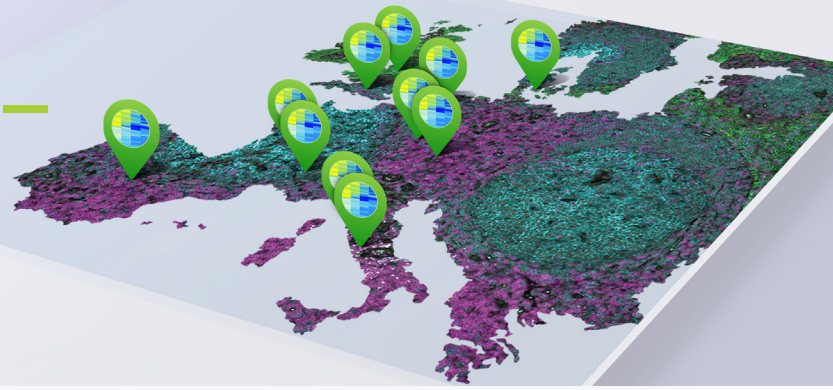
New Frontiers in Digital Spatial Profiling—GeoCast Event



During our conversation on Spatial Discovery we received lots of questions but did not have time to answer them all. Please find answers below.

Learn more about GeoMx™ Digital Spatial Profiler, the new platform that leverages nCounter® Technology to both spatially resolve and digitally quantify up to 96 proteins or 1000+ RNA targets with non-destructive sample processing at nanostring.com/DSP

Can both paraffin and frozen samples be analyzed?	Yes, both FFPE and frozen tissue samples are compatible for DSP.
Will a conjugation kit be released?	We are evaluating the development of a conjugation kit but cannot currently comment on timelines for availability.
Will the panels support in-situ PAM50 analysis for breast cancer?	We are currently working on our high plex RNA panel design for NGS readout, which contains some PAM50 probes. Future content development may include the full gene set.
Could DSP be used to personalize treatments against different types of tumors for individual patients based on their profile? In addition, could DSP be further used to later track how the treatment works?	As our panel has shared, clinical translation is a key objective in using the GeoMx DSP. The platform's multiplex and high throughput nature is designed to facilitate a wide range of experiments that enable discoveries for the clinic, given the right tissue samples and information. A key consideration raised by Aubrey Thompson, PhD, during the panel is the size and kind of tissue samples that are available and the degree to which these samples are annotated. Tissue sample availability and annotation are critical to making clinically-relevant conclusions, and they're especially important for precision medicine.
What happens to the unbound probes? Will they be washed away before the UV light exposure?	Yes. The instrument performs a wash immediately after slides are loaded and between every cleavage event.
Can DSP be used with acetone fixed tissue?	Currently, DSP is compatible for FFPE and frozen tissue samples.

How are the probes validated?	NanoString® performs extensive QC on the assays provided. For protein, antibodies are screened via IHC before and after oligo conjugation to ensure quality and specificity. Next, sensitivity is tested via cell line titration experiments. Following this sensitivity and specificity screening, antibodies are screened to ensure no antibody-antibody interactions in multi-plex. Final Protein Assays are run on a TMA to evaluate performance in tissue. For RNA, we first screen probes bioinformatically to test specificity. Next designed probes are pooled in equimolar concentrations. We then analyze probe performance and screen out any outliers followed by sensitivity and specificity testing on cell lines and finally a TMA screen.
What is the antibody performance with respect to affinity and density?	Compared to standard IHC assays where primary and secondary antibodies are required to amplify and detect signal, with DSP, only primary antibodies are used in the assay which is less prone to antigen crowding effects. Moreover, in validation, we routinely perform "leave one out" experiments to test antibodies by themselves and in a panel and eliminate antibodies that don't behave well.
Post mortem delay and time in fixative are known to interact with RNA and protein detection. Do you know whether these 2 parameters will impact the proteins and gene detection by your technology?	We recognize that parameters such as cold/warm ischemic time, fixation, storage, and others can play major roles in performance. For DSP, we have validated robust protein and RNA detection for FFPE samples under 3 years of age prepared from tissue with a cold ischemic time of less than 1 hr using 10% neutral-buffer formalin or a similar fixative.
How would you suggest pre-treating the FFPEs? Would it be in the same way as pre-treating for FISH?	Slide preparation for FFPE follows standard protocols for pre-treatment for IHC for protein and FISH for RNA.
Will there be any contamination at the border of a single cell ROI by probes bound to the adjacent cells?	The digital micro mirror device used to focus the light uses 1 micron mirrors to highlight regions of interest, which is less than a cell. Having said that, sometimes It is possible to have contamination at the border, and drawing clear margins of each cell can be difficult when cells are clumped together. Our recommendation is that, when possible, users find cells that are relatively isolated to allow drawing smaller and clear ROIs for single cells.
Will the results of this technology need the interpretation of the pathologist or can the analysis be done by biologists and clinicians?	DSP data can be interpreted by a variety of users, including pathologists, biologists, and clinicians.
Do you have experience in the detection of miRNAs?	We currently do not have experience in detecting miRNAs, though we intend to explore this application further for product development.

New Frontiers in Digital Spatial Profiling—GeoCast Event

What is the reason for separating the tissue first into segments, and then profiling the tissue instead of profiling the whole tissue? Is single cell resolution really possible? If yes, for how many cells?	The system offers whole tissue imaging at single cell resolution for up to 4 markers. This allows users to identify biological regions of interest for deeper characterization at significantly high plex. The segmentation feature allows users the flexibility to tease out heterogeneous regions with near 100% cellularity. For deep characterization through high plex spatial profiling, most users will select 10 cells or higher for protein analysis and 100 cells or higher for RNA analysis.
Was the automated Leica system used in the pilot studies? Or was the staining performed manually?	We have used the Leica Autostainer for some of our Technology Access Program projects. To date, our beta testers have utilized manual workflows.
Are there protocols available for other automated platforms, Roche-Ventana for example?	The DSP slide prep chemistry is designed to be compatible with commercially available autostainers. However, we have not validated the protocols on other automated slide preparation platforms. We expect this to be available in the near future.
How does DSP deal with FFPE-related autofluorescence?	Our multicolor IHC morphology visualization panels have been validated and designed for best signal isolation. Additionally, the technology automatically calculates appropriate thresholds for each channel in order to optimize signal.
Is DSP ready for clinical applications in lung cancer?	Through our Technology Access Program, the GeoMx DSP has been used in a variety of studies involving lung cancer samples with the objective of biomarker discovery. As shared by our panel in the GeoCast, a key application is biomarker discovery with the goal of translation into the clinic. The multiplex and high throughput nature of the GeoMx platform enables simultaneous investigation of multiple key targets that are either already immunotherapy targets or have the potential to be. Translating these discoveries into the clinic is a goal for many of our users.
Does the DSP work with murine markers?	Yes, we are launching with a murine panel for Immuno-Oncology applications. We will also continue to build our panel portfolios to contain murine markers of interest to our customers. Additionally, customers will have the option to introduce custom modules that contain targets specific to their needs.
To what extent is the cost of the assay dependent on the area analyzed? With particular reference to Aubrey Thompson's work on breast cancer, is the entire slide examined or multiple selected fields?	Multiple fields were selected for analysis in Aubrey's data. The cost does not depend on the size of an ROI, only the number of ROI and the number of targets in the panel.