

Human Multiplex Immunofluorescence Panels

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Overview

The CIIA provides comprehensive, full-service multiplex immunofluorescence (mIF) support from experimental design through to panel development, staining and scanning, and image and data analysis and interpretation. Our team uses mIF to detect up to six surface or intracellular markers within a single tissue section, enabling spatial characterization of multiple immune cell types and their tissue microenvironment (**Page 2, Table 1**).

Explore our established panels (**Pages 3-7**) or connect with us at ciiia@uhn.ca to design a custom panel that supports your discovery, translational and clinical research needs.

Sample Requirements

Please refer to our [Sample Requirements and Submission Guidelines](#).

Panel Selection and Costs

A subset of markers can be selected from any panel, to reduce costs while focusing the analysis on the most relevant markers. The final cost will depend on the chosen markers/panel(s), batch size, and level of analysis support required.

Instrument(s) and Kits

The panels listed in this document are compatible with the Quanterix 6-Plex Detection Kit and Vectra 3 Imaging System.

Image Analysis Software

The CIIA uses InForm (Quanterix) software for routine mIF image analysis including tissue and cell segmentation and phenotyping. Additional spatial analyses can be performed using HALO (Indica Labs), QuPath, and pipelines developed in R and Python.

Data Delivery and File Formats

Data can be delivered in a range of formats including summary reports, publication-ready figures, cell densities, spatial statistics, and QPTIFF or TIFF files compatible with downstream image analysis platforms.

Table 1. Immune Cell Types

Immune Cell Type ^(a)	mIF Markers	Main Functions
Conventional T cells	CD3, CD4, CD8	Provide adaptive, antigen-specific immunity including direct killing of infected and/or malignant cells (CD8+ cytotoxic T cells) and helper functions through cytokine secretion (CD4+ helper T cells).
Regulatory T cells (Tregs)	CD3, CD4, FOXP3	Suppress immune responses through cytokine secretion to maintain self-tolerance and prevent autoimmunity.
$\gamma\delta$ T cells	CD3, TCR- $\gamma\delta$	Innate-like T cells that respond to stress, infection, and malignant cells without classical MHC restriction.
B cells	CD19, CD20, CD21, CD23	Produce antibodies in response to antigen and differentiate into memory B cells or plasma cells.
NK cells	CD16, CD94, NKp46	Innate-like cells of lymphoid origin that kill infected and/or malignant cells that lack expression of MHC-I.
Macrophages	CD68, CD163	Tissue-resident cells that phagocytose pathogens, dead cells and debris, produce immunomodulatory cytokines and support tissue repair.
Neutrophils	CD15	Rapid innate effector response; phagocytosis and acute inflammation.

^(a) Please contact us at cija@uhn.ca regarding detection of immune cells and non-immune cells that are not listed in this table.

General Immune Panels

Our general immune panels (6 markers) enable quantification of major immune cell subsets (T, B, and NK cells, macrophages, granulocytes) and epithelial/tumor cells.

These panels have been optimized for 4–5-micron thick FFPE sections, and are suitable for a range of applications including:

- Immune profiling of the tissue microenvironment across disease settings.
- Assessment of immune populations in epithelial and stromal compartments.
- Assessment of PD-L1 (checkpoint) and Ki67 (proliferation) expression.

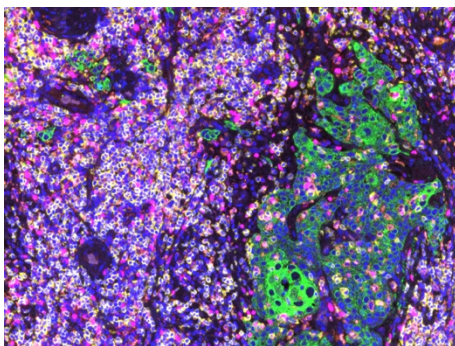
General Immune Panels	
Panel ^(a)	Markers
1	CD3, CD8, CD20, FOXP3, CD68, PanCK ^(b)
2	CD3, CD8, CD20, FOXP3, NKp46, PanCK ^(b)
3	CD3, CD8, CD68, CD163, PD-L1, PanCK ^(b)
4	CD3, CD15, CD68, CD163, PD-L1, PanCK ^(b)
5	CD3, CD8, TCRγδ, PD-1, Ki67, PanCK ^(b)
6	CD3, CD8, NKp46, TCRγδ, PD-1, PanCK ^(b)
7	CD3, CD8, CD68, CD11c, FOXP3, PanCK ^(b)

^(a) Please contact us at cija@uhn.ca to discuss panel customization.

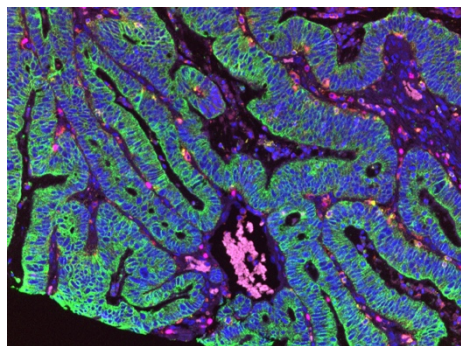
^(b) For melanomas, PanCK can be replaced with a melanoma cocktail and SOX10.

General Immune Panel 1 – Staining in Cancer Tissues

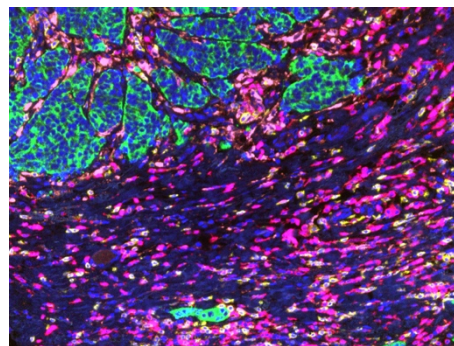
Head & Neck Cancer



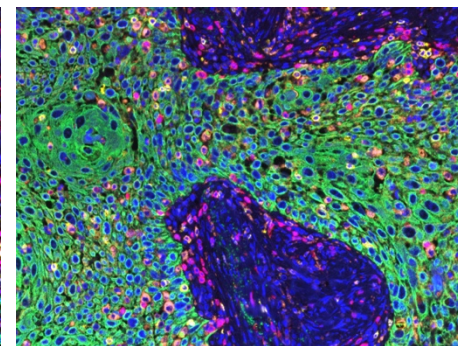
Colorectal Cancer



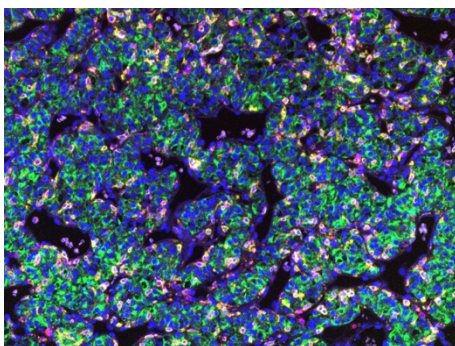
Breast Cancer



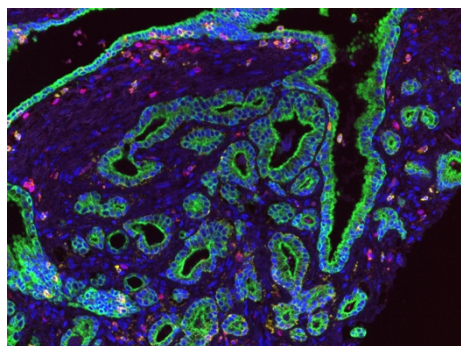
Lung Cancer



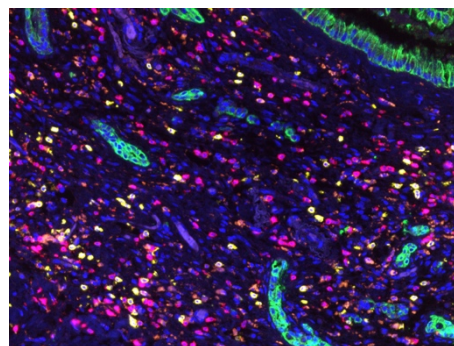
Renal Cancer



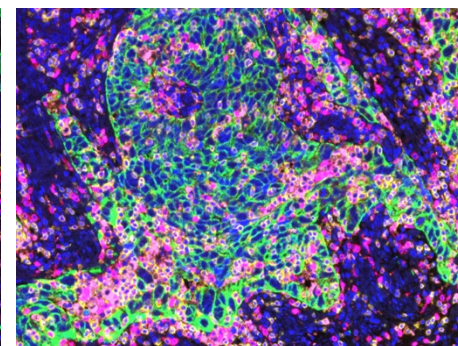
Prostate Cancer



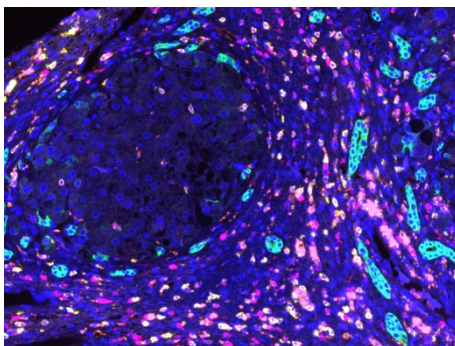
Pancreatic Cancer



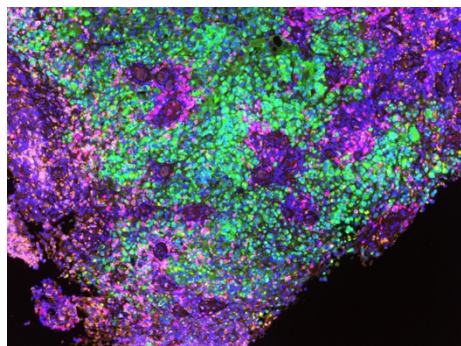
Ovarian Cancer



Liver Cancer



Melanoma



- DAPI (nuclei)
- ● immune cells (T cells)
- pan-cytokeratin -or- melanoma cocktail



>1000 cases stained to date
for the Marathon of Hope
Cancer Centres Network.

Immune Activation Panels

Our immune activation panels (5-6 markers) enable assessment of T cell activation, exhaustion, and cytotoxic activity, immune checkpoint expression, and macrophage-driven inflammation within and surrounding epithelial/tumor regions.

These panels have been optimized for 4–5-micron thick FFPE sections, and are suitable for a range of applications including:

- Immune profiling of the tissue microenvironment across disease settings.
- Assessment of immune populations in epithelial and stromal compartments.
- Assessment of immune activation and checkpoint marker expression in tumor and stromal compartments.

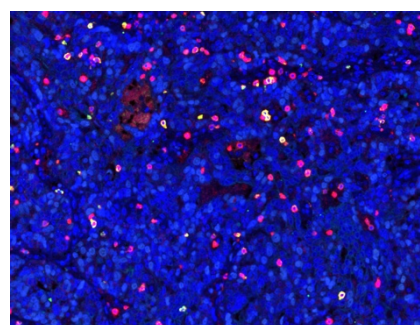
Immune Activation Panels

Panel ^(a)	Markers
1	CD3, CD68, CD163, IDO1, PD-L1, PanCK ^(b)
2	CD3, CD8, CD103, PD-1, PanCK ^(b)
3	CD3, CD8, NKp46, CD103, Granzyme B, PanCK ^(b)
4	CD3, CD8, CD68, HLA-I, PD-L1, PanCK ^(b)
5	CD3, CD8, CD31, PD-L1, ISG15, PanCK ^(b)
6	CD3, CD8, CD68, CD163, Caspase 1, PanCK ^(b)
7	CD8, PD-1, TIM-3, LAG-3, PanCK ^(b)

^(a) Please contact us at cija@uhn.ca to discuss panel customization.

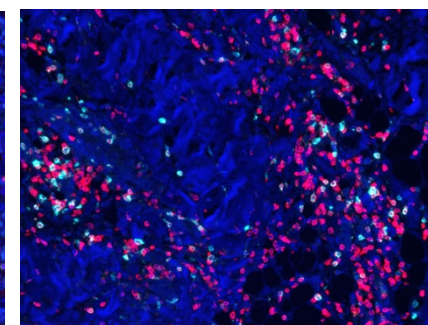
^(b) For melanomas, PanCK can be replaced with a melanoma cocktail and SOX10.

PD-1+ T Cells in Breast Cancer



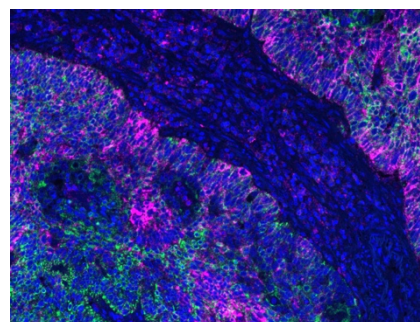
● CD3 ● PD-1+ T cells

CD103+ T Cells in Breast Cancer



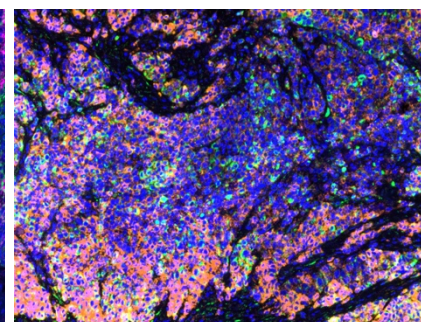
● CD3 ● CD103

PD-L1 in Cervical Cancer



● PanCK ● PD-L1

HLA-I in Cervical Cancer



● PanCK ● HLA-I

Tertiary Lymphoid Structure (TLS) Panels

Our TLS panels (6 markers) enable assessment of organized immune aggregates, including T cell and B cell organization, B cell maturation, and immune cell proliferation within and surrounding epithelial/tumor regions.

These panels have been optimized for 4–5-micron thick FFPE sections, and are suitable for a range of applications including:

- TLS identification and profiling across different disease settings.
- Assessment of T and B cell organization and the expression of BCL6.
- Assessment of T and B cell proliferation (Ki67 expression).

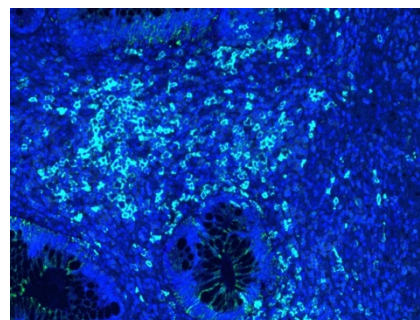
TLS Panels

Panel ^(a)	Markers
1	CD3, CD20, CD23, Ki67, BCL6, PanCK ^(b)
2	CD3, CD20, CD21, CD23, BCL6, PanCK ^(b)

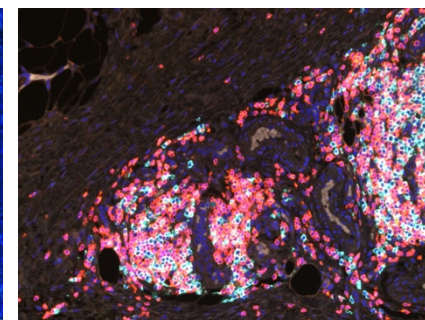
^(a) Please contact us at cija@uhn.ca to discuss panel customization.

^(b) For melanomas, PanCK can be replaced with a melanoma cocktail and SOX10.

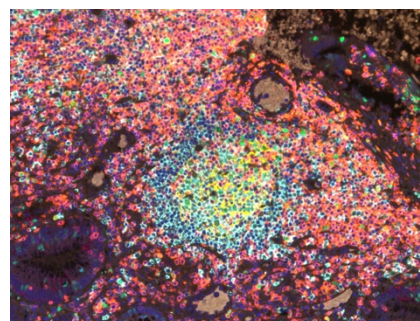
B Cell Aggregate in PDAC



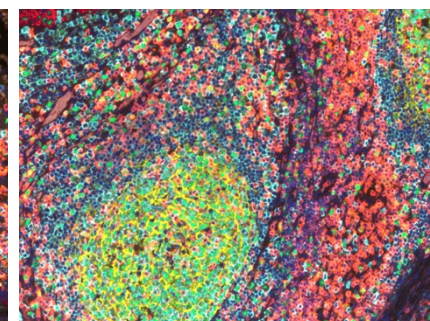
TLS in PDAC



Mature TLS in PDAC



Mature TLS in PDAC



● CD20
 ● CD3
 ● CD23
 ● Ki67

Other Panels

Our team has experience working with several tissue types including brain and assessing tissue architecture including endothelial cells. These panels have been optimized for 4–5-micron thick FFPE sections, and are suitable for:

- Characterization of brain tissues.
- Characterization of endothelial cells across different disease settings.

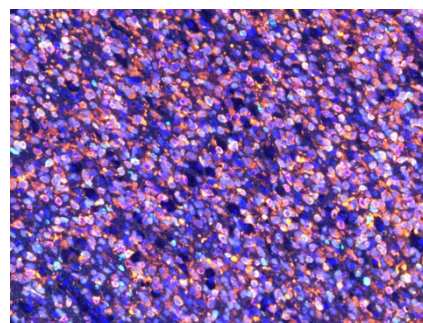
Other Panels

Panel ^(a)	Markers
Glioma 1	ASCL1, MAP2, DLL3, GFAP+CD44, SOX2, OLIG2
Glioma 2	JAB1, Iba1, p53, β -TRCP, BLBP, IFIT3
Endothelial 1	CD3, CD31, PNA ^d , PD-L1, LYVE1, PanCK ^(b)

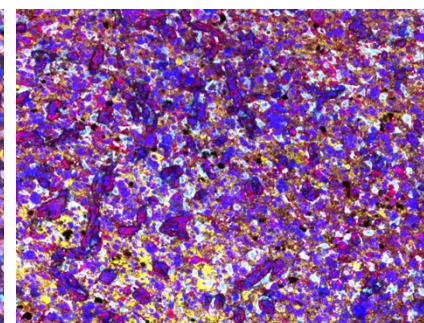
^(a) Please contact us at cija@uhn.ca to discuss panel customization.

^(b) For melanomas, PanCK can be replaced with a melanoma cocktail and SOX10.

Glioma Panel 1

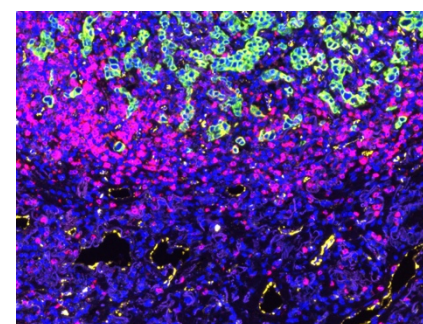


Glioma Panel 2



● DLL3
 ● MAP2
 ● OLIG2
 ● Iba1
 ● BLBP
 ● IFIT3

Endothelial Panel



● PanCK
 ● CD3
 ● CD31