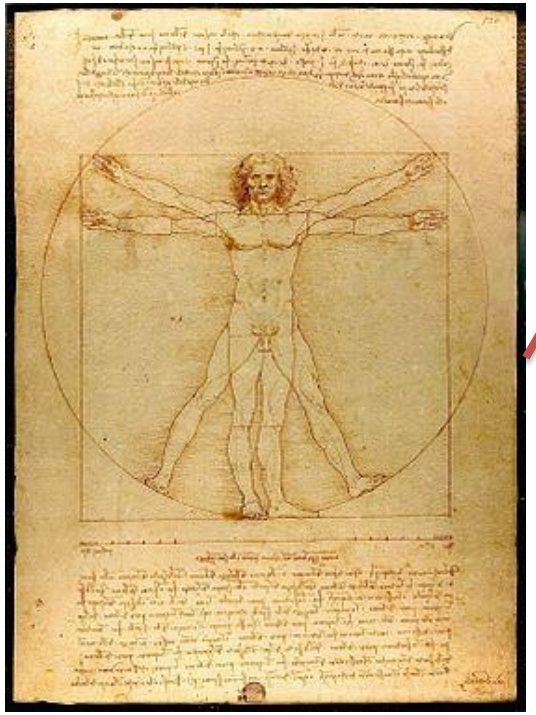
Traitement et analyse des données CARS hyperspectrales pour l'imagerie cellulaire

D. Boildieu, A. Magnaudeix, P. Leproux, D. Helbert, P. Carré

Contexte biologique

Les différentes échelles du vivant

Anatomie



Organisme pluricellulaire

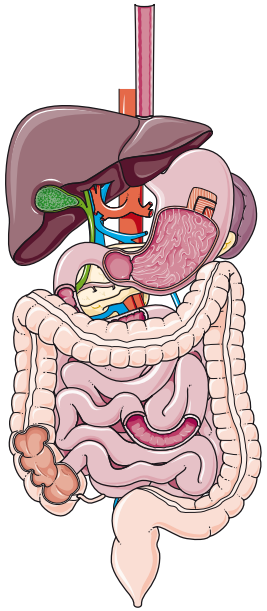
Ex : humain

Systèmes Appareils

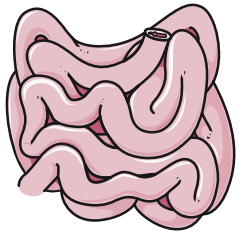
Physiologie
Histologie

Organes

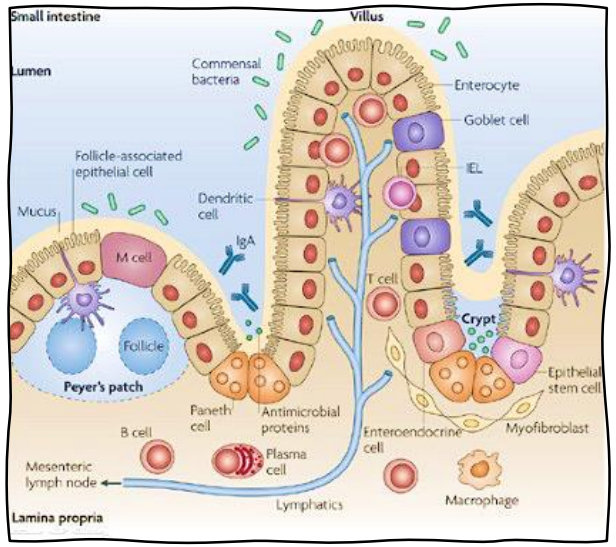
Tissus



Ex : appareil digestif



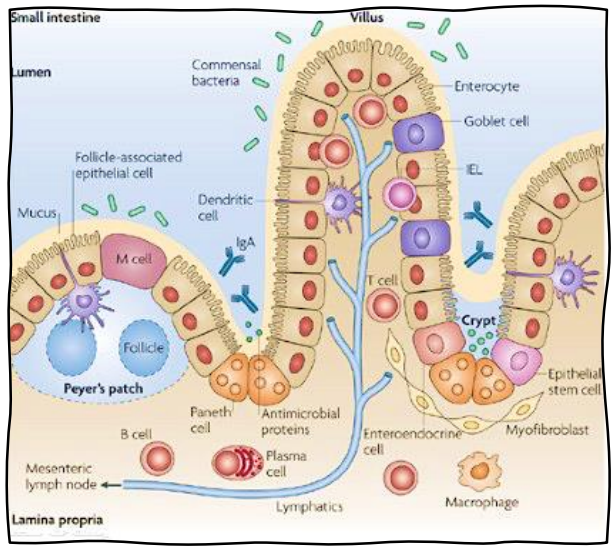
Ex : intestin grêle



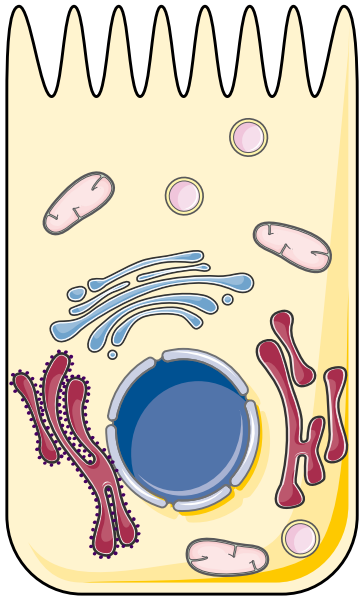
Ex: épithélium intestinal

Les différentes échelles du vivant

Tissus → **Cellules** → **Organites**

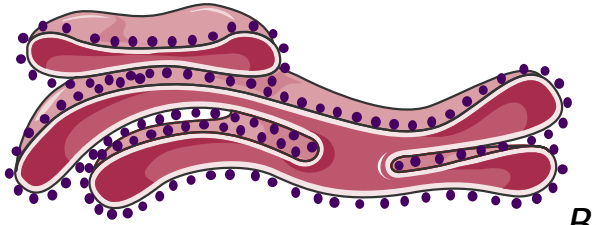


Ex : épithélium intestinal



Ex: entérocyte

Physiologie cellulaire
Biologie cellulaire
Biologie moléculaire

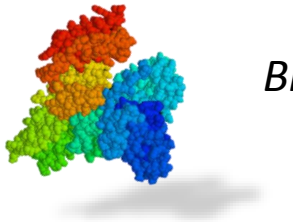


Ex: réticulum endoplasmique rugueux

Biologie cellulaire
Biologie moléculaire

Macromolécules, molécules

Ex : protéines

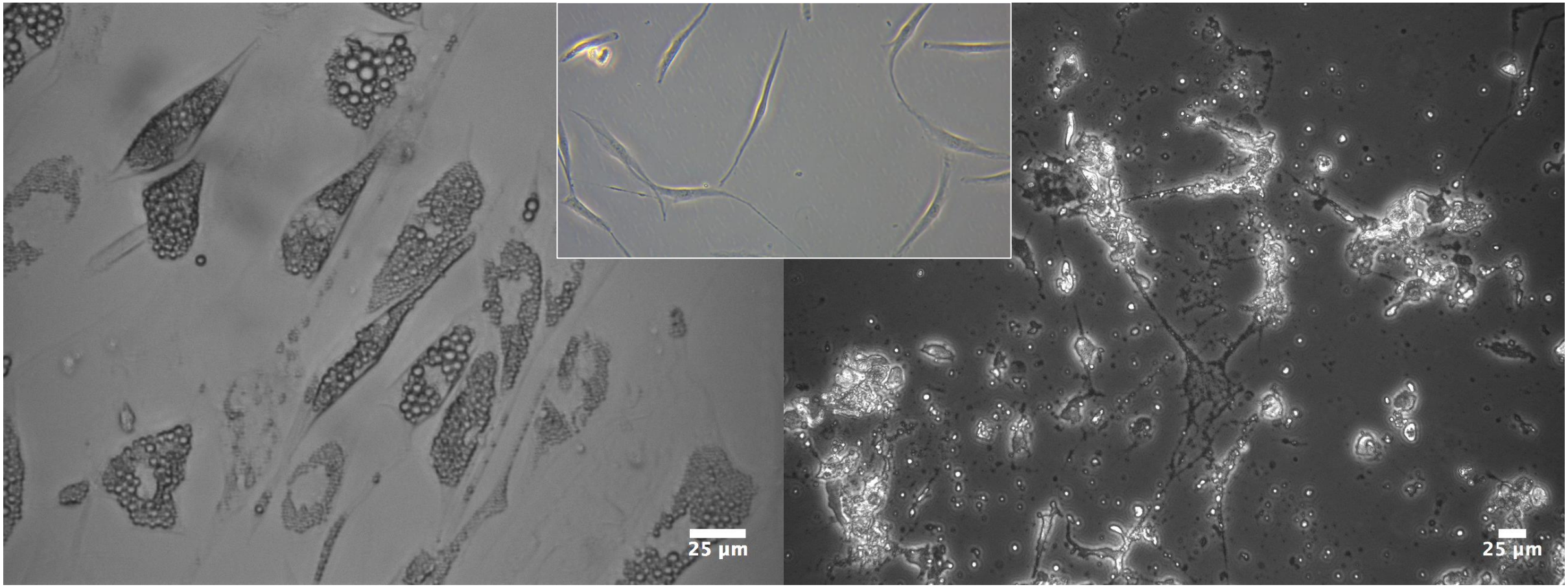


Biochimie

Microscopie optique en lumière blanche

=> morphologie cellulaire

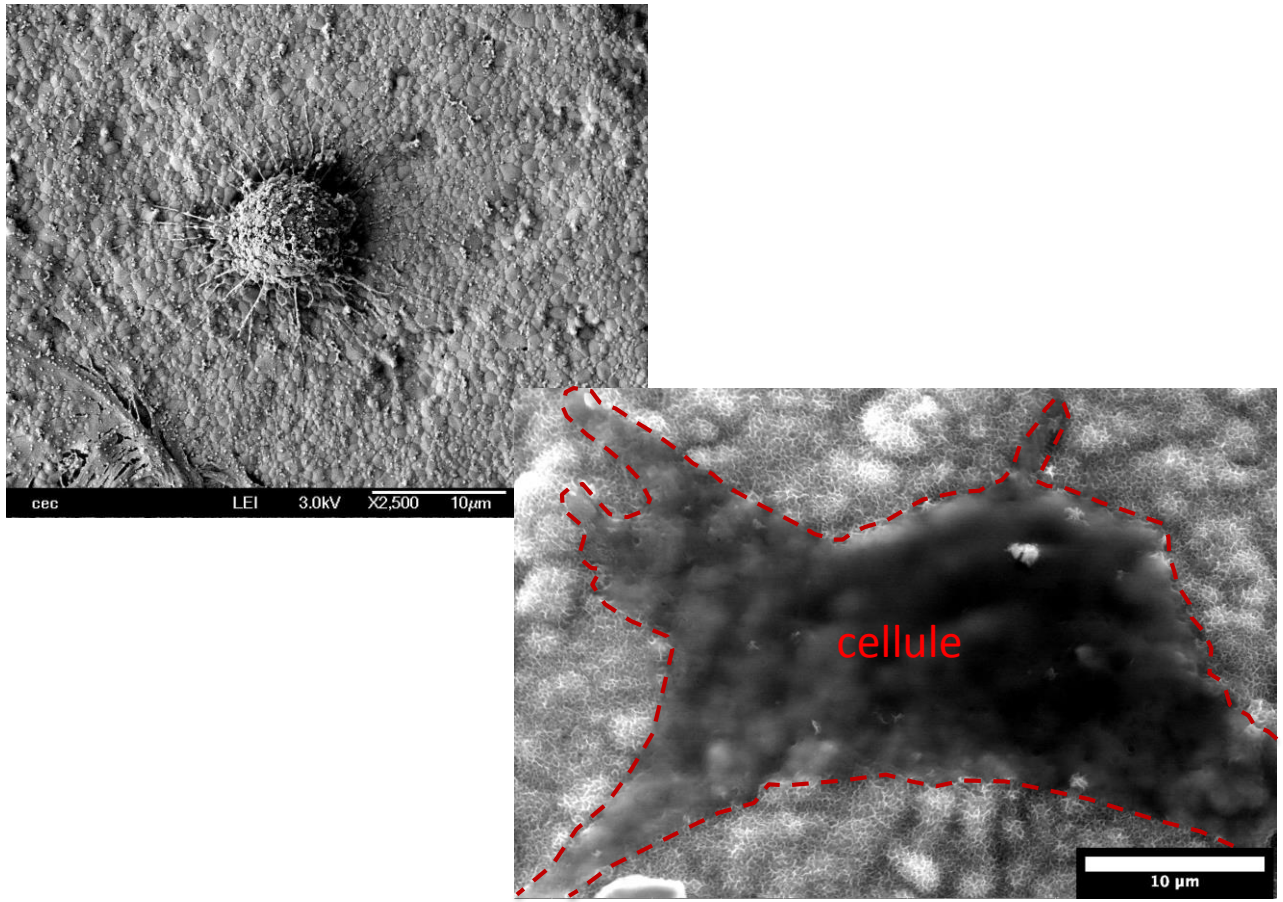
Cellules souches mésenchymateuses



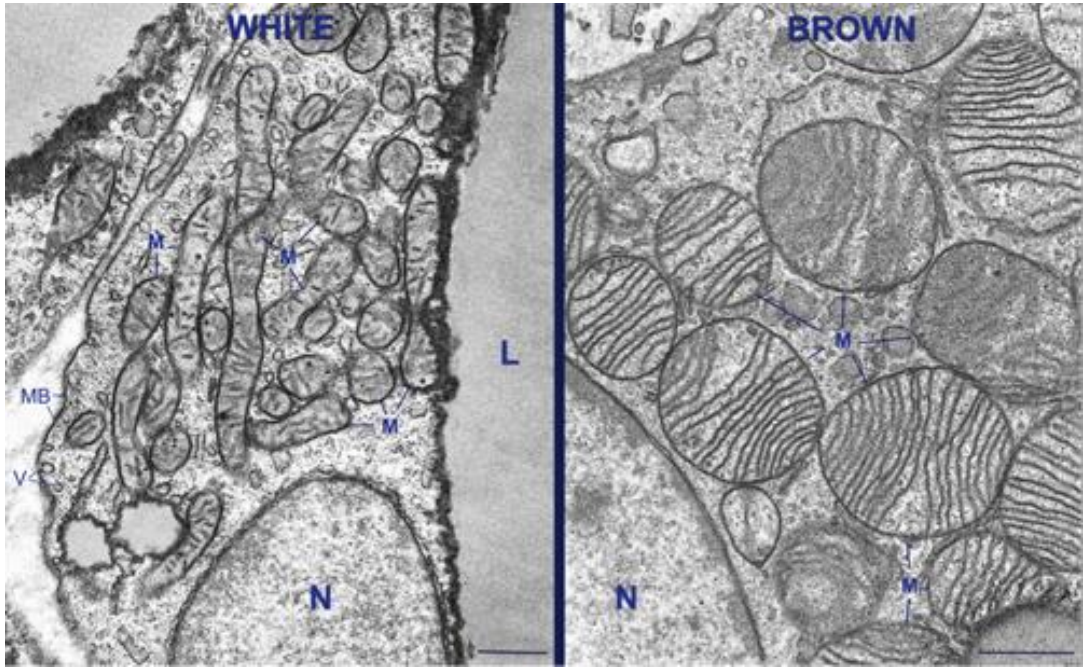
Adipocytes

Ostéoblastes

Microscopie électronique



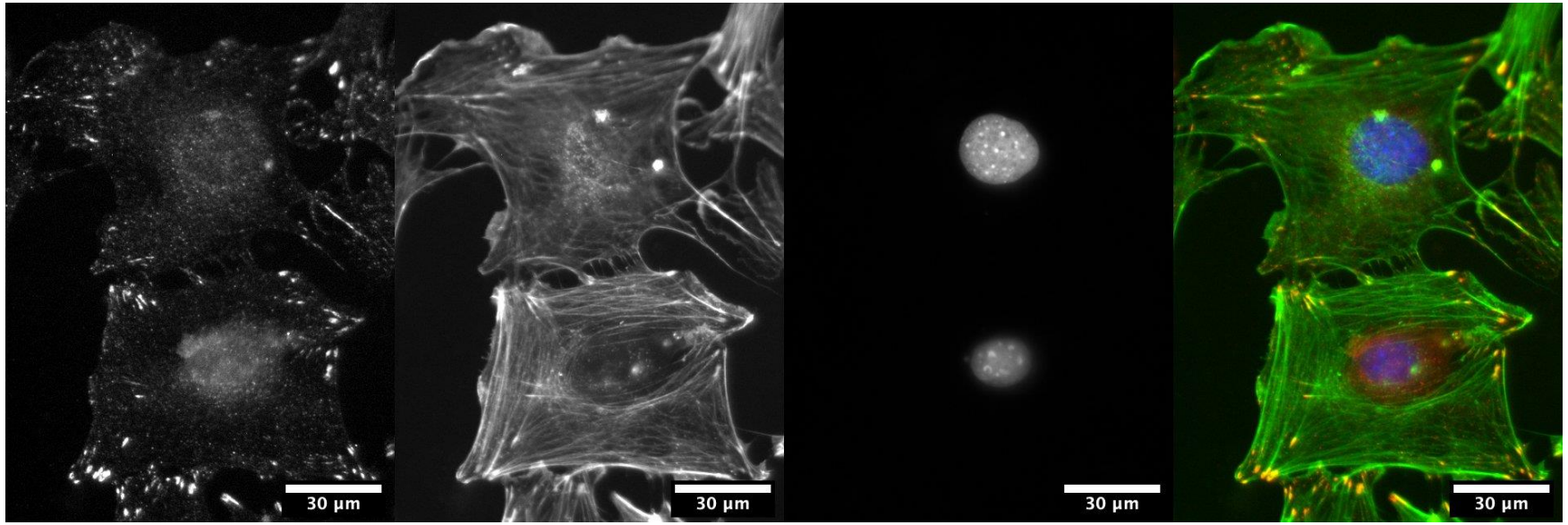
Microscopie électronique à balayage
=> morphologie cellulaire



Cinti S. "The Adipose Organ" Kurtis, Milan 1999

Microscopie électronique en transmission
=> organites cellulaires (morphologie)

Microscopie optique en fluorescence



MC3T3-E1 cells on dense HA pellets
Staining of focal adhesions: Vinculin - Actin - Nuclei
A. Magnaudeix - own work

Acquisition / type de données

Microspectroscopie CARS

(*coherent anti-Stokes Raman scattering*)

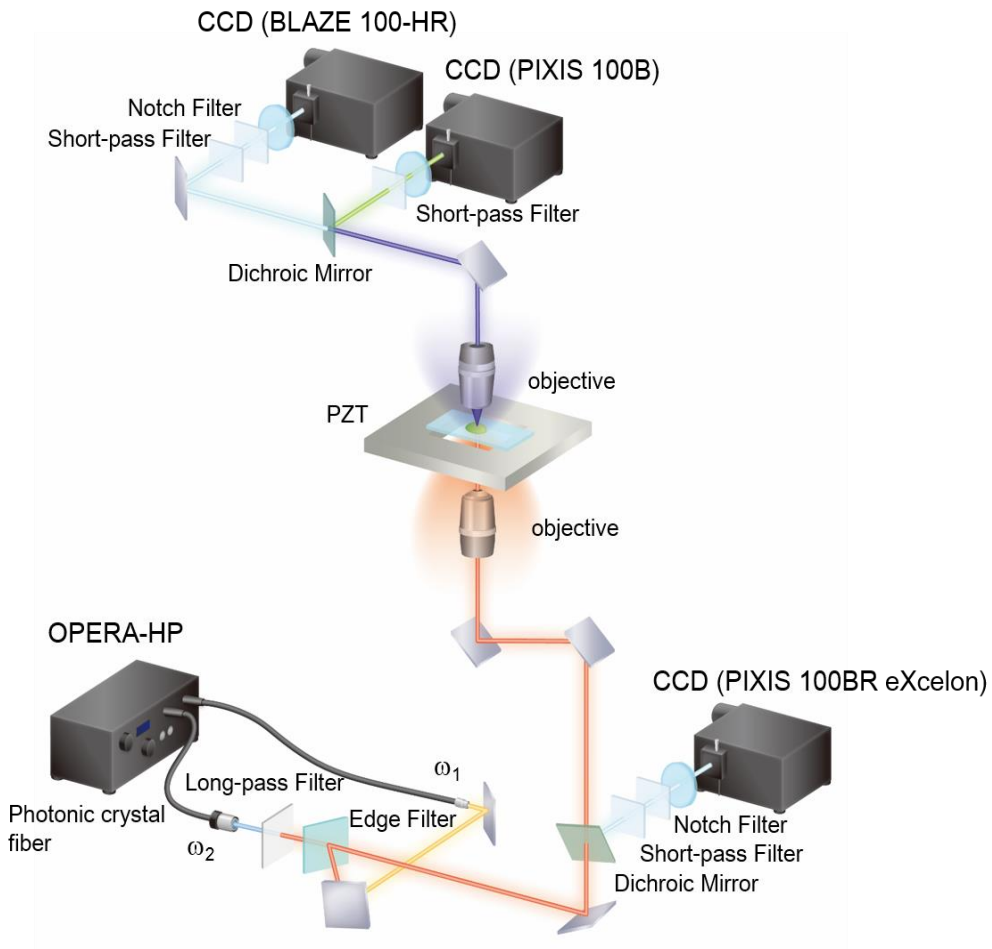
- Exploite un mécanisme **d'optique non linéaire** (le mélange à quatre ondes) mettant en jeu les états vibrationnels du matériau (**spectroscopie vibrationnelle**)

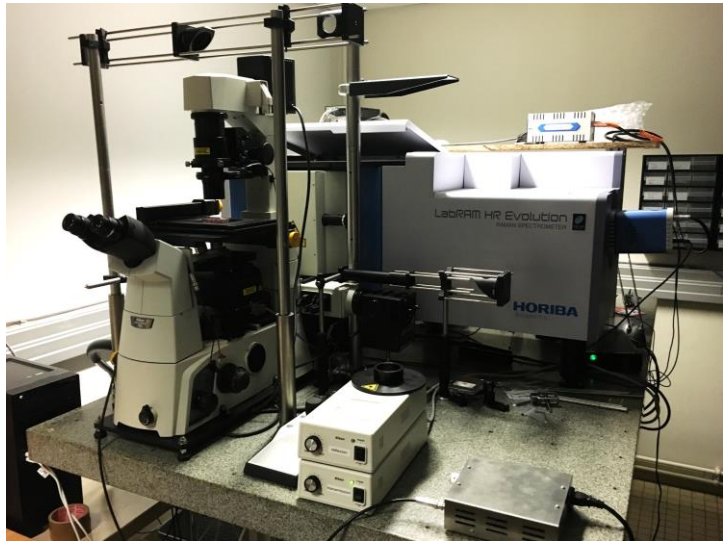
- Méthode d'imagerie sans marquage, **chimiquement sélective**, pour l'étude d'échantillons biologiques

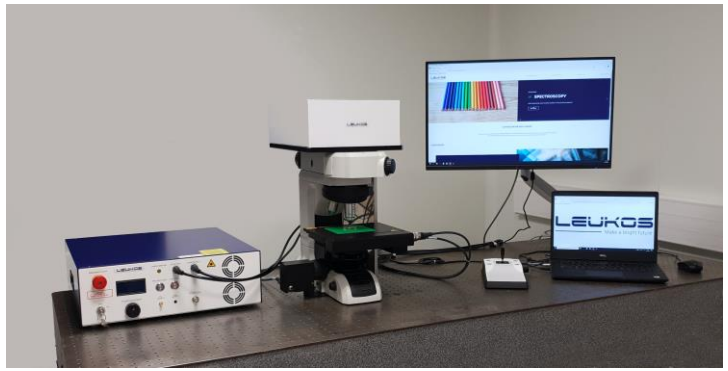
- Permet d'obtenir des images « multicomposantes » de l'échantillon (composante <=> famille de composés chimiques, i.e. lipides, protéines, ADN/ARN, eau, etc.)

Microspectroscopie CARS

(*coherent anti-Stokes Raman scattering*)







Données CARS x,y,(z),λ

Longueur d'onde

Coordonnées (pixels)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	699.9920	700.3650	700.7380	701.1120	701.4850	701.8580	702.2310	702.6040	702.9770	703.3500	703.7230	704.0960	704.4690	704.8420	705.2150	705.5870	705.9600	706.3330
2	831	836	833	832	832	828	829	830	832	833	834	829	834	831	833	832	838	829
3	834	833	831	831	833	831	835	831	833	827	830	835	835	834	836	831	834	832
4	833	836	828	833	833	831	834	826	834	832	830	831	833	831	834	828	833	831
5	835	832	835	829	833	834	832	832	833	830	834	831	830	829	828	832	832	832
6	833	831	835	829	829	830	834	829	832	832	830	832	830	833	831	836	828	831
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8	834	832	827	833	829	834	830	833	832	833	830	835	829	837	834	831	831	831
9	835	834	832	832	831	834	827	831	829	831	833	833	829	830	834	831	831	833
10	832	833	829	834	832	835	831	833	830	829	833	832	832	834	835	831	831	828
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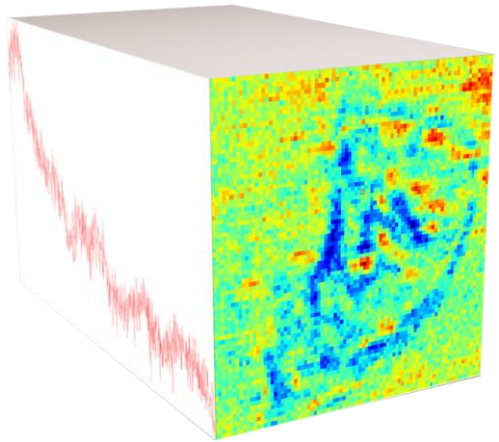
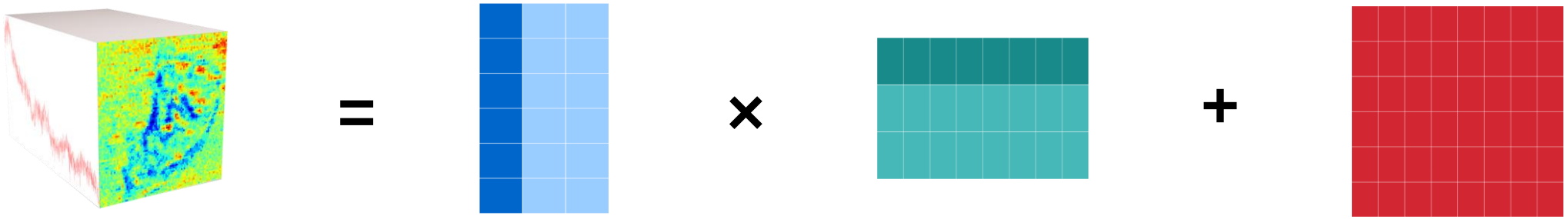


Ex : 500 px * 500 px * 1000 ch => 2,5x10^{8} éléments (images 2D)

Traitement / analyse

Multivariate curve resolution (MCR)

$$D = CS^T + E$$



MCARS cube linearized
$D \in \mathbb{R}^{M \times N}$

Concentration matrix
(quantitative information)
$C \in \mathbb{R}^{M \times K}$

Spectra matrix
(qualitative information)
$S \in \mathbb{R}^{N \times K}$

Error matrix
$E \in \mathbb{R}^{M \times N}$

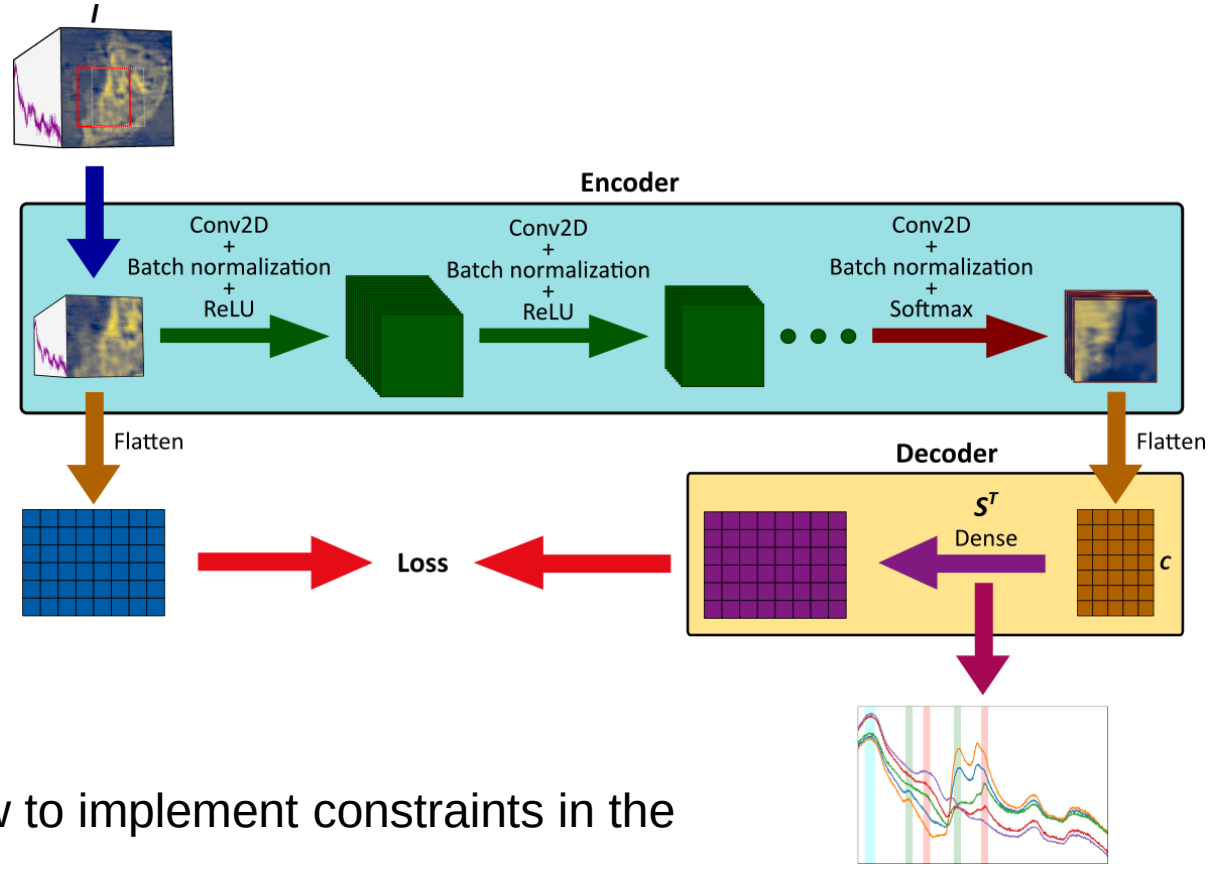
- Can be solved using least squares alternatively
- Need an initial estimation for one of the matrices
- Need to apply constraints consistent with studied phenomenom to reduce the amount of solutions:
  - Non-negativity on S
  - Non-negativity and sum to one on C

M: Number of pixels
N: Number of spectral values
K: Number of components

MCR solved with autoencoders

- Autoencoder: NN made of two blocks
  - Encoder $\mathfrak{E}$
  - Decoder $\mathfrak{D}$
- The encoder maps input data into a latent space
- The decoder represents the spectra
- With a dense layer with weights ϕ as $\mathfrak{D}$:

$$D = \mathfrak{E}(D)\phi^T + E$$



Using autoencoders for MCR allows:

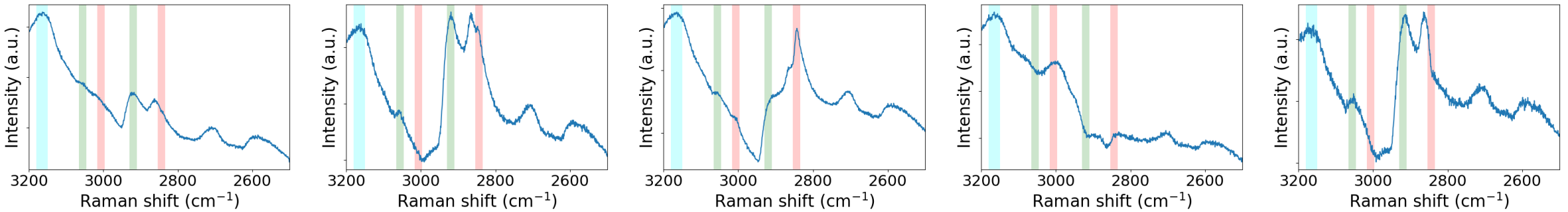
- Non-linear operations
- Spatial and spectral convolutions
- A large choice of loss function

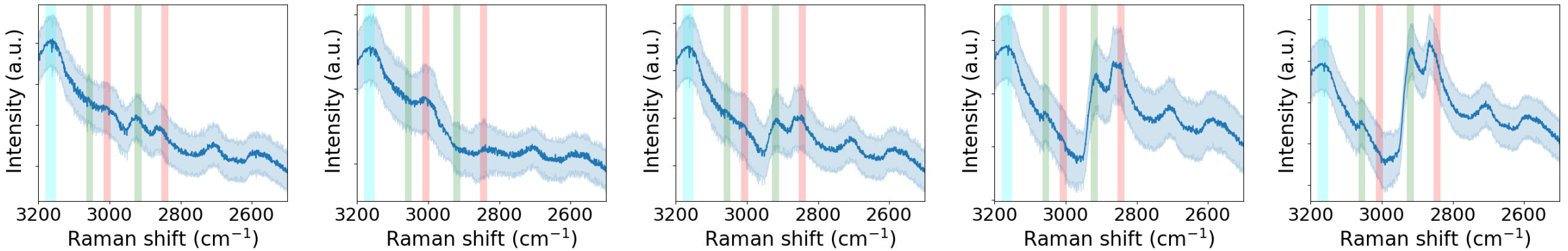
But requires:

- Define how to implement constraints in the network
- Hyperparametrization
- Repeat trainings to validate found solutions

MCR-ALS compared to MCR-AE: Spectra

MCR-ALS

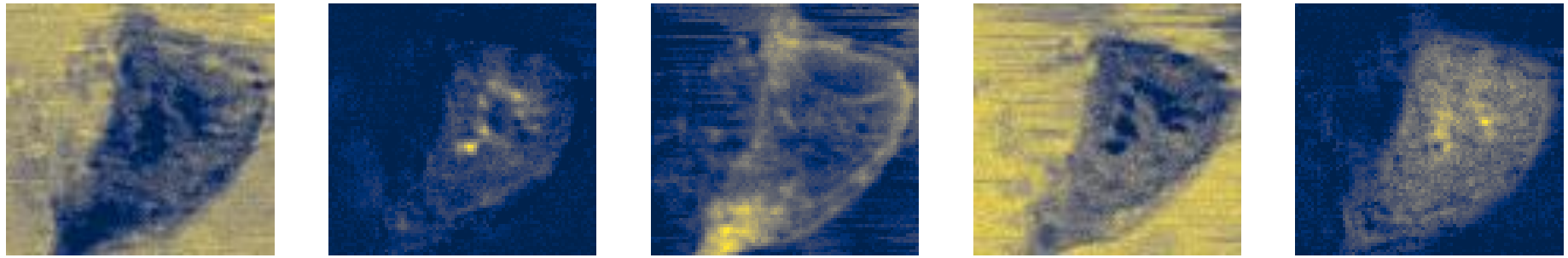




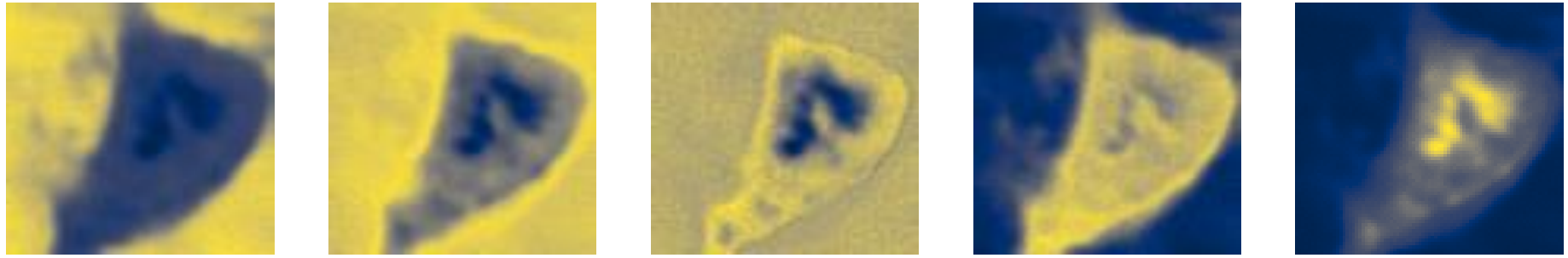
MCR-AE

MCR-ALS compared to MCR-AE: Concentration

MCR-ALS

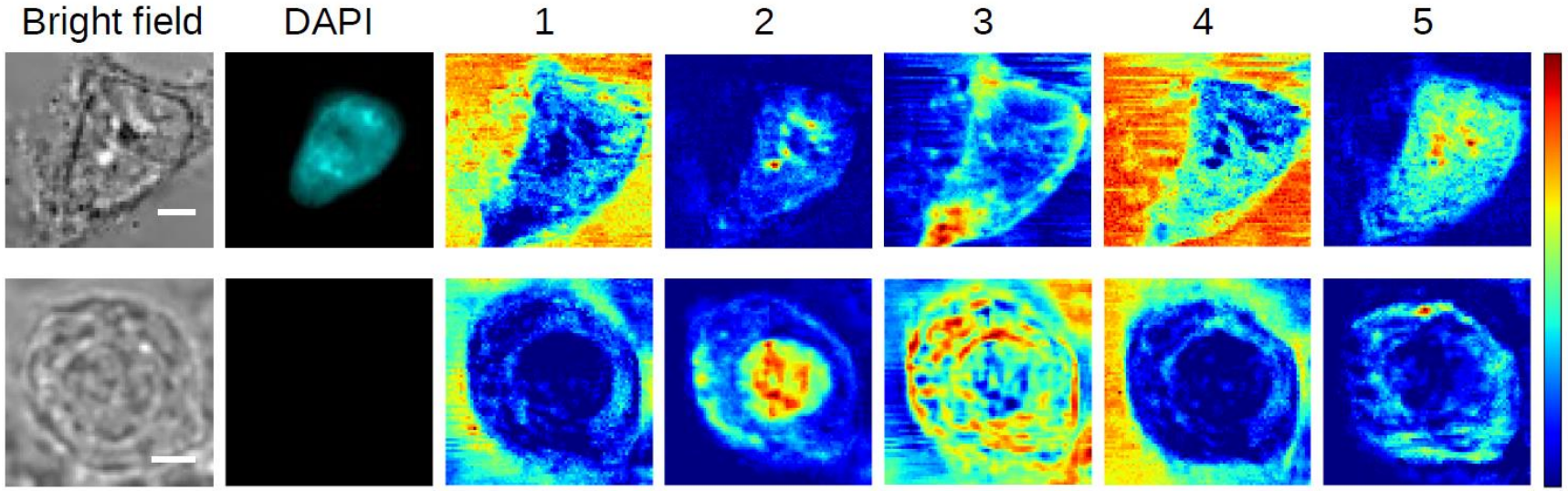


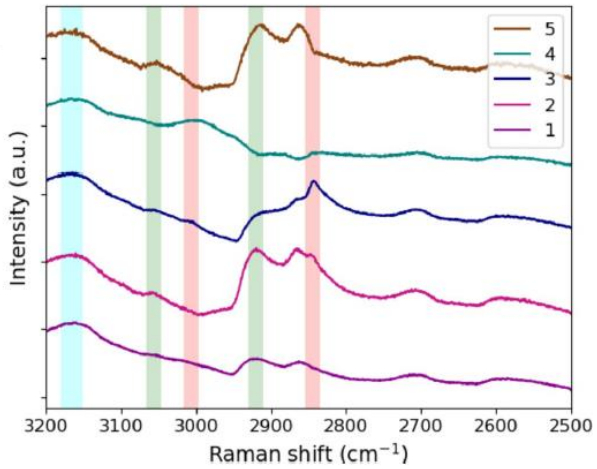
MCR-AE

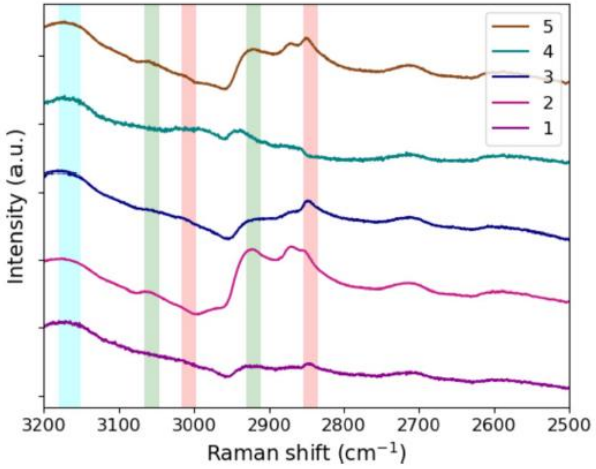


Quelques résultats (MCR)

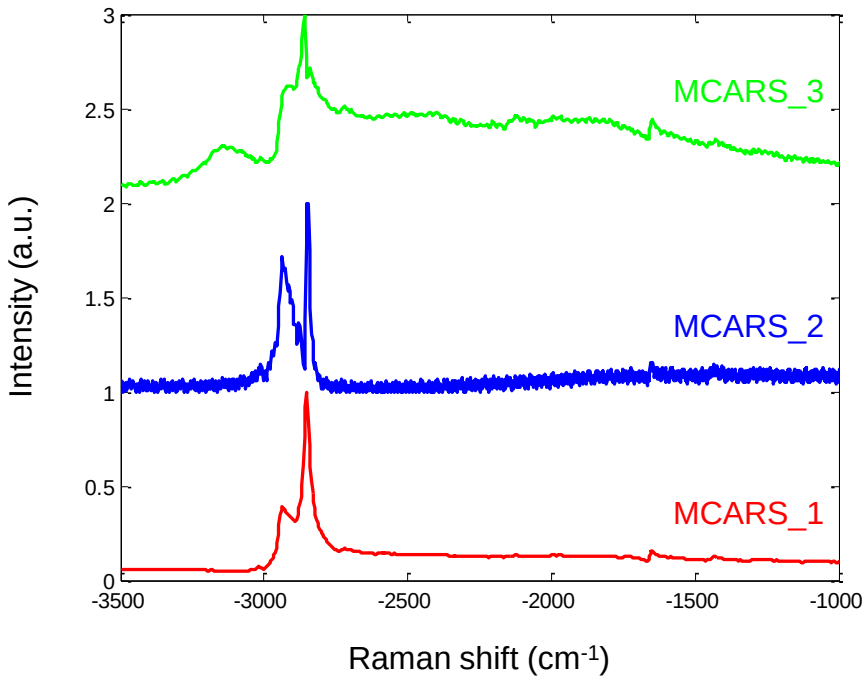
Study of the cell cycle

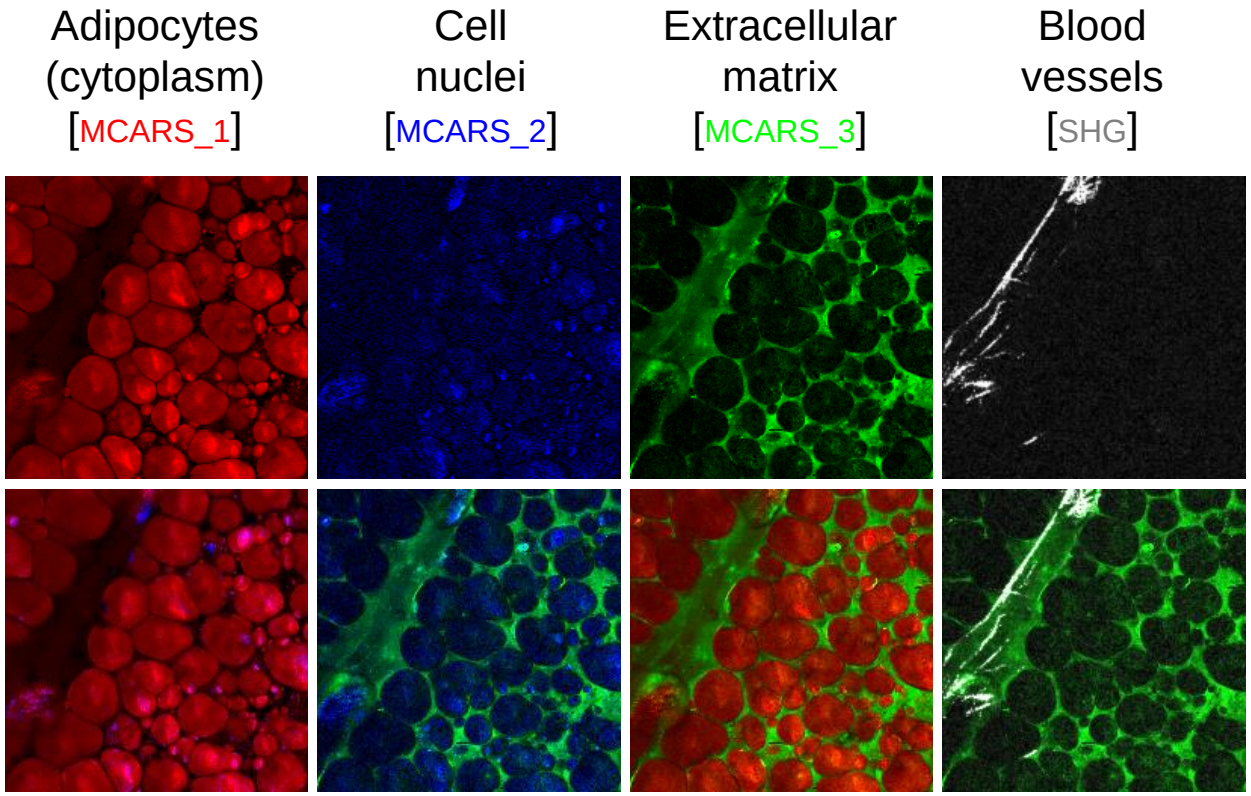






Hyperspectral imaging of adipose tissue





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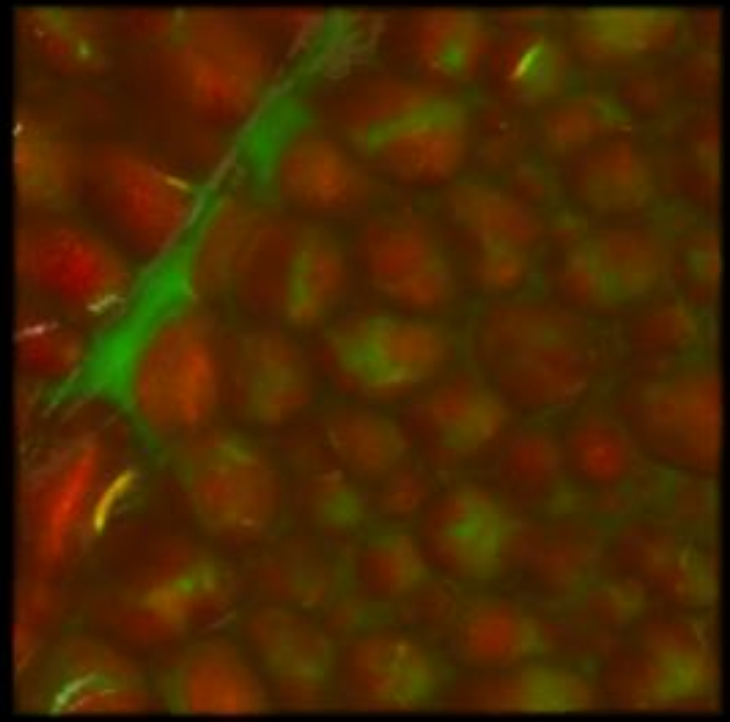
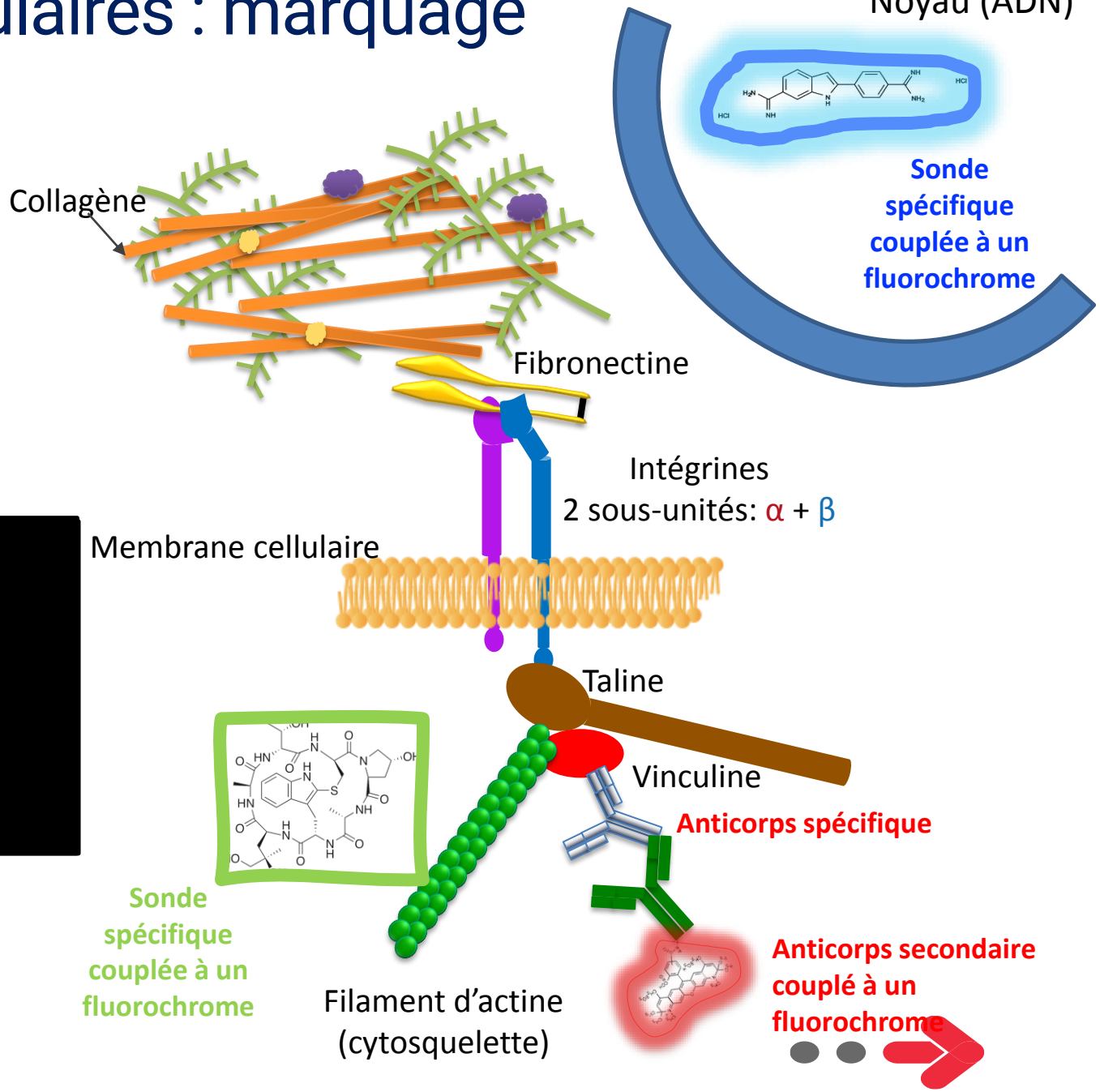


Annexes

Visualisation d'éléments cellulaires : marquage

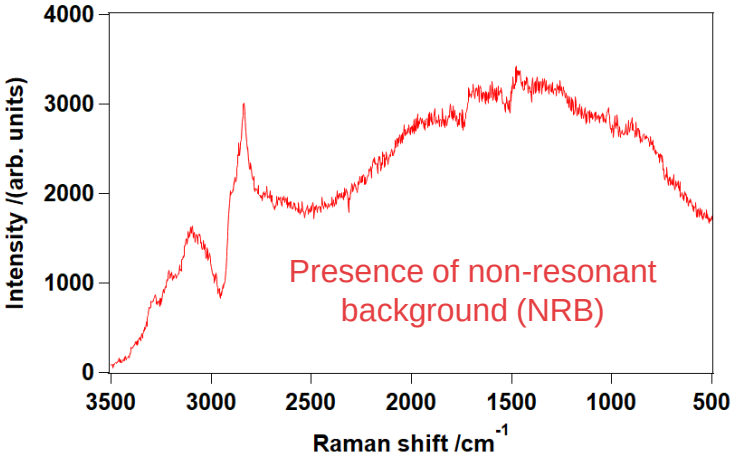
→ (trop) spécifique
→ Couteux en réactifs, temps, échantillons

MC3T3-E1 cells on dense HA pellets
Fluorescence microscopy
Staining of focal adhesions : Vinculin – Actin – Nuclei
A. Magnaudeix – own work



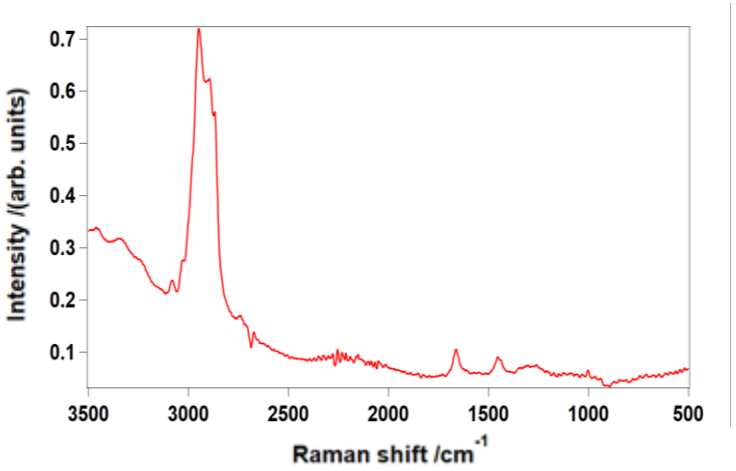
Data analysis: maximum entropy method (MEM)

Raw CARS spectrum



$$S(\nu) = \left| \frac{\beta}{1 + \sum_{k=1}^{M} a_k \exp(-2\pi i k \nu)} \right|^2$$

Im[$\chi^{(3)}$] spectrum



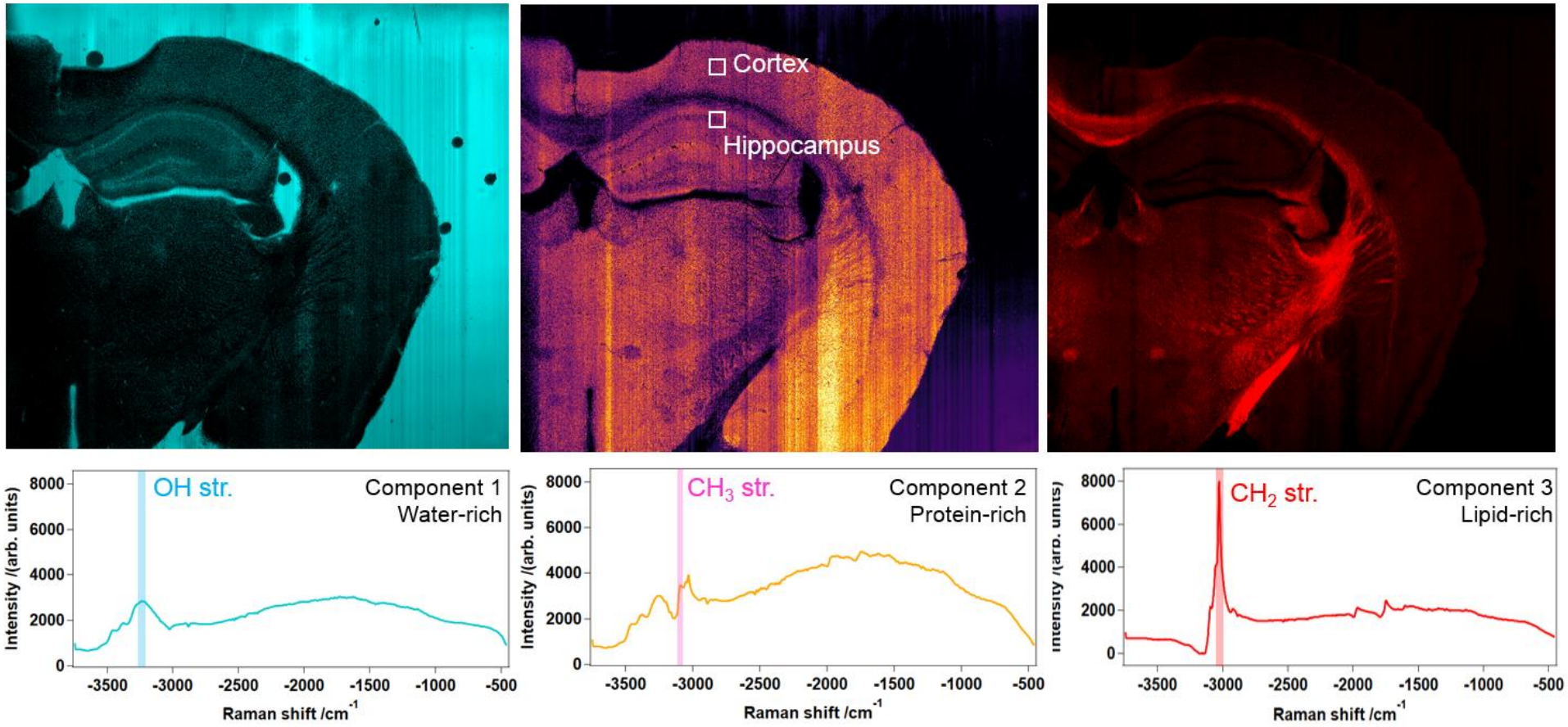
Spectrum-by-spectrum extraction of the pure vibrational signal and recovery of conventional Raman-like spectra

[Vartiainen et al., Optics Express 14, 3622-3630, 2006]

Brain label-free molecular imaging

MCARS + MCR

Macroscale imaging



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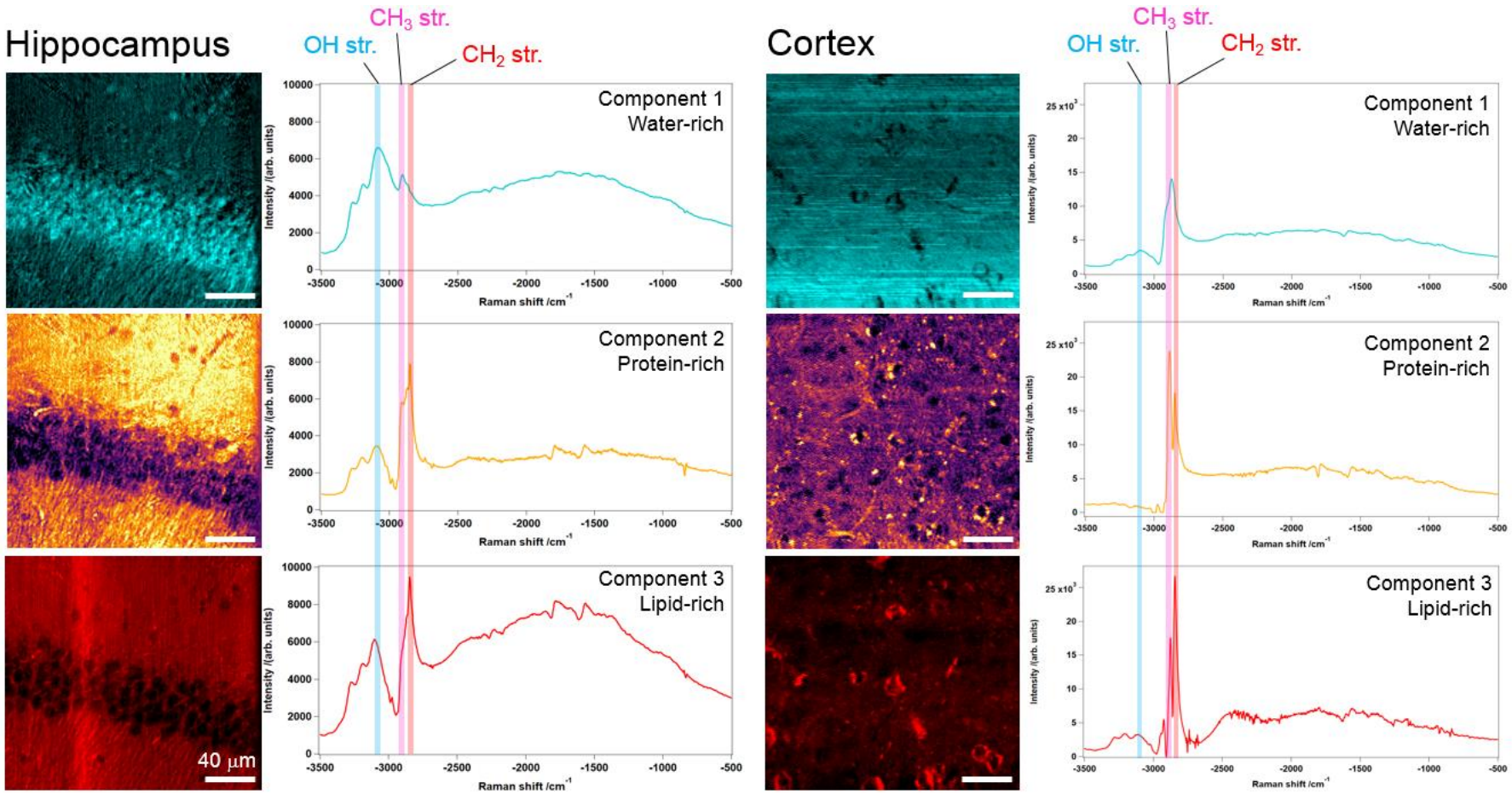
Ph.D.Program in Humanics

Brain label-free molecular imaging

MCARS + MCR

Microscale imaging



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