

# Spatial Single-Cell Transcriptomics Technology and Analytics

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# Outline

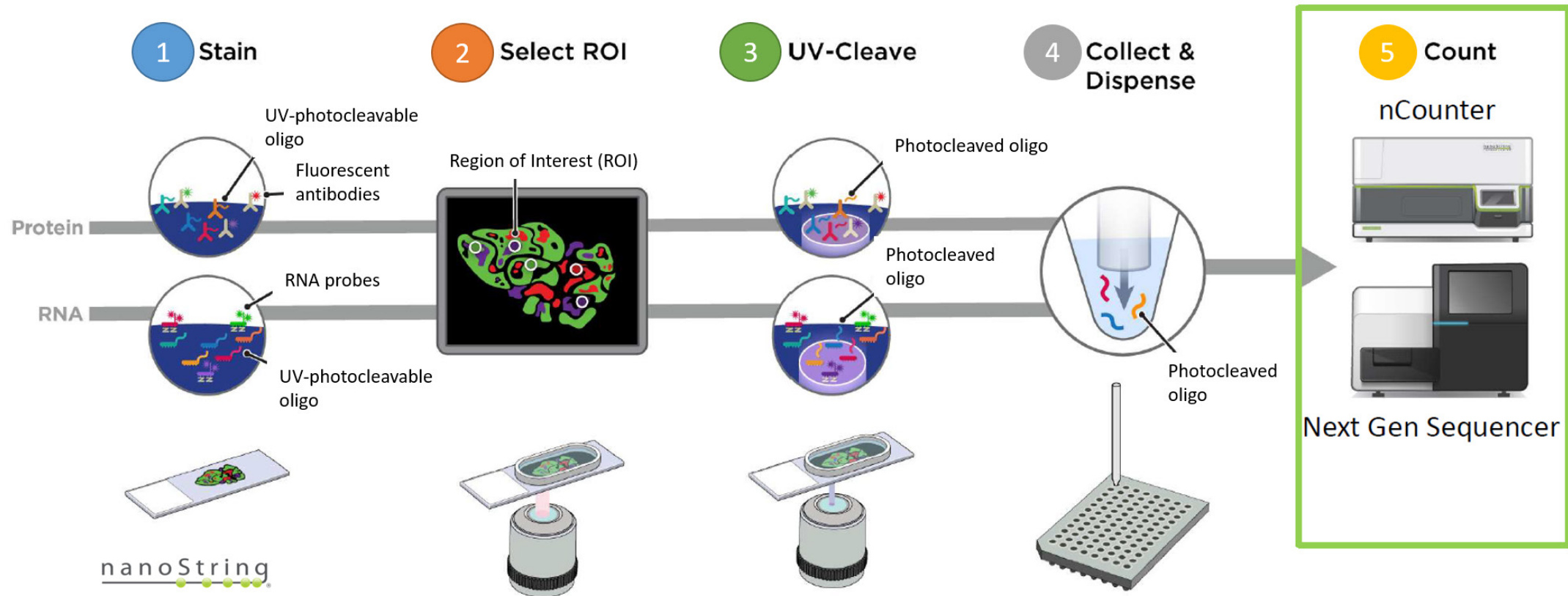
## Lecture

- NanoString spatial omics platforms
- Case study in Non-Small Cell Lung Cancer (NSCLC)
- Introduction to MC-SOLVE (Mayo in-house developed analytic framework)

# Introduction to NanoString Spatial Platforms

- **GeoMx** Digital Spatial Profiler (DSP): High resolution (10 microns)
- **CosMx** Spatial Molecular Imaging (SMI): True single cell and subcellular resolution
  
- Additional platforms:
  - ✓ 10x Genomics: Visium and Xenium
  - ✓ Vizgen: MERSCOPE

# GeoMx Digital Spatial Profiler (DSP):

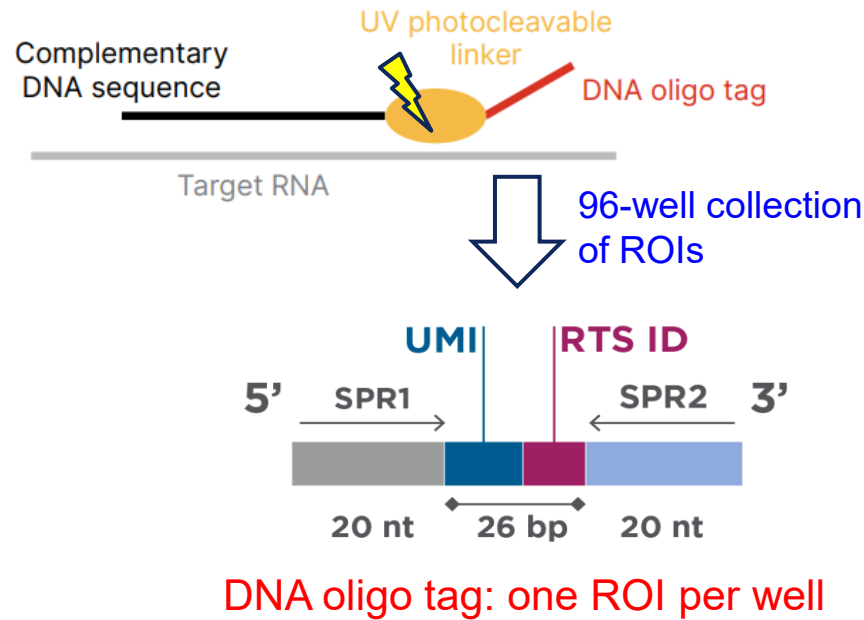


- Select distinct tissue compartments or cell types
- Use micromirrors to expose the ROI to UV
- Release barcodes for quantification: 18000+ protein coding genes

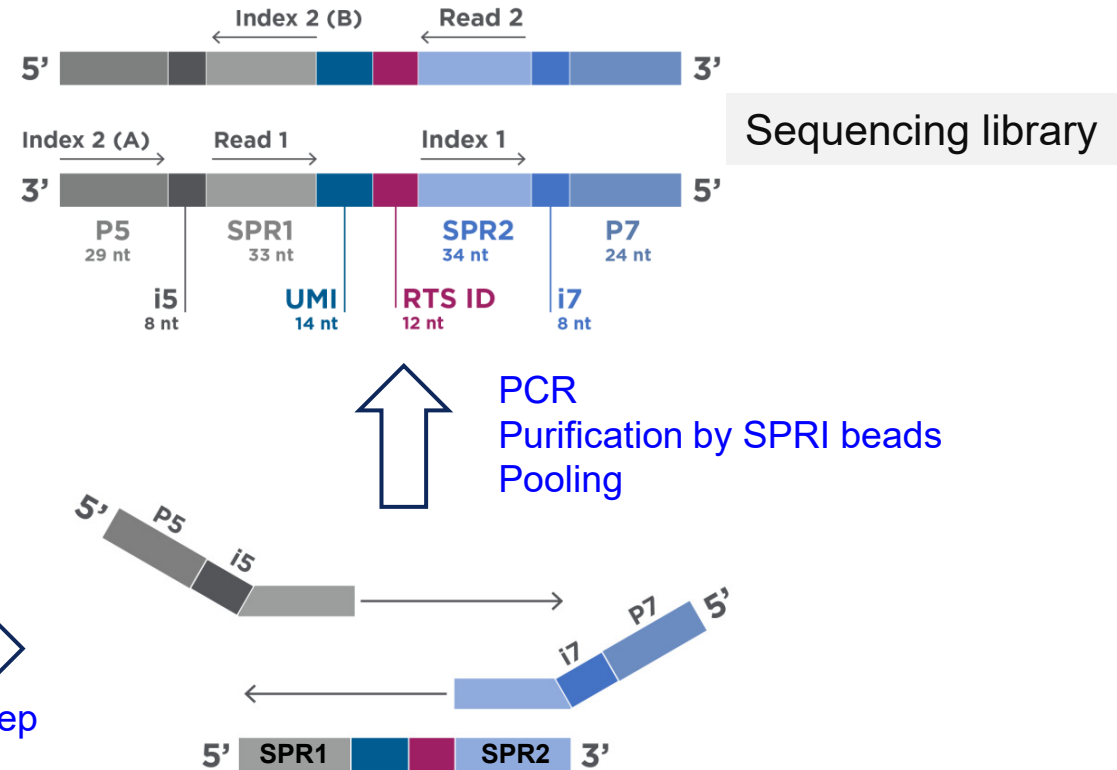
# GeoMx Digital Spatial Profiler (DSP):

## One ROI per well (96 well plate)

Oligo-labeled RNA probes



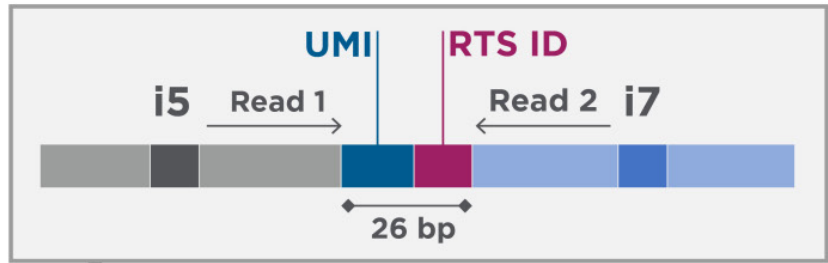
Sequencing library Prep



- Readout tag sequence identifier (RTS ID): biological targets
- Unique molecular identifier (UMI): identify PCR artifacts
- SPR: sequencing primers

- i5/i7: uniquely identifies ROI
- P5/P7: binding to Illumina flowcell
- P5/P7: PCR primers

# GeoMx NGS Pipeline

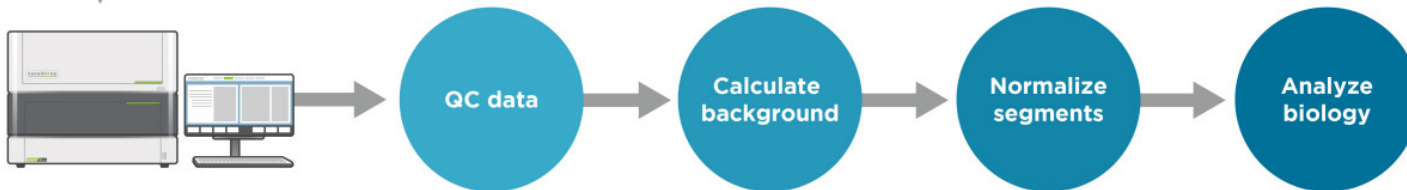


- A gene count table per ROI
- Compare ROIs of the same sample: e.g., Tumor adjacent to immune cells vs. others
- Compare similar ROIs between samples

## AUTOMATED DATA PROCESSING PIPELINE



## INTERACTIVE DATA ANALYSIS AND VISUALIZATION

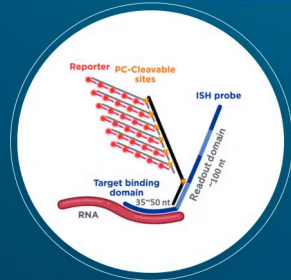
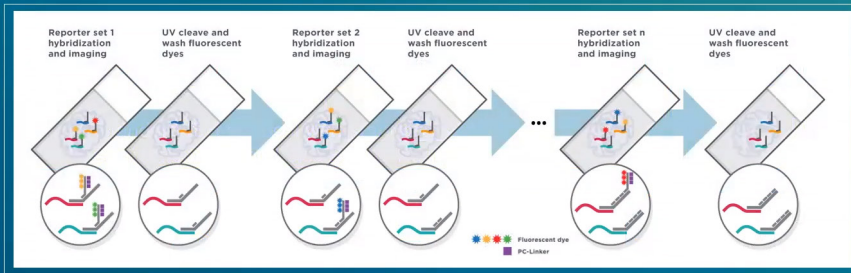


# GeoMx Digital Spatial Profiler Summary

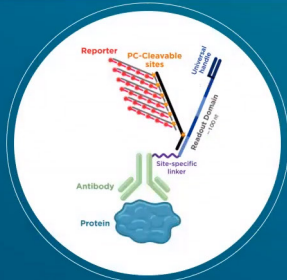
- Pseudo-spatial: via selection of ROIs
- Resolution: 10 microns
  - ✓ cell size: 10-20 microns in diameter
  - ✓ not conducive to single-cell analysis: need at least 20-300 cells per ROI for reliable quantification of transcripts
- Throughput: full transcriptome, plus more targeted panels
  - ✓ cancer transcriptome atlas (>1800 RNAs)
  - ✓ TCR profiling add-on

# CosMx Spatial Molecular Imaging (SMI): True Single Cell Resolution, True Spatial

## CosMx™ Uses Single Molecule In Situ Hybridization Chemistry with Cyclic Imaging



The Smallest Readout Domain Uniquely Enables Both FFPE and Multiomics

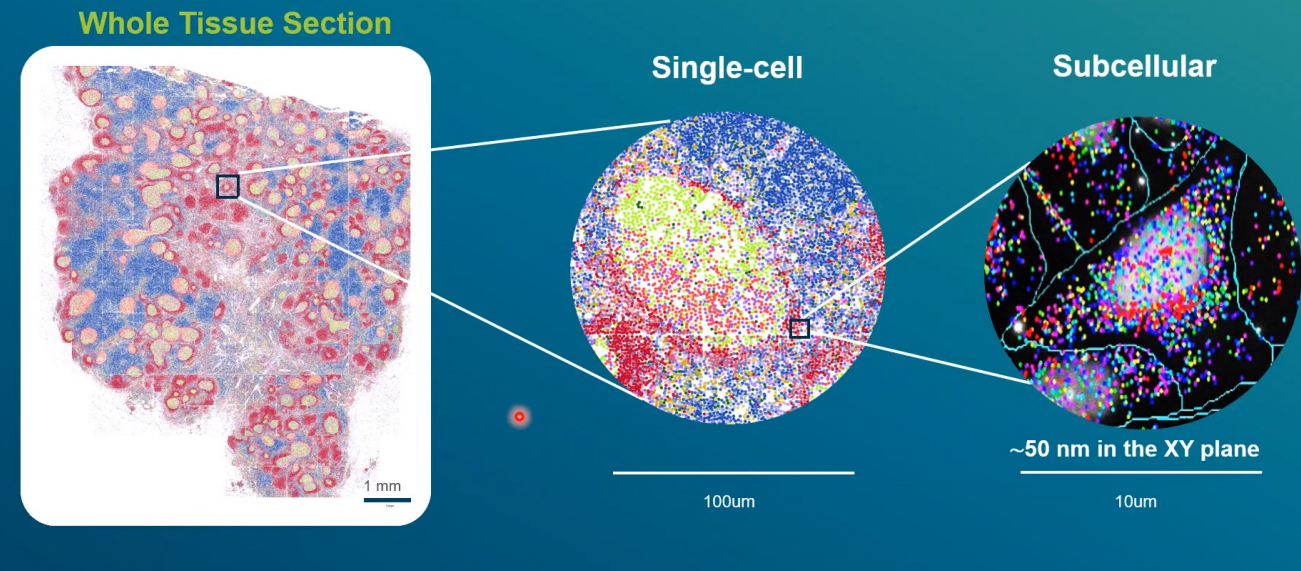


nanoString  
UF

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

CosMx  
Spatial Molecular Imager

## Highest-Plex Spatial Analysis with Subcellular Resolution



**In Situ Chemistry: protein, RNA**

**Subcellular Resolution**

# GeoMx vs. CosMx

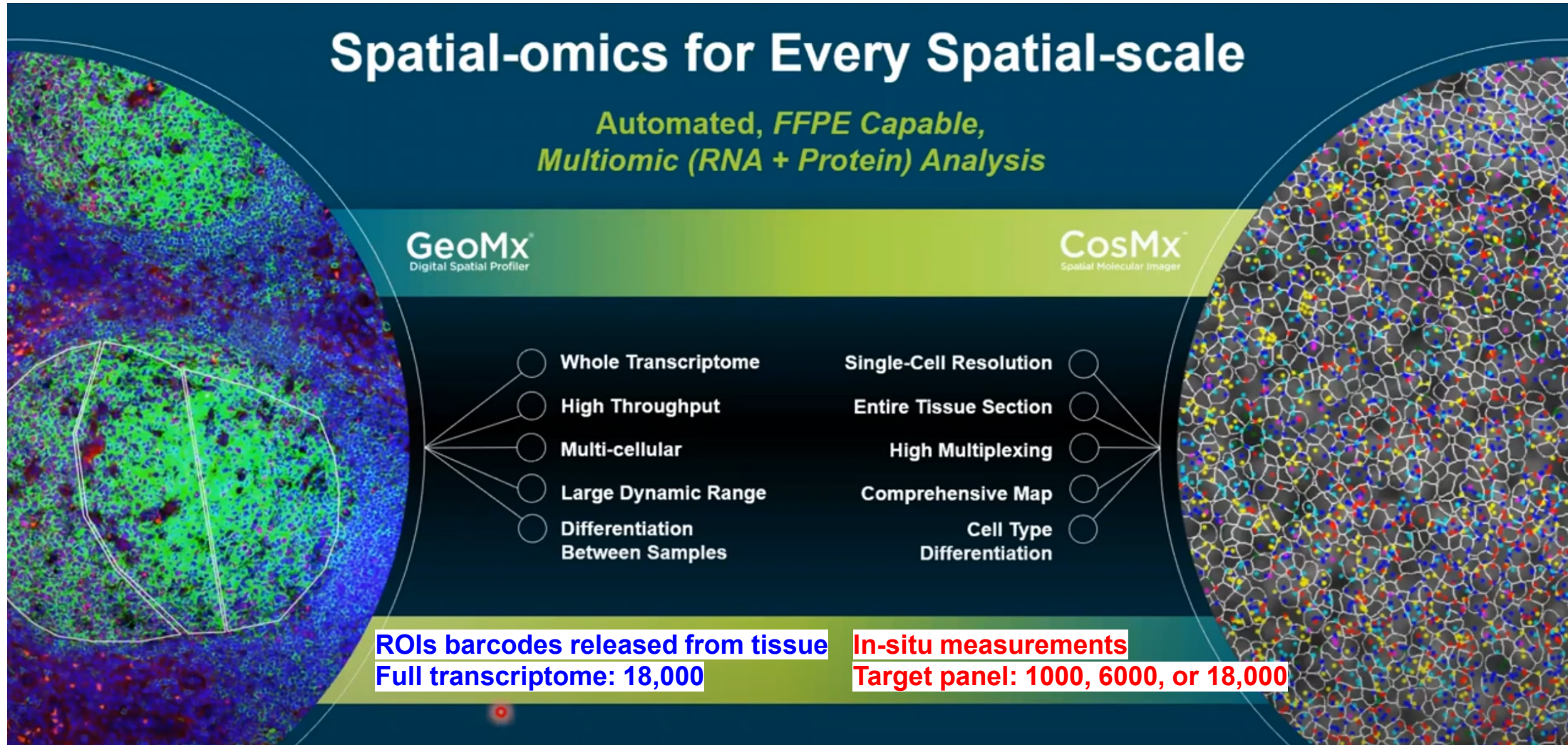
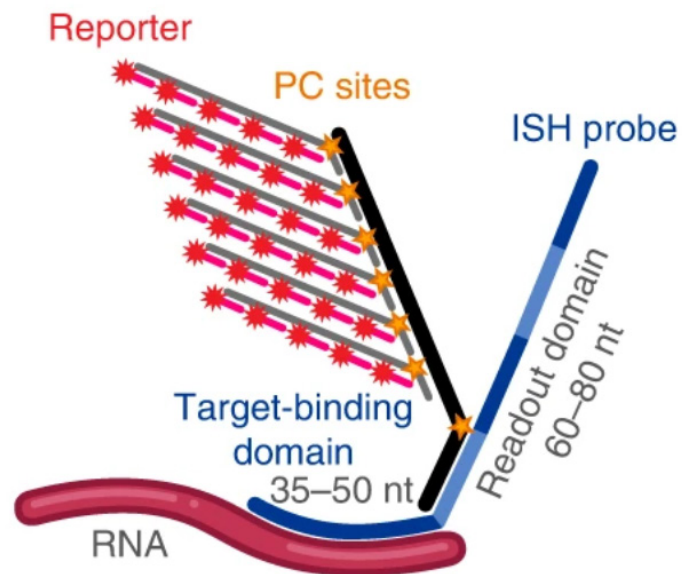
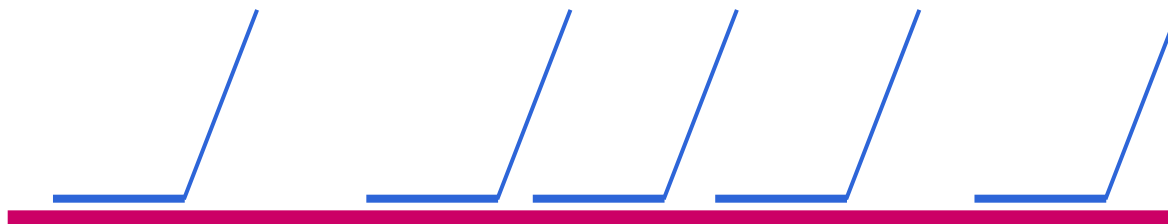


Figure from Nanostring

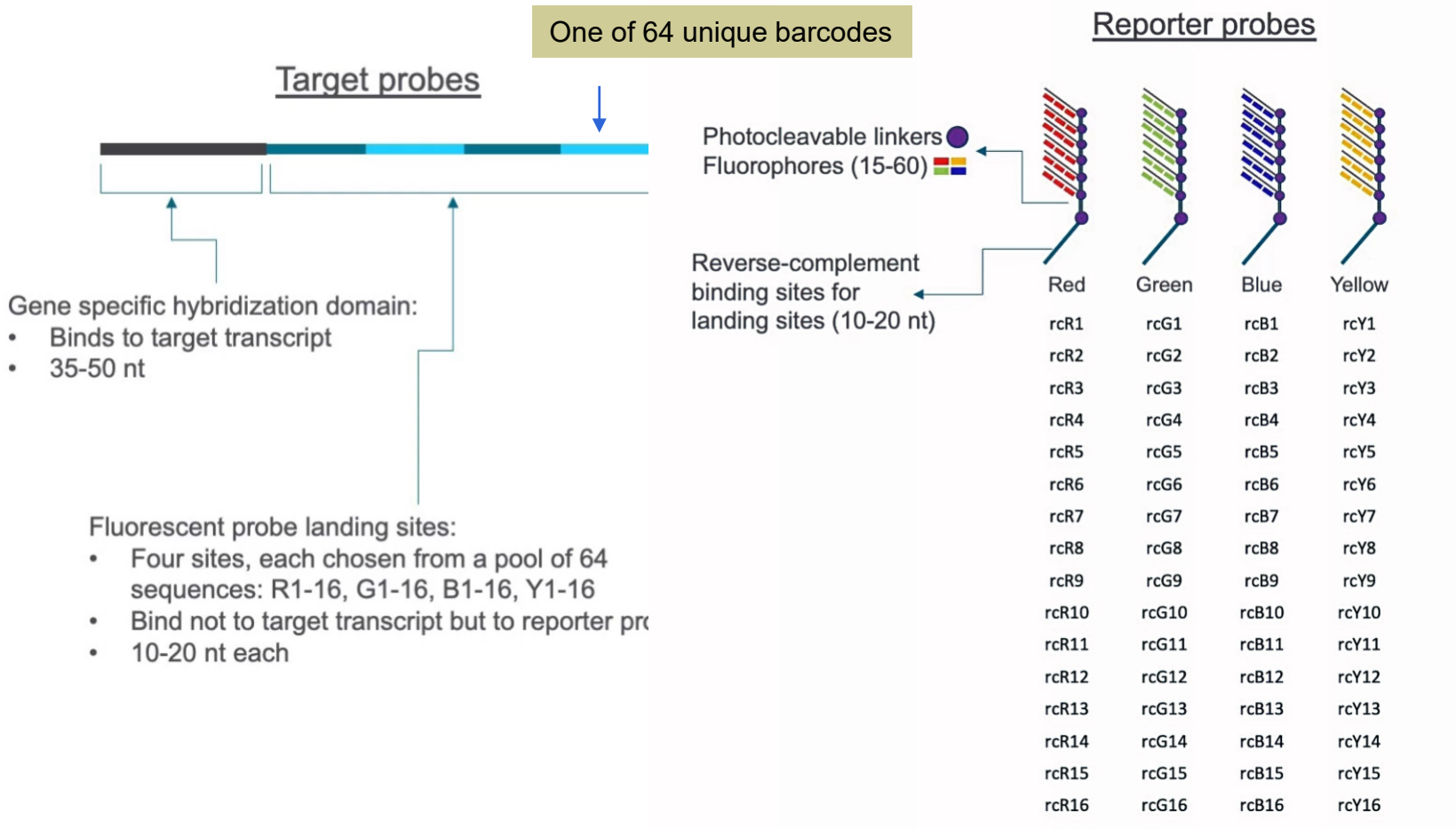
# CosMx SMI chemistry and workflow



- **in situ hybridization (ISH) probes**
  - ✓ Target binding domain (35-50 nt)
  - ✓ Read out domain (60-80 nt)
  - ✓ 4 consecutive 10-20 nt sequences that are individual landing sites of 4 reporter probes
- **fluorescent readout probes called reporters**
  - ✓ photocleavable sites to remove reporters (★)
  - ✓ The reporter landing domain still occupied after PC
- **detect RNAs in intact tissue**
  - ✓ 5 ISH probes are designed per gene to detect different regions of the RNA target,

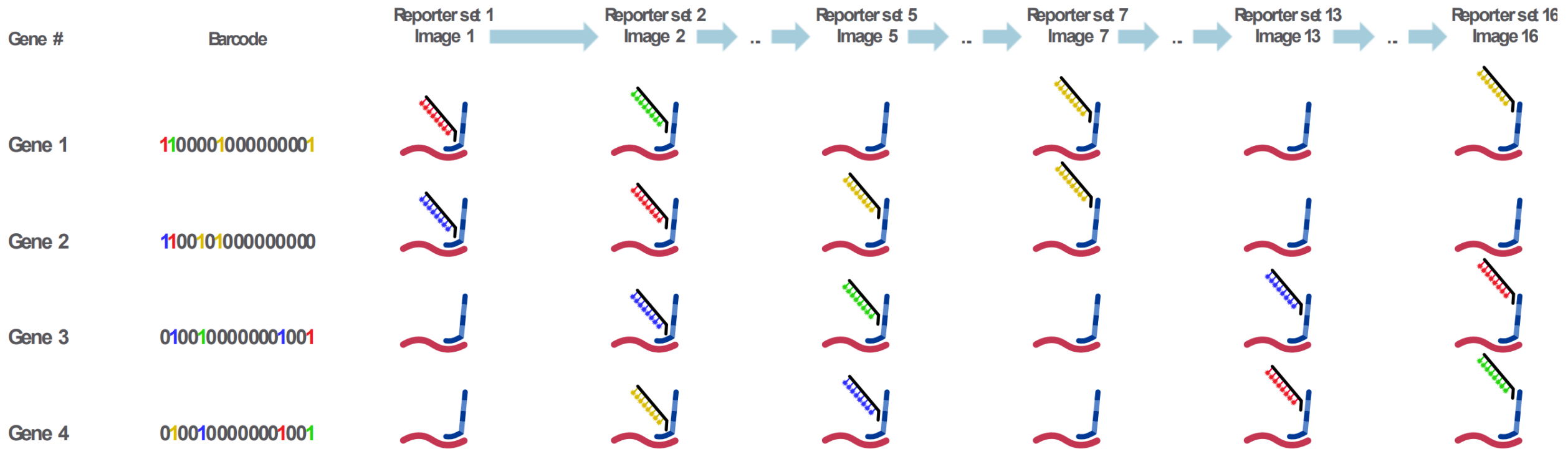


# CosMx SMI chemistry and workflow



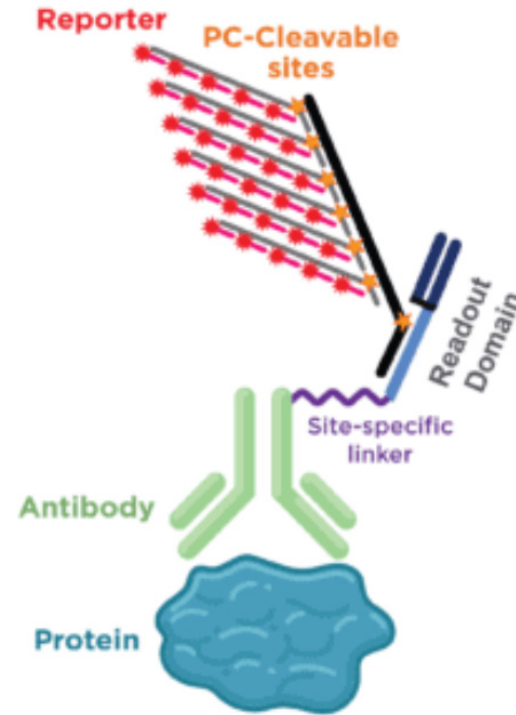
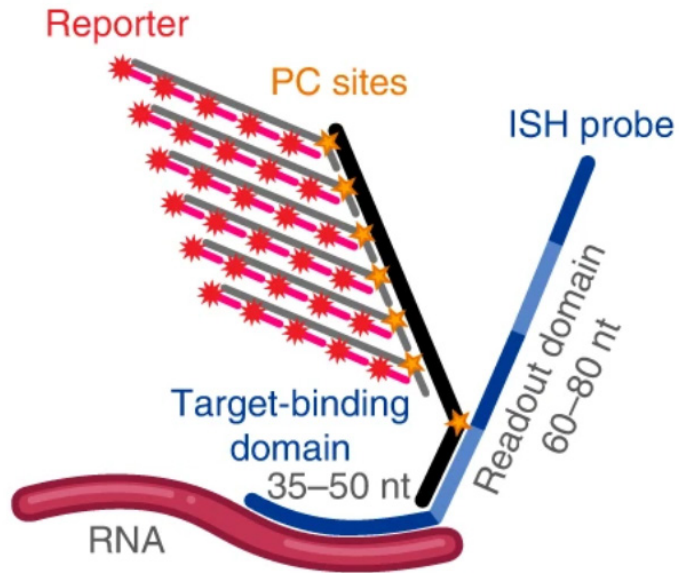
[Watch Nanostring University Video](#)

# CosMx SMI chemistry and workflow



- Every gene has a 16-digit binary barcode (with four 1s, and 12 0s)
- with Hamming distance 4 (HD4): every barcode is separated by an HD of at least **four** from all other barcodes to maximally suppress RNA decoding error.
- Images will localize a gene to a subcellular location: one “lit-up” location is one copy of the transcript.

# CosMx SMI chemistry and workflow: RNA vs. Protein



## Throughput

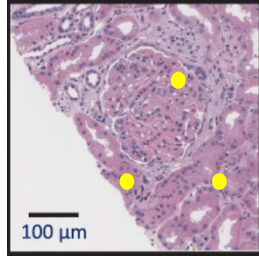
- 64, 68, or 72 proteins

## Protein Barcode Chemistry

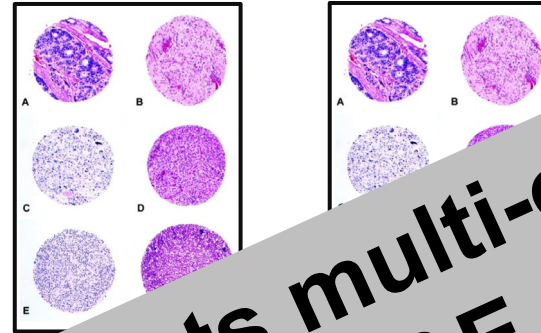
- Each antibody has a specific linker with a readout domain
- Protein chemistry is similar, but with a single readout domain for a single-color reporter to quantify a protein target

# CosMx SMI: Lab Workflow

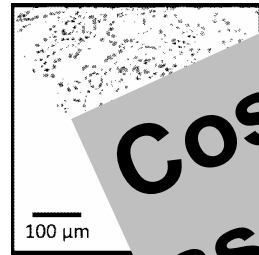
May or may not be Full-Slide



**FOV (Fields of View)**  
Selection (10s – 100s)



**H&E**



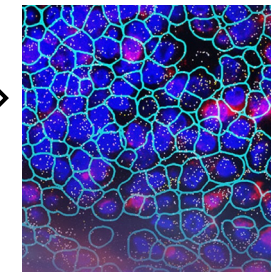
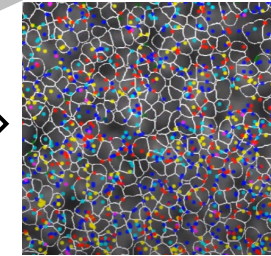
**Unstained**

**CosMx currently supports multi-omics assays: RNA, protein, and H&E can be done on the same tissue section.**

RNA Assay

**Panels:**

- Human 1K/6K Discovery RNA
- Human Immuno-Oncology
- Mouse Neuroscience
- Custom Assays ...
- Whole Transcriptome (~18K genes)



**Measurements:**

- Segmentation
- Cell typing
- RNA expression
- RNA cellular coordinates
- Niche or Neighborhood
- Cell/FOV level data

**Data Size:**

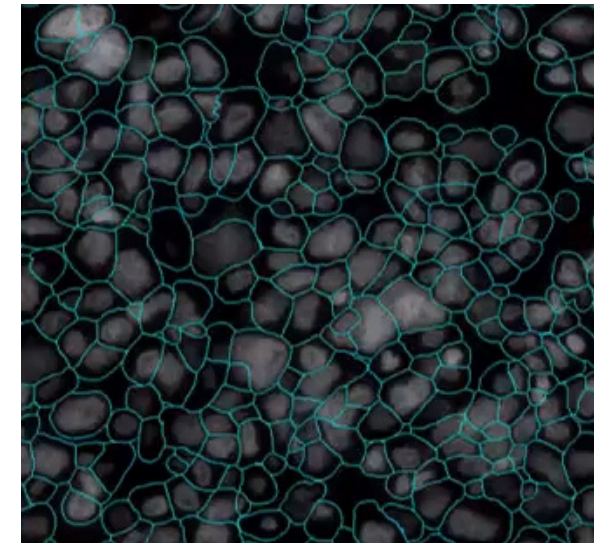
per FOV: ~2GB (10-300  
FOV per project)



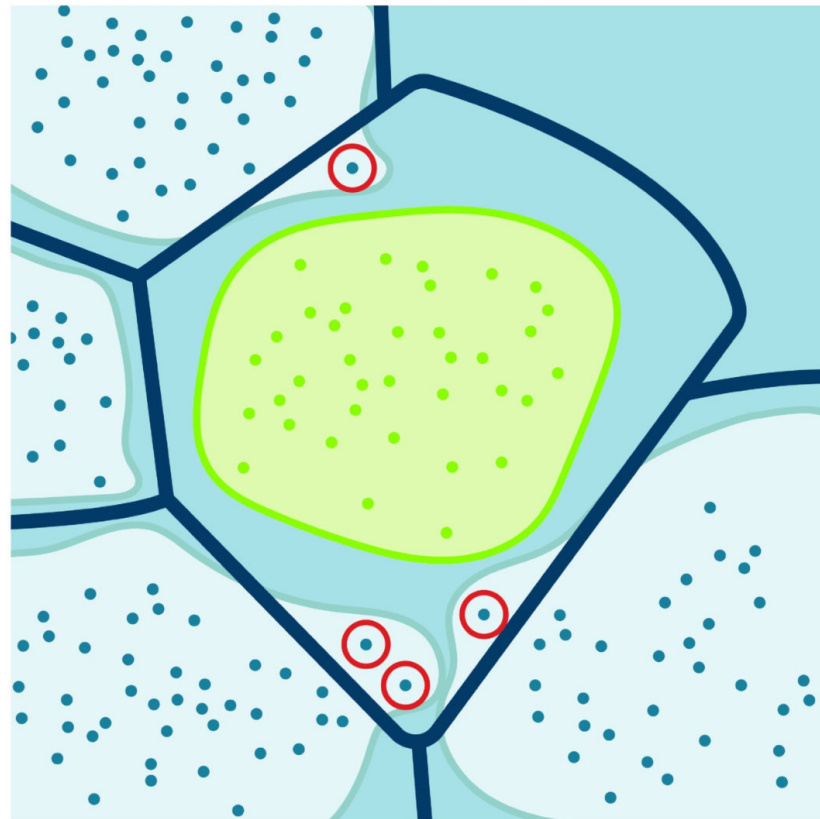
# Single cells identified by segmentation

## Accurate cell segmentation is challenging:

- [Heterogeneous Shapes](#): nearly impossible to define mathematical shape models.
- [Variation in Size and Shape](#): Unlike nuclei, the cytoplasm exhibits significant variations in shape and size.
- [Weak Boundary Gradients](#): Cells that are in close proximity can have weak boundary gradients
- [Makeshift Nature of Segmentation Approaches](#): dataset constraints, including differences in staining or imaging modality, artifacts in image capture, or morphological differences.



# Single cells identified by segmentation



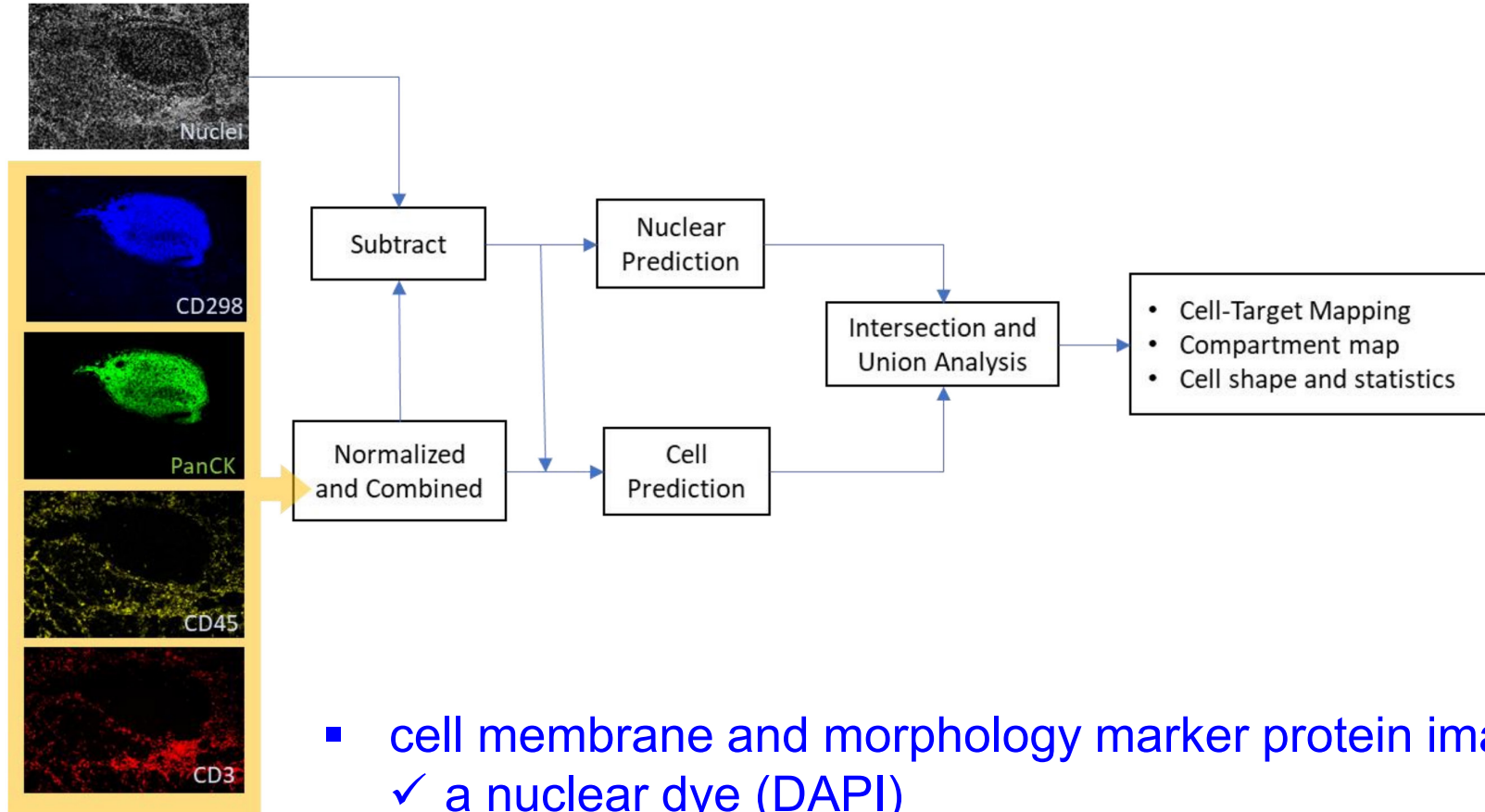
- Tumor mRNA
- T-cell mRNA
- Segmentation boundaries

○ Tumor mRNA falsely attributed to T-cell after mild segmentation errors.

Precise cell segmentation is the most important parameter when determining data accuracy. An imager's ability to identify accurate cell boundaries to minimize segmentation errors provides the confidence to draw biologically impactful conclusions from your spatial data.

Consequence of minor segmentation error

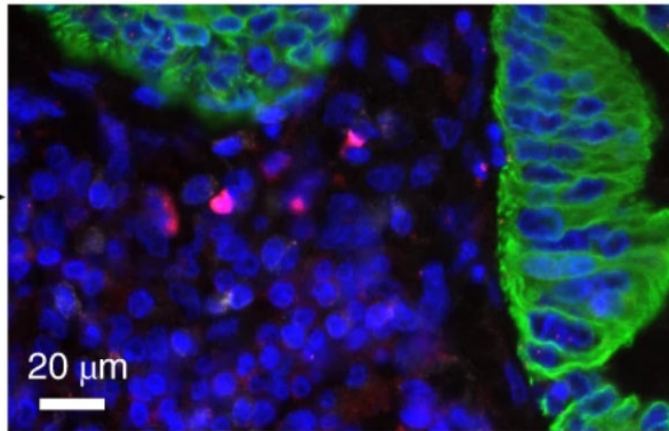
# Single cells identified by segmentation



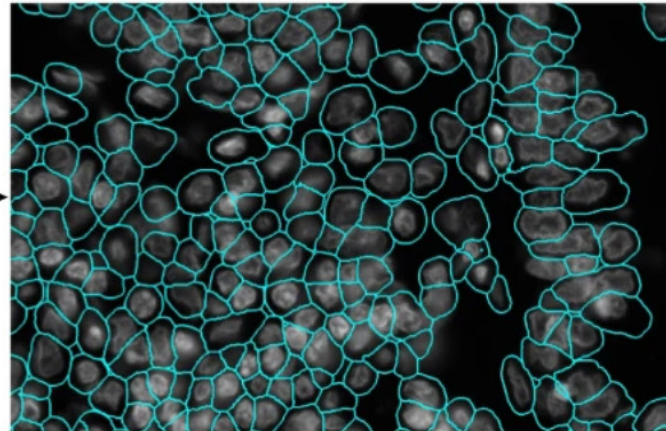
- cell membrane and morphology marker protein images
  - ✓ a nuclear dye (DAPI)
  - ✓ Protein markers: Membrane (CD298), epithelial cells (PanCK), and T cells (CD3), ...
- machine-learning augmented cell segmentation (Cellpose neural network models)
- transcript-based segmentation refinement

# Single cells identified by segmentation

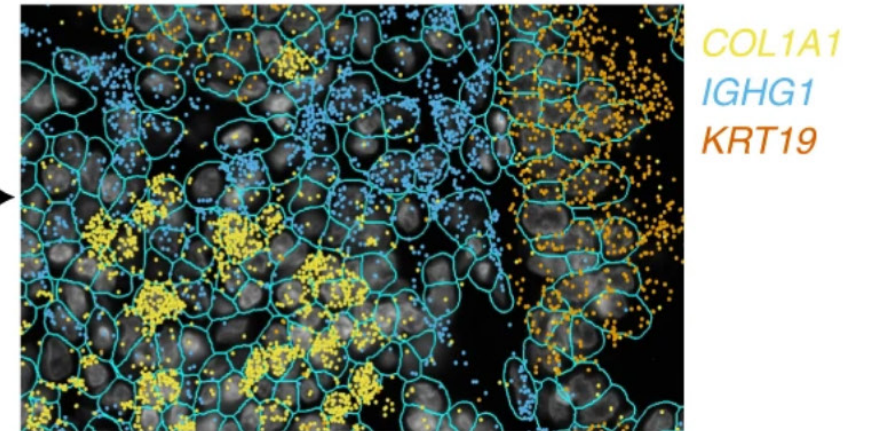
Morphology



Segmentation



Cell assignment



Most genes/transcripts  
have a cell assignment

# Data

- A table per FOV: genes, counts, cell ID, annotation
- A Seurat object: similar to that from a single-cell RNA-Seq data set (cell typing, U-map, differential expression, etc.)
- Nanostring AtoMx Pipeline Results: inspection of images, QC etc.

# CosMx Spatial Omics: an example in NSCLC

Category	Total*	Lung 5_rep 3	Lung 5_rep 4	Lung 5_rep 5	Lung 6	Lung 9_rep 1	Lung 9_rep 2	Lung 12	Lung 13
Tissue type	FFPE human lung	FFPE human lung	FFPE human lung	FFPE human lung	FFPE human lung	FFPE human lung	FFPE human lung	FFPE human lung	FFPE human lung
Panel	980 plex	980 plex	980 plex	980 plex	980 plex	980 plex	980 plex	980 plex	980 plex
Number of slides analyzed	8	1	1	1	1	1	1	1	1
Total tissue area analyzed ( $\mu\text{m}^3$ )	753,480,217	97,014,620	97,014,620	97,014,620	97,014,620	64,676,413	145,521,930	90,546,979	64,676,413
Number of Field of Views (FOVs)	233	30	30	30	30	20	45	28	20
Total number of cells	800,327	100,292	106,660	100,264	93,795	91,972	150,504	73,997	82,843
% Cells passed QC ( $\geq 20$ transcripts)	96.1	94.7	99.3	97.6	96.2	95.3	92.8	96.6	98.1
Number of cells analyzed	769,114	94,977	105,903	97,898	90,193	87,677	139,713	71,489	81,286
Transcripts detected	262,649,897	36,505,900	42,342,772	31,583,902	35,952,059	26,404,493	33,597,576	26,074,273	30,188,922
% of transcripts assigned to cells	79.2	81.7	83.3	82.1	80.9	85.7	71.2	69.1	77.7
Cellular transcripts/ $\mu\text{m}^3$	0.446	0.523	0.59	0.456	0.423	0.456	0.28	0.393	0.547
Mean transcripts/cell	260	297	331	259	310	246	159	244	283
Mean negatives/cell/target	0.0429	0.0323	0.0611	0.0283	0.0238	0.0415	0.0412	0.076	0.0463
Genes detected	850	874	763	865	849	805	724	629	736
% Genes detected	88.5	91.0	79.5	90.1	88.4	83.9	75.4	65.5	76.7
Mean false call/cell/target	0.0092	0.0058	0.0096	0.0076	0.0100	0.0105	0.0083	0.0090	0.0144

## Data Size:

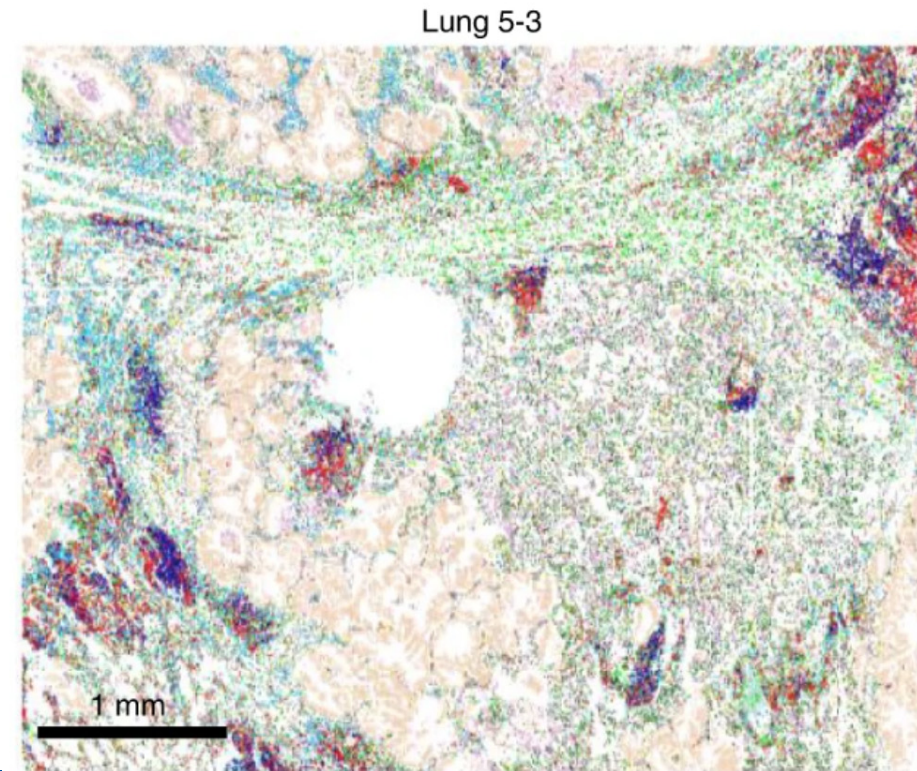
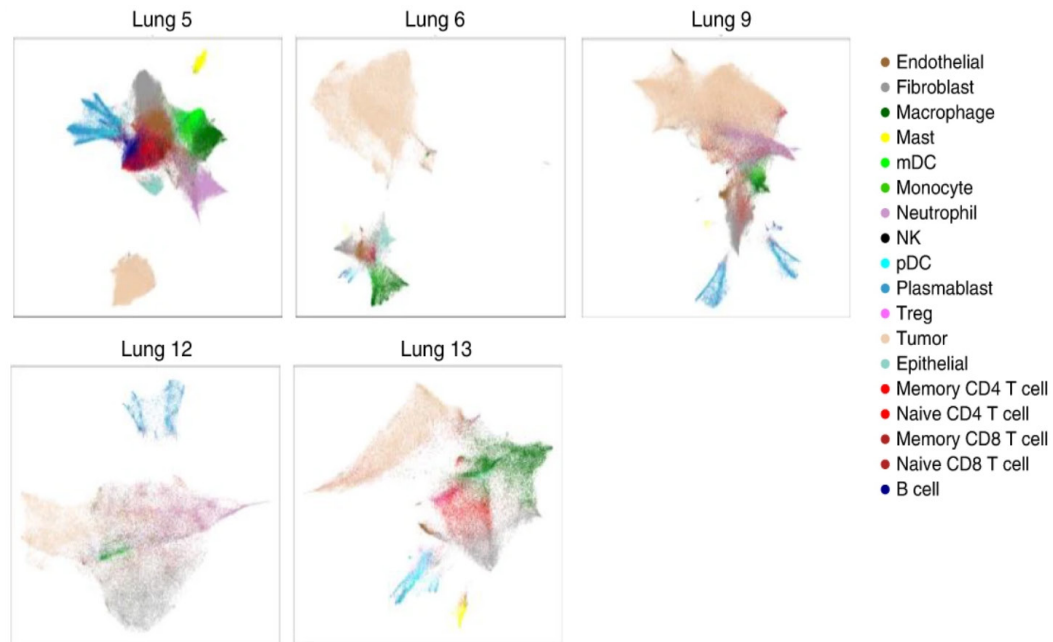
per FOV: ~2GB (233 FOVs: 466 GB)

5 patients, 8 samples

He S, et al. Nat Biotechnology. 2022, 40(12):1794-1806.

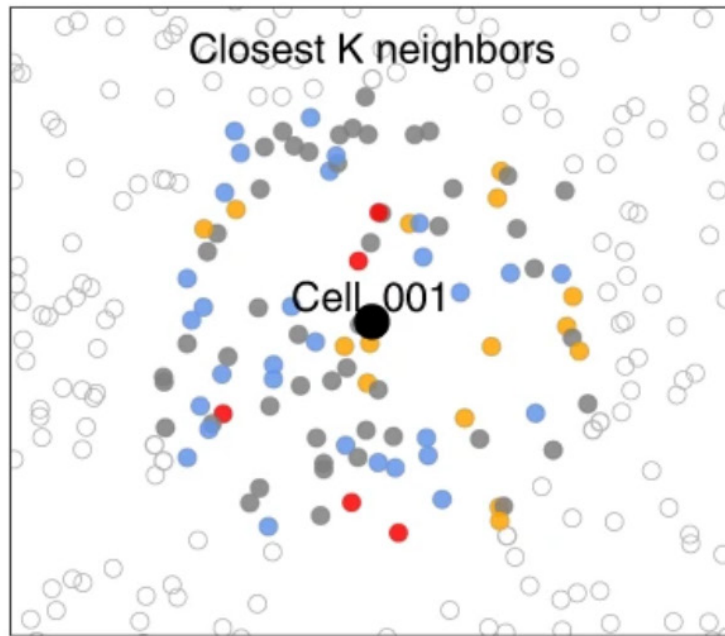
# Cell Typing:

- Similar to scRNA-Seq's cell typing: using cell type-specific markers to define the cell type of any cell
- Challenges: 1000 RNA targets only, may not be sufficient for cell typing of all cells
  - ✓ 1000 RNA targets were partially optimized for cell typing
  - ✓ *de novo* clustering without assigning cell types?
- Cell typing + **spatial information**



B/T Cells  
co-localization

# cell neighborhoods: from spatial information



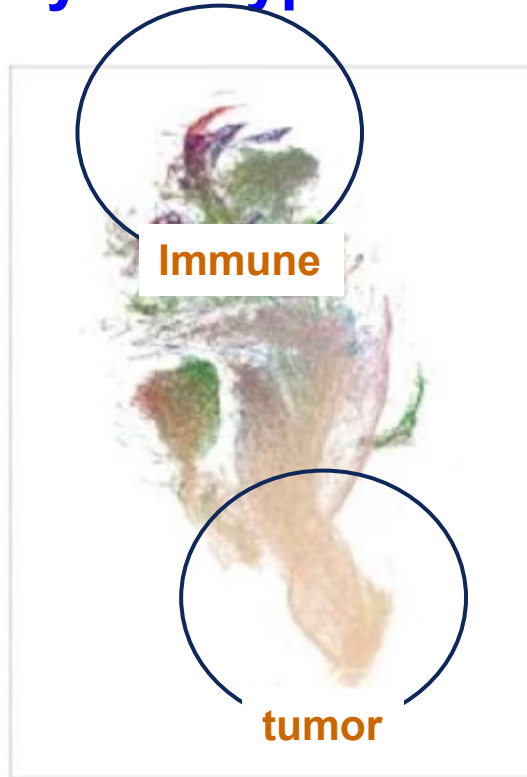
Neighborhood matrix:  
number in closest K neighbors

	●	●	●	●
Cell 001	5	15	30	50
Cell 002	...	...	...	...

For **every cell**, the nearest K neighbors are identified, and a summary of those neighbors is recorded (e.g., abundance of each cell type) → matrix of cells and **neighborhood characteristics**

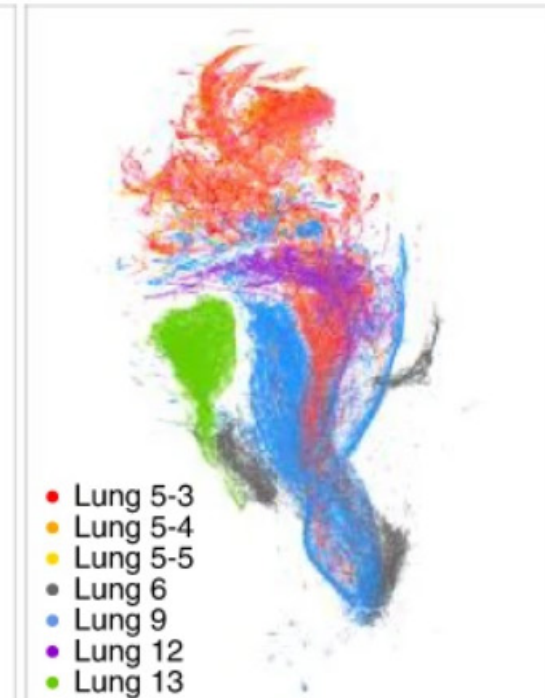
# UMAP illustration of the neighborhood matrix: of all cells, all FOVs, across all samples (769,114 cells)

by cell type



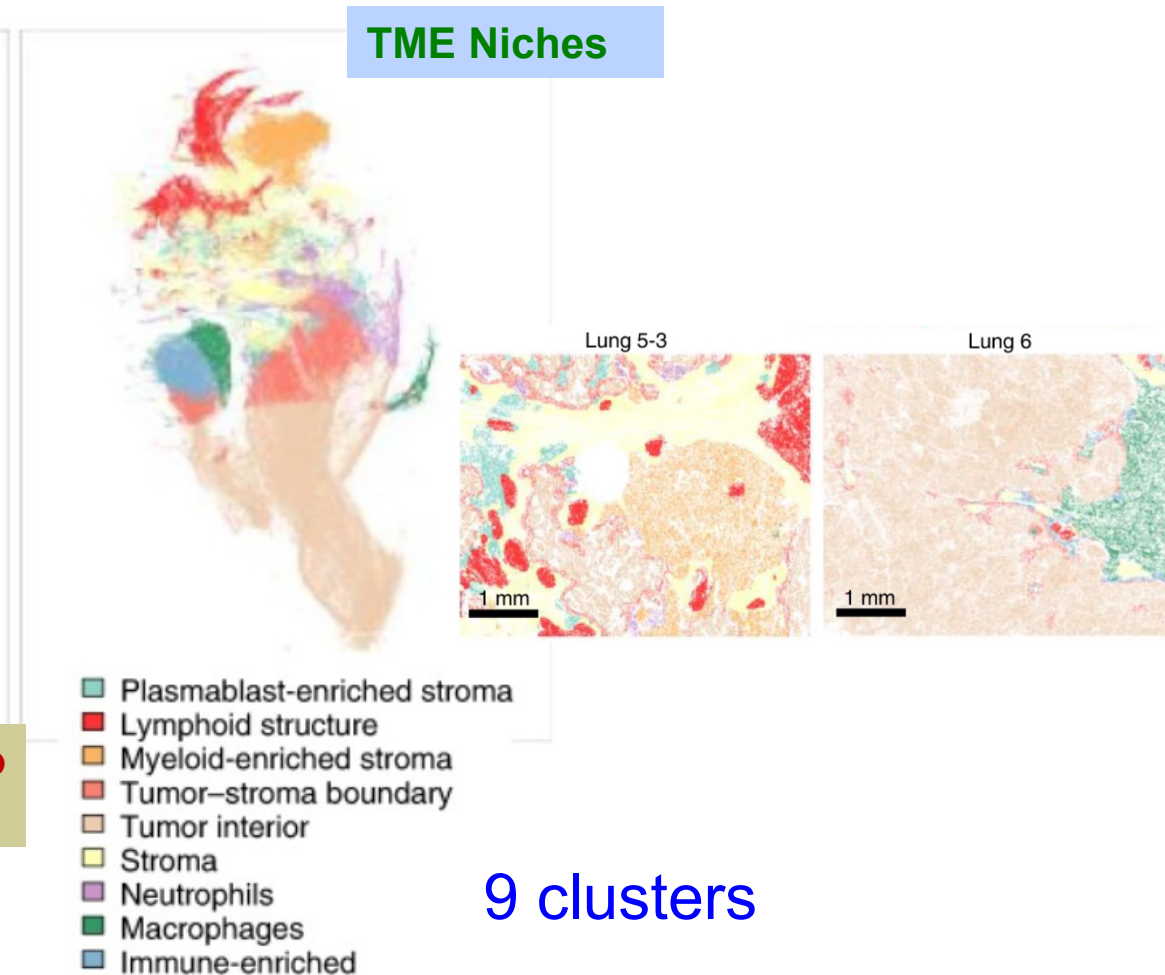
- Endothelial
- Fibroblast
- Macrophage
- Mast
- mDC
- Monocyte
- Neutrophil
- NK
- pDC
- Plasmablast
- Treg
- Tumor
- Epithelial
- Memory CD4 T cell
- Naive CD4 T cell
- Memory CD8 T cell
- Naive CD8 T cell
- B cell

by sample

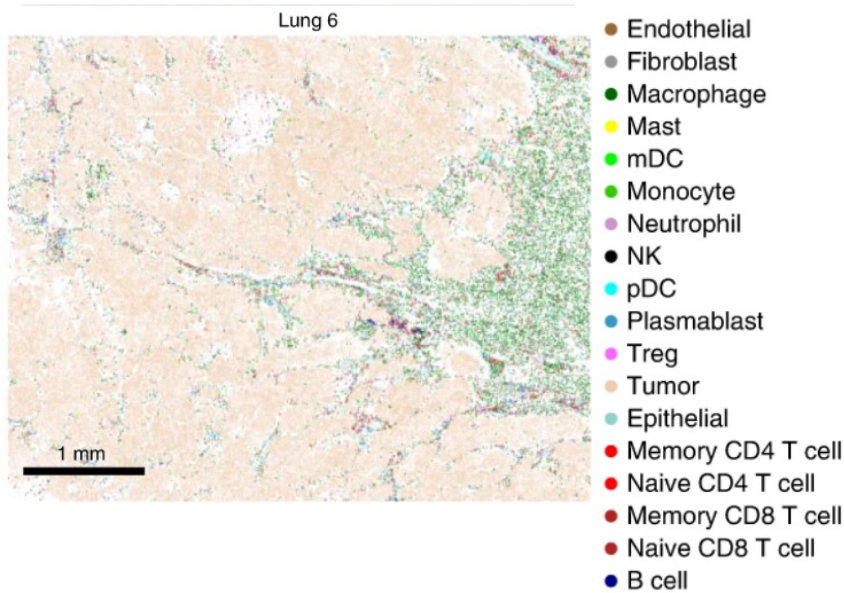


**Neighborhoods unique to or shared by samples**

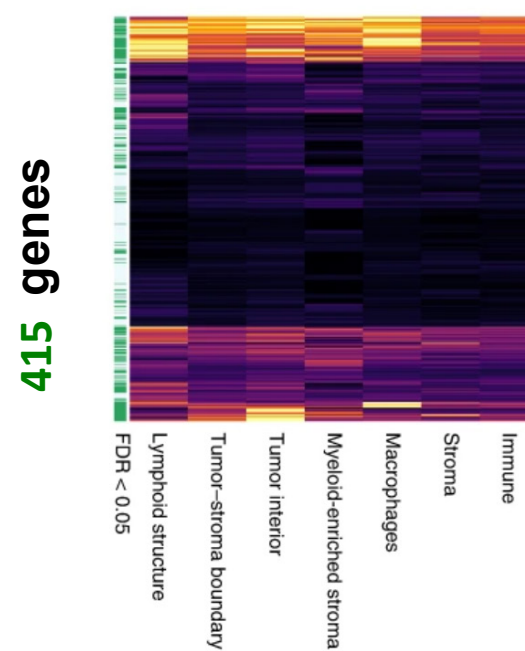
by neighborhood clusters



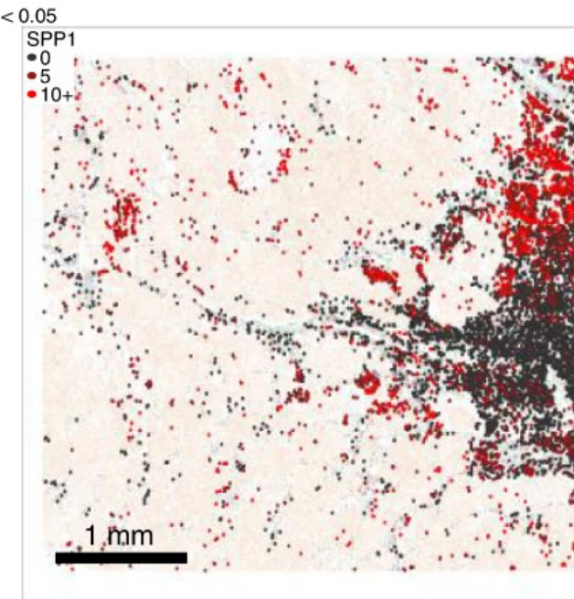
# Biological questions that can be answered by neighborhoods or niches



Mean gene expression of macrophages in each niche



*SPP1* ( $p = 5 \times 10^{-61}$ ): two sub-types of macrophages

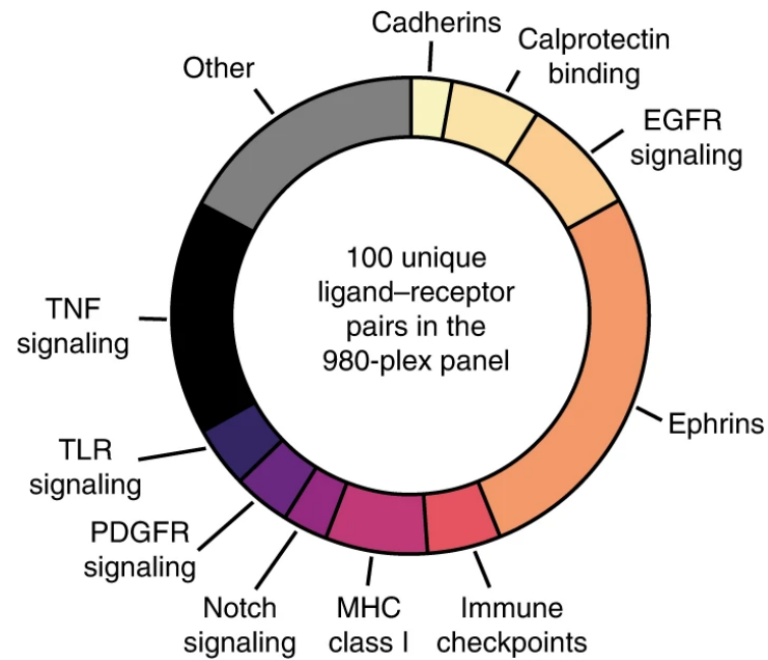


**SPP1**: mediates macrophage polarization and upregulate PD-L1 expression

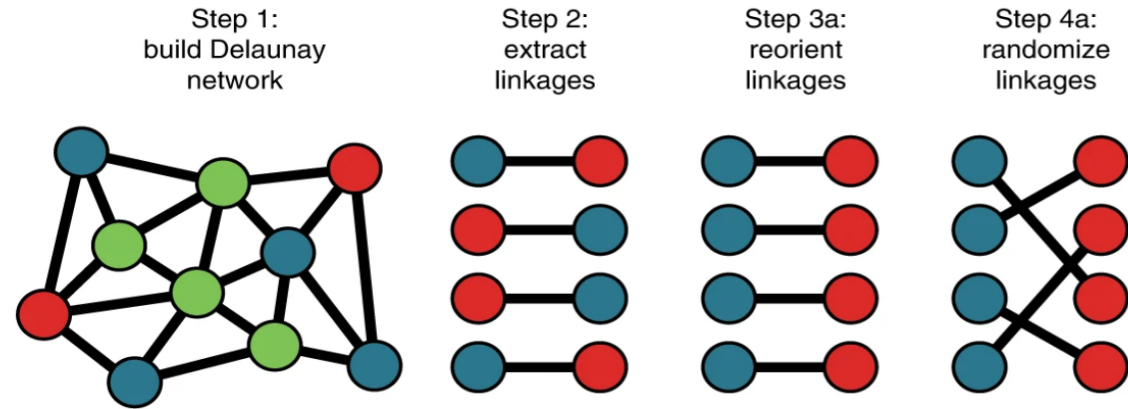
Changes of gene expression in macrophages between niches in Lung 6

Does a cell change expression profile in response to neighbors?

# Interactions between Tumor and T-cells



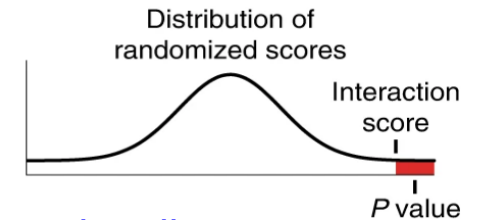
Distribution of ligand-receptor pairs between 980 RNA targets: including many tumor-immune interface pairs



Steps 3b and 4b: calculate score across linkages

$$\text{LR interaction score} = \frac{\sum \sqrt{L_i R_i}}{n_{\text{linkages}}}$$

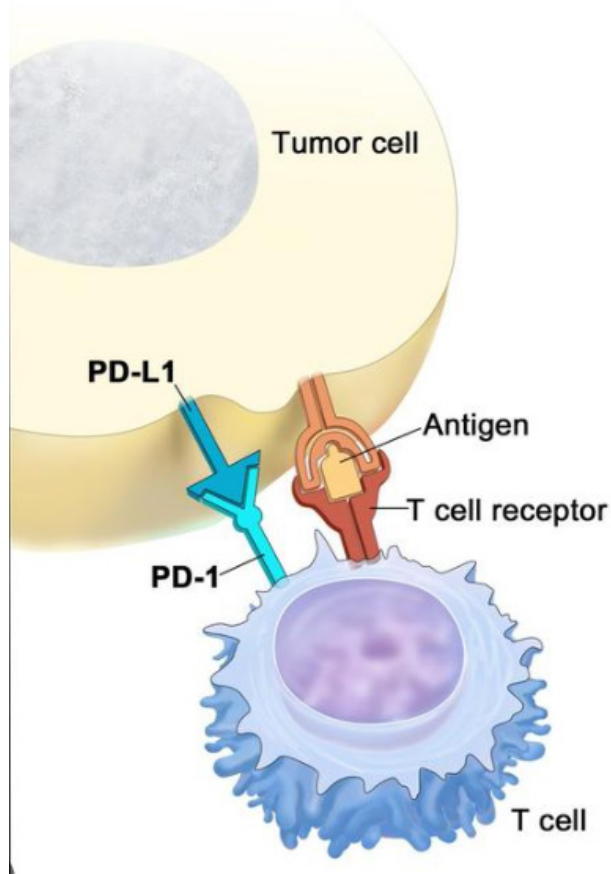
Step 5: calculate  $P$  value



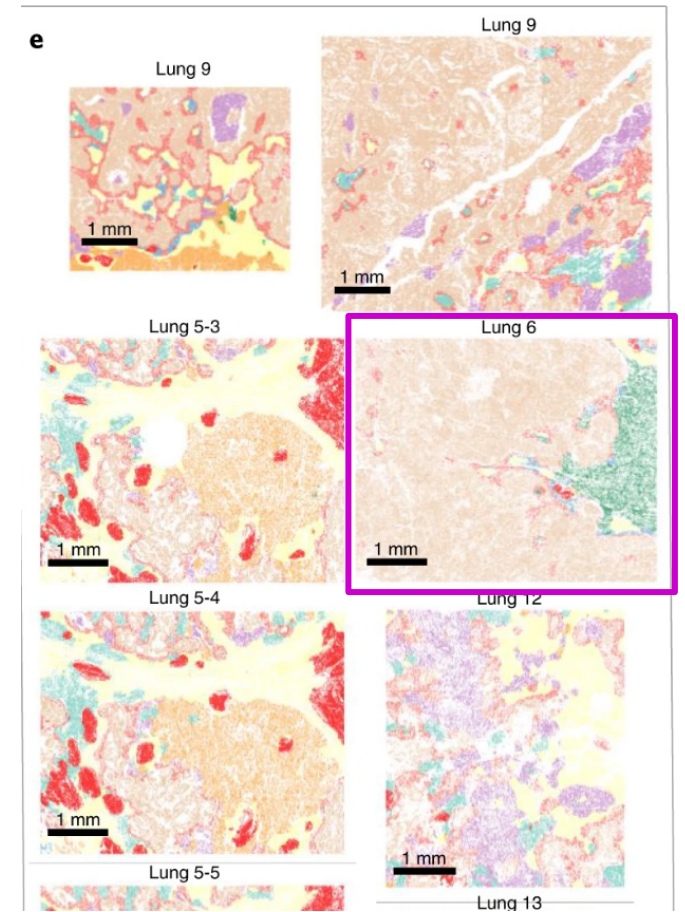
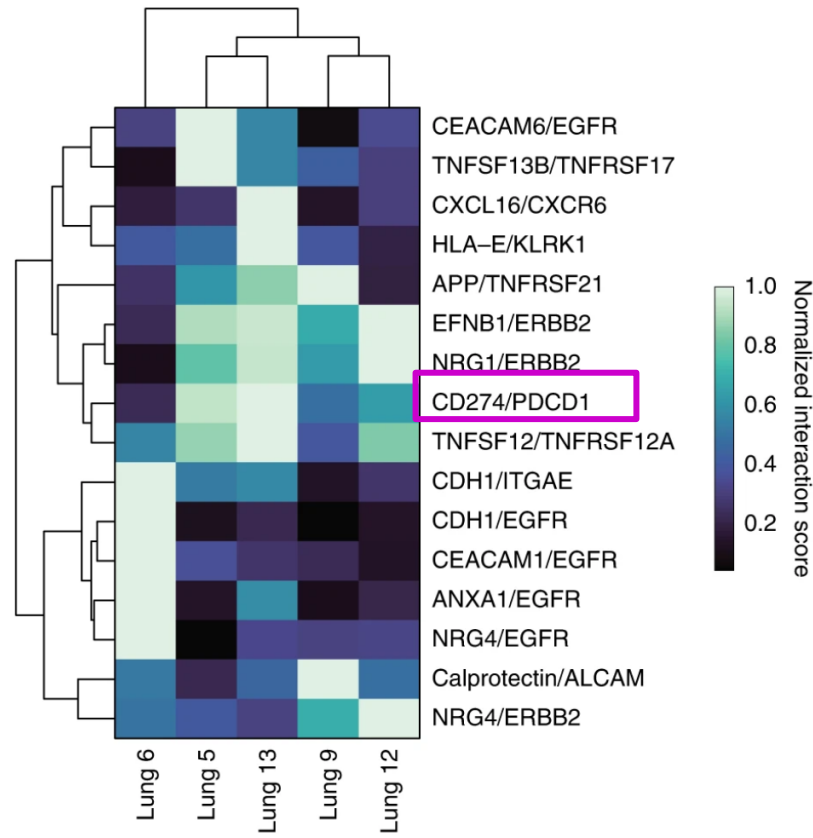
- a spatial **adjacency** network, given the spatial locations of each cell.
- For each pair of adjacent tumor -T cells: **an interaction score** using the geometric mean of their ligand and receptor expression.
- LR interaction score was calculated across all adjacent cell pairs for 100 distinct LR pairs
- An average score was calculated for each LR pair.
- Each average score was tested to determine whether it was enriched by the spatial arrangement of cells within the adjacency matrix, compared to a null distribution of simulated average scores calculated using randomized adjacency networks.

# Interactions between Tumor and T-cells

PD-L1 binds to PD-1 and inhibits T cell killing of tumor cell



PD-L1/PD-1 (CD274/PDCD1): Low interaction in Lung 6, higher in Lungs 5, 13, 9, 12



LR interactions change across space and between samples

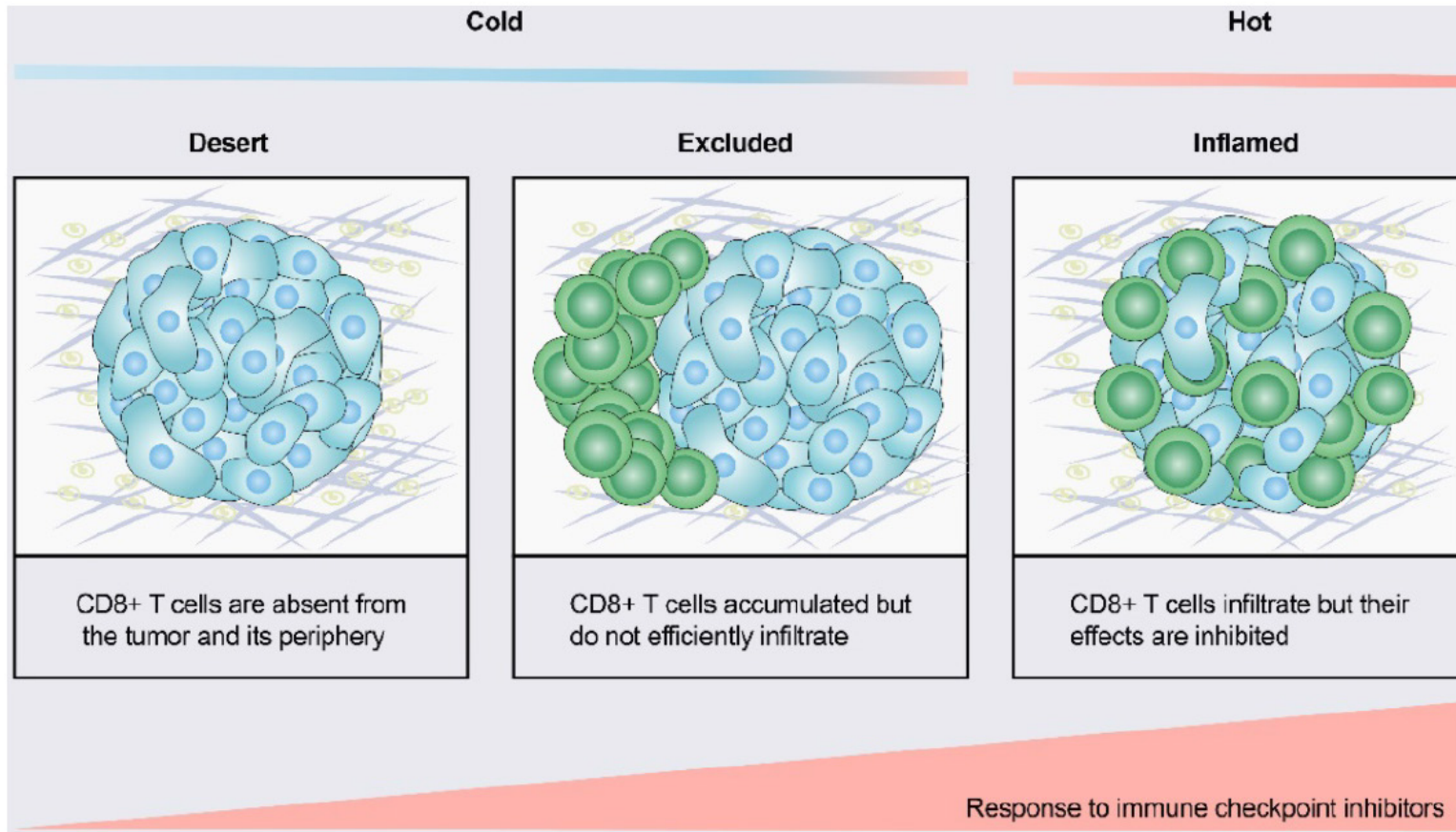
# CosMx SMI Summary

- Spatial platform with true single cell and sub-cellular resolution
- Biggest challenge: cell segmentation (foundation of all analyses)
- Throughput:
  - ✓ Older panels: ~1000 RNA, and 6000 RNA panels
  - ✓ Full transcriptome (~18K genes, 2<sup>nd</sup> half of 2025)

# Clinical Application of spatial transcriptomics

- Mulholland EJ, et al., *Redefining clinical practice through spatial profiling: a revolution in tissue analysis*. Ann R Coll Surg Engl. 2024;106(4):305-312.
- Zhang L, et al., *Clinical and translational values of spatial transcriptomics*. Signal Transduct Target Ther. 2022;7(1):111
- Hu W, et al. *Spatial transcriptomics in human biomedical research and clinical application*. Curr Med 2, 6 (2023).

# Clinical Application of spatial transcriptomics



## Immunotherapy

- Activate patient's own immune system
- Hot tumors are more likely to respond to immunotherapy
- e.g., lung 6 vs. others

Tumor immune phenotypes and immunotherapy outcome

# MC-SOLVE: an Interactive Dashboard for Spatial Single Cell Analyses

Mayo Clinic's Spatial Omics Landscape Visualization  
and analysis Engine

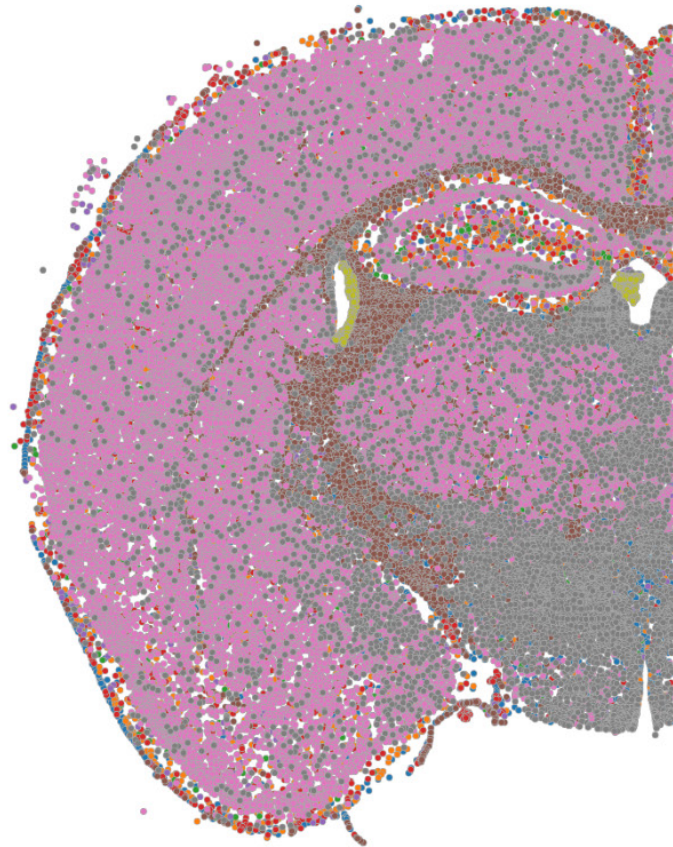
Nicholas Dove, PhD; and Yi Liu, PhD

**Motivation:** Few analytic frameworks allow users to engage directly with data by visually selecting or labeling cells and regions, a functionality **essential for refining downstream analyses with expert knowledge**. Interactive annotation is increasingly recognized as critical in spatial single-cell analysis. **Automated algorithms often struggle to define cell types or tissue regions that are readily apparent to human experts through morphology or context**. For instance, a pathologist can delineate tumor margins or anatomical sub-regions by eye and identify artifactual cells and expression signals for exclusion, which are subtleties that generic clustering or segmentation algorithms may overlook. **Incorporating domain knowledge requires the ability to visually select or exclude cells and regions during analysis.**

**“human expert in the loop”**

# Use Case: Not all Neurons are the same

Spatial Coordinates



UMAP



## Slide

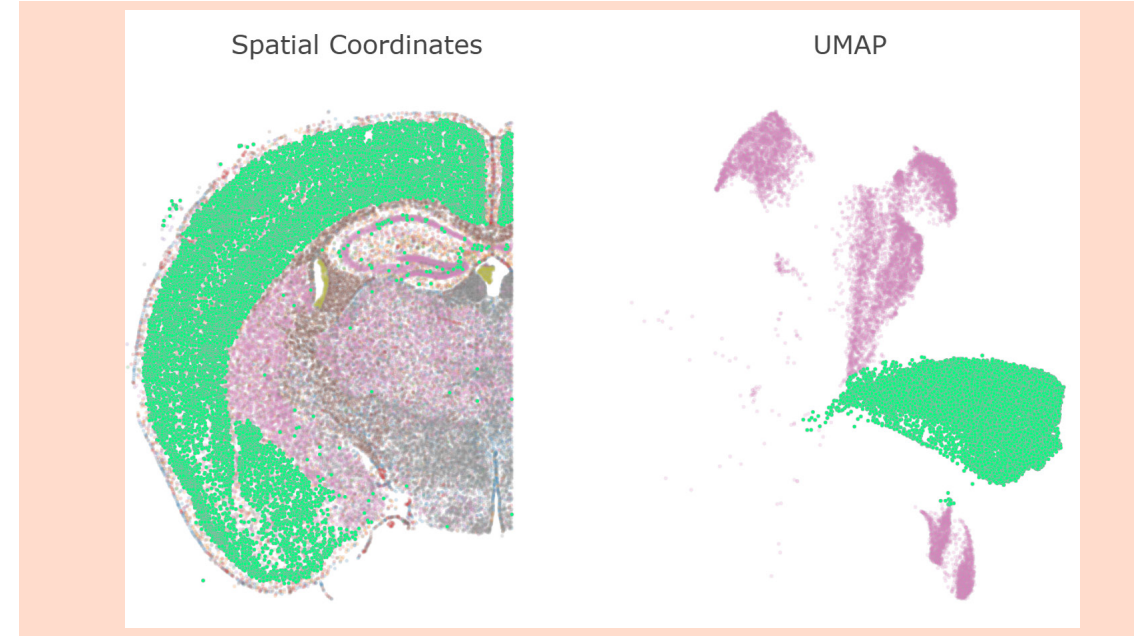
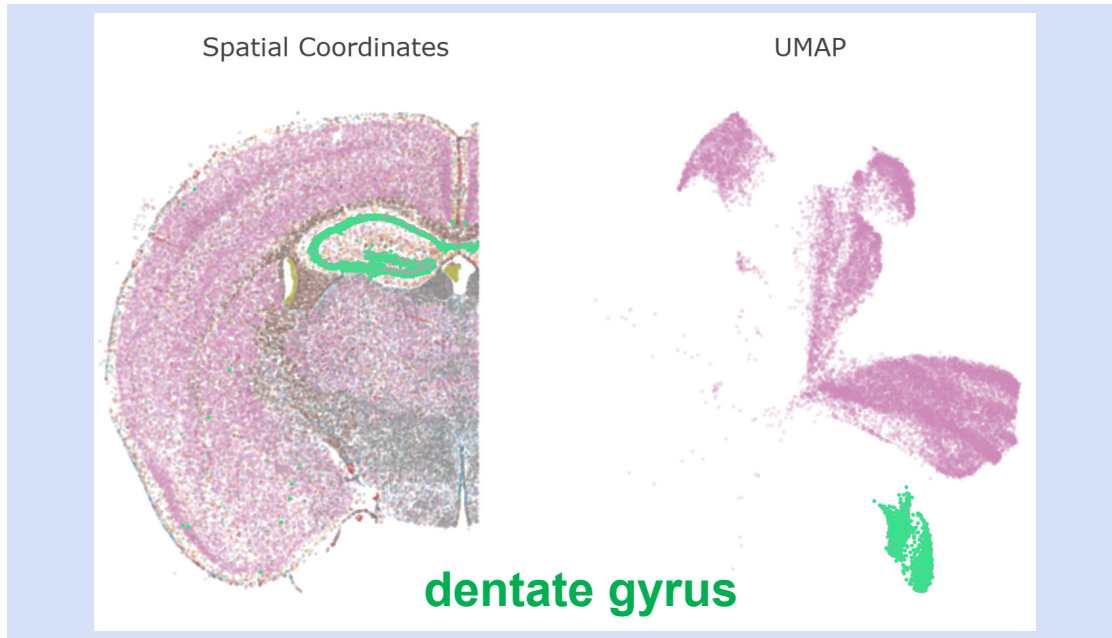
- Astrocyte.Atp1b2.high
- Astrocyte.Mfe8.high
- Macrophage.Klf2.high
- Macrophage.Lyz2.high
- Microglia
- Myelinating.oligodendrocyte
- Neuron
- Oligodendrocyte.precursor
- Schwann.cell

## UMAP

- Astrocyte.Atp1b2.high
- Astrocyte.Mfe8.high
- Macrophage.Klf2.high
- Macrophage.Lyz2.high
- Microglia
- Myelinating.oligodendrocyte
- Neuron ←
- Oligodendrocyte.precursor
- Schwann.cell

Mouse Brain: CosMx

# MC-SOLVE: Lasso Selection Tool: to highlight group of cells



## Shopping Cart and Waste Basket to select or remove cells for downstream analyses

🛒 Shopping Cart

+ Add selected points   - Remove selected rows   📄 Download shopping cart metadata   📄 Download shopping cart transcript counts   📄 Upload shopping cart metadata

---

🗑️ Waste Basket

+ Add selected points   - Remove selected rows   📄 Download waste basket metadata   📄 Download waste basket transcript counts   📄 Upload waste basket metadata

**Save annotations in metadata**  
**-- Sub-setting the “neuron” cells**

# Summary

- Operating principles, spatial resolution, and analytical outputs of GeoMx DSP and CosMx SMI for spatial transcriptomic profiling.
- Cell segmentation, cell typing, neighborhood and niche analysis, and ligand-receptor modeling, and the interpretation of spatial single-cell data in a case study using data from non-small cell lung cancer (NSCLC)
- How spatial transcriptomics can support tumor immune phenotyping and the translational assessment of immunotherapy response in clinical research.
- the importance of human expertise and domain knowledge in examining, annotating, and designing downstream analyses.

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