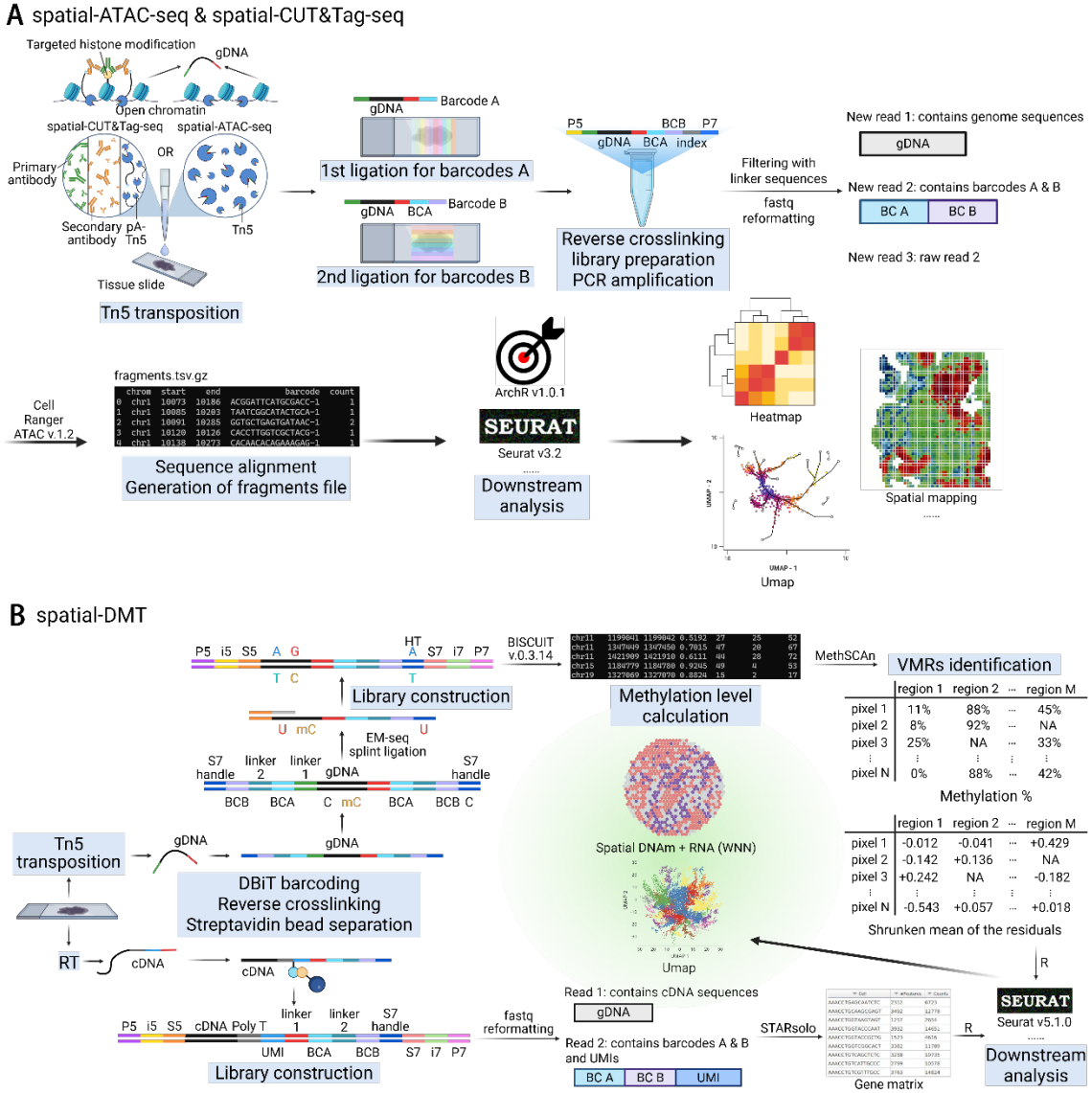

Supplementary Figure 1. The principles and data processing pipelines of spatial-ATAC-seq, spatial-CUT&Tag and spatial-DMT. BCA: Barcode A; BCB: Barcode B.

Supplementary Table 1. Cost, Applicability and Applications of spatial omics technologies

Part I: Imaging-based spatial omics technologies

Technology	Cost	Applicable sample types	Existing applications
MERFISH	Independent of gene/loci number; cost per unit decreases as multiplexity increases(35) ~$0.01 per cell at 10,000-gene scale (106)	Fresh frozen tissue (115); FFPE tissue (118)	1. Mapping the specific distribution of active promoters (66) and Whole-transcriptome-scale expression profiling (107) in the mouse brain 2. Identification of cell types and spatial structure in healthy murine kidney (115) 3. Spatially resolved cell typing of 17 tumor and 16 normal tissue types (118)
seqFISH (108)	Medium-High	Fresh frozen tissue	Defining injury specific microenvironments and cell-cell interactions in kidney regeneration and disease
seqFISH+	Medium-High	Fresh frozen tissue (109)	1. Whole genome imaging of mouse brain (38) 2. ST profiling of mouse brain (109)
Raman-based platform (24)	High	Fresh frozen tissue	Lipidome and other Metabolome in both normal and DN kidney tissues

Part II: Solid-phase array (bead/chip) spatial omics technologies

Technology	Cost	Applicable sample types	Existing applications
Stereo-seq V2 (10)	Medium-High	Fresh frozen tissue; FFPE tissue	1. Simultaneous host-pathogen transcriptome profiling in human tuberculous lung tissue 2. Spatial atlas of lncRNA in mouse brain. 3. Mapping tumor subtypes and alternative splicing in clinical breast cancer FFPE specimens archived for 9 years
Slide-seq	Medium	Fresh frozen tissue (40,82)	1. ST in mouse normal brain (82) 2. Tumor clones and genomic mutations in mouse model of liver matastasis and human metastatic colon cancer (40) 3. Spatially defining injury states and fibrotic niches in kidneys (119)
Slide-tags (112)	Medium	Fresh frozen tissue	1.ST in embryonic mouse brain and human cerebral cortex 2. Immune microenvironment and BCR–ligand interactions in the human tonsil 3. TME of human metastatic melanoma.
SM-omics (68)	High	Fresh frozen tissue	ST and proteomics in mouse brain, spleen and colorectal cancer model
Spot-based spatial-ATAC-seq (56)	High	Fresh frozen tissue	1. Mouse organogenesis 2. spatiotemporal atlas of epigenetic reprogramming in the mouse brain 3. TME epigenetic signatures in HER2+ human breast cancer

Part III: Solid-phase array (bead/chip) spatial omics technologies

Technology	Cost	Applicable sample types	Existing applications
MAGIC-seq (110)	Low; $0.11 per mm^2 for the chip fabrication	Fresh frozen tissue	High-throughput spatial transcriptomics of distinct mouse tissues

DBiT-seq (9,114)	Low-Medium; $1000 per tissue section	Fresh frozen tissue	ST and proteomics in mouse embryo sections
xDBiT (113)	Low-Medium; 125€ per tissue section	Fresh frozen tissue; FFPE tissue	ST in mouse cerebellum, liver, kidney, spleen and heart
Patho-DBiT (30)	Medium	FFPE tissue	Spatial whole RNA landscape, spatial SNV, and spatial distribution of splicing isoforms in human lymphoma FFPE samples
Spatial-CITE-seq (12)	High	Fresh frozen tissue	Spatial profiling of whole transcriptome and proteome in mouse tissues, human tonsil and skin.
Microfluidics-based spatial-ATAC-seq (13,58,60)	Medium	Fresh frozen tissue	1. Identifying of tissue domains and regulatory elements in mouse embryos, mouse brains, human hippocampi, and human tonsil (13,58,60) 2. Cell identification in mouse embryos and developmental trajectory tracing in the mouse brain (14)
Spatial-CUT&Tag (14,58,60)	Medium	Fresh frozen tissue	
spatial-DMT (15)	High	Fresh frozen tissue; FFPE tissue	1. Lineage tracing of mouse embryos 2. DNA methylation landscape of the mouse brain
spatial ARP-seq & spatial CTRP-seq (61)	High	Fresh frozen tissue	1. Spatiotemporal atlas of the epigenome, transcriptome, and proteome in the mammalian cerebral cortex 2. Developmental patterns of the corpus callosum 3. Dynamic pathophysiological changes in the focal demyelination injury model
spatial-Mux-seq (62)	High	Fresh frozen tissue	Multi-omics analysis of mouse embryo, brain, and EAE models

Part IV: Imaging-free computational array reconstruction

Technology	Cost	Applicable sample types	Existing applications
Imaging-free computational array reconstruction (106,111)	Low	Fresh frozen tissue	Identifying spatial cell clustering on a 1.2 cm^2 P1 mouse cranial section

Part V: Spatial metabolomics

Technology	Cost	Applicable sample types	Existing applications
scSpaMet (80)	Very high	Fresh frozen tissue; FFPE tissue	Metabolic heterogeneity in human lung cancer, tonsil and endometrium

Abbreviations: MERFISH, multiplexed error-robust fluorescence in situ hybridization; FFPE, formalin-fixed paraffin-embedded; seqFISH (+), sequencing fluorescence in situ hybridization (+); ST, spatial transcriptomics; FISSEQ, fluorescence in situ sequencing; MAGIC-seq, microfluidics-assisted grid chips for spatial transcriptome sequencing; Stereo-seq, spatial enhanced resolution omics sequencing; TCR, T cell receptor; BCR. B cell receptor; TME, tumor microenvironment; DBiT-seq, deterministic barcoding in tissue for spatial omics sequencing; Patho-DBiT, pathology-compatible deterministic barcoding in tissue; SNV, single nucleotide variant; spatial-CITE-seq, spatial cellular indexing of transcriptomes and epitopes by sequencing; SM-omics, spatial multiomics; spatial-ATAC-seq, spatially resolved assay for transposase-accessible chromatin using sequencing; spatial-CUT&Tag, spatially resolved profiling of histone modifications using in situ cleavage under targets and tagmentation; spatial-DMT, spatial DNA methylome–

transcriptome sequencing; spatial-ARP-seq, spatial-ATAC–RNA–protein-seq; spatial-CTRP-seq, spatial-CUT&Tag–RNA–protein-seq; EAE, experimental autoimmune encephalomyelitis; DN, diabetic nepfropathy.

Supplementary Table 2. Comparison of conventional transcriptomics and representative commercial spatial transcriptomics platforms

Feature	Bulk RNA-seq	scRNA-seq	Visium HD	Xenium
Spatial information	No	No	Yes (2D tissue mapping)	Yes (2D tissue mapping)
Resolution	Tissue	Single cell	~2 μm high-density grid (computational cell-typing required)	Subcellular (~0.2 μm optical resolution)
Transcript coverage	Whole transcriptome	Whole transcriptome	Whole transcriptome	Targeted panel
Detection strategy	Sequencing	Sequencing	Spatial barcoded sequencing	Imaging-based ISS
Cell morphology	NO (tissue homogenate)	NO (dissociated cells)	Histology-assisted	Direct imaging
Cell segmentation	Not required	Dissociation	Computational inference	Required
FFPE compatibility	Yes (with kit)	Limited (low RNA integrity)	Yes (probe-based)	Yes (optimized)
Throughput (per run)	High (multiple samples)	High (thousands of cells)	High (millions of spots, 2 areas/slide)	Moderate (area-limited, ~6.5×6.5 mm max)
Major strengths	Comprehensive profiling	Cell-type resolution	Genome-wide spatial profiling	High-resolution localization
Major limitations	No spatial context	Loss of spatial context	Cost; limited by segmentation/deconvolution methods	Cost; non-whole-genome; imaging time and segmentation challenges
Representative applications	Differential expression	Cell atlas	Tissue architecture	Cell-cell interaction