A fully optimized end-to-end solution for I/O multiplex immunofluorescence staining using Opal Polaris 7-Color PD1/PD-L1 Panel Kits for Lung Cancer and Melanoma

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1 Background

Understanding cellular heterogeneity and spatial relationships between biomarkers within the tumor microenvironment is a key component to translational research in immuno-oncology. A reproducible, quantitative, easy-to-use, and standardized multiplex fluorescent IHC assay is required for quantitative assessment of these relationships *in situ* for current I/O clinical trials and translational researches. In this study, we demonstrate a fully developed, yet flexible, end-to-end workflow solution for tissue biomarker discovery in lung cancer and melanoma. This newly developed Phenoptics™ solution provides an integrated MOTiF™ workflow including optimized RTU reagents plus image analysis algorithms enabling a more comprehensive and specific tumor microenvironment analysis with minimal user tuning.

2 Methods

FFPE samples from human lung cancer and melanoma were stained using MOTiF PD1/PD-L1 Panel: Auto LuCa Kit and MOTiF PD1/PD-L1 Panel: Auto Melanoma Kit. Staining was performed on the Leica BOND RX™ automated stainer with the preloaded MOTiF protocol. Multispectral scans were acquired on Vectra Polaris® with pre-optimized acquisition parameters and analyzed with a pre-configured phenotyping algorithm in inForm®. Spatial analyses and visualizations were performed in R using phenoptr and phenoptr Reports [1].

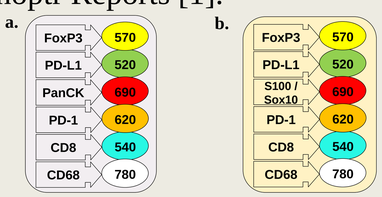

Fig. 1 Antibody-Opal pairs for MOTiF PD1/PD-L1 Panel: Auto LuCa Kit (a) and MOTiF PD1/PD-L1 Panel: Auto Melanoma Kit (b).

3 MOTiF Panel Kit Workflow

MOTiF Panel Kit	BOND RX	Vectra Polaris	InForm Software
Quality Reagents	Optimized protocol	Standardized imaging protocol	Pre-configured analysis algorithms

Fig. 2 Akoya new 7-color MOTiF PD1/PD-L1 Panel Kits along with whole slide multispectral imaging workflow provide the only validated end-to-end solution for unparalleled quantitative data for translational immuno-oncology research.

4 Results

Pathologist-verified Antibody Performance

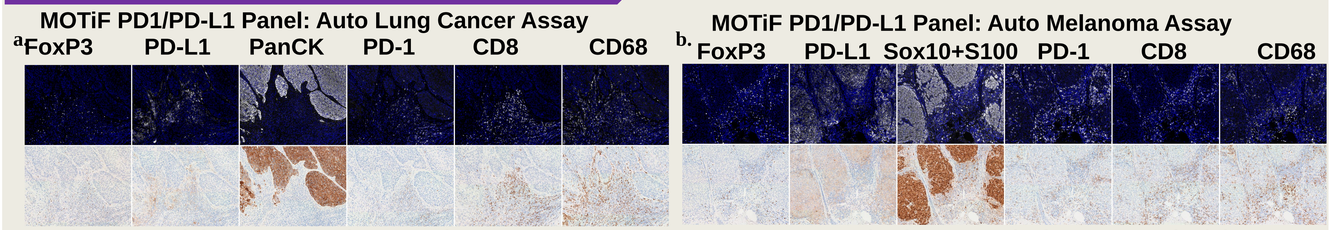

Fig. 4 Chromogenic and Fluorescence Concordance Matrix for MOTiF PD1/PD-L1 Panel Assay. Consecutive sections from lung cancer and melanoma are stained with the MOTiF PD1/PD-L1 Panel Kits or Leica BOND Polymer Refine Detection Kit. Representative Fields comparing DAB and Unmixed Monoplex view from the MOTiF PD1/PD-L1 Panel Assay from Lung Cancer (a) and Melanoma (b).

Rules-based Phenotyping Analysis

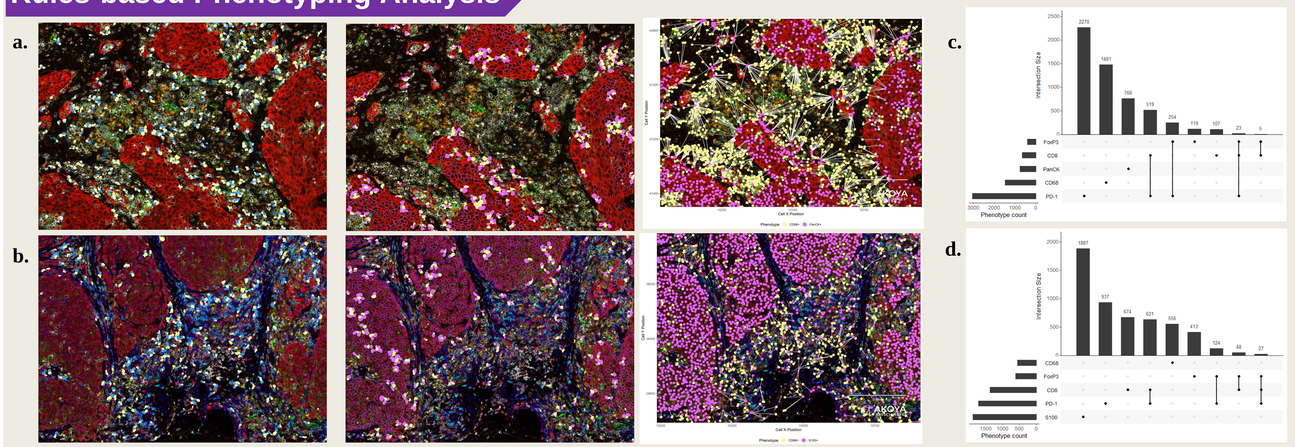

Fig. 5. Touching cell (left and middle) and nearest neighbor (right) plots from phenotprReports for lung cancer (a) and melanoma (b). Each plot shows two phenotypes (CD8+ is blue, CD68+ is yellow, and tumor+ is pink). In touching cell plots, a cell outline is filled if it is touching a cell of the paired phenotype. In nearest neighbor plots, the nearest PanCK+ cell to each CD8+ cell is connected by a white line. (c) and (d) are summaries of cell phenotype counts for the fields in (a) and (b), respectively. Horizontal bars show counts of individual positivities. The vertical bars show counts of specific phenotype combinations present in the data. The central matrix shows the combinations graphically.

Reproducibility of MOTiF PD1/PD-L1 Panel Kits

Luca 1177664	CD8+ >= 75%ile Membrane Opal 480 Mean	PD-L1+ >= 75%ile Membrane Opal 520 Mean	PD-1+ >= 75%ile Membrane Opal 620 Mean	FoxP3+ >= 75%ile Nucleus Opal 570 Mean	PanCK+ >= 75%ile Membrane Opal 690 Mean	CD68+ >= 75%ile Entire Cell Opal 780 Mean
Field 1	10.9	10.9	16.7	6.8	16.5	13.0
Field 2	19.5	15.1	17.3	9.0	5.0	11.5
Field 3	6.2	9.7	6.1	5.0	8.2	12.0
Field 4	10.6	12.9	9.2	19.9	5.0	3.3
Field 5	11.5	5.7	6.0	7.2	6.1	10.7
Average %CV	11.7	10.8	11.1	9.6	8.2	10.1

LuCa 44483	CD8+ >= 75%ile Membrane Opal 480 Mean	PD-L1+ >= 75%ile Membrane Opal 520 Mean	FoxP3+ >= 75%ile Nucleus Opal 570 Mean	PD-1+ >= 75%ile Membrane Opal 620 Mean	PanCK+ >= 75%ile Membrane Opal 690 Mean	CD68+ >= 75%ile Entire Cell Opal 780 Mean
Field 1	18.0	9.4	18.6	11.9	6.9	19.6
Field 2	19.6	12.4	15.9	20.0	6.0	18.9
Field 3	5.6	14.6	20.4	13.8	4.6	6.2
Field 4	4.9	9.0	16.2	6.8	5.4	7.9
Field 5	9.6	14.2	15.4	9.5	4.6	6.7
Average %CV	11.5	11.9	17.3	12.4	5.5	11.9

LuCa 44762	CD8+ >= 75%ile Membrane Opal 480 Mean	PD-L1+ >= 75%ile Membrane Opal 520 Mean	FoxP3+ >= 75%ile Nucleus Opal 570 Mean	PD-1+ >= 75%ile Membrane Opal 620 Mean	PanCK+ >= 75%ile Membrane Opal 690 Mean	CD68+ >= 75%ile Entire Cell Opal 780 Mean
Field 1	19.5	8.7	19.9	15.7	10.0	18.7
Field 2	9.1	7.3	17.8	29.4	6.7	10.3
Field 3	29.6	5.0	23.4	10.0	29.2	27.5
Field 4	9.9	24.8	25.6	10.7	5.2	5.6
Field 5	41.0	24.1	17.3	8.2	22.3	27.8
Average %CV	21.8	14.0	20.8	14.8	14.7	18.0

Mela 1262227	CD8+ >= 75%ile Membrane Opal 480 Mean	PD-L1+ >= 75%ile Membrane Opal 520 Mean	FoxP3+ >= 75%ile Nucleus Opal 570 Mean	PD-1+ >= 75%ile Membrane Opal 620 Mean	S100+ >= 75%ile Membrane Opal 690 Mean	CD68+ >= 75%ile Membrane Opal 780 Mean
Field 1	15.8	22.4	8.5	13.4	10.0	30.8
Field 2	12.9	7.8	6.4	8.9	18.6	13.7
Field 3	13.1	3.9	9.5	14.4	15.9	16.4
Field 4	16.0	6.6	6.8	7.4	17.7	6.1
Average %CV	14.5	9.7	7.8	11.0	15.6	16.8

Mela 1275166	CD8+ >= 75%ile Membrane Opal 480 Mean	PD-L1+ >= 75%ile Membrane Opal 520 Mean	FoxP3+ >= 75%ile Nucleus Opal 570 Mean	PD-1+ >= 75%ile Membrane Opal 620 Mean	S100+ >= 75%ile Membrane Opal 690 Mean	CD68+ >= 75%ile Membrane Opal 780 Mean
Field 1	8.7	7.5	8.3	11.1	19.1	9.3
Field 2	10.7	15.5	11.3	22.6	16.4	23.2
Field 3	10.4	7.8	14.9	22.6	17.8	14.9
Field 4	19.0	8.7	8.3	28.1	22.1	9.5
Average %CV	12.2	9.9	10.7	21.1	18.9	14.2

Mela 132397	CD8+ >= 75%ile Membrane Opal 480 Mean	PD-L1+ >= 75%ile Membrane Opal 520 Mean	FoxP3+ >= 75%ile Nucleus Opal 570 Mean	PD-1+ >= 75%ile Membrane Opal 620 Mean	S100+ >= 75%ile Membrane Opal 690 Mean	CD68+ >= 75%ile Membrane Opal 780 Mean
Field 1	14.6	7.5	16.5	16.8	7.1	16.9
Field 2	29.4	15.5	21.8	8.1	4.6	25.8
Field 3	34.1	7.8	35.3	18.4	15.7	20.6
Field 4	16.0	8.7	26.8	6.1	8.1	7.8
Average %CV	23.5	9.9	25.1	12.4	8.9	17.8

Lung Cancer	CD8 Opal Polaris 480	PD-L1 Opal 520	FoxP3 Opal 570	PD-1 Opal 620	PanCK Opal 690	CD68 Opal Polaris 780
Overall Avg %CV	15.0	12.2	15.9	12.7	9.5	13.3

Melanoma	CD8 Opal Polaris 480	PD-L1 Opal 520	FoxP3 Opal 570	PD-1 Opal 620	S100+SOX10 Opal 690	CD68 Opal Polaris 780
Overall Avg %CV	16.7	9.8	14.5	14.8	14.4	16.3

Fig. 6 Top Quartile results from Phenotyping analysis on serial section slides from 3 different tissues stained with MOTiF PD1/PD-L1 Panel kits. Phenotyping algorithm applied to all tissues and top 25% of positive cells reported. CV's calculated by taking the mean and standard deviation of individual fields across five different slides (StDev/Mean * 100). CV's were then averaged between fields (lower table). A scoring threshold was used to determine PD-L1+ cells for analysis.

7-Color Whole Slide Multispectral Imaging

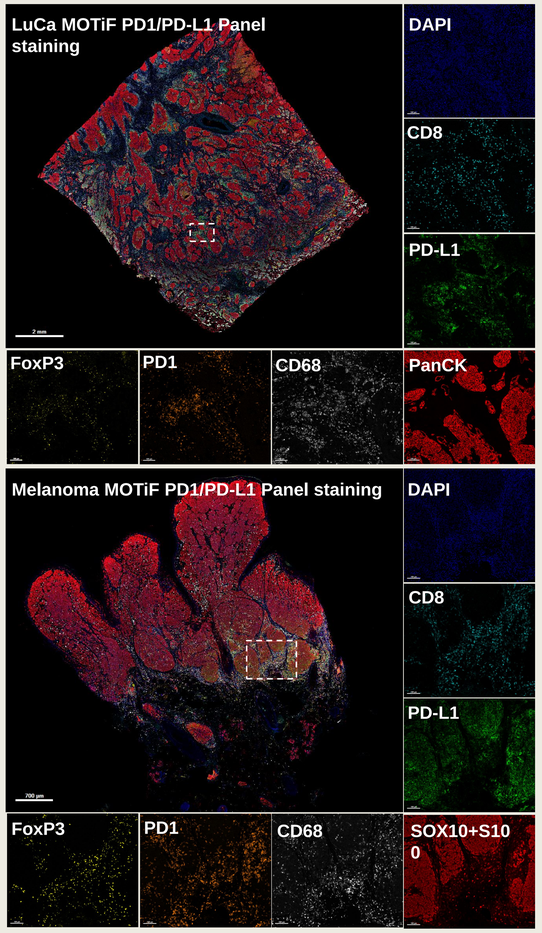

Fig. 3 Whole slide scan of MOTiF PD1/PD-L1 Panel assay on lung cancer (upper) and melanoma (lower) samples.

5 Conclusions

With new MOTiF PD1/PD-L1 Panel Kits, we have demonstrated an easy-to-use yet comprehensive end-to-end Phenoptics research workflow. We have radically simplified the Opal method and facilitated the development and optimization of translational multiplex fluorescent assays by providing pre-defined staining conditions while still giving researchers the flexibility to balance signals based on their tissue samples. Complementary pre-configured imaging protocol and analysis algorithm provide researchers faster access to quantitative data across study samples.

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/

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