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Spatial insights into tumor immune evasion illuminated with 1,000-plex RNA profiling with CosMx Spatial Molecular Imager

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Abstract

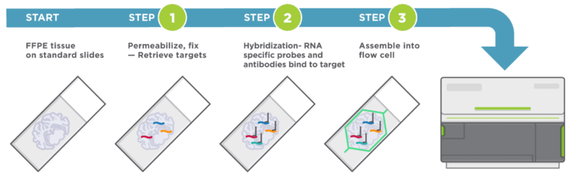
Background: Patient response to immunotherapy is limited due to the inability to convert excluded or cold tumors into ones which would be permissive to therapeutic intervention. To treat patients that evade immune therapy, comprehensive understanding of their tumor microenvironment (TME) is needed. To date, most profiling efforts have lacked the ability to capture high-plex 'omics data while retaining the spatial architecture of the TME. We developed the CosMx™ Spatial Molecular Imager (SMI) for analyzing formalin-fixed paraffin-embedded (FFPE) or fresh-frozen (FF) tissue and capturing the expression of over 1,000 RNA targets simultaneously with subcellular resolution from a single histopathology slide.

Methods: We profiled a cohort of 10 patient samples with CosMx SMI using the Human Universal Cell Characterization (UCC) Panel across a range of solid tumors. This cohort represents a diverse array of patients which include both infiltrated and excluded tumors with breast, lung, liver and colon tumors represented. We included technical replicates for 4 of the samples to better understand reproducibility of the assay. We were able to characterize over 4.5 million cells and 1.5 billion transcripts, representing detection of on average over 80% of the panel per patient and assigning more than 95% of the transcripts profiled to unique cells across these samples.

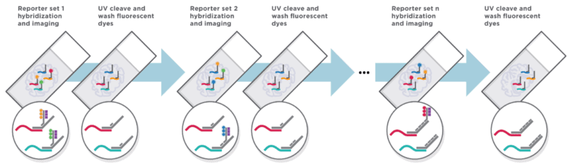
Results: In each of the cancer types we tested we were able to map and identify both previously identified cell types as well as novel ones associated with malignancy. We demonstrate robust delineation of critical immune cell populations from across lymphoid and myeloid lineages, as well as stromal cell populations inclusive of cell types frequently missed using dissociated cell sequencing, such as vascular endothelium associated with immune cell migration into the tumor bed. We leveraged 450+ genes from our panel dedicated to cell lineage, cell-cell interaction, and ligand-receptor signaling to identify unique interactions occurring at different scales between the tumor and the TME. We find that diverse mechanisms of immune evasion can be captured, and a clear role for cell types such as SPP1+ macrophages emerges in multiple cancer types.

Conclusions: Utilizing the CosMx platform to profile tissues allows for robust resolution of critical immunogenic signaling cascades and cellular interactions that are necessary to truly understand the tumor architecture. By maintaining the tissue structure, we can directly measure cellular interactions and capture cells commonly missed during dissociative studies. With this new platform, we are better poised than ever to truly understand the molecular mechanisms that drive tumor response to intervention.

Profiling Diverse Cancers with CosMx Spatial Molecular Imager



A streamlined and simple workflow that integrates with a standard ISH protocol with no need for tissue expansion or clearing, cDNA synthesis, or amplification. Go from sample to result faster.



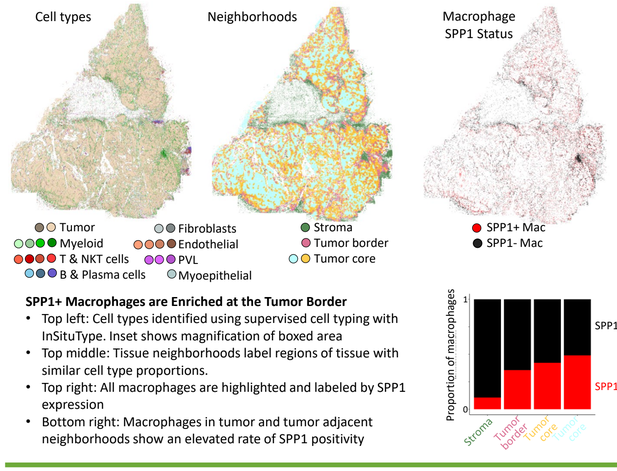
The robust hybridization chemistry provides higher sensitivity and supports high-plex assays for tissue samples to uncover deeper biological insights.

CosMx SMI is the first high-plex, *in situ* spatial multiomics platform for formalin-fixed paraffin-embedded (FFPE) and fresh frozen (FF) tissue samples with single-cell and subcellular resolution
CosMx SMI is an integrated system with mature cyclic fluorescent in situ hybridization (FISH) chemistry, high-resolution imaging readout, and interactive data analysis and visualization software.

Tissue Type	Slides (#)	Area Profiled (mm²)	Cells Profiled	Transcripts
Breast Cancer	2	87 – 91 mm²	530k – 543k	149.7M
Colon Cancer	2	100 mm²	889k – 910k	171.7M –173.8M
Liver Cancer	1	78 mm2	333k	183M
Normal Liver	1	100 mm2	533k	404M
Lung Cancer	8	13 – 29 mm²	74k – 151k	26M – 40M
Total	**14**	**709 mm²**	**4.54M**	**1.52B**

Patient samples profiled with CosMx SMI. A cohort of cancer samples was collected from biobanks and imaged to capture large areas across tumor, stoma, and adjacent normal tissue

SPP1+ Macrophages Invade TME Boundary of HER2+/ER+ Breast Cancer

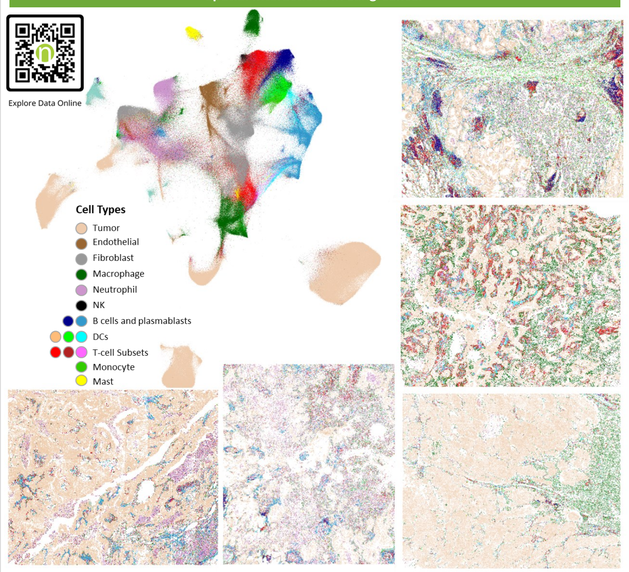


SPP1+ Macrophages are Enriched at the Tumor Border

- Top left: Cell types identified using supervised cell typing with InSituType. Inset shows magnification of boxed area
- Top middle: Tissue neighborhoods label regions of tissue with similar cell type proportions.
- Top right: All macrophages are highlighted and labeled by SPP1 expression
- Bottom right: Macrophages in tumor and tumor adjacent neighborhoods show an elevated rate of SPP1 positivity

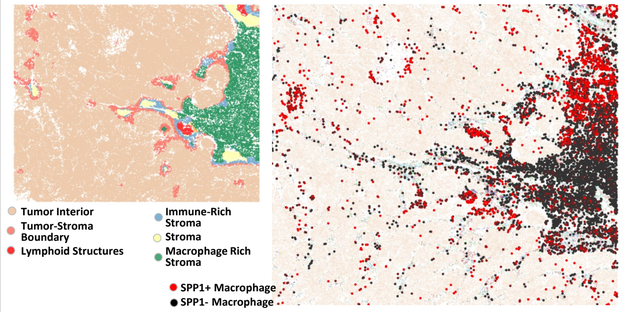
Spatial Interactions in Lung Cancer

Explore Data Online



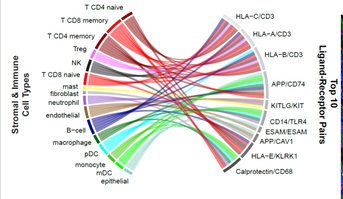
Spatial Mapping of Immune Cell Types from Cohort of 8 NSCLC Samples. UMAP and physical representation of samples profiled with the Human Universal CosMx RNA Panel, which captures 1,000 RNA targets at subcellular resolution. Examples of images from 5 sections are shown. Scan QR code above to explore this data online or download data for analysis.

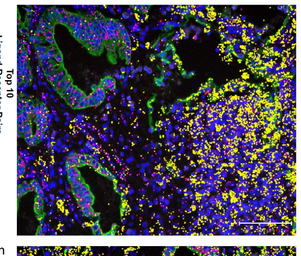
SPP1+ Macrophages invade TME boundary of NSCLC

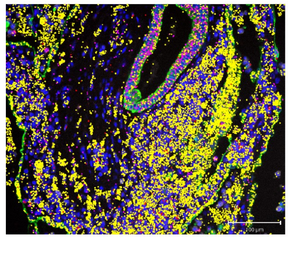


SPP1+ Macrophages Identified Using Differential Expression Analysis Between Those Near and Far from Tumor
Tumor nests were classified based on cell proportions in a given radius. By classifying both the tumor and stromal neighborhoods, we explored which genes were differentially enriched near or far from the tumor in unique immune subsets. SPP1 was significantly (FDR < 0.01) enriched near the tumor while SPP1- macrophages were farther from the tumor itself.

Ligand-Receptor Analysis of Lung Cancer Cohort

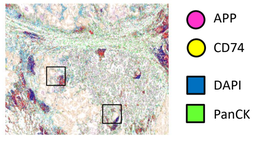




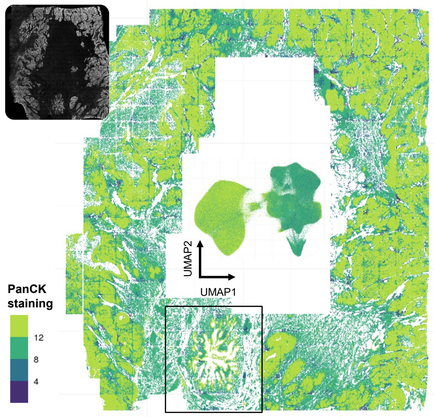


Ligand-Receptor Analysis Enabled with High Plex Panel

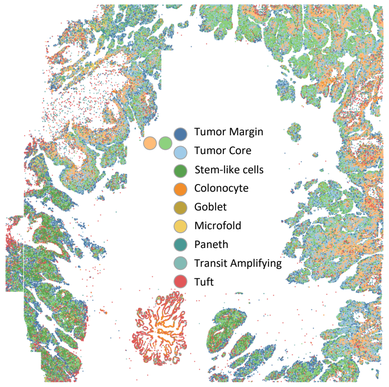
- Top: 100 juxtacrine ligand-receptor pairs are included in the UCC panel. Chord diagram between all non-tumor cell types – tumor pairs and the top 10 enriched ligand-receptor (LR) pairs.
- Right: Immunofluorescence (IF) images for two field of views in Lung 5 where detected RNAs for the LR pair APP/CD74 are plotted atop the image.
- Bottom: Location of inset panels on the right.

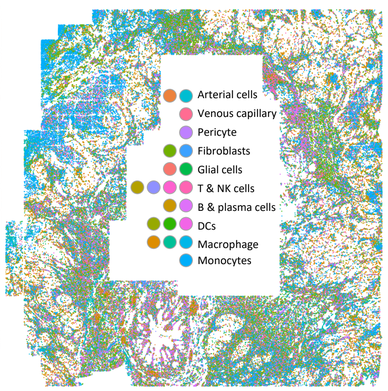


Mapping Colorectal Cancer to Identify Mechanisms of Tumor-Associated Immune Surveillance and Escape

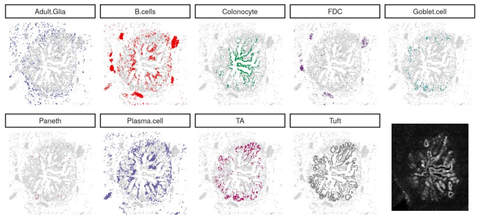


Profiling Whole CRC Resections with CosMx SMI
Autofluorescence (top left) scan compared to PanCK IF staining with CosMx platform. Epithelial (light green, high MFI) vs stromal (dark green, low MFI) tissue. UMAP inset middle.





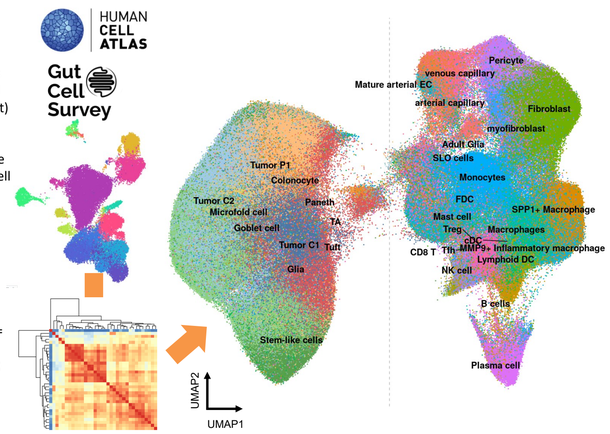
Accurate Cell Typing within Adjacent Normal



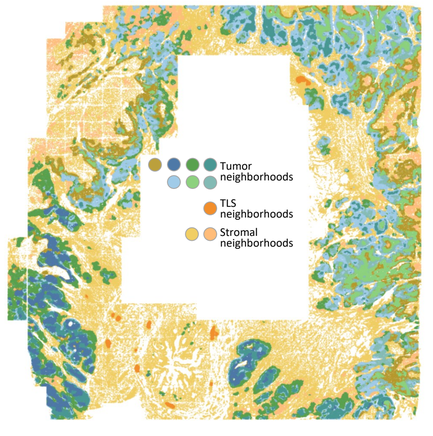
Example cell type maps of adjacent normal tissue found within the sample tissue block (inset box in panel above) confirms accuracy of cell assignment.

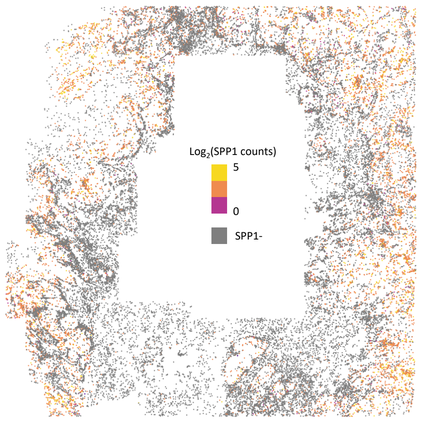
Mapping Healthy & Disease States of Colorectal Cancer

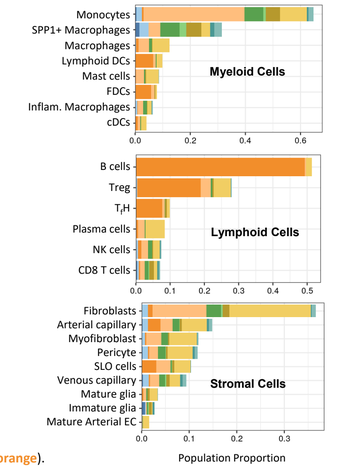
- Top: Physical locations of cell types identified of either epithelial (left) or stromal (right) lineage
- Bottom Left: Reference profile from the Gut Cell Survey was used to identify known cell types in the digestive tract.
- Bottom Right: Semi-supervised clustering (InSituType, bottom) allowed matching previous cell types (n = 28) and identifying novel cell types (n = 6) not captured by the input matrix.



SPP1+ Macrophages Invade Tumor Nests within CRC







Tumor Associated Macrophages Polarize to SPP1+ Phenotype Invading into CRC Tumor Nests
Left: Neighborhood analysis automates classification of tumor nests (**blues**, **greens** and **brown**), surrounding stroma (**yellows**), and lymphoid structures (**orange**).
Center: SPP1 Expression shown for all cells classified as any subset of macrophage
Right: Enrichment of specific lineages in certain compartments. SPP1+ Macrophage subset was enriched specifically in tumor compartment

Conclusions

- **Spatial profiling of 1,000 RNA species at subcellular resolution enables unprecedented views into the dynamics occurring across and within tumors.**
- **In breast, lung, and colon cancer samples, SPP1+ macrophages were associated with tumor nests more consistently than other macrophage subsets.**
- **Ligand-receptor analysis, enabled by the inclusion of 100s of targets captured in spatially resolved data, revealed pockets of elevated tumor-immune engagement within individual tumors.**

Links and References

- He S *et al. High-plex imaging of RNA and proteins at subcellular resolution in fixed tissue by spatial molecular imaging.* Nature Biotechnology 2022
- Danaher *et al. Insitutype: likelihood-based cell typing for single cell spatial transcriptomics.* BioRxiv preprint available
- **InSituType** Package (visit NanoString's Github page for more information)

CosMx SMI Publications