High resolution imaging of maize stem with time-of-flight secondary ion mass spectrometry

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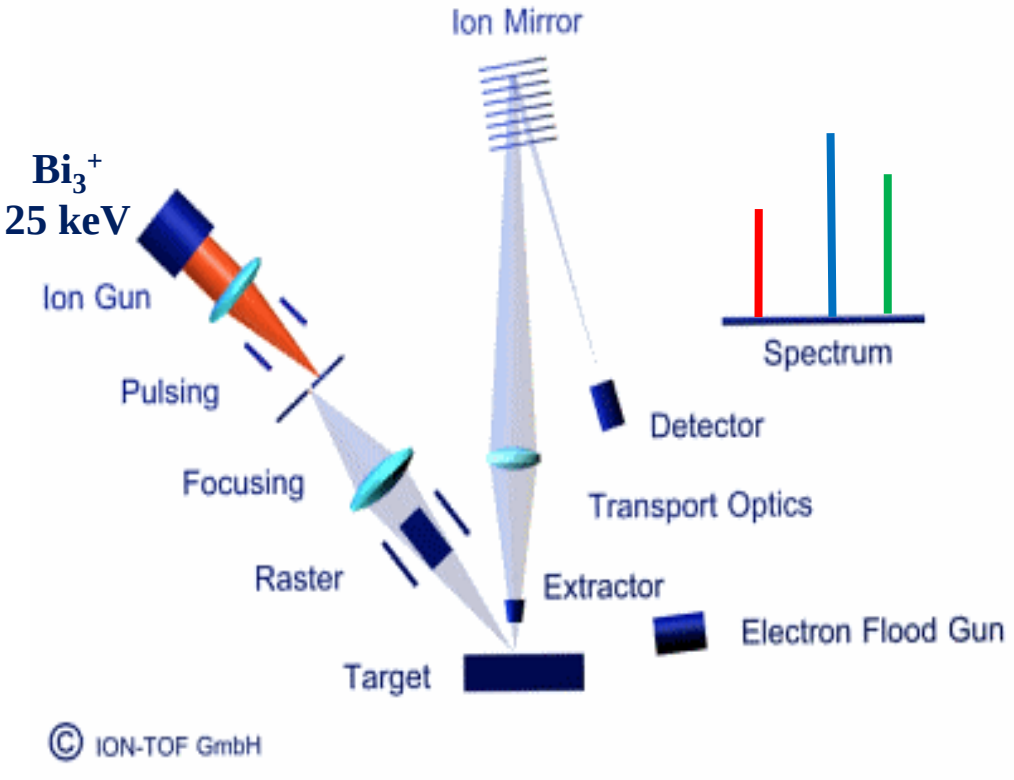
Time-of-Flight Secondary Ions Mass Spectrometry

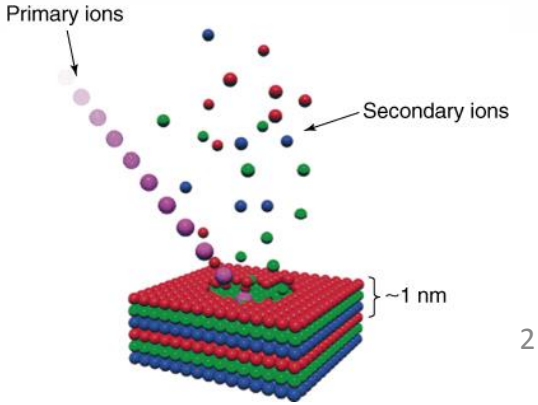
(TOF-SIMS)

$$t_{tof} = \frac{L}{\sqrt{2eV}} \times \sqrt{\frac{m}{z}}$$



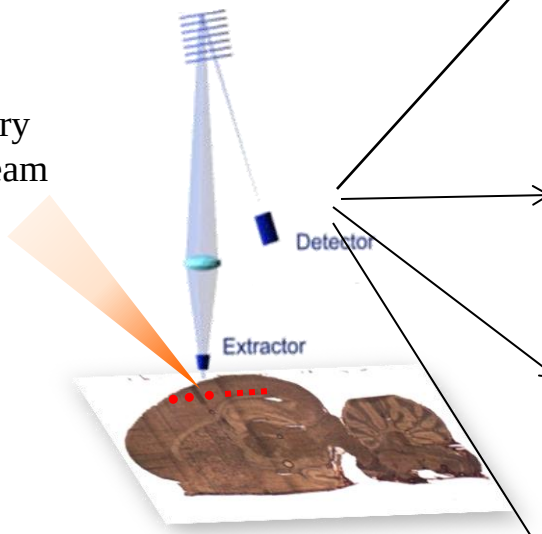
- High spatial resolution: 400 nm~2μm
- No matrix coating
- Suitable for analysis of lipids, matabolites, inorganics…

(Peptides, proteins)

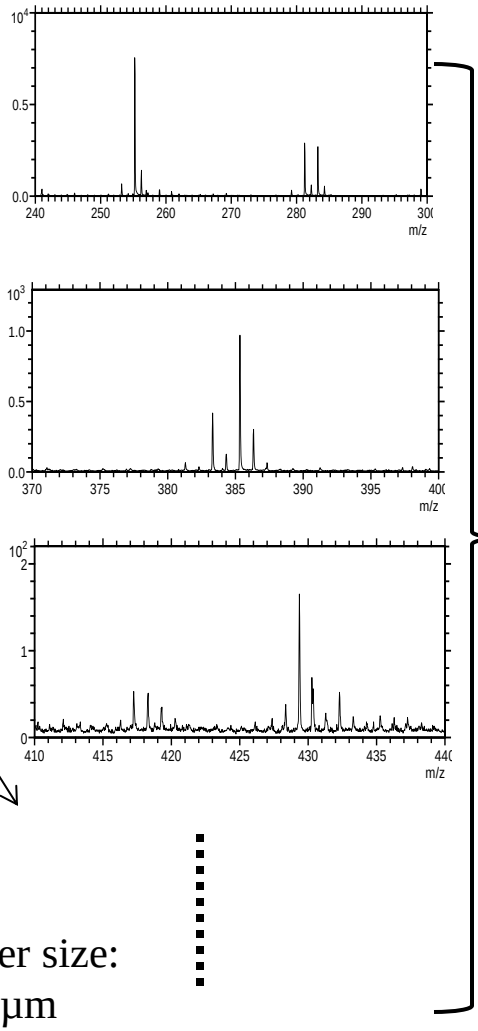


Imaging principle of TOF-SIMS

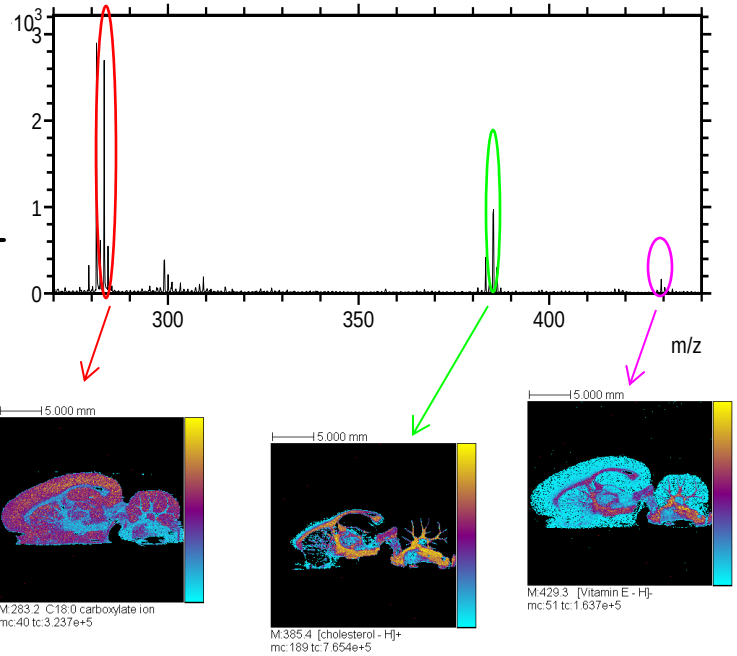
Primary ion beam



Maximum raster size:
500 µm × 500 µm



Total mass spectra



22.4 mm × 22.4 mm

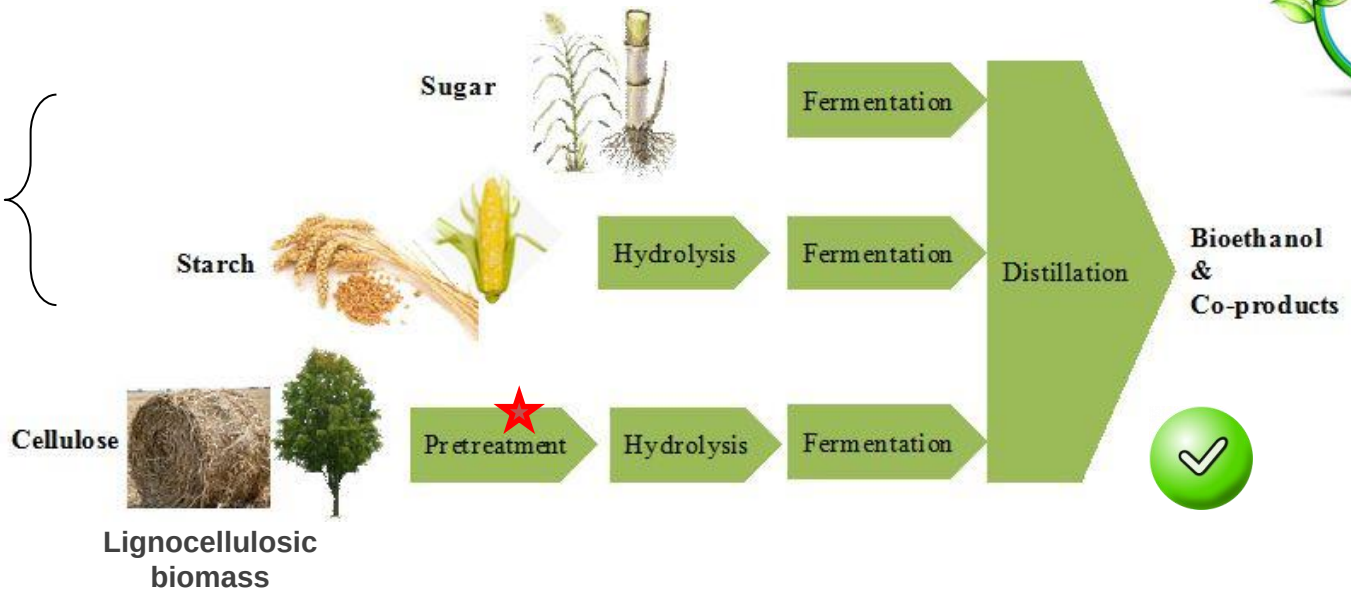
256x256 pixels= 65 536 pixels

= 65 536 mass spectra!!!

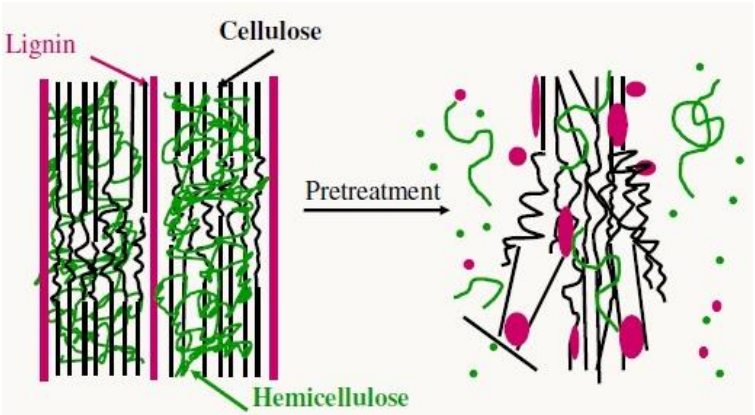
Context: conversion of biomass to bioethanol

First generation bioethanol production

Secondary generation bioethanol production



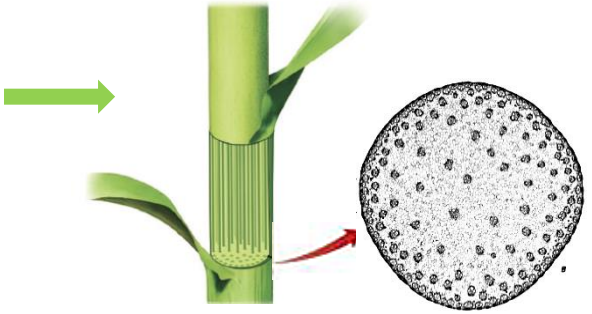
Low conversion efficiency



High temperature, acid or alkaline penetration…

Genetic engineering

Lignin distribution ?

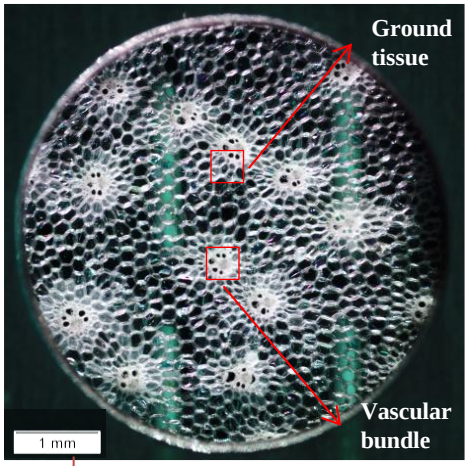


Ragauskas, A. J.; *et al. Science* **2006**, *311*, 484.

Chen, F.; *et al. Nat. Biotechnol.* **2007**, *25*, 759.

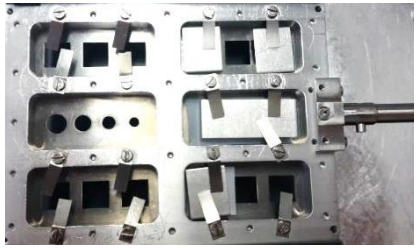
Sample preparation

Vibratoming

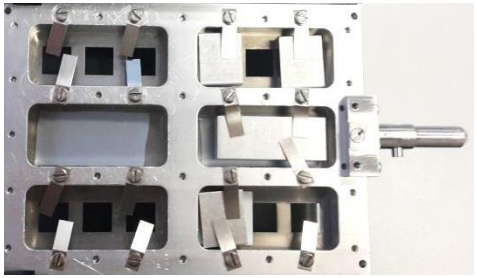


Analyzed area:
400 µm × 400 µm
1024 × 1024 pixels
~0.4 µm/pixel

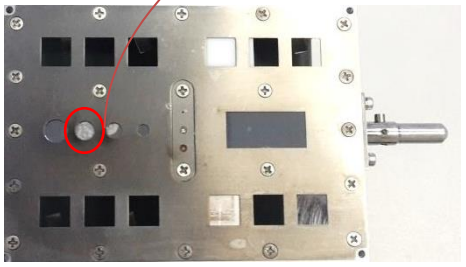
Thickness: 150 µm



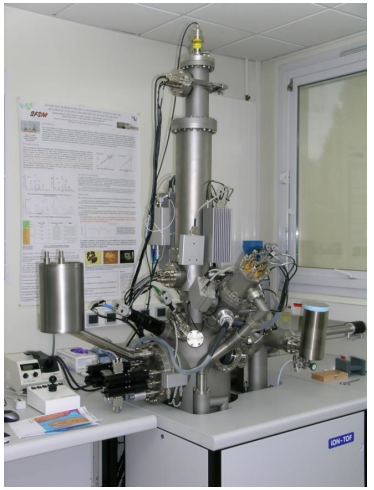
backside



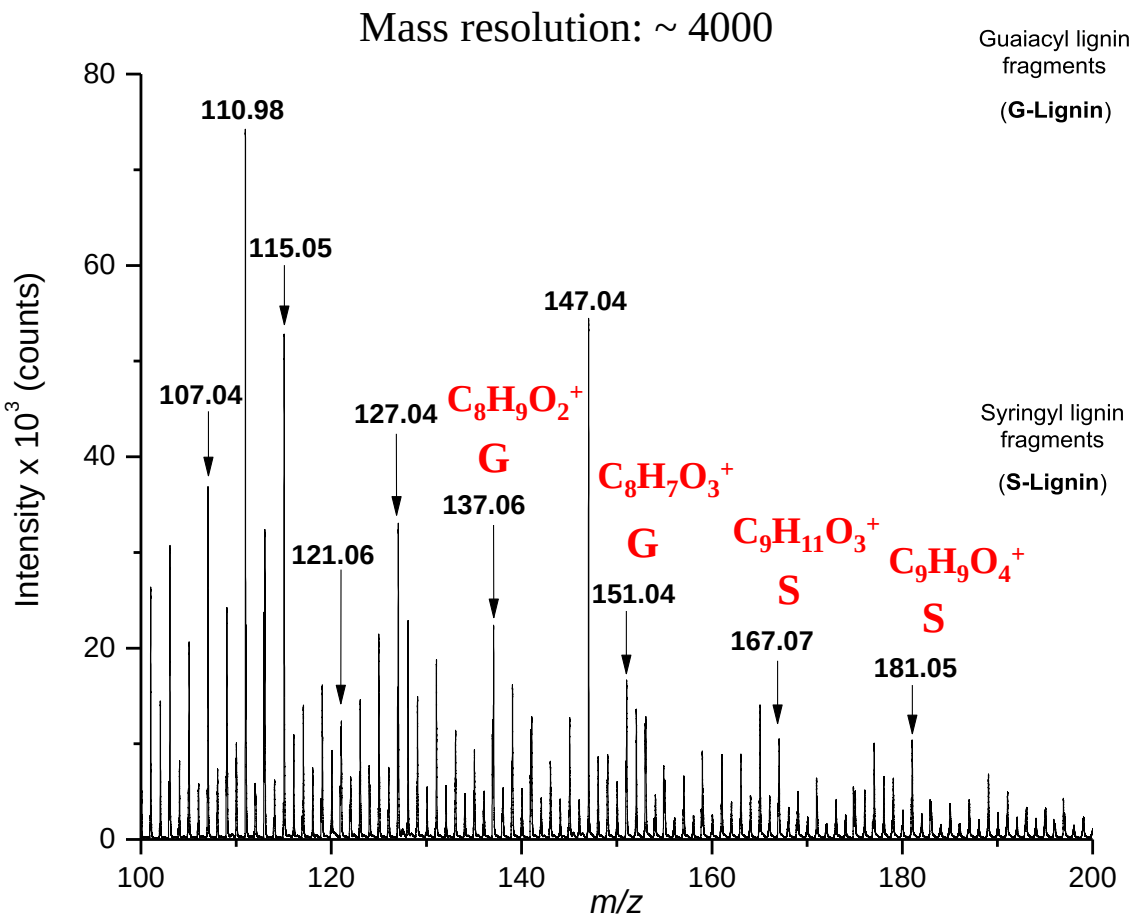
backside



topside

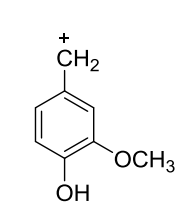


Characteristic lignin fragments in TOF-SIMS

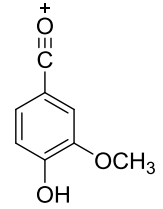


Mass spectrum of ground tissue

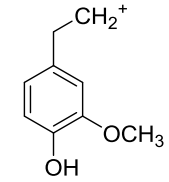
Guaiacyl lignin fragments (**G-Lignin**)



$C_8H_9O_2^+$
m/z 137.060
C_6-C_1

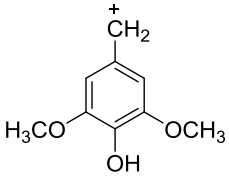


$C_8H_7O_3^+$
m/z 151.039
C_6-C_1 benzoyl

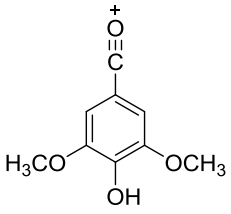


$C_9H_{11}O_2^+$
m/z 151.075
C_6-C_2

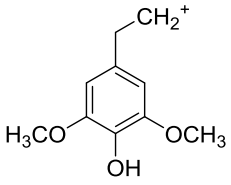
Syringyl lignin fragments (**S-Lignin**)



$C_9H_{11}O_3^+$
m/z 167.070
C_6-C_1



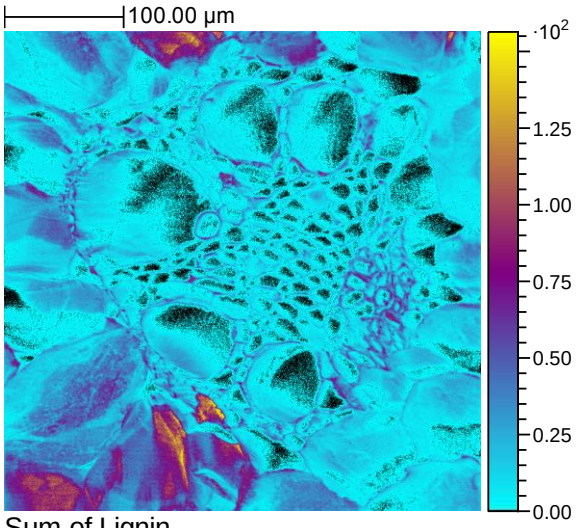
$C_9H_9O_4^+$
m/z 181.049
C_6-C_1 benzoyl



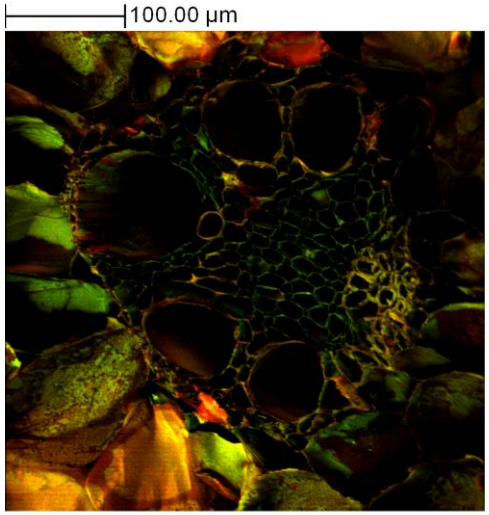
$C_{10}H_{13}O_3^+$
m/z 181.086
C_6-C_2

Lignin	Chemical formula	Measured *m/z*	Calculated *m/z*	Standard deviation (ppm)
G	$C_8H_9O_2^+$	137.056	137.060	-29
	$C_8H_7O_3^+$	151.037	151.039	-13
S	$C_9H_{11}O_3^+$	167.065	167.070	30
	$C_9H_9O_4^+$	181.045	181.049	-22

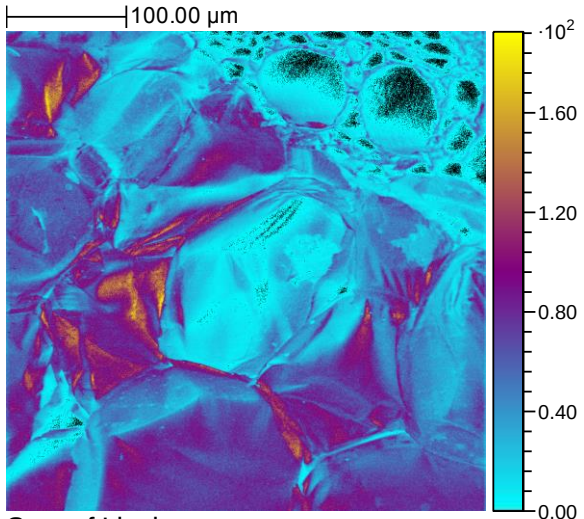
Lignin distribution in vascular bundle and ground tissue



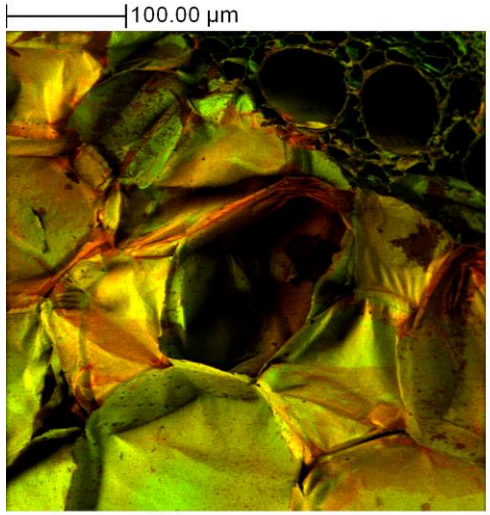
Sum of Lignin
MC: 156; TC: 1.912e+007



Red: sum of Lignin
Green: sum of PS



Sum of Lignin
MC: 193; TC: 5.060e+007



Red: sum of Lignin
Green: sum of PS

Fragments	Chemical formula	Measured Mass (m/z)	Calculated mass (m/z)	Standard deviation (ppm)
Lignin	$C_4H_3^+$	51.022	51.023	-20
	$C_5H_5^+$	65.043	65.039	61
	$C_6H_5^+$	77.041	77.039	26
	$C_6H_7^+$	79.056	79.054	25
	$C_7H_7^+$	91.055	91.054	11
	$C_8H_9O_2^+$	137.056	137.060	-29
	$C_8H_7O_3^+$	151.037	151.039	-13
	$C_8H_8O_3^+$	152.056	152.047	59
	$C_9H_9O_3^+$	165.059	165.055	24
	$C_9H_{11}O_3^+$	167.065	167.070	-30
	$C_9H_9O_4^+$	181.045	181.049	-22
	$C_{11}H_9O_3^+$	189.057	189.055	11
PS	$CH_3O_2^+$	47.013	47.012	21
	$C_2H_5O_2^+$	61.030	61.028	33
	$C_4H_5O_2^+$	85.030	85.029	12
	$C_5H_5O_2^+$	97.028	97.029	-10
	$C_4H_5O_3^+$	101.025	101.024	-9.9
	$C_5H_5O_3^+$	113.025	113.024	8.8
	$C_6H_7O_3^+$	127.039	127.040	-7.9
	$C_6H_9O_4^+$	145.048	145.050	-14

Saito, K.; *et al. Biomacromolecules* **2005**, *6*, 678.

Saito K.; *et al. Appl. Surf. Sci.* **2006**, *252*, 6734.

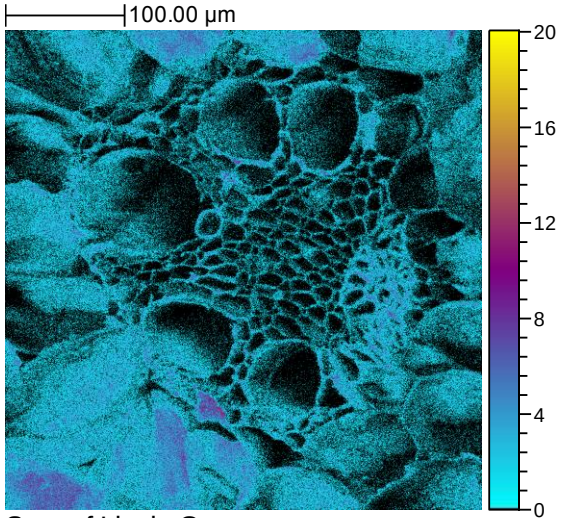
Goacher, R. E.; *et al. Anal. Chem.* **2011**, *83*, 804.

Tokareva, E. N.; *et al. J. Wood Chem. Technol.* **2011**, *45*, 767.

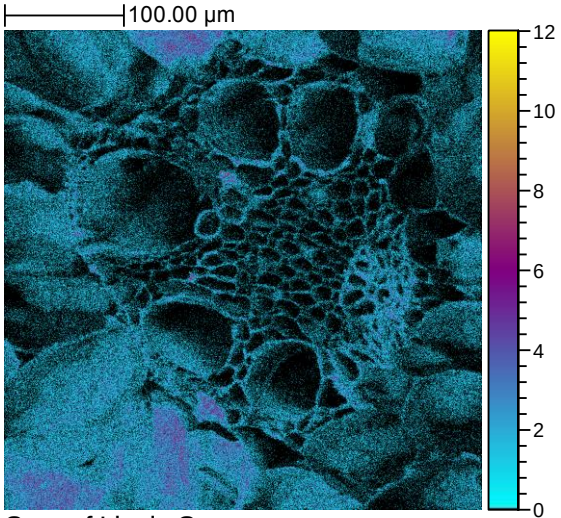
PS: Polysaccharides

Distribution of G- and S-lignin

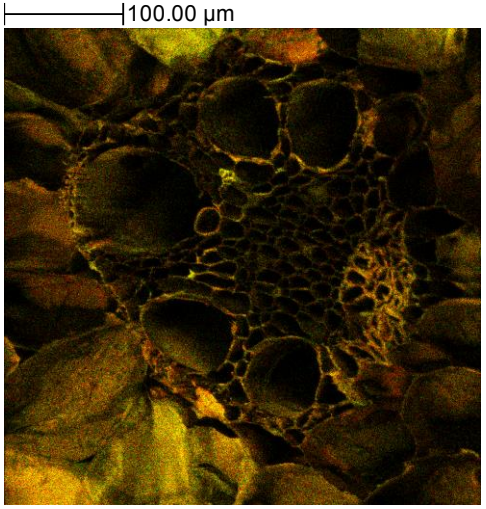
Identical localization with different proportions



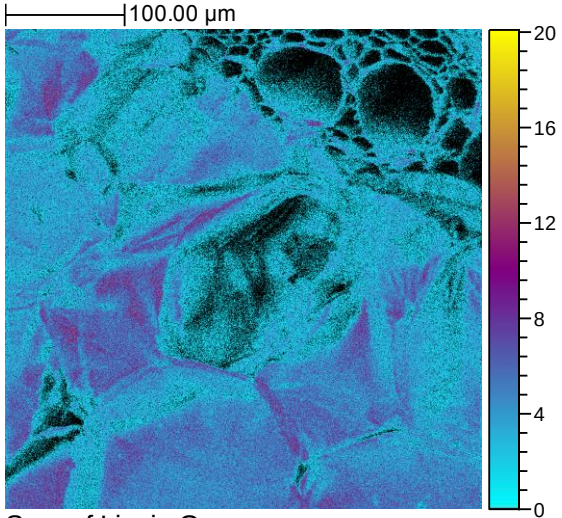
Sum of Lignin G
MC: 20; TC: 1.206e+006



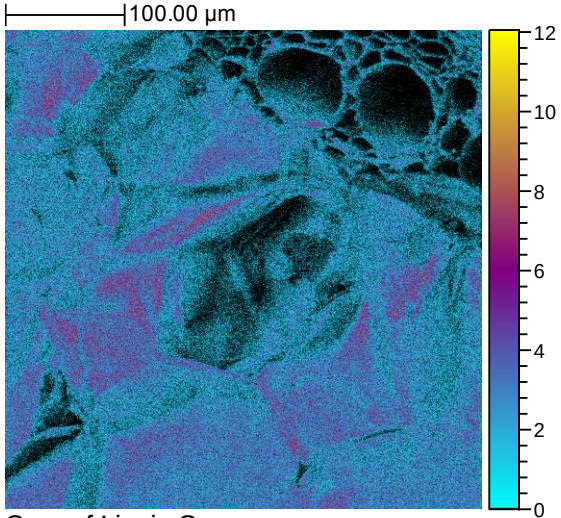
Sum of Lignin S
MC: 12; TC: 7.397e+005



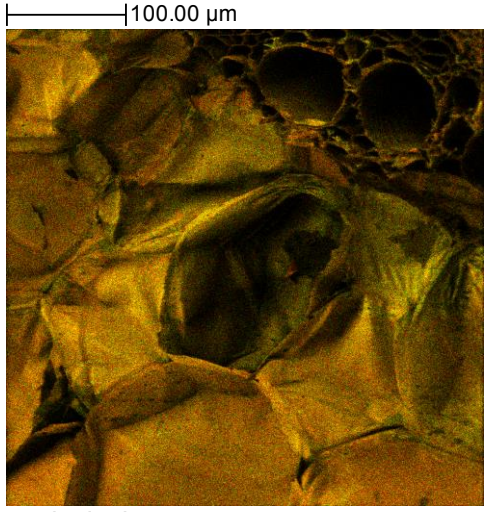
Red: Lignin G
Green: Lignin S



Sum of Lignin G
MC: 20; TC: 3.299e+006



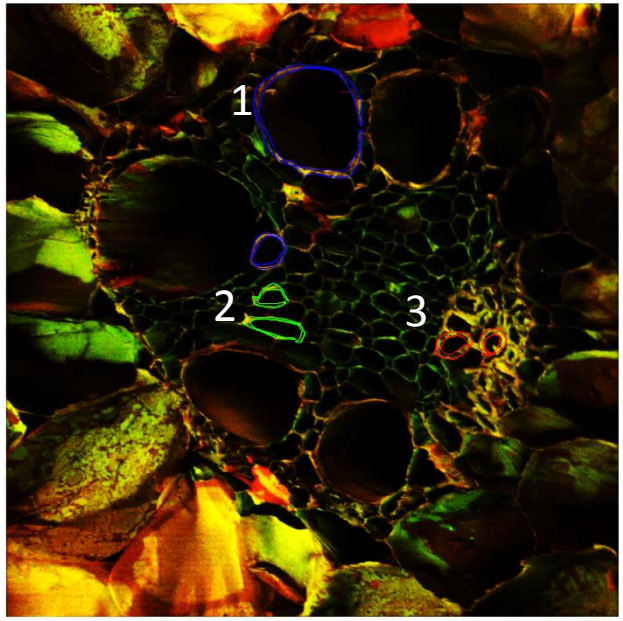
Sum of Lignin S
MC: 12; TC: 2.023e+006



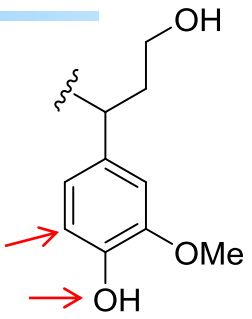
Red: Lignin G
Green: Lignin S

G/S ratios in vascular bundle

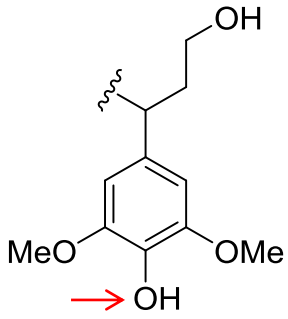
Region of interest (ROI):



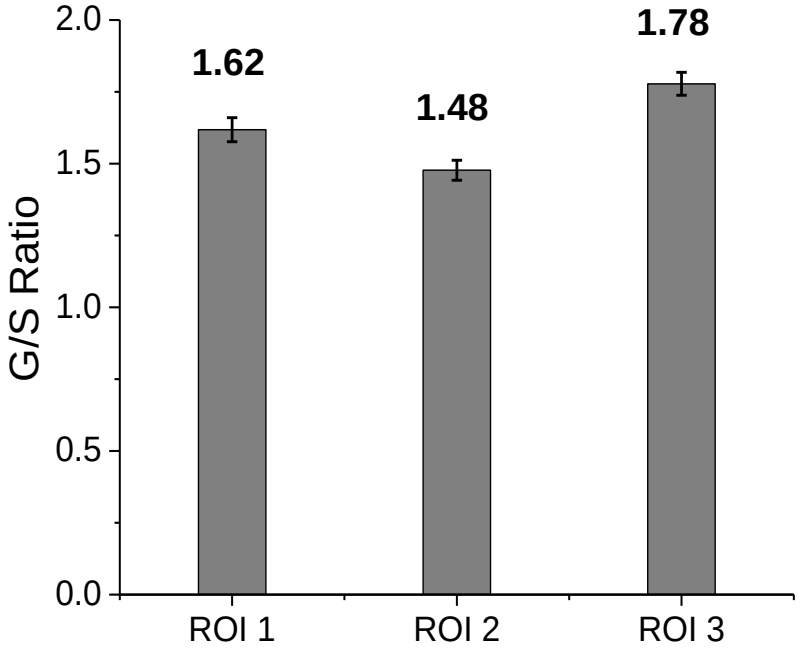
1. Xylem vessels
2. Trachaid cells
3. Sclerenchyma cells



G subunit

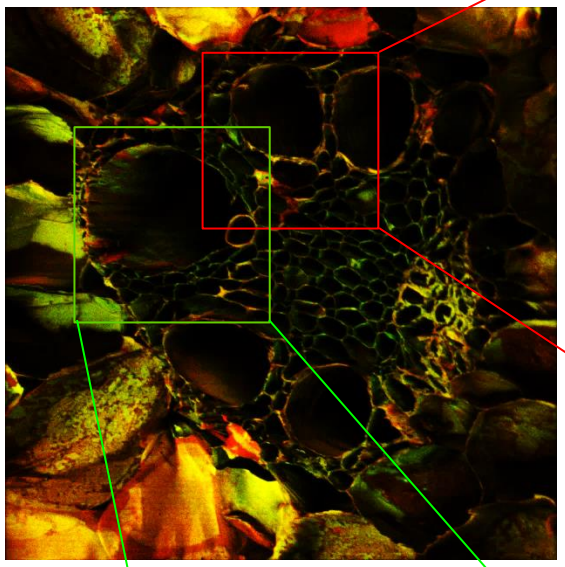


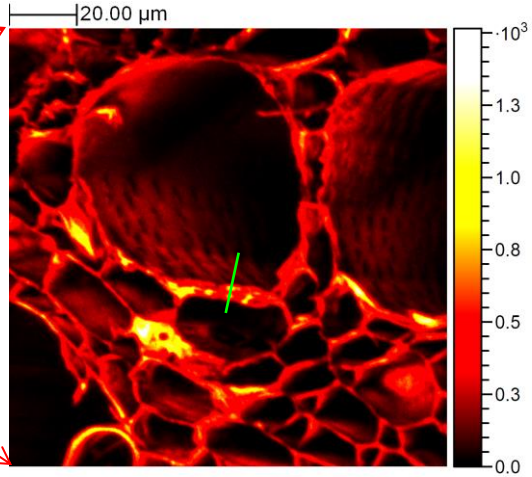
S subunit



Highest degree of cross-linking in sclerenchyma cells

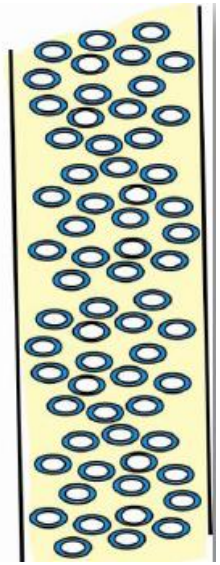
Lignin deposition in metaxylem vessel

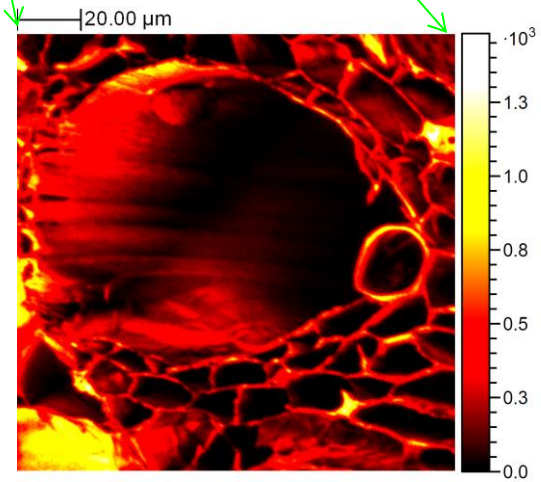




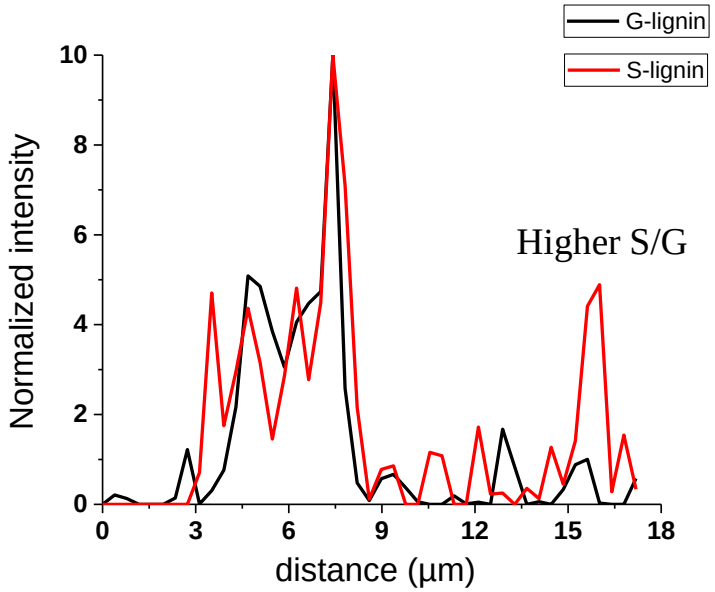
Metaxylem vessel
(total ion image)

Pitted lignin deposition pattern





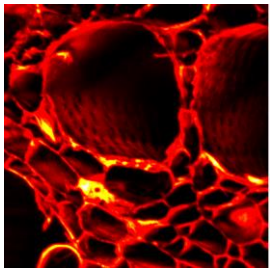
Lysigenous cavity
(total ion image)



TOF-SIMS analysis of maize stem

Conclusion

- A mature maize stem has been mapped by TOF-SIMS with high mass and spatial resolution
  - Heterogeneous lignin distribution in ground tissue and vascular bundle
  - G/S ratios in different cell types in vascular bundle; relatively higher degree of cross-linking of lignin in sclerenchyma cells
  - Direct visualization of pitted side wall in metaxylem vessel and no lignified lysigenous cavity



Perspectives

- Analysis of potential genetic phenotypes for bioethanol generation
- Studying maize stem maturation process (lignin and other constitues distribution in different growing stages)

Acknowledgement

Alain Brunelle
David Touboul
Nicolas Elie
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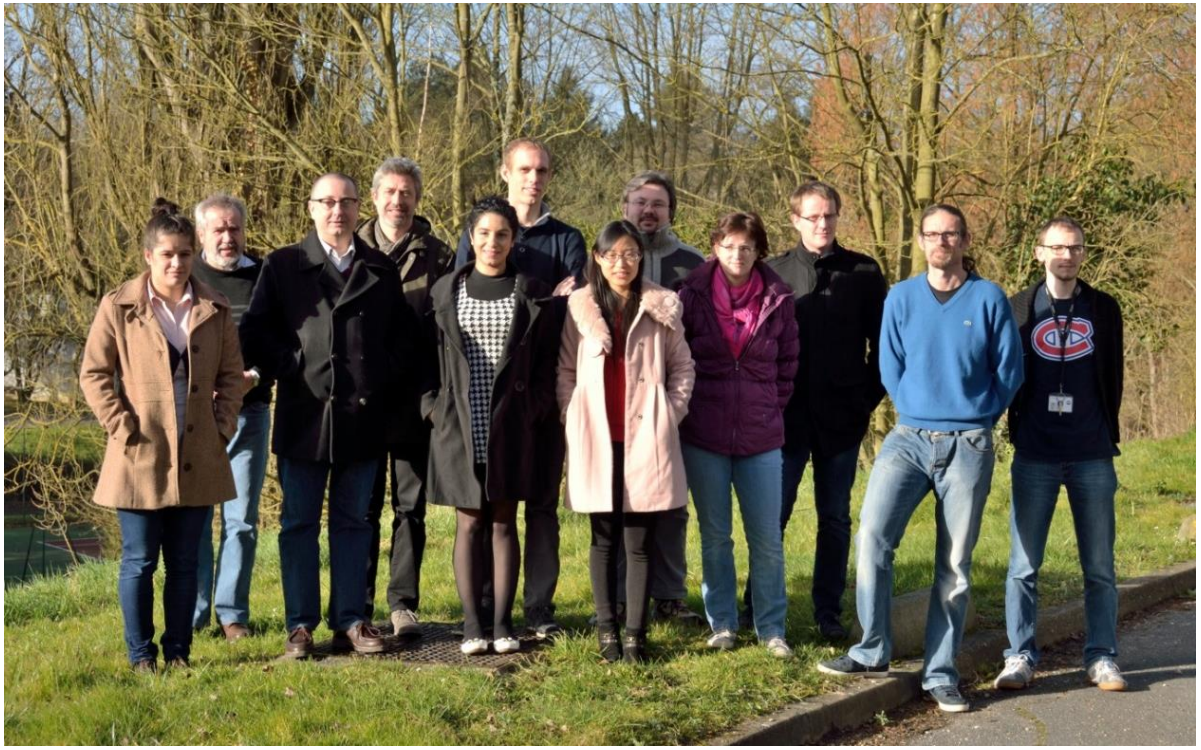
Serge Della-Negra



Valérie Méchin