

Spatial Transcriptomics Technology & Analysis

Corinne Strawser, PhD

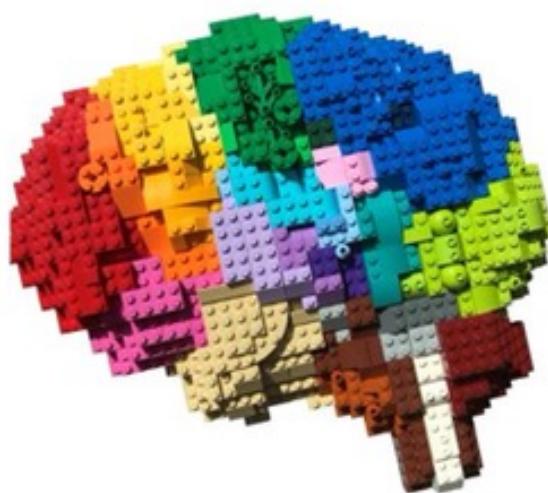
Bioinformatics Scientist

Dr. Elaine Mardis & Dr. Katie Miller Labs

Institute for Genomic Medicine



Why spatial?



Complex Tissue

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



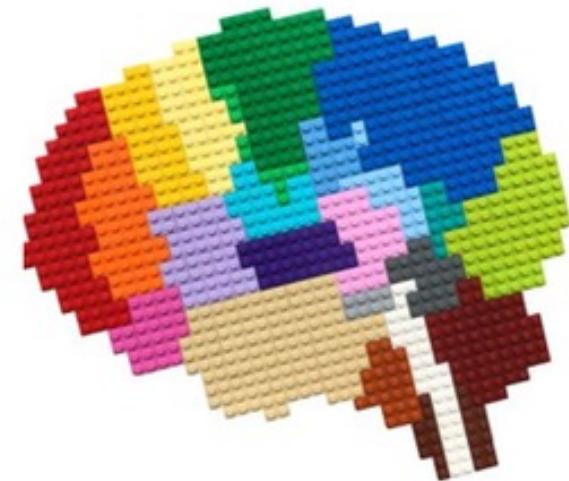
Bulk RNA-seq

tissue-average gene expression



Single-cell RNA-seq

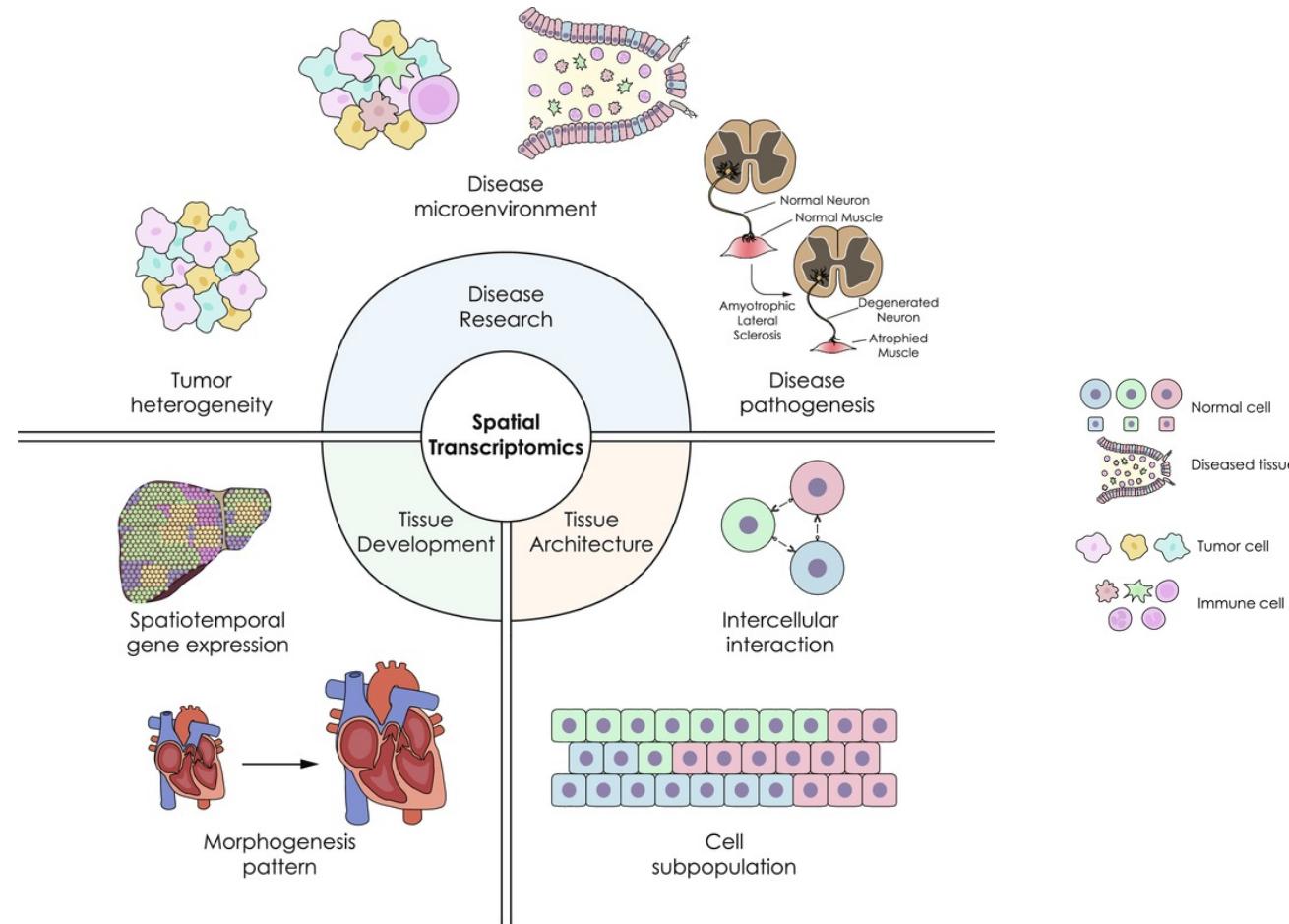
Cell-specific gene expression



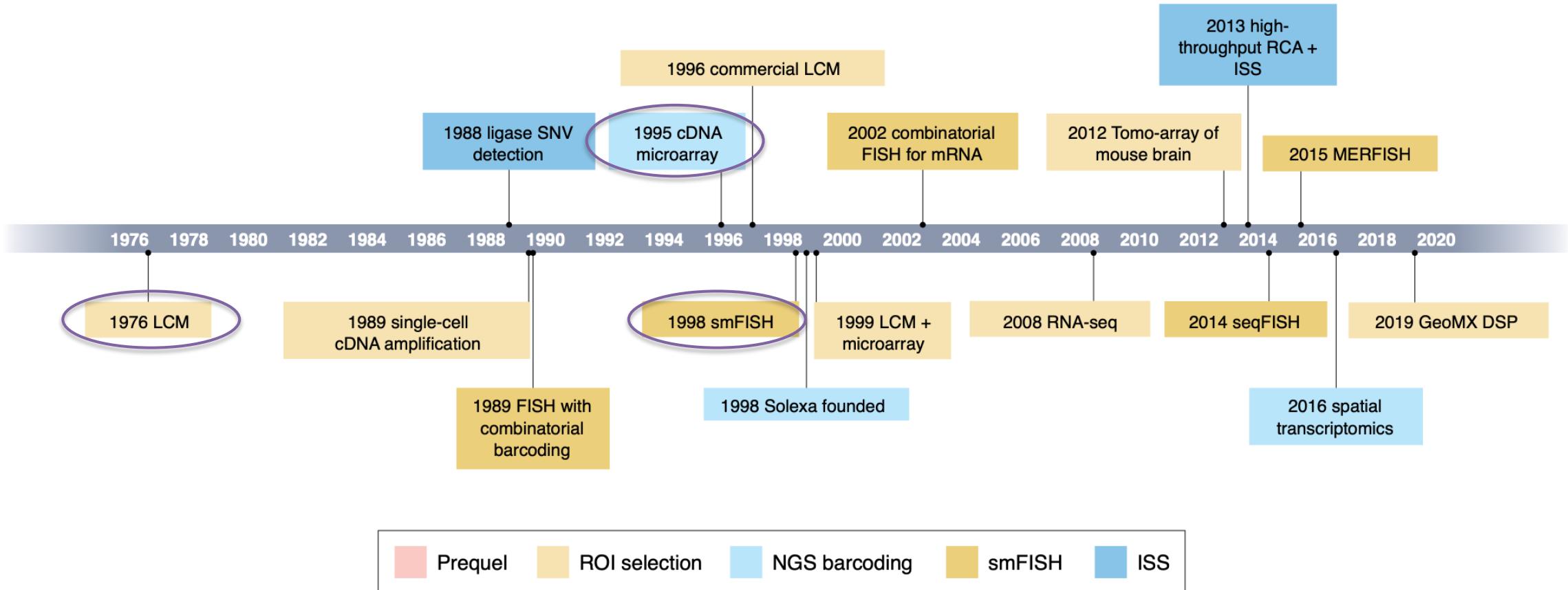
Spatial Transcriptomics

region-, cell-, or subcellular-specific gene expression with spatial context

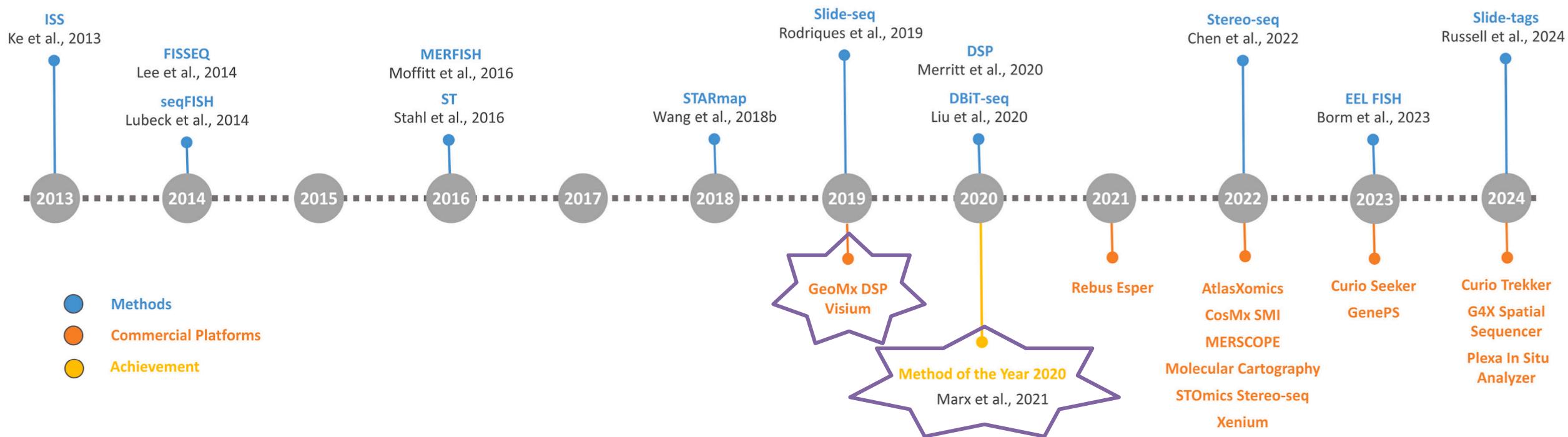
Spatial transcriptomics has broad applications

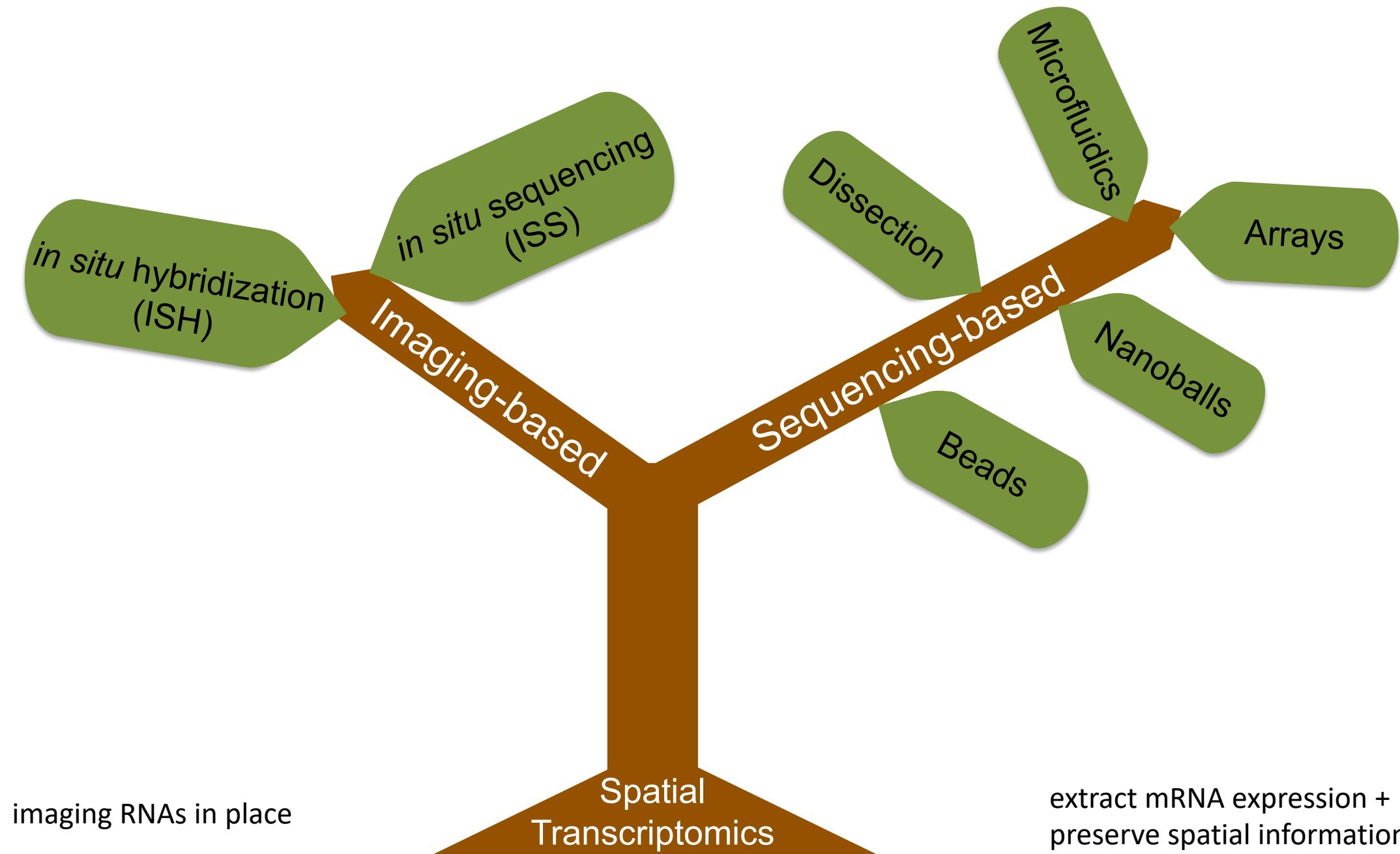


Spatial transcriptomics is not “new”...



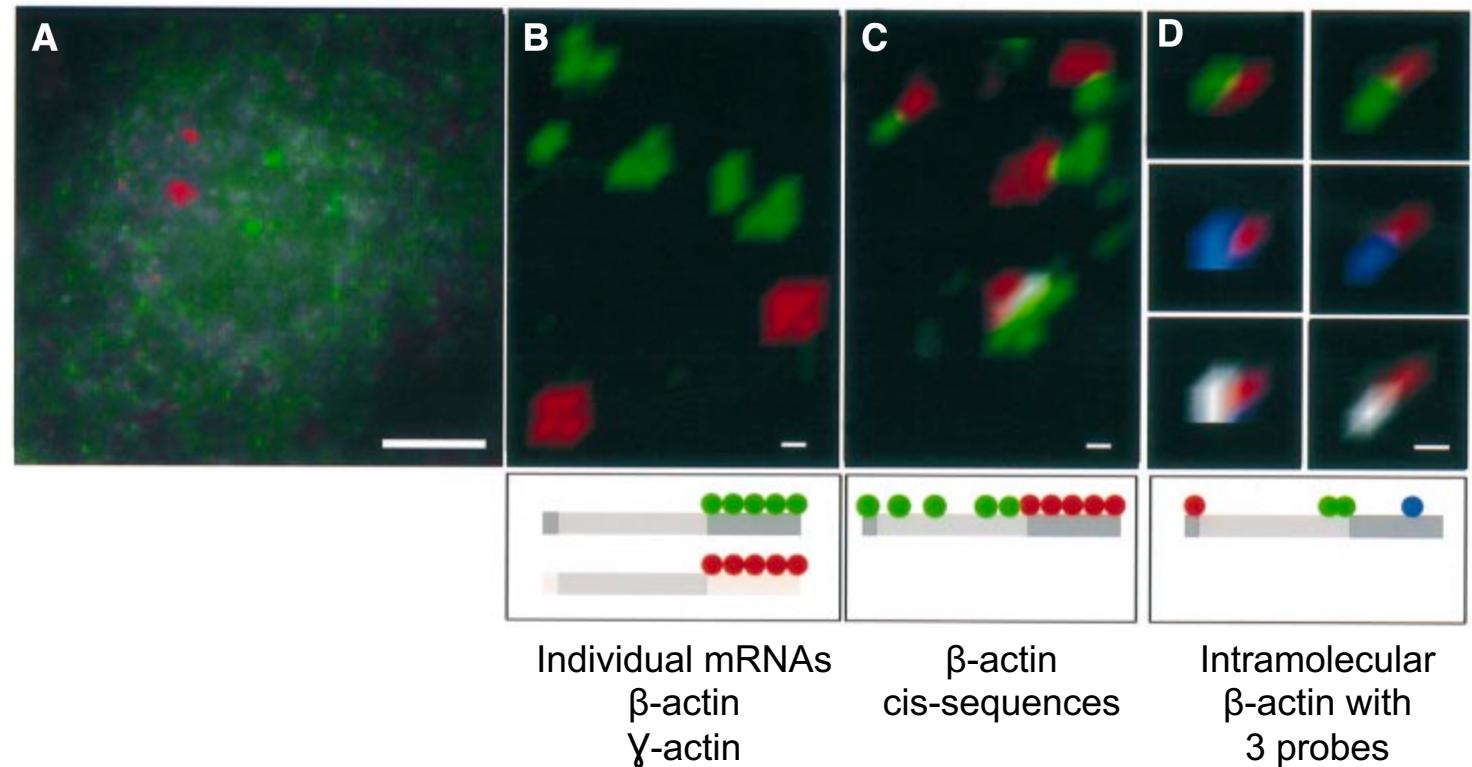
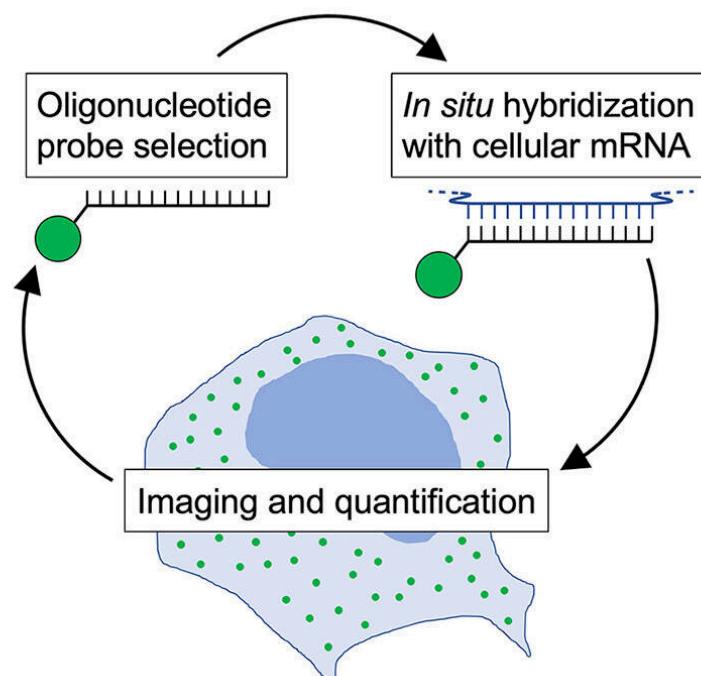
...but it's expanding!





Imaging-based spatial transcriptomics prequels

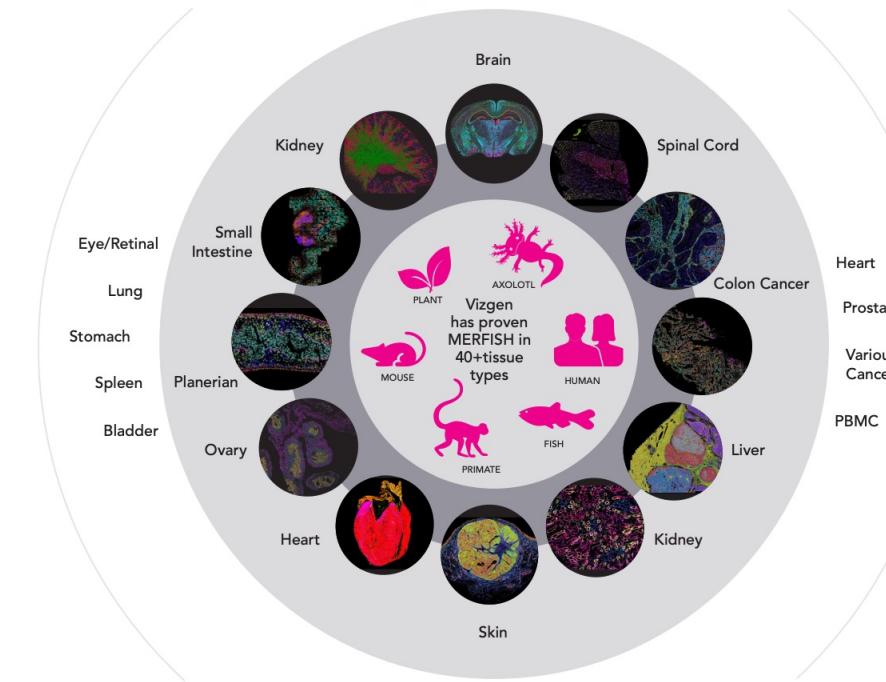
single-molecule fluorescence *in situ* hybridization (smFISH)



in situ hybridization (ISH): Vizgen MERSCOPE (MERFISH)



Flexibility in species and gene samples is critical



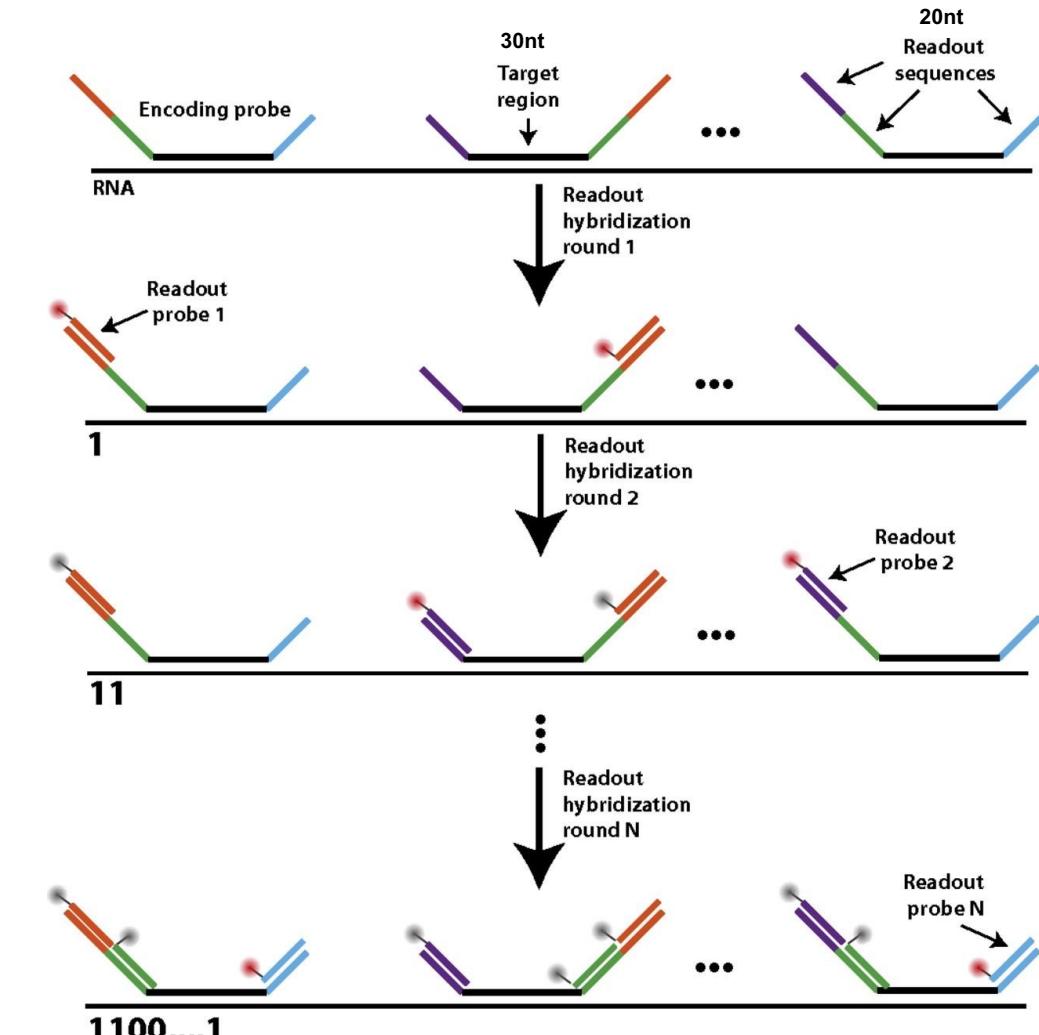
Compatible sample types include fresh or fixed frozen, adherent or suspended cells and FFPE

Multiplexed Error-Robust Fluorescence in situ Hybridization

VIZGEN

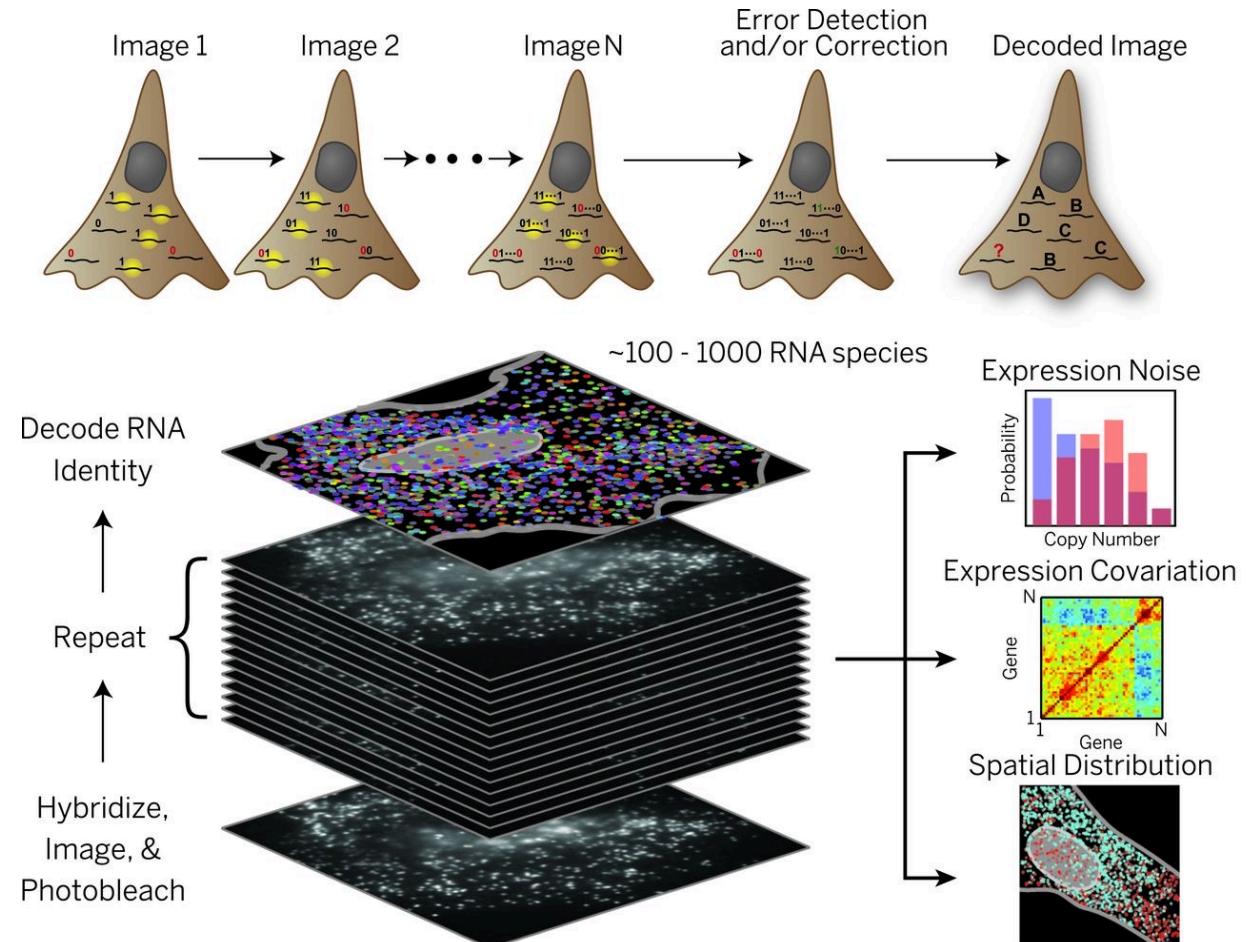
Combinatorial fluorescent labeling of error-robust barcodes

- Encoding probes have two parts:
 1. 30 nt target region (black)
 2. 3 x 20 nt readout sequences (purple, green, blue orange)
- Combination of readout probes determines the barcode used to identify given RNA sequence



Combinatorial fluorescent labeling of error-robust barcodes

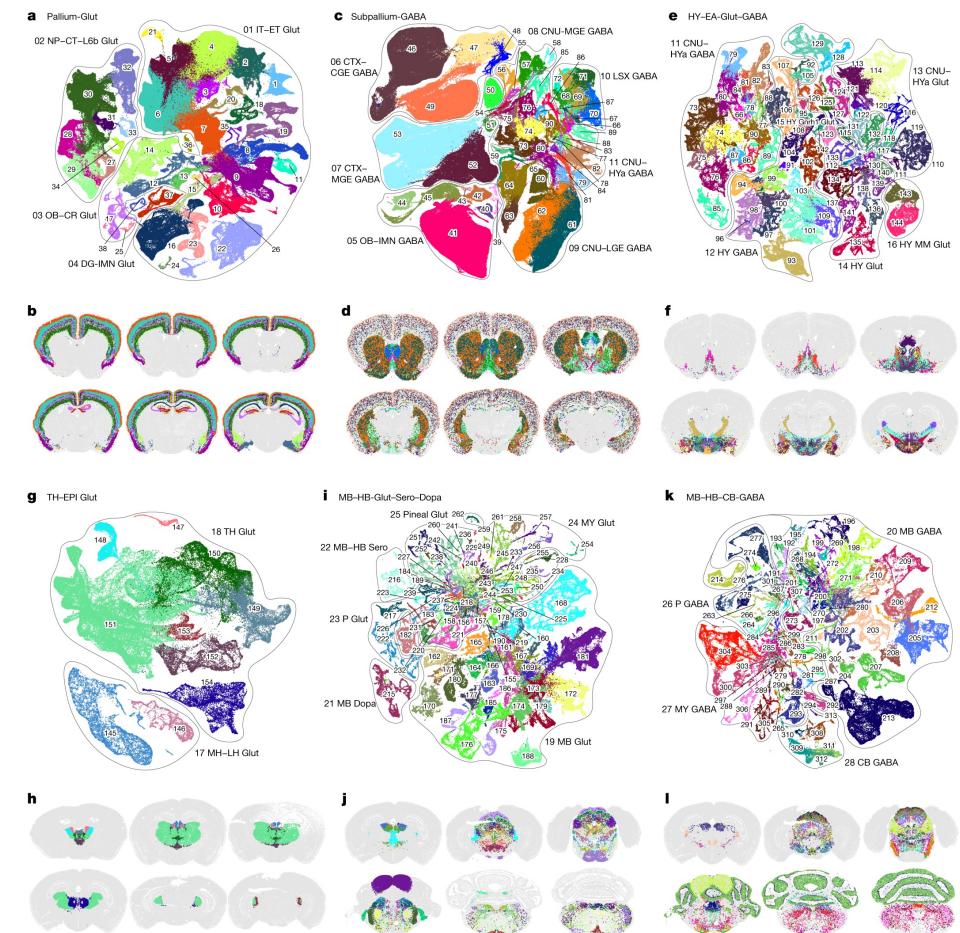
1. Single readout probe complementary to a given readout sequence conjugated to a dye is hybridized to the sample
2. The sample is imaged
3. Probe binds (fluorescence) bit = “1”; otherwise bit = “0”
4. Fluorescence signal is eliminated.
5. Repeated for N rounds of readout hybridization.
6. A registered stack of images for each sample produces a stack of fluorescence spots with on/off patterns that define binary barcode
7. Barcode “code book” was developed in such a way that at least four bits must be read incorrectly to change one valid code word into another
8. Each barcode is then an individual RNA species to be identified



VIZgen

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!!



	MERSCOPE / MERSCOPE Ultra
Company	Vizgen
Method	MERFISH
Resolution	<100 nm
# Features	1000 genes
# Areas (per slide)	1
Tissue Area	1 cm ² / 1.25 cm ² or 3 cm ²
Sample Type	FFPE, fresh or fixed frozen, adherent or suspended cells
Species	human, mouse, reference
Time	⌚⌚
Customizable	Yes
Cost (instrument)	\$\$\$
Cost (per sample/slide)	\$\$
Destructive	Yes
Required Instrumentation	MERSCOPE or MERSCOPE Ultra
Multiomics Compatibility	Protein (antibody)

PRO:

1. highly multiplexed
2. can correct errors

CON:

1. high start-up costs
2. limited RNA species

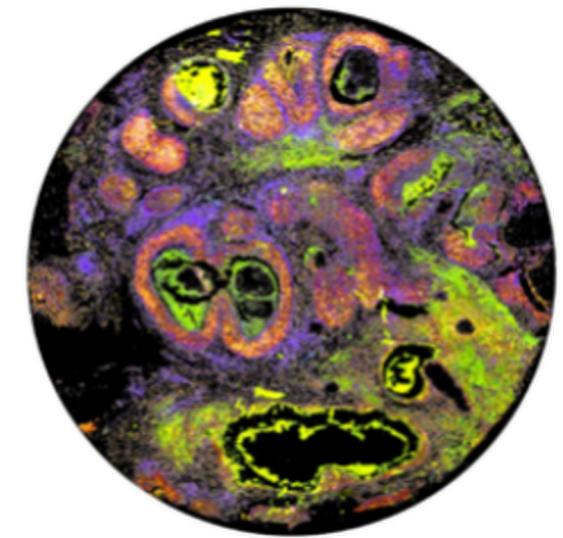
Cost (instrument): ≥ \$400k (\$\$\$), \$100k-399k (\$\$), < \$100k (\$)

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

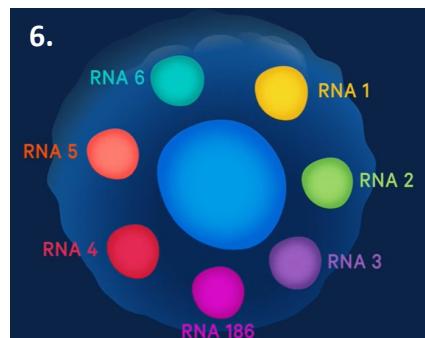
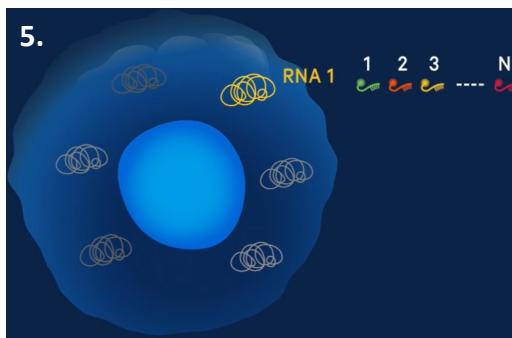
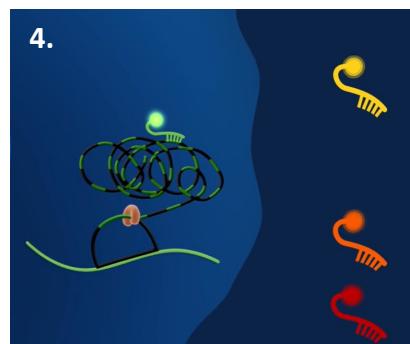
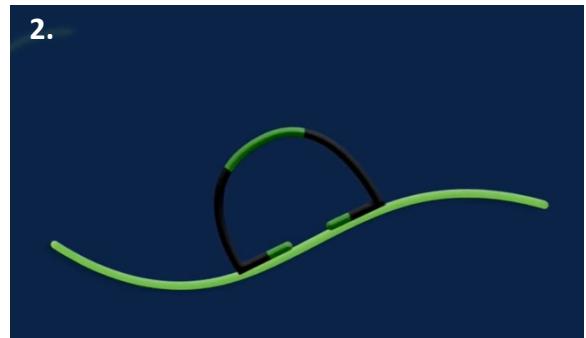
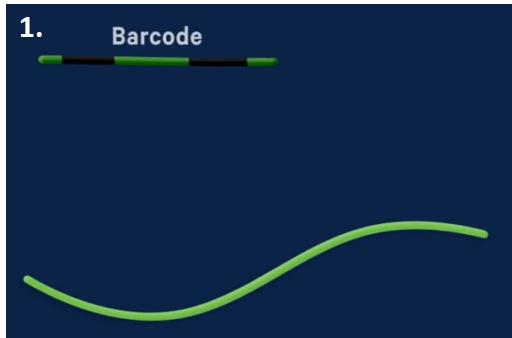
Time (per run): ≥ 3 days (⌚⌚⌚), 1-2 days (⌚⌚), < 1 day(⌚)

* for poly-A-based capture

in situ sequencing (ISS): 10X Genomics Xenium

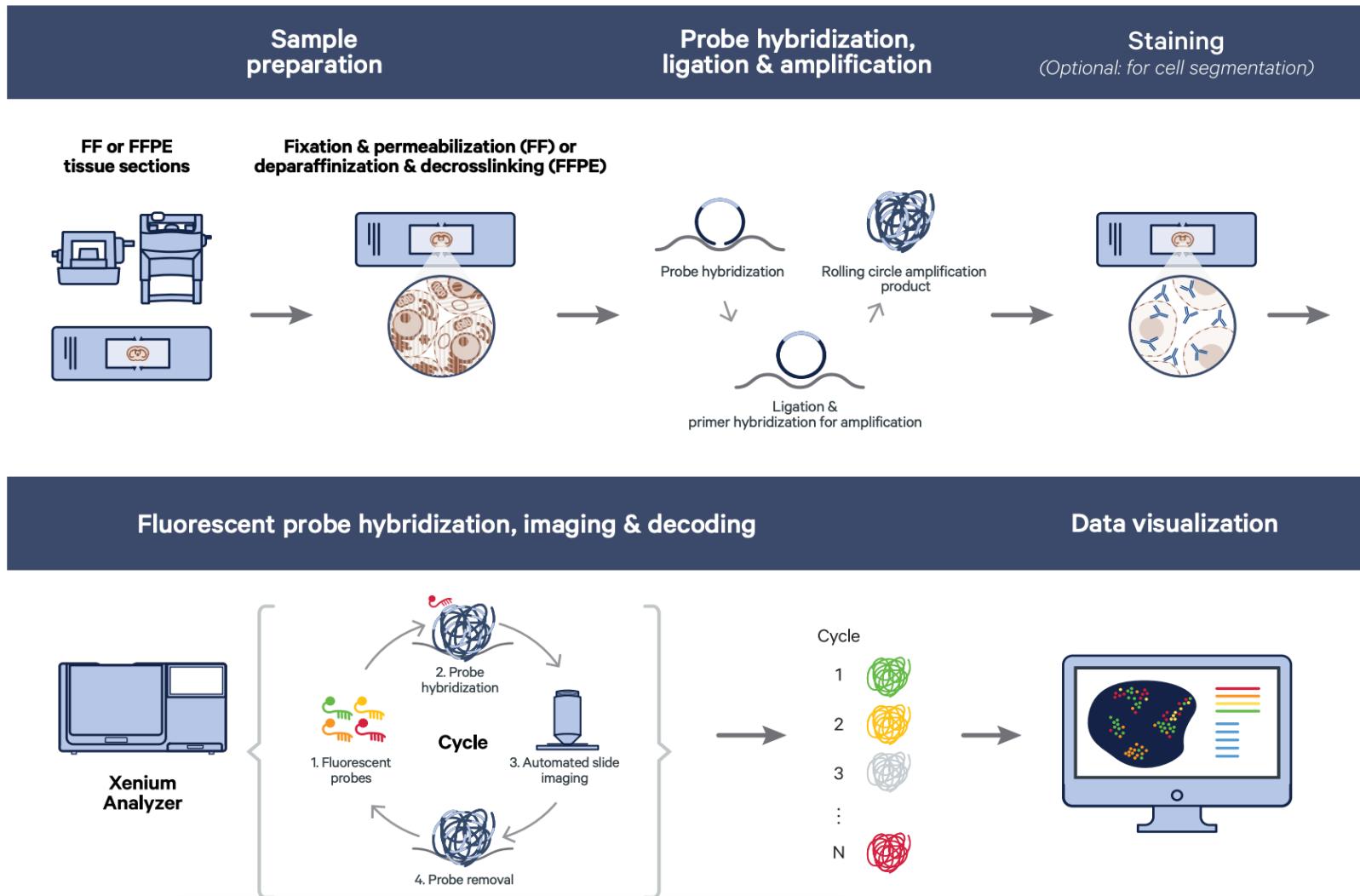


Xenium padlock probes



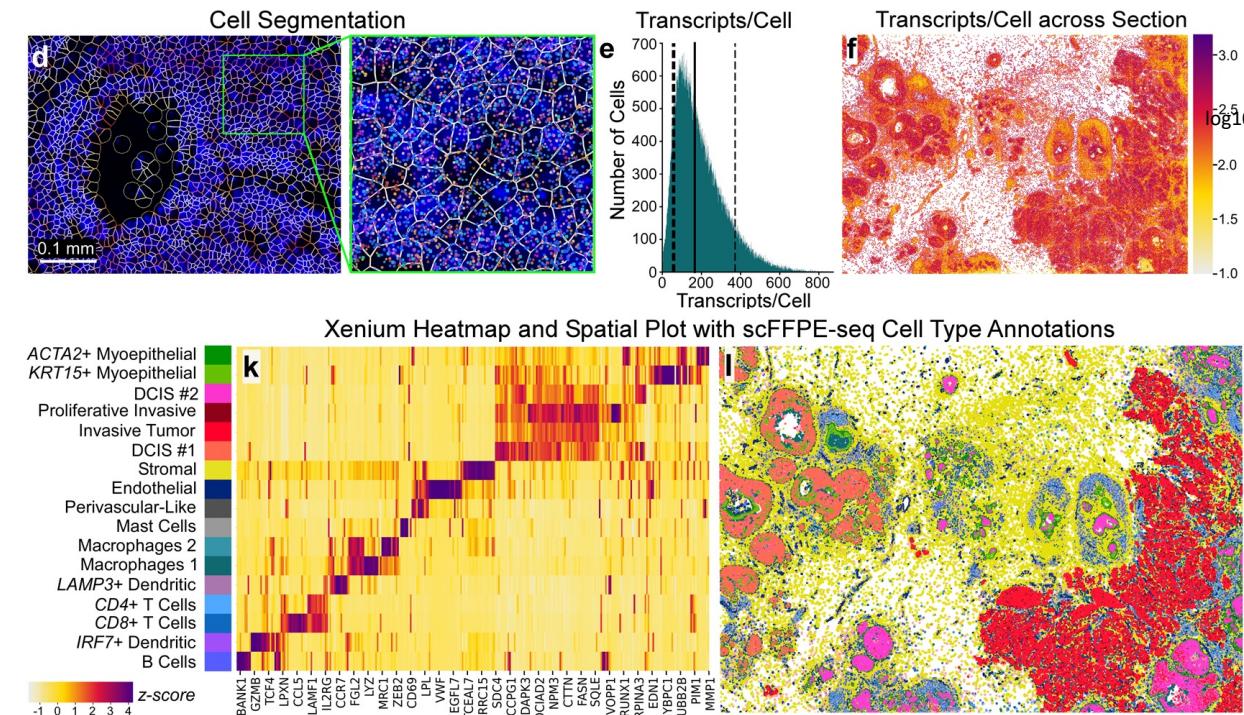
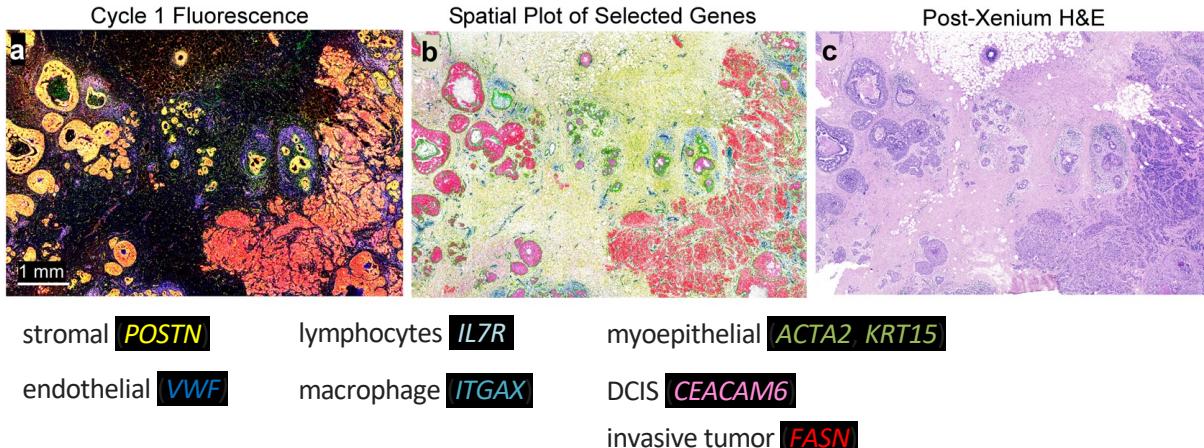
1. Circularizable DNA padlock probes contain two regions that bind specific target RNA sequence and a third region with gene-specific barcode sequence
2. Probes bind RNA and ligate
3. Probes are enzymatically amplified to produce many copies of gene-specific barcode.
4. Fluorescent oligos bind to the amplified barcode sequence
 - ❖ very bright signal due to amplification (high signal:noise)
 - ❖ ideal for samples with lots of background fluorescence like FFPE
5. Successive rounds of fluorescent oligo attachment, imaging, and removal for highly specific targeted optical signature for genes of interest
6. The images are then stitched and identified with the instrument software

Xenium *in situ* analysis workflow



High resolution mapping of the breast TME using integrated single-cell and Xenium

- Xenium Human Breast Panel (280 genes) + 33 add-on genes in breast tumor
- 15 cycles of hybridization and imaging



	MERSCOPE / MERSCOPE Ultra	Xenium
Company	Vizgen	10X Genomics
Method	MERFISH	ISS + ISH
Resolution	<100 nm	<100 nm
# Features	1000 genes	5000 genes
# Areas (per slide)	1	1
Tissue Area	1 cm ² / 1.25 cm ² or 3 cm ²	236 mm ²
Sample Type	FFPE, fresh or fixed frozen, adherent or suspended cells	FFPE, fresh frozen
Species	human, mouse, reference	human, mouse
Time	⌚⌚	⌚⌚⌚
Customizable	Yes	Yes
Cost (instrument)	\$\$\$	\$\$\$
Cost (per sample/slide)	\$\$	\$\$\$
Destructive	Yes	No
Required Instrumentation	MERSCOPE or MERSCOPE Ultra	Xenium Analyzer
Multiomics Compatibility	Protein (antibody)	Protein (in dev)

PRO:

1. high accuracy w/ padlock probe

CON:

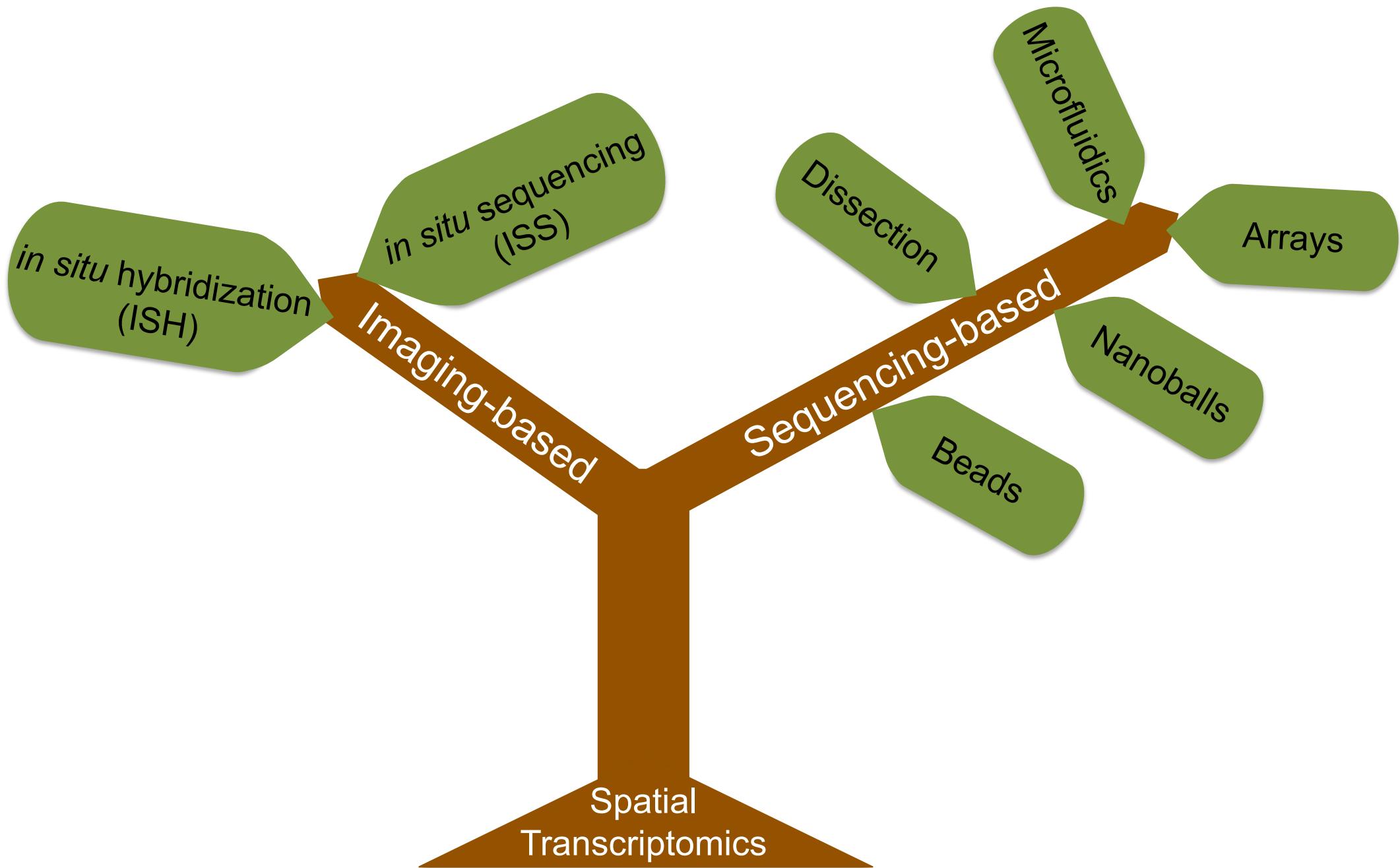
1. pre-designed probes
2. limited RNA species

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

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

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

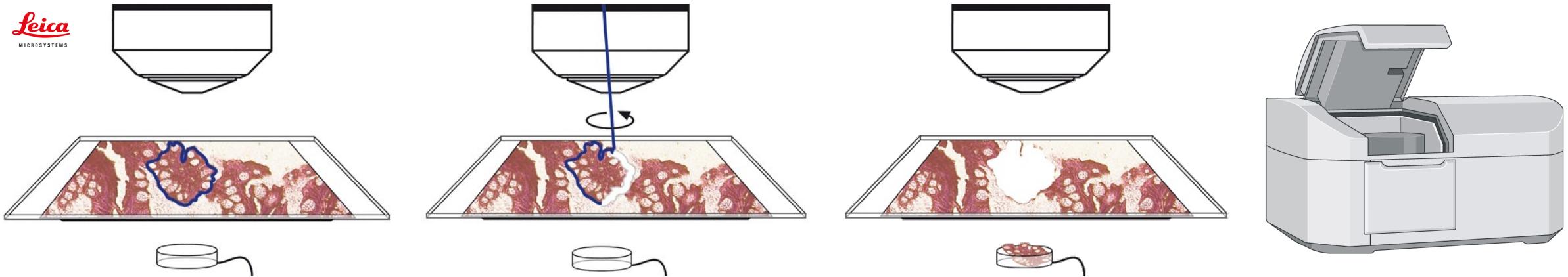
* for poly-A-based capture



Sequencing-based spatial transcriptomics prequel:

laser capture microdissection (LCM) + microarray analysis

Leica
MICROSYSTEMS



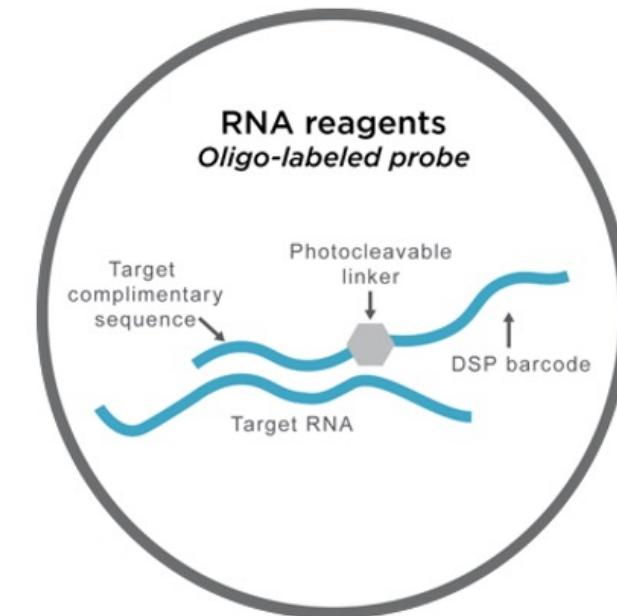
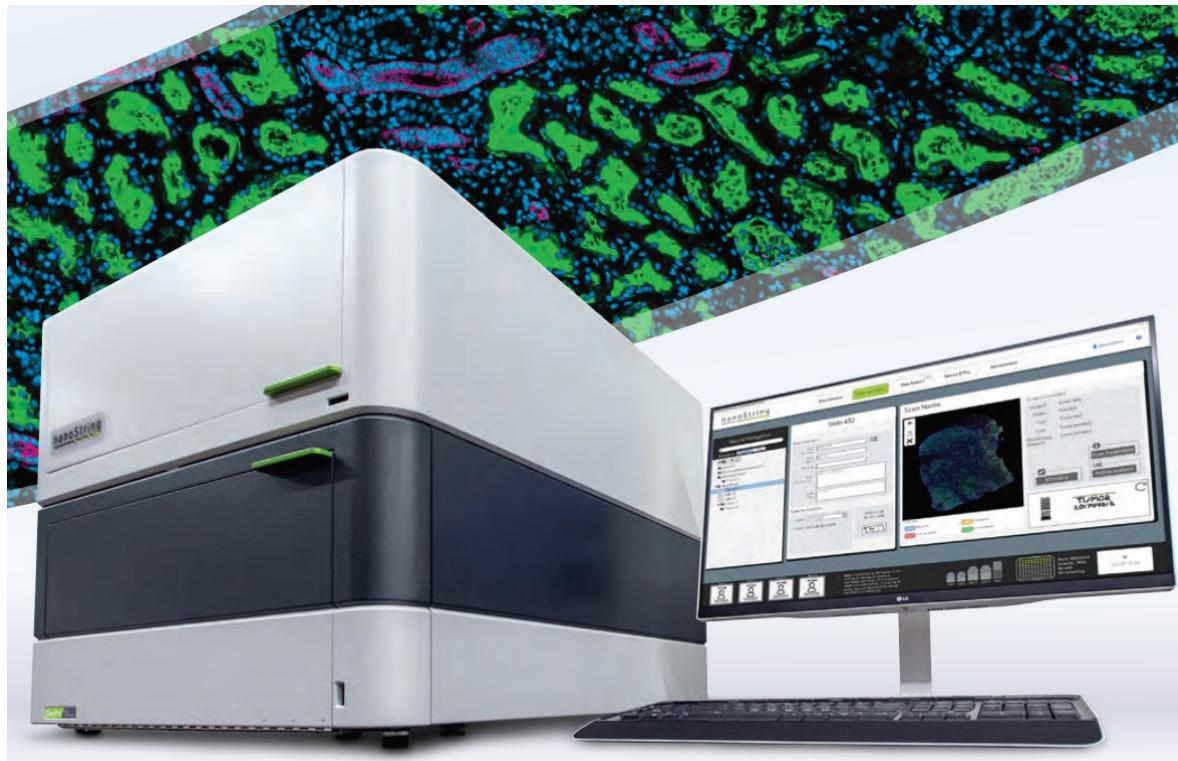
Step 1. Define
region of interest (ROI)

Step 2. Laser beam
precisely steered by
prisms along ROI
definition

Step 3. Dissectate is
collected by gravity

Step 4. Process for
microarray analysis

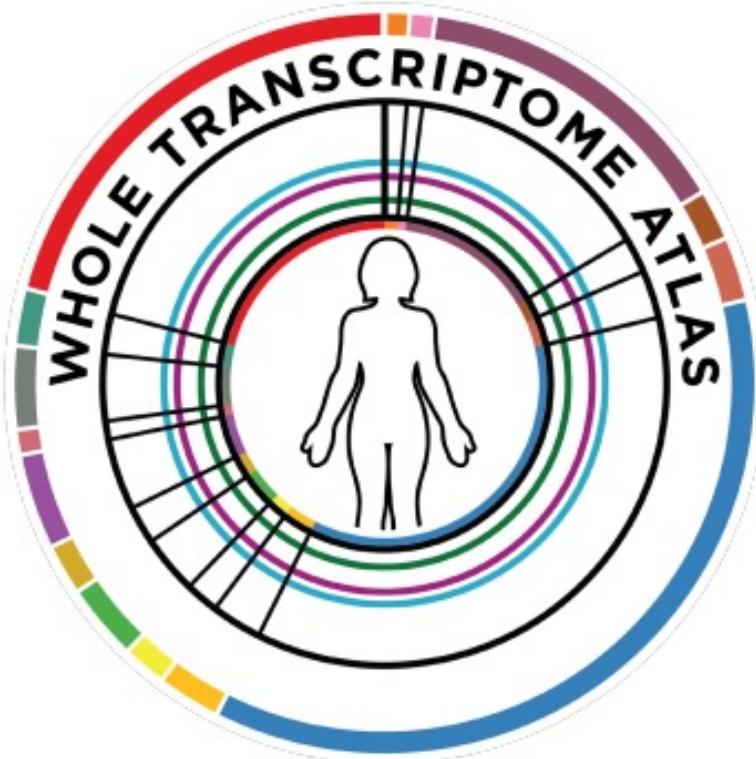
Dissection: nanoString GeoMX DSP



nanoString[®]

GeoMx DSP

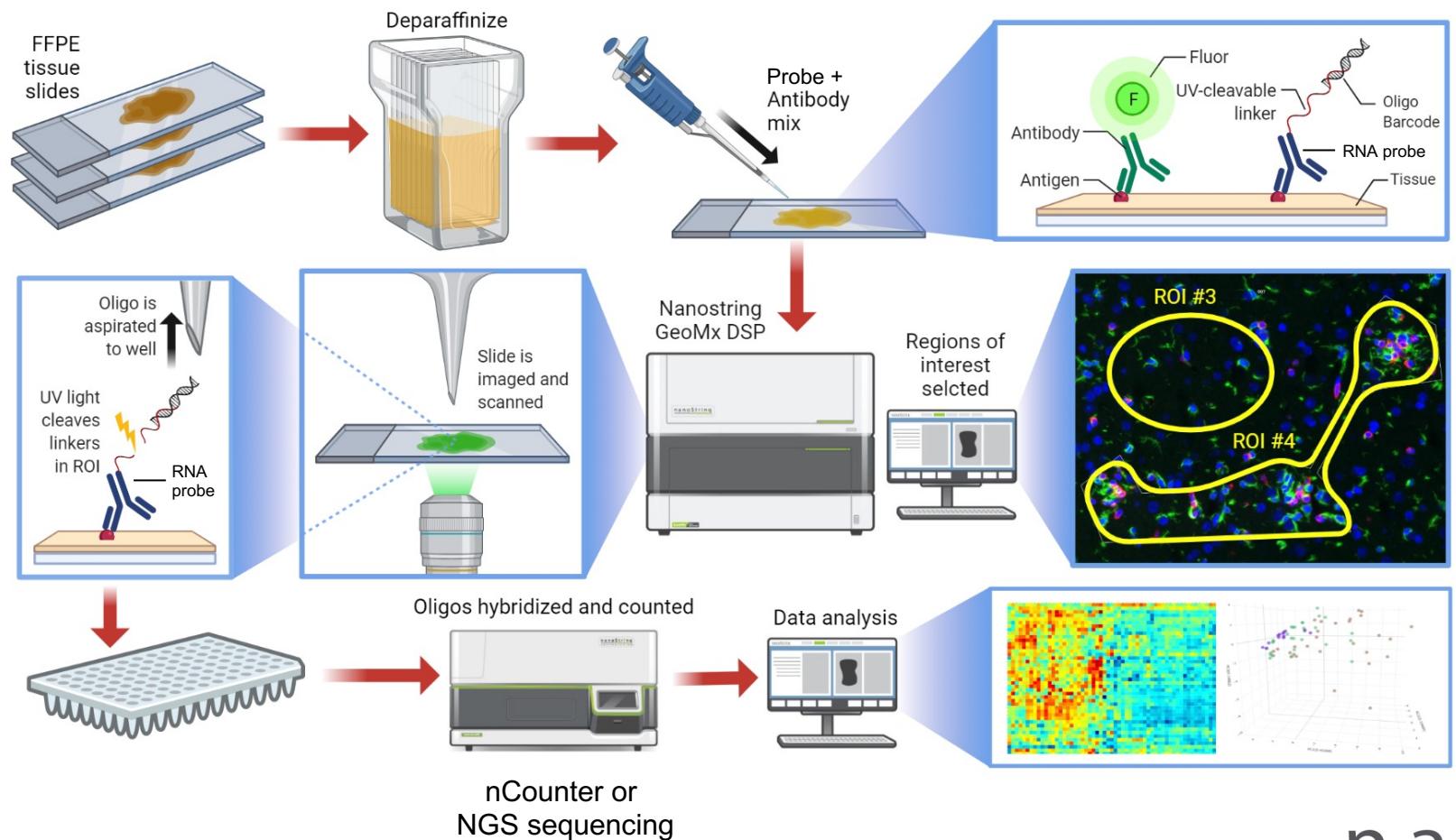
Whole Transcriptome Atlas (WTA)



Product Highlights

- Whole transcriptome coverage with probes specific to protein coding mRNA sequences
- Superior sensitivity to detect 1000s of unique human genes in <50 µm regions
- Robust performance across sample types including FFPE with high concordance with RNA-seq and RNAscope™
- Map single cell RNA-seq populations to their tissue location
- Integrated Illumina library prep protocols and streamlined NGS analysis pipelines
- Flexibility to customize with up to 60 additional targets to assay non-coding RNA, synthetic DNA or exogenous genes
- Visualization and statistical analysis using GeoMx Data Suite and bioinformatics toolset

Dissection: nanoString GeoMX DSP



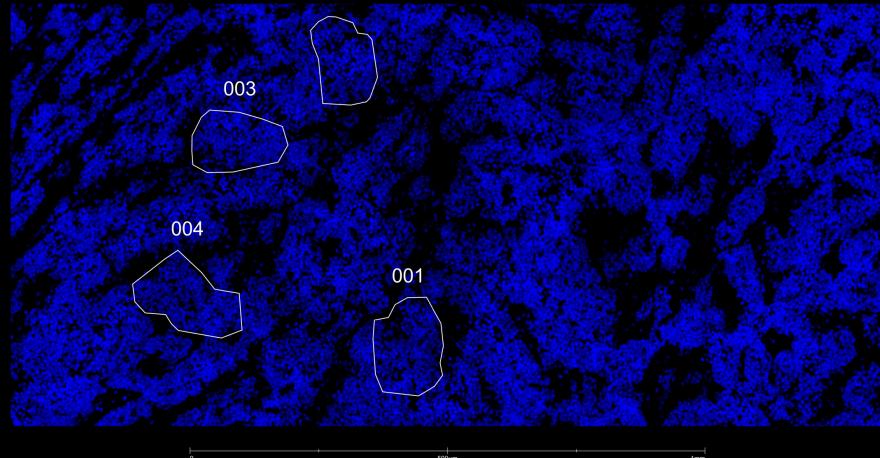
nanoString

GeoMx Profiling of central nervous system (CNS) melanocytosis

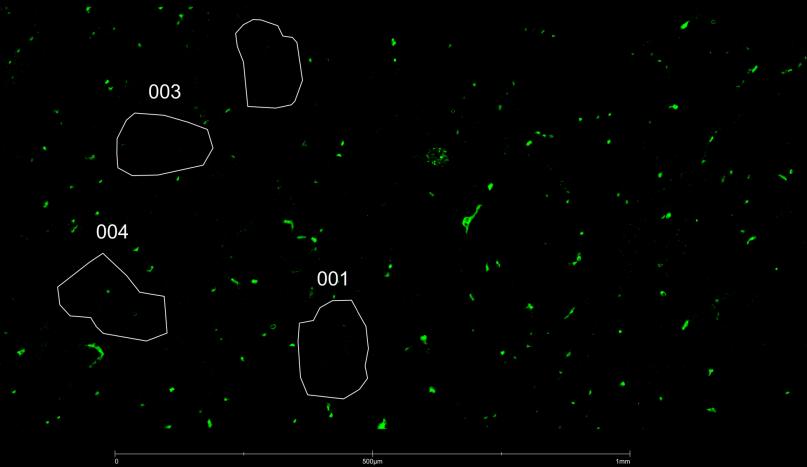
- Very rare
- Originate from melanocytes that are derived from the neural crest and migrate to the leptomeninges during embryonic development
- Mutational profile may be similar to cutaneous melanoma
- Non-specific clinical symptoms
- Median survival from diagnosis is only 4 months
- 2 patients at Nationwide Children's Hospital
- Questions:
 - Are there any transcriptomic differences in immune infiltrated areas of the neoplasm?
 - Patient-specific differences?

Patient #1 CD45 “low”

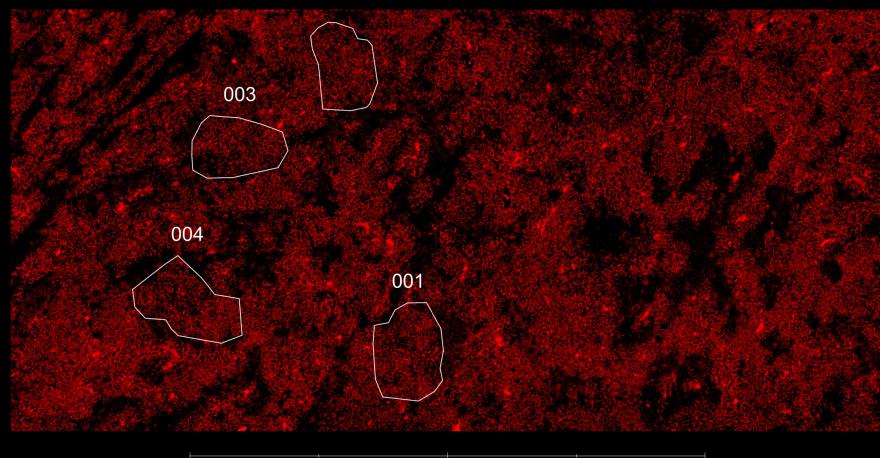
FITC/525nm : SYTO 13 : DNA (Blue)



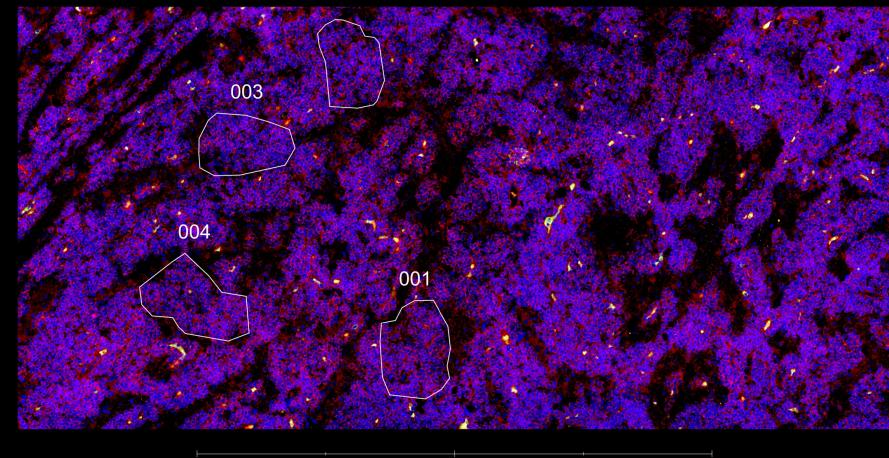
Texas Red/615nm : Alexa 594 : CD45 (Green)



Cy5/666nm : AF647 : Custom : MART-1 (Red)

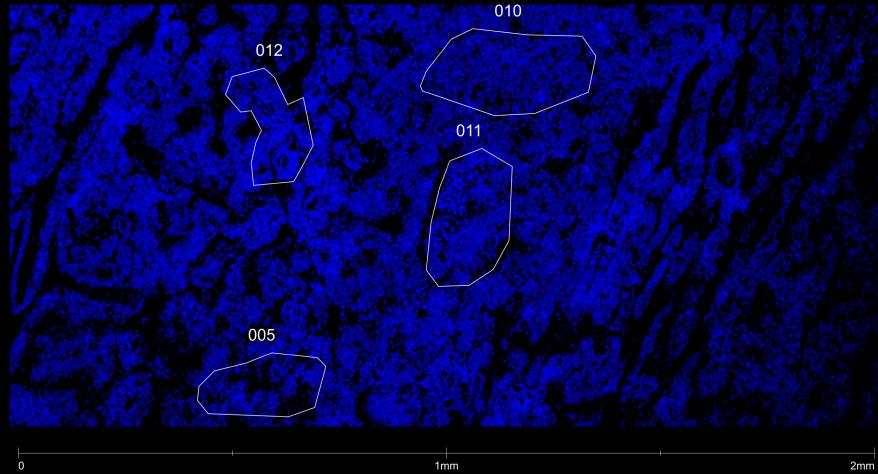


FITC/525nm : SYTO 13 : DNA (Blue)
Texas Red/615nm : Alexa 594 : CD45 (Green)
Cy5/666nm : AF647 : Custom : MART-1 (Red)

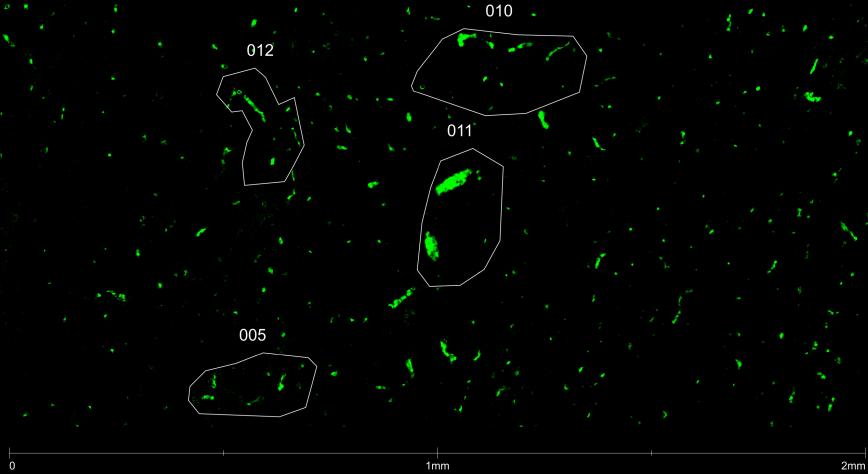


Patient #1 CD45 “high”

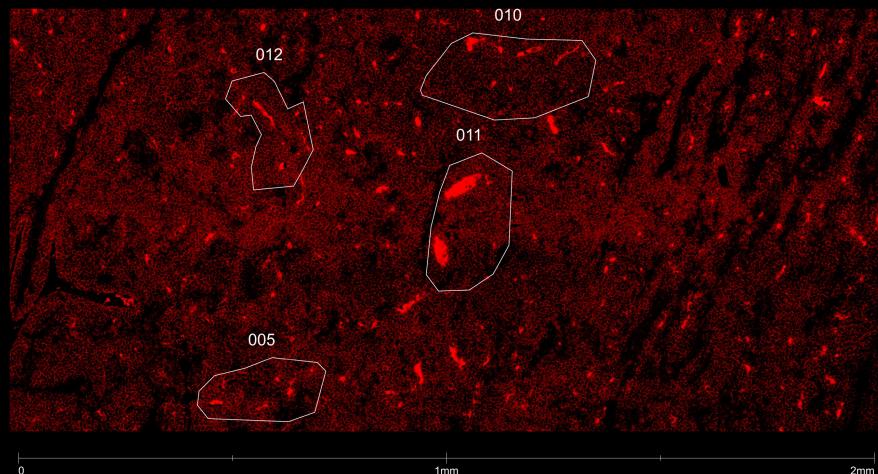
FITC/525nm : SYTO 13 : DNA (Blue)



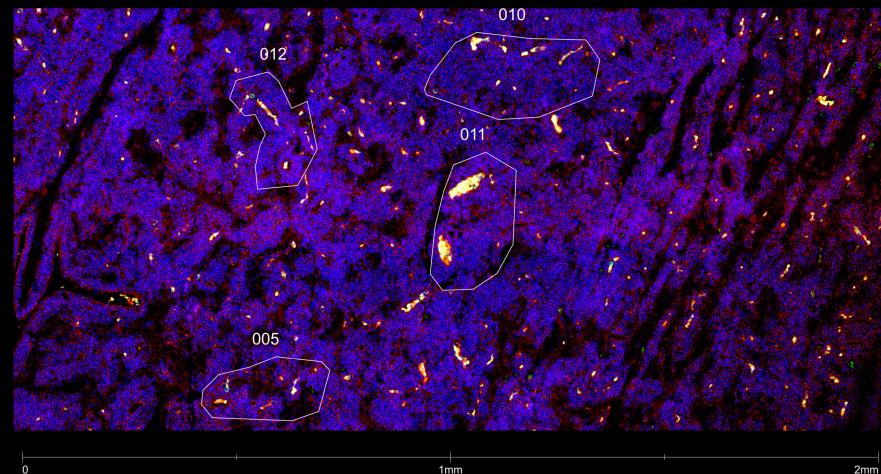
Texas Red/615nm : Alexa 594 : CD45 (Green)



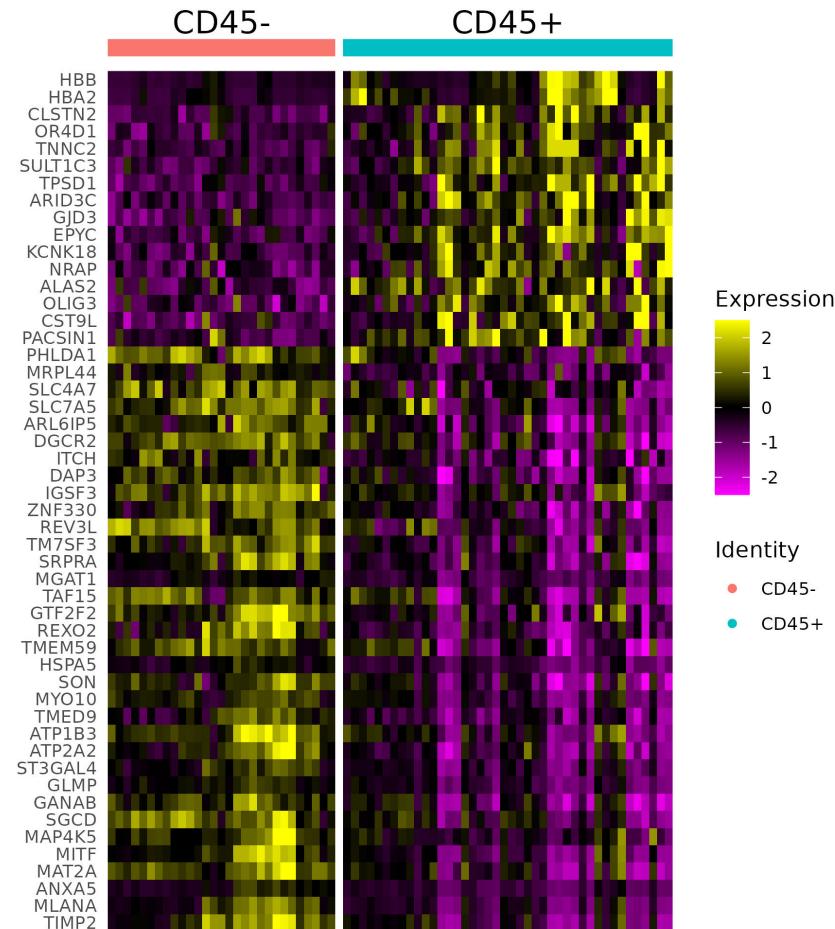
Cy5/666nm : AF647 : Custom : MART-1 (Red)



FITC/525nm : SYTO 13 : DNA (Blue)
Texas Red/615nm : Alexa 594 : CD45 (Green)
Cy5/666nm : AF647 : Custom : MART-1 (Red)



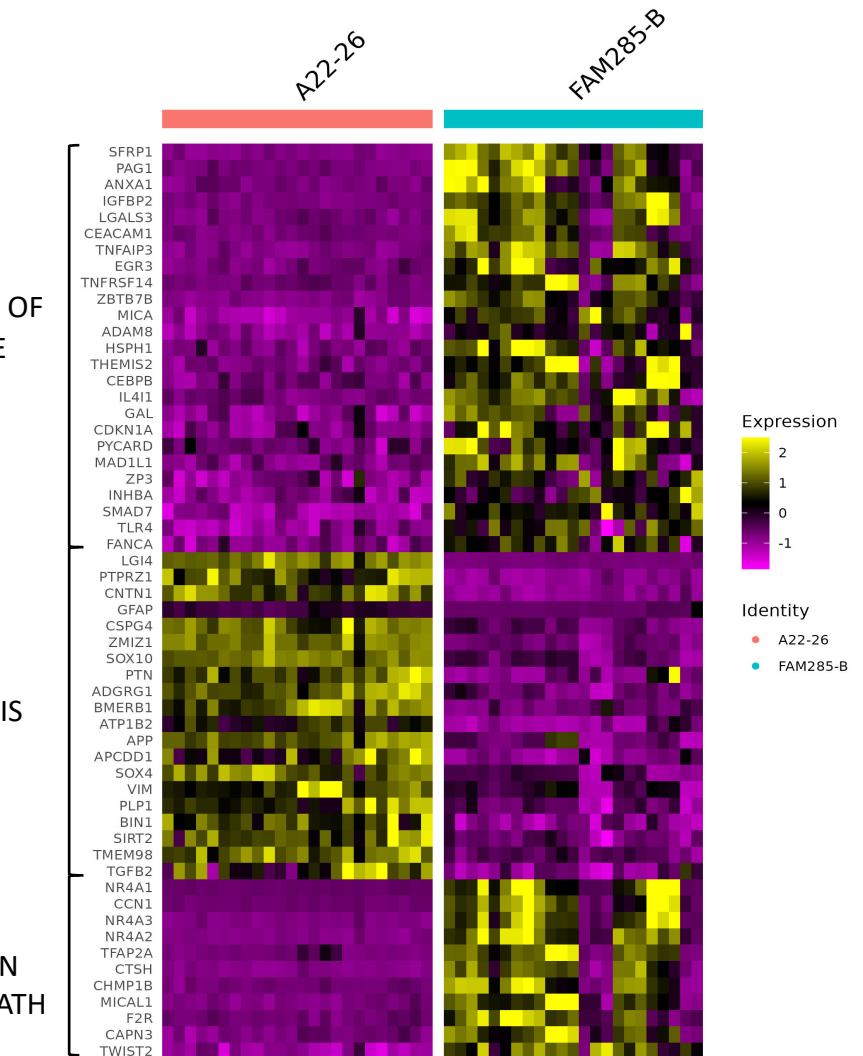
Immune-infiltration and patient-specific transcriptomic profiles



REGULATION OF LYMPHOCYTE ACTIVATION

GLIOGENESIS

NEGATIVE REGULATION OF CELL DEATH



	MERSCOPE / MERSCOPE Ultra	Xenium	GeoMx
Company	Vizgen	10X Genomics	Nanostring
Method	MERFISH	ISS + ISH	Light dissection
Resolution	<100 nm	<100 nm	< 50 µm
# Features	1000 genes	5000 genes	WT
# Areas (per slide)	1	1	96
Tissue Area	1 cm ² / 1.25 cm ² or 3 cm ²	236 mm ²	100 - 490,000 µm ²
Sample Type	FFPE, fresh or fixed frozen, adherent or suspended cells	FFPE, fresh frozen	FFPE, fresh frozen
Species	human, mouse, reference	human, mouse	human, mouse, canine
Time	⌚⌚	⌚⌚⌚	⌚⌚⌚
Customizable	Yes	Yes	Yes
Cost (instrument)	\$\$\$	\$\$\$	\$\$
Cost (per sample/slide)	\$\$	\$\$\$	\$\$
Destructive	Yes	No	No
Required Instrumentation	MERSCOPE or MERSCOPE Ultra	Xenium Analyzer	GeoMx DSP
Multiomics Compatibility	Protein (antibody)	Protein (in dev)	Protein (antibody)

PRO:

1. high plexity
2. non-destructive

CON:

1. bias in ROI selection
2. many cells in ROI

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

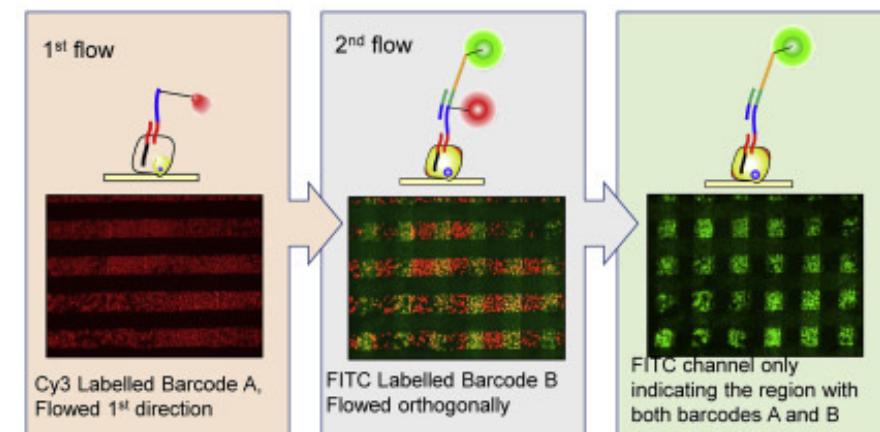
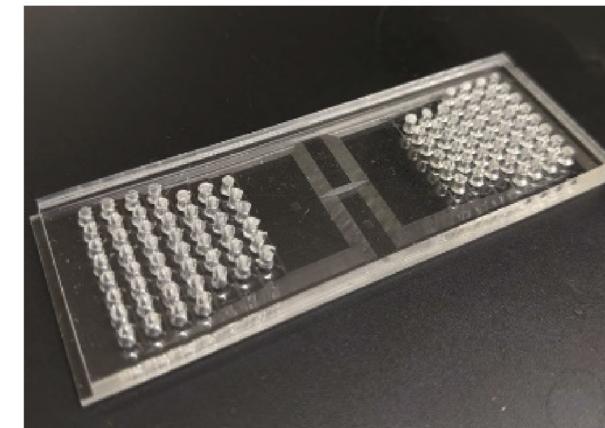
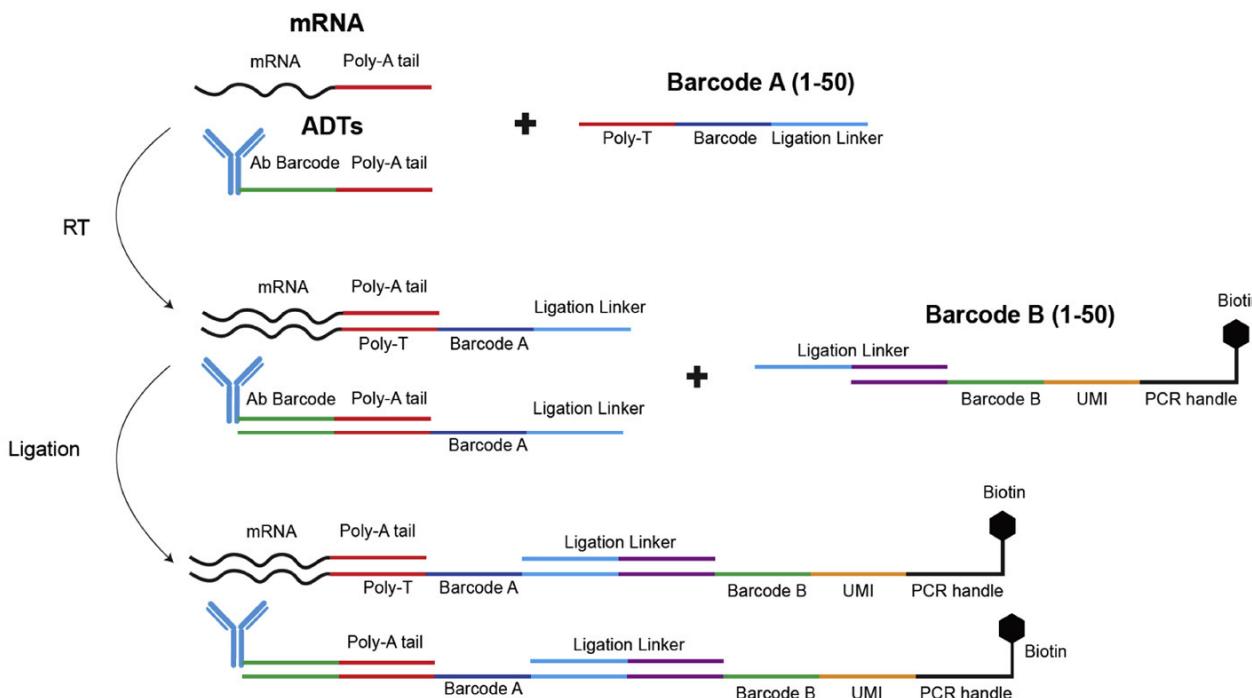
Cost (per sample): ≥ \$5000 (\$\$\$), \$1000-4999 (\$\$), < \$1000 (\$)

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

* for poly-A-based capture

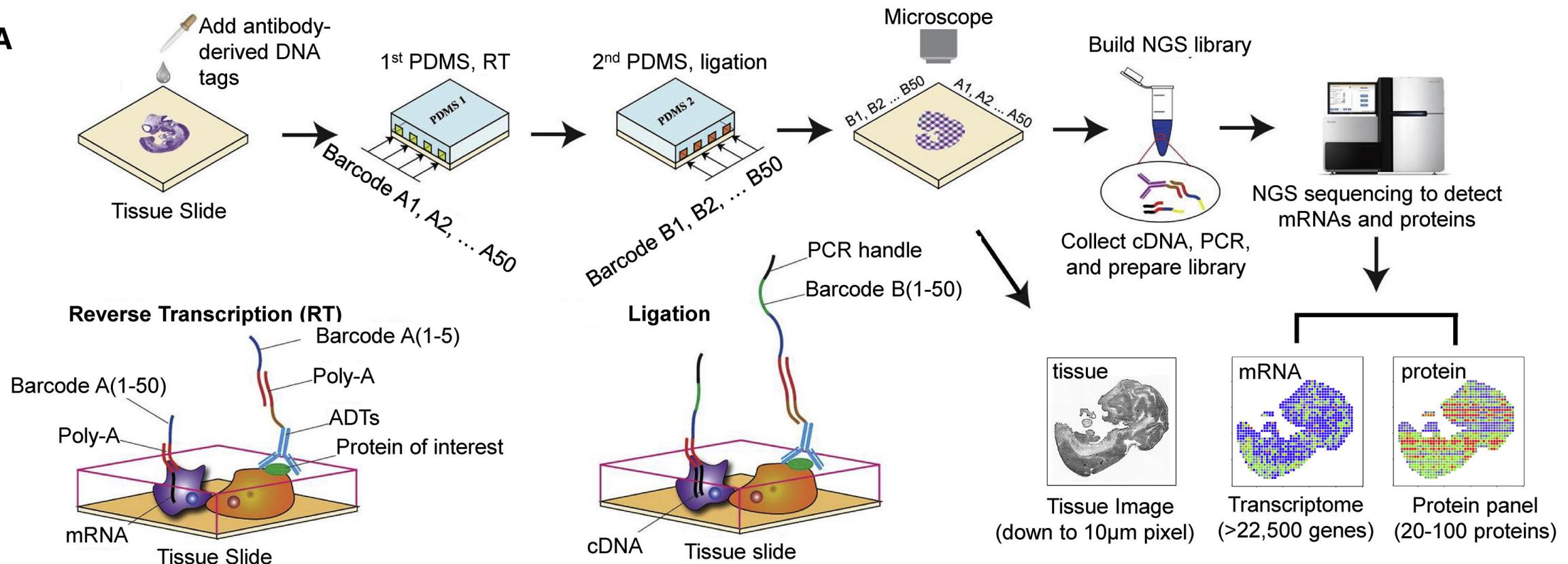
Microfluidics: AtlasXomics FlowGel (DBiT-seq)

Deterministic Barcoding in Tissue (DBiT)



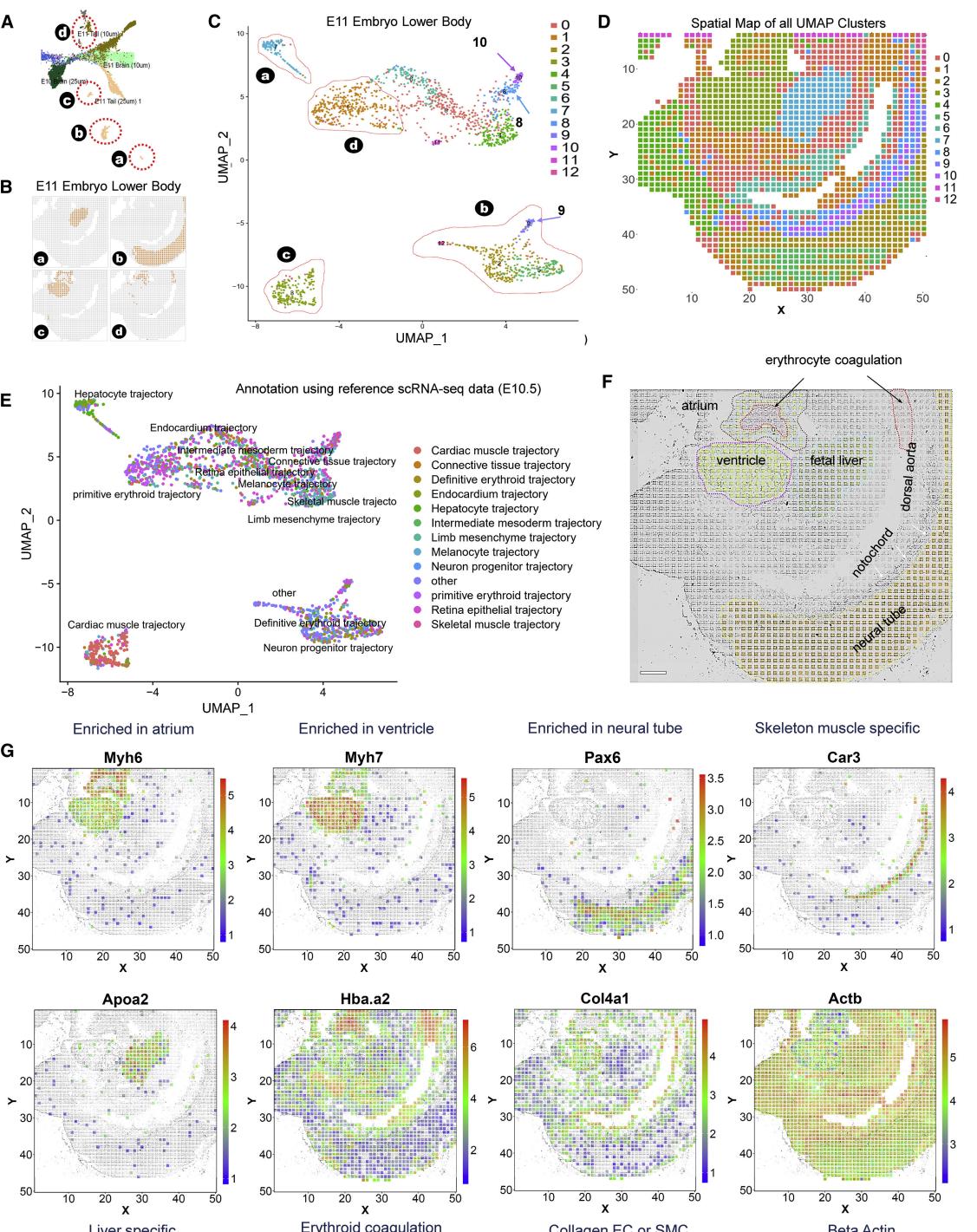
DBiT-seq workflow

A



Spatial mapping of internal organ development in E11 mouse embryo

- DBiT-seq data can be clustered and annotated like scRNA-seq data
- Mapping of clusters back to tissue image reveals distinct spatial patterning
- Clusters annotated with scRNA-seq data
- Spatial patterns are associated with expression of genes involved in organ development



	MERSCOPE / MERSCOPE Ultra	Xenium	GeoMx	FlowGel
Company	Vizgen	10X Genomics	Nanostring	AtlasXomics
Method	MERFISH	ISS + ISH	Light dissection	DBiT-seq
Resolution	<100 nm	<100 nm	< 50 µm	10 µm
# Features	1000 genes	5000 genes	WT	WT
# Areas (per slide)	1	1	96	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
Sample Type	FFPE, fresh or fixed frozen, adherent or suspended cells	FFPE, fresh frozen	FFPE, fresh frozen	FFPE, fresh or fixed frozen
Species	human, mouse, reference	human, mouse	human, mouse, canine	reference
Time	⌚⌚	⌚⌚⌚	⌚⌚⌚	⌚
Customizable	Yes	Yes	Yes	Yes
Cost (instrument)	\$\$\$	\$\$\$	\$\$	N/A
Cost (per sample/slide)	\$\$	\$\$\$	\$\$	\$
Destructive	Yes	No	No	No
Required Instrumentation	MERSCOPE or MERSCOPE Ultra	Xenium Analyzer	GeoMx DSP	Microfluidics Stamp Scanning microscope Incubator
Multiomics Compatibility	Protein (antibody)	Protein (in dev)	Protein (antibody)	Protein (antibody) Epigenome (ATAC-seq + CUT&Tag)

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

PRO:

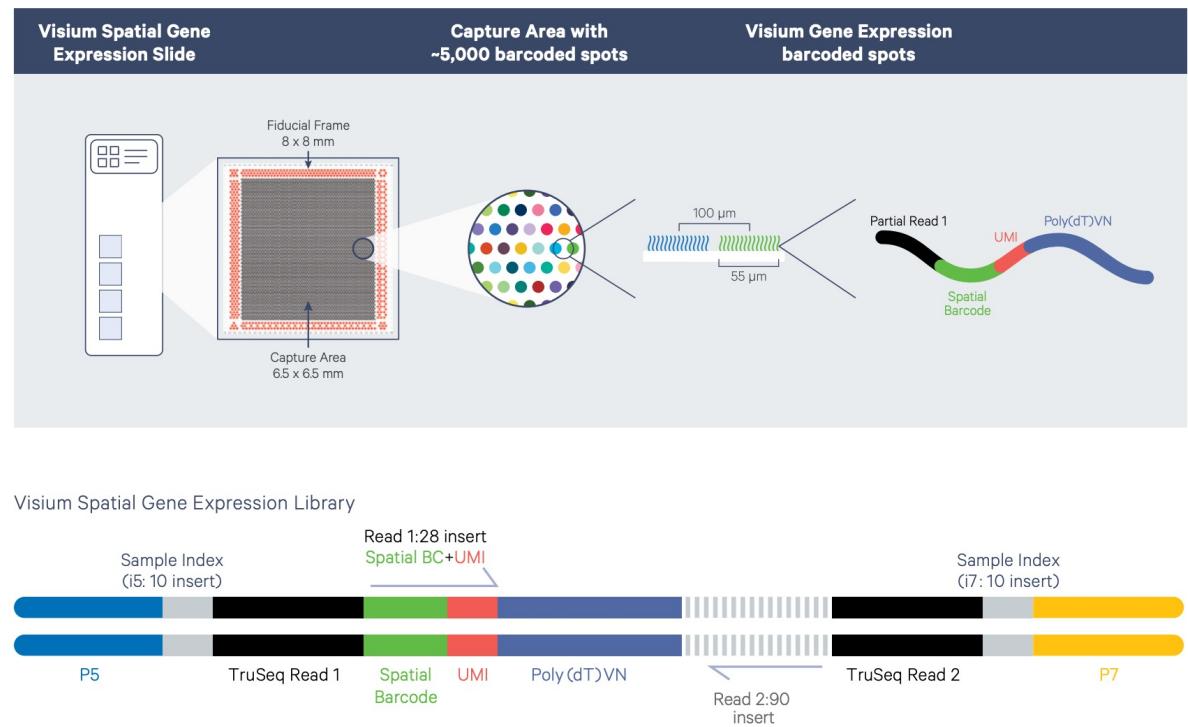
1. good spatial resolution
2. relatively inexpensive
3. multi-omics compatible

CON:

1. limited # of flow channels

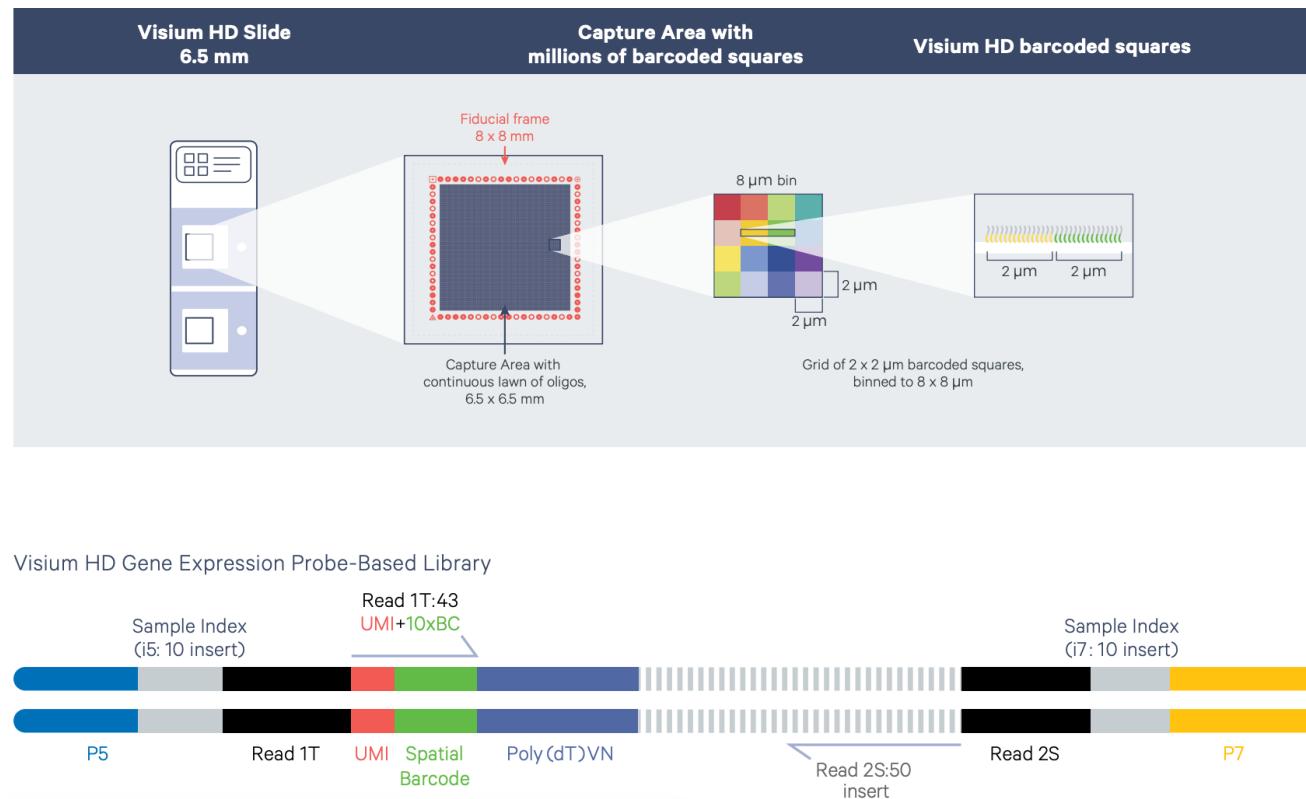
Arrays: 10X Genomics Visium

- Visium slides has 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, which is shared by all oligonucleotides within each individual gene expression capture spot



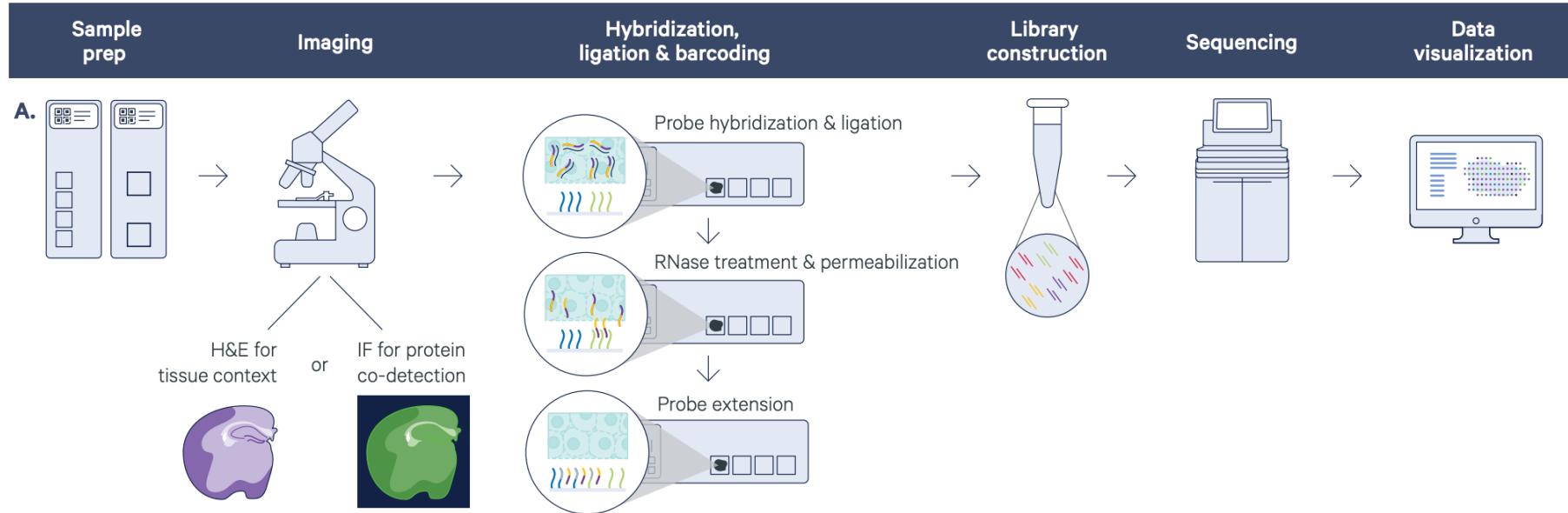
Arrays: 10X Genomics Visium HD

- The HD slide has two capture areas, each 6.5 x 6.5 mm
- ~ 11 million 2 x 2 μm barcoded squares without gaps
- Oligonucleotides each have
 - poly(dT) sequence for capture of the ligation product
 - UMI for the identification of duplicate molecules that arise during the library preparation and sequencing process
 - Spatial Barcode

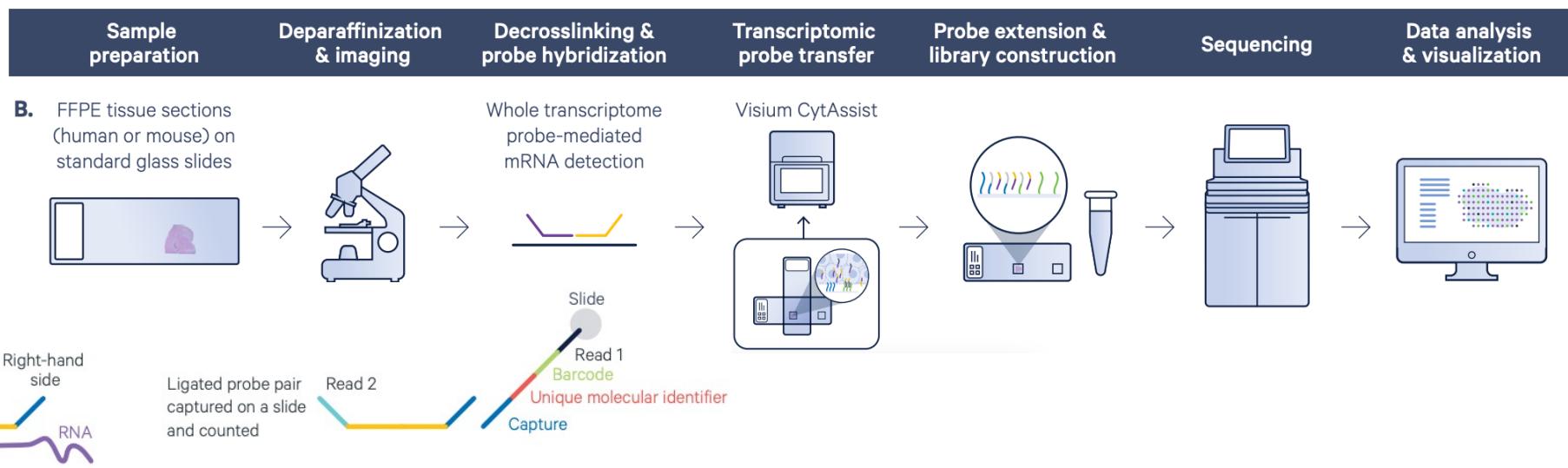


10X Genomics Visium Workflows

**poly-A
Visium
Workflow**

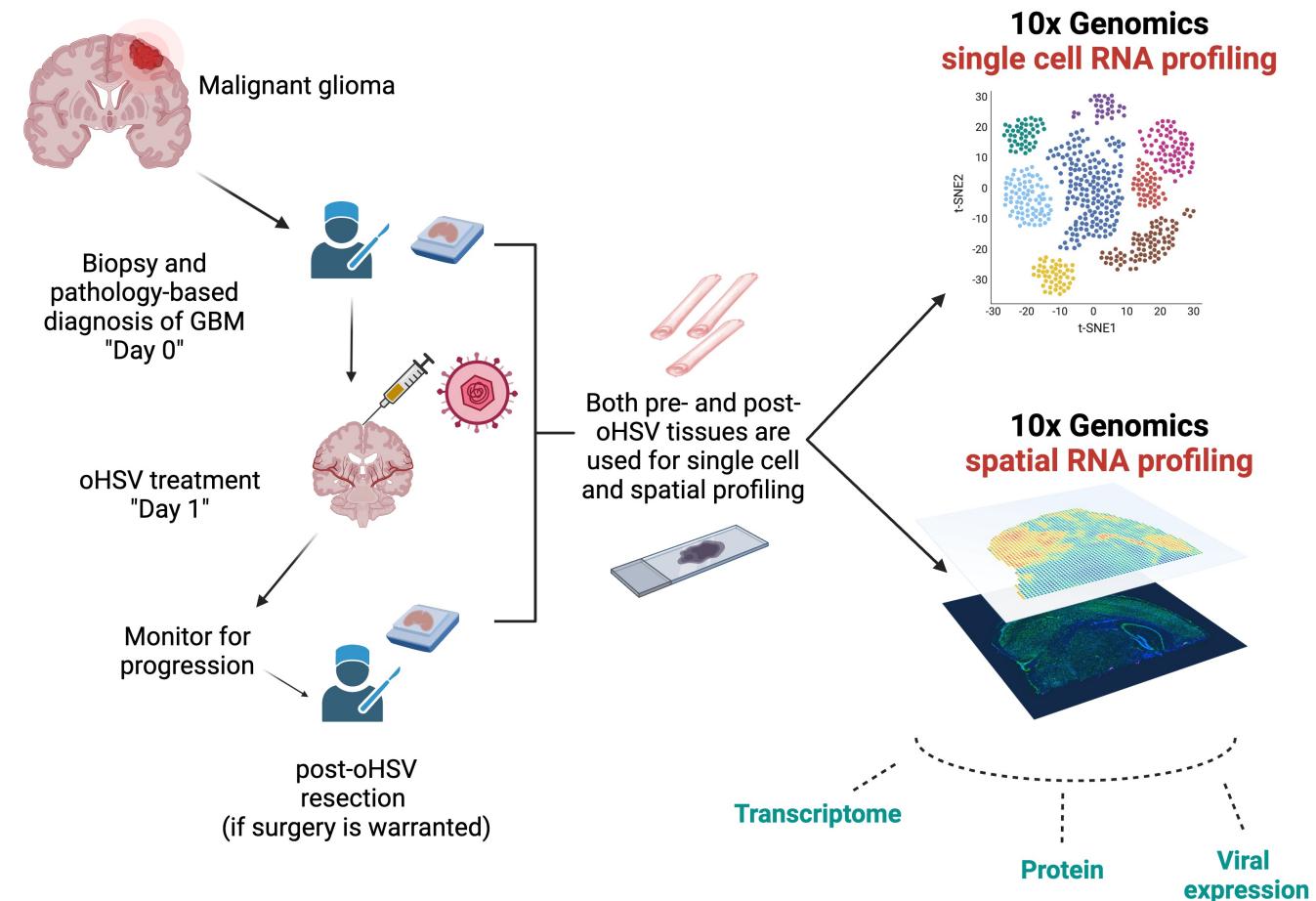


**Probe-based
Visium &
Visium HD
workflow**

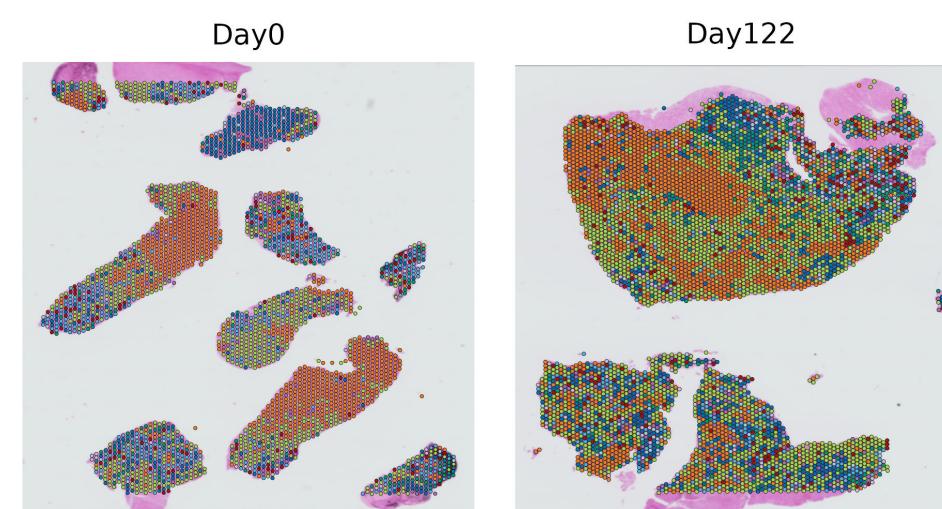
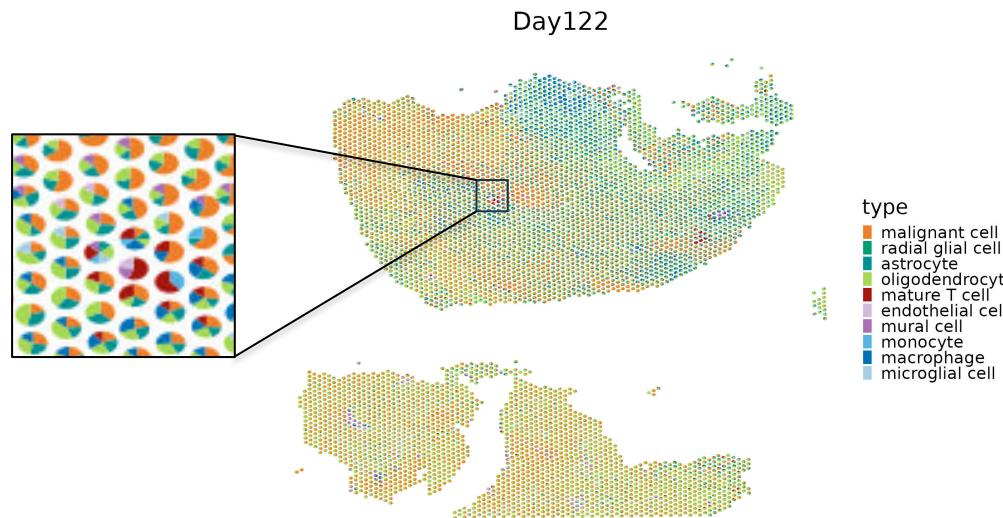
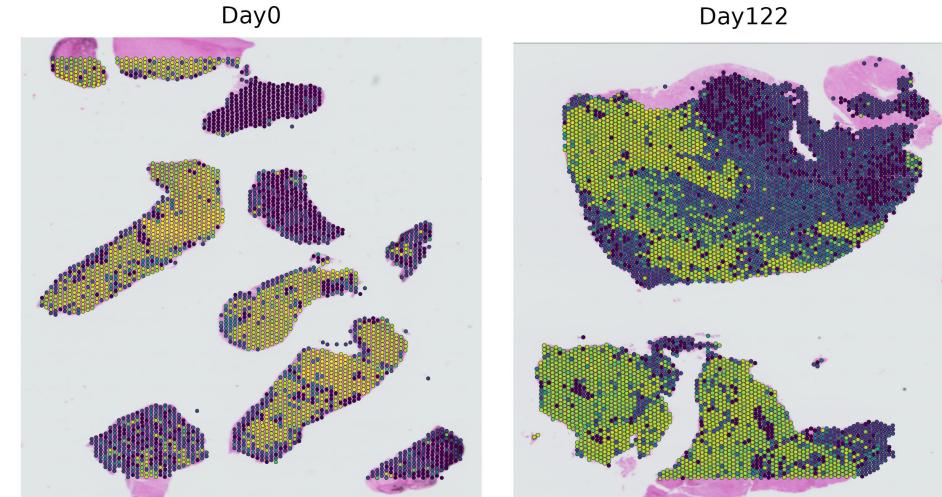
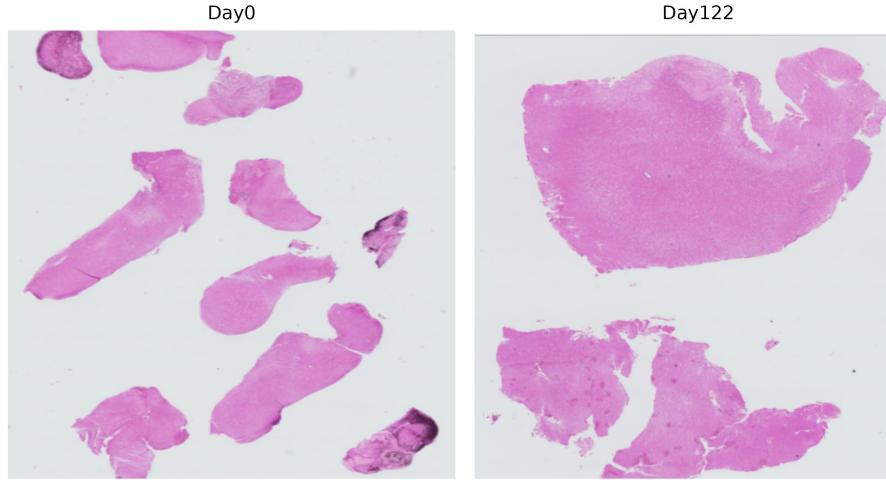


Visium profiling of glioblastoma tumors prior to and following oncolytic herpes simplex virus (oHSV) treatment

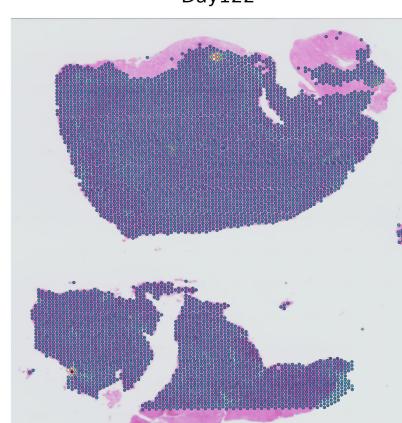
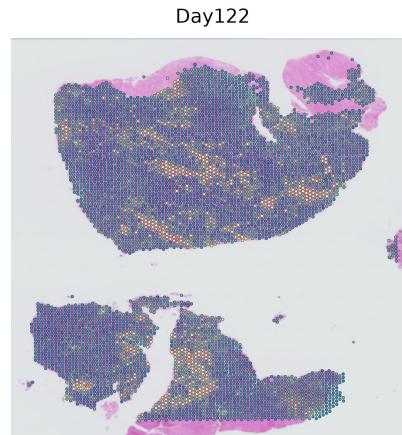
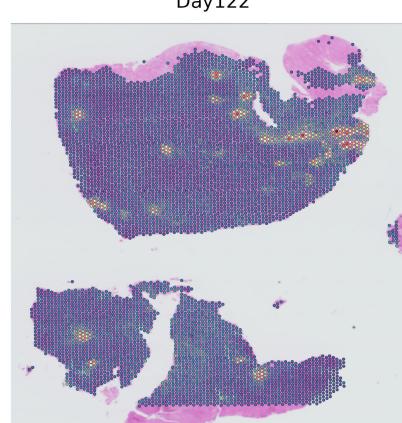
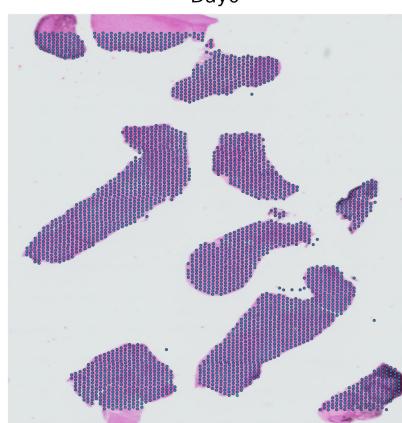
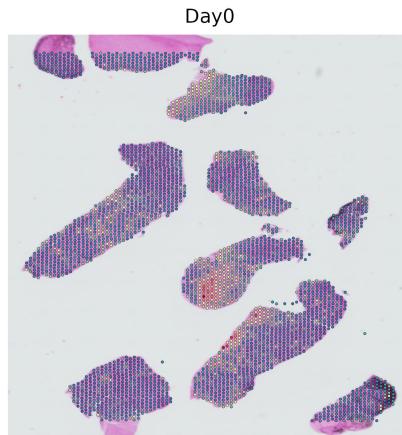
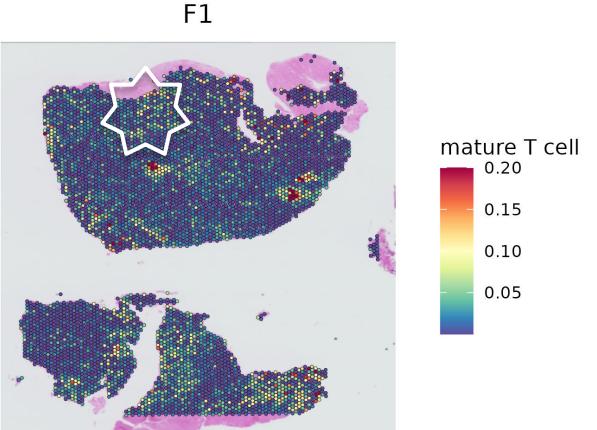
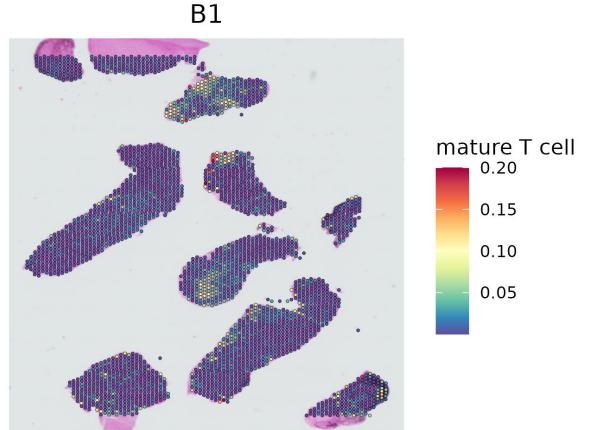
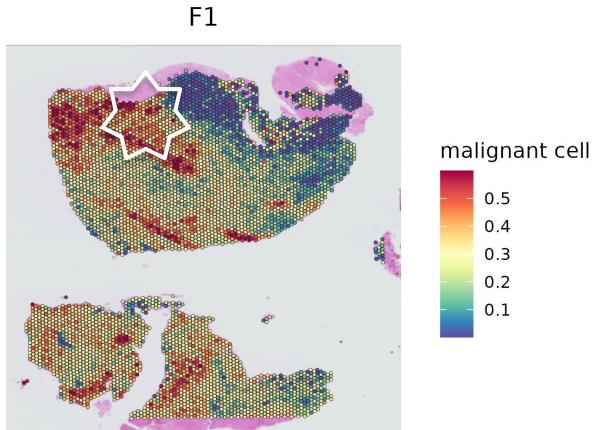
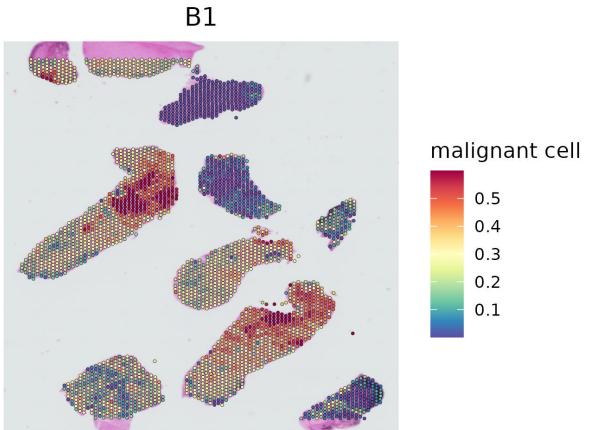
- One of the most common type of brain tumor in adults
- 5-year survival rate of only 5%
- oHSV treatment may be a therapeutic avenue for patients that have failed standard of care chemotherapy



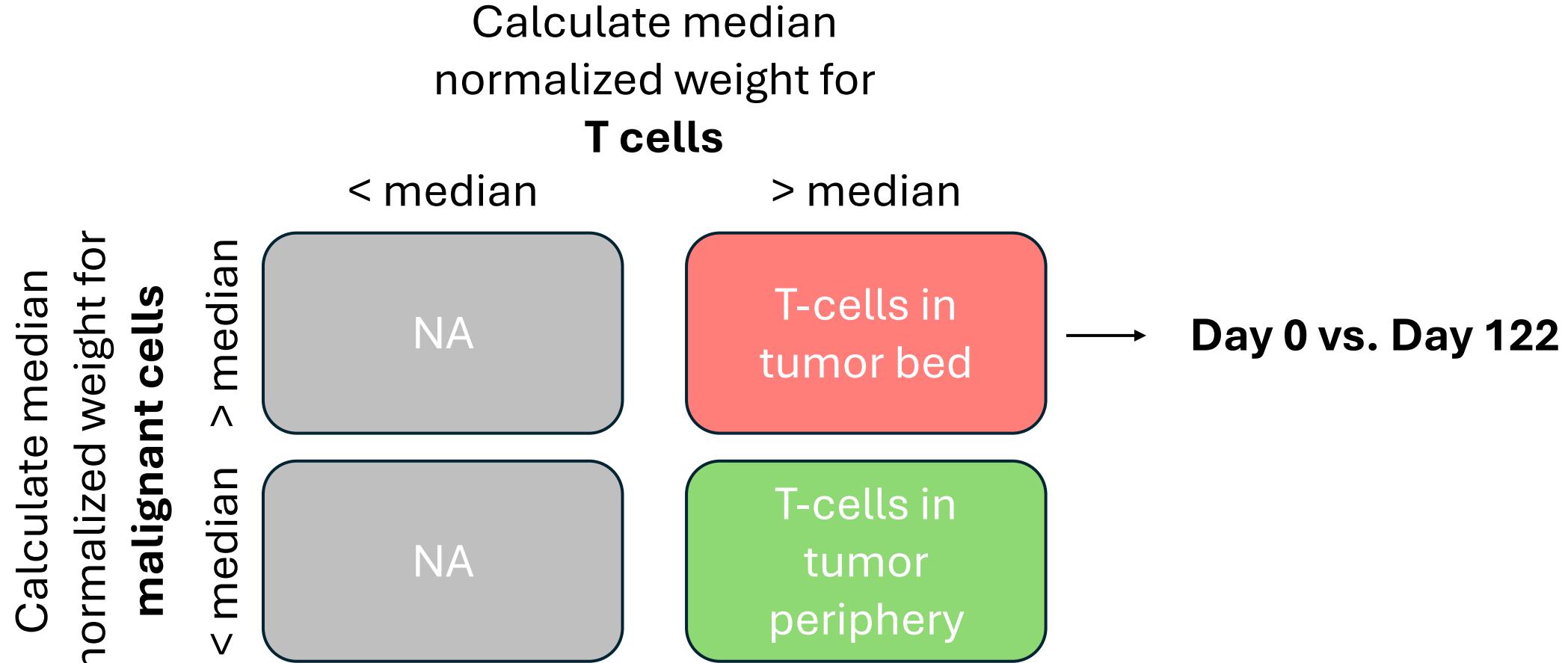
Incorporated InferCNV information to confirm annotation

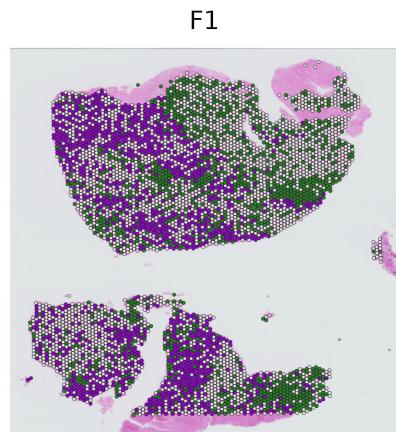
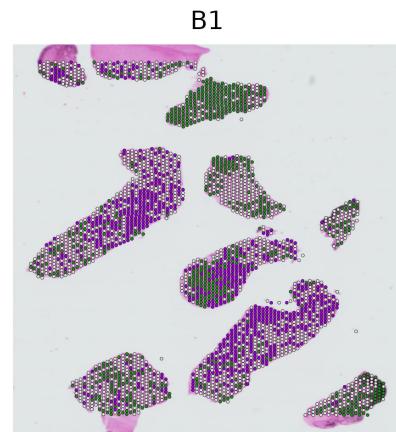
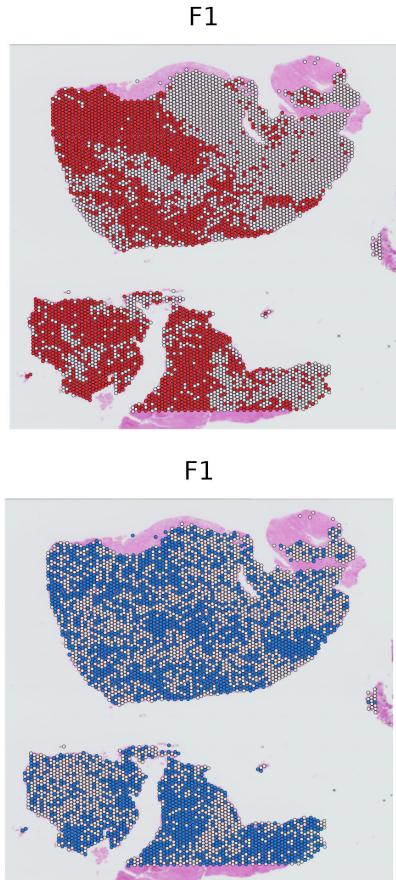
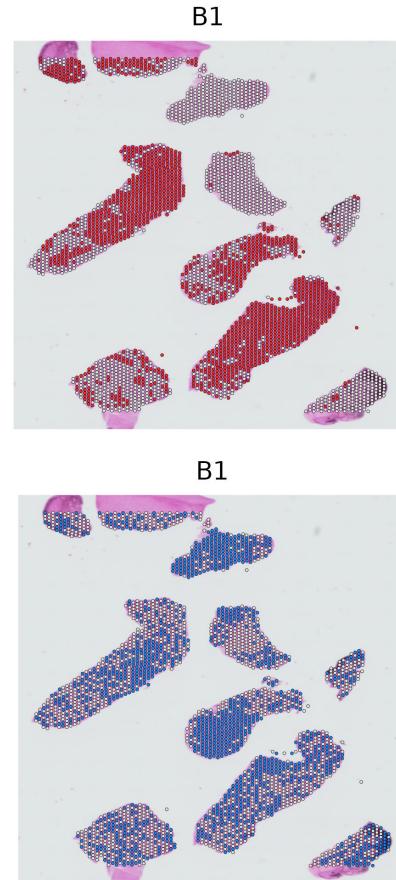
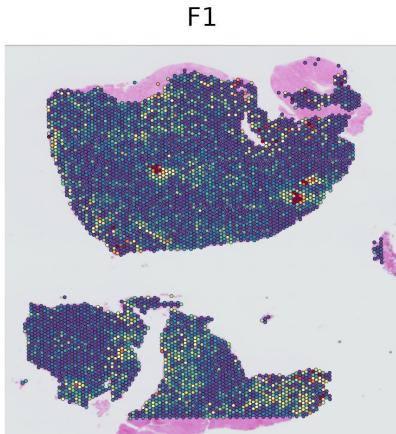
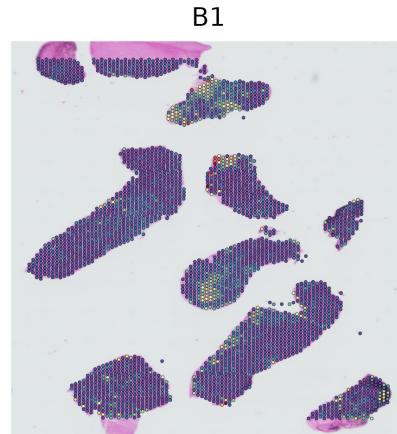
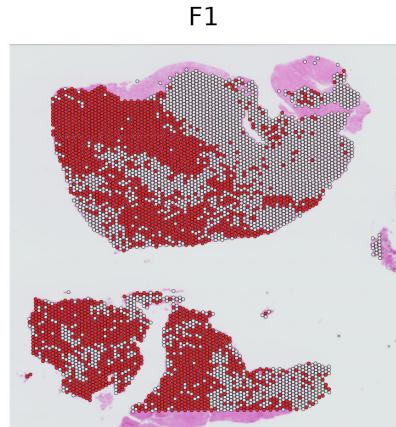
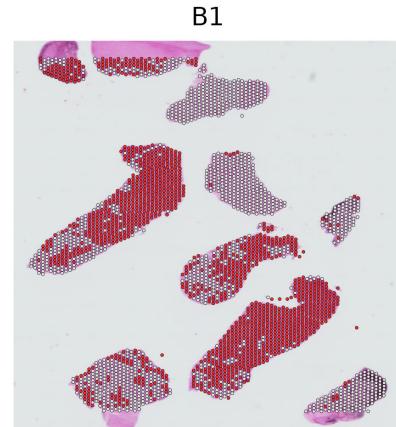
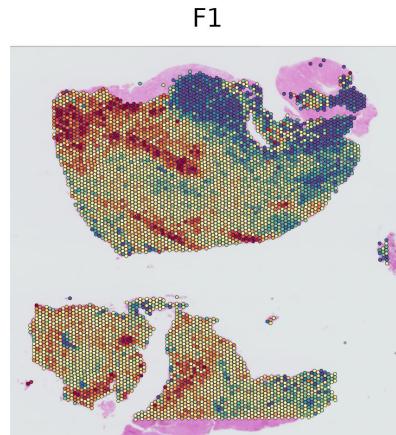
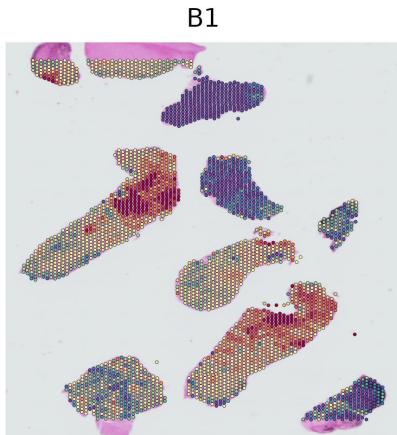


Some areas of the tumor have infiltrating T-cells



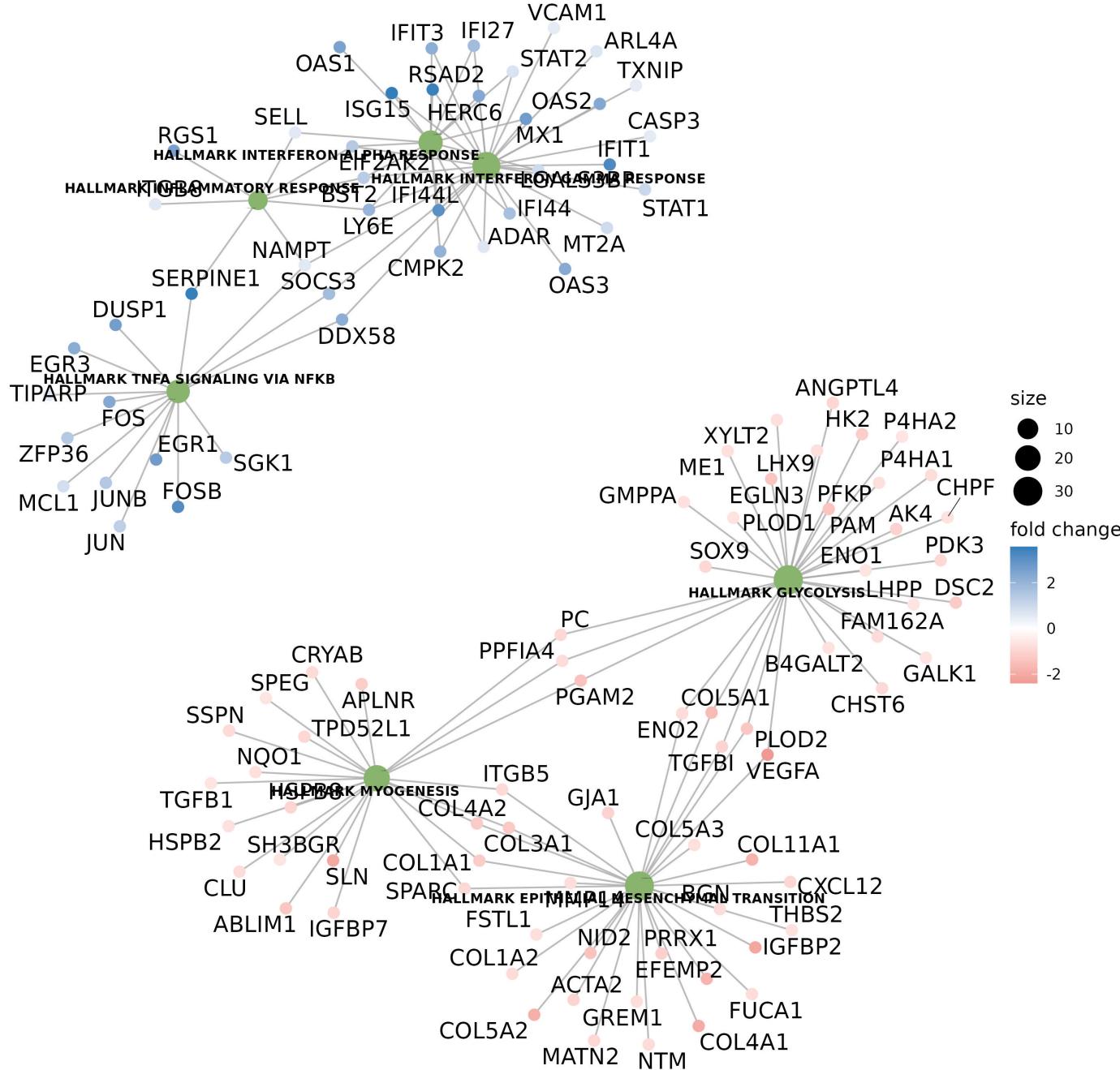
Question: What changes in gene expression occur in areas of the tumor with T-cell infiltration following oHSV treatment?



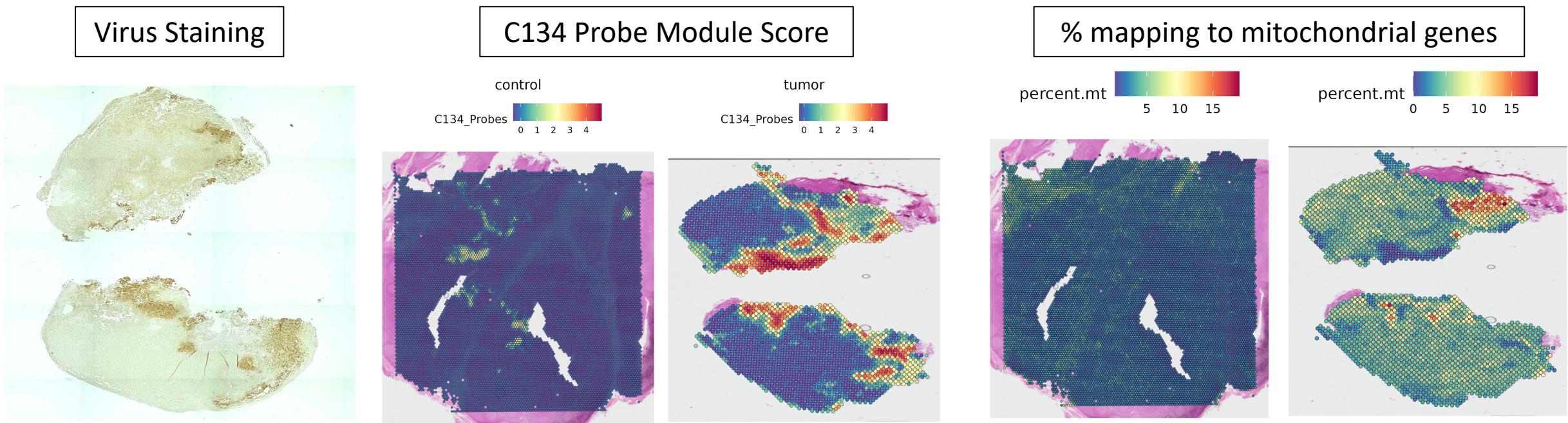


Many immune response transcriptional regulators are **upregulated** in the tumor bed when T-cells are present following oHSV treatment

Genes associated with glycolysis and EMT are **downregulated** in the tumor bed when T-cells are present following oHSV treatment



High probe module score overlaps with histology and high mitochondrial gene expression



Visium allows you to spike in custom probes, including to other genomes, such as the HSV genome!

	MERSCOPE / MERSCOPE Ultra	Xenium	GeoMx	FlowGel	Visium / Visium HD
Company	Vizgen	10X Genomics	Nanostring	AtlasXomics	10X Genomics
Method	MERFISH	ISS + ISH	Light dissection	DBiT-seq	Array
Resolution	<100 nm	<100 nm	< 50 µm	10 µm	55 µm / 2 µm
# Features	1000 genes	5000 genes	WT	WT	WT
# Areas (per slide)	1	1	96	1	2 or 4
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
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)
Species	human, mouse, reference	human, mouse	human, mouse, canine	reference	human, mouse, reference*
Time	⌚⌚	⌚⌚⌚⌚	⌚⌚⌚⌚	⌚	⌚⌚
Customizable	Yes	Yes	Yes	Yes	Yes
Cost (instrument)	\$\$\$	\$\$\$	\$\$	N/A	\$
Cost (per sample/slide)	\$\$	\$\$\$	\$\$	\$	\$\$
Destructive	Yes	No	No	No	No
Required Instrumentation	MERSCOPE or MERSCOPE Ultra	Xenium Analyzer	GeoMx DSP	Microfluidics Stamp Scanning microscope Incubator	Microscope Visium CytAssist
Multomics Compatibility	Protein (antibody)	Protein (in dev)	Protein (antibody)	Protein (antibody) Epigenome (ATAC-seq + CUT&Tag)	Protein (antibody)

PRO:

- ## 1. well documented

CON:

1. can have several cells per spots
 2. Requires Visium slides

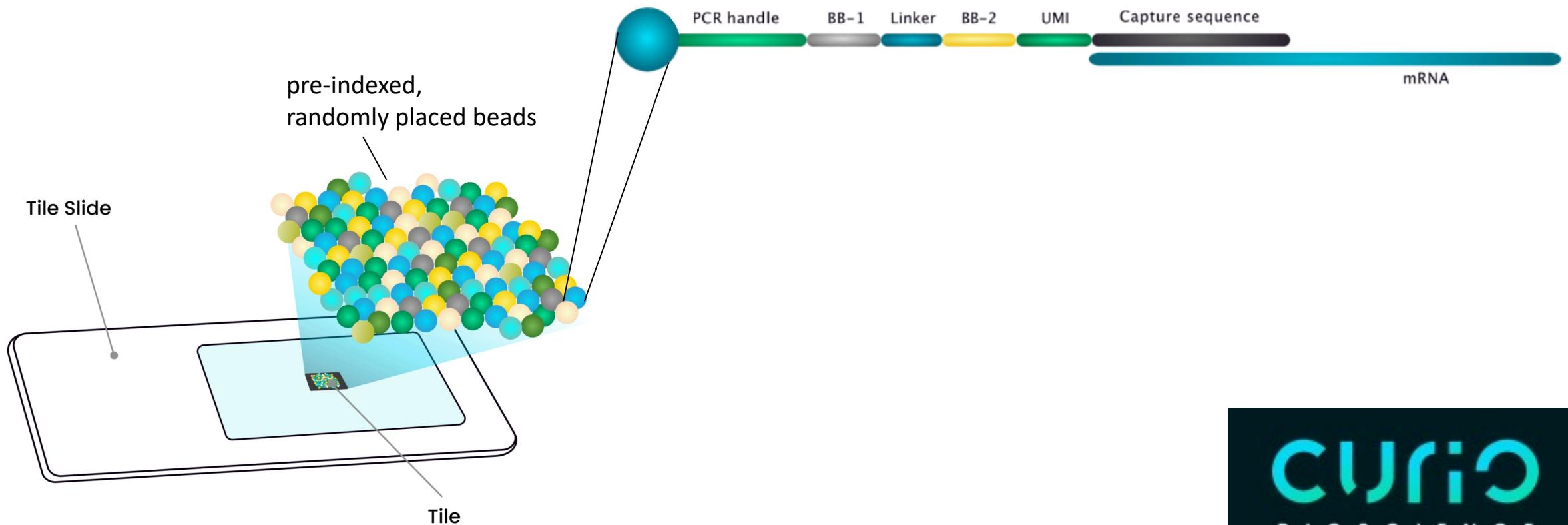
Cost (instrument): ≥ \$400k (\$\$\$), \$100k-399k (\$\$), < \$100k (\$)

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

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

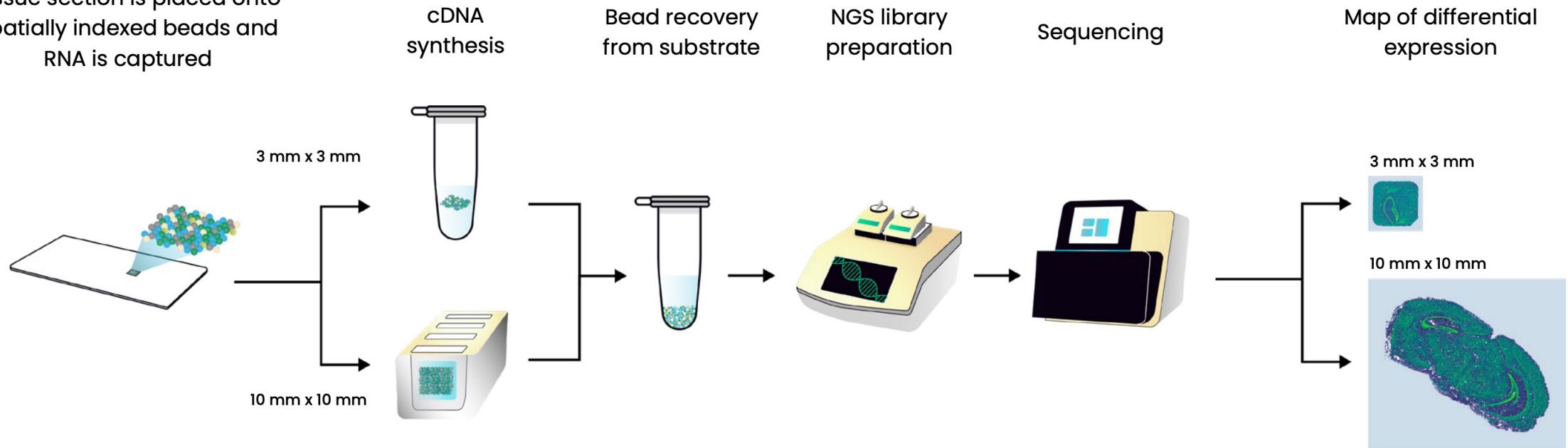
* for poly-A-based capture

Beads: Curio Seeker (Slide-seq v2)



Curio Seeker Workflow

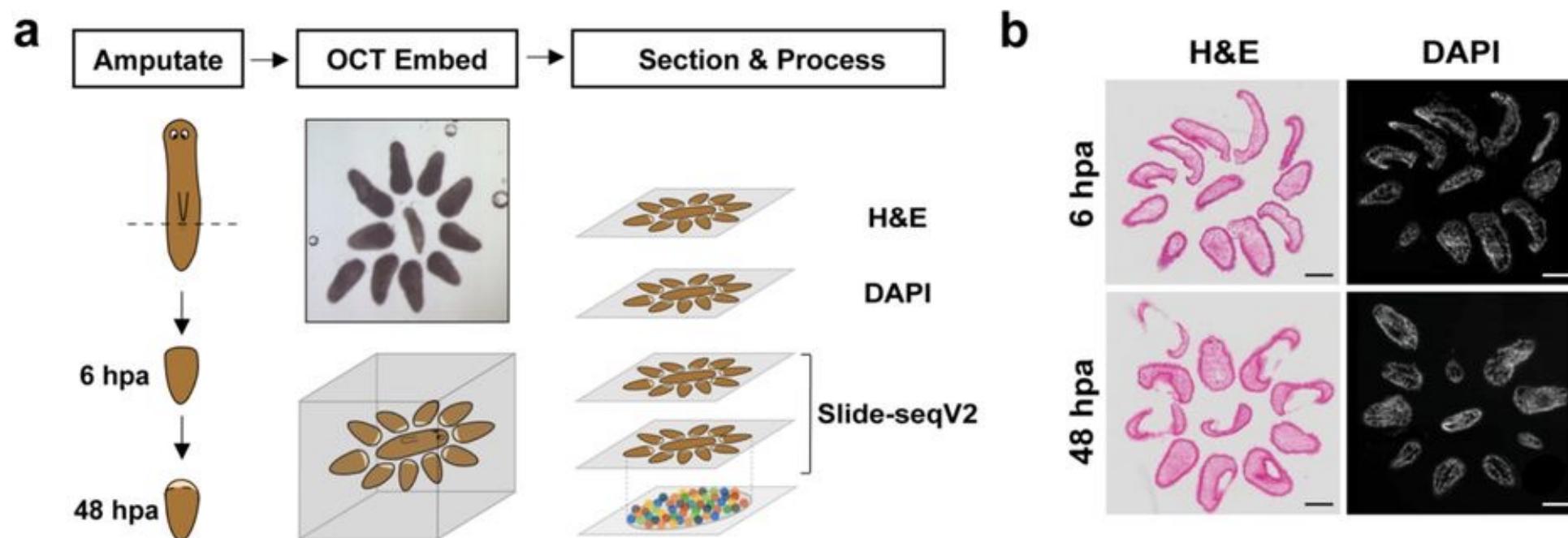
Tissue section is placed onto spatially indexed beads and RNA is captured



Note: imaging must be performed prior to sectioning onto slide!

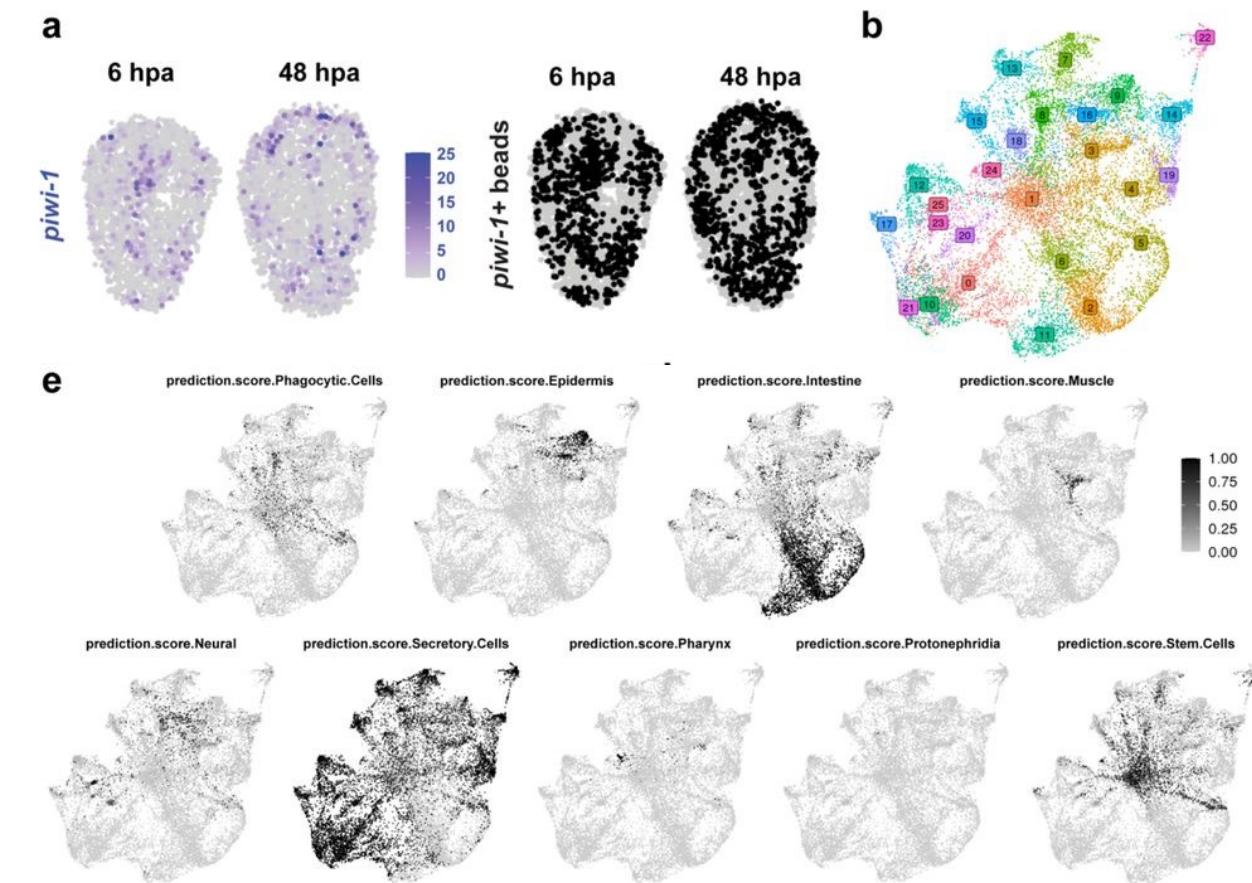


Use of Curio Seeker to define the cellular environments that support pluripotency in the highly regenerative freshwater planarian



Planarian stem cells are near secretory cells

- Hypothesized that cell types in close proximity to stem cells (*piwi-1+*) would co-deposit mRNA onto the same beads
- Only 11% of the *piwi-1+* beads had a high stem cell signature
- Prior studies have shown that stem cells are intercalated between gut branches and intestinal signatures (high in a subset of beads)
- Majority of beads were dominated by a secretory signature suggesting proximity to parenchymal cells



	MERSCOPE / MERSCOPE Ultra	Xenium	GeoMx	FlowGel	Visium / Visium HD	Curio Seeker
Company	Vizgen	10X Genomics	Nanostring	AtlasXomics	10X Genomics	Curio Biosciences
Method	MERFISH	ISS + ISH	Light dissection	DBiT-seq	Array	Slide-Seq
Resolution	<100 nm	<100 nm	< 50 µm	10 µm	55 µm / 2 µm	10 µm
# Features	1000 genes	5000 genes	WT	WT	WT	WT
# Areas (per slide)	1	1	96	1	2 or 4	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
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
Species	human, mouse, reference	human, mouse	human, mouse, canine	reference	human, mouse, reference*	human, mouse, reference
Time	⌚⌚	⌚⌚⌚	⌚⌚⌚⌚	⌚	⌚⌚	⌚
Customizable	Yes	Yes	Yes	Yes	Yes	No
Cost (instrument)	\$\$\$	\$\$\$	\$\$	N/A	\$	N/A
Cost (per sample/slide)	\$\$	\$\$\$	\$\$	\$	\$\$	\$\$
Destructive	Yes	No	No	No	No	Yes
Required Instrumentation	MERSCOPE or MERSCOPE Ultra	Xenium Analyzer	GeoMx DSP	Microfluidics Stamp Scanning microscope Incubator	Microscope Visium CytAssist	none
Multiomics Compatibility	Protein (antibody)	Protein (in dev)	Protein (antibody)	Protein (antibody) Epigenome (ATAC-seq + CUT&Tag)	Protein (antibody)	none

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

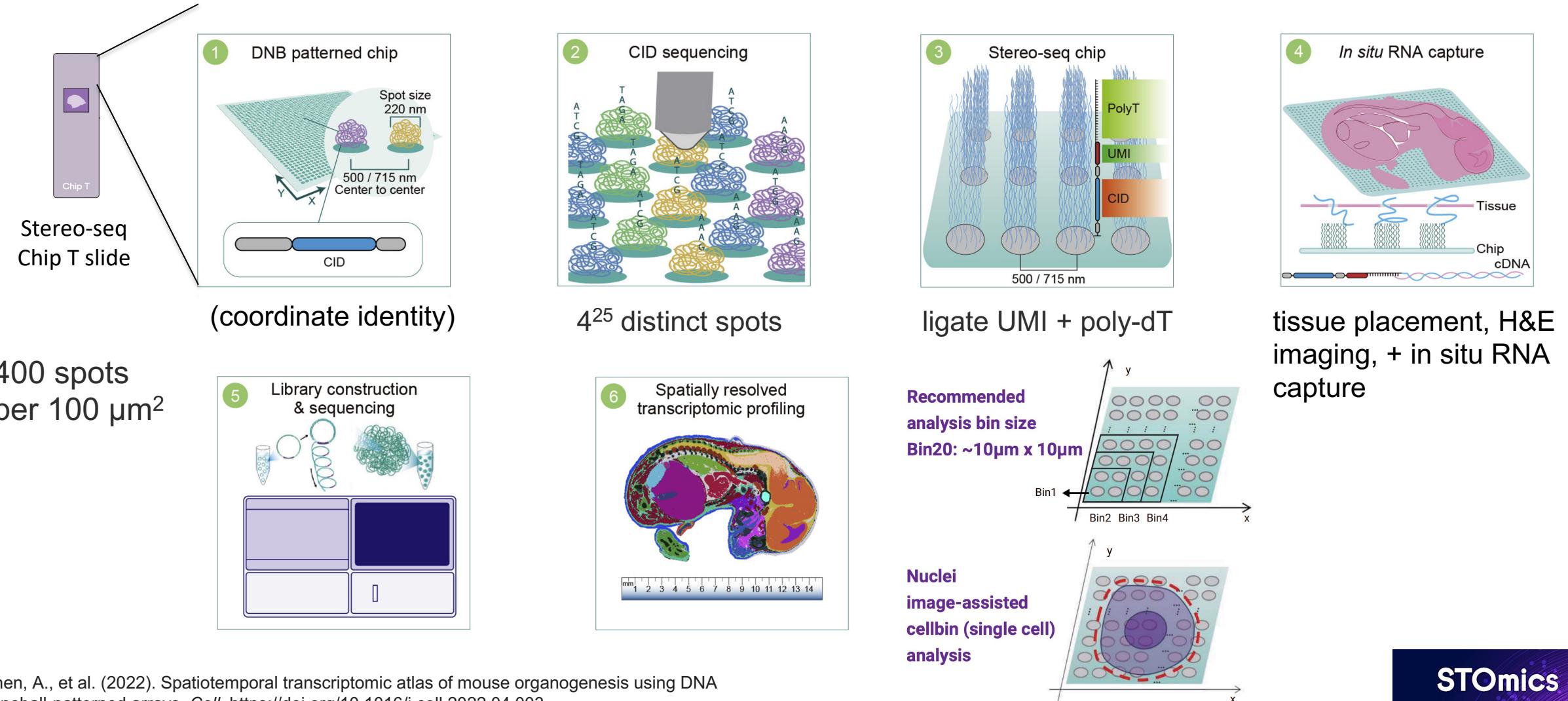
PRO:

- ## 1. high resolution

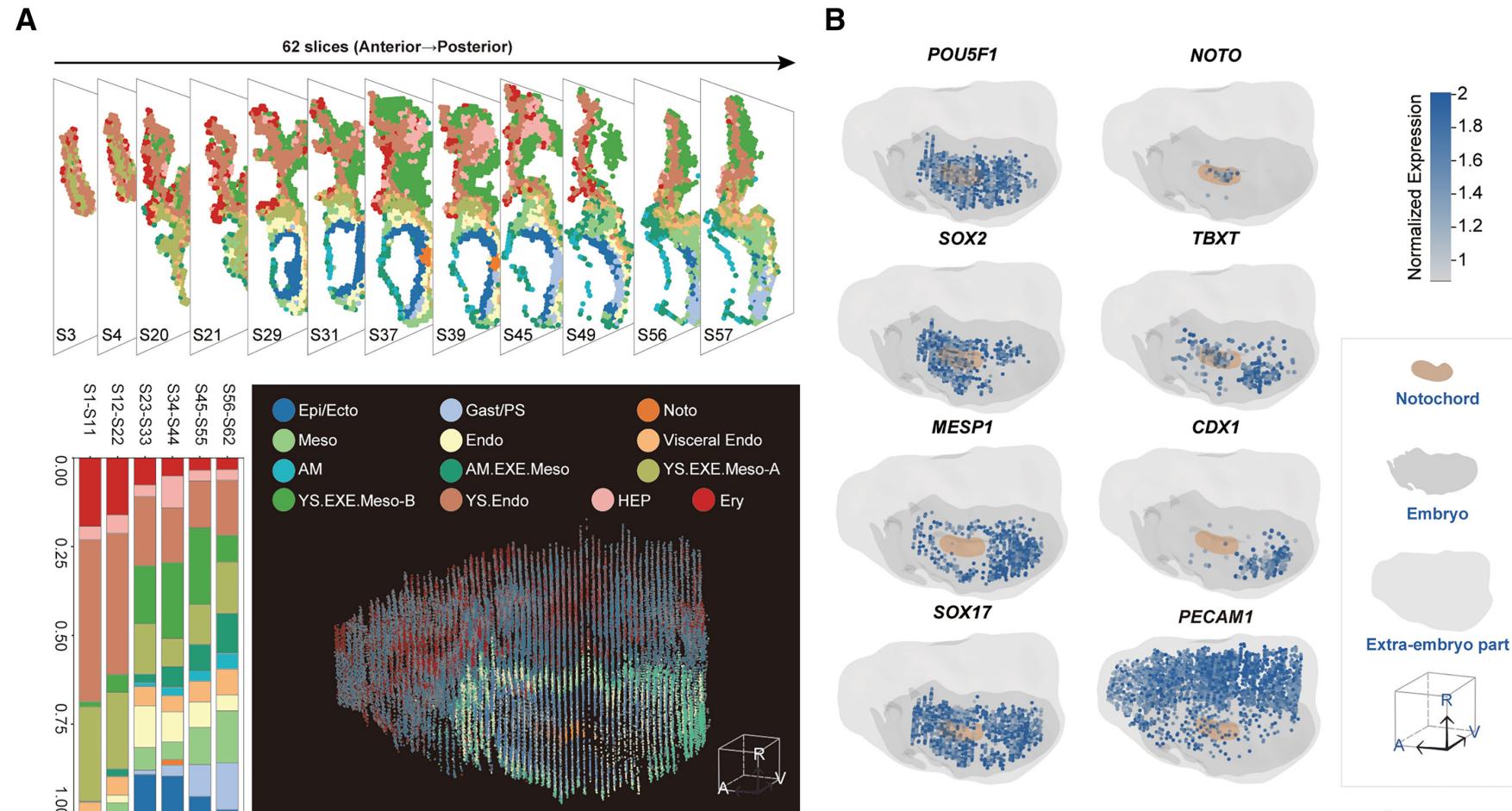
CON-

1. may capture transcripts from neighboring cells

DNA Nanoballs (DNB): STOmics Stereo-seq



3D reconstruction of a gastrulating human embryo with Stereo-seq



	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 (\$)

* for poly-A-based capture

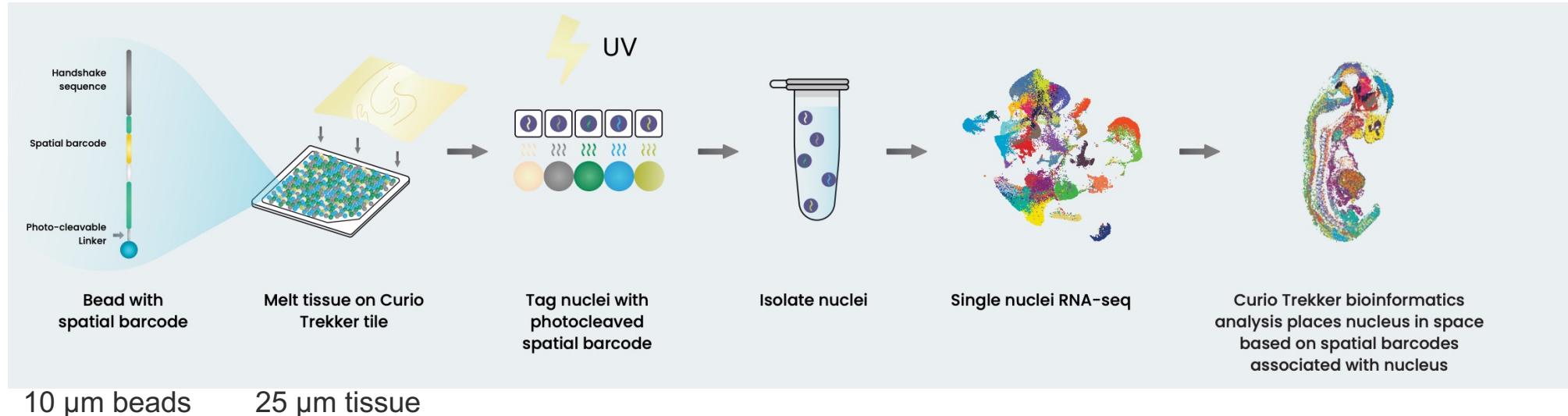
PRO:

1. no major instrument
 2. high res and sensitivity

CON:

- ## 1. sequencer-specific

Truly single cell spatial transcriptomics: Curio Trekker (Slide-tags)



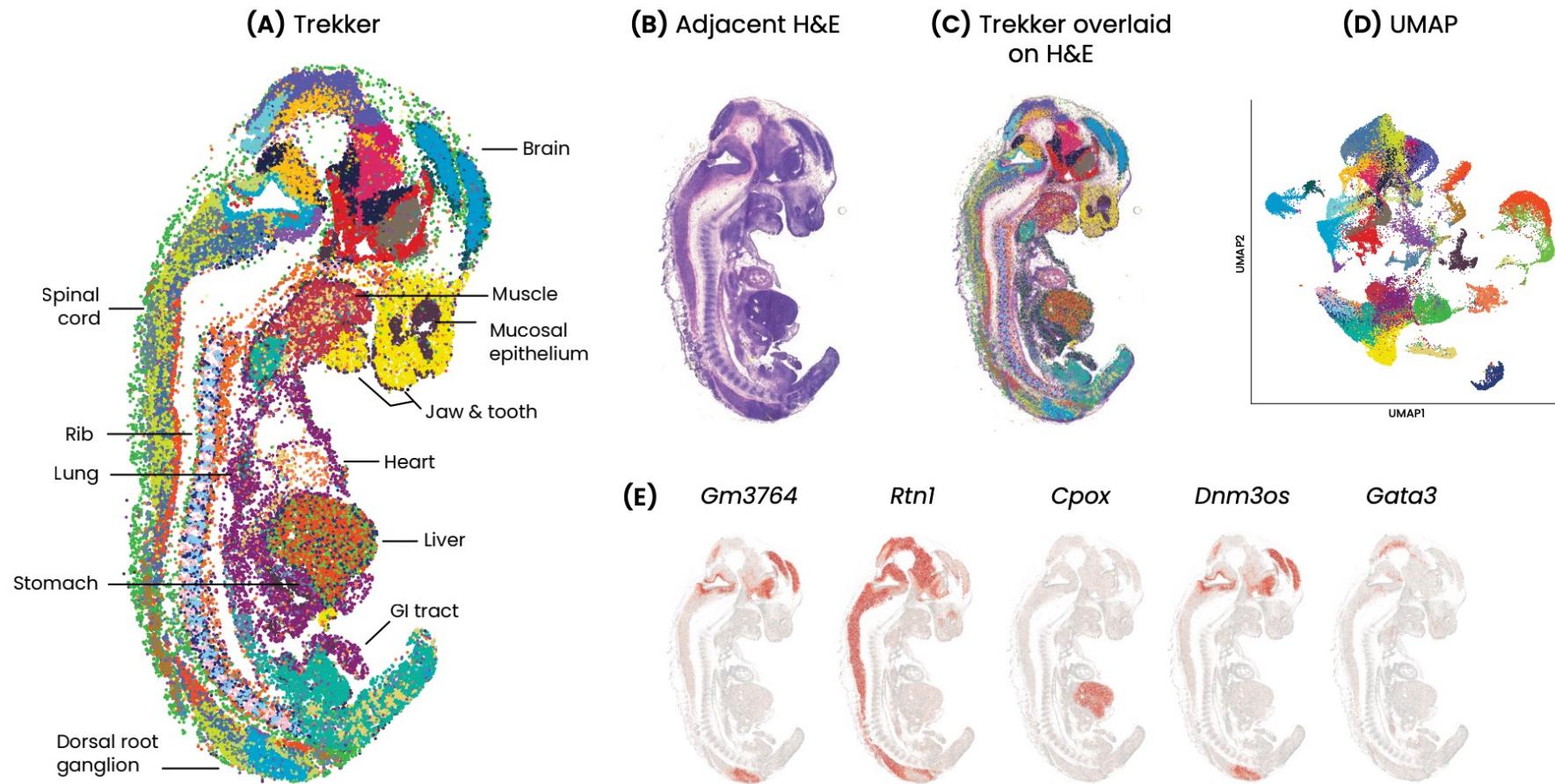
Product features	
Spatial resolution	True single-cell
Sample type	Fresh frozen tissues
Tile Size	10 mm x 10 mm
Specialized capital equipment	None required
Required auxiliary equipment	Cryostat, single-cell sequencing platform, NGS sequencer
Workflow duration	1 hour upstream of single-cell workflow
Sensitivity	Same as the molecule capture sensitivity of the single-cell workflow of choice

Single-cell workflow compatibility	
Supported	User Demonstrated

- 10x Chromium™ 3' RNA v3.1, v4
- BD Rhapsody™ WTA

- Fluent PIPseq™ V
- 10x Chromium™ Multiome ATAC + Gene Expression
- ScaleBio™ Single-Cell RNA Kit

Spatial mapping of nuclei from an E11 mouse embryo using Curio Trekker



Spatial Transcriptomics Analysis

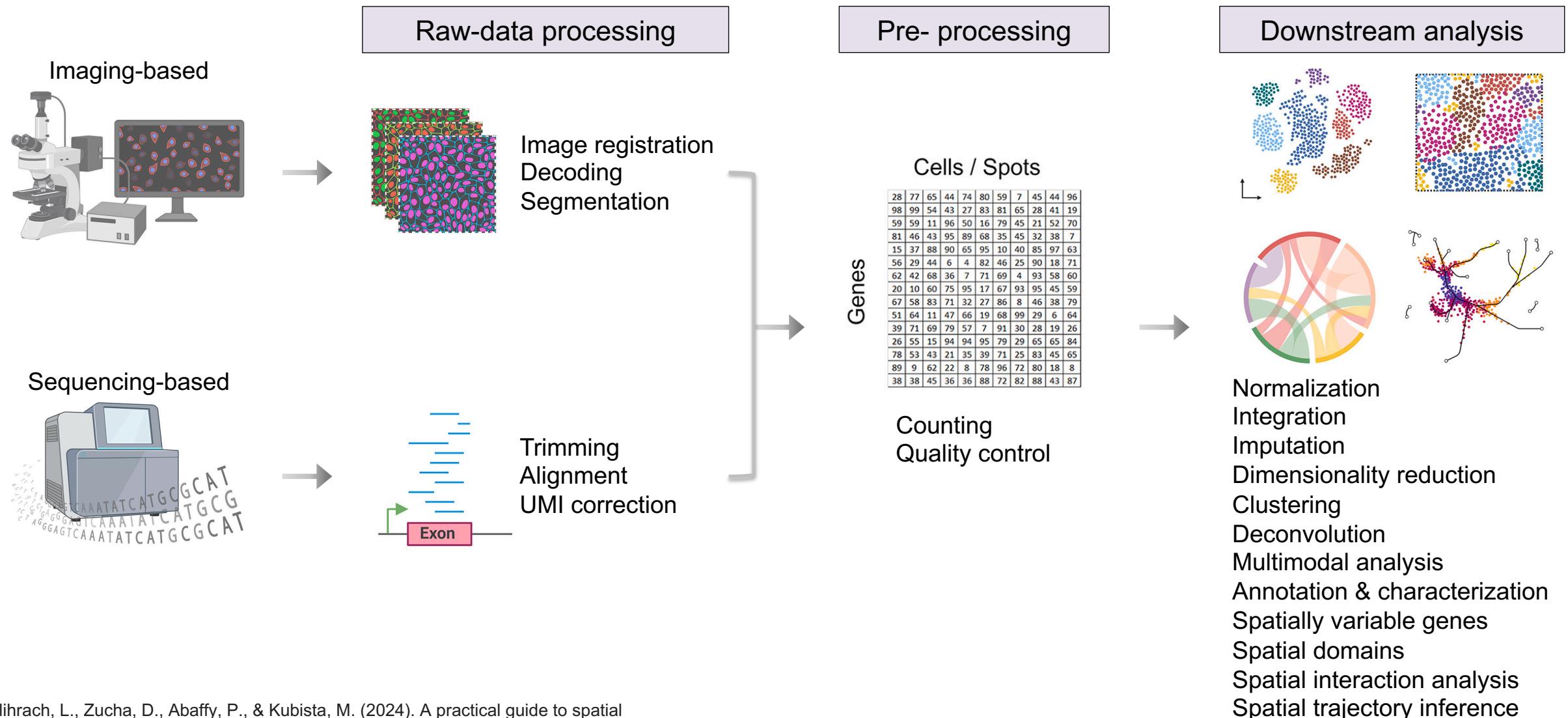
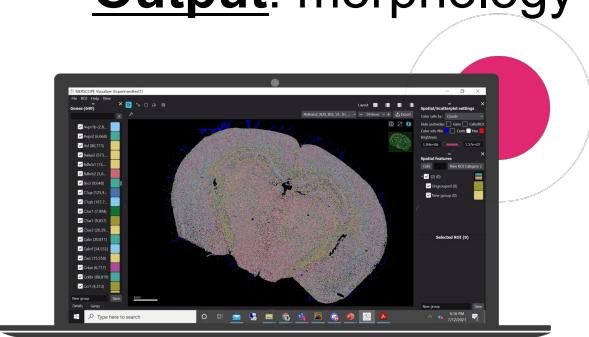


Image-based processing

Input: Tbs of image data

1. Image registration: merge images through stitching, background corrections & re-alignments
 - makes use of stable image features (fluorescent beads, nuclei staining, etc.)
2. Decoding & counting: convert signal to mRNA quantification using modified Hamming distance 4 (MHD4) codebook (MERSCOPE) or multi-focus image fusion (Xenium)
3. Segmentation: using complex statistical models and machine learning approaches based on nuclei staining (DAPI) – this is hard!
4. Quality control: Phred-style calibrated quality score (Q-score)

Output: morphology images + cell x feature matrix



VIZgen

MERSCOPE Visualizer +
Vizgen Post Processing
Tool (VPT)

**10X
GENOMICS®**

Xenium Analyzer +
Explorer

Space Tx

Consortium for imaging-
based spatial transcriptomics
standardization

Sequencing-based processing

Input: morphology images + BCL or FASTQ files

1. Trimming: remove non-template sequence from each read
2. Alignment: to probe set reference or transcriptome
3. UMI correction and filtering: compare observed barcodes to whitelist barcodes and make any corrections; filter UMIs using barcode, UMI, gene, and number of supporting reads
4. Barcode filtering: manually or computationally determine spots/bins under tissue

Output: morphology image + spot/cell x feature matrix



GeoMx Digital Spatial Profiler



AtlasXBrowser

https://github.com/atlasxomics/ATX_epigenomics



Curio Seeker
NextflowPipeline



Space Ranger

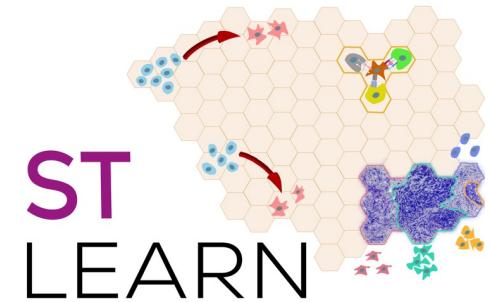


ImageStudio
SAW
StereoMap

Downstream analysis: choose your own adventure!



squidpy

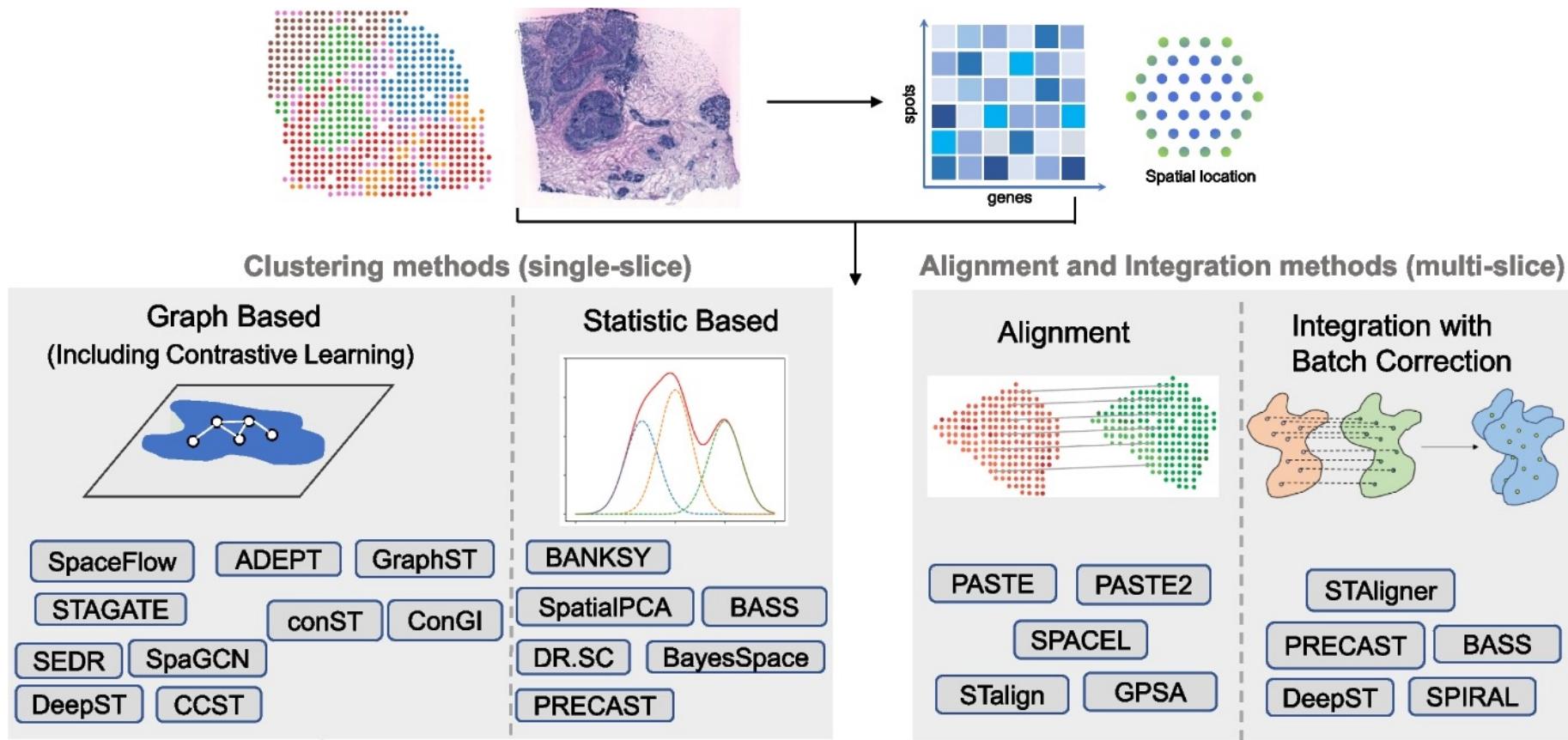


(formerly STUtility)

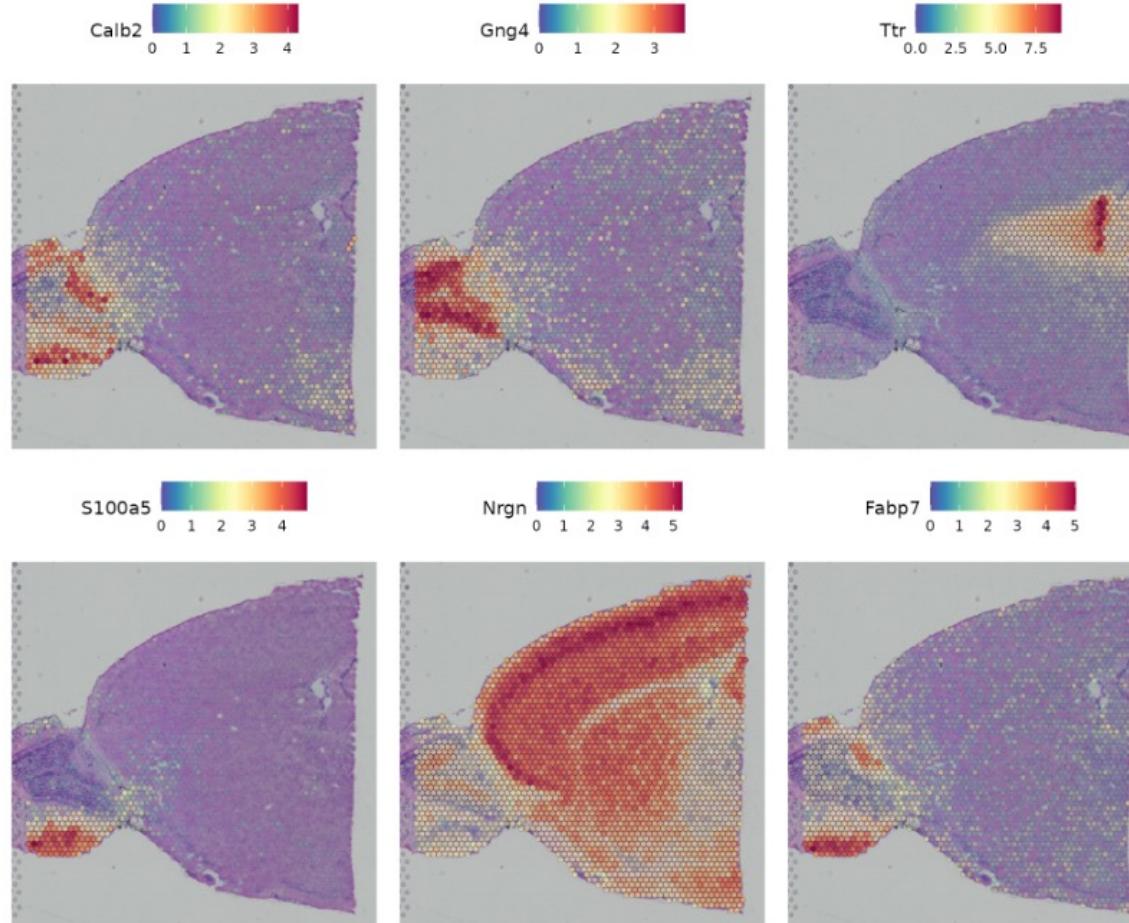
Normalization methods

- Goal: account for differences in mRNA capture across tissue
- 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”)
- Option #2: normalize cells/spots based on groups of genes with similar abundance, one group at a time (“SCTransform”)
- Option #3: smooth library sizes in cells/spots based on library size in nearby cells/spots within a radius and their morphological similarity using deep learning of histological features (**spatial and morphological expression** “SME”)
- Several other methods out there!

Data integration and clustering



Identifying spatially variable features in absence of cluster annotation



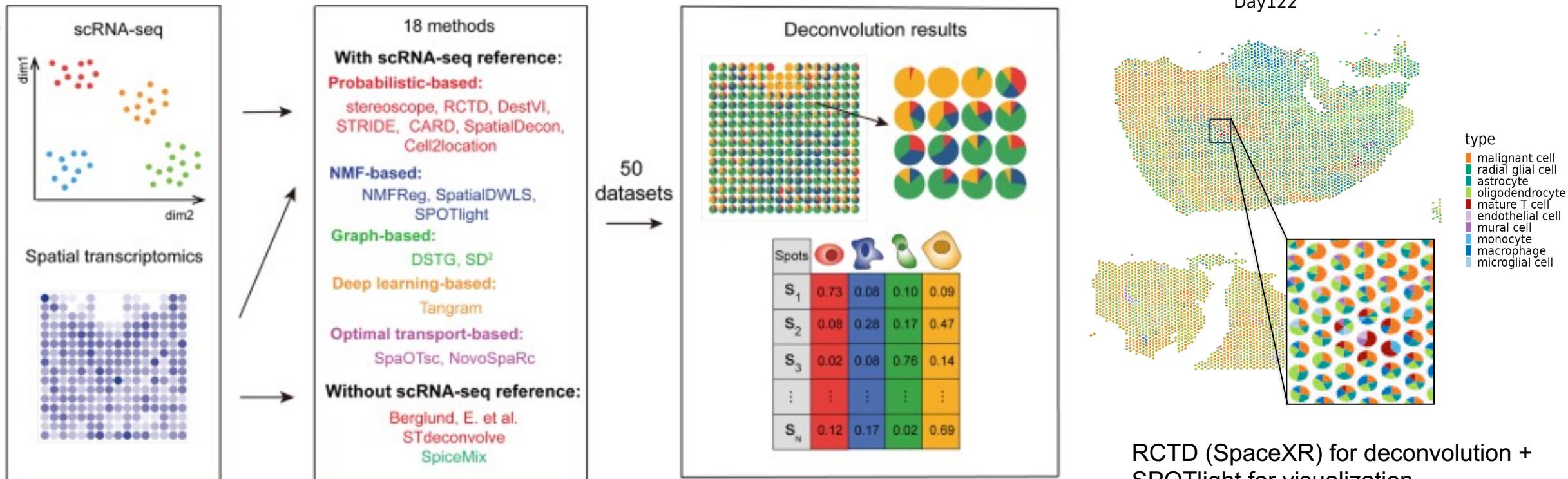
Seurat (*built-in*)
Giotto (*built-in*)
trendsseek
SPARK and SPARK-X
SpatialDE
Hotspot
nnSVG

*no one-size-fits all approach!

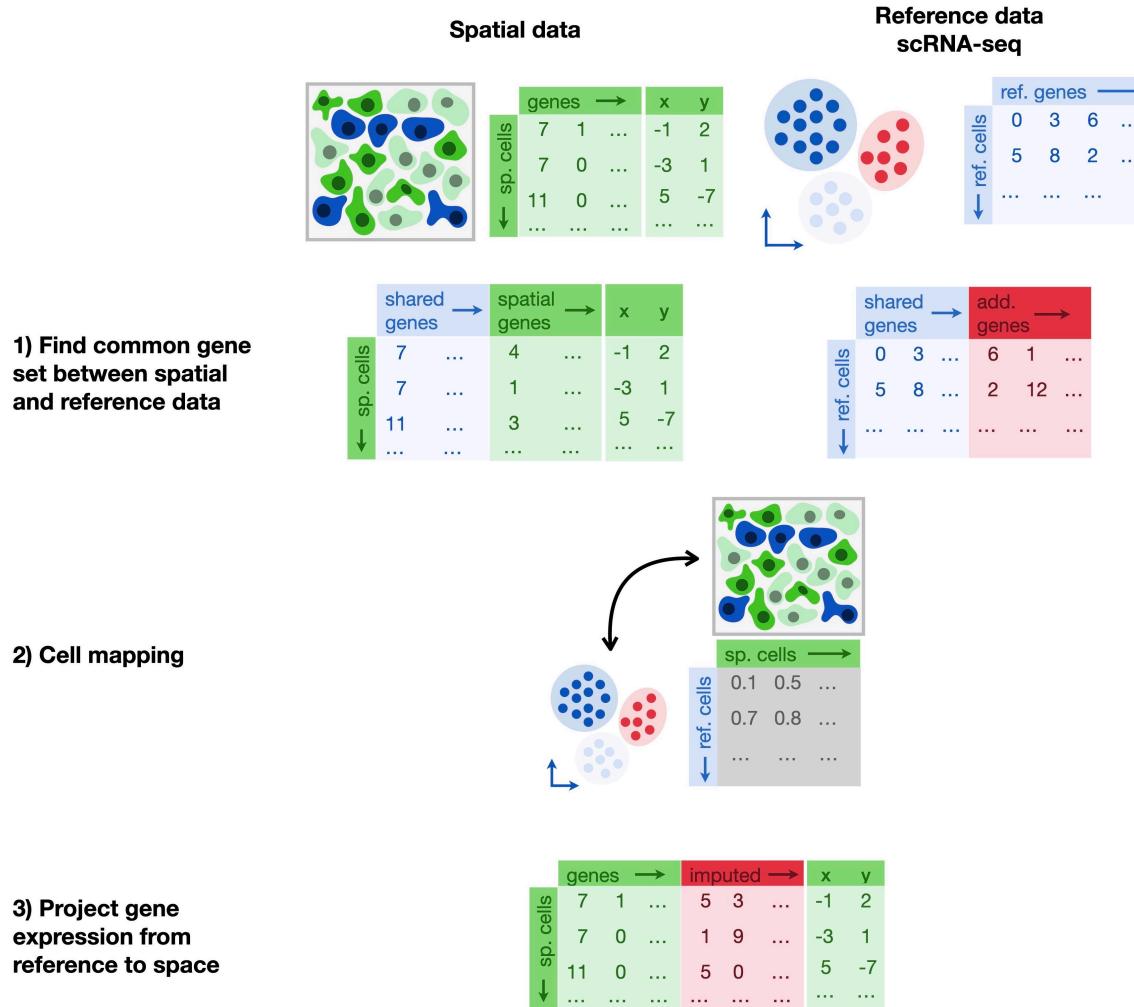
Spot deconvolution

Problem: a single spatial unit may be overlaid by multiple cells; thus, a single barcode may detect expression from a heterogeneous population of cells

A



Imputation of missing genes



Problem: a given approach may be targeted to a limited number of genes or have low capture sensitivity

“Fill in the blanks” with:

Seurat (*built-in*)

Tangram

SpaGE

gimVI

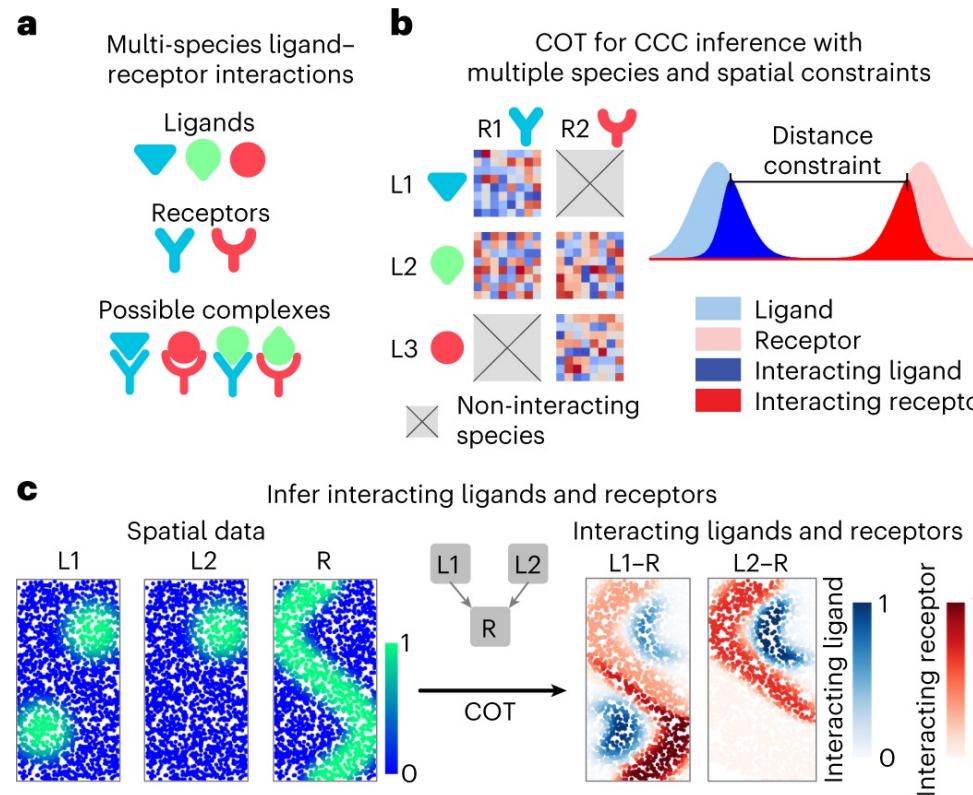
LIGER

NovoSpaRc

spARC

Identifying cell-cell interactions

COMMunication analysis by Optimal Transport (COMMOT)



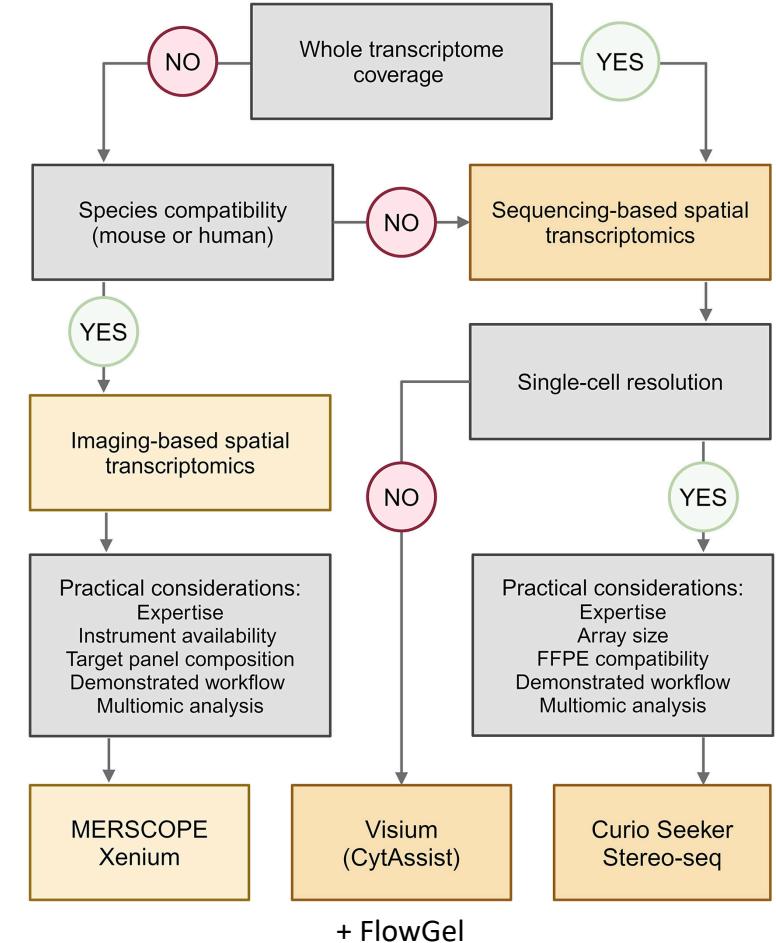
Use database of genes that encode interacting proteins along with models (with or without spatial info) to infer an interaction probability

COMMOT*	CellChat
MISTy*	SoptSC
DIALOGUE*	NicheNet
NCEM*	CytoTalk
CellPhoneDB	scTensor
ICELLNET	

*developed with ST in mind

Experimental Considerations

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

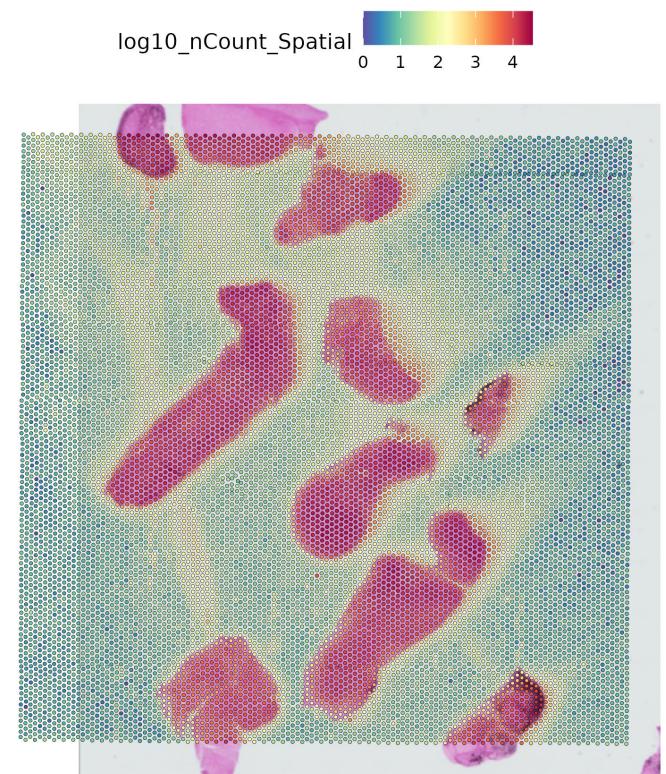


Other spatial -omics

- **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!*

What challenges remain?

- Compatibility with wider range of tissues
- Optimize data storage and processing speed
- Optimize spatial resolution and capture efficiency
- Reduce signal diffusion
- ST-specific normalization and batch correction
- Registration across slices
- Integration with multi-omics
- Increased throughput, reduced cost, greater accessibility



Questions?



Mardis/Miller Lab

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Katie Miller, PhD

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Jaye Navarro

Lakshmi Prakruthi Rao Venkata

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