Investigating chronic liver diseases and cancer using multimodal spectroscopy

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IR Workshop on Spectro-Microspectroscopy

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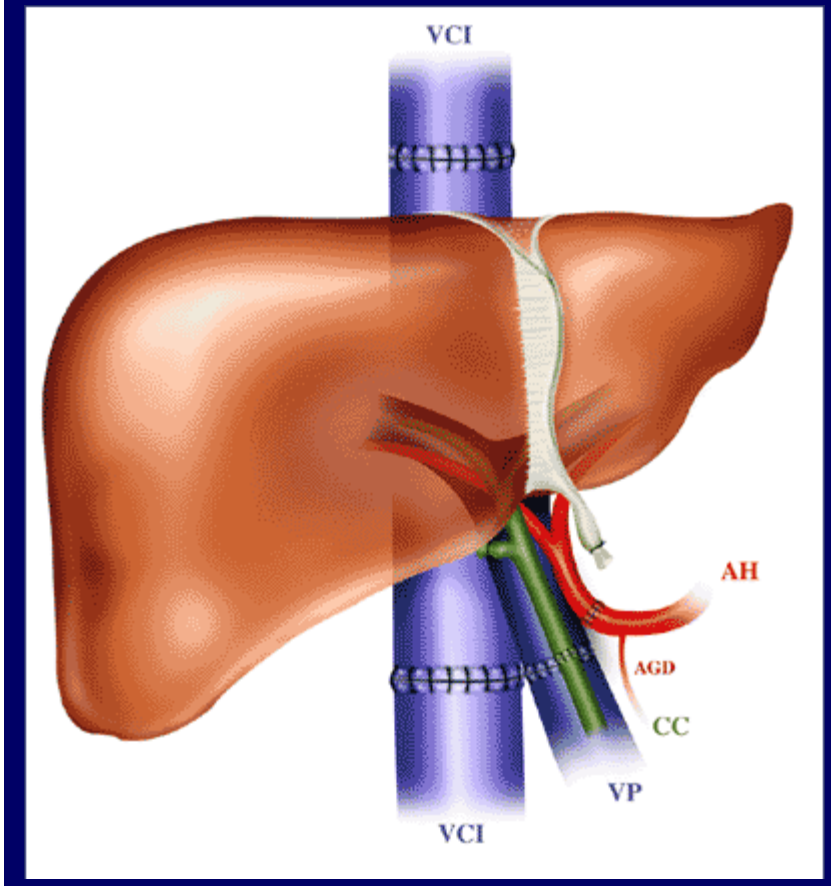
The liver



Glycogen synthesis & catabolism

Lipids: cholesterol synthesis & catabolism, production of triglycerids, lipoprotein synthesis

Destruction of old red blood cells & leucocytes

Production of coagulation factors (I, III, V, VII, IX & XI)

Metabolism of toxins and drugs

Urea synthesis

Storage of vitamins (A, B12, D, K, E)

Storage of elements (Fe, Cu)

The liver is the organ with the most important activity in metabolism

Chronic liver diseases and cancer

Normal liver

Fatty liver
/ steatosis

Cirrhosis

Obesity / Diabete / Drugs

Alcohol

Viral hepatitis
(HBV, HCV)

CANCER

Agressive

Low survival

Chronic liver diseases and cancer

Normal liver

Fatty liver
/ steatosis

Cirrhosis

CANCER

Agressive

Low survival

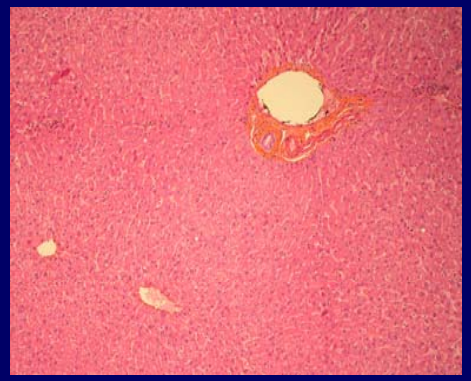
→ Crucial need of markers for diagnosis and prognosis

A multimodal spectroscopy-based approach at synchrotron SOLEIL

Liver

Cryomicrotome

Tissue section



Microspectroscopy

The synchrotron SOLEIL

Brillance

Accordability

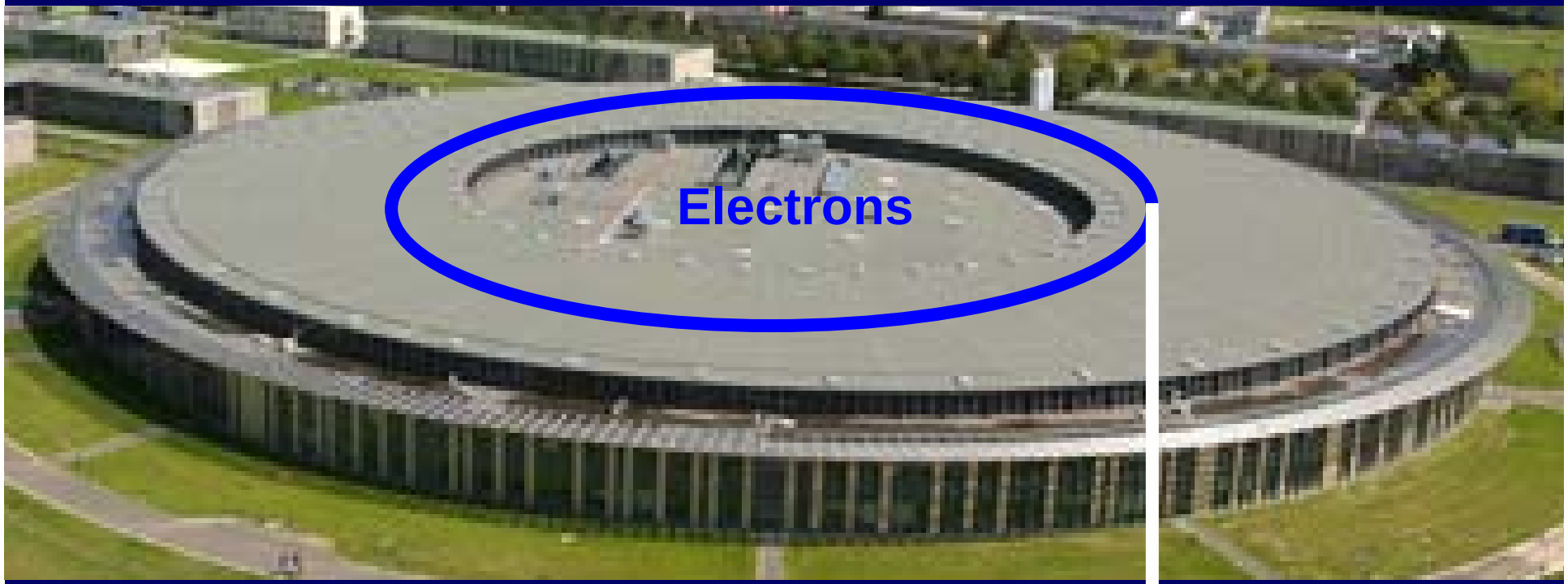
IR

UV

X-ray

Spectral markers

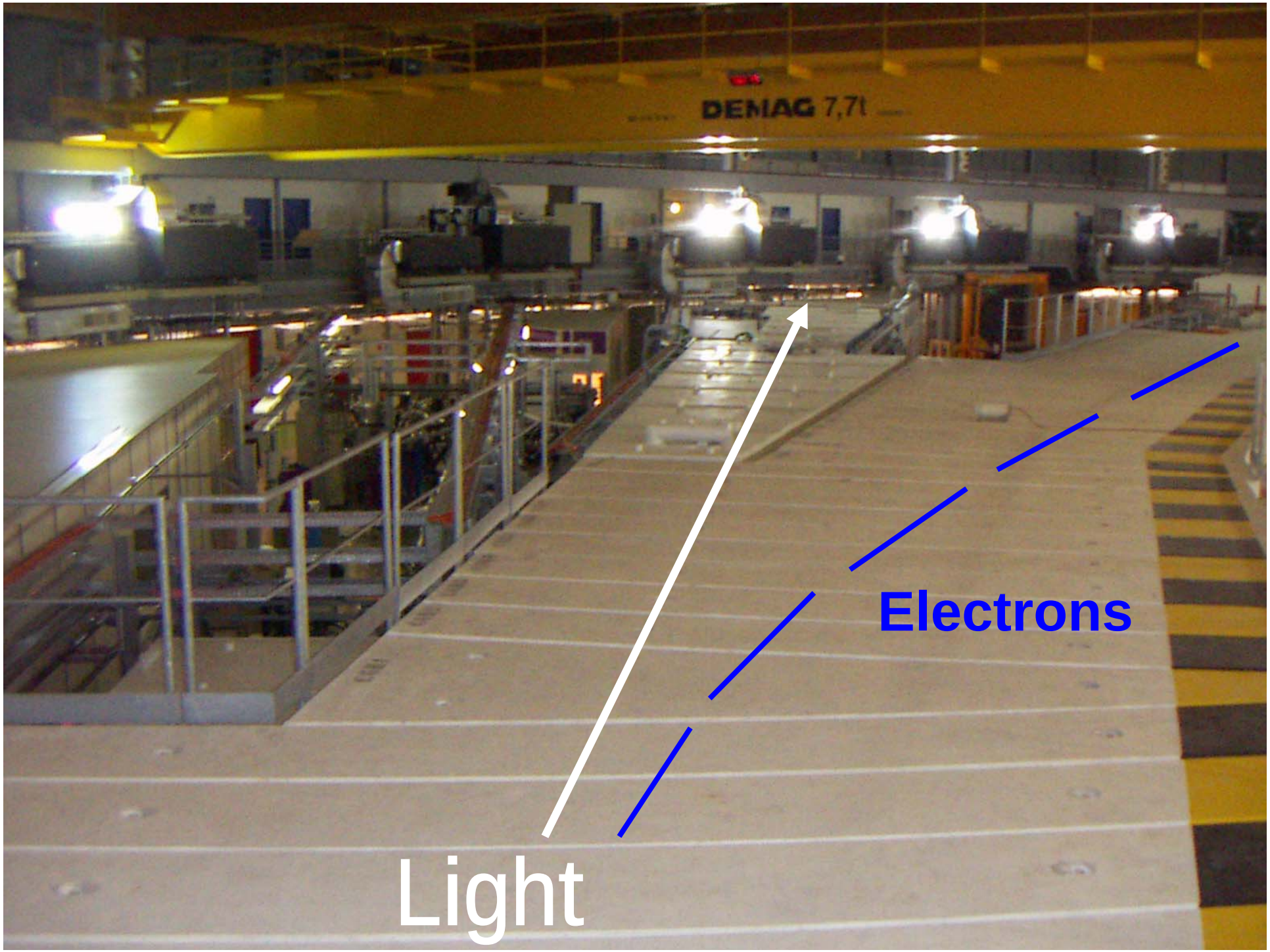
The synchrotron SOLEIL

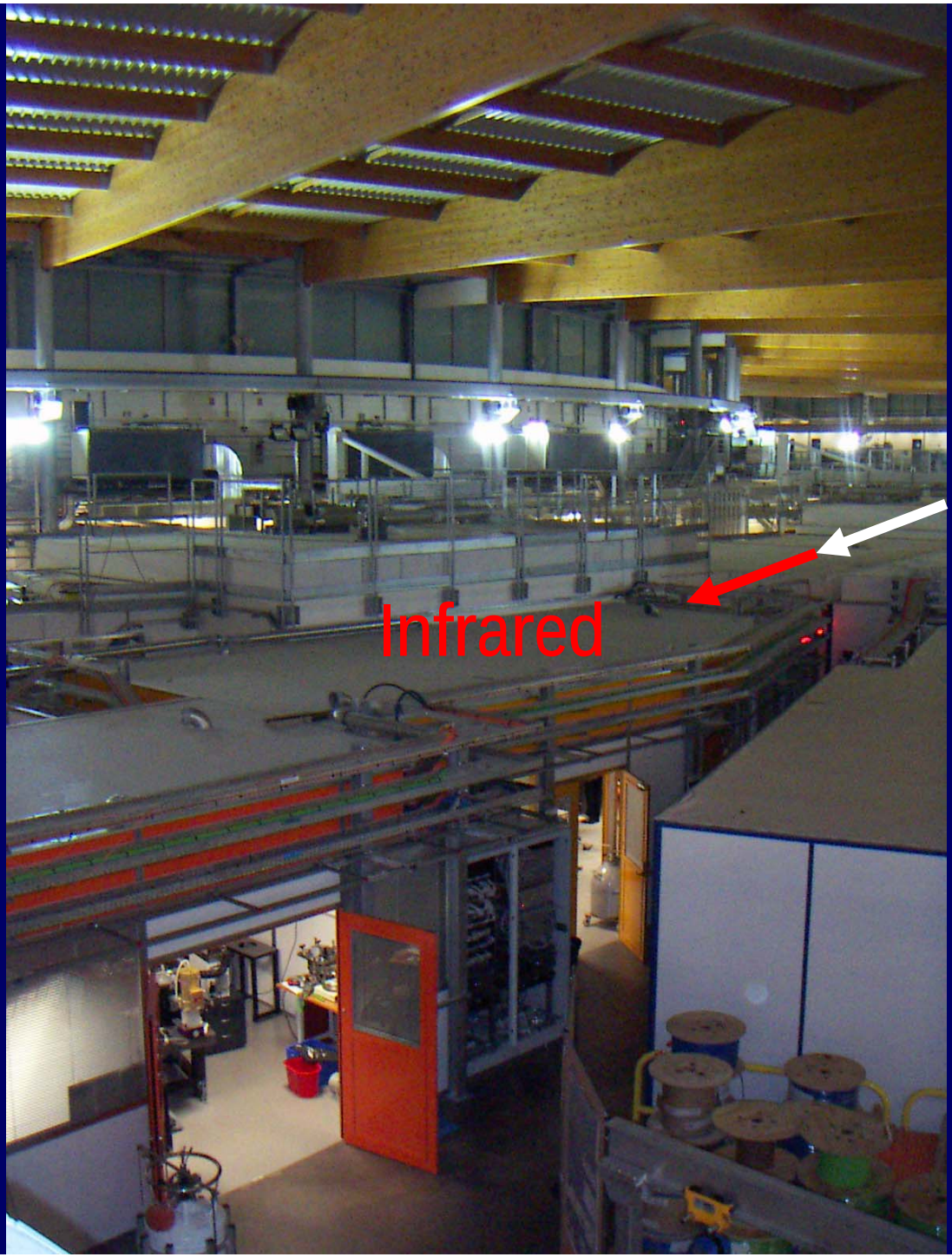


Characteristics

- **Brightness**
- **Accordability**

IR **UV** **RX**

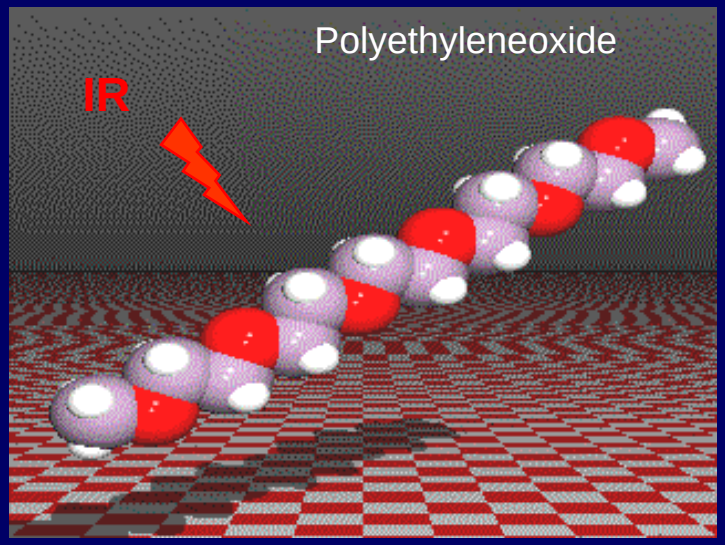

DEMAG 7,7t
Electrons
Light

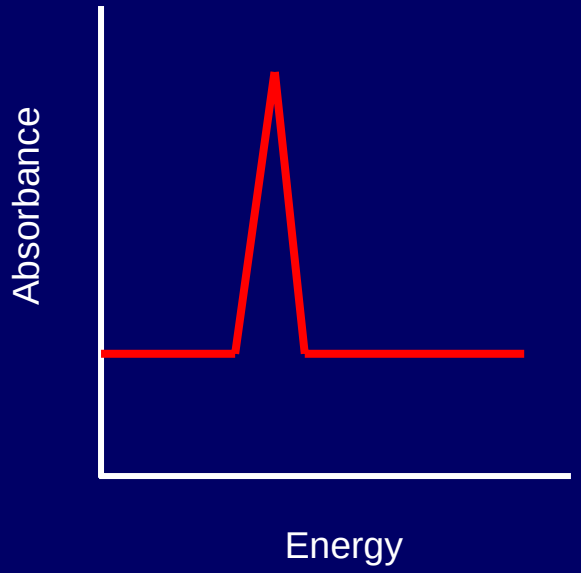

Infrared

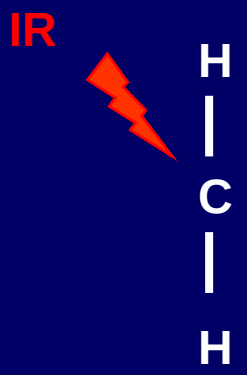
Infrared microspectroscopy at SMIS beamline

Infrared

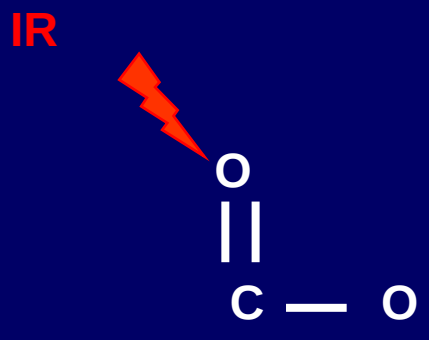
Infrared spectroscopy



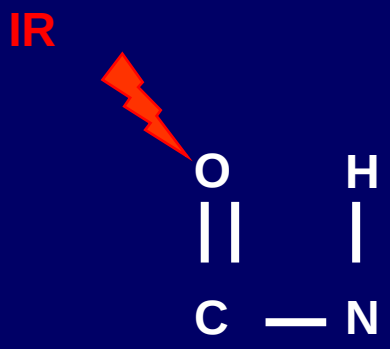




3000-2800 cm-1

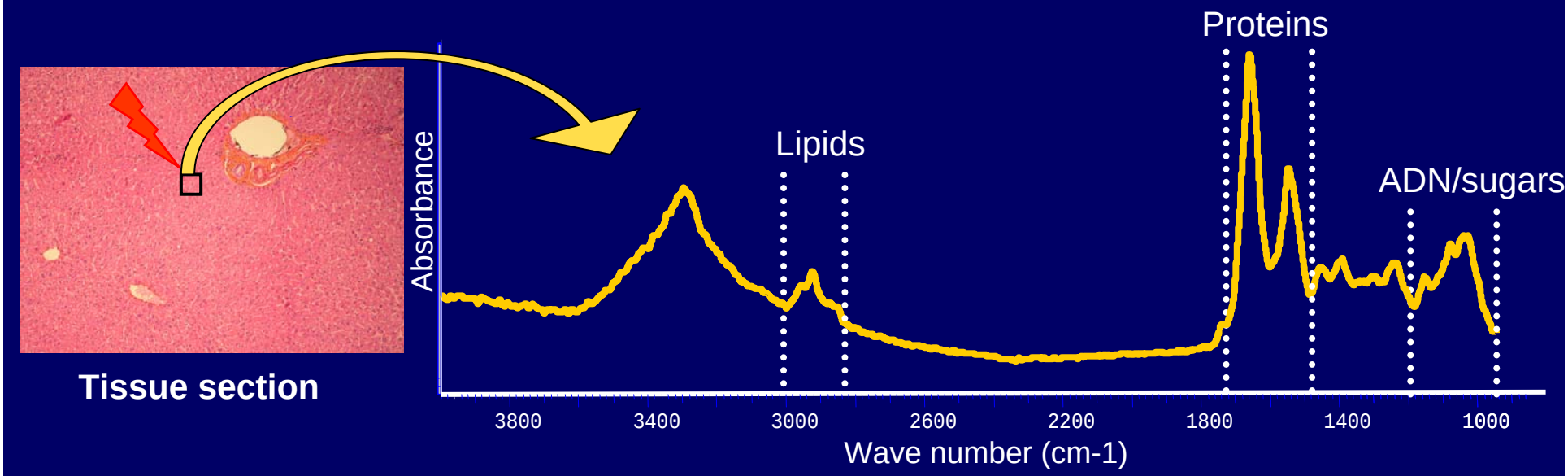


1740 cm-1



1650 / 1540 cm-1

Infrared spectroscopy on tissues

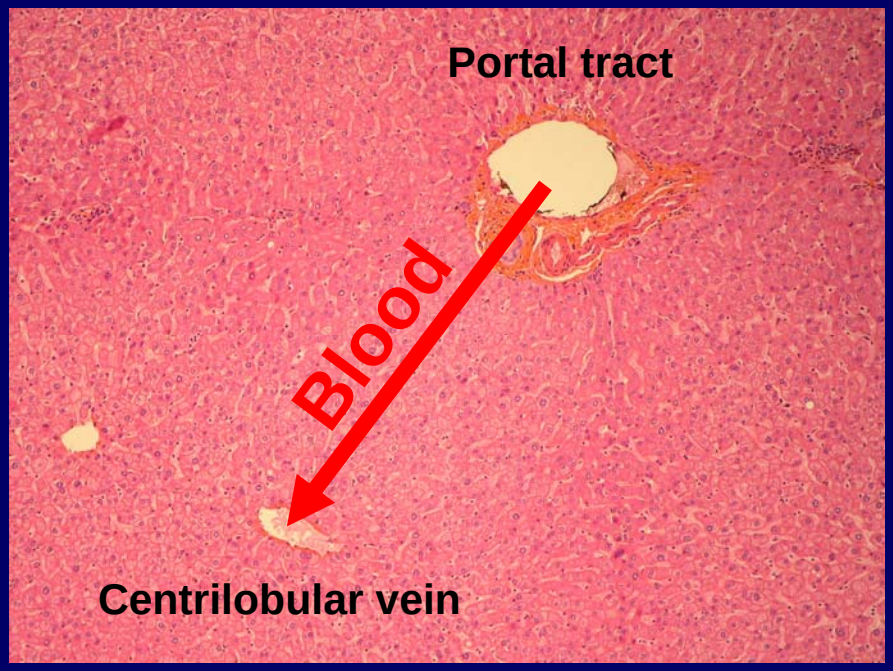


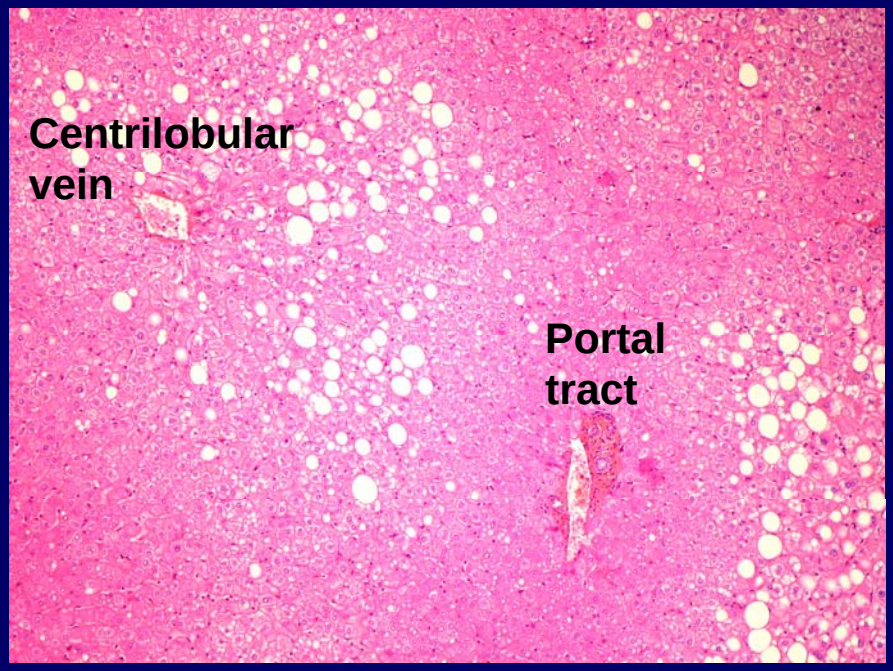
The spectrum is resulting of the global biochemical composition

Liver steatosis

Normal liver

Steatosis

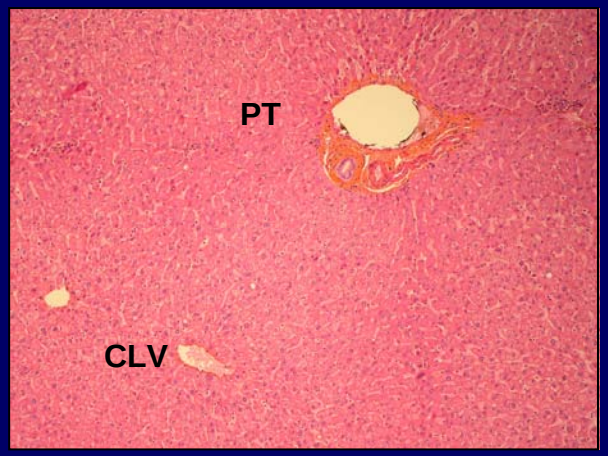




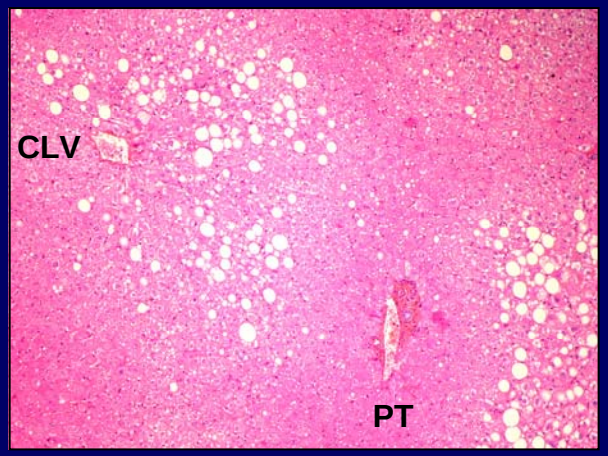
Steatosis is characterized by the formation of vesicles enriched in lipids

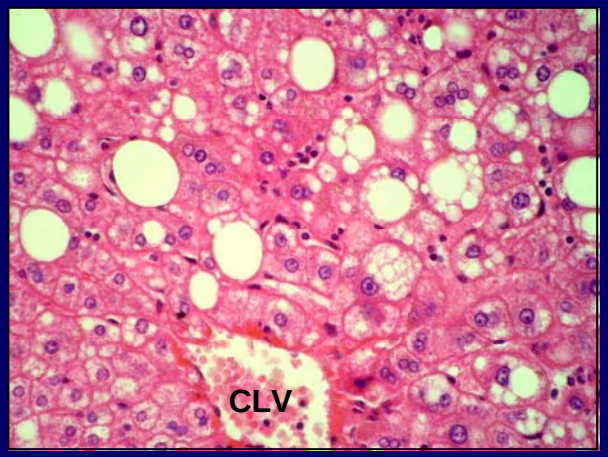
Liver steatosis

Normal liver



Liver steatosis



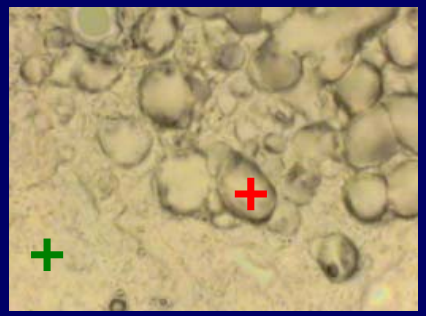


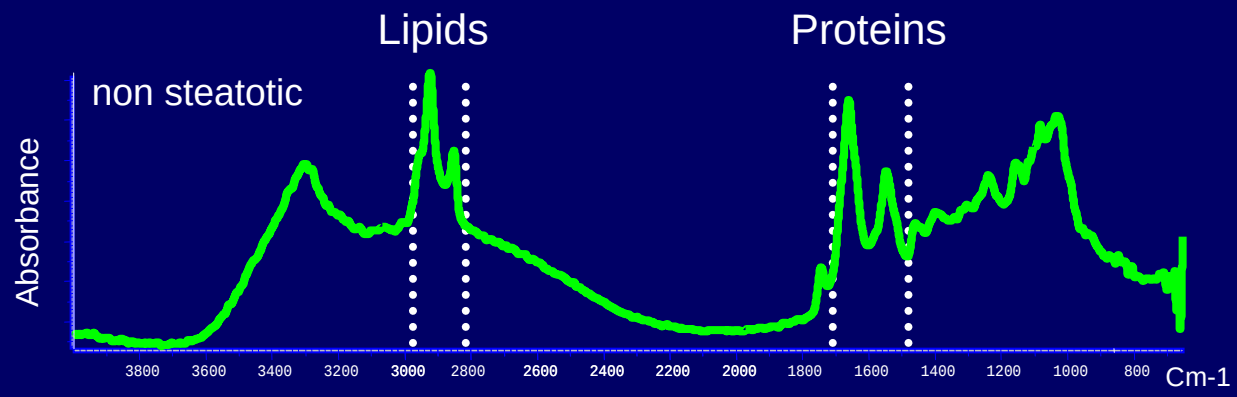
PT: portal tract

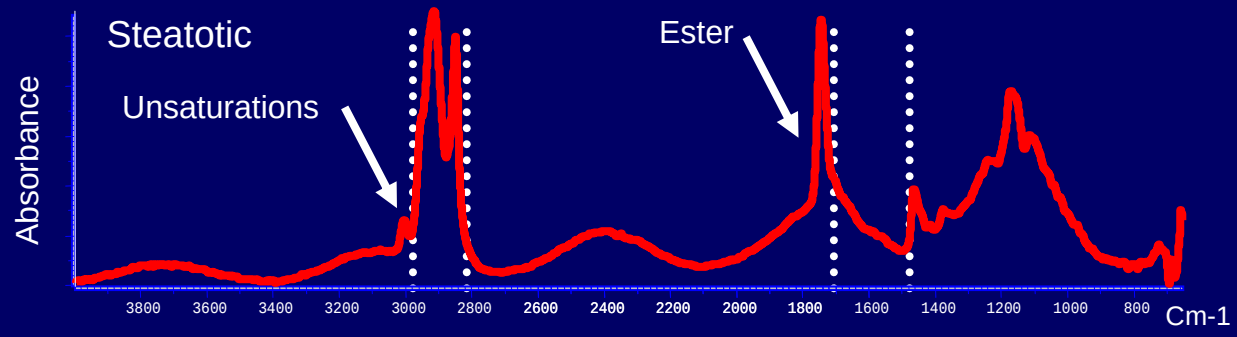
CLV: centrilobular vein

Infrared microspectroscopy on steatosis

Steatosis

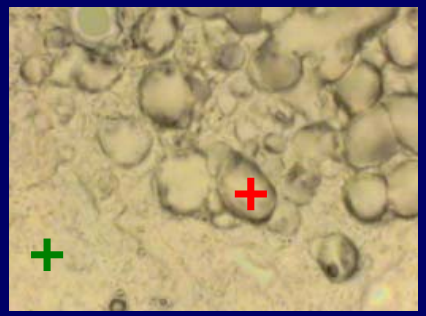


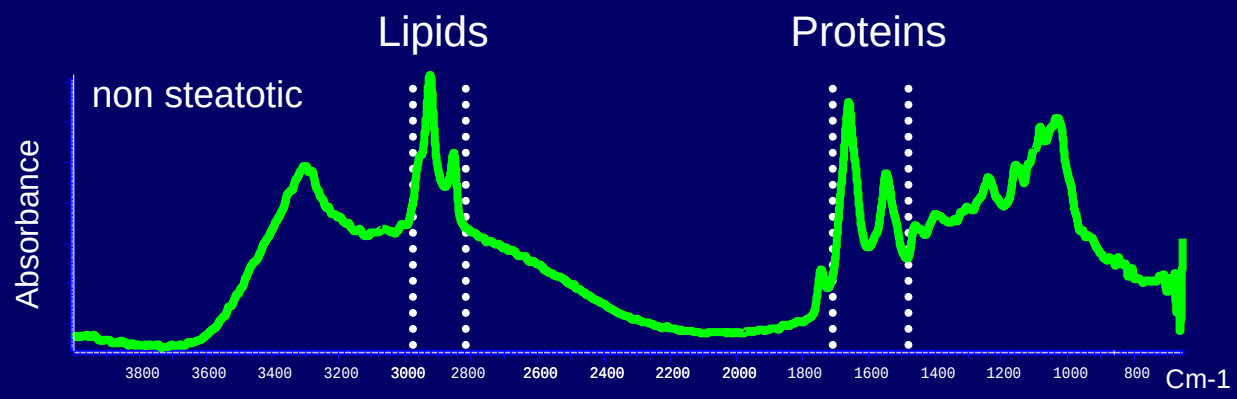




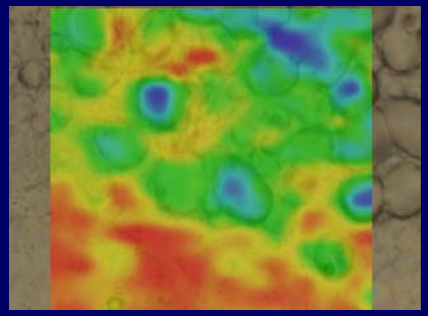
Infrared microspectroscopy on steatosis

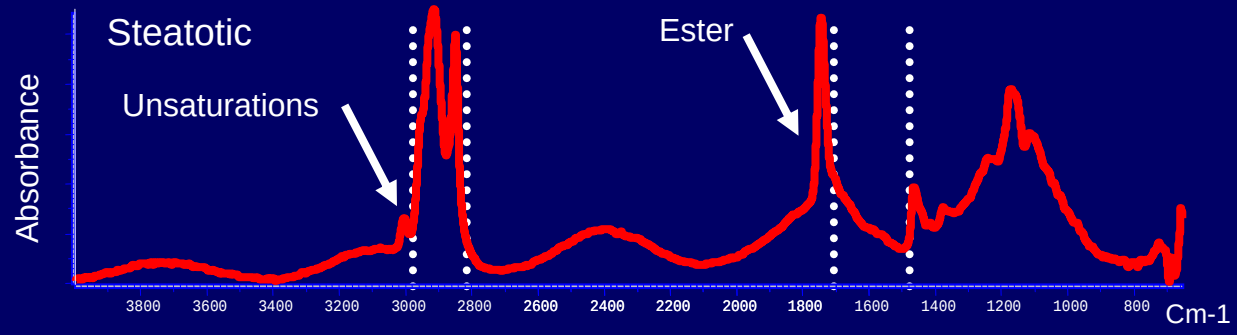
Steatosis



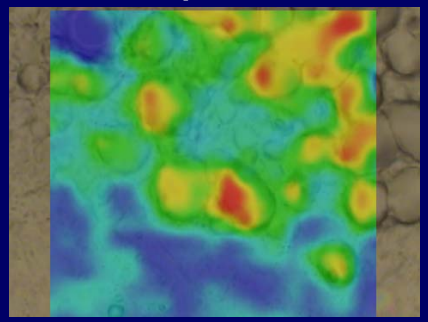


Proteins

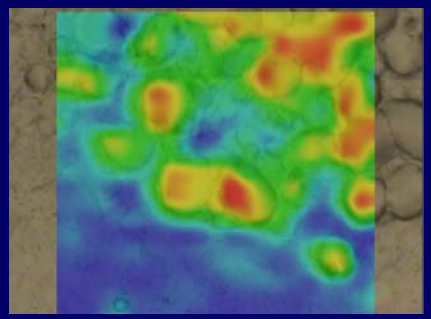




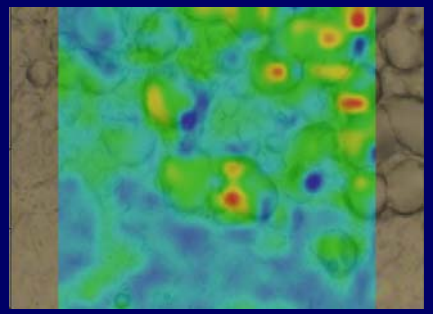
Lipids



Ester

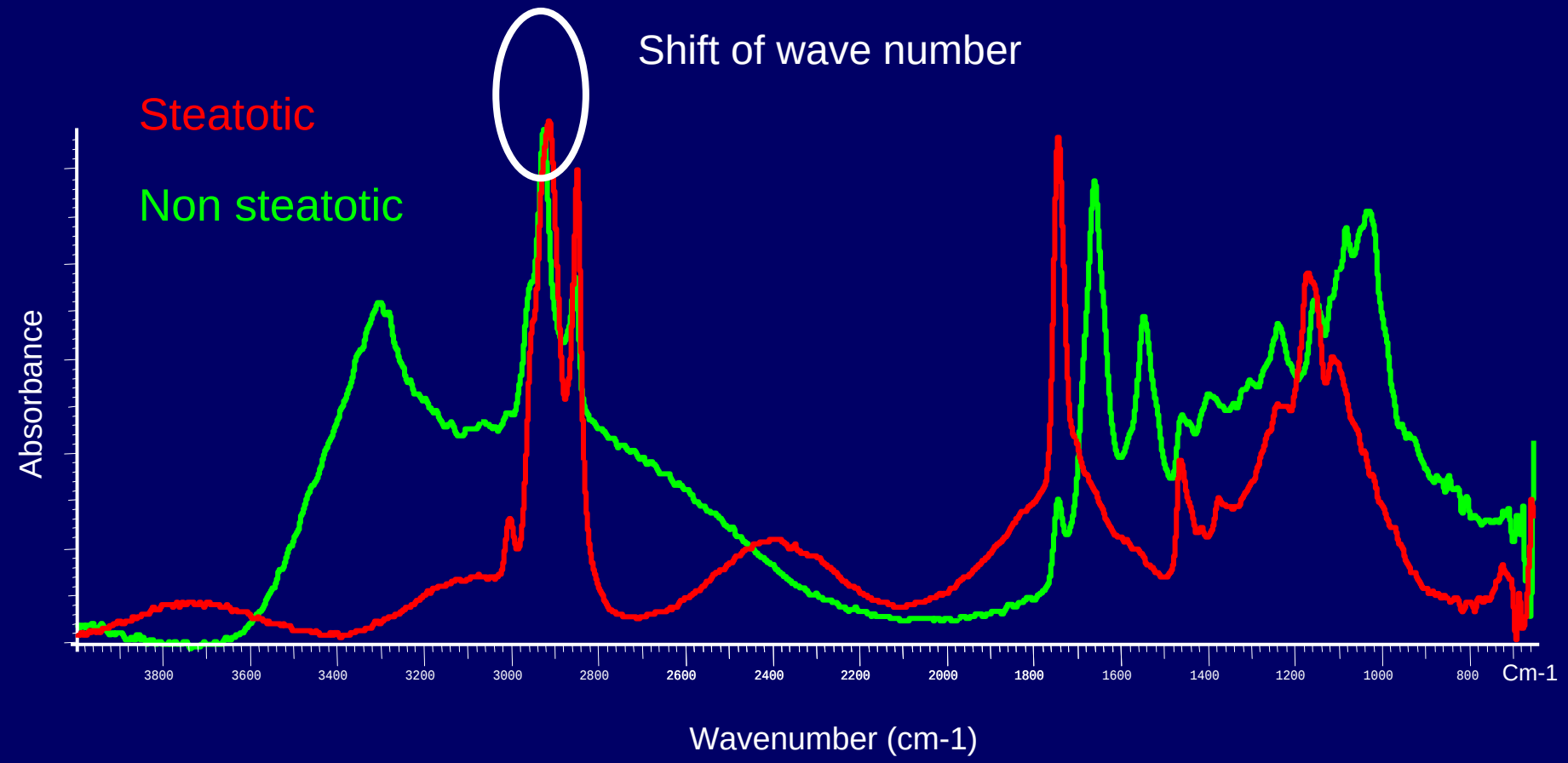


Unsaturations

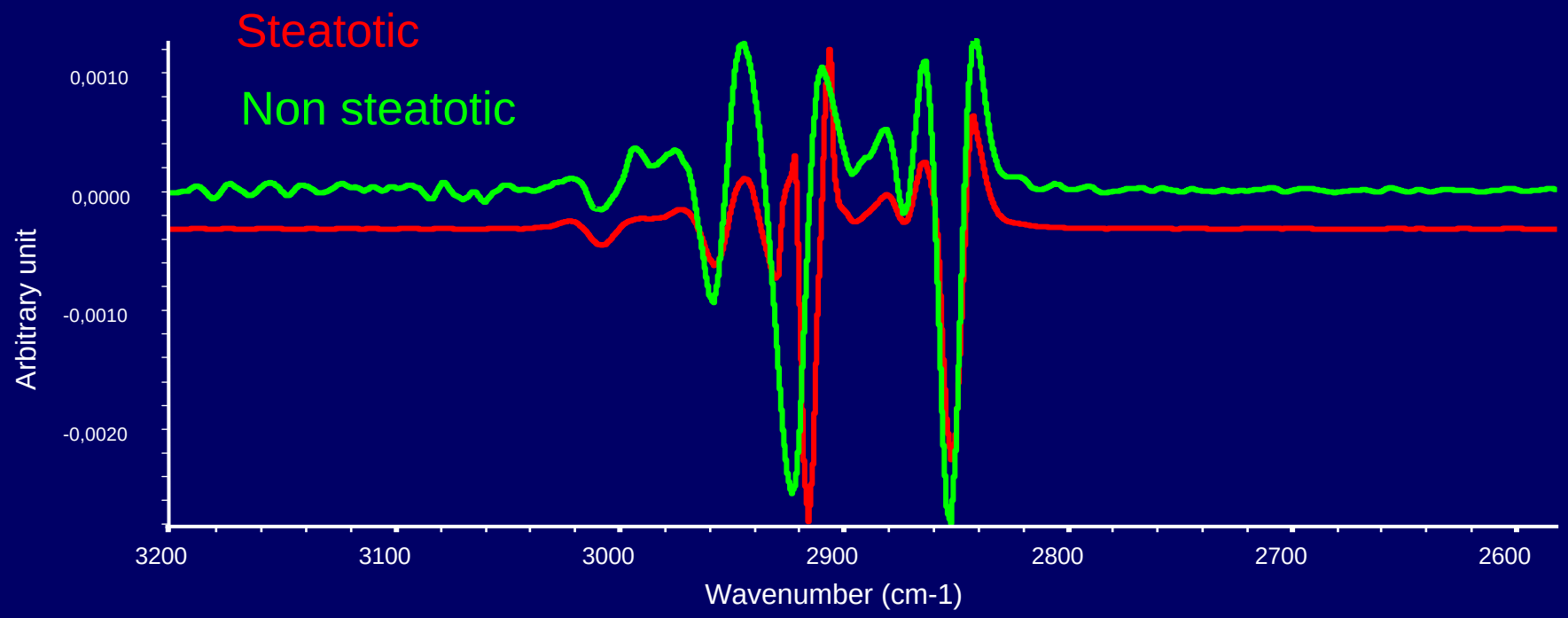


Min — Max

Infrared microspectroscopy on steatosis



Infrared microspectroscopy on steatosis
second derivation



→ Variation in lipid composition or environment

Conclusions

Infrared microspectroscopy

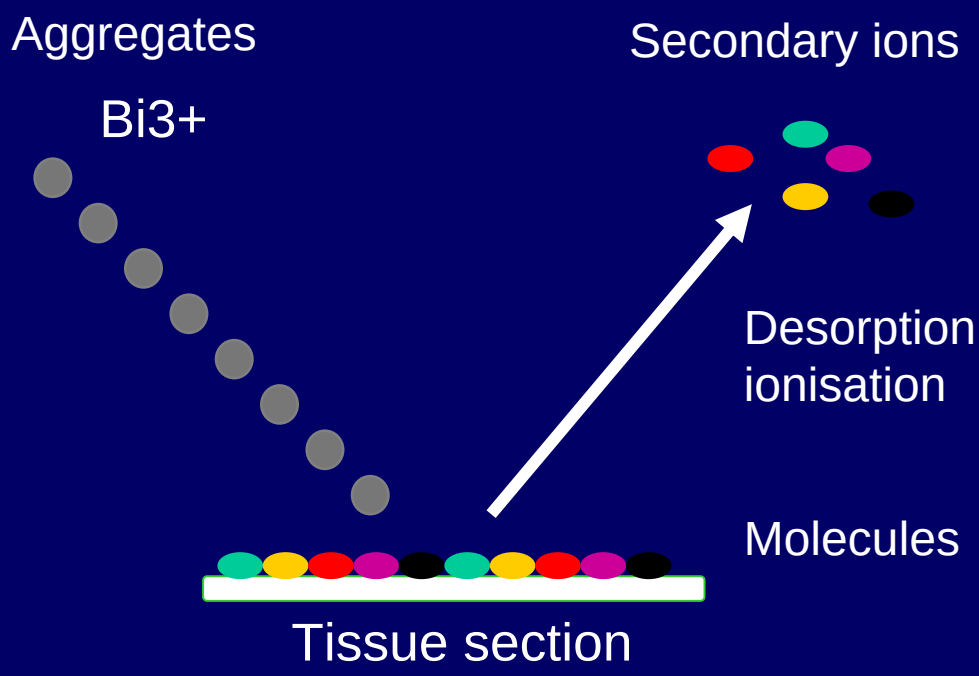
↓

Global biochemical composition of steatotic vesicles

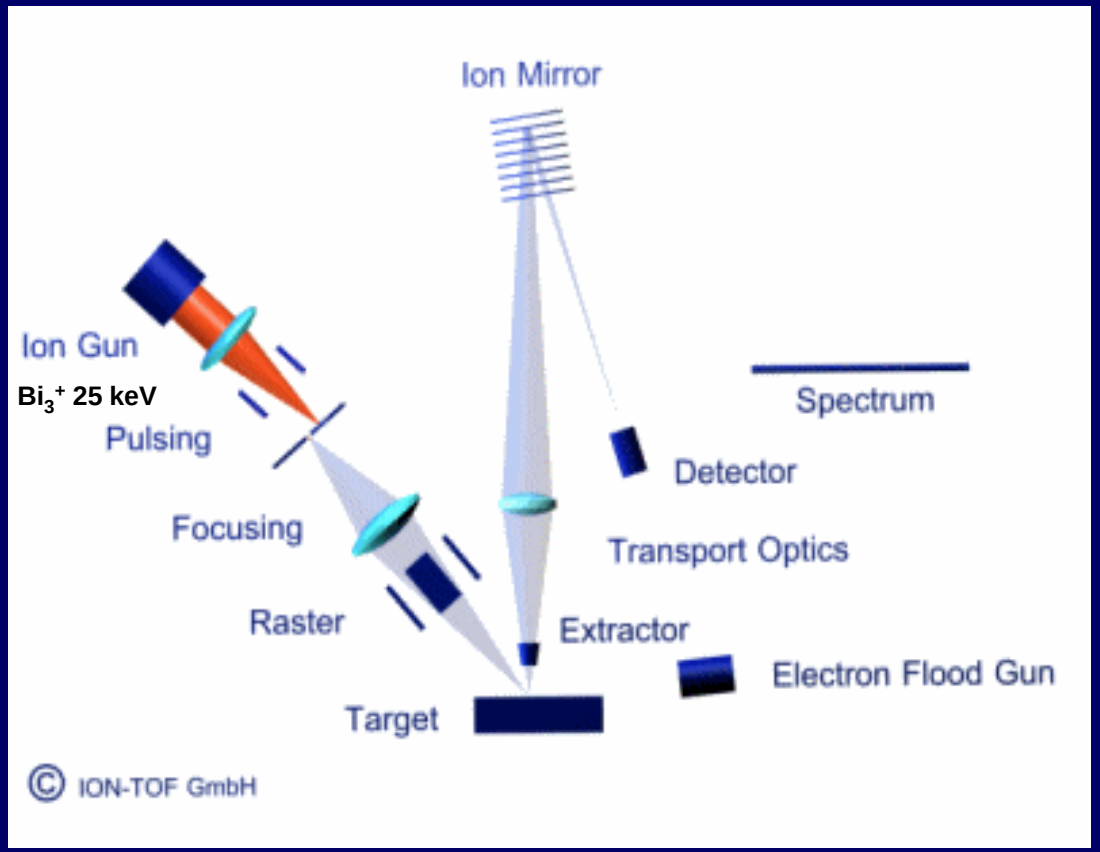
- Enrichment in esters
- Enrichment in unsaturated lipids
- Variation of the lipid environment

→ Investigating the molecular composition *in situ*

TOF-SIMS
Time of flight-secondary ion mass spectrometry



TOF-SIMS
Time of flight-secondary ion mass spectrometry

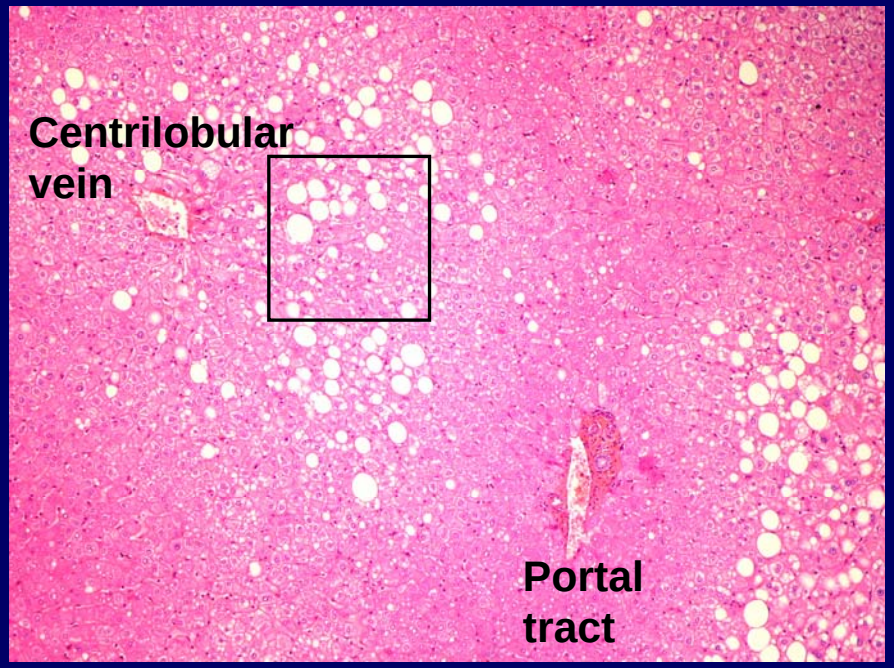


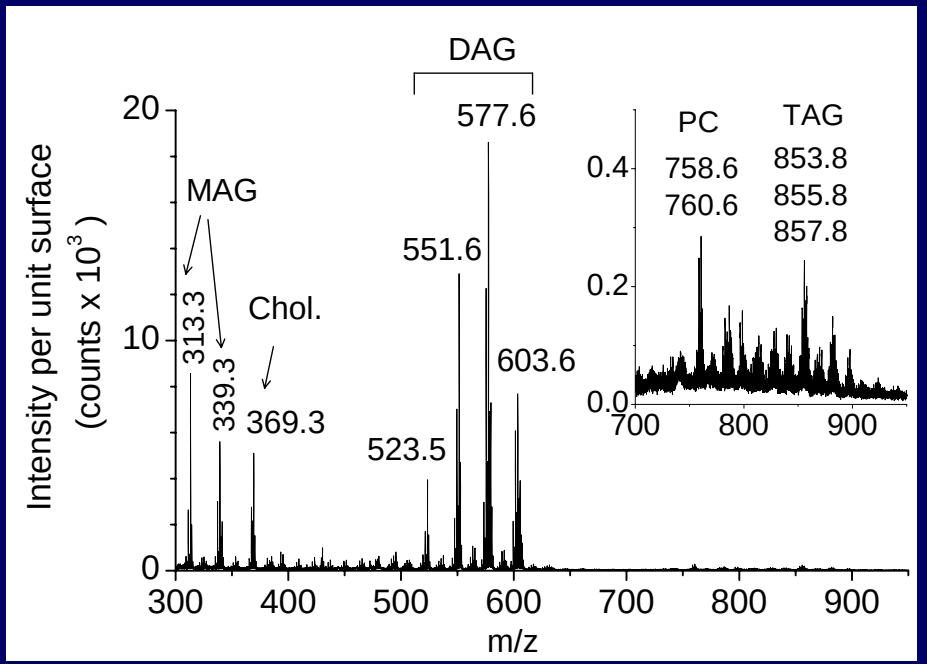
Without any treatment of the sample

Lateral resolution : 1-2µm

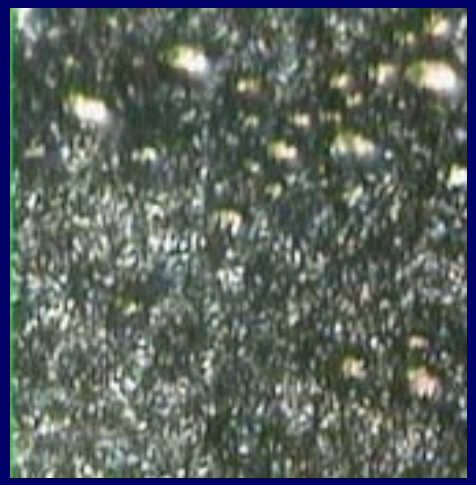
Mass <1500 Da → Lipids

Lipidomic *in situ* of steatosis using ToF-SIMS

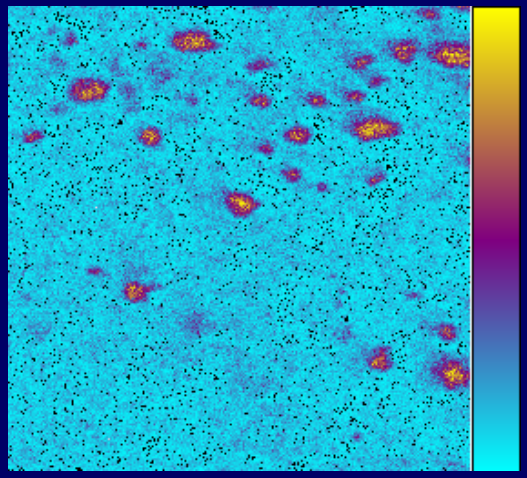




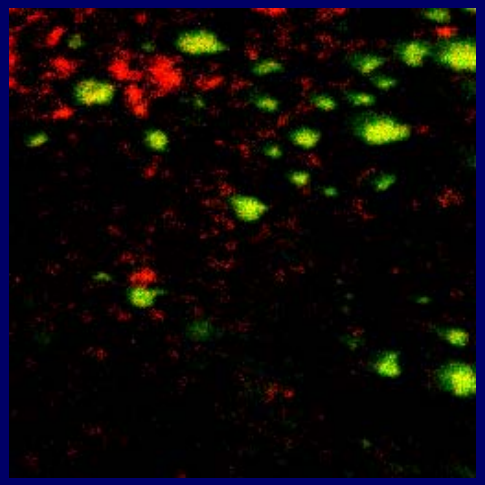
Lipidomic *in situ* of steatosis using ToF-SIMS



Optical image



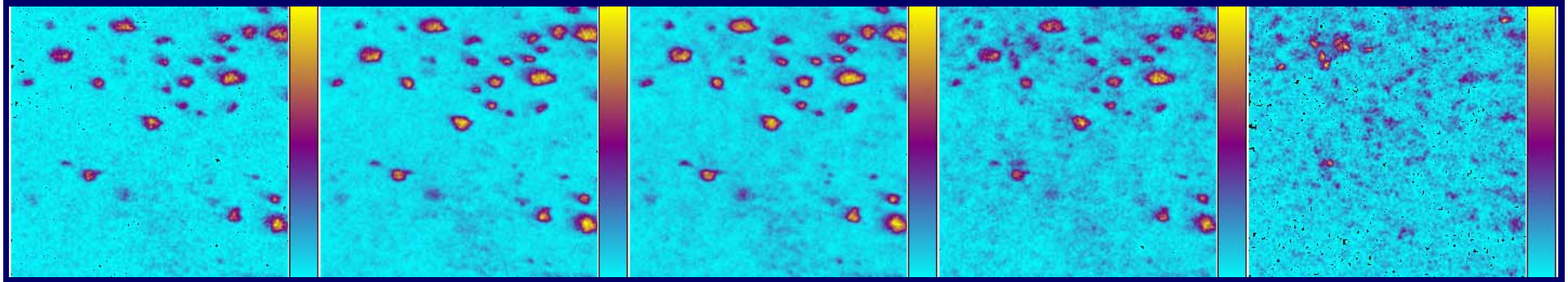
Cholesterol



Red = DAG C30
Green = DAG C36

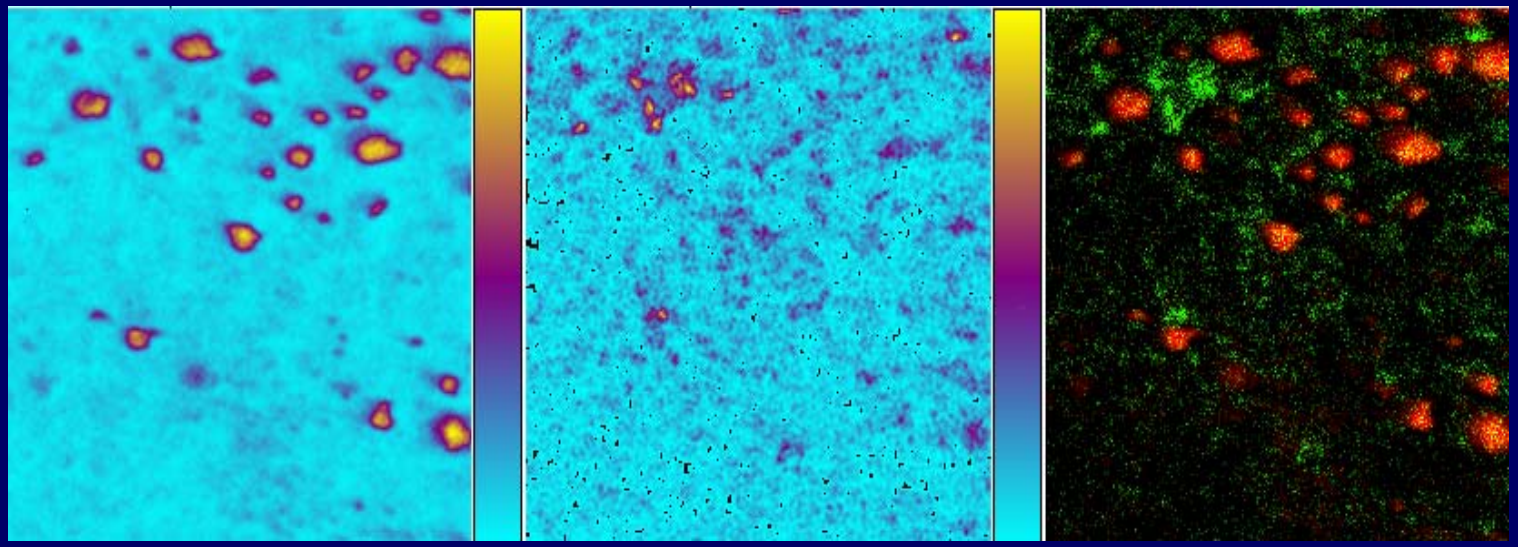
Lipidomic *in situ* of steatosis using ToF-SIMS

DAG : diacyl glycerol



C36:4 C36:3 C36:2 C36:1 C36:0

Lipidomic *in situ* of steatosis using ToF-SIMS



DAG C36
unsaturated

DAG C36
saturated

Red = Unsaturated
Green = Saturated

Conclusions

Infrared microspectroscopy and ToF-SIMS

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Composition of steatotic vesicles

- Enrichment in cholesterol
- Lipids with longer acyl chains
- Enrichment in unsaturated lipids

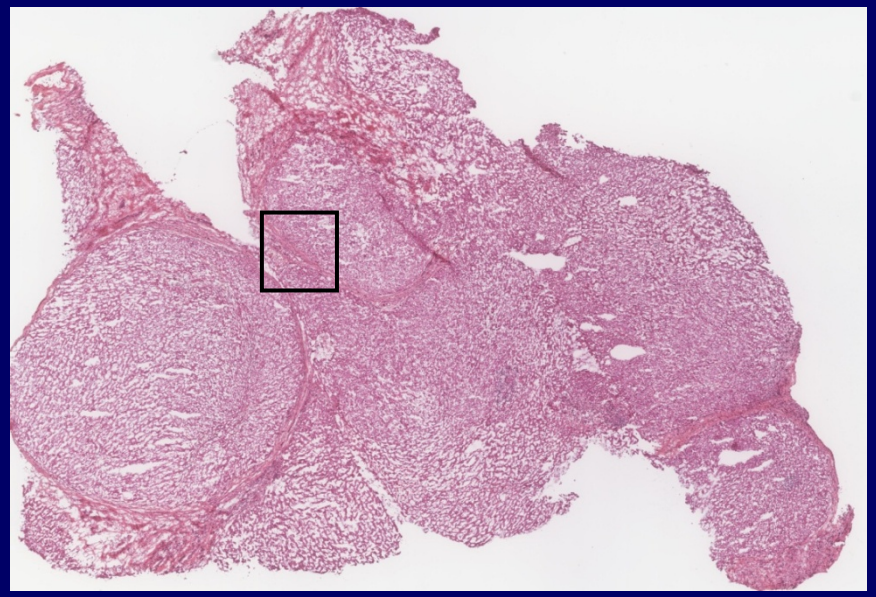
Conclusions

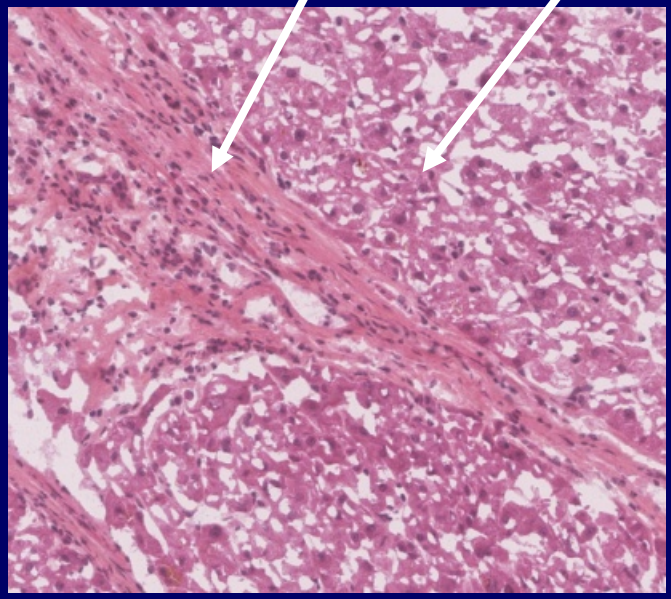
Biomedical relevance

→ The steatotic vesicle is potentially highly reactive

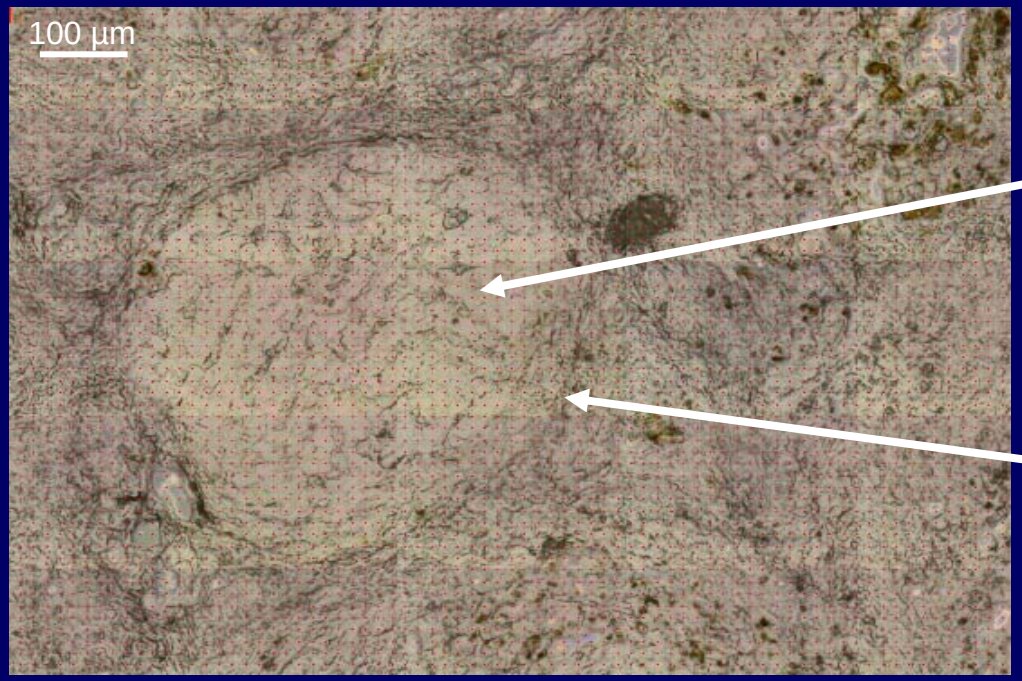
→ The mechanisms of the selective enrichment are not known

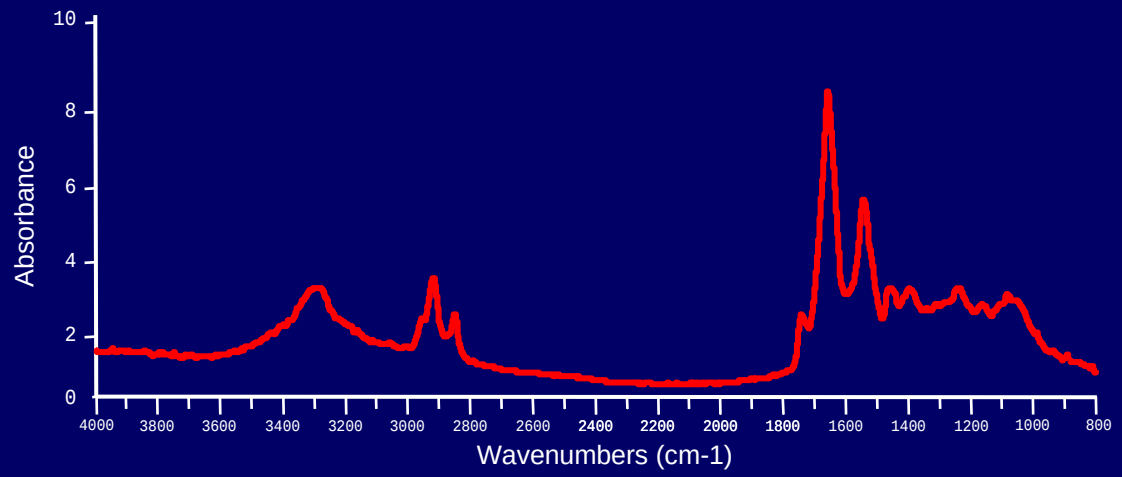
Liver cirrhosis



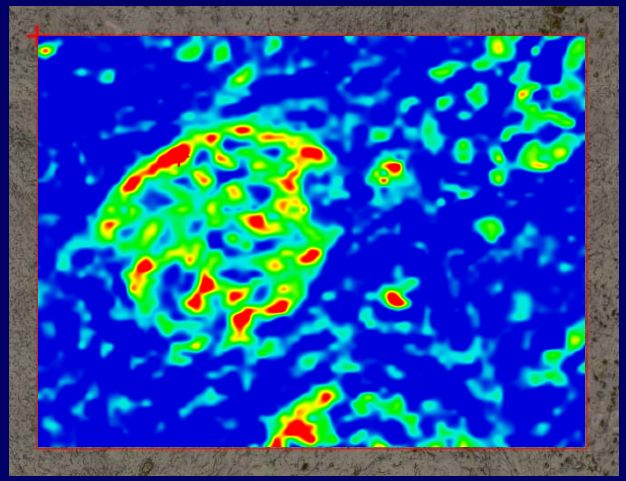


Infrared microspectroscopy on cirrhosis

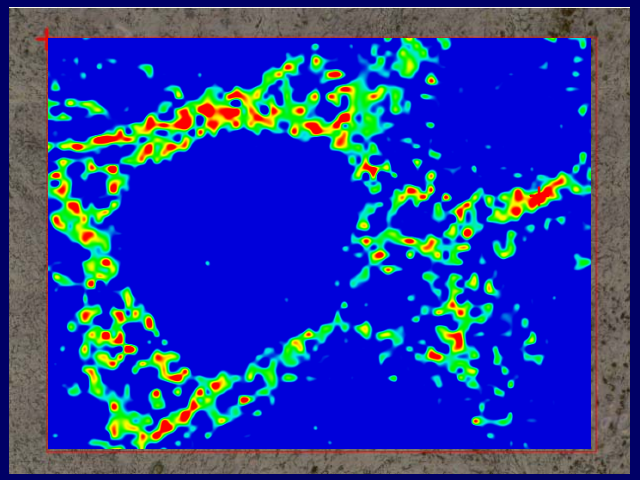




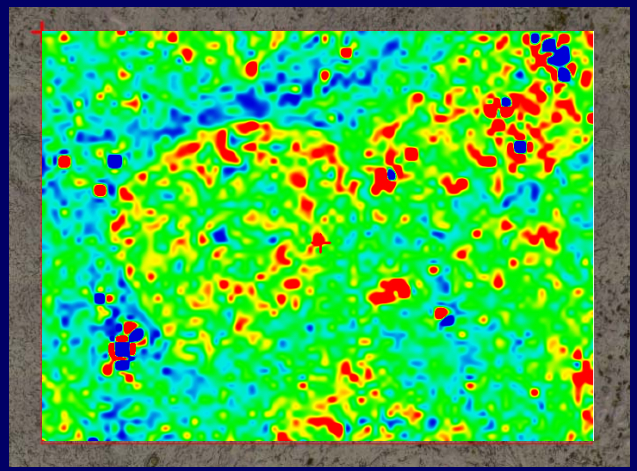
Infrared microspectroscopy on cirrhosis



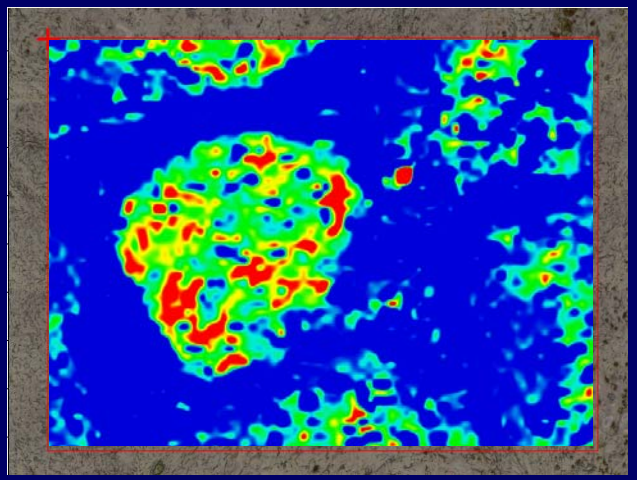
Glycogen



Collagen



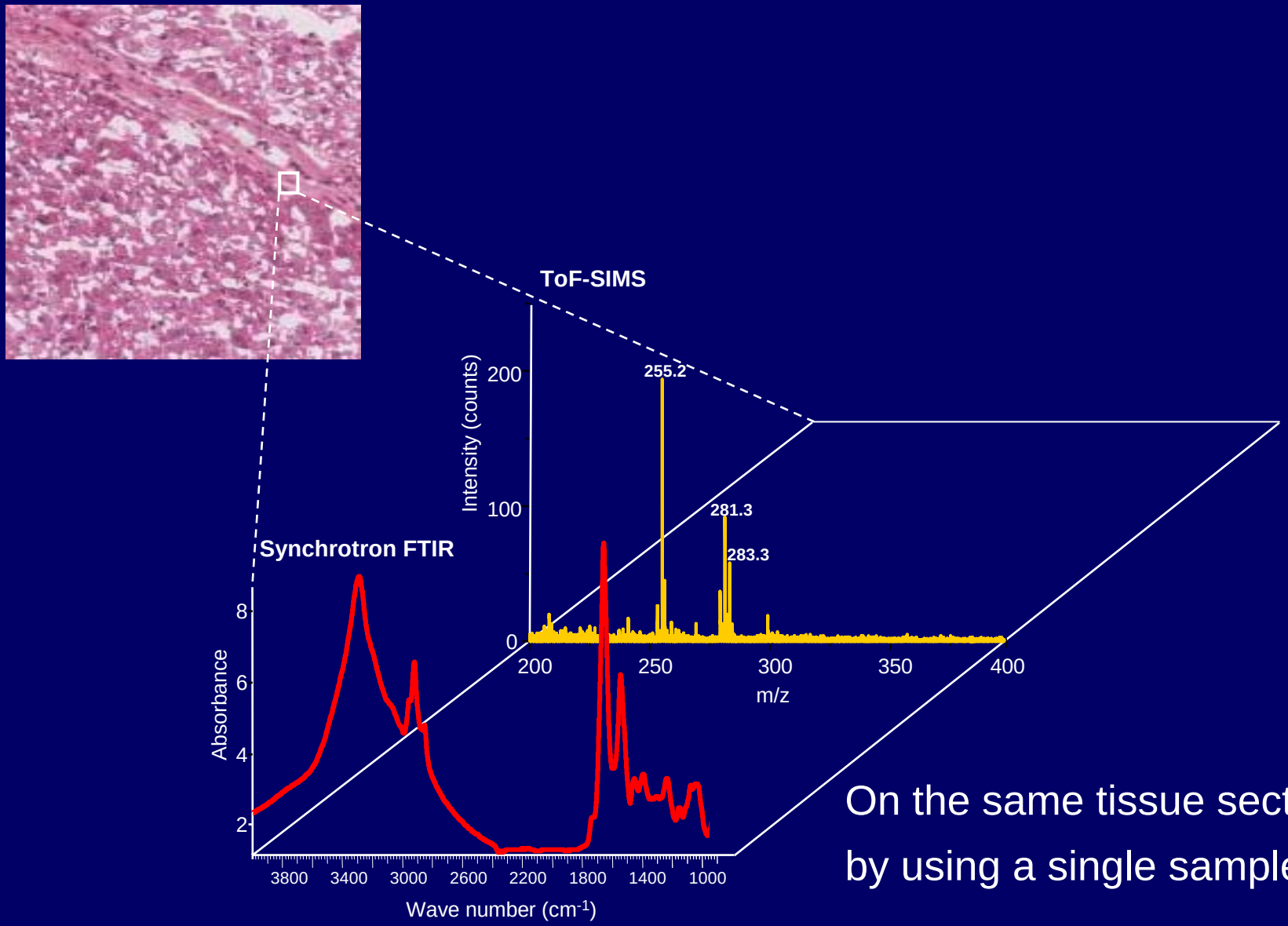
Long chain lipids



Lipid esters

Multimodal Spectroscopy

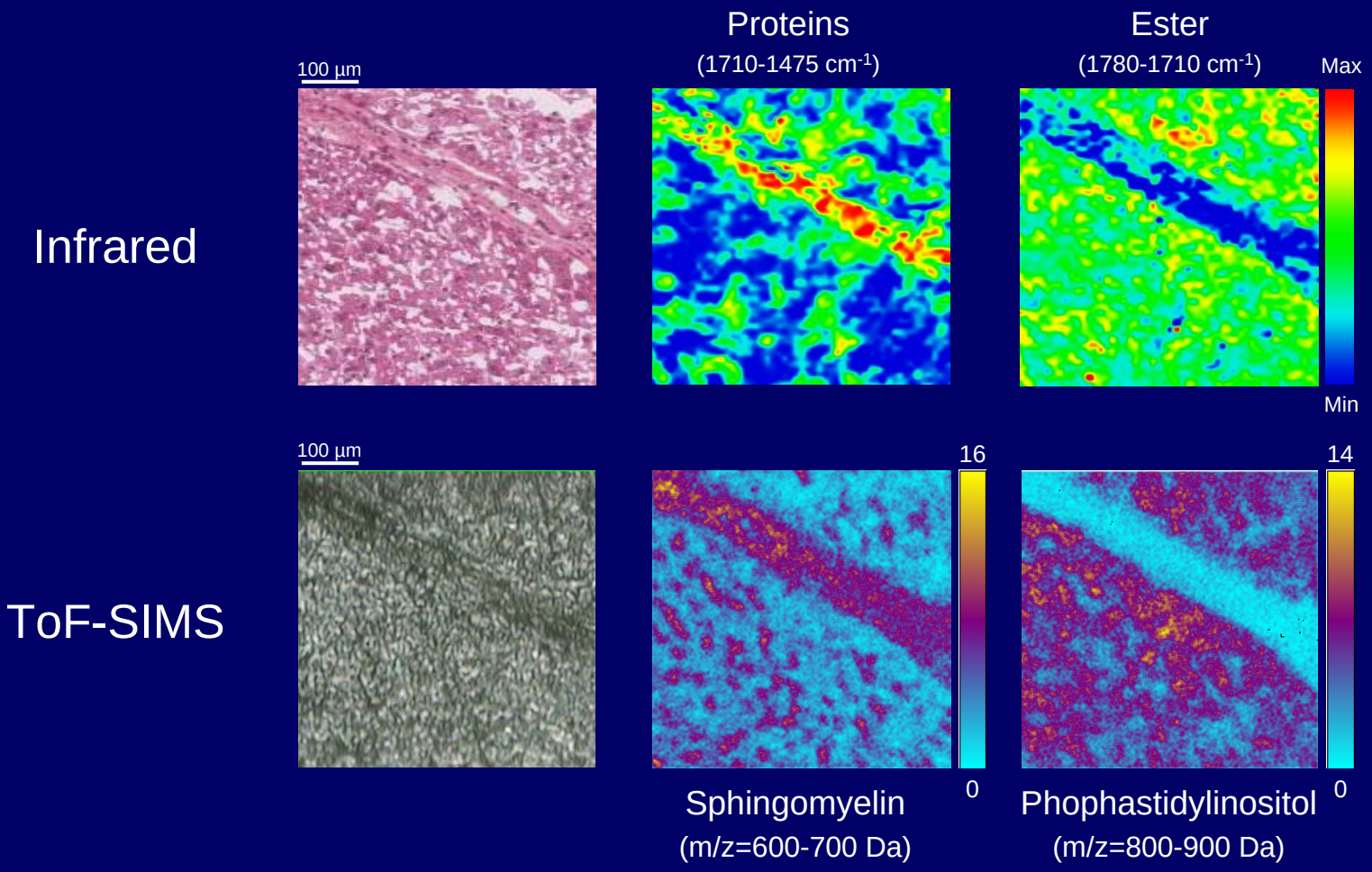
combining synchrotron-FTIR and ToF-SIMS



On the same tissue section

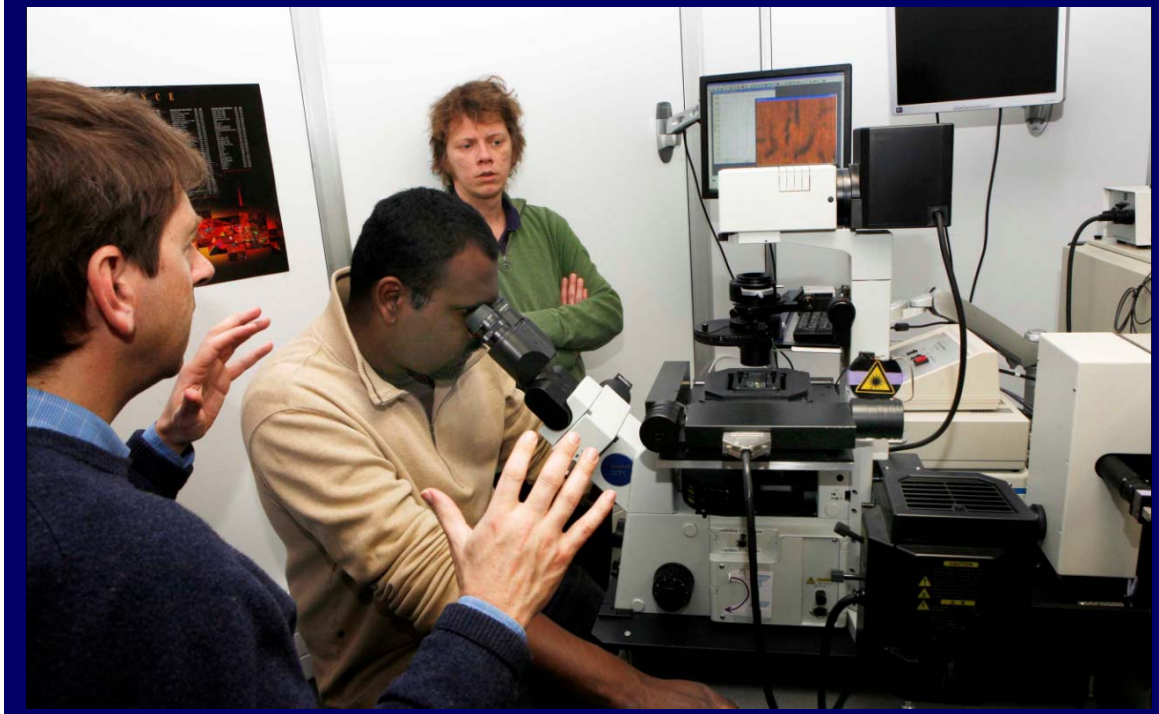
by using a single sample holder

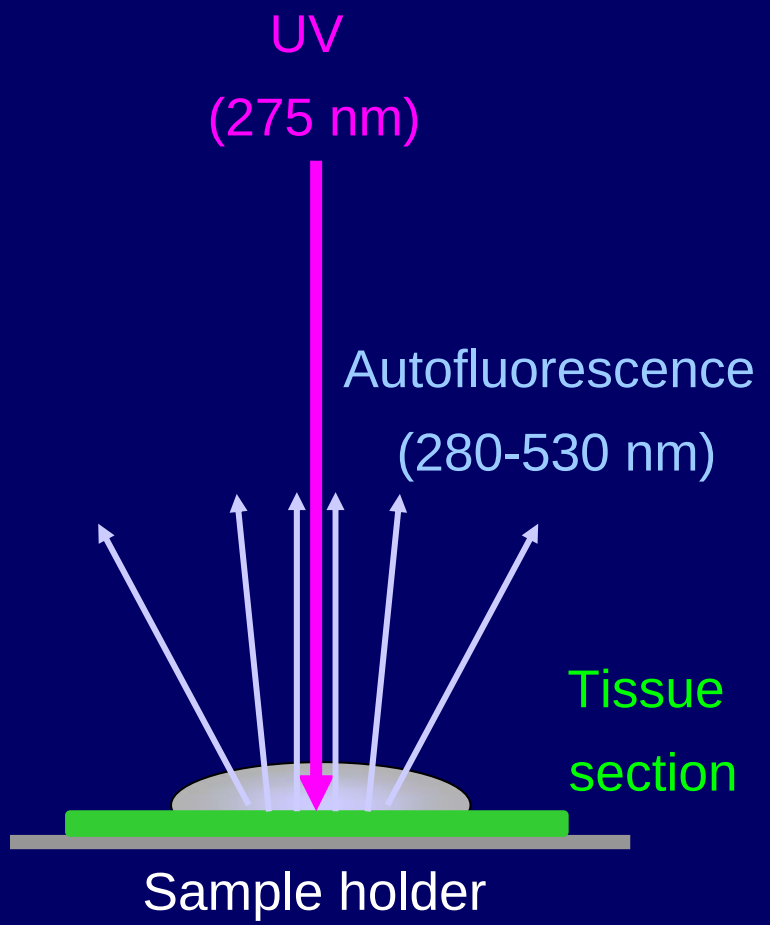
Coupling IR and ToF-SIMS microspectroscopies on the same tissue section



UV microspectroscopy
Autofluorescence of tissues

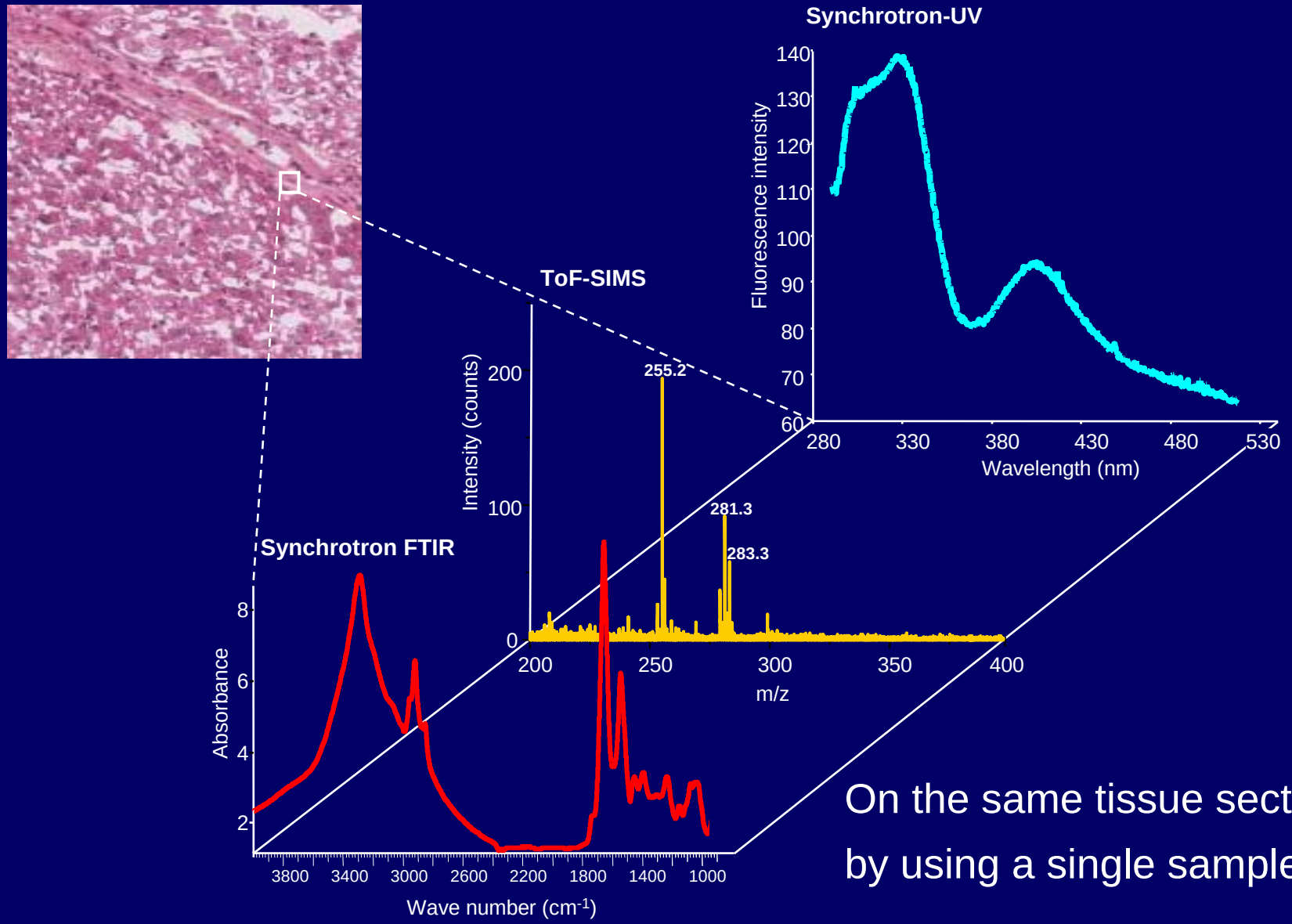
The beamline DISCO at SOLEIL





Multimodal Spectroscopy

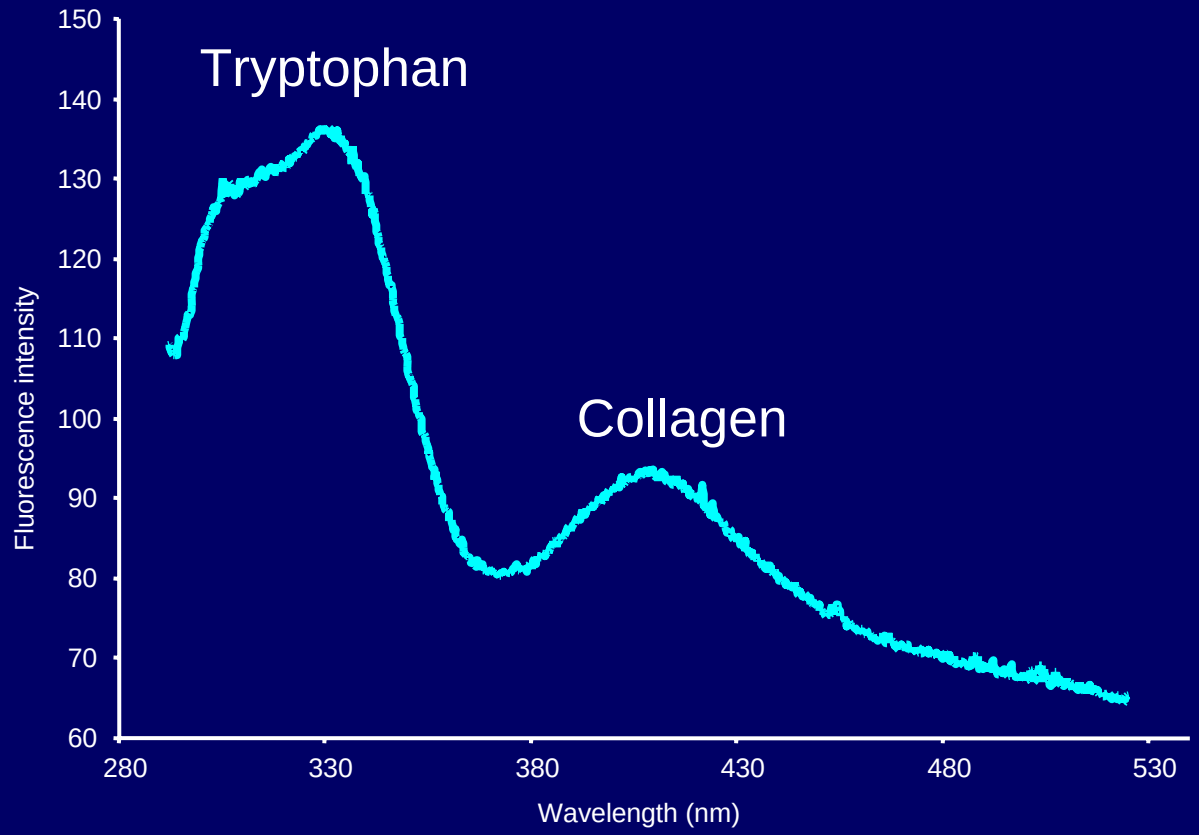
combining ToF-SIMS, synchrotron-FTIR and –UV microspectroscopies



On the same tissue section

by using a single sample holder

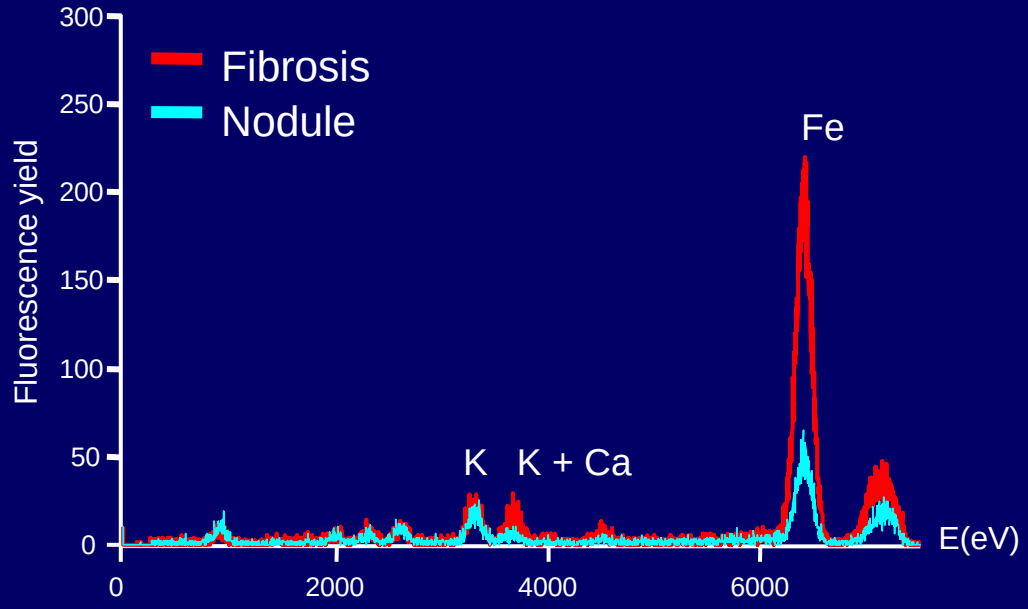
UV microspectroscopy
Autofluorescence of liver cirrhosis

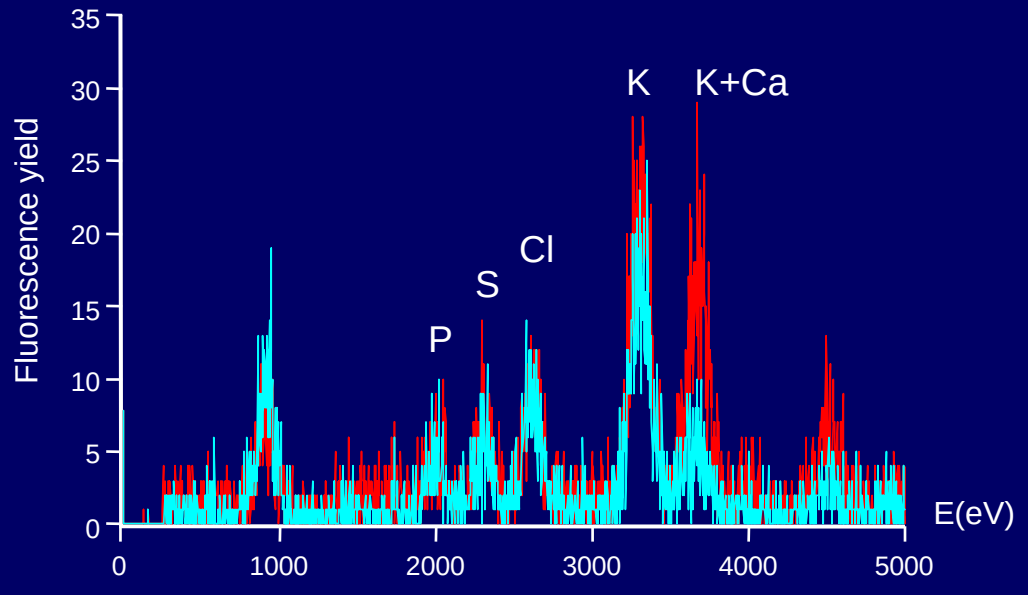


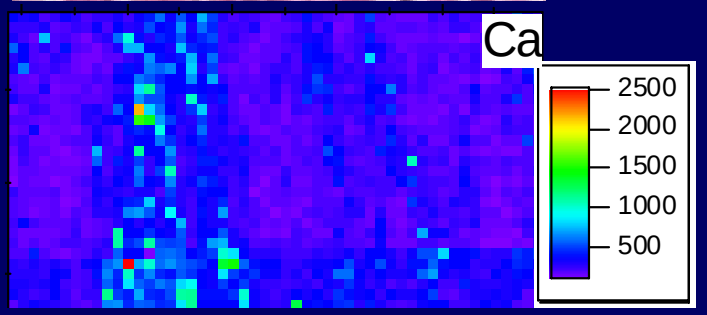
Fibrosis is enriched in collagen

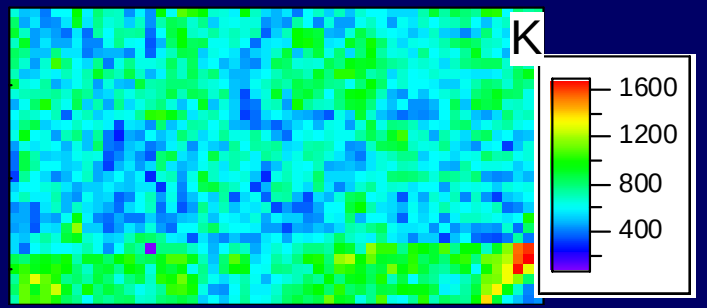
Elemental composition of cirrhosis by X-ray fluorescence

Beamline LUCIA at SOLEIL









Conclusions

Spectroscopy	*Composition*	*Fibrosis*
IR	**chemical**	**proteins**
ToF-SIMS	**lipids**	**sphingomyelin**
UV	**autofluorescence**	**collagen**
X-ray	**elemental**	**Ca**

Multimodal spectroscopy allows a complete characterization of the composition of a tissue

Investigating early stages of cirrhosis on clinical series

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