#P350 Constructing comprehensive single-cell spatial atlases across multiple FFPE human tissue using a universal 6,000 plex RNA panel

Rudy van Eijsden, Michael Patrick, Shanshan He, David Kroeppler, Olivia Ho-shing, Patrick Danaher, Jason Reeves, Melanie Moon, Jim Forrester, Trieu My Van, Julian Preciado, Haiyan Zhai and Joseph Beechem

nanoString
A BRUKER COMPANY
NanoString Technologies
530 Fairview Avenue North, Seattle, WA 98109

Abstract

Understanding the single-cell atlas and molecular organization of single-cell spatially resolved tissue is crucial to uncover underlying organ development processes and disease mechanisms. A high-plex RNA panel that has high coverage and works universally on various tissue types provides a powerful tool for researchers to study distinct molecular characteristics at spatial single-cell resolution. We have developed a universal 6K Discovery RNA panel that covers broad biological areas of interest with special emphasis on oncology, immunology, and neuroscience. This panel generates a high number of transcripts-per-cell (often over 1000 transcripts/cell) in intact formalin-fixed paraffin-embedded (FFPE) human tissue from many tissue types.

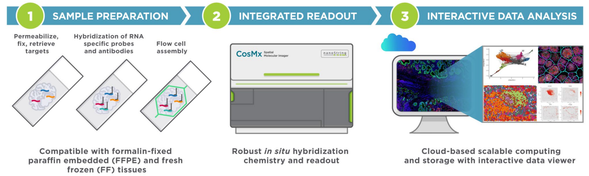
In this study, we used the 6K Discovery RNA panel to characterize eight different FFPE tissue types, including brain, skin, lung, breast, liver, colon, pancreas, and kidney from humans. Four protein markers and DAPI were co-detected on the same tissue slide to identify the morphology in tissues as well as to improve the accuracy of cell segmentation. Also, tertiary analysis algorithms were developed for cell typing, co-localization of genes and ligands, cell-cell interaction, and pathway analysis.

Hundreds of millions of transcripts were simultaneously detected with a Spatial Molecular Imager (SMI) with high sensitivity and specificity on an FFPE tissue section with up to 1 cm^2 scan area. Thousands of genes were detected above the limit of detection (LOD) across each tissue, with single-cell and subcellular resolution. We also constructed the methods to investigate sample-specific spatial neighborhoods, defined by cell types, cell states, nearly a full-reactome set of biological pathways, and over 160,000 ligand-receptor pairwise interactions in each tissue type. Finally, we created the cell type and spatial neighborhood atlas of eight tissue types.

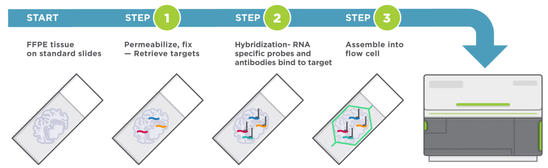
Single-cell spatial measurements at 6,000-plex in a large viewing area on archival tissue with the CosMx™ SMI, coupled with comprehensive tertiary analysis workflow, help researchers in every field to gain a global perspective of spatial transcriptional landscape across multiple FFPE tissue types, enabling the next level of biological discovery and translational research.

Methods

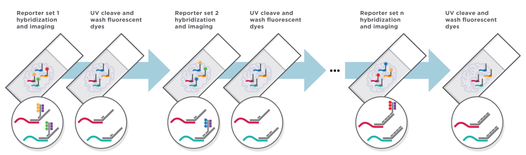
CosMx™ Spatial Molecular Imager (SMI) was used for single-cell imaging



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

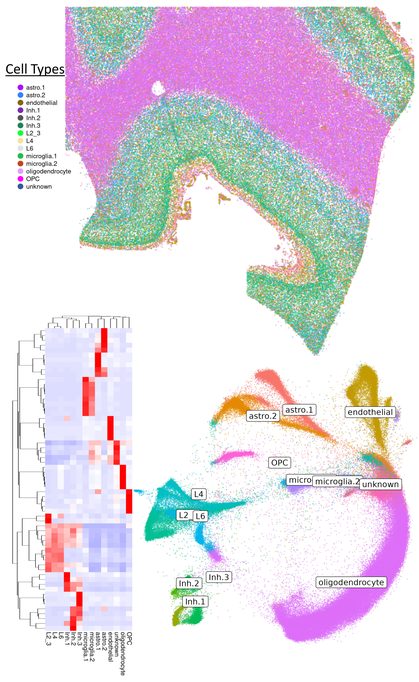


CosMx assay enables efficient single-cell spatial transcriptome profiling in intact FFPE tissue with automatable sample preparation.



Automated Cyclic Chemistry for the *in situ* detection of transcripts

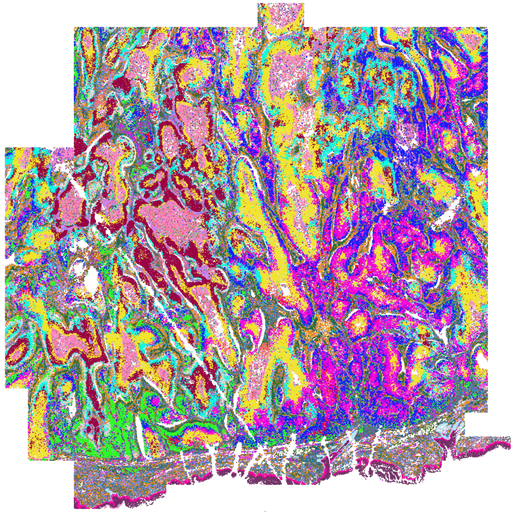
Brain – Frontal Cortex

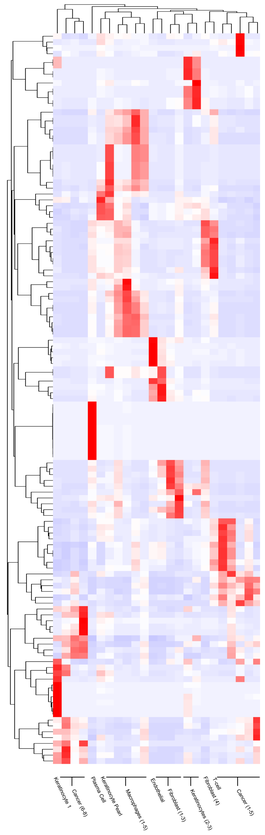


Cortical layering clearly evident in spatial cell typing

Excitatory neurons can be seen in a layered pattern along gray matter. White matter shown to be composed mostly of oligodendrocytes. Astrocytes and microglia are seen dispersed throughout the tissue.

Squamous Cell Carcinoma

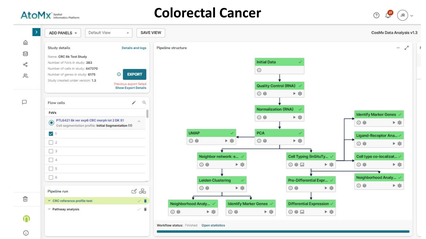


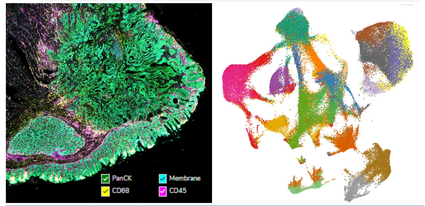


Healthy and cancerous tissue clearly distinguished

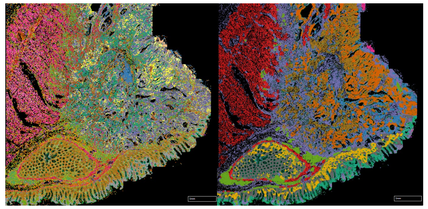
Cancerous cells subcluster into seven distinct spatial population. Epidermal layering of keratinocytes observed in healthy layer of tissue. Cancer forms rings around keratinous pearls.

Data Analysis Natively Enabled with AtoMx

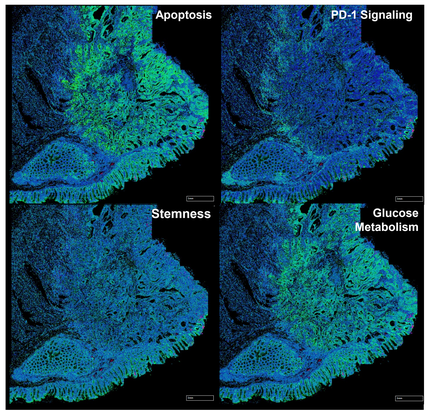




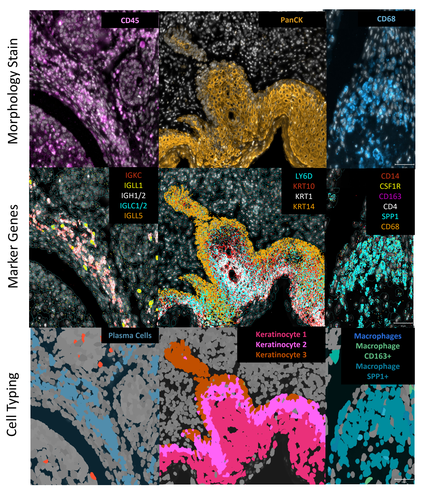
Cell Typing and Niche Analysis



Pathway Enrichment Mapping with 6k

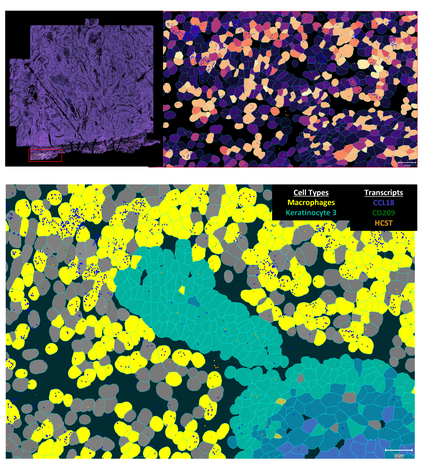


Robust cell typing to enable downstream analysis



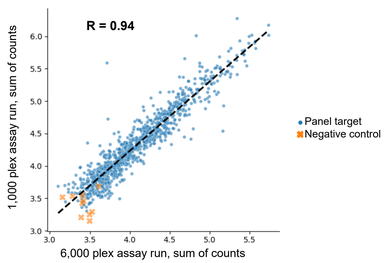
Cell typing is confirmed by the spatial distribution of cell types, marker genes that most differentiate groups of cells, and alignment with protein stains. The figure below demonstrates the colocalization of cell types, marker genes, and stained proteins for three major cell types in cancerous skin.

InSituCor reveals macrophage cytotoxic response to inflamed epidermis



InSituCor identifies gene “hotspots” by organizing the normalized values of gene expression across each cells nearest 50 neighbors, and then grouping spatially correlated genes. This hotspot shows an uptick in genes responsible for antigen presentation, lymphocyte chemotaxis, and cytotoxicity around a cluster of keratinocytes.

6k Assay Performance Across 8 FFPE Tissue Types



Performance of overlapping targets in the 1k and 6k on a CPA with more than 30 cell pellets shows strong concordance between the two assays. The chart on the right shows performance across multiple test tissues

	Mean Counts Per Cell	Genes Detected Per Cell	Genes Above LOD	# Cells
Kidney Cancer	861	531	4,429	388,333
Lung Cancer	695	443	4,087	131,815
Colon Cancer	838	531	4,573	125,588
Frontal Cortex	897	571	3,404	207,261
Skin Cancer	1,656	765	3,632	522,163
Normal Pancreas	939	512	4,354	231,036
Normal Liver	895	490	2,092	59,014
Breast Cancer	1,218	781	4,791	133,569

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