#4146 MultiOmyx™ multiplexed tumor infiltrating lymphocyte panel provides comprehensive immunophenotyping from a single FFPE slide

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Background

Immune checkpoint therapies target immune regulatory pathways to enhance anti-tumor immune response. These therapies have contributed to important clinical advances, and are a promising approach to combat cancer. Development of effective immune checkpoint therapies requires an understanding of the host immune response within the tumor microenvironment. Clarient Diagnostics Service Inc., has developed a multiplexed Tumor Infiltrating Lymphocyte (TIL) panel consisting of 12 key cancer immune markers: CD3, CD4, CD8, CD20, CD45RO, CD56, CD68, CTLA4, FOXP3, PD1, PD-L1 and Pan-CK. MultiOmyx (MO) TIL panel identifies algorithmically individual T_{helper} (CD3+CD4+), $T_{cytotoxic}$ (CD3+CD8+), $T_{regulatory}$ (CD3+CD4+FoxP3), memory T-cells (CD3+CD4+CD45RO), anergic T-cells (PD1+CD8+), natural killer cells (CD3-CD56+), macrophages (CD68+), and B-cells (CD20+) within the tumor and the stromal regions and differentiate PD-L1 expression in tumor (PanCK+PD-L1+) and macrophages (CD68+PD-L1+). Utilizing the MO TIL panel, immune responses in the tumor microenvironment were profiled in melanoma, lung, colorectal, prostate, and breast cancer.

Overview of MultiOmyx™ Technology Workflow

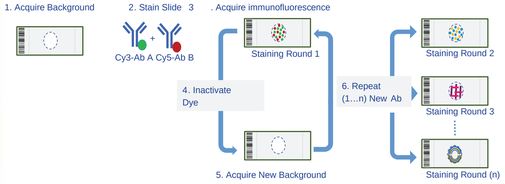
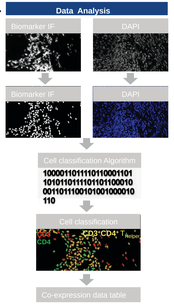


Figure 1. MultiOmyx IF multiplexing scheme from a single tissue section. Conjugated fluorescent antibodies were applied to a slide, followed by whole slide imaging. The dye was chemically inactivated, enabling a second round of staining with another fluorescent antibody. The process is repeated multiple times from a single slide until all biomarkers of interest are multiplexed.

A.



B.

TIL Panel BioMarkers

CD3, CD4, CD8, CD45RO, CD20, CD68, CD56, FoxP3, CTLA-4, PD1, PD-L1, PanCK

Coexpression	Phenotype
CD3+CD4+	T helper
CD3+CD4+FoxP3+	T regulatory
CD3+CD4+FoxP3+PD-L1	T reg expressing PD-L1
CD3+CD4+CD45RO	Memory T helper
CD3+CD4+PD1	Anergic T helper
CD3+CD4+CTLA4	Downregulate activated T helper
CD3+CD8+	T cytotoxic
CD3+CD8+CD45RO+	Memory T cytotoxic
CD3+CD8+PD1+	Anergic T cytotoxic
CD3+CD8+CTLA4	Downregulate activated T cytotoxic
CD3-CD68+	Macrophage
CD3-CD68+PD-L1+	Mφ expressing PD-L1
CD3-CD20+	B cell
CD3-CD20+PD-L1+	B cell expressing PD-L1
CD3-CD56+	Natural Killer cell
CD3-CD8+CD56+	Natural Killer T cell
PanCK+PD-L1+	Tumor expressing PD-L1

Figure 2. MultiOmyx data analysis workflow and TIL panel coexpression phenotypes. A. For each biomarker, the AF-removed immunofluorescence image is transformed into a biomarker segmentation map via proprietary algorithms that take into account staining pattern for each specific biomarker. Nuclear segmentation algorithms are applied to the DAPI image to identify location of nuclei. The biomarker segmentation map and the nuclear segmentation image are superimposed digitally, and proprietary algorithms compared areas of overlap and determined whether to call a given cell positive or negative. The result of this process is a classification label map and biomarkers coexpressions were obtained by overlapping individual classification label maps. B. Coexpression phenotypes algorithmically classified.

Conclusion

MultiOmyx TIL Panel was utilized to profile immune response in the tumor microenvironment within solid tumors including breast cancer, lung cancer, colorectal cancer, esophageal cancer, prostate cancer, and melanoma. The results shown in figure 4 revealed two distinct immunologic phenotypes, high TIL (Prostate & Breast), and Low TIL (Colorectal). The high TIL samples showed enhanced T cell population within the tumor and in the peritumoral stroma including CD8+ cytotoxic T cells, CD4+ helper T cells and CD45RO+ memory T cells. The low TIL sample showed reduced population of T cells and cells co-expressing different immune phenotypes. In the lung sample shown, PD-L1 is expressed primarily in the tumor while in the breast sample, PD-L1 is expressed primarily in the macrophages. Immunophenotyping analysis offered by the MultiOmyx TIL panel enabled unambiguous identification of $T_{cytotoxic}$, T_{helper}, $T_{regulatory}$, macrophages, B cells, PD-L1 expressing cells and concise spatial relationship between immune cells and immune cells to the tumor.

MultiOmyx TIL panel immune cells phenotypes

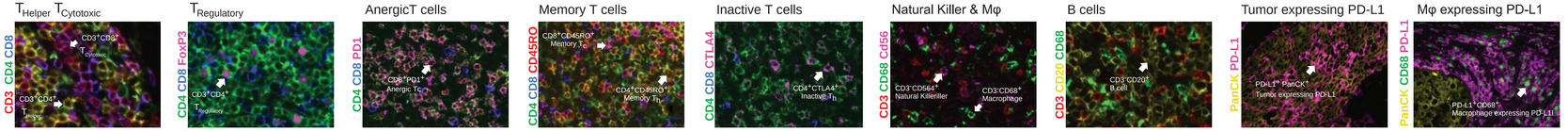
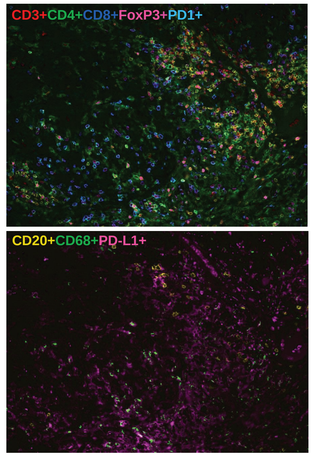


Figure 3. MultiOmyx TIL panel coexpression phenotypes. Autofluorescence removed (AFR) gray scale images of individual biomarkers were color blended based on biomarkers coexpressions to differentiate multiple immune phenotypes. Each phenotype is indicated by an arrow and an individual biomarker color code is indicated above for different combinations of coexpressions.

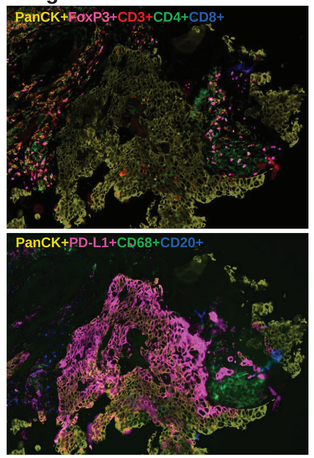
MultiOmyx TIL panel immuno profiling in solid tumors

Melanoma



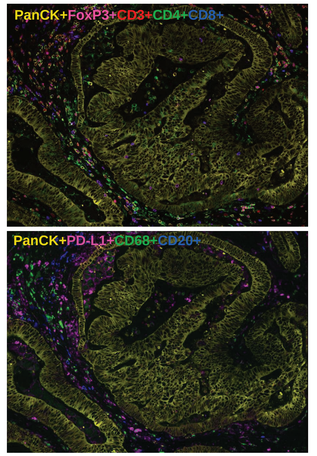
Co-expression	Cell counts # of cells	Percentage of cells
CD3+CD4+	345	9.5%
CD3+CD4+FoxP3+	79	2.2%
CD3+CD4+CD45RO	333	9.2%
CD3+CD4+PD1	50	1.4%
CD3+CD4+CTLA4	73	2.0%
CD3+CD8+	506	14.0%
CD3+CD8+CD45RO+	467	12.9%
CD3+CD8+PD1+	243	6.7%
CD3+CD8+CTLA4	54	1.5%
CD3-CD68+	299	8.3%
CD3-CD68+PD-L1+	127	3.5%
CD3-CD20+	13	0.4%
PanCK+PD-L1+	0*	0%
DAPI (total # of cells)	3618	-

Lung Cancer



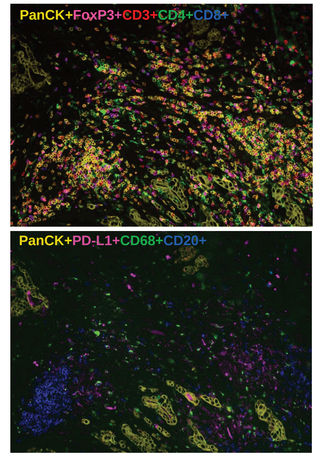
Co-expression	Cell counts # of cells	Percentage of cells
CD3+CD4+	537	15.5%
CD3+CD4+FoxP3+	131	3.8%
CD3+CD4+CD45RO	446	12.9%
CD3+CD4+PD1	336	9.7%
CD3+CD4+CTLA4	147	4.2%
CD3+CD8+	144	4.2%
CD3+CD8+CD45RO+	118	3.4%
CD3+CD8+PD1+	91	2.6%
CD3+CD8+CTLA4	35	1.0%
CD3-CD68+	61	1.8%
CD3-CD68+PD-L1+	33	1.0%
CD3-CD20+	11	0.3%
PanCK+PD-L1+	1024	29.6%
DAPI (total # of cells)	3464	-

Colorectal Cancer



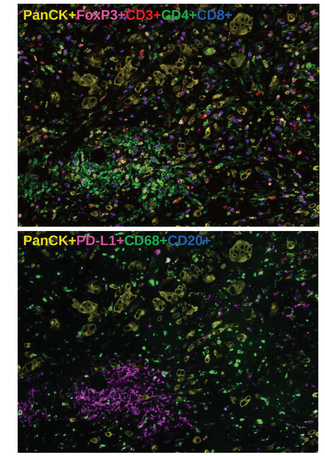
Co-expression	Cell counts # of cells	Percentage of cells
CD3+CD4+	244	3.7%
CD3+CD4+FoxP3+	114	1.7%
CD3+CD4+CD45RO	231	3.5%
CD3+CD4+PD1	72	1.1%
CD3+CD4+CTLA4	73	1.1%
CD3+CD8+	131	2.0%
CD3+CD8+CD45RO+	111	1.7%
CD3+CD8+PD1+	38	0.6%
CD3+CD8+CTLA4	9	0.1%
CD3-CD68+	276	4.2%
CD3-CD68+PD-L1+	156	2.4%
CD3-CD20+	10	0.2%
PanCK+PD-L1+	81	1.2%
DAPI (total # of cells)	6620	-

Prostate Cancer



Co-expression	Cell counts # of cells	Percentage of cells
CD3+CD4+	1671	33.4%
CD3+CD4+FoxP3+	304	6.1%
CD3+CD4+CD45RO	1564	31.3%
CD3+CD4+PD1	439	8.8%
CD3+CD4+CTLA4	48	1.0%
CD3+CD8+	500	10.0%
CD3+CD8+CD45RO+	442	8.8%
CD3+CD8+PD1+	101	2.0%
CD3+CD8+CTLA4	5	0.1%
CD3-CD68+	150	3.0%
CD3-CD68+PD-L1+	68	1.4%
CD3-CD20+	224	4.5%
PanCK+PD-L1+	64	1.3%
DAPI (total # of cells)	4996	-

Breast Cancer



Co-expression	Cell counts # of cells	Percentage of cells
CD3+CD4+	476	15.8%
CD3+CD4+FoxP3+	173	5.7%
CD3+CD4+CD45RO	425	14.1%
CD3+CD4+PD1	278	9.2%
CD3+CD4+CTLA4	81	2.7%
CD3+CD8+	207	6.9%
CD3+CD8+CD45RO+	153	5.1%
CD3+CD8+PD1+	56	1.9%
CD3+CD8+CTLA4	4	0.1%
CD3-CD68+	687	22.8%
CD3-CD68+PD-L1+	189	6.3%
CD3-CD20+	5	0.2%
PanCK+PD-L1+	19	0.6%
DAPI (total # of cells)	3019	-

Figure 4. MultiOmyx TIL panel immuno profiling in solid tumors. Representative color blended multiplexed images are shown in melanoma, lung, colorectal cancer, prostate, and breast cancer. All 12 biomarkers were multiplexed on a single slide and displayed as two sets of images to improve visualization. Summary tables list the number of cells classified algorithmically based on co-expression of multiple immune markers. For each phenotype, percentages of cells are calculated using total number of cells based on DAPI staining.