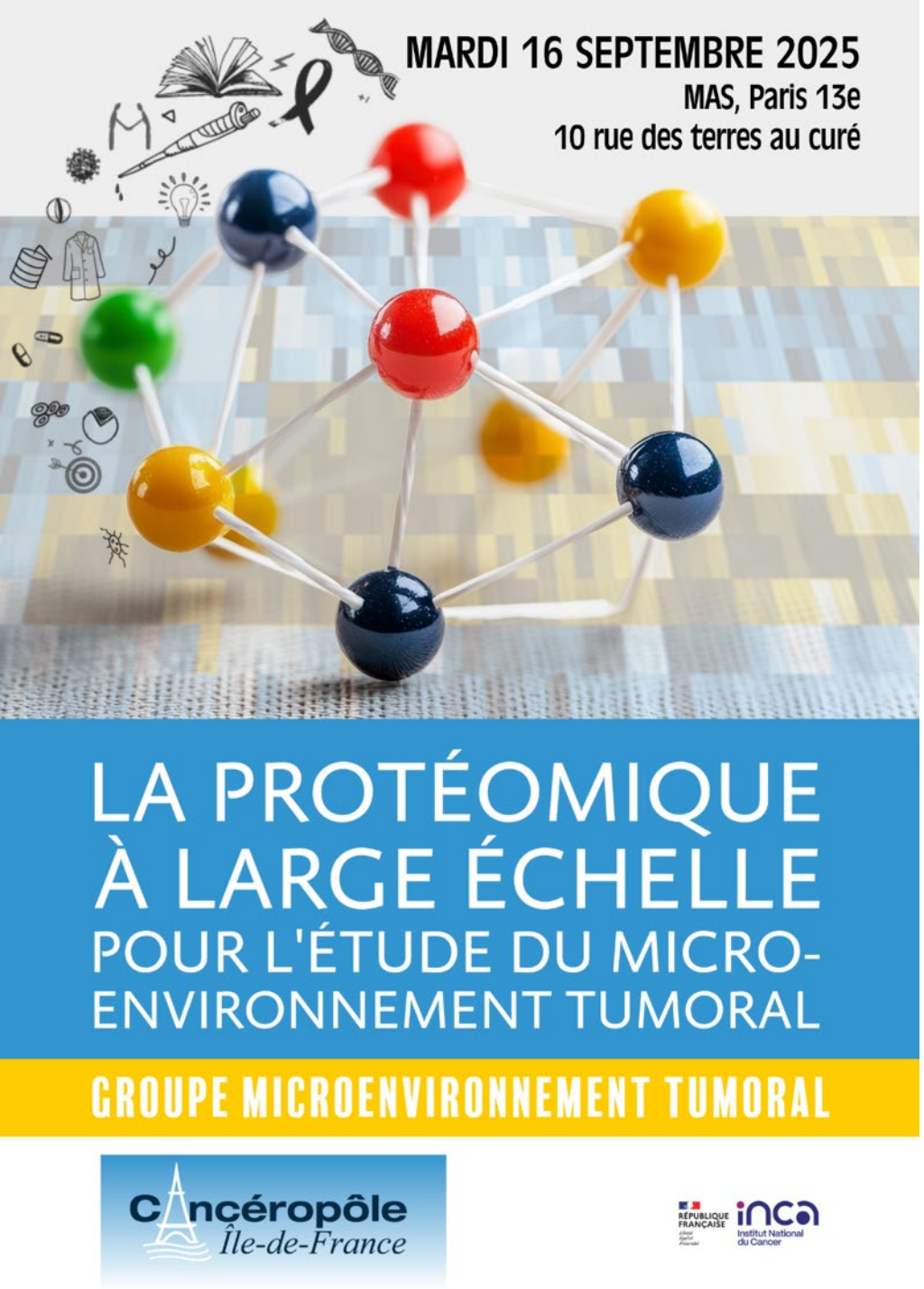
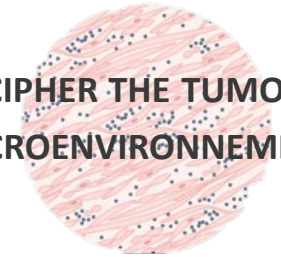


SPATIAL PROTEOMIC

Mass Spectrometry Imaging

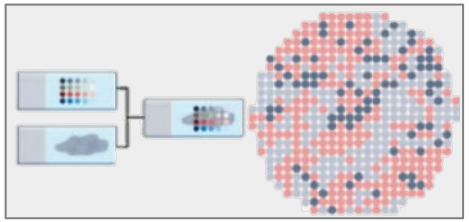
Hélène Cazier and Rémy Nicolle

Decipher the tumoral microenvironnement
Toward spatial proteomic approaches

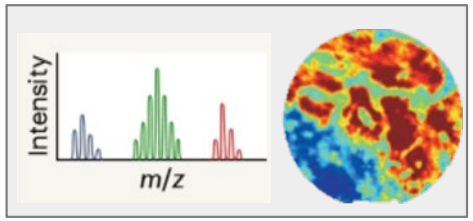


Bulk approaches	Spatial approaches	Single Cell approaches
Homogenized molecular information	Regional level heterogeneity	Cellular level heterogeneity
High coverage information with PTMs information	Low coverage information	Full repertorie molecular state
"Quick and cheap" sample prep Specialized equipment	Specialized equipment Time consuming sample prep	Current cost are restricting

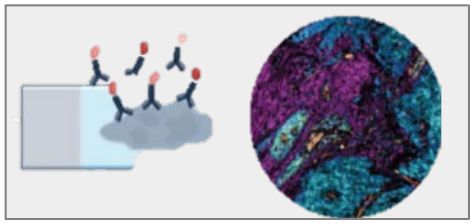
SPATIAL OMICS APPROACHES



TRANSCRIPTOMICS



METABOLOMICS AND LIPIDOMICS



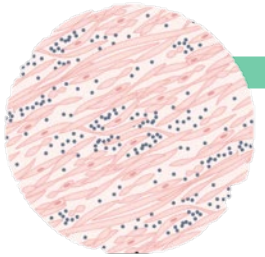
PROTEOMICS

L. A. Walsh et D. F. Quail, « Decoding the tumor microenvironment with spatial technologies », *Nat Immunol*,

Towards spatial proteomics

Spatial proteomics approaches

DECIPHER THE TUMORAL MICROENVIRONNEMENT



Why proteins ?

Proteins are the functional molecules carrying most cellular functions

Post-translational modifications critical for activity, localization and interactions

Many biomarkers and drug targets are actually proteins

TARGETED PROTEOMIC

Immunofluorescence

IHC multiplex

CyTOF
MALDI HiPLEX-IHC

UNTARGETED PROTEOMIC

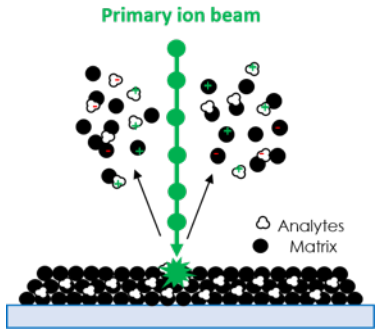
Mass spectrometry Imaging

Top down (Protein Intact)

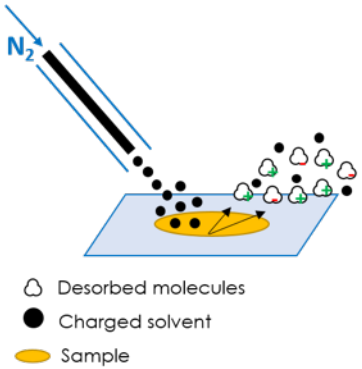
Bottom up (digested peptide)

Towards spatial proteomics in mass spectrometry

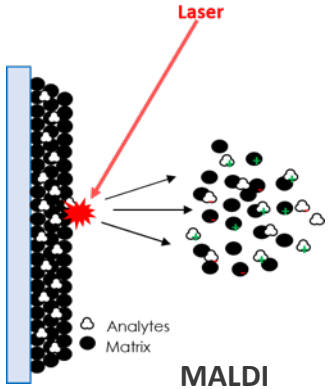
Samples and methods



SIMS
Secondary **I**on **M**ass **S**pectrometry



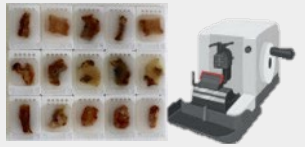
DESI
Desorption **E**lectro**S**pray **I**onisation



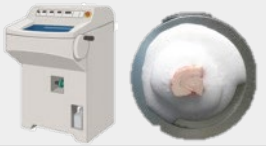
MALDI
Matrix **A**ssisted **L**aser **D**esorption **I**onisation

Protocols for sample conservation

FFPE tissues
= Formalin Fixed Parafin embedding tissue



Fresh frozen tissues
(snap frozen : nitrogen/isopentane, stored at – 80°C



Samples treatments on slides

Delipidation (chloroform)

Desalting (ammonium sulfate)

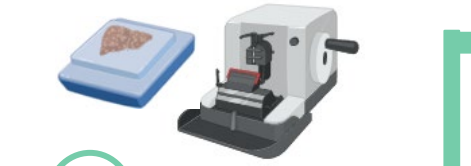
Antigenic retrieval

Derivatization : aromatic ring or charge addition

On tissue digestion (Trypsin, PNGase …)

Towards spatial proteomics in mass spectrometry

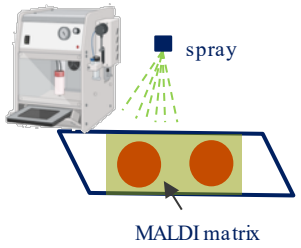
MALDI Mass spectrometry imaging

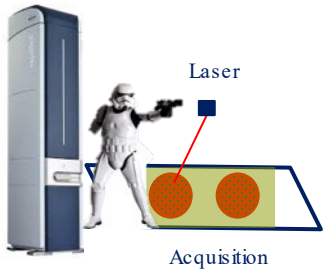


Microtome (Leica)

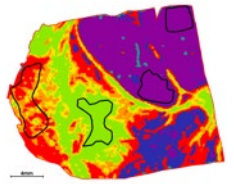
1

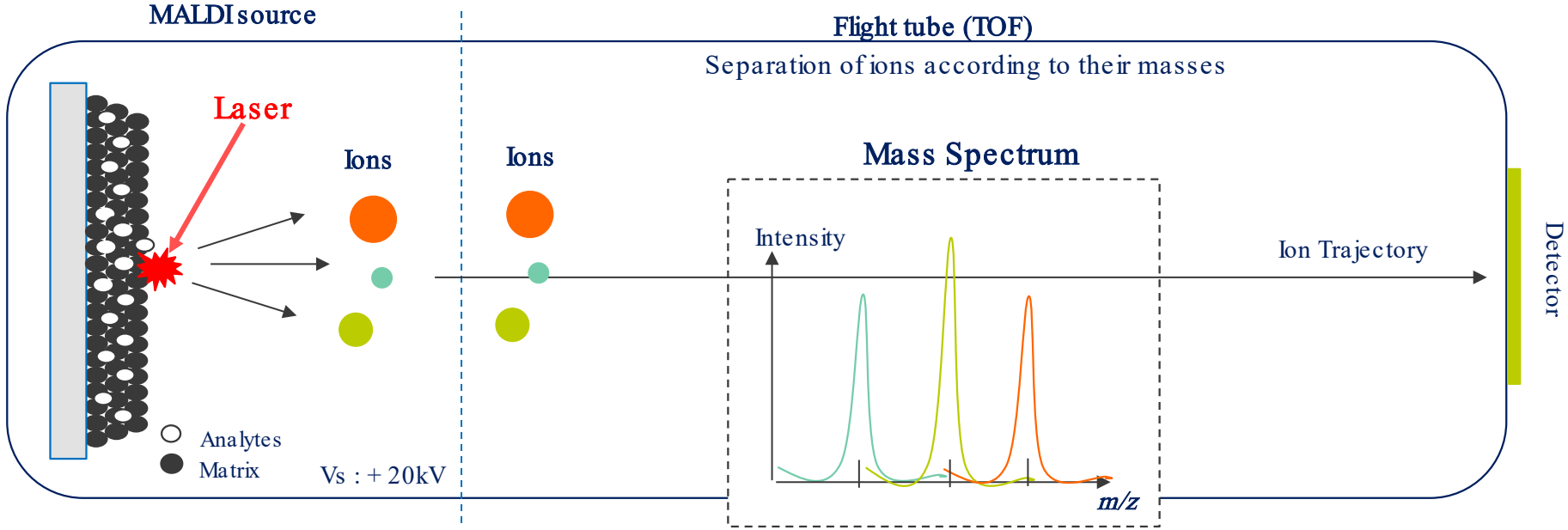
Tissue slicing





Scils Lab Pro





1. Co-crystallization of the matrix with the sample
2. Proton/electron exchange
3. Desorption and ionization of molecules

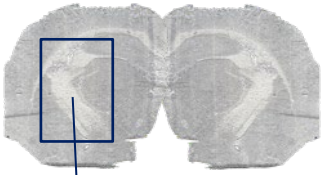
Molecular composition of the sample

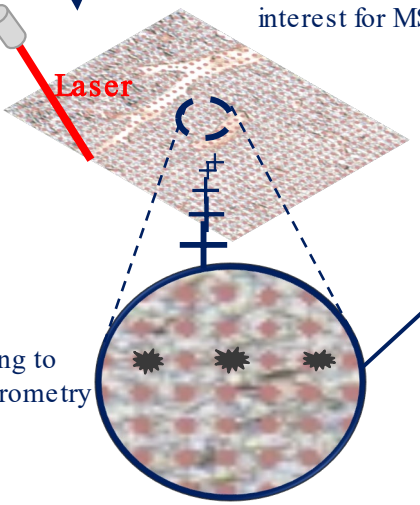
Towards spatial proteomics in mass spectrometry

MALDI Mass spectrometry imaging

1 Microtome (Leica)

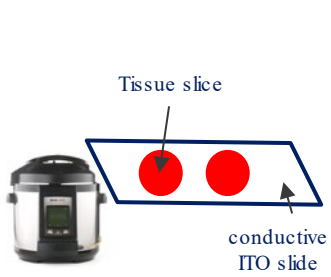
Tissue slicing

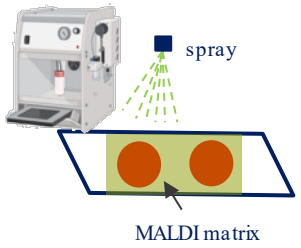


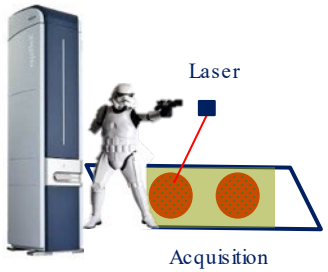


1. Creation of regions of interest for MSI analysis

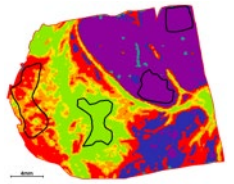
2. Laser travel according to predefined mass spectrometry parameters

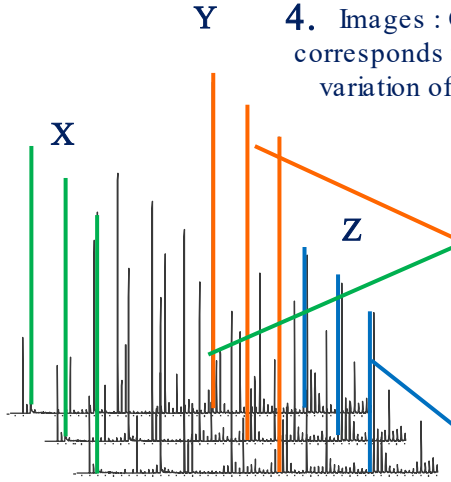






Scils Lab Pro





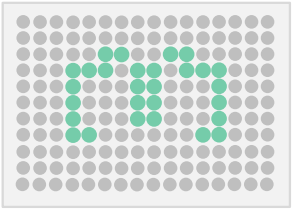
3. Each laser impact will correspond to an acquired spectrum

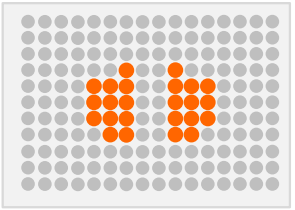
4. Images : One image corresponds to the intensity variation of a single m/z

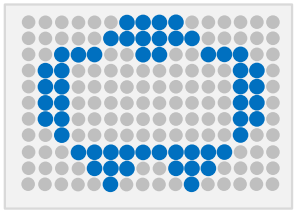
Ions X

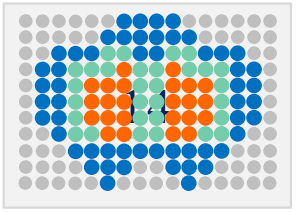
Ions Y

Ions Z





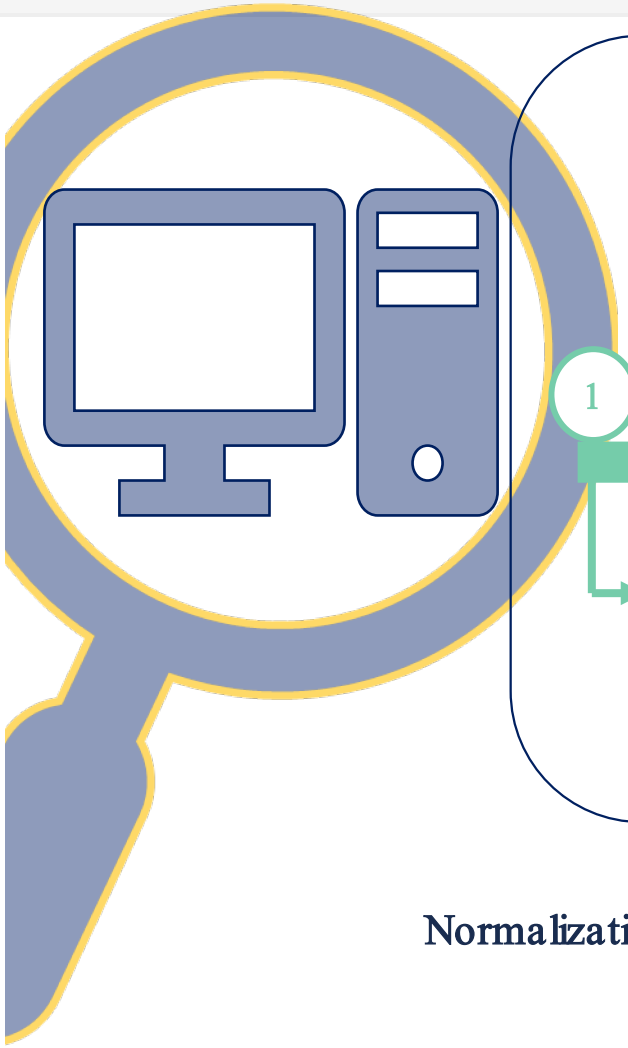


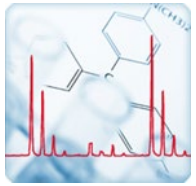


5. Overlayed of molecular images on tissue sections

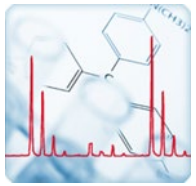
Towards spatial proteomics in mass spectrometry

Software and important parameters





flexcontrol



flexImaging

SCiLS

Metadata
Mass spectra information
Multimodal imaging

1 Acquisition parameters

- Mass range (m/z)
- Laser power (%)
- Number of Data point / spectra
- Calibration
- Noise removal

2 Imaging files

- Spatiale resolution (µm)
- Region of interest
- Data point reduction

3 Data analysis file

- Baseline reduction
- Normalisation
- Segmentation
- PCA
- Statistical analysis
- Correlation between images

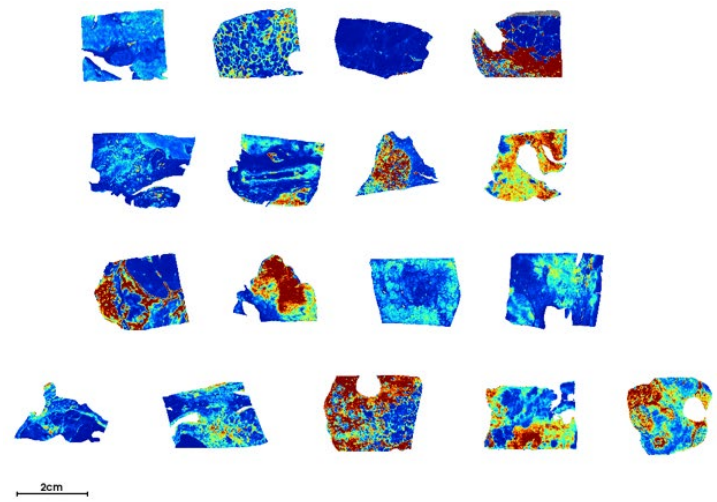
3 The exports

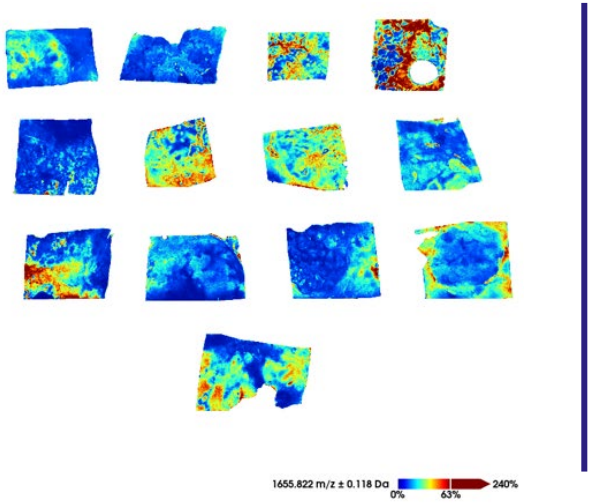
- .mis
- .imzml
- .ibd
- .TIFF
- .svs

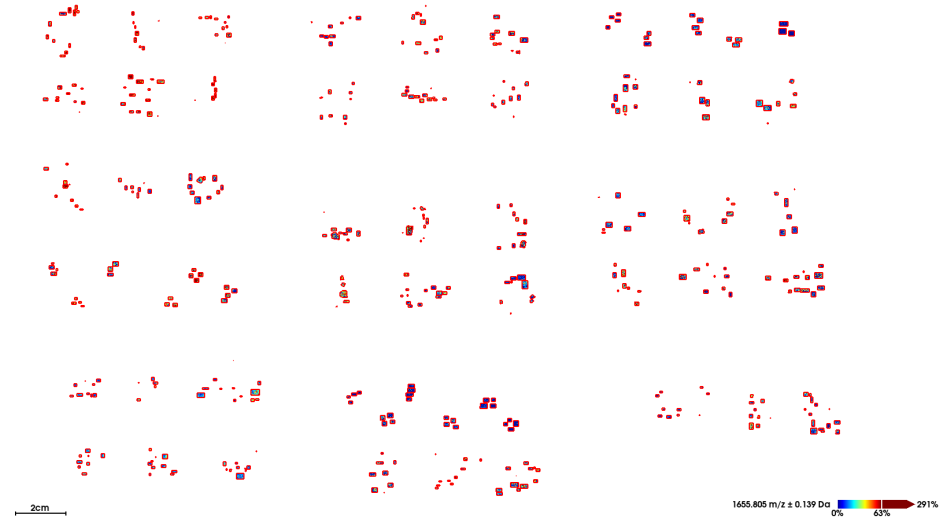
Normalization -> Smoothing -> Baseline reduction -> Peak detection and alignment

Images superposition : MALDI Scan, HES and or IHC

Towards spatial proteomics in mass spectrometry
Analytical challenge and imaging data integration







Projet MAIA : 30 slices of cHCC-CCA
Acquisition : 100 µm on whole slice
Raw = 360 Go
Processed data = 1,656 To
Scan HES + IHC = 665 Go

Mean of 39 000 pixel / slices
1,17 millions of pixels

Projet CHRIS : 50 slices of ADK
Acquisition : 50 µm on whole slice
Raw = 2,5 To
Processed data = 7 To
Scan HES = 150 Go

Mean of 120 000 pixel / slices
6 millions of pixels

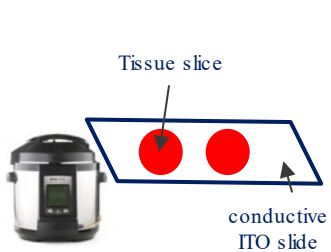
61 slices of lung
Acquisition : 35 µm on partial slice
Raw = 482 Go
Processed data = 677 Go
Scan HES = 100 Go

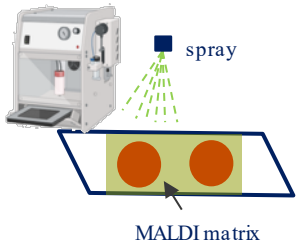
Mean of 26 000 pixel / slices
1,59 millions of pixels

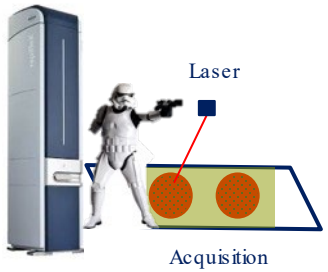
Towards spatial proteomics in mass spectrometry

MALDI Mass spectrometry imaging

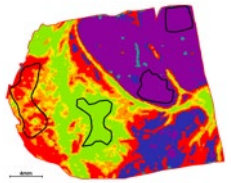
Microtome (Leica)







Scils Lab Pro



1

Tissue slicing

Benefits

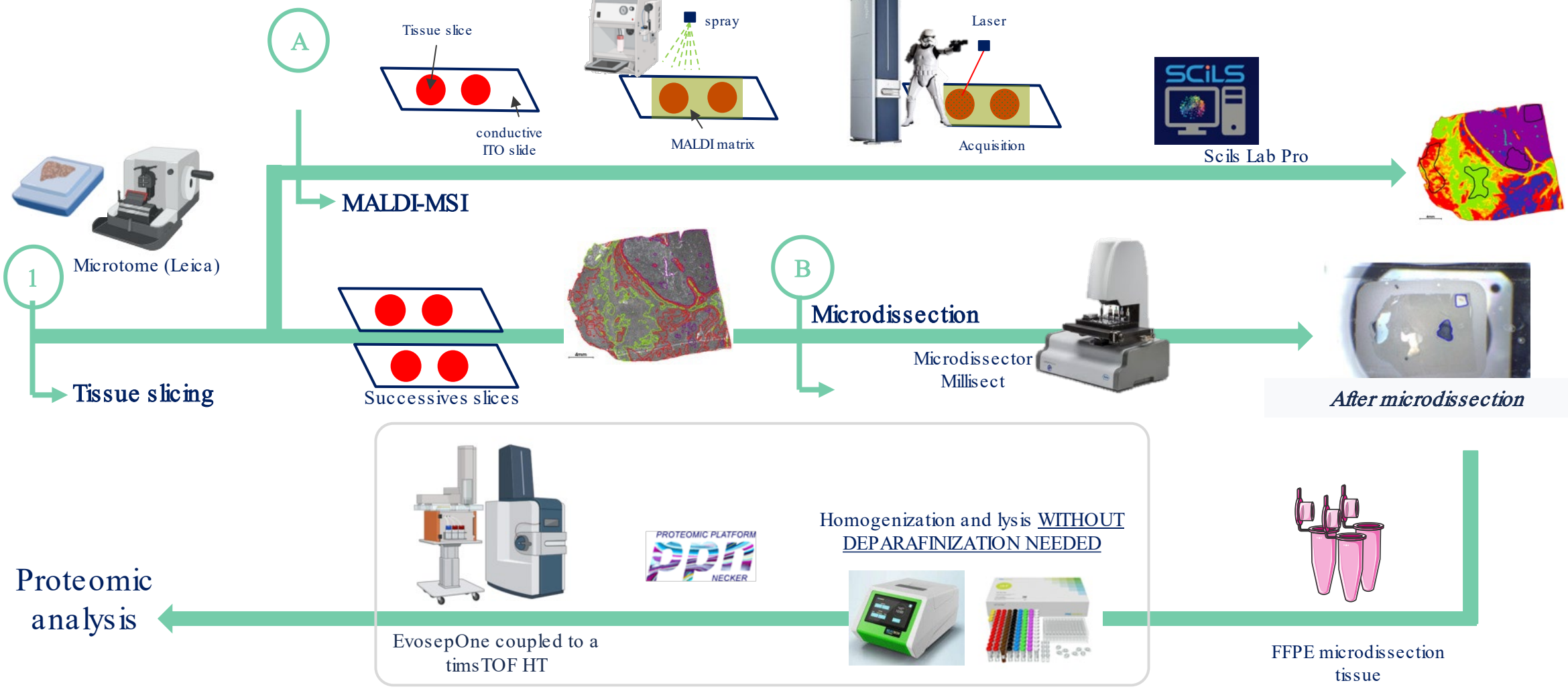
- ✓ Molecular biodistribution of protein
- ✓ Images acquisition at high spatial resolution
- ✓ Knowledge's about molecules co-localization on tissues
- ✓ Detection in a label-free manner
- ✓ Provide complementary information's with other imaging techniques as autoradiography, immunohistochimestry and immunofluorescence

Drawbacks

- ✓ Time consuming workflow
- ✓ Big amount of data generated
- ✓ Successive slides to overlayed according approaches
- ✓ Tissue morphology sometimes difficult to preserve
- ✓ Low sensitivity or sample characterization compare to LCMSMS

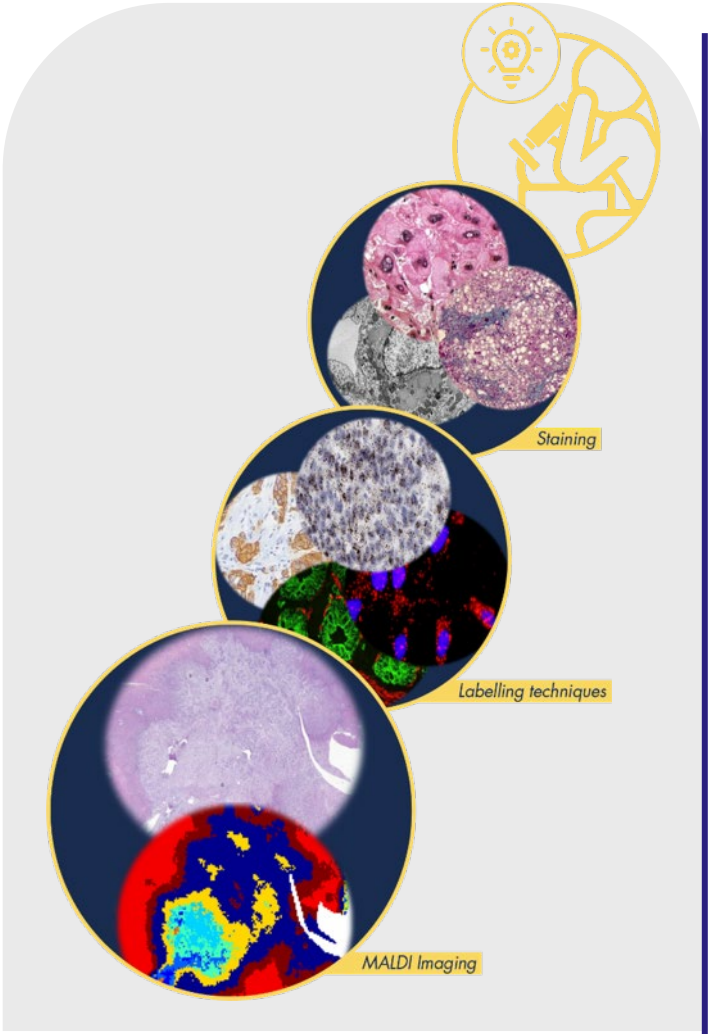
Towards spatial proteomics in mass spectrometry

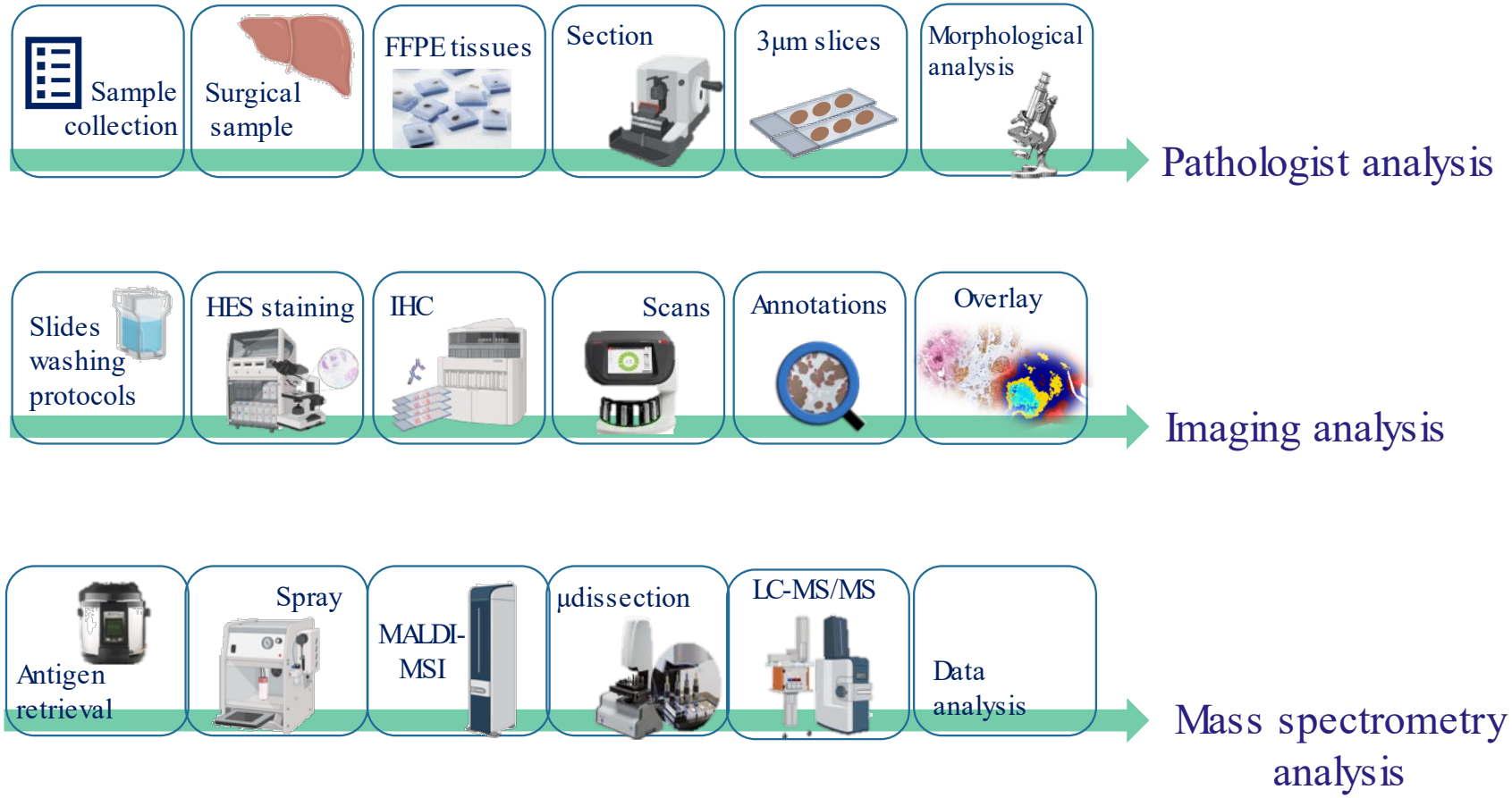
Microdissection based on MALDI imaging



Towards spatial proteomics in mass spectrometry

Integration of MALDI-MSI in pathology routine





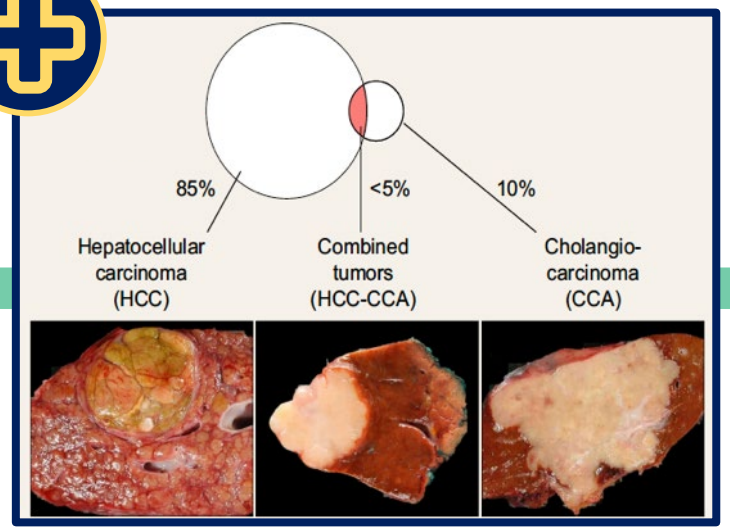
Bioinformatics needed to combine all of those data

Towards spatial proteomics in mass spectrometry

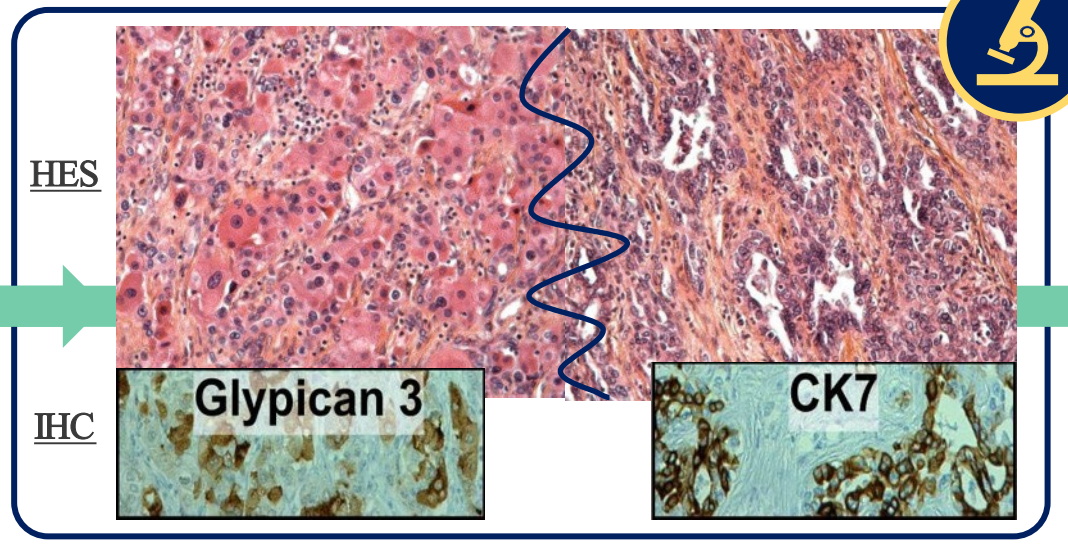
cHCC-CCA analysis with mass spectrometry

Hepatocytic and cholangiocytic contingent sometimes associated with a “intermediate" contingent within the same tumor

Primary Liver Carcinomas (PLC)

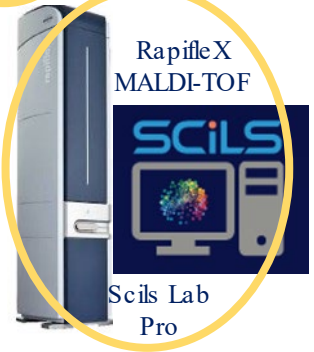


HCC *cHCC-CCA* *CCA*



cHCC-CCA

MALDI-MSI



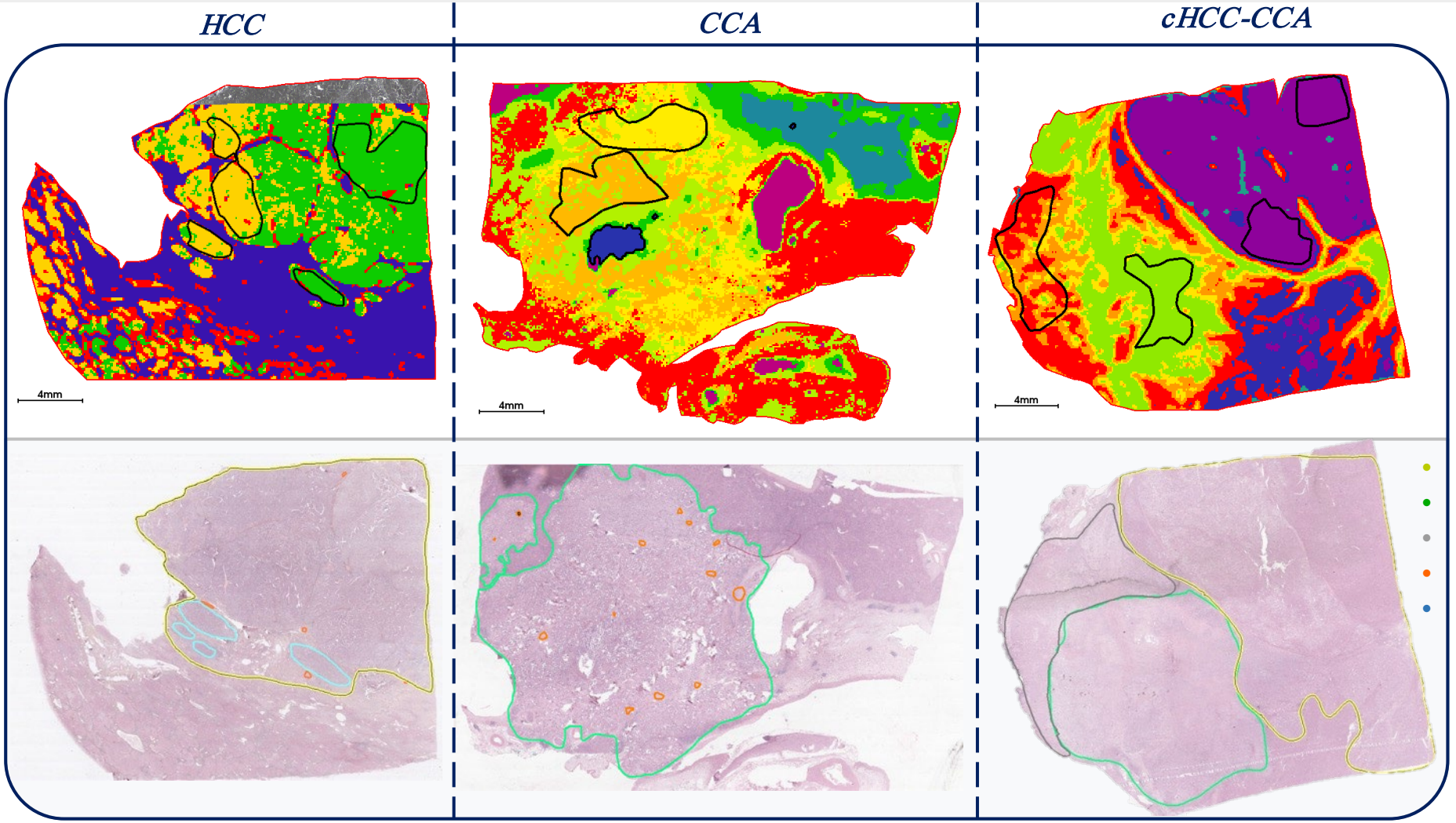
BETTER CHARACTERIZATION OF MIXED TUMORS FOR IMPROVED DIAGNOSIS AND TREATMENT OF CHCC-CCA TUMORS

Towards spatial proteomics in mass spectrometry imaging

cHCC-CCA analysis with mass spectrometry

Segmentation performed on MALDI MSI data

Pathologist annotations on HES

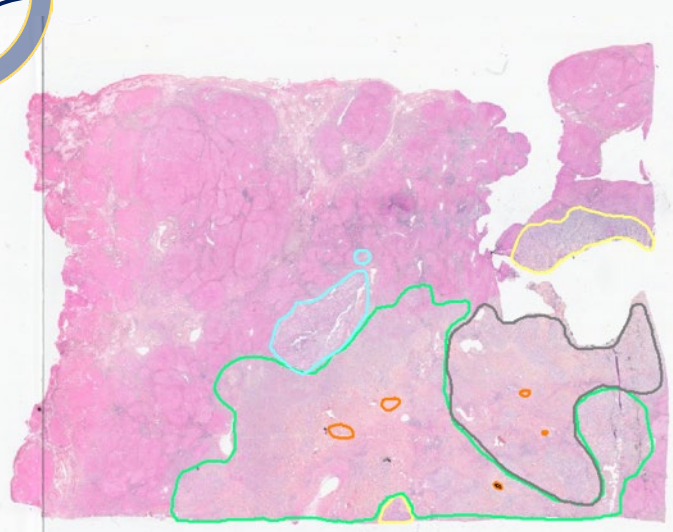


MORE HETEROGENEITY BASED ON MOLECULAR INFORMATION THAN ON MORPHOLOGY

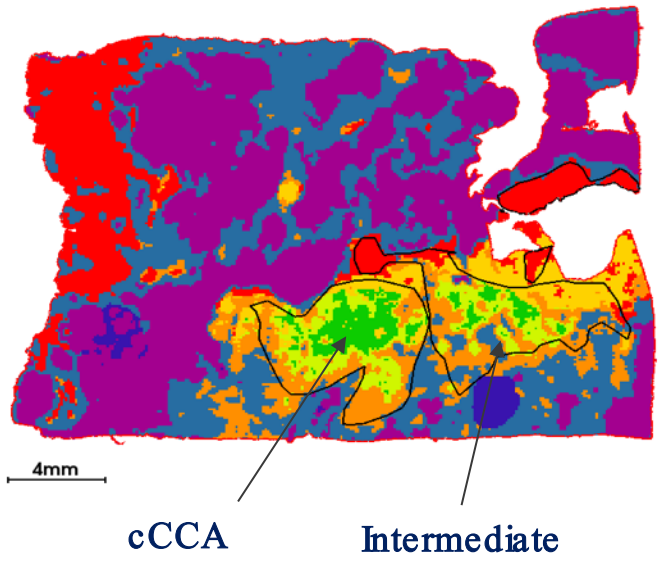
Towards spatial proteomics in mass spectrometry

cHCC-CCA analysis with mass spectrometry

HES with pathologist annotations

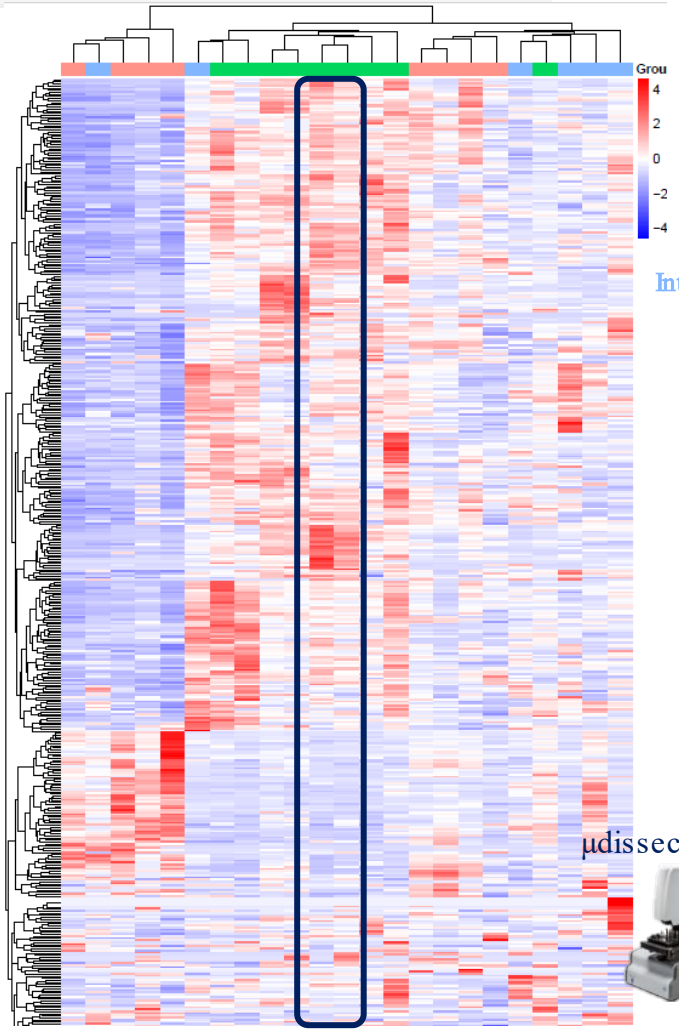


MALDI MSI segmentation with microdissected ROIs annotations



Hepatocholangiocarcinoma

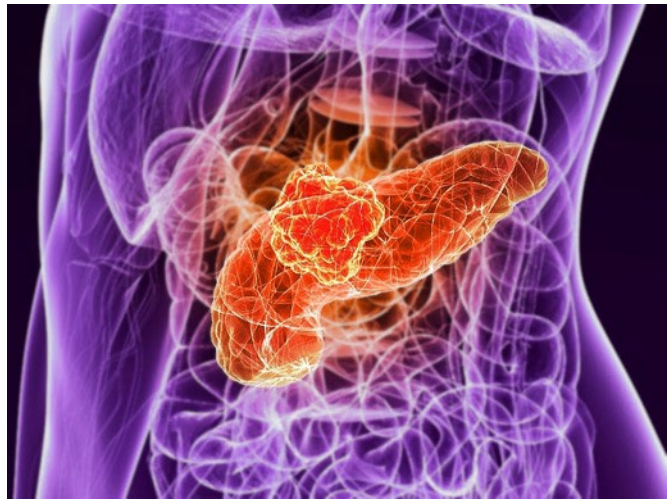
MALDI imaging analysis is correlated with LC/MS-MS proteomic rather than morphology for CCA and intermediate contingents



µdissection

Heatmap performed on 465 proteins with pvalue < 0.01 after ANOVA analysis

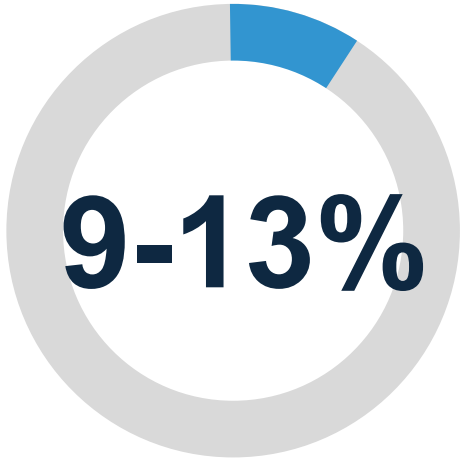
Pancreatic adenocarcinoma



Frequent late-stage diagnosis

High invasive potential

Broad chemoresistance



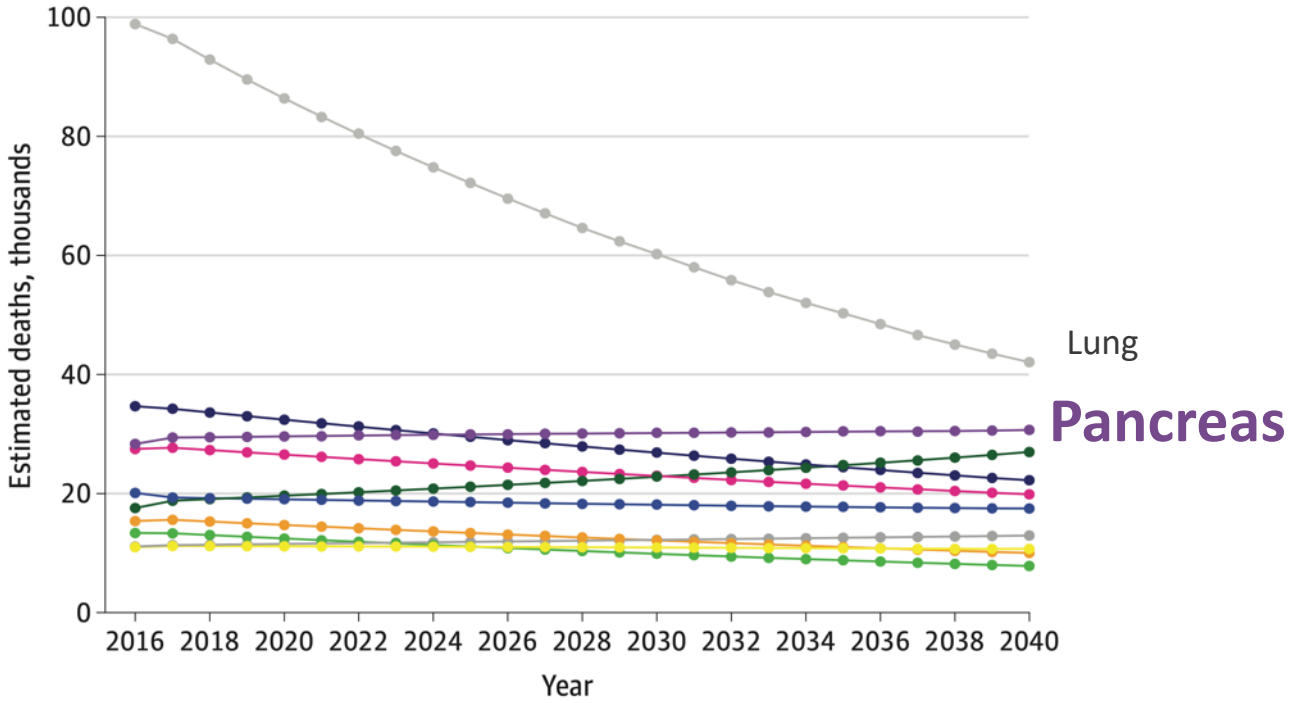
5 year survival

510 992 cases diagnosed worldwide in 2022

In France **4 500** in 1997, **15 991** in 2023

A growing burden

Projected Cancer-related Deaths



+1,6% per year in men
+2,1% per year in women

2040 projections :

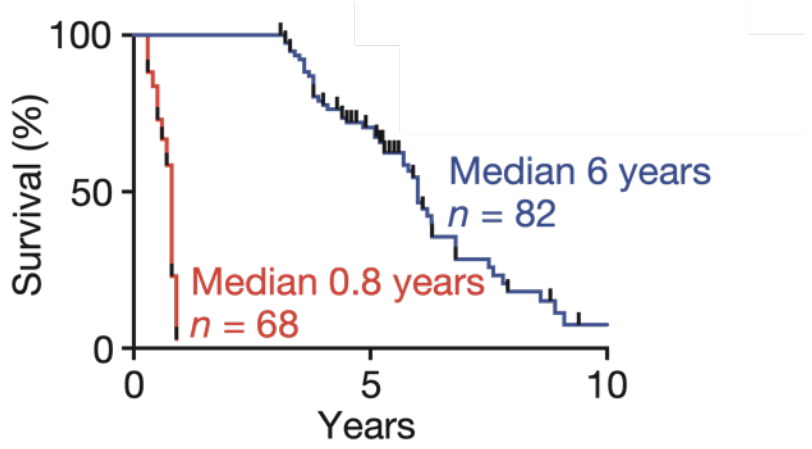
11% of all cancer-related deaths

Rahib, L., Wehner, M. R., Matrisian, L. M. & Nead, K. T. (2021). Estimated Projection of US Cancer Incidence and Death to 2040. *JAMA Network Open*, *4*(4), e214708.

High level of heterogeneity

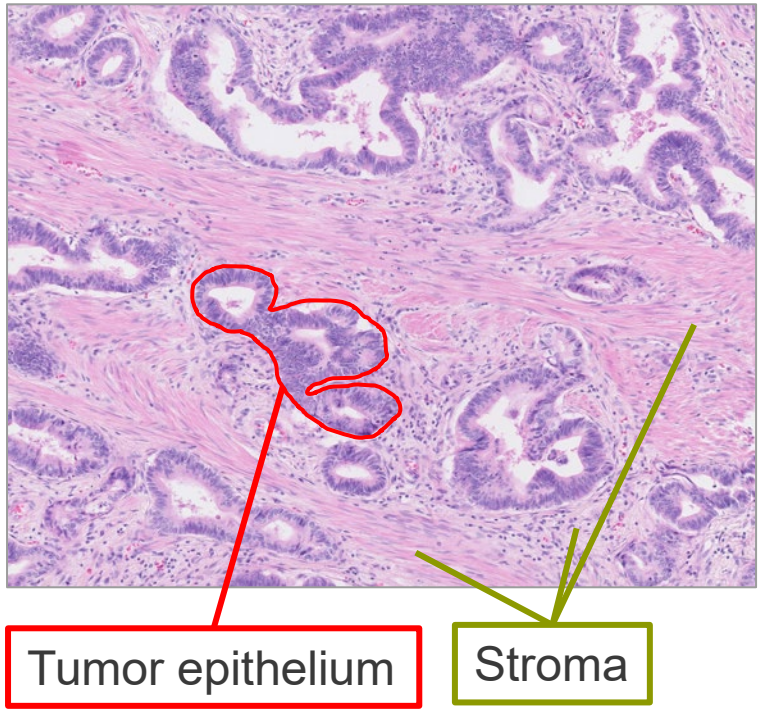
Inter-tumor heterogeneity

- Long survivors
- 10% to 30% initial responders to chemo
- …



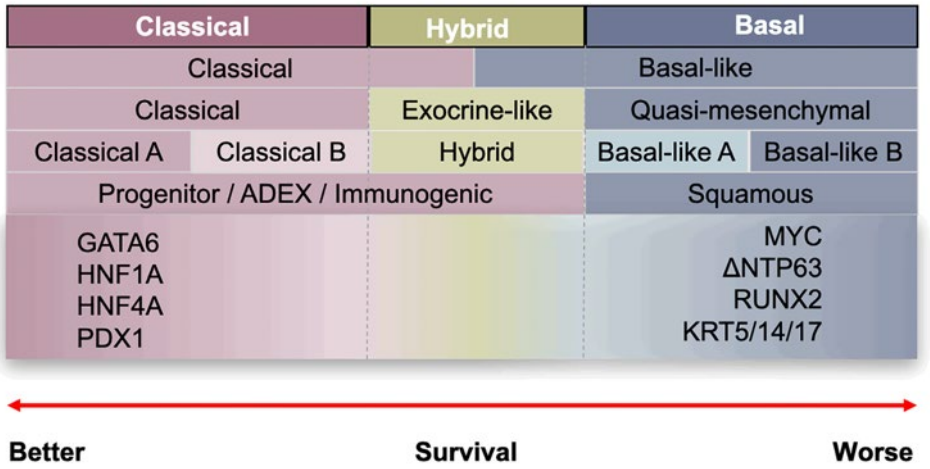
Balachandran et al. Nature 2017;551:512–516.

Intra-tumor heterogeneity



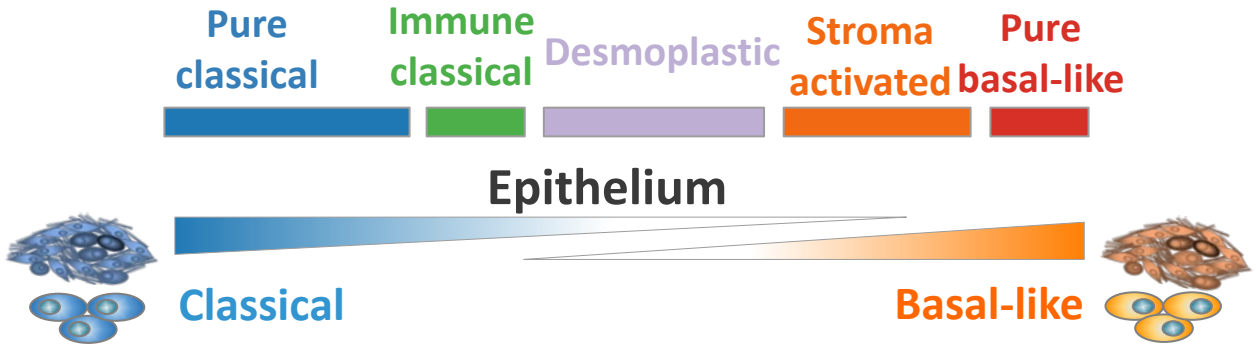
Epithelial and stromal classifications of PDAC

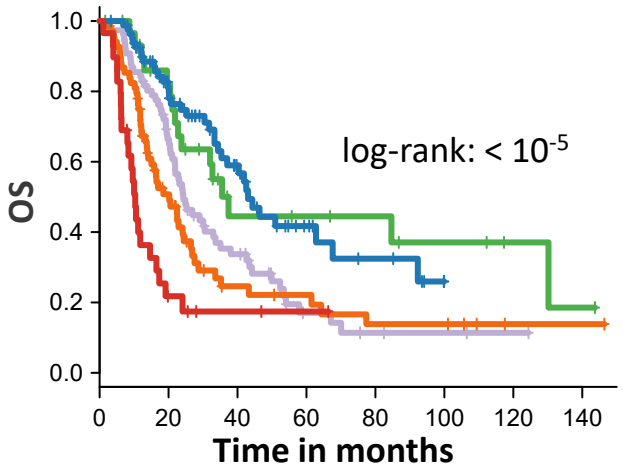
Molecular classifications



Bailey et al , Function 2023

Moffitt et al
Collisson et al
Chan-Seng-Yue et al
Bailey et al



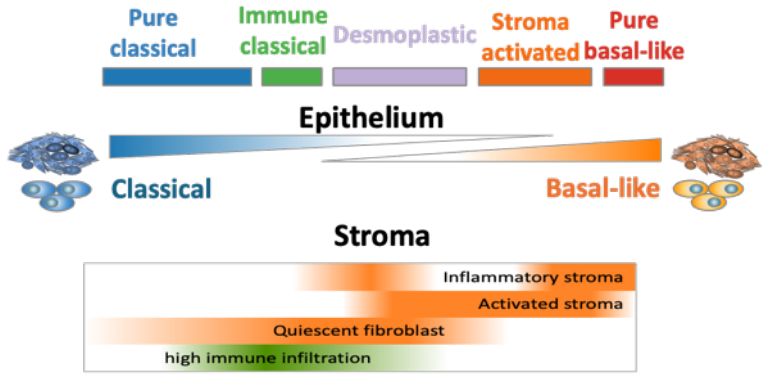


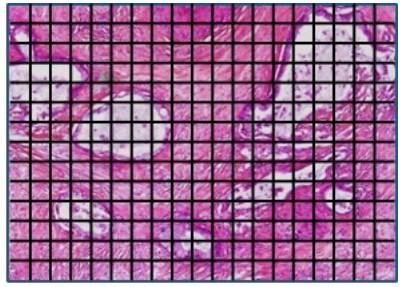
Puleo, Nicolle et al,
Gastroenterology 2018

AI on histology to *paint* PDAC: PACpAInt

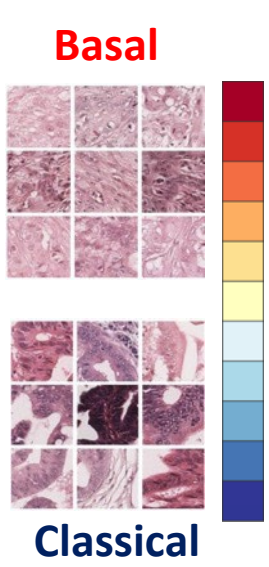
451 patients, 6.3 million tiles

Transcriptome-based subtyping

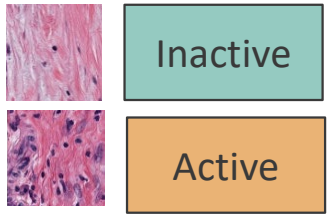


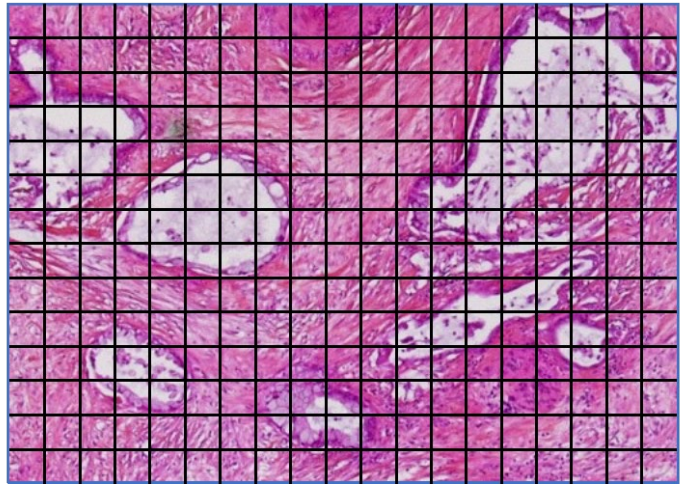


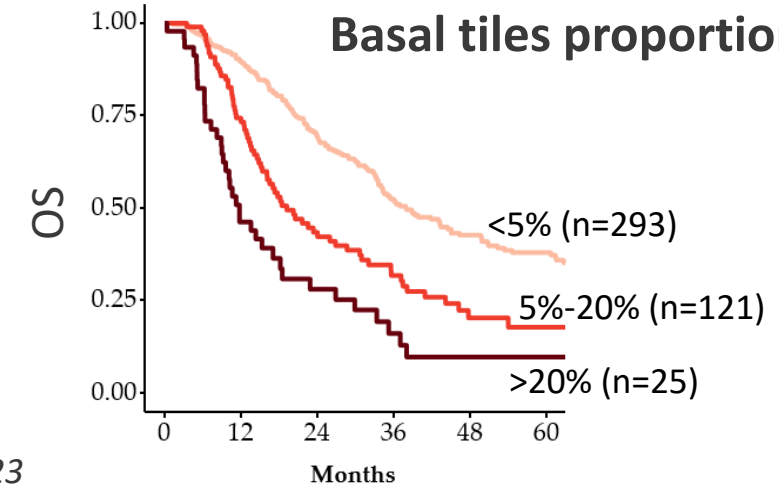
Transfer to histology



Stroma



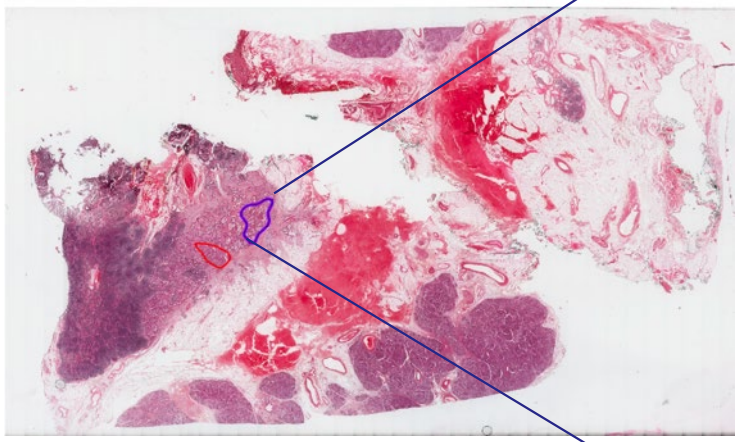




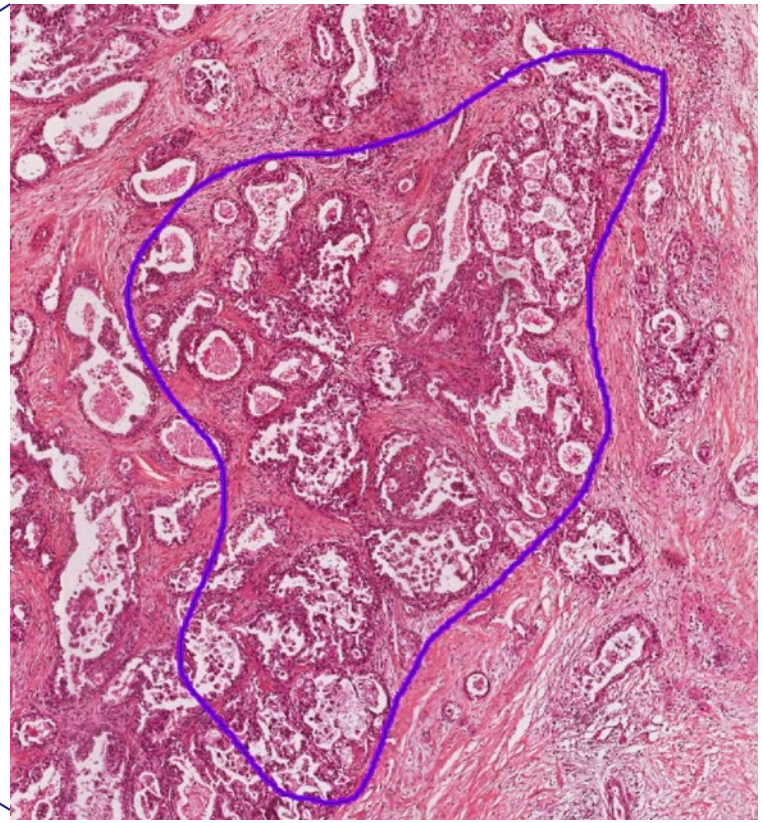
Saillard et al, Nat com, *2023*

Morphomolecular patterns: associate the transcriptome to histology

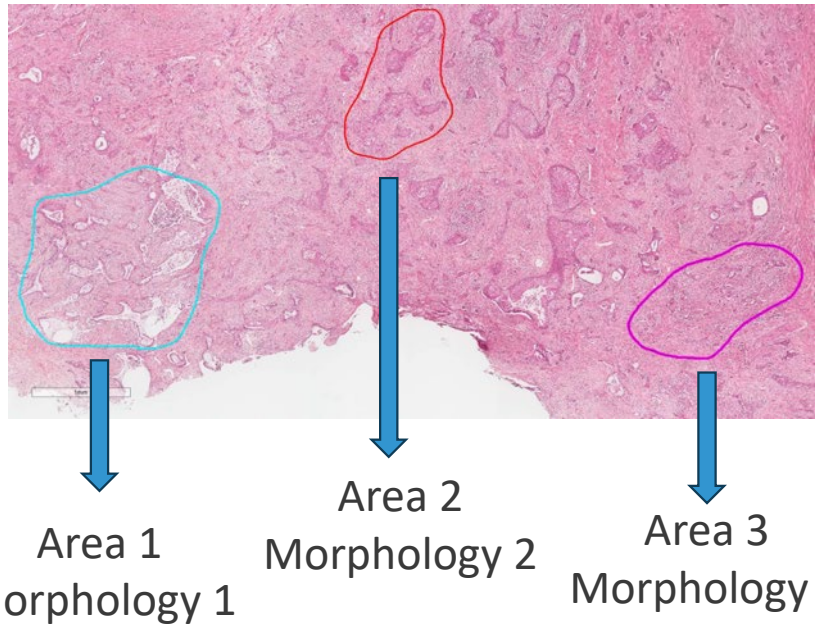
Pr. Jerome Cros Julien de Martino



100 patients
1 019 tissue blocks



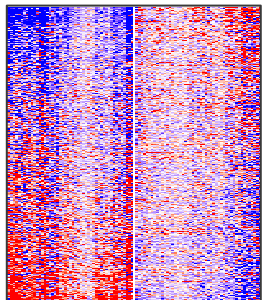
Ex: 1 slide, 3 differents histological patterns



Area 1
Morphology 1

Area 2
Morphology 2

Area 3
Morphology 3

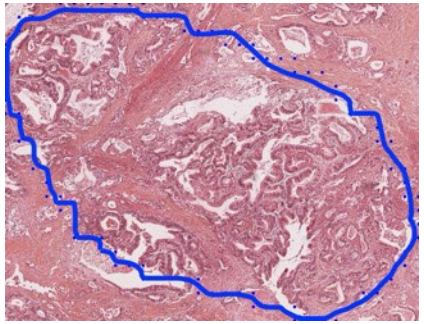


407 matched transcriptome & morphology

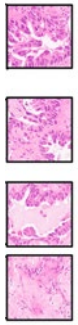
Tile-level gene expression prediction

Taib Bourega

PhD student

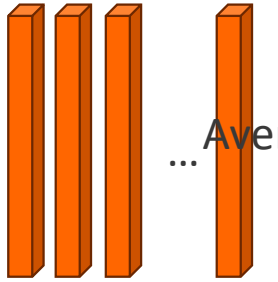


ROI



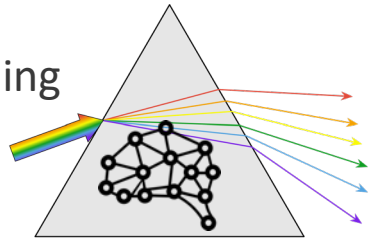
tiles

Feature extraction

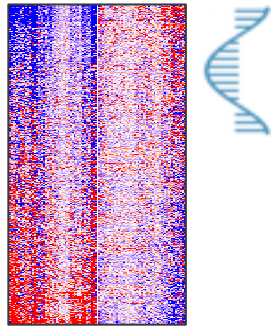


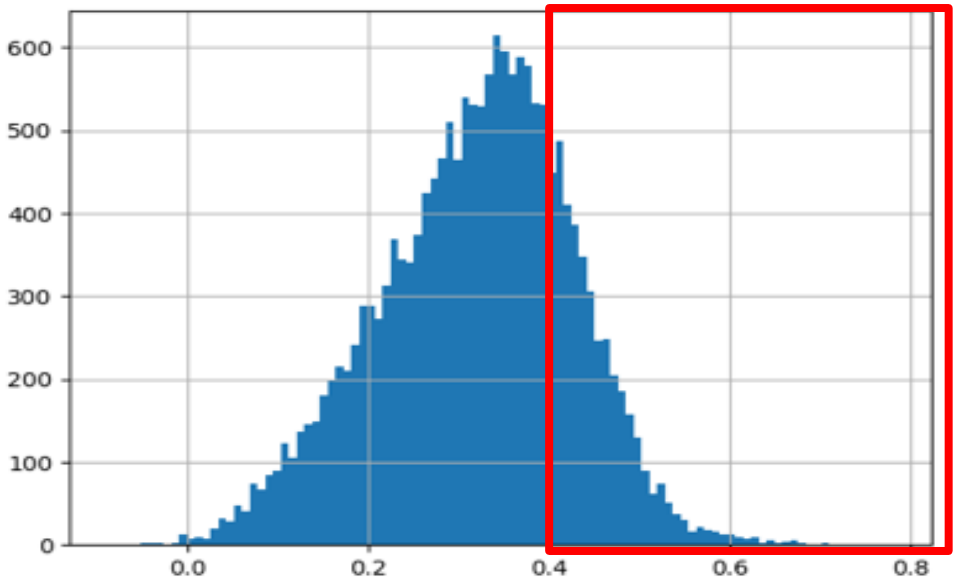
Feature Vectors

... Average pooling



Transcriptome



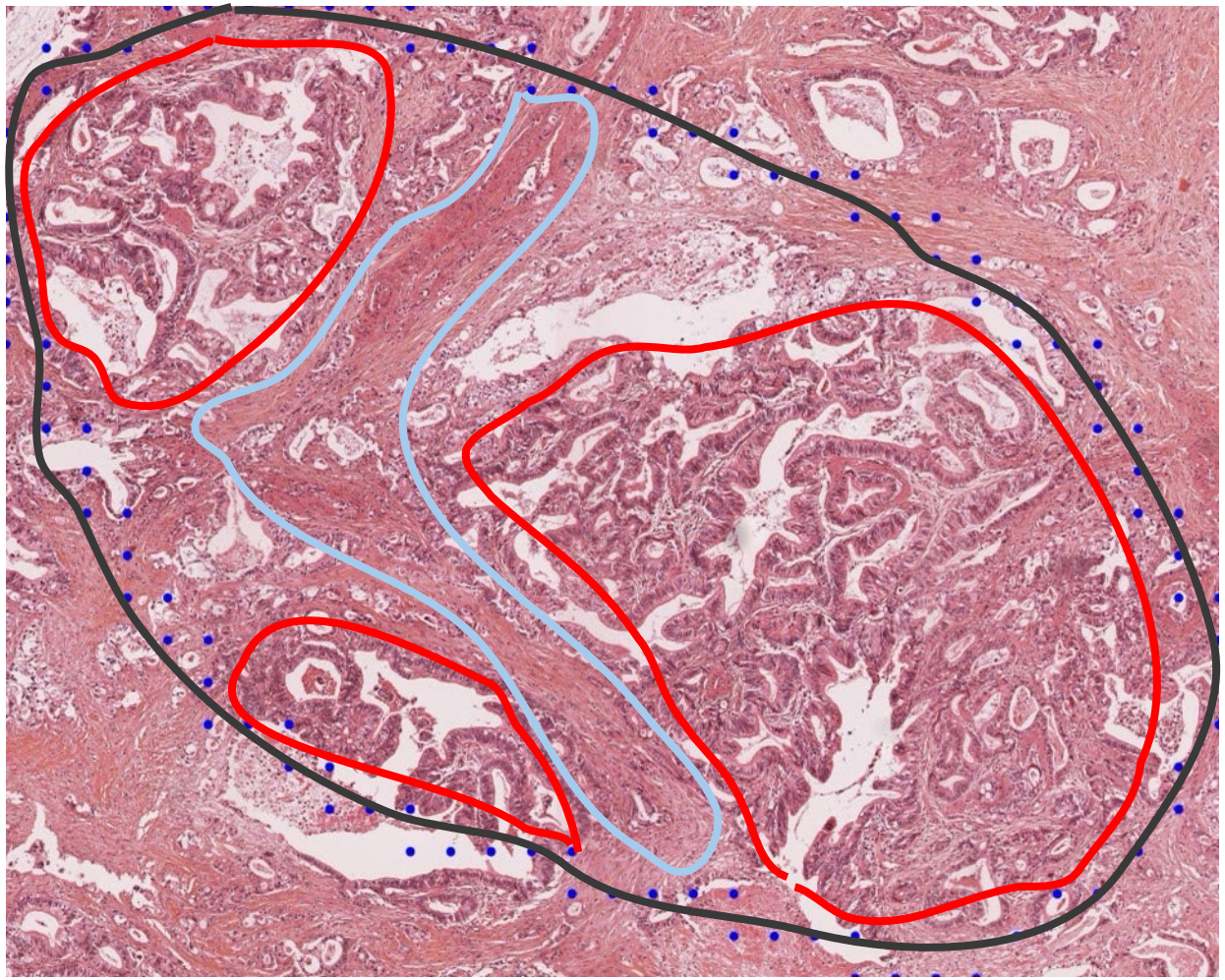


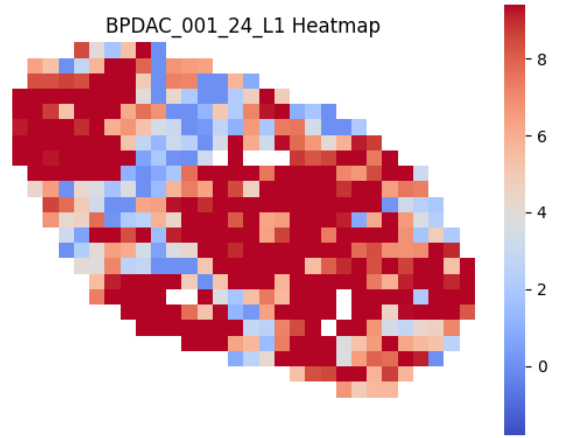
Pearson correlation

3789 genes

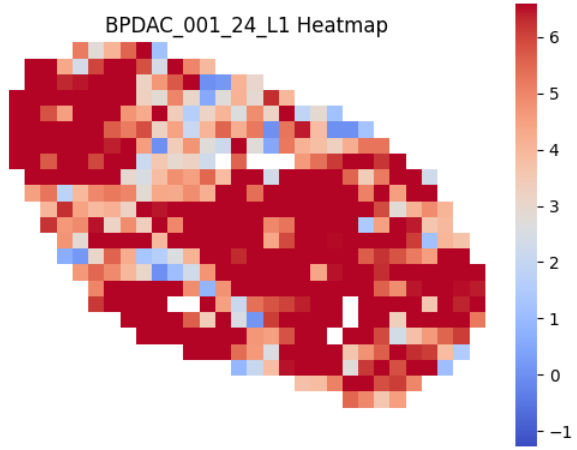
>17 000 genes tested

Marker gene prediction





TFF1

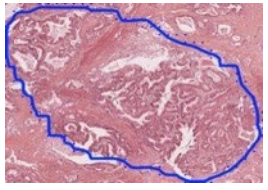


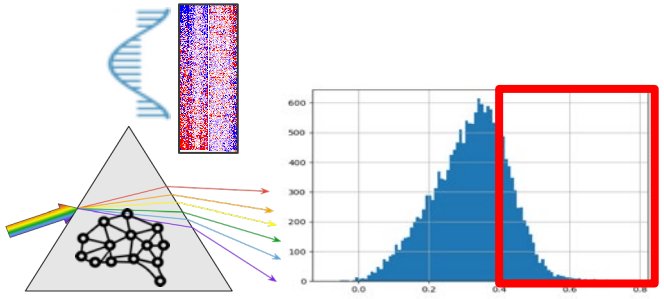
CLDN18

Marker genes of *Classical* tumor phenotype

Delineate morpho-molecular patterns

Stromas

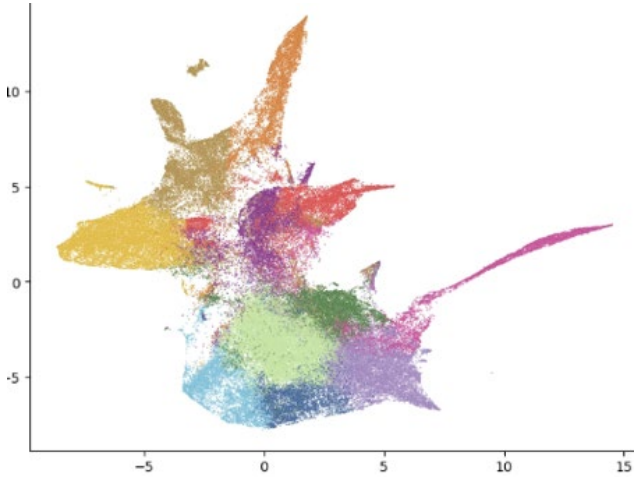




ROI
n=407

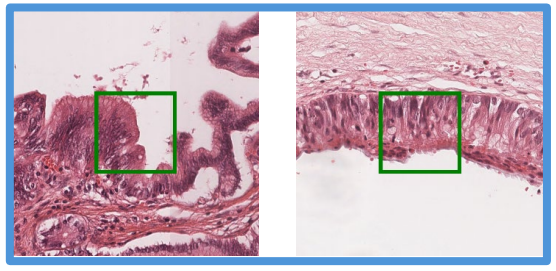
Tile-level

Unsupervised analysis

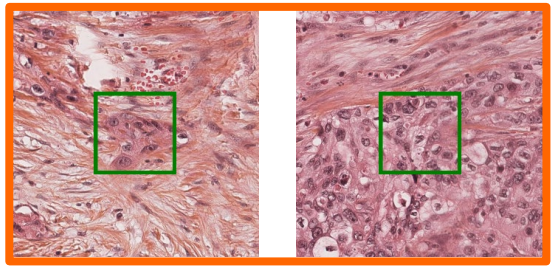


12 Patterns

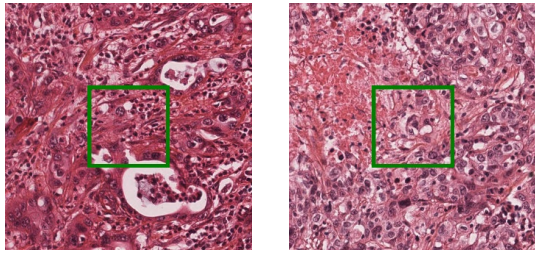
Tumor : classical

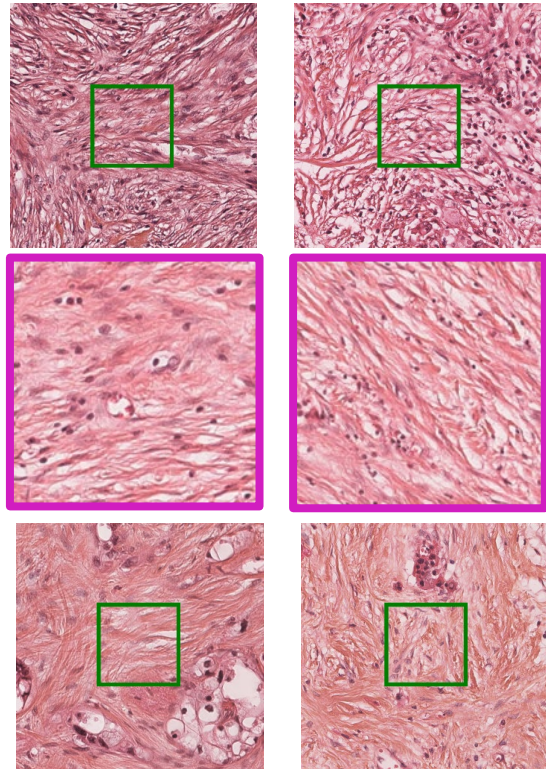


Tumor: Basal-like

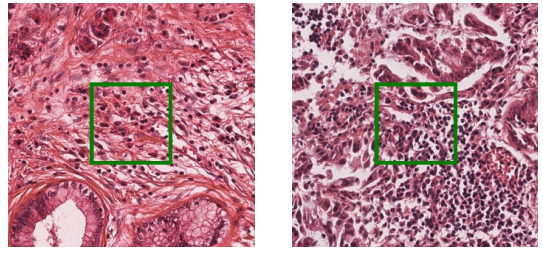


Tumor: inflammatory “Basal”





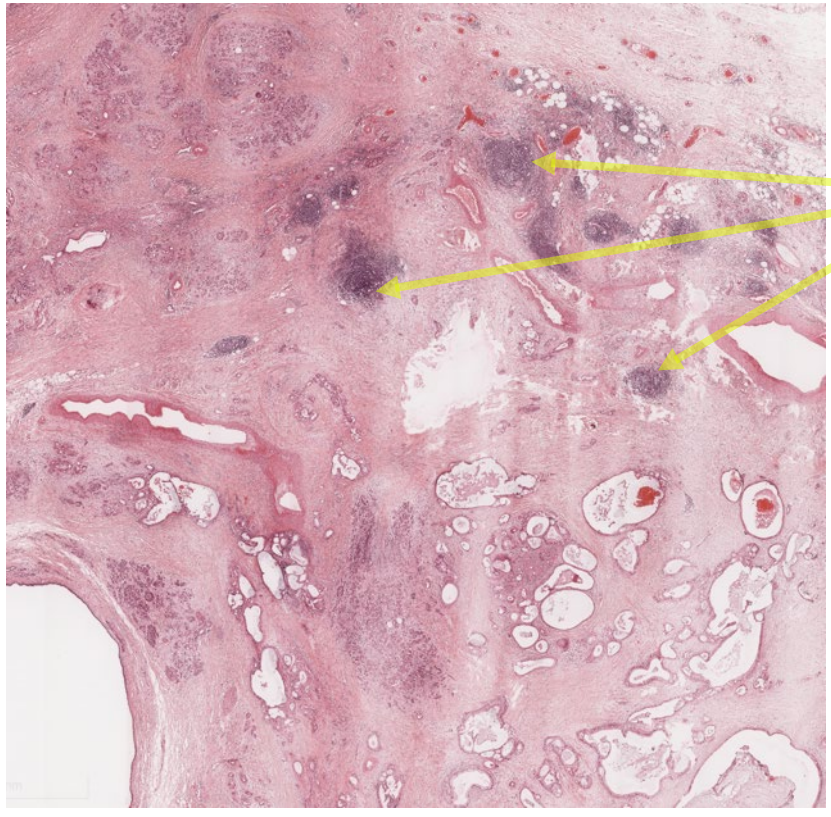
Lymphoid pattern



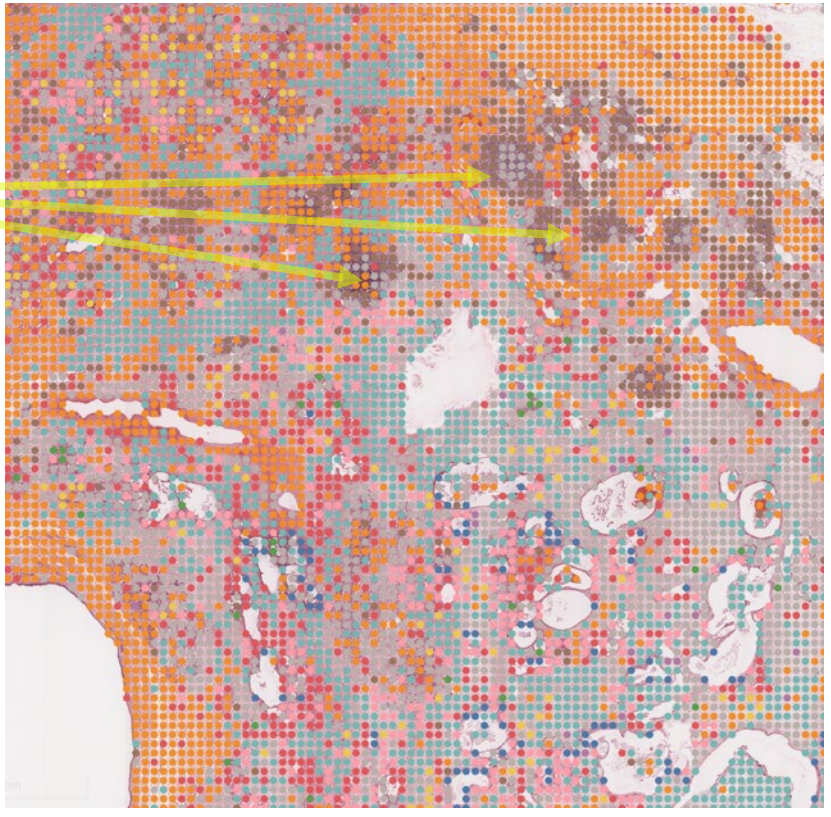
Spatial distribution and co-occurrence

Trained on 407 small ROI

Projected on 1’019 whole tumor blocks

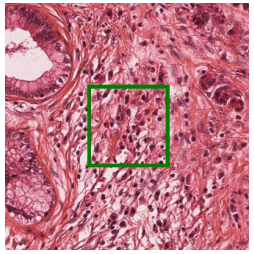


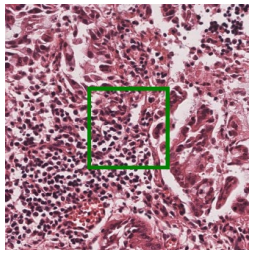
Lymphoid
dense areas

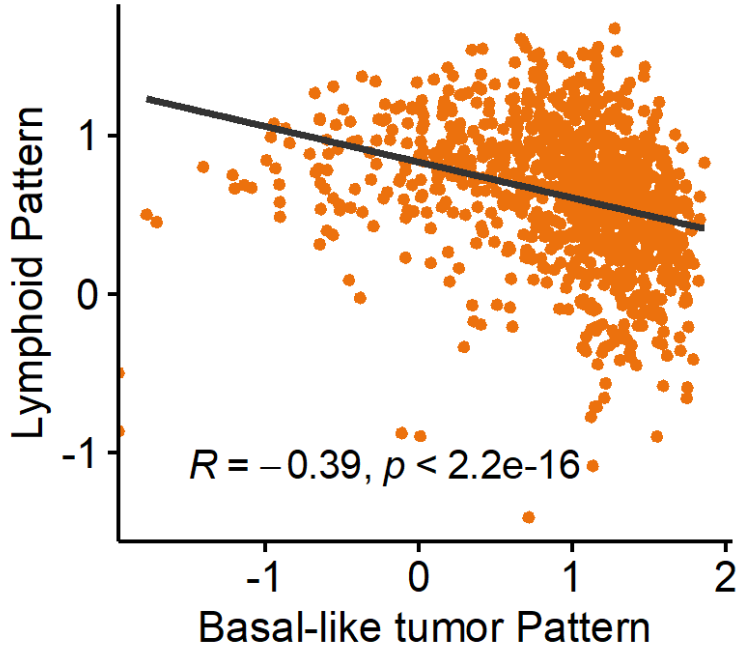


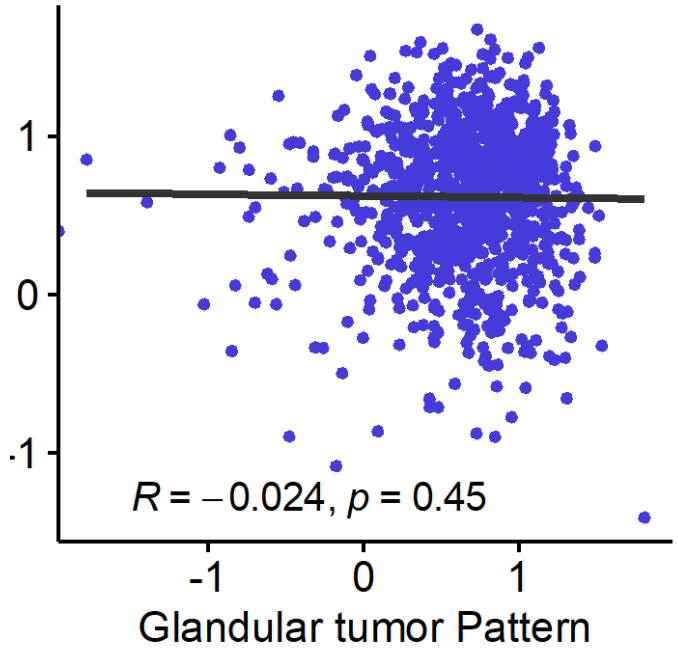
Association with Lymphoid infiltration

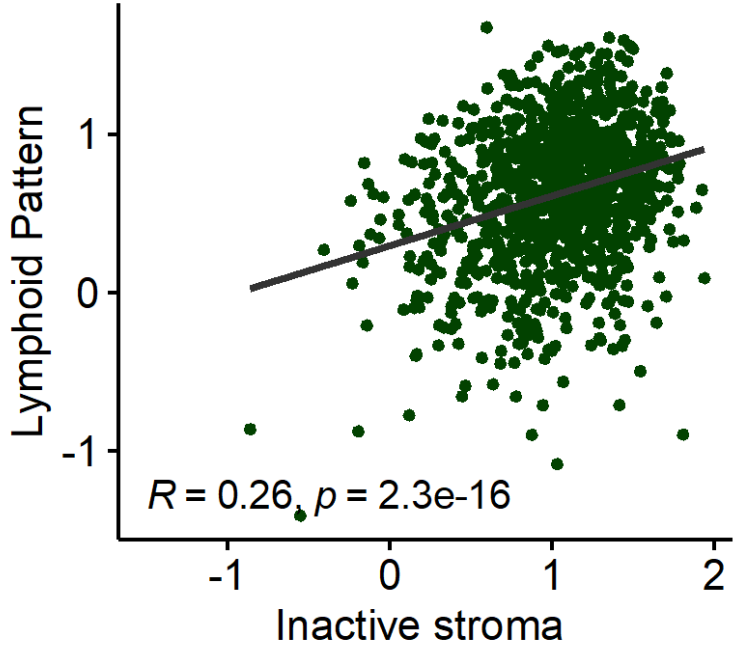
n = 1 019

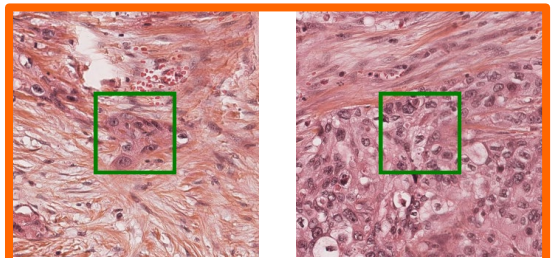


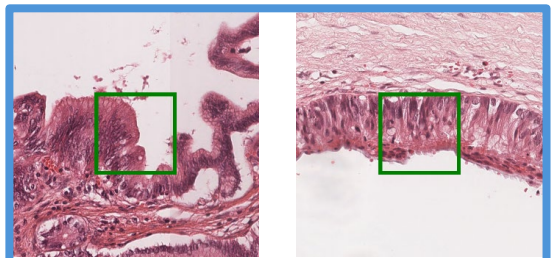


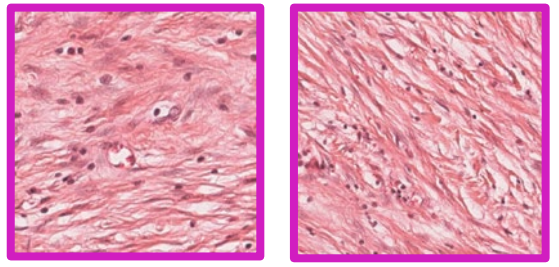






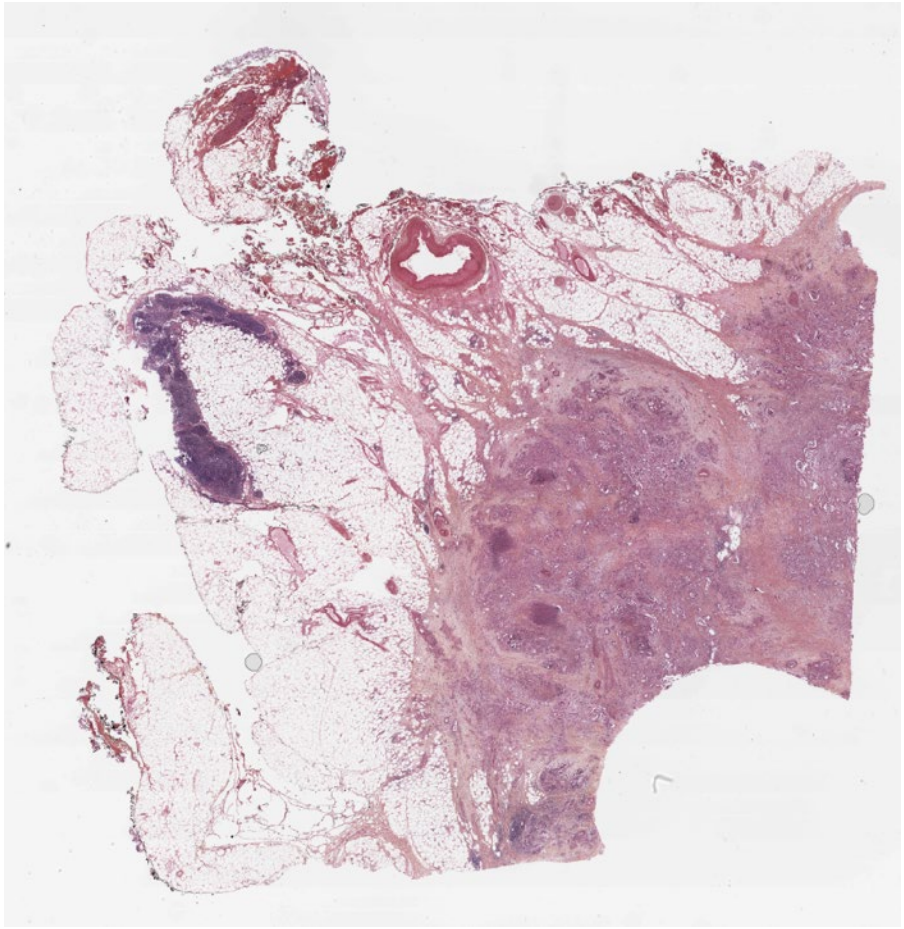




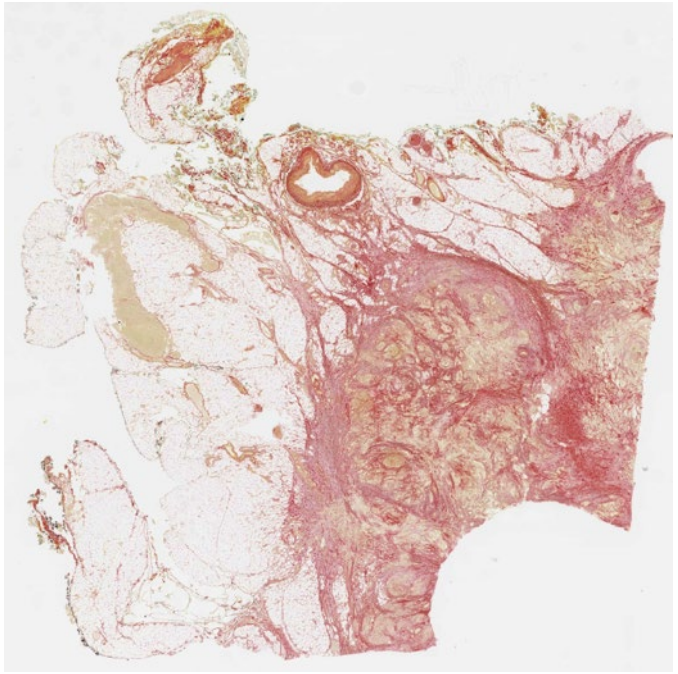


What makes up a CD8 T cell-compliant stroma?

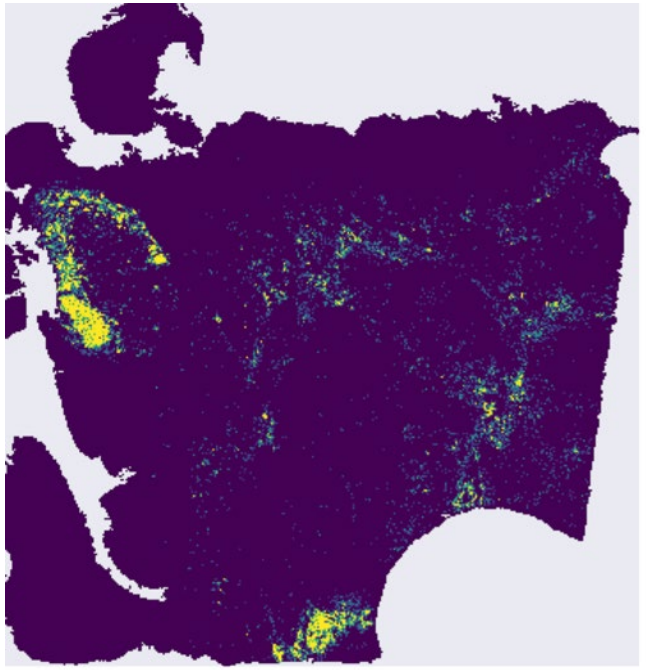
C. JEAN



Collagen

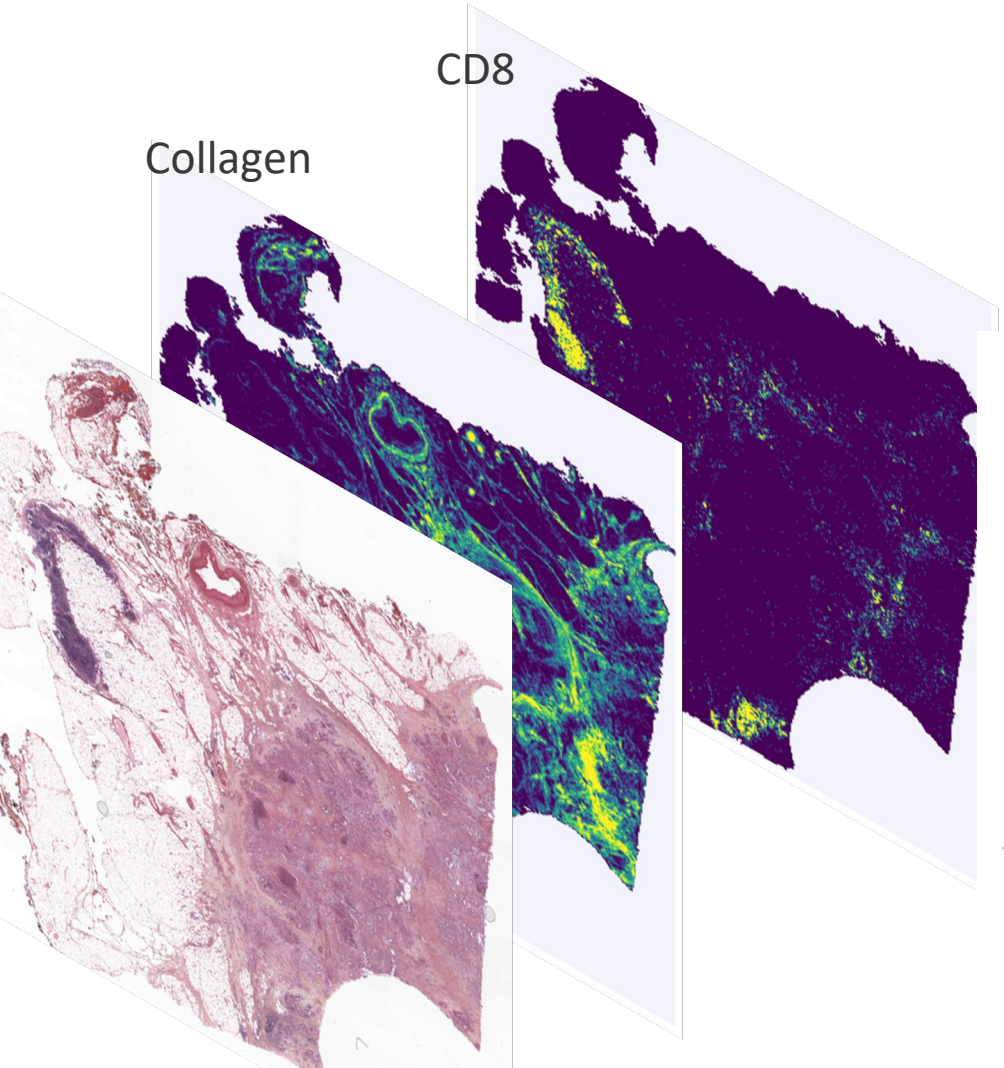


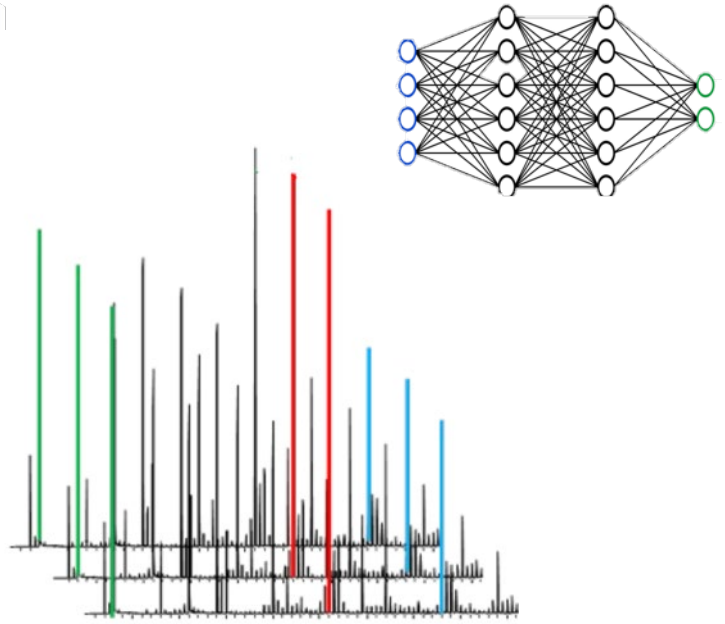
IHC CD8



Can protein content identify a CD8 stroma?

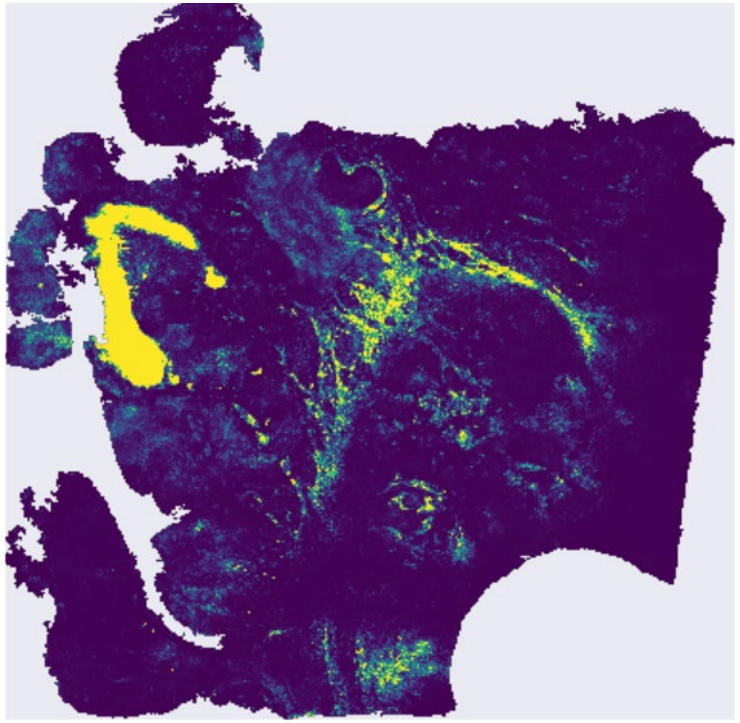
A. Abdelrahman
postdoc





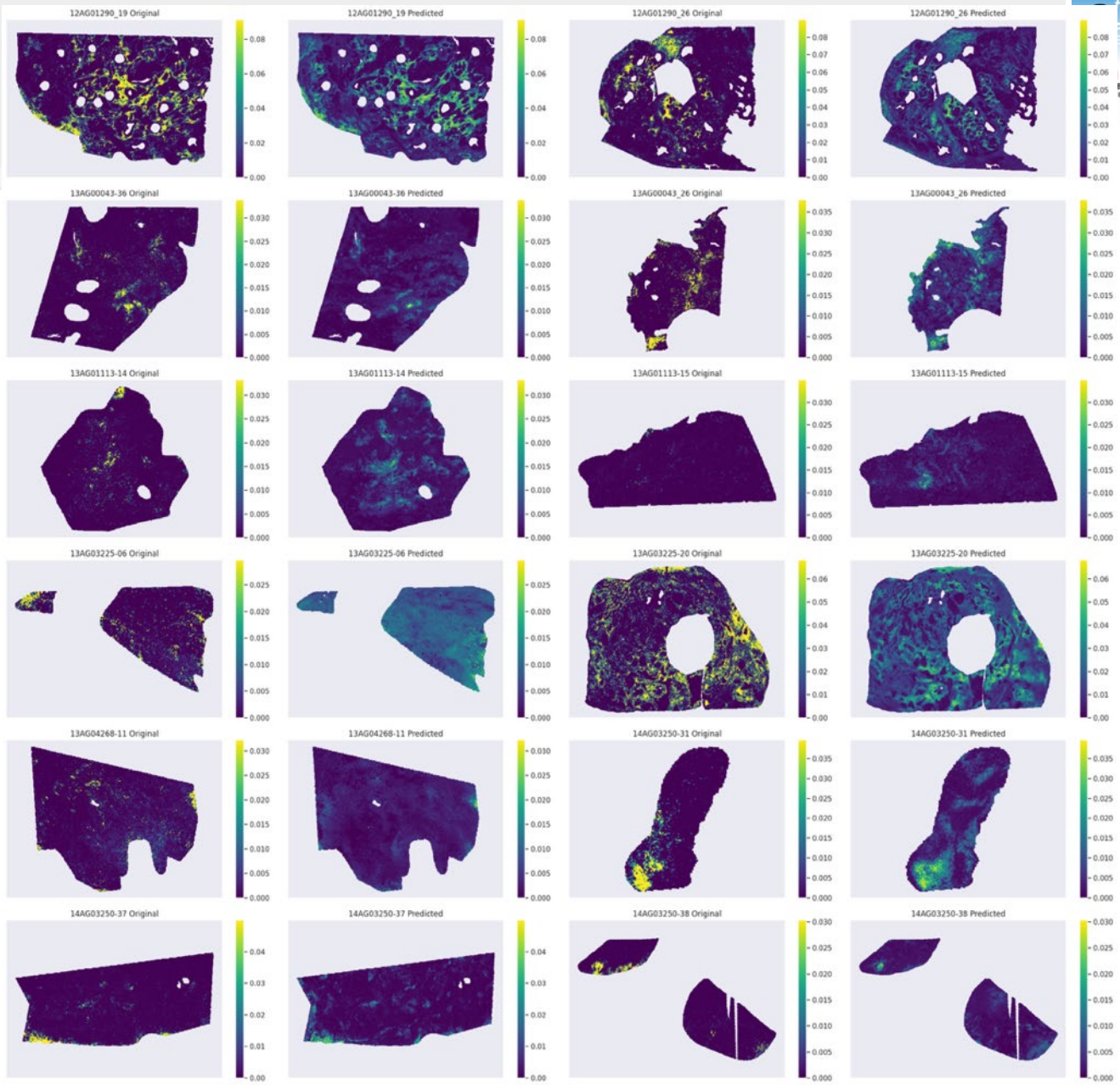
MALDI imaging (50 µm)

Lymphoid-compliant stroma

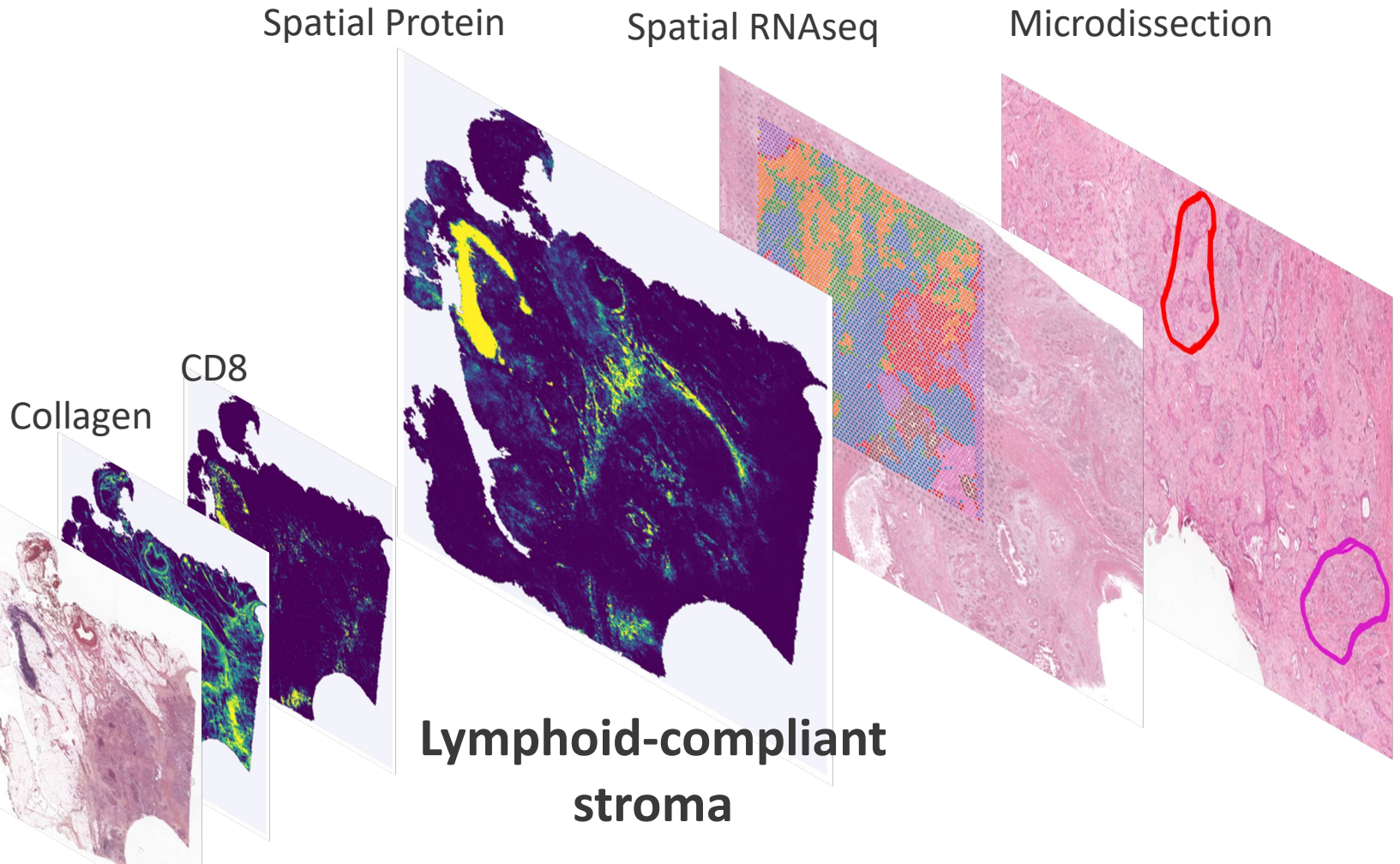


40 PDAC

CD8-compliant stroma found in every tumor around CD8 infiltration



Characterize CD8-related stroma



CD8 excluding stroma

COL1A1/2, COL11A1, COL12A1, COL5A1, Fibronectin

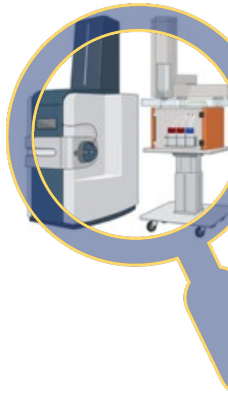
CD8 infiltrated stroma

COL4A3/4, COL19A1, Reelin

Towards spatial proteomics in mass spectrometry

To conclude …

- ✓ Molecular heterogeneity are more important than their histological heterogeneity
- ✓ We have demonstrated the usefulness of mass spectrometry imaging to highlight intra-tumoral heterogeneity using proteomic approaches.
- ✓ Mass spectrometry imaging identifies novel diagnostic and biological biomarkers



- ✓ MALDI-MSI combined with targeted microdissected areas analyzed by LC-MS/MS = optimal method for FFPE tissues

- ✓ Bioinformatics is essential for big data analysis, as it provides the computational tools and algorithms needed to process, integrate, and interpret complex biological datasets

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Rémy Nicole

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Joanna Lipecka
Chiara Guerrera

Equipe MicMac
Samira Laouirem
Adel Hammoutene
Etienne Becht

Plateforme iMAP
Ghalia Ben Ghedifa
Rovelyne Pissa
Meriem Marref
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Arnaud Senecaut
Elia Gigante