

Review

Spatiotemporal omics for biology and medicine

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SUMMARY

The completion of the Human Genome Project has provided a foundational blueprint for understanding human life. Nonetheless, understanding the intricate mechanisms through which our genetic blueprint is involved in disease or orchestrates development across temporal and spatial dimensions remains a profound scientific challenge. Recent breakthroughs in cellular omics technologies have paved new pathways for understanding the regulation of genomic elements and the relationship between gene expression, cellular functions, and cell fate determination. The advent of spatial omics technologies, encompassing both imaging and sequencing-based methodologies, has enabled a comprehensive understanding of biological processes from a cellular ecosystem perspective. This review offers an updated overview of how spatial omics has advanced our understanding of the translation of genetic information into cellular heterogeneity and tissue structural organization and their dynamic changes over time. It emphasizes the discovery of various biological phenomena, related to organ functionality, embryogenesis, species evolution, and the pathogenesis of diseases.

INTRODUCTION

The advancements in molecular biology throughout the 20th century, particularly the formulation of the Central Dogma,¹ revealed how our genetic information is transcribed into messenger RNA (mRNA) and then translated into protein, uncovering the core processes behind genetic inheritance, evolution, adaptation, and functional expression. Building on these foundational principles, the Human Genome Project (HGP) embarked on a mission to identify and map the three billion DNA base pairs and the estimated 25,000 genes that make up the human genetic code, ushering in a new era of research on how our genetic information determines biological functions.² One of the major challenges arising from the HGP is understanding the translation of genomic sequences into complex biological systems.³ This is exemplified by how a fertilized egg, containing a complete genome, ultimately develops into a fully formed organism with a vast array of different cell types, organized into tissues with distinct functions, through the process of gene expression regulation⁴ (Figure 1A). Another knowledge gap is understanding how variations in our genome affect our phenotypes and drive pathogenesis of diseases. For example, large-scale genomic analyses based on extensive populations, such as the 1000 Genomes Project⁵ and genome-wide association studies (GWASs),⁶ have uncovered numerous variants associated with human phenotypes and diseases. However, a critical question remains about how these variants influence the functions of specific organs or cells.⁶ Addressing these challenges necessitates a comprehensive understanding of how molecular-level sequence information

translates to a single cell within a network of communicating and interacting cells that form functional biological systems. This encompasses (1) qualitative—the diversity of cell types generated through the regulation of gene expression; (2) quantitative—the count, proportion, and density of each cell type; (3) space—the spatial arrangement of each cell type and their interactions and cellular ecosystem; (4) time—the specific timing of changes in cell types and states; and (5) direction—the potential transformational paths each cell type may undergo (Figure 1B). Understanding these multifaceted dimensions of cellular diversity and organization is crucial for comprehending the connections between the genome and specific biological processes, as well as the regulatory mechanisms involved.

The advances of sequencing technologies, particularly the massively parallel sequencing technology, has enabled large-scale, genome-wide multi-omics analyses of tissues.⁷ These methods have revealed pronounced tissue and cell specificity in genetic transcription. However, multi-omics analyses based on bulk tissue primarily capture average-level signals, failing to fully comprehend the heterogeneity of different cell types within tissues.⁸ The emergence of single-cell sequencing technology in 2009, and its rapid development in terms of throughput, cost, and multimodal capabilities, has provided unprecedented tools for understanding the cellular heterogeneity within tissues.⁹ Currently, single-cell omics enables the definition of cell types and states across multiple dimensions, including genomic, transcriptomic, epigenomic, and proteomic profiles.¹⁰ This has become a crucial technological means for answering how genomic information is transcribed and channeled into specific



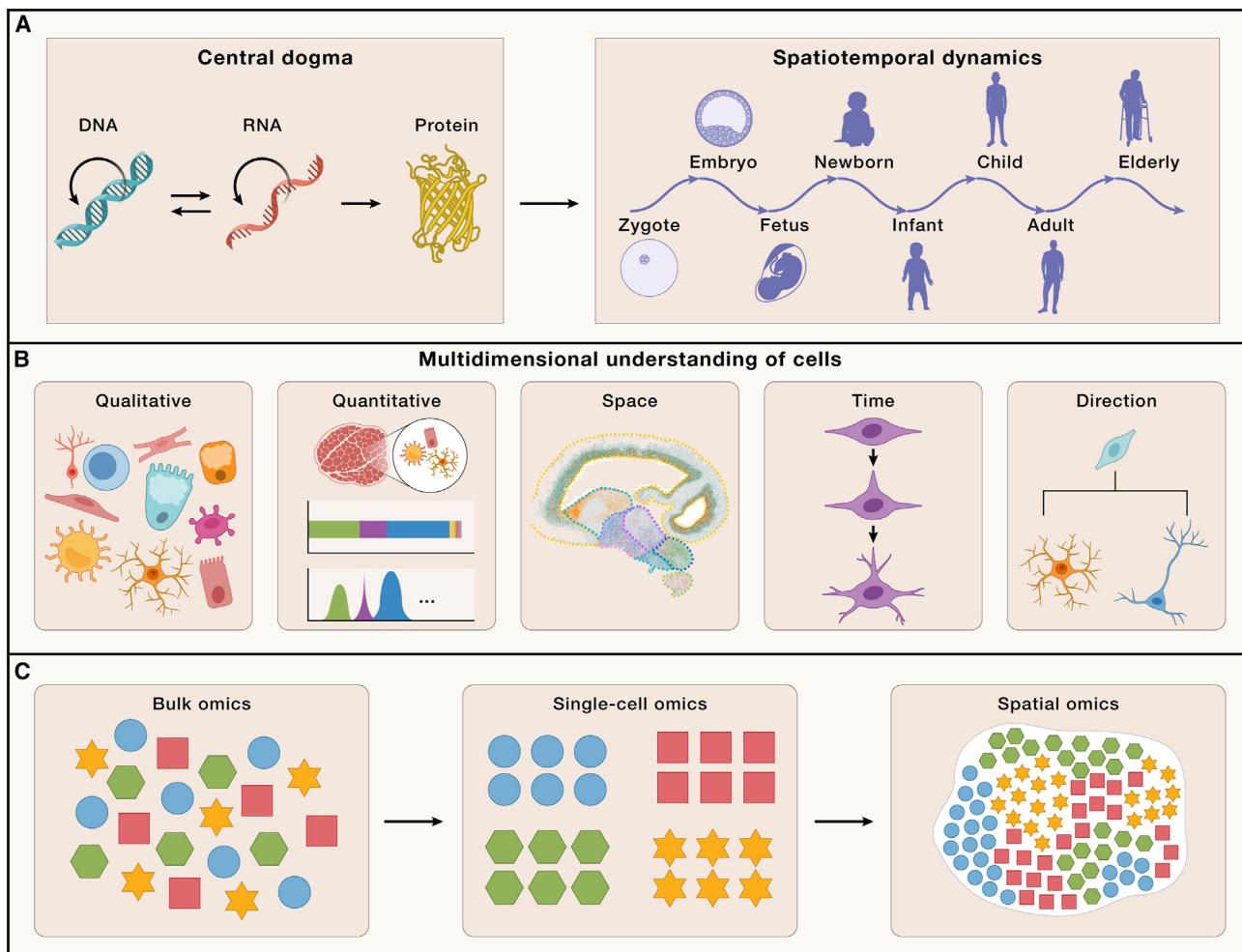


Figure 1. Multi-dimensional measurements of cells

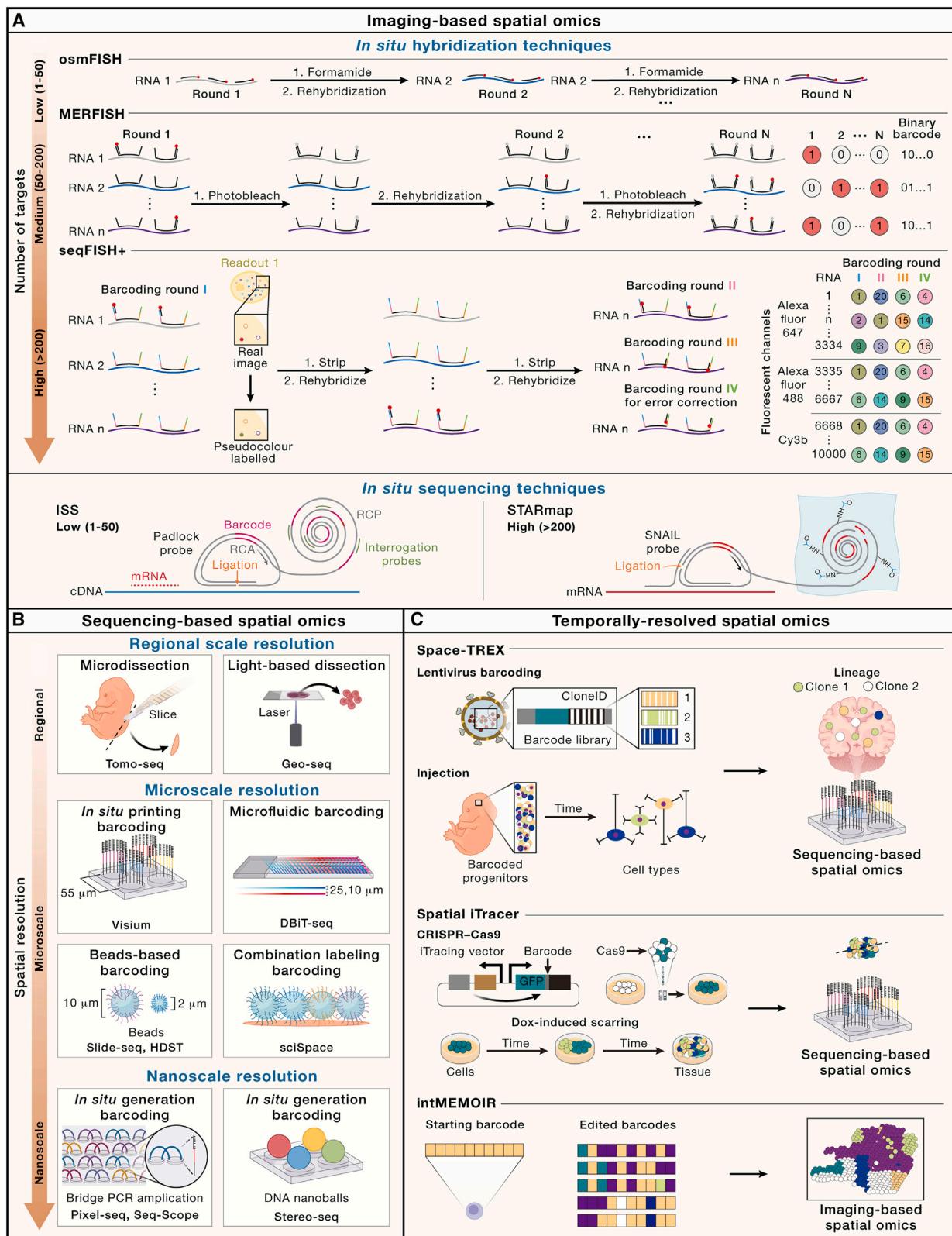
(A) Schematic diagram showing that life sciences are progressing from understanding the Central Dogma to understanding the spatiotemporal dynamics of life.
 (B) Multi-dimensional understanding of cells.
 (C) Evolution of genomic measurement tools from bulk omics to spatial omics.

cell-type information. However, it remains challenging to get a good representation of all tissue-resident cell types and cell states. In particular, the capture of rare cells requires a large number of analyzed cells (unbiased) or cell-selection (biased) approaches to reach the level of statistical power to compare between cell types. Furthermore, the specific spatial context of cells within tissues, forming organs with distinct functions, poses additional complexity. Traditional imaging methods, such as X-rays, computed tomography (CT) scans, and magnetic resonance imaging (MRI) scans, have enabled visualization of the three-dimensional (3D) structure of tissues and organs but lack molecular and cellular resolution.¹¹ Immunohistochemistry (IHC) or *in situ* hybridization (ISH) allow for spatial localization of specific genes or proteins but can only detect a limited number of targets.¹² Recent developments in spatial omics technologies address these limitations by providing solutions for acquiring high content omics data on tissues while retaining spatial localization information. Spatial omics technologies

enable comprehensive mapping of cellular composition, localization, cell-cell interactions, and spatial dynamics of cellular ecosystems¹³ (Figure 1C). From a functional perspective, these variables are essential for understanding morphogenesis during development, the structure of different organs, and their subsequent functionality and cellular microenvironment changes associated with disease processes.¹⁴

AN OVERVIEW OF SPATIAL OMICS METHODS

The fundamental essence of spatial omics technology lies in its aptitude for the simultaneous detection of molecular constituents at exact spatial coordinates. Predominant technological advances encompass methodologies reliant on either imaging-based detection or indirect interrogation through massively parallel sequencing. The former, an advanced derivative of single-molecule hybridization techniques, provides a high degree of resolution, transitioning the scope of gene detection from



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single-gene analysis toward the simultaneous quantification of hundreds to thousands of pre-selected gene targets.¹⁵ In contrast, the latter, inherently capable of unbiased whole-genome analysis, has witnessed a progressive refinement in its precision, advancing from regional to cellular and ultimately to subcellular resolution.¹⁶

In situ imaging-based spatial omics

This collection of methods intrinsically consists of two distinct steps. The first step is the design of nucleic acid oligonucleotide probes, exemplified by fluorescent probes or nucleotide sequence-tagged probes, which target a designated set of genes through the process of hybridization. The second step entails the implementation of image capturing, potentially encompassing techniques such as fluorescence ISH (FISH) imaging or fluorescence *in situ* sequencing (FISSEQ) imaging.¹⁷ Concomitant with technological advancements, particularly in the realms of probe labeling, signal amplification, signal-to-noise, and augmentation of sensitivity and resolution in microscopic imaging, there has been a notable increment in the plethora of genes that can be effectively detected simultaneously¹⁸ (Figure 2A; Table 1).

In situ hybridization techniques

As the earliest spatial transcript detection technique, single-molecule FISH (smFISH) can detect the expression level of RNA molecules within cells. It generally utilizes multiple short single-stranded oligonucleotide probes with fluorescent labels, which specifically bind to target RNA molecules. Through fluorescent microscopy imaging, each bound probe's fluorescent signal is detected as a bright spot, representing a single RNA molecule, allowing for quantitative analysis of RNA abundance and location within individual cells. As a spatial omics technique, smFISH's inherent advantage is single-molecule level resolution, with limited options to increase the number of genes detected simultaneously. Original smFISH could only detect a single target.^{64–66} Through simultaneous use of multiple differently fluorescently labeled probes, a multiplexed version can detect multiple transcripts in the same cell.⁶⁷ However, due to the limited number of distinguishable fluorescent labels, this method can only detect a small number of targets simultaneously.

One approach to extend beyond spectral limitation involves the utilization of balanced fluorophore combinations to annotate a single gene, with unique combinations delineating different targets. For instance, the employment of three distinct fluorophores could potentially differentiate 27 unique RNAs.⁶⁸ However, due to the limited variety of distinguishable fluorescent groups, this technique is constrained to the detection of dozens of genes. A pivotal advancement in this domain is the amplification of the detectable gene repertoire through successive iterations of hybridization imaging. For example, ouroboros smFISH (osmFISH) uses this strategy, recording the location of multiple transcripts in each round by fluorescence imaging. After each image acquisition, probes are eluted to prepare for the next round of hy-

bridization. The number of targets is determined by the number of fluorescent channels and the number of hybridization cycles. Employing this methodology, osmFISH can detect the expression of 33 transcripts simultaneously.⁶⁹

The above approaches independently increase the number of detectable transcripts to a certain extent but fall short of achieving whole-transcriptome goals. By simultaneously using both strategies, designed fluorescent expression combination matrices and multiple rounds of hybridization, techniques like multiplexed error-robust FISH (MERFISH) and sequential FISH (seqFISH) or seqFISH+ have achieved simultaneous detection of hundreds to thousands of transcripts.^{20,22,70,71} seqFISH employs sequential FISH and probe stripping in serialized rounds to barcode-encoded cellular mRNA. Each mRNA transcript is specifically targeted by a complement of FISH probes, each labeled with a unique fluorophore. Following imaging, these probes are systematically removed to prepare for the sequential rounds. The strategic employment of distinct fluorophores in each round incrementally amplifies the barcode complexity, thereby permitting the identification of a multitude of genes, extending into several thousand.⁷⁰ seqFISH+ can image more than 10,000 genes at cellular resolution, with high precision and sub-diffraction-limit resolution. The key to seqFISH+ is the use of sequential hybridization to expand the barcode base color palette, extending the original four to five colors to a larger "pseudo-color" palette. By utilizing 60 pseudo-color channels, mRNA molecules are dispersed into 60 separate images, allowing each mRNA spot to be below the diffraction limit, enabling ultra-high-throughput gene-number detection.²² In MERFISH, RNA is recognized through a combinatorial labeling method, using error-robust barcodes to encode RNA species. These barcodes are readout using sequential smFISH rounds. MERFISH uses an encoding scheme to address cumulative error issues caused by single-molecule labeling and detection errors. These schemes are designed based on coding schemes used in digital electronics (like extended Hamming codes) and modified for error characteristics in FISH measurements. Through these schemes, each RNA is assigned a binary word and encoded with a combination of readout sequences based on this binary word. Using combinatorial FISH labeling and sequential imaging, MERFISH can detect thousands of RNA species at cellular resolution.²⁰

Advantages of *in situ* hybridization imaging techniques include high gene detection efficiency and single-molecule resolution, with single-round smFISH achieving nearly 100% detection efficiency. However, the processes of multiple-round hybridization and barcode encoding in MERFISH and seqFISH or seqFISH+ are relatively complex, requiring the synthesis of a large variety of primary probes and fluorescently labeled readout probes, entailing significant costs. Moreover, these technologies involve many cycles of primer hybridization, image acquisition, and dissociation, and are time consuming and labor intensive. The repeated reaction cycles also substantially decrease detection

Figure 2. Spatial omics technologies

- (A) The image-based spatial omics technologies.
- (B) The sequencing-based spatial omics technologies.
- (C) Examples of temporally resolved spatial omics.

Table 1. Summary of the existing spatial omics technologies

Date	Method	Sample	Target	Resolution	Single cell	Probe	Area	Throughput (cells)	Throughput (genes)
Transcriptomics Imaging-based spatial technologies									
2013	ISS ¹⁹	cells, tissue sections	targeted RNA	–	yes	padlock probe	222 × 166 μm ²	450 cells covering an area of 0.16 mm ²	39 genes
2014	FISSEQ ¹⁷	cells, tissue sections	untargeted RNA	–	yes	fluorescent probe	10 mm × 10 mm	–	4,171 genes with size >5 pixels
2015	MERFISH ²⁰	cells, tissue sections	targeted RNA	–	yes	oligonucleotide probes	scales with acquisition time	~100, up to 40,000 cells	130 genes, up to 10,050 genes
2018	STARmap ²¹	tissue sections	targeted RNA	–	yes	padlock probe	1.7 mm × 1.4 mm × 0.1 mm	>30,000 cells	160 to 1,020 genes per sample
2019	seqFISH+ ²²	cells, tissue sections	targeted RNA	–	yes	oligonucleotide probes	Scales with acquisition time	2,963 cells	10,000 genes
2021	ExSeq ²³	cells, tissue sections	untargeted/targeted RNA	–	yes	padlock probe	0.933 mm × 1.140 mm × 0.02 mm	~2,000 cells	–
2021	EASI-FISH ²⁴	tissue sections	targeted RNA	0.23 × 0.23 × 0.42 μm	yes	–	–	80,000 cells	–
2022	EEL FISH ²⁵	tissue sections	targeted RNA	–	yes	primary probe	Scales with acquisition time	128,000 cells	883 genes
2023	STARmap PLUS ²⁶	tissue sections	targeted RNA and antibody-based protein	–	yes	padlock probe	194 mm × 194 mm × 345 nm	–	1,022 genes
Transcriptomics sequencing-based spatial technologies									
2014	Tomo-seq ²⁷	fresh-frozen	transcriptome-wide	–	no	–	–	–	12,000 genes per section
2016	Visium	fresh-frozen and FFPE	A-tailed RNA transcripts and targeted genes	100 μm	no	print	6.5 mm × 6.5 mm	20,000–40,000 cells	~1,700 genes per spot
2017	Geo-seq ²⁸	fresh-frozen	transcriptome-wide	–	yes	–	–	5–40 cells per section	~8,000 genes
2019	Slide-seq/V2 ²⁹	fresh-frozen	A-tailed RNA transcripts	10 μm	no	beads	Φ3.0 mm	–	550 UMI (unique molecular identifier) per bead
2019	HDST ³⁰	fresh-frozen	A-tailed RNA transcripts	2 μm	no	beads	5.7 mm × 2.4 mm	~60,000 cells per individual hexagonal	44 UMI per 5× beads
2020	DBiT-seq ³¹ (FFPE)	FFPE	A-tailed RNA transcripts	25 μm	no	free probe	2.5 mm × 2.5 mm	~50,000 cells	1,000 genes per pixel

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Table 1. *Continued*

Date	Method	Sample	Target	Resolution	Single cell	Probe	Area	Throughput (cells)	Throughput (genes)
2021	Seq-Scope ³²	fresh-frozen	A-tailed RNA transcripts	~0.6 μm	yes	<i>in situ</i> synthesize	0.8 mm × 1 mm	–	~1,617 genes per cell
2021	PIC ³³	fresh-frozen	transcriptome-wide	75–5,000 μm	yes	photo-caged oligonucleotides	scales with acquisition time	10 or more cells in tissue sections	~8,000 genes
2021	sci-Space ³⁴	fresh-frozen	A-tailed RNA transcripts	222 μm	yes	beads	18 mm × 18 mm	121,909 cells	1,231 genes per cell
2022	Stereo-seq ³⁵	fresh-frozen	A-tailed RNA transcripts	0.5 μm	yes	<i>in situ</i> synthesize	up to 132 mm × 132 mm	100,000–16,900,000 cells	1,910 UMI and 792 genes per cell
2022	Pixel-seq ³⁶	fresh-frozen	A-tailed RNA transcripts	~1 μm	yes	<i>in situ</i> synthesize	75 mm × 25 mm	15,000 cells per section	~500 genes per cell
2023	Slide-tags ³⁷	fresh-frozen	A-tailed RNA transcripts	10 μm	yes	beads	Φ3.0 mm	81,000 cells	2,377 UMI per cell
2024	Visium HD ³⁸	fresh-frozen FFPE	targeted transcriptomics	2 μm	yes	print	6.5 mm × 6.5 mm	20,000–40,000 cells	7,605 probes
2024	Open-ST ³⁹	fresh-frozen	A-tailed RNA	0.6 μm	yes	<i>in situ</i> synthesize	6.3 mm × 89 mm	–	~1,000 UMI per cell
multi-omics									
2018	CODEX ⁴⁰	cells, tissue sections	antibody-based protein	–	yes	–	–	–	–
2020	OligoFISSEQ ⁴¹	cells, tissue sections	genomics	–	no	barcoded oligopaint probes	–	–	–
2020	DBiT-seq ⁴²	Fresh-frozen	A-tailed RNA transcripts and antibody-based protein	10/25/50 μm	no	free probe	2.5 mm × 2.5 mm	~50,000 cells	~4,000 genes per 50-μm pixel
2021	DNA seqFISH+ ⁴³	Cells	genomics	N/A (5,616.5 ± 1,551.4 dots per cell)	yes	primary probe	scales with acquisition time	446 cells	3,660 chromosomal loci per cell
2021	IGS ⁴⁴	cells, tissue sections	genomics	400–500 nm	yes	hairpin DNA	–	113 cells	–
2021	slide-DNA-seq ⁴⁵	fresh-frozen	genomics	10 μm	yes	beads	Φ3 mm	2,274 cells	–
2022	CAD-HCR ⁴⁶	cells	targeted RNA and antibody-based protein	–	yes	padlock probe	–	–	–
2022	MOSAICA ⁴⁷	cells, FFPE	targeted RNA and antibody-based protein	–	yes	primary probe	–	–	–

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Table 1. *Continued*

Date	Method	Sample	Target	Resolution	Single cell	Probe	Area	Throughput (cells)	Throughput (genes)
2022	SM-Omics ⁴⁸	fresh-frozen	A-tailed RNA transcripts and antibody-based protein	55 μm	no	print	6.5 mm × 6.5 mm	–	–
2022	Spatial-CUT&Tag ⁴⁹	fresh-frozen	epigenomics	20 μm	no	–	–	–	H3K27me3: 9,735; H3K4me3: 3,686 per 20-μm pixel size
2022	Space-TREX ⁵⁰	fresh-frozen	A-tailed RNA transcripts and ClonetIDs	single cell/55 μm	yes	–	–	65,160 cells	5,000–10,000 genes
2022	Perturb-map ⁵¹	fresh-frozen	A-tailed RNA transcripts and CRISPR-targeted genes	55 μm	yes	–	–	–	–
2022	slide-TCR-seq ⁵²	fresh-frozen	A-tailed RNA transcripts and targeted T cell receptor genes	10 μm	no	beads	Φ3.0 mm	–	–
2022	SmT ⁵³	fresh-frozen	host transcriptome- and microbiome-wide	55 μm	no	print	6.5 mm × 6.5 mm	–	–
2022	RIBOmap ⁵⁴	cells, tissue sections	ribosome-bound mRNAs	–	yes	tri-probes (splint DNA probe, padlock probes, primer probes)	–	60,481 cells	5,413 genes
2022	Epigenomic MERFISH ⁵⁵	cells, tissue sections	epigenomics	–	yes	oligonucleotide probes	scales with acquisition time	~5,400 cells	3 histone modifications, H3K4me3: 127 genes/loci; H3K27ac: 142 target genomic loci
2023	spatial ATAC ⁵⁶	fresh-frozen	epigenomics	55 μm	no	splint oligonucleotide, spatially barcoded surface oligonucleotides	–	–	–
2023	spatial-CITE-seq ⁵⁷	fresh-frozen	A-tailed RNA transcripts and antibody-based protein	50 μm	no	free	2.5 mm × 2.5 mm	37,500–50,000 cells	411 genes and 153 proteins per pixel
2023	Stereo-CITE-seq ⁵⁸	fresh-frozen	targeted RNA and antibody-based protein	500 nm	yes	<i>in situ</i> synthesize	up to 132 mm × 132 mm	100,000–16,900,000 cells	4.05K UMI per bin50

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Table 1. Continued

Date	Method	Sample	Target	Resolution	Single cell	Probe	Area	Throughput (cells)	Throughput (genes)
2023	Spatial-ATAC-RNA-seq ⁵⁹	fresh-frozen	A-tailed RNA transcripts and Transposase chromatin	20/50 µm	no	free	2.5 mm × 2.5 mm	37,500–50,000 cells	–
2023	Ex-ST ⁶⁰	fresh-frozen	A-tailed RNA transcripts	20 µm	no	print	6.5 mm × 6.5 mm	8,000–16,000 cells	~500 genes per spot
2023	immuno-SABER ⁶¹	cells	antibody-based protein	–	yes	–	–	–	–
2023	scDVP ⁶²	fresh-frozen	proteins	–	no	–	–	–	1,700 proteins
2023	scSpaMet ⁶³	fresh-frozen, FFPE	metabolomics and targeted multiplexed protein	–	yes	–	–	–	–

sensitivity. Additionally, because probe-based methods rely on the specific affinity between the probe and its target, which varies among different probes, direct comparisons between various transcripts are challenging. Furthermore, technologies based on multiple-round *in situ* hybridization struggle to detect shorter transcripts and have limited scalability for large tissue areas.

In situ sequencing techniques

An alternative methodology to *in situ* imaging is *in situ* sequencing (ISS), which involves reading specific sequences of target nucleic acids *in situ* within cells, achieving spatial resolution for multi-gene detection. Similar to *in situ* hybridization imaging, a key issue in ISS is generating a sufficiently strong detectable fluorescent signal on a nucleic acid sequence. The solution in ISS is through rolling-circle amplification (RCA) to produce clonally amplified rolling-circle products (RCPs) containing numerous copies of the target sequence, then reading the target sequence through sequencing by ligation. However, ISS can only read a limited length of nucleotides, with the first-generation ISS detecting the expression of 39 genes using 4 nucleotide bases.¹⁹

A major issue with the first-generation ISS was low detection efficiency, primarily due to the low efficiency of *in situ* reverse transcription forming cDNA. To address this, STARmap (spatially resolved transcript amplicon readout mapping) designed a method using double primers to form DNA nanospheres, bypassing the reverse transcription step, thus significantly enhancing detection efficiency. Additionally, STARmap also made optimizations including utilizing hydrogel-tissue chemistry and a new two-base sequencing scheme with error reduction, increasing the number of detectable genes to over a thousand.²¹ ISS can perform targeted detection and achieve non-targeted whole-transcriptome detection. For example, FISSEQ uses adapter sequence-tagged random hexamers for non-targeted cDNA synthesis, theoretically achieving whole-transcriptome coverage.¹⁷

Regardless of employing ISH or ISS imaging techniques, the simultaneous detection of a large number of genes invariably encounters the challenge of molecular crowding. This situation arises when the density of fluorescent molecules exceeds the diffraction limit of the microscope and individual molecules cannot be distinguished. An effective solution is the use of expansion microscopy,⁷² which combines biological samples with expandable polymer gels, then chemically induces gel expansion, physically magnifying the sample. This expansion makes microstructures, originally indistinguishable due to optical diffraction limits, visible. By combining with expansion microscopy, techniques like expansion FISH (ExFISH)⁷³ and expansion sequencing (ExSeq)²³ have significantly improved in both gene number detection and detection efficiency. Compared to ISH imaging technology, ISS sequencing imaging can provide single-nucleotide resolution, thus obtaining more information on target genes. However, ISS imaging technology also has many limitations, the most critical being the complex intracellular environment and limited enzymatic reaction efficiency (such as the reverse-transcription process), significantly impacting reaction efficiency. Another limitation is the limited intracellular space. The required RCP number for current ISS is limited, making it difficult to increase detection throughput.

Sequencing-based spatial omics

With the rapid development of high-throughput massively parallel sequencing technology, it is now feasible to concurrently acquire spatial location and molecular information at the cellular level. The essence of sequencing-based spatial transcriptomics rests on the capture and labeling of molecules with spatial location information. As capture strategies have evolved, there has been a gradual improvement in resolution, transitioning from tissue-level to subcellular precision (Figure 2B; Table 1).

Regional-scale resolution

The initial spatial transcriptomics technology obtained spatial location information of cell clusters through physical cutting, such as Tomo-seq (RNA tomography for spatially resolved transcriptomics),²⁷ based on tissue sectioning, and Geo-seq (geographical position sequencing),²⁸ based on microdissection. These methods involve physically dividing and barcoding the tissue at preset positions for separate transcriptomics sequencing, followed by 3D structural assembly based on spatial information. However, these methods, limited by the extremes of physical operations, could only achieve tissue regional spatial resolution. Subsequent technological developments, such as NICHE-seq⁷⁴ and ZipSeq,⁷⁵ used photosensitive groups to pre-modify capture probes, allowing detection of RNA molecules with spatial barcoding. These methods improved resolution to some extent but were restricted to the level of cell groups or regional scale.

Microscale resolution

Over the past 10 years, the development of spatial barcoding technologies allows microscale resolution detection of molecules within tissues. These methods involve preparing and recording probes with spatial location information on a physical carrier, capturing mRNA *in situ*, adding spatial information to the captured mRNA molecules, and finally obtaining transcript identity with spatial location information through sequencing. ST (spatial transcriptomics)⁷⁶ was the first technology to implement this method, using clustered barcoding and polyT probes with known sequences printed at intervals on a glass surface to form a capture carrier. The original ST resolution was 100 μm,⁷⁶ later optimized to 55 μm.⁷⁷ However, this physical *in situ* printing technique, limited by the physical mechanical planting distance, faced challenges in fundamentally breaking through cellular spatial resolution. Later technologies in this direction attempted different barcoding strategies to reduce the distance between spots and enhance resolution. Slide-seq or Slide-seqV2^{29,78} innovatively fixed barcoded magnetic beads randomly on a chip, then recorded the spatial location information of barcodes through a round of ISS, followed by RNA capture sequencing, thereby achieving 10-μm resolution. Using Slide-seq barcoded beads, Slide-tag³⁷ enables spatially barcoded single-nucleus sequencing. This method tags single nuclei within an intact tissue section with bead-derived spatial barcodes, which are then followed by single-nucleus sequencing. In addition, high-definition ST (HDST),³⁰ by switching to smaller magnetic beads, further improved resolution to 2 μm. Visium HD⁷⁹ also utilizes a whole-transcriptome probe panel and resolves data at 2-μm scale within intact tissue sections.

Recent advances in microfluidics and joint labeling strategies can achieve micron-level spatial transcriptomics resolution. The deterministic barcoding in tissue for spatial omics sequencing

(DBiT-seq)⁴² technique, for instance, applies microfluidic channel-guided molecular barcodes on tissue sections to spatially locate and quantitatively analyze mRNA. This technique uses two sets of DNA barcodes, forming a unique barcode combination of 2D tissue pixels through cross-flow and *in situ* linkage on the tissue section, thereby constructing spatial expression atlases at a resolution of 10 μm. Limited by the channel width, its resolution is difficult to further enhance. A similar strategy is sci-Space³⁴, which arranges encoded hashing oligonucleotides grids on glass slides to capture spatial information, transferring these oligonucleotides into the nuclei of cells on tissue sections covering the slides. These oligonucleotides, after being associated with sci-RNA-seq (single-cell combinatorial indexing RNA sequencing) profiles, can capture the approximate tissue coordinates of each cell during sequencing. The advantage of sci-Space lies in its ability to resolve spatial heterogeneity over a larger range while maintaining single-cell resolution, but its spatial resolution is currently limited by the encoded oligonucleotide pattern array, approximately 200 microns.

Nanoscale resolution

A significant advancement for resolution enhancement is the use of nano-array sequencing chips as capture carriers, which can achieve nanometer-level spatial transcriptomics. Such technologies undergo two rounds of sequencing, with the first round for obtaining spatial label sequences and their location information, then capturing RNA molecules, adding spatial label information to RNA, and obtaining transcripts with spatial label information through a second round of sequencing. By integrating information from both rounds, spatial transcriptomics information with nanometer-level coordinates can be obtained. Technologies such as SeqScope³², which leverages Illumina sequencing chips, and Stereo-seq (spatial enhanced resolution omics sequencing),³⁵ which utilizes nanoball array chips from DNA nanoball sequencing (DNBSEQ) platforms, offer resolutions as fine as 500 nm. Pixel-seq³⁶ employs a similar strategy, first planting capture probes at a 1-μm interval on a gel surface, then sequencing to identify the sequence of each position probe. Its advantage lies in using polony gels for DNA replication and spatial barcode mapping, undergoing only one round of first-stage sequencing, allowing reuse of layout information for spatial group sequencing of multiple samples, significantly reducing the costs. In addition, Open-ST³⁹ addresses the need for high-resolution, user-friendly, cost-efficient, and 3D-scalable methods by providing open-source experimental and computational resources. These resources are designed to overcome these challenges and enable the study of the molecular organization of tissues in both 2D and 3D at nanoscale resolution.

Non-transcriptomic spatial omics

While spatial transcriptomics has significantly advanced our understanding of gene expression and regulation, it represents only one layer of the complex molecular landscape within cells and tissues. Other omics layers, such as genomics, epigenomics, translomics, proteomics, and metabolomics, provide crucial insights into the underlying DNA sequences, regulatory elements, post-transcriptional regulations, protein functions, and metabolic pathways. These diverse molecular layers collectively influence cellular states and behaviors, offering a more comprehensive

understanding of biological processes and disease mechanisms.¹³

Spatial genomics has significant applications in studying the clonal evolution of tumors, highlighting key DNA variations in tumor development and progression. An example of spatial genomics technology is slide-DNA-seq⁴⁵ which captures DNA sequences directly from fixed tissue sections onto slides coated with polystyrene beads carrying sequencing adapters and unique spatial barcodes. This technology has been applied to tissue sections from mouse models of metastasis and primary human cancers, allowing the identification and spatial mapping of different tumor clones based on their DNA copy-number profiles.⁴⁵

Spatial epigenomics involves techniques like spatial ATAC-seq (assay for transposase-accessible chromatin sequencing)⁸⁰ and spatial chromatin immunoprecipitation techniques (CUT&Tag).⁴⁹ Spatial ATAC-seq maps chromatin accessibility, revealing regions of open chromatin associated with active gene regulation, while spatial CUT&Tag identifies histone modifications, providing insights into the epigenetic landscape of tissues. These techniques use microfluidic barcoding to capture spatial information about epigenetic modifications, uncovering cell-type-specific regulatory mechanisms that influence gene expression.

Spatial proteomics technologies, such as co-detection by indexing (CODEX)⁴⁰ and immunostaining with signal amplification by exchange reaction (Immuno-SABER)⁸¹ utilize DNA-barcoded antibodies to detect and quantify proteins across tissue sections. Another method, single-cell deep visual proteomics (scDVP),⁶² integrates high-content imaging, laser microdissection, and multiplexed mass spectrometry, allowing single-cell proteomics analysis at spatial resolution. These methods enable multiplexed analysis of protein expression and spatial distribution, linking protein functions to specific cellular contexts and states. This is crucial for understanding protein interactions and their roles in cellular processes and disease mechanisms. To analyze the process of protein synthesis, spatial translomics, such as RIBOmap,⁵⁴ capture spatial information about ribosome positioning and mRNA translation, presenting a new way that accelerates our understanding of protein synthesis in the context of subcellular architecture, cell types, and tissue anatomy.

Spatial metabolomics is emerging as a powerful approach to map the spatial distribution of metabolites within tissues, providing a direct link between metabolic activity and tissue function. Techniques such as mass spectrometry imaging (MSI)⁸² and scSpaMet⁶³ allow for the visualization and quantification of metabolites at high spatial resolution, offering insights into metabolic pathways and their roles in health and disease (Table 1).

Spatial multi-omics

The integration of multiple omics layers within the same spatial context has led to the development of spatial multi-omics technologies, providing a holistic view of the molecular complexity within tissues. Spatial cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq)⁵⁷ and Stereo-CITE-seq⁵⁸ combine transcriptomics and proteomics by using

DNA-barcoded antibodies alongside RNA sequencing at spatial resolution. This allows for the simultaneous measurement of gene expression and protein abundance within individual cells, revealing intricate details about cellular phenotypes and interactions. Besides, spatial ATAC- and RNA-seq⁸³ combine chromatin accessibility mapping with RNA sequencing, offering a dual perspective on epigenetic regulation and gene expression within the same cells. This method captures both ATAC-seq and RNA-seq data using microfluidic barcoding, elucidating the relationship between chromatin state and transcriptional activity in spatially resolved tissue sections. This integrated approach provides comprehensive insights into the regulatory mechanisms controlling gene expression. Moreover, spatial CUT&Tag-RNA-seq⁵⁹ integrates epigenomic profiling with transcriptomics by combining CUT&Tag with RNA sequencing. This enables the simultaneous mapping of histone modifications and gene expression, offering insights into how epigenetic changes influence transcriptional programs in specific cellular contexts. This method is particularly useful for understanding the dynamic interplay between epigenetic modifications and gene expression during development and disease (Table 1).

Temporally resolved spatial omics

Temporal information is crucial for studying the dynamic changes in biological processes. Single-cell omics, combined with techniques, such as lineage tracing,⁸⁴ metabolic labeling,⁸⁵ molecular recording,⁸⁶ and *in vivo* labeling using fluorescent timestamps,⁸⁷ have enabled researchers to investigate cell developmental lineages and disease state alterations at the single-cell level. However, most current spatiotemporal studies are limited to consecutive sampling, generating only snapshots of the underlying temporal progression. Despite this limitation, there have been reports demonstrating that DNA barcode-based temporal information can be integrated with either imaging- or sequencing-based spatial omics. For example, intMEMOIR (integrase-editable memory by engineered mutagenesis with optical *in situ* readout)⁸⁸ utilizes a synthetic DNA-based recording system with integrase enzymes to capture cell lineage information. This method involves inserting engineered DNA sequences, or barcodes, into the genome, which are modified over time through integrase activity. These modifications create a temporal record of cellular events. Sequential imaging of these barcodes reveals the temporal sequence of lineage changes, providing precise tracking of cell differentiation and development. In contrast, Spatial iTraCeR⁸⁹ combines reporter barcodes with inducible CRISPR-Cas9 scarring to map lineage relationships. In this method, unique barcodes are integrated into the genome and CRISPR-Cas9 is used to create permanent scars at specific stages of development. Sequencing these barcodes and scars allows tracing cell lineages and identifying critical periods of fate restriction. Space-TREX⁵⁰ also employs a sequencing-based approach, utilizing a lentiviral system to introduce unique genetic barcodes into cells, which are inherited by daughter cells. This technique combines single-cell RNA sequencing with spatial transcriptomics to reveal clonal relationships within tissues, such as the mouse brain (Figure 2C; Table 1). Although these technologies show promise in studying cell fate

dynamics within specific biological processes, challenges related to sensitivity and resolution remain. These limitations are often due to technical factors such as the efficiency of barcode integration and the accuracy of sequential imaging or sequencing steps. Improving these aspects is essential for advancing the reliability and applicability.

Further improvement of spatial omics technologies

The advantage of spatial omics technology lies not only in its ability to provide spatial information for molecules or cells but also in its potential to achieve more comprehensive and unbiased information capture. This is due to its reliance on tissue sections compared to single-cell omics techniques, which often lose cells during the cell dissociation process or underestimate specific cell types due to the size of droplets/microwells. Nonetheless, there is still room for optimization. Future technological developments should aim toward higher spatial precision and multi-dimensional profiling with better accessibility. Specifically, improvements may include

- (1) Higher sensitivity and precision. To further enhance the signal capture efficiency of spatial omics technologies and address the current issue of low sensitivity,¹⁶ significant improvements in region segmentation and cell typing can be achieved. Besides, lateral diffusion of RNA transcripts occurs during the process of RNA capturing, leading to slight changes in spatial gene expression patterns.⁹⁰ Reducing RNA diffusion by either technical or computational approaches to improve spatial localization accuracy is crucial for analyzing cell interactions and is a prerequisite for achieving true subcellular-resolution analysis.
- (2) Enhanced multi-omics detection. Although some relevant technologies already exist, we are still in the early stages, and developing more robust multi-omics techniques remains a significant challenge in the field. Integration of multiple omics dimensions, such as genomics, epigenomics, transcriptomics, and proteomics within a single tissue section, promises to provide needed tools for understanding tissue molecular characteristics at all relevant modalities. This holistic approach would enable a more complete exploration of complex multi-modal biological systems.
- (3) Long-read sequencing. Leveraging advanced sequencing technologies, such as single-molecule long-read sequencing,⁹¹ could enable the sequencing of full-length mRNA transcripts. This would encompass the detection of alternative splicing, single-nucleotide variations, and structural variations, providing a more detailed and accurate genetic analysis.
- (4) Compatibility with a broader range of tissue types. The majority of currently available spatial transcriptomics technologies are limited to fresh-frozen tissue. Given that the standard processing of clinical samples involves formalin-fixation and paraffin-embedding (FFPE), which has a better preservation of cell and tissue morphology, improving compatibility with such samples is imperative. Enhancing the versatility of spatial omics technologies

to accommodate FFPE tissues could significantly advance the diagnostic tools available for clinical disease analysis.⁹²

- (5) Increased throughput, reduced costs, and greater accessibility. Advancements that achieve higher throughput and lower costs, while enhancing accessibility and experimental design options, are crucial. Such improvements would ensure that spatial omics technologies become fundamental tools beneficial to researchers across various domains of biology. These directions not only highlight the potential for future innovations in spatial omics but also underscore the importance of continued research and development to fully exploit the capabilities of these technologies for comprehensive biological and clinical investigations.

DATA ANALYSIS TOOLS FOR SPATIAL OMICS

Spatial omics is revolutionizing our understanding of tissue organization and function, demanding sophisticated analysis through a diverse spectrum of data to decipher its complexities. This discipline extends beyond the analysis of cell-type-level heterogeneity, a hallmark of single-cell omics, by accurately pinpointing cell locations. Such an advancement considerably broadens the scope of input signal dimensions and modalities, mapping the biological landscape from cell density and local spatial configurations to intercellular connections, communication, and cell morphology. While early data analyses focused on cellular decomposition of spots from multi-cellular resolution spatial transcriptomics techniques, current cellular- or subcellular-resolution spatial transcriptomics methods necessitate an in-depth examination of cell types and their interrelationships across various biological scales, including molecular, cellular and subcellular, regional, and spatiotemporal levels.

The standard workflow for spatial omics data analysis starts with preprocessing to filter and normalize raw data, enhancing its quality. This is followed by spatial domain segmentation to delineate regions of interest within the tissue. For cellular resolution spatial omics data, cell-type annotation is performed using either reference profiles or unbiased clustering methods. Subsequent analysis investigates spatial patterns relevant to the biological question, such as gene expression patterns, spatial distribution of cells, differential expression in specific regions or cell types, and cell-cell communications. Our previous review article has summarized the methods, tools, and challenges of spatial omics data analysis.⁹³ Here, we further discuss these issues from molecular, cellular, and tissue levels, and also cover analysis methods for 3D reconstruction and time-resolved spatial omics studies (Table 2).

Molecular-level analysis

Spatial omics assays, by meticulously exploring the localization of molecules, facilitate the study of the spatial distributions of specific molecules that orchestrate tissue functions. A significant aspect of this analysis is the identification of spatially variable genes (SVGs)—genes exhibiting variable expression across different locations.¹²⁴ SVGs are instrumental in defining tissue

domains and driving functional specialization, as evidenced in recent studies.^{94,95} This advancement paves the way for more comprehensive analyses, such as exploring the coordinated activities of gene sets, as well as encompassing gene signatures or gene programs linked to specific cell types, regions, or functions. These investigations are key to uncovering the regulatory mechanisms of gene expression, where gene clusters collaborate to execute distinct functions. On a broader scale, spatial transcriptomics serves as a gateway to understanding gene regulatory networks (GRNs), shedding light on spatial regulatory interactions within signaling and metabolic pathways that are pivotal in tissue development, functionality, and disease response.⁹⁹

However, under-sampling of transcripts can significantly obscure crucial gene-gene relationships. Imputation techniques address this issue with statistically informed predictions, thereby ensuring a comprehensive understanding of the spatial profile of gene expression. Two primary approaches have emerged: one involves mapping single-cell RNA sequencing (scRNA-seq) cells onto the spatial map, akin to solving a puzzle, and the other entails direct imputation of gene expression in spatial contexts. For *in situ* imaging-based spatial omics techniques, which do not capture the entire transcriptome, scRNA-seq data can provide a valuable supplement. Mapping utilizes a set of landmark genes,¹²⁴ applying methods such as probabilistic inference¹²⁴ or optimal transport theory for cell placement.¹²⁵ Conversely, direct imputation projects spatial and scRNA-seq data into a shared latent space, estimating missing values for specific spatial locations. Variational inference imputes missing values in a statistically sound manner,¹²⁶ whereas Markov-affinity-based methods have shown robust performance¹⁰¹ as evidenced in single-cell datasets.¹²⁷ Notably, these methods can also integrate spatial context into single-cell multi-omics data, creating a powerful link between modalities. Consequently, the synergy between spatial transcriptomics and scRNA-seq contributes to a more complete and nuanced understanding of spatial omics profiles.

Cellular- and subcellular-level analysis

Cell segmentation and annotation are the initial steps in the analysis of spatial transcriptomics data. Therefore, cellular or subcellular spatial transcriptomics signals need to be convolved to individual cells, while multi-cellular resolution spatial transcriptomics signals need to decompose cell-type mixtures for each of the spots. For imaging-based spatial transcriptomics techniques, where cells are stained and imaged simultaneously with captured molecules, cell segmentation is usually performed on stained images.^{128–130} However, all these image-based segmentations struggle with high cell density and nucleus-biased spatial underrepresentation. For sequencing-based spatial transcriptomics, cells are *in situ* imaged while RNA molecules are *ex situ* sequenced, and the inconsistency between these two modalities restrains the application of segmentation methods.

Approaches to measure the neighborhood composition vector,¹⁰² which quantifies the similarity in gene composition between different locations, provide a universally applicable concept for both imaging- and sequencing-based spatial transcriptomics.^{103,104} However, these methods grapple with challenges of data sparsity and background noise, currently limiting

Table 2. Summary of existing computational tools for different tasks in spatial omics

Type	Toolkit	Advantages	Disadvantages
SVG recognition	SpatialDE ⁹⁴	a statistical framework to identify genes that show spatial variability, which can be crucial for understanding tissue organization	may not perform well with noisy data or when spatial patterns are subtle
	SPARK ⁹⁵	designed to work with large datasets, improving scalability	require fine-tuning for optimal performance on datasets with different characteristics
	SPARK-X ⁹⁶	uses a nearest-neighbor approach to improve detection of SVGs	performance may suffer if the spatial distribution of cells is highly irregular
	nnSVG ⁹⁷	identifies hotspots of coordinated gene expression, which can indicate biological significance	requires parameter tuning and a good understanding of the underlying biology to interpret results effectively
GRN inference	GCNG ⁹⁹	applies graph convolutional networks to represent the spatial relationships	hard to interpret the extracellular interactions between genes
Imputation for spatial transcriptomics	NovoSpaRc ¹⁰⁰	adds spatial information to scRNA-seq data to achieve imputation for ST	high computational cost; low performance in mapping ST-absent transcripts
	spARC ¹⁰¹	denoises spatial omics count matrix by adjusting affinity graph	potentially overlooks platform-specific technical differences
Transcripts-based cell segmentation	BaysoR ¹⁰²	incorporates local neighborhood information for better segmentation accuracy	lack of generalization for sequencing-based ST datasets
	SSAM ¹⁰³	uses spatial information and association maps to improve cell-type classification	lack of single-cell cell segmentation; requires pre-defined cell types
	ClusterMap ¹⁰⁴	clustering-based approach allows for the identification of cell types and states	clustering resolution may affect the accuracy of segmentation
	SCS ¹⁰⁵	combines imaging and gene vector signals to define cell shapes	expensive, time-consuming in scalable datasets; difficulty in interpreting the results
Deconvolution	Cell2location ¹⁰⁶	allows for the mapping of cell types from scRNA-seq data onto spatial data	performance is sensitive to hyperparameters; relies on fine-tuning
	SPOTlight ¹⁰⁷	can deconvolute mixed signals to predict cell-type compositions	dependent on the quality and resolution of the spatial transcriptomics data
	Tangram ¹⁰⁸	employ dual optimization function for aligning spatial distribution and gene expression	Limited application due to its registration based on common coordinate framework (CCF)
	RCTD ¹⁰⁶	robust for cross-platform data; fast processing based on optimization for maximum likelihood estimation (MLE)	Poisson distribution for spot may not be true for all ST data; dependent on reference scRNA-seq data
Annotation transfer from single-cell transcriptomics	Spatial-ID ¹⁰⁹	facilitates the transfer of annotations from scRNA-seq to spatial transcriptomics data, potentially saving time and resources	the accuracy is highly dependent on the representativeness of the reference scRNA-seq data.

(Continued on next page)

Table 2. Continued

Type	Toolkit	Advantages	Disadvantages
Spatial domain recognition	SpaGCN ¹¹⁰	integrates graph convolutional networks to identify spatial domains, leveraging both spatial and gene expression data	may be computationally intensive and require significant training time
	STAGATE ¹¹¹	uses attention mechanisms to capture complex relationships between genes and spatial locations	complexity of the model may lead to overfitting if not properly regulated
	SpatialPCA ¹¹²	applies principal component analysis to reduce dimensionality and highlight key spatial patterns	may overlook non-linear relationships that are important for understanding spatial structures
	BayesSpace ¹¹³	employs a Bayesian framework to enhance resolution and interpret spatial heterogeneity	model assumptions and priors may not be suitable for all types of data
Spatial cell-cell interaction	COMMOT ¹¹⁴	designed to analyze and model the spatial interaction networks between cells	analysis may be complex and require careful interpretation
	MISTy ¹¹⁵	provides a framework for modeling and interpreting intercellular communication from spatial data	relies on existing knowledge of signaling pathways, which may not be comprehensive
	DIALOGUE ¹¹⁶	focuses on the dynamics of cell-cell interactions over time, which is critical for understanding development and disease progression	focuses on the dynamics of cell-cell interactions over time, which is critical for understanding development and disease progression
	NCEM ¹¹⁷	neural conditional expectation model that can infer cell-cell interactions in a spatial context	may require large datasets for training to achieve accurate predictions
3D reconstruction	PASTE ¹¹⁸	no need for prior knowledge of annotation	GPU (graphics processing unit) consuming; FGW (fused Gromov-Wasserstein) requires every spot to be in the transportation, which leads to possible mismatch
	SPACEL ¹¹⁹	recognize spatial domains based on cell types; high precision	rely highly on reference single-cell dataset and hyperparameters
Alignment across time points	MOSCOT ¹²⁰	allowing non-rigid registration	non-rigid alignment deconstructs spatial domains
	SLAT ¹²¹	combining spatial graph convolution and adversarial matching	optimal transport may pose difficult in modeling the distant alignment
Lineage tracing and cell fate inference	stLearn ¹²²	integrates spatial transcriptomics with lineage tracing to understand cell development trajectories	lack of fined trajectory in single slice
	Spateo ¹²³	targets the analysis of spatial and temporal expression data to infer cell fate and cell mobility during biological processes	the predicted morphological changes do not directly infer alterations in gene expression profile

their practicality primarily to imaging-based spatial transcriptomics. Recent advancements in determining gene composition similarity using transformer models¹⁰⁵ may unlock new potential in this area, offering a promising direction for future research in sequencing-based spatial transcriptomics.

In multi-cellular resolution techniques, the primary objective is to infer the cell-type composition for each spatial spot. The success of these methods hinges largely on the quality and quantity of the associated scRNA-seq data.^{106–108,131} They operate under the strong assumption that technical variances between single-cell and spatial omics can be reconciled through these cell-type deconvolution methods. However, challenges arise due to the absolute expression differences between cell types, which often lead to the underrepresentation of cell types with fewer transcripts, such as T cells and B cells in cancer studies.¹³¹ Additionally, variable cell density presents another intractable biological factor affecting performance.¹³² Spatial transcriptomic analyses under multi-cellular resolution are more suitable at the regional level.

Regional-level analysis

Cell behaviors, such as developmental trajectories and microenvironment formation, contribute to the concept of spatial domains that represent spatially organized and functionally distinct anatomical structures. This biological phenomenon resembles the principle observed in natural images, where neighboring pixels tend to have similar values. Algorithms that seek neighborhoods in both expressional and physical spaces have largely addressed these challenges.^{112,133} Furthermore, the implementation of graph neural networks has integrated auxiliary stained tissue images (hematoxylin and eosin [H&E]) with anatomical features to enhance performance.^{110,111,122} However, there is a significant concern regarding the over-fitting problem in this field, as most algorithms^{110,111,134–138} benchmark their performance using data from 12 sections of the human dorsolateral prefrontal cortex (DLPFC).¹³⁹ Therefore, expanding the database to include a diverse range of biological ground truths and re-benchmarking the generalization capability for these algorithms has become an urgent need.

Spatial transcriptomics opens new venues to study microenvironments, going beyond merely verifying spatial proximity relationships for ligand-receptor inference. This field is rapidly expanding with a constant development of novel innovative strategies aimed at unraveling more complex cell-cell communications. Notable among these are the exploration of many-to-many ligand-receptor networks¹¹⁴ and the analysis of cell-cell dependencies.¹¹⁷ The latter involves predicting a target cell's expression profile based on the profiles of its neighboring cells,¹¹⁵ especially within specific spatial niches. Furthermore, novel algorithms are broadening the horizon of intercellular interaction research, transitioning from a local niche perspective to a more comprehensive, tissue-level view.¹¹⁵ Current research in cell-cell communications is increasingly integrating spatial programming of multicellular patterning, with a focus on the engineered assembly of the microenvironment.¹¹⁶ This advancement in research methodology is a promising stride toward a deeper understanding of the complexities inherent in cellular interactions across diverse spatial contexts, and it holds significant potential for break-

throughs and discoveries on the pathogenesis of diseases,¹⁴⁰ improved tissue engineering, and adequate disease modeling.

3D reconstruction and spatiotemporal cell trajectory analysis

Most biological tissues and cells exist and interact within a 3D environment. Adopting a 3D approach in spatial transcriptomics provides a more accurate and realistic representation of these structures, enhancing our understanding of cellular interactions, tissue organization, and the overall architecture of biological systems. There have been some attempts at 3D reconstruction algorithms in imaging or spatial transcriptomics. For instance, based on H&E-stained tissue sections, CODA¹⁴¹ has achieved the reconstruction of large tissues (up to multi-centimeter cubes) at subcellular resolution. Using spatial transcriptomics data, PASTE (probabilistic alignment of ST experiments)¹¹⁸ and SPACEL (spatial architecture characterization by deep learning)¹¹⁹ facilitate the joint analysis of multiple spatial transcriptomics slices, aligning them to construct a 3D stack of the tissue. However, significant challenges remain in this field. There is a need for batch correction to address technical effects on detected raw gene expression levels across sections. Current workflows often utilize Pearson residuals¹⁴² for 3D batch correction or slightly adjust gene expression using deep learning methods when considering spatial domains. Genes exhibiting gradient changes across sections, particularly morphogen-related genes, could potentially be used as benchmarks for smoothing expression patterns across sections.¹²⁴ This will enhance the correlation and comparison of gene expression patterns and spatial domains, leading to a more comprehensive understanding of tissue organization. Additionally, achieving a balance between global optimization and local feature-based alignment becomes critical,^{118,120} especially when the sections are relatively sparse. This balance assumes even greater importance when aligning tissues across a timescale to reconstruct developmental trajectories. Such alignment must faithfully represent the dynamic interplay among time, space, and gene expression. Two methods that stand out in this context are MOSCOT (multi-omics single-cell optimal transport)¹²⁰ and SLAT (spatial-linked alignment tool),¹²¹ which serve as pioneering tests in this field.

When spatial resolution is accurate to the cellular level, time-resolved analyses, like lineage tracing and cell fate inference, become viable techniques for studying the dynamics of cellular identities and developmental trajectories. In spatial transcriptomics, it is essential to consider changes in both physical and expression spaces simultaneously. Recent endeavors in this field have seen innovative attempts to merge these multiple dimensions. Notable examples include the use of minimum weight spanning arborescence,¹²² the application of morphological vector fields,¹²³ and the spatial transition tensor.¹⁴³ These methods mark significant advancements in spatial transcriptomics, offering new insights and enhancing our understanding of complex biological processes, particularly in embryology and etiopathology.

Databases for spatiotemporal omics

The rise of spatial omics technologies has also led to an increasing demand for large-scale online databases that

Table 3. Summary of online databases for spatial omics

Database	Link	Download/upload	Analysis tools
HCA ¹⁴⁷	https://data.humancellatlas.org/	yes	interactive visualization
HuBMAP ¹⁵⁵	https://portal.hubmapconsortium.org/	yes	interactive visualization
SODB ¹⁵²	https://gene.ai.tencent.com/SpatialOmics/	yes	interactive visualization
STOmicsDB ¹⁴⁹	https://db.cngb.org/stomics/	yes	interactive visualization
SpatialDB ¹⁵⁰	http://www.spatialomics.org/SpatialDB/	yes	interactive visualization
SOAR ¹⁵¹	https://soar.fsm.northwestern.edu/	yes	interactive visualization
HTAN ¹⁵³	https://humantumoratlas.org/	yes	interactive visualization
Allen Brain Map ¹⁵⁴	https://portal.brain-map.org/	yes	interactive visualization, programmatic access, multi-omics analysis

facilitate data sharing, exploration, and analysis. Early efforts were limited to imaging and focused on model organisms, with resources like the e-Mouse Atlas and Gene Expression^{144,145} and Xenbase¹⁴⁶ cataloging anatomical and *in situ* hybridization data. Large-scale single-cell omics initiatives such as the Human Cell Atlas¹⁴⁷ and Human BioMolecular Atlas Program (HuBMAP)¹⁴⁸ compiled single-cell datasets spanning diverse human tissues and organs. More recently, databases dedicated to spatial transcriptomics data have been developed, including STOmicsDB,¹⁴⁹ SpatialDB,¹⁵⁰ SOAR,¹⁵¹ and SODB,¹⁵² hosting datasets generated using spatial multi-omics technologies. There are also databases for specific areas like cancer research, exemplified by the Human Tumor Atlas Network (HTAN),¹⁵³ and neuroscience in multiple species, represented by the Allen Brain Map.¹⁵⁴ All the datasets offer web-based interfaces for data submission, retrieval, and basic analysis functionalities (Table 3).

However, as the field continues to evolve, there is a growing need for more comprehensive databases that can accommodate multi-modal spatial omics data, including proteomics, metabolomics, and imaging data. Accommodating diverse data types and seamless data integration across modalities and studies, as well as implementing robust metadata annotations, user-friendly interfaces, and scalable computational infrastructure to handle large-scale spatial datasets, are other key areas requiring improvement. Developing robust, comprehensive, and user-friendly spatial omics databases with state-of-the-art analytical capabilities remains an important challenge to fully leverage the potential of these rich datasets.

BIOLOGICAL AND CLINICAL APPLICATIONS OF SPATIAL OMICS

Biological systems are inherently spatial, with distinct cellular microenvironments and tissue structures playing critical roles in determining gene expression patterns and cellular functions. Capturing the spatial dimension of gene expression is crucial for obtaining a complete picture of the cellular ecosystem. Spatial omics technologies enable the precise mapping of molecular signals to specific locations within a tissue, providing invaluable insights into how cells interact with their immediate surroundings and contribute to the overall function of the tissue. By integrating spatial and temporal contexts, researchers can now uncover the intricate relationships

between cellular behavior, tissue organization, and molecular regulation in various biological processes, thereby advancing our understanding of organ functionality, developmental biology, species evolution, disease mechanisms, and therapeutic interventions.¹⁵⁶

Elucidating organ organization and function at the molecular and cellular levels

Although single-cell multi-omics technologies have facilitated the mapping of cellular atlases at organ and even individual levels,^{157–164} past efforts have largely focused on the types and proportions of cells, overlooking the important functions cells play as part of specific spatial environments and cellular networks. At the molecular level, spatial transcriptomics methods can identify spectra of molecular signals with specific spatial distribution characteristics, aiding in our understanding of the molecular network features and functions of different tissue domains. At the cellular level, these methods not only detect cell types and population proportions but also assess the spatial characteristics of cell densities and cell interactions. At the regional level, spatial transcriptomics enables spatial clustering of distinct areas with similar cellular composition and functions based on molecular signatures, thus obtaining molecular-level anatomical structural features. All this information provides powerful assistance in understanding organ function (Figure 3A).

For example, the spatial distribution atlas of the entire macaque brain based on the Stereo-seq method is among the largest primate brain cell atlas effort to date. The first results mapping the cerebral cortex¹⁶⁵ produced spatial transcriptomic data of 161 large field-of-view coronal sections, segmenting over 42 million single cells. Leveraging more than 1.4 million single-cell transcriptomic data for cortical regions, the study identified 23 major cell types and 264 subtypes, encompassing excitatory neurons, inhibitory neurons, non-neuronal cells, and 143 cortical regions. It annotated the spatial distribution of cell types, finding almost all had spatial preferences. Focusing on the cortex, the study observed significant regional specificity in cell density, correlated with the hierarchical functions of the cerebral cortex. For example, in the visual and somatosensory systems, cell density and hierarchical structure showed negative correlations, with most cell subtypes having either positive or negative correlations within the hierarchical structure. Additionally, neighborhood analysis of different subtypes of inhibitory neurons like the chandelier cells (CHCs) revealed differences in surrounding

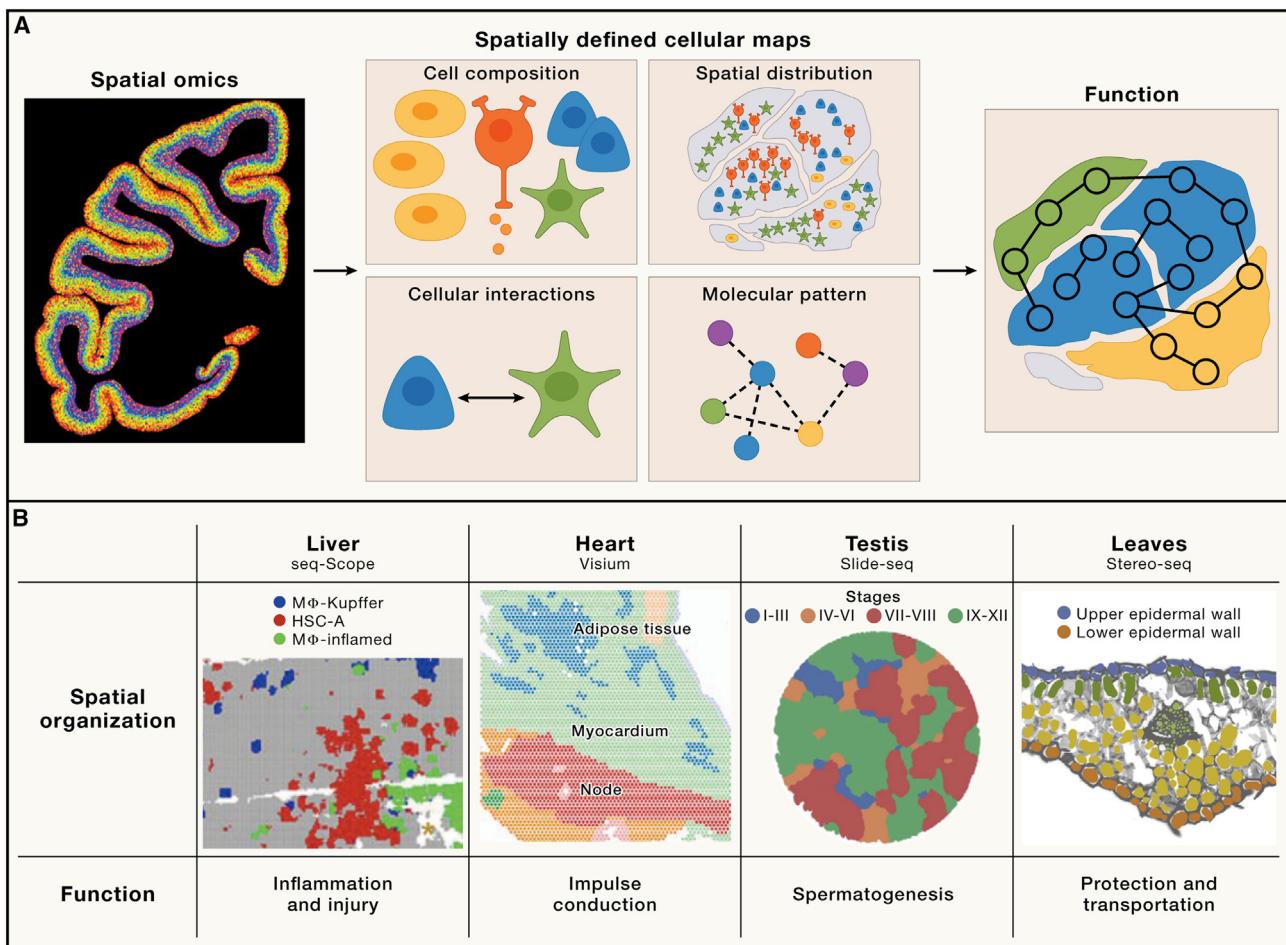


Figure 3. Spatially defined cellular maps elucidate organ structure and function

(A) Spatial transcriptomic analyses reveal cellular composition, cellular distribution, cellular interactions, and molecular networks.

(B) Examples of spatial mapping to elucidate the structure and function of the organs including liver, heart, leaves, and testis. (Part of the image is adapted from Cho et al., Chen et al., Kanemaru et al., Chen et al., Xia et al., and Hao et al. ^{32,165–169})

excitatory neuron types, suggesting potential spatial specificity in neuronal circuits. The number of spatial cell distribution atlases of the brain is growing rapidly, covering different species such as humans,^{139,170–172} non-human primates,¹⁷³ mice,^{26,174–176} and goldfish¹⁷⁷ and analyzing various brain regions, revealing the brain's molecular characteristics in detail at cellular and molecular resolutions. For example, the brain-wide spatial atlases, such as the BICCN mouse atlas,¹⁷⁸ not only present comprehensive resources to draw the map of mammalian brains but also allow for unbiased comparisons of cell-type proportions between regions, which is essential for a deeper understanding of the cellular and molecular components of systems that drive distinct functions in specific regions of the brain.

Spatial transcriptomics technology has also been extensively utilized in the analysis of various organs across multiple species, offering profound insights into the intricate relationship between spatial cellular distribution and function. For instance, comprehensive studies at the whole-lobe scale of the liver have elucidated molecular and cellular gradients, as well as specific cell

microenvironments—such as macrophage niches—that play pivotal roles in modulating liver cell function, revealing diverse cellular interactions and mechanisms of homeostatic regulation within the liver.³² Similarly, in cardiac research, spatial transcriptomics has been instrumental in uncovering functional regulatory networks associated with ion channels, linking specific genes exclusive to the rare cardiac Purkinje cells that are critical for impulse conduction.¹⁶⁶ These experiments provided unprecedented insights into cardiac anatomy, enhancing our comprehension of heart physiology. Moreover, the application of spatial transcriptomics in lung research has led to the discovery and characterization of novel respiratory bronchiole cell types, such as airway-associated LGR5+ fibroblasts, significantly advancing our understanding of lung physiology and the underlying mechanisms of lung regeneration and disease.¹⁷⁹ Spatial transcriptomics accurately localized cell types in seminiferous tubules and uncovered its corresponding stage in adult mouse testis¹⁶⁷ (Figure 3B). The technology also illustrates how the spatial arrangement of cells is intricately linked to their external environments. For example, spatial transcriptomic profiling has

revealed the differentiation patterns of lower and upper epidermal cell subtypes in *Arabidopsis* leaves¹⁶⁸ (Figure 3B), the medium proximal and distal epidermal cell subtypes in tomato callus, and the specialized role of flavonoid metabolism in onion epidermal cells.¹⁶⁹ These discoveries have substantially enriched our comprehension of the complexity within plant epidermal cell classification and their specific functional differentiation, crucial for regulating light response, ion transport, water loss prevention, and pest resistance.

Even though recent developments in spatial omics have provided novel insights on spatial structures of organs and tissues, some challenges remain. Due to technological limitations, recent studies are more focused on transcriptomics compared to other molecular modalities. Future multi-omics analyses, such as epigenomics and proteomics, are needed to comprehensively understand how genomic regulation affects organ physiological function. Additionally, advances and standards in constructing 3D atlases will facilitate visualization of cellular interactions, the microenvironment, and complex biological processes at the whole-organ level.

Unveiling the black box of spatiotemporal dynamics during development

The recent explosion in single-cell omics technologies has boosted our abilities to study massive numbers of individual cells, revealing cell dynamic events that are particularly relevant for developmental biology. This has led to the generation of comprehensive atlases, ranging from single organs up to entire embryos, and from early zygotes to late development postnatal stages.^{180–185} However, it remains unclear how induction triggers (or morphogens) determine lineage specification, which is typically provided by an organized niche microenvironment within specific spatiotemporal windows.

Spatial transcriptomics have been applied to dissect the spatiotemporal dynamics of developing organs such as brain,¹⁸⁶ heart,¹⁸⁷ intestine,¹⁸⁸ and spinal cord,¹⁸⁹ as well as embryo from multiple species including human,¹⁹⁰ mouse,^{35,42,191} zebrafish,¹⁹² and drosophila¹⁹³ (Figure 4A). These atlases provide detailed insights into organogenesis including the formation of functionally distinct regions in the human embryonic brain¹⁸⁶ and the tangential migration trajectory of interneurons from the ganglionic eminence to cerebral cortex,³⁵ the spatial archetypes and cellular heterogeneity of human heart development from 4.5 to 9 post-conception weeks (PCW)¹⁸⁷ and the human intestinal development from 12 PCW to adult stage¹⁸⁸ (Figure 4B). These studies also reveal the presence of heterogeneous cell populations with domain-specific expression patterns, which subsequently contribute to regional specification. In addition to normal development, spatiotemporal atlases are expanding to tissue degeneration and regeneration. These studies include the characterization and mapping of the appearance and migration of early-development-like neuron stem cells in the regenerating axolotl brain after injury,¹⁹⁴ lobular zone-specific responses in the mammalian liver upon injury and subsequent repair,^{195,196} and the 3D transcriptomic landscape of regenerating planarians¹⁹⁷ (Figure 4C). These studies focus more on spatial distribution characteristics of gene expression, receptor-ligand binding and crosstalk between different cells, regional heterogeneity,

and developmental trajectories of cells, providing new insights into development (Figure 4D).

Current studies primarily focus on transcriptomics, which limits the opportunities for understanding the molecular hierarchy underlying developmental events. However, by combining DNA-tagged antibodies for protein profiling,¹⁹⁸ spatial omics will be able to profile the local cell-intrinsic and -extrinsic molecular gradients controlling tissue morphogenesis. Additionally, integrating epigenetic information, including DNA methylation, chromatin accessibility, and histone modifications with transcriptome data, will enable a refined, spatially resolved GRN underlying cell differentiation. Besides, incorporating additional modalities that currently remain uncharted, such as profiling of small RNAs and the epitranscriptome, would further provide fundamental insights in development regulation. It is important to note that current time-resolved spatial omics are the joint analysis of individual time points in a sequential manner, which essentially combines static state measurements. However, this approach solely captures the snapshots of gene expression at given time points, thereby disregarding the crucial details pertaining to dynamic transcriptional changes occurring within the time frame of minutes to hours. To achieve this, spatial omics will require the integration of methods that offer enhanced temporal resolution, such as molecular recording,¹⁹⁹ metabolic labeling,²⁰⁰ and lineage tracing techniques.⁸⁶ These approaches not only capture the static states but also the ancestral states during transition. In this regard, by coupling spatial transcriptomics with genetic label-based lineage tracing, techniques such as intMEMOIR,⁸⁸ Spatial iTTracer⁸⁹ and Space-TREX⁵⁰ demonstrate the potential to understand the clonality and lineage dynamics from the developing brain organoid *in vitro*, as well as mouse and *Drosophila* brain *in vivo*. While these techniques represent a promising future direction, they continue to face numerous technical hurdles that lead to incomplete lineage information, which include (1) limited barcoding efficiency by the current genetic approaches, (2) suboptimal sensitivity for capturing barcode information in current spatial transcriptomic techniques, and (3) limited availability of computational tools for resolving the lineage information. Therefore, continued research and innovation in both the experimental and computational aspects will be required to realize the full potential of these approaches. Additionally, introducing mitochondrial variant lineage tracing may also enhance the capabilities of current spatially resolved methods.

Understanding evolution at spatiotemporal resolution

At the heart of evolutionary studies, the examination of genes and genomes is pivotal in unraveling the mechanisms of adaptability in species. Genomic analyses provide detailed insights into the genetic changes linked to phenotypical characteristics of organisms during evolution.^{201–203} Central to this research field are comparative genomics approaches, which employ methods like genome comparison, phylogenetic analysis, and gene positive selection analysis to uncover evolutionary events and phylogenetic relationships^{204–206} (Figure 5A). However, adaptation of species, imprinted in DNA sequence alterations, is represented from molecular architecture to cellular composition, resulting in morphological

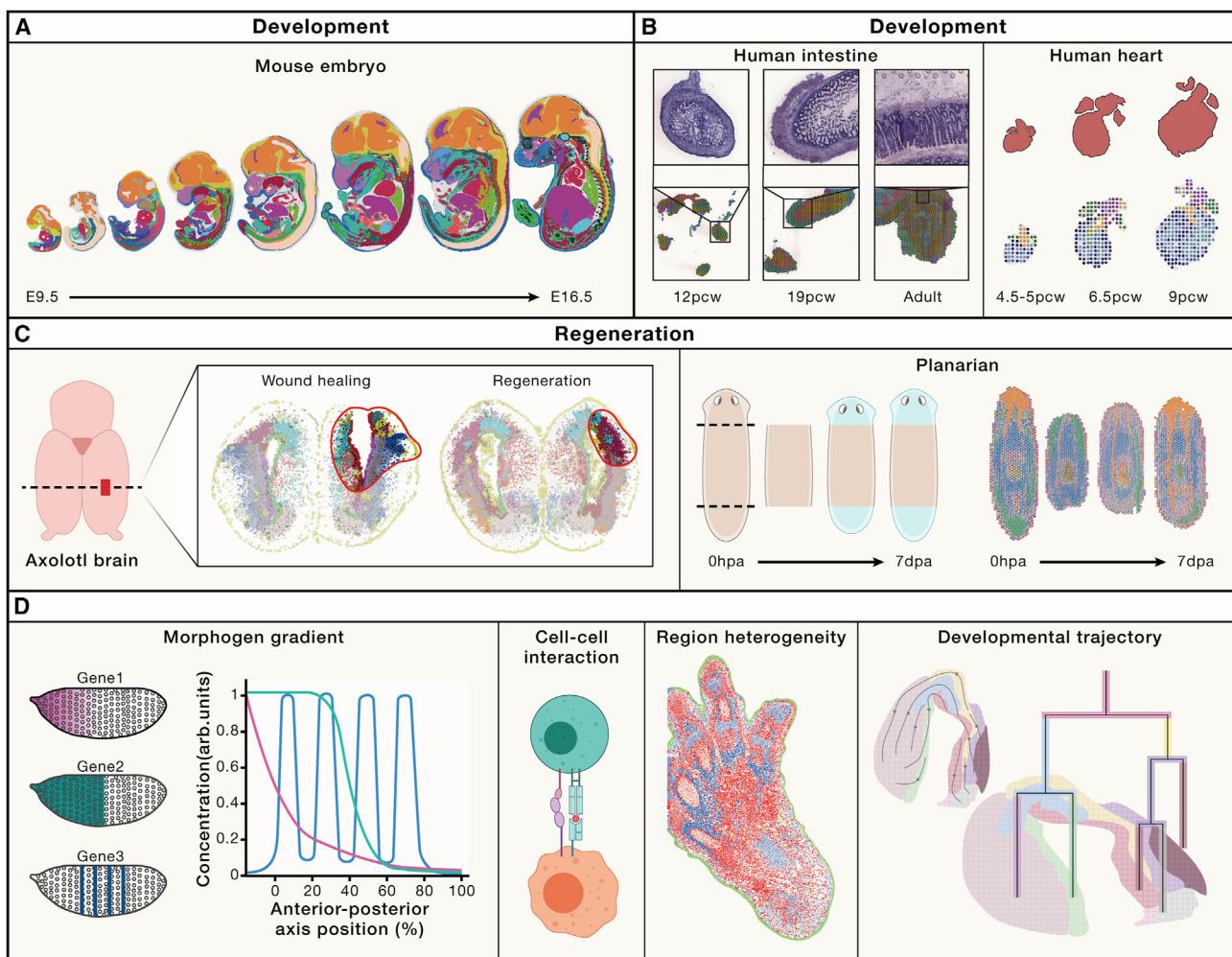


Figure 4. The spatiotemporal landscape of development and regeneration

(A) The spatiotemporal atlas of mouse showing organogenesis in the embryonic development process.³⁵

(B) The spatiotemporal landscape of human intestine and heart development.^{187,188}

(C) Spatial visualization demonstrates how induced progenitor cells regenerate lost neurons following injury in the axolotl telencephalon and the planarian regeneration process.^{194,197}

(D) The time-series spatial transcriptome can be used to analyze gradient gene expression at the spatiotemporal level, illustrating how molecular distributions (such as morphogens) and cell-cell interactions regulate complex and delicate morphogenesis. This analysis also traces the dynamic cell fate transition process from stem cells to terminally differentiated cells.

and functional attributes. Significant knowledge gaps in understanding the intricate association between genotype and phenotype during the adaptation process are a major limiting factor. Advances in single-cell and spatial omics technologies are poised to play a crucial role in bridging these gaps, offering unique insights into the nuanced mechanisms of species adaptation.²⁰⁷

By analyzing gene expression at cellular resolution across species such as humans,²⁰⁹ non-human primates,²¹⁰ mammals,^{211–213} and lower organisms,²¹⁴ researchers can trace significant transitions and the emergence of new cell types during the course of evolution.²¹⁵ For example, this approach has been instrumental in uncovering a multitude of human-specific cell types within the brain, shedding light on the cellular and molecular characteristics that distinguish the human brain from

those of other species.^{216–218} Besides, multimodal analysis by integrating single-cell gene expression and other layers of omics can help expose how genetic alterations drive cellular changes during evolutionary processes. For example, single-cell atlases for zebrafish and Mexican tetra uncovered the divergence of cell types associated with gene family evolution in the hypothalamus.²¹⁹ Moreover, integrating single-cell transcriptomics with *cis*-regulatory elements revealed primate-specific gene expression networks that may explain our advanced brain development as compared to mice.²²⁰ These studies illustrate how changes at the genomic level affect cellular gene regulation, ultimately determining the distinct traits of cells and tissues and of species (Figure 5B).

Compared to single-cell analysis, advancements in spatial transcriptomics have now provided more detailed insights into

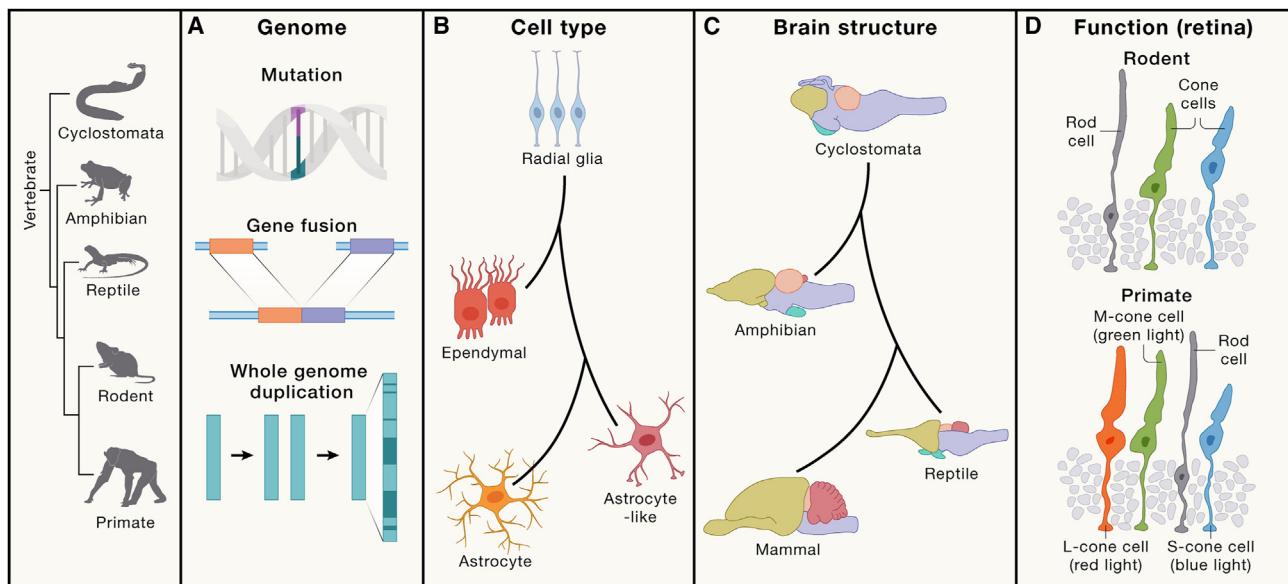


Figure 5. Spatial omics enhances understanding of evolutionary mechanisms from genomes to structure and function

The process of species evolution can be broadly divided into four stages:

- (A) DNA sequence variation: This includes genomic sequence changes such as mutations, gene fusion, whole genome duplication, etc., which can be studied using genome-sequencing technologies.
- (B) Changes in cell types: Variations in sequences lead to changes in cell types, with ancestral cell types evolving into a greater variety of cell types responsible for different functions, and changes occurring in their cellular morphology and function, which can be studied using single-cell sequencing technologies.
- (C) Changes in tissue or organ structure: The increase in cell diversity, along with changes in morphology, function, and spatial distribution, leads to structural changes in tissues or organs, which can be studied using spatial omics technologies.
- (D) Functional changes (illustrated with the retina): The diversity of cell types, their spatial distribution, and changes in tissue morphology collectively contribute to functional changes, ultimately facilitating the adaptive evolution of the experimental species. For example, the retinas of different species of amniotes vary in cellular composition, spatial distribution, and tissue morphology, leading to differences in visual functions such as color recognition and photosensitivity. (Part of the image is adapted from Lamb et al.²⁰⁸)

evolution at the tissue structural level. For example, utilization of both single cell and spatial transcriptomics technology demonstrated the cerebellar nuclei evolve by duplicating and diverging all cell types within the fundamental archetypal cerebellar nucleus.²²¹ Spatial transcriptomics technologies, due to their capability to *de novo* characterize the anatomical structures, provide the scientific community with tools and opportunities to study organ evolution of all species with a paucity of anatomical knowledge. For example, vesicular glutamate transporters (VGLUTs), a family of key proteins in glutamatergic neurons, have a large number of homologous proteins present in different species. Through the combination of genomics, single-cell omics, and spatial transcriptomics in the amniote brain, species-specific preferences in the usage of paralogous genes adjacent to VGLUTs was observed, which may be associated with the evolution of brain structures in amniotes.²¹⁰ These studies integrated genomic information with cell taxonomy and their spatial distribution to provide a multi-dimensional understanding of species evolution (Figures 5C and 5D).

Evolutionary biology still grapples with many unsolved fundamental questions, and the advent of single-cell and spatial technologies offers new avenues for exploration. For instance, the origin and evolution of the first nervous system is one of the fundamental questions in evolutionary biology, with several invertebrate phyla like Choanoflagellates, Porifera, Ctenophora, and Cnidaria being considered the first species with a nervous

system.^{222–226} Beyond single-cell technology, spatial transcriptomics technology could reveal homologous cell types and molecular foundations across species *in situ*, offering a more systematic understanding of nervous system evolution. In addition, spatial transcriptomics also provides new opportunities in understanding plant evolution. For example, C₃ and C₄ plants utilize different photosynthetic pathways, with C₄ plants having specialized leaf anatomy and cell types that concentrate CO₂ around the enzyme rubisco, reducing photorespiration and increasing efficiency in high-temperature and low-CO₂ environments. Spatial transcriptomics can map the expression patterns of genes involved in these pathways, identifying specific changes in cell types, their spatial arrangement, and functional zones within the leaves. This detailed insight can elucidate the evolutionary steps and adaptations that have led to the distinct C₃ and C₄ photosynthetic mechanism.^{227,228} Overall, we anticipate a new era of comparative spatial genomics that will broaden our understanding of evolutionary processes.

Deciphering the mechanisms of disease onset and progression

Population-based genome sequencing studies identify numerous genomic variants associated with diseases. However, charting the exact cellular or tissue transcription activity of these genomic loci has proven to be a challenge, hindering the understanding of the impact of genetic variance on biological

functions. Bulk transcriptomic, epigenomic, or proteomic approaches have significantly advanced our understanding of gene expression and regulation linked to diseases. These methods have been instrumental in uncovering abnormalities and facilitating the identification of additional targets for disease detection or treatment. However, it is important to note that these approaches can hardly identify alterations in specific cell types and tissue environments relevant to disease development. Single-cell sequencing provides a comprehensive in-depth analysis of various diseases and diseased conditions including cancer, identifying numerous disease-related cell types and their altered microenvironments. However, single-cell sequencing is unable to achieve the desired detailed resolution of the structural features of lesions and the interaction within the microenvironment.

Spatial omics provides a more detailed understanding of disease-related genetic variations and epigenomic and transcriptomic alterations within tissues and microenvironments.^{229,230} On one hand, it provides distributions of genes and signaling pathways associated with features such as hypoxia, epithelial-mesenchymal transition, or inflammation that are associated with relevant pathological characteristics *in situ*.^{229,231,232} In cancer, identifying different mutational subclones on tumor sections enabled the study of spatial patterns in relation to histological progression, contributing to the recognition of molecular characteristics of tumor subpopulations leading to metastasis.²³³ Other studies combining the spatial distribution of gene and protein expression explored the association between tumor heterogeneity and spatial organization in glioblastomas, unraveling drivers of tumor progression and treatment resistance.^{234,235} Spatial transcriptomics also enables the study of cell-cell interactions within the tumor microenvironment, including the relationship between tumor, stromal, and immune cells. Cancer-associated fibroblasts (CAFs) have been uncovered in the tumor nests of patients with lung cancer, and an enriched presence of MYH11+ αSMA+ and FAP+ αSMA+ CAFs at the tumor border correlated with significant fibrous tissue deposition, impacting T cell infiltration and potentially influencing immunotherapy responses.²³⁶ Additionally, invasive liver cancer has been characterized by spatial transcriptomics, revealing the recruitment of macrophages by damaged liver cells as a crucial factor in liver cancer progression.²³⁷ Analyzing cell interactions within spatial structures, such as tertiary lymphoid structures, has also provided insights into therapy response²³⁸ or resistance.²³⁹

In addition to cancer research, spatial omics is pivotal in chronic and infectious disease studies.^{240–244} For instance, interactions between microglial cells and astrocytes in the amyloid plaque cell niche could contribute to Alzheimer's disease development. In infectious diseases like COVID-19, spatial omics offers insights into molecular processes during infection, providing valuable spatial information for understanding the virus's impact on different tissues. By analyzing the spatial location of cells and gene expression patterns, researchers propose that SARS-CoV-2 may infect sustentacular cells in the olfactory epithelium via angiotensin-converting enzyme 2 (ACE2), avoiding direct infection of olfactory sensory neurons. The loss of olfactory function in COVID-19 patients may be due to dysregulated transcrip-

tomes in infected sustentacular cells, resulting in insufficient support for olfactory sensory neurons, which impairs their function.²⁴⁵

Taken together, the emergence of spatial omics technologies has enabled the simultaneous study of diseases at molecular, cellular, and tissue levels. Integrating these layers of information will enhance our understanding of the mechanism of disease progression, thereby accelerating the development of novel clinical diagnosis and interventions (Figure 6). Nevertheless, there are still several challenges when applying spatial omics to clinical research. These include (1) multi-omics information—to comprehensively understand the impact of genetic variations and effective integration of various omics data, such as genomic variations, epigenetic signals, and post-translational modifications with spatial multi-omics features in genetic diseases, is still a challenge; (2) monitoring temporal information—the initiation and development of diseases are dynamic and evolving processes, and thus, temporal information during disease development, progression, and treatment is critical to fully comprehend diseases and develop corresponding treatment strategies; (3) pathogen detection—in infectious diseases, pathogen detection remains a significant technical challenge as simultaneously detecting molecular features of pathogens and hosts within tissues is difficult to achieve. In order to tackle the aforementioned challenges, forthcoming advancements are imperative in the domains of technology, algorithms, and clinical study design.

CLINICAL APPLICATION OF SPATIAL OMICS: FROM BENCH TO BEDSIDE

Spatial omics contributes significantly to decoding the tumor microenvironment and facilitating the understanding of onset and progression of disease, which are the foundation of precision medicine. Spatial omics technology can associate manifested tissue architecture with high-resolution molecular features and there is potential for the use of spatial molecular profiles as a diagnostic tool in clinical settings, improving stratification of diseases with high heterogeneity that require personalized complex therapeutic strategies.²⁴⁶ However, the clinical access of such powerful tools is still limited by stability, reproducibility, rationality, practicality, high cost, and accessibility.

Recent advancements in immunotherapies, including immune checkpoint inhibitors, cell-based therapies, and mRNA vaccines, have successfully fulfilled regulatory requirements, significantly enhancing the treatment options and outcomes of numerous advanced cancers. This breakthrough, however, introduces substantial challenges in developing efficient stratification methods due to the complex sense-and-respond processes within the tumor microenvironment. Spatial omics testing has played a pivotal role in identifying biomarkers with high diagnostic and prognostic accuracy, thereby informing both monotherapy and combination immunotherapy strategies. Despite these advances, spatial omics face limitations such as high costs, prolonged turnaround times, inconsistencies across different platforms, and the requirement for sophisticated bioinformatics, which collectively restrict its clinical adoption.

Spatial omics assays also offer a promising platform to correlate morphological features of tissues and cells with gene

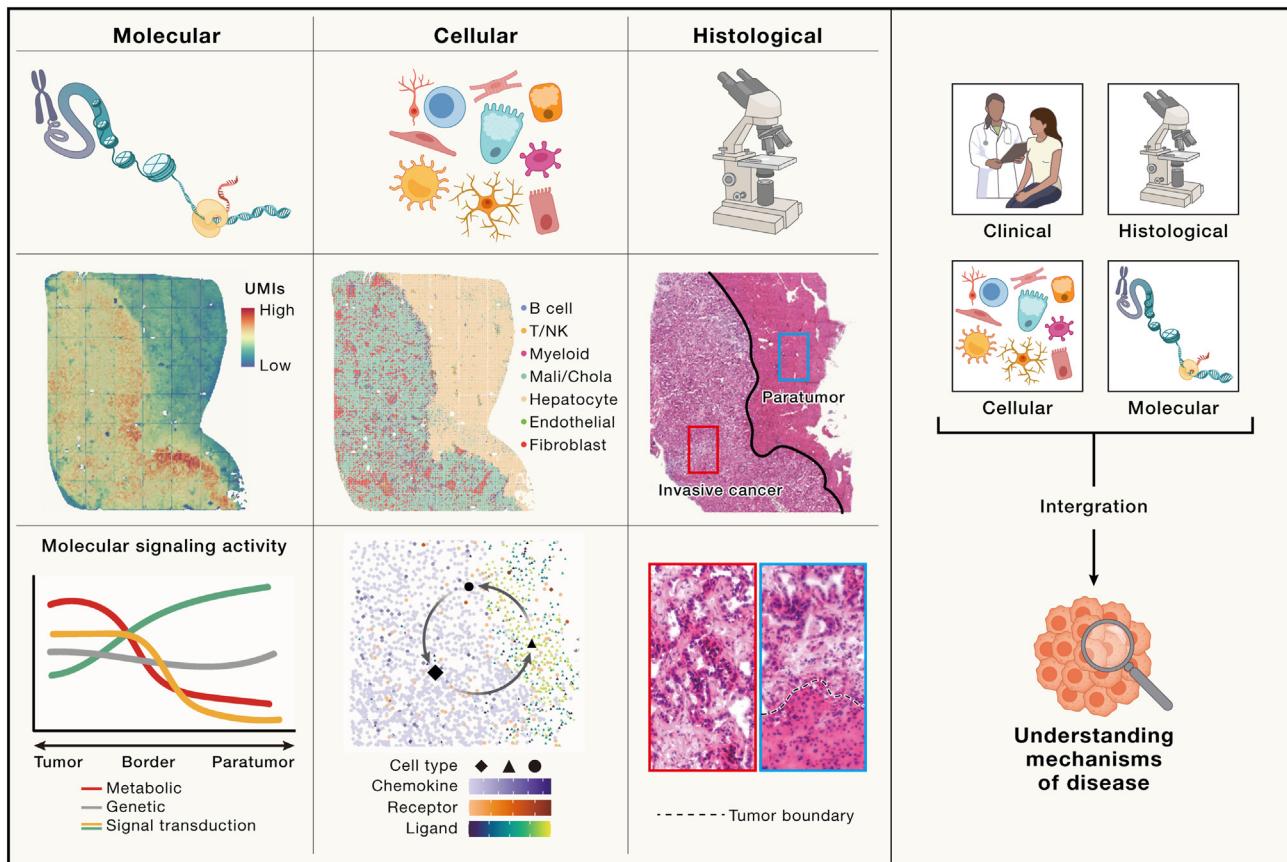


Figure 6. Role of spatial omics in uncovering pathogenesis of diseases

The emergence of spatial omics technologies has enabled the simultaneous study of diseases at molecular, cellular, and histological levels. Integrating these layers of information will enhance our understanding of the mechanism of disease progression. (Part of the images adapted from Wu et al.²³⁷)

expression through *in situ* spatial information. This approach enables the training of AI algorithms to link H&E characteristics with cellular states, inter-cellular communication, and other biological activities based on gene expression profiles. The predictive and prognostic validity of H&E-based biomarkers has been established, presenting a significant opportunity.²⁴⁷ Given that H&E staining is the most prevalent modality in pathology, AI analyses trained on spatially resolved transcriptomic data could be integrated into clinical practice. This integration promises to reduce costs and data storage requirements and improve accuracy. However, the translational application of these technologies faces hurdles, including the absence of detailed protocols and evaluations by pathology experts, challenges in data quality control, and the lack of prospective, randomized, multicenter trials with sufficient sample sizes to objectively assess their impact on both pathologists and patients. Addressing these challenges is crucial for realizing the full potential of H&E-based AI analysis in clinical settings and enhancing cancer diagnosis, prognosis, and treatment efficacy.

In addition to treatment decisions, another clinical challenge is the diagnosis of diseases with high complexity. Routine diagnosis procedures for highly heterogeneous diseases, like undifferentiated tumors and carcinoma of unknown primary origin,

require more than 12 IHC stains and reserved sections for genomic subtyping,²⁴⁸ still resulting in a high risk of misclassification. Statistically, the diagnosis of classification changed in 19.7% of patients with lymphomas between first diagnosis and expert second opinion, negatively impacting patient care.²⁴⁹ Nowadays, diagnosis and treatment regimens tend to rely largely on minimally invasive biopsies and diagnosis based on selective panels, without comprehensive evaluation due to limited samples.²⁵⁰ Single-plex staining and molecular assays without aligned tumor architecture struggle to achieve satisfactory stratification of patients with cancer. Spatial multiplexing of gene or protein analysis overcomes such difficulties by integrating multiple biomarkers and their co-localization in a single section.²⁴⁶ Implementation of new technologies with multiplexing ability and high sensitivity into clinical practice is under urgent demand to facilitate precise diagnoses with high sensitivity.

Recent studies indicate that diagnosis or prognosis resulting from multiplexed methods associate more closely with the patient outcome when compared to single-plexed methods. For example, a systematic review of predictive power among different companion assays shows that spatial phenotyping by multiplexing immunofluorescence imaging provides the highest accuracy in predicting the outcome of PD-1 treatment in patients with

cancer.²⁵¹ The population of proliferating CD8+TCF1+ T cells and MHCII+ cancer cells by imaging mass cytometry can predict immunotherapy response in triple-negative breast cancer.²⁵² However, pitfalls of spatial-multiplexing technology like unreliable quantitation under brightfield in multiplex IHC (mIHC), opaque information captured by specific wavelengths in multiplex immunofluorescence (mIF), and limited targets have led to low clinical utility.²⁵³ A potential solution could be provided by sequencing-based multiplexing methods that enable simultaneous gene detection and staining of protein markers with near-to-single cell resolution.^{42,58} In addition, concurrent detection of transcriptomic and proteomic profiling allows quantitative performance and spatial analyses. Taking a lung cancer patient waiting for a treatment decision based on a needle biopsy as an example, Stereo-CITE-seq can determine the routine diagnostic panels of TTF1, napsin, p63, and cytokeratin for diagnosis, and additional biomarkers like PD-L1, PD-1, CD68, CD45, CD8, and CD3 to determine the eligibility for immune-checkpoint therapy to guide the optimal treatment strategy.⁵⁸

To become clinically routine, spatial omics methods need to be implemented in clinical laboratories using standardized and fail-proof protocols, self-explainable and reproducible results, and digital pathology workflows. Validation of ethical, legal, and social implications of the assay is essential to prove validity and robustness. Reasonable instrument requirements, lower costs, reduction of manpower, and minimal to no need for experienced specialists can improve access to spatial omics in the clinical setting, including in low-resource sites. In conclusion, spatial omics assays can unlock a new era of interpreting clinically relevant information to improve outcomes in patient care based on integrated knowledge.

GLOBAL EFFORTS IN LARGE-SCALE SPATIOTEMPORAL OMICS STUDIES

The complexity of life processes and the vast amount of data has made large-scale, cross-disciplinary research a prevailing trend. Following the HGP, large-scale genomic research has evolved from genome decoding (such as the International HapMap,²⁵⁴ the 1000 Genomes Project,⁵ and the UK Biobank²⁵⁵) to functional genomics analysis (such as the Encyclopedia of DNA Elements [ENCODE],²⁵⁶ International Human Epigenome Consortium [IHEC],²⁵⁷ Genotype-Tissue Expression [GTEx],²⁵⁸ and Human Protein Atlas [HPA]²⁵⁹) and ultimately to the era of cellular genomics analysis.

The Human Cell Atlas (HCA)¹⁴⁷ is the first international collaborative research consortium aiming to map all cell types in the body, from development to adulthood and old age. The global HCA community has so far profiled tens of millions of cells from nearly 10,000 individuals. Building on this, the HuBMAP¹⁵⁵ aims to integrate cell atlas data to construct detailed and comprehensive biomolecular maps of human tissues. Additionally, the SpatioTemporal Omics Consortium (STOC)²⁶⁰ focuses on understanding biological questions from spatiotemporal dynamics, aiming to accelerate our comprehension of cellular complexity and interactions at the tissue scale in development, physiology, and disease through large-scale spatially resolved multi-omics analyses.

In various fields, large-scale collaborative research is prominent, particularly in brain and tumor research. In brain science, the BRAIN Initiative Cell Census Network (BICCN)¹⁷⁸ is a notable success, aiming to build reference brain cell atlases that provide a molecular and anatomical foundation for studying brain function and disorders. Various national brain initiatives include the EU's Human Brain Project (HBP),²⁶¹ the China Brain Project,²⁶² and Japan's Brain/MINDS.²⁶³ In tumor research, the Human Tumor Atlas Network¹⁵³ aims to chart tumor transitions across space and time at single-cell resolution, enhancing our understanding of cancer biology and potentially improving cancer detection, prevention, and therapeutic discovery for precision medicine treatments of cancer patients.

Single-cell and spatial omics are rapidly advancing in throughput, cost, and accessibility, enabling increasingly widespread global scientific collaboration. However, achieving more efficient team collaboration to maximize the value of genomic big data production and analysis remains challenging. Further considerations may include (1) the formation of collaborative groups for technology and algorithm development, involving interdisciplinary cross-sector partnerships that necessitate the union of teams from diverse backgrounds; (2) the creation of a global multicentric omics platform to ensure consistency among different teams; (3) the establishment of data collaboration groups to promote multicentric data sharing, shared analysis methods, and databases; and (4) the development of technical, platform, and data standards for harmonization across teams.

CONCLUSION

The emergence and rapid development of spatial omics technology, combined with advancements in sequencing and single-cell omics, will enable us to create a “Google Maps” of the human body at cellular resolution across temporal and spatial dimensions. This will enhance our understanding of the cellular and molecular foundations underlying biological processes. Concurrently, the rapid progress in AI and computational biology will significantly drive the integration of cell atlas data, imaging, and clinical phenotype data with algorithms and greatly advance methods for disease diagnosis, treatment, and prognosis, accelerating the clinical application of precision medicine.

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DECLARATION OF INTERESTS

X.X., L.L., A.C., and Y.L. are the co-inventors of Stereo-seq technology. The chip, procedure, and applications of Stereo-seq are covered in pending patents.

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