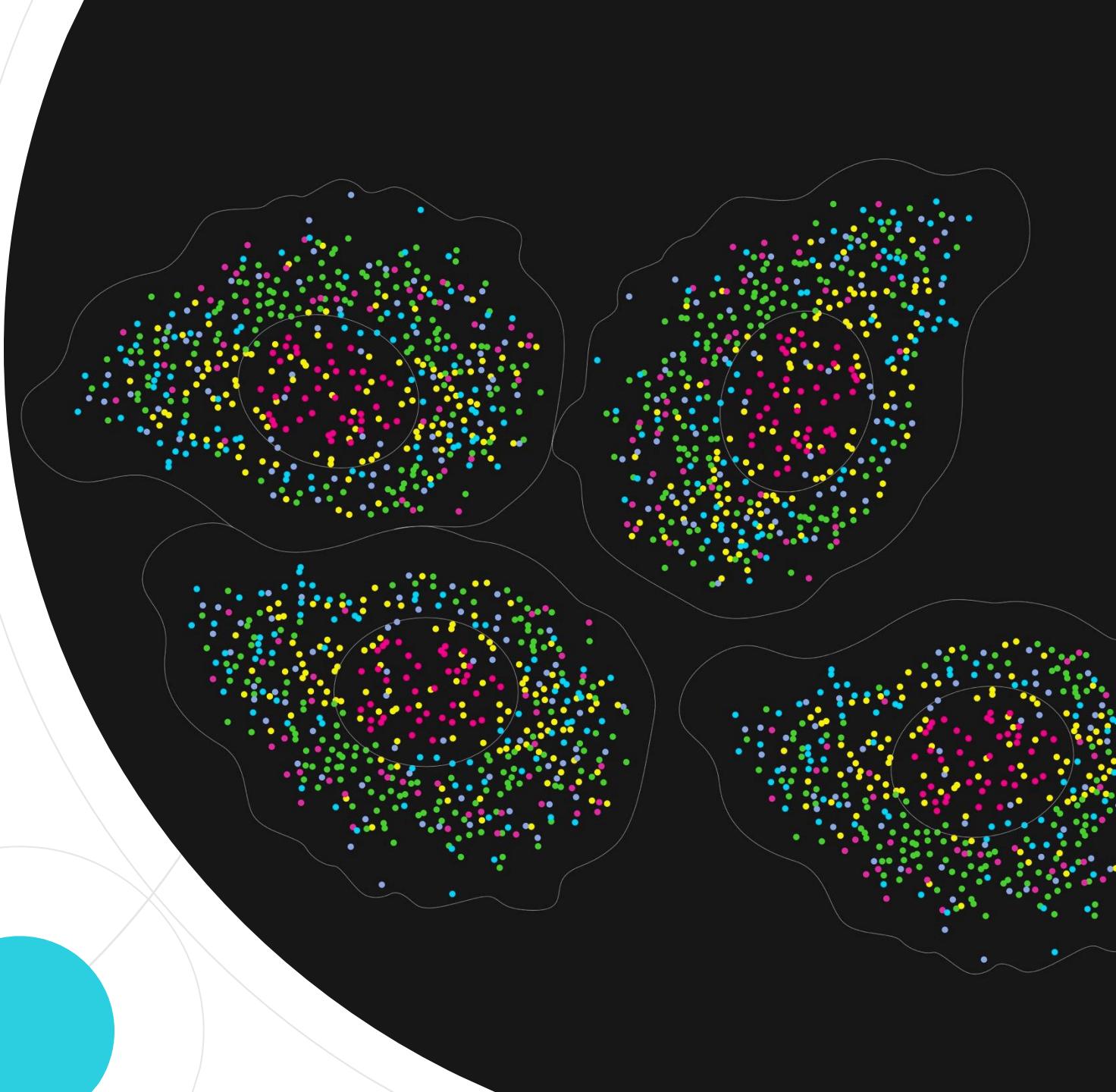


# Mapping the Future with Spatial Genomics

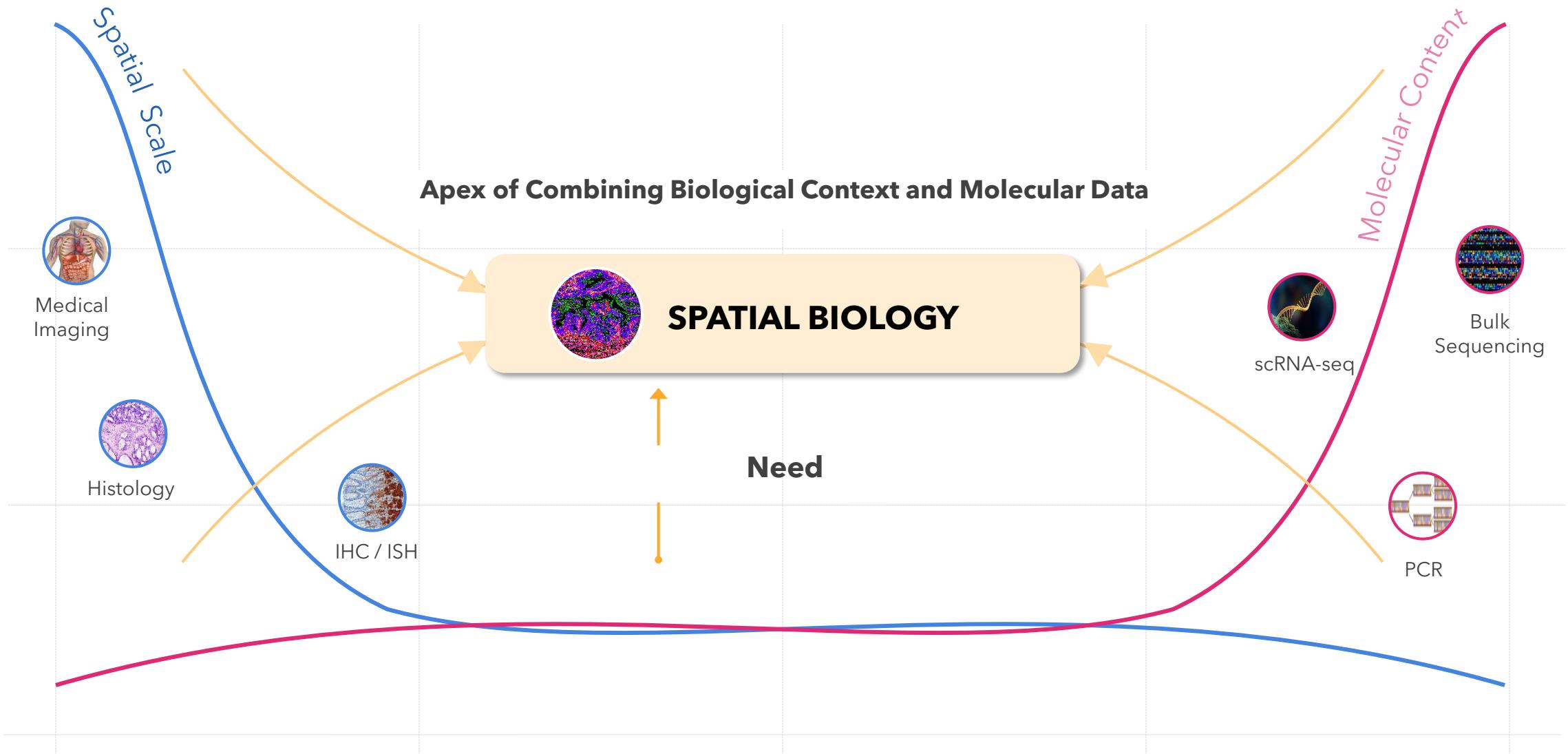
Cong Luan Nguyen

Business Manager, APAC

Vizgen Ltd.



# What is Spatial Biology Technology?

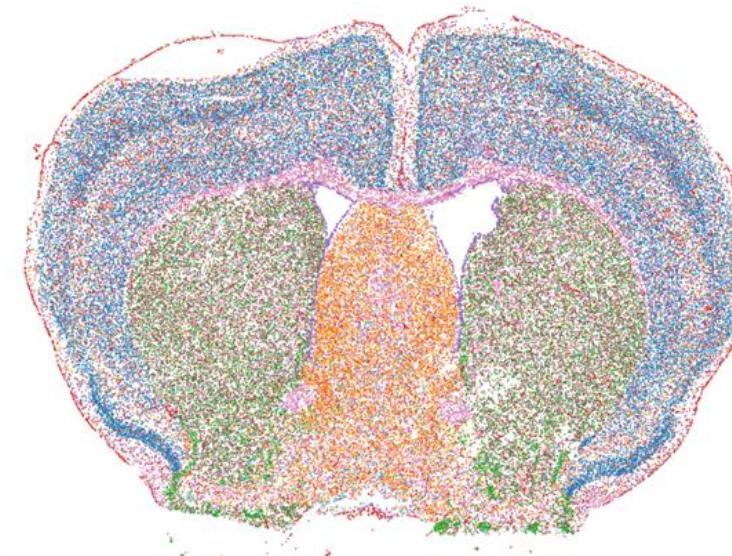


Bulk and single cell sequencing are fundamentally limited

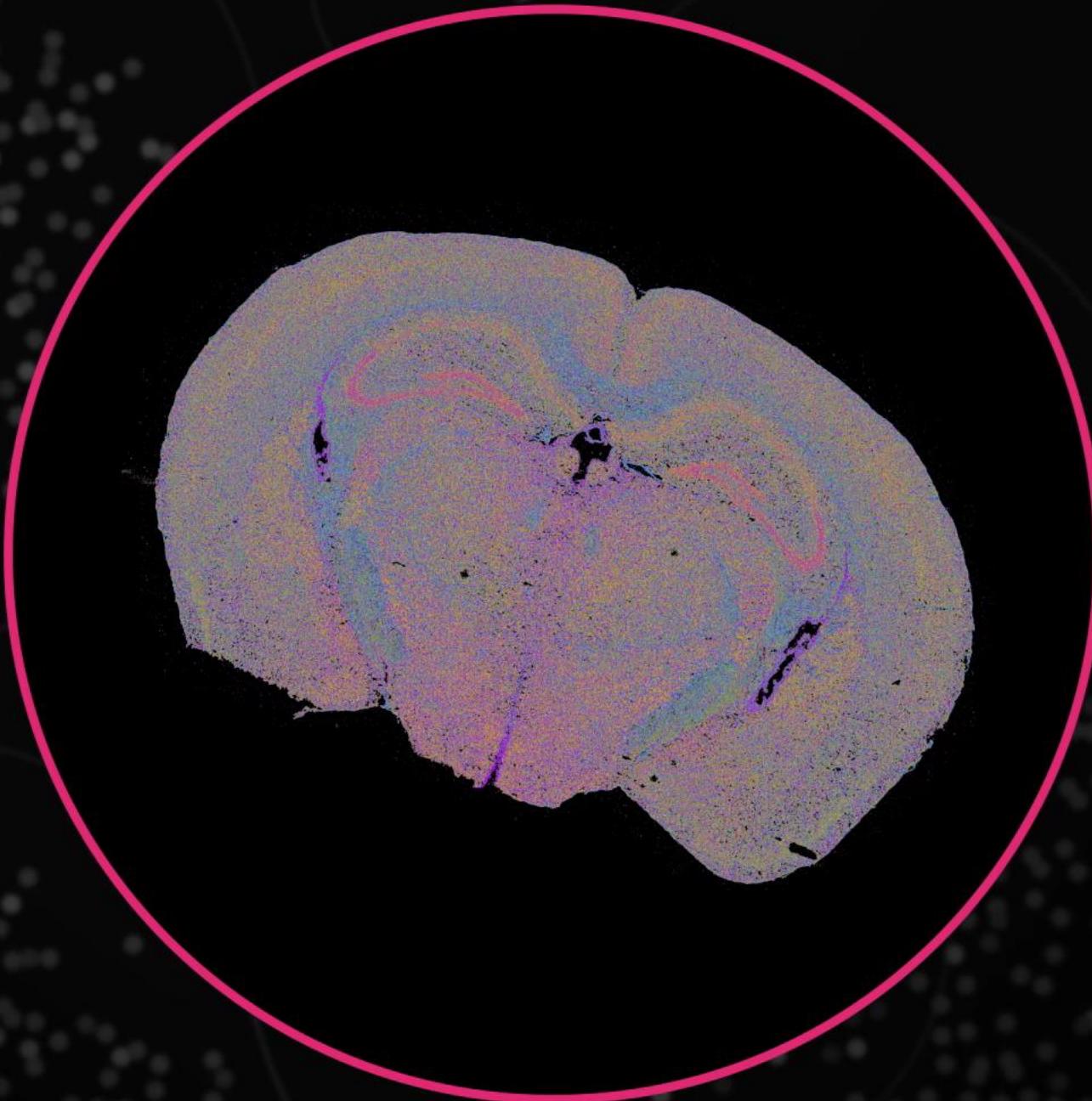
# Biological systems are intricately spatially organized



**Bulk and single cell sequencing**  
can show us parts



**Spatial genomics with true single cell resolution**  
offers highly multiplexed direct *in situ* detection  
and valuable insight into the bigger picture





Headquarters  
in Cambridge,  
Massachusetts



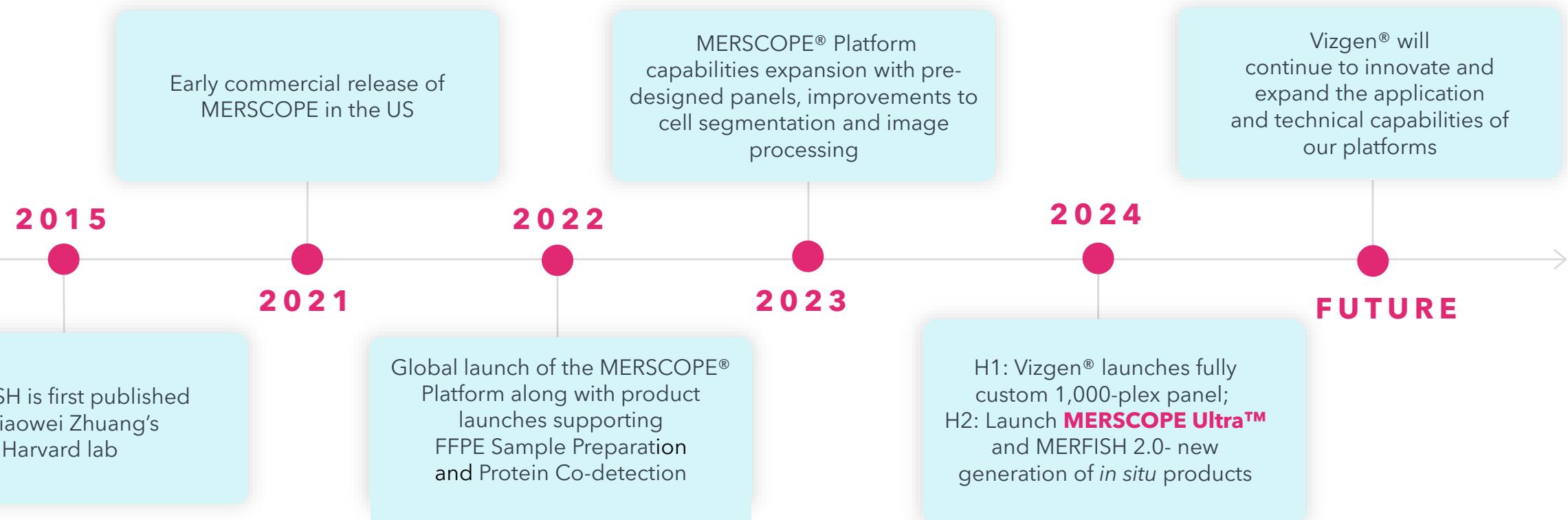
## MISSION

To work tirelessly with researchers  
to deliver the best tools for spatial  
biology that enable discoveries to  
improve human life.

## VISION

A world where every disease has a  
map, and every cure has a path.

# Enabling groundbreaking research with spatial genomics

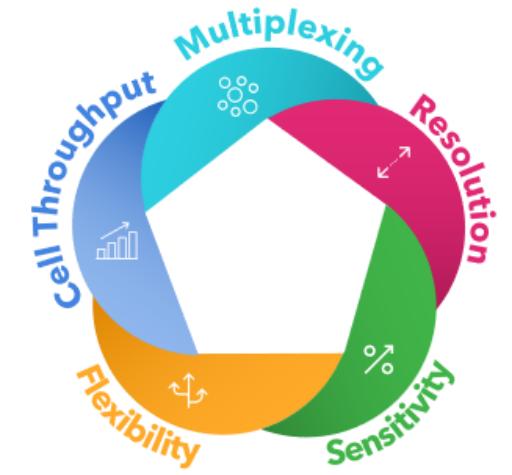
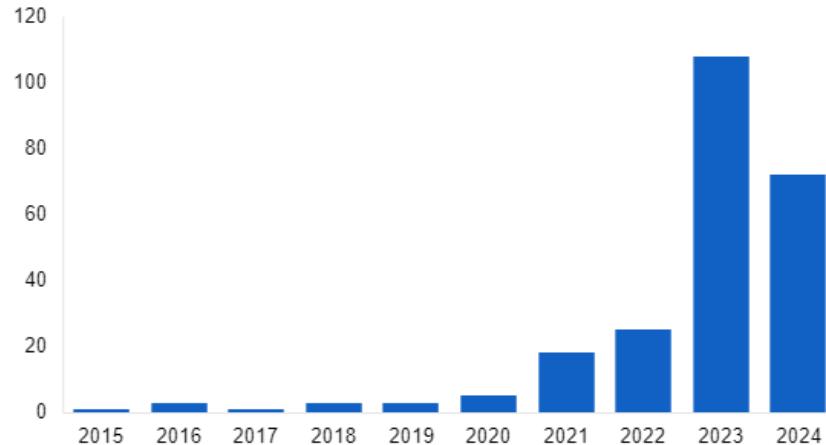


Established as the only provider of commercial MERFISH instruments

**MERSCOPE Ultra™ is an end-to-end solution with an exceptional range of applications across interests and industries**

# MERFISH is the leading single-cell resolution *in-situ* high-plex spatial transcriptomics technology with over 260 published papers & preprints

MERFISH publication by year



Proceedings of  
the National Academy of Sciences  
of the United States of America

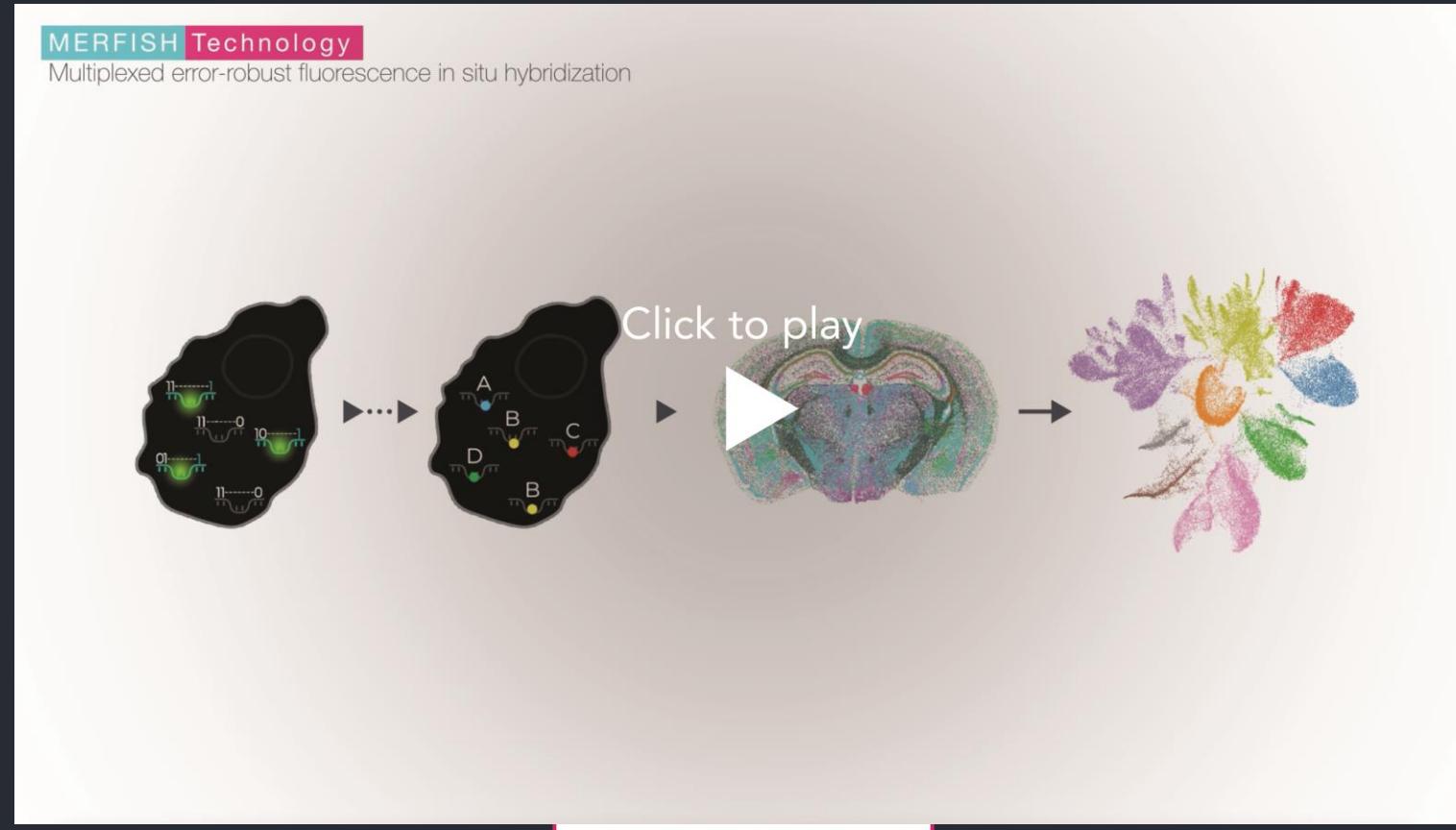


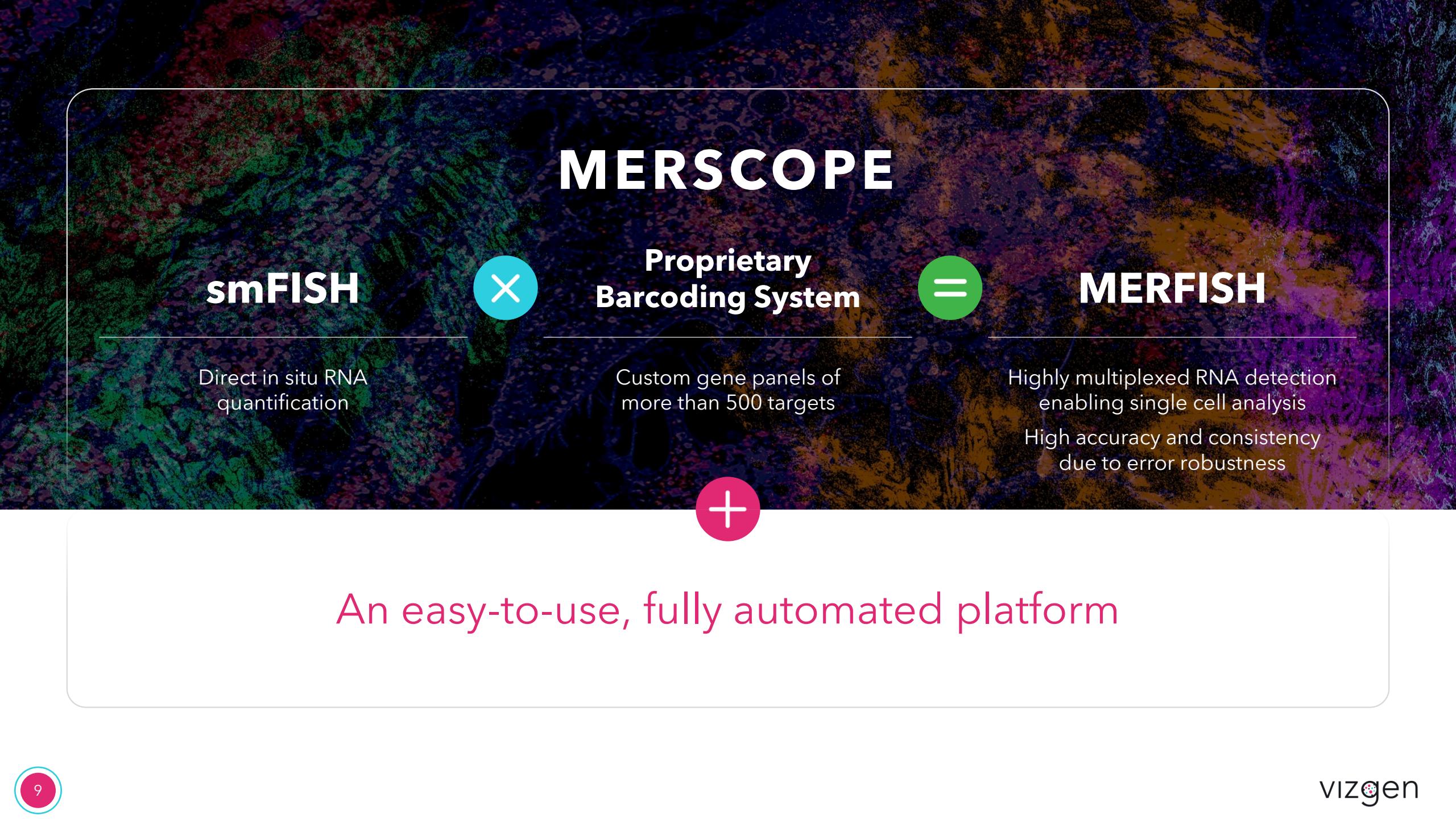
**260+** peer reviewed publications and preprints across many therapeutic areas such as neuro, oncology, cardio and infectious disease; in high impact journals

**100s** of researchers in academia, pharma and service organizations are using MERSCOPE to further their research and drug development

# How MERFISH Works

Multiplexed Error-Robust Fluorescence *in situ* Hybridization (MERFISH) is a spatially resolved single-cell transcriptome profiling technology. It combines the power of single-cell transcriptomics with spatial biology by directly visualizing and counting up to a billion RNA transcripts from 100's to 1000's of genes across whole tissue slices with single-cell resolution.





# smFISH

Direct *in situ* RNA quantification

# MERSCOPE

Proprietary  
Barcoding System



# MERFISH

Custom gene panels of  
more than 500 targets

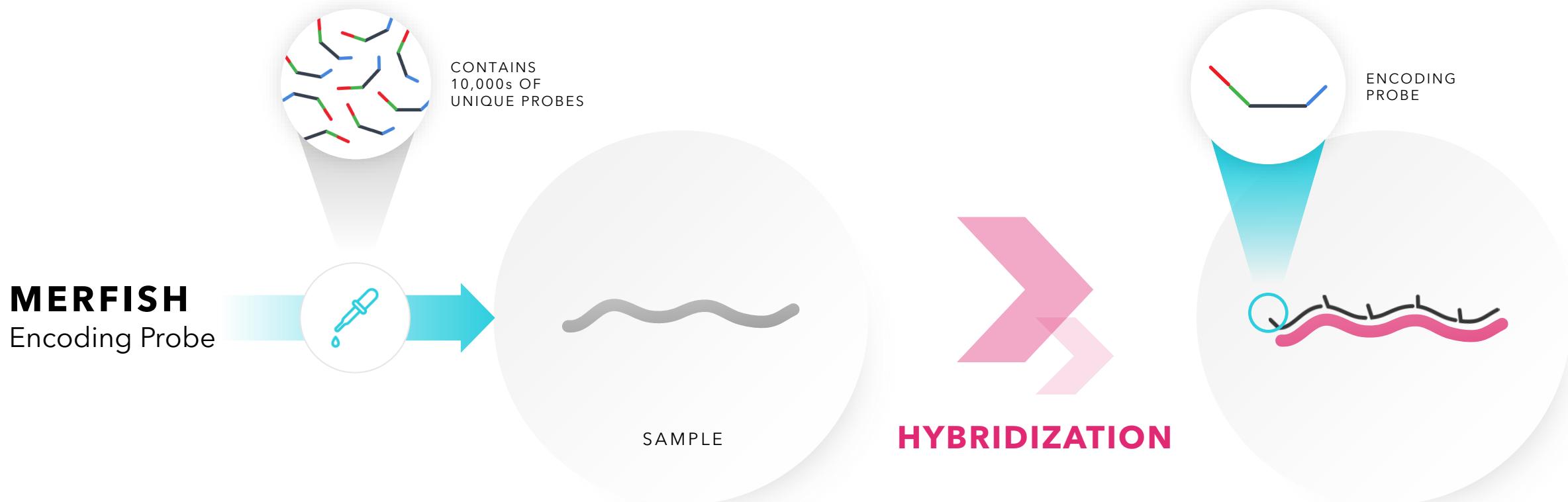
Highly multiplexed RNA detection  
enabling single cell analysis  
High accuracy and consistency  
due to error robustness



An easy-to-use, fully automated platform

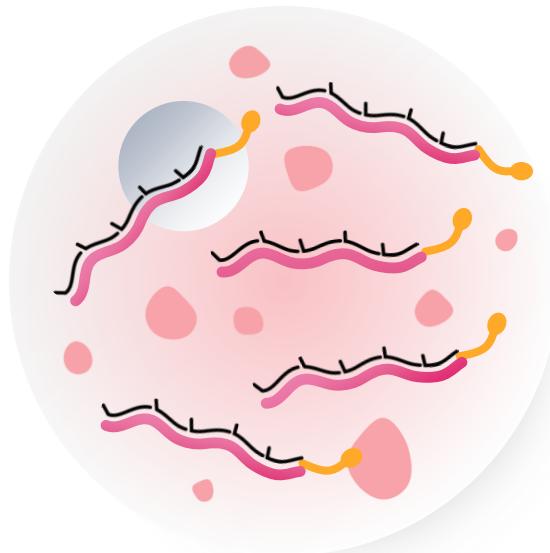
# STAGE 1

## Hybridization

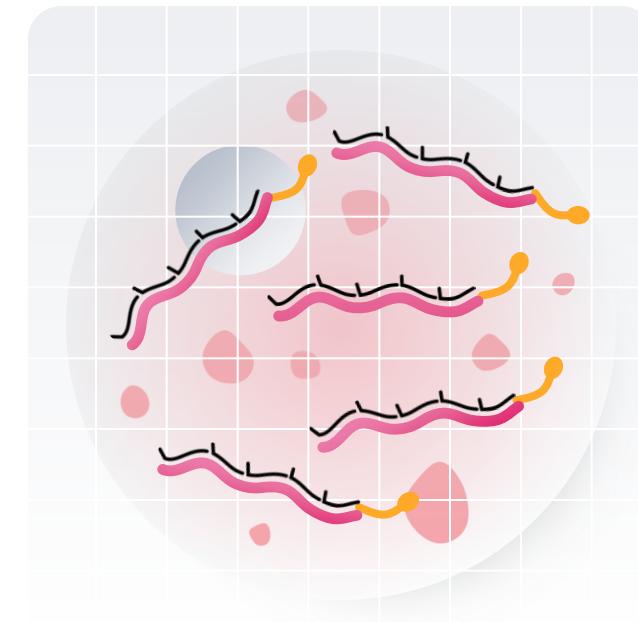


# STAGE 2

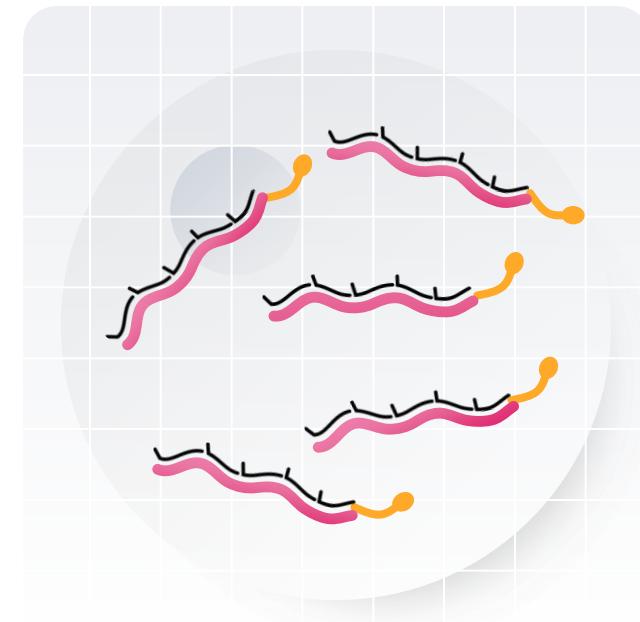
## Clearing



EMBED

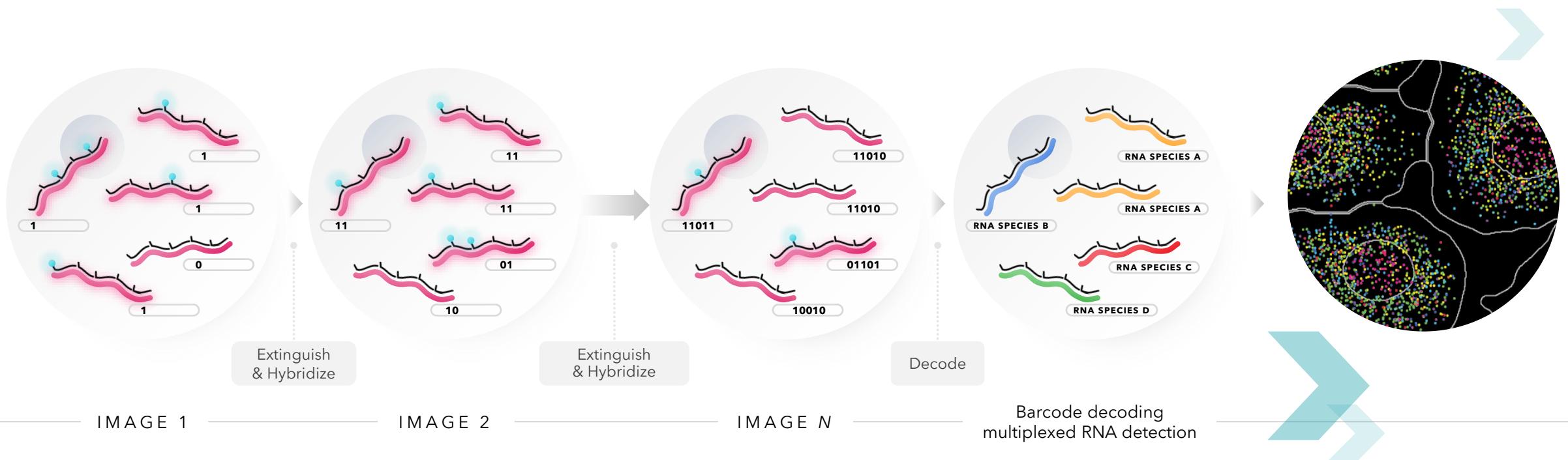


DIGEST



# STAGE 3

## Imaging



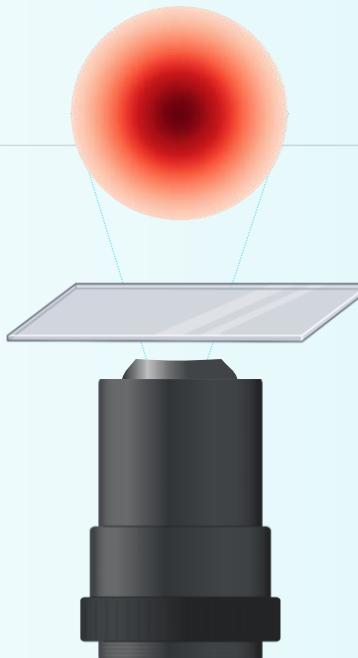
Chen et al, bioRxiv, 2021; Chen et al, Science, 2015; Emanuel et al, Nature Methods, 2017; Favuzzi et al, Cell, 2021; Hara et al, Cancer Cell, 2021; Lu et al, Cell Discov, 2021; Miller et al, CVPR, 2021; Moffitt et al, PNAS, 2016; Moffitt et al, Science, 2018; Park et al, Nat Commun, 2021; Su et al, Cell, 2020; Wang et al, BioRxiv, 2020; Wang et al, PNAS, 2019; Wang et al, Sci. Rep, 2018; Xia et al, PNAS, 2019; Xia et al, Sci. Rep, 2019; Zhang et al, bioRxiv, 2020

# High Spatial Resolution: Optimized Optics for Correct Transcript Count Per Cell

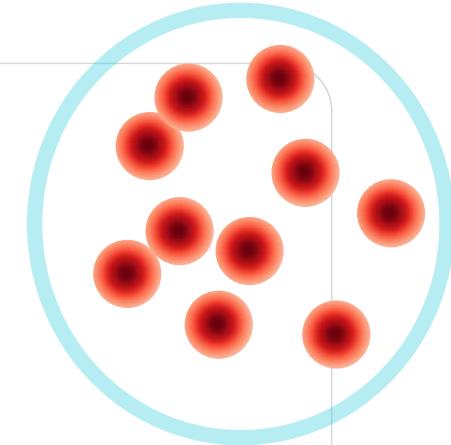
MERSCOPE



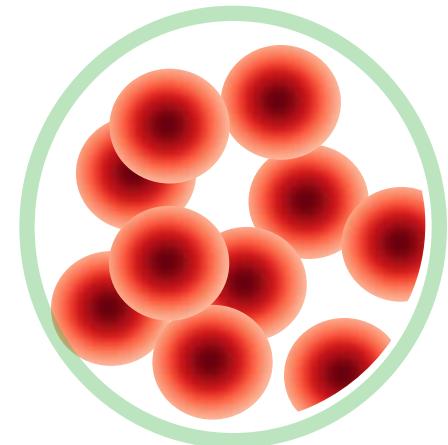
Alternative Technologies



MERSCOPE



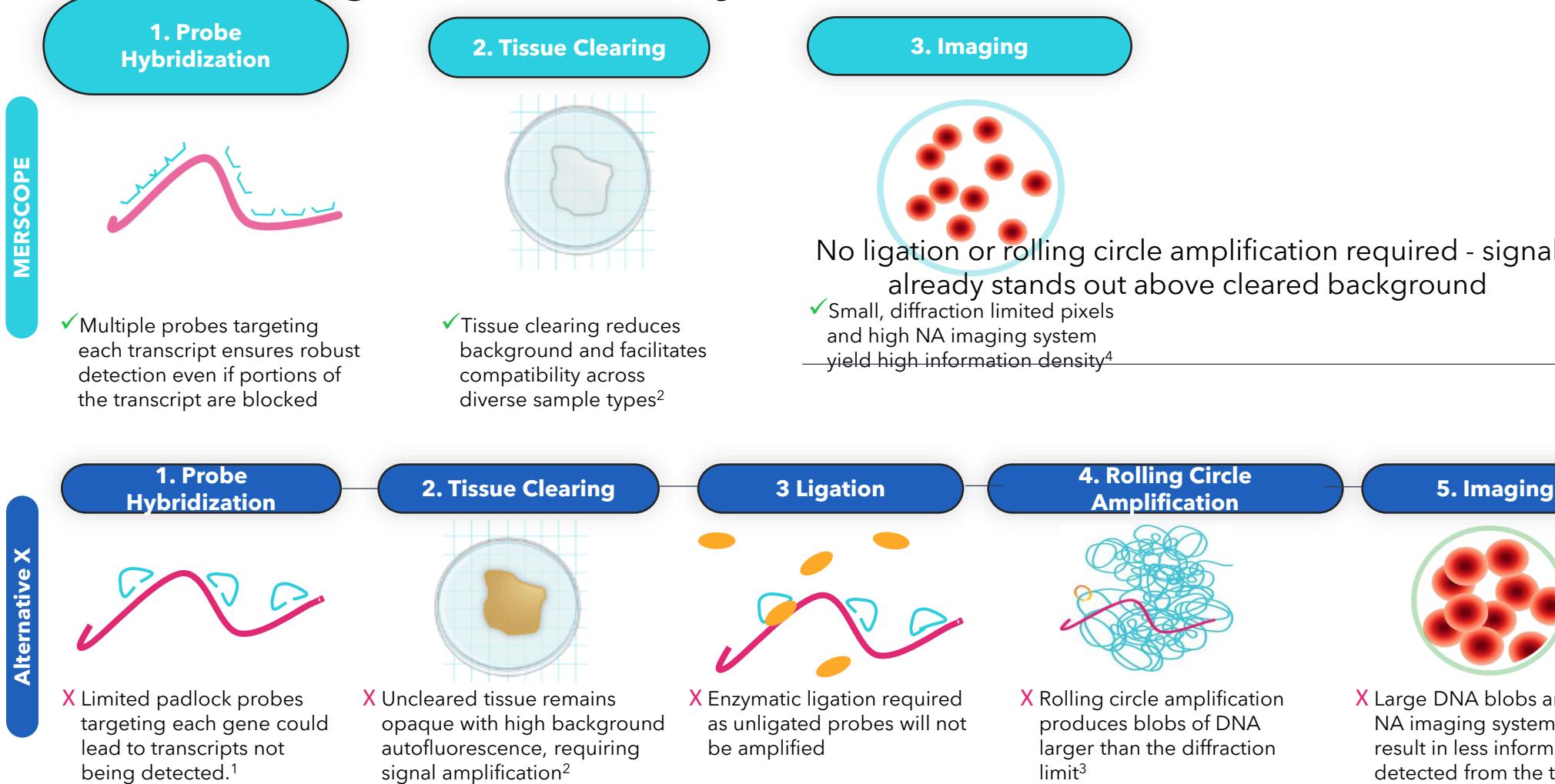
Alternative Technologies



MERSCOPE's higher resolution optical design enables greater discrimination to detect signals more accurately. Smaller spot size means more spots detected per cell.

Cellular volume is limited. Resolving more transcripts within a limited cellular volume provides more information for characterizing cellular state.

# MERSCOPE® designed to efficiently extract the most information from tissues



"With each amplicon being around 400–800 nm in diameter ([45](#)), the number of reads that can be obtained from each cell is likely limited to a couple of hundreds to lower thousands" Liu et al, NAR 2021

Not a Head to Head Study – Depicts Selected steps

<sup>1</sup>Xenium human breast cancer panel v1 uses between 2 and 16 probes per gene. <sup>2</sup>Moffitt et al, PNAS, 2016. <sup>3</sup>Liu et al, NAR, 2021 "Spatial crowdedness could be a challenge when targeting many genes, which is common to all RCA-based approaches" <sup>4</sup>Difference in NA is supported by MERSCOPE using an oil immersion microscope lens; applies to dense samples with high quality RNA.



vizgen

Analyzer:

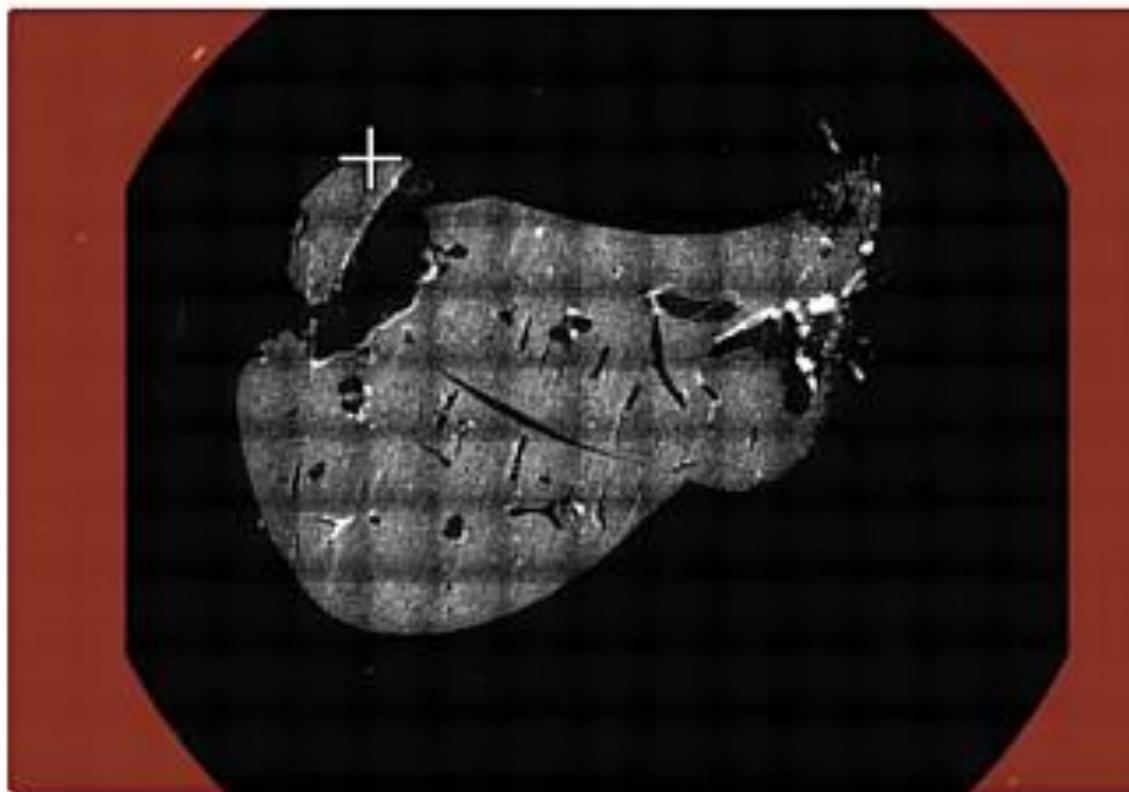
Configure Load Select Batch Experiment Open

Abort experiment



### Select Regions 2 of 2 – Select regions for MERFISH imaging

Experiment Demo



Visible Intensity Range: 0-65535



PolyT

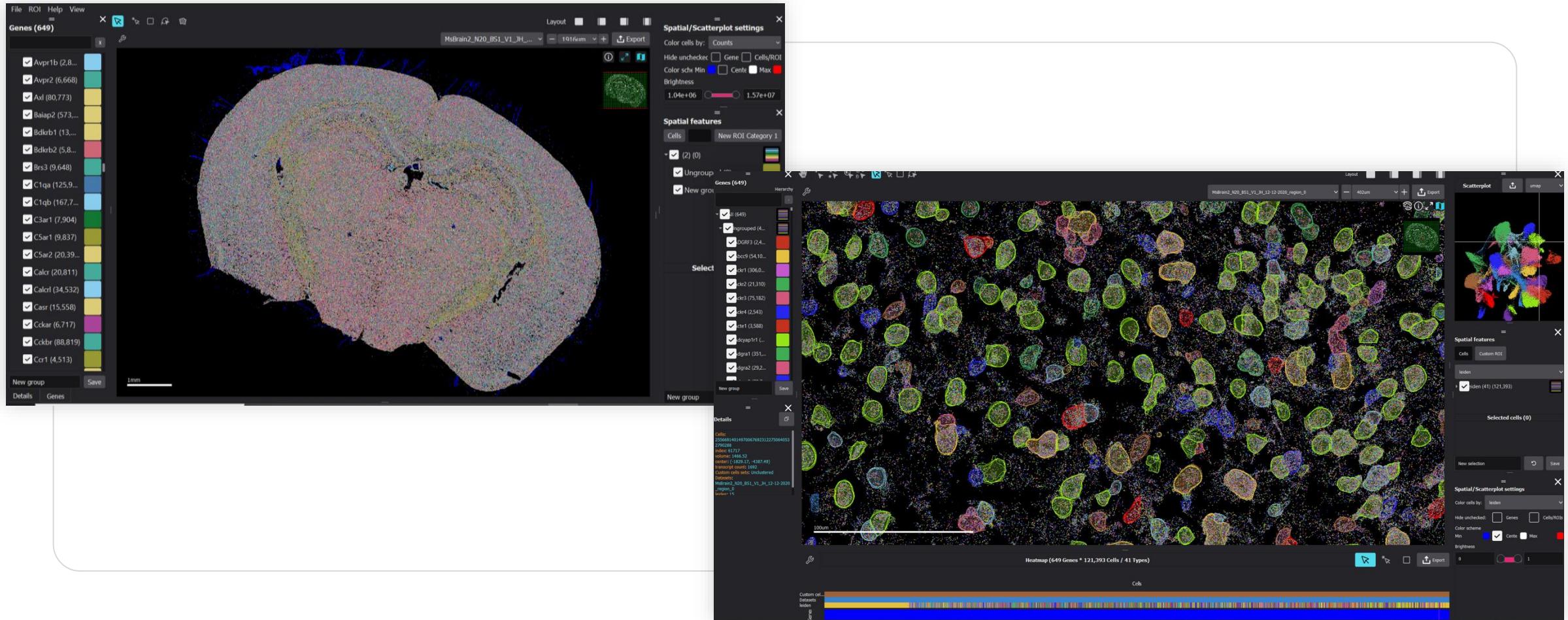
DAPI

Draw boundaries on the image to define regions for MERFISH imaging

Next

launch your experiment on the Mercospe UI / select the region of interest

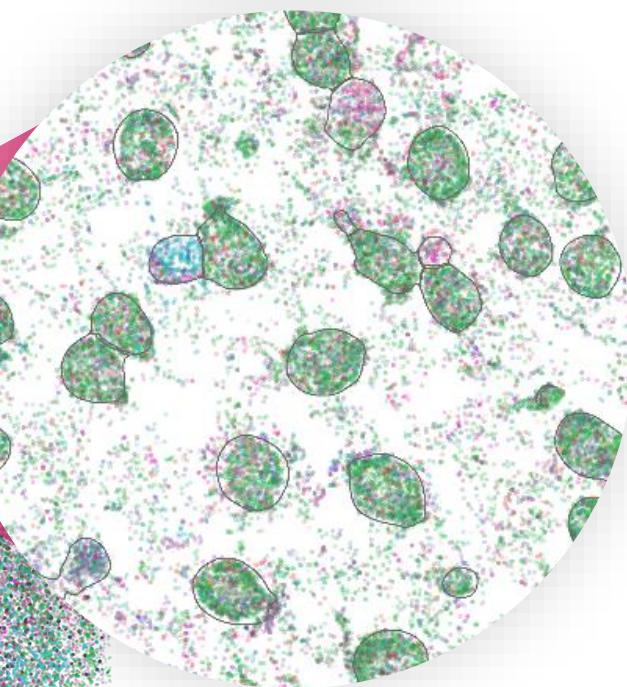
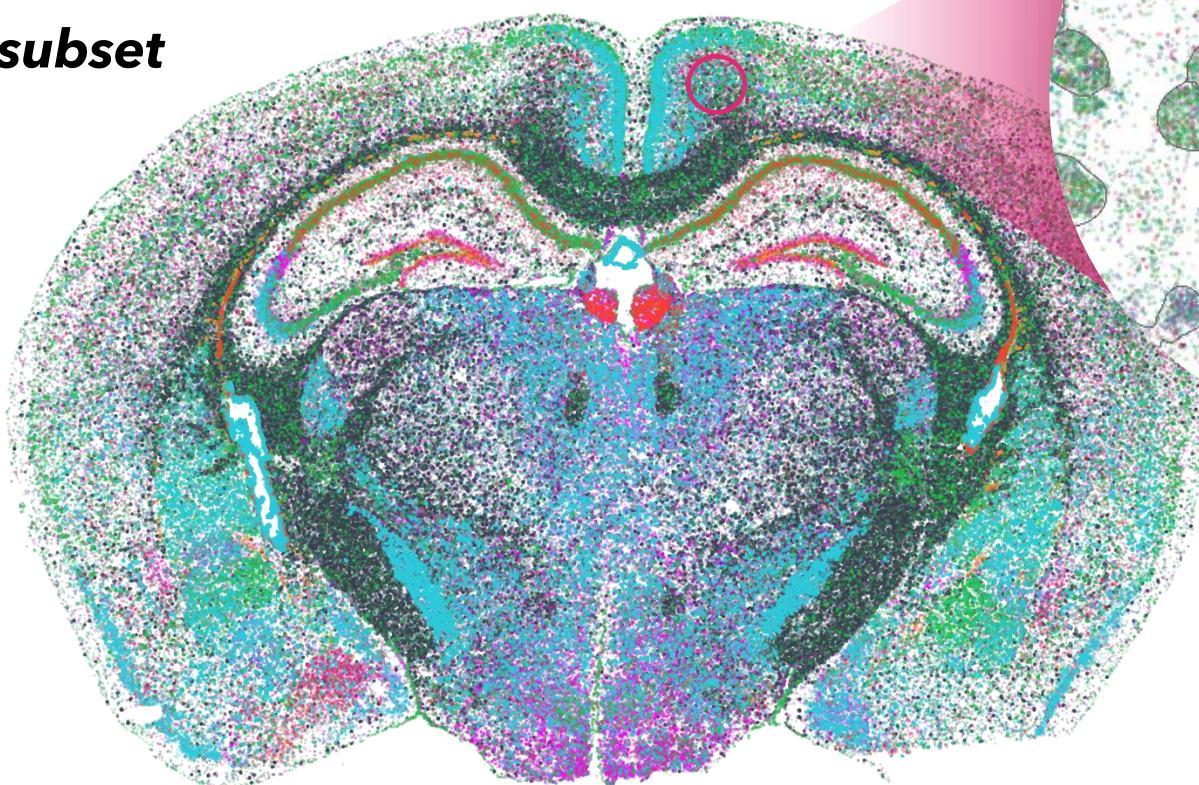
# Our visualization software provides the answers



# Increased cell throughput enables rare cell detection and neighborhood analysis

Profile a square centimeter sized tissue, with hundreds of thousands of cells in a single experiment

**8 RNA subset**



**483 RNA species**

200 x 200 micron

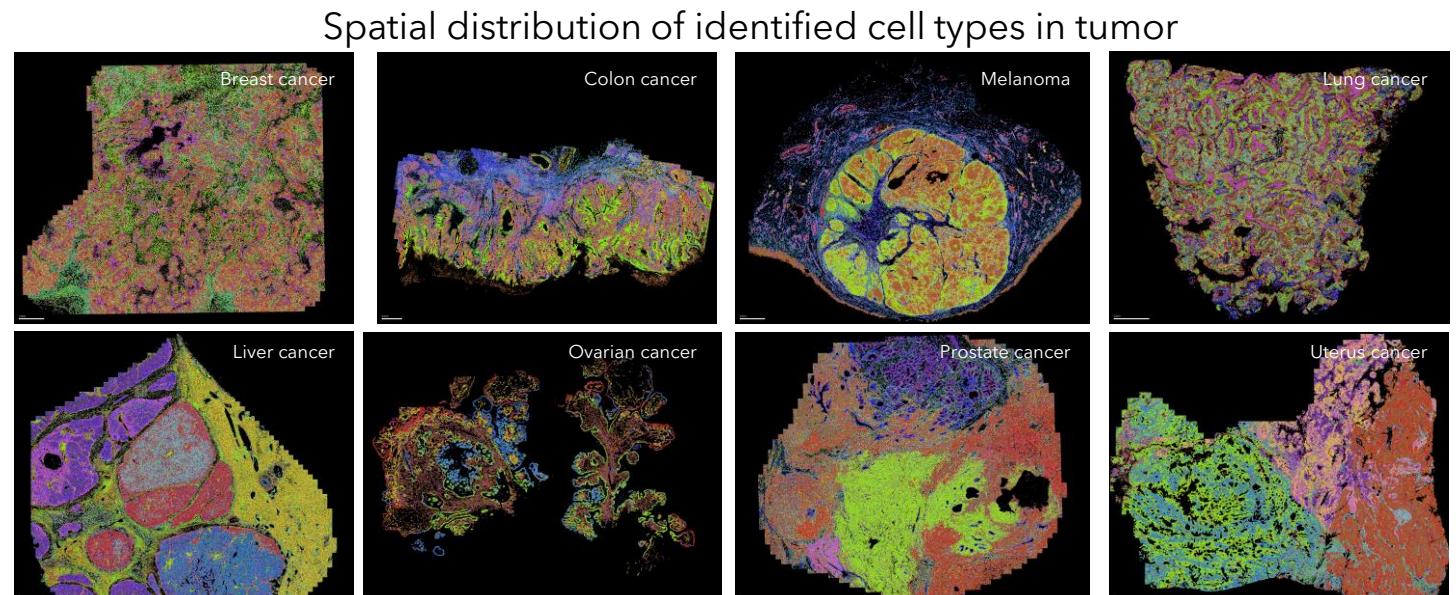
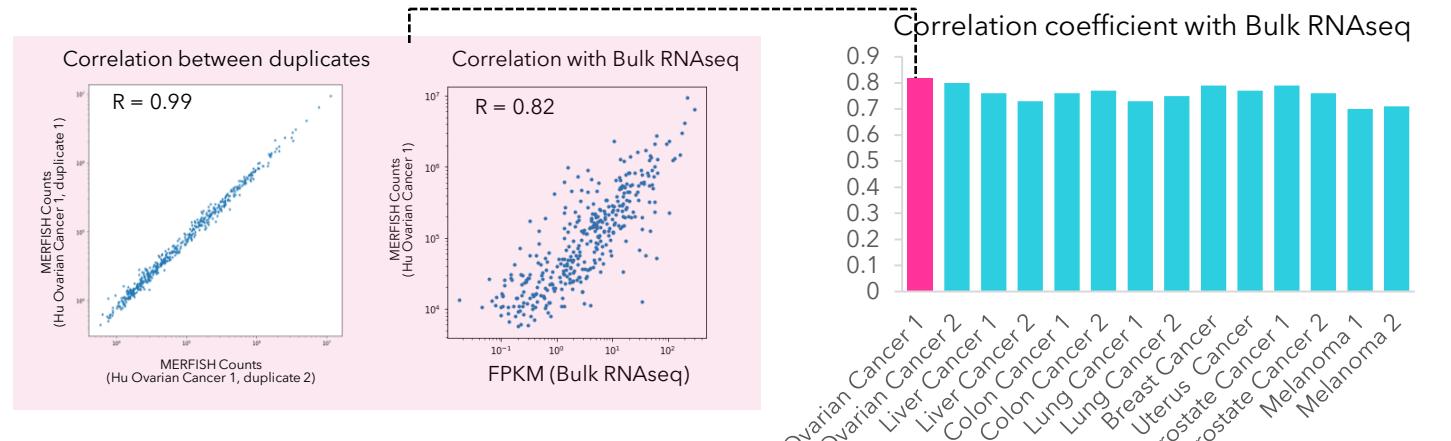
# Single cell, spatially resolved transcriptomic profiling in FFPE human Tumors with MERSCOPE

## Data Summary:

Sample Type	Total Transcripts	Total Cell Number
Colon cancer 1	411,716,053	677,451
Colon cancer 2	507,576,479	817,588
Liver cancer 1	272,021,991	568,355
Liver cancer 2	283,068,068	598,141
Melanoma 1	160,181,929	468,138
Melanoma	75,617,432	207,869
Ovarian cancer 1	197,365,319	358,485
Ovarian cancer 2	121,798,559	254,347
Ovarian cancer 3	32,054,444	71,381
Ovarian cancer 4	119,742,527	212,425
Prostate cancer 1	291,996,280	721,668
Prostate cancer 2	221,331,615	993,825
Lung cancer 1	144,388,044	353,762
Lung cancer 2	425,594,806	836,739
Breast cancer	490,398,542	713,121
Uterus cancer	374,580,211	843,285
<b>Total</b>	<b>4,129,432,299</b>	<b>8,978,174</b>

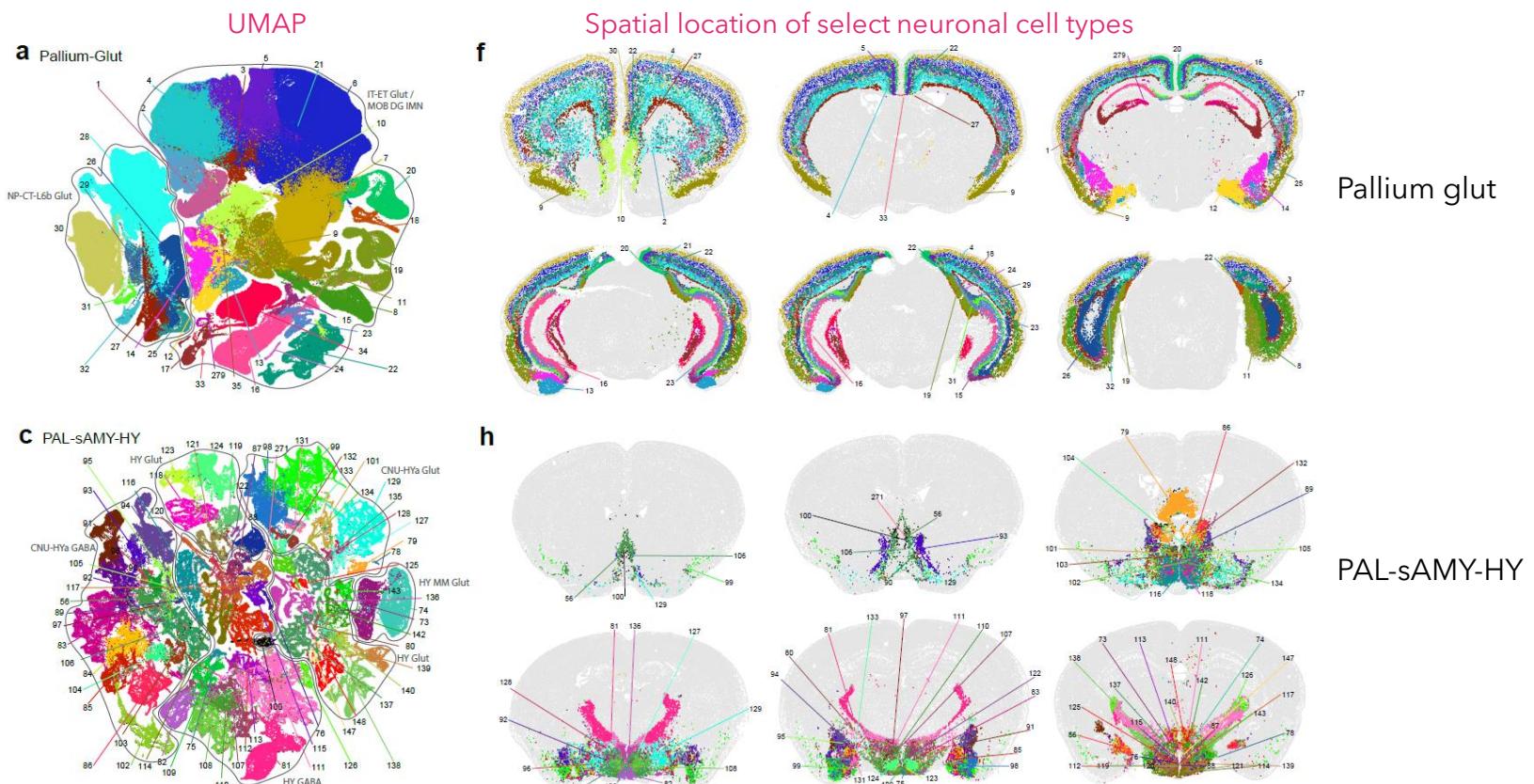
8 sample types, 16 datasets, 500 genes, ~4 billion transcripts, ~9 million cells.

<https://info.vizgen.com/merscope-ffpe-solution>



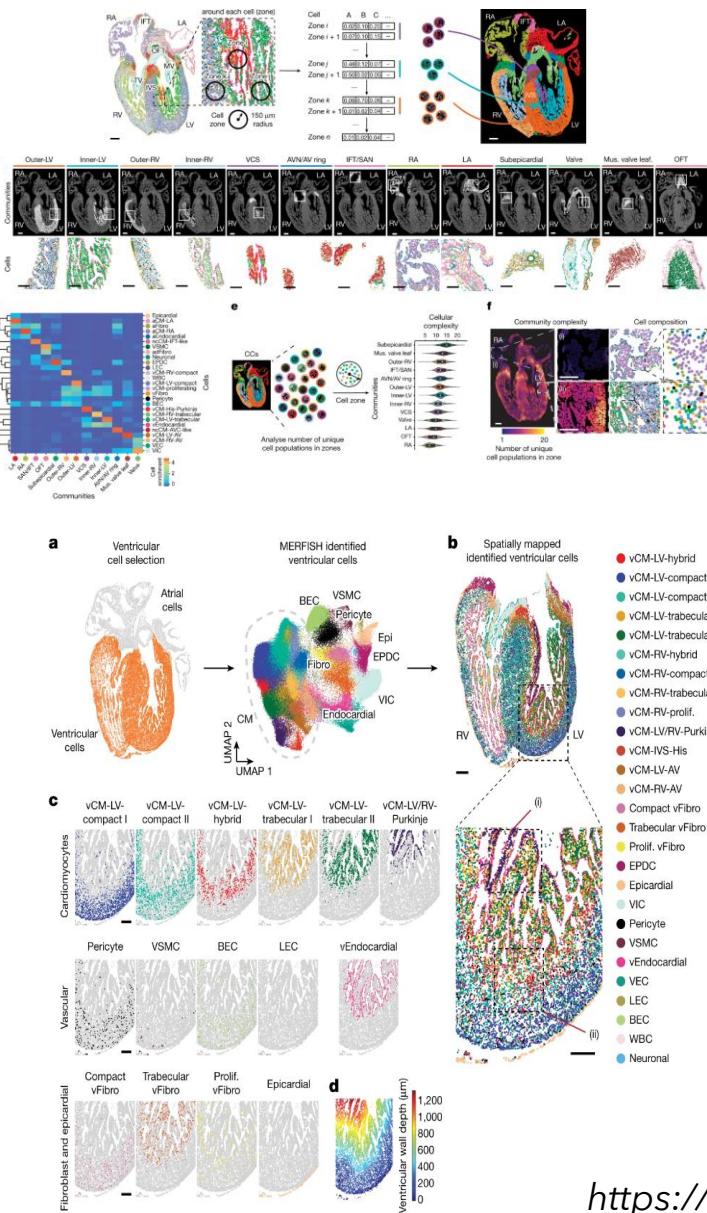
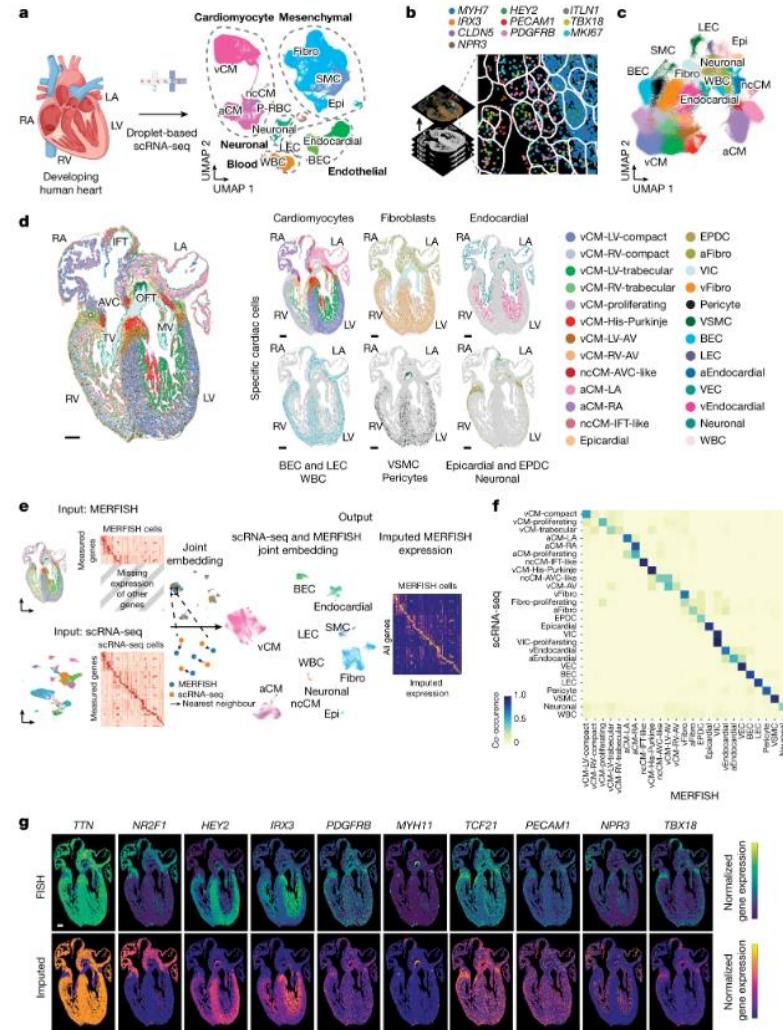
# MERSCOPE enables creation of a high-resolution whole mouse brain transcriptomic cell atlas

7 divisions, 32 classes, 306 subclasses, 1,045 supertypes and 5,200 clusters



- **High degree of correspondence between transcriptomic identity and spatial specificity**, with every subclass, all supertypes and many clusters, having a unique and specific spatial localization pattern
- **Dichotomy of cell type organization in different brain regions**, with the dorsal part containing fewer yet highly divergent neuronal types, whereas the ventral part contains more neuronal types closely related to each other

# Spatially organized cellular communities form the developing human heart



Institute of Engineering in Medicine, University of California San Diego, La Jolla, studying human heart development

- spatial mapping of individual cells that enables illumination of their organization into cellular communities that form distinct cardiac structures
- many of these cardiac cell types further specified into subpopulations exclusive to specific communities, which support their specialization according to the cellular ecosystem and anatomical region
- ventricular cardiomyocyte subpopulations displayed an unexpected complex laminar organization across the ventricular wall and formed, with other cell subpopulations, several cellular communities
- Interrogating cell-cell interactions within these communities using in vivo conditional genetic mouse models and in vitro human pluripotent stem cell systems revealed multicellular signaling pathways that orchestrate the spatial organization of cardiac cell subpopulations during ventricular wall morphogenesis

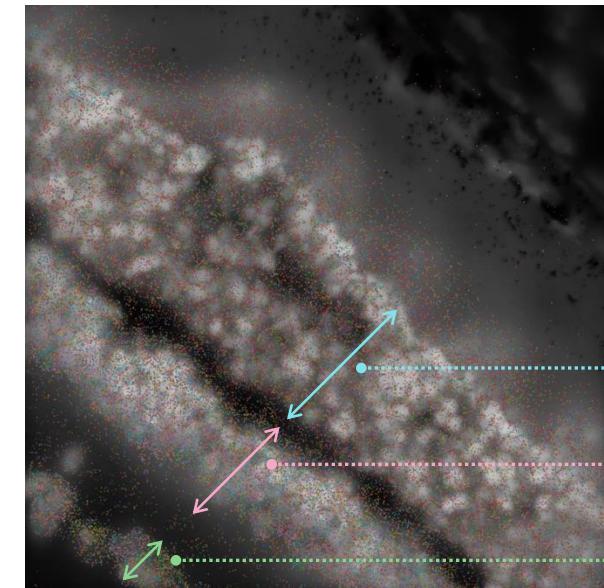
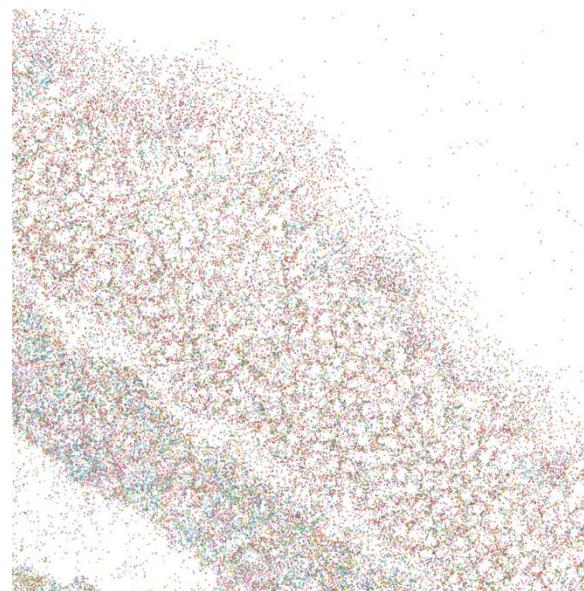
# Industry-leading Multiplexing Power

100s to 1000s of genes measured simultaneously in a single sample

Demonstrates potential to measure 10,000 RNA species

Future in protein imaging proteomics spaces

MERFISH WITH A PANEL OF 368 MARKER GENES ON THE MOUSE RETINA

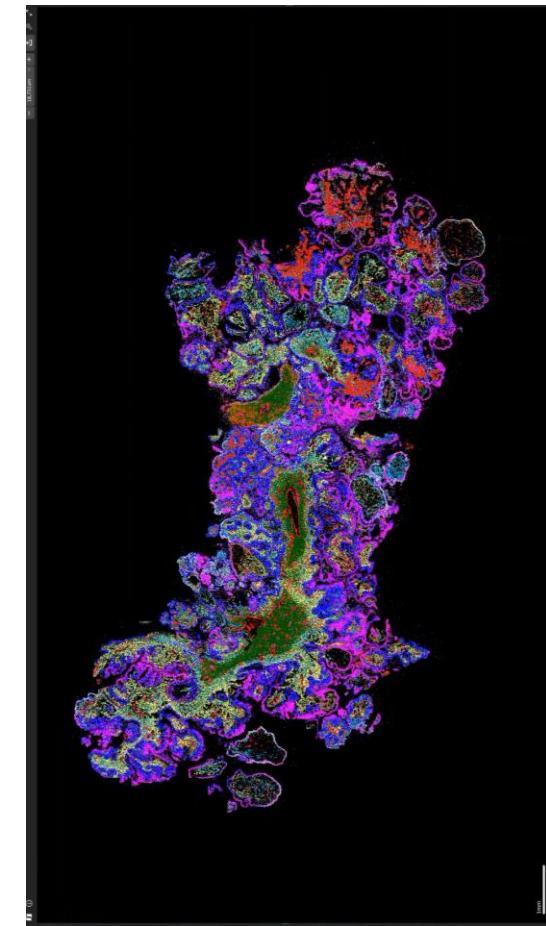
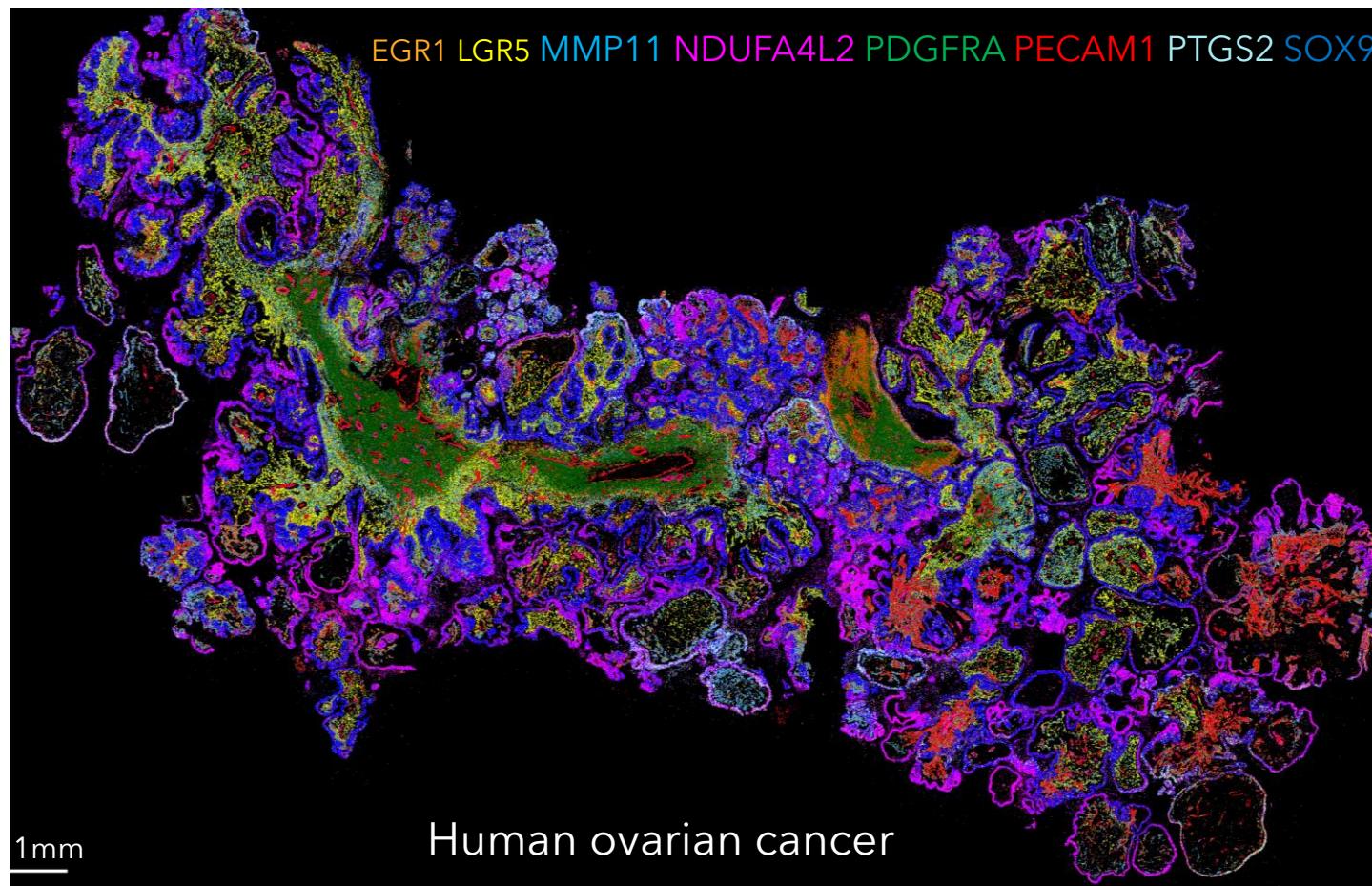


ONL

INL

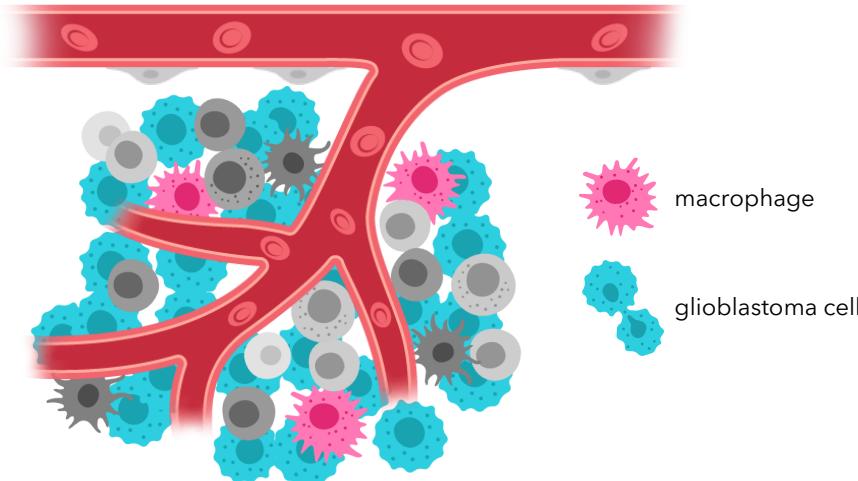
RGC

# Profile gene expression *in situ*, from whole tissue, to sub-cellular

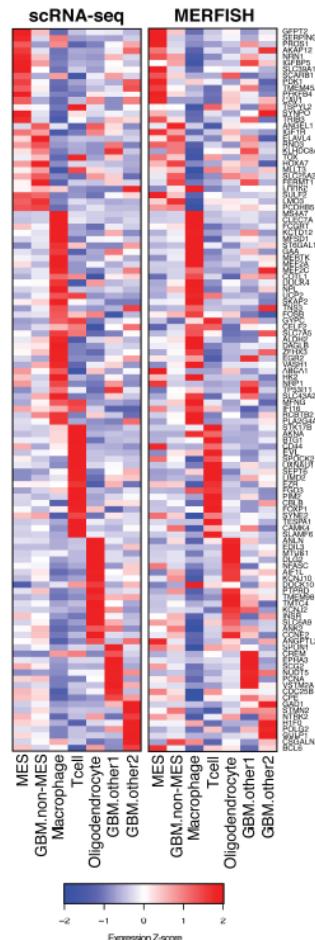


500 gene panel, 174 million RNA transcripts detected

# Demonstrating connection and impact on the cellular level, *in situ*

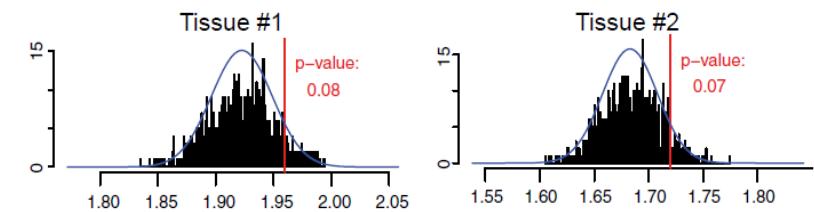


## Single cell characterization

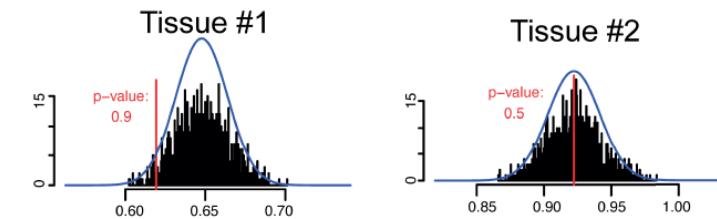


## Spatial co-localization

Mean number of macrophages in 30 nearest cells to each MES-like glioblastoma



Mean number of oligodendrocytes in 30 nearest cells to each MES-like glioblastoma

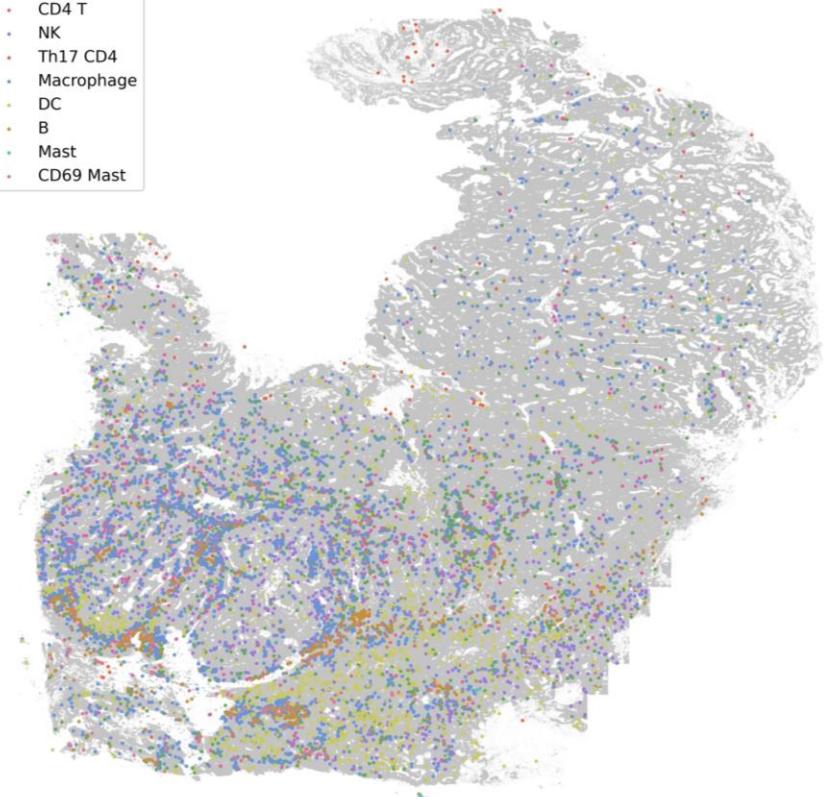


**The study found enrichment of macrophages adjacent to MES-like glioblastoma cells *in situ*.**

# Mapping different immune cell types within the tumor microenvironment

By performing cell clustering analysis, we can identify transcriptionally distinct cell types within the tissue, including B cells, CD8T cells, NK cells, and macrophages.

- CD8 T
- CD4 T
- NK
- Th17 CD4
- Macrophage
- DC
- B
- Mast
- CD69 Mast

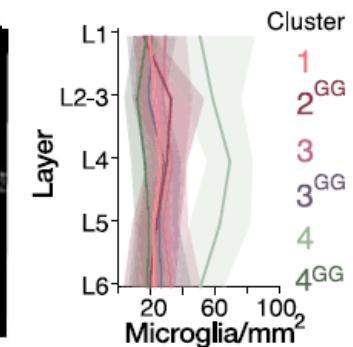
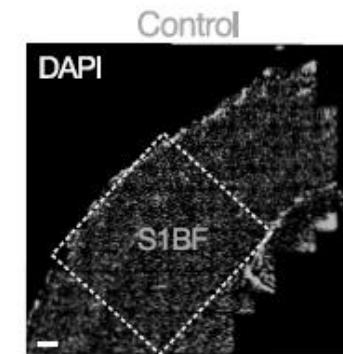
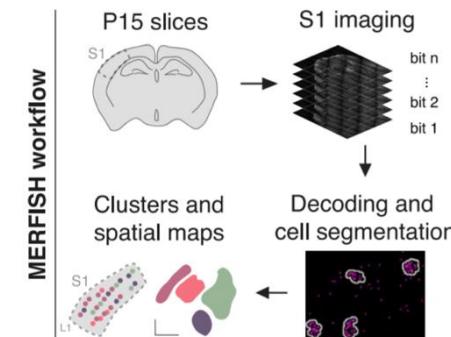


A 417 gene measurement with single cell resolution across full tissue.

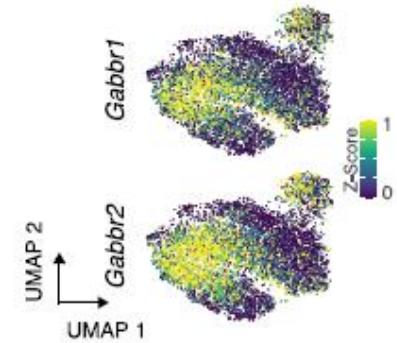
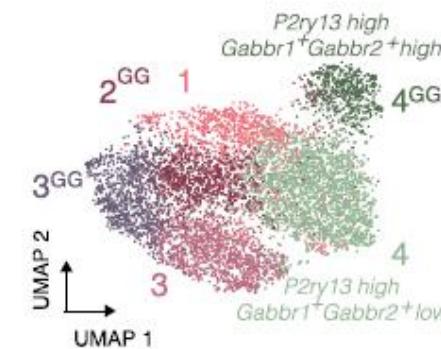
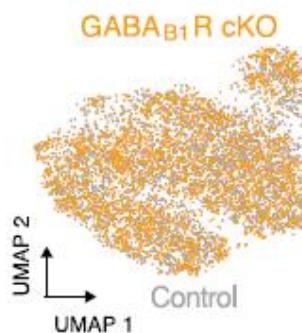
# Gaining a deeper understanding of cell types, lowly expressed genes, and differential gene expression patterns

Without MERFISH, the co-expression of Gabbr1 and Gabbr2 in microglia would not have been completely detected and the changes gene expression patterns would have remained undiscovered.

## MERFISH enables spatial gene expression profiling



**GABA-receptive microglia highly enriched in three clusters**  
with their own gene signatures

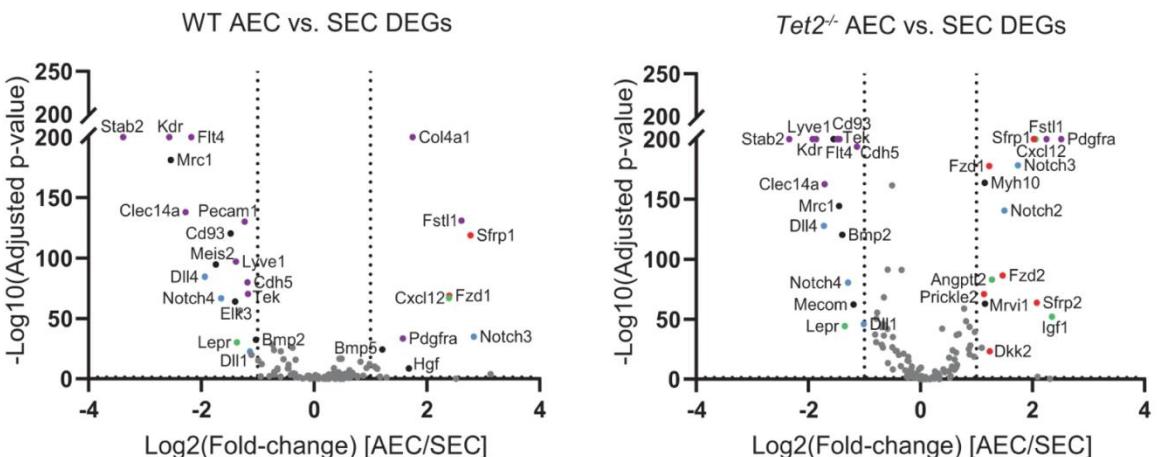
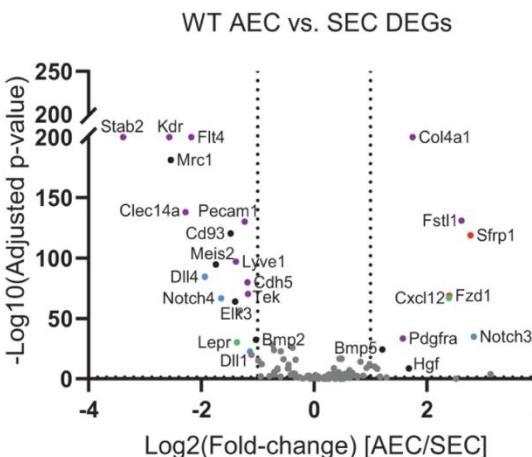
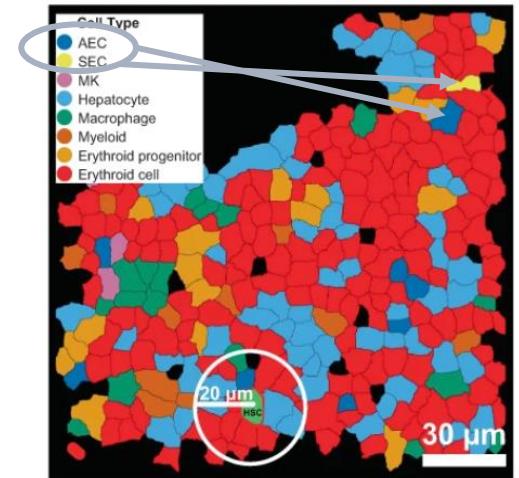
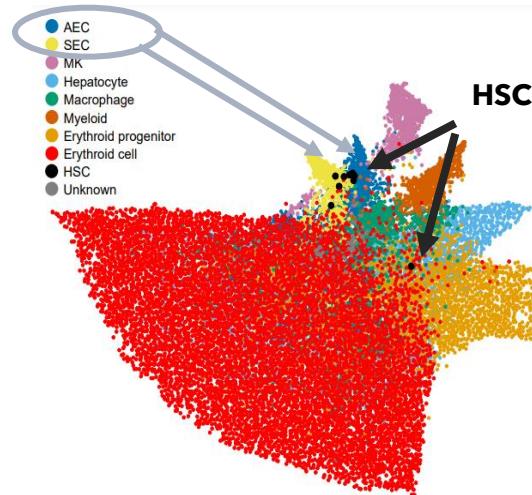


# Investigating tissue architecture to identify rare cell types and understand cell signaling

Researchers turned to MERFISH to profile 140 genes, image thousands of tightly packed cells, identify 8 major liver cell types, and determined their spatial locations.

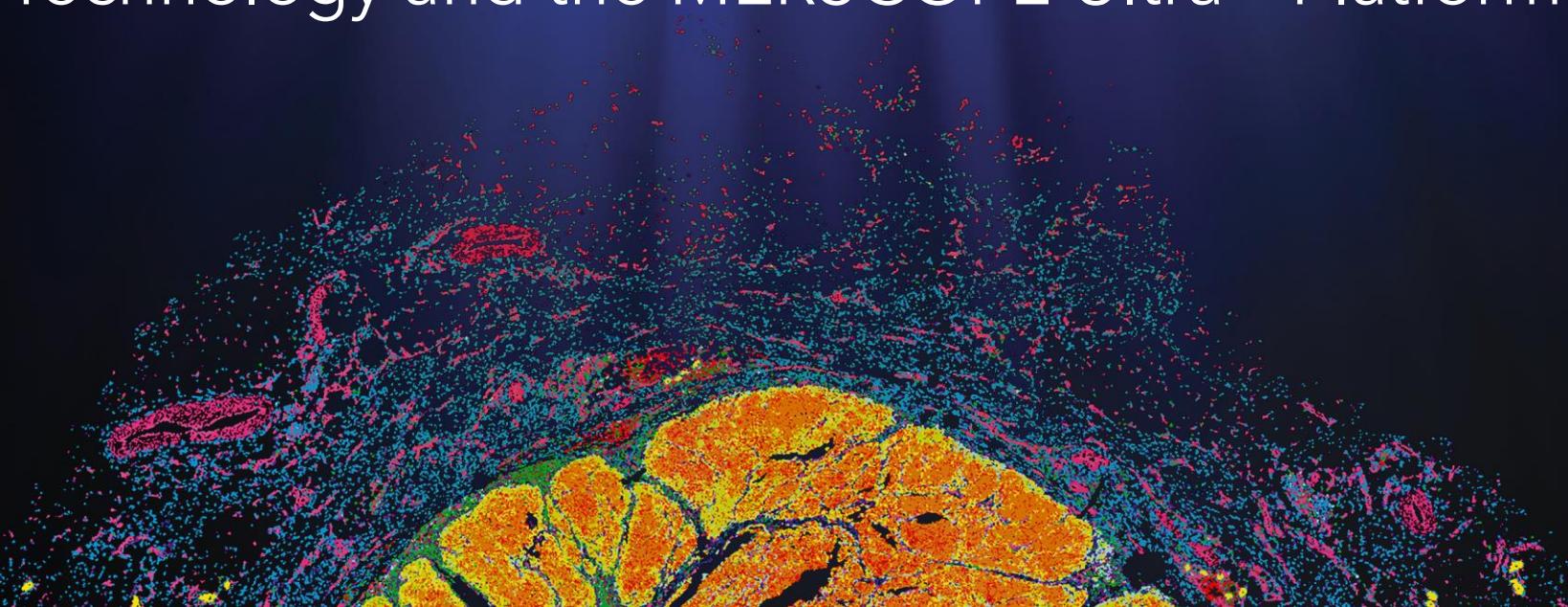
Without using MERFISH, researchers would have missed the AEC and SEC cell types.

**EC Subtypes AEC and SEC were identified by MERFISH and not identified by RNA-seq**



# ultra

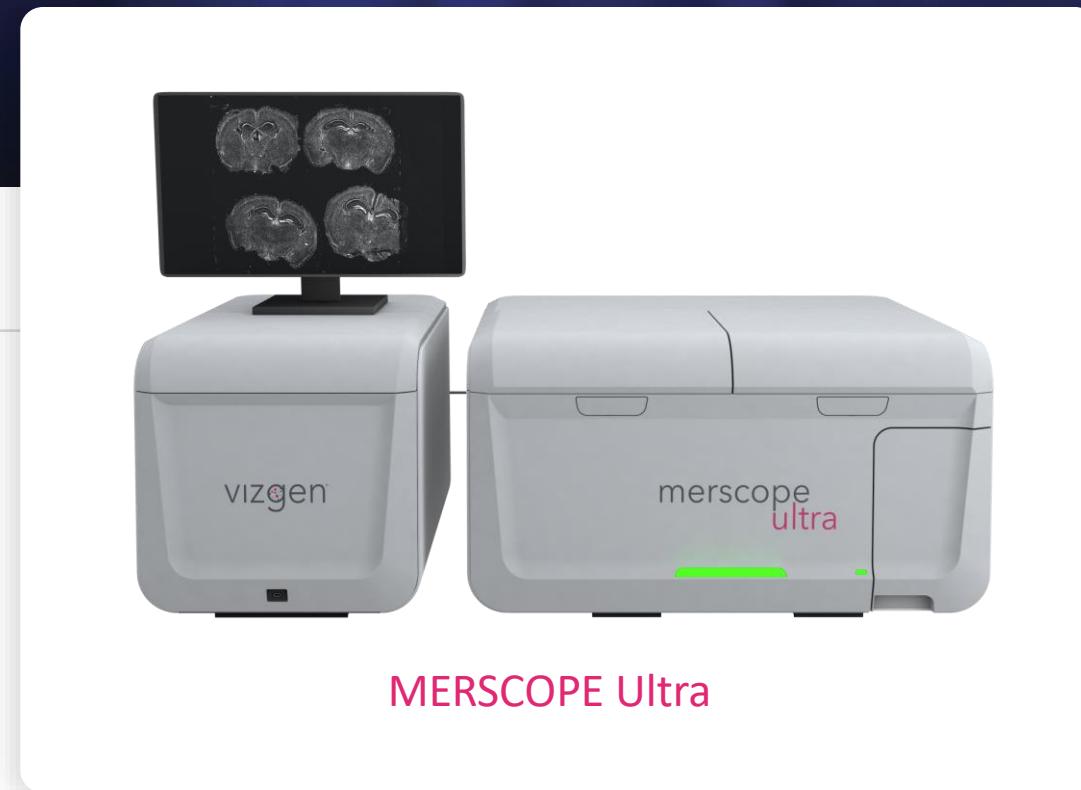
Deep Dive into Spatial Biology with MERFISH  
Technology and the MERSCOPE Ultra™ Platform



# The MERSCOPE Ultra™ Platform is an end-to-end MERFISH solution:

A custom targeted gene panel via our interactive web-based platform

Reagents and consumables for sample preparation and imaging



An analysis computer to process massive amounts of data and take the burden off the instrument, making it available for immediate use

Proprietary visualization and analysis software to help make the most of your data

# MERFISH: Highly multiplexed, Spatially Resolved, Single-cell Gene Expression Profiling by RNA Imaging

Platform technology from Harvard: [Multiplexed Error-robust Fluorescence \*in situ\* Hybridization \(MERFISH\)](#)

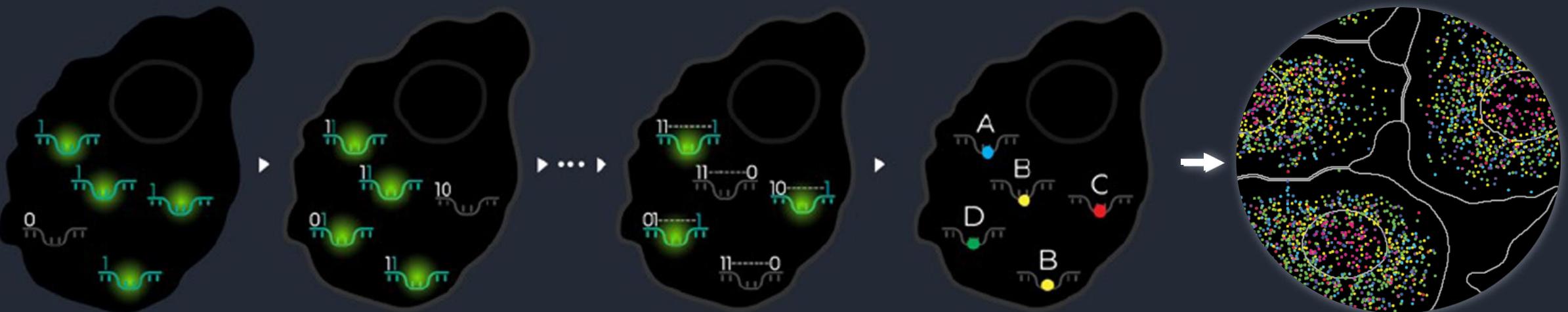
COMBINATORIAL LABELING | SEQUENTIAL IMAGING | ERROR ROBUST BARCODING

Image 1

Image 2

Image N

Barcode decoding  
Multiplexed RNA Detection

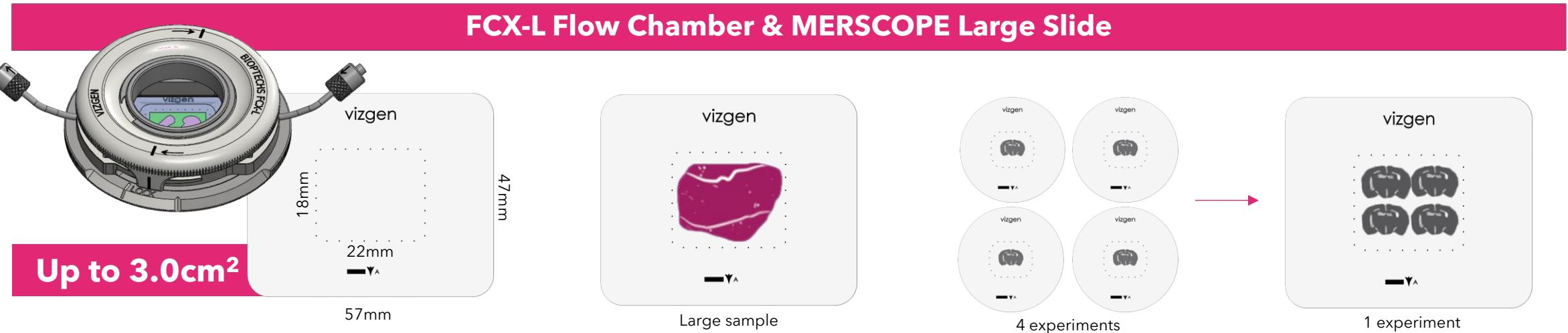


*“Method of the Year 2020: spatially resolved transcriptomics”* ---- Nature Methods

Chen et al, Science, 2015; Moffitt et al, PNAS, 2016; Moffitt et al, PNAS, 2016; Emanuel et al, Nature Methods, 2017; Wang et al, Sci. Rep, 2018; Moffitt et al, Science, 2018; Wang et al, PNAS, 2019; Xia et al, Sci. Rep, 2019; Xia et al, PNAS, 2019;

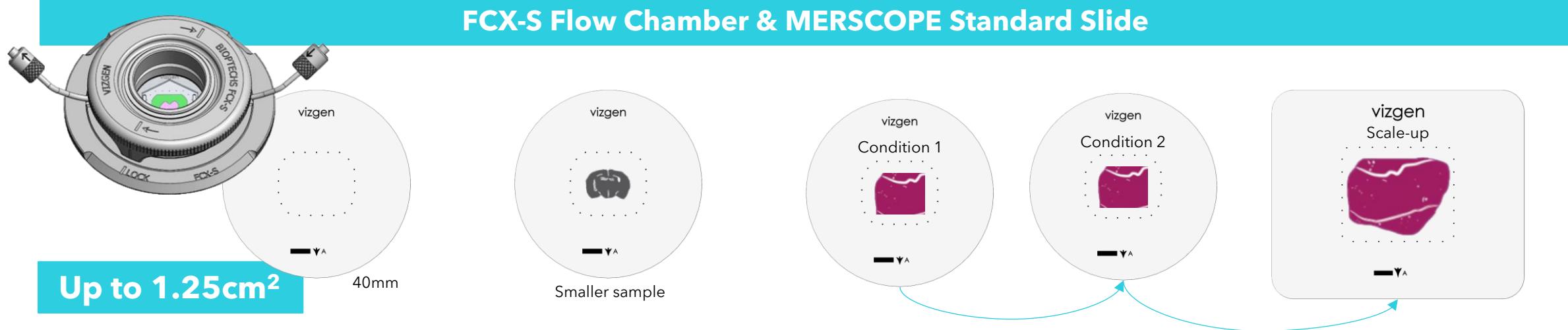
VIZgen

# Expansive imaging area enables streamlined analysis of larger samples and reduces cost per sample with multiple tissues on a single slide



- FCX-L flow chamber supported by our new MERSCOPE Large Slide consumable
- Large, rectangular tissue placement area is ideal for running larger samples reducing the need for scoring or separation or multiple smaller samples reducing cost per sample and hands-on time by combining multiple experiments on a single slide
- Slide markings help users visualize imageable area and provide guidance for tissue placement during sectioning. The marked area exceeds the 3.0cm<sup>2</sup> providing flexibility in sample placement and orientation to help ensure the full 3.0cm<sup>2</sup> area can be imaged every run.

# Scale down imaging area with FCX-S to conserve reagent when imaging smaller samples or optimizing new tissues



- FCX-S flow chamber supported by our 40mm, round MERSCOPE Standard Slide
- Reduced reagent volume requirements compared to 3.0cm<sup>2</sup>, large flow chamber
- Standard slide imaging area ideal for smaller sample types or initiating optimization of new tissues
- Swapping between flow chambers requires minimal setup and both slide sizes share the same sample prep workflow steps

## Rich data output from MERSCOPE Ultra measurements include:

List of all detected transcripts

**.csv  
.parquet**

Mosaic images

**.tiff**

Transcripts per cell matrix

**.csv**

Cell metadata

**.csv**

Cell boundaries

**.parquet**

Cell clustering annotations

**.csv  
.h5ad**

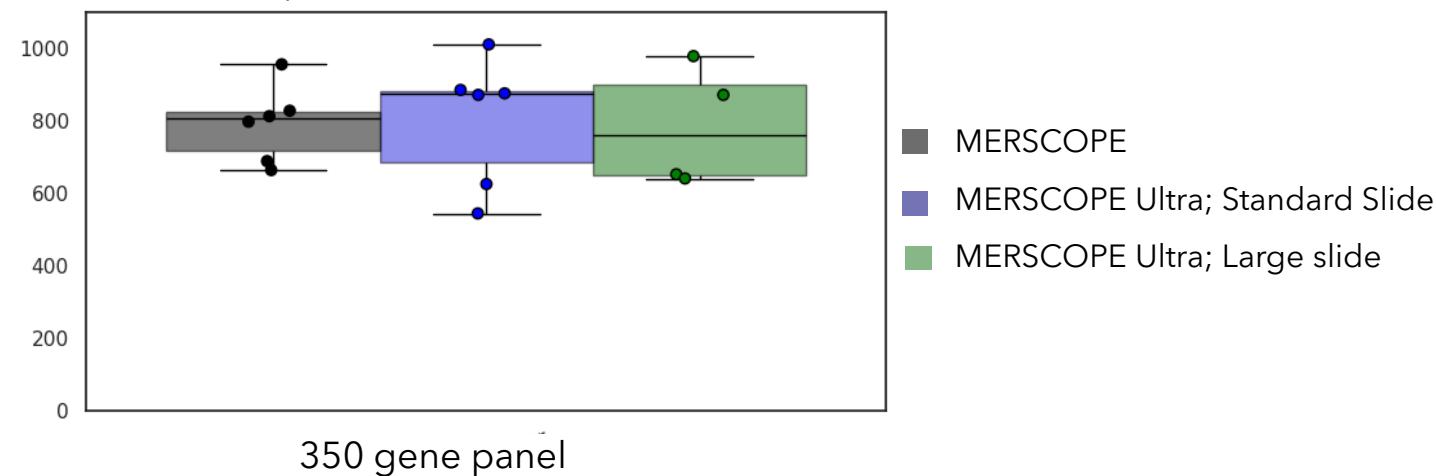
Data compatible with single-cell gene expression analysis software platforms

**S E U R A T**

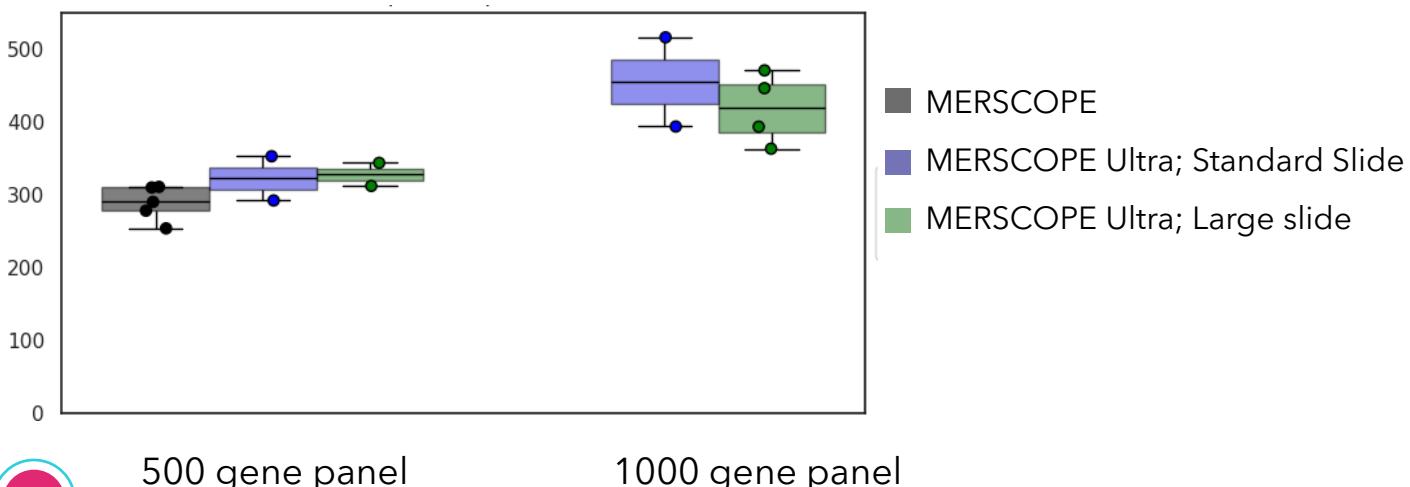


# 3X the imageable area with the same high quality MERSCOPE performance

Counts/100 $\mu\text{m}^2$  for FFPE Mouse Liver



Counts/100 $\mu\text{m}^2$  for FF Mouse Brain

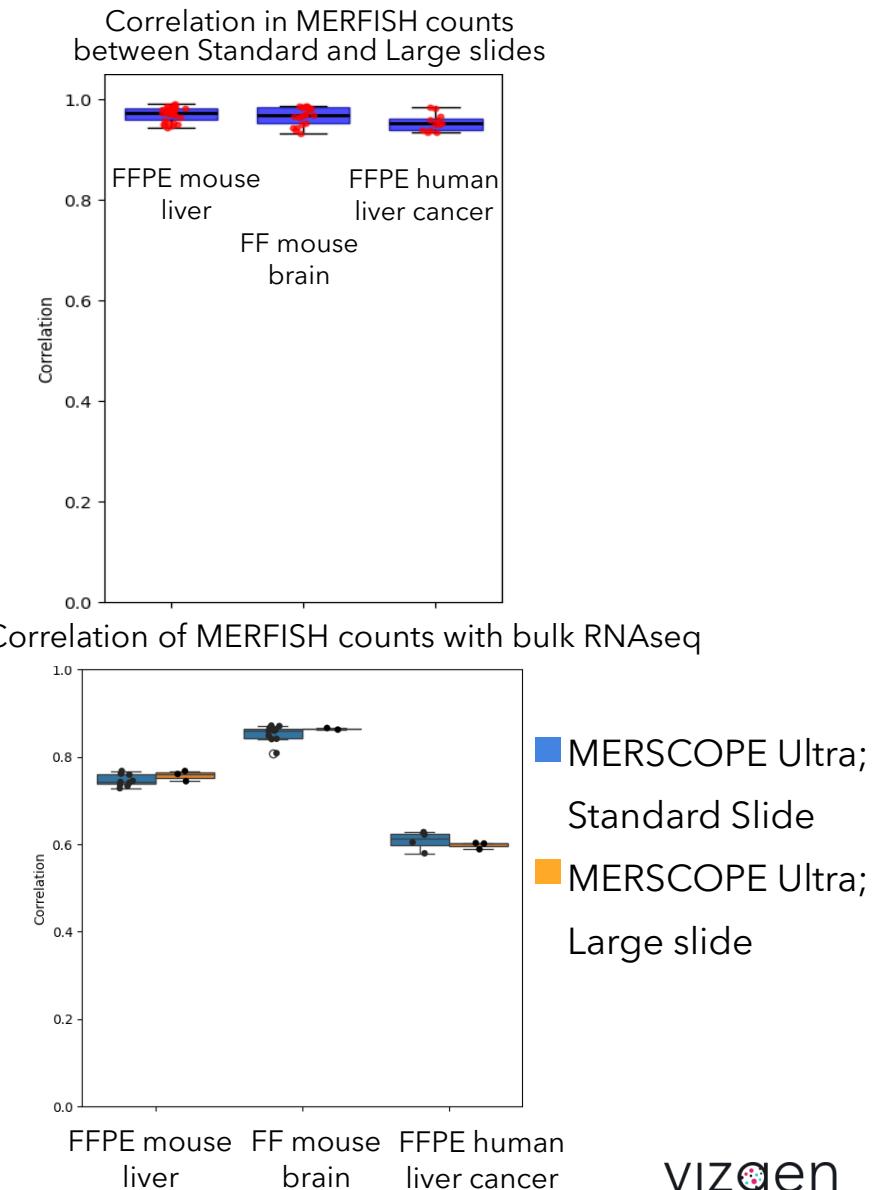
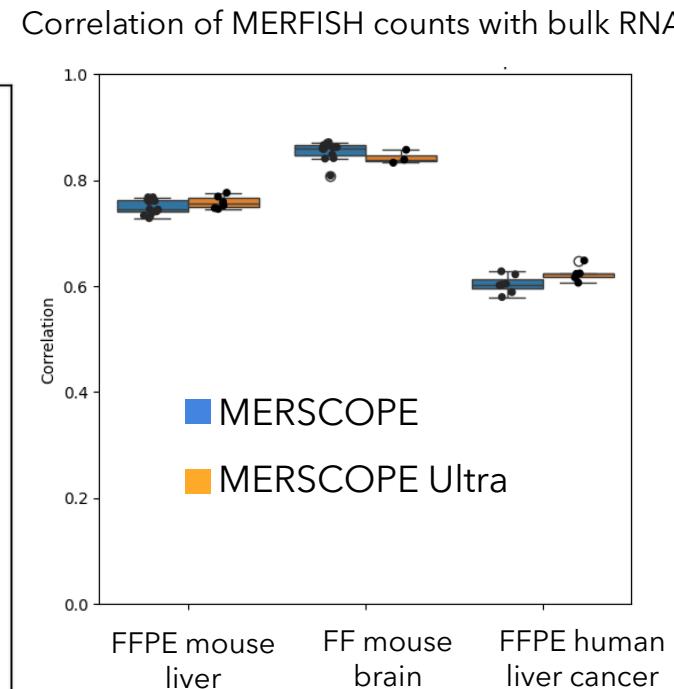
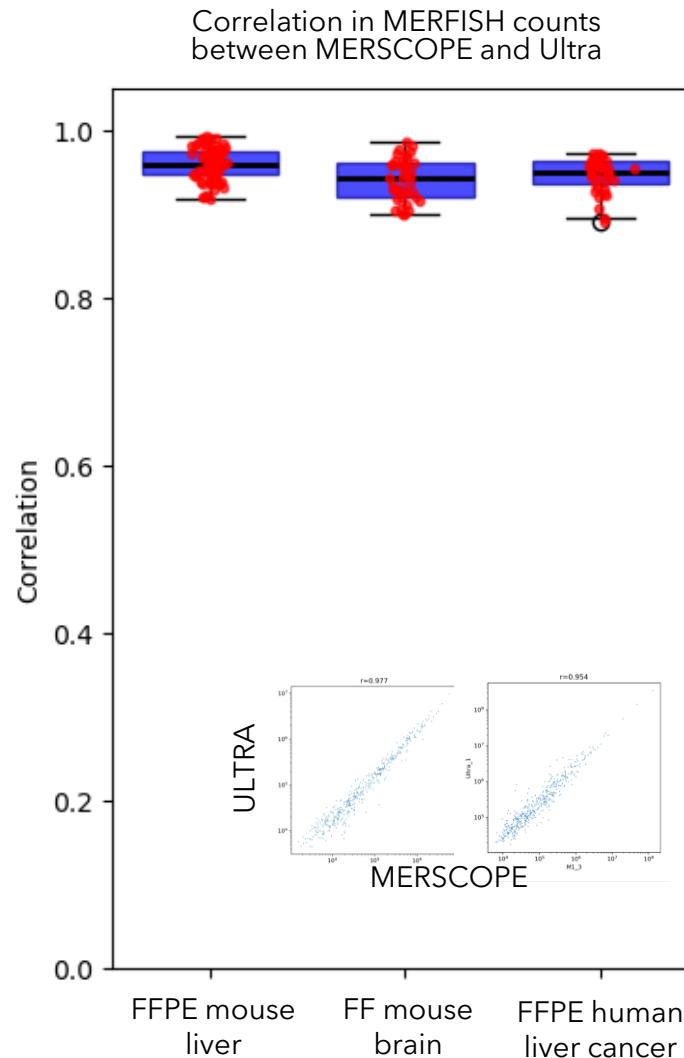


13 FFPE Mouse Livers over 3cm<sup>2</sup>



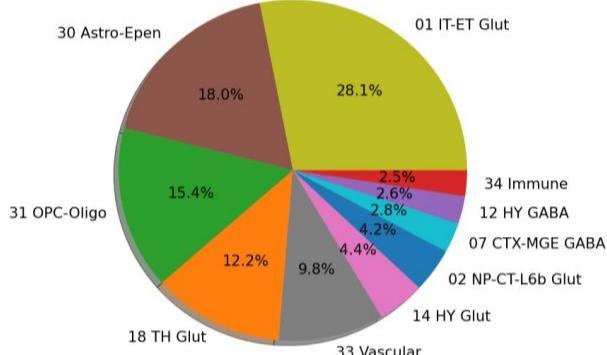
- 2.4 million cells in one measurement
- 1.5 billion total transcripts
- 730 transcripts/cell

# MERSCOPE and MERSCOPE Ultra data is comparable across tissues

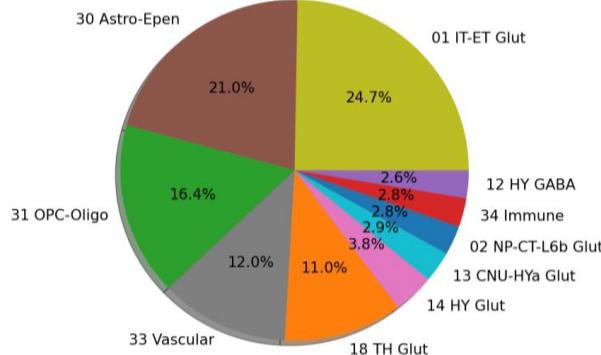


# Consistent biological insights between MERSCOPE and MERSCOPE Ultra

Predicted cell type composition

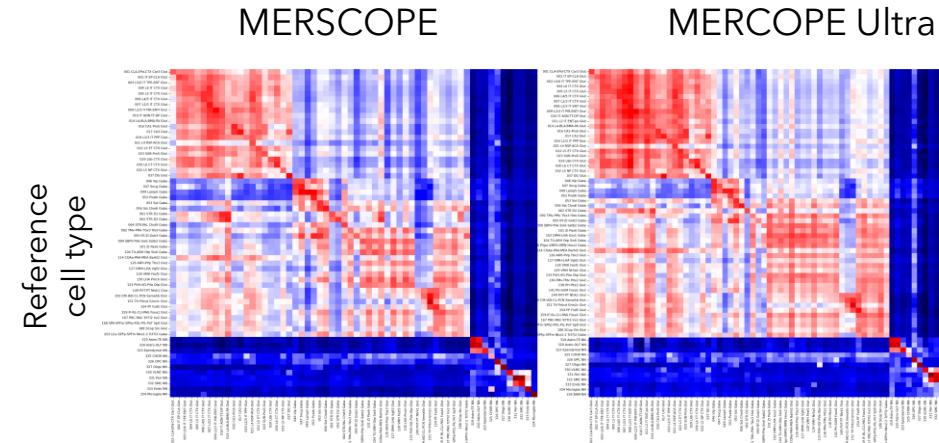


MERSCOPE



MERSCOPE Ultra

Predicted cell type

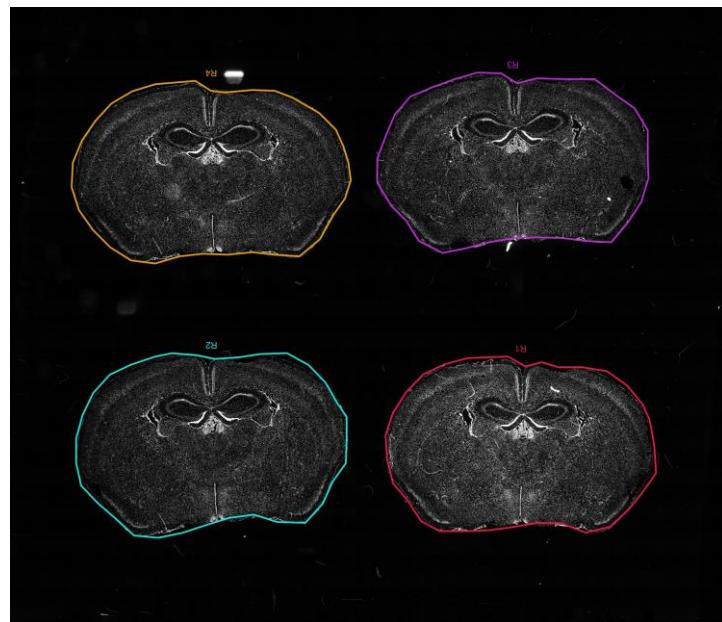


Mouse brain cell types were identified in similar abundance using MERSCOPE and MERSCOPE Ultra

Similar expression profile correlations between predicted and reference cell type

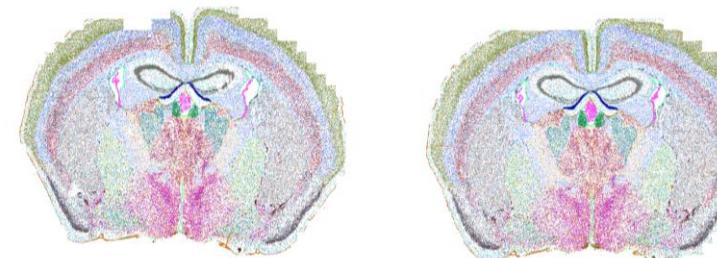


# Consistent performance across multiple sections - 4 mouse brain example



Low magnification DAPI  
image of 4 adult mouse  
brains placed on 1 large  
MERSCOPE Ultra slide

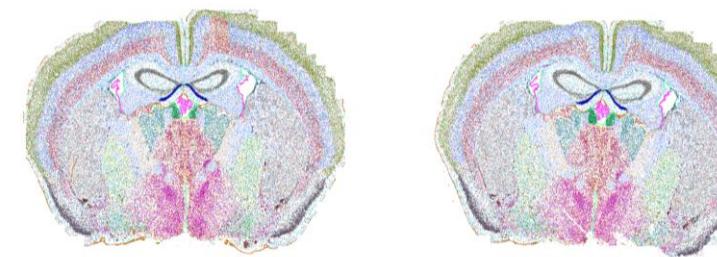
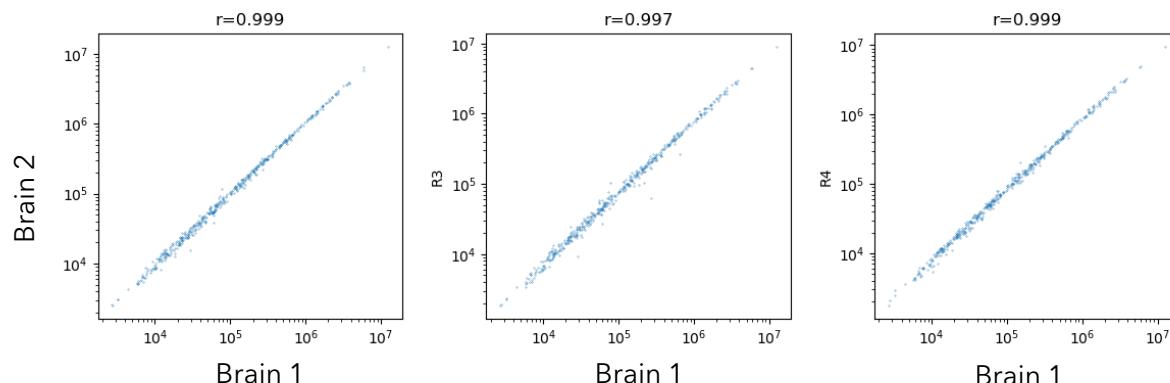
Consistent gene expression



Legend of genes:

- ACNT2
- ACTE2
- CHOR
- COP1
- DECHO2
- DEGLU1
- DEGLU3
- DEINH1
- DEINH3
- DEINH7
- DGGRC2
- EPEN
- HYPEP5
- MGL1
- MOL1
- MSN2
- MSN4
- PER1
- SZNLB
- TECHO
- TEGLU1
- TEGLU2
- TEGLU3
- TEGLU8
- TEINH1
- TEINH15
- TEINH18
- VECA
- VECV

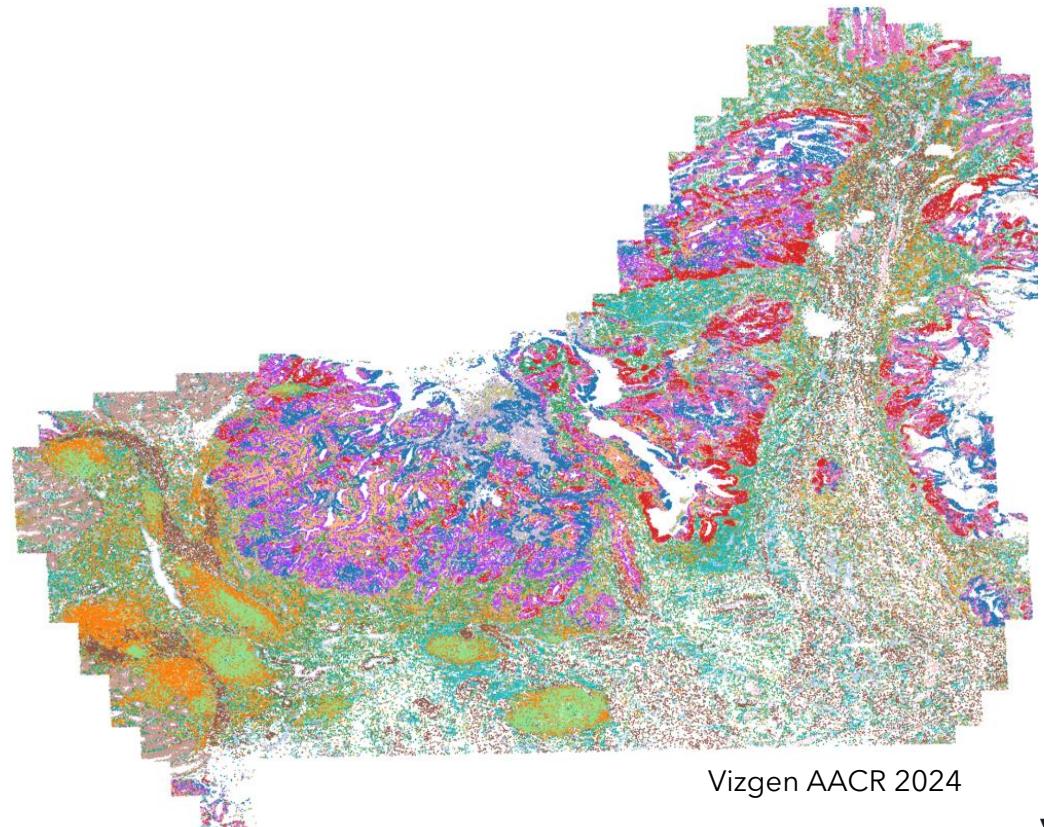
Pairwise correlations in gene expression approaching 1



# Vizgen's MERFISH 2.0

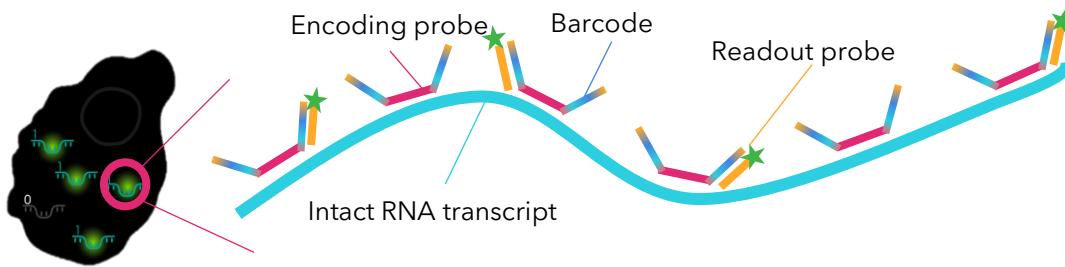
Unlock rare biological insights with Vizgen's enhanced MERFISH 2.0 chemistry. Designed for researchers facing challenges with RNA-compromised samples or seeking to push the boundaries of spatial transcriptomics, MERFISH 2.0 delivers improved sensitivity and reproducibility with a streamlined, optimized workflow

- Detect a **greater number of transcripts**
- Better informed **cell identification**
- Enhanced **spatial resolution**



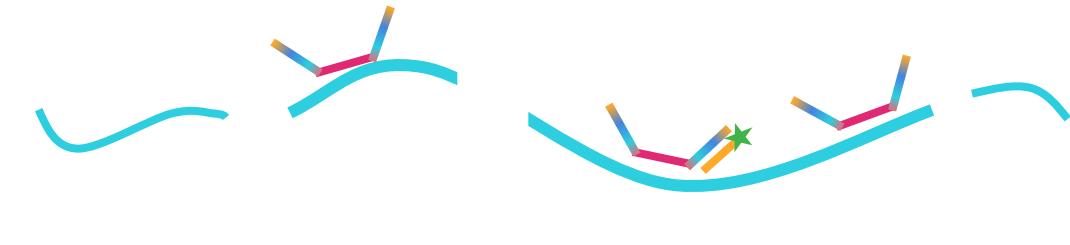
# The MERSCOPE Platform and Enhanced MERFISH Method

## High Quality RNA Samples and traditional MERFISH



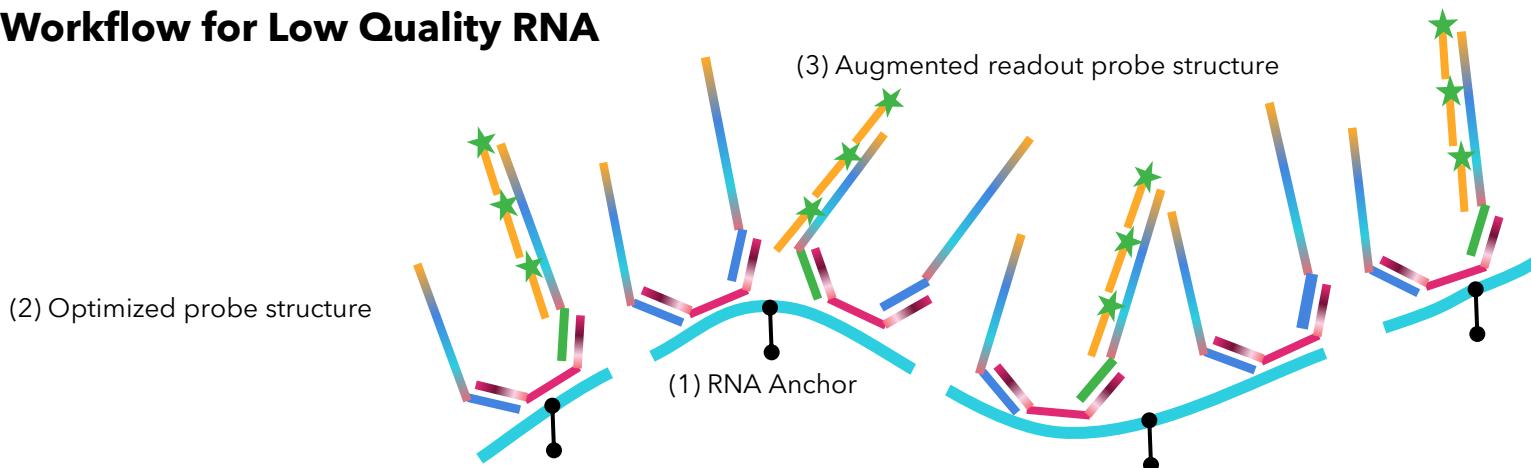
In intact RNA samples, multiple encoding probes bind along the transcript, generating strong fluorescent signals at the '1' barcode positions.

## Low Quality RNA Samples and traditional MERFISH

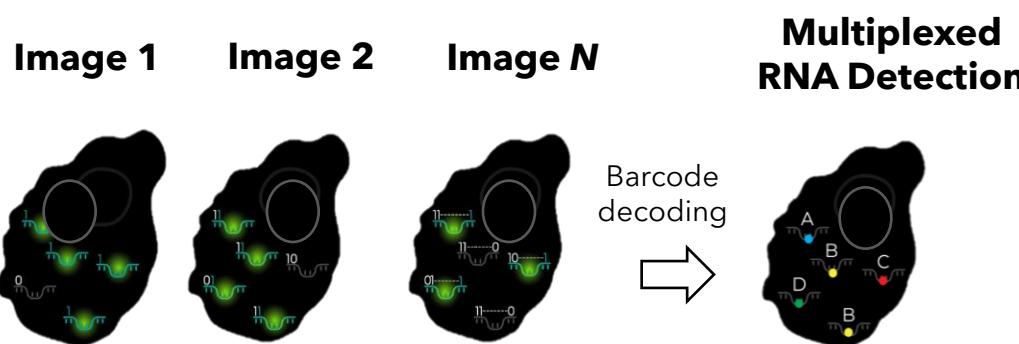


In degraded samples, such as archival FFPE tissues, RNA fragmentation leads to fewer probe binding sites, reducing signal intensity. At lower RNA quality, background noise can obscure signals, making transcript quantification more challenging.

## Enhanced MERFISH Workflow for Low Quality RNA



# The MERSCOPE Platform and Enhanced MERFISH Method

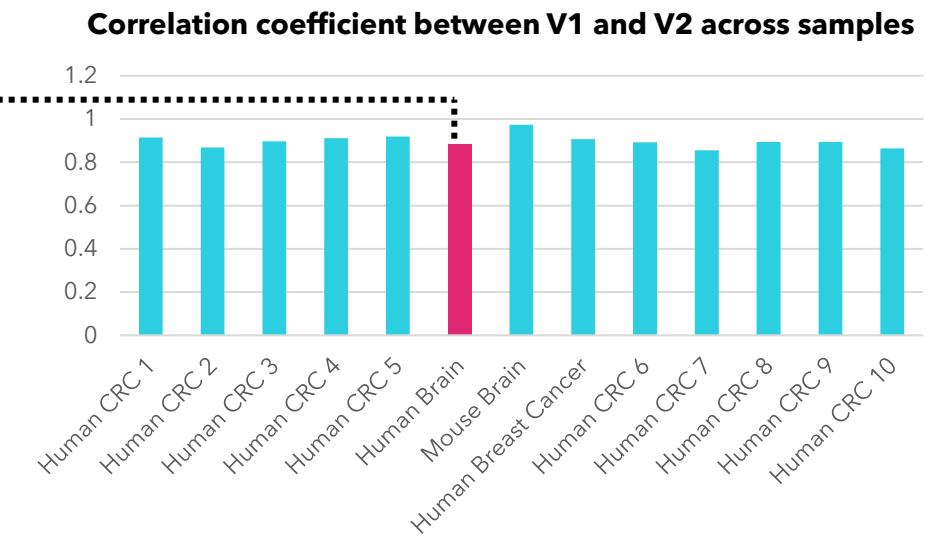
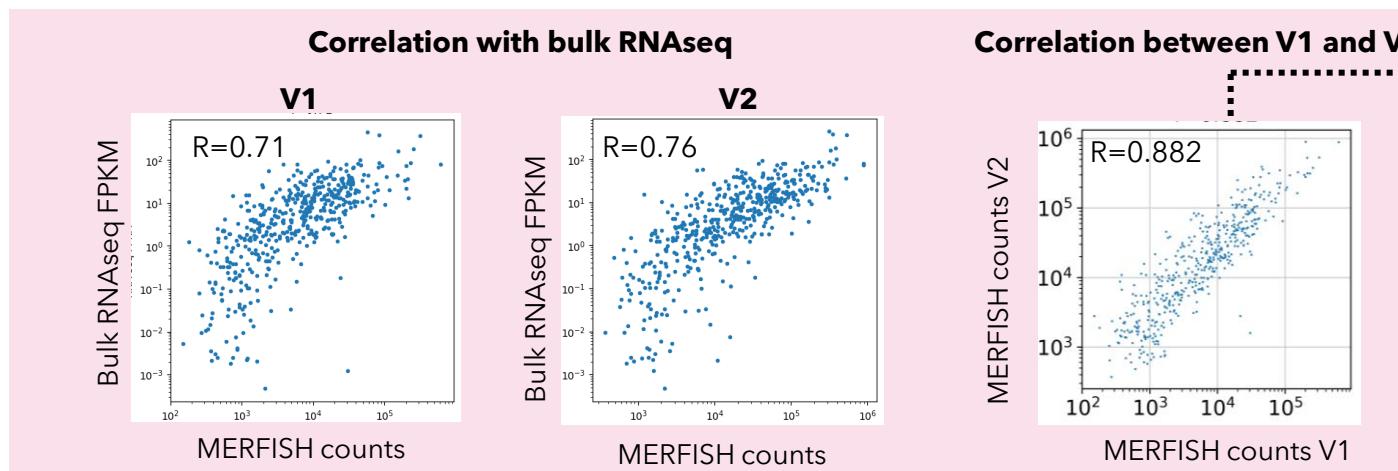
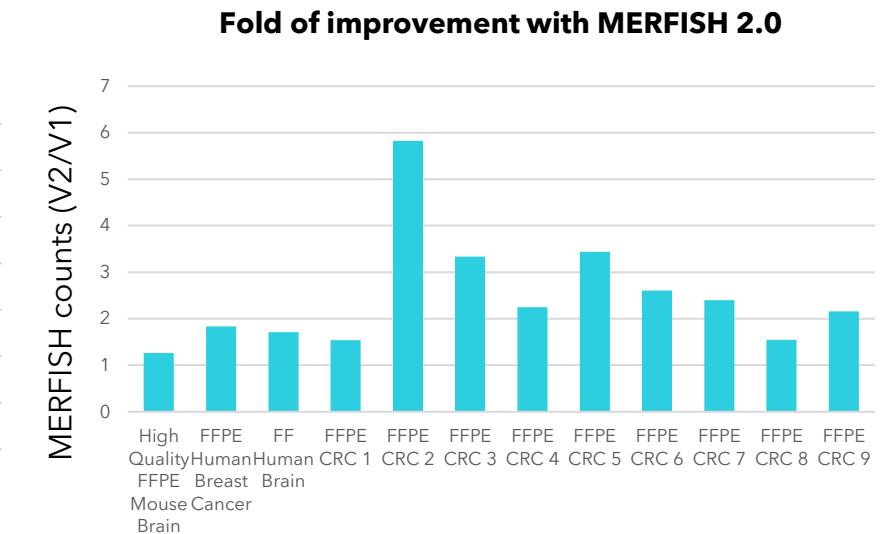
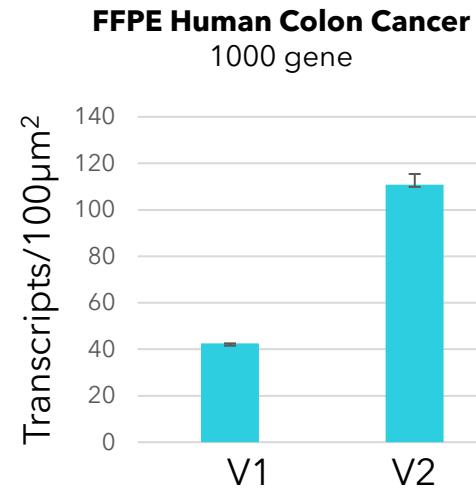
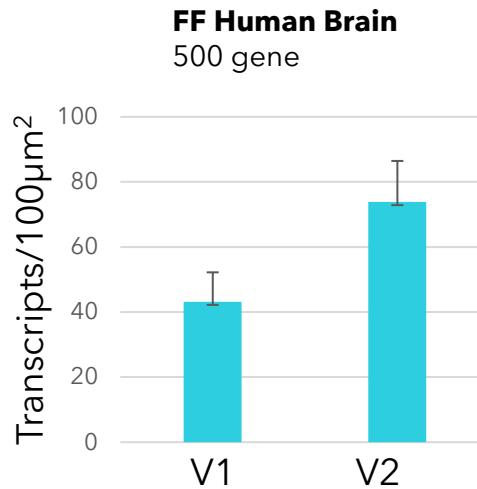
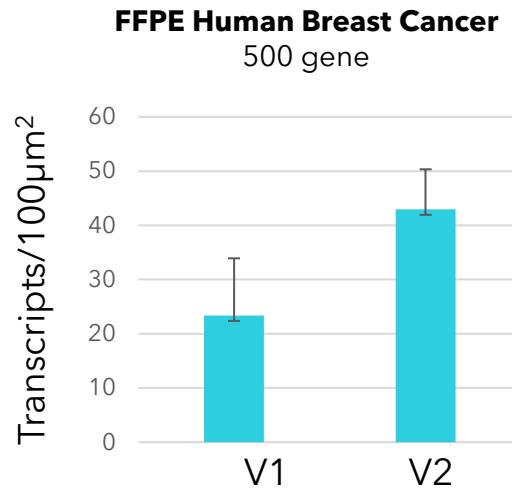


MERFISH uses binary barcodes to encode different mRNA species, which enables *in situ* profiling of hundreds to thousands of genes at single-molecule resolution



The MERSCOPE Platform provides an end-to-end solution for the MERFISH technique, from sample preparation to data analysis and visualization.

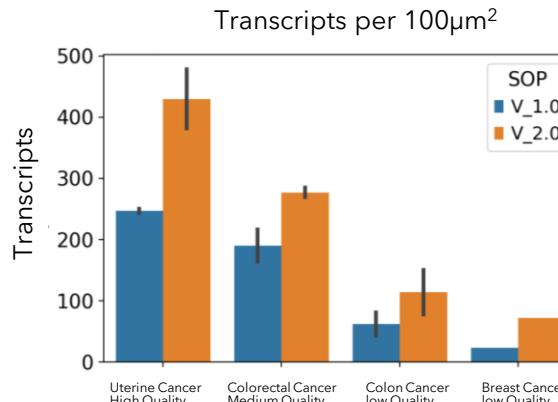
# Improved sensitivity in a wide range of samples with MERFISH 2.0



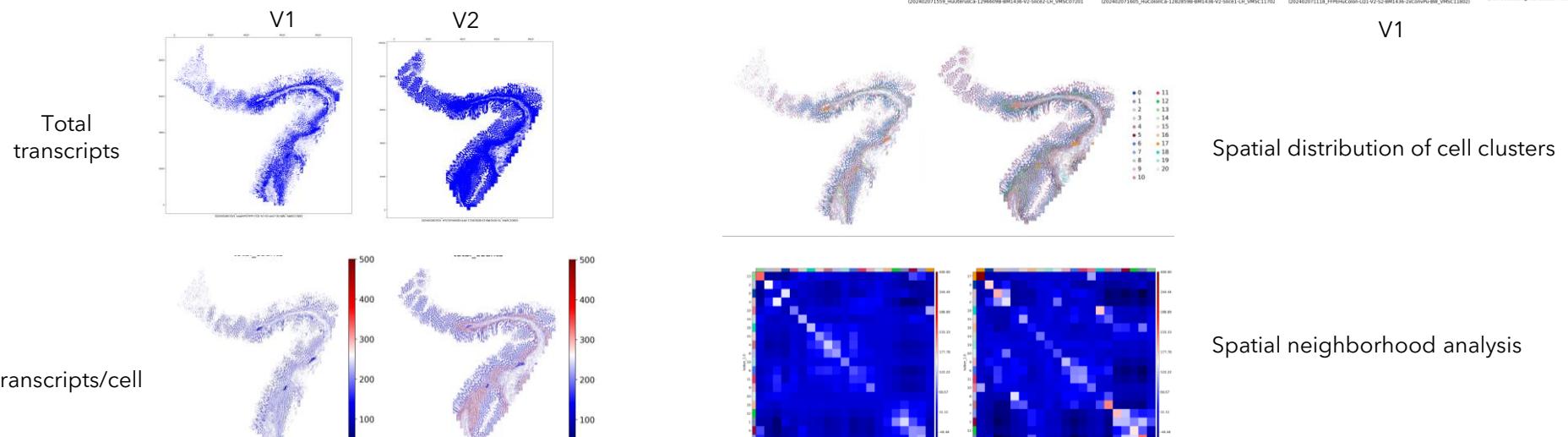
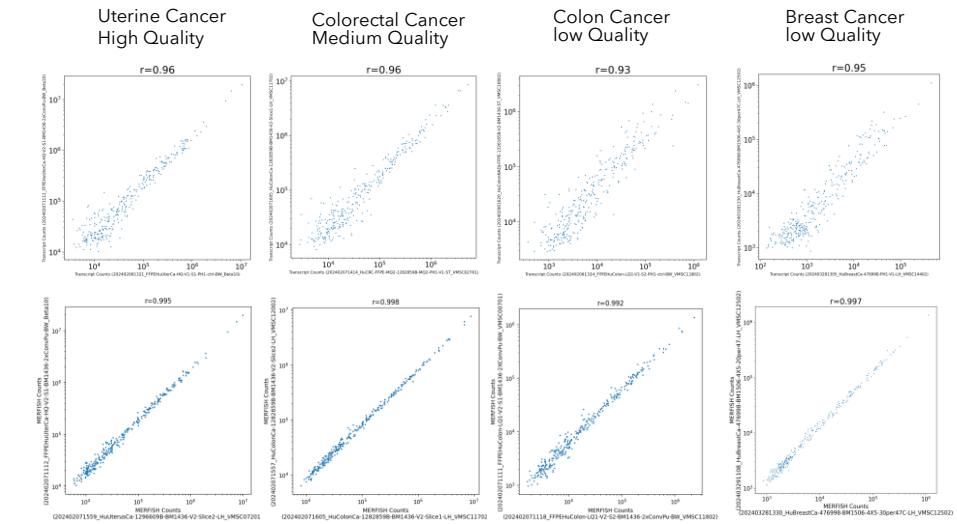
# MERSCOPE sensitivity was increased in both low and high quality FFPE samples with the enhanced workflow

## Human FFPE samples tested

Sample type	Species	Preservation	DV200
Uterine Cancer (HQ)	Human	FFPE	59.71
Colorectal Cancer (MQ)	Human	FFPE	55.09
Colon Cancer (LQ)	Human	FFPE	29.51
Breast Cancer (LQ)	Human	FFPE	51.21



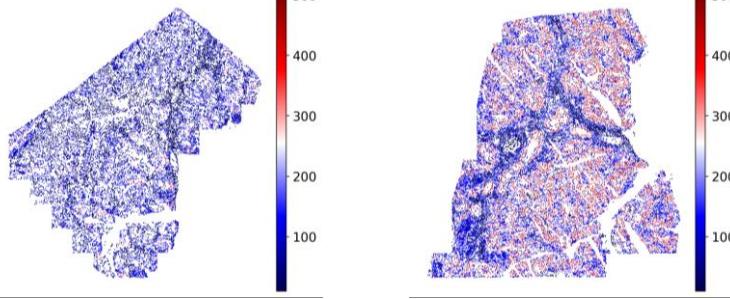
## Enhanced MERFISH has high correlation with the standard protocol



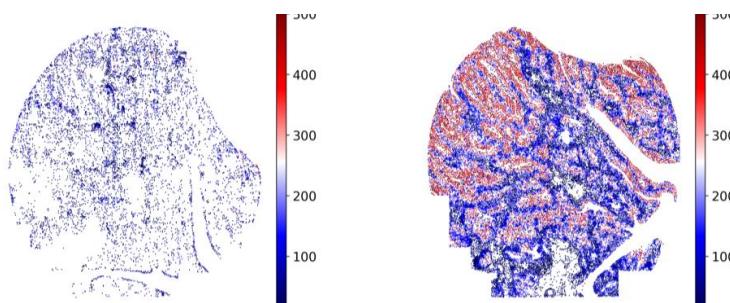
# Enhanced workflow improves counts up to 7x in colon cancer FFPE tissue

## Transcript density

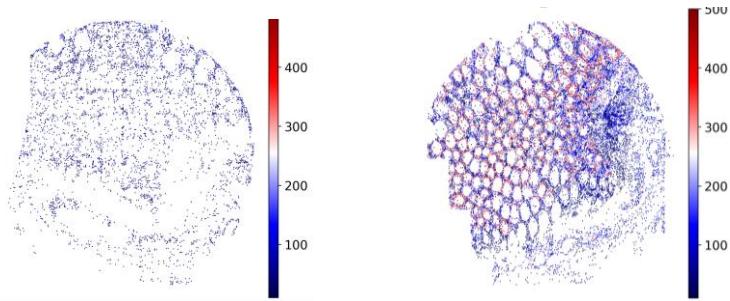
Sample 1



Sample 2



Sample 3

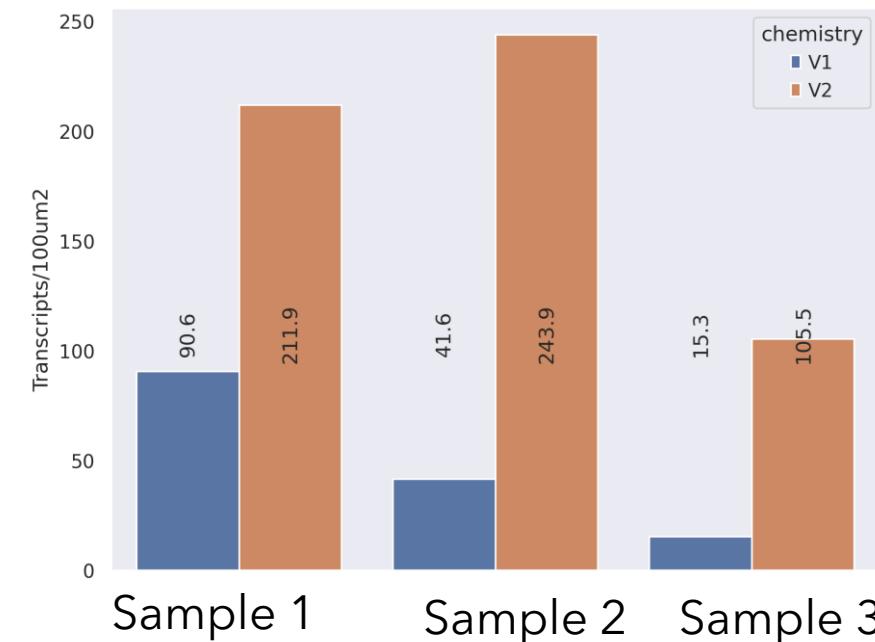


V1

V2

Internally generated data. Validated performance specifications not available until launch.

## Transcripts per 100 $\mu\text{m}^2$

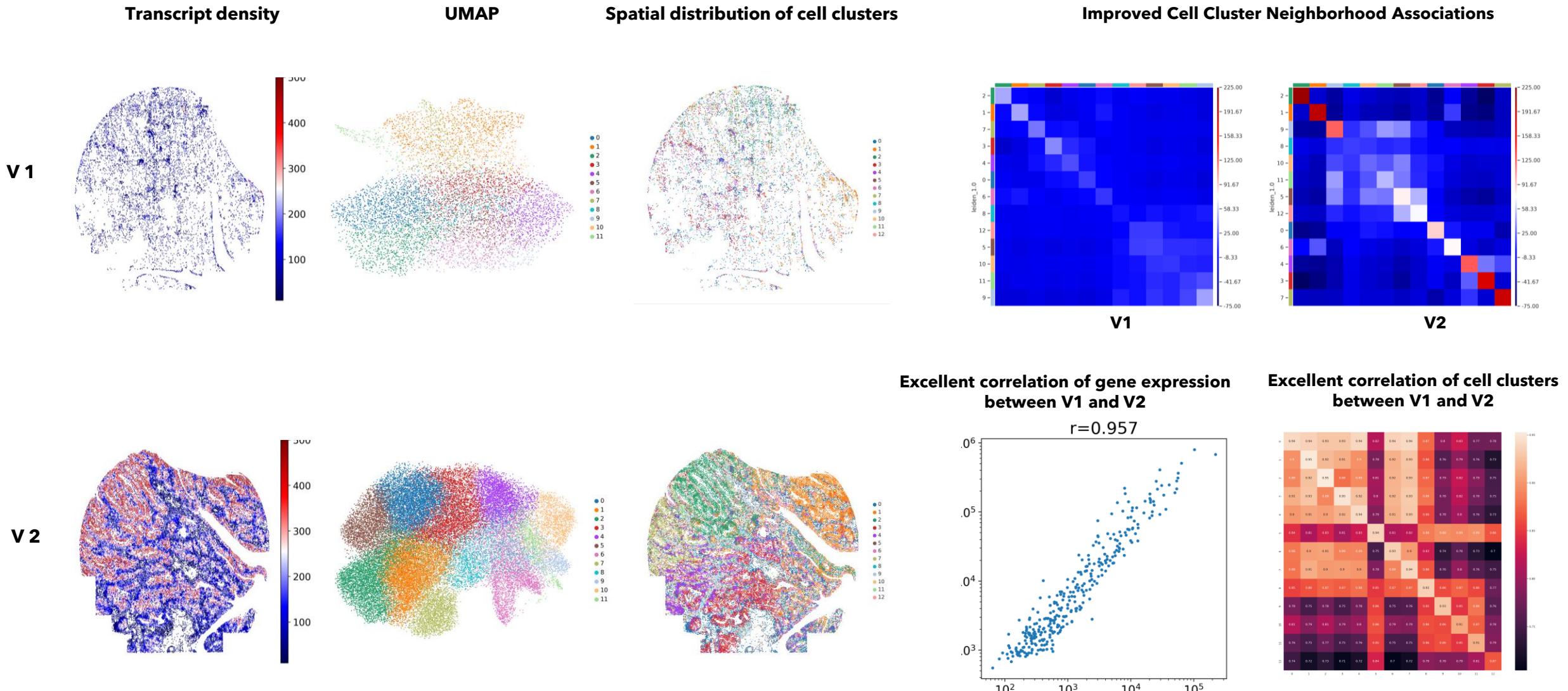


## Transcripts per cell

Sample	Median Transcripts/cell v1	Median Transcripts/cell v2
Sample 1	64	161
Sample 2	35	141
Sample 3	26	111



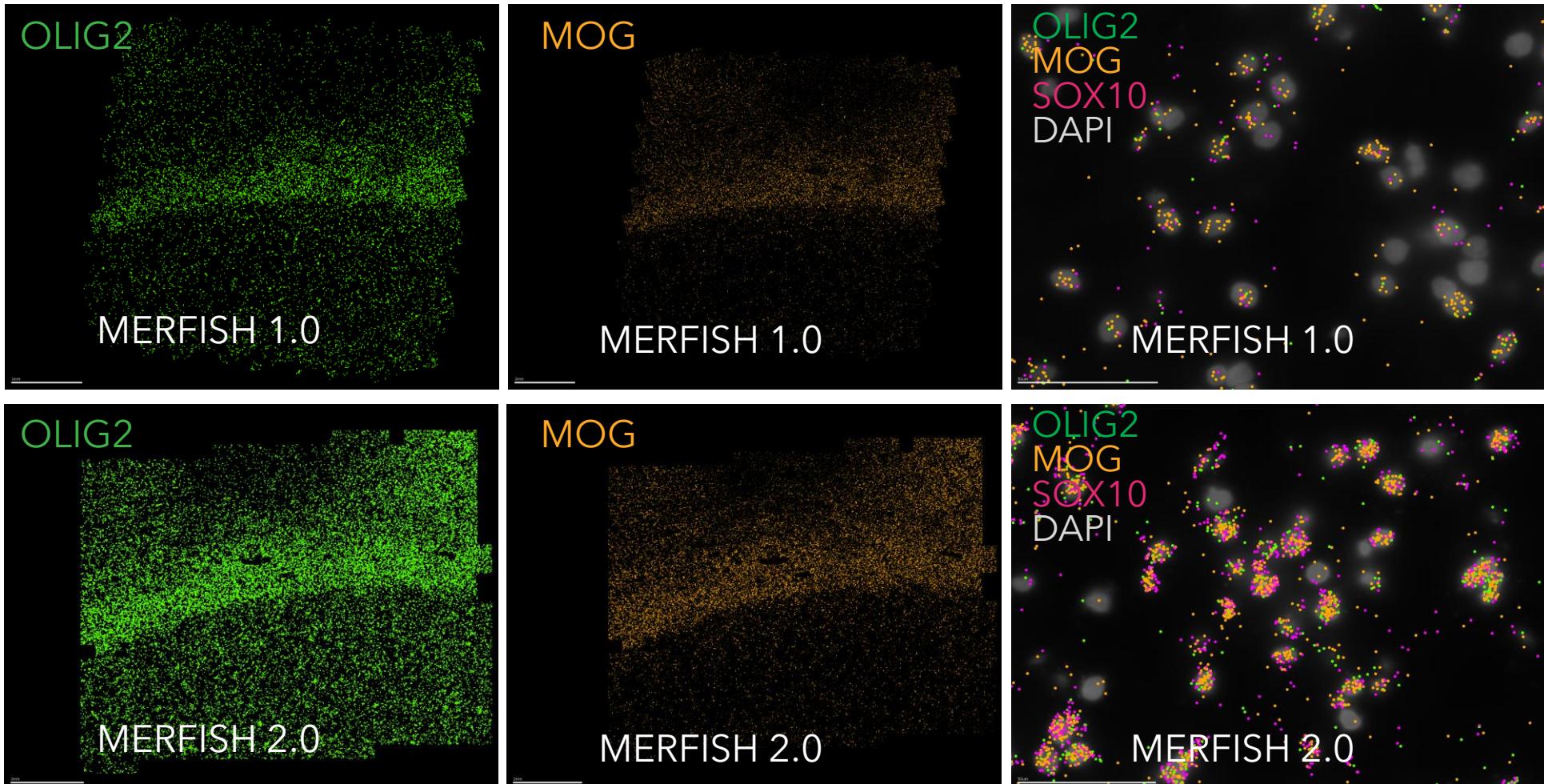
# MERFISH 2.0 greatly improves spatial analysis for tumor samples



Internally generated data. Validated performance specifications not available until launch.

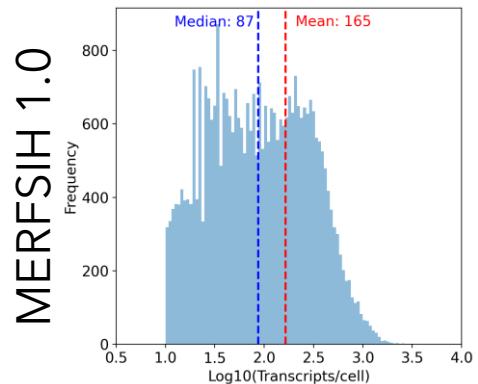
VIZgen

# MERFISH2.0 enhances *in situ* gene expression profiling in human brain

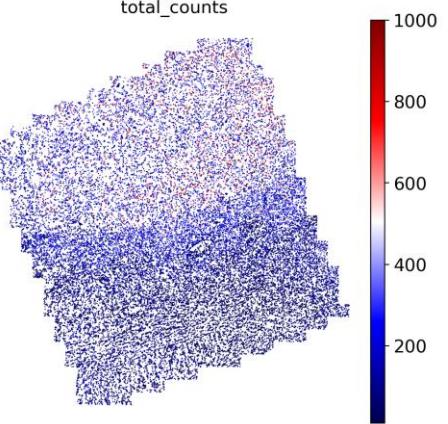


# MERFISH2.0 greatly improves single cell analysis in human brain

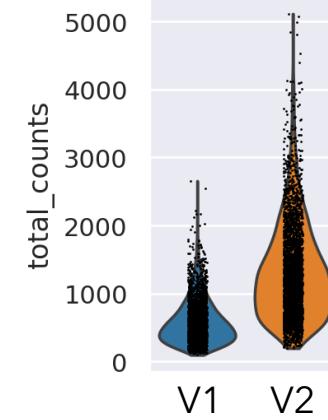
Transcripts/cell



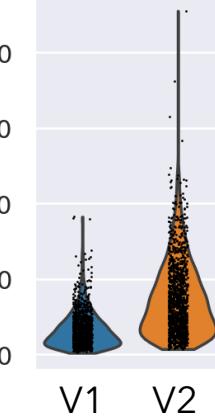
Transcripts/cell



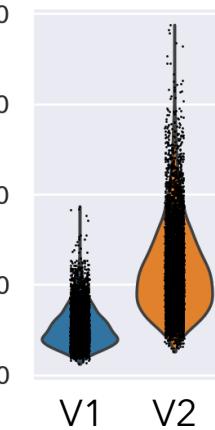
Glutamatergic neuron



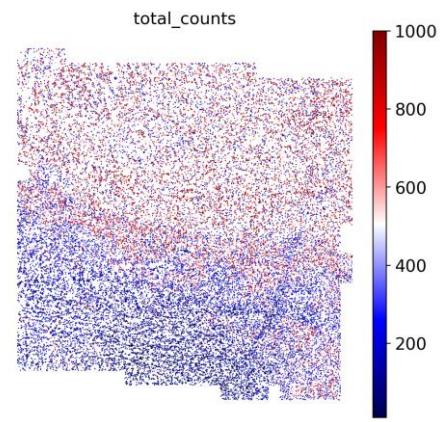
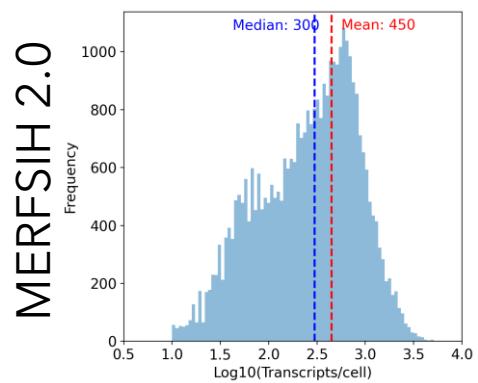
GABAergic neuron



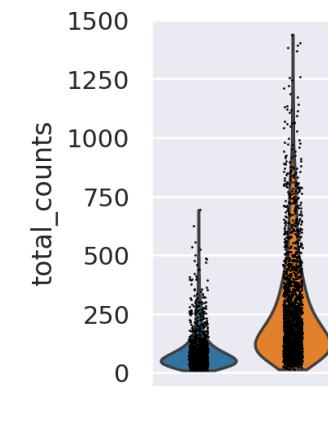
Oligoden-drocyte



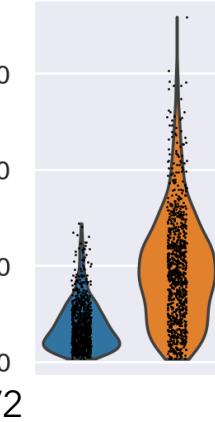
MERFSIH 2.0



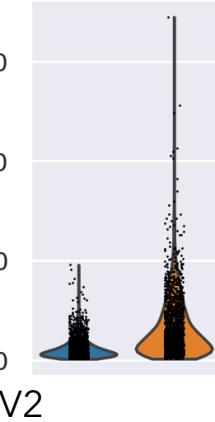
Astrocyte



Vascular cell



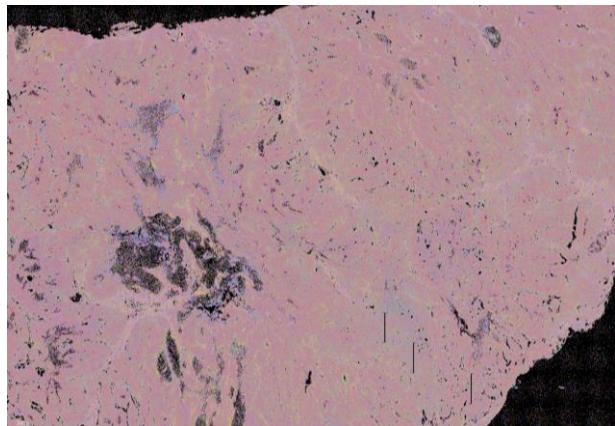
Microglia



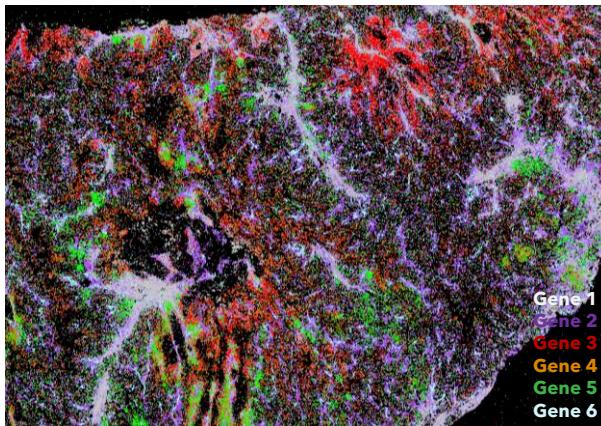
# MERFISH 2.0 Case Studies

# MERFISH 2.0 enables *in situ* spatial transcriptomic imaging in FFPE mouse mammary gland

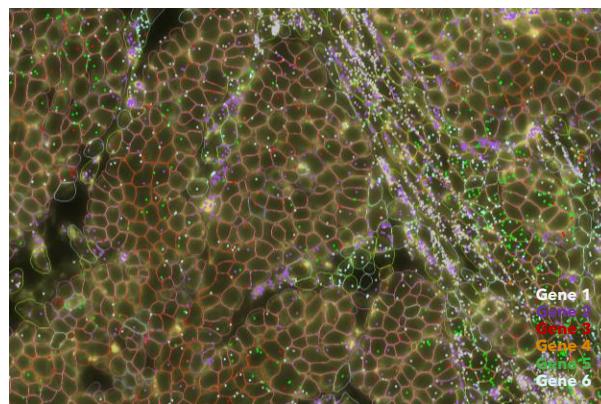
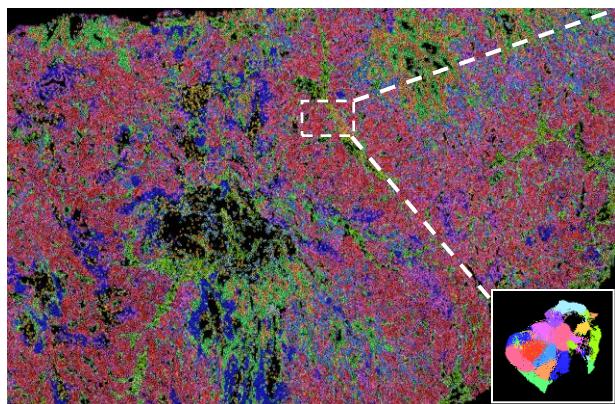
All transcripts  
(377 transcripts/100 $\mu\text{m}^2$ )



Spatial distribution of select genes



Spatial distribution of cell clusters



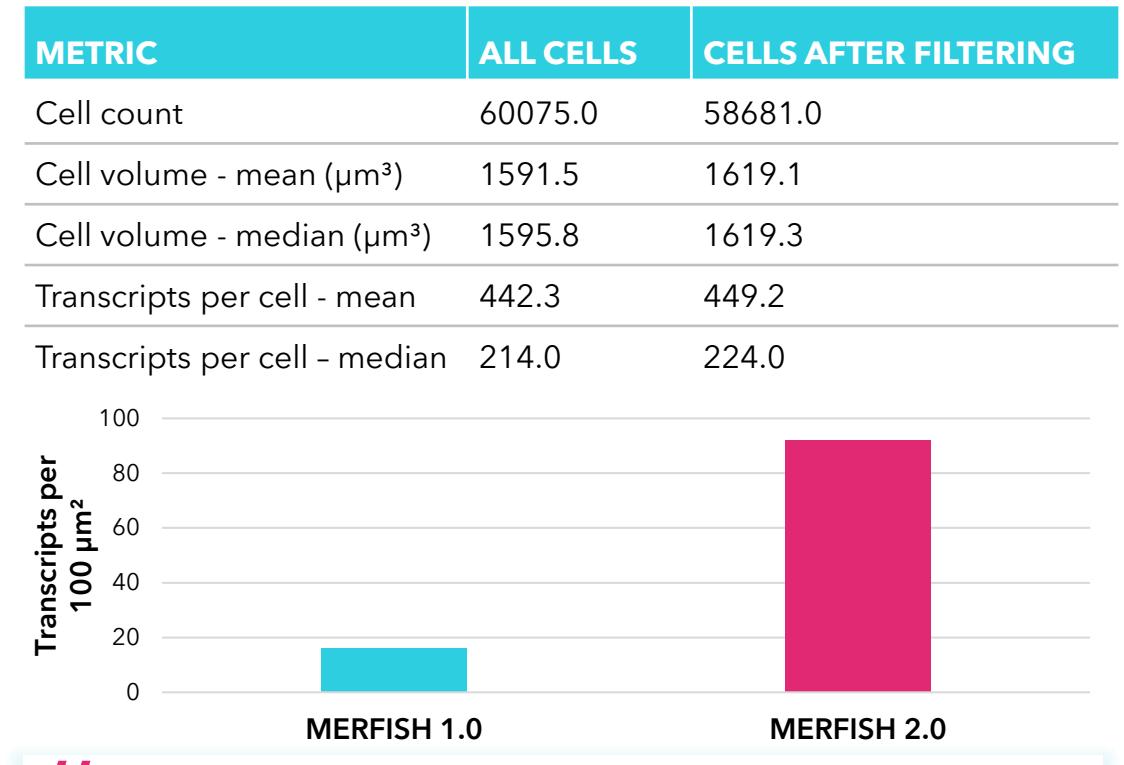
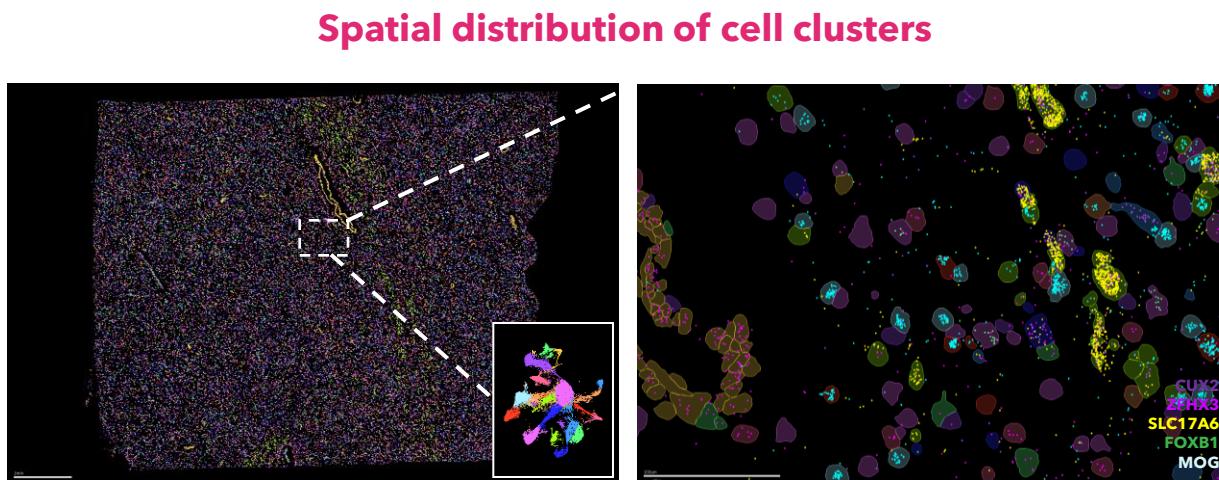
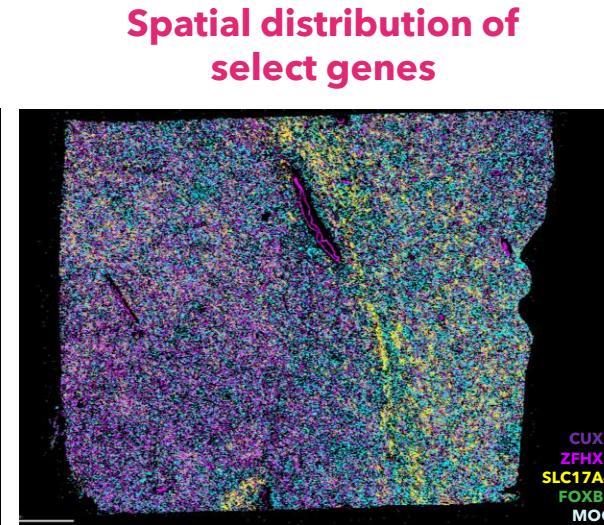
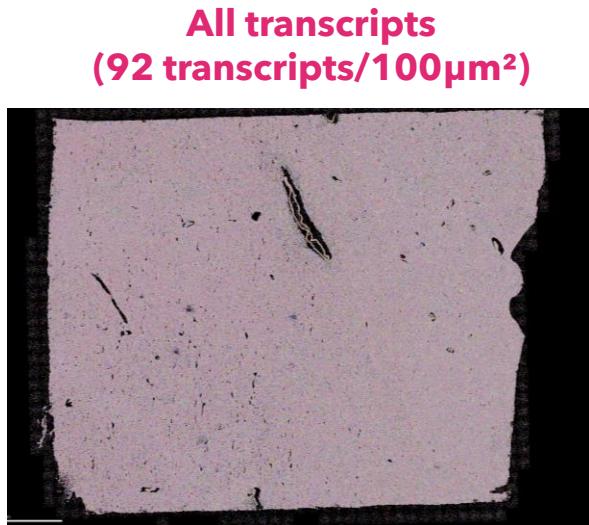
METRIC	ALL CELLS	CELLS AFTER FILTERING
Cell count	245582.0	242767.0
Cell volume - mean ( $\mu\text{m}^3$ )	697.0	700.6
Cell volume - median ( $\mu\text{m}^3$ )	633.8	636.6
Transcripts per cell - mean	396.4	397.4
Transcripts per cell - median	353.0	353.0

//

The data really amazed us with the extraordinary number of transcripts we could detect in sub-optimal samples—almost 10-fold higher than MERFISH 1.0!

Raymond Yip, WEHI, Australia

# MERFISH 2.0 significantly improved transcript detection efficiency in low quality human brain

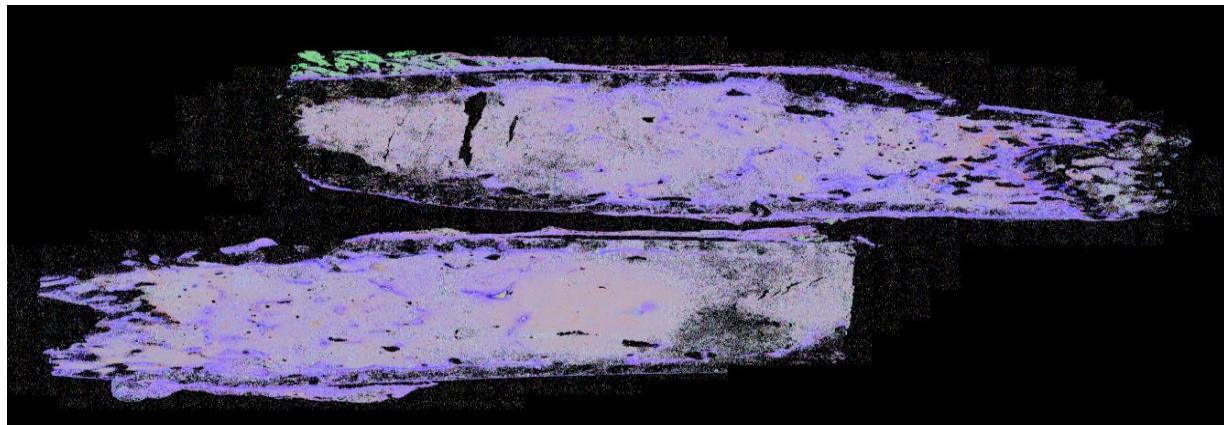


The MERFISH 2.0 chemistry further enhances detection efficiency and sensitivity, offering substantial improvements. In matched low-quality human brain samples, we have seen MERFISH 2.0 improves the sensitivity by >6 fold.

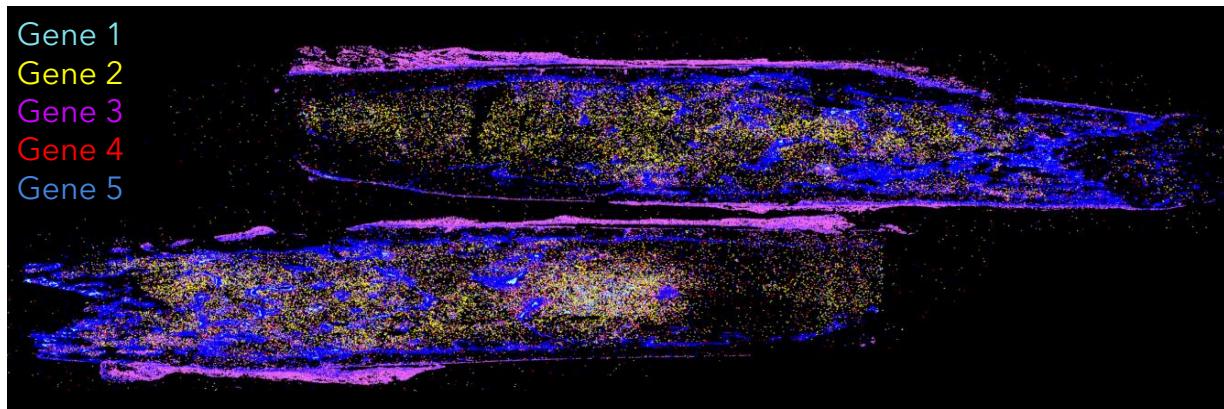
Academic Customer, USA

# MERFISH 2.0 enables *in situ* spatial transcriptomic imaging in FFPE mouse bone marrow

All transcripts (70 transcripts/ $100\mu\text{m}^2$ )



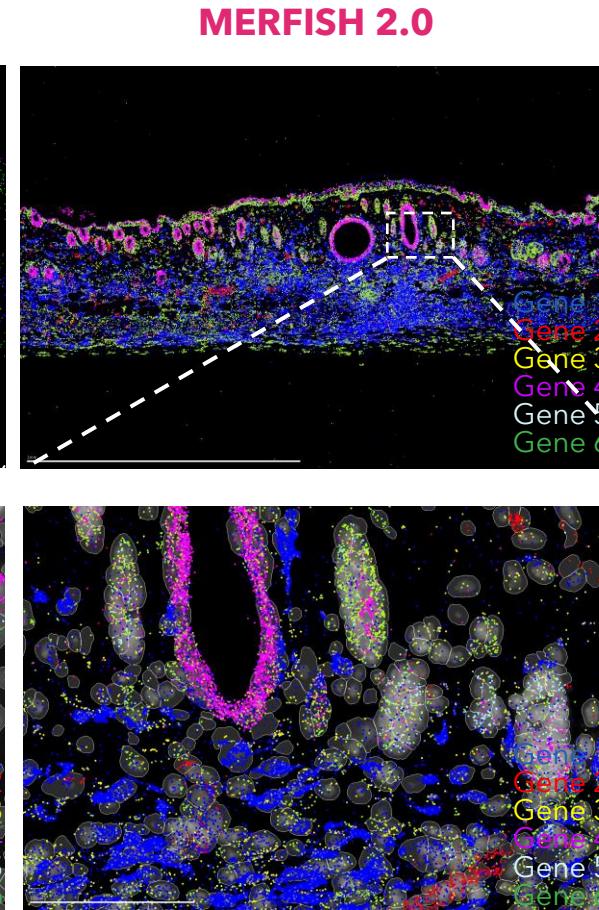
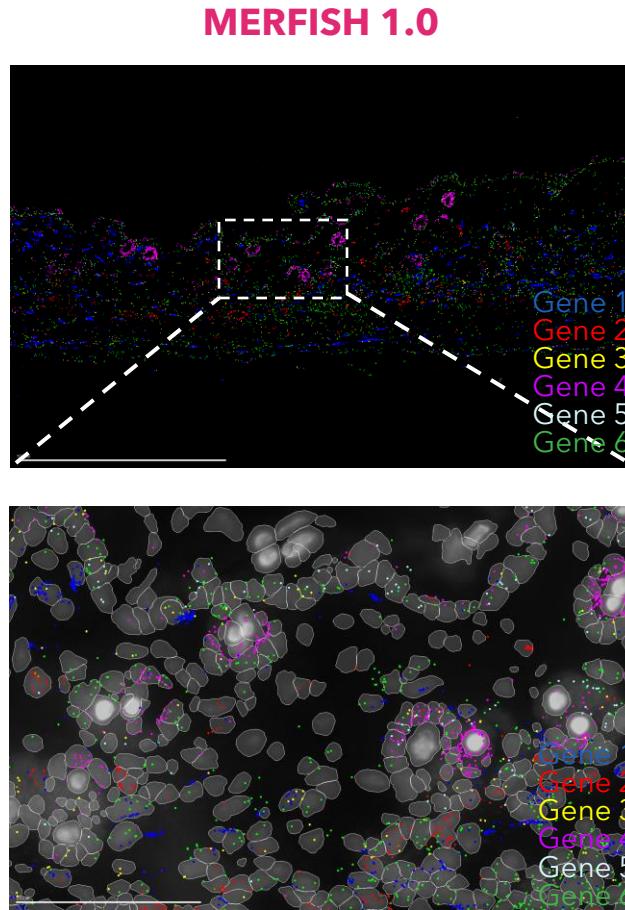
Spatial distribution of select genes



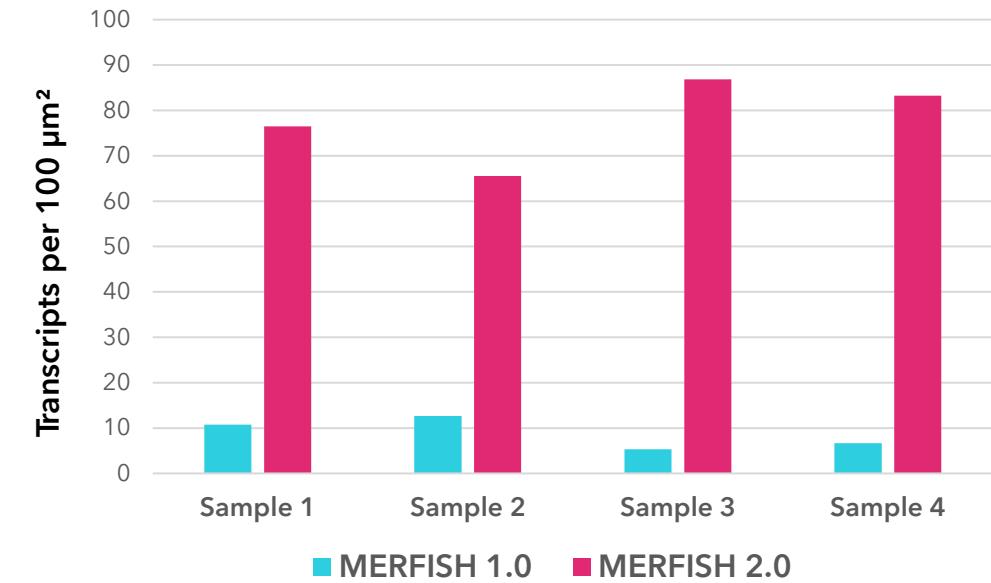
METRIC	ALL CELLS	CELLS AFTER FILTERING
Cell count	104259.0	84124.0
Cell volume - mean ( $\mu\text{m}^3$ )	636.5	679.6
Cell volume - median ( $\mu\text{m}^3$ )	555.8	597.2
Transcripts per cell - mean	92.7	112.1
Transcripts per cell - median	39.0	56.0

Centre de Recherche en Cancérologie de Marseille, France

# MERFISH 2.0 substantially improves transcript detection efficiency in FFPE mouse skin

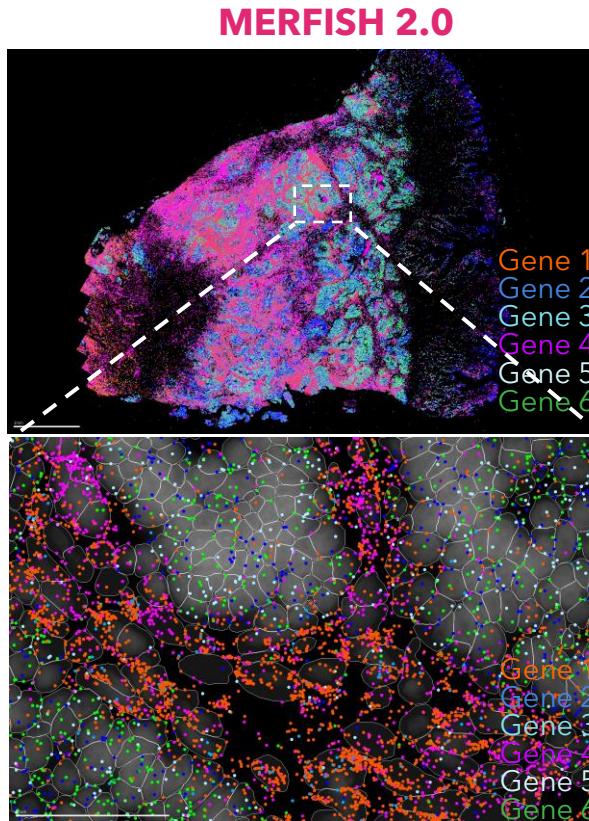
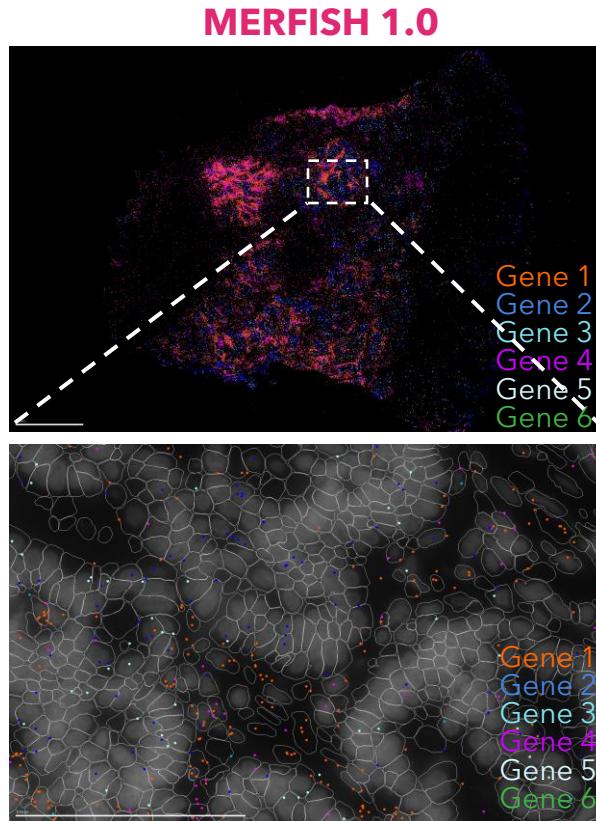


**Comparison of detection efficiency**

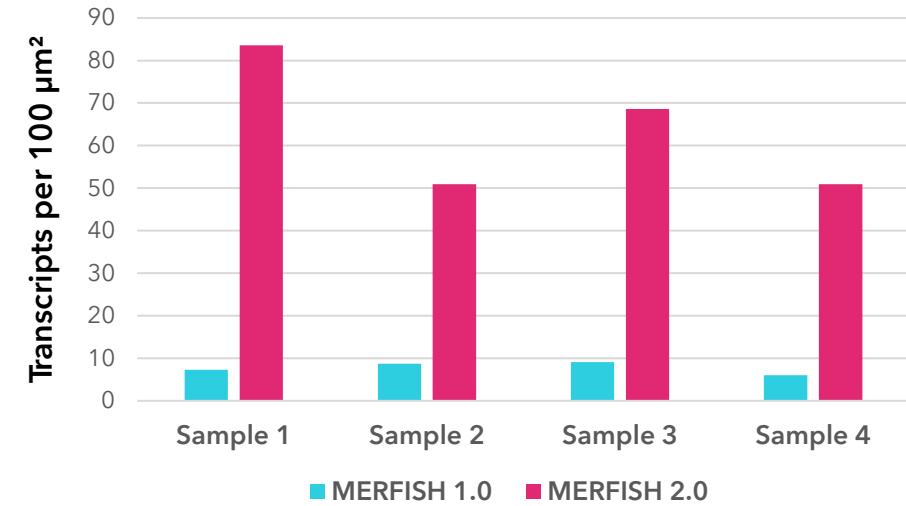


Biotech Customer, USA

# MERFISH 2.0 substantially improves transcript detection efficiency in FFPE human colorectal cancer samples



**Comparison of detection efficiency**

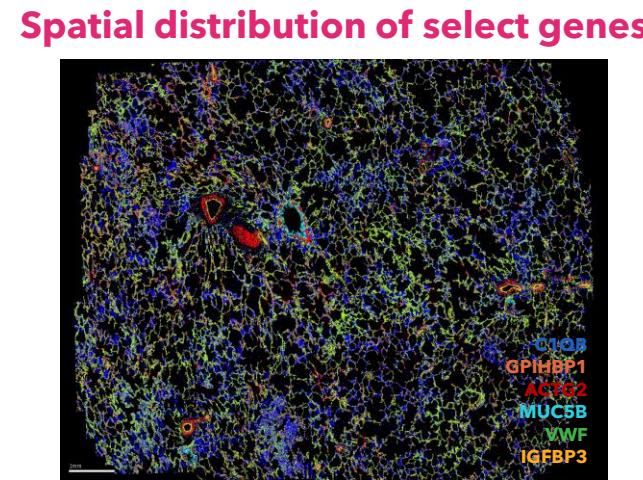
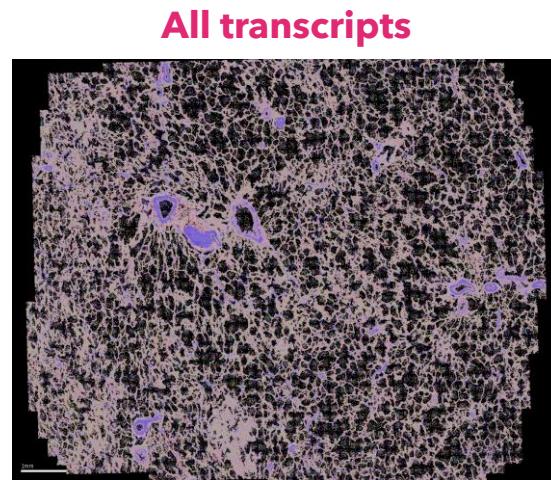


//

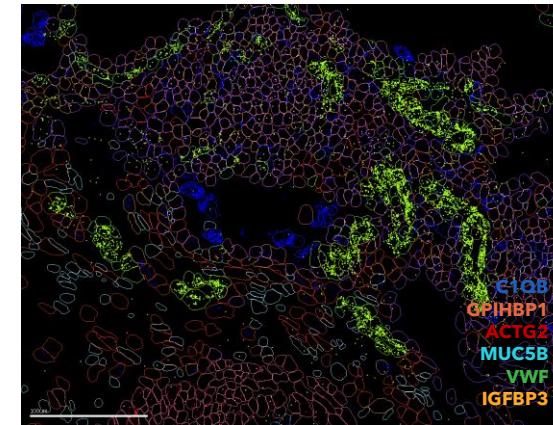
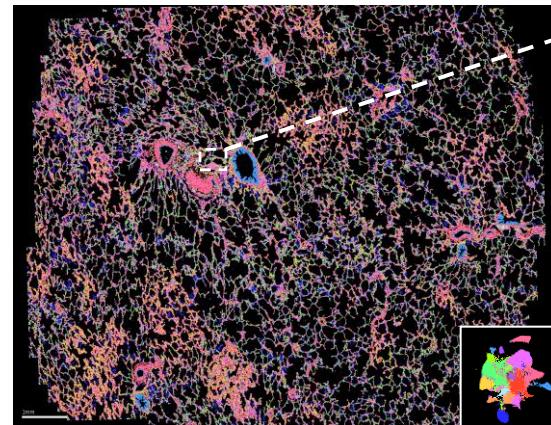
We decided on MERFISH due to the high sensitivity and the relatively low requirements for tissue samples. Our expectations were greatly surpassed by the astonishing (subcellular) resolution of MERFISH 2.0, especially when compared to MERFISH 1.0. This will allow us to mechanistically test the main hypothesis of the project using this data alone, Thank you very much!!!"

University Hospital Tübingen, Germany

# MERFISH 2.0 enables *in situ* spatial transcriptomic imaging in FFPE human lung



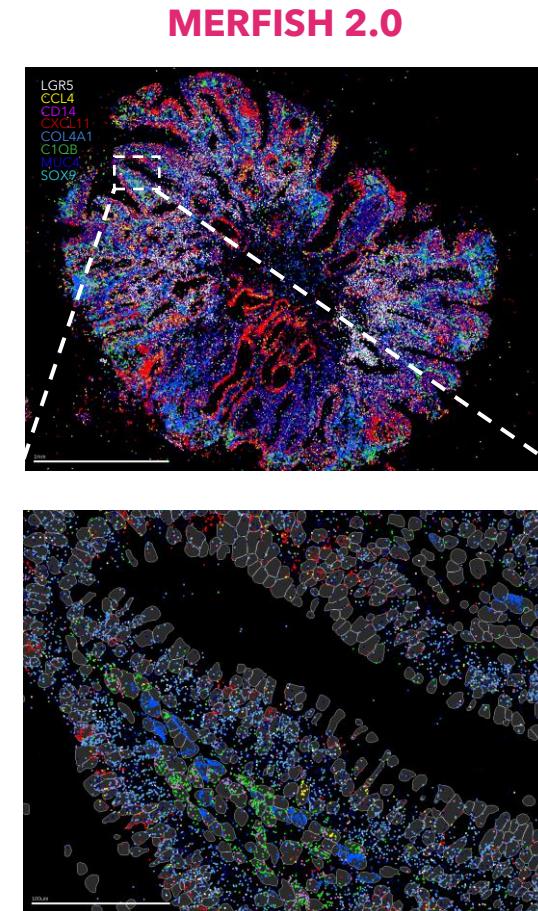
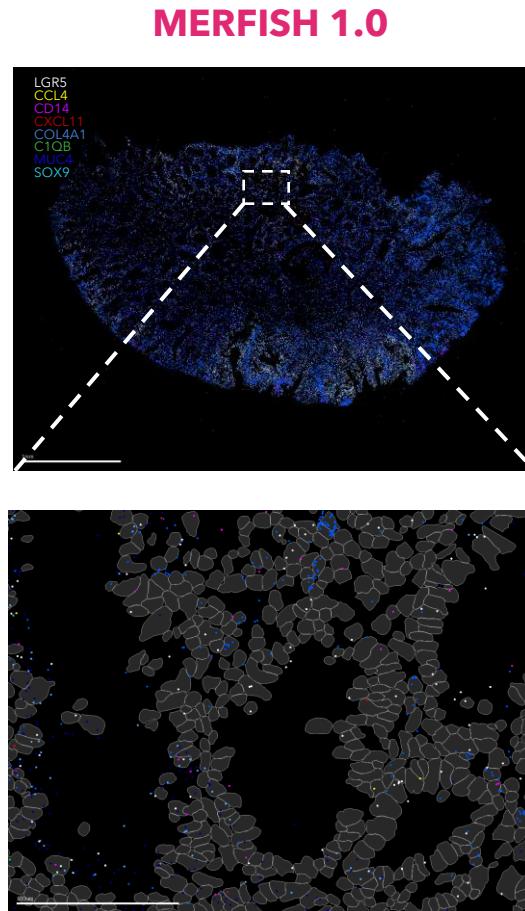
Spatial distribution of cell clusters



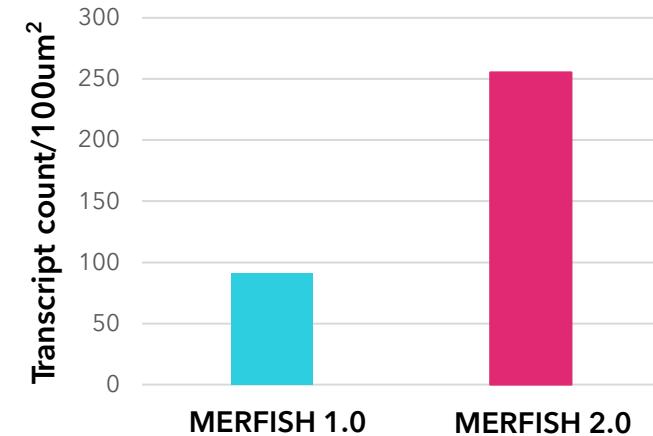
METRIC	ALL CELLS	CELLS AFTER FILTERING
Cell count	301377.0	292065.0
Cell volume - mean ( $\mu\text{m}^3$ )	897.2	912.5
Cell volume - median ( $\mu\text{m}^3$ )	812.4	824.5
Transcripts per cell - mean	112.8	115.5
Transcripts per cell - median	95.0	97.0

Pascal Barbry, Institute of Molecular and Cellular Pharmacology, France

# MERFISH 2.0 enables *in situ* spatial transcriptomic imaging in FFPE human colorectal polyps



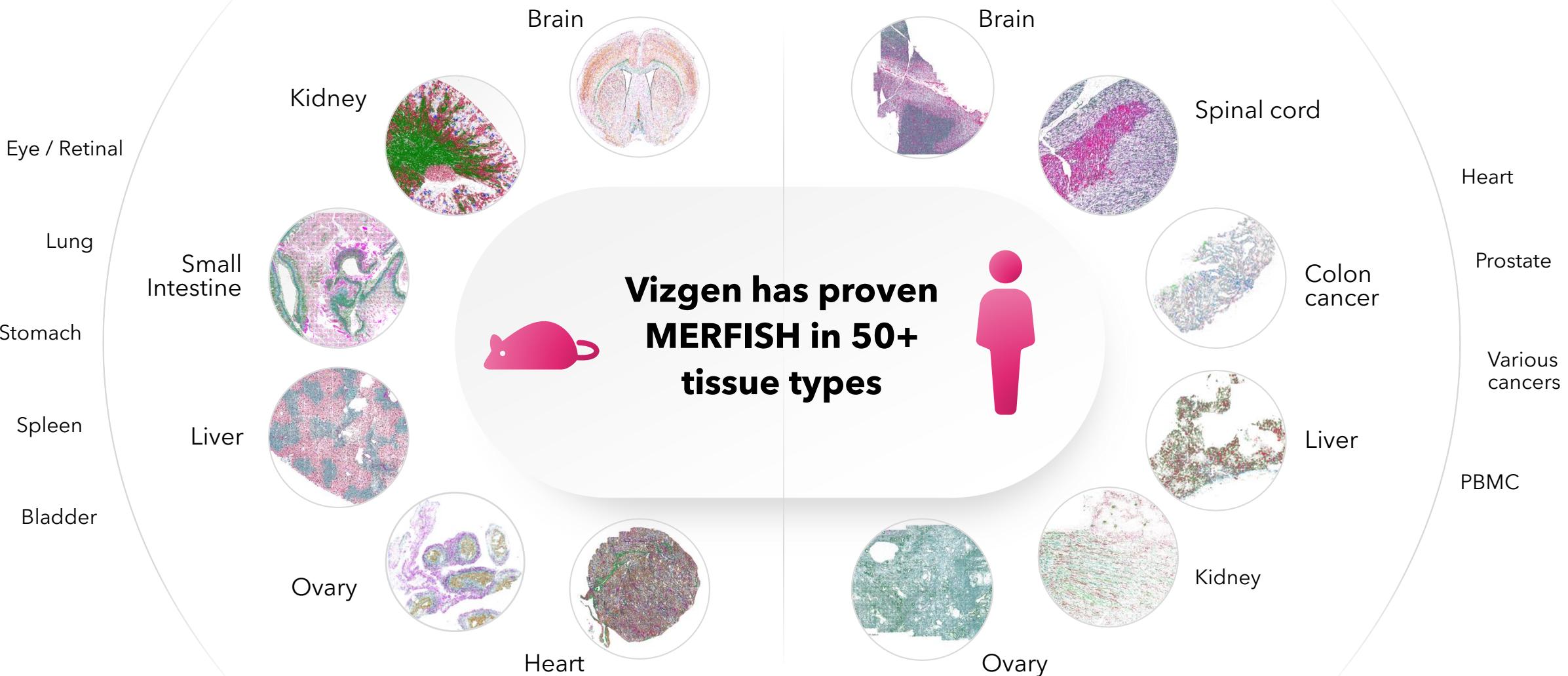
Comparison of detection efficiency



METRIC	ALL CELLS	CELLS AFTER FILTERING
Cell count	83380.0	78865.0
Cell volume - mean ( $\mu\text{m}^3$ )	666.6	671.8
Cell volume - median ( $\mu\text{m}^3$ )	560.8	565.3
Transcripts per cell - mean	176.5	185.1
Transcripts per cell - median	124.0	133.0

Alison Simmons, Director of the MRC Translational Immune Discovery Unit, Oxford University, UK

**Vizgen has proven  
MERFISH in 50+  
tissue types**



**Compatible sample types include cells, fresh frozen / fixed tissue blocks**

# Highly Multiplexed Spatial Transcriptomics in Bacteria

Ari Sarfatis, Yuanyou Wang, Nana Twumasi-Ankrah,  Jeffrey R. Moffitt

doi: <https://doi.org/10.1101/2024.06.27.601034>

This article is a preprint and has not been certified by peer review [what does this mean?].



Abstract    **Full Text**    Info/History    Metrics     Preview PDF

## Abstract

Single-cell decisions made in complex environments underlie many bacterial phenomena. Image-based transcriptomics approaches offer an avenue to study such behaviors, yet these approaches have been hindered by the massive density of bacterial mRNA. To overcome this challenge, we combine 1000-fold volumetric expansion with multiplexed error robust fluorescence *in situ* hybridization (MERFISH) to create bacterial-MERFISH. This method enables high-throughput, spatially resolved profiling of thousands of operons within individual bacteria. Using bacterial-MERFISH, we dissect the response of *E. coli* to carbon starvation, systematically



Jeffrey Moffitt, Harvard University

Bacterial-MERFISH accurately profiles thousands of operons in *E. coli*

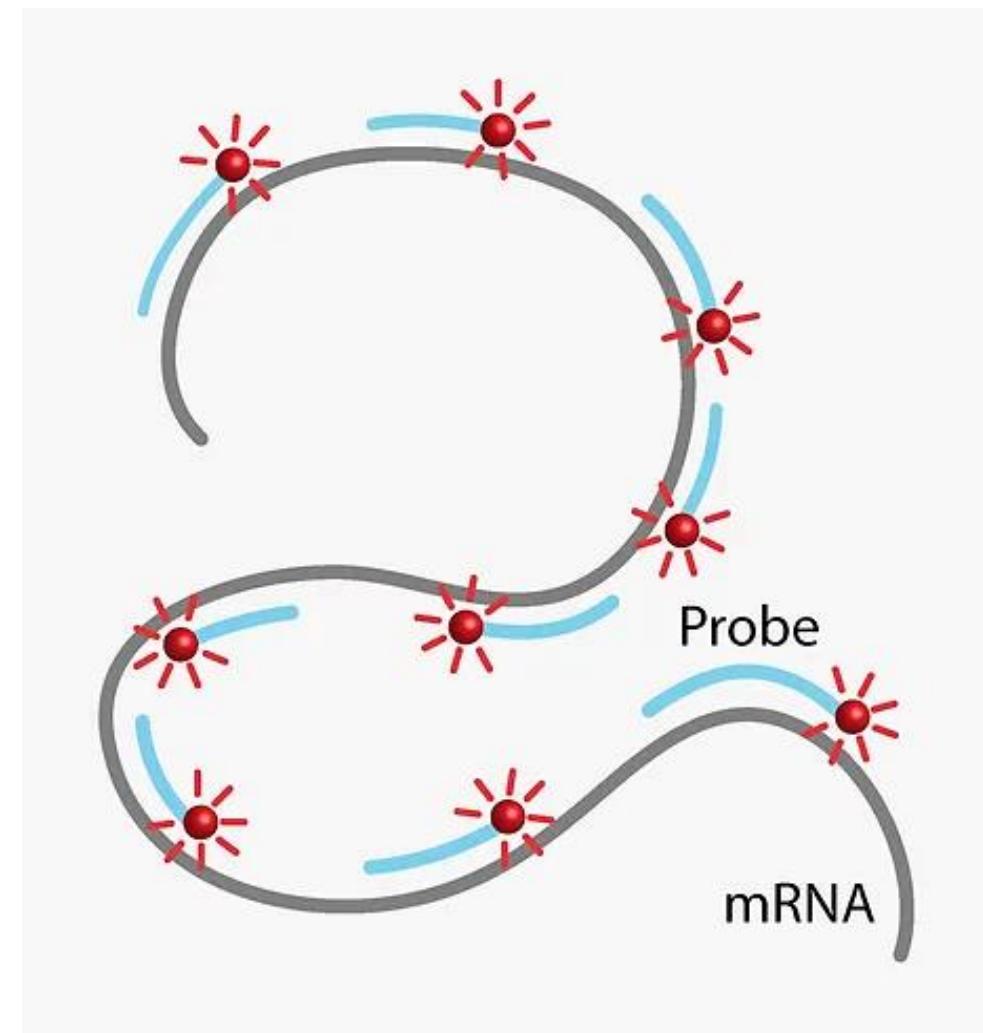
MERFISH enables the identification of thousands of different mRNA molecules by using combinatorial, error-robust, fluorescent optical barcodes built from repetitive rounds of single-molecule FISH (smFISH) (28). However, to decipher barcodes, the fluorescent signal from different molecules must be optically resolvable. For conventional high-resolution optical microscopy, only a few molecules per  $\mu\text{m}^3$  can be distinguished (29). For eukaryotic systems, large transcriptome fractions can be targeted while satisfying this limit (28). By contrast, a log-phase *E. coli* cell contains ~8,000 mRNA molecules in a cell volume of  $\sim 3 \mu\text{m}^3$  (30), producing a total mRNA



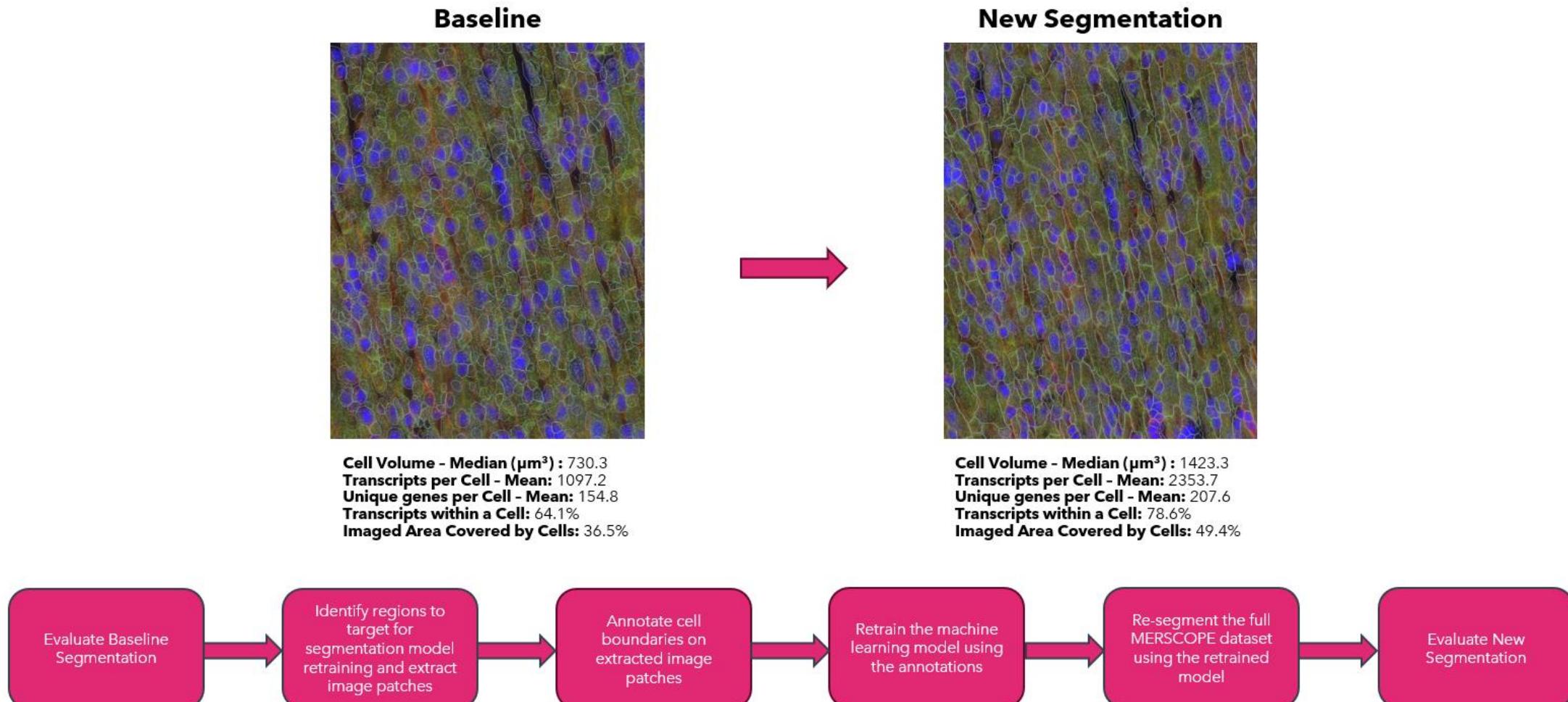
# What is the RNA (Sample) Verification Kit?

## Vizgen Verification Kit:

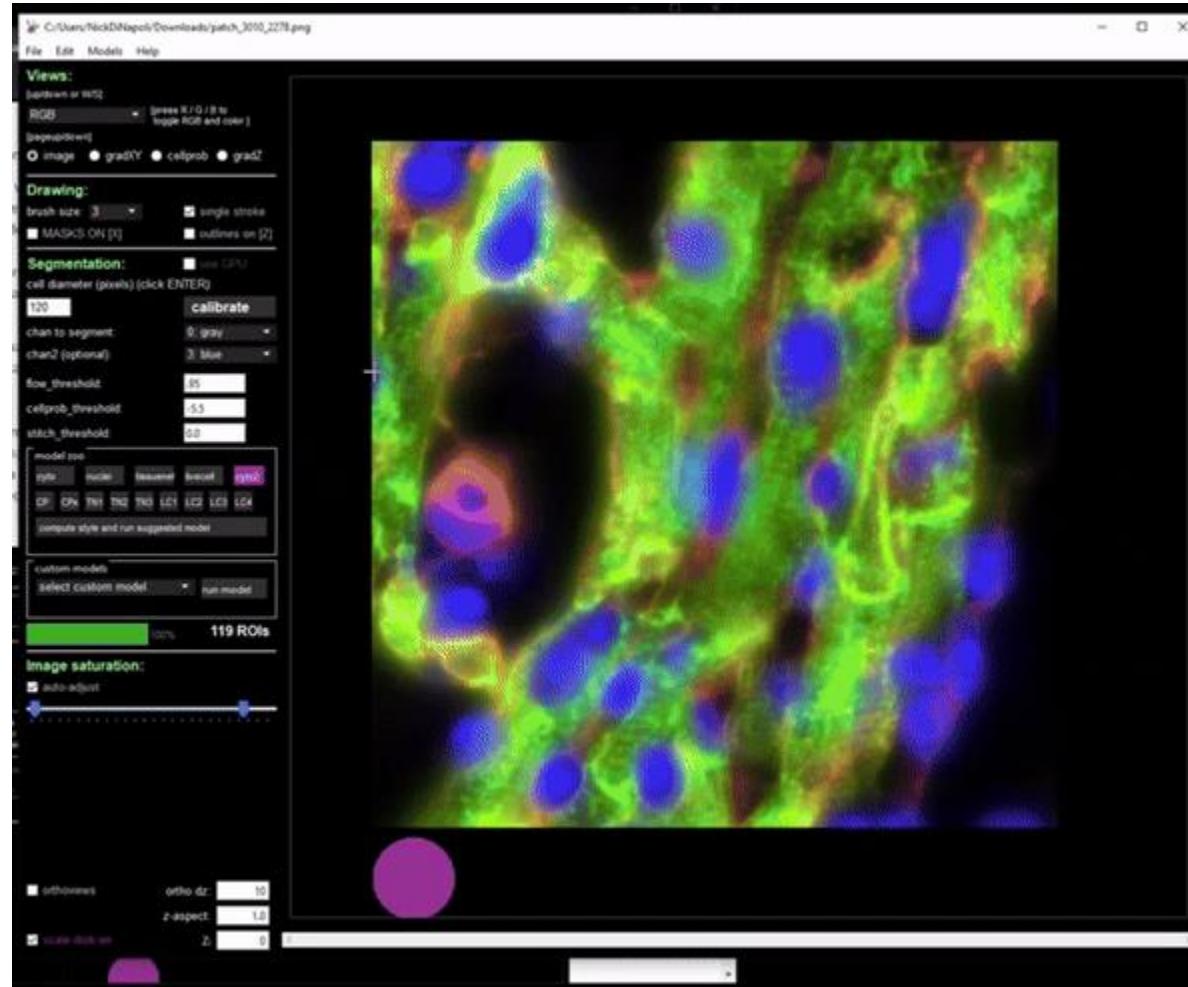
- Uses single-molecule fluorescence *in situ* hybridization (smFISH) methods to tile along the RNA target.
  - smFISH probe: small oligo conjugated to fluorescent dye
- Co-Hybridization of two probe sets with different fluorophores
- Data assessed via co-localization of smFISH spots
- Less expensive and time consuming than running MERFISH panel



# How does it work?



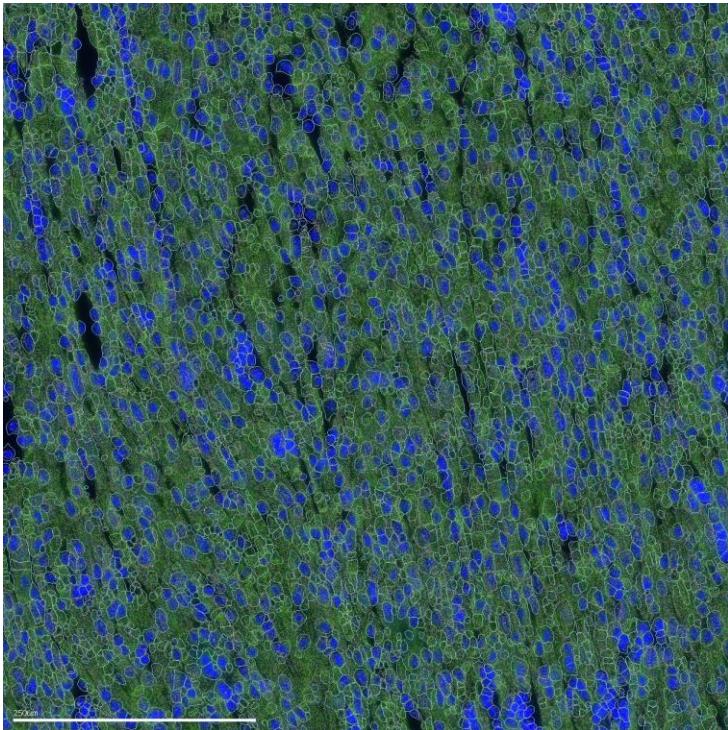
# Provide input to improve the segmentation model



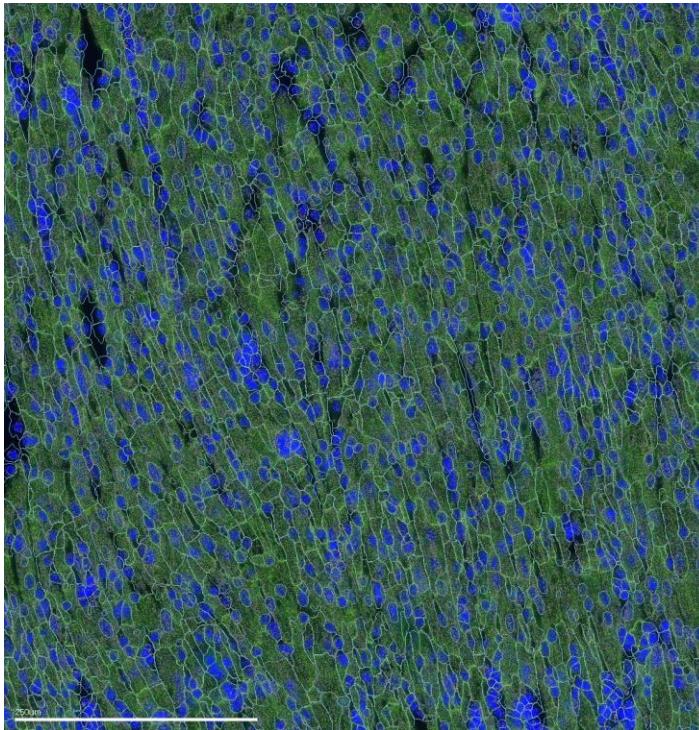
# End Results

- Dataset: Mouse Heart

Cellpose 1



Cellpose 2



Cellpose 1

## Segmentation Metrics

METRIC	ALL CELLS	CELLS AFTER FILTERING
Cell count	120178.0	107020.0
Cell volume - mean ( $\mu\text{m}^3$ )	876.2	920.7
Cell volume - median ( $\mu\text{m}^3$ )	730.3	770.7
Transcripts per cell - mean	1097.2	1221.6
Transcripts per cell - median	891.0	995.0
Unique genes per cell - mean	154.8	170.7
Unique genes per cell - median	150.0	162.0
Transcripts within a cell (%)	64.1	63.6
Imaged area covered by cells (%)	36.5	34.1
Filtered out cell density (1/100 $\mu\text{m}^2$ )	0.0	0.0

Cellpose 2

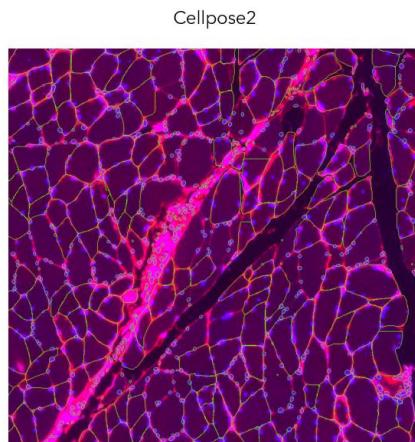
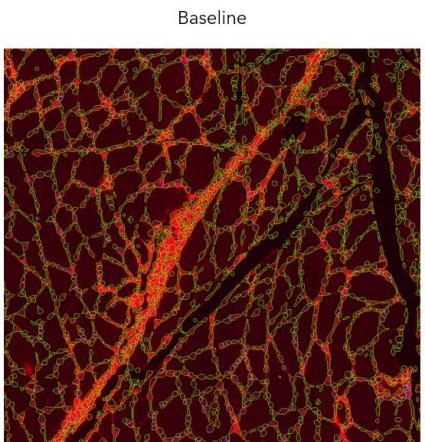
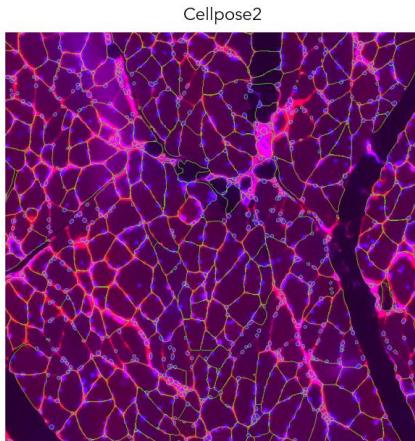
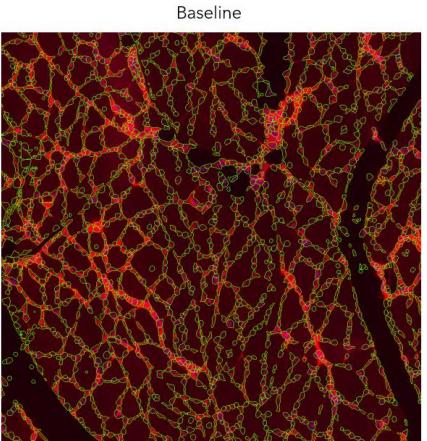
## Segmentation Metrics

METRIC	ALL CELLS	CELLS AFTER FILTERING
Cell count	68657.0	58731.0
Cell volume - mean ( $\mu\text{m}^3$ )	2075.4	2136.7
Cell volume - median ( $\mu\text{m}^3$ )	1423.3	1547.7
Transcripts per cell - mean	2353.6	2748.6
Transcripts per cell - median	1514.0	1805.0
Unique genes per cell - mean	207.6	241.6
Unique genes per cell - median	219.0	237.0
Transcripts within a cell (%)	78.6	78.5
Imaged area covered by cells (%)	49.4	43.5
Filtered out cell density (1/100 $\mu\text{m}^2$ )	0.0	0.0



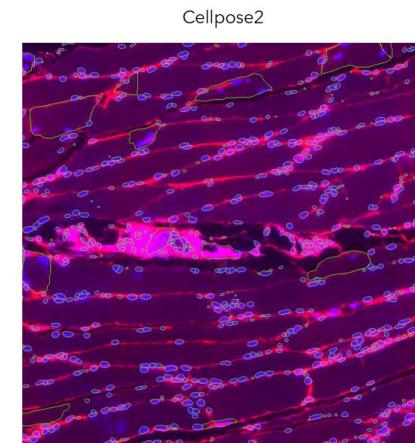
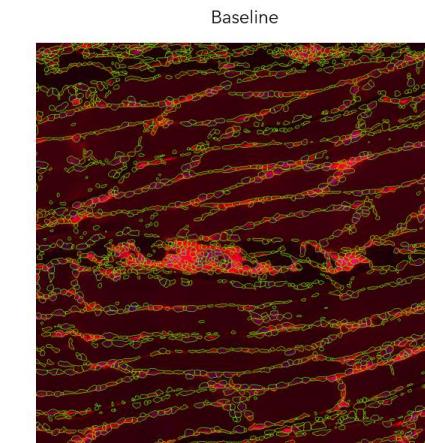
# End Results

- Dataset: Human Muscle



Segmentation Metrics		
METRIC	ALL CELLS	CELLS AFTER FILTERING
Cell count	218649.0	76533.0
Cell volume - mean ( $\mu\text{m}^3$ )	1049.6	1607.3
Cell volume - median ( $\mu\text{m}^3$ )	878.8	1477.9
Transcripts per cell - mean	96.0	227.6
Transcripts per cell - median	45.0	195.0
Unique genes per cell - mean	30.5	65.4
Unique genes per cell - median	19.0	60.0
Transcripts within a cell (%)	46.3	38.4
Imaged area covered by cells (%)	20.6	11.0
Filtered out cell density (1/100 $\mu\text{m}^2$ )	0.0	0.13

Segmentation Metrics		
METRIC	ALL CELLS	CELLS AFTER FILTERING
Cell count	82867.0	34594.0
Cell volume - mean ( $\mu\text{m}^3$ )	3920.0	8445.1
Cell volume - median ( $\mu\text{m}^3$ )	636.8	1143.1
Transcripts per cell - mean	289.9	644.3
Transcripts per cell - median	79.0	195.0
Unique genes per cell - mean	52.3	99.3
Unique genes per cell - median	36.0	70.0
Transcripts within a cell (%)	53.0	49.2
Imaged area covered by cells (%)	29.1	26.2
Filtered out cell density (1/100 $\mu\text{m}^2$ )	0.0	0.05



# Why are custom gene panels so critical?

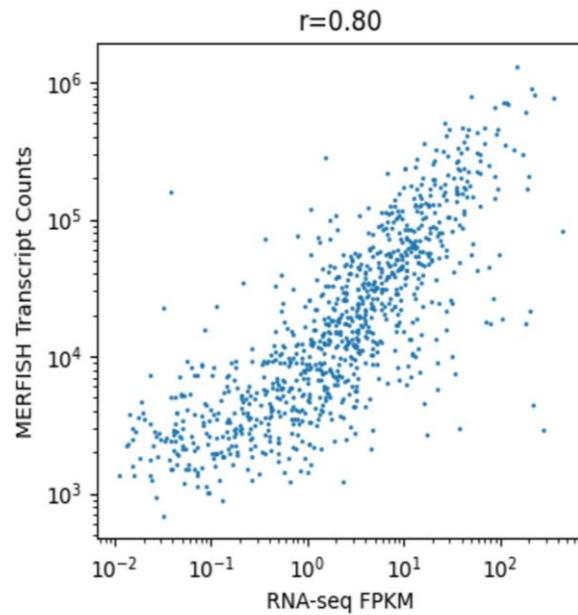
- **Species Flexibility:** No competitors have announced >1000plex panels for any species other than human
  - Vizgen allows you to customize any species, any gene list, any disease for research beyond human cancer and mouse brains
  - Move from preclinical animal models through trials on a single seamless platform
- **Research Flexibility:** Specific questions require specific content
  - By trying to be everything everyone, competitor high plex panels are missing key content for specific research
  - Example: Vizgen Liver HCC panel: of 400 genes, 223 (56%) are **NOT** in competitor's 1k panel. 80 (20%) not in 6k
- **Seamless transition from Single Cell Gene Expression**
  - View differentially expressed genes in single cell experiments in space, without the constraints of panels
  - Define highest quality cell type markers using YOUR single cell data and map these cell types spatially
  - Example: Allen Brain mouse atlas: Used 500 gene MERSCOPE panel to recapitulate >5000 distinct mouse brain cell states

		Not included in competitor N premade panels			
Vizgen Panel	Gene number	Number		Percentage	
		6k	1k	6k	1k
Immuno Oncology	500	40	199	8%	40%
Liver	400	80	223	20%	56%
Pan Cancer	500	78	351	16%	70%

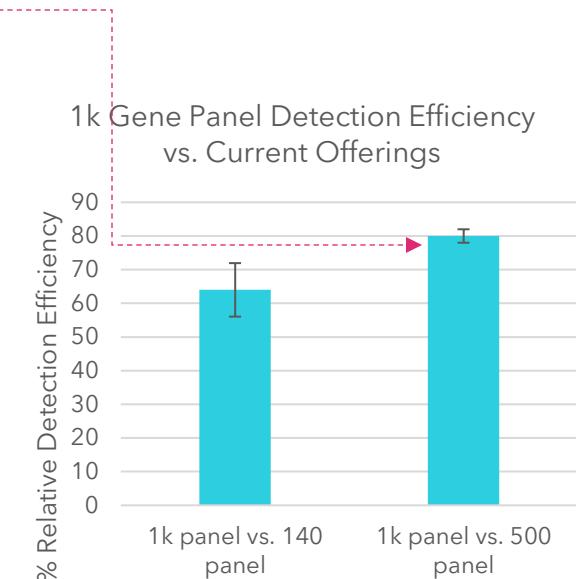
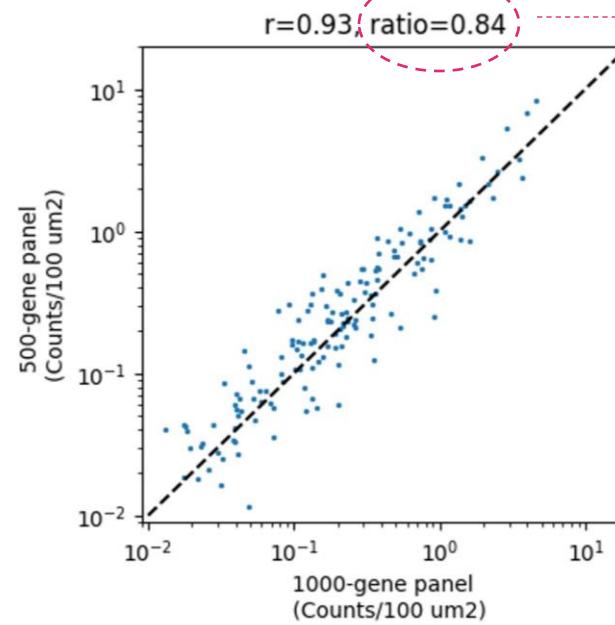
# MERSCOPE 1000 Gene Panel Maintains High Specificity

Vizgen's MERSCOPE 1000 Gene Panel and MERSCOPE 1000 Gene Imaging Kit will provide researchers with the ability to expand their gene targets while maintaining high sensitivity and specificity

## MERSCOPE 1000 Gene correlation to Bulk RNA sequencing



## MERSCOPE 1000 Gene Experiment correlation to MERSCOPE 500 Gene Experiment



Generate High Quality Data at High Plex

Data from mouse brain with 500 and 1000 gene panels

Minimal drop in detection efficiency at high plex

# Customizable Panels

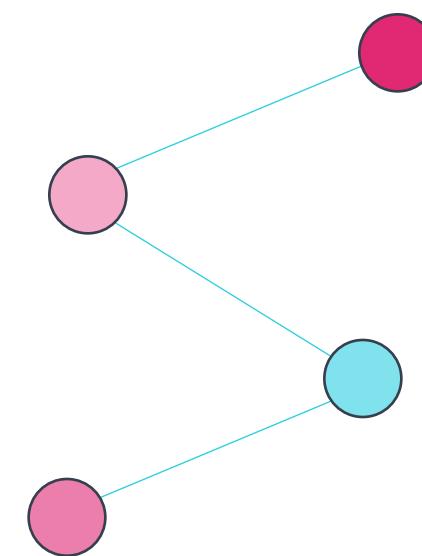
## Vizgen Customizable Panels

- Total panel size of 1000 (960)
- Predesigned component of 860 genes
- Reservation of 100 genes for customization



## Extensive Panel Library

Aggressive roadmap of panels targeting major neurology, oncology, tissues and applications





VIZgen®

Leader in single-cell spatial transcriptomics

*"dedicated to pioneering the next generation of genomics, providing tools that demonstrate the possibilities of *in situ* single-cell spatial genomics"*



Ultivue

Leader in mid-plex spatial proteomics

**Stronger  
Together**

*"powering insights for the development of precision cancer therapies through AI-driven, quantitative spatial proteomics"*



VIZgen

## For More Information



### Website

[www.vizgen.com](http://www.vizgen.com)



### Email

[info@vizgen.com](mailto:info@vizgen.com)



### LinkedIn

[linkedin.com/company/vizgen](https://linkedin.com/company/vizgen)



### Twitter

@vizgen\_inc



vizgen

The logo consists of the word "vizgen" in a large, white, sans-serif font. The letter "i" has a circular graphic with a grid pattern inside it. The background is a solid pink color with a subtle circular gradient and scattered small, semi-transparent pink and orange dots.