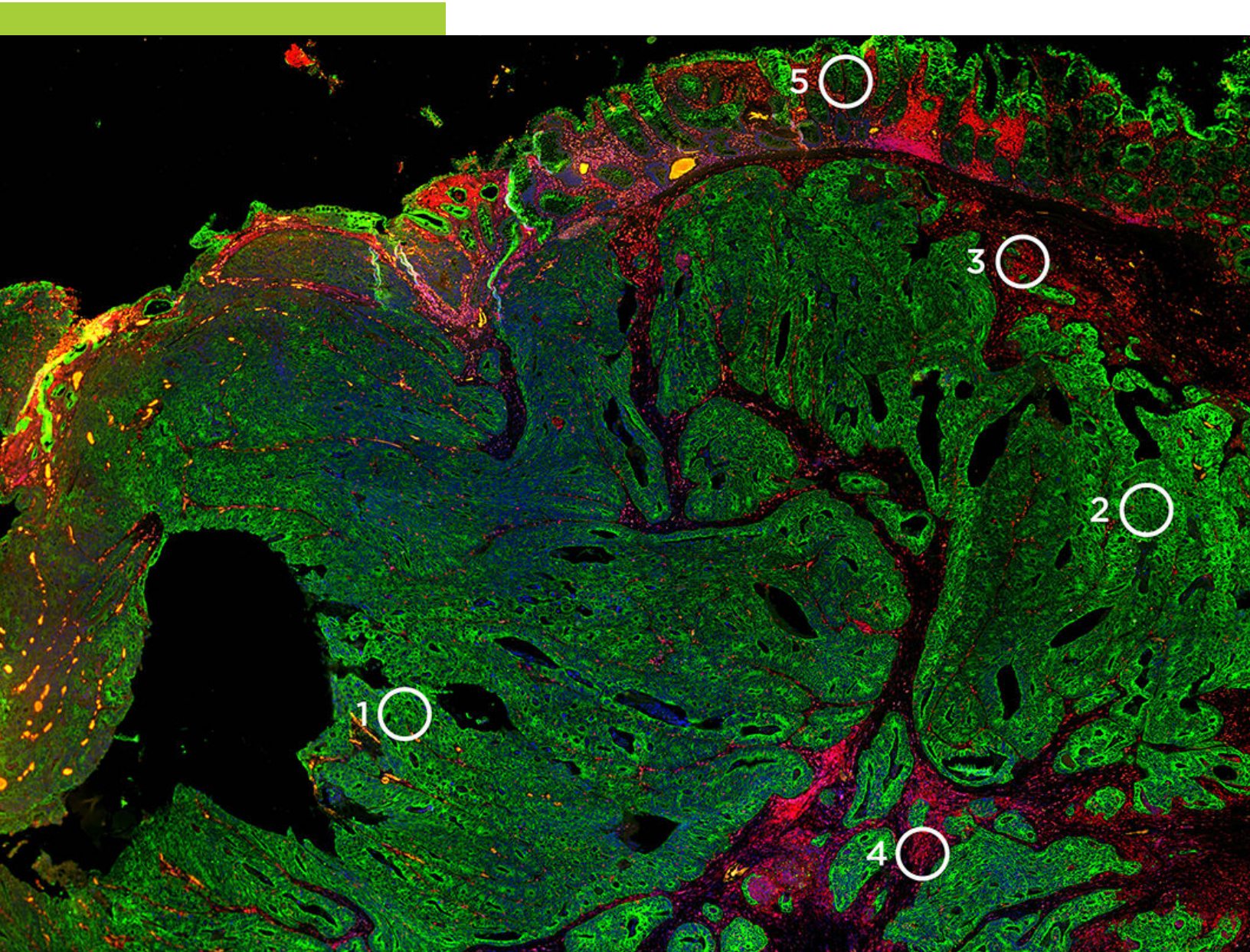# GeoMx®
Digital Spatial Profiler

Grant Application Package

Section 1: Information to provide in grant application

Platform Overview

GeoMx® Digital Spatial Profiler (DSP) is the spatial biology solution.

DSP enables morphology-driven, high-plex profiling on a single FFPE or fresh frozen tissue section. DSP technology combines standard immunofluorescence techniques with nCounter optical barcoding quantification. The system specifications are as follows:

1. Throughput: >10 sections per day

2. Multi-analyte: RNA and Protein

3. Single Cell Segment Resolution

4. Minimum Segment Size Recommendation:
   - Target detection is dependent on the size and number of cells in segment and expression level
   - For small cells (e.g., T Cells or B cells): 10-20 cells for protein, 50-200 cells for RNA
   - For large cells (e.g., astrocytes Neurons): 1-5 cells for protein and 5-20 RNA

5. Multiplexing capability:
   - 96 targets with nCounter readout
   - 20,000+ targets with NGS readout
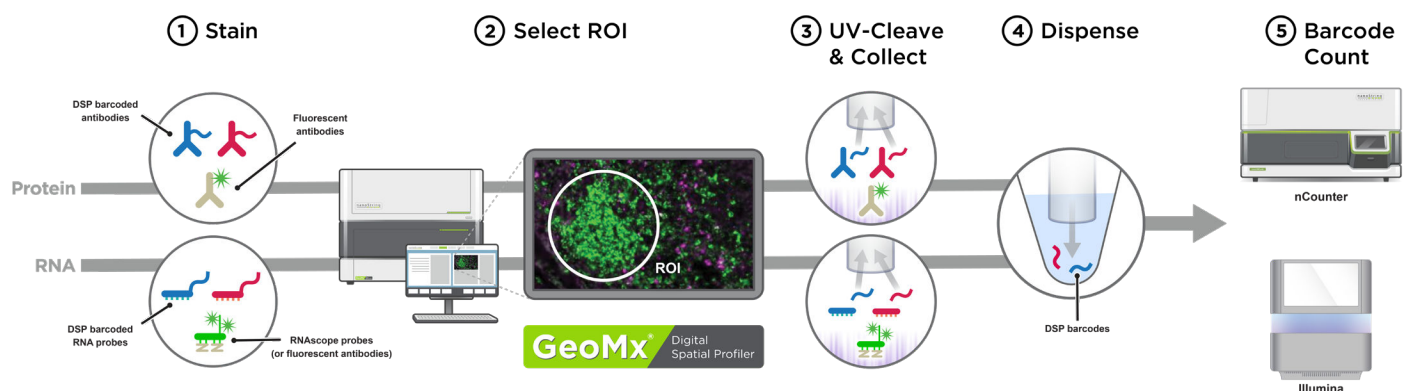6. Quantitative: 6 logs of dynamic range

Technology Overview & Workflow

GeoMx® Digital Spatial Profiler (DSP) is a novel platform developed by NanoString Technologies. DSP enables spatially resolved, high-plex (10s -10,000s) digital quantitation of proteins and mRNA in tissue. The assay utilizes unique reagents (antibodies or RNA probes) coupled to UV photocleavable oligonucleotide barcodes. After incubation/hybridization of the GeoMx reagents along with visualization reagents to slide-mounted formalin fixed paraffin-embedded (FFPE) or fresh frozen tissue sections,

the DSP scans the slides and presents a high quality image for region of interest selection. The oligonucleotide tags are released from user-selected regions of the tissue with focused UV light based on the user region and segmentation selections. The digital micromirror device of the DSP instrument tunes the UV light with 1 micron resolution. This allows great flexibility for region selection including irregularly shaped and non-continuous segments. Released tags are quantitated with nCounter optical barcodes or an Illumina® next-generation sequencer (NGS). Finally, counts are mapped back to the tissue location by the software, resulting in a spatially-resolved digital profile of protein or mRNA abundance.

1. A 5-10µm thick formalin-fixed, paraffin-embedded (FFPE) or fresh frozen tissue section is stained with a cocktail of standard morphology markers (i.e. fluorescent antibodies) combined with either oligo-tagged antibodies (protein) or oligo-tagged ISH probes (RNA). Oligo tags are attached to the antibody and ISH probes via a UV-photocleavable linker. Staining can be performed manually or an automated protocol is available on the Leica BOND RX.

2. Regions of interest (ROI) are selected by the user on the visualized tissue. Using fluorescent morphology markers and our auto-segmentation algorithm, users can further divide an ROI into individual segments, such as specific tissue compartments or cell populations. (Please refer to section: GeoMx DSP ROI selection).

3. After ROI/segment selection, oligos from the selected regions are released upon focused exposure to UV light.

4. The photocleaved oligos are aspirated via a microcapillary tube and stored in microplate wells.

5. Lastly, the photocleaved oligos from the spatially-resolved ROIs in the microplate are (1) quantified using standard NanoString nCounter® or next-generation sequencing Illumina® workflows.

GeoMx DSP Workflow

① Stain   ② Select ROI   ③ UV-Cleave & Collect   ④ Dispense   ⑤ Barcode Count

Protein — DSP barcoded antibodies / Fluorescent antibodies

RNA — DSP barcoded RNA probes / RNAscope probes (or fluorescent antibodies)

ROI

GeoMx® Digital Spatial Profiler

DSP barcodes

nCounter

Illumina

GeoMx DSP Instrumentation and Assays

Spatial biology experiments with GeoMx require the DSP instrument for slide scanning, ROI selection, and oligo collection. Downstream of the DSP, there are two instrumentation platforms for quantitative readout: NanoString nCounter® platform or a standard library preparation workflow for analysis on Illumina® NGS platforms.

The GeoMx DSP platform is commercially available from NanoString Technologies. DSP results correlate with those from immunohistochemistry high concordance is observed between DSP results generated using both antibodies or RNA probes. Oligo conjugation does not interfere with antibody or RNA probe specificity and once conjugated ensures robust quantification of protein and RNA abundance. Additionally, analytical validation data has demonstrated multiplexing compatibility. Lastly, any commercially-available antibody can be custom-labeled with oligo tags and used in a DSP protocol. (reference- internal data)

GeoMx DSP ROI Selection

The DSP software enables the user tremendous flexibility in the ROI selection strategy to profile tissue. In particular, highlighted below are five unique profiling modalities designed to interrogate tissue samples.

The segmentation and cell-type specific approaches are achieved through the use of masks in software based on the fluorescent marker staining of the tissue (e.g. PanCK+ vs. PanCK-). UV light then specifically targets the ROI with multiple illumination events, enabling the separate profiling of complex and interdigitated cell populations within a selected ROI (e.g. tumor vs. stroma).
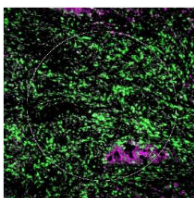
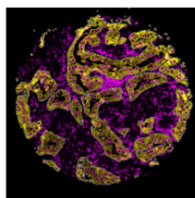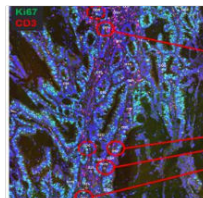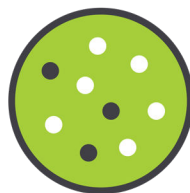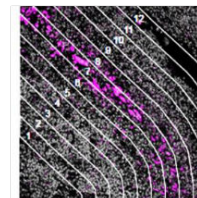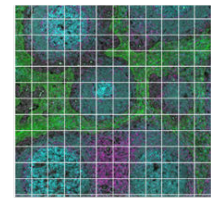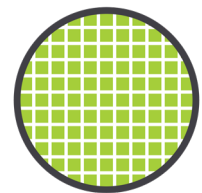| Geometric	Segmentation	Cell-Type Specific	Contour	Gridded
What is the heterogeneity of expression in different regions of my tissue?	What is the expression profile of distinct biological compartments (e.g., Tumor-TME)?	What is the expression profile of a specific cell population in my tissue?	How does the immune environment change on either side of an infiltrate boundary?	What novel targets are uncovered with deep mapping of a specific tissue region?

GeoMx DSP Data Analysis

The GeoMx DSP comes with built-in data analysis software that enables users to easily visualize and interpret their data. The intuitive interface displays high-resolution images side-by-side with quantitative profiling data. The connection between the image and profiling data is preserved allowing users to interact with either dataset in real time. For example, users can select a data point within a data visualization and the software will automatically highlight the ROI or segment from which the expression data was generated. The data analysis workflow includes QC and normalization operations, as well as several data visualization methods for various types of scientific and statistical inquiries.

Users can auto-generate figures such as heatmaps, box plots, and bar graphs, to visualize the distribution of protein or gene expression across multiple tissue compartments (e.g., tumor versus stroma); discover statistical changes in expression between experimental groups (e.g., t-tests, linear mixed models) that can also be visualized in volcano and PCA plots; visualize large data matrices using dendrograms and clustering analyses to discover biomarkers; and, perform pathway analyses to understand disease mechanisms of action and identify novel targets.

Image Data

Profiling Data
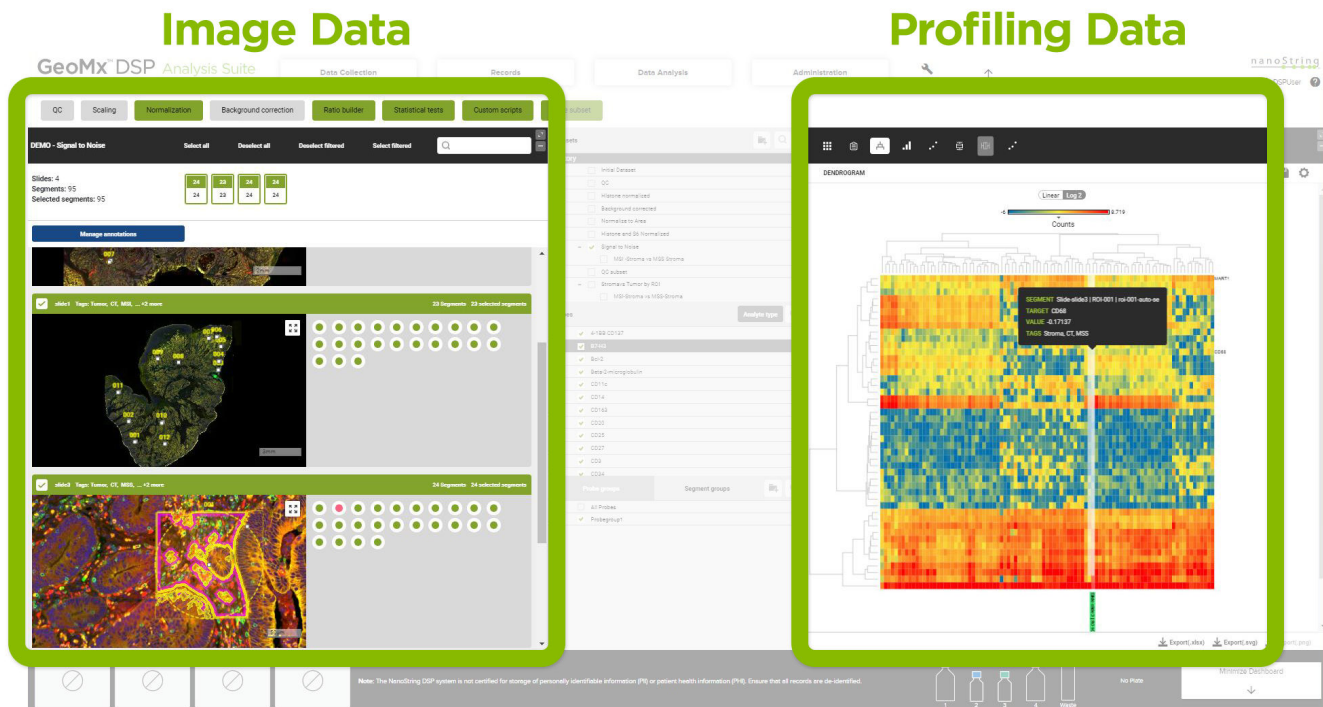
GeoMx DSP Consumables
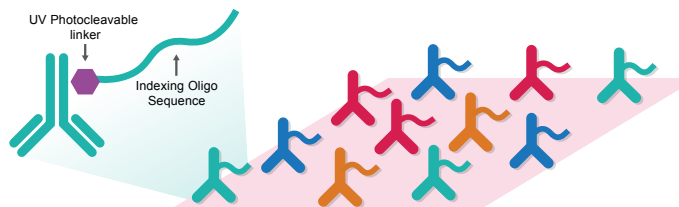
The GeoMx DSP is a multi-analyte platform that can spatially resolve both protein and RNA from two serial sections. The available and highly validated content covers Immuno-Oncology, Immunology, and Neuroscience.

Protein content is designed for flexibility with a ~20-plex core panel that includes additional positive and negative controls. Additional ~10-plex modules are available and can be mixed and matched with the core based on an individual researcher's needs (up to 96-plex total). Users can also add up to 5 custom targets through Abcam (>6000+ antibodies to select from) or up to 10 custom targets of interest using
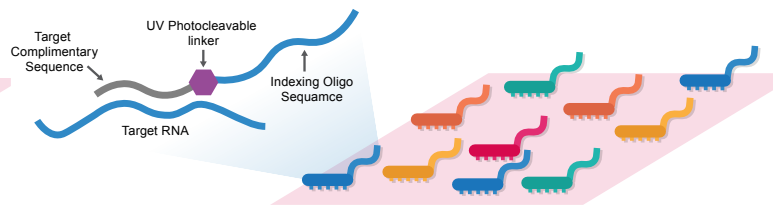
NanoString's Protein Barcoding Service (more details for custom antibodies below). A Core panel must always be run.

The following content is commercially available on nCounter readout:

1. Human IO Protein – Immune Cell Profiling Core, IO Drug Target Module, Immune Activation Status Module, Immune Cell Subtyping Module, Pan-Tumor Module, Cell Death Module, AKT/PI3K Signaling Module, and MAPK Signaling Module

2. [Mouse IO Protein](#) - Immune Cell Profiling Core, IO Drug Target Module, Immune Activation Status Module, Immune Cell Subtyping Module, and Pan-Tumor Module

3. [Human Neuroscience Protein](#) – Neural Cell Profiling Core, Alzheimer's Pathology Module, Parkinson's Pathology Module, Alzheimer's Pathology Extended Module, Autophagy Module, and Glial Cell Subtyping Module

All NanoString antibody panels are validated in a pre- and post-conjugation screen, a sensitivity/specificity screen using FFPE cell pellets, an interaction screen, and a TMA screen using normal and tumor TMAs (see Supporting Data).

Custom antibodies:
Custom Antibodies from Abcam: Abcam provides 1-5 custom antibodies to extend GeoMx protein assays. To add custom antibodies to a GeoMx protein assay, users place the order directly through Abcam, using one of Abcam's IHC-validated recombinant rabbit monoclonal antibodies, and the form found at: www.abcam.com/dsp. See the table below for order requirements. Abcam custom antibodies are tested in a pre- and post-conjugation screen.

Custom Antibodies from NanoString: NanoString provides 1-10 custom antibodies through the NanoString Protein Barcoding Services (PBS). To add custom antibodies of interest to a GeoMx protein assay, users submit a PBS Antibody Submission Form found at: www.nanostring.com/pbs Users purchase IHC-validated antibodies directly from a vendor and ship them to NanoString for barcoding. NanoString custom antibodies are tested for the presence of the conjugated tag, but are not tested with IHC or further validated.

Guidance on further validating custom antibodies can be provided upon request.

RNA Content:
1. [Human IO RNA for nCounter](#)– Immune Cell Pathways Panel with 84 targets (including controls) and room to add up to 10 custom targets (+2 additional controls) of interest for a total of up to 96 targets. Fixed panel content covers characterization of the global immune response, microenvironment immune activity, tumor reactivity, and the Tumor Inflammation Signature.

2. [Human Cancer Transcriptome Atlas (CTA) for NGS](#): 1,800+ mRNA gene targets that cover important aspects of immune response, tumor biology and the microenvironment. The CTA curated content is deeply annotated with 55 major pathways, immune cell subsets, and clinically-derived gene sets such as the Tumor Inflammation Signature (TIS) and PAM50 tumor subtype geneset. Users can add up to 30 custom mRNA gene targets of interest.   CTA launch expected 2020 H2.

3. Human Whole Transcriptome Atlas (HuWTA) for NGS: 18,000+ protein encoding mRNA targets encompasses the whole human transcriptome and is relevant for any type of research. The HuWTA takes a targeted approach to quantify transcripts in every biological pathway and across all gene expression levels. It has been pruned of uninformative high expressors like ribosomal proteins from the target list, so that reads are comprised of informative protein coding genes.   Users can add up to 30 custom targets of interest to count unique transcript variants such as isoforms, lnRNA and exogenous transcripts. HuWTA launch expected 2021 H1.

4. Mouse Whole Transcriptome Atlas (MuWTA) for NGS: 18,000+ protein encoding mRNA targets encompasses the whole mouse transcriptome and is relevant for any type of research. The MuWTA takes a targeted approach to quantify transcripts in every biological pathway and across all gene expression levels. The probes have been designed toward BalbC, C57B6, and NOD strains and has considerable cross-reactivity with other strains. It has been pruned of uninformative high expressors like ribosomal proteins from the target list, so that reads are comprised of informative protein coding genes. Users can add up to 30 custom targets of interest to count unique transcript variants such as isoforms, miRNA and exogenous transcripts. MuWTA launch expected 2021 H1.

All NanoString RNA probes are validated in a bioinformatic screen and undergo technical QC(see Supporting Data).

Training and support
Upon purchase and acquisition of the GeoMx DSP instrument, NanoString will provide on-site installation and calibration of the instrument by a trained field engineer. Once the instrument has been qualified, a Product Application Scientist (PAS) will be on site to provide a comprehensive introduction to the GeoMx DSP platform. The introduction will cover routine use of the instrument, experimental design, project consultations, and data analysis. In addition, the PAS will train the lab scientist to ensure that they have hands-on experience in operating the instrument, implementing the selected GeoMx protocol, and running an experiment from end-to-end . Lastly, the PAS will provide data analysis training with the GeoMx Data Analysis software so that the customer is familiar with all data analysis methods to process, analyze and interpret their data. Upon successful completion of the training, the customer can access support through the NanoString GeoMx Customer Experience Team regarding experimental design, instrumentation, consumables, and software.

Tips to maximize success with the grant application process

Emphasize Collaboration

The GeoMx DSP software enables collaboration and secured remote-access. The GeoMx instrument makes it possible to securely access your data from anywhere and at anytime through a web browser. The software also provides administrators the ability to assign roles and permissions to their team members and collaborators. The platform allows multiple users within large labs, core facilities, or contract research organizations to securely manage and share their data.

Enabling the DSP workflow in your research environment will facilitate deep and meaningful collaborations between scientists, pathologists, and biostatisticians. Hypothesis-driven study design, pathology-driven ROI selection, and data-driven analyses will enable translational discoveries and contributions to the fields of science and medicine. Acknowledging these collaborative opportunities and providing details on how the working relationships will be fostered can increase the success of your grant application.

Benefits to local research environment

The digital spatial profiler (DSP) quantifies biology with spatial context. Any tissue that can be sectioned including Formalin-Fixed Paraffin Embedded (FFPE) and fresh frozen tissues can benefit from the platform. Our reagents are designed towards human and mouse, but our reagents have appreciable and useful cross-reactivity with non-human primate and rattus respectively. Information on cross-reactivity is available upon request. The DSP is relevant to any individual using tissue slides to study biology, making the technology a valuable addition to the local research ecosystem.

Publications

The utility of the GeoMx DSP system is demonstrated already in over a dozen peer-reviewed publications, many in leading journals. Please click here to view current GeoMx DSP Publications.

Supporting Data

Reproducibility of GeoMx DSP

All GeoMx antibodies and RNA Probes go through a robust validation process to ensure they will perform as expected and researchers can use GeoMx reagents off the shelf without having to spend time validating panels. Additionally, serial sections analyzed on the GeoMx DSP for protein (Figure 1) or RNA (Figure 2) expression demonstrate high reproducibility. For additional reproducibility data see: Gupta et al., 2020 (Rimm); Merritt et al., 2020
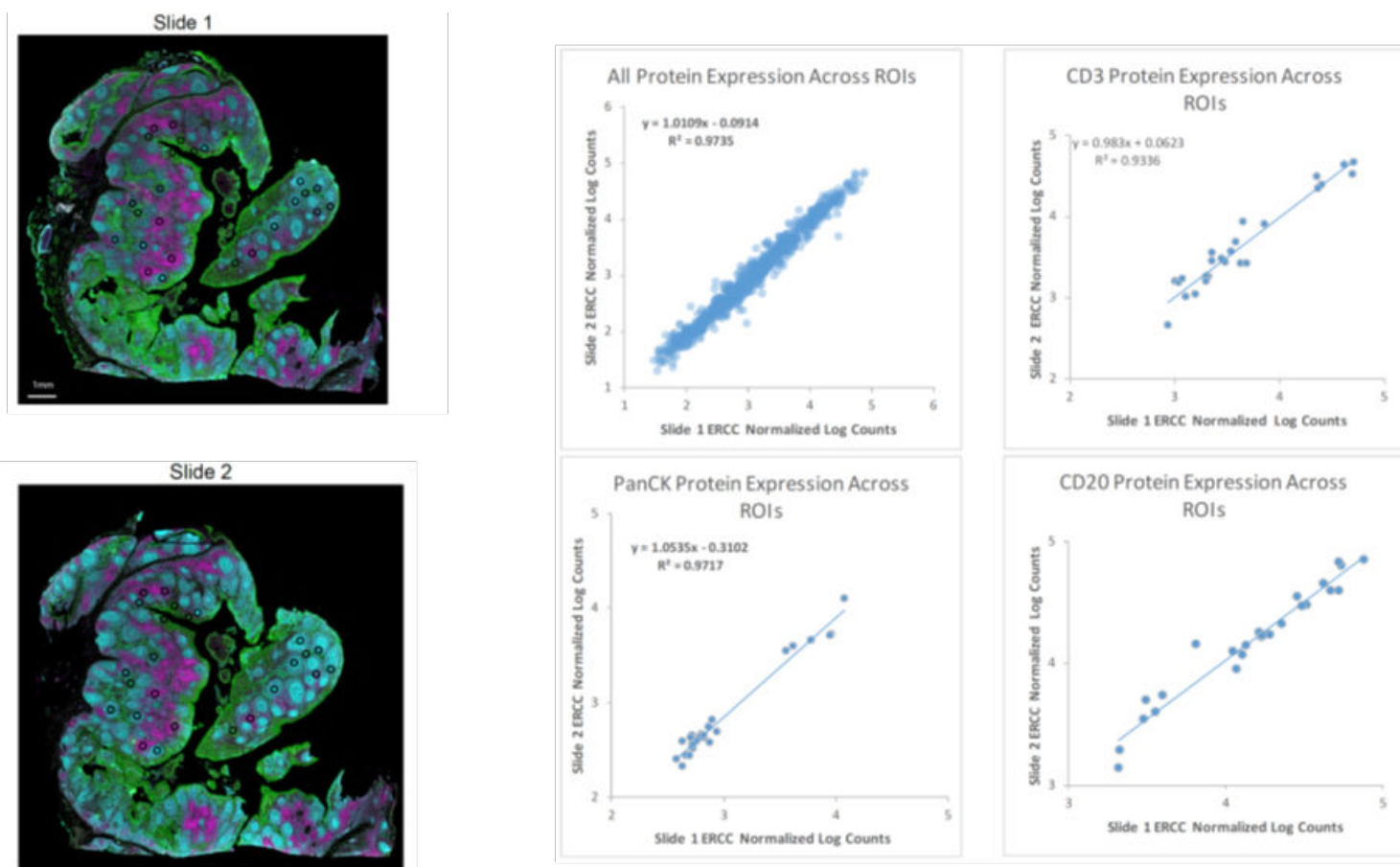

FIGURE 1: Two serial tonsil sections demonstrate high concordance with 24 co-registered ROIs
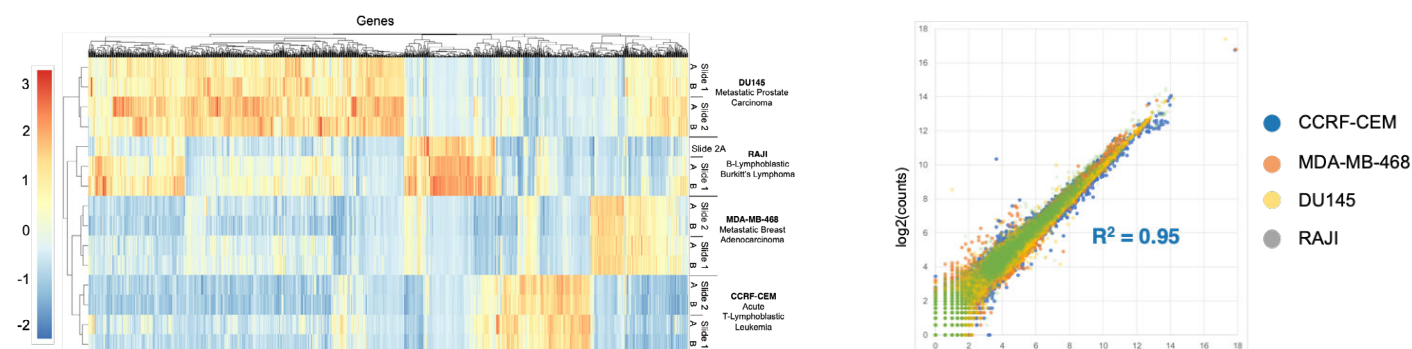

FIGURE 2: GeoMx® DSP High-Plex RNA was performed on 5 μm FFPE sections. High reproducibility was demonstrated between experimental replicates with serial sections with a pre-commercial panel of 4,998 probes.

Ordering Information

Ancillary Products

Product	Description	Catalog Number
GeoMx™ Digital Spatial Profiler Slide Tray	Additional 4-slide holder	GMX-DSP-TRAY

GeoMx Digital Spatial Profiler

Product	Description	Catalog Number
GeoMx Digital Spatial Profiler	GeoMx™ Digital Spatial Profiler Analysis Instrument. Includes 1 year manufacturers warranty.	GMX-DSP-1Y
	GeoMx™ Digital Spatial Profiler Analysis Instrument. Includes 1 year manufacturers warranty and 1 year service contract.	GMX-DSP-2Y
	GeoMx™ Digital Spatial Profiler Analysis Instrument. Includes 1 year manufacturers warranty and 2 year service contract.	GMX-DSP-3Y
	GeoMx™ Digital Spatial Profiler Analysis Instrument. Includes 1 year manufacturers warranty and 3 year service contract.	GMX-DSP-4Y
	GeoMx™ Digital Spatial Profiler Analysis Instrument. Includes 1 year manufacturers warranty and 4 year service contract.	GMX-DSP-5Y

Materials Included

Item	Quantity
Logitech MK540 Advanced Keyboard, Mouse set	1
LED Monitor 4K 8MP Color 27"	1
Slide Holder Assembly	1
96 Well Plate Adapter	1
Uninterruptible Power Supply	1

Request a quote: GeoMx@nanostring.com

References

1   Gupta S, Zugazagoitia J, Martinez-morilla S, Fuhrman K, Rimm DL. Digital quantitative assessment of PD-L1 using digital spatial profiling. Lab Invest. 2020; epub ahead of print.

2   Merritt CR, Ong GT, Church SE et al. Multiplex digital spatial profiling of proteins and RNA in fixed tissue. Nat Biotechnol 202;38:586â€"599.