International Society for Plant Spectroscopy

ISPS

4th INTERNATIONAL PLANT SPECTROSCOPY CONFERENCE

IPSC 2024

2024 September 24-27

Program & Book of Abstracts

University of Natural Resources and Life Sciences
Vienna, Austria

BOKU UNIVERSITY

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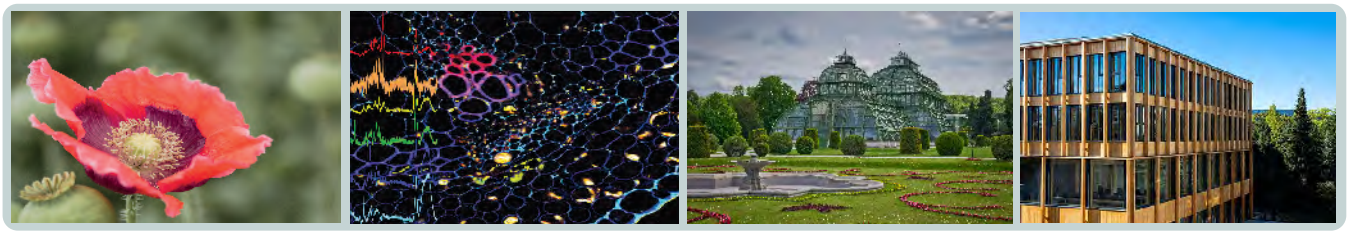
4th INTERNATIONAL PLANT SPECTROSCOPY



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4th INTERNATIONAL PLANT SPECTROSCOPY CONFERENCE

PROGRAM

Tuesday	24.09.2024
10:00 - 18:00	**REGISTRATION & RECEPTION**
11:00 - 12:00	**Company workshop** "Multimodal imaging: Sub-micron, Simultaneous IR (O-PTIR) and Raman with co-located Fluorescence imaging" by **Photothermal Spectroscopy Corp.**
13:00 - 13:20	**OPENING CEREMONY**
13:20 - 14:40	**Session: Infrared Spectroscopy I (Chair: B. Hinterstoisser)**
13:20 - 14:00	**L. Salmén: Why dynamic FTIR for studies on wood and other complex materials?**
14:00 - 14:20	A. Ponzecchi: Investigating the development of heartwood in Quercus robur in Denmark
14:20 - 14:40	L. Sommerauer: NIR integration for the evaluation of compost quality with extruded wood fibers
14:40 - 15:10	Coffee break
15:10 - 15:50	**Session: Infrared Spectroscopy II (Chair: MF Devaux)**
15:10 - 15:30	M. Zohmann-Neuberger: Characterizing food items of grouse species via FT-IR
15:30 - 15:50	B. Hinterstoisser: How infrared spectroscopy conquered wood research
15:50 - 16:30	**POSTER FLASH presentations (Chair: S. Rosner)**
16:30 - 18:00	**POSTER SESSION with Beer & Snacks**

Wednesday	25.09.2024
09:00 - 12:40	**Session: Raman Spectroscopy (Chair: N. Gierlinger)**
09:00 - 09:40	**P. Matousek: Non-invasive Probing of Opaque Materials with Raman Spectroscopy**
09:40 - 10:00	R. Elbaum: Polymerization of silica on a lignin matrix modifies the chemistry of the associated lignin
10:00 - 10:20	C. Distefano: Raman determination of nitrate content in spinach leaves: from single descriptors to multivariate data analysis
10:20 - 10:40	G. Tiloca: Raman microspectroscopy uncovers the chemical adaptations of alpine azalea leaves
10:40 - 11:10	Coffee break
11:10 - 12:00	**J. Pilátová: Newly found cellular structures in the spotlight of Raman microscopy**
12:00 - 12:20	B. Bakan: RAMAN-AFM mapping highlighted chemical clustering associated to heterogenous mechanical properties within the fruit cutin polymer matrix
12:20 - 12:40	A.D. Orlando: Linear unmixing of a series of hyperspectral Raman images to reveal variations of outer layers during wheat grain development
12:40 - 13:40	Lunch break
13:40 - 15:10	**Session: Nano & Physical Spectroscopy (Chair: J. Huss)**
13:40 - 14:10	**G. Ramer: Label-free chemical analysis at nanoscale spatial resolution**
14:10 - 14:30	C.M. Johnson: Infrared nanospectroscopy of cellulose-based materials
14:30 - 14:50	M. Weinberger: How mosses cope with heavy-metal stress: Applying µ-SRXRF to moss samples to determine and quantify heavy-metal distribution
14:50 - 15:10	M. Vasiljevic: Wood Moisture Monitoring by Impedance Spectroscopy
15:10 - 15:40	Coffee break
15:40 - 16:30	**Session: Mass based spectrometries (Chair: F. Guillon)**
15:40 - 16:10	**D. Ropartz: New dimensions in the characterization of carbohydrates by emerging technologies in mass spectrometry**
16:10 - 16:30	J. Kubásek: Stable isotopes in plant science: A general fairy tale subtly focusing on the plant cuticle
17:00 - 18:30	BOKU Tour
19:30 - 21: 00	Heuriger **s´Pfiff** (Rathstraße 4, 1190 WIEN)

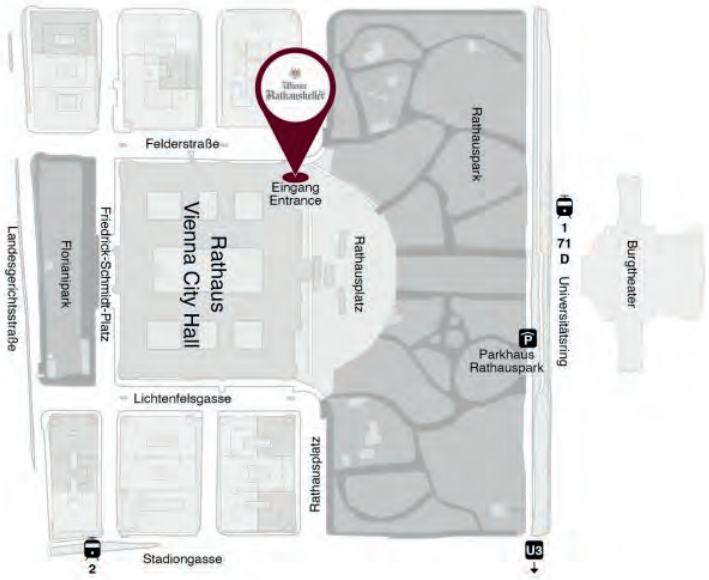
4th INTERNATIONAL PLANT SPECTROSCOPY CONFERENCE

PROGRAM

Thursday	26.09.2024
09:00 - 10:00	**Session: Data analysis (Chair: A. Gorzsás)**
09:00 - 09:40	**A. Mosig: Theory is dead, long live theory: Hypothesis-centric machine learning in vibrational spectroscopy**
09:40 - 10:00	M.F. Devaux: A tool for comparing large multispectral images: PCA score distributions
10:00 - 10:40	**A. Gorzsás: A chemometrics approach to plant cell walls**
10:40 - 11:10	Coffee break
11:10 - 12:20	**Session: NMR spectrometry (Chair: C. Deborde)**
11:10 - 12:00	M. Musse: MRI and Time Domain NMR: Opportunities and Challenges in Plant Investigations
12:00 - 12:20	C. Drießlein: Dandelion species for rubber yield enhancement evaluated by NMR metabolite profiles using artificial intelligence methods
12:20 - 13:20	Lunch break
13:20 - 14:20	*Discussion: Future activities of the IPSC?*
	FREE AFTERNOON
20:00 - 23: 00	Conference Dinner **RATHAUSKELLER** (Lehársaal), Rathausplatz 1, 1010 WIEN

Friday	27.09.2024
09:00 - 10:20	**Session: Fluorescence Spectroscopy (Chair: C. Barron)**
09:00 - 09:40	**L. Donaldson: Auto-fluorescence-based techniques in plant sciences**
09:40 - 10:00	G. Paës: Automated autofluorescence-based intensity and morphological quantification of plant cell wall
10:00 - 10:20	F. Guillon: Multi-hyperspectral and multimodal imaging to map the distribution of cell wall compounds in *Brachypodium distachyon* mutants
10:20 - 10:50	Coffee break
10:50 - 11:10	**Poster prize**
11:10 - 11:30	**Closing ceremony**

Conference Dinner:
Rathauskeller, Rathausplatz 1, 1010 Wien



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ABSTRACTS

TALKS

Infrared Spectroscopy

Why dynamic FTIR for studies on wood and other complex materials?

L. Salmén

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Infrared spectroscopy (FTIR) is a powerful analytical tool as molecular structures leave specific traces of absorbance. However, for complex materials the IR absorbance peaks may overlap making interpretations challenging. To tackle this the FTIR may be combined with another variable, here a mechanical perturbation. The deformation stretches the molecular bonds of the load-carrying substances shifting the position of the absorbance peaks. Peak shifts are small and require very precisely controlled measurements to be accurate. To enhance resolution dynamic deformations may be applied where time resolved absorbance changes are averaged. Correlation of the absorbance changes with time enables associations between components to be determined. Such measurements have given enhanced knowledge with regard to the structural complexity of wood [1]. As deformations are small and the measuring time rather long, very stable conditions are required. For wood materials this poses a problem due to its hygroscopic nature, for which moist conditions are often of more interest, why FTIR compatible moisture chambers has to be used. Such measurements have for instance revealed the association between lignin and cellulose in the primary wall as opposed to its non-existence in the secondary wall; see Figure 1. Using dynamic FTIR, changes in the association between components during for instance pulping as well as the complex interactions between cellulose and water during loading, i.e., mechano-sorptive creep may be investigated.

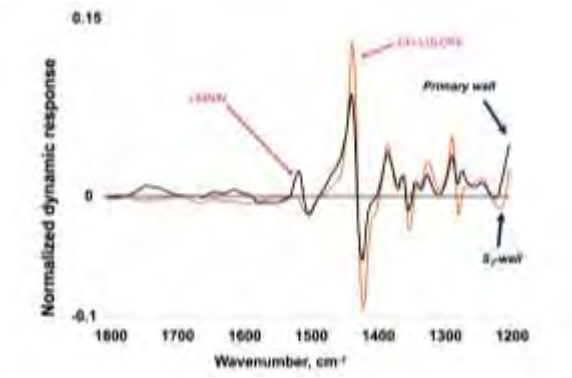

Figure 1: Dynamic FTIR spectra at 0% RH, 30 ^{o}C of the primary and secondary walls of softwood spruce showing the association of lignin and cellulose contributing to stress transfer (split peaks) in the former.

References

[1] - L.Salmén, M.Åkerholm and B. Hinterstoisser, Two-Dimensional Fourier transform Infrared Spectroscopy applied to cellulose and paper. In ”Polysaccharides: Structure diversity and functional versatility. sec.ed.” Ed. S Dumitriu, Marcel Dekker, New York (2004) p.159-187

Investigating the development of heartwood in *Quercus robur* in Denmark

Andrea Ponzecchi1, Albin Lobo2, Jill Katarina Olofsson2, Jing Xu2, Erik Dahl Kjær2, Lisbeth Garbrecht Thygesen2

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Quercus robur, commonly known as English oak, is celebrated for its extraordinary adaptability to diverse climates and its production of high-quality wood. Its heartwood is particularly esteemed for its strength and durability. However, the details of heartwood formation and aging in oak remain unknown, in particular which factors determine how much heartwood is formed, and whether formation rate and the resulting quality such as durability or colour of the heartwood formed are interrelated. In this study, wood cores were extracted from 300 20-year-old *Q. robur* trees in Denmark, representing 100 different maternal lineages (half-sib families), to investigate the factors affecting heartwood quantity and quality. The heartwood area, measured as the cross-sectional area of the heartwood in the trunk at 50 cm above ground, showed significant variation among families. Non-destructive assessments using UV-VIS reflectance and ATR-FTIR spectroscopy proved useful for analysing heartwood formation and aging, though no straightforward inheritance patterns were identified. This study highlights the importance of genetic research on heartwood formation to inform the selection of planting material for future afforestation initiatives.

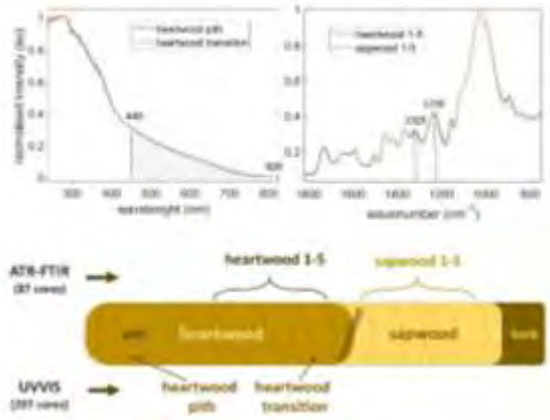

Figure 1: (a) Average UV-VIS spectra measured at the pith (heartwood pith) and at the first ring following the transition zone (heartwood transition), highlighting the specific areas used to compute the UV-VIS score, informing on heartwood aging. (b) ATR-FTIR spectra, averaged from five distinct rings within both the heartwood (heartwood rings 1-5) and sapwood (sapwood rings 1-5) for all analyzed cores, detailing the peak locations marked by numbers and dotted lines, which are crucial for deriving the ATR-FTIR score informing on heartwood formation. (c) Schematic diagram of a wood core indicating the specific sections subjected to analysis using ATR-FTIR and UV-VIS spectroscopy.

NIR integration for the evaluation of compost quality with extruded wood fibers

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Extruded wood fibers are an environmentally friendly alternative to peat in horticulture and are suitable to produce high-quality compost[1]. Extrusion increases the homogeneity and stability, which enables more consistent compost products[2]. An essential quality component of this compost is nitrogen stability, determined by the availability of ammonium (NH_4^+) and nitrate (NO_3^-). Traditionally, nitrogen stability is measured using the Zöttl test, but this is time-consuming and can give inconsistent results[3,4]. Therefore, near infrared spectroscopy (NIR) in combination with partial least squares (PLS) modeling is being investigated to provide a faster and more accurate assessment of compost wood fiber quality. Four sample batches of extruded wood fibers (Ehrensberger GmbH, Tenneck, AT) were prepared under different conditions regarding sample preparation, enrichment and incubation. NIR spectra were recorded, and NH_4^+ and NO_3^- were determined using steam distillation. The NIR data were analyzed using PLS methods to develop predictive models for the nitrogen contents and to check the correlations with the chemical analyses. Initial analyses showed significant correlations between the NIR spectra and the chemically determined NH_4^+ and NO_3^- values, confirming the potential of the NIR technique to replace the Zöttl test. The raw data models showed good agreement between predicted and actual values for NO_3^-, while there was potential for improvement for NH_4^+ at higher concentrations. Adjustments to the spectra pretreatment and modeling methodology further increased the prediction accuracies. The integration of NIR into compost wood fibre quality control offers advantages such as time and cost efficiency as well as the possibility of continuous monitoring. The results confirm the suitability of NIR in combination with PLS modeling as a reliable method for predicting nitrogen stability in compost substrates made from extruded wood fibers.

References

[1] - Kharazipour, A.; Ludwig, K.; Chaisaena, W.; Polle, A.; Kües, U. Wood and Other Plant Fibres in the Production of Peat Substitutes and Pot Plant Containers. In Wood Production, Wood Technology, and Biotechnological Impacts; 2007.
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Characterizing food items of grouse species via FTIR

M. Zohmann-Neuberger[1], J. Tintner-Olifiers[2], E. Inselsbacher[3], A. Hromatka[3], M. Schaefer[3], M. Kriechbaum[4], Ólafur K. Nielsen[5], J.S. Forbey[6] and U. Nopp-Mayr[1]

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Studies on the foraging ecology of wildlife species are of fundamental ecological interest. As the availability and quality of particular plant parts might distinctly drive population dynamics of vertebrate herbivores [1] techniques are needed that can assess both diet composition and quality at different spatial and temporal scales. Analyses with Fourier Transform Infrared spectroscopy (FTIR) support the determination of specific parts of a plant or phytochemical composition. Using grouse species as model system, we applied FTIR to explore the diet composition of plant taxa and plant parts consumed by vertebrate herbivores. We analyzed crop contents of 247 hunted rock ptarmigan *Lagopus muta* individuals, being collected from 2006-2014 in northeast Iceland [2]. The thawed vegetation was manually separated to species or higher taxonomic levels accounting for different plant parts. This yielded a set of pure fractions of plant parts per taxa from the crop samples. For our study, we used a sample set (n=312) of different plant parts (berries, leaves, catkins etc.), of *Empetrum nigrum, Vaccinium* spp., *Dryas octopetala*, *Salix herbacea*, *S. phylicifolia*, *Betula nana* and *B. pubescens*. FTIR spectra of crop contents were recorded on a Bruker® FTIR spectrometer (Tensor 27) in the Attenuated Total Reflectance (ATR) mode. The replications were vector-normalized and averaged with the integrated software OPUS® 7.2. Spectral interpretation was based on reference literature for lignocellulosic material [3,4]. PCA of selected spectral regions was performed with The Unscrambler® X 10.1 software. The FTIR spectra of the plant samples yielded a distinct separation of plant species and plant parts in terms of PCA scores, indicating the existence of spectral fingerprints that can distinguish species and parts with expected differences in quality. These spectral fingerprints allow for rapid non-invasive analyses on foraging ecology that could further link availability and quality of reproductive parts of plants to body condition and population dynamics of long-lived vertebrate herbivores.

References

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How infrared spectroscopy conquered wood research

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The 1950s can be seen as the early years of the triumph of infrared spectroscopy in analytical organic chemistry. It quickly became a useful tool for lignin and cellulose chemists, although pure organic chemists were very skeptical of those who dealt with natural products and even more so of those who used spectroscopic methods for their analysis. The reason for this may have been the complexity of the problems to be solved, i.e. hardly any pure substances, diversity of substances, seasonal and tissue-dependent fluctuations in the composition of macro- and low-molecular substances, etc.

A famous, often cited paper was published in 1954 by H.J. Marrinan and J. Mann [1], who investigated hydrogen bonds in cellulose. A year earlier (1953) [2], a cooperation between chemists from the University of Vienna and botanists from BOKU University published a paper on infrared spectra of microscopic wood sections. This unique cooperation led to the well-known Viennese wood research group around the organic chemist Karl Kratzl. The dispersive instruments of the time became a limiting factor, and it took several years until the 1970s for Fourier transform infrared technology to become established in this field. Famous wood researchers associated with IR spectroscopy include Oskar Faix [3] and Dietrich Fengel [4] as well as K.V. Sarkanen [5] and R. H. Marchessault [6] and many others.

The development of infrared devices went hand in hand with the development of computer technology and the possibility of using statistical methods to evaluate and interpret spectra. At the same time, the triumphant advance of near-infrared spectroscopy began.

The presentation will use examples to show how IR and NIR spectroscopy has developed over the years into a very useful tool in wood research - a path that has only been made possible by researchers with an inter- and transdisciplinary thinking.

References

[1] - H.J. Marrinan and J. Mann, J.appl.Chem. **4**, 204-211 (1954)
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[3] - O. Faix, Holzforschung **40**, 273-280 (1986)
[4] - D. Fengel, Holzforschung, **46** (4), 283-288 (1992)
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Raman Spectroscopy

Non-invasive Probing of Opaque Materials with Raman Spectroscopy

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This presentation will provide an overview of recent developments in the area of subsurface probing of diffusely scattering (turbid) samples using Spatially Offset Raman Spectroscopy (SORS) [1]. SORS is an approach in which the illumination and collection areas on sample surface are mutually displaced by spatial offset Δs (see Fig. 1). This enables deeper sensing than possible with conventional Raman spectroscopy where the illumination and collection zones are overlapped. SORS facilitates the non-invasive interrogation of turbid samples such as biological tissues, opaque containers (paper/plastics/glass) and pharmaceutical powder formulations (tablets/capsules) at depths typically up to several mm's to cm's [1]. The method yields chemical information on the inner content of materials or containers as well as it enables the characterisation of their physical nature (e.g. temperature).
The talk will cover the description of underlying physical phenomena, emerging SORS variants and applications including disease diagnosis and security screening.

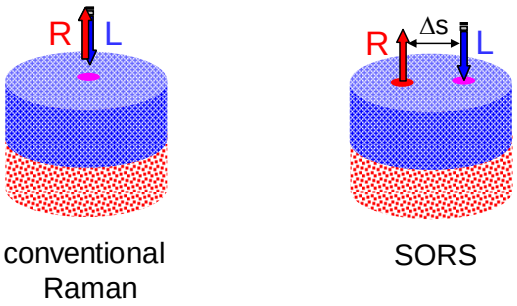

Figure 1: Schematics of conventional Raman and SORS sampling configurations.

References

[1] S. Mosca, C. Conti, N. Stone and P. Matousek. Nat. Rev. Methods Prim. **1**, 21 (2021).

Polymerization of silica on a lignin matrix modifies the chemistry of the associated lignin

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Plants produce silica in their bodies, by polymerizing silicic acid, a common solute in soil water. Most of the mineralization occurs in cell walls, but the specific chemistry that controls the deposition is mostly unknown. One model system for silica biomineralization is the formation of silica aggregates at the endodermis of sorghum roots (Figure 1A). Aggregation follows a spotted pattern of locally deposited lignin at the inner tangential cell walls (Figure 1B,C). To highlight variations in lignin chemistry, we mapped sorghum root endodermis by Raman microspectroscopy in plants grown hydroponically with or without Si amendment [1]. Cluster analysis of Raman maps collected from +Si samples indicated concentric variation in the aggregate (Figure 1D). The centre was characterized by lignin monomer bands, lignin with a low level of polymerization, in addition to silica bands. Farther from the centre, polysaccharide concentration increased together with soluble silicic acid. In -Si samples, the main band at the spot centre was assigned to lignin radicals and highly polymerized lignin. Mapping of lignin *in situ* without extraction allowed us to show that in -Si samples, the monomers diffuse in the wall and crosslink with cell wall polymers, forming a ring of dense lignified cell wall. In contrast, in +Si roots, short lignin polymers bind to the silicic acid silanols, and nucleate silica aggregates, while leaving unpolymerized silicic acid in locations far from the lignin deposition locus. Our results suggest that binding of silicic acid to the polymerizing lignin inhibits its extensive polymerization. An *in vitro* system of silica formation in the presence of synthetic lignin also indicates that Si inhibits lignin extension. At the same time lignin nucleates nanometric silica particles (Figure 1E), [2]. Our results show that lignin can nucleate silica deposition *in planta*. Further studies are needed to illuminate the specific chemistry involved in this common biomineralization process.

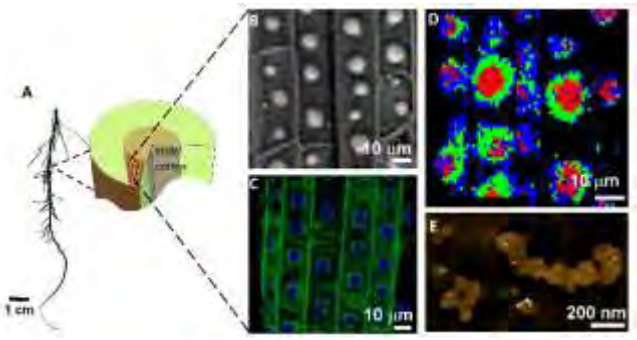

Figure 1: **A.** sorghum root (left) and root section (right). **B.** Scanning electron microscopy of the inner tangential cell wall of the root endodermis, as shown in panel A. silica aggregations are seen as bright structures. **C.** Fluorescence microscopy indicates lignin at the silica deposition sites, by its blue autofluorescence. **D.** Clustering of Raman maps show that the silica deposition spots have concentric symmetry, indicating on variation in the cell wall chemistry [1]. **E.** Lignin was synthesized *in vitro* in the presence of silicic acid. A slice from transmission electron tomography showing silica nanoparticles in white deposited in the synthetic lignin particles, shown in brown [2].

References

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[2] - S. Palakurthy, L. Houben, M. Elbaum, R. Elbaum, Biomacromolecules **xx**, (2024).

Raman determination of nitrate content in spinach leaves: from single descriptors to multivariate data analysis

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In recent years, the environmental impact of excessive nitrogen-based fertilizer use in vegetable crop cultivation has driven a shift towards sustainable fertilization strategies. Leafy greens, particularly spinach, are prone to nitrate accumulation, raising health and food security concerns. This study explores the application of Raman spectroscopy for non-destructive quantification of nitrate levels in greenhouse-grown spinach leaves. Using a portable Raman system working with a 785 nm laser excitation wavelength, we obtained Raman profiles and applied multiple linear regression (MLR) and partial least squares regression (PLSR) analyses. The results demonstrated that Raman spectroscopy, combined with advanced regression models, predicts nitrate levels with high accuracy (R^2 ~0.8-0.9), surpassing the precision of popular portable meters. The comparative analysis revealed that the PLSR model slightly outperformed MLR due to its ability to capture complex spectral data interactions.

The successful use of a portable Raman spectrometer highlights its practical feasibility for on-field assessments, providing rapid and reliable nitrate measurements to support real-time nitrogen management decisions. Integrating Raman spectroscopy with other optical sensors, such as the Dualex, presents a synergistic approach to monitoring plant health and nutrient status, enhancing the accuracy of nitrate predictions. The study underscores the limitations of single-band descriptors for nitrate quantification and emphasizes the necessity of multivariate techniques to fully exploit spectral data. Future research should focus on expanding datasets to include diverse plant varieties and growing conditions, as well as exploring additional machine learning algorithms to improve predictive capabilities.

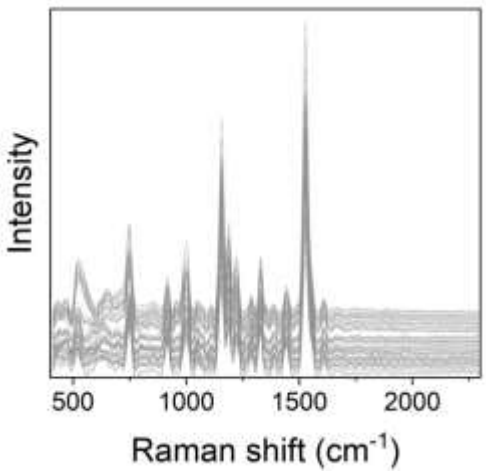

Figure 1: Stacked Raman spectra of spinach leaves

Raman microspectroscopy uncovers the chemical adaptations of alpine azalea leaves

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Vibrational spectroscopic techniques, such as Raman spectroscopy, offer a non-destructive analysis of plant tissues and provide insights into chemical composition[1]. In combination with microscopy, chemical (spectral) information is linked to spatial (mapping) position[2], thereby imaging plant microchemistry in context with plant anatomy. Novel multivariate data analysis approaches and plant reference spectra databases increased our knowledge gain from the acquired hyperspectral data maps[4, 5] and so shed new light on the intricate structure of plant cuticles, the outer thin surface layer of plants[3,4]. Yet, the comprehensive characterisation of cuticles, including functional adaptations like stomata and trichomes, remains underexplored as well as reactions on environmental adaptations. In this study, we used high-resolution Raman imaging to investigate the leaf cuticle, trichomes and stomata of the alpine azalea (*Kalmia procumbens)*, a perennial evergreen shrub adapted to survive in harsh high mountain regions. Our research elucidates the chemical adaptations, with a focus on flavonoids, terpenoids and epicuticular waxes, which protect against water loss, heat/frost and high radiation levels. Additionally, we observed in stomata guard cells and the cuticle unsaturated lipids, which can serve as precursors for various bioactive molecules and stocks for cutin polymerisation. Our findings demonstrate the effectiveness of Raman spectroscopy in chemically profiling different leaf structures and correlating chemical composition to environmental adaptability. By advancing the capability for in-depth, *in-situ* analysis of plant organs, this study contributes to a broader understanding of plant leaf strategies to enhance ecosystem resilience, also with respect to climate change.

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Newly found cellular structures in the spotlight of Raman microscopy

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Single-celled eukaryotes (algae and protists) represent 70% of eukaryotic biodiversity. They are significant producers of the oxygen we breathe, play important roles in global cycles of biogenic elements and food webs, and are key to understanding the early evolution of life. They are biotechnologically exploited in biofuels, food supplements, and cosmetics, yet we know little about their biology. In my work, I focused on identifying neglected cellular structures in these organisms using in situ in vivo Raman microscopy. To demonstrate the revolutionary potential of Raman microspectroscopy for biology, I will present two case studies in which chemical identification of cell inclusions directly in living cells brought unexpected discoveries.

Our serendipitous discovery of purine crystalline inclusions in dinoflagellates and other microalgae led us to further investigation. We found that purine crystals act as dynamic high-capacity nitrogen storage [1]. This has great implications for future revisiting of cellular nitrogen metabolism as well as its global cycling. To test how widespread this phenomenon is, we screened more than 200 species of microscopic eukaryotes across all the supergroups of eukaryotic diversity coming from various habitats. We found biogenic crystals in freshwater algae, endosymbionts of reef-building corals, deadly parasites, anaerobes in termite guts, slime molds, including model organisms and biotechnologically important species. Compared to the formerly described oxalates, calcite, or silica, we shifted the paradigm of eukaryotic biocrystallization in favour of purines to be the most widespread type of intracellular biocrystals [2]. Apart from that, purine crystals might be possibly the most ancient cell crystalline inclusions, likely present in the last eukaryotic common ancestor. Moreover, we introduced the first hypothetical scheme of purine crystal metabolism inside the membrane-bounded compartment.

In collaboration with experts on diplonemids, we used Raman microscopy to distinguish the contents of crystalline inclusions of the newly isolated and described species. Diplonemids are marine heterotrophic flagellates recently found to be one of the most abundant and diversified protists in the oceans. We have found their great potential for massive accumulations of Ba and Sr trace elements [3]. Thus, we proposed they could be the long-missing players in Ba/Sr cycling in oceans that can explain the long-missing causal link in Ba/Sr correlations with marine productivities, the proxies for the paleoceanographic reconstructions of the past climate.

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RAMAN-AFM mapping highlighted chemical clustering associated to heterogenous mechanical properties within the fruit cutin polymer matrix

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Covering the plant's surface, cuticle, is a natural hydrophobic composite barrier protection from environmental stress. During fruit development, cuticles have to adapt to different mechanical constraints, combining extensibility and rigidity. Little is known about the structural data governing these properties. Yet understanding the architecture of plant cuticles is a prerequisite for controlling their functionality with a view to sustainable crop production and processing. Within the chemically complex cuticle, the lipid polymeric scaffold is the cutin, an insoluble polyester of oxygenated fatty acids including also phenolic compounds and polysaccharides.

Using the tomato as a model from the beginning of the expansion phase through to maturity, an experimental scheme was designed to enable chemical composition information (RAMAN imaging combined with multivariate data analysis) to be imaged [1] and coupled with nanometric-scale mechanical property measurements (atomic force microscopy) [2].
Within the cuticle, the analyses clearly identified chemical clusters with different contributions from the components (i.e. cutin, polysaccharide, phenolic compounds). In addition, these zones are finely adjusted during the development of the fruit. Besides, unprecedented heterogeneities in mechanical properties were revealed (Fig.1).
This fine-tuning of cuticle architecture and mechanical properties offers new prospects for plant improvement and the design of bioinspired functional materials

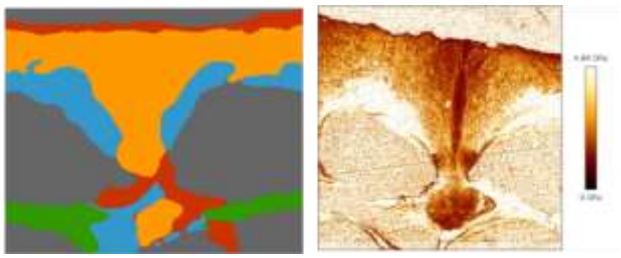

Figure 1: RAMAN chemical clustering and AFM mechanical mapping within the fruit cutin

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Linear unmixing of a series of hyperspectral Raman images to reveal variations of outer layers during wheat grain development

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To overcome the current stagnation in wheat yields, it is essential to understand how the grain develops, particularly as regards the external tissues that help to limit how it grows [1]. To this end, we are conducting a multiscale and multimodal hyperspectral imaging project including deep UV fluorescence and Raman to study changes in the composition of the outer tissues of the wheat grain during its development.

A common sample preparation suitable for all imaging methods and all development stages was required. In the Raman spectra, this resulted in the presence of signals due to the freezing medium used to section the grains and to the quartz lamella required for deep UV imaging. We propose here an unmixing approach using Multivariate Curve Resolution - Alternating Least Squares (MCR-ALS [2]) for separating the contribution of freezing medium and quartz lamella in the Raman spectra of wheat grain outer layers. The method combined with the spectral-spatial normalisation [3] allows recalculation of wheat spectra and the comparison of wheat grain polymer concentration maps for different tissues, grain regions and development stages.

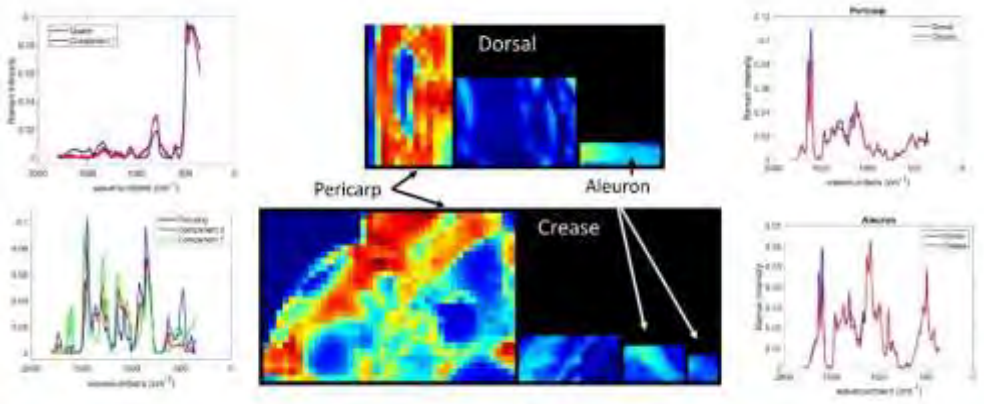

Figure 1: Left: quartz and freezing medium pure spectral signatures extracted by MCR compared to reference spectra. Middle: concentration maps of two grain regions after normalisation. Right: spectra of pericarp and aleurone recalculated without freezing medium, quartz lamella and residual contributions.

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Nano & Physical Spectroscopy

Labelfree chemical analysis at nanoscale spatial resolution

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Vibrational spectroscopy (mid-infrared and Raman spectroscopy) is without a question a powerful tool for molecular analysis and has therefore found wide acceptance for label free chemical imaging of organic materials and plant and animal tissues. However, as with any optical imaging technique, the spatial resolution of infrared and Raman spectroscopy are limited by diffraction to roughly around the employed wavelength.
We can circumvent the diffraction limit by building a nearfield imaging system, i.e. by moving the detector and/or the light source as close to our specimen as possible. One approach to move the "detector" closer that has found wide acceptance for mid-IR spectroscopy is to use photothermal expansion induced by a tuneable pulsed laser for detection of local absorption. This AFM-IR (atomic force microscopy induced resonance) technique reads the local thermal expansion using an AFM tip to enable nanometre scale spatial resolution chemical imaging. AFM-IR can be used to collect mid-IR absorption spectra from nanoscale samples that resemble conventional bulk spectra [1].
An exciting aspect of AFM-IR is that it can be used to apply multivariate chemical imaging techniques that are well established for IR microscopy at the nanoscale. These enable to combine information from multiple spectra or multiple single wavelength images into actual maps of chemical composition, which can reveal inclusion bodies in cells. We can also leverage full spectra to create images showing us – secondary structure specific - intracellular protein distribution at the nanoscale [2].

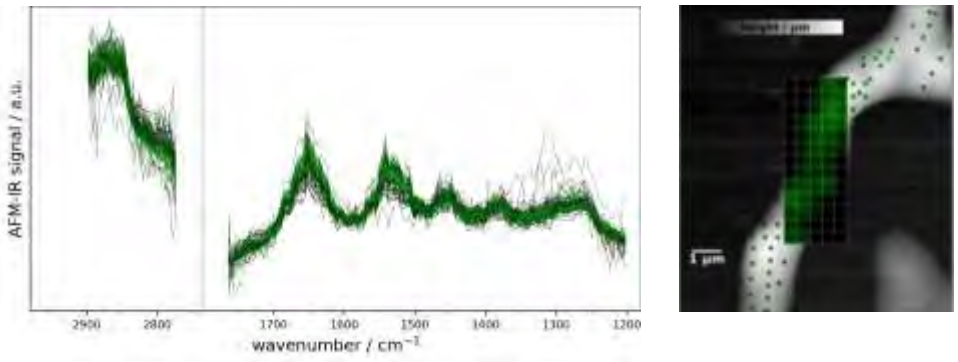

Figure 1: Left panel: AFM-IR spectra of a *T. reesei* hypha. Right panel: local fluorescence brightness calculated from AFM-IR spectra via partial least squares model.

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Infrared nanospectroscopy of cellulose-based materials

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Understanding the chemistry on the nanoscale is of importance to explain numerous properties of materials originating from wood, such as nanocellulose and pulp fibers, as well as understanding how wood cell walls are constructed.

With infrared nanospectroscopy, which is a combination of atomic force microscopy (AFM) and infrared (FTIR) spectroscopy, it is possible to obtain chemical information in terms of IR spectra and IR images with a lateral resolution being a few tens of nanometers, hence significantly below the diffraction limit [1]. In these studies we have used infrared nanospectroscopy (sSNOM, scattering-type scanning near-field optical microscopy) to obtain chemical information with a lateral resolution being around 20 nm in studies of wood cell walls, pulp cellulose fibers, as well as nanocelluloses.

Wood cell walls consist of several thin layers, and infrared nanospectroscopy has been used to examine how cell wall constituents such as cellulose, lignin, and hemicellulose are distributed within the cell wall on the nanoscale. When producing pulp, the fibers in the wood cell walls are separated from each other, which can be done in different ways, and hence the fibers will have a different chemistry. Here we have used infrared nanospectroscopy to study the nanoscale chemistry of fibers produced in different ways to reveal their homogeneity. Different types of nanocelluloses, such as cellulose nanofibrils (CNFs) and cellulose nanocrystals (CNCs) have been studied to reveal their morphology on the nanoscale. By acquiring nano FTIR spectra along individual nanocellulose particles and comparing them with reference spectra of crystalline and amorphous cellulose, we have investigated the local crystallinity in different types of domains such as straight segments and kinks.

Most nano FTIR studies of biomolecules have for simplicity been performed in the dry state. However, such examinations should in many cases preferably be performed in an aqueous environment that is the natural environment for many types of biomolecules, and the feasibility of this type of experiments will be demonstrated.

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How mosses cope with heavy-metal stress: Applying μ-SRXRF to moss samples to determine and quantify heavy-metal distribution

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Bryophytes are frequently utilized as biomonitors to investigate environmental pollution due to their capacity to absorb essential minerals and water, as well as harmful heavy metals, throughout their entire surface [1]. Additionally, the fact that many moss leaflets consist of a single layer of cells [2] is advantageous for analysing metal transport and accumulation. The moss *P. patens* is a prime model organism for non-vascular plants. In a recent study, we observed that different metals present in growth agar have a different harmful effect on this moss [3]. The specimen used in this study were cultivated in a laboratory under stable conditions, allowing for precise control over the samples. In the oral presentation and the corresponding poster, we will discuss our approach to quantify stacked micro–Synchrotron X-ray Fluorescence (μ-SRXRF) data measured at the PETRA III P06 beamline at DESY in Hamburg, Germany. From a physical perspective, areal concentrations are the only valid approach for X-ray fluorescence spectra quantification. However, biological samples have volume concentrations. Therefore, we propose a possible thickness normalization method using the region of interest (ROI) of the Compton scattering peaks of each pixel to correct our concentration results.

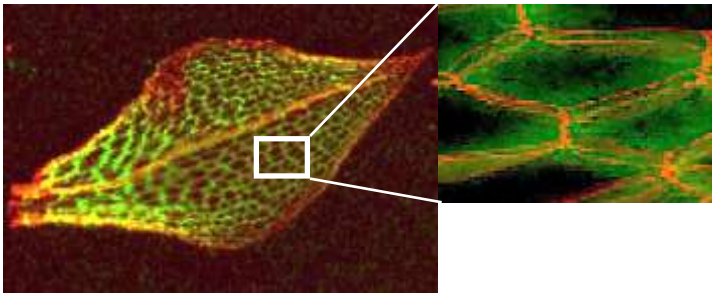

Figure 1: Distribution of Mn (red) and Cl (green) within a moss leaflet. Inlay: subcellular resolution shows Mn also in lamina cell walls; Cl (green) in the protoplast.

Figure 2: Gametophore of *P. patens*, bar=1 cm [4].

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Wood Moisture Monitoring by Impedance Spectroscopy

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To unravel wood's full potential as a sustainable construction material, we aim to investigate its electrical properties to enhance engineered wood products and prevent moisture-related damage [1–3]. Current methods for measuring wood moisture content provide single values and are often based on destructive test procedures [4, 5]; therefore, we employ electrochemical impedance spectroscopy (EIS) to collect AC conductivity data over a large frequency range, even from weak signals. By analyzing AC conductivity through EIS, we seek to gain more information than traditional DC measurements provide [6–9].
We conditioned wood specimens, including common construction woods like spruce and beech, to specific moisture levels using climate chambers. Measurement consistency was achieved with a specialized sample holder and a climatized glovebox. First results on spruce, beech, larch, and oak revealed significant impedance differences based on anatomical direction, with the lowest impedance in the axial direction correlating with water transport pathways. As moisture content increased, impedance decreased and phase shift curves changed. Furthermore, we examined the impact of changing the wood chemistry by selective extraction or chemical modifications on the electrical conductivity of wood, presenting preliminary results from selected tests.

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Mass based spectrometries

New dimensions in the characterization of carbohydrates by emerging technologies in mass spectrometry

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Unlike other high molecular weight biomolecules, polysaccharides are extremely heterogeneous in terms of size, structure and chemical functions, posing a clear challenge to the field of analytical chemistry. Mass spectrometry (MS), with its remarkable sensitivity, speed and high information content, is a forefront method for deciphering biopolymers. However, classical MS approach fails in many cases to achieve definitive structural assignments of carbohydrates. The methodology commonly used for MS based approach involved digesting polymers, using specific enzymes, into a complex mixture of oligosaccharides, which include numerous isoforms. With this approach, in order to avoid the ubiquity of small major structures, the challenge is to obtain the largest possible oligosaccharides while developing efficient separation techniques compatible with these molecule sizes and approaches for their structural characterizations.
Our work explores the use and development of two innovative MS-based approaches for characterizing the fine structure of carbohydrates derived from plant and algal cell walls.
The first use a new generation ion mobility (IM)-MS that allows provide unprecedented resolutions on all stages of fragmentation using a cyclic cell [1]. We introduce notably the concept of IMSn sequencing [2,3] and molecular networking to preprocess the data [4].
The second is based on recently introduced methods using high-energy activation pathway [5-7] called charge transfer dissociation (CTD) and electron activated dissociation (ExD) that lead to a variety of diagnostic fragments to solve isomerism questions based on the positioning of modifications and the branching pattern between subunits. Our results illustrate the potential of these emerging techniques to push back the existing analytical barriers in the field of structural chemistry of carbohydrates.

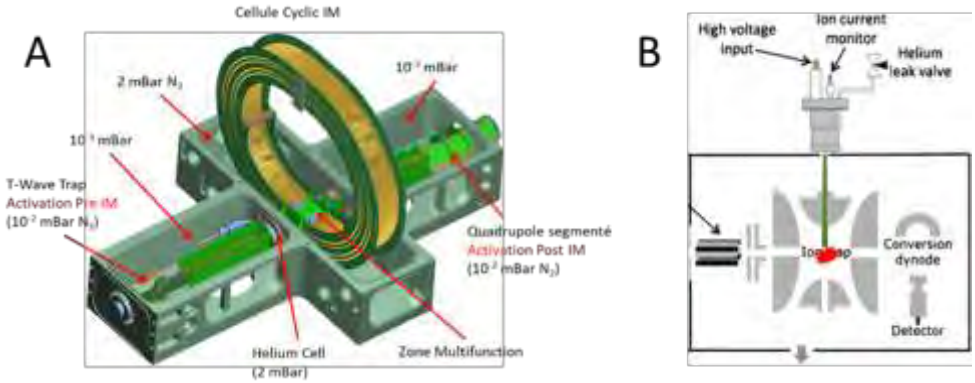

Figure 1: Scheme of the experimental setups used for A. IM-MS (Cyclic IMS Cell), B. CTD-MS.

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Stable isotopes in plant science: A general fairy tale subtly focusing on the plant cuticle

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Stable isotopes (SI) have become respectful tool in many industrial and research areas, including plant science [1]. The natural abundance studies and the isotope labelling open new avenue to answer many questions that are difficult to answer or inaccessible by other techniques. At the same time, SI possess substantial analytical challenge since only several isotopically different particles per 100 000 atoms may be result of important natural processes. In the first part of the presentation, I will introduce the power of SI in physiological and (paleo)ecological research. I am going to review briefly available methods for SI analyses as well. In the second part, I would like to focus on our recent interest – the plant cuticle development and regeneration. The coupling of separation techniques (gas chromatography) and Isotope Ratio Mass Spectrometry (IRMS) allow us to follow the fate of assimilated carbon in different plant compounds and substructures, as well as, plant cuticle. In turn, heavy carbon (^{13}C) labelling provides a nice temporal resolution that stems from the scarcity of minor carbon isotope (^{13}C) on Earth. Thus, we obtained new results that were previously unknown. For example that cuticular wax (in contrast to cutin matrix) is also synthetised and deposited even on mature evergreen leaves of *Prunus laurocerasus* and *Clusia rosea* [2] and that wax removal from the leaf surface has no effect on subsequent wax regeneration rate (MS in preparation).

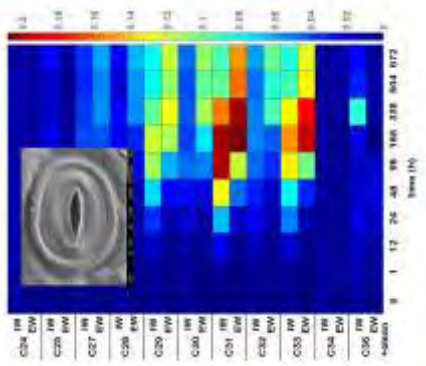

Figure 1: Stoma of *Prunus laurocerasus* leaf and illustration of different cuticular compounds dynamics.

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Data analysis

Theory is dead, long live theory: Hypothesis-centric machine learning in vibrational spectroscopy

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In recent years, artificial intelligence approaches have contributed to significant progress in life science research, ranging from the prediction of 3D protein structures to breakthrough developments in medical image analysis. These developments have also reached the application of microscopy, including infrared and Raman microscopy. In this context, our work adresses a key problem: Training deep neural networks to localize relevant patterns in imaging data conventionally requires pixel precise annotations, which are notoriously difficult to obtain for vibrational microspectroscopic images, because visual inspection and investigation of hyperspectral infrared or Raman microscopic images is limited when relying on our human sense of vision. To overcome this problem, we introduce an approach based on the so-called comparative segmentation network (CompSegNet) that requires weak, coarse grained labels only, while pixel-precise localization patterns are being inferred during the process of training the neural network. We demonstrate the validity of our approach in several applications, where the CompSegNet successfully localizes disease patterns in infrared microscopic images. The CompSegNet can be understood as an explainable neural network approach: While deep neural networks are inherent black boxes that lack transparency, the localization map inferred by the CompSegNet makes its output interpretable and explainable. Driven by the question of what constitutes a scientifically valid explanation, we introduce a hypothesis-based framework for falsifiable explanations of machine learning models. In this framework, a falsifiable explanation is a hypothesis that connects the interpretable output inferred by a neural network with the sample from which the data originate. As we demonstrate, this framework provides answers to some fundamental questions in machine learning, and identifies explainable machine learning as the missing link between machine learning and the scientific method.

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A tool for comparing large multispectral images: PCA score distributions.

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Using today's microscopes and macroscopes, multispectral autofluorescence images of whole sections of organs can be acquired with a spatial resolution of around 2 µm and a field of view of 1 to 2 cm². Large multispectral image series can be easily obtained, allowing statistical histological analysis. Here, we propose to quantify the differences between images based on pixel score distributions after Principal Component Analysis (PCA).
Series of 30-60 large images may contain millions to billions of pixels. Principal Component Analysis can be applied to image series by iteratively computing the variance-covariance matrix and the score images [1]. It is called *large PCA*. For each principal component, the pixel score distribution across the entire image series and its percentiles are determined. Local PCA score distributions are then calculated for each image using these percentiles. These local score distributions are regarded as quantitative characteristics enabling multispectral images to be compared.
Two examples of large autofluorescence multispectral image series are presented for which UV and visible fluorescence variability are studied. In the first study, 40 maize stem sections from 4 inbred lines were compared. Figure 1 shows an example of a score zoom image for each inbred line highlighting vascular bundles and parenchyma regions, the average score distributions for the 4 lines and finally the Principal Component Analysis of the score distributions showing the overall comparison of the 40 images. In the second example, a series of 40 images of wheat grains sections at 4 development stages was analysed. The score distributions of external tissues of wheat grain sections were compared after image segmentation and revealed an unsuspected level of spatio-temporal variability.
Pixel score distributions were found to be a promising tool for statistical comparison of multispectral images. The method is easy to implement, can be used as a first descriptive analysis of the image series, and can be easily extended to other spectral imaging techniques.

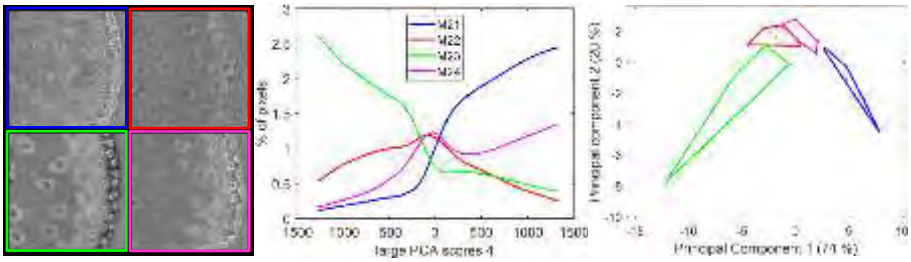

Figure 1: left: example of a *large PCA* score zoom images for four maize inbred lines. Middle: inbred line average score distributions. Right: Comparison of the 40 large images by Principal Component Analysis of pixel score distributions.

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A chemometrics approach to plant cell walls

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Spectroscopic analyses of plant cells provide large amount of data. Multivariate approaches have proven ideally suited for mining information from such rich veins of data. However, there is a wide variety of multivariate analysis methods, and it is not always easy to identify which one is best suited for the problem at hand. This lecture will outline a strategy for choosing the right multivariate tool for the right task. A common plant biological question (comparing two types of plants: a control / wild type and a modified (either genetically, or via treatment / environmental factors) will be used as an illustration to make the abstract methods and discussions easier to follow. Fourier-transform infrared (FTIR) and Raman spectroscopic datasets will be used as examples, but the demonstrated strategies are generic enough to be applied to any spectroscopic dataset. The techniques covered will include principal component analysis (PCA), spectral unmixing (such as multivariate curve resolution – alternating least squares, MCR-ALS), segmentation (e.g. k-means clustering), and supervised discriminant analysis (based on partial least squares (PLS) or orthogonal projections to latent structures (OPLS)). Advantages and disadvantages of each of the used multivariate methods will be discussed, along with common pitfalls and general guidelines regarding their use. Specific issues regarding sampling, spectral data characteristics and processing will be discussed as well, as these can have profound effects on the outcome of the analyses.

NMR spectrometry

Time Domain NMR and MRI: Opportunities and Challenges in Plant Investigations

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Time-domain (TD)-NMR and MRI provide an efficient approach to characterize living plants and plant tissues due to their ability to non-destructively access quantitative parameters related to water status and dynamics at multiple scales. Indeed, NMR signals are used to study key information about plant tissues, including compartmentalization, cell size, membrane permeability, and cell fluid transfer and composition. TD-NMR and MRI have therefore been used to address a range of questions in plant physiology, agriculture, post-harvest management and processing. These approaches also provide new opportunities for fine phenotyping.

To fully exploit these approaches in plant and food sciences, questions remain regarding interpretation of NMR data in terms of structure, composition and water dynamics in plant tissues undergoing various physiological or technological changes. There is also a need to improve the NMR/MRI methods and instrumentation required to obtain more accurate quantitative data. To extend plant characterization and phenotyping from laboratory experiments to plants in their natural environment, innovative approaches are also needed.

This talk will give a non-exhaustive overview of recent developments and illustrate some applications.

Analysis of the combination suitability between different dandelion species for rubber yield enhancement by evaluation of NMR metabolite profiles using artificial intelligence methods

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This project aims to establish a connection between certain properties of dandelion species, such as high rubber content, and their metabolite profiles using multivariate and machine learning methods. The calculated models shall help to gain insights into relevant individual metabolites and metabolite networks and, thus, to understand the underlying biochemical mechanism [1]. The metabolite profiles required for the analysis are calculated automatically from ^{1}H NMR spectra of the dandelion plants with the help of a self-written computer program. The significance of this work lies in the potential for dandelions to become an alternative source of natural rubber production [2], mitigating environmental concerns associated with traditional rubber production, while creating significant regional value chains. So far, only molecular approaches have been pursued to identify relevant genetic markers in dandelions for rubber content and root morphology [2], while the metabolome has not been considered. Plant material, from leaves and roots, underwent optimized sample preparation and one-dimensional ^{1}H NMR measurements were conducted using a 600 MHz Bruker NMR spectrometer. Metabolites were automatically identified and quantified from spectra using a self-written identification algorithm, non-linear optimization methods and an extensive database. No precise details can be given about the further data analysis, as these studies had just begun at the time of submission. As part of this project, 142 metabolites were measured in dandelion matrices. Among these, 34 known metabolites were confirmed, while 22 new metabolites were identified. Together, these 56 metabolites account for most signals in dandelion spectra, while the remaining identified metabolites are present in smaller concentrations and thus intensity. Automated identification processes demonstrated high accuracy, providing a basis for subsequent analyses. Initial results of statistical evaluations are presented and their significance for a deeper understanding of the underlying biochemical processes is discussed. This project represents a crucial step towards understanding the biochemical mechanisms underlying rubber yield enhancement in dandelion. The use of advanced data analysis methods highlights the importance of continued research in this field for further advancements in biotechnology and sustainable agriculture.

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Fluorescence Spectroscopy

Auto-fluorescence-based techniques in plant sciences

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Most plant tissues autofluoresce with UV or visible light excitation [1]. The two most abundant fluorophores are lignin in stems and chlorophyll in leaves, but many other fluorophores have also been studied. Autofluorescence can be used for phenotyping either using spectral analysis or by imaging and provides a label-free method for compositional imaging. Some methods can use autofluorescence as a signal to evaluate physical properties or for colocalization of fluorescent labels such as in immunolocalization or together with stains or chemical labels. Autofluorescence may often be due to multiple colocalized fluorophores that are amenable to spectral unmixing and deconvolution. Autofluorescence can be further characterized in terms of fluorescence lifetime.
This presentation will examine several applications for autofluorescence in imaging plant tissue and plant-based biomaterials.

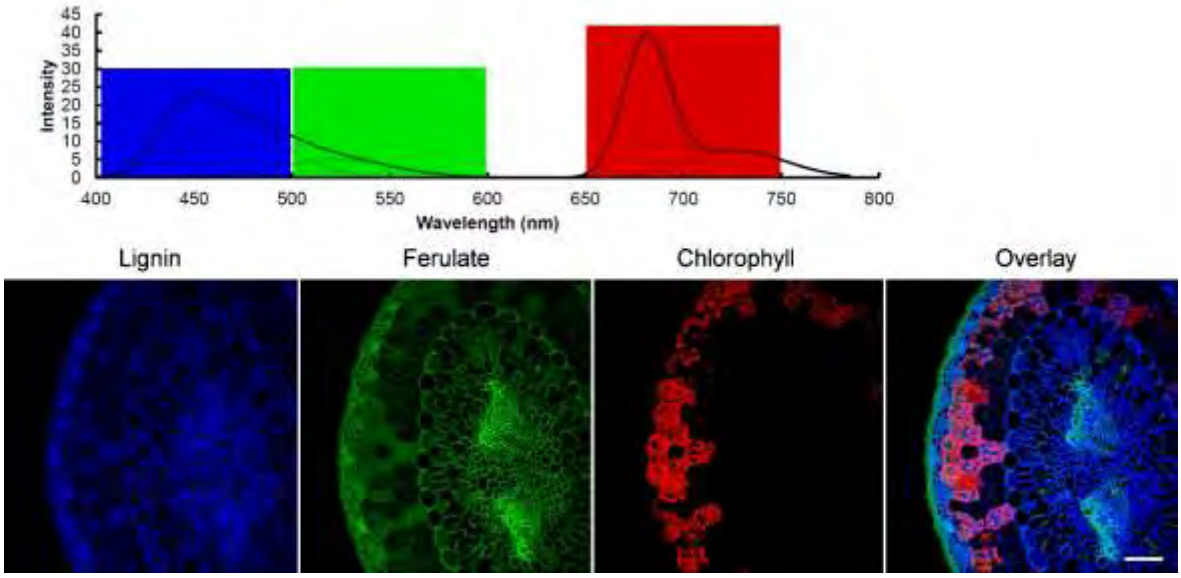

Figure 1: Autofluorescence of pine needle, spectral imaging, and deconvolution. Excitation 355 nm. Scalebar = 100 µm.

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Automated autofluorescence-based intensity and morphological quantification of plant cell wall

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Lignocellulosic biomass is a complex network of polysaccharides and lignin that requires a pretreatment step to overcome its recalcitrance and optimize its bioconversion into chemicals and materials. Pretreatment of biomass is associated to important chemical and morphological changes at different scales, so that quantification of these modifications is critical to understand biomass recalcitrance and to predict its reactivity.
We have thus developed a complete and automated image processing method (Figure 1) for rapid and easy quantification of both fluorescence intensity and morphological parameters of plant cell walls imaged by fluorescence macroscopy. It was applied on wood samples (spruce and beechwood) pretreated by steam explosion at different severities.
Concerning autofluorescence of cell walls, the intensity decreased with higher severity of pretreatment: it was directly negatively correlated with pretreatment temperature, and could be related to lignin properties.
Regarding the evolution of morphological parameters with the severity of pretreatment, we observed that while cell perimeter showed no significant changes, area and circularity of cells were significantly reduced when applying the most severe pretreatment conditions. Consequently, these two parameters can be considered as complementary markers of alteration of cells: cell area as a marker of cell size reduction and cell circularity as a marker of cell wall alteration.
This approach [1] can be applied to fluorescence macroscopy as well as other imaging techniques and provides a relevant method towards the understanding of native and pretreated cell wall architecture.

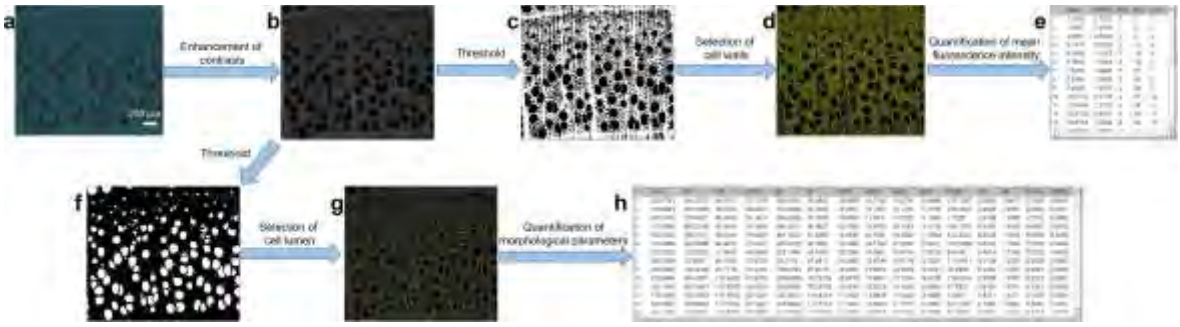

Figure 1: Automated procedure for autofluorescence quantification of wood cell wall. **a** Stack of original images. **b** Enhanced grey level average image. **c** Threshold for fluorescence intensity quantification. **d** Application to the initial stack. **e** Table of results for fluorescence intensity. **f** Threshold for morphological parameters quantification. **g** Selection of the vessels. **h** Table of results for morphological parameters.

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Multi-hyperspectral and multimodal imaging to map the distribution of cell wall compounds in *Brachypodium distachyon mutants*

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The bioconversion of lignocellulosic biomass into high-value molecules, materials or energy is severely hampered by lignins. Lignins are mainly composed of *p*-hydroxyphenyl, guaiacyl and syringyl units, which spontaneously couple to form the lignin polymer after oxidation by laccase or peroxidase. In addition to the major monolignols, several other molecules can be incorporated in the polymer. In particular, grass incorporates ferulate and *p*-coumarate [1]. Structural evidence suggests that ferulates may act as nucleation sites for lignin formation. The role of lignin acylation by coumarate remains unclear. Similarly, little is known about the relative distribution of these phenolic compounds at the tissue, cell and cell wall levels.
Here, multispectral fluorescence macroimaging and mid-infrared microspectroscopy were used to reveal differences in the composition of stems from *Brachypodium distachyon* plants, wild type, enriched and depleted in coumarate [2].
Multispectral autofluorescence macroimaging was used to quantify variations in cell wall phenolic composition in sections. Images of full cross-sections were acquired with a spatial resolution of less than 0.56 µm. Two UV and two visible excitation conditions were combined. Large principal component analysis was applied to the analysis of the full series of 9 images [3]. The loadings and score images showed variations in tissue fluorescence and according to genotypes.
Hyperspectral images were acquired using an FTIR microspectrophotometer equipped with a 64 x 64 focal plane array (FPA) detector and an x15 objective. After baseline correction and spatial spectral normalisation [4], the 29 hyperspectral images were merged into a single data structure and analysed by Multivariate Curve Resolution - Alternating Least Squares (MCR-ALS) [5]. Two components corresponding to aberrant spectra (pixels of the epidermis located at the border between the window and the section) were not taken into account. [5]. The 4 other components could be assigned to cellulose + phenolics, proteins, xylans and esterified acetyl groups. The concentration maps allow the differentiation of tissues according to their relative amounts of proteins, cellulose+phenolics and xylans. However, differences between genotypes were difficult to detect using this approach.

Funding: French ANR agency, PEPR B-BEST FillingGaps project (ANR-23-PEBB-0006)

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POSTERS

(Alphabetical order)

P1 Stable iotopes as proxies for hydro-climatic conditions and stress-related metabolite biosynthesis in Norway spruce and European beech

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The analysis of stable isotopes (SIs) of major biogenic elements in different plant organs is being increasingly utilized to understand the impact of hydro-climatic conditions on plant physiological processes. Notably, there is a well-established theoretical link between the $\delta^{13}C$ isotopic signal and Water Use Efficiency (WUE), a key physiological parameter representing the interaction between carbon and water cycles in plants and ecosystems. Recent analyses [e.g., 1] have demonstrated that $\delta^{13}C$ and $\delta^{18}O$ values in tree rings are more reliable proxies for temperature and precipitation, respectively, than traditional tree-ring metrics like width or wood density.

In this work, we provide (*1*) further evidence for the exceptional efficacy of $\delta^{13}C$ and $\delta^{18}O$ as proxies for current hydro-climatic conditions, including temperature, precipitation, evapotranspiration, vapour pressure deficit, and (*2*) investigate the relationships between SI abundances and the biosynthesis of selected stress-related metabolites. We found high correlations (up to $R = 0.9$) between specific hydro-climate indicators and $\delta^{13}C$ and $\delta^{18}O$ values in needles and leaves of Norway spruce and European beech trees growing at elevations ranging from 445 to 1001 m a.s.l. across the Czech Republic (Fig. 1). Generally, hydro-climate variables showed stronger associations with $\delta^{18}O$ than $\delta^{13}C$, particularly for Norway spruce. Additionally, the representation of SIs and metabolite contents was influenced by the age of spruce needles. Finally, we observed strong correlations (up to $R = 0.90$) between $\delta^{18}O$ and the contents of GABA (γ-aminobutyric acid) and salicylic acid (Fig.1) — compounds associated with occurrence of drought stress.

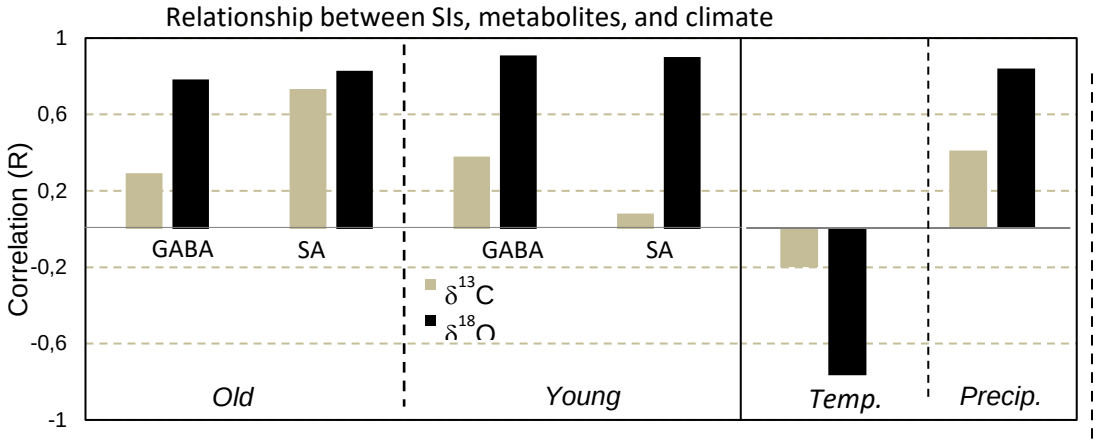

Figure 1: Correlation coefficients between carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotopes, stress metabolites (GABA, salicylic acid), and climate variables (temperature, precipitation) in old (>100y) and young (<65y) spruce trees (n=10).

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Acknowledgement: This work was available thanks to GA ČR project number: 23-07583S.

P2 UV excitation multispectral imaging to explore Poaceae plant cell walls diversity

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Poaceae plant cell walls are composed of phenolic compounds such as lignin and hydroxinnamic acids which are known to affect the potential uses of such feedstock. They are not uniformly distributed within plant organs and this distribution could explain differences in key properties such as their milling behaviour or hydrolysis patterns. Based on the autofluorescence properties of these components, specific spectral signatures of plant cell walls have been assessed according to plant tissues or organ localisation and related to known differences in tissue composition [1,2]. Indeed, autofluorescence spectra could be relevant for the presence of different components (ferulic acid/para-coumaric acid, lignin) and their amount or relative proportions, but also for their structure and interactions in the cell walls. The UV wavelength range is of great importance and the fluorescence signal of ferulic acid in maize stem after UV excitation has been shown to be in shorter excitation wavelength than para coumaric acid [2]. The aim of this work was to focus on UV excitation fluorescence to investigate the heterogeneity of plant cell walls at the microscopic scale, both in excitation and emission mode. Two well-studied reference samples (wheat grain and maize stem) were used to cover a wider range of variability. Cross sections were imaged with a filter microscope to obtain multispectral images. Large field of view at high spatial resolution (pixel size=1.06 µm) were investigated in accordance to plant structure size and heterogeneity. In this case, acquisition is carried out to obtain pseudo-excitation spectra, focusing in the UV range. The fluorescence emission was then recovered in four wavelength bands in blue and green for 4 excitation ranges in UV, centred around 340 nm, 360 nm, 380 nm and the last one covering the whole UV range. The possible influence of pH was also evaluated in relation to known fluorescence shift [3].

Funding: French ANR agency, PEPR B-BEST FillingGaps project (ANR-23-PEBB-0006)

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P3 Cellulose Microfibril Orientation in Moss Cell Morphogenesis using Polarized Raman Spectroscopy

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Morphogenesis intricately relies on dynamic cytoskeleton, where especially microtubular structures should serve as a scaffold indispensable for orchestrating the cellular shape changes. While KO lines of Fass along with Ton1 were firstly described as preprophase band-less mutants [1,2], later on, strong defects also on interphase microtubule nucleation and reorientation dynamics has been described [3]. While the mutant still manages to undergo normal histogenesis, this made the FASS protein also an important contributor to the post-cytokinetic complexity in cell morphogenesis. The coordination of the cellulose microfibrils along the changing microtubule cytoskeleton is thought to play a pivotal role in determination and directionality of the cell elongation. While trying to explore this paradigm, we looked at the cell surface fiber arrays anisotropy and their orientation changes in dwarfish *fass* mutants and elongating wild types. Using FibrilTool plugin for image analyses, imaging using different fluorescent stains for cellulose and High-Resolution Scanning Electron Microscopy revealed some considerable differences in the orientation of cellulose microfibril patterns their and anisotropy in mutants compared to wild type. Confocal Raman spectroscopy has provided valuable insights into the intricate organization and composition of plant cell walls non-destructively. Thus, shedding light on some interesting variations of cell wall organization between WT and the KO lines. This work is supported by GACR/CSF project 23-05564S.

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P4 Insights into structure and composition of a cellulosic nanocomposite (kombucha pellicle) by Raman imaging

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Through the symbiotic interaction of yeast and the bacterium *Komagataeibacter xylinus*, a pellicle forms during fermentation of kombucha tea [1-2]. A thin layer initially forms and continues to thicken and finally results in a pellicle of a fibrous network of bacterial cellulose on top of the kombucha tea [3-4]. To reveal the structural and chemical changes throughout the formed kombucha pellicle we cut thin cross-sections by cryo-microtomy and scan them by Confocal Raman microscopy. Based on the different Raman signature of cellulose and bacteria, we were able to image *in-situ* the cellulose network in the wet stage together with the underlying bacterial machinery (Figure 1). While the spectra reflecting the bacteria have higher background and bands at e.g. 1658 cm^{-1}, 1577 cm^{-1} and 1475 cm^{-1}, the produced bacterial cellulose has the typical strong bands at 1097 cm^{-1} and 1122 cm^{-1} amongst other typical cellulose bands (Figure 1, inset). The visualized fibre network itself becomes denser from the bottom (right side) to the middle (left side, Figure 1), but on the bottom thicker layering of fibrils is observed (arrow, Figure 1). Accordingly also the amount of bacteria increases from bottom to the middle part (red, Figure 1). Based on the Raman spectra of cellulose, insights into crystallinity can be gained and thus in future research also changes after drying and/or further processing of the pellicles can be followed. As kombucha pellicles are a waste material from kombucha fermentation, the produced bacterial cellulose is considered as interesting resource for new sustainable nanocomposites, e.g. mixed with nutshell waste. Understanding the natural built fibre cellulosic network is important to develop new composite materials based on sustainable waste resources.

Figure 1: Based on the different Raman spectra, the bacteria (red) and the fibrous cellulose network (white) can be visualized within the kombucha pellicle by scanning a cross-section. The left side reflects the middle part and the right side the bottom of the pellicle.

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Funding: PUZZLE MATERIALS project ERC-POC (grant agreement 101113395).

P5 Deciphering Microchemical Degradation Patterns in Waterlogged Archaeological Wood by Confocal Raman Microscopy

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The properties of waterlogged archaeological wood differ significantly from those of healthy wood due to environmental degradation and biodegradation during prolonged burial and excavation [1, 2]. In oxygen-depleted burial environments, wood is susceptible to bacterial attack, while soft rot fungi thrive in more oxygen-rich aquatic conditions [3, 4]. Despite lignin's reputation for greater resistance to biological decay compared to cellulose, its eventual degradation is acknowledged [5, 6]. However, the scarcity of studies on the microchemical changes in severely decayed wood samples underscores the challenges in their preparation [7]. In this study, we used Confocal Raman microscopy to elucidate the chemical composition of the different cell wall layers of waterlogged archaeological pine (*Pinus* sp.) wood. Our investigation shows that the chemical structure of the residual tracheid cell wall closely mirrors that of cell corners after degradation. Additionally, we observed a consistent increase in relative lignin content with degradation, corroborating traditional chemical composition analyses [8]. Analysing the different cell layer during degradation reveals that initially methoxy groups of the lignin in the cell corners breakdown, along with the cleavage of some covalent bonds linking lignin to carbohydrates. Subsequently, lignin side chains degrade progressively until complete breakdown, leaving residual aromatic ring structures. Eventually, cellulose undergoes complete degradation in the final stage. Similar patterns of lignin degradation, including the cleavage of covalent bonds between lignin and carbohydrates, were observed in the S1-and S3 layers. In the S1 layer, carbohydrates degrade gradually, while in the S2 layer, initial carbohydrate degradation is sluggish and accelerates upon the rupture of covalent bonds between lignin and carbohydrates. In the S3 layer, hemicellulose is fully degraded before cellulose undergoes partial degradation. We anticipate that our findings will contribute to a deeper understanding of microchemical distribution patterns in archaeological wood. Moreover, comprehending changes in the cell wall chemistry of waterlogged archaeological wood aids in the selection of appropriate preservation methods to safeguard this fragile cultural heritage.

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P6 Magnetic resonance imaging of node and internode in maize stalk

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The maize stalk is roughly cylindrical in shape, with many longitudinally oriented vascular bundles in the pith and a succession of nodes and internodes with a leaf inserted at each node, alternately on either side of the stalk. The internode number and length vary according to genotype, development stage, environment and crop management. The stalk structure has an impact on its use properties for animal feed and its degradability for the biofuels production. The maize internode histology has been investigated using microscopic methods [1], but the study of the node heterogeneous structure has been hampered by the difficulty of preparing microtome sections. Magnetic resonance imaging at the microscopic scale was chosen to investigate non-destructively maize node structure.

The internode under ear with a node, sampled from the stem, carefully wiped and wrapped to prevent desiccation was placed in a 30 mm tube along with a 5 mm one containing doped water for NMR signal amplitude normalization. A vertical wide bore 400 MHz NMR spectrometer equipped with microimaging accessory was used. Images were acquired at room temperature, using a multi-slice multi-echo pulse sequence in order to obtain water mobility image (T_2 images) and water distribution image (normalized proton density images). Two axial slices packages were selected, one within and the second above the node region, and collected with a slice thickness of 500 µm and an in-plane resolution of 100 × 100 µm^2.

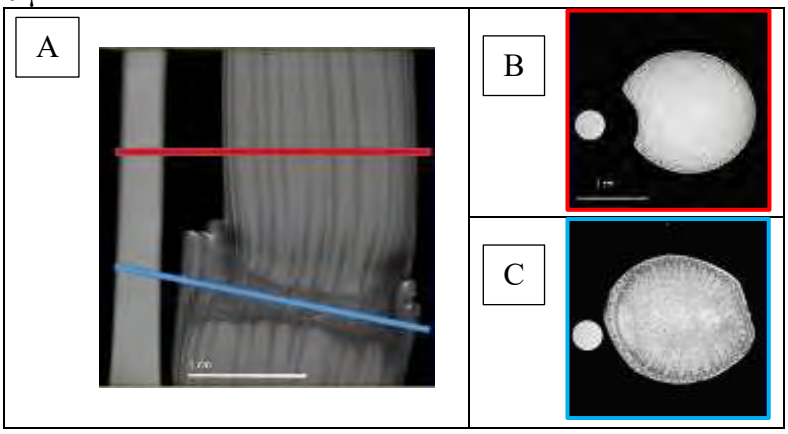

Figure 1: Examples of the first echo images of coronal (A) and axial (above (B) and within (C) node) slices from a maize stalk.

Currently other imaging modalities, such as MALDI mass spectrometry imaging, are used to add chemical/metabolite information. The ultimate aim will be to produce a 3D model of the stalk by combining morphological, water dynamics and chemical information in order to gain a better understanding of maize nodes and internodes.

Funding: French ANR agency, PEPR B-BEST FillingGaps project (ANR-23-PEBB-0006)

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P7 Analysis of the combination suitability between different dandelion species for rubber yield enhancement by evaluation of NMR metabolite profiles using artificial intelligence methods

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This project aims to establish a connection between certain properties of dandelion species, such as high rubber content, and their metabolite profiles using multivariate and machine learning methods. The calculated models shall help to gain insights into relevant individual metabolites and metabolite networks and, thus, to understand the underlying biochemical mechanism [1]. The metabolite profiles required for the analysis are calculated automatically from ^{1}H NMR spectra of the dandelion plants with the help of a self-written computer program. The significance of this work lies in the potential for dandelions to become an alternative source of natural rubber production [2], mitigating environmental concerns associated with traditional rubber production, while creating significant regional value chains. So far, only molecular approaches have been pursued to identify relevant genetic markers in dandelions for rubber content and root morphology [2], while the metabolome has not been considered. Plant material, from leaves and roots, underwent optimized sample preparation and one-dimensional ^{1}H NMR measurements were conducted using a 600 MHz Bruker NMR spectrometer. Metabolites were automatically identified and quantified from spectra using a self-written identification algorithm, non-linear optimization methods and an extensive database. No precise details can be given about the further data analysis, as these studies had just begun at the time of submission. As part of this project, 142 metabolites were measured in dandelion matrices. Among these, 34 known metabolites were confirmed, while 22 new metabolites were identified. Together, these 56 metabolites account for most signals in dandelion spectra, while the remaining identified metabolites are present in smaller concentrations and thus intensity. Automated identification processes demonstrated high accuracy, providing a basis for subsequent analyses. Initial results of statistical evaluations are presented and their significance for a deeper understanding of the underlying biochemical processes is discussed. This project represents a crucial step towards understanding the biochemical mechanisms underlying rubber yield enhancement in dandelion. The use of advanced data analysis methods highlights the importance of continued research in this field for further advancements in biotechnology and sustainable agriculture.

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P8 Evaluation of food ingredient using fluorescence spectroscopy

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Fluorescence spectroscopy is a simple and rapid technique that is proving very useful in answering various INRAE research questions. The data generated by the spectroscope used in our laboratory enables us to study the overall compositions in food samples of plant or animal origin. To obtain a global characterization of all fluorescent molecules, we simultaneously scan a wide range of excitation and emission wavelengths to build 3D maps with excitation wavelengths on the ordinate and emission wavelengths on the abscissa (Figure 1). The main fluorescent molecules studied are proteins and other fluorescent molecules such as phenolic compounds which appear as spot of high color intensity on the 3D maps generated. Methodological developments on sample preparation have been carried out to optimize acquisition conditions.
To achieve this, we are using R programming and have set up an interface for reprocessing the raw data obtained by fluorescence spectroscopy, enabling any user, whether familiar with the R language or not, to render more reliable and reproducible interpretations on fluorescence evolution.
Our results in fluorescence spectroscopy will be illustrated in the poster through 2 examples: the characterization of pigeon pea flours which led to the identification of minor compounds [1], and the monitoring of the conservation of insect flours from Cameroon.

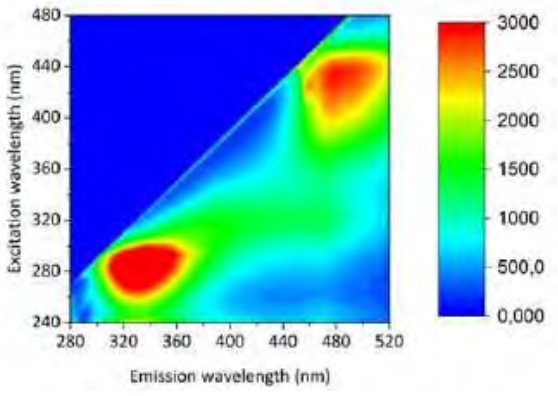

Figure 1: Three-dimensional excitation-emission matrix (3D-EEM) of pigeon pea flour produced with raw seeds in dry state.

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P9 Mapping biochemical networks in living plants using spectral FLIM

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By leveraging spectral and lifetime components, Spectrally Resolved Fluorescence Lifetime Imaging Microscopy (sFLIM) enables multiplexed imaging of several fluorophores in living plant tissue, maximizing biochemical resolution. This technique can monitor multiple endogenous autofluorescent molecules, such as phenolic compounds, in combination with second harmonic generation from cellulose fibers, without the need for exogenous staining. Additionally, sFLIM serves as a powerful tool for imaging multiple FRET sensors, allowing for the simultaneous biochemical multiplexing of several molecular pathways and interactions within living cells and tissues [1]. We will present our advancements in two-photon (2P) sFLIM imaging of living plants and introduce a multidimensional phasor demixing approach for data analysis [1,2].

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P10 Raman Imaging of rafting kelp from California

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Brown macroalgae of the species *Macrocystis pyrifera* and *Egregia menziesii* often form large kelp forests along the Pacific shore [1]. While being sessile organisms, thalli also frequently detach from the seafloor and drift ashore sometimes for large distances, eventually reaching intertidal zones and beaches. As such, we collected live and seemingly healthy kelp in La Jolla, California in July 2023. Punched out discs of the sporophyte blades were chemically fixed for high resolution histological observations. Semithin sections illustrate the outer meristoderm and the central medulla region containing acidic polysaccharides (Fig. 1a). For Raman spectroscopy, fresh material was lyophilized and rehydrated for cross-sections (10 µm) by cryo-microtomy. Raman mapping opened the view on microchemistry and visualized cell walls containing mainly cellulose, but also fucoidan and alginate [2] (Fig. 1b-c, blue). The latter particularly filled the space between the cells in the medulla. In the lumen of the medulla cells, spectrally different compounds were visualized as well as in the apoplast between the cell walls of the meristoderm (Fig. 1b-c, orange). In the cell lumen of the meristoderm cells a chemically similar component (Fig. 1b-c, red) was detected. Meristoderm cells were reported to be filled with photopigments, including the special carotenoid fucoxanthin [3]. The strong carotenoid chain bands (1160 cm^{-1} and 1530 cm^{-1}) were very weak or absent, probably due to photo-bleaching by laser irradiation. Nevertheless, a weak band was found at 1927 cm^{-1}, which can be assigned to the allene group in fucoxanthin. Additionally, the Raman signature included membrane constituting lipids (e.g. 1453 cm^{-1}, CH_2/CH_3 bending) and proteins (e.g. 1237 cm^{-1}, 1672 cm^{-1}). The aromatic contributions (~1600 cm^{-1} and high fluorescence background) might come from phlorotannins, which are also part of cell membranes, produced by polymerization of phloroglucinol and acting as a shelter against environmental stress in brown algae. In summary this study shows that rafting kelp show intact structures and microchemical compositions.

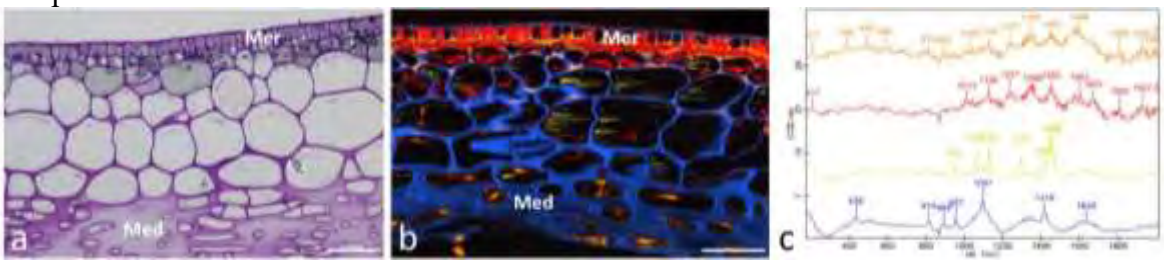

Figure 1: **a)** *Macrocystis pyrifera* histological section stained with 0.3% toluidine blue shows meristoderm (Mer) and medulla (Med), **b)** Raman image showing the distribution of the four spectrally most different components: orange/red includes cell membranes, pigments (fucoxanthin) and gallotannin with highest amounts in the meristoderm, yellow represents lipids in the middle part and *blue* the cell wall polymers, **c)** Average spectra with characteristic Raman bands corresponding to the different components. Scalebars 50 µm.

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P11 Widefield super-resolution photothermal infrared spectroscopy of fluorescently labeled and autofluorescent biological materials

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Optical Photothermal Infrared (O-PTIR) spectroscopy has established itself as a breakthrough vibrational microspectroscopy tool, offering significant advantages over the traditional FTIR/QCL & Raman spectroscopy, providing submicron simultaneous IR+Raman and fluorescence imaging. However there still exists a demand for rapid and high-resolution widefield (snapshot) IR imaging. To this end, we have developed a novel widefield super-resolution IR imaging technique, powered by Fluorescence Detected Photothermal IR (FL-PTIR) that utilizes changes in fluorescent emission intensity directly for IR chemical information extraction. As the fluorescent signal is captured with a 2D fluorescence camera, this generates, simultaneously, widefield IR as well as widefield fluorescence images. The key enabling factor here, is that when the wavelength of the IR pulses is tuned to a molecular vibration of fluorescently labeled molecules, the absorbed heat causes a modulation in the amount of fluorescent light emitted from the fluorophores or autofluorescence. Coupled with the parallel data acquisition via the 2D (512 x 512) visible fluorescence camera, using a standard glass objective of 50x, 0.8NA, single field of view for IR of 66 x 66µm with 130nm pixels are possible. Compatibility with other standard visible glass objectives such as those with higher NA, or even immersion objectives open up further possibilities for widefield super-resolution IR imaging. Widefield O-PTIR thus allows the IR spectroscopic analysis of specifically labelled or autofluorescent regions of biological cells and tissue, for example to study conformational stages of a specifically labeled class of target proteins or protein misfolding associated with neurodegenerative diseases. Various examples from these applications will be provided.

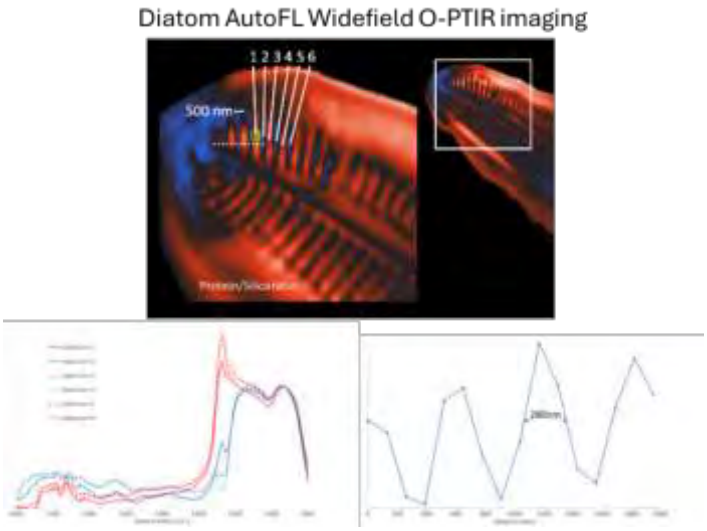

Figure 1: Diatom Autofluorescent Widefield O-PTIR Chemical imaging with extracted IR spectra and spatial resolution assessment

P12 Identification and quantification of carotenoids from paprika extracts using Thin Layer Chromatography coupled with Raman spectroscopy

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Carotenoids represent a big and important group of plant secondary metabolites that give yellow, orange, and red colour to fruits, seeds, some of the roots, and leaves. More than 1000 different carotenoids are identified so far, out of which around 500 are completely characterized based on physicochemical properties [1]. In human nutrition, there are usually 40 different types of carotenoids [2]. Intake of carotenoids is related to improvement of functioning of the immune system and reducing the risk of development degenerative chronic [3]. Paprika (*Capsicum annuum* L.) is one of the most popular vegetables in the world and a rich source of carotenoids [4].

The aim of this paper is to monitor biosynthesis of selected carotenoids (capsanthin, β-cryptoxanthin and β-carotene) during paprika fruit maturation (genotype Kurtovska kapija in five stages of ripening) and its traditional Balkan product (prepared from roasted paprika in final stage of ripening) - ajvar. For this purpose, Thin Layer Chromatography and Raman spectroscopy were applied as follows: extracts of paprika fruit at different maturation stages were separated in TLC separation chamber; after development of TLC plates, selected carotenoids bands were scratched, purified, dissolved, transferred to quartz microcapilar tube and analysed by Raman spectroscopy. Besides, concentration series of investigated carotenoids were also prepared and analysed by Raman spectroscopy to form calibration curve. The HPTLC method (High Performance Thin Layer Chromatography) was used as a control (standard method) for both qualitative and quantitative analysis. The analyses were performed using the Witec Alpha 300 R system equipped with a 532 nm laser and a CAMAG semi-automatic HPTLC system (carotenoids were detected at a 440 nm wavelength). The statistical analysis was carried out using The Unscrambler X software.

Results of Raman spectroscopy displayed that all investigated carotenoids increased during maturation and slightly decreased in ajvar. Relative concentration of investigated carotenoids in fresh paprika was as following: capsanthin ranged from 0.11-0.30 g/100 g DW, β-carotene 0.09-0.99 g/100 g DW while β-cryptoxanthin has not been detected in first four maturation stages while concentration in the last stage of maturity was 0.06 g/100 g DW). When it comes to ajvar concentration was 0.29, 0.05 and 0.98 g/ 100 g DW for capsanthin, β-cryptoxanthin and β-carotene, respectively. All results were highly correlated with results obtained by standard analytical method – HPTLC.

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P13 Seasonal changes in bark anatomy and chlorophyll content of five temperate tree species

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Bark photosynthesis contributes to the carbon balance of the whole tree and varies throughout the growing season. Specific anatomical features in the bark play a role in transport and assimilation of photosynthetic substances. The inner bark of young stems is protected by the periderm and houses living cells that contain photosynthetic pigments, which are used for assimilation in the stem. The relationship between the anatomical characteristics and the photosynthetic properties of the living bark is still poorly understood. Moreover, little is known about the effect of changes in chlorophyll content and tissue morphology during the growing season.

Bark tissue of five temperate tree species was dissected to investigate how tissue structure and chlorophyll content vary in response to seasonal changes. Seasonal effects of the changing bark structures were quantified on freshly cut cross-sections by means of image analysis. The concentration of photosynthetic pigments was investigated by determining the concentration of chlorophyll a and b, and carotenoids in both the bark and wood of fresh material by DMSO extraction. Subsequently absorption spectra were measured using a UV/Vis spectrophotometer and compared to those of leaves.

Our findings reveal widespread variation in chlorophyll content between different stem tissues and between different seasons. We show how chlorophyll content correlates with the expected light levels in the bark, and discuss how seasonal changes may explain the observed variation.

P14 Tomato drying investigated by MRI, single sided NMR and conventional techniques

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Drying is a common method of fruit preservation, slowing microbial activity and chemical spoilage reactions. In addition to these benefits, drying induces histological and compositional changes (alteration of cellular metabolism in addition to water loss) and affects cell wall properties. It can therefore be expected that the characteristics of the fruit at harvest, its behaviour during drying and the final quality of the product are related. This final quality is largely determined by the organisation of water in the tissues. A more comprehensive understanding of this organisation and its role in the structural changes in the tissues during drying is therefore required in order to adapt the drying process to the variability of fresh fruits, with the aim of obtaining high-quality dried products in terms of nutritional and textural properties.

In this study, MRI and single-sided NMR were employed to assess the alterations in water quantity and organisation during the drying of tomato slices. Concurrently with the MRI and NMR experiments, tomato tissues were observed by optical microscopy and their texture was characterised. Tomatoes from two industrial cultivars differing in terms of histological properties were studied, and their slices were analysed both fresh and dried at three defined degrees. The drying kinetics were also studied *in situ*, by performing MRI analyses on tomato slices subjected to dehydration inside the MRI device.

The MRI (Figure 1) and NMR approaches were shown to be effective to reveal differences in drying kinetics of the two cultivars analysed. Additional microscopy and texture data enabled to improve the understanding of the dynamics of water loss and the final quality of dried tomatoes, thus offering a future for the use of MRI and NMR for improving fruit drying processes.

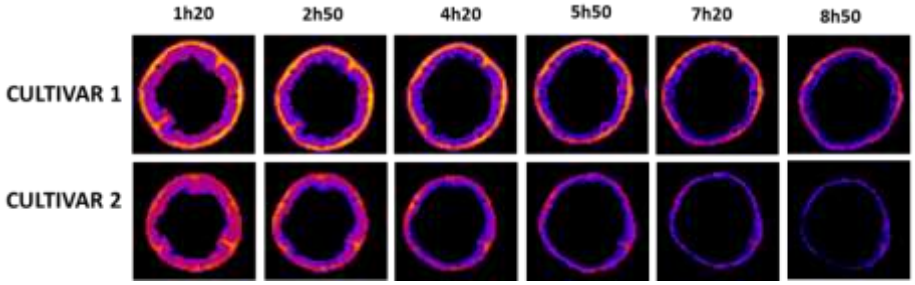

Figure 1: MRI transverse relaxation (T_2) maps of tomato slices during drying. The 24 mm thick slices were prepared by cutting the upper and lower parts of the fruits and removing the locular tissue, leaving only the pericarp and skin.

P15 Feeding ecology and food selection – the FTIR way to capercaillie's choice

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Understanding food selection of wildlife species is one crucial target when studying feeding ecology. Herbivores might be highly selective in their choice of certain plant species and also in terms of individual plants and plant parts within a particular species [1]. This selection can be driven by secondary plant components, nutrients and other factors [2] and it might differ between wildlife individuals. Fourier Transform Infrared spectroscopy (FTIR) is an approved technique to determine chemical and physio-chemical properties of biomaterials and might thus be used to analyse food items of wildlife species. Analyses with FTIR might hold a clear advantage to DNA metabarcoding approaches [3] as they support the determination of specific parts of a plant or the phytochemical composition, which is helpful to answer questions of food selection at different temporal and seasonal scales. We analyzed crop contents of juvenile and adult male capercaillie individuals (n=19) as well as samples from trees, provided from a previous study in Scandinavia [4]. At the study sites in Norway, capercaillies´ dominating winter diet is Scots pine (*Pinus sylvestris*), consisting of needles (N) and twigs with needles and cones (TNC), which differ in terms of secondary metabolite content and nutrients. For our analyses, 117 samples were available including 1) crop contents, 2) samples from trees that were selected by capercaillie for feeding, 3) non-browsed neighbor trees and 4) non-browsed trees at a distance of 200 m [4]. FTIR spectra of crop contents, tree samples and N vs. TNC were recorded on a Bruker® FTIR spectrometer (Tensor 27) in the Attenuated Total Reflectance (ATR) mode. We compared the spectra of crop contents (N, TNC) with the one of sampled trees. Addressing age-specific feeding ecology, we also compared spectral signals of juvenile vs. adult capercaillie males.

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P16 Rapid Nondestructive Assessment of the Botanical Origin of Honey Using FT-Raman Spectroscopy and chemometrics model

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Honey is product created by honey bees, primarily from collected floral nectar, but also from the excretions of plant sucking insects in the case of the forest honey. Honey contains a large amount of carbohydrates, followed by much less proteins, vitamins and minerals, pigments, phenolic acids, flavonoids and enzymes. The composition and quality of honey varies depending on a number of factors, but above all on the plant from which the nectar originates. The physico-chemical parameters of honey are determined by a complex of different methods [1], while the determination of the botanical origin of honey is usually challenging and relies on melissopalynological analysis [2]. This method is time-consuming and is highly dependent on the expertise and experience of the person performing the analysis. Therefore, the present research aimed to develop a model for effective, rapid and specific determination of honey origin by combined use of FT-Raman spectroscopy and chemometrics. During two years, over 100 samples of the six most common monofloral and multifloral honey types from seven European countries were analysed. The Raman spectra of the honey samples were recorded with a laser at a wavelength of 1064 nm in the range of 176-1500 cm^{-1}. In order to correctly classify different honey samples, QDA and SVM classification models were used. Sucrose molecules consisting of dominant skeletal vibration δ(C–C–C), δ(C–C–O), δ(C–O) and τ(C–C) in glucose and fructose. In the Raman spectra, the bands with higher intensity at 418, 515, 623 and 1064 cm^{-1} could be assigned to the skeletal vibration of α-glucose and the ring deformation of fructose, probably due to their highest content in the acacia, lime and forest honey samples. These bands are in lower intensities in sunflower, Mediterranean, and mountain honey samples together with bands at 913 and 1370 cm^{-1} attributed to bending mode of the glucose and sucrose [3]. QDA displayed classification accuracy 94.72 % in average for training and test sets of data, while SVM showed around 95% of precision. These results show that Raman spectroscopy, combined with statistical analysis, have a great potential as a toll for determining quality of honey. The advantages of applying this model are that it requires small amounts of sample and the method is very fast, reliable and repeatable.

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P17 Spectroscopic assessment of mechanical stimuli response in juvenile Serbian spruce (*Picea omorika* (Pančić) Purkyne)

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The reaction to gravitropic stress in trees is the formation of reaction wood, in conifers known as compression wood (CW). It forms on the lower side of the leaning stem. Opposite wood (OW) is formed on the opposite side to the compression wood in the same growth ring, while wood from growth rings not containing any CW is termed normal wood (NW). In slow-growing conifer species, CW typically occurs in a severe form. There are several physical and chemical indicators of CW.

Aiming to understand the reaction wood response in a slow-growing conifer species we studied CW formation on the stem sections in juvenile *P. omorika* trees subjected to one-year static bending using microscopic and spectroscopic techniques. The sections were imaged using a Leica SP5 II confocal microscope. The analysis of fluorescence emission spectra was performed by the non-linear fitting using the Nelder–Mead simplex algorithm implemented in MATLAB. Since lignin is the primary fluorescent compound in wood cell walls, the emission spectra reveal the structural properties of lignin and their changes as a consequence of mechanical stimulation. The results indicate a change in lignin structure including flurophore type along the stem, from the base to the top segment. This change varies across the growth rings. The Pyrolysis-GC analysis of individual phenolic and sugar compounds from the three stem segments, as well as brightness data for 488 nm excitation confirmed a change in fluorophore type and variation in compression severity from the basal to the top segment.

P18 How does a tolerant moss develop in presence of heavy metals?

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The most common pollutants in Europe are heavy metals which affect 35% of the arable soils [1]. The increasing contamination in the environment necessitates the exact assessment of metal accumulation in plants which are at the base of the food chain. Mosses are commonly used as biomonitors for the presence of heavy metals in soil, air, and water, and they also make suitable models for evaluating metal adsorption [2, 3]. The studies were performed on mosses grown in controlled conditions on solid agar contaminated with heavy metals. We analysed the reaction of a tolerant moss, *Pohlia drummondii* to different heavy metals, $CuCl_2$, $MnCl_2$, $FeCl_2$, and *Sb-acetate*, alone and in combinations [4, 5]. We assessed growth and physiological parameters over a period of several weeks. First results show that even a tolerant moss like *P. drummondii* is seriously affected by $CuCl_2$.

Figure 1: Habitus of the moss, *Pohlia drummondii*.

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P19 Characterization of the antioxidant activity of *Helichrysum arenarium* leaf extract using EPR spectroscopy

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Helichrysum arenarium (L.) Moench, commonly known as dwarf everlast or immortelle, is a fascinating plant that has been cherished for centuries due to its various medicinal and aromatic properties. One of the most notable features of *Helichrysum arenarium* is its ability to thrive in harsh, rocky environments with poor soil quality and intense sunlight. The leaves of *Helichrysum arenarium* exhibit remarkable antioxidative activity, making them valuable in various therapeutic applications and as a potential source of natural antioxidants. This antioxidative activity is primarily attributed to the presence of bioactive compounds such as flavonoids, phenolic acids, and other polyphenols found within the leaves [1]. However, there's no information available regarding the utilization of Electron Paramagnetic Resonance (EPR) spectroscopy in examinations of the antioxidant activity of *H. arenarium*. In this study, we utilized supercritical CO_2 extraction to obtain a polyphenol-rich extract from *H. arenarium* leaves. Supercritical CO_2 extraction, recognized as a green technology, replaces traditional solvent-based methods, allowing for the isolation of plant compounds in their purest form without sample degradation [2]. To assess the extract's ability to scavenge free radical species (DPPH and hydroxyl radicals), EPR spectroscopy was employed due to its high sensitivity and specificity. Additionally, the spin-trapping technique was utilized to detect activity against short-lived hydroxyl radicals.

The results indicate that the extract obtained from *H. arenarium* leaves is an effective scavenger of free radicals. It demonstrated impressive specificity in neutralizing hydroxyl radicals (72.1%, compared to 26.6% for DPPH). This is particularly significant since hydroxyl radicals are known as the most reactive oxygen species with biological relevance and involvement in numerous pathologies. These findings underscore the potential therapeutic value of pomegranate peel in addressing oxidative stress-related medical conditions. Moreover, they prompt further exploration into its mechanism of action and potential use in pharmaceutical or nutraceutical products. Incorporating these leaves or their extracts into dietary supplements, skincare products, or functional foods may help bolster antioxidant defenses, promote health, and mitigate the effects of oxidative stress-related disorders.

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P20 Classification of THC and CBD contents in cannabis plants by Raman spectroscopy of fresh leaves

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We present an innovative approach to the in-vivo classification of THC/CBD-rich cannabis plants through a novel miniaturization strategy for Raman spectroscopy. By developing compact Raman spectrometers that utilize patented technology based on cheap non-stabilized laser diodes, densely-packed optics, and small pixel size sensors without cooling, the study achieves performance comparable to more expensive, research-grade systems. This miniaturization is facilitated by real-time calibration of Raman shift and intensity using a built-in reference channel. The miniRaman spectrometer effectively records high-quality Raman spectra of fresh cannabis and its products without the need for sample or environment preparation, identifying characteristic peaks of primary phytocannabinoids such as THC, CBD, and CGB and avoiding time-consuming HPLC analysis.

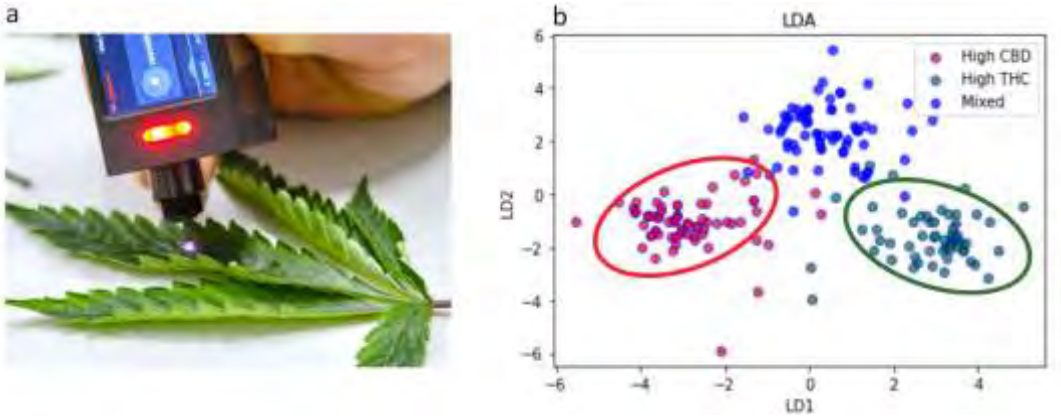

Figure 1: Measurement procedure (a) and results of the classification of THC and CBD in cannabis leaves.

Through spectral deconvolution and chemometrics, quantitative analysis becomes possible, significantly reducing the influence of fluorescence for more precise analysis [1]. The application of this technology allows for the identification of THC or CBD-rich plants with a high accuracy rate of 92%, demonstrating the potential of Raman spectroscopy aided by machine learning for rapid, non-destructive cannabis classification.

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P21 Scanning through Plant Surfaces: 3D-Raman Imaging of epidermal Peels of *Arabidopsis arenosa*

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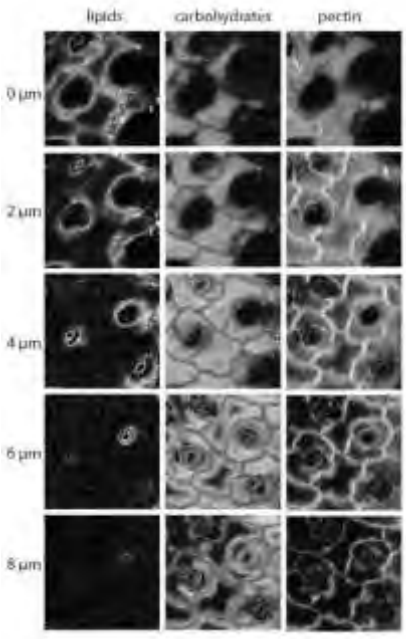
Raman microscopy is a non-destructive technique widely employed for molecular and structural analysis of biological samples [1]. Its ability to provide detailed chemical (spectral) information on the micro-scale and in context with anatomical structures makes it particularly valuable in plant sciences [2]. However, the complex three-dimensional structure of plant surfaces, as well as the presence of diverse molecular components, often complicates spectra acquisition. These challenges can lead to increased noise, lower spectral quality, and difficulty in interpreting the data, particularly when trying to analyse intricate features like thin layers or microstructures like stomata. To overcome these limitations, a novel combination of sample preparation (peeled plant surface) and 3D-Raman stacking has been applied to the leaves of the alpine plant *Arabidopsis arenosa*. By generating Raman images at different depth, we were able to visualize the spatial organisation of the leaf surface throughout the epidermis.



The distribution of lipids, cellulose, and pectin was mapped by band integration from the top of the surface (0µm) through the epidermal layer (Figure 1). Lipids were visualized on top (0-2µm) and on the border of the stomata, whereas carbohydrates reflect the epidermal cells. In the space between the cells high pectin signal was observed as well as pointwise lipids (Figure 1). True component analysis gives additionally more detailed insights, as e.g that the lipids on the stomatal aperture were unsaturated. By elucidating such a comprehensive view of the leaf epidermis and cuticle (including hairs and stomata), this 3D-Raman imaging approach will become an important *in-situ* tool to investigate plant surfaces and their complex interactions with the environment.

Figure 1: 3D-Raman images (100 µm x 100 µm x 8µm) of a peeled surface layer of *Arabidopsis arenosa*, visualizing lipids, carbohydrates and pectin by band integration from the upper surface (0µm) through the epidermal cells (2 µm, 4 µm, 6 µm, 8µm).

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P22 Raman microscopic imaging of pea seed hilum chemical composition

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On a mature seed, the hilum is visible as a scar or mark at the position formerly attached to the ovary wall or the funiculus. During growth and maturation the hilum plays a pivotal role in the transfer of nutrients and water from the parent plant to the seed and upon detachment it acts as a water gateway to reactivate the seed's metabolic processes and initiate germination. Some pea genotypes have a dark pigmented hilum and in our previous work we have identified that polyphenol oxidase gene activity is involved in the oxidation of phenolics in dark coloured hila [1]. However, the chemical composition of the dark pigmented hilum in comparison to more pale hilum remains underexplored.

To reveal the chemical composition of wild (*Pisum elatius*, JI1794) and domesticated (*Pisum sativum* cv. Trendy, ATC7025) on the microscale, we used Confocal Raman microscopy (Alpha300RA, WITec, Germany) equipped with a 532 nm laser and a high-numerical aperture (NA) objective (Nikon, 100x NA=1.4). The spatial distribution of key compounds (lignin, cellulose, pectin, lipids and aromatic compounds) was visualized on transversal cryosections of the hilum (8 - 14 μm) and the corresponding extracted average spectra were investigated in detail. The dark pigmented hilum spectra of the wildtype genotype had a higher fluorescence background with broad aromatic contributions around 1570 cm^{-1} and 1340 cm^{-1}, pointing towards quinones (melanin). The hilum spectra of the domesticated genotypes gave multiple aromatic and more sharp Raman bands (e.g. at 1581 cm^{-1}), but also in the lower wavenumber region around 600 cm^{-1}, pointing to flavonoid structures (e.g. Tricetin, Quercetin). The high 1622 cm^{-1} band together with the 850/830 cm^{-1} doublet and the 645 cm^{-1} band might come from tyrosine [2]. Tyrosinase, a copper-containing polyphenol oxidase, can induce browning by the formation of melanin based on flavonoids (flavones and flavonols) [3].

The findings of this study offer valuable insights into the composition of the hilum and contribute to further research on the browning mechanism of pea seed hilum. Complementary techniques, such as MALDI imaging and LC/MS, will be employed to enhance the understanding of this process.

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