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DATA SHEET

Illumina StrataMap™ Spatial

Whole-transcriptome spatial profiling at cellular resolution

Profile full tissue sections on a single slide without limiting analysis to selected regions

Resolve gene expression at cellular resolution across tissue, without predefined panels

Integrate spatial analysis with Illumina sequencing systems and analysis tools

Introduction

Spatial transcriptomics profiles gene expression within intact tissue, revealing how biological activity is organized across cells and tissue structures. By preserving spatial context, it connects expression patterns to location, making it possible to examine cellular interactions, tissue organization, and disease biology directly within the native tissue environment. This combined molecular and spatial information supports applications such as tissue atlasing, tumor microenvironment characterization, and cell-type mapping.[1-3]

Spatial technologies have expanded the ability to profile gene expression *in situ*; however, many approaches still require balancing transcriptome coverage, spatial resolution, and tissue area. Imaging-based methods can achieve high spatial resolution but are typically limited to predefined gene panels, restricting discovery. In addition, imaging-based approaches require access to expensive, specialized instruments. Sequencing-based approaches provide broader, unbiased profiling but may be constrained by capture area or scalability, and often involve trade-offs in spatial resolution. These limitations require researchers to compromise between resolution, coverage, and scale within a single experiment.[4-6]

Illumina StrataMap Spatial brings together whole-transcriptome analysis through next-generation sequencing (NGS), cellular-resolution mapping, and large-area tissue profiling (Figure 1). Gene expression can now be examined across entire tissue sections while retaining tissue architecture and spatial organization, revealing cellular organization and biological structure that may be missed when resolution, transcriptome coverage, or tissue context are limited.

By capturing genome-wide expression directly from tissue, StrataMap Spatial supports hypothesis-free spatial discovery, allowing analysis of gene expression across diverse cell populations and tissue regions within a single experiment.

With StrataMap Spatial, spatial gene expression profiling is integrated with standard histopathology techniques and existing Illumina sequencing systems. This allows laboratories to expand into spatial transcriptomics without requiring specialized instrumentation, opening new opportunities for discovery.

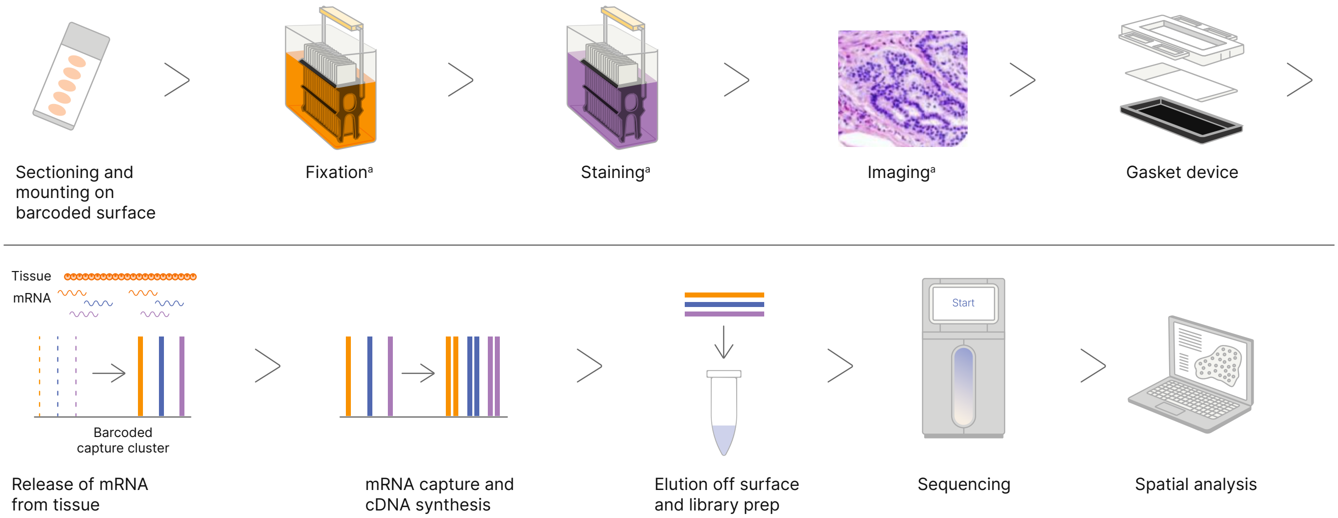

Figure 1: Illumina StrataMap Spatial integrates whole-transcriptome sequencing from tissue to analysis

Fresh-frozen tissue sections undergo histology workflow steps, including fixation, staining, and imaging to preserve morphology and establish a spatial reference. StrataMap Spatial captures RNA across continuous 1 µm surface features through poly(A)-based hybridization and generates sequencing-ready libraries. Sequencing data are processed with the DRAGEN spatial transcriptomics pipeline to generate spatially resolved gene expression matrices for interpretation and visualization in Illumina Connected Multiomics.

a. Requires third-party, user-supplied equipment (cryostat/microscope) and reagents.

Integrated spatial transcriptomics with Illumina NGS

StrataMap Spatial combines histology, spatial barcoding, and whole-transcriptome sequencing in an NGS-based workflow to generate spatially resolved gene expression data from intact tissue sections (Figure 1). The workflow integrates with standard histology processes, allowing incorporation into established laboratory practices. The workflow also integrates with existing high-throughput Illumina sequencing systems and Illumina data analysis and visualization tools, allowing adoption without the need for specialized spatial instrumentation.

Step 1: Tissue preparation and imaging

- Fresh-frozen tissues are cryosectioned and mounted onto a barcoded capture surface that accommodates full tissue sections or multiple sections or samples
- Tissue is fixed, stained, and imaged to preserve morphology and establish a spatial reference

Step 2: RNA capture and library preparation

- Tissue is permeabilized to release RNA while preserving spatial organization
- Poly(A)-based capture hybridizes RNA to spatially barcoded oligonucleotides across continuous 1 µm surface features
- Spatially indexed cDNA is generated, linking transcripts to specific tissue locations
- cDNA is recovered and prepared as sequencing-ready libraries using standard workflows

Step 3: Sequencing on Illumina systems

- Libraries are sequenced on Illumina high-throughput sequencing systems (eg, NovaSeq™ or NextSeq™ sequencing systems)
- Sequencing depth is adjusted based on tissue complexity and study design

Step 4: Data analysis

- Sequencing data are processed using the DRAGEN™ spatial transcriptomics pipeline to generate spatially resolved gene expression matrices
- Data are interpreted and visualized in Illumina Connected Multiomics with tools for clustering, cell-type identification, exploration of gene expression within tissue context, and more

Whole-transcriptome analysis for unbiased discovery

Unbiased spatial profiling of gene expression requires access to the full transcriptome rather than predefined gene sets. StrataMap Spatial uses sequencing-based capture of poly(A) RNA for genome-wide interrogation of transcripts directly from tissue, supporting discovery-driven analysis across diverse biological contexts.

Whole-transcriptome spatial profiling enables characterization of cellular organization and biological interactions across complex tissue environments. In high-grade invasive ductal carcinoma (IDC) tissue, StrataMap Spatial resolved distinct expression patterns associated with proliferating tumor cells, stromal fibroblasts, and macrophage populations across the tumor microenvironment (Figure 2).

Compared with targeted, probe-based panel approaches, whole-transcriptome spatial profiling provides broader detection of differential gene expression without restricting analysis to predefined targets. In IDC tissue, StrataMap Spatial identified differentially expressed biomarkers not captured by commonly used 5000- and 18,000-gene panels, expanding opportunities for unbiased and comprehensive discovery (Figure 3).

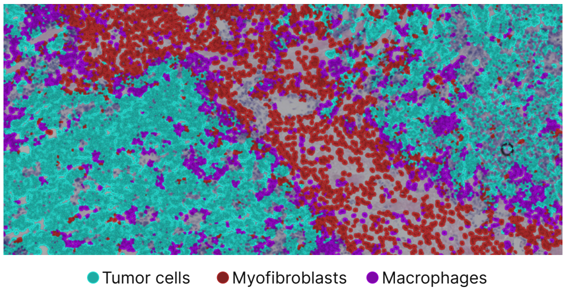

Figure 2: StrataMap Spatial reveals cellular diversity within the tumor microenvironment

Whole-transcriptome spatial profiling of a high-grade invasive ductal carcinoma (IDC) sample resolves spatially organized gene expression patterns across the tumor microenvironment. The image highlights expansion of proliferating tumor cells (aqua), cancer-associated fibroblasts within the desmoplastic stroma (red), and pro-tumorigenic macrophages (purple) located at the tumor-stromal interface and dispersed throughout the tumor tissue.

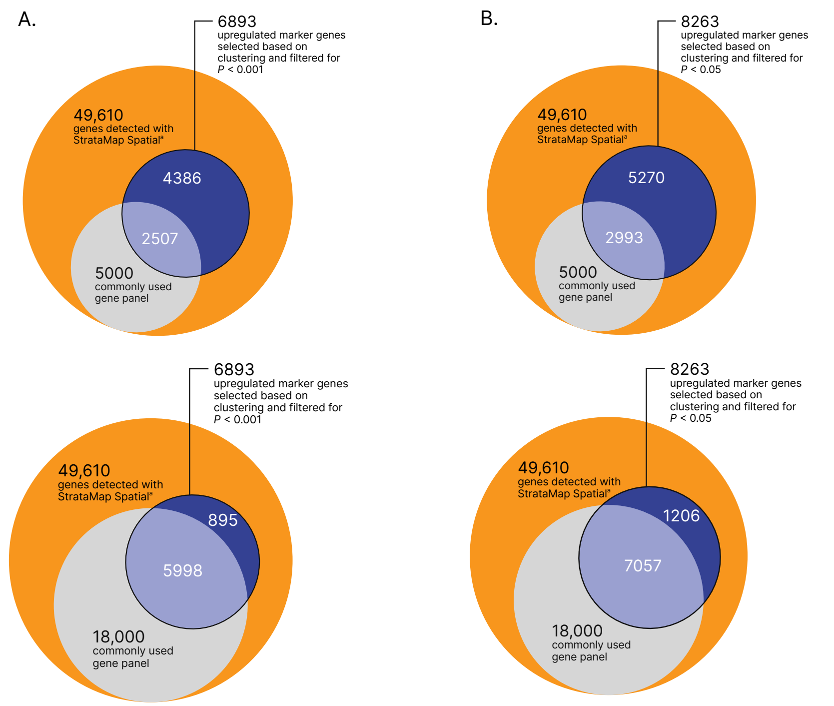

Figure 3: Whole-transcriptome spatial profiling expands detection of differential gene expression in invasive ductal carcinoma (IDC)

The Venn diagrams illustrate comparisons of the number of genes identified by whole-transcriptome spatial profiling using StrataMap Spatial and targeted panel approaches using 5000 (top) and 18,000 (bottom) commonly used gene panels. (A) Comparisons using a significance threshold of $P < 0.001$ show that 63.6% and 13.0% of upregulated biomarkers identified by StrataMap Spatial were not captured by the 5000 and 18,000 targeted panels, respectively. (B) Comparisons using a significance threshold of $P < 0.05$ show that 63.8% and 14.6% of upregulated biomarkers identified by StrataMap Spatial were not captured by the 5000 and 18,000 targeted panels, respectively.

a. Based on GRCh38 Genome, Gencode version 41.[7]

High-resolution mapping for cellular characterization

In StrataMap Spatial, cellular-resolution spatial mapping is achieved through continuous 1 µm surface features that preserve spatial detail without signal aggregation across larger regions. This resolution reflects the integration of the capture surface architecture, spatially encoded transcript capture, and computational analysis. Fiducial markers on the slide surface ensure accurate alignment of histology images with sequencing-derived spatial data for precise registration of tissue morphology and gene expression. Integrated cell segmentation within the analysis pipeline further refines spatial assignment, enabling gene expression to be localized at cellular resolution.

The high spatial resolution of StrataMap Spatial allows visualization of organized cell populations alongside localized gene expression patterns within the same section, as shown in ductal carcinoma *in situ* (DCIS) tissue (Figure 4). Cell type classification and transcript-level heatmaps aid interpretation of cellular composition and spatial organization across complex tissue regions. This level of spatial detail resolves boundaries between

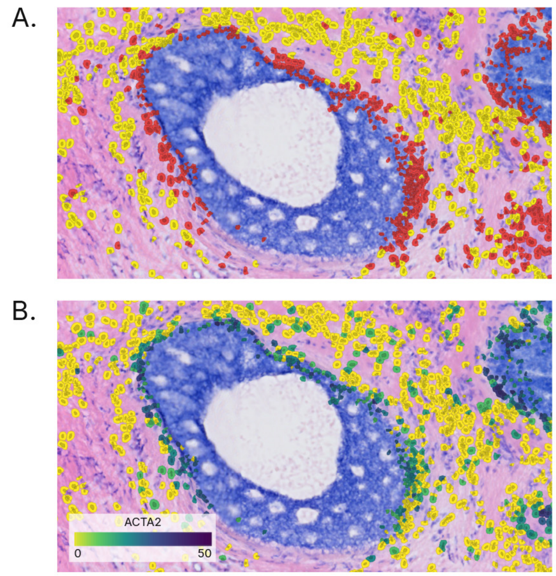

Figure 4: Cellular-resolution spatial mapping reveals cell populations and localized gene expression

Localized gene expression patterns mapped at cellular resolution are shown in ductal carcinoma *in situ* (DCIS) tissue. (A) Cell-type visualization shows spatially resolved distribution of fibroblasts (yellow) in normal stroma and myofibroblasts (red) at the tumor–stromal interface. (B) Spatial expression of *ACTA2*, a biomarker of myofibroblasts, across the same tissue section.

adjacent cell populations and captures localized expression patterns that may be obscured when signals are averaged across larger regions.

High sensitivity for transcript detection

Spatial transcriptomic profiling of breast cancer tissue samples spanning DCIS, grade 1 IDC, and grade 2 and grade 3 IDC using StrataMap Spatial shows consistent detection of transcripts across varying levels of tissue complexity (Figure 5). Histological images aligned with spatial expression heatmaps link tissue morphology with gene expression patterns across disease states and grades. Quantitative analysis shows increasing numbers of unique molecules and genes detected per cell with increasing sequencing depth (Figure 6). Across all tissue types and grades, gene and molecule detection scales predictably with read depth, indicating efficient capture and consistent performance across tissues with differing cellular composition and RNA content. This sensitivity supports detection of both abundant and lower-expressed transcripts while resolving heterogeneous cell populations within complex tissue environments.

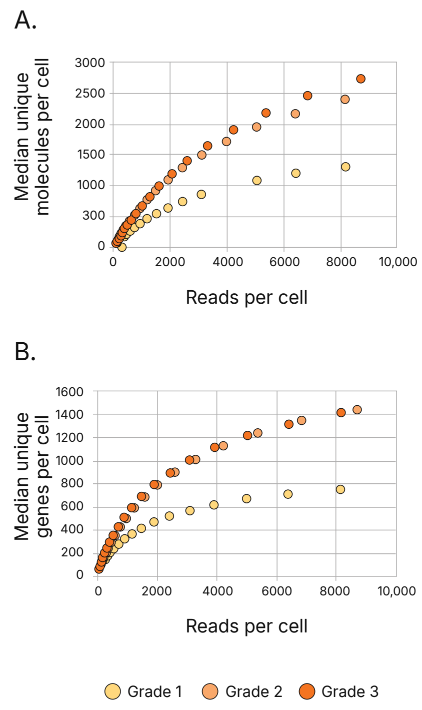

Figure 6: Consistent transcript detection across sequencing depths

Across ductal carcinoma *in situ* (DCIS) samples spanning grades 1–3, unique molecules and genes detected per cell increase with sequencing depth, demonstrating robust and consistent transcript detection performance across tissue states.

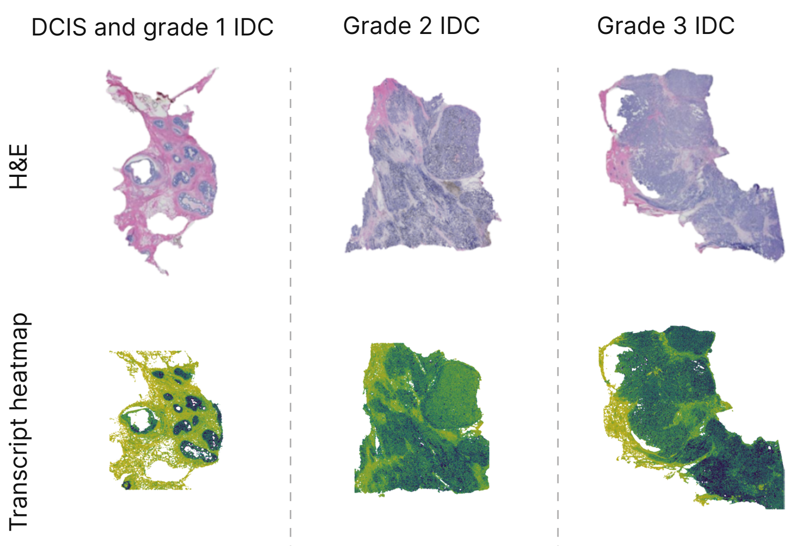

Figure 5: Sensitive transcript detection across breast cancer tissue states

Spatial transcriptomic profiling of breast cancer tissue samples, including ductal carcinoma *in situ* (DCIS), grade 1 invasive ductal carcinoma (IDC), and grade 2 and grade 3 IDC demonstrates consistent transcript detection across varying tissue states. Hematoxylin and eosin (H&E)-stained histological images aligned with images showing spatial gene expression demonstrate concordance between tissue morphology and spatial gene expression patterns across disease states and grades.

Large capture area for full tissue profiling

StrataMap Spatial uses a 750 mm^2 capture area that accommodates full tissue sections and multiple sections or samples on a single slide. For example, multiple mouse heart tissue sections can be placed across the capture area, enabling spatial transcriptomic profiling across all tissue sections while preserving spatial context (Figure 7).

As spatial gene expression patterns are maintained across large tissue areas, analysis of cellular heterogeneity and regional variation can be performed within a single experiment. The expanded capture area increases experimental flexibility for studies requiring high sample throughput or broad tissue coverage, including large-scale tissue atlasing and spatial reconstruction workflows.

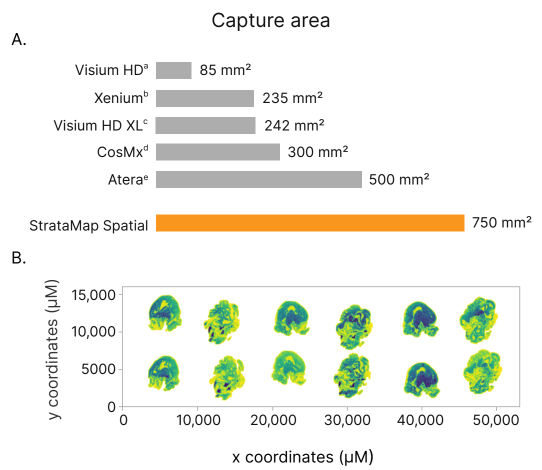

Figure 7: Large-format spatial capture supports multi-sample and full tissue profiling
StrataMap Spatial provides a 750 mm^2 capture area for large-format spatial profiling, providing broader tissue coverage and reducing the need for tissue selection. (A) Comparison of spatial capture areas across spatial transcriptomics platforms. (B) Transcriptional changes between virgin and pregnant mouse heart tissue sections. From left to right, tissue sections alternate between virgin and pregnant mice; the bottom and top rows represent replicates.

a. Based on a capture area of 6.5 × 6.5 mm, 2 per slide.[8]
b. Available sample positioning area of 235 mm^2.[9]
c. Based on a capture area of 11 × 11 mm, 2 per slide.[8]
d. Flexible scan area of up to 300 mm^2 per slide.[10]
e. Imageable area per slide.[11]

Integrated analysis for cell-state identification

Interpreting spatial transcriptomic data requires integration of gene expression, spatial context, and cell-type identity within a unified analytical environment. Illumina Connected Multiomics provides a cloud-based platform for spatial data integration, visualization, and interpretation, linking sequencing output to biologically meaningful insights.

Illumina Connected Multiomics supports comparison of spatial domains, cell-type annotations, and transcriptional patterns within the same tissue section (Figure 8). Relationships between tissue organization, cell populations, and gene expression can be explored through linked analytical views, including clustering, dimensionality reduction, and spatial transcript mapping.

The key capabilities of Illumina Connected Multiomics include:

- Integration of spatial data sets for joint analysis
- Clustering and cell-type identification
- Visualization of gene expression within tissue context

The interactive graphical user interface allows direct exploration of spatial transcriptomic data, reducing reliance on specialized bioinformatics workflows. This integrated approach streamlines interpretation of complex data sets without requiring multiple tools or custom pipelines.

Species-agnostic workflow for diverse applications

By using poly(A)-based capture, StrataMap Spatial directly interrogates transcripts from tissue without reliance on species-specific probes. This approach enables spatial gene expression profiling across a wide range of eukaryotic species without assay redesign or predefined probe sets.

StrataMap Spatial captures gene expression across multiple sample types from both model and nonmodel organisms (Table 1), supporting applications ranging from comparative biology to xenotransplant research. This broad species compatibility removes the need for bespoke probe design or limiting analysis to species with available probe panels, extending spatial transcriptomics beyond commonly studied organisms.

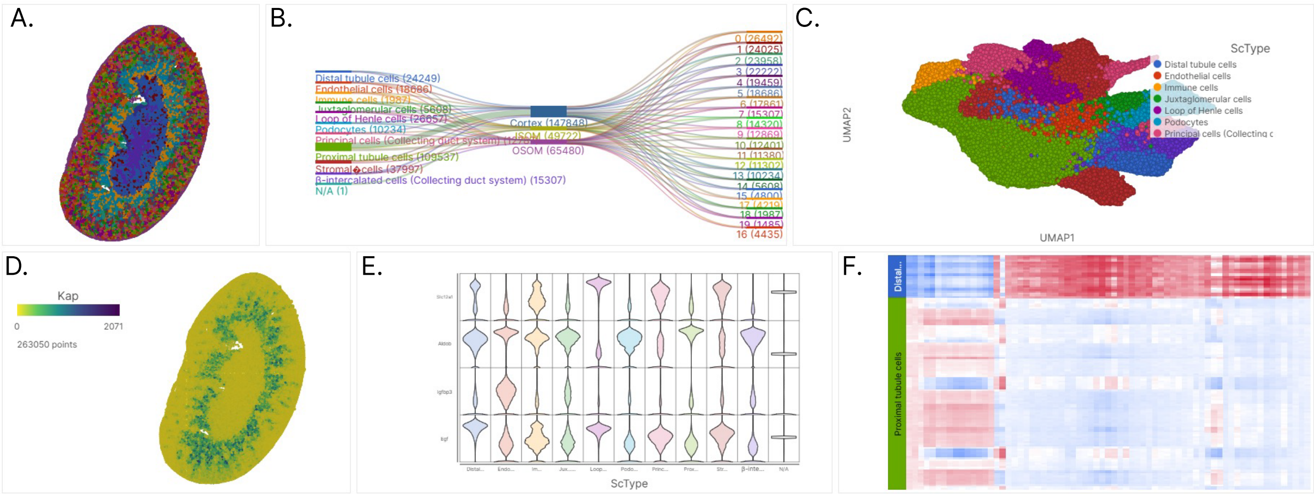

Figure 8: Illumina Connected Multiomics supports integrated spatial visualization and transcriptomic analysis

Spatial analysis outputs generated in Illumina Connected Multiomics from a mouse kidney sample. (A) Spatial domains across the tissue section. (B) Sankey diagram showing relationships between cell type annotations, manually annotated tissue regions, and spatial domains. (C) UMAP plot colored by cell type. (D) Spatial transcript heatmap showing localization of *KAP* expression. (E) Violin plots displaying marker gene expression across cell types. (F) Heatmap of gene expression patterns across cell types. The integrated analysis environment supports spatial mapping, clustering, dimensionality reduction, and visualization of transcriptomic patterns across tissue regions and cell populations.

Table 1: Species-agnostic spatial transcriptomic profiling with StrataMap Spatial

Example organisms profiled[a]	
Human	Pig
Mouse	Marmoset[b]
Monkey (Rhesus)	Corn[b]
Rhino[b]	Fruit fly[b]

a. The examples shown are representative and do not constitute a comprehensive list of supported species or applications.
b. Organisms were tested by early access collaborators.

Summary

Illumina StrataMap Spatial delivers sequencing-based, whole-transcriptome spatial profiling across large tissue areas, allowing gene expression to be examined within intact tissue architecture. Full tissue sections can be profiled on a single slide, with cellular-resolution mapping that links expression patterns to tissue structure and organization.

At its core, StrataMap Spatial integrates a large capture area, continuous 1 µm surface features, and poly(A)-based RNA capture to support sensitive, genome-wide analysis at cellular resolution. By capturing genome-wide expression without predefined panels and eliminating trade-offs between resolution, coverage, and scale, StrataMap Spatial provides a more complete view of tissue biology.

Laboratories with existing high-throughput Illumina sequencing systems can adopt the workflow without dedicated instrumentation, supporting routine integration of spatial transcriptomics into research workflows.

Learn more →

Illumina StrataMap Spatial

Illumina Connected Multiomics

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