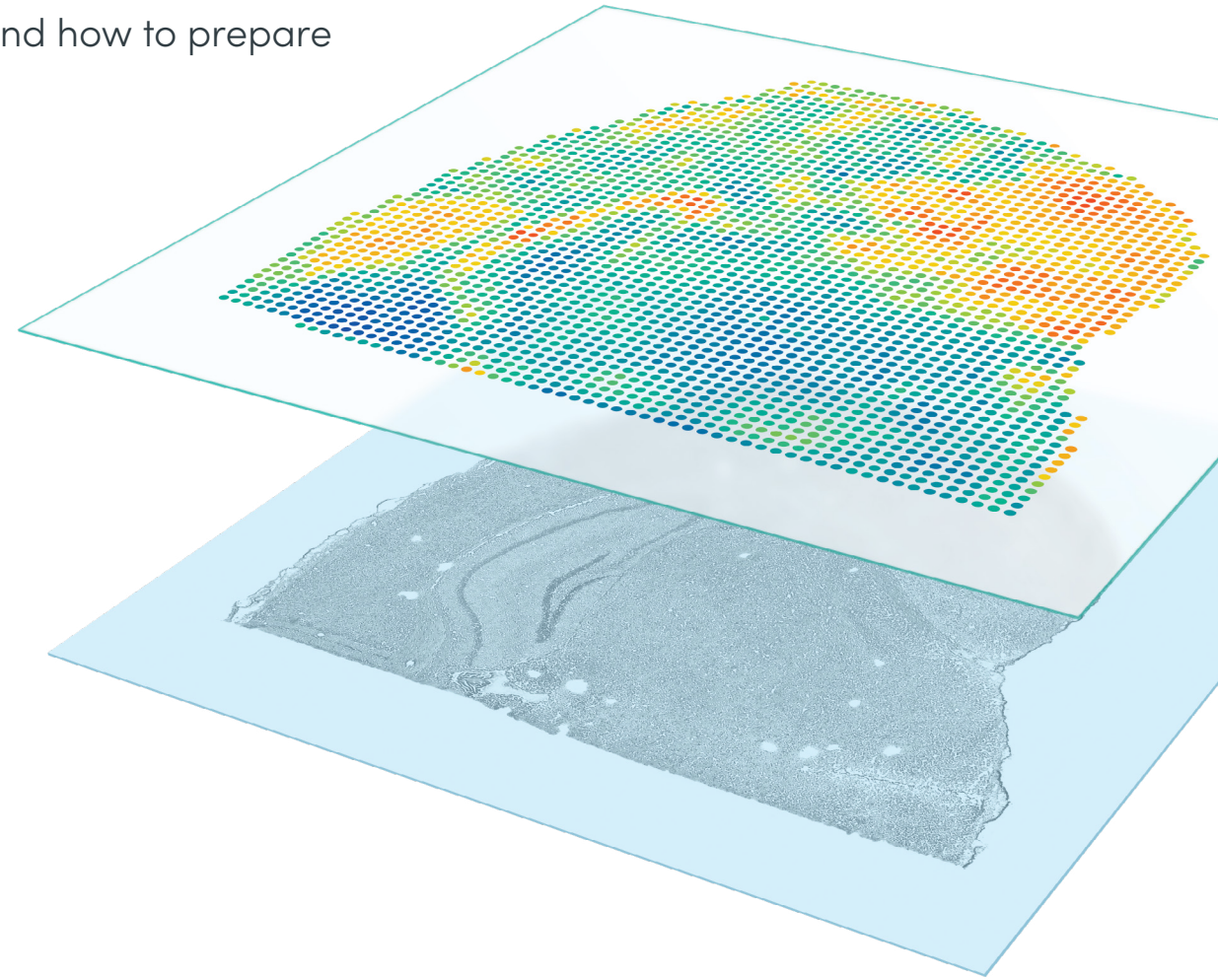
10x GENOMICS

Inside Visium Spatial Technology

Introduction and how to prepare



10xgenomics.com/spatial-gene-expression

The power of two: Mapping and measuring gene expression

Visium Spatial Gene Expression incorporates unbiased total mRNA analysis for intact tissues sections with morphological context. Bringing these two complementary methods together to study tissue offers a previously inaccessible view of tissue biology.

More than a tissue slide

Map the spatial gene expression of complex tissue samples with slides that utilize poly(A) capture and novel spatial barcoding technology for library preparation.

Visium Spatial Gene Expression slides are powered by spatially barcoded mRNA-binding oligonucleotides (Figure 1). To capture gene expression information, mRNA is released from processed tissue sections allowing the mRNA bind to capture oligos from a proximal location on the tissue.

Reverse transcription occurs while the tissue is still in place, generating a cDNA library that incorporates the spatial barcodes and preserves spatial information. Barcoded cDNA libraries are mapped back to a specific spot on the Capture Area. This gene expression data is subsequently layered over a high-resolution microscope image of the tissue section (Figure 2), making it possible to visualize the expression of any mRNA, or combination of mRNAs, within the morphology of the tissue in a spatially resolved manner.

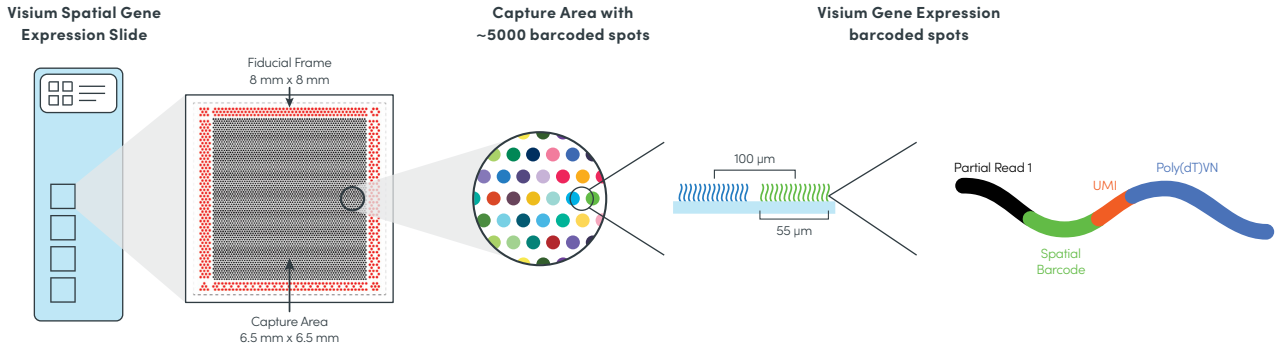


Figure 1. Composition of Visium Spatial Gene Expression Slide. Each slide can contain either two or four Capture Areas with approximately 5000 barcoded spots, containing millions of spatially barcoded capture oligos. Released tissue mRNA binds to these oligos enabling capture of gene expression information.

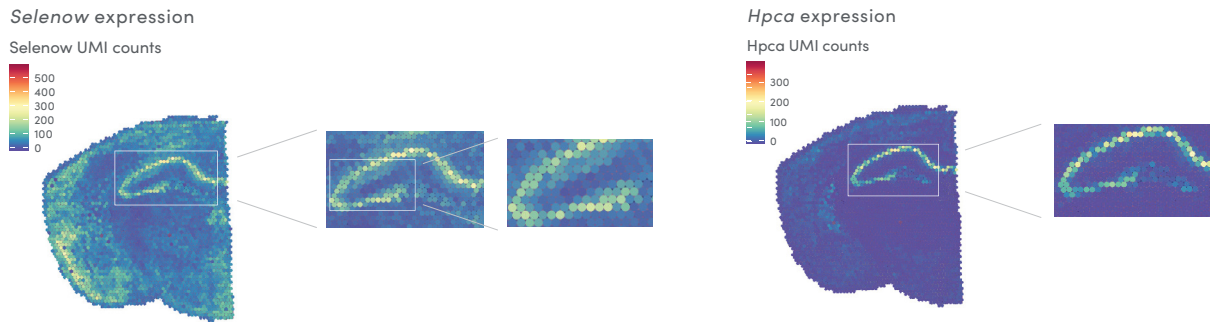


Figure 2. In situ gene expression patterns. This is a coronal mouse brain section with overlaid spatial gene expression information. The spots correspond to localized mRNA of *Selenow* and *Hpca*, both known to have predominant hippocampal expression.

Efficient workflow: Utilize standard laboratory methods

The Visium Spatial Gene Expression workflow makes it easy to implement spatial transcriptomics technology into standard tissue sectioning and staining methods.

Streamlined, ready-to-use assay

Prepare your sample
Embed, section, and place fresh-frozen tissue onto a Capture Area of the gene expression slide. Each Capture Area has thousands of barcoded spots, each containing millions of capture oligonucleotides with spatial barcodes unique to that spot.

Stain and image the tissue
Utilize standard fixation and staining techniques, including hematoxylin and eosin (H&E) staining, to visualize tissue sections on slides using a brightfield microscope and immunofluorescence staining to visualize protein detection in tissue sections on slides using a fluorescence microscope.

Permeabilize tissue and construct library
Permeabilize the tissue to release mRNA from the cells, which binds to the spatially barcoded oligonucleotides present on the spots. A reverse transcription reaction produces cDNA from the captured mRNA. The barcoded cDNA is then pooled for downstream processing to complete a sequencing-ready library.

Sequence
The resulting 10x barcoded library is compatible with standard NGS short-read sequencing on Illumina sequencers for massive transcriptional profiling of entire tissue sections.

Analyze and visualize your data
Use our Space Ranger analysis software to process your spatial gene expression data and interactively explore the results with our Loupe Browser visualization software.

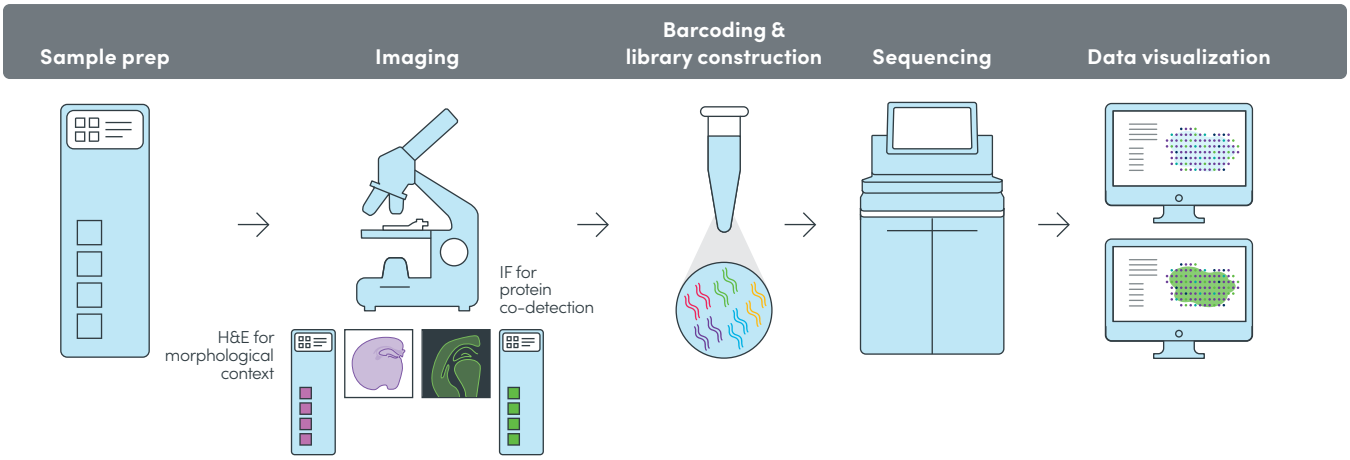


Figure 3. Workflow diagram for the Visium Spatial Gene Expression. Fresh-frozen tissue is sectioned, placed onto a library preparation slide, then fixed, stained with either H&E or immunofluorescence (IF), and imaged, followed by spatial barcoding and library construction. The libraries are then sequenced and data visualized. The workflow from sample to sequencing-ready library prep can be completed in < 1 day.

Library construction: How it works

Visium Spatial Gene Expression uses standard tissue analysis methods and tools, making it easily adoptable within existing lab infrastructures. Complete your entire spatial gene expression library construction in a day, or pause at strategic points and continue later.

Stepwise library construction

Fresh-frozen tissue samples are sectioned and placed in the four Capture Areas on the Visium Spatial Gene Expression slide.

Utilizing standard fixation and staining techniques, including H&E or immunofluorescence staining, tissue sections are visualized on slides. Microscope recommendations can be found under "Preparing my lab" on page 6.

The tissue is then permeabilized to release mRNA from the cells. mRNA binds with spatially barcoded oligonucleotides present on the spots. A reverse transcription reaction produces cDNA from captured mRNA. The second strand of cDNA is then synthesized and denatured. Note, if you're using a tissue for the first time with the Visium solution, you will need to perform tissue optimization beforehand. The barcoded cDNA is then pooled for downstream processing, library preparation, and cDNA amplification. Subsequent steps are taken to fragment and process cDNA to complete a sequencing-ready library. This is followed by a final sample index PCR.

The Visium Spatial Gene Expression library is sequenced using standard short-read sequencers, and data is processed and visualized using 10x Genomics software: Space Ranger Analysis Pipelines and Loupe Browser.

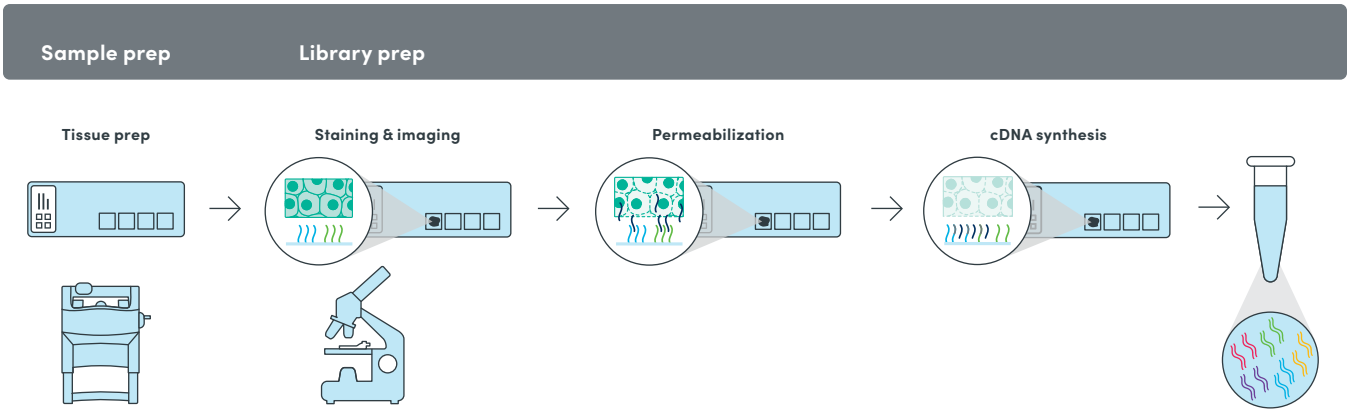


Figure 4. Constructing a sequencing library with Visium Spatial Gene Expression. Fresh-frozen tissue is sectioned, placed onto a library preparation slide, then fixed, stained with either H&E or immunofluorescence, and permeabilized, releasing mRNA which binds to spatially barcoded capture probes, allowing for the capture of gene expression information. cDNA is then synthesized from captured mRNA, and sequencing libraries prepared.

Gain a new perspective: Simultaneous gene and protein spatial profiling

Visium Spatial Gene Expression with Immunofluorescence lets you combine immunofluorescence protein detection and spatial gene expression in the same tissue section alongside histological analysis, providing a new perspective on tissue complexity.

Spatially resolve multiple analytes

Visualize spatial patterns of gene expression together with protein detection by immunofluorescence (IF) on the same tissue section. The combined Visium Spatial Gene Expression with Immunofluorescence workflow allows the simple incorporation of spatial transcriptomics into standard tissue sectioning and IF staining methods. The combined workflow is simple, streamlined, and readily adoptable using your current IF antibodies (Figure 5). Get the most out of precious samples by combining protein, total mRNA, and histological analyses on the same tissue section: discover new tissue biomarkers and drug targets with cell-type specificity; characterize immune cells and engineered T cells and their activity states in the tissue microenvironment; interrogate gene expression profiles in "hot" and "cold" tumor regions; identify the cellular source of secreted molecules; and spatially map neuronal subtypes and their gene expression profiles.

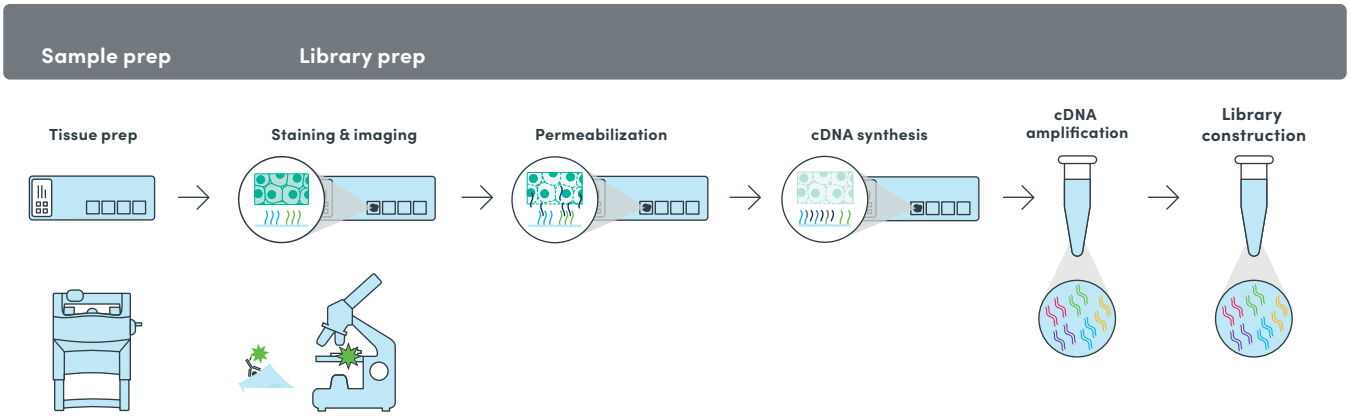


Figure 5. Workflow diagram for Visium Spatial Gene Expression with Immunofluorescence. Fresh-frozen tissue is sectioned, placed onto a library preparation slide, fixed, stained with IF, and imaged under a fluorescent microscope. The tissue is then permeabilized, releasing mRNA which binds to spatially barcoded capture probes. cDNA is synthesized from captured mRNA and amplified, and sequencing libraries prepared.

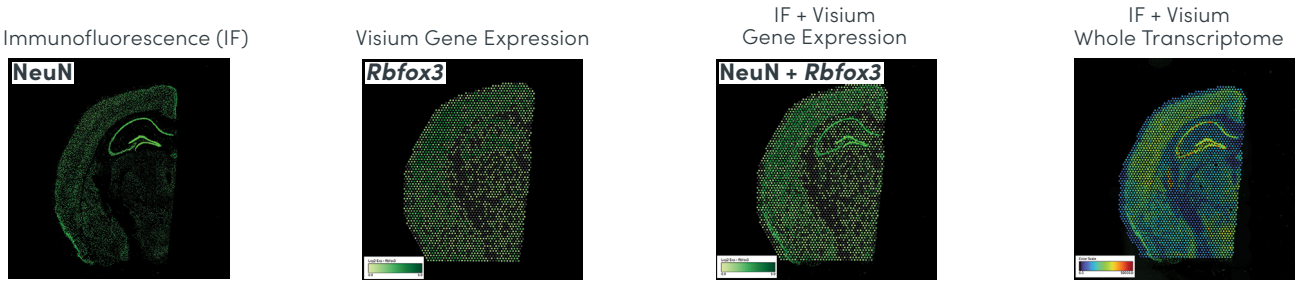


Figure 6. Co-detection of protein and RNA using Visium Spatial Gene Expression with Immunofluorescence. A coronal mouse brain section was stained for NeuN by IF, imaged, and processed through the Visium Spatial Gene Expression workflow. Shown left to right: NeuN IF, Visium Spatial mRNA expression of *Rbfox3* (gene encoding NeuN), overlay of NeuN IF image and Visium Spatial *Rbfox3* mRNA data, and NeuN IF image overlaid with total UMI count data obtained by Visium.

Targeted gene expression: Focus on the genes that matter most

Visium Targeted Gene Expression combines crucial spatial insights with the ease and breadth of targeted panels. Accelerate your understanding of human health and disease with a more refined picture of the biology captured on a tissue slide.

Streamlined targeted workflow

Comprehensively targeting the relevant genes and biomarkers in your tissue sections gives you a complete view of the biology most important to your research. Compatible with Visium Spatial Gene Expression, you can profile a defined set of transcripts with pre-designed cancer, immunology, neuroscience, and gene signature panels. First, select one of our comprehensive, pre-designed panels, with the option to add up to 200 genes with the Custom Panel Designer. Second, enrich your Visium Spatial library with the selected gene panel. Next, sequence the resulting 10x barcoded, target-enriched library for efficient transcriptional profiling of tissue sections. Convert the raw sequencing data to biologically meaningful insights with Space Ranger's targeted analysis pipelines. Then, visualize using Loupe Browser to interactively explore your spatial data. Perform differential gene expression analysis to identify cell types, distinguishing genes, and more.

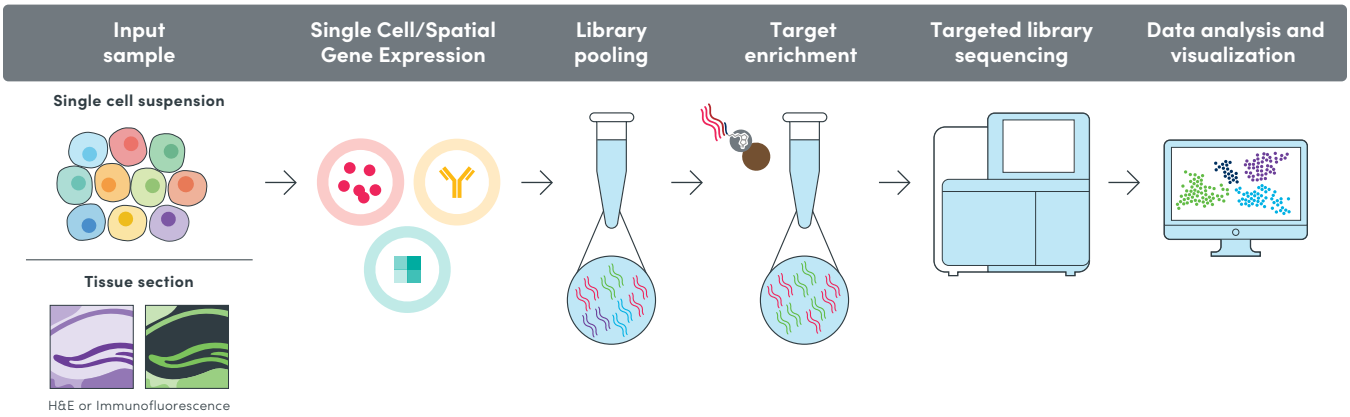


Figure 7. Targeted Spatial Gene Expression workflow with Visium Spatial solutions. Targeted Spatial Gene Expression enables the enrichment and analysis of a targeted set of mRNAs prepared from tissue sections. Starting with a final, barcoded 10x Genomics library, the workflow allows whole transcriptome and targeted gene expression on the same samples, while simultaneously examining morphology or co-detecting proteins.

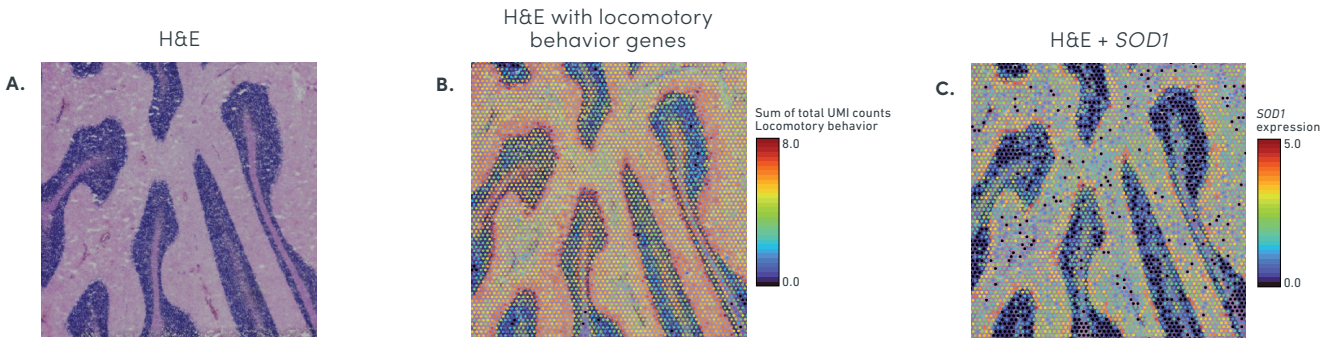


Figure 8. Spatially resolved targeted gene expression profiling with Visium Spatial solutions. A human cerebellum tissue section was H&E stained and processed using the Visium Spatial Gene Expression workflow, then enriched using Targeted Gene Expression with the Human Neuroscience Panel. Shown are the H&E image (A), H&E image overlaid with total UMI counts for 36 locomotory behavior genes from the neuroscience panel (B), and H&E image overlaid with *SOD1* expression level (C).

Preparing my lab

10x Genomics is committed to making adoption of Visium Spatial Gene Expression as easy and efficient as possible for you and your research team. Here are some microscope considerations to transition seamlessly to the Visium workflow.

Imaging systems & specifications	
Microscopes Any equivalent system with the listed features may be used for imaging.	
Nikon	Nikon Eclipse Ti2 with brightfield and fluorescence capacity (TRITC)
Molecular devices	ImageXpress Nano Automated Cell Imaging System
Hamamatsu	NanoZoomer S60
Keyence	Keyence BZX800
BioTek	Cytation 7
Thermo Fisher Scientific	EVOS M7000
Leica	Leica DMi8 Versa 8
Microscope features	
Objectives	• 4X (Plan APO λ; NA 0.20) • 10X (Plan APO λ; NA 0.45) • 20X (Plan APO λ; NA 0.75)
Automated scanning stage	Microscope tile scanning functionality is required for imaging tissue sections placed on a Capture Area of a Visium Spatial slide.
Brightfield features (for H&E staining only)	• Color camera (3 x 8 bit, 2,424 x 2,424 pixel resolution) • White balancing functionality • Minimum Capture Resolution 2.18 µm/pixel • Exposure times 2–10 milli sec

Table continued on next page

Imaging systems & specifications (cont'd)

Microscope features (cont'd)	
Fluorescence features* **Only required for Visium Spatial Tissue Optimization protocol & Visium Imaging Test Slide verification and if performing Immunofluorescence Staining prior to Tissue Optimization and Gene Expression protocols.*	• Light source (or equivalent) with a wavelength range of 380–680 nm • Monochrome camera (14 bit, 2,424 x 2,424 pixel resolution) • DAPI filter cube (Excitation 392/23, Emission 447/60) • Cy5 filter cube (Excitation 618/50, Emission 698/70) • TRITC filter cube (Excitation 542/20, Emission 620/52) (required for Immunofluorescence Staining & Tissue Optimization protocols only) • Minimum Capture Resolution 2.18 µm/pixel • Exposure times 100 milli sec–2 sec
Additional specifications	
Image format	Save image as tiff (preferred) or jpeg
Computer	Computer with sufficient power to handle large images (0.5–5 GB)
Software	Image stitching software (microscope's software or equivalent, like Image J)

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LIT000060 *Rev C Inside Visium Spatial Technology Brochure*

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