Mapping cell type, cell state, and cell-cell interactions with 1000-plex single cell gene expression assay using spatial molecular imaging

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Abstract

One of the great enduring mysteries of biology resides in the intricate conversations between cells, as the cell initiating and the cell receiving any given signal are of high interest but are rarely determinable.
The novel spatial molecular imager (SMI) has the capability to measure transcript abundance with resolution down to the single-cell level. To demonstrate the ability of this platform to interrogate complex cellular interaction, **we have developed a 993-plex gene expression assay that investigates the biology of single cells and their interactions across tissues**.
A comprehensive bioinformatics process was undertaken to curate potential targets using HUGO-compiled gene families, the KEGG BRITE ontology, cross-referencing against Human Cell Landscape scRNA-Seq data, and expert curation based on published studies. To enable cell-type classification across a broad range of tissue types, we mined publicly available scRNA-seq data, finding 243 genes that maximized the contrasts between all pairs of cell types found within each of 14 tissue types.
In cancer tissues, we demonstrate the **ability to robustly define key immune cell types**. Moreover, while this technology is compatible with common clustering or spatial differential expression methodologies, we leverage **knowledgebase-driven analytics to directly detect cell-cell communication and regionally defined interactions**.

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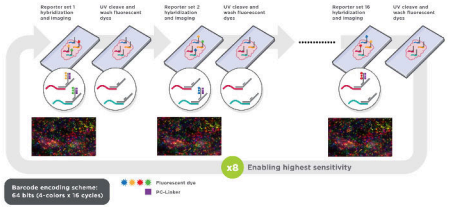
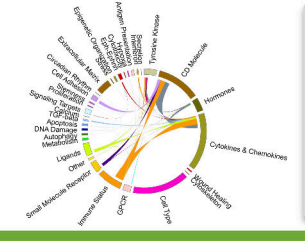
Introduction to Spatial Molecular Imager



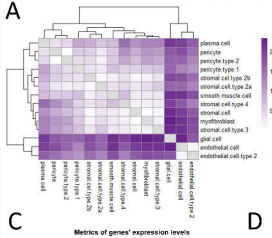
For more information on SMI, see Beechem et al, AGBT 2021

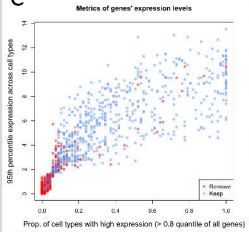
Design of a High Plex Assay for SMI



The design of the ~1000-plex content was divided in two portions: biological content focused on cell signaling and cell state, and cell type content. The Circos diagram summarizes the overlapping genes between the panel's gene sets. Cell type is designed to increase contrast between cell types beyond the biological content.

Design of 1000-Plex RNA Panel for SMI



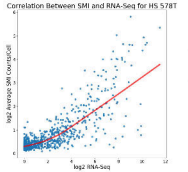


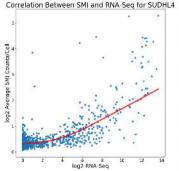
D

Application	# Genes
Cell typing & Mapping	243
Cell state & function	269
Cell-cell interaction	435
Hormone activity	46

A Example of starting point for gene selection, demonstrating which cell types were selected for additional discrimination after accounting for biologically-relevant genes. **B** Example of cell type profiles over complete gene list (per kidney scRNA-seq). Most genes discriminate between cell types. **C** Using scRNA-seq average cell type profiles from 76 Human Cell Landscape datasets, we scored genes for expression level across cell types based on level of expression and consistency of expression across cell types. Genes selected based on scoring metrics are shown. Other genes were considered required based on strong biological relevance. **D** Final gene counts by purpose, categories not mutually exclusive.

SMI Assay Demonstrates High Concordance with RNA-Seq





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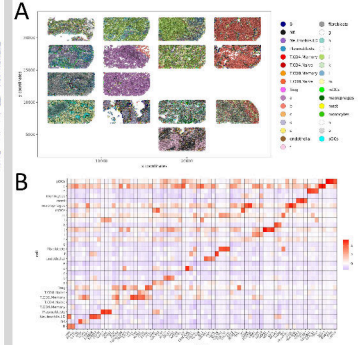
Cell Line	Correlation
HS 578T	0.823
CCRF-CEM	0.760
COLO 205	0.765
HCT-116	0.778
EKVX	0.806
HOP-92	0.808
SUDHL4	0.742
MDA-MB468	0.760
All	0.754

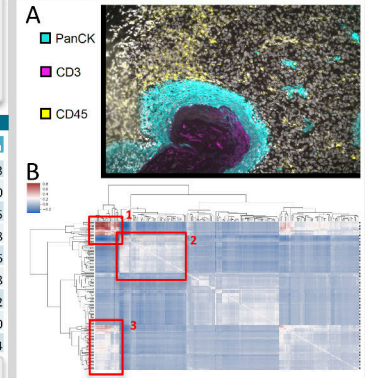
The 1000-plex panel was run against 8 different cell lines from the NCI-60 or CCLE. SMI counts were background-subtracted, averaged, and log2-transformed. These were compared to the log2 transformed, normalized counts from each matched RNA-seq dataset. Scatterplot showing correlation between SMI and RNA-seq for **A** HS-578T and **B** SUDHL4, with Lowess line plotted. **C** Table showing correlation (Pearson's R) between counts for each cell line.

Cell Type Profiling from NSCLC Tissue Microarray



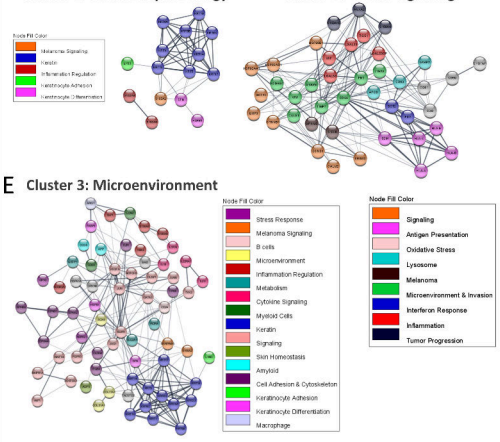
The 1000-plex panel was run against a TMA of NSCLC samples. Cell typing was performed, using methods described in Yang et al, AGBT 2021. **A** Pseudo-colored images demonstrating cell type distributions across the TMA. Robust populations of immune cell types are detected, and new cell type clusters are identified. **B**. Heatmap showing marker genes for each cell type.

Spatially Resolved Gene Networks in Melanoma



A Melanoma sample for SMI assessed by immunofluorescence. **B** Clusters of spatially correlated genes as determined by Lee's L, a measure of bivariate spatial association, are denoted.

Spatially Resolved Gene Networks in Melanoma



Genes from the clusters in **B** were associated using STRING. **C** The first cluster consists exclusively of genes expressed in keratinocytes. This includes keratins, regulators of inflammation known in keratinocytes, molecules involved in differentiation or adhesion of keratinocytes, or melanoma-specific signaling in this cell type. **D** The second cluster shows hallmarks of MAPK, TGF-beta, and Wnt Signaling, including transcriptional targets of those pathways. Tumor progression-related gene ANXA2 and 2 of its binding partners are present: Class I Antigen presentation, Inflammation & interferon response genes are notable. **E** The third cluster is shared between the genes from cluster 1 (keratinocyte related) and others that demonstrate both arms of the immune response, metabolism, and others. Cell adhesion & Cytoskeleton contains a clear signal from the Eph-Ephrin family of receptors. This also contains amyloid precursor protein (APP) and one of its receptors, CD36. The signaling component shows aspects of MAPK, TGF-beta, Wnt, and Notch.

Conclusions

- The SMI 1000-plex assay for assessing tissue neighborhoods was designed to understand interactions at the single cell level and to facilitate cell typing over a broad array of tissue types.
- The SMI 1000-plex assay shows **excellent concordance with publicly available bulk RNA-seq data across a panel of 8 cell types.**
- We have developed methods **capable of localizing cell types across tissues** which accurately capture immune cell types, and provide markers to positively identify others with less certain expression profiles.
- SMI enables **detection of spatially related genes**, highlighting important cell types and functionalities within specific substructures of tissue. This technology helps identify not just what is happening in a tissue, but which cells or regions of tissue are responsible for key functions.