Custom morphology markers allow better tissue stratification to study tumor heterogeneity using NanoString® GeoMx® DSP Platform

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Background

- Understanding tissue heterogeneity is a key goal in oncology research and new spatial technologies are now available to address this.
- NanoString GeoMx DSP uses fluorescently labeled morphology markers to guide selection of regions of interest on tissue samples so molecular profiling can be done with spatial context.
- NanoString's standard markers broadly target tumor and immune cells within tissue.
- Canopy Biosciences' ROI Selection markers enable more precise morphological analysis of tissue samples, specific for sample type, disease indication, cell type and study goal.
- To evaluate the capability of GeoMx DSP to selectively enrich specific cell types, tissue samples were analyzed with relevant markers and transcriptional profiles were compared.
- Custom morphology markers enable better tissue stratification providing more meaningful gene expression analysis data.

Methods

- Here we present the analysis of human tonsil and lung carcinoma FFPE samples analyzed using the NanoString® GeoMx® DSP platform using the Whole Transcriptome Atlas (WTA) panel for molecular profiling.
- Samples were stained with fluorescent antibodies for relevant tissue markers and regions of interest (ROIs) were selected based on indication and segmentation goals.
- Probes from these ROIs were collected and analyzed using an Illumina Novaseq NGS instrument.
- Data was generated in the GeoMx DSP Software Analysis Suite using pre-determined comparisons for specific targets or cell types.

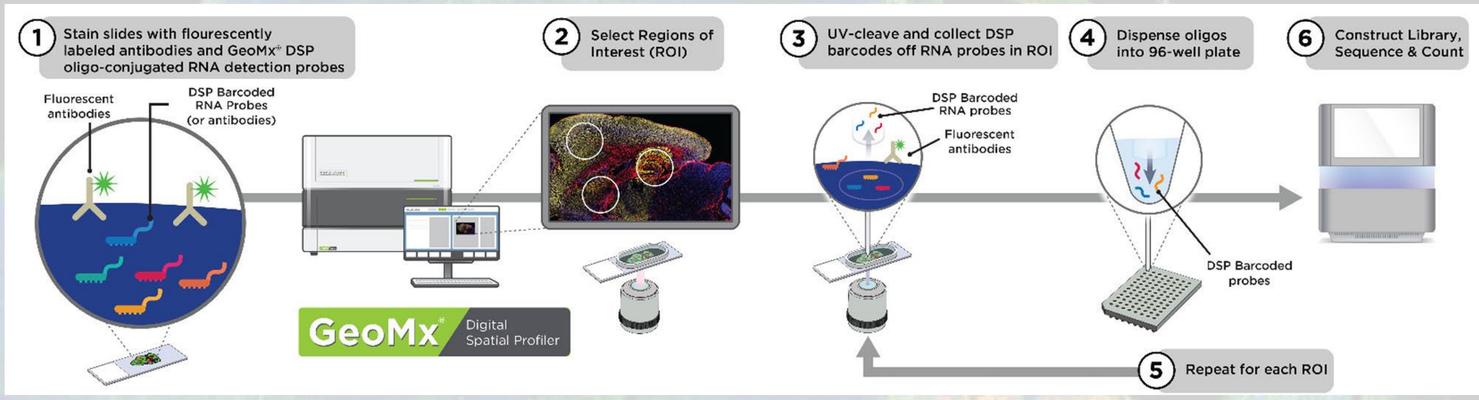

Figure 1. NanoString GeoMx workflow. Slides are stained with morphology markers to highlight tissue architecture and marker-specific regions. ROIs are selected based on imaging. Probes are collected from ROIs for molecular profiling via NGS.

Table 1. Sample list with segmentation strategy and comparison list for molecular profiling analysis

Sample	Tonsil #1	Tonsil #2	Lung Carcinoma
Segmentation Strategy	CD3+ (T cells) CD20+ (B cells) CD68+ (Macrophages) DNA+ (Remaining cell types)	CD4+ (T helper cells) CD8+ (Cytotoxic cells) CD20+ (B cells) DNA+ (Remaining cell types)	TTF-1+ (Adenocarcinoma cells) p40+ (Squamous cell carcinoma) CD45+ (Immune cells) DNA+ (Remaining cell types)
Transcriptional Profiling Comparisons	Each cell type vs all other cell types	Each cell type vs all other cell types	Adenocarcinoma vs Squamous cell carcinoma Immune cells vs other cells

Results

- Fluorescence imaging and segmentation on the GeoMx system (Figure 1 and Figure 2) can effectively select and enrich for cell types, even in cell-dense environments.
- Compartments selecting for CD8+ and CD4+ cells in the same tonsil areas show differences in expression of numerous genes (Figure 3). This includes key T-cell maturation factors, the CD3 complex.
- Using the WTA expression profile of segments in each selected area, a spatial deconvolution in the GeoMx analysis suite determined the most likely representation of cell types for each segment (Figure 4). This showed enrichment for the expected cells types (e.g. CD3: T-cells, CD20: B-cells, CD68: macrophages).
- Adenocarcinoma and squamous cell carcinoma in a single lung tissue, selected through TTF-1 or p40 staining show differential RNA expression (Figure 5).

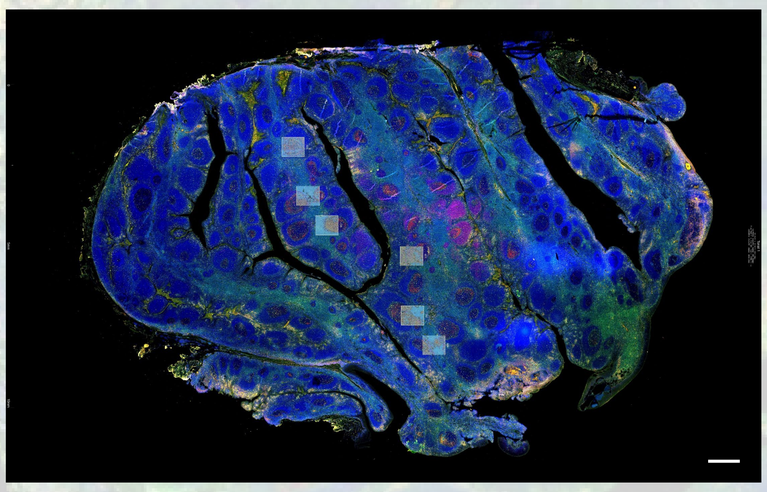

Figure 1. Tonsil #1 whole tissue fluorescence image with biomarkers: DNA (blue), CD3 (green), CD20 (red), CD68 (yellow). White scale bar is 1 mm. Greyed boxes are ROI selections.

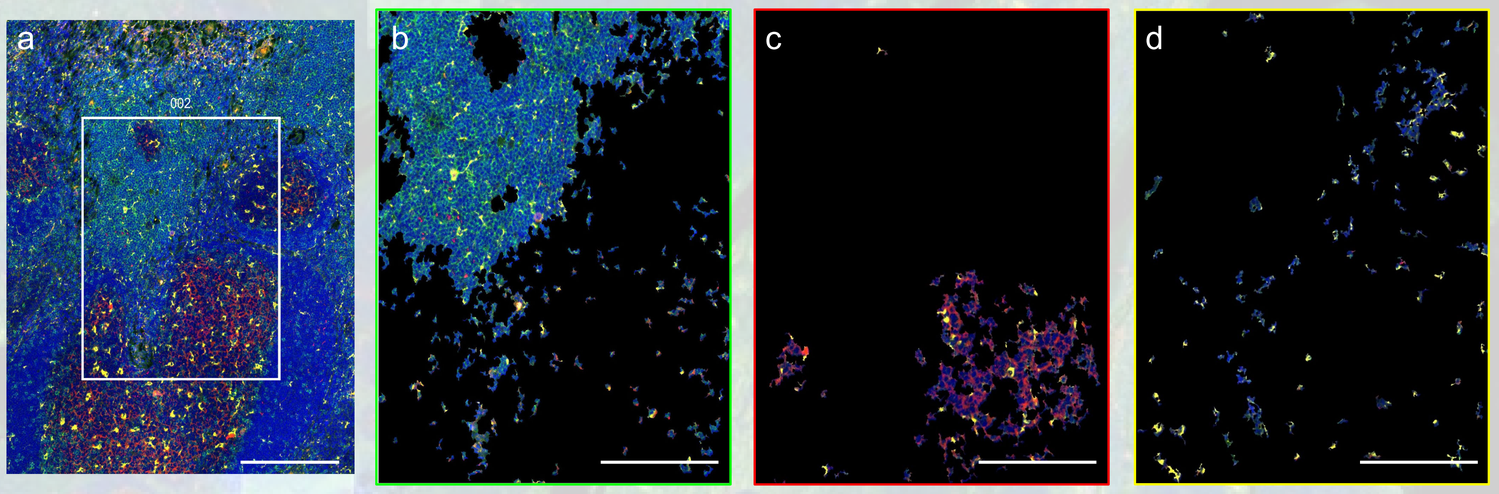

Figure 2. (a) Representative tonsil #1 ROI outlined within the white box. Fluorescence colors are conserved from Figure 1. White scale bar = 300 µm, **(b)** CD3+ segment of ROI in (a). Scale bar = 200µm, **(c)** CD20+ segment of ROI in (a). Scale bar = 200µm, **(d)** CD68+ segment of ROI in (a). Scale bar = 200µm.

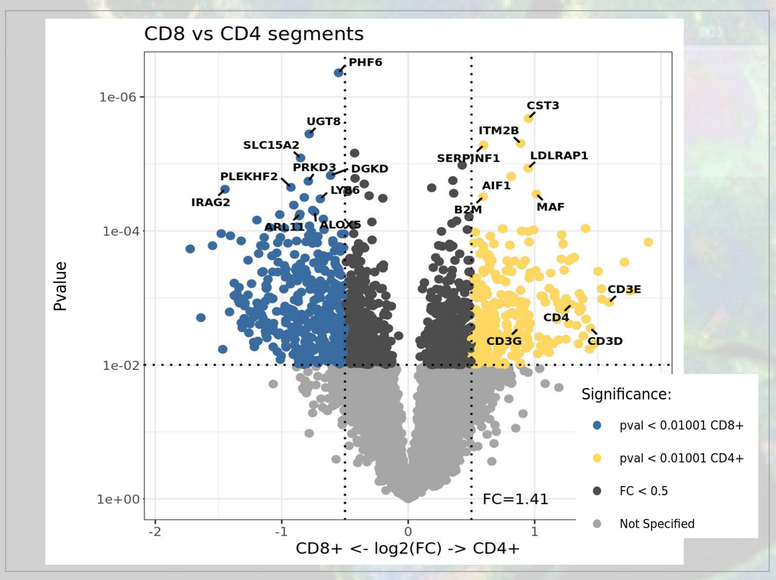

Figure 3. Volcano plot showing differentially expressed RNAs between CD8+ and CD4+ cell compartments in Tonsil #2. Labeled genes are the twenty most significant with altered expression between compartments and four genes essential to T-cell maturation.

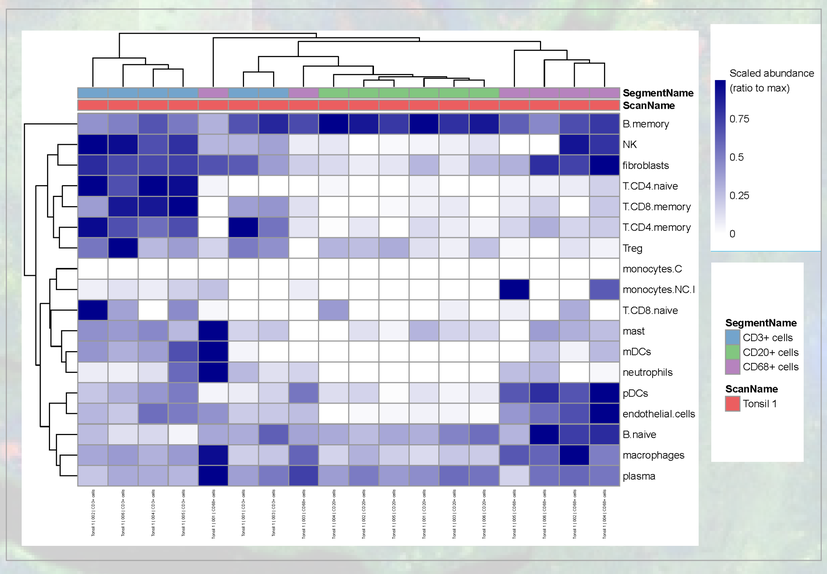

Figure 4. Spatial deconvolution heat map used to compare relative representations of cell types within CD3+, CD20+, and CD68+ compartments of Tonsil #1. All three compartments show enrichment of expected cell types. Matrix is comprised of separate ROIs for each segment, and cell type.

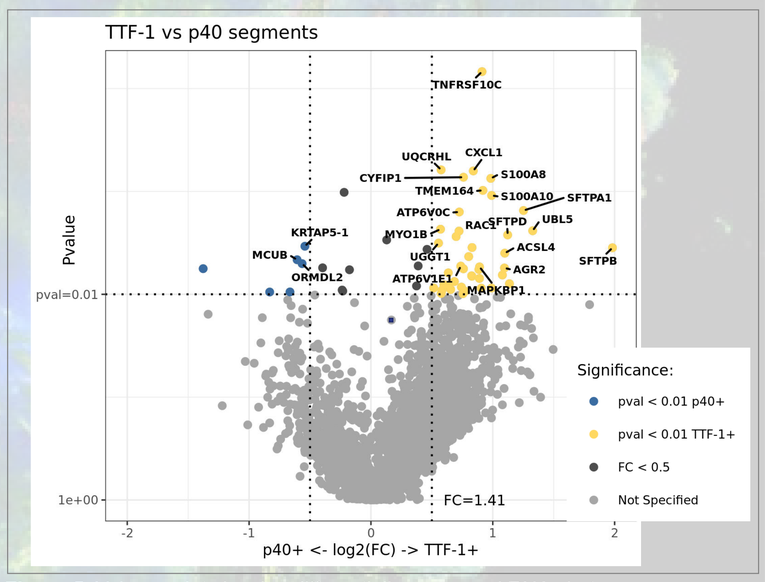

Figure 5. Volcano plot showing differentially expressed RNAs between adenocarcinoma (TTF-1 positive) and squamous cell carcinoma (p40 positive) compartments in Lung sample.

Conclusions

- Identification of CD8+ and CD4+ compartment RNA expression in a tonsil sample reveals differential RNA levels for many genes. Recognition of known lymphocyte differentiation factors, genes with disease causing variants, and as-of-yet unimplicated genes in immunity all show differential expression levels. The identification of these unimplicated genes highlights the potential of GeoMx DSP to guide research, and to answer questions that non-spatial techniques can not.
- Use of the Spatial Deconvolution within the GeoMx DSP analysis suite allowed identification and confirmed enrichment of expected cell types based on markers selected for each compartment. This demonstrates the utility of GeoMx DSP in characterizing the cellular makeup of tissue beyond the markers used for compartment and ROI selections.
- The GeoMx DSP was able to show that two different types of carcinoma, present within the same tissue displayed significantly different expression of genes. The ability to study specific tumor types within single samples rather than at the patient or organ level can expand therapy development, and tie survivability markers to expression of specific genes/cell types.

Presenter Bio

Jessica Runyon is the Director of Product Management for Canopy Biosciences, specializing in CRO Services including NanoString GeoMx and nCounter, single-cell RNA Seq, IHC, Hisopathology and Gene Expression Analysis. She received her Masters in Biochemistry from Boston University School of Medicine focusing on epigenetics.