Grant application resources for Visium products

Summary statement
The Visium platform from 10x Genomics combines histology, protein detection, and spatially resolved whole transcriptome gene expression profiling to localize and quantify gene expression in the tissue context. It is based, in part, on the Spatial Transcriptomics methodology (1). Visium was commercialized in 2019 and has been used in groundbreaking papers demonstrating the breadth of its applications, including cancer (3,4), neuroscience (5), immunology (6), and developmental biology (7). The assay has been well adopted, being utilized in over 68 peer-reviewed publications and 130 pre-prints. Currently, Visium Spatial Gene Expression is compatible with fresh frozen (FF) tissue sections from any species. This assay utilizes poly(A) capture and novel spatial barcoding technology for library preparation. 10x Genomics also offers Visium Spatial Gene Expression for FFPE, which is compatible with human and mouse formalin-fixed paraffin-embedded (FFPE) tissue sections. This assay utilizes RNA-templated ligation (RTL) of pairs of gene target probes for highly specific and sensitive detection of the whole transcriptome. Both assays leverage the same suite of analysis tools and pipelines (i.e., Space Ranger, Loupe Browser) to process and visualize Visium data. Additionally, researchers have access to 10x Genomics technical experts and tissue specialists who can provide support through scientific and technical consultations, workflow optimization, and methodology troubleshooting.

Overview
The ability to detect and count transcripts and proteins by sequencing has led to significant advances in our understanding of biology (2), as well as the development of clinical applications. However, traditional sequencing suffers from a loss of spatial information. Researchers typically extract analytes from tissue and sequence it in bulk. Data regarding the type of cells expressing a given transcript, the location of these cells within the tissue, and co-expression of transcripts in the tissue geography are all lost by this bulk preparation. Alternatively, researchers can study gene and protein expression from dissociated cells, however, the location of individual cells within the tissue architecture is also lost with this methodology.

10x Genomics has developed a workflow for sequencing mRNA and protein while preserving spatial information. The Visium platform allows for multiomic analysis using high-throughput sequencing then subsequently maps gene and protein expression patterns to entire tissue sections using high-resolution whole-slide scans. The workflow surveys global spatial gene expression in tissue sections, giving researchers the ability to profile the whole transcriptome or a defined set of transcripts via targeted gene panels. To further streamline this workflow, 10x Genomics has also developed the new Visium CytAssist instrument, which allows researchers to process samples using standard glass slides and histology protocols for FFPE tissue samples, enabling spatial profiling insights to be gained from even more samples.
Visium platform

The Visium workflow allows for whole transcriptomic analysis of FF and FFPE tissue sections without the loss of spatial information. This provides gene expression data within the context of tissue architecture, tissue microenvironments, and cell groups.

Spatial capture technology

Gene expression capture on the Visium platform relies on the use of Visium slides, each of which has two or four Capture Areas. Each Capture Area is arrayed with ~5,000 capture spots, each containing millions of oligonucleotides with the following features: a 30 nucleotide poly(dT) sequence for the capture of polyadenylated mRNA molecules; a 12 nucleotide unique molecular identifier (UMI) for the identification of duplicate molecules that arise during the library preparation and sequencing process; a 16 nucleotide Spatial Barcode, which is shared by all oligonucleotides within each individual gene expression capture spot; and a partial TruSeq Read 1 sequence, for use during the library preparation and sequencing portions of the workflow.

Components of a Visium Spatial Gene Expression Slide. A Visium Spatial Gene Expression Library comprises standard Illumina paired-end constructs which begin and end with P5 and P7. The Visium Spatial Gene Expression Slide includes two or four Capture Areas, each defined by a fiducial frame. Each Capture Area has ~5,000 gene expression capture spots, each containing millions of oligonucleotides that include a TruSeq Read 1 sequence, Spatial Barcode, UMI, and poly(dT) sequence.
Visium for FFPE spatial gene expression capture (manual method)

FFPE-sectioned tissues are placed on each Capture Area of the slide, where they are deparaffinized and stained using either hematoxylin and eosin (H&E)- or immunofluorescently (IF)- tagged antibodies. Each section is then imaged using the appropriate microscopy technique, the results of which are ultimately used to overlay gene expression patterns onto the image. The stained tissue is then de-crosslinked to release RNA that was sequestered by formalin fixation. Human or mouse whole transcriptome probe panels, consisting of a pair of specific probes for each targeted gene in the transcriptome, are then added to the tissue to capture the free mRNA targets. Together, probe pairs hybridize to their complementary target RNA. After hybridization, a ligase is added to seal the junction between the probe pairs that have hybridized to RNA, forming a ligation product. The single-stranded ligation products are released from the tissue upon RNase treatment and permeabilization and then captured on the Visium slides. Once ligation products are captured, probes are extended by a polymerase, thereby creating ligated probe products that incorporate a complement of the Spatial Barcode sequence and UMI. The spatially barcoded, ligated probe products are released from the slide by denaturation and PCR amplified using common sample-indexing primers. The final library is sequenced at a recommended depth of 25K read pairs per spot covered with tissue in most FFPE samples. For some samples, fewer reads will be sufficient, while more complex samples may require more reads.

Workflow overview for the manual Visium Spatial Gene Expression for FFPE assay.

Visium for FFPE with facilitated analyte transfer using the Visium CytAssist instrument

The new Visium CytAssist is a compact, benchtop instrument that enables the transfer of transcriptomic and proteomic analytes from tissues on standard glass slides to Visium slides. Compatible with H&E- or IF-stained FFPE tissue sections, the CytAssist instrument allows pre-sectioned tissues to be used for the Visium workflow and eliminates the need to directly section onto Visium slides.

Sectioning, deparaffinization, staining, and imaging (H&E or IF) take place on a standard glass slide in the Visium CytAssist workflow. After probe hybridization (Step 3), two standard glass slides and a two–Capture Area Visium slides are placed in the CytAssist instrument so that the tissue sections on the standard slides can be aligned on top of the two Visium Capture Areas. Within the instrument, a brightfield image is captured to provide spatial orientation for data analysis, followed by hybridization of transcriptomic and proteomic analytes to the Visium slide (Step 4). The remaining steps, starting with probe extension, follow the standard Visium for FFPE workflow outside of the instrument (Steps 5–6).
### Workflow overview for the Visium for FFPE assay using Visium CytAssist for facilitated transfer of transcriptomic and proteomic analytes in FFPE samples from standard glass slides to Visium slides.

**Visium for fresh frozen tissues**

Fresh frozen–sectioned tissues are placed on each Capture Area of the slide, where they are fixed and stained using either (H&E)- or IF-tagged antibodies. Each section is then imaged using the appropriate microscopy technique. The tissue sections are then permeabilized, and the mRNA molecules within cells are captured by the poly(dT) sequence on the slide surface. The captured mRNA molecule is reverse transcribed by extending the oligo bound on the slide surface, thereby creating a cDNA molecule with the Spatial Barcode sequence and UMI covalently attached to the slide. The captured mRNA molecule is denatured and removed, which allows for a second-strand copy, containing the complement of the Spatial Barcode and UMI, to be synthesized. The newly synthesized second strand is denatured and PCR amplified using common sequences. The cDNA is further processed into a sequencing library through enzymatic fragmentation, end repair, ligation of sequencing adapters, and enrichment of sequenceable molecules using sample barcoded primers targeting the adapter ends. The final library is sequenced at a recommended depth of 50K read pairs per capture spot covered by tissue. For some samples, fewer reads will be sufficient, while more complex samples may require more reads.

### Fresh frozen

**Workflow for Visium Spatial Gene Expression for fresh frozen tissues.**
Data analysis
During the Visium workflow, two main data types are captured: a tissue image and sequencing data. 10x Genomics provides two software tools to process and visualize these Visium data types, Space Ranger and Loupe Browser. Space Ranger processes the input file types to align the Visium sequencing data with the image. Each Spatial Barcode with the associated UMIs captured during the Visium workflow is assigned a spatial location in the tissue image. Space Ranger produces a variety of output files that can be used in Loupe Browser or third-party tools to visualize and apply spatial analysis methods to the data.

Data benchmarking
The Spatial Transcriptomics assay, a precursor to Visium, has been validated using laser capture microdissection as well as single molecule fluorescence in situ hybridization (ISH) (2). Comparison to data generated for the Allen Brain Atlas using ISH determined that Spatial Transcriptomics can detect twice as many transcripts (Figure S5 from Ref. 2). Spatial Transcriptomics studies examining gene expression among tissue replicates have found very high reproducibility ($r = 0.97$; Figure S3, Panel E from Ref. 2). High reproducibility was also observed when compared to RNA in solution ($r = 0.94$, Figure S3D from Ref. 2).

Applications
Visium Spatial Gene Expression is tissue and species agnostic and Visium for FFPE is suitable for profiling of human and mouse tissues, allowing for their use in numerous applications in both healthy and diseased tissues. Among many applications, the technology in its current and previous versions has been used to examine:

- Tumor heterogeneity in human prostate cancer (3)
- Spatial architecture in human squamous cell carcinoma (4)
- Spatial topography of the human dorsolateral prefrontal cortex, an area implicated in a number of neuropsychiatric disorders (5)
- Anatomical organization of the fibroblast response to influenza (6)
- Spatiotemporal analysis of human intestinal development (7)
- Spatial mapping of cells in the human endometrium and myometrium (8)
- Spatial characterization of human nociceptors (9)
- Characterization of B-cell responses within intratumoral tertiary lymphoid structures in renal cell carcinoma (10)
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**Justification for using the Visium platform for your research**

Visium offers many advantages, making it an optimal product for spatial transcriptomics. These include:

- **Spatially resolved whole transcriptome detection**—Visium Spatial Gene Expression for fresh frozen tissues captures polyadenylated mRNA molecules, reducing the bias introduced by targeted amplicon sequencing or sequence-specific hybridization techniques. Visium Spatial Gene Expression for FFPE tissues utilizes RNA-templated ligation of pairs of gene target probes for highly specific and sensitive detection of the whole transcriptome in human and mouse FFPE tissue sections.

- **Demonstrated technology**—Visium has been used as a cornerstone technology in many peer-reviewed papers in high-caliber journals, including *Science, Cell, Nature Neuroscience, Nature Communications, and Nature Protocols*.

- **Comprehensive data analysis solution**—The Visium platform includes a data analysis pipeline as well as state-of-the-art software for data visualization. The latter is compatible with most desktop computers and includes tools for differential gene expression analysis.

- **High reproducibility and sensitivity**—Publications using Visium’s core technology have determined that data reproducibility between adjacent tissue sections is $r = 0.97$ (2). Comparison between sequenced mRNA from the Visium workflow and mRNA from traditional RNA-seq found that 95% of transcripts can be found in both assays, highlighting the workflow’s sensitivity of detection.

- **High spatial resolution**—Each Capture Area on the Visium slide contains thousands of 55-µm barcoded spots, with an average of 1–10 cells captured per spot depending on tissue type. Visium Capture Areas are available in two sizes, 11 x 11 mm or 6.5 x 6.5 mm, providing the flexibility to study many different organisms and tissue types.

- **Streamlined sample preparation with Visium CytAssist**—The compact, benchtop CytAssist instrument enables spatial profiling insights to be gained from even more samples by facilitating the transfer of transcriptomic and proteomic analytes from standard glass slides to Visium slides.

- **Optimized conditions for numerous tissues**—The Visium Spatial Gene Expression workflow for fresh frozen tissue has been optimized for healthy and diseased fresh frozen tissues in diverse organisms, including human, mouse, rat, and zebrafish. For an up-to-date list of fresh frozen tissues optimized for the Visium assay, please visit our support website. The Visium Spatial Gene Expression workflow for FFPE tissues does not require individual tissue optimization and has been tested on a number of human and mouse healthy and diseased tissues. For an up-to-date list of tested FFPE tissues, please visit our support site.

- **Broad support resource**—10x Genomics provides comprehensive support resources, ranging from its technical specialists trained in all Visium offerings to freely available videos and documents that guide new users through the Visium workflow.

- **Certified Service Providers**—Get streamlined access to the complete Visium workflow through Certified Service Providers, third-party facilities specially trained and verified by 10x Genomics to support a wide variety of spatial biology research applications.

- **Certified product quality**—10x Genomics product development and manufacturing processes are ISO 9001:2015 certified.
References


Publications

The utility of Visium is demonstrated in numerous peer-reviewed publications, many in top journals. Visit 10xgenomics.com/publications to see the most current list of Visium publications.

Resources

- Product information
  10xgenomics.com/spatial-gene-expression

- Technology overview
  10xgenomics.com/spatial-transcriptomics

- Spatial gene expression support
  support.10xgenomics.com/spatial-gene-expression