Aspect Analytics

Dedicated Software to Support High Throughput Spatial Multi-Omics Applications

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Co-founder and Chief Executive Officer
marc.claesen@aspect-analytics.com

AGENTSCHAP INNOVEREN & ONDERNEMEN
Vlaanderen is ondernemen
eit Accelerated by Health
NVIDIA INCEPTION PROGRAM
NIH
THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

Presentation outline

1. Introduction
2. Our spatial multi-omics approach
3. Case studies
4. Conclusion

Aspect Analytics in a nutshell

Bioinformatics solutions provider for spatial biology

- **Weave cloud platform** for spatial biology data management and analysis
  - **Fit-for-purpose tools**: customizable to fit core lab workflows
- **Bioinformatics services**: multi-omics integrations, data-driven QC, software co-development, …

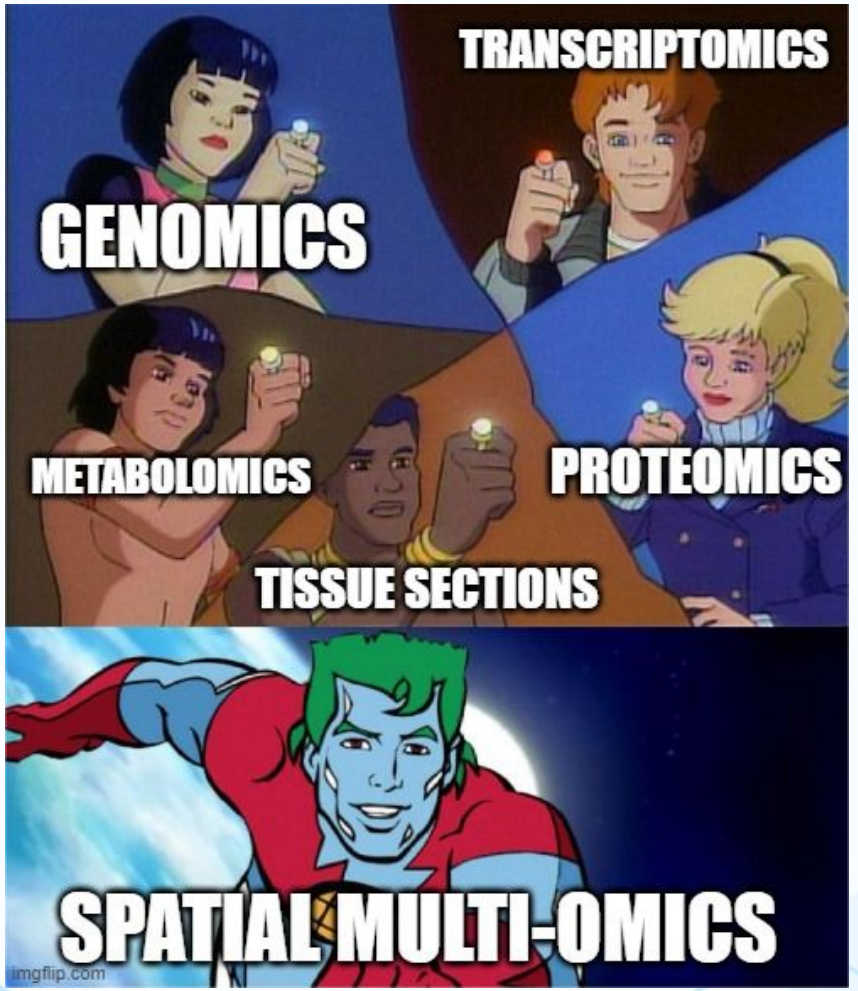
Our mission

- Provide a **unified platform solution** for spatial biology data analysis
- Unlock full potential of **spatial multi-omics in high-throughput contexts**

Spatial multi-omics

Improving our understanding of tissue biology

- **Spatial context is a key driver** of complex biology
- **Multi-omics needed** to capture full biological process



Spatial multi-omics

Improving our understanding of tissue biology

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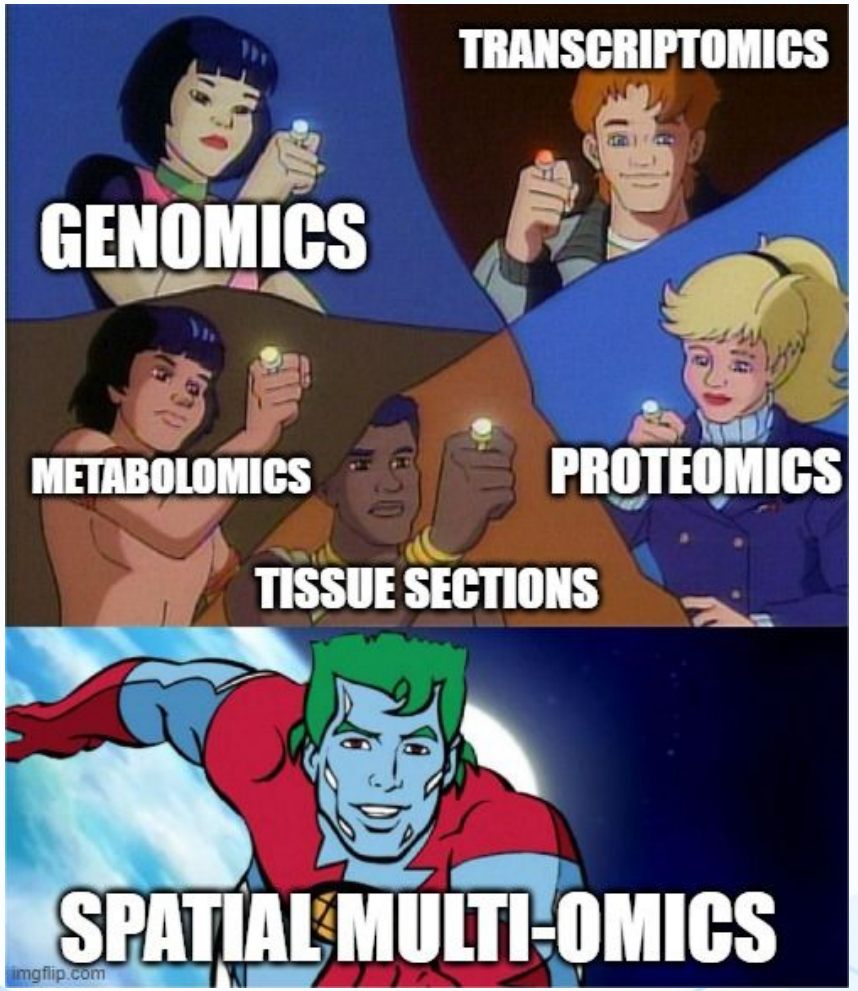
Data analysis challenges in spatial multi-omics

- **Multiple assays needed to target all omics layers**
  - Integrate data across sections and vendor formats
- **Large data volumes and complexity**
  - Multi-petabyte storage & on-demand computations
- **Interdisciplinary collaboration** with many stakeholders
  - Team communication drives data analysis efficiency



Broad assay coverage

Spatial transcriptomics

- **Subcellular assays:** Xenium, CosMx, MERSCOPE
- **Minibulk assays:** Visium, Visium HD, GeoMx, Seeker

Spatial proteomics

- **mIF based:** COMET, PhenoCycler, CellDIVE, CellScape
- **Other approaches:** Hyperion (IMC), MALDI-IHC

Mass spectrometry imaging

- **instruments:** timsTOF fleX, solariX, scimaX, MRT, Orbitrap, Synapt, iMScope (+ imzML)
- **analytes:** drugs, lipids, glycans, metabolites, peptides, proteins

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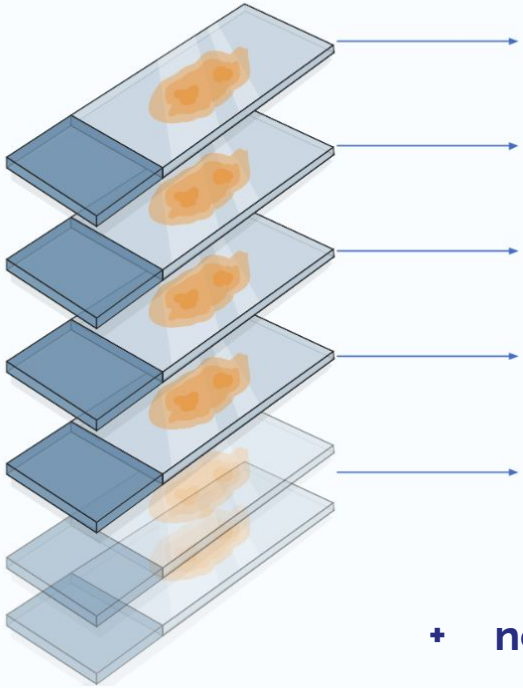
Spatial multi-omics data integration across any combo of these assays

Check booth #3 for live demos!

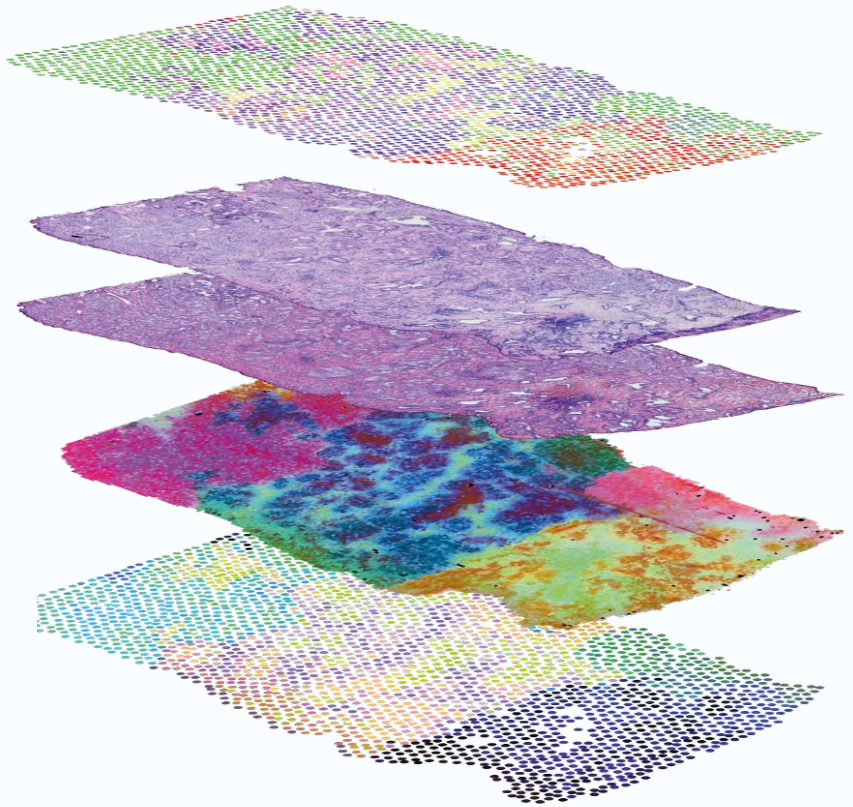
Our spatial multi-omics approach

Spatial multi-omics data comes in *stacks*



- morphology & pathologist annotations
- spatial transcriptomics
- spatial proteomics
- spatial metabolomics
- other new technologies …

\+ non-spatial (bulk proteomics, scRNAseq, …)



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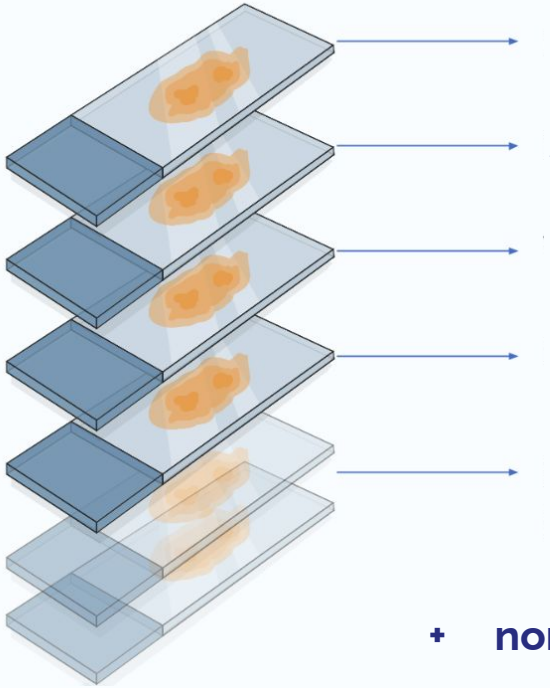

morphology & pathologist annotations

spatial transcriptomics

spatial proteomics

spatial metabolomics

other new technologies …

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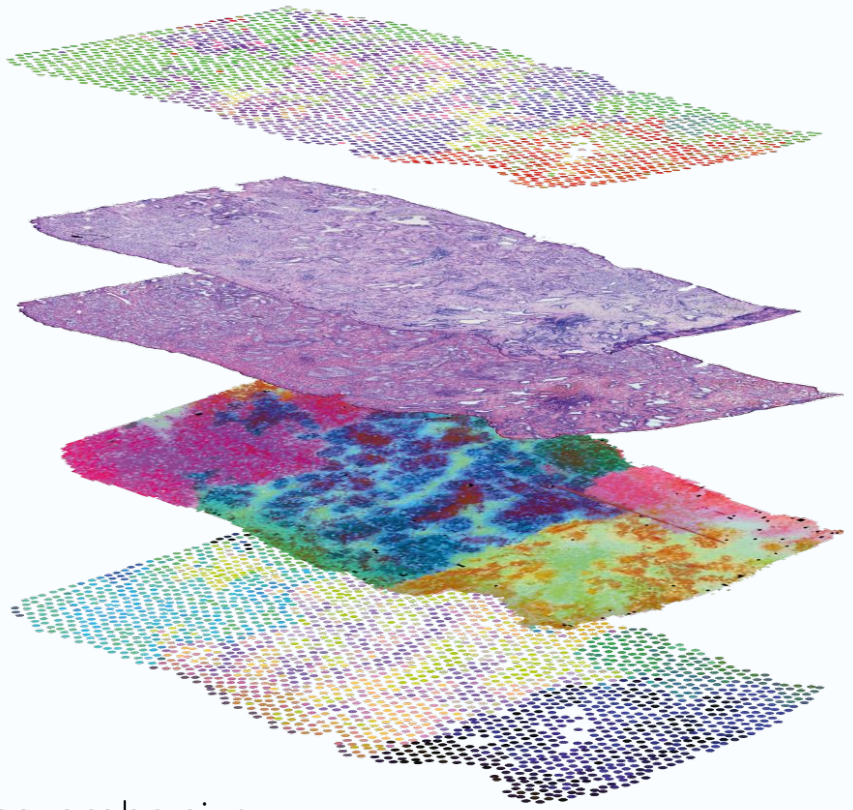
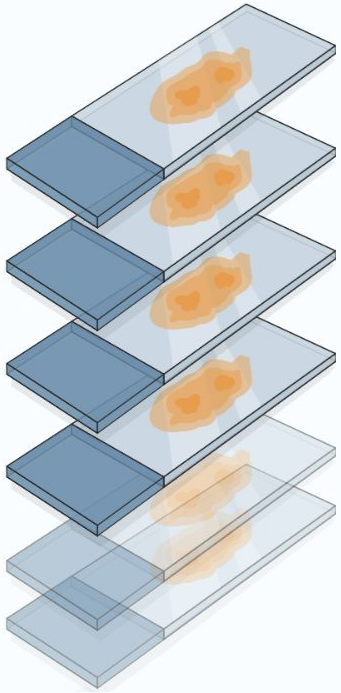


Data integration is challenging due to differences in

- **Coordinate systems** due to spatial resolution, different sections, probe size, …
- **Data formats**: each assay has its own proprietary vendor format

weave platform for spatial biology

Aspect Analytics



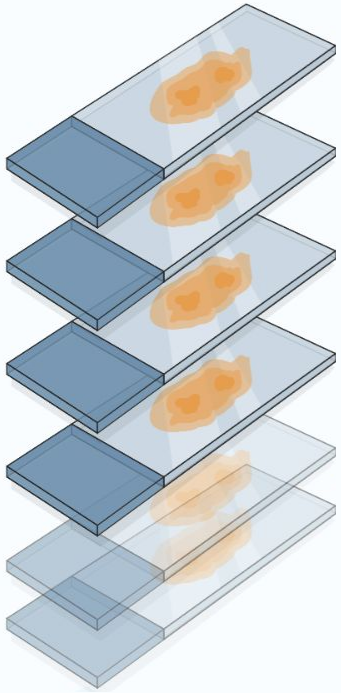
morphology	pathologist annotations	spatial transcriptomics	spatial proteomics	spatial metabolomics	other data

Stack *fusion*

- Integrate readouts across assays and sections to obtain unified, holistic dataset
- Enables true spatial multi-omics using any number and combination of assays

weave platform for spatial biology

Aspect Analytics



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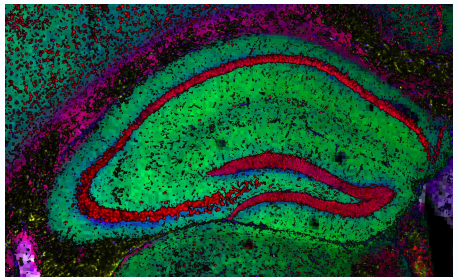
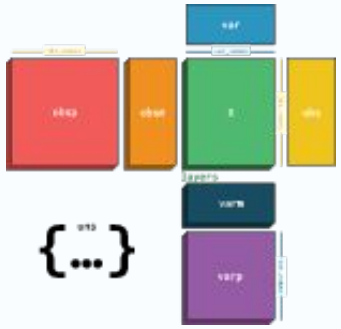
Stack *fusion*

- Integrate readouts across assays and sections to obtain unified, holistic dataset
- Enables true spatial multi-omics using any number and combination of assays
- Facilitate downstream analysis and interactive visualization via dataframe-like interfaces

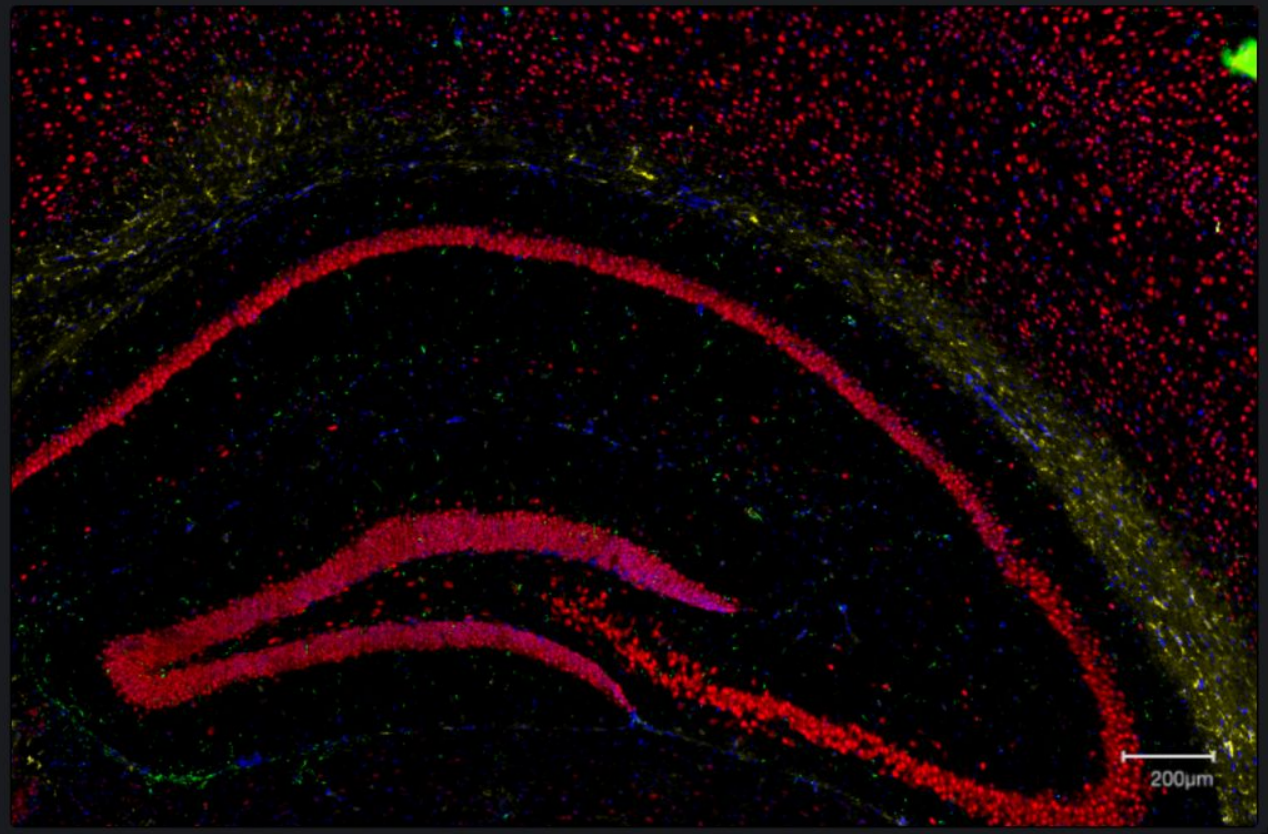
multimodal visualization



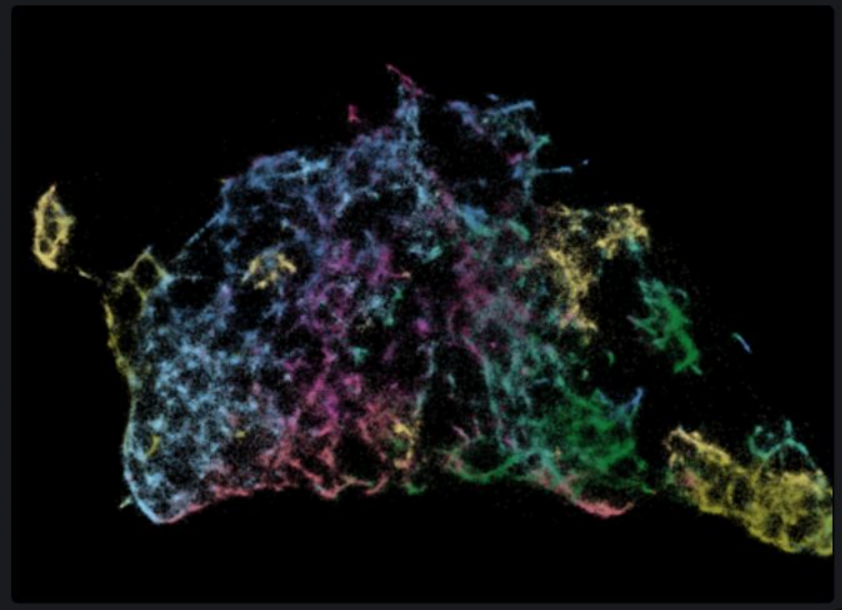
unified data structure



Spatial 169,441 Cells



Scatterplot (UMAP) 169,441 Cells



Spatial Layers

Cell Seg...
Opacity:

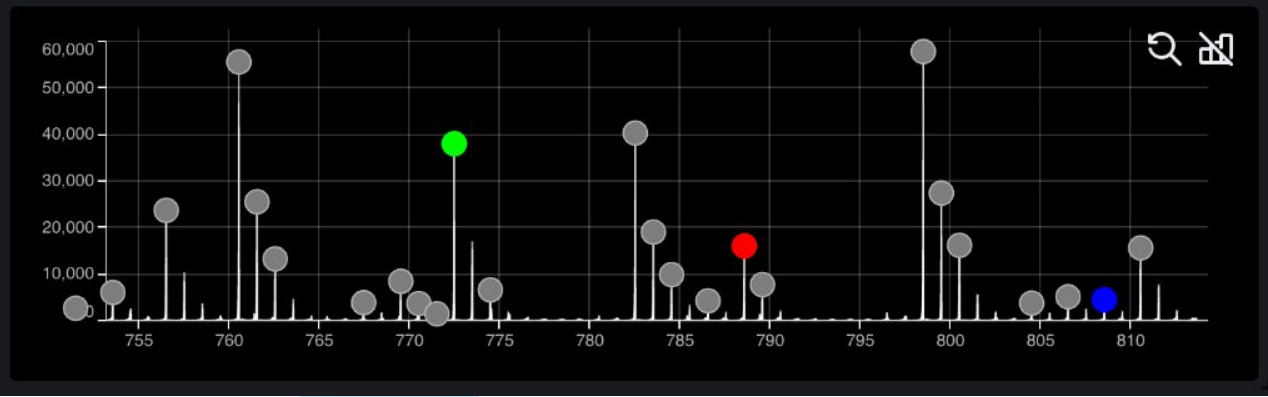
MALDI ...
Opacity:

H&E (Ma...
Opacity:

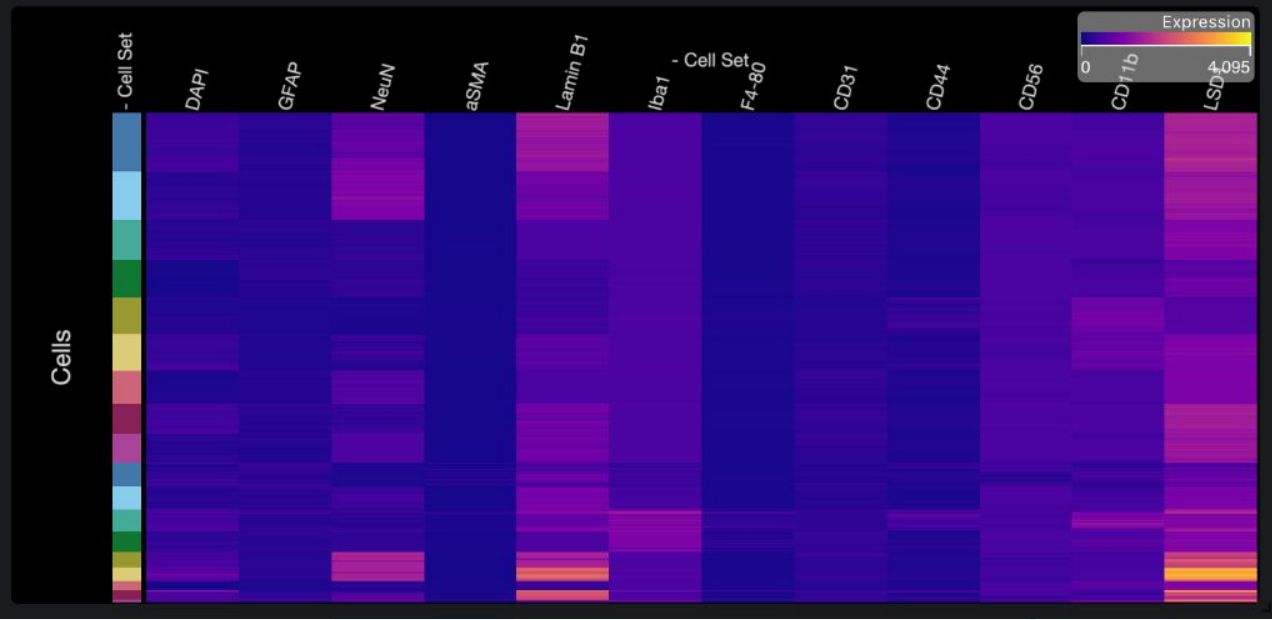
H&E Mo...
Opacity:

H&E (M...

Spectrum Viewer



Heatmap 169,441 Cells × 12 Markers, with 169,441 Cells selected



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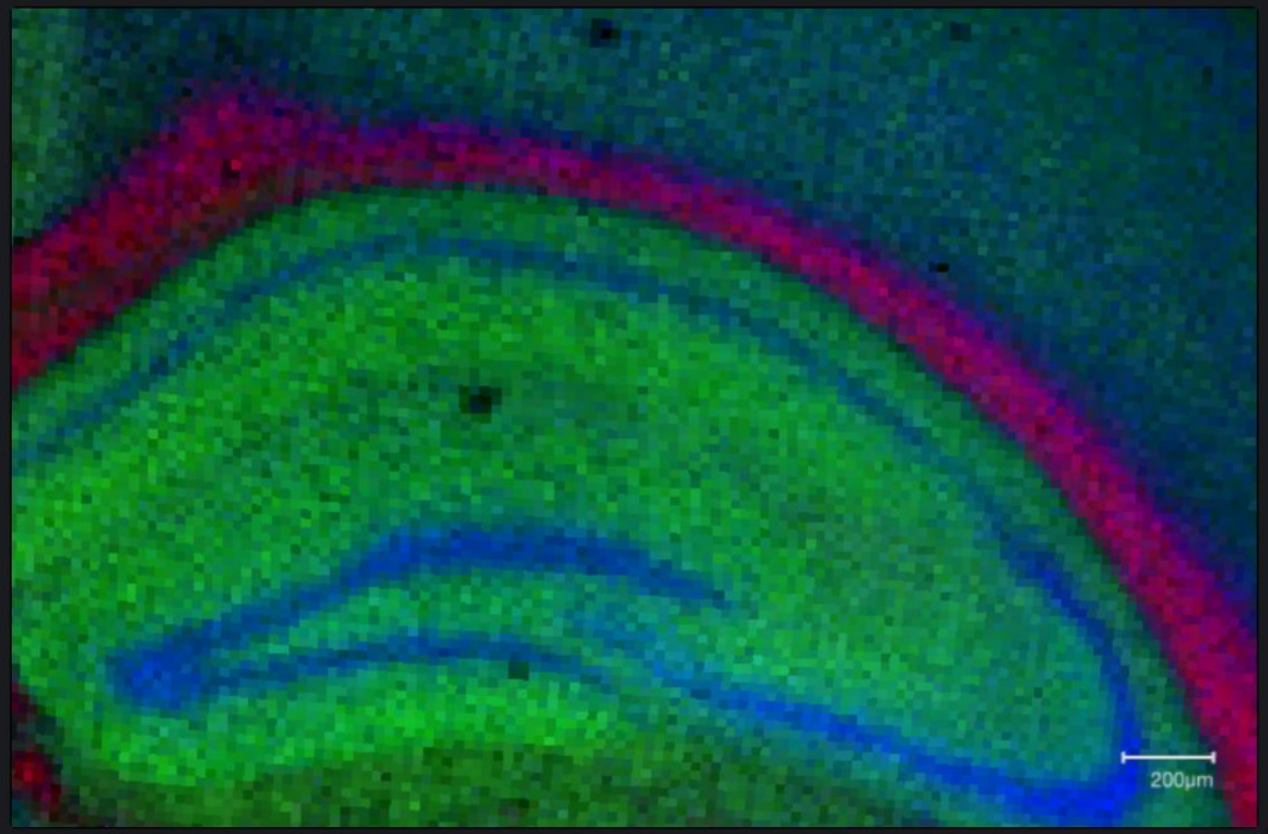

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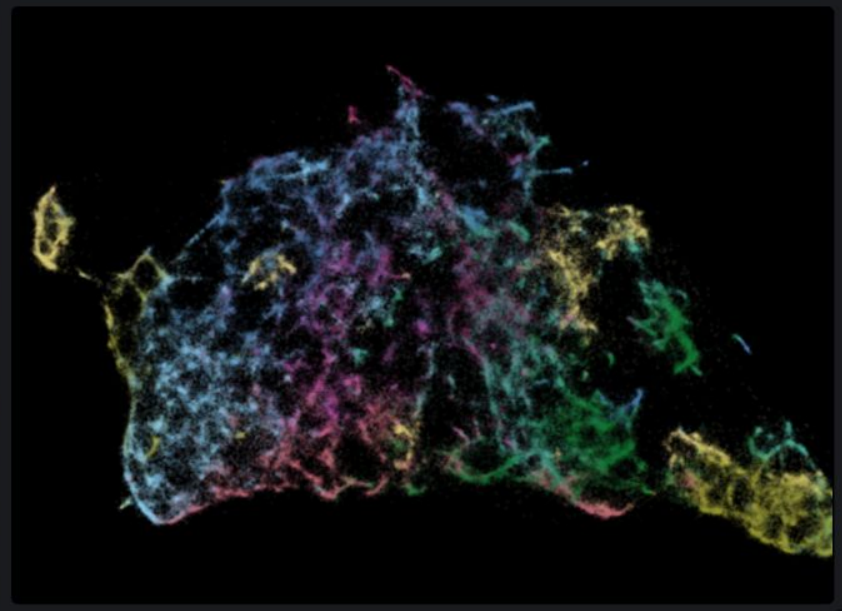

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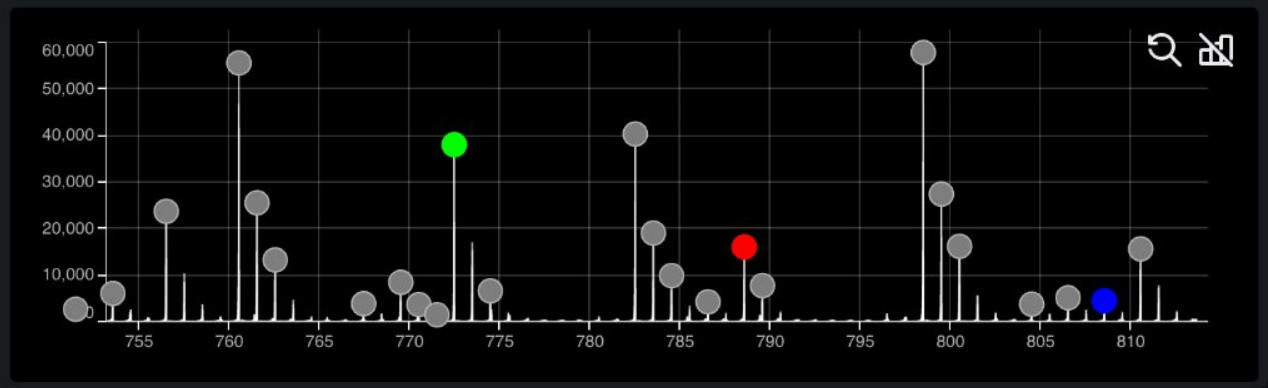

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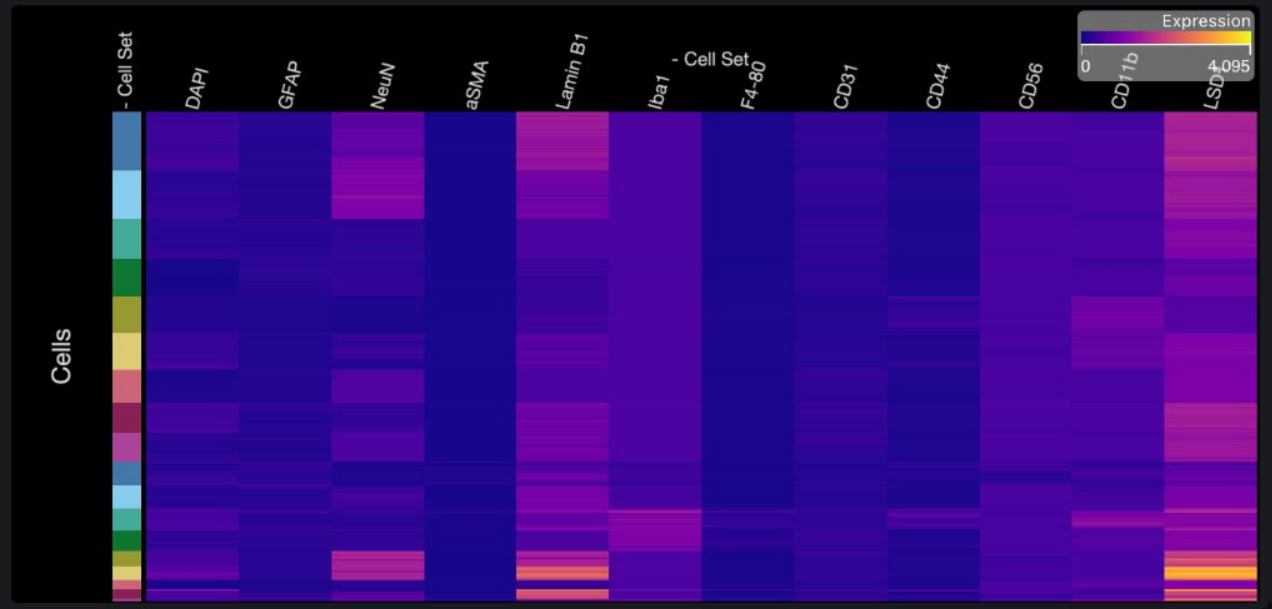

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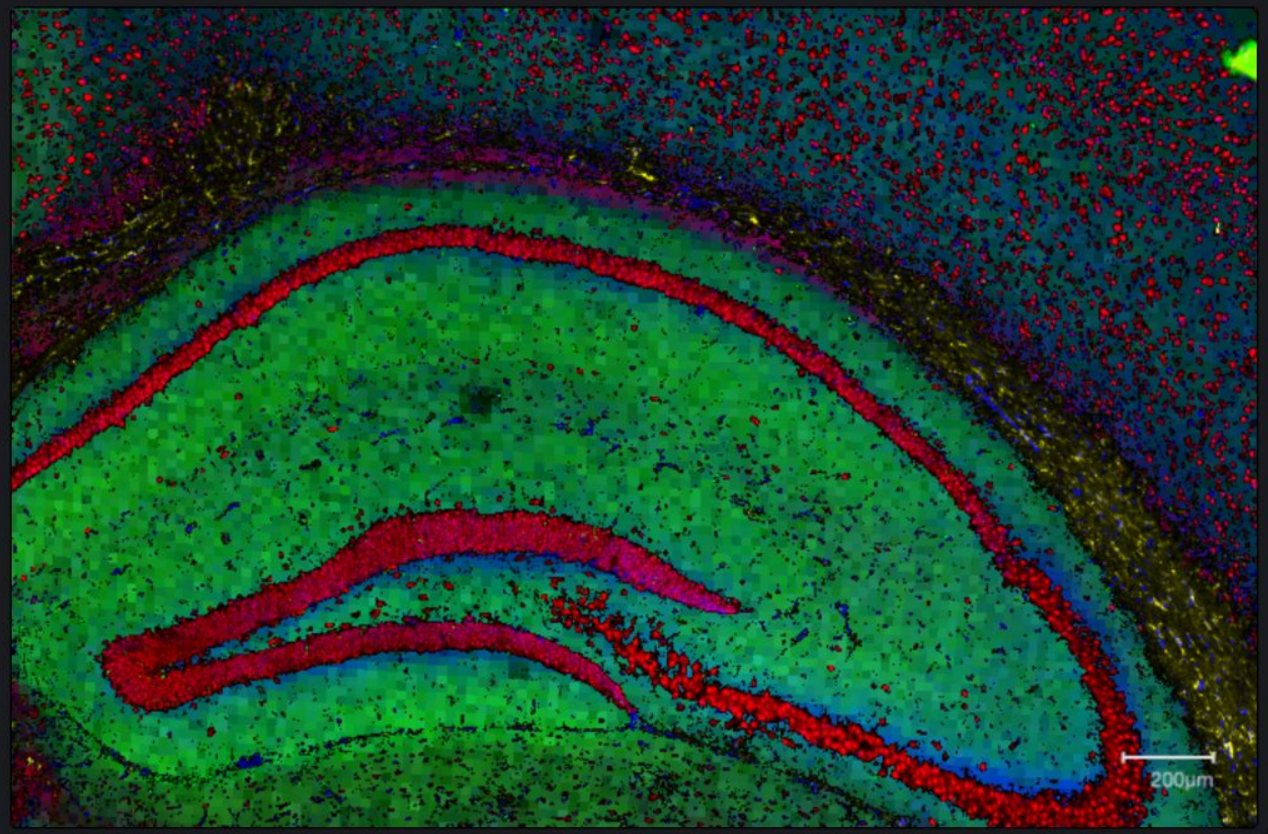

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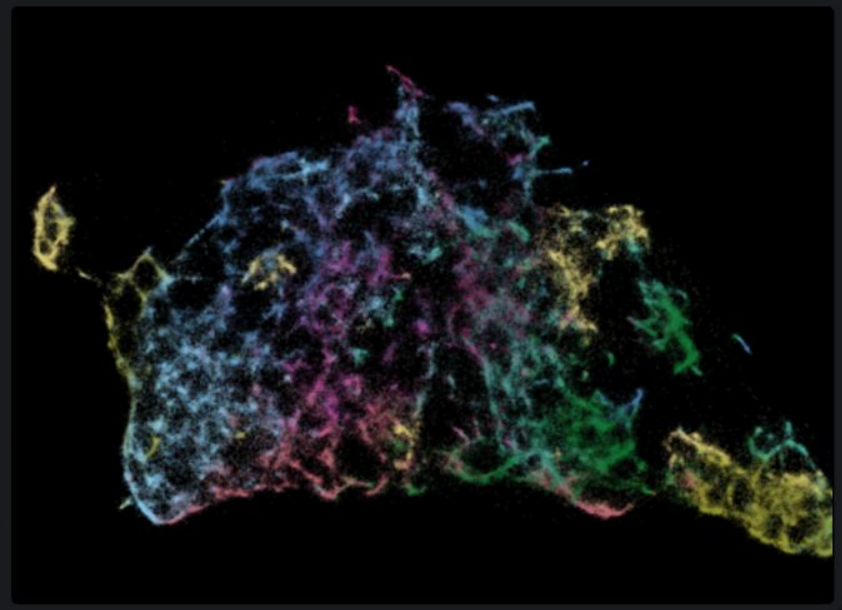

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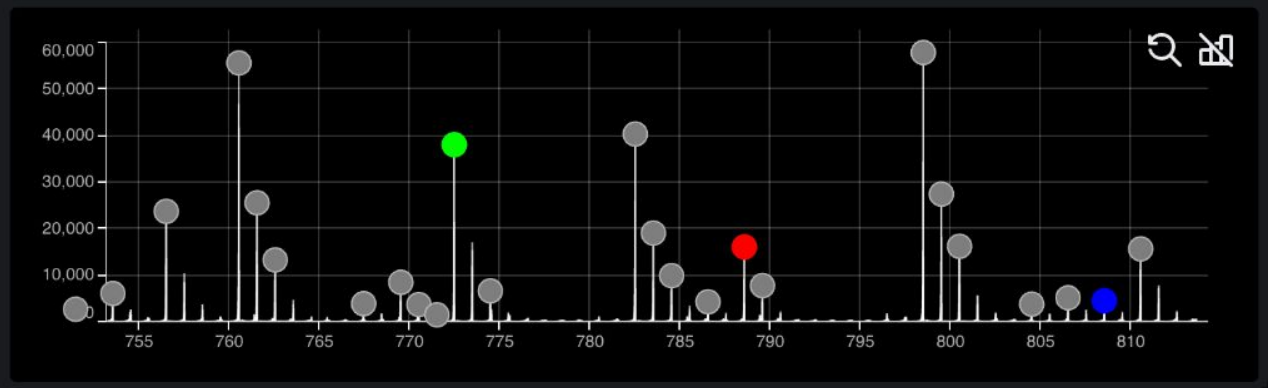

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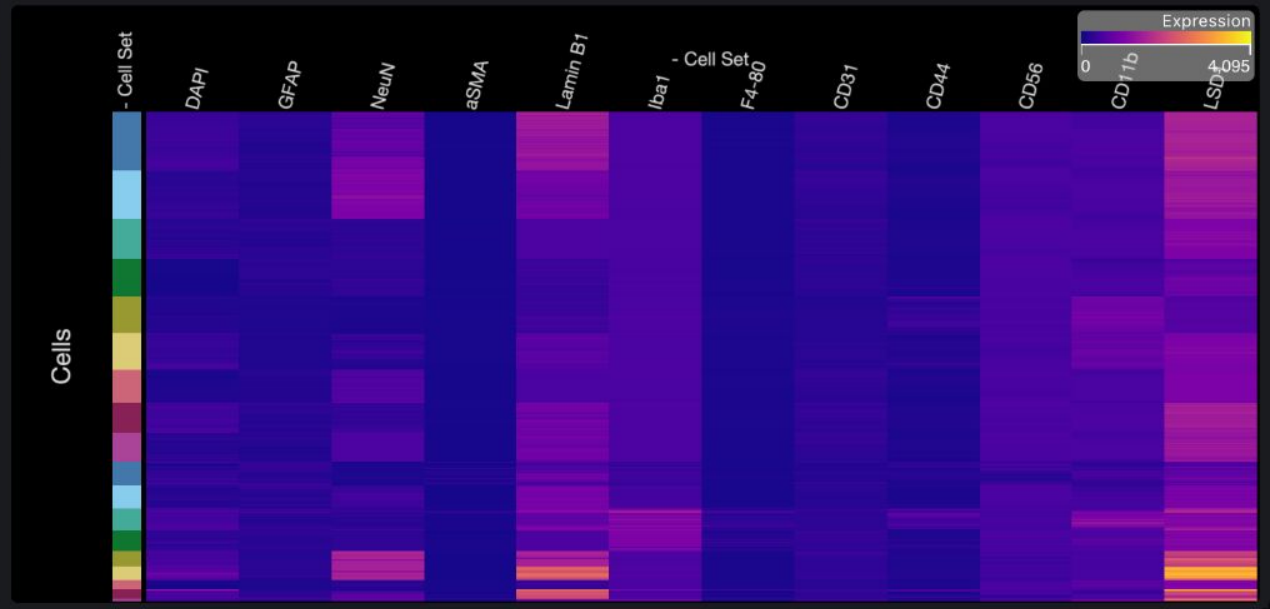

Cloud platform for spatial biology analysis

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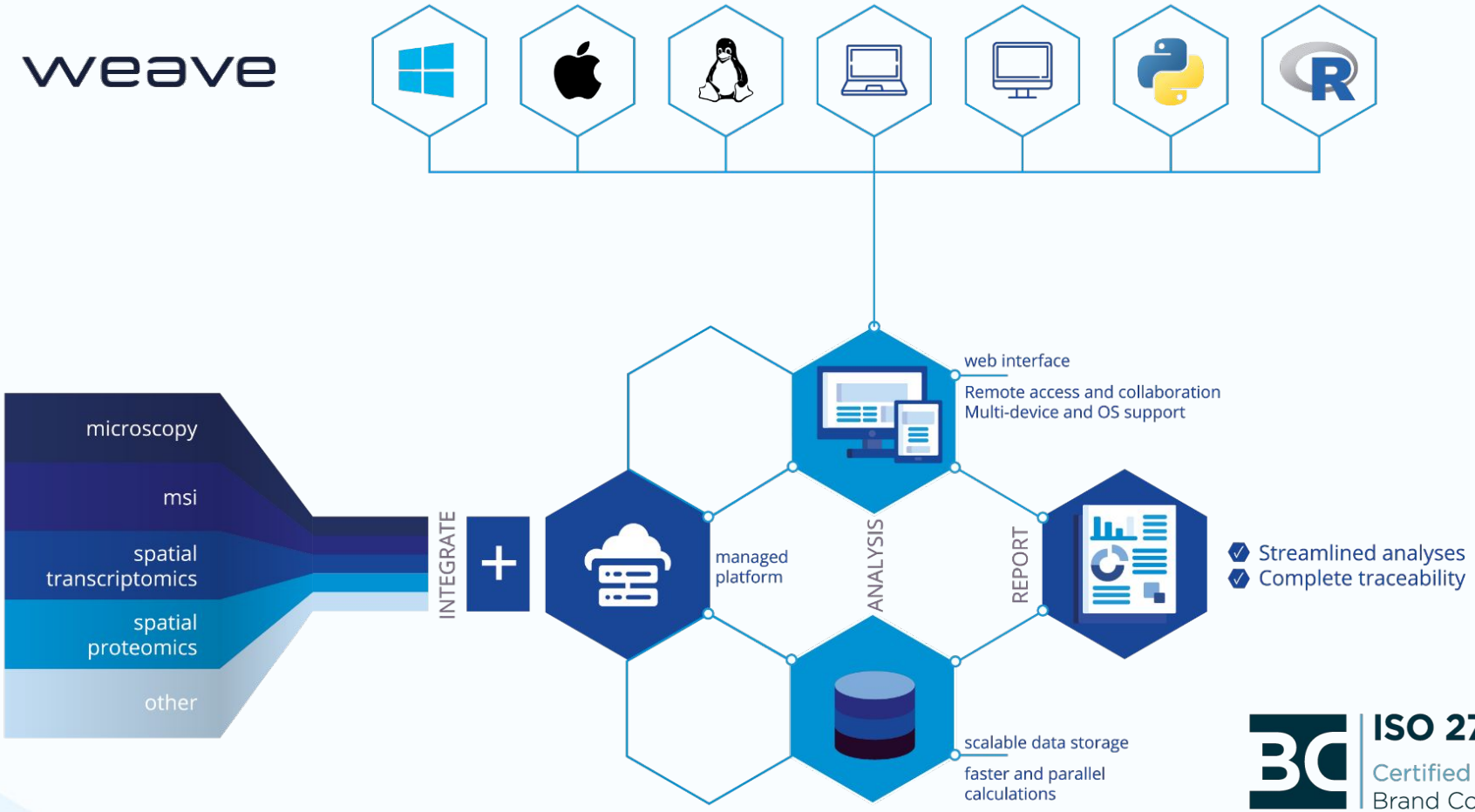
weave



ISO 27001:2017
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Case studies

Spatial profiling of prostate cancer (PCa)

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KU Leuven Institute for Single Cell Omics

Weill Cornell Medicine

UOW AUSTRALIA

THE UNIVERSITY of ADELAIDE

Goal: improving understanding of PCa biology via spatial biology

- Roughly **1 in 8 men will be diagnosed with PCa** during their lifetime
- **PCa is a very complex disease**
  - high inter -and intra-tumoral heterogeneity
  - multiple morphological disease states in single section
  - involves molecular changes across multiple omics classes

Zhang et al., bioRxiv 2023.08.28.55505
https://doi.org/10.1101/2023.08.28.555056

Wanqiu Zhang
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Sebastiaan Vanuytven
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Fabio Socciarelli
Katy Vandereyken
Jonas Dehairs
Jakub Idkowiak
David Wouters
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Gabriele Partel
Alice Ly
Vincent de Laat
Maria José Q Mantas
Thomas Gevaert
Wout Devlies
Chui Yan Mah
Lisa M Butler
Massimo Loda
Steven Joniau
Bart De Moor
Alejandro Sifrim
Shane R. Ellis
Thierry Voet
Marc Claesen
Nico Verbeeck
Johannes V. Swinnen

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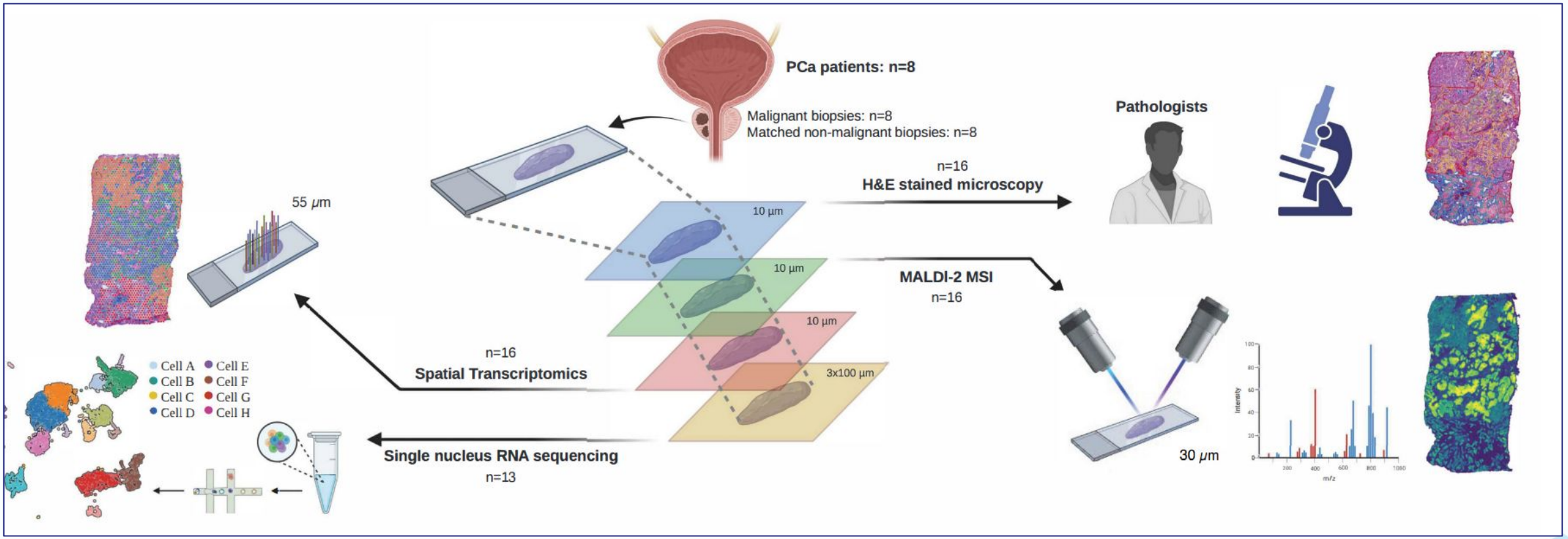
High-level design:

- 16 samples coming from 8 patients - 8 malignant and 8 non-malignant biopsies
- Histology and multi-omics combination:
  - Spatial analysis: **spatial transcriptomics** (Visium) + **spatial lipidomics** (Orbitrap)
  - Bulk: **snRNAseq** + **lipidomics** (LC-MS)

Stack design and experimental approach



Step 1: multimodal registration pipeline

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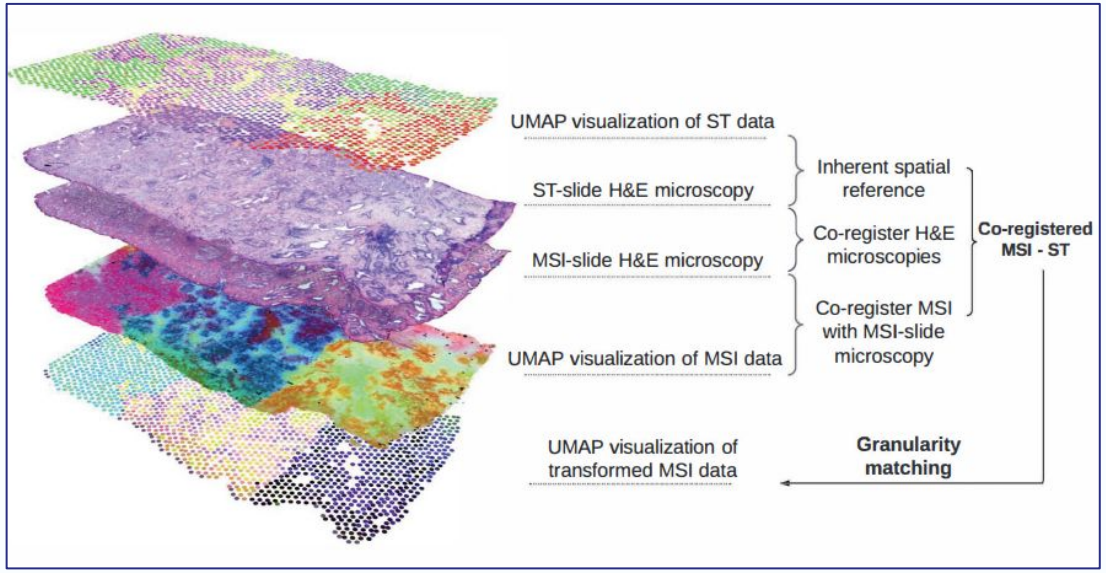
Approach

- **Inter-section registration via H&E's**
  - H&E available for both Visium & MSI sections
  - Shared modality facilitates registration
- **Registrations per section of omics and H&E**
  - MSI to MSI H&E, Visium to Visium H&E
- All **registrations are non-rigid** to account for serial sections and complex sample deformation



Outcome

- **Full coordinate transformation available from between any layer combination in the stack**

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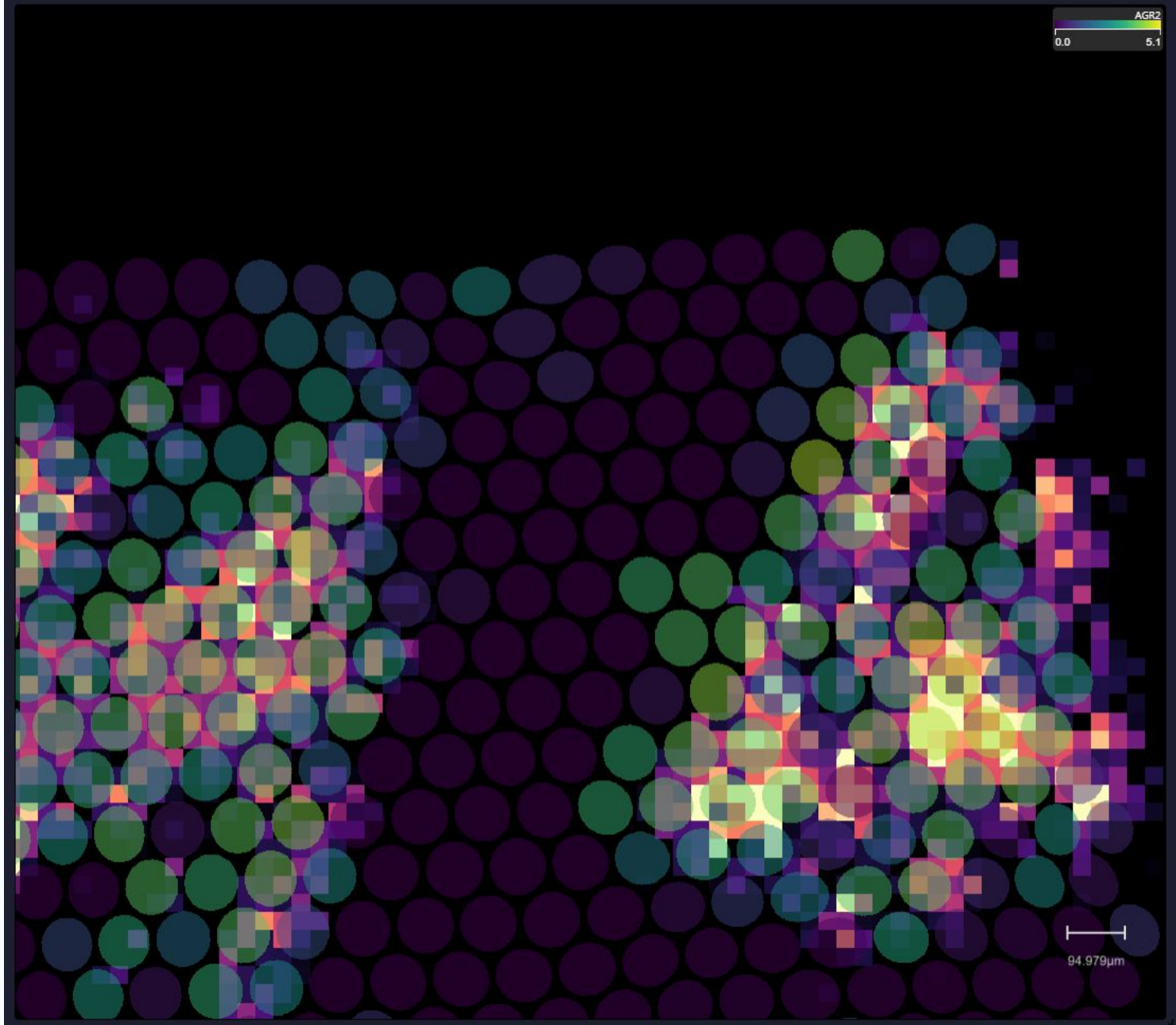
Spatial

5,622 Visium Spots



Spatial Layers

Visium Assay
Opacity:

H&E (MALDI MSI Tissue Section)
Opacity:

H&E (Visium Tissue Section)
Opacity:

MALDI Mass Spectrometry Imaging of Lipids
Colormap: magma
Domain: Min/Max
Opacity:
Zero Transparent:

PE 40:3 [M+Na]+ - m/z 820.5836 - ppm e
PE O-34:2 / PE P-34:1 [M+H]+ - m/z 702.
PE 42:7 [M+H]+ - m/z 818.5716 - ppm er

Gene List

18,950 Genes

Search

AGPAT5
AGPS
AGR2
AGR3
AGRN
AGT
AGTPBP1
AGTR1
AGTRAP
AHCTF1
AHCY
AHCYL1

Cell Sets

Visium Spot Labelling

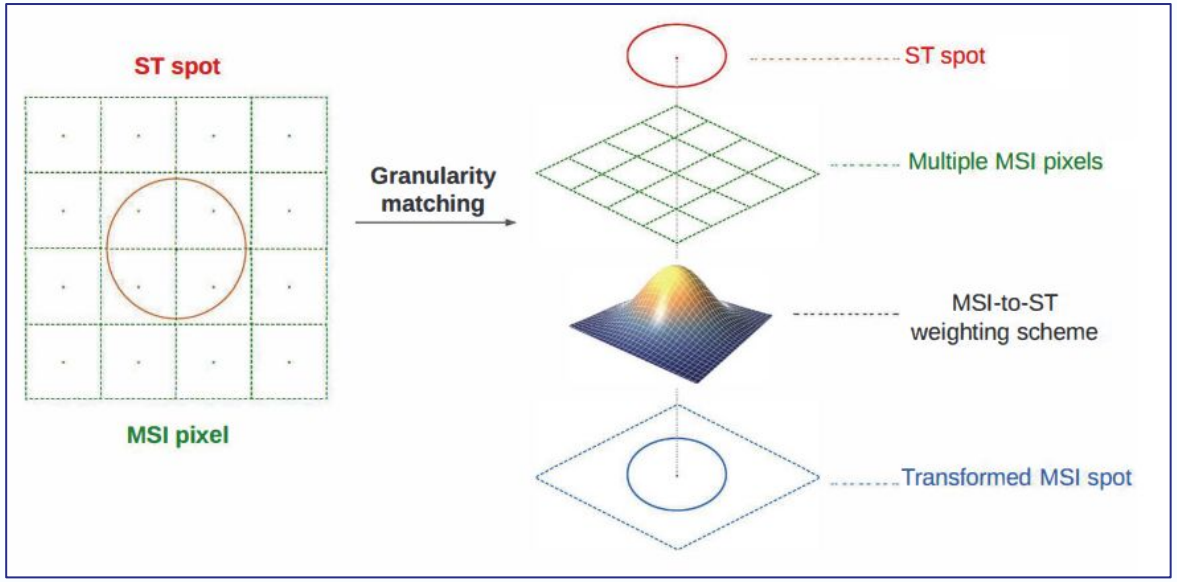
KU Leuven Institute for Single Cell Omics | Aspect Analytics | Weill Cornell Medicine | University of Wollongong Australia

In this project a collection of human prostate samples has been analyzed using spatial multi-omics, namely spatial lipidomics via MALDI MSI (Thermo), spatial transcriptomics using Visium (10x Genomics) and traditional histology.

Step 2: data integration to Visium spots

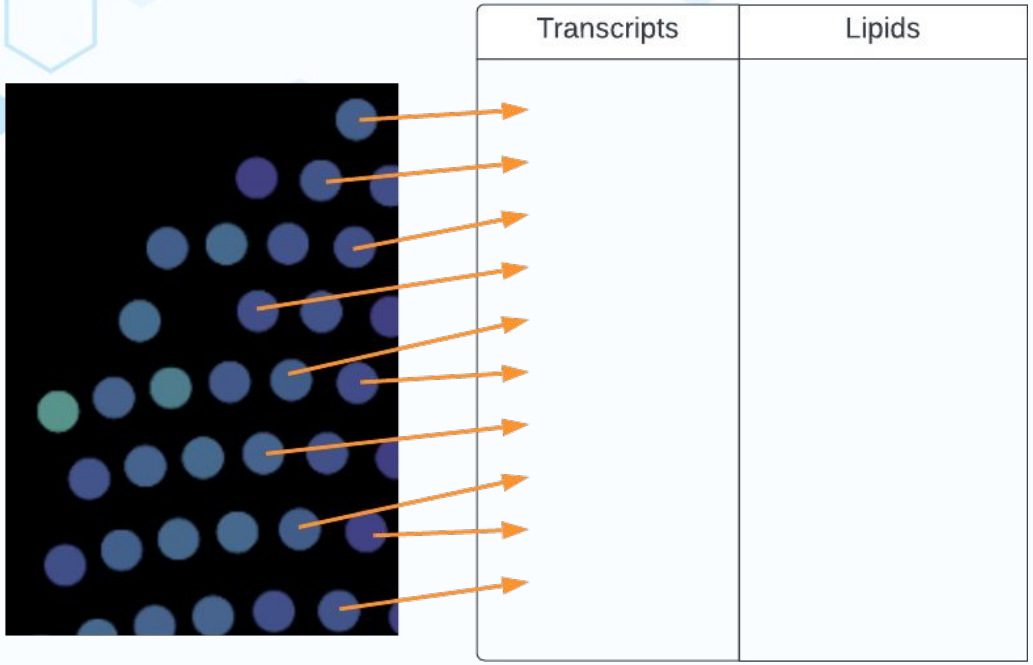
Principled strategy for data integration

- **Different spatial footprints** between assays
  - MSI: 30 micrometer per pixel
  - Visium: 55 micrometer per spot
- We matched MSI pixels to Visium spots by **weighing their spatial overlap**



Outcome

- **Integrated data structure for downstream analysis**
  - Aggregated, representative mass spectrum for each Visium spot



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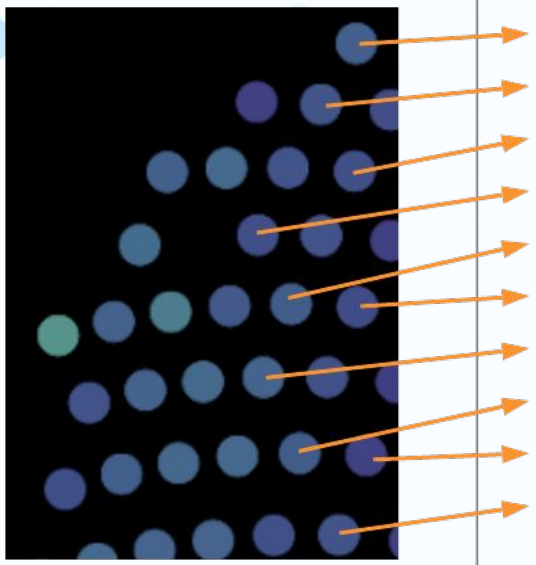
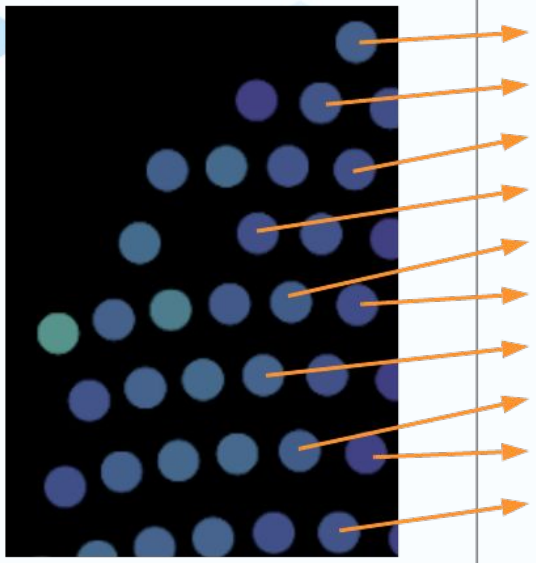


Transcripts	Lipids	Proteins	Cell types	...

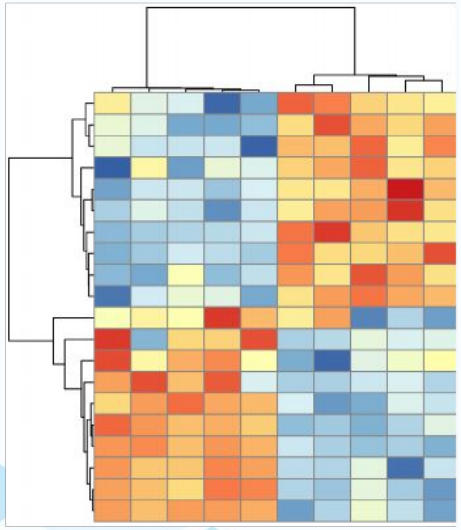
Aspect Analytics



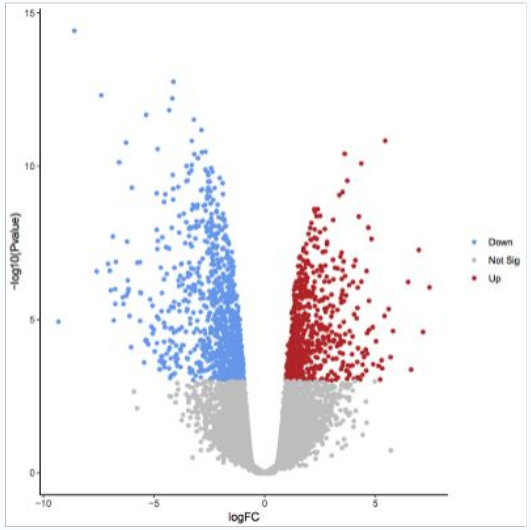
Transcripts	Lipids	Proteins	Cell types	...

Aspect Analytics

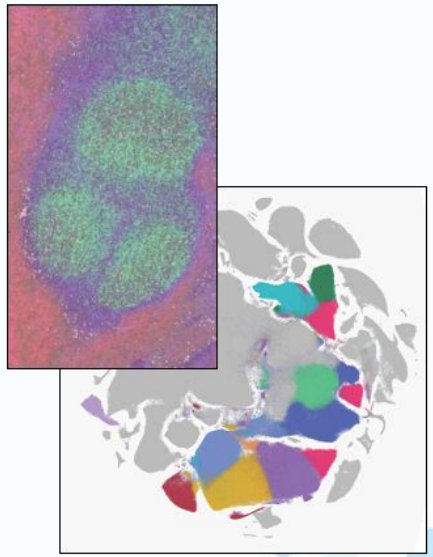
Correlation



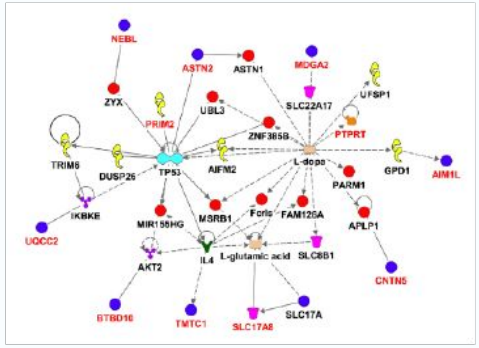
Differential expressions



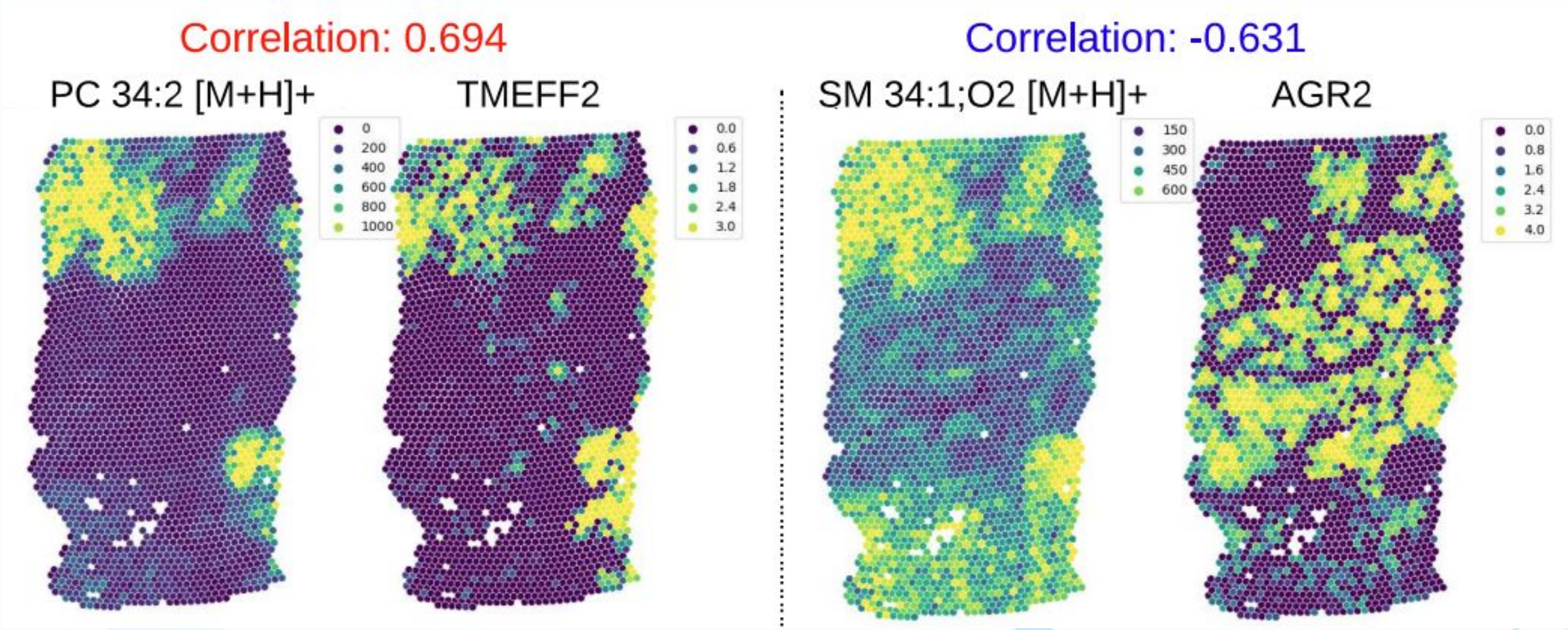
Unsupervised analysis



Pathways



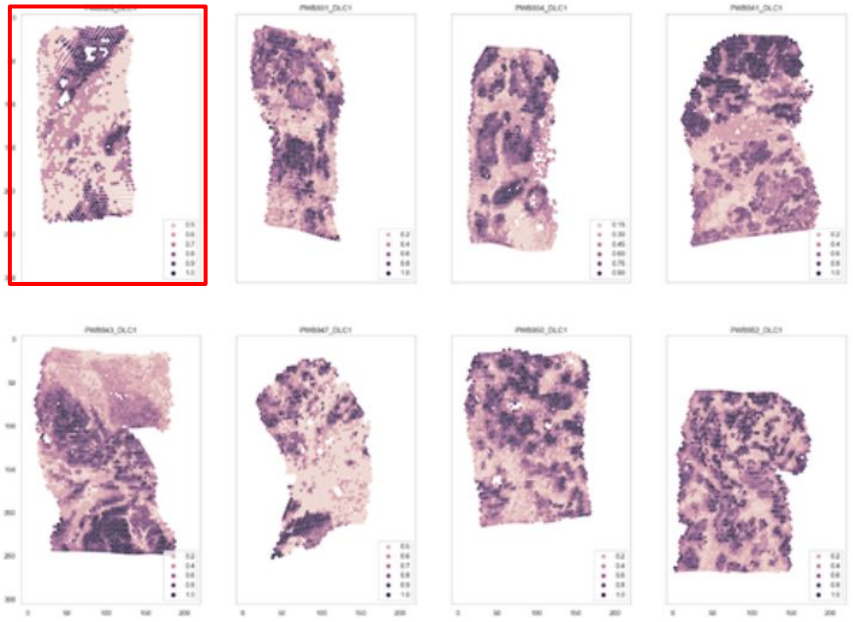
Spatial correlation / anticorrelation



0.5
0.0
−0.5
KLK3
KLK2
PCA3
DHRS7
DEGS1
LRIG1
GOLM1
FOLH1
ABCC4
AMACR
TRPM8
HPGD
NUCB2
KLK11
AGR2
MIA3
IDH1
CD164
TMEM45B
MIPEP
ACSM1
TFF3
ALDH1A3
DSCAM-AS1
ENTPD5
PLAT
SMS
CLGN
TMSB15A
ZNF350-AS1
DNAH8
NEFH
MDK
EEF1A2
RLN1
TMEFF2
Genes
PC 30:1 [M+H]+
PC 32:1 [M+H]+
733.5573
PE 40:6 [M+K]+
PE 38:0 [M+Na]+
831.5163
797.5907
PE 38:6 [M+K]+
PE O-44:8 / PE P-44:7 [M+Na]+
PC 36:1 [M+H]+
PC 40:5 [M+H]+
PE 38:3 [M+H]+
771.5731
800.6616
772.6318
PE 40:3 [M+Na]+
PE 40:6 [M+H]+
793.5564
821.5904
PE 42:7 [M+H]+
819.5746
PE 40:4 [M+H]+
PE 38:6 [M+H]+
765.528
PE 40:5 [M+H]+
795.5717
PE 38:2 [M+H]+
773.5898
PE 38:1 [M+H]+
775.6067
PC 38:2 [M+H]+
815.6354
PC O-36:7 / PC P-36:6 [M+Na]+
934.6813
928.5687
836.5437
962.7141
822.6032
773.6355
PE 40:2 [M+H]+
963.7183
994.7706
743.5425
PE 34:2 [M+H]+
855.5613
856.568
857.5721
710.521
904.76
704.5511
701.531
PE O-34:2 / PE P-34:1 [M+H]+
703.5468
PE O-36:3 / PE P-36:2 [M+H]+
729.5637
924.5621
DG 34:2 [M+H-H2O]+
LPE 18:2 [M+H]+
781.5571
PC 36:5 [M+H]+
878.5562
933.5726
1070.5934
895.5892
894.5861
546.2586
509.3059
PE O-36:2 / PE P-36:1 [M+H]+
508.3034
PC 34:2 [M+H]+
759.5742
PE O-38:7 / PE P-38:6 [M+H]+
PG 34:1 [M+H]+
788.5455
789.5486
PE O-36:6 / PE P-36:5 [M+H]+
731.5777
PE O-34:3 / PE P-34:2 [M+H]+
530.2868
SM 34:1;O2 [M+K]+
SM 34:1;O2 [M+Na]+
SM 34:1;O2 [M+H]+
704.5777
PC 36:4 [M+K]+
PG 38:4 [M+Na]+
LPE 16:0 [M+H]+
455.2955
LPE 20:3 [M+H]+
LPE 18:0 [M+H]+
483.3288
LPE 16:0 [M+K]+
LPE 16:0 [M+Na]+
Lipids

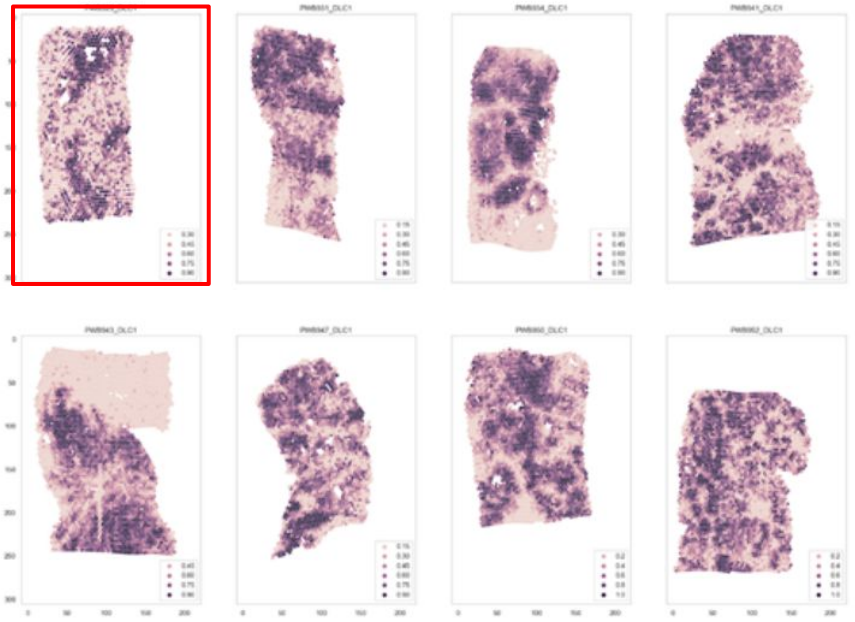
NORMAL SAMPLES

Lipid: PE 40:2 [M+H]+



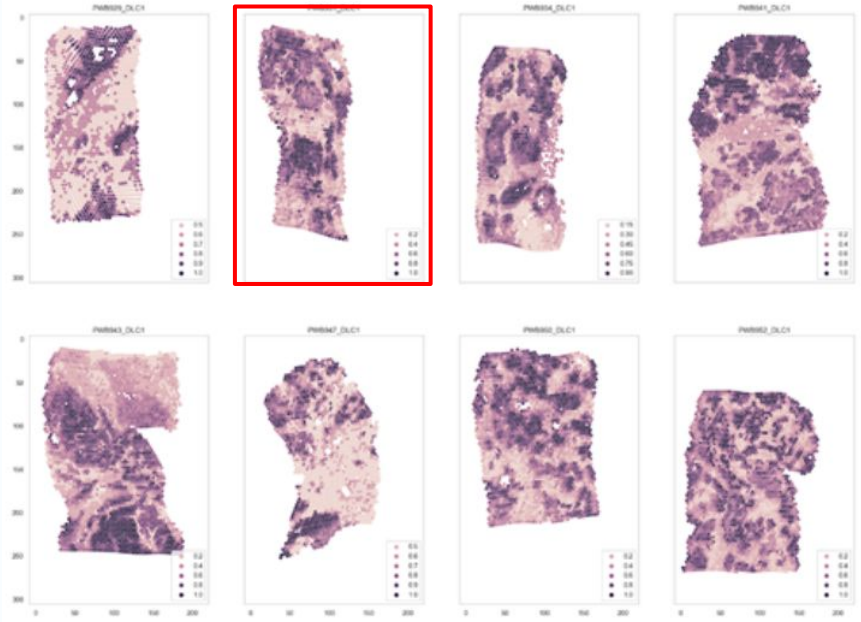
High positive correlation in normal samples

Gene: MSMB



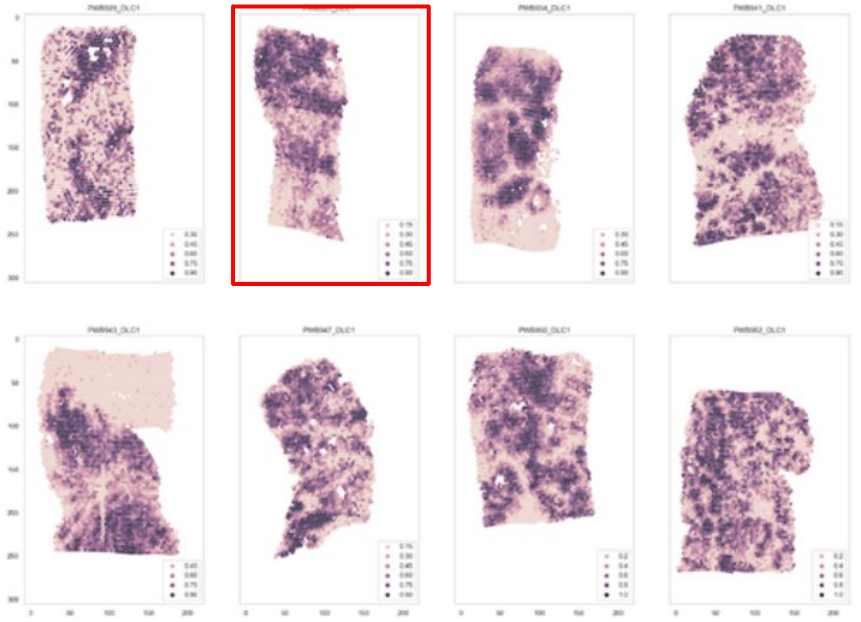
NORMAL SAMPLES

Lipid: PE 40:2 [M+H]+



High positive correlation in normal samples

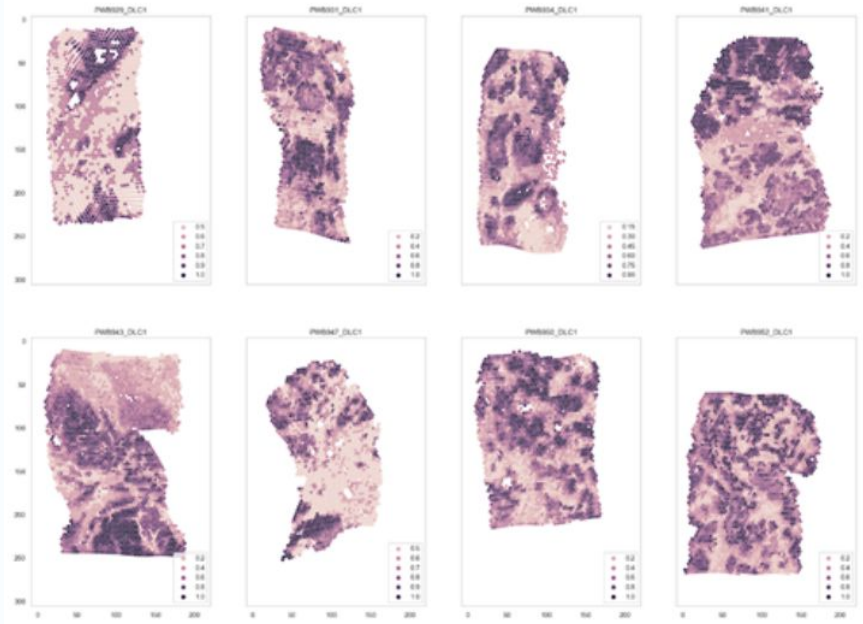
Gene: MSMB

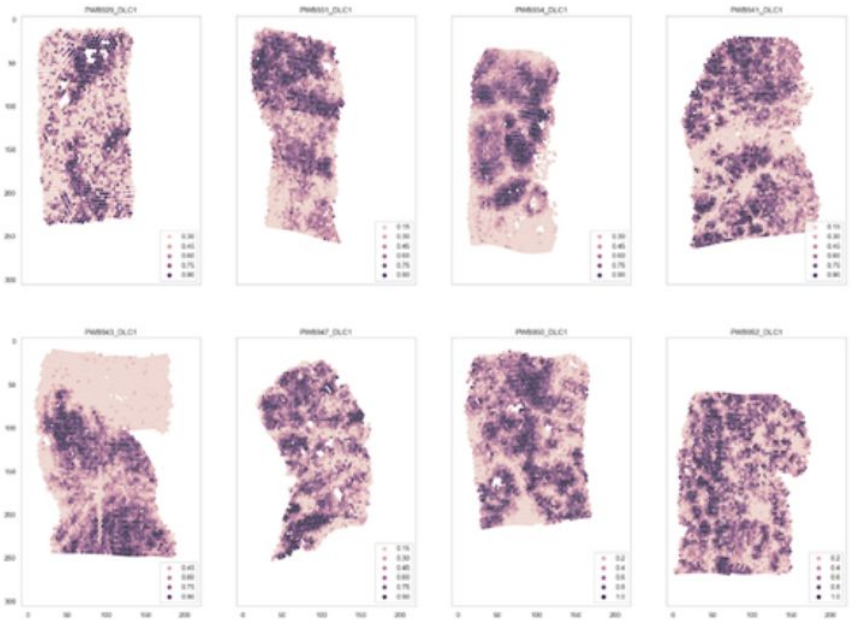


Lipid: PE 40:2 [M+H]+

Gene: MSMB

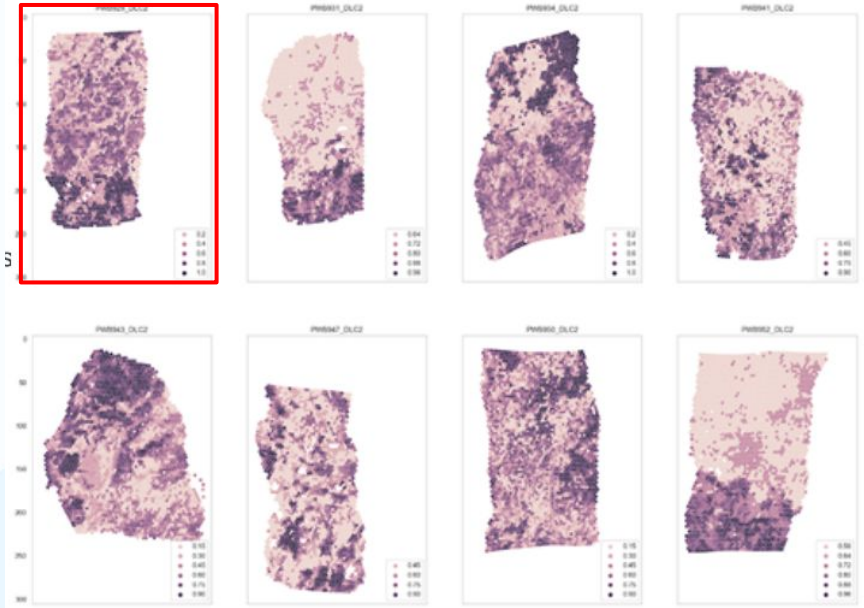
NORMAL SAMPLES

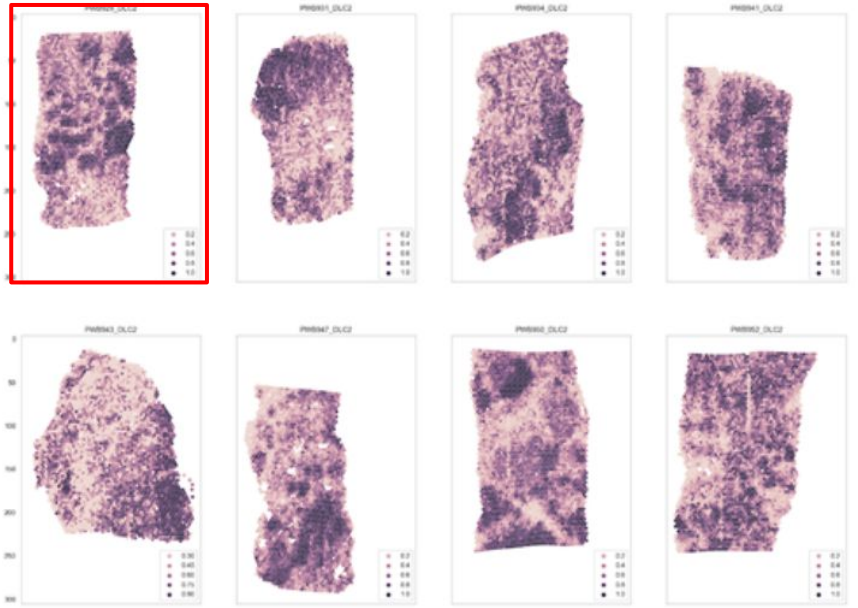




High positive correlation in normal samples

CANCER SAMPLES



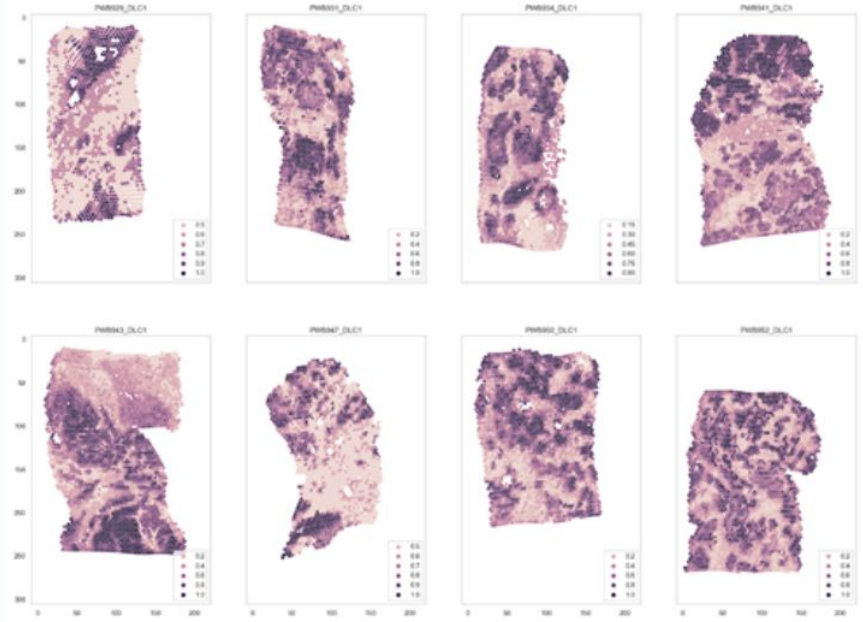


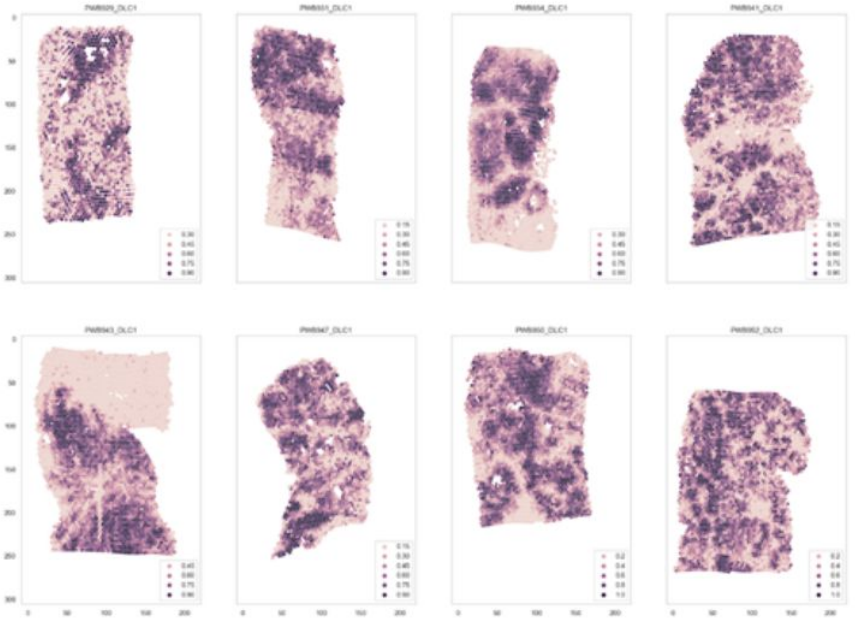
Almost no correlation in cancer samples

Lipid: PE 40:2 [M+H]+

Gene: MSMB

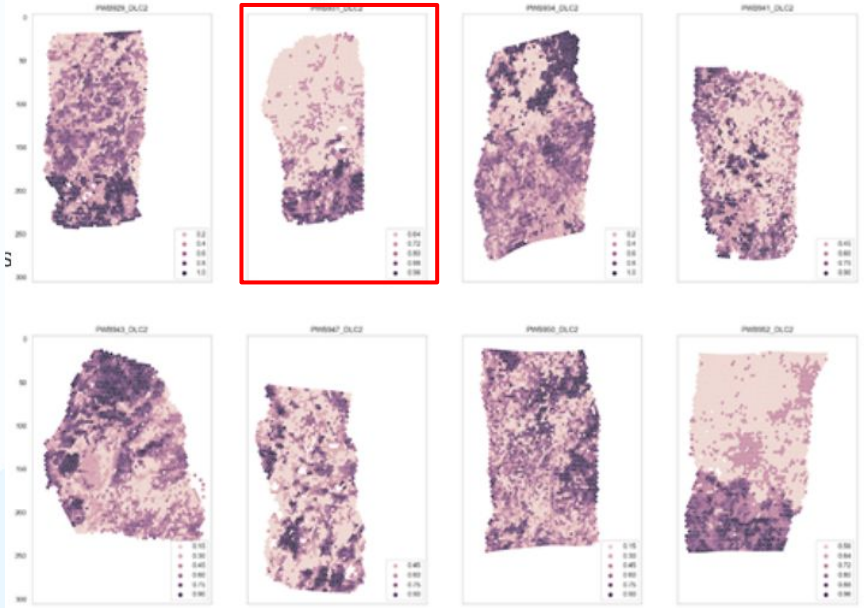
NORMAL SAMPLES

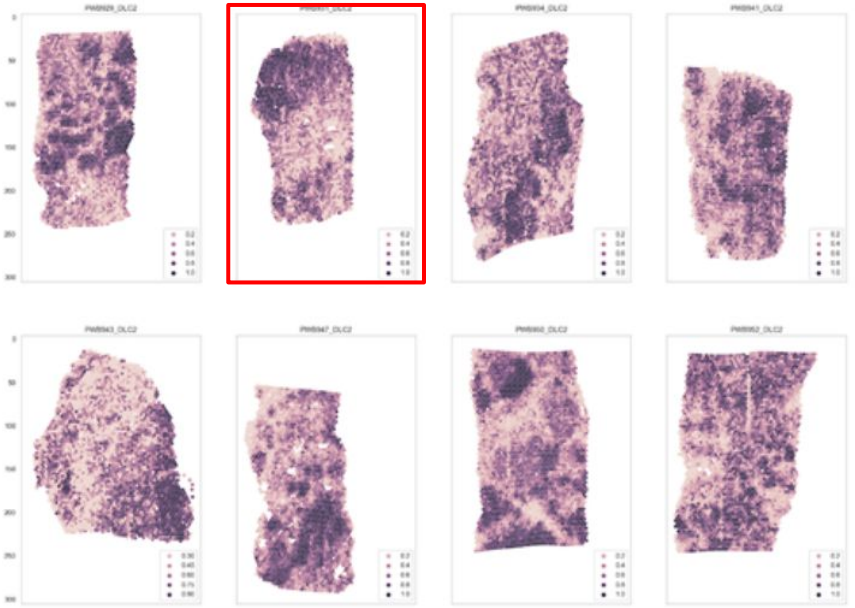




High positive correlation in normal samples

CANCER SAMPLES



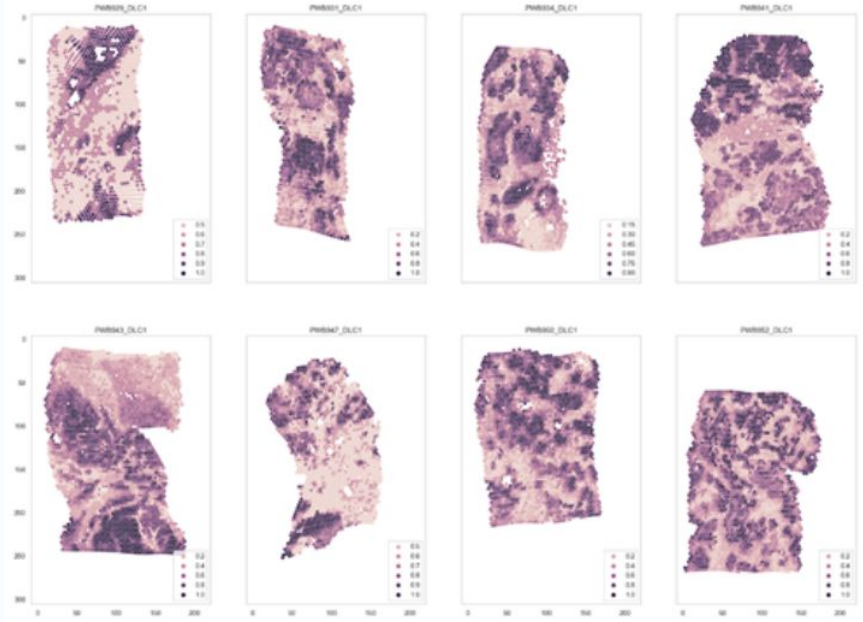


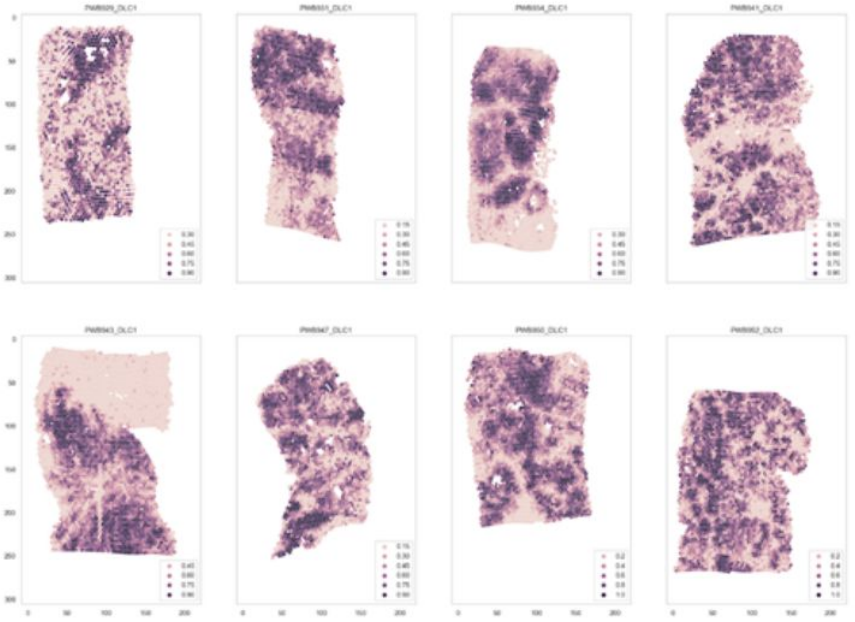
Almost no correlation in cancer samples

Lipid: PE 40:2 [M+H]+

Gene: MSMB

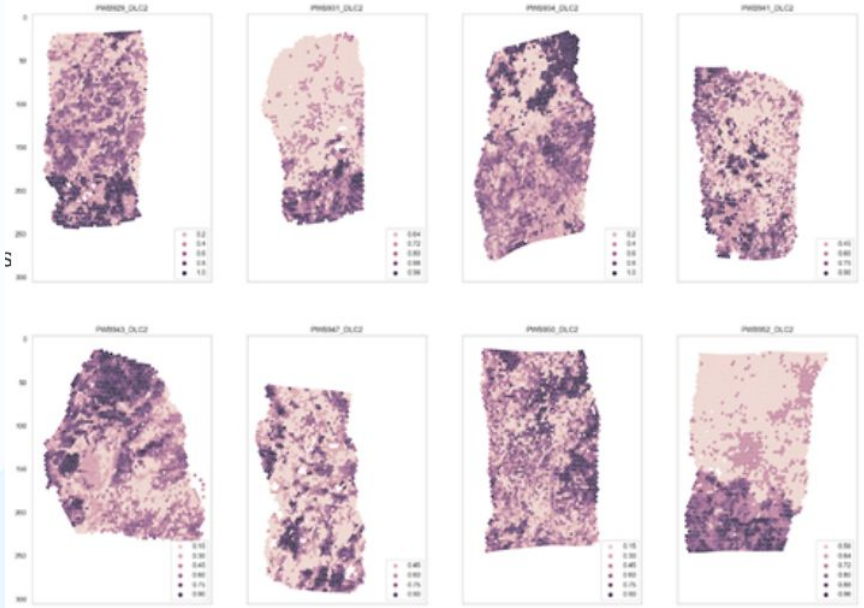
NORMAL SAMPLES

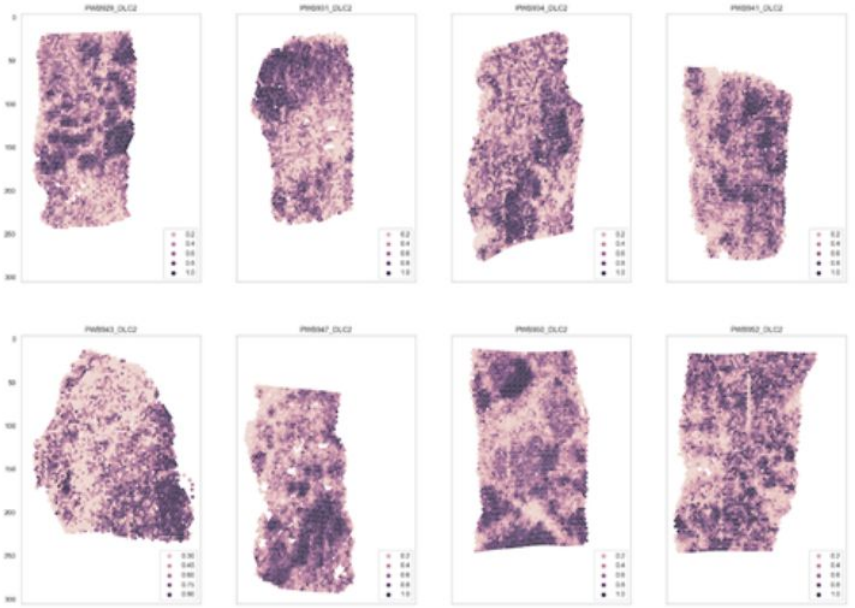




High positive correlation in normal samples

CANCER SAMPLES





Almost no correlation in cancer samples

High-grade serous ovarian cancer (HGSC)

Aspect Analytics

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

TEXAS
The University of Texas at Austin

Jared Burks
Erin Seeley
Sammy Ferri Borgogno

Ovarian cancer is the most lethal gynecologic malignancy

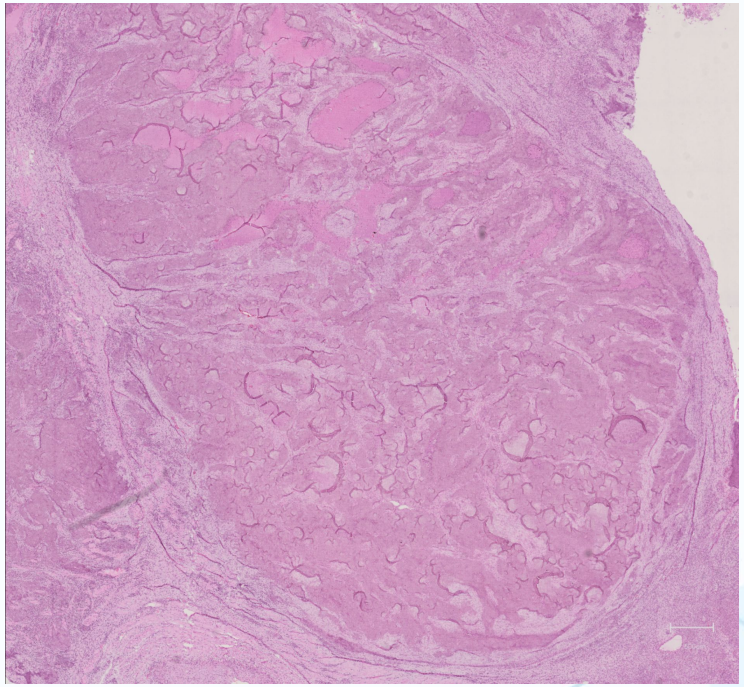
- HGSC is the most common histotype, accounting for over 70% of all ovarian cancer deaths

Current treatment regime:

- various combinations of surgery and 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**

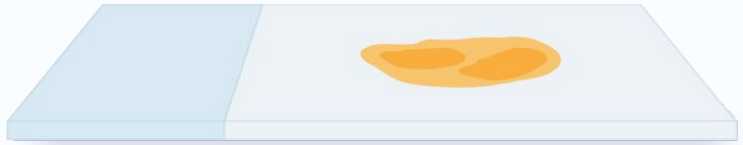


Materials + Methods - Tissue stack generation

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

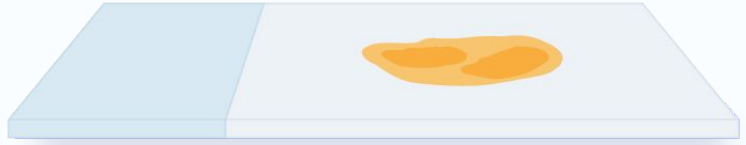
Visium (~55 µm)

- ➔ + H&E
- ➔ Spatial Transcriptomics

Lunaphore COMET

Multiplex IF (<1 µm)

- ➔ 20 markers
- ➔ + DAPI



Bruker timsTOF fleX

MSI (20 µm) - Erin Seeley

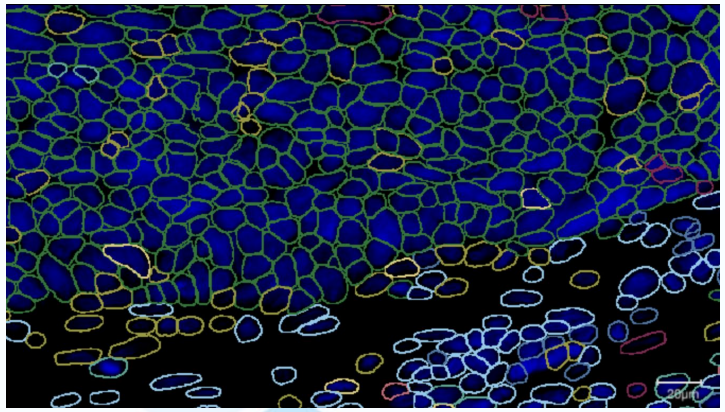
- ➔ metabolites
- ➔ glycans
- ➔ peptides
- ➔ post -measurement H&E

Why combine modalities?

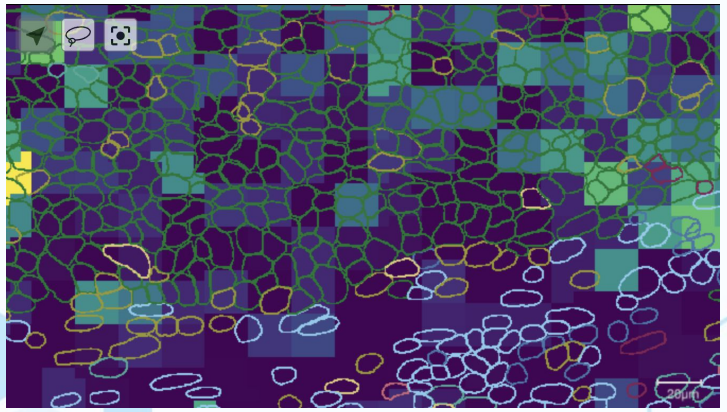
Immunofluorescence (IF) provides high resolution images where we can **identify** and spatially segment **individual cells**

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

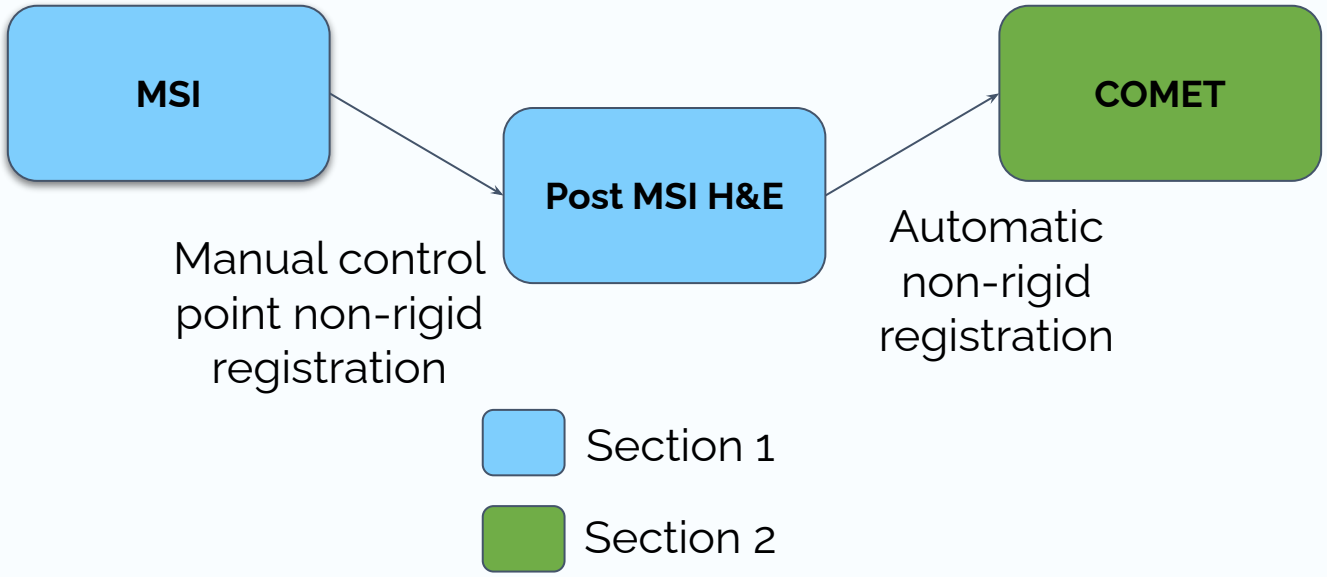
MSI provides **novel molecular information** that can only be obtained using the technique

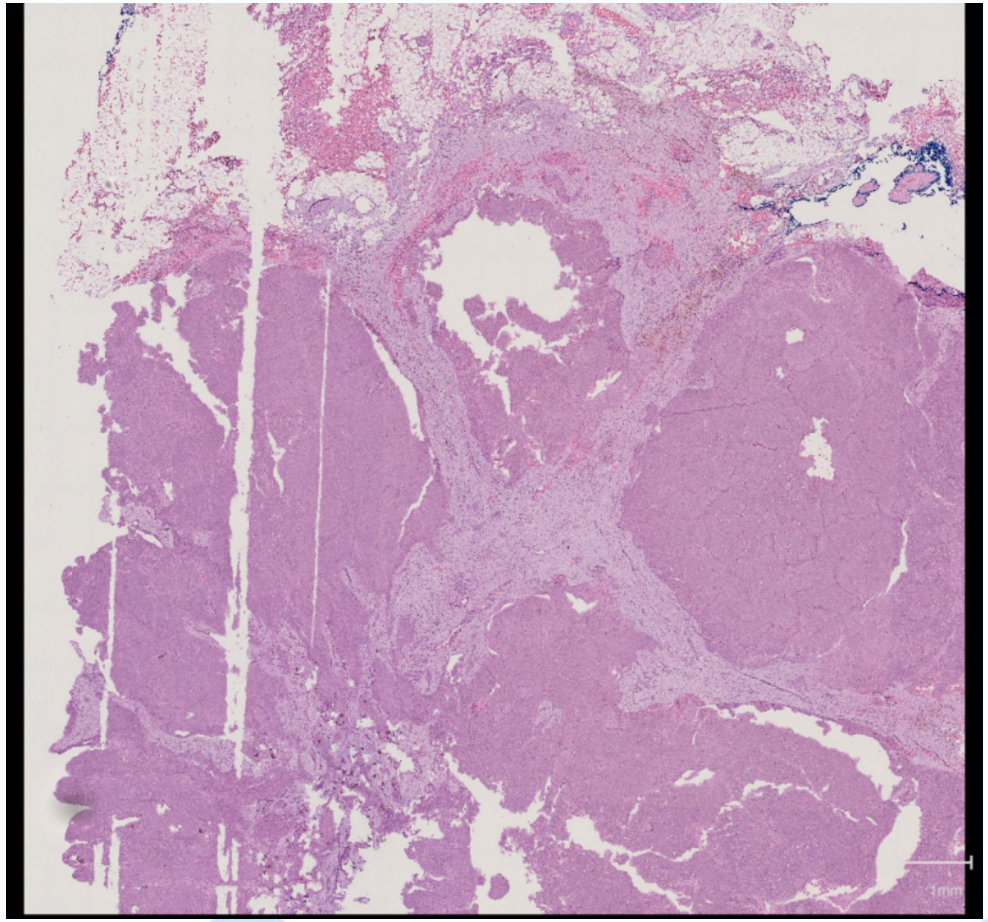


Describe novel spatial environments by molecular signals

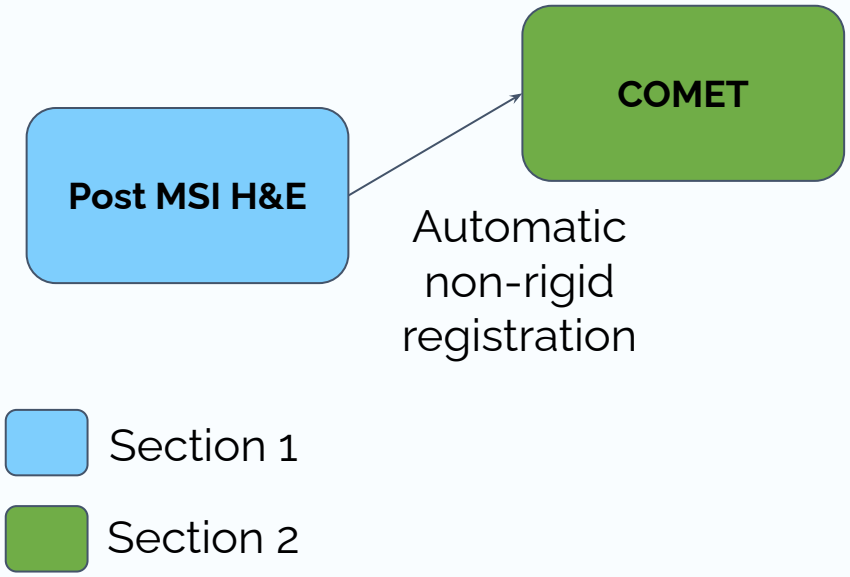


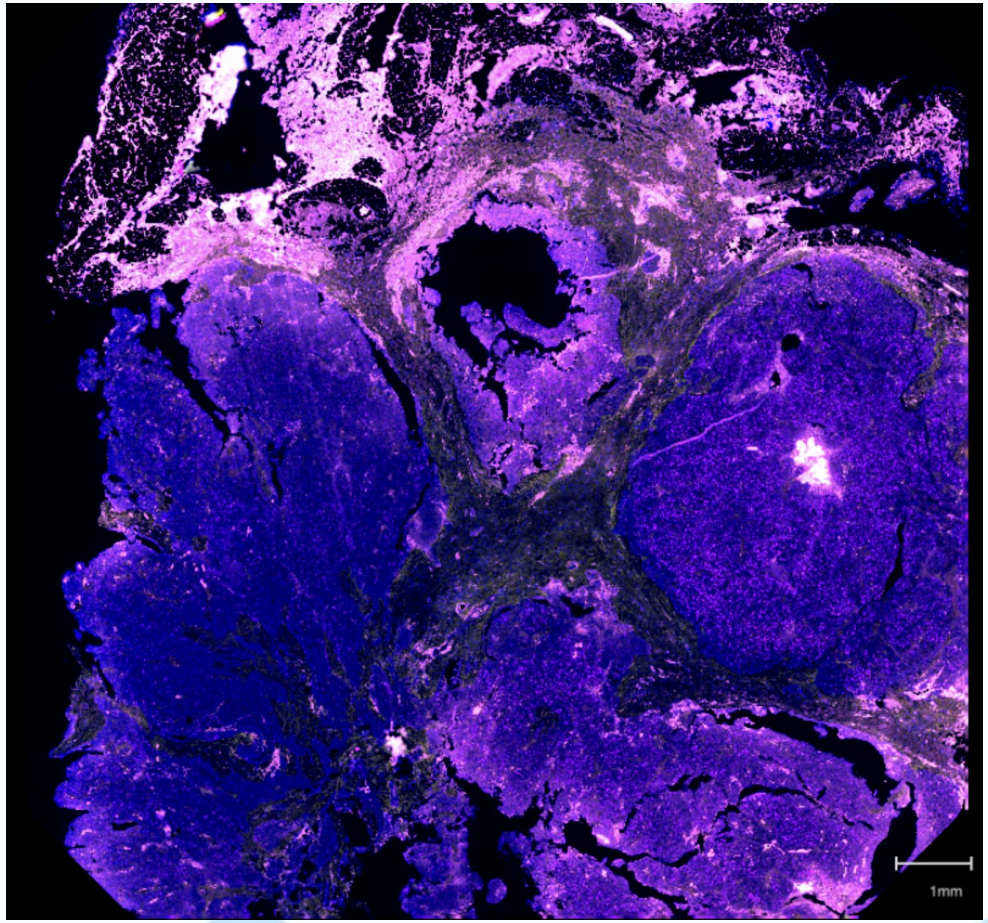
Step 1: multimodal co-registrations



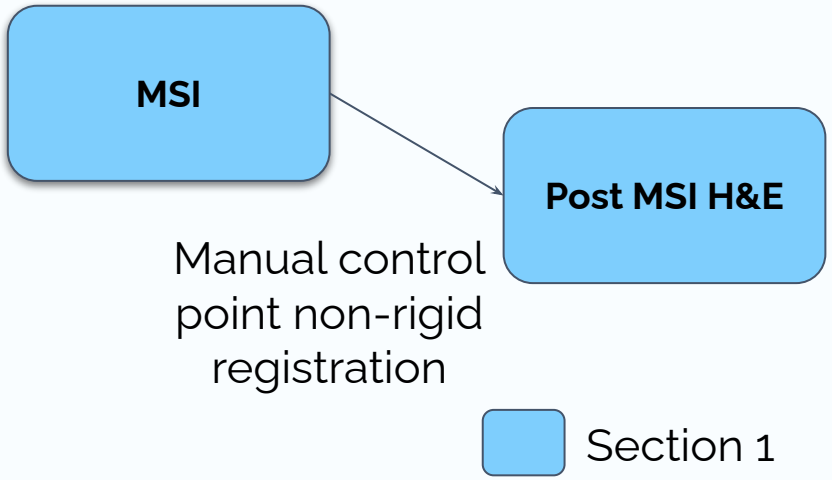


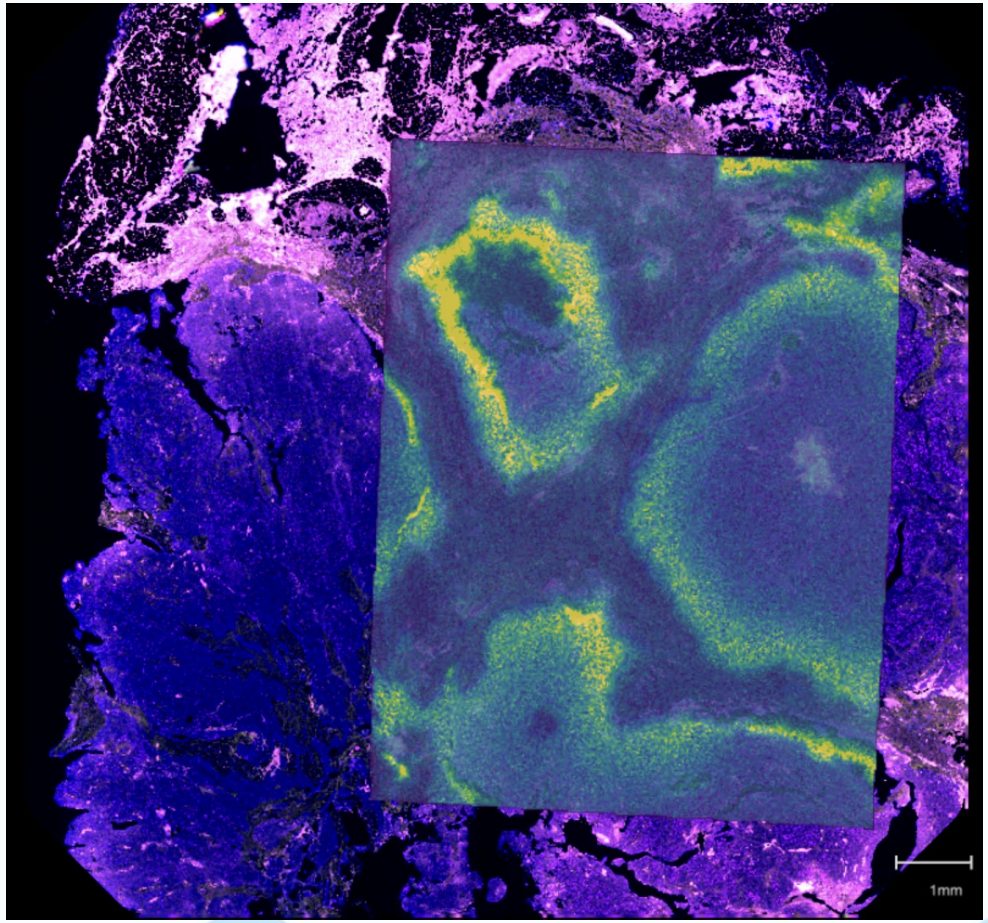
Step 1: multimodal co-registrations



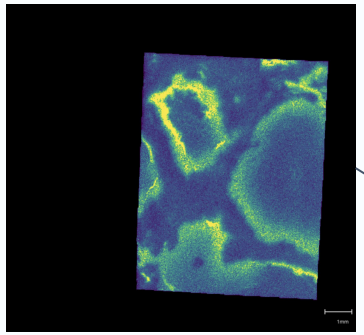


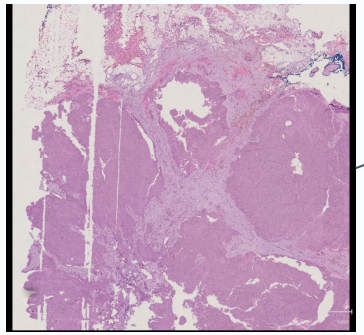
Step 1: multimodal co-registrations

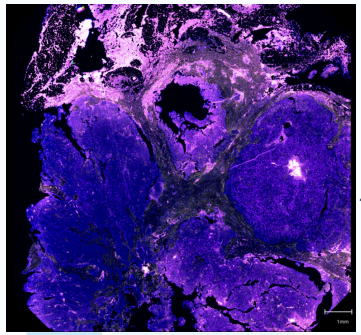




Step 2: matching MSI pixel footprint

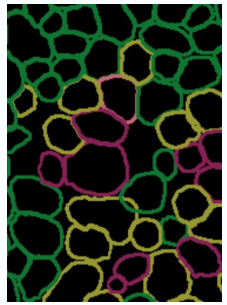
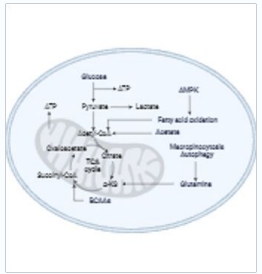
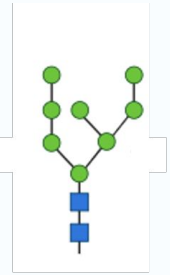






MSI pixels 1-n…

Aggregation to the MSI pixel level…

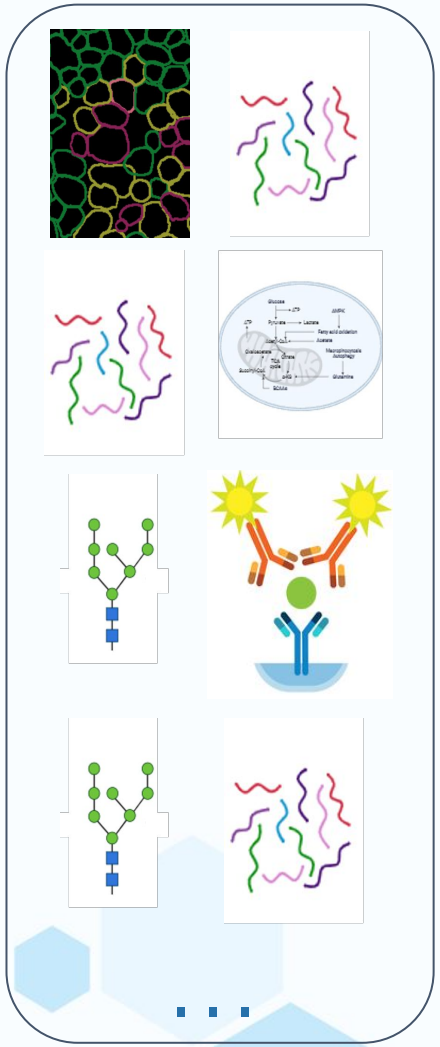
cell type proportion	antibody markers	MSI peptides	MSI metabolites	MSI glycans
				

weave

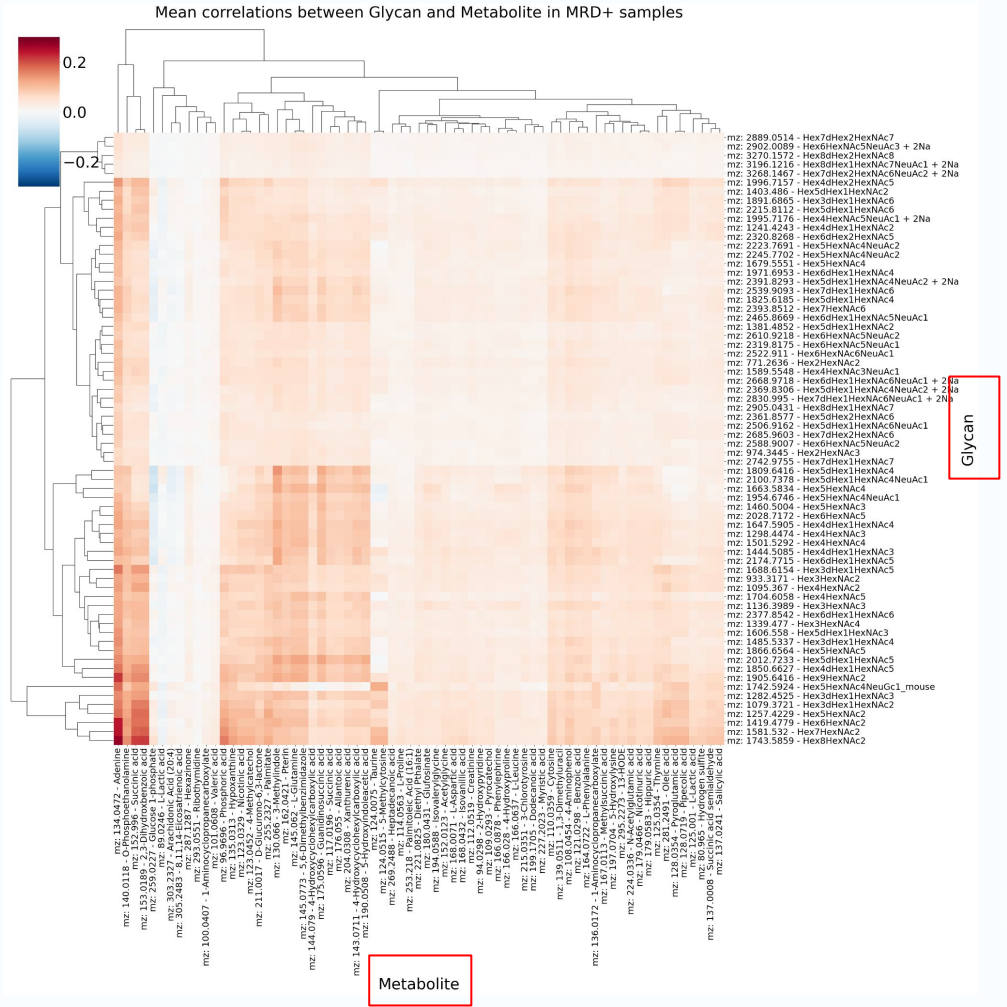
Correlating molecular readouts

Aspect Analytics

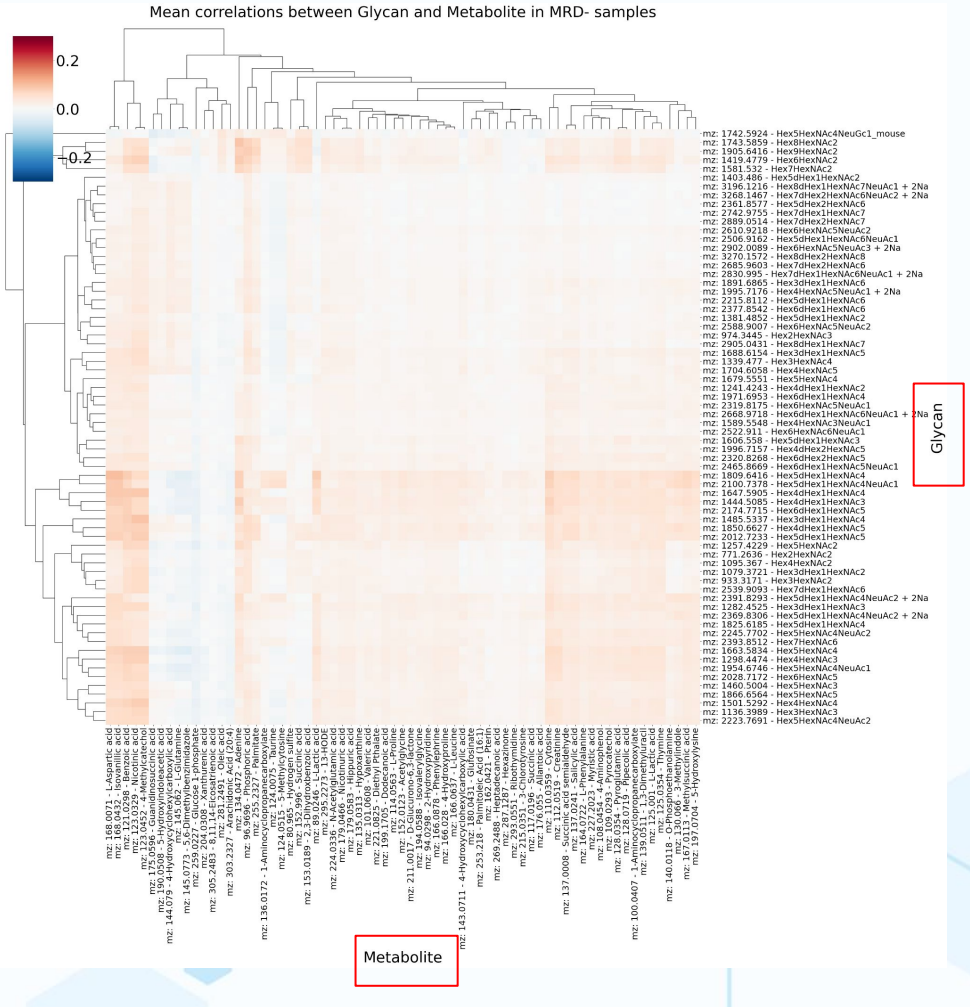
Data Pairs



MRD+



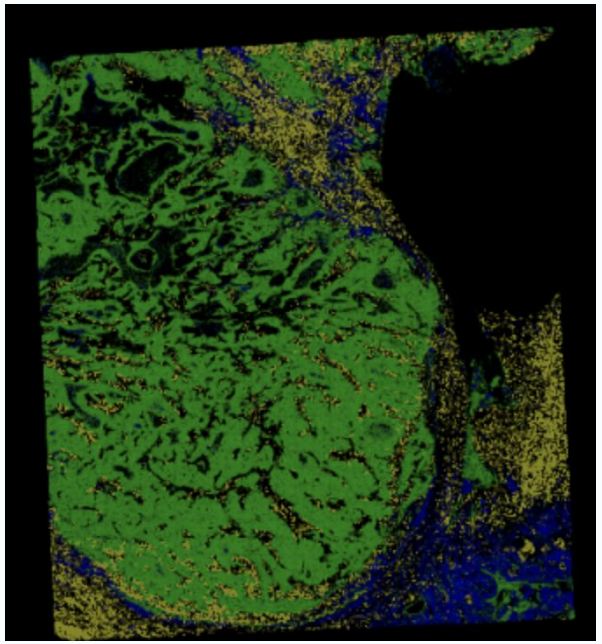
MRD-



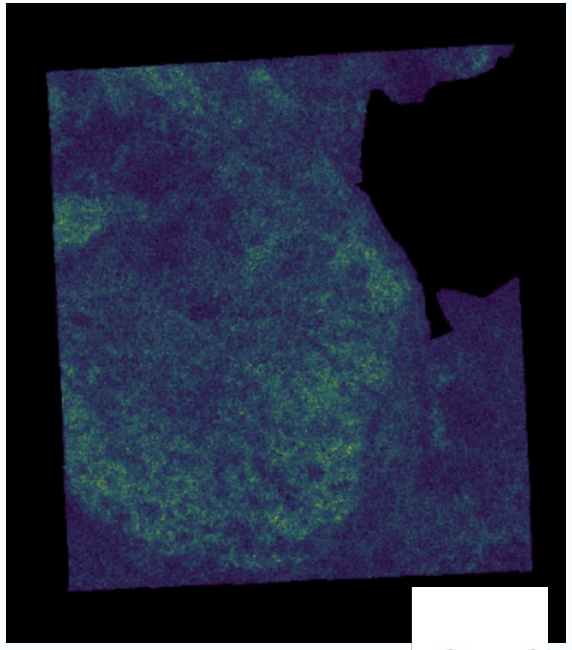
Differential MSI signals by cell type aggregation (tumor vs CAFs)

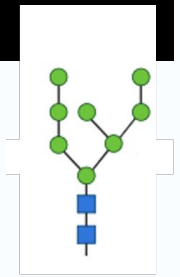
Aspect Analytics

Tumor



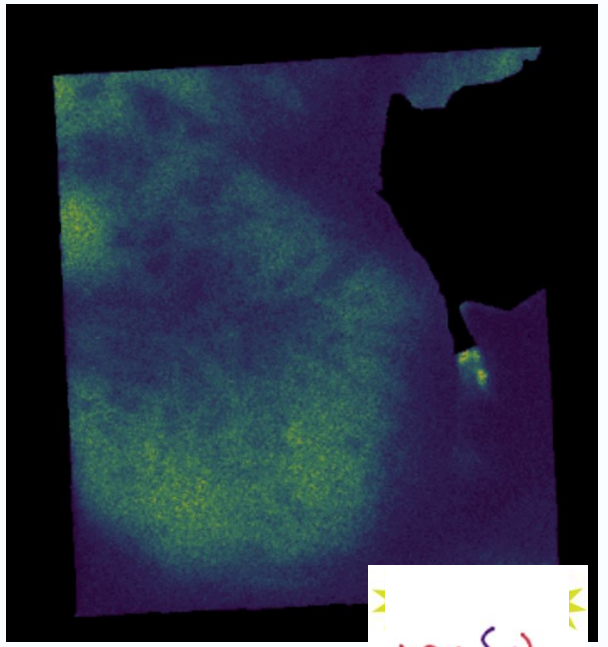
m/z 1742.5924 - Hex5HexNAc4NeuGc1_mouse

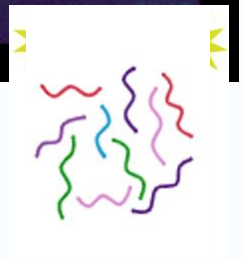




logfc ~0.53

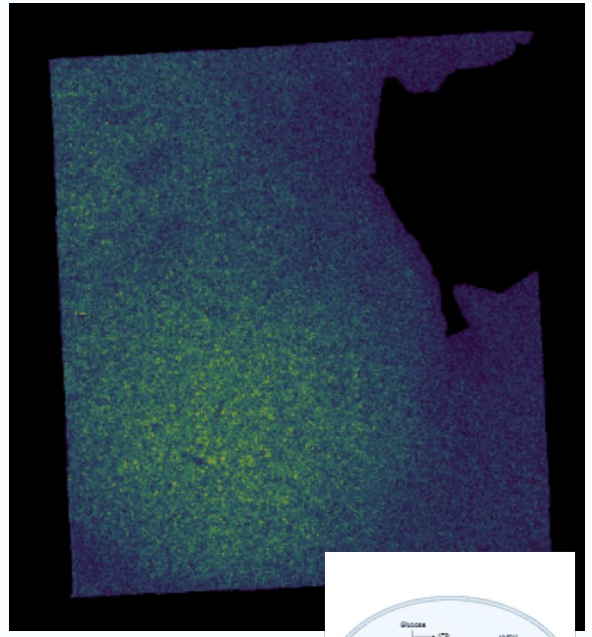
m/z 1325.757 - histone H4





logfc ~0.92

m/z 128.0354 - Pyroglutamic acid



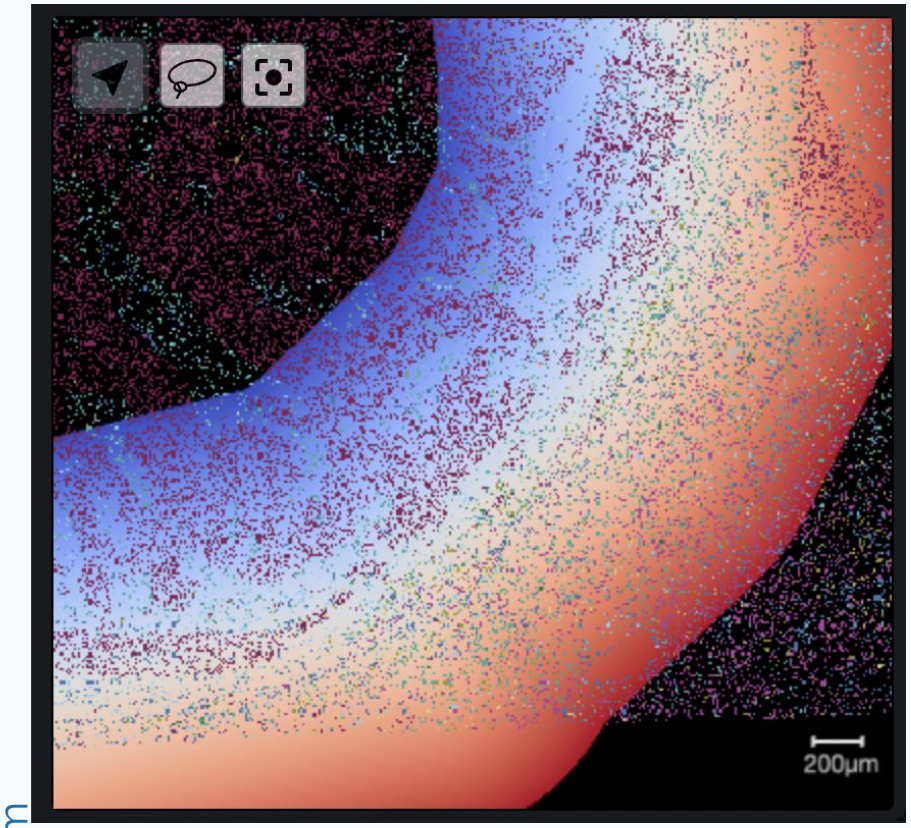
logfc ~0.69

Tumor

Cancer Associated Fibroblasts

Immune cells

Spatial exploration of Tumor-Stroma interface



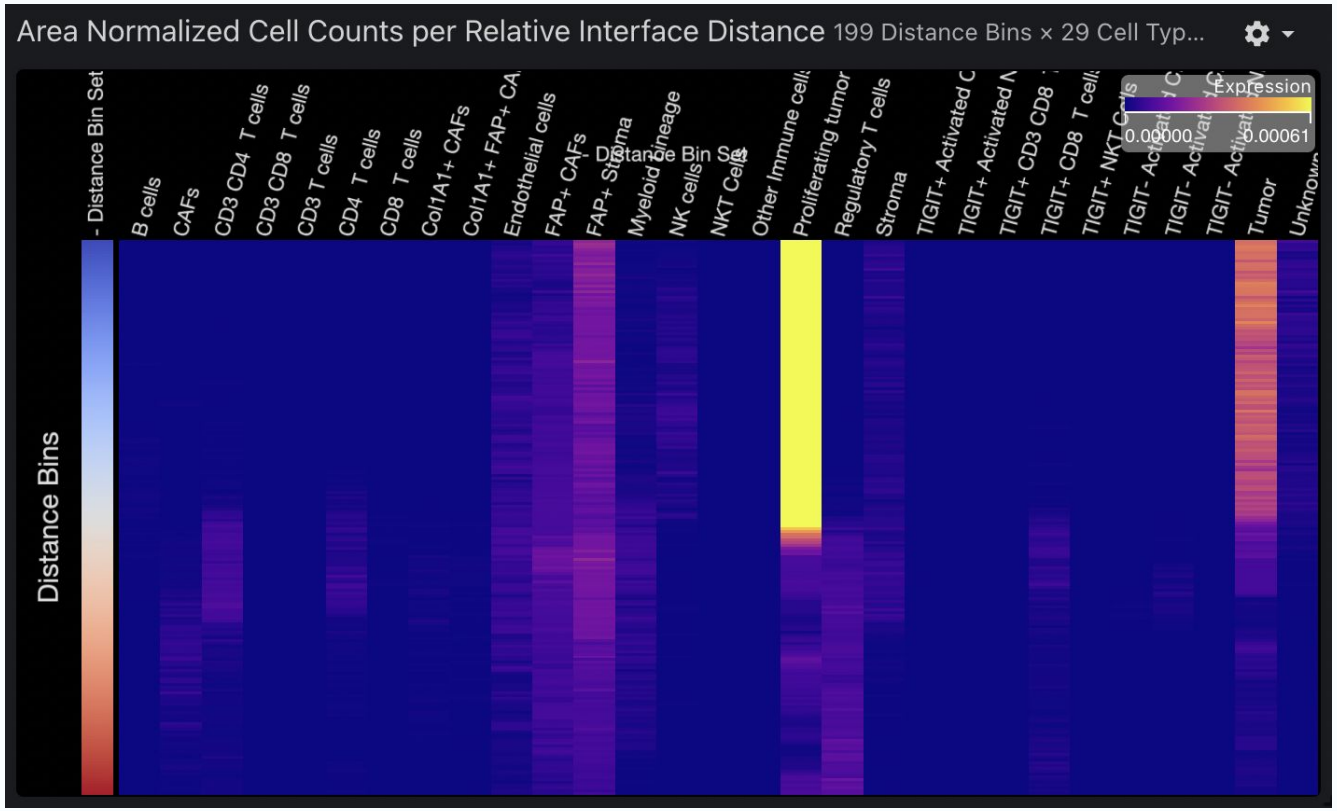
-1000 µm

Tumor

Stroma

+1000 µm

Interface



Xenium reproducibility analysis

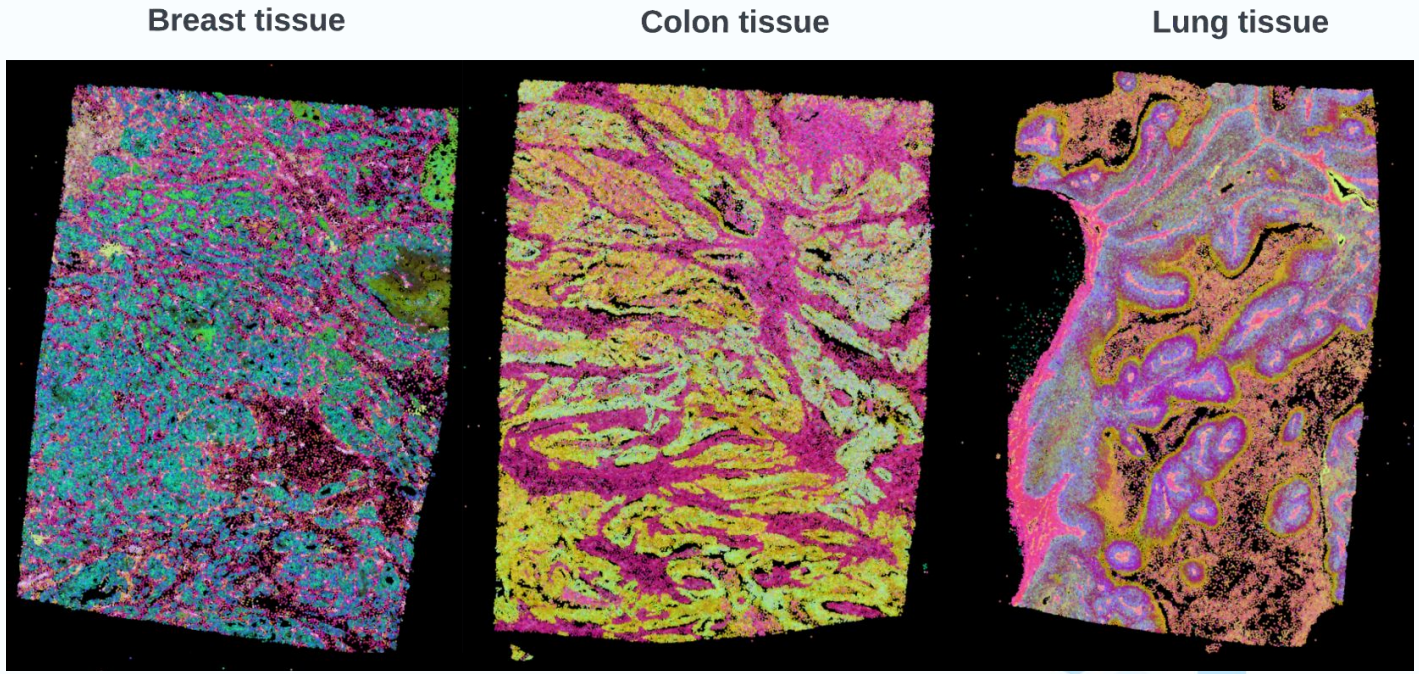
Aspect Analytics

BioChain®

Vidyodhaya Sundaram
Rikita Gakhar

Goal: assess reproducibility of Xenium assay

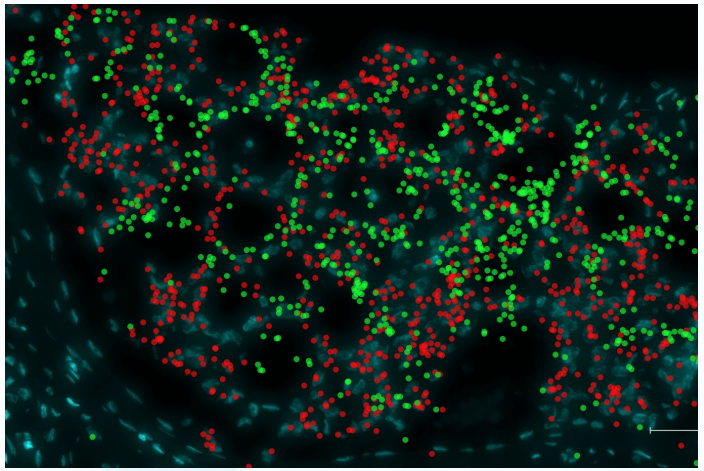
- Analyzed **Xenium data acquired from 2 serial sections**
  - Experiments run by different operators
- Performed the analysis on **3 human carcinoma FFPE tissue types**



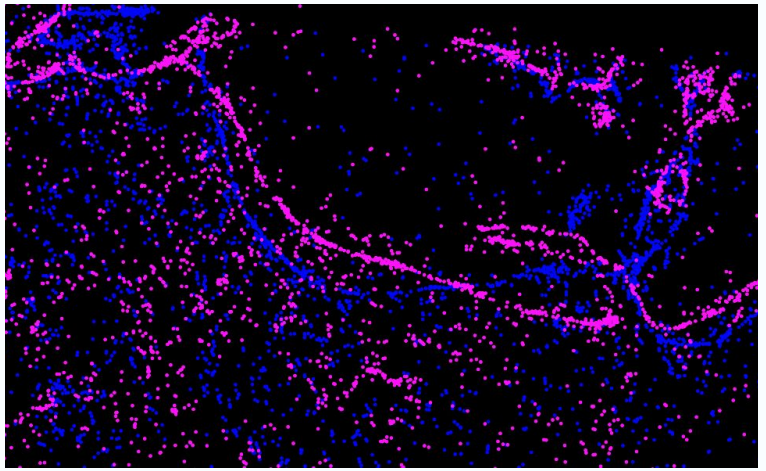
Xenium reproducibility analysis

Leveraging Weave

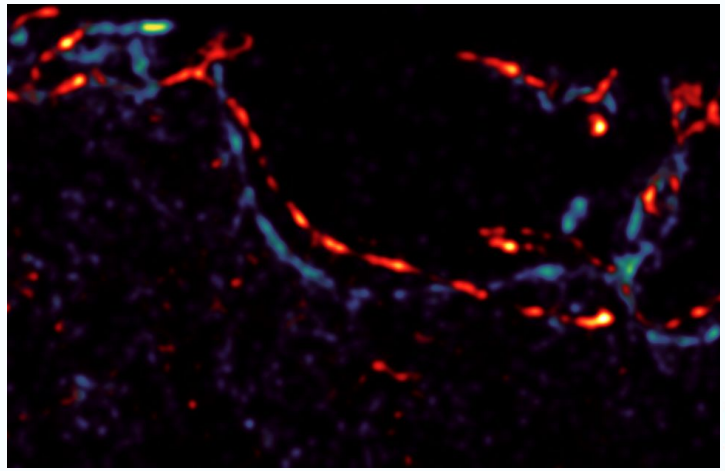
- **Automatic registration between each pair of Xenium data sets**
  - Per section, DAPI to H&E stained microscopy
  - Between sections, H&E to H&E
- **Joint data visualization of each pair into a single view**



ABCC11 for each section (red/green)



MYLK transcripts per section



MYLK density per section

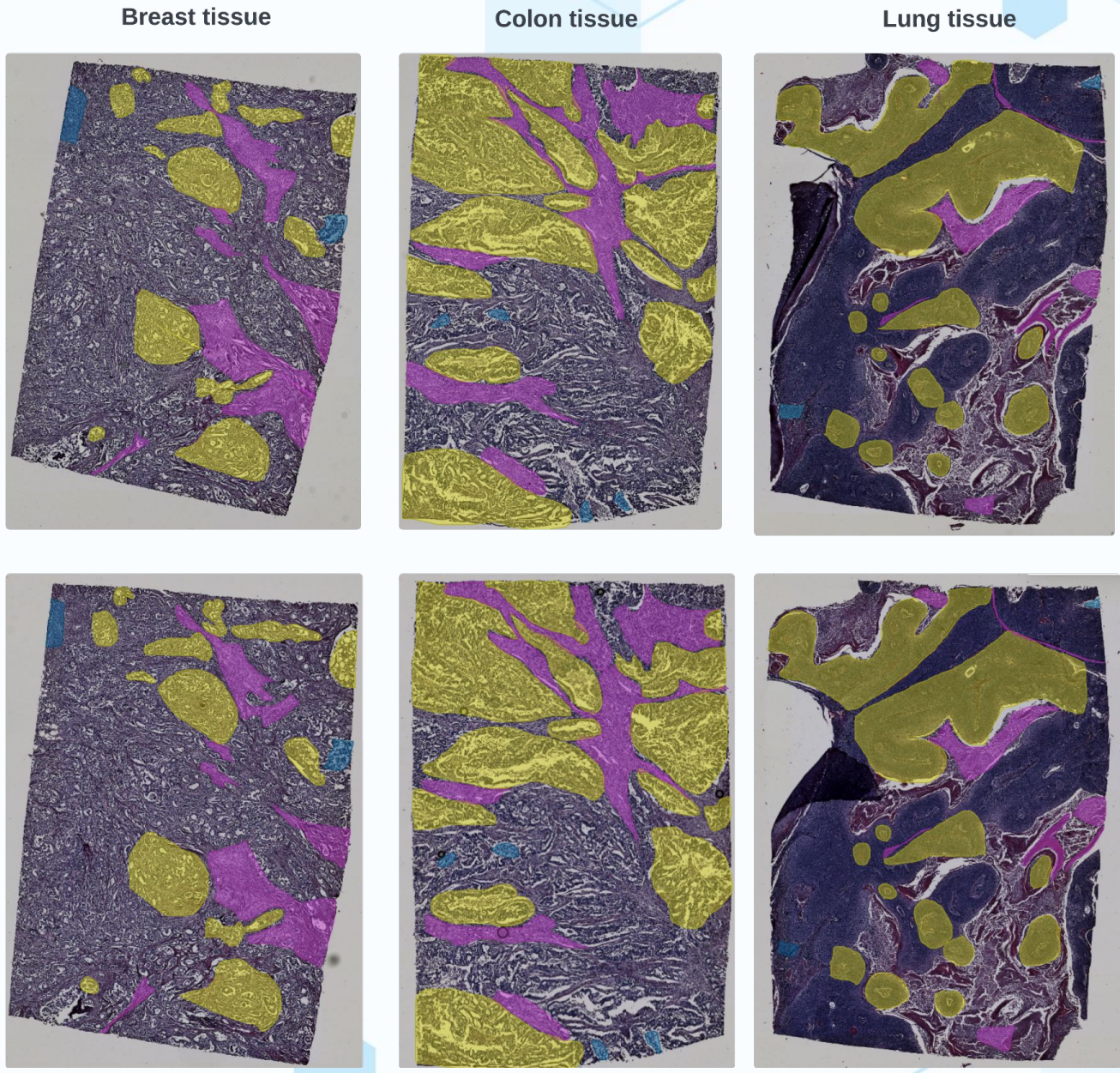
Statistical check

Analysis 2: assess whether transcript count distributions are comparable

- Stratified across gross morphological regions defined by expert pathologists
- Non-parametric KS test for distribution similarity per region x gene pair

Tissues	Tumor region	Stroma region	Immune region
Breast	90%	90%	89%
Colon	98%	94%	99%
Lung	94%	100%	99%

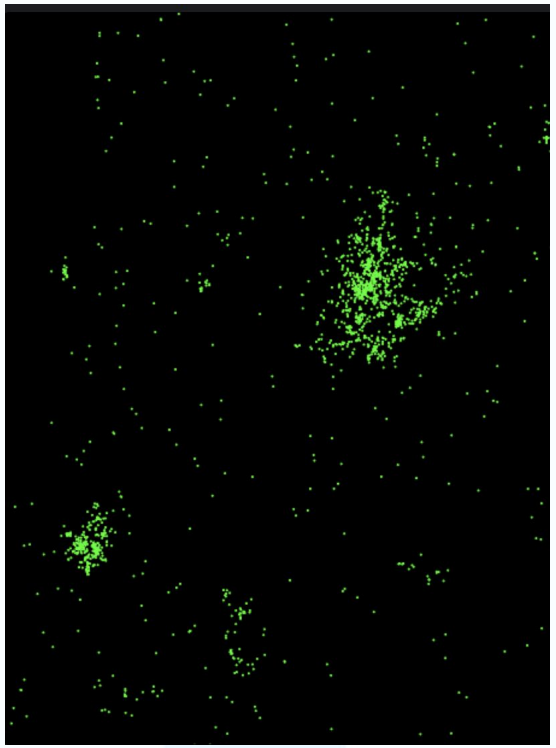
Percentage of genes with equivalent distribution per region.



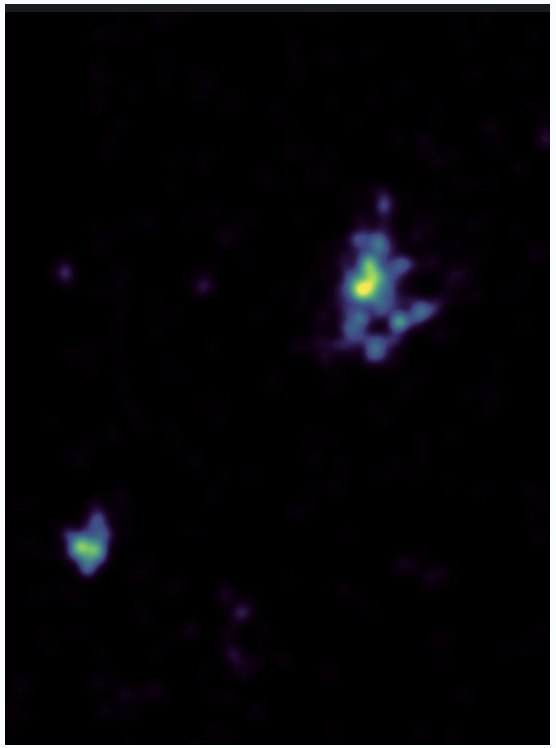
Xenium - Visium comparison

Analysis 3: check concordance of Xenium & Visium on same section

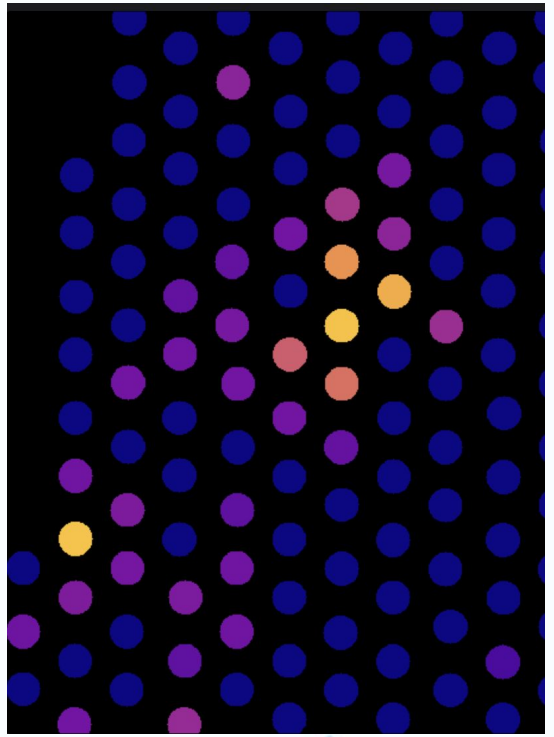
- Investigate gene expression in same area (here CXCL9)



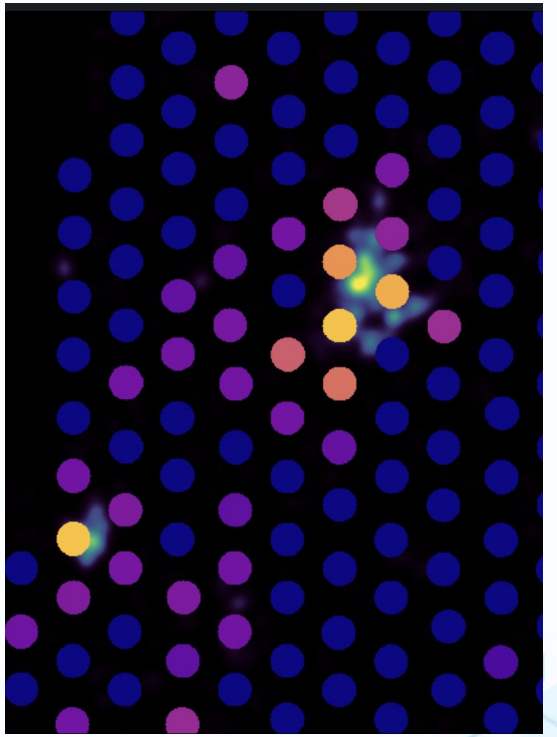
CXCL9 Xenium (spots)



CXCL9 Xenium (density)



CXCL9 Visium (spots)



CXCL9 overlay

Conclusion

Conclusion

Cloud platform for spatial multi-omics

- Foundation for data management and analysis
- Customized fit-for-purpose data workflows
- Vendor neutral

Collaboration & services

- Research partner for high-end bioinformatics
- Development of new assays and applications
- Deep integration with vendors

Addressing spatial multi-omics challenges

- Integration across analytical platforms
- Supporting high-throughput workflows
- Effective communication of spatial omics insights

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