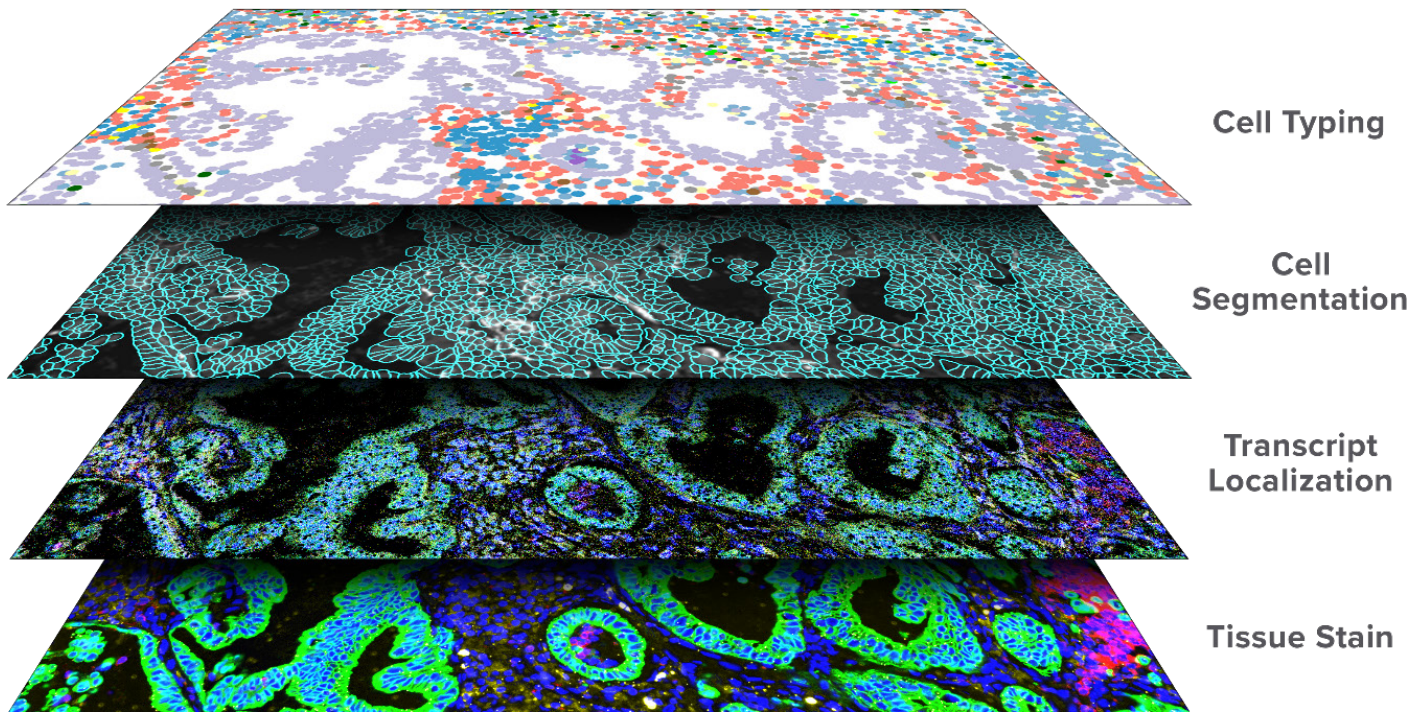


# CosMx™ Spatial Molecular Imager

## Grant Support Document

Experience the power of spatial biology with the most comprehensive, fully integrated single-cell spatial solution.



## Information to provide in grant application

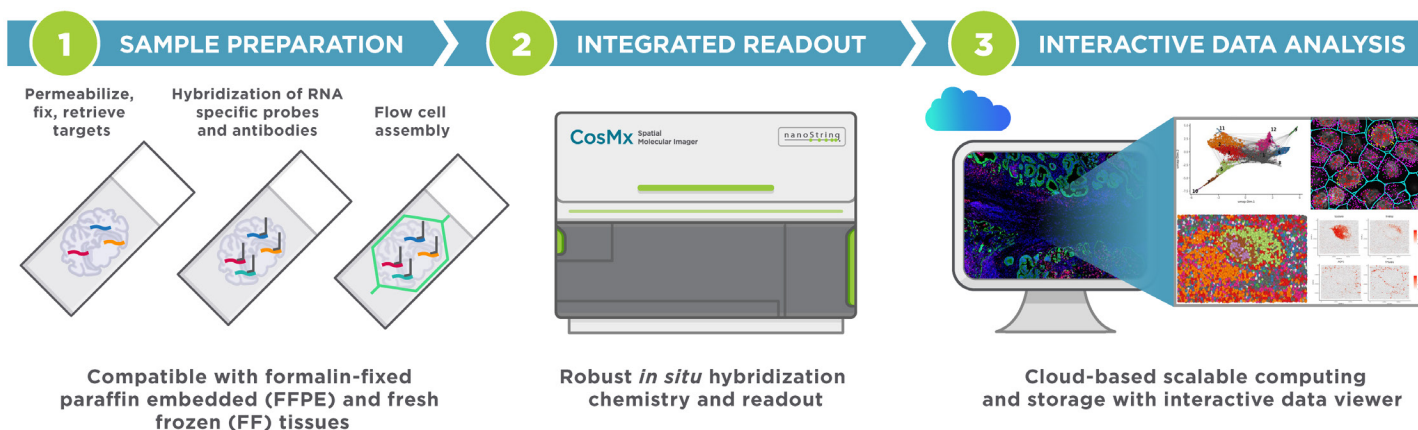
### Platform Overview

CosMx Spatial Molecular Imager (SMI) is the first high-plex in situ analysis platform to provide spatial multiomics with formalin-fixed paraffin-embedded (FFPE) and fresh frozen (FF) tissue samples at cellular and subcellular resolution.

The CosMx SMI platform is based on high sensitivity in situ technology (proprietary to NanoString) that combines the power of high plex profiling with super resolution imaging and high throughput capabilities. The platform allows researchers to visualize and quantify targeted protein and gene expression on Formalin-Fixed, Paraffin-Embedded (FFPE) or Fresh Frozen (FF) tissue sections on a microscope slide.

### System Specifications for CosMx™ Spatial Molecular Imager

Resolution	Subcellular resolution (120 nm) with transcript localization precision < 50 nm
Throughput	Up to 3 million cells/slide, up to 4 slides/run, Up to 26 slides/week
Tissue compatibility	FFPE, Fresh Frozen, organoids, cultured cells
Multiplexing capability (option to customize)	<ul style="list-style-type: none"><li>• Up to 6,000-plex RNA panels</li><li>• Up to 76-plex Protein</li></ul>
Flexible scan area	Up to 300mm <sup>2</sup> per slide
Platform Offering	Instrumentation, validated panels and reagents, with cloud data storage and analysis through AtoMx™ Spatial Informatics Platform (SIP)



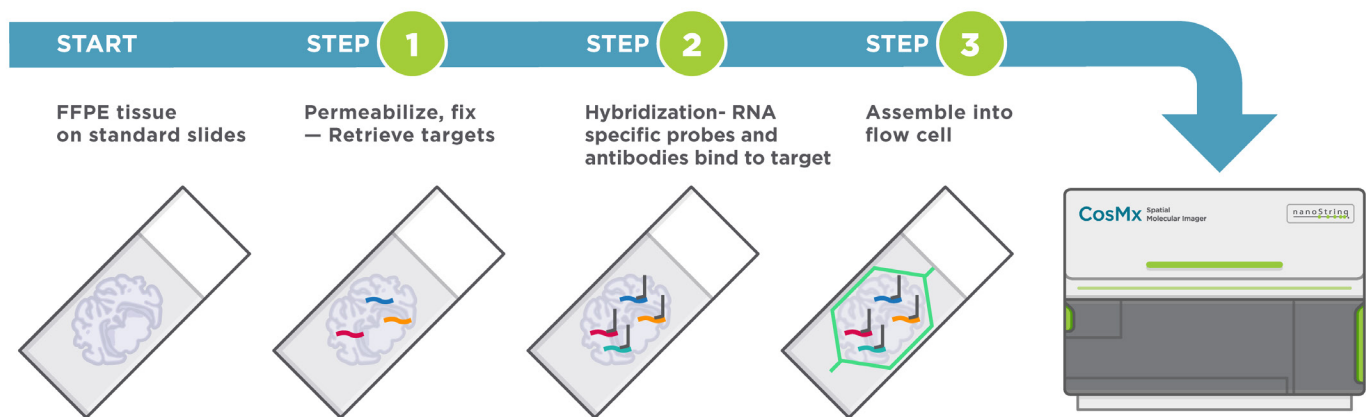
CosMx SMI for single-cell imaging delivers a comprehensive package which includes validated reagents, instrument, and data analysis software for seamless sample-to-result.

### Technology Overview & Workflow

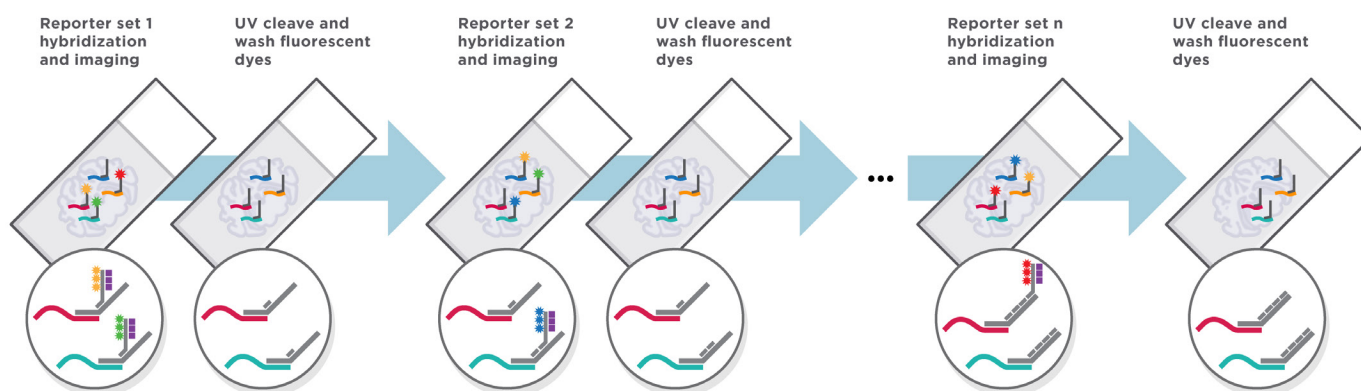
The CosMx SMI platform is an integrated system with mature cyclic in situ hybridization chemistry, an ultra-high-resolution imaging readout instrument, and an interactive data analysis and visualization software. Just three steps will take you from sample to result using CosMx SMI: 1) Sample preparation, 2) Automated readout on the SMI instrument, and 3) Interactive data analysis using AtoMx™ Spatial Informatics Platform (SIP). Sample preparation involves common in situ hybridization (ISH) processing steps. The protocols are compatible with standard glass pathology slides, and do not require complicated tissue expansion or clearing, nor cDNA synthesis or amplification. RNA or protein targets in individual cells are identified via hybridization or binding with highly specific probes or antibodies labeled with a unique barcode system. Barcode readout occurs through multiple rounds of reporter probe

binding and fluorescence imaging using the CosMx SMI instrument. Each RNA appears as a single bright spot in the sample and is digitally quantified in the image. The data is then migrated to cloud storage for analysis and visualization. Users can also incorporate custom analysis workflows.

The CosMx SMI is the first platform to demonstrate simultaneous single-cell and sub-cellular detection of 76 proteins on standard, bio-banked, FFPE tissue samples. Rather than conventional cyclic exchange methods, the CosMx Protein Assays uses a unique antibody-oligonucleotide conjugate. CosMx SMI decodes the 4-color readout to detect each protein's subcellular localization and quantify its expression level. CosMx oligo-labeled antibodies undergo rigorous QC testing, and site-specific labeling chemistry to select for pure imaging reagents with no unconjugated antibody or free oligonucleotide contamination, which could lead to background noise.



Simple and streamlined sample prep workflow.



Robust hybridization chemistry that provides higher sensitivity and supports high plex assay.

## CosMx SMI Workflow

1. A 5µm FFPE or fresh frozen tissue section is fixed and permeabilized.
2. RNA probes or protein antibodies are hybridized to their targets in the tissue sample.
3. The tissue sample is washed, then incubated with oligo-labeled antibodies for cell segmentation staining.
4. After washing, the flow cell is assembled and loaded onto the CosMx SMI instrument for imaging.
5. The desired imaging area(s) on the tissue (up to 300 mm<sup>2</sup>) are selected.
6. The instrument automates rounds of reporter binding and fluorescent imaging to read out the barcodes on each imaged RNA probe or protein antibody.

## CosMx SMI Instrumentation and Assays

CosMx SMI is a complete end-to-end single cell spatial biology solution. Data acquisition is done on the instrument and all data storage and analysis is completed within each user's AtoMx SIP account. The CosMx SMI instrument is used for slide scanning, FOV selection, and automated hybridization chemistry to detect target molecules. CosMx SMI provides a high-resolution subcellular image-based readout by identifying the X, Y, and Z coordinates of each target molecule, which is translated to spatial location data and exported to the cloud in AtoMx SIP. With AtoMx SIP, the user can collaboratively interact with, analyze, and visualize their data.

The optical system is an epi-fluorescent configuration that uses custom water immersion optics with 1.1 NA and 22.78X magnification. The FOV size is 0.51 mm x 0.51 mm. Illumination is widefield with a mix of lasers and LEDs to allow for UV cleavage (385 nm) and imaging of five different fluorophores: DAPI, FITC, TRITC, Texas Red and Cy5.



## AtoMx SIP Data Analysis

The CosMx SMI includes one year access to AtoMx Spatial Informatics Platform (SIP), the only cloud-based, fully-integrated platform for spatial biology. No coding experience is required to use AtoMx SIP. Researchers can analyze and visualize spatial multiomics data with ease while utilizing NanoString-configured analysis modules and pipelines. For researchers with computational experience, custom analysis modules and pipelines can effortlessly be created and executed all while leveraging the compute power of the cloud.

With advanced analytics enabling global data sharing and collaboration, AtoMx SIP helps researchers analyze large amounts of spatial multiomics data anytime, anywhere.

## CosMx SMI Consumables

The following content is commercially available for CosMx SMI:

### CosMx RNA Assays

1. CosMx 6k Discovery Panel: designed to provide coverage across all major pathways in biology. Profile expression of 6000 RNA targets at subcellular resolution and customize with up to 200 of your own targets. Will be available in early 2024 for human.
2. CosMx Universal Cell Characterization Panel: designed to provide robust cell typing, cell-cell interaction analysis, and more in a wide range of human tissues and disease states. Profile expression of 1000 highly curated targets at subcellular resolution and customize with up to 50 of your own targets. Currently available for human and mouse will be available by end of 2023.
3. CosMx Neuroscience Panel: designed to provide robust cell typing, cell-cell interaction analysis, and more in mouse brain and other neuronal tissues. Profile expression of 1000 highly curated targets at subcellular resolution and customize with up to 50 of your own targets. Currently available for mouse.
4. CosMx Human Immuno-Oncology Panel: optimized for cell typing of key immune and stromal cells and evaluation of immune infiltration and tumor microenvironments. Profile expression of 100 transcripts covering immune cells, their activities, and aspects of tumor biology relevant to IO therapies, and customize with up to ten of your own targets.
5. CosMx Custom RNA Barcoding Service: enables you to create customized content for use with the CosMx SMI. Add targets of your choice to any of our pre-validated CosMx RNA assays or build your own entirely custom CosMx RNA panel for spatial analysis.

### CosMx Protein Assays

1. CosMx Human Immuno-Oncology Protein panel: designed to provide comprehensive IO coverage for evaluation of immune cell typing and function, checkpoint surveillance, and more in a wide variety of human tissues and disease states. Profile protein expression of 64+ targets at subcellular resolution using the panel's validated antibodies today, and customize with up to 8 custom-conjugated antibodies starting Q3 2023.
2. CosMx Mouse Neural Cell Typing and Alzheimer's Pathology Protein panel: designed to provide comprehensive coverage for evaluation of neural cell typing and function and Alzheimer's pathology in mouse brains and other neuronal tissues. Profile protein expression of 64+ targets at subcellular resolution using the panel's validated antibodies.
3. CosMx Custom Protein Barcoding Service: enables you to add up to 8 custom-conjugated antibodies to any existing CosMx protein panel.

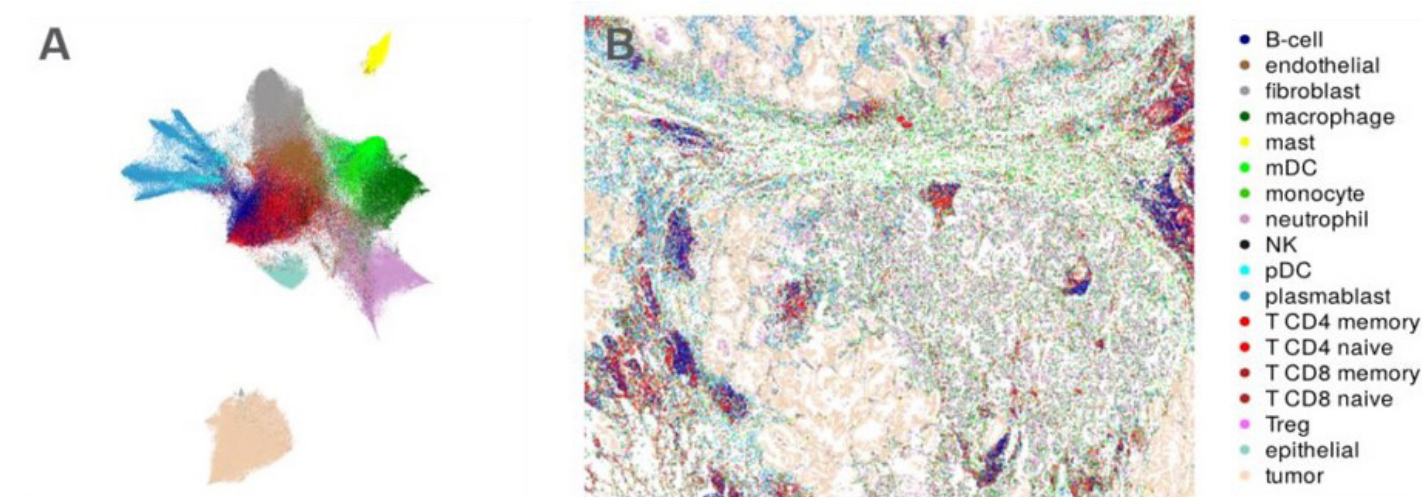
### Consumables

1. Cell Segmentation kits with options to customize with a la carte options for:
  - Universal Cell Characterization
  - Immuno-oncology
  - Neuroscience
2. Sample prep kits for FFPE and Fresh Frozen (FF) tissues
3. Flow cells and Imaging trays for readout
4. Instrument buffer kits

## Training and Support

Upon purchase and acquisition of the CosMx SMI system, NanoString will provide on-site installation and calibration of the instrument by a trained field engineer. Once the instrument has been qualified, a Product Application Scientist (PAS) will be on site to provide a comprehensive introduction to the CosMx SMI platform. The introduction will cover routine use of the instrument, experimental design, project consultations, and data analysis using AtoMx SIP. In addition, the PAS will train the lab scientist to ensure that they have hands-on experience in operating the instrument,

## Applications



**Figure 1.** Cell type map of NSCLC tissues. The map displays 135,707 cells across ~20 mm<sup>2</sup> tissue section. Color denotes cell type. (A) UMAP projection. (B) Spatially resolved cell-type map.

cell ID	B2M	CE3E	GZMB	HLA-A
1	0	0	1	0
2	4	2	0	0
3	2	0	0	5
4	1	0	0	1

cell ID	T-cells	B-cells	mDCs	Tumor cells
1	25	50	5	15
2	25	2	0	10
3	1	0	1	130
4	3	0	1	150

**Figure 2.** Matrix of gene expression (left) compared to the matrix of neighboring cell types (right).

implementing the selected CosMx workflow, and running an experiment from end-to-end. Lastly, the PAS will provide data analysis training with AtoMx SIP so that the researcher is familiar with all data analysis methods to process, analyze and interpret their data. Upon successful completion of the training, the researcher can access support through the NanoString CosMx Customer Experience Team regarding experimental design, instrumentation, consumables, and software.

## Supporting Data

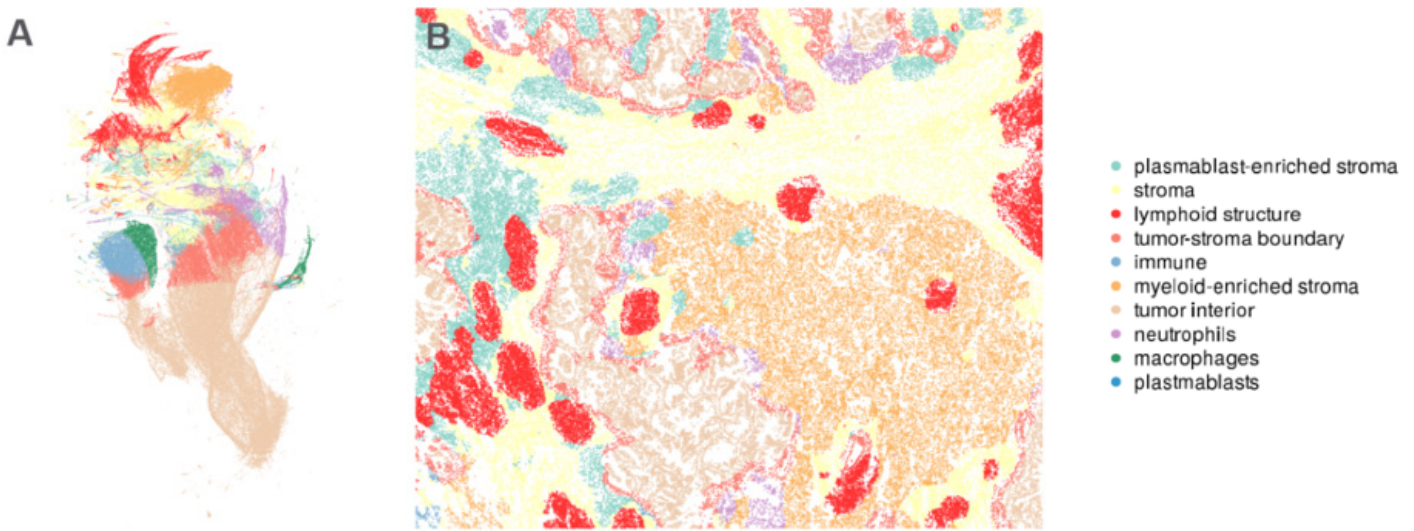
This dataset was generated using a 960-plex gene expression assay on Non-Small Cell Lung Cancer (NSCLC) FFPE tissues that classified >800,000 cells over 8 slides from 5 tumors to investigate the biology of single cells and their interactions across tissues. The content focuses both on cell type deconvolution of multiple tissue types and key aspects of cell signaling and cell state, with a focus on ligands and receptors that enable communication between cells.

### 1. Cell typing and discovery

To classify the cells in the intact NSCLC tissue, an expectation-maximization algorithm used reference profiles to assign cells to known cell types while also discovering new clusters. In Figure 1, immune and stroma cells were identified based on pre-defined profiles, making it possible to discover a new cluster representing tumor cells.

### 2. Neighborhood clustering

This spatial information also allows detailed analysis of cell “neighborhoods”, which reveals complex location-based cell-cell interaction and relationship insights. A “neighborhood matrix” (Figure 2) can be defined by encoding the number of each cell type among each cell’s 200 closest neighbors. Neighborhood matrices can be tailored to answer a wide range of different biological questions. For example, these neighborhoods can be defined over smaller or larger distances and used to calculate the average gene expression levels of specific cell populations.

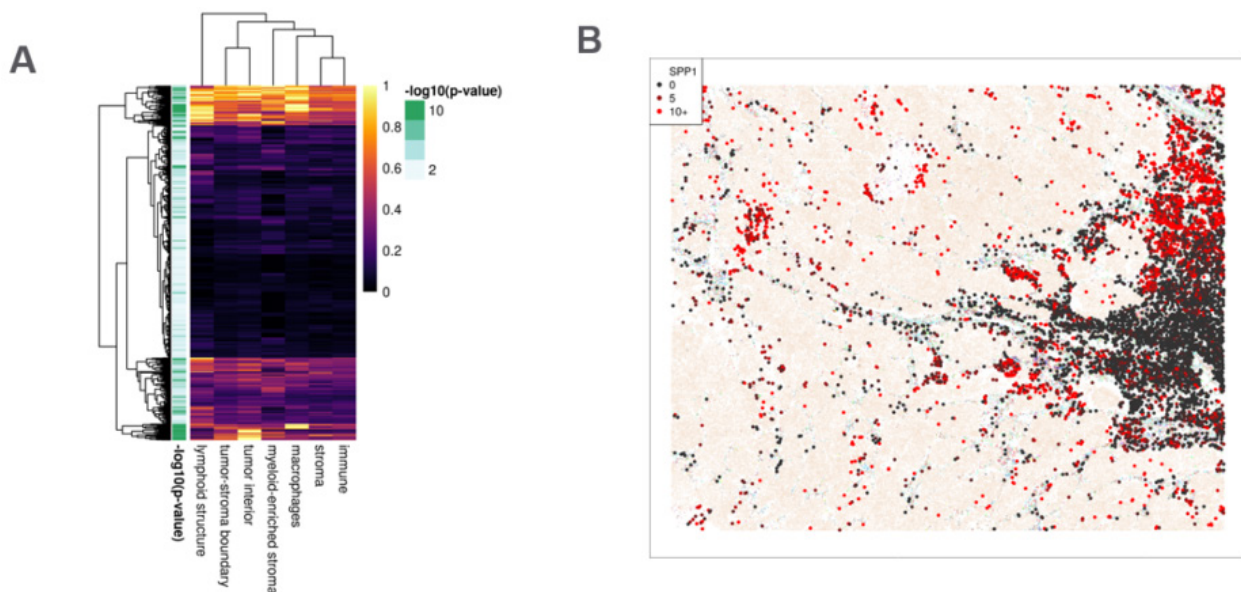


**Figure 3.** Organizational map of NSCLC tissue. Color denotes neighborhood cluster, “niche”. (A) UMAP projection. (B) Spatially resolved neighborhood cluster map.

### 3. Phenotyping of the tissue microenvironment

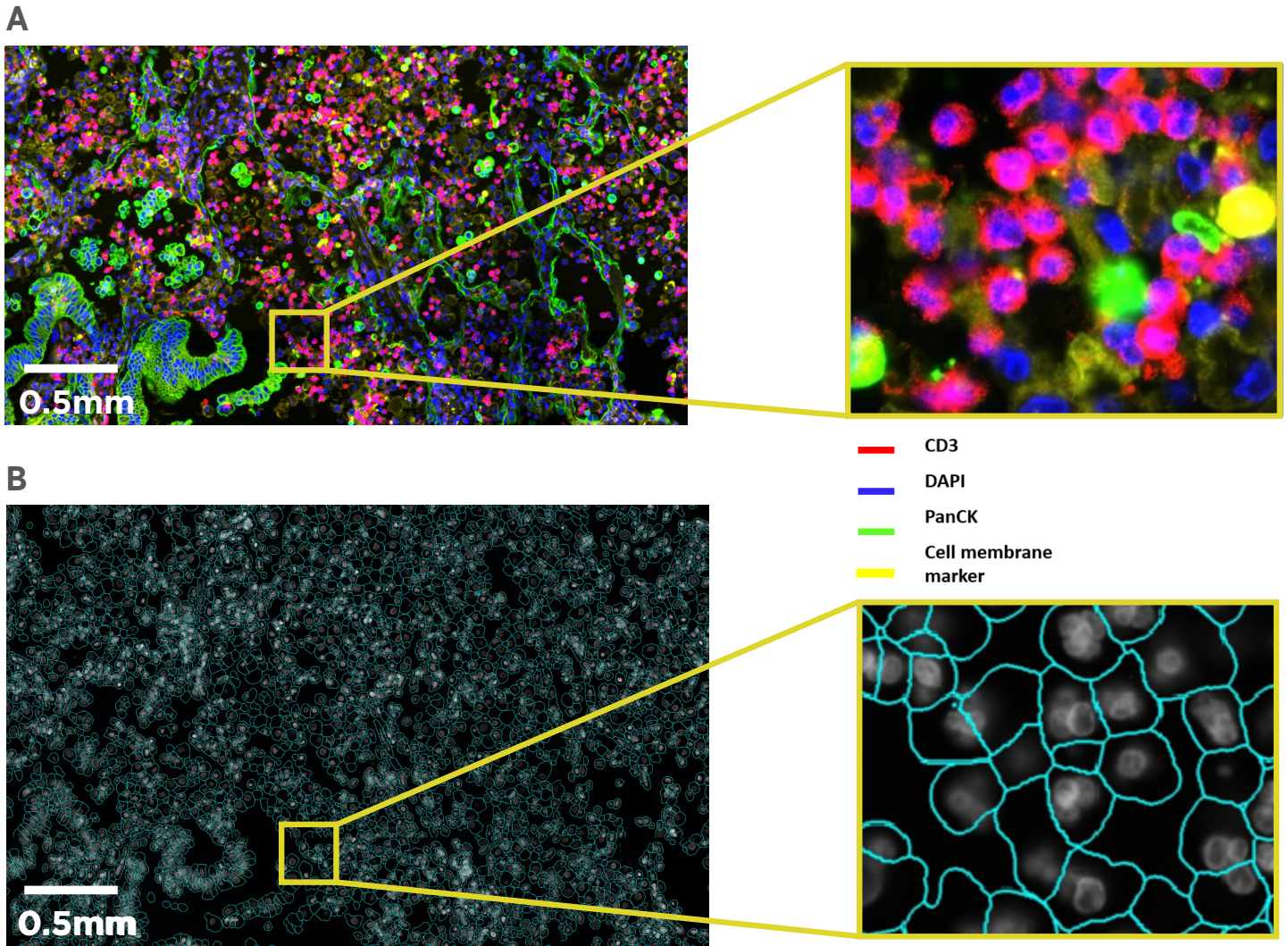
Once a neighborhood matrix was defined, it was subjected to traditional single-cell analyses. A UMAP projection of the neighborhood matrix shows the diverse microenvironment states within these tumors. Figure 3 shows neighborhoods of tumor cells with very low levels of macrophage and T-cell infiltration. In contrast, some neighborhoods were dominated by single-cell types, like macrophages, neutrophils, plasmablasts and myeloid dendritic

cells (mDCs). A third category of cell neighborhood shows mixed immune populations of B-cells with T-cells, macrophages with T-cells and plasmacytoid dendritic cells (pDCs), and macrophages with neutrophils and sporadic lymphoid cells. The neighborhood matrices of these various tumor microenvironments were clustered and partitioned into distinct niches and plotted based on spatial location. Plotting niches in physical space clarified the spatial organization within and the contrasts between these tumors.



**Figure 4.** Gene expression of macrophages based on spatial context. (A) Heat map for expression of 960 genes across all niches. (B) Spatial map for expression of SPP1. Color shows SPP1 expression level.





**Figure 5.** Nuclear and Morphology staining on the NSCLC FFPE tissue (A). Cell segmentation boundaries with nucleus in gray (B).

#### 4. Differential expression pattern of a cell type based on spatial location

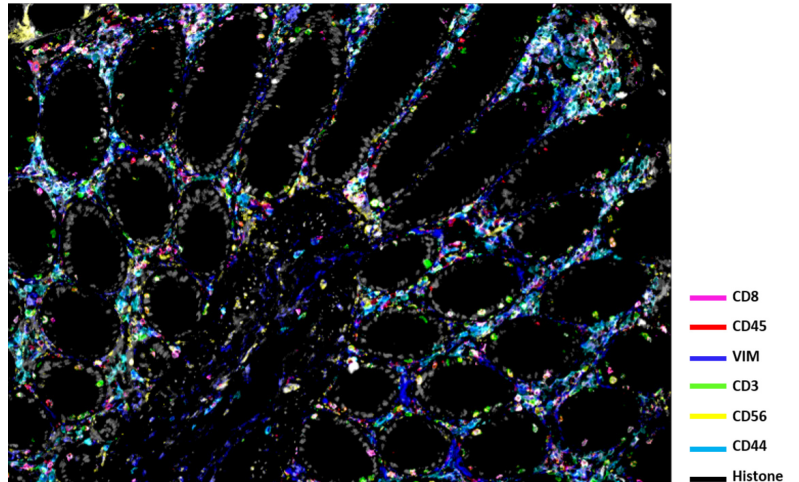
The SMI data can be used to analyze changes in the gene expression patterns of any given cell type based on spatial context. Figure 4 shows that macrophages express more SPP1 in the tumor interior and tumor-stroma boundary than they do in more immune-rich settings. SPP1 has a p-value of  $5 \times 10^{-61}$  and has been shown to mediate macrophage polarization and up-regulate PD-L1 (Zhang 2017).

#### 5. True cell segmentation using protein imaging

Accurate cell segmentation is critical to data quality but very challenging for tissue sections where cells are tightly packaged with shared, 3D boundaries and uneven morphology staining. CosMx uses a multi-modal cell segmentation process to provide accurate cell boundary detection (Figure 5). The method uses cell membrane and morphology marker protein images, machine-learning augmented cell segmentation algorithm and transcript-based segmentation refinement to achieve precise single-cell segmentation in morphologically intact tissue.

## 7. Detect and quantify proteins simultaneously at subcellular resolution

The CosMx protein assay reagents were validated on multi-organ FFPE tissue microarrays and 45 human FFPE cell lines, including cell lines overexpressing key targets such as GITR, CD278, PD-L1, and PD-1. Figure 6 shows seven protein targets on a healthy colon FFPE tissue sample imaged using a 100-plex protein panel. Benchmarking to multiple orthogonal datasets (e.g., the Human Protein Atlas and low-plex IHC) demonstrates that the assay is highly sensitive and specific.



**Figure 6:** Visualizing 7 protein targets on a healthy colon FFPE tissue sample probed with a 100-plex protein panel.

### Reference:

1. Zhang, Y., Du W., Chen Z., Xiang C., Upregulation of PD-L1 by SPP1 mediates macrophage polarization and facilitates immune escape in lung adenocarcinoma. *Experimental Cell Research*, 359, 449-457 (2017)

## Ordering Information

Product	Description	Catalog Number
CosMx Spatial Molecular Imager	CosMx Spatial Molecular Imager Instrument. Includes 1 year manufacturers warranty.	CMX-SMI-1Y
	CosMx Spatial Molecular Imager Instrument. Includes 1 year manufacturers warranty and 1 year service contract.	CMX-SMI-2Y
	CosMx Spatial Molecular Imager Instrument. Includes 1 year manufacturers warranty and 2 year service contract.	CMX-SMI-3Y
	CosMx Spatial Molecular Imager Instrument. Includes 1 year manufacturers warranty and 3 year service contract.	CMX-SMI-4Y
	CosMx Spatial Molecular Imager Instrument. Includes 1 year manufacturers warranty and 4 year service contract.	CMX-SMI-5Y

For more information, please visit [nanostring.com](https://www.nanostring.com)

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