Yale
SCHOOL OF MEDICINE

Validation of novel high-plex protein spatial profiling quantitation based on NanoString's Digital Spatial Profiling (DSP) technology with quantitative fluorescence (QIF)

Maria I. Toki[1], Chris Merritt[2], Giang Ong[2], Joseph M. Beechem[2], Daniel E. Carvajal-Hausdorf[1], Yalai Bai[1], Sarah Warren[2], David L. Rimm[1,3]

[1]Department of Pathology, Yale School of Medicine, New Haven, CT; [2]NanoString Technologies, Seattle, WA;
[3]Department of Internal Medicine, Yale School of Medicine, New Haven, CT

Poster Number: 3810

BACKGROUND

Protein expression in formalin-fixed, paraffin-embedded (FFPE) tissue is routinely measured by Immunohistochemistry (IHC) on only one protein, or with quantitative fluorescence (QIF) on a handful of proteins on a single section. NanoString's Digital Spatial Profiling (DSP)* technology can detect and quantify proteins at significantly higher multiplex (currently 30 plex, potential for up to 800 plex) with spatial resolution using molecular digital color-coded "barcodes", within specific regions of interest on FFPE tissue. Here, we compare Nanostring DSP technology to automated QIF (AQUA), for accurate and reproducible measurement of protein expression on a continuous scale. Additionally, using the multiplexing potential of Nanostring technology, we did a pilot study to assess cold ischemic time as a variable to monitor tissue quality by assessment of epitope degradation in Non-Small Cell Lung Cancer (NSCLC).

METHODS

The DSP technology uses a cocktail of primary antibodies conjugated to indexing DNA oligos with a UV Photocleavable linker that can be counted on the nCounter™ platform. Regions of interest (ROI) on the tissue are selected with fluorescently labeled antibodies, and oligos from that region are released via UV mediated linker cleavage. Free oligos are captured via microcapillary fluidics into a microtiter plate and then quantitated on the nCounter. The comparator for this technology was the AQUA method of QIF. We examined a breast cancer tissue microarray with a range of HER2 expression, and a NSCLC time to fixation standardization array with timepoints from 20 min to 48 hrs.

RESULTS

Multiple markers were assessed and a high correlation was found between Nanostring DSP counts and QIF scores (R^2>0.7), when the measurements were performed in the same region of interest (defined by cytokeratin expression). The dynamic range of DSP exceeded the quantification range of QIF (nearly 4 logs vs about 2 logs). When the 28 protein markers' expression was compared at different fixation timepoints, most were found to be stable over different cold ischemic timepoints. Two markers, including phospho-ERK (Extracellular Signal-Regulated Kinases) and phospho-GSK (Glycogen Synthase Kinase) showed epitope loss as a function of delay to fixation (R^2=0.0064 and R^2=0.05 respectively).

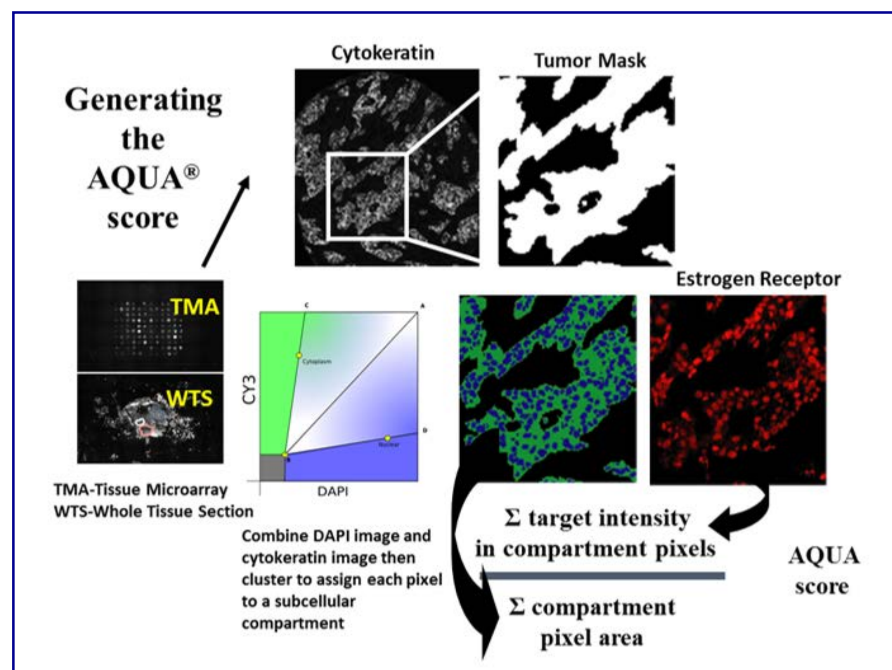


Figure 1: Overview of Automated Quantitative Immunofluorescence (AQUA) scoring. AQUA calculates protein expression on a continuous scale by quantifying immunofluorescence pixel intensity per unit area and has been proven to provide objective and reproducible measurement of targets.

RESULTS

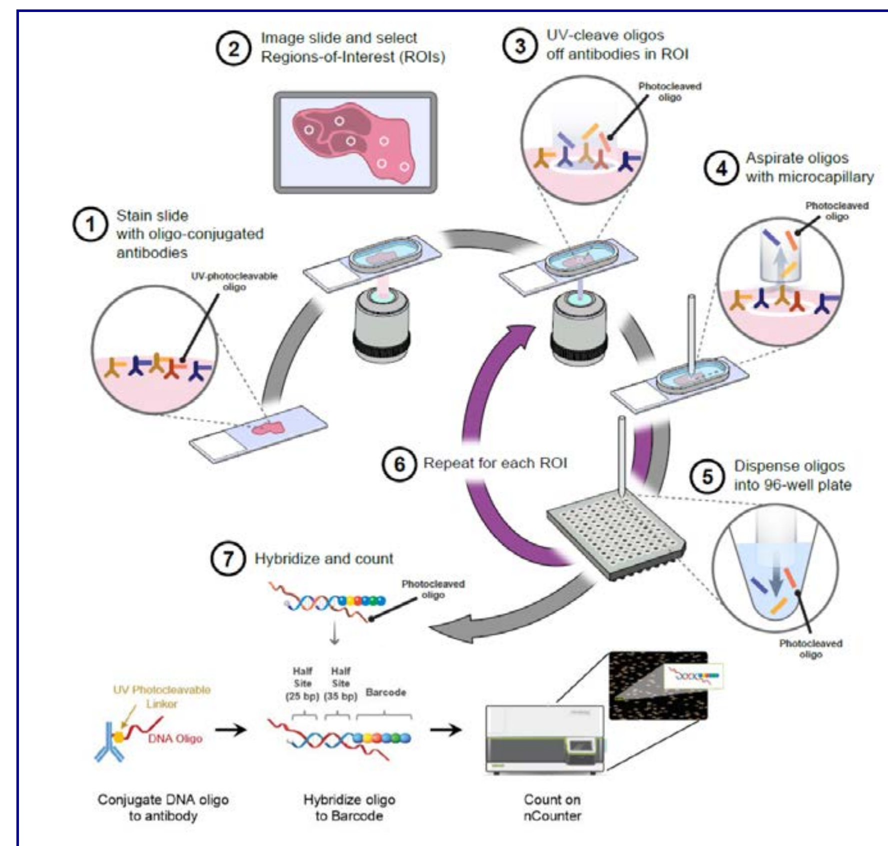


Figure 2: Overview of Nanostring in situ protein profiling workflow.
(1) Process: FFPE tissue slide incubated with a cocktail of oligo conjugated antibodies (2) View: Regions of interest (ROIs) are identified with visible lightbased imaging (3) Profile: Selected ROIs are chosen for high-resolution multiplex profiling, and oligos from the selected region are released upon exposure to UV light (4) Collection: Photocleaved oligos are then collected via a microcapillary tube and stored in a microplate well (5) Digital counting: Photocleaved oligos from the spatially-resolved ROIs in the microplate are hybridized to 4-color, 6-spot optical barcodes, enabling up to ~1 million digital counts of the protein targets (distributed across all targets) in a single ROI using standard NanoString nCounter® instruments

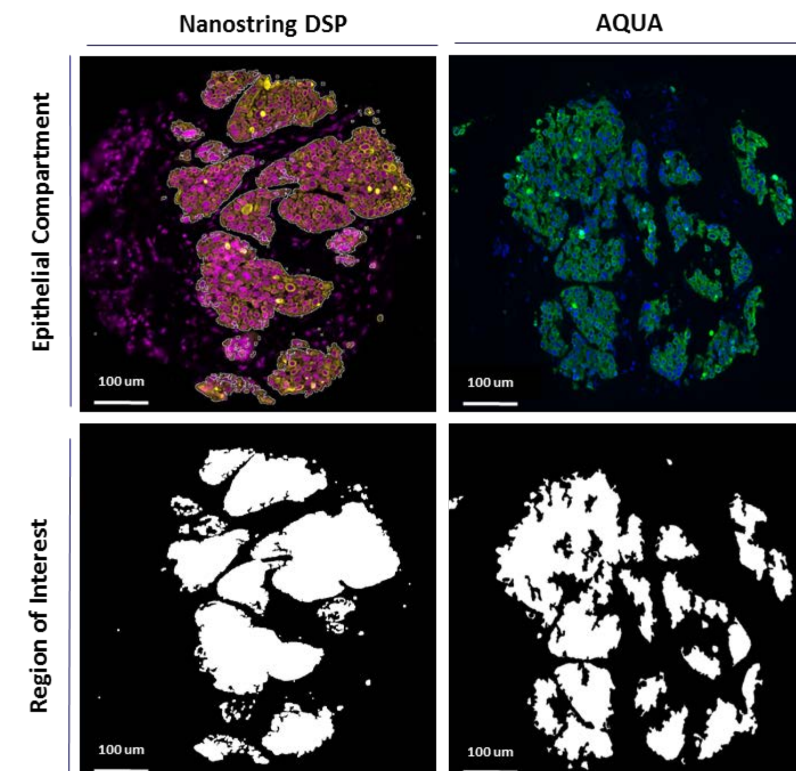


Figure 3: Nanostring DSP Regions of Interest (ROIs) and AQUA Tumor Mask generation. The epithelial compartment was defined by cytokeratin positivity (yellow by Nanostring DSP, green by AQUA) and allowed the measurement of targets by both assays in the same compartment.

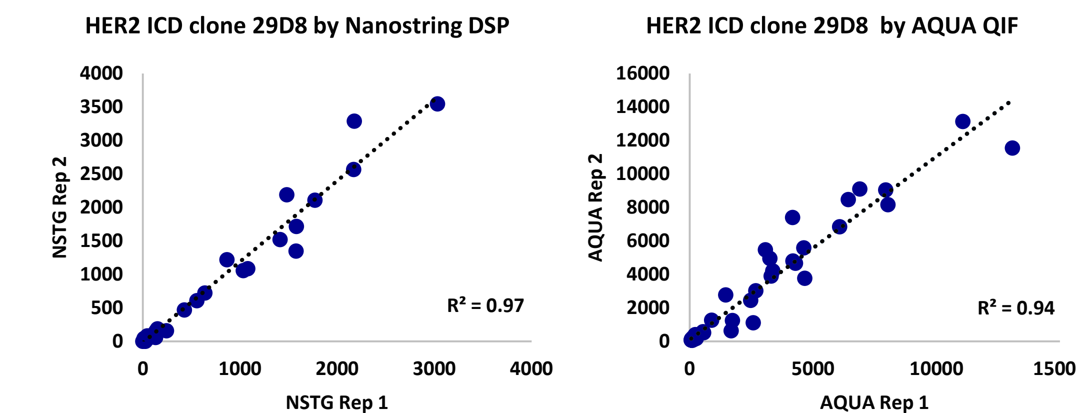
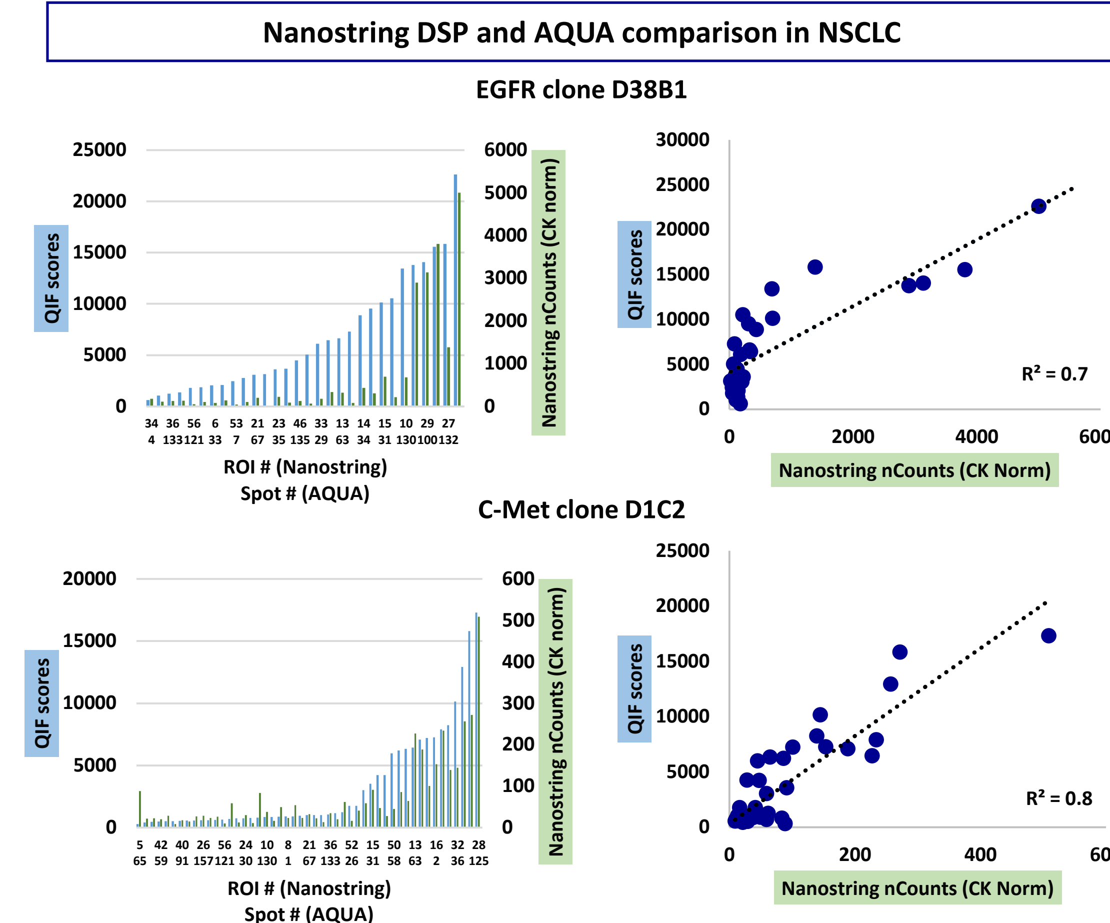


Figure 4: Nanostring DSP and AQUA Reproducibility. Regression chart of two independent experiments by both assays. Nanostring DSP and AQUA QIF are highly reproducible methods for target measurement on a field of view averaged basis.

Nanostring DSP and AQUA comparison in NSCLC



Nanostring DSP and AQUA comparison in Breast Cancer

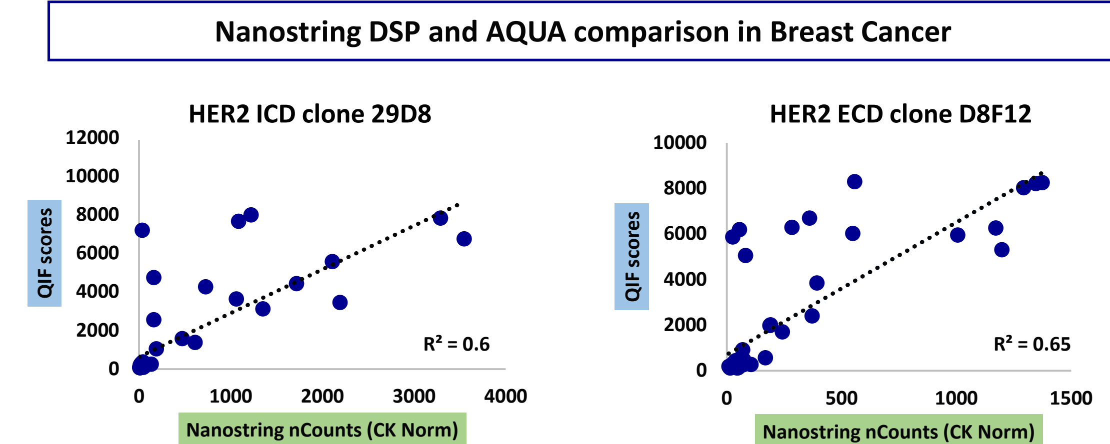
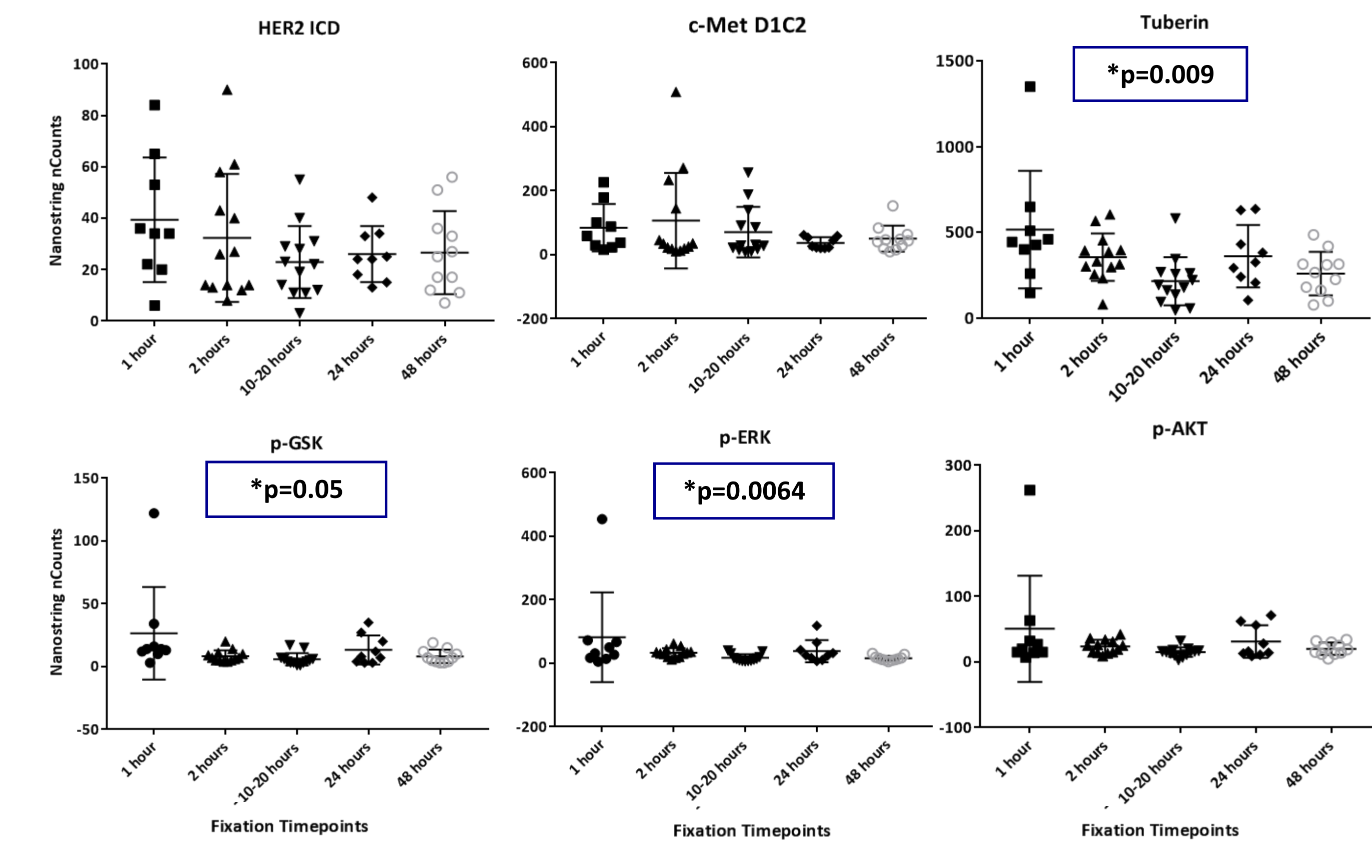
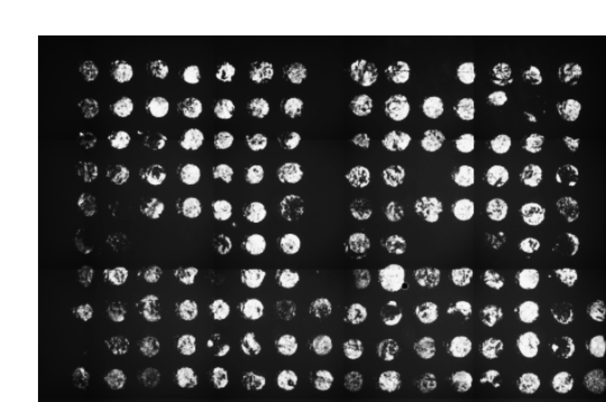


Figure 4: Nanostring DSP and AQUA comparison. Nanostring nCounts and AQUA scores were compared for multiple markers including EGFR and c-Met in NSCLC and HER2 in a breast cancer array. There is a high concordance between the two assays when the measurements are performed in the same compartments, on a field of view averaged basis.





Other targets with no statistically significant difference across time-to-fixation timepoints

EGFR Histone H3	p-EGFR p-Histone H3	Ki67 Pan Keratin	p-PRAS p-P70 S6 Kinase
Ribosomal S6	p-Ribosomal S6	Progesterone Receptor	p-PKD1
AKT HER2 ICD/ECD	p-AKT p-HER2	cMet 4E-PB1	p-cMet p-4E-PB1
ERK	p-ERK p-AmpK	GSK	p-GSK

Figure 6: Nanostring nCounts on different time to fixation (Cold Ischemic) timepoints in NSCLC. No statistically significant difference in the majority of markers on different fixation timepoints. Tuberin, p-GSK and p-ERK have a statistically significant difference across time-to-fixation timepoints. One way ANOVA (Kruskal-Wallis test). Bars: Mean with SD.

CONCLUSIONS

- Nanostring DSP is a reproducible method that offers highly multiplexed measurements of protein expression on a field of view averaged basis.
- There is a high concordance of Nanostring nCounts with the QIF scores generated by AQUA, an extensively validated technique for protein quantification.
- Most protein markers are stable across fixation timepoints.
- The high-plex capacity of DSP allows inclusion of markers that are sensitive to time to fixation as an intrinsic control for tissue quality.

This work was supported by grants including the Yale SPORE in Lung Cancer, Stand Up to Cancer (SU2C) and Breast Cancer Research Foundation (BCRF)

*FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

www.nanostring.com | info@nanostring.com | @nanostringtech

© 2016 NanoString Technologies, Inc. All rights reserved. Patents pending.
NanoString, NanoString Technologies, the NanoString logo, nCounter, 3D Biology, and nSolver are registered trademarks or trademarks of NanoString Technologies, Inc., in the United States and/or other countries.