Linking spatial to functional proteomics on heterogeneous samples: The “pixel-by-pixel” shotgun proteomic approach

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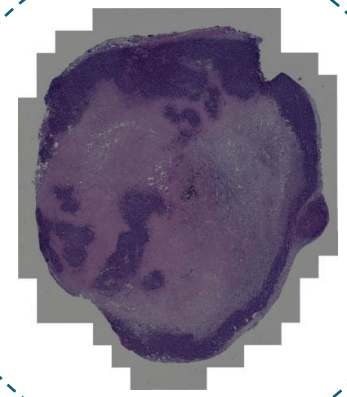
Belgian Proteomics Association Conference, Ghent, 05/12/24

Spatial and fuctional proteomics



Context and Biological Challenges

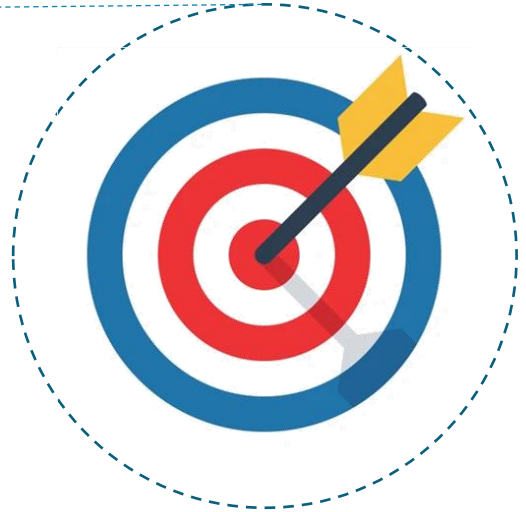
Tissue = complex functional assembly of cells and structures

- Cellular/phenotypic composition
- Impact of the microenvironment
- Complexity and interpretation of molecular signatures

Strategic Approach

Spatial and Functional Proteomics

- Mapping of cellular subpopulations
- Understanding biological mechanisms (physiological and pathological)



Spatial and fuctional proteomics

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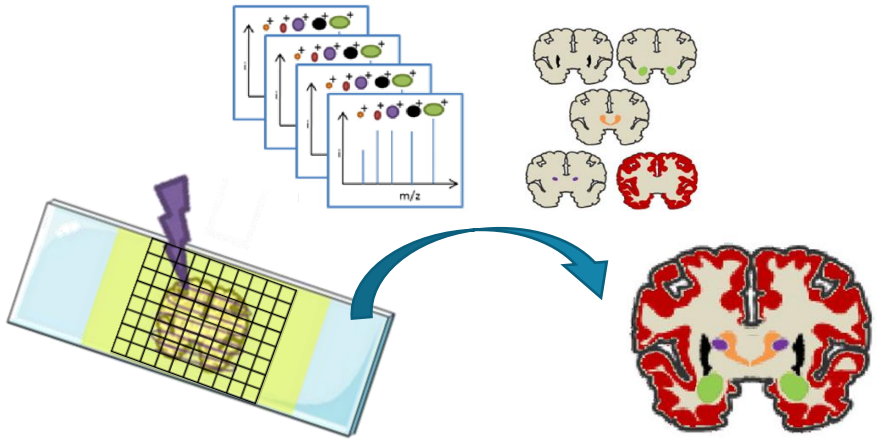
Spatial proteomics

Mass Spectrometry Imaging (MSI)



- ✓ High spatial resolution
- ❑ Low functional information

➢ Spatial molecular localization

Functional proteomics

Laser capture Microdissection (LCM) / LC-MS

Region Of Interest

Shotgun Proteomic

- ❑ Low spatial resolution
- ✓ High functional information

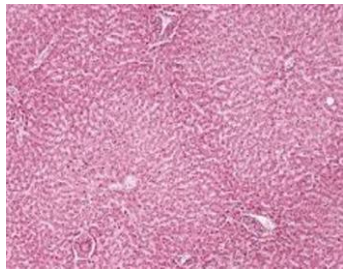
➢ Proteins function in region of interest

Strategy

Develop spatial and functional proteomic method combining MALDI-MSI and LCM-LC-MS

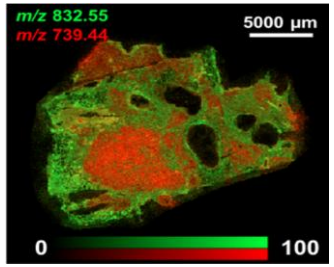
Serial tissue sections

Histology



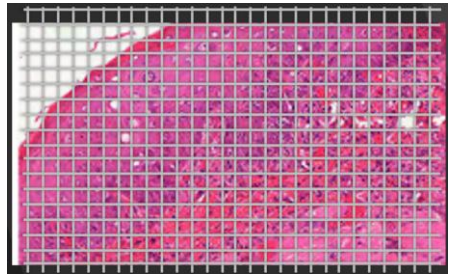
+

MALDI Imaging



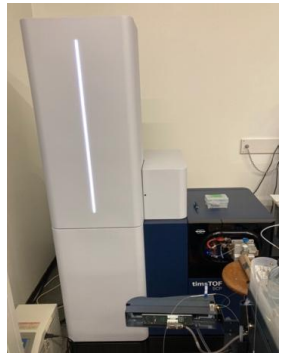
Spatial information

New LCM approach: Pixel-by-pixel LCM



Functional and spatial information

Bioinformatics



Functional Spatial proteomics

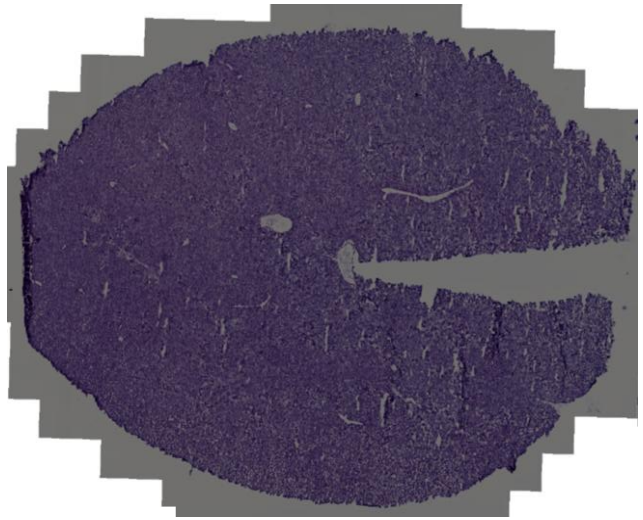
Pixel-by-pixel LCM concept: unsupervised LCM proteomics at high spatial resolution

- **Require method development and optimisation**

Biological models

Homogenous tissue

Mouse liver

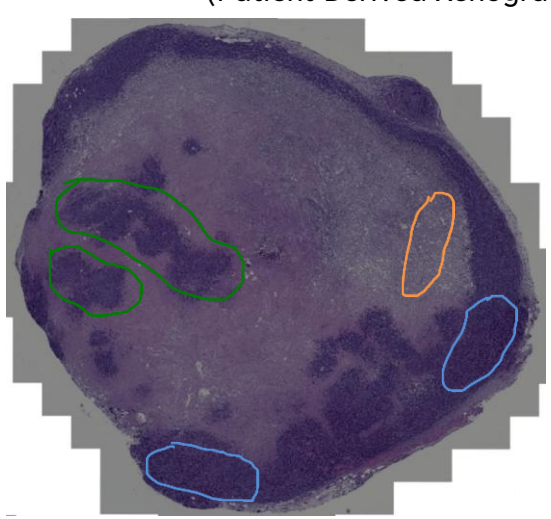


Methods optimisation

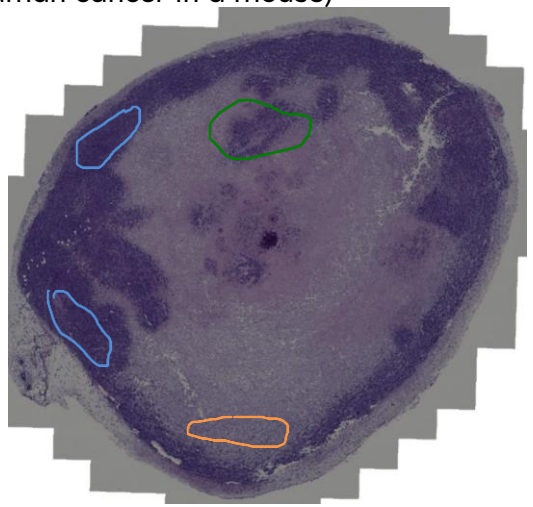
Heterogenous tissue

Triple negative breast cancer (TNBC)

(Patient Derived Xenograft (PDX) from human cancer in a mouse)



Non-treated



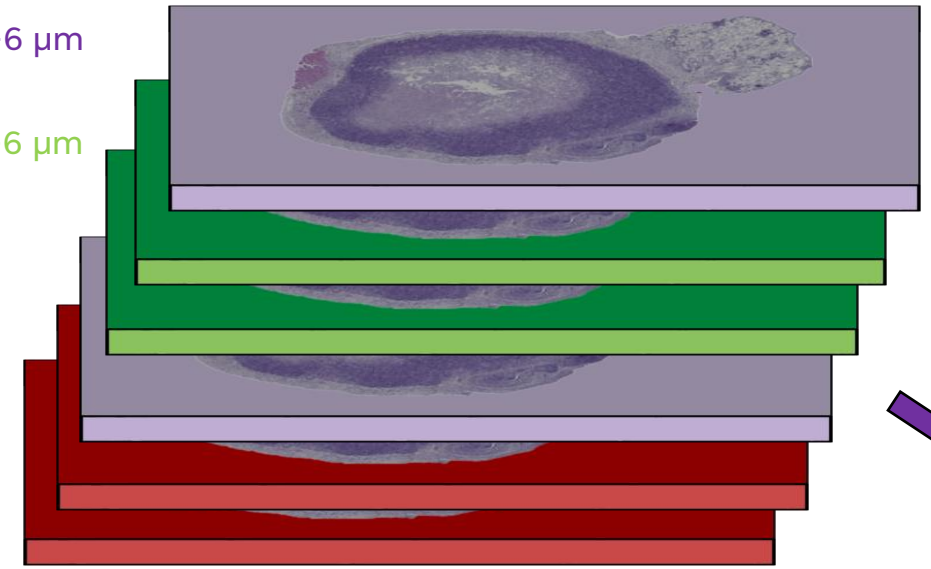
Anti-cancer drug treated

Peripheral region Hypoxic region Inflammatory region

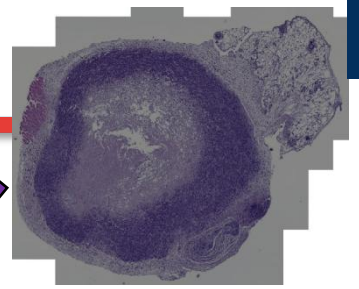
Methods evaluation

- Heterogenity study
- Region differential study
- Non-treated vs treated comparison

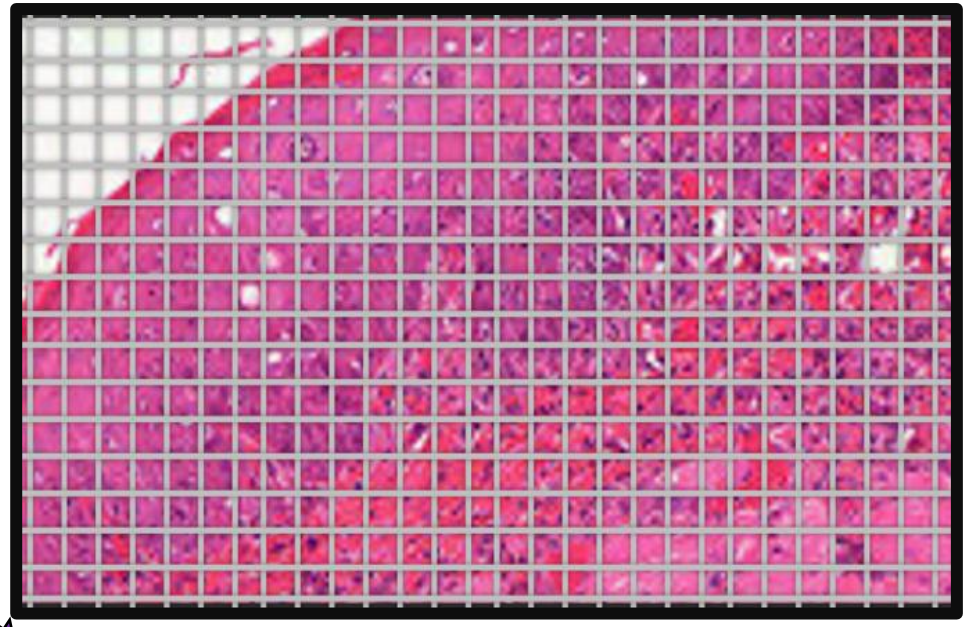
Serial sections strategy



H&E

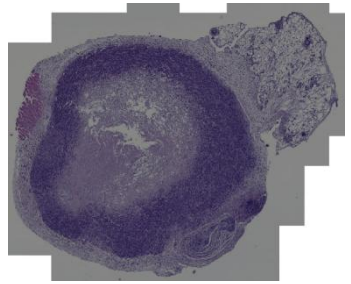


Pixel by pixel proteomics



H&E

Molecular MALDI imaging



- A section for each experiment
- H&E sections, to visualise tissue structure variation

Analytical and bioinformatics workflow

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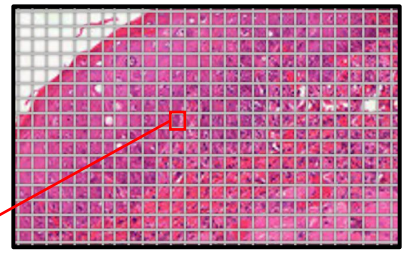
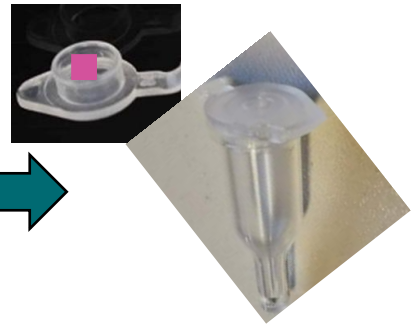
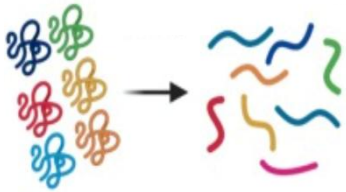
Systematical Laser Microdissection



FFPE and FF (Fresh Frozen) Tissue Sections



Reduction, alkylation, and trypsin digestion

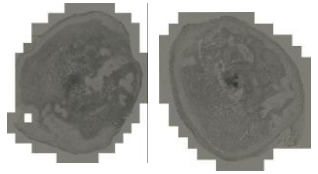


LC-MS/MS

60 minutes, 15cm Aurora column, DIA run

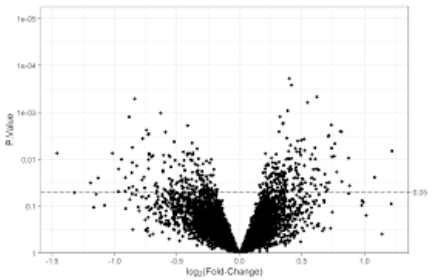
DaSER

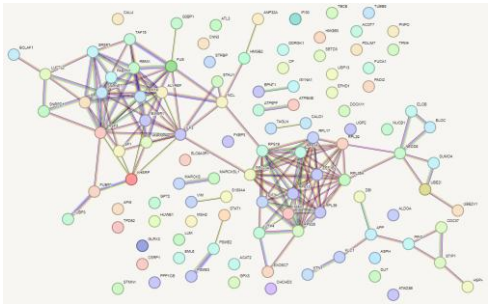
In-house spectral library



- DDA analysis of whole sections in triplicate
- 25 cm Aurora column and 100 min run

→ New spectral library with 12000 proteins



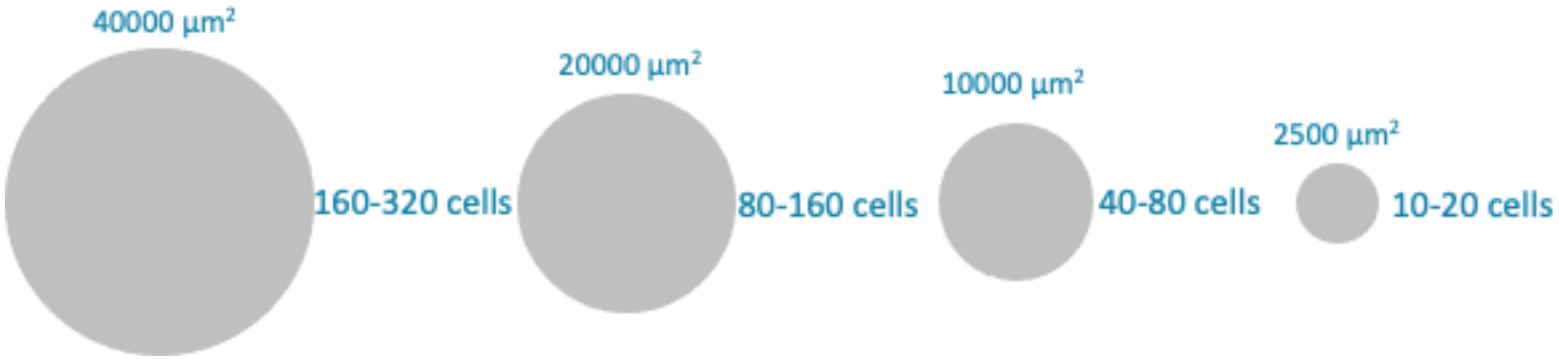


STRING

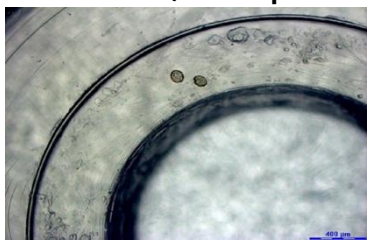
Biological interpretation

Pixel-by-pixel LCM: LCM optimisations

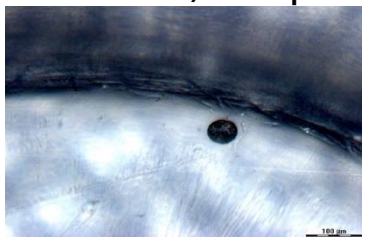
Best spatial resolution (smallest pixel size) maintaining high proteomics performance



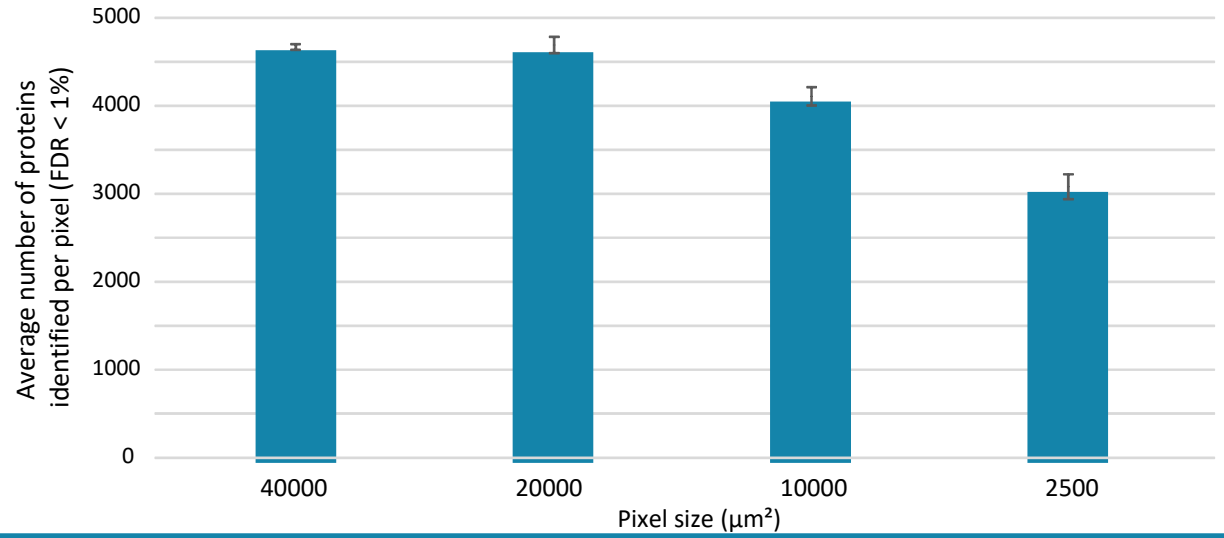
10,000 µm²



2,500 µm²



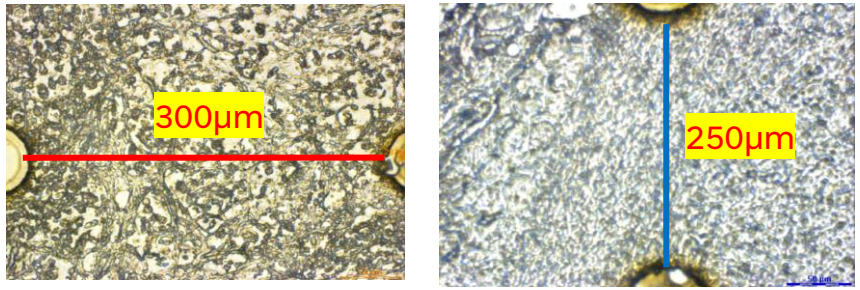
Proteomic performance vs Pixel size



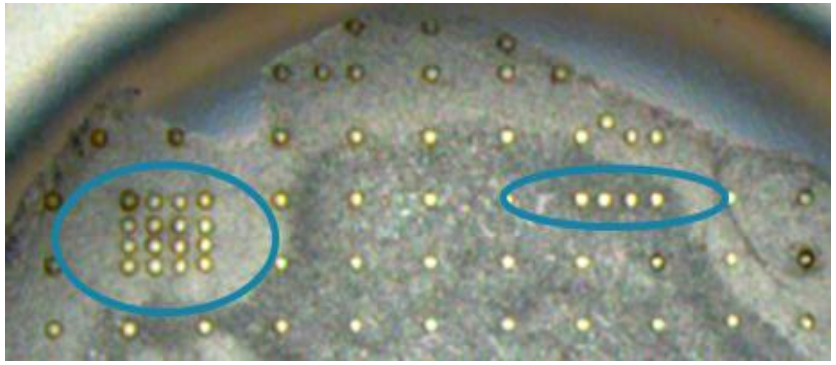
- Pixel surface 16 times smaller.
- Decrease in terms of identified proteins does not even correspond to a factor of 2.
- Biological material sufficient to achieve a fairly deep proteome.

Pixel-by-pixel LCM: Two approches of pixel sampling

1) Systematic - grid

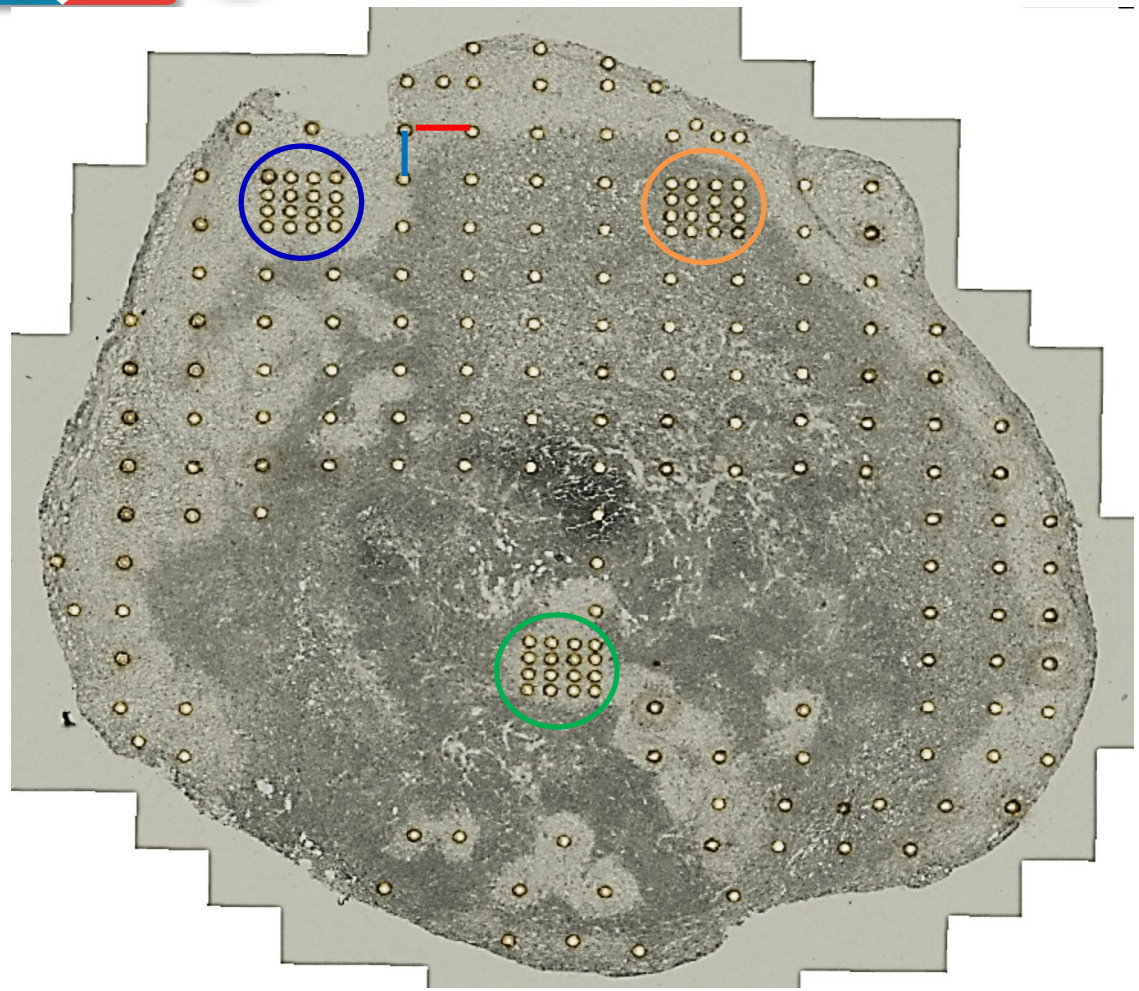


2) Areas of interest



- Pixels density higher in areas of histological interest
  distance = 25 microns

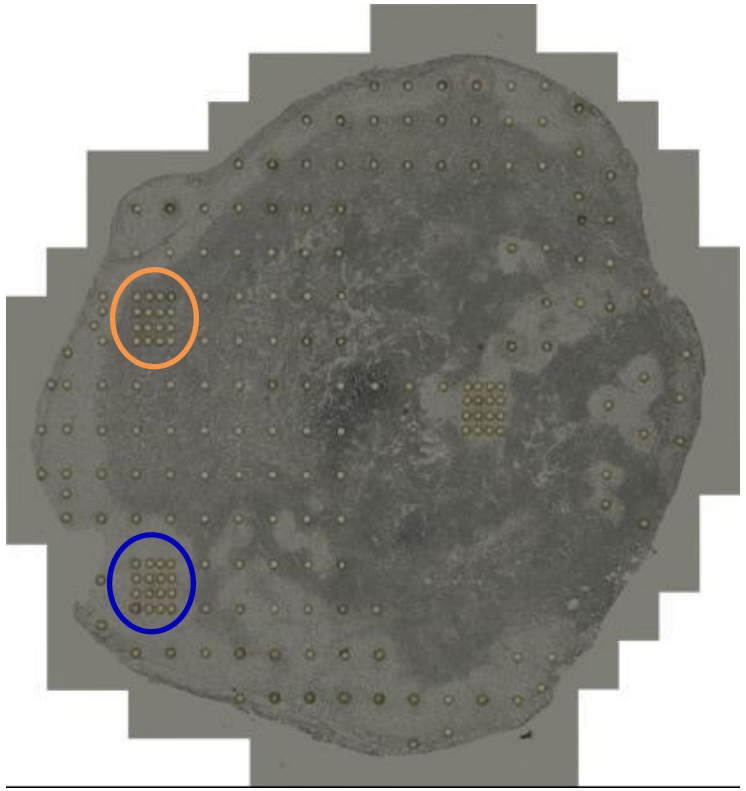
Best approach (best resolution), but challenging without automation



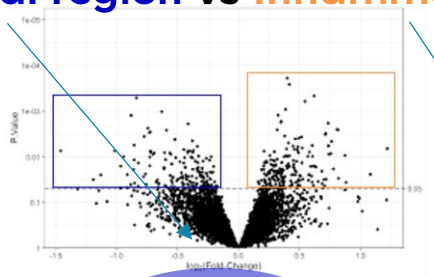
- Number of proteins ranges between 800 and 3000 per pixel (2500µm² - 10 to 20cells)
- **Supervised analysis between areas of biological interest**

Pixel-by-pixel LCM: Differential analysis on regions

Differential protein abundances have been detected in the specific regions of biological interest :



Peripheral region vs **Inflammatory region**



- Structural proteins
- mRNA translation
- RNA metabolism

High proliferative activity

Characteristics of the peripheral zone

- Presence of Fibrinogene
- Plasma protein

Hemorrhagic phenotype
Highly vascularized region

Characteristics of the inflammatory zone

Perspectives: towards single-cell spatial resolution

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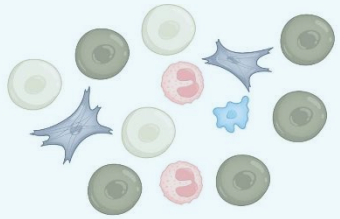
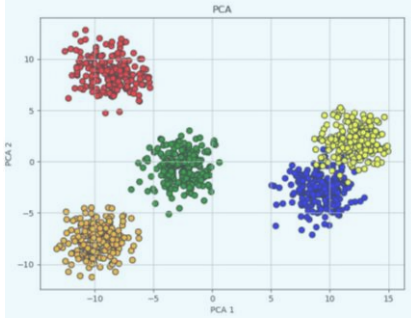
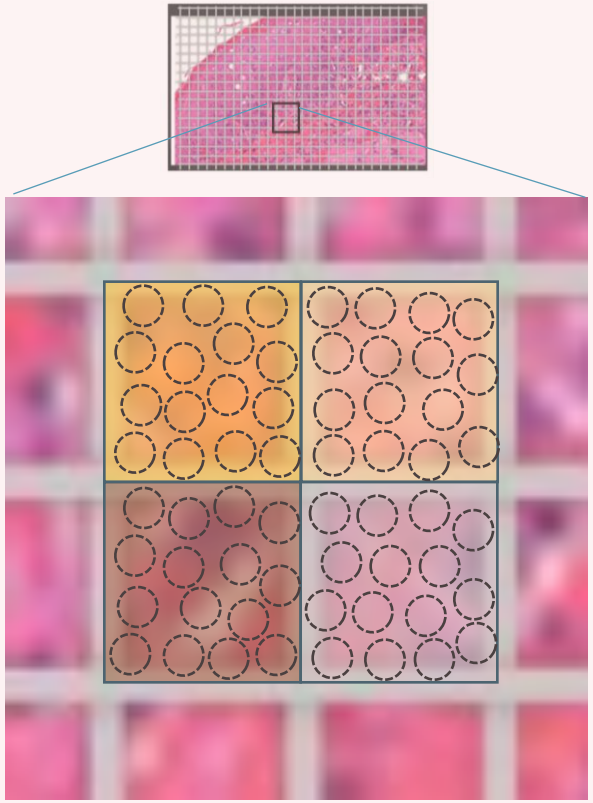
SCP

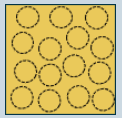
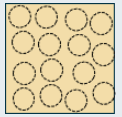
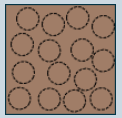
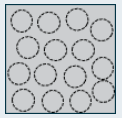




Phénotype cellulaire
A
B
C
D
E

P2P



Pixel	Cellular phenotypes
	
	
	
	

Deconvolution

Cellular Spatio-functional

Take-home messages

- Development of a spatial method giving functional information with a systematic LCM sampling grid.
- Pixel size = 10-20 cells = from 800 to 3000 identified proteins

- 120 pixels /tissue section, 2 tissue sections analyzed in the current proof of concept study

- A novel approach to reach single-cell spatial resolution

→ Ongoing work: Bioinformatic pipeline development to combine single-cell experiment and the pixel-by-pixel approach

The End

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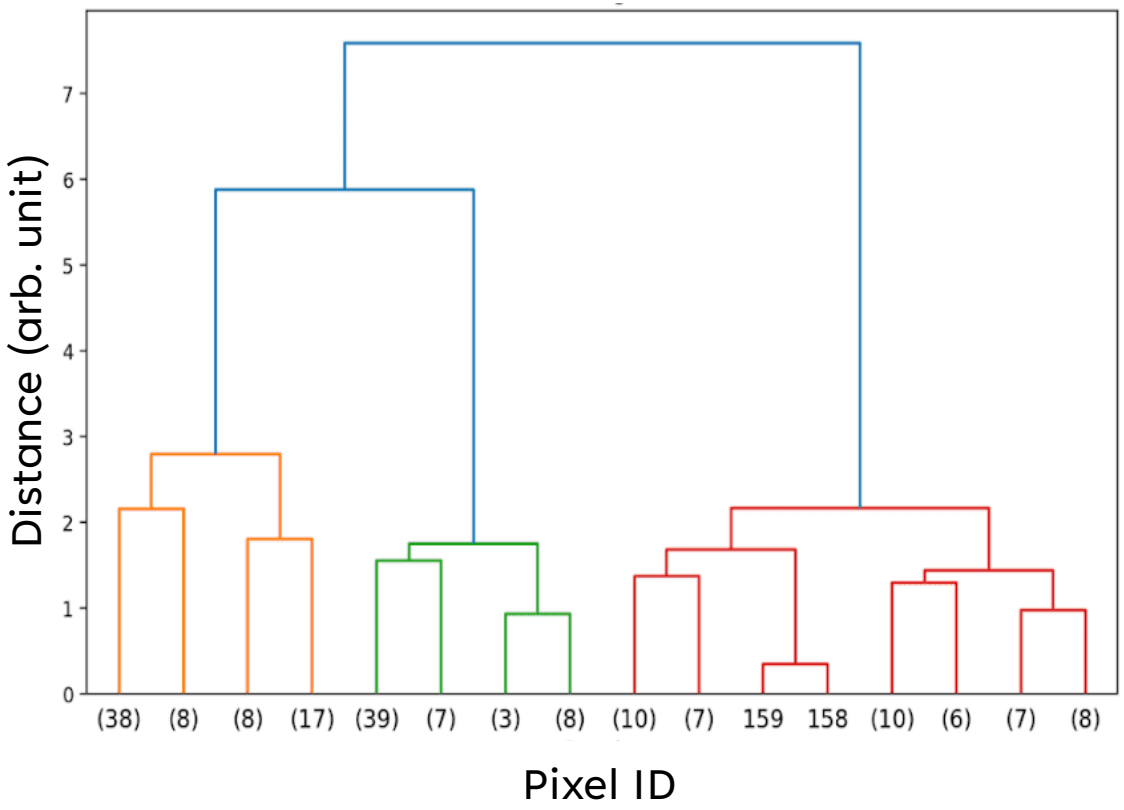
Thank you for your attention!

Do you have any questions?

BACK-UP

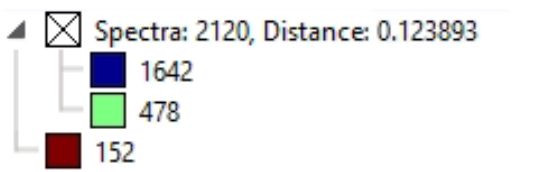
MALDI MSI Results: Hierarchical clustering

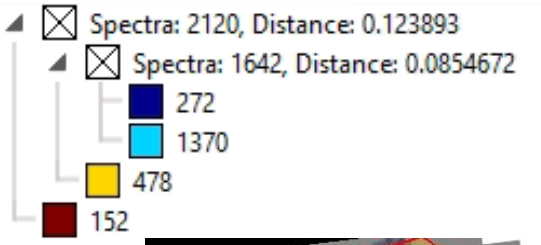
Hierarchical clustering concept :

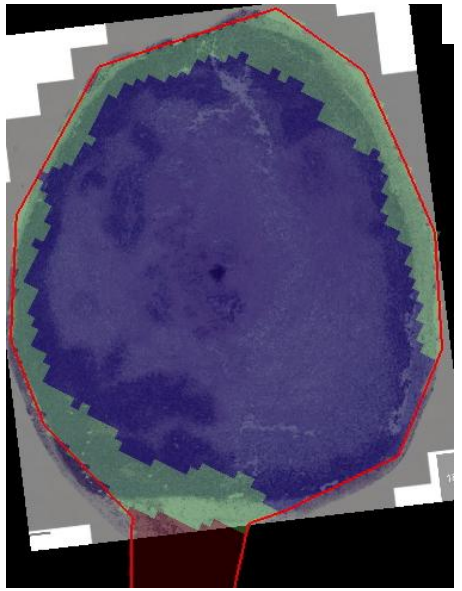


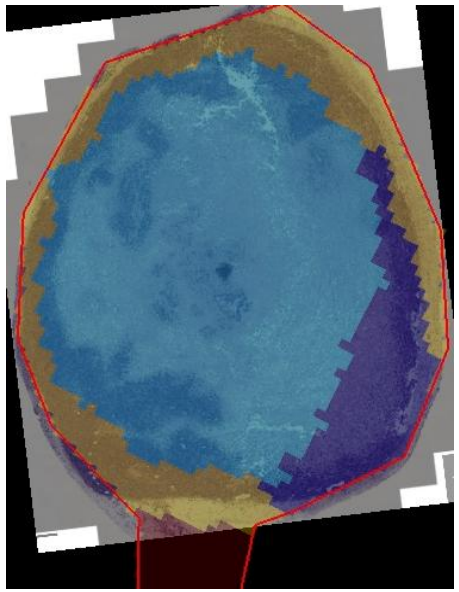
Pixel are clustered based on the difference on their MS spectrum

Pixel are colored based on their group



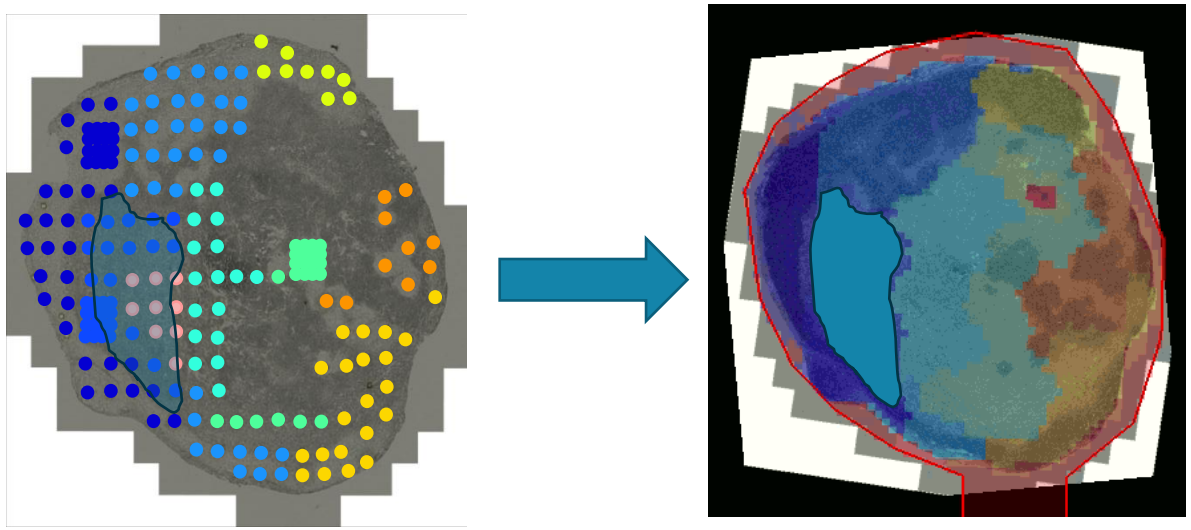






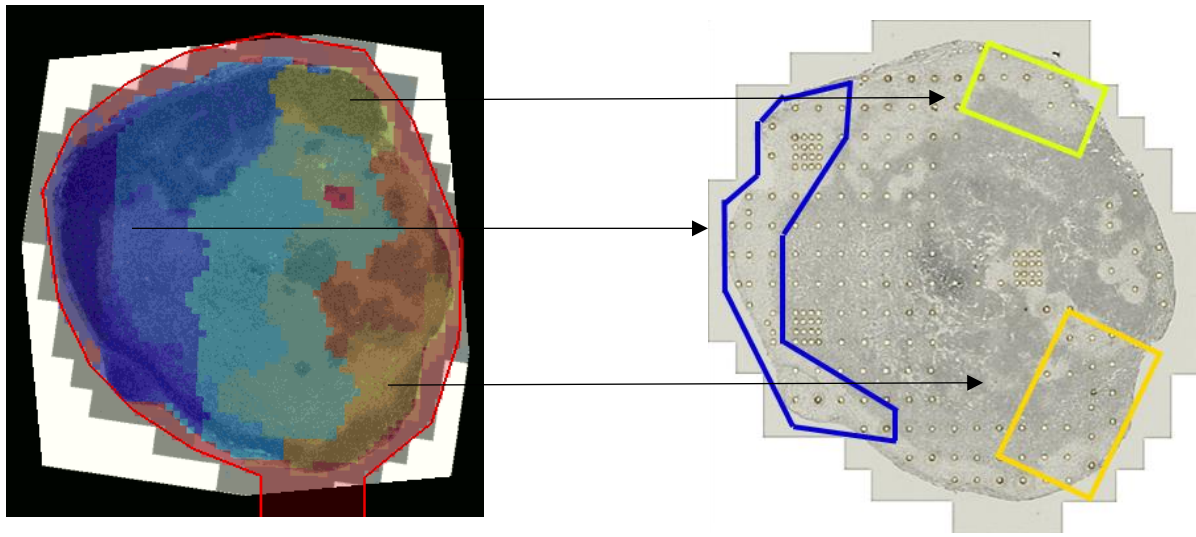
Perspectives

Unsupervised LCM pixel-by-pixel proteomics



- Obtaining zones by clustering LCM data with functional interpretation
- Compare with MALDI MSI molecular clustering

Supervised LCM pixel clustering



- Clustering pixels based on the zones sharing similar molecular MALDI profiles
- Supervisied differential study based on MALDI imaging
- Allow futur revisiting of LCM-data

Objectif et approche

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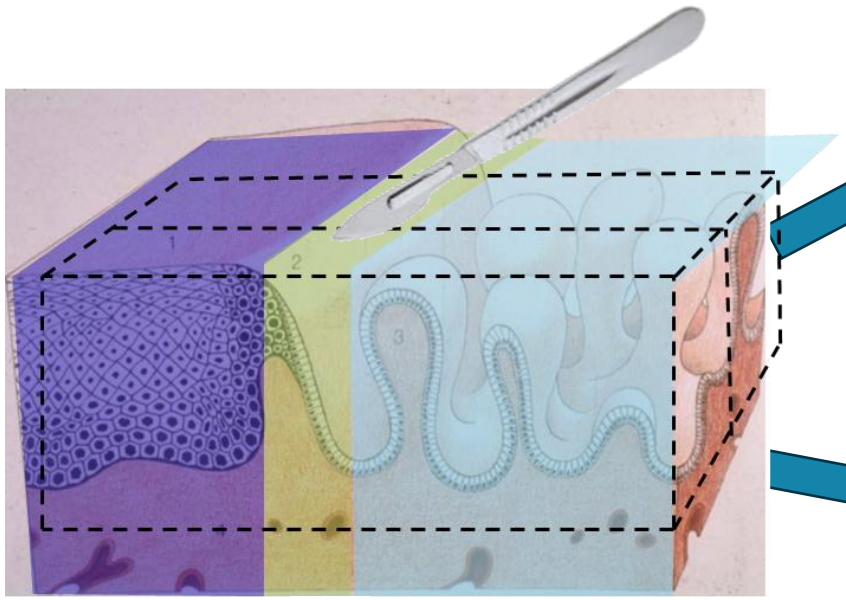
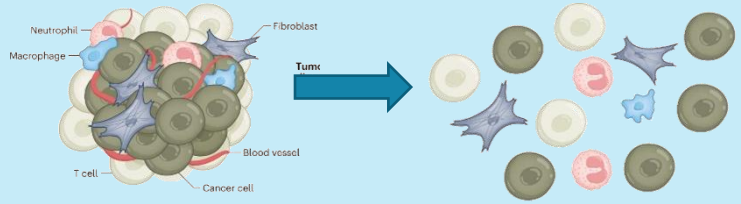
Méthode d'analyse de protéomique spatiale systématique à échelle cellulaire



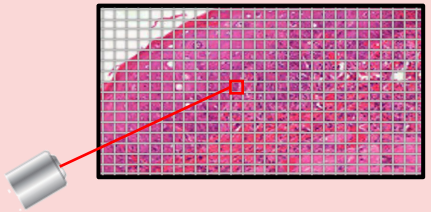
Approche fonctionnelle

Single-cell Protéomique (SCP)

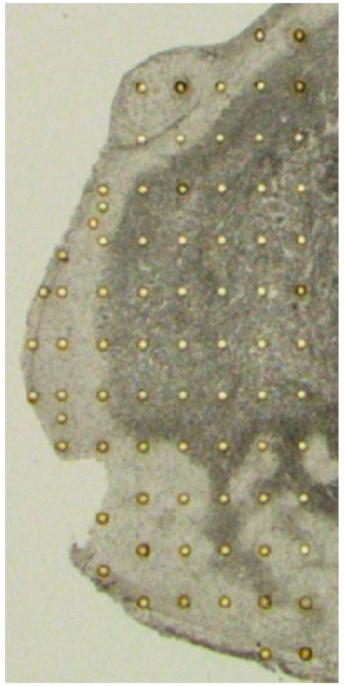


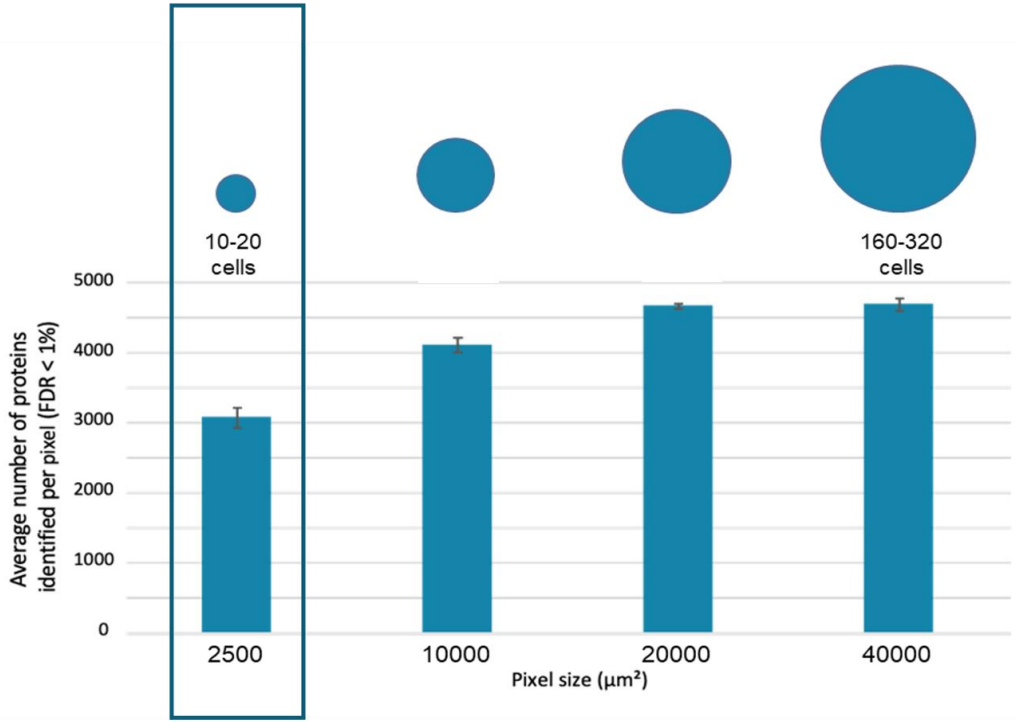
Approche spatiale

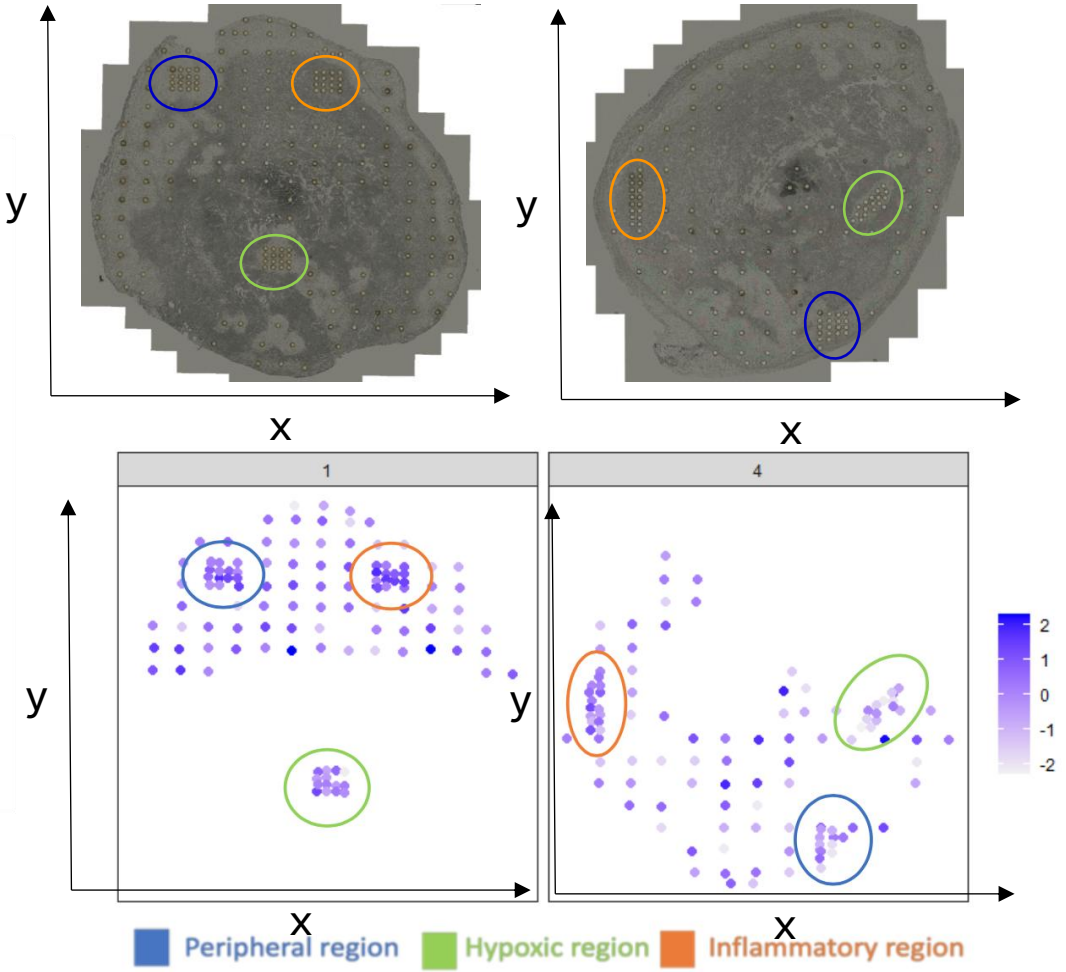
Pixel par pixel Protéomique (P2P)



Résultats préliminaires







✓ Microdissection systématique

↗ Densité pixels

✓ Protéomique spatiale (P²P)