Carrefour Pathologie 2017
Palais des Congrès de Paris
23 novembre 2017

Imagerie multimodale *in situ* des lipides et métaux sur coupes tissulaires de foie

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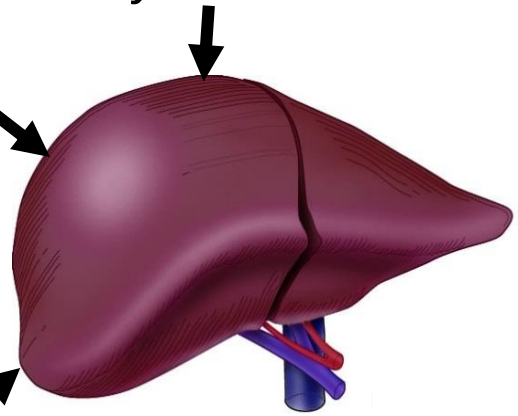
Inserm UMR-S 1193, Hôpital Paul Brousse, Villejuif

Chronic liver diseases and cancer

Dysmetabolic syndrome

Virus
HBV, HCV



Alcohol

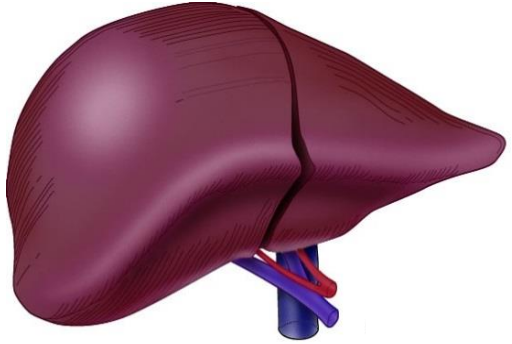
Normal liver

Chronic liver disease

Cirrhosis &
Hepatocellular carcinoma

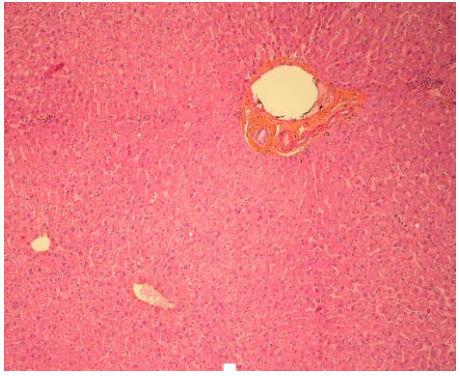
A multimodal spectroscopy-based approach

Liver



Cryomicrotome

Tissue section



The synchrotron SOLEIL

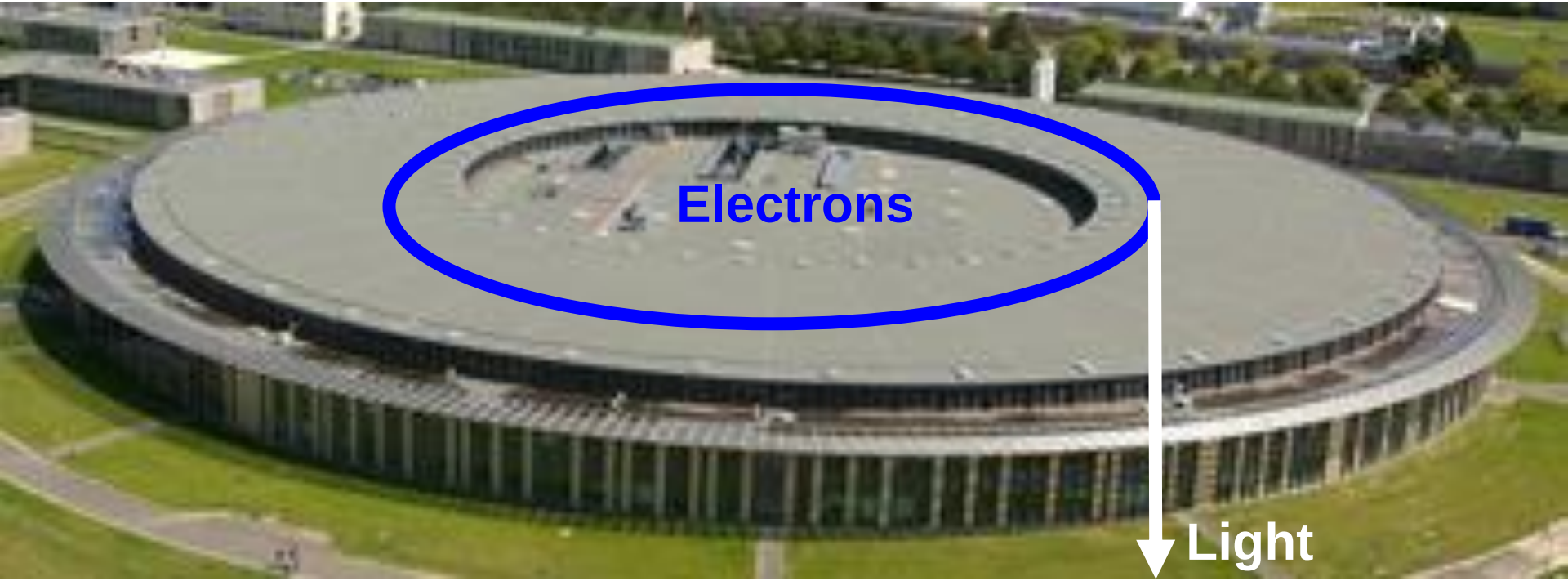
Brillance

Accordability

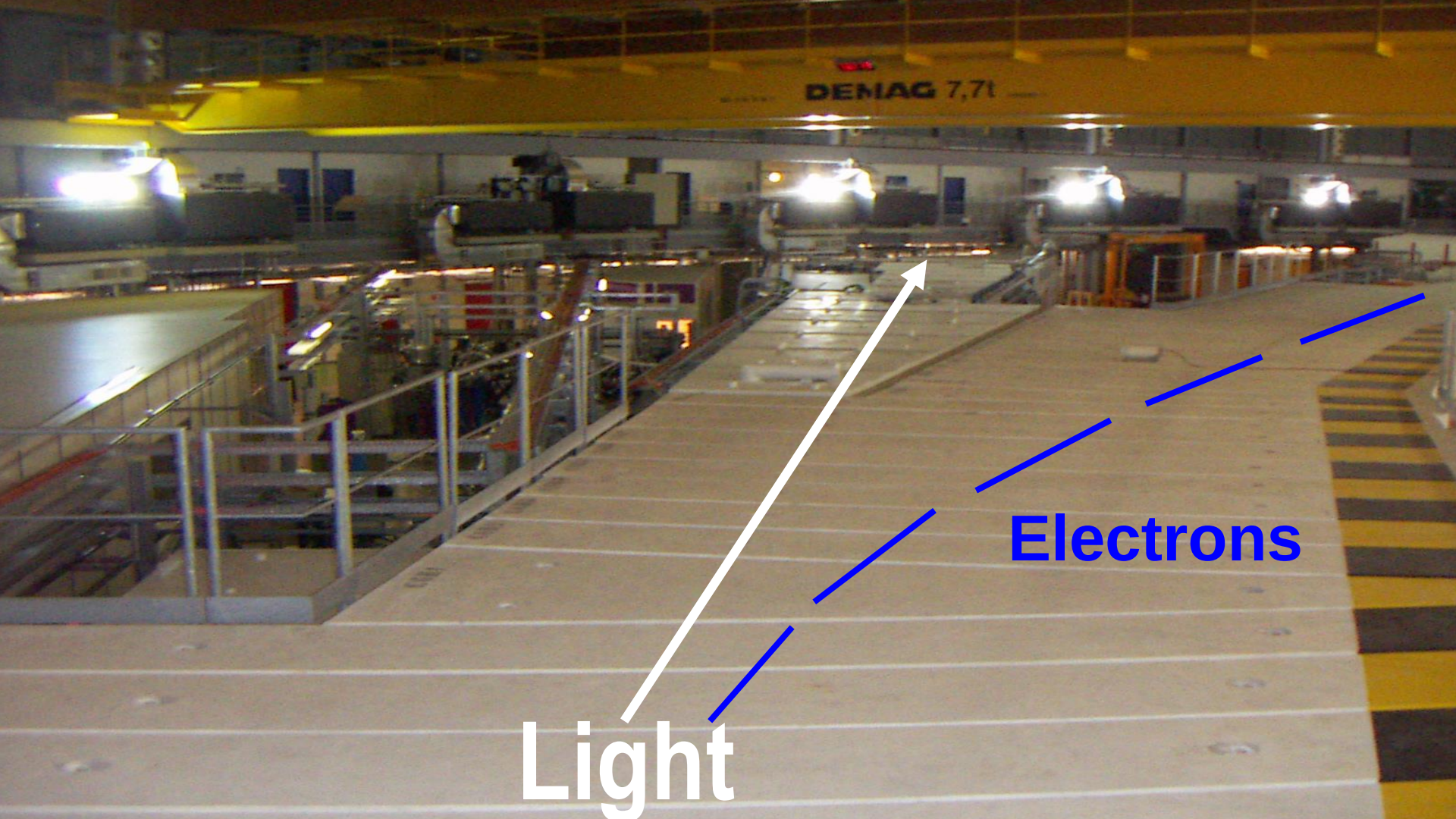
IR UV X

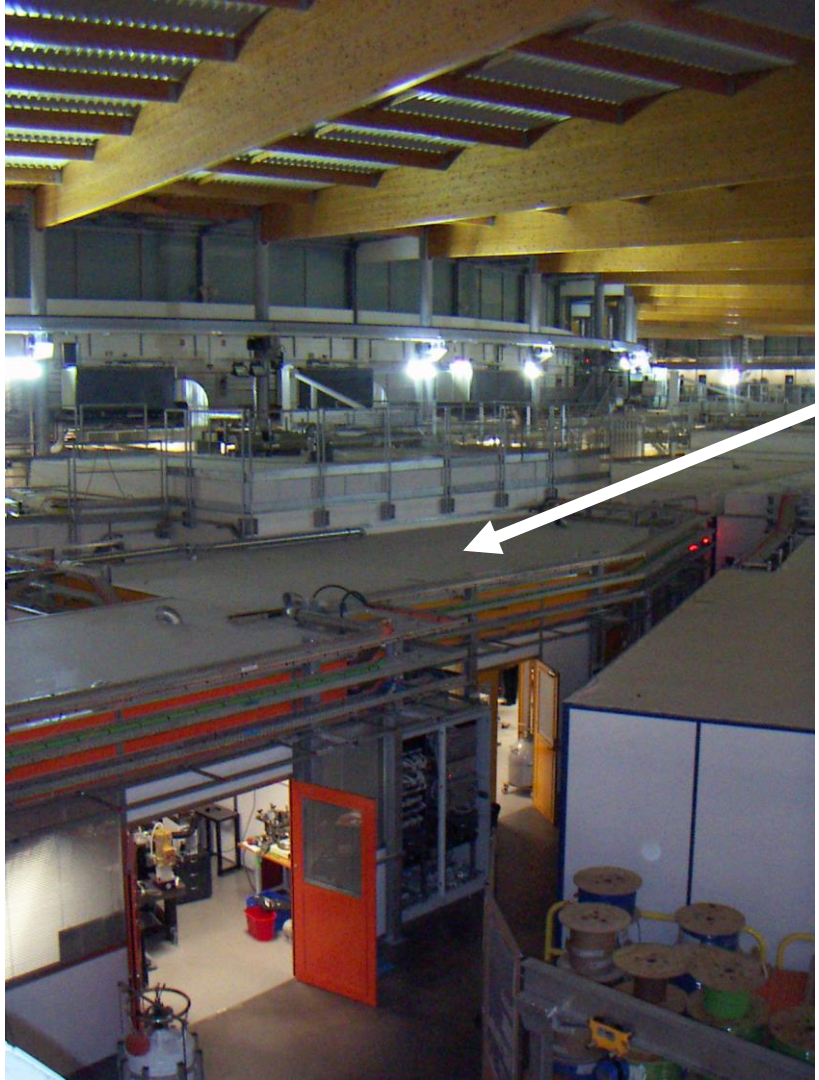
Microspectroscopy

The synchrotron SOLEIL



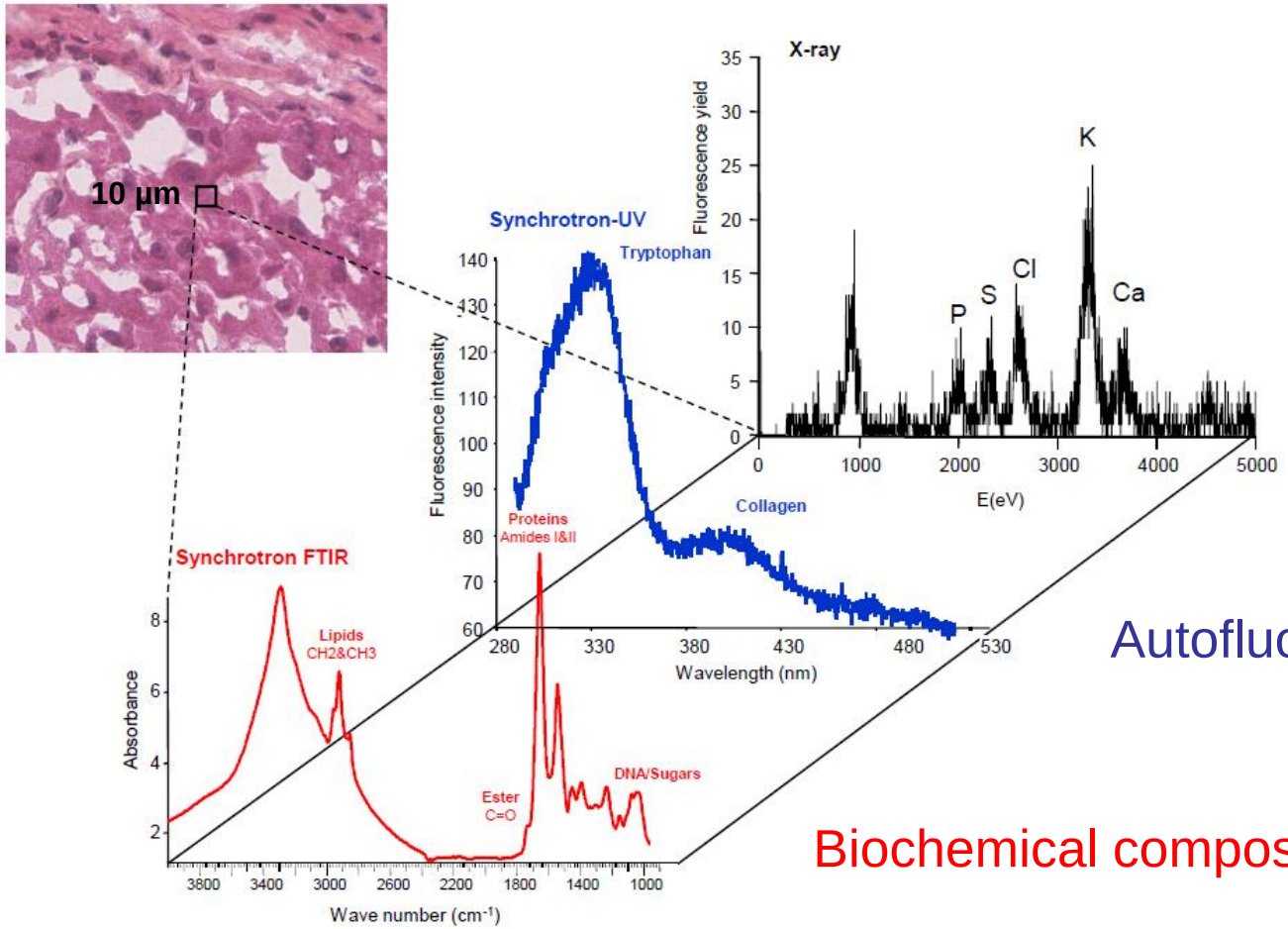
IR UV X


DEMAG 7,7t
Electrons
Light



Light

Multimodal spectroscopy



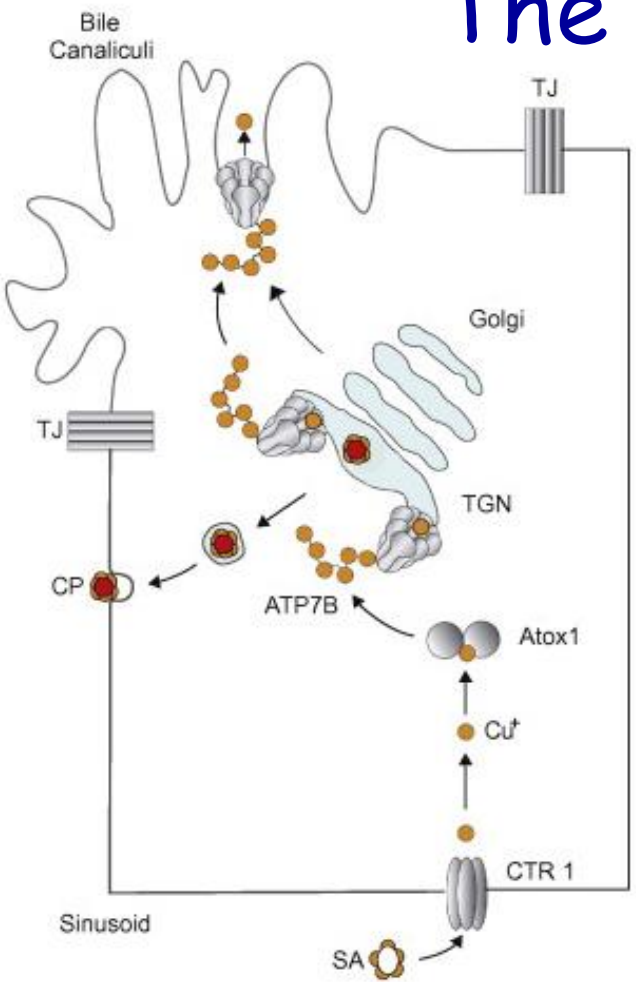
Elemental composition

Autofluorescence

Biochemical composition

Diagnosis of Wilson disease using X-ray fluorescence

The Wilson's disease



ATP7B is a translocase involved in Cu excretion

Wilson's disease

Mutations in ATP7B → Accumulation of Cu

Rare genetic disease (1/30 000)

The Wilson's disease

Clinical symptoms

Hepatic symptoms (45%)
Chronic hepatitis / cirrhosis

Haemolytic anaemia

Neurologic symptoms (35%)

Psychiatric troubles (10%)

<u>False positive</u>
Autoimmune hepatitis
Sever hepatic insuffiency
Familial aceruloplasminaemia

Diagnosis

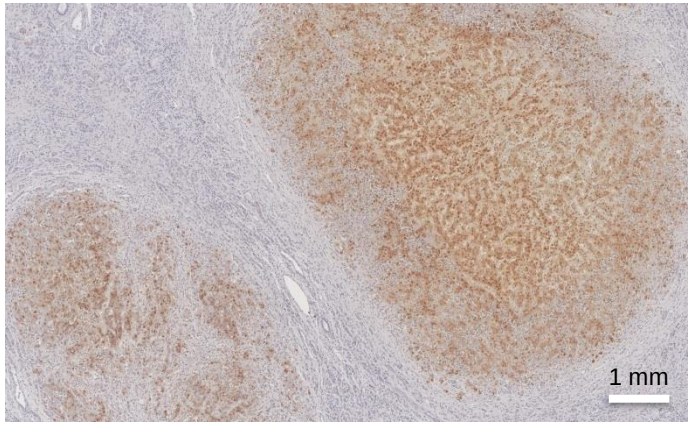
Numerous mutations (>500) on ATP7B
(long and expensive)

Kayser-Fleischer ring (50%)

Histological examination
(rhodanine staining)

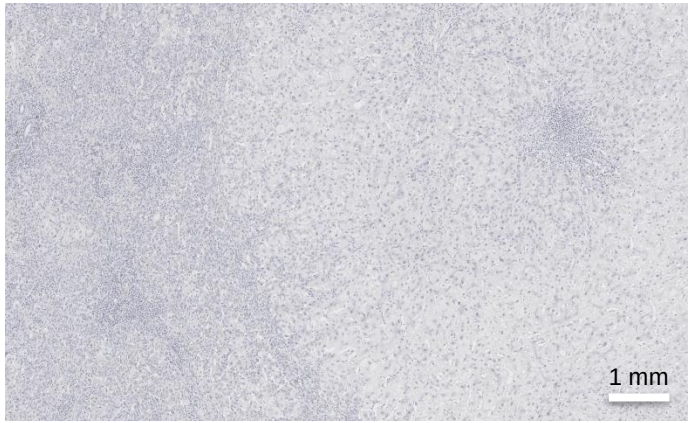
Diagnosis of the Wilson's disease

Patient #55
Wilson's disease



Rhodanine staining
positive

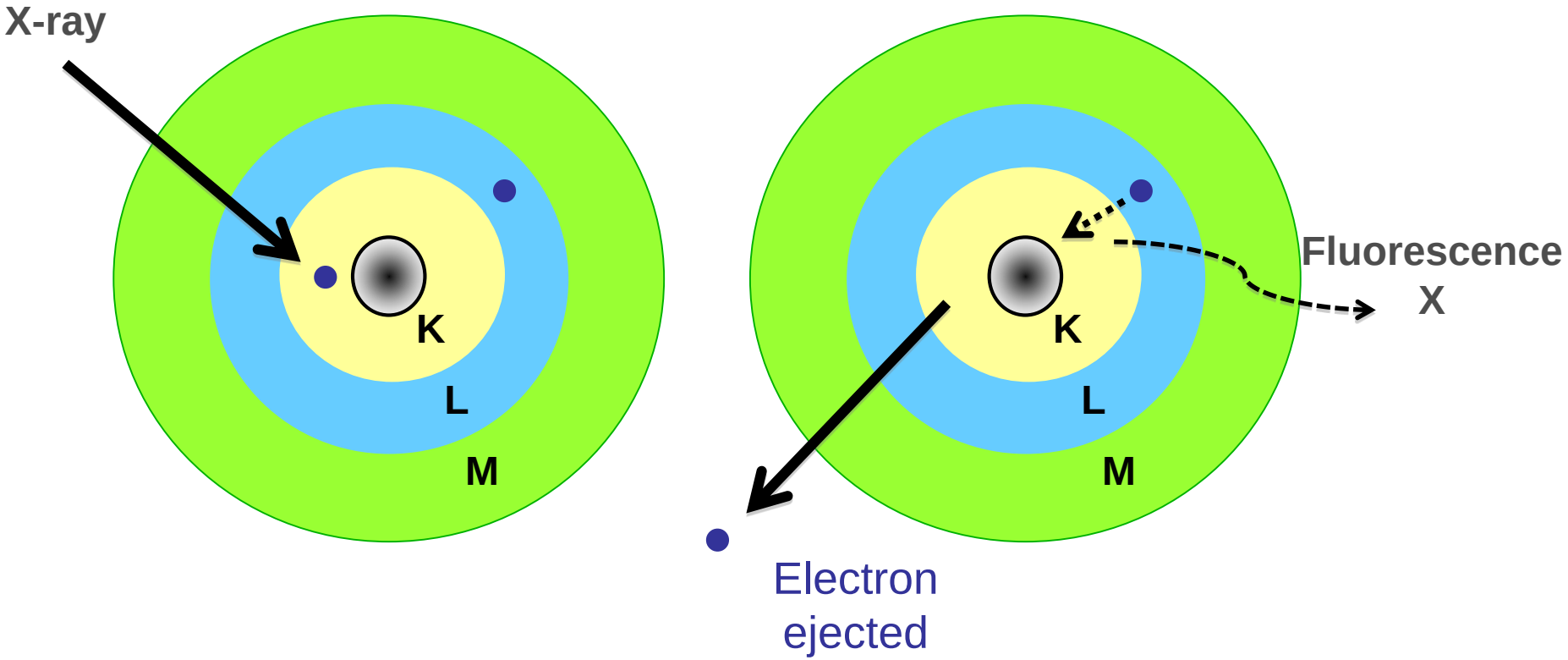
Patient #53
Wilson's disease



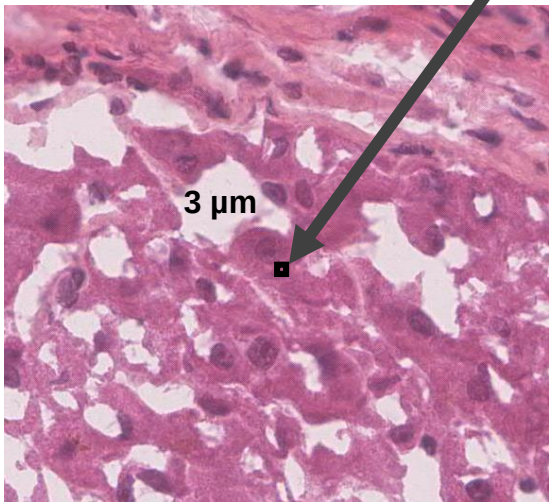
Rhodanine staining
negative

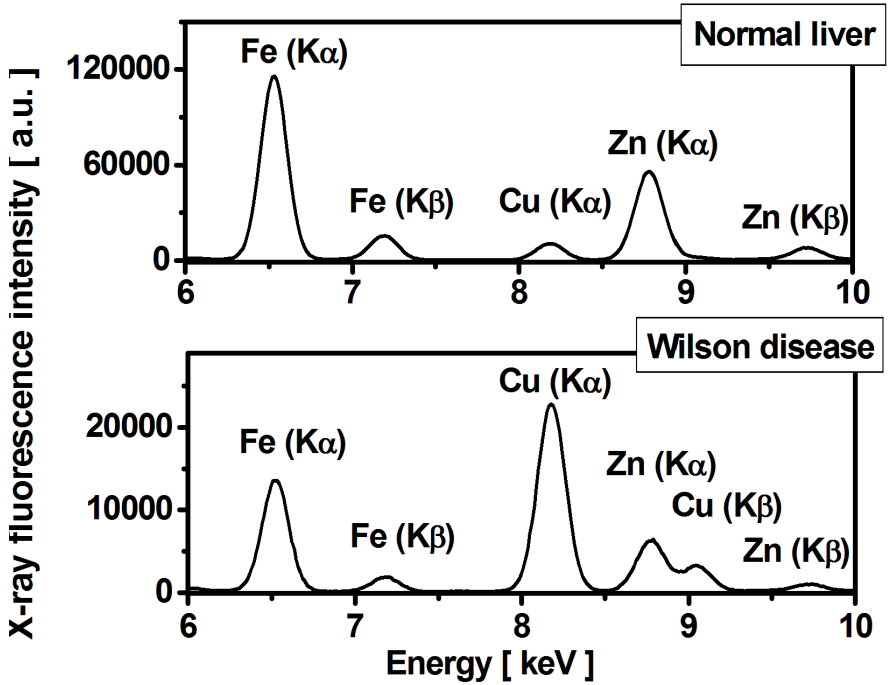
FALSE NEGATIVE

X-ray fluorescence

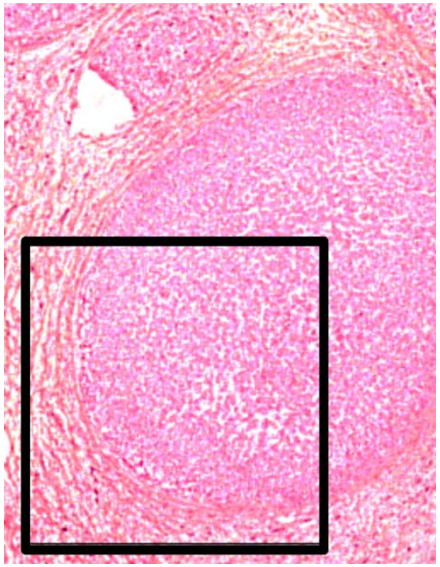


X-ray fluorescence on Wilson's disease

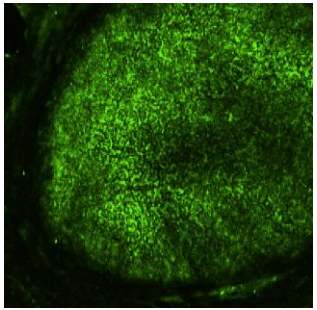




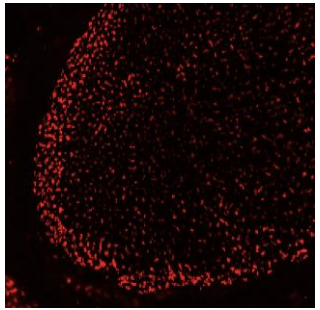
X-ray fluorescence on Wilson's disease



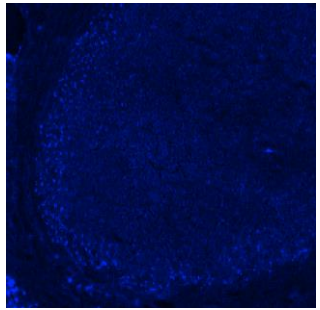
Fe



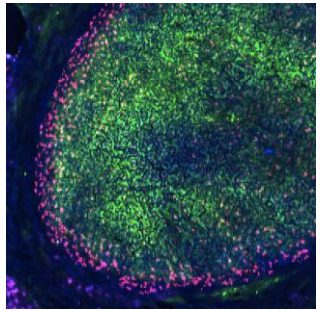
Cu



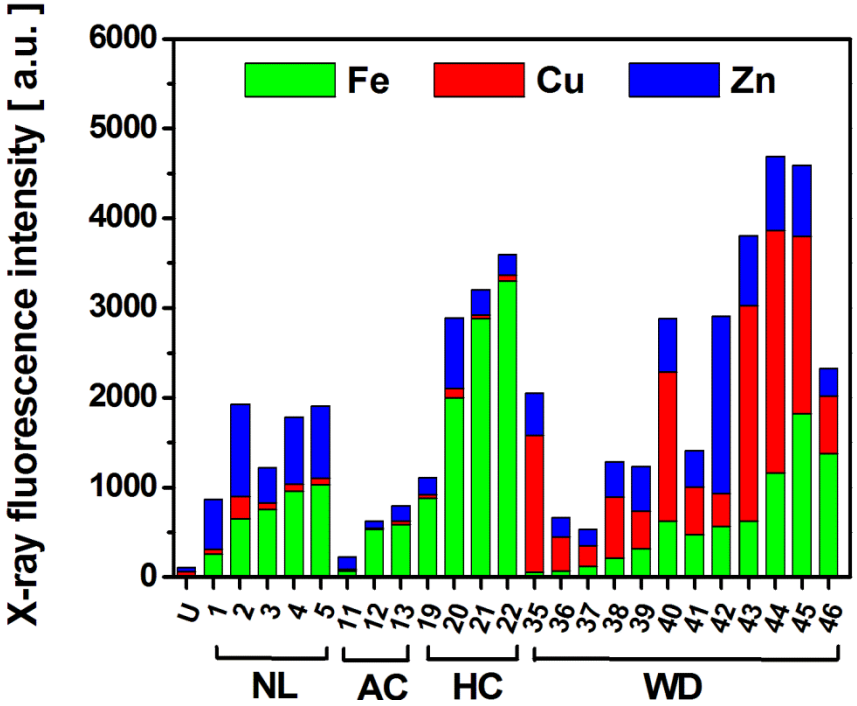
Zn

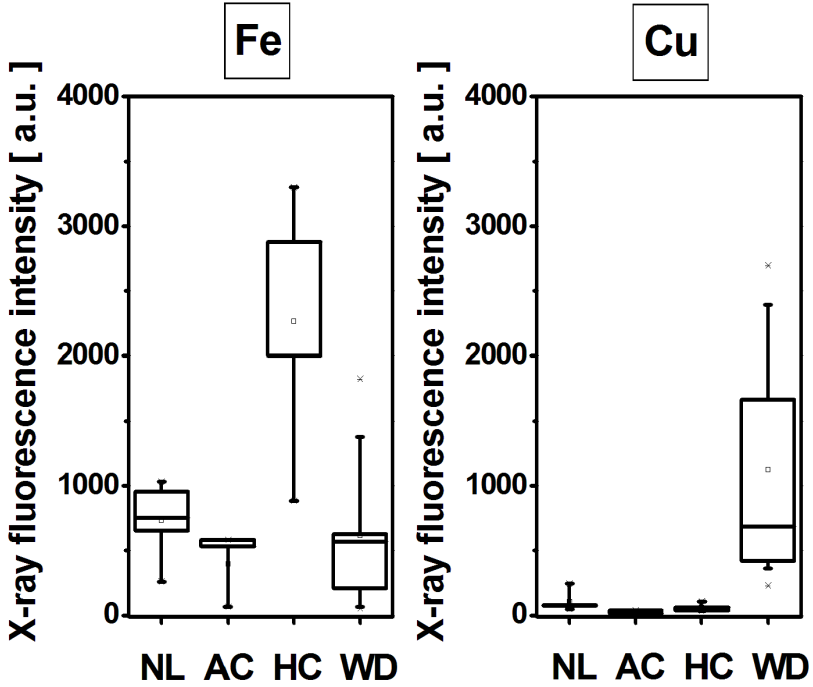


Merge



Diagnosis of Wilson's disease on tissue sections using X-ray fluorescence



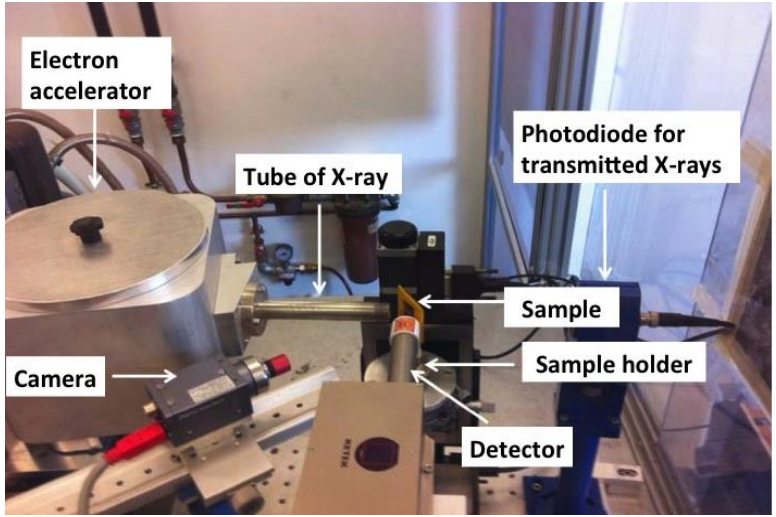


Diagnosis in 2h

NL: normal liver AC: alcoholic cirrhosis HC: hemochromatosis WD: Wilson's disease

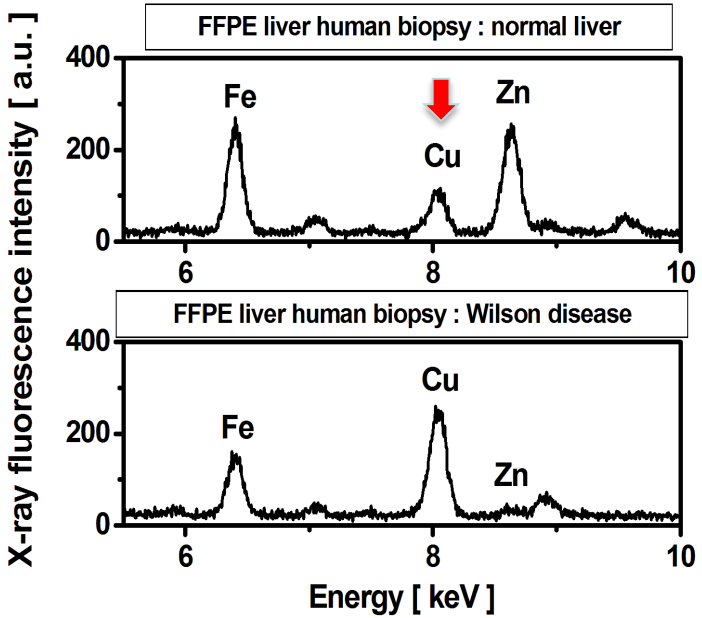
From synchrotron to the hospital

Laboratory X-ray source



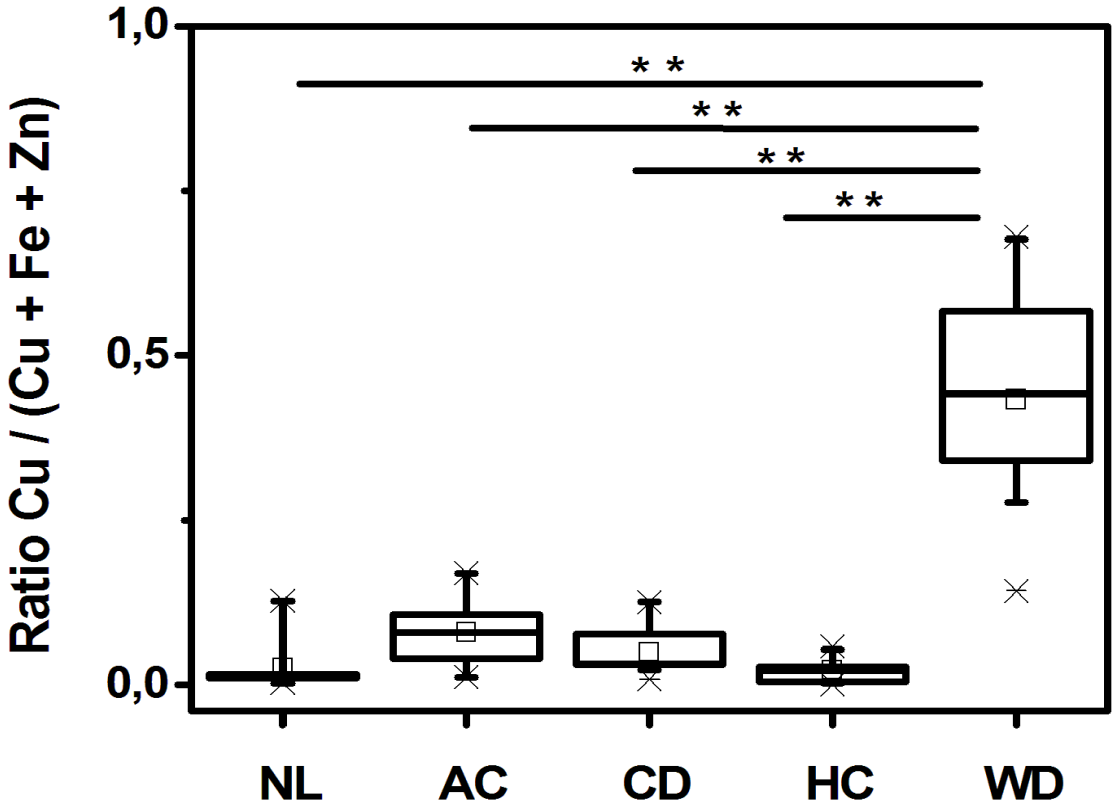
Needle biopsy (FFPE)

Beam size : Ø 150 µm



Diagnosis in 30 min

Diagnosis of Wilson's disease on needle biopsies using X-ray fluorescence



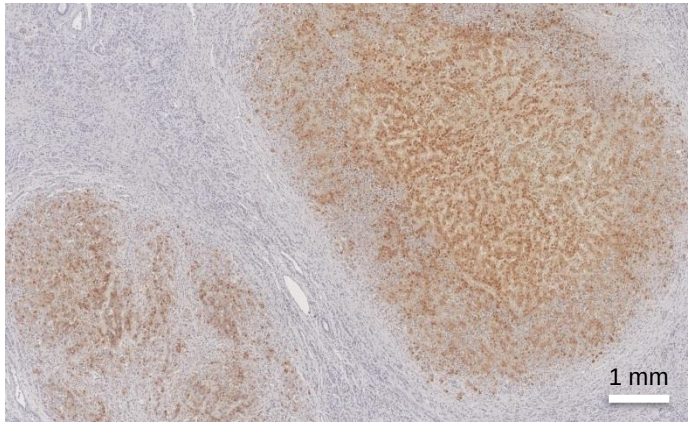
Specificity 97.6%

Sensitivity 100%

NL: normal liver
AC: alcoholic cirrhosis
CD: cholestase disease
HC: hemochromatosis
WD: Wilson's disease

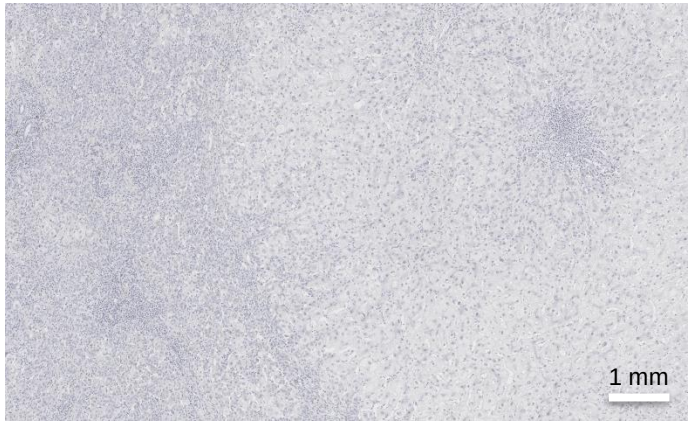
Diagnosis of the Wilson's disease

Patient #55
Wilson's disease



Rhodanine staining
positive

Patient #53
Wilson's disease

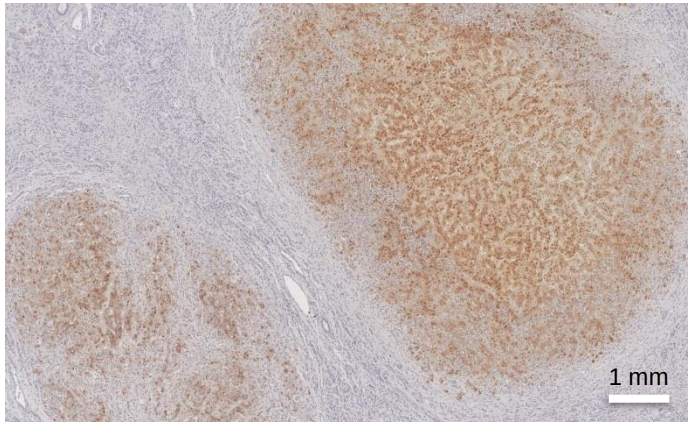


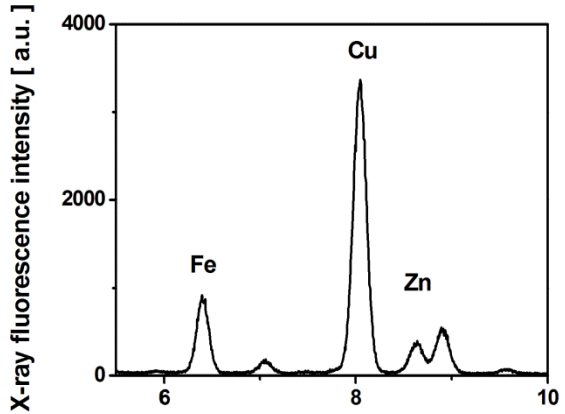
Rhodanine staining
negative

FALSE NEGATIVE

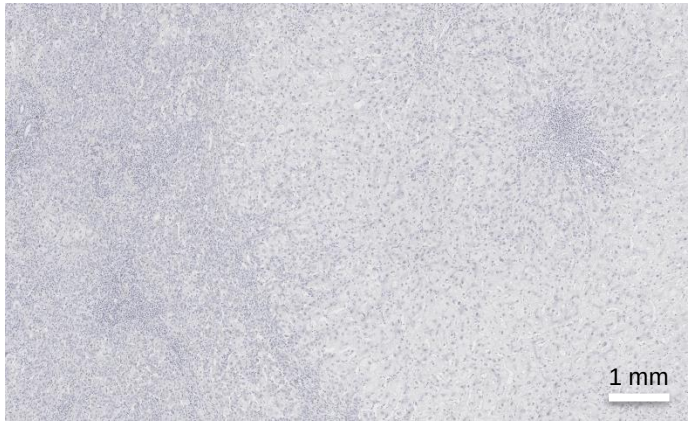
Diagnosis of the Wilson's disease

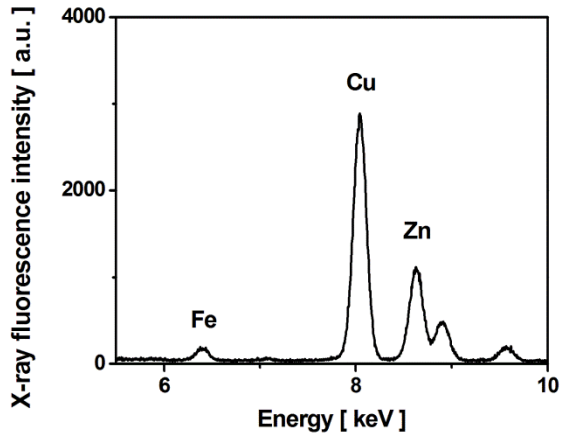
Patient #55
Wilson's disease





Patient #53
Wilson's disease





Conclusion

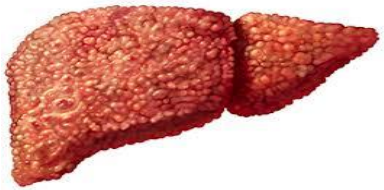
- Versatile (compatible with fresh, frozen or parrafin embedded tissues)
- Compatible with tissue sections and needle biopsies
- No staining & no specific preparation
- Non destructive
- Quantitative
- Rapidity: diagnosis in 30 min!

→ Rapid and reliable diagnosis of Wilson disease using X-ray fluorescence

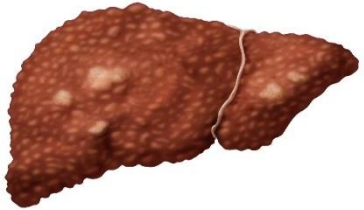
Chronic liver diseases and cancer

Normal liver

Cirrhosis



Hepatocellular Carcinoma



Alcohol

Dysmetabolic syndrome
(diabete, obesity)

Viral Hepatitis

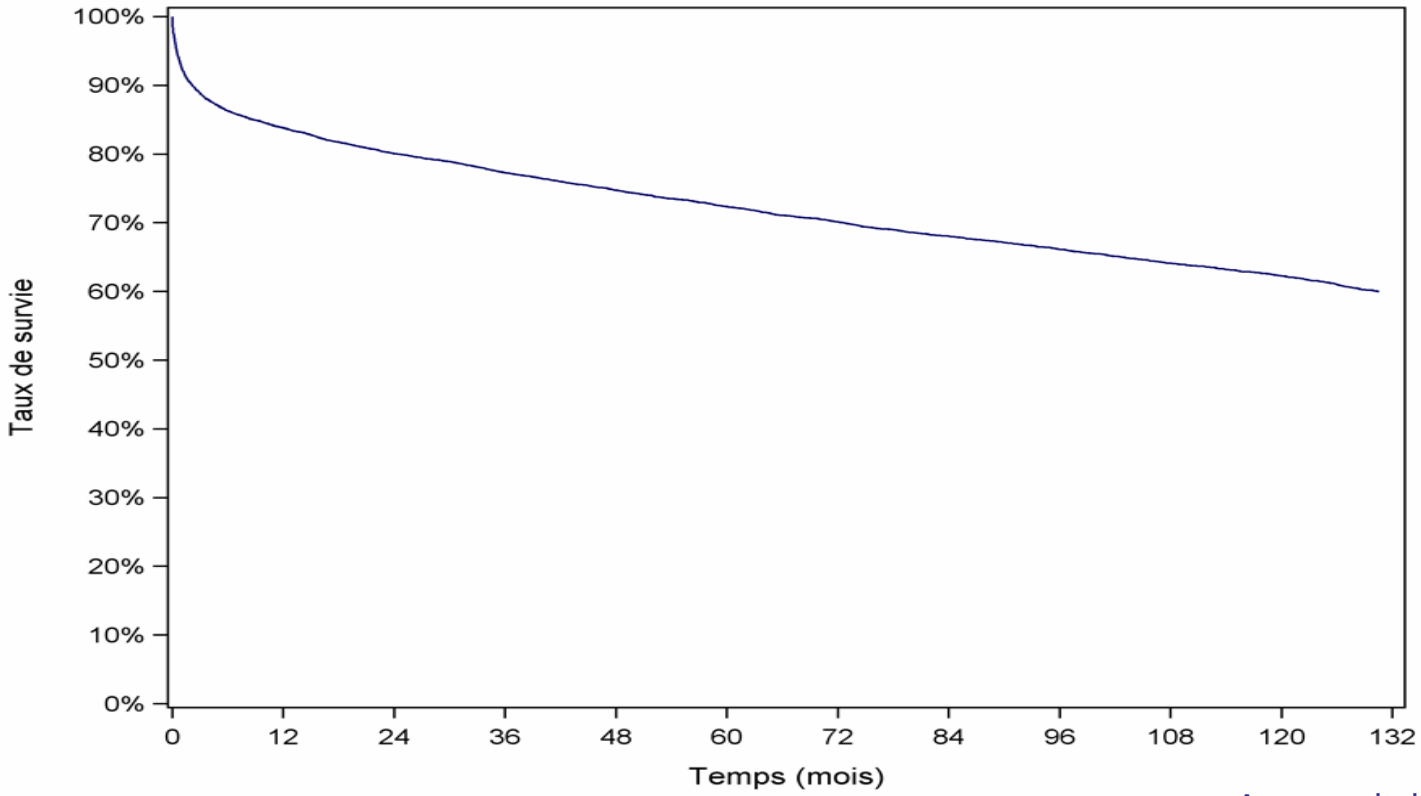
6th cause of cancer worldwide

2nd cause of death by cancer

Poor prognosis (survival < 1 year)

TRANSPLANTATION

Liver transplantation : a major treatment for liver diseases



Agence de la Biomédecine

Limitation of liver transplantation
Shortage of grafts

Year	Transplantation	Waiting patients
2005	1024	474
2006	1037	486
2007	1061	540
2008	1011	574
2009	1047	669
2010	1092	806
2011	1164	932
2012	1161	941

Agence de la Biomédecine

Limitation of liver transplantation
Shortage of grafts

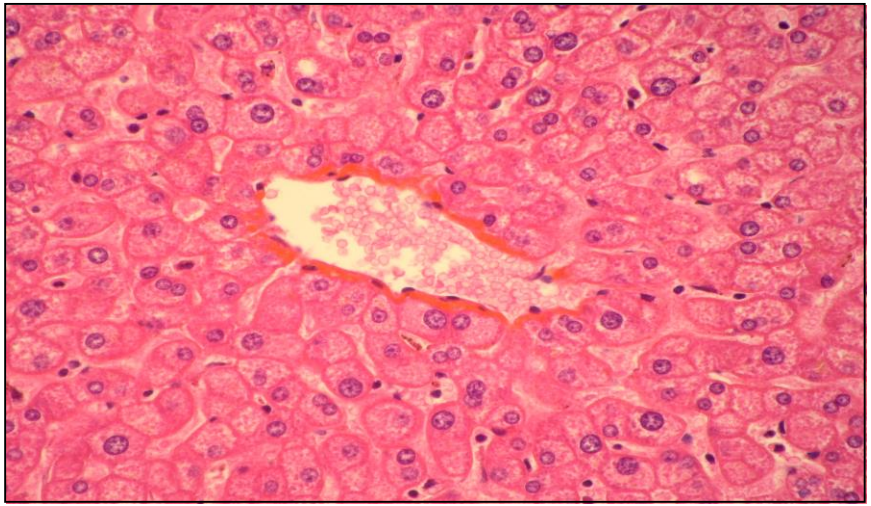
- Additional grafts: living donors, graft splitting, domino program, use of marginal grafts, non-heart-beating donors

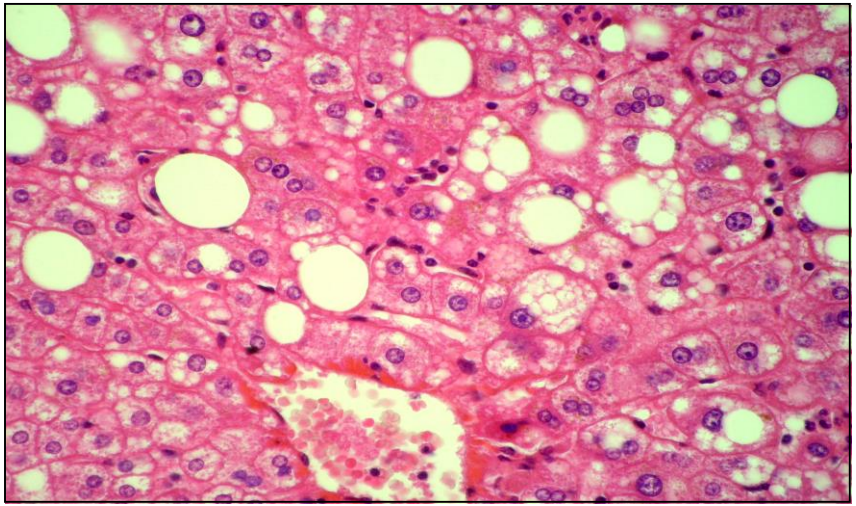
Additional grafts BUT increased risk…

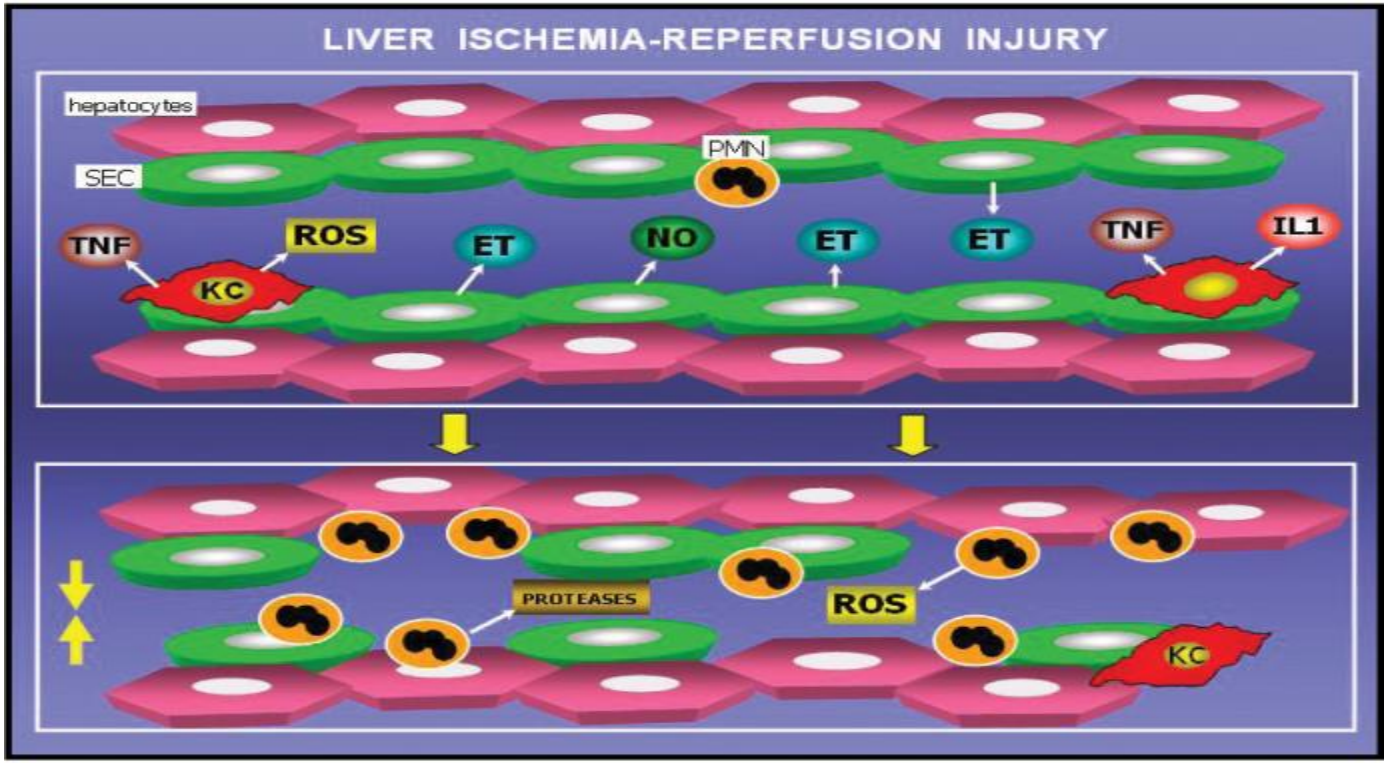
→ Quality of the graft: Steatosis

Liver steatosis

Steatosis: excess of fat in hepatocytes (TG) Lipid droplets in at least 5% of cells

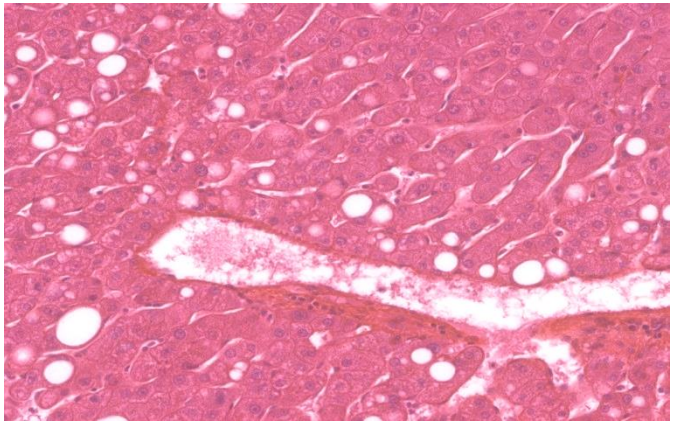


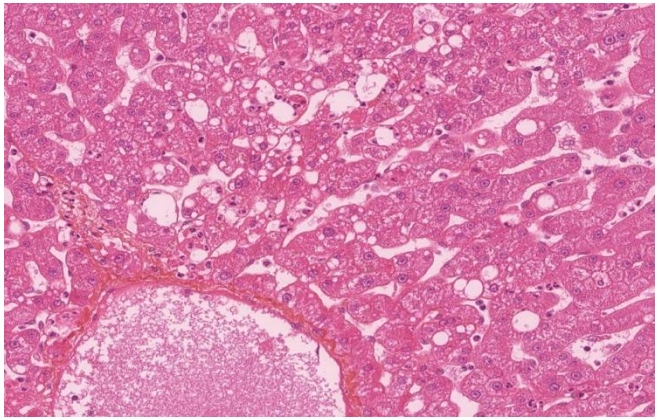


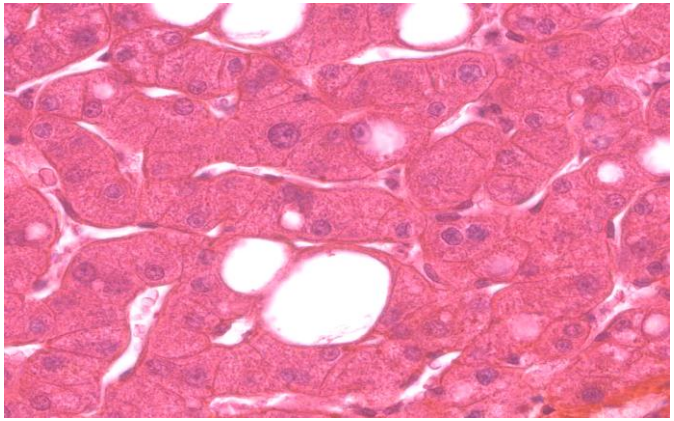


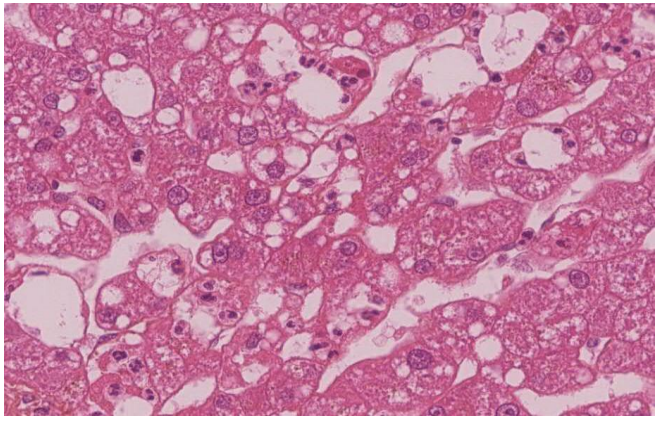
Steatosis sensibilizes liver to ischemia-reperfusion lesions through altered blood flow in liver microcirculation and enhanced peroxydation of membrane lipids

Ischemia-Reperfusion lesions in liver graft









Liver graft & steatosis

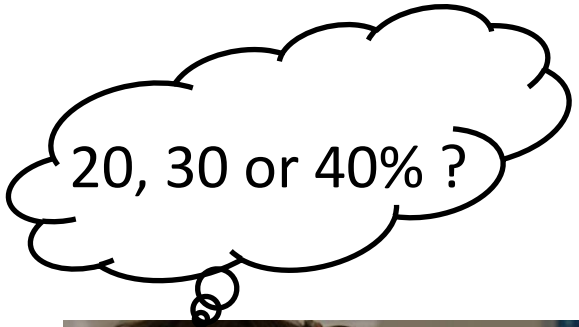
- Steatosis <30%
  low incidence

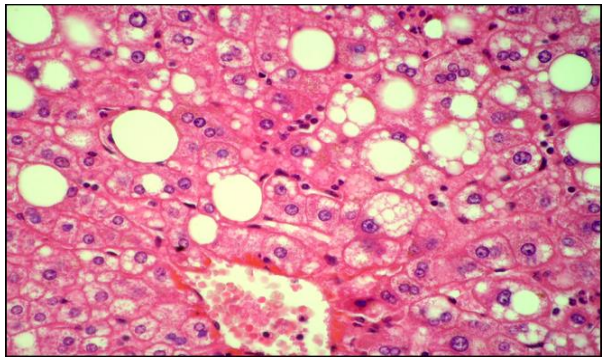
- Steatosis 30-60%
  35% delayed function
  15% non primary function

- Steatosis >60%
  80% non primary function

Quantification of steatosis

Estimation of steatosis





Assessment of Hepatic Steatosis by Expert Pathologists: The End of a Gold Standard

El-Badry, et al Annals of Surgery. 2009; 250(5):691-697

TABLE 3. The Pathologists' Quantitative Estimation of Total, Micro- and Macrosteatosis

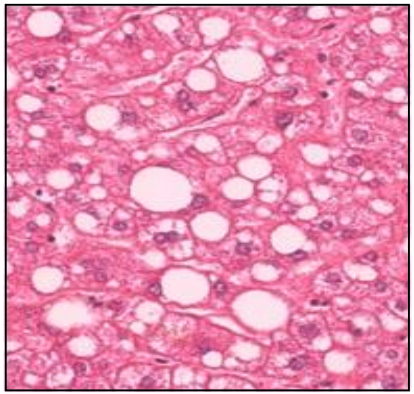
	Pathologist 1	Pathologist 2	Pathologist 3	Pathologist 4	ICC
Total steatosis (%)	20	12.5	20	10	0.57
Microsteatosis (%)	10	5	0	0	0.22
Macrosteatosis (%)	10	5	12.5	10	0.55

Median values of the assessment of total, micro- and macrosteatosis hepatocytes were collected. The intra-class correlation coefficients (ICCs) indicate poor agreement among the pathologists regarding the quantitative (total steatosis) and qualitative (micro- and macrosteatosis) evaluations.

Poor inter-observer reproducibility

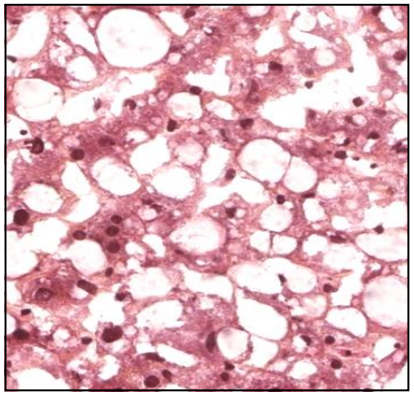
Estimation of steatosis in the context of emergency

Paraffin



> 24h

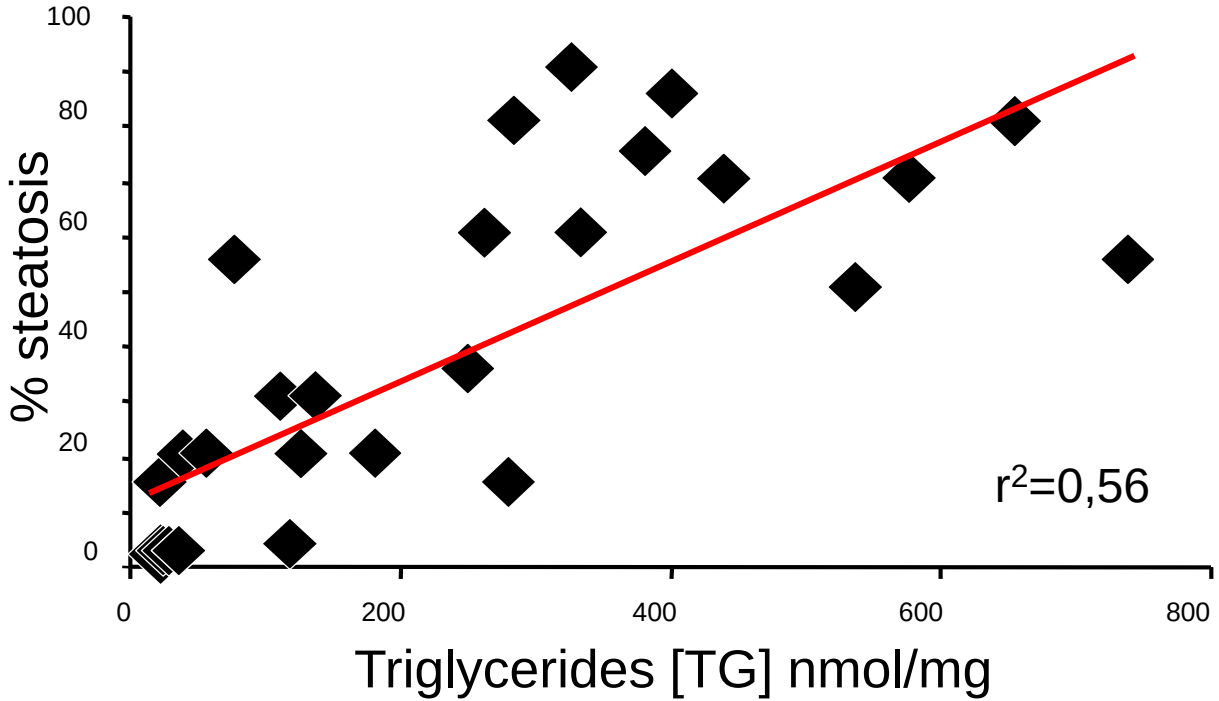
Frozen



< 15 min

A liver transplantation has to be performed in less than 8h…

Histological estimation of steatosis is not correlated to the lipid content



Histological estimation of steatosis

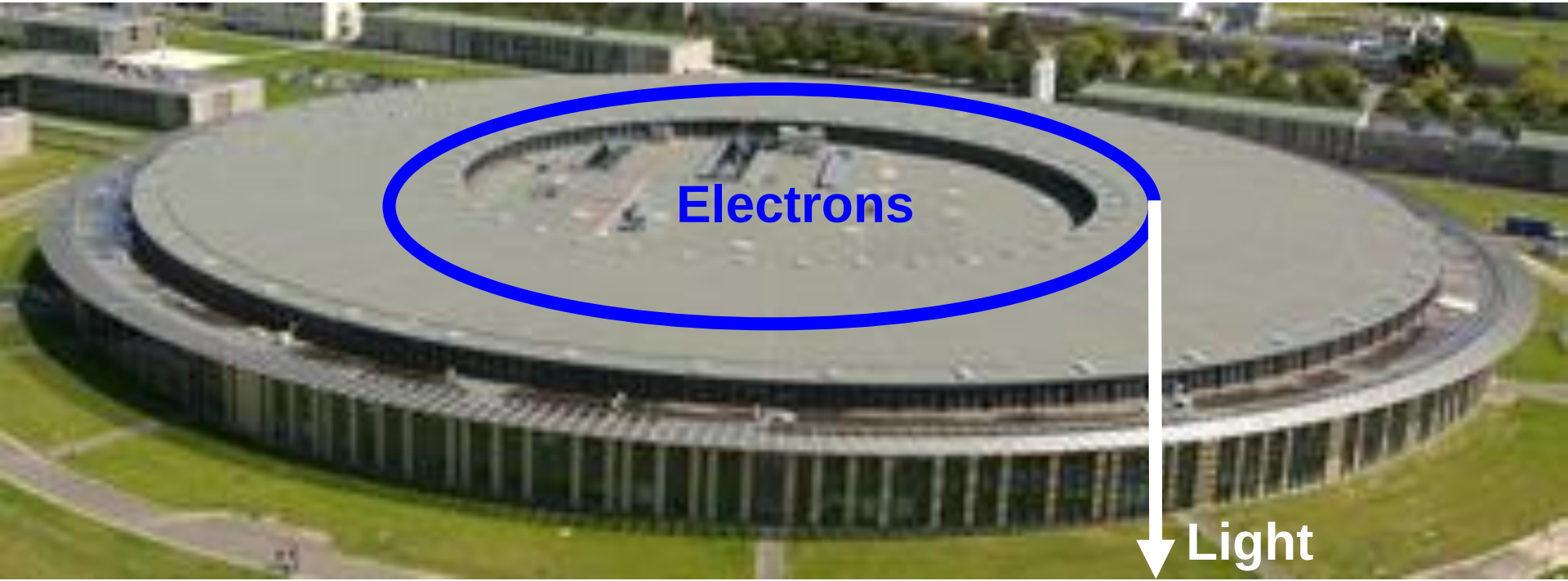
- Poor inter-observer reproducibility

- Poor conservation of the morphology on frozen tissue sections

- Not correlated to the true lipid content

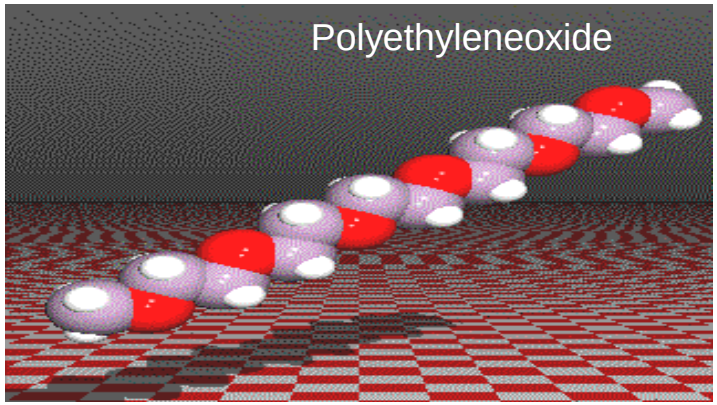
→ A need for a new tool

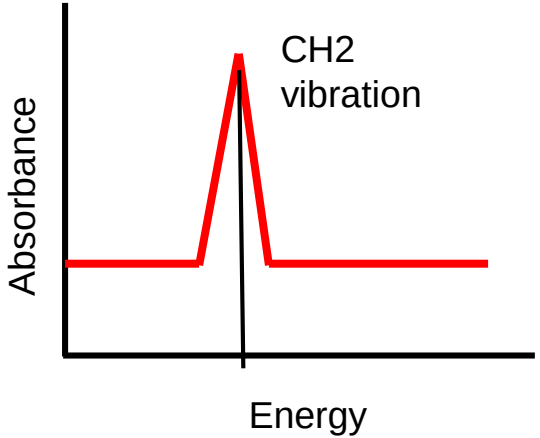
The synchrotron SOLEIL



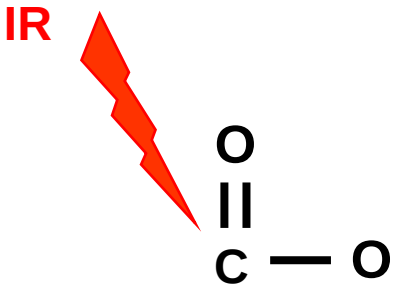
IR UV X

Infrared spectroscopy

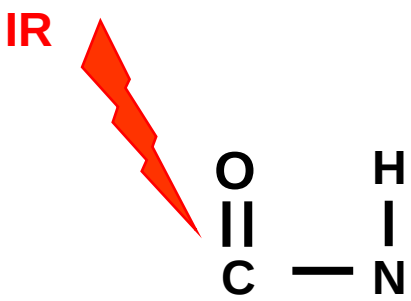




3000-2800 cm-1

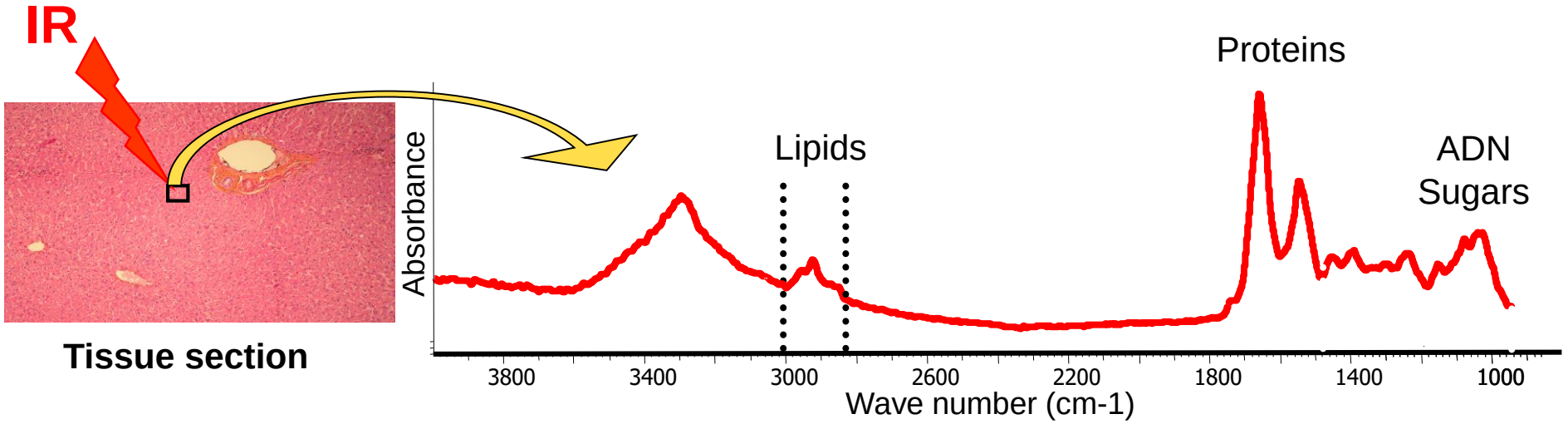


1740 cm-1



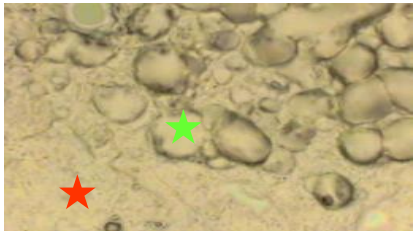
1650 / 1540 cm-1

Infrared spectroscopy on tissues

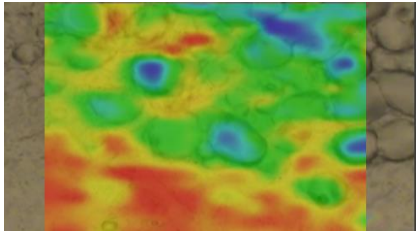


The spectrum is resulting of the global biochemical composition

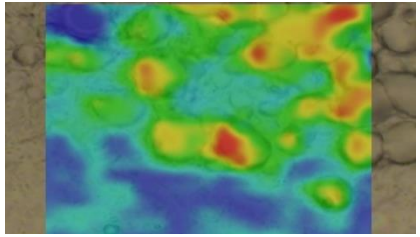
Infrared microspectroscopy on steatosis



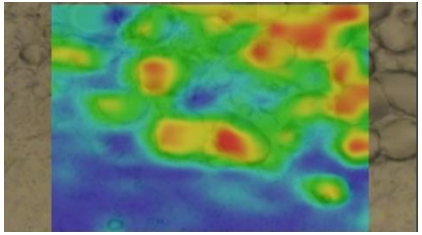
1475-1710 cm^{-1} **Proteins**



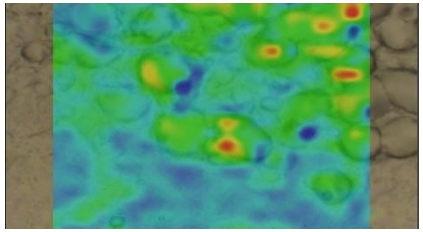
1710-1780 cm-1 **Esters**



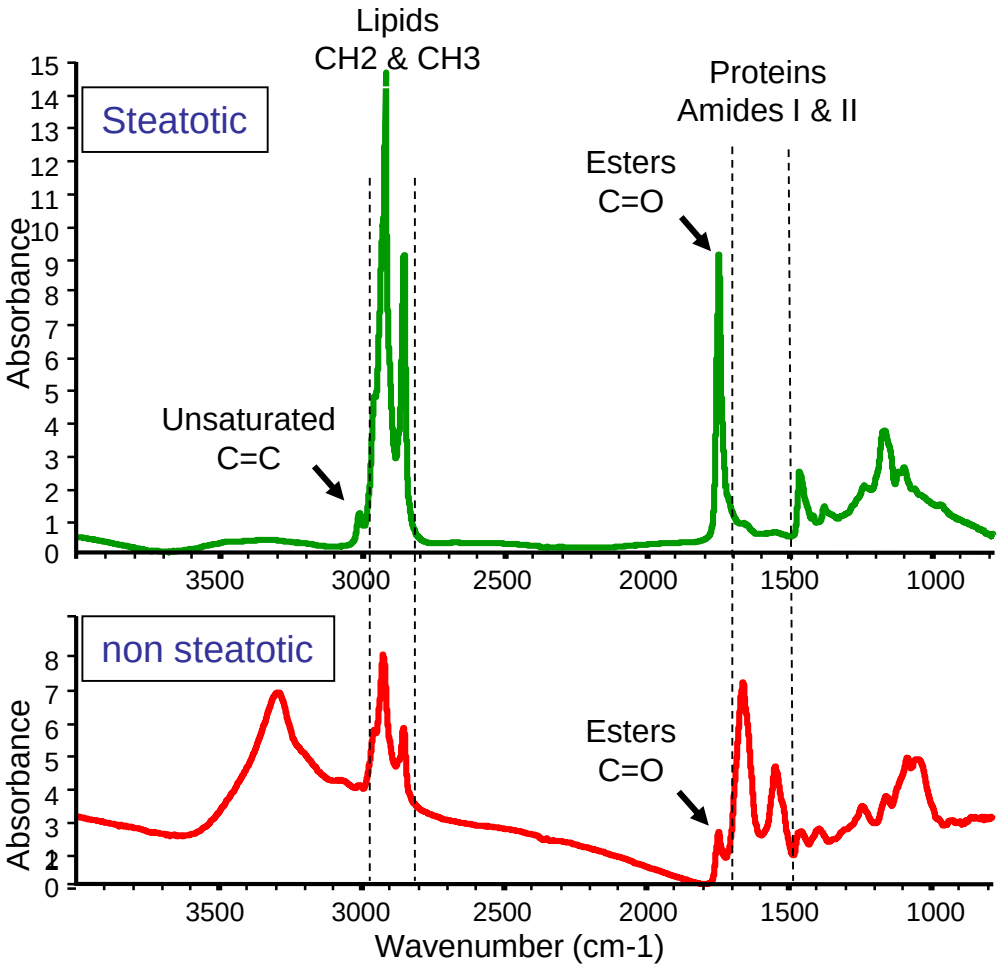
3000-2800 cm-1 **Lipids**



3000-3060 cm-1 **Unsaturated**

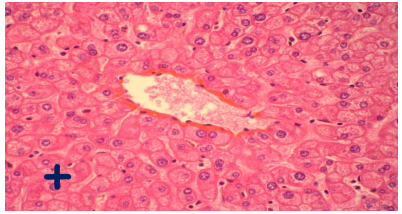


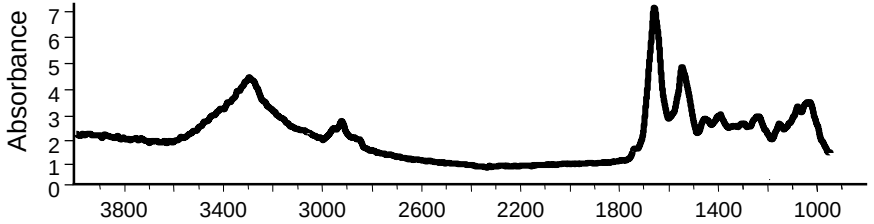
Min Max



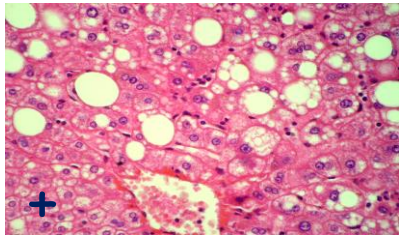
Analysis of the non-steatotic tissue

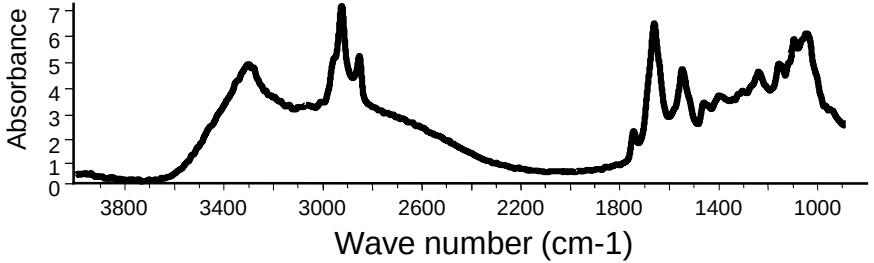
Normal liver





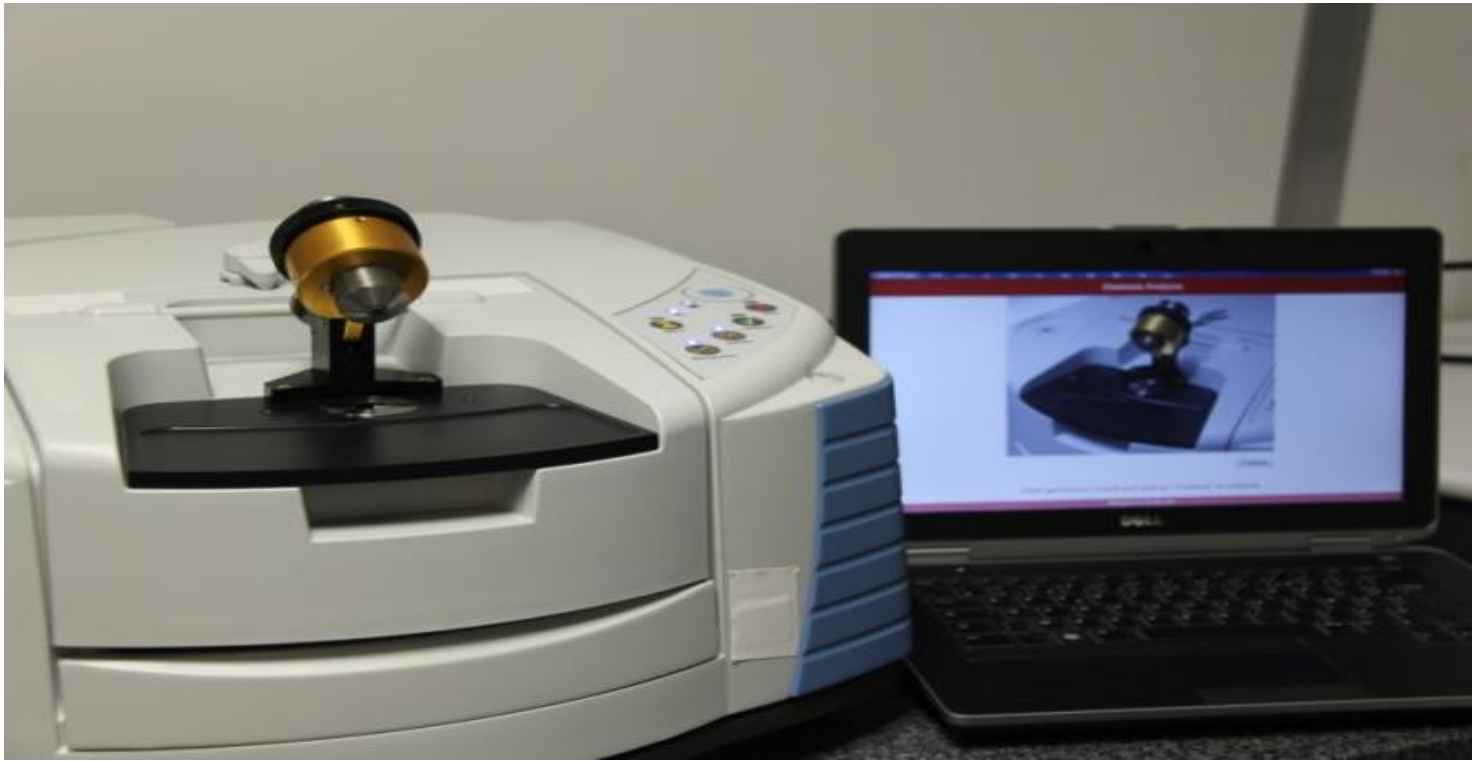
Steatosis



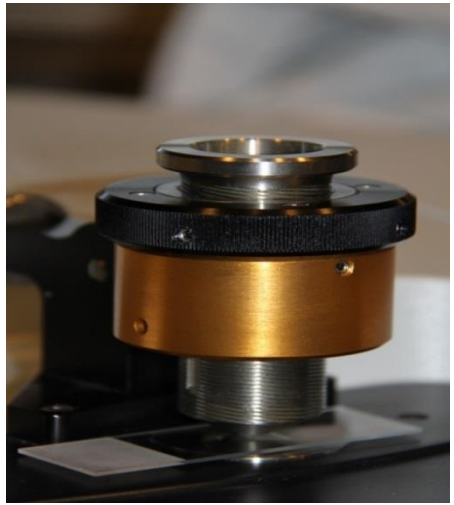


The non-steatotic part of the tissue is NOT normal

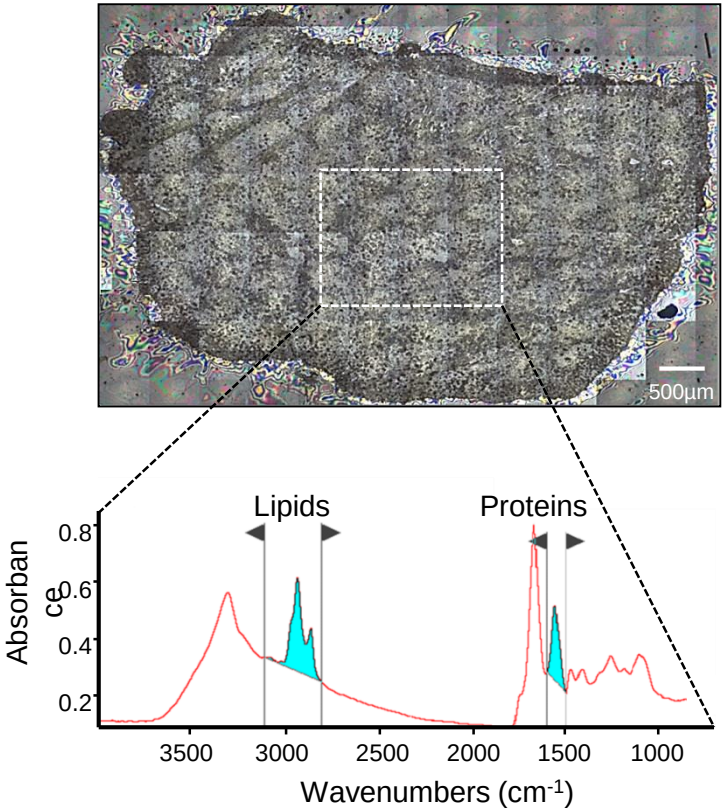
From synchrotron to the hospital



ATR infrared spectroscopy



Quantification of steatosis using IR



Pixel 2 mm x 2 mm

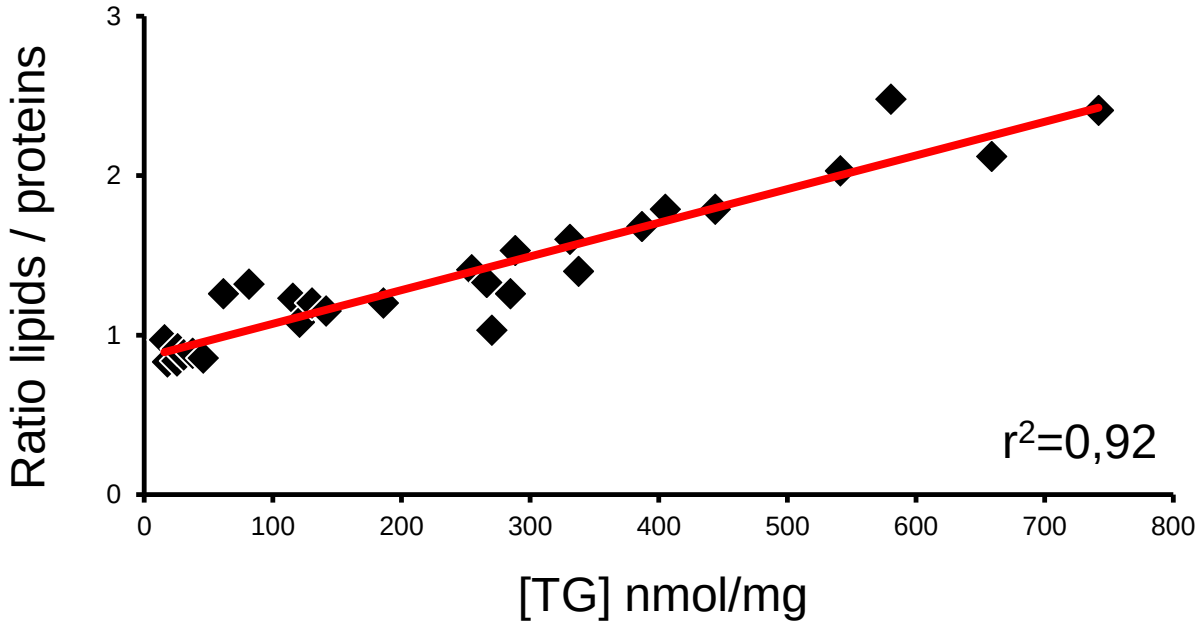
Time acquisition : 1 min

Ratio: lipids / proteins

Estimation of lipids

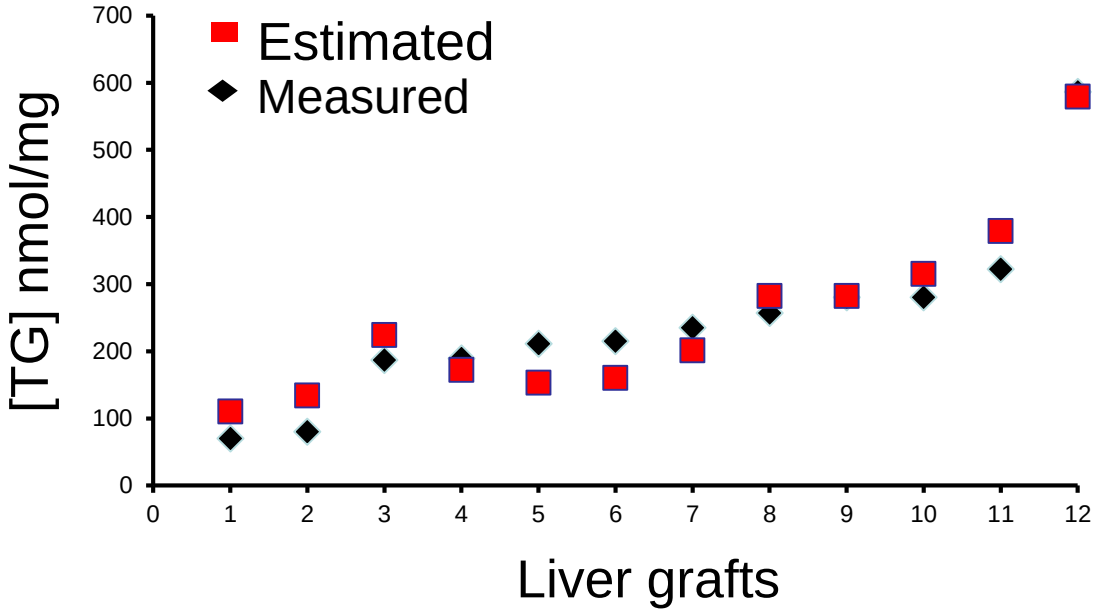
Quantification of steatosis using IR

25 tissue samples from liver surgical specimens - Steatosis : 5 to 90%



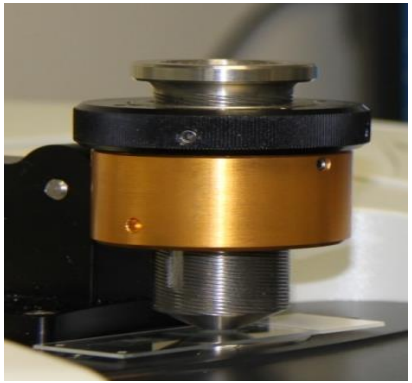
Infrared spectroscopy allows the quantification of lipids on tissue section

Lipid evaluation by IR microspectroscopy



Validation on additional 21 liver graft biopsies

Estimation of steatosis on liver graft



Conclusion

- Quantitative
- Reproducible
- Compatible with clinical practise (frozen sections on glass slides)
- Non destructive
- Non expansive
- Rapid – quantification of steatosis in 1 min!

→ Assesment of liver graft quality

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C Kewish

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