10x GENOMICS

Empowering Impactful Science with Leading Edge Single Cell, Spatial, and In Situ Technologies

Owen Kuo
Application Scientist
20 June



Chromium

10x GENOMICS

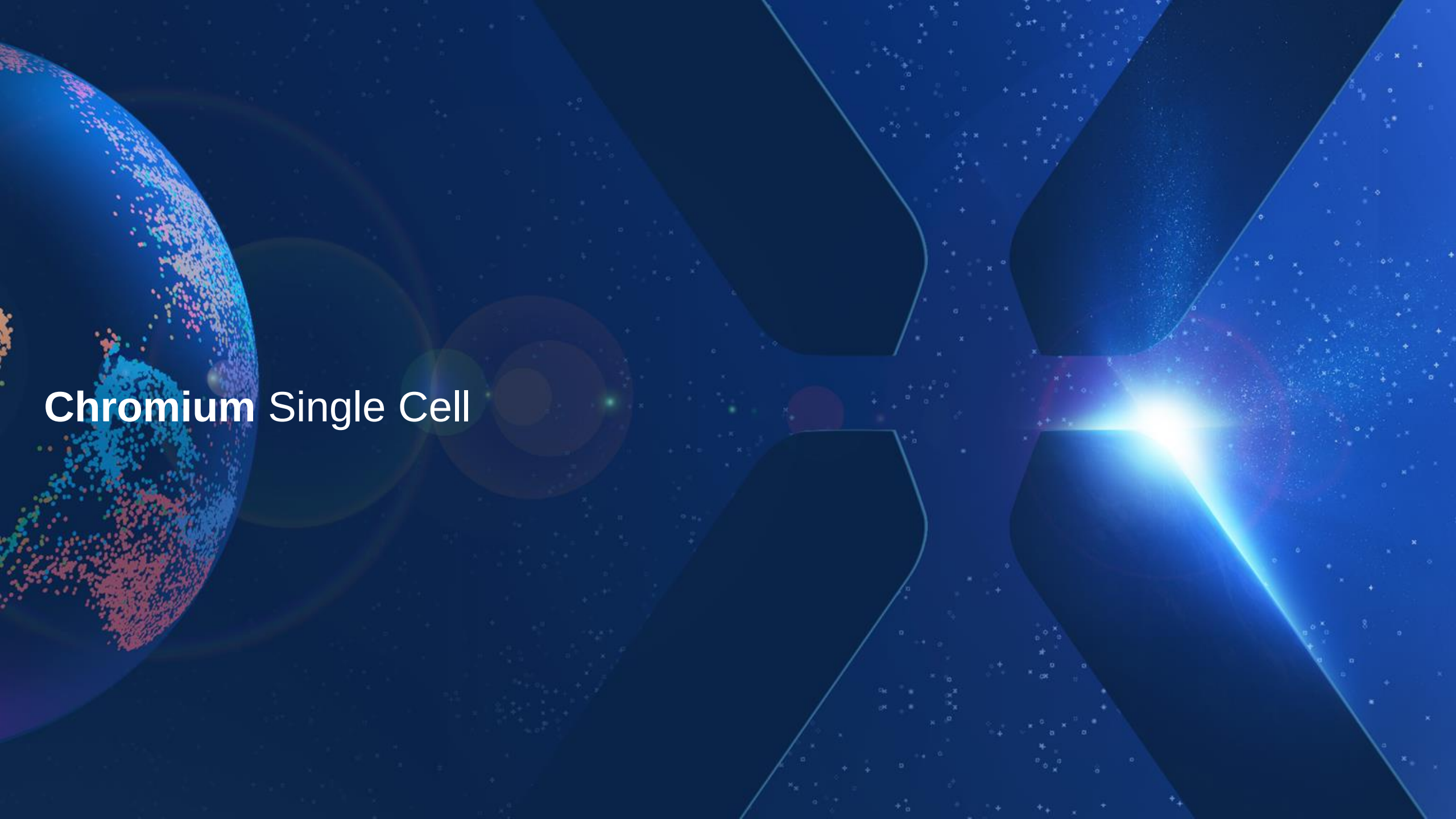
Toolkit



Visium



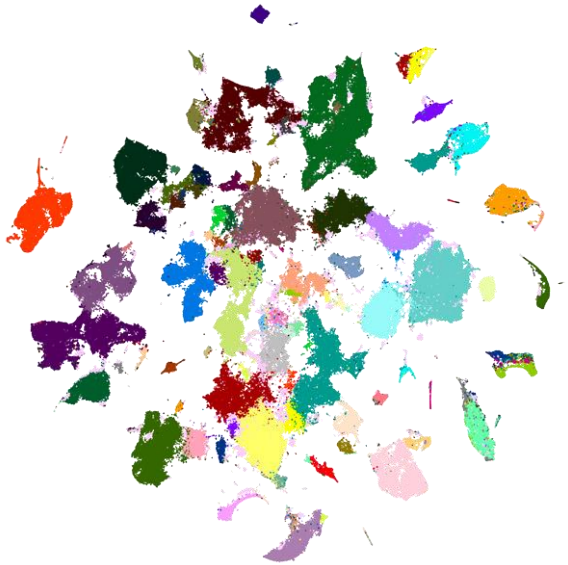
Xenium


Chromium Single Cell

Leading in Single Cell Breadth, Scale, and Access

Breadth and scale





- Gene Expression 3’
- Immune Profiling 5’
- Gene Expression Flex

~1M cells per chip

- ATAC
- Multiome

Braun E, et al. *bioRxiv,* 2022.

Access and robustness



- Sample preparation kits
- Validated protocols
- FFPE access
- Market-leading technical support

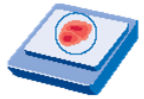
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Gene Expression Flex – Any Sample at Any Scale

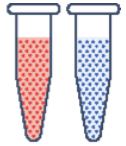
Broad sample compatibility



Fresh tissue
Frozen tissue



FFPE tissue



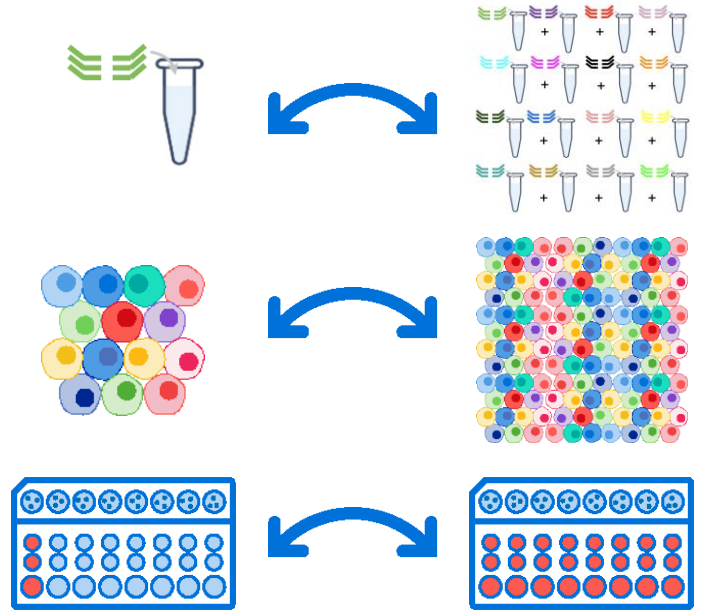
Cell suspensions
Nuclei suspensions

In-line multiplexing



5-fold increase in scale over other multiplexing techniques

Flexible sample and cell input number



- Up to 128 samples per chip
- Up to 1M cells per chip

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Despite Chromium advances, certain challenges can impact the decision to perform single cell

Costs can be high and multiplexing can be difficult

Working with fresh cells remains challenging

Accessing certain sample types is very difficult

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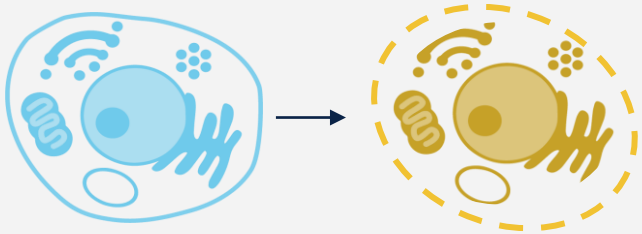
Working with living fresh cells can remain challenging

Limited flexibility



- Hard to collect at different times
- May want to decide which samples to run later

Sample degradation



- Fast sample prep is needed
- Some cells cryopreserve better than others

Logistic constraints



- Unavailability of cryopreservation
- Transport can compromise quality

Sample stabilization is crucial for large and complex projects

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Additionally, accessing certain sample types has been a challenge

Low quality samples



- Associated with damaged RNA
- Reproducibility of downstream analyses is unknown

Whole tissues



- Requires expertise
- Variability in acquisition can lead to loss, errors, and inconsistencies

FFPE Samples



- Challenging to extract intact cells or nuclei
- Heavily degraded, low quantity RNA

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Fixation stabilizes samples and eases sample constraints



Increased flexibility

- No sample prep constraints
- Decide which samples you want to use later
- Batch and multiplex samples to increase efficiency



Retain sample quality

- Lock in biological states at point of collection
- Analyze cells that may not survive storage or dissociation

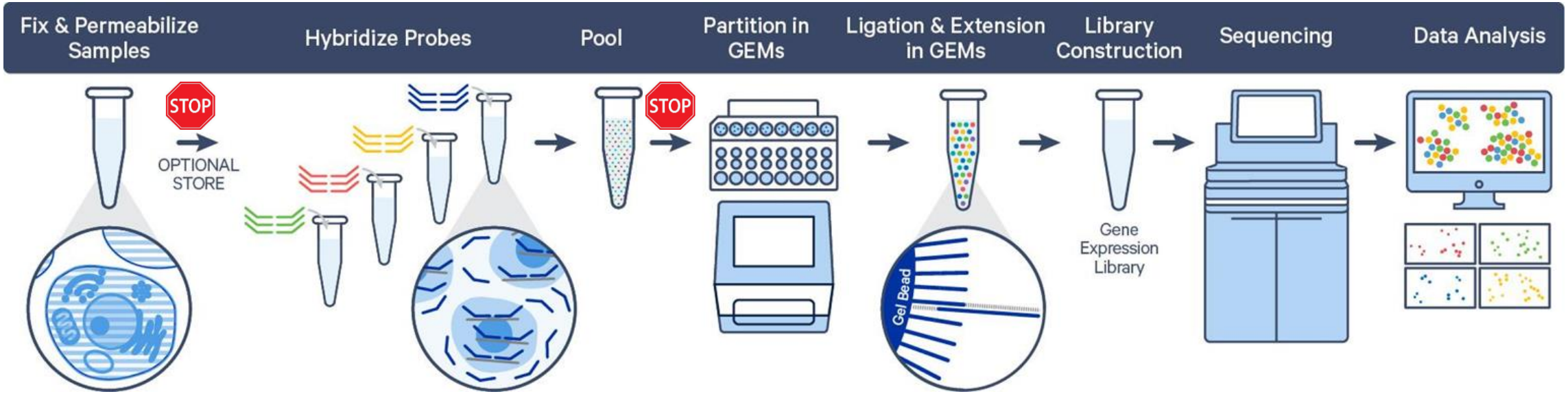


Ease of transport and storage

- Store in the fridge/freezer or transport until ready to use
- Enables large multi-site projects w/o cryopreservation

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Simple and fast workflow with 4hrs. Hands On Time



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Species and Sample Compatibility for Gene Expression Flex

Species compatibility


Human


Mouse

Demonstrated protocols and guidance for preparation of a **wide range of sample types** are available on the 10x Genomics Support Site

Sample type compatibility:


Cell lines


Primary cell suspensions


Isolated nuclei


Dissociated tissue samples

Fresh or frozen tissue

FFPE



size reduction <1 mm before fixation

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Internally tested tissues with Gene Expression Flex

Range of samples tested including single cell+nuclei suspensions, fresh+frozen tissue, and FFPE

HUMAN SAMPLES

PBMCs and Cell Lines

Fixed Dissociated Tissue	Tissue Nuclei	FFPE
Breast	Colon	Brain (healthy)
Colon	Jejunum	Brain (Alzheimer's)
Duodenum	Liver	Brain (glioblastoma)
Ileum	Spleen	Breast cancer
Jejunum	Testes	Colorectal cancer
Kidney	Tonsils	Heart
Liver		Kidney
Lung		Lung (cancer, healthy)
Melanoma		Ovarian cancer
Ovary		Lymph Node (reactive, healthy)
Pancreas		Liver (cancer, healthy)
Spleen		Pancreas
Testes		Prostate cancer
Tonsils		Skin melanoma
		Testis
		Thymus
		Tonsil
		Spleen

MOUSE SAMPLES

PBMCs and Cell Lines

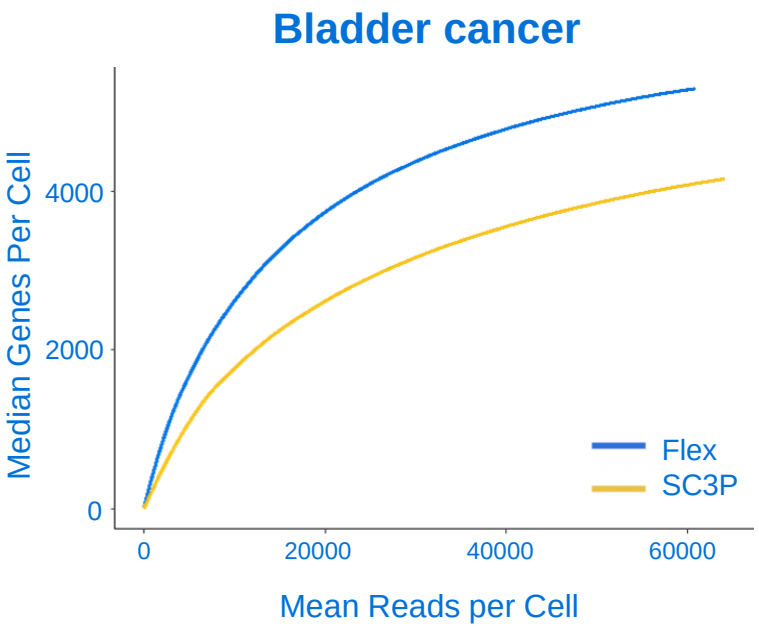
Fixed Dissociated Tissue	Tissue Nuclei	FFPE
Adipose*	Adipose	Brain
Bladder	Bladder	Heart
Brain	Brain	Kidney
Colon	Colon	Liver
Eye	Eye	Spleen
Heart	Heart*	Thymus
Intestine	Intestine	
Kidney	Kidney	
Liver	Liver	
Lung	Lung	
Muscle	Muscle*	
Ovary	Ovary	
Pancreas	Pancreas	
Spinal cord*	Spinal cord*	
Spleen	Spleen*	
Stomach	Stomach	
Testis	Testis	
Thymus	Thymus*	
Tongue	Tongue	

**challenging sample type*

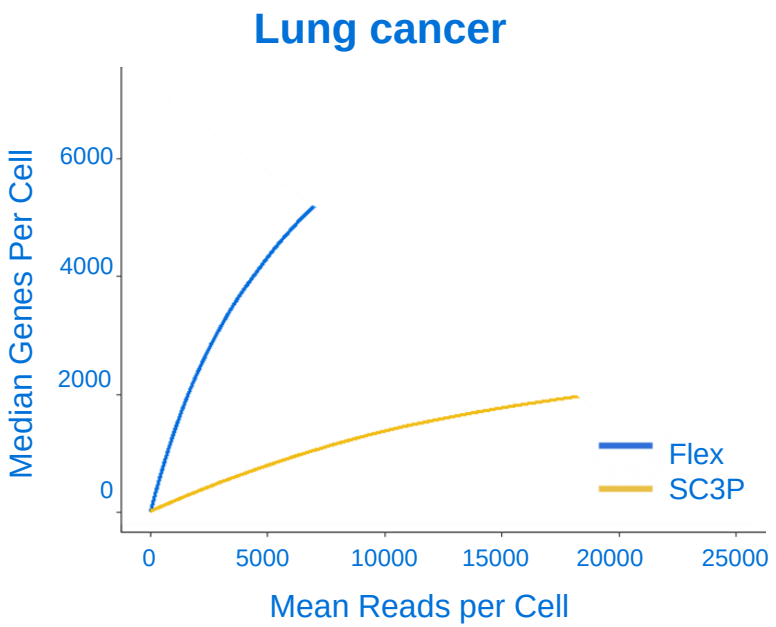
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Gene Expression Flex – High Performance in Challenging Samples

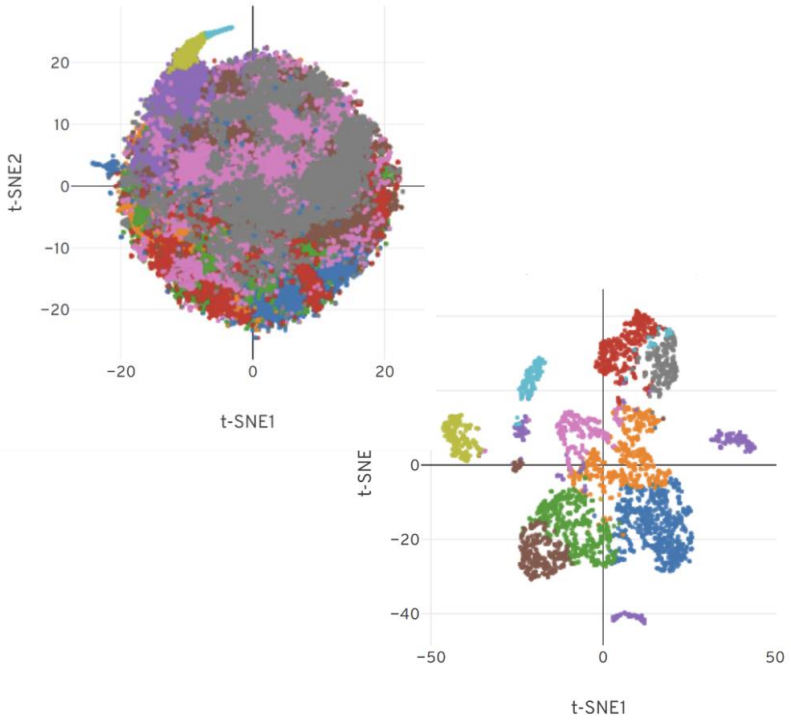
Cryopreserved tumor cells



Frozen lung tumor nuclei



Fresh human endometrium



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Visium Spatial

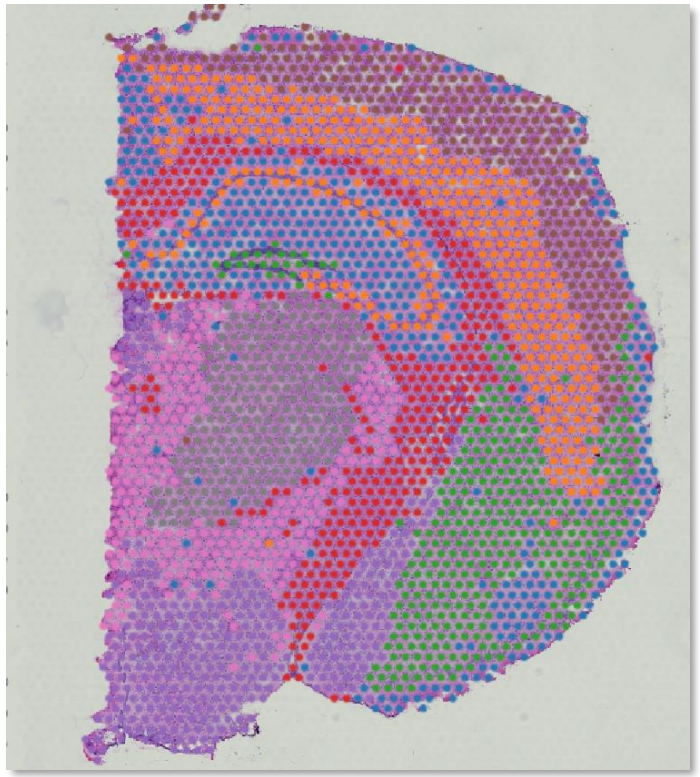
Two Types of Information Make Up Visium Data

Tissue image and gene expression

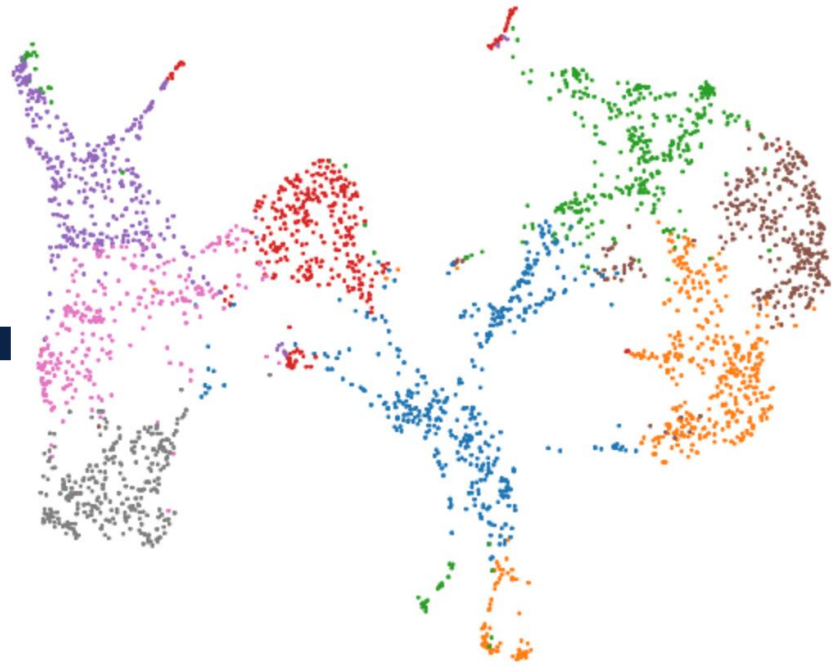
Histology image



Histology + Gene expression



Gene expression



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CytAssist is the Future of Visium



Improved FFPE performance

Co-detection of gene and protein expression

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CytAssist Simplifies Visium Prep & Broadens Sample Access

Precision analyte transfer and spatial barcoding



Technical Note CG000618 | Rev A

Visium CytAssist Spatial Gene Expression for FFPE: Reagents, Workflow, & Data Comparison

- Improved gene sensitivity and specificity
- Broader tissue type compatibility
- Access to archival samples
- Improved spatiality
- Streamlined workflow

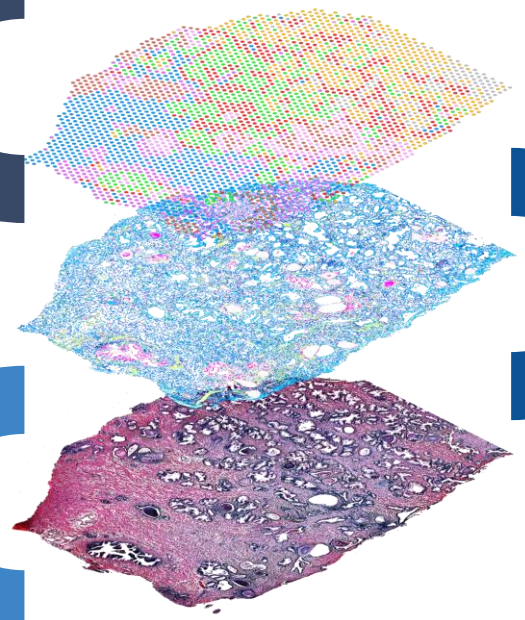
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Spatial Gene and Protein Overview

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

Whole Transcriptome

Whole transcriptome detection via probe-based RNA Template Ligation (RTL) technology



Multiplexed Protein

Co-detect intracellular and extracellular immune markers using oligo-tagged antibody panel*

Tissue Morphology

Utilize H&E or IF staining to profile tissue biology

**Additional targets may be detected by spiking in oligo-tagged antibodies*

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Product Highlights

Visium CytAssist Spatial Gene and Protein Expression solution

Visium CytAssist Spatial
Gene and Protein Expression Solution



Spatially resolved gene expression data

- Resolve biological architecture in normal and diseased tissue with Hematoxylin and Eosin (H&E) or Immunofluorescence (IF) staining
- Unlock high-throughput, whole transcriptome and highly multiplexed protein analysis of human FFPE sections

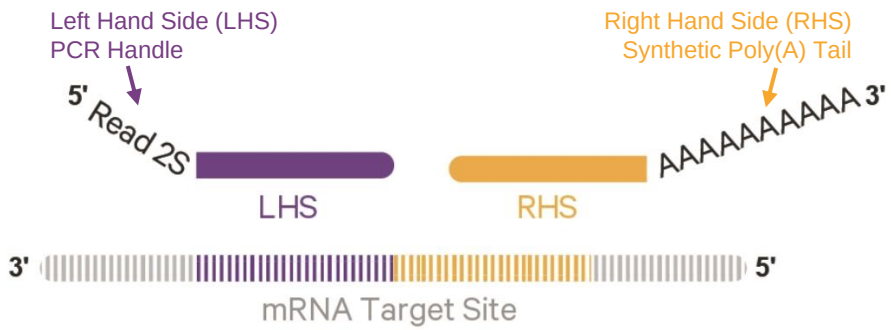
Move through the workflow and analysis easily

- Flexible workflow with integrated stopping points
- End-to-end workflow from sections on slides to multiomic libraries within three working days
- Compatible with 10x Genomics turnkey analysis and visualization software

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Critical Assay Components

Visium Human Transcriptome Probe Kit v2



Probe Pairs and Handles

- LHS probes contain a partial Read 2S sequence (for PCR)
- RHS probes contain a synthetic poly(A) tail (for slide capture)
  - Both probe pairs need for mRNA detection

Designed to maximize specificity, sensitivity, and sequencing efficiency

- Target all annotated isoforms
- Avoid common sequence variants
- Avoid polymorphic regions
  - >18,000 coding genes specifically detected
  - >55,000 probe pairs
    - 3-fold coverage for most genes
    - 1-fold coverage for top high expression genes
  - <10% genes have 1-fold coverage

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Critical Assay Components

Human Immune Cell Profiling Panel antibodies



Antibody Panel Composition

- Lyophilized 35-plex antibody panel
  - Targets intracellular and extracellular antigens
- 4 isotype controls
  - Used for normalization
- Antibodies sourced from BioLegend and Abcam

CytAssist Panel Antibody Tag

- Read 2
  - Illumina TruSeq Read 2
- Antibody Barcode
  - Unique sequence per unique antibody
- Capture Sequence 1
  - Sequence for antibody tag capture

Reactivity

- Antibodies reactive to *human* proteins from *FFPE tissue* that are retrieved as per our documentation

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Human Immune Cell Profiling Panel

Composition – 35 antibodies sourced from Abcam and BioLegend

Target	Cell Type or Marker	Clone ID	Vendor
alphaSMA	Structural marker	EPR5368	Abcam
CD3E	T cells	SP162	
CD4	T cells	EPR6855	
CD11b	Myeloid cells	EP1345Y	
CD11c	Dendritic cells	EP1347Y	
CD14	Myeloid cells	EPR3652	
CD16	Granulocytes/NK cells	EPR16784	
CD20	B cells	EP459Y	
CD27	Functional B cells	EPR8569	
CD31	Endothelial cells	EPR3094	
CD40	T and B lymphocytes	EPR20735	
CCR7/CD197	Functional B cells	EPR23192-57	
CXCR5	T cells	EPR23463-30	
EPCAM	Epithelial cells	EPR20532-222	
PD1	Checkpoint marker	NAT105	
PDL1	Checkpoint marker	73-10	

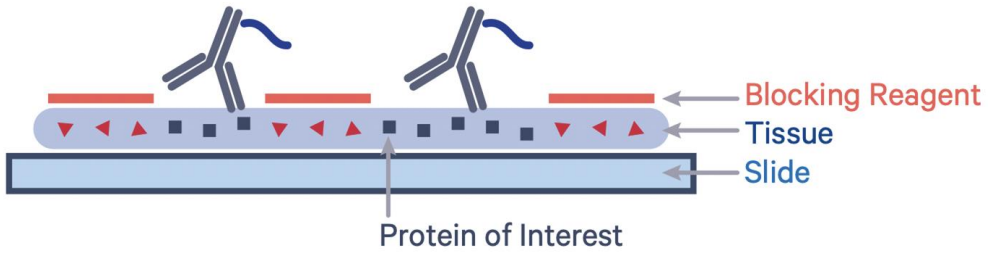
Target	Cell Type or Marker	Clone ID	Vendor
BCL2	Apoptotic marker	100	BioLegend
CD8A	T cells	C8/144B	
CD19	B cells	R109	
CD21	Dendritic and B cells	Bu32	
CD45RA	Functional T cells	HI100	
CD45RO	Functional T cells	UCHL1	
CD66b	Neutrophils	6/40c	
CD68	Macrophages	BL13756	
CD163	Macrophage marker	QA19A16	
CD138	Plasma cells	DL-101	
HLA-DR	Myeloid cells	LN3	
PanCK	Structural marker	AE-1/AE-3	
PAX5	B cells	1H9	
PCNA	Cell proliferation marker	PC10	
Vimentin	Mesenchymal cells	O91D3	
IgG2a	Isotype controls	MG2a-53	BioLegend
IgG1 k		MOPC-21	
IgG2b k		MPC-11	
IgG2a		RTK2758	

View Abcam or BioLegend website for IHC images and other details about antibody clones in Human Immune Cell Profiling Panel

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Summary of User Guide Steps

Tissue Blocking and Protein Labeling



Tissue Blocking, Protein Labeling, and Washes

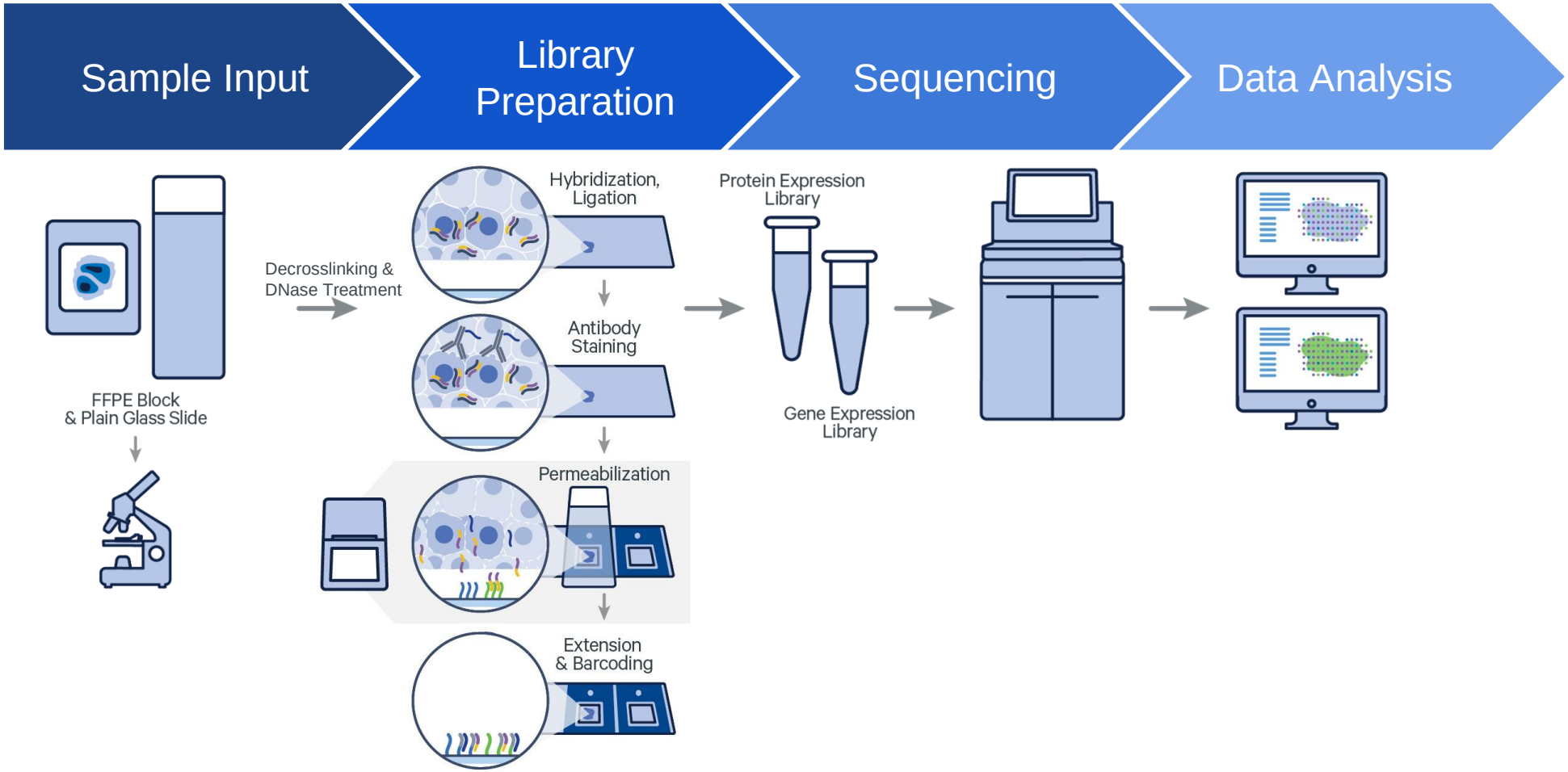
- Block to reduce non-specific antibody binding
  - **RNase Inhibitor** and **Block & Stain Buffer**
- Spin down lyophilized antibody panel
- Reconstitute **Immune Cell Profiling Panel**
  - Utilize **RNase Inhibitor** and **Block & Stain Buffer**
- Pellet antibody aggregates
- Apply supernatant to tissue
- Overnight Antibody Labeling
- After incubation, perform Washes

*When aspirating supernatant from tube containing spun down antibody panel, **do not** place pipette tip at bottom of tube as this will introduce antibody aggregates and impact data quality*

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Spatial Gene and Protein Expression for FFPE

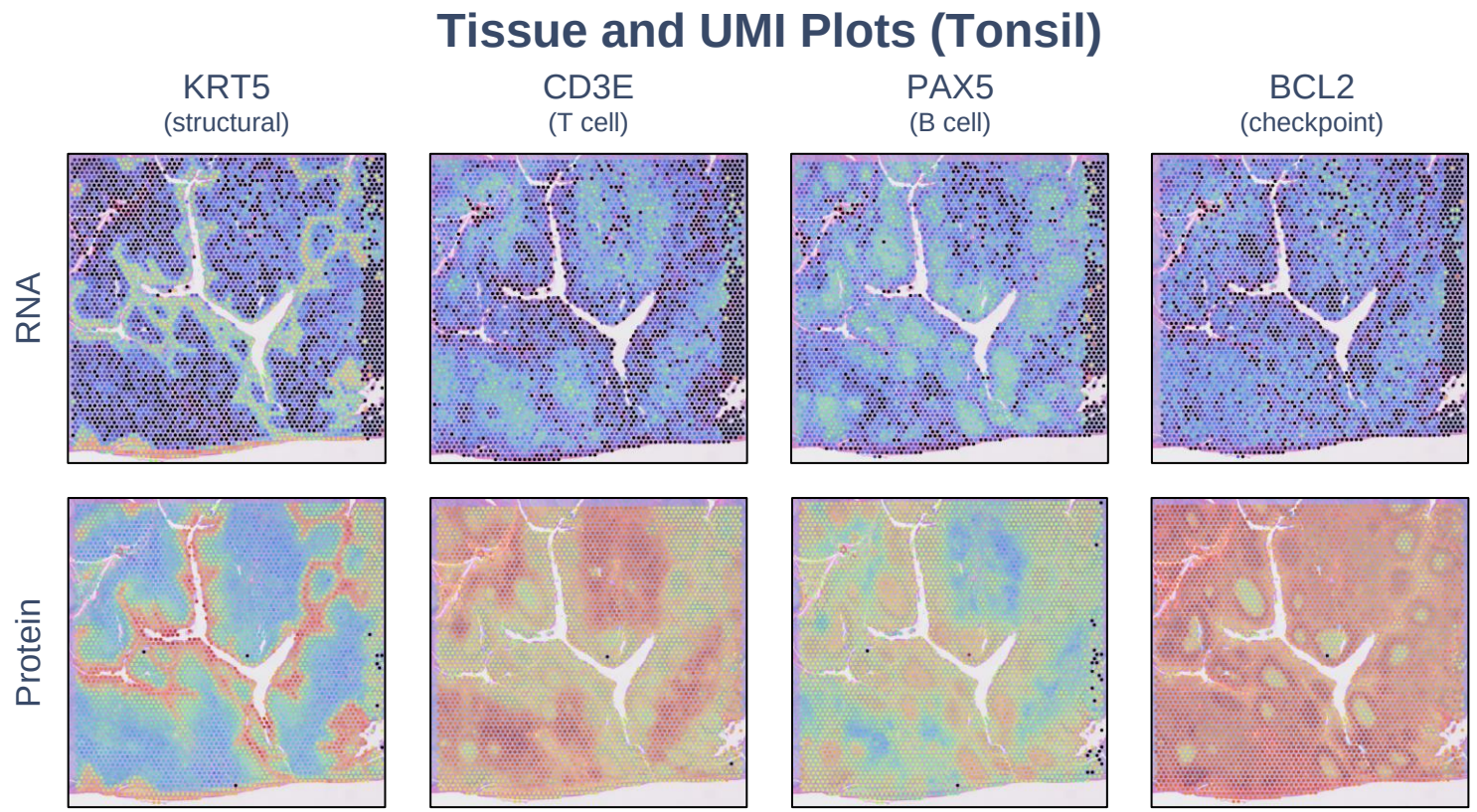
High-level steps to generate multiomic libraries



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Gene and Protein Data Agree with Tissue Morphology

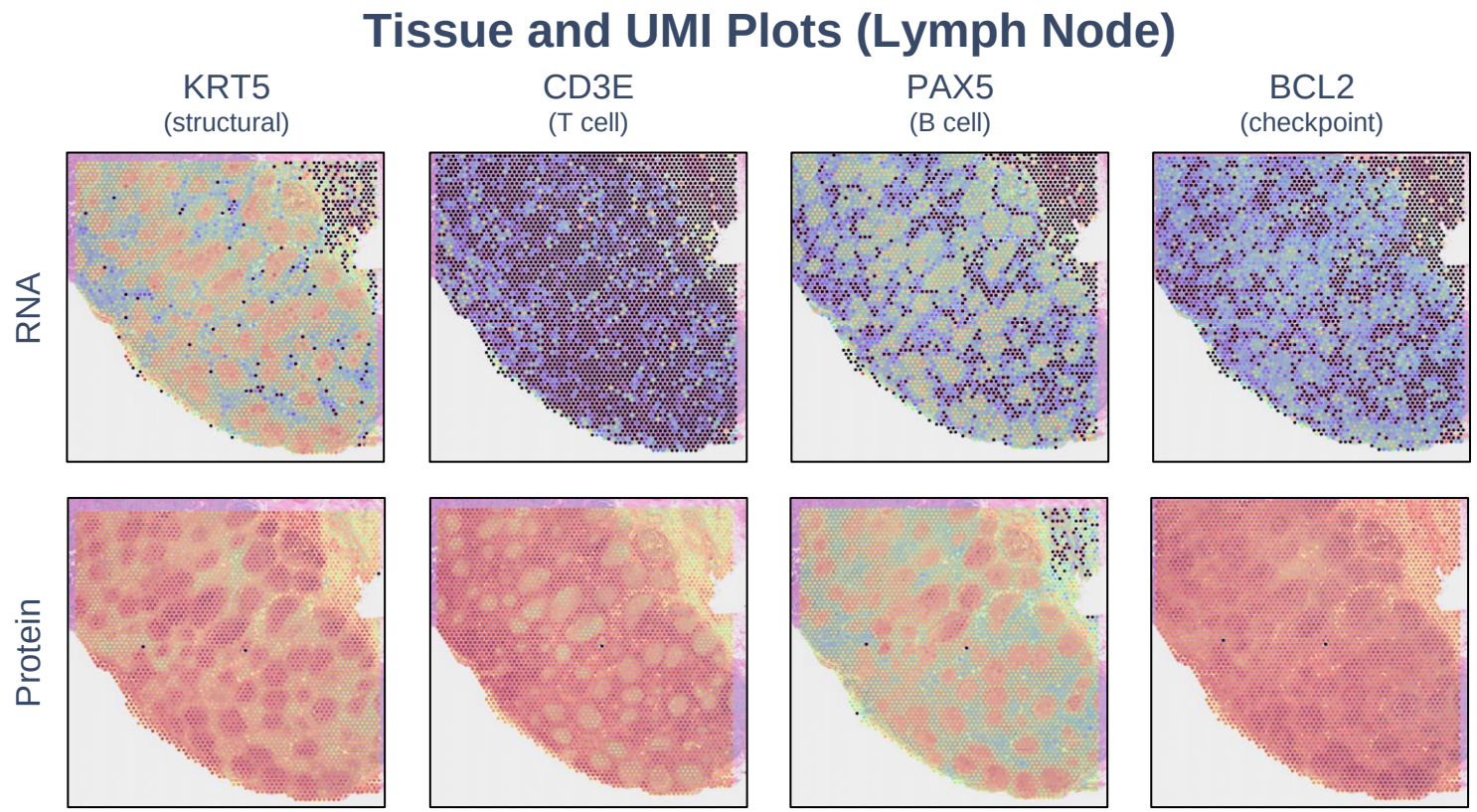
Gene and Protein Expression markers are positively correlated



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Gene and Protein Data Agree with Tissue Morphology

Gene and Protein Expression markers are positively correlated



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Xenium In Situ

Xenium

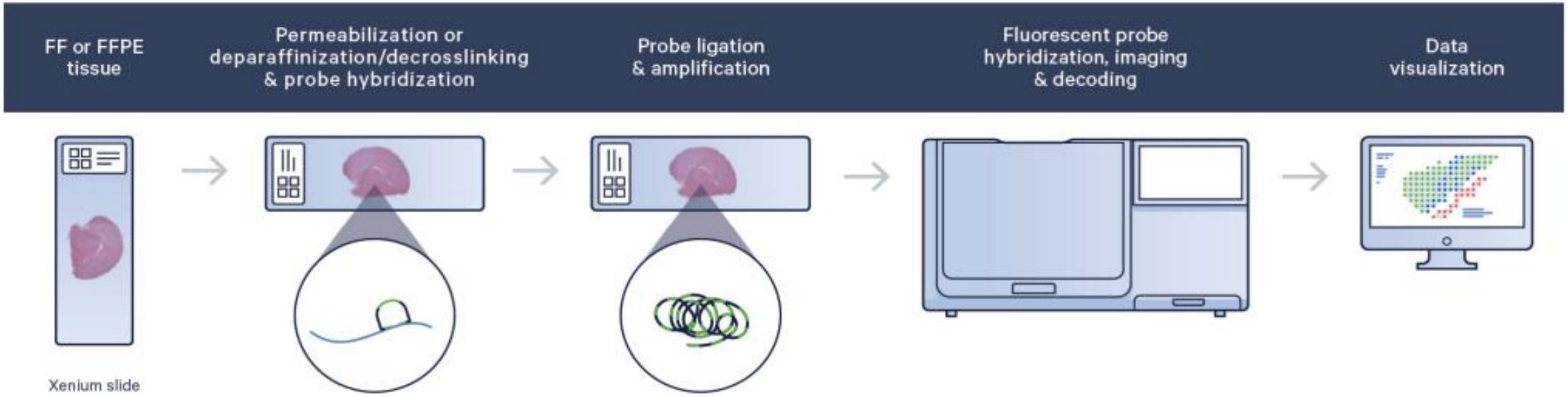
Industry Leading Subcellular In Situ From the Single Cell Leader



- Highly sensitive and specific
- Customizable, biologically relevant content
- Industry-leading speed and throughput
- Easy-to-run workflow with intuitive analysis software
- H&E & IF on the same section
- Best-in-class service and support
- Future-proofed platform

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A Platform Designed to Make In Situ Work for You

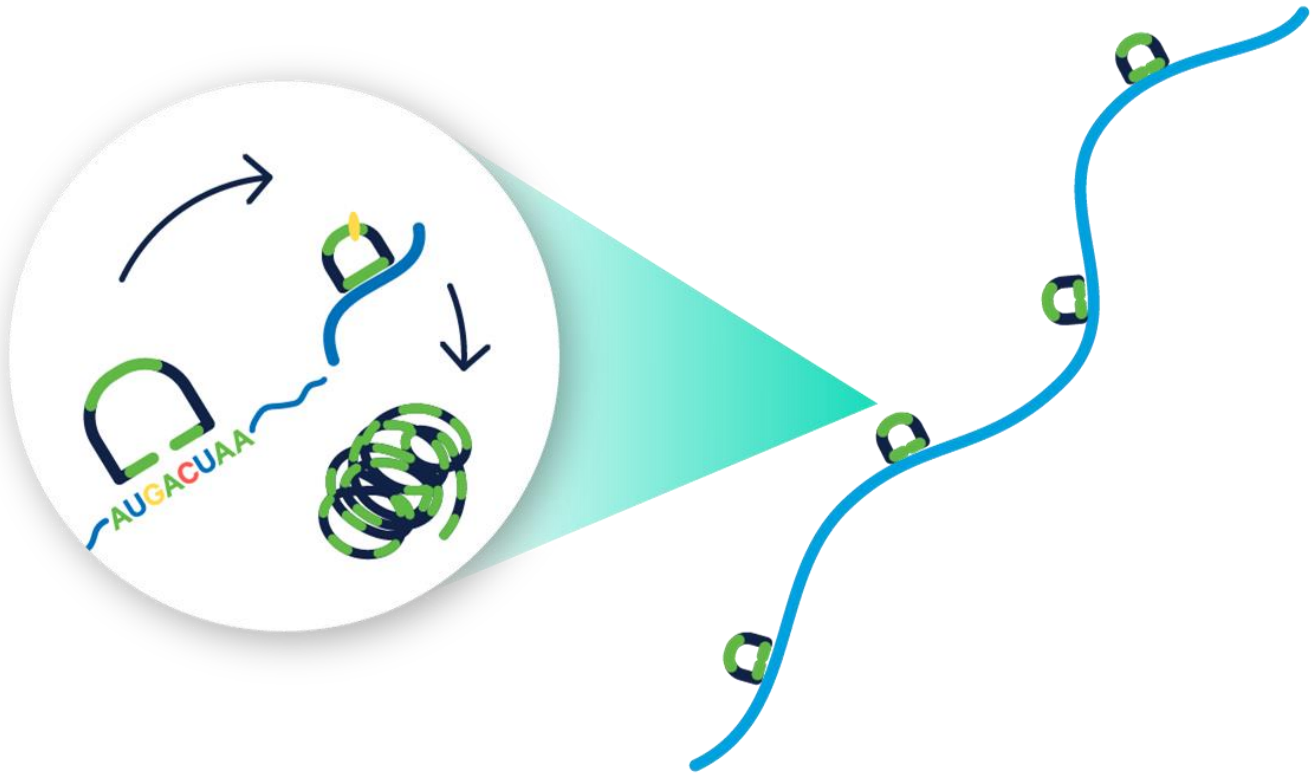


- Easy to implement with only standard tissue preparation techniques needed
- Simple and fast with low hands-on time

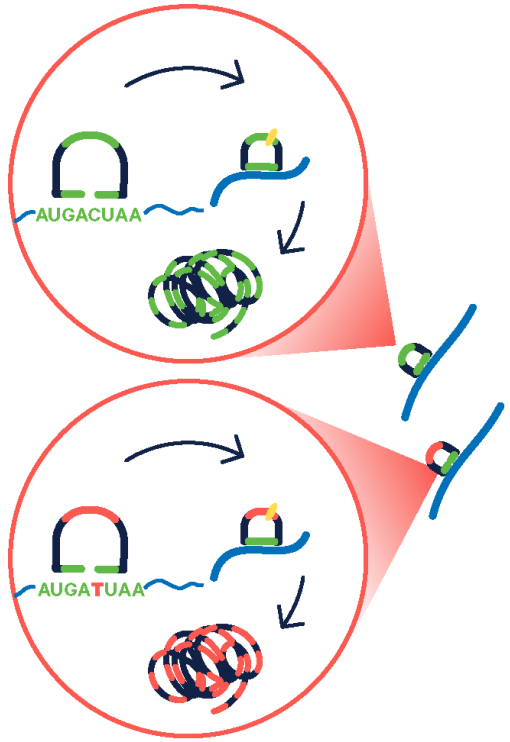
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Xenium Chemistry is Powerful & Highly Flexible

- Minimal gene length requirements
- Tunable detection
- Individual probes bright enough for robust detection



Beyond expression:
Isoform and SNV detection



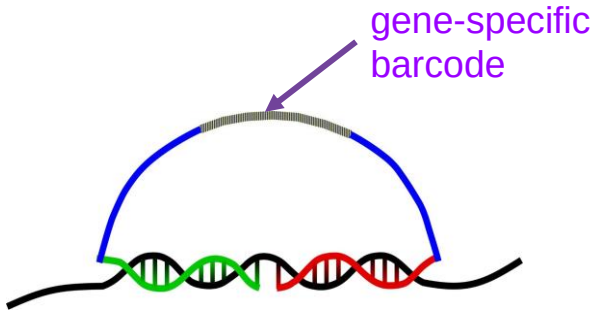
Padlock ligation ensures
high specificity

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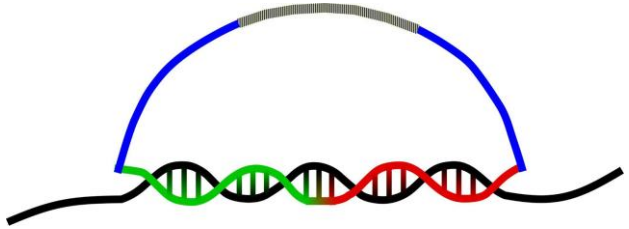
Before Xenium Workflow



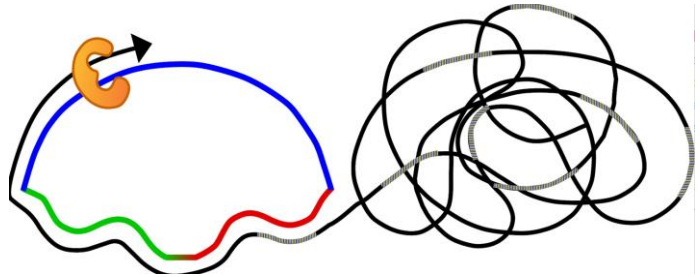
Section tissue



DNA probes hybridized to target RNA



Highly specific ligation



Multiple copies of barcode generated

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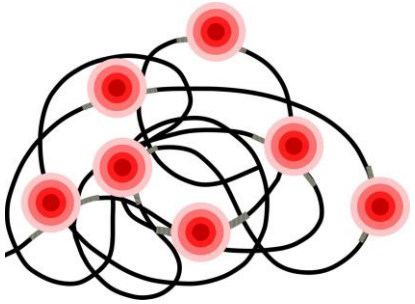
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On Instrument Workflow Includes In-line Decoding

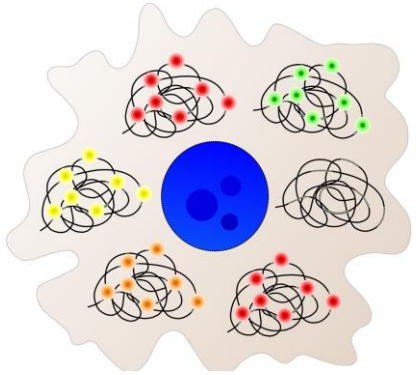
Chemistry and Imaging







Fluorescent oligos hybridize to amplified probes



Multiple cycles of probe binding and stripping

In-line decoding



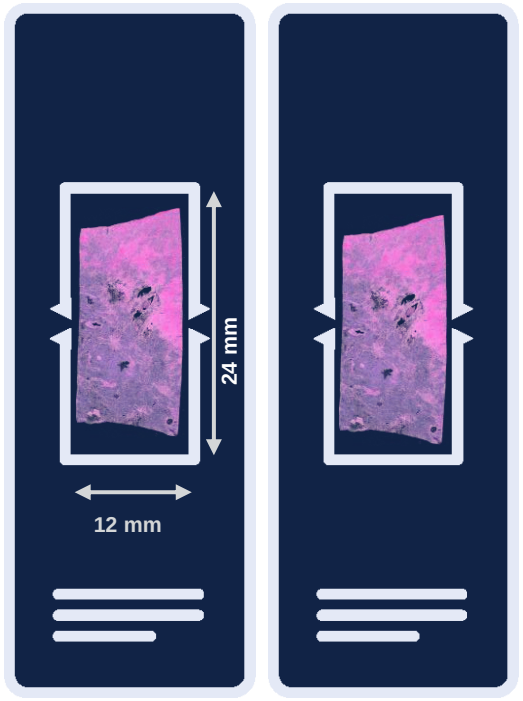
Onboard analysis decodes fluorescent signal across cycles to transcripts

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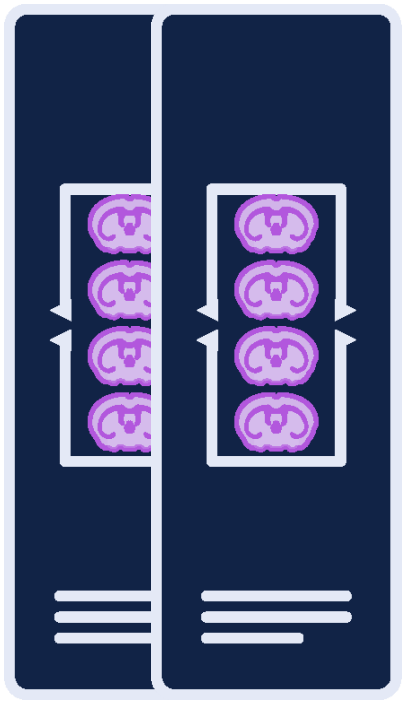
Class Leading Throughput and Cycle Time

Large imageable area maximizes experimental flexibility



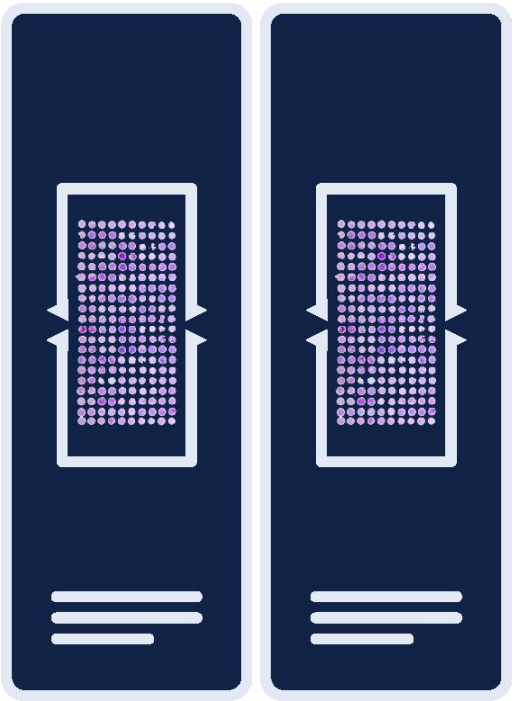
Two 12 x 24 mm sections

~2M cells



Eight 6 x 10 mm
mouse brain sections

~1M cells

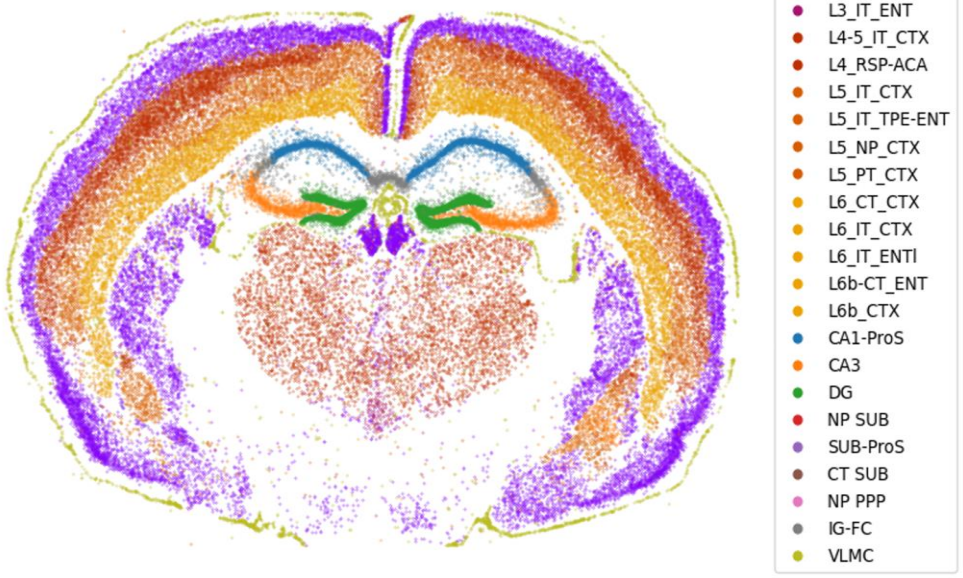


~Hundreds of 1 x 1 mm
biopsies in TMA

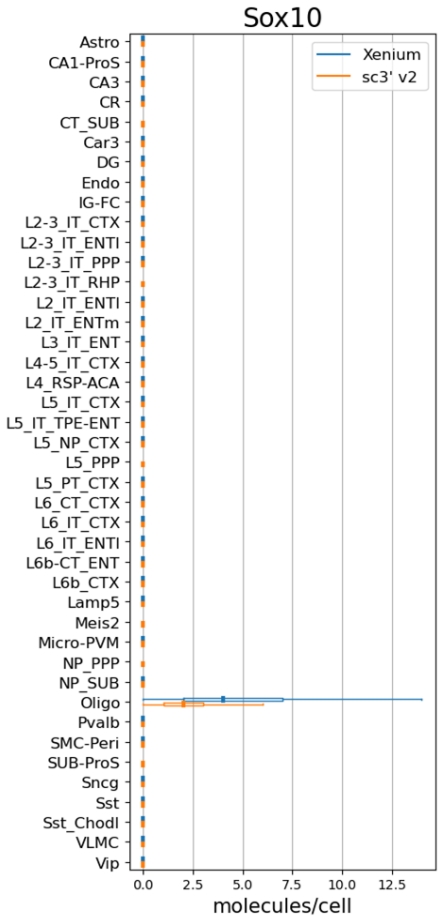
~1M cells

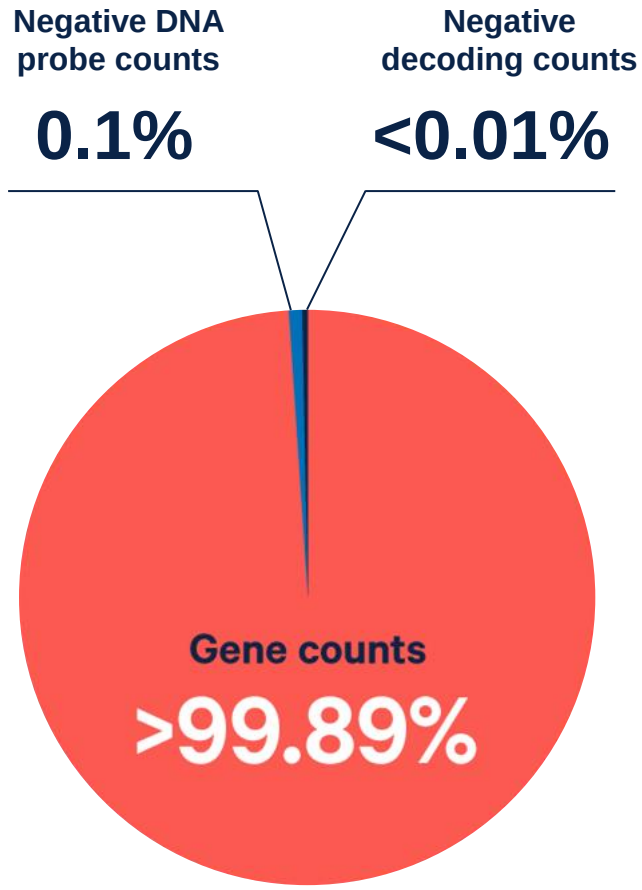
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Xenium Chemistry Shows Strong Specificity



Excitatory cells in fine layers

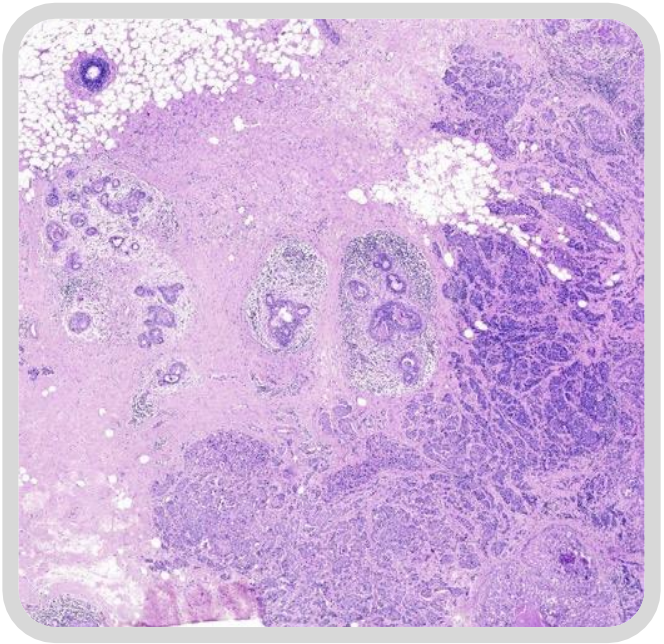




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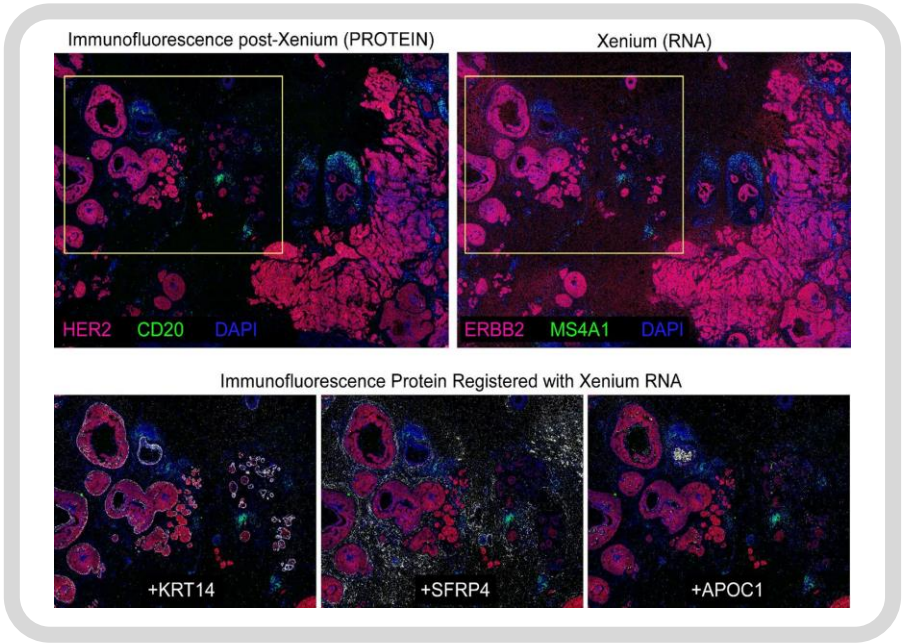
Non-Destructive Workflow Allows for Additional Assays

Perform Xenium In Situ, H&E, and IF staining from a single section



H&E staining

+



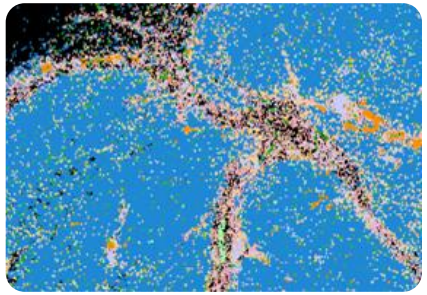
IF staining

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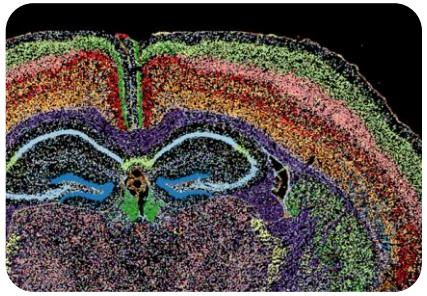
Fit for Purpose, Validated & Customizable Panel Content

Compatible with FF and FFPE

Human breast
280 genes
+100 custom



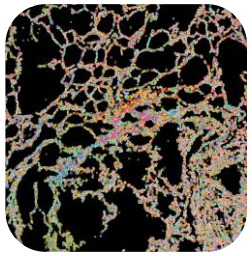
Mouse brain
248 genes
+100 custom



Available Now

Tissue Specific

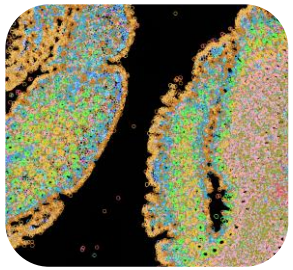


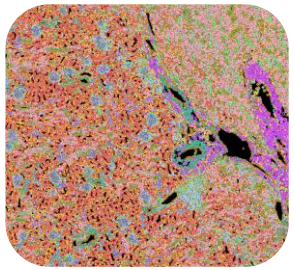


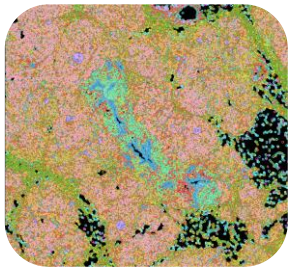
Human brain 266 genes
+100 Custom

Human lung 289 genes
+100 custom

Multi Tissue Human, Multi Tissue Mouse







Breast; Lung; Skin; Gut; Pancreas; Liver; Kidney; Heart; Lymph Nodes*

Coming Soon

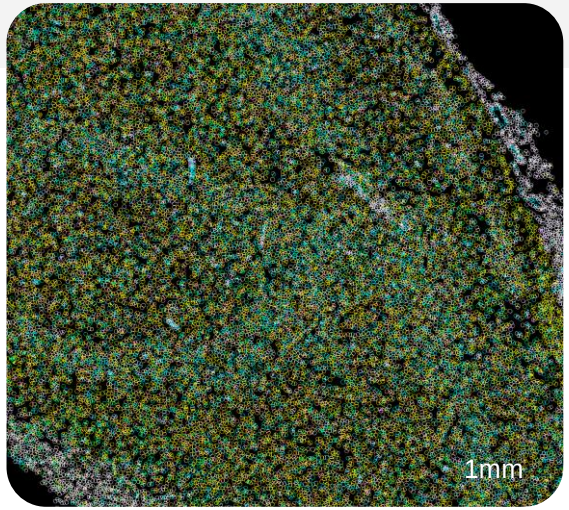
and stand-alone custom of up to 300 genes

*In development; final specs may vary

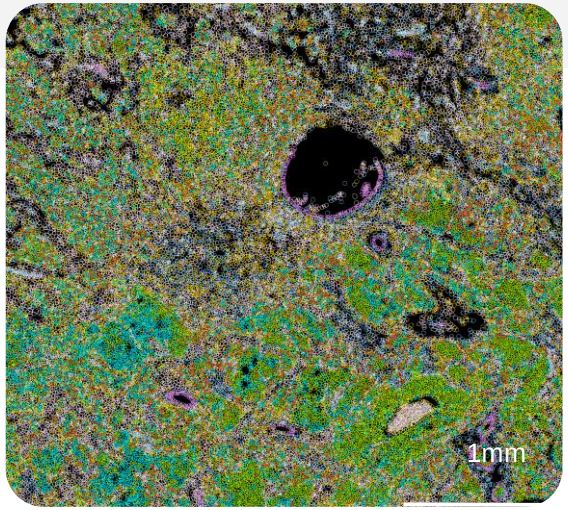
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Xenium is Compatible with a Broad Range of FFPE Tissues

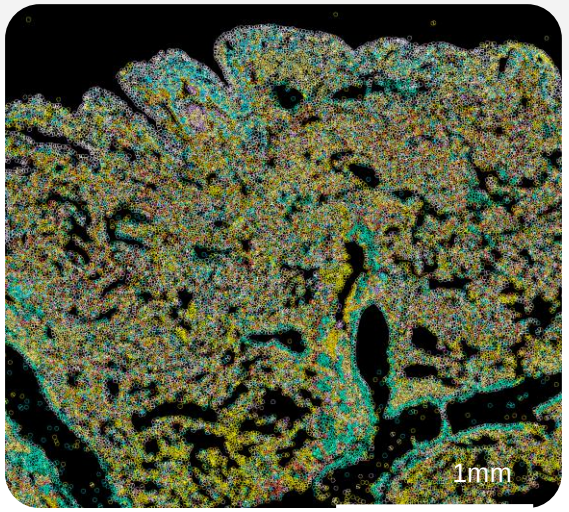
Brain - cancer



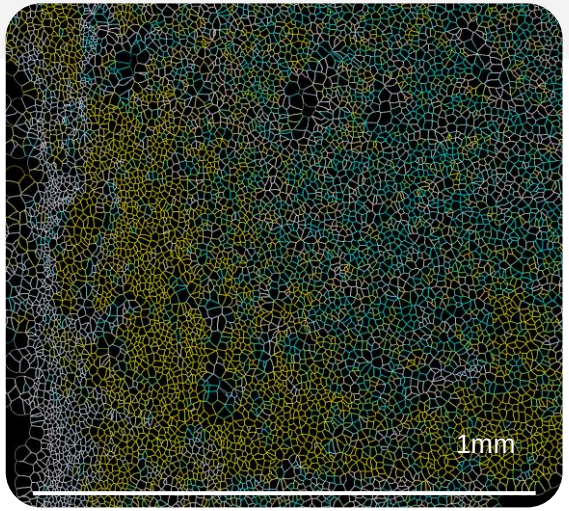
Breast - cancer



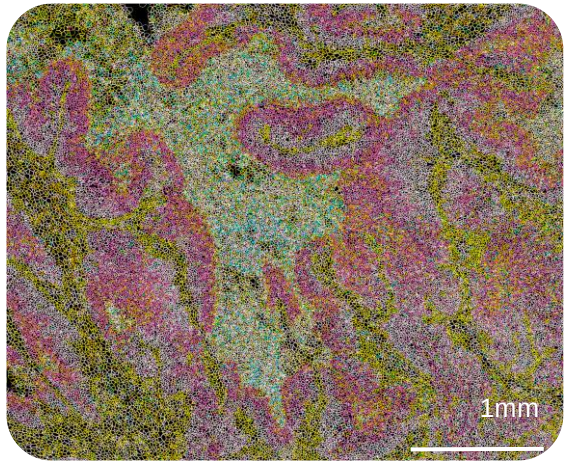
Lung - normal



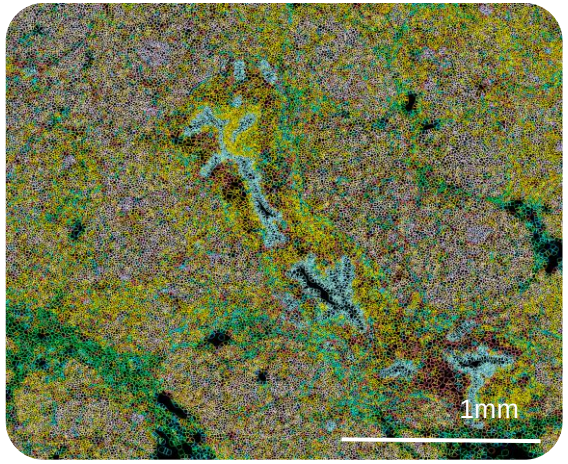
Skin - cancer



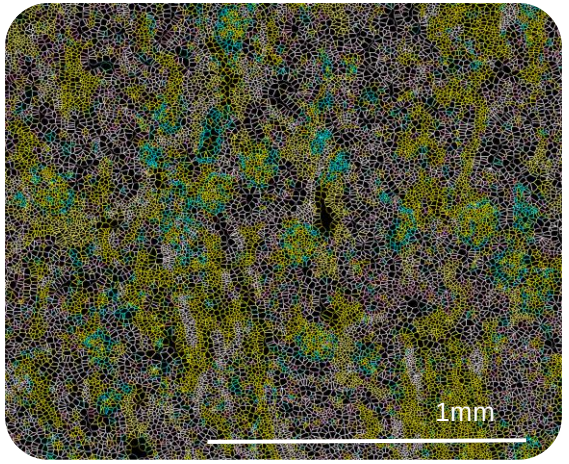
Colon - normal



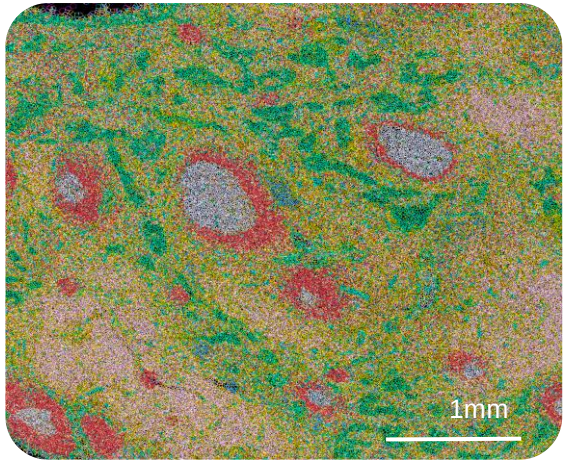
Pancreas - normal



Kidney - normal



Lymph node - normal

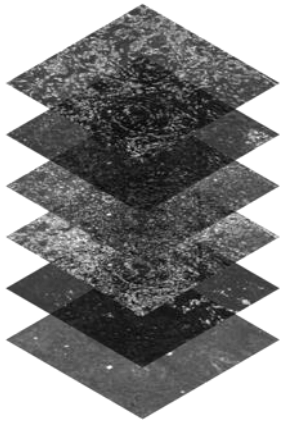


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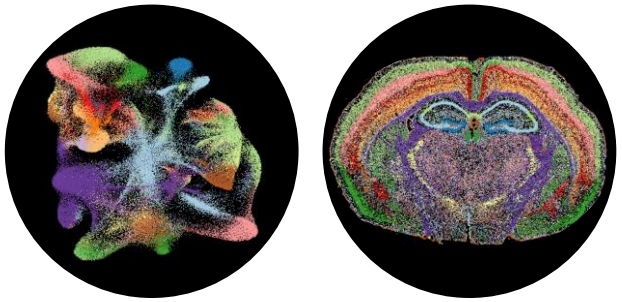
Xenium Onboard Analysis Shortens the Path to Insight

Exploration-ready data output

Comprehensive on instrument analysis

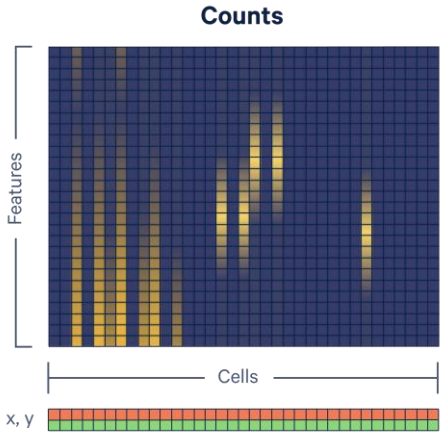


Decoding in line with biochemistry and imaging cycles



On-instrument secondary analysis, including cell segmentation

Visualization & downstream analysis



Ready to use standard file formats for further analysis in community tools



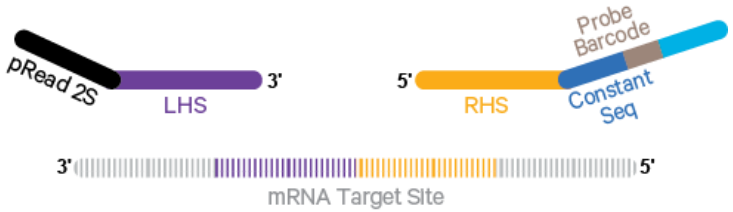
Xenium Explorer for interactive visualization analysis off instrument

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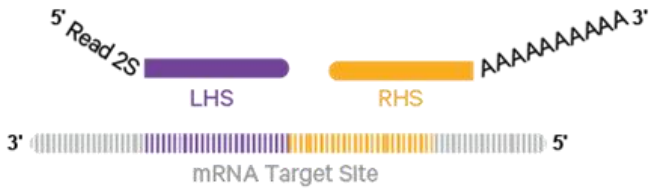
10x Probe-Based Chemistries Provide Highly Specific and Customizable Workflows

10x Probe-Based Chemistries Provide Highly Specific and Customizable Workflows

Chromium Gene Expression Flex



Visium for FFPE



Xenium



Technical Note

CG000621 | Rev A

Custom Probe Design for Visium Spatial Gene Expression and Chromium Single Cell Gene Expression Flex

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Integrated Single Cell, Spatial and In Situ Analysis of the Breast Cancer Microenvironment

Highly Synergistic Platforms Enable the Deepest Biological Insights

Shared Sample Compatibility, Technologies, Workflows, and Software



Xenium



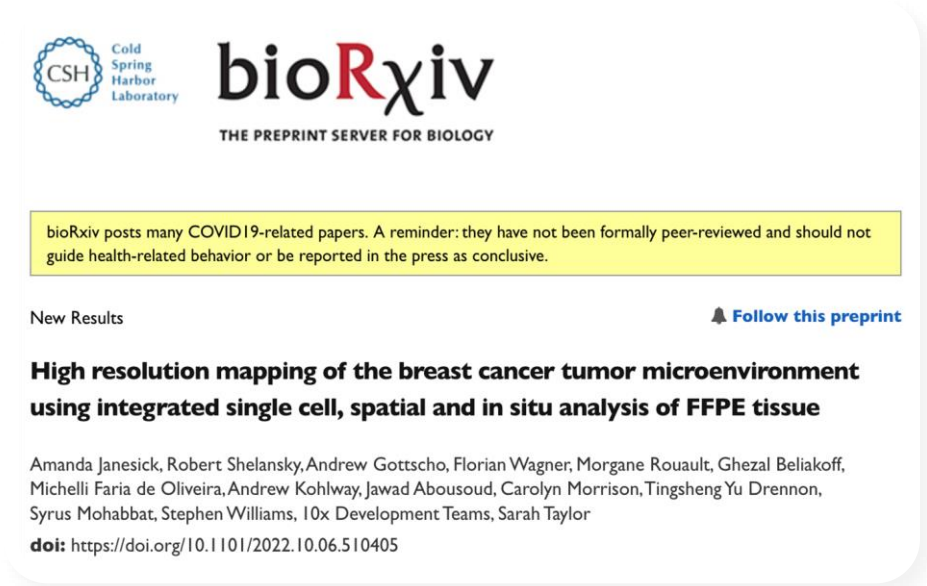
Visium

10x GENOMICS

Toolkit



Chromium



Cold Spring Harbor Laboratory

bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

New Results

Follow this preprint

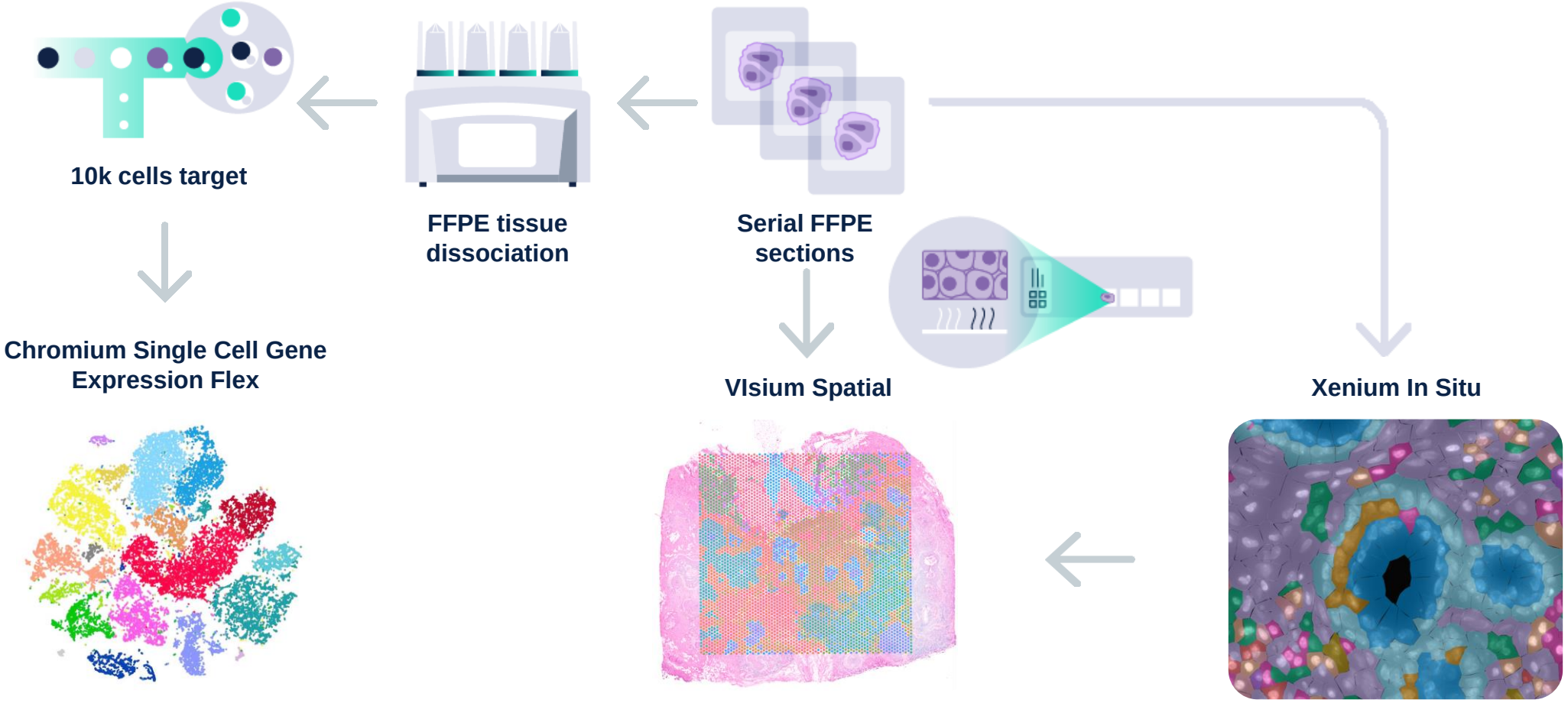
High resolution mapping of the breast cancer tumor microenvironment using integrated single cell, spatial and in situ analysis of FFPE tissue

Amanda Janesick, Robert Shelansky, Andrew Gottscho, Florian Wagner, Morgane Rouault, Ghezal Beliakoff, Michelli Faria de Oliveira, Andrew Kohlway, Jawad Abousoud, Carolyn Morrison, Tingsheng Yu Drennon, Syrus Mohabbat, Stephen Williams, 10x Development Teams, Sarah Taylor

doi: https://doi.org/10.1101/2022.10.06.510405

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Exploring Breast Cancer Biology with 10x Genomics

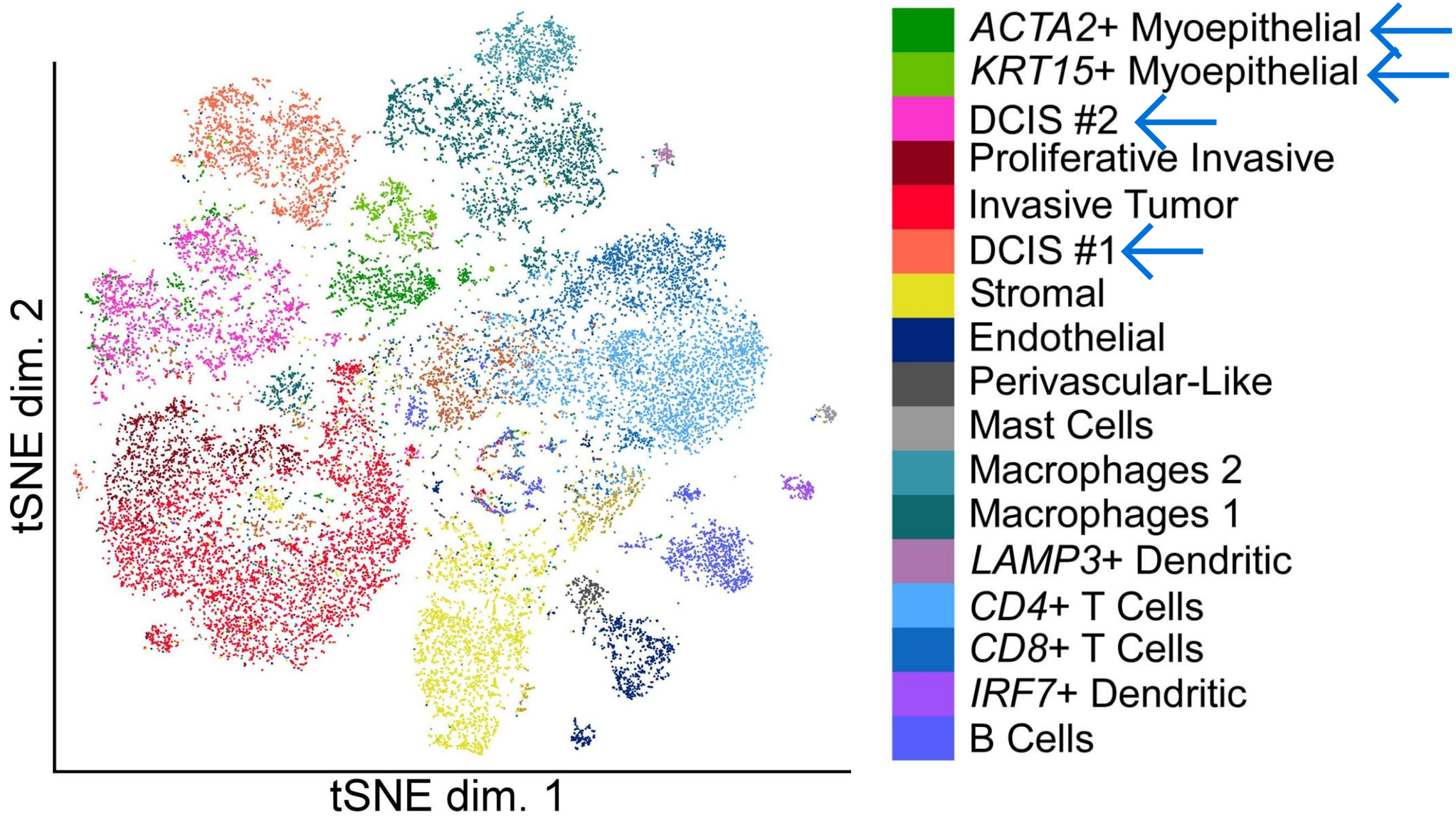


Whole transcriptome

In Situ gene expression

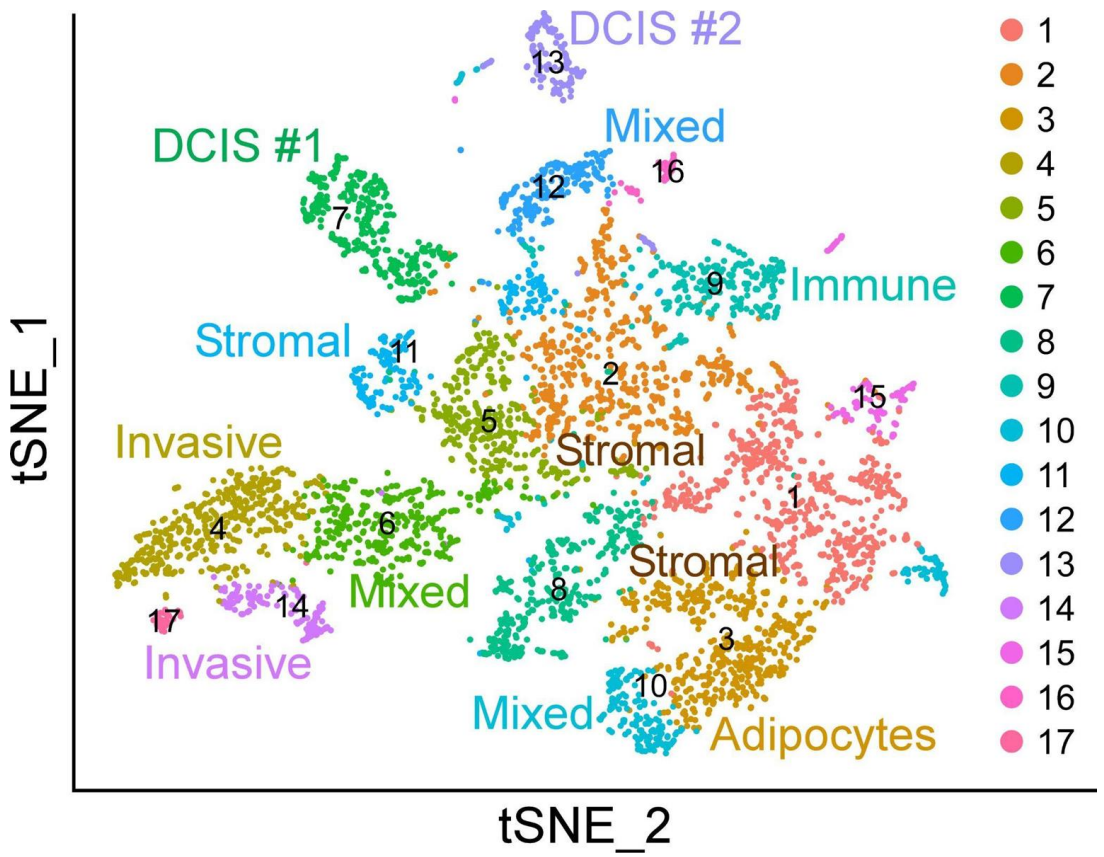
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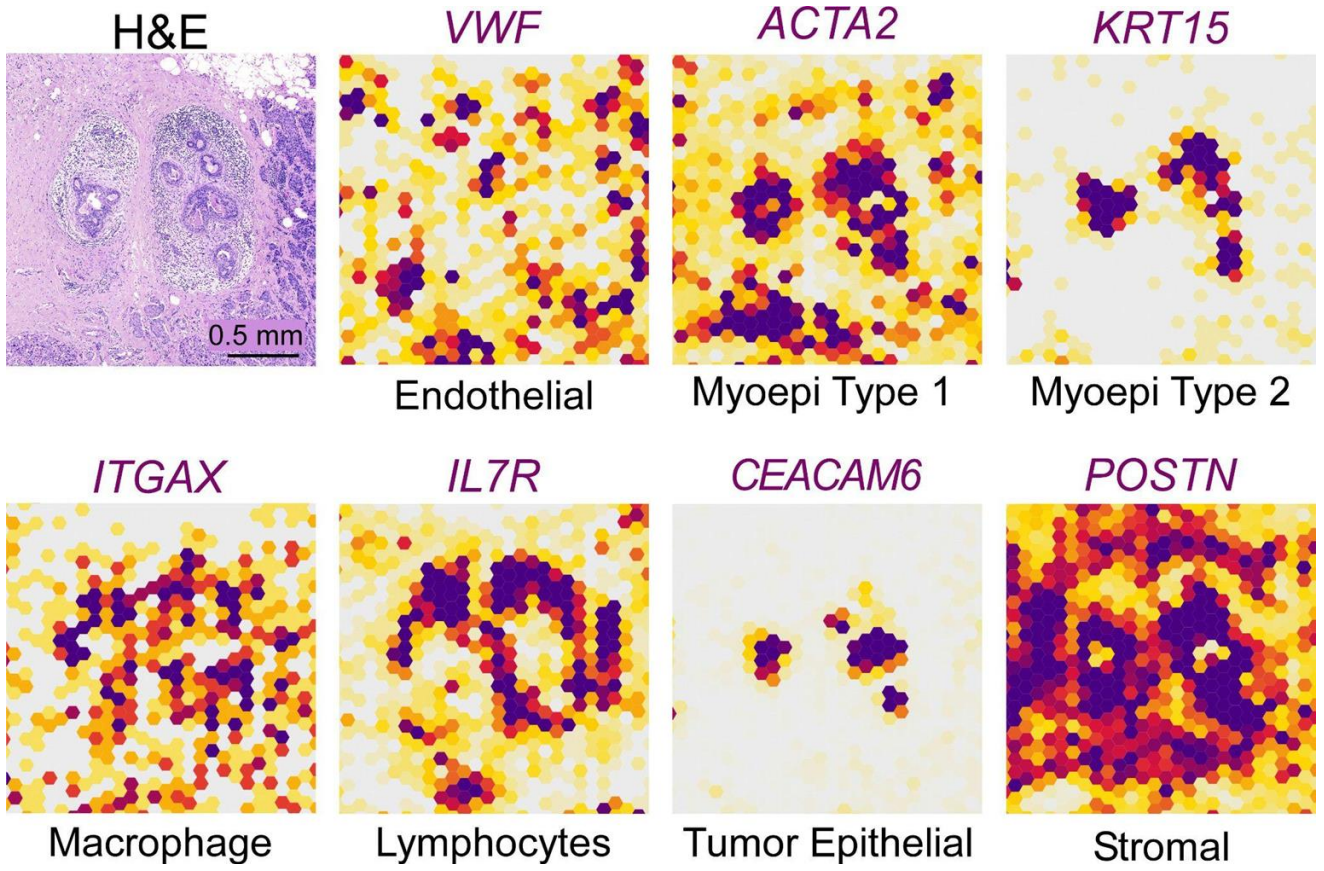
Comprehensively Annotated scFFPE-seq Data



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17 Unique Clusters Identified Using Visium

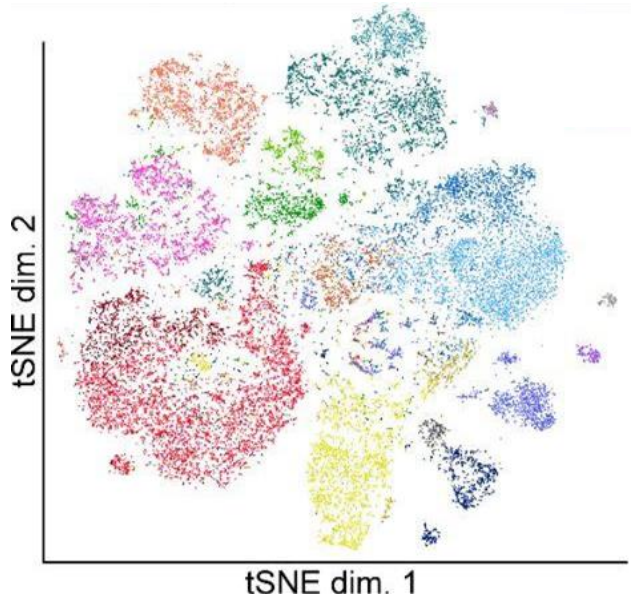




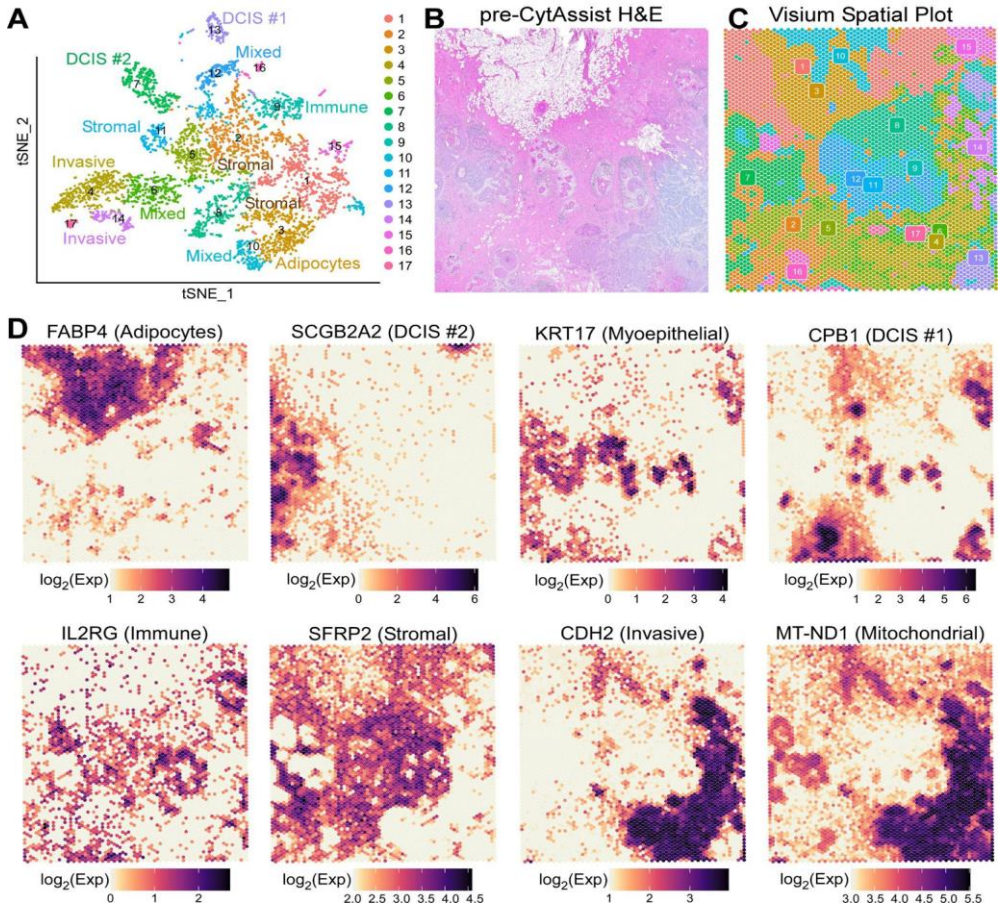
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Chromium and Visium Resolve Main Cellular Components and Reveal Molecularly Distinct Cancer-Associated Cells

Single Cell Whole Transcriptome

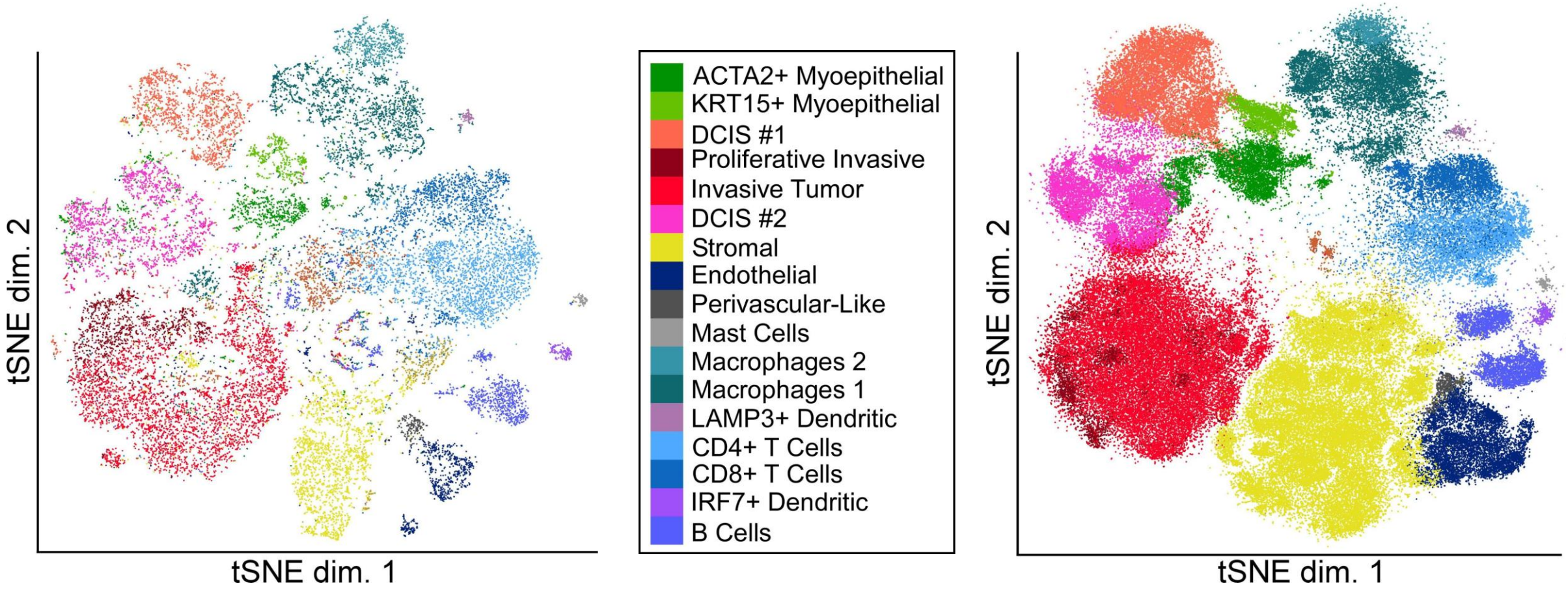


Spatial Whole Transcriptome



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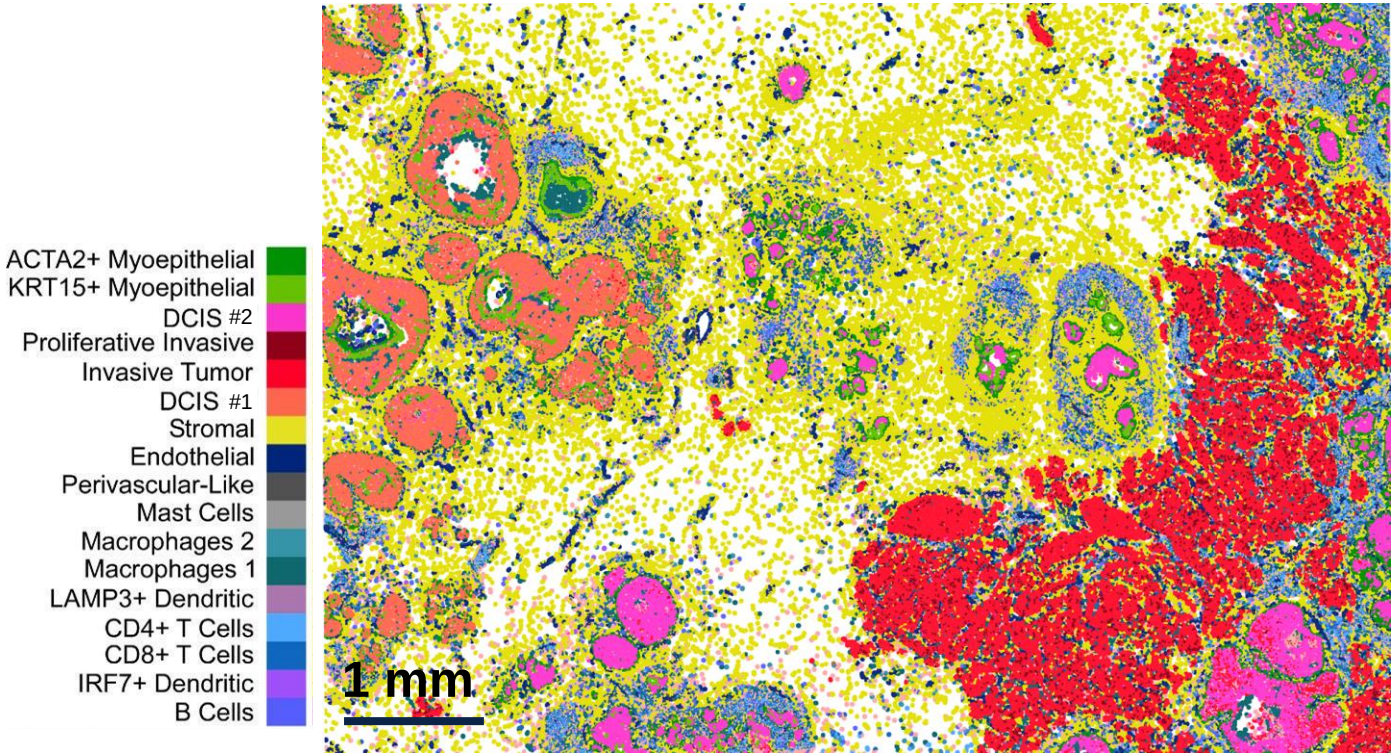
Cell Types in Xenium Data Identified Using scFFPE-seq Annotations



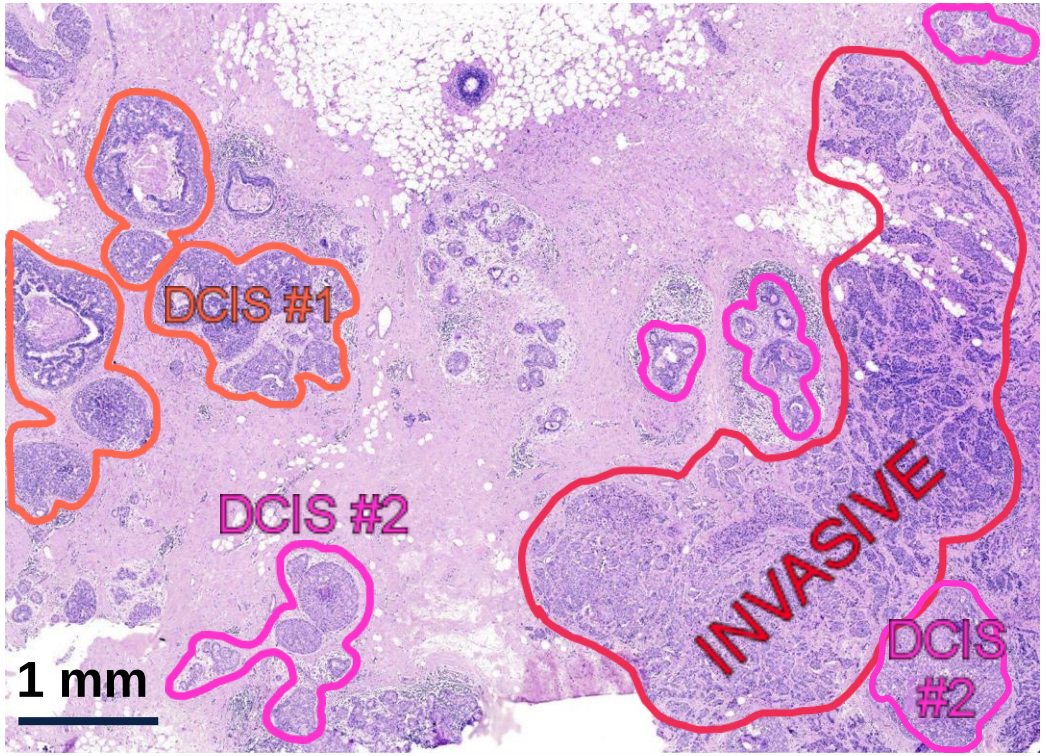
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Xenium Used to Identify the Cellular Composition and Molecular Markers of Tumor Subtypes

Xenium Cell Annotations

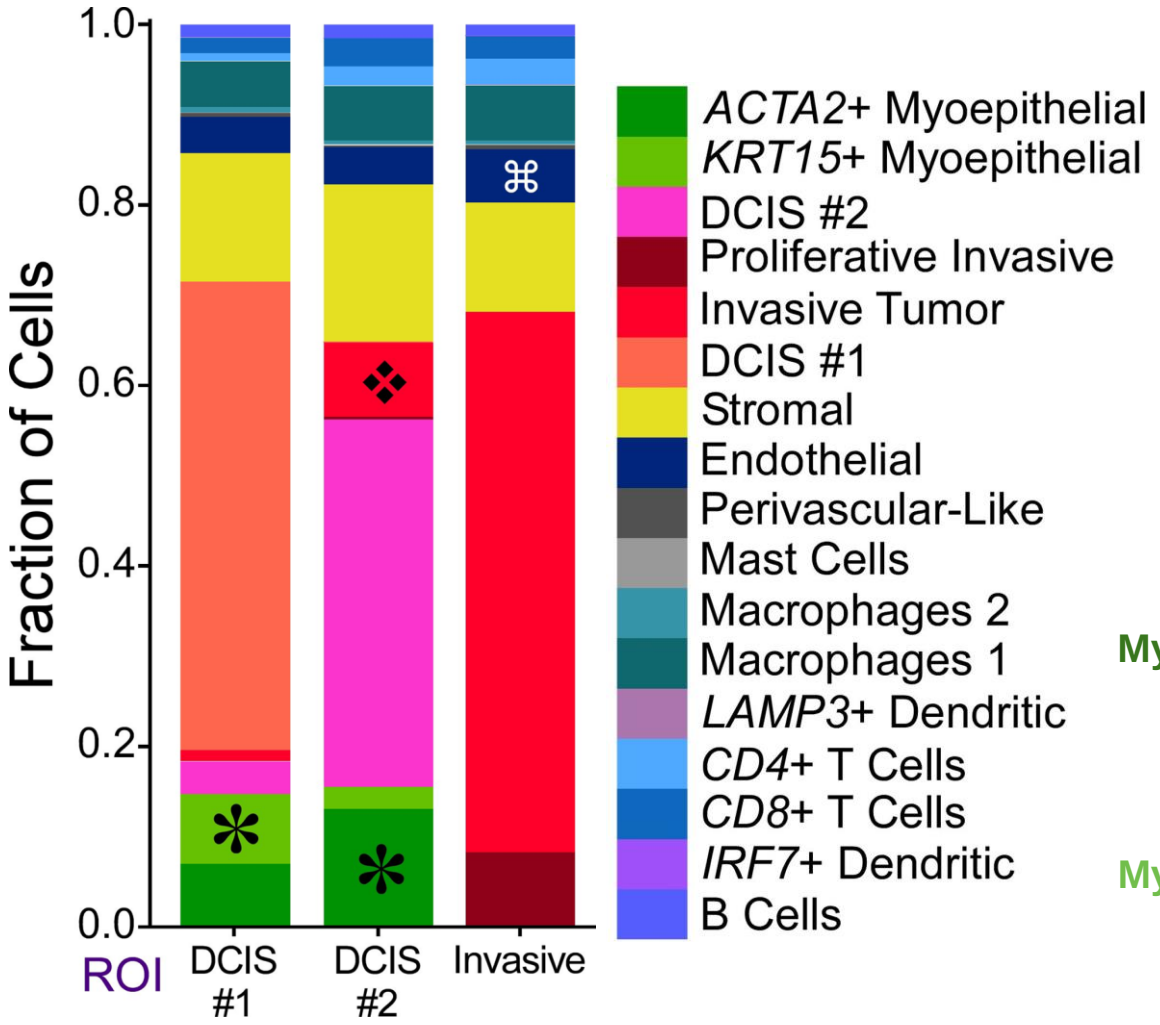


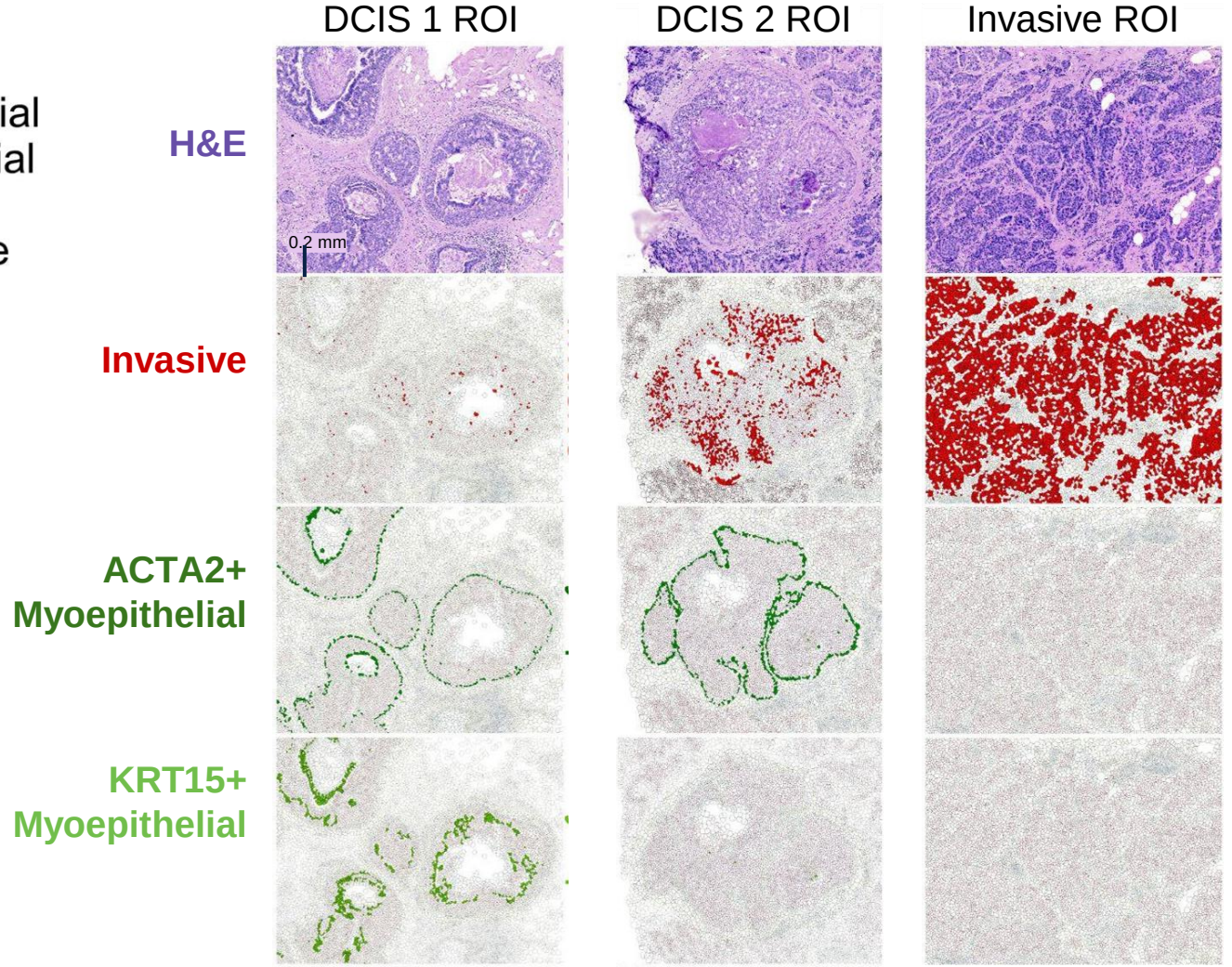
Selection of Tumor ROIs



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Xenium Localizes Cancer-Associated Cell Subtypes on Breast Cancer FFPE Sample



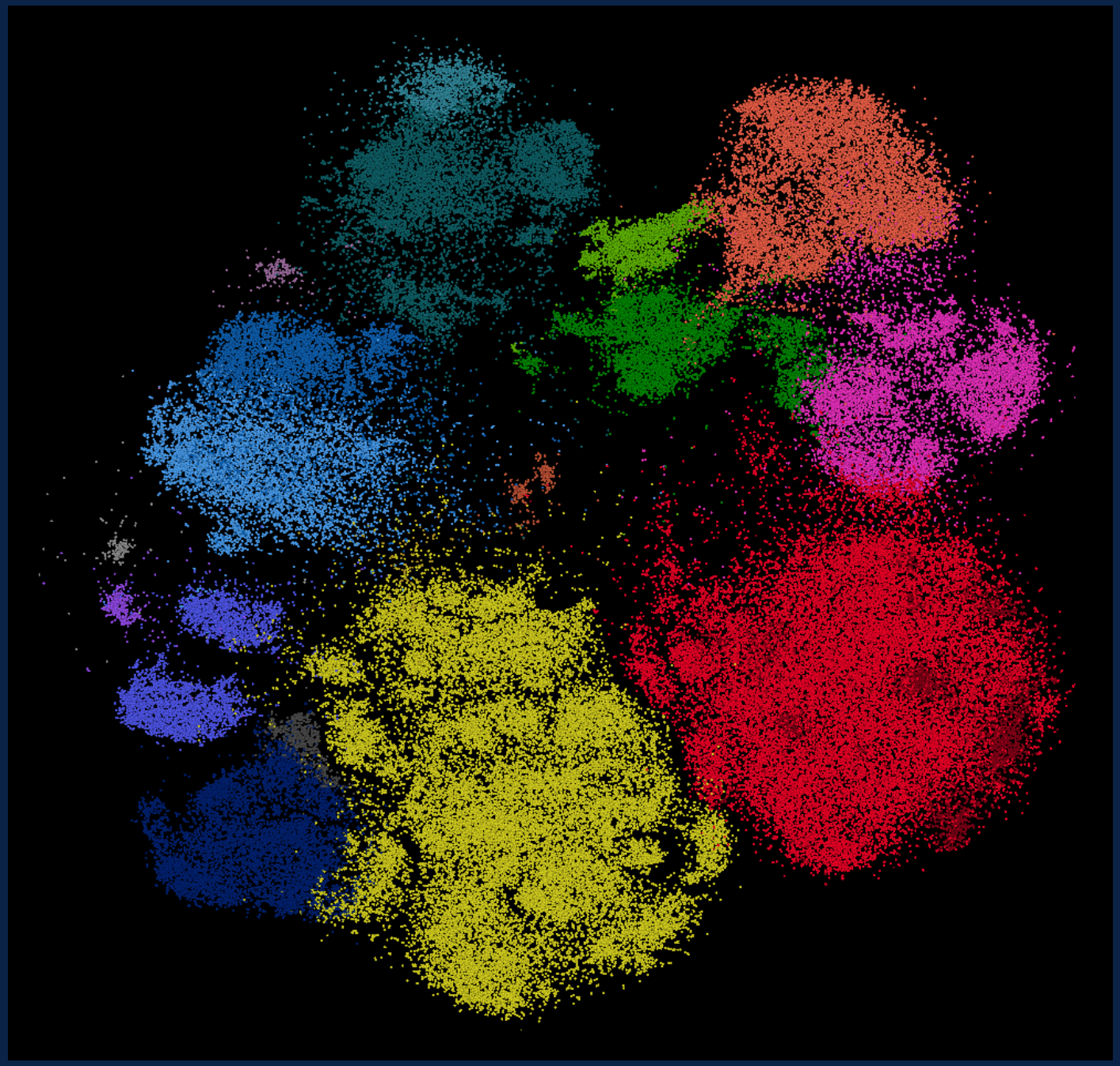


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10x GENOMICS

Thank you!

Questions?



Accelerating Breakthroughs in Cell Therapies with Single Cell Resolution

Owen Kuo, Application Scientist
Pharmigene, Inc

HEMATOLOGICAL MALIGNANCIES →

50%

patients treated with commercial CAR T cells show durable responses

Labanieh et al. CAR immune cells: design principles, resistance and the next generation. Blood Cancer Journal. *2021.*

SOLID TUMORS →

Zero

approved cell therapies for solid tumors

HEMATOLOGICAL MALIGNANCIES →

20-93%

incidence of severe toxicity after commercial CAR T therapy

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20-93%

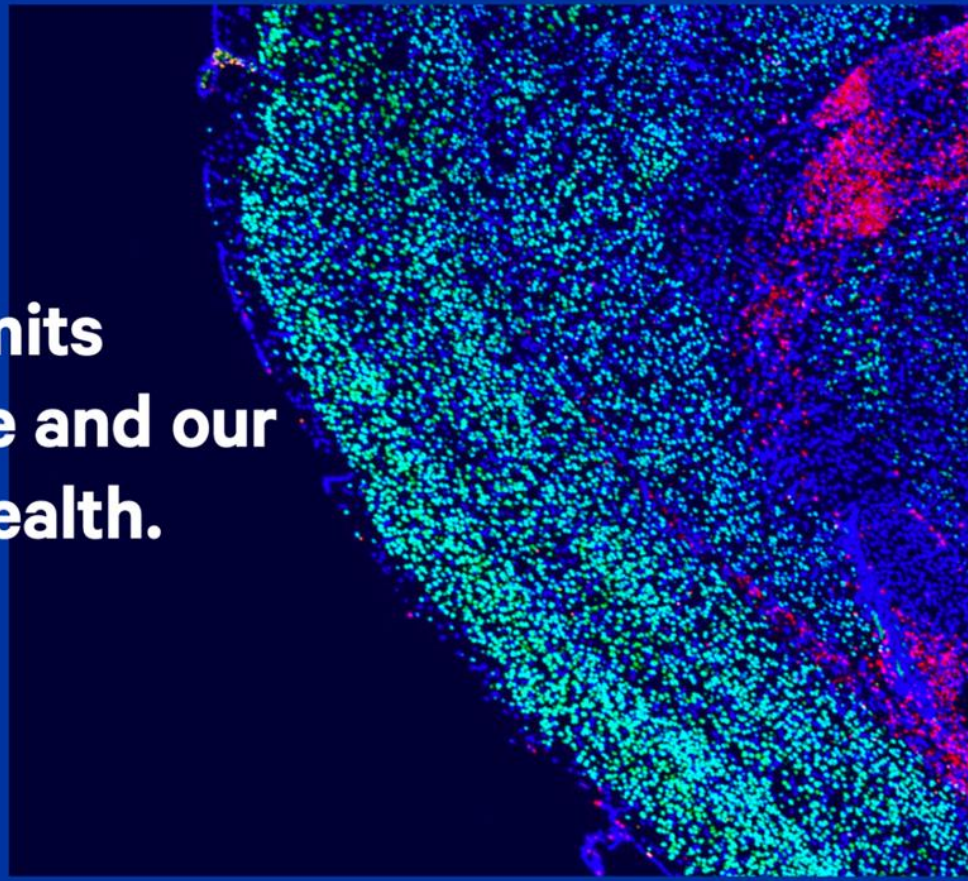
incidence of severe toxicity after commercial CAR T therapy

Santomasso et al. Management of Immune-Related Adverse Events in Patients Treated With Chimeric Antigen Receptor T-Cell Therapy: ASCO Guideline. Journal of Clinical Oncology. 2022.

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10x GENOMICS

The complexity of biology limits our understanding of disease and our abilities to improve human health.



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Chromium

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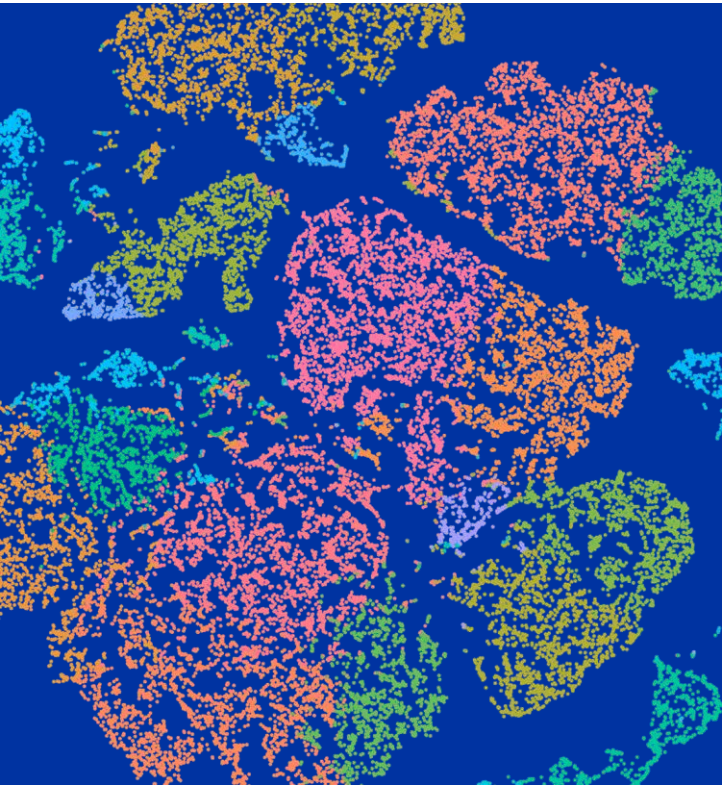
Toolkit



Visium



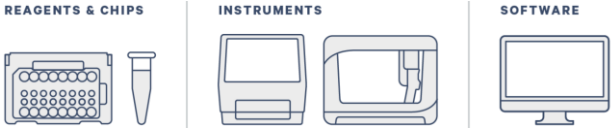
Xenium



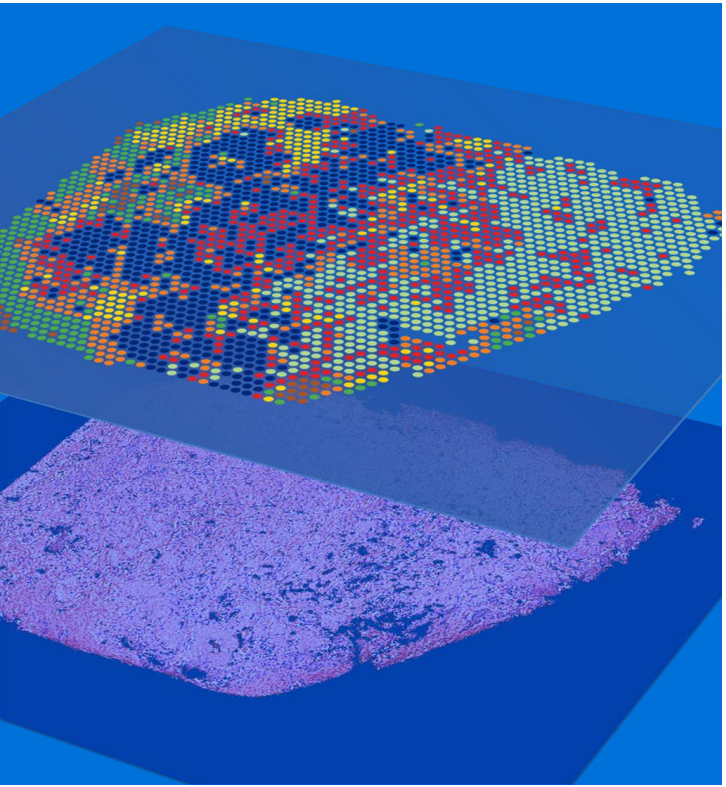
Chromium

RESOLVE TUMOR HETEROGENEITY, CELL BY CELL

- Partitions and barcodes cells in minutes
- Analyzes 100s to millions of cells
- Leverages scale of NGS while preserving cellular identity
- FFPE compatible *(Gene Expression Flex)*
- Perform Single Cell:
  - Gene expression
  - Protein expression
  - Functional genomics by CRISPR
  - Immune repertoire and corresponding antigens
  - Epigenetics



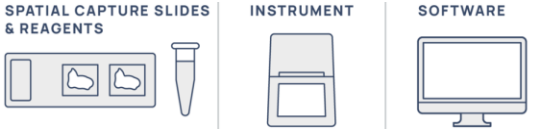
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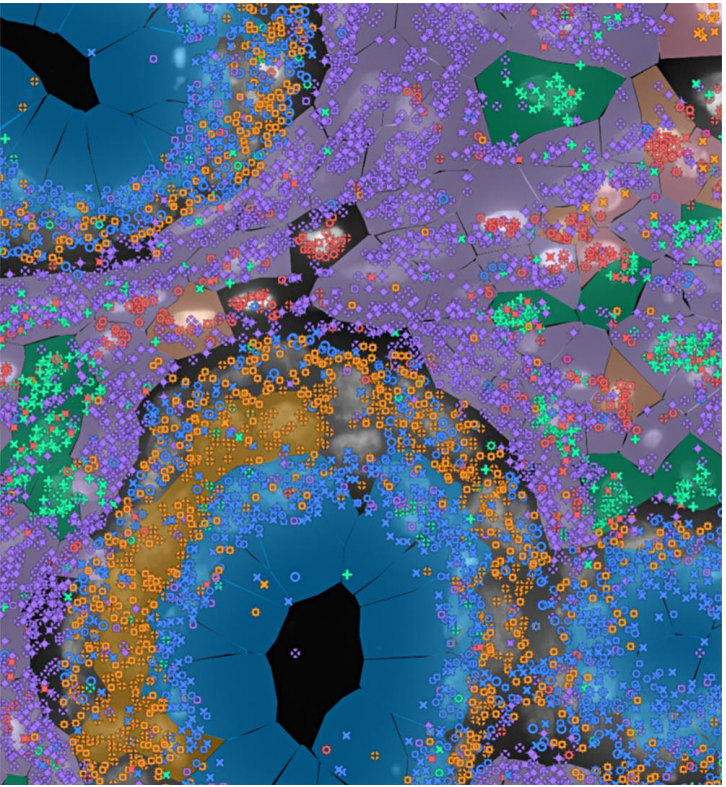
Visium

EXPLORE SPATIAL ARCHITECTURE OF CANCER

- Histological & whole transcriptomic information from an entire tissue section
- Slide contains arrayed barcoded oligos representing spatial locations
- Leverages NGS while preserving spatial location
- Unbiased gene expression in tissue context
- Compatible with Fresh Frozen and FFPE



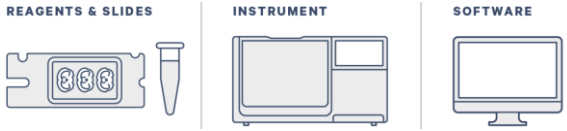
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Xenium

UNCOVER CELL-CELL INTERACTIONS DRIVING FUNCTION

- Reveals the relationship between cellular structure and function
- End-to-end in situ platform with imaging readout (no NGS required)
- Hundreds of RNA targets with subcellular resolution
- Highly specific and sensitive
- High throughput, fast time to results
- Compatible with Fresh Frozen and FFPE

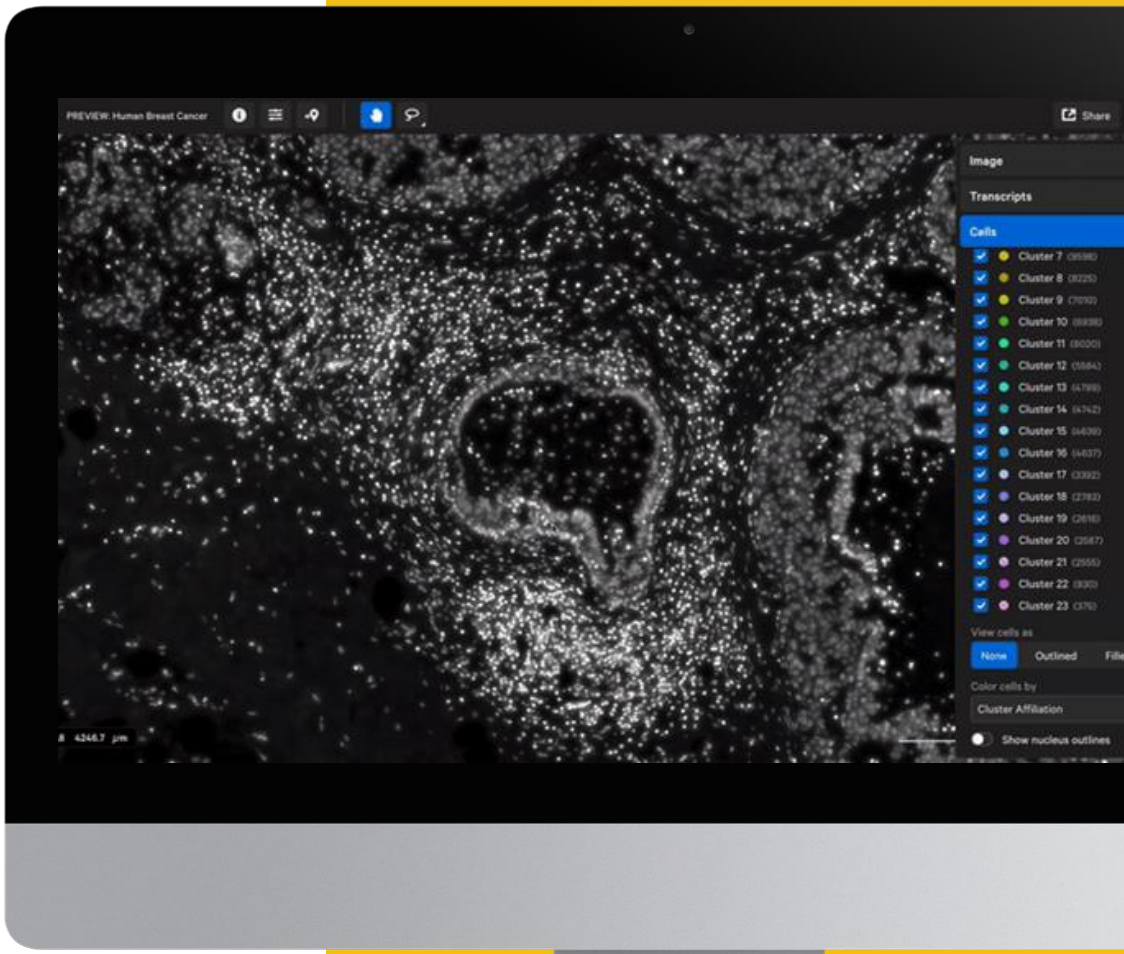


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SOFTWARE:

Multi-faceted

- Included with our products
- Precompiled, easy to install pipelines
- Output compatibility with third party tools
- Desktop visualization with intuitive interface
- Informatics background not necessary
- Backed by 10x Genomics support

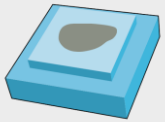


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New technological innovations are unlocking insights from clinical samples at **scale**

Table Stakes

FFPE Compatible



Unlock single cell and spatial insights in archival tissue

High-Throughput



Sufficiently power insights across heterogeneous populations

Cost Efficient



Multiplexing, automation, and throughput reduce cost/insight

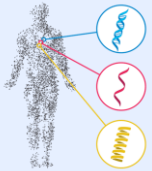
10x Enabled

Cellular Resolution



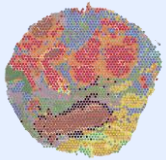
Discover cell-type-specific biomarkers of CAR function

Multiple Features Per Cell



Simultaneously measure multiple features from each CAR T cell

Spatial Context



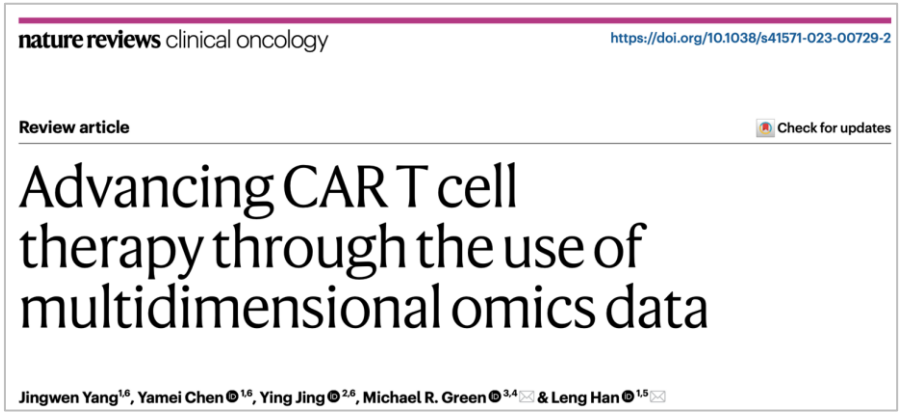
Resolve cell product trafficking and function in tissue

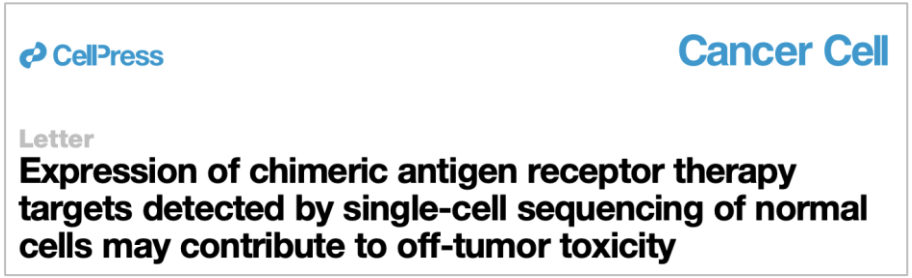
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Single cell profiling is poised to **accelerate** the development of cell therapies across blood and solid cancers







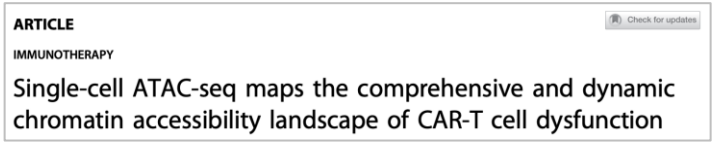


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Case Studies: unlocking **novel insights** to unanswered clinical questions

Therapeutic Development → **Product Characterization** → **Therapeutic Monitoring** → **Resistance**

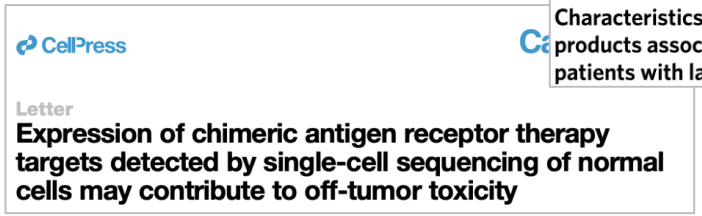








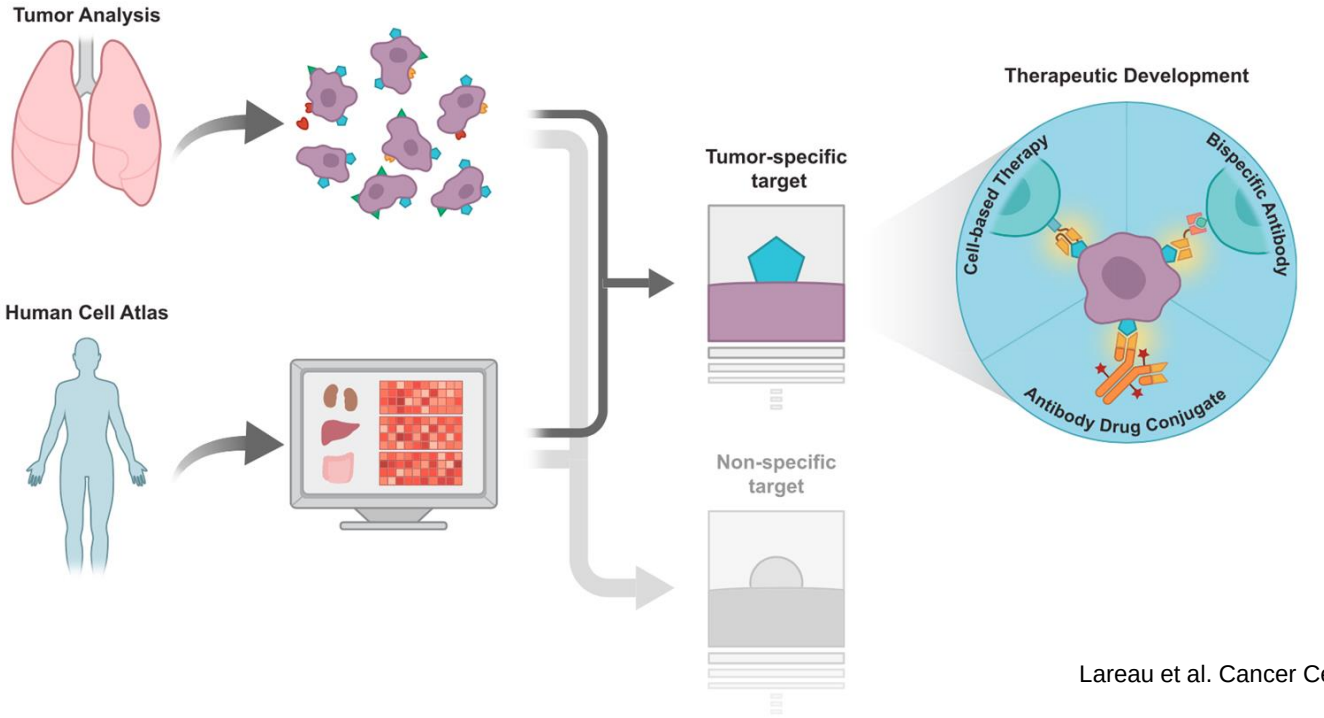






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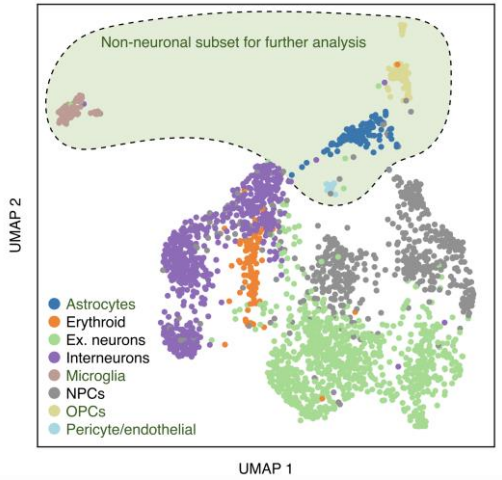
Therapeutic Development: What **antigen targets** are highly tumor-specific and lowly expressed in normal tissue?



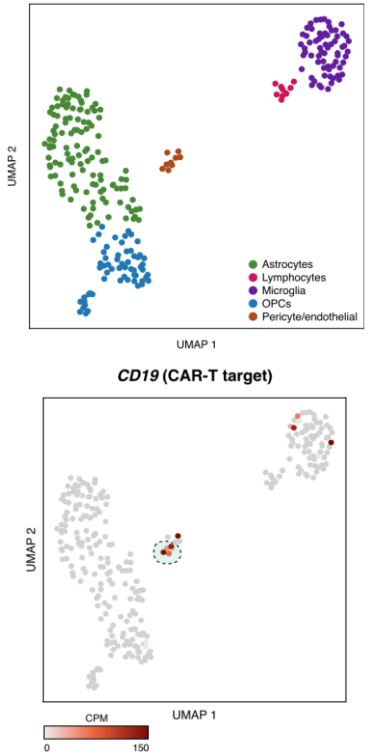
Lareau et al. Cancer Cell. 2021

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scRNA-seq identifies CD19-expressing **pericyte** population in brain



Parker et al. Cell. 2020



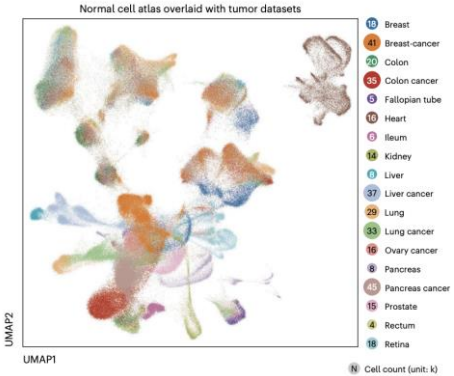
"It's only through single cell sequencing that we're able to identify that there's this very small percentage of cells in the brain that also contain this molecule"

Avery Posey, PhD. UPenn

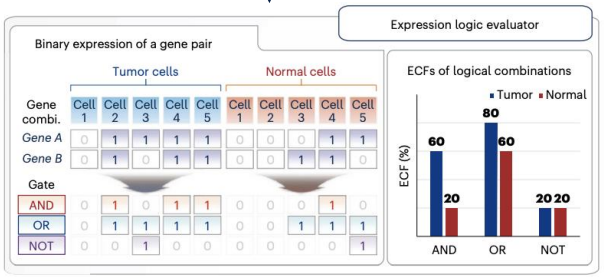
Penn Medicine News "Penn Researchers Discover Potential Cause of Immunotherapy-Related Neurotoxicity, Chart Path Forward"

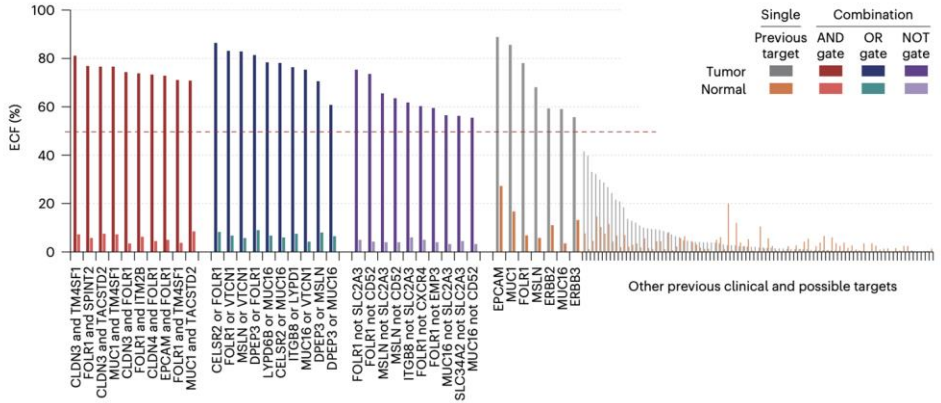
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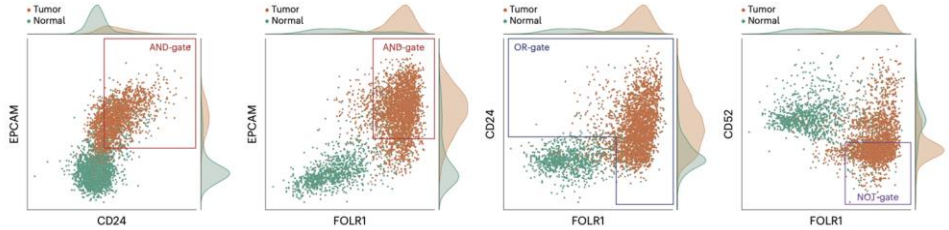
scRNA-seq atlas reveals optimal target antigens for **logic-gated CARs** in solid tumors



1.4 million cells



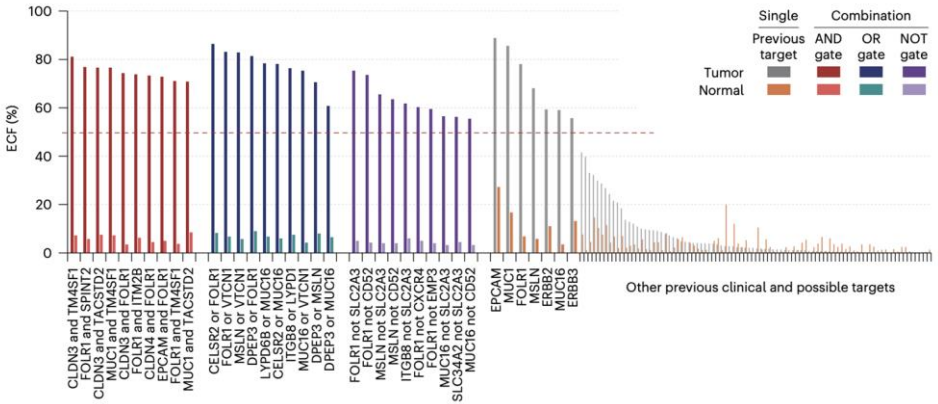




Kwon et al. Nature Biotechnology. 2023

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scRNA-seq is a powerful tool for discovering **effective** and **safe** antigen targets



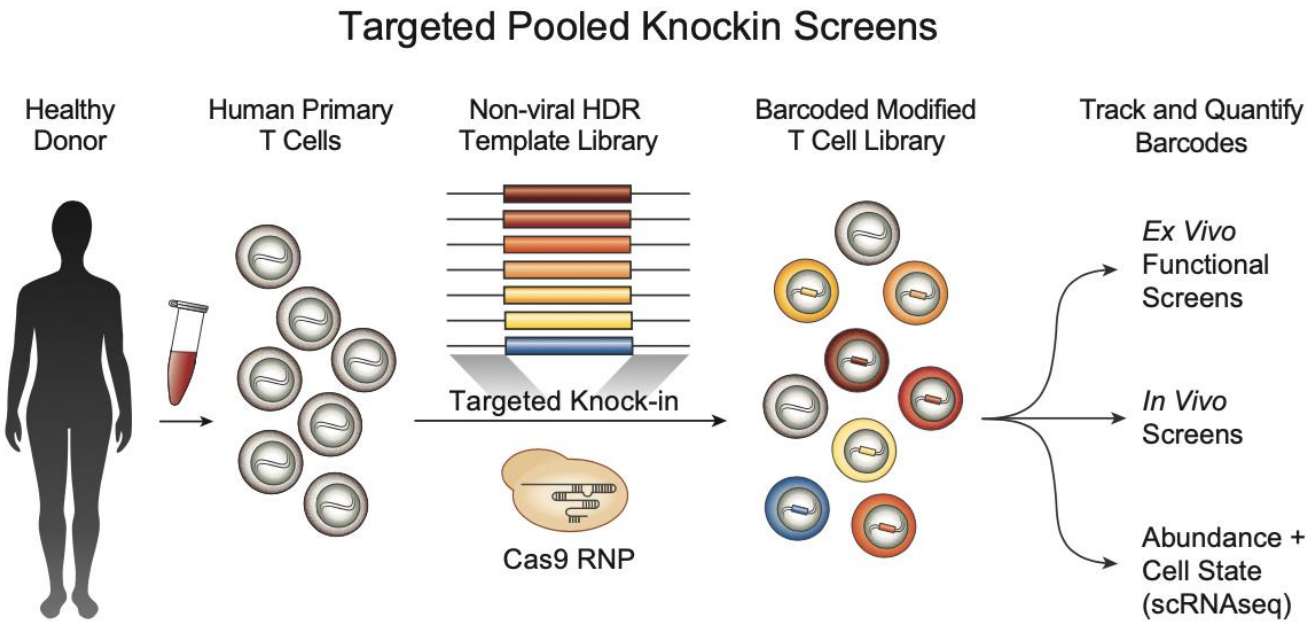
Kwon et al. Nature Biotechnology. 2023

Key Takeaways:

- scRNA-seq reveals efficacy and safety of CAR therapy based on target expression in rare cell subpopulations in normal tissue
- CD19 expression in rare pericyte population in the brain may explain neurotoxicity in CD19 CAR T therapy
- scRNA-seq atlas identifies optimal combinations of antigen targets for CAR T therapy in solid tumors – with greater specificity than targets in clinical trials or identified by bulk RNA-seq

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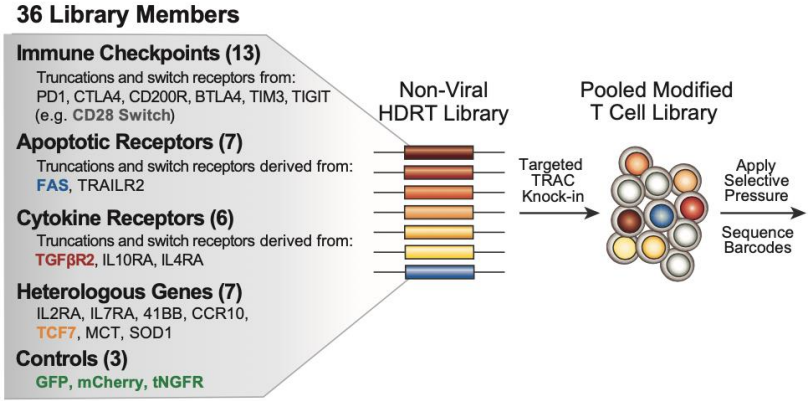
Therapeutic Development: What genetic programs can be engineered to improve CAR T function?



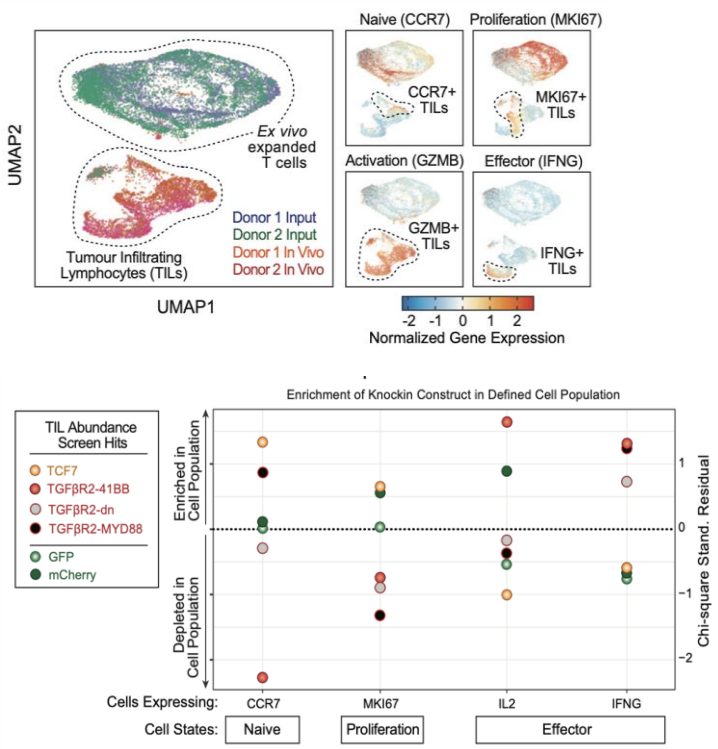
Roth et al. Cell. 2020

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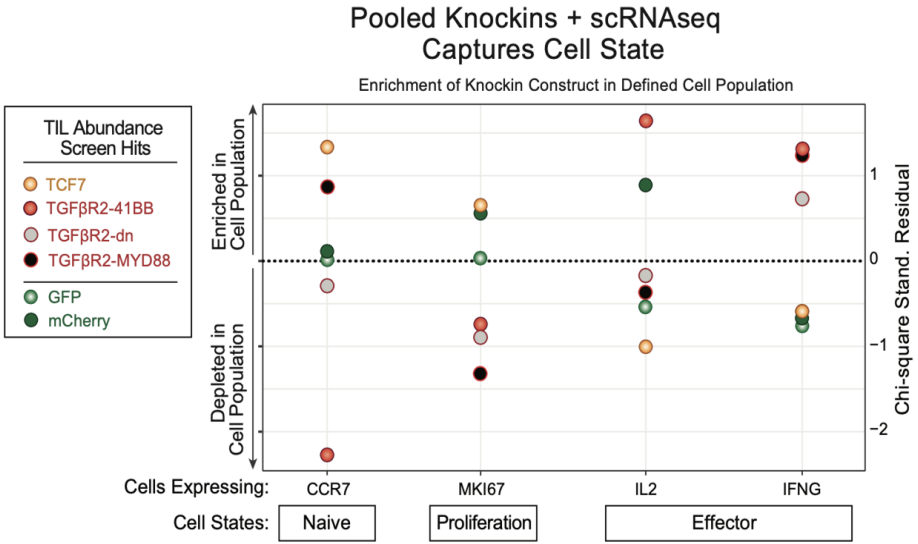
Pooled knockin sequencing (PoKI-seq) combines single cell transcriptome analysis and pooled knockin screening



Roth et al. Cell. 2020



High-throughput **functional screen** identifies knock-in genetic programs enhancing T cell function



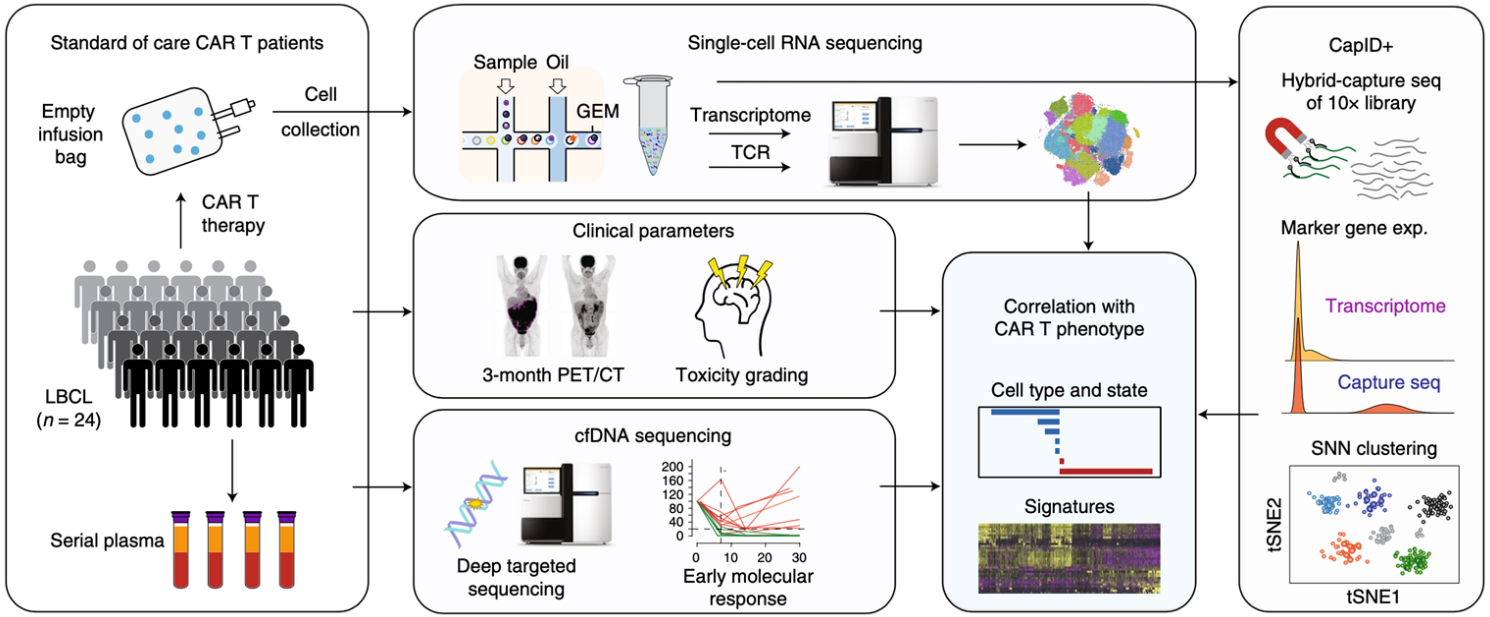
Roth et al. Cell. 2020

Key Takeaways:

- Pooled knockin sequencing (PoKI-seq) utilizes single-cell transcriptome analysis to accelerate screening of genetic engineering approaches for cell therapy
- PoKI-seq measures cell abundance and cell state in vitro and in vivo to parallelize functional assessment of knockin genetic programs to enhance T cell function
- Showed ability of exogenous TGF-BR2-derived receptors to convert a suppressive signal in the tumor microenvironment into a proliferation and effector state-inducing signal

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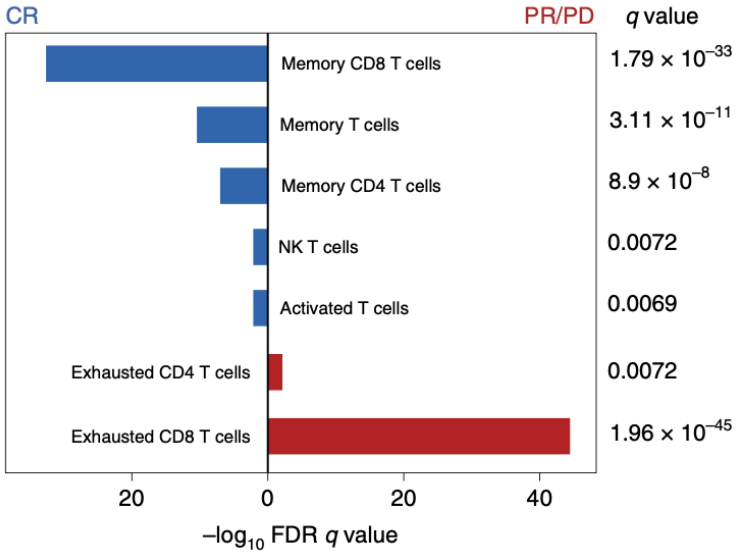
Product Characterization: What features of the infusion product are associated with clinical response in lymphoma?

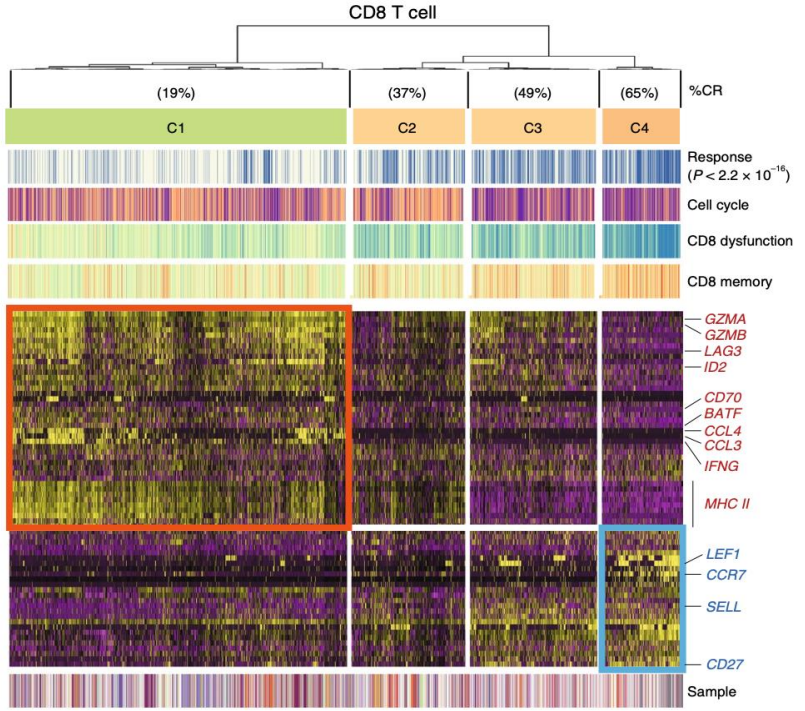


Deng et al. Nature Medicine. 2020

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Memory CD4+ and **CD8+ T** cell enriched in patients with clinical response

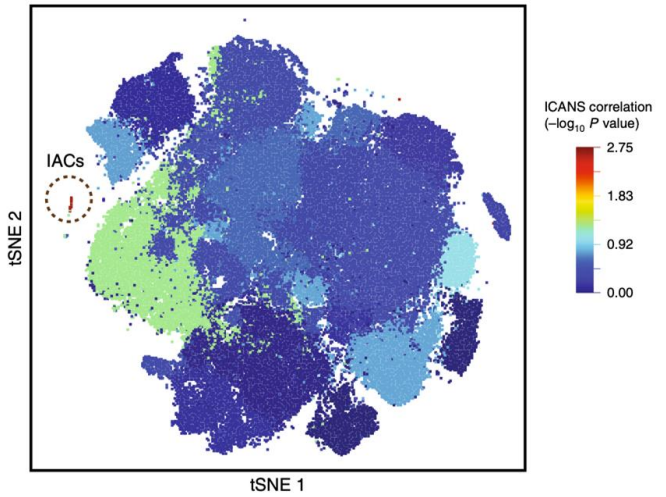


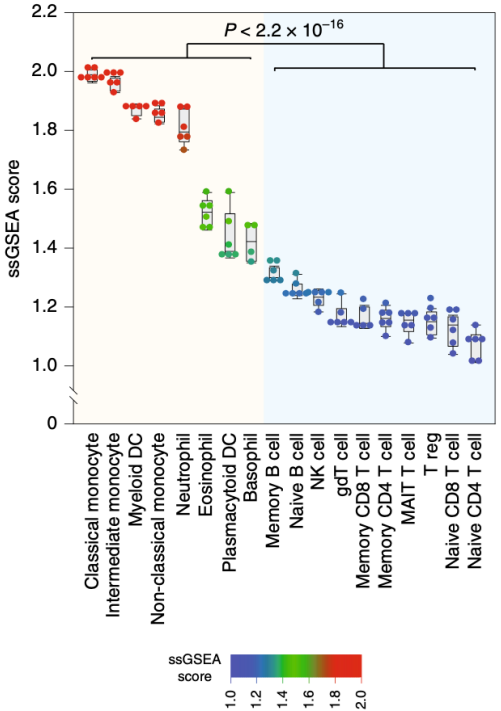


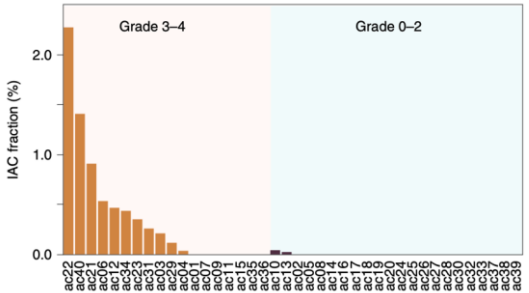
Deng et al. Nature Medicine. 2020

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Cells with **monocyte signature** are associated with high-grade immune effector cell-associated neurotoxicity syndrome (**ICANS**)



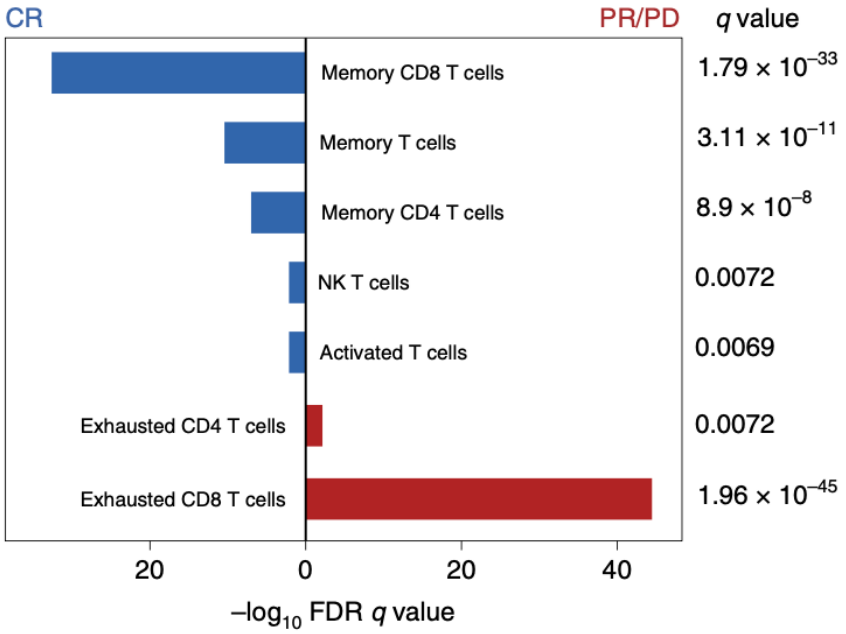




Deng et al. Nature Medicine. 2020

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Single Cell Immune Profiling resolves phenotypes associated with **efficacy** and **toxicity** to CAR T therapy in lymphoma



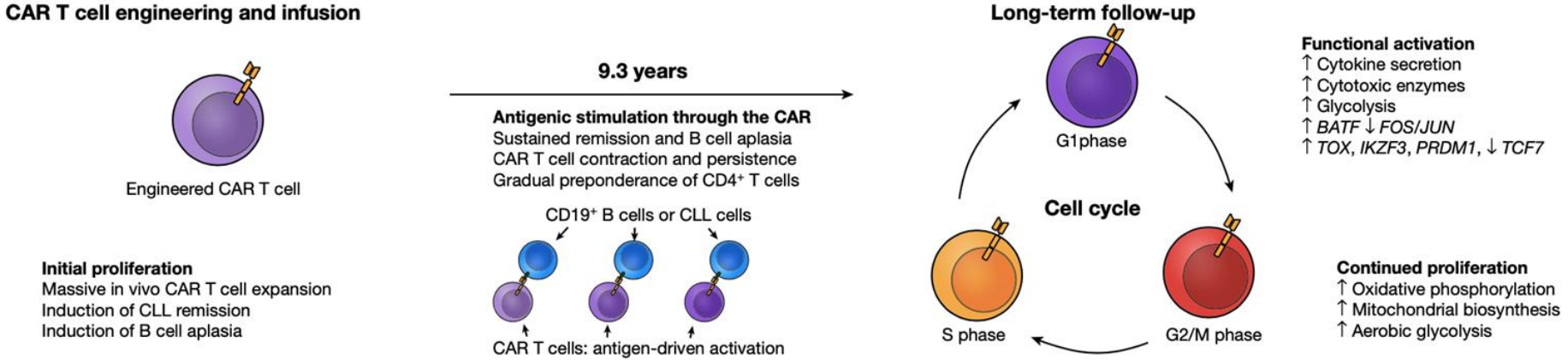
Deng et al. Nature Medicine. 2020

Key Takeaways:

- Single Cell Immune Profiling reveals features of the infusion product associated with clinical response in large B cell lymphoma treated with autologous CD19 CAR T therapy
- Memory CD4+ and CD8+ T cells enriched in patients with complete response
- Monocyte-like populations in infusion product is associated with high-grade immune effector cell-associated neurotoxicity (ICANS)

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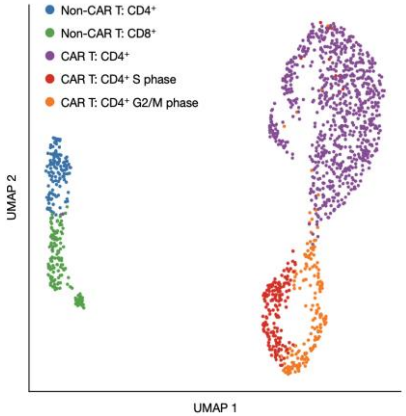
Therapeutic Monitoring: What are the characteristics of CAR T cells in leukemia patients in remission **10 years post-infusion**?

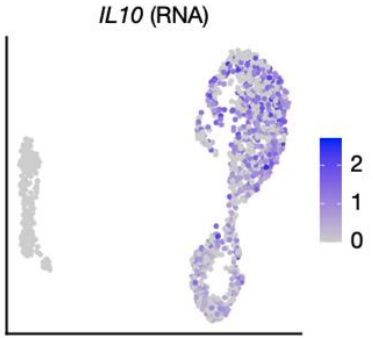


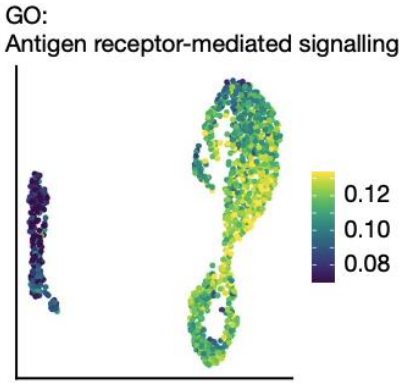
Melenhorst et al. Nature. 2022

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Single Cell Immune Profiling revealed **long-persisting** cytotoxic **CD4+ CAR T cells** with activation and proliferation signatures







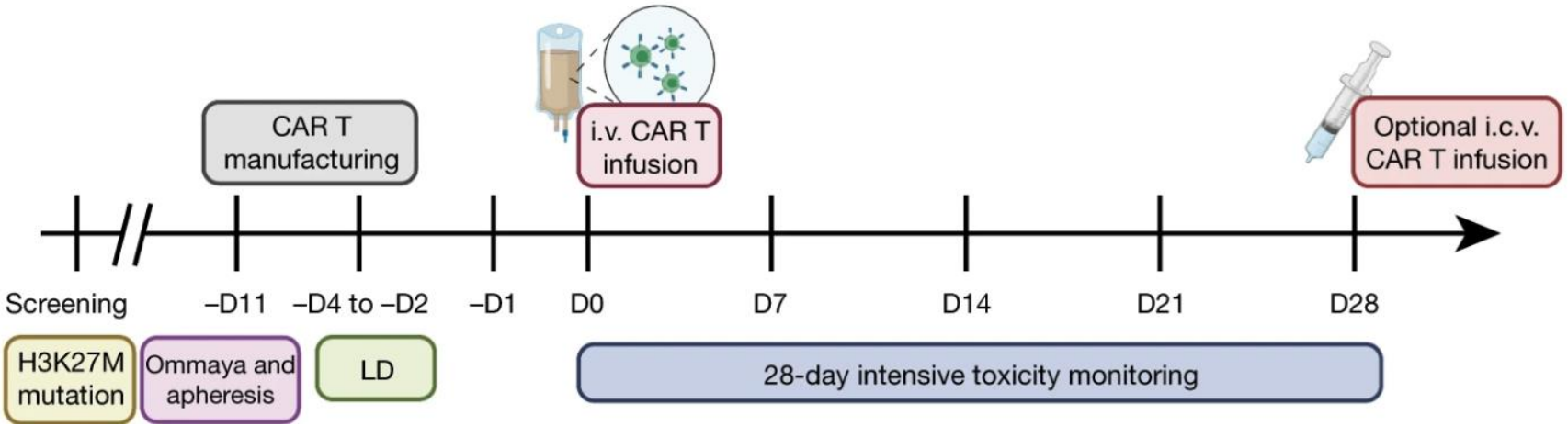
Melenhorst et al. Nature. 2022

Key Takeaways:

- Single Cell Immune Profiling characterizes clonality, protein expression, and transcriptome of 1150 CAR T cells from a patient in remission ~10 years post-infusion
- Emergence of dominance of a few clonal populations over time
- Long-persisting CD4+ CAR T cells exhibited cytotoxic characteristics along with ongoing functional activation and proliferation

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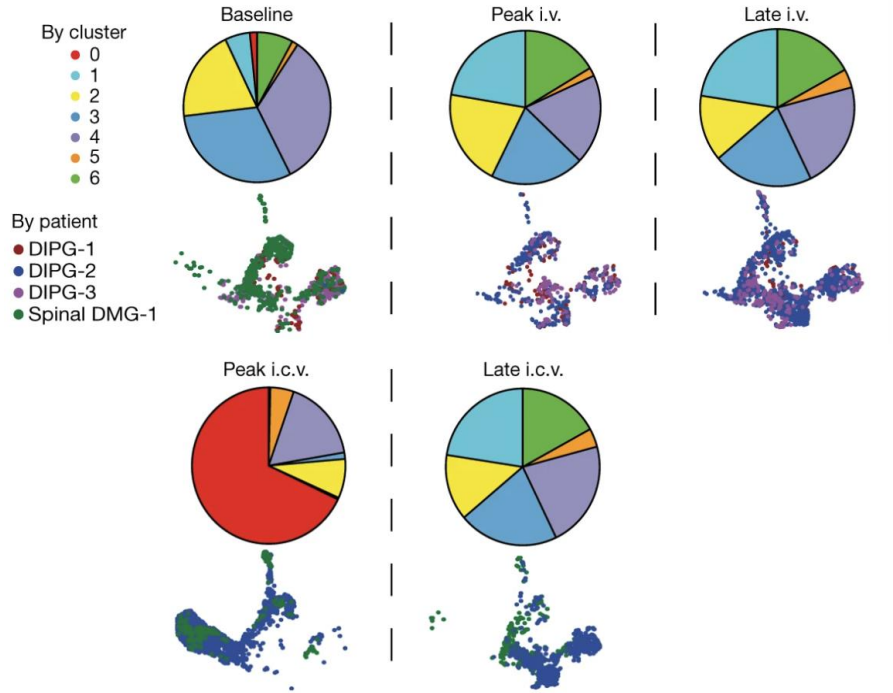
Therapeutic Monitoring: Are there biomarkers of response in a first-in-human GD2-CAR T trial for diffuse midline gliomas?



Majzner et al. Nature. 2022

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scRNA-seq of CAR T cells from infusion product and serial CSF identified **myeloid** population at peak inflammation



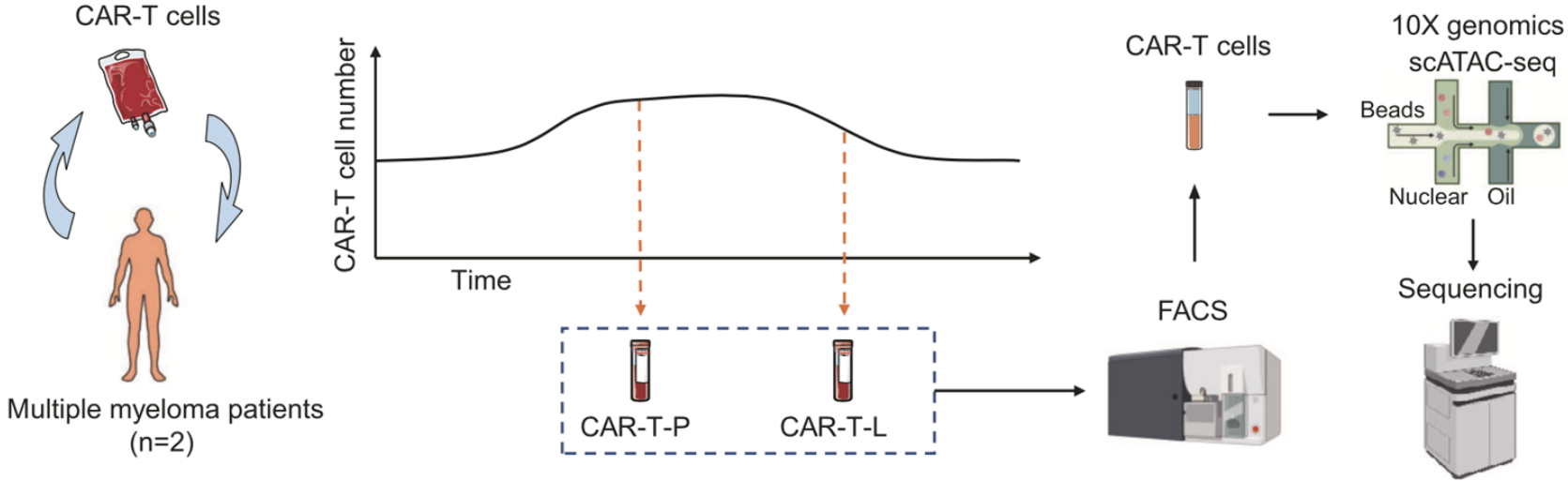
Majzner et al. Nature. 2022

Key Takeaways:

- scRNA-seq of 65,000 GD2 CAR T cells from the infusion product and serial cerebrospinal fluid (CSF) samples resolves the cellular composition associated with clinical features
- Increased myeloid activation at peak inflammation timepoint was correlated with favorable response
- Increased activation of tonic signalling pathways was associated with reduced CAR T cell activity and poor response

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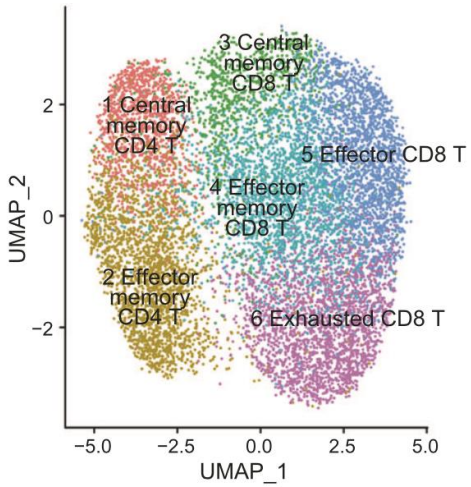
Resistance: What epigenetic regulators modulate CAR T dysfunction and exhaustion?

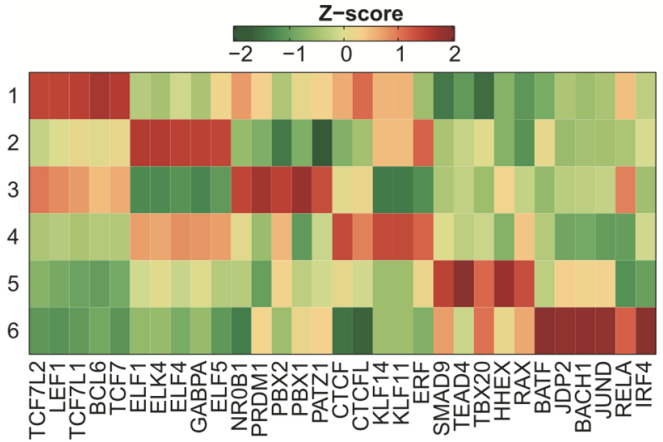


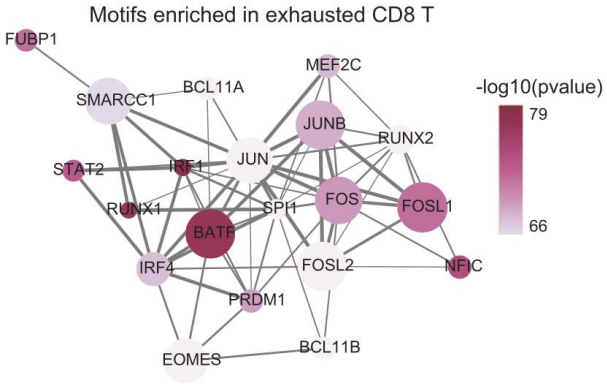
Jiang et al. Nature Leukemia. 2022

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scATAC-seq revealed cell type-specific changes in chromatin accessibility in BCMA CAR T cells from Multiple Myeloma patients



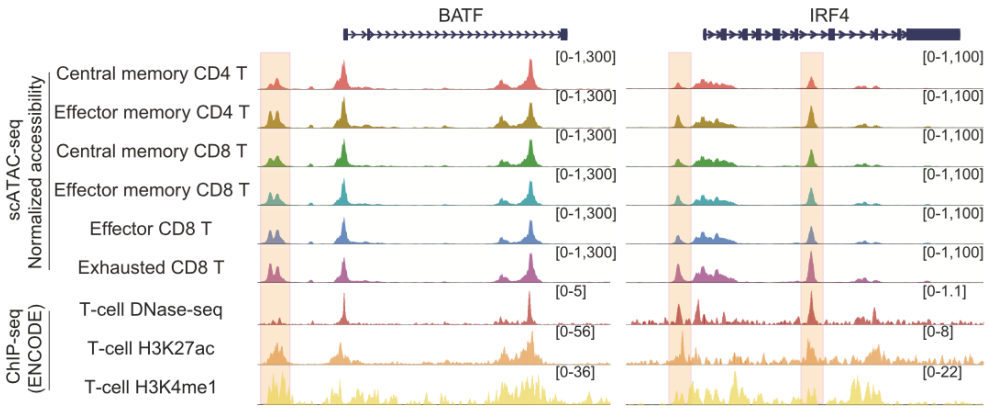




Jiang et al. Nature Leukemia. 2022

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scATAC-seq in patient-derived CAR-T cells identified BATF and IRF4 as pivotal **regulators** of **CAR-T cell exhaustion**



Jiang et al. Nature Leukemia. 2022

Key Takeaways:

- scATAC-seq resolves the global, dynamic, and cell type-specific changes of chromatin accessibility of CAR-T cells during tumor antigen stimulation
- BATF and IRF4 transcription factors were highly activated in terminal exhausted CAR-T cells and regulated the expression of genes related to cytotoxicity and exhaustion
- Reducing the expression of BATF or IRF4 improved antitumor potency of CAR-T cells

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Toolkit



Visium



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