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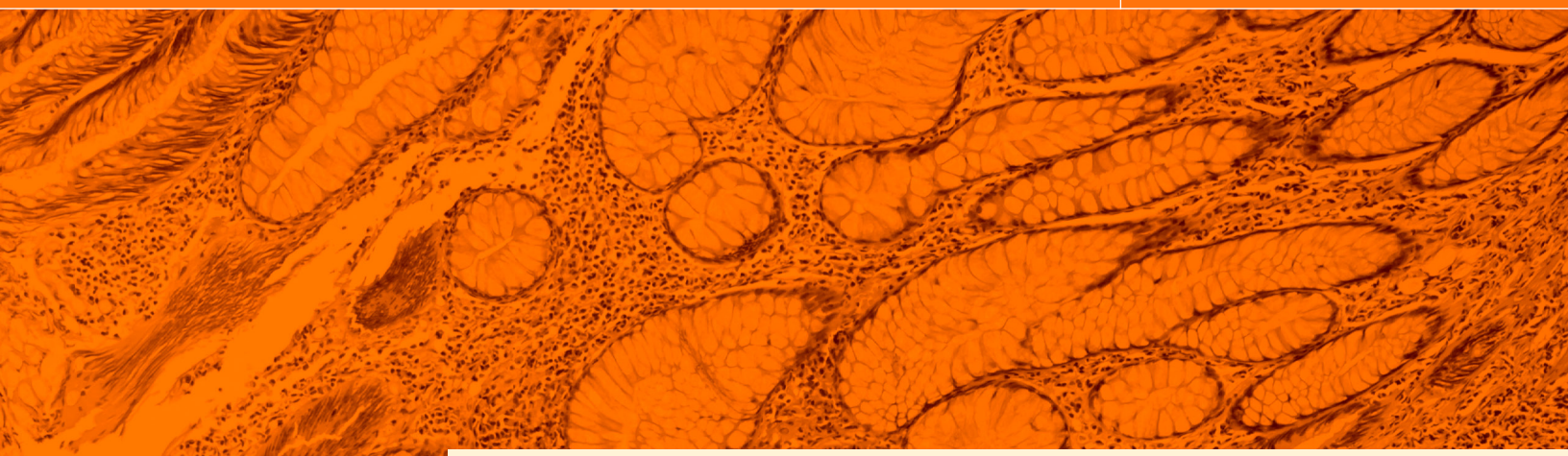

Illumina Spatial Technology: research planning resource

Spatial gene expression analysis through sequencing-based approaches

Technology overview

Learn how Illumina spatial technology enables broad spatial profiling while balancing resolution and sensitivity

Workflow integration

Navigate integration with Illumina sequencing systems and data analysis workflows

Research planning

Explore resources for study planning, methodology evaluation, and proposal development

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Purpose

As adoption of spatial technologies accelerates, researchers face increasingly complex decisions around platform selection, procurement justification, and data integration. This document provides guidance for research teams to evaluate, plan, and implement spatial transcriptomics studies.

Introduction

Spatial transcriptomics is transforming how gene expression is studied, providing both transcript identity and spatial context within intact tissue. By providing biologically contextualized gene expression data, it enables applications such as tissue atlasing, biomarker discovery, tumor microenvironment analysis, and cell-type classification.[1-3] Recent advances have expanded spatial technologies beyond array-based methods to include high-resolution, sequencing-based methodologies capable of capturing transcriptomes across large tissue areas.[4-6] While these innovations open new discovery pathways, they also introduce technical and logistical challenges that require careful planning.

Illumina spatial technology provides a comprehensive workflow for high-throughput, whole-transcriptome spatial profiling. The methodology uses a polyA-capture approach to enable hypothesis-free analysis and is designed for flexible implementation across a wide range of tissue types and research applications. Data processing and visualization are supported through integrated workflows that facilitate segmentation, spatial mapping, and multiomic interpretation.

This guide outlines the challenges faced by researchers performing spatial transcriptomics studies and illustrates how Illumina spatial technology helps researchers navigate these challenges with clarity. It also provides an overview of the spatial transcriptomics workflow, data analysis, and integration capabilities to support planning and implementation.

Current challenges

Different spatial transcriptomics methodologies offer trade-offs in resolution, gene coverage, and scalability.[7] In particular, a trade-off is commonly made between spatial resolution and transcriptomic breadth. Imaging-based approaches can achieve subcellular resolution but are generally limited to small gene panels and restricted tissue areas. Whole-transcriptome approaches enable broad, unbiased gene expression profiling across intact tissue sections, capturing spatial variation in gene activity and cellular diversity. However, this approach typically comes at the expense of single-cell or subcellular resolution, especially in densely packed microenvironments, making it less suited to applications requiring precise cellular localization. Sequencing depth and capture density must also be carefully balanced to preserve sensitivity across large tissue areas.[7-10]

Tissue coverage introduces additional complexity. Capturing large tissue areas is essential for resolving spatial gradients in complex organs such as brain, tumor, or developing systems. However, broader capture areas require higher sequencing depth to maintain transcript sensitivity, increasing cost and infrastructure demands and posing practical limits when scaling across samples, replicates, or timepoints.[11,12]

Additional limitations include:

- Technical variability introduced during tissue handling, staining, or RNA preservation
- The need for advanced tools for spatial deconvolution and integration with other omics modalities

Illumina spatial technology

The Illumina spatial transcriptomics methodology addresses many of the technical and practical barriers associated with whole-transcriptome spatial studies. It draws on core strengths in sequencing technology to deliver a balanced solution for resolution, scalability, and accessibility.

Resolving the resolution–coverage trade-off

The Illumina spatial solution combines whole-transcriptome breadth with high spatial resolution by using advanced flow cell technology adapted from Illumina sequencing systems. This technology provides a dense, non-patterned capture surface that maximizes transcript recovery while maintaining spatial fidelity across large tissue areas. By aligning transcript capture with standard imaging and Illumina sequencing, the workflow allows researchers to access broad transcriptomic profiles alongside localized gene expression patterns without compromising resolution or coverage breadth.

Managing sequencing depth and scaling costs

To support cost-effective implementation, the assay is optimized for use with high-throughput systems like the NovaSeq™ X Series. Researchers can capture extensive tissue regions on fewer slides, reducing per-sample costs. This configuration enables scalability from pilot studies to high-throughput cell atlas efforts.

Preserving spatial context with large-area tissue capture

The assay accommodates capture areas up to 750 mm², supporting full tissue sections or large anatomical regions. This broad field of view reduces sampling bias and preserves spatial heterogeneity, which is critical for tissues with structured or zonated architecture, such as brain, kidney, and tumor margins.

Simplifying spatial data analysis

To meet the demands of spatial deconvolution and multiomic integration, DRAGEN™ secondary analysis and Illumina Connected Multiomics work together to streamline these processes, transforming raw sequencing data into interpretable outputs and enabling flexible downstream analysis and visualizations

Workflow

The Illumina spatial transcriptomics workflow enables whole-transcriptome analysis within intact tissue architecture (Figure 1). It integrates established molecular biology techniques with spatial barcoding, histological imaging, and downstream computational analysis. The approach supports a range of study designs, from pilot experiments to high-throughput spatial profiling across large tissue cohorts.

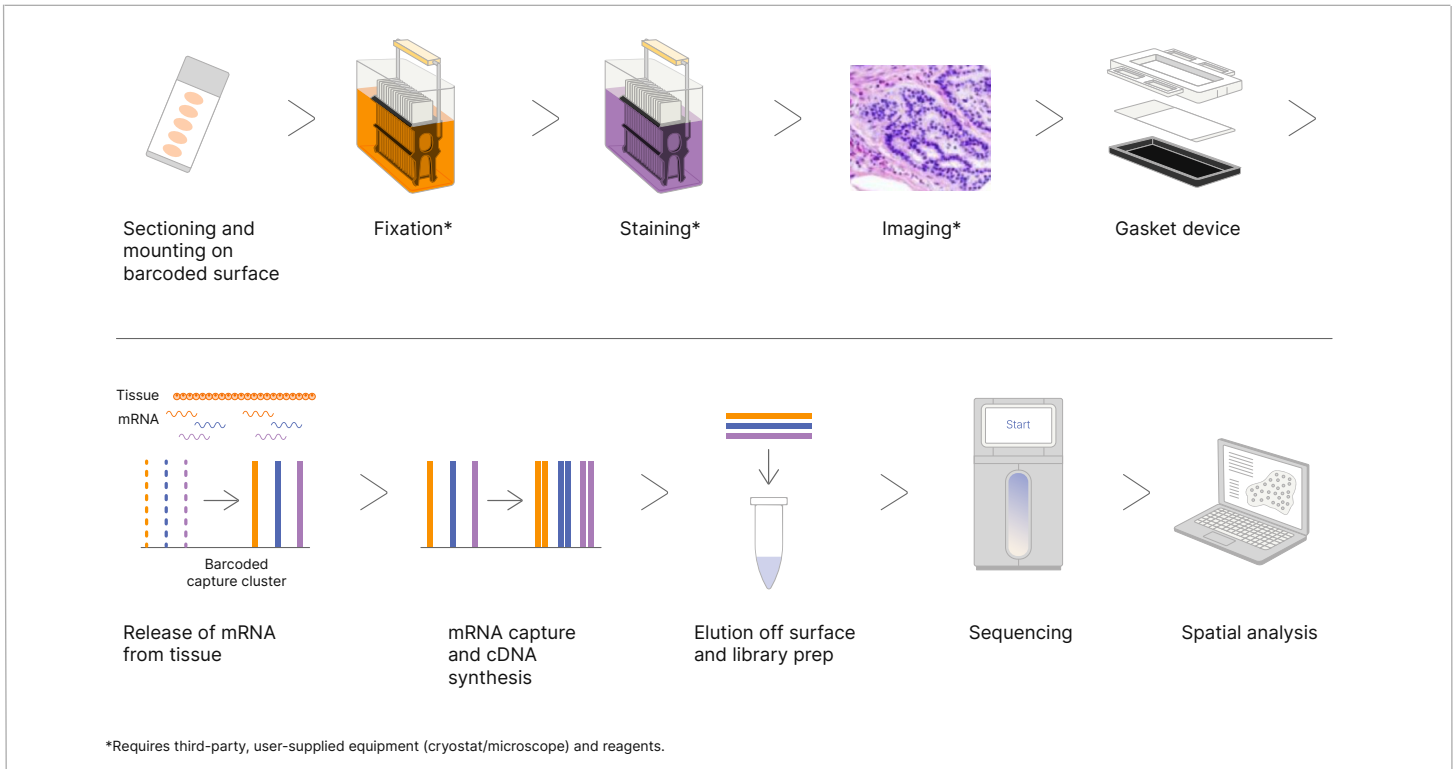

Figure 1: Overview of the Illumina spatial transcriptomics workflow from tissue sectioning through spatial analysis.

Sample preparation

Tissue sections are mounted onto custom capture slides that support a 750 mm² active area (50 × 15 mm) (Figure 2). The current assay is optimized for fresh-frozen tissue. Following cryosectioning (10 µm recommended), tissues are fixed, stained, and prepared for downstream histology and spatial barcode capture.

Histological imaging

After tissue fixation and hematoxylin and eosin (H&E) staining, high-resolution slide scanning is performed (Figure 3). The imaging step preserves cellular morphology and allows mapping of expression data onto tissue structure. Most standard brightfield slide scanners (20× to 40× magnification) are compatible. Captured images serve as the spatial reference during data visualization and cluster overlay.

mRNA capture and library preparation

After imaging, tissues are permeabilized to release RNA, which hybridizes to spatially barcoded oligonucleotides on the slide. Each capture oligo includes molecular identifiers and poly(dT) sequence for capturing polyadenylated RNA. Reverse transcription is performed *ex situ* on the slide, incorporating spatial barcodes into the resulting cDNA. The cDNA is then extracted and used to construct sequencing libraries through standard RNA-Seq library prep steps, including fragmentation, end repair, adapter ligation, and PCR amplification.

Sequencing

Illumina spatial technology is compatible with the Illumina NovaSeq X Series, NovaSeq 6000 System, and NextSeq™ 2000 System. Sequencing can be performed using 100-cycle flow cell reagent kits. Sequencing depth requirements vary by tissue complexity, resolution goals, and experiment type. High-throughput sequencing systems are recommended for multi-slide or large-cohort designs.

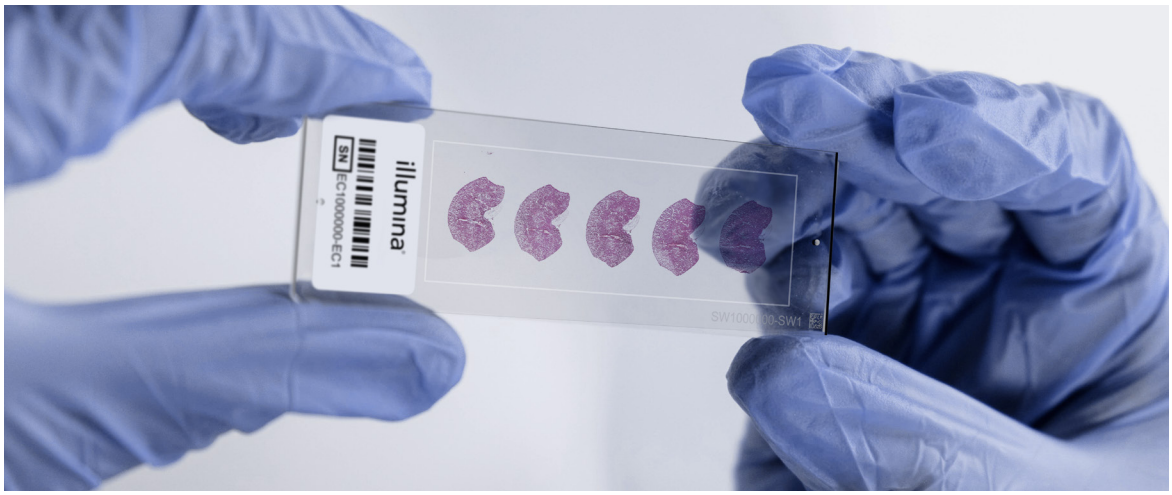

Figure 2: Tissue placement and capture slide layout
Fresh-frozen tissue sections (10 µm) are cryosectioned and mounted onto custom capture slides with a 750 mm² active area. Sections are then fixed, stained, and processed for histological imaging and spatial barcode capture.

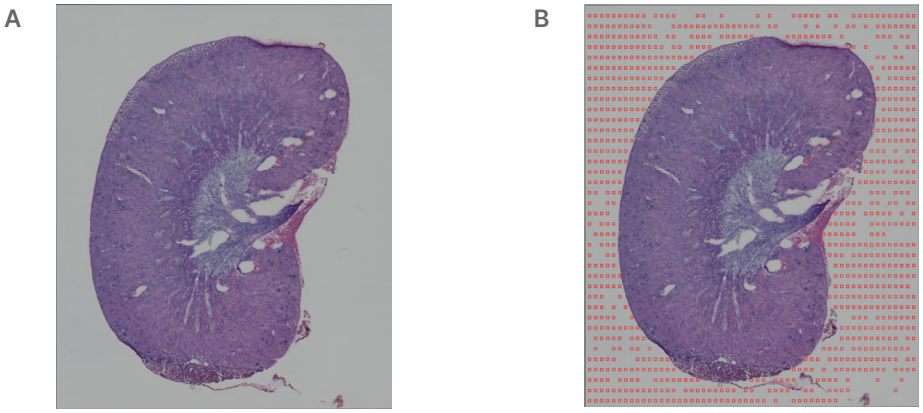

Figure 3: Illumina spatial technology enables broad coverage with high precision resolution
(A) H&E-stained section of mouse kidney highlighting key tissue architecture. (B) The same section with fiducial markers overlaid. Fiducial markers enable accurate alignment of gene expression measurements to the histological image, ensuring spatial data can be precisely localized within the tissue context.

Spatial Analysis

Raw sequencing data are processed using the DRAGEN spatial transcriptomics pipeline, which performs read alignment, molecular identifier deduplication, and generation of spatial feature barcode matrices. These matrices are imported into Illumina Connected Multiomics for downstream visualization* and tertiary analysis (Figure 4). Illumina Connected Multiomics supports spatial overlay of gene expression maps, clustering, cell segmentation, and other analysis (Figure 5). For advanced users, data can be exported to third-party bioinformatic tools such as cell2location[13] and Robust Cell Type Decomposition[14] (RCTD).

Use case examples

Spatial transcriptomics enables insights that are difficult to capture with conventional methods, particularly in studies involving complex tissues, disease states, or cellular heterogeneity. The following examples highlight how researchers have applied Illumina spatial technology to investigate biological mechanisms and inform study design across diverse research contexts.

Neurodevelopment

In a comparative neurodevelopmental study, spatial transcriptomics was used to examine pregnant versus virgin mouse brains, focusing on region-specific and cell type–specific gene expression changes.[15] This investigation revealed pronounced shifts in the expression of genes related to mood regulation, neuroendocrine signaling, and white matter plasticity. Critically, these findings could be obscured in bulk or single-cell approaches due to the regional confinement and layered architecture of the brain. The ability to visualize transcriptional activity across broad tissue areas provided critical insights into the spatial orchestration of gene programs during pregnancy.

Nephrology

Another prominent example involves a large-scale spatial map of the mouse kidney, presented at the 2025 AGBT General Meeting.[16] In this study, researchers profiled 12 million cells across 37 tissue sections using Illumina spatial technology. The resulting data captured regional organization, cell-specific remodeling, and fine-scale transcriptional differences that reflected functional compartmentalization within the kidney. The resolution and breadth of this map enabled the identification of spatially distinct expression patterns tied to nephron structure and epithelial-stromal interactions, supporting downstream analyses of renal physiology and disease.

* Illumina Connected Multiomics incorporates Partek™ Flow™ software, which supports spatial clustering, cell segmentation, and transcriptomic data visualization within an interactive analysis environment.

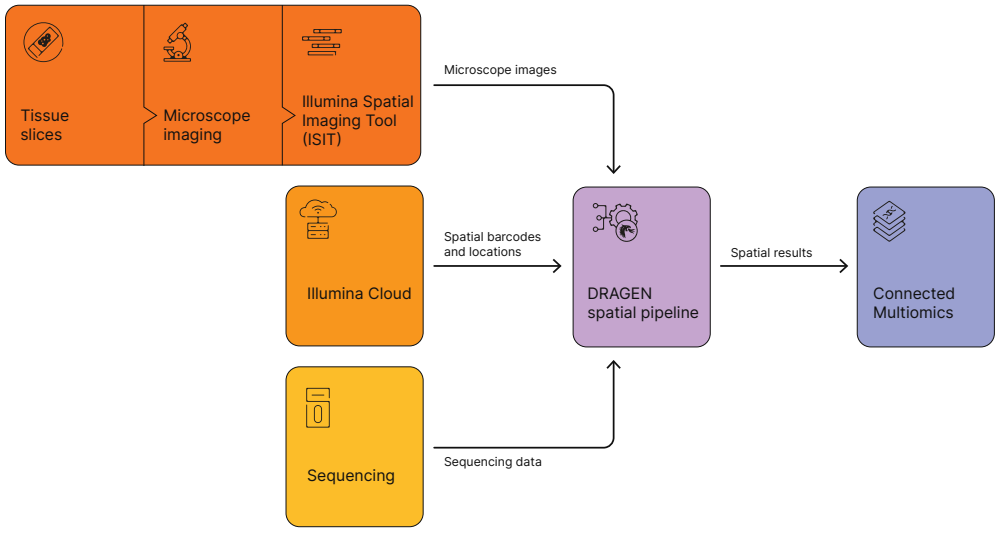

Figure 4: Illumina spatial transcriptomics software workflow

Tissue images and spatial barcodes are processed through the DRAGEN spatial pipeline and visualized in Illumina Connected Multiomics. The integrated workflow supports segmentation, clustering, and spatial mapping from raw imaging to transcriptomic analysis.

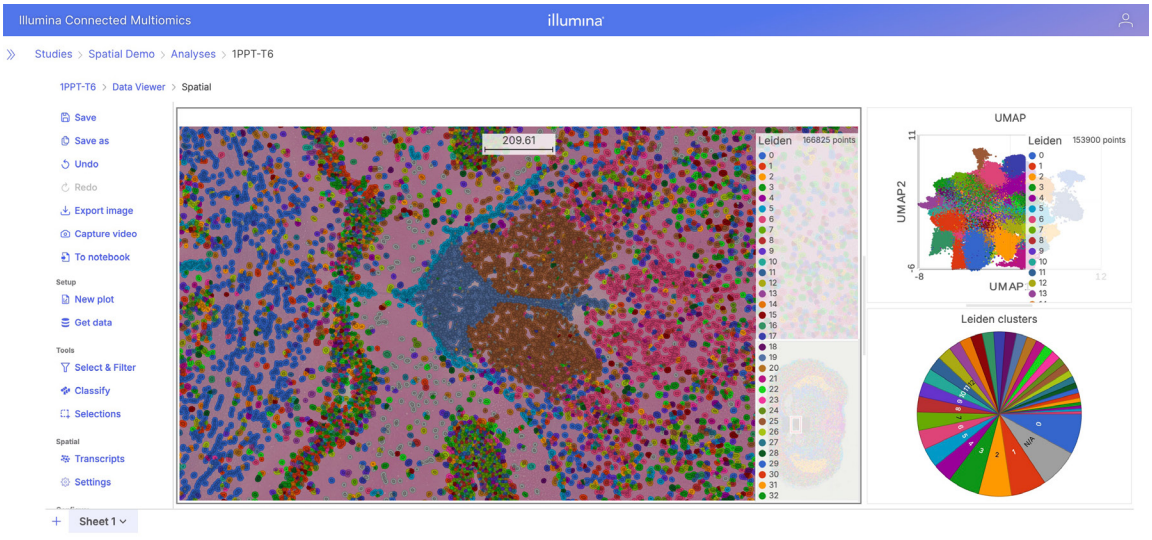

Figure 5: Illumina Connected Multiomics interactive analysis environment for interpreting and visualizing multiomic data

The intuitive software supports spatial and multiomic data exploration with tools for dimensionality reduction, clustering, expression heatmaps, spatial mapping, and cell segmentation. It enables flexible, interactive investigation of biological patterns across diverse samples and modalities.

Oncology

In oncology, spatial transcriptomics has proven especially powerful in characterizing intratumoral heterogeneity and resistance mechanisms.[17,18] A recent study of advanced prostate cancer demonstrated how spatial profiling using Illumina spatial technology revealed therapy-induced changes that could otherwise go undetected.[19] Researchers mapped over four million cells from prostate tumor samples, recovering more than 60,000 RNA features, including low-abundance transcripts and long noncoding RNAs. These included key prostate cancer markers such as *PCA3*, a gene that outperforms prostate-specific antigen (PSA) as a biomarker in early recurrence detection. Spatial data uncovered that androgen receptor–targeted therapy may promote a shift toward neuroendocrine cell states, which is critical for understanding resistance pathways that evade conventional treatments. Notably, these resistant states were distributed patchily across the tumor landscape and could be missed by single-point sampling or single-cell RNA sequencing (scRNA-Seq) due to their rarity and spatial confinement.

Future directions

As spatial transcriptomics becomes integral to systems biology, oncology, and developmental research, expectations around resolution, throughput, and multiomic integration continue to grow. Illumina is actively expanding the capabilities of Illumina spatial technology in response to researcher input and ongoing collaborations.

Current areas of innovation include:

- **Increased spatial resolution**: Illumina is developing a next-generation methodology offering four times greater resolution than current *ex situ* versions. This improvement will allow researchers to resolve fine tissue architecture, localize rare or transitioning cell states, and improve statistical power in spatial analyses[6]
- **Expanded sample type compatibility**: While the current workflow is optimized for fresh-frozen samples, support for formalin-fixed, paraffin embedded (FFPE) tissue is actively in development. This development will facilitate access to archival specimens and extend applications in retrospective cohort studies[20]
- **Improved transcript recovery**: Updates to capture chemistry and library prep are aimed at increasing sensitivity, particularly for low-abundance transcripts and partially degraded RNA. These improvements will support deeper profiling and more accurate detection of regulatory and noncoding elements[21]
- **Advanced bioinformatics integration**: Continued development of DRAGEN spatial analysis and Illumina Connected Multiomics is focused on streamlining analysis. New features include built-in cell segmentation, spatially informed clustering, and tools for coregistering spatial data with histology and external modalities[22]

Setup, integration, and access

Illumina spatial technology is designed for streamlined adoption in research environments, with minimal instrumentation requirements and integration into existing histopathology and Illumina sequencing workflows.

- **System setup and integration**: The spatial capture workflow is based on a slide-mounted array compatible with standard cryosectioning methods and requires a brightfield microscope for histological imaging. Data are processed through the cloud-based Connected Software Suite, including DRAGEN spatial analysis and Illumina Connected Multiomics
- **Sequencing system compatibility**: Libraries generated from the spatial workflow are compatible with high-throughput Illumina sequencing systems, including the NovaSeq X Series and NovaSeq 6000 System, and the NextSeq 2000 System for moderate-throughput needs
- **Product access and availability**: The initial version is optimized for fresh-frozen tissue with commercial availability expected in the first half of 2026. A version supporting analysis of FFPE tissue is in development, with future plans aimed at expanding capability and broadening access

Summary

Illumina spatial technology offers a high-resolution, sequencing-based approach for whole-transcriptome analysis in intact tissue. It provides broad transcriptomic coverage across large tissue areas, a key advantage over targeted or imaging-based methods. Ongoing technological development and workflow integration support a range of research goals, with tools and resources that guide experimental design, streamline data generation, and help uncover spatial patterns in gene expression, enabling researchers to move from complexity to clarity with confidence.

Learn more →

Introduction to Spatial Transcriptomics

Advances in Genome Biology and Technology (AGBT) 2025 poster presentation

Videos and webinars

Illumina Spatial Technology – Whole-Transcriptome Profiling of Large Tissue Areas

How to analyze spatial data in Illumina Connected Multiomics

High Resolution Spatial Sequencing Reveals Changes in Prostate Tumor Environment

Characterizing alveolar dysregulation in pulmonary fibrosis using Illumina Spatial Technology

Cell atlasing human intestine using Illumina spatial technology

Web resources

Analyzing spatial transcriptomic data in Partek Flow

Illumina Publications

Seq-Scope: repurposing Illumina sequencing flow cells for high-resolution spatial transcriptomics[23]

Spatial transcriptomics identifies molecular niche dysregulation associated with distal lung remodeling in pulmonary fibrosis[24]

Comparison of the Illumina NextSeq 2000 and GeneMind Genolab M sequencing platforms for spatial transcriptomics[25]

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