Development of a custom high-plex GeoMx digital spatial profiler breast cancer protein biomarker assay

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Background

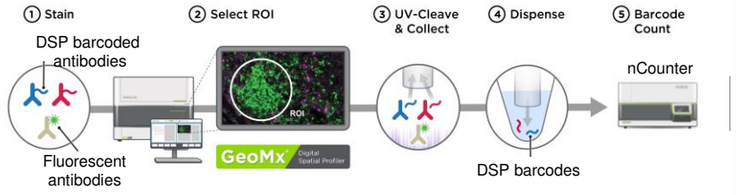
The goal of this study was to develop a high-plex assay to simultaneously quantitate 27 established and novel breast cancer (BC)-related, immune protein and phosphoprotein biomarkers using the GeoMx® Digital Spatial Profiler (DSP). The custom assay performance was compared to standard, immunohistochemistry-based clinical BC biomarker assays (e.g.ER, PR, HER2) across the spectrum of BC subtypes and in multiple laboratories.

Methods

Commercially available antibodies to 27 BC-related protein biomarkers, including ER, PR, HER2, Ki-67, AR, immune-related targets (e.g. PD-L1) and several cell cycle/proliferation markers were oligonucleotide-tagged and verified by immunohistochemistry for performance against untagged antibodies. The tagged antibodies were combined with 3 isotype controls and 2 housekeeping proteins into a custom BC high-plex assay for DSP. Confirmation of target specificity was done on a custom tissue microarray (TMA) (Run control) composed of cancer cell lines (+/- drug treatment) and normal tissues. For clinical BC samples, four 600 µm regions of interest were selected by pathologists and segmented into pan-Cytokeratin+ tumor cells and pan-Cytokeratin-negative adjacent stromal segments. With targeted UV light, oligonucleotides were collected from each segment sequentially and quantitated with nCounter. Raw counts were geomean normalized for analysis.

GeoMx DSP Overview

Figure 1: Overview of DSP Workflow
Samples profiled be GeoMx enable spatial resolution of high-plex protein readout. Regions of interest (ROI) are selected guided immunofluorescence and tissue compartments are segmented by thresholding IF channels. Tagged barcodes are cleaved from individual antibodies by UV excitation within the segments defined by the GeoMx DSP. The DNA barcodes are then quantified downstream on the NanoString nCounter® platform.



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Multi-site Assay Concordance

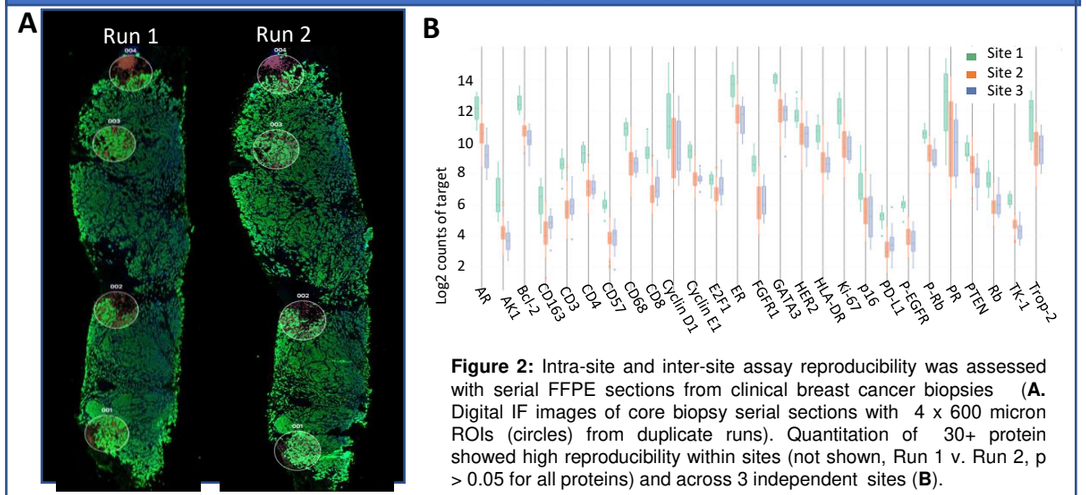

Figure 2: Intra-site and inter-site assay reproducibility was assessed with serial FFPE sections from clinical breast cancer biopsies (**A.** Digital IF images of core biopsy serial sections with 4 x 600 micron ROIs (circles) from duplicate runs). Quantitation of 30+ protein showed high reproducibility within sites (not shown, Run 1 v. Run 2, p > 0.05 for all proteins) and across 3 independent sites (**B**).

HER2 Quantification

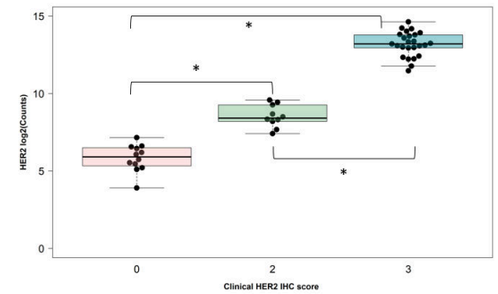

Figure 3: HER2 protein quantitation using DSP with the Custom BC Panel. 5-micron FFPE sections of diagnostic BC biopsies were tested on a set of HER2-negative (HER2 IHC scores of 0, left) and HER2+ positive BC biopsies (HER2 IHC 3+, right) or HER2 IHC 2+/HER2 FISH-amplified, middle). Clinical HER2 IHC assays were scored per CAP/ASCO guidelines using a digital algorithm. The DSP-based custom BC Panel reproducibly quantitated HER2 protein within IHC score categories and could discriminate between clinical HER2 IHC scores (*p <0.005 for all pairwise comparisons).

Estrogen Receptor Quantification

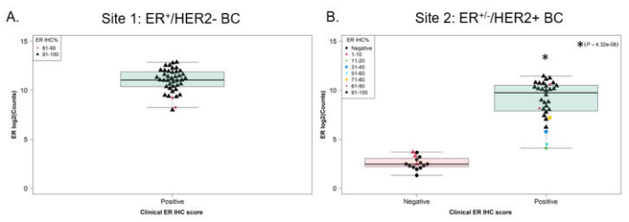

Figure 4: Estrogen receptor (ER) protein quantitation using DSP with the Custom BC Panel. 5-micron FFPE sections of diagnostic BC biopsies were tested at 2 sites: Site 1 tested Luminal ER+ BC (ER+/HER2-)with ER positivity defined as a clinical immunohistochemical assay score of >10% tumor nuclei staining (**Panel A**); site 2 tested a set of HER2-negative (defined as HER2 IHC scores of 0,1 or 2 with negative HER2 FISH) and HER2+ positive BC (HER2 IHC 3+ or HER2 IHC 2+/HER2 FISH-amplified) with variable ER and PR status (**Panel B**). Clinical ER IHC assays were scored in deciles, using ASCO/CAP guidelines. The Custom BC Panel reproducibly quantitated ER protein levels in ER+ BC across the 2 testing sites, and could discriminate ER+ BC from ER- BC (*p <0.005).

Progesterone Receptor Quantification

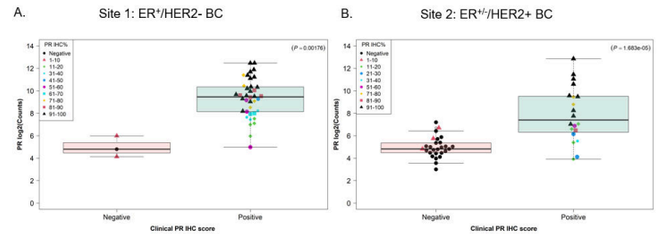

Figure 5: Progesterone receptor (PR) protein quantitation using DSP with the Custom BC Panel. 5-micron FFPE sections of diagnostic BC biopsies were tested at 2 sites: Site 1 tested Luminal ER+ BC (ER+/HER2-) and variable PR status with PR positivity defined as a clinical immunohistochemical assay score of >10% tumor nuclei staining (Panel A). Site 2 tested a set of HER2-negative (defined as HER2 IHC scores of 0,1 or 2 with negative HER2 FISH) and HER2+ positive BC (HER2 IHC 3+ or HER2 IHC 2+/HER2 FISH-amplified) with PR status (Panel B). Clinical PR IHC assays were scored in deciles. The Custom BC Panel reproducibly quantitated PR protein levels across the 2 testing sites, and could discriminate PR+ BC from PR- BC (*p <0.005); however, there was less robust discrimination in PR+ BC at lower clinical PR IHC scores (e.g. PR IHC decile scores of 11-20%, 21-30%, 31-40%).

Panel Contents

ER alpha	**Her2**	**Cyclin D1**	**p16**	**Trop2**	**CD3**
PR	**PTEN**	**Cyclin E1**	**E2F1**	**PD-L1**	**CD4**
AR	**Phospho-EGFR**	**RB**	**Thymidine Kinase -1**	**CD57**	**CD8**
Ki67	**FGFR1**	**Phospho-RB (T252)**	**Aurora Kinase A**	**CD163**	**CD68**
GATA3	**Bcl-2**	**HLA-DR**			

Table 1: The GeoMx DSP breast cancer (BC) panel has 32 antibodies, including 3 IgG controls and 2 housekeeping proteins (S6 and histone H3) and includes clinical BC biomarkers and key targets for BC subclassification,

Conclusions

Our preliminary data demonstrate that this custom high-plex BC assay can quantitate protein biomarkers across a wide dynamic range with high intra-lab and inter-lab reproducibility. The assay requirement of a single 5-µm tissue section facilitates complex biomarker profiling in biopsies with limited material. The custom assay alone or in combination with other targeted DSP protein modules can simultaneously interrogate standard breast biomarkers, other drug target markers, and the immune microenvironment of BC specimens, providing a novel approach for actionable tumor subtyping.