

Technology transfert and Industrial relations in Research Infrastructures

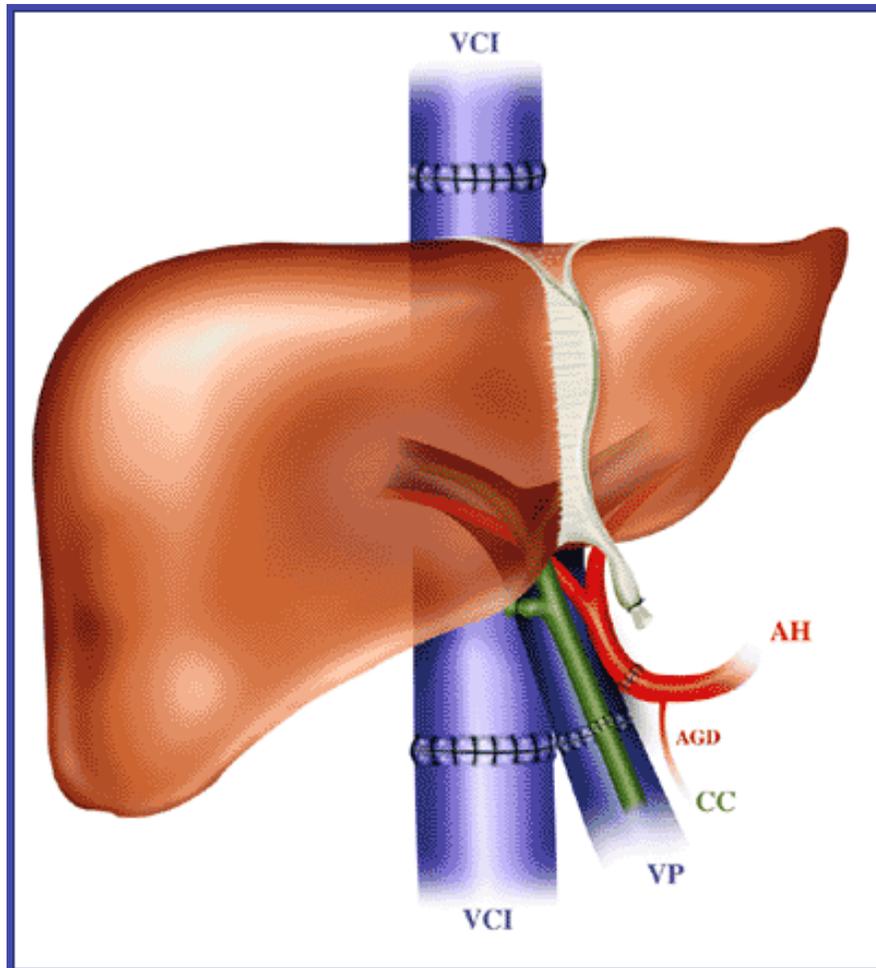
**" Quantitative assessment
of steatosis by infrared microspectroscopy
for liver graft quality control «**

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INSERM U785**

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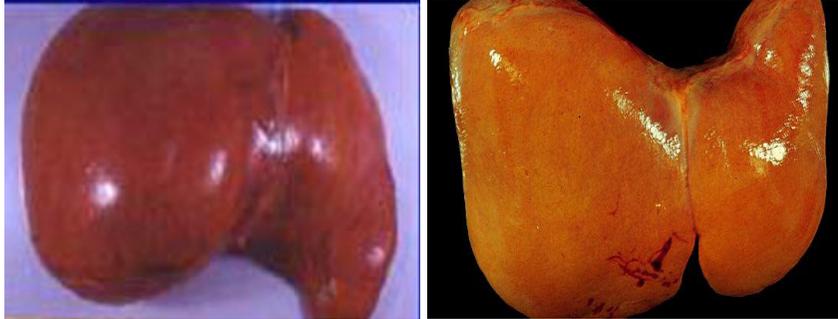
The liver



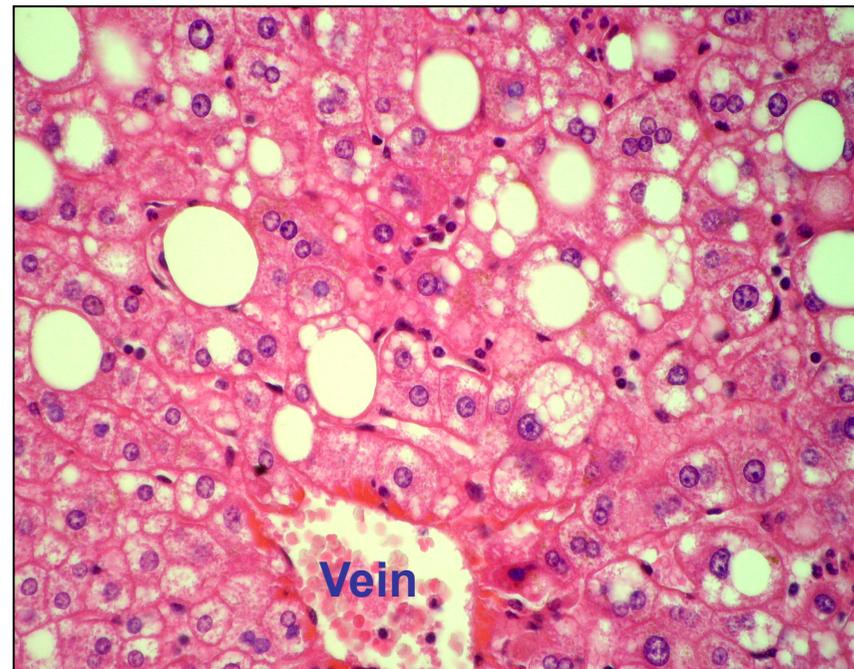
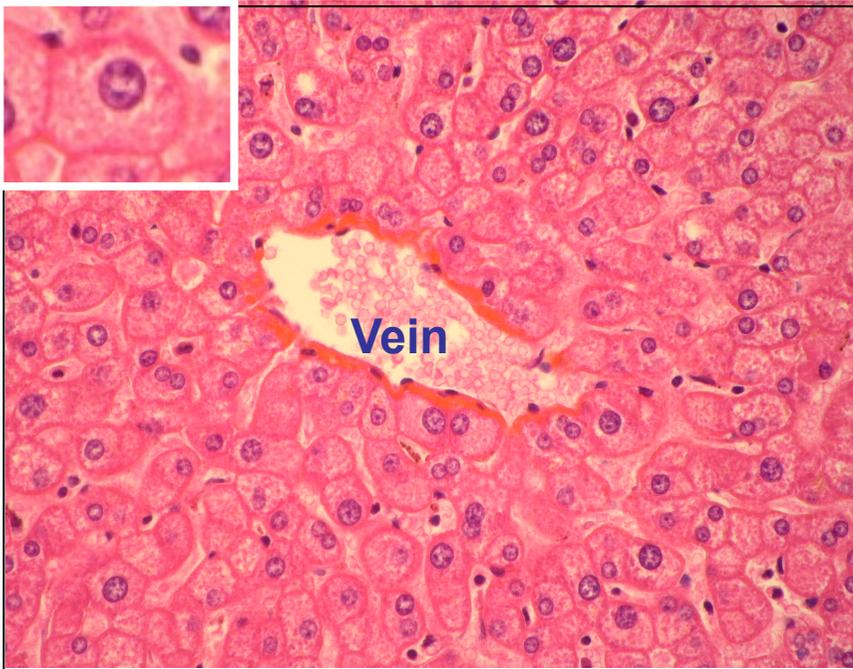
- Glycogen synthesis & catabolism
- Lipids: cholesterol synthesis & catabolism, production of triglycerids, lipoprotein synthesis
- Destruction of old red blood cells & leucocytes
- Production of coagulation factors (I, III, V, VII, IX & XI)
- Metabolism of toxins and drugs
- Urea synthesis
- Storage of vitamins (A, B12, D, K, E)
- Storage of elements (Fe, Cu)

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

Liver steatosis



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

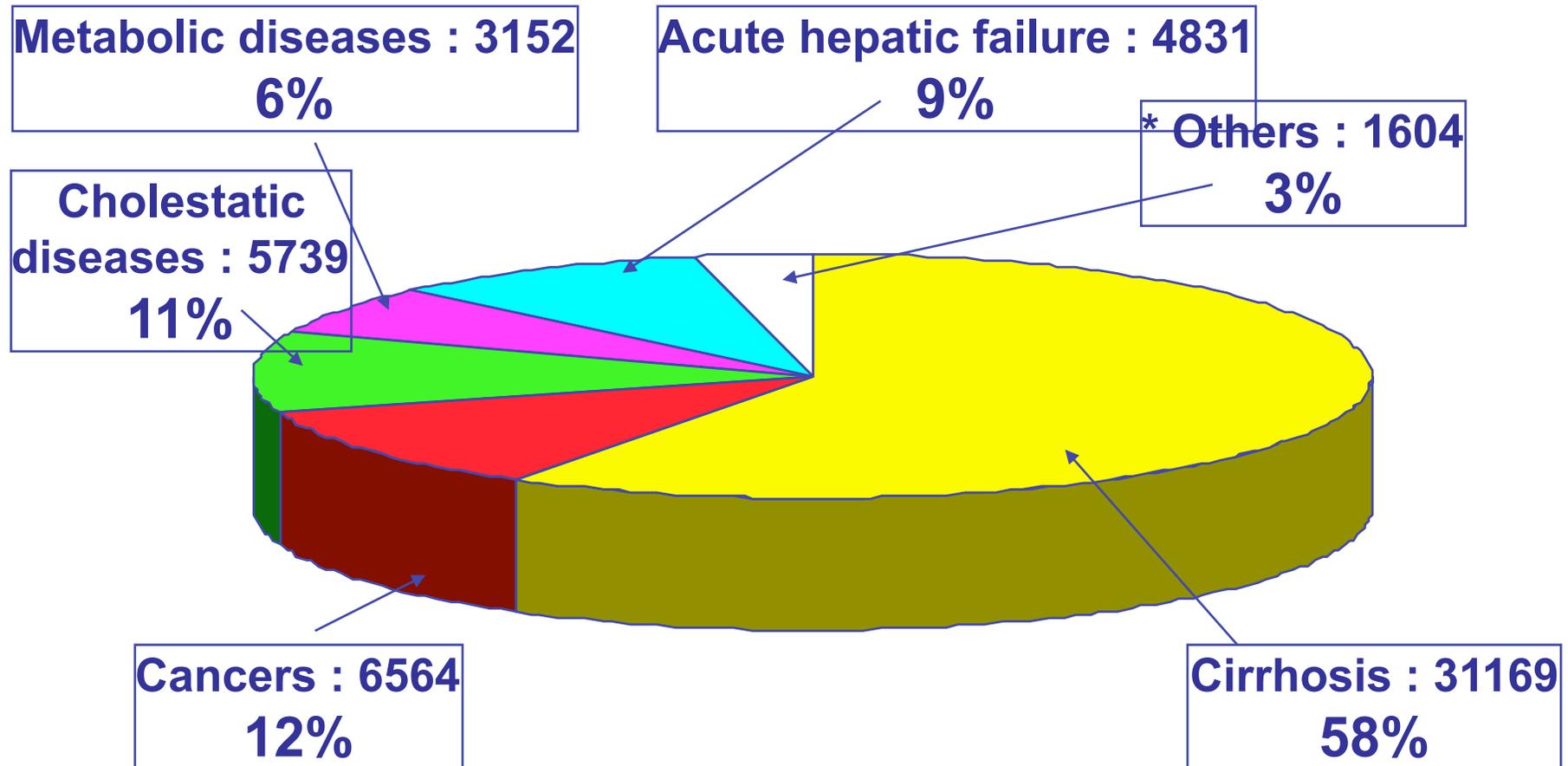


Liver steatosis: a frequent lesion

- Alcoholic liver disease
- Chronic hepatitis C
- Non alcoholic fatty liver disease: 30% of the general population in Western country

Steatosis and Liver transplantation

Liver transplantation : a major treatment in liver diseases

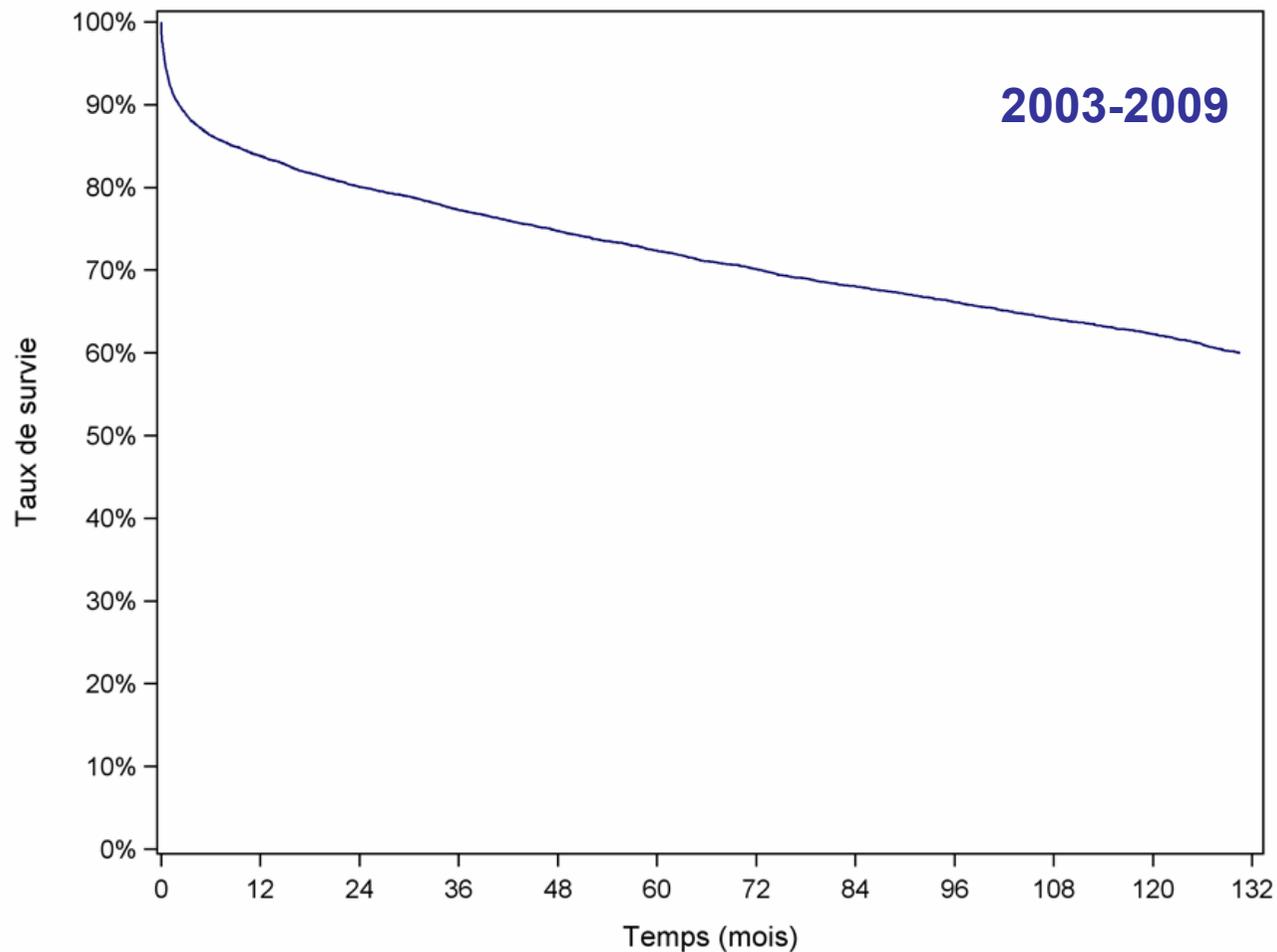


Liver transplantation : a major treatment in liver diseases

Année de greffe	Total greffe
1990	658
1991	698
1992	680
1993	662
1994	624
1995	646
1996	625
1997	621
1998	693
1999	699
2000	806
2001	802
2002	883
2003	833
2004	931
2005	1024
2006	1037
2007	1061
2008	1011
2009	1047
2010	1092

**Cumulated number
18 851**

Liver transplantation : a good treatment for liver diseases



Liver transplantation

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

Tableau F1. Evolution de la liste d'attente et devenir des candidats en greffe hépatique

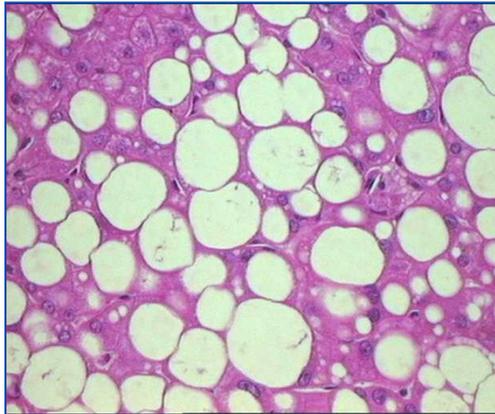
	2005	2006	2007	2008	2009	2010	2011
Liste d'attente							
Malades restant en attente au 1er janvier de chaque année	474	486	540	574	669	806	932

- Quality of the graft: Steatosis

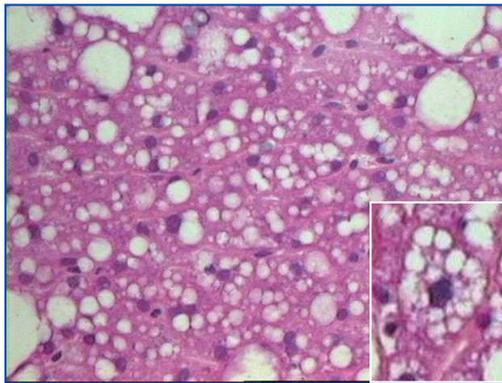
Graft steatosis

- Primary non-function
 - 80% with steatosis > 60%
 - 15% with steatosis 30-60%
- Delayed function
 - 35% with steatosis 30-60%
- Long term poorer evolution of the graft: fibrosis, survival

Quantification of liver steatosis on tissue section



Macrovesicular steatosis



Microvesicular steatosis

Histological examination of the graft before transplantation

Frozen section*

- HES staining
- Oil-RedO

Microscopic evaluation

- % d'hépatocytes

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

- Technique-induced changes (frozen/paraffin sections)
- Poor inter-observer reproducibility

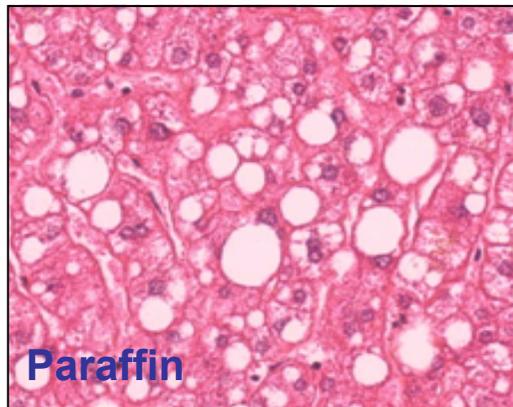
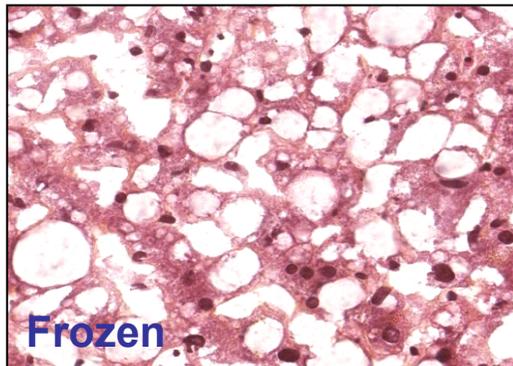


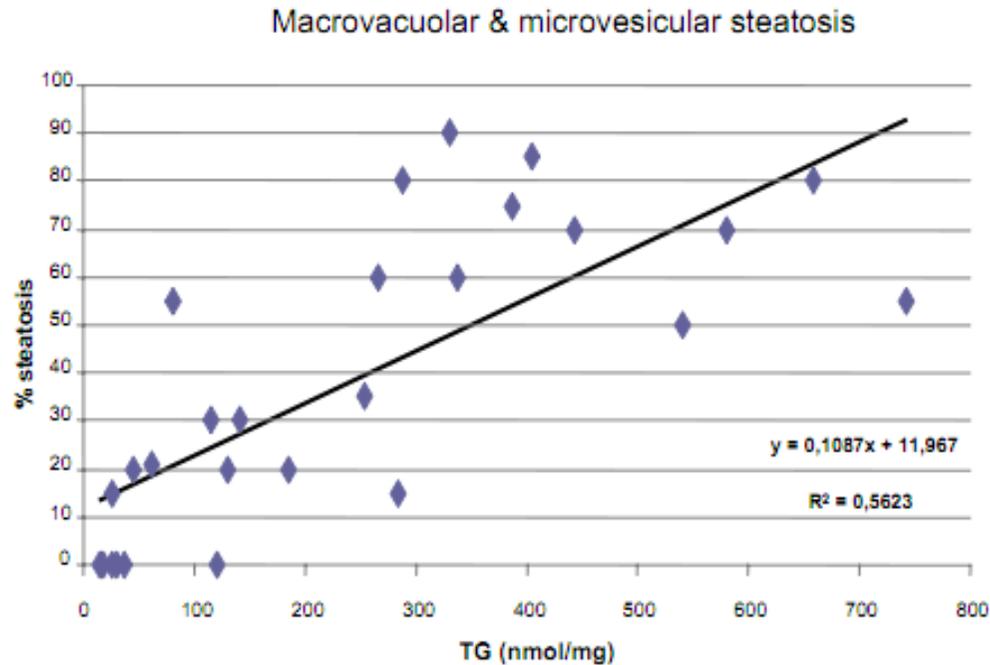
TABLE 3. The Pathologists' Quantitative Estimation of Total, Micro- and Macrosteatosis

	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.

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

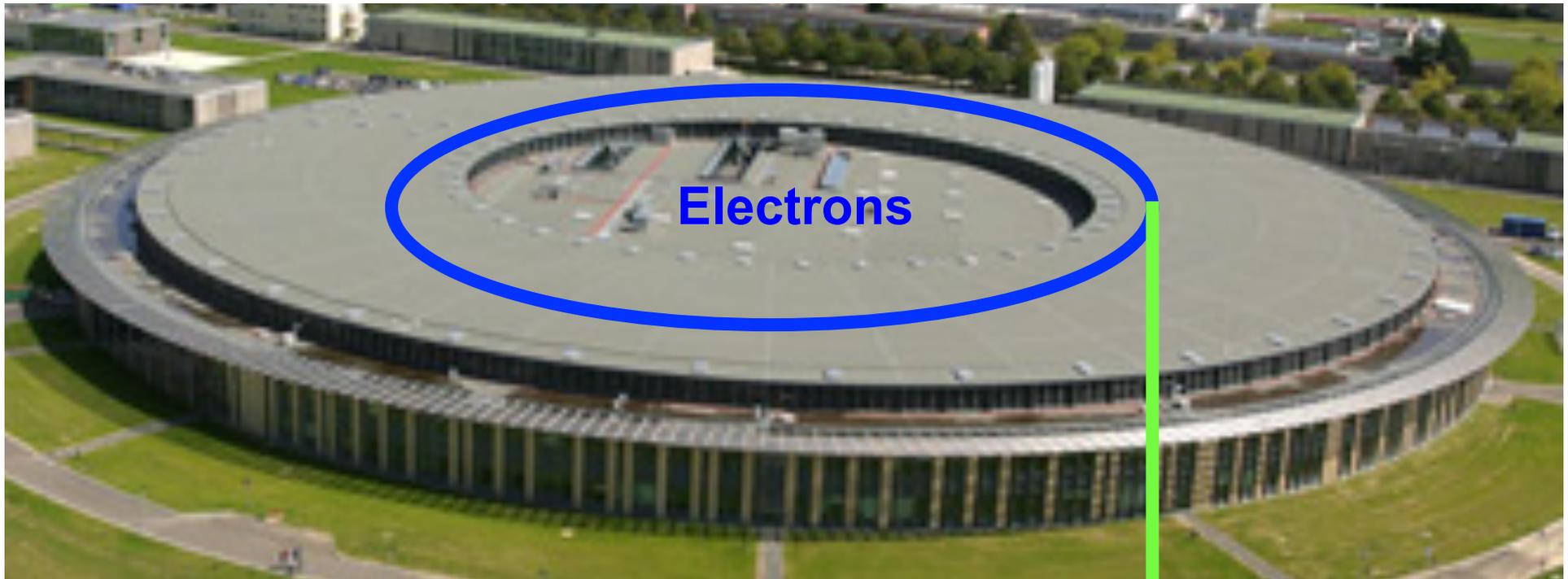
An imperfect evaluation



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

Histological evaluation on frozen sections / Biochemical assay for triglycerides from frozen tissue samples :

A need for a new tool



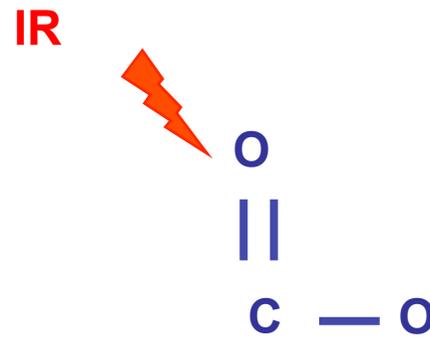
InfraRed source
with high brilliance
achieving subcellular
resolution

**IR
SMIS**

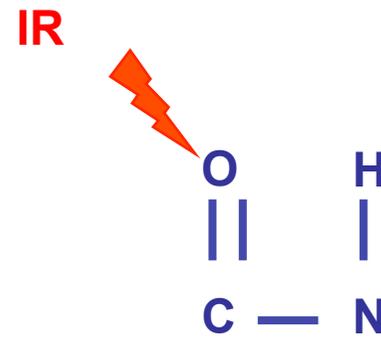
InfraRed Spectroscopy



3000-2800 cm⁻¹

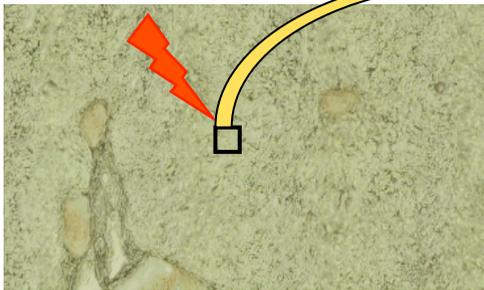


1740 cm⁻¹

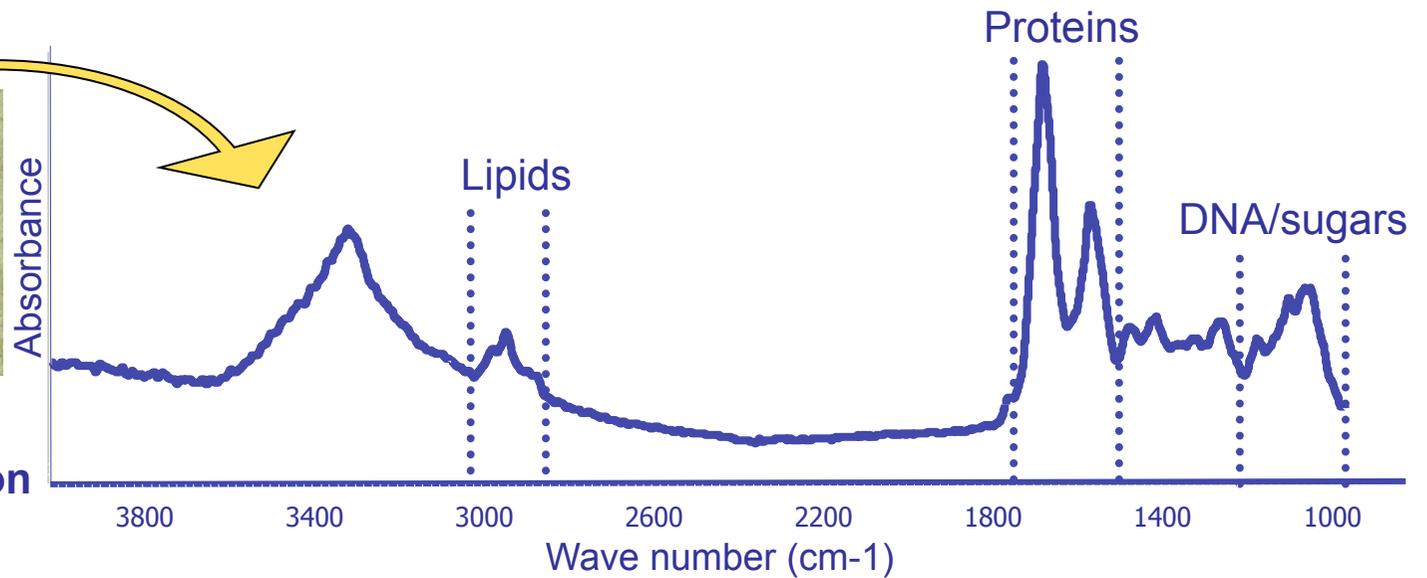


1650 / 1540 cm⁻¹

10 x 10 μm²



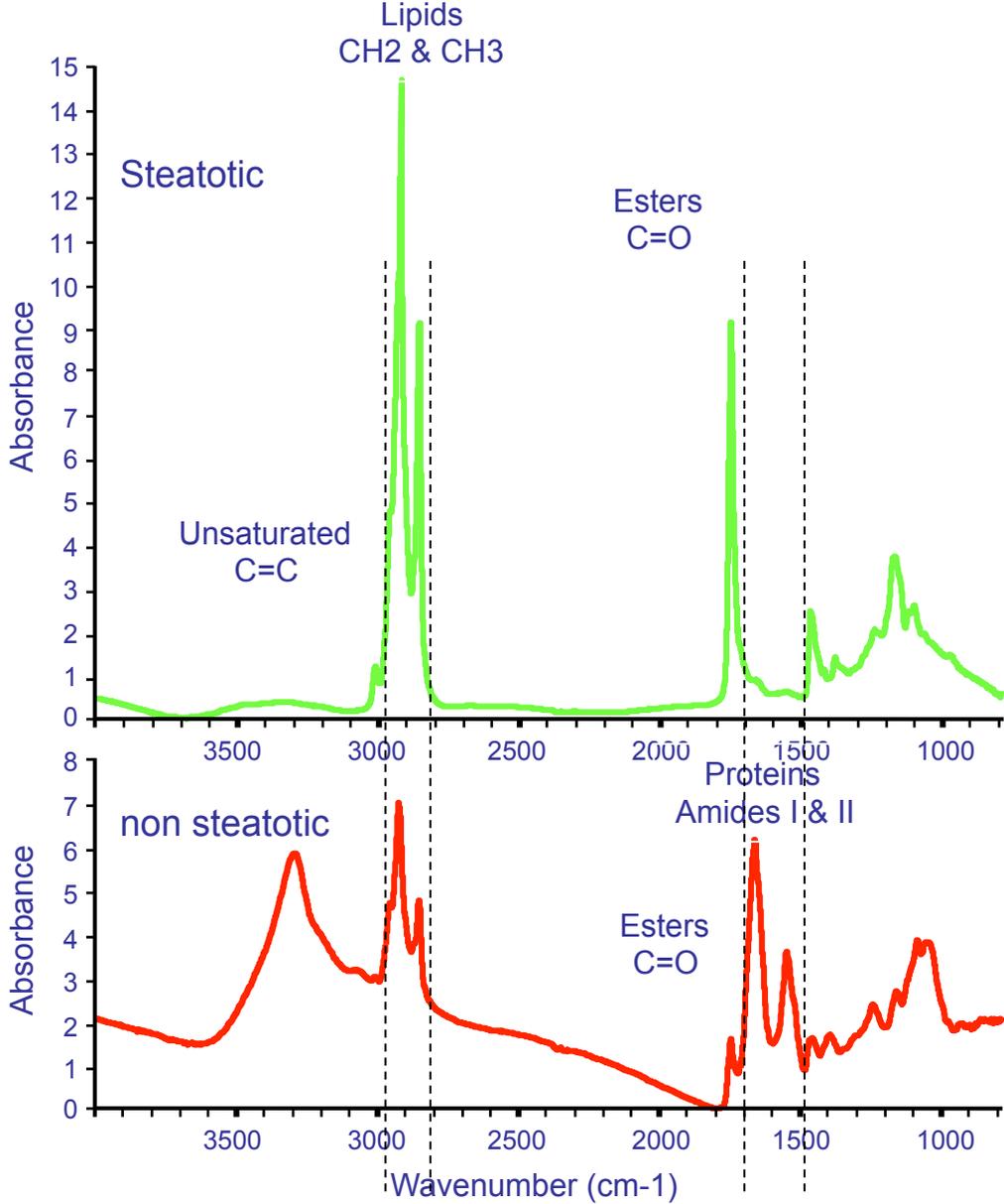
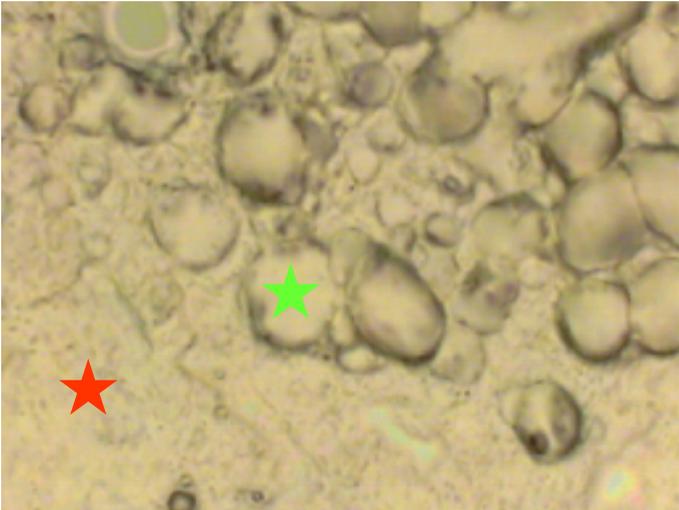
Unstained frozen section
On Gold-coated slide



The absorption spectrum reflects the global biochemical composition at each pixel

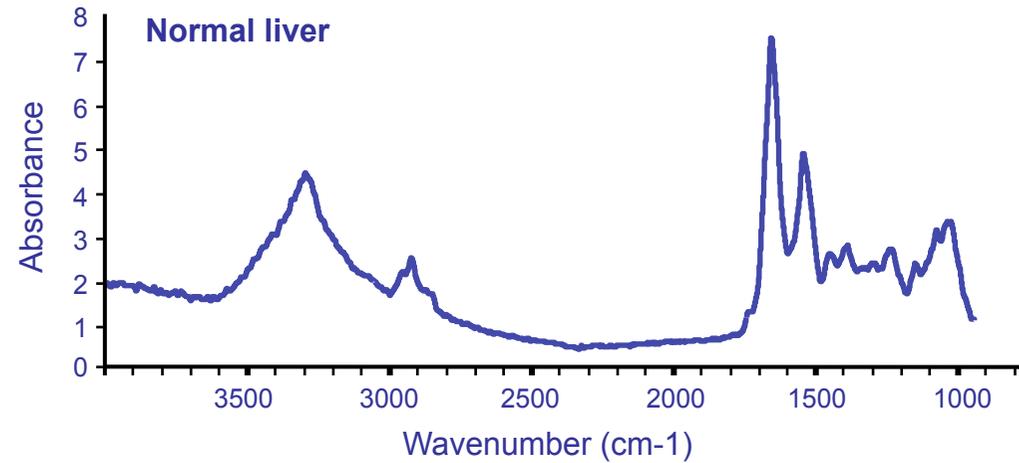
Infrared microspectroscopy on steatosis

Unstained frozen section

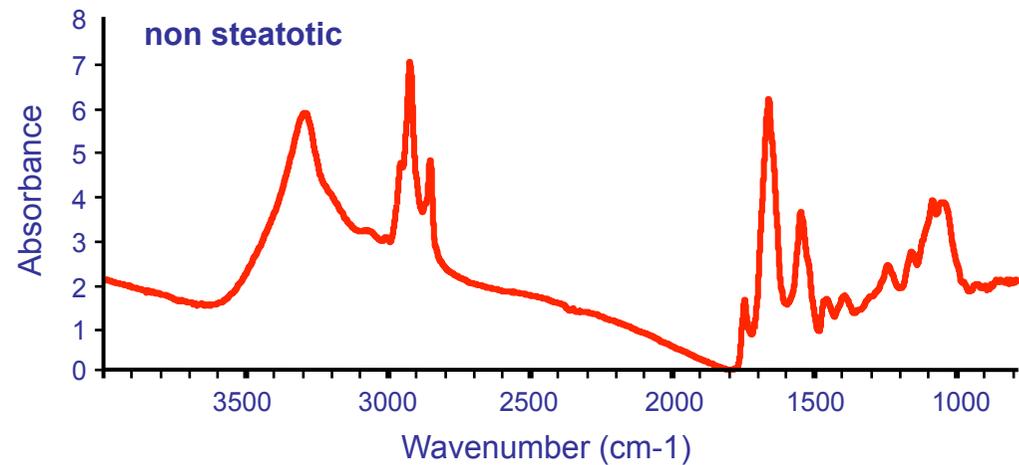
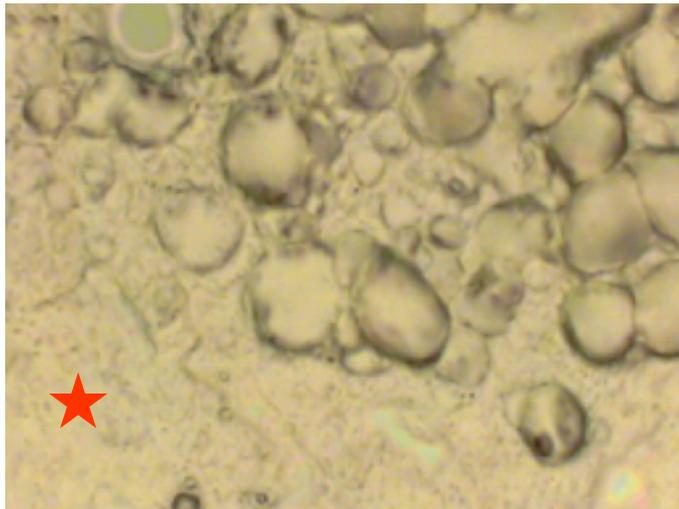


Normal liver and non steatotic regions

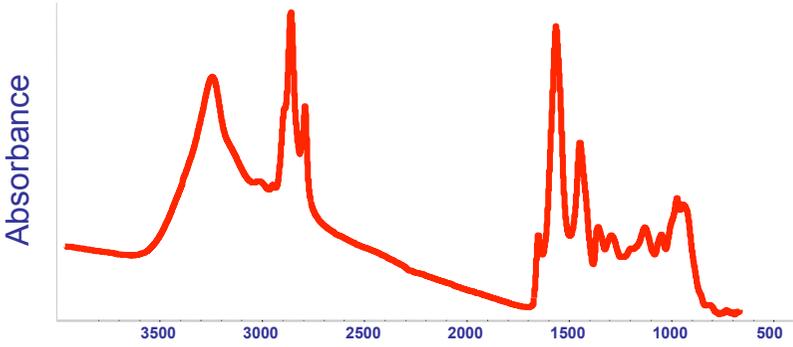
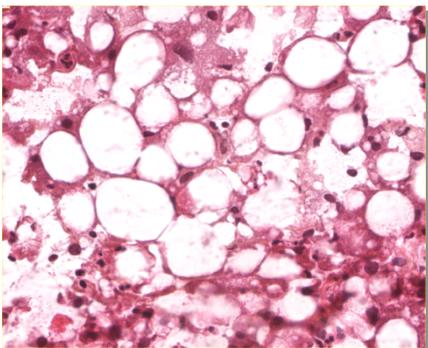
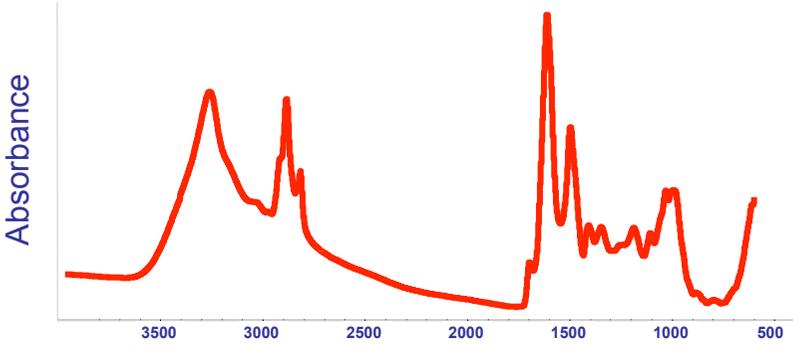
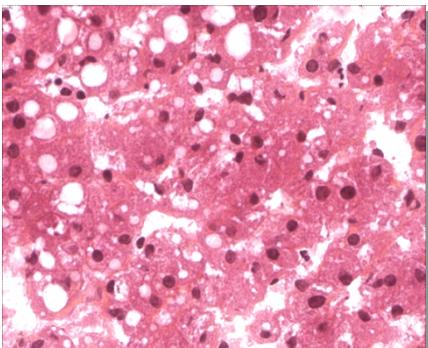
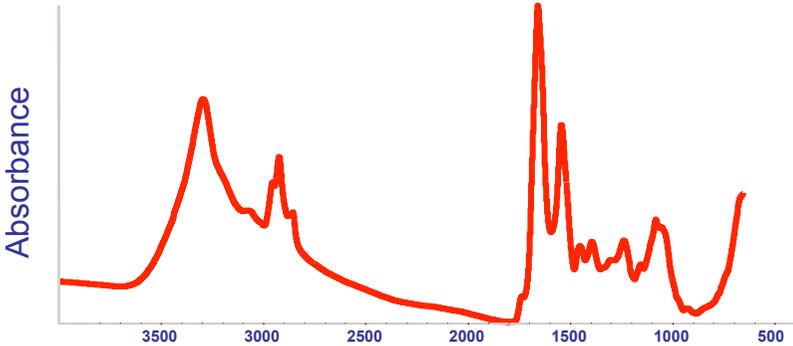
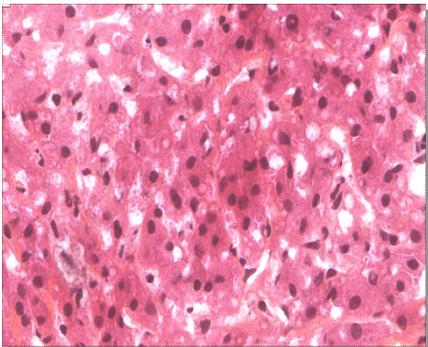
Normal liver



Steatotic liver



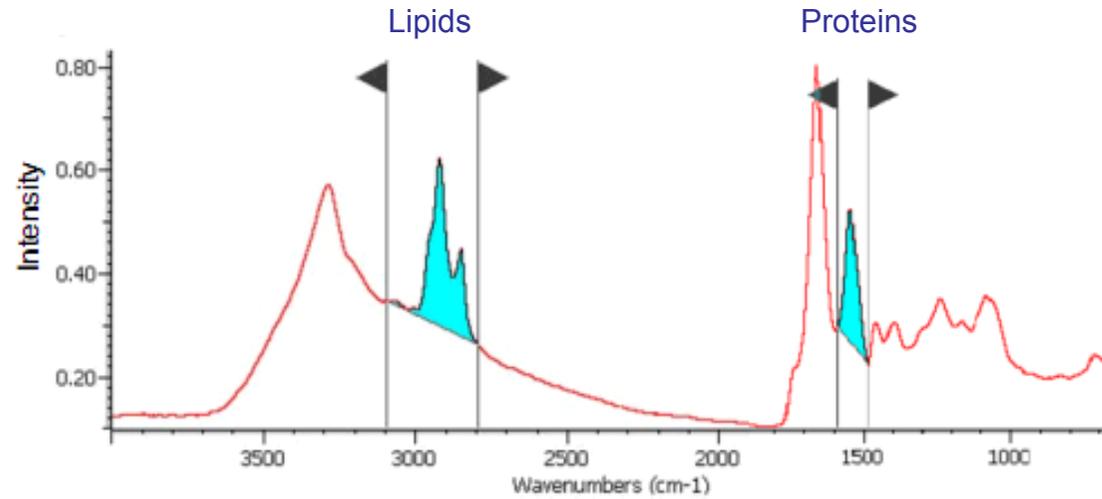
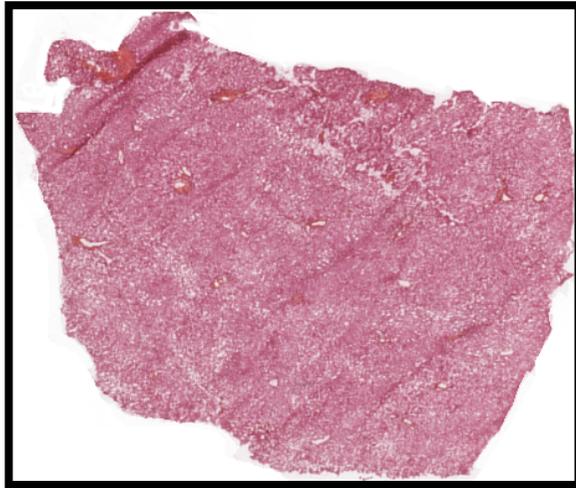
IR microspectroscopy on various grades of steatosis



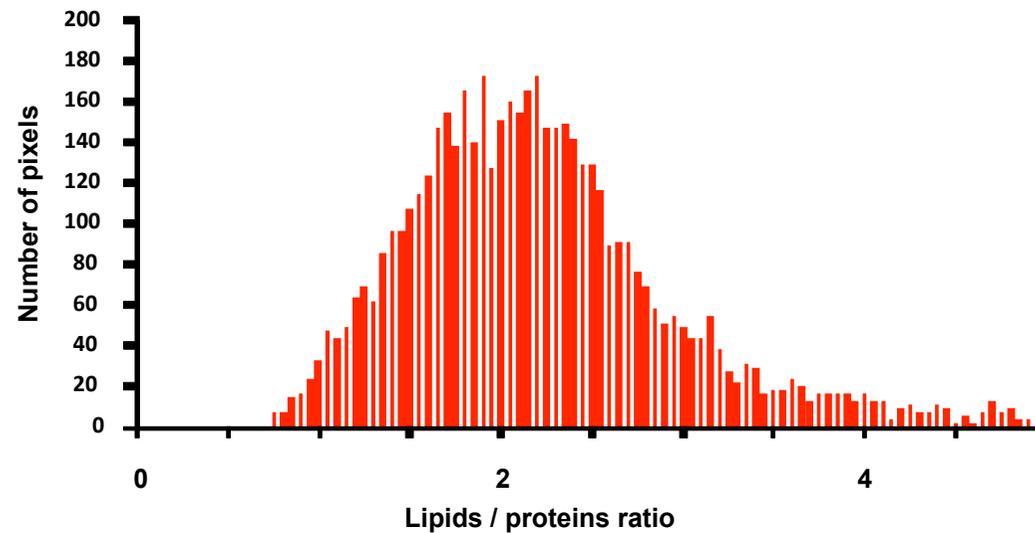
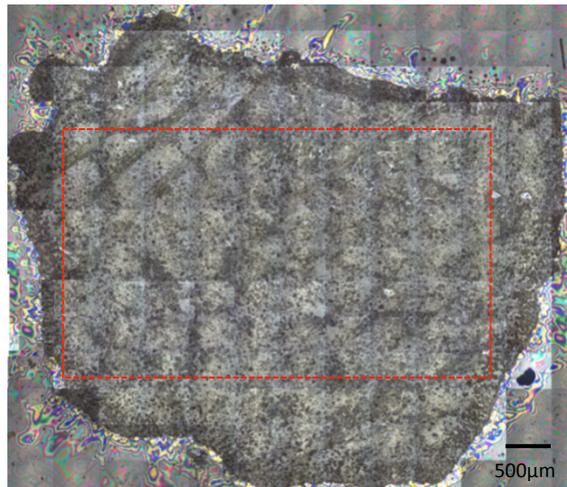
Quantification of steatosis

InfraRed microspectroscopy

HES staining

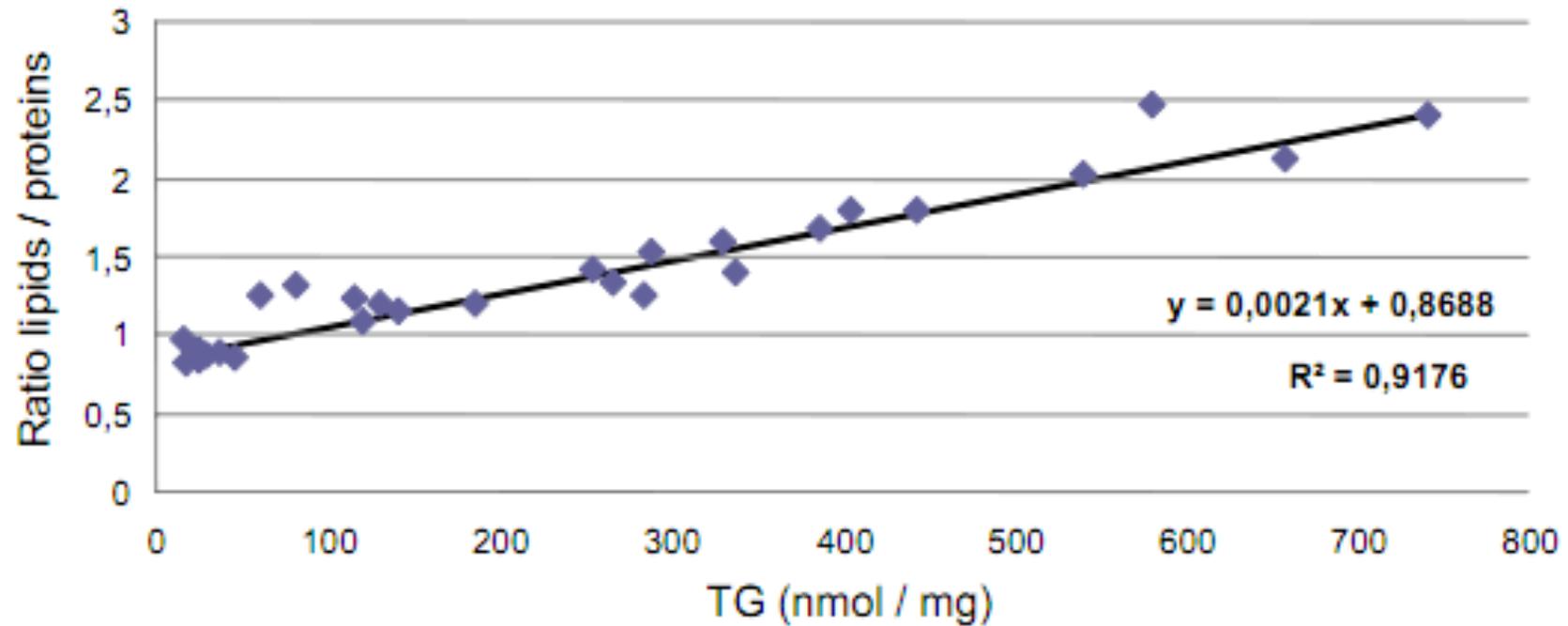


Unstained section



Quantification of steatosis

InfraRed microspectroscopy



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

Translational Research

Synchrotron IR

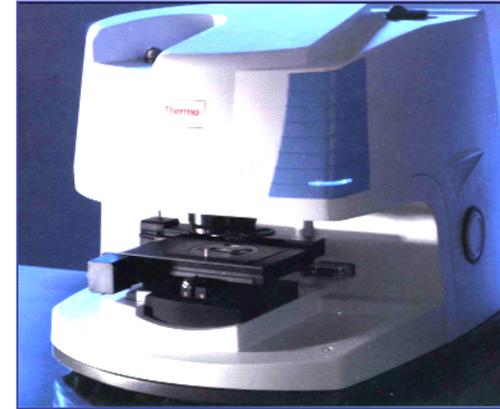


High resolution $10 \times 10 \mu^2$

Transfert



Classical InfraRed

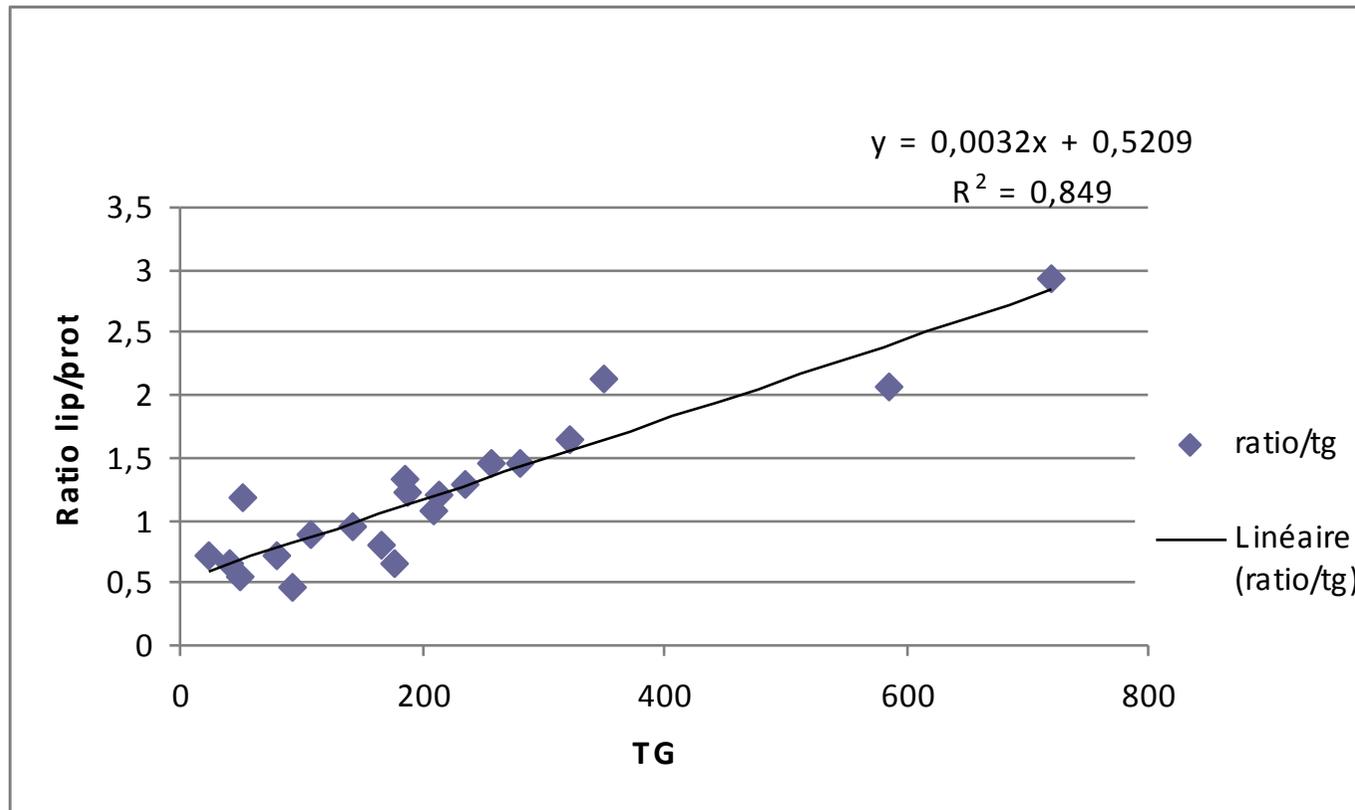


Low resolution $50 \times 50 \mu^2$

Aperture size (μ^2)		Lipids / proteins
10 x 10	Synchrotron	1.1962
100 x 100	IN10	1.1927

Clinical application at hospital

Liver graft evaluation by IR microspectroscopy



21 liver graft biopsies

- Precise quantification of liver steatosis
- Reproducible assessment of liver graft
- Rapid method (15mn)
- Non expensive procedure



Inserm



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