

Grant application resources for Visium products

Summary statement

The Visium platform* from 10x Genomics combines histology 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). Many researchers have adopted the assay, as evidenced by the over 850 peer-reviewed publications and pre-prints utilizing the technology. Visium Spatial assays are compatible with a variety of tissue types and species. 10x Genomics offers two Visium capture chemistries, each with a distinct chemistry, to suit the needs of every researcher. One chemistry utilizes poly(A) capture and novel spatial barcoding technology for library preparation, offering diverse species applicability. The other chemistry utilizes RNA-templated ligation (RTL) of pairs of gene target probes for highly specific and sensitive detection of the whole transcriptome. Both chemistries leverage the same suite of analysis tools and pipelines (i.e., Space Ranger pipelines, Loupe Browser software) 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 significantly advanced our understanding of biology and the development of clinical applications (2, 11). 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. The Visium HD platform developed by 10x Genomics offers a workflow for sequencing mRNA while preserving spatial information. The platform enables gene expression analysis using high-throughput sequencing, then subsequently maps gene patterns to entire tissue sections using high-resolution imaging. The workflow surveys global spatial gene expression in tissue sections, giving researchers the ability to profile the whole transcriptome at single cell scale. To further streamline the gene expression workflow, 10x Genomics also developed the Visium CytAssist instrument,* facilitating the transfer of transcripts or transcriptomic probes from standard glass slides to Visium slides. The unique workflow supported by Visium CytAssist enables best-in-class spatial fidelity via controlled analyte transfer.

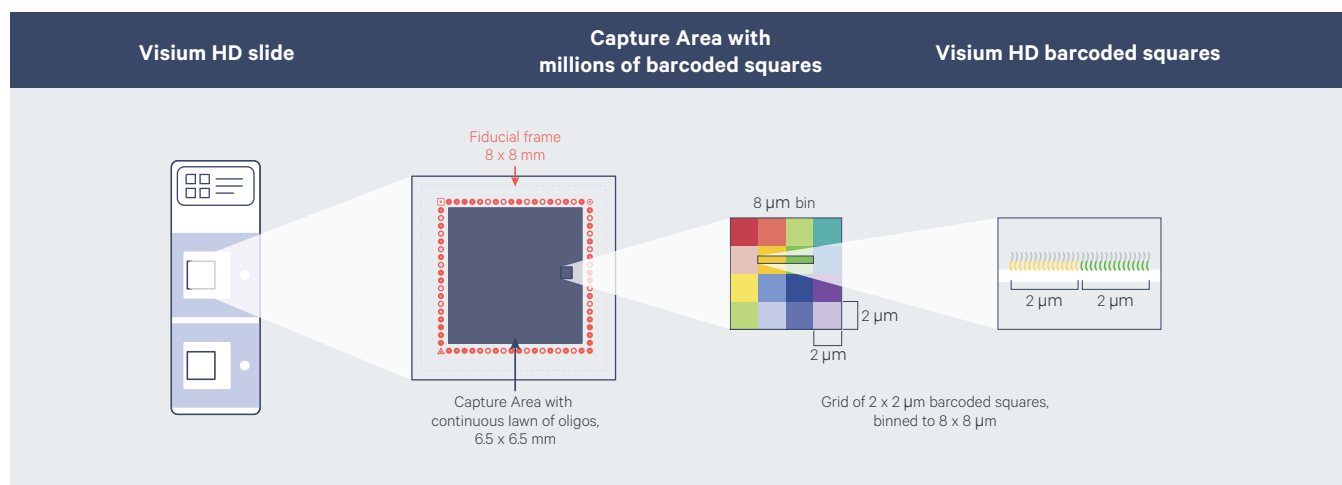
**The Visium platform may be subsequently referred to as “Visium” for the remainder of this piece. The Visium CytAssist instrument may be referred to as “Visium CytAssist” or “CytAssist” for the remainder of this piece.*

Visium platform

The Visium workflow enables whole transcriptome analysis of entire tissue sections within the context of tissue architecture, tissue microenvironments, and cell groups.

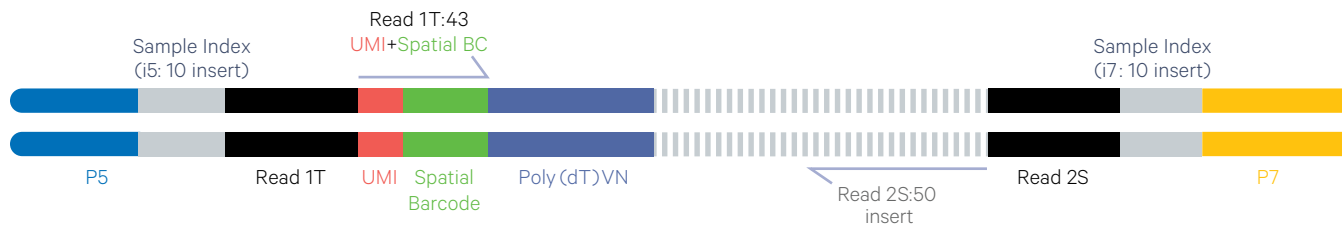
Visium HD spatial capture technology

Visium HD enables whole transcriptome spatial analysis at single cell scale with no gaps in tissue coverage. Featuring a redesigned slide architecture, each slide has two 6.5 x 6.5 mm Capture Areas. Gene expression capture relies on Visium HD slides. Each Capture Area is defined by a fiducial frame (fiducial frame + Capture Area is 8 x 8 mm). Capture Areas are arrayed with a continuous lawn of oligonucleotides organized into millions of 2 x 2 μm barcoded squares without gaps. Oligonucleotides have the following features: a 30 nucleotide poly(dT) sequence for capture of the ligation product; a unique molecular identifier (UMI) for the identification of duplicate molecules that arise during the library preparation and sequencing process; a Spatial Barcode, which is shared by all oligonucleotides within each barcoded area; and a partial Illumina TruSeq Read 1 sequencing primer, for use during the library preparation and sequencing portions of the workflow.



Components of a Visium HD Spatial Gene Expression slide. The HD array consists of a continuous lawn of oligonucleotides, arranged into ~11 million 2 x 2 μm barcoded squares without gaps. The data is output at 2 μm , as well as multiple bin sizes. The 8 x 8 μm bin is the recommended starting point for visualization and analysis.

Visium HD Gene Expression Library



Visium CytAssist

Visium CytAssist is a benchtop instrument that creates a controlled environment for probe or transcript transfer from glass tissue slides to the Visium slide, minimizing processes such as diffusion and fluidic flow and enabling accurate barcode assignment. This ensures that maximal transcriptomic probes or transcripts are captured and that they are localized with high spatial fidelity. The Visium CytAssist workflow follows standard histological practices, integrating seamlessly with H&E or IF staining, and is compatible across FFPE, fresh frozen, or fixed frozen tissues with no optimization required. Additionally, the CytAssist instrument allows you to pre-screen and orient tissue sections to align the most desired region of tissue onto the Capture Area of the Visium slide.

Components of the Visium CytAssist workflow. Two standard glass slides and a Visium slide with two Capture Areas are loaded into the CytAssist instrument so that the tissue sections on the standard slides can be aligned on top of the Capture Areas. Within the instrument, a brightfield image is captured to provide spatial orientation for data analysis, followed by transcript or probe transfer from the tissue onto the Visium slide.



Visium assays

Visium assays offer the highest data quality and utmost flexibility for your spatial discovery studies with CytAssist-enabled transcript localization and two robust chemistries compatible across diverse species, tissue preparations, and histological staining protocols.

Visium HD 3’ Gene Expression

The Visium HD 3’ assay is species agnostic and compatible with H&E-stained fresh frozen tissue sections. Tissue slides are generated via tissue sectioning, staining, and imaging on a glass slide prior to using the Visium CytAssist. Two tissue slides and a two-Capture Area Visium HD slide are then placed in the CytAssist instrument such that the tissue slides are 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 permeabilization of the tissue and capture of mRNA molecules by the poly(dT) sequence on the Visium HD slide surface. The captured mRNA molecules are reverse transcribed and, following second-strand synthesis, transferred to a tube to initiate the process of constructing a gene expression library. The final gene expression library is sequenced at a recommended minimum depth of 550 million read pairs for 6.5 mm x 6.5 mm Capture Areas fully covered by tissue.

Workflow for Visium HD 3’



Visium HD WT Panel Gene Expression

Visium HD WT Panel utilizes probe-based gene expression chemistry, and requires the use of the Visium CytAssist to facilitate the transfer of transcriptomic probes from tissues on glass slides to Visium HD slides. Visium HD WT Panel is compatible with H&E- or IF-stained fresh frozen, fixed frozen, and FFPE tissue sections from human, mouse, and rat samples. Tissue slides are generated via tissue sectioning, deparaffinization (for FFPE tissue), staining, and imaging on a glass slide prior to loading onto the Visium CytAssist. Two tissue slides and a two-Capture Area Visium HD slide are then placed in the CytAssist instrument such that the tissue slides are 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 permeabilization of the tissue and transfer of transcriptomic probes to the Visium HD slide. After the probes are extended, the sample is eluted and transferred to a new tube to initiate the process of constructing a gene expression library. The final gene expression library is sequenced at a recommended minimum depth of 275 million read pairs for 6.5 mm x 6.5 mm Capture Areas covered fully by tissue.

Workflow for Visium HD WT Panel



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 pipelines and Loupe Browser software. Space Ranger pipelines process 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 pipelines produce a variety of output files that can be used in Loupe Browser software or third-party tools to visualize and apply spatial analysis methods to the data.

Data benchmarking

The Spatial Transcriptomics assay, a precursor to Visium, was 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 were highly reproducible ($r = 0.97$; 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

The Visium HD 3' assay for fresh frozen tissue is species agnostic, while the Visium HD WT Panel assay is suitable for profiling human, mouse, and rat tissues; either technology is applicable to spatial discovery applications in both healthy and diseased tissues. Among its 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)
- B-cell responses within intratumoral tertiary lymphoid structures in renal cell carcinoma (10)
- Tumor-intrinsic biomarkers and putative drug targets in patient tumor biopsies (11)

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**—The Visium HD WT Panel assay offers whole transcriptome analysis in FFPE, fixed frozen, or fresh frozen tissues utilizing RNA-templated ligation of pairs of gene target probes. The Visium HD 3' assay for fresh frozen tissues captures polyadenylated mRNA molecules to enable unbiased whole transcriptome analysis.
- **Demonstrated technology**—Visium has been used as a cornerstone technology in > 850 peer-reviewed papers, 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**—Visium HD assays achieve single cell-scale spatial resolution through Visium HD slides. The features on the Visium HD slide are $2 \times 2 \mu\text{m}$ barcoded squares with no gaps between squares. Novel cell segmentation software enables true single cell resolution.
- **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 probes from standard glass slides to Visium slides.
- **Optimized conditions for numerous tissues**—The Visium HD 3' assay for fresh frozen tissues does not require individual tissue optimization and has been tested across healthy and diseased tissues and species, including human, mouse, rat, non-human-primate, and more. An up-to-date list of tested tissues for the [Visium HD 3' assay](#) is available on the support site. The Visium HD WT Panel assay for FFPE, fresh frozen, and fixed frozen tissues do not require individual tissue optimization and have been tested on a number of human, mouse, and rat healthy and diseased tissues. For an up-to-date list of tested tissues for the [Visium HD WT Panel](#) assay, 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

1. Ståhl PL, et al. Visualization and analysis of gene expression in tissue sections by Spatial Transcriptomics. *Science* 353: 78–82 (2016). doi: [10.1126/science.aaf2403](https://doi.org/10.1126/science.aaf2403)
2. Stark R, Grzelak M and Hadfield J. RNA sequencing: The teenage years. *Nat Rev Genet* 20: 631–656 (2019). doi: [10.1038/s41576-019-0150-2](https://doi.org/10.1038/s41576-019-0150-2)
3. Berglund E, et al. Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity. *Nat Commun* 9: 2419 (2018). doi: [10.1038/s41467-018-04724-5](https://doi.org/10.1038/s41467-018-04724-5)
4. Ji AL, et al. Multimodal analysis of composition and spatial architecture in human squamous cell carcinoma. *Cell* 182: 497–514.e22 (2020). doi: [10.1016/j.cell.2020.05.039](https://doi.org/10.1016/j.cell.2020.05.039)
5. Maynard KR, et al. Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex. *Nat Neurosci* 24: 425–436 (2021). doi: [10.1038/s41593-020-00787-0](https://doi.org/10.1038/s41593-020-00787-0)
6. Boyd DF, et al. Exuberant fibroblast activity compromises lung function via ADAMTS4. *Nature* 587: 466–471 (2020). doi: [10.1038/s41586-020-2877-5](https://doi.org/10.1038/s41586-020-2877-5)
7. Fawcner-Corbett D, et al. Spatiotemporal analysis of human intestinal development at single-cell resolution. *Cell* 184: 810–826.e23 (2021). doi: [10.1016/j.cell.2020.12.016](https://doi.org/10.1016/j.cell.2020.12.016)
8. Garcia-Alonso L, et al. Mapping the temporal and spatial dynamics of the human endometrium in vivo and in vitro. *Nat Genet* 53: 1698–1711 (2021). doi: [10.1038/s41588-021-00972-2](https://doi.org/10.1038/s41588-021-00972-2)
9. Tavares-Ferreira D, et al. Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. *Sci Transl Med* 14: eabj8186 (2022). doi: [10.1126/scitranslmed.abj8186](https://doi.org/10.1126/scitranslmed.abj8186)
10. Meylan M, et al. Tertiary lymphoid structures generate and propagate anti-tumor antibody-producing plasma cells in renal cell cancer. *Immunity* S1074-7613(22)00081-4 (2022). doi: [10.1016/j.immuni.2022.02.001](https://doi.org/10.1016/j.immuni.2022.02.001)
11. Lyubetskaya A, et al. Assessment of spatial transcriptomics for oncology discovery. *Cell Rep Methods* 2: 100340 (2022). doi: [10.1016/j.crmeth.2022.100340](https://doi.org/10.1016/j.crmeth.2022.100340)

Publications

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

Resources

Product information

10xgenomics.com/platforms/visium

HD assay information

10xgenomics.com/platforms/visium/product-family

Spatial gene expression support

10xgenomics.com/support

Contact us

10xgenomics.com | info@10xgenomics.com