Enhancing spatial signature development of immuno-oncology biomarkers in Lung Cancer tissue with new multiplexed immunofluorescence staining method using novel protein chemistry

Rachel Schaefer, Linying Liu, Michael McLane, Oscar Perez, Jacob Circelli, Carla Coltharp, Yi Zheng

1 New Multiplex Panels for Immune Checkpoint Research - Powered by Novel Protein Chemistry

To accelerate biomarker development efforts to more reliably predict response for mono- and combination immune checkpoint inhibitor therapies from discovery to the clinic, we have adopted the barcode-based antibody chemistry used on our Akoya PhenoCycler platform and integrated it with the signal amplification capabilities of our Opal chemistry used on our Akoya PhenoImager platform. This novel protein chemistry enables staining efficiencies by the application of a single primary antibody cocktail incubation step of six antibodies while retaining the sensitivity required for translational and clinical research.

Utilizing this universal chemistry, we have developed complementary antibody panels which enable comprehensive mapping of the tumor micro-environment. These pre-validated panels are designed to provide the easy addition of a single biomarker, making them ideal to rapidly develop spatial phenotypic signatures for mono- or combination immune checkpoint therapies. Here, we showcase the first of these panels, the immuno-contexture panel.

Panel of Immune Markers	Assessment of Tumor Microenvironment
Immune Profile / Tumor Microenvironment	What are the immune cells in the tumor?
Immuno-Contexture	Is the tumor “hot” or “cold”?
Activated Tumor Infiltrating Lymphocyte Status	Are tumor cells proliferating or lymphocytes activated?
Tumor Associated Macrophages	Where are the TAM’s in proximity to the tumor margin or tumor cells?
Immune Resistance	Where are the exhausted and regulatory T cells?

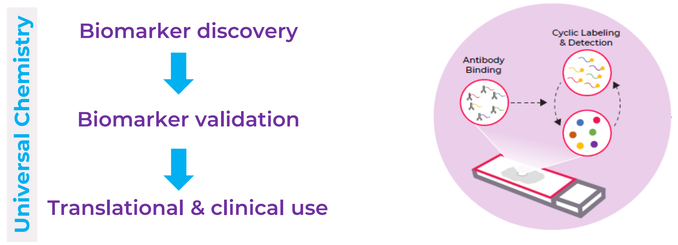
Fig. 1 Key Panels Enable Immune Profiling of the Tumor Microenvironment (TME)

2 Methods

Formalin-fixed paraffin-embedded (FFPE) Lung Cancer (LuCa) tissue samples were stained on the Leica BOND RX™ automated staining system using our new multiplex procedure and compared to 3,3'-Diaminobenzidine (DAB) chromogenic immunohistochemistry assay. Multispectral scans were acquired on the PhenoImager® whole-slide scanning system with optimized acquisition parameters. Image analysis was performed with a phenotyping algorithm in inForm® analysis software and intensity analysis was performed in R using phenoptr and phenoptrReports.

3 Universal Chemistry Establishes a Continuum from Discovery to Translational and Clinical Research

This universal protein chemistry can be applied from discovery through translational and clinical research, thus enabling the efficient discovery and development of predictive spatial signatures. The diagram below highlights the use of this detection methodology for staining tissue.



4 Immune Profiling – Assessment of Cell Phenotypes

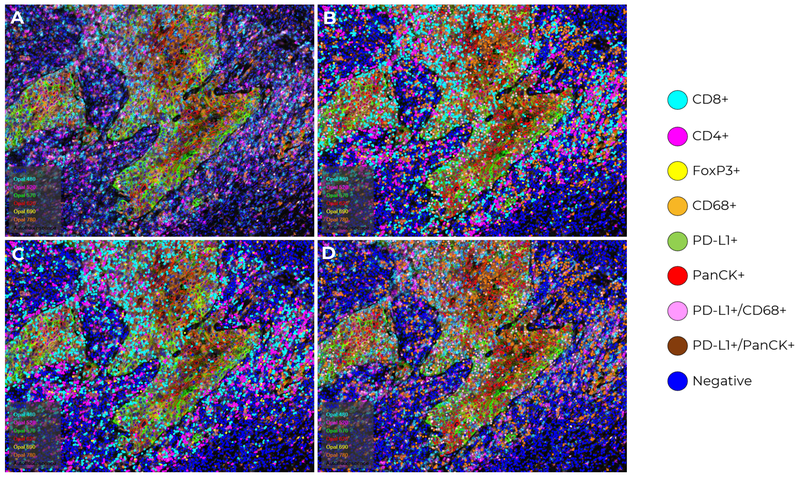


Fig. 2 Phenotype combinations assessed with 6-plex Immuno-Contexture panel; region of interests (ROIs) in LuCa tissue using Multispectral scan in Inform®
A. Composite image
B. Phenotyping of all markers
C. Subgroup of B displaying CD8+, CD4+, FoxP3+, and Negative cells
D. Subgroup of B displaying CD68+, PD-L1+, PanCK+, PD-L1+ /CD68+, PD-L1+/PanCK+, and Negative cells

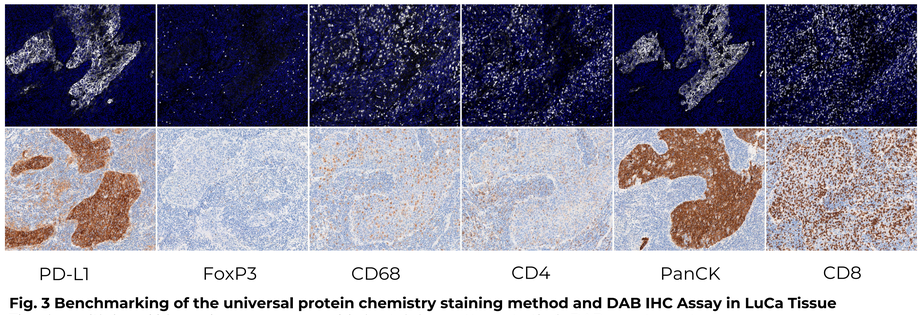


Fig. 3 Benchmarking of the universal protein chemistry staining method and DAB IHC Assay in LuCa Tissue
The six multiplexed biomarkers are comparable in staining patterns to their DAB counterparts.

5 Qualitative and Quantitative Analysis of 6-Plex mIF Staining Quality

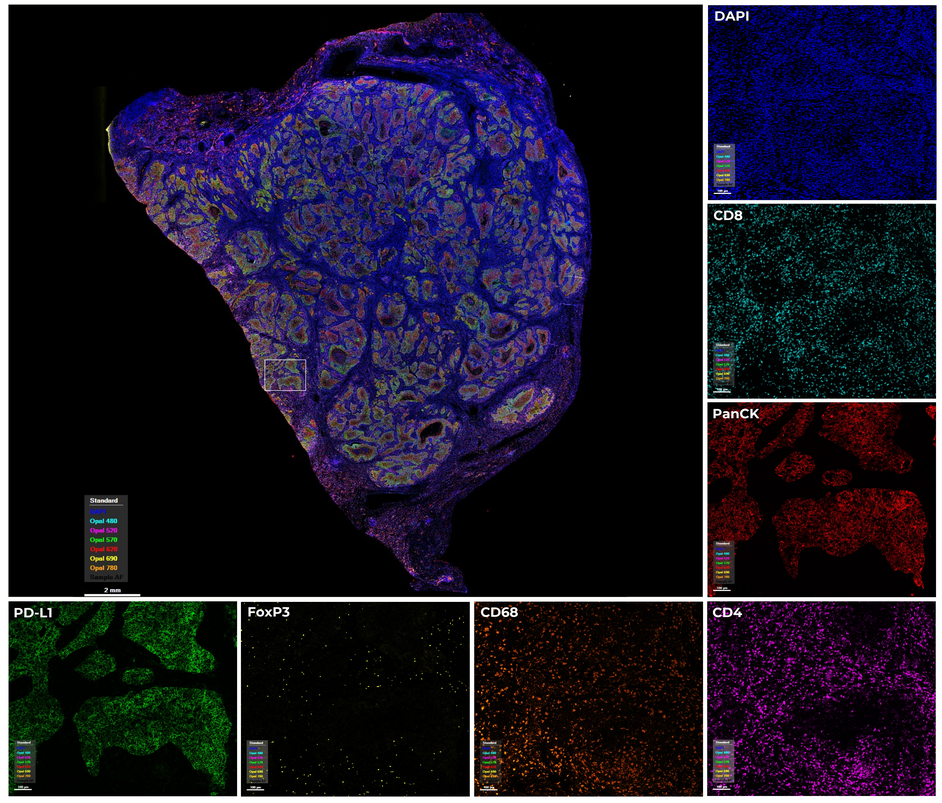


Fig. 5 Whole Slide Scan of 6-Plex Staining
Whole slide image scans were successfully unmixed and treated as individual monoplexes, but combined to observe the spatial relationships in LuCa tissue.

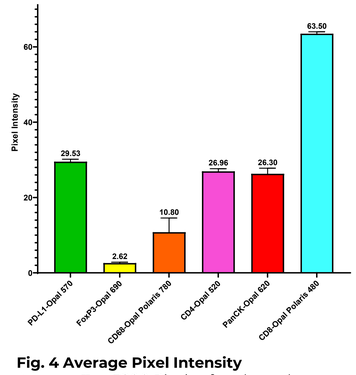


Fig. 4 Average Pixel Intensity
Average Top 3200 Pixels of each marker determined from 5 ROIs each from 3 sections of LuCa tissue

6 Summary

We have demonstrated a novel protein chemistry that integrates our PhenoCycler and PhenoImager chemistries to simplify panel development. This easy-to-use and efficient staining method:

- Shows equivalence with gold standard: benchmarked to chromogenic DAB
- Accurately identifies multiple cell phenotypes: powered by Akoya’s MSI technology and data analysis workflow

The ability to rapidly develop panels to investigate the immune landscape of the TME will accelerate the development of spatial phenotypic signatures that reliably predict response for both mono- and combination immune checkpoint inhibitor therapies.

Akoya Biosciences, Inc., 100 Campus Drive, 6th Floor, Marlborough 01752, MA USA (855) 896-8401 www.akoyabio.com