Spatial molecular imager captures melanoma cells' response to their surroundings

Zachary Reitz[1], Patrick Danaher[1], Shanshan He[1], Isabel Lee[1], Kaitlin James[1], Aster Wardhani[1], Dae Kim[1], Joseph Beechem[1]
1. Nanostring Technologies, Seattle WA 98109

nanoString
NanoString Technologies
530 Fairview Avenue North, Seattle, WA 98109

Abstract

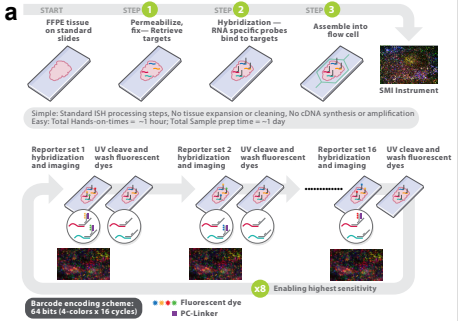
It has thus far been difficult to dissect the behavior of immune cells as a function of the varying conditions within the TME. **We use the NanoString spatial molecular imager (SMI) to profile melanoma FFPE tissue, measuring up to 1000 RNAs for 12000 cells with corresponding spatial locations.** We obtain the following results:

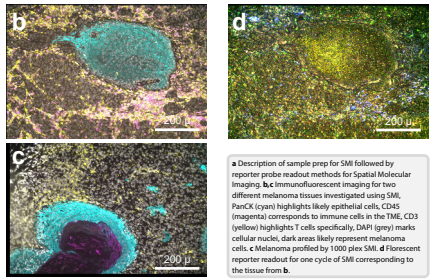
- Epithelial cells remain well segregated from other cell types.
- Immune cells are spatially distinct from melanoma cells.
- Myeloid cells express PDCD1LG2 away from tumor cells.
- T cells express PDCD1 and CTLA4, markers of immune exhaustion.
- Mast cells appear but remain close to nearby endothelial cells.

Spatial molecular imaging reveals the behavior of tumor, stroma, and immune cells in unprecedented detail. **This technology is uniquely capable of detecting pathways of immune modulation employed by only small subsets of cells in precise spatial contexts.**

For research use only. Not for use in diagnostic procedures.

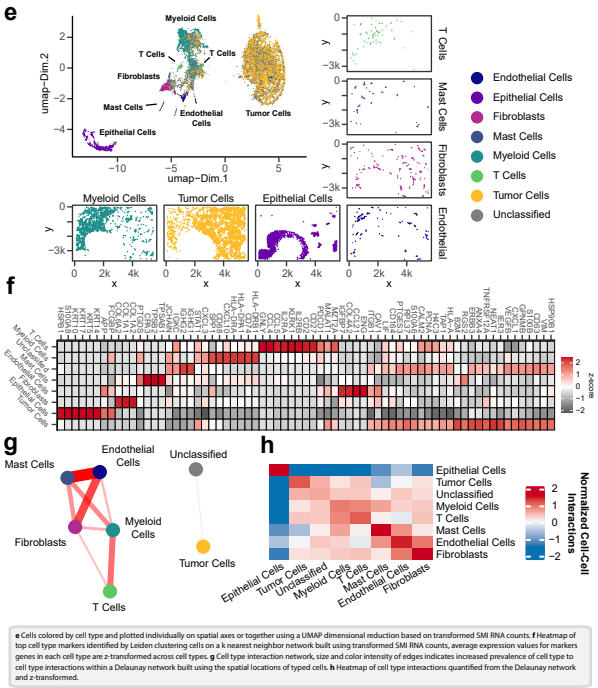
1. SMI Quantifies 1000 RNAs in FFPE Melanoma Tissue





a Description of sample prep for SMI followed by reporter probe readout methods for Spatial Molecular Imaging. **b, c** Immunofluorescent imaging for two different melanoma tissues investigated using SMI, PanCK (cyan) highlights likely epithelial cells, CD45 (magenta) corresponds to immune cells in the TME, CD3 (yellow) highlights T cells specifically, DAPI (grey) marks cellular nuclei, dark areas likely represent melanoma cells. **c** Melanoma profiled by 1000 plex SMI. **d** Florescent reporter readout for one cycle of SMI corresponding to the tissue from **b**.

2. High Plex SMI Enables Cell Typing and Cell-Cell Interaction Analysis



e Cells colored by cell type and plotted individually on spatial axes or together using a UMAP dimensional reduction based on transformed SMI RNA counts. **f** Heatmap of top cell type markers identified by Leiden clustering cells on a k nearest neighbor network built using transformed SMI RNA counts, average expression values for markers genes in each cell type are z-transformed across cell types. **g** Cell type interaction network, size and color intensity of edges indicates increased prevalence of cell type to cell type interactions within a Delaunay network built using the spatial locations of typed cells. **h** Heatmap of cell type interactions quantified from the Delaunay network and z-transformed.

SMI Allows Single Cell Spatial Interactions to be Quantified in Melanoma

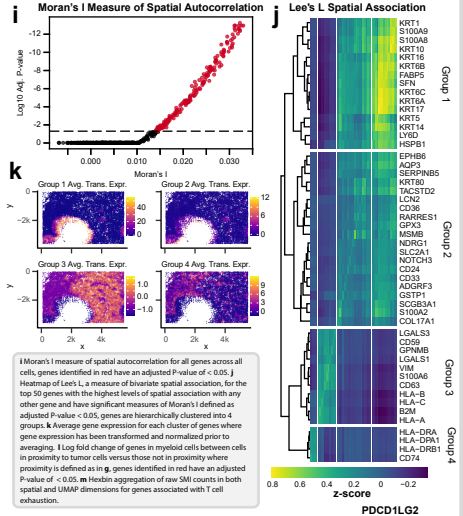
SMI as a platform integrates traditional immuno-fluorescent imaging with high plex, spatially resolved gene expression data.

- PanCK indicates normal epithelial cells. (**fig b & c**)
- Epithelial cells bordering a dark area may denote a hair follicle. (**fig c**)
- CD45 and/or CD3 indicate immune infiltration within these melanomas. (**fig b & c**)
- **High plex single cell RNA quantification is performed on the same tissue. (fig c)**

The melanoma TME contains a diverse number of cell types that are often spatially segregated.

- Cells are clustered and labeled by their most probable cell type. (**fig e**)
- Epithelial cells remain highly segregated in physical and UMAP space. (**fig c & e**)
- Immune cells are distinct in UMAP space and physically interdigitated amongst melanoma cells (**fig c & e**)
- **Cell type interactions can be quantified**, confirming the above results (**fig g & h**)

3. SMI Empowers Spatial Expression Analysis in the TME



i Moran's I measure of spatial autocorrelation for all genes across all cells, genes identified in red have an adjusted P-value of < 0.05. **j** Heatmap of Lee's L, a measure of bivariate spatial association, for the top 50 genes with the highest levels of spatial association with any other gene and have significant measures of Moran's I defined as adjusted P-value < 0.05, genes are hierarchically clustered into 4 groups. **k** Average gene expression for each cluster of genes where gene expression has been transformed and normalized prior to averaging. **l** Log fold change of genes in myeloid cells between cells in proximity to tumor cells versus those not in proximity where proximity is defined as in **g**, genes identified in red have an adjusted P-value of < 0.05. **m** Hexbin aggregation of raw SMI counts in both spatial and UMAP dimensions for genes associated with T cell exhaustion.

SMI Identifies Spatially Variable Genes

Given the spatial segregation of cells by type, unbiased approaches of spatial expression analysis are likely to detect genes that vary by cell type. In this melanoma tissue we found:

- **626 significantly autocorrelated genes**. (**fig i**)
- Four **groups of genes clustered by spatial association.** (**fig j & k**)
- Groups 1 and 2 align to different layers of the epithelium. (**fig k**)
- Groups 3 and 4 align to tumor cells or immune cells respectively. (**fig k**)
- Many of these genes overlap with identified cell type markers. (**fig f & j**)

Spatial expression analysis needs to incorporate cell type information to probe biology that goes beyond cell type.

- **When we classify myeloid cells based on their proximity to melanoma cells, we find 216 significant differentially expressed genes.** (**fig l**)
- PDCD1LG2, an immune checkpoint gene, is expressed in myeloid cells away from tumor cells. (**fig l & m**)
- PDCD1 and CTLA4, markers of immune exhaustion, are expressed in T cells in immune dense regions. (**fig m**)

Methods

SMI was carried out as in (fig a) and elsewhere (Beechem et. al AGBT 2021). 960 genes remained after removing high expressors from measurement. Cell typing using SCTransform (Hafemeister & Satija 2019), Leiden clustering, scran (Lung et al. 2016), Giotto (Dries et al. 2020), and PanglaoDB (Franzén 2019). Spatial expression analysis was done using Moran's I and Lee's L (Lee 2001) as well as Giotto.

Conclusions

SMI applied to melanoma facilitates the following conclusions:

- SMI measures **high plex single cell gene expression** in melanoma tissue
- Epithelial, immune, and tumor cell typing and **marker identification matches immuno-fluorescent images**
- Spatial cell type interactions can be qualitatively observed and quantified
- Unbiased spatial expression identifies gene expression patterns driven by cell type
- **PDCD1LG2-expressing myeloid cells may influence T cell exhaustion** in immune rich regions of melanoma