



# Spatial omics: applications and utility in profiling the tumor microenvironment

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## Abstract

Spatial transcriptomics has emerged as a transformative technology in biomedical research, offering unprecedented insights into gene and protein expression within their native tissue context. Unlike conventional bulk or single-cell sequencing approaches, spatial omics has the advantage of preserving the spatial structure of tissues, allowing researchers to directly map molecular information onto histological structures. This review provides an overview of the current state of spatial omics technologies, highlighting their application in cancer research. Spatial omics has enabled detailed characterization of the tumor microenvironment (TME), revealing spatial heterogeneity, immune cell infiltration patterns, and complex mechanisms of tumor progression and therapy resistance across various cancer types. The review covers future directions, including artificial intelligence-driven analytics, improved standardization, and cost reduction to accelerate clinical translation. Ultimately, spatial omics is poised to play a central role in precision oncology, enabling a deeper understanding of tumor biology and informing more effective individualized treatment strategies.

**Keywords** Spatial transcriptomics · Cancer research · Tumor microenvironment · Tumor heterogeneity · Tumor-immune interactions · Precision medicine

## 1 Introduction: spatial omics technologies

### 1.1 The evolution of spatial omics technology

Traditional genomic approaches, such as bulk RNA sequencing, have been instrumental in analyzing RNA to infer overall tissue characteristics. However, these methods lack spatial resolution, which limits their ability to identify where specific transcripts originate within the tissue architecture and hinders the understanding of cellular interactions *in situ*. The advent of single-cell transcriptomics marked a major advance, enabling the dissection of transcriptomes at the level of individual cells. This allowed for finer classification of cell states and types, and provided new insights into inter-cellular dynamics. Yet, despite this granularity, single-cell

approaches could not definitively resolve spatial context or confirm physical interactions between cells within native tissue environments [1, 2].

Spatial transcriptomics technologies were developed to bridge this gap. Initial methods such as laser-capture microdissection and targeted region-specific RNA sequencing enabled transcriptomic analysis in defined tissue areas, but were limited in throughput and scalability [3–5]. The commercial release of sequencing-based platforms expanded access to spatially resolved transcriptomics with standardization for broad use.

High-resolution imaging-based platforms have significantly advanced spatial omics. Techniques such as multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) allow for direct visualization of RNA transcripts within individual cells, preserving spatial integrity at subcellular resolution [6]. Similarly, other high multiplexing *in situ* hybridization (ISH) and *in situ* sequencing (ISS) systems have adopted single-molecule FISH-like chemistries to subcellularly profile hundreds to thousands of genes within tissue [7, 8]. These innovations have dramatically expanded the utility of spatial transcriptomics and catalyzed

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a wave of global research activity focused on understanding tissue organization, cell–cell communication, and disease processes in unprecedented detail.

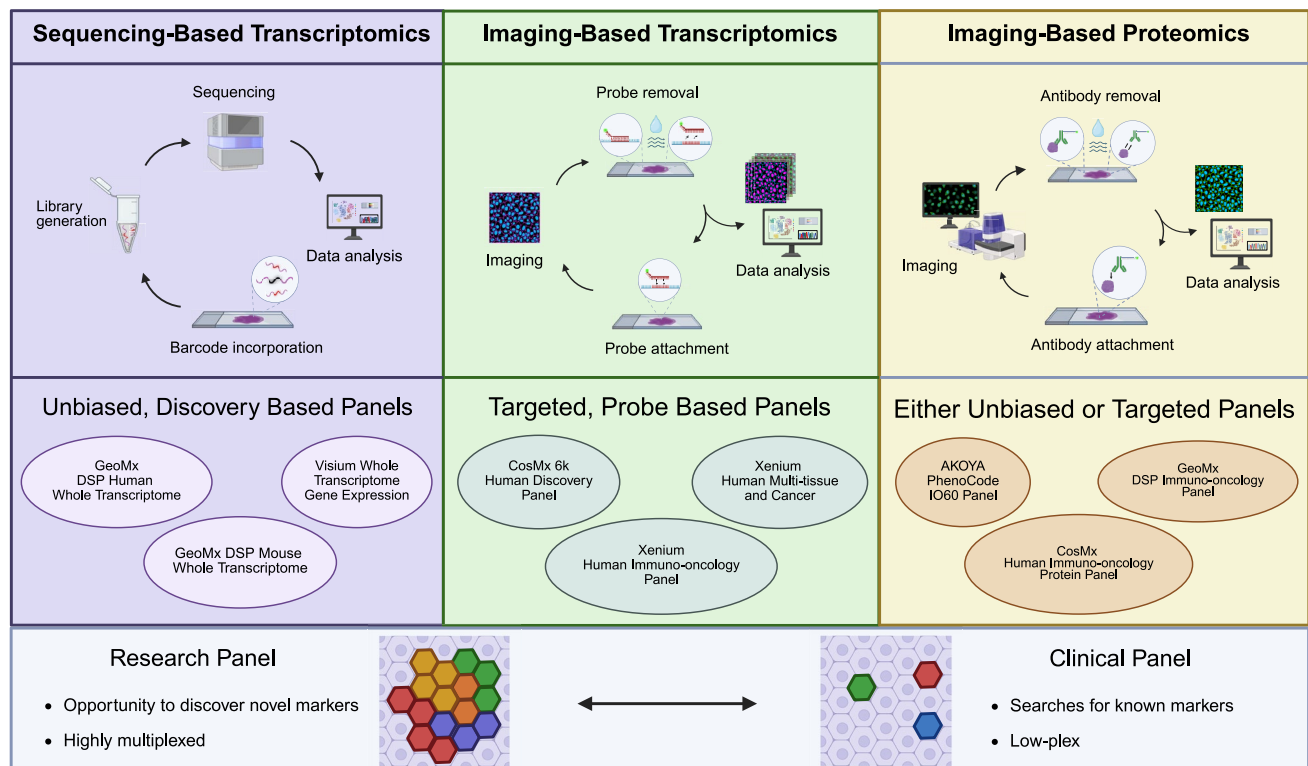
## 1.2 Sequencing-based spatial omics

Sequencing-based spatial transcriptomics applies the core principles of single-cell RNA sequencing to spatially resolved tissues, enabling transcriptome-wide profiling across intact tissue sections. These methods rely on spatially indexed surfaces—such as barcoded glass slides (e.g., 10× Genomics Visium), patterned DNA nanoball arrays (e.g., BGI Stereo-seq), or barcoded beads (e.g., Curio Slide-seq)—to capture RNA molecules in a spatially informed manner [9]. Tissue sections are mounted onto these surfaces, followed by mRNA capture using barcoded oligonucleotides that correspond to known positions on the array. After reverse transcription and library preparation, sequencing reads are mapped back to both the reference genome and the spatial coordinates from which they originated. This enables unbiased, high-throughput transcriptome analysis across large regions of tissue without requiring prior knowledge of target genes. The result is a rich spatial map of gene expression that preserves anatomical context while delivering single-cell or near-cellular resolution. Platforms

like GeoMx Digital Spatial Profiler—combining sequencing with region-specific profiling—and DBIT-seq—using fluidic delivery of the barcodes, allowing for multimodal spatial profiling assays (e.g., RNA, protein and epigenomics)—further expand the utility of sequencing-based approaches [10, 11]. Together, these technologies have positioned sequencing-based spatial omics as a powerful tool for discovery-driven research in cancer, neurobiology, and developmental biology (Fig. 1, Table 1).

## 1.3 Imaging-based spatial transcriptomics

Imaging-based spatial transcriptomics multiplex techniques (ISH, ISS) localize RNA molecules within intact tissues using iterative cycles of hybridization and high-resolution fluorescence imaging [12]. These methods require prior knowledge of target transcripts, as they rely on the design of specific, barcoded oligonucleotide probes that hybridize to selected mRNA species. Following each hybridization cycle, fluorescent imaging captures the spatial position of bound probes, and the signal is then decoded through sequential imaging rounds to reconstruct transcript identity and abundance. Platforms such as MERFISH (multiplexed error-robust fluorescence *in situ* hybridization) and SMI (spatial molecular imaging)



**Fig. 1** Overview of spatial omics methodologies. Schematic diagram of three fundamental methodologies in spatial omics and high multiplex tissue profiling (generated using Biorender)

**Table 1** Comparison of spatial omics technologies. Overview of key characteristics and distinctions between sequencing-based and imaging-based spatial omics approaches. (*FF* fresh frozen, *FFPE* formalin fixed paraffin embedded, *FxF* fixed, frozen, *seq* sequencing, *DBiT* deterministic barcoding in tissue)

| Platform         |                   | Resolution                                       | Gene throughput                                      | Tissue type   | Advantages                                                                                                  | Limitations                                        |
|------------------|-------------------|--------------------------------------------------|------------------------------------------------------|---------------|-------------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| Sequencing-based | <b>GeoMx</b>      | Dependent on ROI size (20–300 cells)             | Whole transcriptome + proteins                       | FFPE, FF, FxF | Flexible ROI selection, non-destructive (slides can be used for other studies after the assay is completed) | Not single-cell resolution, coarse spatial context |
|                  | <b>Stereo-seq</b> | ~0.5–2 μm spot (single-cell, subcellular)        | Whole transcriptome                                  | FFPE, FF      | High spatial resolution, large area capture                                                                 | Limited instrument access, complex data analysis   |
|                  | <b>Visium</b>     | ~55 μm spot (10–20 cells)                        | Whole transcriptome                                  | FFPE, FF, FxF | Whole-transcriptome coverage, standardized workflow                                                         | Not single-cell resolution, coarse spatial context |
|                  | <b>Visium HD</b>  | ~2 μm pixel (single-cell, subcellular)           | Whole transcriptome                                  | FFPE, FF, FxF | High spatial resolution                                                                                     | Large data size                                    |
|                  | <b>Slide-seq</b>  | ~10 μm bead (single-cell)                        | Whole transcriptome                                  | FF            | Low cost, scalable                                                                                          | Not FFPE compatible                                |
|                  | <b>DBiT-seq</b>   | ~10 μm pixel (single-cell)                       | Whole transcriptome + proteins+epigenetic marks      | FFPE, FF      | Multi-omics (RNA + protein)                                                                                 | Complex microfluidic setup                         |
| Imaging-based    | <b>CosMx</b>      | ~0.5–1.2 μm pixel (single-cell, subcellular)     | Targeted panels (~1000–whole transcriptome+proteins) | FFPE, FF      | High spatial resolution, preserve cell integrity                                                            | Limited gene count, long imaging time              |
|                  | <b>Xenium</b>     | ~200 nm pixel (single-cell, subcellular)         | Targeted panels (~5000 genes)                        | FFPE, FF      | High spatial resolution                                                                                     | Not whole-transcriptome, high cost                 |
|                  | <b>MERSCOPE</b>   | ~100–200 nm localization precision (subcellular) | Targeted panels (~ 10,000 genes)                     | FFPE, FF      | High spatial resolution, molecular-level precision                                                          | Complex imaging and analysis, high cost            |

utilize combinatorial barcoding schemes to increase multiplexing capacity while minimizing imaging errors [13, 14]. These approaches enable the simultaneous detection of hundreds to thousands of transcripts from whole transcriptomes at subcellular resolution, offering insights into both gene expression patterns and the fine structure of cellular neighborhoods.

ISS-based platforms such as Xenium offer an alternative to ISH-only approaches by enabling direct readout of RNA sequences within the tissue using reverse transcription of RNA into cDNA, ligation of padlock probes, rolling circle amplification, and fluorescent sequencing-by-ligation. A significant advantage of imaging-based transcriptomics is the preservation of tissue architecture and single-cell spatial resolution, which is particularly valuable for resolving fine-grained structures such as tumor margins, immune cell niches, and developmental gradients [15]. These technologies are well-suited for both hypothesis-driven and exploratory research in contexts where spatial precision is essential. Overall, imaging-based spatial transcriptomics has emerged

as a powerful tool for studying gene expression within its native spatial context (Fig. 1, Table 1).

#### 1.4 Imaging-based spatial proteomics

Imaging-based spatial proteomics leverages similar principles to transcriptomic assays but focuses on detecting and spatially mapping protein expression across tissues. These approaches utilize antibodies or affinity reagents conjugated to either fluorescent tags or unique oligonucleotide barcodes, which are then decoded through iterative imaging or sequencing-based methods. Sequential immunofluorescence (seqIF-COMET) and other cyclic immunofluorescence (CycIF) methods expand on traditional immunohistochemistry by performing repeated rounds of staining, imaging, and signal removal to profile up to 100 protein targets in the same tissue section [16, 17]. One of the most widely used platforms, CODEX (CO-Detection by indEXing), relies on antibody-conjugated DNA barcodes that are visualized through repeated cycles of hybridization with complementary fluorescent reporters. Each imaging round reveals a

subset of proteins, and after multiple cycles, a highly multiplexed protein expression map can be reconstructed across the tissue [18].

Other platforms, such as MIBI (multiplexed ion beam imaging) and IMC (imaging mass cytometry), use metal isotope-tagged antibodies and time-of-flight mass spectrometry to detect dozens of proteins in parallel, offering exceptional multiplexing without the limitations of spectral overlap in fluorescence-based systems. These technologies are especially powerful in oncology, where spatially resolved immune profiling and tumor heterogeneity are critical to understanding disease mechanisms and treatment responses [19, 20].

Proteomic assays are often integrated with transcriptomic spatial data to provide a more comprehensive, multimodal view of tissue organization and cellular phenotypes. While proteomics is inherently limited by the availability and specificity of antibodies, advances in antibody validation and new multiplexing chemistries continue to expand target panels. Imaging-based proteomics also benefits from high spatial fidelity, making it ideal for mapping protein expression at the level of cell–cell contacts and tissue microenvironments (Fig. 1).

### 1.5 Computational methods for spatial data analysis

Spatial omics technologies generate large-scale, high-dimensional data that combine transcriptomic and/or proteomic profiles with spatial coordinates, necessitating specialized computational approaches for analysis. The analytical pipeline generally begins with preprocessing steps such as image alignment, tissue segmentation, and spatial barcode demultiplexing to associate molecular counts with spatial positions or individual cells. For sequencing-based platforms like Visium, GeoMX, DBitseq, or Slide-seq, this involves aligning sequencing reads to a reference genome and mapping transcripts to spatial barcodes; for imaging-based platforms, image registration and spot decoding are used to identify transcript locations. For most spatial assays, including image-based transcriptomics and proteomics, cell segmentation is a critical component in imaging-based analyses, requiring precise delineation of cell boundaries using DAPI staining or membrane markers, often supported by deep learning algorithms (e.g., Cellpose, Stardist) [21] (Table 2).

Once molecular features are assigned to spatial units (cells, spots, or pixels), dimensionality reduction techniques such as principal component analysis (PCA), uniform manifold approximation and projection (UMAP), or t-distributed stochastic neighbor embedding (t-SNE) are used to visualize cellular heterogeneity. Clustering methods—such as Leiden or Louvain—group cells or regions based on transcriptomic similarity, and spatially

aware algorithms (e.g., BayesSpace [22], SpaGCN [23], or stLearn [24]) integrate spatial proximity into the clustering to preserve local structure. To annotate cell types, reference-based label transfer approaches (e.g., Seurat’s anchor-based integration, Tangram, or InsitutypeML) leverage existing single-cell RNA-seq atlases to infer identities based on transcriptional similarity and spatial consistency [25].

Beyond clustering, spatial transcriptomics enables unique downstream analyses, including inference of spatially variable genes using tools like SpatialDE [26], Trendsceek [27], or SPARK [28]. These genes help define anatomical structures, tumor boundaries, or microenvironmental niches. Moreover, ligand–receptor interaction inference tools such as NicheNet [29], CellPhoneDB [30], and Giotto’s spatial interaction modules enable predictions of cell–cell communication by incorporating spatial colocalization into signaling models [31]. Trajectory inference methods adapted for spatial contexts (e.g., Monocle 3 with spatial anchoring or spatial pseudotime models) can reveal developmental or pathological gradients across tissue landscapes [31, 32].

While these computational tools have significantly advanced spatial omics analysis, they rely on distinct assumptions that influence their performance and interpretation. For example, statistical models such as SpatialDE [26] and SPARK [28] assume smooth spatial variation, which may not fully capture abrupt transitions in heterogeneous tissues such as tumors. Graph-based methods like SpaGCN [23] and BayesSpace [22] more effectively model spatial topology but are sensitive to graph construction parameters and resolution scale. Similarly, reference-based mapping frameworks such as Tangram and InsitutypeML depend heavily on the quality and robustness of single-cell reference atlases, potentially including biased cell-type annotation in poorly characterized tissue areas. These considerations underscore the need for careful tool selection, balancing interpretability, robustness, and biological relevance.

Recent integrative frameworks have emerged to address these limitations by combining multiple spatial layers and modeling intercellular relationships more comprehensively. SiGra [33] and xSiGra [34] employ graph neural networks to integrate spatial transcriptomics and proteomics information, revealing latent spatial domains and higher-order cellular interactions. SpaCI [35] leverages spatial gene expression with inferred cell–cell communication networks to capture context-dependent signaling. These methods represent a shift toward multi-modal, predictive spatial OMICs analysis, expanding its analytical depth and translational potential.

Visualization is essential for interpreting spatial omics data and is supported by platforms like Squidpy [36], Giotto [31], Napari [37], and Vitesce [38], which offer interactive spatial plots, expression overlays, and neighborhood graph

**Table 2** Computational pipeline tools for spatial omics processing and analysis. Summary of computational tools and pipelines supporting spatial omics data processing and analysis, with concise descriptions of their functionalities and approaches. (AI) artificial intelligence, *CNN* convolutional neural network, *CODEX* CO-Detection by indEXing, *Cycle* cyclical immunofluorescence, *DL* deep learning, *FISH* fluorescence *in situ* hybridization, *GNN* graph neural network, *GPU* graphics processing unit, *GRN* gene regulatory network, *H&E* hematoxylin and eosin, *LLM* large language model, *MERFISH* multiplexed error-robust fluorescence *in situ* hybridization, *PCA* principal component analysis, *scRNAseq* single cell RNA seq, *ST* spatial transcriptomics)

| Category                              | Tool/pipeline               | Pipeline type           | Primary function(s)                                | Core computational methods          | Input/output data formats        | Unique features/highlights                               | Reference |
|---------------------------------------|-----------------------------|-------------------------|----------------------------------------------------|-------------------------------------|----------------------------------|----------------------------------------------------------|-----------|
| Spatial transcriptomics core analysis | <b>Seurat + STUtility</b>   | Preprocessing/analysis  | Spatial mapping, clustering, visualization         | PCA, multimodal integration         | 10 × Visium, Slide-seq/R objects | Widely used R ecosystem, easy integration with scRNA-seq | [59]      |
|                                       | <b>Giotto</b>               | Analysis/visualization  | Spatial clustering, neighborhood, 3D visualization | Spatial graphs, enrichment analysis | 10 × Visium, seqFISH/HDF5, CSV   | Interactive 2D/3D visualization and network inference    | [31]      |
|                                       | <b>BayesSpace</b>           | Analysis                | Subspot resolution, clustering                     | Bayesian mixture model              | 10 × Visium/R objects            | Enhances spatial resolution beyond spot level            | [22]      |
|                                       | <b>SpatialDE/SpatialDE2</b> | Statistical testing     | Detect spatially variable genes                    | Gaussian process regression         | Generic count matrices/CSV       | Foundational spatial variability model                   | [26, 60]  |
|                                       | <b>SPARK/SPARK-X</b>        | Statistical testing     | Identify spatially variable expression             | Spatial generalized linear model    | Visium, Slide-seq/CSV            | High computational efficiency for large datasets         | [28, 61]  |
|                                       | <b>SpatialScope</b>         | Integration/enhancement | Map high-res histology with gene expression        | Transformer + GNN                   | Stereo-seq, Visium-HD/HDF5       | Learns cell-resolution spatial features                  | [55]      |
|                                       | <b>Spiral</b>               | Integration             | Harmonize multi-resolution spatial datasets        | Diffusion-based smoothing           | seqFISH, MERFISH/AnnData         | Robust to irregular sampling and noise                   | [54]      |
|                                       | <b>Spacia</b>               | Interaction modeling    | Quantify ligand-receptor signaling in space        | Probabilistic spatial regression    | Visium + scRNA-seq/CSV           | Ligand receptor interactions                             | [62]      |
|                                       | <b>SpatialCell/SpaCell</b>  | Integration             | Integrate histology + expression                   | Deep CNN + graph embedding          | H&E + ST/AnnData                 | Joint morphology–transcriptomics learning                | [63]      |
|                                       | <b>Cell2location</b>        | Mapping                 | Map scRNA-seq to spatial transcriptomics           | Bayesian hierarchical modeling      | scRNA-seq + Visium/AnnData       | Quantifies cell abundance per spot                       | [64]      |
|                                       | <b>Tangram</b>              | Integration             | Align scRNA-seq and spatial data                   | Deep learning                       | scRNA-seq + Visium/HDF5          | Cross-modality alignment of transcriptomes               | [32]      |
|                                       | <b>Stelar</b>               | Spatiotemporal Modeling | Track spatial dynamics                             | Latent factor decomposition         | Time-series ST/AnnData           | Models tissue evolution across timepoints                | [65]      |
|                                       | <b>CellPhoneDB</b>          | Analysis                | Ligand–receptor interaction inference              | Statistical modeling                | scRNA-seq/CSV                    | Gold-standard reference of signaling pairs               | [30]      |
|                                       | <b>NicheNet</b>             | Analysis                | Predict ligand-target effects                      | Bayesian regulatory network         | scRNA-seq, ST/CSV                | Links ligands to downstream gene programs                | [29]      |
|                                       | <b>Transceek</b>            | Integration             | Spatially aware signaling inference                | Transformer context learning        | scRNA-seq + ST/PyTorch           | Combines ligand-receptor and morphology context          | [66]      |
| Spatial cell–cell communication       | <b>InsituTypeML</b>         | Annotation              | Cell-type classification                           | Machine learning ensemble           | Visium, MERFISH/AnnData          | Trained across multiple tissue types                     | [67]      |

Table 2 (continued)

| Category                                | Tool/pipeline       | Pipeline type                | Primary function(s)                                       | Core computational methods          | Input/output data formats          | Unique features/highlights                            | Reference |
|-----------------------------------------|---------------------|------------------------------|-----------------------------------------------------------|-------------------------------------|------------------------------------|-------------------------------------------------------|-----------|
| Spatial proteomics/<br>imaging-based    | <b>QuPath</b>       | Preprocessing/quantification | Segmentation, feature extraction                          | Modular Nextflow workflow           | CODEX, CyCIF/OME-Tiff              | Scalable and reproducible image analysis              | [68]      |
|                                         | <b>histoCAT</b>     | Visualization/analysis       | sc feature quantification                                 | PCA/t-SNE, neighbor analysis        | Imaging mass cytometry/CSV         | GUI-based IMC data exploration                        | [69]      |
|                                         | <b>Vitesse</b>      | Preprocessing/quantification | High-throughput image segmentation                        | GPU-accelerated                     | Multiplex imaging/HDF5             | Real-time large-image processing                      | [38]      |
|                                         | <b>CellPose</b>     | Segmentation                 | Universal cell/nucleus segmentation                       | Deep CNN (U-Net)                    | Microscopy, CODEX, H&E/TIFF        | Robust across imaging modalities                      | [21]      |
|                                         | <b>StarDist</b>     | Segmentation                 | Nuclear segmentation                                      | Star-convex polygon model           | Microscopy/TIFF                    | Accurate in dense tissue contexts                     | [70]      |
| Image segmentation and<br>visualization | <b>Napari</b>       | Visualization                | Multi-dimensional viewer                                  | Plugin-based architecture           | TIFF, OME-Tiff/interactive UI      | Open-source ecosystem for spatial data                | [37]      |
|                                         | <b>Monocle3</b>     | Analysis                     | Trajectory inference, pseudotime                          | Graph learning + manifold embedding | scRNA-seq, ST/RDS                  | Supports spatial-temporal lineage analysis            | [71]      |
|                                         | <b>ScFoundation</b> | Foundation model             | Cross-modal representation learning                       | Transformer pretraining             | Multimodal datasets/PyTorch models | Generalizable pre-trained model for sc + spatial data | [72]      |
|                                         | <b>scGPT</b>        | Foundation model             | Generative                                                | Large scale transformer             | scRNAseq + ST/Pytorch              | Adapts LLM to biological tokens                       | [73]      |
|                                         | <b>Sigra/XSigra</b> | Regulatory network           | Infer spatial GRNs                                        | GNN + causality inference           | scRNA-seq + ST/CSV                 | Predicts gene regulatory interactions in tissue       | [33, 34]  |
| AI, DL, ML models                       | <b>SpaGCN</b>       | Integration/analysis         | Detect spatial domains, integrate histology               | Graph convolutional network         | Visium + H&E/AnnData               | Combines image and gene expression context            | [23]      |
|                                         | <b>scGPT</b>        | Foundation model             | Generative representation learning                        | Large-scale transformer             | scRNA-seq, ST/PyTorch              | Adapts LLMs to biological tokens                      | [73]      |
|                                         | <b>spaLLM</b>       | LLM                          | Multi-omics domain discovery using pre-trained embeddings | LLM embeddings + attention fusion   | Multimodal datasets                | Pretrained LLMs for spatial                           | [74]      |
|                                         | <b>STAGATE</b>      | Integration/clustering       | Spatial domain detection, batch correction                | Graph attention autoencoder         | Visium, Slide-seq/AnnData          | Preserves topology across tissue sections             | [75, 76]  |
|                                         | <b>SpatialGlue</b>  | Multimodal integration       | Integrate ST + proteomics/epigenomics                     | GNN                                 | Multimodal datasets                | Multimodal integration                                | [76]      |
|                                         | <b>stLearn</b>      | Integration                  | Integrate morphology with expression                      | ML based                            | Multimodal                         | Integrates spatial, morphology and expression         | [24]      |

visualizations. As data complexity increases—especially with 3D spatial data or multi-omics integration—scalable, interpretable, and biologically grounded computational frameworks will be vital to fully capitalize on the power of spatial omics.

## 2 Application of spatial omics technology to cancer research

Spatial omics is most effectively applied in fields that require detailed analysis of cellular activities. In cancer, spatial omics can be used to map the tumor microenvironment (TME) at the single-cell and subcellular level, revealing the spatial distribution of biomarker expression and interactions of various cell types, including cancer cells, immune cells, fibroblasts, and endothelial cells. This is crucial to understanding how the TME supports tumor evolution, metastasis, and immune evasion, and to identify potential therapeutic targets. In 2023, Xia *et al.* demonstrated the importance of spatial omics for understanding the TME of primary central nervous system lymphoma (PCNSL) [39]. By combining spatial omics with single-cell transcriptomics, high-resolution maps of the TME can be generated to determine cellular composition and spatial location. Spatial omics uniquely revealed how immune and tumor cells were organized and interacted within the PCNSL TME—specifically, the location-dependent immune suppression and signaling patterns that traditional single-cell or bulk analyses could not detect. This study identified distinct tumor subpopulations, such as “defenders,” “attackers,” and “aggressors,” illustrating the influence of immune cell infiltration on tumor behavior, offering insights for targeted therapies based on TME characteristics. These findings represent important progress in the study of PCNSL and contribute to the development of precision treatment strategies. Another typical example is provided by Croizer *et al.*, who mapped the TME and characterized the diversity and plasticity of FAP + cancer-associated fibroblasts (CAFs) and identified 10 CAF-associated EcoCellTypes [40]. This integrated single-cell and spatial omics approach revealed how different CAF clusters interact with immune and cancer cells, offering a deeper understanding of TME organization and suggesting potential therapeutic strategies targeting FAP + CAFs to enhance immunotherapy efficacy. Immunosuppressive myCAF clusters as well as immunosuppressive macrophages (TREM2<sup>+</sup> TAM) and regulatory lymphoid cells (FOXP3<sup>+</sup> Tregs, NKG2A<sup>+</sup> NKregs) were consistently found in close proximity to tumor cells. These spatial patterning—distance-based segregation of cell clusters and EcoCellTypes

enrichments—correlate with patient outcomes. These studies demonstrate that spatial omics goes beyond conventional bulk or single-cell analyses to understand cellular identity and interactions within the TME in a spatial context. Spatial information allows researchers to understand not only the role of each cell, but also where and how they communicate with one another, providing crucial insights for a deeper understanding of cancer behavior and treatment response.

Beyond structural and cellular mapping, spatial omics captures treatment-induced changes in tissue and surrounding the TME, providing an epidemiological understanding of drug action and helping to evaluate drug efficacy and mechanisms at the single cell level. The SpaRx model explores the therapeutic responses of diseases such as non-small cell lung cancer and hepatocellular carcinoma by combining pharmacogenomics with CosMx and MERSCOPE data [41]. This approach reveals spatially distinct drug response patterns, such as scattered cisplatin-sensitive cells and clustered resistant cells in lung cancer and core-localized resistant cells in liver cancer. By identifying spatially relevant regions within cancer, SpaRx uncovered drug resistant mechanisms enabling personalized treatment strategies and also facilitating target validation, biomarker discovery, and drug repurposing across various cancers. These findings demonstrate that spatial omics enhances mechanistic understanding and serves as a translational bridge, linking molecular insights to clinical phenotypes and variability in drug response. Such high-resolution profiling of molecular heterogeneity across different tumor regions within the TME allows the identification of different cancer cell subtypes within a single tumor, providing insights into tumor progression, drug resistance, and metastasis potential. An investigation on intratumoral heterogeneity in pancreatic ductal adenocarcinoma has highlighted the effects of spatial gene expression on tumor behavior and treatment outcomes [42]. Sun *et al.* mapped spatial gene expression in hypoxic and normoxic tumor regions and found that hypoxia induced significant changes in tumor cell subpopulations that increase resistance and promote invasion, particularly in hypoxic areas. This study highlights the potential of targeting hypoxia-related pathways like glycolysis with PI3K inhibitors identified as potential therapeutics for hypoxic regions using spatial omics as a discovery tool. This study emphasizes that spatial context reveals TME-specific vulnerabilities that are critical for designing targeted interventions. Extending this approach to early stage lung adenocarcinoma, a recent study revealed distinct molecular features of lepidic and acinar subtypes, including subtype specific gene signatures with prognostic significance [43]. Spatial profiling further revealed that PD-L1 + endothelial cells in acinar regions contribute to immune evasion and tumor progression by inhibiting CD8 + T-cell infiltration. In highly heterogeneous

tumors, spatial omics analysis improves diagnostic accuracy, which has been shown in two rare breast cancer subtypes, claudin-low (CL) and metaplastic breast carcinoma (MpBC) studies [44]. By conducting spatial omics analysis on 11 triple-negative tumors, including 3 CL and 4 MpBC samples, Coutant *et al.* identified tumor-specific markers like BMPER, POPDC3, and SH3RF3 despite stromal contamination. These results reveal the limitations of bulk molecular profiling in these highly plastic tumors and demonstrate the value of spatial approaches in improving subtype classification and establishing treatment strategies. In high-grade serous ovarian carcinoma originating from serous tubal intraepithelial carcinoma (STIC), early disease progression was investigated by Wang *et al.*, who identified IGFBP2, a key gene that is upregulated in STICs in their native micro-environment through DNA hypomethylation using sequencing-based spatial omics [45]. By spatially mapping epithelial cells and their adjacent stroma, they confirmed that IGFBP2 promotes tumor cell proliferation in the postmenopausal fallopian tubes when knockdown IGFBP2 inhibits tumor growth by blocking the AKT pathway, suggesting the potential mechanism of early cancer development and its therapeutic target potential. Overall, spatial omics holds great potential as a translational tool to guide precision oncology and target discovery, not only by mapping molecular and cellular heterogeneity across tumors but also by providing mechanistic insights into drug response, resistance, and tumor progression at single-cell resolution.

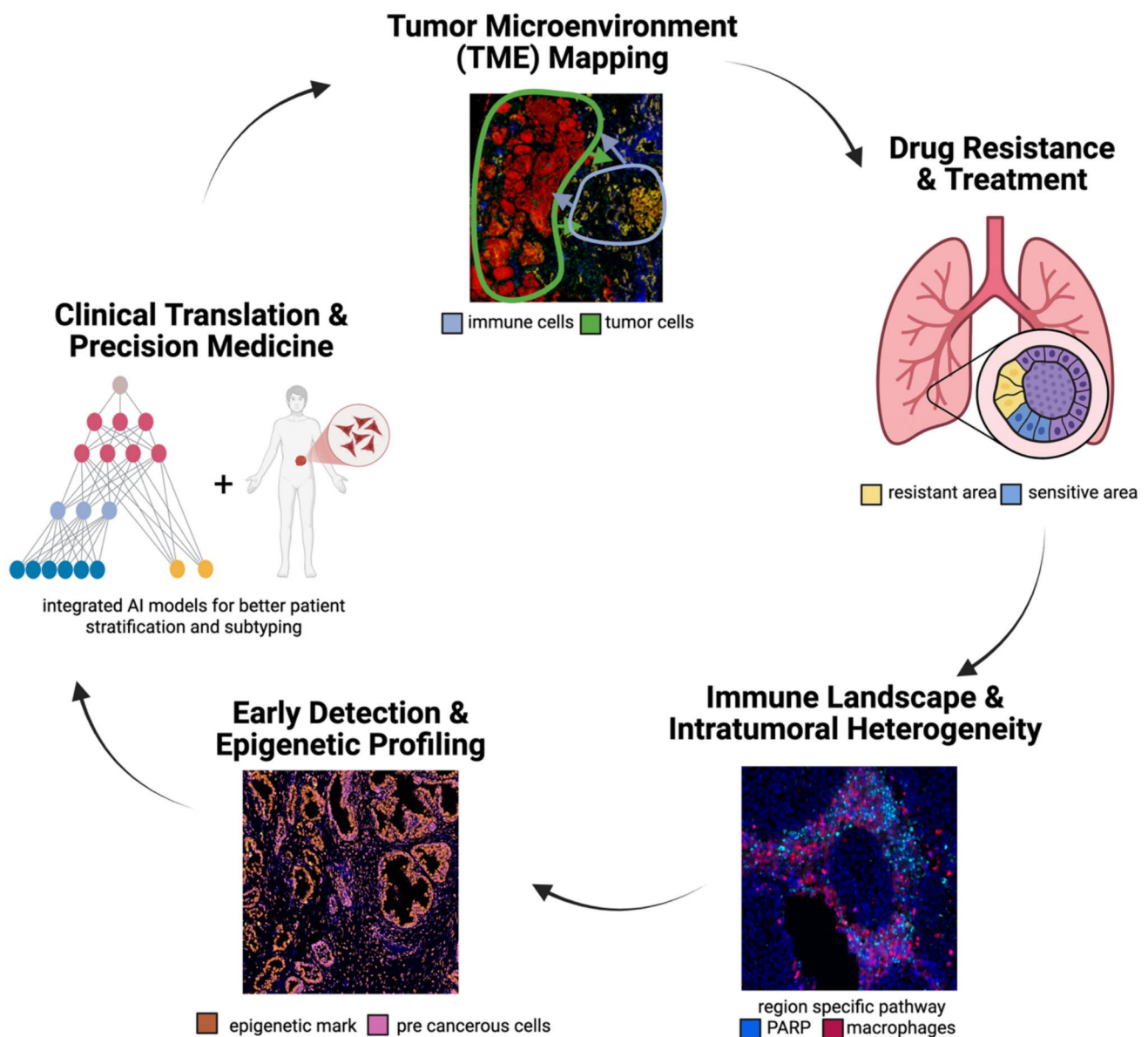
Spatial omics has emerged as an innovative tool to analyze immune cell infiltration and tumor-immune interactions, providing important insights in the development of immunotherapy. Cai *et al.* compared ductal carcinoma *in situ* and invasive ductal carcinoma (IDC), confirming increased tumor aggressiveness and immune evasion during breast cancer progression [46]. Pseudotime spatial omics analysis showed that the activation of oncogenic pathways and higher copy number variations were observed in IDC, and T cells were converted to exhaustion through co-inhibitory interactions such as NECTIN2–TIGIT. These findings demonstrate how tumor–immune interactions shape the immunosuppressive TME. With the increasing success of immune checkpoint inhibitors, the understanding of tumor–immune interactions has become more essential. Spatial omics has become indispensable for interpreting immune checkpoint inhibitor responses by revealing how immune cell distribution and tumor-immune interactions affect treatment outcomes. Iwasa *et al.* identified the mechanisms of acquired resistance to immunotherapy in oral squamous cell carcinoma through spatial omics [47]. By comparing tumor and TME samples before and after immunotherapy, the authors found a shift from immune-related to epigenetic pathways, including increased activity of PRMTs, EZH2, HDACs, and DNMT1. These changes associated with decreased MHC

class I expression and impaired antigen presentation show the possibility of overcoming resistance in combination with epigenetic inhibitors and immunotherapy. Another study applied spatial omics to characterize the immune landscape of head and neck angiosarcomas, a rare tumor type associated with UV exposure [48]. By integrating spatial data with genomic and transcriptomic analyses, Loh *et al.* discovered topological heterogeneity, including immune-hot tumors with inflammation excluded from tumor regions and immune-cold tumors harboring inflammatory foci. These findings reveal the spatial complexity of immune infiltration and suggest that spatial profiling can improve the accuracy of immunotherapy strategies in angiosarcoma. These studies demonstrate that spatial omics provide mechanistic insights into how the spatial organization of immune cells and tumor-immune interactions influence immunotherapy outcomes, enabling the identification of actionable targets and the design of more precise and context-specific immunotherapy strategies.

Spatial omics offers a comprehensive, single-cell level perspective on tumor biology, enabling the identification of specific molecular signatures and guiding precision medicine. By mapping how different tumor regions respond to therapy, spatial omics can guide the selection of targeted therapies tailored to individual patients. Wang *et al.* used spatial omics approaches to map the tumor architecture and TME in 92 triple-negative breast cancer (TNBC) patients, identifying nine spatial archetypes linked to clinical outcomes [49]. The study revealed distinct molecular and immune features, including a novel tertiary lymphoid structure gene signature predictive of immunotherapy response, emphasizing the value of spatial profiling in refining TNBC classification and guiding personalized treatment strategies. Spatial omics can also help identify new drug targets by revealing which genes and pathways are activated in specific regions of the tumor or TME. This helps guide the development of targeted therapies aimed at disrupting cancer-promoting signals in the TME or cancer cells themselves. Arora *et al.* conducted a study on HPV-negative oral squamous cell carcinoma using spatial omics to reveal distinct gene expression and cellular profiles between the tumor core (TC) and leading edge (LE) [50]. The LE signature, linked to invasiveness and poor prognosis, was conserved across cancers, while the TC signature correlated with better outcomes. The study also suggested that drugs reversing LE-like states could improve therapy, suggesting that spatial omics can precisely identify the LE as a promising treatment target. Vahid *et al.* performed spatial proteomics profiling of tumor and stromal compartments in 102 non-small-cell lung cancer (NSCLC) patients to identify protein signatures associated with overall survival rates [51]. This study has shown that stromal CD56 expression correlates with better survival, whereas tumoral B-cell lymphoma extra large (BCLXL)

and B7-H3 were linked to poorer outcomes. These results suggest the possibility of spatial omics to discover prognostic biomarkers and develop targeted treatments in NSCLC. Spatial omics goes beyond simply classifying molecular features and transforms spatially analyzed molecular information into insights applicable to precision medicine. By systematically linking specific molecular features to tumor architecture, immune environment, and clinical outcomes, spatial omics enhances personalized treatment strategies and highlights previously unknown prognostic factors and therapeutic targets (Fig. 2).

Spatial omics technologies are transforming cancer research by enabling high-resolution insights into tumor architecture and cellular interactions. As these technologies evolve beyond simple mapping tools into integrated platforms, they are revealing that core functional characteristics of tumor biology such as subclonal evolution, immune evasion, metabolic adaptation, and therapeutic resistance are intimately linked to spatial organization. By capturing these spatially resolved processes, spatial omics establishes a conceptual framework that links molecular alterations to their spatial and functional consequences within the tumor microenvironment. This framework supports



**Fig. 2** Applications of spatial omics in cancer research and clinical translation. Schematic diagram depicting spatial omics utility in cancer research and future clinical applications. Microscopy images

were generated by Plummer lab and schematic images were generated using BioRender

spatial epigenetic profiling for precision oncology and early diagnosis, enabling the rational design of personalized therapeutic strategies. These applications are reshaping our understanding of tumor biology and accelerating the translation of spatial insights into clinical practice.

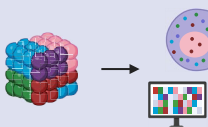
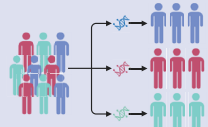
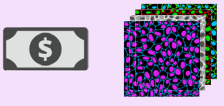
### 3 Unanswered questions and challenges in cancer research with spatial omics

Although spatial omics has significantly advanced our understanding of cancer, several challenges and unanswered questions remain that still hinder its full potential in cancer research and clinical application. A major technical challenge with current spatial omics platforms is their limited resolution, especially when it comes to distinguishing individual cells. For these applications, because it is not assigned at an individual cell level, it becomes computationally challenging to separate gene activity accurately and restricts the analysis of cellular heterogeneity, which is important for cancer research. High-resolution imaging-based transcriptomic approaches offer greater resolution but are limited in scale and gene coverage and require predefined probe panels, which may limit the discovery of novel transcripts or rare cell populations. Developing platforms that balance high resolution, comprehensive transcriptome coverage (which was recently launched by the CosMX platform) and scalability is still a significant challenge, particularly when studying tumors where the spatial distribution of rare cell populations is important. Both sequencing-based and imaging-based approaches are limited in the range of available morphology markers. The addition of validated antibodies for staining could alleviate challenges associated with distinguishing important cell types. Tissue degradation and adherence to the slide also present a technical challenge for all spatial omics assays. Many tissue types that have not been validated may require additional tissue optimization, which can be problematic when a sample is limited in supply. Smaller pieces of tissue, such as those found in tissue microarrays, are more likely to detach from the slide during tissue processing, leading to the loss of samples.

Another challenge is the integration of spatial omics with other omics data. Tumors are formed for various reasons, including gene expression, mutations, chromatin state, protein expression, and metabolic state, each of which has its own impact on cancer progression and treatment resistance. Integrating spatial omics with multi-omics data can provide a more comprehensive understanding of cancer biology. However, spatial omics data tend to be sparse and noisy, and in certain cases, genes can be under-detected due to molecular capture limitations, which limit biological interpretability and reduce the accuracy of

integration with other modalities at single-cell resolution [52]. Moreover, spatial datasets may contain novel or rare cell types that are not present in scRNA-seq references, and assuming complete overlap in cell populations during data integration can introduce bias or misinterpretation [53]. Few standardized protocols and analytical frameworks exist for integrating multi-modal datasets, which hinder effective data alignment among data sets. SPIRAL, developed by Guo *et al.*, is an analytical tool that performs well in batch integrations and tissue structure delineation across samples from different spatial technologies, but the integration of datasets with varying spatial resolution is still a challenge and has limitations under particular experimental conditions and platforms [54]. Methods like SpatialScope, developed by Wan *et al.*, use deep generative models to integrate scRNA-seq and spatial transcriptomics platforms for single-cell resolution and transcriptome-wide imputation, but such frameworks are time-consuming and the model's assumption that neighboring cells have the same cell type is not always valid in all tissue contexts [55]. There is a need for robust and generalizable algorithms and integration tools capable of handling multi-omics data at the single-cell level across different experimental settings and biological contexts [56].

The absence of these standardized protocols reduces reproducibility across platforms and laboratories, preventing spatial omics from being effectively implemented in the clinical area [57]. Moreover, the clinical predictive or prognostic utility of spatial omics has not been sufficiently validated through large-scale clinical trials to date. For spatial omics to be a routine part of precision oncology, further studies are essential to link spatial gene expression patterns with clinical outcomes across various cancer types and patient populations. In addition, the high cost of spatial omics experiments is a hurdle to clinical translation. It requires infrastructure such as specialized equipment, consumables, and data storage solutions, as well as personnel trained in sample preparation, imaging, and data analysis. These economic constraints limit the scale of studies that include heterogeneous patient cohorts and ultimately slow the clinical translation of spatial omics in cancer research. Efforts to reduce costs, such as targeted gene panels, improved barcoding, and the design of low-cost imaging methods, may facilitate widespread adoption of spatial omics in clinical practice in the future (Fig. 3). To move toward clinical translation, spatial omics assays must meet stringent regulatory and technical standards akin to those that facilitated the clinical adoption of next-generation sequencing (e.g., MACQC guidelines). However, spatial proteomics has a clear path to clinical practice because it is uniquely positioned with current diagnostic use antibody-based panels. Achieving this will require not only analytical reproducibility and assay robustness but also streamlined workflows and interpretability. In this context, artificial

| Current Challenges                                                                                                                                                                                                                                | Future Directions                                                                                                                                                                                                          |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Technical challenges</b> <ul style="list-style-type: none"> <li>Limited spatial resolution</li> </ul>                                                         |  <b>Technical advancements</b> <ul style="list-style-type: none"> <li>Advanced AI and machine learning</li> </ul>                        |
| <b>Integration challenges</b> <ul style="list-style-type: none"> <li>Lack of standard analysis pipelines</li> </ul>                                              |  <b>Development of standardization methods</b> <ul style="list-style-type: none"> <li>Creation of standard analysis pipelines</li> </ul> |
| <b>Clinical Implementation challenges</b> <ul style="list-style-type: none"> <li>Insufficient validation through large-scale clinical trials to date</li> </ul>  |  <b>Advancements in Clinical Practice</b> <ul style="list-style-type: none"> <li>Enhanced detection and diagnosis</li> </ul>             |
| <b>Economic Constraints</b> <ul style="list-style-type: none"> <li>High imaging costs with low accessibility</li> </ul>                                          |  <b>Low cost imaging methods</b> <ul style="list-style-type: none"> <li>Affordable technologies with wider access</li> </ul>             |

**Fig. 3** Challenges and future directions in spatial omics for cancer research. Current challenges and future directions of applying spatial omics to cancer research: from technological, analytical, and biological perspectives. Schematic images were generated using Biorender

intelligence (AI) and machine learning (ML) are poised to play a transformative role (Figs. 2, 3). New frameworks such as *Spacia*, a multi-instance learning model, exemplify how AI can infer biologically meaningful cell–cell interactions from spatial omics data, even in complex settings like TMEs. *scGPT-spatial*, a transformer-based large language model with a Mixture-of-Experts decoder for protocol-aware integration, and *Novae*, a self-supervised graph attention network that encodes local microenvironmental context into spatial representations, demonstrate the power of AI to integrate multimodal spatial transcriptomics data while uncovering biologically meaningful cell–cell interactions and TME heterogeneity in cancer tissues. By modeling one-to-many communication networks, these tools provide new insight into tissue ecology that would be difficult to achieve through manual annotation alone. Foundation models like *CellLM* and *scFoundation* link cellular states to drug response and metastatic potential, highlighting the increasingly important role AI and ML play as predictive engines for precision oncology [58] (Table 2).

## 4 Conclusion and future perspectives

Spatial omics technologies have rapidly evolved from niche experimental tools to powerful platforms capable of resolving cellular organization and communication within

intact tissues. Continued advances in chemistry, imaging, and sequencing are expected to enhance both spatial resolution and molecular sensitivity, enabling the detection of low-abundance targets and rare cell states with greater precision. A major frontier lies in the development of standardized protocols for integrating multi-omics modalities—such as spatial transcriptomics, proteomics, epigenomics, and metabolomics—which would allow for a more holistic understanding of tumor biology and disease progression. Importantly, spatial omics not only identifies molecular features but also uncovers functional landscapes within tumors, including spatial gradients of tumor evolution, immune–tumor crosstalk, and localized microenvironmental influences on therapy response. By linking these spatial patterns to clinical outcomes, spatial omics provides a conceptual framework for interpreting how cellular organization drives disease progression and therapeutic resistance. Such integration could yield robust, spatially informed biomarkers for early cancer detection, prognosis, and therapy selection.

As accessibility and throughput continue to improve, the impact of spatial omics is likely to extend well beyond oncology into areas such as neurodegeneration, immunology, infectious disease, and developmental biology. Ultimately, the convergence of high-resolution spatial measurements, multi-modal integration, and advanced computational tools will redefine how we study human disease—bringing spatial biology from bench to bedside.

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## Declarations

**Ethics approval** Authors have nothing to declare.

**Competing interests** The authors declare no competing interests.

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