#6312: Subcellular characterization of over 100 proteins in FFPE tumor biopsies with CosMx™ Spatial Molecular Imager

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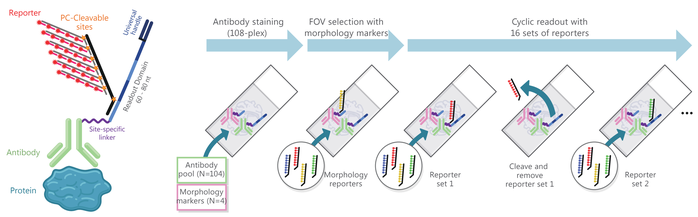
Abstract

The spatial interactions between the immune system and tumor cells greatly influence antitumoral immunity. Characterization of immune cell composition and infiltration within the tumor niche informs prognosis, drug delivery efficiency, and therapeutic efficacy. However, few methods exist to query large numbers of immune biomarkers at subcellular spatial resolution. The CosMx™ Spatial Molecular Imager is the first platform to demonstrate simultaneous single-cell and subcellular detection of over 100 proteins on standard, biobanked, FFPE tissue samples. This high-plex protein panel detects key drivers of cancer progression and immune cell activation states. Here, we apply the CosMx 100-plex immuno-oncology assay on a set of breast cancer biopsies and demonstrate its quantitative and spatial capabilities. The CosMx system uses a fully automated, cyclic microfluidics imaging system, high-resolution optics and 3D capability. CosMx SMI produces protein localization maps for each target, which characterizes tissue microenvironment heterogeneity while providing spatial information. Additionally, accurate segmentation of individual cells enables spatial single-cell protein expression analysis, facilitating further mining and analyses of cellular subpopulations. The CosMx protein assay reagents were validated on multi-organ FFPE tissue microarrays and 35 human FFPE cell lines, including overexpression lines for key targets and cellular activation states, such as GITR, CD278, PD-L1, and PD-1. Benchmarking to multiple orthogonal datasets (e.g., the Human Protein Atlas, Cancer Cell Line Encyclopedia, and low-plex IHC) demonstrates that the assay is highly sensitive and specific. CosMx SMI protein assay can be coupled with SMI's 1000-plex RNA-detection assay; together, such a multi-omics platform can generate an unprecedented information-rich view of spatial biology that could usher in novel discoveries about health and disease.

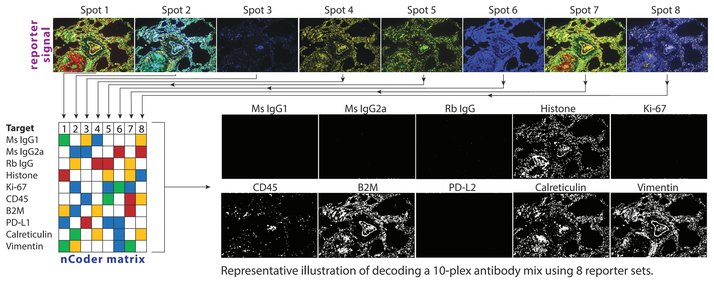
FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

108-plex protein imaging with a cyclic encoded readout



The Spatial Molecular Imaging platform uses automated fluidics and imaging to hybridize and visualize **16 sets of four fluorescent reporters**, enabling >100-plex protein readout.

Least squares decoding of protein expression patterns



Representative illustration of decoding a 10-plex antibody mix using 8 reporter sets.

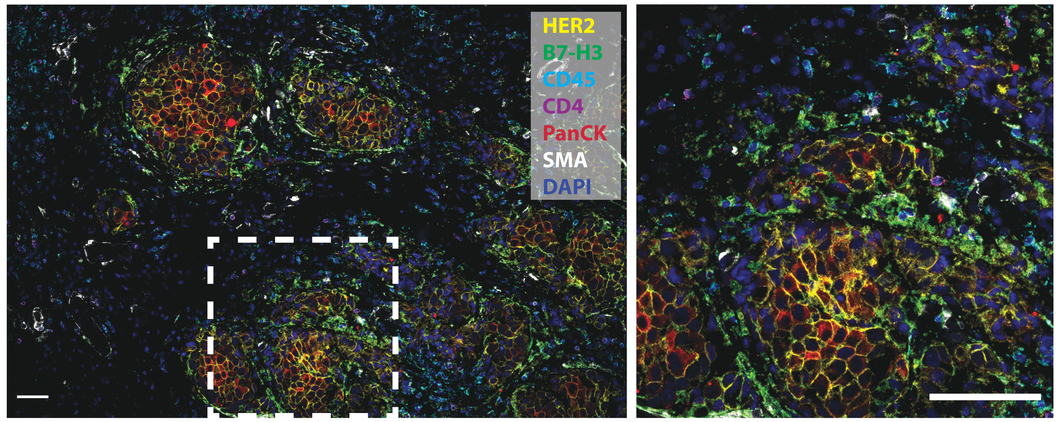
$$\underset{\text{Measured reporter signal}}{\mathbf{Y}} = \underset{\text{Protein nCoder matrix}}{\mathbf{X}} \; \underset{\text{Protein expression values}}{\boldsymbol{\beta}}$$

$$\begin{bmatrix} b_1 \\ g_1 \\ \vdots \\ y_5 \\ \vdots \\ y_{16} \\ r_{16} \end{bmatrix}_{64*N} = \begin{bmatrix} 1 & 0 & \dots & 0 \\ 0 & 0 & \dots & 1 \\ 0 & 1 & \dots & 1 \\ \vdots & 0 & \dots & 0 \\ 0 & 1 & \dots & 0 \end{bmatrix}_{64xP} \begin{bmatrix} Protein_1 \\ Protein_2 \\ \vdots \\ Protein_{57} \\ \vdots \\ Protein_P \end{bmatrix}_{P*N}$$

Where **N** is the number of pixels and **P** is the number of encoded proteins

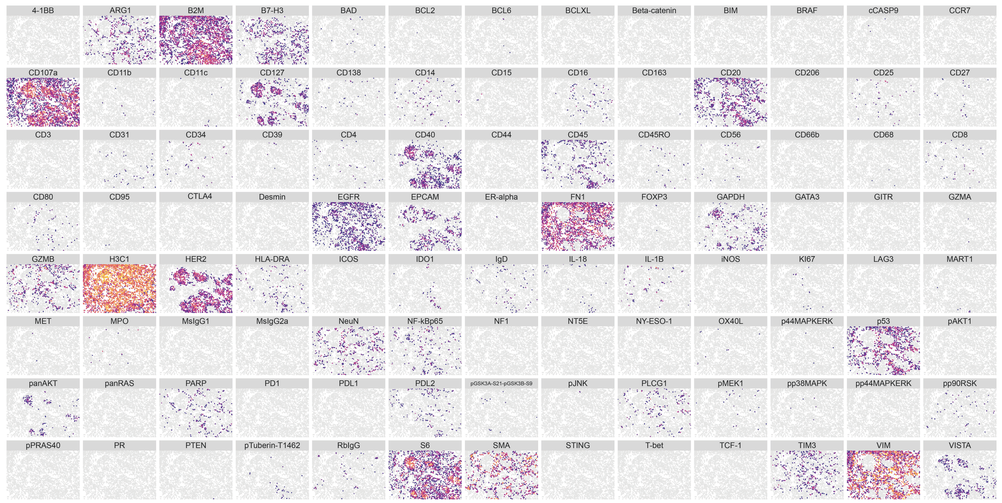
Protein expression patterns are computed by **projecting 3D stacks to 2D then unmixing** using the pseudo inverse of the protein nCoder matrix for each pixel.

Protein expression patterns can be visualized and overlayed

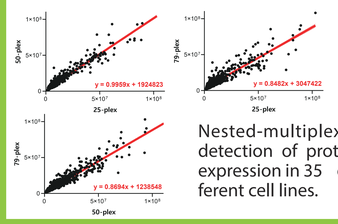


Multi-channel overlay of six protein targets detected in a breast cancer biopsy (HER-2 positive invasive carcinoma) from a 108-plex assay (104 encoded targets and 4 morphological markers)

Single-cell expression profiles across 104-encoded protein targets

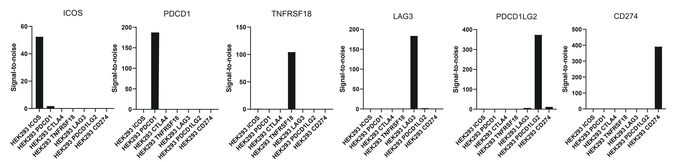


Protein level readouts remain reproducible as plex size increases



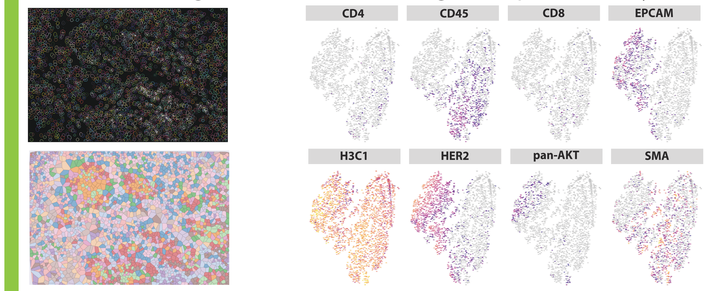
Nested-multiplexed detection of protein expression in 35 different cell lines.

Decoding accuracy is validated on overexpressing cell lines



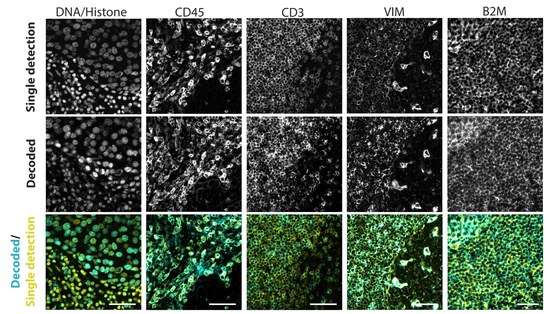
Key immunooncology targets are detected on target-specific over-expression cell lines to demonstrate specificity and antibody functionality.

On-board cell segmentation enables single-cell protein analysis



Automated machine-learning-based multichannel cell segmentation delivers accurate cell boundary detection. Cell segmentation allows single-cell spatial analysis of protein expression and cellular neighborhoods.

Multiplexed decoded protein localization is consistent with target localization detected by traditional singe-channel immunofluoescence



Conclusions

108 protein targets were imaged using CosMx SMI detection chemistry via oligonucleotide-conjugated antibodies.

108-plex exceeds the **highest reported panel size** used for spatial imaging of proteins.

HER-2+ breast cancer was profiled for protein expression, as a function of fluorescence intensity.

High-plex protein detection is possible through cyclic imaging of encoded protein signals.

Decoded **protein localization** patterns can be visualized individually or mapped to **single cells** using CosMx cell segmentation results.

Encoding protein localization patterns reduces on-instrument turnaround time, **preserving tissue stability** and **increasing throughput**.

CosMx SMI reagents support readout of **both RNA and protein** expression on the same commercial platform with the same consumable reagents.

He et al., 2022. **High-plex Multiomic Analysis in FFPE at Subcellular Level by Spatial Molecular Imaging.** bioRxiv. https://doi.org/10.1101/2021.11.03.467020

Voronoi plot generated with: Nirmal et al., SCIMAP: Spatial single-cell analysis toolkit. https://scimap.xyz