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# Reveal spatial signatures of tumor microenvironment and oncogenic pathways using 6,000-plex single-cell spatial molecular imaging on FFPE tissue

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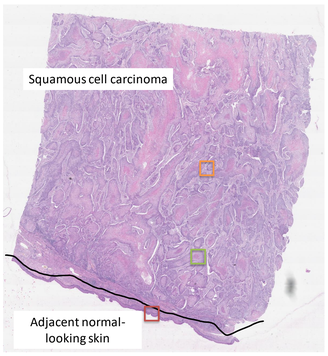
## Background

Tumor progression and therapeutic response are regulated by the tumor microenvironment. Understanding the spatial association and architecture of molecular characteristics and composition of tumor immune microenvironment at single-cell and subcellular resolution encourages improvements in clinical prognosis and immunotherapy benefits. Single-cell spatial profiling technologies permit the study of transcriptional activity at the spatial, single-cell level and provide abundant, high-resolution information required for the identification of clinical-related features in immuno-oncology.

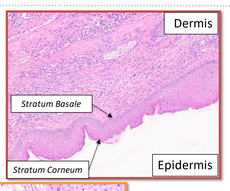
## Method

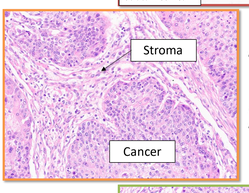
### FFPE cancerous tissue sections were stained with H&E to study tissue structure

Skin squamous cell carcinoma — Grade 2, T3N2bM0, Stage IV



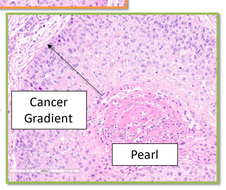

*Adjacent normal-looking epidermis stratified by a gradient of cornification.*



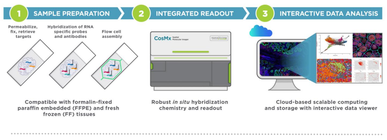

*Complex tissue microenvironment with cancerous growths separated by interstitial stroma made up primarily of fibroblasts.*

*Keratinous pearl with a gradient of cancerous cells radiating outwards as concentric rings.*

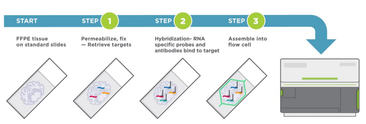



### CosMx™ Spatial Molecular Imager (SMI) was used for single-cell imaging

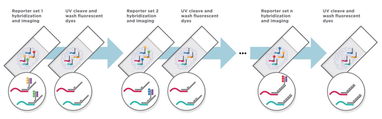
CosMx SMI delivers a comprehensive package which includes validated reagents, instrument, and data analysis software for a seamless sample-to-result workflow.




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

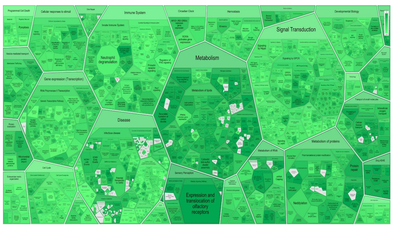



Automated Cyclic Chemistry for the *in situ* detection of transcripts




### 6,000-plex RNA panel was used for in-situ transcripts detection

This high-plex RNA panel provides the broadest coverage available on biological areas with special emphasis on oncology, immunology, and neuroscience.

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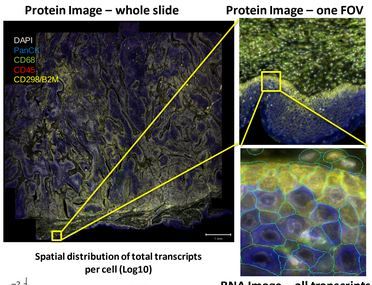
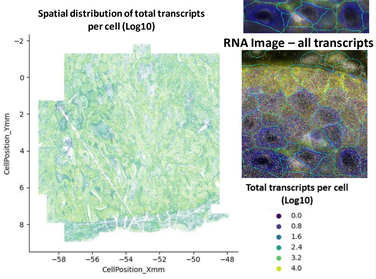
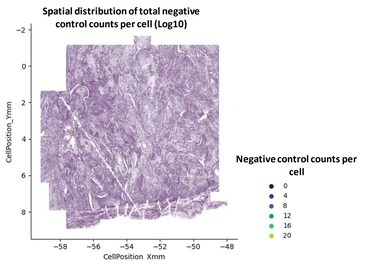
## Results

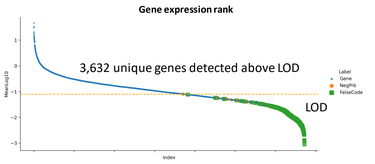
### Co-detection of 6,000 RNAs and 4 proteins on the same intact tissue slide on skin squamous cell carcinoma

~0.5 million cells (522,163) were profiled in the entire 100 mm² intact FFPE skin squamous cell carcinoma tissue within days of the run time. Four protein markers (CD298/B2M, CD45, PanCK, CD68) and DAPI were co-detected with 6,000 unique genes on a single 5 µm section. Up to 5,000 transcripts were detected in single cells.

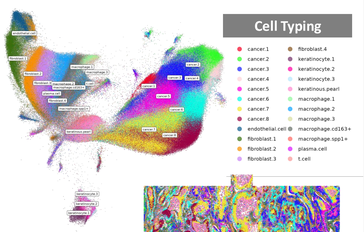


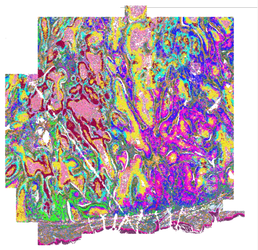



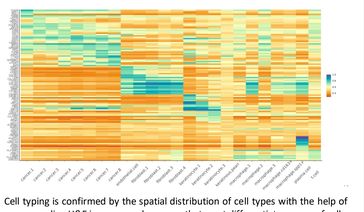

## Tertiary Analysis



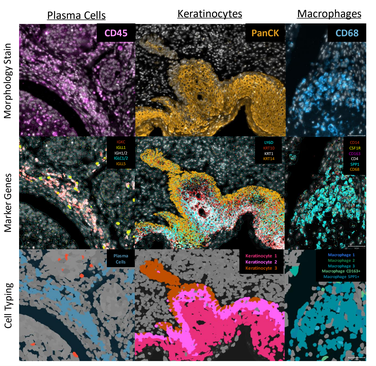



Cancerous cells subcluster into seven distinct spatial population. Spatial transcriptomics allows for the visualization of novel cell type patterns in the complex tissue microenvironment.

### Marker Gene Heatmap



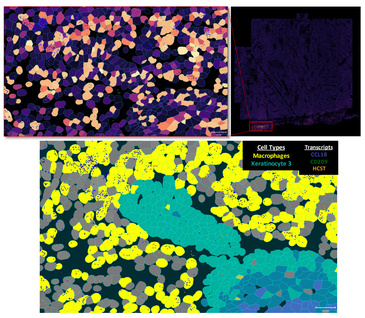
Cell typing is confirmed by the spatial distribution of cell types with the help of corresponding H&E images, marker genes that most differentiate groups of cells, and alignment with protein stains. The figure below demonstrates the colocalization of cell types, marker genes, and stained proteins for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



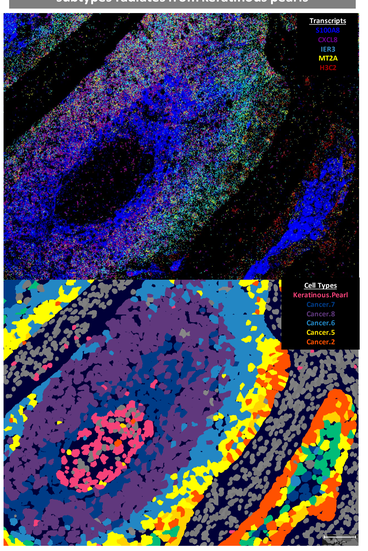

## Downstream Analysis

### 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.

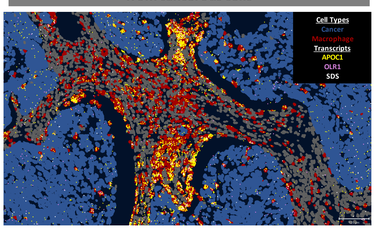



### The gradient of genetic expression among cancer subtypes radiates from keratinous pearls




Cell typing revealed many cancerous subpopulations, which resolved spatially from cells that lacked any defining marker genes. Matched the structure of keratinous pearls, also identified in the H&E staining. Visual inspection of these cancer populations revealed concentric rings, which is underscored by visualizing the most distinct marker genes from each cancer cluster.

### Data analysis enhanced with spatial coordinates of cell types reveals gene expression correlated with distance from cancerous cells




Macrophages were defined as near-cancer if at least one of the ten nearest cells was annotated with a cancer label from cell typing. Average gene profiles of near-cancer and far-from-cancer macrophages were compared, along with profiles of near-cancer macrophages and cancerous cells to eliminate bias from segmentation error. APOC1, OLR1, and SDS were identified by finding the intersection of targets that favored macrophages near cancer relative to both profiles and confirmed with spatial plotting.

## Conclusions

CosMx 6,000-plex panel enables spatial measurements of 6,000 genes simultaneously, with protein co-detection on single archived FFPE tissue with high efficiency and low noise. Along with this panel, the CosMx platform also offers large throughput, profiling 1 million cells across multiplex tissue sections in days. CosMx high-plex technology provides a novel tool to reveal the spatial signature of tumor microenvironment and oncogenic pathways, facilitating the next level of cancer and therapeutic research.

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