Approches de protéomique spatiale pour l'étude des mécanismes moléculaires de pathologies

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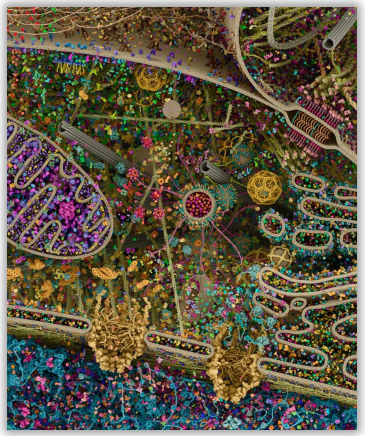
Workshop *Transcriptomique et Protéomique spatiale : des avancées technologiques pour la recherche en santé*, 28/04/2026

We are interested in PROTEINS, the workhorses of life

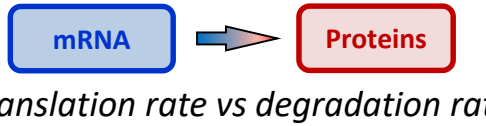


https://gaelmcgill.artstation.com/projects/Pm0JL1

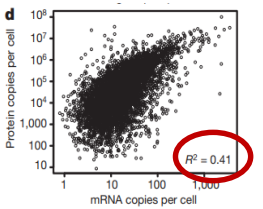
- Proteins = **functional** and **dynamic** units, carrying most cellular functions

- **Post-translational modifications** = critical for protein functions, activities, localizations, and interactions
  - *They cannot be predicted from transcriptomics or metabolomics data*
  - *In humans, ~20,000 genes → >1 million predicted proteoforms!*

- **Poor correlation** between mRNA and protein abundance



Translation rate vs degradation rate

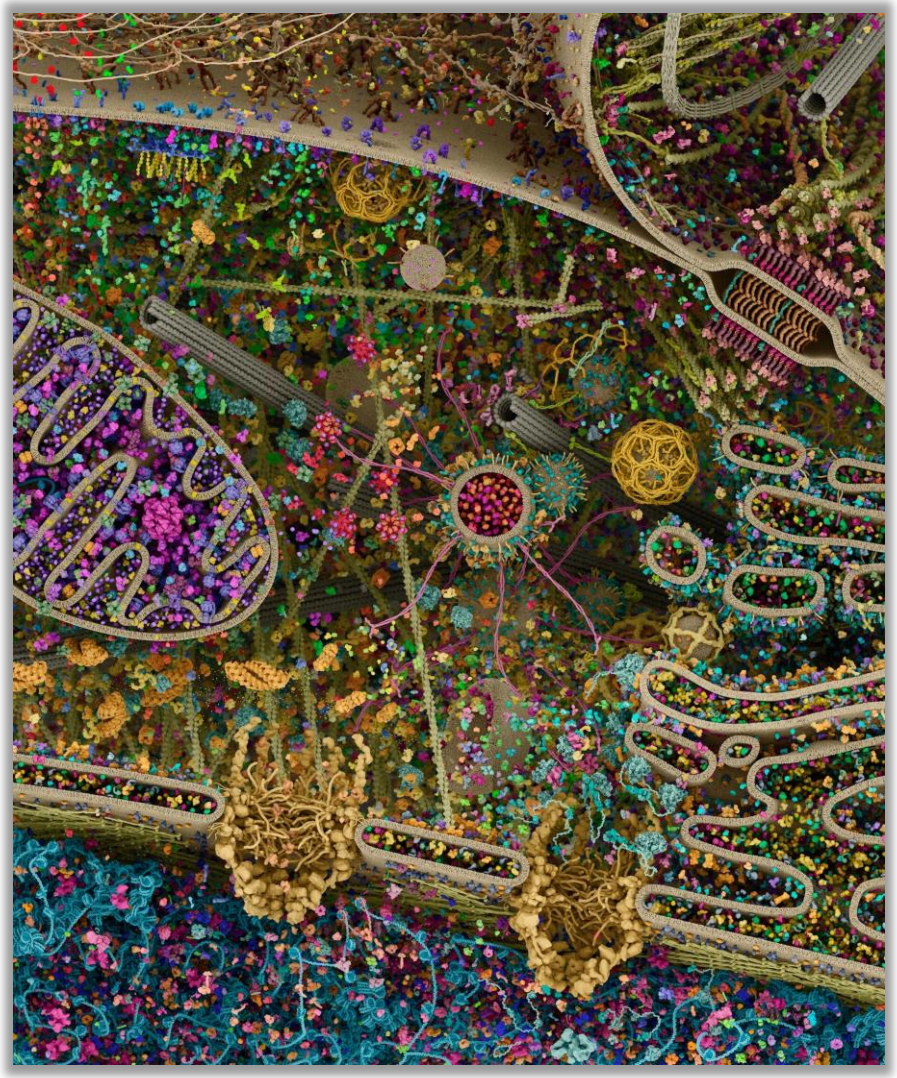


$R^2 = 0.41$

Schwanhaüsser B *et al., Nature*, 2011

- Many **biomarkers** and **drug targets** are proteins!

Two routes to study the proteome



Gael McGill
https://gaelmcgill.artstation.com/projects/Pm0JL1

Proteome

Antibody-based analysis

IHC

SomaScan

Olink

CyTOF

MALDI HiPLEX-IHC

Targeted proteomics

Mass Spectrometry-based analysis

LC-MS/MS

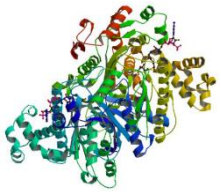
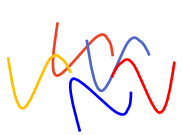
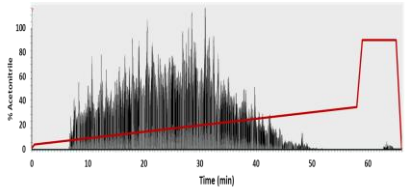
Bottom-up proteomics

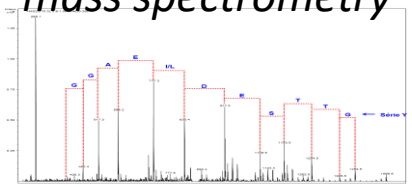
MS imaging

Untargeted proteomics

25 years of progress towards higher sensitivity & throughput

Protein mixture → *Enzymatic digestion* → *Peptide mixture* → *Separation* → *Liquid chromatograhy* → *MS/MS analysis* → *Tandem or MS/MS mass spectrometry*

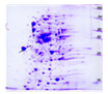


2001	2008	2011	2014	2018	2019	2023
Washburn *et al.* **1500 yeast proteins** 68h	Mann *et al.* **4000 yeast proteins** 144h	Thakur *et al.* **3000 yeast proteins** 8h	Hebert *et al.* **4000 yeast proteins** 1h	Meier *et al.* **6000 human proteins** 2h	Muntel *et al.* **10500 human proteins** 2h	Astral (Thermo) Tims Ultra (Bruker) **>10000 human proteins** 0,5h

Instrumentation
→ Sensitivity
→ Acquisition speed is crucial

1 Hz | 2 Hz | 7 Hz | 22 Hz | 40 Hz | 100 Hz | 200-300 Hz → **300 MS/MS spectra/sec**

Sample preparation
→ Miniaturization
→ Automation


2D gel

1D gel


MudPIT

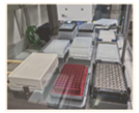
Stacking gel

Liquid digestion

FASP

S-trap

PreOmics iST

SP3

Automation

Data analysis tools
→ AI-prediction tools

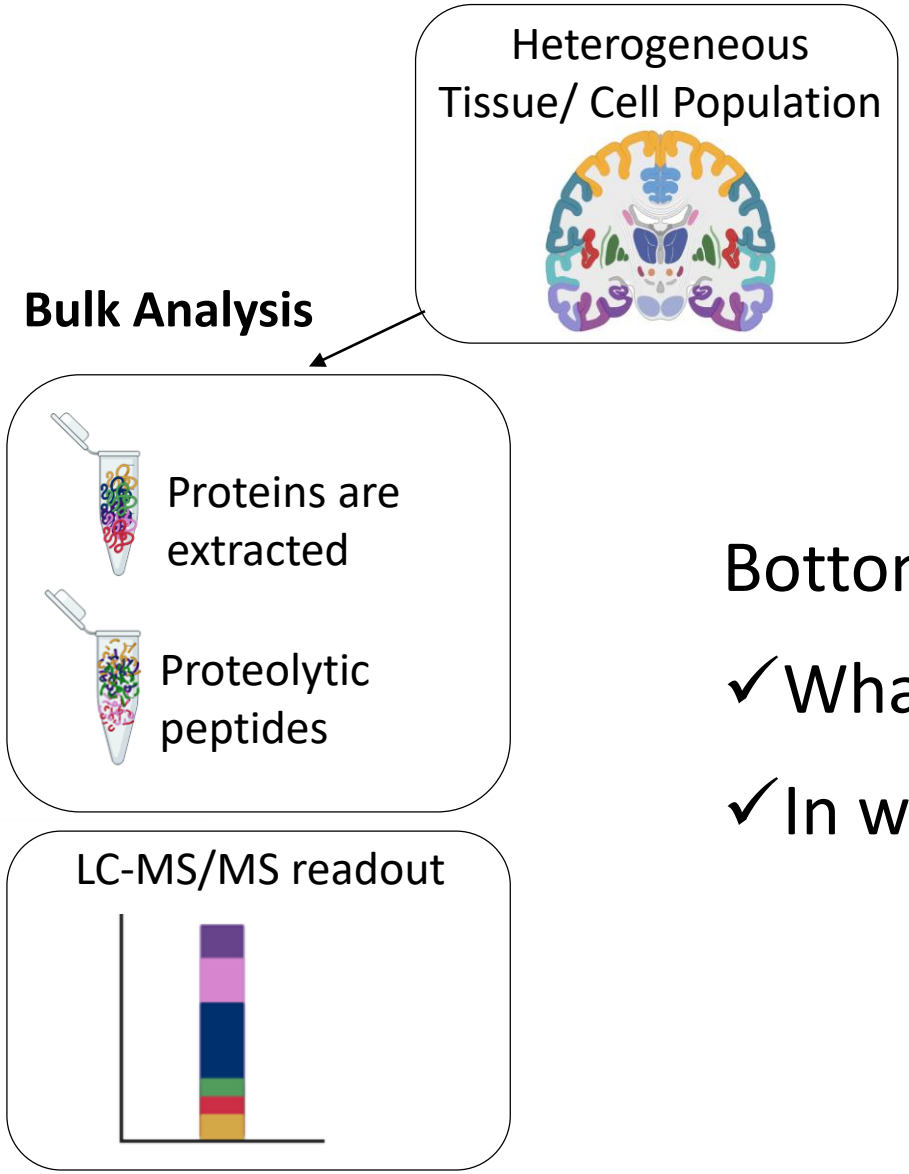
MATRIX SCIENCE Sequest


OpenSWATH
+AI


DIA-NN


Confidence PTMs

Bottom-up proteomics for identification and quantification



Bottom-up proteomics tells us

✓What proteins are present

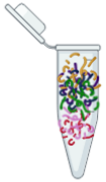
✓In what quantity proteins are present

But spatial information is missing

Heterogeneous
Tissue/ Cell Population

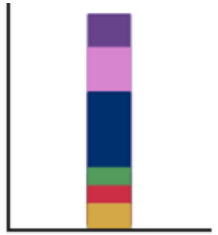
Bulk Analysis

Proteins are
mixed together



Subpopulations
not defined

« Averaged » readout



Bottom-up proteomics tells us

✓What proteins are present

✓In what quantity proteins are present

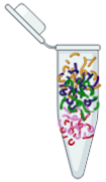
But **not** where proteins are localized

We need to analyze proteins *spatially*

Heterogeneous
Tissue/ Cell Population

Bulk Analysis

Proteins are mixed together



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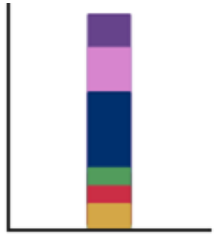
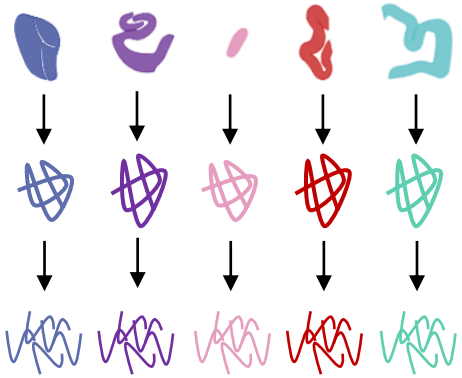


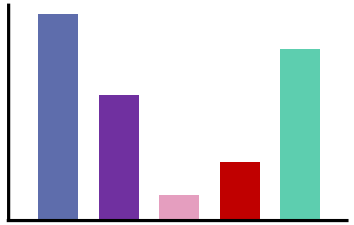
Spatial Analysis



Proteins from each region are analyzed

Subpopulations defined

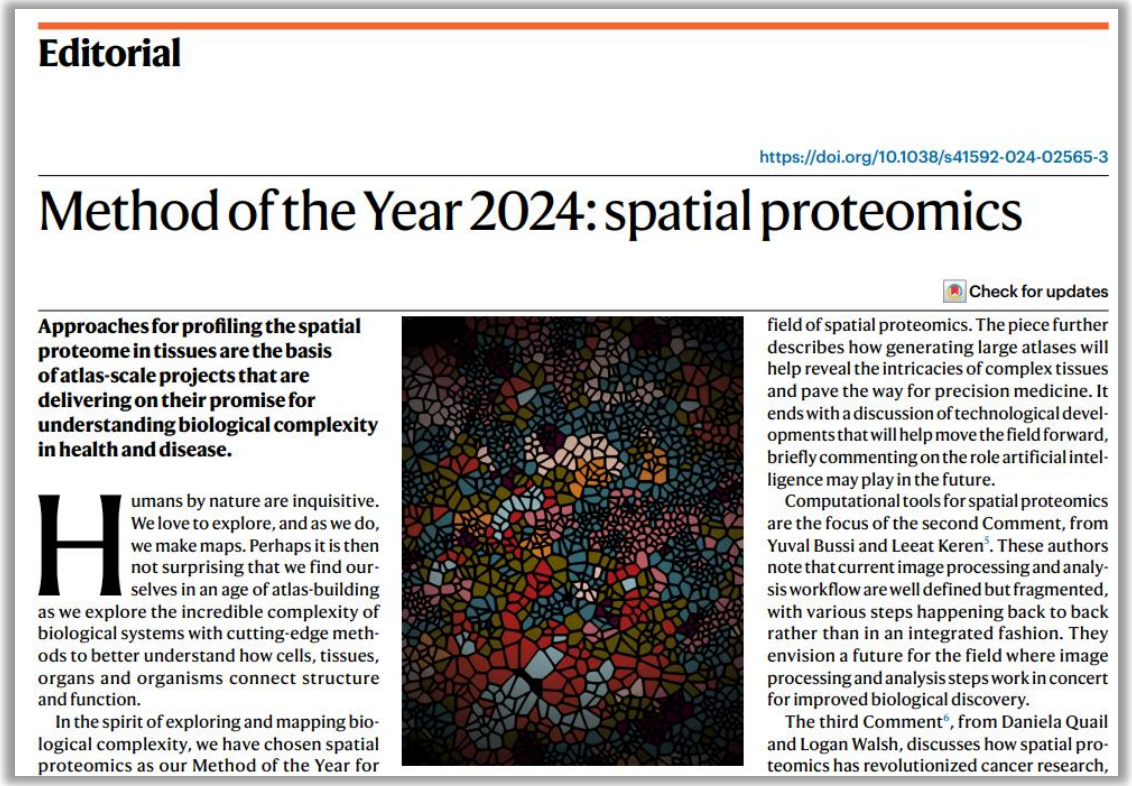
Region-specific readout



spatially-resolved ‘molecular profile’

Spatial proteomics

- Mapping proteins within their native environment by capturing both protein **identity and location** profiles within defined structures



Editorial

https://doi.org/10.1038/s41592-024-02565-3

Method of the Year 2024: spatial proteomics

Check for updates

Approaches for profiling the spatial proteome in tissues are the basis of atlas-scale projects that are delivering on their promise for understanding biological complexity in health and disease.

Humans by nature are inquisitive. We love to explore, and as we do, we make maps. Perhaps it is then not surprising that we find ourselves in an age of atlas-building as we explore the incredible complexity of biological systems with cutting-edge methods to better understand how cells, tissues, organs and organisms connect structure and function.

In the spirit of exploring and mapping biological complexity, we have chosen spatial proteomics as our Method of the Year for

field of spatial proteomics. The piece further describes how generating large atlases will help reveal the intricacies of complex tissues and pave the way for precision medicine. It ends with a discussion of technological developments that will help move the field forward, briefly commenting on the role artificial intelligence may play in the future.

Computational tools for spatial proteomics are the focus of the second Comment, from Yuval Bussi and Leeat Keren[5]. These authors note that current image processing and analysis workflow are well defined but fragmented, with various steps happening back to back rather than in an integrated fashion. They envision a future for the field where image processing and analysis steps work in concert for improved biological discovery.

The third Comment[6], from Daniela Quail and Logan Walsh, discusses how spatial proteomics has revolutionized cancer research,

Spatial proteomics

- Mapping proteins within their native environment by capturing both protein **identity and location** profiles within defined structures

Why is it important?

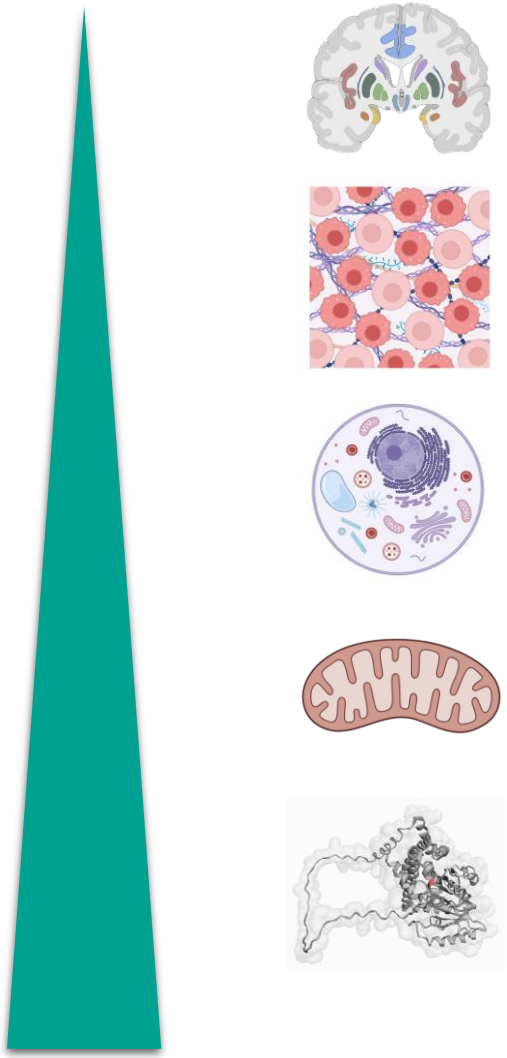
- Biological processes are spatially arranged
- Protein function may depend on its location
- Connecting molecular functions and cellular architecture/tissue heterogeneity
- Necessary to better understand physiology and diseases

Mou M *et al.*, *Journal of Chemical Information and Modeling*, 2022

Spatial proteomics operates across multiple scales

Complexity

Resolution



Tissue

Whole tissue or regions

Region/Micro-environment

Defined regions, specific niches

Cell

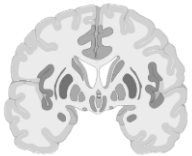
Different cell populations or individual cells

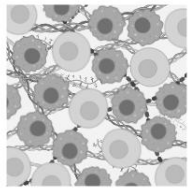
Subcellular

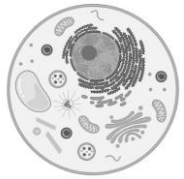
Compartments/organelles, protein complexes

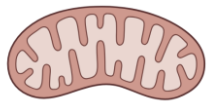
Spatial proteomics operates across multiple scales

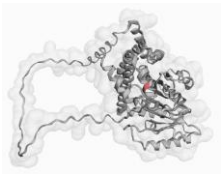
Resolution











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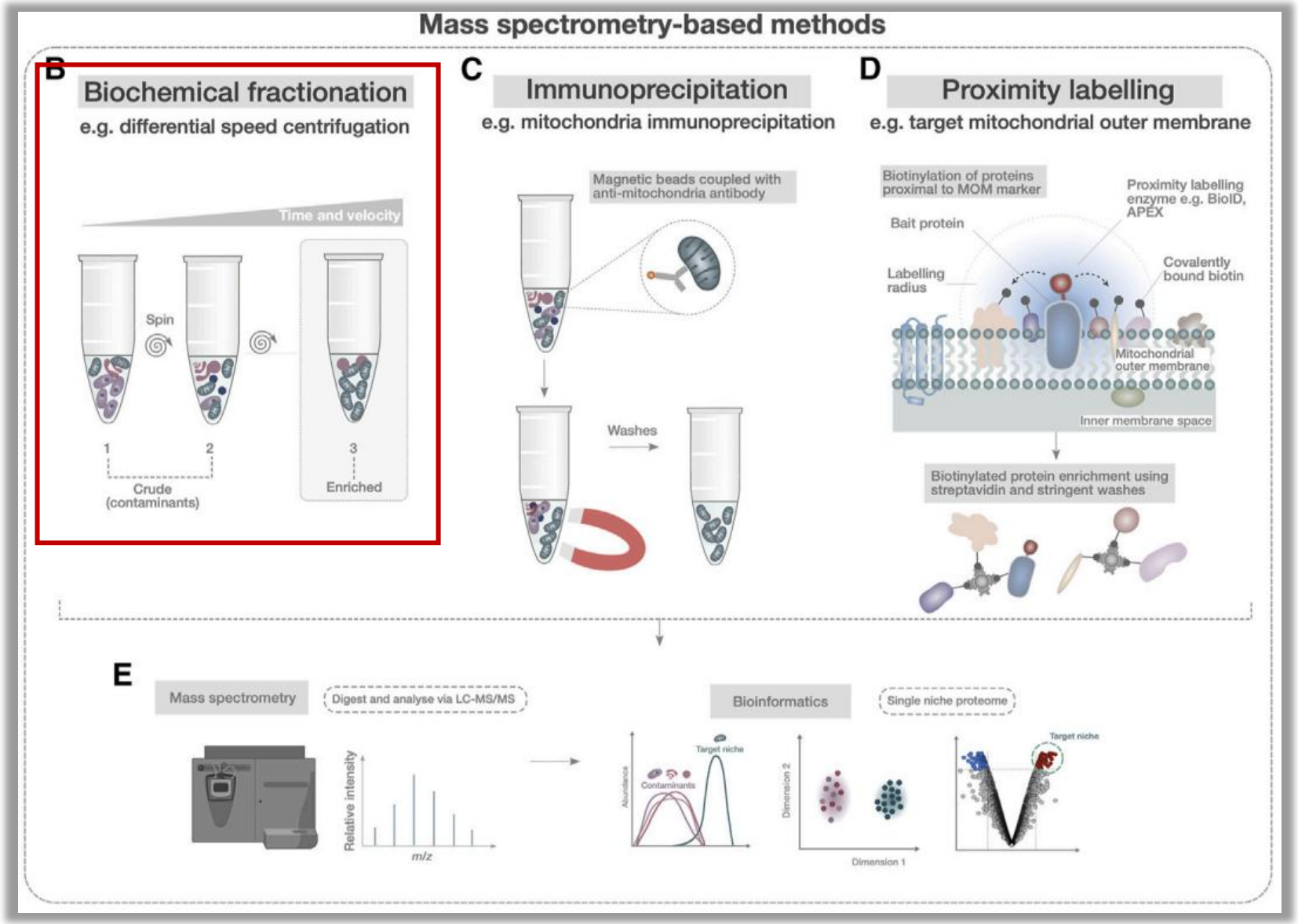
Strategies for subcellular proteomics

Compartment isolation by differential centrifugation (LOPIT-DC*)

→ Currently being implemented @LSMBO



*Geladaki A., Lilley K. *et al.*, *Nature Comm, 2019*

Breckels L. *et al.*, *Cell Chemical Biology*, 2024

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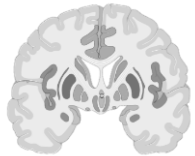
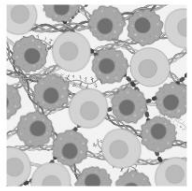
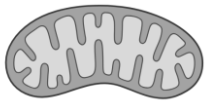
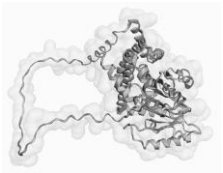
Resolution









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Single cell proteomics

Christine Carapito

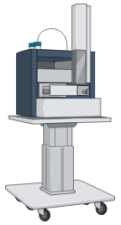
Isolation → **Lysis** → **Liquid chromatography** → **Tandem mass spectrometry** → **Data processing**

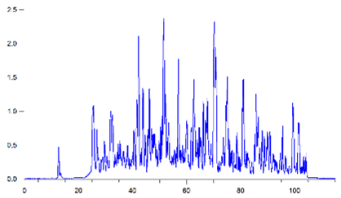
Cells bulk

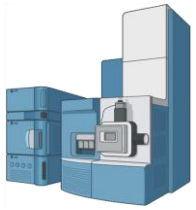
Protein lysate from isolated cell

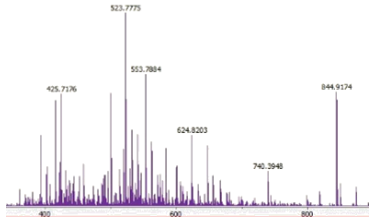
Digestion

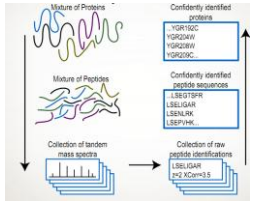
Peptide mixture











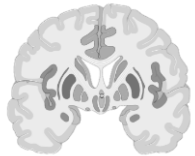
CellenOne (Cellenion)
Cell sorting & sample preparation robot

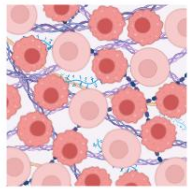
TimsTOF Ultra (Bruker → September 2023)
Orbitrap Astral (Thermo → August 2024)
DIA-PASEF & FAIMS-DIA MS/MS

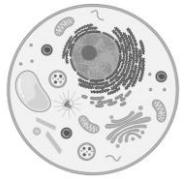
>5000 unique proteins on single HeLa cells

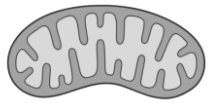
Spatial proteomics operates across multiple scales

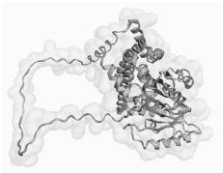
Resolution











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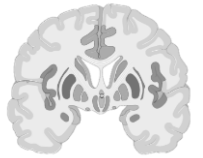

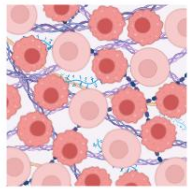

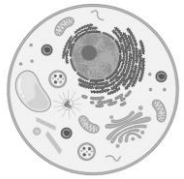

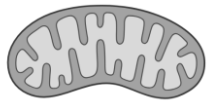

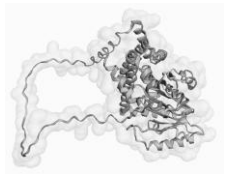

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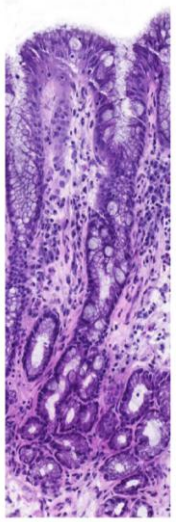
Case study:

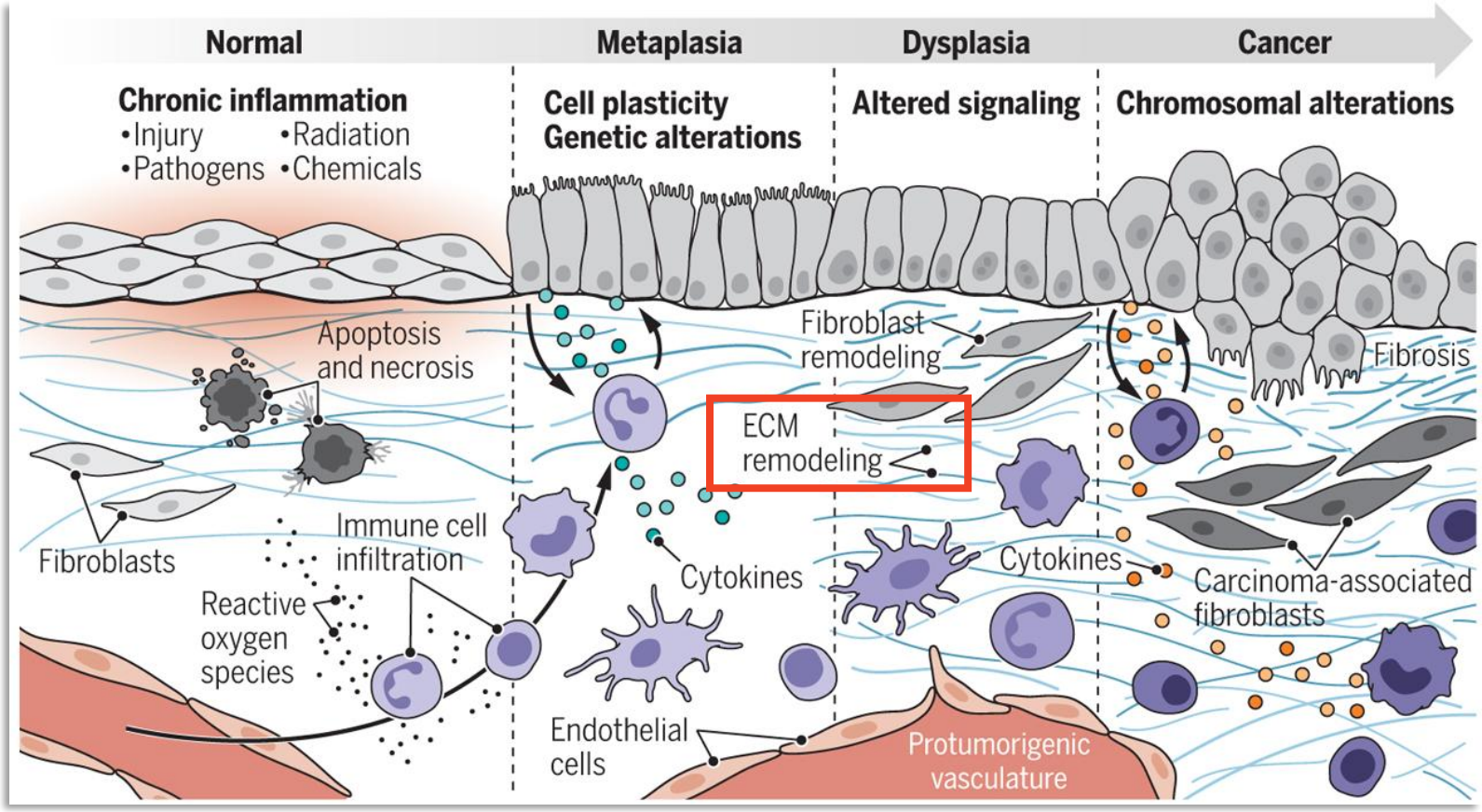
Investigate how the extracellular matrix is remodeled in lung squamous cell carcinoma (LSCC)

Stroma contributes to CIAC development

Thea Tlsty
Project Leader
University of California at San Francisco



Chronic inflammation-associated cancer (CIAC)

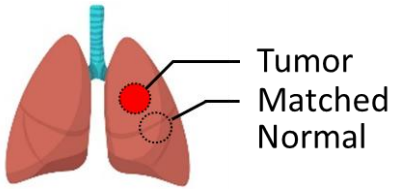


Tlsty T.D. and Gascard P., 2019, *Science*

- Address the **unmet clinical needs of CIACs** – stromal-targeted therapy for prevention and intervention
- We need to gain insights into **extracellular matrix (ECM) proteome remodeling**

Tumor-specific marker candidates in LSCC

Prospective sample collection



From 10 patients with lung squamous cell carcinoma (**LSCC**)

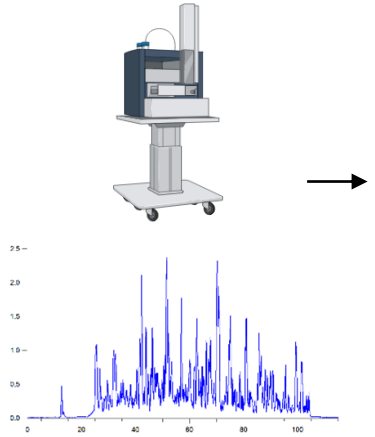
ECM proteins

Enriched, solubilized

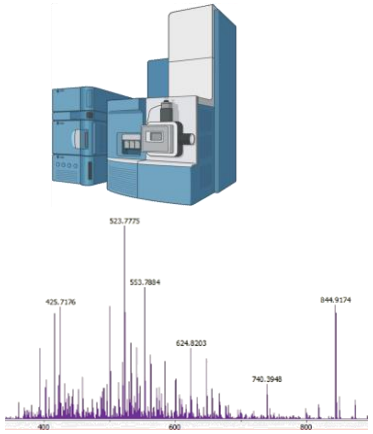
Digestion

Peptide mixture

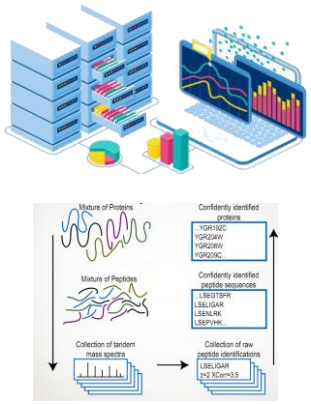
Liquid chromatography



Tandem mass spectrometry



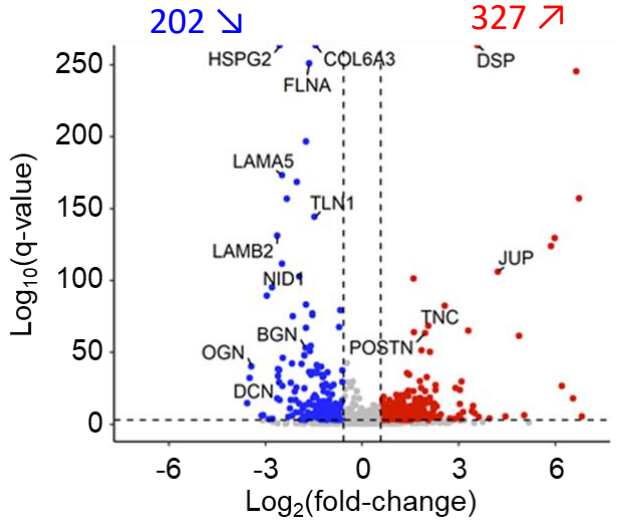
Quantification & statistics



In-depth ECM proteome profiling

Total = 1,802
quantifiable protein groups
(≥2 unique peptides)

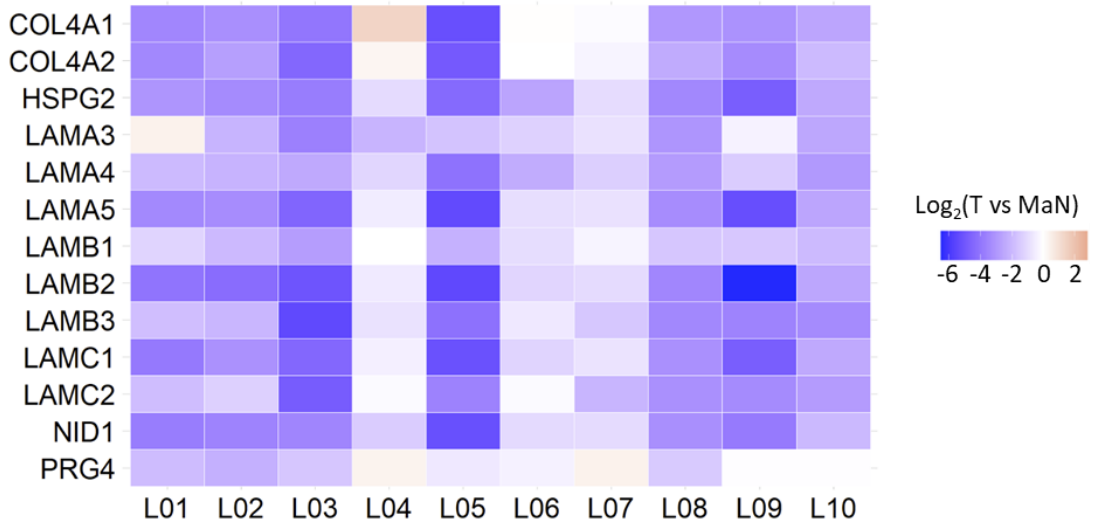
Tumor vs Matched Normal



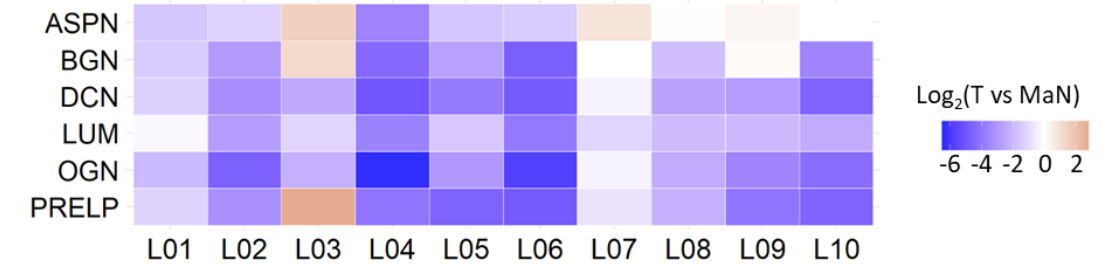
Bons J. *et al.*, *Proteomics*, 2023

Conserved ECM remodeling signature in LSCC

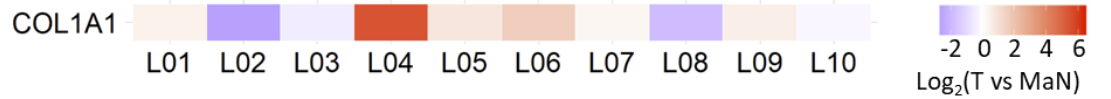
Basement membrane in Lung SCC



Small leucine-rich proteins in Lung SCC

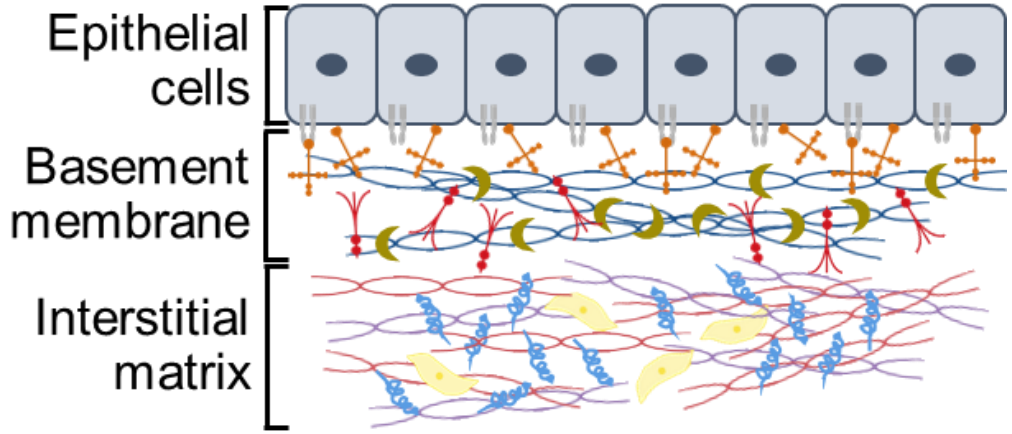


Type I collagen in Lung SCC



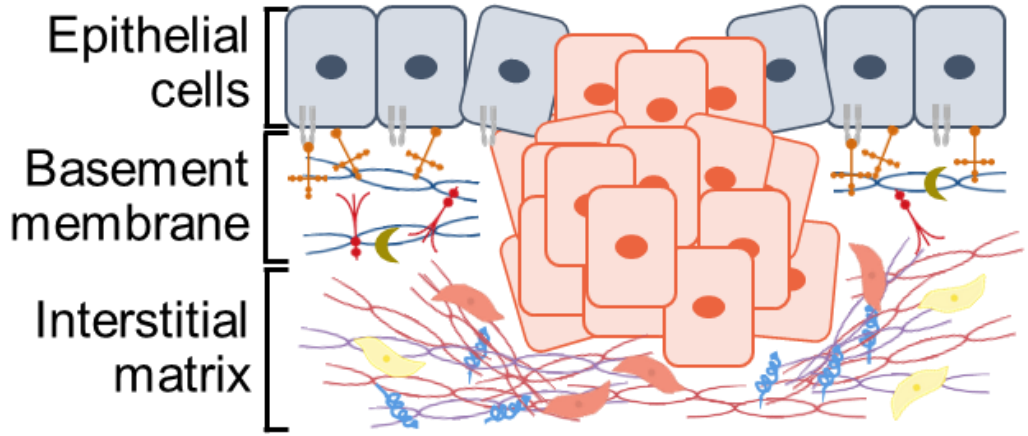
Bons J. *et al.*, *Proteomics*, 2023

Normal ECM



ECM remodeling in LSCC

Tumor ECM



SERPINH1/Hsp47 as a potential biomarker for CIACs?

Tumor vs Matched Normal
(same patient)

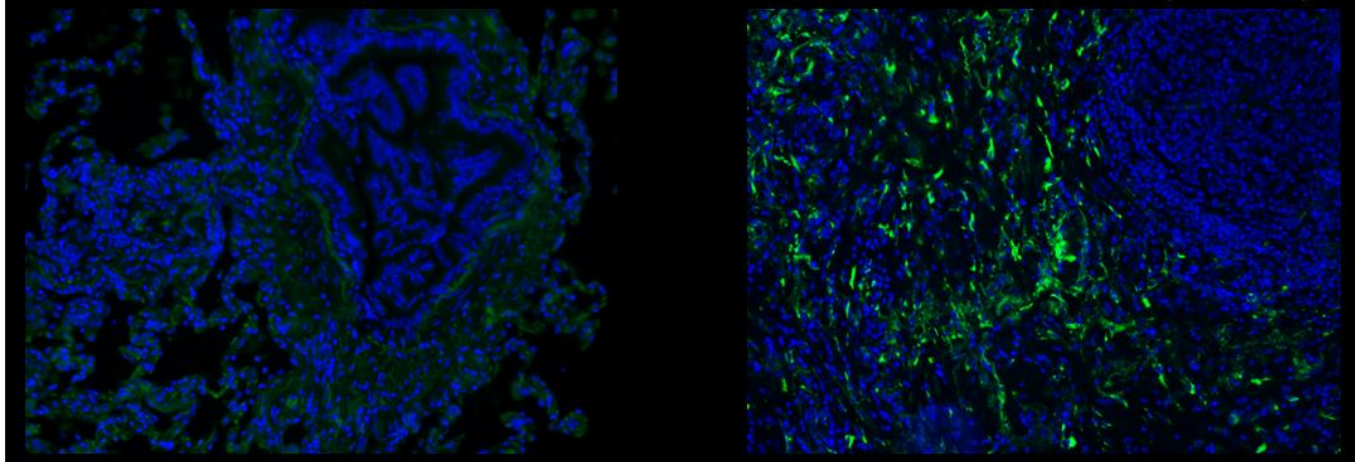
	Lung	Oesophagus	Stomach
SERPINH1	•	•	•
Log_2(T vs MaN)	1.23	1.44	0.79
Q-value	8 e-15	8 e-2	2 e-2

Profiling of SERPINH1/Hsp47
by immunofluorescence:

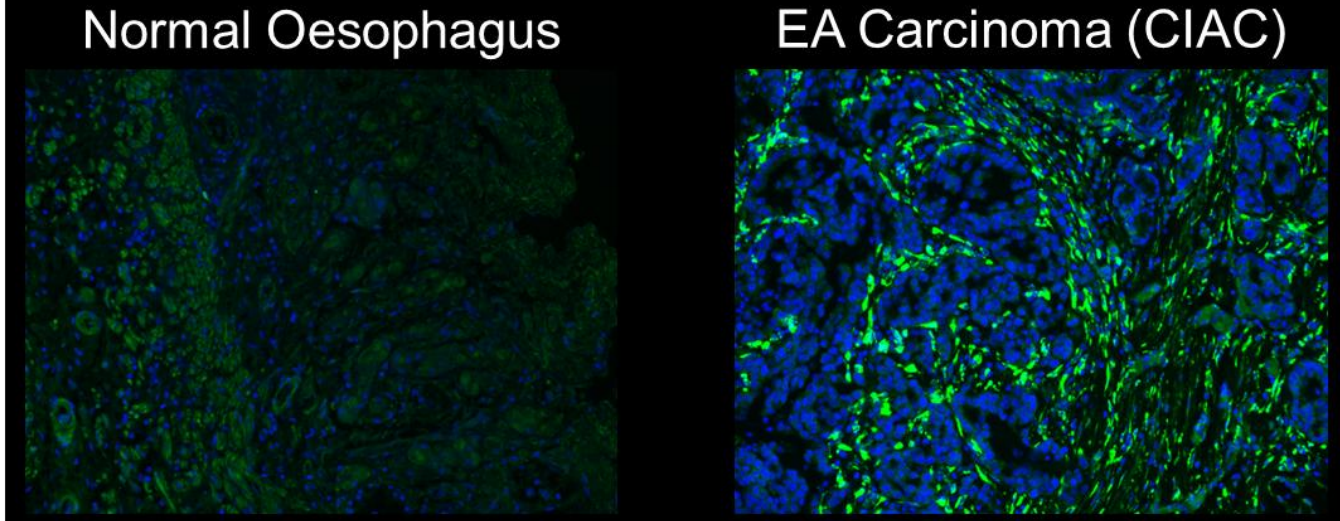
Dramatic increase
in cancer-associated fibroblasts (CAF) in

- **Lung** Squamous Cell Carcinoma, LSCC
- **Oesophageal** Adenocarcinoma, EAC

Detection of SERPINH1/Hsp47 In Lung



Detection of SERPINH1/Hsp47 In Oesophagus



Spatial proteomics operates across multiple scales

Resolution

Tissue
Whole tissue or regions

Region/Micro-environment
Defined regions, specific niches

Cell
Different cell populations or individual cells

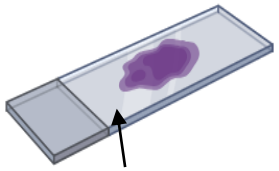
Subcellular
Compartments/organelles, protein complexes

Mass spectrometry imaging as a powerful tool to study tissues or regions of interest in pathologies

MALDI-Mass spectrometry imaging (MSI) workflow

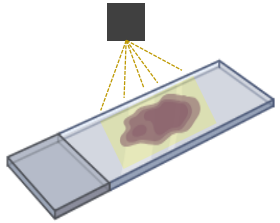
MALDI: Matrix-assisted laser desorption ionization

Tissue section (~10 µm)

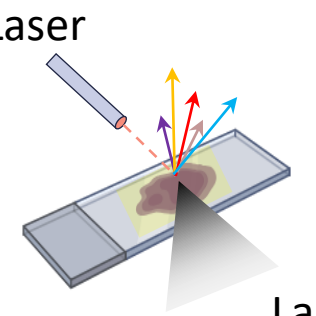


Conductive ITO slide

MALDI matrix deposition



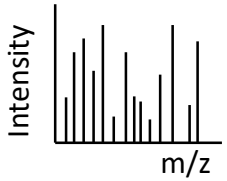
MALDI ionization



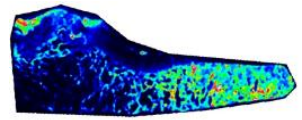
MS Imaging

Data processing

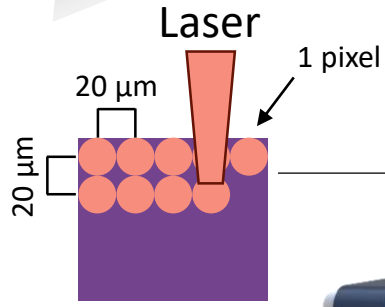
One spectrum per pixel



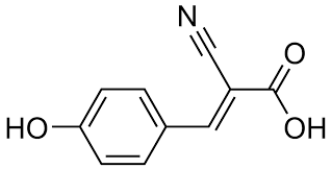
Distribution map of one analyte



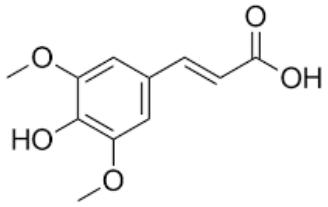
- ✓ **Label-free**
- ✓ ***In situ* co-localization of analytes**



Matrix depends on the investigated analytes and polarity.
For instance: α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid



CHCA



Sinapinic acid

Today

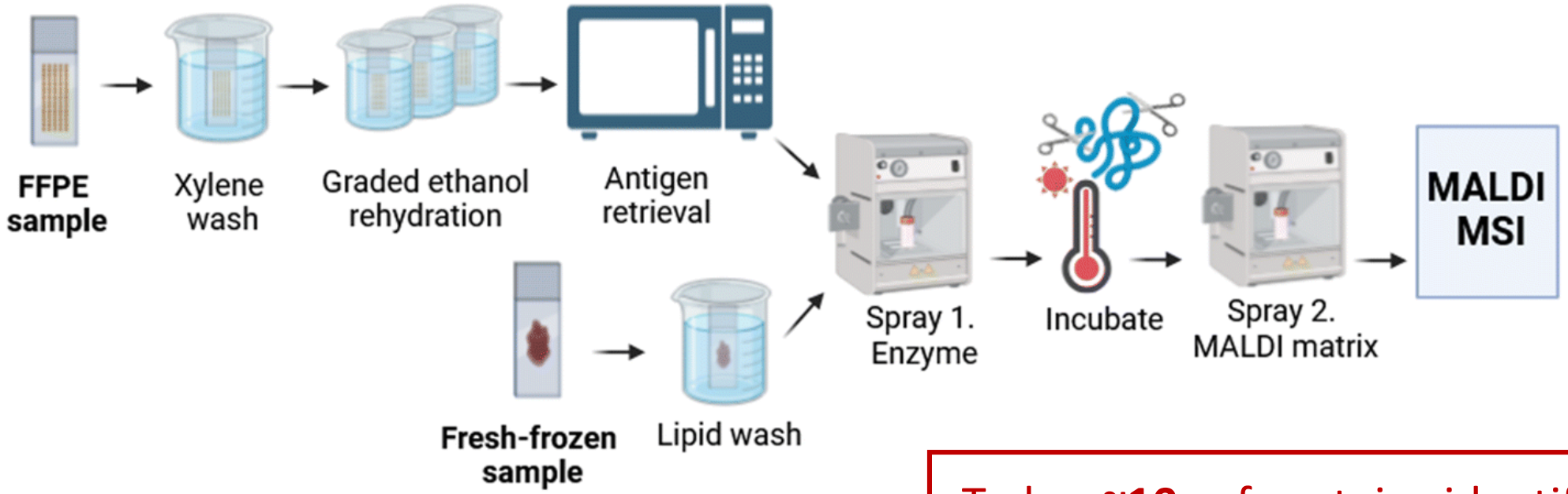
- ➢ 10 kHz, up to 20 pixel/s
- ➢ **Spatial resolution:**
  - **routine: 20 µm**
  - **down to 5 µm (~single cell)**

Spatial proteomics by bottum-up MALDI-MSI

➢ **On-tissue protein digestion** compatible with FFPE and fresh-frozen samples

➢ Additional steps need to be performed beforehand:

- **FFPE samples:** deparaffinization → antigen retrieval → enzymatic digestion
- **Fresh-frozen samples:** lipid and salt washing → enzymatic digestion

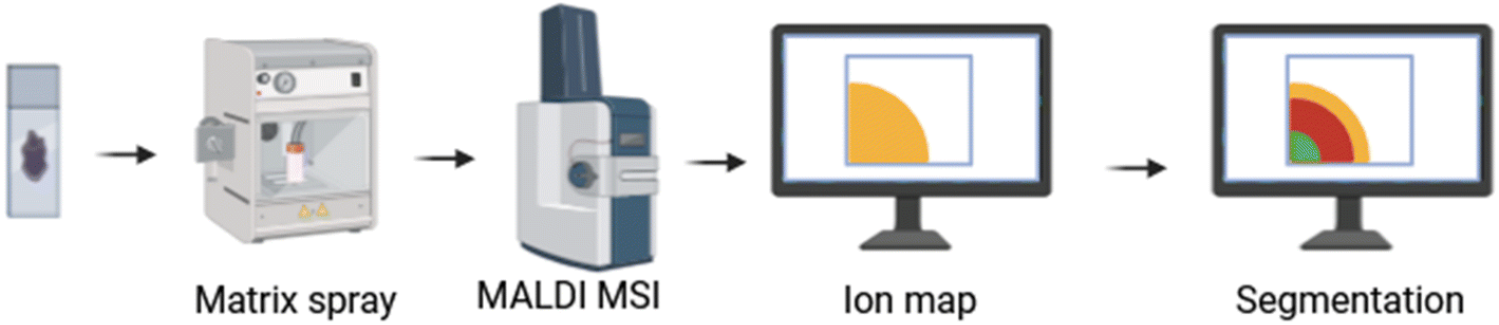


Today, **~10s** of proteins identified *in situ* by **bottom-up MALDI-MSI**

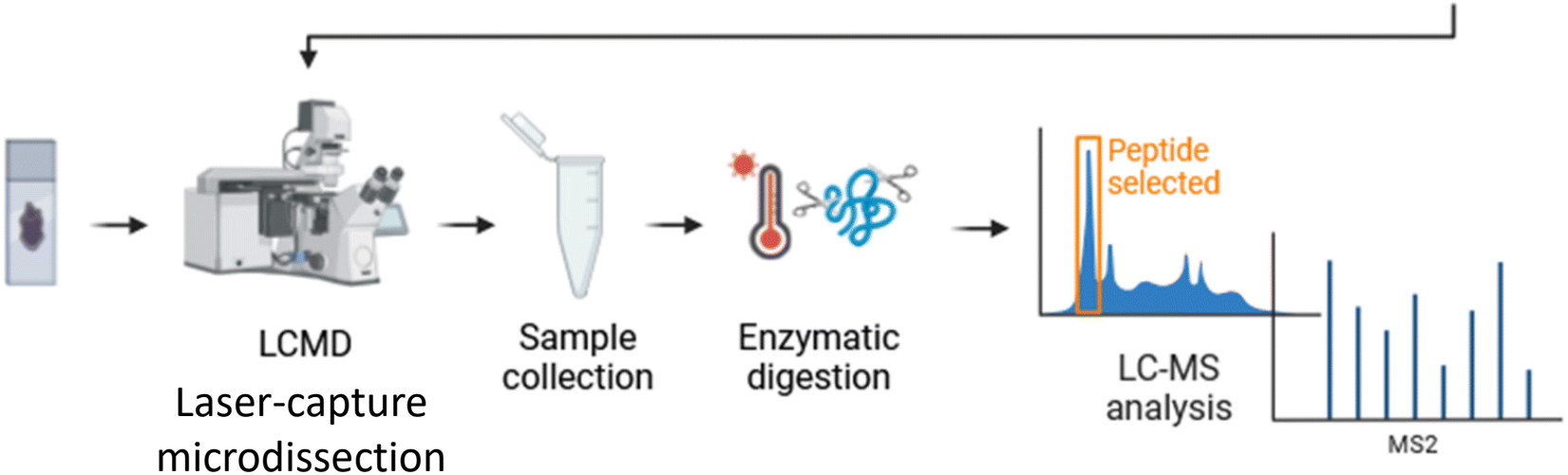
Lopez A, Hummon AB, *Anal Methods*, 2026

MSI-guided spatial proteomics

1) MALDI-MSI experiment to determine regions of interest – **IMAGING**



2) Microsampling followed by LC-MS/MS for in-depth spatially-resolved protein identification – **PROFILING**



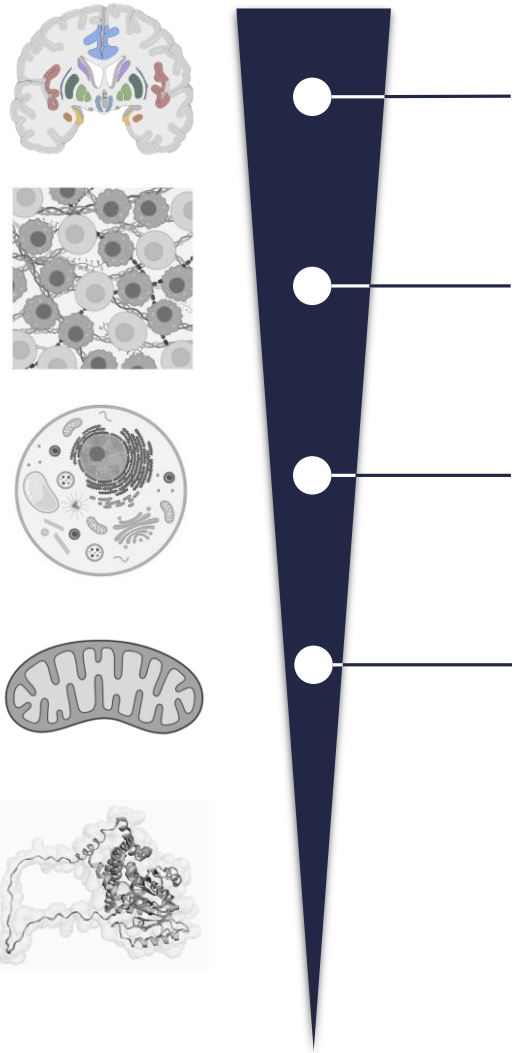
→ **Several 1000s** of proteins identified by high-throughput **LC-MS/MS**

Other excision techniques are available.

Adapted from Lopez A, Hummon AB, *Anal Methods*, 2026

Spatial proteomics operates across multiple scales

Resolution



Tissue

Whole tissue or regions

Case study:

Unveil the molecular heterogeneity of glioblastoma tumor microenvironment

Duhamel M., Fournier I., Salzet M., *et al.*, *Nat Commun,* 2022

→ MALDI MSI approaches under development @LSMBO

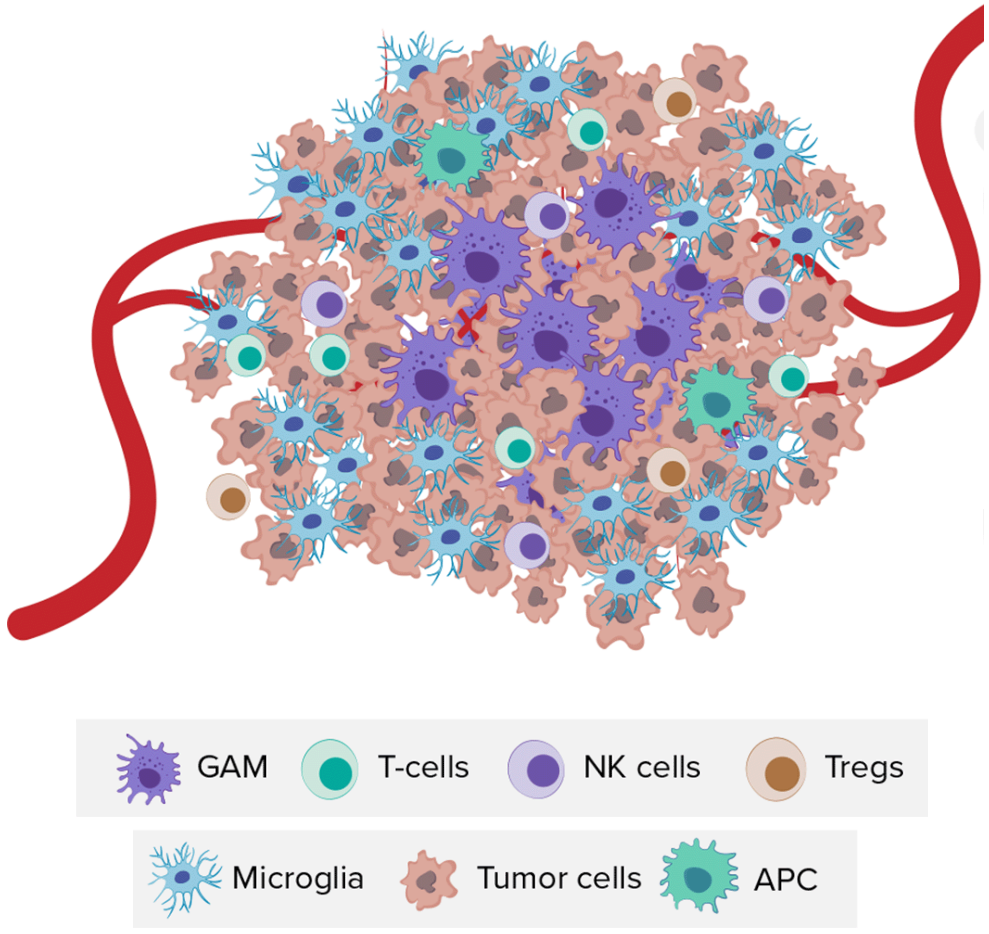
Glioblastoma heterogeneity

- Glioblastoma
  - ➢ Main malignant primary brain tumor
- Survival:
  - ➢ Median survival ~12-16 months
  - ➢ ~5% of patients survive more than 5 years
- **Highly heterogeneous tumors**:
  - ➢ Across microenvironments and within each microenvironment
  - ➢ Poor patient stratification and discrepancies in patient survival

Spatial proteomics of clinical samples of glioblastoma to characterize the molecular heterogeneity of glioblastoma tumors

Duhamel M *et al.*, *Nat Commun,* 2022

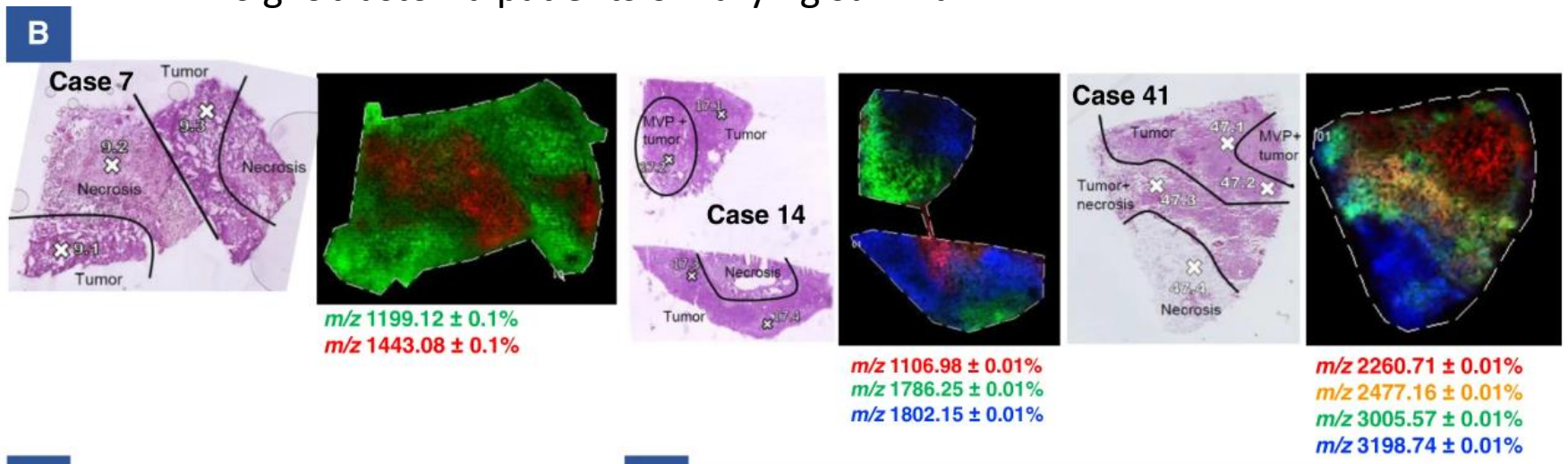
Tumor microenvironment of glioblastoma

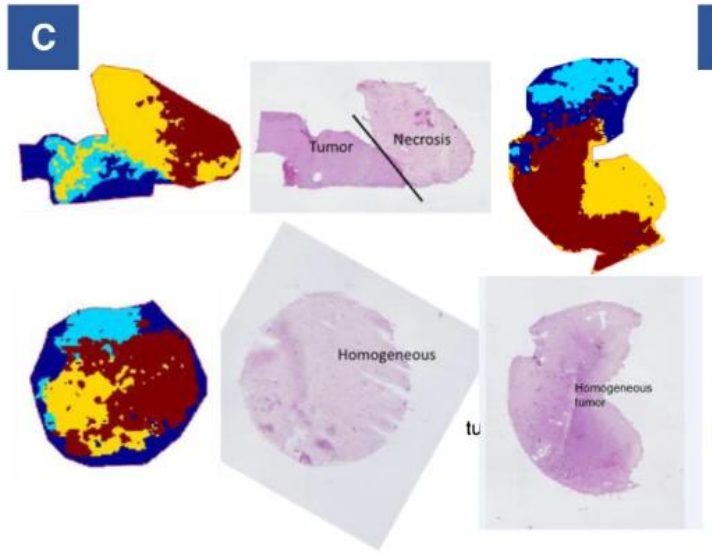


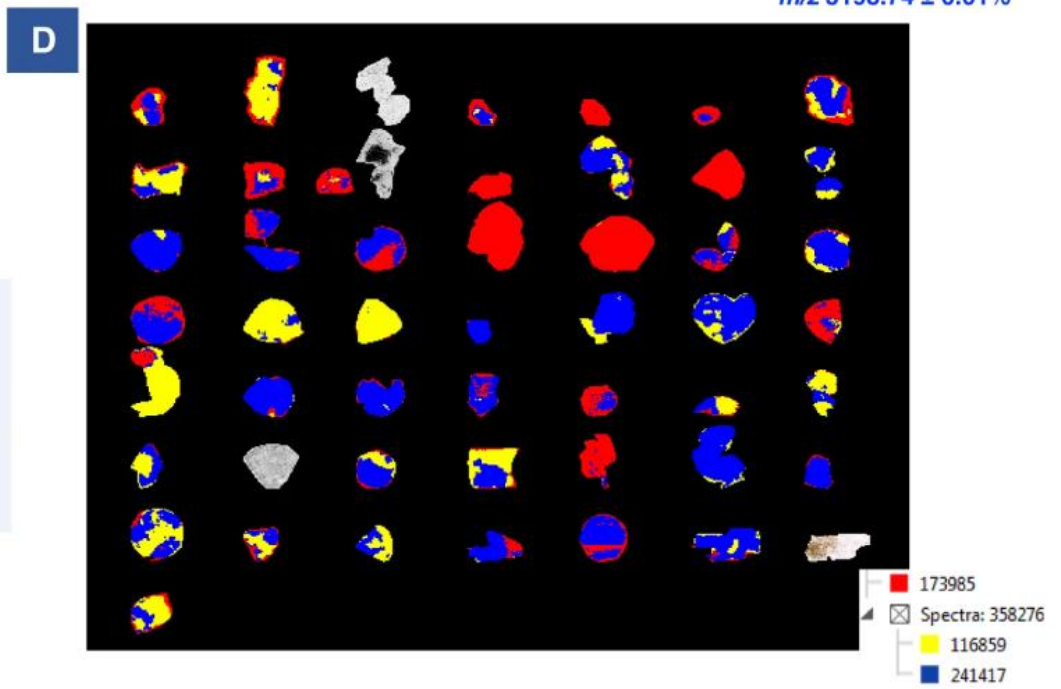
Adapted from Perdyan A *et al.*, *F1000Research,* 2022

MSI-guided spatial proteomics of glioblastoma

46 glioblastoma patients of varying survival





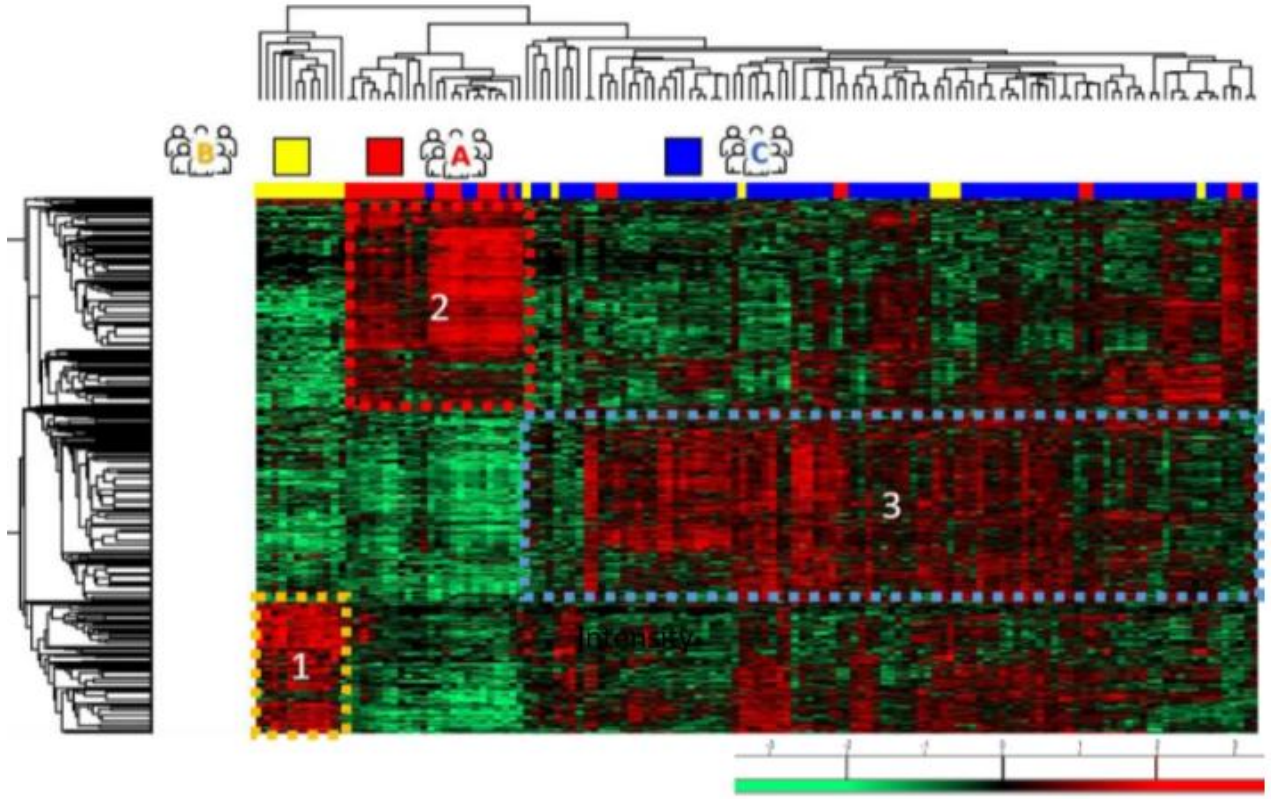


- Correlation between m/z features and histological annotations
- Segmentation maps identify 3 main molecular clusters
- Microextraction followed by LC-MS/MS analysis to determine proteomic profiles

Duhamel M *et al.*, *Nat Commun,* 2022

Identification of three molecular regions in glioblastoma

Proteins with differential profiles across the three regions:



Neurotransmission and synaptogenesis (neurogenesis and axon guidance)

Tumorigenesis (MAPK3, PRKCA, CRMPs, GAP43, Tau)

RNA processing and metabolism, tumor growth (HDGF, DRG2), virus infection

Bad prognosis indicators (EIF2AK2, ZC3HAV1)

Immune infiltration (microglial activation, immune system activation), iron transport

Histology: intense proliferation of capillary endothelial cells with inflammation and hemorrhage

- Integration with survival data → identification of 5 prognostic survival markers
- **Identification of specific molecular phenotype of the glioblastoma tumors informing on the intratumoral molecular heterogeneity**

Duhamel M *et al.*, *Nat Commun,* 2022

Conclusions

Spatial proteomics using mass spectrometry = powerful approaches:

- ✓ Map proteins *in situ* from fresh and FFPE tissues – from 10s (bottom-up MALDI) to several 1000s (microsampling coupled to LC-MS/MS) proteins.
- ✓ Uncover regionally-defined molecular profiles, at different and complementary scales, down to 5 µm resolution.

Molecular heterogeneity is more important than histological heterogeneity.

Spatial proteomics enables

- ➢ Gaining insights into **proteome complexity** and **processes/signaling pathways** associated with **pathogenesis** and **progression, notably in cancer.**
- ➢ Identifying **novel biomarker candidates** and potential **therapeutic targets.**

Thank you for your attention!

Questions?