

Spatial Transcriptomics: Technologies and Analysis

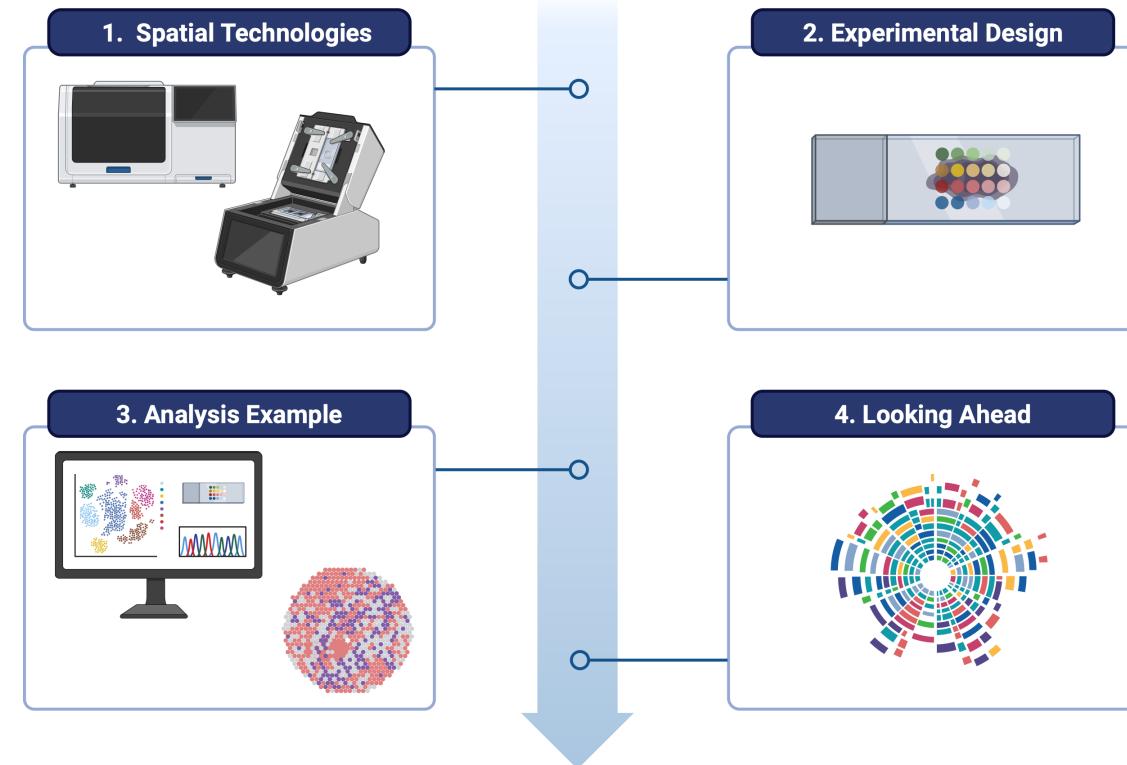
Elizabeth Garfinkle, PhD
Research Scientist

Dr. Elaine Mardis and Dr. Katherine Miller Lab
Institute for Genomic Medicine

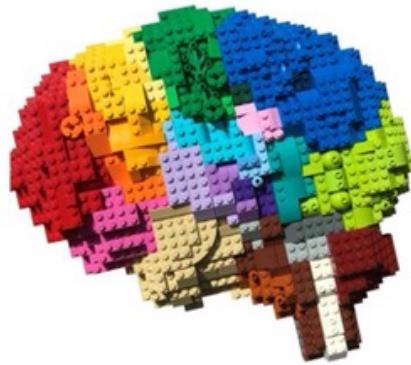
November 10, 2025



Outline



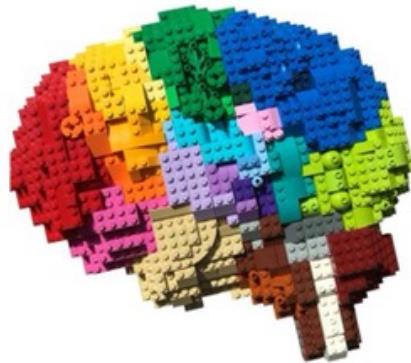
What can spatial transcriptomics tell us?



Complex Tissue

*Network of many cells each
with individual gene
expression patterns that
regulate interactions and
ultimately tissue function*

What can spatial transcriptomics tell us?



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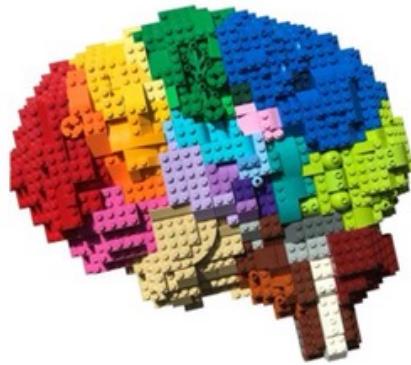
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Bulk RNA-seq

Tissue-average gene expression; lose individual cell expression patterns and spatial context

What can spatial transcriptomics tell us?



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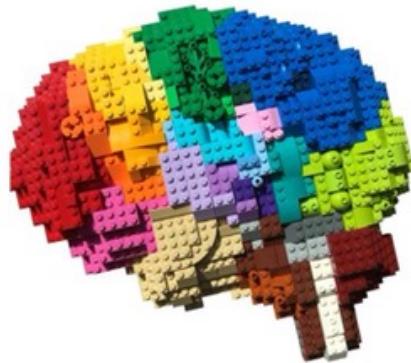
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Single cell RNA-seq

Cell-specific gene expression; still no spatial context but can infer cell-cell interactions

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Network of many cells each with individual gene expression patterns that regulate interactions and ultimately tissue function



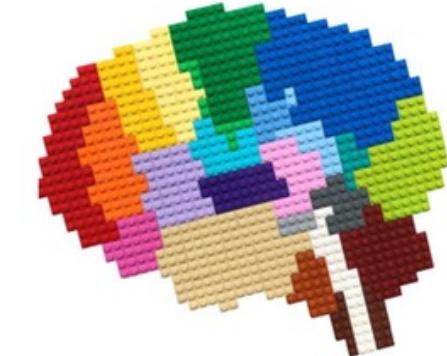
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Single cell RNA-seq

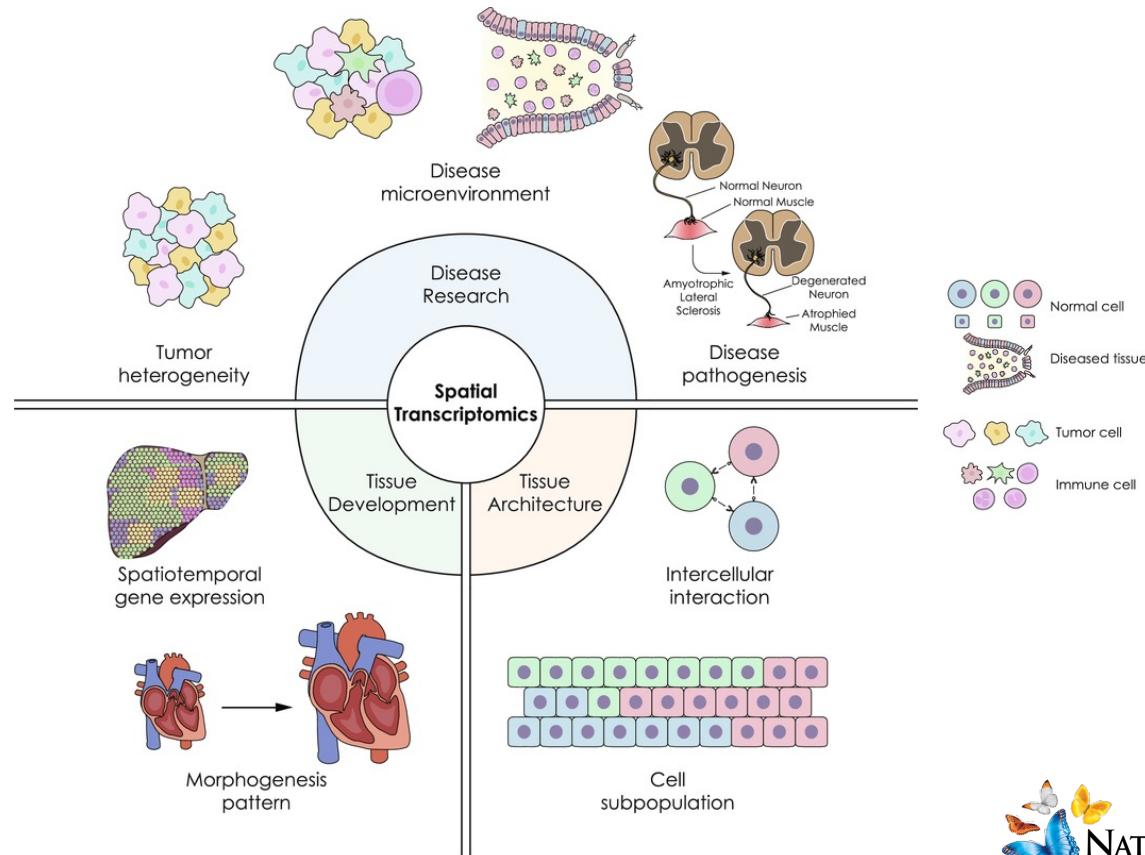
Cell-specific gene expression; still no spatial context but can infer cell-cell interactions



Spatial Transcriptomics

Region-, cell-, and/or subcellular-specific gene expression with spatial context

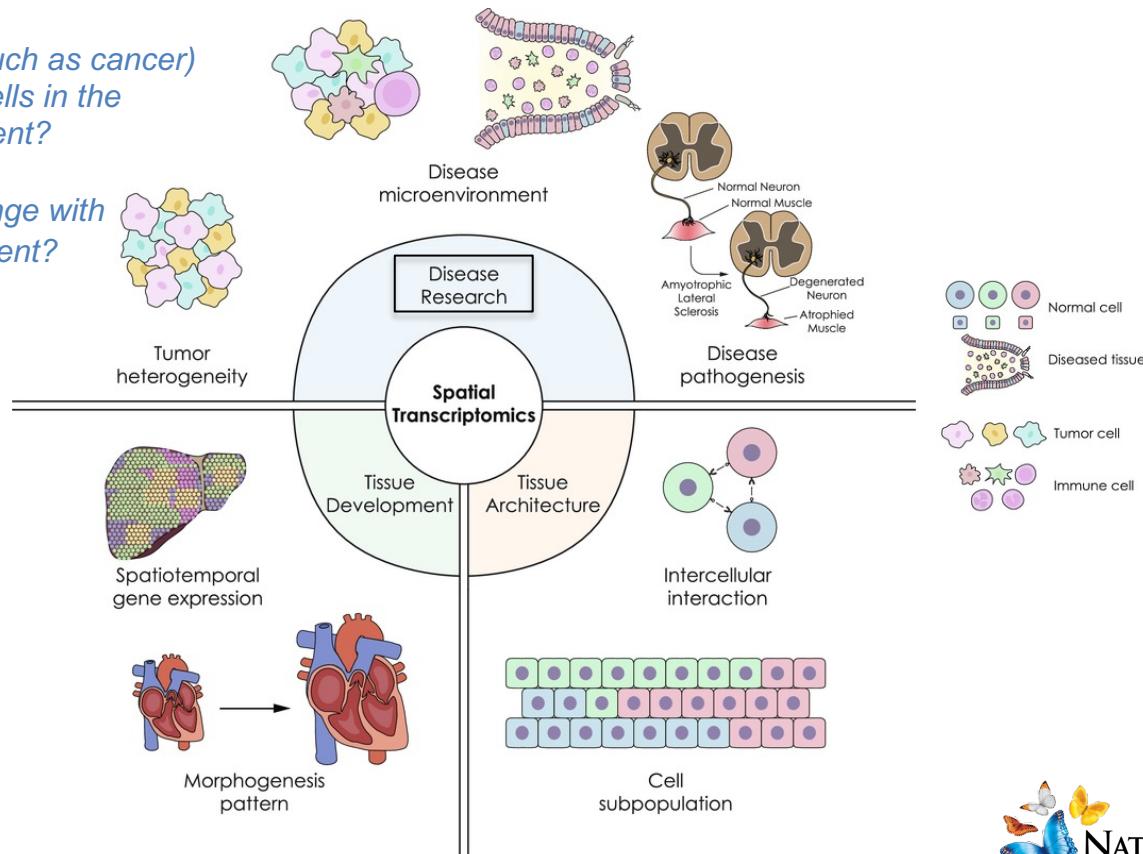
What can spatial transcriptomics tell us?



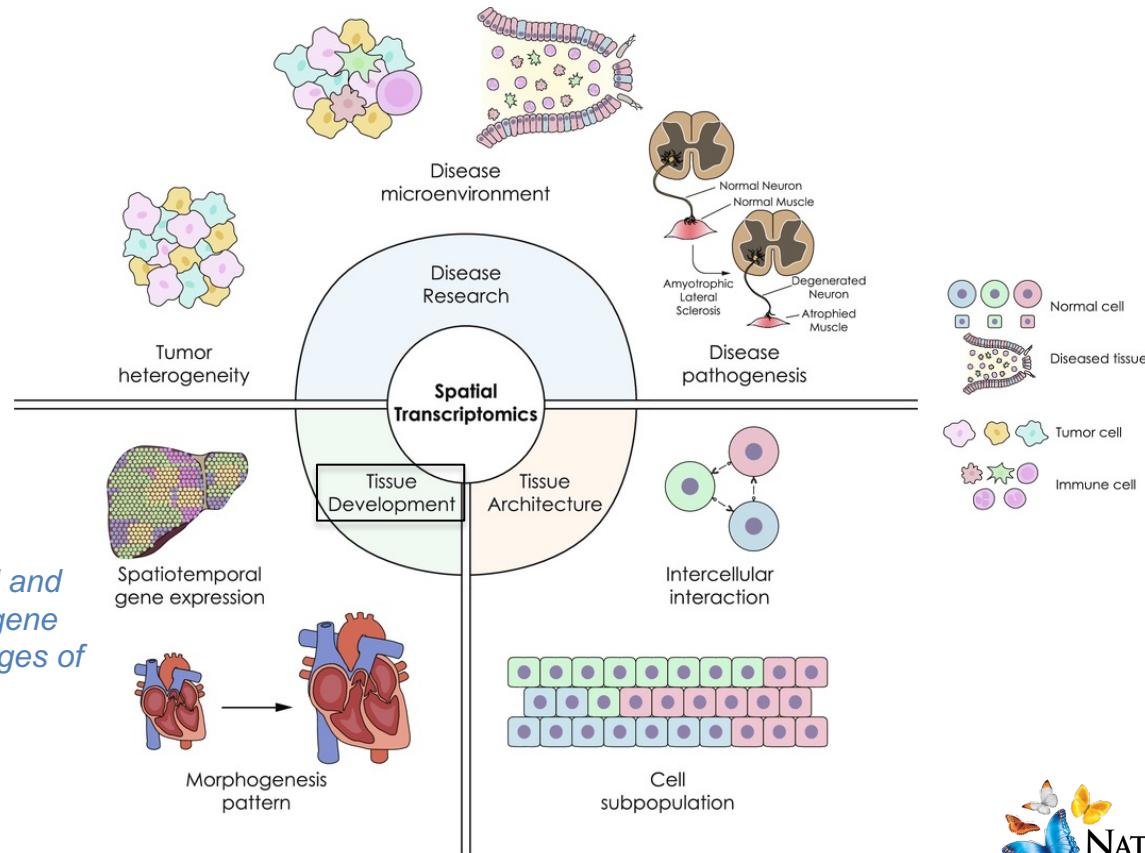
What can spatial transcriptomics tell us?

How do diseased cells (such as cancer) interact with other cells in the microenvironment?

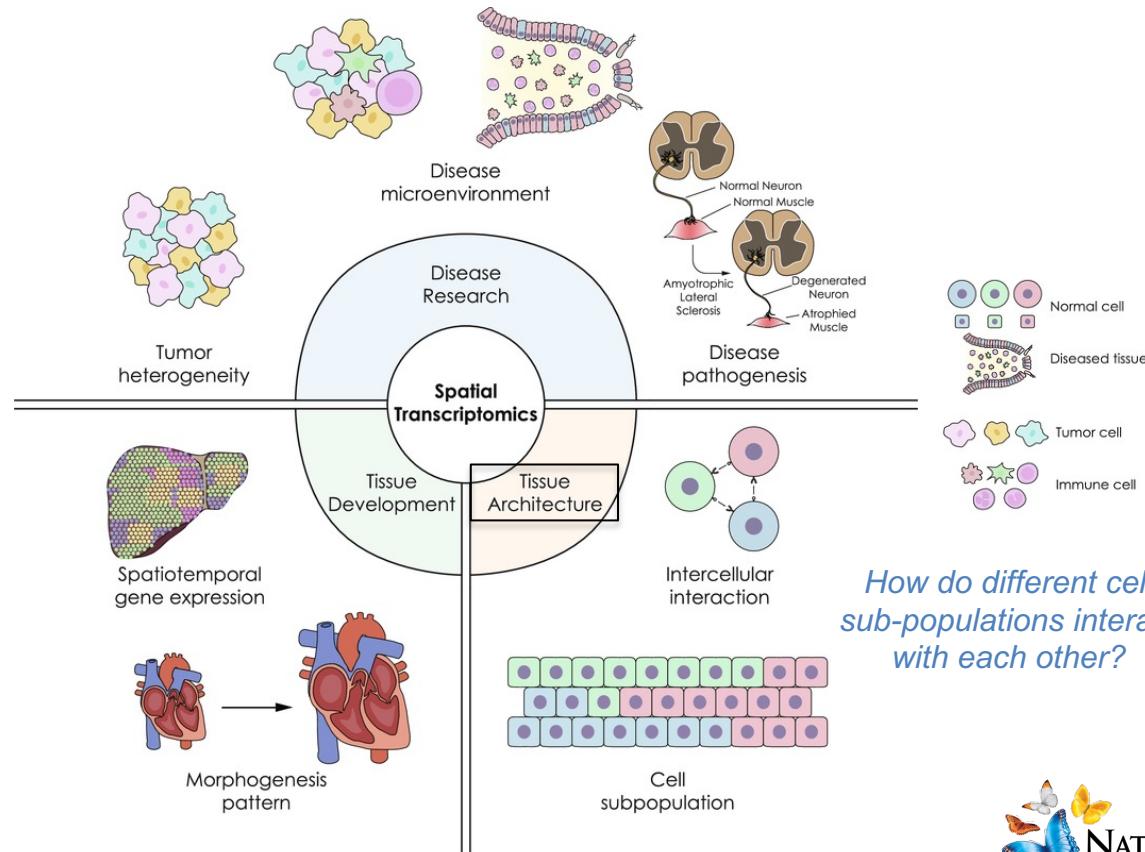
How does this change with time and treatment?



What can spatial transcriptomics tell us?

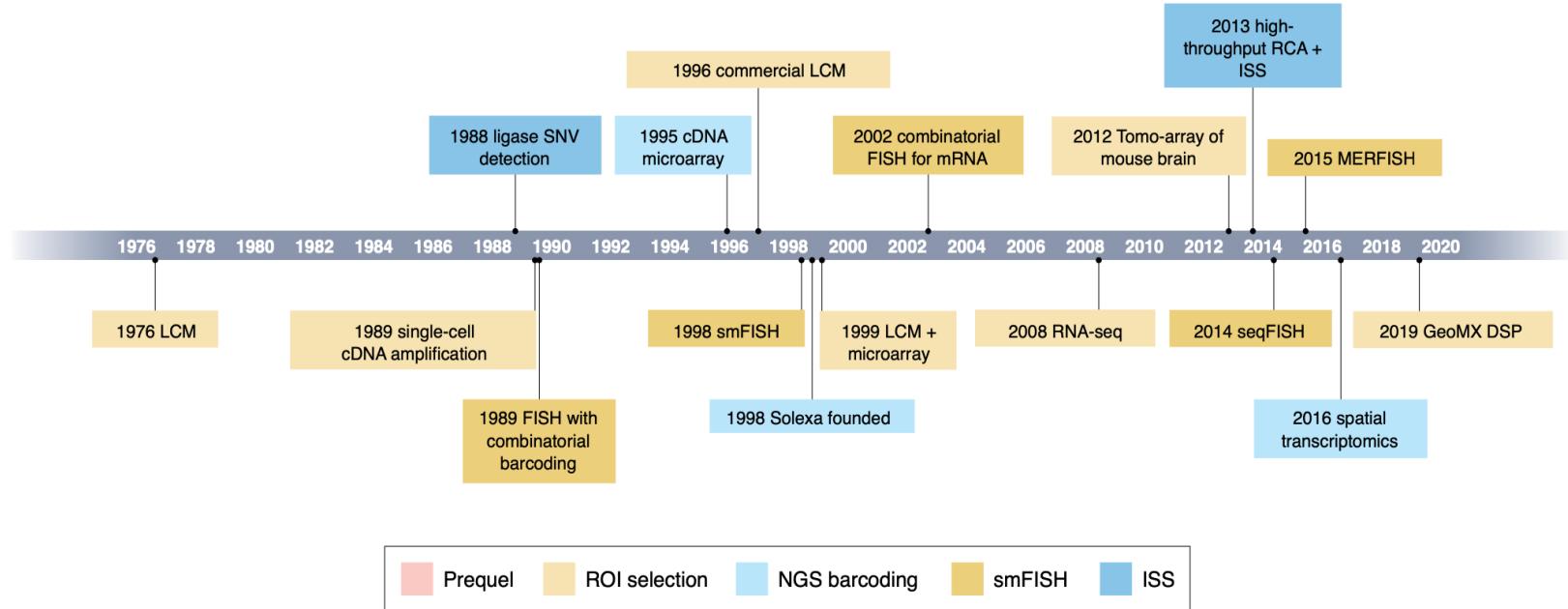


What can spatial transcriptomics tell us?

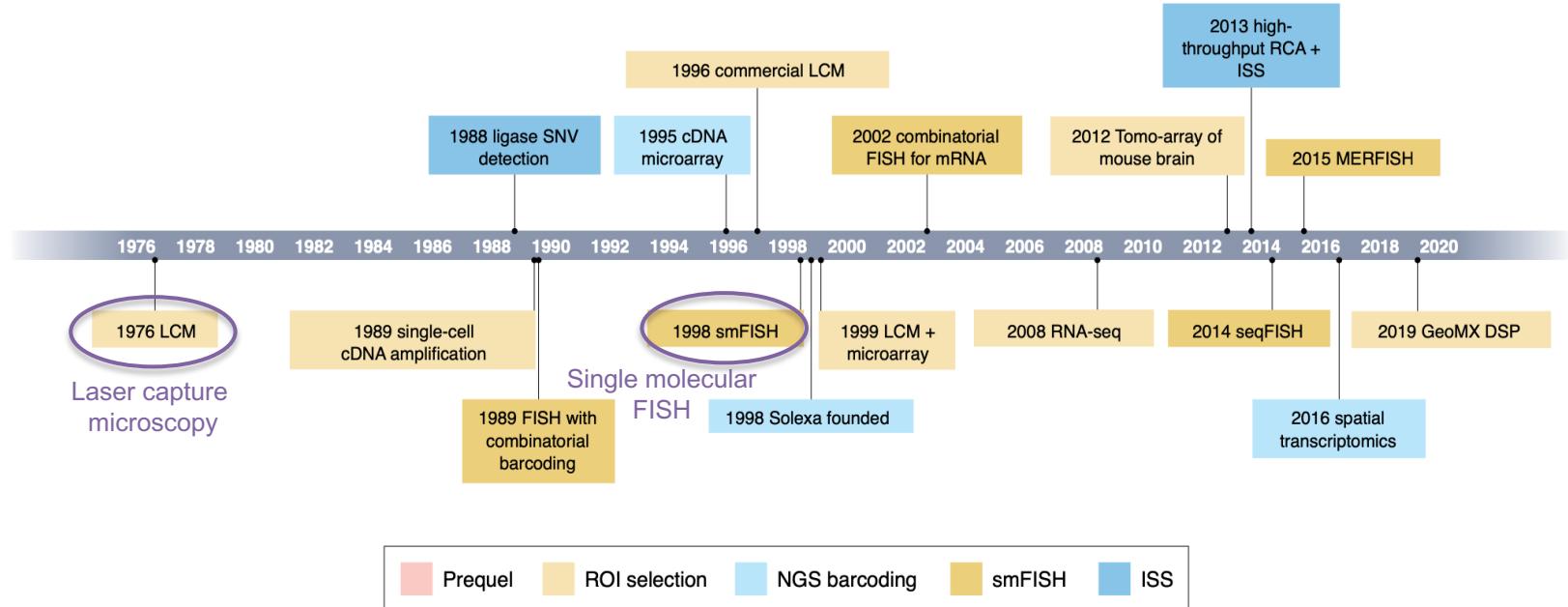


How do different cell sub-populations interact with each other?

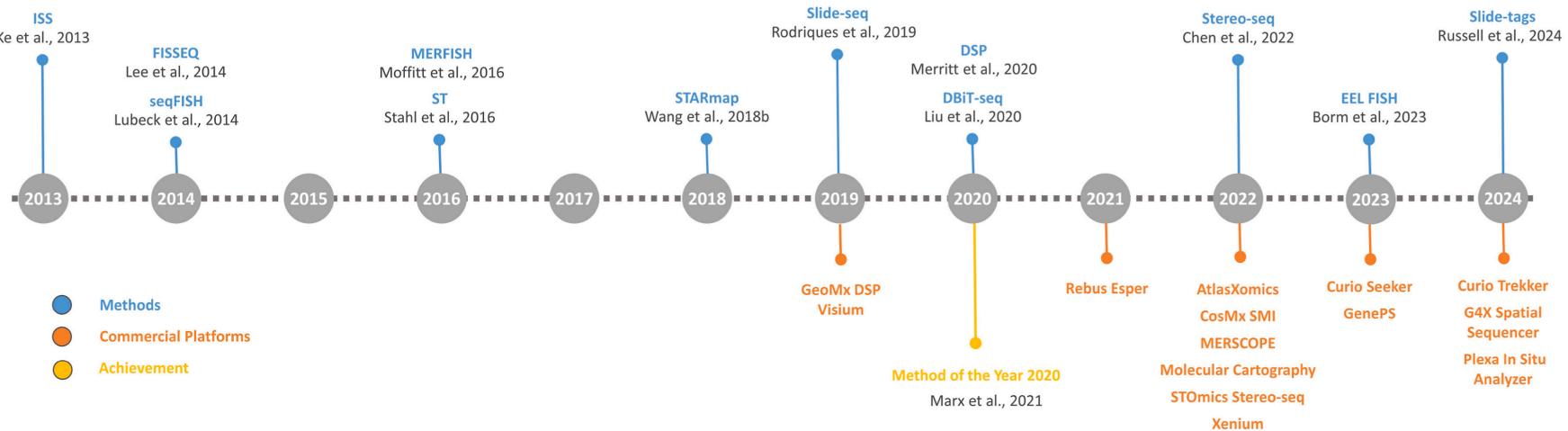
A brief history of spatial transcriptomics



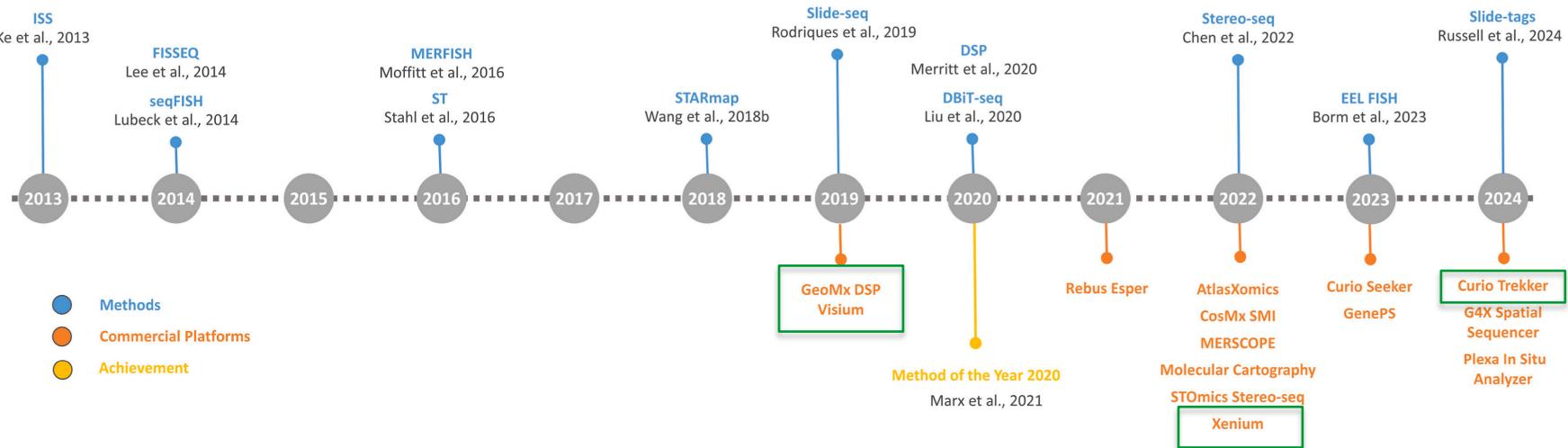
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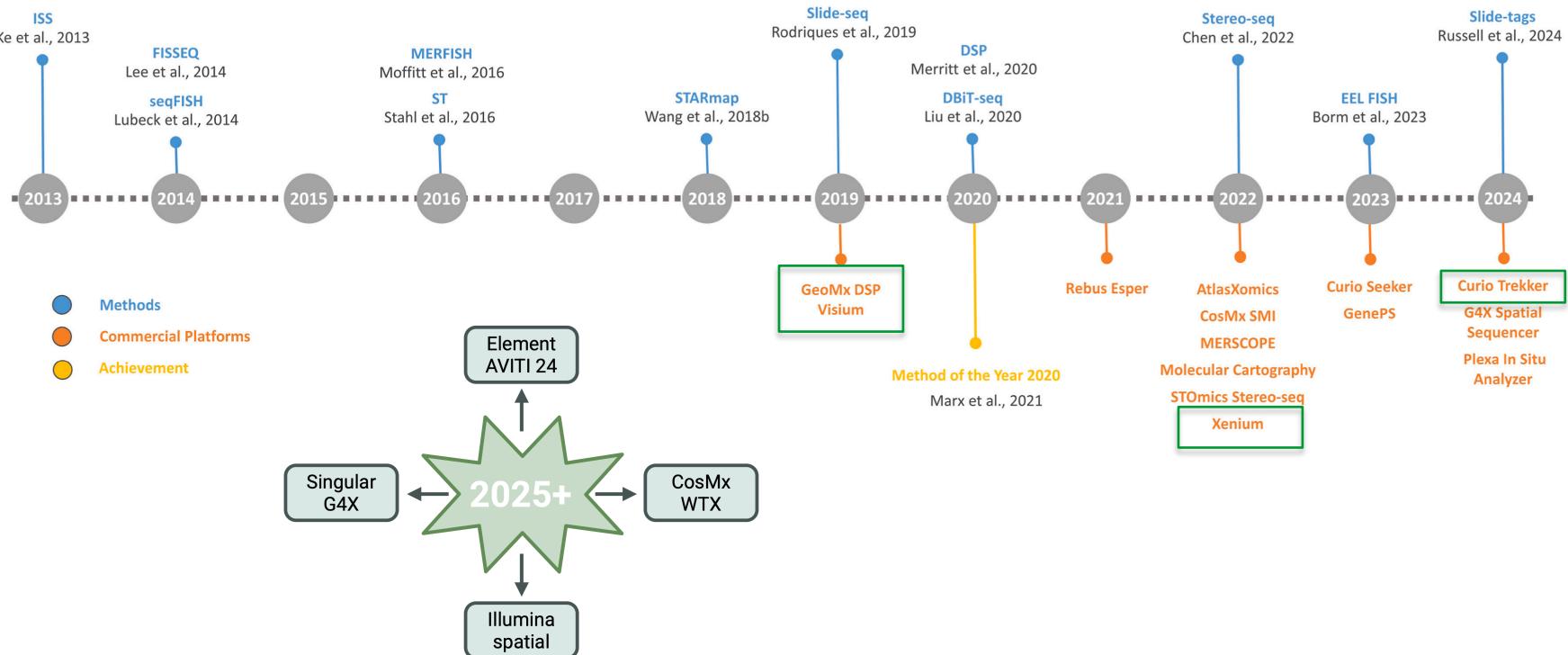
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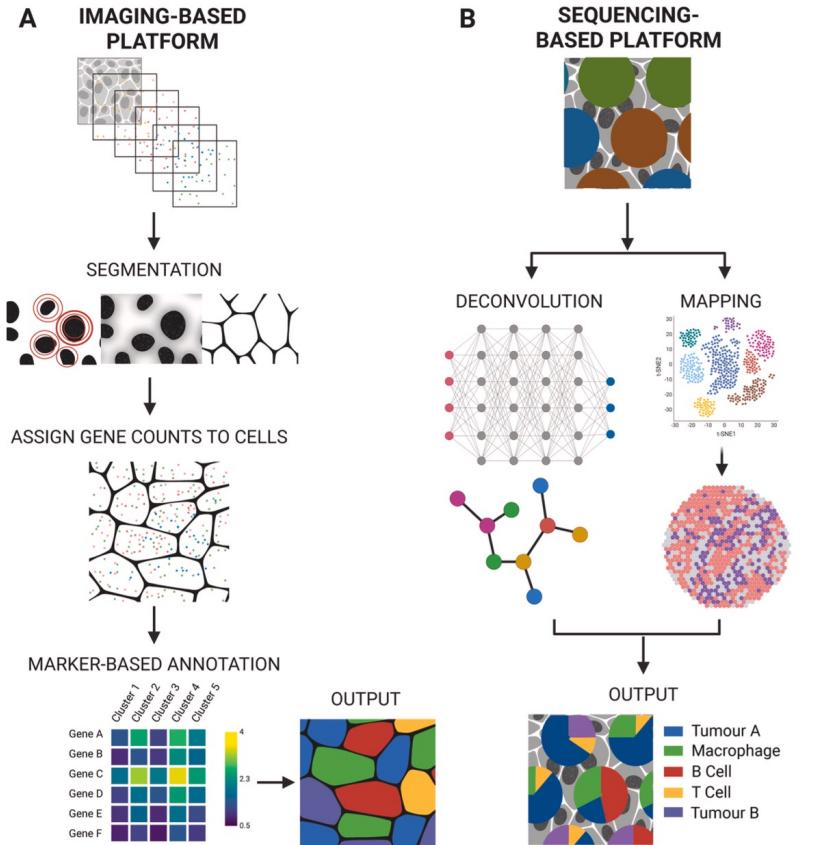


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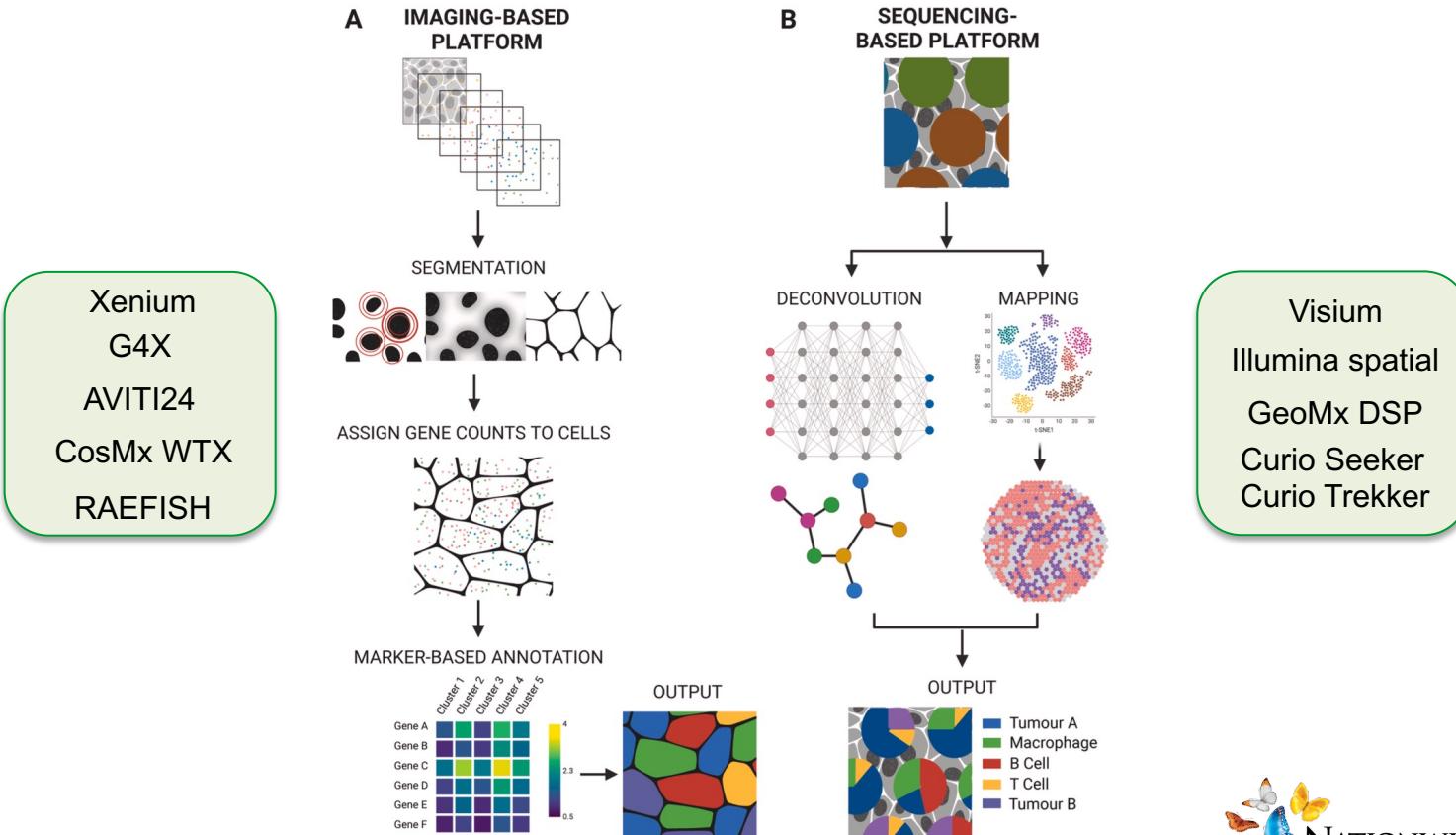


NATIONWIDE CHILDREN'S®
When your child needs a hospital, everything matters.

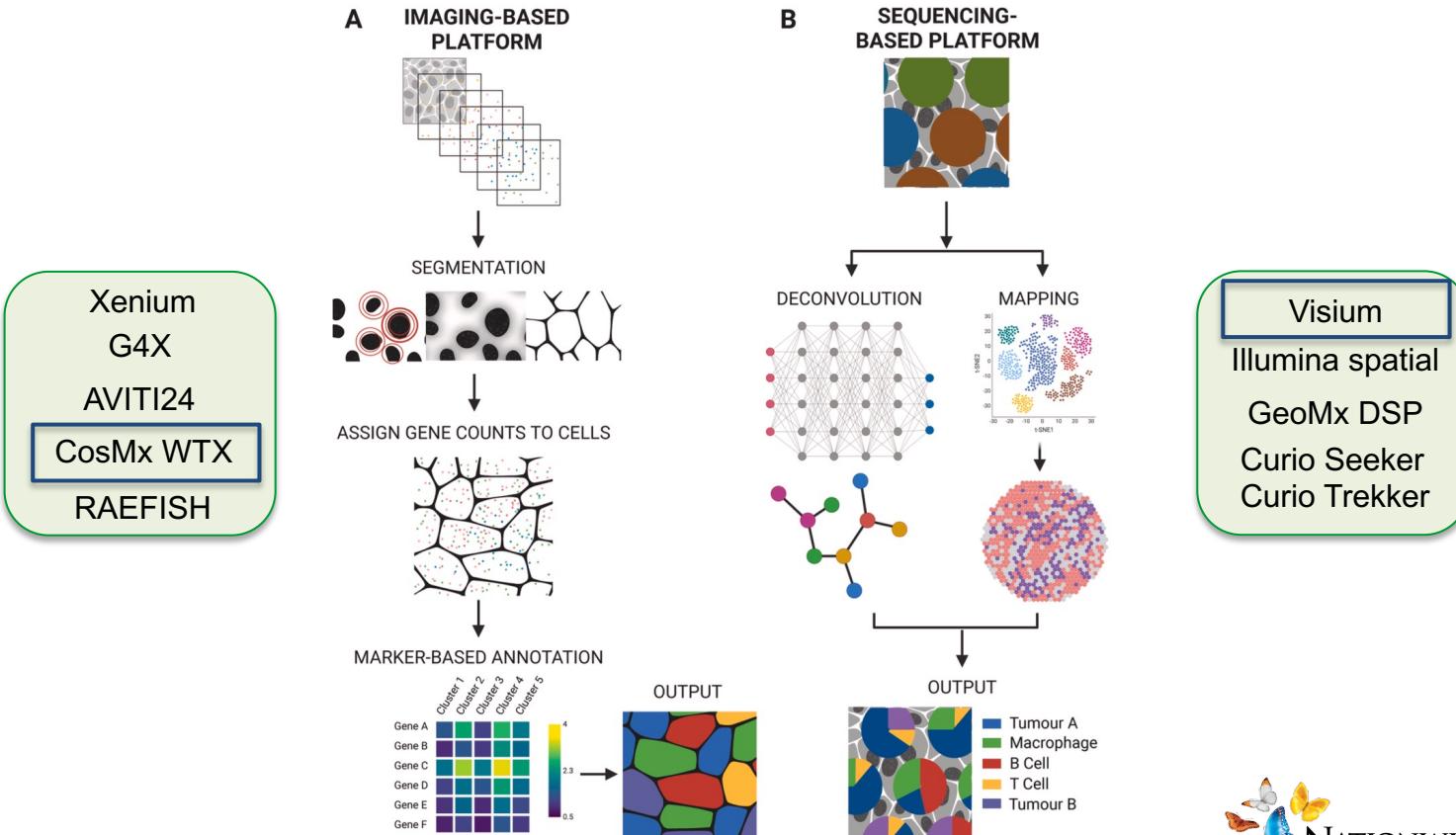
2 main types of spatial transcriptomics

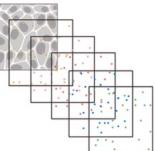


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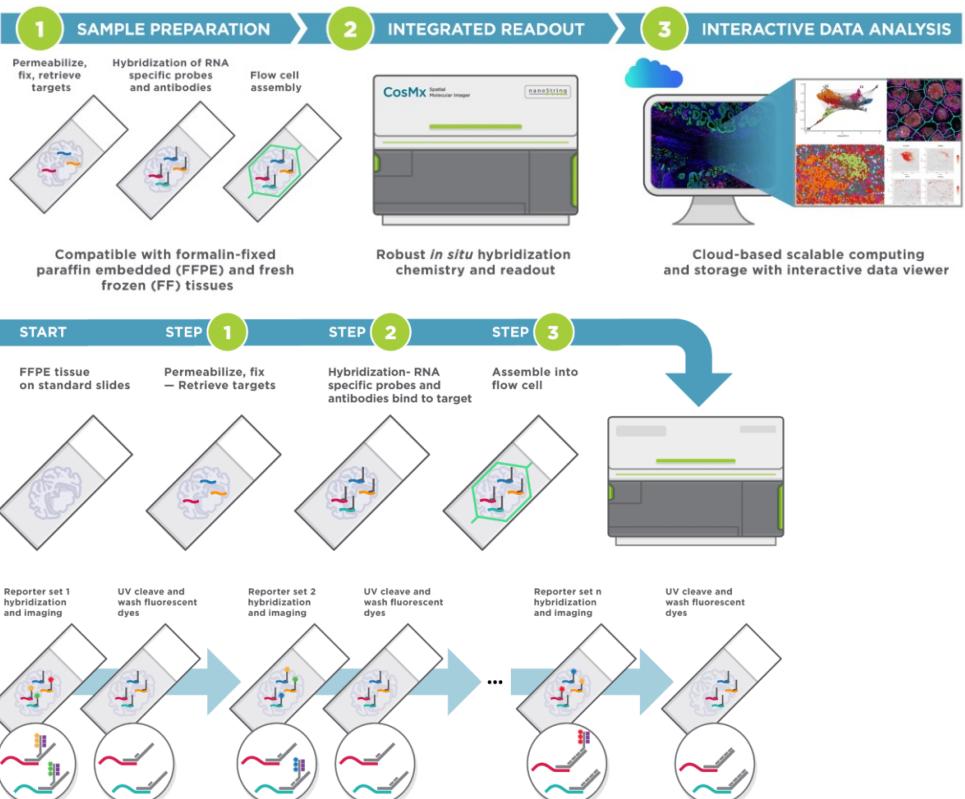
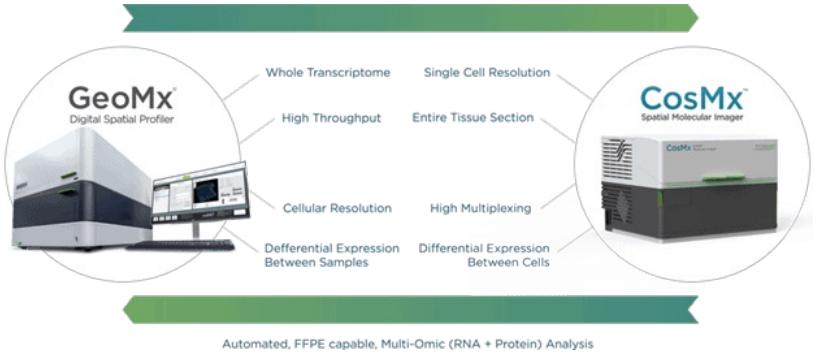


2 main types of spatial transcriptomics





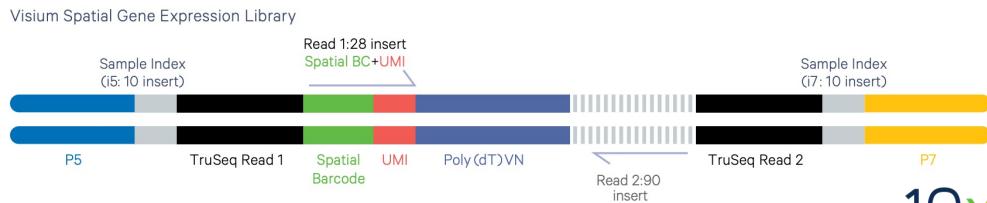
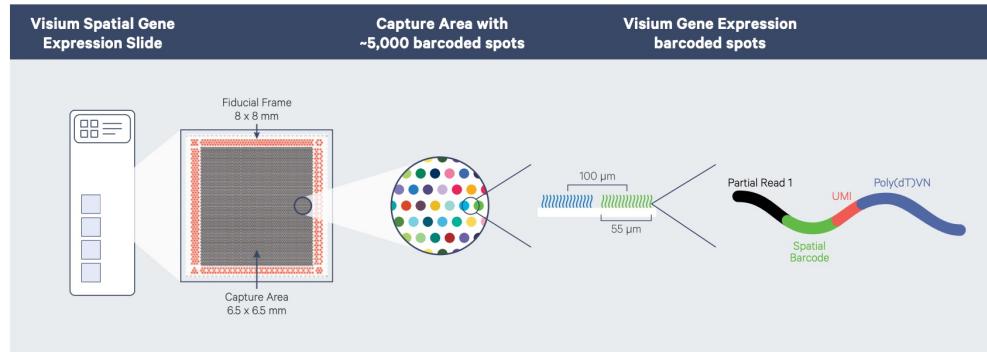
Bruker CosMx Spatial Molecular Imager





10x Genomics Visium

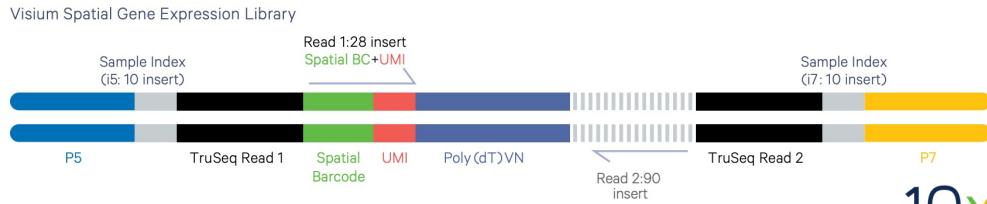
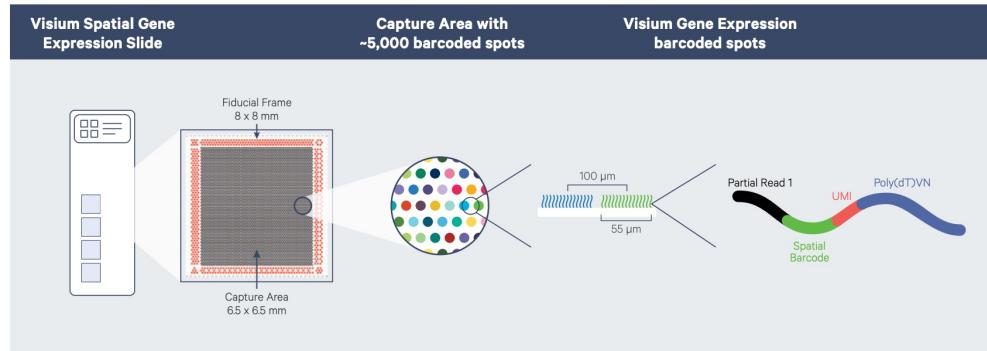
- Visium slides have two or four capture areas of either 6.5 x 6.5 mm or 11 x 11 mm.
- ~5,000 capture spots (6.5 x 6.5 mm capture area)
- ~14,000 barcoded spots (11 x 11 mm capture area)
- Each spot has millions of oligonucleotides with:
 - poly(dT) sequence:** for the capture of polyadenylated molecules
 - UMI:** used for the identification of duplicate molecules that arise during the library preparation and sequencing process
 - Spatial Barcode:** shared by all oligonucleotides within each individual gene expression capture spot





10x Genomics Visium

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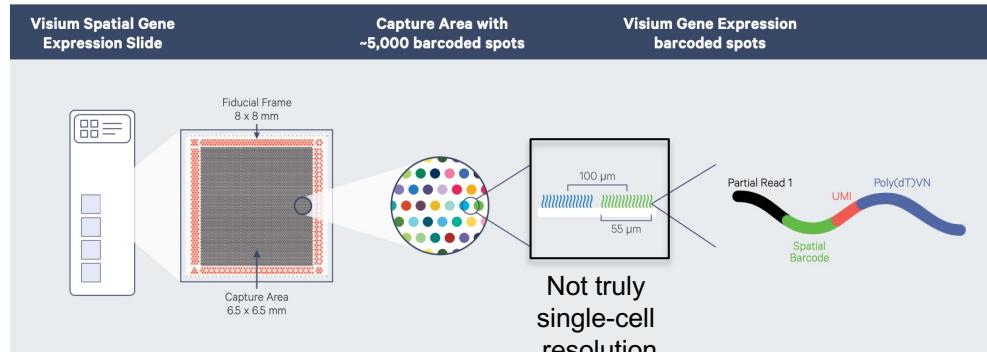


- Option to place tissue directly on Visium slide or blank tissue slide and transfer probes to Visium slide via CytAssist instrument
- Option to add 35-plex CytAssist Panel Antibodies
 - Targets intracellular/extracellular antigens (mostly immune)



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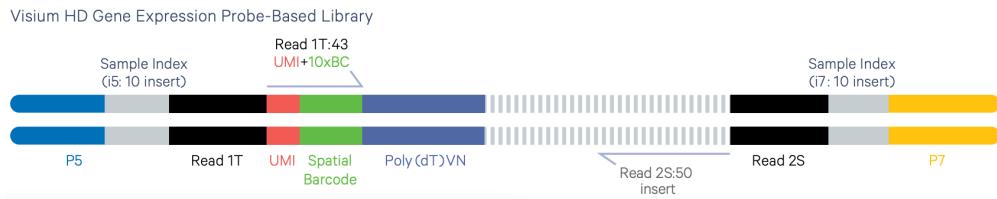
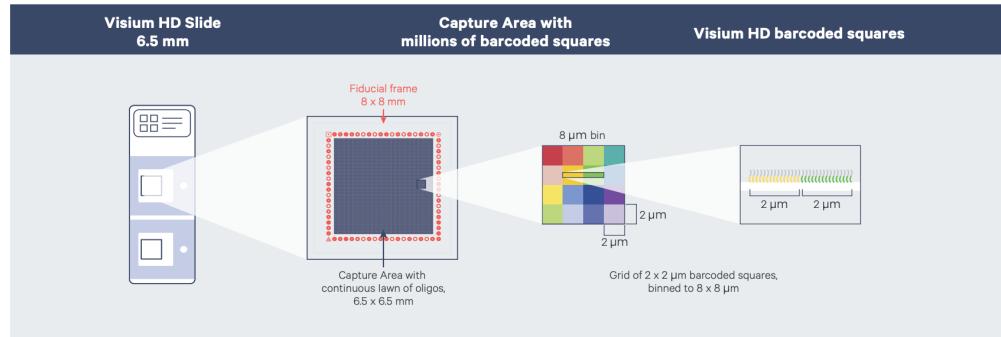


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10x Genomics Visium HD

- The HD slide has two capture areas, each 6.5 x 6.5 mm (11 x 11 mm coming soon!)
- ~ 11 million 2 x 2 µm barcoded squares without gaps (binned to 8 x 8 µm)
- Oligonucleotides each have
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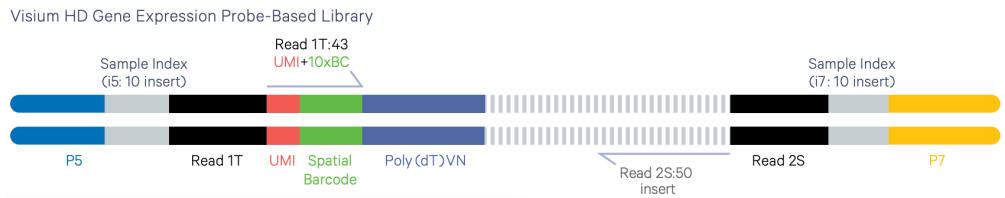
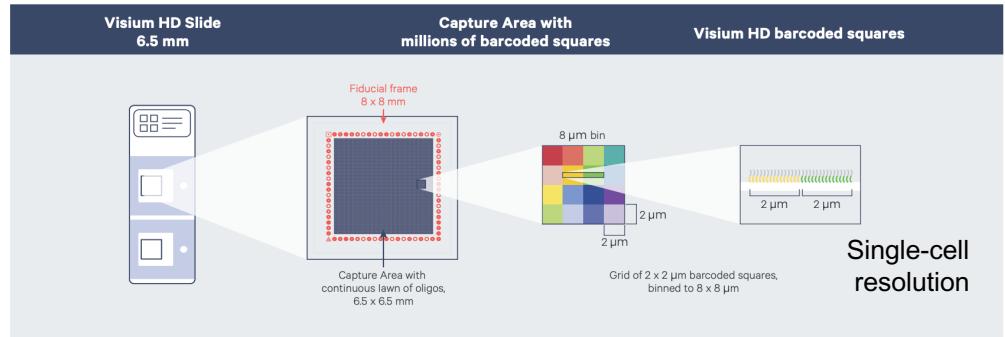


10X
GENOMICS®

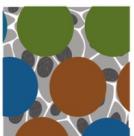


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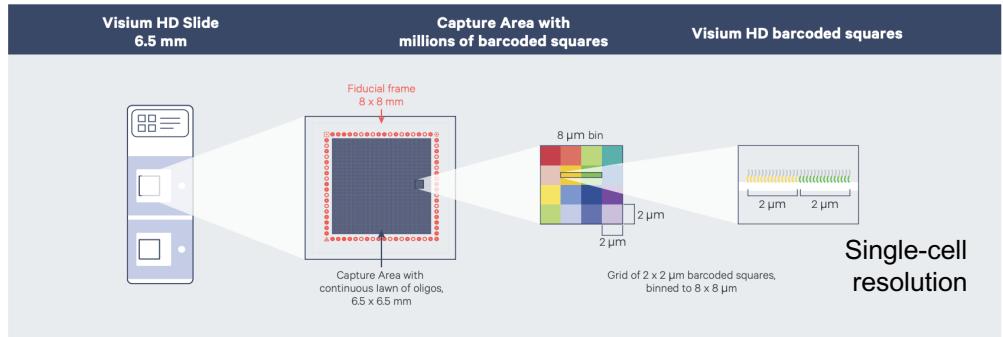


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- Probe-based: human (works with chimpanzee, too!) and mouse
 - Can spike-in custom probes of interest
- Fresh-frozen polyA capture species agnostic coming soon!





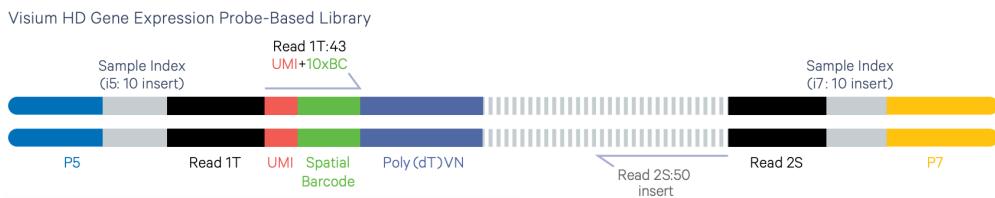
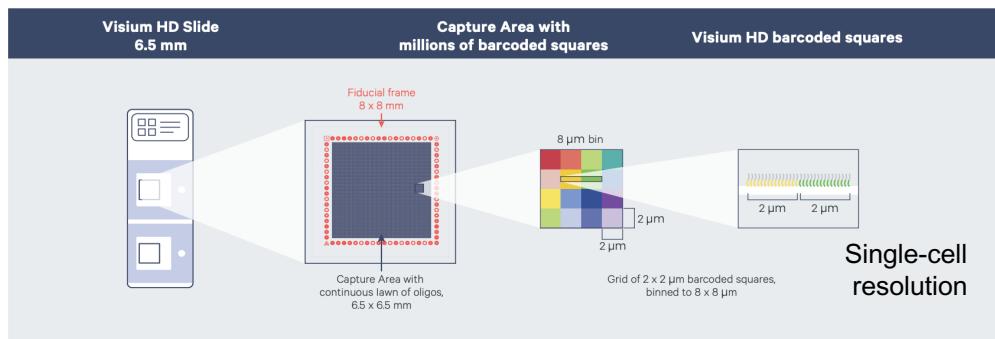
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- Requires use of CytAssist instrument
- Transcriptome only for now

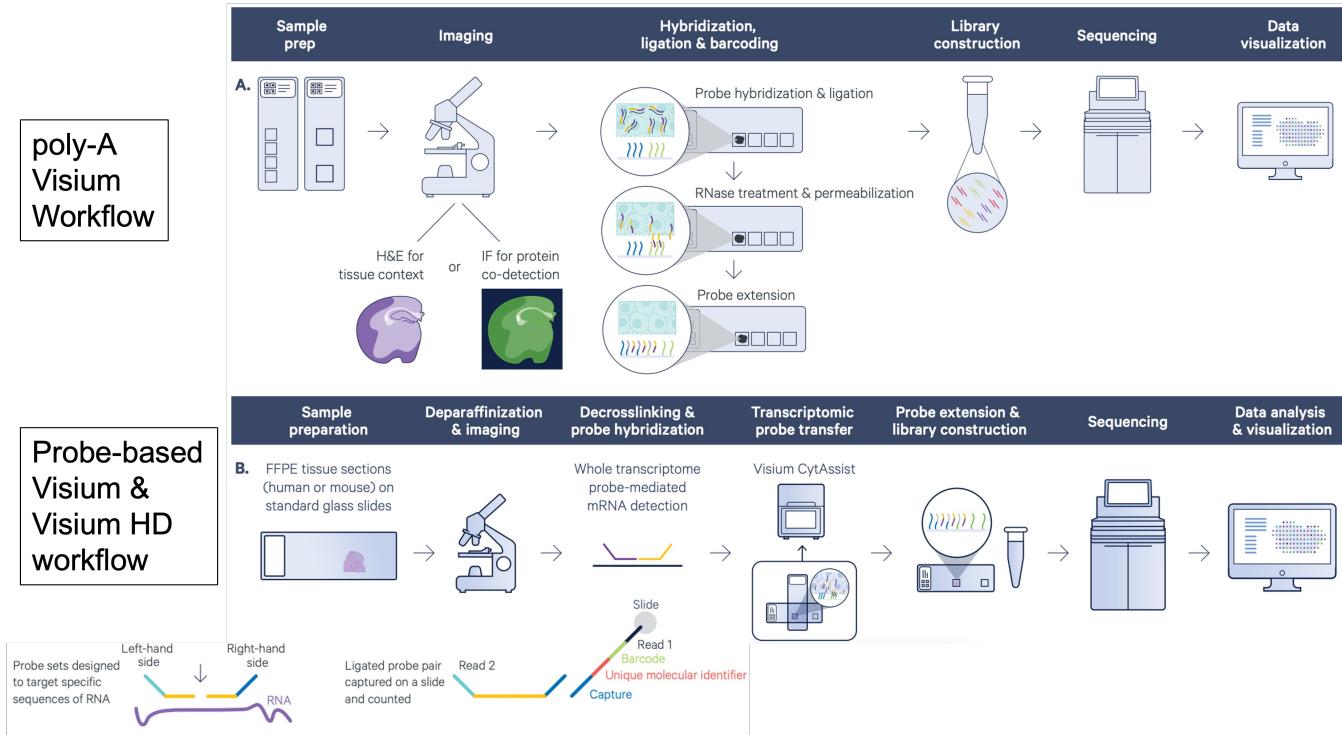
<https://www.10xgenomics.com/platforms/visium>



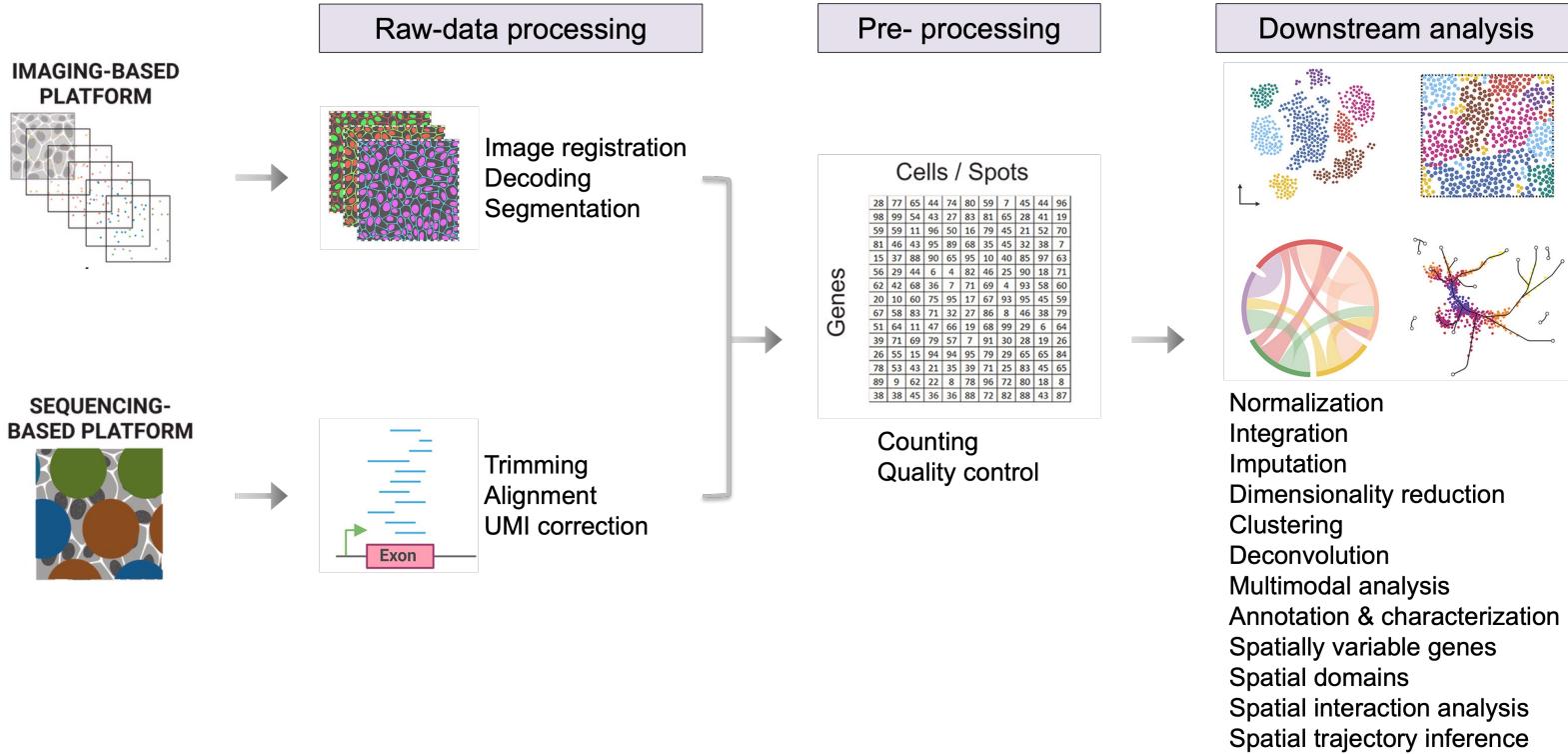
Slide modified from Corinne Strawser, PhD (NCH)



10x Genomics Visium Workflows

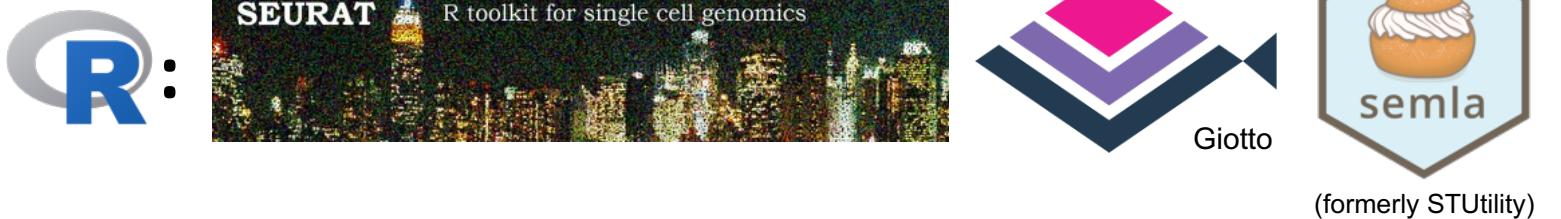


Spatial Transcriptomics Analysis



Spatial Transcriptomics Analysis

Choose your own adventure!



How do I design my spatial experiment?

1. Experimental design

- Hypothesis generation or testing?

2. Target (gene or cell) detectability

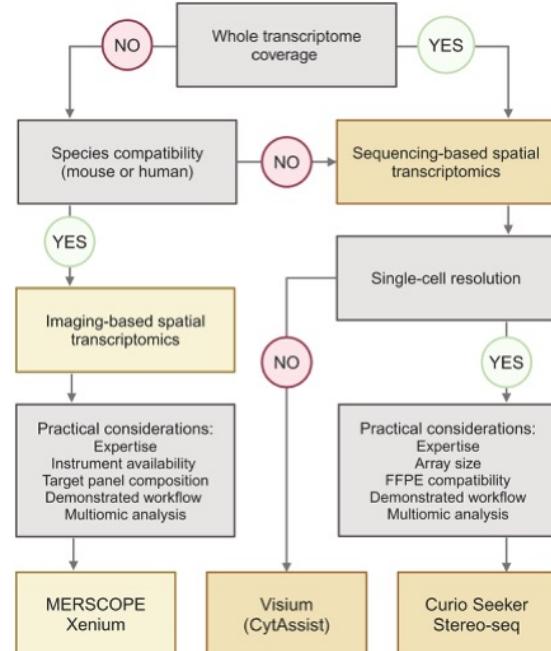
- Rare cells will be hard to identify with a low-res technology
- Transcripts with low expression may be difficult to profile with a technology that has low capture efficiency

3. Tissue type and species

4. Availability of reference data

- For cell deconvolution

5. Tissue section type and quality



Valihrach, L., Zucha, D., Abaffy, P., & Kubista, M. (2024). A practical guide to spatial transcriptomics. *Molecular aspects of medicine*, 97, 101276. <https://doi.org/10.1016/j.mam.2024.101276>

What platform should I use?

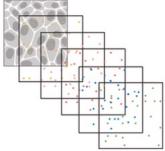
	MERSCOPE / MERSCOPE Ultra	Xenium	GeoMx	FlowGel	Visium / Visium HD	Curio Seeker	Stereo-seq / Stereo-seq OMNI
Company	Vizgen	10X Genomics	Nanostring	AtlasXomics	10X Genomics	Curio Biosciences	STOmics
Method	MERFISH	ISS + ISH	Light dissection	DBiT-seq	Array	Slide-Seq	DNA Nanoballs
Resolution	<100 nm	<100 nm	< 50 µm	10 µm	55 µm / 2 µm	10 µm	500 nm
# Features	1000 genes	5000 genes	WT	WT	WT	WT	WT
# Areas (per slide)	1	1	96	1	2 or 4	1	1
Tissue Area	1 cm ² / 1.25 cm ² or 3 cm ²	236 mm ²	100 - 490,000 µm ²	0.3 cm ²	42.25 mm ² or 121mm ² / 42.25 mm ²	9mm ² or 100mm ²	0.25cm ² or 1 cm ²
Sample Type	FFPE, fresh or fixed frozen, adherent or suspended cells	FFPE, fresh frozen	FFPE, fresh frozen	FFPE, fresh or fixed frozen	FFPE, fresh or fixed frozen (Visium only)	fresh frozen	FFPE, fresh frozen
Species	human, mouse, reference	human, mouse	human, mouse, canine	reference	human, mouse, reference*	human, mouse, reference	human, mouse, reference
Time	⌚⌚	⌚⌚⌚	⌚⌚⌚⌚	⌚	⌚⌚	⌚	⌚⌚
Customizable	Yes	Yes	Yes	Yes	Yes	No	Yes
Cost (instrument)	\$\$\$	\$\$\$	\$\$	N/A	\$	N/A	N/A
Cost (per sample/slide)	\$\$	\$\$\$	\$\$	\$	\$\$	\$\$	\$\$
Destructive	Yes	No	No	No	No	Yes	No
Required Instrumentation	MERSCOPE or MERSCOPE Ultra	Xenium Analyzer	GeoMx DSP	Microfluidics Stamp Scanning microscope Incubator	Microscope Visium CytAssist	none	Stereo-seq Chip T Slide Microscope Complete Genomics Sequencer
Multiomics Compatibility	Protein (antibody)	Protein (in dev)	Protein (antibody)	Protein (antibody) Epigenome (ATAC-seq + CUT&Tag)	Protein (antibody)	none	Protein (antibody)

Cost (instrument): ≥ \$400k (\$\$\$), \$100k-399k (\$\$), < \$100k (\$)

Cost (per sample): ≥ \$5000 (\$\$\$), \$1000-4999 (\$\$), < \$1000 (\$)

Time (per run): ≥ 3 days (⌚⌚⌚), 1-2 days (⌚⌚), < 1 day(⌚)

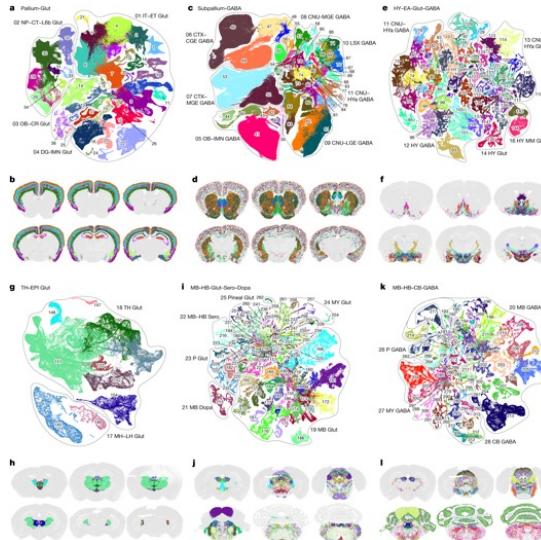
* for poly-A-based capture



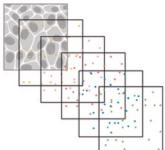
What references are currently available?

Allen Brain Cell Atlas: A high-resolution transcriptomic and spatial atlas of cell types in the whole mouse brain.

- 4 million cells profiled by scRNA-seq
- 4.3 million cells profiled by MERFISH
- Identified 6 neighborhoods of neuronal cell types with high regional specificity
- Integration of scRNA-seq and MERFISH data show a high correspondence between transcriptomic specificity and relatedness and spatial specificity and relatedness in neuronal neighborhoods
- Great reference data!!



Yao, Z., et al. (2023). A high-resolution transcriptomic and spatial atlas of cell types in the whole mouse brain. *Nature*. <https://doi.org/10.1038/s41586-023-06812-z>



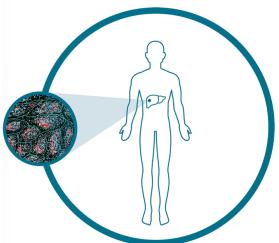
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Discover More from Precious FFPE Samples

Using [CosMx® SMI](#), we characterize liver FFPE tissue from normal and hepatocellular carcinoma patients and generated open-source dataset. This data showcases the best class data quality of the CosMx SMI. The dataset highlights CosMx SMI's high sensitivity with the capability to detect and quantify an average of 1,150 transcripts per cell and maximize biological insights by detecting 676 genes. The dataset also represents CosMx SMI's high specificity, with more than 99% of identified counts on targeted genes.

The CosMx SMI human liver data provides a subcellular expression map of 1,000 genes and a single cell tissue atlas that categorizes each cell in the tissue as one of 18 unique cell types. The complete dataset consists of over 800,000 single cells and ~700 million transcripts, and a single-cell tissue atlas across a ~180 mm² area of liver tissue. The complex analysis provided deep insight into the cell and tissue changes that occur in cancer, including infiltration of diverse immune cells.



LIVER DATA SUMMARY

INTERACTIVE VISUALIZATIONS

DOWNLOAD DATA

All Data Files

Download Links	Description	Size
Normal Liver Raw Data Files	Normal Liver datasets (does not include Seurat Object, TileDB Array or Vignette files)	349 GB
Liver Cancer Raw Data Files	Liver Cancer datasets (does not include Seurat Object, TileDB Array or Vignette files)	467 GB
Seurat Object	For analysis and visualization using the Seurat package	19.7 GB
Seurat Object (no transcripts)	For analysis and visualization using the Seurat package (transcript coordinates are not included)	16.6 GB
TileDB Array	For analysis and visualization using the TileDB structure	23.2 GB
Liver Data Set Vignette (.html)	Intro to Liver dataset and data objects	26.2 MB



What references are currently available?

Nucleic Acids Research, 2025, 53, gkae1298
<https://doi.org/10.1093/nar/gkae1298>
 Advance access publication date: 16 January 2025

Data Resources and Analyses



Mapping the spatial atlas of the human bone tissue integrating spatial and single-cell transcriptomics

Weiqiang Lin ^{①,†}, Yisu Li ^{②,†}, Chuan Qiu ¹, Binghao Zou ³, Yun Gong ¹, Xiao Zhang ¹, Di Tian ⁴, William Sherman ⁵, Fernando Sanchez ⁵, Di Wu ¹, Kuan-Jui Su ¹, Xinyi Xiao ⁶, Zhe Luo ¹, Qing Tian ¹, Yiping Chen ², Hui Shen ¹ and Hongwen Deng ^{①,*}

¹Tulane Center for Biomedical Informatics and Genomics, Deming Department of Medicine, School of Medicine, Tulane University, 1440 Canal Street, Downtown, New Orleans, LA 70112, USA

²Department of Cell and Molecular Biology, School of Science and Engineering, Tulane University, 6823 St. Charles Avenue, Uptown, New Orleans, LA 70118, USA

³Department of Structural and Cellular Biology, School of Medicine, Tulane University, 1430 Tulane Avenue, Downtown, New Orleans, LA 70112, USA

⁴The Molecular Pathology Laboratory, Department of Pathology and Laboratory Medicine, School of Medicine, Tulane University, 1430 Tulane Avenue, Downtown, New Orleans, LA 70112, USA

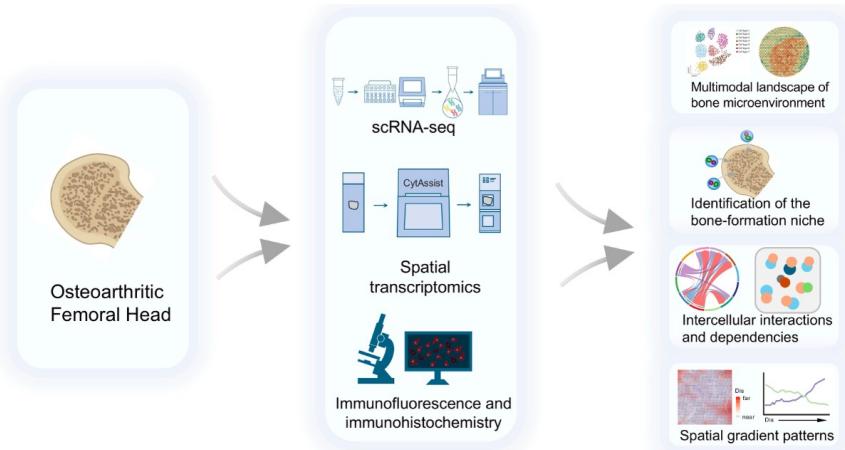
⁵Department of Orthopaedic Surgery, School of Medicine, Tulane University, 1430 Tulane Avenue, Downtown, New Orleans, LA 70112, USA

⁶Department of Epidemiology, School of Public Health and Tropical Medicine, Tulane University, 1440 Canal Street, Downtown, New Orleans, LA 70112, USA

*To whom correspondence should be addressed. Tel: +1 504 988 1310; Email: hdeng2@tulane.edu

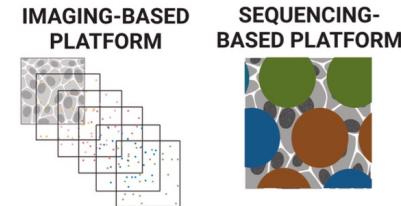
†The first two authors should be regarded as Joint First Authors.

- Human femoral head sample collected from hip replacement surgery
- Integration of 10x scRNA-seq and Visium



What references are currently available?

LatchBio



25M Cell Human Spatial Atlas on Latch

25M spatial atlas for human anatomy // 45 tissue types, 63 diseases and 11 spatial technologies // white-labeled spatial portals for kit providers // view large AnnData counts without a server



HANNAH LE, HARIHARA MURALIDHARAN, AND KENNY WORKMAN

SEP 05, 2025



New capabilities are added to Latch for spatial biology:

- Release of a 25 million cell spatial atlas spanning all 11 major spatial vendors: including 10X Genomics Visium, Visium HD, Xenium, Bruker Spatial CosMx, STOmics Stereo-seq, Vizgen MERSCOPE, Takara Bio Seeker, AtlasXomics DBiT-seq, Spatial Genomics GenePS (seqFISH), Element Biosciences AVITI24, and Singular Genomics G4X.
- A public data portal with annotated H5ADs ready to visualize and download
- A spatial vignette to allow researchers to continue with downstream steps, such as cell type annotation, ligand-receptor analyses, or multi-sample differential gene expression

To tackle the curation challenge, the LatchBio engineering team aggregated 25M spatial transcriptomes from public human studies covering 45 tissue types, 63 diseases and 11 spatial technologies. To our knowledge, this is the largest open access sequencing based spatial atlas available to date.

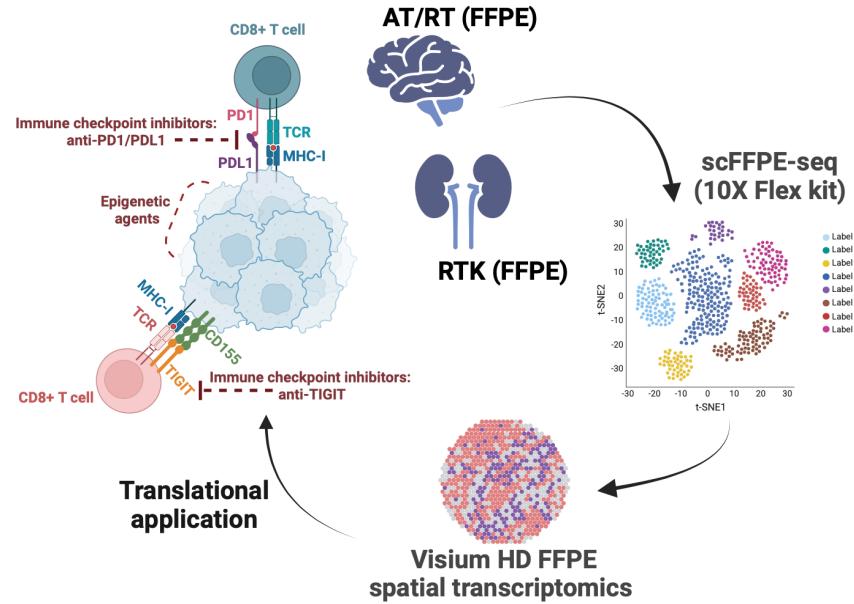


Let's walk through an example of Visium HD

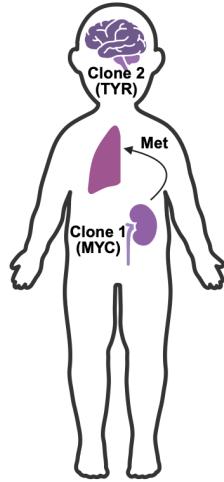
- Rhabdoid tumors (RT) can arise in the brain (atypical teratoid/rhabdoid tumor; AT/RT) or kidney (RTK)
- Aggressive, often diagnosed in children less than 3 years old
- 5-year survival of only 20-30% despite surgery, radiation, chemotherapy
- Majority of cases harbor a germline LOF mutation in *SMARCB1*
- Tumor immune microenvironment (TIME) of RT not well characterized

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- Tumor immune microenvironment (TIME) of RT not well characterized



Samples



Subject 1

Female

10 mo at diagnosis

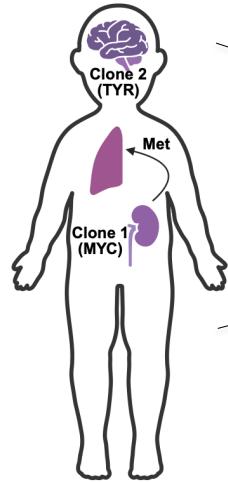
Primary AT/RT

Primary RTK with
met to lung

Germline
SMARCB1

p.Pro215Leufs*14

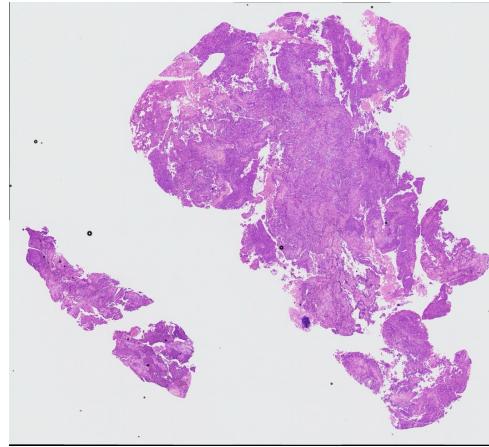
Samples



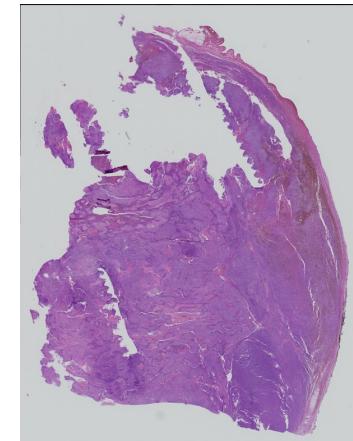
Subject 1
Female
10 mo at diagnosis
Primary AT/RT
Primary RTK with
met to lung

Germline
SMARCB1
p.Pro215Leufs*14

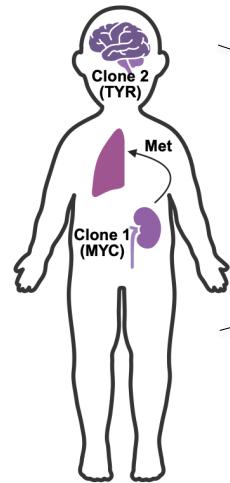
Primary AT/RT – H&E



Primary RTK – H&E



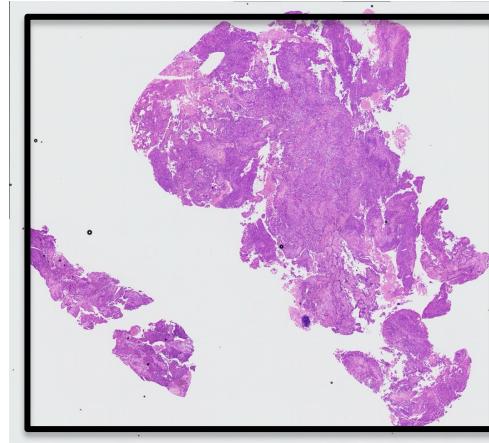
Samples



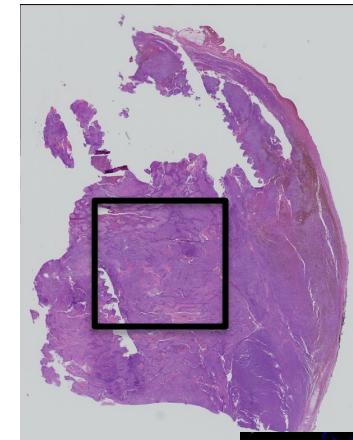
Subject 1
Female
10 mo at diagnosis
Primary AT/RT
Primary RTK with
met to lung

Germline
SMARCB1
p.Pro215Leufs*14

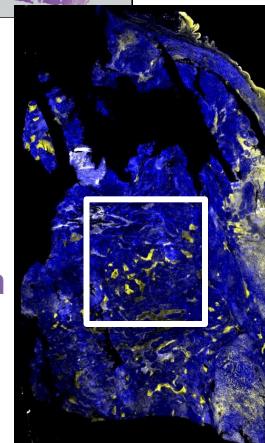
Primary AT/RT – H&E



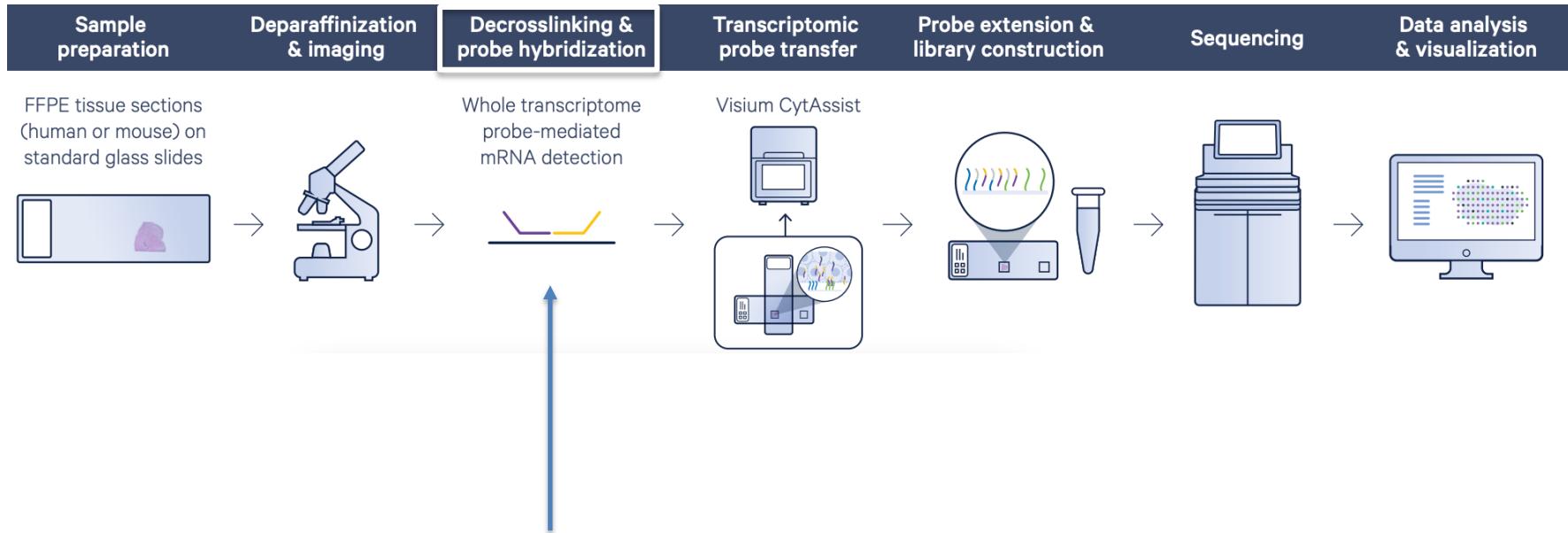
Primary RTK – H&E



Primary RTK – GeoMx CD45 Stain
"Sister section"
(CD45 = yellow; nuclei = blue)



Lab workflow



Required modification for human (not mouse) brain (healthy and tumor):
increase decrosslinking temperature from standard 80 °C to 95 °C

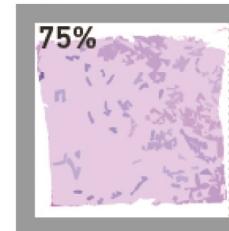
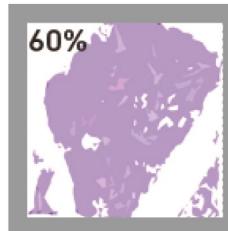
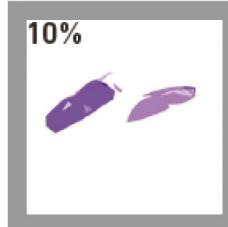
Sequencing

10x Recommended Sequencing Depth:

Example: Sequencing Depth for a Sample

- Estimate the approximate Capture Area (%) covered by the tissue section.
- Calculate total sequencing depth =
(Coverage Area x 275,000,000 read pairs)
- Example calculation for 60% coverage:
 $0.60 \times 275,000,000$ read pairs =
165,000,000 total read pairs for that sample

Estimated Coverage Area (%) Examples



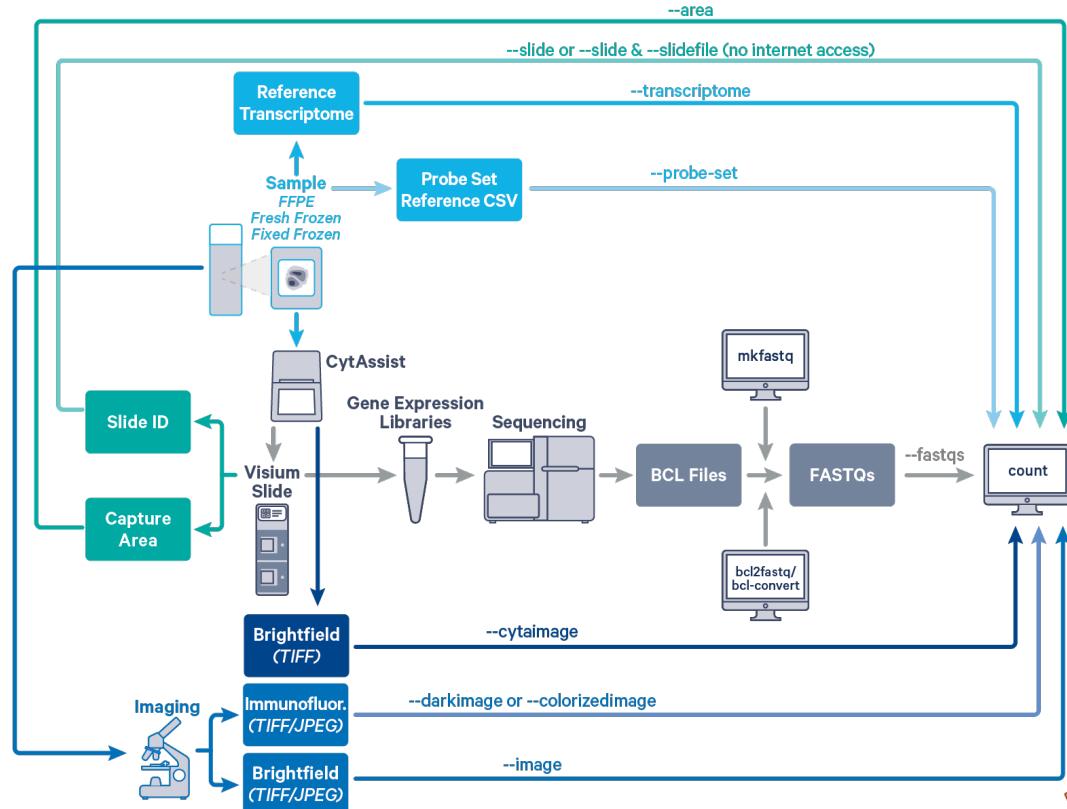
Singular G4 F3 Flow Cell:



1 Visium HD library per F3 flow cell
~ 450 M reads on average



Bioinformatics Workflow



Bioinformatics Workflow

SpaceRanger 3.1.1 command line shell script

Path to SpaceRanger

Sample ID

Transcriptome (human or mouse)

FASTQ path

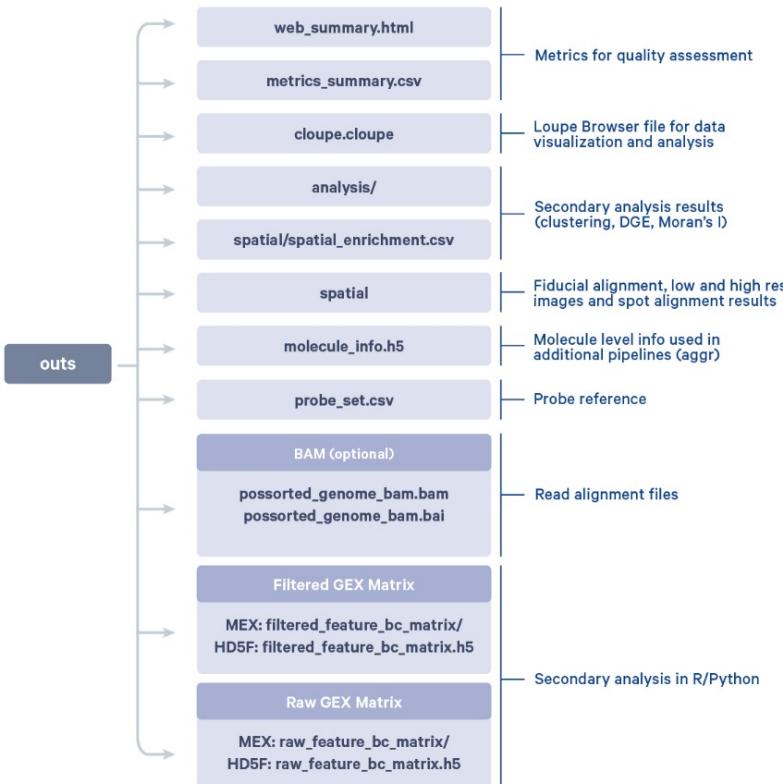
Probe set (human or mouse)

Slide ID

Area (CytAssist position)

Cytassist image

High-res H&E image



2 um, 8 um, and 16 um bins

Bioinformatics Workflow

SpaceRanger 4.0.1 via 10x Genomics Cloud Analysis (web-based)

**10x Genomics
Cloud Analysis**

Upload FASTQs, images, and Loupe files after sequencing a 10x Genomics library, analyze the data using 10x analysis pipelines, and download the results.



Upload:

- FASTQs
- CytAssist Image
- High-res H&E image
- Select appropriate transcriptome/probes (human or mouse)

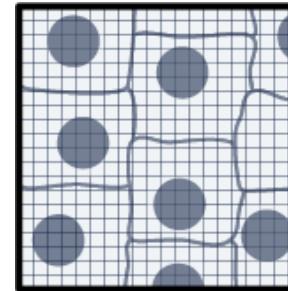
Output:

```
<run-id>/  
└─ outs/  
    ├─ metrics_summary.csv  
    ├─ web_summary.html  
    ├─ cloupe.clope  
    ├─ binned_outputs/  
    │   ├─ square_002um/  
    │   ├─ square_008um/  
    │   └─ square_016um/           (symlink for 8 µm bin  
    │           :contentReference[oaic  
    └─ segmented_outputs/  
        ├─ cell_segmentations.geojson  
        ├─ nucleus_segmentations.geojson  
        ├─ graphclust_annotation_cell_segmentations.geojson  
        ├─ graphclust_annotation_nucleus_segmentations.geojson  
        ├─ filtered_feature_cell_matrix/ (and .h5)  
        ├─ raw_feature_cell_matrix/     (and .h5)  
        ├─ spatial/  
        └─ analysis/  
            ├─ spatial/  
            └─ <slideID>/  
                ├─ tissue_hires_image.png  
                ├─ tissue_lowres_image.png  
                └─ scalefactors_json.json  
        └─ feature_slice.h5           (for 2 µm resolution
```

2 um, 8 um, and 16 um bins
+ cell segmentation

Cell Segmentation

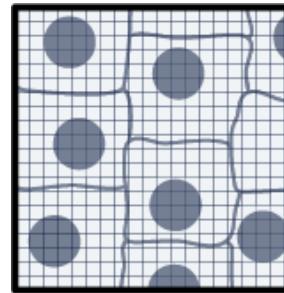
- Visium HD slide spatial barcodes patterned in a continuous grid of 2x2 um squares



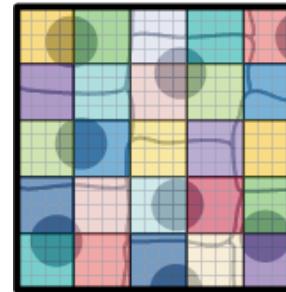
Tissue over 2x2um
Visium HD slide

Cell Segmentation

- Visium HD slide spatial barcodes patterned in a continuous grid of 2x2 um squares
- By default, SpaceRanger v3 creates 8x8 um and 16x16 um bins
- Each bin contains the gene—wise summation of the UMI counts of multiple 2x2 um squares



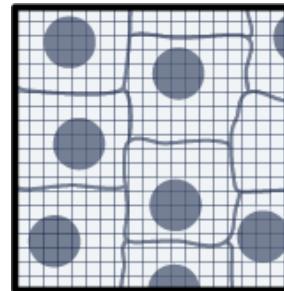
Tissue over 2x2um
Visium HD slide



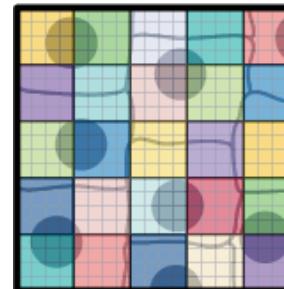
Binning based on
widening of 2x2um
barcoded spots

Cell Segmentation

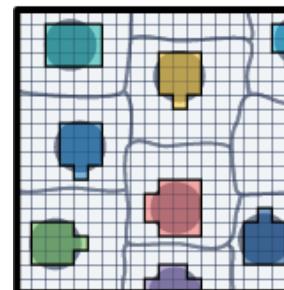
- Visium HD slide spatial barcodes patterned in a continuous grid of 2x2 um squares
- By default, SpaceRanger v3 creates 8x8 um and 16x16 um bins
- Each bin contains the gene—wise summation of the UMI counts of multiple 2x2 um squares
- Alternate approach:
 - Use the high-res microscope image of the tissue to create custom bins
 - The nuclei staining partitions barcodes into bins based on the nuclei they correspond to
 - This partitioning into nuclei-specific bins (“cell segmentation”) mimics true single cell data since counts are now reported on a per-cell basis



Tissue over 2x2um
Visium HD slide



Binning based on
widening of 2x2um
barcoded spots



H&E image is used to
identify nuclei and
estimate diameter of
cell and then bin spots
based on single cell
coverage

SpaceRanger QC

SpaceRanger 4.0.1 via 10x Genomics Cloud Analysis (web-based) Web Summary

Summary	Image Alignment	Bin-Level Metrics	Cell Segmentation
Key Metrics			
672,918 Number of 8 µm binned Squares under tissue			721.6 Mean reads per 8 µm bin
139.1 Mean UMIs per 8 µm bin			18,032 Total genes detected
Mapping			
Reads Mapped to Probe Set 98.7%			
Reads Mapped Confidently to Probe Set 98.5%			
Reads Mapped Confidently to the Filtered Probe Set 96.9%			
Reads Half-Mapped to Probe Set 0.1%			
Reads Split-Mapped to Probe Set 0.0%			
Sequencing			
Number of Reads 485,567,208			
Valid Barcodes 90.2%			
Valid UMI Sequences 100.0%			
Sequencing Saturation 76.8%			
Q30 Bases in Barcode 94.3%			
Q30 Bases in Probe Read 94.7%			
Q30 Bases in UMI 91.3%			
Fraction of Bins Under Tissue 8 µm 95.8%			
UMIs per sq mm of Tissue 2,173,676			
Reads per sq mm of Tissue 10,047,071			
Fraction Reads in Squares Under Tissue 98.6%			
Cell Segmentation Metrics			
Number of Cells 314,843			
Reads in Cells 90.0%			
UMIs in Cells 96.2%			
Mean Reads per Cell 1,335.8			
Median Genes per Cell 204.0			
Median UMIs per Cell 234.0			
Median Cell Area (µm ²) 116.0			
Median Nucleus Area (µm ²) 40.0			
Maximum Nucleus Diameter (pixels) 256			



Unpublished

SpaceRanger QC

SpaceRanger 4.0.1 via 10x Genomics Cloud Analysis (web-based) Web Summary

Summary Image Alignment Bin-Level Metrics Cell Segmentation

Key Metrics

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Number of 8 µm binned
Squares under tissue

721.6

Mean reads per 8 µm bin

139.1

Mean UMIs per 8 µm bin

18,032

Total genes detected

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Unpublished

SpaceRanger QC

SpaceRanger 4.0.1 via 10x Genomics Cloud Analysis (web-based) Web Summary

Summary Image Alignment Bin-Level Metrics Cell Segmentation

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Unpublished

SpaceRanger QC

SpaceRanger 4.0.1 via 10x Genomics Cloud Analysis (web-based)
Web Summary

Summary Image Alignment Bin-Level Metrics Cell Segmentation

Key Metrics

672,918	Number of 8 µm binned Squares under tissue
721.6	Mean reads per 8 µm bin
139.1	Mean UMIs per 8 µm bin
18,032	Total genes detected

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Mean Reads per Cell	1,335.8
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Median Cell Area (µm ²)	116.0
Median Nucleus Area (µm ²)	40.0
Maximum Nucleus Diameter (pixels)	256



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Importance of de-crosslinking temperature modification for FFPE human brain samples

Standard: 80°C de-xlink

Alerts		
The analysis detected ⚠ 3 warnings.		
Alert	Value	Detail
⚠ High Fraction Reads Half-Mapped to Probe Set	32.0%	Ideal < 20%. This can indicate low RNA content in the sample, poor washing after probe hybridization, deviation from recommended protocol during probe hybridization, or suboptimal sample preparation.
⚠ High Estimated gDNA Content	21.7%	Ideal < 20%. High gDNA levels were detected. Confirm that workflow was performed correctly and check tissue block quality.
⚠ Low Fraction Gene Expression Reads in Squares	36.6%	Ideal > 75%. Application performance may be affected. Many of the reads were not assigned to tissue covered squares. This could be caused by high levels of ambient RNA resulting from inefficient permeabilization, because the incorrect image was used, or because of poor tissue detection. The latter case can be addressed by using the manual tissue selection option through Loupe. <i>Ignore this alert if you manually selected a subset of the tissue in the capture area, such as a single core punch in a TMA.</i>

Summary Image Alignment Bin-Level Metrics

Key Metrics

360,485

Number of 8 μm binned squares under tissue

387.0

Mean reads per 8 μm bin

0.3

Mean UMIs per 8 μm bin

8,639

Total genes detected

Mapping

Reads Mapped to Probe Set	96.6%
Reads Mapped Confidently to Probe Set	63.7%
Reads Mapped Confidently to the Filtered Probe Set	62.3%
Reads Half-Mapped to Probe Set	32.0%
Reads Split-Mapped to Probe Set	0.9%

Summary Image Alignment Bin-Level Metrics

Bin Metrics Overview

Bin Size (μm)	8 μm	16 μm
Number of Bins Under Tissue	360,485	94,659
Mean UMI Counts per Bin	0.3	1.1
Mean Genes per Bin	0.1	0.5

UMIs from Genomic DNA

Estimated UMIs from Genomic DNA

21.7%

No alerts

Modified: 95°C de-xlink

Summary Image Alignment Bin-Level Metrics

Key Metrics

244,213

Number of 8 μm binned squares under tissue

1889.3

Mean reads per 8 μm bin

161.7

Mean UMIs per 8 μm bin

18,011

Total genes detected

Mapping

Reads Mapped to Probe Set	98.4%
Reads Mapped Confidently to Probe Set	97.7%
Reads Mapped Confidently to the Filtered Probe Set	95.5%
Reads Half-Mapped to Probe Set	0.2%
Reads Split-Mapped to Probe Set	0.5%

Summary Image Alignment Bin-Level Metrics

Bin Metrics Overview

Bin Size (μm)	8 μm	16 μm
Number of Bins Under Tissue	244,213	63,037
Mean UMI Counts per Bin	161.7	628.8
Mean Genes per Bin	153.1	549.3

Sequencing

Number of Reads

461,385,091

Valid Barcodes

92.9%

Valid UMLs

100.0%

Sequencing Saturation

89.3%

Q30 Bases in Barcode

94.3%

Q30 Bases in Probe Read

94.2%

Q30 Bases in UMI

91.6%

Fraction of Bins Under Tissue 8 μm

34.8%

Fraction Reads in Squares Under Tissue

93.3%

UMIs from Genomic DNA

Estimated UMIs from Genomic DNA

9.7%

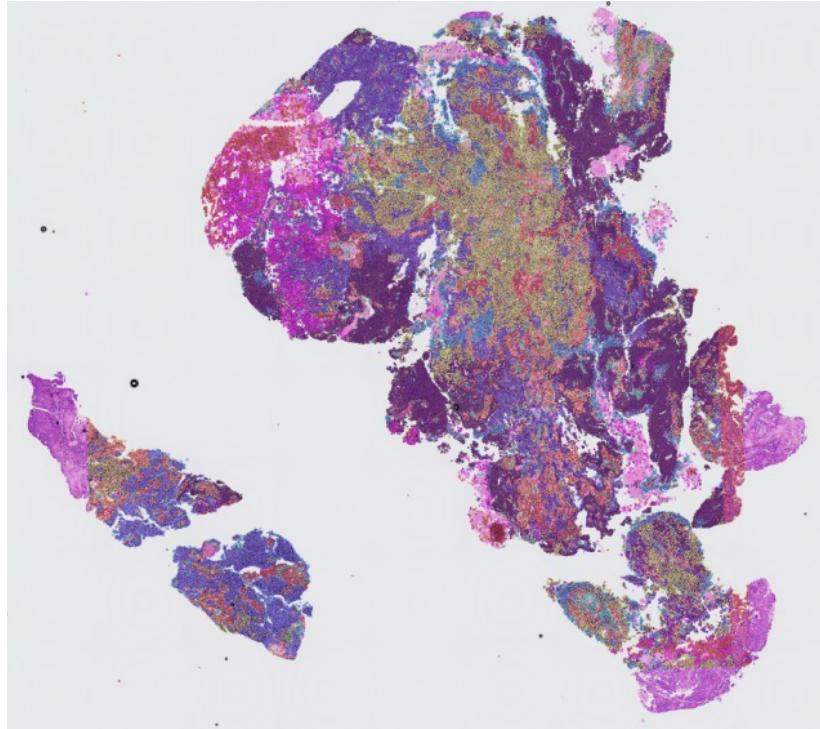


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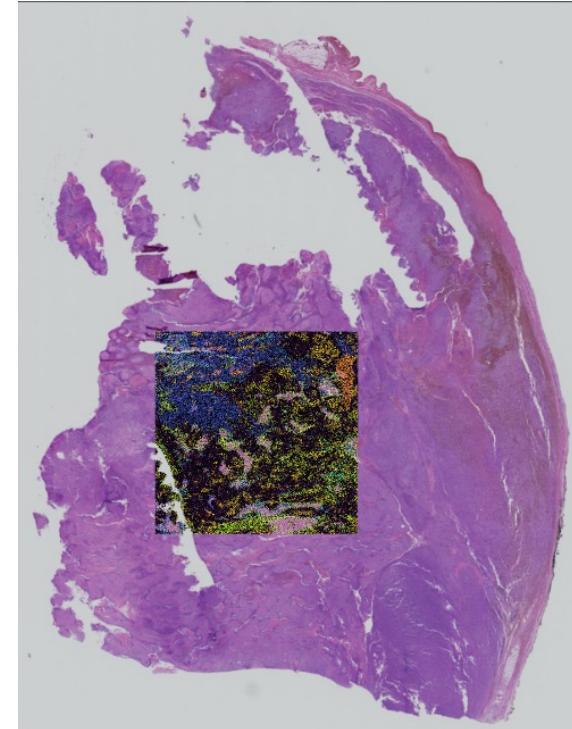
Unpublished

Visualize your data: 10X Genomics Loupe Browser 9.0.0 with cell segmentation

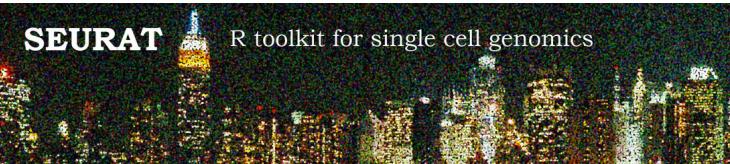
Primary AT/RT



Primary RTK



Incorporating cell segmentation into Seurat analysis



R toolkit for single cell genomics

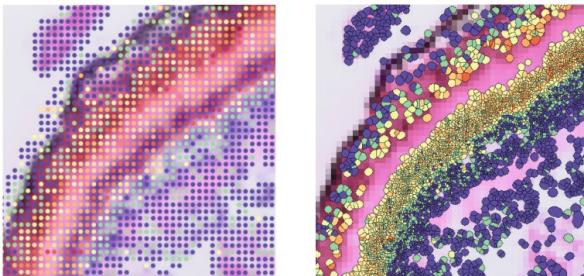
A screenshot of a GitHub repository page for "satijalab/seurat". The repository has 277 issues and 34 pull requests. A pull request titled "Add support for Space Ranger cell segmentation data #10028" is shown, which has been merged. It includes a conversation with 46 messages, 27 commits, 1 check, and 14 files changed. The pull request details show anashen merging 27 commits from "spaceranger-4.0" into "main" last month.

Add support for Space Ranger cell segmentation data #10028

Merged anashen merged 27 commits into main from spaceranger-4.0 last month

A screenshot of the GitHub commit history for pull request #10028. It shows a list of 27 commits, each with a small preview icon and a link to the commit details. The commits are part of the merge from "spaceranger-4.0" into "main".

Support for Space Ranger 4.0 Spatial Datasets



16um cell binnings

Nuclei-expansion cell binnings

<https://github.com/satijalab/seurat/pull/10028>
(Requires Seurat beta version)

Space Ranger Cell Segmentation Folder Structure

```
<run-id>/  
└─ outs/  
    └─ metrics_summary.csv  
    └─ web_summary.html  
    └─ cloupe.clope  
        ( symlink for 8 µm bin )  
    └─ binned_outputs/  
        └─ square_002um/  
        └─ square_008um/  
            └─ square_016um/ :contentReference[oaic]  
    └─ segmented_outputs/  
        └─ cell_segmentations.geojson  
        └─ nucleus_segmentations.geojson  
        └─ graphclust.annotated_cell_segmentations.geojson  
        └─ graphclust.annotated_nucleus_segmentations.geojson  
        └─ filtered_feature_cell_matrix/ (and .h5)  
        └─ raw_feature_cell_matrix/ (and .h5)  
        └─ spatial/  
            └─ analysis/  
    └─ spatial/  
        └─ <slideID>/  
            └─ tissue_hires_image.png  
            └─ tissue_lowres_image.png  
            └─ scalefactors_json.json  
    └─ feature_slice.h5 (for 2 µm resolution)
```

Read in spatial data and
retain 8 and 16 um bins and segmentation

```
ch112_atr <-  
  Load10X_Spatial(data.dir = data.dir,  
                  slice = "slice1",  
                  bin.size = c(8,16, "polygons"))
```

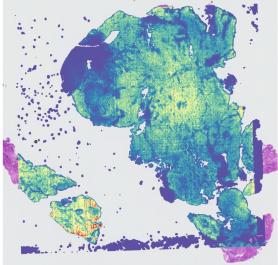


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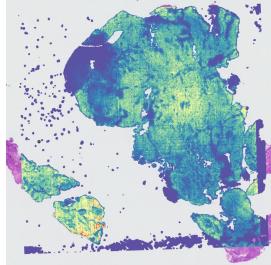
Seurat QC – 8 um vs 16 um vs cell segmentation polygons

8 um binning

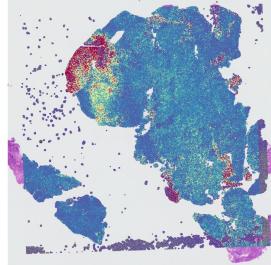
nCount_Spatial.008um
0 250 500 750 1000



nFeature_Spatial.008um
0 250 500 750 1000

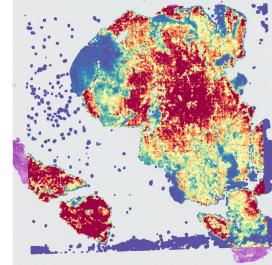


percent.mt_Spatial.008um
0 5 10 15 20

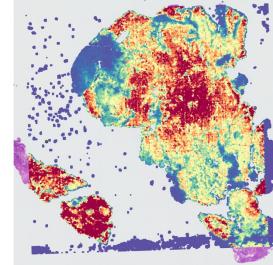


16 um binning

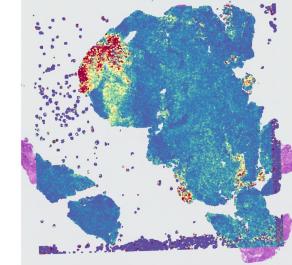
nCount_Spatial.016um
0 250 500 750 1000



nFeature_Spatial.016um
0 250 500 750 1000

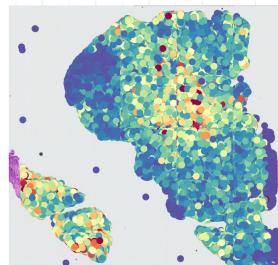


percent.mt_Spatial.016um
0 5 10 15 20

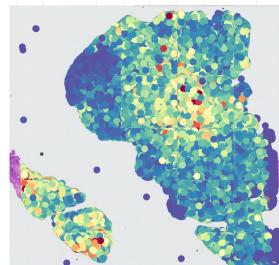


cell segmentation polygons

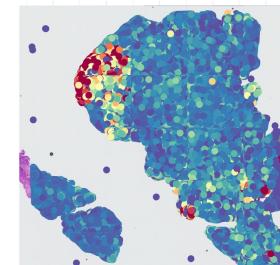
nCount_Spatial.Polygons
0 250 500 750 1000



nFeature_Spatial.Polygons
0 250 500 750 1000



percent.mt_Spatial.Polygons
0 5 10 15 20



nCount

Total number of molecules in each spot

nFeature

Total number of genes detected in each spot

Percent mito

Percent of mitochondrial reads



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Unpublished

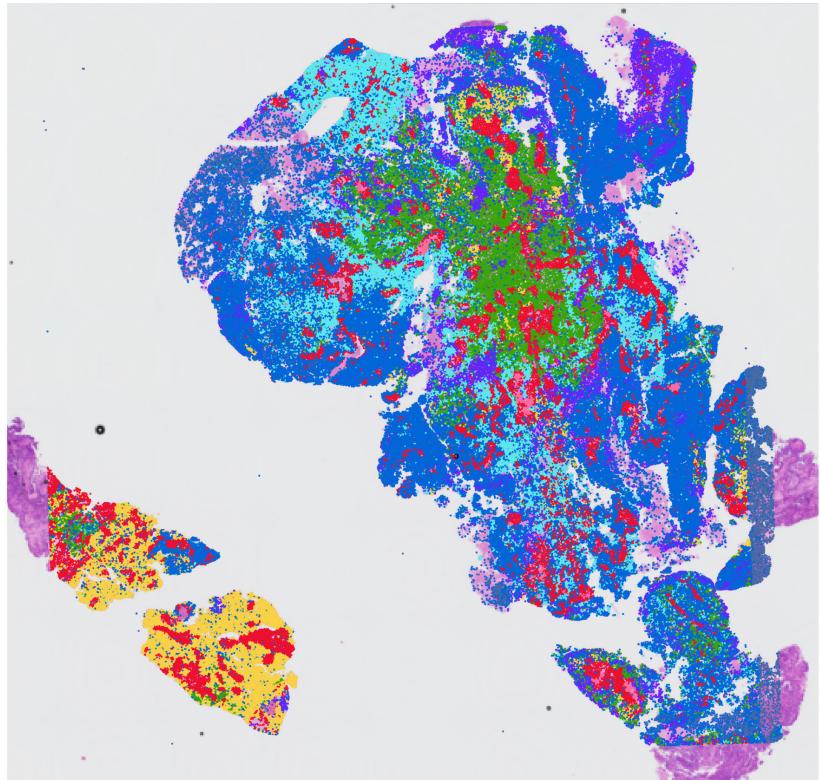
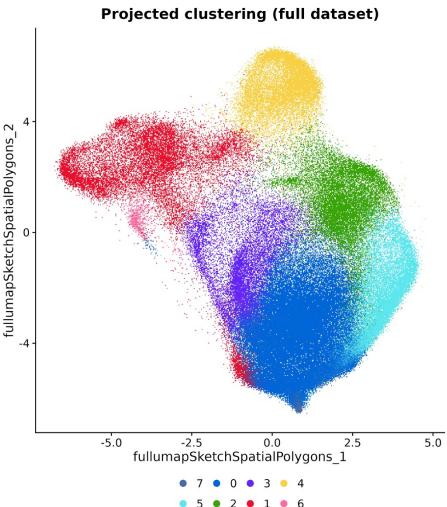
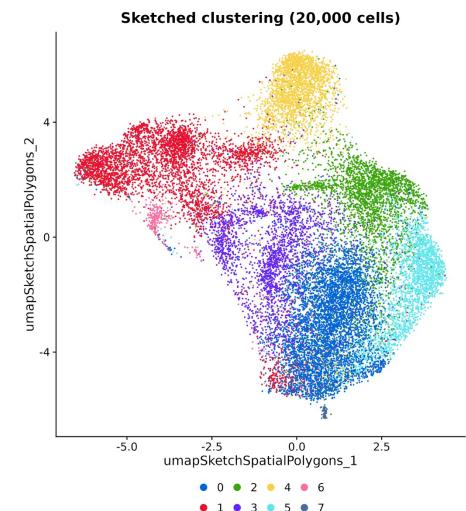
Normalization	Sketch-based Clustering
<p>Standard log-normalization so far...best normalization method for spatial data still being developed and evaluated</p> <p>Goal: account for differences in mRNA capture across tissue</p> <p>Option #1: divide each cell/spot by the total counts for that cell/spot and multiple by a scale factor and natural-log transform ("log-normalize")</p> <p>Option #2: normalize cells/spots based on groups of genes with similar abundance, one group at a time ("SCTransform") ~ good for samples with varying sequencing depth</p>	<p>Select a subset ("sketch") of bins</p> <p>Uses "leverage score" to sample cells rather than sampling cells with equal probability</p> <p>In linear regression, this leverage is the influence of an individual point in determining the best least-squares fit</p> <p>Reflects the magnitude of its contribution to the gene-covariance matrix</p> <p>High leverage scores = largest contribution = important!</p> <p>Oversamples rare populations, retaining biological complexity</p> <p>Perform clustering on sketched data</p> <p>Project sketched clusters onto full data</p>

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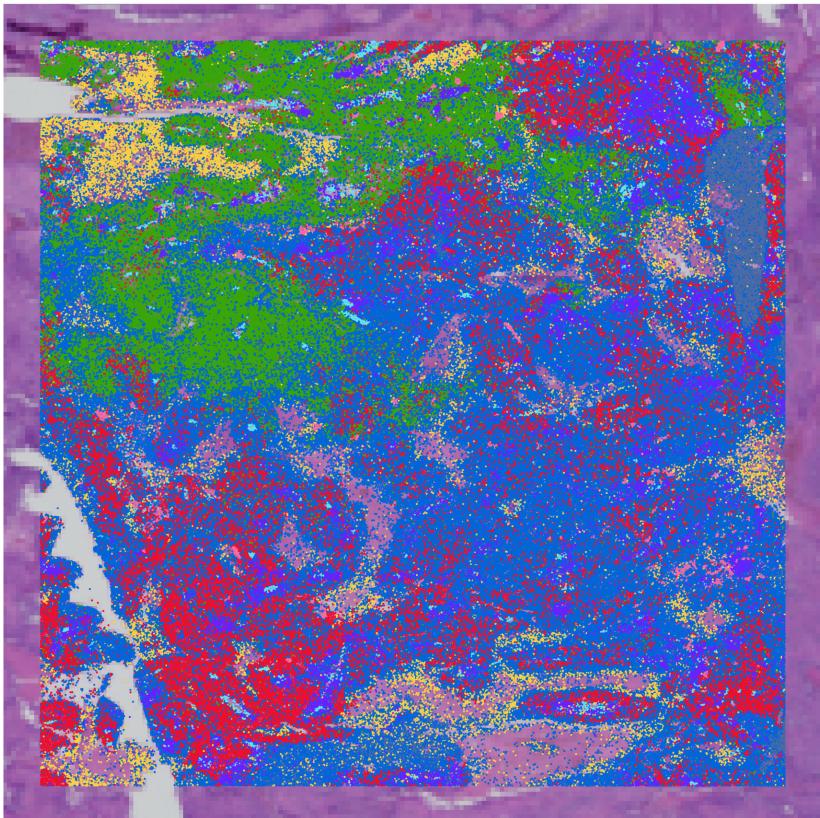
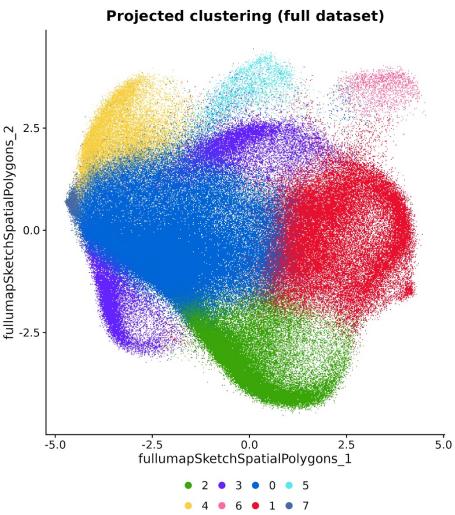
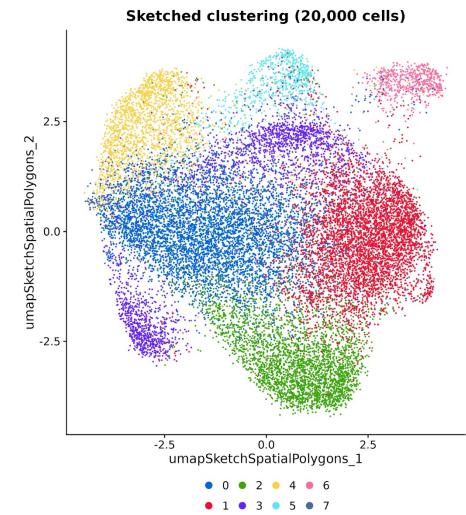
[^]For now, as long as sequencing depth per bin of choice is relatively consistent across samples, no need to integrate for comparisons

<https://www.10xgenomics.com/analysis-guides/tutorial-visium-hd-multi-sample-r-collab>

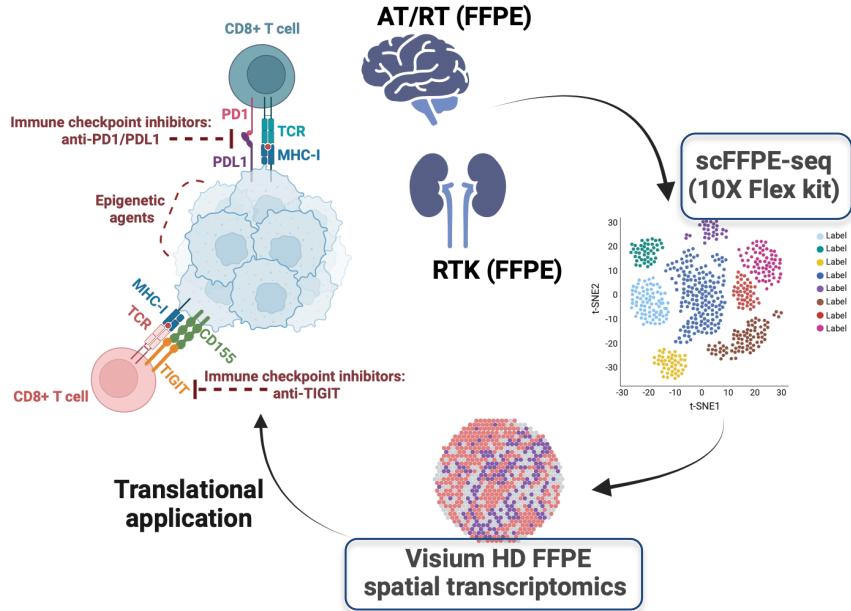
Normalization and sketch clustering - cell segmentation polygons – AT/RT



Normalization and sketch clustering - cell segmentation polygons – RTK



Cell typing using scFFPE-seq reference



Article | Published: 18 February 2021

Robust decomposition of cell type mixtures in spatial transcriptomics

Dylan M. Cable, Evan Murray, Luli S. Zou, Aleksandrina Goeva, Evan Z. Macosko, Fei Chen

& Rafael A. Irizarry

Nature Biotechnology 40, 517–526 (2022) | Cite this article

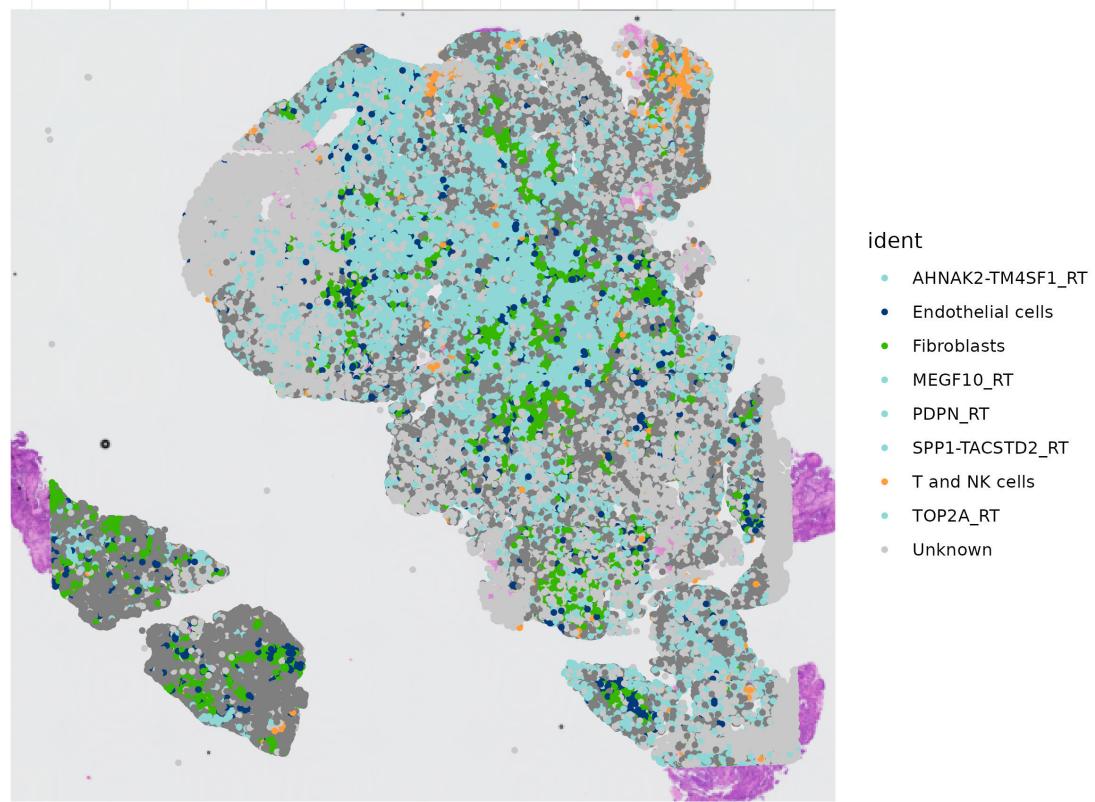
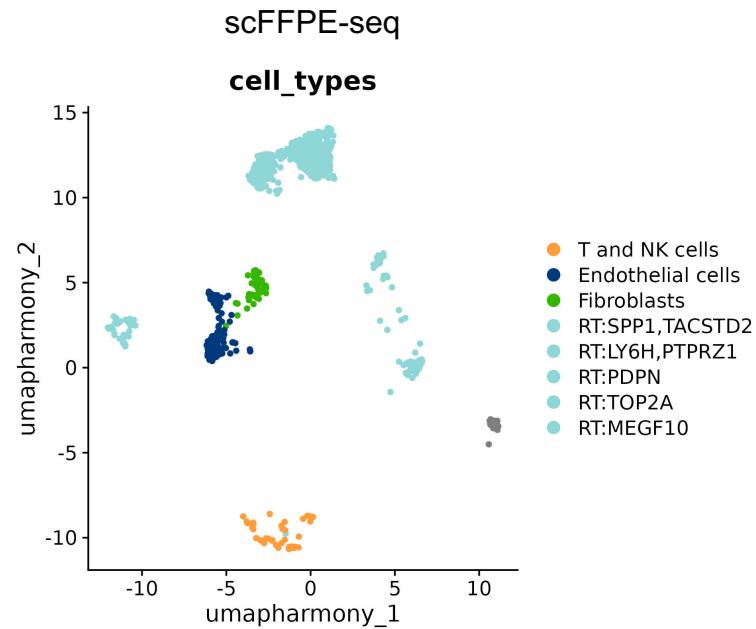
64k Accesses | 973 Citations | 115 Altmetric | Metrics

- Computational approach to deconvolute spot-level data for spatial datasets when provided with scRNA-seq reference
 - Note: requires at least 25 cells per cell type
- Example use case:
 - scFFPE-seq from scrolls cut from same block used for Visium HD



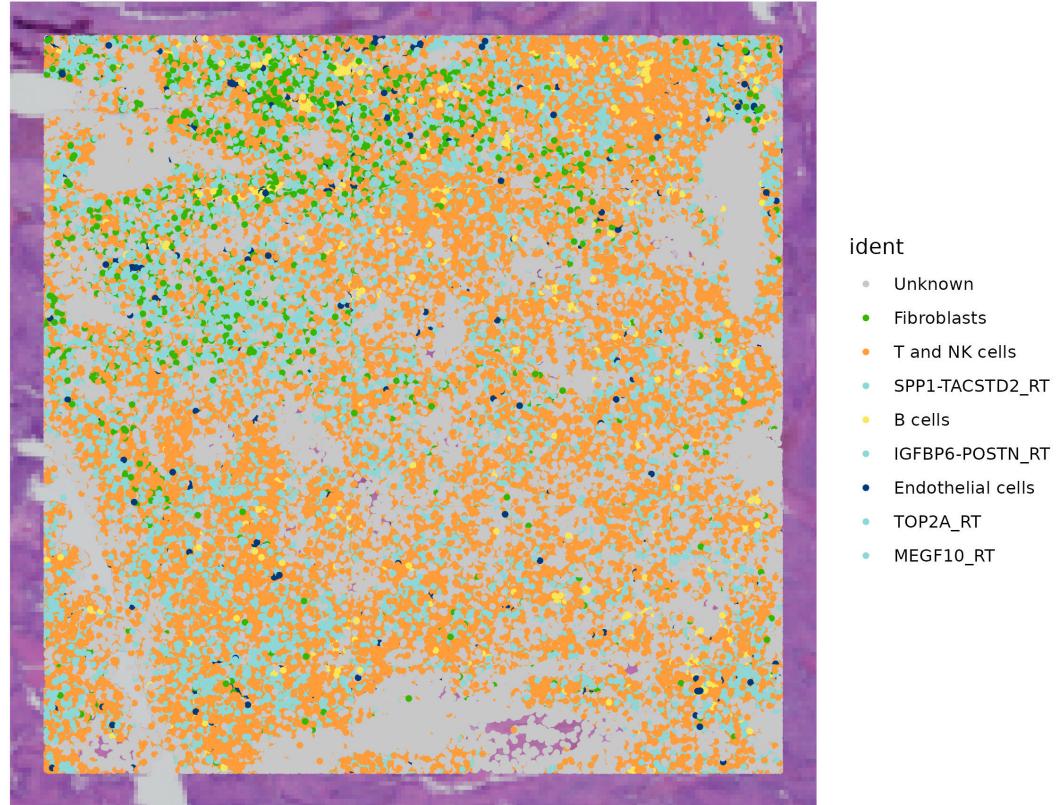
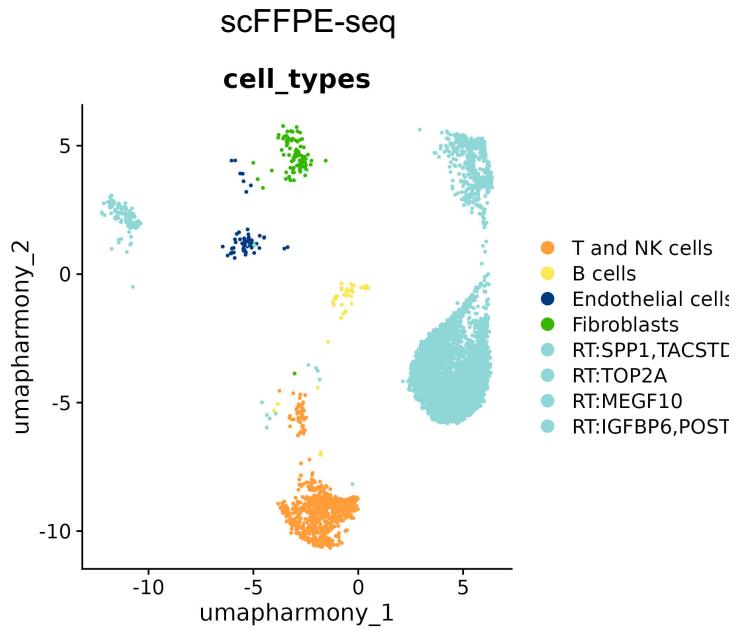
Unpublished

Cell typing using scFFPE-seq reference – AT/RT



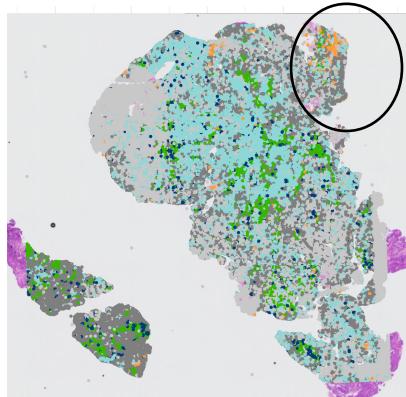
Unpublished

Cell typing using scFFPE-seq reference – RTK



Unpublished

Selecting region of interest with Loupe Browser

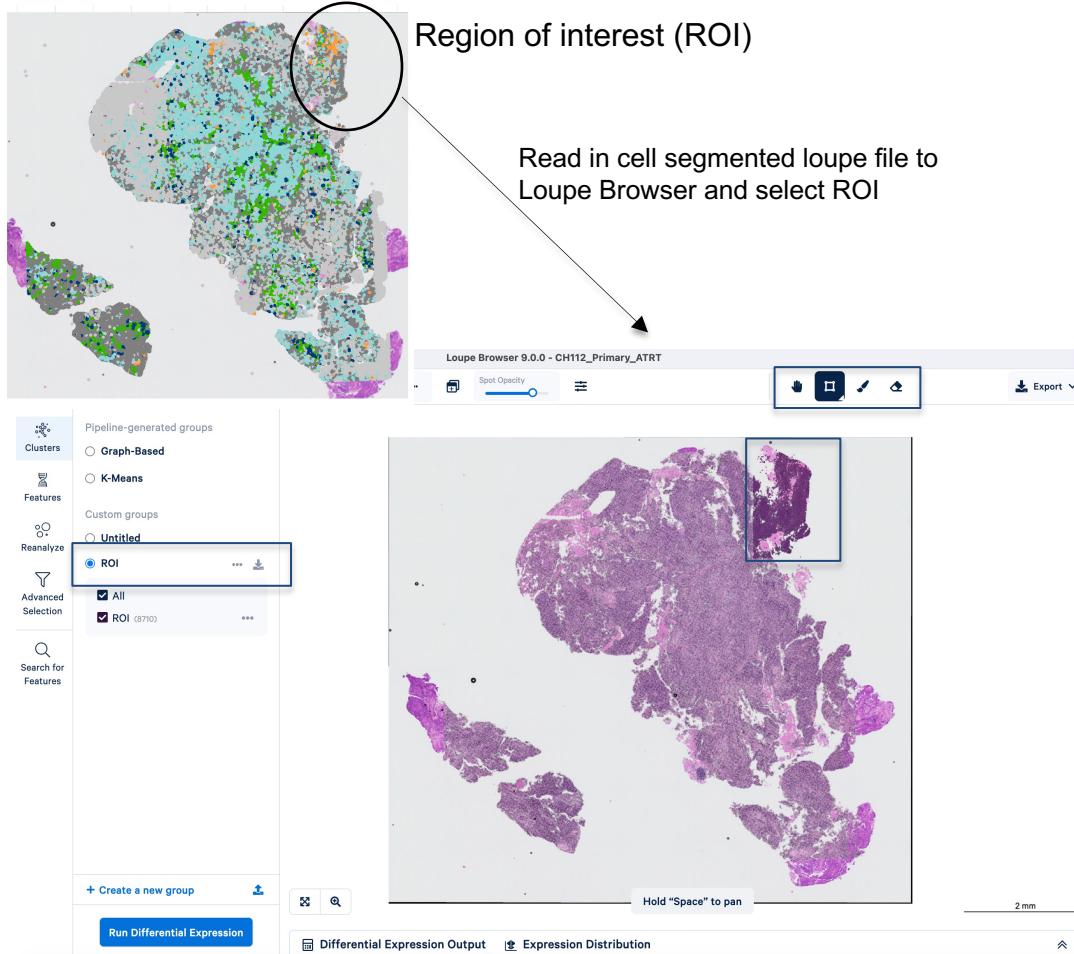


Region of interest (ROI)

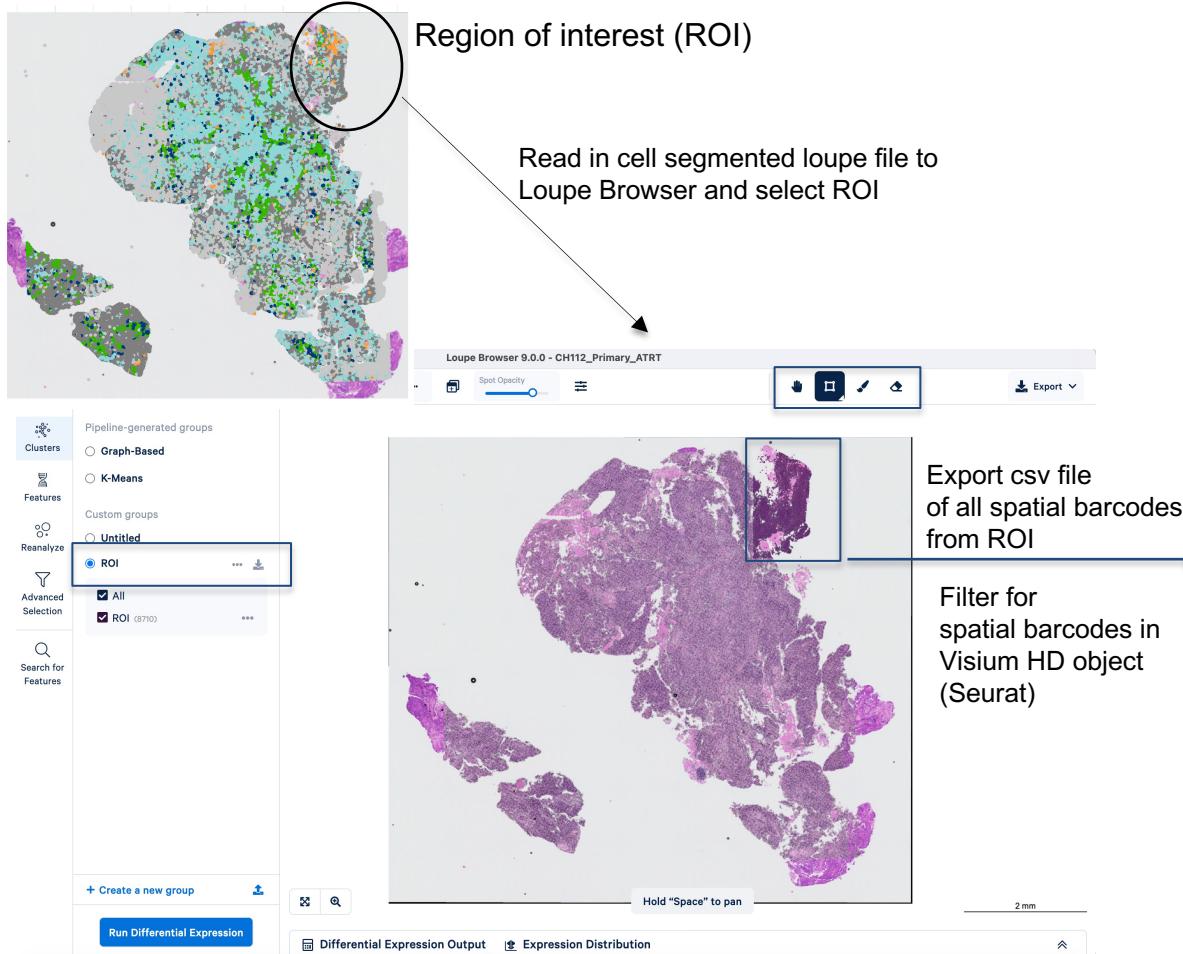


Unpublished

Selecting region of interest with Loupe Browser

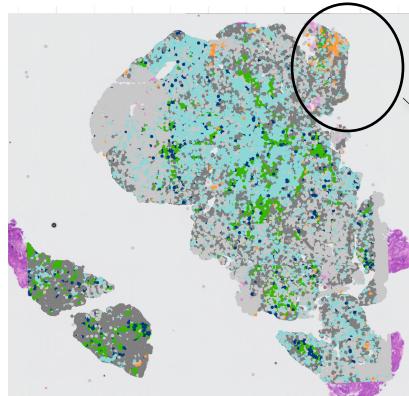


Selecting region of interest with Loupe Browser



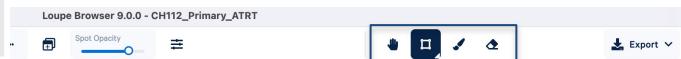
Unpublished

Selecting region of interest with Loupe Browser



Region of interest (ROI)

Read in cell segmented loupe file to
Loupe Browser and select ROI



Clusters

- Pipeline-generated groups
 - Graph-Based
 - K-Means
- Custom groups
 - Untitled
 - ROI

Features

Reanalyze

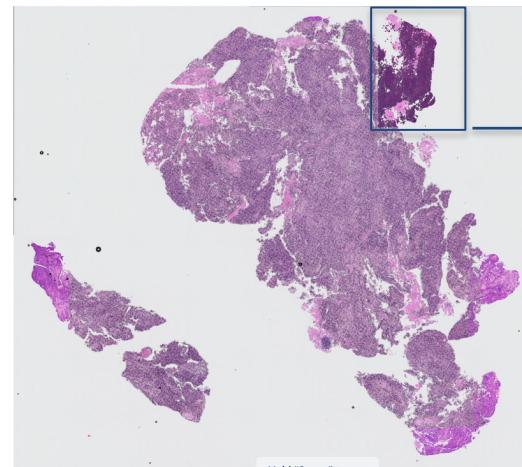
Advanced Selection

Search for Features

Find

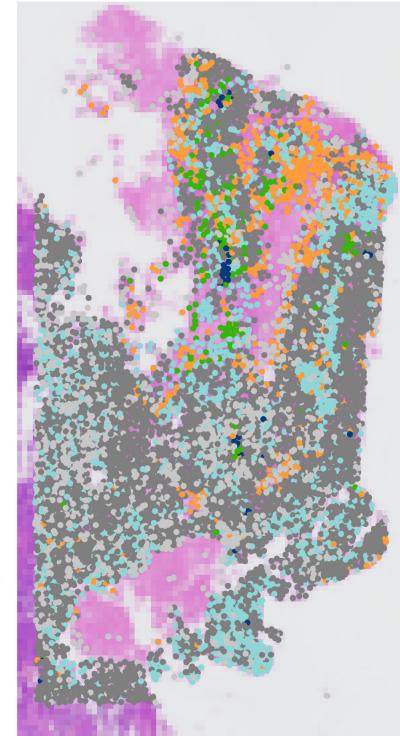
+ Create a new group

Run Differential Expression



Export csv file
of all spatial barcodes
from ROI

Filter for
spatial barcodes in
Visium HD object
(Seurat)



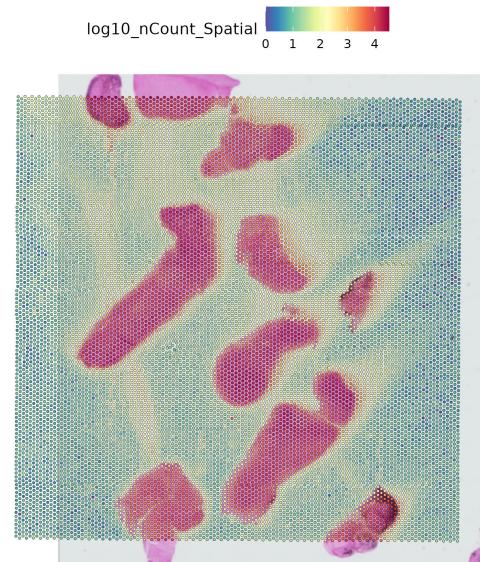
Subsetted Visium HD object for downstream analyses
Sub-cluster, differential expression, etc.



Unpublished

What challenges remain?

- Compatibility with wider range of tissues
- Optimize data storage and processing speed
- Optimize spatial resolution and capture efficiency
- Improve normalization methods and batch correction
- Increased throughput, reduced cost, greater accessibility
- Reduce signal diffusion
- Integration with multi-omics
 - Spatial TCR/BCR-seq or genotyping (long-read)



What's next?

- **DNA sequencing:** slide-DNA-seq (*Zhao et al., 2022*)
- **Epigenomics:** ATAC-seq (*Deng et al., 2022*) and CUT&TAG (*Deng et al., 2022*) both available with FlowGel assay by AtlasXomics
- **Proteomics:** CODEX (*Goltsev et al., 2018*), Immuno-SABER (*Saka et al., 2019*), scDVP (*Rosenberger et al., 2023*), RIBOmap (*Zeng et al., 2023*), CITE-seq (*Liu et al., 2023*), Stereo-CITE-seq (*Liao et al., 2023*)
- **Metabolomics:** MSI (*Unsihuay et al., 2021*) and scSpaMet (*Hu et al., 2023*)
- **Temporally-resolved:** intMEMOIR (*Chow et al., 2021*), Spatial iTracer (*He et al., 2022*), Space-TREX (*Ratz et al., 2022*)
 - *Enable lineage tracing using CRISPR-Cas9 scarring or lentiviral barcoding!*

Just this week...

nature portfolio

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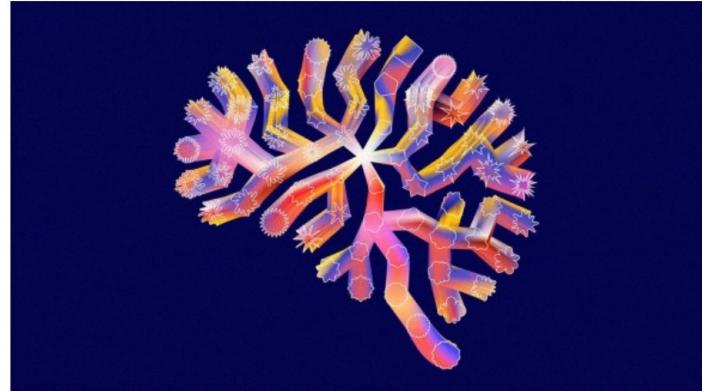
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[nature](#) > collection

Collection | 05 November 2025

BICAN: A cell census of the developing human brain

Building on their landmark efforts to create cell-type atlases of adult brains using single-cell and spatial genomics technologies, researchers in the BRAIN Initiative Cell Atlas Network (BICAN) have unveiled their next endeavour, to create comprehensive and multimodal cell-type atlases of the developing human, mouse and non-human primate brains.



Mardis/Miller Lab

Elaine Mardis, PhD

Katie Miller, PhD

Corinne Strawser, PhD

Jaye Navarro, BS

Lakshmi Prakruthi Rao Venkata, MS

Adithe Rivaldi, BS

Sophie Paxton, BS

Maha Hussein, MS

Srestha Chattopadhyay

Scott Friedland, MD/PhD

Emily Ringwalt, PhD



Thank you

Contact:

Elizabeth.Garfinkle@nationwidechildrens.org



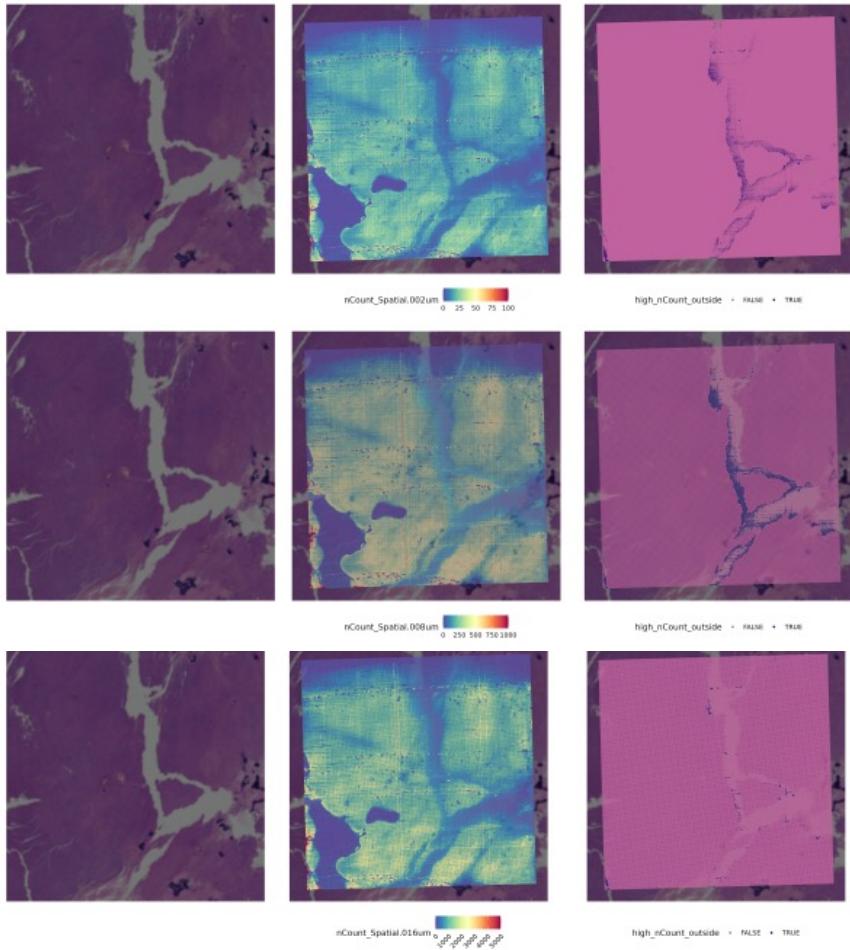
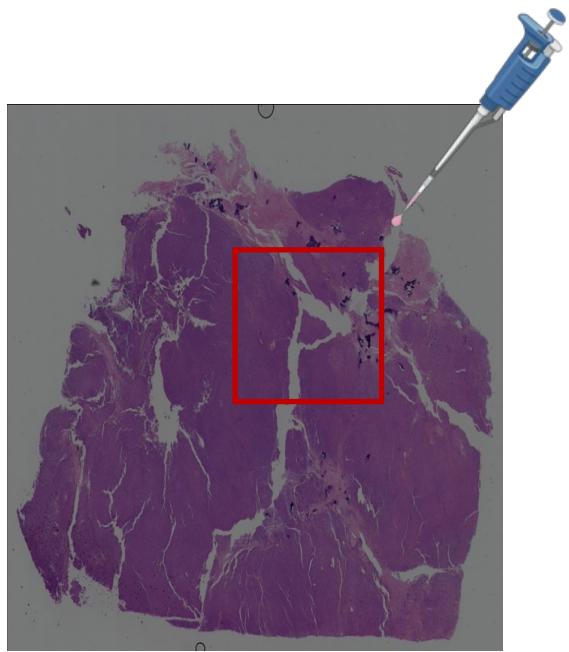
Questions?



Mark Foundation Momentum Fellowship

	Visium	Visium HD
Company	10X Genomics	10X Genomics
Method	Array	Array
Resolution	55 µm	2 µm
# Features	Whole Transcriptome	Whole Transcriptome
# Areas (per slide)	2 or 4	2
Tissue Area	6.5 x 6.5 mm 11 x 11 mm (FFPE)	6.5 x 6.5 mm
Sample Type	FFPE (CytAssist) or fresh frozen (direct)	FFPE, fresh frozen, or fixed frozen
Species	human or mouse (probe), any reference species (poly-A)	human or mouse
Time	⌚⌚ ~ 2 days	⌚⌚ ~ 2 days
Customizable	Yes (Probes)	Yes (Probes)
Destructive	No	No
Required Instruments / Materials	Microscope and CytAssist	Microscope and CytAssist
Multiomics Compatibility	Protein (antibody)	none
Microscopy Compatibility	Yes	Yes
Instrument Cost	\$ (CytAssist)	\$ (CytAssist)
Kit Cost (per sample)	\$1,500	\$2,800
Additional Materials Cost (per sample)	\$200	\$200
Total Prep Cost (per sample)	\$1,700	\$3,000
Sequencing Reads	250M	275M
Sequencing Cost (Full SP)	\$2,400	\$2,400
Total Cost (per sample)	\$4,100	\$5,400

Diffusion



Diffusion

