A Spatial Molecular Imager study of Non-Small Cell Lung Cancer FFPE samples; Largest Spatially Resolved High-Plex RNA Data Release Ever in Cancer

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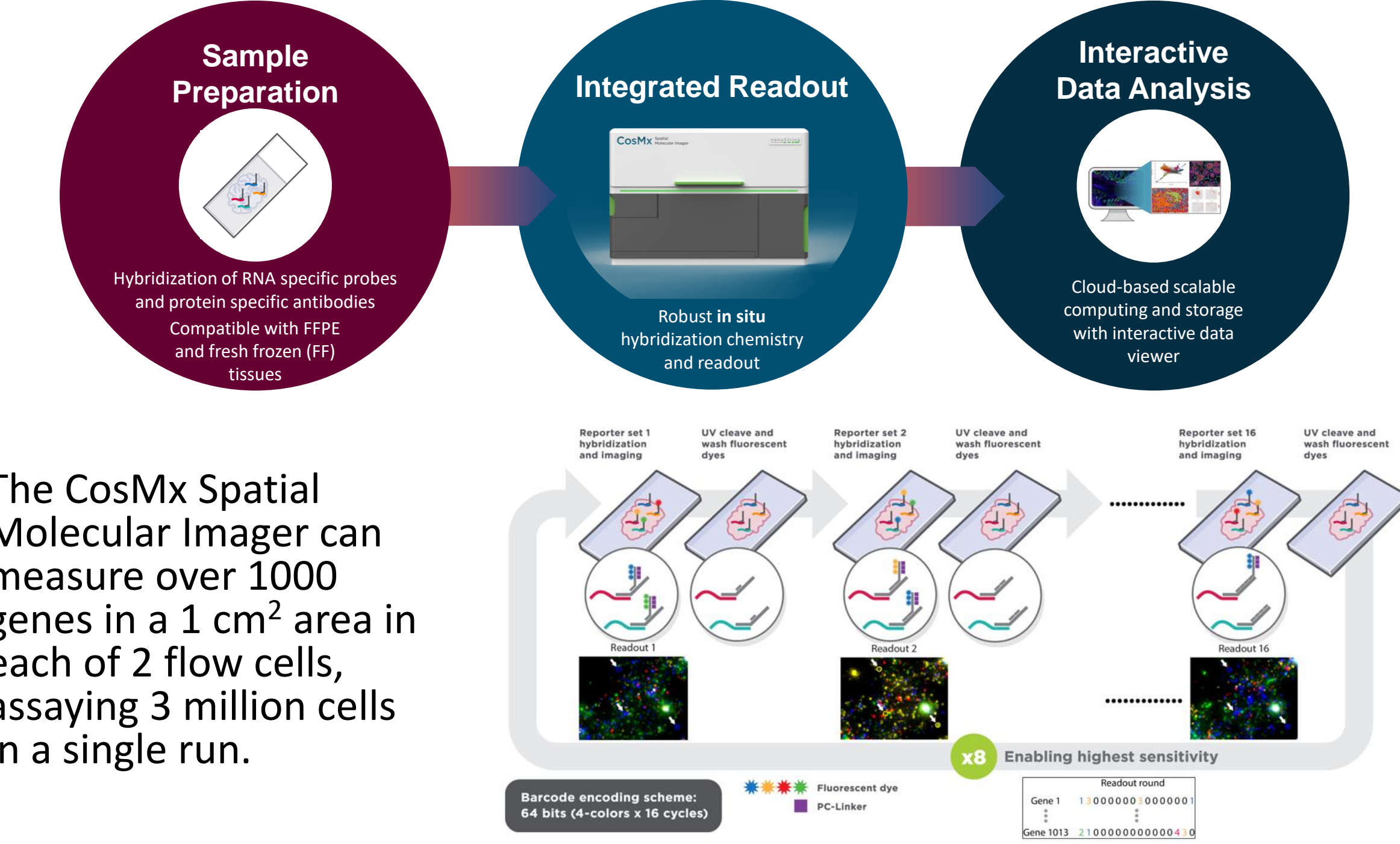
Abstract

We used CosMx™ Spatial Molecular Imager to profile 960 genes across 5 non-small cell lung cancer (NSCLC) samples, one in triplicate, for 7 total slides and 771,236 cells.

We have made the data available online as a resource for cancer researchers, algorithm inventors and software developers.

Here we review the data and demonstrate some of its capabilities.

The CosMx Spatial Molecular Imager

Sample Preparation
Hybridization of RNA specific probes and protein specific antibodies
Compatible with FFPE and fresh frozen (FF) tissues

Integrated Readout
Robust in situ hybridization chemistry and readout

Interactive Data Analysis
Cloud-based scalable computing with interactive data viewer

The CosMx Spatial Molecular Imager can measure over 1000 genes in a 1 cm² area in each of 2 flow cells, assaying 3 million cells in a single run.

High Sensitivity vs. scRNA-seq

To assess sensitivity, we compared mean signal in CosMx to mean signal in a scRNA-seq dataset from CD45+ cells from NSCLC tumors[1]. Only immune cells were used in the comparison.
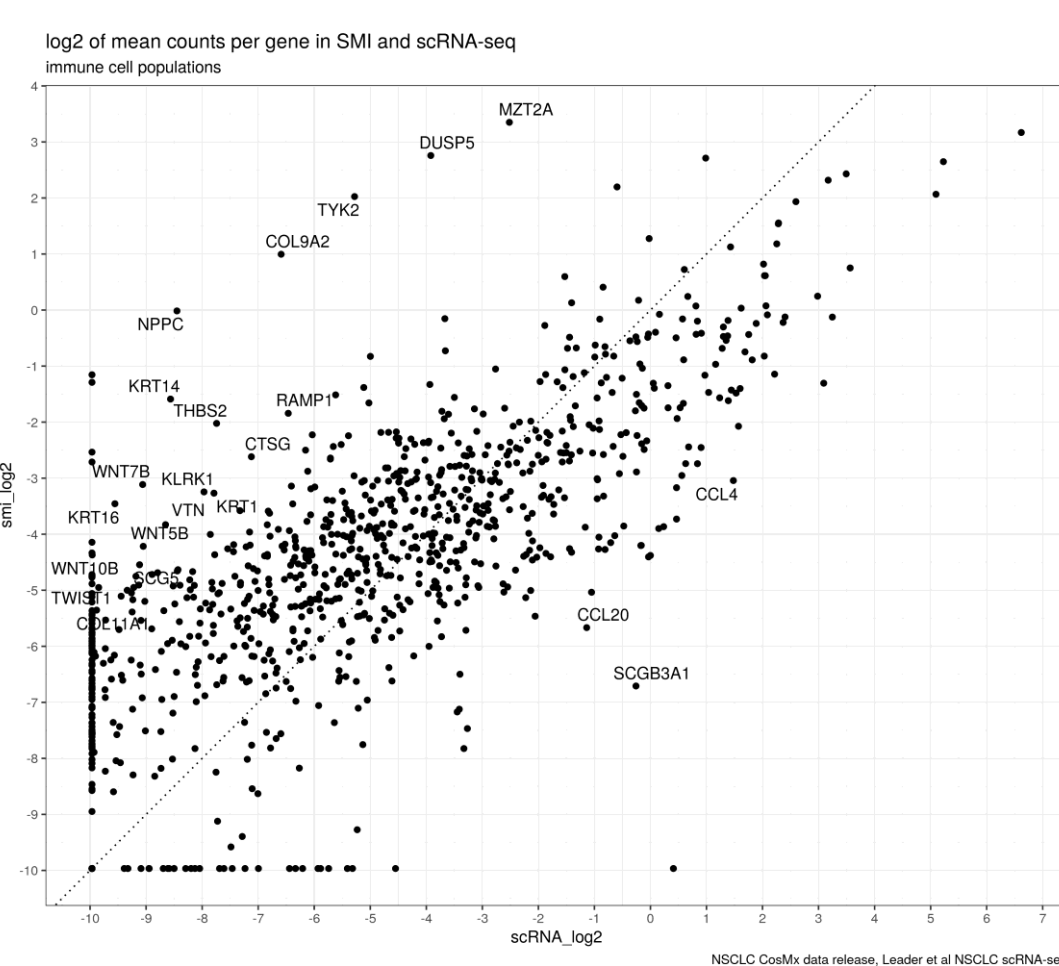
Right: Mean per-cell expression in CosMx vs. scRNA-seq. Genes below the line had higher average counts in scRNA-seq; genes above the line had higher average counts in CosMx.

1. Leader AM, Grout JA, Maier BB, Nabet BY, Park MD, Tabachnikova A, et al. Single-cell analysis of human non-small cell lung cancer lesions refines tumor classification and patient stratification

High Concordance with RNA-seq in cell lines

**16 Cell lines were profiled with CosMx and bulk RNA-seq.**

A: RNA-seq vs. "bulk" CosMx profile. Red lines show breakpoint regression; orange lines mark the breakpoint between the background-dominated data and the signal-dominated data.
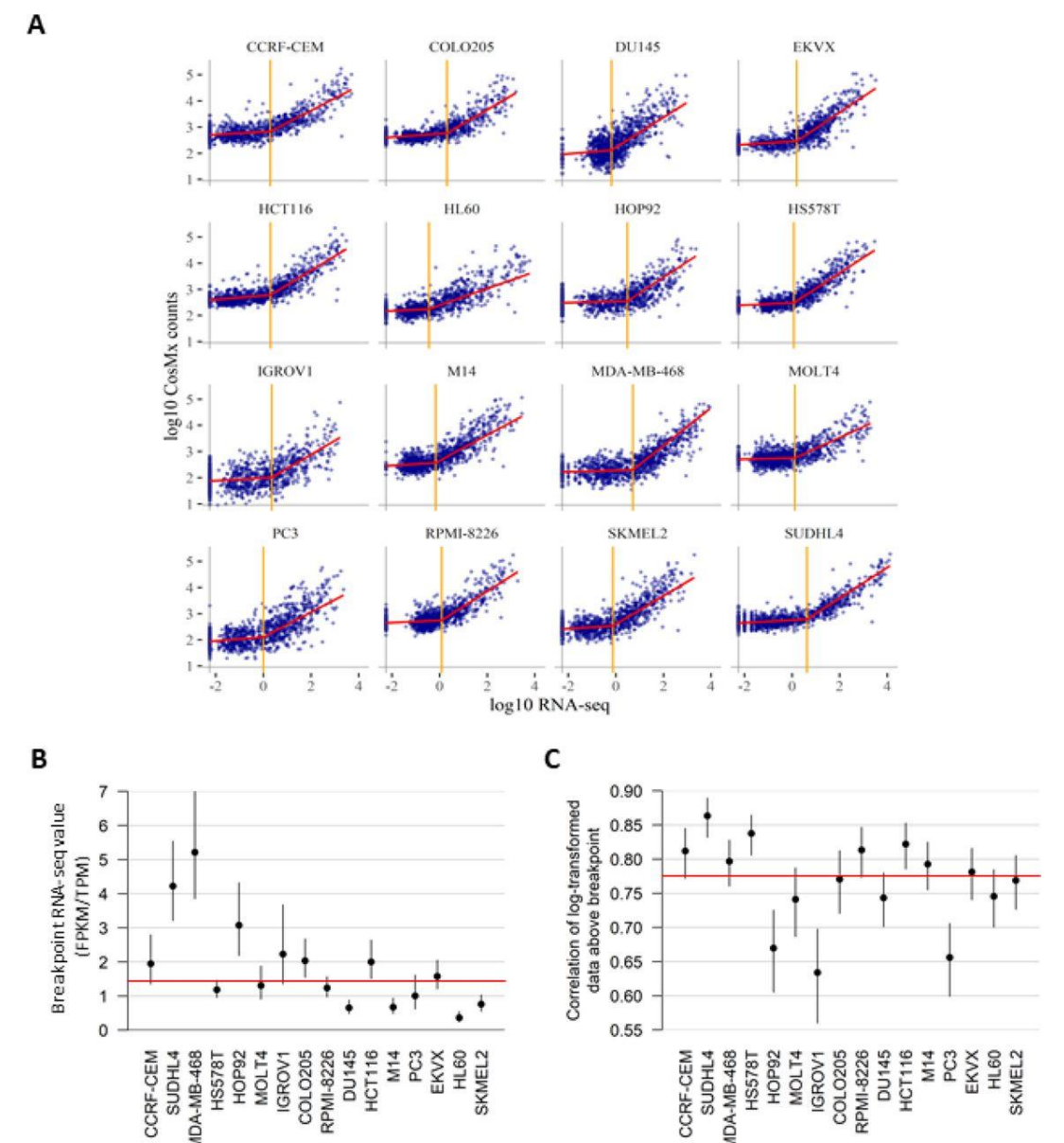
B: FPKM values of the breakpoint above which CosMx and RNA-seq are linear.

**C:** Correlation between RNA-seq and CosMx above the breakpoint.

## Reproducibility study in serial sections

Lung5 rep 3
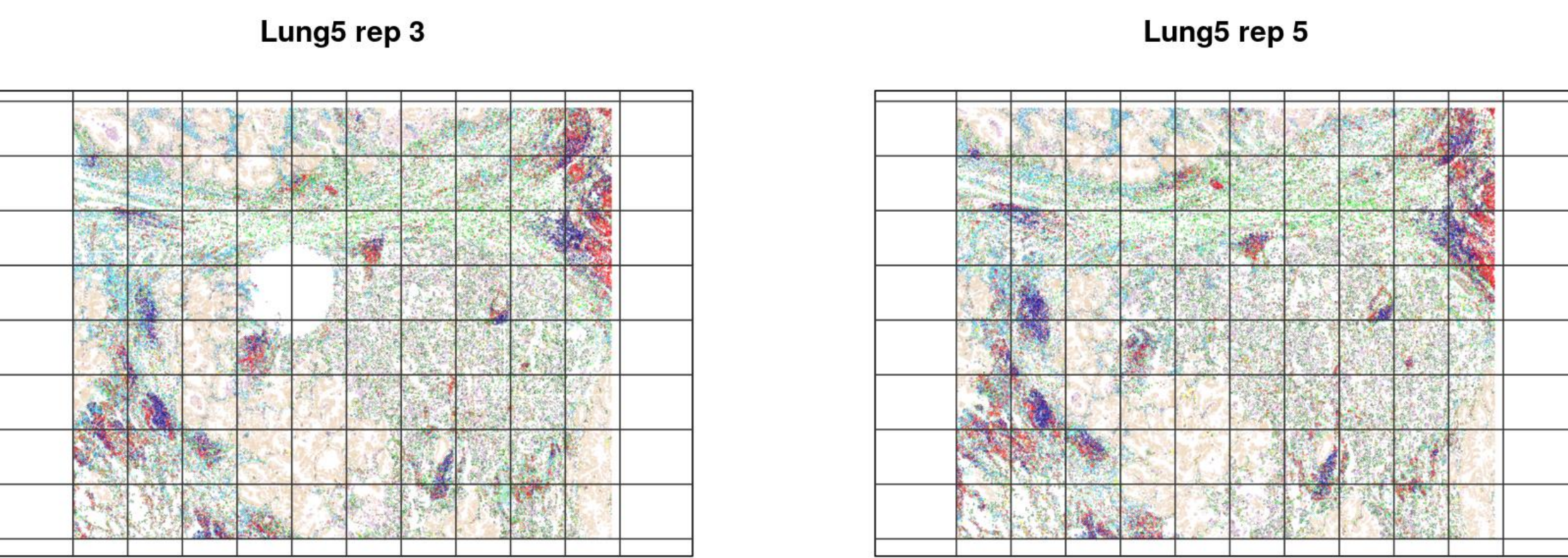
Lung5 rep 5

Above: Two serial sections of FFPE lung tissue (Lung 5 replicates 3 and 5) was partitioned into a grid. Squares held between 600 and 2,000 cells.

Right: Concordance between the 980-gene expression profiles of matching grid squares.

**Below:** concordance between "bulk" profiles of 3 replicate sections.
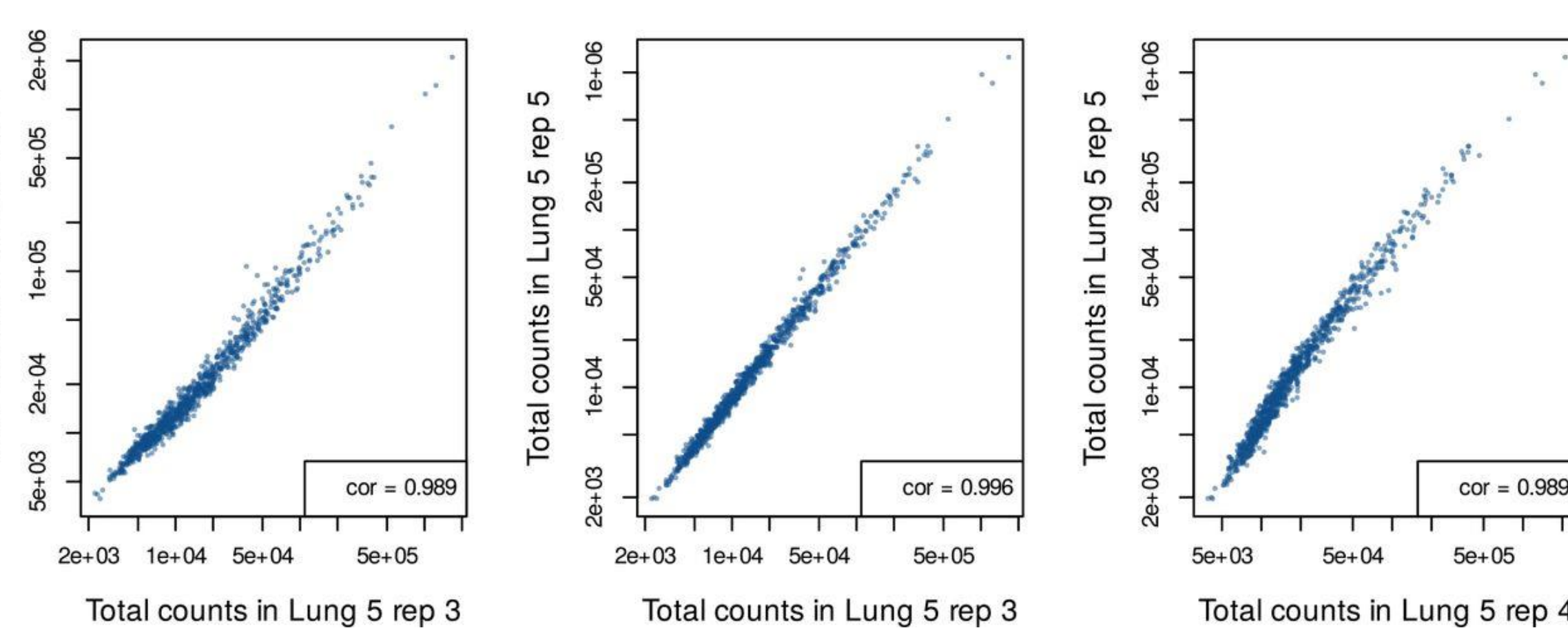
NSCLC cells in expression space and physical space

Lung5 Lung6 Lung9
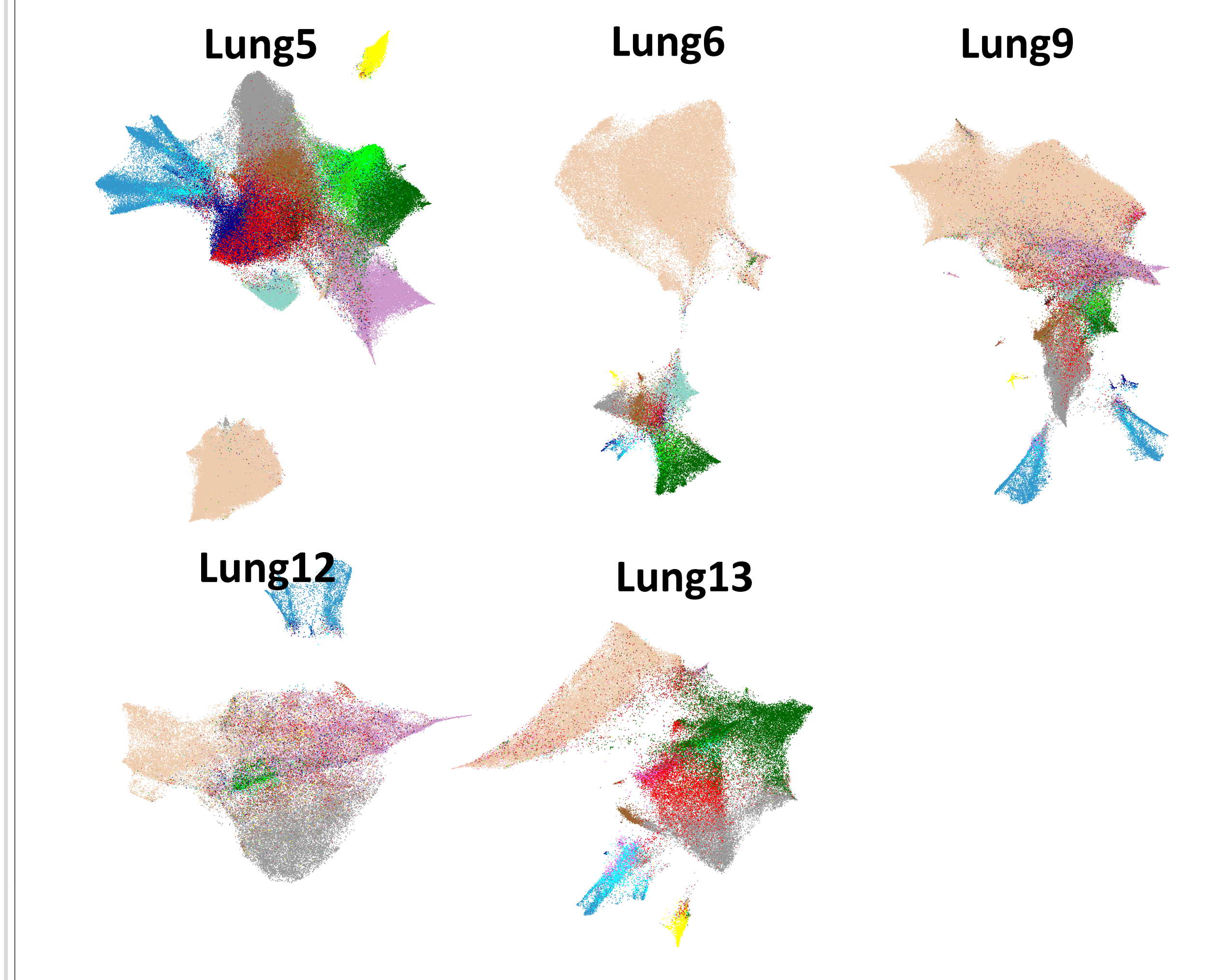
Lung12 Lung13

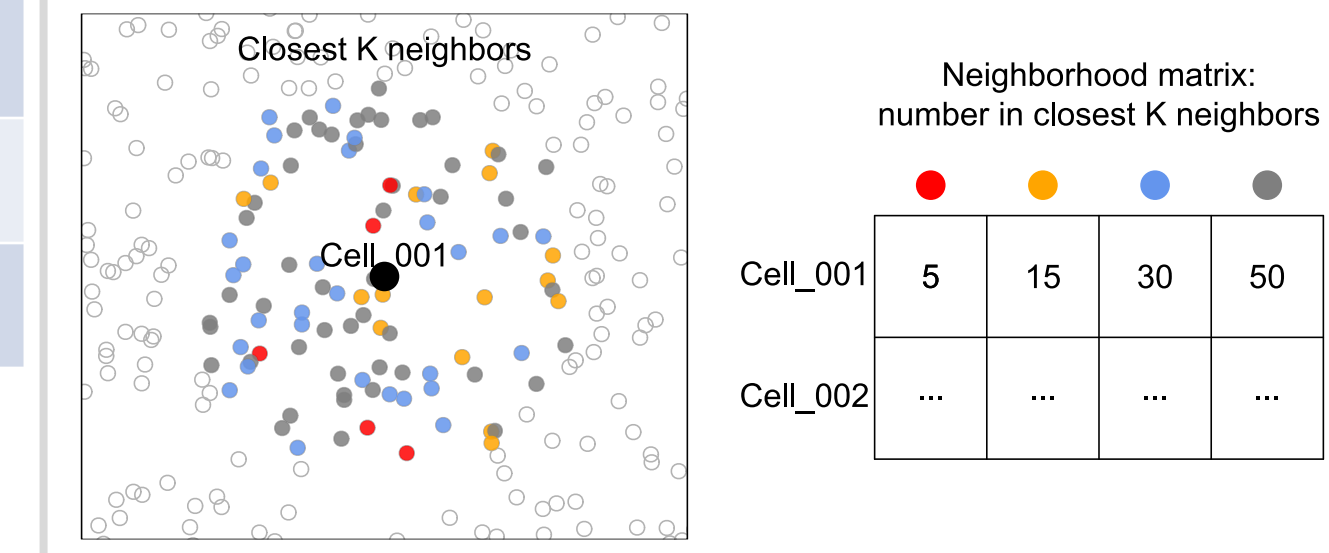| FFPE sample	RIN score	DV 200 (%)	Cells passing QC (%)
Lung 5	Unmeasurable	21	89.1
Lung 6	NA	NA	96.0
Lung 9	2.3	65	94.1
Lung 12	2.4	64	96.6
Lung 13	1.8	23	98.1

Legend:
- 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
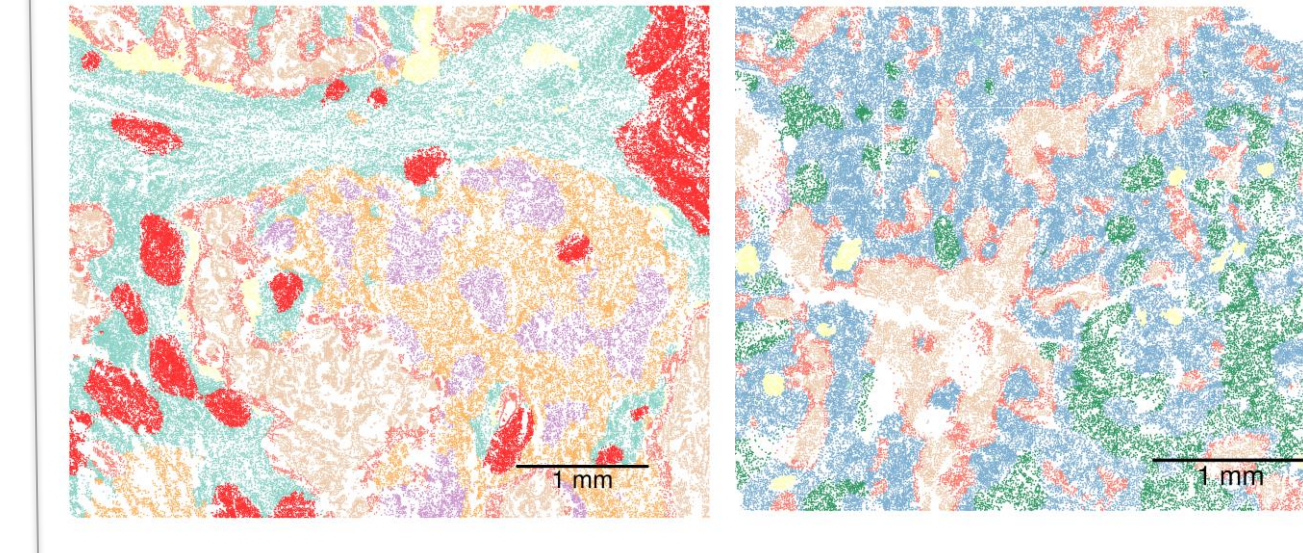- Naive CD8 T-cell
- B.cell

Lung5-5

Lung13

1 mm

Lung12

1 mm

Lung6

Box 1

1 mm

Box 2

Lung9

1 mm
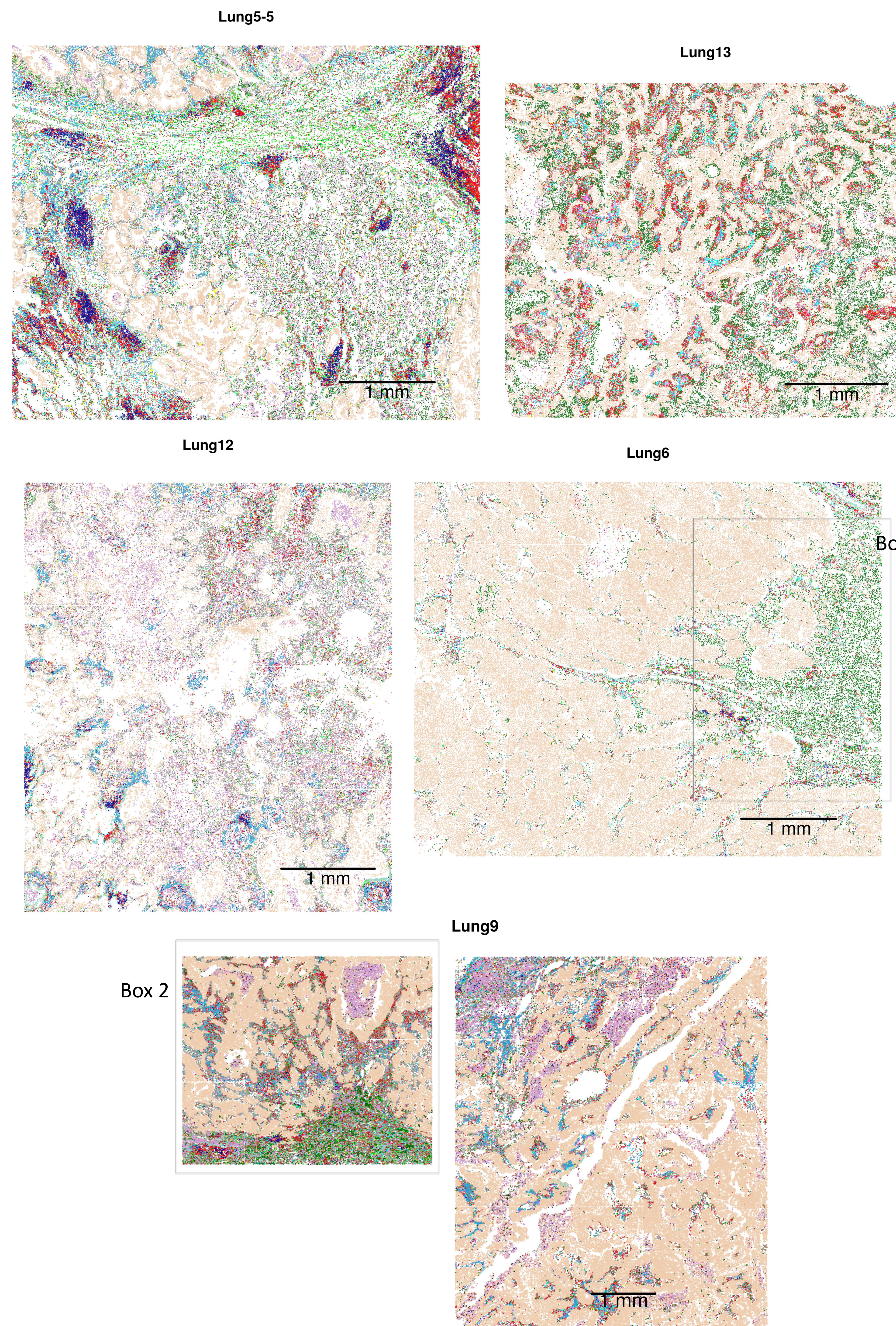
What questions can you ask with CosMx?

What ecosystems exist within tissues?

Neighborhood clustering
- Characterize each cell's environment based on the cell types in its neighborhood.
- Cluster cells based on their neighborhood compositions.

Closest K neighbors

Neighborhood matrix: number in closest K neighbors

	5	15	30	50
Cell_001	5	15	30	50
Cell_002	...	...	...	...

Neighborhood clustering results in two tissues

Legend:
- plasmablast-enriched stroma
- lymphoid structure
- myeloid-enriched stroma
- tumor-stroma boundary
- tumor interior
- stroma
- neutrophils
- macrophages
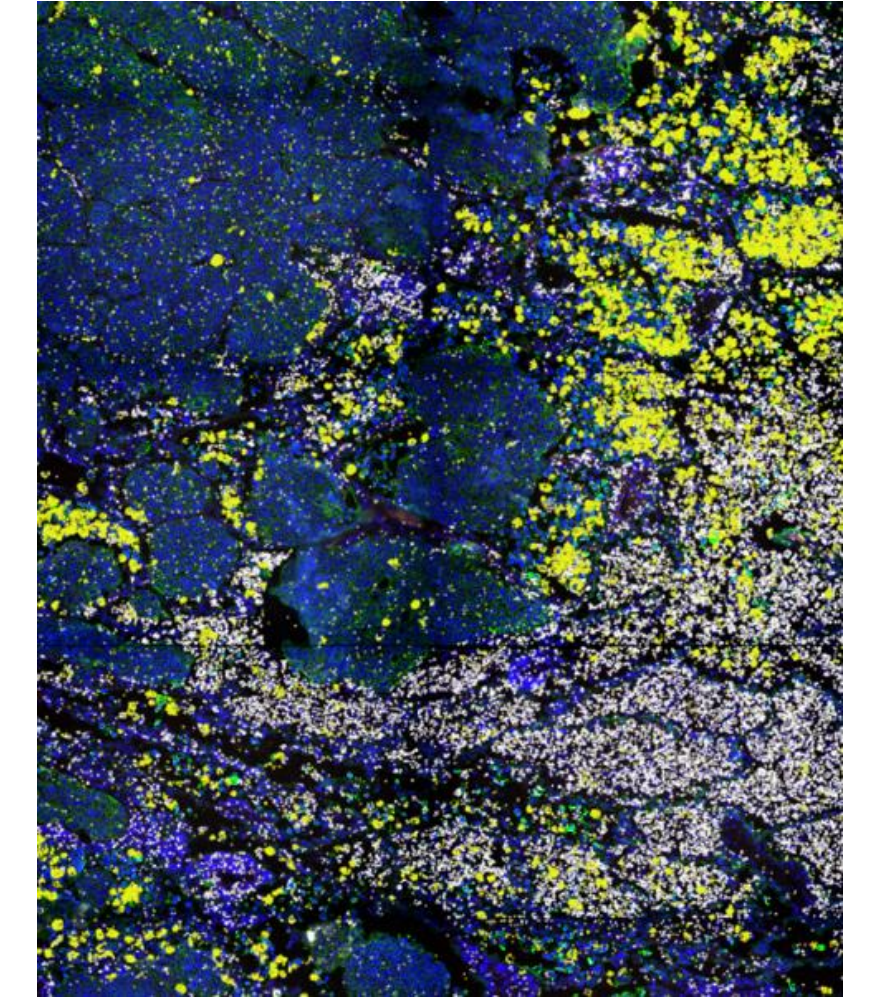- immune-enriched

Lung5 Lung13

How do cells respond to their environments?

Macrophage gene expression changes across the span of tumor "Lung 6"

Yellow dots: SPP1, a driver of macrophage polarization, up-regulates PD-L1

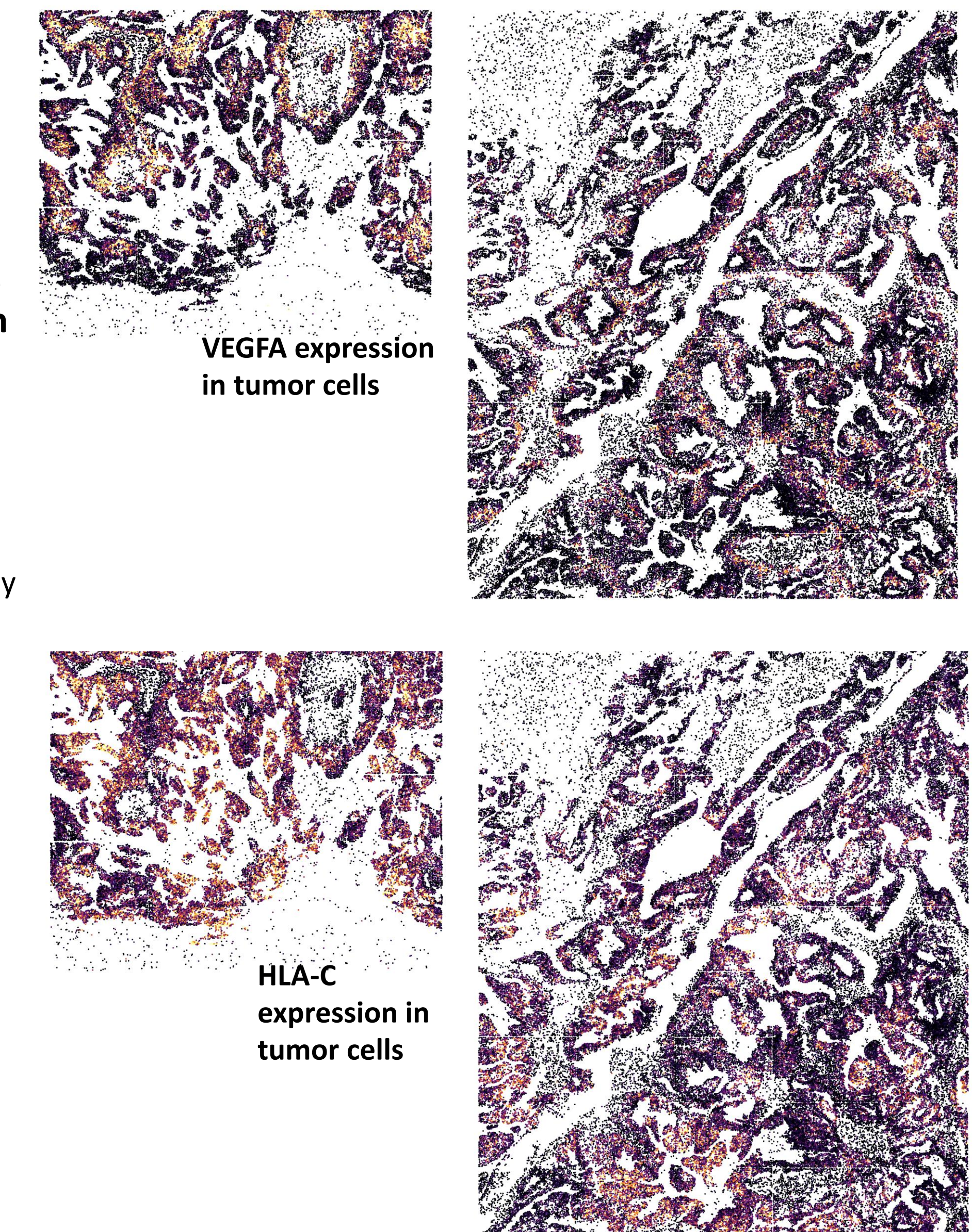White dots: HLA-DQA1, needed for MHC-II antigen-presentation

Data from Lung 6, Box 1

Spatial dependence of tumor expression

Plots show only tumor cells from Lung 9.

Expression patterns of VEGFA and HLA-C are both complex and highly spatially ordered.

VEGFA expression in tumor cells

HLA-C expression in tumor cells

How do cells interact with their neighbors?

Ligand-receptor signaling analysis

Macrophages were scored for APP -> CD74 signaling.

Grey: CD74- macrophages
Blue: CD74+ macrophages with only APP- neighbors
Red: CD74+ macrophages with APP+ neighbors

Data from Lung 9; image from Box 2.
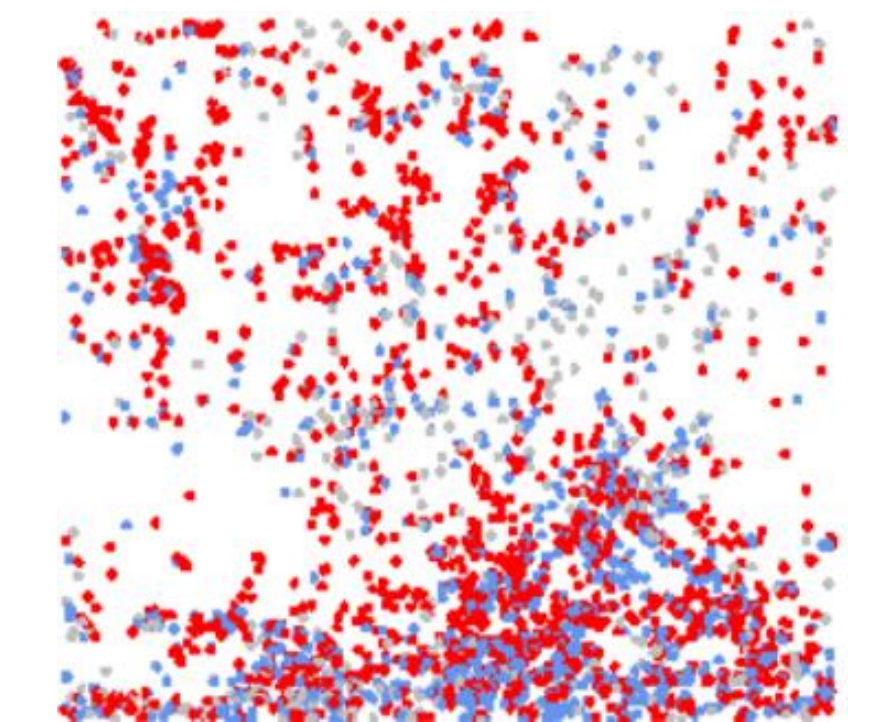
Cellular response to ligand-receptor signaling

Follow-up question:
How do macrophages react to APP->CD74 signaling?

Approach:
Perform differential expression comparing CD74+ macrophages with/without APP+ neighbors.

See more Ligand-Receptor analyses in Poster 213

Down in presence of APP+ neighbors

Up in presence of APP+ neighbors

Mean transcripts per cell: R=L+R+S
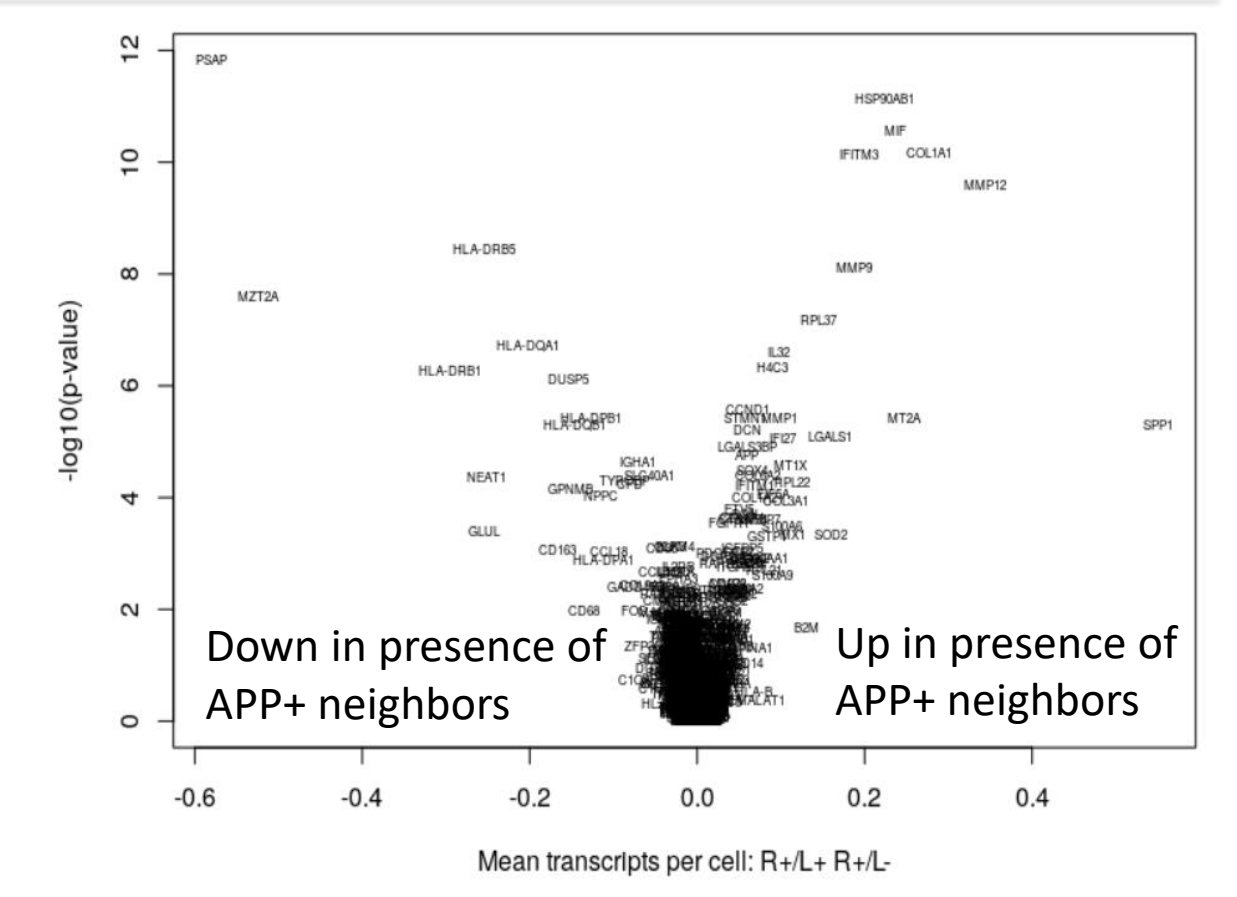
Accessing the data release

The NSCLC showcase dataset can be downloaded at
https://nanostring.com/products/cosmx-spatial-molecular-imager/ffpe-dataset/

See it used by the open-source community:
- Seurat: https://satijalab.org/seurat/articles/spatial_vignette_2.html
- Jerby-Arnon, Livnat, and Aviv Regev. "DIALOGUE maps multicellular programs in tissue from single-cell or spatial transcriptomics data." *Nature Biotechnology* (2022): 1-11.

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