Study Report

nCounter® Digital Spatial Profiling

Technology Access Program

[*Study Title*]

Prepared for (Customer Institute Name)

[PTL-#]

SAMPLE

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Approvals

Role	Name	Organization	Signature	Date
Principal Investigator	*Yan Liang, Ph.D.* *Director, Pathology*	*NanoString Technologies*		
Management	*Joseph M. Beechem, Ph.D.* *Senior Vice President of R&D*	*NanoString Technologies*		

1. Definitions & Abbreviations

Term	Definition
DSP	Digital Spatial Profiling
FFPE	Formalin-Fixed, Paraffin-Embedded
NS	NanoString Technologies
QC	Quality Control
ROI	Region of interest
Ab	Antibody
H&E	Hematoxylin and Eosin stain
Digital Spatial Profiler	Platform under development, upon which the Digital Spatial Profiling Assay is run
TAP	Technology Access Program; For Research Use Only. Not for use in diagnostic procedures
ERCC Positive	Positive assay control
ERCC Negative	Negative assay control

2. Report Summary

Full Title	Study Report for nCounter Digital Spatial Profiling Assay	
Sponsor	Company XYZ	
Sample Size	20 FFPE unstained sections / up to 24 ROI per section	
Study Objective	nCounter Digital Spatial Profiling	
Study Design	20 FFPE unstained tissue sections were stained with 2-3 fluorescently labelled antibodies to visualize the tissue morphology. A panel of up to 20 antibody cocktail will then be applied to the FFPE unstained section and processed through NanoString's DSP platform followed by quantitative detection using the nCounter analysis system.	
Study Duration	Study Initiation Date	
	Study Completion Date	
Test/Assay/Device	Custom antibody panel or standard 20 Ab cocktail-nCounter, protein tag set, nCounter analysis system.	

3. Overview

The goal of the Digital Spatial Profiling Technology Access Program (TAP) is to provide a service offering to NanoString's multiplexed digital spatial profiling IHC technology, which is currently being developed on NanoString's DSP technology platform. This program is offered to provide early access to customers to leverage the principles of single molecule optical barcoding to enable detection of multiplexed proteins from the surface of FFPE tissue and enable protein quantitation from a defined region of the interest (ROI).

4. Purpose

The purpose of this report is to document the experimentation and data analysis performed according to protocol number PTL-M-XXX, executed to utilize NanoString's DSP technology platform to enable digital characterization of protein distributed on the surface of FFPE tissue sections.

5. Equipment, Materials and Methods

5.1. Equipment

Equipment
Auto-stainer
nCounter Analysis System
Digital Spatial Profiler Prototype Instrument

5.2. FFPE Samples

Twenty unstained sections (4-6µm) will be used in this study.

Sample Type	Section #	Tissue Type	Section thickness

5.3. Material and reagents requested for multiplex IHC assay

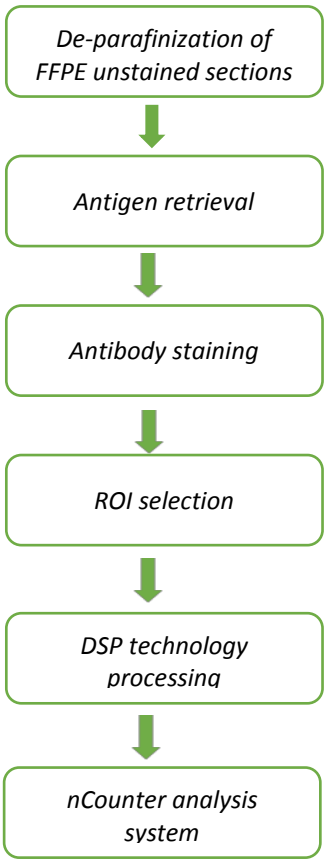
Materials
Pipettes for 5–1,000 µL
Benchtop Centrifuge
Plastic Coplin Jars
Hydrophobic pen
Thermal Cycler
Salmon Sperm DNA
Dextran Sulfate
TintoRetriever Pressure Cooker
CitriSolv
Ethyl Alcohol, 200 Proof, Absolute
ImmEdge Hydrophobic Barrier Pen
Digital Spatial Profiler Instrument
Humidity Chamber
Citrate Buffer pH6
SignalStain® Antibody Diluent
Tris Buffered Saline with Tween 20 (TBST-10X)
Phosphate Buffered Saline with Tween® 20 (PBST-20X)
Goat Serum
Protein TagSet

5.4. Antibody Panel

Table xx [Example of probable Antibody cocktail used in study]

Target Name
AKT
B7-H3 (CD276)
Bcl-2
Beta-2-microglobulin
Beta-Catenin
CD14
CD19
CD3
CD4
CD44
CD45
CD45RO
CD56
CD68
CD8A
FOXP3
GZMB
Histone H3
IgG Rabbit Isotype Control
Ki67
Mouse IgG Control
MS4A1 (CD20)
P-AKT
Pan-Cytokeratin
PD1
PD-L1
P-STAT3 (phospho Y705)
PTEN
S6
STAT3
VISTA

6. Assay Work Flow [Procedure]



7. Data Analysis

7.1. Data Quality Control

[Positive and Negative Hybridization Control Probes (POS and NEG A-F): nCounter Assays include a set of 6 Positive and Negative control probes to monitor hybridization efficiency, Prep Station purification, and imaging. POS control targets are built into the CodeSet reagent, and therefore will reflect systematic variability between assays]

Positive Control Performance: Linearity of Counts vs. protein tag set concentration: Protein tag concentrations in the hybridization for protein tag positive control targets are listed in parentheses in the data file, and range from 128fM (POS_A) to 0.125fM (POS_F) in a 4-fold titration. The expected correlation of Positive control counts to Protein tag target concentration is R2>0.95

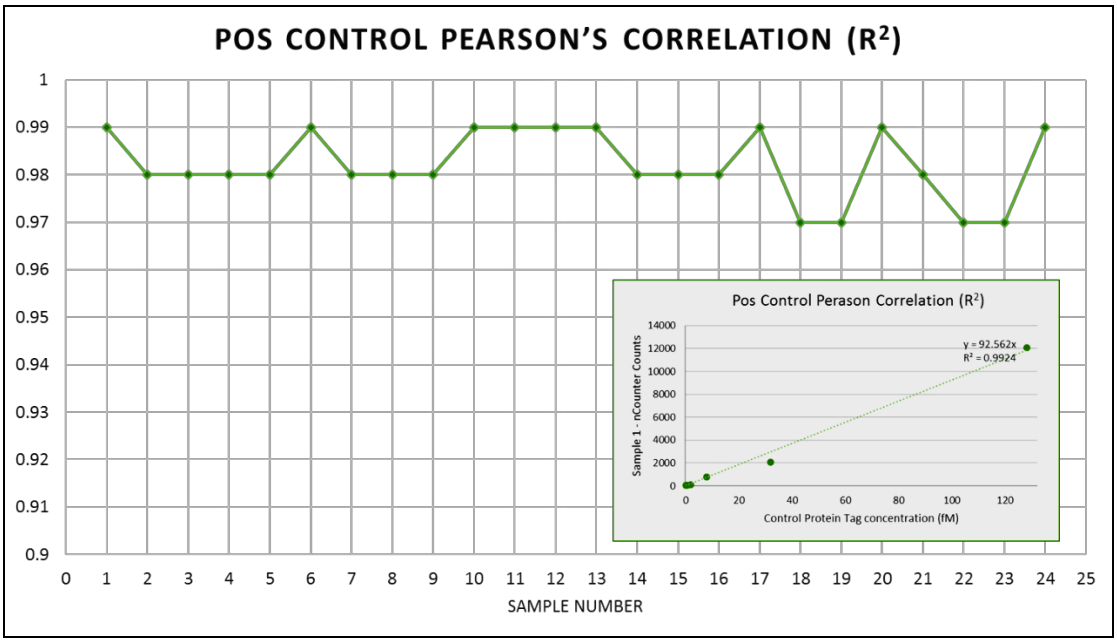


Figure 1. This graph demonstrates the linearity of the nCounter platform. The Pearson Correlation (R^2) of target concentration vs. counts is plotted for the 6 Positive control probes across 24 assays. Correlations are in expected range (R^2> 0.95).

NanoString recommends using endogenous protein tag counts to evaluate sample quality and data normalization.

7.2. Positive Protein Tag Normalization:

Data has been normalized using the geometric mean of the protein tag positive control in each sample. NanoString recommends that for optimal results, normalization factors range between XX and XX for all assays

7.3. House keeper normalization:

S6 Ribosomal Protein and Histone 3 will be used for house keeper.

7.4. Area normalization:

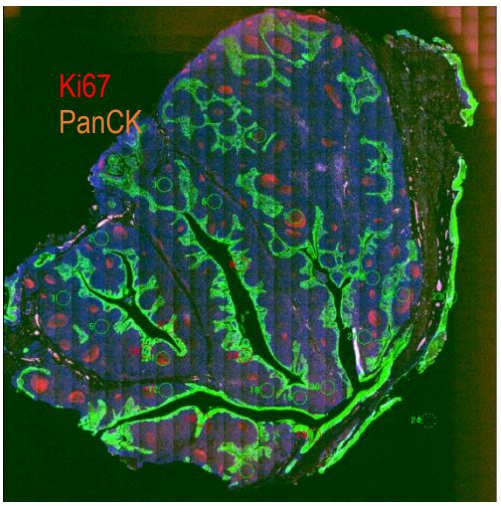
If custom ROI or different ROI size will be used, area normalization will be applied (see attached excel data sheet).

8. Results

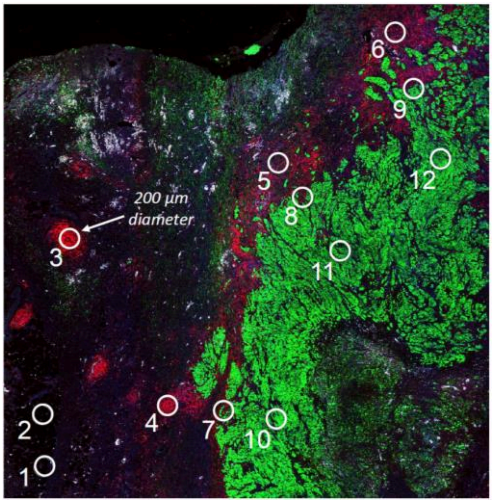
8.1. ROI selection

8.2. Size: 100-500um diameter circle, rectangle ROIs (for example 300x400um), custom ROI and single cell auto-selection ROIs et.al.

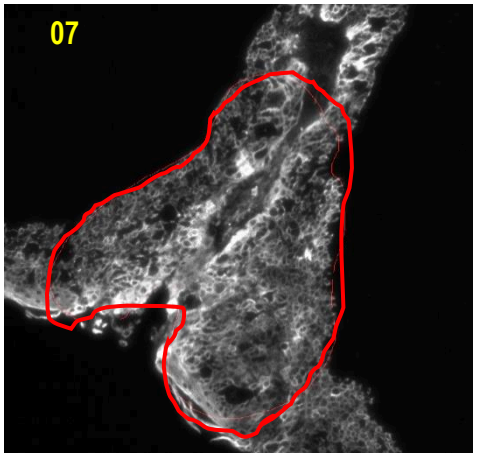
8.3. Images: All images from 20 samples with ROI selected area will be provided



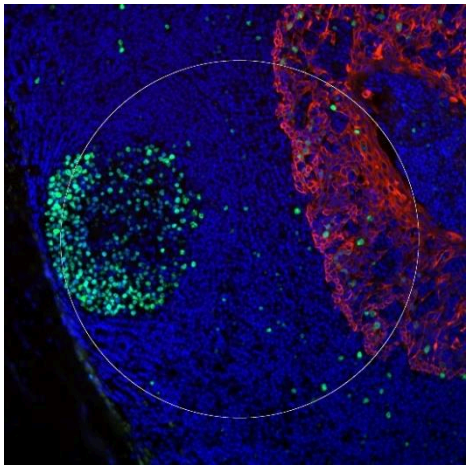
Human tonsil, DSP microscope scanned image. Stained with PanCK (green), Ki67 (red) and nuclear staining(blue) to visualize the section morphology



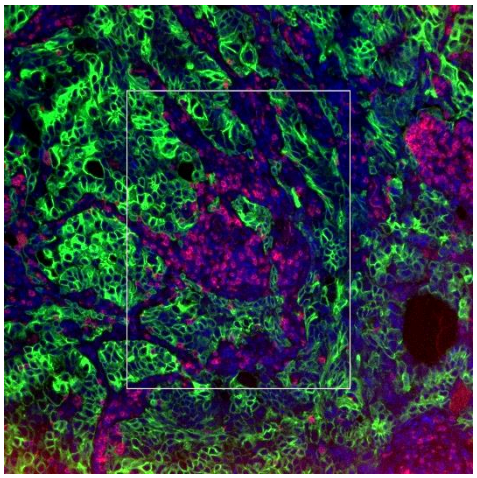
Human colon cancer, DSP microscope scanned image. Stained with PanCK (green), CD45 (red) and nuclear staining (blue) to visualize the section morphology



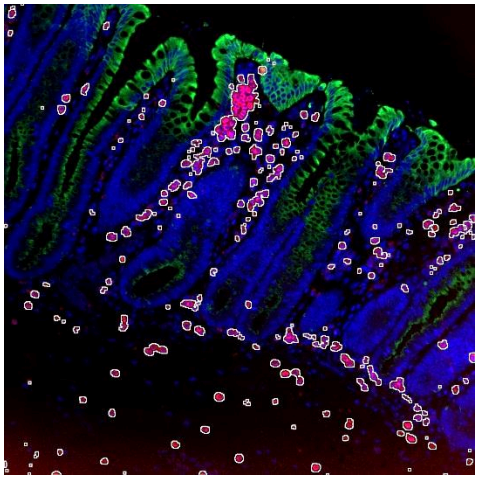
Human tonsil, DSP microscope scanned image. Custom ROI: PD-L1 high expression area.



Human tonsil, DSP microscope scanned image. ROI: 500um diameter. panCK (red), Ki67 (green) and nuclear staining(blue) to visualize the section morphology

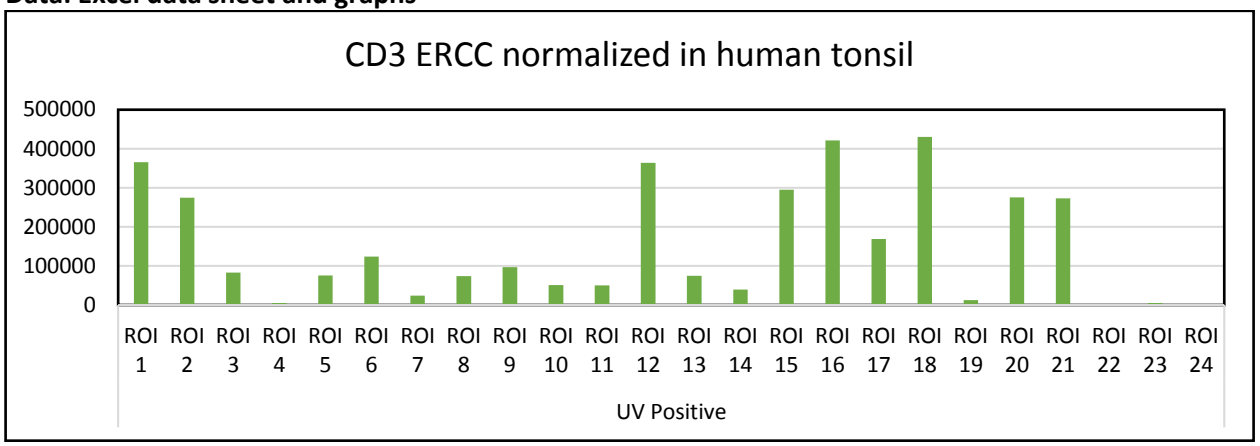


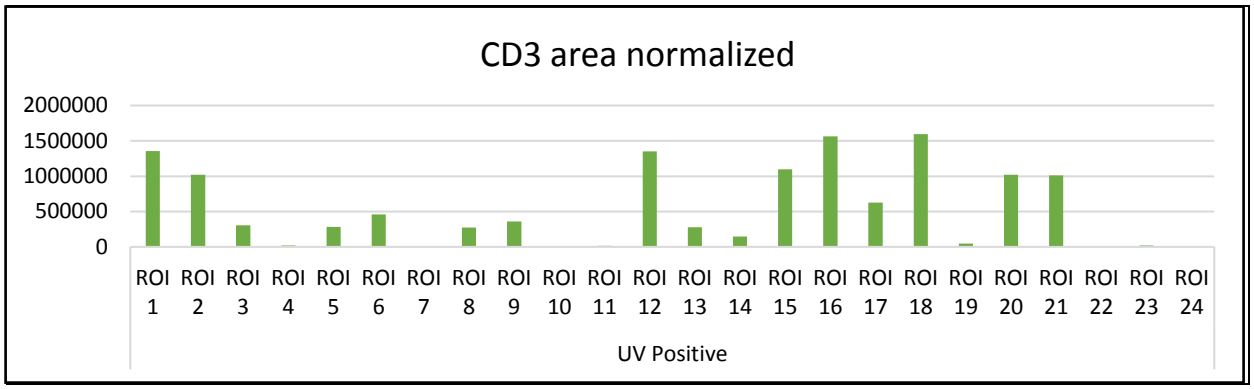
Human NSCLC, DSP microscope scanned image. ROI: 300um x 400um. Stained with PanCK (green), CD3 (red) and nuclear staining(blue) to visualize the section morphology.

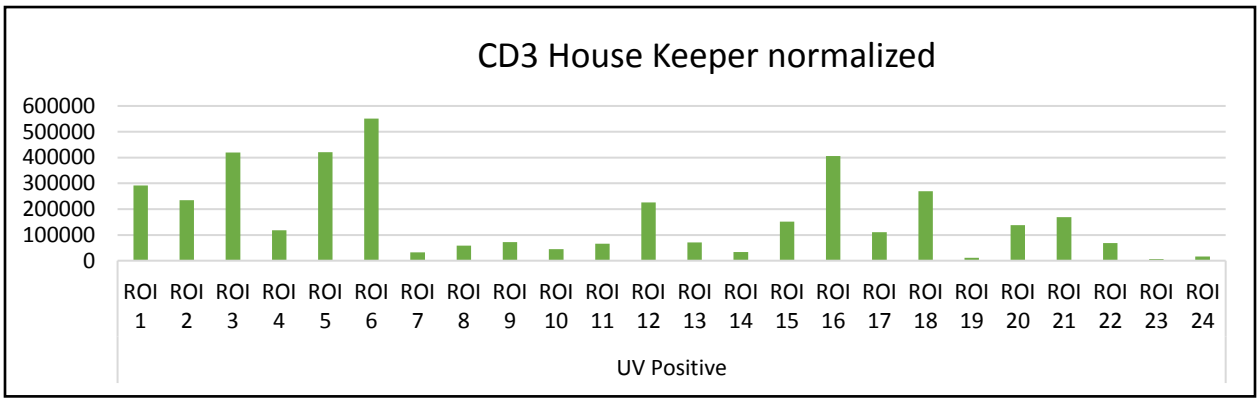


Human colon, DSP microscope scanned image. ROI: custom selected CD3 positive cells. Stained with PanCK (green), CD3 (red) and nuclear staining(blue) to visualize the section morphology.

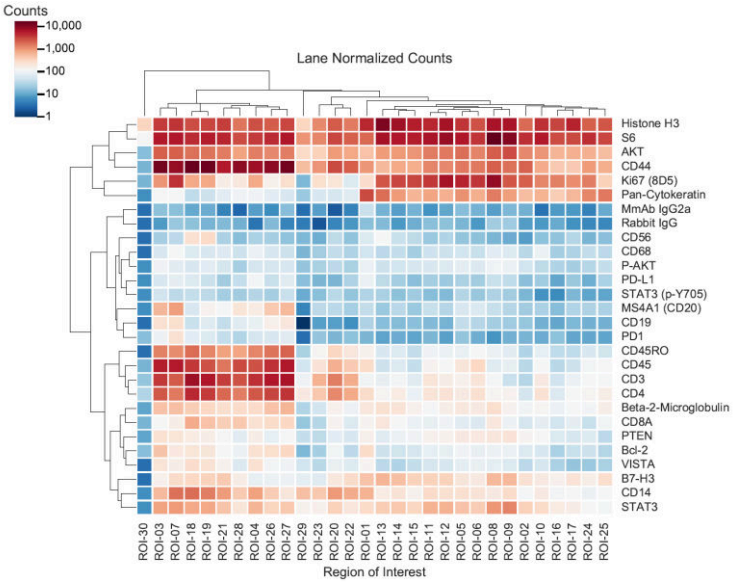
8.4. Data: Excel data sheet and graphs





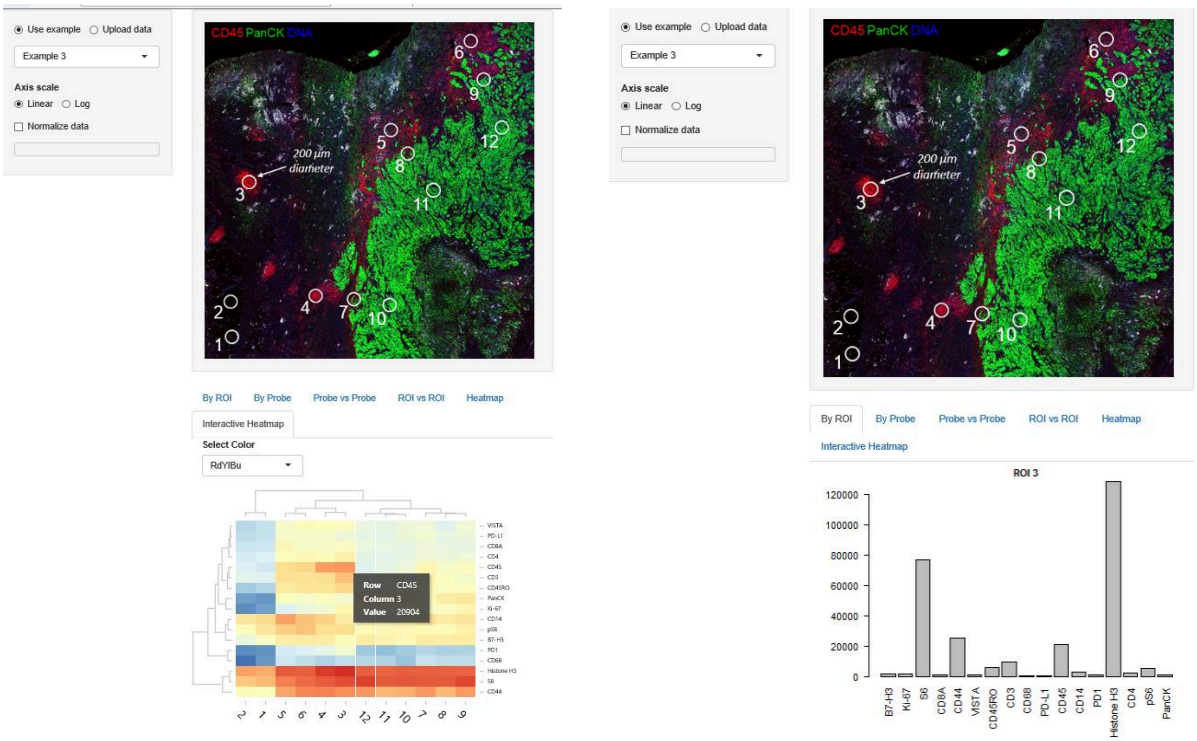


8.5. Heat map [optional]



8.6. DSP App, an interactive data visualization tool:

Visualize data and images in web browser. Preprocess with custom options, explore data on ROIs and targets, and create figures with simple clicks.



9. Conclusions

[This section will contain a summary of the results of the study using the nCounter Digital Spatial Profiling Assay.]

10. Deviations

Any protocol deviations will be detailed.

11. Data Collection & Storage

Raw data (excel data sheet), images (whole tissue images (low magnification), individual ROI high magnification images) from all samples, power point documentation and DSP App (a live data visualization tool) will be provided to customers.

Sample Archiving: Stained sections and remaining tissue blocks will be returned to the customer.

Data will be archived at NanoString for 12 months after completion of the study.

12. References

Appendix 1

Excel file “project number”. This file contains multiple sheets:

- Raw Data: raw counts for nCounter assay
- Normalized Data: Raw counts adjusted using the normalization methods.
- All images
- Power point documentation
- DSP App (a live data visualization tool).