Spatial Whole Transcriptome Analysis of Human Kidney Histological Structures using Digital Spatial Profiling

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NanoString Technologies

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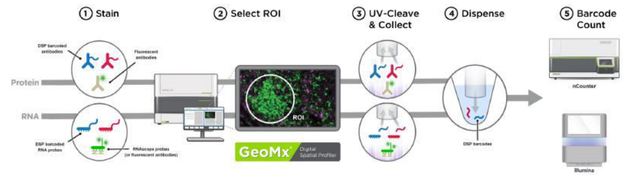
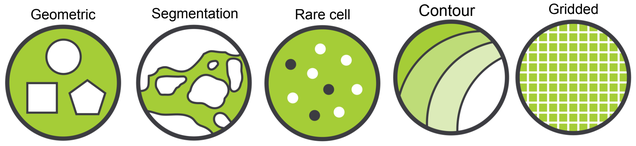
Project Overview

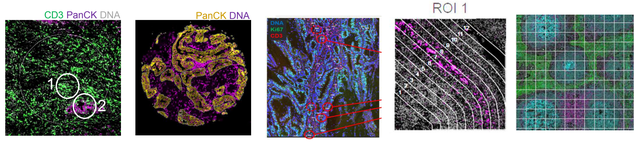
A thorough understanding of normal tissue biology is crucial to advances in disease treatment. In this study, we report deep profiling of kidney function by analyzing whole transcriptomes of histological structures. This work corroborates decades of detailed molecular studies and reveals novel insights into organ physiology.
Using the GeoMx Digital® Spatial Profiler (DSP) and accompanying Whole Transcriptome Assay, we analyzed four non-diseased kidneys as a proof of principle for spatial organ profiling studies. We profiled fundamental functional structures within kidney nephrons: glomeruli, glomerular filtration membrane, proximal and distal convoluted tubules, loops of Henle, and collecting ducts. GeoMx® DSP allowed the selection of each of the histological structures for specific profiling of the whole transcriptome with high precision. These data can be used as standards to inform future profiling studies in normal and diseased kidney tissue.

Automated workflows with Flexibility to Dissect a Variety of Tissue Features and Substructures



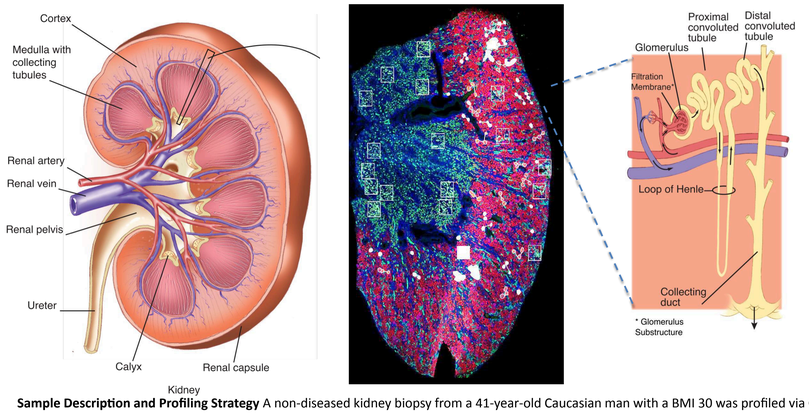
GeoMx DSP enables spatially resolved high plex profiling of either proteins or RNA transcripts from FFPE tissue sections. It uses target binding reagents (antibodies or ssRNA) labeled with photocleavable indexing oligos to bind targets of interest on a slide mounted tissue section, and in parallel tissue architecture is visualized with immunofluorescent imaging reagents. Regions of interest (ROI) are selected for molecular profiling, and those regions are sequentially exposed to UV light which cleaves the photocleavable linker and releases the indexing oligos, which are collected and stored off tissue. Indexing oligos are subsequently enumerated via nCounter® or next gen sequencing





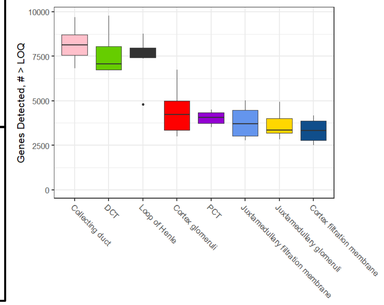
By using the visualization markers to mask specific areas within the ROI, the ROI can be further compartmentalized and molecular profiles of the different compartments profiled independently. This enables, for example, differential profiling of tumor vs stroma, or various immune cell types, within an ROI. NanoString® has developed panels of protein and RNA detection reagents for use with the GeoMx platform. Most recently, we have commercialized the Whole Transcriptome Atlas (WTA), an 18,000+ gene panel that enables characterizing the spatial biology of any human system.

Biology Driven Profiling of the Kidney



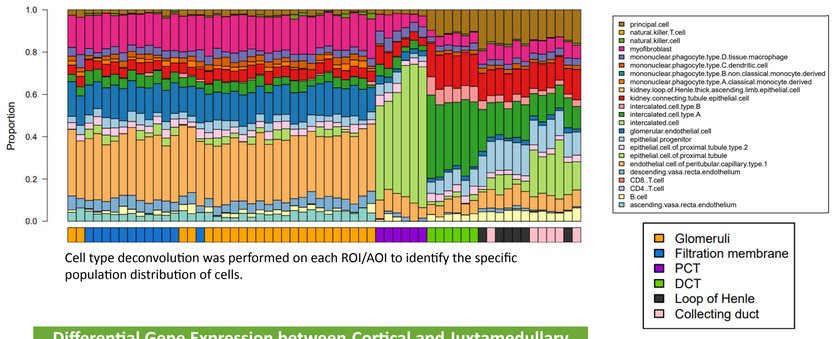
Sample Description and Profiling Strategy A non-diseased kidney biopsy from a 41-year-old Caucasian man with a BMI 30 was profiled via GeoMx DSP using the Whole Transcriptome Atlas (18,000 genes). The tissue was stained with fluorescent antibodies to guide region of interest selection to functional units within the tissue. Morphology markers were used to illuminate **PanCK** (epithelial cells), **CD10+CD31** (proximal nephrons and endothelial cells), and **Syto13** (DNA). Regions of interest were placed on substructures within glomeruli and geometric and segmentation AOIs were used to specifically profile substructures.

High Sensitivity Detection of Genes Across All Tissue Compartments



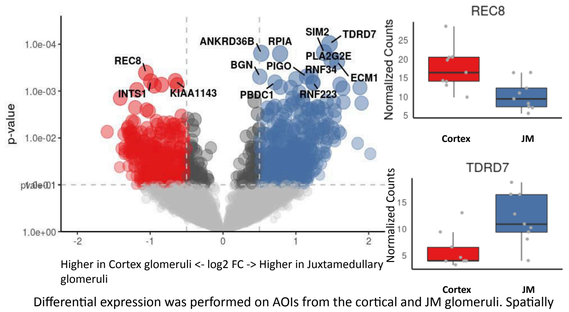
14,319 genes were detected in any segment collected. Further, **9,779 genes** detected above limit of quantitation in >10% of segment collected

Deconvolution of Cell types within ROIs



Cell type deconvolution was performed on each ROI/AOI to identify the specific population distribution of cells.

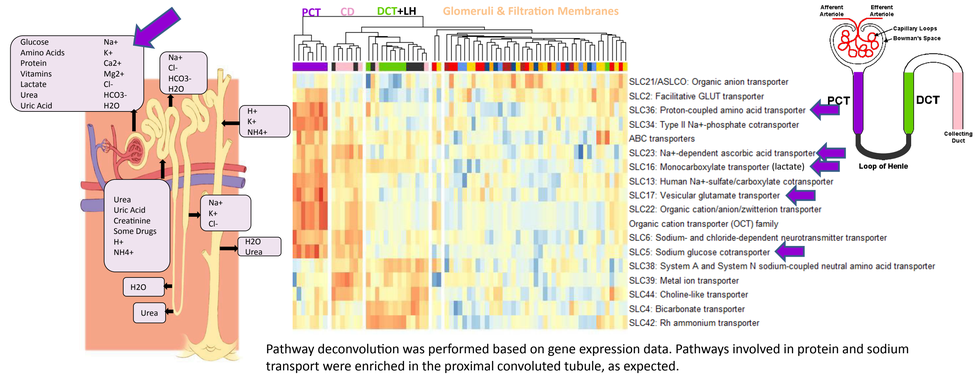
Differential Gene Expression between Cortical and Juxtamedullary Glomeruli



Higher in Cortex glomeruli <- log2 FC -> Higher in Juxtamedullary glomeruli

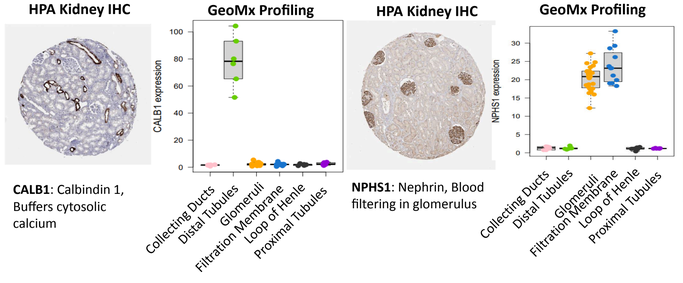
Differential expression was performed on AOIs from the cortical and JM glomeruli. Spatially resolved gene expression was observed between the two locations.

Pathway Analyses Confirm Known Biology



Pathway deconvolution was performed based on gene expression data. Pathways involved in protein and sodium transport were enriched in the proximal convoluted tubule, as expected.

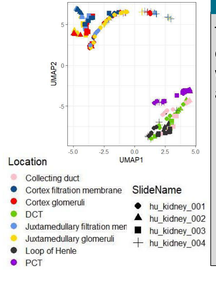
Independent and Orthogonal Confirmation of Target Expression with the Human Protein Atlas



CALB1: Calbindin 1, Buffers cytosolic calcium

NPHS1: Nephrin, Blood filtering in glomerulus

Cohort Analysis



Accessing the Data and Contributing to the Spatial Organ Atlas

These data can be accessed and freely downloaded at the Spatial Organ Atlas webpage: www.nanostring.com/spatial-organ-atlas

The Spatial Organ Atlas is growing! If you are interested in participating in the project, please contact geomx@nanostring.com

SPATIAL ORGAN ATLAS

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