

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 400 peer-reviewed publications and pre-prints utilizing the technology. Visium Spatial Gene Expression is 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, 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

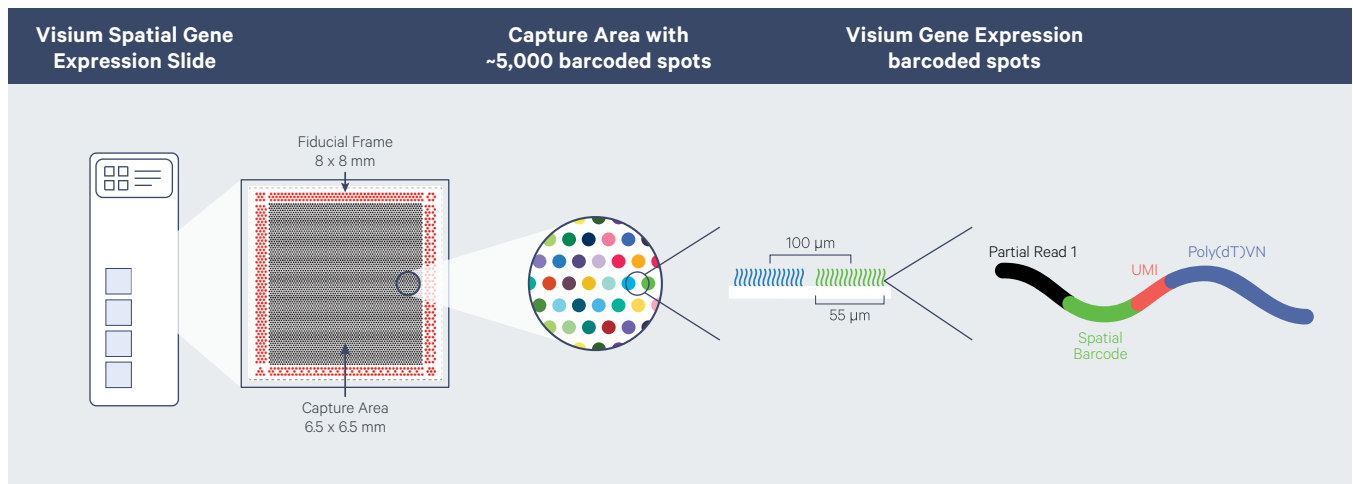
The ability to detect and count transcripts and proteins by sequencing has significantly advanced our understanding of biology and the development of clinical applications (2, 11). However, traditional sequencing suffers from a loss of spatial information. Researchers typically extract analytes from 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 platform developed by 10x Genomics offers a workflow for sequencing mRNA while preserving spatial information. The platform enables multiomic analysis using high-throughput sequencing, then subsequently maps gene and protein expression 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 or a defined set of transcripts. To further streamline the gene expression workflow, 10x Genomics also developed the Visium CytAssist instrument, facilitating the transfer of transcriptomic probes or antibody oligos from standard glass slides to Visium slides. The unique workflows supported by the Visium CytAssist platform enable spatial profiling of whole transcriptome and targeted protein expression across entire tissue sections to yield key insights from more sample types than ever before. The new Visium HD Spatial Gene Expression assay further enhances the discovery power of the Visium platform by enabling whole transcriptome spatial gene expression at single cell scale.

Visium platform

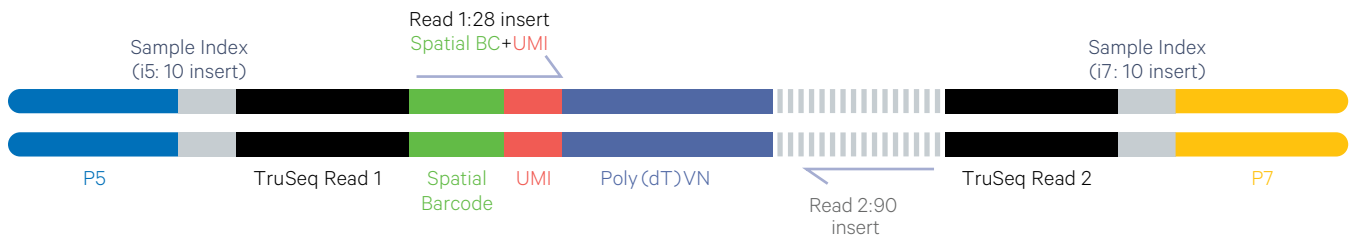
The Visium workflow enables whole transcriptome analysis of entire tissue sections within the context of tissue architecture, tissue microenvironments, and cell groups.

Visium 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 of either 6.5 x 6.5 mm or 11 x 11mm. Each Capture Area is arrayed with ~5,000 capture spots (for the 6.5 x 6.5 mm capture area) or ~14,000 barcoded spots (for the 11 x 11mm capture area), each containing millions of oligonucleotides with the following features: a 30 nucleotide poly(dT) sequence for the capture of polyadenylated molecules; a 12 nucleotide unique molecular identifier (UMI) used 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.



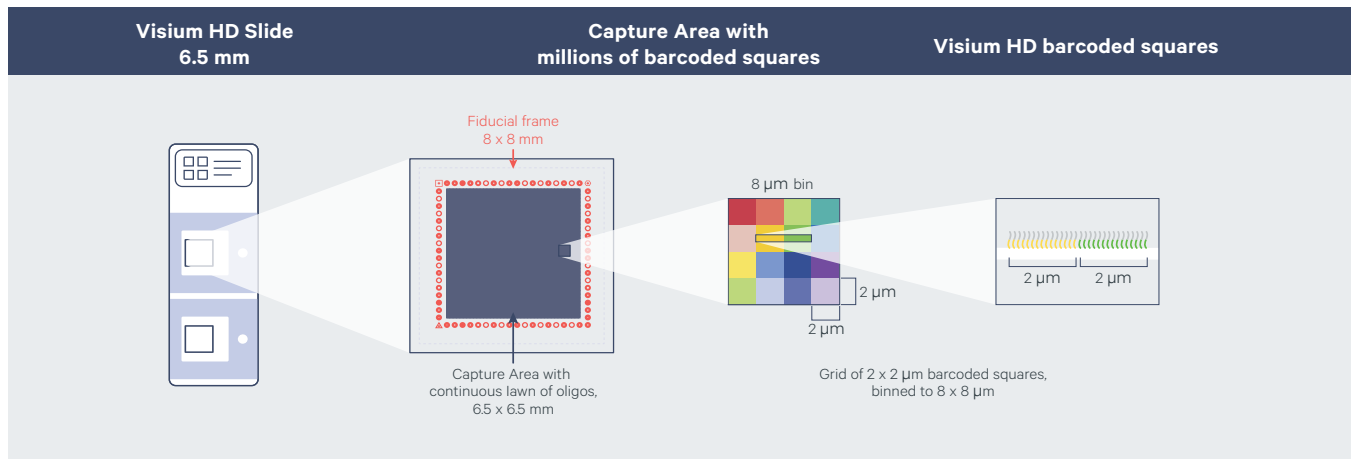
Visium Spatial Gene Expression Library



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.

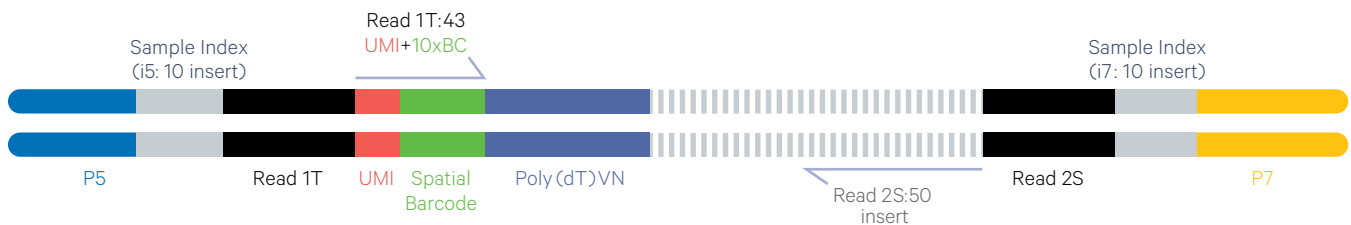
Visium HD spatial capture technology

Gene expression capture for our Visium HD Spatial Gene Expression assay relies on Visium HD slides. The HD slide has two Capture Areas, each 6.5 x 6.5 mm, 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.



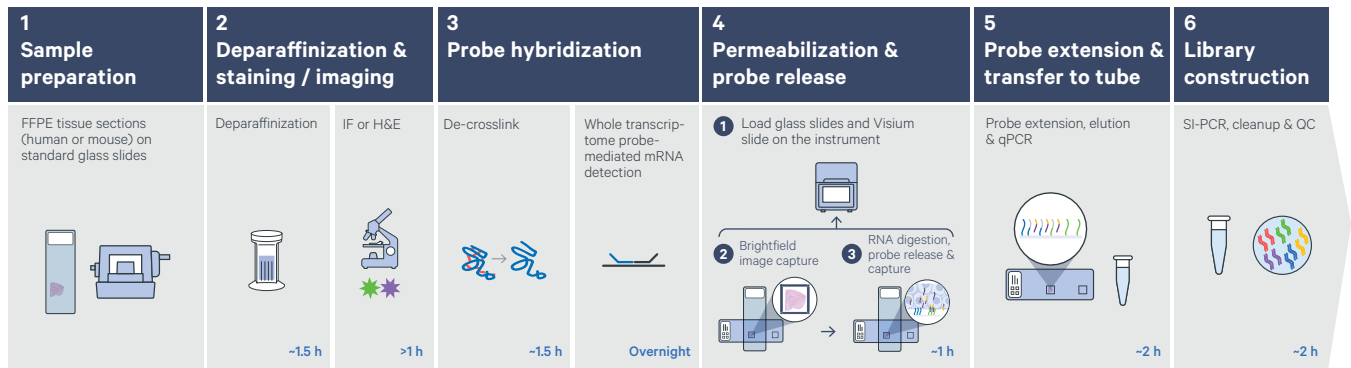
Components of a Visium HD Spatial Gene Expression slide. The HD array consists of a continuous lawn of oligonucleotides, arranged into ~11 million 2 x 2 µm barcoded squares without gaps. The data is output at 2 µm, as well as multiple bin sizes. The 8 x 8 µm bin is the recommended starting point for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

Visium HD Gene Expression Probe-Based Library



Visium CytAssist Spatial Gene Expression

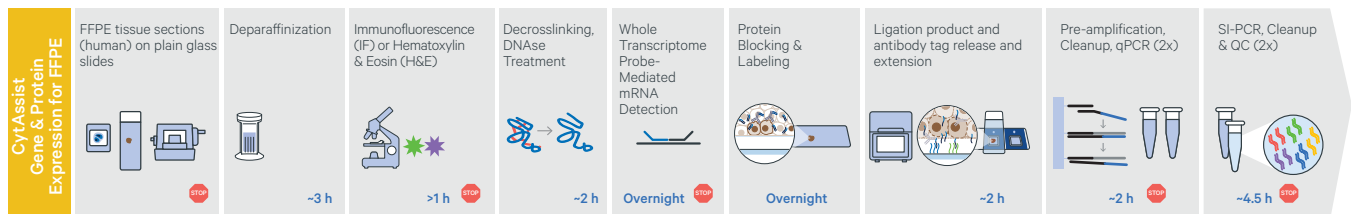
The Visium CytAssist is a compact, benchtop instrument that enables the transfer of transcriptomic probes or antibody oligo tags from tissues on standard glass slides to Visium slides. Compatible with hematoxylin and eosin (H&E)- or immunofluorescently (IF)-stained FFPE tissue sections, and H&E-stained fresh or fixed frozen samples, the CytAssist instrument allows pre-sectioned tissues to be used for the Visium workflow. 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 CytAssist gene expression slide 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 permeabilization of the tissue and transfer of transcriptomic probes to the Visium slide (Step 4). 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 depth of 25K read pairs per capture spot covered by tissue.



Workflow overview for the Visium for FFPE assay using Visium CytAssist for facilitated transfer of transcriptomic probes in FFPE samples from standard glass slides to Visium slides.

Visium CytAssist Gene and Protein Expression

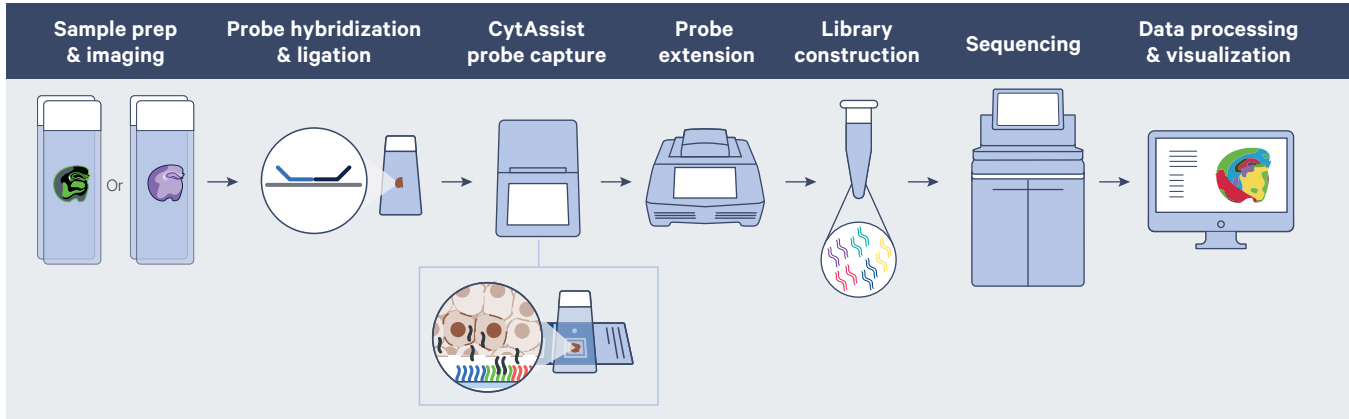
The Visium CytAssist Gene and Protein Expression solution is a set of specialized reagents that enables the transfer of transcriptomic probes and antibody oligo tags from tissues on standard glass slides to Visium slides. Compatible with H&E- or IF-stained human FFPE tissue sections, the CytAssist instrument allows pre-sectioned tissues on standard glass slides or H&E-stained, permanently mounted and coverslipped sections (“archived slides”) to be used for the Visium workflow. The Visium CytAssist Gene and Protein Expression solution features a pre-validated, 35-plex antibody panel optimized for use on human FFPE tissues. The assay enables protein and whole transcriptome RNA mapped together in a single experiment, from a single tissue section, while preserving H&E staining patterns before any chemical treatment of input tissue sections. Our pre-validated, 35-plex antibody panel for human immune cell profiling lets you hit the ground running and will provide a specialized protocol to combine the panel with additional antibodies against proteins of interest. The final gene expression library is sequenced at a recommended depth of 25K read pairs per capture spot covered by tissue, and the final protein expression library is sequenced at a recommended depth of 5K read pairs per capture spot covered by tissue. For some samples, fewer reads will be sufficient, while more complex samples may require more reads.



Workflow for Visium CytAssist Gene and Protein Expression.

Visium HD

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. The assay utilizes the same probe-based gene expression chemistry used in other Visium CytAssist assays, 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 is compatible with H&E- or IF-stained FFPE tissue sections from human and mouse samples. Tissue slides are generated via tissue sectioning, deparaffinization, 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 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 reads for Capture Areas covered fully by tissue.



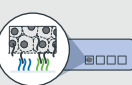




Workflow for Visium HD Spatial Gene Expression using Visium CytAssist for facilitated transfer of transcriptomic probes in FFPE samples from glass slides to Visium HD slides.

Visium Spatial Gene Expression for Fresh Frozen (Direct placement)

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

1 Sample preparation	2 Staining / imaging	3 Permeabilization & barcoding	4 Transfer to tube	5 Library construction
Snap-frozen & OCT-embedded tissue sections on Visium slide Fresh frozen 	IF or H&E  >1h	RT reaction, 2nd strand synthesis & denaturation  -2 h	qPCR, cDNA amplification & QC  -2 h	Fragmentation, end repair, A-tailing, SI-PCR, cleanup & QC  -4 h

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, 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

Direct Placement Spatial Gene Expression for fresh frozen is species agnostic and Visium with CytAssist is suitable for profiling of human and mouse 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**—Visium CytAssist-enabled assays offer unbiased whole transcriptome analysis in FFPE, fixed frozen, or fresh frozen tissues utilizing RNA-templated ligation of pairs of gene target probes. This includes the new Visium HD assay, which is compatible with FFPE tissues. The direct placement Visium assay for fresh frozen tissues captures polyadenylated mRNA molecules to enable whole transcriptome analysis.
- **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**—The Visium HD Spatial Gene Expression assay achieves single cell-scale spatial resolution through Visium HD slides. The features on the Visium HD slide are $2 \times 2 \mu\text{m}$ barcodes squares with no gaps between squares.

- **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 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 site](#). Visium CytAssist-enabled workflows for FFPE tissues do not require individual tissue optimization and have 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.

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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

Technology overview

10xgenomics.com/spatial-transcriptomics

Spatial gene expression support

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

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