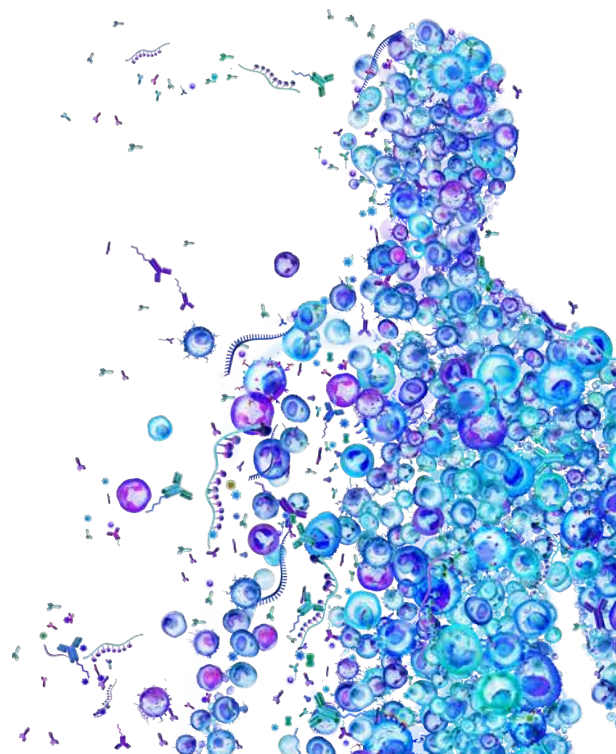


Biology at true resolution: Resolving biological complexities with single cell and spatial solutions

Daphne Cooper, PhD
Science & Technology Advisor, 10x Genomics
May 24, 2022
Portland Multiomics Discovery Symposium

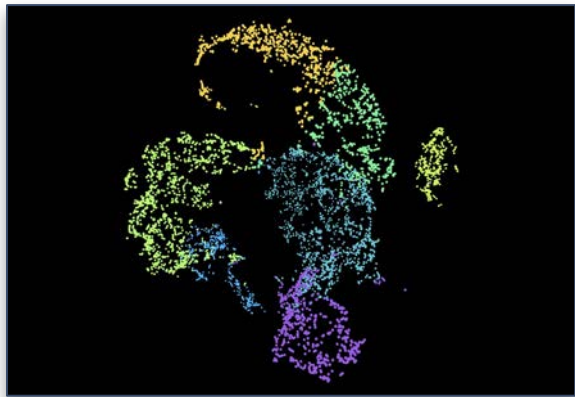
Biology is Complex



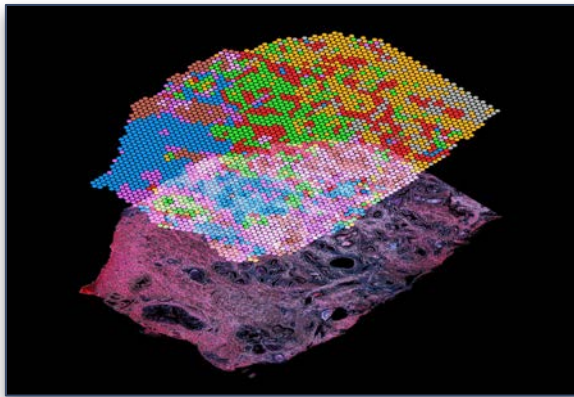
- Organisms are composed of diverse tissues and organs
- Tissues and organs are composed of organized, heterogeneous groups of distinct cell types
- Cell types and states are defined by gene expression and protein function
- Comprehensive and detailed analysis of cell types enables an understanding health and disease

Our Three Platforms

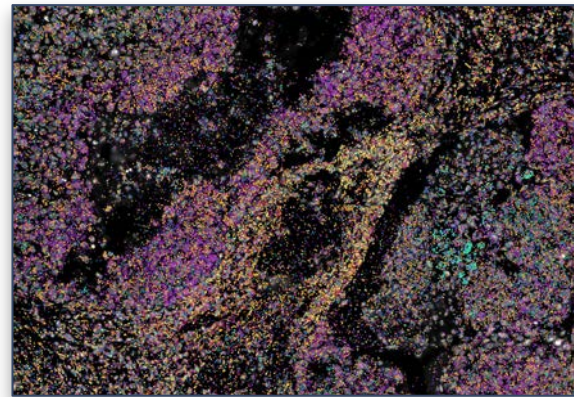
Chromium Single Cell



Visium Spatial



Xenium In Situ



Single Cell Gene Expression – Unbiased and scalable

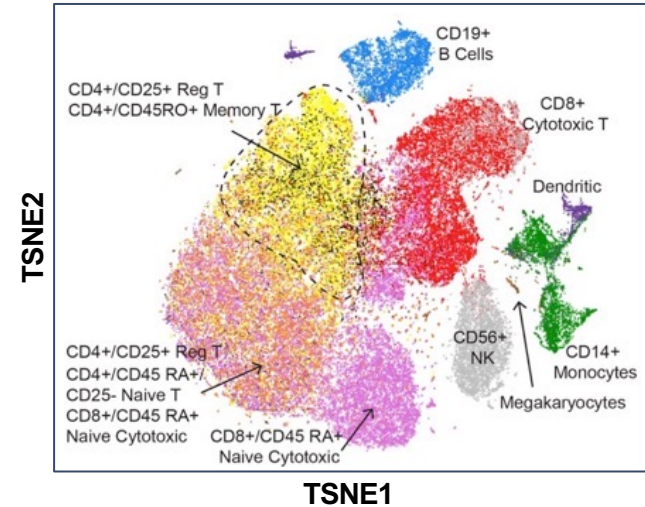
Conventional gene expression



One "average" data point from a mixture of cells



10x Genomics



Capture cells with:

- Diverse sizes
- Broad transcriptional activity
- Rare frequencies

Zheng et. al Nature Communications **volume8**, Article number: 14049 (2017)

Chromium Single Cell Gene Expression with Feature Barcode technology

Input



User-supplied
cells or nuclei

Library Creation



**Chromium X
and Next GEM Single Cell 3' HT Reagents**

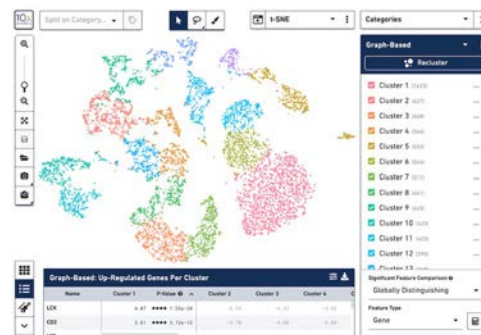
- Chromium Next GEM Single Cell 3' HT Kit v3.1
- Chromium Next GEM Chip M Single Cell Kit
- Dual Index Kit TT Set A

Sequence



Illumina sequencer
compatibility

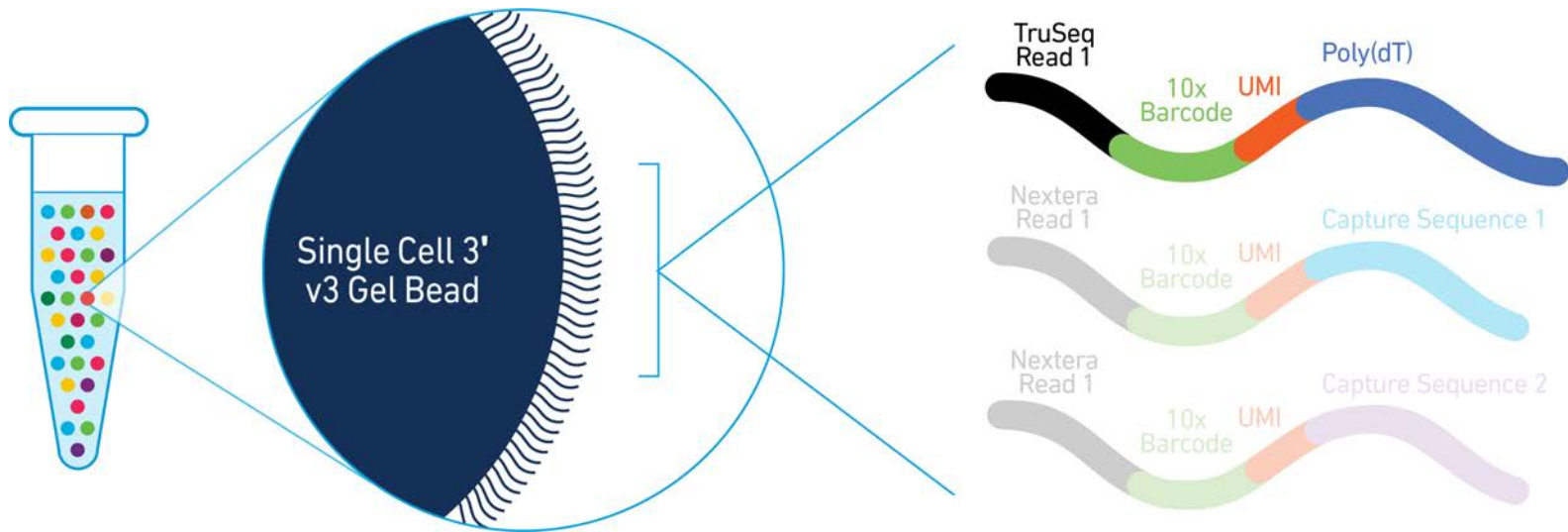
Data Analysis and Visualization



**Cell Ranger
Loupe Browser
Cloud Analysis (US-only)**

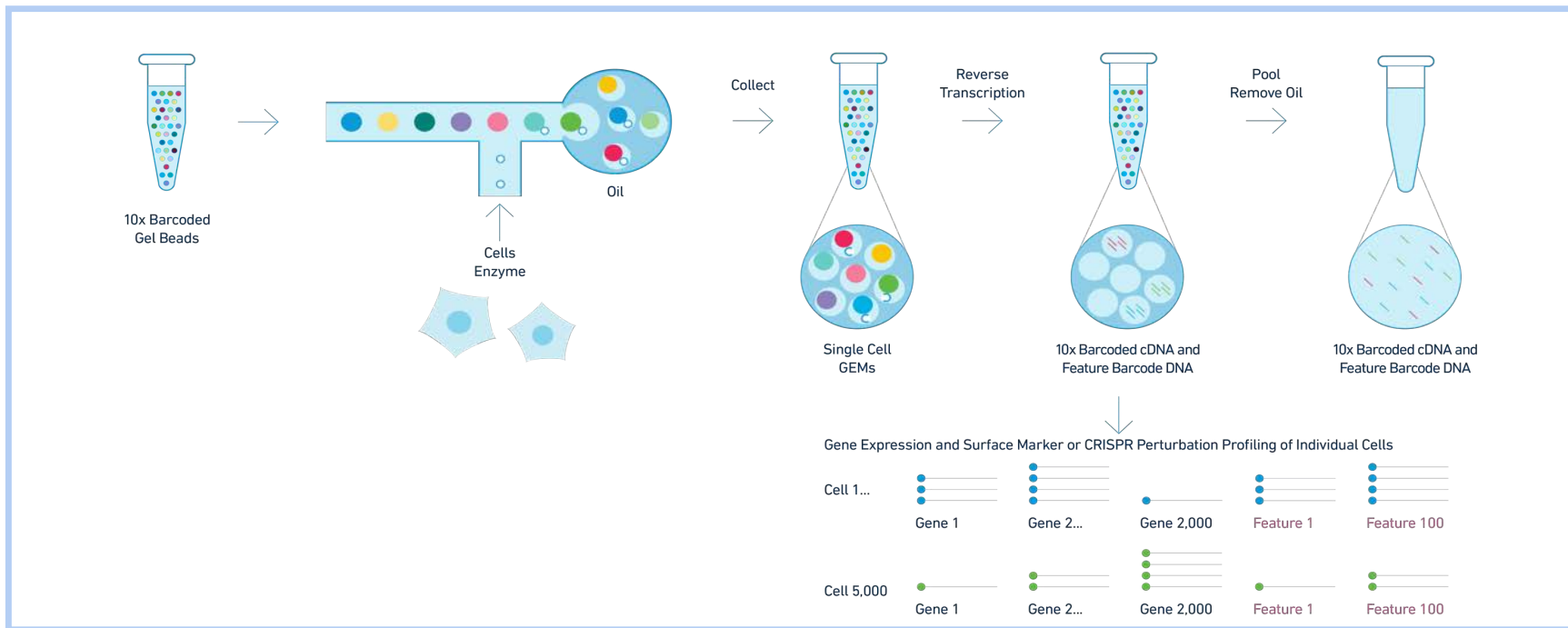
10x Genomics Single Cell Technology

Barcoding single cell transcriptomes and other readouts



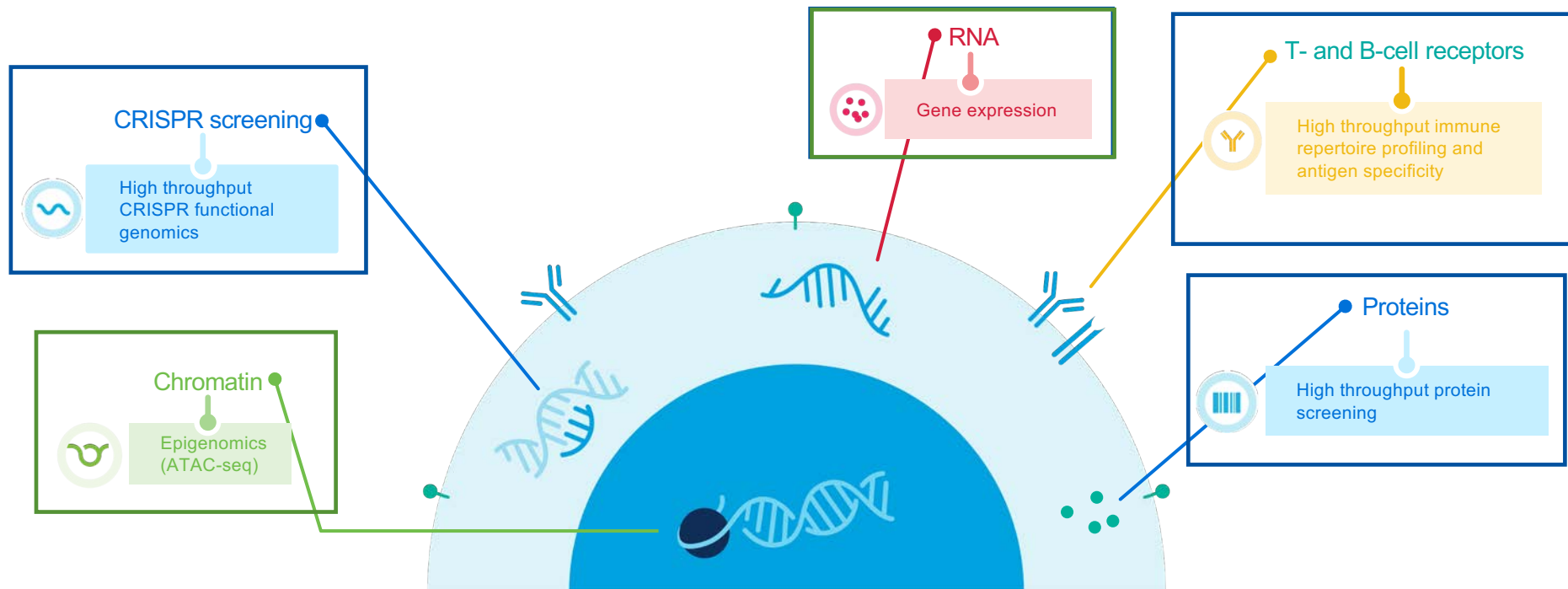
Library creation process

Workflow overview

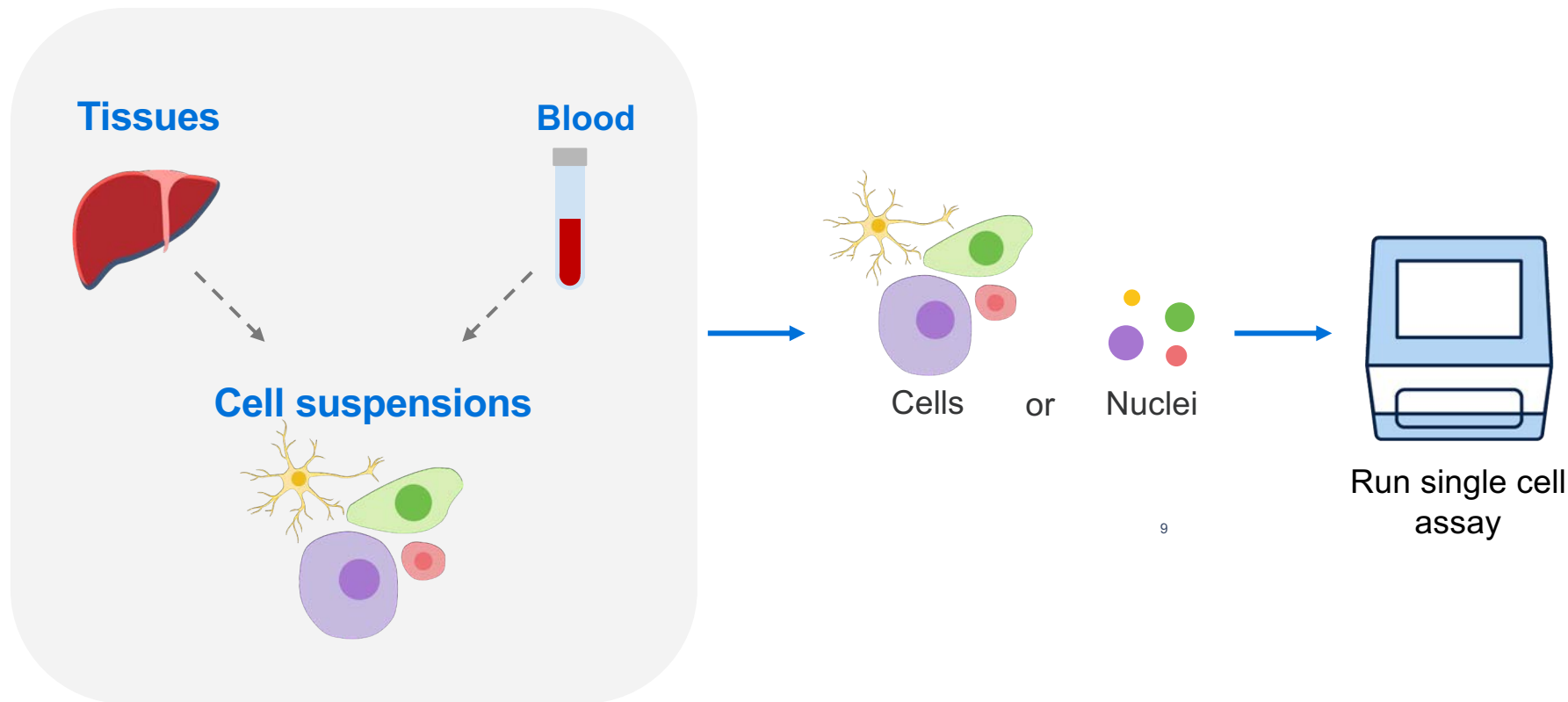


Single cell multiomic cytometry

Simultaneous measurements of different cellular analytes

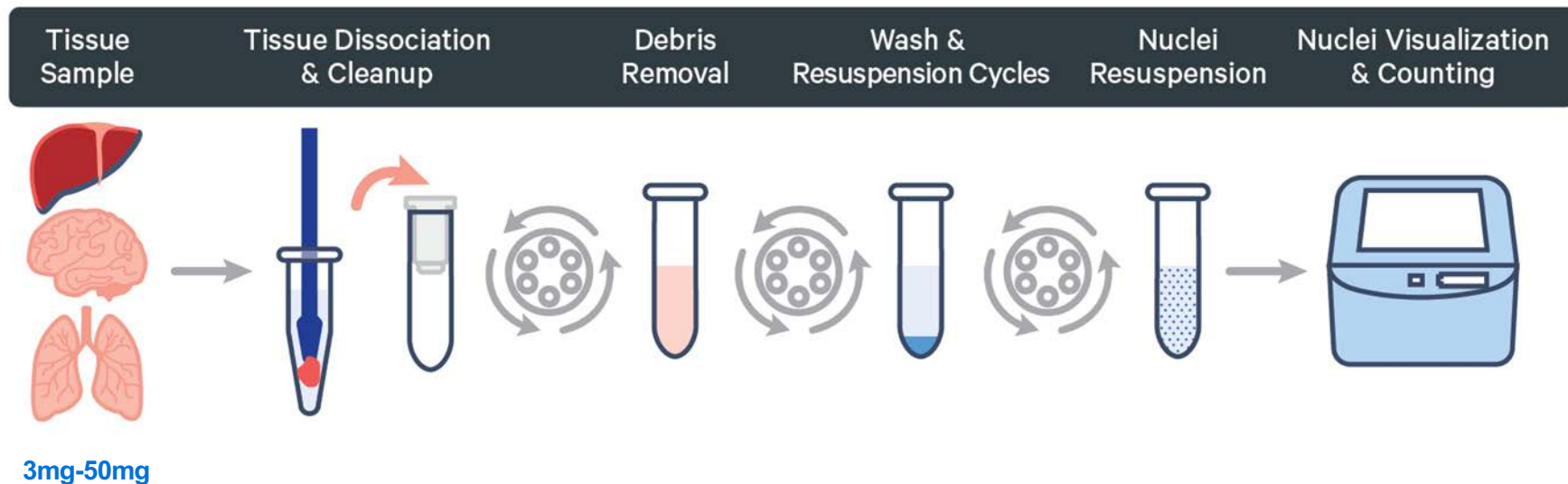


Chromium Single Cell sample inputs



Chromium Nuclei Isolation Kit

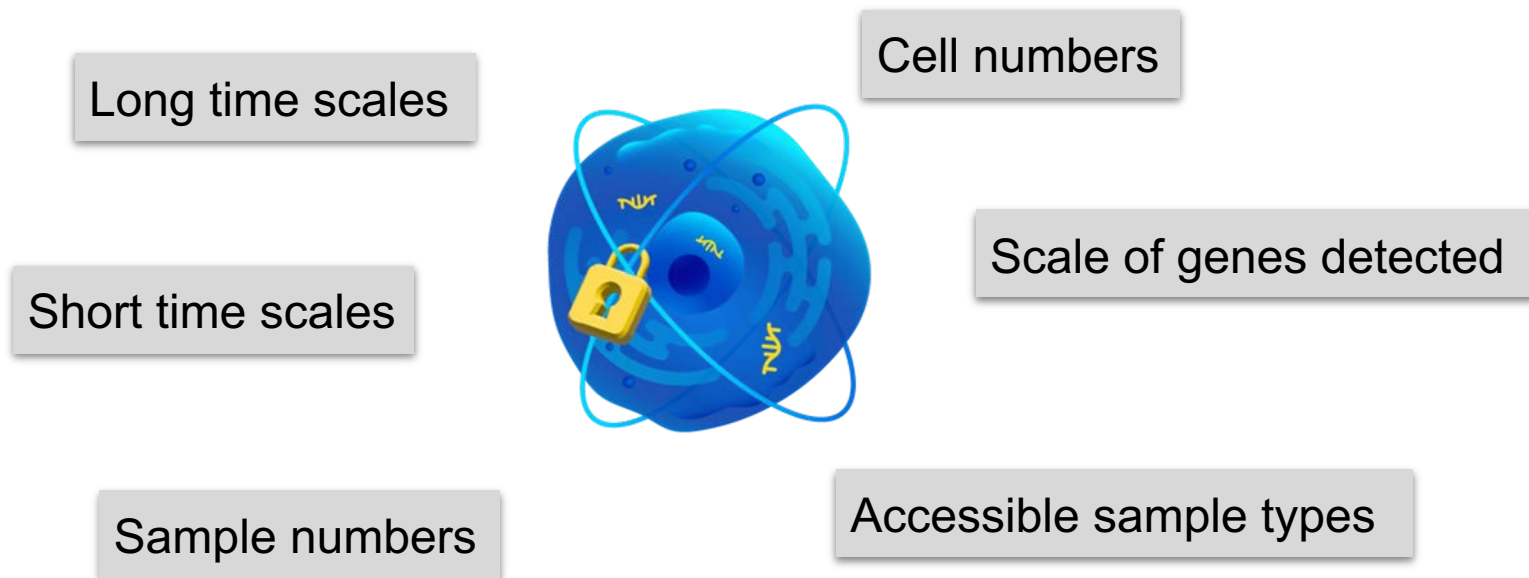
Streamlined sample preparation workflow



All you need is an hour of lab time, a benchtop centrifuge, and an interesting frozen sample!

The Next Generation Single Cell Assay

Enabling a new scale of experiments



Single Cell Fixed RNA Profiling

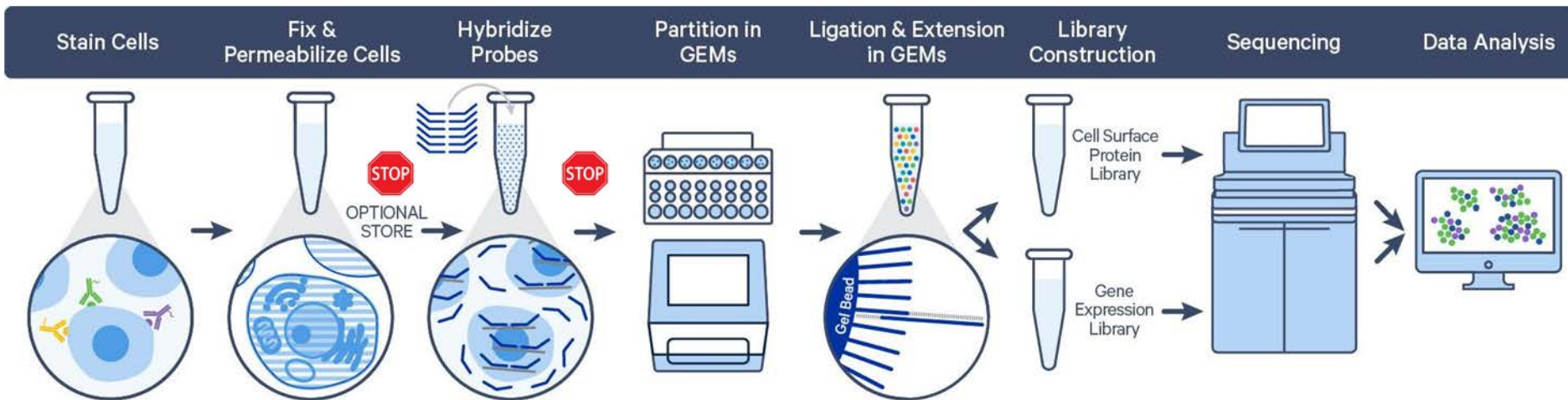
Unlock constraints from single cell workflows



- Runs on Chromium X series of instruments with new Chip Q
- Recover 500-10,000 cells per channel in singleplex format
- Maximize cell and sample throughput: multiplex up to 16 samples and 128,000 cells in a single channel
- Compatible with Feature Barcode Technology for Cell Surface Protein in singleplex format

Chromium Fixed RNA Profiling Workflow

Singleplex workflow overview - compatible with Feature Barcode Technology



*optional: Cell
Surface
Protein
Labeling prior
to fixation

- Each sample has a probe barcode.
- No multiplexing

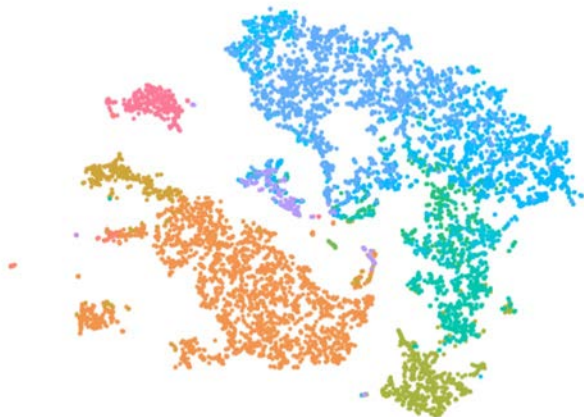
STOP **Optional Sample Prep storage:**

- Validated stopping points during sample preparation - Post-Fix and Post-Hyb

Cell Surface Protein with Feature Barcode Technology

Generate multiomic data at single cell resolution without time constraints

Gene Expression



Surface Protein Expression



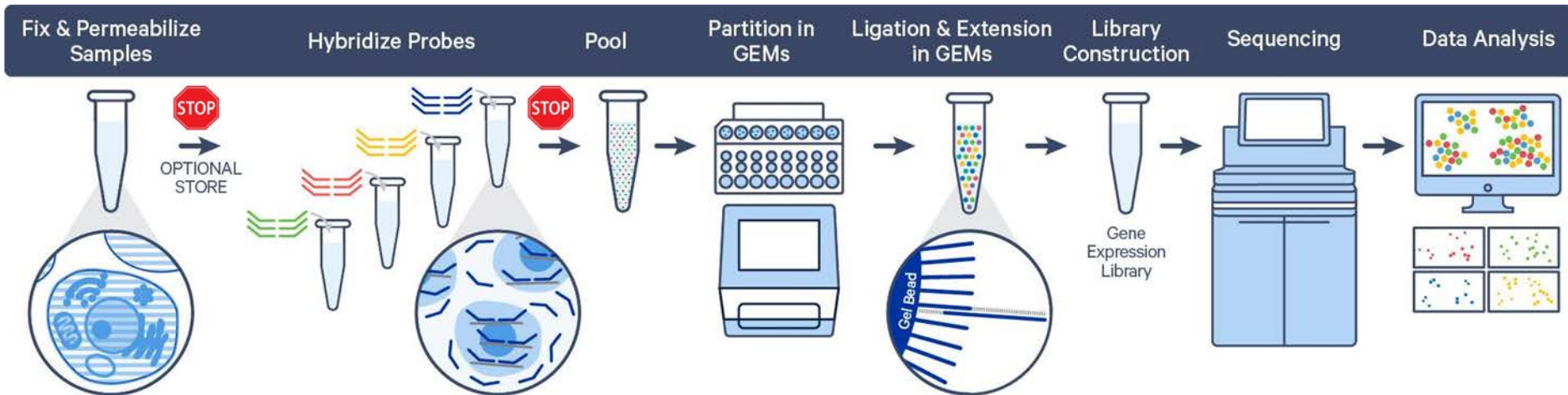
Human PBMCs stained with BioLegend TotalSeq B antibodies before fixation

- B cells
- Dendritic cells
- CD14 monocytes
- CD16 monocytes
- NK cells
- Platelets
- Double-negative T cells
- Effector T cells
- Memory T cells
- Naïve T cells
- Memory helper T cells
- Naïve helper T cells
- Undetermined

Simultaneous analysis of cell surface protein expression is compatible with running Fixed RNA Profiling in singleplex format

Chromium Fixed RNA Profiling Workflow

Multiplex workflow overview

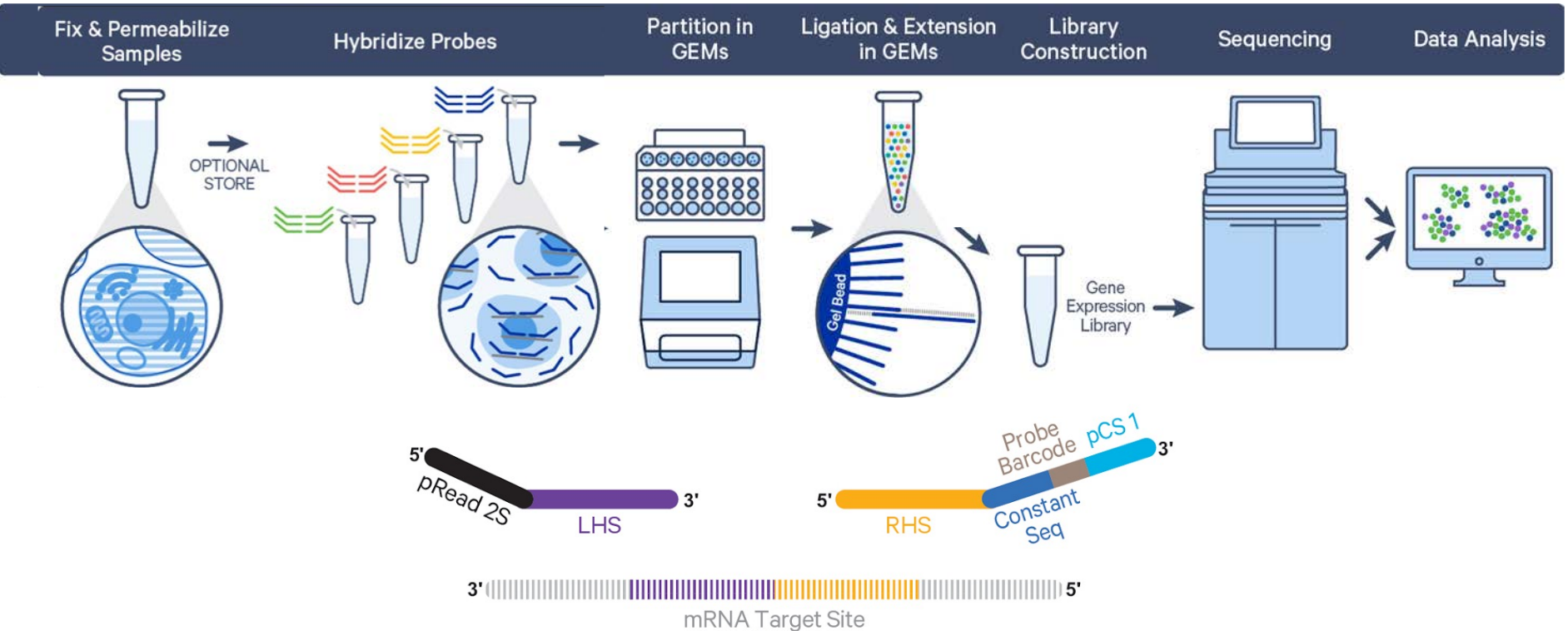


- Multiplex up to 4 or 16 samples.
- Each sample has a unique probe barcode.

STOP Optional Sample Prep storage:

- Validated stopping points during sample preparation - Post-Fix and Post-Hyb

Similar Streamlined Workflow for Singleplex & Multiplex FRP



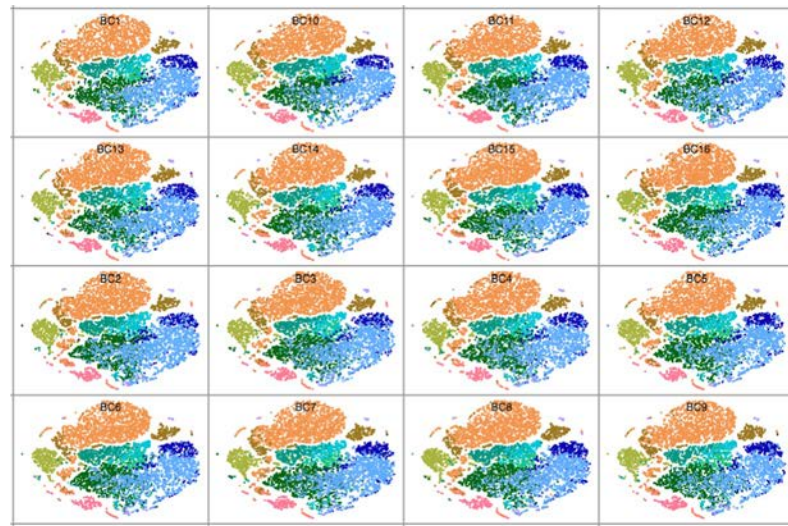
Multiplexed Data from a Single Channel

Can be used to maximize unique samples or cells loaded



80,000 PBMCs from a single donor,
split into 16 pools and overlaid

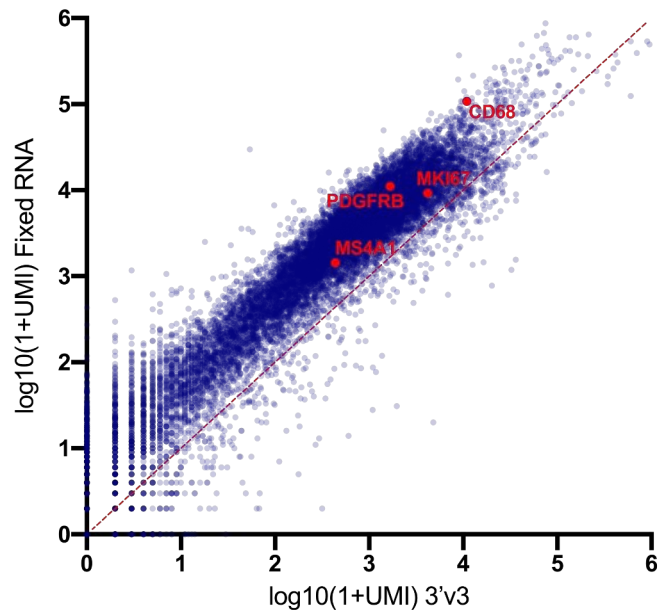
- B cells
- Dendritic cells
- CD14 monocytes
- CD16 monocytes
- NK cells
- Platelets
- Double-negative T cells
- Effector T cells
- Memory T cells
- Naive T cells
- Memory helper T cells
- Naive helper T cells
- Undetermined



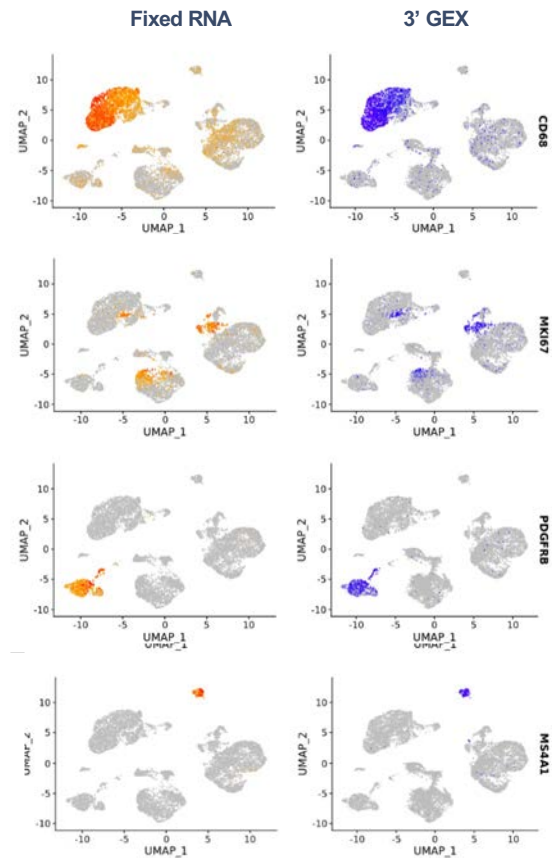
Sample sample, split by
probe barcode

Fixed RNA Profiling

Data highly correlated with 3' GEX assay

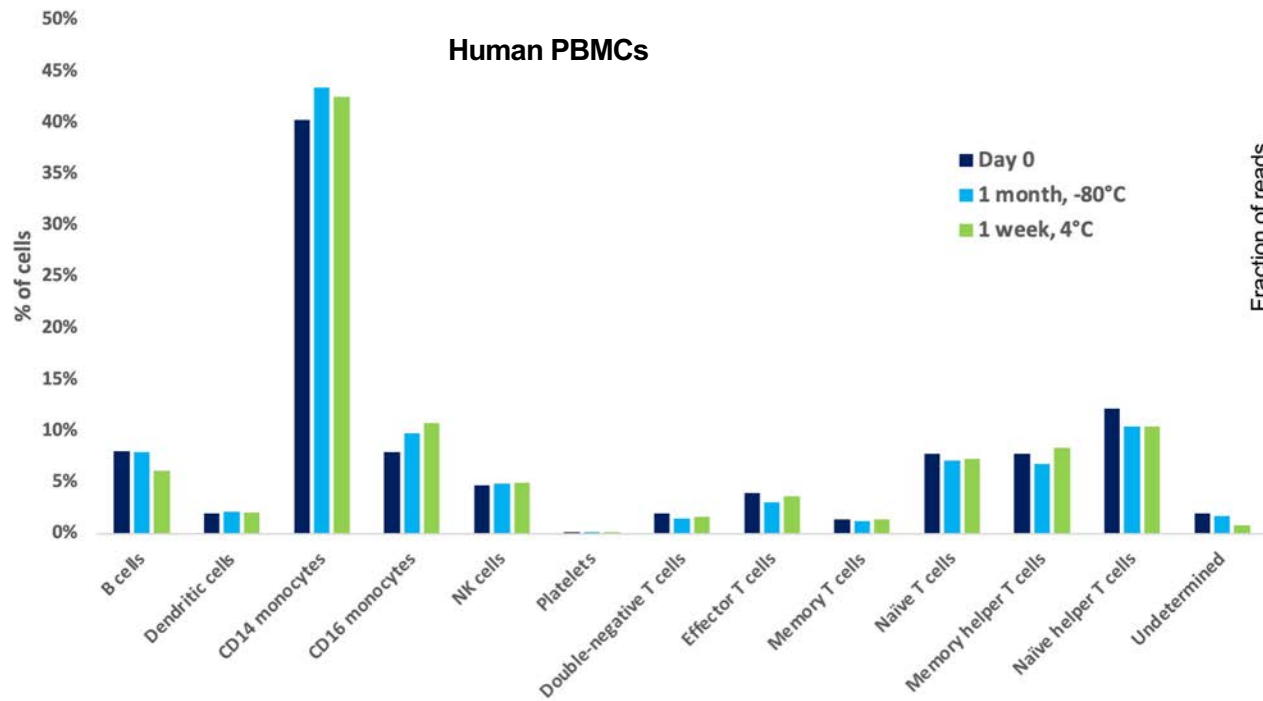


Marker	Cell Type
MKI67	Cycling
CD68	Myeloid
MS4A1	B cell
PDGFRB	Mesenchymal

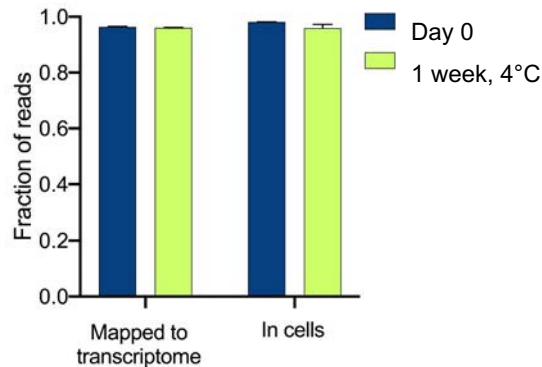


Fixed Cell Storage Preserves Samples

Enables sample batching, transport, and storage

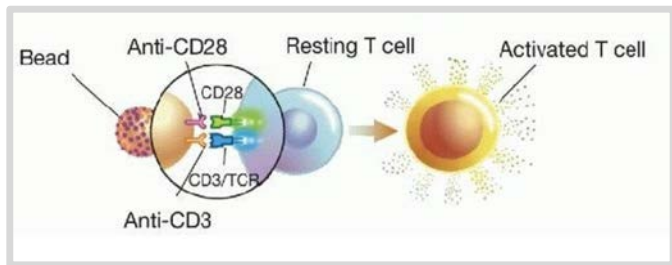


Bladder DTCs



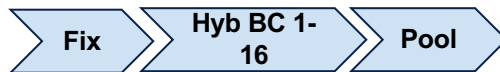
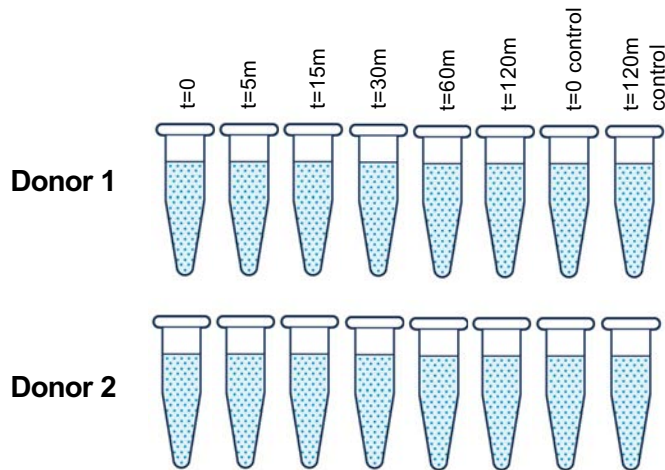
Interrogating Dynamic Systems Through Fixation

CD3/CD28 activates T cells

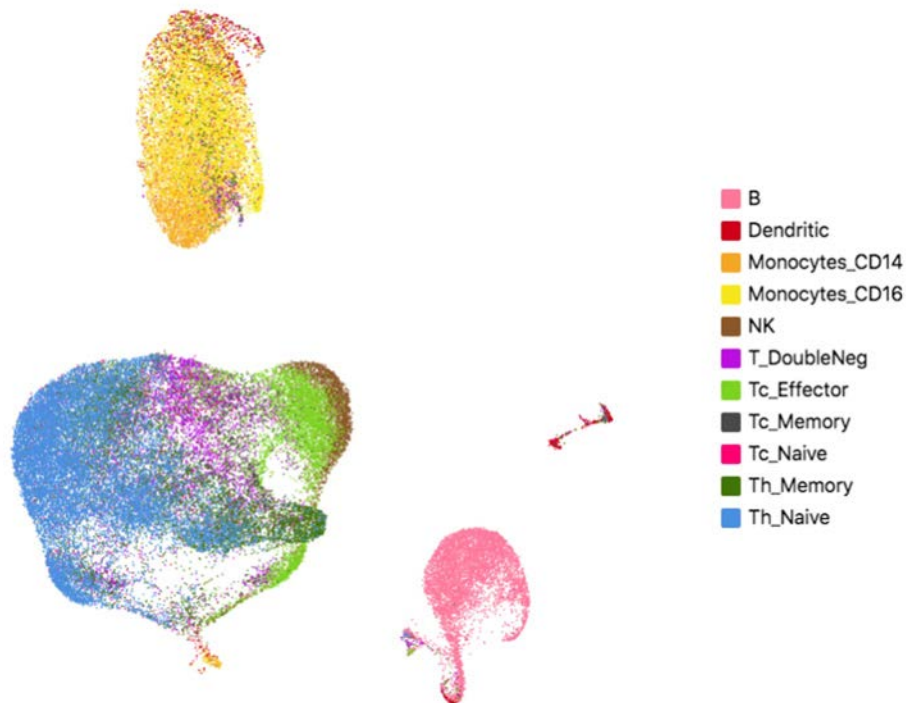


Thermo Fisher Scientific

- Two independent donor PBMC samples, cultured overnight
- Stimulated for up to 2h using anti-CD3 and anti-CD28
- Sampled over six time points, plus two media-only controls
- 16 samples multiplexed into a single channel of Chip Q
- Sequencing: 15k reads per cell
- 64,000 total cells recovered



Cell Types Identified and Cell Recovery



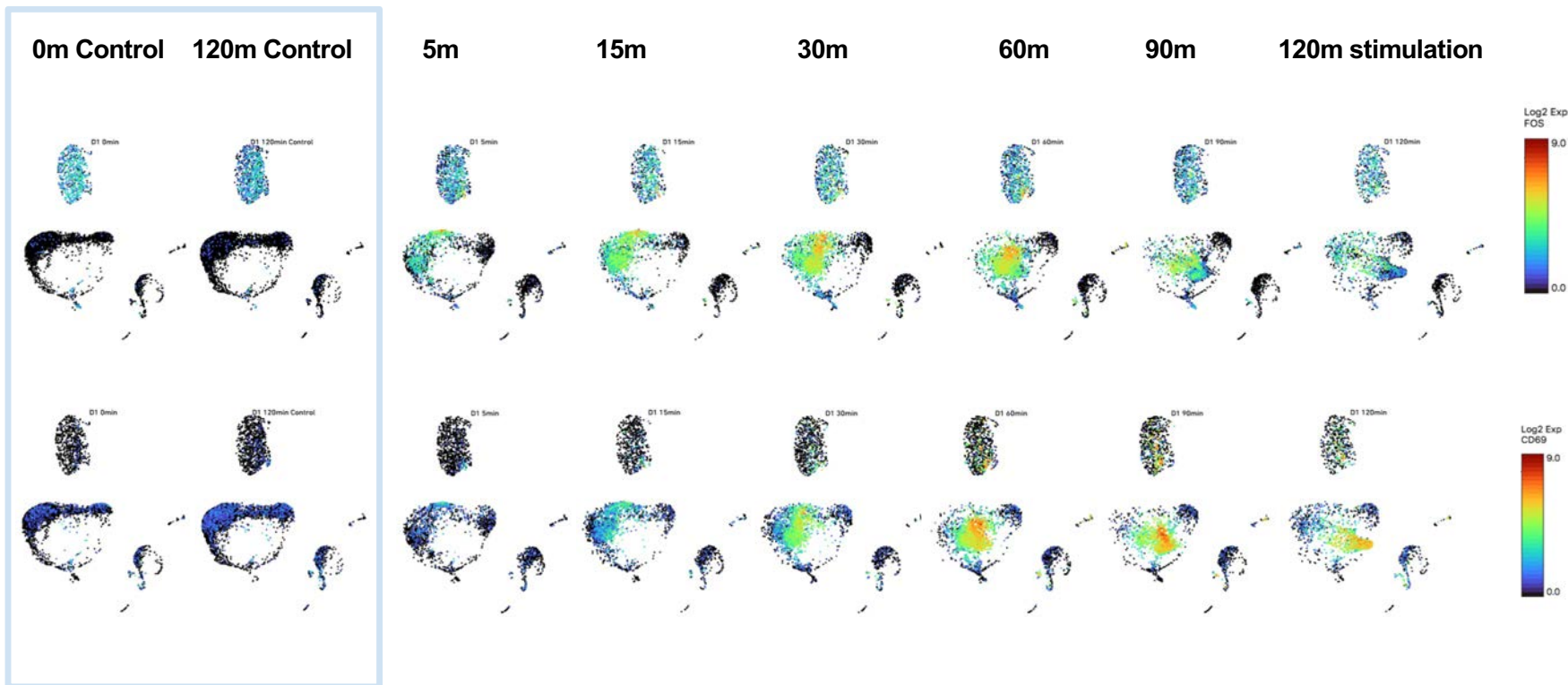
Cell recovery (single GEM lane, 16-plex)

	Donor 1	Donor 2
0 min control	4360	4442
120 min control	6919	4285
5 min	2946	3909
15 min	2953	3676
30 min	3365	4558
60 min	4064	4089
90 min	3615	3706
120 min	2661	4740

Capturing Changes in Gene Expression in Minutes Scale

FOS (member of AP-1 TF)

CD69 (Early T-cell activation marker)



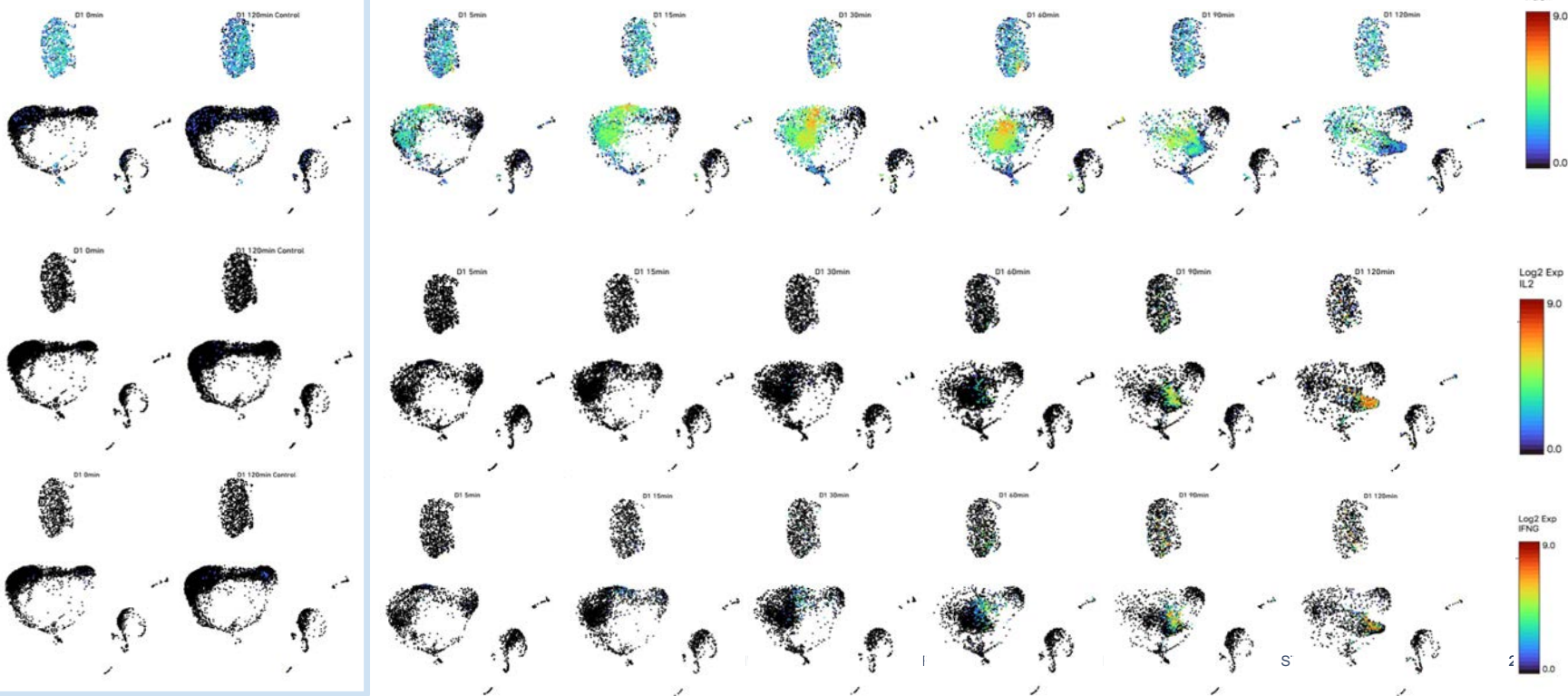
Capturing Changes in Gene Expression in Minutes Scale

FOS (member of
AP-1 TF)

IL2

IFN- γ

0m Control 120m Control 5m 15m 30m 60m 90m 120m stimulation



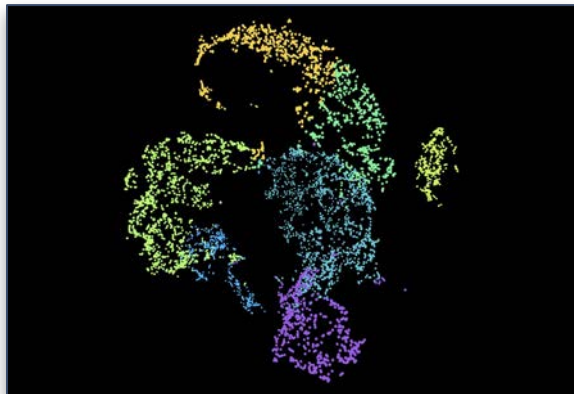
Fixed RNA Profiling



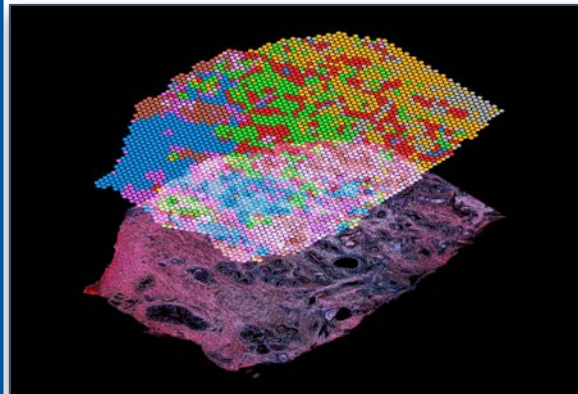
- Enables a whole new scale of single cell experiments
- A full, end-to-end workflow including sample prep protocols, validated reagents, and software for data analysis and visualization

Our Three Platforms

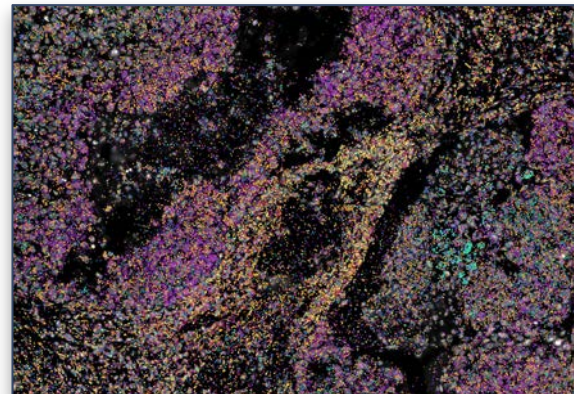
Chromium Single Cell



Visium Spatial



Xenium In Situ



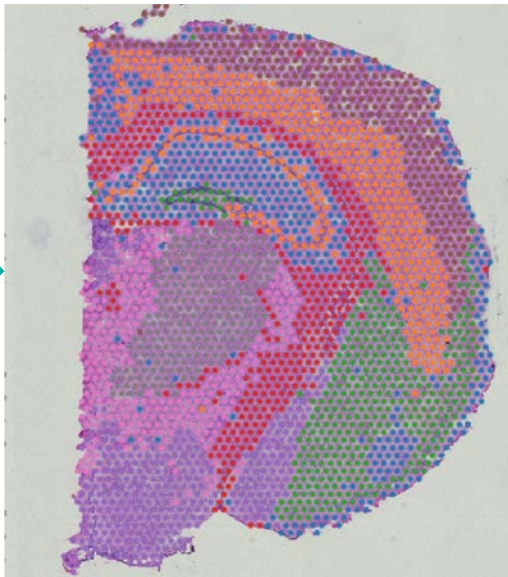
Two types of information comprise Visium data

Tissue image and gene expression

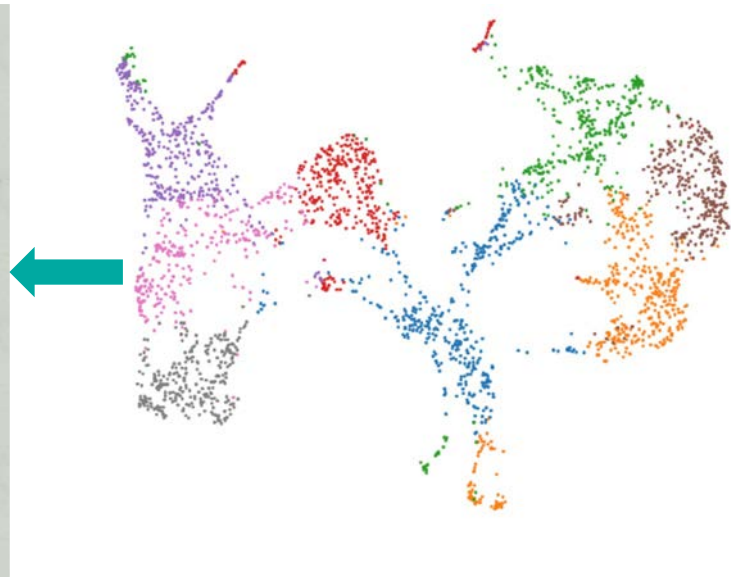
Histology image



Histology + Gene expression



Gene expression



Visium Spatial Gene Expression

Whole transcriptome analysis with morphological context

- Gain a complete view of disease complexity
- Discover new biomarkers
- Identify novel cell types and states
- Map the spatial organization of cell atlases
- Identify spatiotemporal gene expression patterns

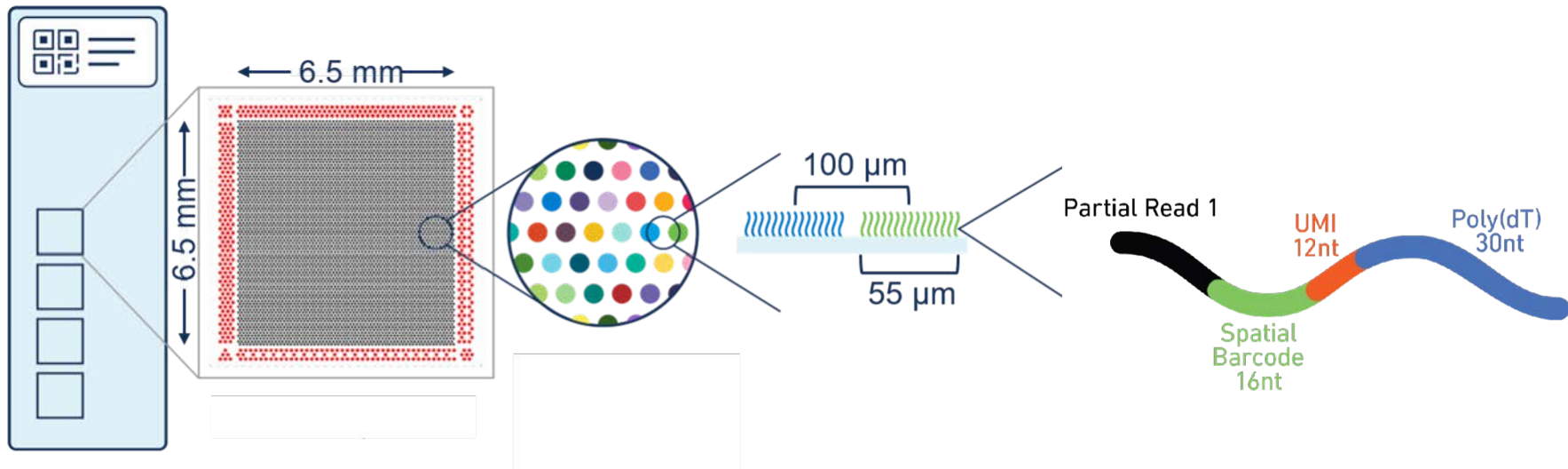


Spatial capture technology for whole transcriptome analysis

Visium Spatial Gene Expression Slide

Capture Area with ~5000 Barcoded Spots

Visium Gene Expression Barcoded Spots



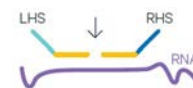
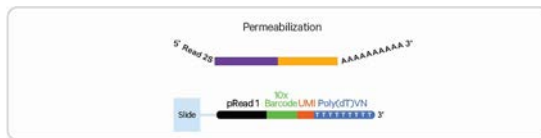
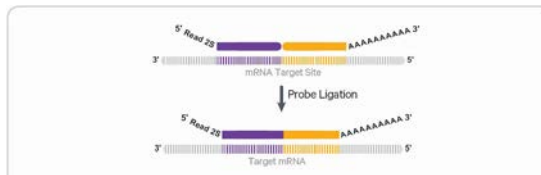
RNA Templated Ligation (RTL) for sensitive, specific RNA detection in FFPE samples

Probe pairs designed against the protein-coding transcriptome, one pair per gene

Probes hybridize to their target sequence, and are ligated to one another

Target RNA is digested, releasing ligated probes

Tissue is permeabilized
Ligated probe pairs are captured on-slide, barcoded, and prepared into a sequencing library



Split two probe chemistry reduces nonspecific signal

~18,000 genes in human

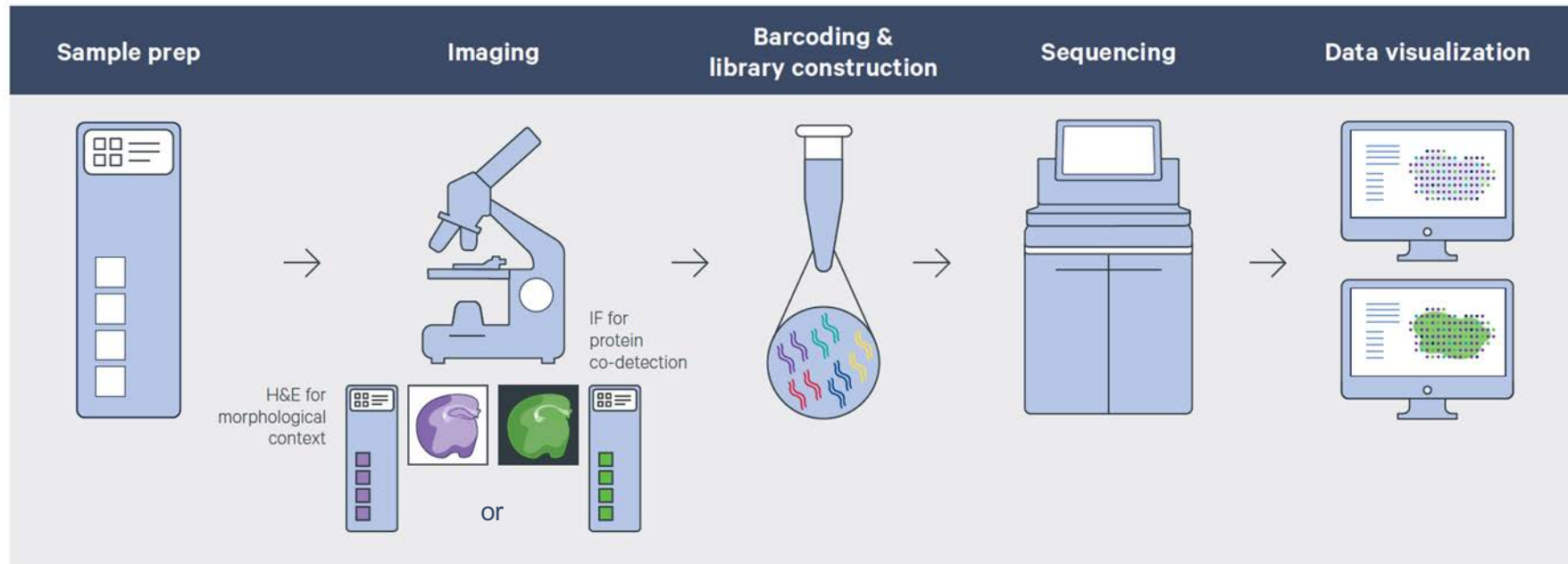


~20,000 genes in mouse



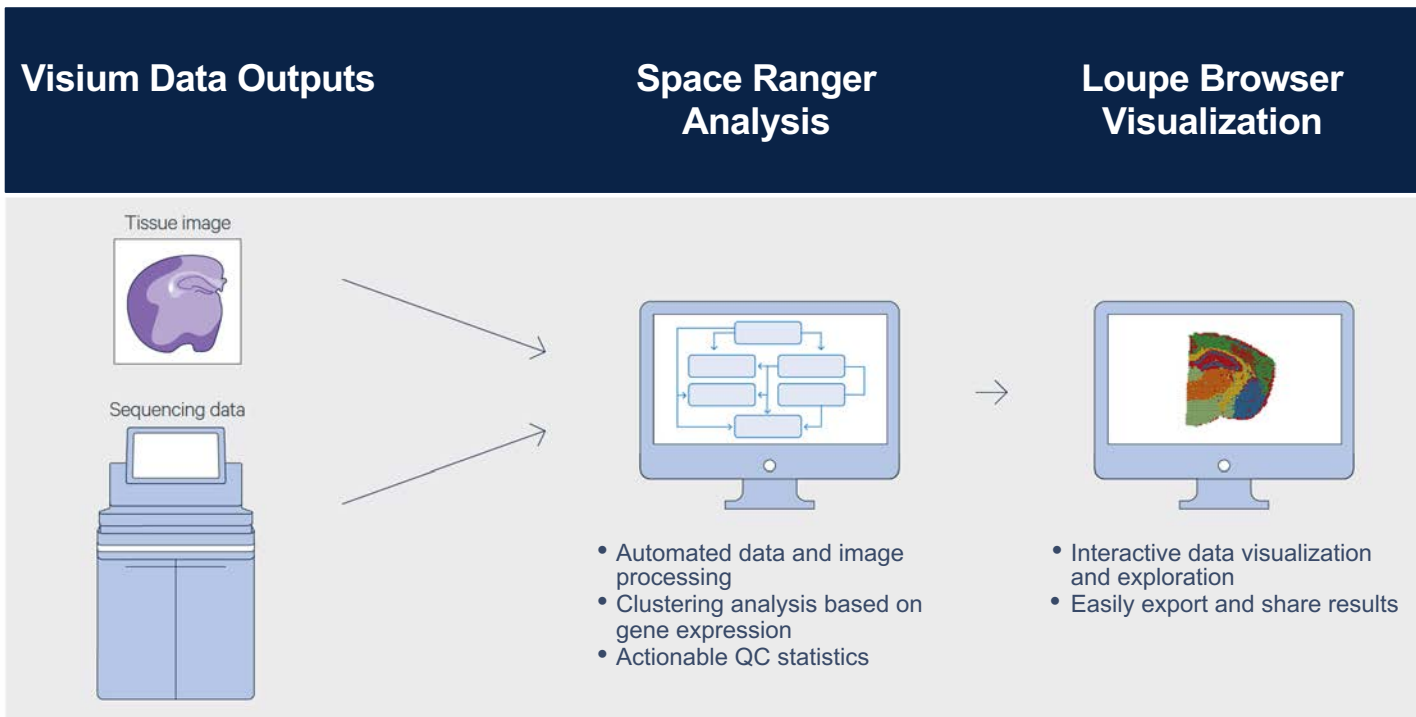
Efficient Workflow

Integrates easily with current laboratory methods and tools for tissue analysis



Easy-to-use software

Combines histological and gene expression data



Visium Spatial Gene Expression



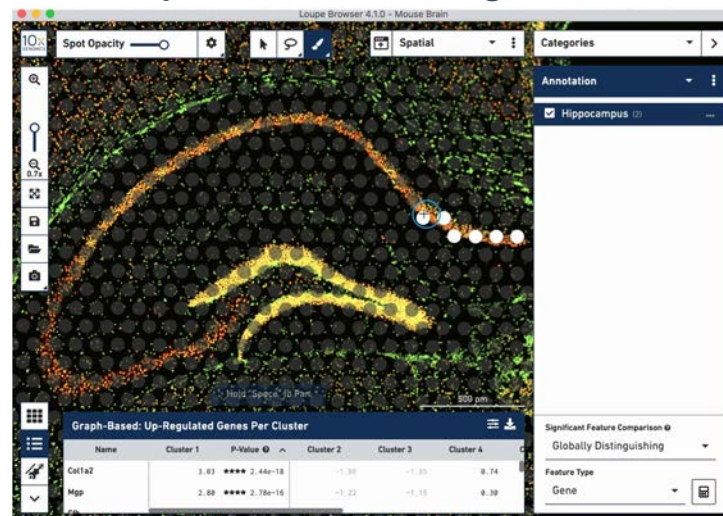
Streamlined Data Analysis

Easy-to-use analysis software that combines histological and gene expression data

Data Exploration in Loupe Browser



Flexible Spot Selection for Region Annotation

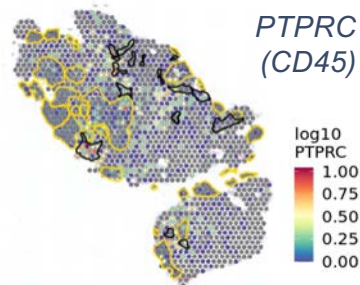


Visium reveals immune infiltration in the microenvironment

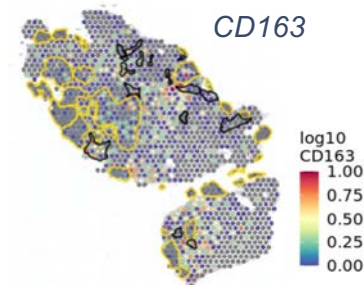
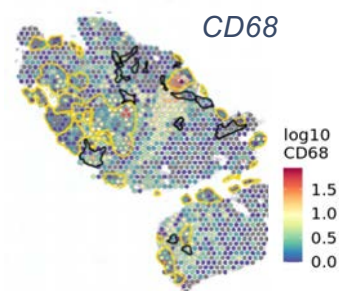
Pathologist
Classification

■ DCIS
■ Immune Cells

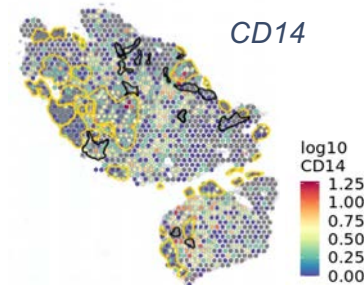
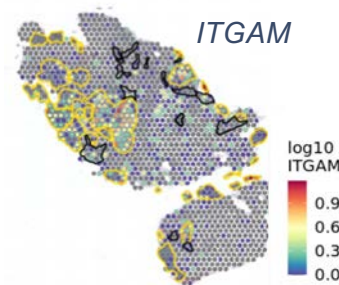
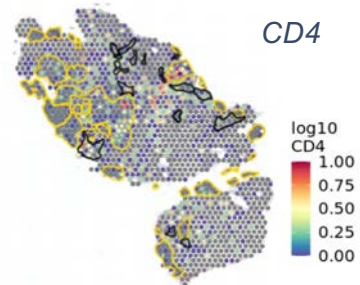
Leukocyte Marker



Macrophage Markers



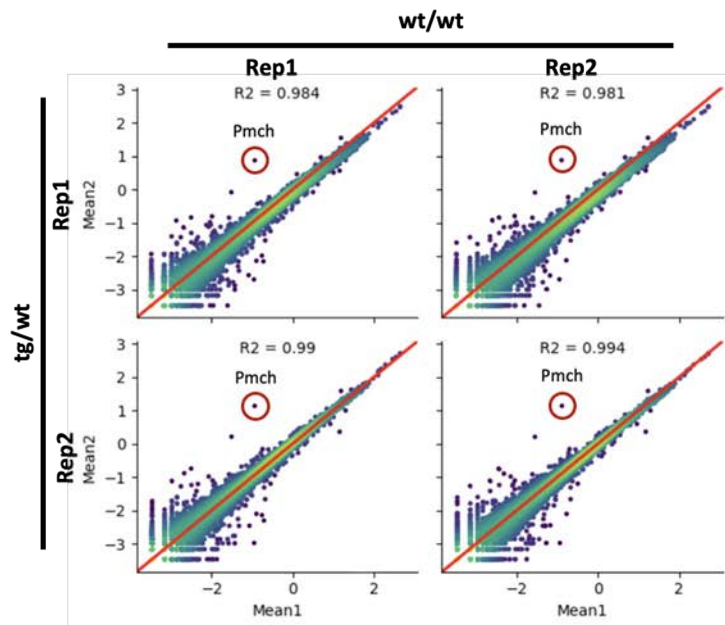
T Cell Marker



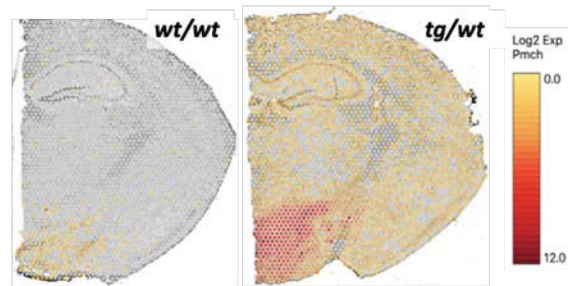
Spatial gene expression of familial Alzheimer's Disease

Demonstrates ability to discover novel targets and/or pathways with whole transcriptome analysis

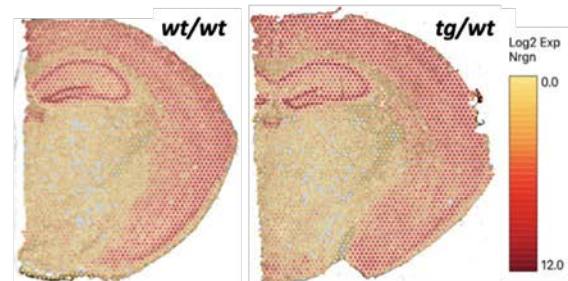
10x Genomics Data



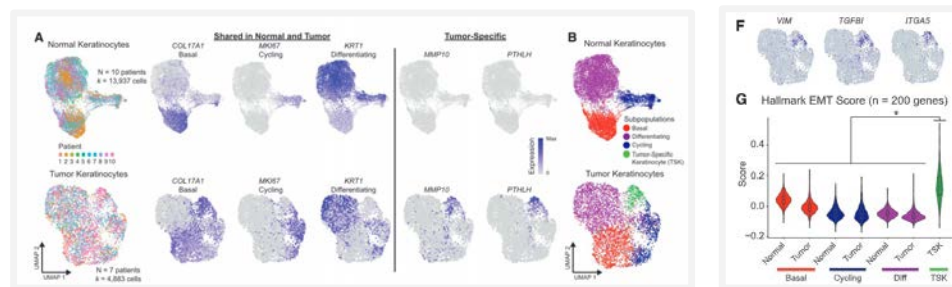
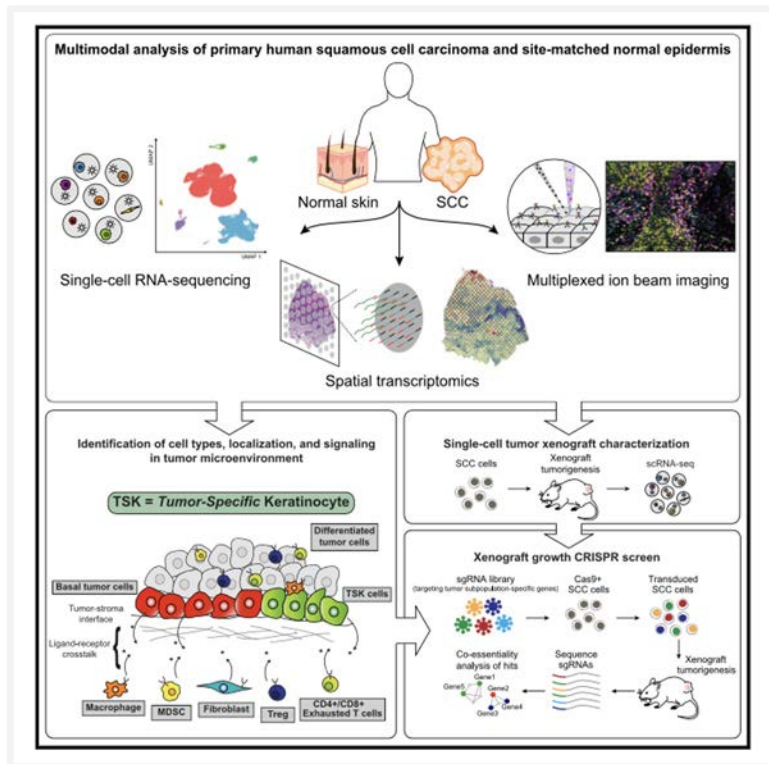
Pmch



Nrgn



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



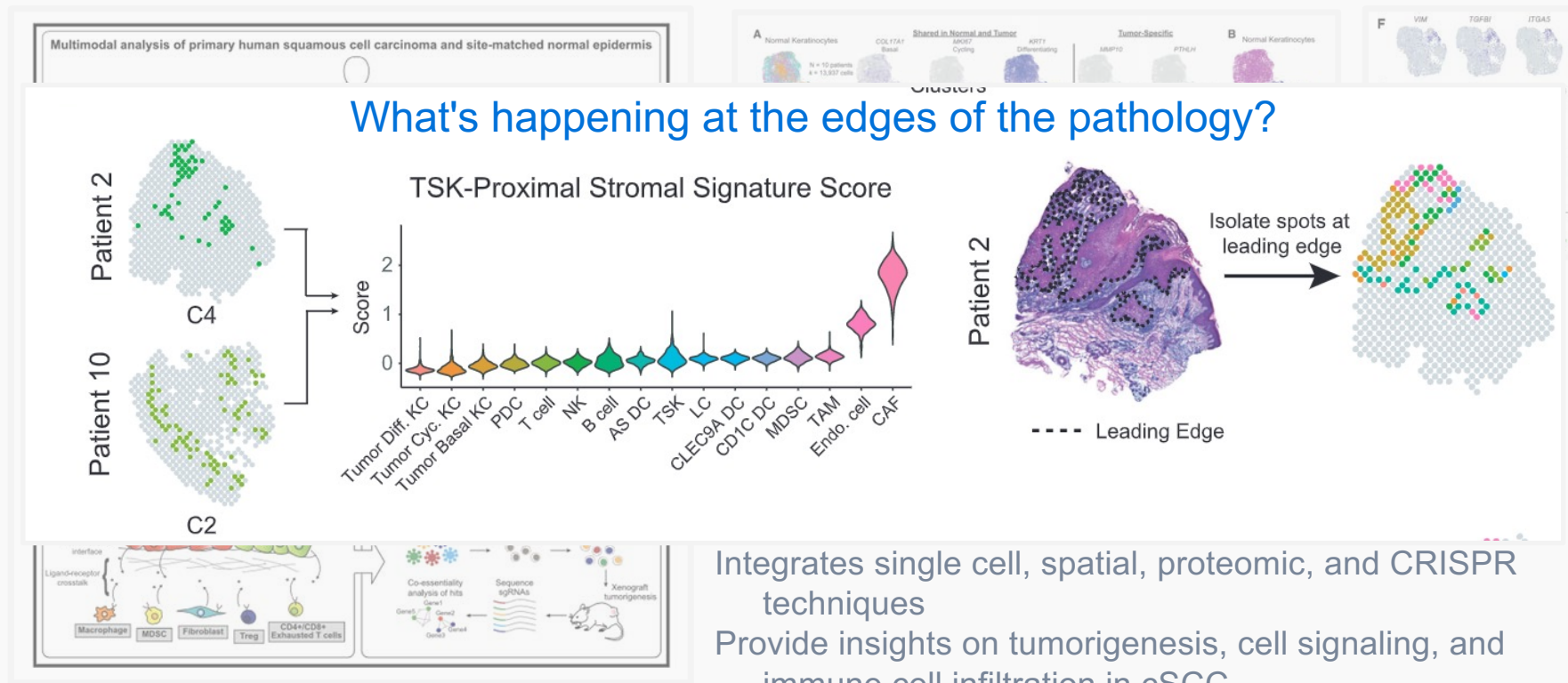
In Brief:

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)



Visium Spatial Gene Expression Features



Whole Transcriptome Analysis in FFPE and Fresh Frozen Tissues



Co-Detect Protein and Whole Transcriptome



High Resolution



Streamlined Data Analysis



True Discovery



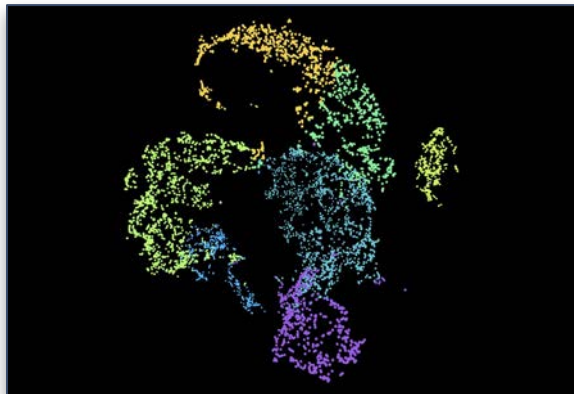
Efficient Workflow



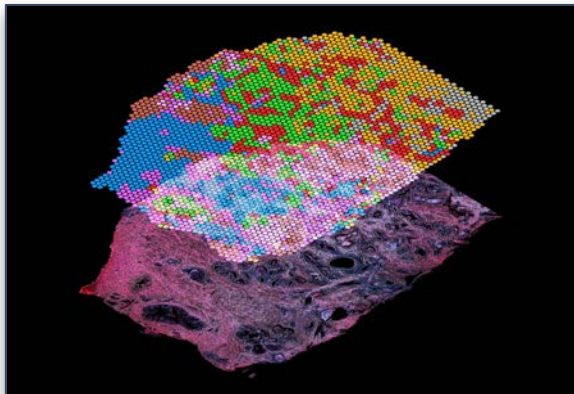
Kitted and Ready to Use

Our Three Platforms

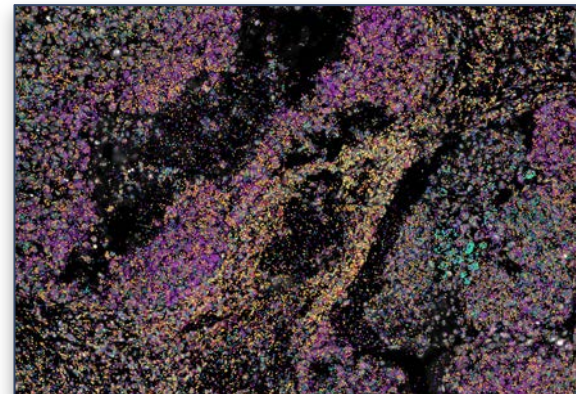
Chromium Single Cell



Visium Spatial



Xenium In Situ



Discovery

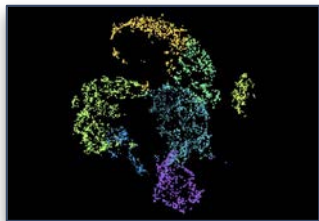
Focused

From discovery to focused with complementary workflows

Discovery

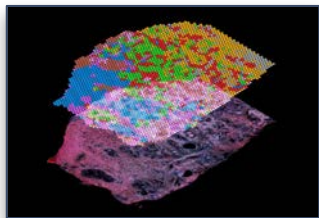
Focused

Chromium Single Cell



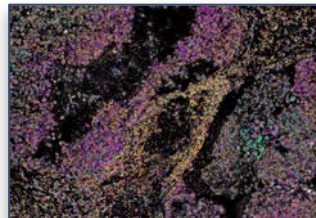
- Entire transcriptome measurement

Visium Spatial



Where are the cells located?
Where can specific combinations of markers be found?

Xenium In Situ



- Targeted gene expression information
- Subcellular resolution
- High sensitivity

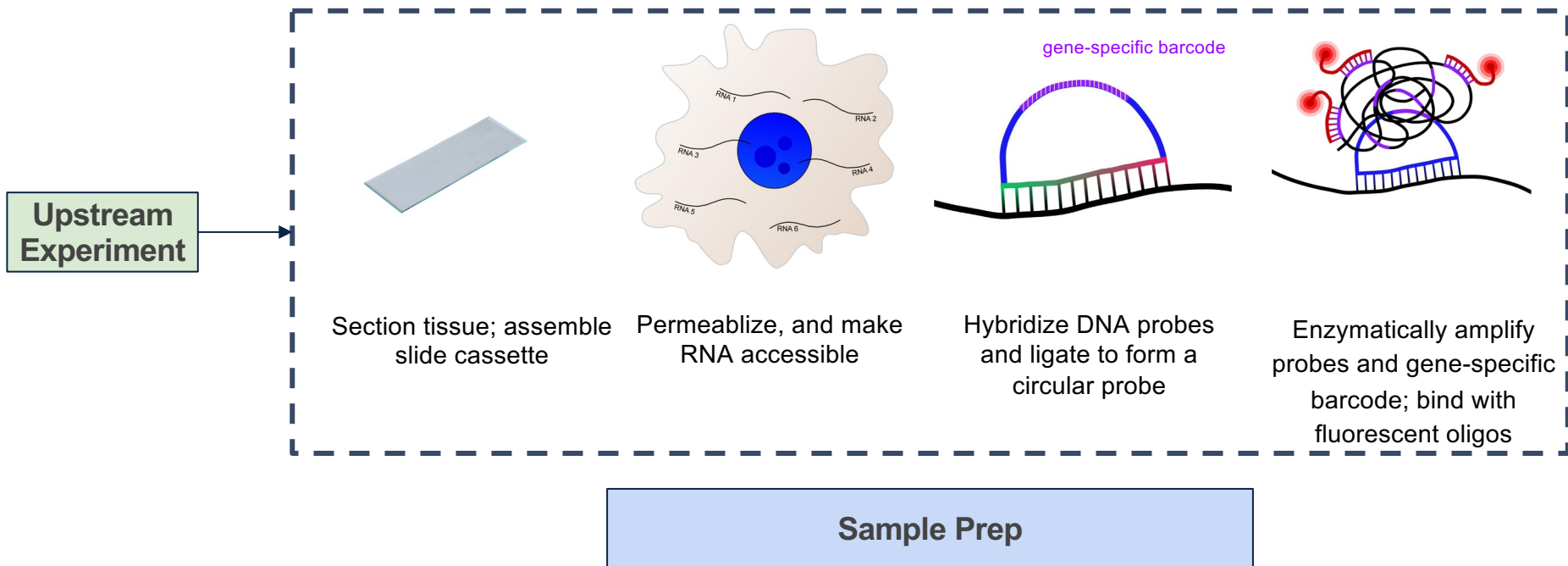
Xenium

In Situ Platform

- Hundreds of gene targets
- Subcellular resolution
- Microscopy based read-out
- Fresh Frozen and FFPE
- Simultaneous RNA and proteins
- Throughput for larger cohorts

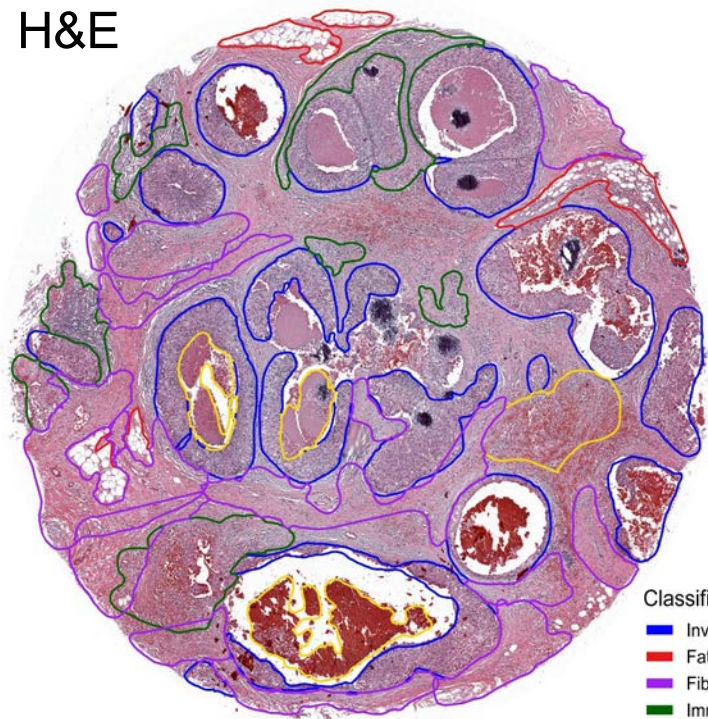


Xenium Workflow Overview



In situ analysis of human FFPE breast cancer

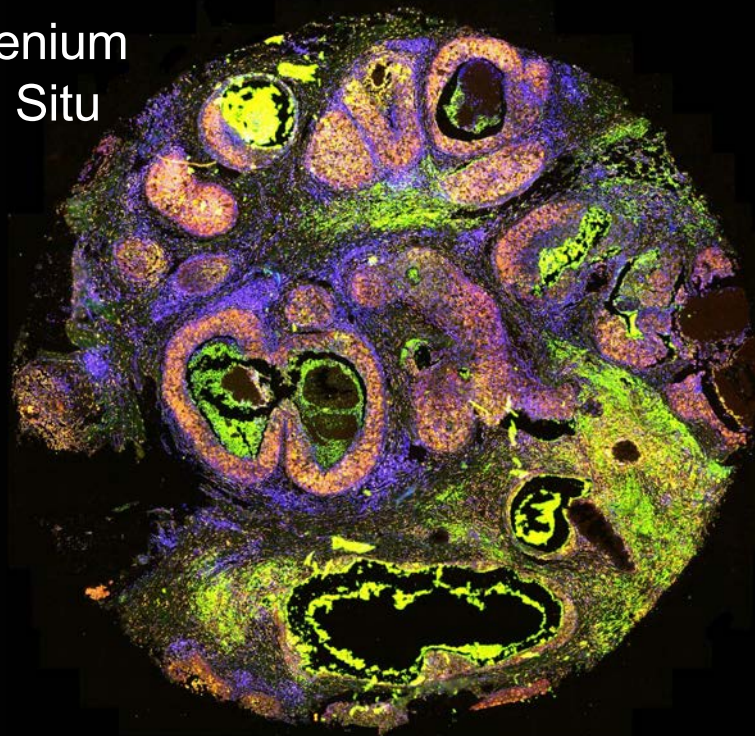
H&E



Classification

- Invasive Carcinoma
- Fat
- Fibrous Tissue
- Immune Cells
- Necrosis

Xenium
In Situ



Correlation between Xenium and Visium

Known *ERBB2* (HER2)-positive tumor

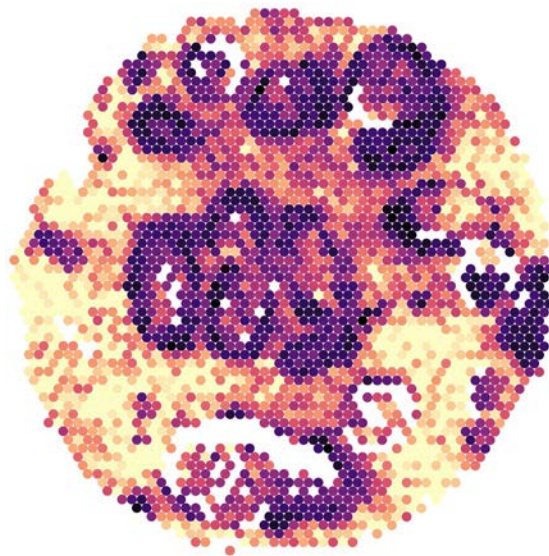
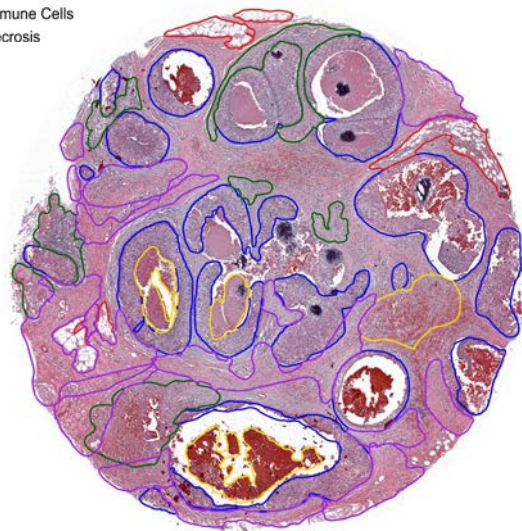
Classification

- Invasive Carcinoma
- Fat
- Fibrous Tissue
- Immune Cells
- Necrosis

H&E

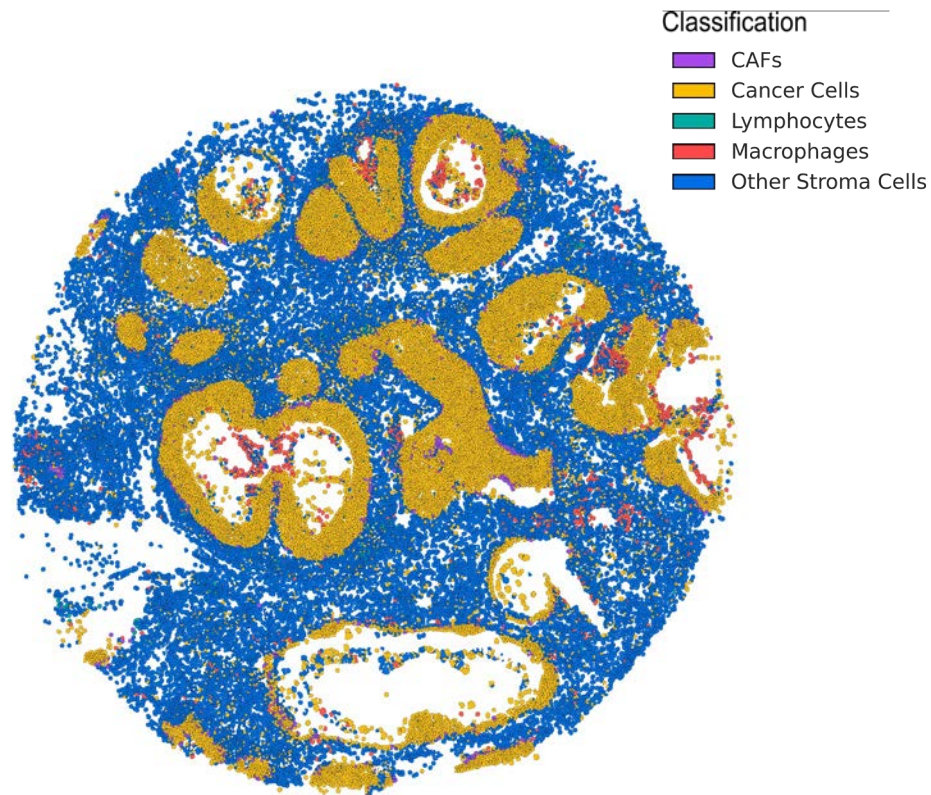
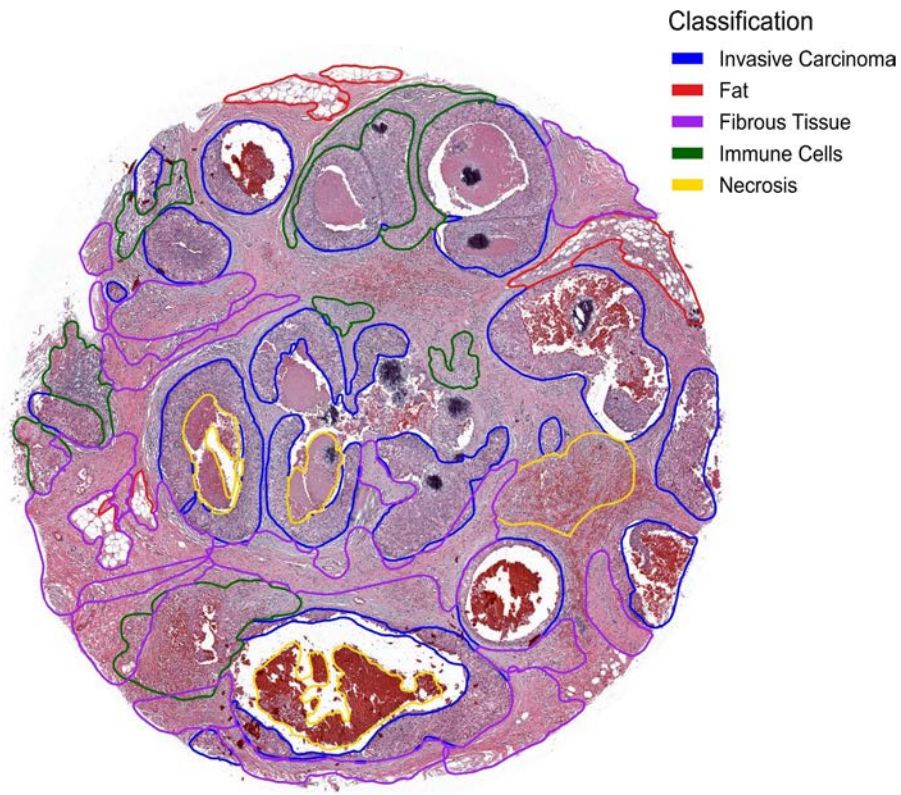
Visium

Xenium

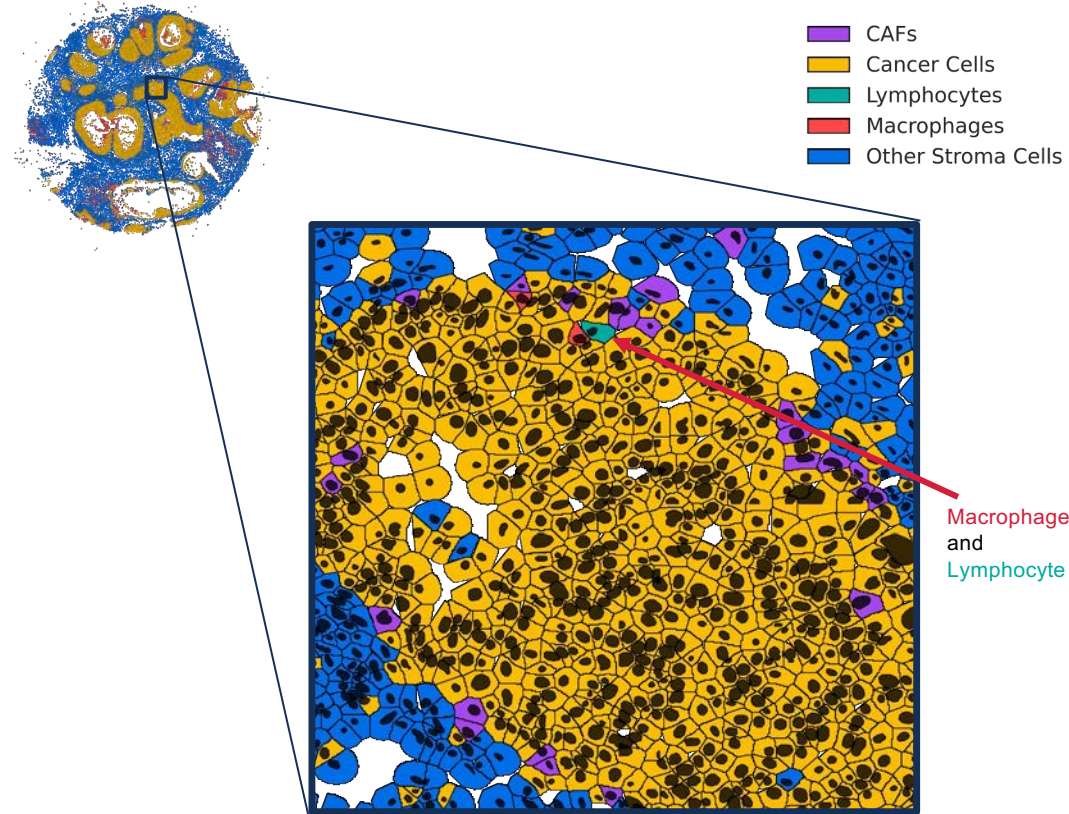
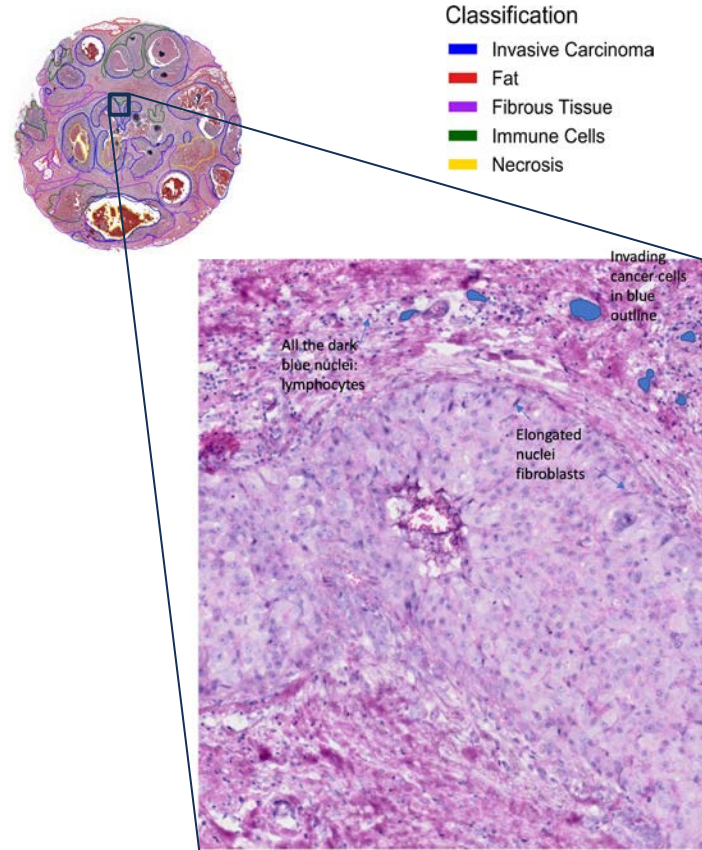


ERBB2
Scaled Exp 2 3 4

Xenium recapitulates pathologist's annotations



Xenium identifies immune cell invasion in tumor region



Xenium In Situ Platform

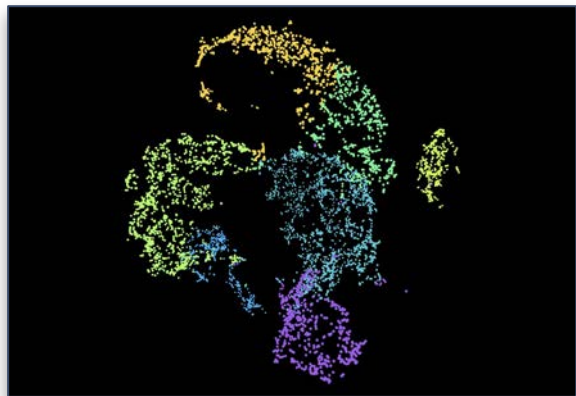
- High throughput, turnkey instrument
- Localize single-molecule RNA and protein targets
- Pre-designed and custom targets

Expected **Late 2022**

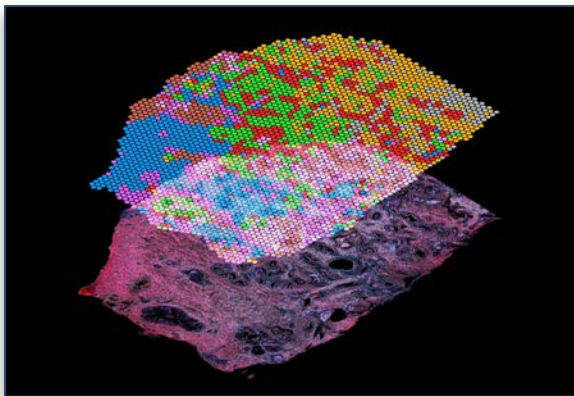


From discovery to focused with three complementary workflows

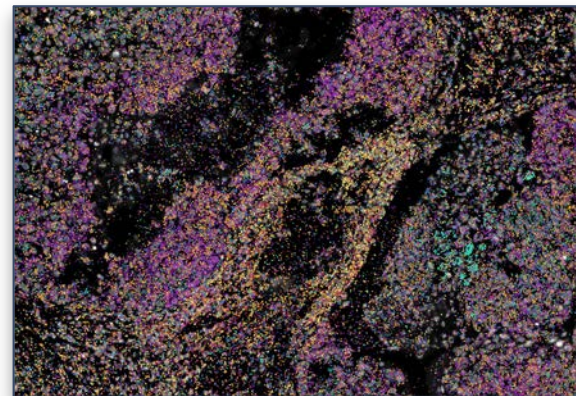
Chromium Single Cell



Visium Spatial



Xenium In Situ



Discovery

Focused

Thank you!

wendy.love@10xgenomics.com (SE) nabiollah.kamyabi@10xgenomics.com (FAS)
nicole.bowman@10xgenomics.com (SA) keven.johnson@10xgenomics.com (STA)
john.manuel@10xgenomics.com (S-STA)

