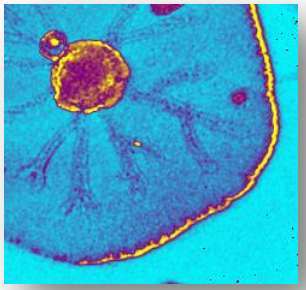
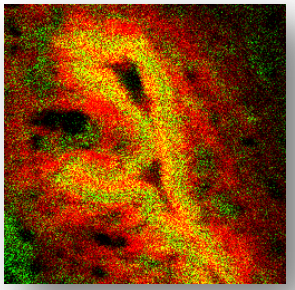
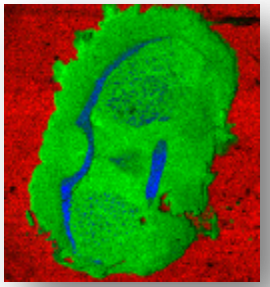
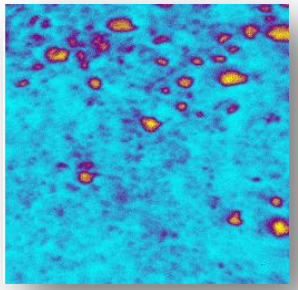



TOF-SIMS et imagerie par MS

David TOUBOUL

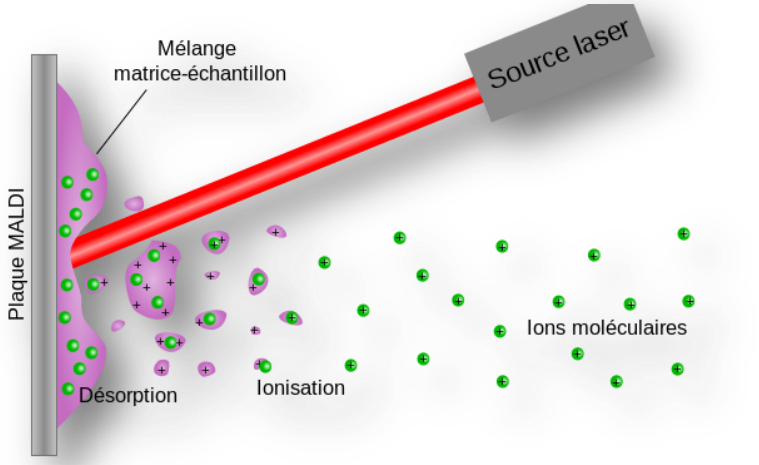
CNRS
Institut de Chimie des Substances Naturelles
Gif-sur-Yvette

David.Touboul@cnrs.fr

MALDI et SIMS

Matrix Assisted Laser Desorption Ionization

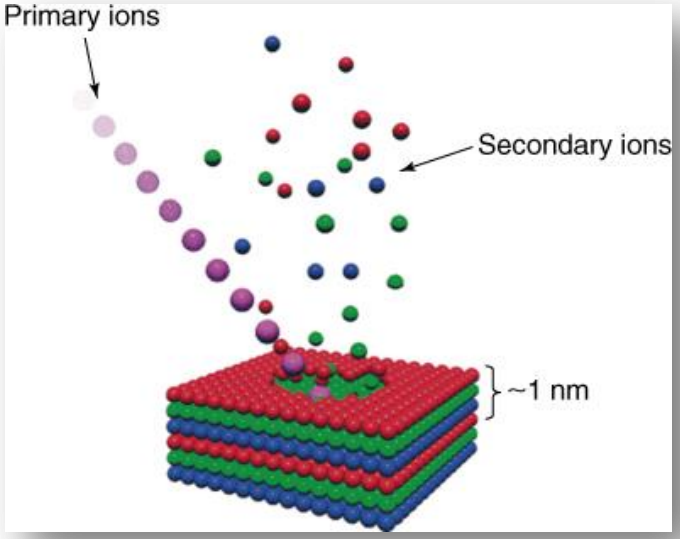
Les molécules (protéines, peptides, sucres, lipides, etc…sont mélangées avec une petite molécule organique appelée matrice. Celle-ci absorbe la lumière du laser UV et permet la désorption et l'ionisation des molécules d'intérêt sans les fragmenter.



M. Karas, F. Hillenkamp, Anal. Chem. 1988, 60, 2299-2301.
K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass Spectrom. 1988, 2, 151-153.

Secondary Ion Mass Spectrometry

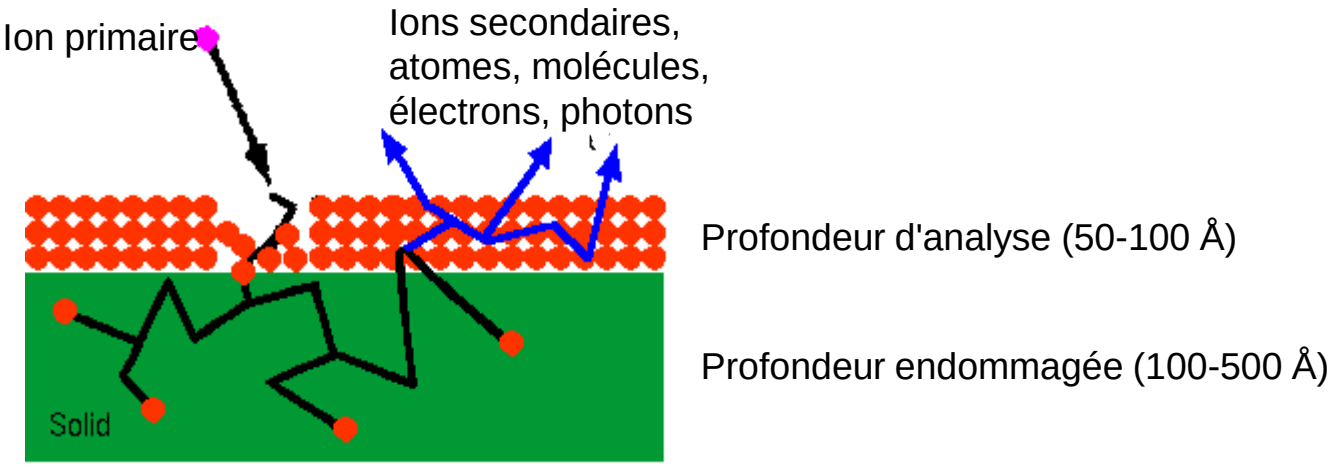
Un faisceau focalisé d'ions, appelés ions primaires, vient irradier la surface d'un échantillon de laquelle des ions, appelés secondaires, et caractéristiques de la surface, sont émis.



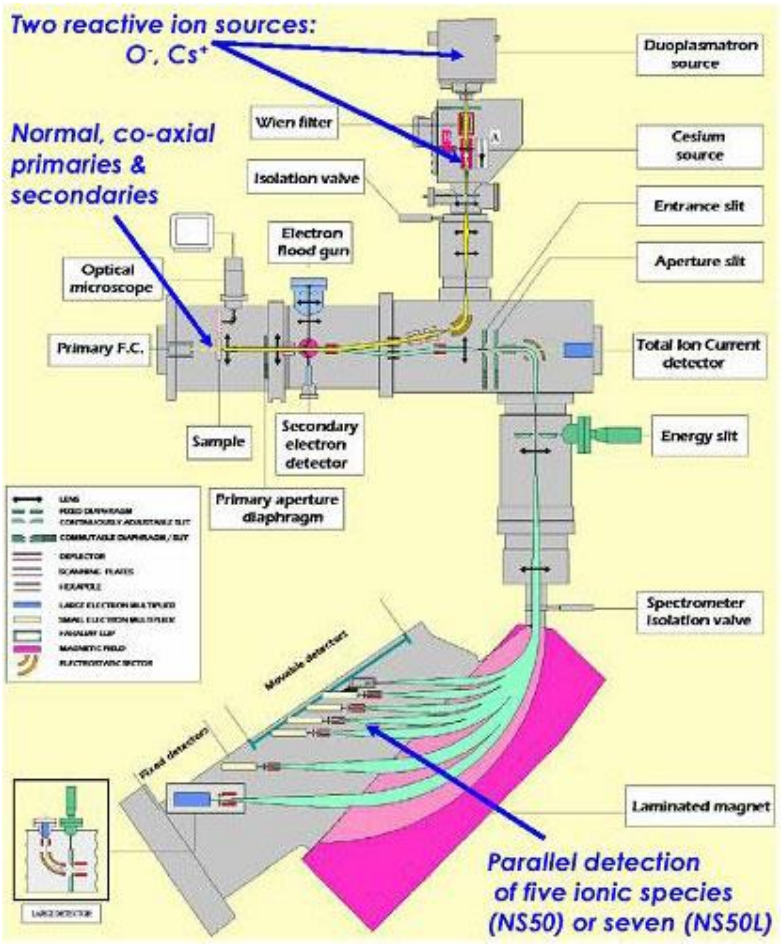
R. Castaing, G. Slodzian, J. Microsc. 1962, 1, 395-410
A. Benninghoven, E. Loebach, Rev. Sci. Instrum. 1971, 42, 49-52.

Le SIMS est une analyse de surface

- Les projectiles (ions primaires) pénètrent sur seulement quelques centaines d'Ångströms.
- Ils *endommagent* la matière sur toute cette profondeur, car ils se *ralentissent* par des successions de *collisions élastiques* avec des atomes de l'échantillon, lesquels sont *déplacés* et des molécules sont donc détruites.
- Les ions secondaires ne proviennent seulement que des premiers 50-100 Å.



Nano SIMS



Caractéristiques du NanoSims50 :

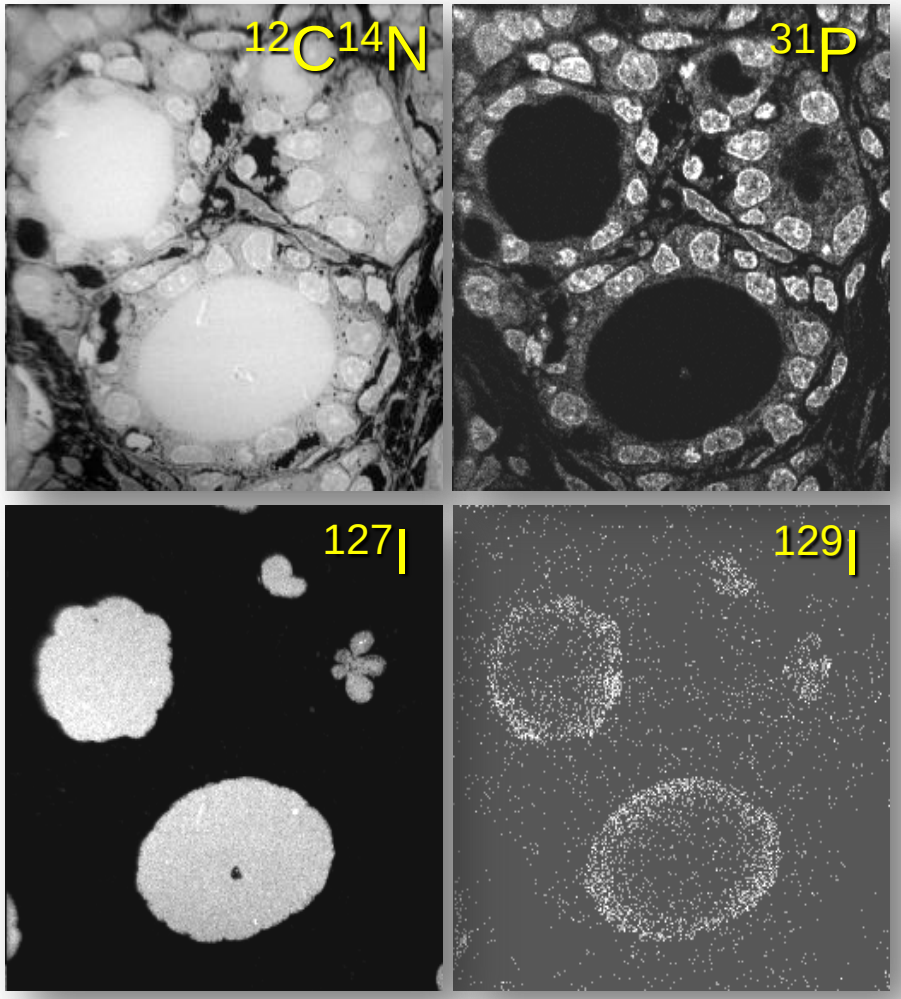
- Analyses élémentaires
- Excellente transmission des ions secondaires (80% avec M/ΔM de 5000)
- Détection en parallèle de cinq images
- Haute résolution latérale (**50 nm** en Cs)

Brevet Université Paris-Sud (P. Slodzian)

CAMECA
SCIENCE & METROLOGY SOLUTIONS

Nano SIMS
Analyse isotopique

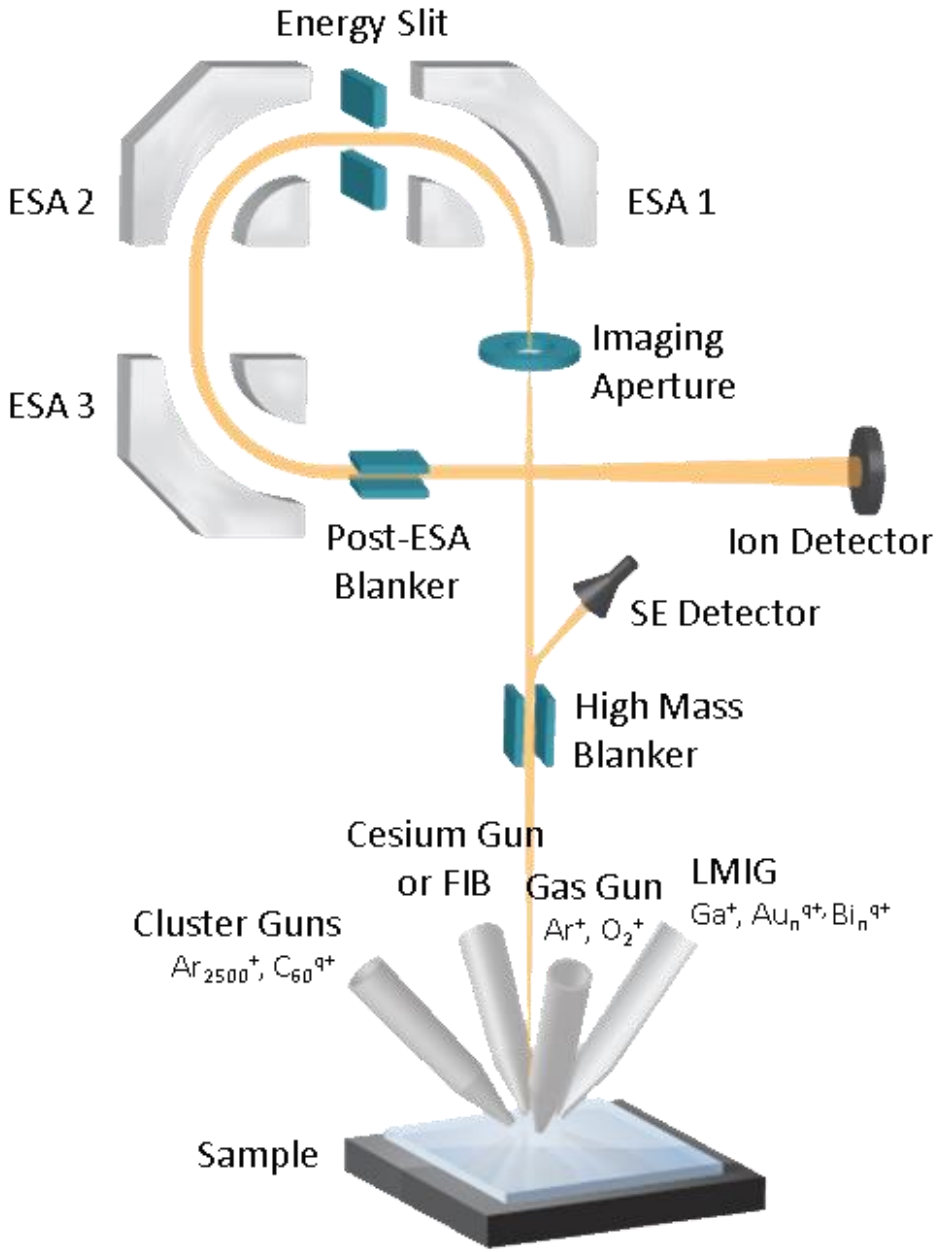
Distribution de l'iode dans la thyroïde
Application pour les simulations de doses absorbées



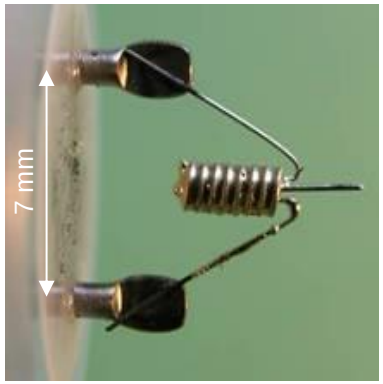
Champ de
60 µm x 60 µm

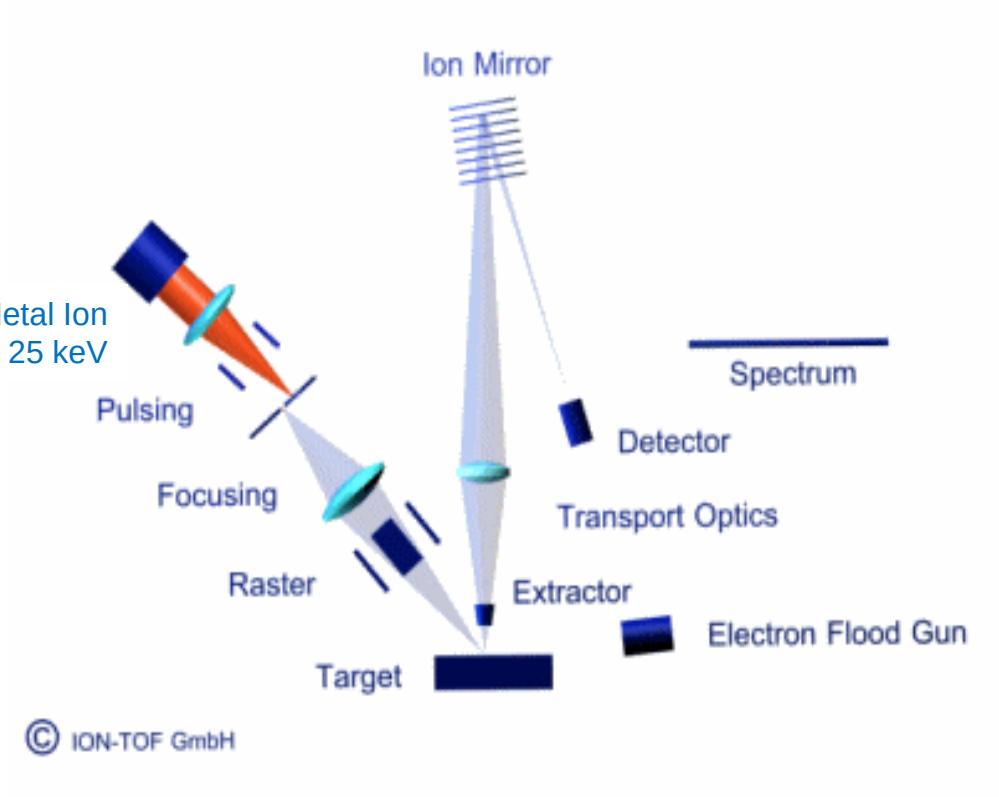
d'après J.-L. Guerquin-Kern, Insitut Curie, Orsay

TRIFT (PHI)


Energy Slit
ESA 2
ESA 1
ESA 3
Imaging
Aperture
Post-ESA
Blanker
Ion Detector
SE Detector
High Mass
Blanker
Cesium Gun
or FIB
Gas Gun
Ar+, O2+
LMIG
Ga+, Aun q+, Bin q+
Cluster Guns
Ar2500+, C60q+
Sample

Instrumentation TOF-SIMS IV (ION-TOF GmbH)

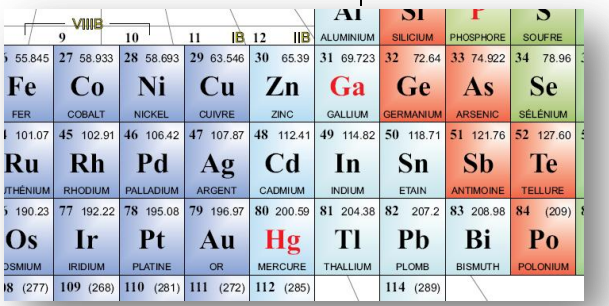




D. Touboul, F. Halgand, A. Brunelle, R. Kersting, E. Tallarek, B. Hagenhoff, O. Laprévote Anal. Chem. 2004, 76, 1550-1559
D. Touboul, O. Laprévote, A. Brunelle Curr. Opin. Chem. Biol. 2011, 15, 725-732

Augmenter l'émission ionique avec des agrégats

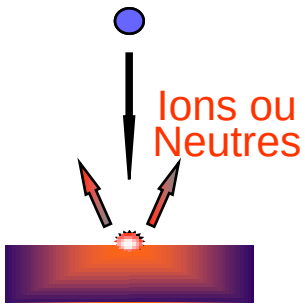
L'impact d'un projectile polyatomique est le moyen unique de bombarder une très petite surface simultanément avec plusieurs atomes



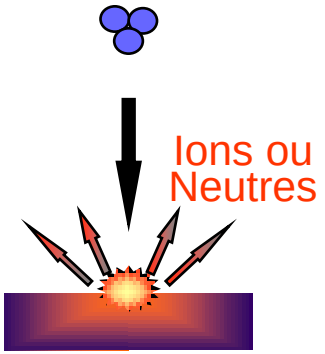
Dépôt d'une grande densité d'énergie très près de la surface

Importante augmentation de l'émission ionique

Projectile Ga_1^+

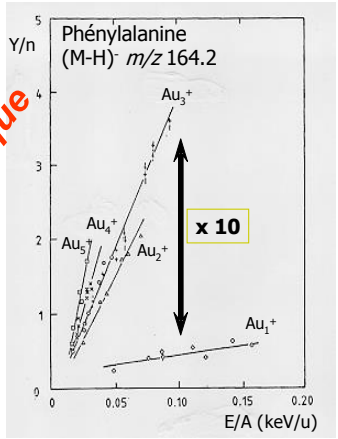


Projectile Bi_3^+



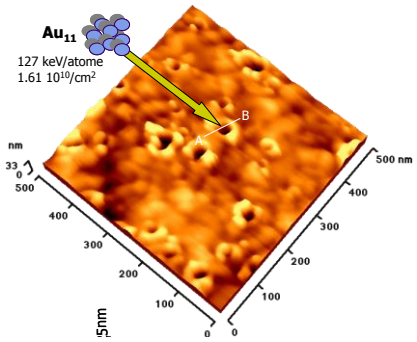
Augmenter l'émission ionique avec des agrégats

Emission ionique



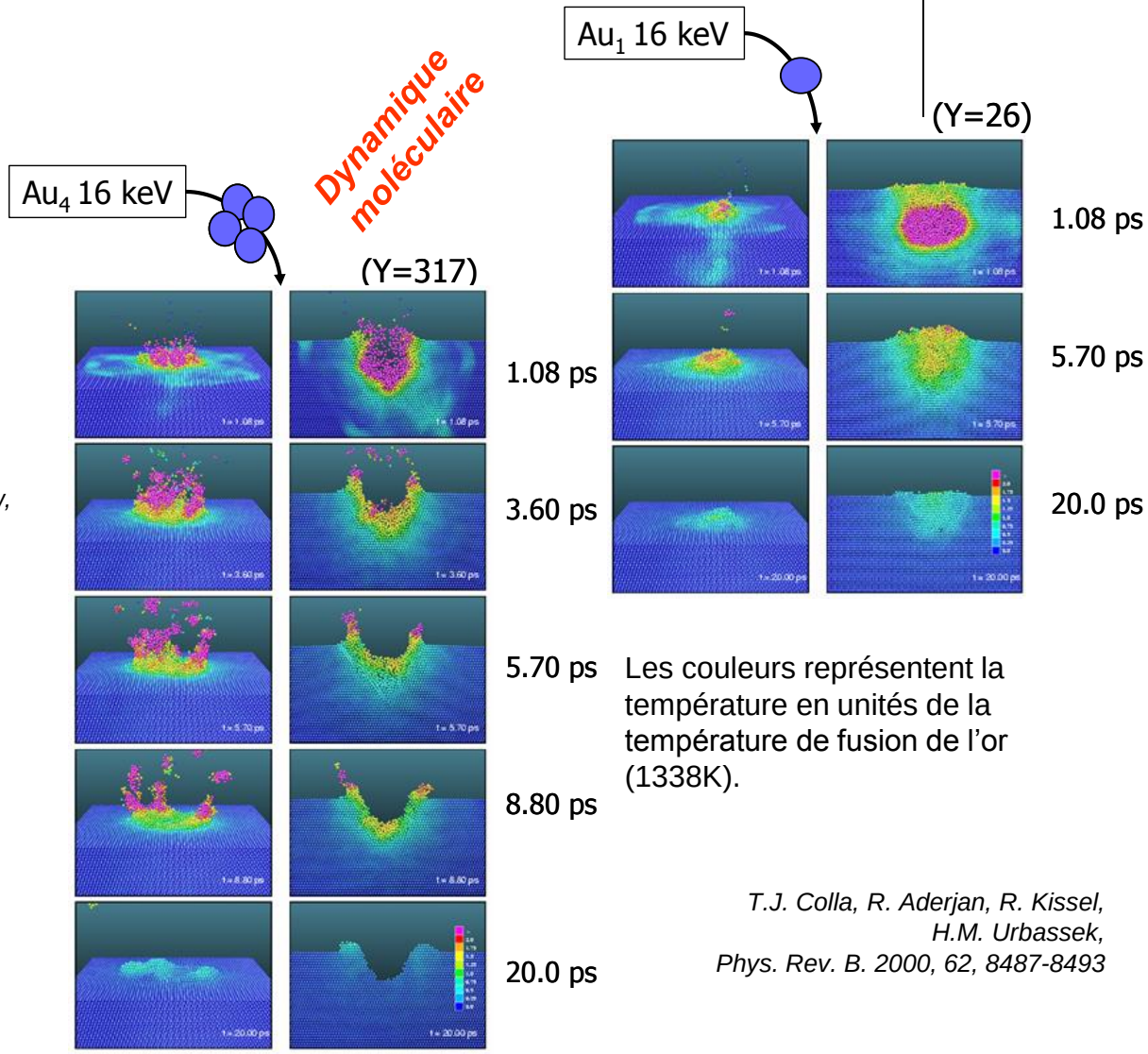
M. Benguerba, A. Brunelle, S. Della-Negra, J. Depauw, H. Joret, Y. Le Beyec, M.G. Blain, E.A. Schweikert, G. Ben Assayag, P. Sudraud
Nucl. Instrum. Methods. Phys. Res. B 1991, 62, 8-22

Pulvérisation



S. Bouneau, A. Brunelle, S. Della-Negra, J. Depauw, D. Jacquet, Y. Le Beyec, M. Pautrat, M. Fallavier, J.C. Poizat and H.H. Andersen,
Phys. Rev. B 2002, 65, 144106 1-10

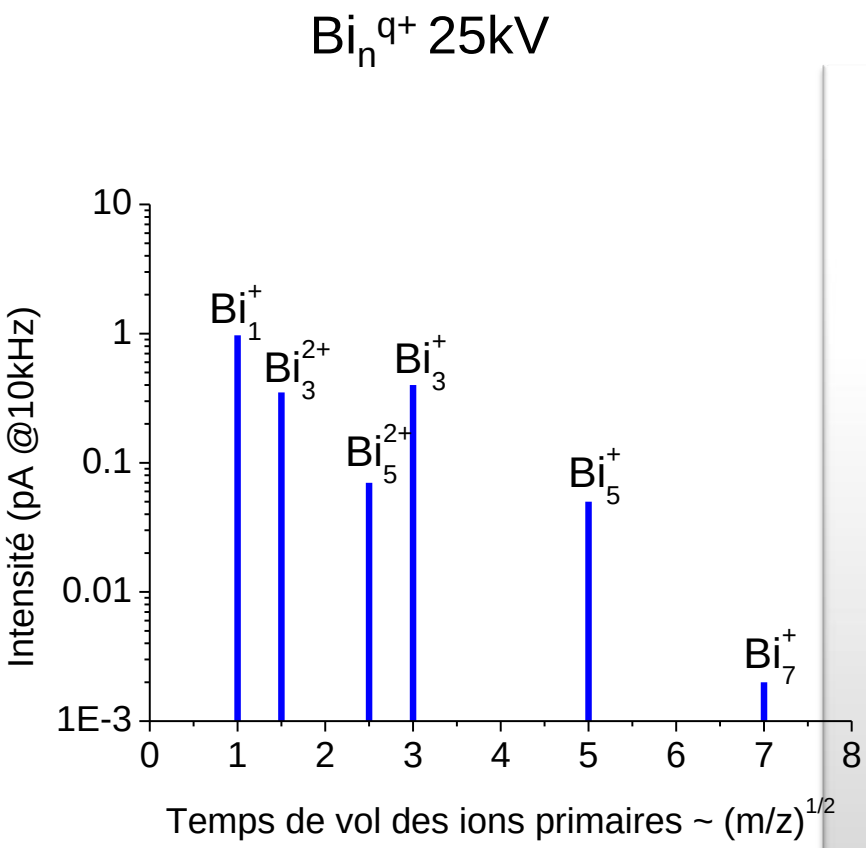
Dynamique moléculaire

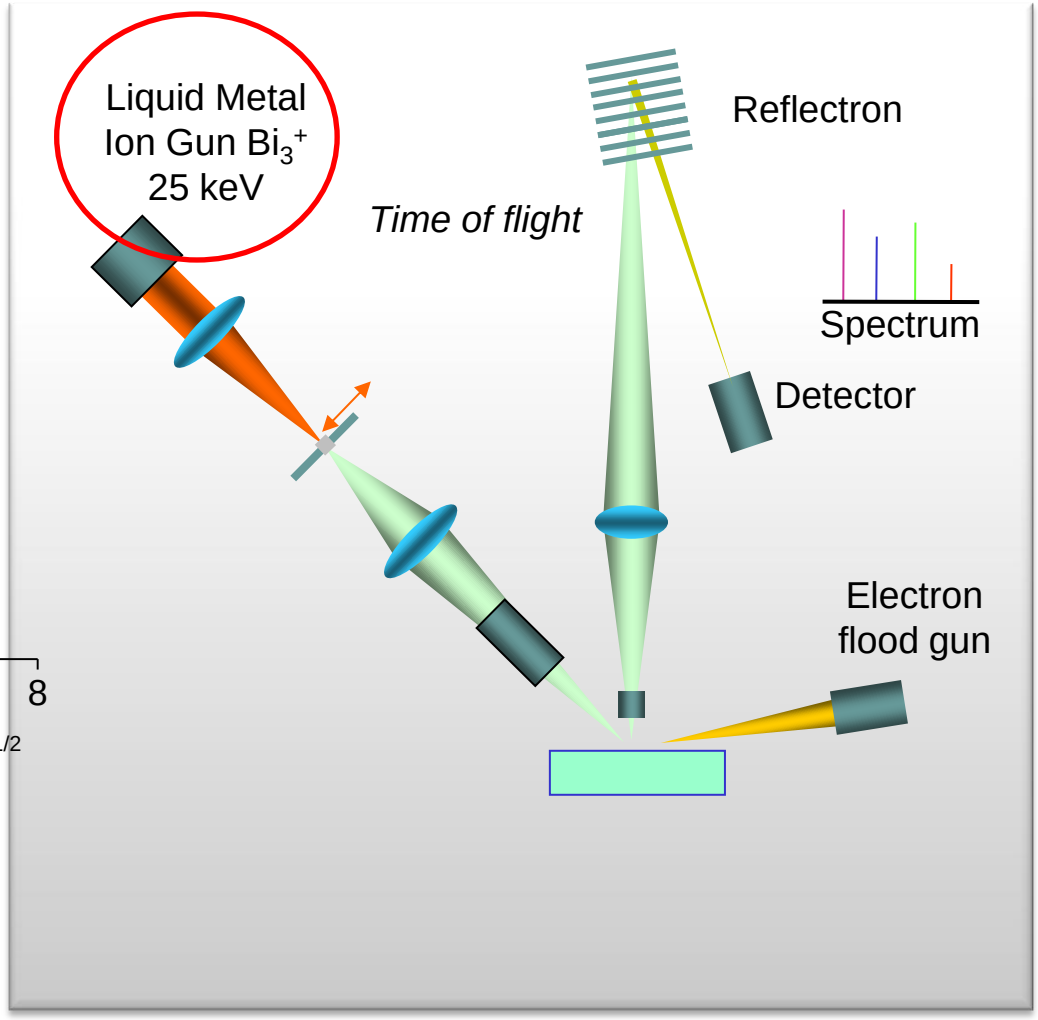


Les couleurs représentent la température en unités de la température de fusion de l'or (1338K).

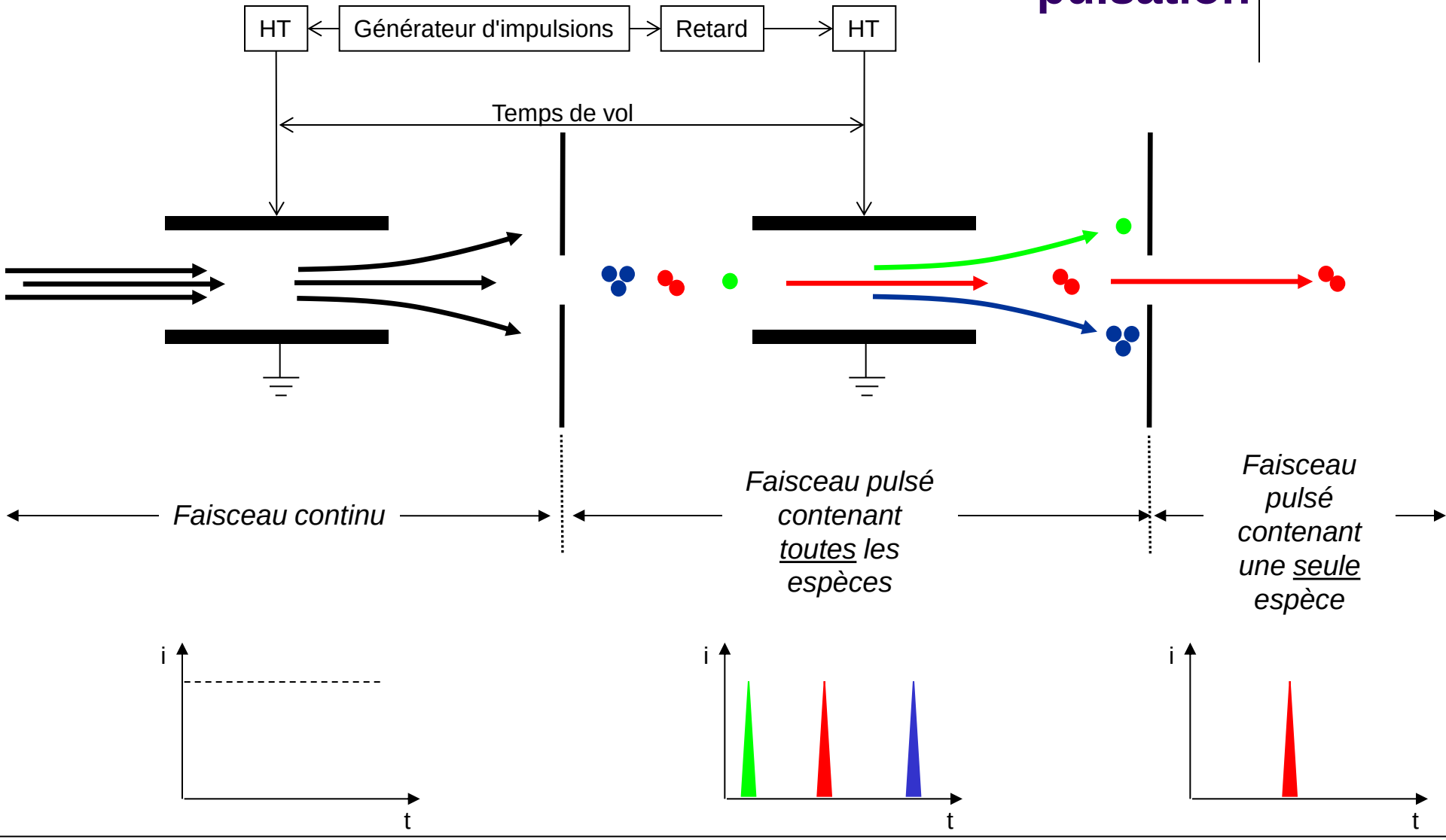
T.J. Colla, R. Aderjan, R. Kissel, H.M. Urbassek,
Phys. Rev. B. 2000, 62, 8487-8493

Composition d'un faisceau d'agrégats de bismuth

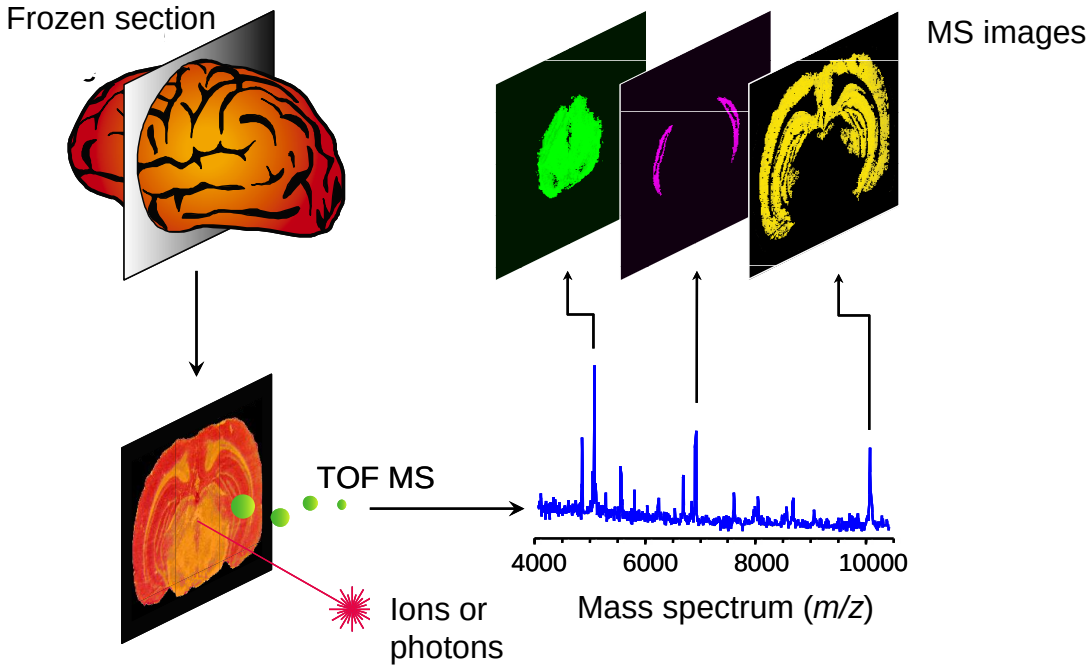




Sélection en masse des ions primaires par leur temps de vol entre deux jeux de plaques de pulsation



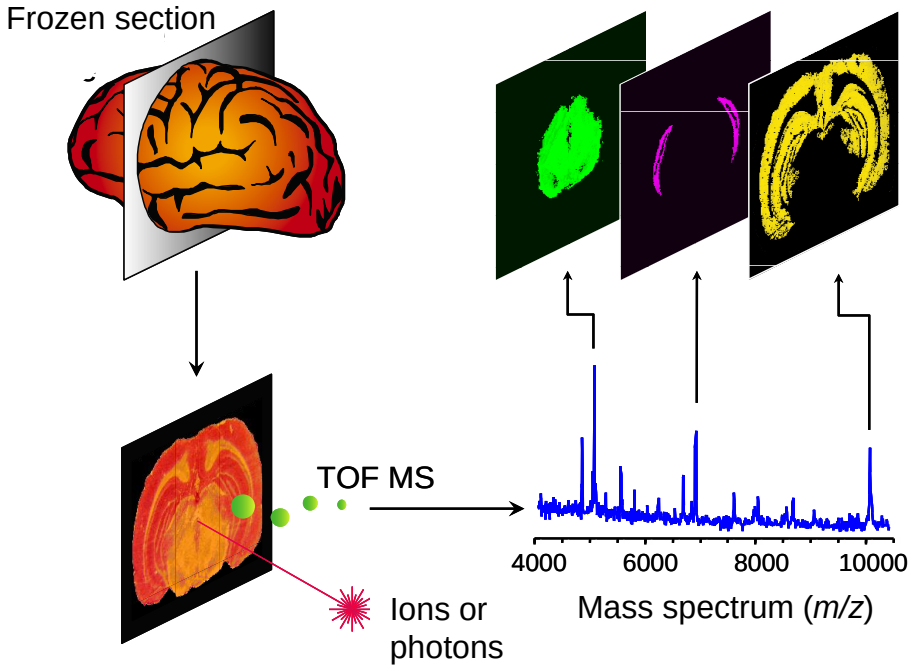
Mass Spectrometry Imaging
MALDI and SIMS



- **MS Images**
  - Ion density maps
  - 1 acquisition = one image for each peak and one spectrum per pixel…
  - Huge amount of data

M. Stoeckli, P. Chaurand, D.E. Hallahan, R.M. Caprioli, Nat. Med. 2001, 7, 493-496.

Mass Spectrometry Imaging
MALDI and SIMS



- **MS Images**
  - Ion density maps
  - 1 acquisition = one image for each peak and one spectrum per pixel…
  - Huge amount of data

	MALDI	SIMS
Desorption	UV Laser	Focused cluster ion beam
Spatial resolution	10-50 µm	400 nm - 2 µm
Sample	Dehydrated, Homogeneous matrix coating	Dehydrated No fixation No matrix
Mass range	$m/z > 200$	$m/z \leq 1500$
Compounds	Proteins, Peptides, **Lipids**, Drugs, Metabolites,…	**Lipids** Glycosphingolipids Cyclopeptides, Drugs, Metabolites, Inorganics,…

Matrix Assisted Laser Desorption Ionization
Secondary Ion Mass Spectrometry

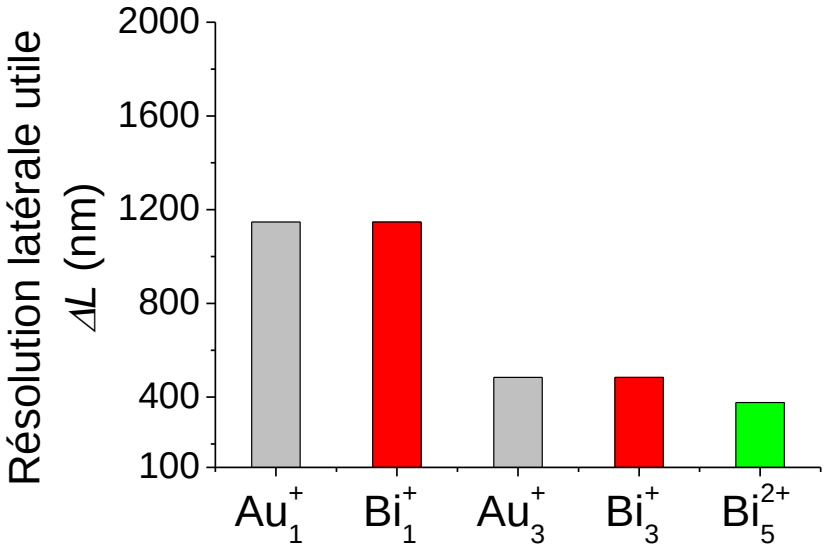
M. Stoeckli, P. Chaurand, D.E. Hallahan, R.M. Caprioli, Nat. Med. 2001, 7, 493-496.

Sensibilité et rendements d'émission ionique secondaire
Résolution latérale utile

Table 1. Estimation of the number of counts per pixel at a total primary ion dose of 10^{13} ions cm^{-2} as a function of pixel size and ion yield.

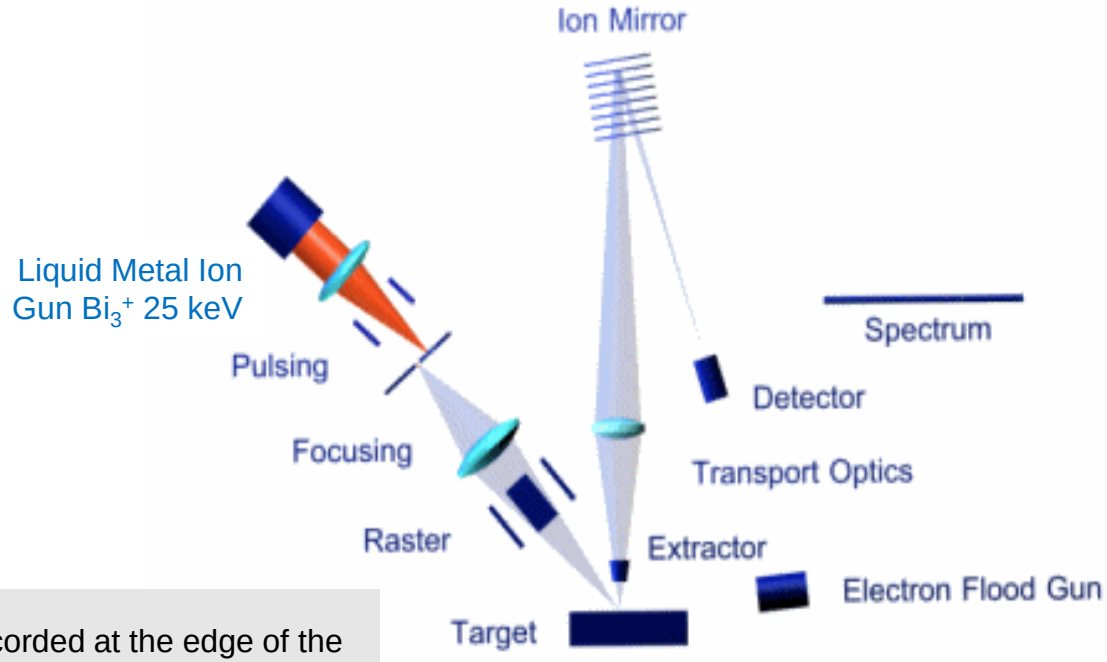
Ion yield	Pixel size	Atoms per pixel	Maximum counts per pixel
1.00E–02	10 µm × 10 µm	1E + 08	100,000
1.00E–02	1 µm × 1 µm	1,000,000	1000
1.00E–02	0.5 µm × 0.5 µm	250,000	250
1.00E–02	0.1 µm × 0.1 µm	10,000	10
1.00E–03	10 µm × 10 µm	1E + 08	10,000
1.00E–03	1 µm × 1 µm	1,000,000	100
1.00E–03	0.5 µm × 0.5 µm	250,000	25
1.00E–03	0.1 µm × 0.1 µm	10,000	1
1.00E–04	10 µm × 10 µm	1E + 08	1000
1.00E–04	1 µm × 1 µm	1,000,000	10
1.00E–04	0.5 µm × 0.5 µm	250,000	3
1.00E–04	0.1 µm × 0.1 µm	10,000	0

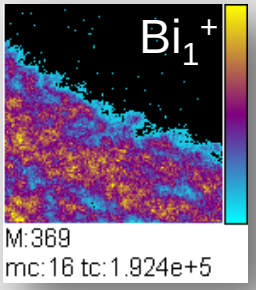
$$\Delta L = \sqrt{\frac{4}{E}} \qquad E = \frac{Y}{\sigma}$$

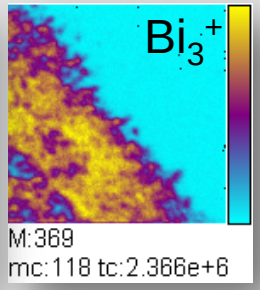


D. Touboul, F. Kollmer, E. Niehuis, A. Brunelle, O. Laprévote, J. Am. Soc. Mass Spectrom., 2005, 16, 1608-1618

Instrumentation TOF-SIMS IV (ION-TOF GmbH)







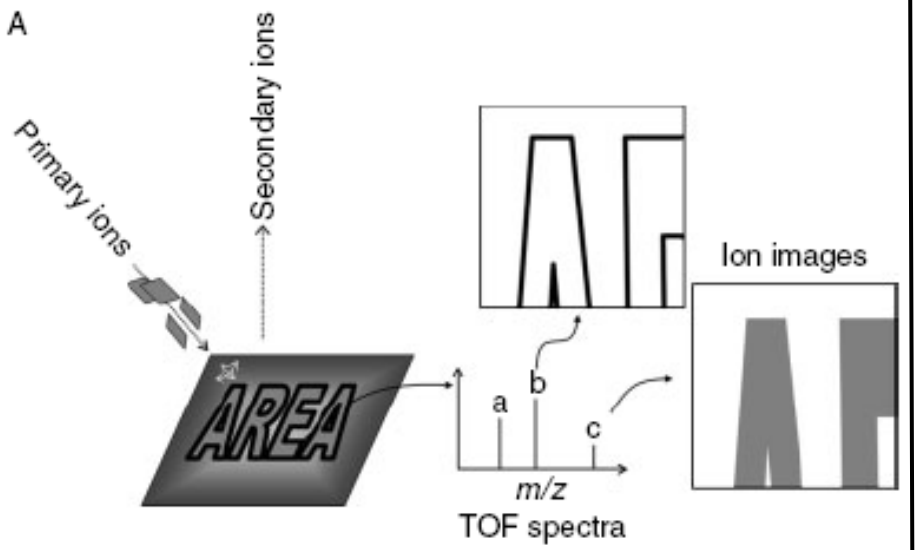
Images recorded at the edge of the *corpus callosum* on a rat brain section
256x256 pixels, 256x256 µm^2
Pixel size 1x1 µm^2
Same fluences 10^{12} ions.cm^{-2}
Acquisition time : 4 and 10 minutes

D. Touboul, F. Halgand, A. Brunelle, R. Kersting, E. Tallarek, B. Hagenhoff, O. Laprévote Anal. Chem. 2004, 76, 1550-1559
D. Touboul, O. Laprévote, A. Brunelle Curr. Opin. Chem. Biol. 2011, 15, 725-732

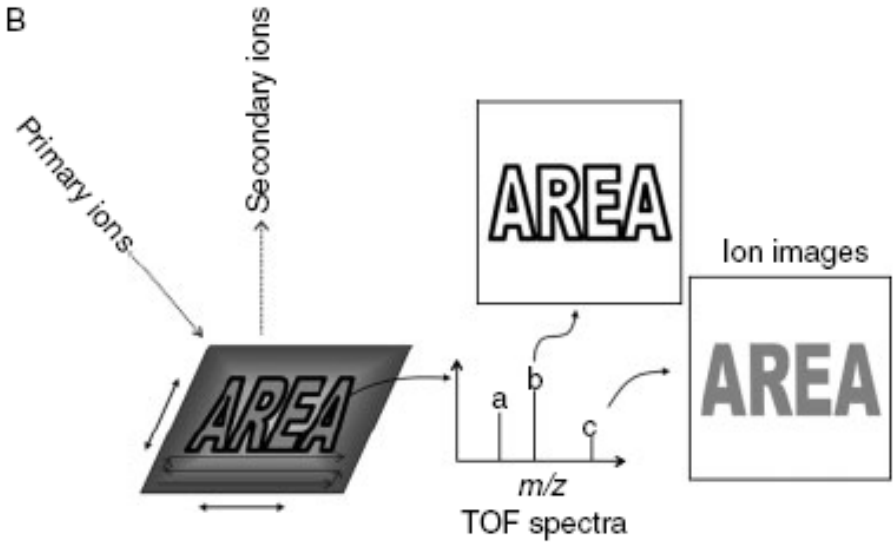
Acquisition des images: Balayage du faisceau d'ions primaires et déplacement de l'échantillon

Champ de l'optique d'extraction
à l'entrée de l'analyseur = 500 x 500 µm^2

- A: Objet ≤ 500 x 500 µm^2
  - Porte échantillon immobile
  - Balayage du faisceau pixel par pixel
  - Image vidéo simultanée

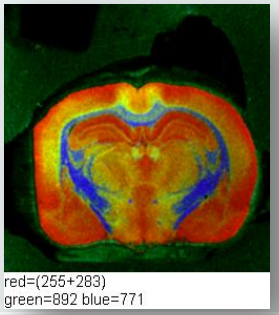
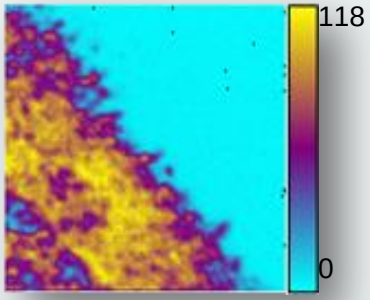
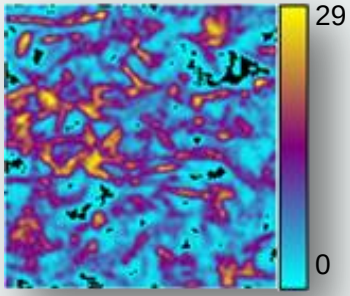


- B: Objet > 500 x 500 µm^2
  - Porte échantillon mobile
  - Le faisceau balaye éventuellement à l'intérieur d'un pixel



Ajustement du nombre de pixels (de 64 x 64 à 2048 x 2048), du nombre de tirs par pixels (ou scans) et de la durée d'analyse (gamme de masse), paramètres conditionnant la durée d'acquisition et la dose (ions.cm^{-2})

TOF-SIMS lipid imaging

Spatial resolution	50 µm	1 µm	200 nm
Acquisition time for a 256x256 pixels image	1 hour	15 minutes	30-60 minutes
Examples on Rat brain sections			
Field of view	18 mm x 18 mm	256 µm x 256 µm	55 µm x 55 µm
	3 colour overlay	edge of *corpus callosum* Cholesterol	inside of *corpus callosum* Cholesterol
➔	Several images (positive and negative ion modes) can always be recorded on the same area		

D. Touboul, F. Kollmer, E. Niehuis, A. Brunelle, O. Laprévote, J. Am. Soc. Mass Spectrom. 2005, 16, 1608-1618

Imagerie biologique et spectrométrie de masse

In vivo :

- Rayon X (absorption)
- TEP (Tomographie par émission de positons)
- IRM (Imagerie par Résonance Magnétique, absorption/émission)

Ex vivo :

- Fluorescence (absorption/émission)
- Marquage radioactif (émission)
- IR (Infra-Rouge, absorption)
- MEB (Microscopie électronique à balayage, molécule)
- MFA (Microscopie à force atomique, atomes)…

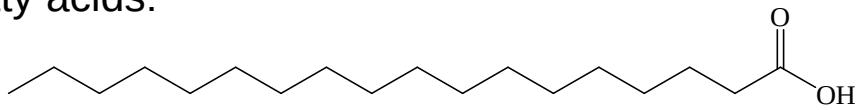
Mais toutes ces techniques sont basées sur des signaux de molécules connues a priori …

Nouvelles techniques d'imagerie biologique permettant d'accéder à la distribution, à la composition et à la structure chimique d'un grand nombre de composés en mélange sur une surface et sans aucun *a priori*

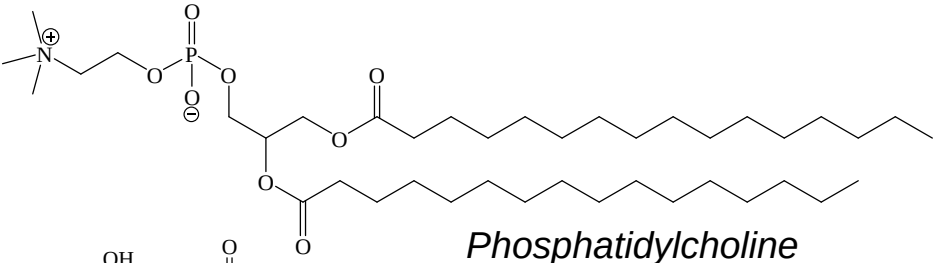
… seule la Spectrométrie de Masse permet d'accéder à ces données grâce aux sources laser (MALDI) ou aux sources d'ions (SIMS) focalisées.

Targeted lipids

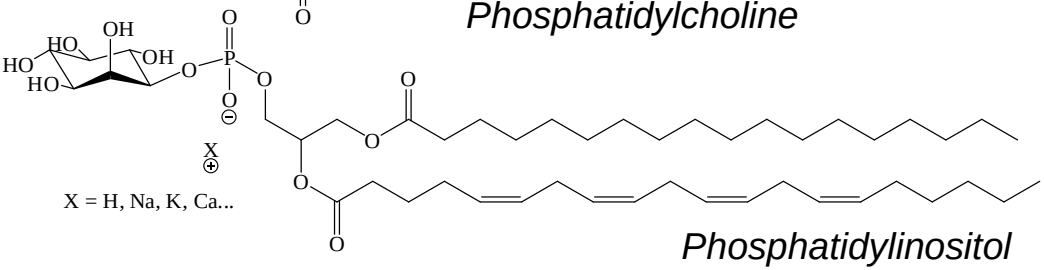
Fatty acids:



Phospholipids:

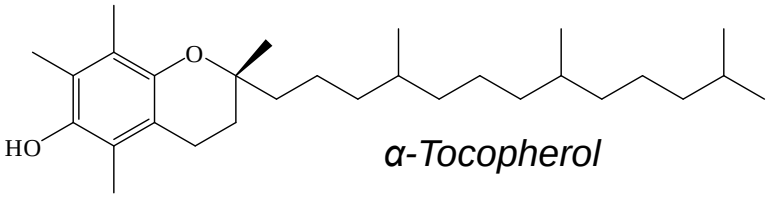


Phosphatidylcholine

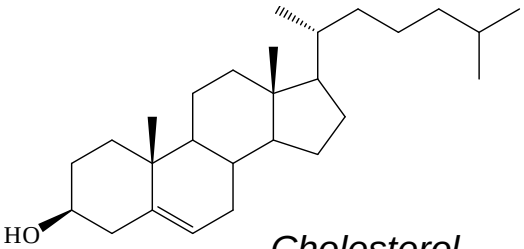


Phosphatidylinositol

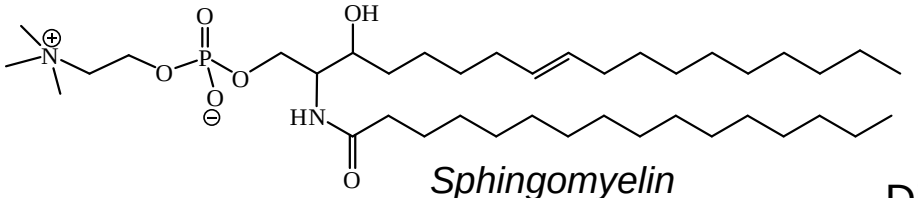
Sterols:



α-Tocopherol

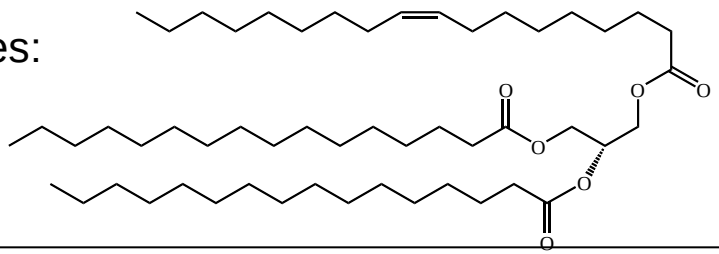


Cholesterol

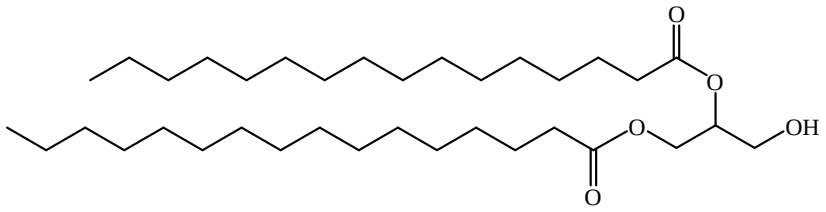


Sphingomyelin

Triglycerides:



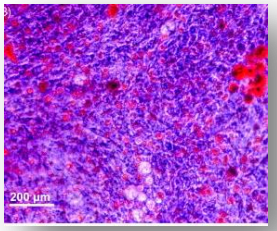
Diglycerides:



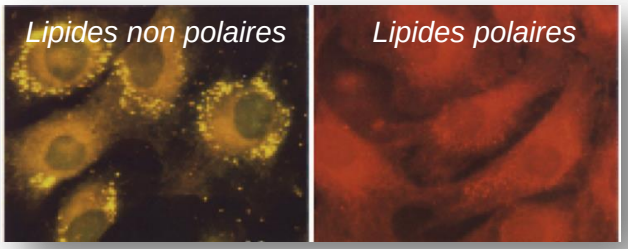
Étude des lipides

- Lipides :
  - Grande variété de fonctions biologiques
    - Composants structurels majeurs des membranes cellulaires
    - Stockage d'énergie
    - Antioxydants
    - Signaux cellulaires
  - Composition et distribution lipidiques qui dépendent:
    - Du type cellulaire
    - De l'état d'un groupe de cellules
    - Des conditions physiologiques
- Imageries chimiques : anatomo-pathologie ➔ colorants lipophiles

Noir Soudan



Rouge de Nil

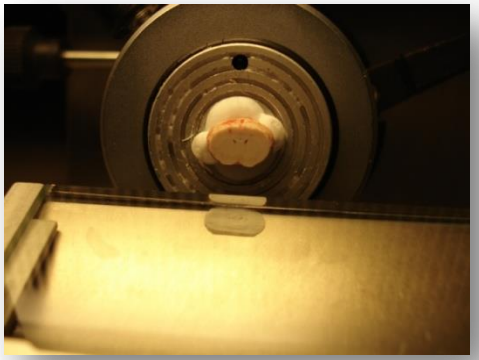


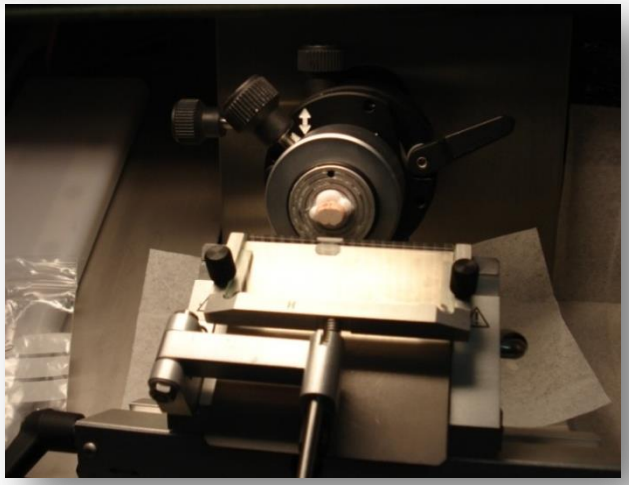
Excitation 450-500 nm
Emission >528 nm

Excitation 515-560 nm
Emission >590 nm

  - Peu spécifiques : tous les lipides, ou seulement des familles de lipides
- Spectrométrie de masse couplée à des chromatographies gazeuses ou liquides :
  - Excellent pour quantification et/ou identification
  - Extraction préalable ➔ perte de la localisation sur le tissu

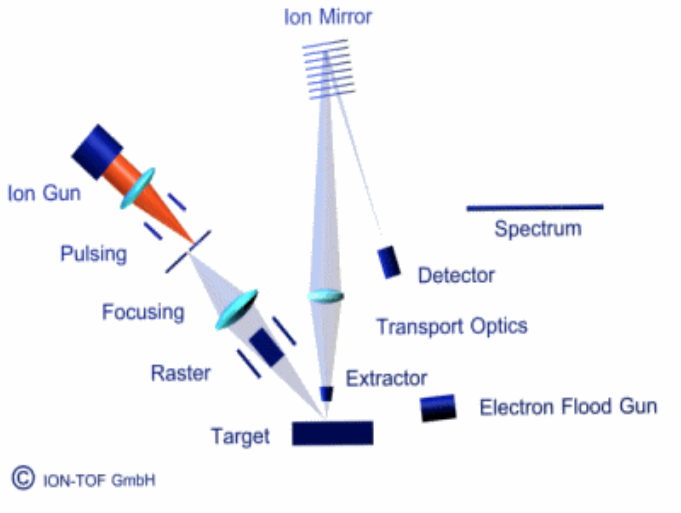
Préparation des échantillons: coupes au cryostat



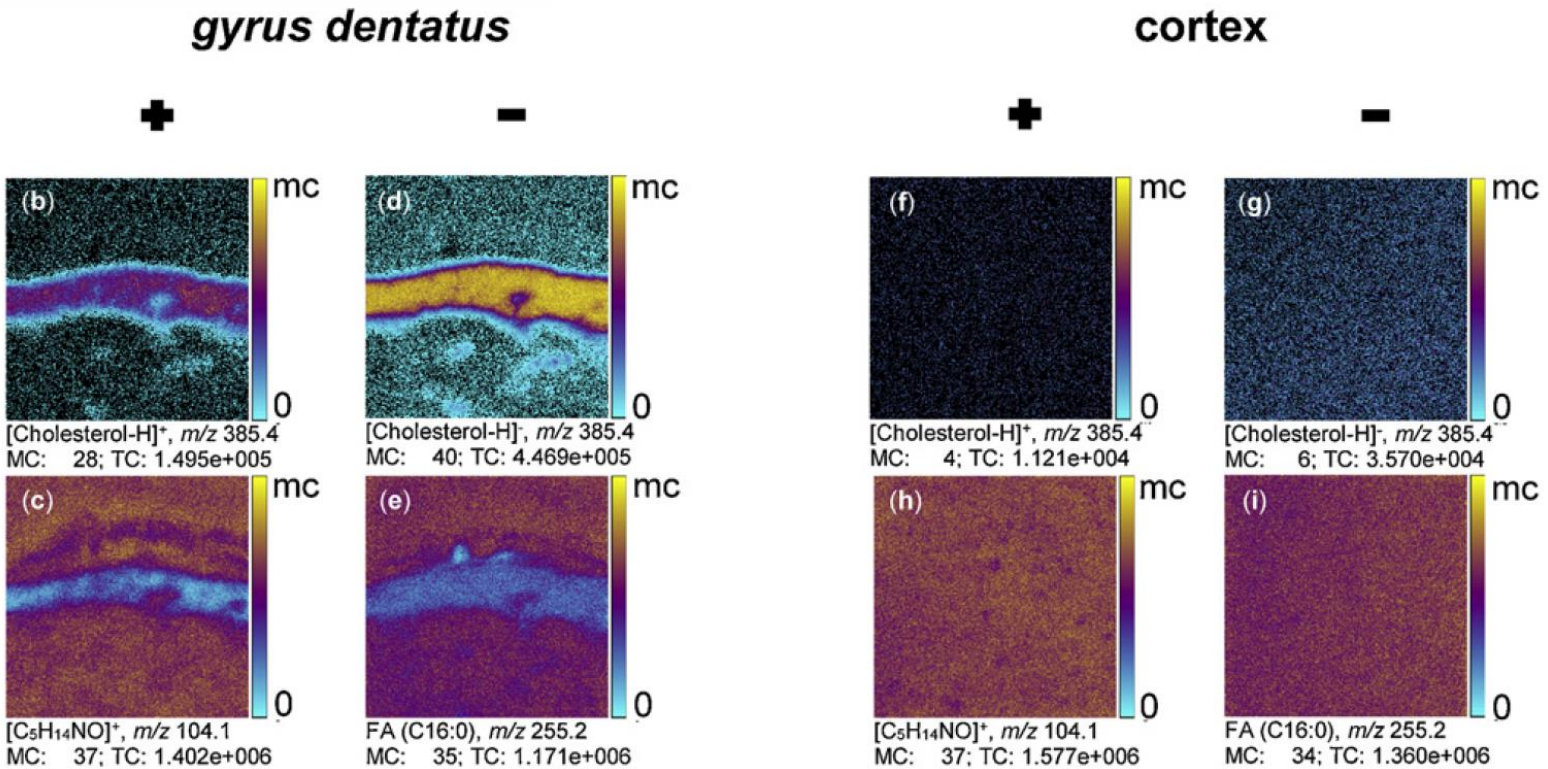


- Après dissection, congélation rapide à -80°C (Azote liquide)
- Montage du tissu sur bloc d'OCT
- Sections de 10-20 µm coupées à l'aide d'un cryostat (-20°C)
- Montage des sections sur une plaque (inox, verre ou silicium)
- Coupes sériées pour SIMS et histologie

Is imaging a reproducible technique ?



Bich C, Touboul D, Brunelle A.
Int J Mass Spectrom. 2013;337:43-49.



Instrumental repeatability

3 adjacent brain sections

m/z		Brain 1					
		L		R		L+R	
		Mean value	CV%	Mean value	CV%	Mean value	CV%
gyrus dentatus							
Positive							
104.1	$[C_5H_{14}NO]^+$	32	0	31	3.2	31.5	2.5
165.1	[Vit E−$C_{19}H_{37}$]$^+$	21	0	24.6	4.4	21.6	4.6
184.1	$[C_5H_{15}NO_4P]^+$	35	0	35	0	35	0
185.1	$[C_5(^{13}C)H_{15}NO_4P]^+$	17	5.8	16	6.2	16.5	6
205.2	[Vit E−$C_{16}H_{33}$]$^+$	8	12.5	7.6	6.6	7.8	8.9
224.1	$[C_8H_{19}NO_4P]^+$	23.6	4.6	24	0	23.8	2.9
369.4	[Cholesterol−H_2O+H]$^+$	33.3	3.3	34.3	1.7	33.8	2.6
385.3	[Cholesterol−H]$^+$	17.3	11.4	19	5.2	18.1	
430.3	[Vit E]$^{+\cdot}$	7.6	19.7	14	0	10.8	33.3
Negative							
241.05	[Phosphinositol−H_2O]	11.6	12.9	11	9	11.3	10.6
255.2	FA C16:0	31.3	1.9	30.6	1.9	31	1.9
281.2	FA C18:1	26	7.7	25.3	2.3	25.6	5
283.2	FA C18:0	23	4.3	22.6	4.8	22.8	3.9
385.4	[Cholesterol−H]$^-$	33.6	1.7	33.6	1.7	33.6	1.4
386.4	[Cholesterol (^{13}C)−H]	19.6	7.6	20	0	19.8	4.5
806.6	[Sulfatide36:1−H]	7	0	7	14.2	7	8.5
Cortex							
Positive							
104.1	$[C_5H_{14}NO]^+$	31	3.22	31.3	1.9	31.1	2.2
165.1	[Vit E−$C_{19}H_{37}$]$^+$	16	6.2	17.3	3.4	16.6	6
184.1	$[C_5H_{15}NO_4P]^+$	35	0	35	0	35	0
185.1	$[C_5(^{13}C)H_{15}NO_4P]^+$	16.6	3.6	16	6.2	16.3	4.9
205.2	[Vit E−$C_{16}H_{33}$]$^+$	6.6	9	7	14.2	6.8	10.2
224.1	$[C_8H_{19}NO_4P]^+$	23.6	8.4	22.3	4.9	23	6.9
369.4	[Cholesterol−H_2O+H]$^+$	5.6	10.7	5.3	11.3	5.5	9
385.3	[Cholesterol−H]$^+$	4.3	13.9	4	0	4.1	9.7
430.3	[Vit E]$^{+\cdot}$	4.3	13.9	4.6	13	4.5	11.1
Negative							
241.05	[Phosphinositol−H_2O]	11	9	10.3	5.8	10.6	7.5
255.2	FA C16:0	31.3	1.9	31	3.2	31.1	2.2
281.2	FA C18:1	24	4.1	24	4.1	24	3.7
283.2	FA C18:0	22.6	4.8	22	0	22.3	3.5
385.4	[Cholesterol−H]$^-$	6	16.7	5.6	10.7	5.8	12
386.4	[Cholesterol (^{13}C)−H]	4	0	4.3	13.9	4.1	9.7
806.6	[Sulfatide36:1−H]	6.3	9.5	6	0	6.1	6.5

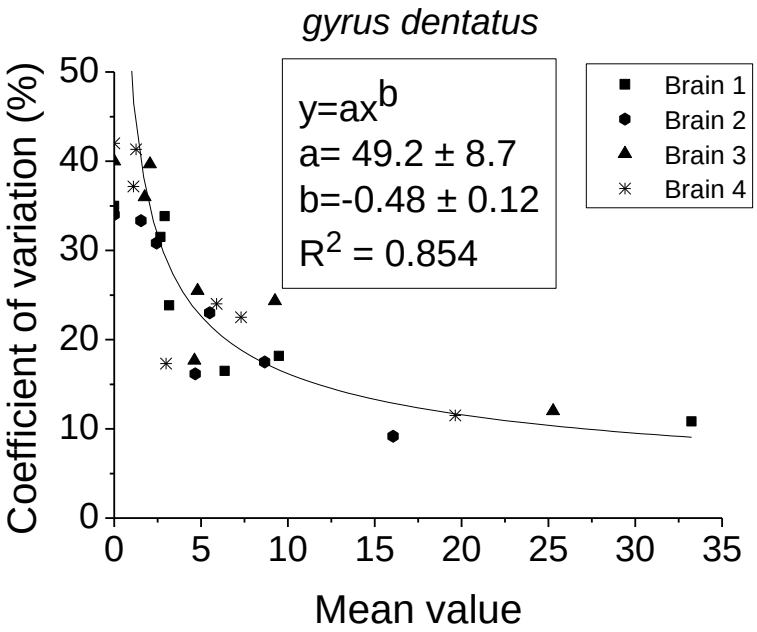
Biological repeatability

3 adjacent brain sections / 4 brains

m/z		Brain 1		Brain 2		Brain 3		Brain 4		Global Mean value	
		L+R		L+R		L+R		L+R		L+R	
		Mean value	CV%	Mean value	CV%	Mean value	CV%	Mean value	CV%	Mean value	CV%
gyrus dentatus											
Positive											
104.1	$[C_5H_{14}NO]^+$	31.5	2.5	30.8	2.7	36	2.7	37.1	1	33.9	8.5
165.1	[Vit E–$C_{19}H$	21.6	4.6	19	4.7	23.6	4.7	24.1	2.9	22.1	10.3
184.1	$[C_5H_{15}NO4P]^+$	35	0	34	0	40	0	42	0	37.8	9.1
185.1	$[C_5(^{13}C)H_{15}NO_4P]^+$	16.5	6	16.1	4.3	17.6	4.3	17.3	2.8	16.9	5.8
205.2	[Vit E–$C_{16}H_{33}$]$^+$	7.8	8.9	7.3	10.9	7.6	10.9	7.8	8.9	7.7	9.2
224.1	$[C_8H_{19}NO_4P]^+$	23.8	2.9	23	5.2	25.5	5.2	24	5.8	24.1	6.0
369.4	[Cholesterol–$H_2O^+H]^+$	33.8	2.6	33.3	1.5	29.6	1.5	41.3	1.2	37.0	9.9
385.3	[Cholesterol–H]$^+$	18.1	9.3	17.5	8.5	24.3	8.5	22.5	7.1	20.6	16.4
430.3	[Vit E]$^{+\cdot}$	10.8	33.3	9.1	15.3	12	15.3	11.5	19.1	10.9	25.3
Negative											
241.05	[Phosphinositol–H_2O]	11.3	10.6	11	8.1	10.8	8.1	10.6	4.7	11.0	8.7
255.2	FA C16:0	31	1.9	29.5	1.6	33.8	1.6	33.8	4.1	32.0	6.6
281.2	FA C18:1	25.6	5	25.3	3.9	27.1	3.9	27.6	4.7	26.5	5.6
283.2	FA C18:0	22.8	3.9	22	4	24.3	4	23.8	4.6	23.3	5.6
385.4	[Cholesterol–H]$^-$	33.6	1.4	33.3	1.5	39.5	1.5	40.8	3.4	36.8	9.6
386.4	[Cholesterol (^{13}C)–H]$^-$	19.8	4.5	18.8	3.7	22.1	3.7	23.1	3	21.0	9.4
806.6	[Sulfatide36:1–H]$^-$	7	8.5	7.1	15.4	6.3	15.4	7.1	5.6	6.9	11.2

Bich C, Touboul D, Brunelle A. Int J Mass Spectrom. 2013;337:43-49.

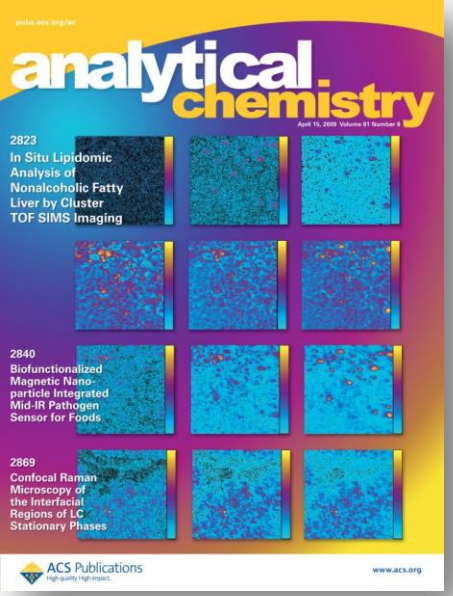
Minimal number of samples



	Number of sample			
C.V.	α≤ 1%	α≤ 5%	α≤ 10%	α≤ 20%
1	7	-	-	-
2	27	-	-	-
3	60	1	-	-
4	106	2	-	-
5	166	4	-	-
6	239	6	1	-
7	325	8	1	-
8	425	10	2	-
9	537	12	2	-
10	664	15	3	-
11	803	19	3	-
12	956	22	4	-
13	1121	26	5	-
14	1301	30	5	-
15	1493	35	6	-
16	1699	39	7	1
17	1918	44	8	1
18	2150	50	9	1
19	2396	55	10	1
20	2654	61	11	2

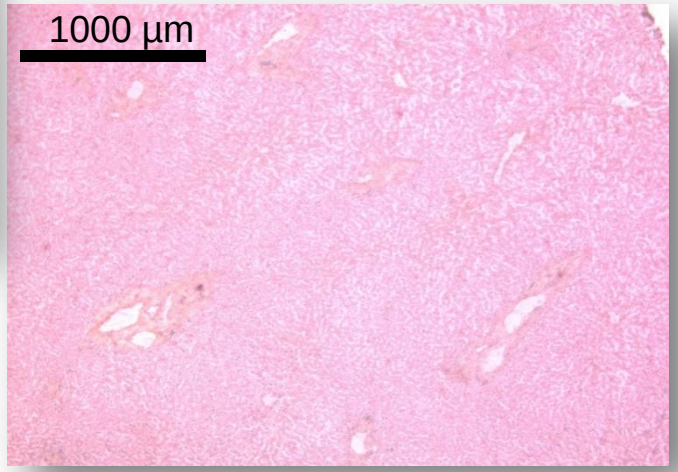
90% of the data measured during an experiment will be in the confidence interval

Bich C, Touboul D, Brunelle A. Int J Mass Spectrom. 2013;337:43-49.

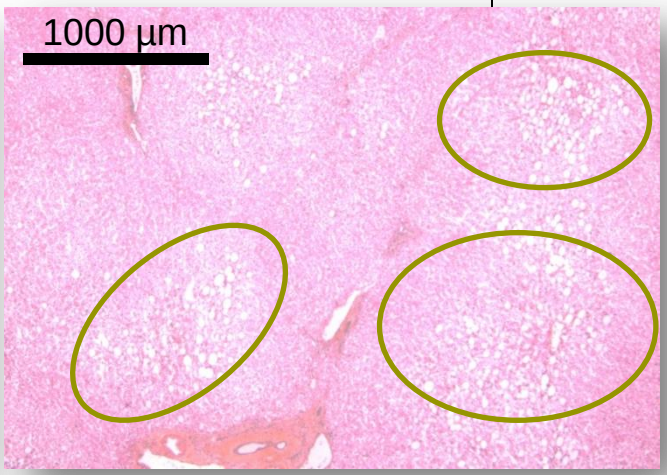


Non-alcoholic fatty liver disease (NAFLD)

H&E staining



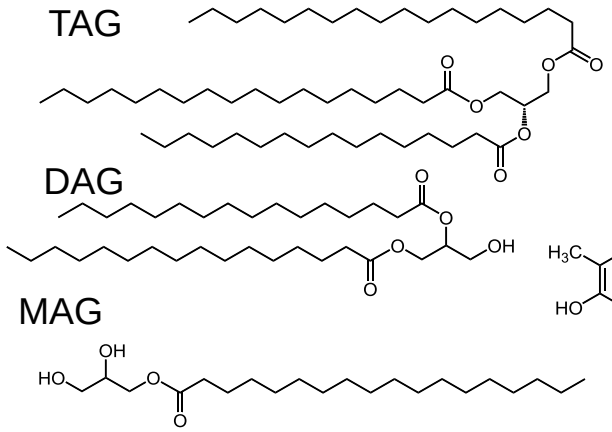
Control liver

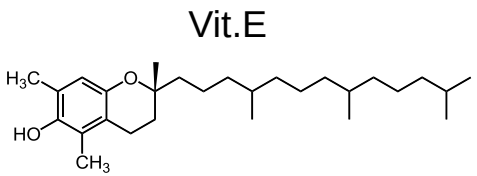


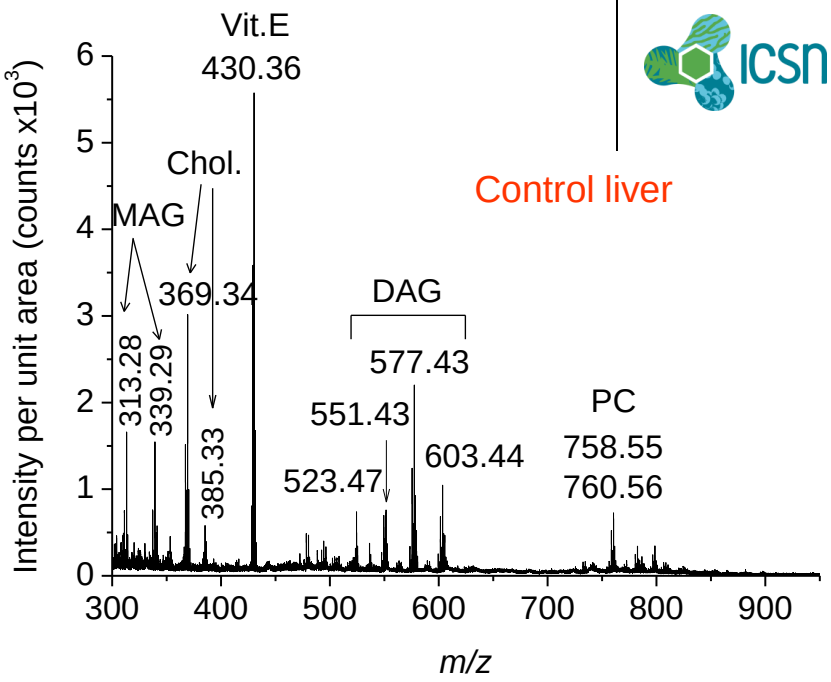
Fatty liver

- Accumulation of lipids (mainly diacyl- and triacylglycerols) in hepatocytes,
- On stained sections, lipid vesicles are observed in fatty liver but are not observable in healthy liver,
- NAFLD represents, in the absence of alcohol abuse, a wide spectrum of conditions ranging from simple steatosis (NAFL) to non-alcoholic steatohepatitis (NASH), that may progress to cirrhosis, cancer and necessitate liver transplantation.
- Collaboration François Le Naour , INSERM, Villejuif

Lipid profile of a fatty liver : Steatotic area, positive ion mode



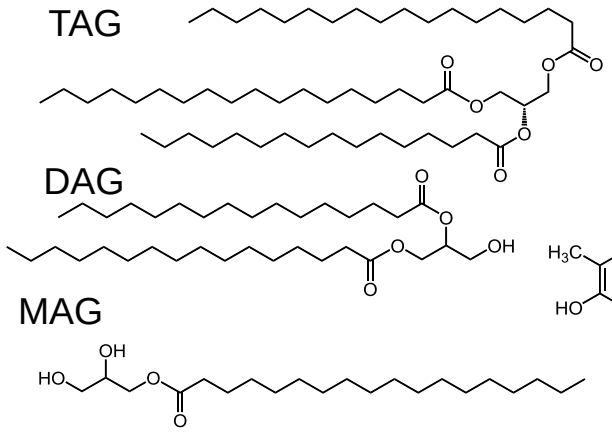


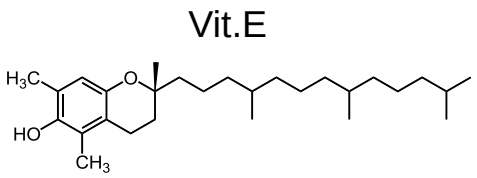


Control liver

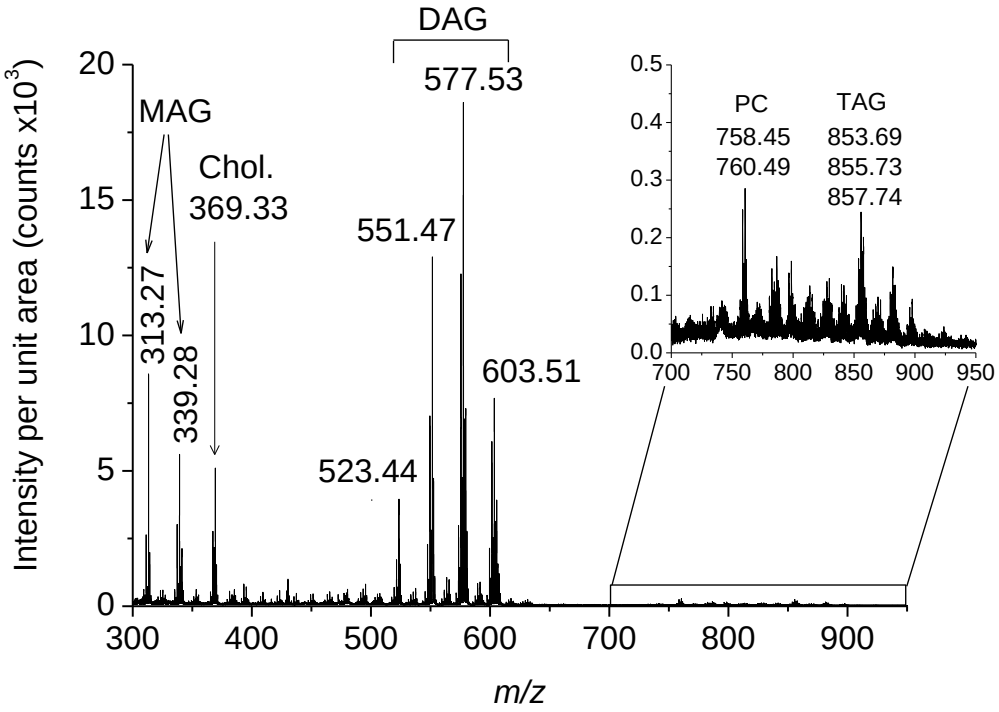
D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote, Anal. Chem. 2009, 81, 2823-2831

Lipid profile of a fatty liver : Steatotic area, positive ion mode

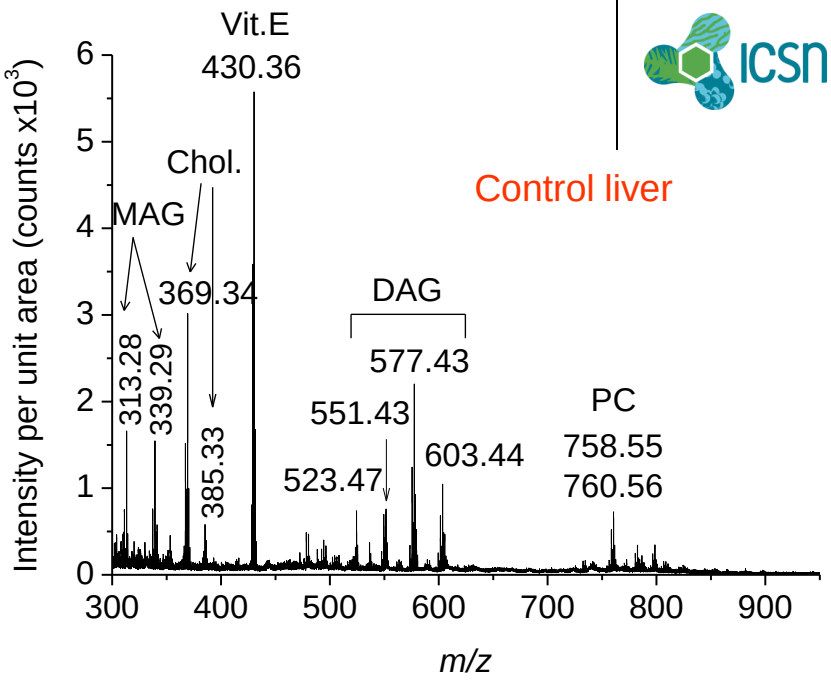




Fatty liver - steatotic area



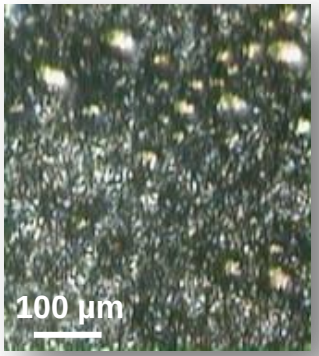
Control liver

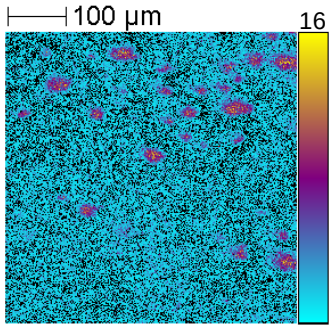


- Increase fo the diacylglycerol signal in the steatotic area
- Strong decrease of vitamin E signal
- Detection of triacylglycerols

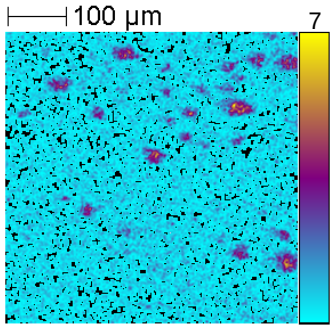
D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote, Anal. Chem. 2009, 81, 2823-2831

Lipid profile of a fatty liver : Steatotic area, positive ion mode

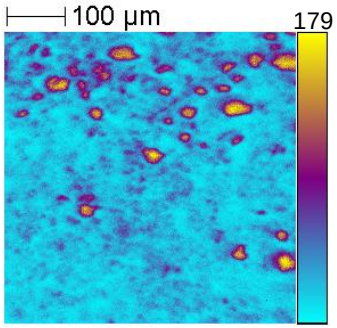




Cholesterol



Sum of TAGs

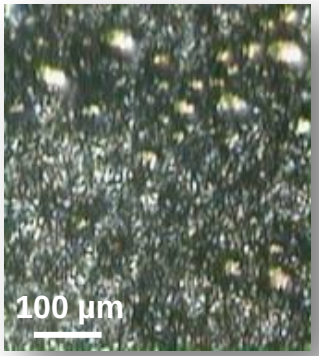


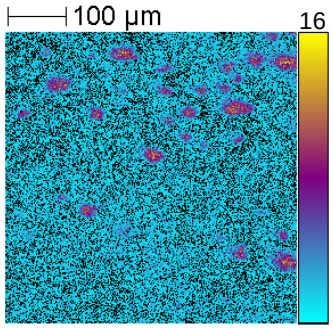
Sum of DAGs

In agreement with the disease description

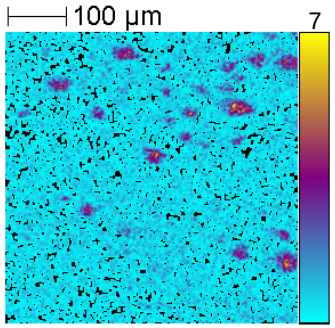
D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote, Anal. Chem. 2009, 81, 2823-2831

Lipid profile of a fatty liver : Steatotic area, positive ion mode

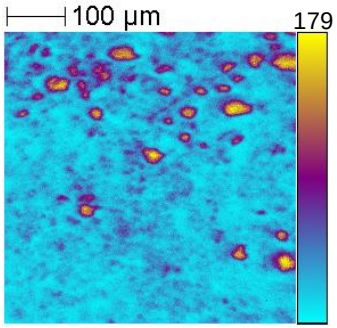




Cholesterol

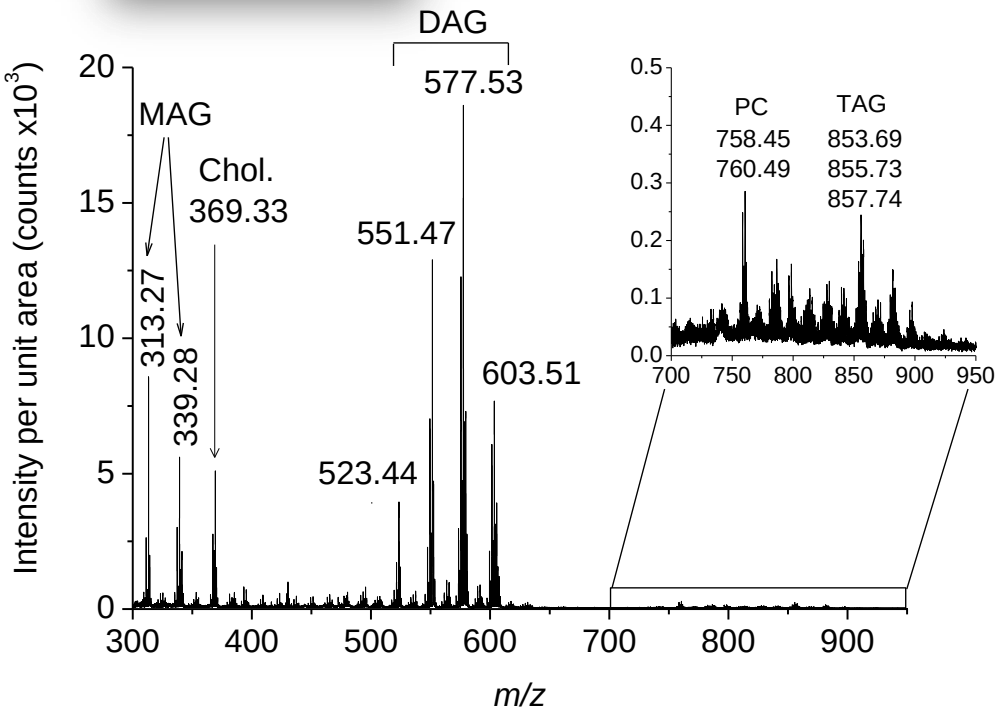


Sum of TAGs



Sum of DAGs

In agreement with the disease description



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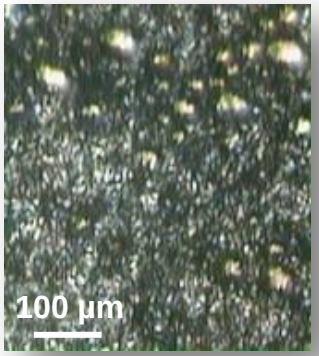
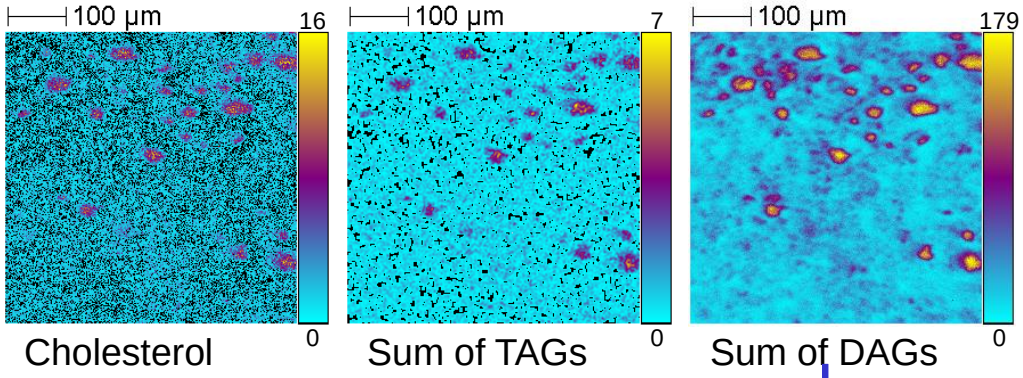
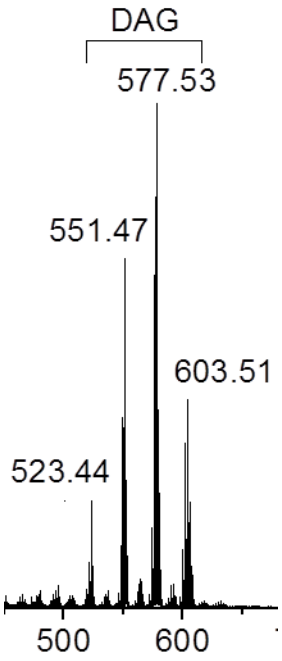
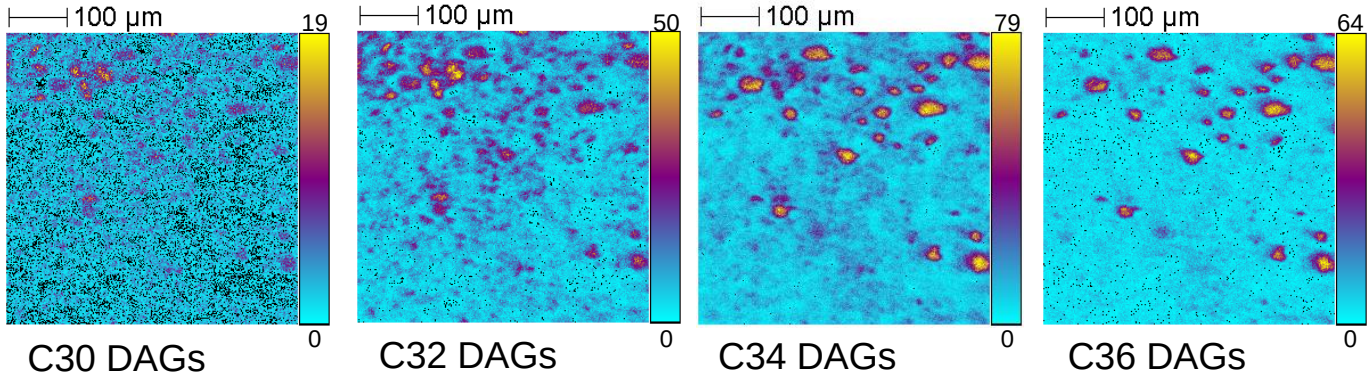
Lipid profile of a fatty liver : Steatotic area, positive ion mode





In agreement with the disease description

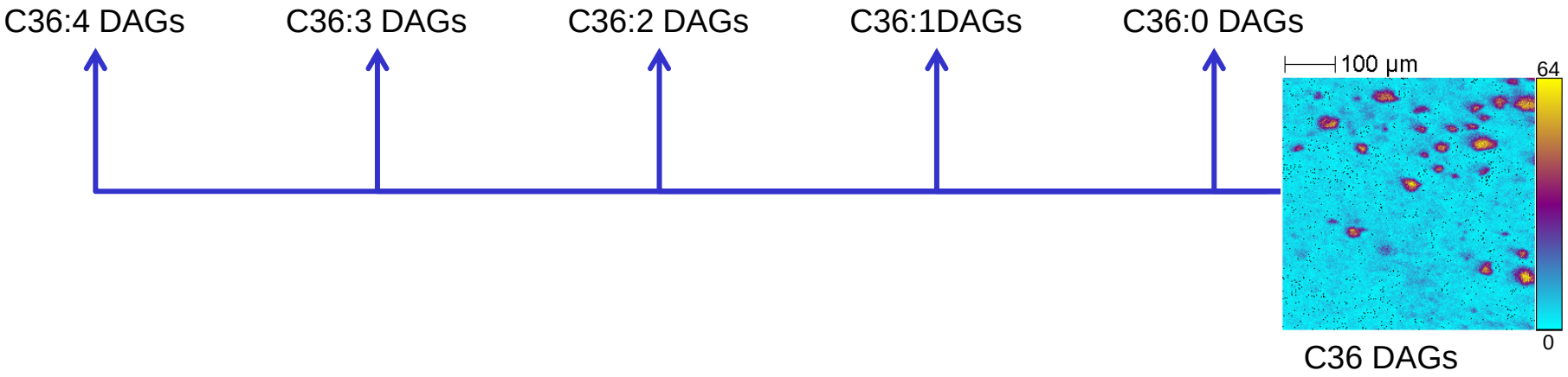




➔ Only mass spectrometry imaging makes possible to distinguish between the different localizations among the same lipid species

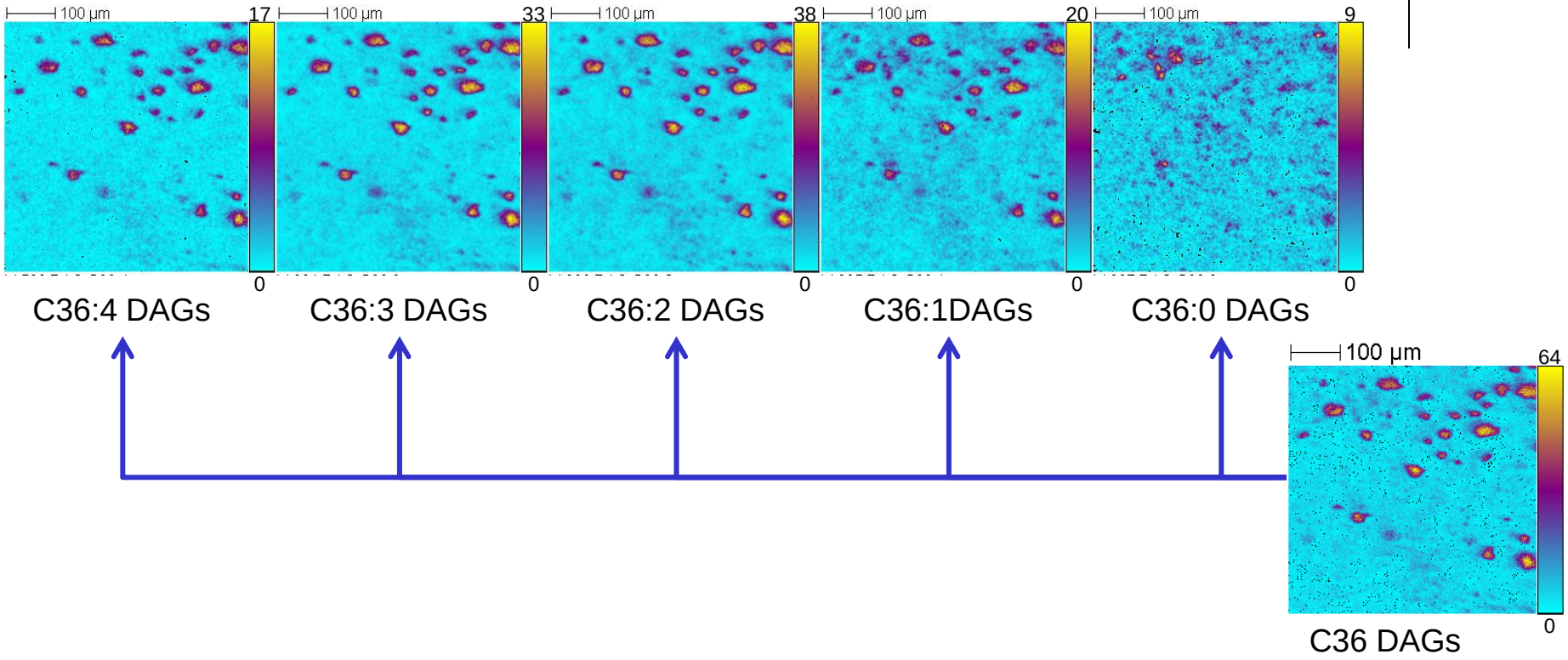
D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote, Anal. Chem. 2009, 81, 2823-2831

Lipid profile of a fatty liver : Steatotic area, positive ion mode



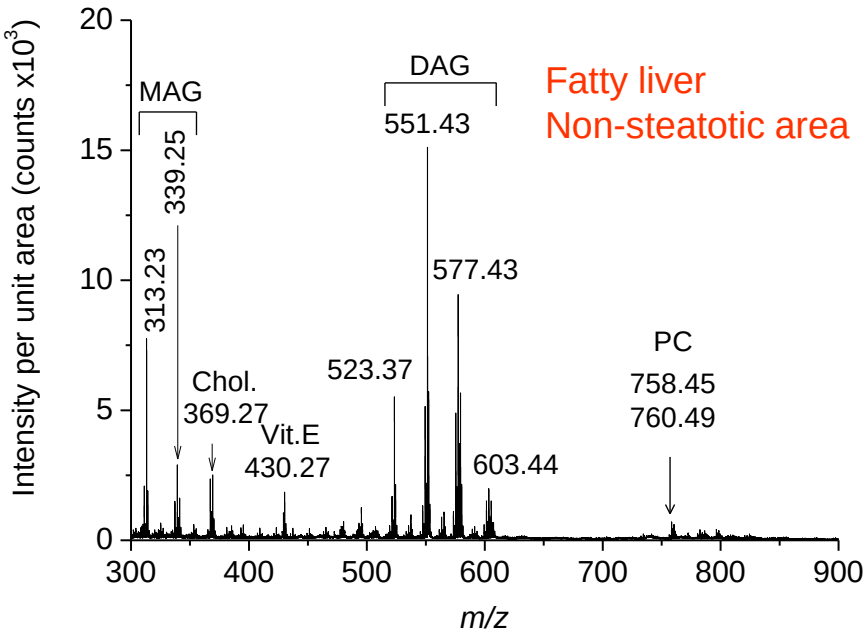
D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote, Anal. Chem. 2009, 81, 2823-2831

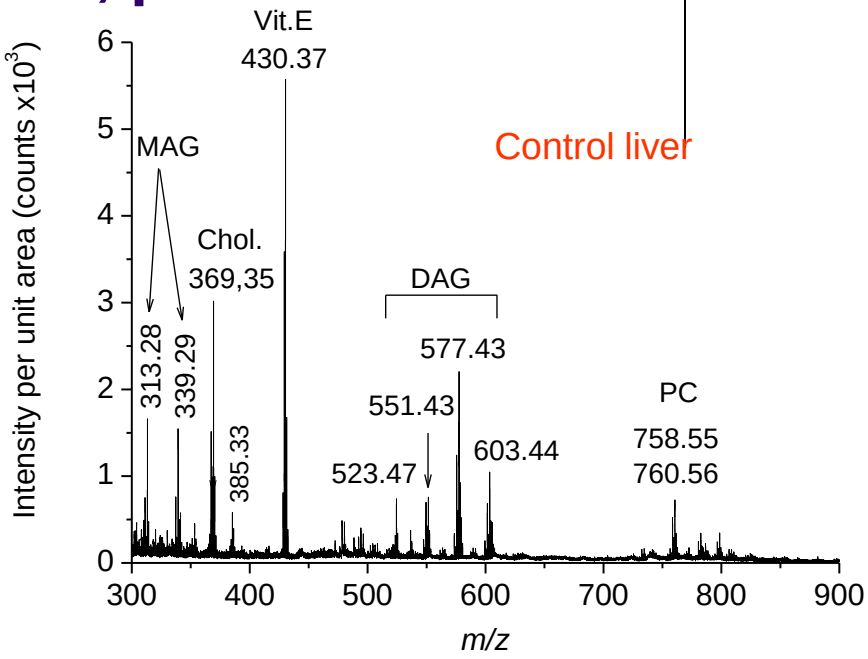
Lipid profile of a fatty liver : <u>Steatotic area</u>, positive ion mode



D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote, Anal. Chem. 2009, 81, 2823-2831

Lipid profile of a fatty liver : Non-steatotic area, positive ion mode





- Decrease of vitamin E signal
- Increase of diacylglycerol signal

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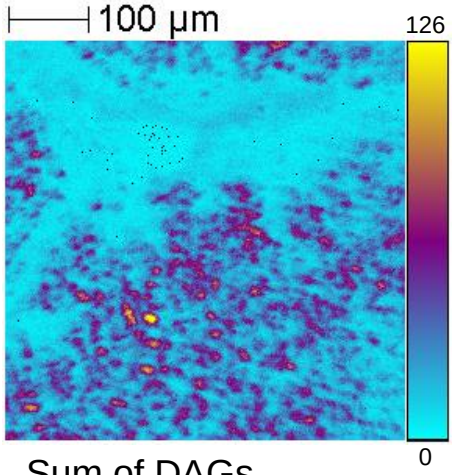
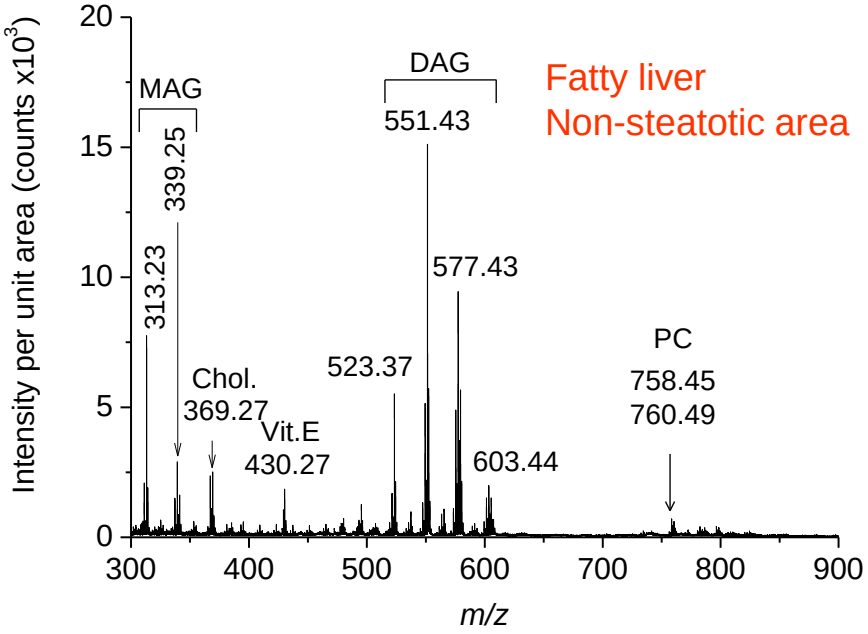
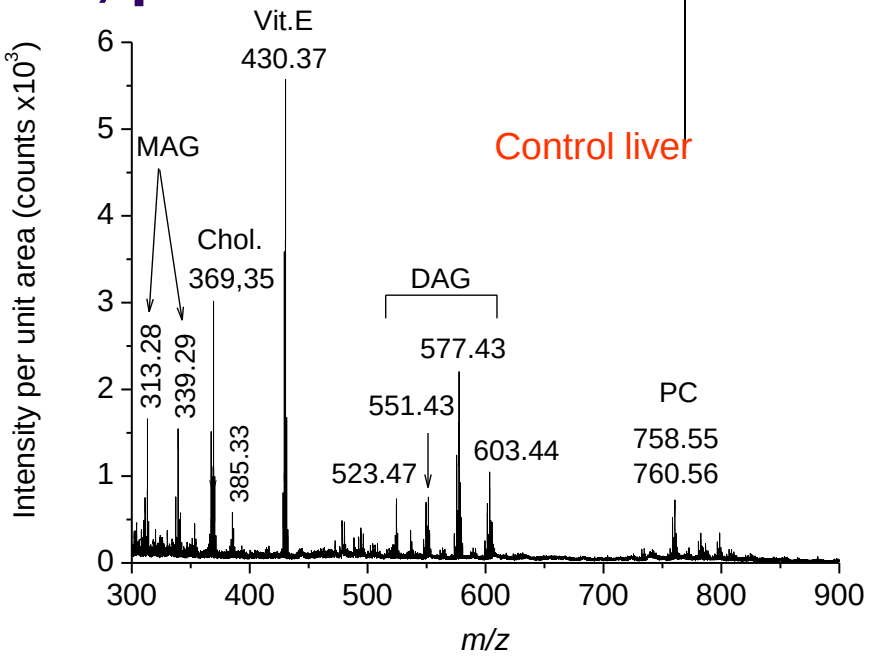


Sum of DAGs



Fatty liver
Non-steatotic area

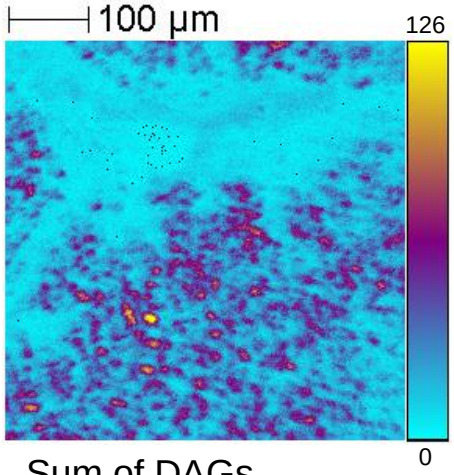


Control liver

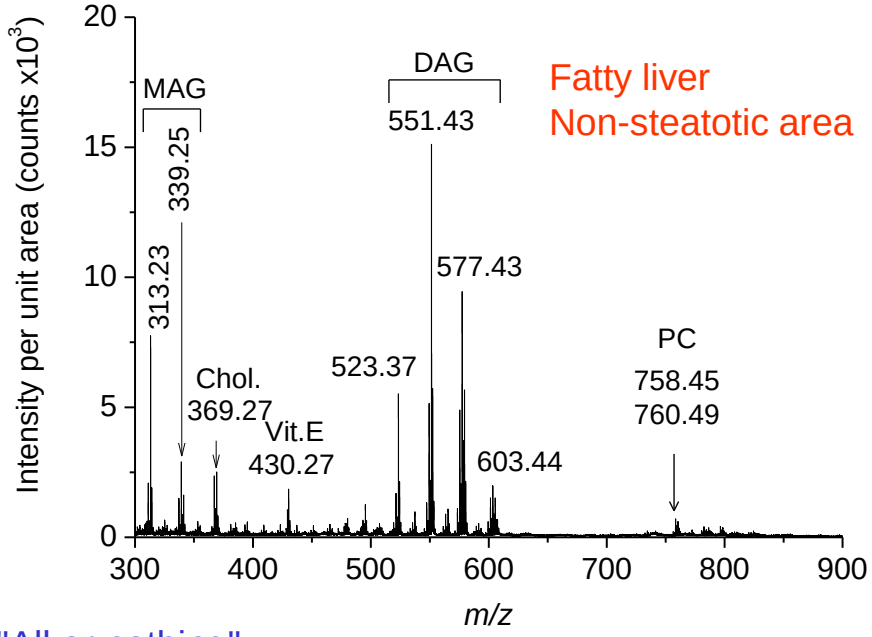
- Decrease of vitamin E signal
- Increase of diacylglycerol signal

D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote, Anal. Chem. 2009, 81, 2823-2831

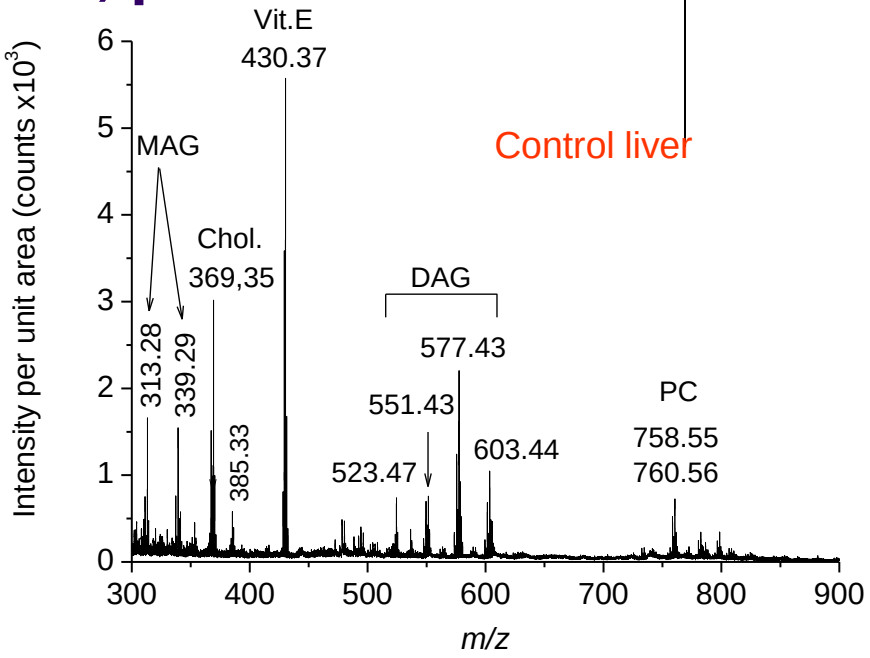
Lipid profile of a fatty liver : Non-steatotic area, positive ion mode



Sum of DAGs



Fatty liver
Non-steatotic area



Control liver

- Decrease of vitamin E signal
- Increase of diacylglycerol signal

"All or nothing":

➔Myristic acid and tricacylglycerols detected only in steatotic liver

Relative intensity variations:

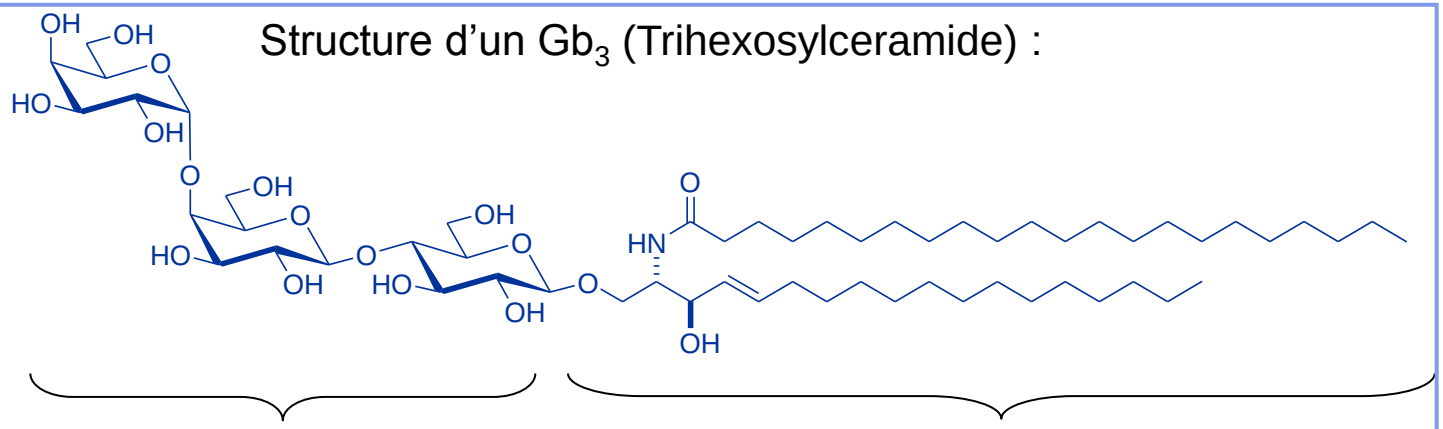
➔Depletion of vitamin E in steatotic liver, better observed in steatotic areas, ➔Accumulation of diacylglycerols in steatotic and non-steatotic areas, although "normal" for histology

D. Debois, M.P. Bralet, F. Le Naour, A. Brunelle, O. Laprévote, Anal. Chem. 2009, 81, 2823-2831

La maladie de Fabry

Maladie lysosomale de la famille des sphingolipidoses due à un déficit en α-D-galactosidase-A (α-GALA) de transmission récessive liée au chromosome X
(1 cas sur 120 000 naissances)
Accumulations de glycosphingolipides Gb_3 et Ga_2

Structure d'un Gb_3 (Trihexosylceramide) :



Chaîne osidique

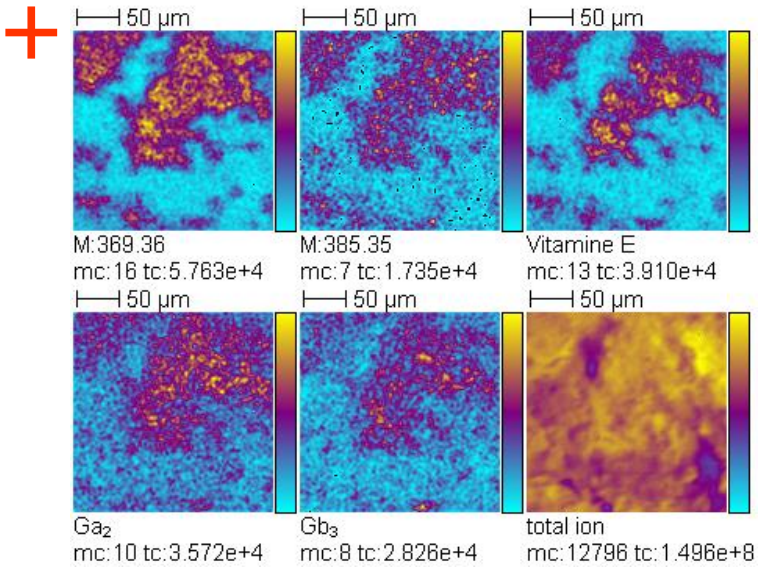
(α-Gal/β-Gal/β-Glu)

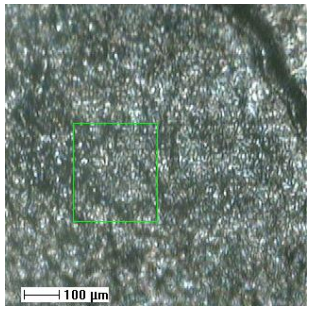
Chaîne acyle (base) variable

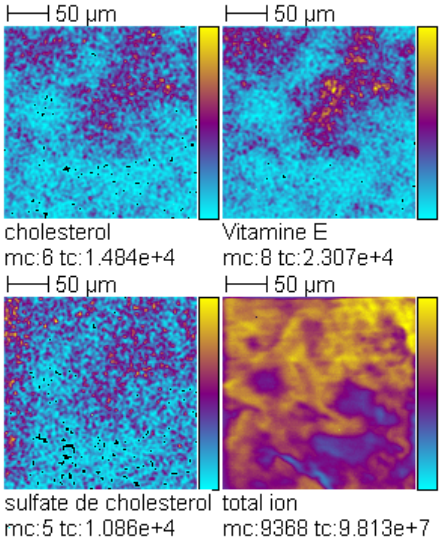
Ga_2 (digalactosylcéramide), enchaînement Gal/Gal

Atteinte multi systémique sévère dominée par une insuffisance rénale inexorable, des lésions neurologiques et cardiaques évolutives et des angiokératomes

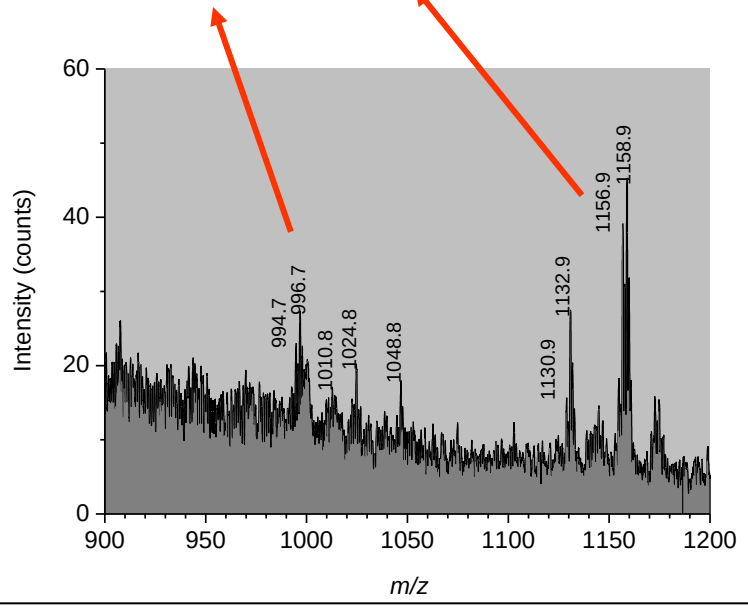
Biopsie cutanée de patient atteint de la maladie de Fabry







Aucun traitement de l ’échantillon
236x236 µm^2
Résolution: 1 µm
15 min d’acquisition
Dose: 1.25 x 10^{12} ions.cm^{-2}

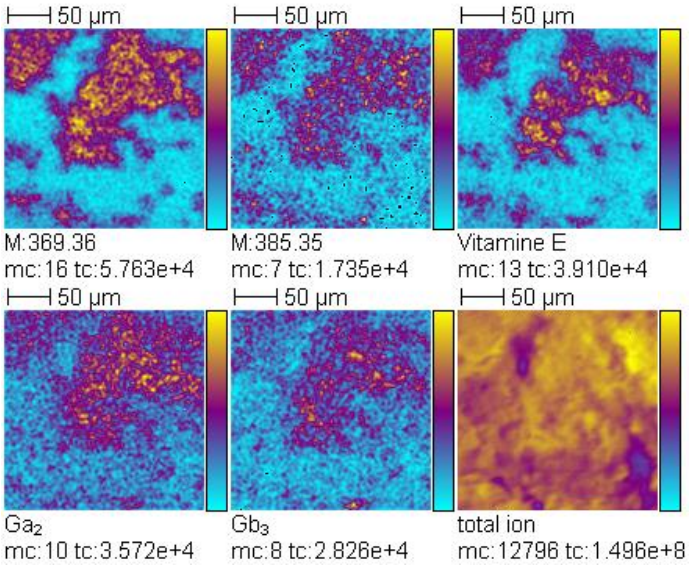


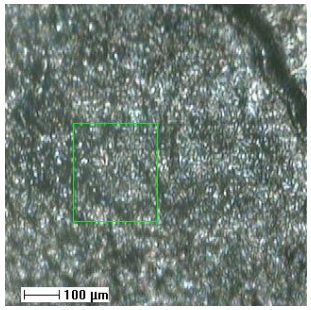
- Détection d’un faible signal de Ga_2 et Gb_3
- Localisation uniquement d’une famille moléculaire avec une résolution de 1 µm
- Colocalisation avec la vitamine E, le cholestérol et le sulfate de cholestéryl

D. Touboul, S. Roy, D.P. Germain, P. Chaminade, A. Brunelle, O. Laprévote, Int. J. Mass Spectrom. 2007, 260, 158-165

Biopsie cutanée de patient atteint de la maladie de Fabry

+





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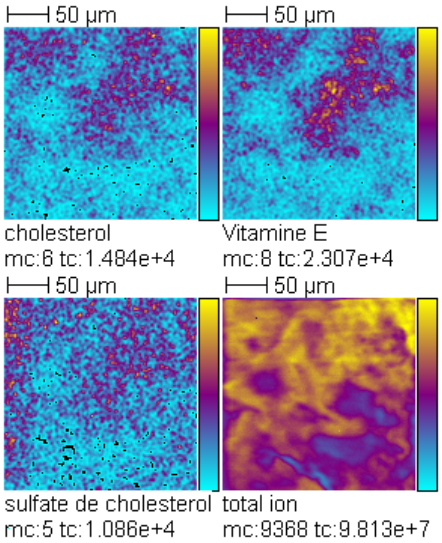
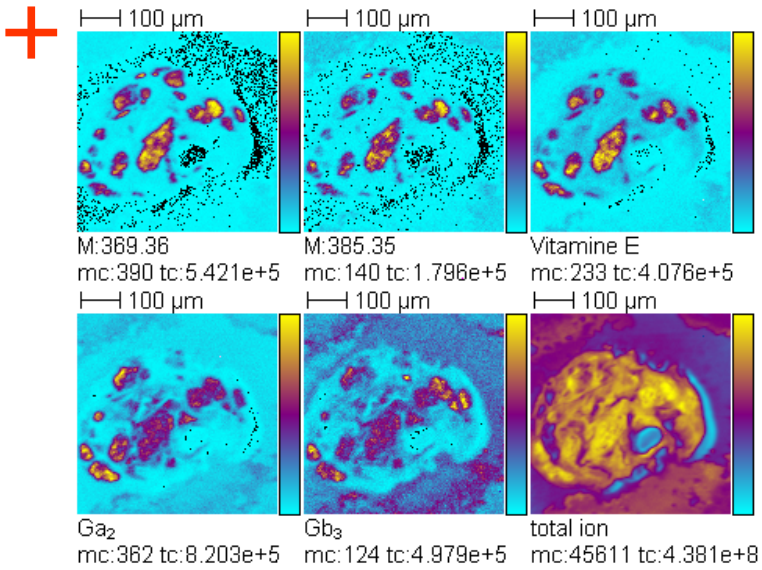
-

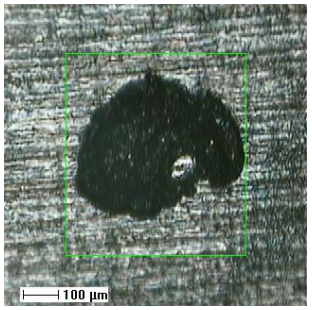


- Pour des cellules de peau en culture, il existe un lien entre l’accumulation de Ga_2 et de cholestérol. Ceci est observé directement sur des coupes de peau de patients atteints par la maladie de Fabry
- La présence de vitamine E est le signe d’une réaction inflammatoire locale et intense. Une dérégulation des NO-synthases, une réduction de la chaîne respiratoire enzymatique et une augmentation de la fréquence de la mutation génétique eNOSG894T ont déjà été démontrées.
- Le sulfate de cholestéryl intervient dans la différenciation cellulaire et dans la formation de la barrière épidermale au niveau de la peau.

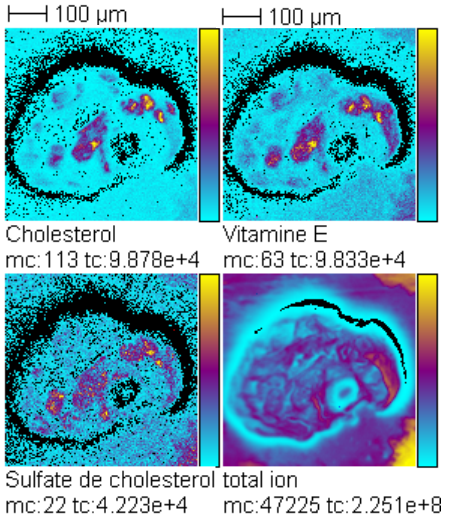
D. Touboul, S. Roy, D.P. Germain, P. Chaminade, A. Brunelle, O. Laprévote, Int. J. Mass Spectrom. 2007, 260, 158-165

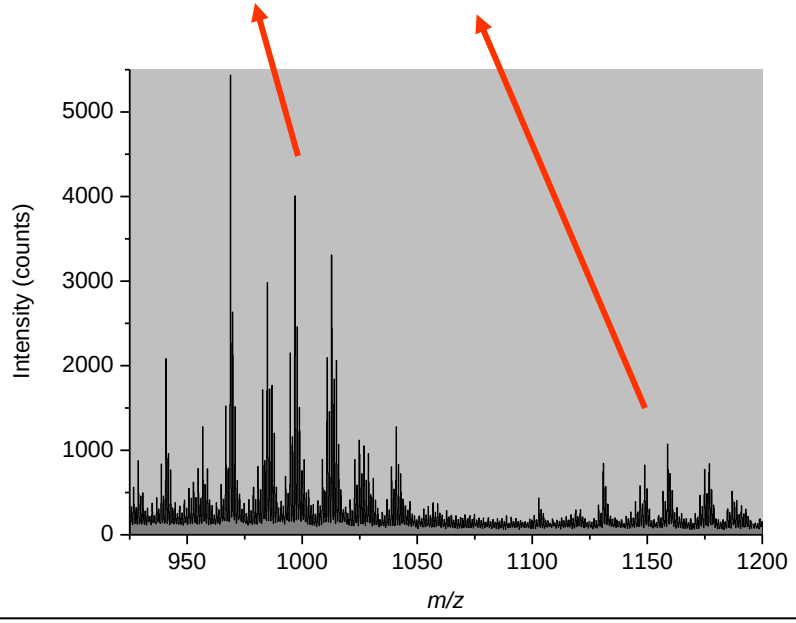
Biopsie rénale de patient atteint de la maladie de Fabry





Aucun traitement de l 'échantillon
500x500 µm²
Résolution: 1 µm
30 min d'acquisition
Dose: 1.06 x 10^{12} ions.cm^{-2}





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- Localisation possible d'une seule espèce moléculaire avec une résolution de 1 µm
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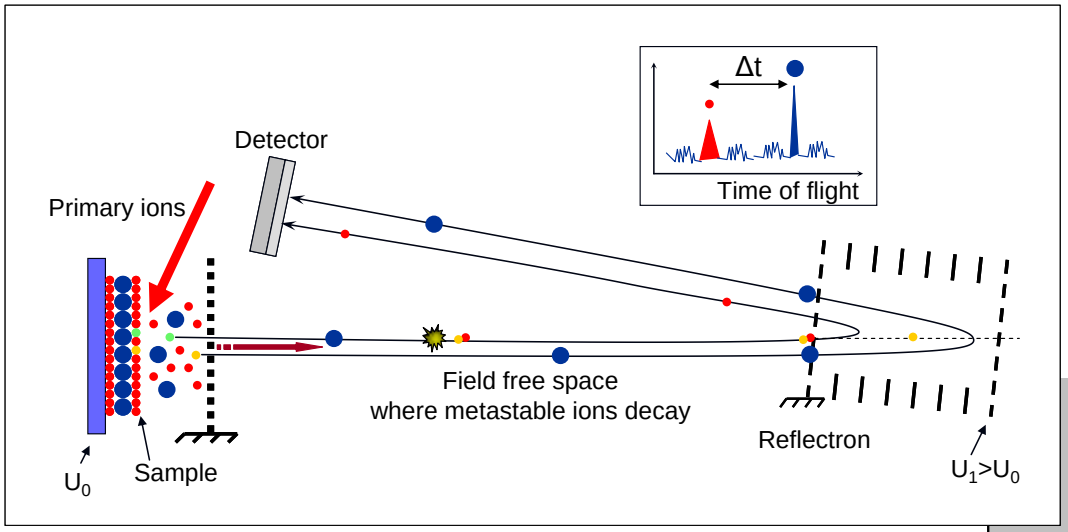
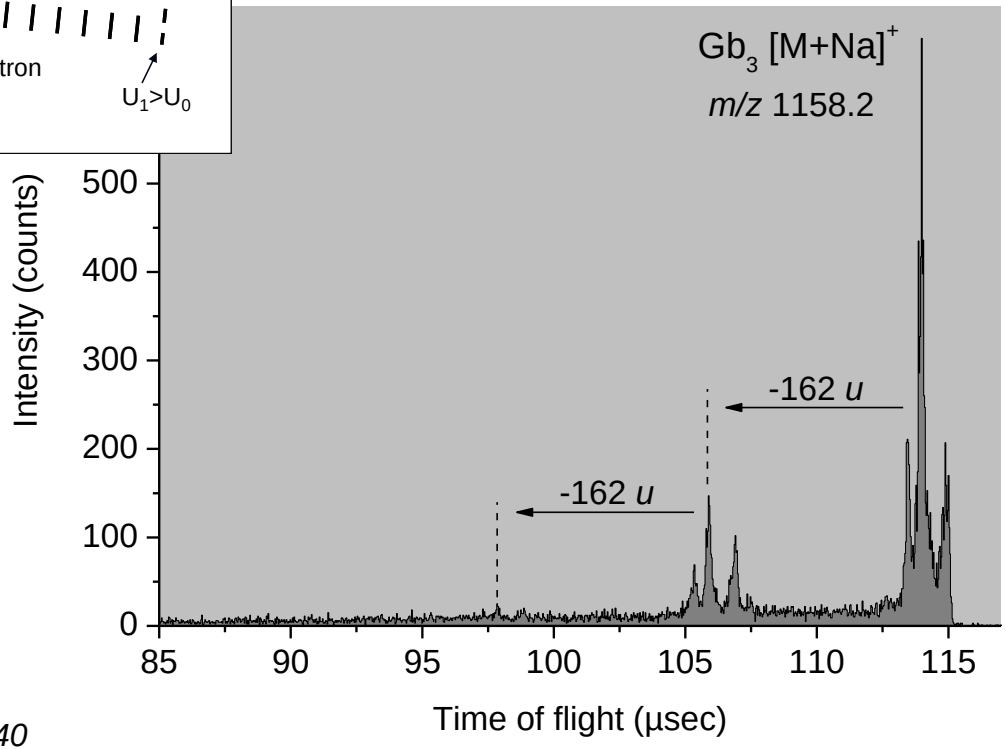
D. Touboul, S. Roy, D.P. Germain, P. Chaminade, A. Brunelle, O. Laprévote, Int. J. Mass Spectrom. 2007, 260, 158-165

MS/MS with a TOF-SIMS: "Post-Source Decay like" method



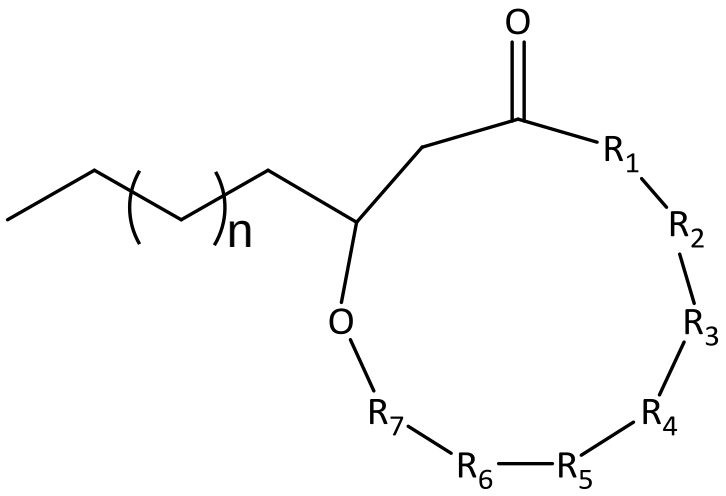
$$m_p - m_f = K \times \sqrt{m_p} \times \Delta T$$

Not a true MS-MS method
➔only confirmation of known structures



D. Touboul, A. Brunelle, O. Laprévote,
Rapid Commun. Mass Spectrom. 2006, 20, 703-709
S. Della-Negra, Y. Le Beyec, Anal. Chem. 1985, 57, 2035-2040

Bacterial peptides: surfactins from *Bacillus subtilis*



Peptide sequence: E L L V D L L or (beta-hydroxy fatty acid)-Glu-Leu-Leu-Val-Asp-Leu-Leu

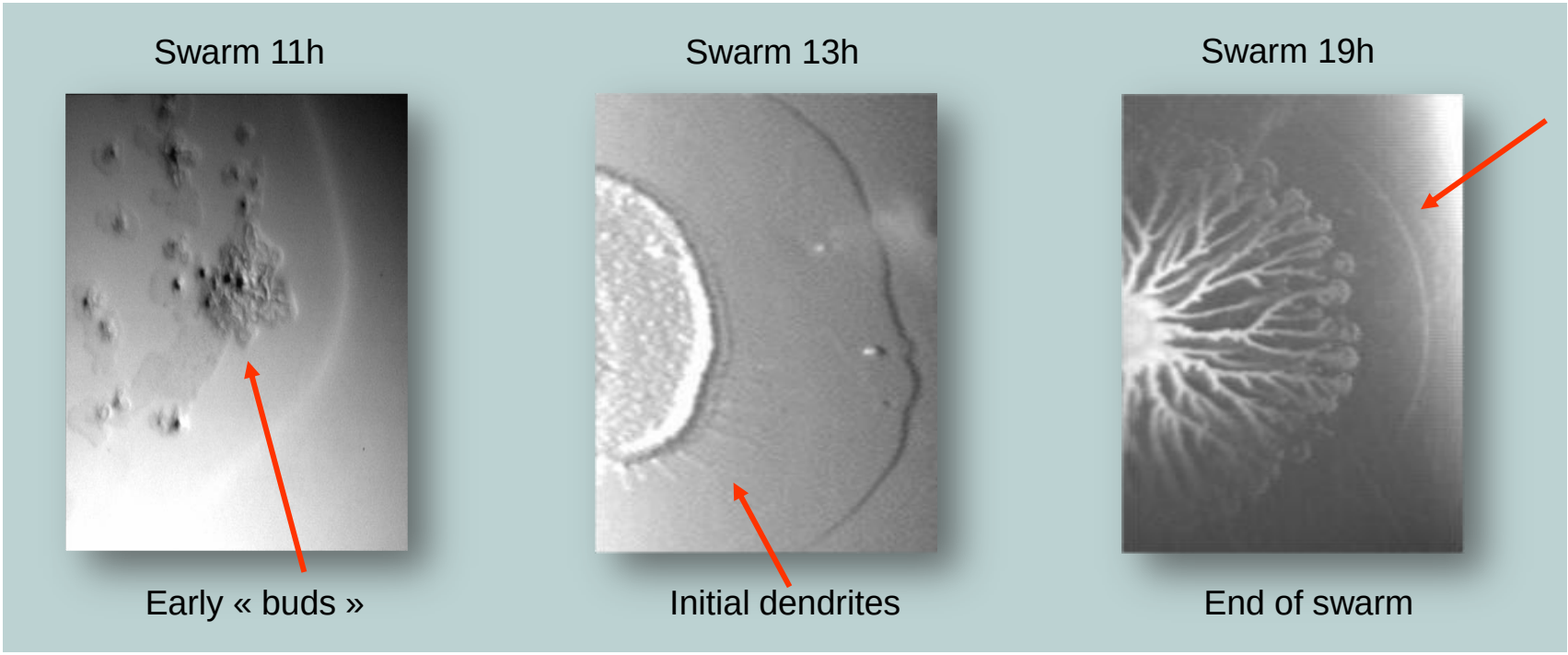
Surfactins: family of heptacyclopeptides in which the *C*-terminal carbonyl is linked with the β-hydroxy group of a fatty acid (12 to 16 carbon atoms long) acylating the *N*-terminal function of a glutamic acid residue.
These compounds secreted by the Gram positive bacterium *Bacillus subtilis* play an important role in the formation of dendritic patterns.

A. Kakinuma et al, Agr. Biol. Chem, 1969, 33: 973-6

Swarming → « Essaimage »

Growing and swarming of a bacterial colony on an agar gel

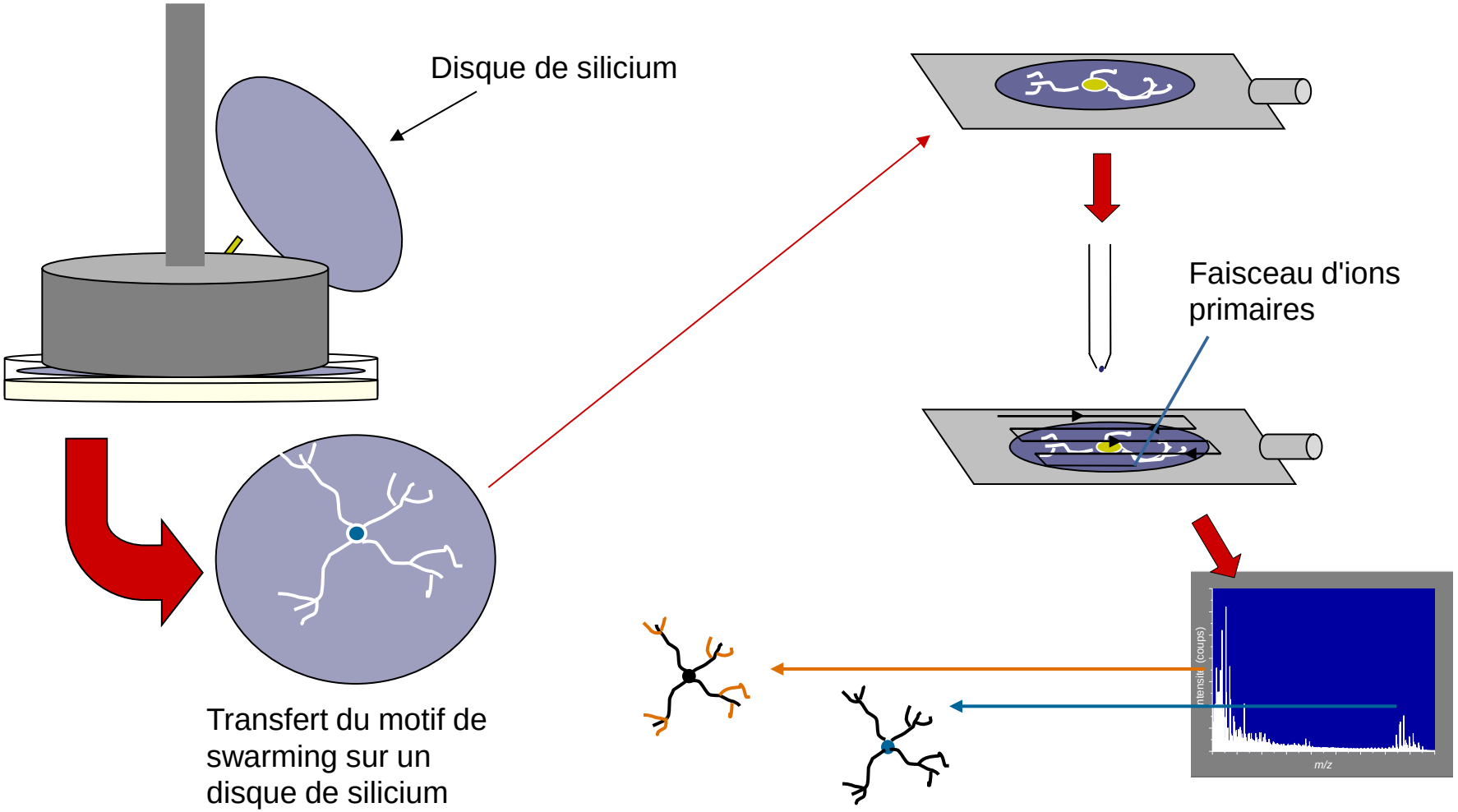
- *Bacillus subtilus* is a model bacteria; swarming is a model of monolayer growing of biofilms
- Linked to medicinal problems: cystic fibrosis, contamination of medical material, dental plaque…



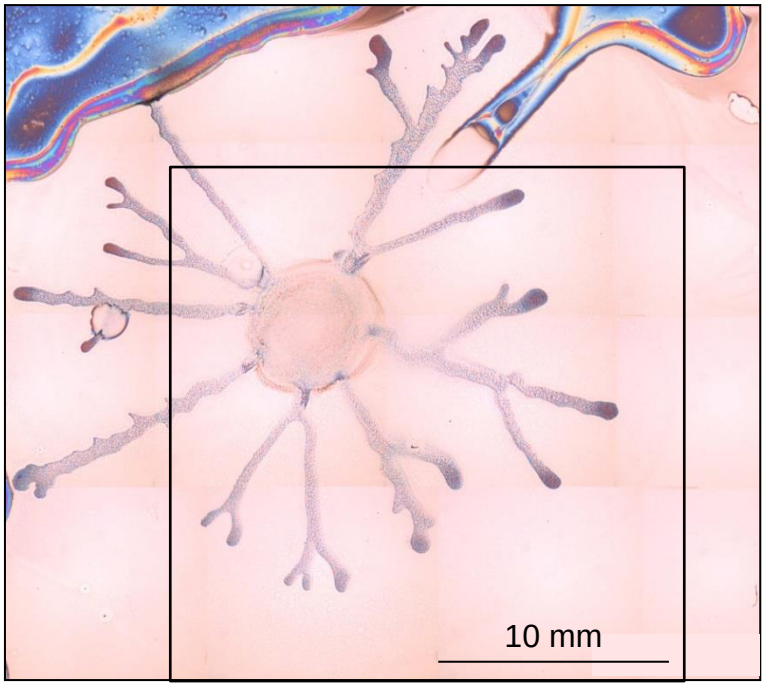
The migration front is always preceded by a “wet” zone due to the excretion of peptidolipids by the bacterial cells

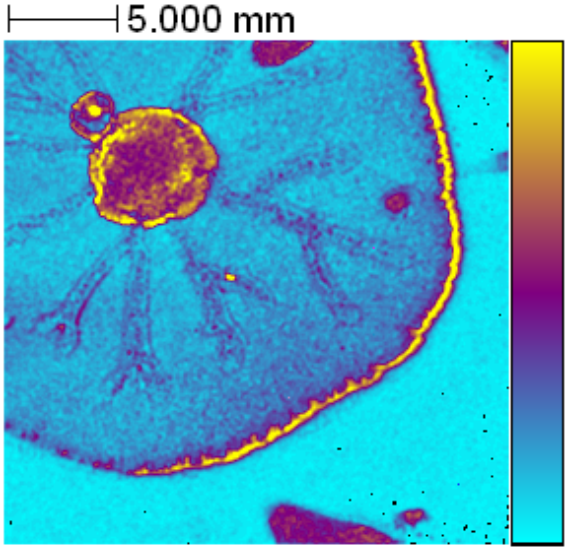
Analyse de profils dendritiques sur une surface chez *Bacillus subtilis*

Préparation d'échantillon de swarming pour l'imagerie TOF-SIMS



Large biological samples: bacterial swarming



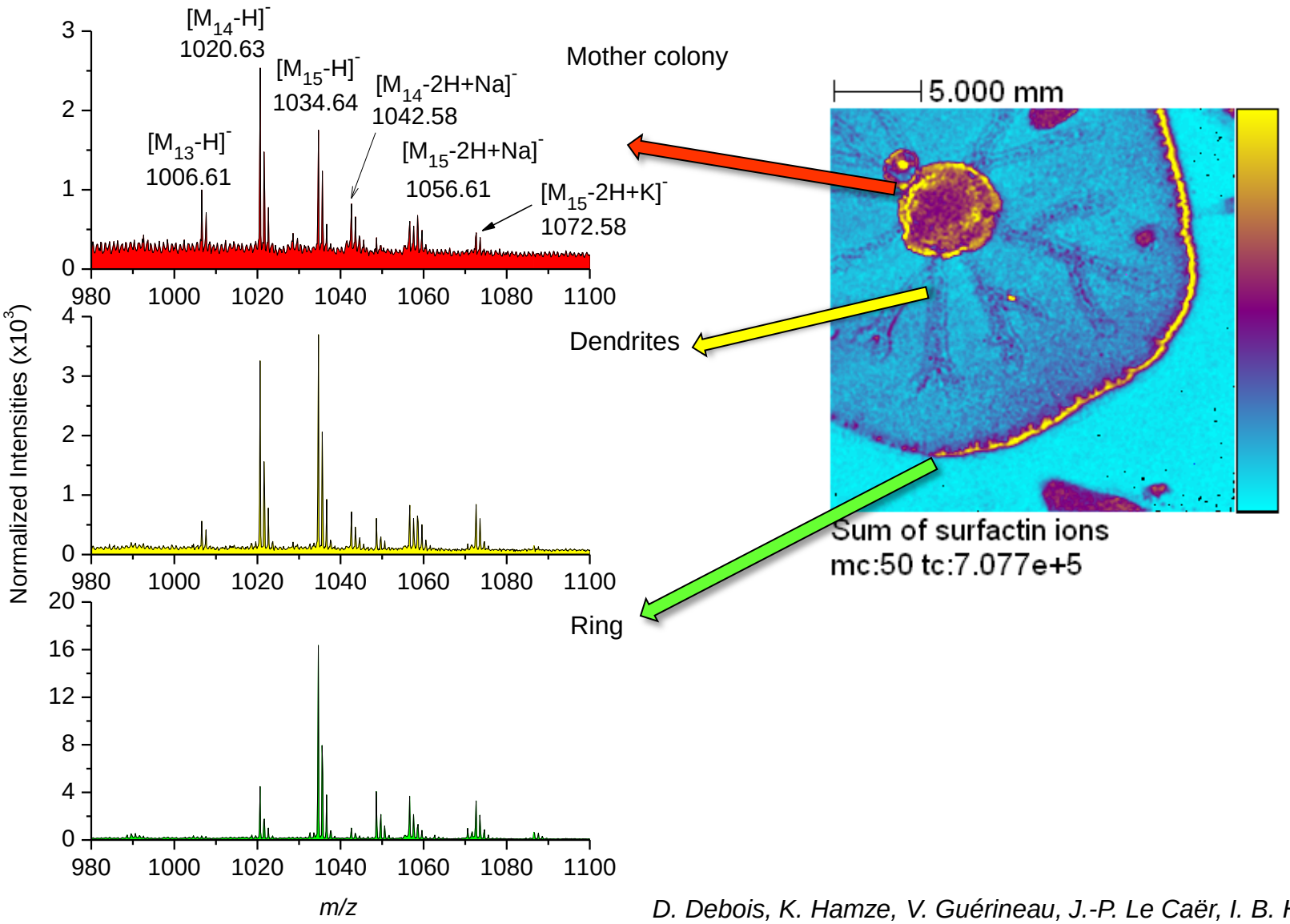


Sum of surfactin ions
mc:50 tc:7.077e+5

Image size: 23 x 23 mm²
Primary ions: Bi_3^+, 25 keV
Negative mode
Fluence: 10^9 ions.cm^{-2}
Pixel size: 90 x 90 µm²
Acquisition time: 4 hours

D. Debois, K. Hamze, V. Guérineau, J.-P. Le Caër, I. B. Holland, P. Lopes, J. Ouazzani, S. J. Séror, A. Brunelle, O. Laprévote, Proteomics, 2008, 8, 3682-3691

Surfactin imaging on *Bacillus subtilis* imprints



Spectra intensities normalized to the smallest ROI area

D. Debois, K. Hamze, V. Guérineau, J.-P. Le Caër, I. B. Holland, P. Lopes, J. Ouazzani, S. J. Séror, A. Brunelle, O. Laprévote, Proteomics, 2008, 8, 3682-3691

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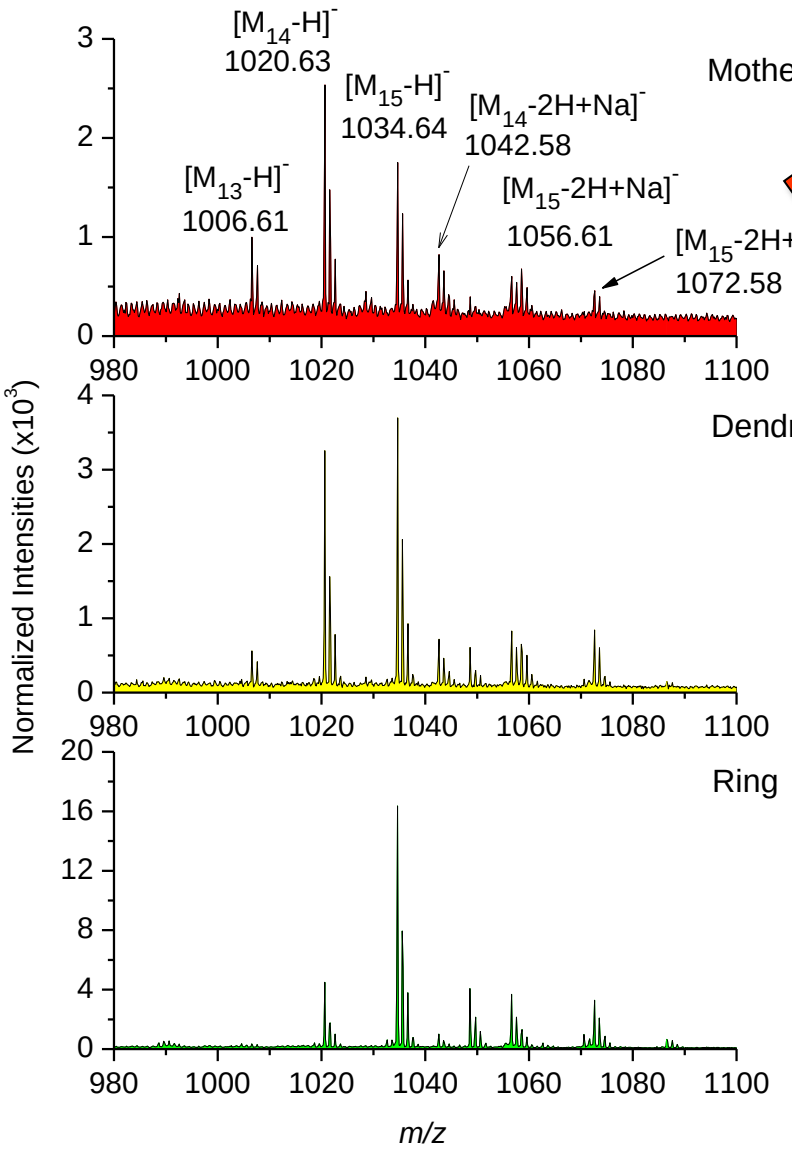
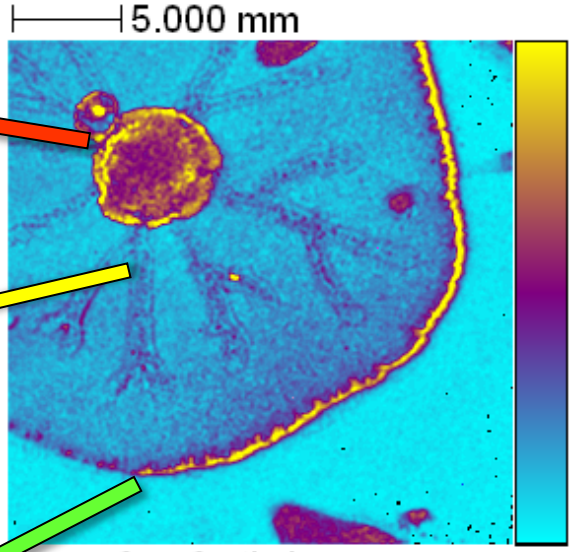


Spectra intensities normalized to the smallest ROI area

Mother colony

Dendrites

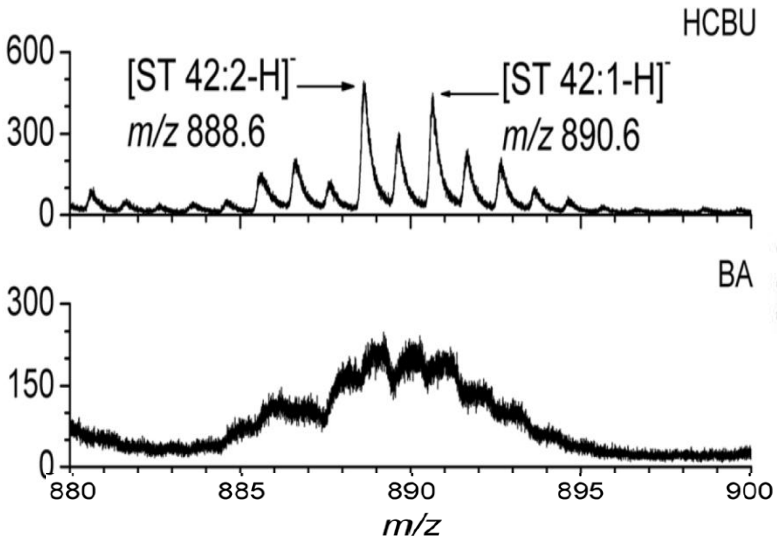
Ring



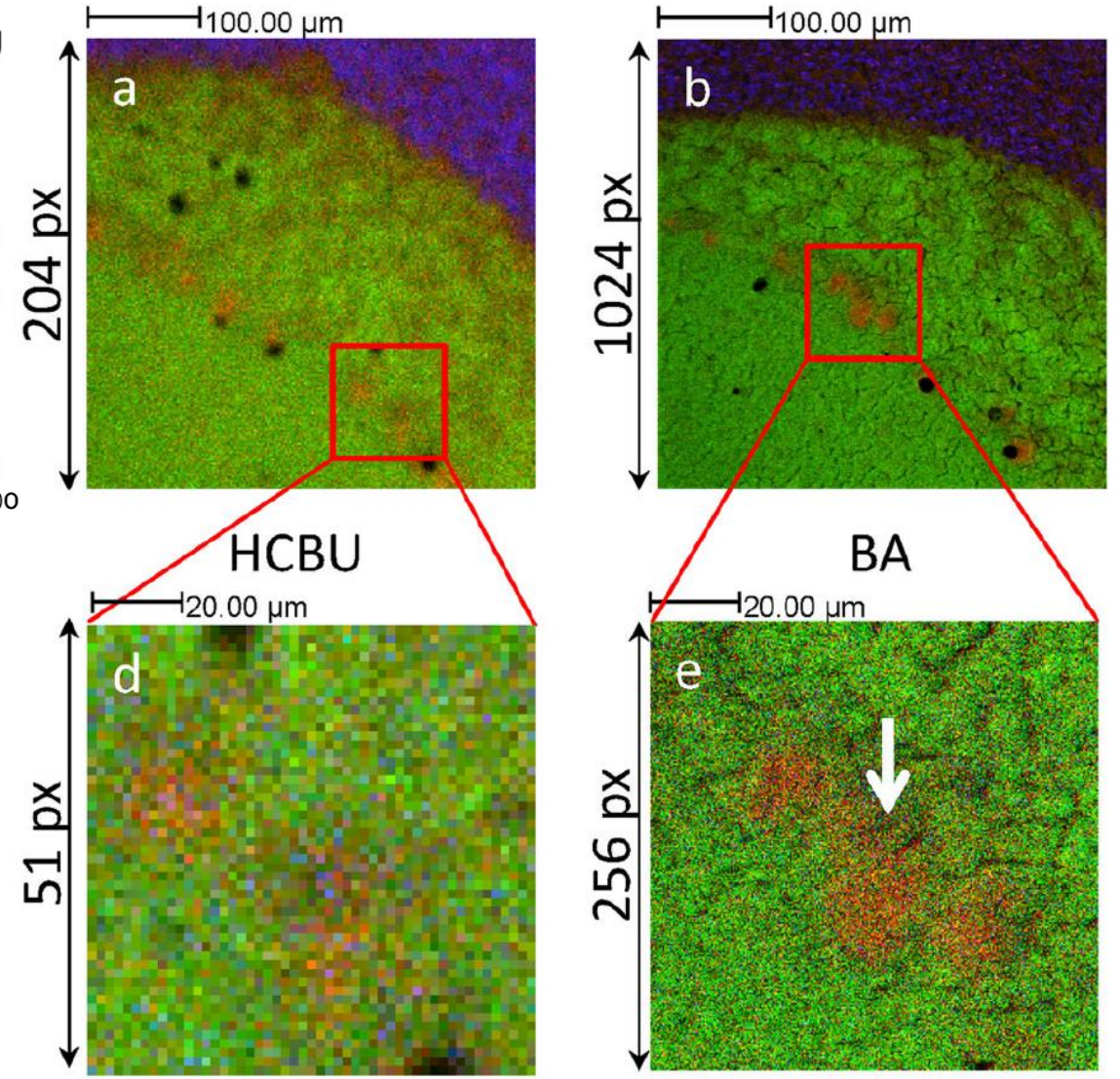
➔Longest fatty acyl chain length = most hydrophobic surfactins,
preferentially released from the mother colony allowing them to spread further than the surfactins with shorter fatty acyl chains.
➔Surfactins with shorter chains (13-15) may remain associated the bacteria.

D. Debois, K. Hamze, V. Guérineau, J.-P. Le Caër, I. B. Holland, P. Lopes, J. Ouazzani, S. J. Séror, A. Brunelle, O. Laprévote, Proteomics, 2008, 8, 3682-3691

How to improve spatial resolution ?

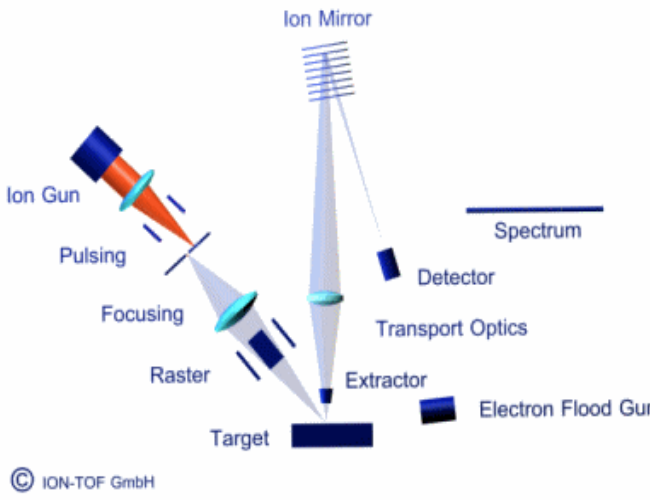


	HCBU	BA
Pulsed primary ion current (pA @10 kHz)	0.28	0.09
Mass resolution at *m/z* 385.4 (M/ΔM, FWHM)	~5000	~300
Beam size (µm)	2.9 ± 0.2	0.45 ± 0.06

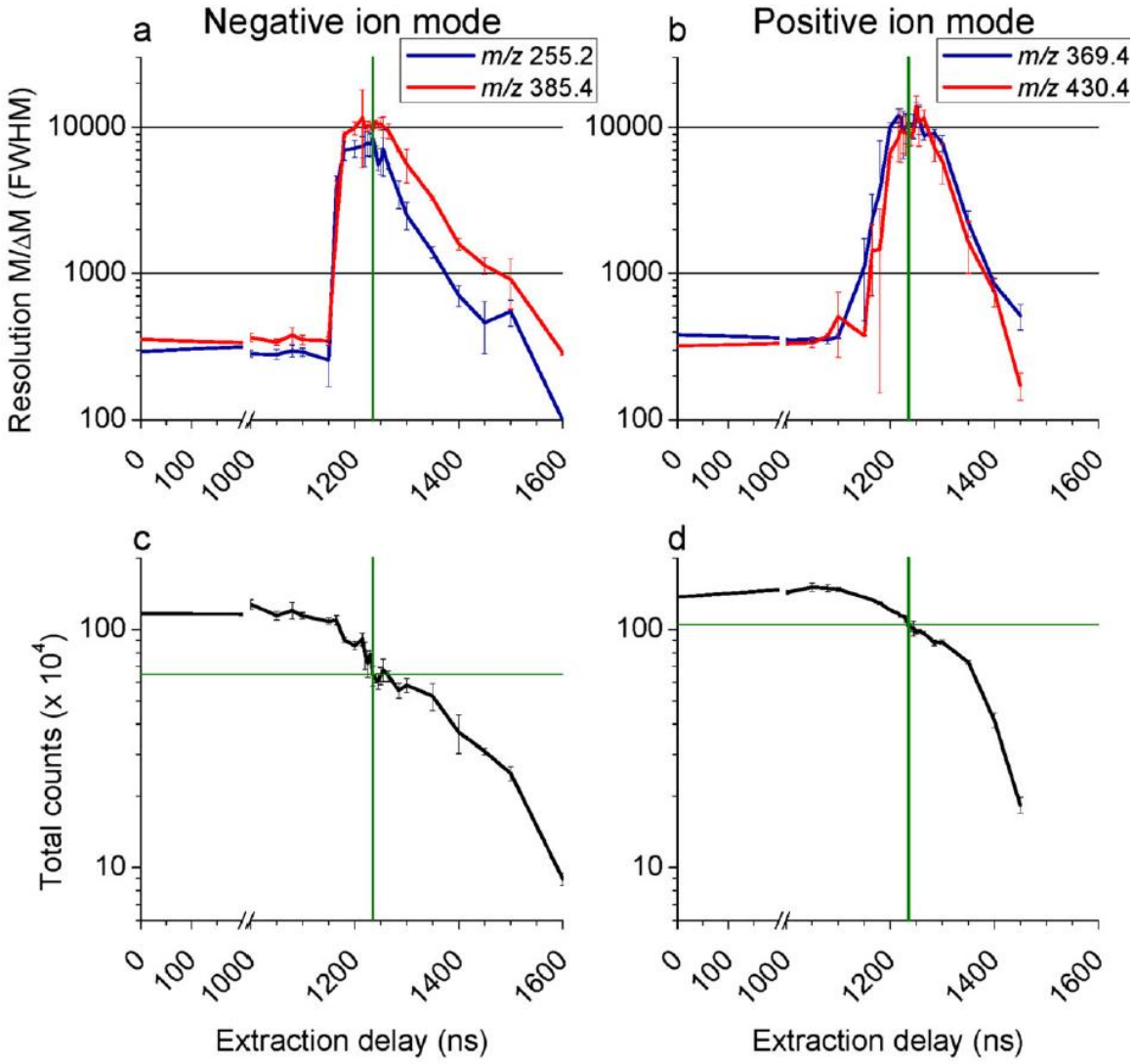


[C18:0–H]⁻ *m/z* 283.3 (red), [C16:0–H]⁻ *m/z* 255.2 (green), and cholesterol [M–H]⁻ *m/z* 385.4 (blue).

How to improve spatial resolution ?

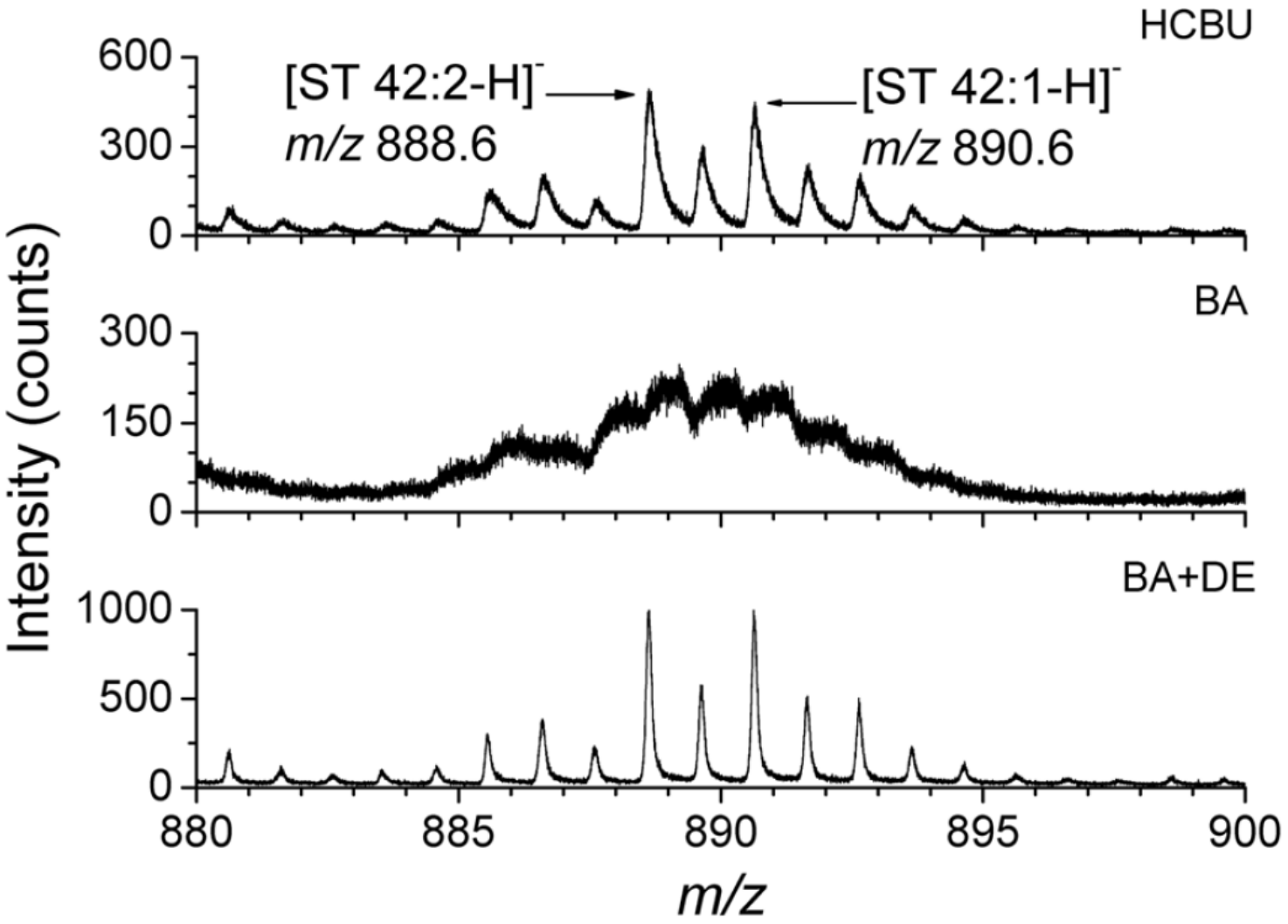


Vanbellingen QP, Elie N, Eller MJ, Della-Negra S, Touboul D, Brunelle A. Rapid Commun Mass Spectrom. 2015; 29(13): 1187-1195.



Optimized extraction delay: 1235 ns

How to improve spatial resolution ?



Vanbellingen QP, Elie N, Eller MJ, Della-Negra S, Touboul D, Brunelle A. Rapid Commun Mass Spectrom. 2015; 29(13): 1187-1195.

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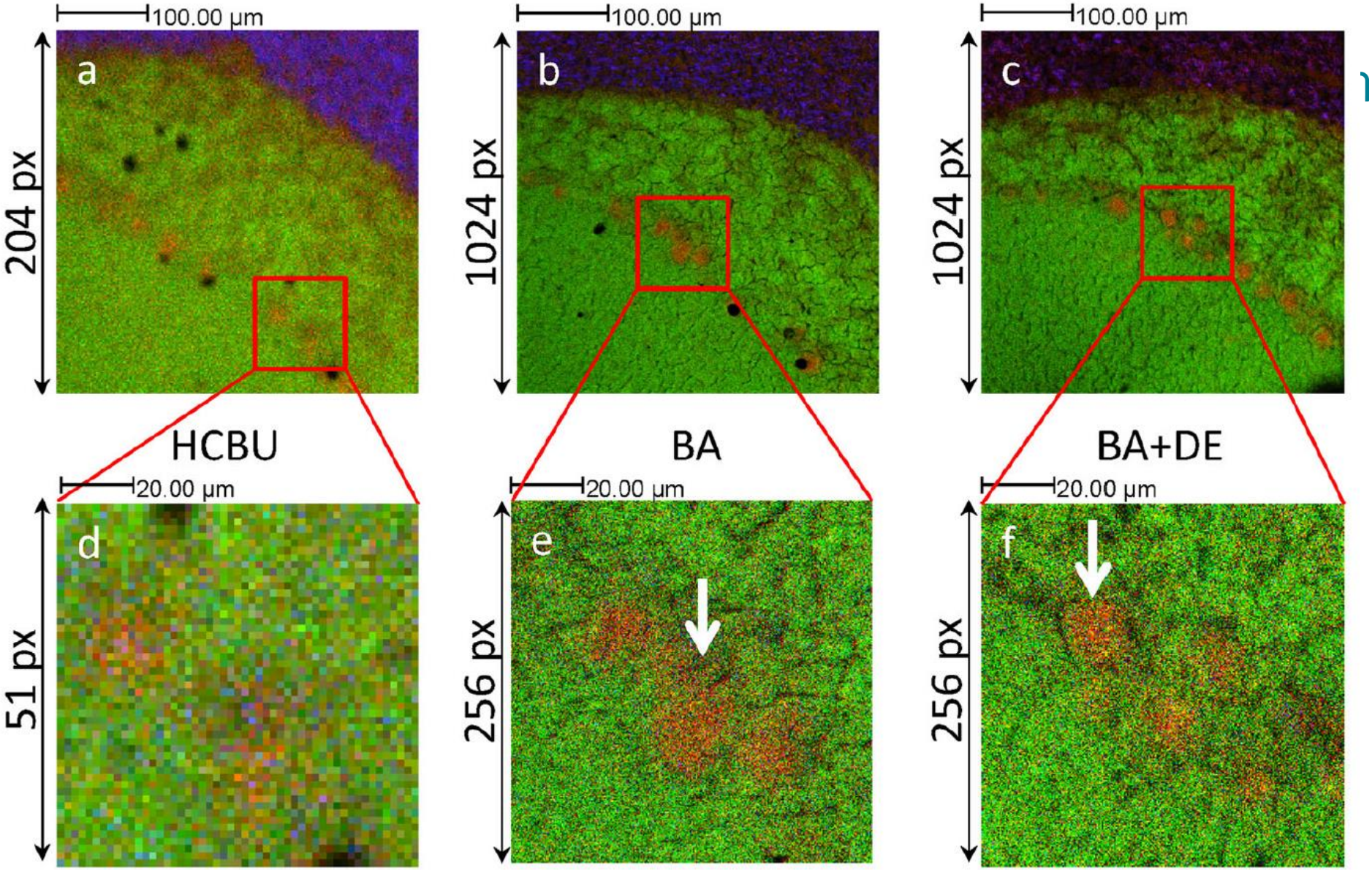


Improvement of sample preparation

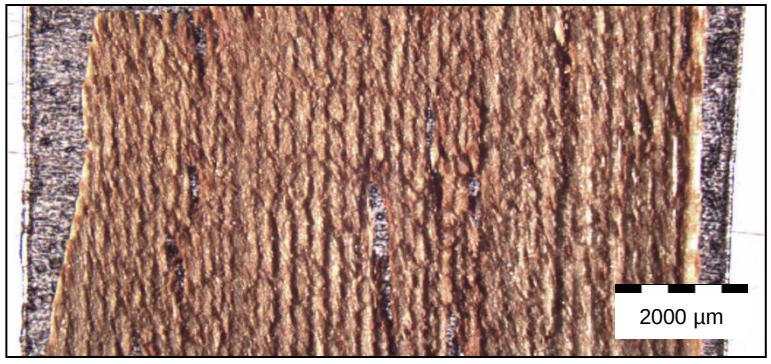
Microtome

Use of a stainless steel blade
Section thickness ~20 µm

Ultramicrotome

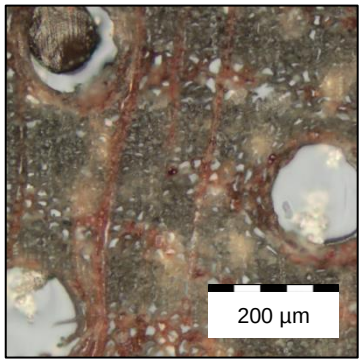
Use of a diamond knife
Section thickness 200 to 500 nm

Dry sample



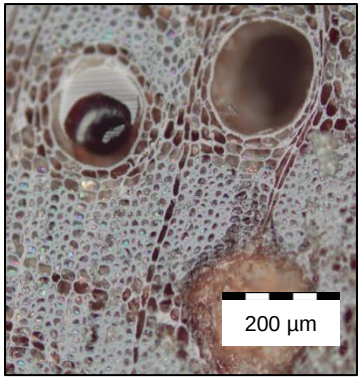
Radial section
Lack of information

Wet sample



Transverse section
Loss of information

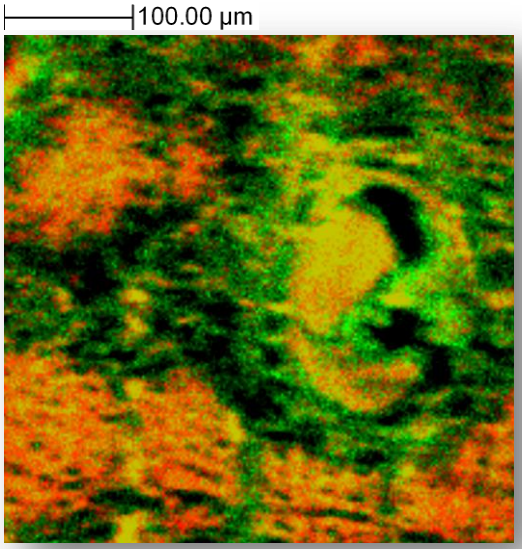
Dry sample

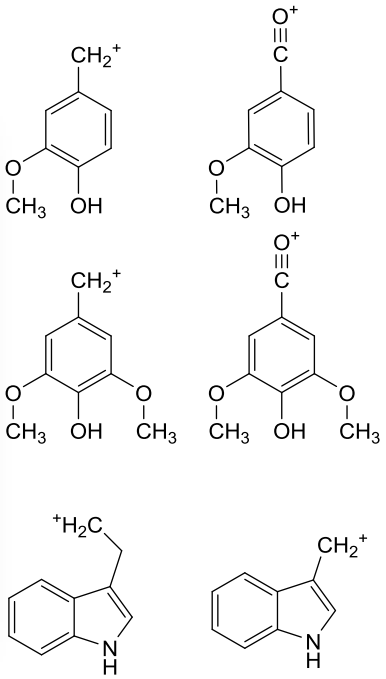


Transverse section

Analysis of a wood (*Dicorynia guianensis*) section

hMr
Beam size ~2.9 µm

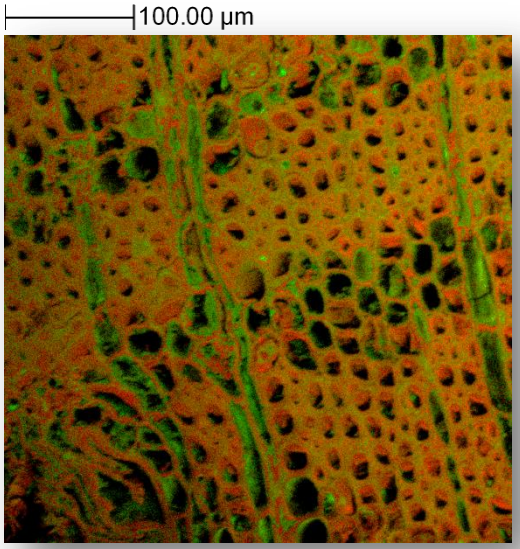




G lignin
fragment ions

S lignin
fragment ions

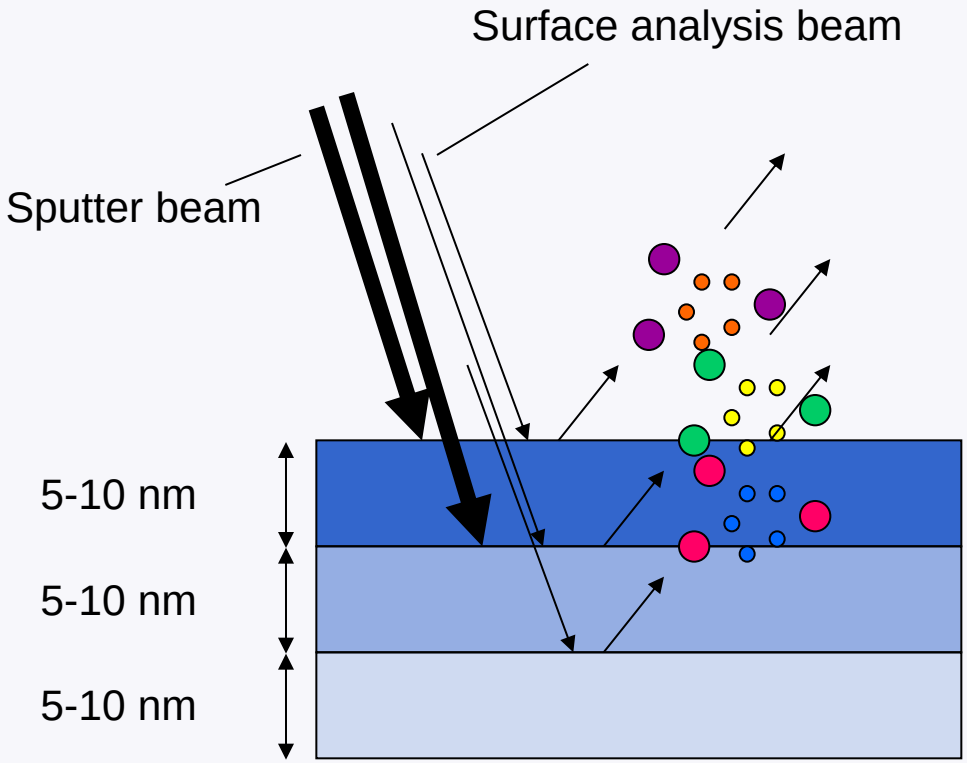
H(M+S)r
Beam size ~0.4 µm



Red **Fragments of lignin**
Green **Fragments of tryptamine**

Collaboration with Didier Stien, Véronique Eparvier et Nadine Amusant , ICSN + Guyane

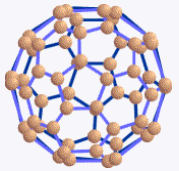
Evaluation and caracterization of the capabilities of a C_{60} ion source for depth profiling



Schematic view of a tissue section

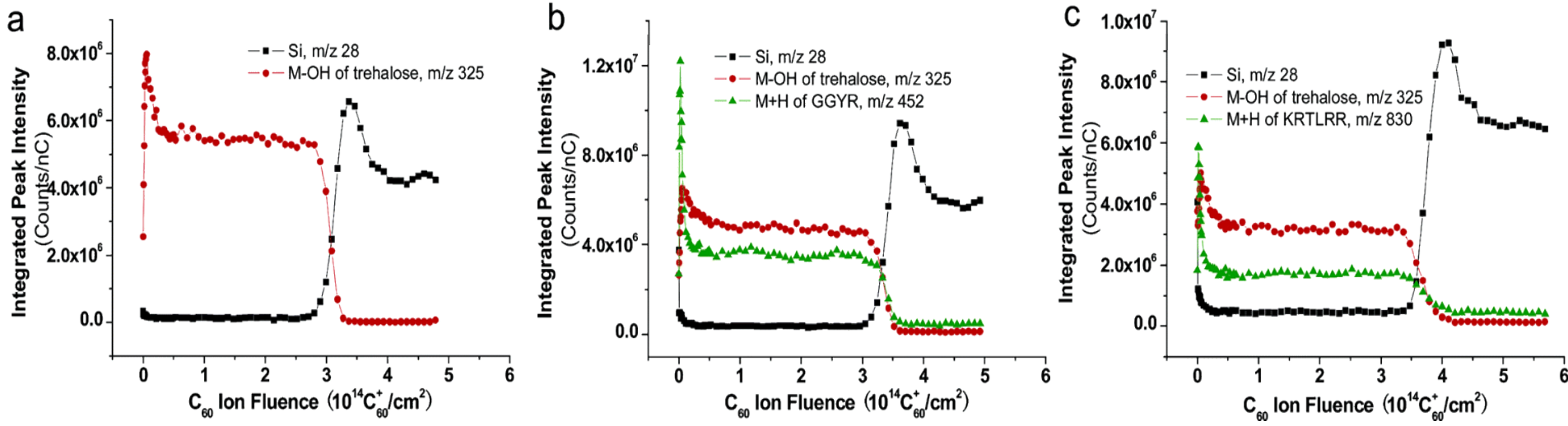
Surface analysis beam:
Bi_3^+ 25 keV (1.5×10^{10} ions/step)

Sputter beam:
C_{60}^+ 10 keV (2.8×10^{13} ions/step)



Evaluation and caracterization of the capabilities of a C_{60} ion source for depth profiling
1. on model layers

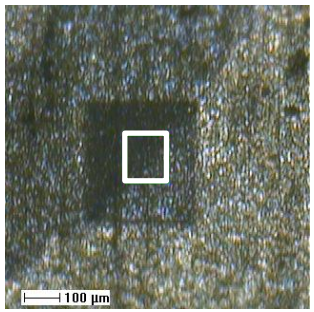
➔~260-270 nm thickness trehalose film doped with synthetic peptides

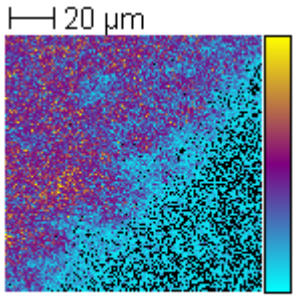


Secondary ion signal intensities versus accumulated C_{60}^+ ion fluence during depth profiling of (a) a pure trehalose film (270 nm), (b) a trehalose film doped with 1% GGYR (263 nm), and (c) a trehalose film doped with 1% KRTLRR (273 nm).

J. Cheng, A. Wucher, N. Winograd, J. Phys. Chem. B, 2006, 110, 8329-8336

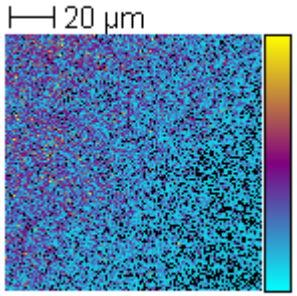
Evaluation and caracterization of the capabilities of a C_{60} ion source for depth profiling 2. directly on a tissue section





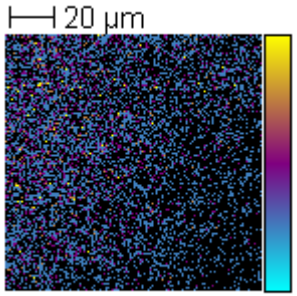
Cholesterol
mc:17 tc:7.588e+4

Profondeur d'analyse:
200 - 300 nm



Cholesterol
mc:11 tc:4.041e+4

Profondeur d'analyse:
1 - 1,6 µm



Cholesterol
mc:4 tc:7.639e+3

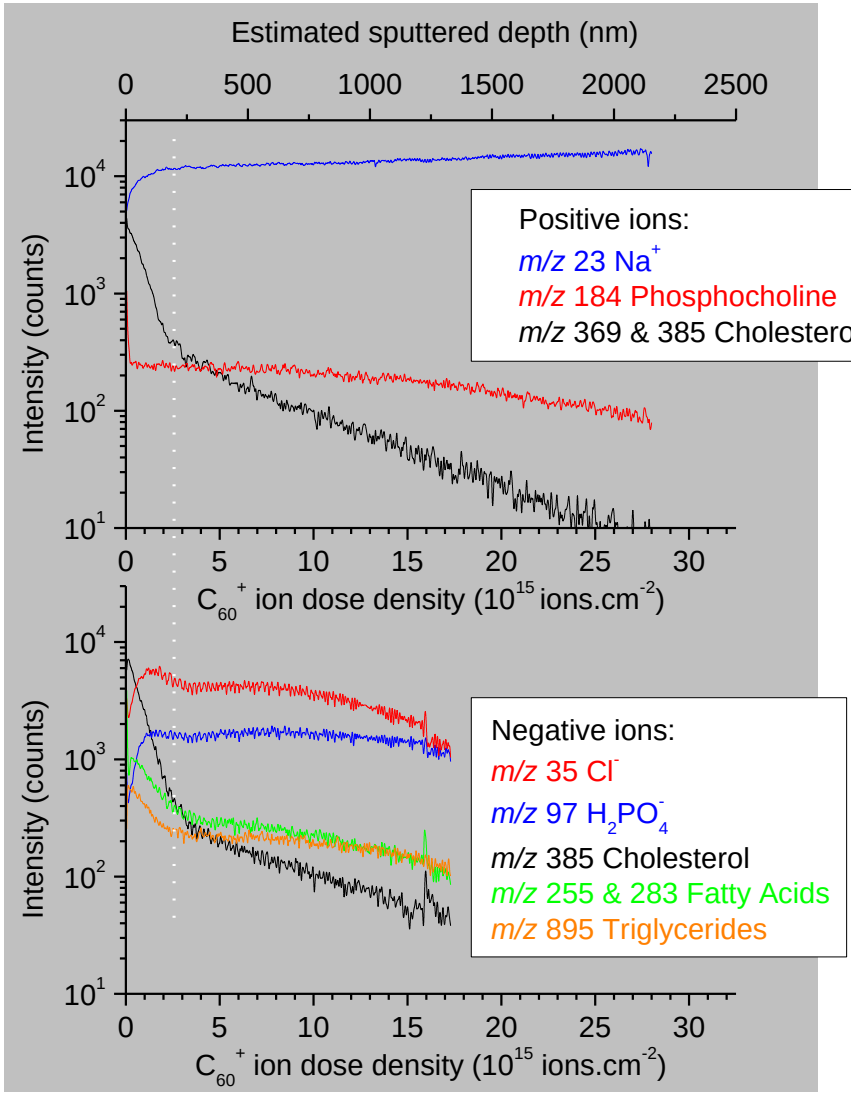
Profondeur d'analyse:
2-3 µm

➔Perte de signal quand on creuse dans la coupe

➔Imagerie 3 D d'une coupe de cerveau de rat impossible

D. Debois, A. Brunelle, O. Laprévote, Int. J. Mass Spectrom., 2007, 260, 115-120

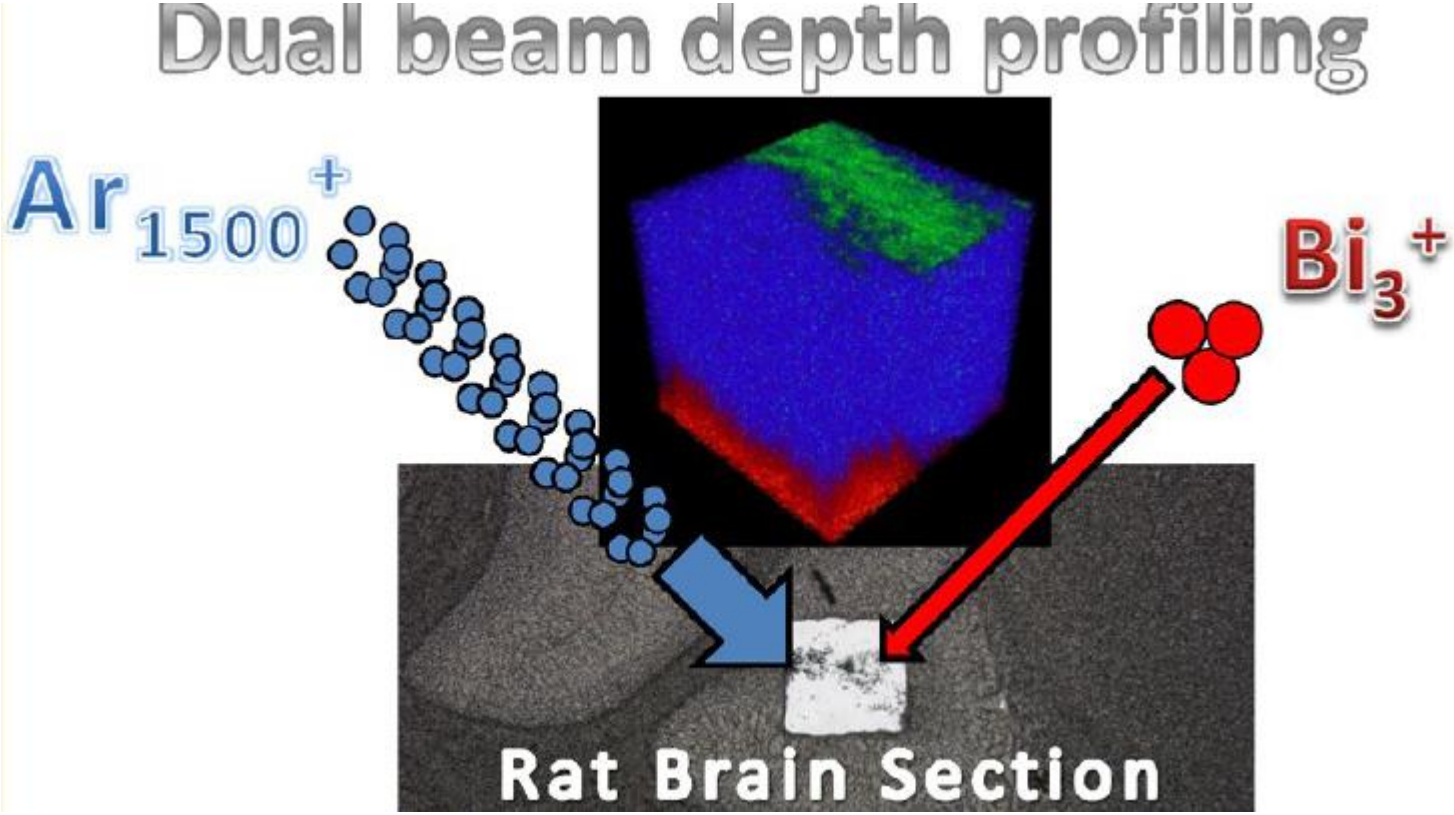
Evaluation and caracterization of the capabilities of a C_{60} ion source for depth profiling 2. directly on a tissue section



- ~ 3 µm have been sputtered at the surface of a rat brain tissue section
- Lipids appear to be concentrated at the surface, in the first 200-300 nm.(P. Sjövall *et al.*, *Appl. Surf. Sci.* 2006, *252*, 6513-6516)
- Damage is still too much important, for heavy molecules, to enable depth profiling with a good sensitivity over several microns.
- The results are not in contradiction with the literature, where model layers of several hundreds of nm only have been successfully profiled.

D. Debois, A. Brunelle, O. Laprévote, Int. J. Mass Spectrom., 2007, 260, 115-120

Dual beam depth profiling



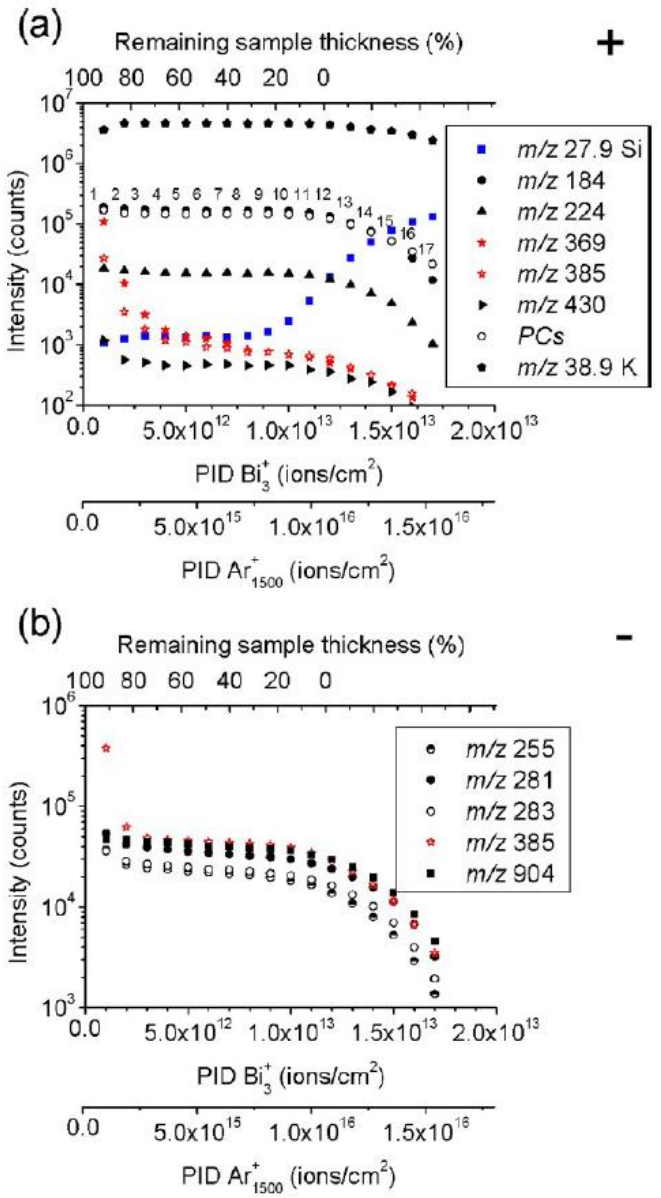
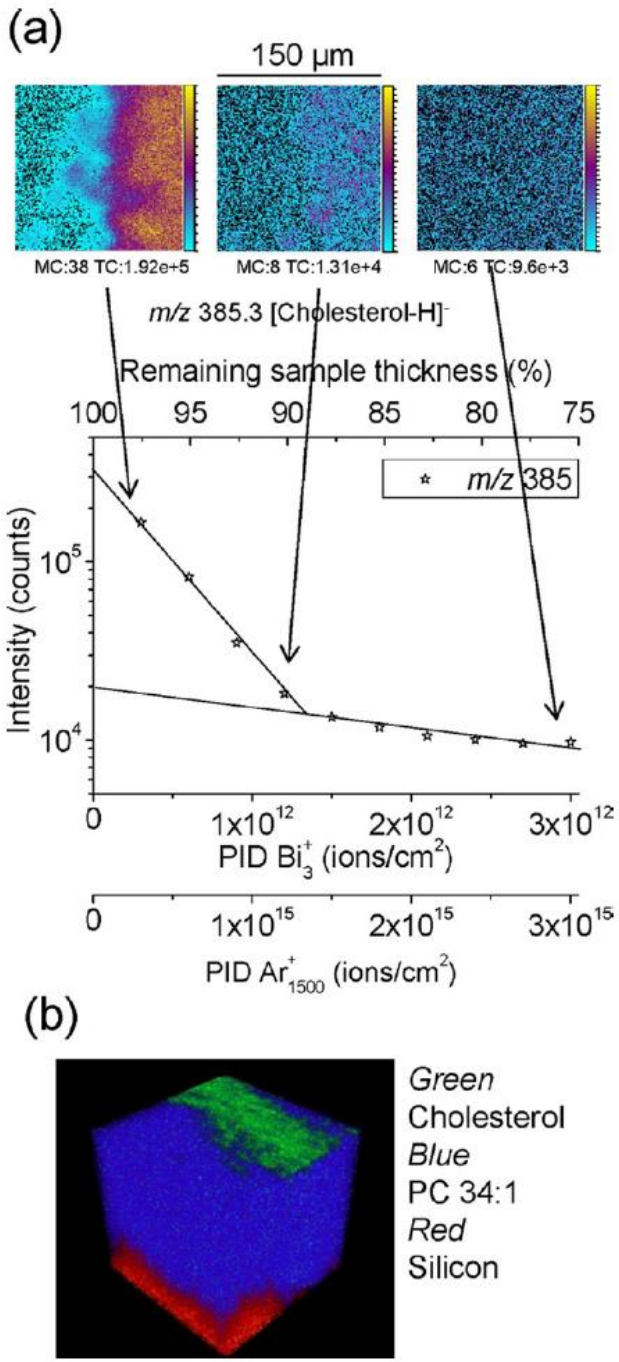


Figure 2. Intensities of several ions as a function of the analysis (Bi_3^+) and sputtering (Ar_{1500}^+) ion doses densities. (a) Positive ion mode; (b) negative ion mode. The error bars, corresponding to $(N)^{1/2}$ (with N the intensity in number of counts) are two small to be reported on the graphic.



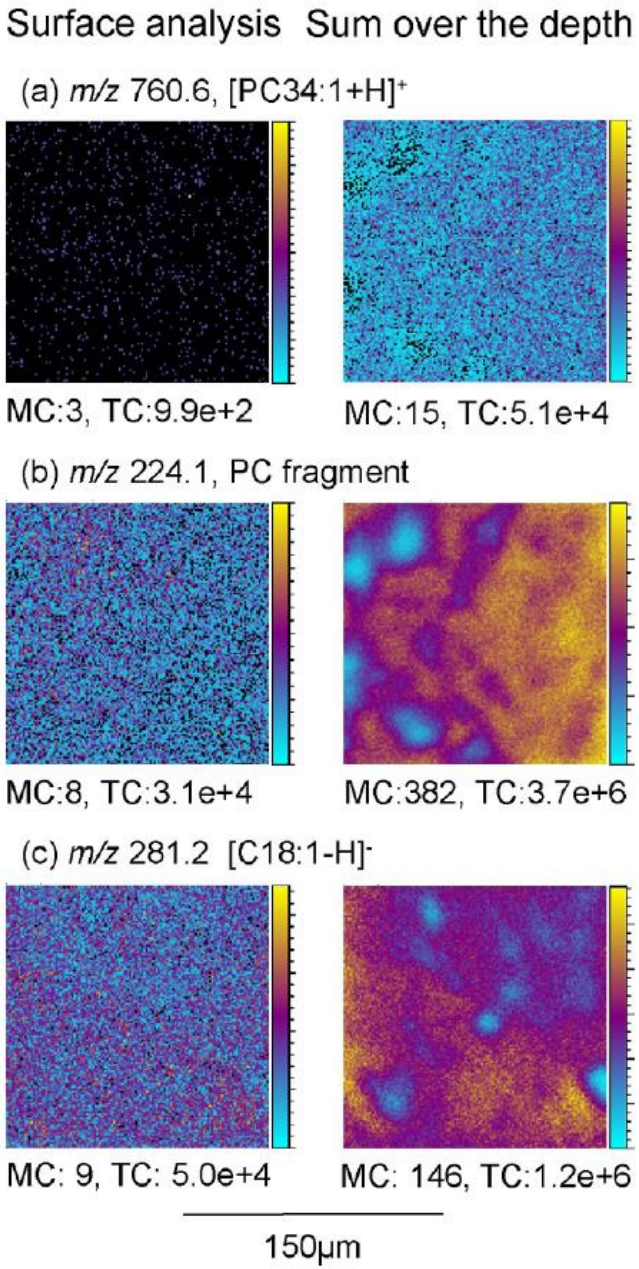
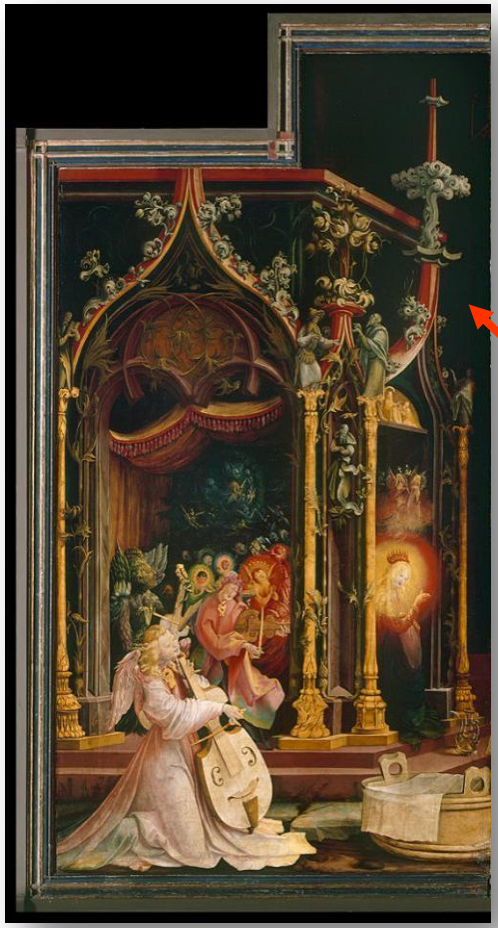


Figure 4. (a) Images of the PC 34:1 ion at m/z 760.6 (b) and images of a PC fragment ion ($C_8H_{19}NO_4P$) at m/z 224.1 in the positive ion mode. (c) Images of the fatty acid C18:1 ion at m/z 281.2 in the negative ion mode obtained at the sample surface and obtained from the sum over the whole sample thickness (right panel).

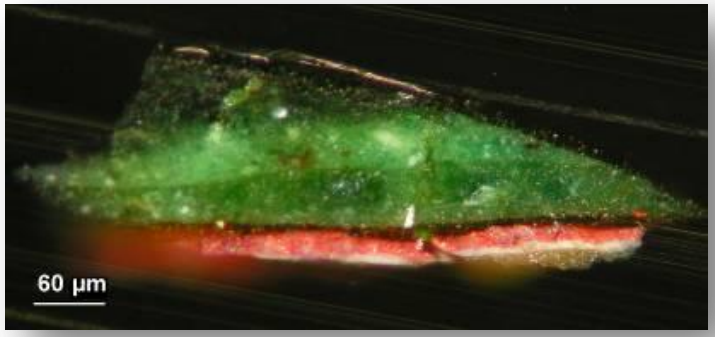
dx.doi.org/10.1021/ac4009513 | Anal. Chem. 2013, 85, 7745−7752

Study of cultural heritage samples

Identification of a copper green pigment



Concert of Angels



In painting, the green colour can be obtained with a mineral pigment:

- malachite (copper carbonate)
- verdigris (copper acetate)
- copper chloride
- green earth (silicates with K, Si, Al, Fe and Mg)

or with an organic pigment:

- copper resine
- breakdown products: interaction between a mineral pigment and a organic binding media (lipids or proteins)…

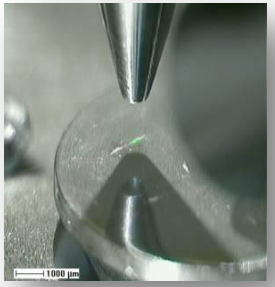
La technique picturale de Grünewald et de ses contemporains, Eds. P. Béguerie-De Paepe, M. Menu, C2RMF CNRS-UMR171 et Musée d'Unterlinden de Colmar, 2007

P. Richardin, V. Mazel, P. Walter, O. Laprévote, A. Brunelle J. Am. Soc. Mass Spectrom. 2011, 22, 1729-1736

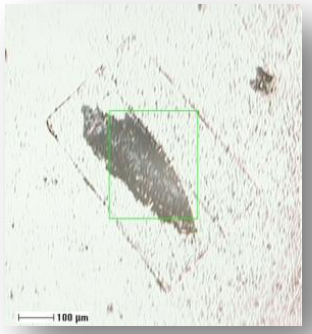
Study of cultural heritage samples

TOF-SIMS analysis of the painting cross section

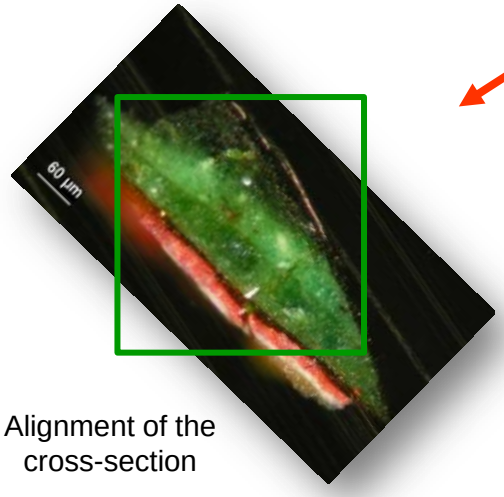
Microscopic image of the cross-section MGN8



Photography of an intact cross-section deposited on a SEM holder



Secondary ion image (Visualisation of the analysed area)



Alignment of the cross-section

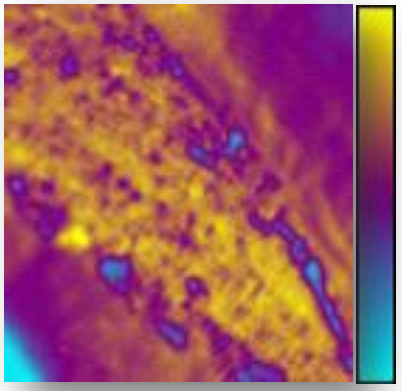
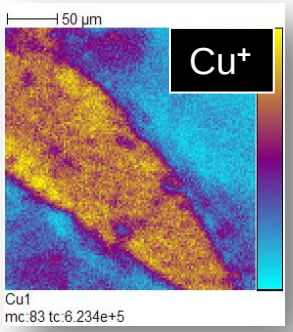


Image of the total ion current

P. Richardin, V. Mazel, P. Walter, O. Laprévote, A. Brunelle
J. Am. Soc. Mass Spectrom. 2011, 22, 1729-1736

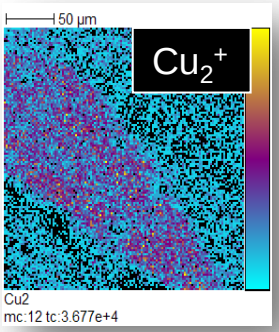
Study of cultural heritage samples

Copper: positive ion mode



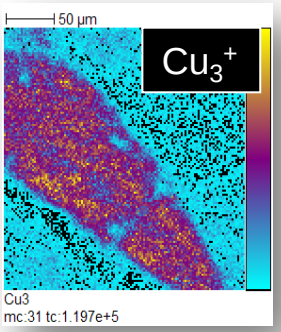
m/z 63 and 65

+



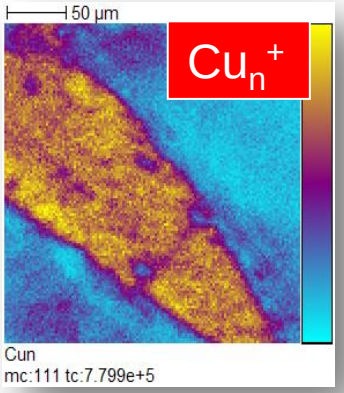
m/z 126, 128 and130

+



m/z 189, 191, 193 and 195

→



Localization of copper

Sum of all the images corresponding to the Cu_n^+ clusters

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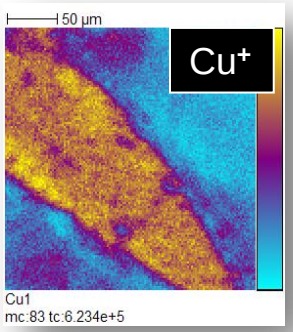
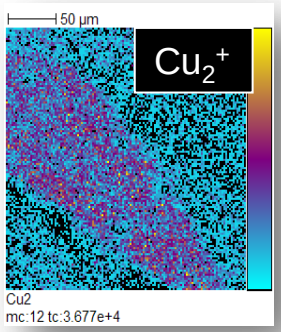
Copper: positive ion mode



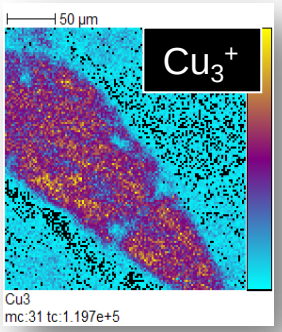
m/z 63 and 65

+



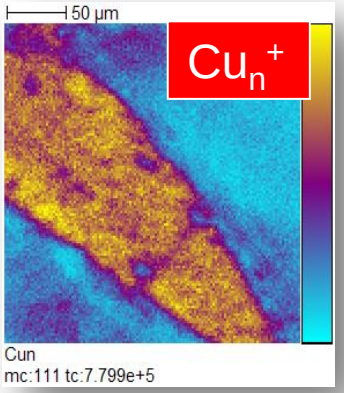
m/z 126, 128 and130

+



m/z 189, 191, 193 and 195

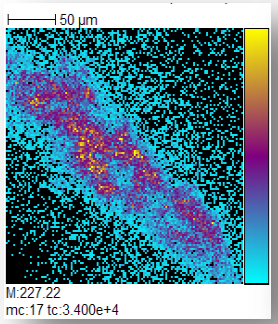
→



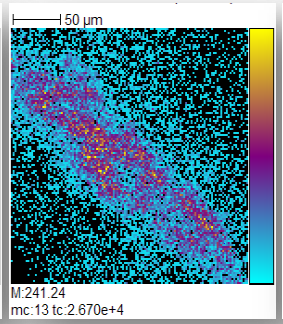
Localization of copper

Sum of all the images corresponding to the Cu_n^+ clusters

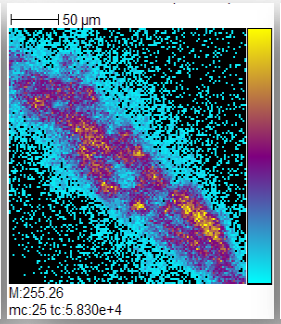
Lipids: negative ion mode



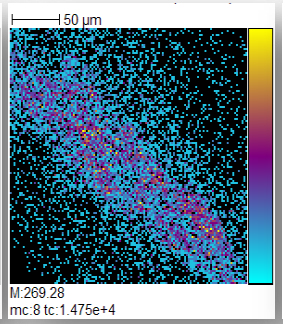
Myristate
(C14:0)



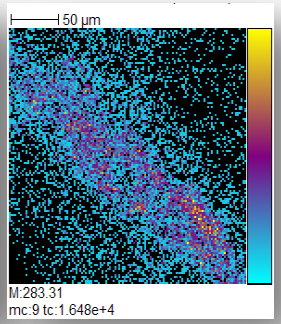
(C15:0)



Palmitate
(C16:0)

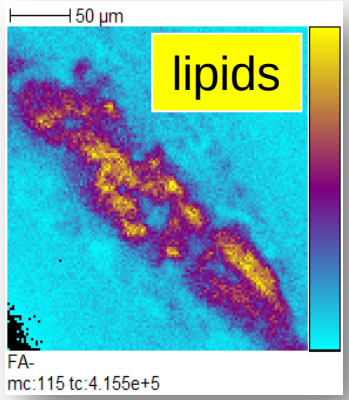


(C17:0)



Oleate
(C18:0)

→



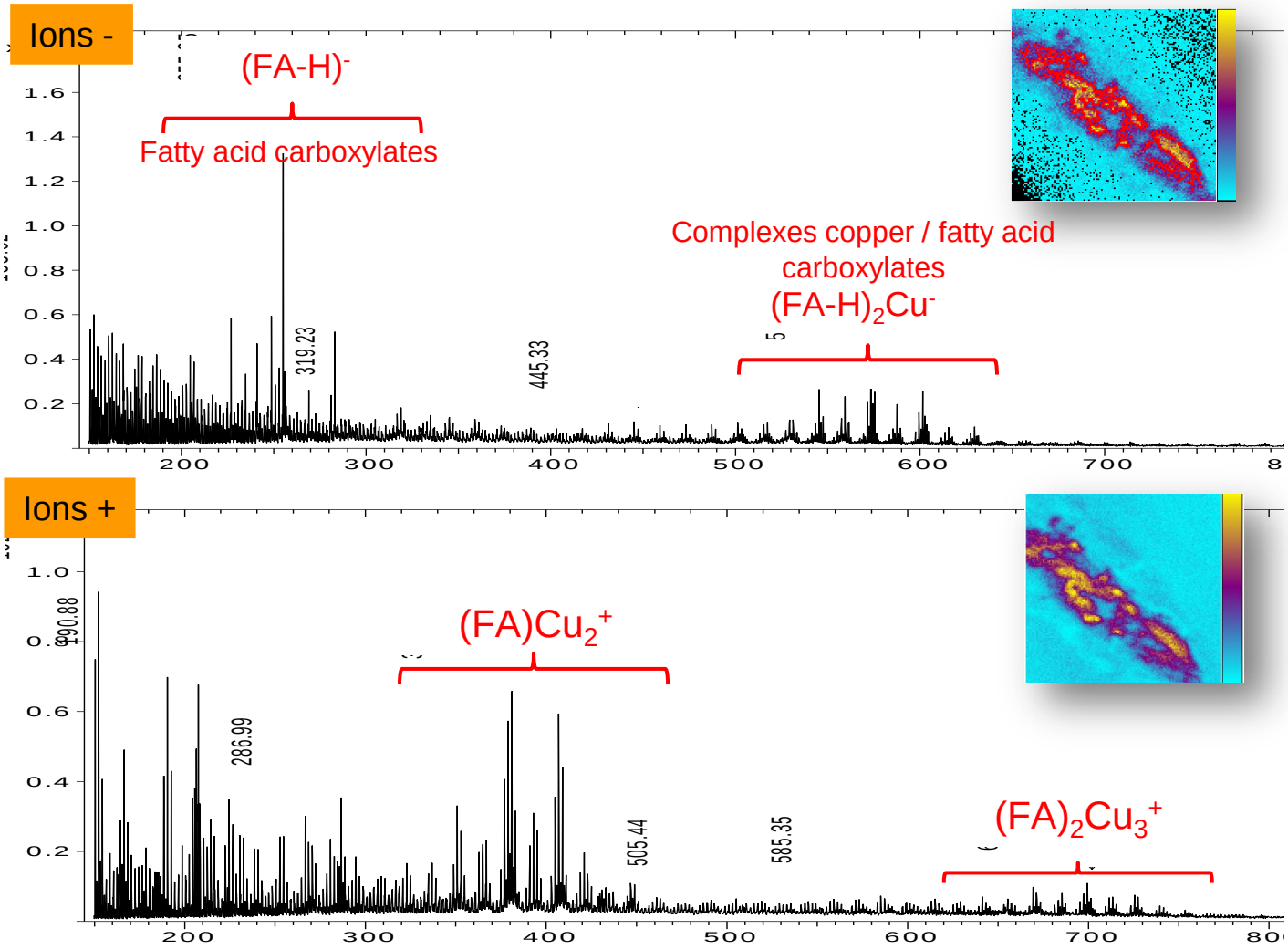
Localization of lipids

Carboxylates $RCOO^-$

P. Richardin, V. Mazel, P. Walter, O. Laprévote, A. Brunelle
J. Am. Soc. Mass Spectrom. 2011, 22, 1729-1736

Study of cultural heritage samples

Mass spectra of the copper / lipid rich area



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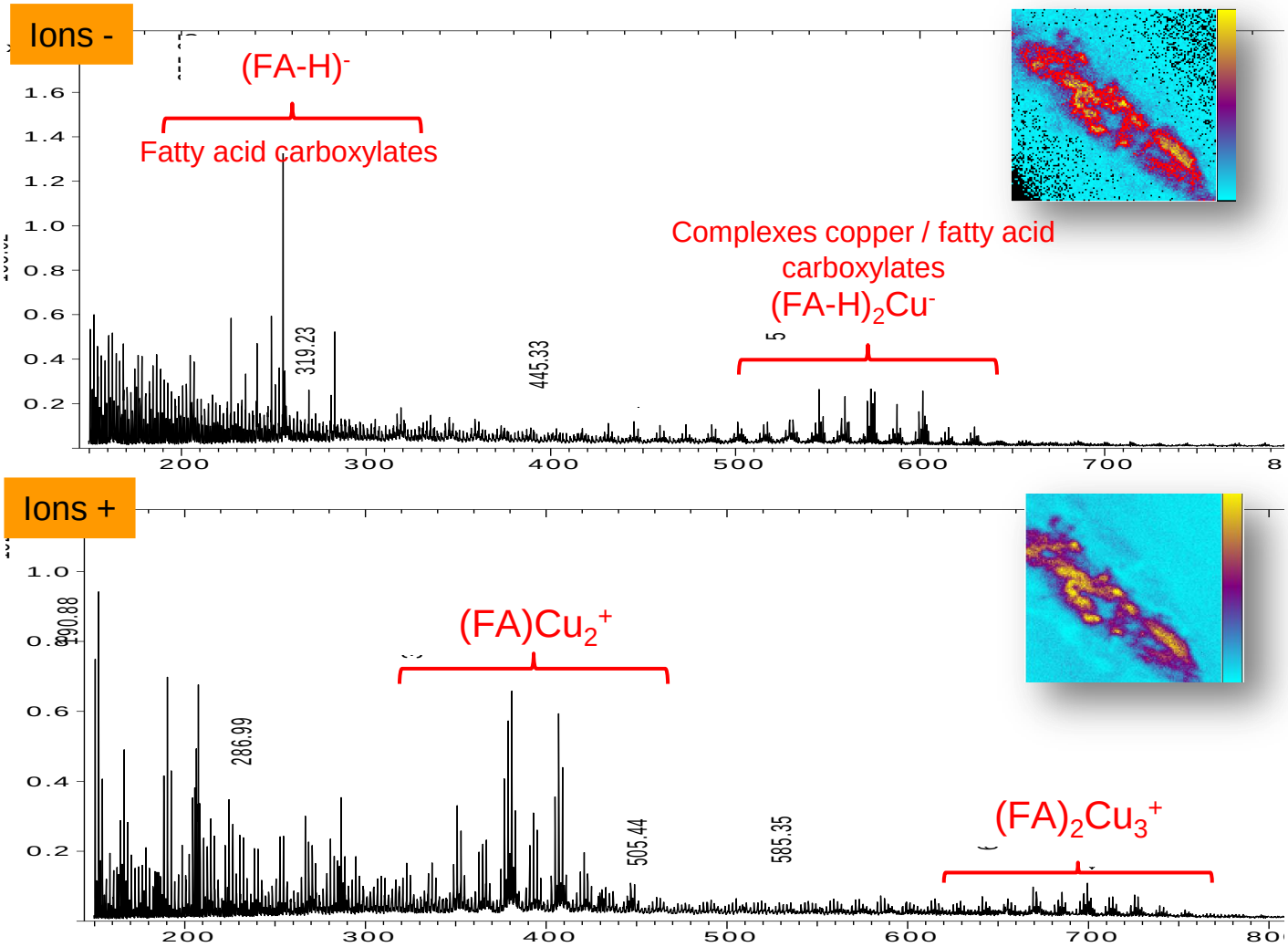
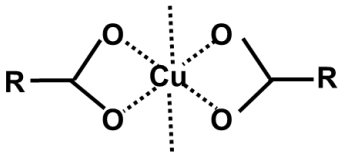


These two mass spectra are compatible with a copper carboxylate pigment (structure not clearly established)



Copper carboxylates ($R = C_nH_{2n+1}$)

This result was confirmed by infrared microscopy (SR-µFTIR)

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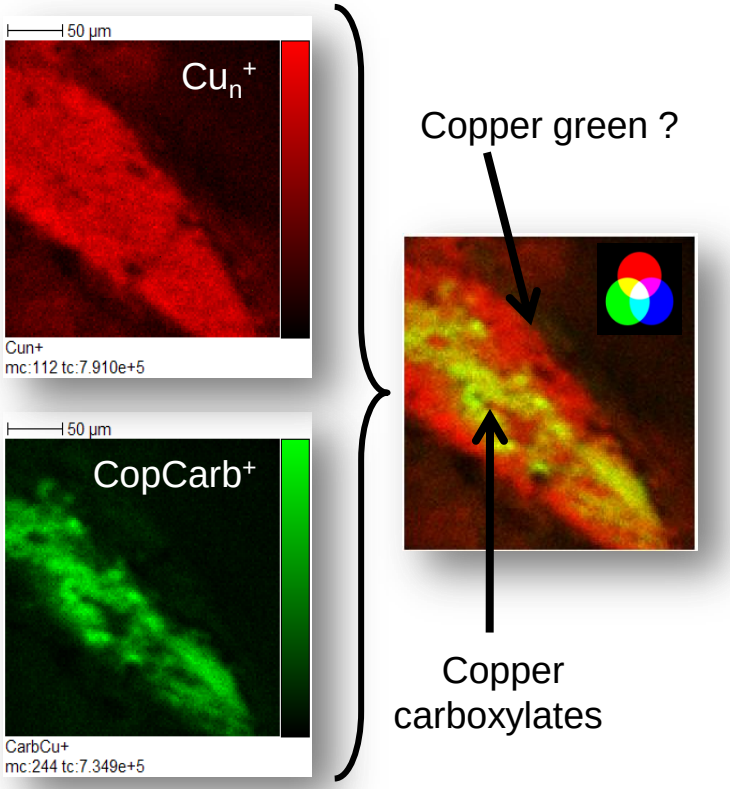
Study of cultural heritage samples

Identification of the copper green pigment



Color overlay shows
the distribution of Cu ions
and Cu carboxylates

This result was also confirmed by infrared microscopy (SR-µFTIR)

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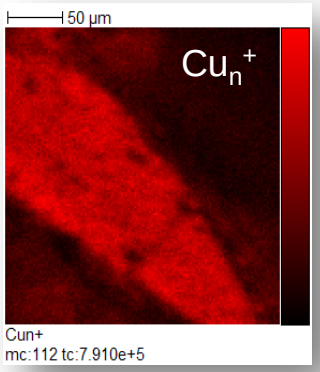
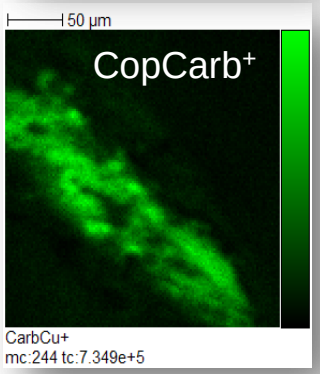




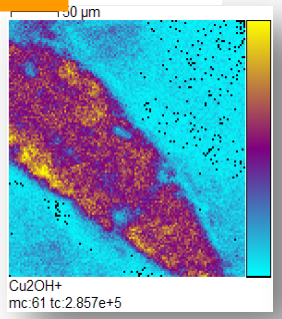
Copper green ?

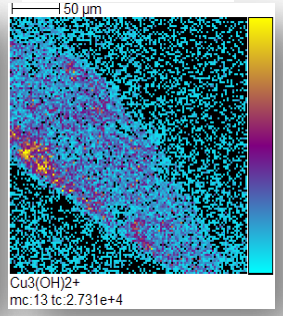
Copper carboxylates

Color overlay shows the distribution of Cu ions and Cu carboxylates

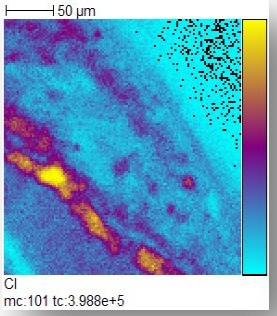
Copper hydroxide ions

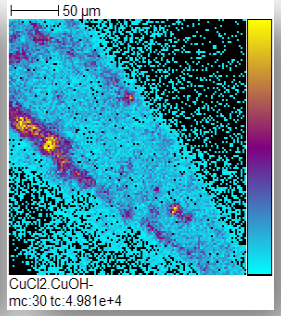
Ions + Cu_2OH^+ $Cu_3(OH)_2^+$





Ions - Cl^- $CuCl_2.CuOH^-$





This result was also confirmed by infrared microscopy (SR-µFTIR)

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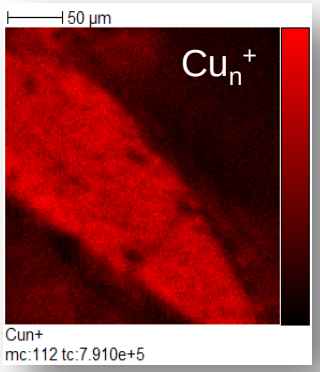
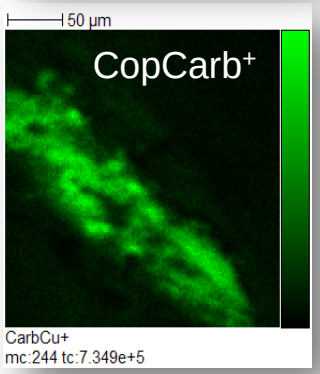
Study of cultural heritage samples

Identification of the copper green pigment





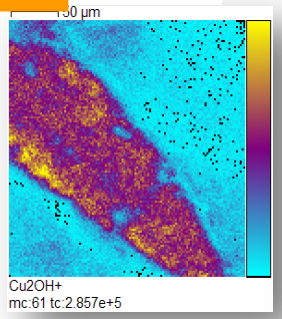
Copper green ?

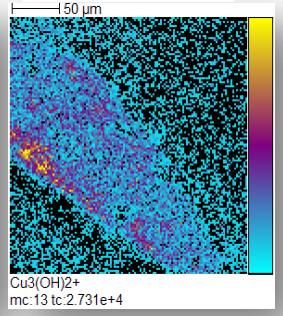
Copper carboxylates

Color overlay shows the distribution of Cu ions and Cu carboxylates

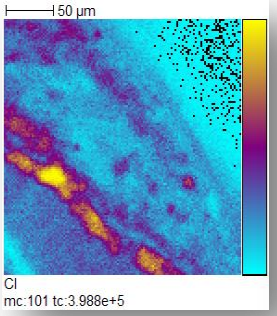
Copper hydroxide ions

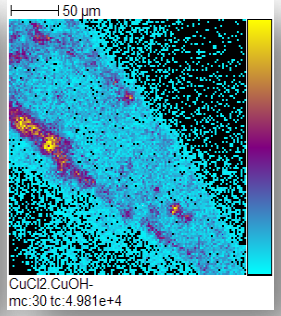
Ions + Cu_2OH^+ $Cu_3(OH)_2^+$



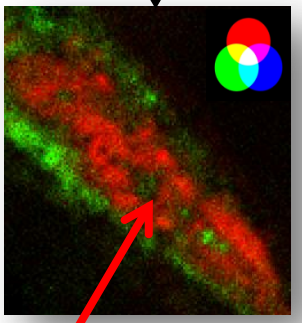


Ions - Cl^- $CuCl_2.CuOH^-$





Atacamite $Cu_2(OH)_3Cl$



$CopCarb^+$

This result was also confirmed by infrared microscopy (SR-µFTIR)

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