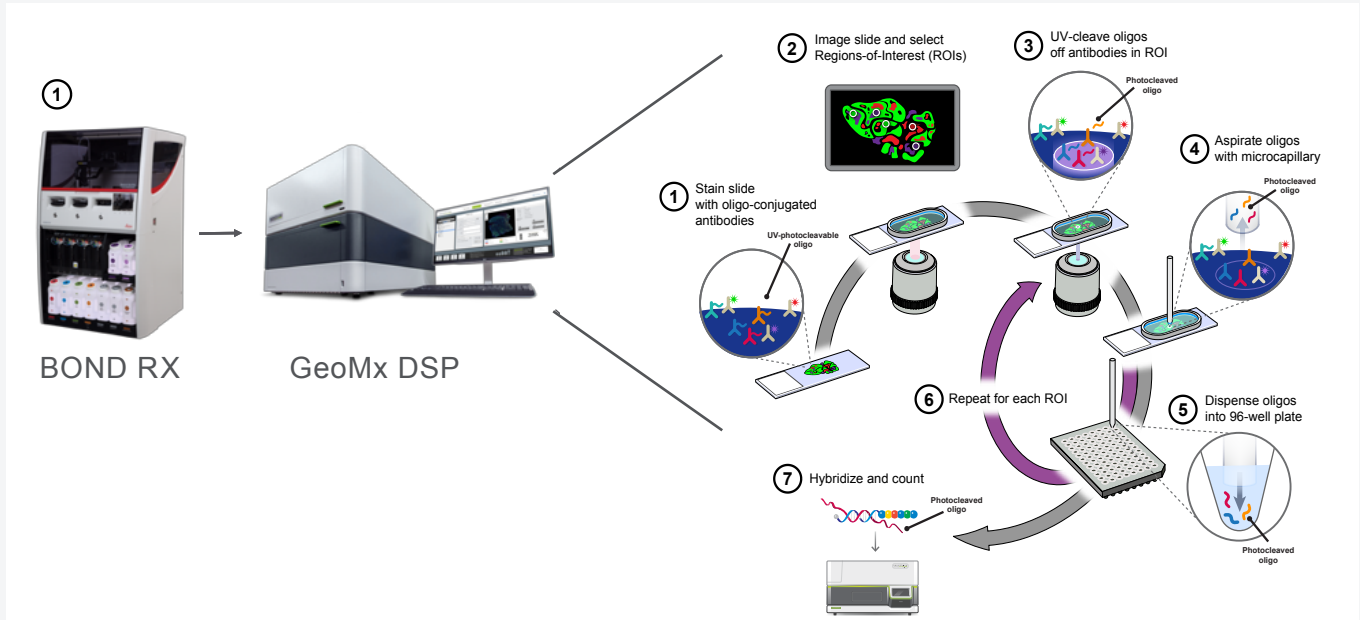# Integrated and Automated Workflow for High Plex RNA and Protein Profiling

BOND RX™ & GeoMx™ Digital Spatial Profiler


FIGURE 1: Workflow Overview

Technology Introduction and Workflow Overview

Spatial analysis has been hampered by low plex and lack of quantitative measures. Alternatively, high plex, quantitative technologies sacrifice critical spatial information. The GeoMx Digital Spatial Profiler (DSP) combines standard immunofluorescence techniques with digital optical barcoding technology to perform highly multiplexed, spatially resolved profiling experiments from challenging samples such as FFPE. A new automated workflow has been developed to combine GeoMx DSP with Leica Biosystems' BOND RX (**FIGURE 1**). Briefly, samples are incubated with up to three fluorescent morphology markers and a nuclear dye plus and a cocktail of 96+ GeoMx RNA or protein probes conjugated to photocleavable indexing oligonucleotides for high-plex RNA or protein analysis. After preparing samples for high plex analysis on the BOND RX, GeoMx DSP performs whole slide imaging to capture tissue morphology for selection of regions of interest (ROI) for high plex profiling. ROI selection is flexible to support a variety of key biological questions (**FIGURE 2**). Once ROI
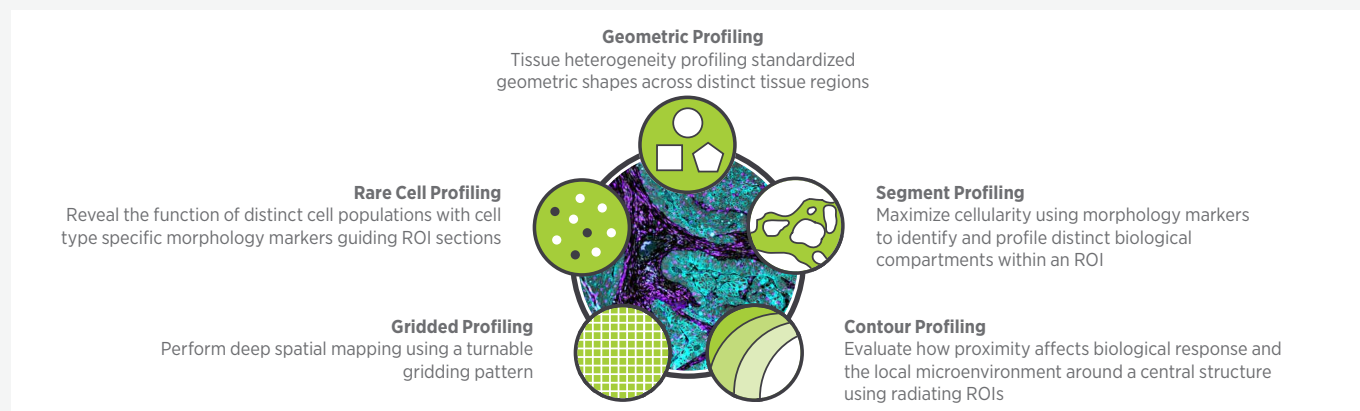
**FIGURE 2:** Application Modalities on GeoMx DSP

have been selected, oligonucleotide tags are released from discrete regions of the tissue via UV exposure. Released tags are collected and quantitated in a standard nCounter® assay via hybridization to digital barcodes and counts are mapped back to tissue location, yielding a spatially-resolved digital profile of analyte abundance (**FIGURE 3**).

## Automated Workflow and Performance

To enable high-throughput and highly reproducible GeoMx DSP data, workflows for sample preparation of GeoMx RNA or Protein assays on the BOND RX have been developed. Briefly, protein analysis, FFPE samples are deparaffinized followed by antigen retrieval and blocking on the BOND RX. Next, the high-plex cocktail of oligonucleotide conjugated antibodies and fluorescent morphology reagents are applied followed by washing, post-fixation, and incubation with nuclear stain reagent for ROI selection. Samples are then ready to be loaded on the GeoMx DSP.

For RNA analysis, samples are deparaffinized followed by EDTA pretreatment, protease digest, and post fixation on the BOND RX. RNA detection reagents are then hybridized off-instrument overnight. The following day, stringent washes are applied, followed by the morphology reagent incubation on the BOND RX. Samples are then ready to be loaded on the GeoMx DSP.

To ensure the automated workflow does not impact performance of the GeoMx DSP assay, sample preparation procedures using the automated workflow were compared to full manual tissue processing. Serial sections of FFPE human colon adenocarcinoma tissue were prepared using automated and manual methods side-by-side up to the point of overnight incubation with antibody mix. Results demonstrate a high correlation in expression of the GeoMx Immune Cell Profiling Panel between the two sample preparation methods (**FIGURE 4**). Therefore, the BOND RX workflow can be easily incorporated into GeoMx DSP experiments for higher throughput analysis of spatially resolved RNA and protein.
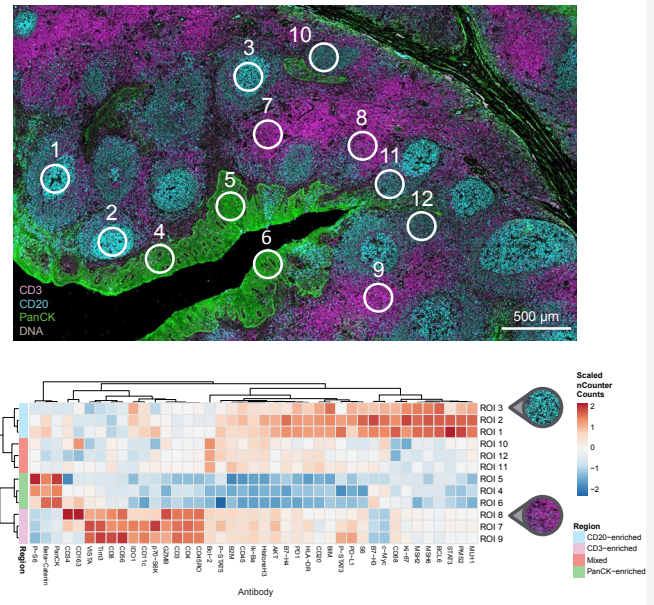
FIGURE 3: GeoMx DSP data demonstrates high plex protein profiling from Geometric ROI
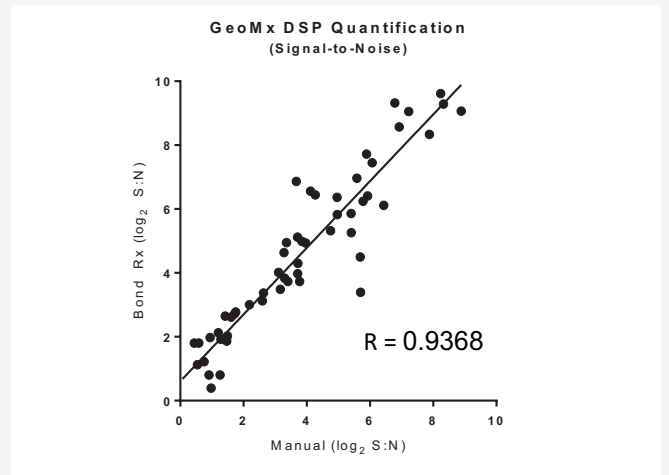
FIGURE 4: Correlation of GeoMx DSP data prepared using manual vs. automated workflows

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