



Les spectres Raman sont associés au matrisome de la fibrose intratumorale dans le carcinome hépatocellulaire humain

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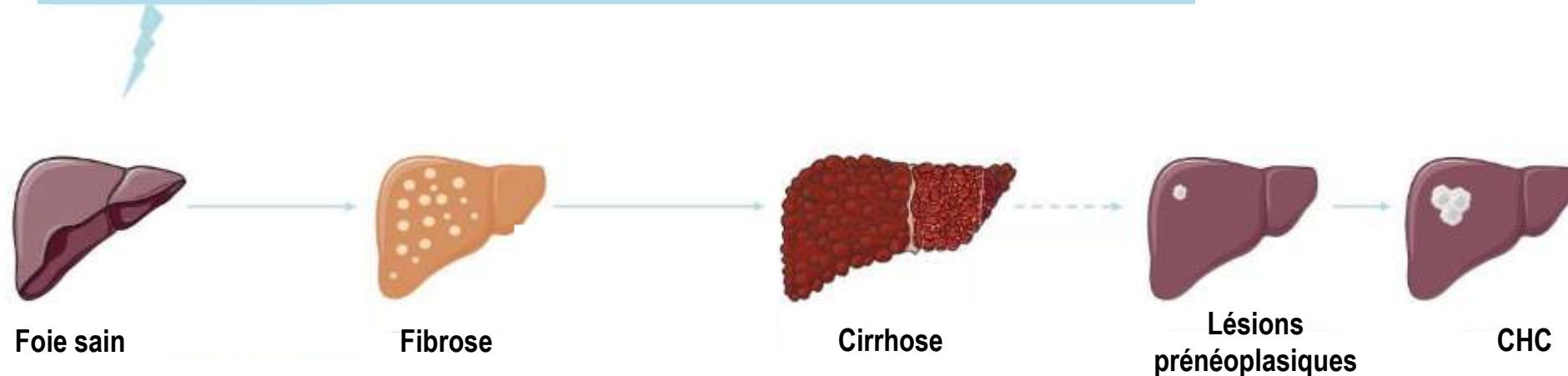
⁵ENSAI, Ecole Nationale de la Statistique et de l'Analyse de l'Information, Bruz, France

Physiopathologie du Carcinome Hépatocellulaire (CHC)

Le CHC survient dans 80% des cas sur un foie fibro-inflammatoire

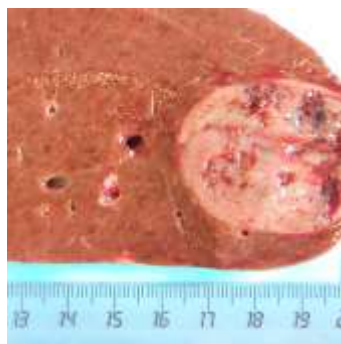
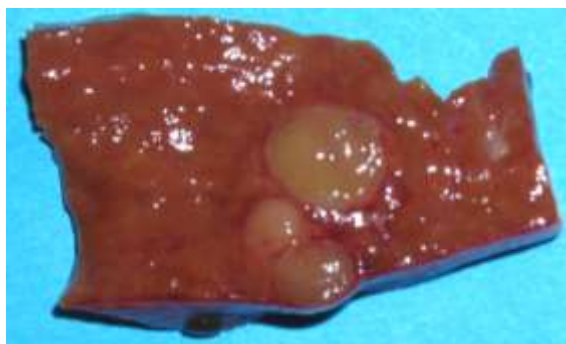
Lésions chroniques fibro-inflammatoires:

- Infections virales
- Abus d'alcool
- MASLD (*Metabolic Dysfunction- Associated Steatotic Liver Disease*)
- Maladies génétiques (e.g., hémochromatose).



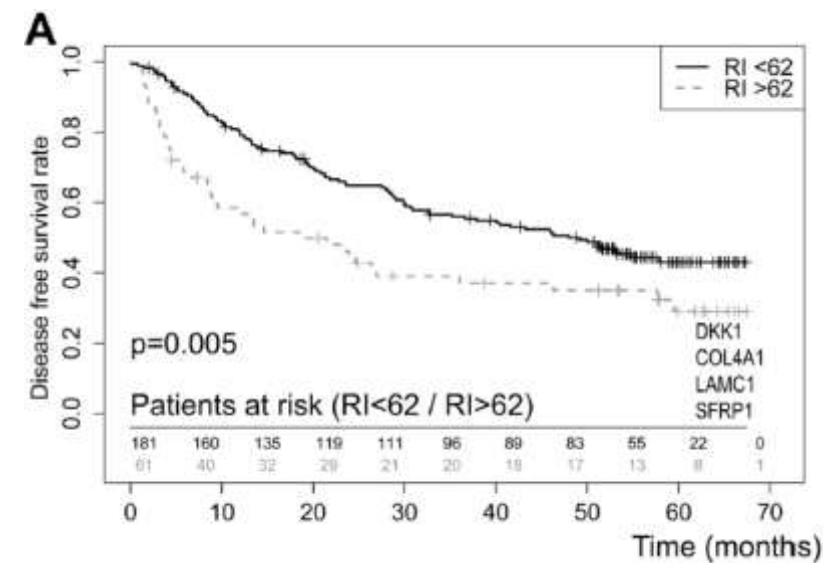
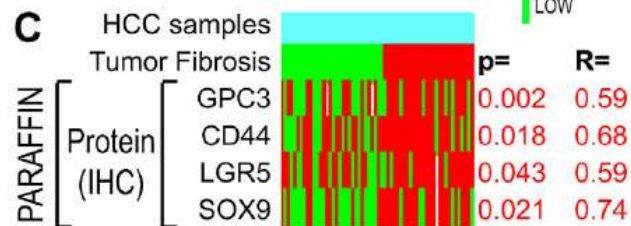
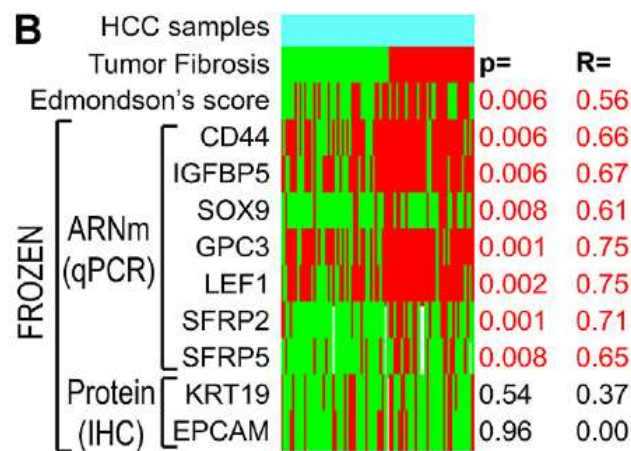
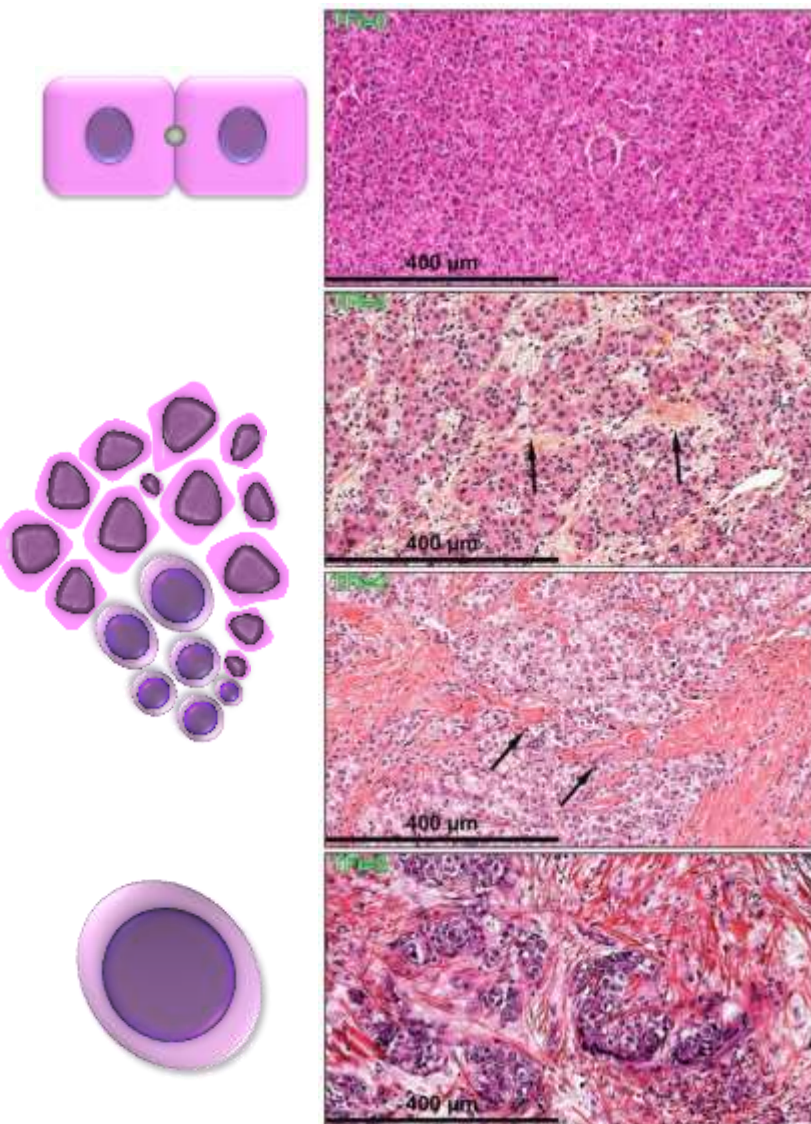
Adapté de Pellicoro et al., *Nat. Rev. Immun.*, 2014

Carcinome hépatocellulaire: hétérogénéité macroscopique



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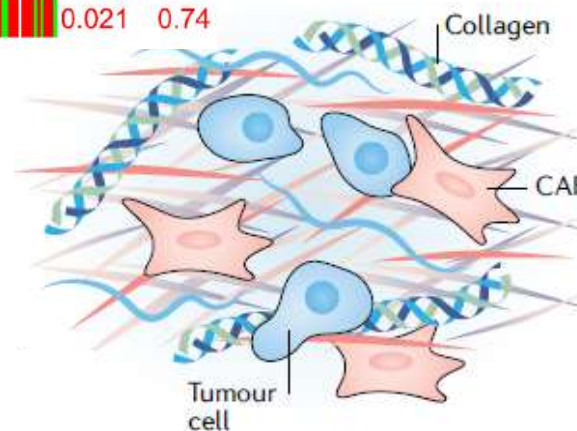
Carcinome hépatocellulaire: hétérogénéité macroscopique



Désert et al. *Int. J. Bioch. Cell Biol.* 2016

Mebarki et al., *Oncotarget* 2016

Stroma tumoral

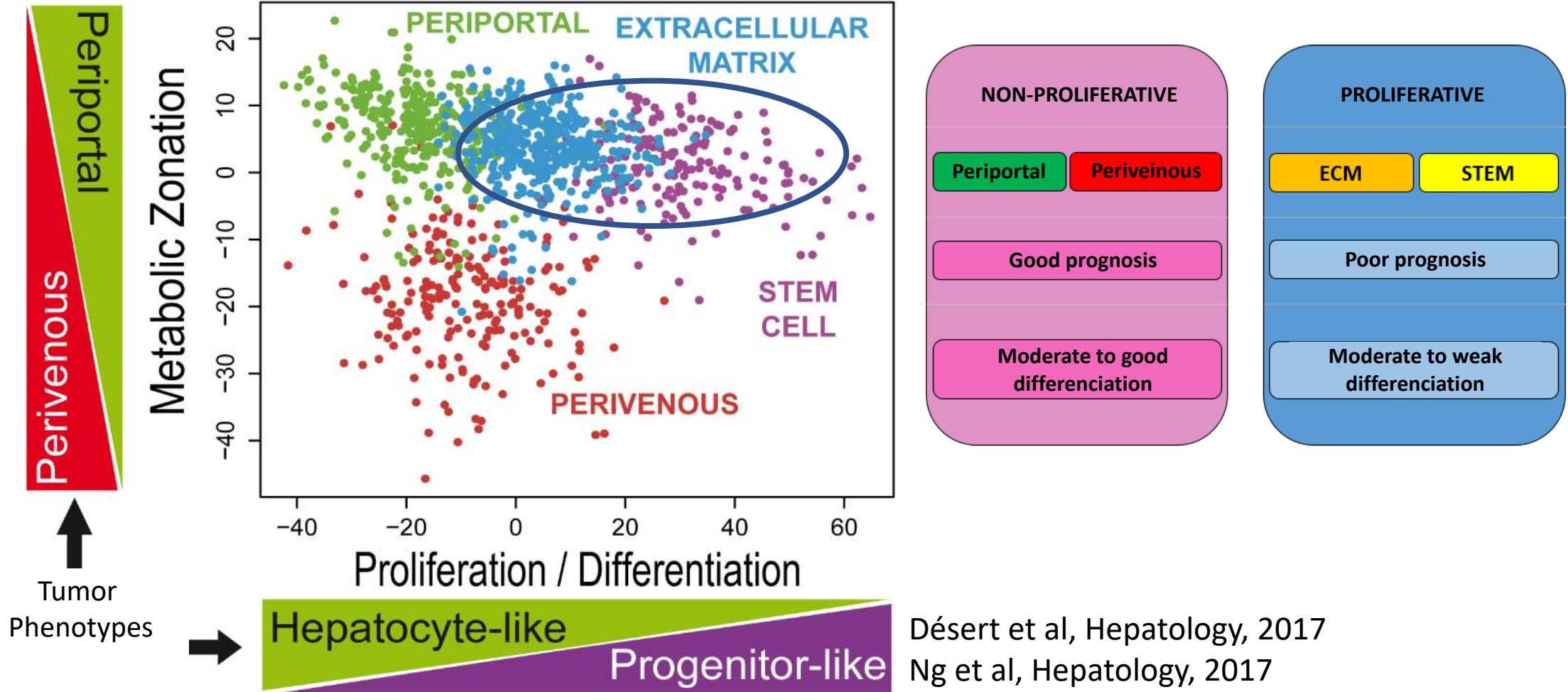


Matrice Extracellulaire (matrisome)

Cox, *Nat Reviews Cancer* 2021

Nids fibreux : Sous-classes ECM et STEM

Hepatocellular Carcinoma Subclasses

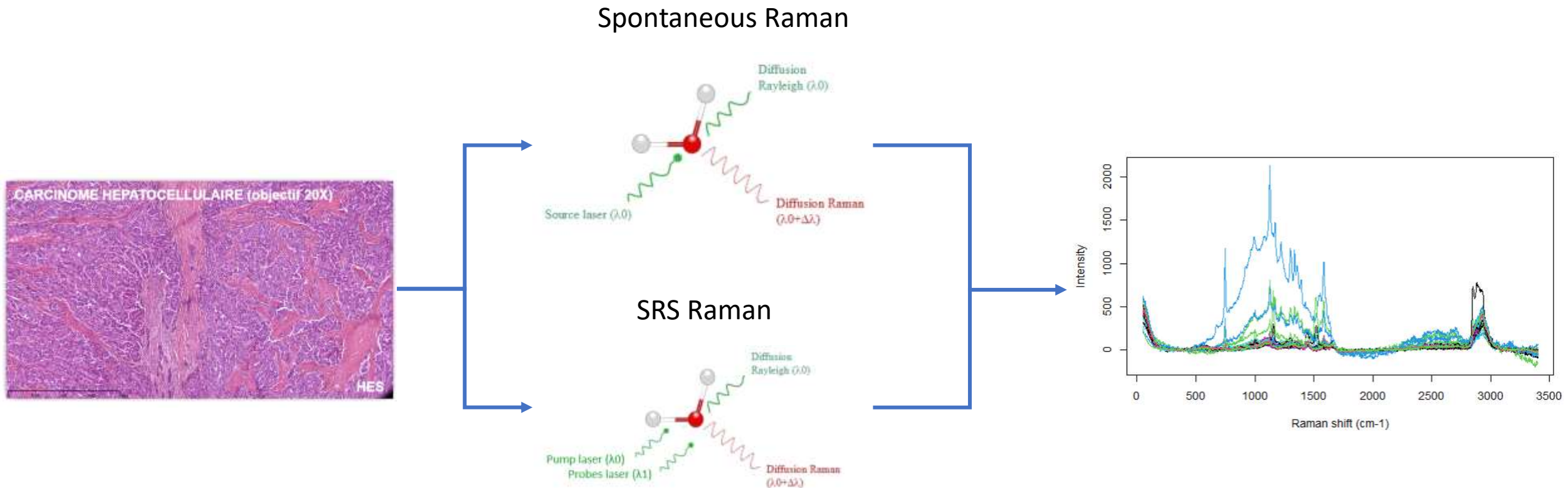


Désert et al, Hepatology, 2017

Ng et al, Hepatology, 2017

Désert et al, World Journal of Gastroenterology, 2018

Spectrométrie Raman : Principe

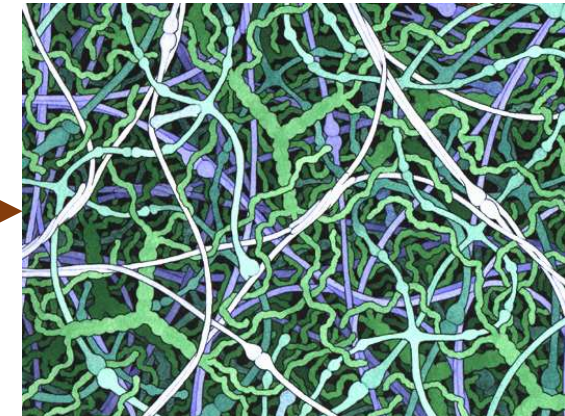
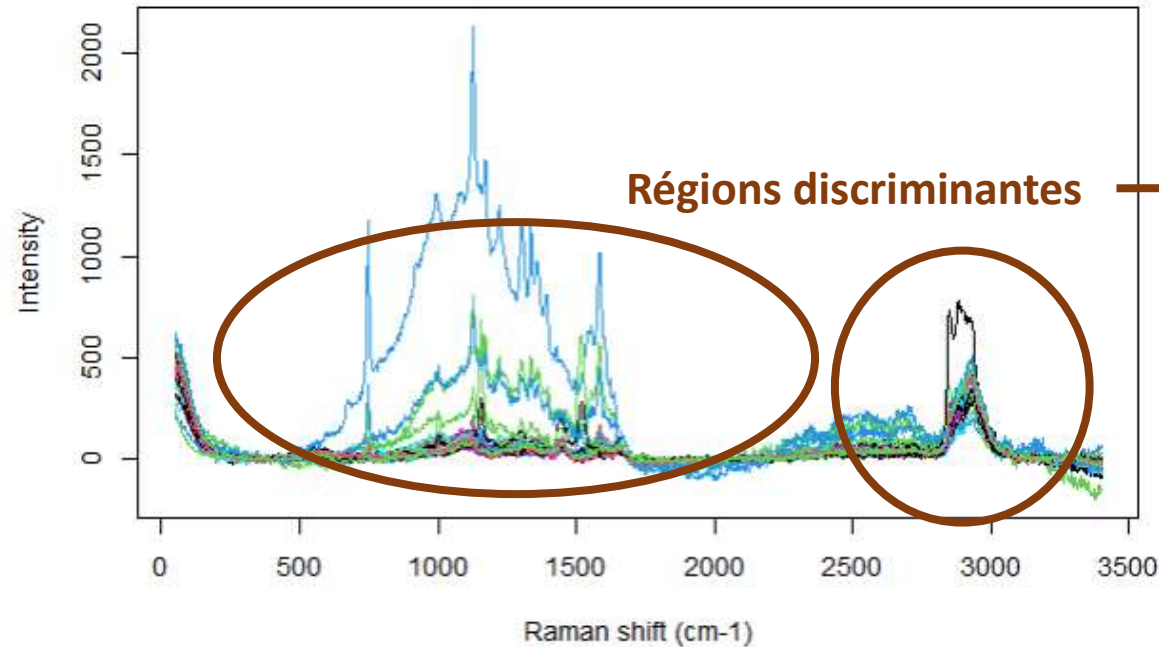


- Méthode non destructive
- Fonctionne sur des tissus frais ou congelés
- Haute sensibilité

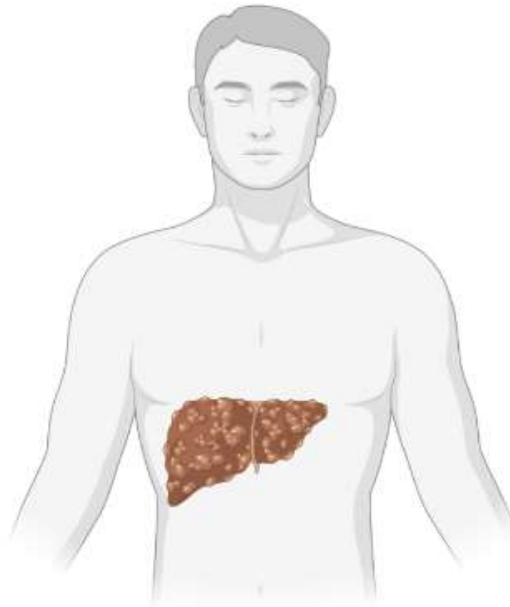
Objectifs



1. Localiser les régions dans les spectres Raman qui discriminent la **fibrose tumorale de haut grade** *versus* **fibrose tumorale de bas grade** (HG vs LG)
2. Recherche de la composition moléculaire sous-jacente de l'ECM



Composition moléculaire de l'ECM



40 échantillons humains

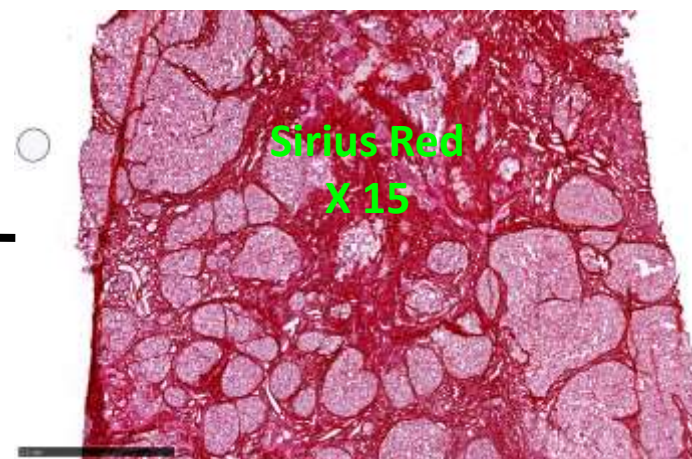
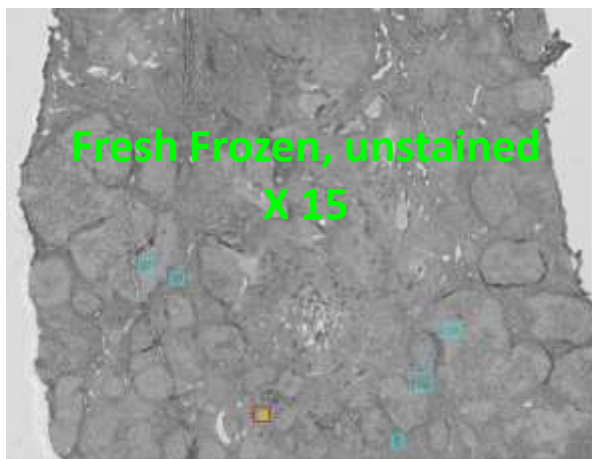
- 10 CHC avec fibrose de haut grade
- 10 foies non tumoraux correspondants
- 10 CHC avec fibrose de bas grade
- 10 foie non tumoral correspondant

Informations réglementaires

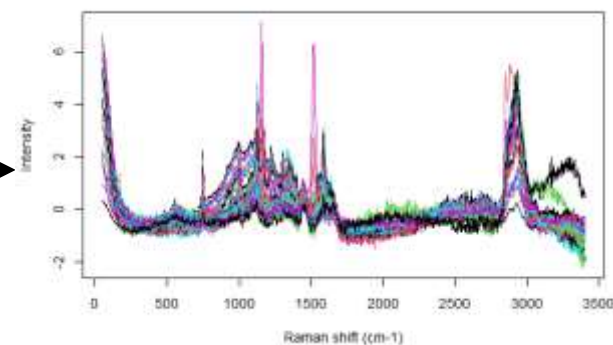
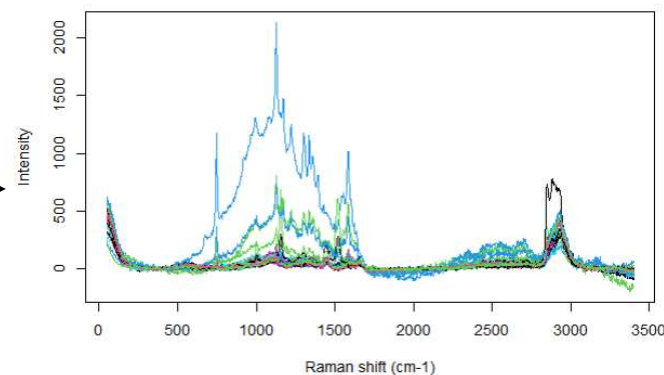
- Avis favorable n° 20-654 du Comité d'évaluation éthique de l'INSERM pour le projet « The molecular landscape underlying mechanical forces in the fibrosis nests of hepatocellular carcinomas ».



Spectroscopie Raman

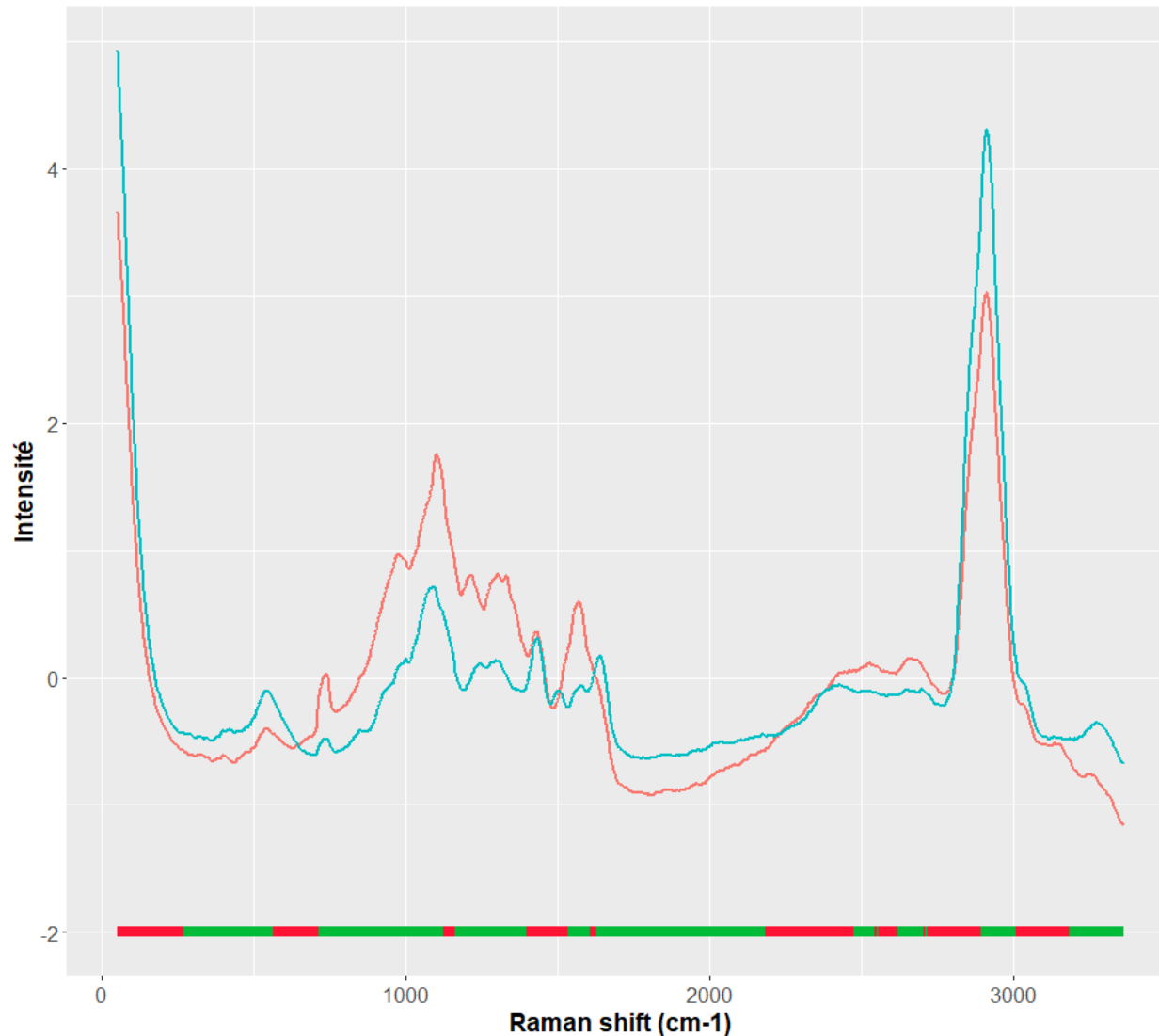


Microspectrometer Imaging Raman DXR2xi
Histopathology Core Facility
SFR UMS CNRS 3480 – INSERM 018



Noise correction

Spectroscopie Raman : fibrose tumorale

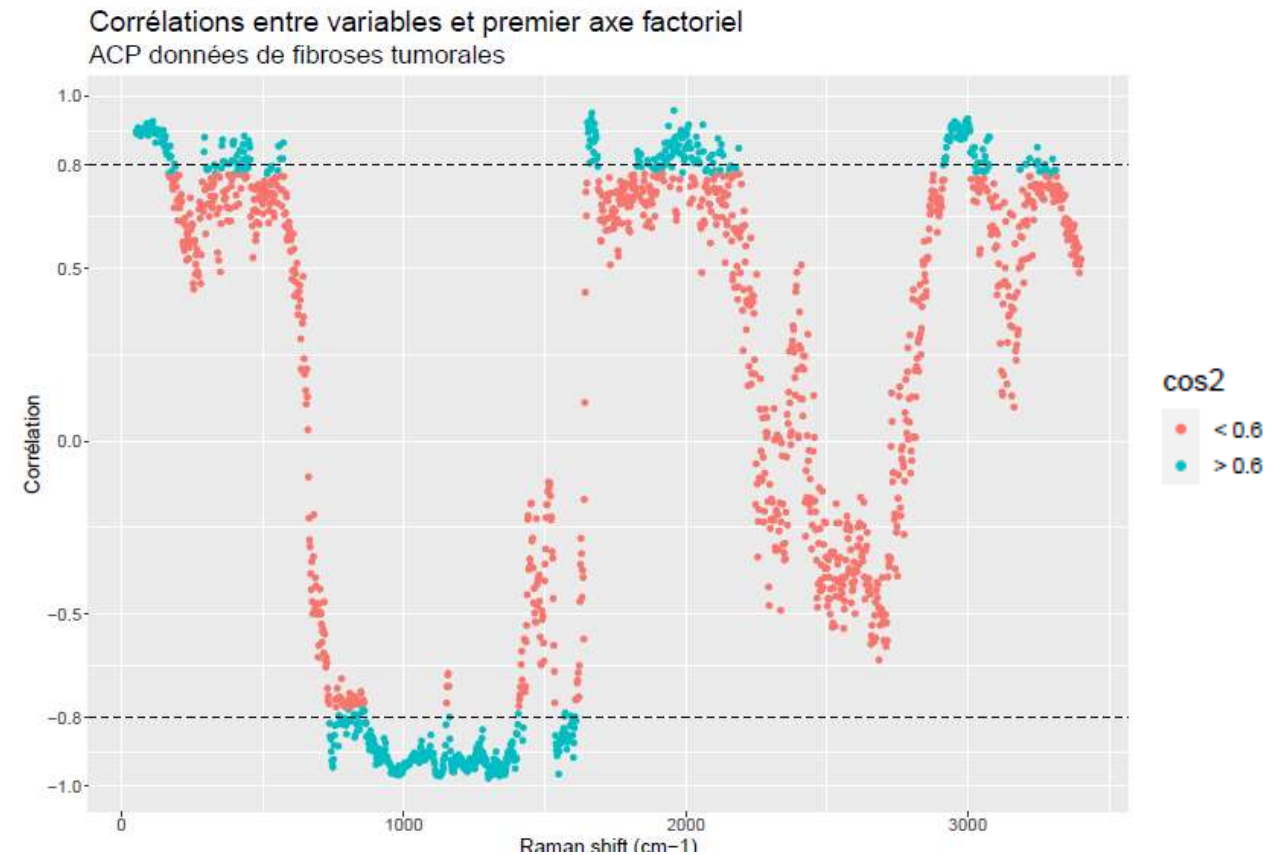
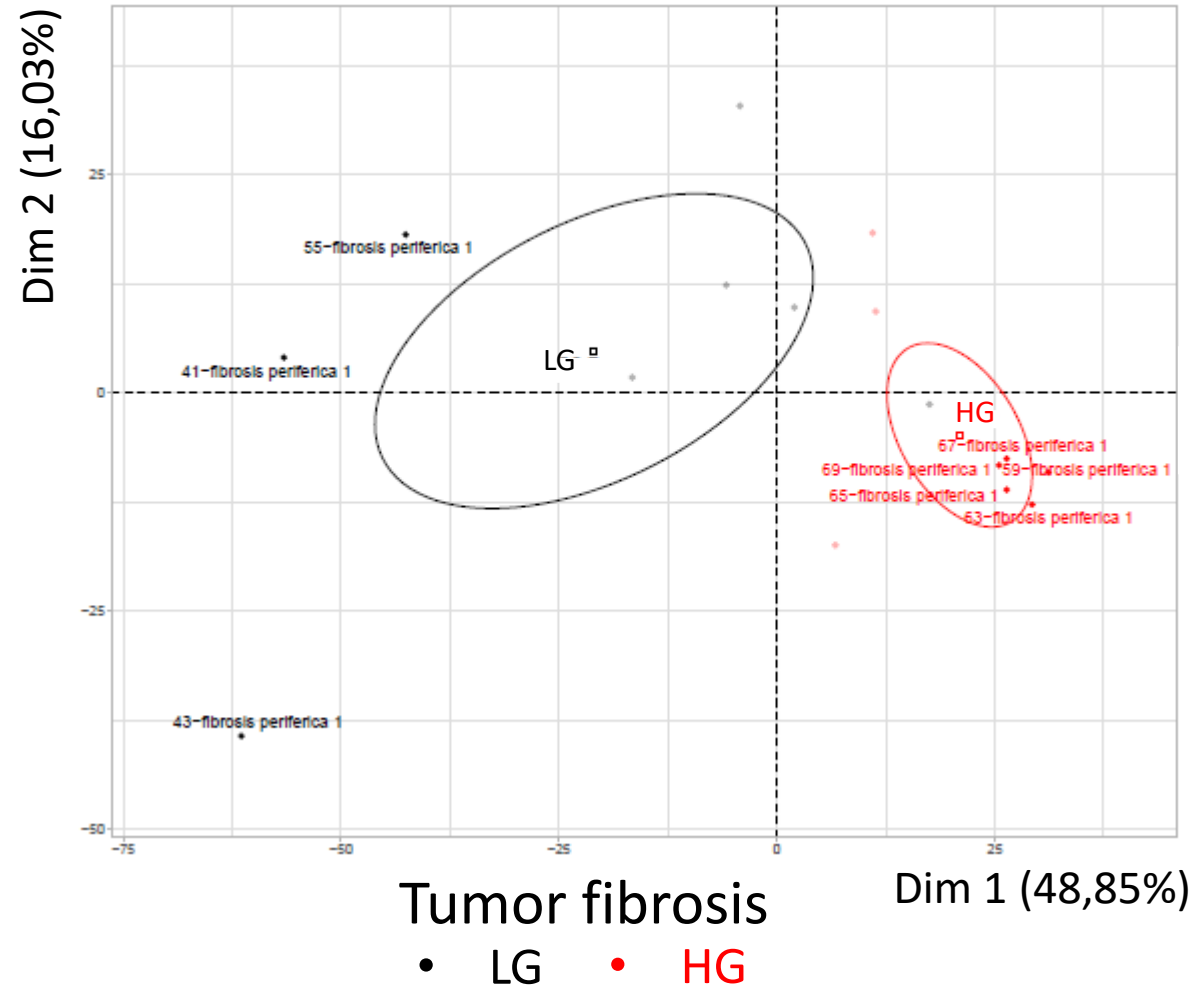


Levels of tumor fibrosis and mean differences :

- Tumor fibrosis of low grade
- Tumor fibrosis of high grade
- Not significant
- Significant with BH^{*} correction at 5% threshold

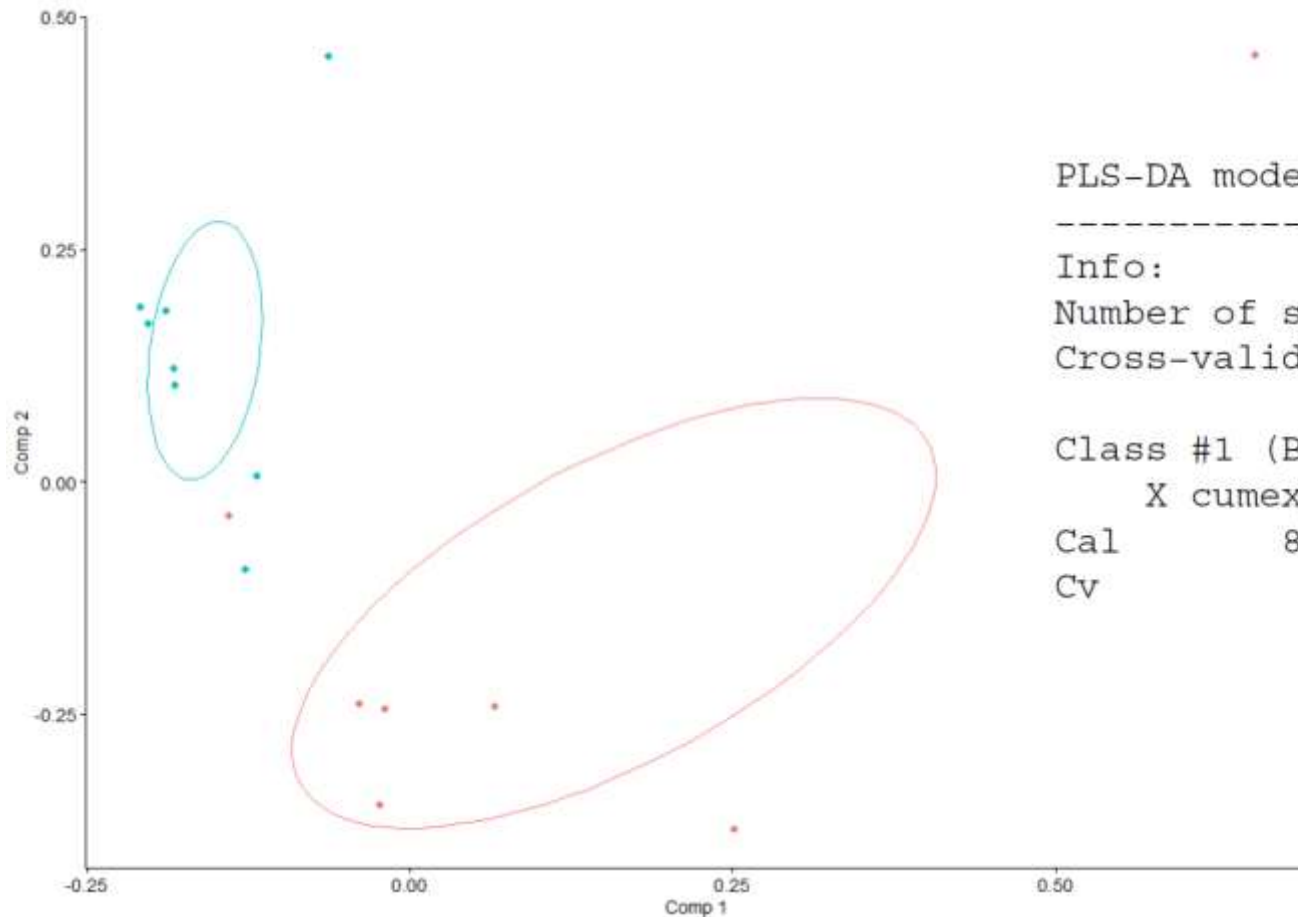
*Benjamini-Hochberg

Régions discriminantes dans les spectres Raman entre la fibrose tumorale de **HG** et **BG**



Régions discriminantes dans les spectres Raman entre la fibrose tumorale de HG et BG

Intratumor fibrosis ● LG ● HG



PLS-DA model (class plsda) summary

Info:

Number of selected components: 2

Cross-validation: full (leave one out)

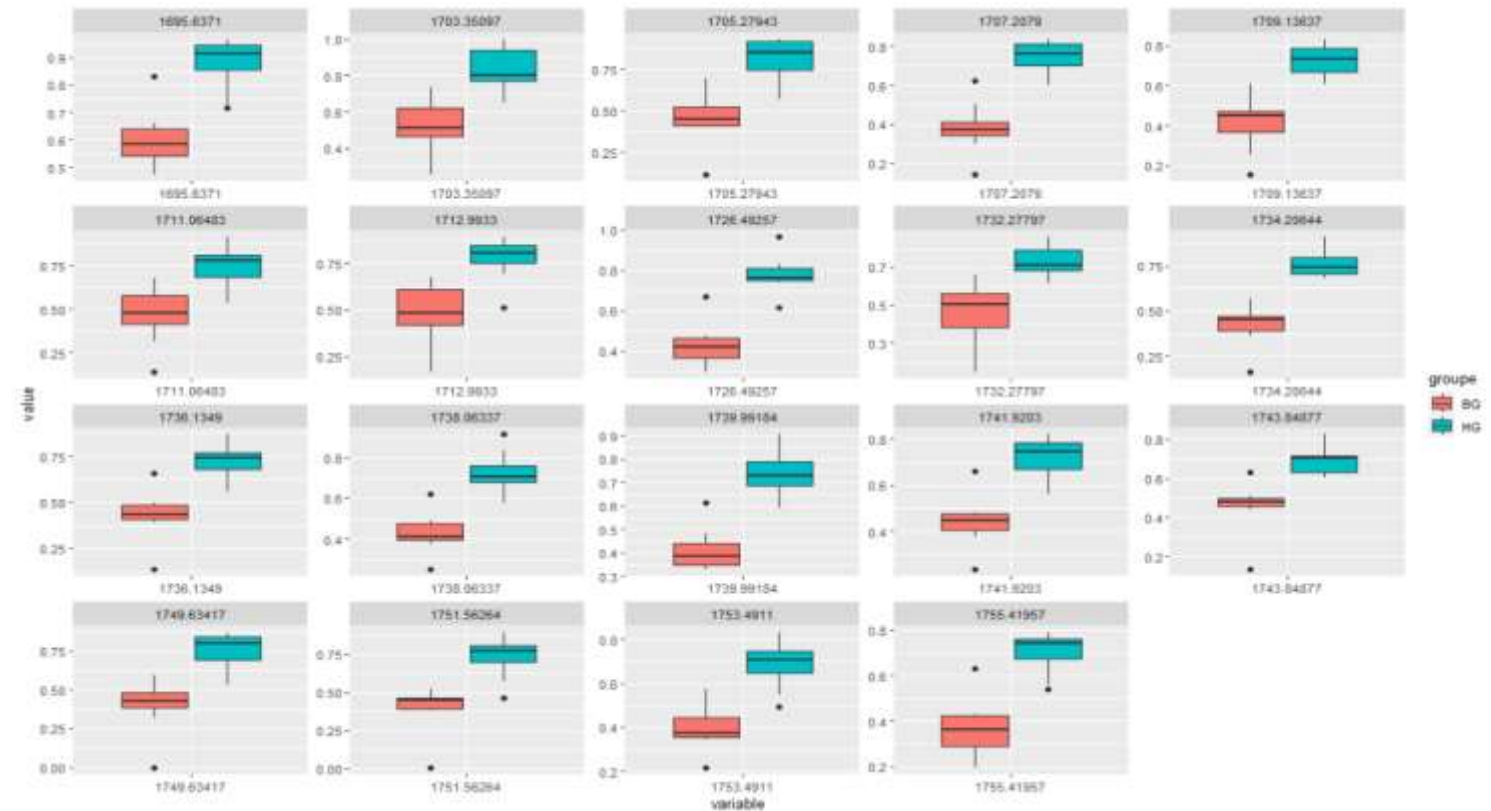
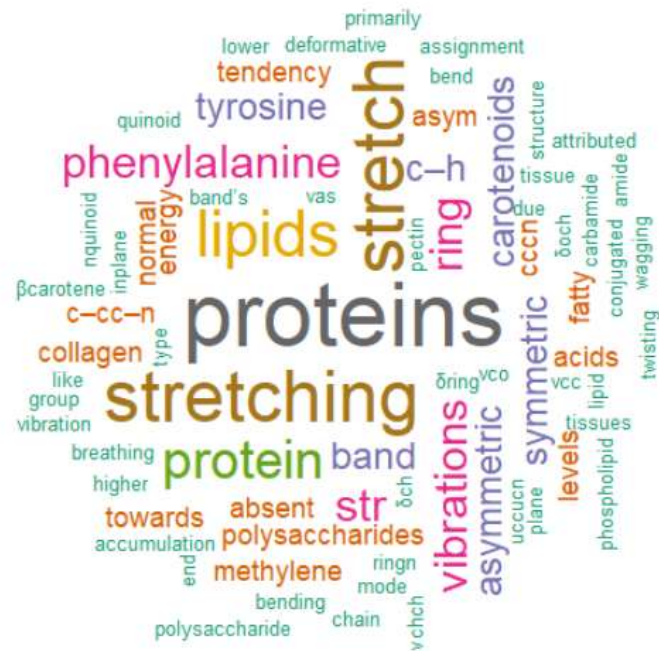
Class #1 (BG T)

	X cumexpvar	Y cumexpvar	TP	FP	TN	FN	Spec.	Sens.	Accuracy
Cal	83.11	72.06	7	0	8	1	1.000	0.875	0.938
Cv	NA	NA	7	1	7	1	0.875	0.875	0.875

Régions discriminantes entre HG et BG (par PLSDA)

Précision du modèle: 0,875

PLSDA

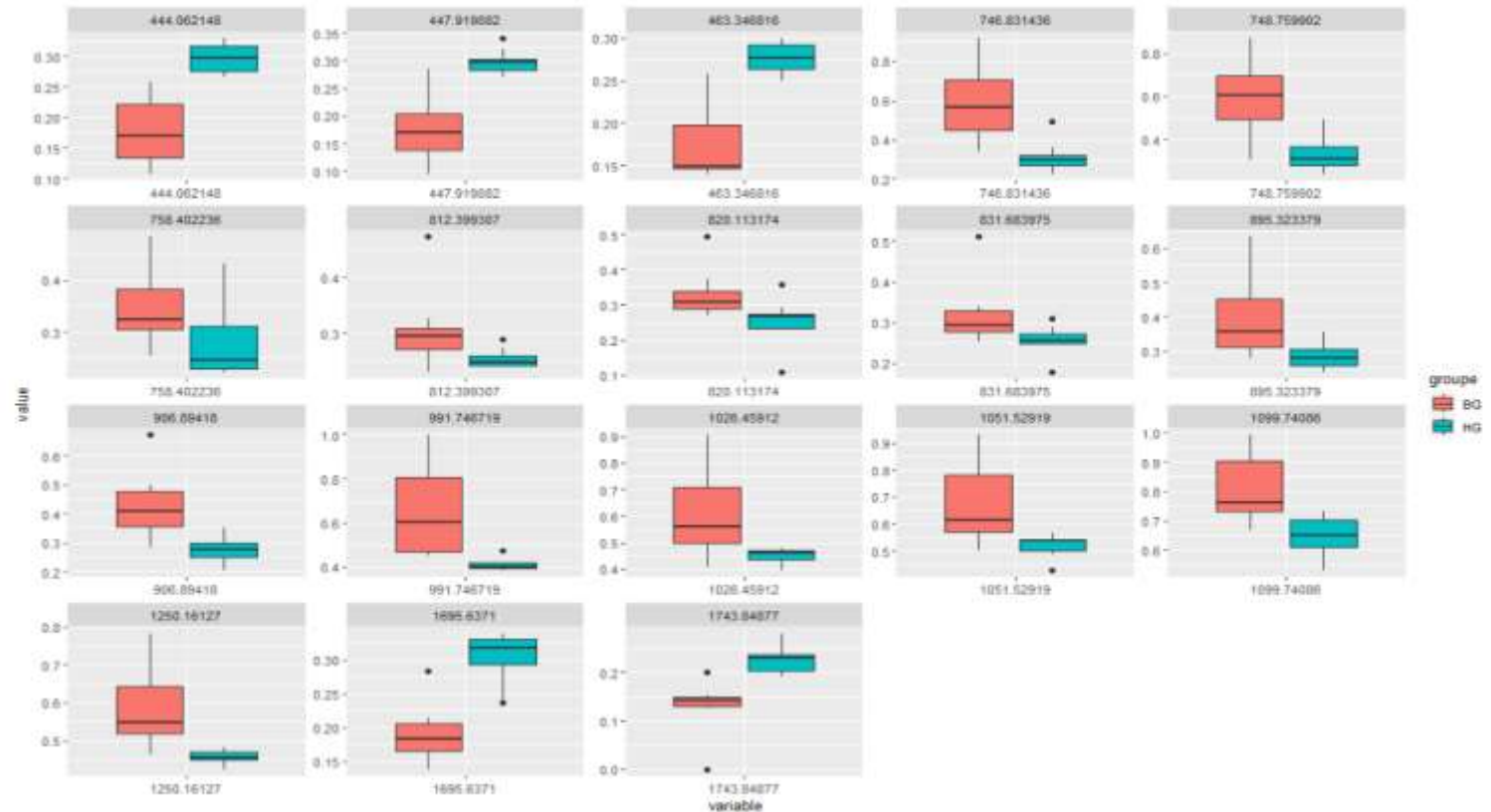
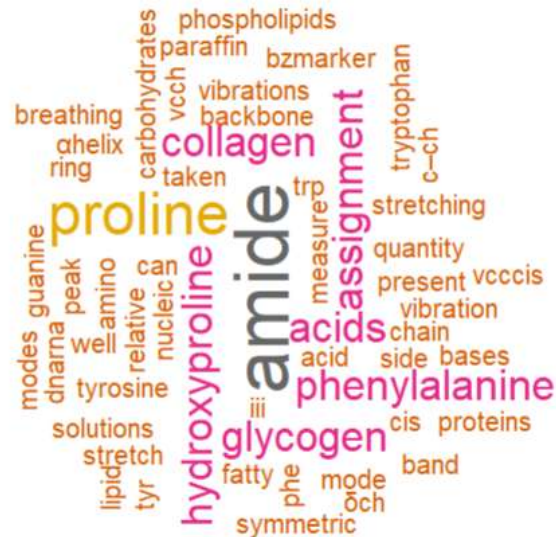


*Talari et al, 2017, Applied spectroscopy reviews

Régions discriminantes entre HG et BG (par Random Forest)

Précision du modèle: 0,875

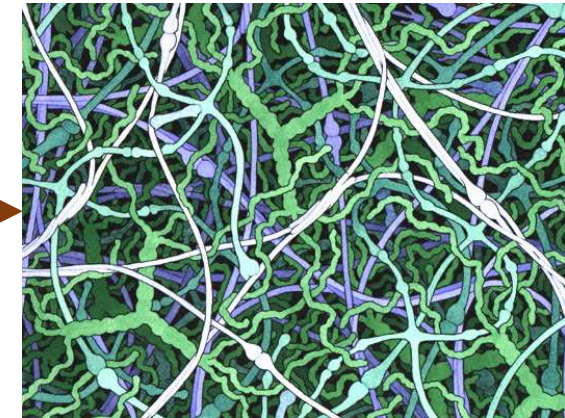
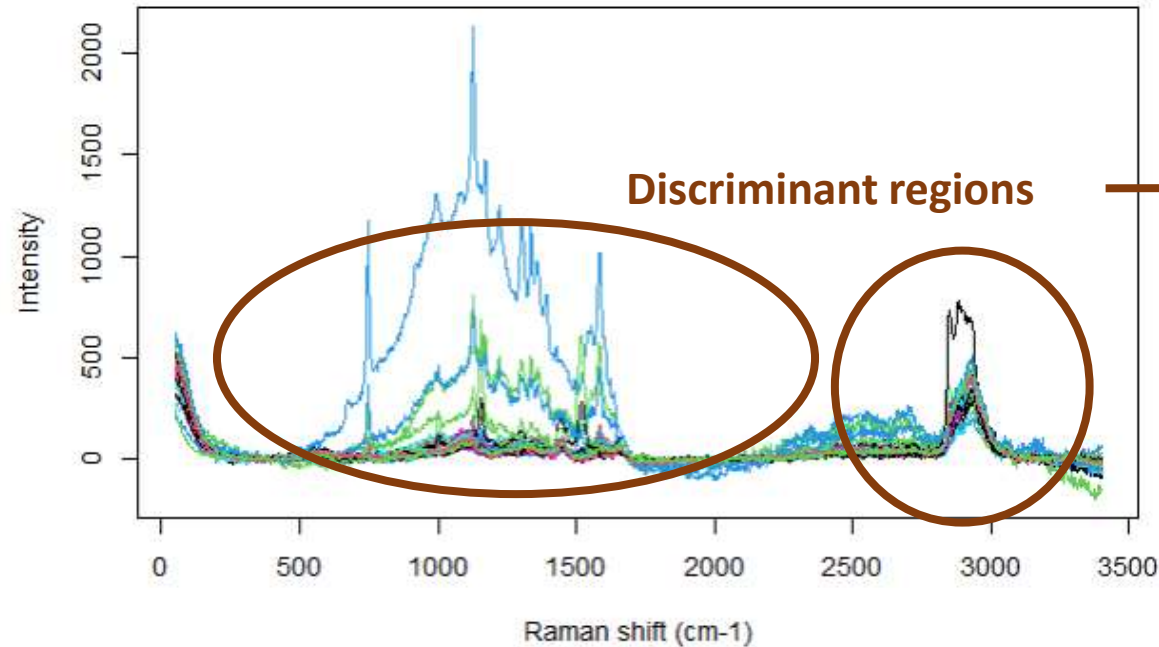
Random Forest



*Talari et al, 2017, Applied spectroscopy reviews

Objectifs

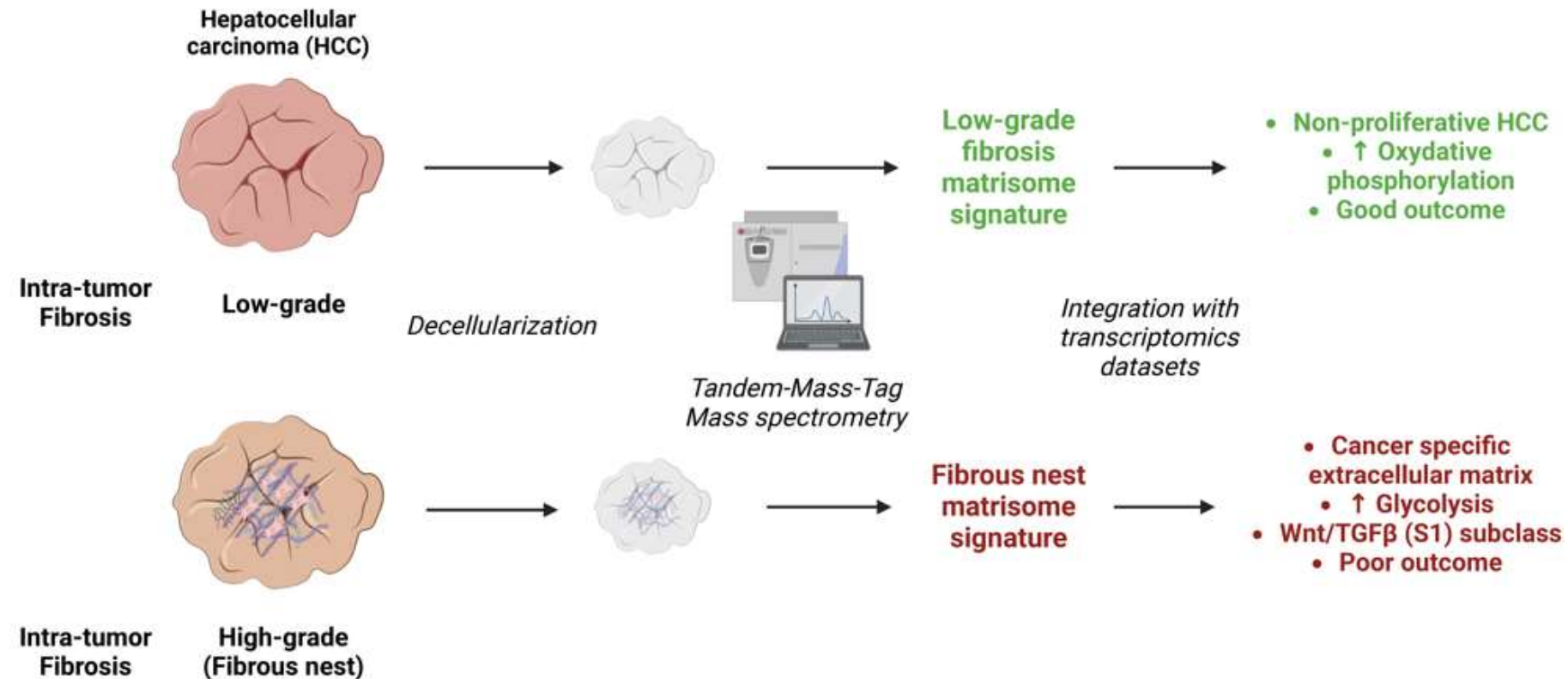
1. Localiser les régions dans les spectres Raman qui discriminent la fibrose tumorale de haut grade *versus* Fibrose tumorale de bas grade (HG vs LG)
2. Recherche de la composition moléculaires sous-jacente de l'ECM



ECM Molecular components

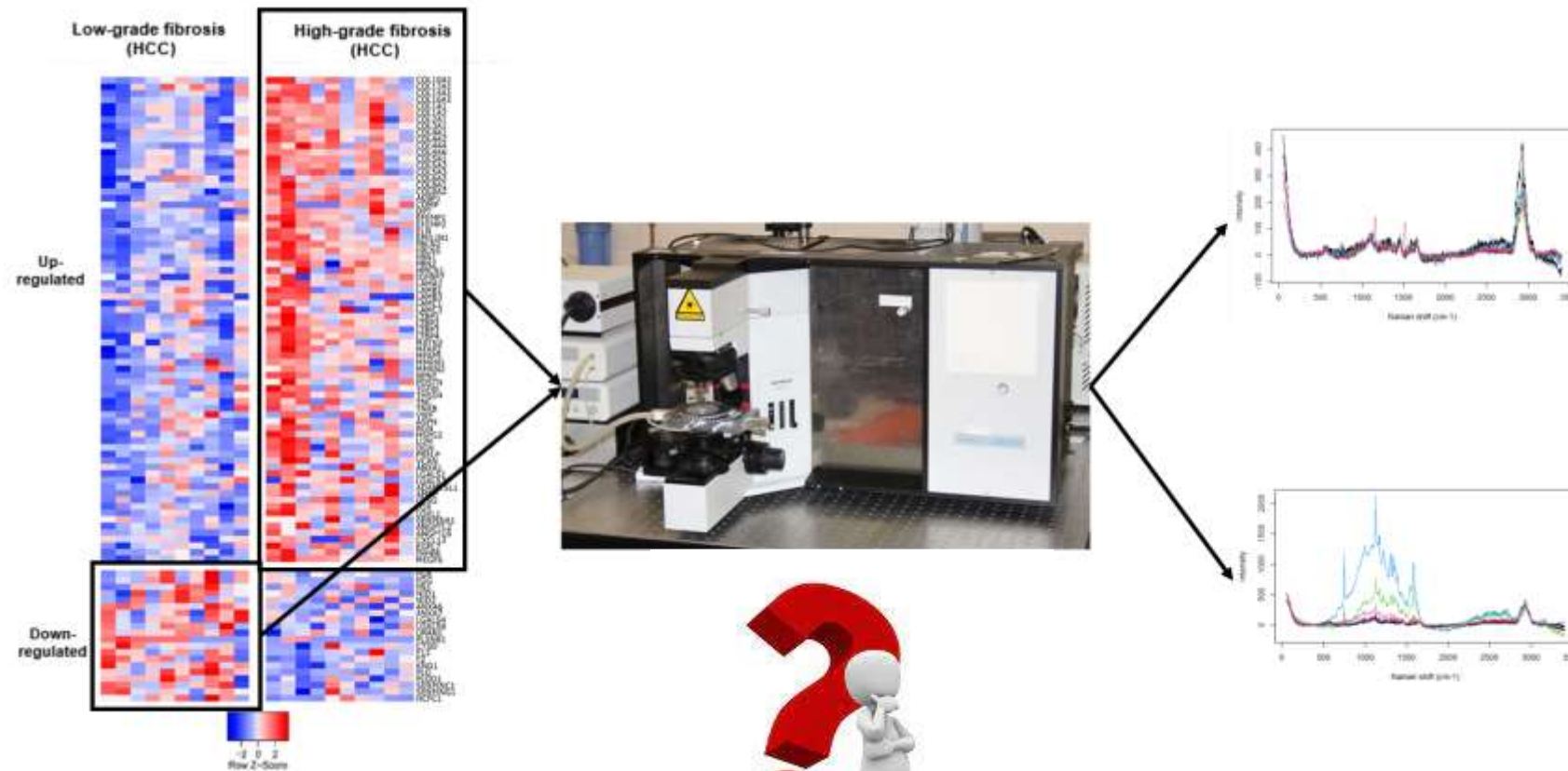


Protéomique quantitative



Désert et al (2023), Hepatology 78: 741.

Intégration Raman/Matrisome (protéomique quantitative)

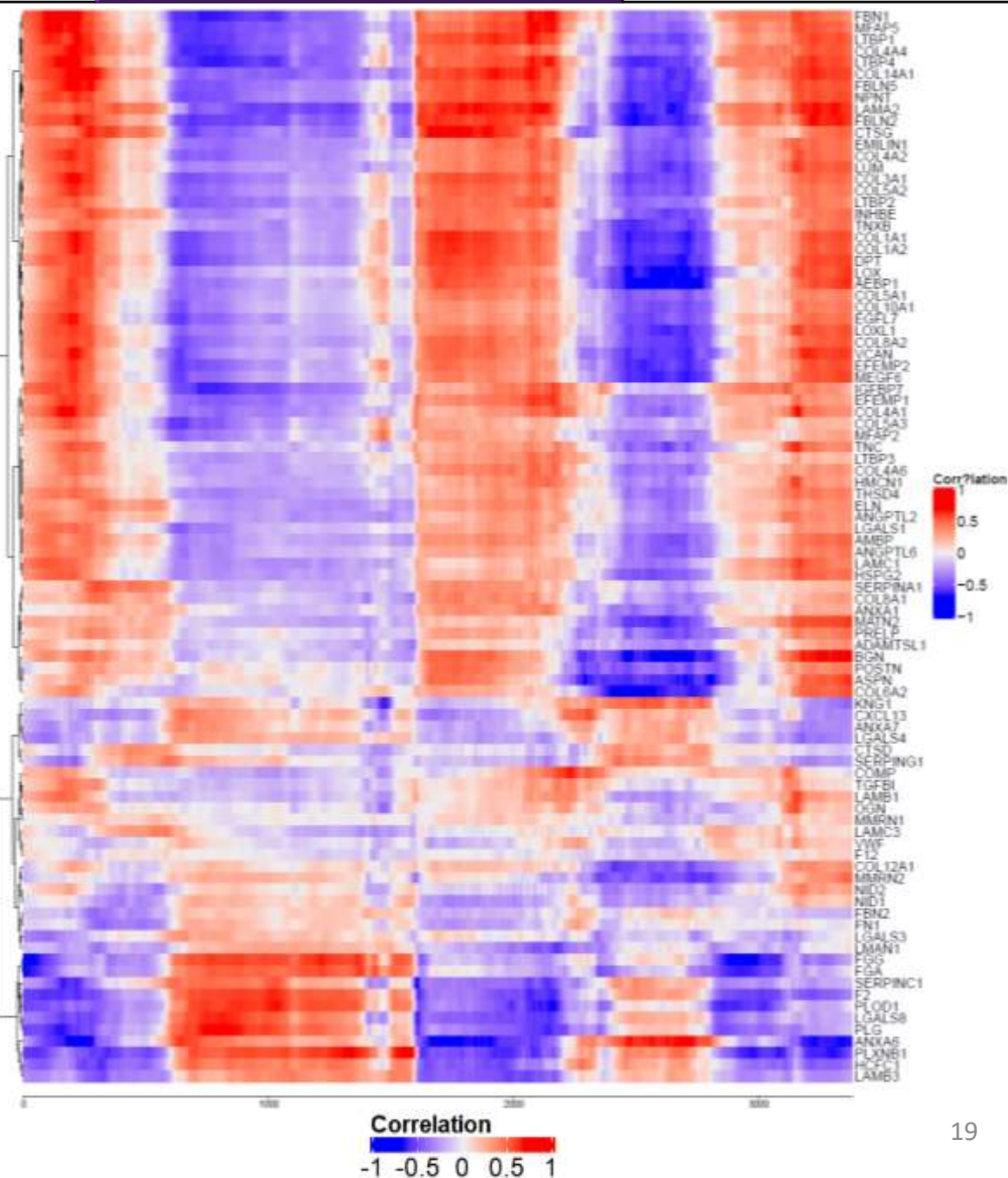


Désert et al (2023), *Hepatology* 78: 741.

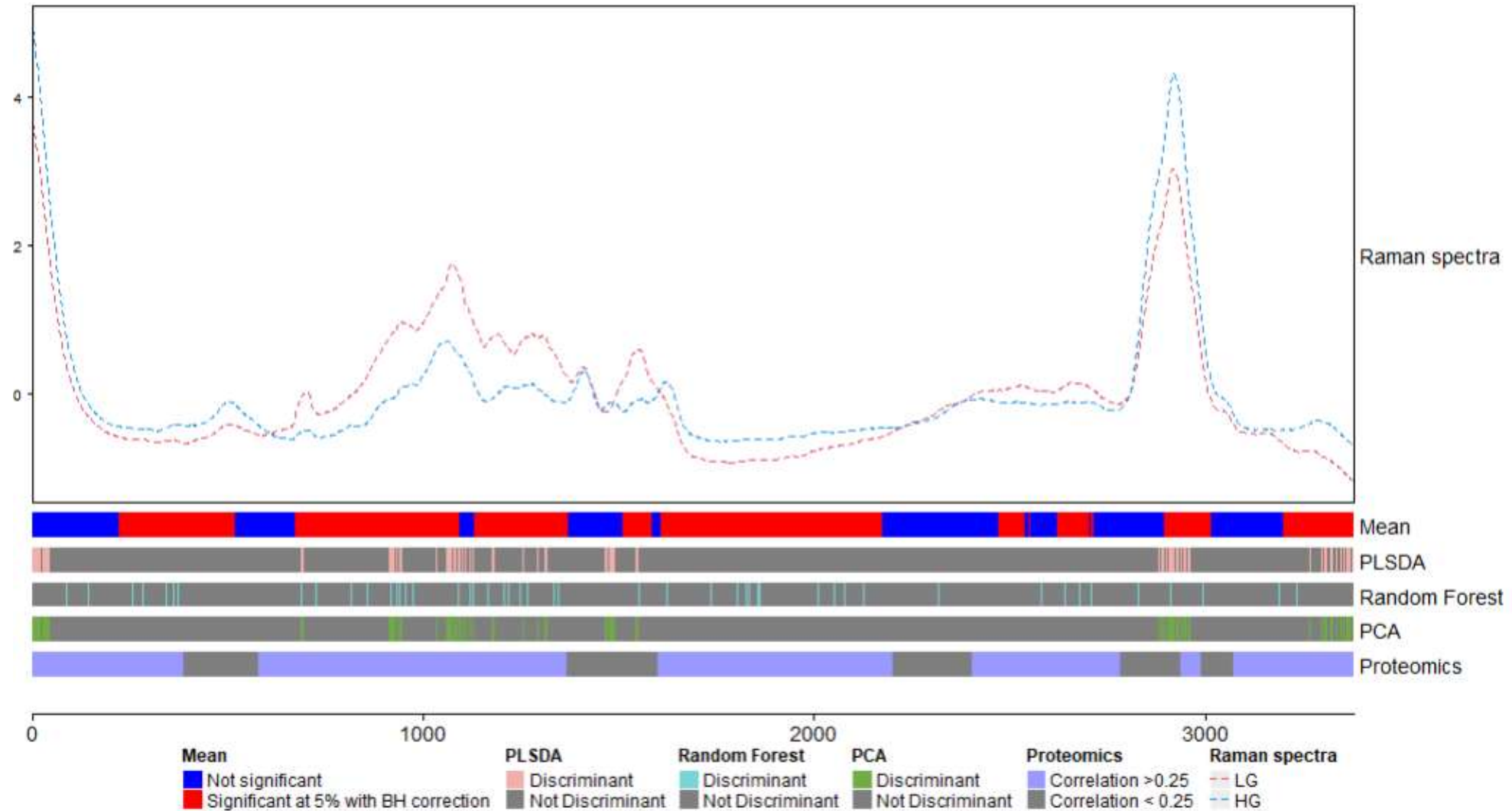
Analyse corrélative Raman-Protéomique

HG

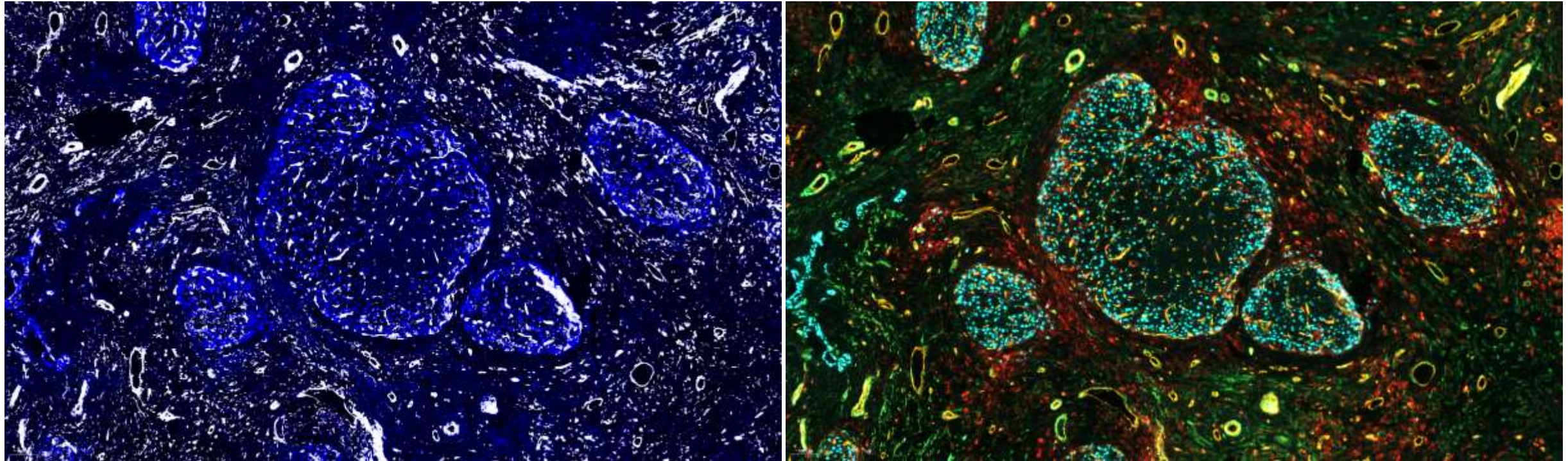
BG



Analyse corrélative Raman-Protéomique



Cellules souches cancéreuses dans les nids fibreux du CHC



● COL1A1
● DAPI

● SOX9 ● CD34
● CD44 ● ACTA2

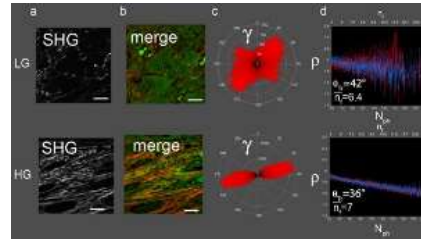
Conclusion

- Les spectres Raman permettent de différencier les nids fibreux de **haut grade** de ceux de **bas grade**.
- Bonne corrélation entre le Raman et la protéomique
- Validation sur une plus grande série de tumeurs en cours (Maëlle Guillout et Anthony Sébillot)

Travaux en cours

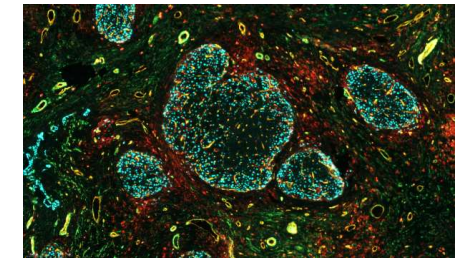
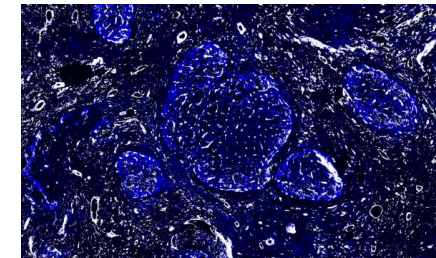
- ❑ Localiser les régions discriminantes *in situ* par RAMAN SRS
- ❑ Corréler les régions discriminantes avec :
Transcriptome

Signatures P-SHG



Détection *in situ* des cellules STEM cancéreuses
 Immunohistochimie multiplex
 Single cell RNA-seq

Quantification de la fibrose intra-tumorale par *deep learning*
 (Maëlle Guillout)

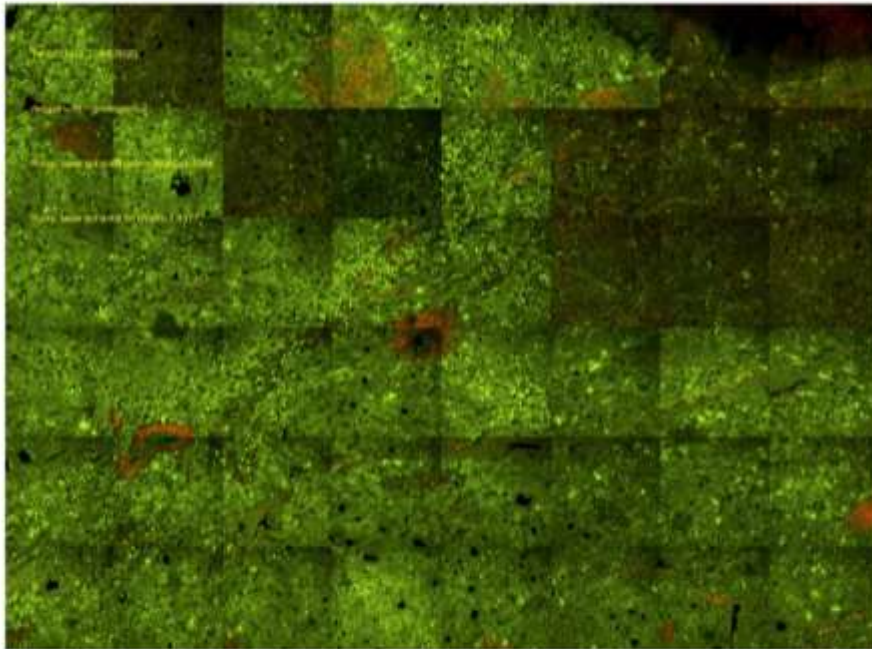


- ❑ Spatial Transcriptomics (Nanostring) sur une collection de 51 CHC (25 de haut grade et 26 de bas grade).

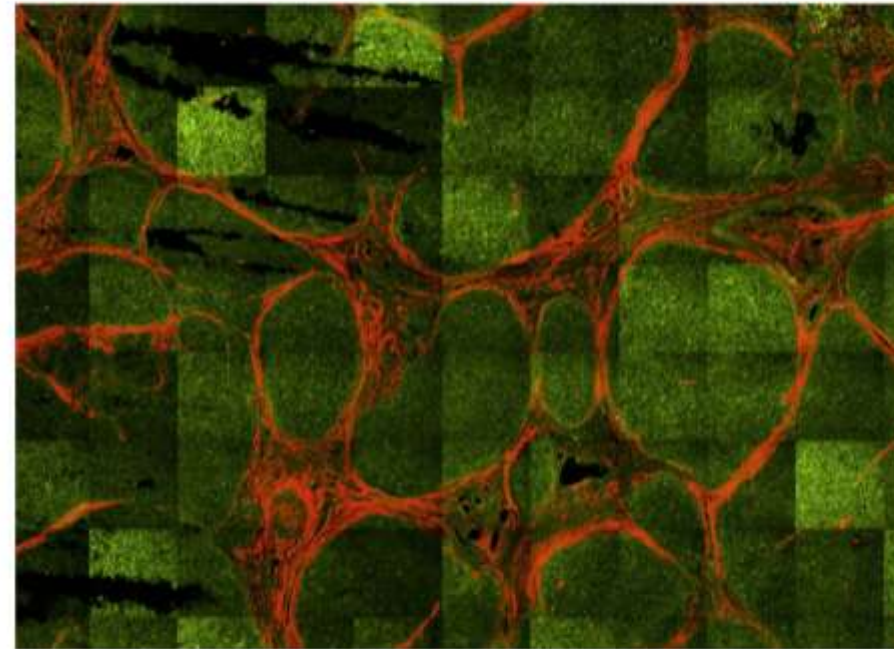
Travaux en cours

Résultats préliminaires SHG:

A: Low fibrotic HCC tumor sample

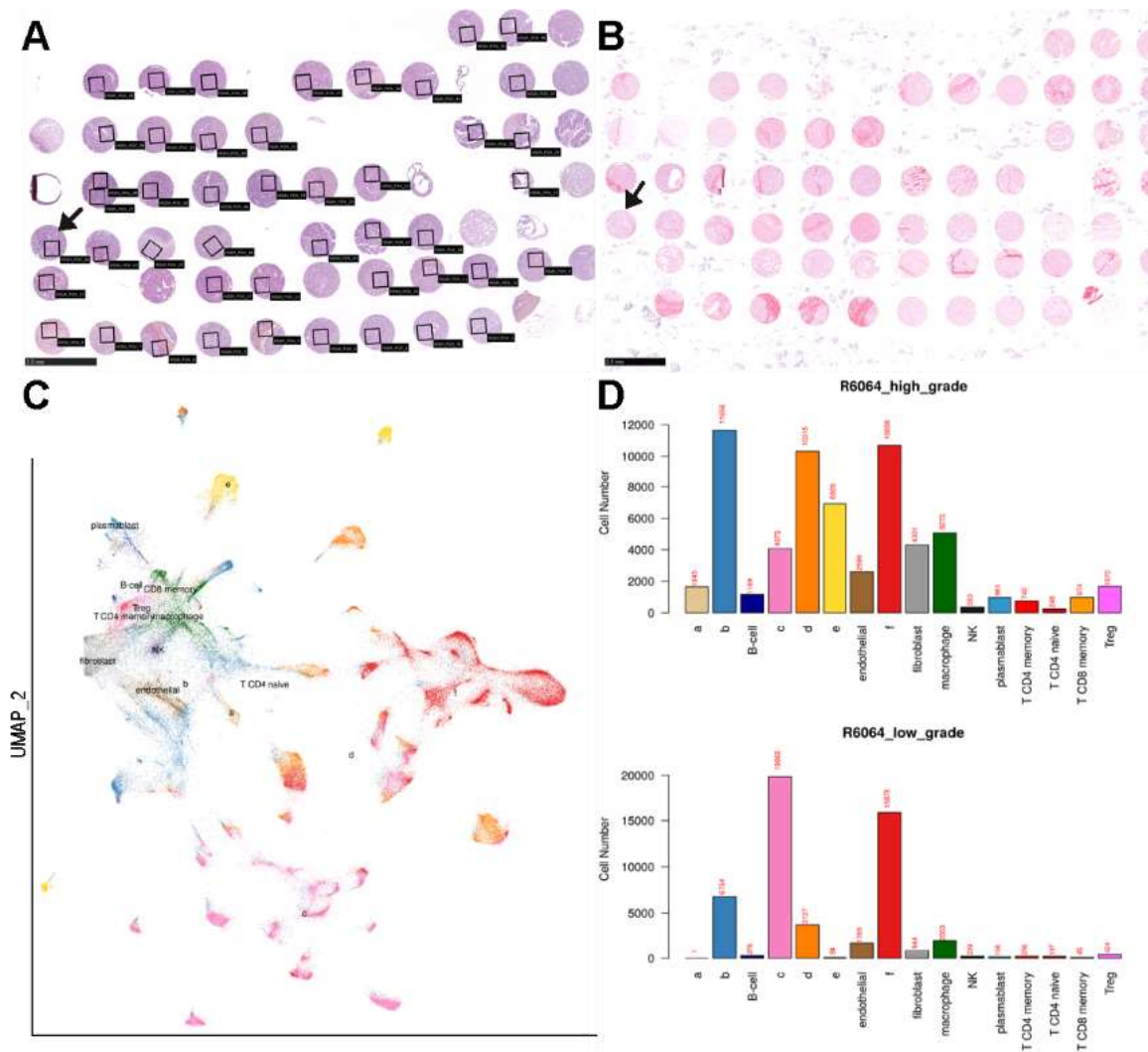


B: High fibrotic HCC tumor sample



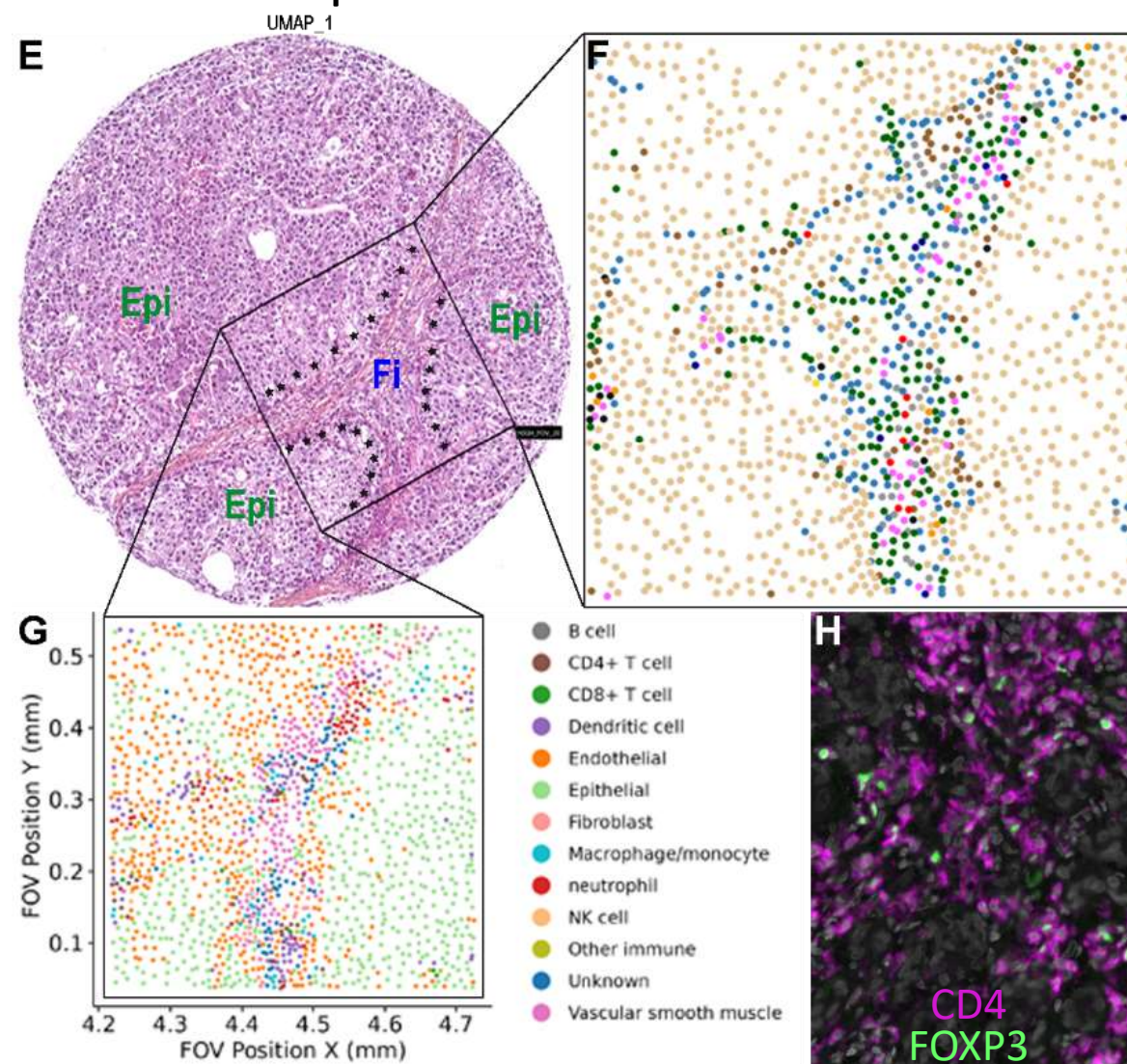
Travaux en cours

Résultats préliminaires spatial transcriptomics:



Travaux en cours

Résultats préliminaires spatial transcriptomics:





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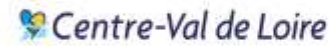


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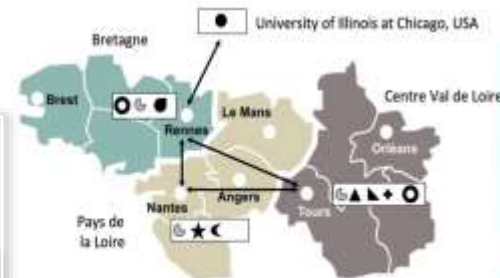
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Stro
Target



coord: O Héroult, PF Cartron