Pathway enrichment analysis of gene expression data from formalin-fixed paraffin embedded (FFPE) samples using the GeoMx® DSP Platform

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Summary

The GeoMx Digital Spatial Profiling (DSP) Platform enables robust detection of high-plex protein and RNA expression from user-defined sections within FFPE samples. As the number of targets detected within such tissues increases, it becomes important to apply systems biology strategies in order to better interpret the complex biology of the tumor microenvironment.

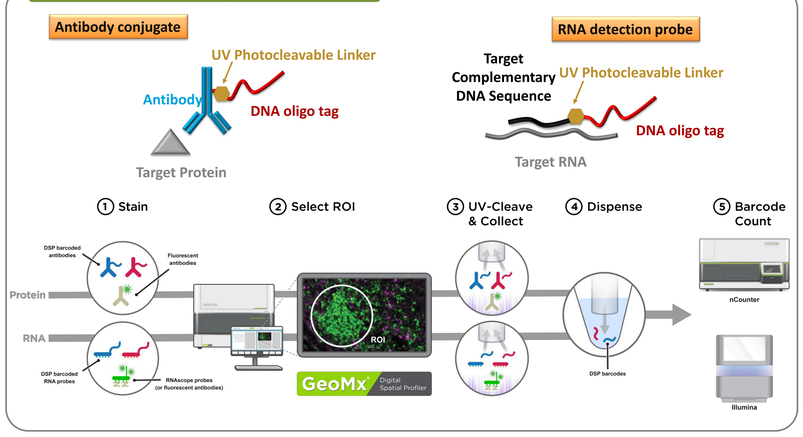
In this study, we investigated the expression profiles of more than 1600 genes by utilizing more than 10,000 DSP-specific in situ hybridized (ISH) probes on FFPE samples. The bioinformatics tools we have development are enabling us to move beyond the single-gene profiling to a better understanding of pathway-based expression. Our study includes colorectal cancer patient samples from which we have matching bulk RNA sequencing and NanoString analysis to compare. The panel of genes profiled have a strong focus around capturing biological signaling along canonical signaling pathways and cell-intrinsic signaling from immune cells and other cell types. We demonstrate the ability to leverage foundational pathway interrogation tools, including Reactome, with the data to capture spatially-resolved pathway interactions and signaling within FFPE tissues.

As we look towards the future of the GeoMx platform and high-plex RNA profiling of tissue samples, these experiments highlight not only the need but the capacity for this platform to derive deep understanding of the biology within and across a single slide of tissue. These experiments are being used to drive development of the software features within the GeoMx ecosystem, which will provide further support for pathway-level exploration of expression when working with highly multiplexed reagents in future platform offerings.

GeoMx DSP technology is for Research Use Only and not for use in diagnostic procedures.

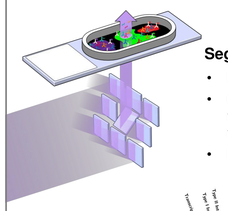
Introduction

GeoMx DSP chemistry and workflow



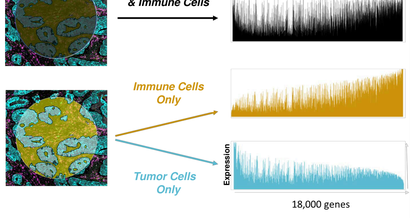
Flexible Illumination Paths Enable Complex Interrogation of TME

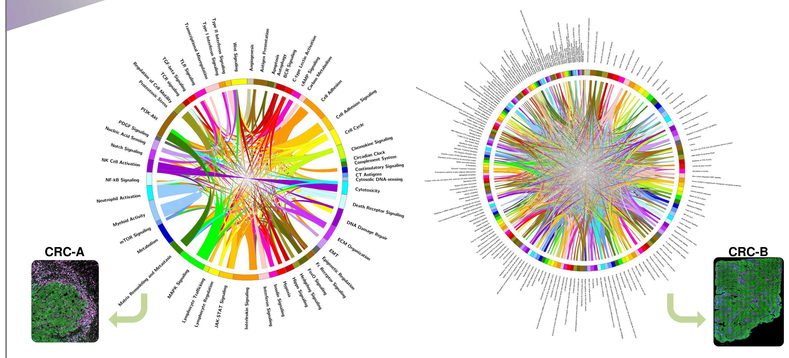
The GeoMx™ DSP contains a tunable micromirror array which enables profiling of distinct tissue compartments and complex morphological structures.



Segments are

- User defined
- Guided by IF channels to specifically sample tissue morphology
- Dynamically adjustable





The **Cancer Transcriptome Atlas (CTA)** contains over 1,800 genes, representing comprehensive coverage of the tumor, immune system, and microenvironment in cancer.

The **Whole Transcriptome Atlas** contains over 18,000 genes, covering an optimized set of protein coding genes in the human transcriptome.

Figure 1. Circos plots of the Cancer and Whole Transcriptome Atlas Content by Pathway
Annotations are sourced from Reactome (reactome.org) and associated with the genes from each panel. Connections between annotations represent shared gene content exceeding 15 genes.

Conclusion

- Pathway analysis methods, including ORA, GSEA, and ssGSEA, can be employed with spatial analysis using the Cancer and Whole Transcriptome Atlas products
- Differential pathway activation is evident within samples, and both across segments and amongst them
- Spatial pathway analysis reveals key immunological activation as cells transit across the invasive margin

Results

Analysis of the Tumor Invasive Margin Demonstrates Dynamic Activation of the Immune System

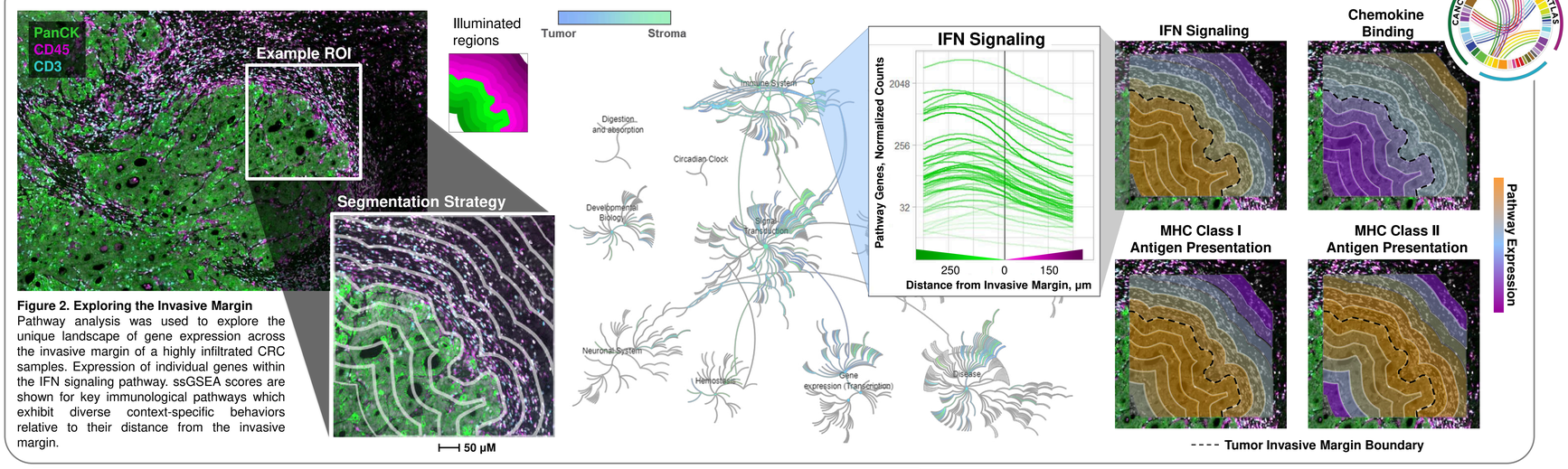


Figure 2. Exploring the Invasive Margin
Pathway analysis was used to explore the unique landscape of gene expression across the invasive margin of a highly infiltrated CRC samples. Expression of individual genes within the IFN signaling pathway. ssGSEA scores are shown for key immunological pathways which exhibit diverse context-specific behaviors relative to their distance from the invasive margin.

GeoMx Transcriptome Profiling of Spatially-resolved Colorectal Cancer Samples Enables Pathway Analysis

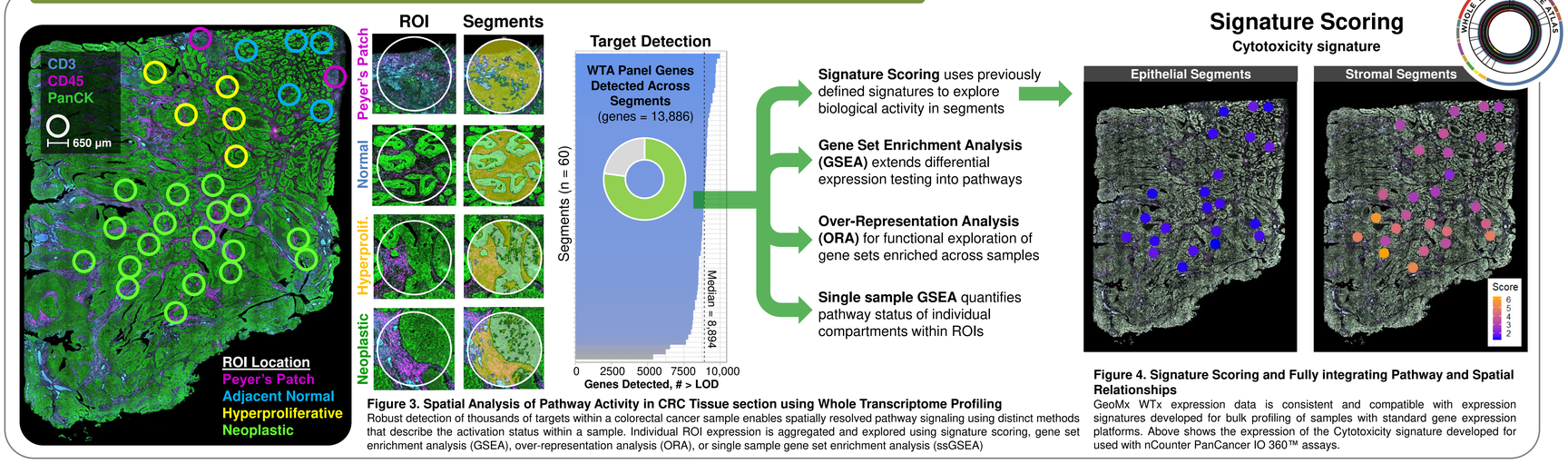


Figure 3. Spatial Analysis of Pathway Activity in CRC Tissue section using Whole Transcriptome Profiling
Robust detection of thousands of targets within a colorectal cancer sample enables spatially resolved pathway signaling using distinct methods that describe the activation status within a sample. Individual ROI expression is aggregated and explored using signature scoring, gene set enrichment analysis (GSEA), over-representation analysis (ORA), or single sample gene set enrichment analysis (ssGSEA)

Figure 4. Signature Scoring and Fully integrating Pathway and Spatial Relationships
GeoMx WTx expression data is consistent and compatible with expression signatures developed for bulk profiling of samples with standard gene expression platforms. Above shows the expression of the Cytotoxicity signature developed for used with nCounter PanCancer IO 360™ assays.

Pathway Analysis Drives Contextual Insight into Spatial Gene Expression Profiles

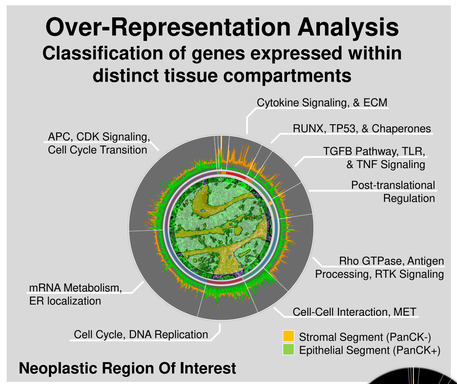
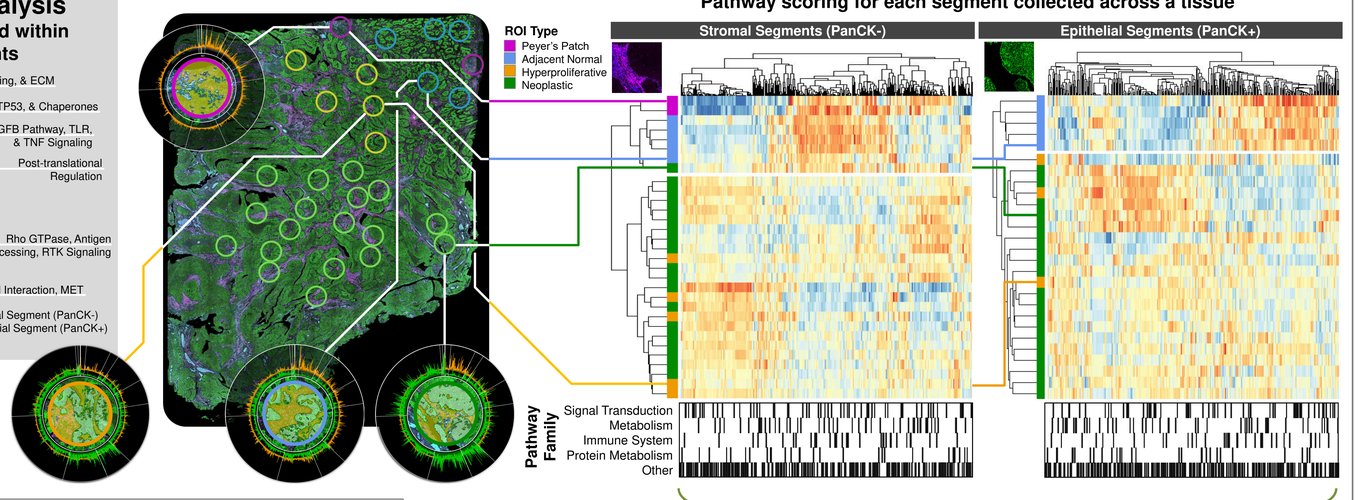


Figure 3. Spatial Overlays and ORA
The top 50% of genes detected based on median expression are clustered around the original ROI. Expression is shown as for each compartment within a tissue based on the segmentation strategy. Gene expression is colored by associated tissue segment.



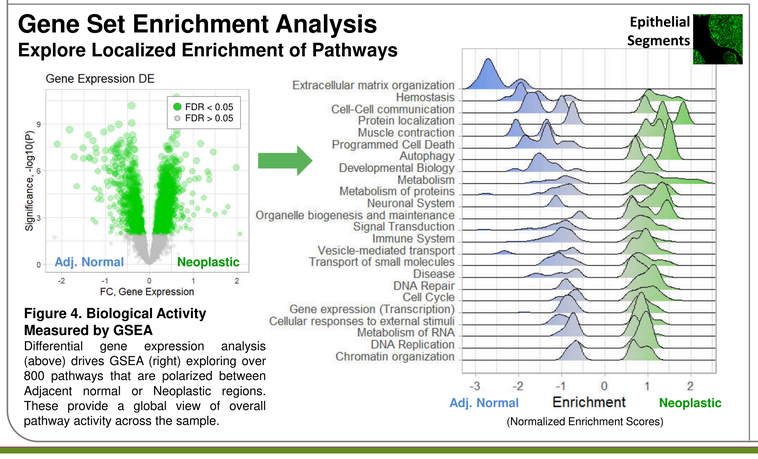


Figure 4. Biological Activity Measured by GSEA
Differential gene expression analysis (above) drives GSEA (right) exploring over 800 pathways that are polarized between Adjacent normal or Neoplastic regions. These provide a global view of overall pathway activity across the sample.

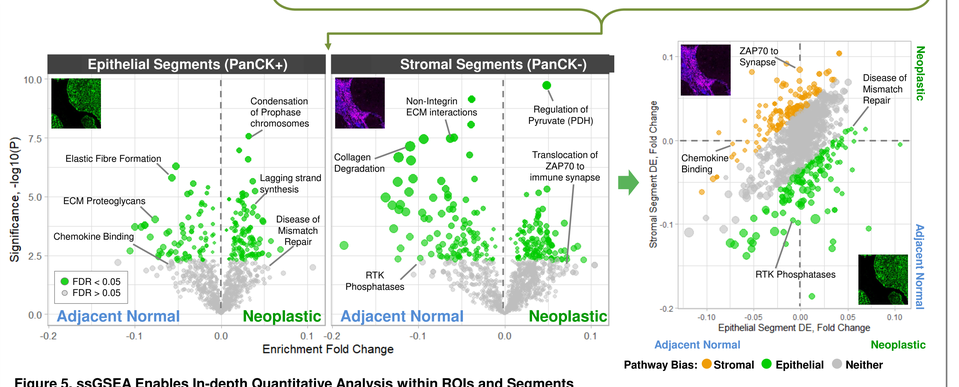


Figure 5. ssGSEA Enables In-depth Quantitative Analysis within ROIs and Segments
ssGESA provides a framework for exploration of the individual activity level of pathways within each segment to quantitatively assess individual sample pathway disregulation. By having segment-level pathway scores, differential expression testing of (above left), exploration of segment pathway activation (top right), as well as coordinated expression analysis (below).

Researchers interested in participating in NanoString's technology access program for its Digital Spatial Profiling technology should contact us at TAP@nanostring.com.

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