APPLICATION NOTE

High-resolution, high-throughput spatial transcriptomics of complex tissues

Revealing the tissue architecture of kidney disease

- The NanoString GeoMx® Digital Spatial Profiler resolves and quantifies transcripts within their *in situ* context
- Illumina sequencing systems radically increase the throughput for spatial methods
- Integrated software tools available in BaseSpace™ Sequence Hub provide streamlined data analysis

Spatially resolved transcriptomics powered by

nanoString

illumina®

Introduction

Traditional sequencing methods using dissociated samples can lose key spatial information present *in vivo*. To understand localized transcriptional changes while maintaining information on tissue architecture, high-plexity spatial technologies are needed for gene and protein profiling. By retaining the precise location of biological molecules within a tissue, spatial methods can further our understanding of mechanisms in health and disease.

The NanoString GeoMx Digital Spatial Profiler (DSP) measures analytes within regions of interest (ROIs) defined by tissue morphology, protein expression, or gene expression (Figure 1). When coupled with readout from Illumina next-generation sequencing (NGS) systems, tens of thousands of targets can be profiled simultaneously, dramatically increasing the throughput of spatial analysis methods.

This application note provides an overview of a comprehensive workflow for spatially resolved transcriptomics with the NanoString GeoMx DSP followed by Illumina sequencing and data analysis (Figure 2). The exceptional performance of this spatial method is demonstrated by analyzing healthy and diseased kidney samples.

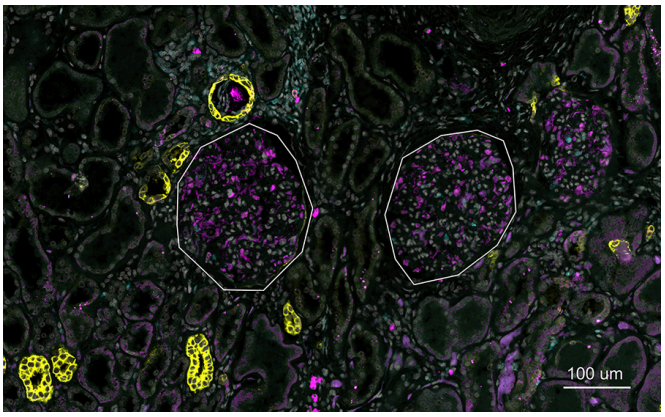

Figure 1: Spatial analysis with the NanoString GeoMx DSP—Example ROI selections drawn around human kidney glomeruli are visualized and profiled by GeoMx morphology markers to identify podocytes with WT1 (magenta), immune cells with CD45 (cyan), distal tubules with PanCK (yellow), and DNA with SYTO13 (grey).

Methods

Prepare samples

Formalin-fixed, paraffin-embedded (FFPE) samples from three normal and four diabetic human kidneys were analyzed. NanoString GeoMx DSP protocols build from standard histology protocols and can be performed manually or on an automated Leica Bond Rx system. The

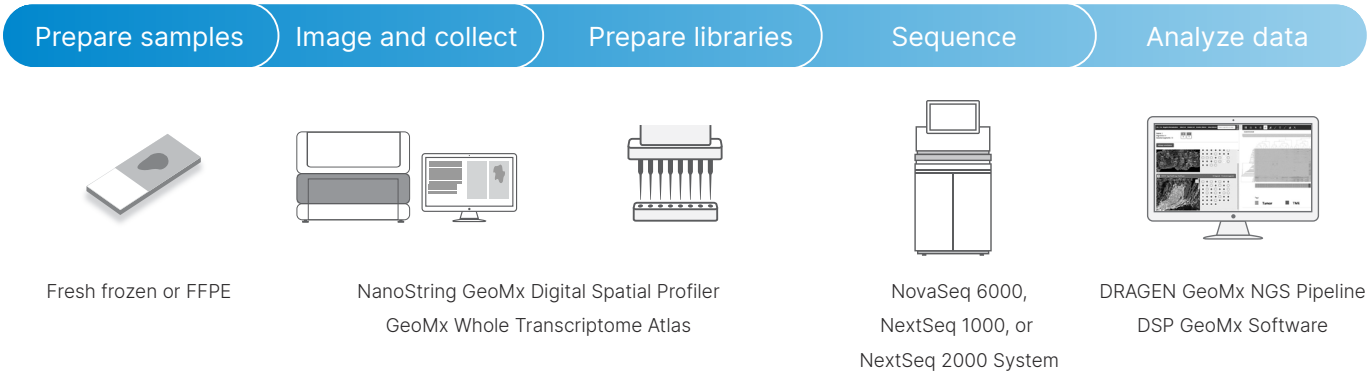

Figure 2: Spatially resolved transcriptomics workflow—The NanoString GeoMx DSP is part of an integrated, sample-to-data workflow for spatially resolved transcriptomics that includes proven Illumina sequencing on a NovaSeq 6000, NextSeq 1000, or NextSeq 2000 System and data analysis with the DRAGEN GeoMx NGS Pipeline and DSP GeoMx software.

NanoString GeoMx DSP is compatible with fresh frozen, fixed frozen, or FFPE samples. See guidelines from NanoString for more details.

Image and collect

GeoMx Whole Transcriptome Atlas (WTA) and GeoMx Protein Assays provide a cocktail of GeoMx RNA *in situ* hybridization probes or protein antibodies, respectively (Figure 3) that are incubated overnight on tissue sections mounted on standard microscope slides. Fluorescently labeled GeoMx morphology markers (up to four) are added during slide preparation for visualization during imaging. The slides are imaged using the NanoString GeoMx DSP and ROIs are selected (Figure 1). These areas are exposed to focused light that releases DSP barcodes and collects them for library preparation and sequencing.

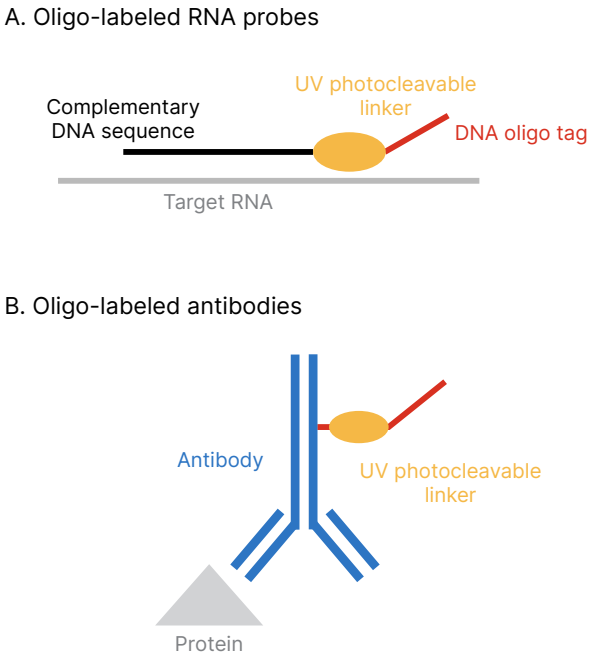

Figure 3: GeoMx imaging probes—GeoMx imaging probes are a cocktail of UV photocleavable oligonucleotides conjugated to (A) *in situ* hybridization probes for RNA or (B) antibodies for proteins.

Prepare libraries

Libraries are prepared using GeoMx Seq Code PCR primers. During library prep, each ROI collected from the tissue section is indexed with a different pair of i7 and i5 unique dual indices. All indexed ROIs are then pooled and purified. The resulting library fragments contain a unique molecular identifier (UMI), target analyte identifier (RTS ID), and the necessary regions for sequencing with Illumina platforms (Figure 4).

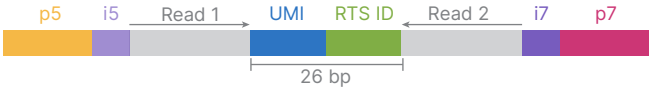

Figure 4: GeoMx library—Prepared library fragments include a 14-bp UMI for molecular counting, a 12-bp RTS ID for target identification, and i5/i7 unique dual indexes and adapters compatible with all Illumina sequencing systems.

Sequence

GeoMx NGS libraries are compatible with any Illumina sequencing system (Figure 2). Higher throughput instruments such as the NextSeq™ 1000, NextSeq 2000, and NovaSeq™ 6000 Systems are recommended to generate the sequencing output required. The number of samples per sequencing run depends on the sequencing system (Table 1) and ROI size and number.

GeoMx NGS libraries are sequenced with 2 × 27 bp paired-end reads using the unique dual index workflow with 8 bp for Index 1 (i7) and Index 2 (i5). The number of samples per sequencing run depends on the sequencing system (Table 1) and the number and size of ROIs per sample. For example, a common WTA workflow of 12 ROIs per sample with each ROI measuring 300 µm x 300 µm in area requires 100M reads per sample.

Analyze data

After sequencing, data processing and analysis begins with the GeoMx NGS Pipeline on Illumina DRAGEN™, available on BaseSpace Sequence Hub.* The software delivers a reliable and efficient method for converting sequencing reads to counts for spatially resolved expres-

* A BaseSpace Sequence Hub subscription (professional or enterprise) is required to launch the app and store data.

Table 1: Example sample throughput for GeoMx libraries on Illumina sequencing systems

Illumina platform	No. of NanoString GeoMx WTA samples (100M reads)	Flow cell	Sequencing kit	Catalog no.	No. of transcripts per flow cell
NextSeq 550 System	4	High Output	NextSeq 500/550 High Output Kit v2.5 (75 cycles)	20024906	400
NextSeq 1000 or NextSeq 2000 System	4	P2	NextSeq 1000/2000 P2 Reagents (100 cycles) v3	20046811	400
NextSeq 2000 System	11	P3	NextSeq 2000 P3 Reagents (50 cycles)	20046810	1100
NovaSeq 6000 System	6.5	SP	NovaSeq 6000 SP Reagent Kit v1.5 (100 cycles)	20028401	650
	13	S1	NovaSeq 6000 S1 Reagent Kit v1.5 (100 cycles)	20028319	1300
	33	S2	NovaSeq 6000 S2 Reagent Kit v1.5 (100 cycles)	20028316	3300
	80	S4	NovaSeq 6000 S4 Reagent Kit v1.5 (35 cycles)	20044417	8000

sion analysis and visualization using GeoMx software. Files generated in the DRAGEN pipeline are then processed further on the NanoString DSP instrument.

The DRAGEN GeoMx NGS Pipeline requires two input files: the configuration file from the NanoString GeoMx DSP and the FASTQ sequencing file from the Illumina sequencing system. While sequencing data streams automatically to BaseSpace Sequence Hub after the run is complete, users need to upload the configuration file manually after selecting the app. Analysis time for a typical experiment containing ~192 ROIs and ~400 M reads is one to two hours. The GeoMx NGS Pipeline outputs digital count conversion (DCC) files that can be uploaded into the GeoMx DSP Data Analysis Suite for interactive visualization on the Nanstring DSP instrument. DCC files are also compatible with open-source analysis scripts on GeoScript Hub.

Results

Spatial analysis of tissue structure

Three fluorescently labeled antibodies targeting epithelial cells (PanCK), immune cells (CD45), and glomeruli (WT1) and a nucleic acid stain (SYTO-13) were used to identify and discriminate sub regions of structural features. Tubules were identified visually and auto segmented into distal (PanCK+) and proximal (PanCK-) tubules. Each distinct tissue compartment was collected independently, yielding two unique whole transcriptome profiles per region, even if the two compartments were adjoining or interdigitated.

The clear globular structure of the glomerulus and positive staining with WT1 enabled identification of each glomerulus independent of the surrounding tissue. Within each sample, individual glomeruli were annotated for pathological features, and data were collected from pathologically healthy and abnormal glomeruli in both normal and diabetic samples (Figure 5A). Further analysis identified pathways that changed expression with diabetic kidney disease (DKD), including upregulation of immune-related pathways, confirming the known effect of increased immune infiltration and inflammation. Many essential

cellular processes, such as translation and mitochondrial respiration, were downregulated, indicating a loss of normal cellular functioning in diseased kidneys (Figure 5B).

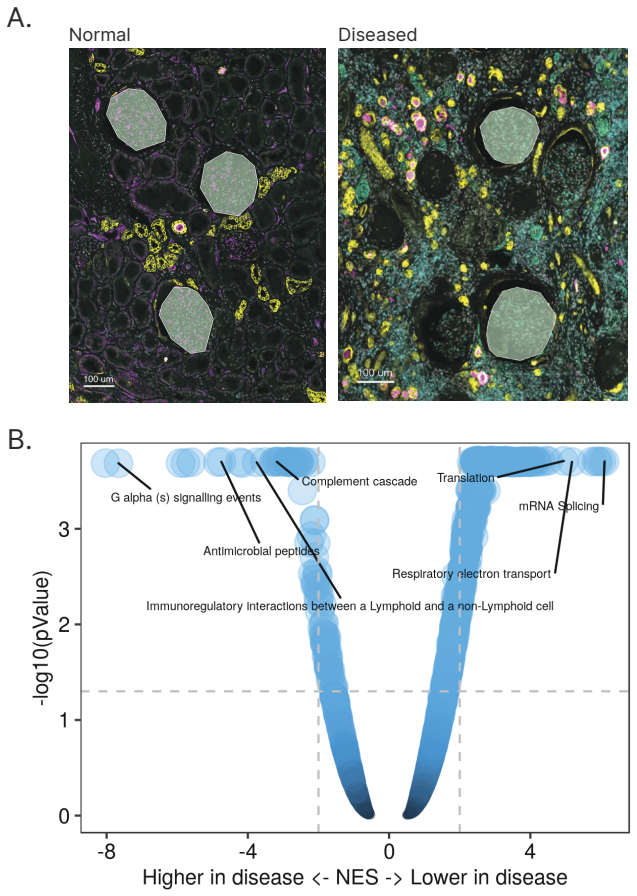

Figure 5: Differential expression analysis reveals pathways that change in disease—(A) Images of ROIs outlining glomeruli in normal and diseased human kidney samples. (B) Analysis identifies pathways that changed expression in glomeruli from DKD compared to normal kidneys, including upregulation of immune-related pathways and downregulation of translation and mitochondrial respiration.

Spatial analysis reveals the heterogeneous nature of disease

Individual glomeruli were annotated by their degree of pathology within each sample and transcriptomes were profiled from both healthy and abnormal glomeruli. Cell types present in each glomerulus were identified using gene signatures from public single-cell data sets. Deconvolution of cell types showed an increase in immune cells

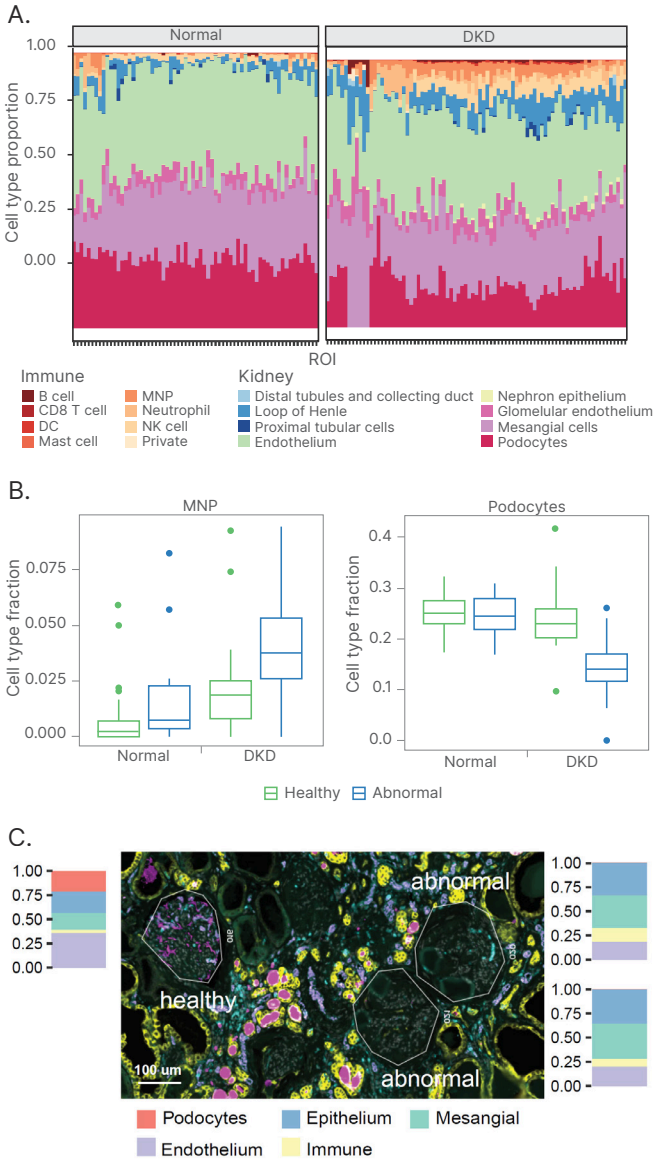

Figure 6: Cell type deconvolution reveals heterogeneity of cell types in glomeruli—(A) Cell type proportions within each glomerulus of healthy and DKD kidney samples. (B) Box plots of distribution of cell type fractions of mononuclear phagocytes (left) and podocytes (right) in pathologically identified abnormal and healthy glomeruli in normal and DKD kidney samples. (C) Image from a DKD kidney showing three example glomeruli with the relative proportion of each cell type plotted alongside. Despite their proximity in space, the healthy glomerulus shows the expected cell type proportions while the two abnormal glomeruli have a complete loss of podocytes and a large increase in immune infiltration.

and loss of podocytes in diseased samples (Figure 6A). These changes were more pronounced in abnormal glomeruli and attenuated in healthy glomeruli, even within a diseased kidney sample (Figure 6B). To investigate further, glomeruli within a diseased kidney were spatially analyzed and the relative proportions of each cell type were determined for each glomerulus. Healthy glomeruli with normal cell type proportions were observed in close proximity to more diseased glomeruli with a complete loss of podocytes and a large increase in immune infiltration (Figure 6C). These results are indicative of the heterogeneous pathology of diabetic kidney disease, with some glomeruli retaining normal cellular architecture even in severe disease.

Summary

Technological advances have enabled new NGS methods that continue to provide insights into complex biological systems. However, analyses performed on dissociated tissues lose key spatial information present *in vivo*. The NanoString GeoMx DSP combined with proven Illumina sequencing provides high-plexity spatial analysis and information on tissue architecture needed for gene and protein profiling. This application note outlines an integrated workflow for spatially resolved transcriptomics and demonstrates the exceptional performance of the solution for profiling the heterogeneous pathology of diabetic kidney disease.

Getting help for your experiment

Beyond simply enabling higher throughput by using NGS as a readout, teams from Illumina and NanoString have been working together to minimze data analysis times and streamline the workflow to improve the customer experience.

For assistance with the GeoMx assay or data analysis with the GeoMx NGS Pipeline BaseSpace App, contact geomxsupport@nanostring.com

For answers to sequencing questions, contact techsupport@illumina.com

Learn more

NovaSeq 6000 System, www.illumina.com/systems/sequencing-platforms/novaseq.html

NextSeq 1000 and NextSeq 2000 Systems, www.illumina.com/systems/sequencing-platforms/nextseq-1000-2000.html

NanoString GeoMx DSP, www.nanostring.com/products/geomx-digital-spatial-profiler/geomx-dsp-overview/

NanoString GeoMx WTA, www.nanostring.com/products/geomx-digital-spatial-profiler/geomx-rna-assays/geomx-whole-transcriptome-atlas/

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