

# Visium Gene Expression Workshop

Josh Talboom, Ph.D. Science & Technology Advisor San Diego & OC, CA

Presented by UCI GRT Hub & 10x Genomics 01/24/2024

# Your Local 10x Genomics Team



#### Christine Kao, MBA Sales Executive christine.kao@10xgenomics.com



Tu Luu Sales Associate tu.luu@10xgenomics.com



#### Josh Talboom, PhD Science & Technology Advisor joshua.talboom@10xgenomics.com



Jay Mehta Xenium Sales Specialist jay.mehta@10xgenomics.com



Hawra Karim, Ph.D. Field Application Scientist hawra.karim@10xgenomics.com



Jess Blake, MBA Tissue Specialist Field Application Scientist jessica.blake@10xgenomics.com

### Outline

- Overview of Visium
- Tissue & Workflow
  - Slides
  - Tissue/Sample Processing Types
  - Stopping Points
- Sample Preparation
  - RNA QC
  - Prep, Sectioning, & Best Practices
- Visium HD
- Conclusion

10 DENOMICS	
CytAssist	

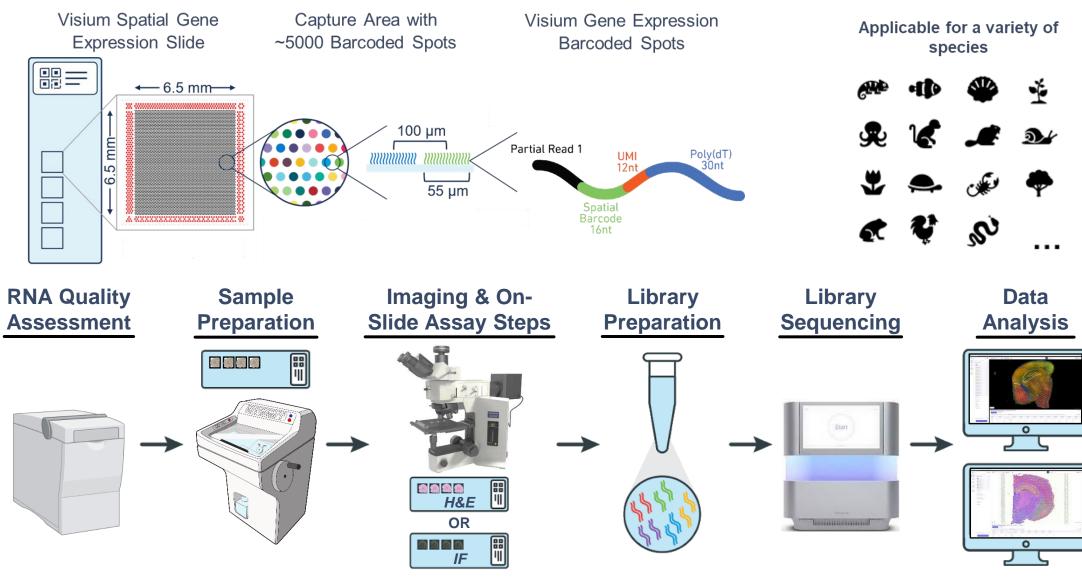




## **Overview of Visium**

### **Visium – Direct Placement**

### No instrument required - Fresh Frozen v1 assay



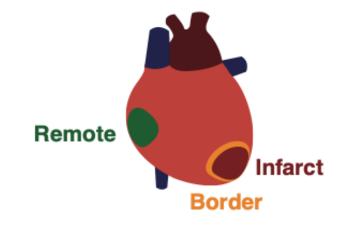
### **Visium – Spatial Gene Expression Provides Deep Insight**

Into Pathological Tissue Architecture - Kuppe et al., (2022), Nature

Cell Repo	orts Open access	sol
Single-Cell	2. ISSUE 3. PRO-610, JANUARY 16, 2018 Transcriptional Profiling Reveals Cellular Diversity and unication in the Mouse Heart	het
	Galen T. Squiers 7 • Micheal A. McLellan 7 • Paul Robson • Nadia A. Rosenthal A <sup>8</sup> • • • • • • • • • • • • • • • • •	
eLIF elifescience		• The
	Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury	bas
	Nona Farbehi <sup>1,2,3,4†</sup> , Ralph Patrick <sup>1,2,5†</sup> , Aude Dorison <sup>1,2</sup> , Munira Xaymardan <sup>1,2,6</sup> , Vaibhao Janbandhu <sup>1,2,5</sup> , Katharina Wystub-Lis <sup>1</sup> , Joshua WK Ho <sup>1,5</sup> , Robert F. Nordon <sup>2,4</sup> *, Richard P. Harvey <sup>1,2,7</sup> *	
	Circulation Volume 142, Issue 19, 10 November 2020; Pages 1831-1847 https://doi.org/10.1161/CIRCULATIONAHA.119.044557	American Heart Association.
	ORIGINAL RESEARCH ARTICLE	
	Single-Cell RNA Sequencing Analysis Revea Role for CTHRC1 (Collagen Triple Helix Rep 1) Cardiac Fibroblasts After Myocardial Infa	eat Containing

Adrián Ruiz-Villalba, PhD<sup>\*</sup>, Juan P. Romero, PhD<sup>\*</sup>, Silvia C. Hernández, PhD ())<sup>\*</sup>, Amaia Vilas-Zornoza, PhD, Nikolaus Fortelny, PhD (), Laura Castro-Labrador, BS (), Patvi

- Several papers using 10x Genomics single cell solutions have unraveled the cellular heterogeneity during Myocardial infarction (MI)
- The next big question is how cells respond based on their distance to the injury



Kuppe et al. (2022) Nature DOI: 10.1038/s41586-022-05060-x

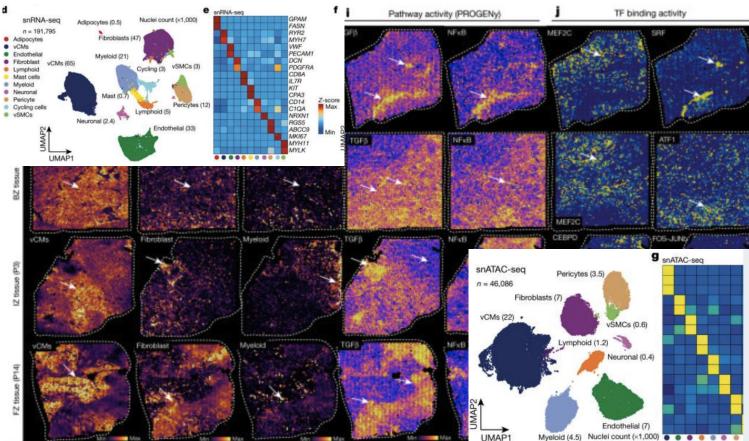


### **Visium – Spatial Gene Expression Provides Deep Insight**

Into Pathological Tissue Architecture - Kuppe et al., (2022), Nature

### • Key Takeaways:

- Integration of Visium, 3', & ATAC generated high-resolution multiomic map after myocardial infarction (MI)
- Integrating 3' & ATAC with Visium identified different cell states and subtypes
  - e.g., Distinct cardiomyocyte cell states associations
- Combining spatial & single-cell data/technologies present a unique opportunity to determine how cell states are influenced by the tissue microenvironment



Kuppe et al. (2022) Nature DOI: 10.1038/s41586-022-05060-x



### **Visium CytAssist – Instrument**

### Visium CytAssist - get MORE from your FF & FFPE samples



Flexible sample formats

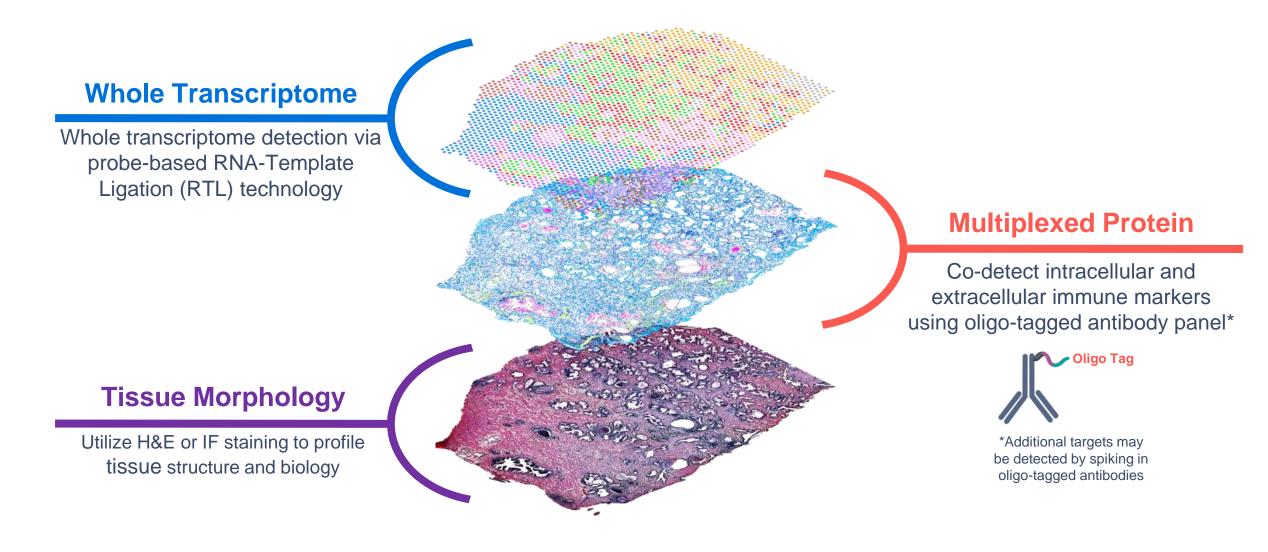
Flexible tissue size

### Easier workflow & better-quality data!



### Visium CytAssist – Spatial Gene and Protein Overview

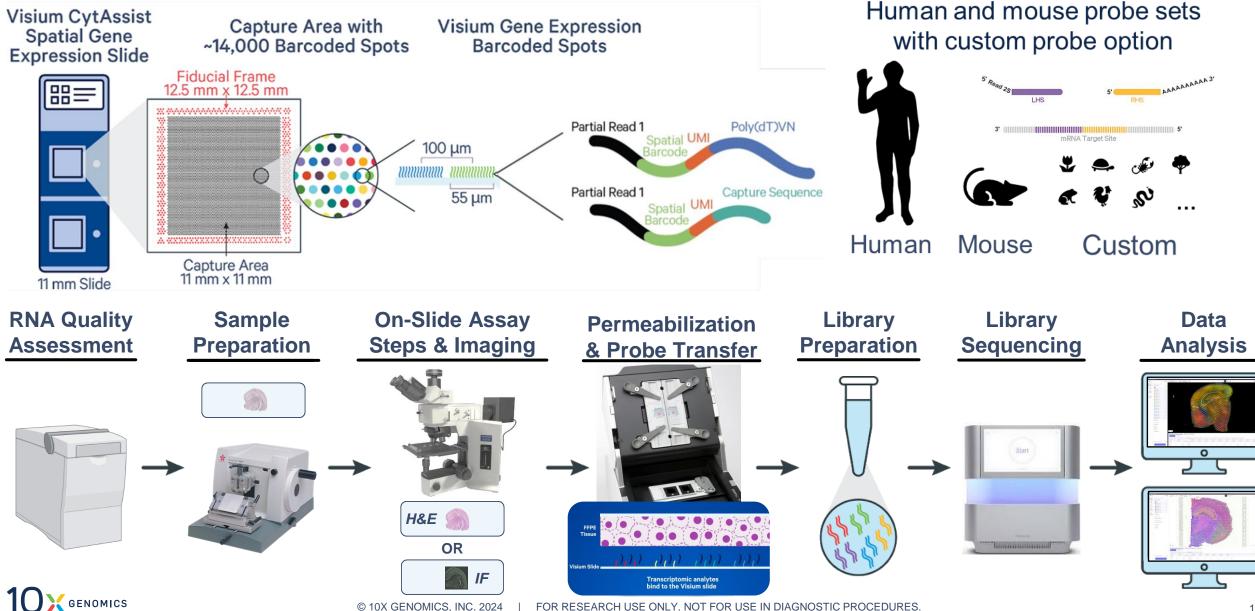
Probes and oligo-tagged antibodies enable RNA and protein co-detection





### Visium CytAssist – Enabled Gene Expression Technology

Visium CytAssist instrument required – probe-based v2 assay



# Visium CytAssist Tissue & Workflow

- Slides
- Tissue/Sample Types
- Stopping Points

### **Tissue Slide**

Best practices



Visit the 10x Genomics Support Site for the latest documentation



#### **Tissue Slide**

- Tissue sections must be placed in specific areas on plain blank glass slide
- To ensure compatibility, use validated positively charged slides (listed below)

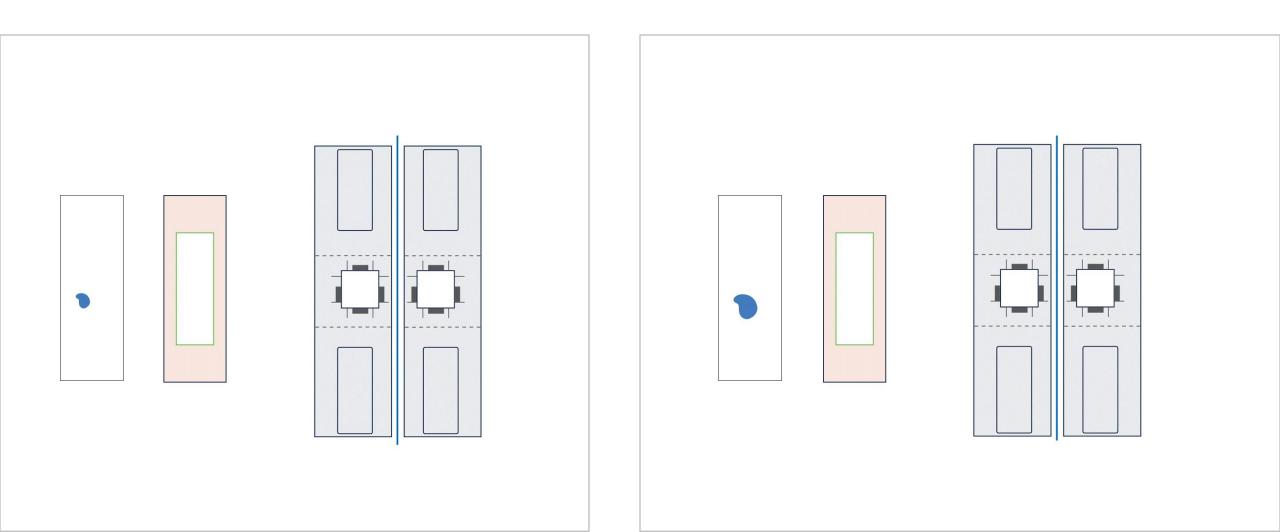
Slide Brand & Name	Catalog Number	Length (mm)	Width (mm)	Thickness (mm)
Epredia Shandon Colorfrost Plus Slides	<u>6776214</u>	75	25	1
Fisher SuperFrost Slides	<u>12-544-7</u>	75	25	1
Sigma-Aldrich Poly-Prep Slides	<u>P0425-72EA</u>	75	25	1
VWR SuperFrost Plus Slides	<u>48311-703</u>	75	25	1

• More information on tissue placement areas and slides are in *Visium CytAssist Tissue Preparation Guide Demonstrated Protocol* and *Visium CytAssist Quick Reference Cards* 



### Visium CytAssist – Section onto Plain Glass Slides

Proper section placement





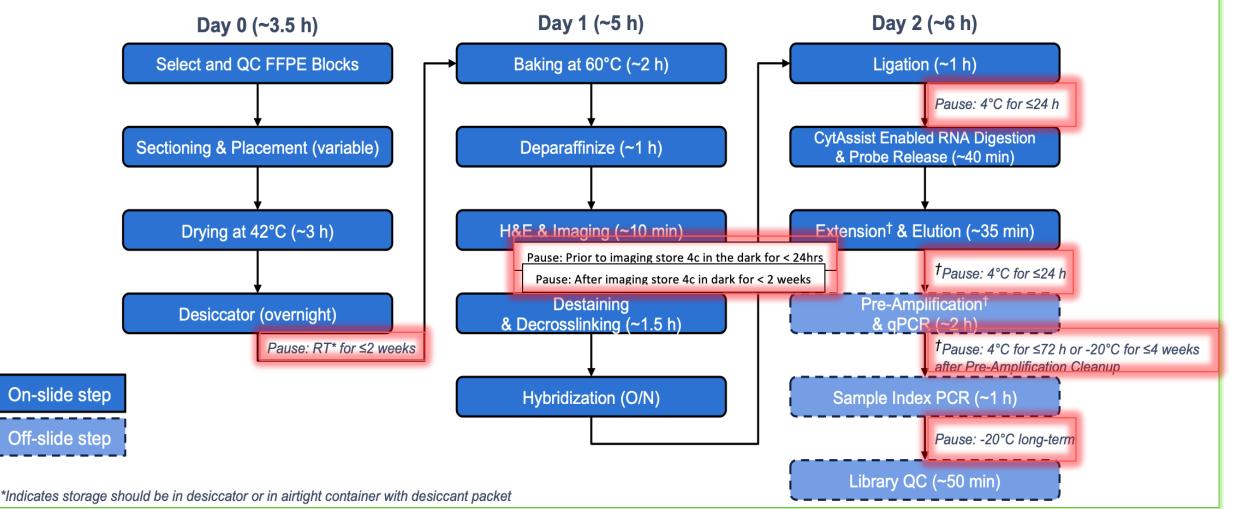
### Visium CytAssist Spatial Gene Expression Comparison

Application	Visium CytAssist for FFPE (FFPE)	Visium CytAssist for Fresh Frozen (FF)	Visium CytAssist for Fixed Frozen (FxF)
Species	Human or Mouse	Human or Mouse	Mouse
Tested Tissues	20+	9	6
Capture Area Size	6.5 mm or 11 mm		
Studies Enabled	Whole transcriptome (WT GEX) WT GEX + Protein detection (IF)	Whole transcriptome (WT GEX)	
Transcript Detection	Probe-based capture using 10x Probe Panels: Human Transcriptome Probe Kit v2 Mouse Transcriptome Probe Kit		Probe-based capture using 10x Probe Panel: Mouse Transcriptome Probe Kit
Sample Quality	DV200 of 30% or greater	RIN of 4 or greater	DV200 of 50% or greater
Sample Input	Fresh cut sections on blank slides H&E or IF Stained (archived) slides	Fresh cut sections on blank slides	
Section Thickness	3 – 10 μm	10 - 20 μm	
Tissue Staining	H&E or Immunofluorescence (IF)	H&E	
Sequencing Depth	25k read pairs per tissue covered spot		



# **CytAssist Spatial Gene Expression for FFPE**

Protocol steps and timing – H&E staining (freshly placed sections)

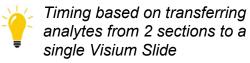




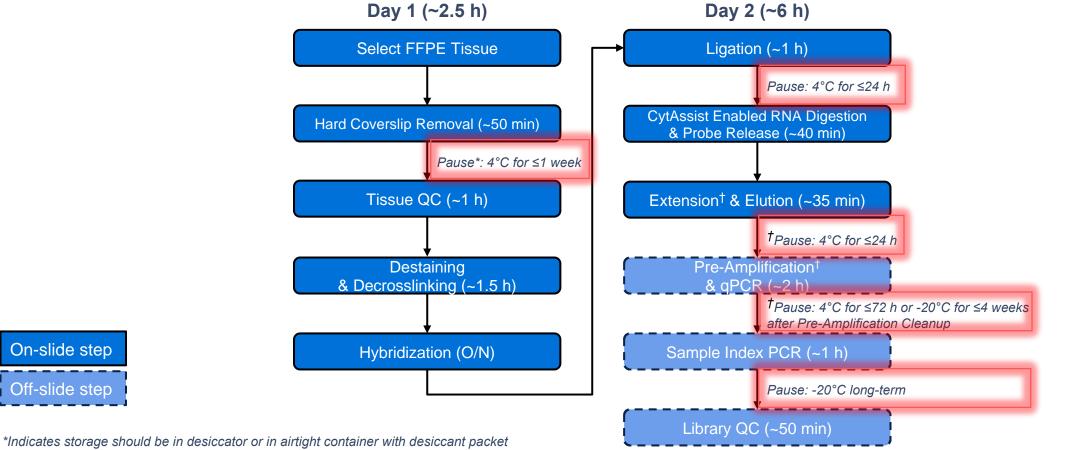
Timing based on transferring analytes from 2 sections to a

single Visium Slide

### **CytAssist Spatial Gene Expression for FFPE**



Protocol steps and timing – H&E staining (archived)\*\*



\*\*Protocol assumes tissue has already been stained and imaged

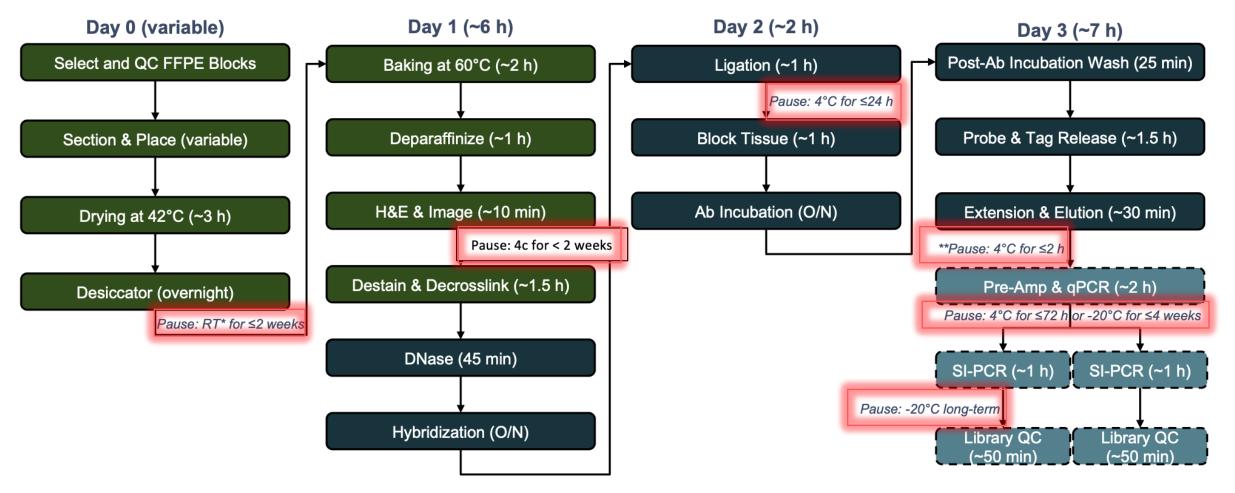


On-slide step

Off-slide step

### **Spatial Gene and Protein Expression for FFPE**

Protocol steps and timing – Tissue Prep, H&E, and User Guide steps



\*Storage should be in desiccator or in airtight container



Timing based on transferring probes & tags from 2 slides a Visium slide

**On-slide UG Step** 

Off-slide UG Step

**On-slide DP Step** 

## **IF Staining Demonstrated Protocol Overview**

### FFPE





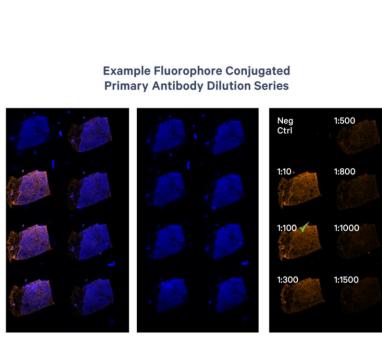
\*Time required for imaging is excluded from time estimation; time range due to direct and indirect staining options



## **IF Staining Optimization**

### FFPE

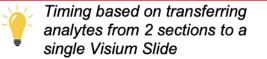
Sectioning & Placement Staining & Imaging



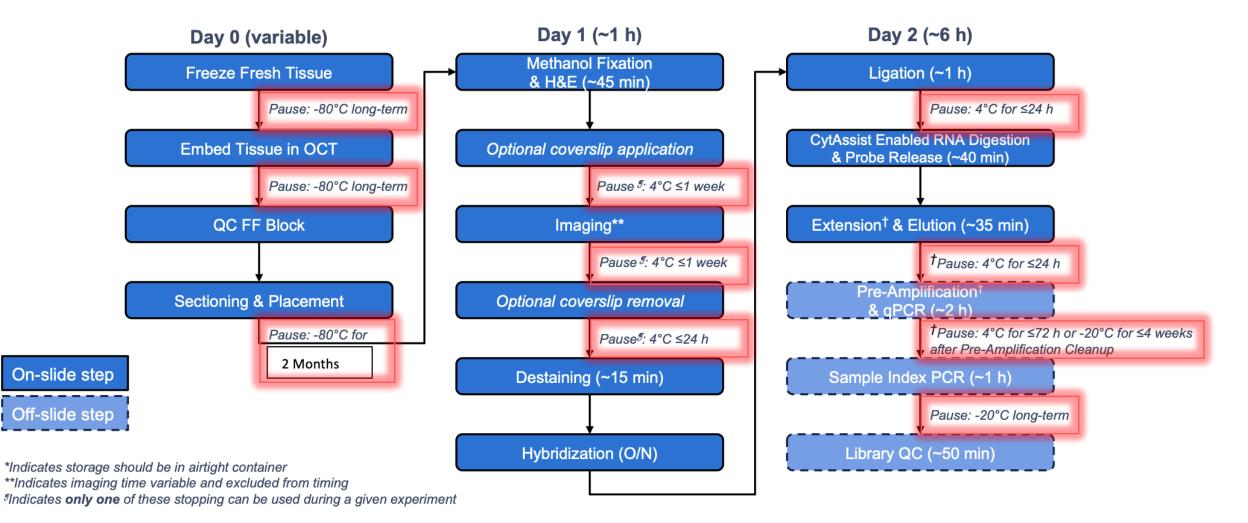
A 1:100 dilution (0.25 ug/sample) was considered optimal in this example

- Visium CytAssist Spatial Gene Expression assay can accommodate reactions for antibody optimization
  - Account for enough reactions for main assay before running optimization experiments
  - Use Visium Slide Cassette, 6.5 mm 4 pack for optimizations, add-on kit will need to be purchased
- To optimize antibody concentration, draw representative frames on the back of a 75 x 25 x 1 mm plain glass slide using the example slide layout
- Execute the Staining Demonstrated Protocol using a range of antibody concentrations
  - A starting concentration of 0.01 µg/µl (0.5 µg/sample) is recommended
- Select antibody concentration that results in specific staining of desired cells, while minimizing nonspecific background staining
  - To reduce autofluorescence, TrueBlack Plus reagent may be added

## **CytAssist Spatial Gene Expression for FF**



### Protocol steps and timing – H&E staining





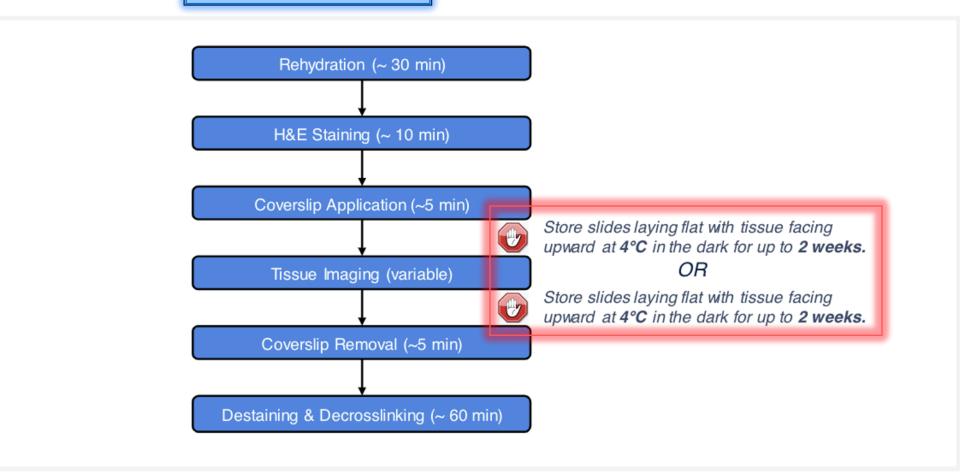
### **Demonstrated Protocol Steps & Timing**

Fixed Frozen



Slides must be coverslipped prior to storage

### Validated Stopping Points



\*Time required for imaging is excluded from time estimation



# Visium CytAssist Sample Preparation

- RNA QC
  - FFPE
  - Fresh Frozen
  - Fixed Frozen
- Prep, Sectioning, & Best Practices
  - FFPE
  - Fresh Frozen
  - Fixed Frozen

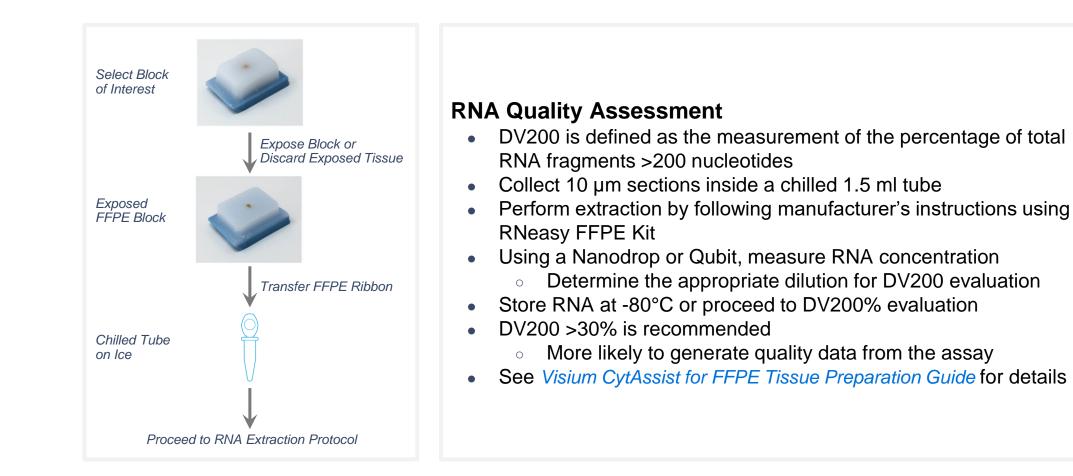




### FFPE – Fresh Cut Sections



GENOMICS



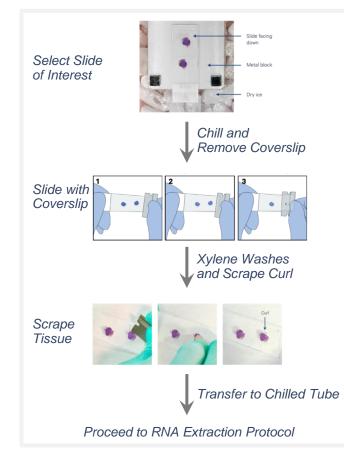


## **RNA quality assessment via DV200%**

### FFPE – Archived Slides



Work slowly in small steps to remove coverslip while keeping slide on metal block; exercise caution with sharp blade



#### **RNA Quality Assessment**

- DV200 is defined as the measurement of the percentage of total RNA fragments >200 nucleotides
- Execute Coverslip Removal, as described in documentation
- Scrape FFPE curl from representative tissue\* and transfer to chilled 0.2-ml in 8-tube strip
- Perform extraction using third-party reagents and consumables
- Using 1 µl of RNA, perform DV200% evaluation following Agilent's instructions
- DV200 >30% is recommended
- See Visium CytAssist for FFPE Tissue Preparation Guide for details

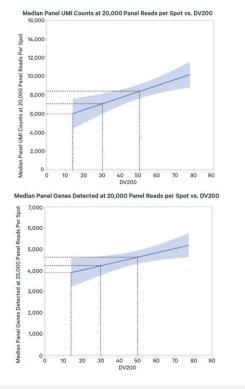
\*Isolate curl from section from same block or serial section as tissue of interest on same slide



#### FFPE

Sectioning & Placement

### Positive correlation between DV200 value and UMIs/Genes per Spot

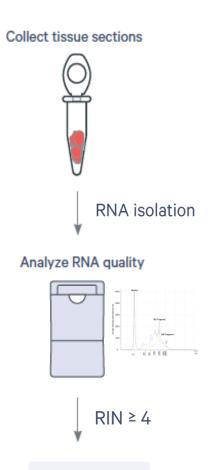


#### **RNA Quality Assessment**

- Measurement of the percentage of total RNA fragments >200 nucleotides (DV200) upstream of library preparation
- Our assay is highly sensitive
  - Fragmented RNA is not a liability unless it's degraded beyond our recommendation
- Recommendation: blocks with DV200 >30%
  - DV200% is not a perfect predictive tool of assay performance
  - Using low DV200-scored blocks will still yield data but likely of lower sensitivity (i.e., lower UMIs and Genes per Spot)
- See Visium CytAssist for FFPE Tissue Preparation Guide for information on factors that can influence DV200

Fresh Frozen

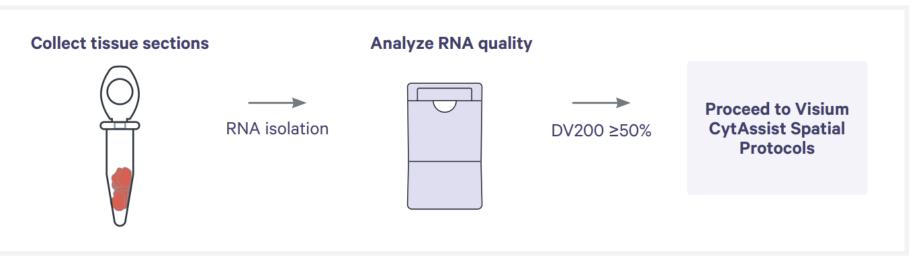
- RNA quality can be assessed by calculating the RNA Integrity Number (RIN)
- Cryosection 20-30 mg of tissue block (~4 x 25 μm)
- Remove excess OCT using chilled forceps
- Transfer sections to pre-cooled microcentrifuge tube on dry ice. Do not allow tissue sections to thaw
  Process immediately or store tube at -80°C
- Extract bulk RNA using a validated kit following manufacturer's protocol. Place RNA on wet ice
- Calculate RIN score by running RNA on a Bioanalyzer RNA Pico chip or similar
- For optimal assay performance, we recommend using blocks with a RIN ≥ 4



Proceed to Visium CytAssist Spatial Protocols



**Fixed Frozen** 



- Measurement of the percentage of total RNA fragments >200 nucleotides (DV200) upstream of library preparation
- Cryosection 20-30 mg of tissue block (~4 x 25 μm)
- Remove excess OCT using razor blade or chilled forceps
- Transfer sections to pre-cooled microcentrifuge tube on dry ice
   Do not allow tissue sections to thaw; process immediately or store tube at -80°C
- Extract bulk RNA using Biotium CELLDATA RNAstorm FFPE RNA Extraction Kit following manufacturer's protocol
   Place RNA on wet ice
- Store purified RNA at -80°C for long-term storage or immediately proceed to DV200 calculation using either BioAnalyzer, TapeStation, or ScreenTape reagents
- Recommendation: blocks with DV200 >50%

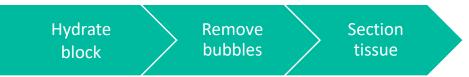








## **Preparing for Tissue Sectioning and Placement**



#### **Minimum requirements:**

- Histology-grade
- Must be able to generate thin, consistent section thickness



Item	Alternatives/Options	Vendor	Part Numbe
Microtome	Epredia HM 355S Automatic Microtome Or any standard histology grade microtome	Fisher Scientific	23-900-672
Microtome blade	Epredia MX35 Premier Disposable Microtome Blades, Low Profile	Fisher Scientific	3052835
Cool-Cut, Optional	Thermo Scientific Cool-Cut, Optional	Fisher Scientific	77-112-0
Section transfer system (STS)	Thermo Scientific Section Transfer System (STS), Optional - If using Section Transfer System	Fisher Scientific	771200
Probes	Fisherbrand Fine Precision Probe	Fisher Scientific	12-000-153
Forceps	Fisherbrand Curved Medium Point General Purpose Forceps	Fisher Scientific	16-100-110
Microscope slides	Fisherbrand Premium Plain Glass Microscope Slides	Fisher Scientific	12-544-4
Water bath	Epredia Digital Round Tissue Section Water Bath Or any equivalent water bath	Fisher Scientific	A84600061
Slide dryer	Epredia High Capacity Section Dryer	Fisher Scientific	A84600051
Brushes	Camel Hair Brushes, Or any equivalent paintbrush	Ted Pella	11859
Additional Materials			
Razor blades		-	-
Ice bucket		-	-
Ultrapure/Milli-Q Water, from Milli-Q Integral Ultrapure V	Nater System or equivalent	-	-

#### Maintenance of workspace:

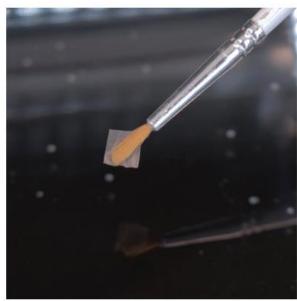
- Clean with RNaseZap RNase Decontamination Solution
- Use a brush for cleaning
- Be sure to wipe off oils with ethanol; let dry before sectioning
- Set clearance angle at 10°



### **Transfer the Section**



# Place section in the water bath





Only float ribbons if the optimal float time has been determined and placement is well-practiced

- Use a heated water bath and float sections
- Ensure the water bath is free from bubbles & particulates
- 42°C works well for most blocks and tissues
- Place it on the surface of the water bath
- Make sure that the brush tip goes underneath and away from the section



## **Floating the Section**

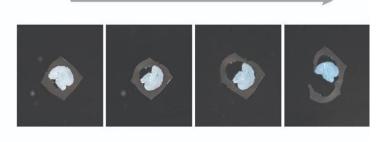




Only float ribbons if the optimal float time has been determined and placement is well-practiced



**Ideal Floating Time** 



Section disintegration due to increased floating time

Section Appearance	Floating Time
Section is flat with no wrinkles	Sufficient floating
Section has wrinkles	Insufficient floating; increase the floating time
Section is torn	Section left too long; decrease the floating time

- Let the section float on the water bath until most of it is flat and without wrinkles
- Optimal floating time depends upon the sample type and block.
- Float the section on a plain glass slide and perform a quality check under a microscope
  - Some sections may never be completely wrinklefree
  - See Visium CytAssist for FFPE Tissue Preparation Guide for troubleshooting section floating

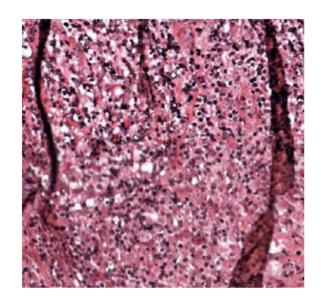


#### **FFPE**



### Common sectioning/tissue artifacts to avoid

#### Wrinkles



#### Causes

- Section compression (due to warm block or dull blade) during sectioning leads to wrinkle formation. These wrinkles become permanent when placed in the water bath.
- Accumulated wax or static electricity on microtome parts also contribute to section compression.
- Incorrect blade/clearance angle may cause compression.

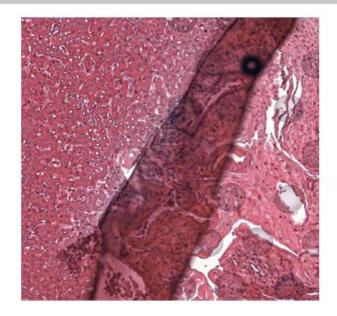
#### Troubleshooting

- · Ensure that the block is well hydrated.
- Adjust temperature down and increase float time.
- Gently and gradually lay FFPE sections onto water bath surface, lengthwise.
- Utilize a new blade.
- Ensure microtome is cleaned with 100% ethanol to minimize static and section compression (bunching on blade).
- Ensure blade/clearance angle is correct prior to sectioning.



### Common sectioning/tissue artifacts to avoid

#### Folds



#### Causes

- Mostly happens when placing the section on the water bath especially when the section is wavy.
- If the fold is at the edge this most likely can happen during sectioning or mounting on the slide.

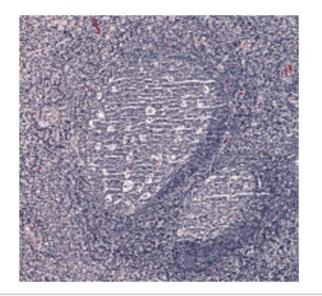
#### Troubleshooting

- Gently and gradually lay FFPE ribbons or sections onto water bath surface, lengthwise.
- If sections curl during sectioning, gently flatten them with a brush before floating.



### Common sectioning/tissue artifacts to avoid

#### Venetian Blinds or Shatter



#### Causes

- Parallel lines in the section mostly appear due to dry tissue because of underhydration of the block in the ice bath.
- Less likely due to dull blade or loose parts of the microtome.

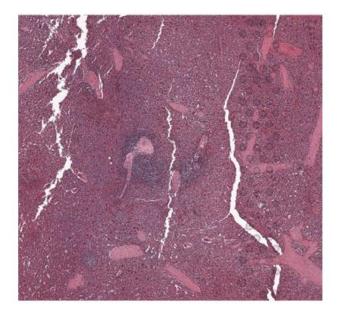
#### Troubleshooting

- Increase incubation time of the block in ice bath.
- Tighten down components of microtome and make sure the blade is at a correct angle.



#### Common sectioning/tissue artifacts to avoid

#### Cracks



#### Causes

- Dry and over-processed tissue can crack during sectioning.
- The cracks that are created before tissue embedding will be filled with wax when section is observed under the microscope after sectioning and wax will be washed away after deparaffinization or H&E staining.

#### Troubleshooting

- Prolonged hydration on the ice bath will most likely reduce the cracks.
- There is no solution for cracks created before tissue was embedded in wax.



## **Prep & Sectioning**

#### **Fresh Frozen**



## **Sample Preparation – Freezing & Embedding**

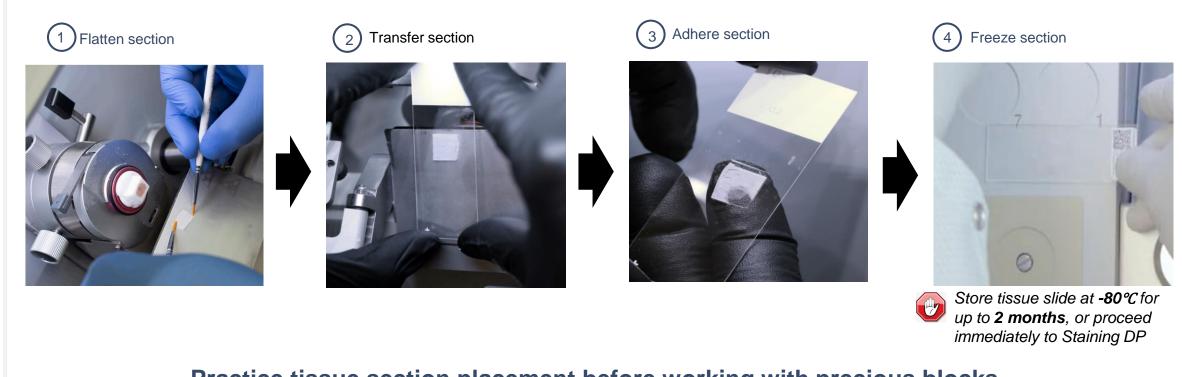
Fresh Tissue is frozen & embedded in Optimal Cutting Temperature Compound (OCT)





### **Sample Preparation – Section Placement**

FF sections are placed on compatible glass slides



#### Practice tissue section placement before working with precious blocks



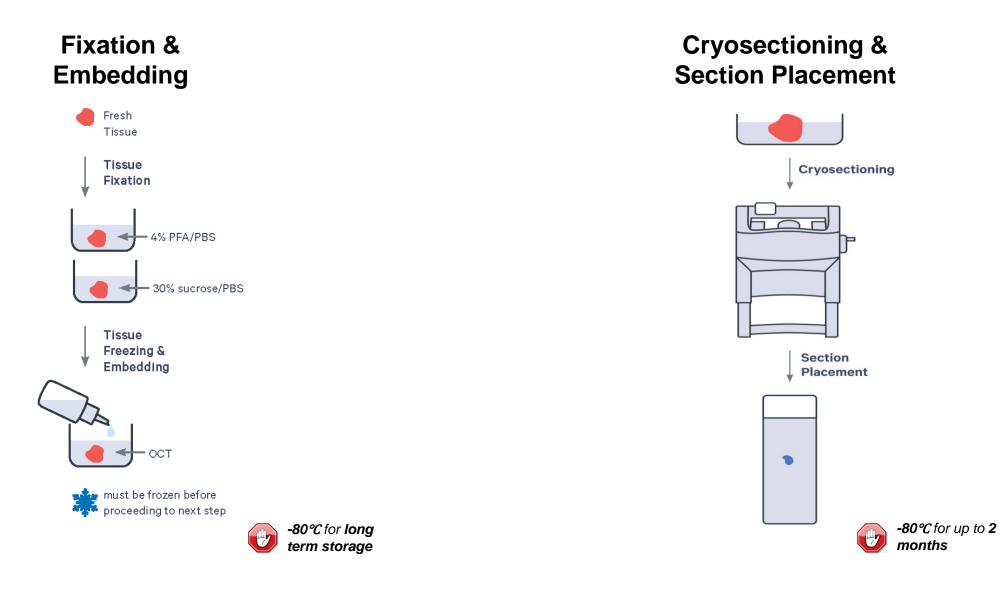
## **Prep & Sectioning**

#### **Fixed Frozen**



### **Sample Preparation Overview**

Fixed Frozen





### **Sample Preparation – Fixation**

Fresh Tissue is fixed in 4% Paraformaldehyde (PFA)



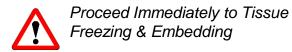
Work quickly between tissue harvest and fixation

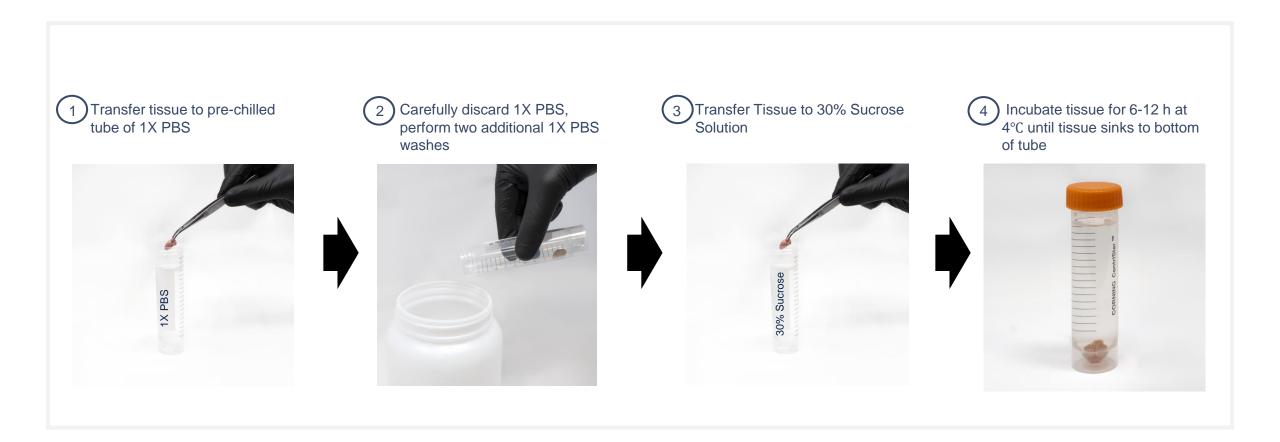




### **Sample Preparation – Cryopreservation**

Fixed Tissue is cryopreserved in 30% Sucrose





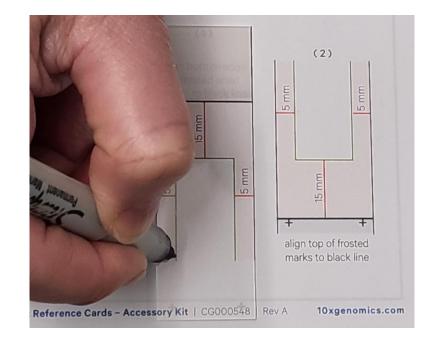


**Fresh & Fixed Frozen** 



- Do not allow OCT embedded tissue block to thaw
- Determine optimal sectioning conditions based upon tissue type
- Draw allowable area based upon slide type on back of blank slides
- Pre-chill blank slide and tools prior to cryosectioning
- Once cold, keep slide in cryochamber throughout placement
- Practice tissue section placement within allowable area
- Transfer tissue slides into individual pre-cooled sealed containers
- Always transport contained tissue slides on dry ice

• Store sealed container containing Tissue Slides at -80°C for up to 2 months





Determine the ideal cutting temperature for your tissue type

Colder sectioning temperatures may result in an increase in tissue cracking

- Optimal sectioning temperatures differ across tissue types
  - High fat content tissues require colder temperatures due to lipid structure

#### Impact of Cryostat Specimen Head Temperatures on Tissue Tearing -14°C -20°C -30°C -10°C



Normal Section



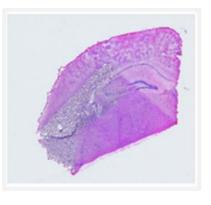






Common sectioning/tissue artifacts to avoid

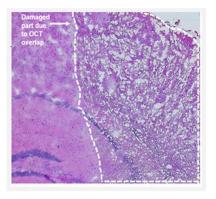
Folded section



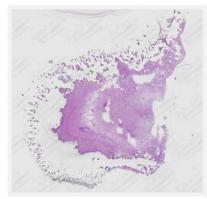
Overlapping sections



OCT overlapping tissue



Condensation formation





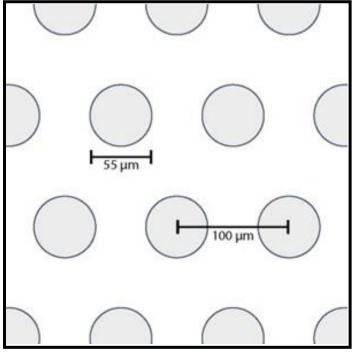
## Visium HD

**Discovering Spatial Biology at High Definition** 

## **Visium HD – Resolution**

#### Putting Visium HD in perspective

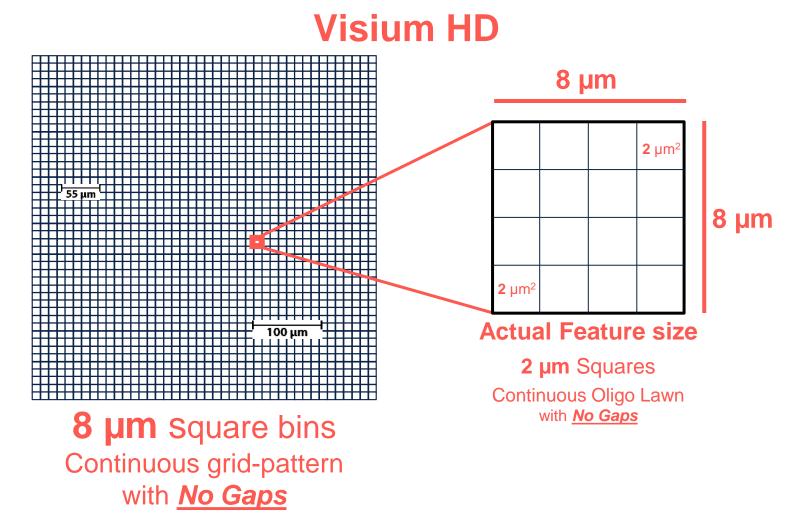
Visium



**55 μm** spots Hexagonally arranged *with 45 μm gaps* 

> **5,000** Features

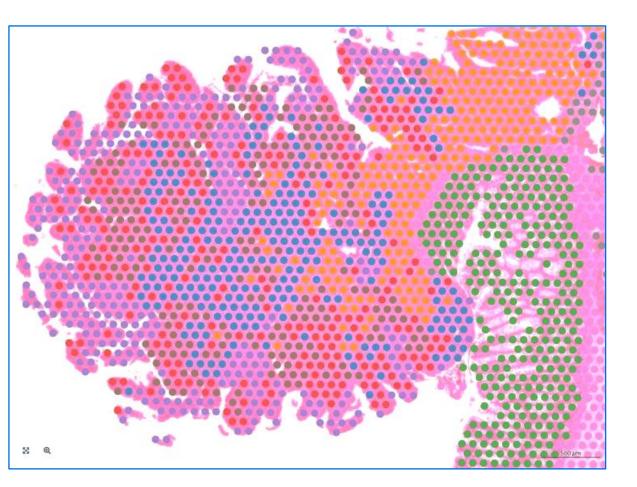
GENOMICS



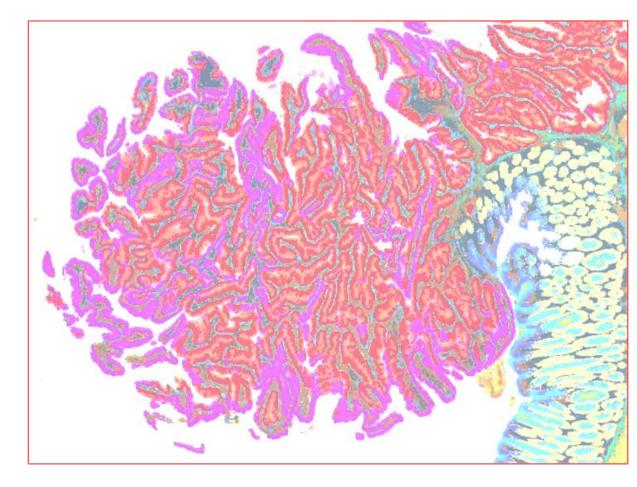


### Visium HD – Comparison to Standard Visium

#### **Visium**



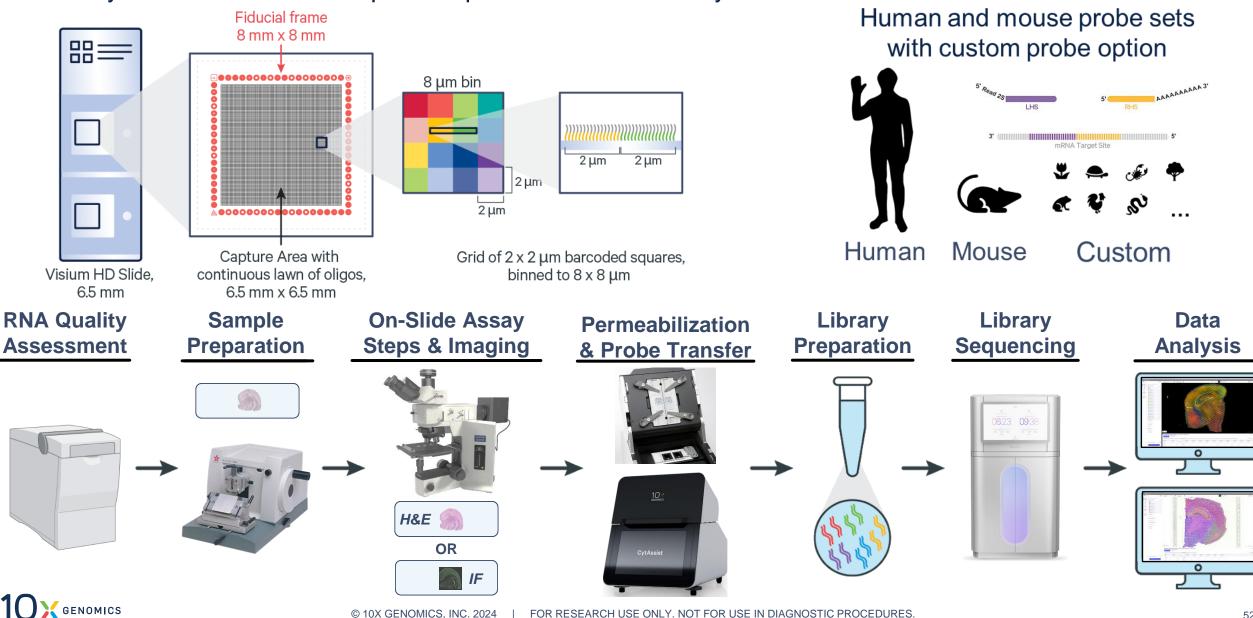
#### Visium HD





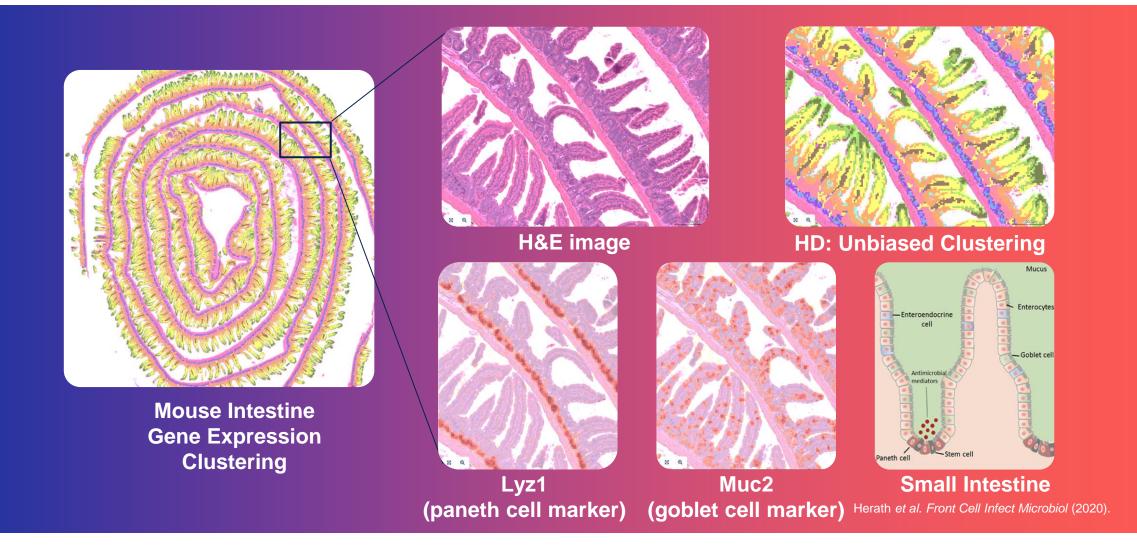
### Visium HD – CytAssist-Enabled Gene Expression Technology

#### Visium CytAssist instrument required – probe-based v2 assay



## Visium HD – Generate Accurate & Specific Spatial Maps

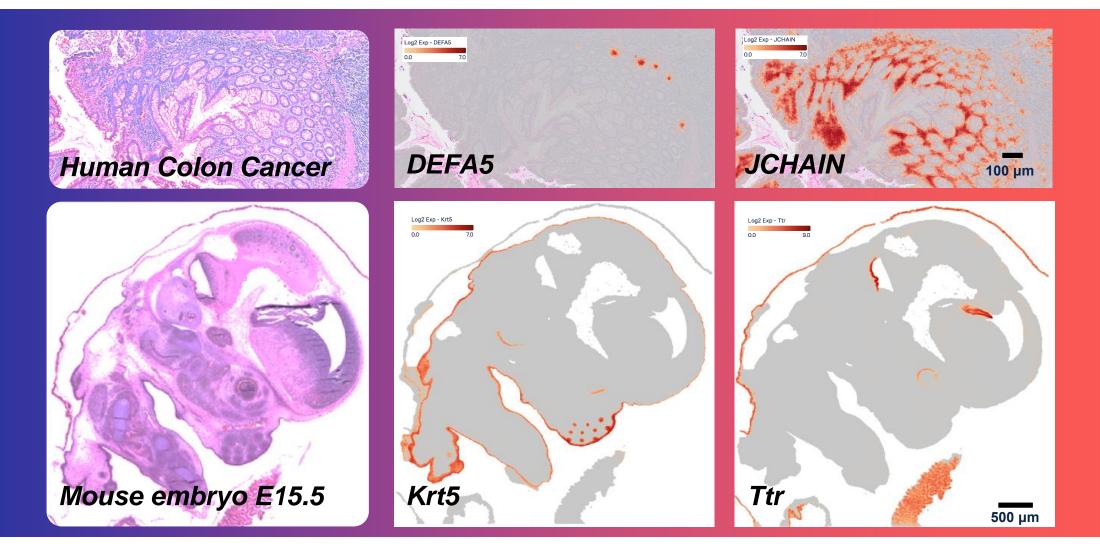
Visium HD precisely replicates known biology





### Visium HD – Achieve High-Quality Data with Confidence

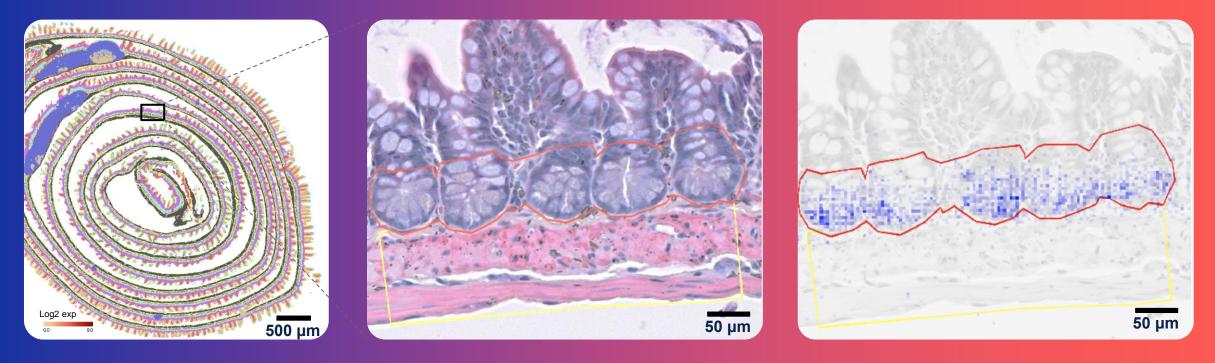
Accurate and precise transcript localization enabled by CytAssist





### Visium HD – Achieve High-Quality Data with Confidence

Accurate and precise transcript localization enabled by CytAssist



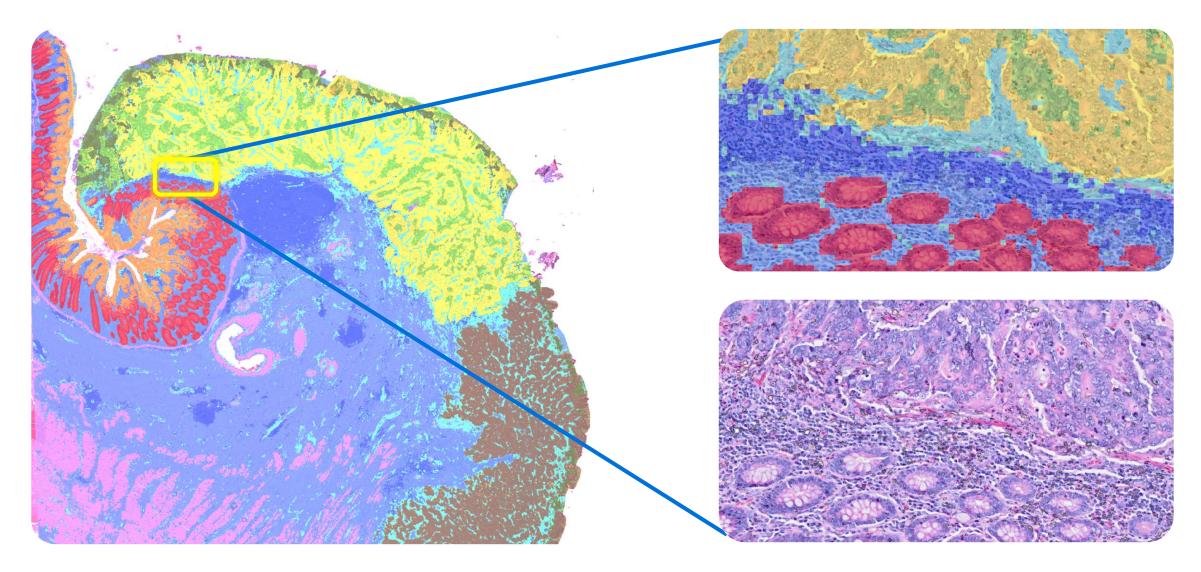
Mouse intestine Graph-based clusters

- Crypts containing paneth cells (Lyz1 + Defa21 expression expected)
- Non-epithelial layers below the crypt (Lyz1 + Defa21 expression not expected)

**95%** of Lyz1 and Defa21 transcripts localized in the crypt region

## Visium HD – Enhanced Histology with Whole Transcriptome

Colon Cancer FFPE: 18,058 genes detected

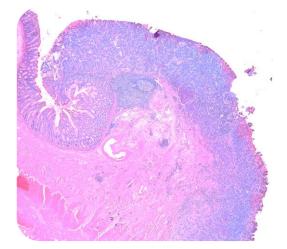




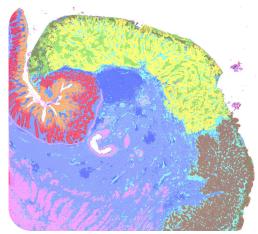
### Visium HD – Transcript Density & Tissue Morphology Correlation

Colon Cancer FFPE: 18,058 genes detected

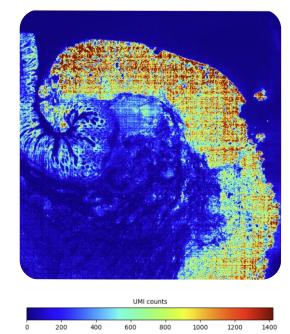
#### H&E Stain

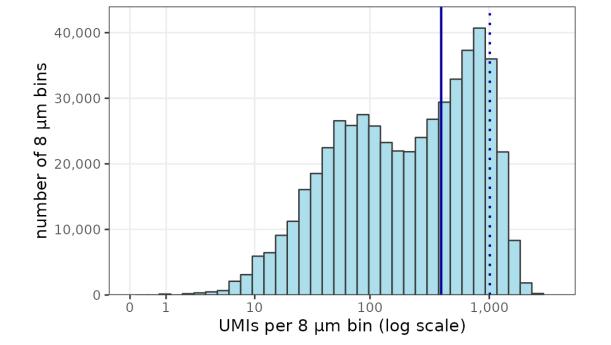


**Gene Expression Clustering** 



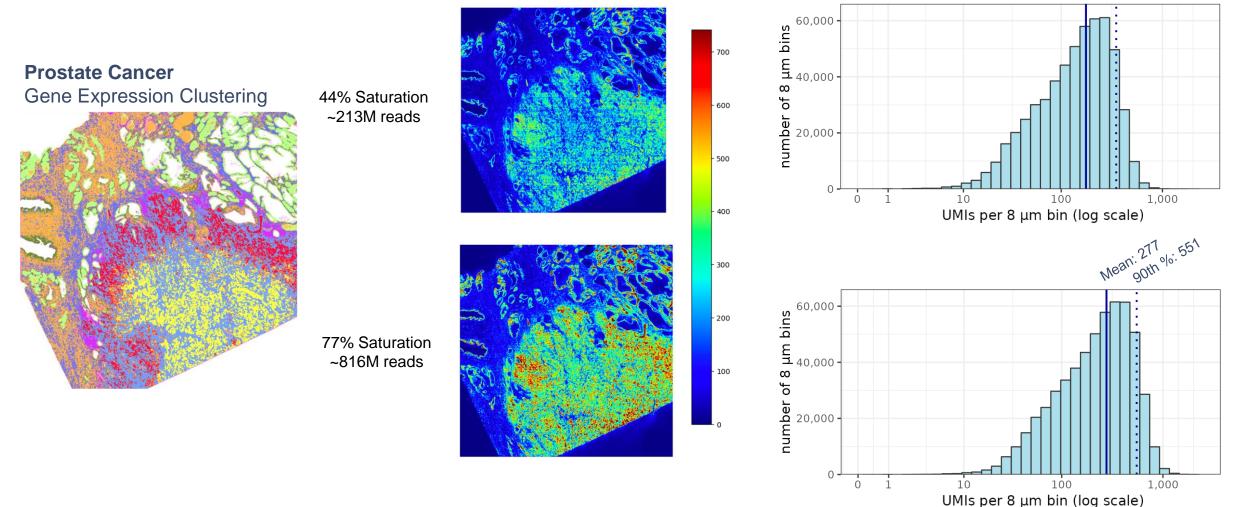
UMI Heat Map (per 8 µm bin)







## Visium HD – Deep Sequencing Increases Transcript Recovery



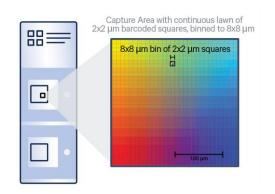
UMI Heat Map (per 8 µm bin)

·din. 100.350

Mean: 176

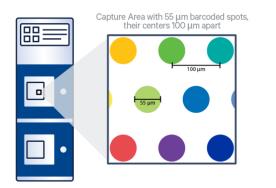
GENOMICS

### Visium HD – Deeper Insight with Higher Resolution

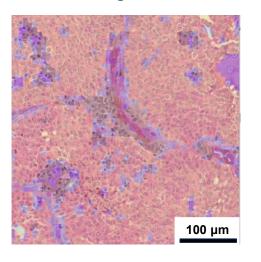


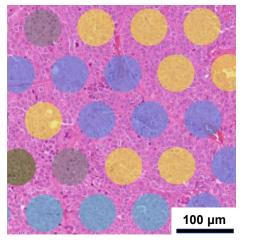
**Visium HD Slide** 

Visium v2 Slide (Visium CytAssist Spatial Gene Expression for FFPE)

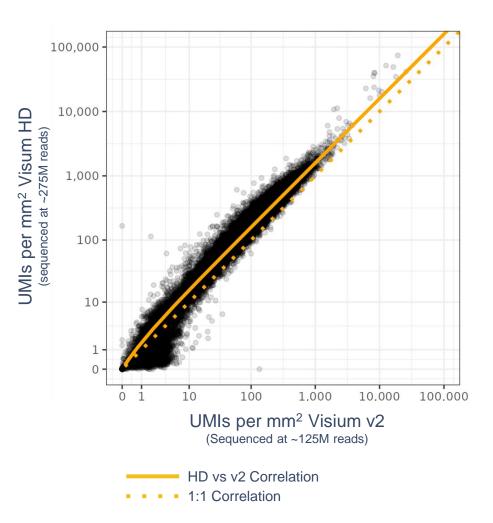


Graph-Based Clustering and H&E Overlay Matched Lung FFPE Sections

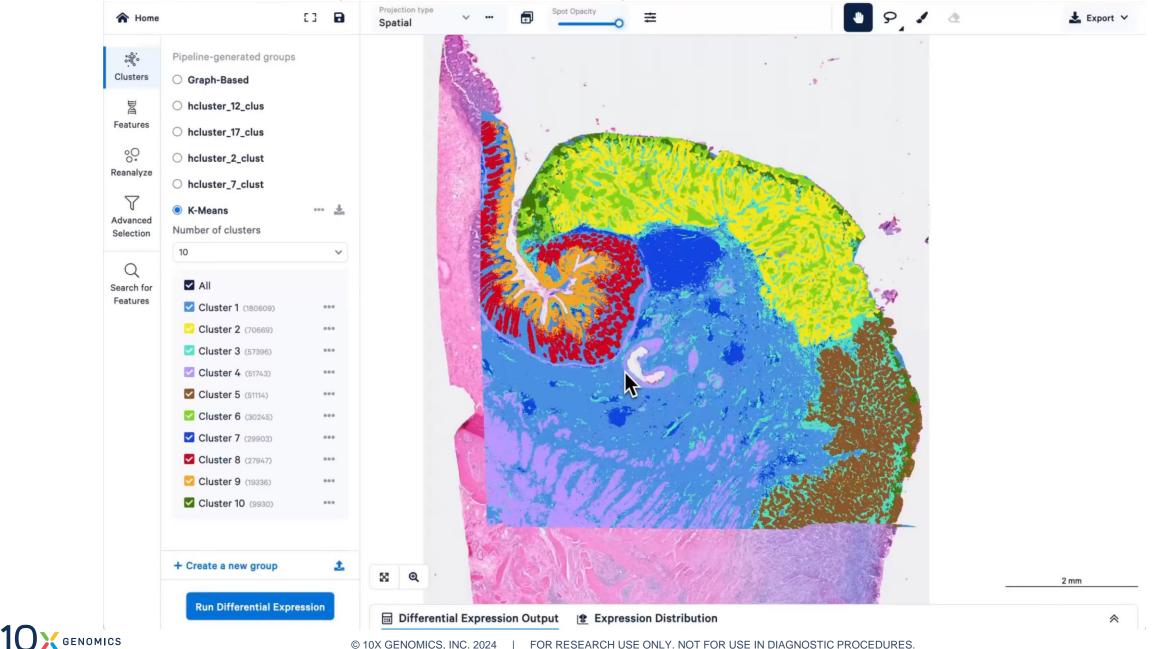




HD and v2 Correlation Matched Lung FFPE Sections



### Visium HD – Easy-To-Use Analysis Tools Hastens Discovery



60

## Visium HD – Visium at High Definition

All the discovery power you want with the resolution you need



Preorder Now!! Ships End of Q1 '24

- Whole transcriptome unbiased analysis from an entire tissue section at single cell scale
- Slide contains arrayed barcoded oligos leveraging NGS while preserving spatial locations

• **FFPE** compatibility at launch

- 6.5 x 6.5 mm capture areas (2 per slide)
- The capture area is grid of 2 x 2 µm barcoded squares (no gaps)

SPATIAL CAPTURE SLIDES & REAGENTS	SOFTWARE





### **Visium Has Powered Impactful Research**

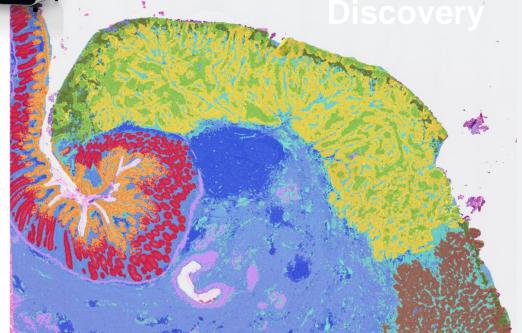
**Cumulative Visium publications** 



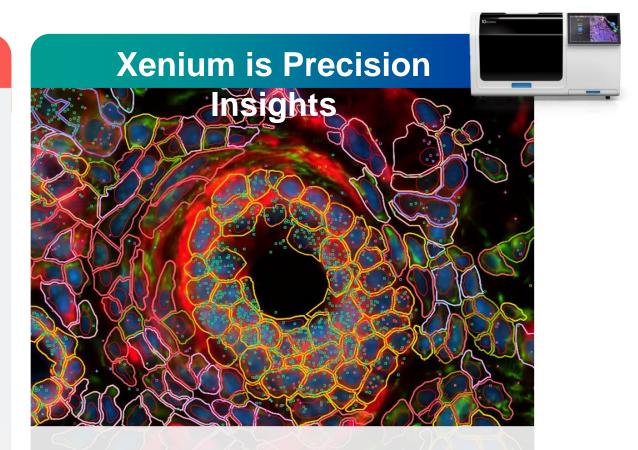


### **Unlock the Full Spectrum of Spatial Biology**

# Visium HD is Unbiased



- Whole transcriptome
- Sequencing based
- Transcripts assigned to multi-micron areas



- 100s–1000s of transcripts
- High-resolution imaging based
- Transcripts assigned to cells



## **Biology's Most Comprehensive Toolkit**





### Visium CytAssist: Ready to Explore More

Simplifying spatial sample preparation and broadening sample access



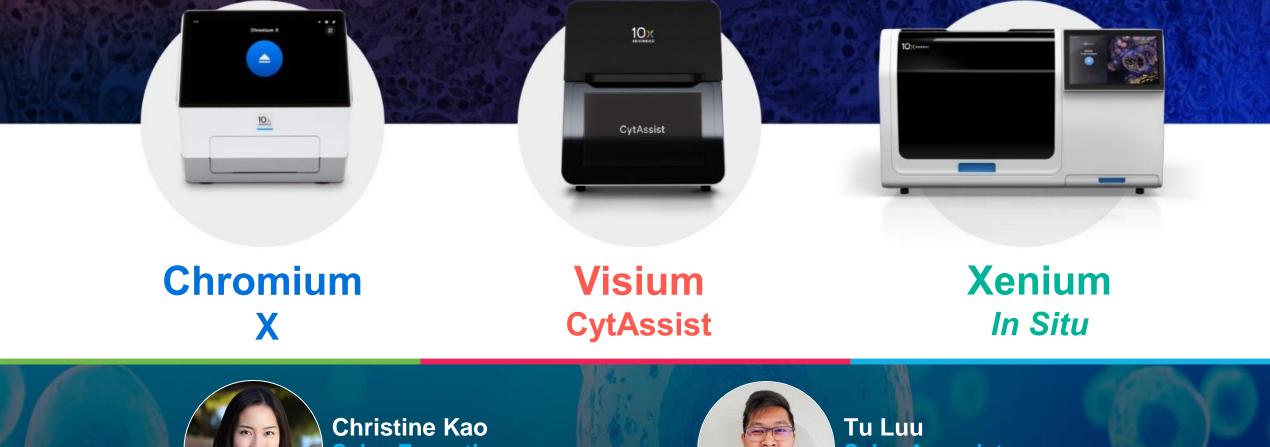
#### Your Sample, Your Spatial Story

Spatial Gene Expression for FFPE Spatial Gene & Protein Expression for FFPE Spatial Gene Expression for Fresh Frozen Spatial Gene Expression for Fixed Frozen Spatial Gene Expression for Tissue Microarrays Spot Deconvolution Validation of Xylene Alternatives Visium HD Spatial Gene Expression

**Coming Soon** Sample Prep Recommendation for Skin & Bone



## Thank you Want to Know More? We are always happy to schedule a meeting!



Sales Executive christine.kao@10xgenomics.co



Sales Associate