#5555: A complete pipeline for high-plex spatial proteomic profiling and analysis on the CosMx[™] Spatial Informatics Platform n a n o S t r i n q

Tiên Phan-Everson, Sangsoon Woo, Zachary Lewis, Giang Ong, Yan Liang, Emily Brown, Dwayne Dunaway, Edward Zhao, Dan McGuire, Alyssa Rosenbloom, Brian Filanoski Rhonda Meredith, Kan Chantranuvatana, Brian Birditt, Hye Son Yi, Erin Piazza, Jason Reeves, John Lyssand, Vikram Devgan, Michael Rhodes, Gary Geiss, Joseph M Beechem NanoString[®] Technologies, Seattle, WA 98109

Abstract

Detecting and analyzing large numbers of proteins using whole-slide imaging is critical for a comprehensive picture of immune response to cancer. Many existing approaches for high-plex proteomics face issues around simplicity, speed, scalability, and big data analysis. Here, we present an integrated workflow from sample preparation through downstream analysis that addresses many key concerns around high plex proteomics. The CosMx[™] Spatial Molecular Imager (SMI) and AtoMx[™] Spatial Informatics Platform (SIP) comprise of a turnkey, end-to-end workflow that efficiently handles highly multiplex protein analysis a plex sizes exceeding 110 targets. We demonstrate an extension of our commercially available 64-plex human immuno-oncology panel to higher numbers of targets and show how the cloud computing-enabled AtoMx[™] SIP allows flexible construction of analytic pipelines for cell typing and spatial analyses.

The CosMx protein assay uses antibodies conjugated with oligonucleotides, which are detected using universal, multi-analyte CosMx readout reagents. The CosMx Human Immuno-oncology panel was optimized to comprehensively profile lymphoid and stromal lineages within the tumor microenvironment as well as markers of cancer signaling and progression. Each CosMx SMI antibody was validated on multi-organ FFPE tissue microarrays covering prevalent solid tumor types with matched controls, and 52 human FFPE cell lines, including overexpression lines for key targets such as GITR, CD278, PD-L1, and PD-1. CosMx SMI uses a deep learning algorithm to segment whole cells and a semi-supervised algorithm to classify cell types. The AtoMx SIP provides full analysis support, including a whole-slide image viewer, and methods for performing built-in or fully customizable analyses for cell typing, ligand-receptor analysis, neighborhood analysis, and spatial differential expression.

Within the cancer sample profiled, we demonstrate that the 100+plex protein panel with the expanded cancer content maps the tumor landscape with superior details without compromising reproducibility in the immune context. Spatial single-cell data output allows us to trace the expression of any marker to any given cell. Here, we report in-depth proteomic profiling across different myeloid cell populations and perform spatial proximity analysis. We also characterize the expression signatures of M1, M2, and cells in M1-M2 transitional states (M1/M2). Notably, we are able to detect rare cellular events using CosMx SMI.

CosMx Spatial Molecular Imager is a high-plex spatial multi-omics platform that enables detection of more than 110 proteins at subcellular resolution in real-world FFPE tissues. The extensibility of the CosMx protein assay to large numbers of protein targets and our flexible, scalable bioinformatic platform provides a straightforward and robust solution for comprehensive immune phenotyping with full spatial context.

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Methods

The CosMx Spatial Molecular Imager (SMI) is an FFPE-compatible, single-cell imaging platform



The CosMx Spatial Molecular Imaging platform uses automated fluidics and imaging to hybridize and visualize 16 sets of four fluorescent reporters, enabling protein imaging of 64 different antibodies.





All CosMx Protein antibodies undergo extensive antibody validation to ensure high quality data



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NanoString Technologies 530 Fairview Avenue North Seattle, WA 98109

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