

# Gain a new perspective on tissue complexity with simultaneous gene and protein spatial profiling

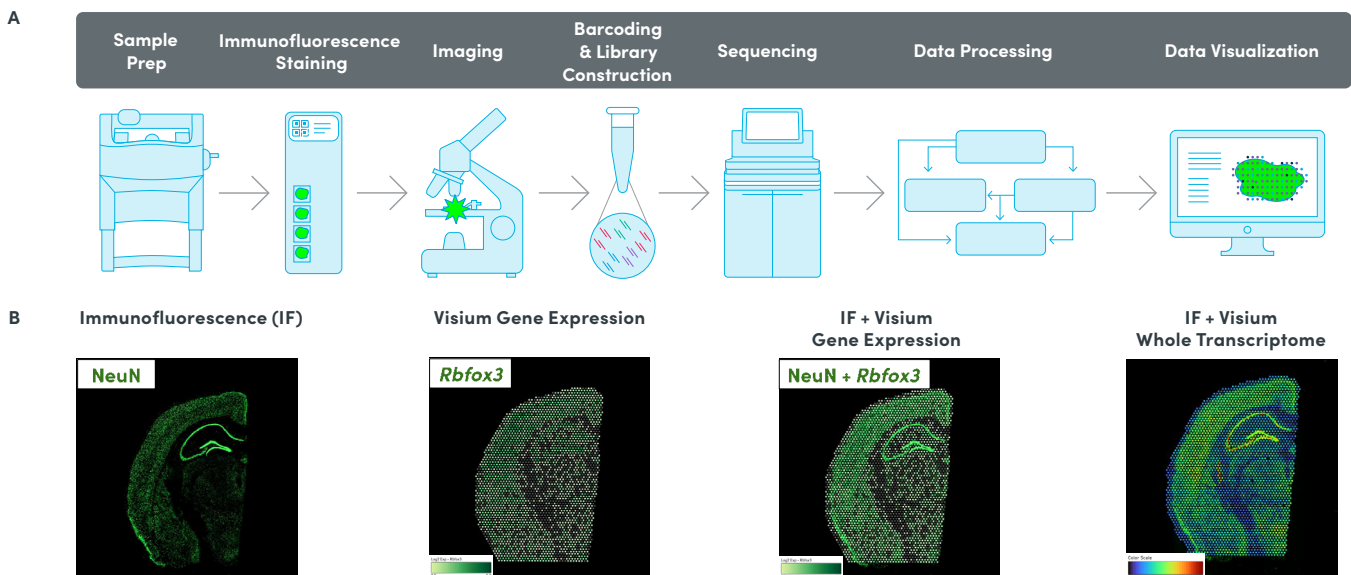
## Spatial Gene Expression with Immunofluorescence

Visualize spatial patterns of gene expression together with protein detection by immunofluorescence on the same tissue section. Histological tools like *in situ* hybridization and immunohistochemistry or immunofluorescence alone are limited in the breadth of analysis they can perform. The Visium Spatial Gene Expression Solution with immunofluorescence enhances these techniques, letting you combine immunofluorescence protein detection and unbiased, spatial gene expression in the same tissue section alongside histological analysis. Localize rare cell subtypes in patient samples, characterize immune cell activation state, or resolve post-translational modifications and cell signaling in intact tissue, without sacrificing whole transcriptome data.

The combined Visium Spatial Gene Expression with Immunofluorescence workflow makes it easy to implement spatial transcriptomics technology into standard methods of tissue sectioning and immunofluorescence staining. The combined workflow is simple, streamlined, and readily adoptable within

### Highlights

- 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 state in the tissue microenvironment
- Interrogate gene expression profiles in “hot” and “cold” tumor regions
- Identify the cellular source of secreted molecules
- Spatially map neuronal subtypes and their gene expression profiles

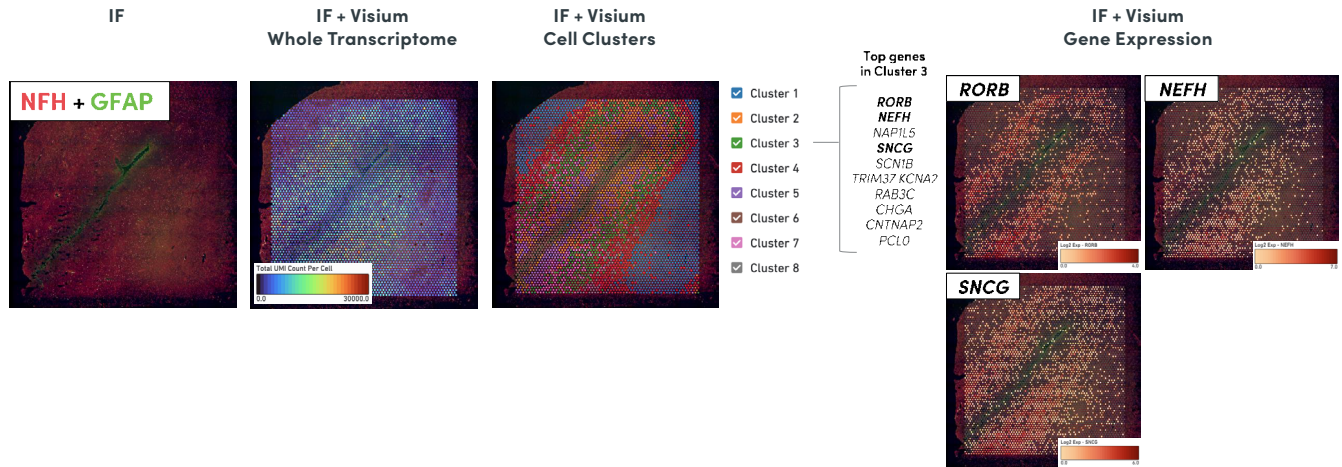


**Figure 1. The Visium Spatial Gene Expression with Immunofluorescence Workflow.** A. Ready-to-use, robust workflow for whole tissue section analysis of both protein and the whole transcriptome that integrates easily with current histological laboratory methods and tools for tissue analysis. B. A coronal mouse brain section was stained for NeuN by immunofluorescence, imaged, and then processed through the Visium Spatial Gene Expression workflow. Shown left to right are NeuN immunofluorescence, Visium spatial mRNA expression of *Rbfox3*, the gene encoding NeuN, NeuN immunofluorescence image overlaid with spatial *Rbfox3* mRNA data obtained by Visium, and NeuN immunofluorescence image overlaid with total UMI count from mRNA data obtained by Visium.

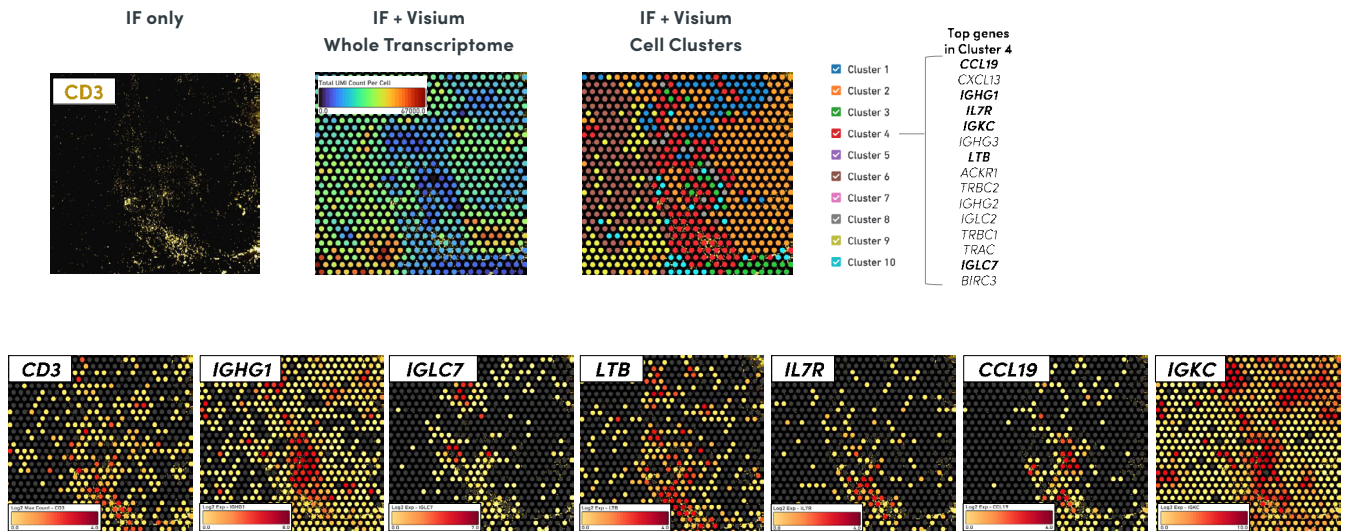
existing lab infrastructure using your current immunofluorescence antibodies. Access multiple streams of information for a single sample with an integrated workflow, and gain the ability

to define regions of interest after you have all the data, so as not to miss out on important or unexpected biological results.

**A Human Brain**



**B Human Tumor**



**Figure 2. Spatially resolved clustering and simultaneous gene expression and protein detection in human tissue samples.** A. A human brain section was stained for NFH and GFAP by immunofluorescence, imaged, and then processed through the Visium Spatial Gene Expression workflow. Shown are an immunofluorescence-only image, an immunofluorescence image overlaid with Visium data containing total UMI counts, and an immunofluorescence image overlaid with Visium data for spatially naive clustering based on total differentially expressed genes. The top 10 genes that are more highly expressed in cluster 3 are shown to the right along with a few example images. B. A human breast cancer tumor section was stained for CD3 by immunofluorescence, imaged, and then processed through the Visium Spatial Gene Expression workflow. Shown are an immunofluorescence-only image, an immunofluorescence image overlaid with Visium data containing total UMI counts, an immunofluorescence image overlaid with Visium data for spatially naive clustering based on total differentially expressed genes. Shown to the right are the top 15 genes that are more highly expressed in cluster 4, which overlaps with a region high in CD3 protein detection. In the bottom row are examples of some of the top differentially expressed genes in cluster 4, including several immunoglobulin, cytokine receptor, and chemokine genes, overlaid with CD3 immunofluorescence image.

## Solution Benefits

- Ready-to-use, robust workflow for whole tissue section analysis
- Easy to integrate with current histological laboratory methods and tools for tissue analysis
- Antibody flexibility: Simultaneously visualize protein and RNA using your own validated antibodies
- Broaden your reach beyond predefined regions of interest to analyze the entire tissue section at once
- Detect more analytes with whole transcriptome plus 2–3 proteins simultaneously

## Solution Features

- Compatible with fresh-frozen samples from most tissue types, including human and mouse tissue types such as brain, tumor, spleen, kidney, intestine, skin, heart, and more
- 4 capture areas each containing ~5,000 barcoded mRNA capture spots on a 6.5mm<sup>2</sup> area (average 1–10 cells captured per spot, dependent on tissue type)
- End-to-end workflow from section to sequencing-ready library, including immunofluorescence staining and imaging
- Easy-to-use and convenient software with Space Ranger and Loupe Browser, enabling alignment of protein, histology, and gene expression images

## Research Areas

- Neuroscience
- Cancer Biology
- Immuno-oncology
- Immunology
- Clinical & Translational Research
- Pathology

## Applications

- Cell-Type Determination
- Cellular Source of Secreted Proteins
- Immune Cell Activation State
- Engineered Cell Therapy Response
- Tumor Microenvironment Profiling
- Post-Translational Modifications

## Additional resources

Demonstrated Protocol

[support.10xgenomics.com/spatial-gene-expression](https://support.10xgenomics.com/spatial-gene-expression)

Datasets

[support.10xgenomics.com/spatial-gene-expression/datasets](https://support.10xgenomics.com/spatial-gene-expression/datasets)

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Publications

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Products	Product Code
Visium Spatial Tissue Optimization Slide & Reagents Kit, 4 samples	1000193
Visium Spatial Gene Expression Slide & Reagents Kit, 4 rxns	1000187
Visium Spatial Gene Expression Slide & Reagents Kit, 16 rxns	1000184
Visium Spatial Gene Expression Accessory Kit	1000194
Visium Spatial Gene Expression Starter Kit	1000200
Dual Index Kit TT Set A, 96 rxns	1000215
Space Ranger	
<a href="https://support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/what-is-space-ranger">support.10xgenomics.com/spatial-gene-expression/software/pipelines/latest/what-is-space-ranger</a>	Download
Loupe Browser	
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