlin JC, Baron M, Hajdu CH, Simeone DM, Yanai I. Integrating microarray-based Spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas. *Nat Biotechnol.* 2020;38:333–42.
- De Zuani M, Xue H, Park JS, Dentro SC, Seferbekova Z, Tessier J, Curras-Alonso S, Hadjipanayis A, Athanasiadis EI, Gerstung M. Single-cell and Spatial transcriptomics analysis of non-small cell lung cancer. *Nat Commun.* 2024;15:4388.
- Gunti S, Hoke AT, Vu KP, London NR Jr. Organoid and spheroid tumor models: techniques and applications. *Cancers.* 2021;13:874.
- Velasco V, Shariati SA, Esfandyarpour R. Microtechnology-based methods for organoid models. *Microsystems Nanoengineering.* 2020;6:76.
- Nath S, Devi GR. Three-dimensional culture systems in cancer research: focus on tumor spheroid model. *Pharmacol Ther.* 2016;163:94–108.
- Ebrahimi A, Icoz K, Didarian R, Shih CH, Tarim EA, Nasser B, Akpek A, Cecen B, Bal-Ozturk A, Güleç K. Molecular separation by using active and passive microfluidic chip designs: a comprehensive review. *Adv Mater Interfaces.* 2024;11:2300492.
- Huang MS, Christakopoulos F, Roth JG, Heilshorn SC. Organoid bioprinting: from cells to functional tissues. *Nat Reviews Bioeng.* 2024;1–17.
- Mandrycky C, Wang Z, Kim K, Kim D-H. 3D Bioprinting for engineering complex tissues. *Biotechnol Adv.* 2016;34:422–34.
- Ravi M, Paramesh V, Kaviya S, Anuradha E, Solomon FP. 3D cell culture systems: advantages and applications. *J Cell Physiol.* 2015;230:16–26.
- Peng Z, Lv X, Sun H, Zhao L, Huang S. 3D tumor cultures for drug resistance and screening development in clinical applications. *Mol Cancer.* 2025;24:93.
- Abuwatfa WH, Pitt WG, Hussein GA. Scaffold-based 3D cell culture models in cancer research. *J Biomed Sci.* 2024;31:7.
- Zauchner D, Müller MZ, Horrer M, Bissig L, Zhao F, Fisch P, Lee SS, Zenobi-Wong M, Müller R, Qin X-H. synthetic biodegradable microporous hydrogels for in vitro 3D culture of functional human bone cell networks. *Nat Commun.* 2024;15:5027.

20. Cougnoux A, Mahmoud L, Johnsson PA, Eroglu A, Gsell L, Rosenbauer J, Sandberg R, Hausser J. Diffusion Smart-seq3 of breast cancer spheroids to explore Spatial tumor biology and test evolutionary principles of tumor heterogeneity. *Sci Rep*. 2025;15:3811.
21. Pierantoni L, Reis RL, Silva-Correia J, Oliveira JM, Heavey S. Spatial-omics technologies: the new enterprise in 3D breast cancer models. *Trends Biotechnol*. 2023;41:1488–500.
22. Tao J-Y, Zhu J, Gao Y-Q, Jiang M, Yin H. Narrative review of 3D Bioprinting for the construction of in vitro tumor models: present and prospects. *Translational Cancer Res*. 2025;14:1479.
23. Zhou Z, Zhong Y, Zhang Z, Ren X. Spatial transcriptomics Deconvolution at single-cell resolution using redeconve. *Nat Commun*. 2023;14:7930.
24. Lim HJ, Wang Y, Buzdin A, Li X. A practical guide for choosing an optimal Spatial transcriptomics technology from seven major commercially available options. *BMC Genomics*. 2025;26:47.
25. Gall JG, Pardue ML. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. *Proceedings of the National Academy of Sciences* 1969;63:378–383.
26. Femino AM, Fay FS, Fogarty K, Singer RH. Visualization of single RNA transcripts in situ. *Science*. 1998;280:585–90.
27. Levisky JM, Shenoy SM, Pezo RC, Singer RH. Single-cell gene expression profiling. *Science*. 2002;297:836–40.
28. Chen K, Boettiger A, Moffitt J, Wang S, Zhuang X. RNA imaging. Spatially resolved, highly multiplexed RNA profiling in single cells. *Science*. 2015;348:aaa6090.
29. Shah S, Lubeck E, Zhou W, Cai L. SeqFISH accurately detects transcripts in single cells and reveals robust Spatial organization in the hippocampus. *Neuron*. 2017;94:752–8. e751.
30. Lubeck E, Coskun AF, Zhiyentayev T, Ahmad M, Cai L. Single-cell in situ RNA profiling by sequential hybridization. *Nat Methods*. 2014;11:360–1.
31. Lee JH, Daugherty ER, Scheiman J, Kalhor R, Yang JL, Ferrante TC, Terry R, Jeanty SS, Li C, Amamoto R. Highly multiplexed subcellular RNA sequencing in situ. *Science*. 2014;343:1360–1363.
32. Biancalani T, Scalia G, Buffoni L, Avasthi R, Lu Z, Sanger A, Tokcan N, Vanderburg CR, Segerstolpe Å, Zhang M. Deep learning and alignment of spatially resolved single-cell transcriptomes with Tangram. *Nat Methods*. 2021;18:1352–62.
33. Vickovic S, Eraslan G, Salmén F, Klughammer J, Stenbeck L, Schapiro D, Åijö T, Bonneau R, Bergenstråhle L, Navarro JF. High-definition Spatial transcriptomics for in situ tissue profiling. *Nat Methods*. 2019;16:987–90.
34. Cho C-S, Xi J, Si Y, Park S-R, Hsu J-E, Kim M, Jun G, Kang HM, Lee JH. Microscopic examination of Spatial transcriptome using Seq-Scope. *Cell*. 2021;184:3559–72. e3522.
35. Greenwald AC, Darnell NG, Hoefflin R, Simkin D, Mount CW, Castro LNG, Harnik Y, Dumont S, Hirsch D, Nomura M. Integrative Spatial analysis reveals a multi-layered organization of glioblastoma. *Cell*. 2024;187:2485–501. e2426.
36. Ren Y, Huang Z, Zhou L, Xiao P, Song J, He P, Xie C, Zhou R, Li M, Dong X. Spatial transcriptomics reveals niche-specific enrichment and vulnerabilities of radial glial stem-like cells in malignant gliomas. *Nat Commun*. 2023;14:1028.
37. Zheng Y, Carrillo-Perez F, Pizurica M, Heiland DH, Gevaert O. Spatial cellular architecture predicts prognosis in glioblastoma. *Nat Commun*. 2023;14:4122.
38. Harwood DSL, Pedersen V, Bager NS, Schmidt AY, Stannius TO, Ařeškevičiūtė A, Josefsen K, Nørøxe DS, Scheide D, Rostalski H. Glioblastoma cells increase expression of Notch signaling and synaptic genes within infiltrated brain tissue. *Nat Commun*. 2024;15:7857.
39. Wu L, Yan J, Bai Y, Chen F, Zou X, Xu J, Huang A, Hou L, Zhong Y, Jing Z. An invasive zone in human liver cancer identified by Stereo-seq promotes hepatocyte–tumor cell crosstalk, local immunosuppression and tumor progression. *Cell Res*. 2023;33:585–603.
40. Zhou P-Y, Zhou C, Gan W, Tang Z, Sun B-Y, Huang J-L, Liu G, Liu W-R, Tian M-X, Jiang X-F. Single-cell and Spatial architecture of primary liver cancer. *Commun Biology*. 2023;6:1181.
41. Magen A, Hamon P, Fiaschi N, Soong BY, Park MD, Mattiuz R, Humblin E, Troncoso L, D'souza D, Dawson T. Intratumoral dendritic cell–CD4+T helper cell niches enable CD8+T cell differentiation following PD-1 Blockade in hepatocellular carcinoma. *Nat Med*. 2023;29:1389–99.
42. Cui Zhou D, Jayasinghe RG, Chen S, Herndon JM, Iglesia MD, Navale P, Wendt MC, Caravan W, Sato K, Storrs E. Spatially restricted drivers and transitional cell populations cooperate with the microenvironment in untreated and chemo-resistant pancreatic cancer. *Nat Genet*. 2022;54:1390–405.
43. Klein L, Tu M, Krebs N, Urbach L, Grimm D, Latif MU, Penz F, Blandau A, Wu X, Samuel RD. Spatial tumor immune heterogeneity facilitates subtype co-existence and therapy response in pancreatic cancer. *Nat Commun*. 2025;16:335.
44. Oliveira MFd, Romero JP, Chung M, Williams SR, Gottschö AD, Gupta A, Pilipauskas SE, Mohabbat S, Raman N, Sukovich DJ. High-definition Spatial transcriptomic profiling of immune cell populations in colorectal cancer. *Nat Genet*. 2025;1–12.
45. Kiviahio A, Eerola SK, Kallio HM, Andersen MK, Hoikka M, Tiihonen AM, Salonen I, Spotbeen X, Giesen A, Parker CT. Single cell and Spatial transcriptomics highlight the interaction of club-like cells with immunosuppressive myeloid cells in prostate cancer. *Nat Commun*. 2024;15:9949.
46. Hirz T, Mei S, Sarkar H, Kfoury Y, Wu S, Verhoeven BM, Subtelny AO, Zlatev DV, Wszolek MW, Salari K. Dissecting the immune suppressive human prostate tumor microenvironment via integrated single-cell and Spatial transcriptomic analyses. *Nat Commun*. 2023;14:663.
47. Chao Y, Xiang Y, Xiao J, Zheng W, Ebrahimkhani MR, Yang C, Wu AR, Liu P, Huang Y, Sugimura R. Organoid-based single-cell Spatiotemporal gene expression landscape of human embryonic development and hematopoiesis. *Signal Transduct Target Therapy*. 2023;8:230.
48. Legnini I, Emmenegger L, Zappulo A, Rybak-Wolf A, Wurmus R, Martinez AO, Jara CC, Boltengagen A, Hessler T, Mastrobuoni G. Spatiotemporal, optogenetic control of gene expression in organoids. *Nat Methods*. 2023;20:1544–52.
49. Ma S, Wang W, Zhou J, Liao S, Hai C, Hou Y, Zhou Z, Wang Z, Su Y, Zhu Y. Lamination-based organoid spatially resolved transcriptomics technique for primary lung and liver organoid characterization. *Proceedings of the National Academy of Sciences* 2024;121:e2408939121.
50. Wahle P, Brancati G, Harmel C, He Z, Gut G, Del Castillo JS, Xavier da Silveira dos Santos, Yu A, Noser Q, Fleck P. JS: Multimodal spatiotemporal phenotyping of human retinal organoid development. *Nat Biotechnol*. 2023;41:1765–1775.
51. Koc-Gunel S, Liu EC, Gautam LK, Calvert B, Murthy S, Harriott NC, Nawroth JC, Zhou BY, Krymskaya VP, Ryan AL. Targeting fibroblast-endothelial cell interactions in LAM pathogenesis using 3D spheroid models and Spatial transcriptomics. *Jci Insight*. 2025;10.
52. Dao L, You Z, Lu L, Xu T, Sarkar AK, Zhu H, Liu M, Calandrelli R, Yoshida G, Lin P. Modeling blood-brain barrier formation and cerebral cavernous malformations in human PSC-derived organoids. *Cell Stem Cell*. 2024;31:818–33. e811.
53. Cappuccio G, Khalil SM, Osenberg S, Li F, Maletic-Savatic M. Mass spectrometry imaging as an emerging tool for studying metabolism in human brain organoids. *Front Mol Biosci*. 2023;10:1181965.
54. Wang Y, Hummon AB. MS imaging of multicellular tumor spheroids and organoids as an emerging tool for personalized medicine and drug discovery. *J Biol Chem*. 2021;297.
55. Onesto MM, Kim J-i, Pasca SP. Assembloid models of cell-cell interaction to study tissue and disease biology. *Cell Stem Cell*. 2024;31:1563–73.
56. Kanton S, Pasca SP. Human assembloids. *Development*. 2022;149:dev201120.
57. Marton RM, Pasca SP. Organoid and assembloid technologies for investigating cellular crosstalk in human brain development and disease. *Trends Cell Biol*. 2020;30:133–43.
58. Kim E, Choi S, Kang B, Kong J, Kim Y, Yoon WH, Lee H-R, Kim S, Kim H-M, Lee H. Creation of bladder assembloids mimicking tissue regeneration and cancer. *Nature*. 2020;588:664–9.
59. Miura Y, Li M-Y, Revah O, Yoon S-J, Narazaki G, Pasca SP. Engineering brain assembloids to interrogate human neural circuits. *Nat Protoc*. 2022;17:15–35.
60. Pasca SP, Arlotta P, Bateup HS, Camp JG, Cappello S, Gage FH, Knoblich JA, Kriegstein AR, Lancaster MA. Ming G-L: A framework for neural organoids, assembloids and transplantation studies. *Nature*. 2025;639:315–20.
61. Kim J-i, Imaizumi K, Jurjut O, Kelley KW, Wang D, Thete MV, Hudacova Z, Amin ND, Levy RJ, Scherrer G. Human assembloid model of the ascending neural sensory pathway. *Nature* 2025;1–11.
62. Sharpe BP, Nazlamova LA, Tse C, Johnston DA, Thomas J, Blyth R, Pickering OJ, Grace B, Harrington J, Rajak R. Patient-derived tumor organoid and fibroblast assembloid models for interrogation of the tumor microenvironment in esophageal adenocarcinoma. *Cell Rep Methods*. 2024;4.
63. Zhang Y, Hu Q, Pei Y, Luo H, Wang Z, Xu X, Zhang Q, Dai J, Wang Q, Fan Z. A patient-specific lung cancer assembloid model with heterogeneous tumor microenvironments. *Nat Commun*. 2024;15:3382.
64. Mei J, Liu X, Tian HX, Chen Y, Cao Y, Zeng J, Liu YC, Chen Y, Gao Y, Yin JY. Tumour organoids and assembloids: patient-derived cancer avatars for immunotherapy. *Clin Translational Med*. 2024;14:e1656.
65. Cacciamali A, Villa R, Dotti S. 3D cell cultures: evolution of an ancient tool for new applications. *Front Physiol*. 2022;13:836480.

66. Fang Y, Eglén RM. Three-dimensional cell cultures in drug discovery and development. *Slas discovery: Advancing Life Sciences R&D*. 2017;22:456–472.
67. Si Y, Lee JS, Jun G, Kang HM, Lee JH. Spatial omics enters the microscopic realm: opportunities and challenges. *Trends Genet*. 2025.
68. Roth JG, Brunel LG, Huang MS, Liu Y, Cai B, Sinha S, Yang F, Pasca SP, Shin S, Heilshorn SC. Spatially controlled construction of assembloids using Bioprinting. *Nat Commun*. 2023;14:4346.
69. Bouchard G, Zhang W, Ilterten I, Li I, Bhattacharya A, Li Y, Trope W, Shrager JB, Kuo C, Ozawa MG. A quantitative Spatial cell-cell colocalizations framework enabling comparisons between in vitro assembloids and pathological specimens. *Nat Commun*. 2025;16:1392.
70. Williams CG, Lee HJ, Asatsuma T, Vento-Tormo R, Haque A. An introduction to Spatial transcriptomics for biomedical research. *Genome Med*. 2022;14:68.
71. Yuan T, Fu X, Hu R, Zheng X, Jiang D, Jing L, Kuang X, Guo Z, Luo X, Liu Y. Bioprinted, spatially defined breast tumor microenvironment models of intratumoral heterogeneity and drug resistance. *Trends Biotechnol*. 2024;42:1523–50.
72. Fang Y, Hao W, Xiong Z. On-orbit 3D Bioprinting for tumor modeling in space. *Trends Biotechnol*. 2025.
73. Lammi MJ, Qu C. Spatial transcriptomics, proteomics, and epigenomics as tools in tissue engineering and regenerative medicine. *Bioengineering*. 2024;11:1235.
74. Wang H, Liu S, Yin X, Huang M, Fu Y, Chen X, Wang C, Sun J, Yan X, Han J. Polar-coordinate line-projection light-curing continuous 3D printing for tubular structures. *Int J Extreme Manuf*. 2024;6:045004.
75. Reynolds DE, Roh YH, Oh D, Vallapureddy P, Fan R, Ko J. Temporal and Spatial omics technologies for 4D profiling. *Nat Methods*. 2025;1–12.
76. Zhang N, Ohlstrom D, Pang S, Bharadwaj NS, Qu A, Grossniklaus H, Coskun AF. Tissue Spatial omics dissects organoid biomimicry. *GEN Biotechnol*. 2023;2:372–83.
77. Byalahunasishetter BI, Pallavi V, Savitha C, Moger SS, Raghavan R. Spatial omics and bioprinting: tissue engineering and precision medicine. *Innovations in precision medicine and genomics*. IGI Global Scientific Publishing; 2025. pp. 85–110.
78. Alonso-Roman R, Mosig AS, Figge MT, Papenfort K, Eggeling C, Schacher FH, Hube B, Gresnigt MS. Organ-on-chip models for infectious disease research. *Nat Microbiol*. 2024;9:891–904.
79. Caballero D, Kaushik S, Corrello V, Oliveira JM, Reis R, Kundu S. Organ-on-chip models of cancer metastasis for future personalized medicine: from chip to the patient. *Biomaterials*. 2017;149:98–115.
80. Mittal R, Woo FW, Castro CS, Cohen MA, Karanxha J, Mittal J, Chhibber T, Jhaveri VM. Organ-on-chip models: implications in drug discovery and clinical applications. *J Cell Physiol*. 2019;234:8352–80.
81. Srivastava SK, Foo GW, Aggarwal N, Chang MW. Organ-on-chip technology: opportunities and challenges. *Biotechnol Notes*. 2024;5:8–12.
82. Lou C, Yang H, Hou Y, Huang H, Qiu J, Wang C, Sang Y, Liu H, Han L. Microfluidic platforms for Real-Time in situ monitoring of biomarkers for cellular processes. *Adv Mater*. 2024;36:2307051.
83. Seder I, Zheng T, Zhang J, Rojas CC, Helalat SH, Téllez RC, Sun Y. A scalable microfluidic platform for nanoparticle formulation: for exploratory-and industrial-level scales. *Nano Lett*. 2024;24:5132–8.
84. Liu Y, Yang M, Deng Y, Su G, Enniful A, Guo CC, Tebaldi T, Zhang D, Kim D, Bai Z. High-spatial-resolution multi-omics sequencing via deterministic barcoding in tissue. *Cell*. 2020;183:1665–81. e1618.
85. Wirth J, Huber N, Yin K, Brood S, Chang S, Martinez-Jimenez CP, Meier M. Spatial transcriptomics using multiplexed deterministic barcoding in tissue. *Nat Commun*. 2023;14:1523.
86. Cheng M, Jiang Y, Xu J, Mentis A-FA, Wang S, Zheng H, Sahu SK, Liu L, Xu X. Spatially resolved transcriptomics: a comprehensive review of their technological advances, applications, and challenges. *J Genet Genomics*. 2023;50:625–40.
87. Shi J, Pan Y, Liu X, Cao W, Mu Y, Zhu Q. Spatial omics sequencing based on microfluidic array chips. *Biosensors*. 2023;13:712.
88. Menon NV, Lee J, Tang T, Lim CT. Microfluidics for morphomics and Spatial omics applications. *Lab on a Chip*; 2025.
89. Zhu J, Pang K, Hu B, He R, Wang N, Jiang Z, Ji P, Zhao F. Custom microfluidic chip design enables cost-effective three-dimensional Spatiotemporal transcriptomics with a wide field of view. *Nat Genet*. 2024;56:2259–70.
90. Jung N, Kim T-K. Spatial transcriptomics in neuroscience. *Exp Mol Med*. 2023;55:2105–15.
91. Cao J, Li C, Cui Z, Deng S, Lei T, Liu W, Yang H, Chen P. Spatial transcriptomics: a powerful tool in disease Understanding and drug discovery. *Theranostics*. 2024;14:2946.
92. Lee Y, Lee M, Shin Y, Kim K, Kim T. Spatial omics in clinical research: A comprehensive review of technologies and guidelines for applications. *Int J Mol Sci*. 2025;26:3949.
93. Alexandrov T, Saez-Rodríguez J, Saka SK. Enablers and challenges of Spatial omics, a melting pot of technologies. *Mol Syst Biol*. 2023;19:e10571.
94. Wan X, Xiao J, Tam SST, Cai M, Sugimura R, Wang Y, Wan X, Lin Z, Wu AR, Yang C. Integrating Spatial and single-cell transcriptomics data using deep generative models with spatialscope. *Nat Commun*. 2023;14:7848.
95. Gong D, Arbesfeld-Qiu JM, Perrault E, Bae JW, Hwang WL. Spatial oncology: translating contextual biology to the clinic. *Cancer Cell*. 2024;42:1653–75.
96. Smith KD, Prince DK, MacDonald JW, Bammler TK, Akilesh S. Challenges and opportunities for the clinical translation of Spatial transcriptomics technologies. *Glomerular Dis*. 2024;4:49–63.
97. Di Mauro F, Arbore G. Spatial dissection of the immune landscape of solid tumors to advance precision medicine. *Cancer Immunol Res*. 2024;12:800–13.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.