Trev and Joyce Deeley Research Centre: Molecular and Cellular Immunology Core

Provincial Health Services Authority

➢ About the Molecular and Cellular Immunology Core (MCIC)

Established in 2004, in partnership with the Tumor Tissue Repository (TTR), the BC Cancer Agency's Trev and Joyce Deeley Research Centre (DRC) MCIC provides a number of services to DRC researchers, external collaborators and external clients worldwide and has been involved in numerous publications. Both our human and murine multicolor Immunohistochemistry (mcIHC) and Immunofluorescence (mcIF) platforms are constantly under development to expand the number of available standard panels and create a diverse and readily available catalogue.

➢ Current Services Offered and Available Equipment

Processing of formalin-fixed tissue and paraffin embedding (FFPE): Leica TP1020 tissue processor and Sakura Tissue-Tek TEC Embedding (fig 1) station

FFPE Tissue sectioning: Microm HM355S Microtome (fig 2); Cryostats available for frozen tissues but not part of routine services offered

Hematoxylin and Eosin Staining: Sakura Tissue-Tek DRS H&E autostainer, useful when planning TMA construction

TMA construction: Beecher Manual Tissue Microarrayer MTA-1 (fig 3)

Consultation: Antibody selection, experimental design, image analysis

Human and Mouse mcIHC and Immunofluorescence staining

Multispectral imaging and automated scoring

Figure 1. Leica TP1020 tissue processor and IP-C Cassette Wriiter

Figure 2. Microm HM355S Microtome

Figure 3. Beecher Manual Tissue Microarrayer MTA-1

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Multicolour IHC and Immunofluorescence

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➢ MCIC utilizes 2 intelliPATH FLX autostainers with combined capacity of 100 slides per run to output consistent runs of multicolour immunohistochemistry and immunofluorescence

➢ Common Human Antibodies Available (not a complete list)

T cell markers
CD3 CD4 CD8

B cells and plasma cells
CD138 CD20 CD79a
CD19 CD21 CD22
PAX5

DC markers
CD1a CD208 CD303
CD11c

NK cell markers
CD56 CD57

Other Immune markers
CD30 CD45 HLA Class 1
A,B,C
HLA-DR+DP+DQ HLA-DR

Vasculature
CD31 CD34 D2-40

Granulocytes
Myeloperoxidase neutrophil elastase

Immunosuppressive markers
B7-H4 PD-L1 IDO-1
CD276 CD273 PD-L2

TLS related
BCL-6 AID PNAd

Tumour markers
B-catenin Her2 E-cadherin p53
folate receptor alpha NY-ESO-1
pan-CK (other CKs) pan-Cadherin
PAX8 PSMA PTEN
SMA Vimentin WT-1

Functional markers
CD103 CD134 CD137 CD25
FoxP3 CD45RO Cleaved Caspase 3
Ki67 Granzyme B TIA-1 PD-1
LAG3 ICOS

Myeloid markers
CD11b CD14 CD15 CD163
CD16a CD206 CD68

➢ Commonly used Brightfield mcIHC panels (not a complete list)

CD3/CD8/CD20 (or CD79a)
CD3/CD8/PanCK
HLA-DR/CD33/CD11b
GranzymeB, Ki67,PD-1, or TIA-1/CD8/CD3 (fig 4)
CD20/CD79a
PD-L1/PD-1/CD8
CD163/CD68/CD79a
CD20/CD79a/PanCK
PD-L1/CD163/PD-1
PD-L1/FoxP3/IDO-1
FoxP3/CD3/CD8

➢ Immunofluorescence panels (not a complete list)

MHCI/MHCII/IDO-1
CD8/CD68/PD-1/IDO-1/PD-L1/Pan-Cytokeratin*
CD68/PD-1/PD-L1/PanCK (Fig 5)
PD-L1/PD-L2/CD276/B7-H4/Vista/CD68/PanCK *
CD8/CD3/CD19 or PAX5/CD11b/FoxP3/PanCK *
FoxP3/CD8/CD79a/CD3/CD20/Pan-Cytokeratin*
CD25/FoxP3/CD8/PanCK
CD8/CD68/PD1/PDL1/IDO-1/PanCK *
PD-1/PD-L1/OX40/CD27/TIM3/CD3/PanCK *

**Panel under development*

Note: Custom IF panels can be constructed but time and cost are challenging to forecast

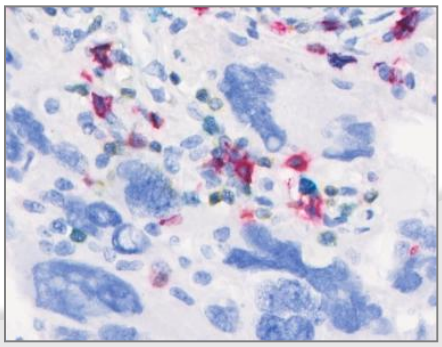

Figure 4. Brightfield composite image: CD8 (red), CD3 (yellow), PD-1 (blue), hematoxylin

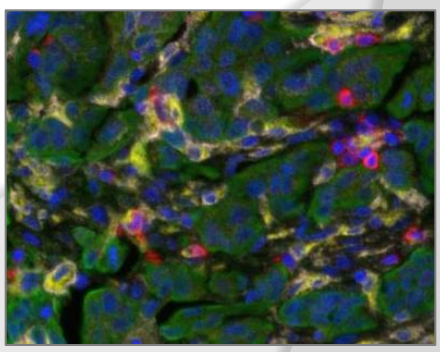

Figure 5. Inform false colour immunofluorescence panel: CD68 (yellow), PD1 (red), PD-L1(pink), PanCK (green), DAPI (dark blue)

Murine multicolour IHC

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➢ Our murine multicolor immunohistochemistry platform is constantly under development to expand the number of available standard panels and create a diverse and readily available catalogue to match staining capabilities of human tissue panels

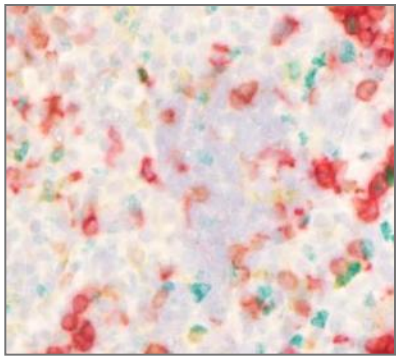

Figure 6. Mouse lymph node inForm composite: Ki67 (green), CD8 (red), CD3 (yellow), hematoxylin

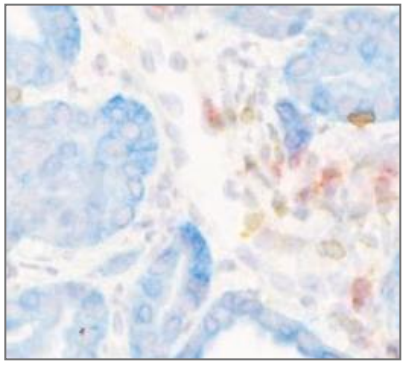

Figure 7. Mouse tumor composite image with hematoxylin: CD8 (red), CD3 (yellow), Ck8+18 (dark blue)

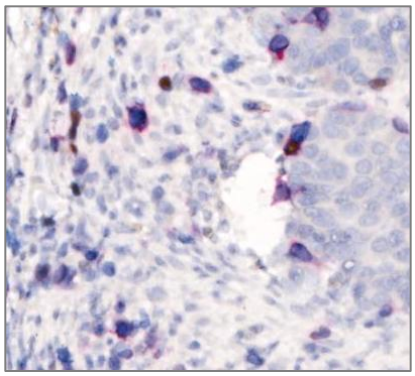

Figure 8. FFPE mouse tumor TMA core Inform composite image; CD3 (blue), CD8 (red), FoxP3 (brown)

➢ Antibodies Available for Mouse Tissue Staining

T-cell markers
CD3 CD8
CD4 FoxP3

B-cell markers
PAX5
CD19

Tumor and endothelial markers
Cytokeratin 8 (Ck8)
Her2
Vimentin
SMA (zinc-fixed only)
PNAd
CD31

Functional markers
Ki67
Cleaved Caspase 3
Granzyme B

Other immune markers
PD-1 CD208
Bcl-6 (FFPE only)

Myeloid markers
F4/80 CD11b
Ly-6G CD163
Ly-6G6C

➢ Common mcIHC Panels in Use*

CD8 CD3 Ck8 (fig. 7)
CD3 CD8
CD4 CD8
CD4 CD3
CD3 Pax5
CD3 CD8 Pax5
GranzymeB CD8 CD3
PD-1 CD3 Pax5

Ki67 CD8 CD3 (fig. 6)
FoxP3 CD3 CD8 (fig. 8)
FoxP3 CD8 Ck8
FoxP3 CD8
FoxP3 CD3
PD-1 PD-L1 CD8

***Custom Panels are available with sufficient workup time**

➢ **Mouse Immunofluorescence panels** are currently under development and custom panels may be available upon request given sufficient workup time

Multispectral Imaging and Automated Scoring

➢ Vectra Multispectral imaging system (fig 13) is compatible with slides stained with both immunoflourescence and multicolor immunohistochemical to image both full tissue sections or TMA cores. Images are converted to be used by Inform software and follow a digital pathology workflow of image spectral separation, tissue segmentation, cell segmentation and cell phenotyping (fig 9).

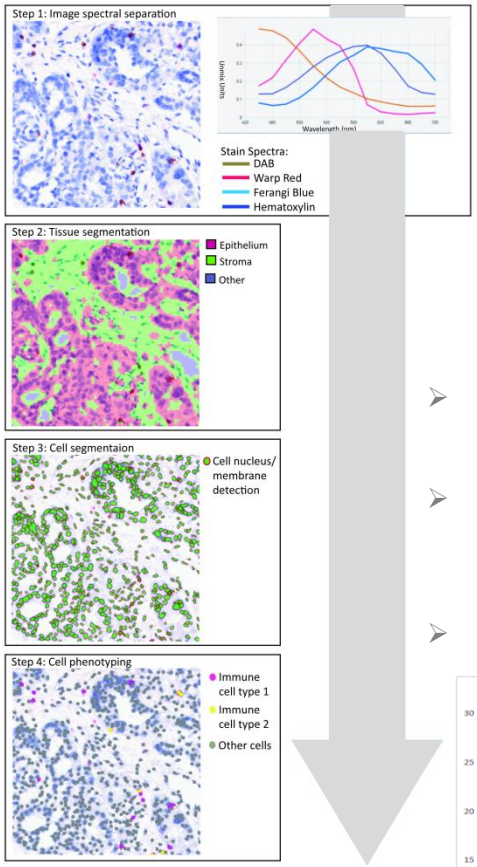

Figure 9. Standard digital pathology work flow using nform analysis software on multispectral images

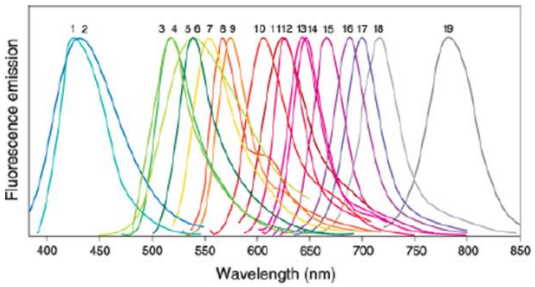

Figure 10. Immunofluorescence wavelength separation allows for inclusion of more markers per panel while maintaining accurate analysis

- Whole section scanning is also available for brightfield IHC using Pannoramic MIDI; uses freely downloadable software
- For imaging requiring spectral unmixing, low powered scans can be shared and areas of interest for 20x image collection annotated remotely
- Data transfer available through SFTP site

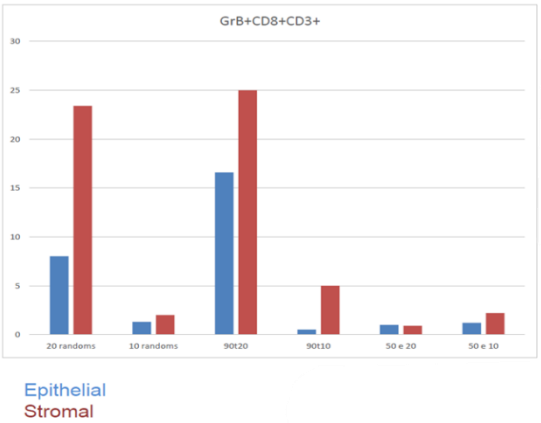

Figure 11. Generated results tissue segmentation and phenotype data of multiple trained Inform algorithms

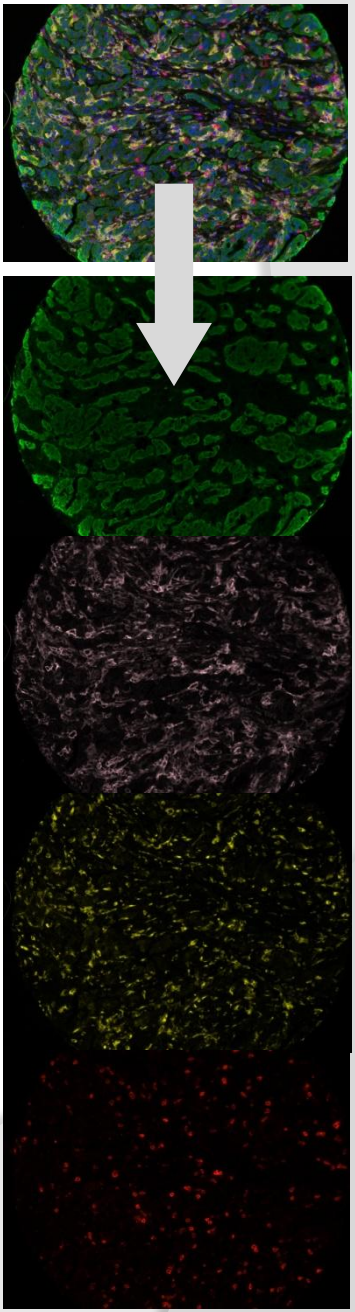

Figure 12. Immunofluorescence Vectra images spectrally separated by inform into compiled composite of colors or can be viewed each fluorophor e independently

Figure 13. Vectra imaging system

Publications

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