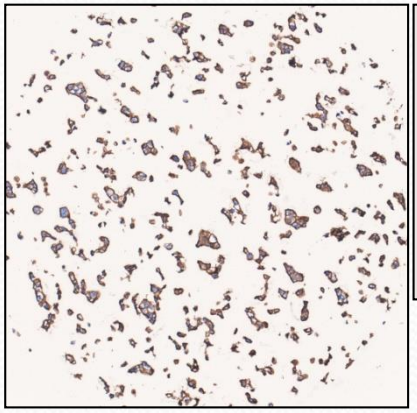
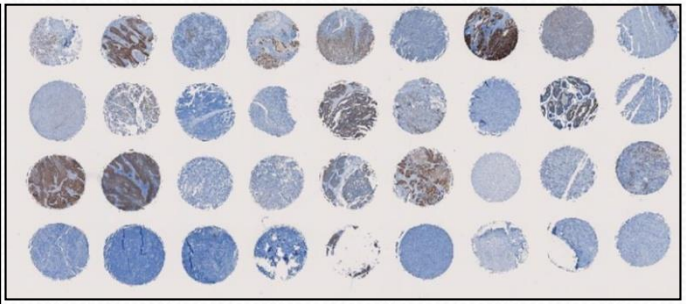
MCIC Histology Services

31Oct2024

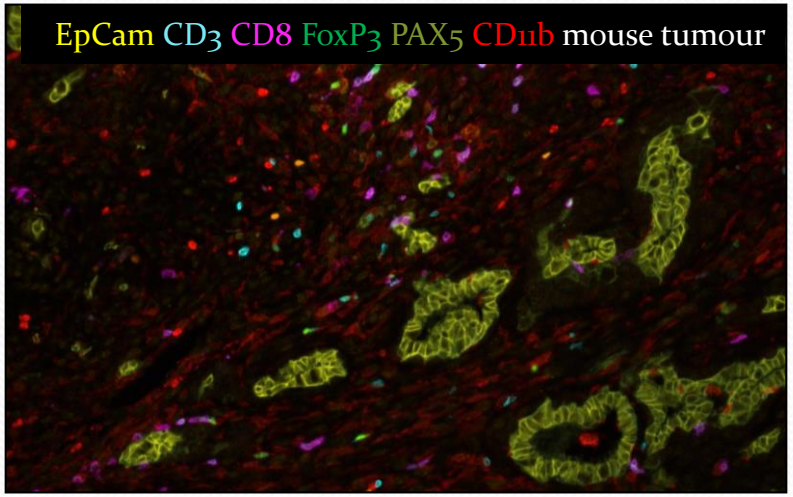
Overview of Projects

- Provide support to a number of internal DRC projects from basic histological preparation to development of complex multicolour immunofluorescent stains
- Provide support to a number of BCCRC researchers in Vancouver, largely processing, serial sectioning and H&Es of mouse tissue up to more complex multicolour IF projects
- Also have a number of national and international collaborations ranging from basic research projects to assessments of immune response in clinical trial tissues (note that we don't hold CAP or CLIA accreditation)
- Numerous projects with biotech companies in biomarker testing and preclinical models
- Experience working with cell pellets, xenografts, mouse tissue and human tissue





Biomarker screening on cell pellet (left) and human tissue TMA (above)



Basic Histology Services

- Leica TP1020 Carousel tissue processor, Leica IP-C cassette writer and embedding station
- 3 Microm HM355 microtomes (two for H&E/IHC/IF, other for molecular)
- 2 Beecher Instruments manual tissue microarrayers for building tissue microarrays
- Sakura DRS 2000 automated H&E stainer

Tissue processor

Cassette Writer

H&E Stainer

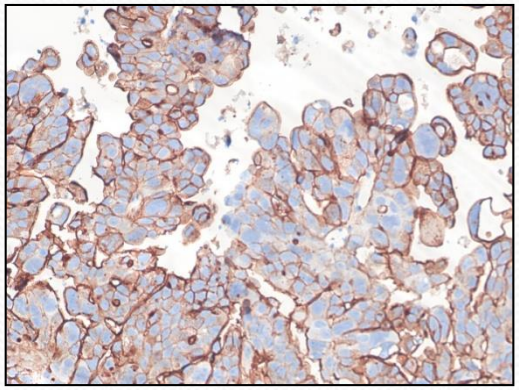
Microtome

Tissue Microarrayer

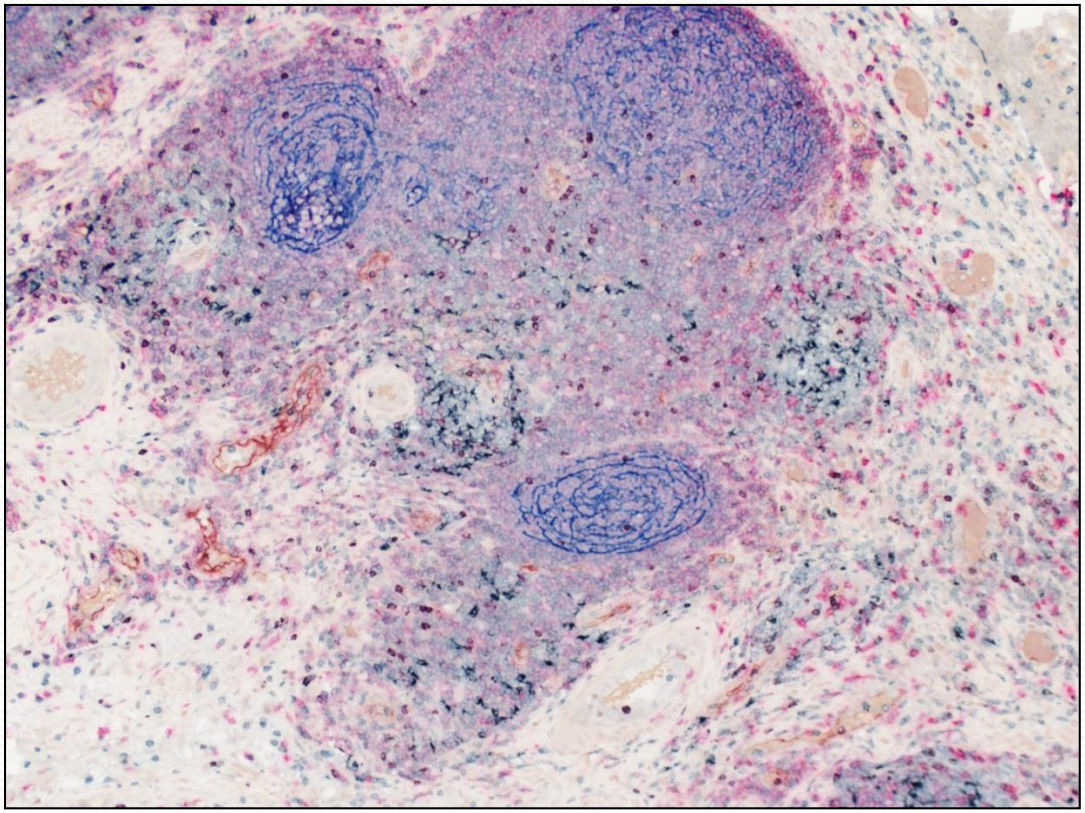
Immunohistochemistry

- 2 Biocare Intellipath FLX autostainers – open system, 50 slides simultaneous run each
- Can do basic one colour IHC with DAB or more complex multicolour stains (do not recommend going above 3 colours if automated scoring is required)

Mesothelin



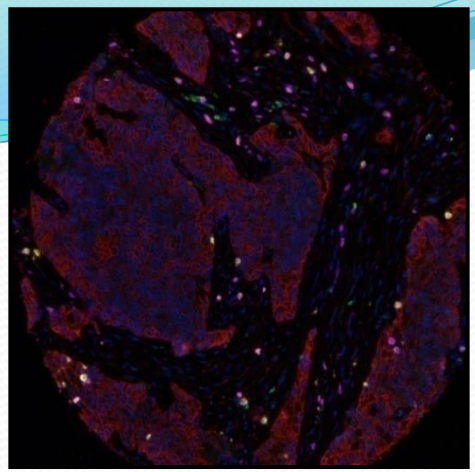
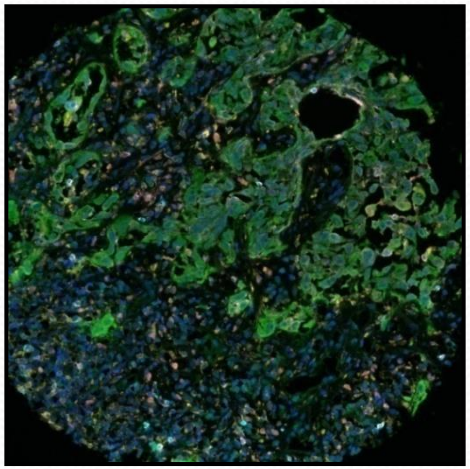
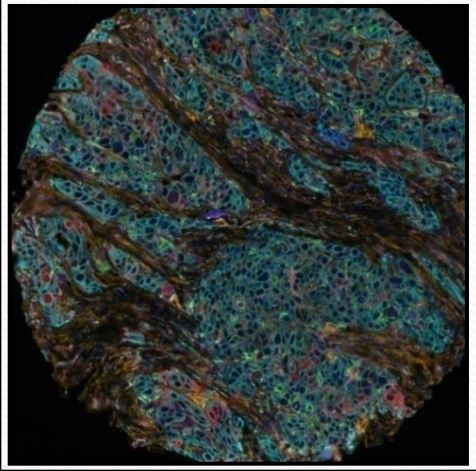
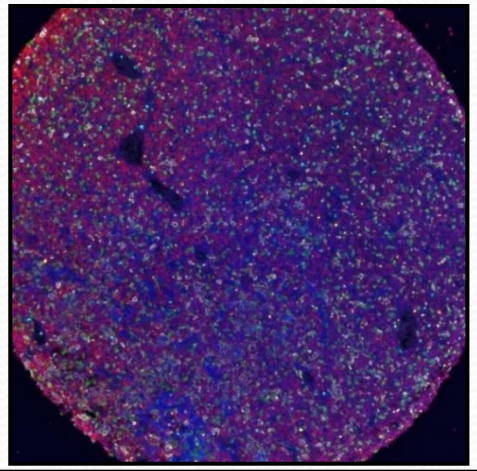
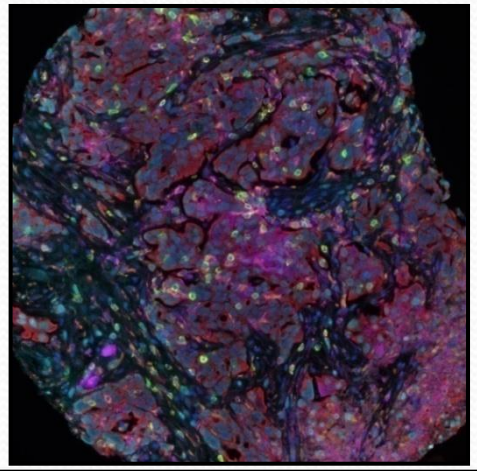
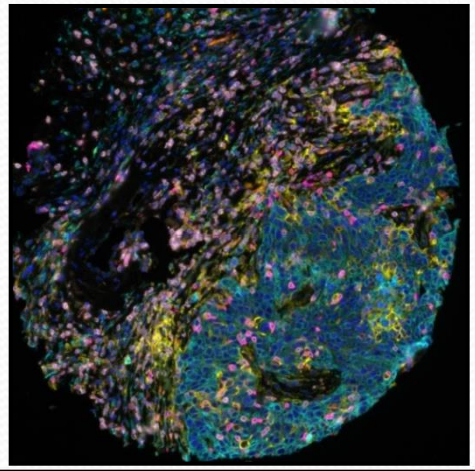
PD1 CD103 CD8



CD21 CD3 CD20 CD8 PNAd CD208

Multicolour Immunofluorescence

- Use Akoya Biosciences's OPAL system
- Can stain up to 8 colours plus DAPI depending on markers
- Developing standardized (predominantly immunological) panels
- Can do custom panel preparation but difficult to precisely forecast cost and length of development time, where possible we lean on existing panels with slight adjustments
- Multispectral Imaging capability allows for removal of autofluorescence from FFPE tissue, no need to do frozen IF

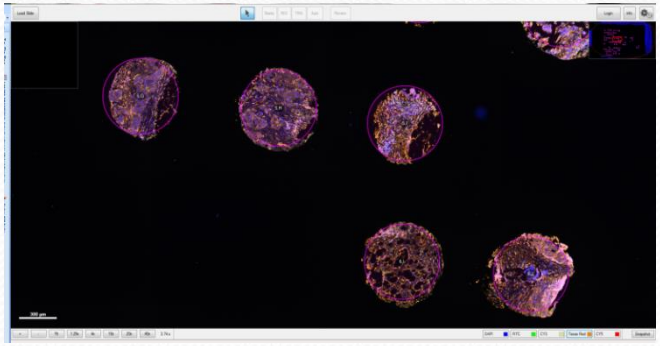
MSI vs Motif

- Vectra 3 scanning involves a low powered scan followed by selection of 20X fields of interest for multispectral image (MSI) acquisition, MSI collection should happen soon after scanning starts to avoid any dimming of signal (ie fields can't be selected a week later then collected), maximum number of colours = 7+DAPI although 6+ DAPI is more common

- Vectra Polaris can capture MSI images like Vectra but can also capture the entire slide multispectrally (Motif) so you can view any area, any channel using freely downloadable Phenochart software(including autofluorescence removal); also allows you to annotate images any time to pull 20X images into inForm; maximum # colours = 8 + DAPI for MSI, 6 + DAPI for Motif, it may also be possible if there is a mix of nuclear and membrane targets to cocktail markers to look at more things

Imaging Options

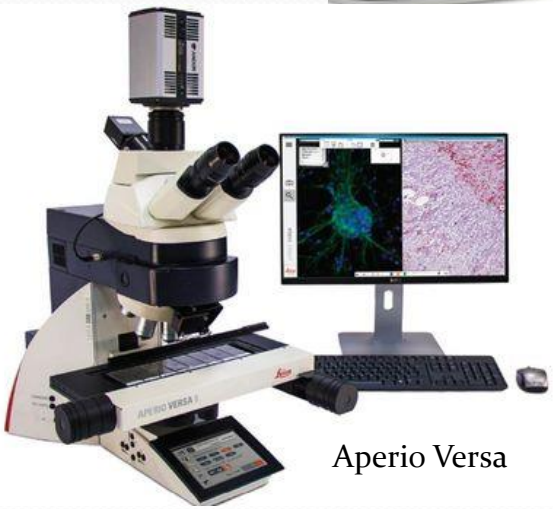
- 3D histech Pannoramic Midi whole slide scanner (Brightfield only)
- Nuance multispectral imaging system
- Vectra multispectral imaging system (plus Phenochart)
- Vectra Polaris multispectral imaging system
- Aperio Versa whole slide scanner (brightfield and IF)



Phenochart display

Vectra

Vectra Polaris

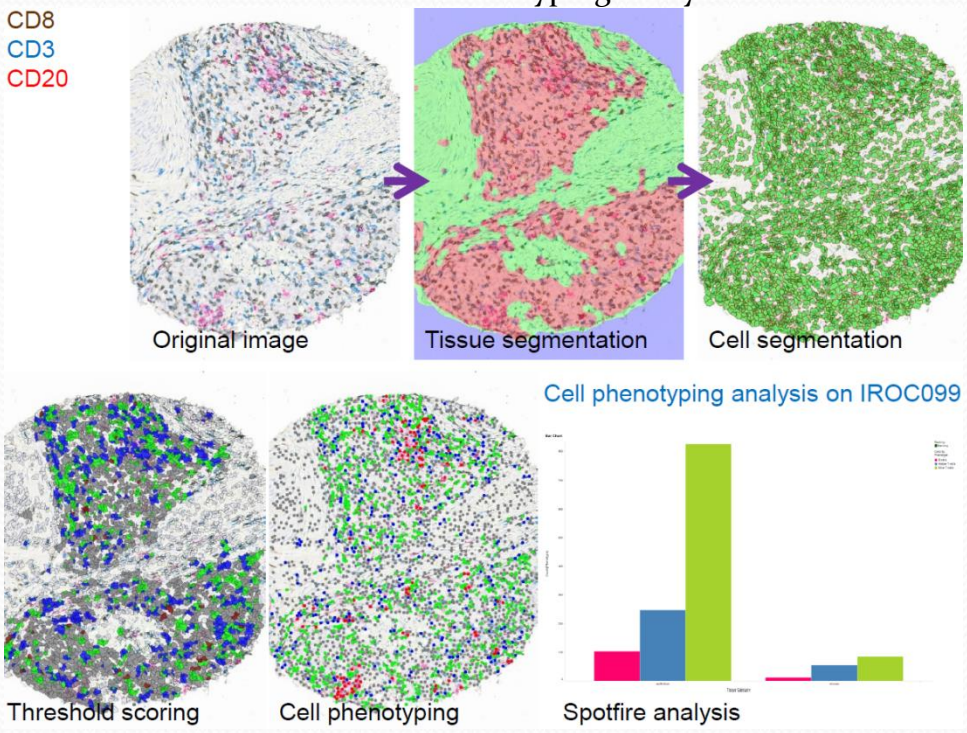


Aperio Versa

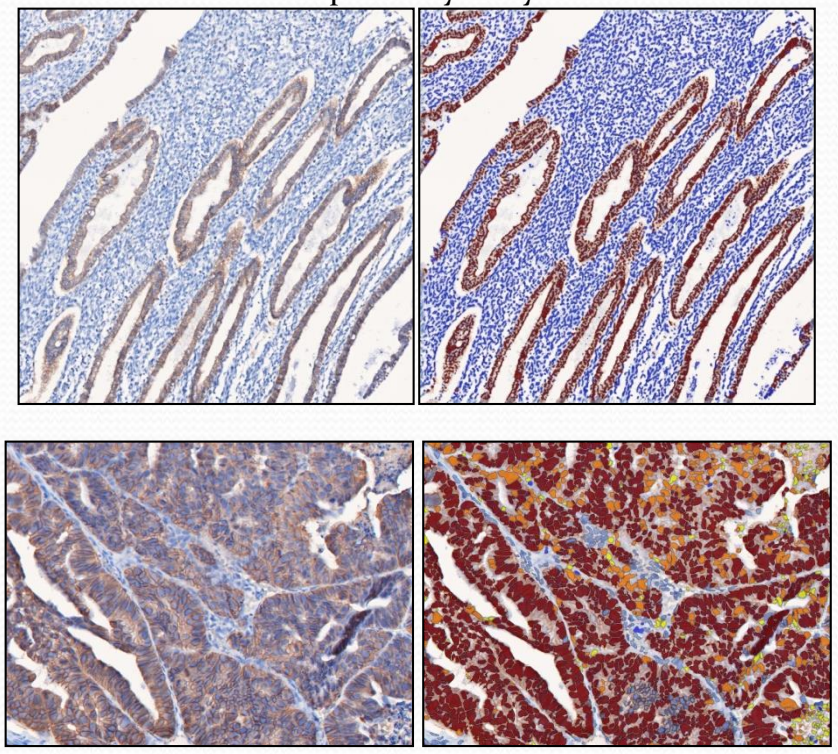
Image Analysis

- Predominantly inForm (Akoya Biosciences)
- Can provide images amenable to analysis by QuPath and other image analysis software

Cell Phenotyping Analysis



Percent positivity analysis



H score analysis

Phenotyping and Spatial Analysis

- For complex phenotyping inForm can be combined with Phenoptr from Akoya to classify cells and perform spatial analysis if desired
- Phenoptr is freely downloadable, typically MCIC will provide the inForm output for the end user to input into Phenoptr although we can help with the classifying part if clear phenotypes are defined
- A set of 10 images is used for training, first the image is broken down into the individual channels, then if desired the image is split into tissue categories (ie epthelial, stroma, other). The cell nuclei are defined and associated with a specific X-Y coordinate. This constitutes the "base algorithm".
- The same base algorithm is used to create single phenotyping algorithms, ie CD3+ vs CD3-. Mutually exclusive markers can be combined in a single phenotyping algotithm, ie CD3+ vs CD68+ vs marker –
- After algorithms are built they are run against the whole set of images, the results of the batch run can be combined in phenoptr and the different phenotypes overlaid to classify the cells; spatial analysis can also be performed based on the X-Y coordinates of each cell

General Workflow

- Discussion of project with Katy to define focus and confirm resources/technical feasibility
- Completion of Statement of Work with Katy (preparation) and Dr David Bond (signatures) - covers REB and budget
- Receipt of materials at MCIC, Katy assigns to whomever may have space in their schedule and will work with them to complete project
- Person working on the project will liaise with collaborator directly with Katy cc'd
- Project completed, materials and slides returned. Data sent via SFTP site or other desired option
- Turnaround is generally rather quick although it depends on volume of projects/staffing/equipment availability and complexity of project – our aim is always to clear things as quickly as possible as we are a high volume lab!
- If the project involves techniques we don't commonly do we're generally pretty adept at figuring out a way to tackle it

Future Technologies

- Recently acquired a PhenoCycler Fusion staining and imaging system
- Up to 40 markers although Akoya has gone up to 100 markers
- Currently working on a 21 colour panel immune panel (CD3, CD8, CD4, FoxP3, CD20, CD79a, CD94, CD16a, CD68, CD163, Ki67, Granzyme B, Tox, TCF-1, HLA-DR, CD31, PD-1, PD-L1, CD45RO, HLA-A and Pan-Cytokeratin) and a 14 colour panel encompassing our most requested targets within that list