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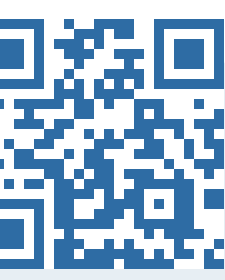
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Development of a multimodal approach to study plant-microorganisms interactions using mass spectrometry imaging, Raman imaging and non-targeted metabolomics



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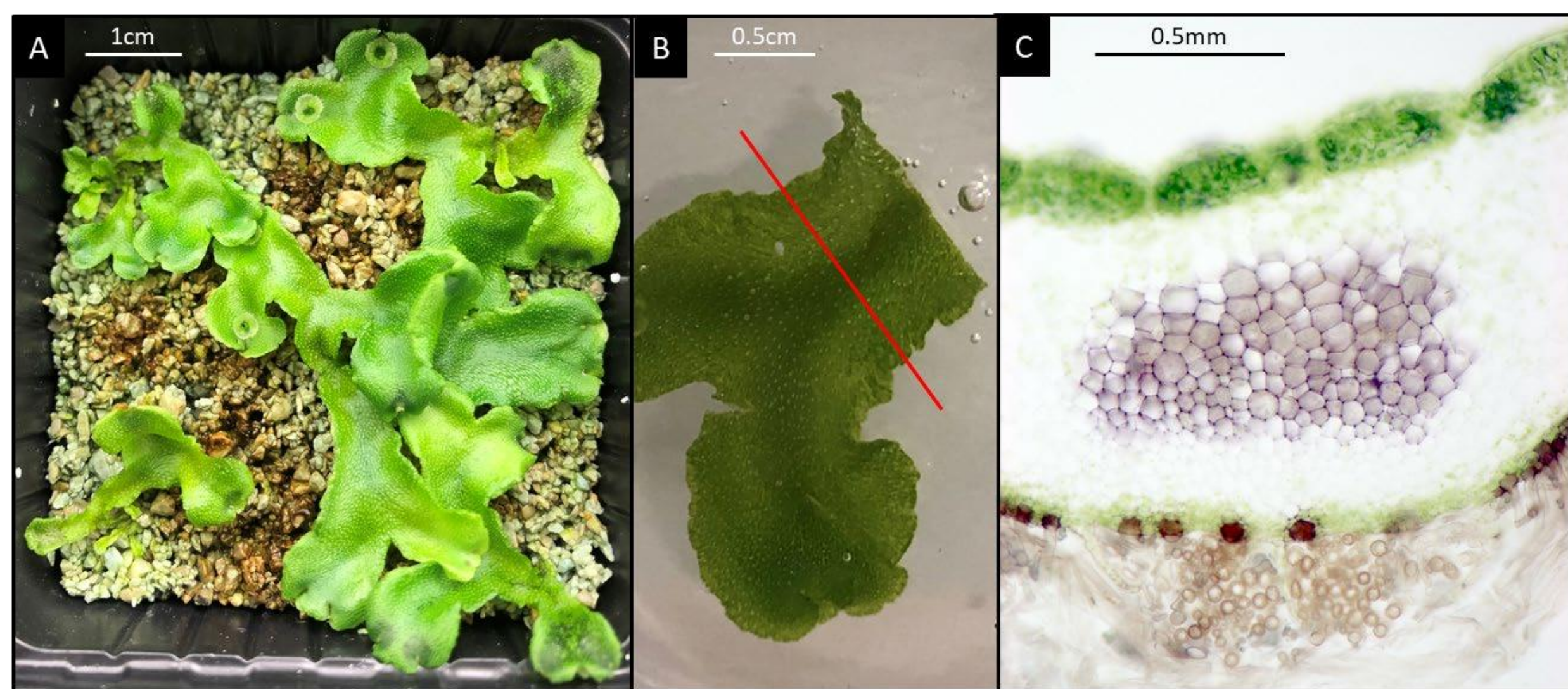
Introduction

450 million years ago, an essential symbiotic relationship called **arbuscular mycorrhizal symbiosis (AMS)** was formed between plants and glomeromycota fungi¹, enabling the first land plants to colonize the soil².

It begins with a molecular dialogue between partners located in the soil, leading to the development of specialized fungal structures in the roots, called **arbuscules**, facilitating the exchange of nutrients³.

Understanding this metabolic dialogue is a challenge, but innovative techniques such as LC-HRMS and MALDI* mass spectrometry imaging enable a complementary localization and quantification of metabolites in AMS. The integration of stimulated Raman microscopy enhances our understanding at the cellular level in a non-invasive way.

The first results of this study concern *Marchantia paleacea* mycorrhized by *Rhizophagus irregularis*.



A : *Marchantia paleacea*
B : Thallus embedded in agarose 7%
C : Transverse section (100µm thickness using vibratome) with arbuscular mycorrhizal fungi (purple area)

Untargeted Metabolomics



Data acquisition

by U-HPLC-HRMS (Thermo Scientific)

UHPLC : Vanquish with CAD and DAD detectors

MS : Q-Exacte Plus ESI +/-

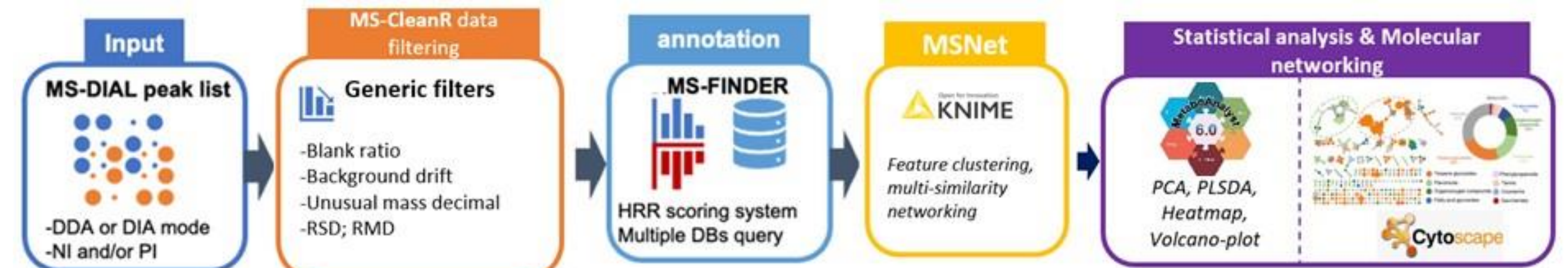
Biphasic extraction following MetaToul AgromiX protocol (MTBE, MeOH, H₂O).

Lipidomic analysis
(apolar compounds)

C18 analysis
(semi-polar compounds)

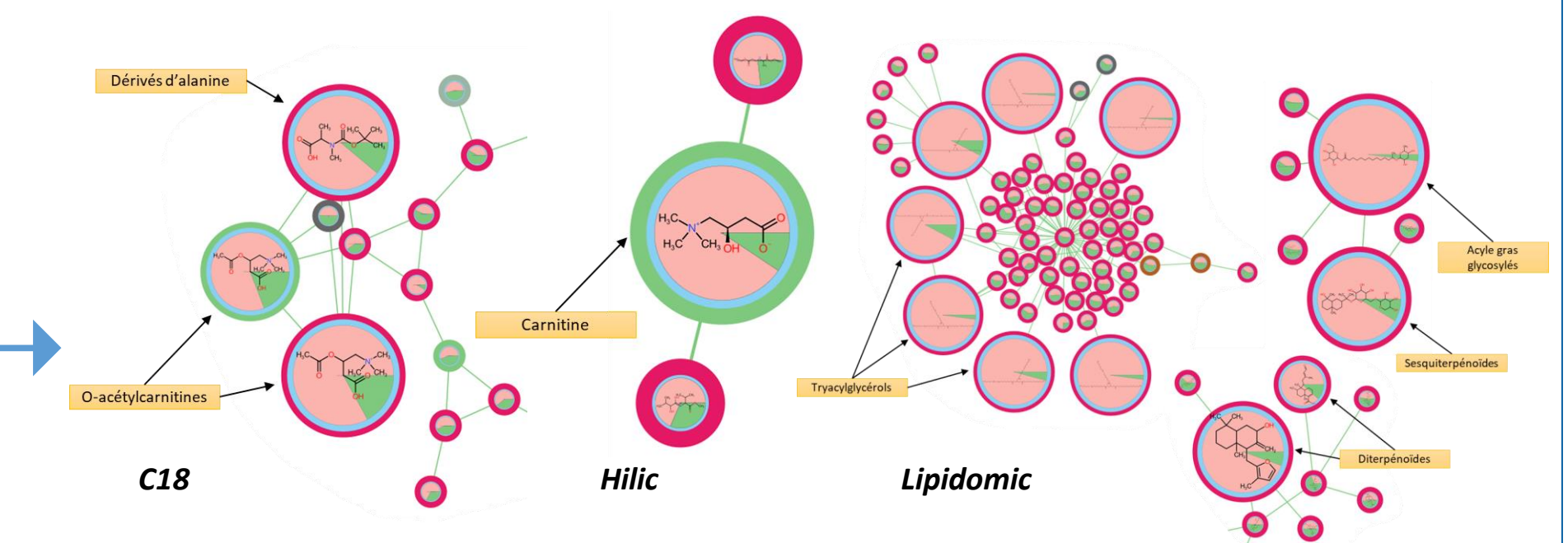
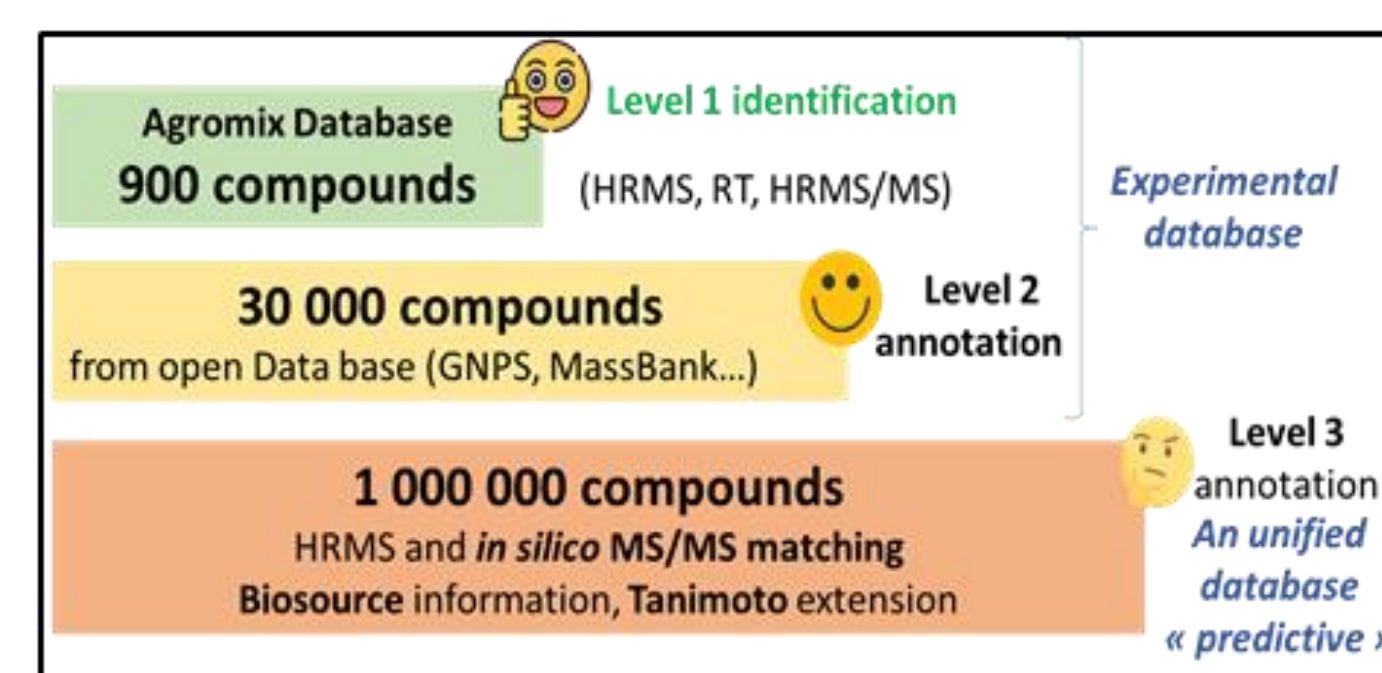
Hilic analysis
(polar compounds)

Data processing



Peak annotation

with dedicated bioinformatic workflow for global metabolomics



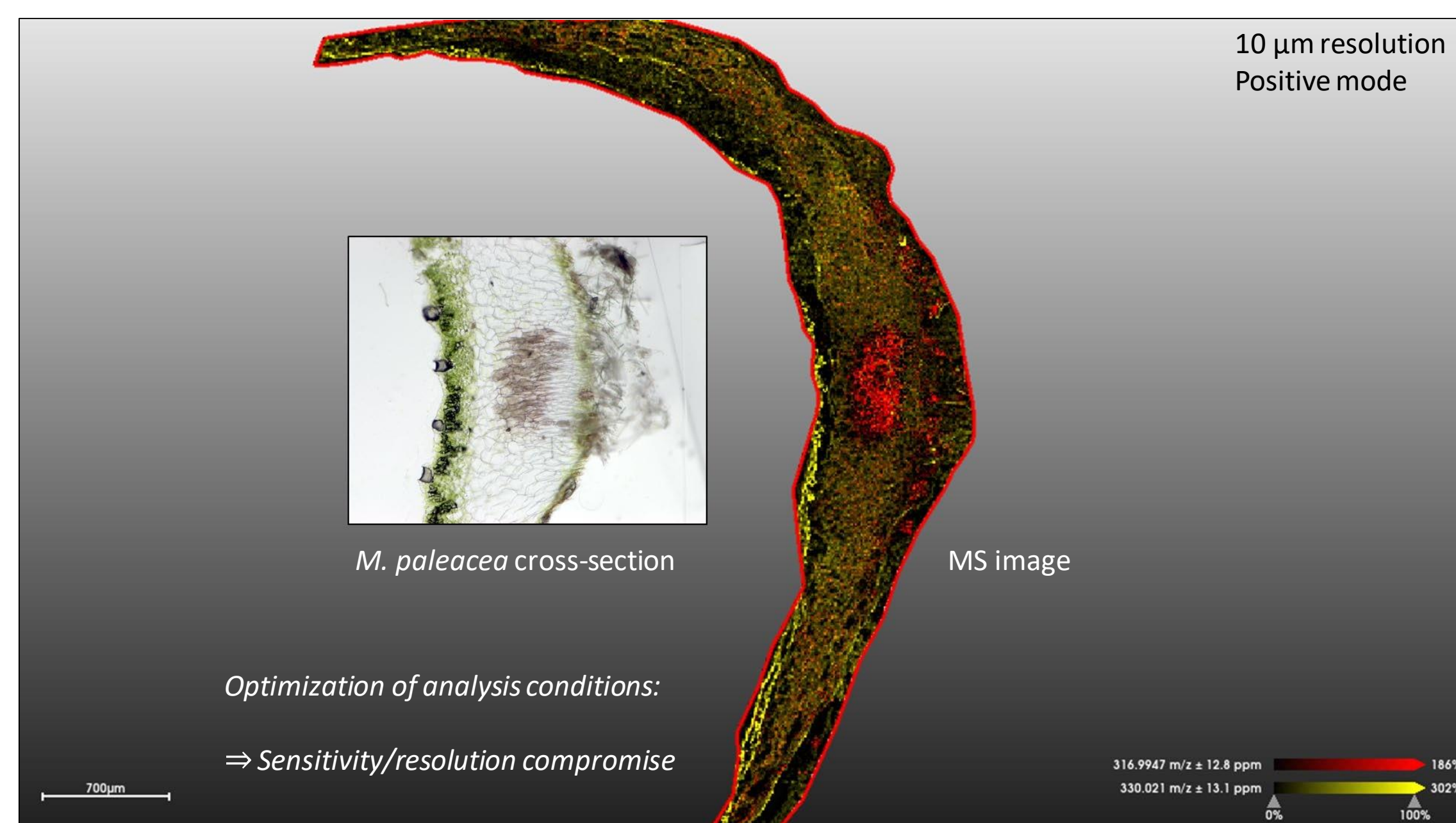
Biomarker identification, lipids involved in Arbuscular Mycorrhizal Symbiosis?

Mass Spectrometry Imaging



Data acquisition

with MALDI-MSI* (timsTOF flex - Bruker)



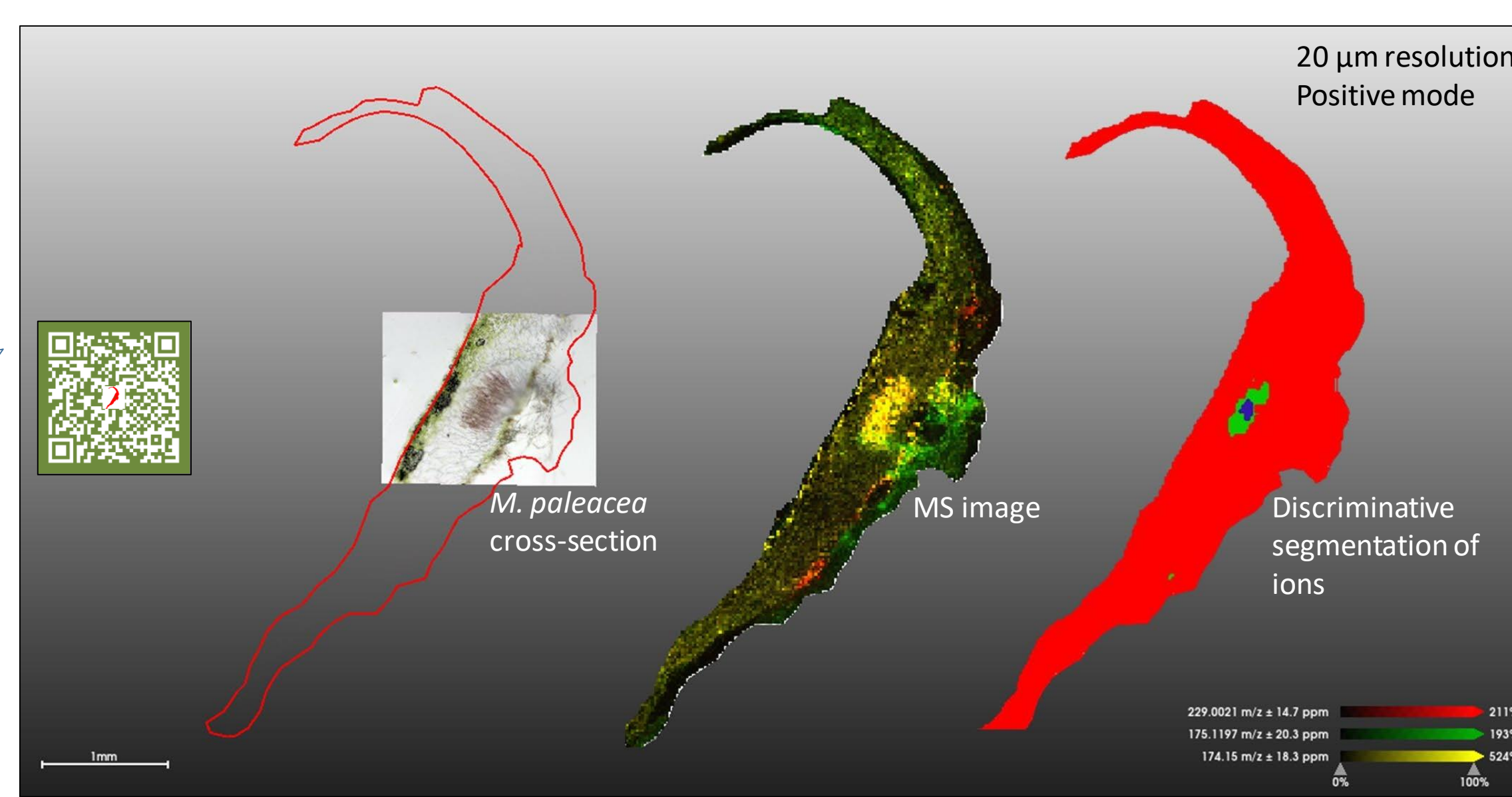
Sample preparation:

- MALDI matrix : DHB
- Deposition with the HTX TM3 sprayer (HTX technologies LLC)

Hey ! Scan me and download the file to see 3D PCA of discriminative segmentation !

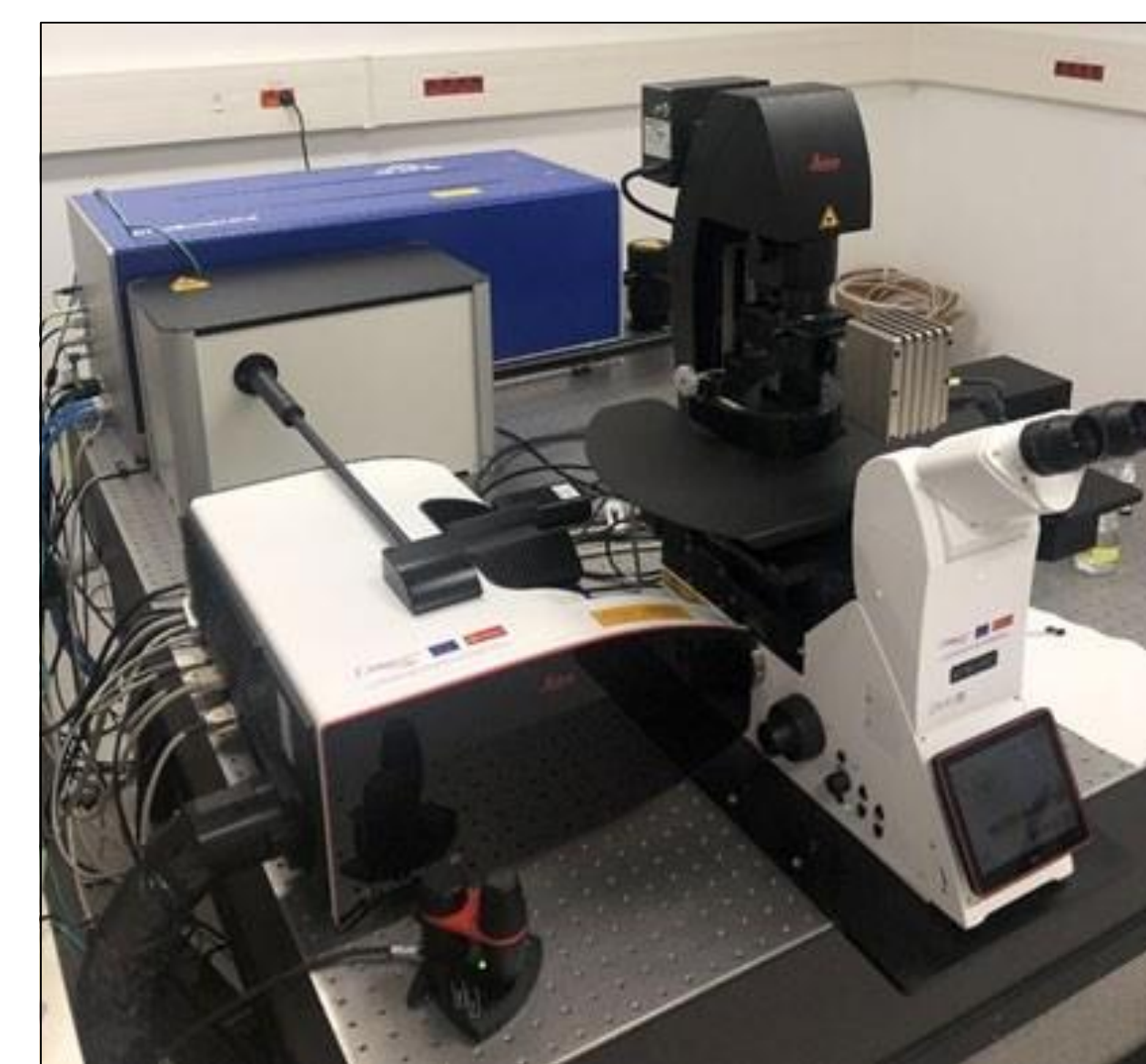
RED = Non mycorrhized area ions
GREEN = Mycorrhized area ions
BLUE = Centre of mycorrhize ions

*Matrix-Assisted Laser Desorption-Ionization



⇒ Targeting of biomarkers identified by LC-HRMS

Raman Stimulated Microscopy



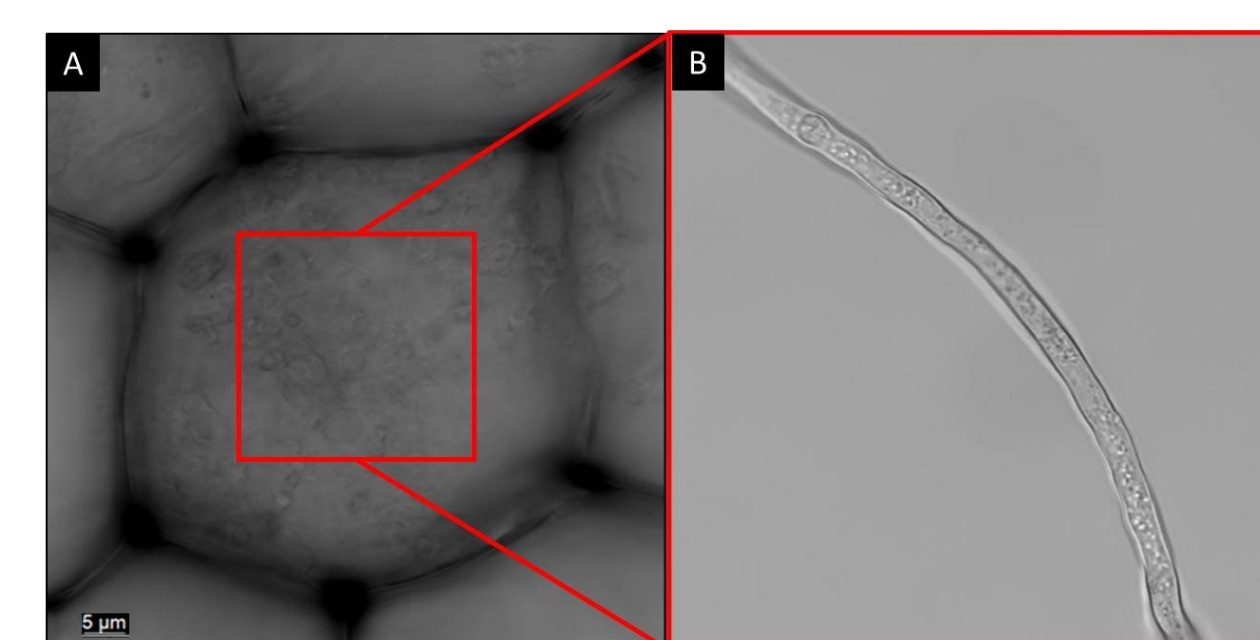
Data acquisition

with Raman stimulated microscopy (STELLARIS 8 CRS - Leica)

⇒ Identification and mapping of Raman signals characteristic of biomarkers identified by LC-HRMS (single cell and hyphae scale) with tunable OPO laser.

Optimization of analysis conditions:

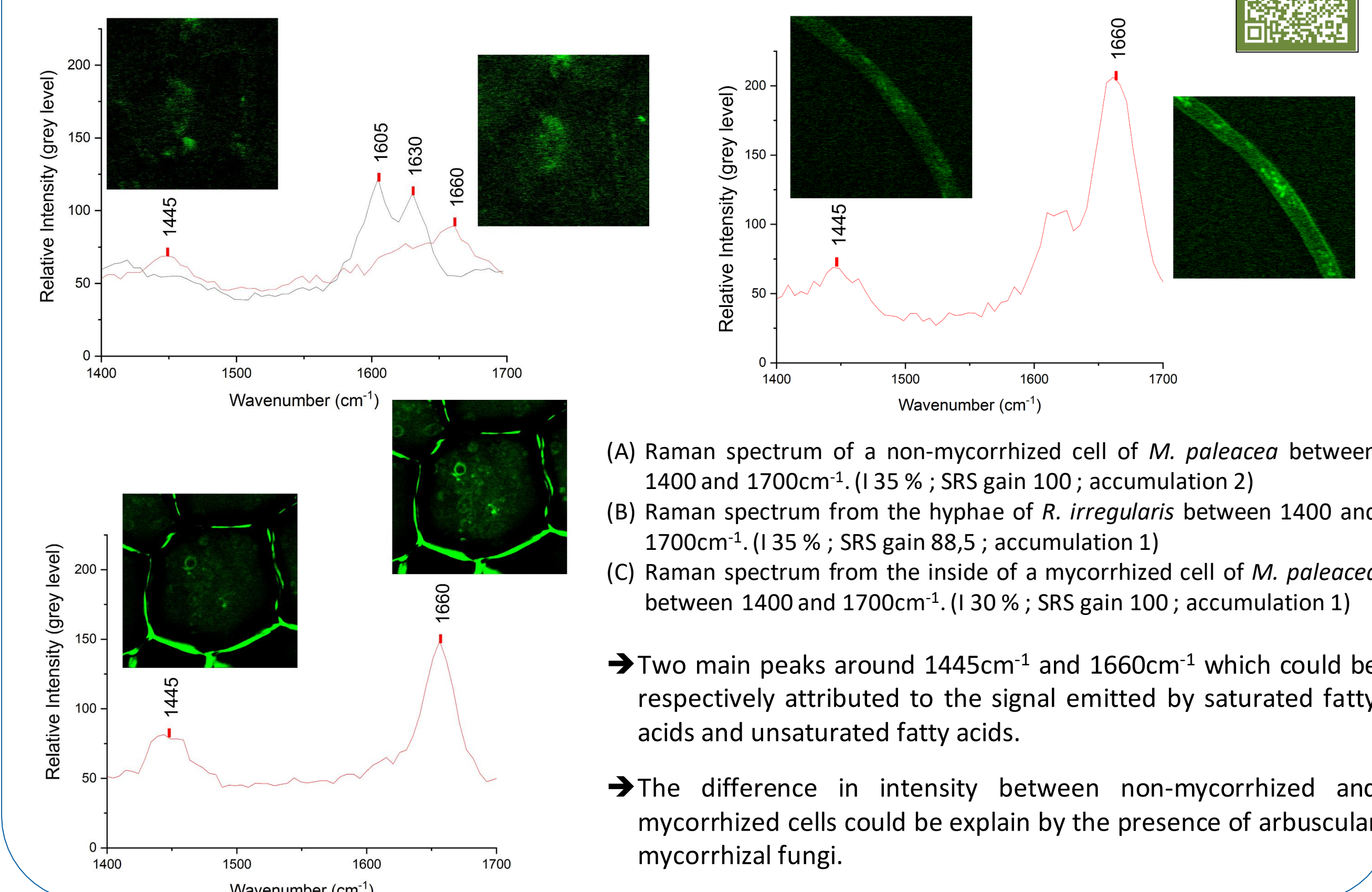
- ⇒ Magnification
- ⇒ Number of accumulations
- ⇒ Signal averaging



(A) BrightField snapshot of a mycorrhized *M. paleacea* cell at a x40 magnification with confocal Raman microscope.

(B) BrightField snapshot of a *R. irregularis* hyphae at a x40 magnification with confocal Raman microscope.

Hi again ! Scan me to see real-time vibrations signals from laser excitation !



(A) Raman spectrum of a non-mycorrhized cell of *M. paleacea* between 1400 and 1700cm⁻¹. (I 35 % ; SRS gain 100 ; accumulation 2)
(B) Raman spectrum from the hyphae of *R. irregularis* between 1400 and 1700cm⁻¹. (I 35 % ; SRS gain 88,5 ; accumulation 1)
(C) Raman spectrum from the inside of a mycorrhized cell of *M. paleacea* between 1400 and 1700cm⁻¹. (I 30 % ; SRS gain 100 ; accumulation 1)

⇒ Two main peaks around 1445cm⁻¹ and 1660cm⁻¹ which could be respectively attributed to the signal emitted by saturated fatty acids and unsaturated fatty acids.

⇒ The difference in intensity between non-mycorrhized and mycorrhized cells could be explained by the presence of arbuscular mycorrhizal fungi.

Conclusions

These results provide a great example on how multimodal approaches can help visualizing biological interactions between different organisms in a more constitutive way:

- Global LC-HRMS analysis helps **unravelling the metabolic segregation** occurring during AMS establishment.
- MALDI imaging helps localizing the metabolites involved at the tissue scale with **high sensibility level**.
- Despite not being as resolute as MALDI, the **high resolution** provided by Raman stimulated microscopy helps us to localize metabolites families. Allowing us to better quantify exchanges occurring during AMS formation.

References

- 1- Malar C.M., Krüger M., Krüger C., Wang Y., Stajich J.E., Keller J., Chen E.C.H., Yildirim G., Villeneuve-Laroche M., Roux C., Delaux P.M., Corradi N., (2021) The genome of Geosiphon pyriformis reveals ancestral traits linked to the emergence of the arbuscular mycorrhizal symbiosis. *Current Biology*, 31:7, pages 1570-1577.
- 2- Kaur S., Suseela V. (2020) Unravelling Arbuscular Mycorrhiza-Induced Changes in Plant Primary and Secondary Metabolome. *Metabolites*, 10: 335.
- 3 - Parniske M. (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* 6; pages 763–775.
- 4- Czamara K., Majzner K., Pilarczyk M., Kochan K., Kaczor A., and Baranska M. (2014) Raman spectroscopy of lipids: A review. *J. Raman Spectrosc.*, 46; pages 4–20.

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