

Developing ROI Selection Markers for GeoMx® Digital Spatial Profiling

Highlights

- NanoString® GeoMx® Digital Spatial Profiler (DSP) enables high-plex spatial profiling of protein or RNA targets
- Morphology markers are fluorescently labeled antibodies that enable ROI-guided analyses of transcriptional and proteomic pathways
- Morphology Marker Kits from NanoString are limited to a few markers that broadly identify immune and tumor compartments
- Canopy Biosciences® ROI Selection Markers expand the available markers to critical targets in immuno-oncology and immunology research for more informed ROI selection

Introduction

A common question for which GeoMx Digital Spatial Profiling (DSP) is employed is the identification of changes in the tissue and tumor microenvironment in relation to therapeutic response. Understanding the structure of the tissue is a critical step in maximizing biological insights for successful spatial transcriptomics experiment (Appelbe et al., 2021). Morphology markers reveal tissue architecture and offer insight into selecting the appropriate ROIs to make meaningful analyses.

However, widespread adoption of GeoMx DSP has revealed a limitation in the approach – namely a lack of morphology markers. Researchers base transcriptional analysis of thousands of RNA targets on the spatial information provided by only a few morphology markers. For example, the Solid Tumor TME Morphology Kit contains only general

lineage markers including Pan-CK and CD45 to broadly distinguish immune cells from epithelial tissue. Researchers have identified a need to expand available morphology markers to further segment tissues to support hypothesis-driven research.

To address this, Canopy Biosciences is developing pre-validated ROI Selection Markers for use with GeoMx DSP. Initially, the catalog will include relevant markers in immuno-oncology and drug target research. Later work will expand the catalog into additional oncology and neuroscience applications. New markers will be available to augment off-the-shelf kits or as part of a complete set of customized markers based on study goals.

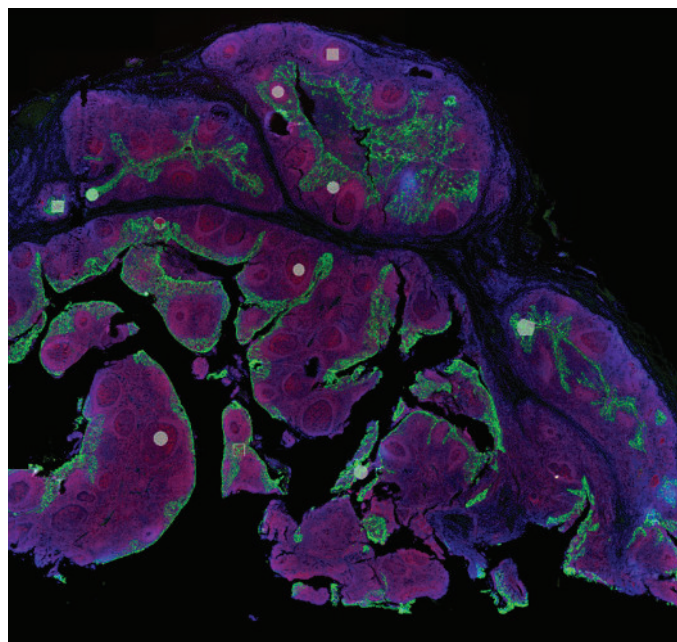


Figure 1. Whole-slide image of tonsil FFPE tissue stained with morphology markers to guide ROI selection in GeoMx DSP assay. (Source: Canopy Biosciences)

Identifying Fluorescently Labeled Antibodies for Validation

GeoMx DSP is a fluorescent imaging system that uses standard filter sets for FITC, Cy3, Texas Red, and Cy5 channels (Figure 2). Antibodies are conjugated to a fluorophore compatible with one of these filter sets, to enable visualization of tissue structures. However, off-the-shelf kits make use of only three channels. NanoString Morphology Marker kits provide the DNA stain detected in the FITC channel and two morphology markers detected in the Cy3 and Texas Red channels, leaving the Cy5 channel open for additional markers.

GeoMx DSP is critical, since the processing protocol for GeoMx RNA assays includes a significant Proteinase K treatment that can reduce the presence of specific epitopes of certain antibody clones.

Markers were tested following the same approach presented in the Morphology Marker Guidelines from NanoString (Appelbe et al., 2021). Qualified markers demonstrate expected staining pattern typical in a single tissue, while verified markers have undergone testing on multiple tissues and were successfully used for ROI segmentation. Our initial testing of immunoncology markers resulted in a mix of both qualified and verified antibodies.

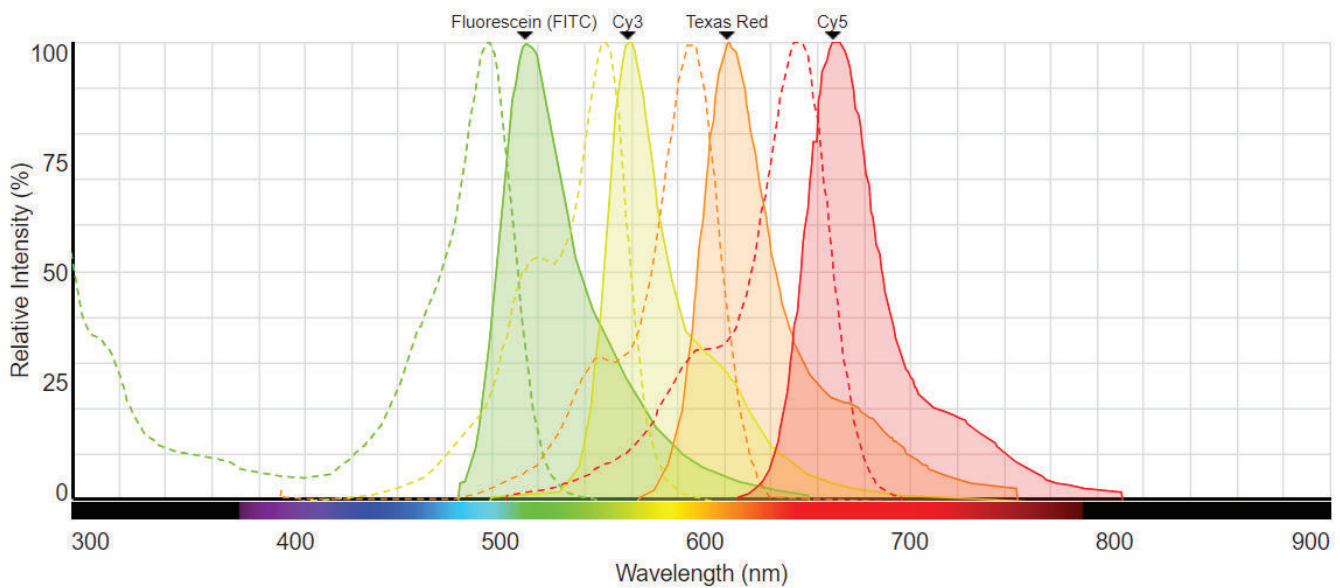


Figure 2. GeoMx DSP is designed for fluorescent imaging in FITC, Cy3, Texas Red, and Cy5 channels. (Source: ThermoFisher SpectraViewer)

Marker Verification and Qualification

Marker development involves sourcing commercially available antibodies – labeled with specific fluorescent tags – and evaluating their suitability for staining tissues with conditions compatible with subsequent transcriptomic analysis. Qualifying custom markers for

Furthermore, target-specific positive tissue staining was verified by an experienced pathologist. Antibody dilution was optimized to assess specificity and reduce background fluorescence. Canopy Biosciences applies a decade’s worth of expertise in assay development and histology experience to develop a catalog of pre-validated ROI Selection Markers for GeoMx DSP.

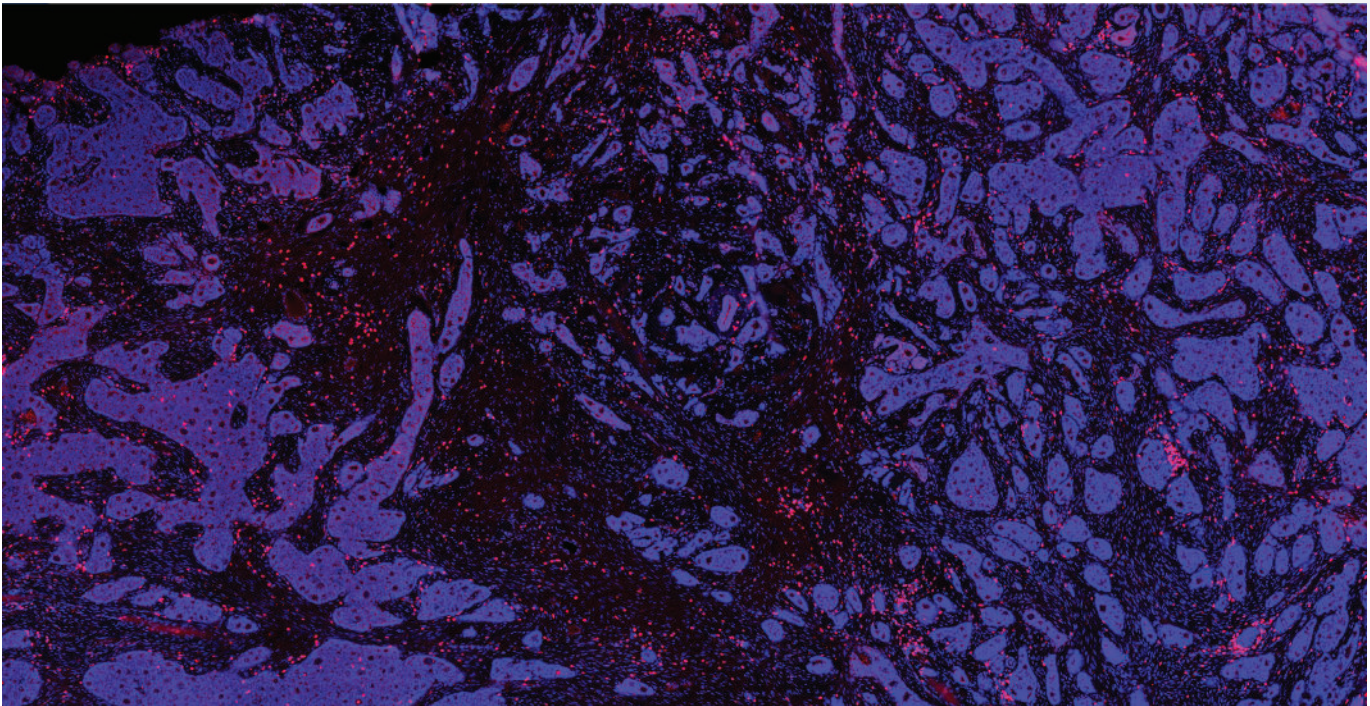


Figure 3. Whole-slide image of CD3 marker in tonsil FFPE tissue. CD3 marker demonstrated expected staining to CD3+ T cells. CD3 in red; SYTO13 DNA dye in blue. (Source: Canopy Biosciences)

Immuno-oncology Markers are the First Added to the Catalog

The initial priority of the project was the addition of a set of qualified immuno-oncology markers, including markers for T-cells (CD3) and macrophages (CD68) among others. These were some of the first markers requested by researchers for custom addition to the Solid Tumor TME Morphology Kit and have been validated in tonsil, breast cancer, and colorectal cancer tissue by Canopy Biosciences. Figure 3 and 4 initial qualification of these markers in tonsil FFPE tissue.

Both CD3 and CD68 differentiate alternate immune cell phenotypes, facilitating ROI selection based on hypotheses about specific cell types. Researchers can now determine whether gene expression of a signaling pathway correlates with T-cell or macrophage-rich tissue regions. Canopy Biosciences® ROI Selection Markers

enable immune cell subtyping beyond what is currently possible with off-the-shelf Morphology Marker Kits from NanoString.

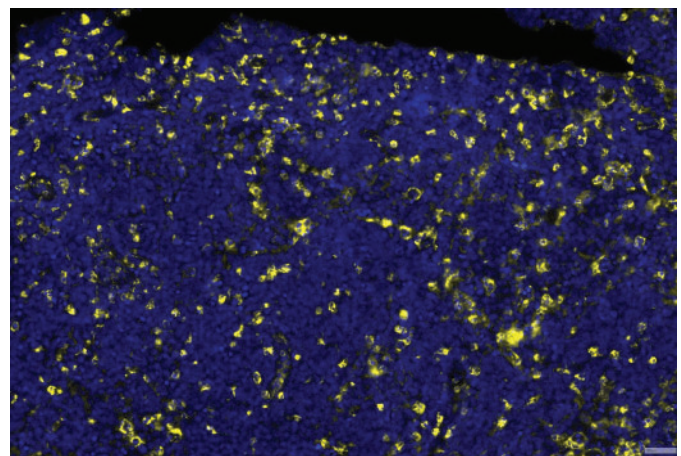


Figure 4. Whole-slide image of CD68 marker in tonsil FFPE tissue. CD68 marker demonstrated expected staining to CD68+ macrophages. CD68 in yellow, SYTO13 DNA dye in blue. (Source: Canopy Biosciences)

Following initial qualification by image review, markers are verified for ROI segmentation. Analysis of Cancer Transcriptome Atlas data shows good correlation between cell type abundance determined by RNA expression in tissue segments where those cell types are expected (Figure 5). The data shows enrichment of macrophages in CD68 segments, T-cells in the CD3 segment, and lack of both cell types in the Pan-CK segment.

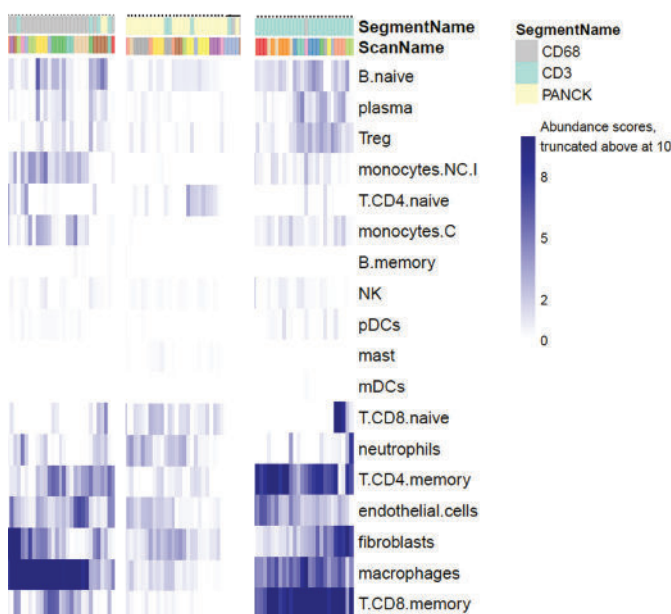


Figure 5. RNA abundance by ROI segmentation in breast cancer tissue. Left panel: CD68 segments. Middle panel: CD3 segment. Right panel: Pan-CK segment. (Source: Canopy Biosciences)

The data reveal that community verified markers are sufficiently sensitive for ROI selection in GeoMx assays. This is an important feature given how few morphology markers are currently available to researchers.

Summary

Molecular and cellular profiling of the tissue microenvironment requires a technology that offers spatial context of cells. NanoString GeoMx Digital Spatial Profiling enables high-plex spatial resolution of protein or RNA targets using fluorescently labeled antibodies as morphology markers for ROI-guided analysis. Yet, researchers have identified a need to expand beyond general lineage markers to further segment tissues for hypothesis-driven research. Here, we describe how Canopy Biosciences applies a decade worth of expertise in antibody validation to develop markers for immuno-oncology applications to augment off-the-shelf kits.

In conclusion, Canopy Biosciences is developing a catalog of markers for use in GeoMx DSP assays. Canopy Biosciences® ROI Selection Markers will enable more researchers to take advantage of spatially resolved, high-plex transcriptomic profiling.

References

1. Appelbe, O. K., Rhodes, M., & Fuhrman, K. (2021). Morphology Marker Guidelines. 8.
2. Bergholtz, H., Carter, J., Cesano, A., Cheang, M., Church, S., Divakar, P., ... on behalf of the GeoMx Breast Cancer Consortium. (2021). Best Practices for Spatial Profiling for Breast Cancer Research with the GeoMx® Digital Spatial Profiler. *Cancers*, 13(17), 4456. <https://doi.org/10.3390/cancers13174456>
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MARCH 2022 V2