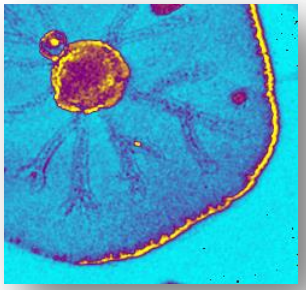
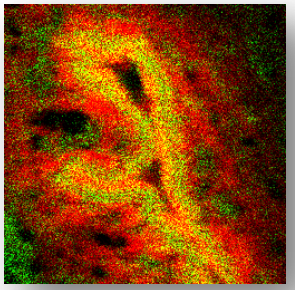
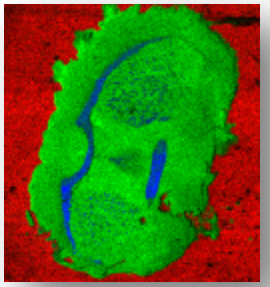
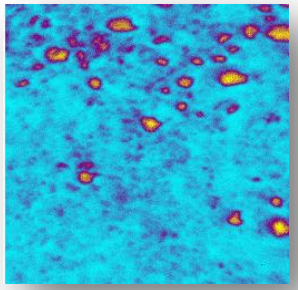



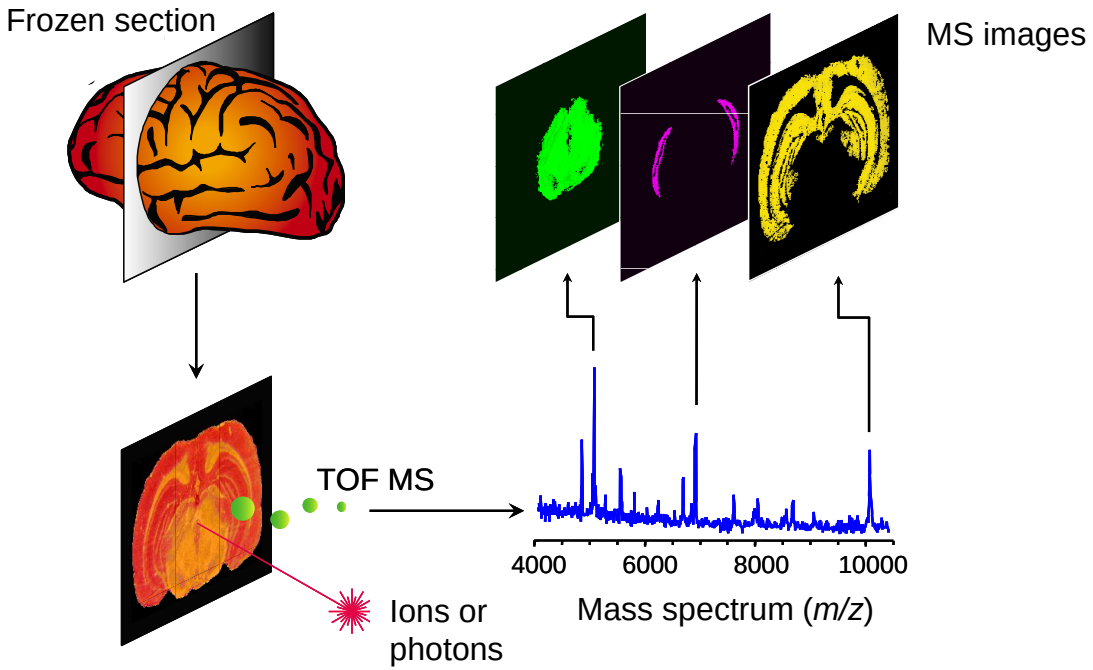
Étude de métabolites de bois tropical à l'échelle micrométrique à l'aide de l'imagerie par spectrométrie de masse TOF-SIMS

11 mai 2016

Alain BRUNELLE

Directeur de Recherche
CNRS
Institut de Chimie des Substances Naturelles
Gif-sur-Yvette
France
Alain.Brunelle@cnrs.fr

Mass Spectrometry Imaging
MALDI and SIMS



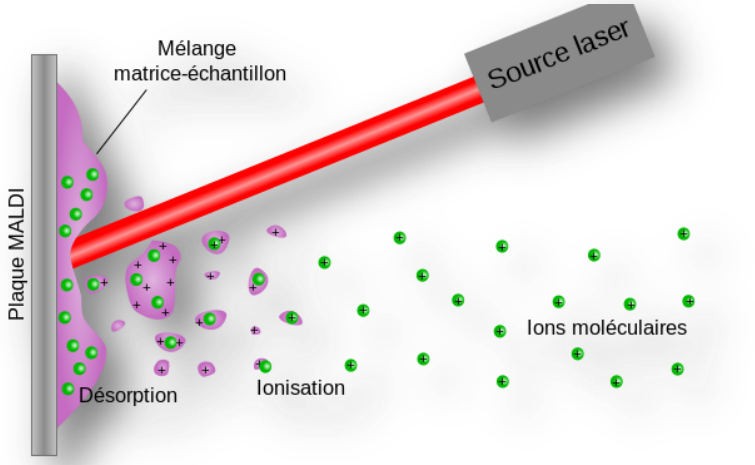
- **MS Images**
  - Ion density maps
  - 1 acquisition = one image for each peak and one spectrum per pixel…

Focused beams of UV photons (MALDI)
and/or cluster ions (SIMS)
make possible to raster a surface

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

Matrix Assisted Laser Desorption Ionization

Proteins, peptides, sugars, lipids, etc… are mixed with a small organic molecule called matrix, which absorbs the UV radiation and enables the soft desorption and ionization of analytes with very little fragmentation.

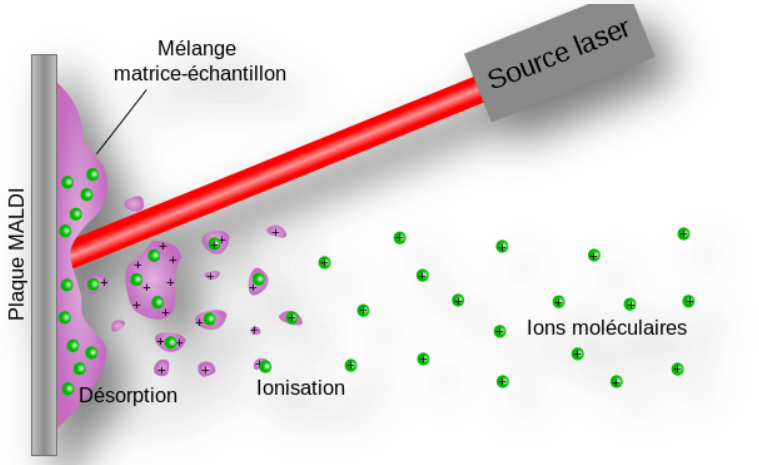


M. Karas, F. Hillenkamp, Anal. Chem. 1988, 60, 2299-2301.
K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida, Rapid Commun. Mass Spectrom. 1988, 2, 151-153.

MALDI and SIMS

Matrix Assisted Laser Desorption Ionization

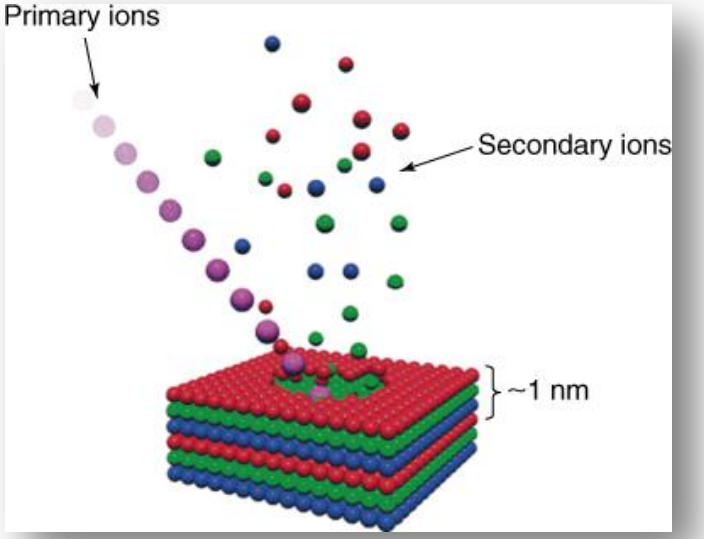
Proteins, peptides, sugars, lipids, etc… are mixed with a small organic molecule called matrix, which absorbs the UV radiation and enables the soft desorption and ionization of analytes with very little fragmentation.



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.

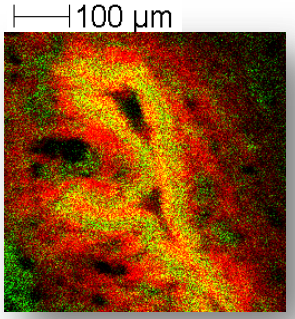
Secondary Ion Mass Spectrometry

A focused ion beam, called primary ions, irradiates the sample surface from which characteristic secondary ions are emitted.

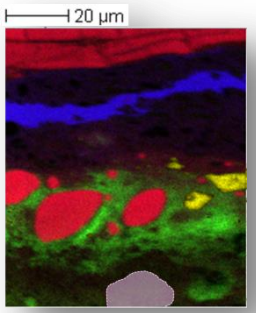


R. Castaing, G. Slodzian, J. Microsc. 1962, 1, 395-410
A. Benninghoven, E. Loebach, Rev. Sci. Instrum. 1971, 42, 49-52.

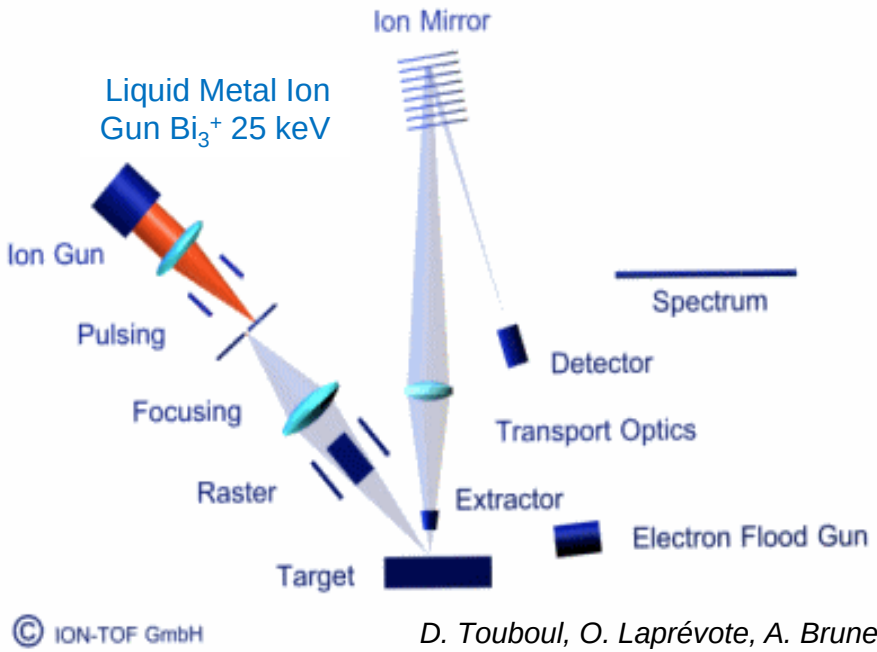
Mass Spectrometry Imaging with a TOF-SIMS



Fornix in rabbit eye section



Rembrandt painting cross-section



Small molecules (lipids, metabolites,…)
Spatial resolution 400 nm – 2 µm

Tissue sections
Cultural heritage samples (organics and non-organics)
Very small samples

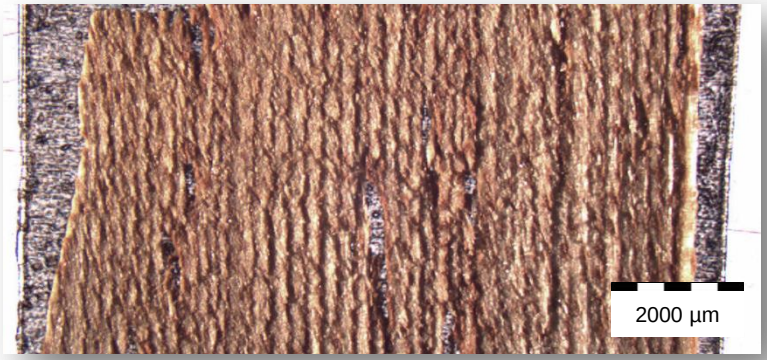
D. Touboul, O. Laprévote, A. Brunelle, Curr. Opin. Chem. Biol. 2011, 15, 725-732
C. Bich, D. Touboul, A. Brunelle, Mass Spectrom. Rev. 2014, 33, 442-451

Sample preparation

Microtome

Use of a stainless steel blade
Section thickness ~20 µm

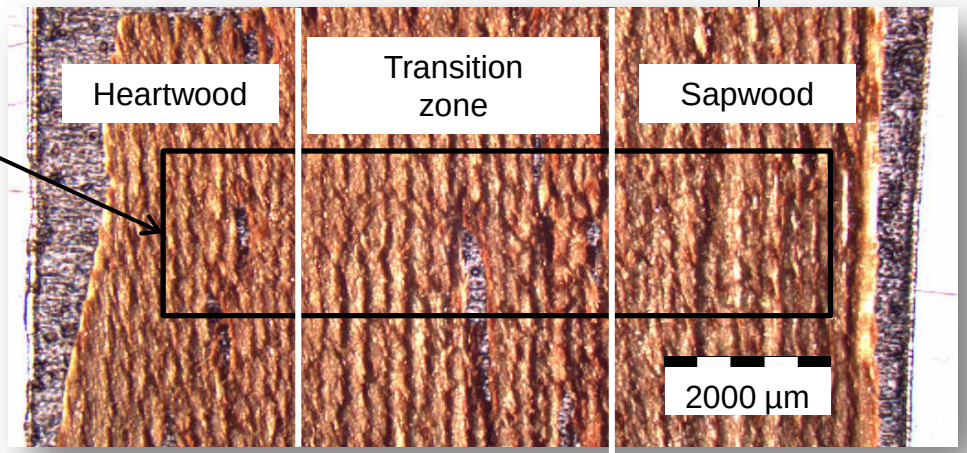
Dry sample



Radial section
Lack of information

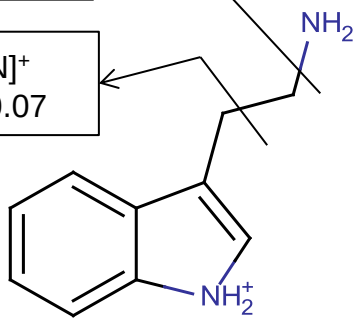
Analysis of a wood (*Dicorynia guianensis*) radial section

Analyzed area
Size: 8000 µm x 2000 µm
1024 x 256 pixels
more than 260 000 spectra
Resolution: 8 µm

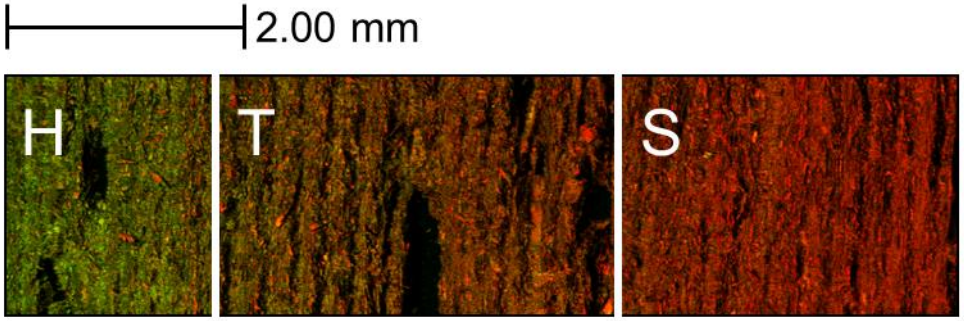


$[C_{10}H_{10}N]^+$
m/z 144.08

$[C_9H_8N]^+$
m/z 130.07



Tryptamine
$C_{10}H_{13}N_2^+$



Green : Tryptamine fragments
Red: Na^+

Q.P. Vanbellingen, T. Fu, C. bich, N. Amusant, D. Stien, S. Della-Negra, D. Touboul, A. Brunelle, J. Mass Spectrom. 2016 DOI 10.1002/jms.3762

Sample preparation

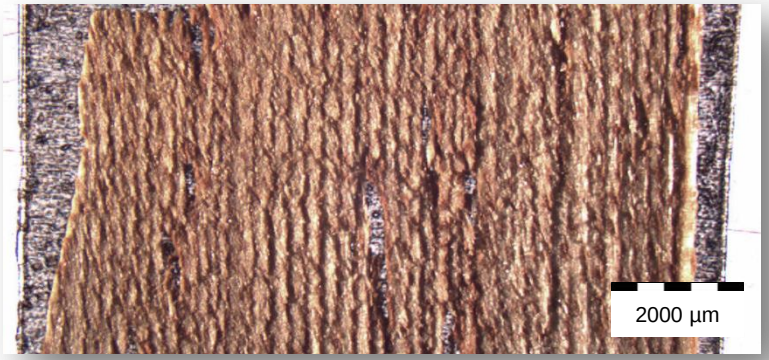
Microtome

Use of a stainless steel blade
Section thickness ~20 µm

Ultramicrotome

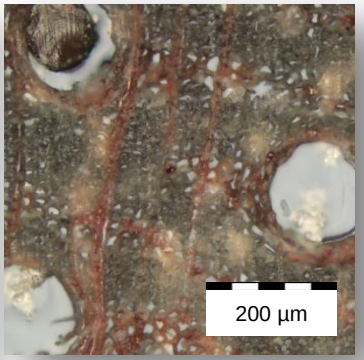
Use of a diamond knife
Section thickness 200 to 500 nm

Dry sample



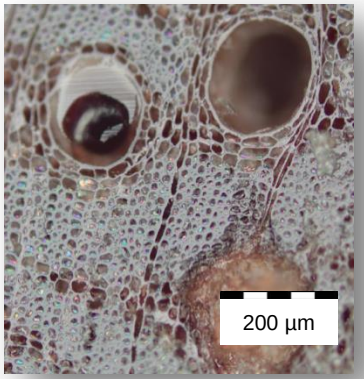
Radial section
Lack of information

Wet sample



Transverse section
Loss of information

Dry sample

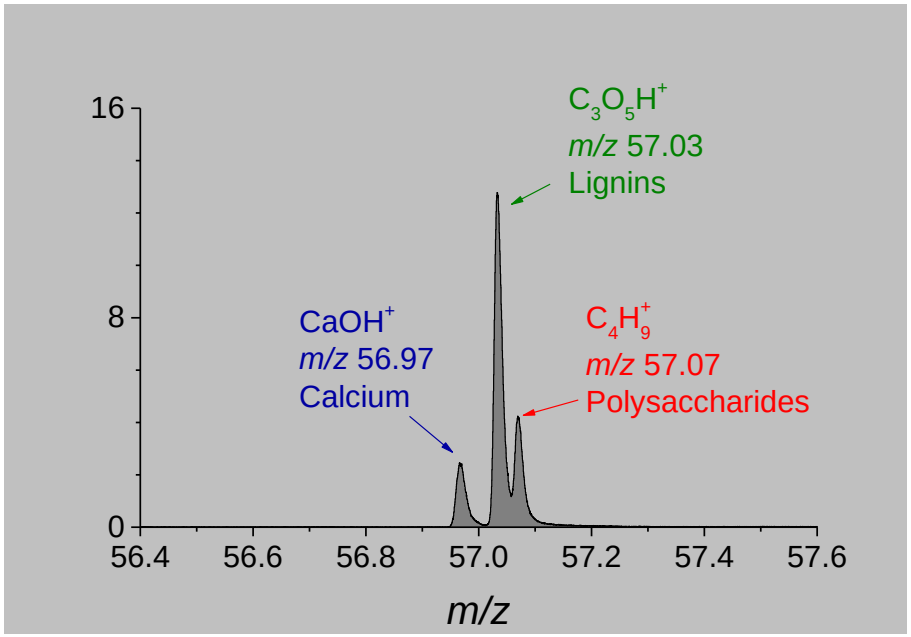


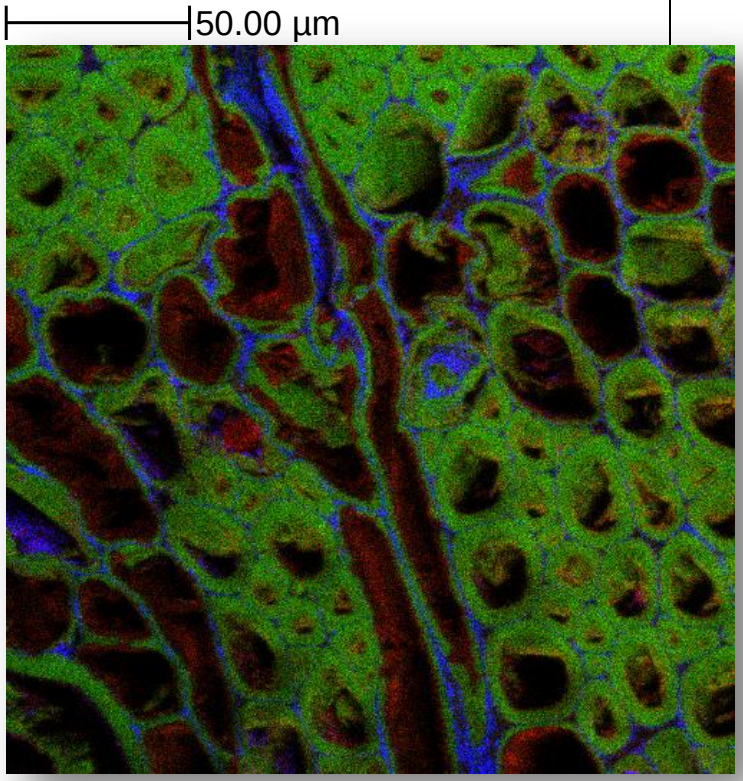
Transverse section

Ion Image at cellular scale

Heartwood

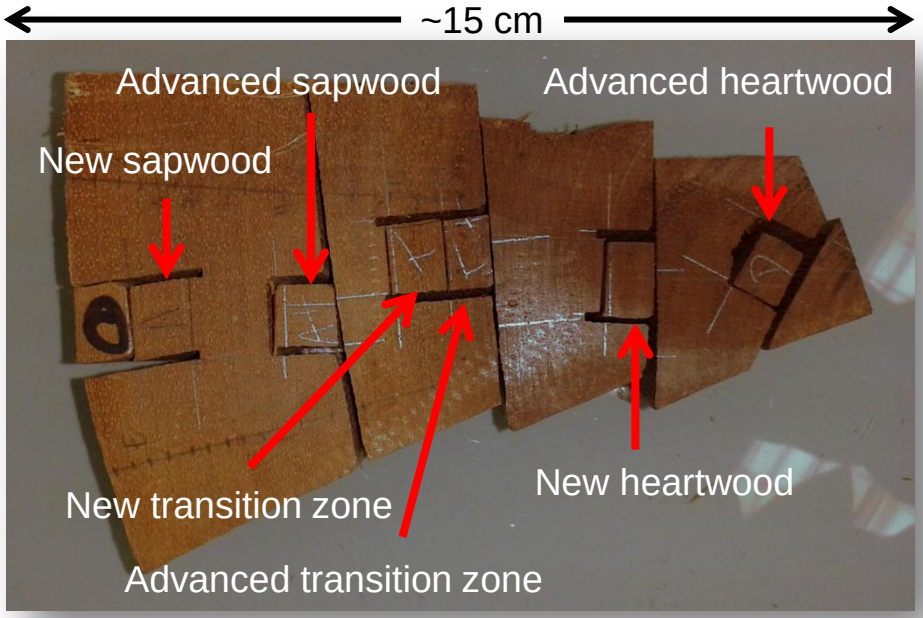
Pixel size 400 nm

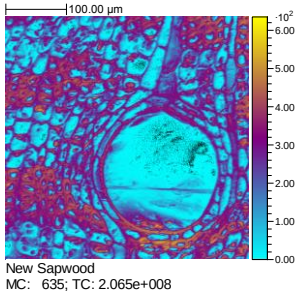




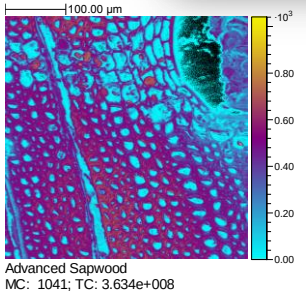
Q.P. Vanbellingen, T. Fu, C. Bich, N. Amusant, D. Stien, S. Della-Negra, D. Touboul, A. Brunelle, J. Mass Spectrom. 2016 DOI 10.1002/jms.3762

Analysis of a wood (*Dicorynia guianensis*) radial section

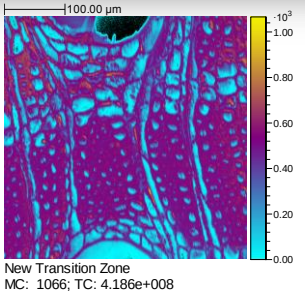




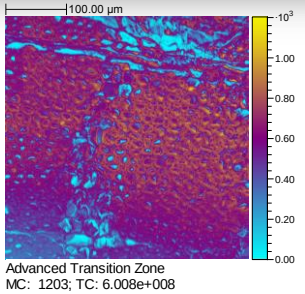
New sapwood



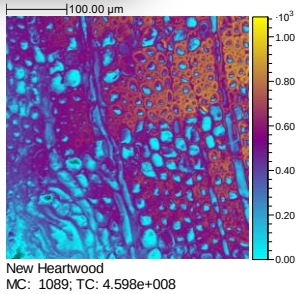
Advanced sapwood



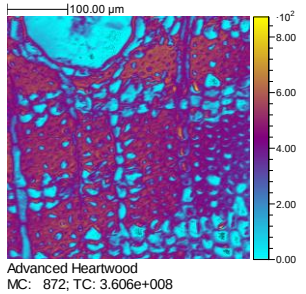
New transition zone



Advanced transition zone



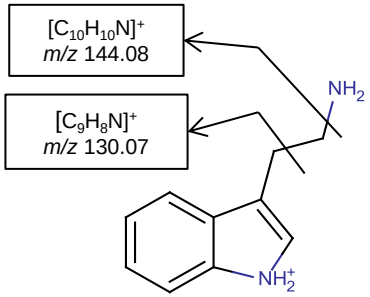
New heartwood



Advanced heartwood

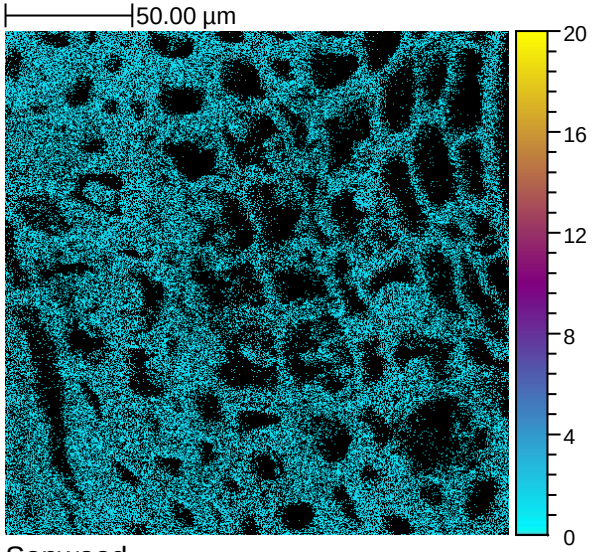
Q.P. Vanbellingen, T. Fu, C. Bich, N. Amusant, D. Stien, S. Della-Negra, D. Touboul, A. Brunelle, J. Mass Spectrom. 2016 DOI 10.1002/jms.3762

Ion Image at cellular scale

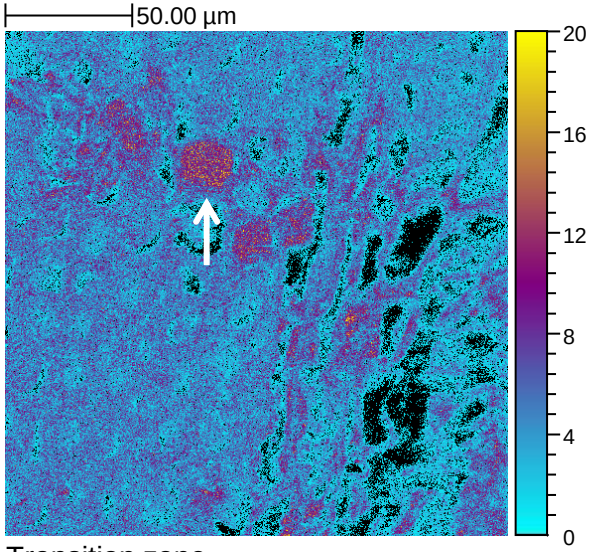


Tryptamine
$C_{10}H_{13}N_2^+$

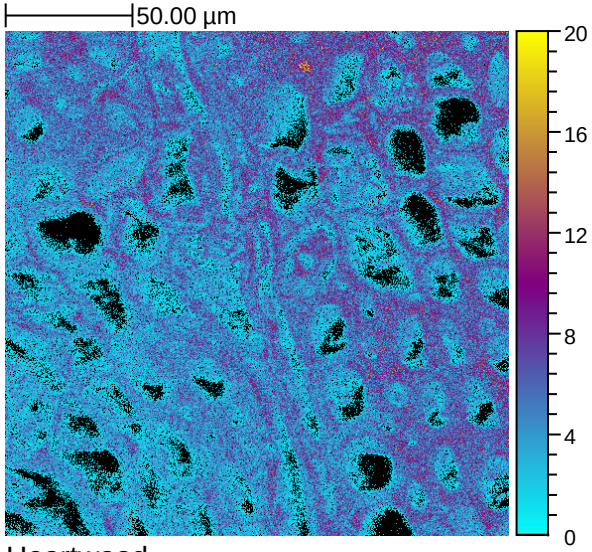
Tryptamine fragment ions



Sapwood
MC: 20; TC: 1.397e+005



Transition zone
MC: 20; TC: 1.092e+006



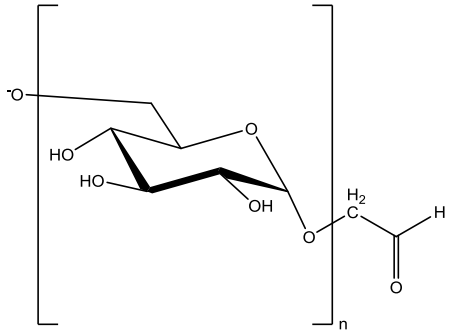
Heartwood
MC: 20; TC: 1.072e+006

Tryptamine is detected in the transition zone in specific cells (axial and radial parenchyma cells)
In heartwood, tryptamine is detected in all the cell walls

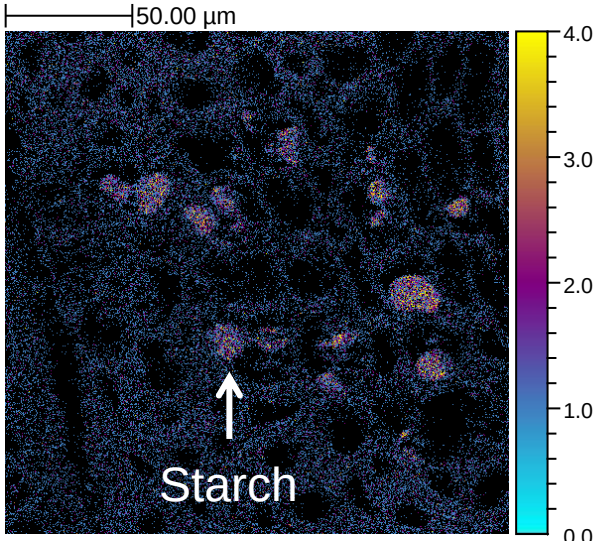
Q.P. Vanbellingen, T. Fu, C. Bich, N. Amusant, D. Stien, S. Della-Negra, D. Touboul, A. Brunelle, J. Mass Spectrom. 2016 DOI 10.1002/jms.3762

A.M. Taylor, B.L. Gartner, J.J. Morrell, Wood Fiber Sci. 2002, 34, 587-611

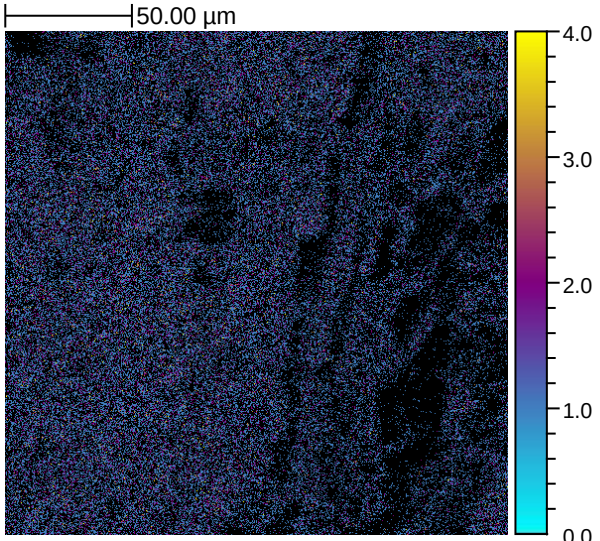
Ion Image at cellular scale



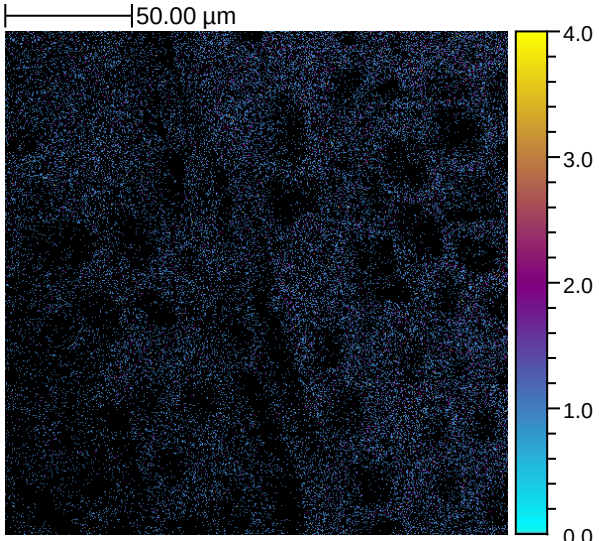
Polysaccharide fragment ions



Sapwood
MC: 4; TC: 6.721e+004



Transition zone
MC: 4; TC: 1.077e+005



Heartwood
MC: 4; TC: 4.681e+004

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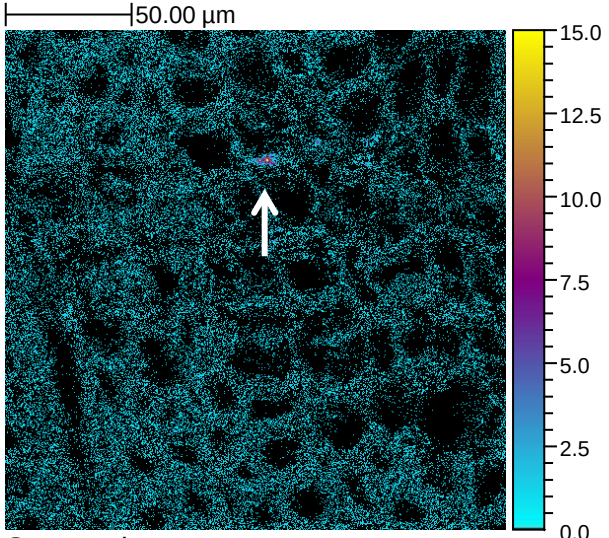
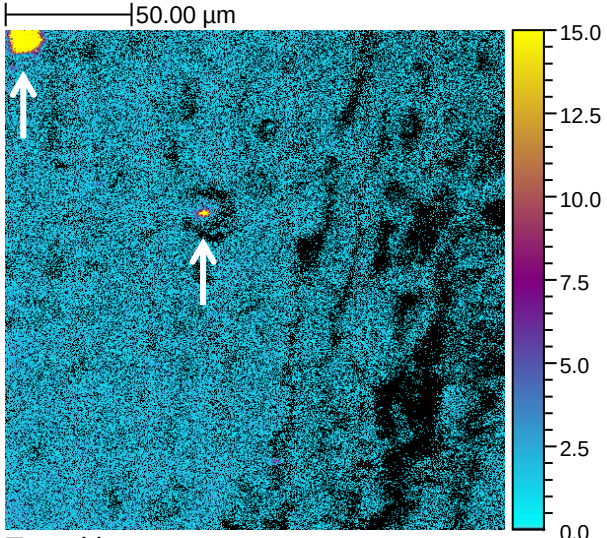
Ion Image at cellular scale

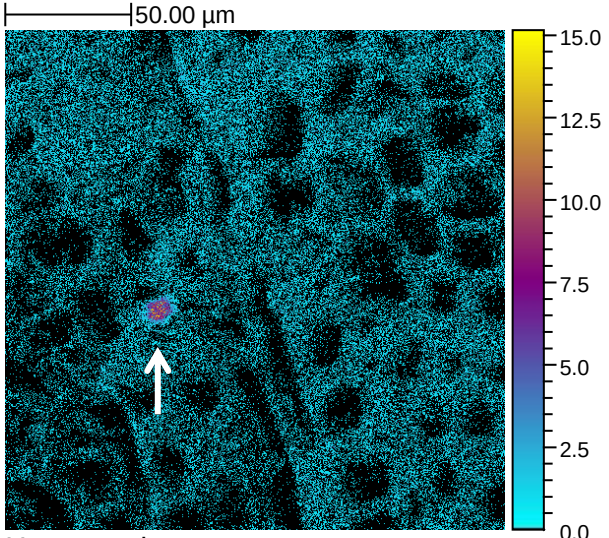
Ions detected from silica structures: Si^-, SiO_2^-, $SiHO_2^-$, SiO_3^- and $(SiO_2)_nOH^-$



Sapwood
MC: 15; TC: 7.339e+004



Transition zone
MC: 15; TC: 2.685e+005



Heartwood
MC: 15; TC: 1.106e+005

Silica structures are detected in all the parts

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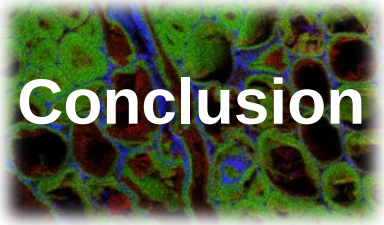
Conclusion



- Optimized acquisition mode in TOF-SIMS imaging
- Optimization of sample preparation
- Three defense modes can be evidenced from the TOF-SIMS analyses:
  - Production of toxic metabolites
  - Removing of nutrients
  - Mineral structures