

Biology at true resolution

Envisioning new dimensions: Visium spatial gene expression



Who 10x is

Resolving biology to advance human health

- 10x Genomics builds products to interrogate, understand and master biology to advance human health.
- Our products have been adopted by researchers around the world and have been cited in over 2,000 research papers on discoveries across the life sciences, including oncology, immunology, and neuroscience.



Serge Saxonov
CEO & Co-Founder



Ben Hindson
CSO & Co-Founder

800+

Number of
employees

2012

Year founded

Pleasanton, CA
USA

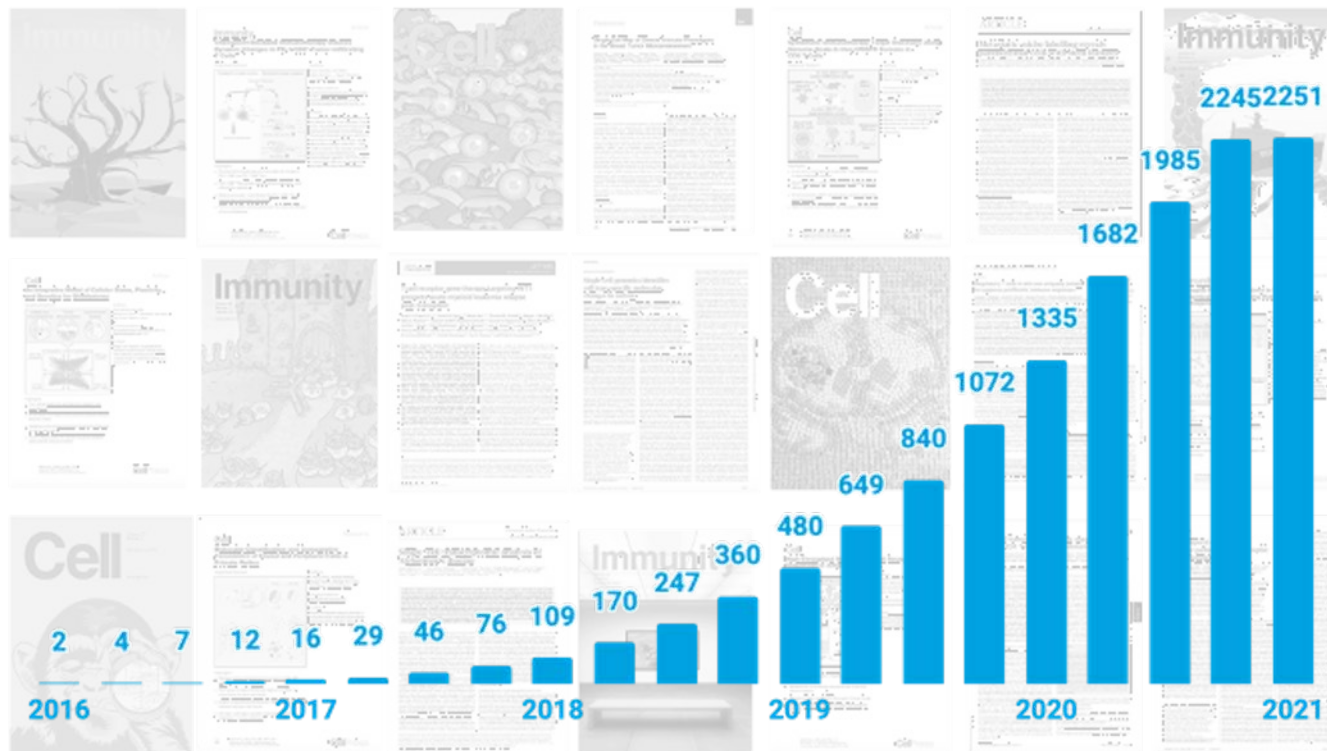
Headquarters

2019

Year IPO

Biology at True Resolution

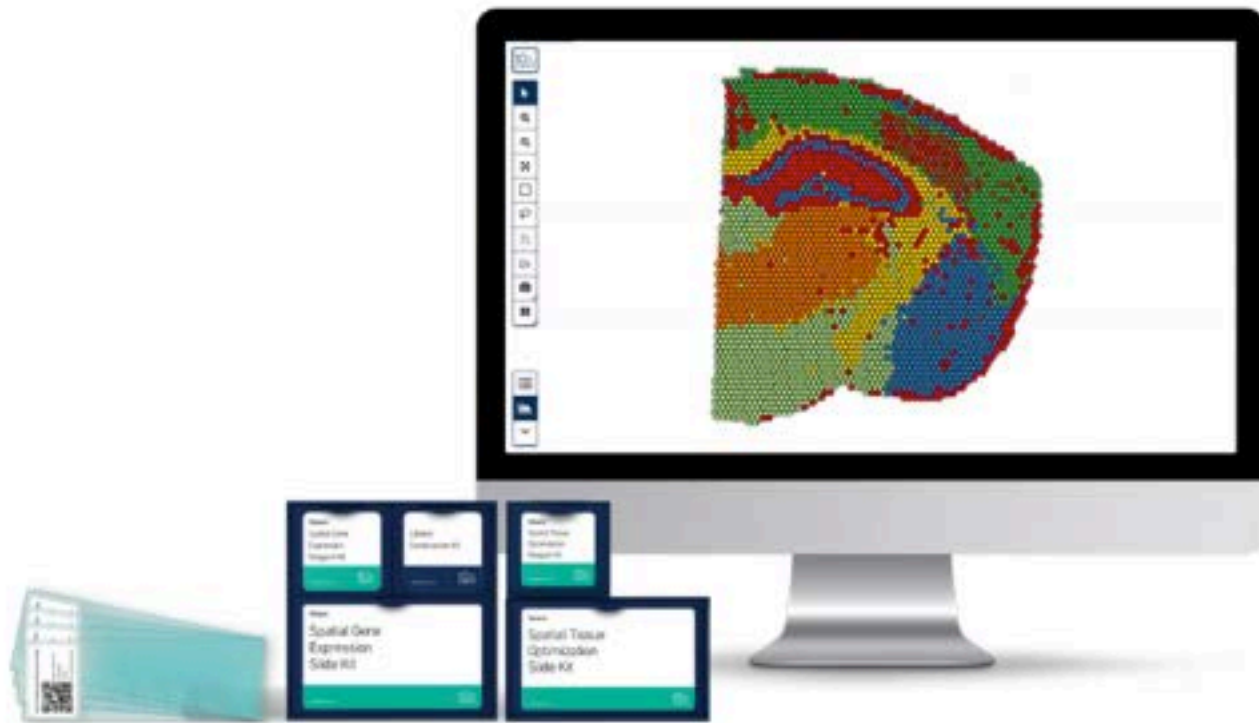
10x Genomics publications continue to scale



Cumulative publications

2,250+
publications

Visium Spatial Platform



TheScientist
TOP 10
INNOVATIONS

2020

Visium Spatial Gene Expression



2020

Spatially Resolved Transcriptomics



“Everyone is waiting for spatial.
Without context, you can only
learn so much”

David Craig, PhD

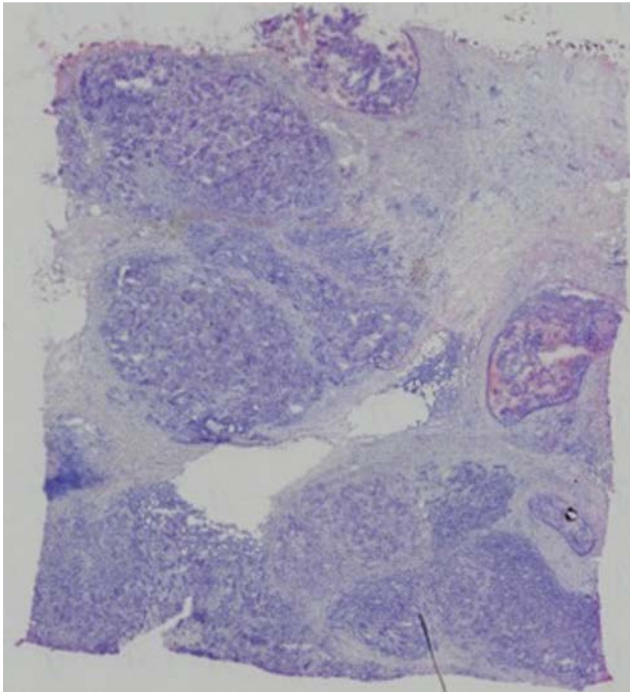
Principal Investigator

UNIVERSITY OF SOUTHERN CALIFORNIA

Two Types of Information Comprise Visium Data

Tissue image and gene expression

Histology image



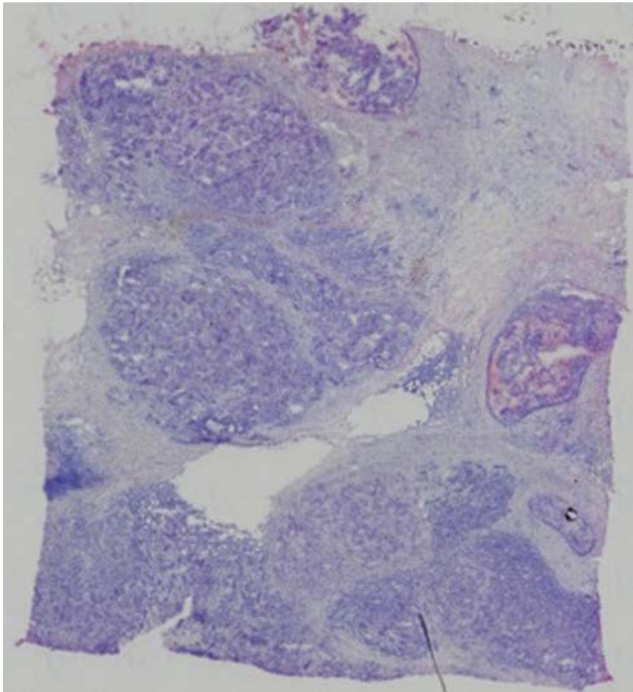
Gene expression



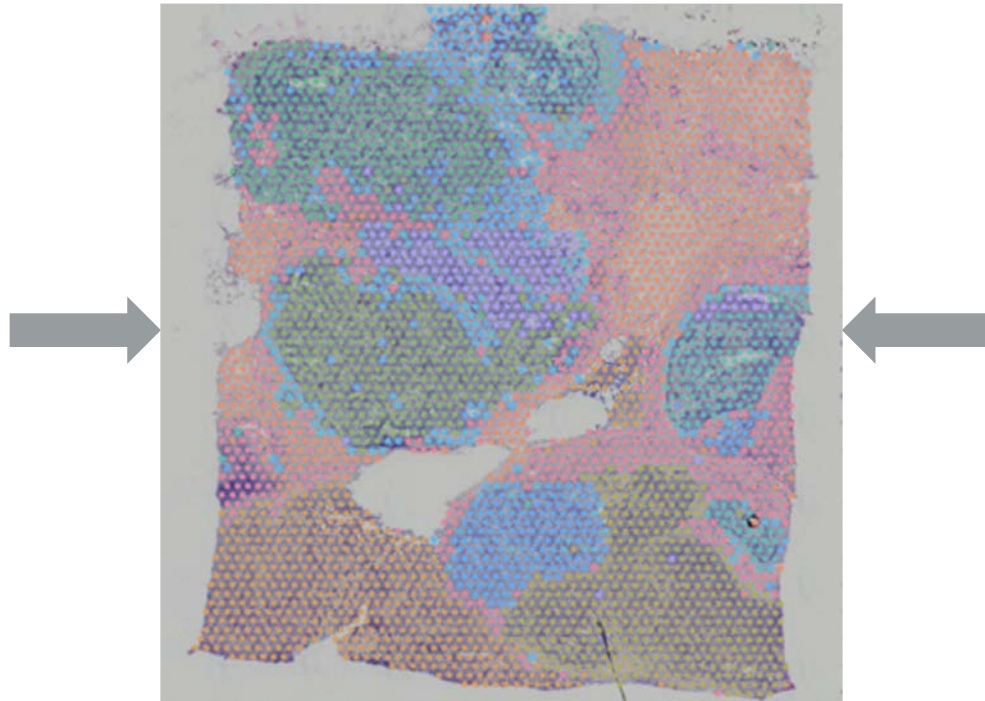
Two Types of Information Comprise Visium Data

Tissue image and gene expression

Histology image



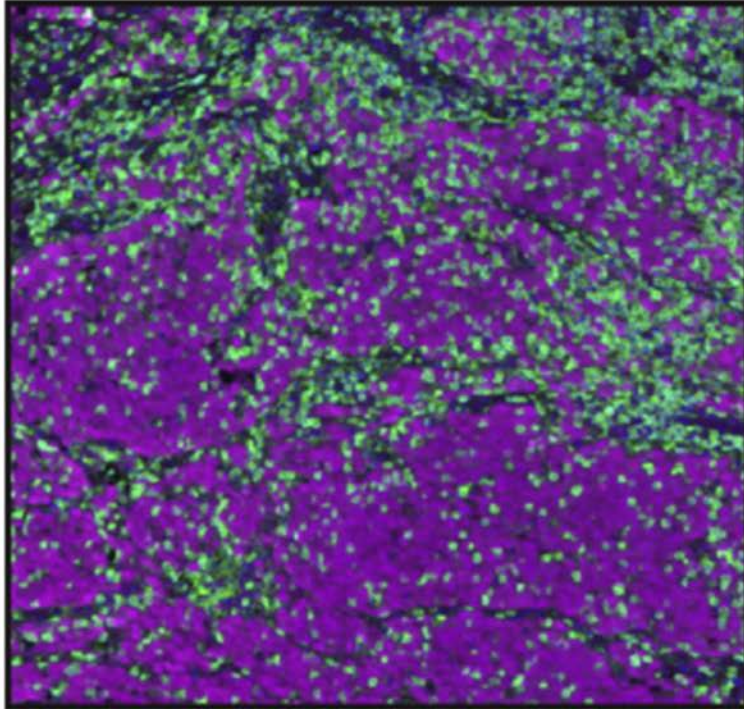
Histology + Gene expression



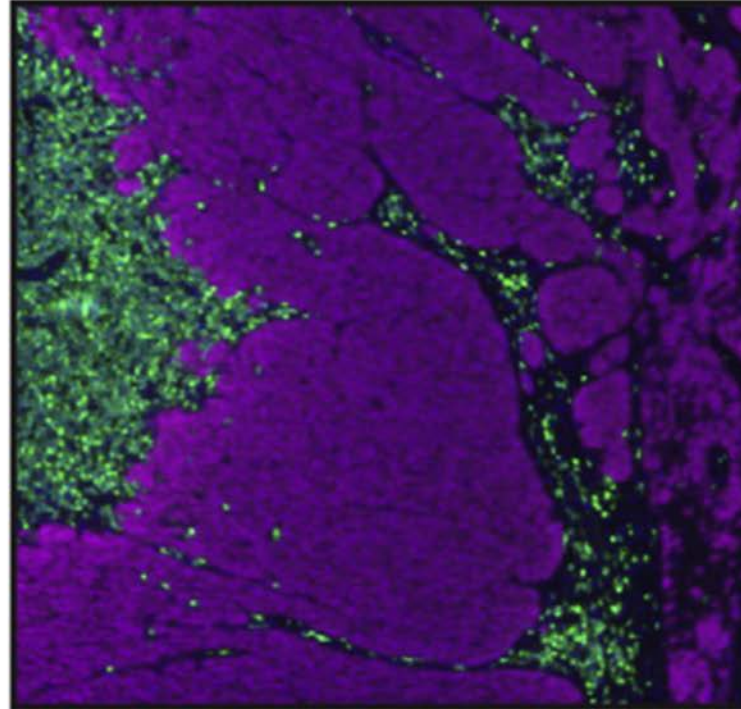
Gene expression



Why Spatial Analysis? Location Matters!



“Hot tumor”
Lymphocytes infiltrating tumor



“Cold tumor”
Lymphocytes stopped at tumor boundary

Tumor cell
Immune cell

The Visium Spatial Gene Expression Solution

Solution Highlights

- Obtain whole transcriptome, high-throughput gene expression analysis for intact tissue sections from a wide variety of sample types
- Unravel biological architecture in normal and diseased tissue
- Discover new tissue biomarkers
- Integrate easily with existing laboratory equipment and tools for tissue analysis
- End-to-end workflow from section to library within one entire working day



Visium Spatial Gene Expression Solution

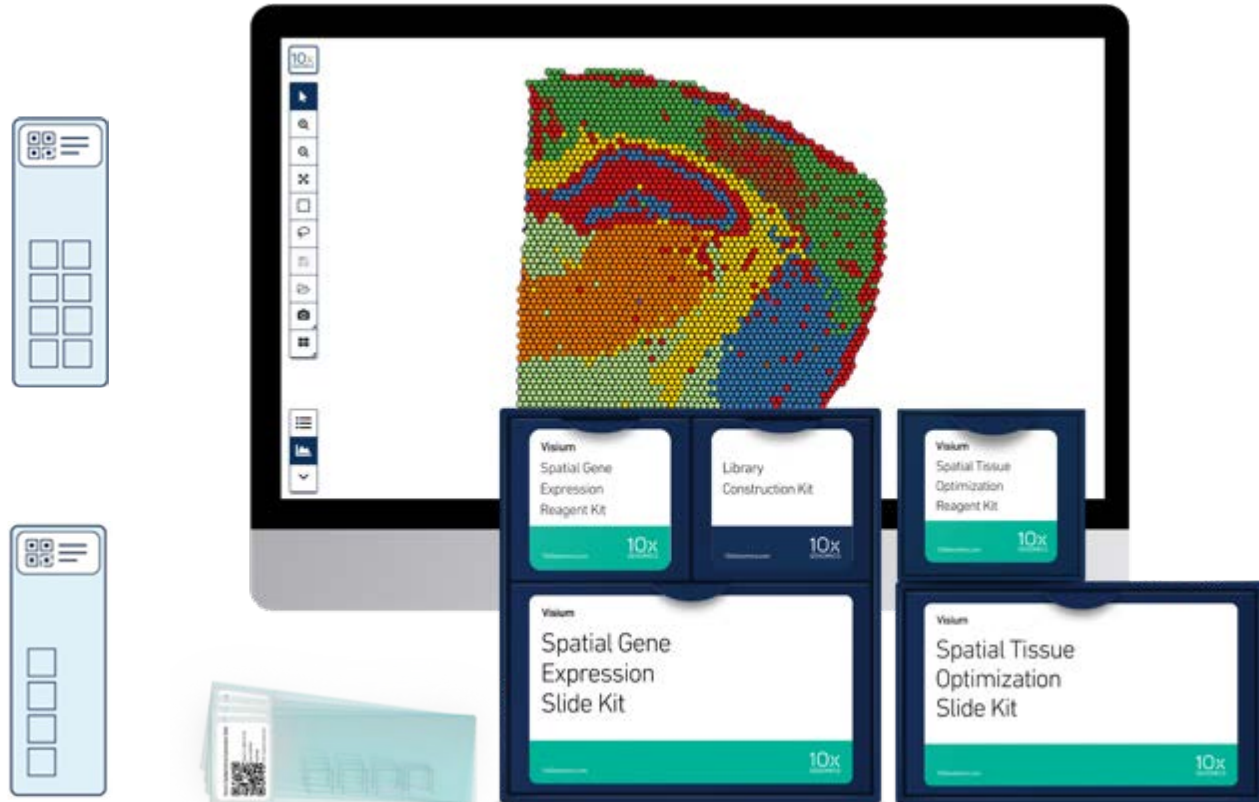
Tissue optimization and library preparation

Visium Spatial Tissue Optimization Slide and Reagent Kit

- **4 sample** TO kit (4 slides)
- Four new tissues can be optimized per TO kit (one new tissue per slide)
- No spatial barcodes

Visium Spatial Gene Expression Slide and Reagent Kit

- **4 reactions** (1 slide) and **16 reactions** (4 slides) LP kit
- Each slide has 4 capture areas containing spatial barcodes



The Visium Spatial Gene Expression workflow

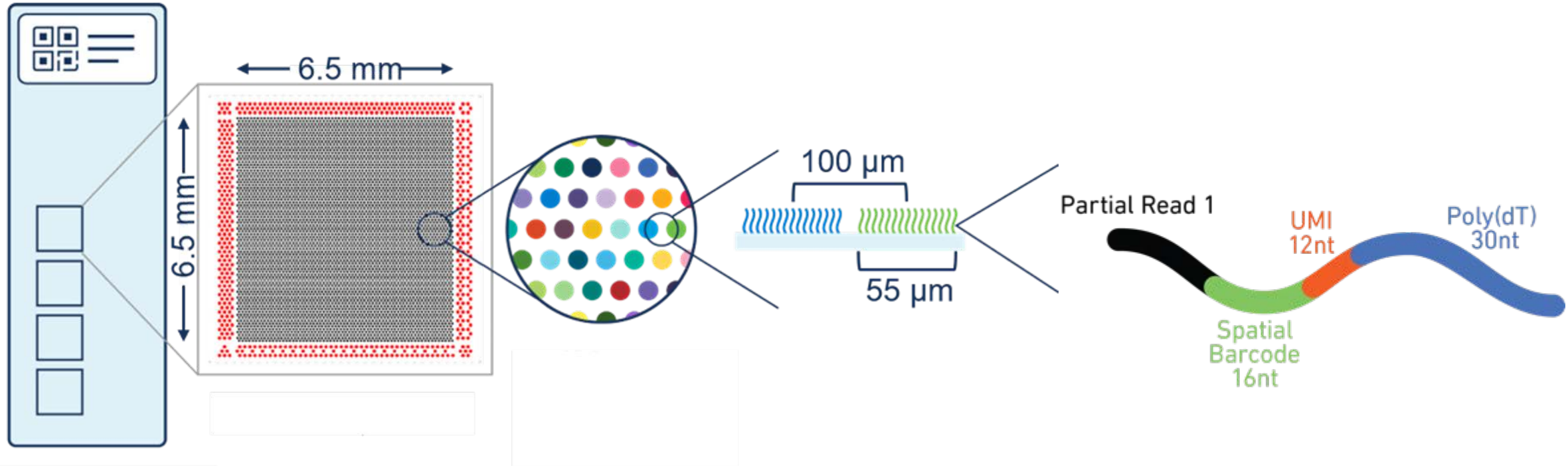
Whole Transcriptome Analysis with Spatial Resolution

Utilizing poly-A capture and unique spatial barcodes

Visium Spatial Gene Expression Slide

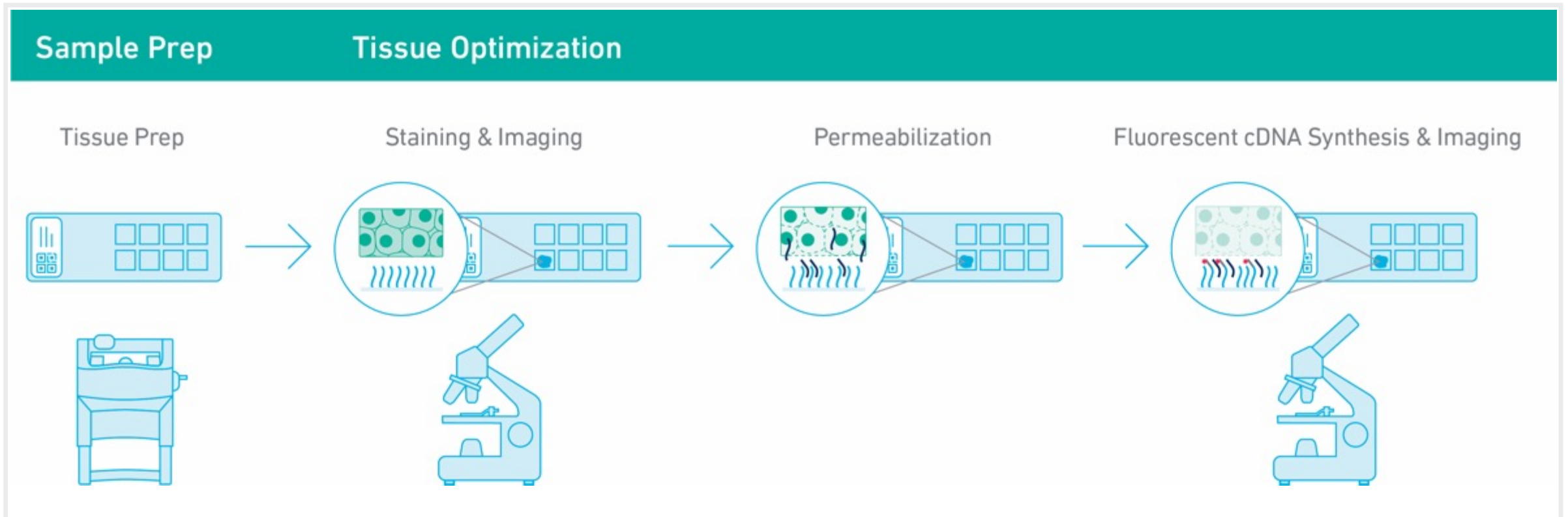
Capture Area with ~5000 Barcoded Spots

Visium Gene Expression Barcoded Spots



Overview of tissue optimization

Getting to know your tissue

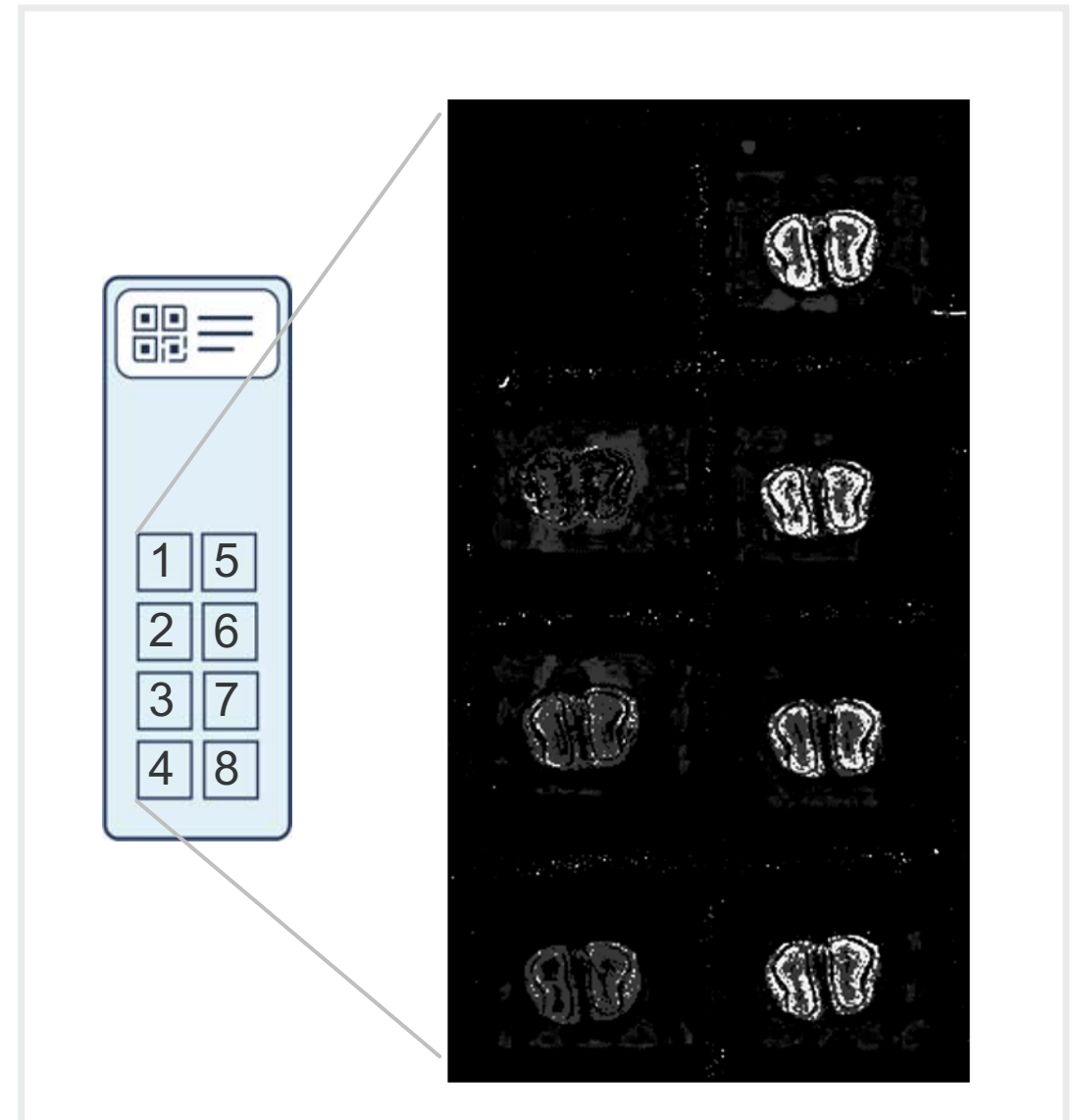


Tissue optimization slides

Tissue optimization

- 8 capture areas per slide
- Capture areas contain an even lawn of poly-dT capture probes without spatial barcodes
- Test a range of permeabilization times (0-36 minutes)
- Reverse transcription incorporates a fluorophore into the cDNA strand
- Looking for the brightest images with the least diffusion

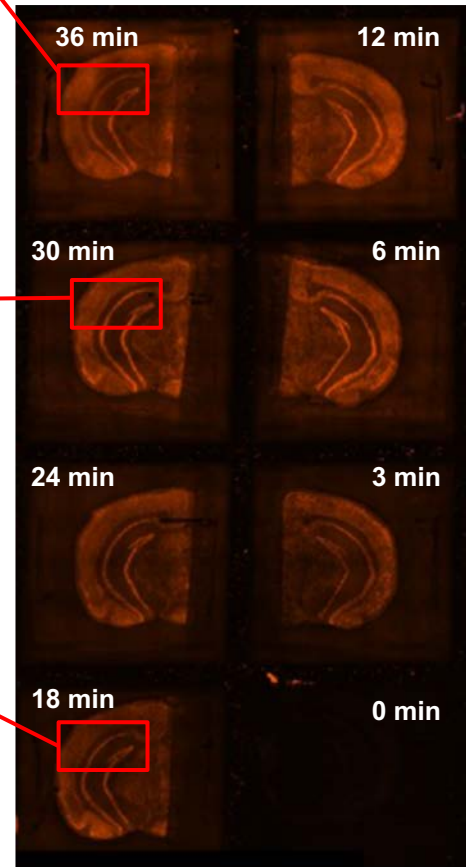
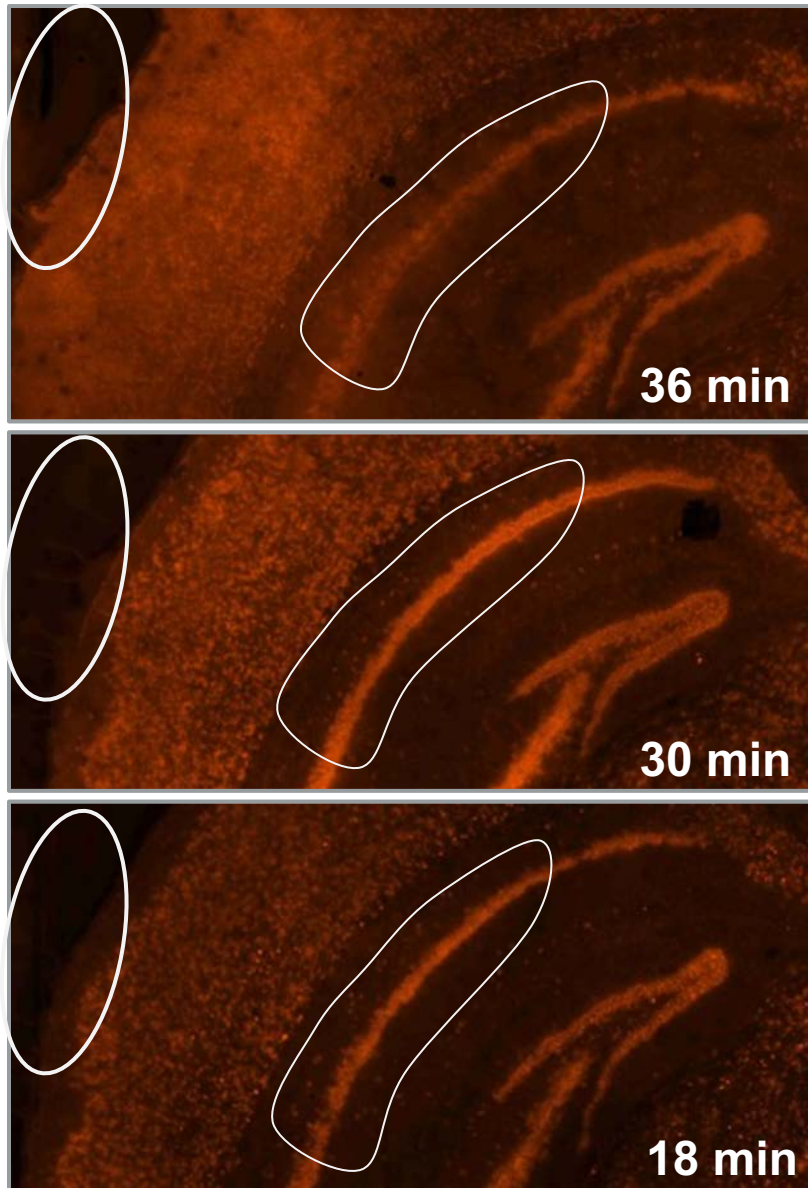
1 – Negative control	5 – 18 minutes
2 – 3 minutes	6 – 24 minutes
3 – 6 minutes	7 – 30 minutes
4 – 12 minutes	8 – 36 minutes or positive control



How do I select the best permeabilization time?

Look for the brightest images with the least diffusion

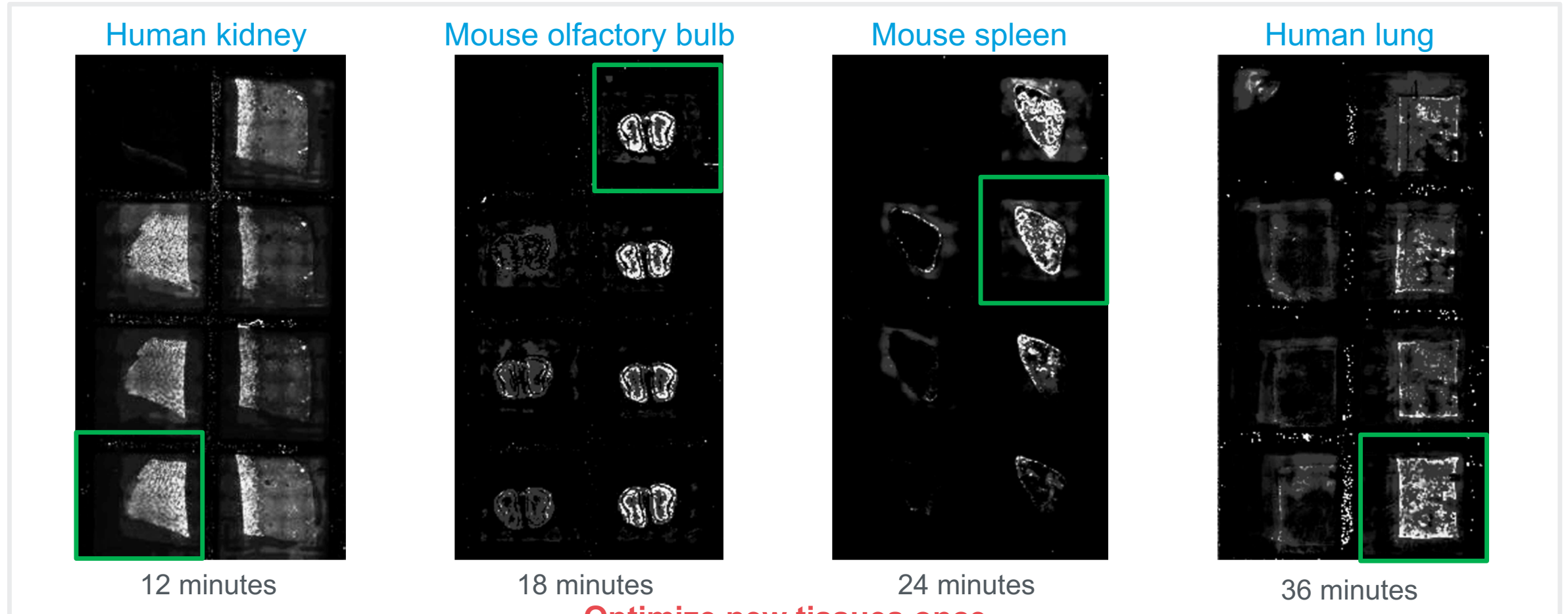
Mouse brain



- Zoom in on the images and check the signal sharpness
- Select the condition where the maximum amount of fluorescence signal with the lowest signal diffusion is obtained.
- If signal is the same at two time points, select the longer time.
- Use best permeabilization condition for Spatial GEX experiment

Tissue optimization

Permeabilization conditions are tissue dependent



Optimize new tissues once

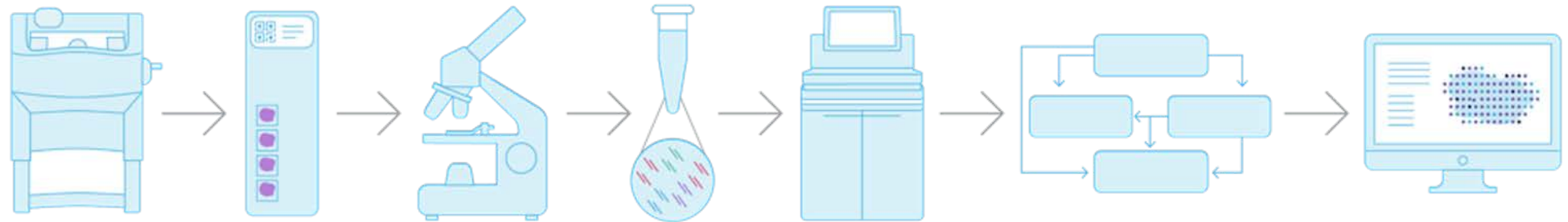
The Visium Spatial Gene Expression Workflow

Streamlined workflow with the choice of H&E or Immunofluorescence



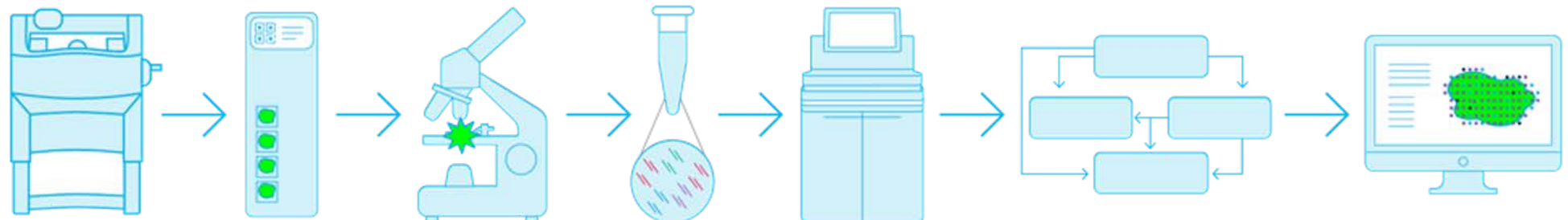
Visium with H&E Workflow

Provides whole transcriptome with morphological context



Visium with IF Workflow

Provides whole transcriptome with protein co-detection

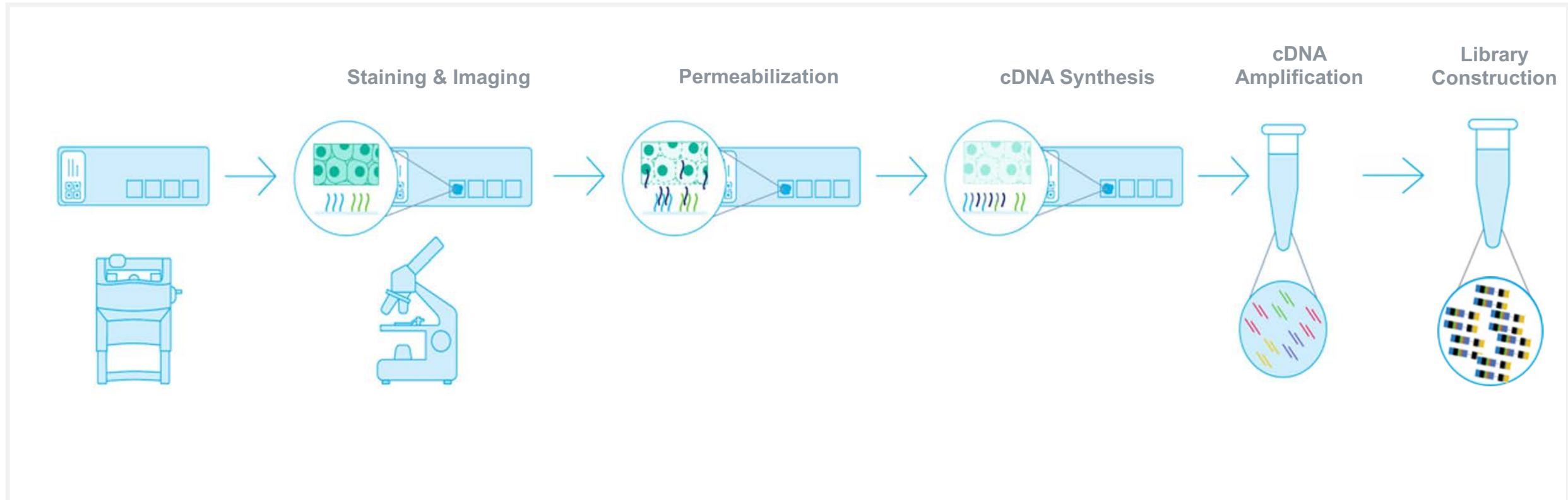


Visium Spatial Gene Expression - Tissue and Library Prep

Complete in 1 Working Day with Standard Tools for Tissue Studies

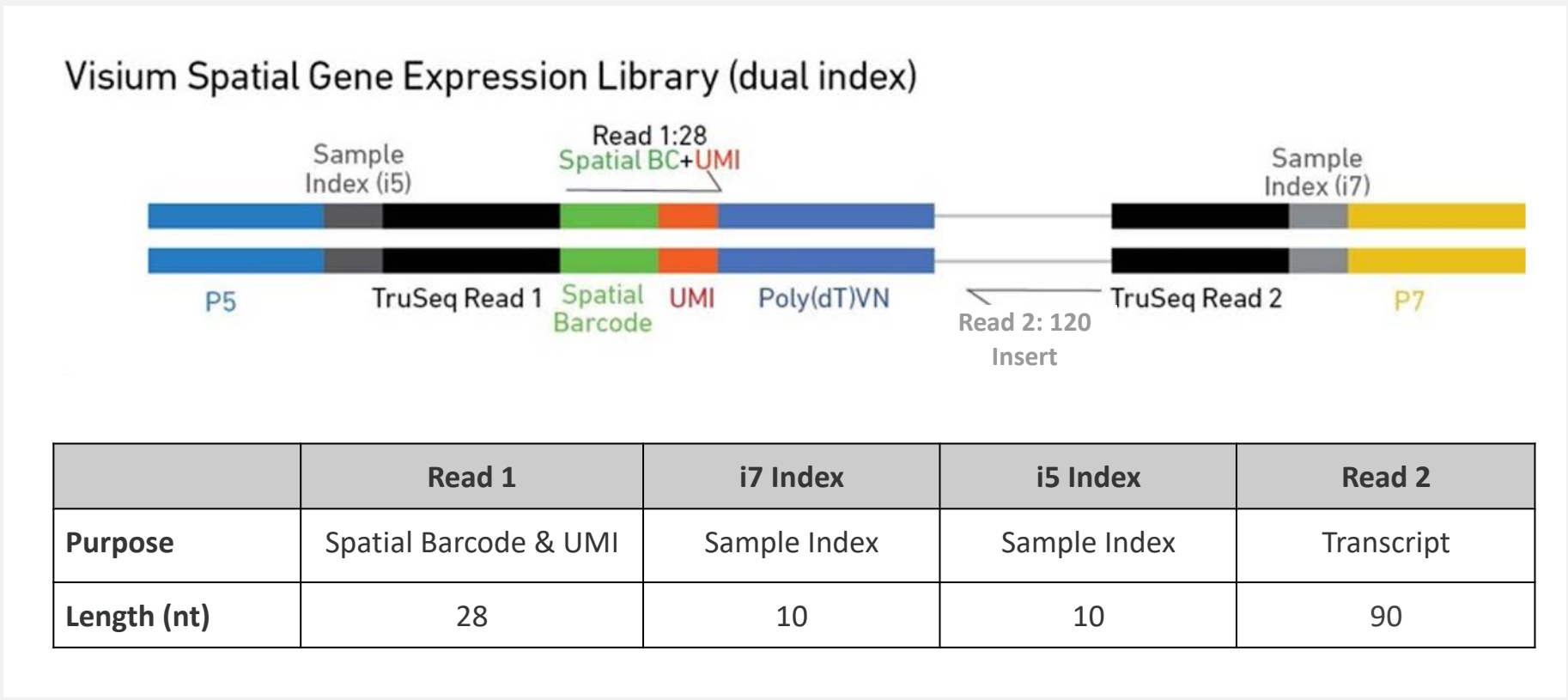
Tissue Prep

Library Prep



Recommended read length

Visium Spatial Gene Expression Libraries

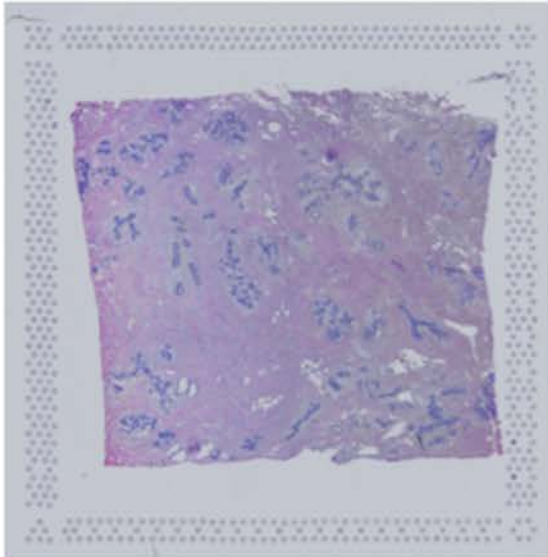


- Paired-end, dual indexed libraries
- Recommended starting point: 50k read pairs per tissue covered spot

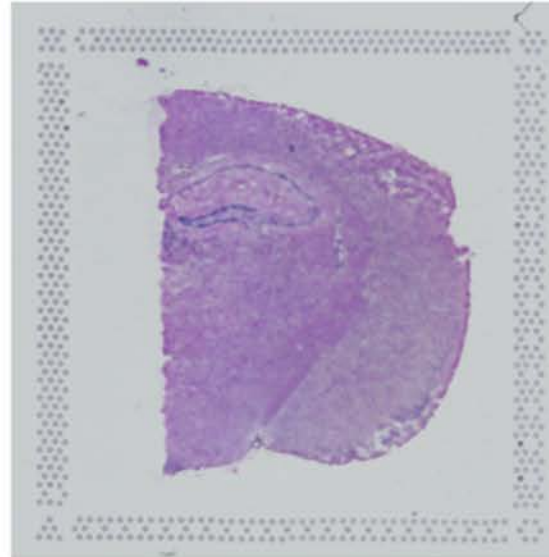
Determining Sequencing Depth

Visium Spatial Gene Expression Libraries

~75%



~50%



~25%



Determining Sequencing Depth


10x GENOMICS Visium Manual Alignment Wizard

Manual Alignment 1 Introduction 2 Input Data 3 Align Image 4 Identify Tissue 5 Export to Space Ranger

Identify the tissue by labeling all spots which overlay the tissue using the lasso or brush tool. The tools can be used multiple times to fine-tune which spots are labeled.

There are currently 3108 spots labeled as tissue.

Continue

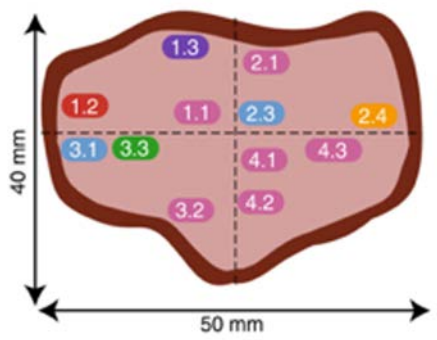


Visium Spatial Gene Expression Solution

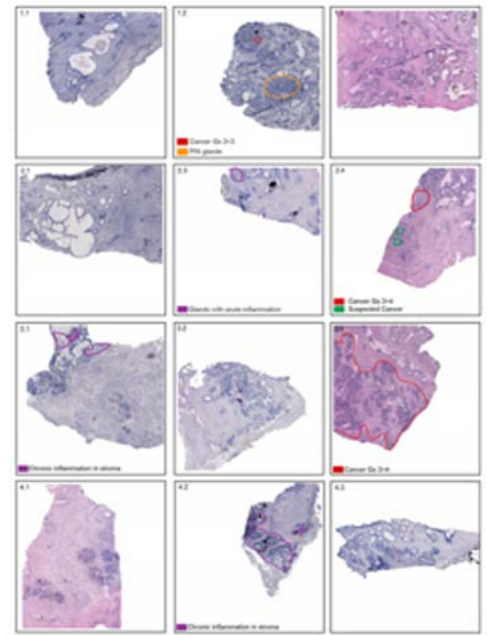
Applications

Spatial maps of prostate cancer transcriptomes

Biopsy & sectioning of prostate

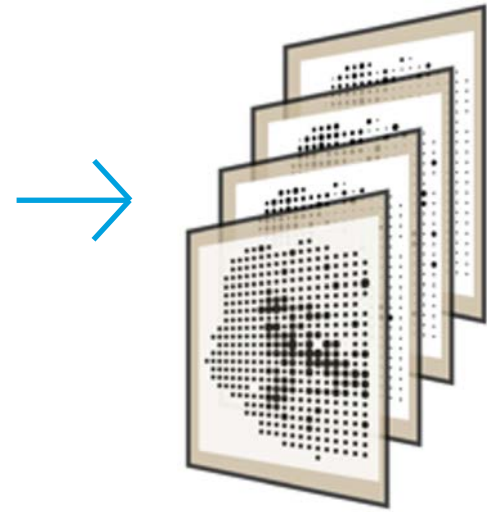


Staining & annotation



Spatial gene expression workflow

Gene expression maps

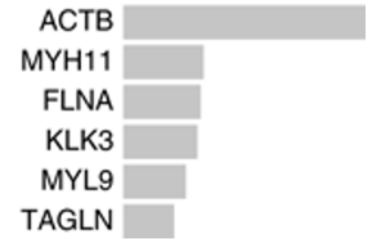


Factor activity maps

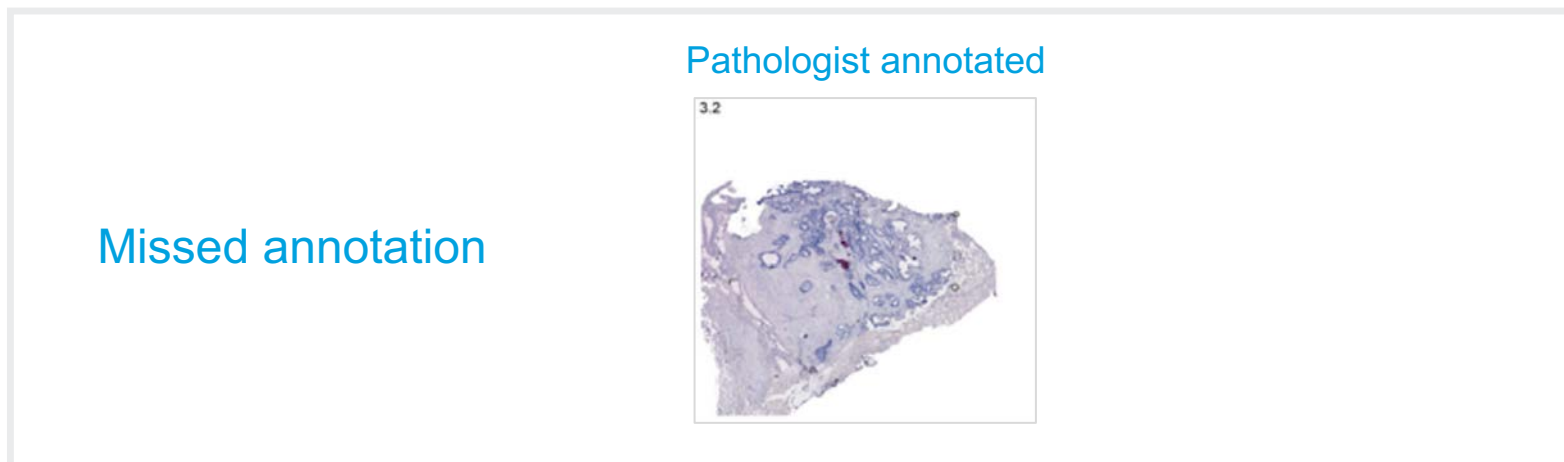
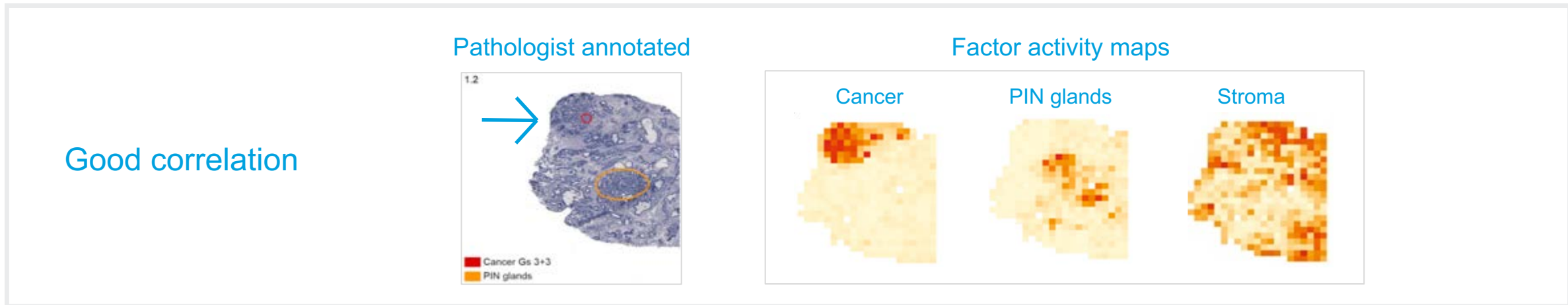


Factor analysis

Factor signatures



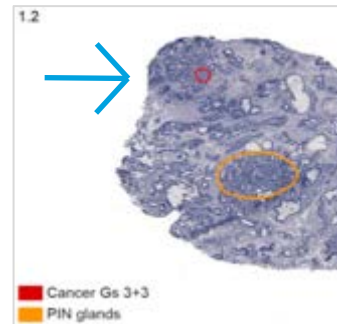
Correspondence between manual and data-driven annotations



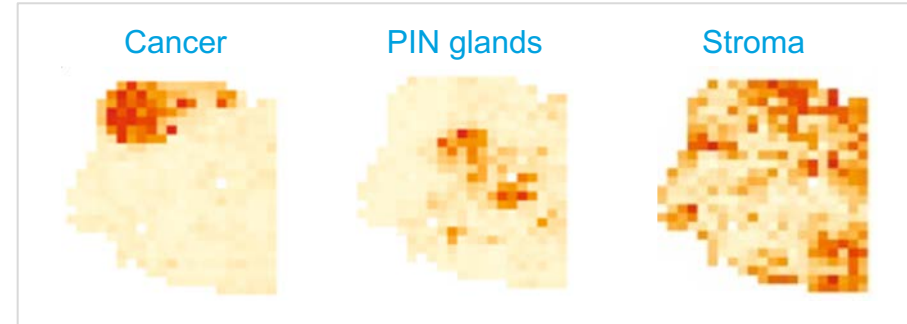
Correspondence between manual and data-driven annotations

Good correlation

Pathologist annotated

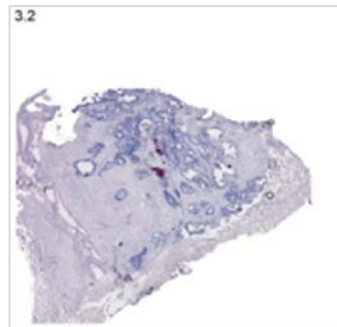


Factor activity maps

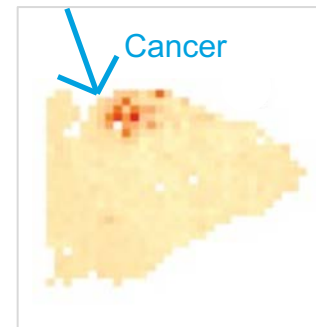


Missed annotation

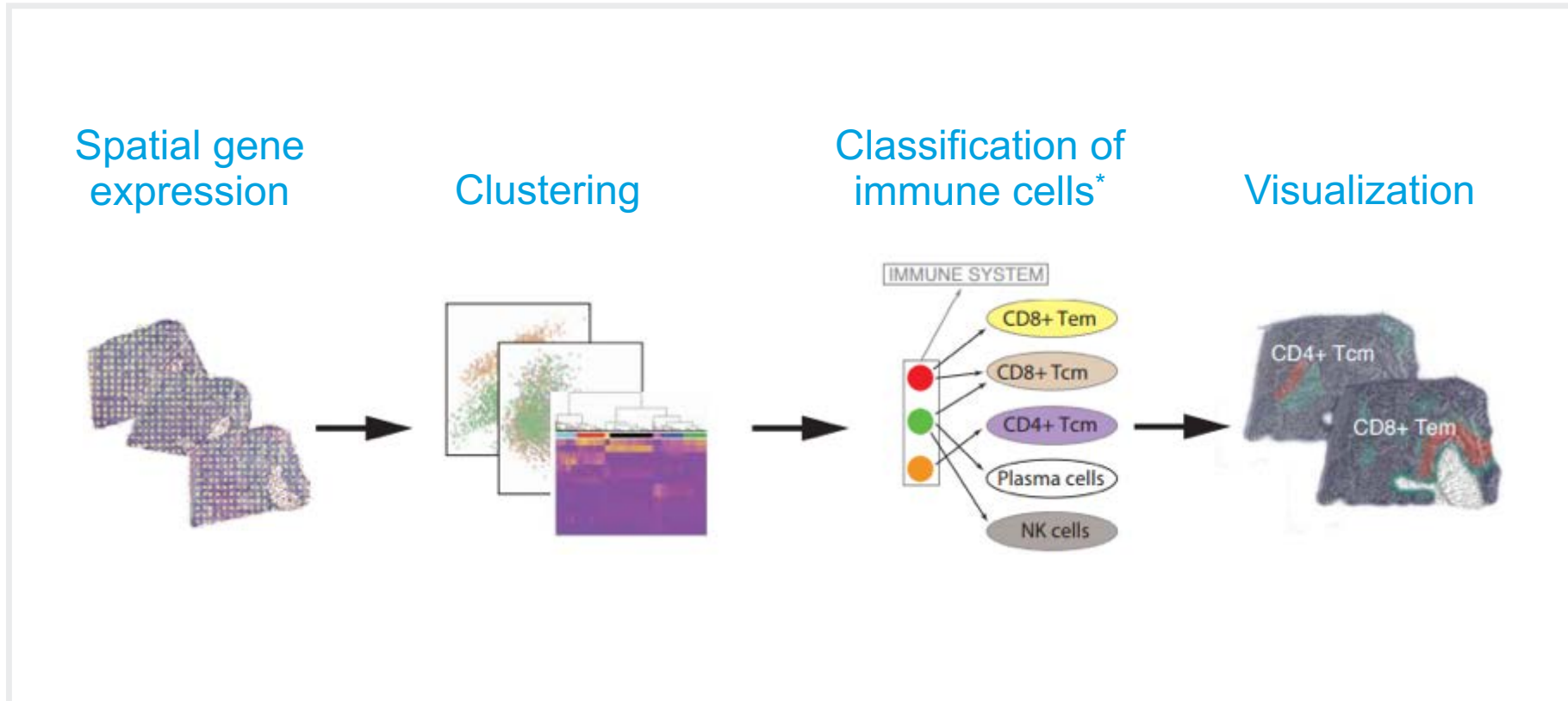
Pathologist annotated



Factor activity map



HER2+ breast tumor microenvironments



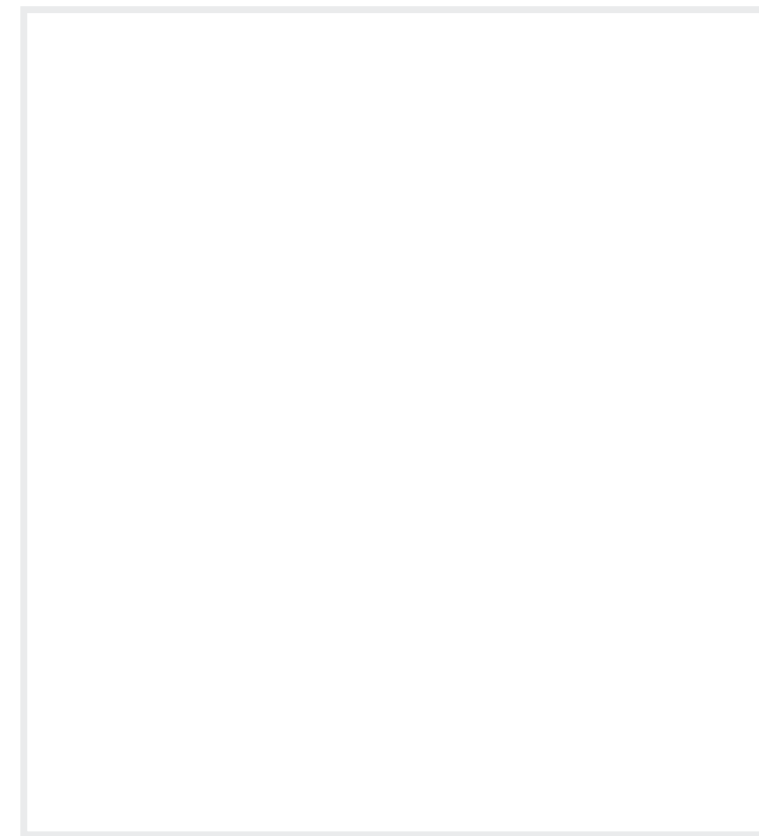
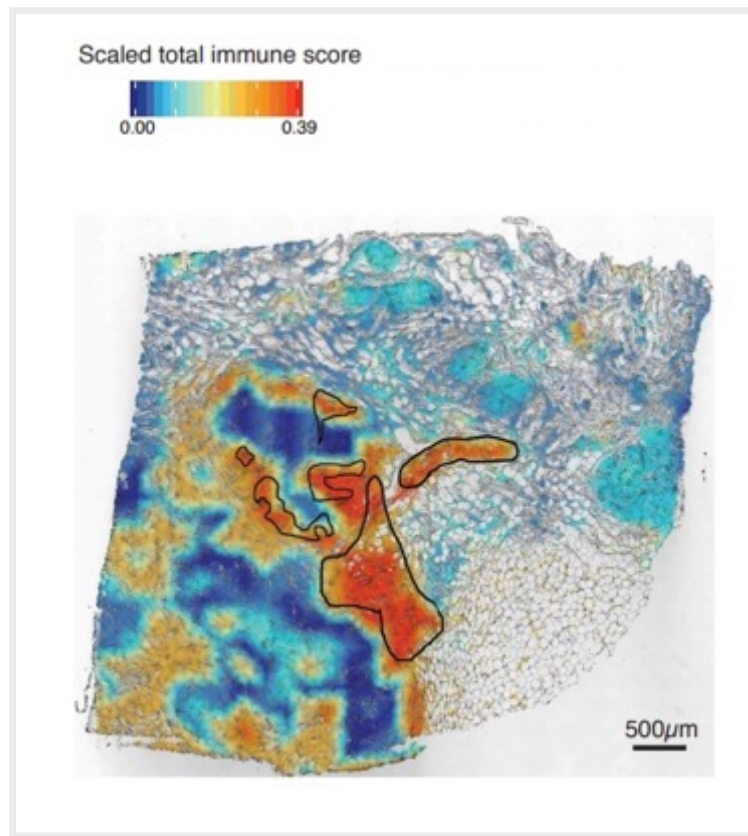
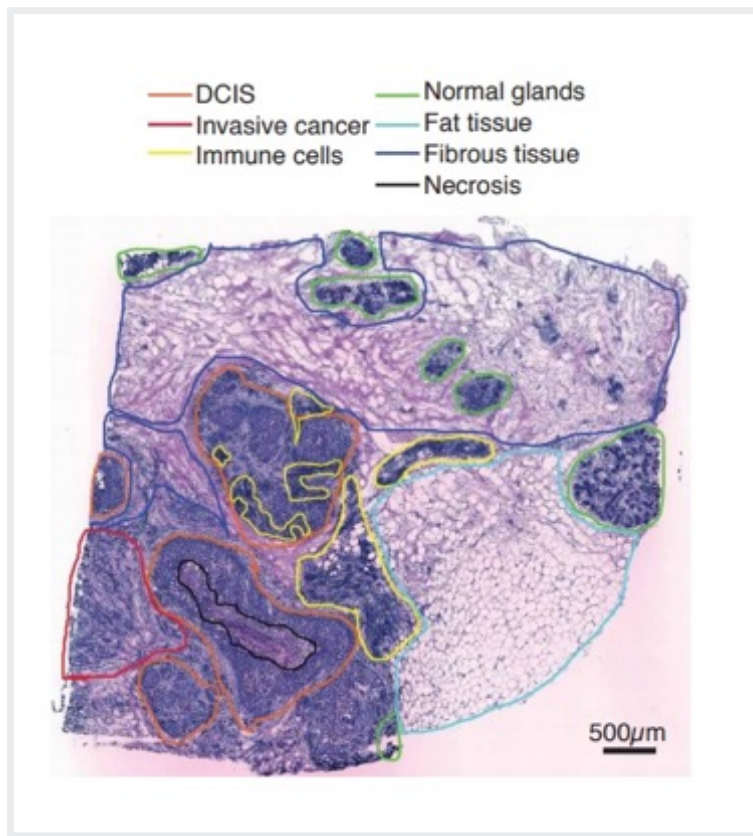
Fredrik Salmén
 Sanja Vickovic
 Ludvig Larsson
 Linnea Stenbeck

SciLifeLab

Salmén F *et al.* *bioRxiv*
 doi.org/10.1101/358937v2

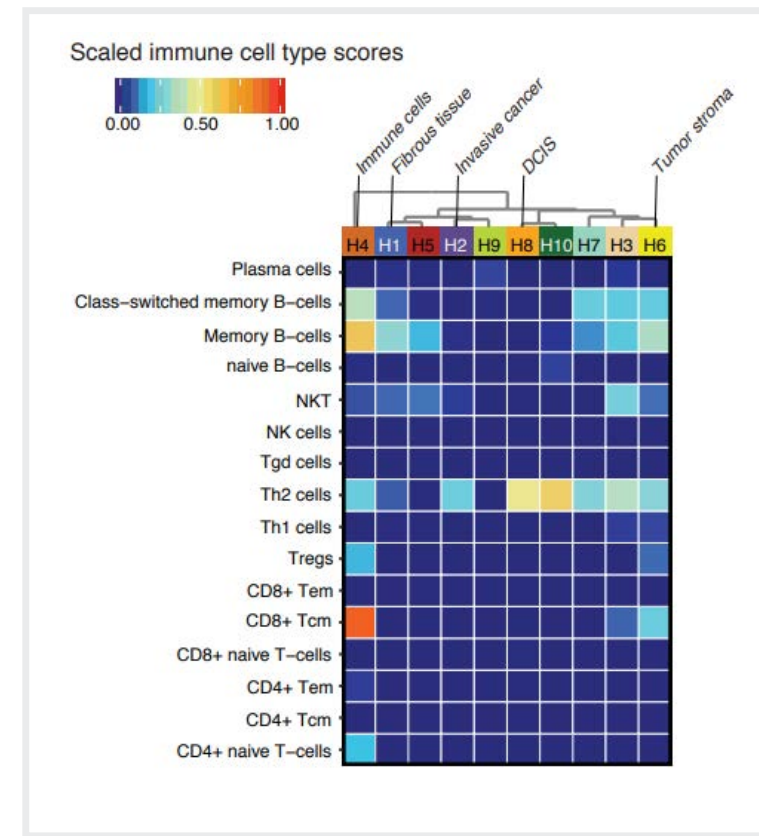
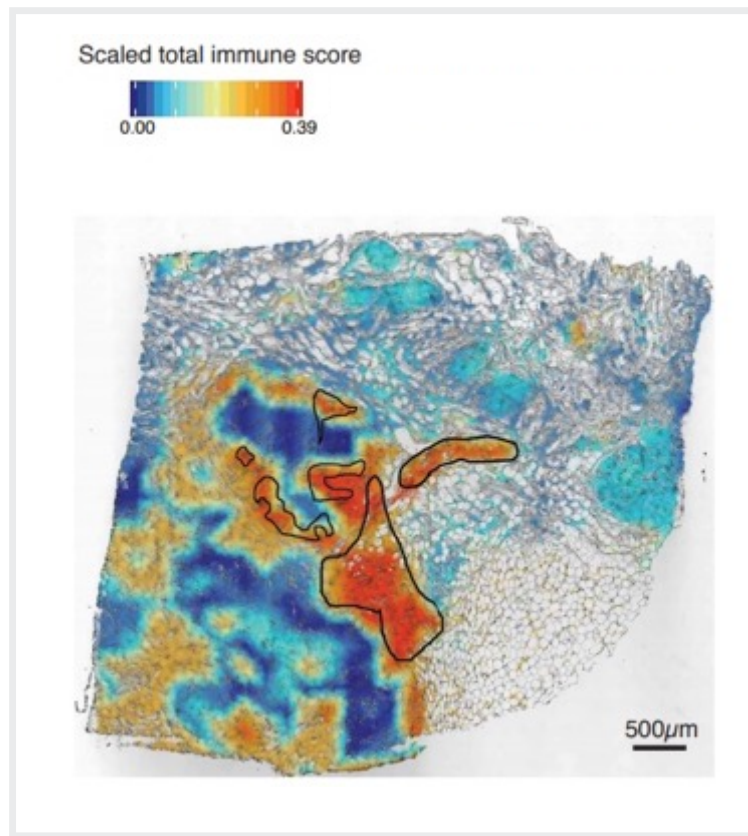
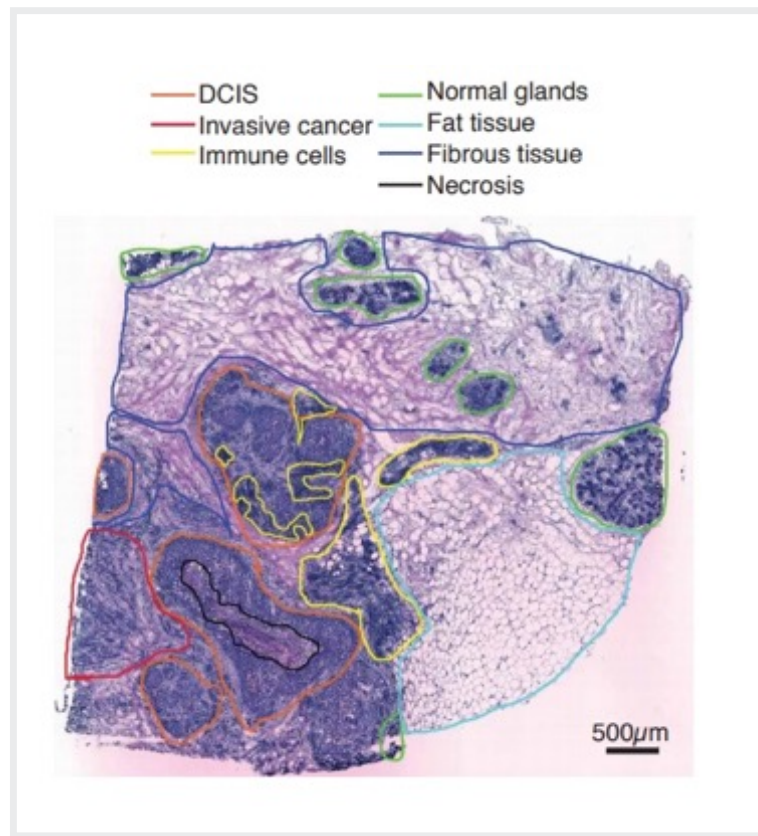
Distribution of infiltrating immune cell types

Pathologist annotated H&E section



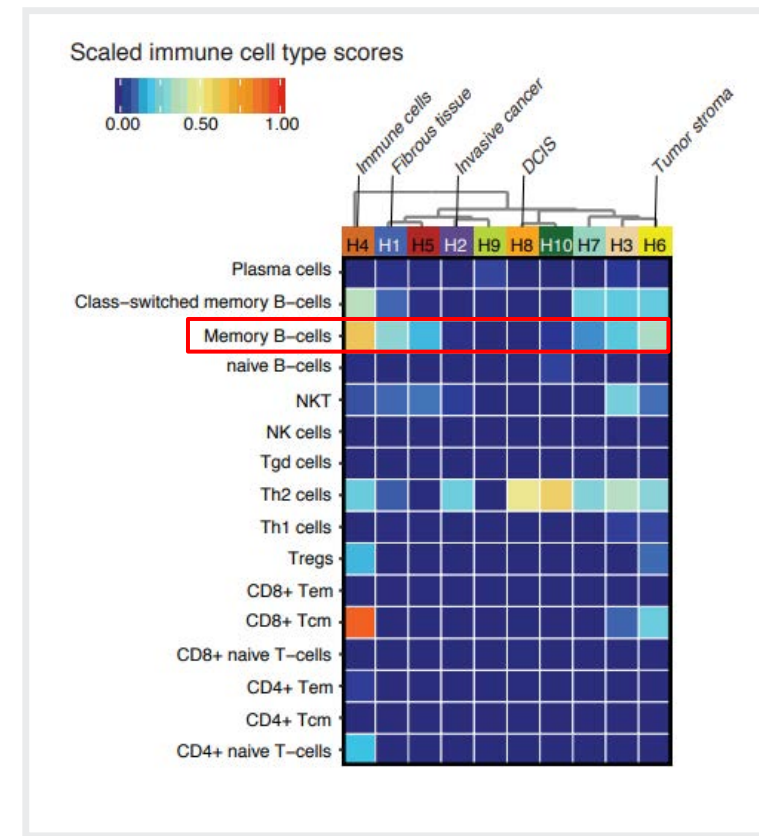
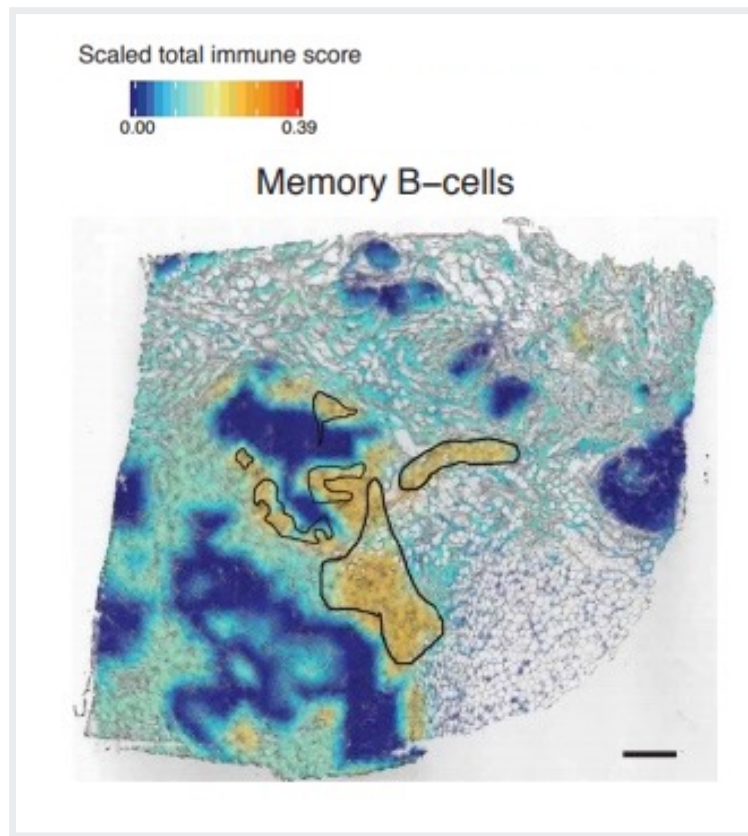
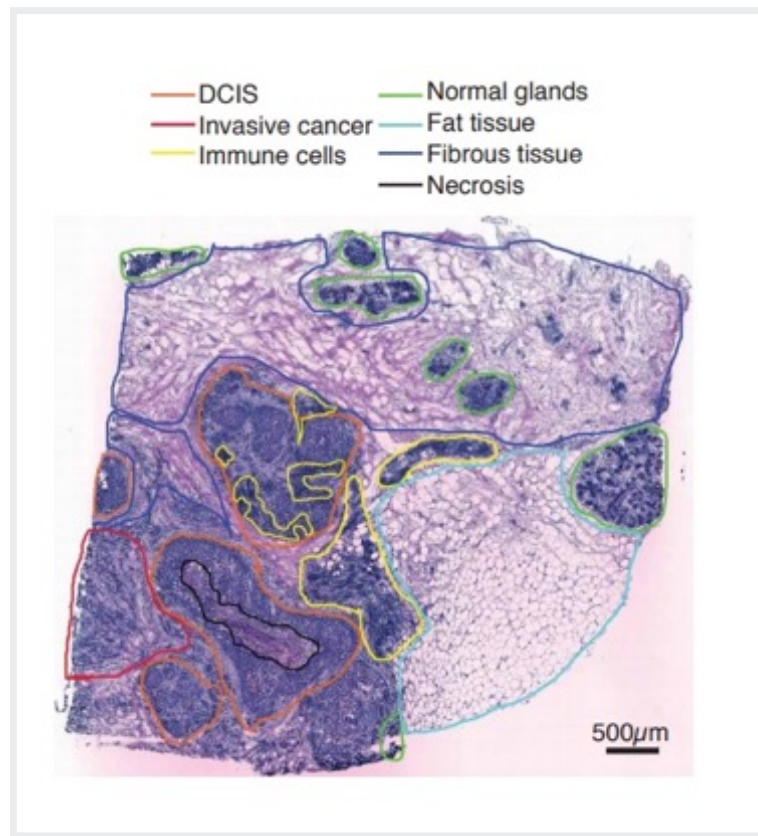
Distribution of infiltrating immune cell types

Pathologist annotated H&E section



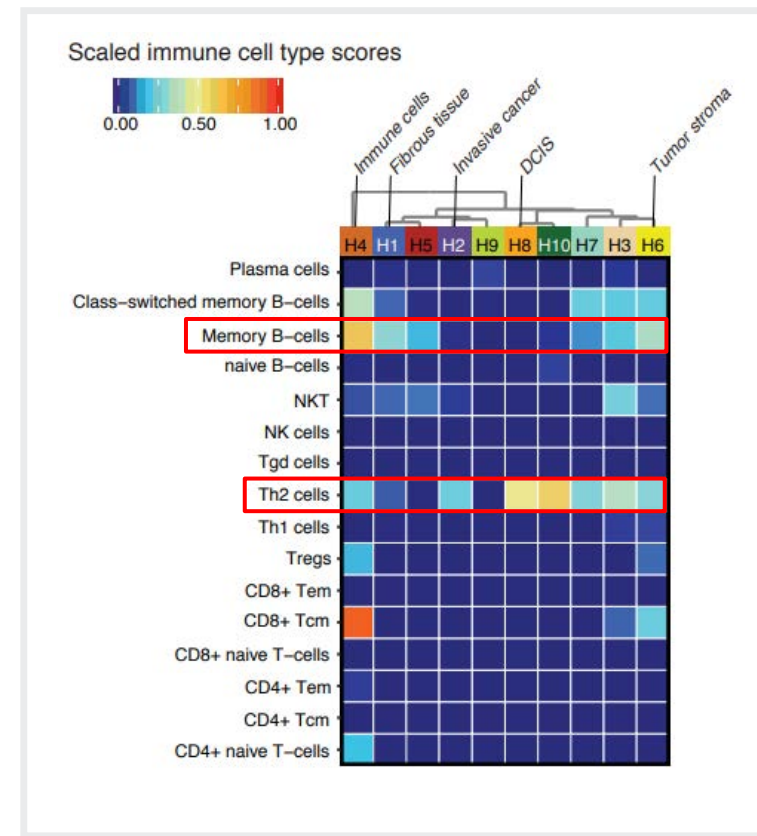
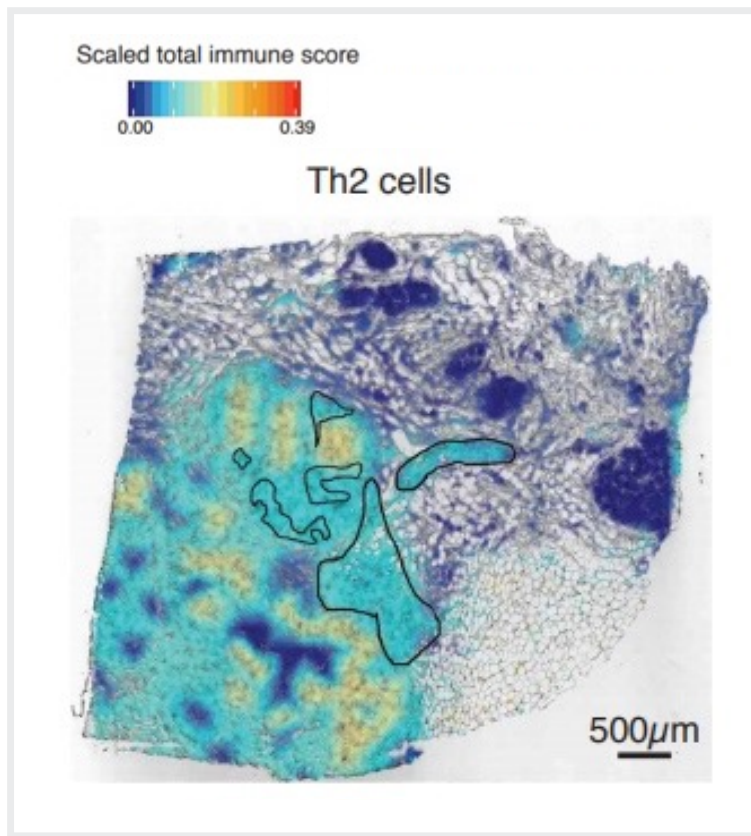
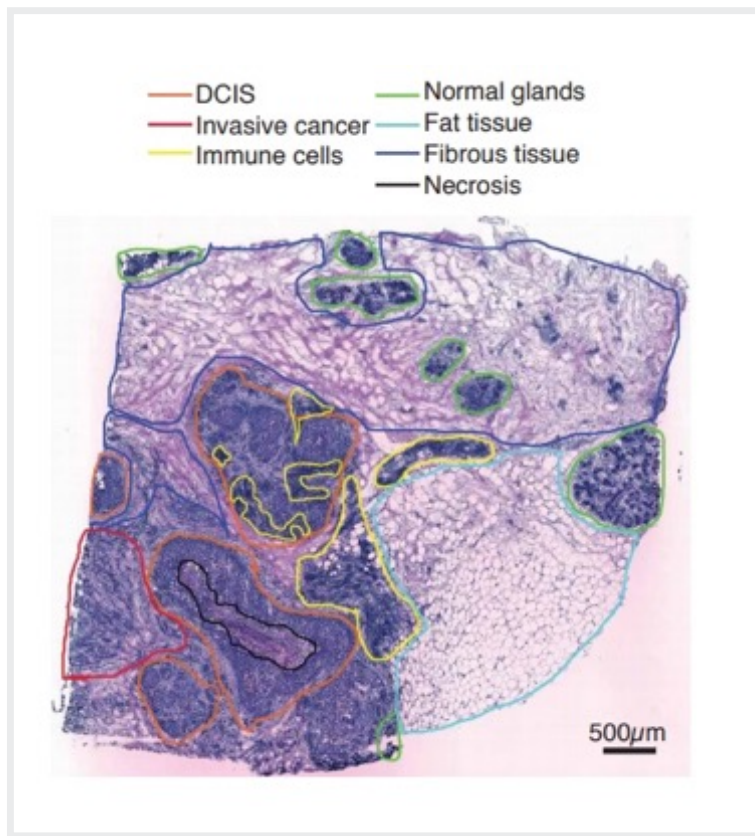
Distribution of infiltrating immune cell types

Pathologist annotated H&E section



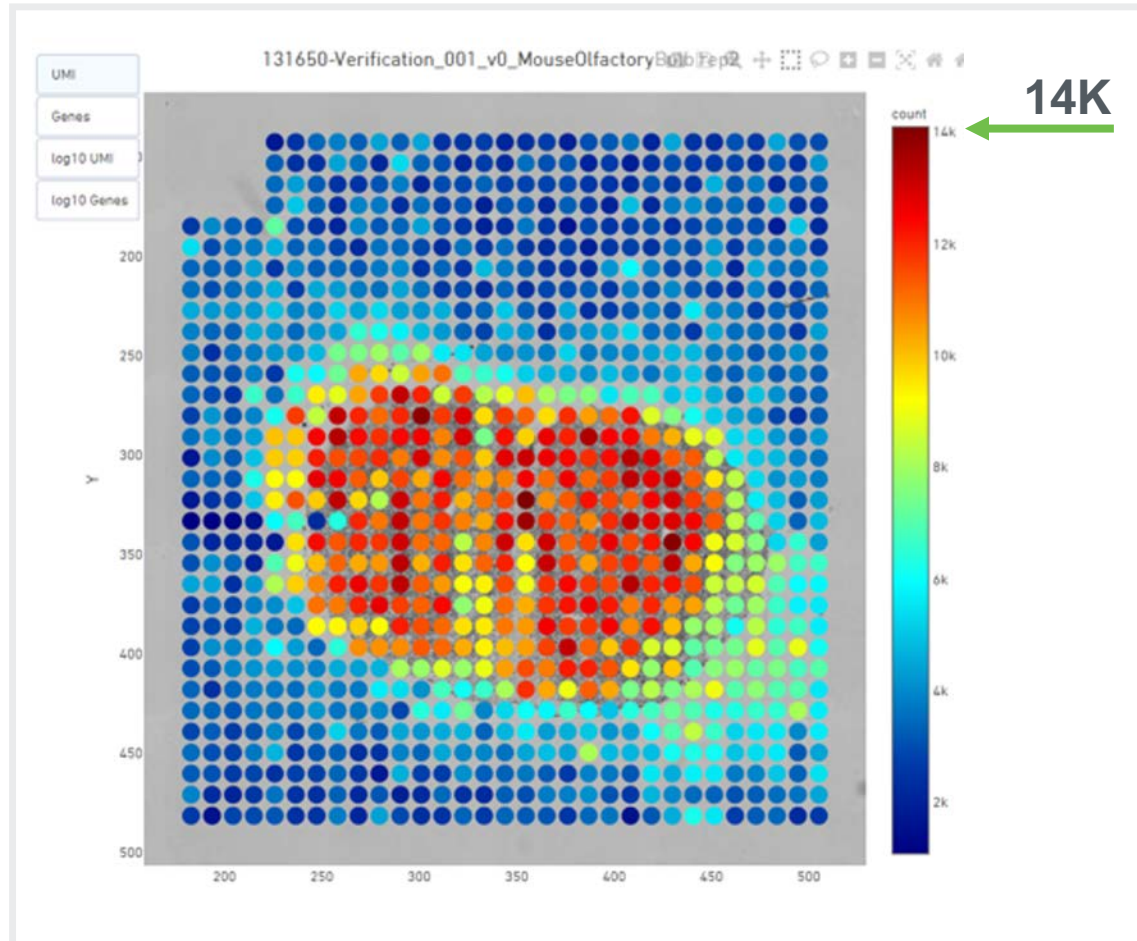
Distribution of infiltrating immune cell types

Pathologist annotated H&E section



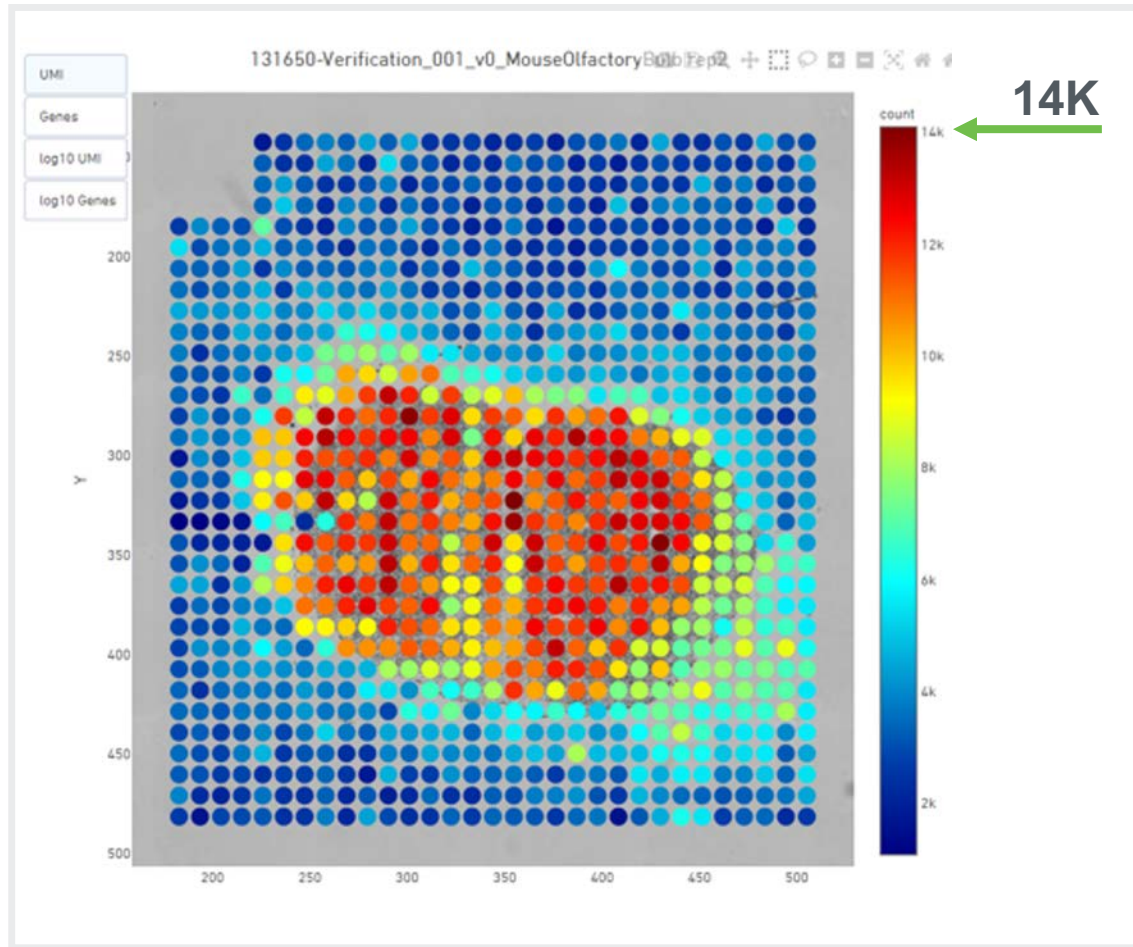
Visium improvements vs Spatial transcriptomics

ST mouse olfactory bulb

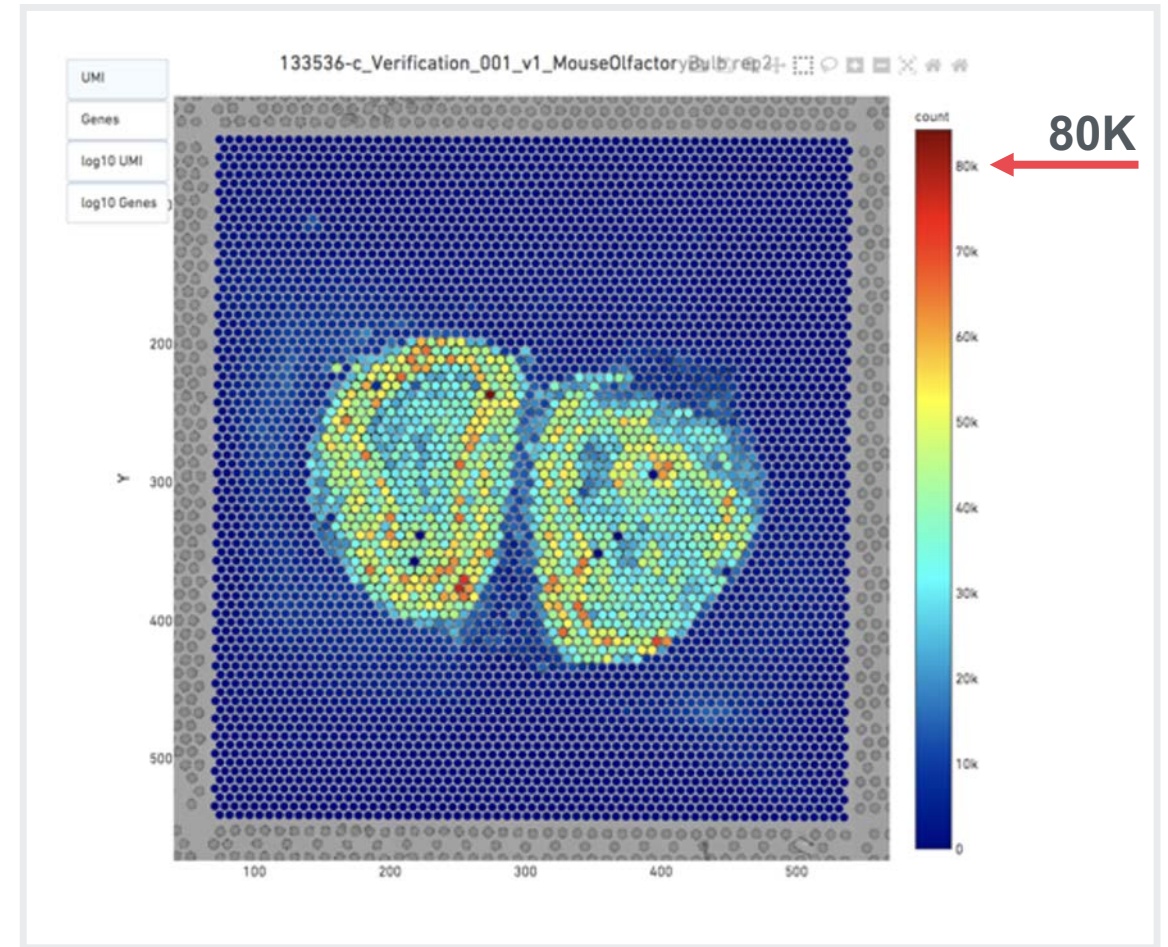


Visium improvements vs Spatial transcriptomics

ST mouse olfactory bulb



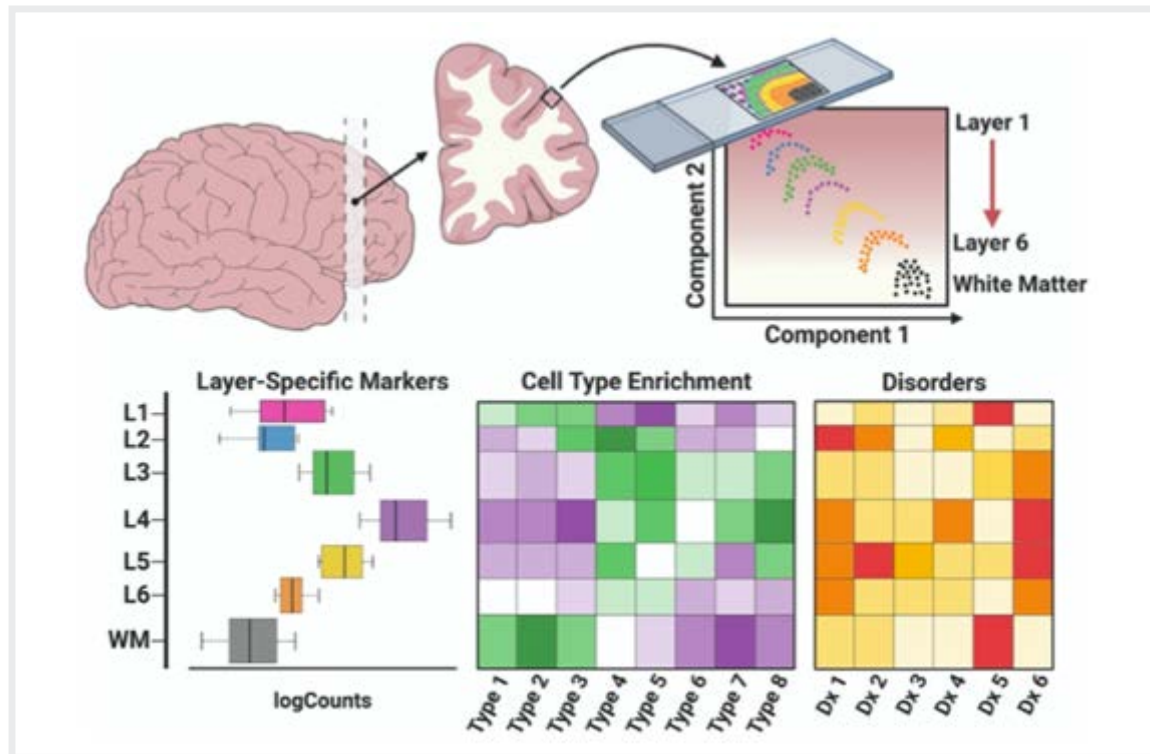
Visium mouse olfactory bulb



Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex

First preprint utilizing Visium

Visual abstract



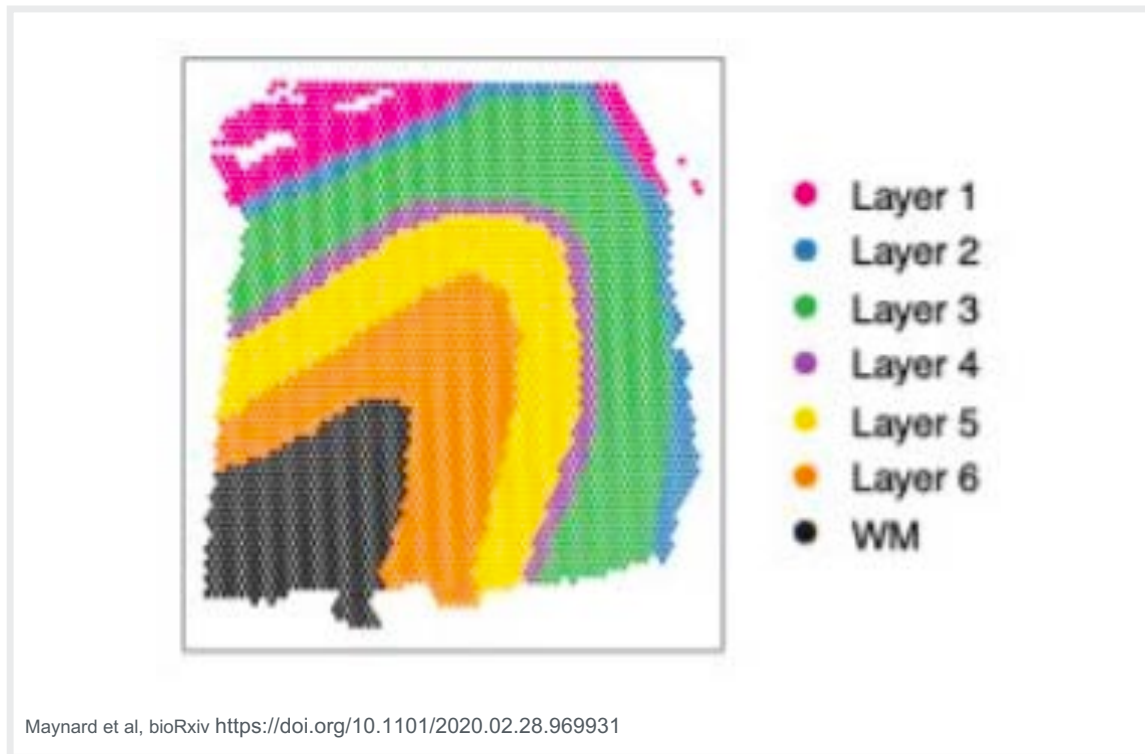
Maynard et al, bioRxiv <https://doi.org/10.1101/2020.02.28.969931>

- Kristen R. Maynard, Leonardo Collado-Torres, Lukas M. Weber, Cedric Uytingco, Brianna K. Barry, Stephen R. Williams, Joseph L. Catallini, Matthew N. Tran, Zachary Besich, Madhavi Tippani, Jennifer Chew, Yifeng Yin, Joel E. Kleinman, Thomas M. Hyde, Nikhil Rao, Stephanie C. Hicks, Keri Martinowich, Andrew E. Jaffe
- Two pairs of ‘spatial replicates’ from three neurotypical adult donors (12 samples total)
- Median depth of 291M reads per sample
- Mean of 1,734 genes per spot

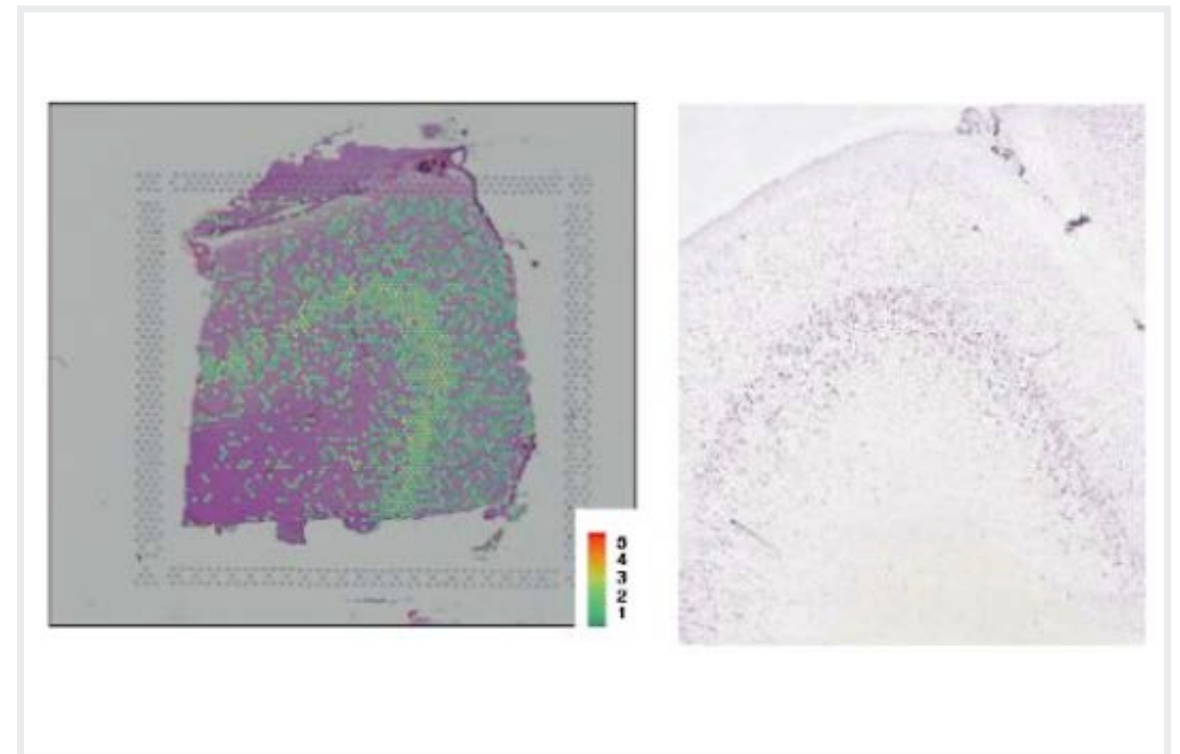
Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex

Visium replicates layer enrichment of previously identified layer marker genes

Detected all six layers and WM

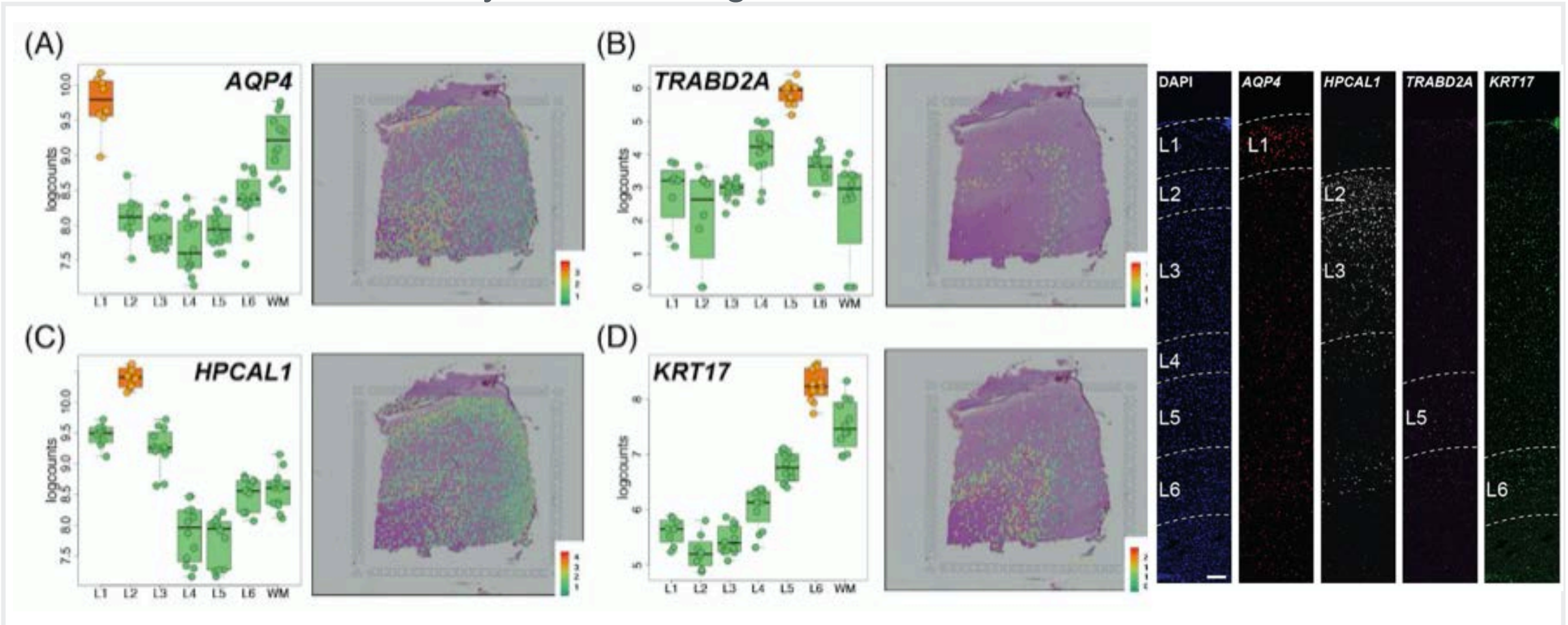


PCP4 of L5



Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex

Identifications of novel layer-enriched genes



Visium webinar from March 19th

<https://bit.ly/prefrontal-cortex-visium-spatial-10x>

LABTOOLS
THE SCIENTIST WEBINAR SERIES

**Transcriptome-Scale Spatial Gene Expression
in the Human Dorsolateral Prefrontal Cortex**

Watch on demand

If you have already registered, follow this link and click on "Already Registered?" to enter your email address and view the webinar.

<https://bit.ly/prefrontal-cortex-visium-spatial-10x>

Whole-Transcriptome Spatial Mapping of the Healthy and Infarcted Human Heart

Christoph Kuppe, M.D.

Department of Internal Medicine, Nephrology and Immunology,
Aachen University, Germany

Jovan Tanevski, Ph.D.

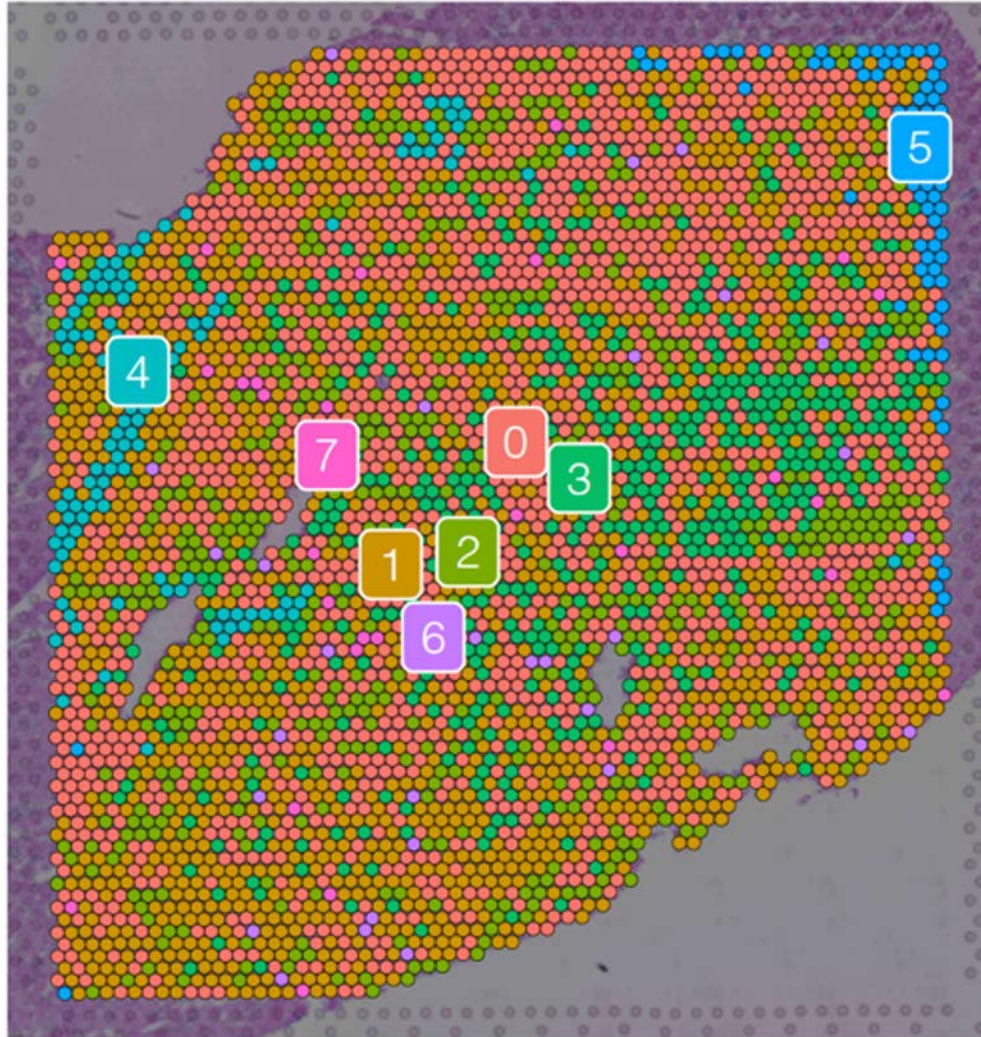
Institute for Computational Biomedicine; Heidelberg University, Germany
Department of Knowledge Technologies; Jožef Stefan Institute, Slovenia



@KuppeChristoph
@saezlab

Control Heart - Vascular Smooth Muscle Cells (VSMCs)

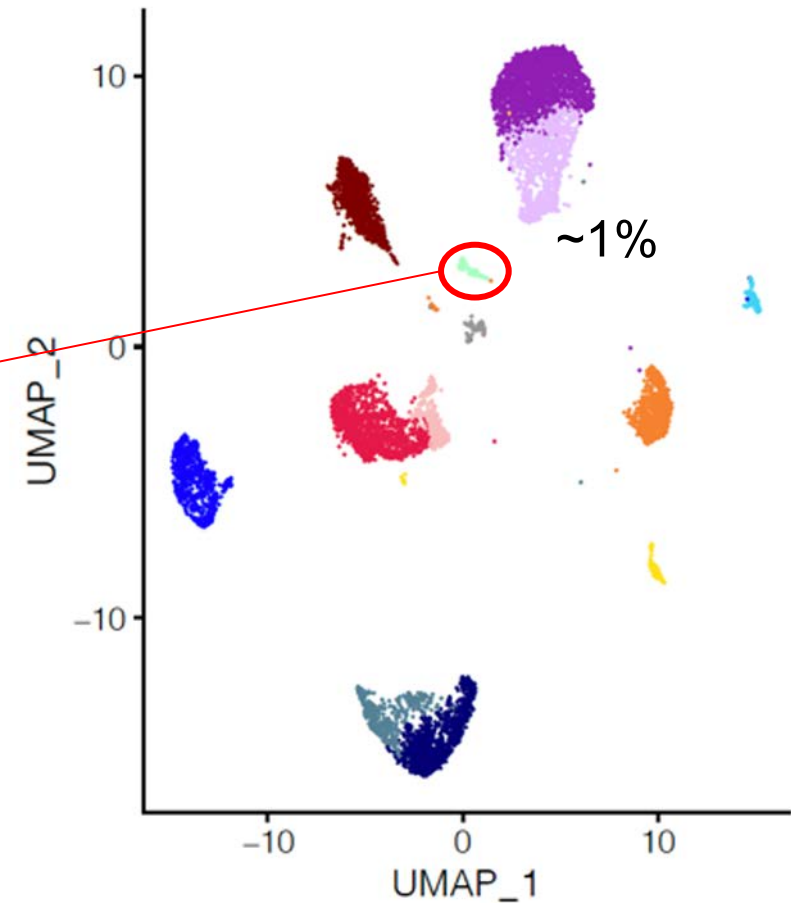
spatial dataset



- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7

VSMCs

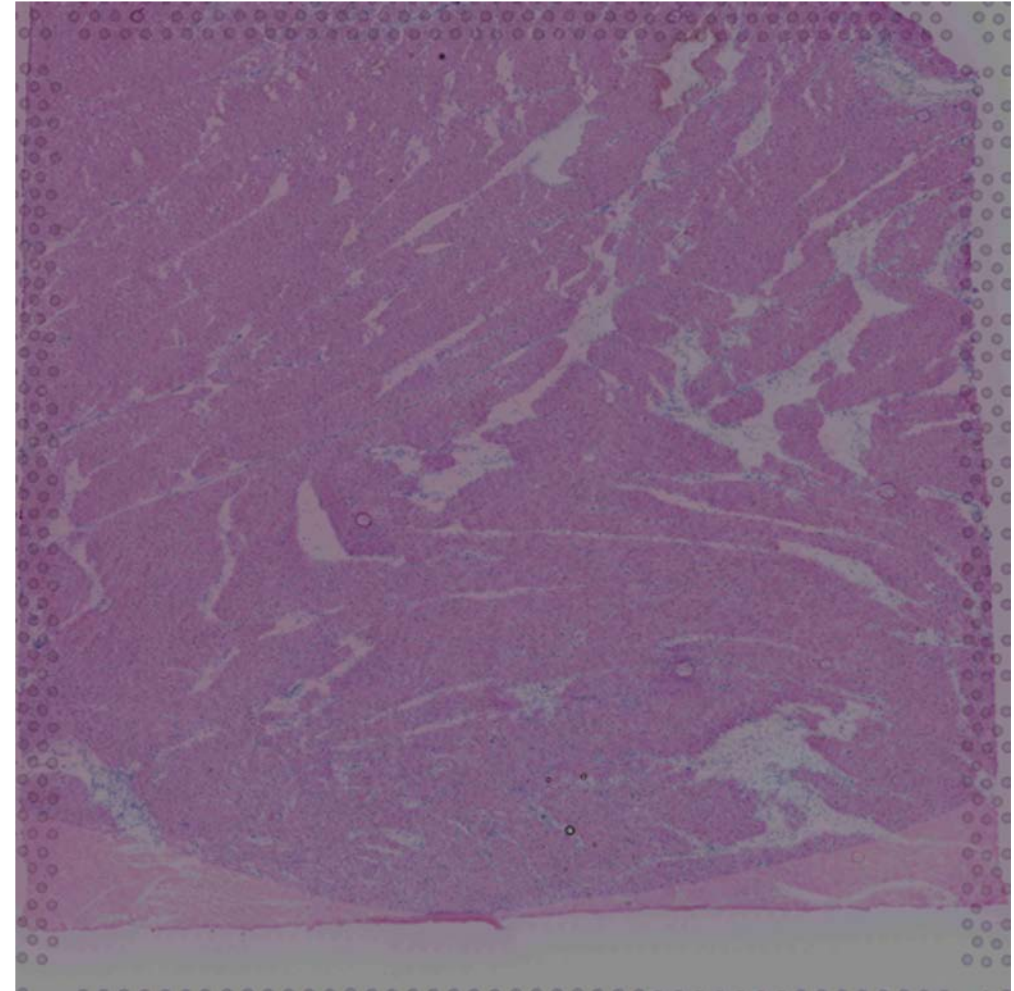
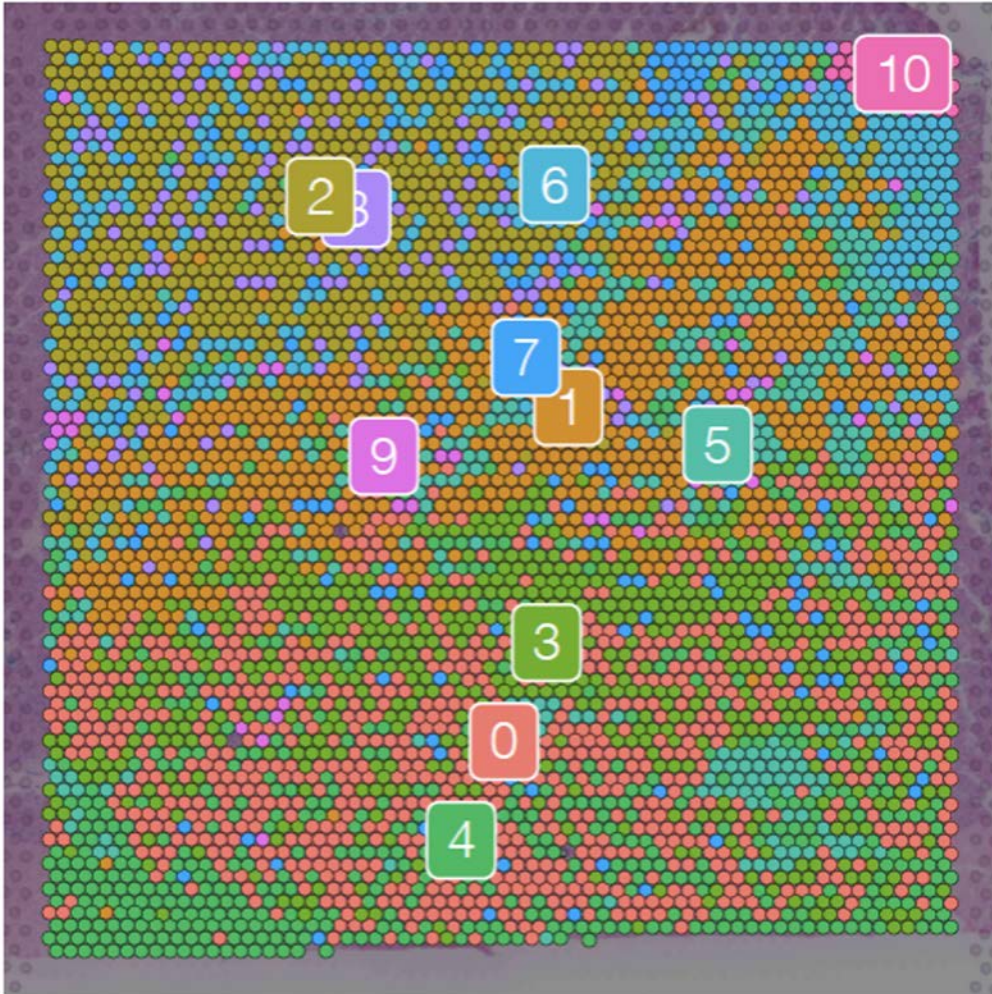
snRNA dataset



n=8335 nuclei

Human Myocardial Infarction – Border Zone

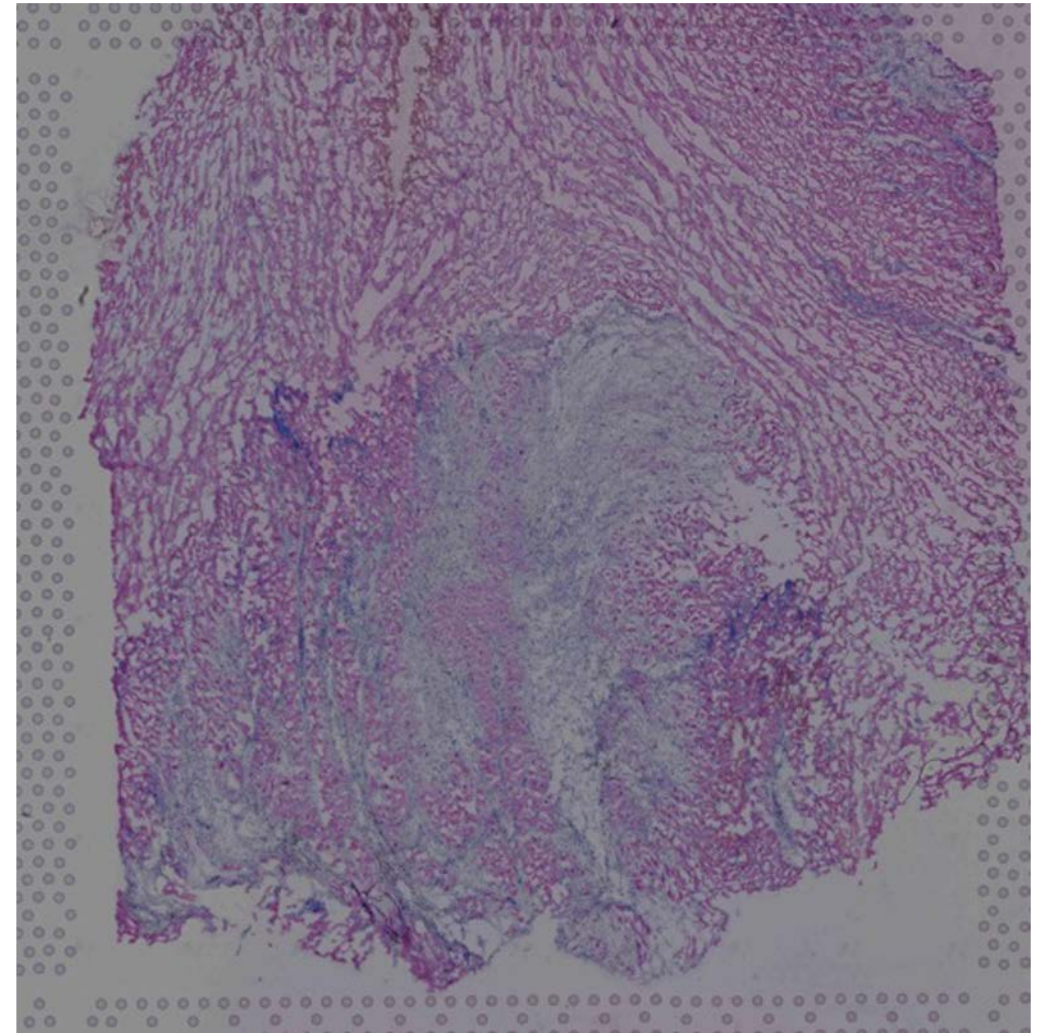
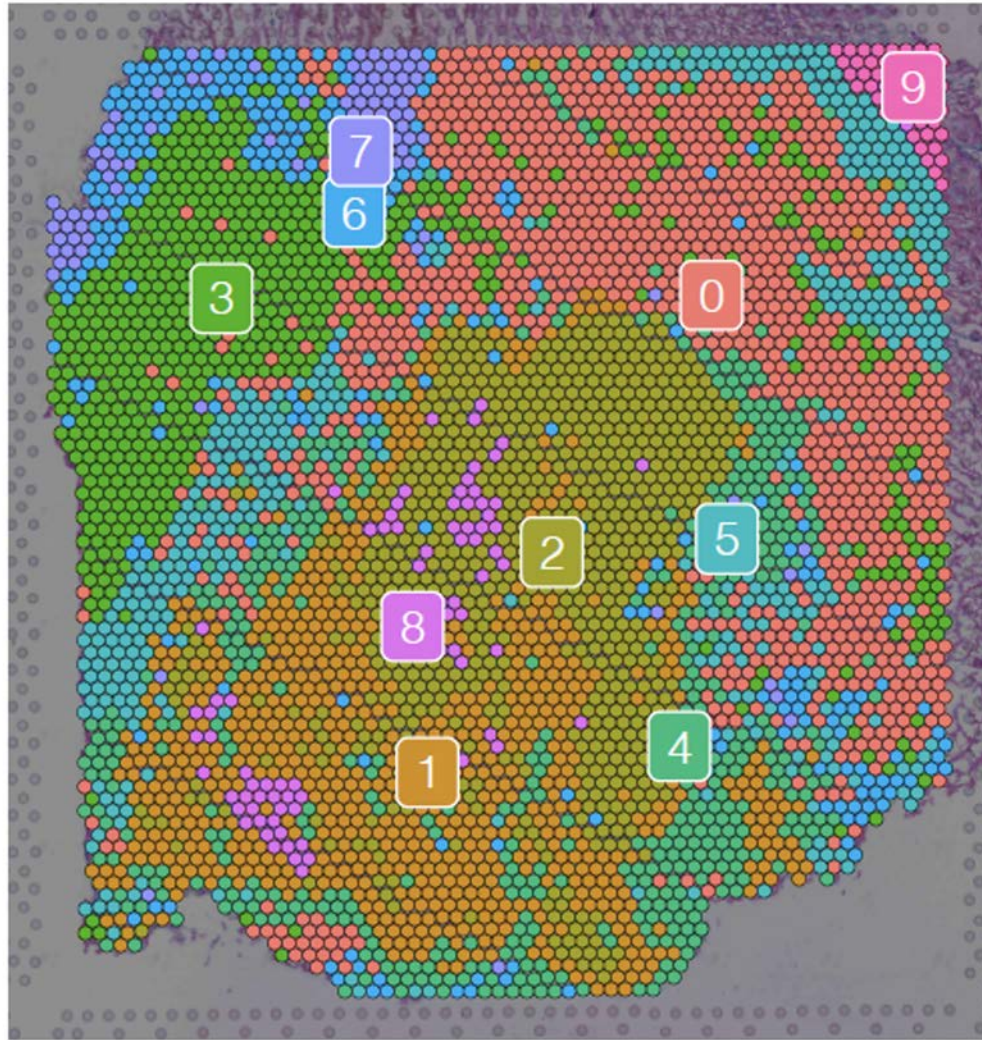
sample	RZ	BZ	IZ
acute MI			



Slides courtesy of:
Dr Christoph Kuppe and Dr. Rafael Kramann
www.kramannlab.com

Human Myocardial Infarction – Chronic Stage – Fibrosis

sample	FZ
chronic (MI)	<input checked="" type="radio"/>

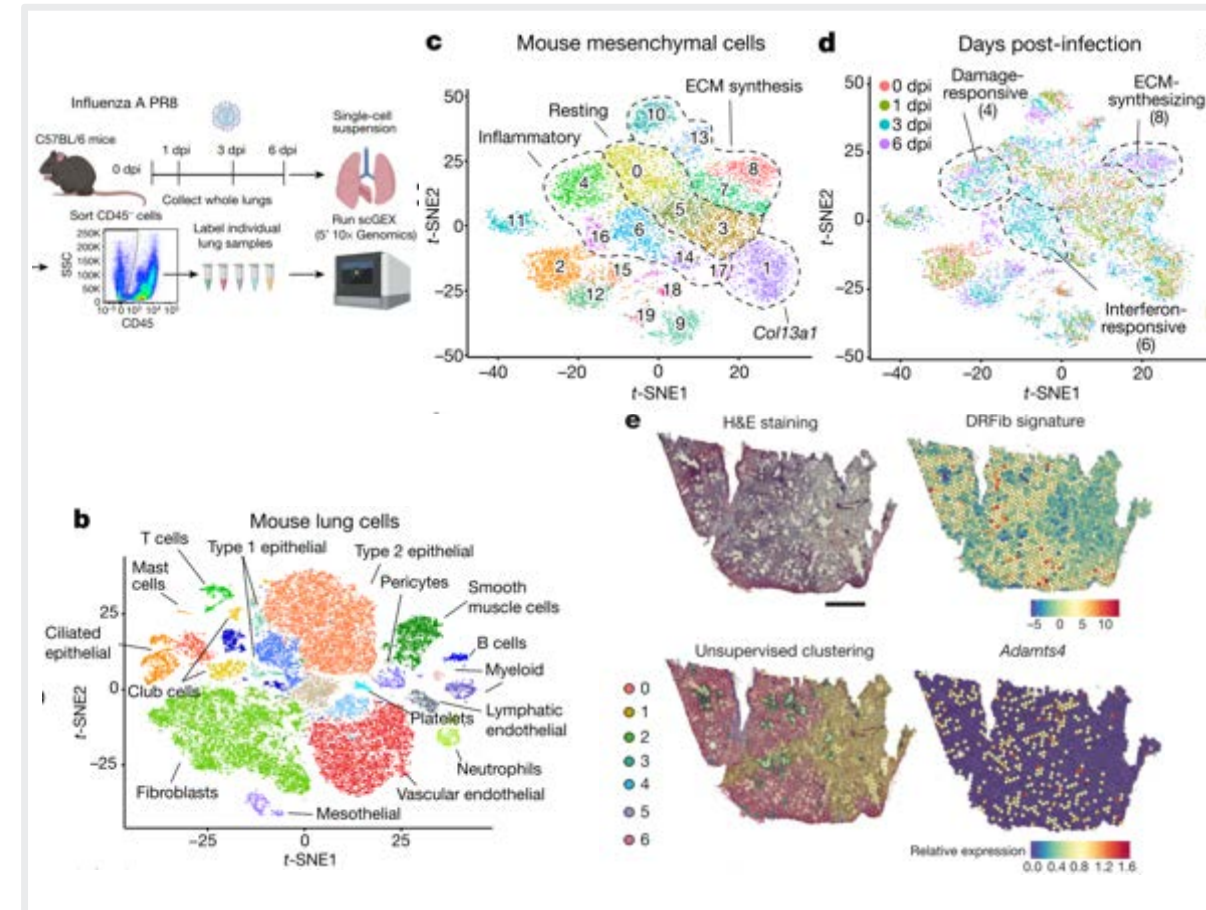


Slides courtesy of:
Dr Christoph Kuppe and Dr. Rafael Kramann
www.kramannlab.com

Exuberant fibroblast activity compromises lung function via ADAMTS4

Boyd et al., 2020, Nature

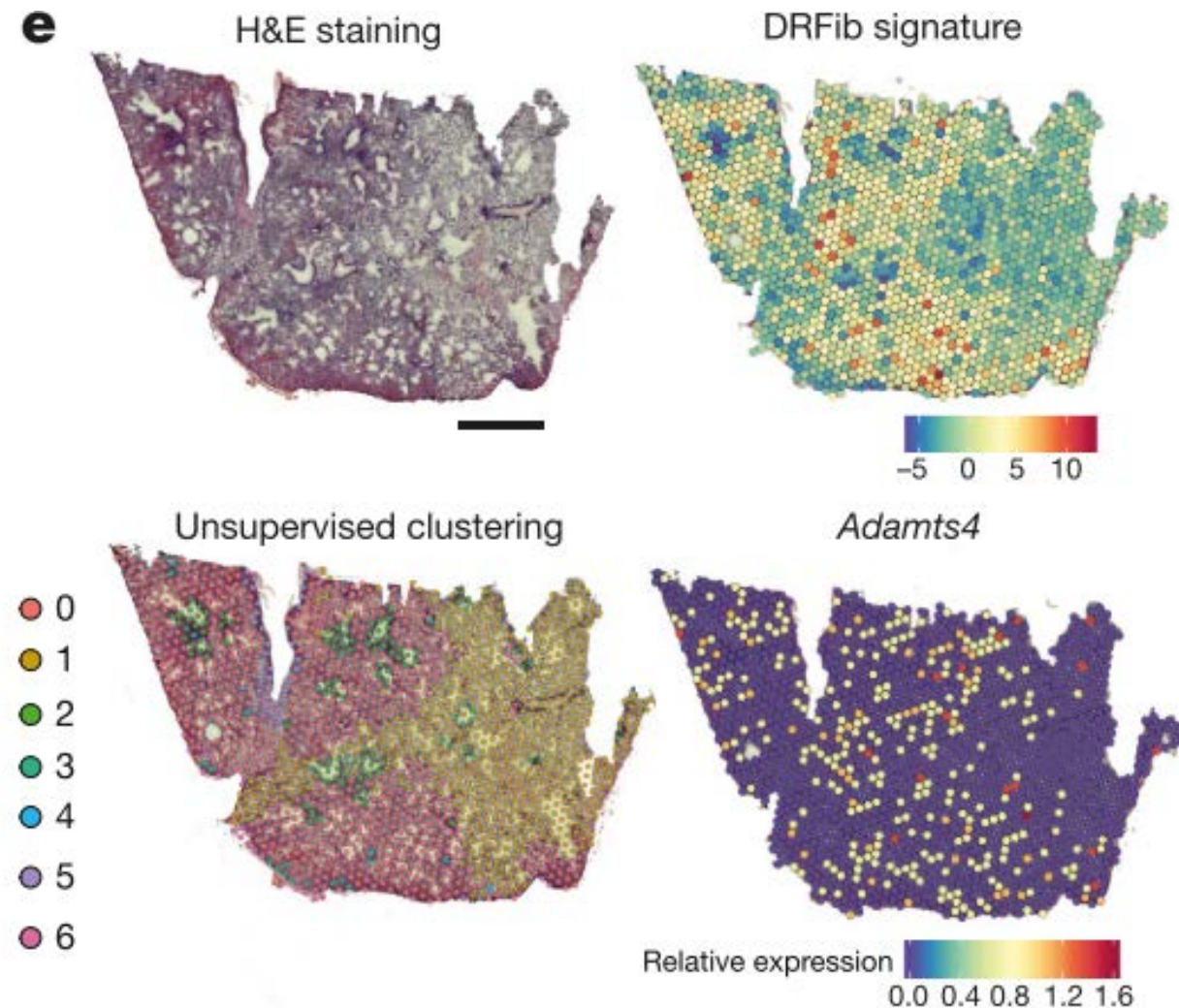
- Chromium Single Cell Gene Expression and Visium Spatial Transcriptomics were implemented to unravel the role of lung stromal cell populations in coordinating host responses to respiratory infections
- Damage-responsive fibroblasts (DRFibs) induced by viral infection are enriched for pathways involved in tissue damage response including *Adamts4*, *Itga5* and *Lox*
- Spatial analysis of infected lung showed that DRFibs were found in the distal airways at a critical inflection point for host outcome
- Targeting cells governing tissue remodeling could provide an avenue for therapeutical management of respiratory viral infections such as Acute Respiratory Syndrome (ARDS).



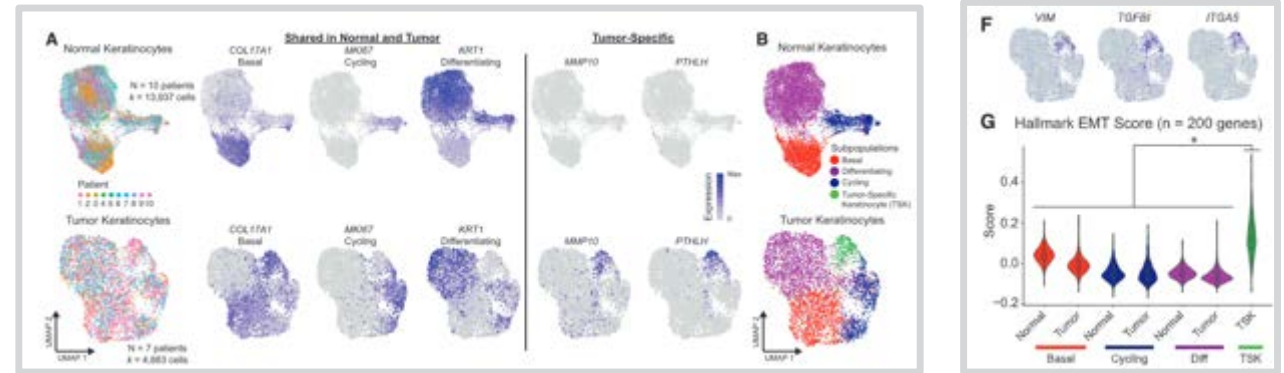
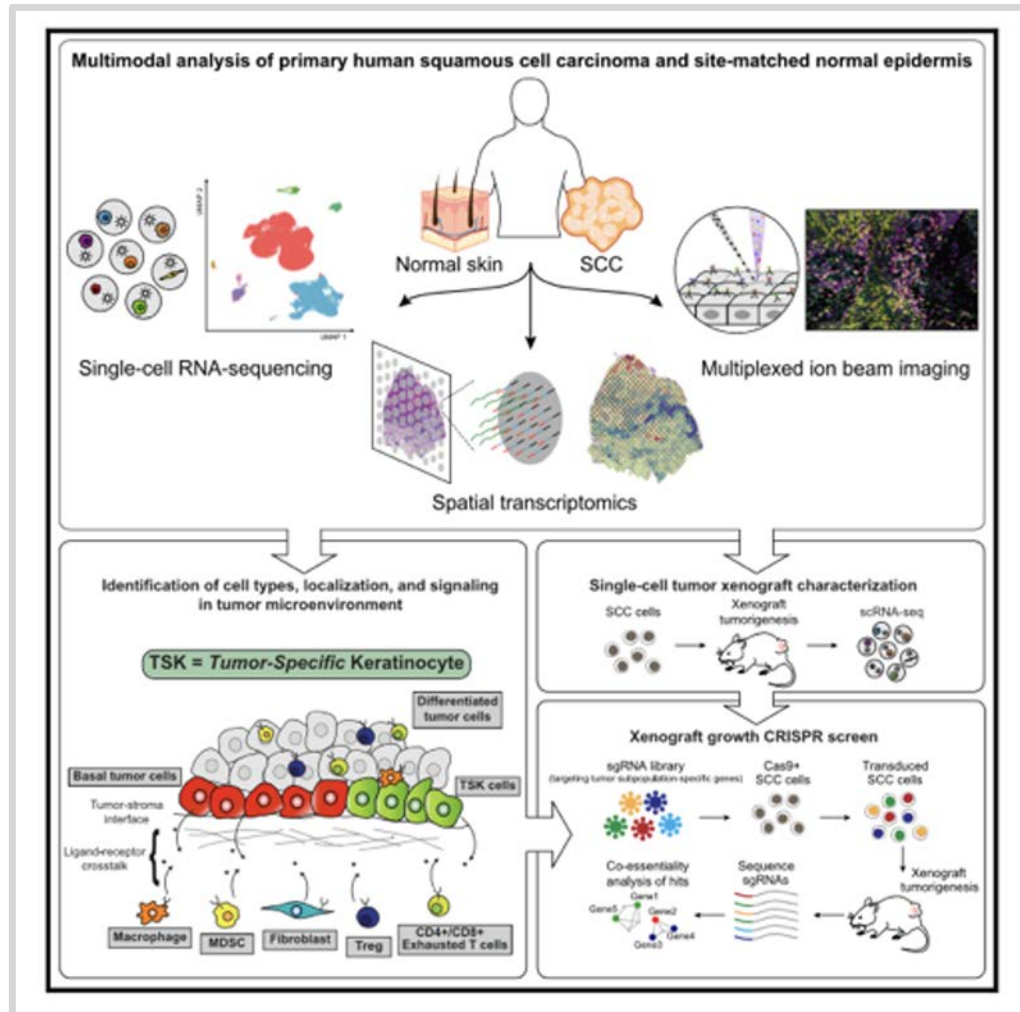
Exuberant fibroblast activity compromises lung function via ADAMTS4

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Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma (Ji, Cell, 2020)



In Brief:

Chromium + Visium (ST) + Proteomics + CRISPR in human **skin** cancer (cSCC) and mouse model

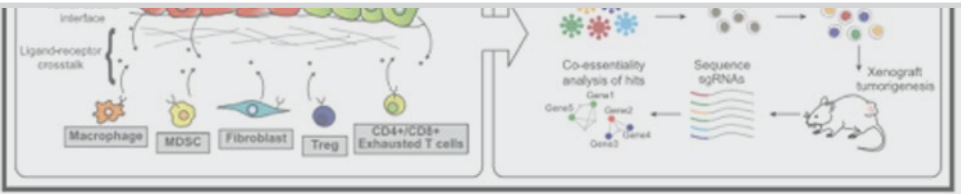
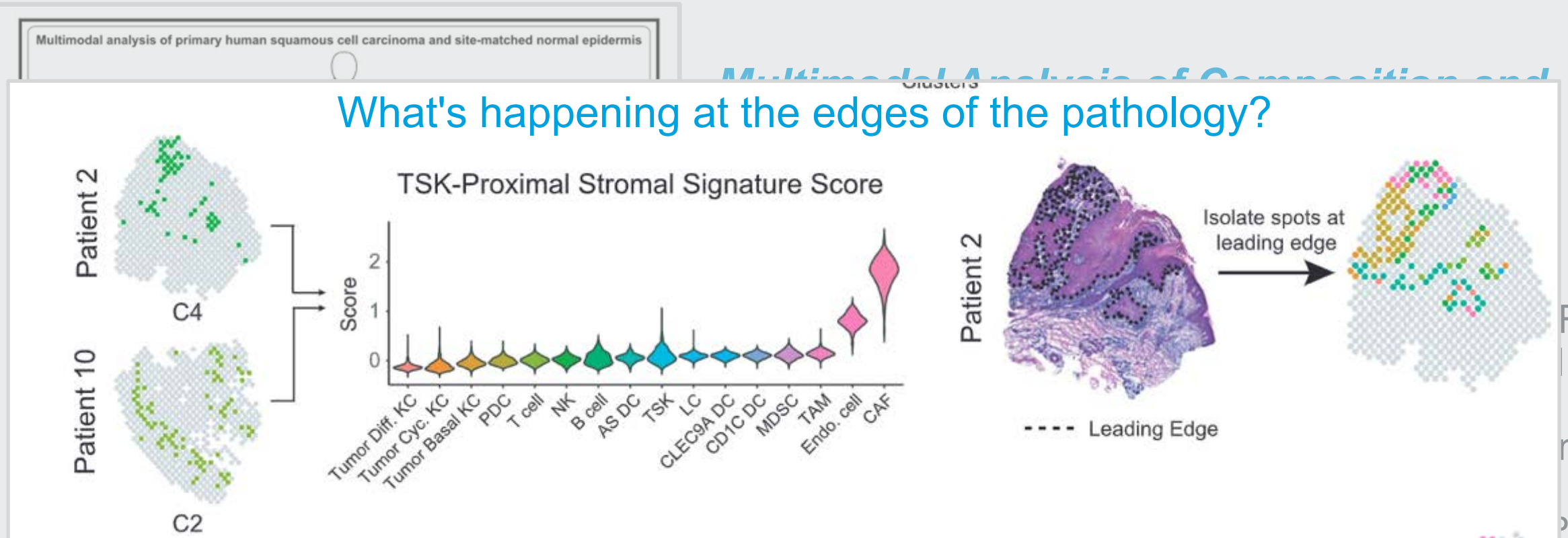
Provides a compelling case for multiomic study in oncology:

- Integrates single cell, spatial, proteomic, and CRISPR techniques
- Provide insights on tumorigenesis, cell signaling, and immune cell infiltration in cSCC

Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma (Ji, Cell, 2020)

Multimodal Analysis of Composition and Spatial Architecture

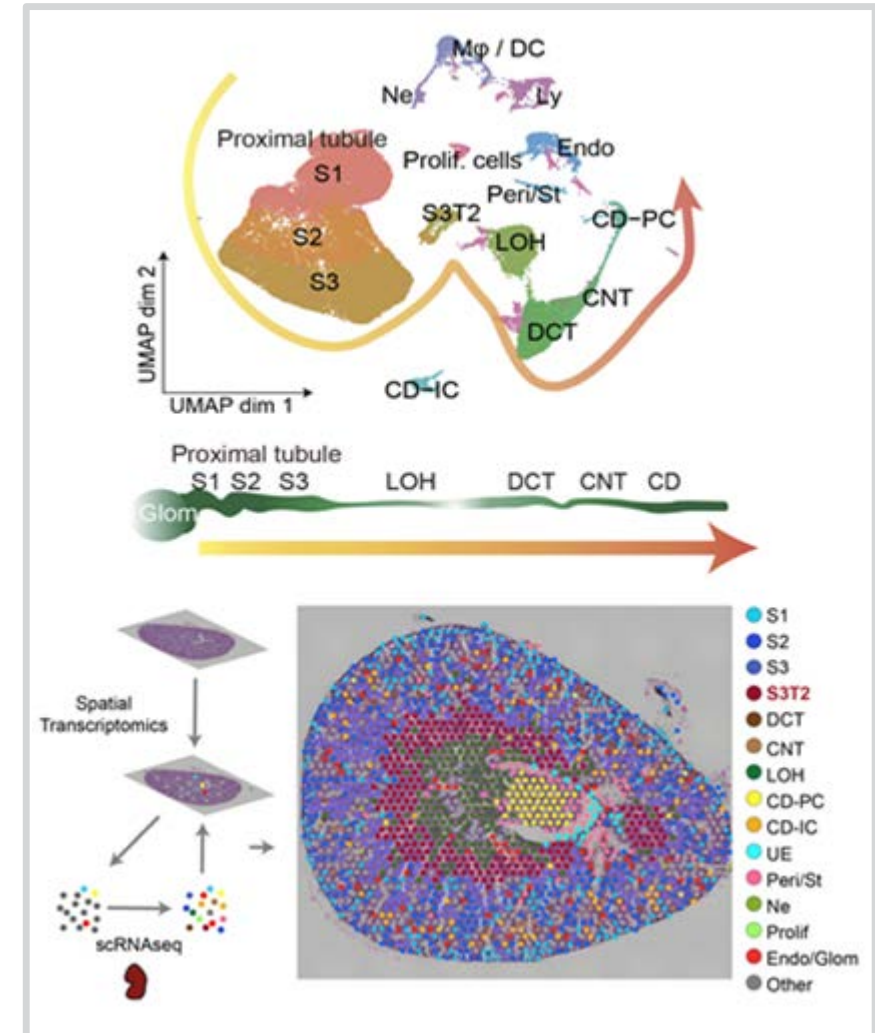
What's happening at the edges of the pathology?



- techniques
- Provide insights on tumorigenesis, cell signaling, and immune cell infiltration in cSCC

The orchestrated cellular and molecular responses of the kidney to endotoxin define the sepsis timeline

- Using Chromium single cell RNA sequencing and *Visium Spatial* the spatial and temporal progression of endotoxin injury to the kidney in a murine sepsis model was examined.
- Authors identified key changes in gene expression that altered cellular functions and cell trajectories which can explain features of clinical sepsis.
- These changes converged towards a remarkable global cell-cell communication failure and organ shutdown at a well-defined point in the sepsis timeline.
- Spatial gene expression profiling enabled the identification of the location of a yet spatially uncharacterized proximal tubular cell type in the kidney tissue.



Visium with IF and targeted gene expression

From discovery to focused transcriptome

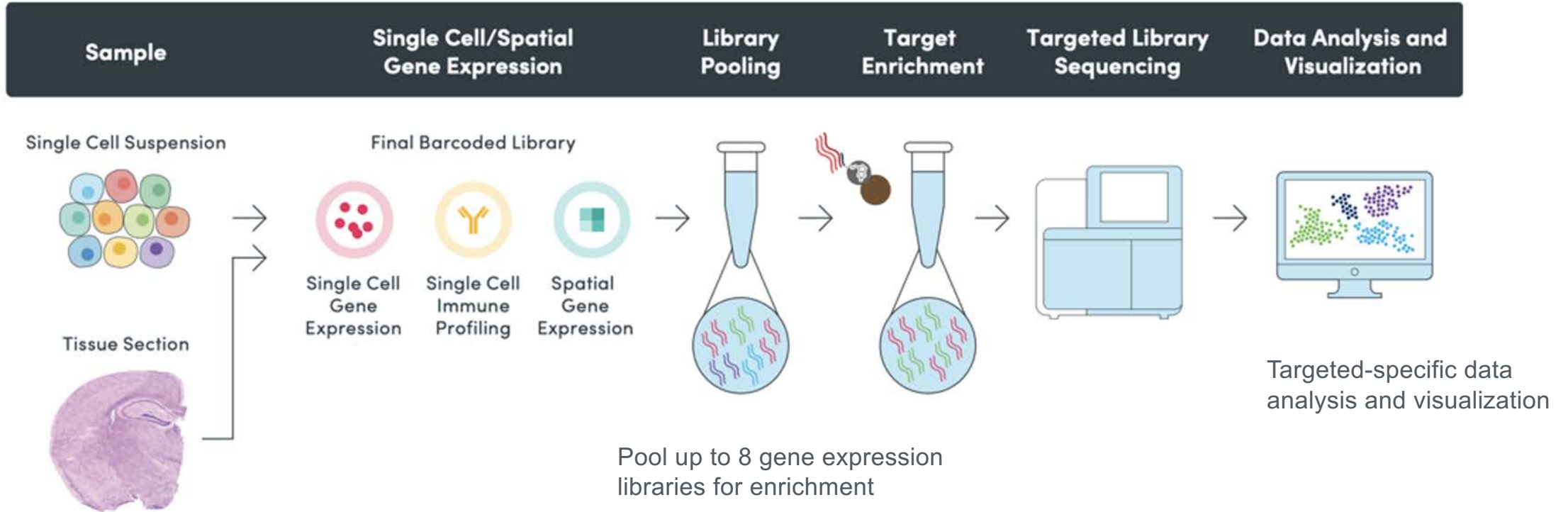
A targeted product built for 10x assays

- Single cell or spatial resolution - use the same validated panels to explore the **morphological context** of relevant biomarkers
- **Whole transcriptome** and **targeted** gene expression from the same samples
- **Reduced sequencing** cost, increased experimental efficiency
- Pair with multiomic measurements
- **Curated content** and **customization**



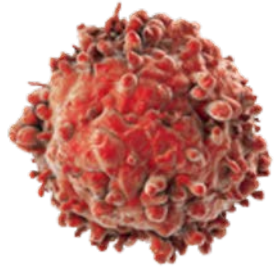
Target enrichment workflow

Versatile, sensitive, customizable targeting with hybrid capture



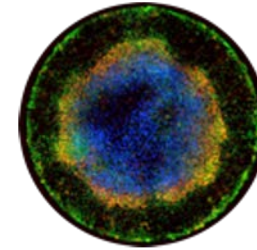
Comprehensive pre-designed panels

Accelerate research in 4 major areas



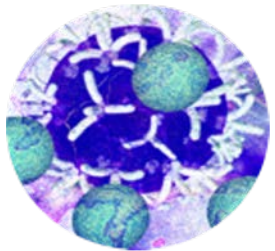
Human Pan-Cancer
1,253 genes

- **33 cancer types**, key biomarkers, pathways, and cellular processes
- Profile tumor microenvironment and heterogeneity, and tumor immune status in a variety of tumors



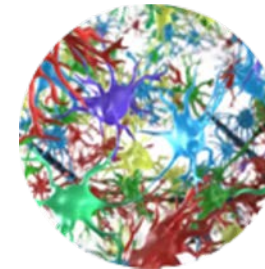
Human Gene Signature
1,142 genes

- **Disease and drug targets**, including kinases, GPCRs, cell cycle/checkpoint
- Analyze the **activation or inhibition** of important signaling pathways, and discover mechanism of action of small molecules



Human Immunology
1,056 genes

- Covers **innate and adaptive immunity, inflammation and immuno-oncology**
- Comprehensively profile the immune response in cells and tissues



Human Neuroscience
1,186 genes

- Covers **neural development, neurogenesis, neurodegenerative diseases and neuro-oncology**
- Characterize changes in gene expression in brain injury and disease

Demonstrated Protocol Available from 10x Genomics

Methanol Fixation, IF Staining & Imaging For Visium Spatial Protocols

CG000312 • Rev A

DEMONSTRATED PROTOCOL

Methanol Fixation, Immunofluorescence Staining & Imaging for Visium Spatial Protocols

Overview

The Visium Spatial Gene Expression Solution measures the total mRNA in tissue sections and requires a Visium Spatial slide with intact tissue sections as input. Immunostaining tissue sections with fluorescently labeled antibodies enables simultaneous protein detection. This protocol outlines methanol fixation, immunofluorescence staining, and imaging of tissue for use with 10x Genomics Visium Spatial protocols. Fixed and stained tissue sections are inputs for the downstream Visium Spatial Tissue Optimization and Visium Spatial Gene Expression workflows.

Additional Guidance

Consult the Visium Spatial Protocols - Tissue Preparation Guide (Document CG000240) for Tips & Best Practices on freezing, embedding, and cryosectioning tissue and placing sections on Visium Spatial Slides. Consult the Visium Spatial Gene Expression Imaging Guidelines (Document CG000241) to verify imaging settings prior to starting this Demonstrated Protocol. Perform this Demonstrated Protocol on tissue sections placed on the correct slide.


- Use a plain glass slide if optimizing antibody concentrations. Refer to the Antibody Optimization section for more information.
- Use a Visium Spatial Tissue Optimization Slide if proceeding with tissue optimization.
- Use a Visium Spatial Gene Expression Slide if proceeding with library construction.

The Tissue Optimization workflow must be performed prior to the Gene Expression workflow to determine the optimal tissue section permeabilization time. Permeabilization times identified using the H&E staining protocol may not be applicable to immunofluorescence staining. After completing this protocol, proceed with either the Visium Spatial Gene Expression Reagent Kits - Tissue Optimization User Guide (CG000238) or the Visium Spatial Gene Expression Reagent Kits User Guide (CG000239).

Visium Slide Selection


Visium Spatial Tissue Optimization Slide (PN-3000394)

- Used with Visium Spatial Gene Expression Reagent Kits - Tissue Optimization User Guide (CG000238) to identify optimal permeabilization time for a specific tissue type.
- Includes 8 Capture Areas, each covered with oligonucleotides for mRNA capture.
- Each Capture Area is 8 x 8 mm and is surrounded by an etched frame.
- A readable label defines the active surface of the slide.



Visium Spatial Gene Expression Slide (PN-2000233)

- Used with Visium Spatial Gene Expression Reagent Kits User Guide (CG000239) to generate Visium Spatial Gene Expression libraries.
- Includes 4 Capture Areas, each with ~5,000 unique gene expression spots.
- Each Capture Area is 6.5 x 6.5 mm and is surrounded by a fiducial frame for a total area of 8 x 8 mm.
- A readable label with a serial number defines the active surface of the slide.

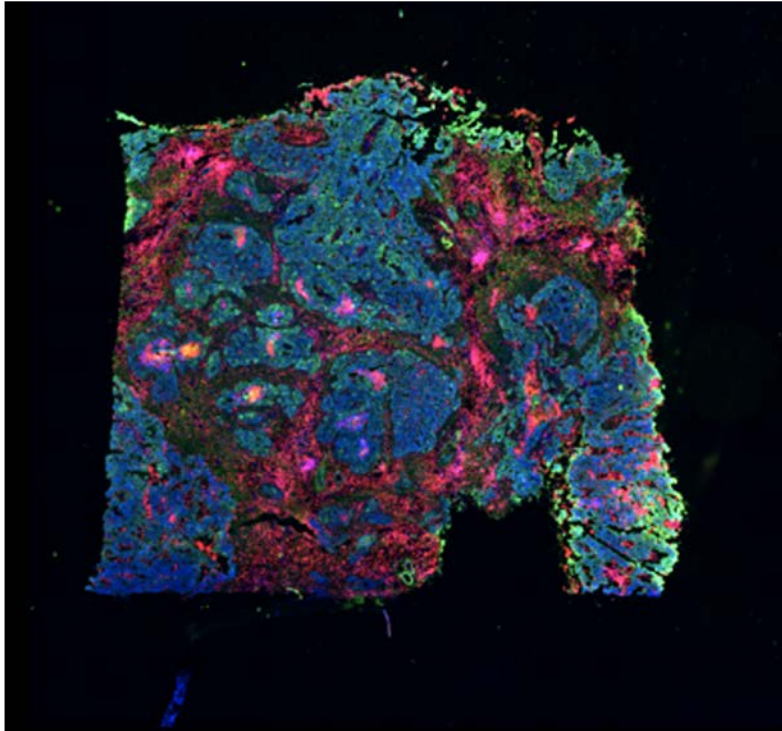


10x GENOMICS

10xgenomics.com Methanol Fixation, Immunofluorescence Staining & Imaging - Visium Spatial Protocols • Rev A

- Demonstrated protocol content includes:
 - Antibody Optimization
 - Tissue Fixation & Immunofluorescence Staining
 - Coverslip Addition & Removal
 - Tissue Imaging
 - Results
 - Troubleshooting
 - Tips & Best Practices

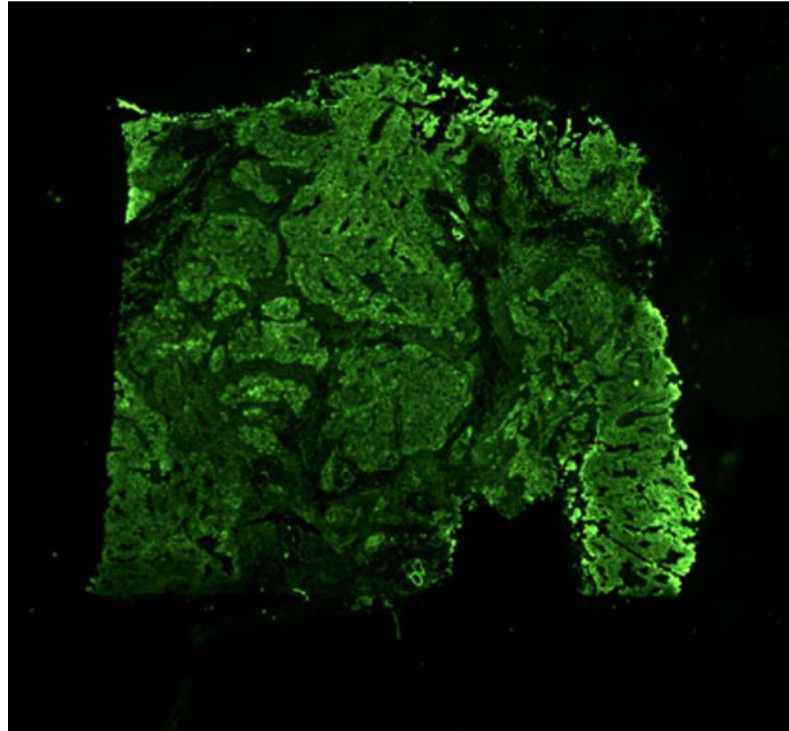
Visium with IF, Human Ovarian Tumor



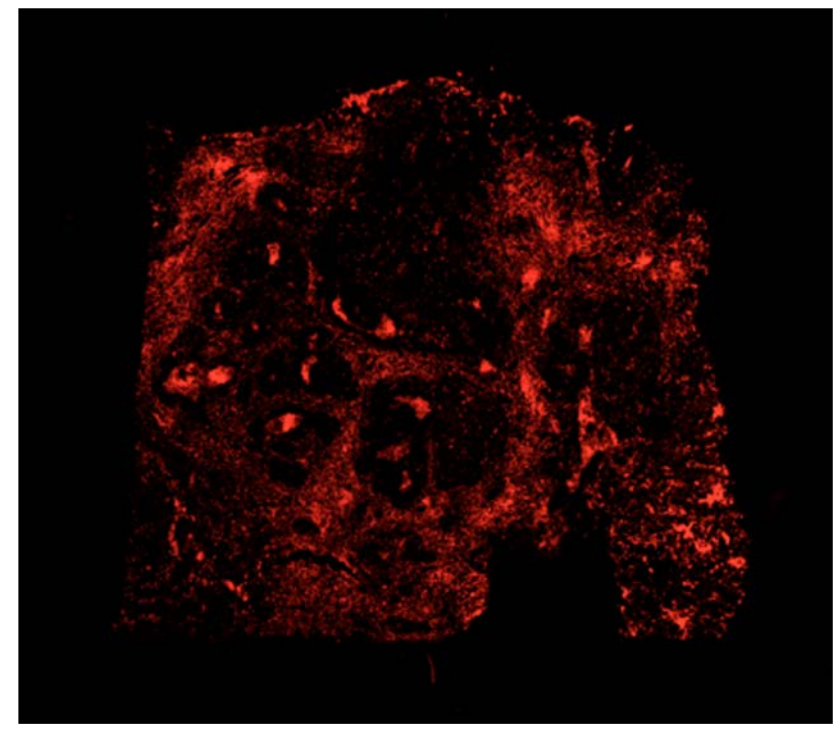
PanCK

CD45

DAPI



PanCK

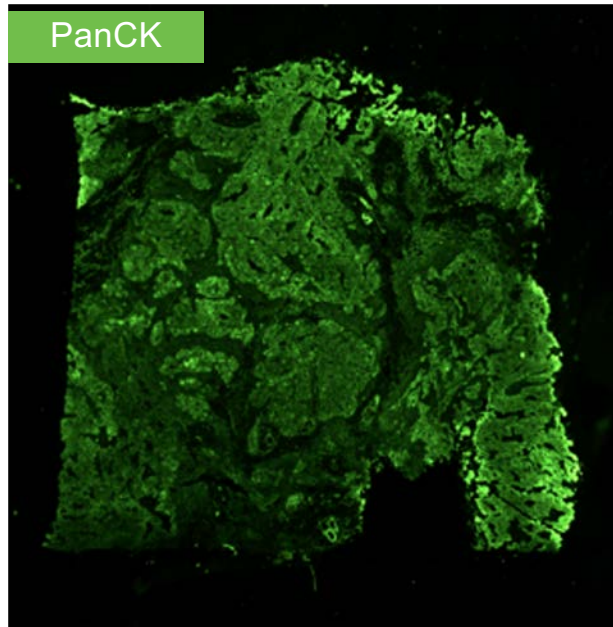


CD45

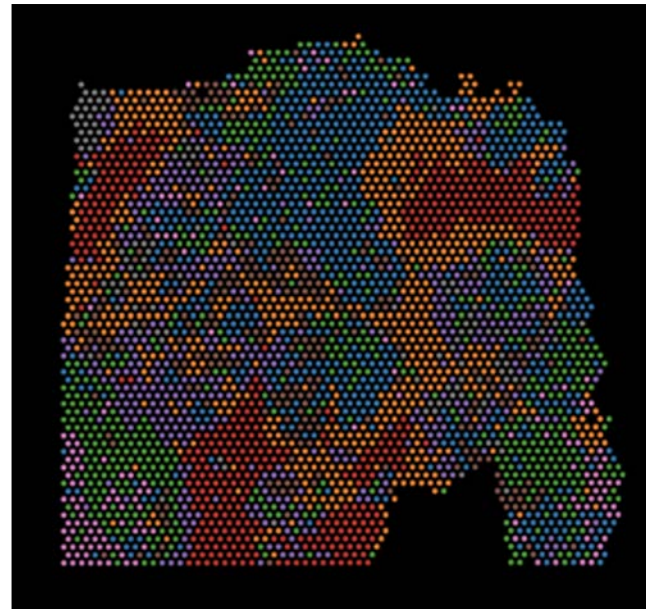
Targeted Spatial Gene Expression of IF Samples

Combining IF and Targeting on Visium samples

Immunofluorescence

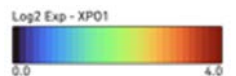
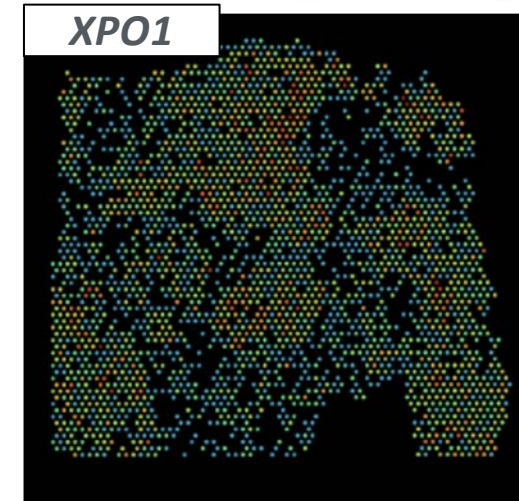
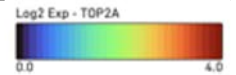
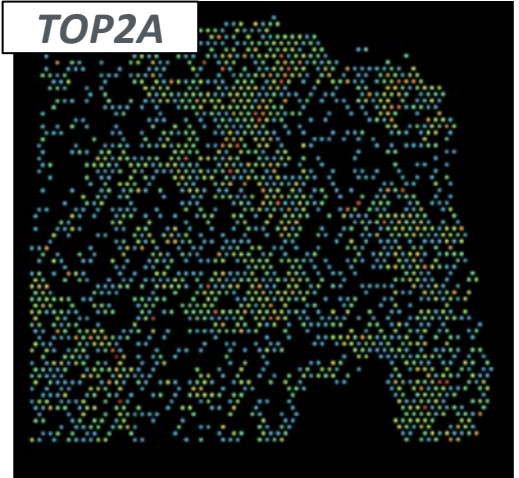


Pan-Cancer Targeted, Clustering
5K reads/spot



- Cluster 1
- Cluster 2
- Cluster 3
- Cluster 4
- Cluster 5
- Cluster 6
- Cluster 7
- Cluster 8

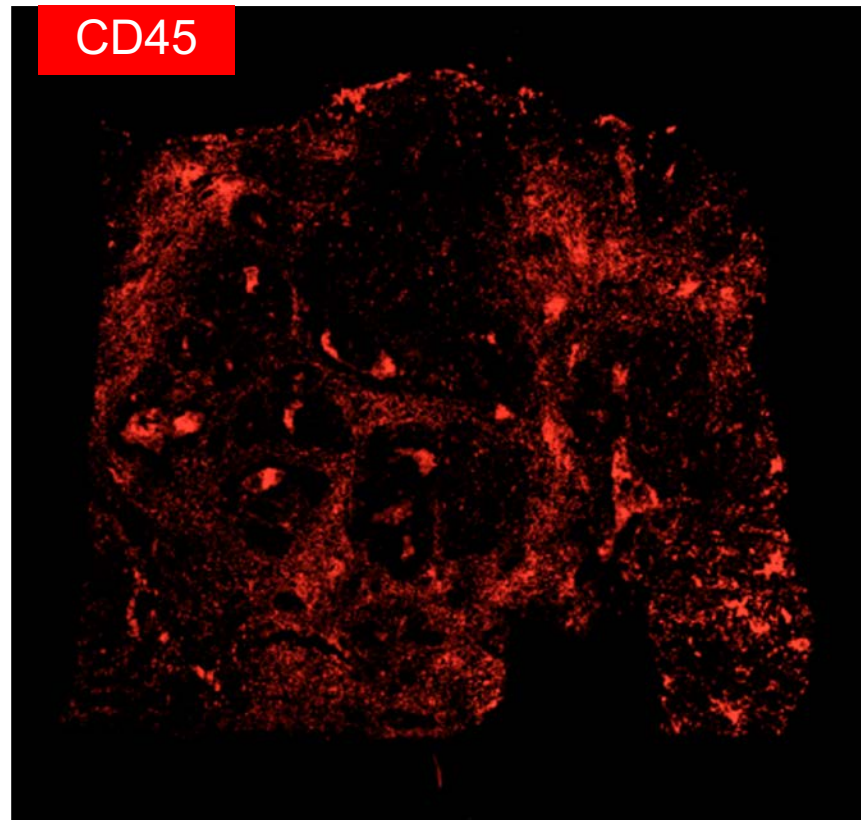
Top genes
Cluster 1
PRKCI
VTCN1
MECOM
TOP2A
SDHD
XPO1
TFRC
FUT8
SOX17
PBX1
EIF42
WT1



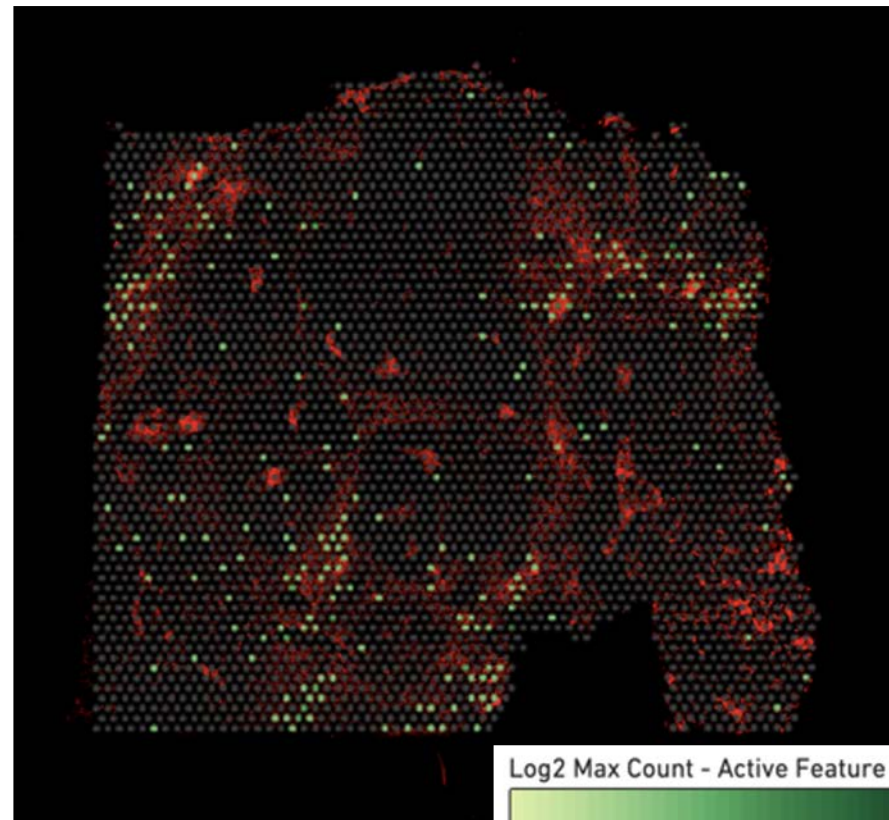
Spatial Identification of Cell Subtypes

Combining IF and Targeting on Visium samples

Immunofluorescence



Immunology Targeted GEX + IF Overlay
5K reads/spot



B-Cell markers

- CD19
Gene · ENSG00000177455
- CD79A
Gene · ENSG00000105369
- CD79B
Gene · ENSG00000007312

Visium with IF, Human Spinal Cord

DAPI + Myelin CNPase + SNAP25

Loupe Browser 4.1.0 - Spinal Cord - Targeted - Parent

Spot Opacity Image Settings Spatial Categories

Graph-Based

- Cluster 1 (515)
- Cluster 2 (499)
- Cluster 3 (435)
- Cluster 4 (418)
- Cluster 5 (325)
- Cluster 6 (242)
- Cluster 7 (218)
- Cluster 8 (160)

Significant Feature Comparison

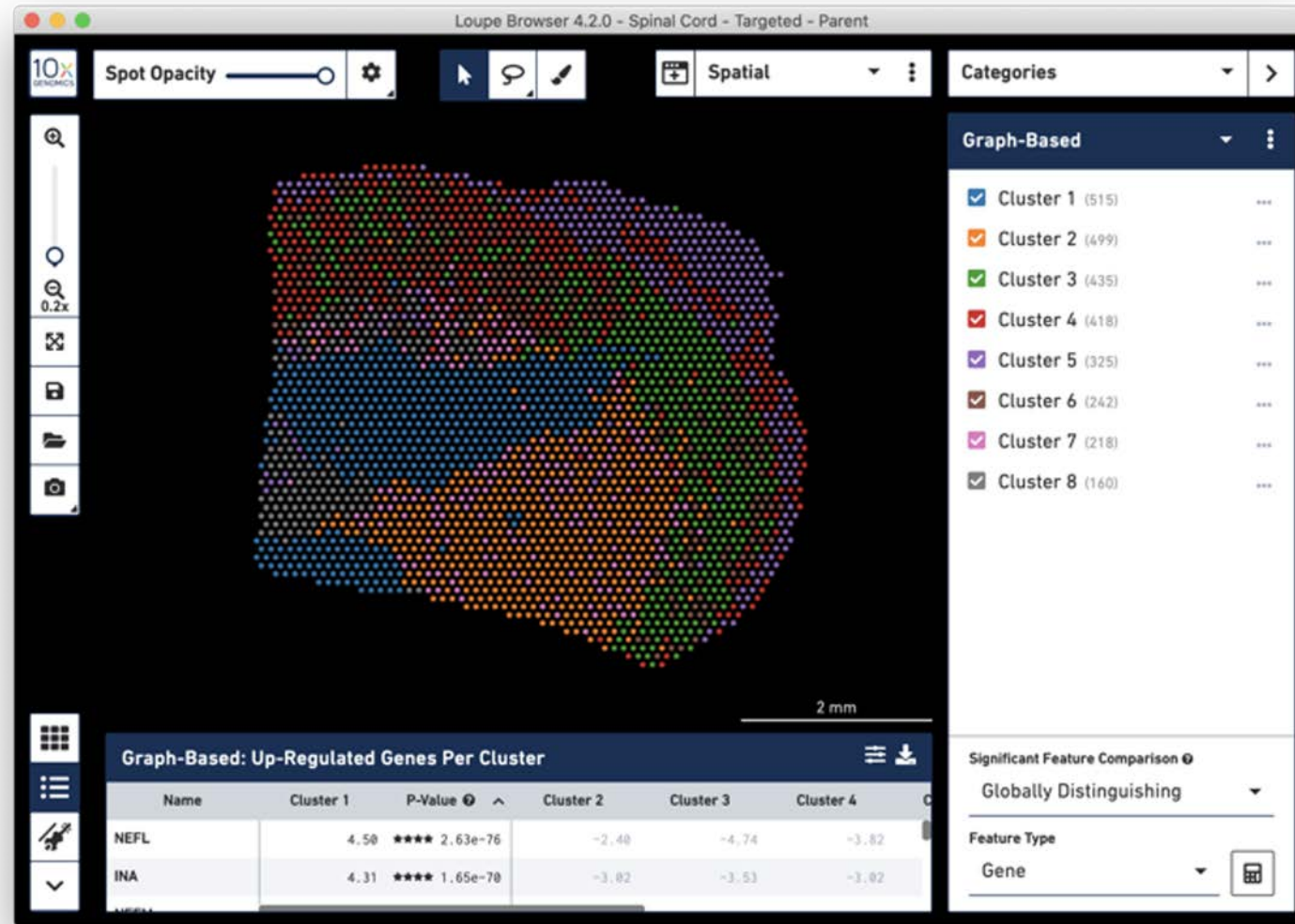
Globally Distinguishing

Feature Type

Gene

Name	Cluster 1	P-Value	Cluster 2	Cluster 3	Cluster 4
NEFL	4.50	★★★★ 2.63e-76	-2.40	-4.74	-3.82
INA	4.31	★★★★ 1.65e-70	-3.02	-3.53	-3.02

Visium with IF, Human Spinal Cord



Coming soon to Visium

Highly Multiplexed Protein Analysis of Tissues

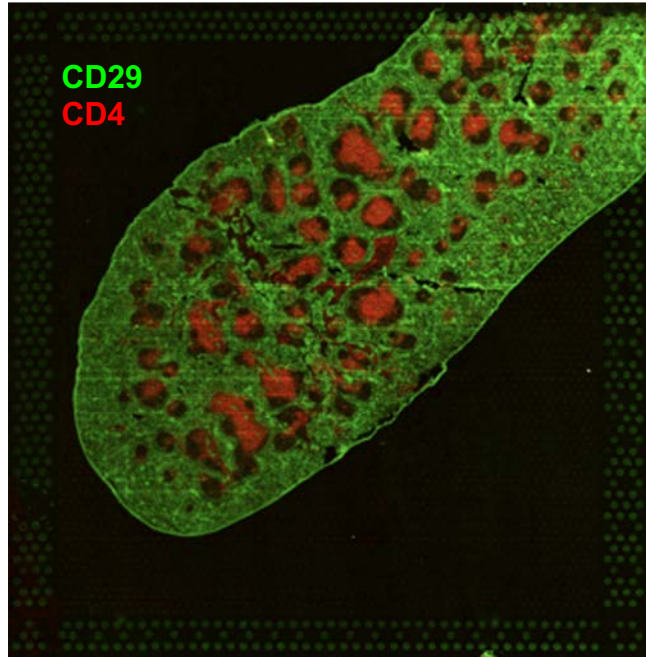
Feature Barcode Technology



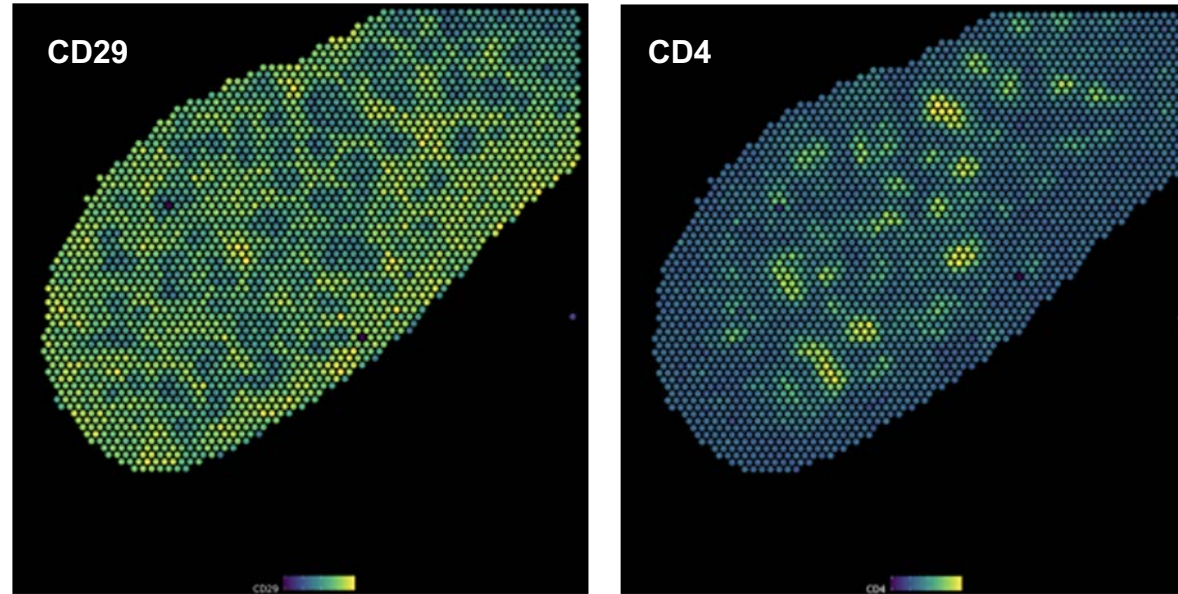
- Tens to hundreds of proteins analyzed at once
- Quantification of protein expression
- Simultaneous whole transcriptome and protein analysis
- No concern of spectral overlap or photobleaching

Feature Barcode Correlates with Immunofluorescence

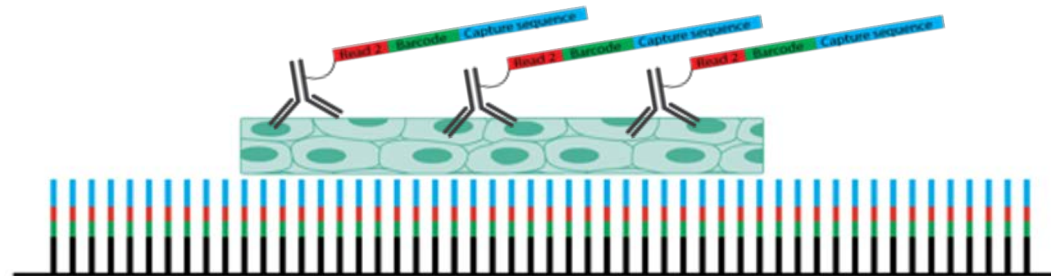
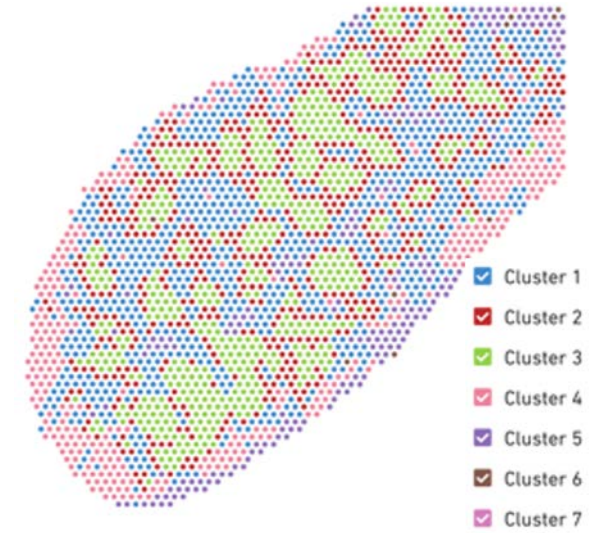
Immunofluorescence on Visium



Feature Barcode Antibody-Barcode counts



Gene expression clustering



Enabling True Discovery on FFPE

Visium for FFPE

Coming Q2 2021



- High sensitivity
- Whole transcriptome
- Full coverage of tissue sample
- Simultaneous co-detection of proteins
- Non-polyA based chemistry

Spatial Gene Expression from Any Sample

Coming 1st
Half 2021

Full tissue coverage with Visium for FFPE

- Whole transcriptome analysis of FFPE samples opens the door to analyzing a plethora of samples, performing retrospective studies, and enabling biomarker discovery



10x Genomics is actively developing a novel non-polyA based solution for whole transcriptome analysis designed for FFPE tissues

Key Features

Unbiased, whole transcriptome analysis

True discovery without the need to select ROIs with predetermined biomarkers

Full tissue section coverage

Not limited to analyzing ROIs in a fraction of the tissue section

High cellular resolution

1 to 10 cells per spot depending on tissue type

Compatible with histological stains

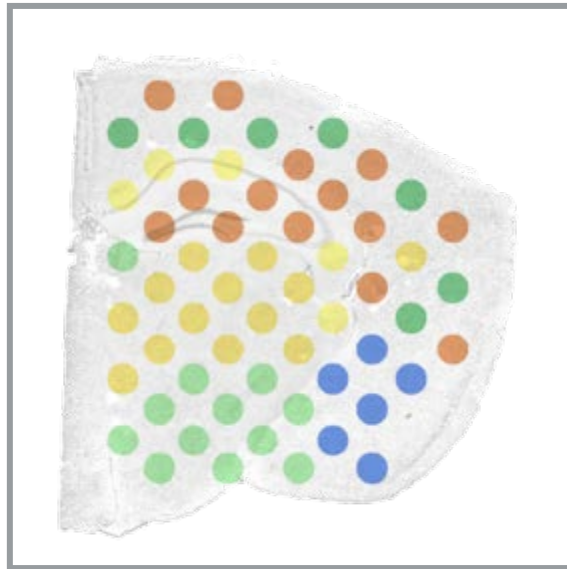
Obtain morphological context with transcriptomic analysis

All kitted reagents and software ready to use

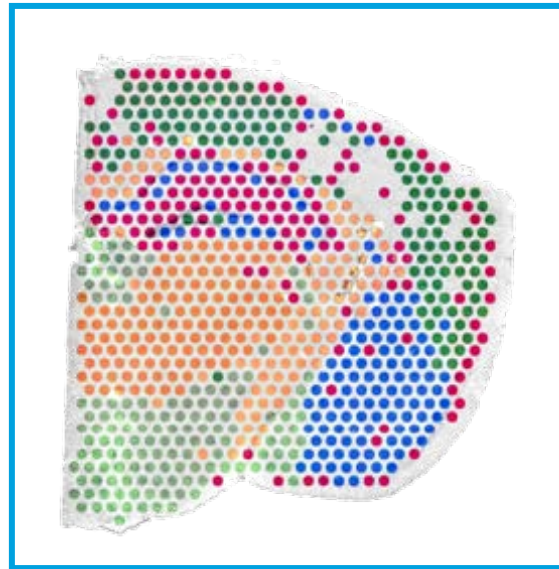
Similar to the existing Visium workflow; no specialized instrumentation required

Visium at Single Cell Resolution

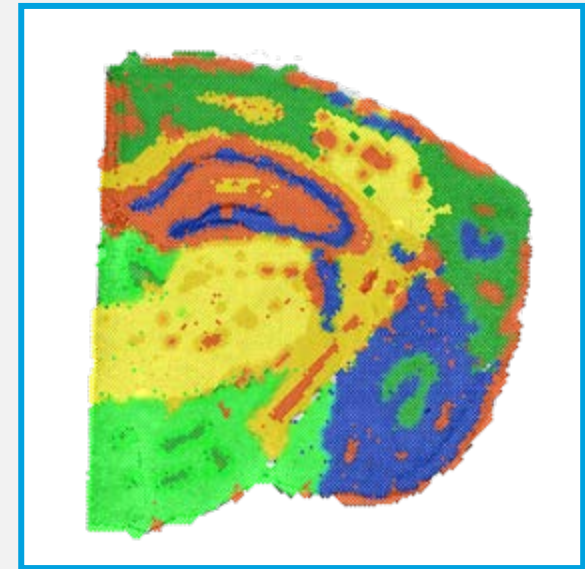
Visium HD
Coming 1H 2022



First Generation



Visium
Resolution 4x



Visium HD
Resolution 1,500x

February 24th



www.10xgenomics.com/xperience

Thank you

spence.fast@10xgenomics.com

