Multispectral Imaging to Detect Immune Phenotypes Within the Tumor Microenvironment in a Multi-Tissue Study:

A Fully Automated 7-color mIF Immuno-Oncology Workflow

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1. Background

Immunotherapy and precision medicine are rapidly developing approaches to cancer therapy. Biomarkers that detect the tumor and tumor microenvironment allow for the development of strategies that accelerate the advancement of treatments to enhance a patient's immune system. Akoya's MOTiF™ PD-1/PD-L1 Panel is a validated, multiplex immunoassay enabling detection of the 6 most clinically relevant immuno-oncology and spatial biomarkers: PD-1, PD-L1, FoxP3, CD8, CD68, and PanCK. The MOTiF™ PD-1/PD-L1 Panel was used to analyze the tumor microenvironment and specifically assess immune phenotypes of different types of cancers: non-small cell lung cancer (NSCLC), colon adenocarcinoma, head and neck squamous cell carcinoma (HNSCC), and breast cancer.

We demonstrate the utility of Akoya's MOTiF™ PD-1/PD-L1 panel kit in studying the cellular diversity of various cancers while retaining spatial context. Stained slides were analyzed using the InForm® and PhenoptrReports image analysis platforms to identify and better understand spatial relationships between a variety of simple and complex cell phenotypes. The MOTiF™ PD-1/PD-L1 panel kit provides reproducibility across different tissue types. These data provide insight into the innate and adaptive immune environment for targeted design of new immunotherapies and have implications for improving the treatment paradigm across many tumor types.

2. Methods

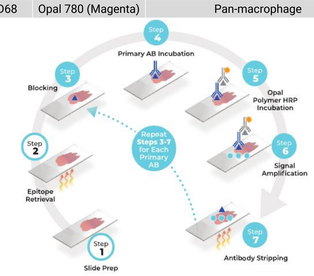
1

MOTiF Panel Kit

Antibody	Opal Pair	Marker
CD8	Opal 480 (Cyan)	T lymphocytes
PD-L1	Opal 520 (Green)	Ligand for programmed cell death protein
FoxP3	Opal 570 (Yellow)	T cells
PD-1	Opal 620 (Orange)	Programmed cell death protein
PanCK	Opal 690 (Red)	Tumor stem cells
CD68	Opal 780 (Magenta)	Pan-macrophage

2

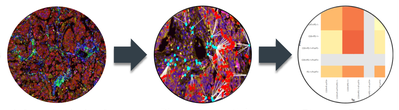
Leica BONDRX



Human FFPE tissues were stained using the MOTiF™ PD1/PD-L1 Auto Lung Cancer kit. Staining was performed on the Leica BOND RX™ automated stainer using the pre-determined protocol.

3

PhenoImager Fusion and InForm Software



Multispectral whole-slide imaging was performed on the PhenoImager-Fusion. Scans were unmixed and analyzed with InForm® software. Spatial analyses and visualizations were performed using Phenoptr and Phenotpr Reports [1].

3. Results

Multispectral imaging of NSCLC, Head and Neck Cancer, Breast Cancer and Colon Cancer

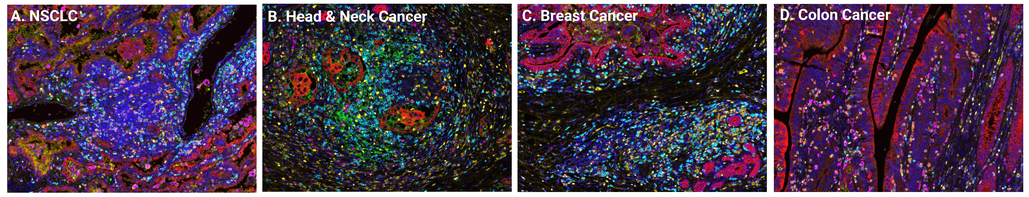


Figure 1. Unmixed 7-color Multispectral Scan of NCSLC, Head & Neck, Breast, and Colon Cancer stained with MOTiF PD-1/PD-L1 Panel. The pre-optimized MOTiF PD1/PD-L1 Panel: Auto LuCa Kit visualized FoxP3 (yellow, Opal 570), PD-L1 (green, Opal 520), PanCK (red, Opal 690), PD-1 (orange, Opal 620), CD8 (cyan, Opal 480), and CD68 (magenta, Opal 780) across a variety of cancer types. **(A)** Detailed 20x view of NSCLC sample. **(B)** Detailed 20x view of Head & Neck cancer. **(C)** Detailed 20x view of Breast Cancer. **(D)** Detailed 20x view of Colon Cancer. (A-D) Scale bar: 50 µm

Cell Phenotyping using Unique NSCLC Samples Shows Distinct Phenotypes

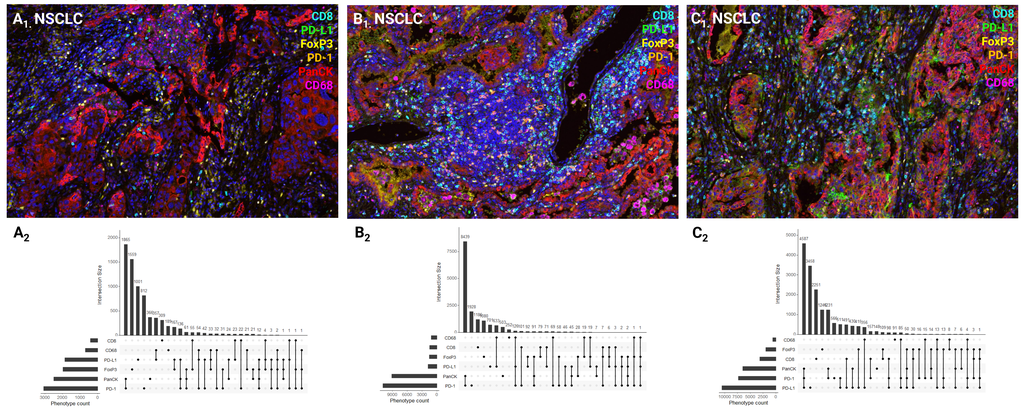


Figure 2. Phenotyping of NSCLC Samples from Unique Patients. (A_1, B_1, C_1) Detailed 20x view of unique NSCLC sample. Summaries of cell phenotype counts for NSCLC (A_2, B_2, C_2) in whole tissue. Horizontal bars show counts of individual phenotypes and vertical bars show counts of specific phenotype combinations. Scale bar: 50 µm

Nearest Neighbor Analysis and Cell Phenotypes of Unique Breast Cancer Samples

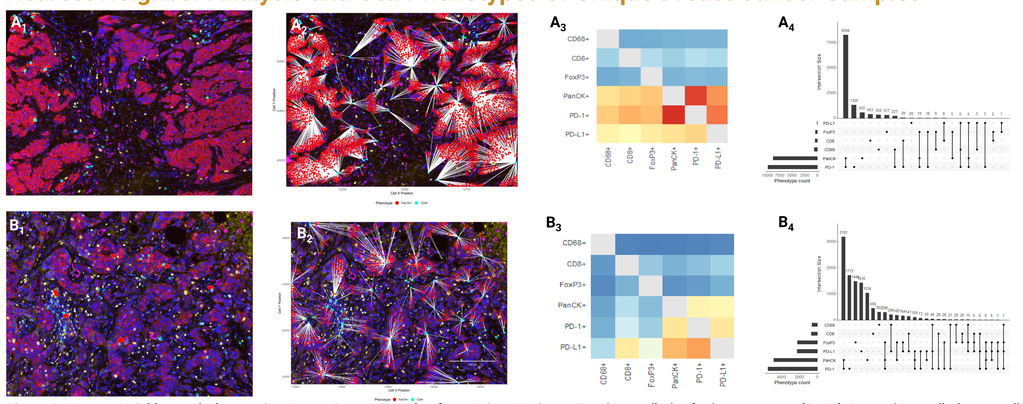


Figure 2. Nearest Neighbor and Phenotyping Breast Cancer Samples from Unique Patients. Touching cell plot for breast cancer **(A_1, B_1)**. In touching cell plots, a cell outline is filled in if touching a cell of a paired phenotype. Nearest neighbor plot for breast **(A_2, B_2)** where the nearest PanCK+ cell to each CD8+ cell is connected with a white line. **(A_3, B_3)** Heat map showing median distances between different cells found in whole tissue. Summaries of cell phenotype counts for breast cancer in whole tissue **(A_4, B_4)**. Horizontal bars show counts of individual phenotypes and vertical bars show counts of specific phenotype combinations in tissue. Scale bar: 50 µm

Nearest Neighbor Analysis for Unique Head and Neck, and Colon Cancer Samples

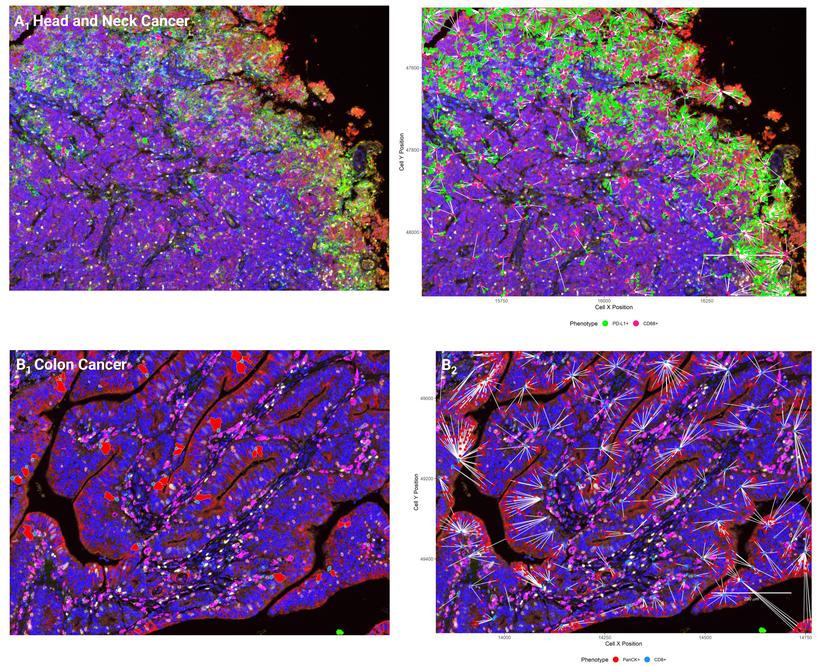


Figure 3. Nearest Neighbor Analysis. Touching cell plot for head and neck cancer **(A_1)**. In touching cell plots, a cell outline is filled in if touching a cell of a paired phenotype. Nearest neighbor plot for head and neck cancer **(A_2)** where the nearest PD-L1+ cell to each CD68+ cell is connected with a white line. Touching cell plot for colon cancer **(B_1)**. In touching cell plots, a cell outline is filled in if touching a cell of a paired phenotype. Nearest neighbor plot for coloncancer **(B_2)** where the nearest PanCK+ cell to each CD8+ cell is connected with a white line. Scale bar: 50 µm.

4. Conclusions

The ready-to-use MOTiF PD1/PD-L1 Lung Cancer kit was used to analyze the immune microenvironment of NSCLC, breast cancer, colon cancer, and head and neck squamous cell carcinoma across multiple patients in order to provide reproducibility data across the different tissue types.

In tumor samples from NSCLC, breast cancer, colon cancer and head and neck squamous cell carcinoma indications, we were able to identify populations of PD-L1, CD8, PD1, FoxP3, CD68, and PanCK positive cells as well as co-localization of multiple phenotypes.

Our goal is to help our clients utilize the customizable MOTiF PD1/PD-L1 lung cancer kit to analyze the immune microenvironment in various cancer indications using the most relevant immuno-oncology biomarkers.

References

[1] Kent S Johnson (2019). phenoptr: inForm Helper Functions. R package version 0.2.3 [software]. Akoya Biosciences. Available from: https://akoyabio.github.io/phenoptr/.