Spatial Molecular Imaging of FFPE Cancer Samples at Unlimited Multi-Omic Spatial Plex allows True Systems Biology Understanding

Joseph Beechem, PhD. SVP and CSO NanoString Technologies Seattle, Washington

Spatial Multi-Omics For Cancer Systems Biology Feb 12, 2024



Notice

The CosMx[™] SMI and decoder probes are not offered and/or delivered to the following UPC member states^{*} for use in these countries for the detection of RNA in a method used for the detection of a plurality of analytes in a cell or tissue sample without the consent of the President and Fellows of Harvard College (Harvard Corporation) as owner of the Unitary Patent EP 4 108 782 B1. The use for the detection of RNA is prohibited without the consent of the President and Fellows of Harvard College (Harvard Corporation). *Austria, Belgium, Bulgaria, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Portugal, Slovenia, Sweden

The CosMx[™] SMI and decoder probes are not offered and/or delivered to the Federal Republic of Germany for use in the Federal Republic of Germany for the detection of cellular RNA, messenger RNA, microRNA, ribosomal RNA and any combinations thereof in a method used in fluorescence in situ hybridization for detecting a plurality of analytes in a sample without the consent of the President and Fellows of Harvard College (Harvard Corporation) as owner of the German part of EP 2 794 928 B1. The use for the detection of cellular RNA, messenger RNA, microRNA, ribosomal RNA and any combinations thereof is prohibited without the consent of the of the President and Fellows of Harvard College (Harvard Corporation) as owner of the German part of EP 2 794 928 B1. The use for the detection of cellular RNA, messenger RNA, microRNA, ribosomal RNA and any combinations thereof is prohibited without the consent of the of the President and Fellows of Harvard College (Harvard Corporation).



Spatial Biology is the Next Life Sciences Revolution





2006



Genome Analyzer

Next Generation Sequencing 2009 Method of the Year



Single Cell

2012



Chromium

Single Cell Genomics 2013 Method of the Year

Adapted from concept and images by Dr. Aviv Regev of The Broad Institute; *Nature Methods*



From very first AGBT Spatial Summit (2019) to Spatial Single-Cell Whole Transcriptome Imaging (2024), pushing multi-omic plex to the absolute maximum, for true systems-biology interrogation of tissue



*Estimated Commercial Launch Year

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CosMx* Whole Transcriptome >18,000 genes

CosMx 6,000 genes

NanoString's CosMx SMI Achieves Whole Transcriptome Imaging at Single-Cell Resolution

Major milestone marked by first public dataset released for CosMx Whole Transcriptome Panel with additiona data forthcoming at the AGBT Meeting

January 7, 2024

SEATTLE--(BUSINESS WIRE)--NanoString Technologies, Inc. (NASDAQ: NSTG), a leading provider of life science tools for discovery and translational research, today announced the release of the first public dataset of the whole transcriptom bbtained at true single-cell resolution on the CosMx™ Spatial Molecular Imager (SMI). The data will be highlighted at the 42nd annual J.P. Morgan Healthcare Conference 2024 in San Francisco during NanoString's corporate presentation or nesday, January 10, 2024, at 5:15 pm ET.

"Using the CosMx Whole

CosMx Whole Transcriptome Panel Achieves Scientific Moonsho

"born" as a sequencing chemistry, and applied to spatial-sequencing

2023



Marco Island, Feb 27 2019: AGBT's very first Spatial Biology Event was NSTG-proposed & Sponsored. NSTG invited all of the spatial technologies to our event, in order to be inclusive and work together to advance the science...



Mapping the brain with MERFISH

The molecular, spatial, and functional organization of the hypothalamic preoptic region via in situ single-cell transcriptomics

Jeffrev R. Moffitt

Program in Cellular and Molecular Medicine Boston Children's Hospital

Department of Microbiology rvard Medical Schoo

Spatial Genomics Summit | February 27, 2019



From tissues to single cells and back again...

Fei Chen Nanostring In Situ Genomics Workshop Feb 27, 2019

Digital Spatial Profiling:

Delivering on the Promise of Spatial Genomics & Proteomics

Joseph M. Beechem, PhD SVP of Research and Development

27-Feb-2019 Spatial Genomics Summit --- AGBT Marco Island FL





NSTG Not Going Anywhere. Accomplished \$47.5M new funding for 2024.



Spatial Genomics Summit | February 27, 2019

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Lawsuit – CosMx, Lawsuit – GeoMx

Digital Spatial Profiling:

Delivering on the Promise of Spatial Genomics & Proteomics

Joseph M. Beechem, PhD **SVP of Research and Development**

Spatial Genomics Summit --- AGBT



Feb 8, 2024: GEN publishes two articles on the problem of the attempt to Monopolize all Spatial Biology Approaches

NanoString CSO Joe Beechem Insists the Company "Not Going Anywhere"

GEN talks to loe Beechem about the decision to file Chapter 11, where the company goes from here, and the future of innovation in the spatial biology field

to advance the science.

Marco Island, Feb 27 2019: AGBT's very first Spatial Biology Event was NSTG-proposed & Sponsored). NSTG invited all of the spatial technologies to our event, in order to be inclusive and work together

By Julianna LeMieux, PhD - February 8, 2024



By Miranda E. Orr, PhD, Arutha Kulasinghe, PhD, Grant R. Kolar, MD, PhD, Holger Heyn, PhD, Jasmine Plummer, PhD, Lasse Sommer Kristensen, PhD, Jorgen Kjems, PhD, Gordon Mills, MD, PhD, Juan J. Garcia-Vallejo, PhD, I.J. Nijman, PhD, Nicholas P. West, PhD, and Amanda Cox, PhD

nanoString

Sounding an Alarm over Spatial Biology



Spatial Biology Demands Multiple Levels of Plex, Resolution, and Throughput

Profilers

TISSUE STRUCTURES ~ 100 µm









SINGLE CELL ~ 10 µm



76-plex Proteins

Imagers

SUB-CELLULAR

~ 1 µm





Up to Whole Transcriptome (>18,000-plex) RNA



GeoMx[®] IO Proteome Atlas

Discover the Next Immuno-Oncology Biomarker

REQUEST A QUOTE



Home » Products » GeoMx Digital Spatial Profiler » GeoMx Protein Assays » GeoMx® IO Proteome Atlas

Spatial Proteomics Redefined

Comprising nearly all Abcam's IHC-validated human antibodies for immuno-oncology (IO), the GeoMx IO Proteome Atlas (IPA) is the highest-plex spatial proteomics panel available for EPA and fresh frozen tissue sections, and enables broad biomarker discovery for the add 40this research. Rapidly discover new protein biomarkers and drug targener YOU can gets to this profiling over 570 proteins in different tissue compartments such a microenvironment, and the immune infiltrate. Get more comprehens per slide than any traditional immunohistochemistry (IHC) or immunon seamlessly integrate GeoMx IPA into your lab with a histology compatible workflow.





Highest-Plex Systems-Biology Approach, Spatially-Resolved, **Cancer-Focused** Panel Ever Developed (by far)



Designing an Immuno Oncology Proteome Atlas

Curated and validated IO Content

- Use Clinical Proteomic Tumor Analysis Consortium (CPTAC) data
- Focus on post-translational modifications
- Use high quality abcam antibodies
- Employ Immuno Oncology subject matter experts
- Human Specific
- 570+ Proteins, Mapped to 556 Unique Genes
- 77 Functional Annotations
- All Hallmarks of Cancer





GeoMx 570-plex Immune-Proteome Atlas Resolves hundreds of proteins on nearly every tissue of the body (normal & cancerous)







Same-Slide, 570-plex Protein (up to 610-plex with Custom) plus 18,000+ RNA in Tissue: Finally a Protein-Plex to get excited about!



CRC FFPE sample



Information on cellular mechanisms and regulation Information on cellular function and phenotypes

GeoMx DS



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dures

Example of same slide 570-plex protein, 18,000-plex RNA in an 8-week (post-gestation) whole human embryo*



* In collaboration with: BS de Bakker, MJB van den Hoff et al. Dutch Fetal Biobank, Amsterdam UMC

Example of same slide 570-plex protein, 18,000-plex RNA in an 8-week (post-gestation) whole human embryo*





CosMx RNA

(single-cell)







* In collaboration with: BS de Bakker, MJB van den Hoff et al. Dutch Fetal Biobank, Amsterdam UMC

Spatial Biology Demands Multiple Levels of Plex, Resolution, and Throughput

Profilers

TISSUE STRUCTURES ~ 100 µm







GeoMx



Whole Transcriptome (18,000+ plex) > 570-plex Proteins

SINGLE CELL ~ 10 µm



76-plex Proteins

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Imagers

SUB-CELLULAR

~ 1 µm





Up to Whole Transcriptome (>18,000-plex) RNA



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CosMx Whole Transcriptome Key Performance Characteristics

From the very beginning of CosMx design, we had one overriding goal: Recapitulate ALL of the capabilities of scRNA-seq, but with sub-cellular spatial resolution

The Performance Specifications that count should be identical to how scRNA-seq is measured

- Sensitivity = maximum number of transcripts per cell = CosMx clearly wins here...
- Sensitivity = largest diversity of transcripts per cell = CosMx clearly wins here...
- For above specifications to be real, best-in-class segmentation \bullet required CosMx clearly wins here...



(in collaboration with Dr. Holger Heyn, CNAG)



CosMx Cell-Segmentation: High-Plex Protein Driven, nVIDIA Accelerated, AI-ML trained, and with 4th Generation Release





High-Plex Panels can be organ/disease agnostic. 200 Million single-cells and 60 publications later now proves this point

Example: Commercial 247-plex Panel



Organ-specific panel coverage of reactome

CosMx 1,000-plex Panel: Covers Single Cell Biology



CosMx SMI 1000-plex Delivered 60+ Publications and Preprints

Tissue Encyclopedia



Patient-to-Patient Heterogenicity

Macrophage and neutrophil heterogeneity at single-cell spatial resolution in human inflammatory bowel diseas

Drug Mechanism of Action

wide Early Insights into Artivity of I

Disease Characterization

3D Cell Atlas

-- Selected Publications --

FOR RESEARCH USE ONLY. Not for use in diagnostic procedure:



CosMx 6,000-plex Panel: Now Shipping Commercially scRNA-seq NOT required, measure all the sample biology in a single slide





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Move beyond "marker-gene" and "individual-gene" analysis with 6,000-plex, directly "project" the full-biology of the reactome onto the tissue

Directly "view" in the tissue, >90% of the Reactome (~ 400 pathways-of-biology)





CosMx 6,000-plex public dataset

Does CosMx 6000-plex work on real-world FFPE samples? ABSOLUTELY, Extended the work that made the cover of *Nature Genetics*



Pancreatic cancer spatial transcriptomics

Single-nucleus and spatial transcriptome profiling of pancreatic cancer identifies multicellular dynamics associated with neoadjuvant treatment. (Aug 2022) William L. Hwang, MD, PhD Assistant Professor, Harvard Medical School Research, Massachusetts General Hospital







Principal Investigator, Center for Systems Biology/ Department of Radiation Oncology/Center for Cancer Associate Member, Broad Institute of MIT and Harvard



HARVARD SB BROAD







CosMx 6000-plex: Amazing Diversity of Transcripts with Real-world FFPE Cancer Tissues



99.5% confidence above both ERCC and FalseCodes

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Spatial Cell-Signaling Ligand Exploration, Spatially Co-expressed Ligands. Discovered a clear participation of NERVE-cells in the tumor microenvironment directly effecting tumor-immune interactions... One cluster:

Profiles of cells' "ligand environments" Correlation matrix of neighborhood expression from 407 x 407 ligands

Ligands **Clusters of ligands** expressed in the same neighborhoods 407 Ligands Ligands

(NOTE: can perform this analysis on 6000 X 6000 genes (36 Million spatial correlations)

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CCL5, CCL8, CXCL9, CXCL10, CD48, SEMA4D, SIGLEC1 Immune cell chemoattractants and activators.

Expressed in sporadic hotspots.

macrophages, nerve cells and malignant cells

250 um

nanoString





Search "InSituCor" Toolbox...

New Results

Follow this preprint

InSituCor: a toolkit for discovering non-trivial spatial correlations in spatial transcriptomics

D Patrick Danaher, Dan McGuire, Michael Patrick, David Kroeppler, Haiyan Zhai, Joachim Schmid, Joseph M. Beechem

doi: https://doi.org/10.1101/2023.09.19.558514

This article is a preprint and has not been certified by peer review [what does this mean?].







No "human being" can examine 36 million spatial correlations, this method provides an ²⁴ automated, systematic method to focus on high-impact first-of-kind spatial discoveries







HLANE, HUM PINLA-G

The "Holy-Grail" of Spatial Biology is Here Explore Single-cell and Subcellular Imaging of the Whole Protein-coding Transcriptome (>18,000-plex)

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PRODUCTS

CosMx Human Pancreas FFPE Dataset

First Single-Cell Spatial Dataset with over 18,000 Genes

REQUEST MORE INFORMATION



Home » Products » CosMx Spatial Molecular Imager » CosMx[™] SMI Datasets » CosMx SMI Human Pancreas FFPE...

CosMx Whole Transcriptome (>18,000-plex) Imaging Panel

Distribution of Transcript Lengths that have CosMx imaging probes





Over 38,000 CosMx imaging probes distributed over 23 chromosomes

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Establishing a Baseline Sensitivity and Specificity for CosMx precommercial WTx >18,000-plex Imaging Panel

(1) Referenced against deep RNA-seq CCLE database (Broad)



(2) Pre-commercial Specs: 62.0% Sensitivity (1 FPKM break-point) 97.5% Specificity

(3) "Bonus": Typical Fixed-cells yield up to 10,000 transcripts per cell at less than 1-cent per cell!







22RV1 769P A172 CCRF-CEM COLO201 DAUDI DU4475 H1648 H596 HDLM2 HEL HEPG2 **HL60** HS5781 HUH7 HUT78 GROV1 KG MALME3N MEC¹ MV411 OPM2 SKLMS1 SKMEL2 SKODW SNU475 SR SUDHL1 SUDHL6 SW948 T47D THP TT U118MG U87MG

First (in the world) FFPE Spatial Single-cell Whole Transcriptome Public Data Release: healthy human pancreas



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GCG (Alpha cells) INS (Beta cells) PPY (Gamma cells) SST (Delta cells) GHRL (Epsilon cells) DAPI PanCK



CosMx WTx Panel: "Listens In" on "Conversations" Between Cells

Pancreas Cell-to-Cell "Conversations" Arrows Represent Receptor-Ligand Interactions



Interactions Detected by CosMx Panel Plex Number of Receptor-Ligand Interactions

CosMx WTx Panel: True single-cell Spatially-resolved Whole Transcriptome with Dr. Holger Heyn (CNAG, Barcelona)

Total single cells = 1.4 Million cells Transcripts = over 1.4 Billion Transcripts per cell > 1,000 Neg-Probes per cell < 1.0Close to snRNA-seq on all key metrics Less than 1-cent per transcriptome

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CosMx WTx – Spatially-Aware Cell State Profiling

cell segmentation

••• epi.TVA•• epi.TVA/N fibroblasts endothelia yeloid B cells – plasma cells – T/NK cells

Spatial Proteomics with CosMx SMI

CosMx™ Human IO Protein Panel Protein Spatial Biology at subcellular resolution

Inflammation	M2	CD8 T Cells		Immune Cell Migration		NK Cells		Tregs		Background		
	Macrophage	CD4 T Cells		Hem	atopoietic	Monocyte		Total Immune	Adipose Endo		ia Fibroblasts	
	Cells	Antigen Presentation		Exhausted T Cells		Interferon		MHC2	Stro			
Checkpoint	Cytotoxicity	Memor T Cell			Noutr	onhile		Plasma	Stronna		u	
					Neutro			Cells	Proliferation		Wnt Signaling	
	Myeloid				T 0-11			A - 11 -	Enithelia	Tum	Tumor Suppressor	
T Cell	Suppressi	on	Na	aive i Cell		IS I		Cells	Epithena	Recepto	ceptor Tyrosine Kinase	
						М		valaid	Apoptosis		ONA Damage	
Activation	B Cell	s	1	Macropha		ge	Act	ivation	Tumor		Antigen	
T Cells			Myeloid						Growth Factor Signaling		МАРК	
									Oncogene			

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HIGH PLEX Visualize 64 protein targets plus 4 segmentation markers

HIGH THROUGHPUT

1 slide/day with 1.0 cm² tissue area

CUSTOMIZABLE

Add up to 8 additional custom protein targets (3Q 2023)

EXPANDING MENU Mouse Neuroscience Panel (3Q 2023)

Explore Subcellular Localization and Post Translational Modifications

Core needle biopsy Clinical trial data Gordon Mills Lab OHSU FN1 SMA **EpCAM** CD45 PanCK **Γ**750 μ

SD2

CD6

total ERK 1/2

Translocation of phosphorylated ERK1/2 to nucleus

Explore Subcellular Localization and Post Translational Modifications

Decreased MAPK/ERK signaling following therapy

-ERK 1/2

First of its Kind Informatics Continuum for Spatial that Scales to any Plex or Sample Number

Iterative, Multi-sample, Custom **Analytics suite**

Machine-learning Cell Segmentation Toolkit

)iameter (µm)

Spatial Informatics Platform

CosMx Spatial

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Spatial-Multi-Omics for Systems-Biology

GeoMx for in-situ FUNCTION at sample high-throughput CosMx for in-situ subcellular resolution at tremendous single-cell number and low single cell cost (< 1cent/cell)

GeoMX[®] Digital Spatial Profiler AtoMx[®] Spatial Informatics Platform

Whole TranscriptomeSingle-Cell ResolutionHigh ThroughputEntire Tissue SectionData matches tissueHighest Multiplex & SensitivityarchitectureBest Cell-SegmentationHighest-protein plexTransformatively lessHighest multi-omicexpensive that sc-RNAseqplexPlane

