THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Spatial multi-omics investigation of high-grade serous ovarian cancer tumor microenvironment provides insight into minimal residual disease and intrinsic chemoresistance

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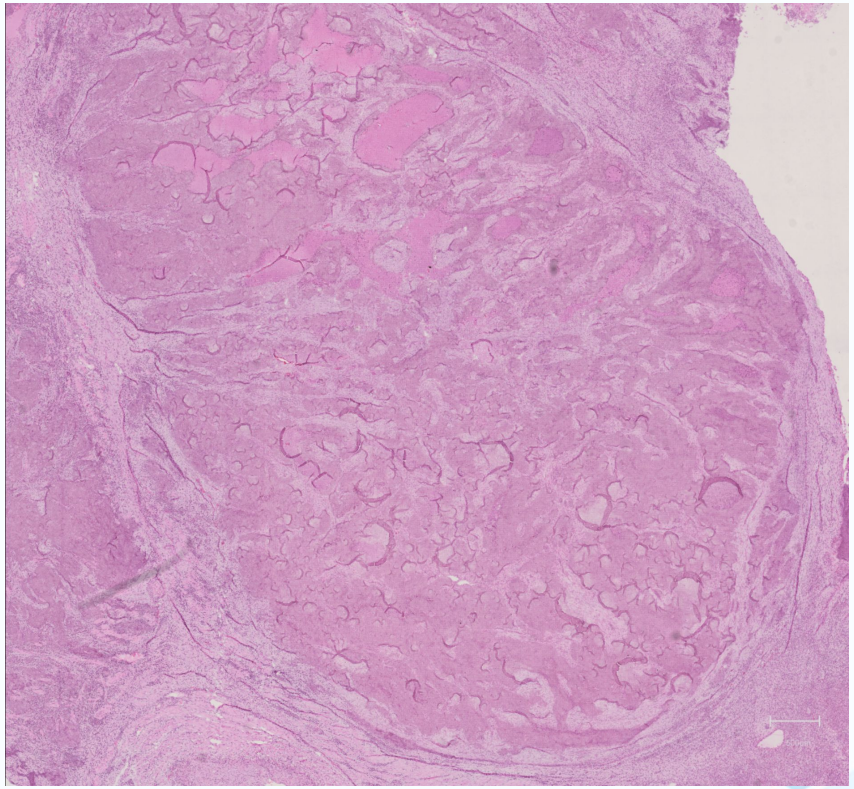
High-grade serous ovarian cancer (HGSC)

Aspect Analytics

- Ovarian cancer is the most lethal gynecologic malignancy
  - ~13,770 deaths in 2021 in the United States (Siegel et al. CA: A Cancer Journal for Clinicians, 2021).
- High-grade serous ovarian cancer (HGSC) is the most common histotype
  - Typically presents as aggressive advanced-stage disease
  - Accounts for over 70% of all ovarian cancer deaths (Coleman et al. Nature Reviews Clinical Oncology, 2013).
  - The current treatment regime of surgery followed by 6+ cycles of chemotherapy, or neoadjuvant chemotherapy followed by interval debulking surgery and adjuvant chemotherapy.
- MD Anderson uses follow up laparoscopy (6-8 weeks) to look for minimum residual disease (MRD)
  - No biomarkers to predict development of MRD or mechanism of chemoresistance



Spatial multi-omics

- Different techniques that enable detailed molecular analyses directly from tissue
  - Spatial transcriptomics
    - Gene expression data
    - e.g. **Visium** (10X), CosMx (NanoString)
  - Multiplexed immunofluorescence (mxIF)
    - Cells and cell typing information
    - e.g. CODEX (Akoya), **COMET** (Lunaphore)
  - Mass Spectrometry Imaging (MSI)
    - Molecular information that isn't detectable using other techniques
    - Metabolites, lipids, glycans…
    - e.g. **timsTOF fleX** (Bruker)

nature TECHNOLOGY FEATURE | 25 January 2022

Seven technologies to watch in 2022

Spatial multi-omics

The explosion in single-cell 'omics development means researchers can now routinely derive genetic, transcriptomic, epigenetic and proteomic insights from individual cells – sometimes simultaneously (see go.nature.com/3nnhooo). But single-cell techniques also sacrifice crucial information by ripping these cells out of their native environments.

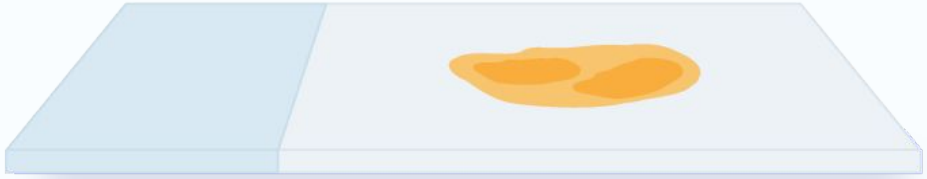
- Spatial multi-omics strategy to examine cellular and molecular heterogeneity of tumour immune microenvironment (Ferri-Borgogno S., Burks J., et al., Cancers 2024 - doi.org/10.3390/cancers16050846)

Materials + Methods - Tissue stack generation

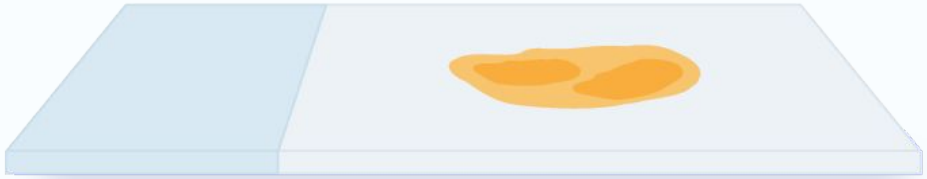
Cohort (8 samples)

- Eight samples
  - Four MRD+
  - Four MRD-
- FFPE
  - 10µm serial sections
  - All sections for analysis collected at same time (“stack”)

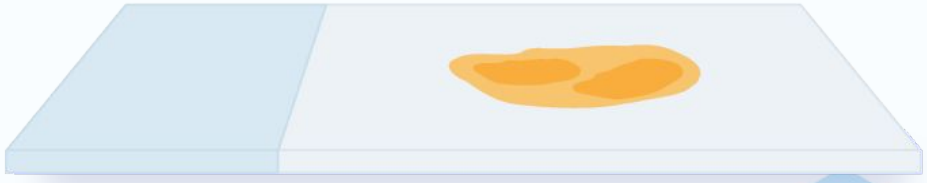
Measurement Stack (6 modalities)



10x Visium



Lunaphore COMET



Bruker timsTOFfleX

Visium (~55 µm) - MD Anderson

- ➔ + H&E
- ➔ Spatial Transcriptomics

Multiplex Immunofluorescence (<1 µm) - MD Anderson

- ➔ 20 markers
- ➔ + DAPI

MSI (20 µm) - performed by Erin Seeley (UTA)

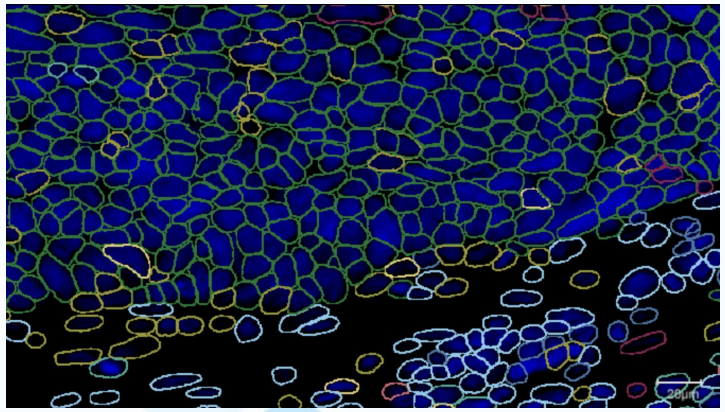
- ➔ metabolites
- ➔ glycans
- ➔ peptides
- ➔ post -measurement H&E

Why combine modalities?

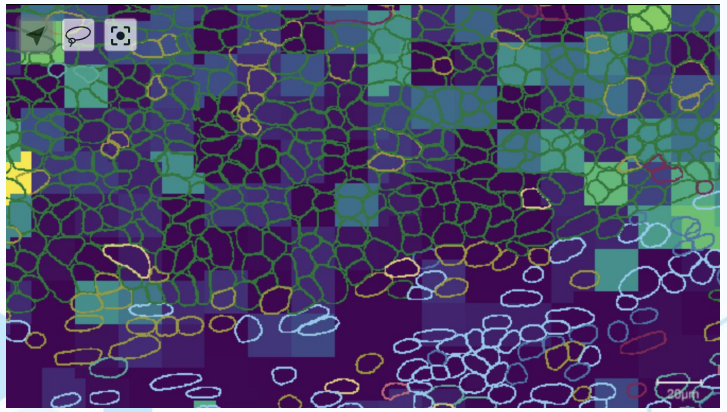
Immunofluorescence (IF) provides high resolution images where we can **identify** and spatially segment **individual cells**

The IF markers allow us to find the **“type”** of the cell using **previous knowledge**

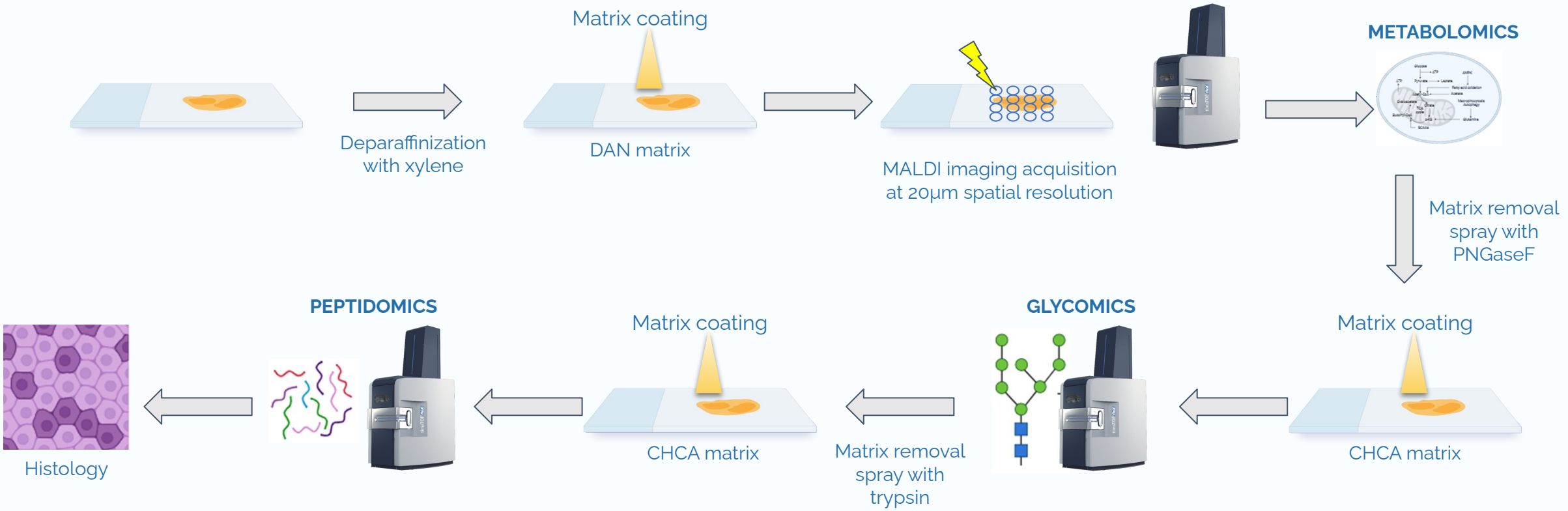
MSI provides **novel molecular information** that can only be obtained using the technique



Describe novel spatial environments by molecular signals

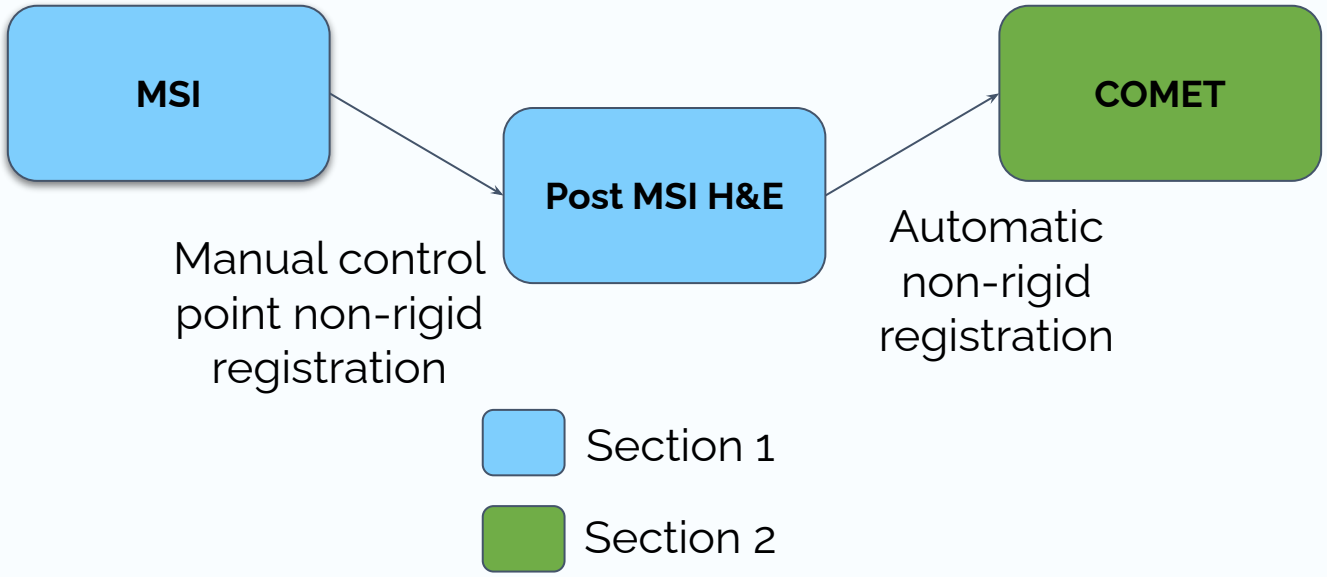


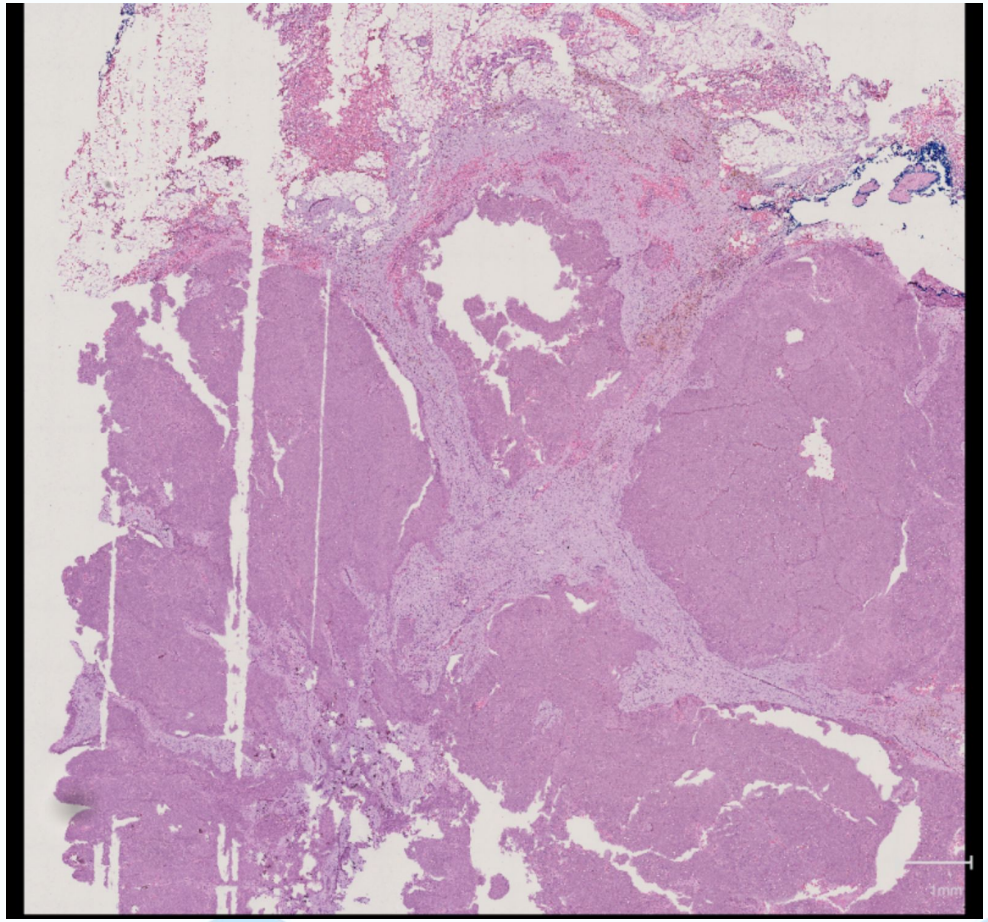
Multi-modal MSI workflow



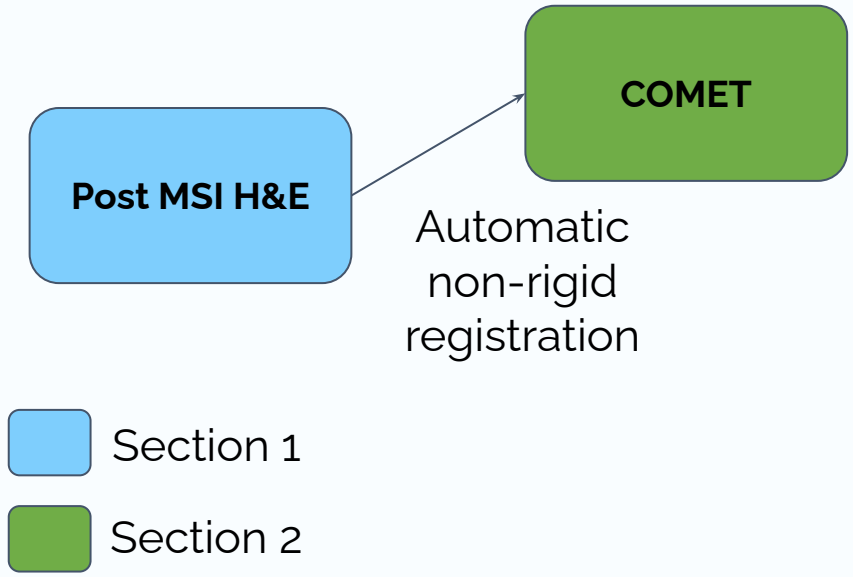
(Ferri-Borgogno S., Burks J., et al., Cancers 2024 - doi.org/10.3390/cancers16050846)

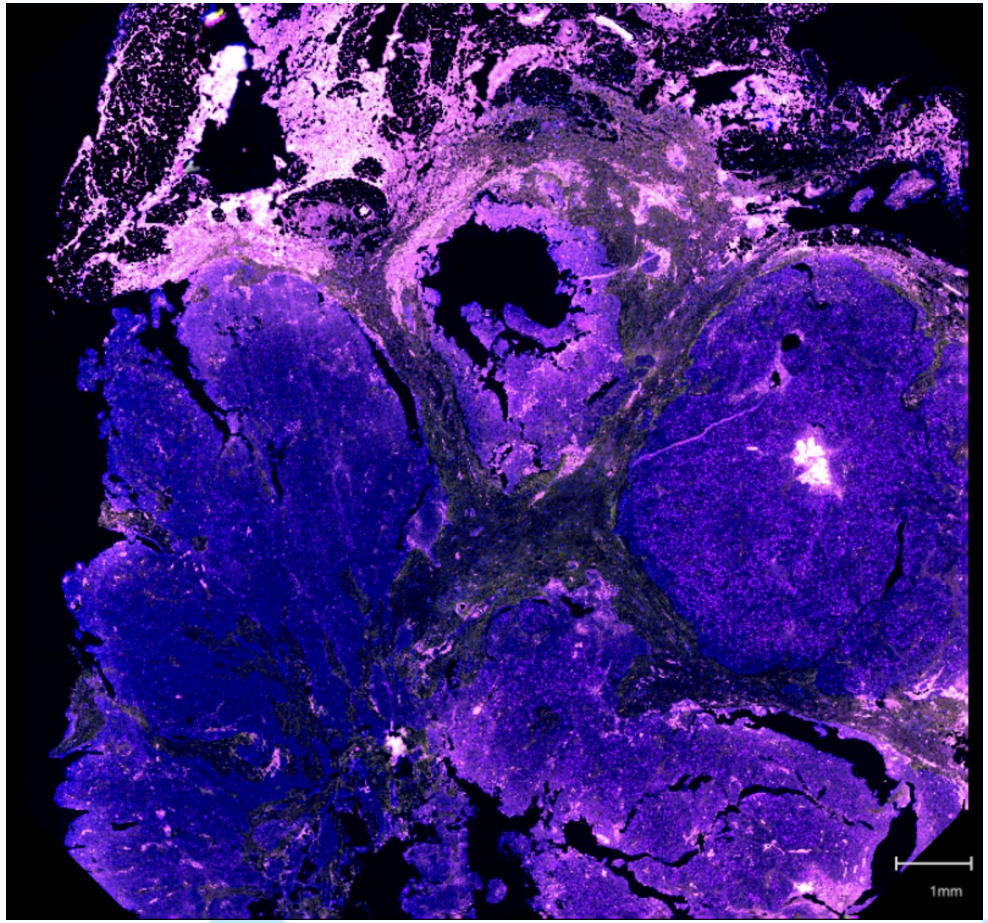
Tissue measurement stack fusion



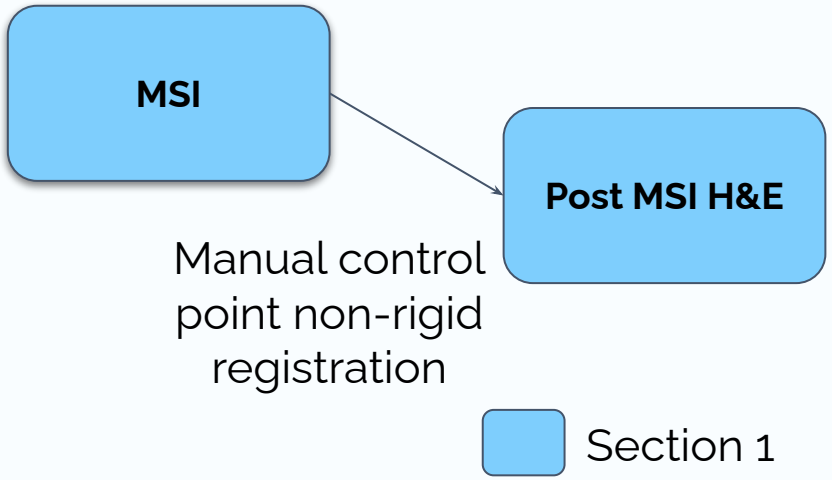


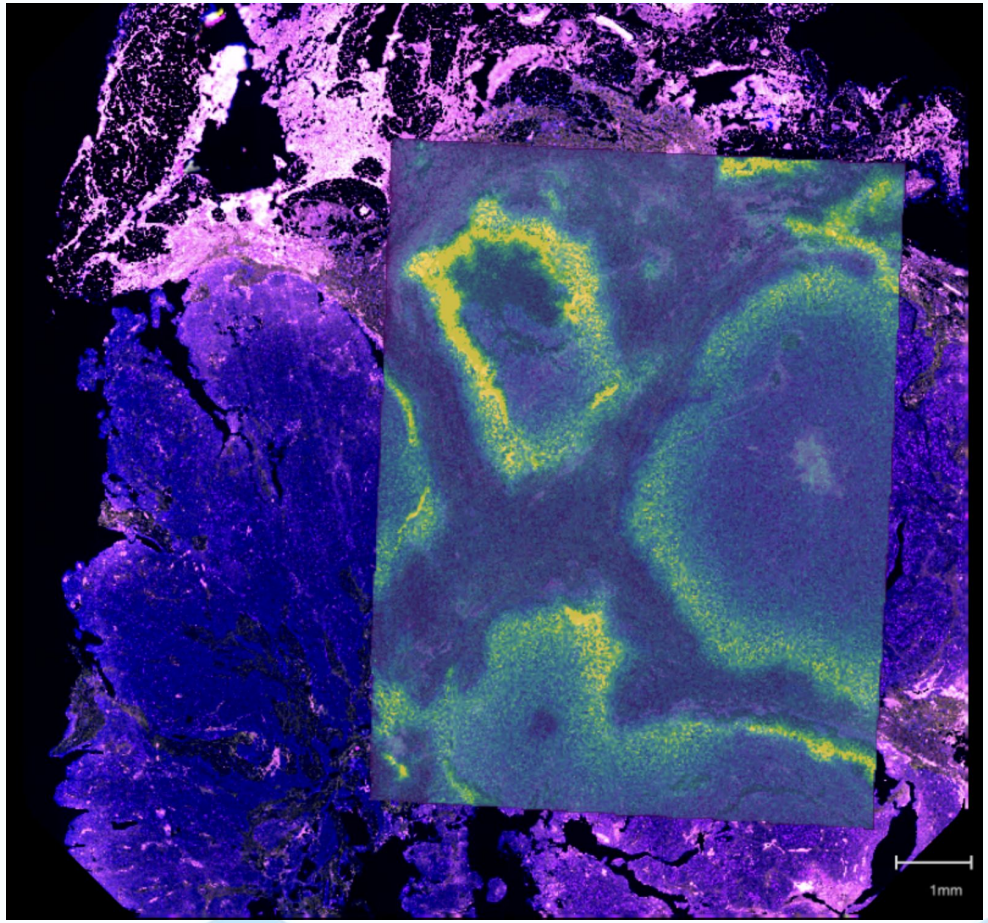
Tissue measurement stack fusion





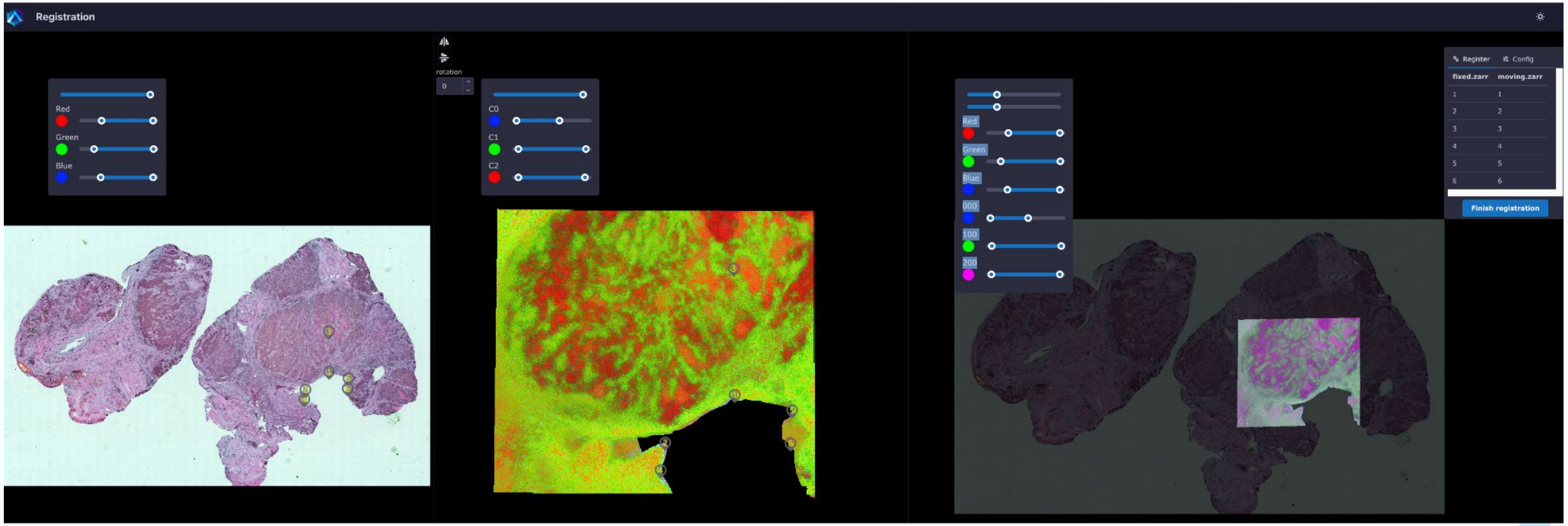
Tissue measurement stack fusion





Weave registration tool for manual, non-rigid registration, MSI → post MSI H&E

Aspect Analytics



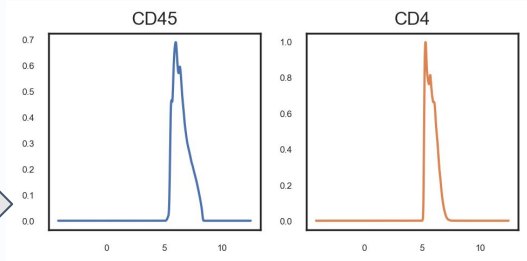
Multiplex immunofluorescence cell typing
22 markers → 29 cell types

Cell phenotyping using Hierarchical Prior Knowledge

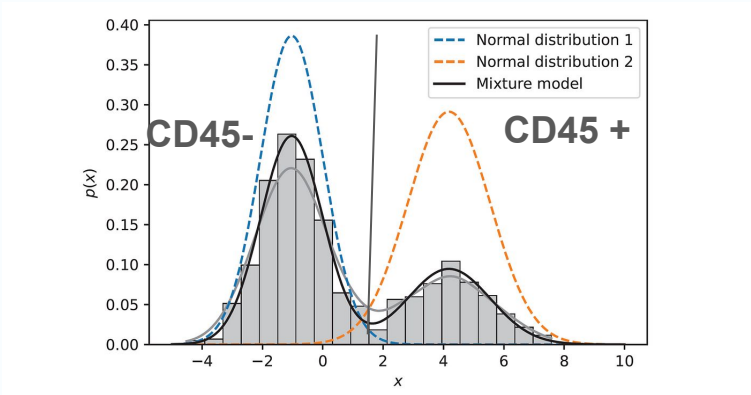
Single cell mean fluorescence intensity

	IF_1	IF_2	…	IF_20
cell_1				
cell_2				
cell_3				

Cell gating (manual/automatic)



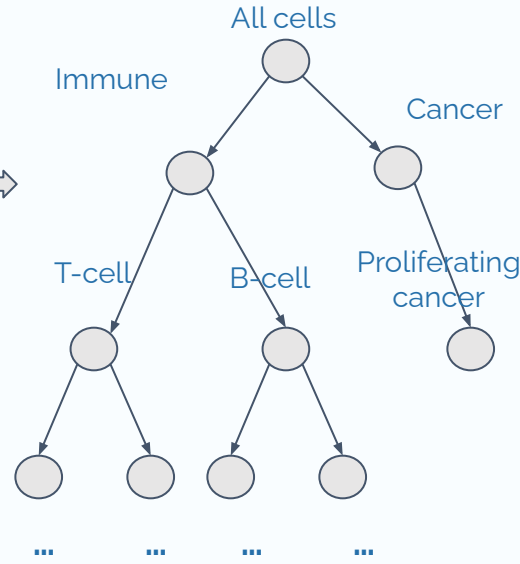
Automatic: Find threshold by fitting **Gaussian Mixture** Model on distribution of IF intensity



Probability that a cell is positive to a marker

	IF_1	IF_2	…	IF_20
cell_1	0.85	0.78		0.20
cell_2	0.55	0.23		0.12
cell_3	0.12	0.23		0.98

Follow phenotype workflow



Multiplex immunofluorescence cell typing
22 markers → 29 cell types

Aspect Analytics

		CD45	Col1A1	aSMA	PanCK	CD31	Ki67	FAP	CD3e	CD8	CD4	CD56	CD11b	CD20	FOXP3	TIGIT	GZMB	CD68	CD66b	CD163	CD86
all	Other Immune cells	pos																			
all	Stroma		pos																		
all	CAFs			pos																	
all	Tumor				pos																
all	Endothelial cells					pos															
Tumor	Proliferating tumor cells						pos														
Stroma	Col1A1+ CAFs			pos																	
CAFs	FAP+ CAFs							pos													
Col1A1+ CAFs	Col1A1+ FAP+ CAFs							pos													
Stroma	FAP+ Stroma							pos													
Other Immune cells	CD3 T cells								pos												
Other Immune cells	CD8 T cells									pos											
Other Immune cells	CD4 T cells										pos										
Other Immune cells	NK cells											pos									
Other Immune cells	Myeloid Lineage												pos								
Other Immune cells	B cells								neg					pos							
CD3 T cells	CD3 CD8 T cells									pos											
CD3 T cells	CD3 CD4 T cells										pos										
CD3 T cells	NKT Cells											pos									
CD4 T cells	Regulatory T cells														pos						
CD8 T cells	TIGIT+ CD8 T cells															pos					
CD8 T cells	TIGIT- Activated CD8 T cells															neg	pos				
NK Cells	TIGIT+ NK Cells															pos					
NK Cells	TIGIT- Activated NK Cells															neg	pos				
Myeloid lineage	Macrophages																	pos			
Myeloid lineage	Neutrophils																		pos		
Myeloid lineage	Myeloid drived suppressor cells (MDSC)																		neg		
CD3 CD8 T cells	TIGIT+ CD3 CD8 T cells															pos					
CD3 CD8 T cells	TIGIT- Activated CD3 CD8 T cells															neg	pos				
NKT Cells	TIGIT+ NKT Cells															pos					
NKT Cells	TIGIT- Activated NKT Cells															neg	pos				
Macrophages	M2 Macrophages																			pos	
Macrophages	M1 Macrophages																				pos
TIGIT+ CD3 CD8 T cells	TIGIT+ Activated CD3 CD8 T cells																pos				
TIGIT+ NK Cells	TIGIT+ Activated NK Cells																pos				
TIGIT+ NKT Cells	TIGIT+ Activated NKT Cells																pos				

A wealth of biological knowledge goes into designing the panel of IF markers

Multiplex immunofluorescence cell typing
22 markers → 29 cell types

Aspect Analytics

		CD45	Col1A1	aSMA	PanCK	CD31	Ki67	FAP	CD3e	CD8	CD4	CD56	CD11b	CD20	FOXP3	TIGIT	GZMB	CD68	CD66b	CD163	CD8
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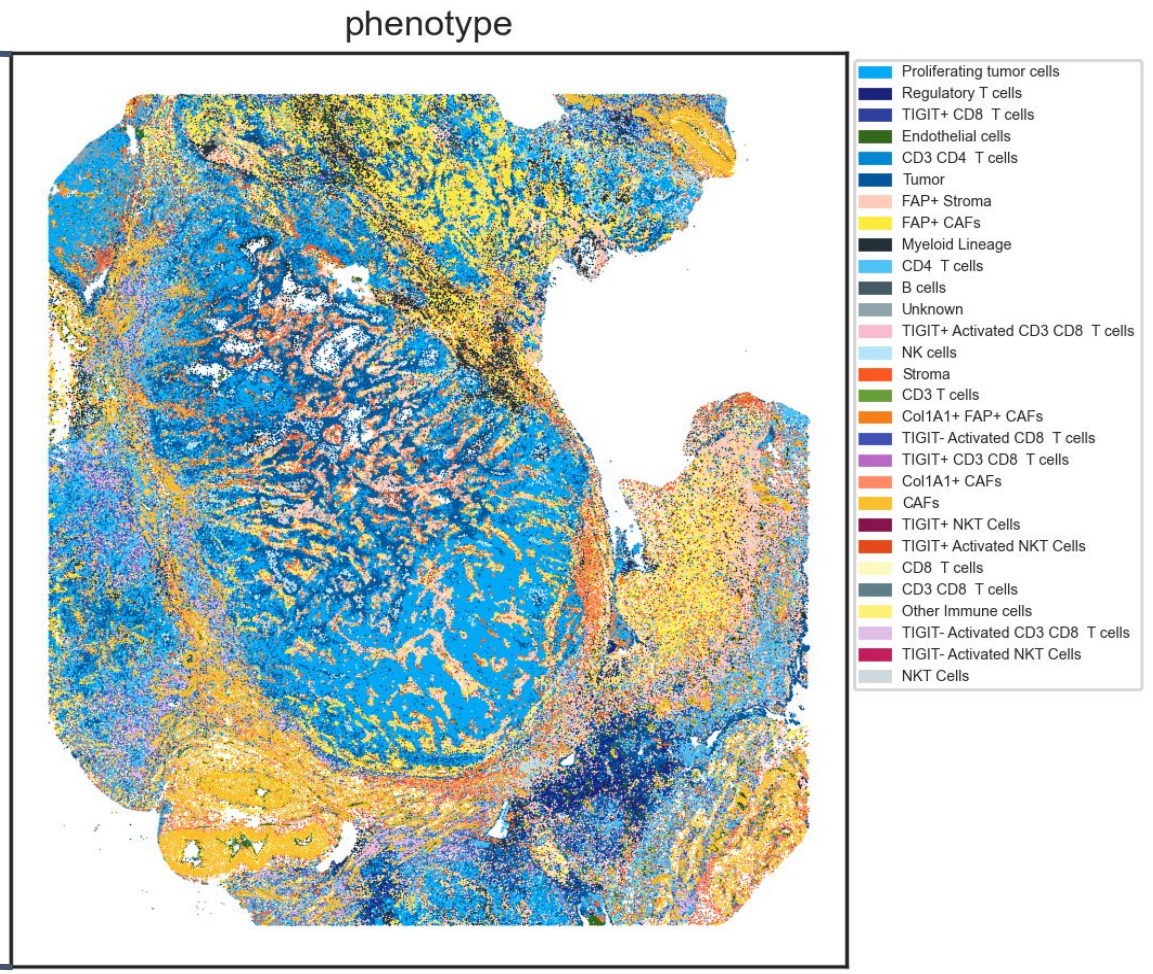
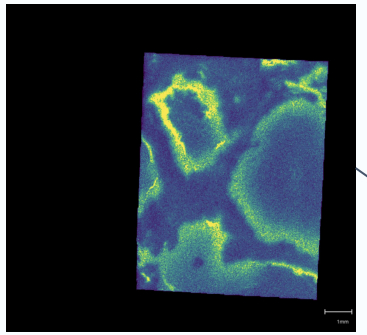
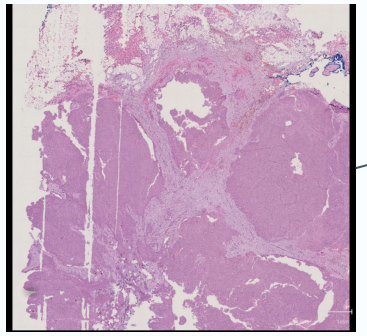
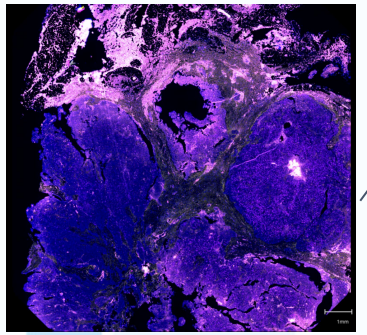
Col1A1+ CAFs	Col1A1+ FAP+ CAFs							pos													
Stroma	FAP+ Stroma							pos													
Other Immune cells	CD3 T cells								pos												
Other Immune cells	CD8 T cells									pos											
Other Immune cells	CD4 T cells										pos										
Other Immune cells	NK cells											pos									
Other Immune cells	Myeloid Lineage												pos								
Other Immune cells	B cells								neg					pos							
CD3 T cells	CD3 CD8 T cells									pos											
CD3 T cells	CD3 CD4 T cells										pos										
CD3 T cells	NKT Cells											pos									
CD4 T cells	Regulatory T cells														pos						
CD8 T cells	TIGIT+ CD8 T cells															pos					
CD8 T cells	TIGIT- Activated CD8 T cells															neg	pos				
NK Cells	TIGIT+ NK Cells															pos					
NK Cells	TIGIT- Activated NK Cells															neg	pos				
Myeloid lineage	Macrophages																	pos			
Myeloid lineage	Neutrophils																		pos		
Myeloid lineage	Myeloid drived suppressor cells (MDSC)																		neg		
CD3 CD8 T cells	TIGIT+ CD3 CD8 T cells															pos					
CD3 CD8 T cells	TIGIT- Activated CD3 CD8 T cells															neg	pos				
NKT Cells	TIGIT+ NKT Cells															pos					
NKT Cells	TIGIT- Activated NKT Cells															neg	pos				
Macrophages	M2 Macrophages																			pos	
Macrophages	M1 Macrophages																				pos
TIGIT+ CD3 CD8 T cells	TIGIT+ Activated CD3 CD8 T cells																pos				
TIGIT+ NK Cells	TIGIT+ Activated NK Cells																pos				
TIGIT+ NKT Cells	TIGIT+ Activated NKT Cells																pos				



Consolidating and coordinating multimodal data

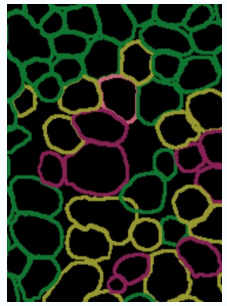
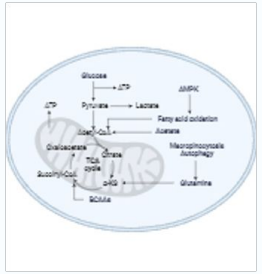
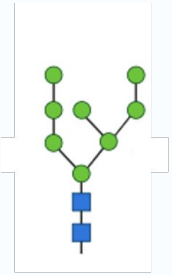






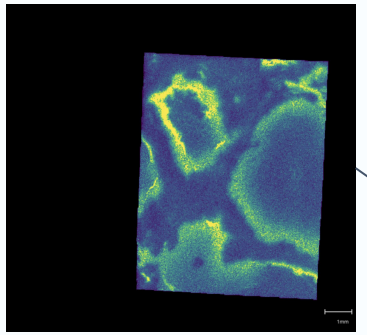
MSI pixels 1-n…

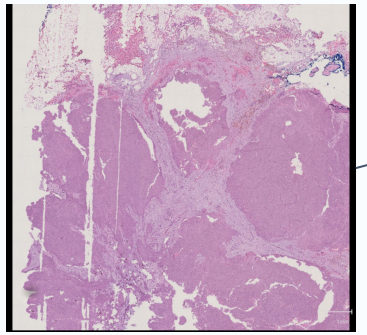
Aggregation to the MSI pixel level…

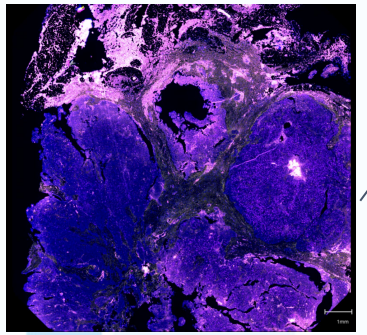
cell type proportion	antibody markers	MSI peptides	MSI metabolites	MSI glycans
				

A consolidated data structure enables making cross modality queries

Consolidating and coordinating multimodal data







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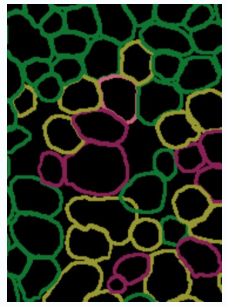
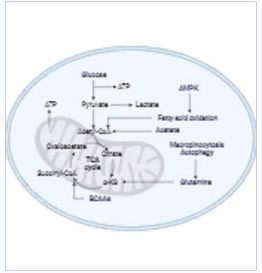
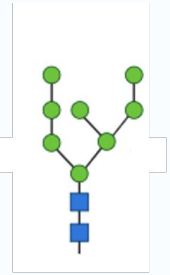
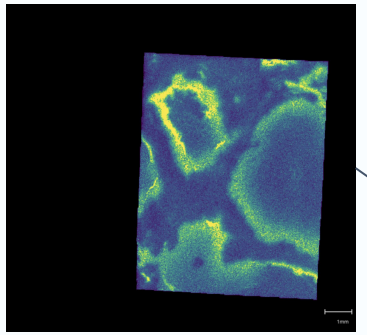
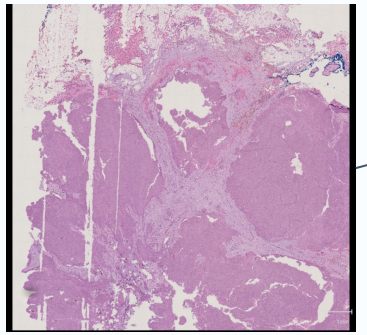
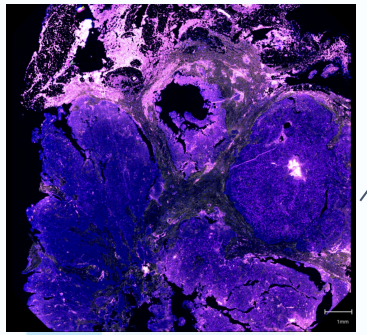
				

Filtering for MSI pixels with purer cell populations

Consolidating and coordinating multimodal data







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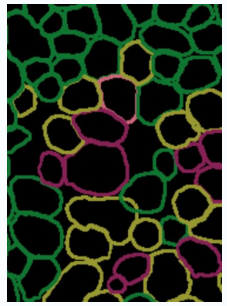
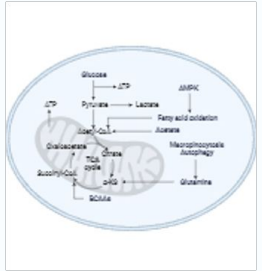
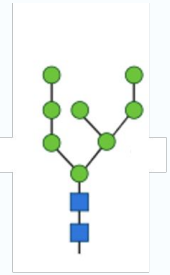
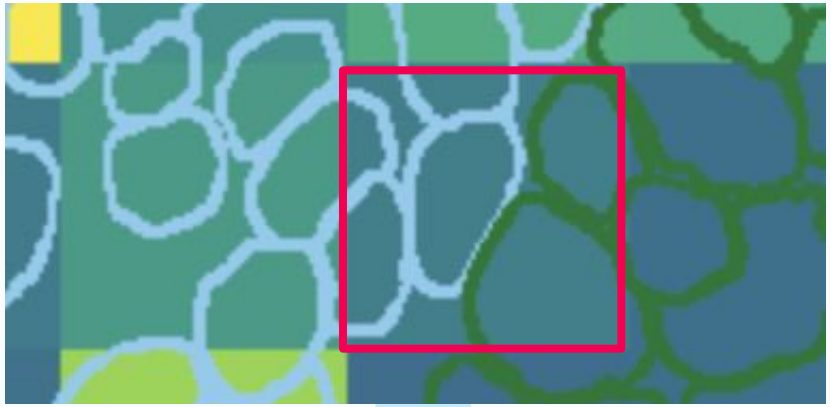
				

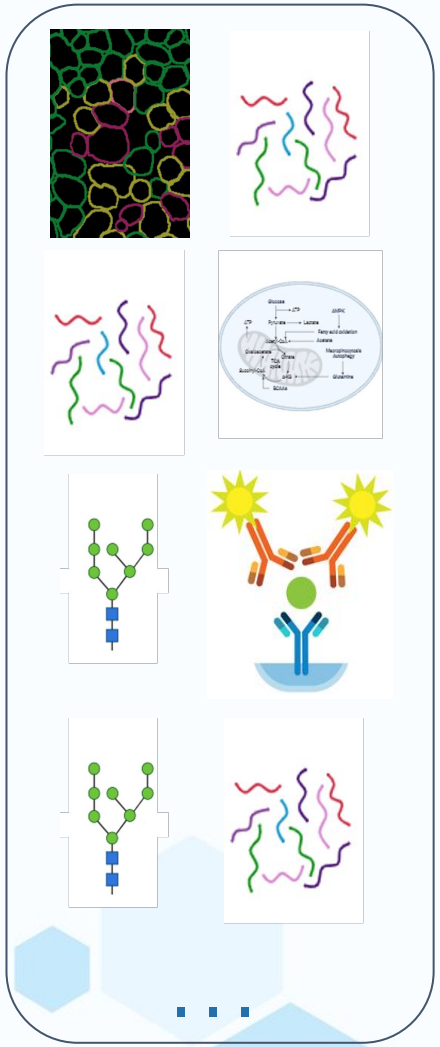


Filtering for MSI pixels with purer cell populations

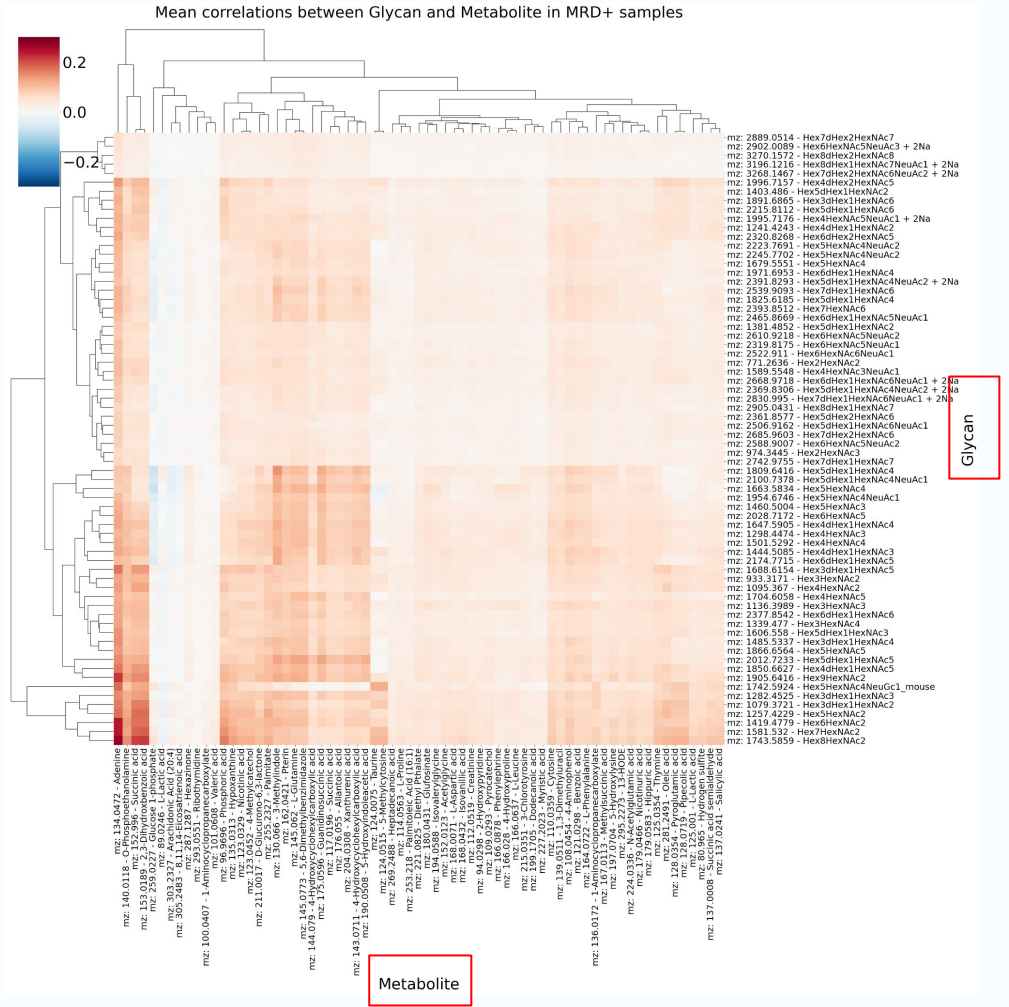
Correlating molecular readouts

Aspect Analytics

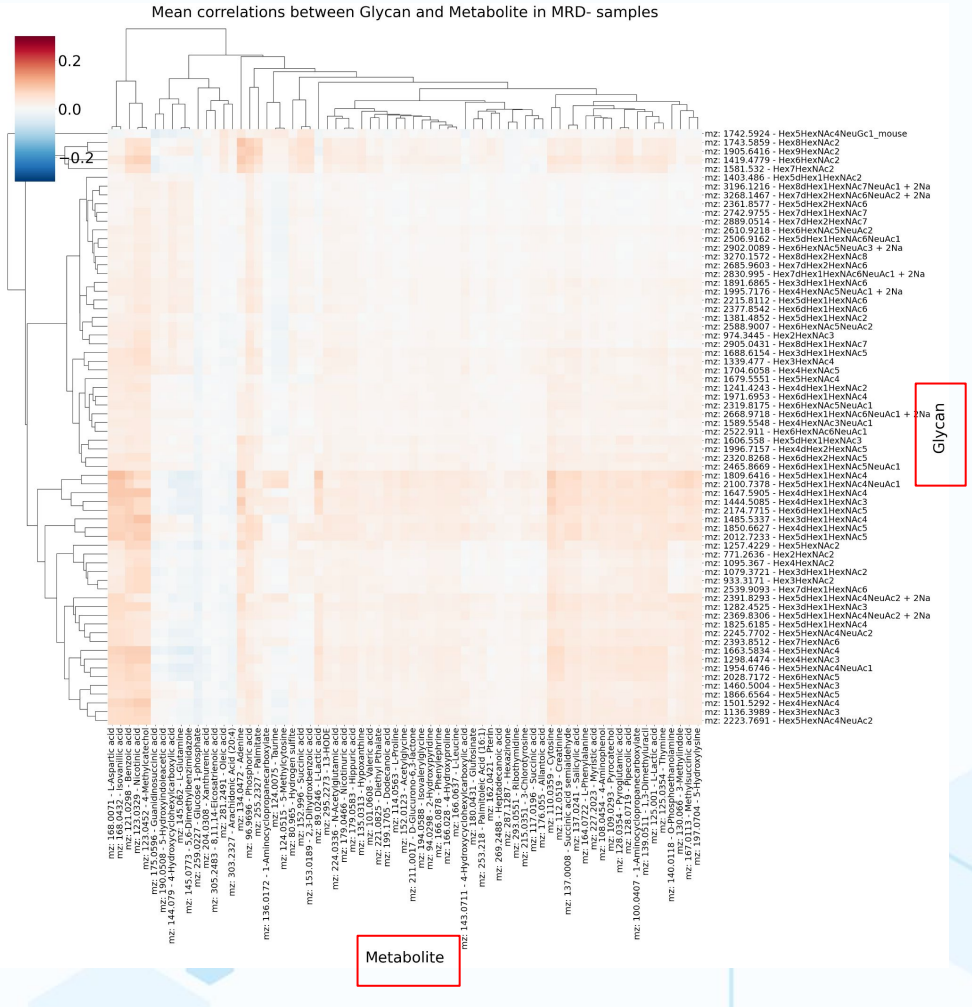
Data Pairs



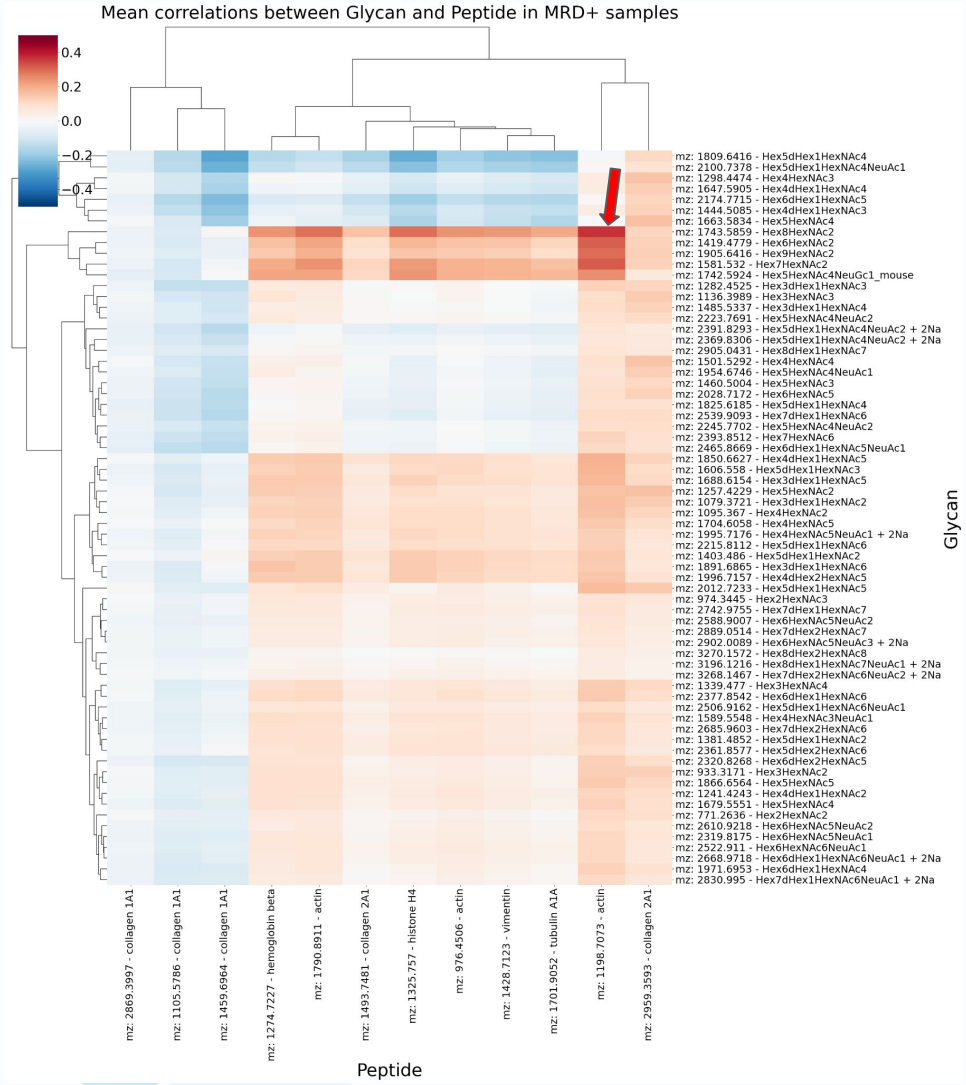
MRD+

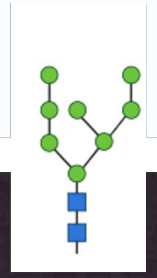


MRD-

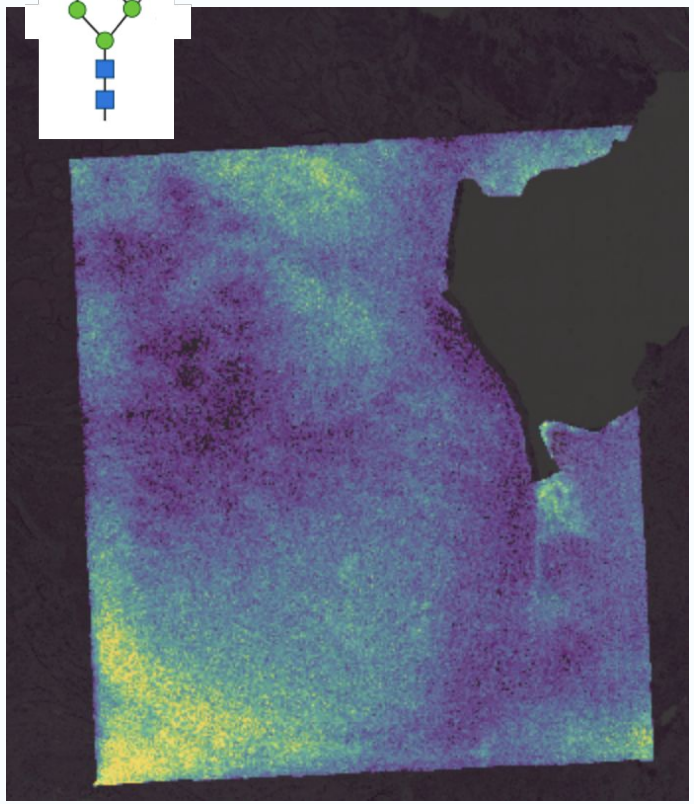


Correlating molecular readouts



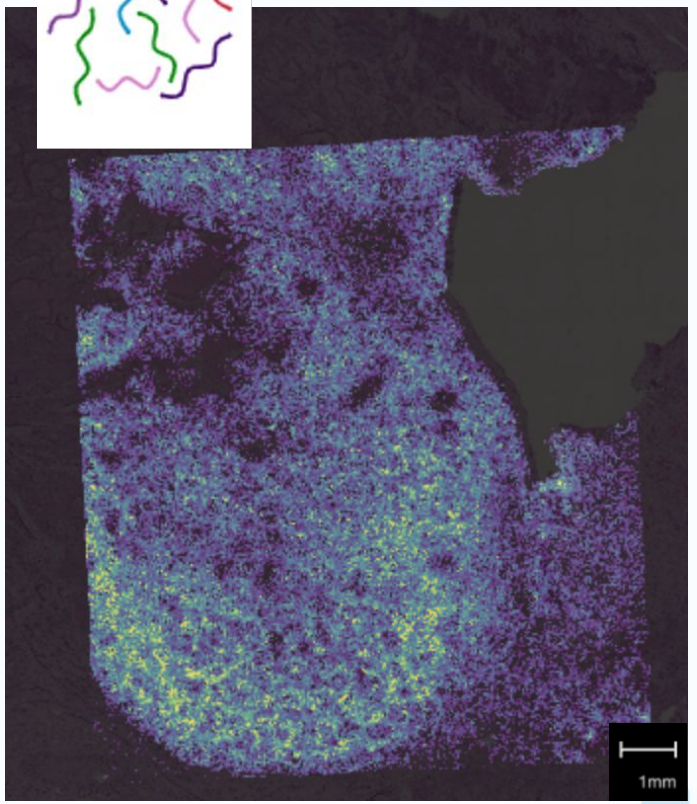


m/z 1809.6416 - Hex5dHex1HexNAc4



5% a.u. 70%

mz: 1198.7073 - Actin

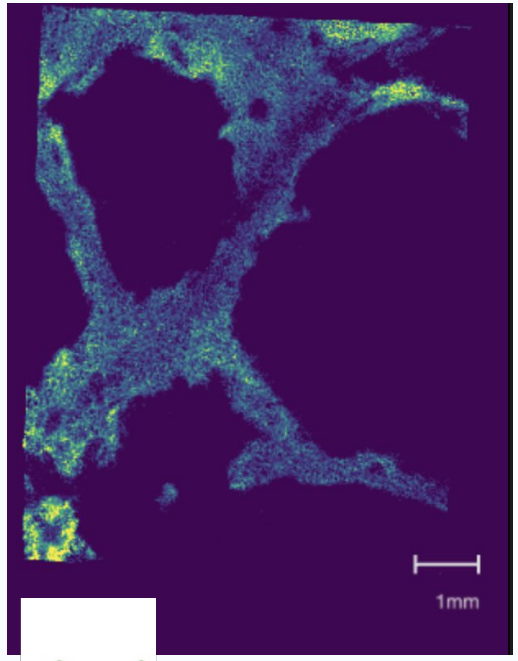


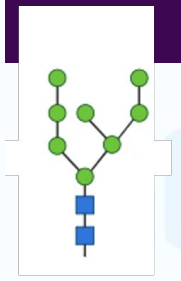
10% a.u. 70%

Differential correlations

MRD+

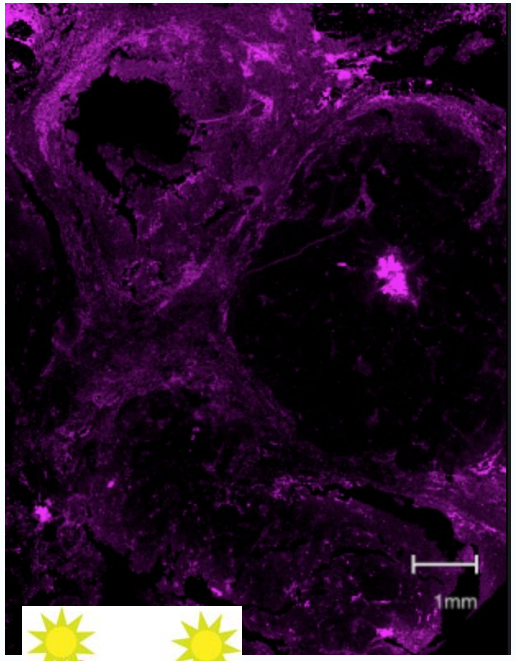
m/z 1809.6416 -
Hex5dHex1HexNAc4



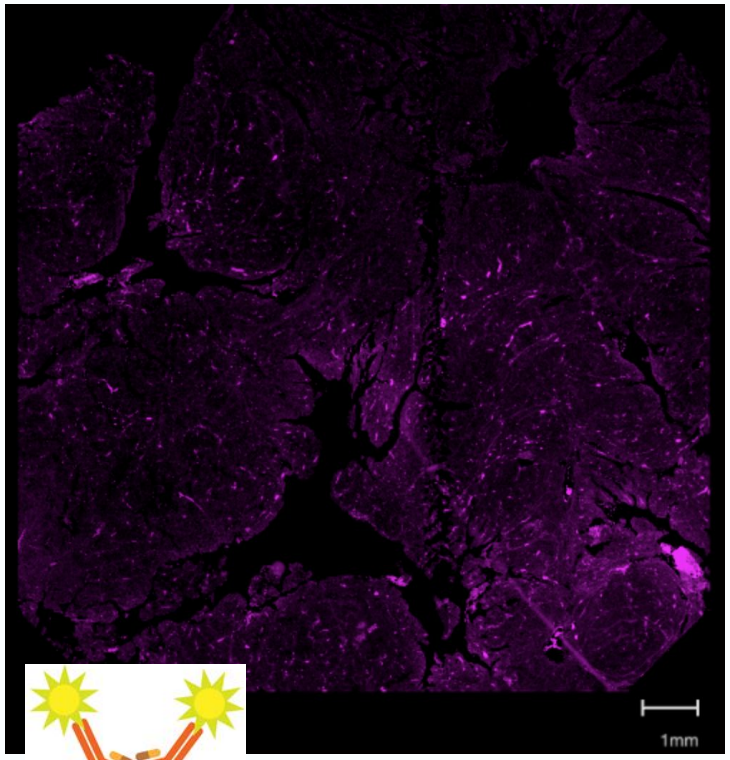


r ~ 0.3

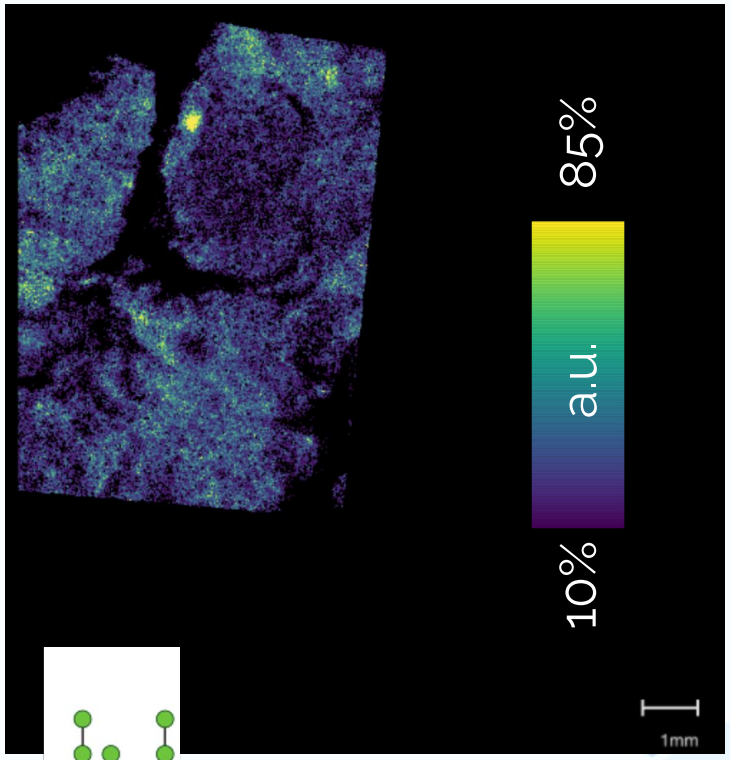
IF Antibody
Fibroblast Activation Protein

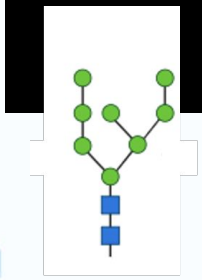


MRD-



r ~ 0.1

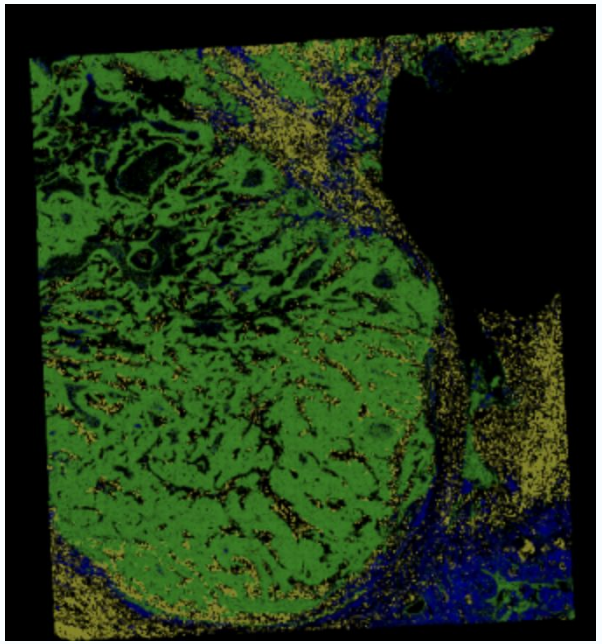




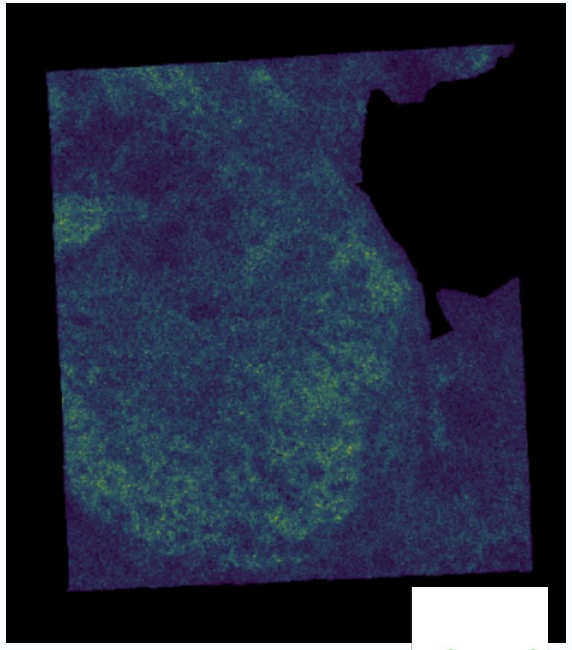
Differential MSI signals by cell type aggregation (tumor vs CAFs)

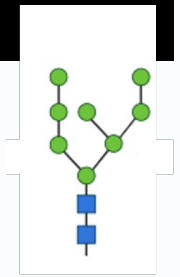
Aspect Analytics

Tumor



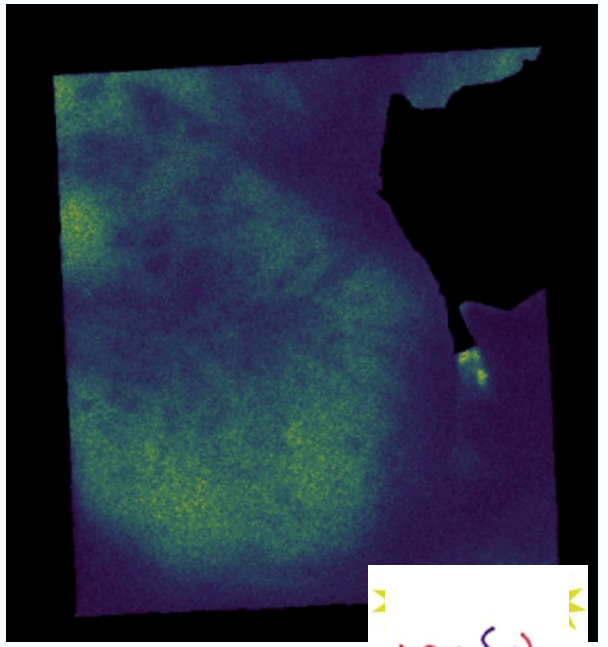
m/z 1742.5924 - Hex5HexNAc4NeuGc1_mouse

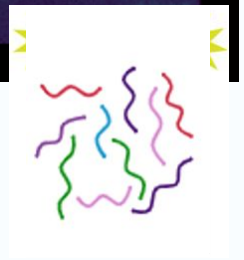




logfc ~0.53

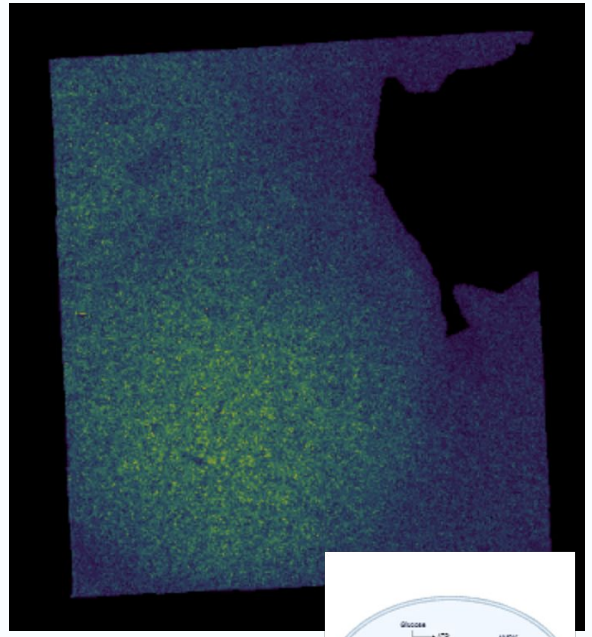
m/z 1325.757 - histone H4





logfc ~0.92

m/z 128.0354 - Pyroglutamic acid



logfc ~0.69

Tumor

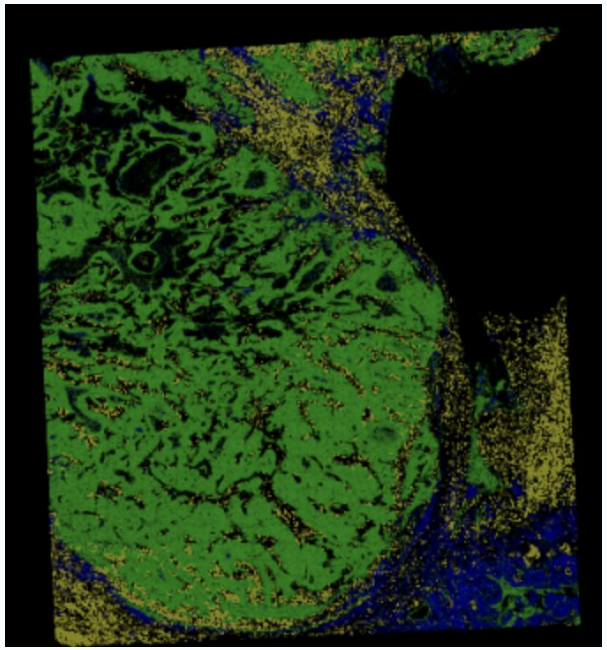
Cancer Associated Fibroblasts

Immune cells

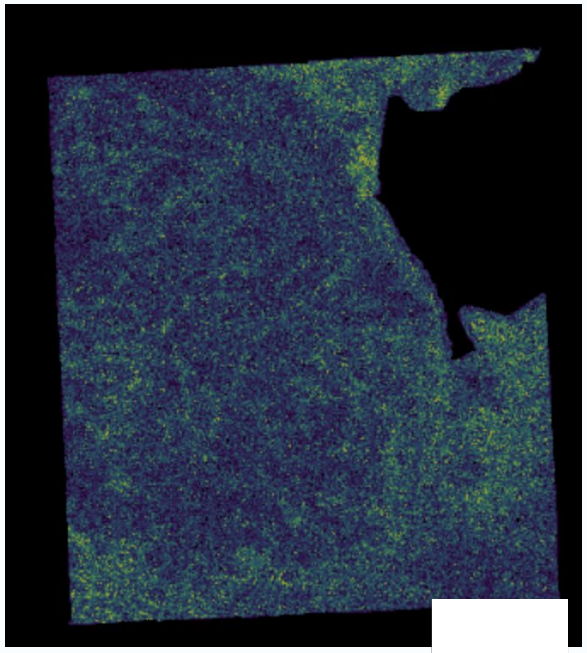
Differential MSI signals by cell type aggregation (CAFs vs Tumor)

Aspect Analytics

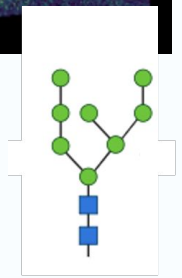
Cancer Associated Fibroblasts



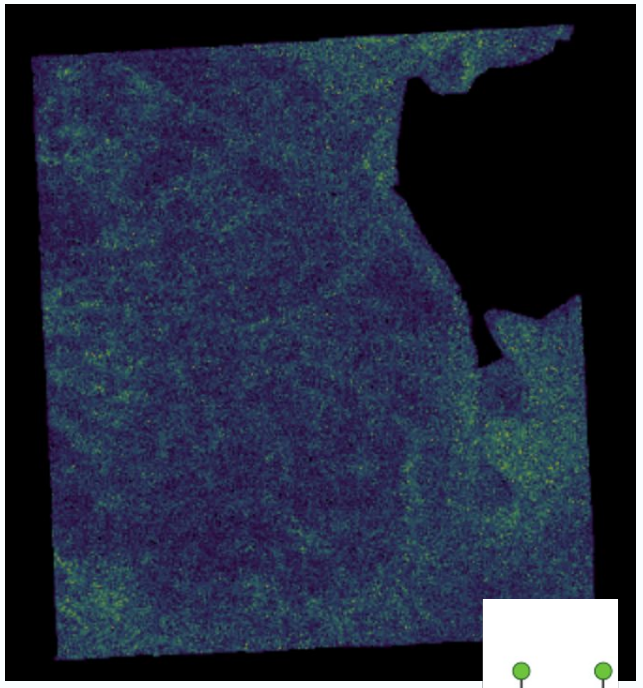
m/z 2369.8306 - Hex5dHex1HexNAc4NeuAc2 + 2Na



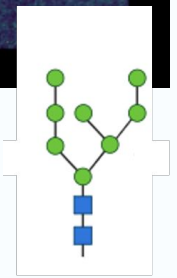
logfc ~1.00



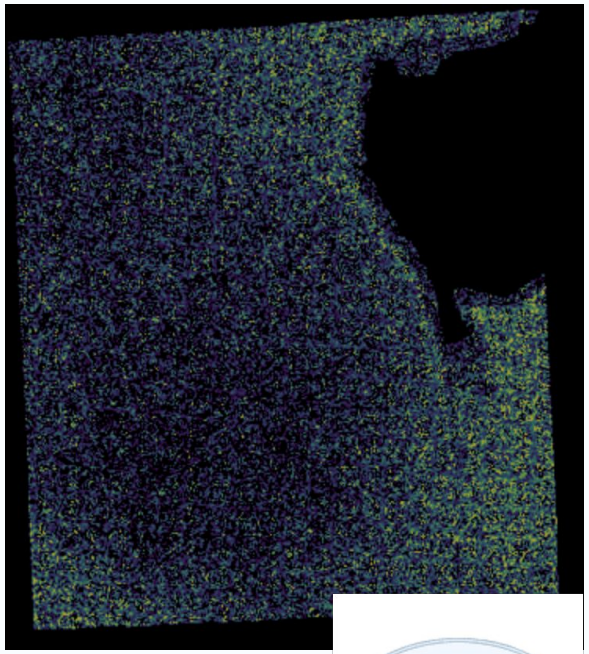
m/z 1825.6185 - Hex5dHex1HexNAc4



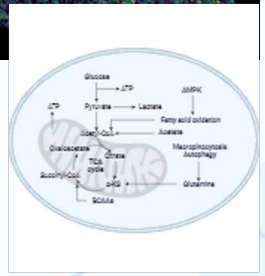
logfc ~0.86



m/z 89.0246 - L-Lactic acid



logfc ~0.66



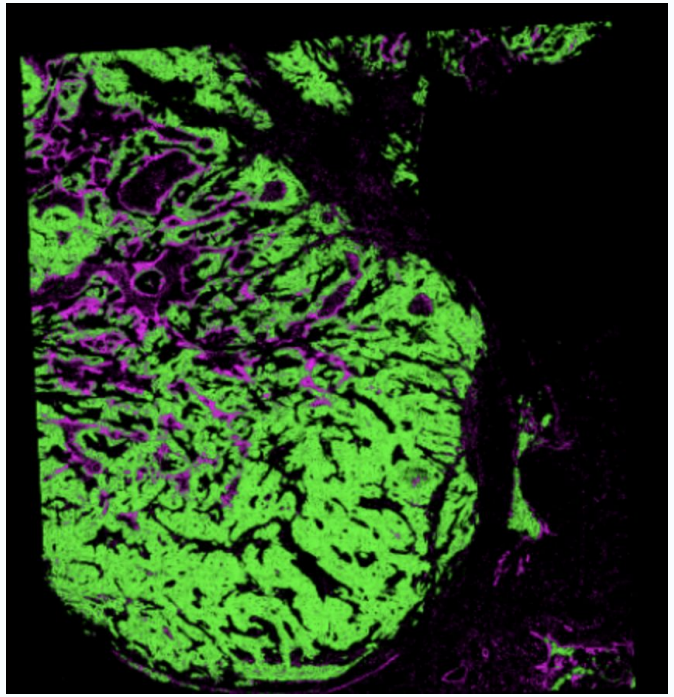
Tumor

Cancer Associated Fibroblasts

Immune cells

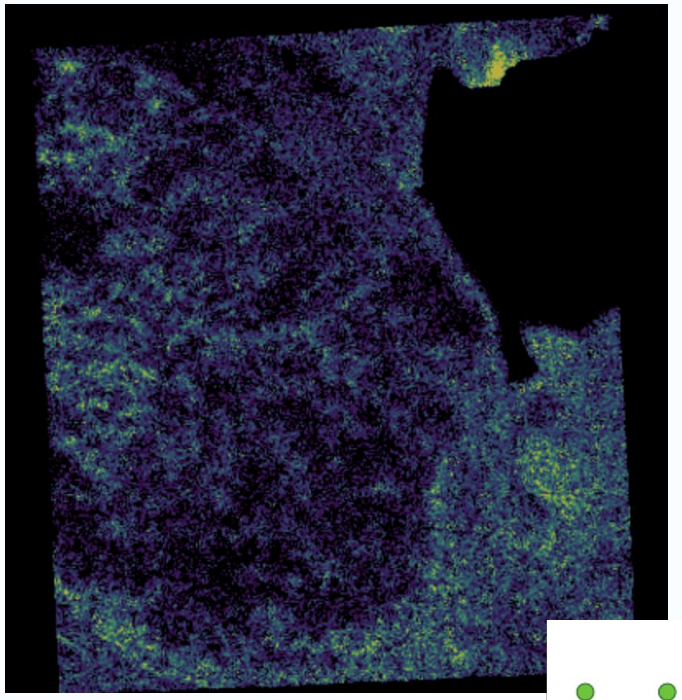
Differential MSI signals by cell type aggregation (proliferating tumor vs tumor)

Aspect Analytics

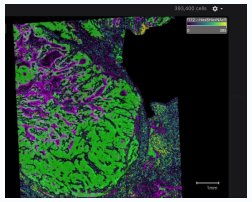


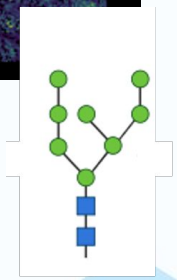
- Proliferating tumor (PanCK+/Ki67+)
- Tumor (PanCK+)

m/z 2028.7172 - Hex6HexNAc5

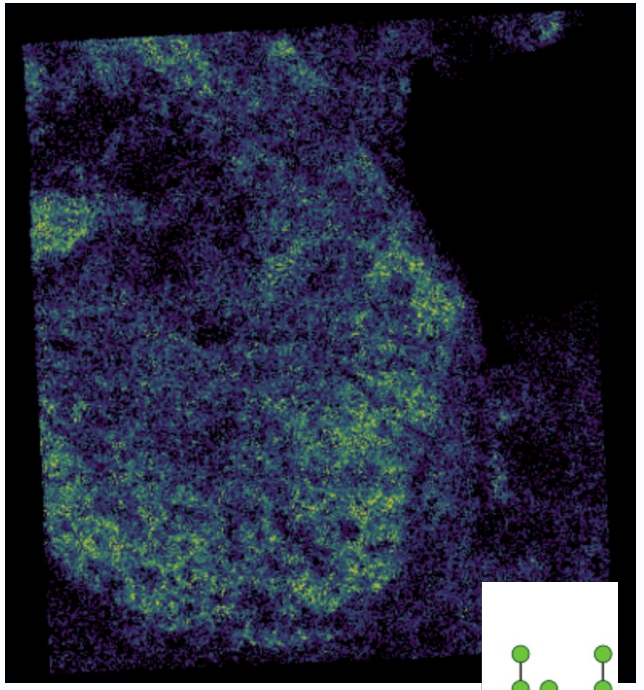


logfc ~0.53

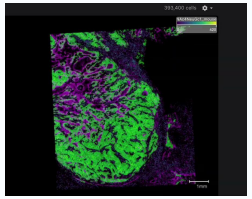


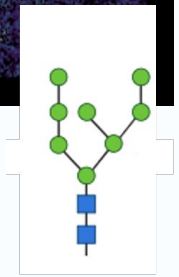


m/z 1742.5924 - Hex5HexNAc4NeuGc1

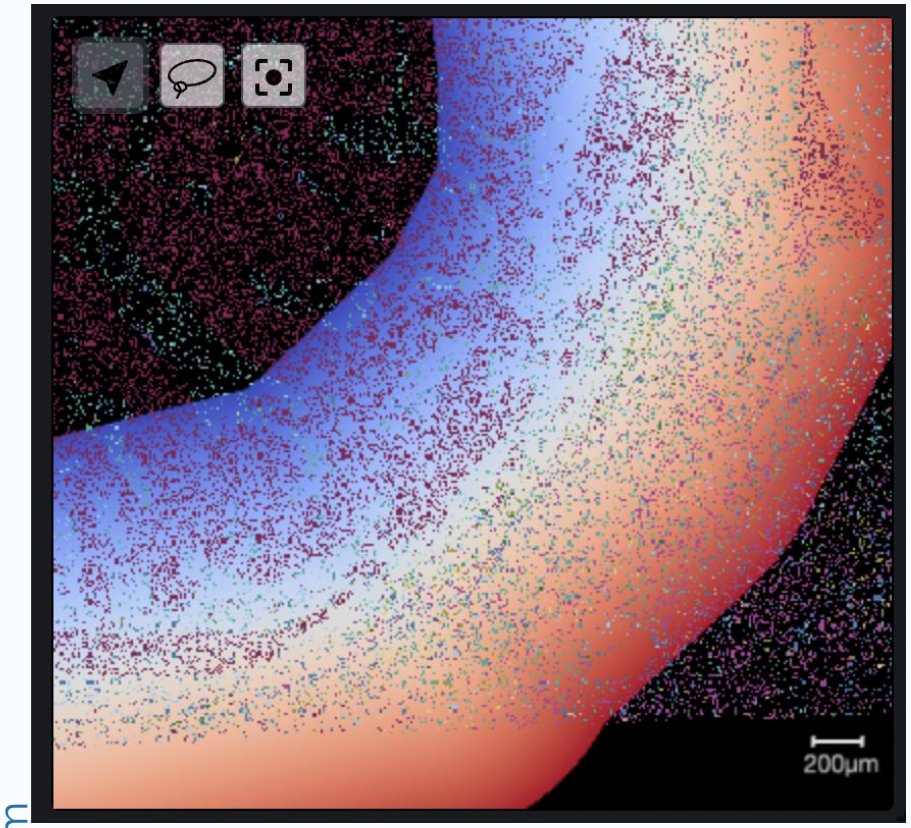


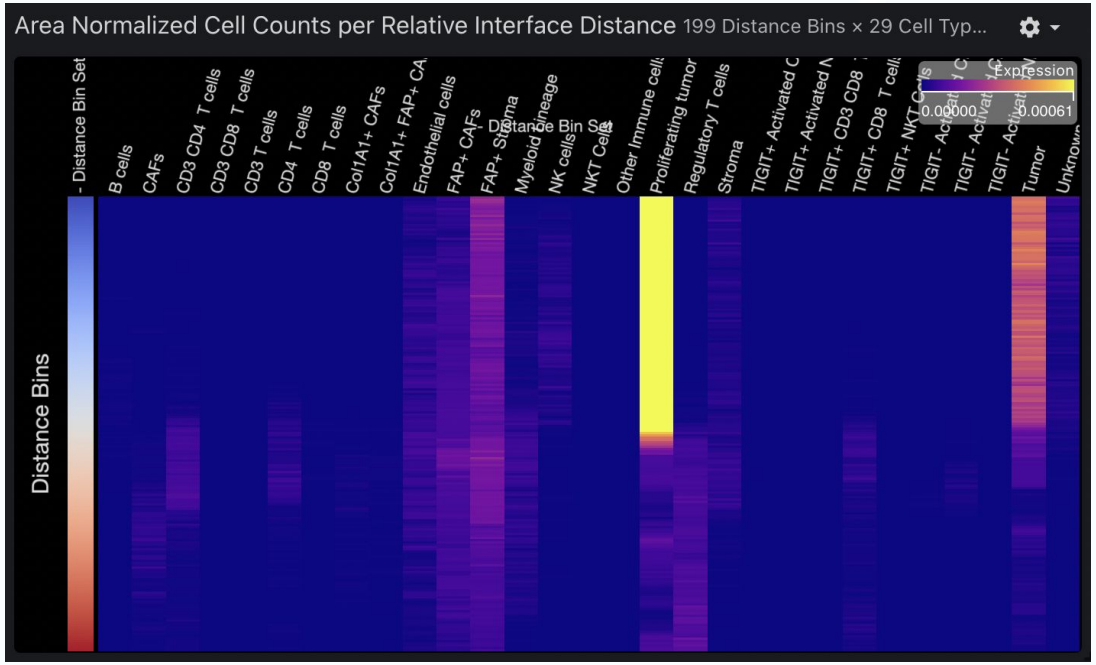
logfc ~0.67



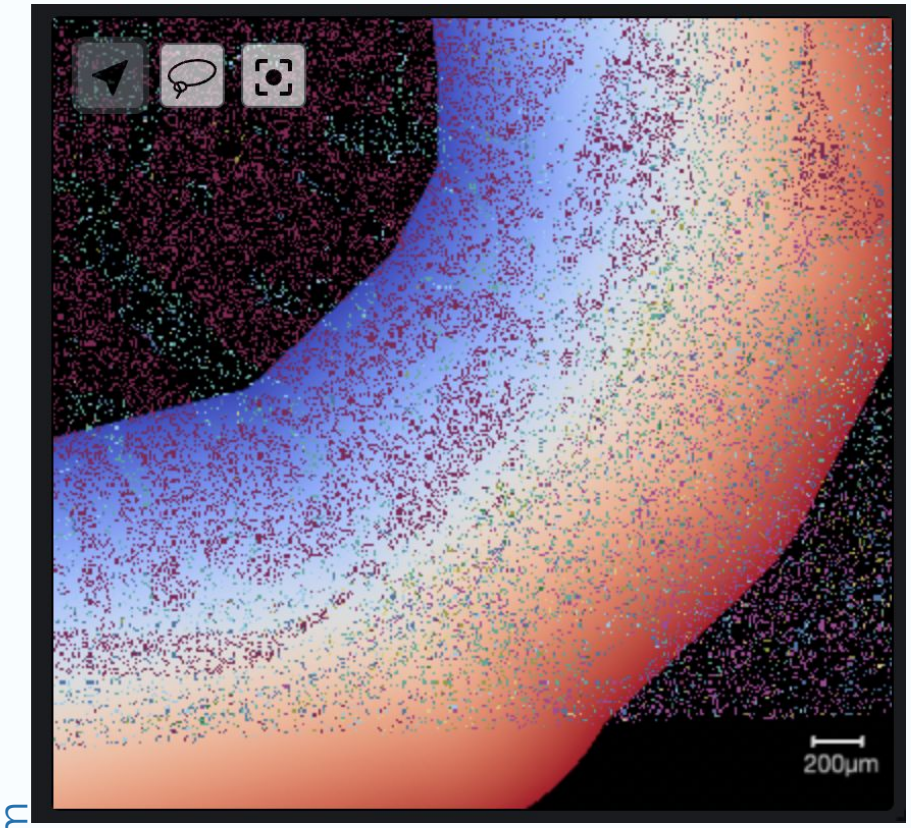


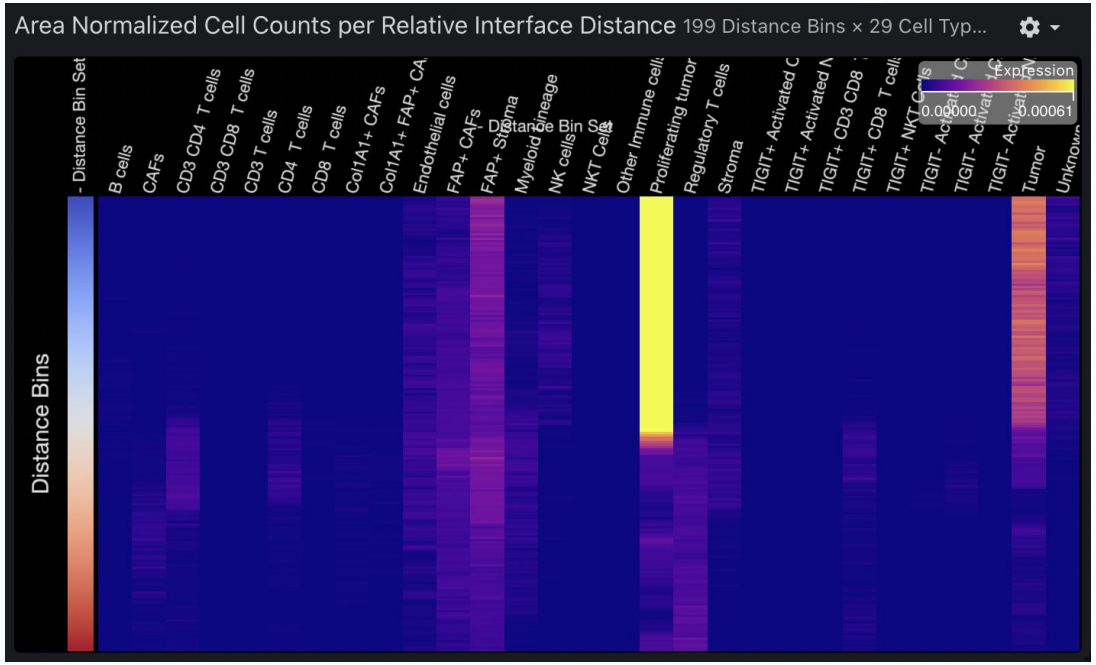
Spatial distance based exploration of Tumor-Stroma interface





Spatial distance based exploration of Tumor-Stroma interface





Conclusion

- We demonstrate a multimodal data acquisition workflow for combined spatial multi-omics
- We describe tooling and a cross modality data structure for correlative, differential, and spatial multimodal data analysis
- Using cell phenotype information, we found interesting preliminary MS markers
- We preliminary describe the tumor microenvironment with spatial analysis of tumor interface using both cell type and MSI data

Thank you for your attention!

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