10x Genomics Scientific Symposium

Copenhagen, Borsen Stock Exchange | 2-3 June

https://10xgenomicsugm-copenhagen.eventbrite.com

- Day 1:
  - Presentations by industry leaders
  - Sample preparation workshop
  - Enjoy an evening social event
  - Book 1:1 meetings with our 10x-perts
  - Visit our troubleshooting stations for expert advice
  - Four juniors researchers (PhD students/early PostDocs) will win a 500 euro travel award to cover their attendance!
  - Enter our Scientific Challenge to win free kits for your projects!
- Day 2 workshops:
  - Getting Started with Chromium Single Cell Gene Expression Data Analysis (by abstract submission)
  - Getting Started with Visium Spatial Gene Expression Data Analysis (by abstract submission)
  - Bioinformatics for Single Cell and Spatial Genomics (bioinformaticians)

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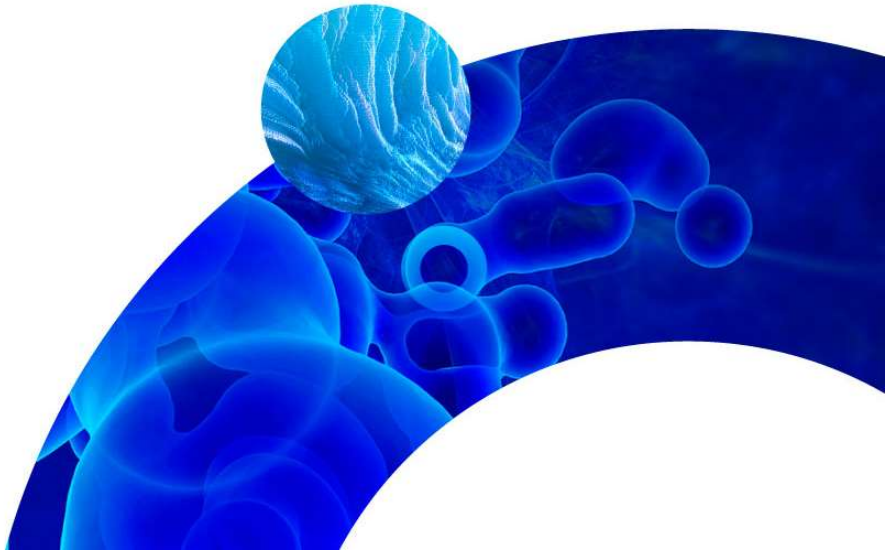
Visium Spatial transcriptomics

From single cell transcriptomics to multi-omics to
spatial transcriptomics

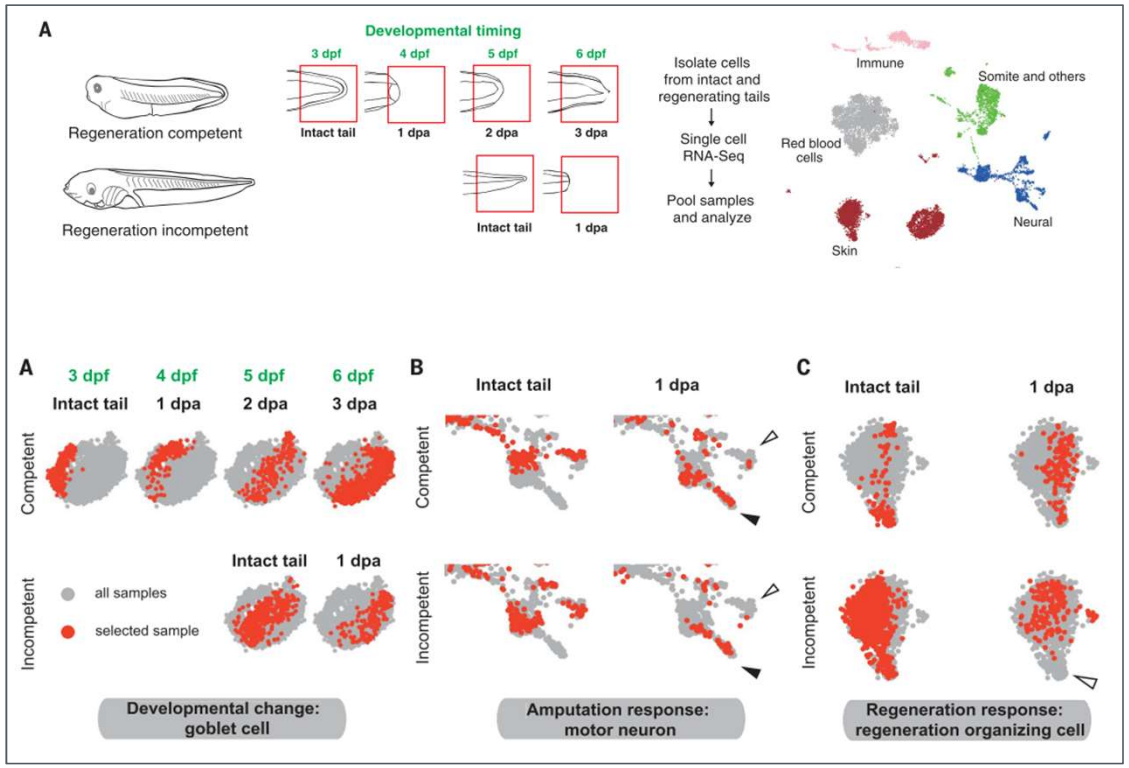


Christophe Fleury, PhD
Science & Technology Advisor

User day Toulouse – 10/03/2020

Identification of a regeneration- organizing cell in the Xenopus tail

Aztekin et al., Science 2019



Single cell sequencing *X.laevis* tail (incompetent vs competent):

- Determine existence of amputation response in both lineage
- Regeneration response different: Identification of a new cell type

  Regeneration organizing cells (ROC)

Where are these cells? Do they relocalise after amputation of the limb?

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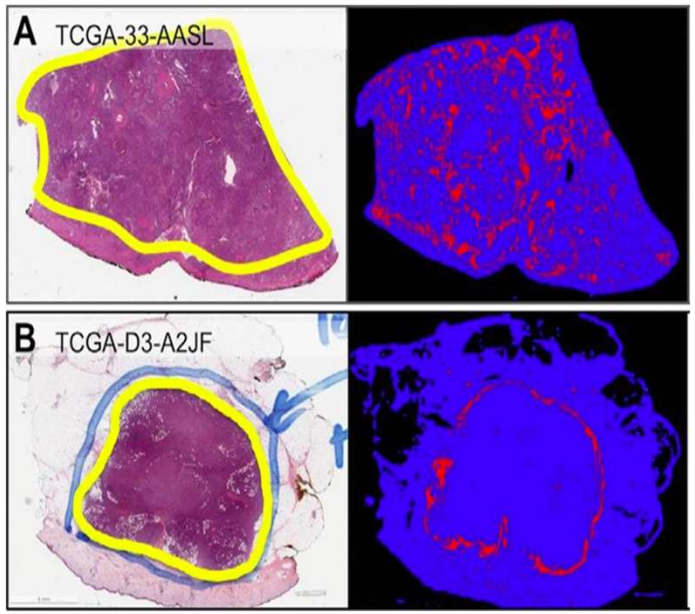
Why spatial analysis?

Gain deep biological insights with 10x Genomics Visium Spatial Gene Expression Platform



Lymphocytes infiltrating tumor

- Immune cells invasion

Lymphocytes stopped at tumor boundary

- Immunce cells at the periphery

Saltz et al. Cell Reports, May 2018

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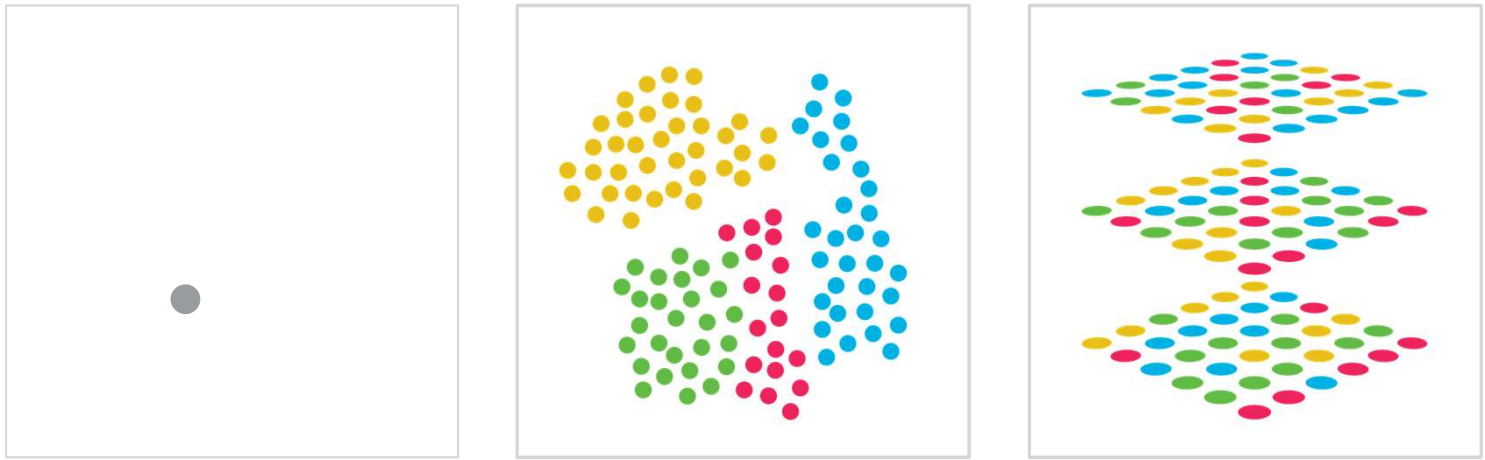
From Bulk to single cell to spatial transcriptomics



Bulk Single Cell Spatial

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Visium Spatial Gene Expression

Applications

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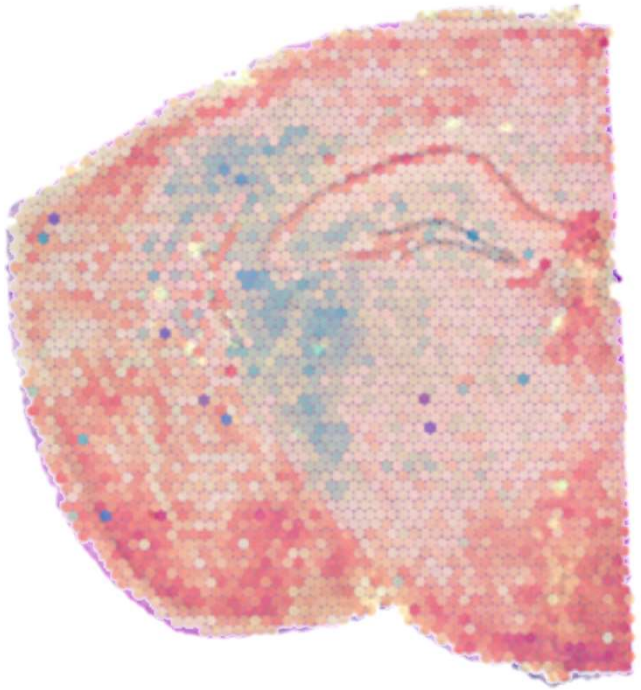
Visium Spatial Transcriptomics

Spatial transcriptomics: Ushering into the era of digital histology



- Capture gene expression information in the context of a tissue section
- Unbiased capture of expressed transcripts (1000s->7000 genes/area)
- Superposition of H&E image and gene expression data
- Simple manipulation of datasets to analyse specific gene expression

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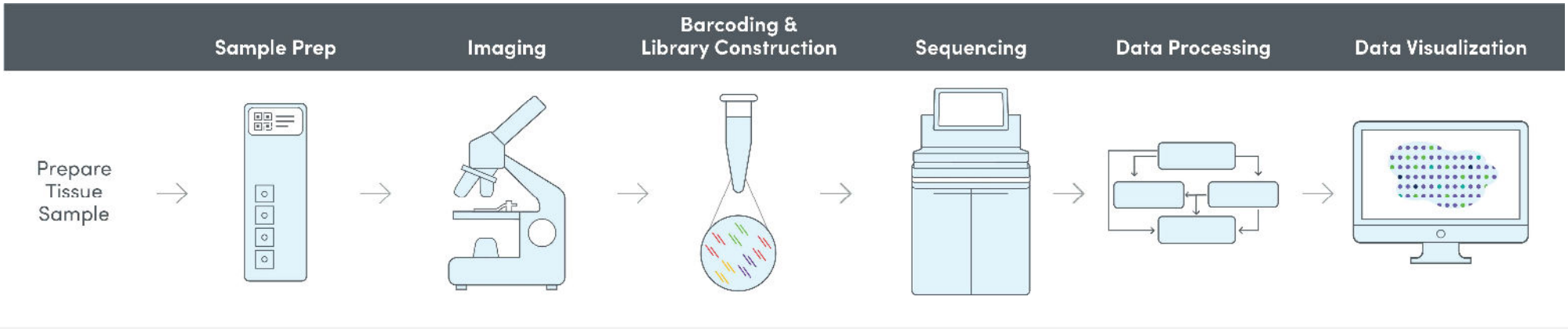
The Spatial Gene Expression Solution

Streamlined Workflow from Sample Prep to Data Analysis



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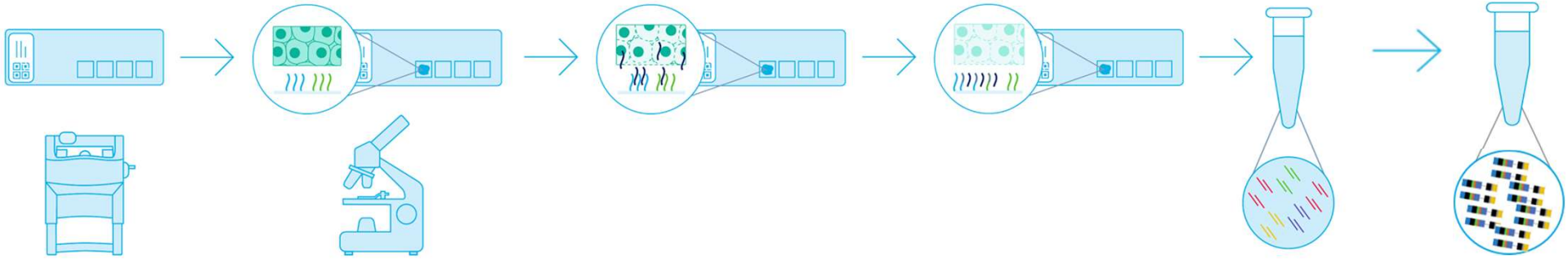
Visium Spatial Gene Expression

Complete in 1 Working Day with Standard Tools for Tissue Studies

Tissue Prep	Library Prep				
	Staining & Imaging	Permeabilization	cDNA Synthesis	cDNA Amplification	Library Construction



- *Section tissue*
- *Place on slide*

- *Fix Tissue*
- *H&E Stain*
- *Brightfield Image*

- *Barcoded Spot Capture*

- *Generate cDNA*
- *Denature cDNA*

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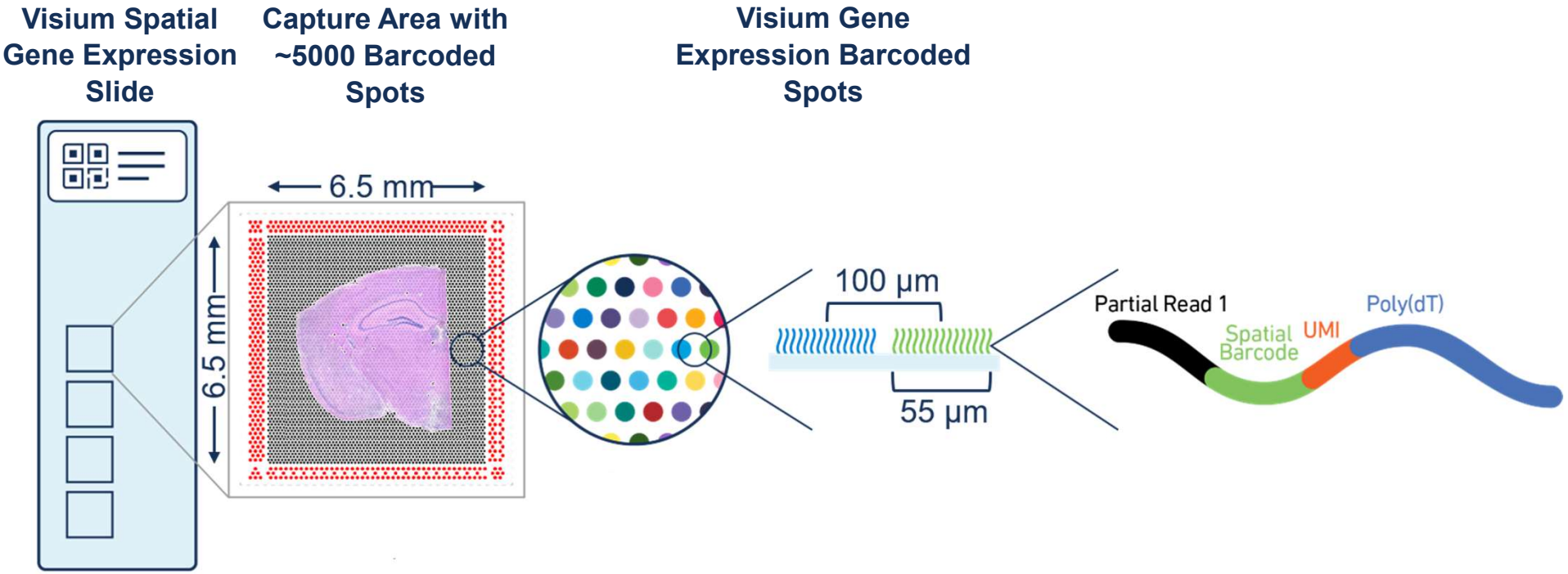
Unbiased Gene Expression at High Spatial Resolution

Utilizing Poly-A Capture and Unique Spatial Barcodes



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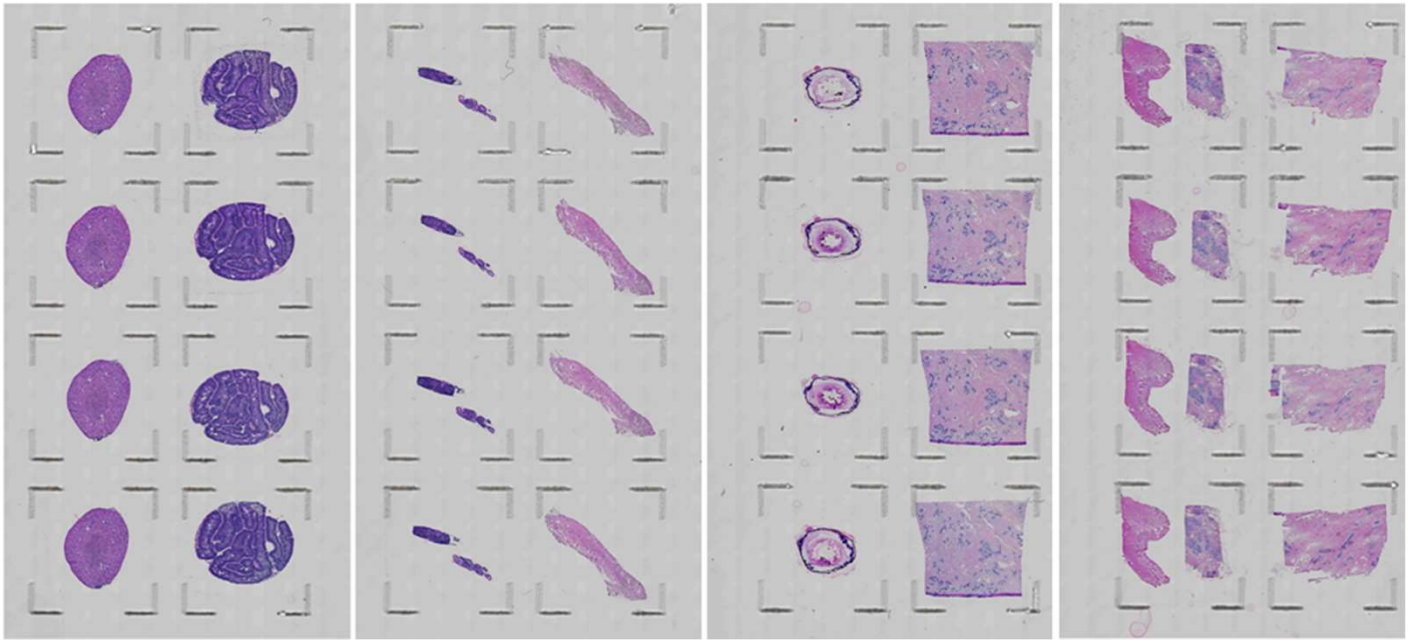
Tissues Tested

Most up to date list: https://support.10xgenomics.com/docs/Spatial_GEX_tissues



HUMAN

Heart, Kidney (Normal & Nephritis),
Ovary, Breast (Normal, Invasive Ductal Carcinoma, Invasive Lobular Carcinoma), Lymph Node (Normal
& Diseased)

MOUSE

Heart, Small & Large Intestine, Stomach, Liver, Kidney, Quadricep, Brain, Lung, Testes, Thyroid, Thymus, Eyes, Tongue, Spleen

RAT

Brain, Kidney, Heart

*Solid tumors for a number of these tissues have also been tested. List will change over time as we are continually testing new tissues. This is not an all inclusive list, if your tissue of interest is not on here it is likely compatible, but we have not tested.

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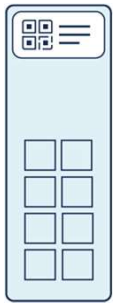
CF2

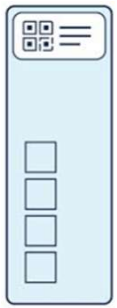
The Visium Spatial Gene Expression Solution

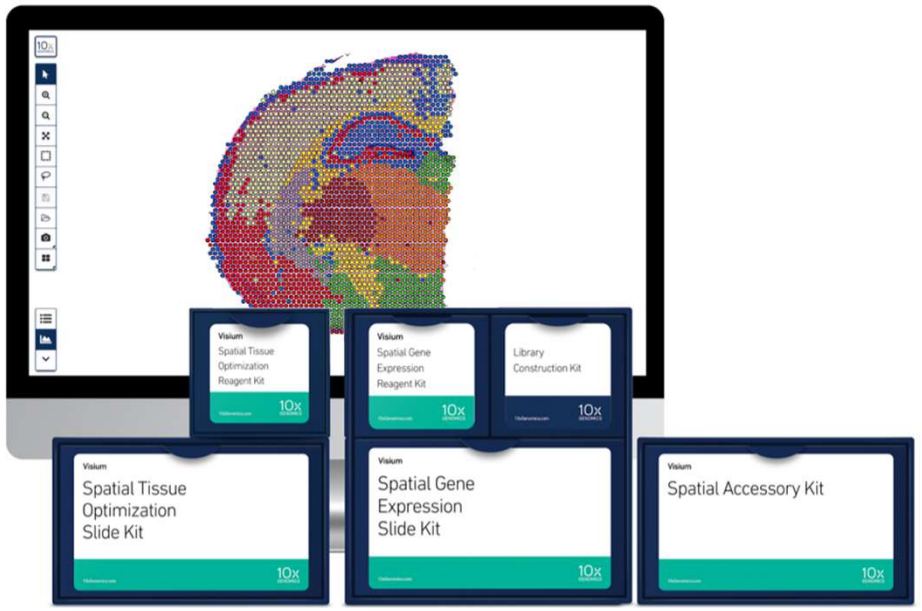
Tissue Optimization and Library Preparation Kits

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





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Slide 12

CF2 Christophe Fleury, 09/03/2020

Visium sensitivity and reproducibility

	Reads per spots	Sequencing Saturation	Number of genes detected
Mouse brain	50 000	41%	4500
Mouse kidney	50 000	48%	3600
Human Lymph Node	50 000	60%	4100
Human Breast Cancer	50 000	63%	4000

https://support.10xgenomics.com/spatial-gene-expression/datasets

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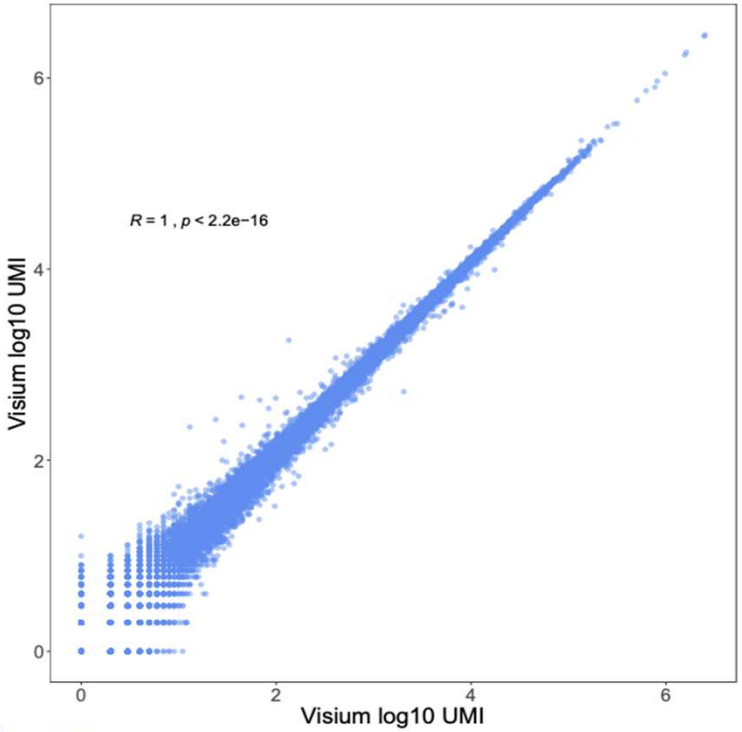
Visium Reproducibility

Pearson Correlation Plot - Mouse Brain Data

4 consecutive slices per week for 6 weeks



Visium Offers an Extremely Robust Workflow

- Repeatability amongst consecutive sections (replicates)
- Reproducible data at different times and days
- UMI and gene counts remain consistent based on Pearson correlation

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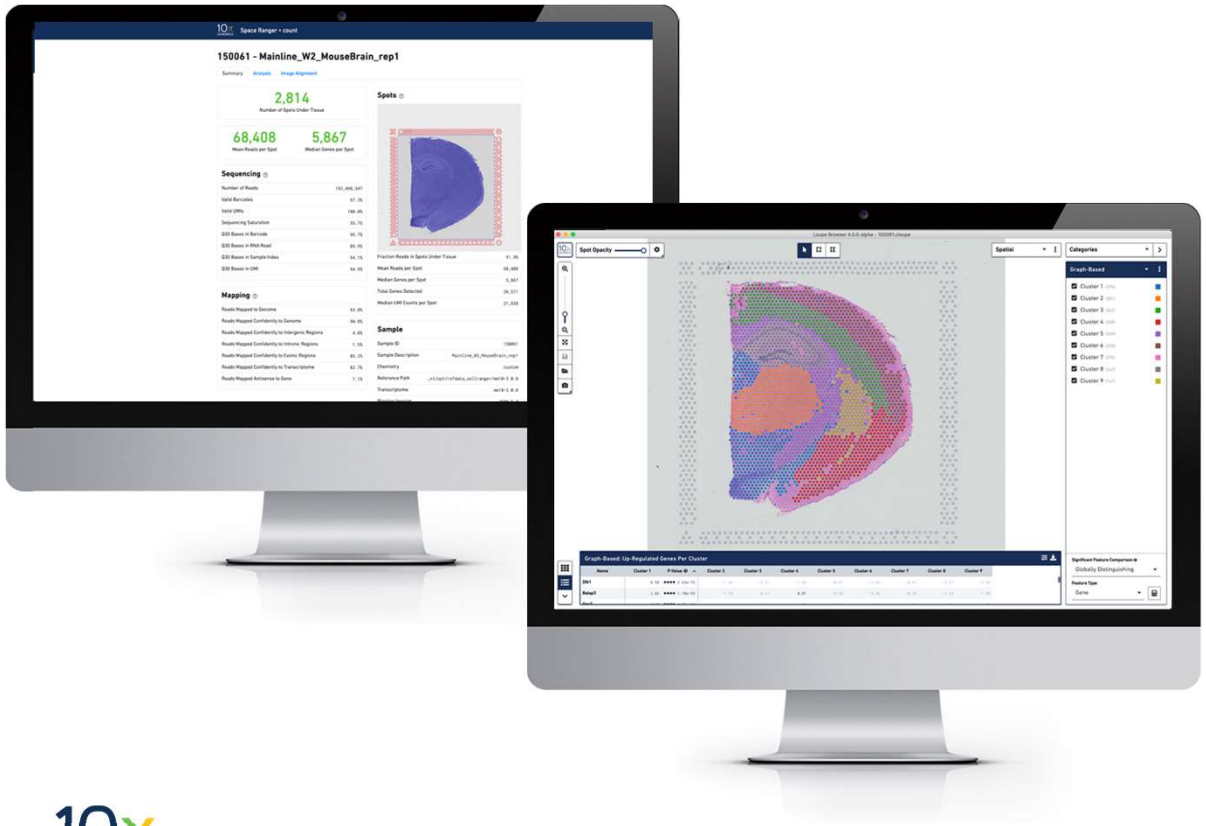
Automated Analysis

Space Ranger and Loupe Browser



- Freely available on support site
- **Space Ranger**
  - Automated data and image processing
  - Clustering analysis based on Gene Expression
  - Actionable QC Statistics
- **Loupe Browser**
  - View spot clusters and expressed genes in the context of the tissue image
  - Manual alignment
  - Interactive data visualization and exploration
  - Easily export and share results

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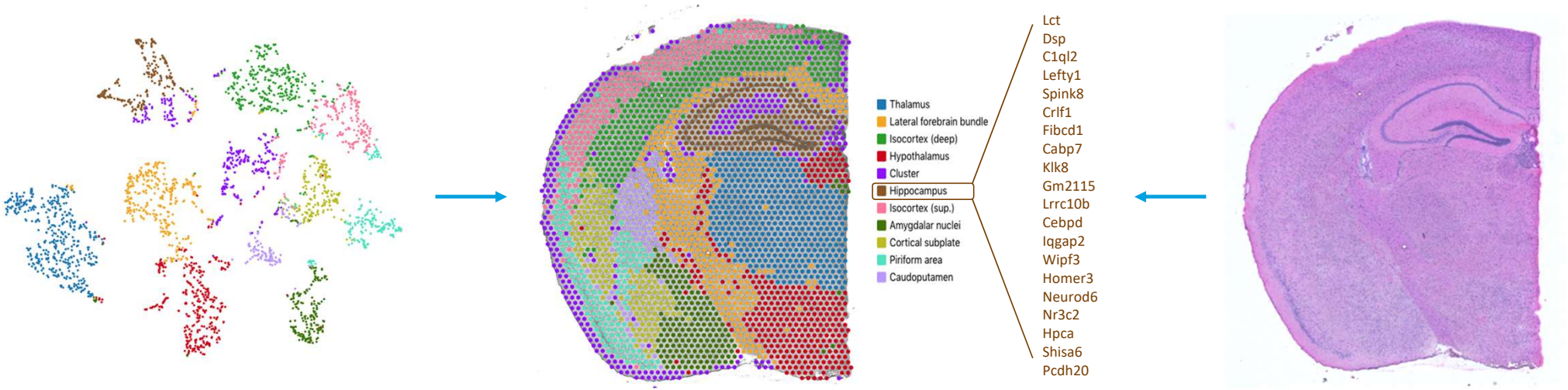
Cluster or Image Driven Analysis of Spatial Data

Start With the Gene Expression Data or microscopy images of the same section



Clustering/classification of glass slide spots based on their gene expression profile

Annotation directly on H&E image

Captured H&E image from the microscope

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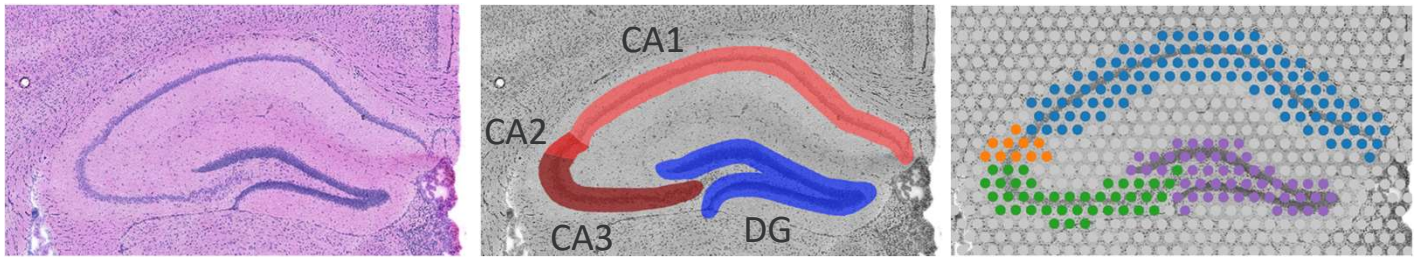
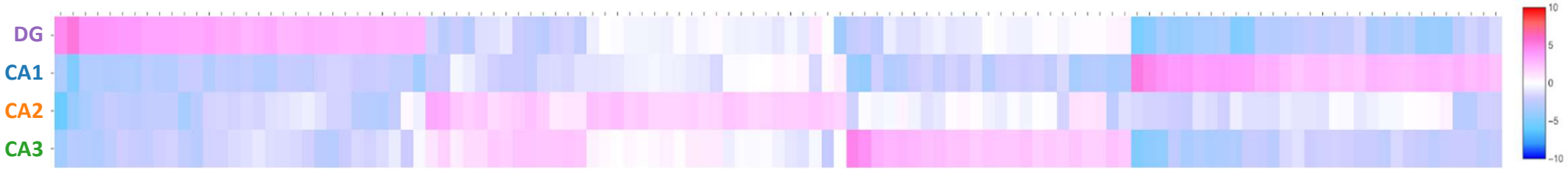
Spatially Resolved Expression in the Mouse Brain

Dissecting Gene Expression within the Hippocampus





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Applications

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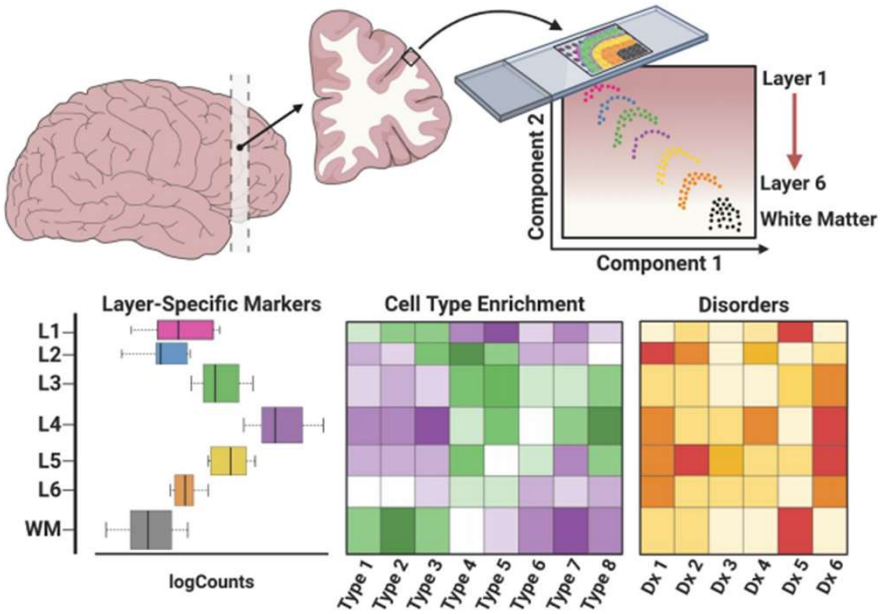
1st preprint in BiorXiv

Maynard et al. 2020 BiorXiv

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

Kristen R. Maynard[1,*], Leonardo Collado-Torres[1,2,*], Lukas M. Weber[3], Cedric Uytingco[4], Brianna K. Barry[1,5], Stephen R. Williams[4], Joseph L. Catallini II[3], Matthew N. Tran[1,6], Zachary Besich[1,6], Madhavi Tippani[1], Jennifer Chew[4], Yifeng Yin[4], Joel E. Kleinman[1,7], Thomas M. Hyde[1,7,8], Nikhil Rao[4], Stephanie C. Hicks[3], Keri Martinowich[1,5,7*], Andrew E. Jaffe[1,2,3,5,6,7,9,*]



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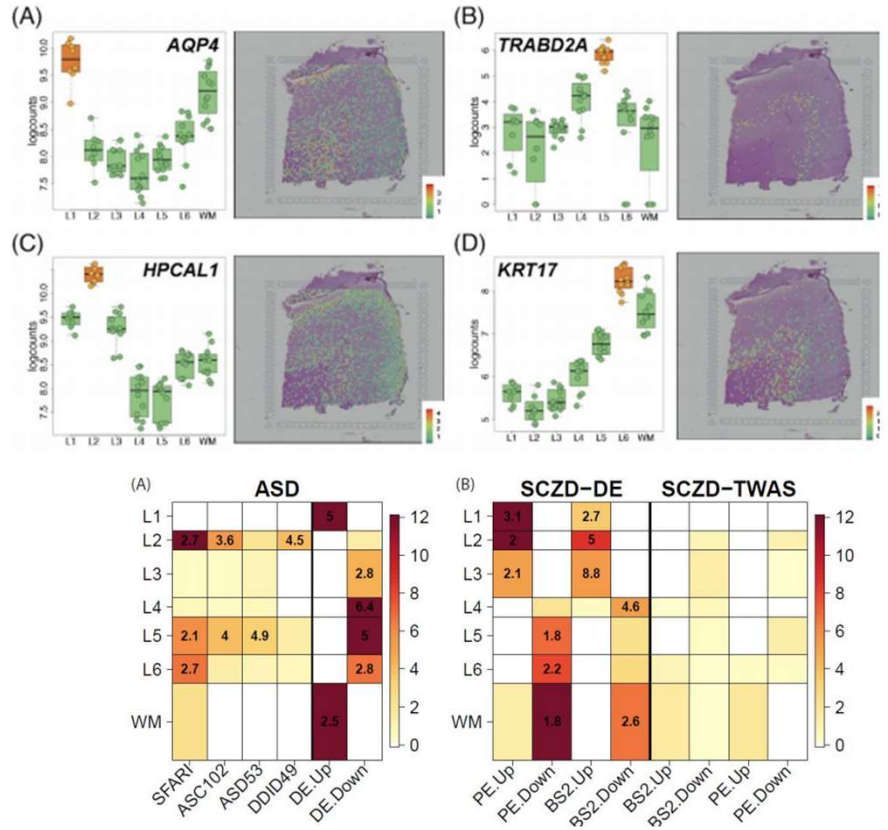
Why using spatial transcriptomics?

Maynard et al. 2020 BiorXiv

- Human neurons are large and fragile and may not be compatible with SnRNA-seq
- Post mortem tissues – SnRNAseq rather than ScRNA-seq
- Differences in pathology and gene expression associated with neuropsychiatric disorders are localized to specific cortical layers
- Topography of human brain dorsolateral prefrontal cortex crucial in some neuropsychiatric disorders



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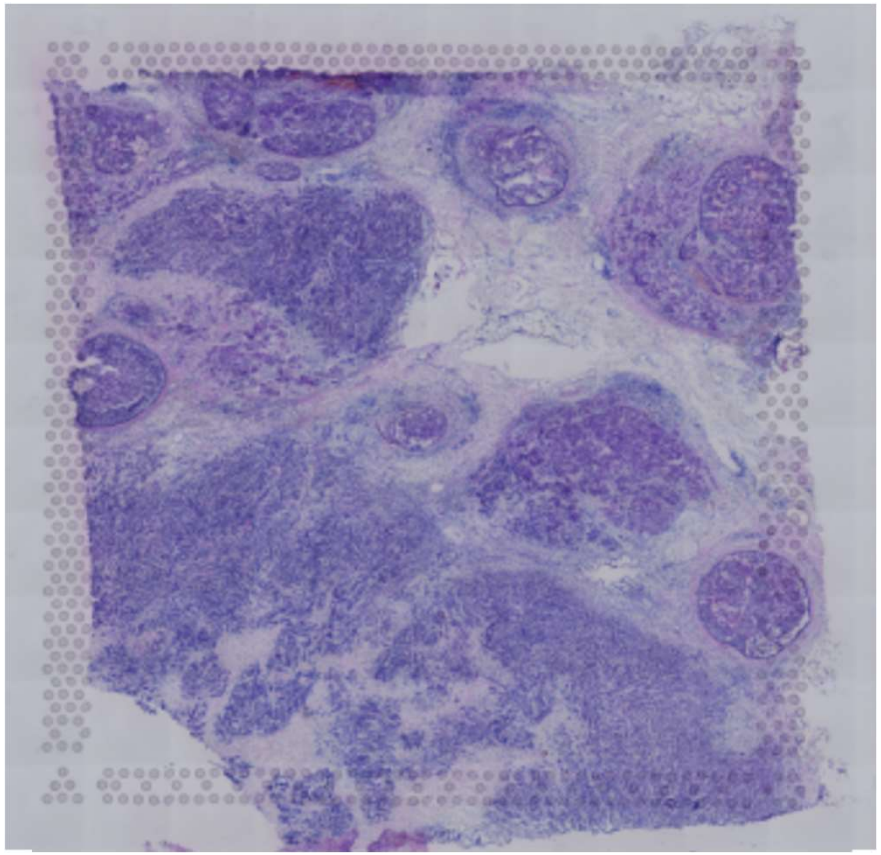
Pathologist Annotations Correlate with GeX Clusters



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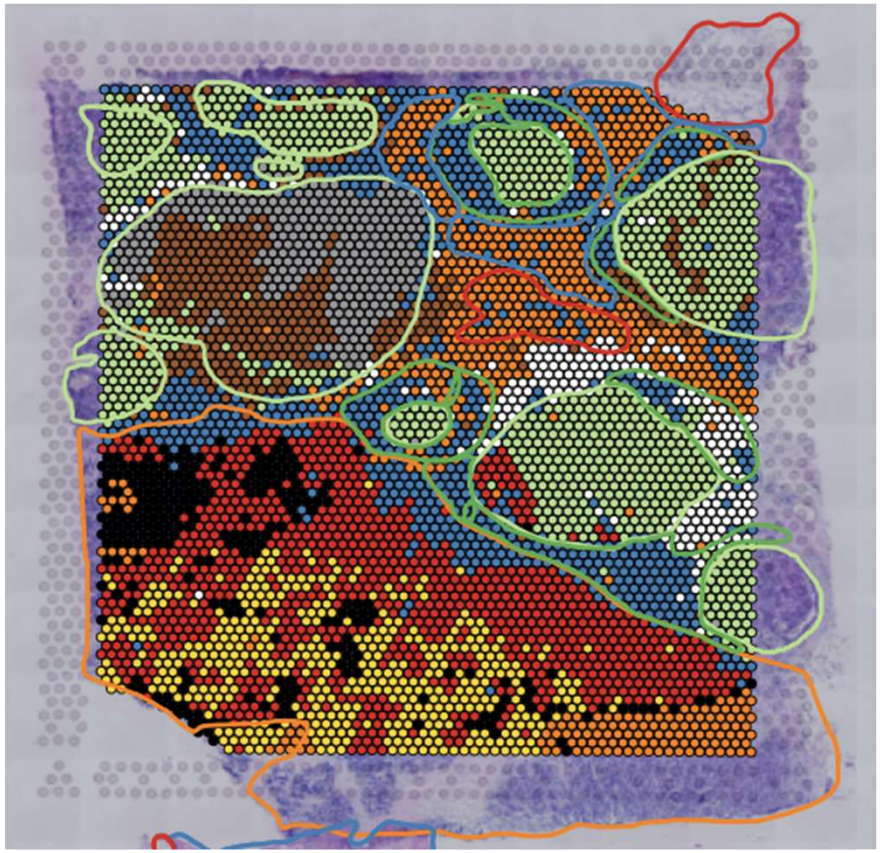
Pathologist Annotations Correlate with GeX Clusters



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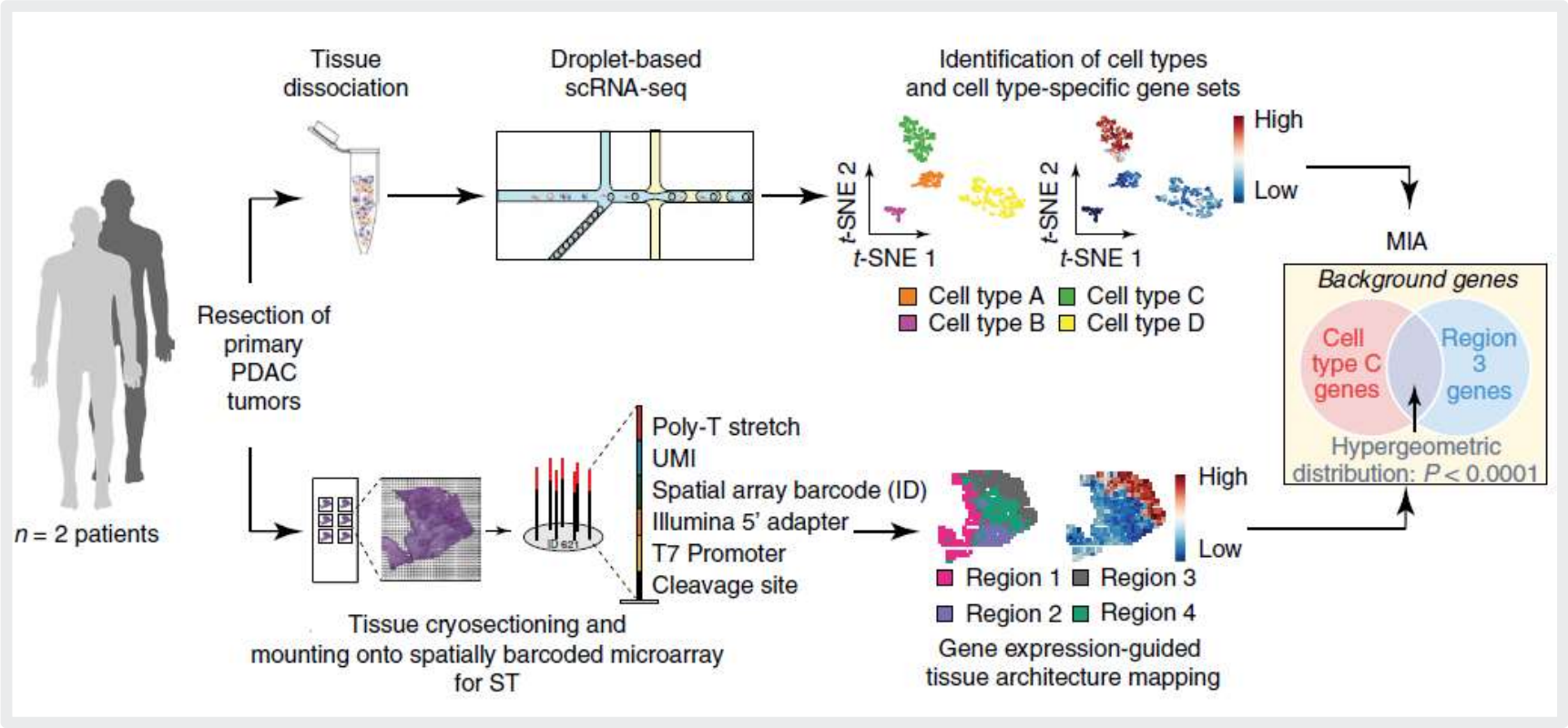
Integrated approach reveals tissue architecture in pancreatic ductal adenocarcinomas

Understanding tissue architecture



Moncada et. Al., 2020, Nature Biotechnology, https://doi.org/10.1038/s41587-019-0392-8

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The Visium Spatial Gene Expression Solution

Features

Sensitivity
Thousands of genes detected per spot

Efficient
1 day workflow time

Resolution
~5000 spots per capture area
Spot size 55 µm diameter
Close packing of spots; 100 µm c-t-c distance

All Inclusive
All slides and reagents included in kit

Unbiased
Assaying all mRNA with polyA capture technique

Cell Resolution
1-10 cells per spot; dependent on tissue type and thickness

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But… This is just the beginning

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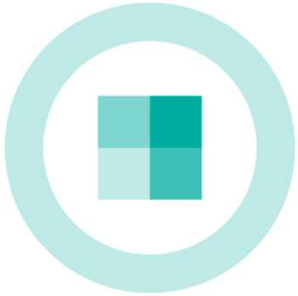
Visium Spatial Portfolio



Targeted Gene Expression

Immuno-fluorescence

Feature Barcode technology

FFPE Solution

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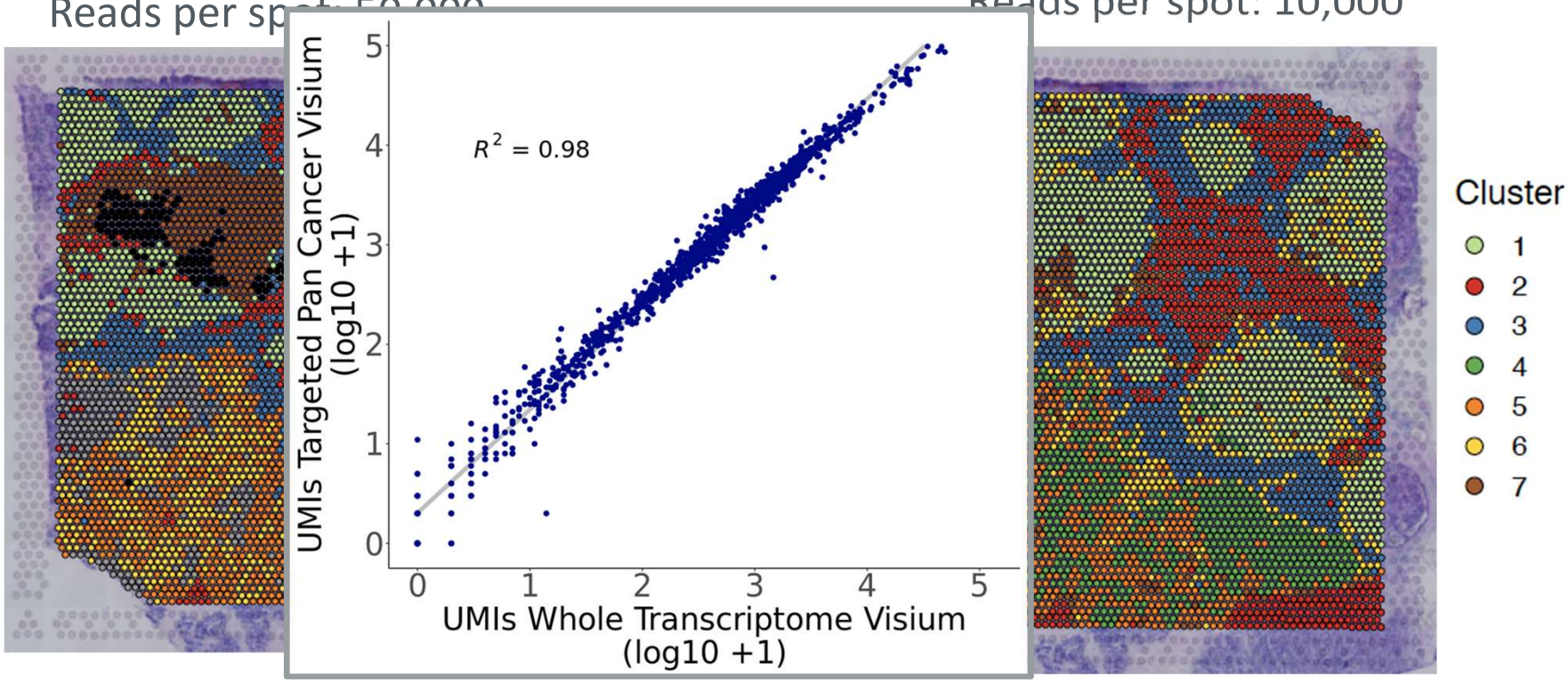
Targeted Gene Expression: Go Deep on What You Care About

Whole Transcriptome, Standard Visium

Reads per spot: 50,000



Pan-Cancer Panel

Reads per spot: 10,000

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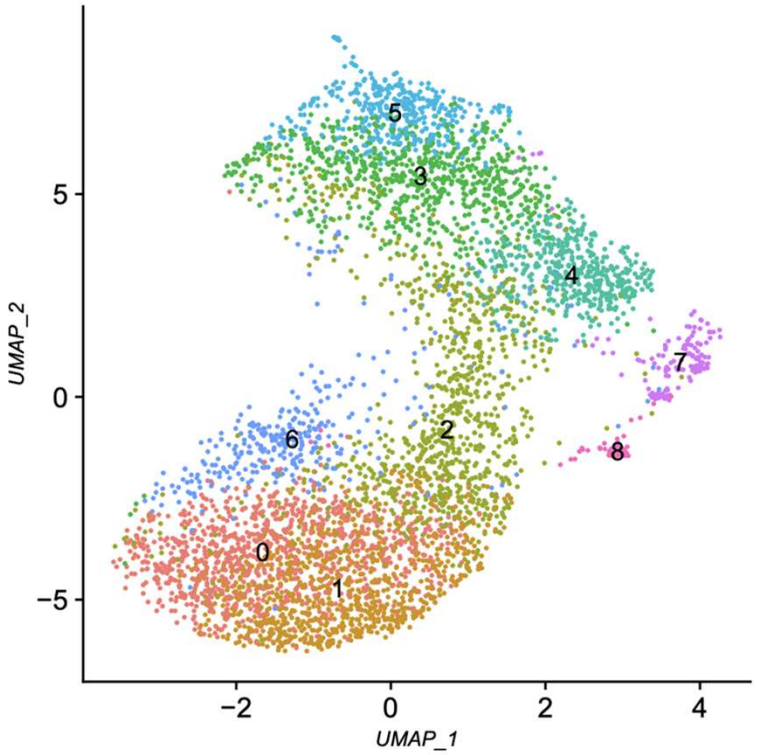
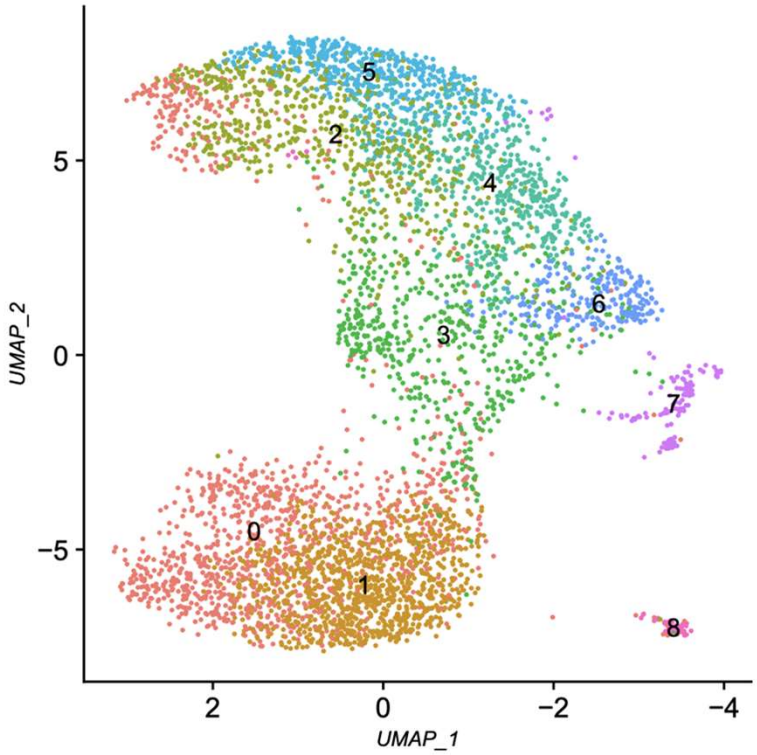
Targeted Gene Expression: Go Deep on What You Care About

Clustering on Pan-Cancer panel genes only

3’ Whole Transcriptome Visium



3’ Targeted Pan Cancer Visium



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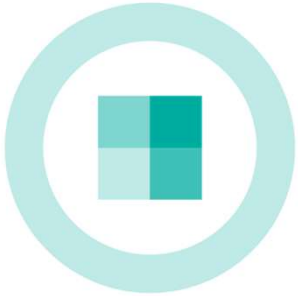
Visium Spatial Portfolio



Targeted Gene Expression

Immuno-fluorescence

Feature Barcode technology

FFPE Solution

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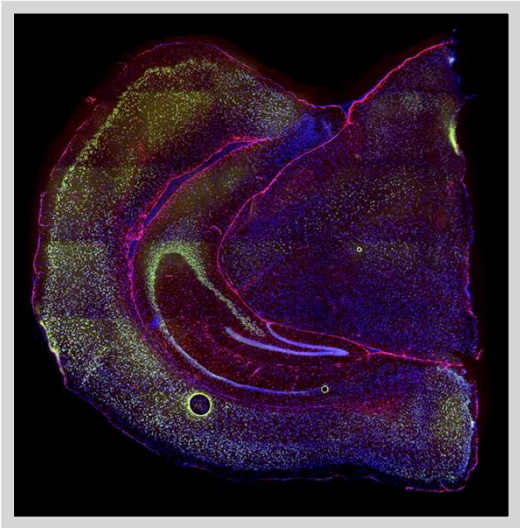
Visium: Immunofluorescence with Gene Expression

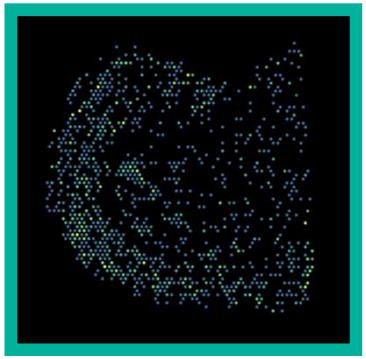
Immunofluorescence (IF)



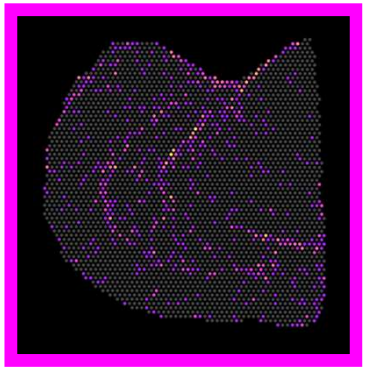
NeuN GFAP DAPI

+

Visium Gene Expression



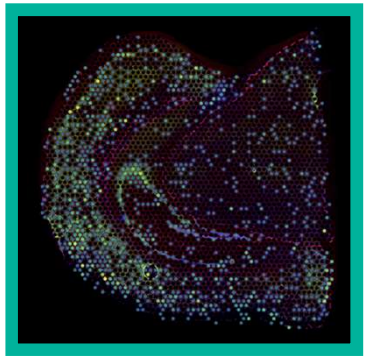
NeuN Selected from a transcriptome



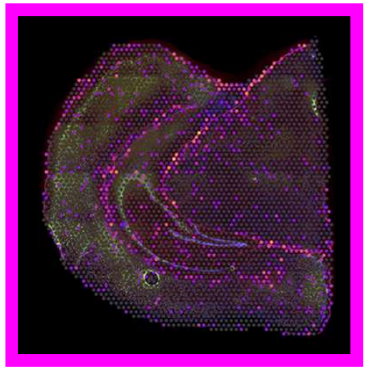
GFAP Selected from a transcriptome

=

IF + Visium Gene Expression



NeuN Merged



GFAP Merged

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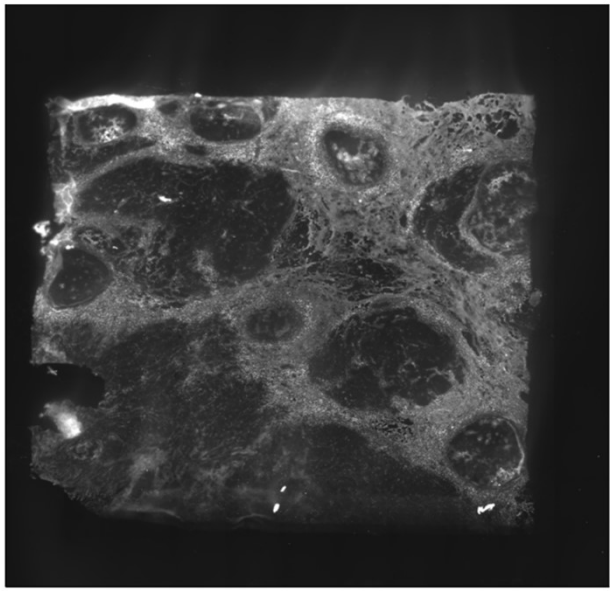
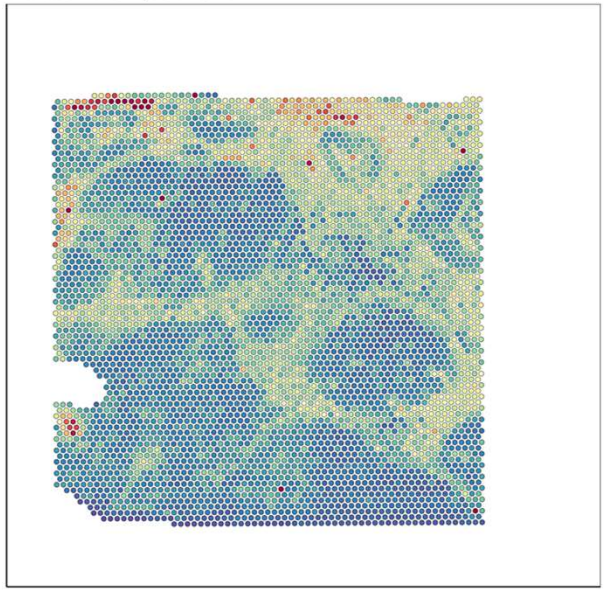
Merge Protein Data into “Spot Space” with mRNA

Match protein and mRNA expression to the exact spot

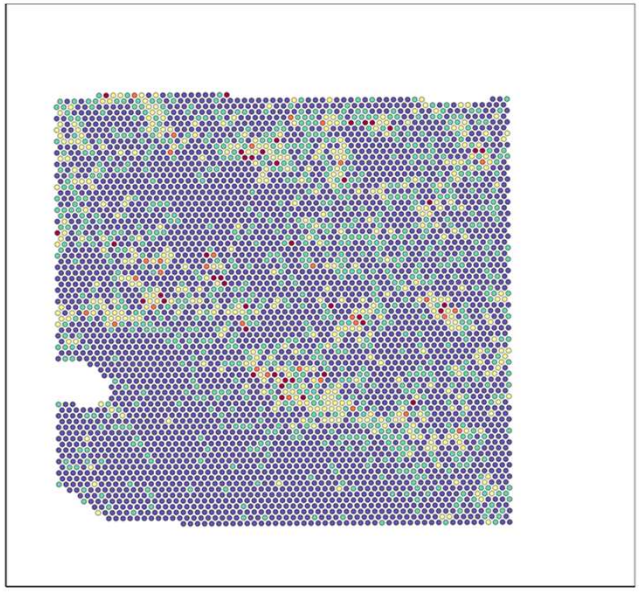
IDC: CD3 Channel



IDC: CD3 Protein Quantification



IDC: T-cell mRNA Quantification



Protein image only

Protein Intensity per spot

T-cell mRNA expression per spot

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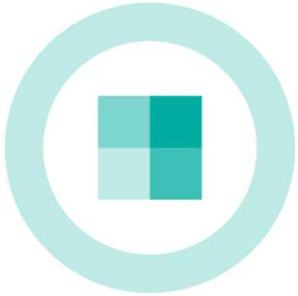
Visium Spatial Portfolio



Targeted Gene Expression

Immuno-fluorescence

Feature Barcode Technology

FFPE Solution

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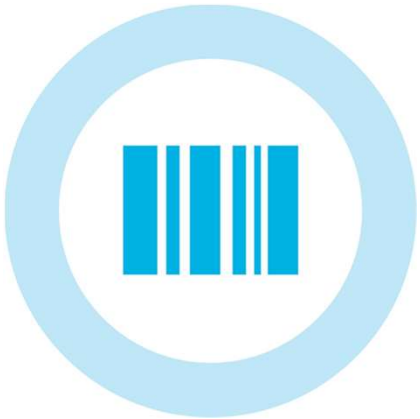
Highly Multiplexed Protein Analysis of Tissues

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

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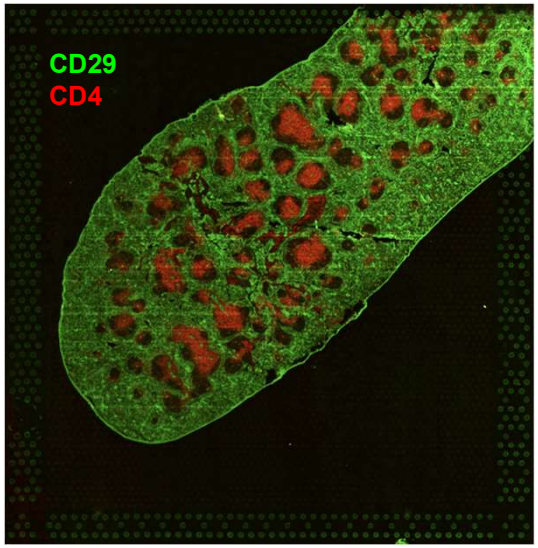
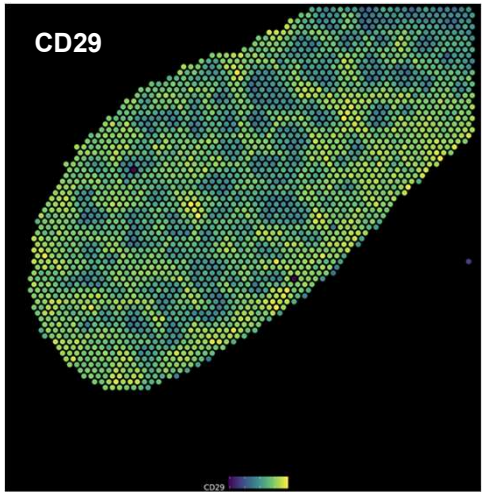
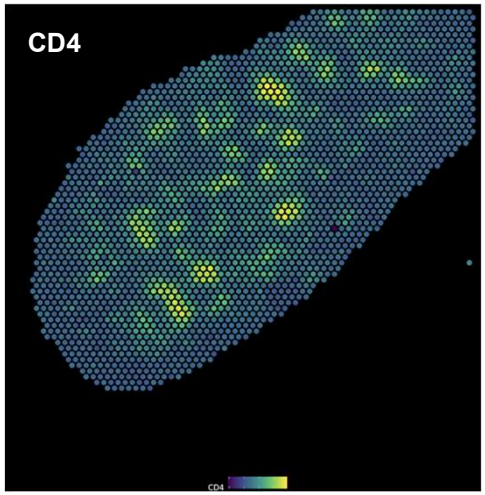
Feature Barcode Correlates with Immunofluorescence

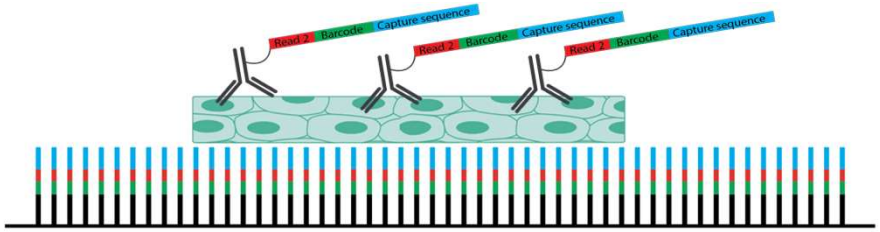
Immunofluorescence on Visium



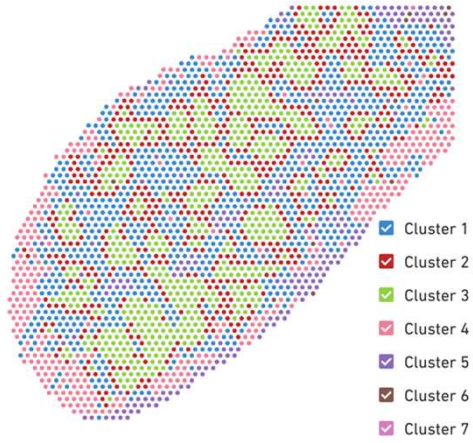
Feature Barcode Antibody-Barcode counts







Gene expression clustering



10x GENOMICS Compatible Products

BioLegend®

10x GENOMICS

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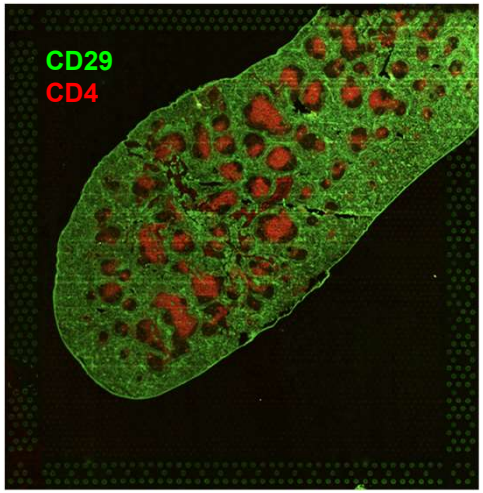
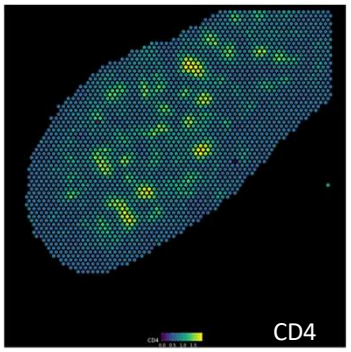
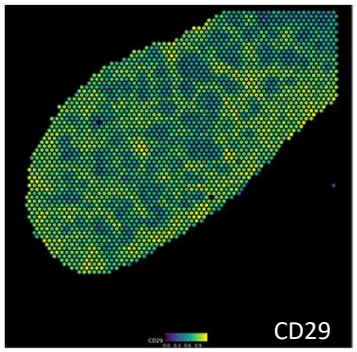
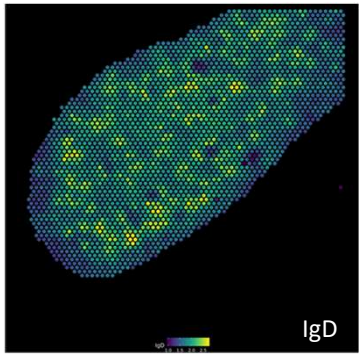
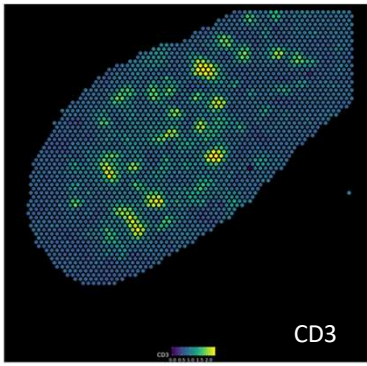
Immunofluorescence on Visium

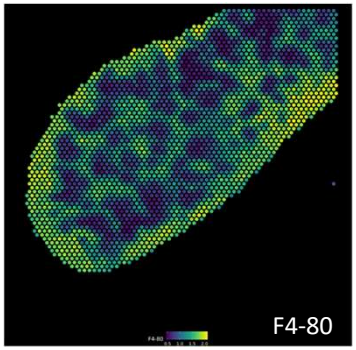


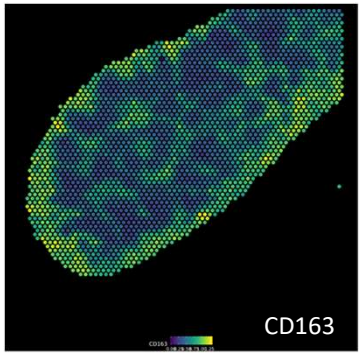


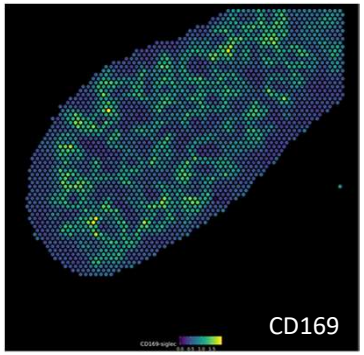


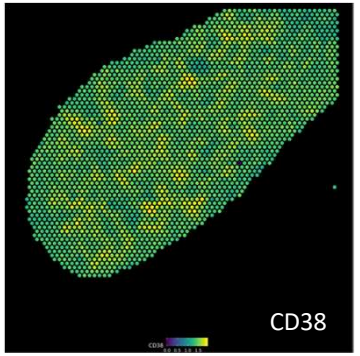


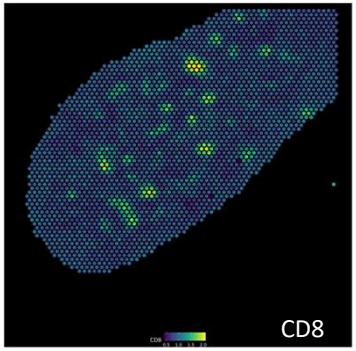


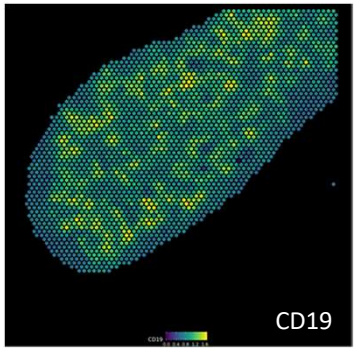


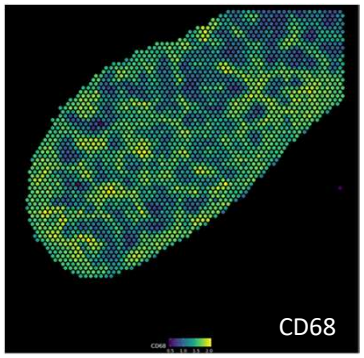


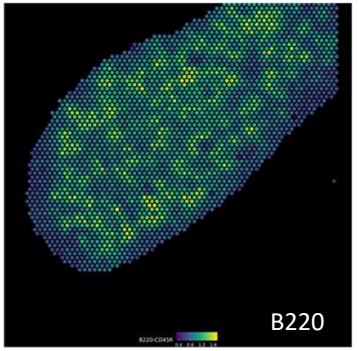












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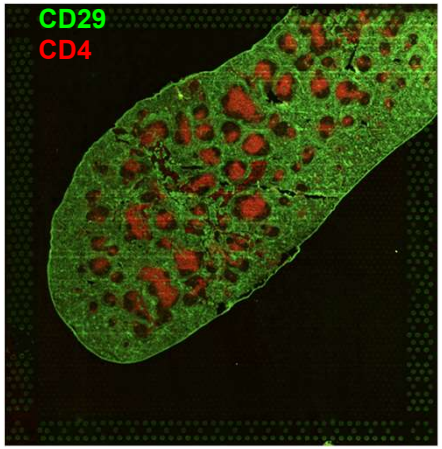
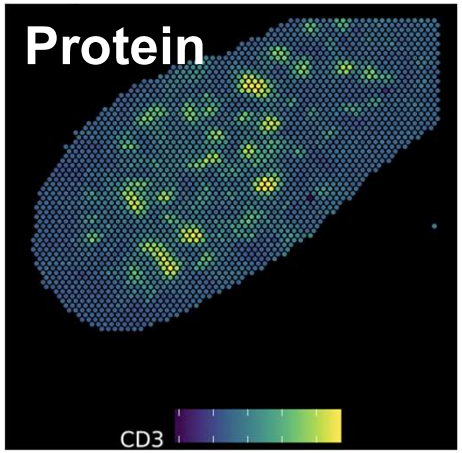
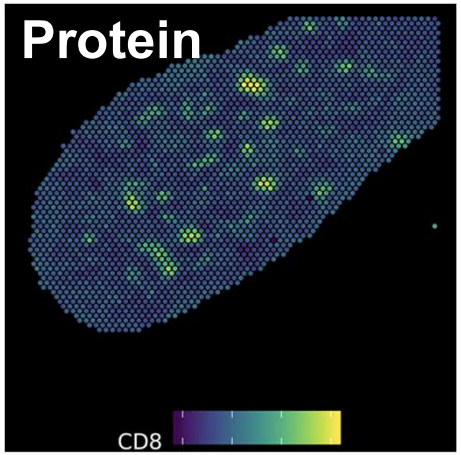
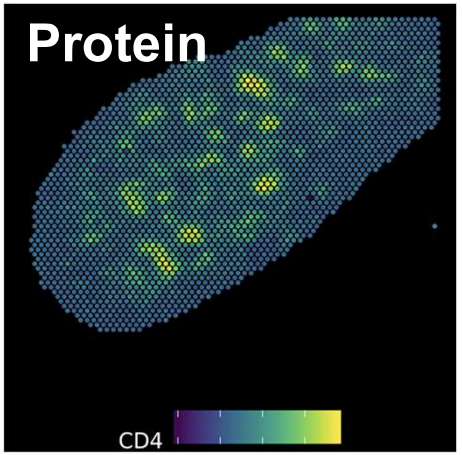
Immunofluorescence on Visium



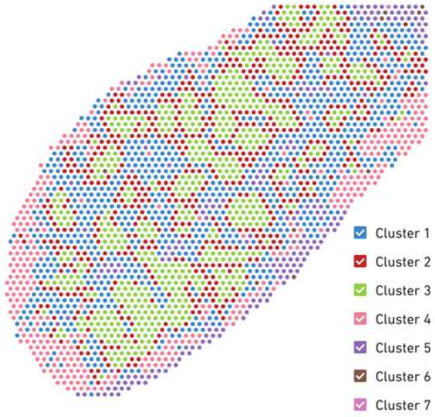
Feature Barcode

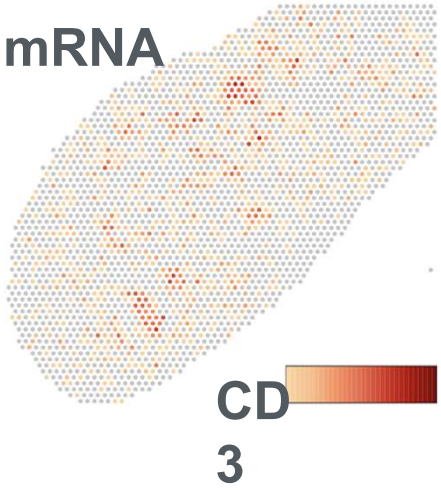


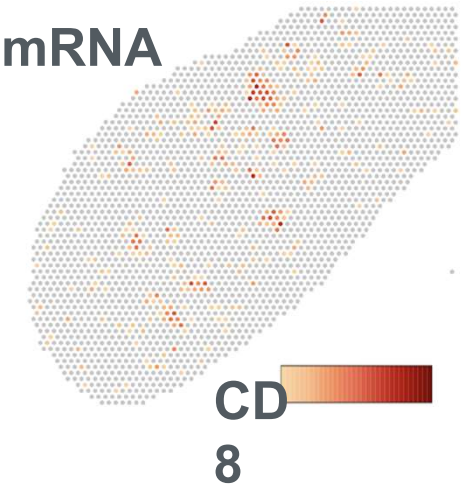


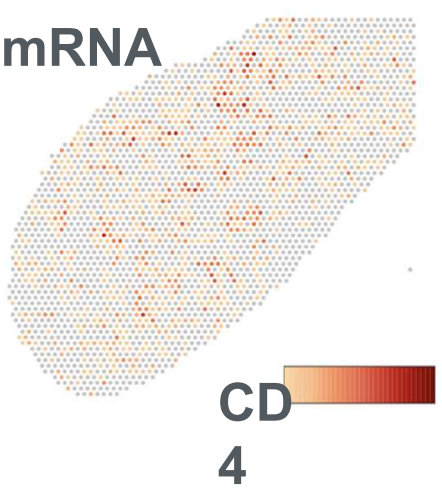


Gene expression clustering









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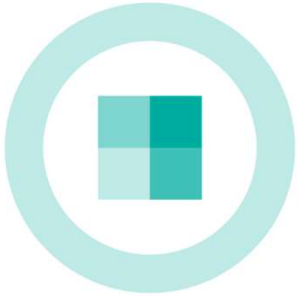
Visium Spatial Portfolio



Targeted Gene Expression

Immuno-fluorescence

Feature Barcode technology

FFPE Solution

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FFPE Enablement

- FFPE is the most commonly used preservation method
- RNA is difficult to recover due to fragmentation and crosslinking

10x FFPE protocol and reagent compatibility on current visium

A 10x solution dedicated for FFPE

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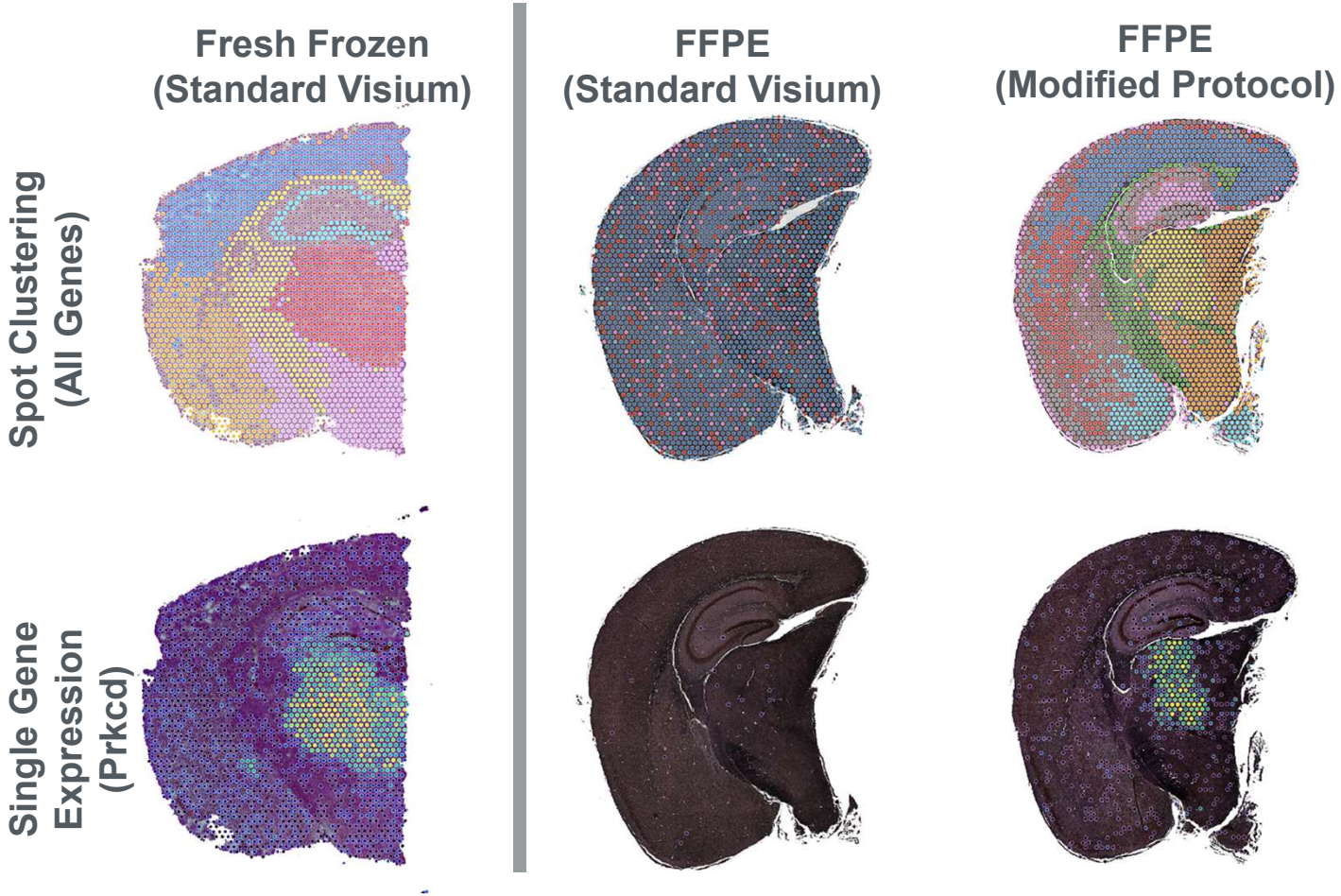
FFPE Enablement



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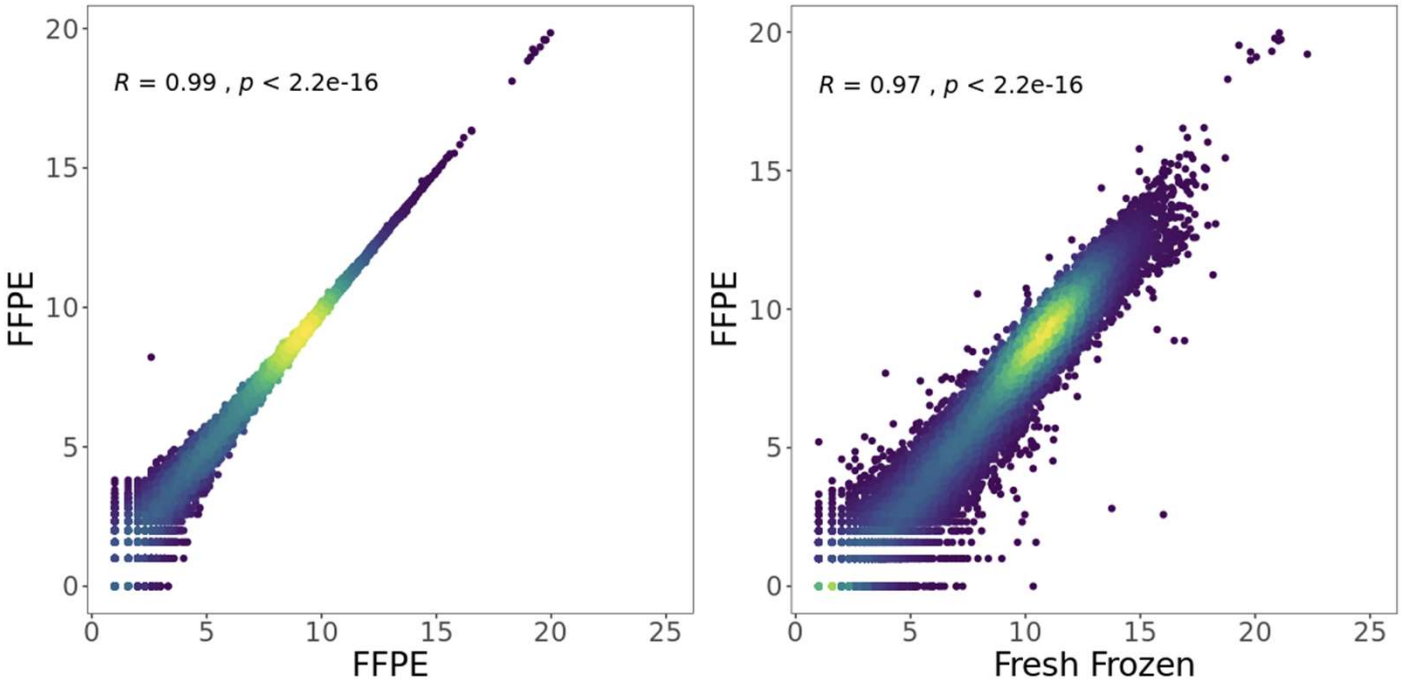
High Correlation Between FFPE and Fresh-Frozen



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Towards a Comprehensive View of Biology

Visium Spatial Assays

Gene Expression

Immunology

DNA

Epigenomics

Proteins

Targeting

Visium Platform

High Resolution

SC Resolution

Visium Samples

Fresh Frozen

FFPE

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Thank You! Questions?

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Science and technology advisor

10x Genomics Team

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Sales Executive

Bashir SADET
Field Applications Scientist

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