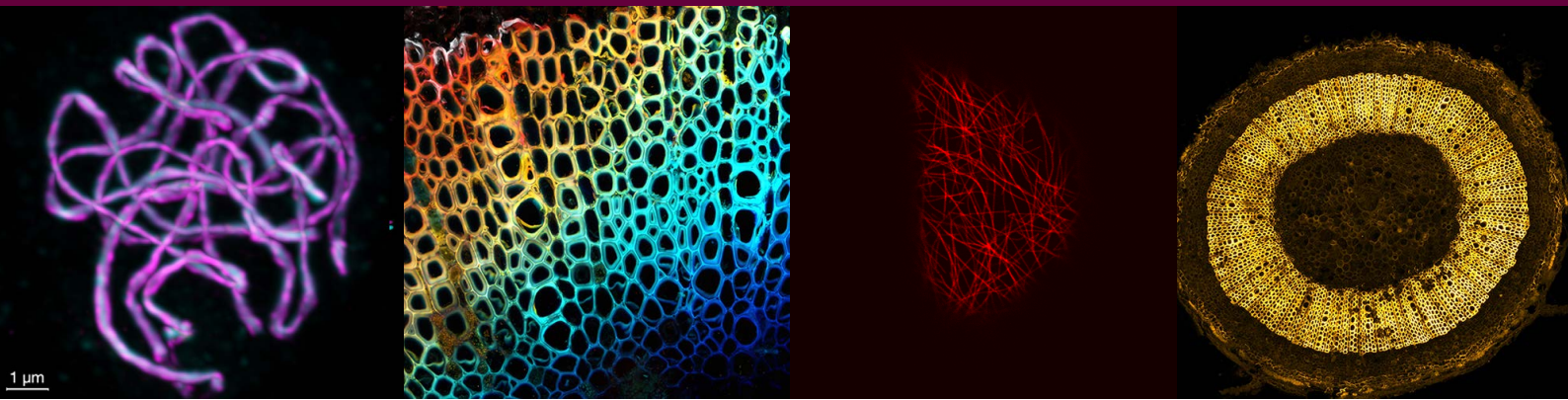


SPS Summer School 2025



**« Advanced Plant Imaging – API:
From super-resolution to fluorescence
lifetime imaging microscopy »**

**June 29th - July 4th, 2025
Versailles, France**

Participant's guide

Contents

Sponsors and partners	2
Venue / Hotel	3
Planning at a glance	4
Practical sessions	5
Program	6
Abstracts of the participants	12
Speakers and organizers	31

Sponsors and partners



Venue

Institute Jean-Pierre Bourgin for Plant Sciences
INRAE Centre Île-de-France Versailles-Saclay
Route de St-Cyr (RD 10)
78000 Versailles

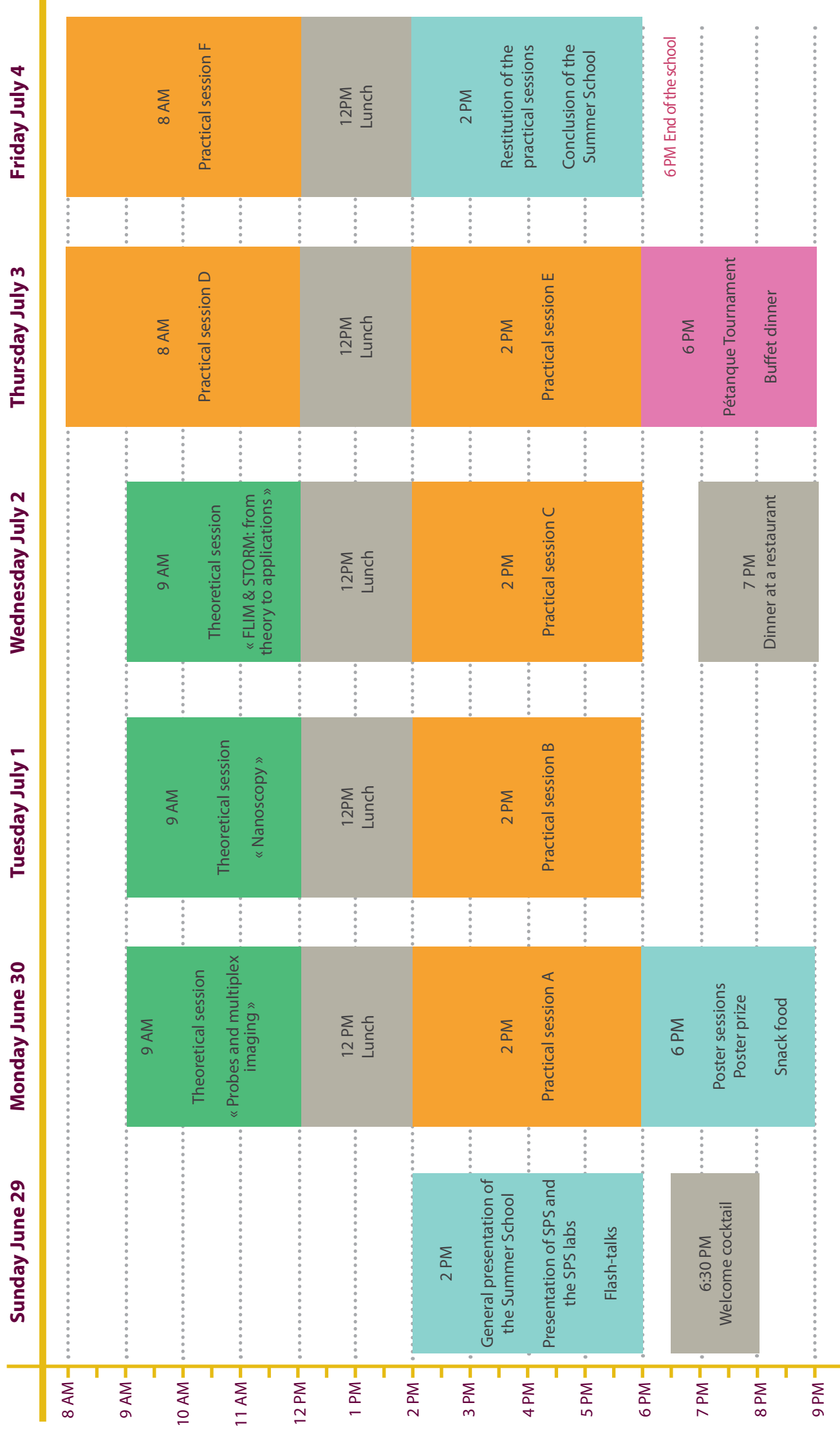
Hôtel

Hôtel Le Bout du Parc
92-94 allée du Cordon Boisé
78000 Versailles
<https://www.hotel-boutduparc.com/>

Summer School e-mail address

SPS-Summer-School@inrae.fr

Planning at a glance



Practical sessions

Workshop 1/ STED super-resolved microscopy

In this hands-on workshop, participants will compare conventional confocal microscopy with Stimulated Emission Depletion (STED) super-resolution imaging. They will learn how to optimize fluorophores and manage phototoxicity while exploring advanced super-resolution techniques, with a focus on Gated STED and TauSTED. Using Fluorescence Lifetime Imaging (FLIM), these modalities enable fast, high-resolution acquisitions while improving the signal-to-noise ratio. The workshop will feature immunolocalization on: (1) protein along the chromosome axes and the synaptonemal complex in 3D meiosis-stage cells, and (2) microtubules in *Arabidopsis* root tips.

Workshop 2 / FRET-FLIM imaging in plant science and Workshop 6 / FLIM analysis

In this workshop, we will discuss the experimental and theoretical foundations of time-domain FLIM. Participants will have the opportunity to work with two FLIM modalities: visible-range white laser excitation FLIM (up to four channels) and two-photon spectral FLIM (sFLIM) with 16 detection channels. We will present the differences and advantages of both systems and compare them using the same sample—a GFP-RFP-based FRET pair localized in *Arabidopsis* roots. During the analysis session, we will introduce time-domain, phasor-based, and pattern-mapping approaches for FRET-FLIM analysis.

Workshop 3 / STORM super-resolved microscopy

During the practical session, we will demonstrate multicolor 3D dSTORM acquisition on plant tissue sections. We will discuss the challenges of single-molecule imaging in highly autofluorescent plant tissues, including immunohistochemistry protocols for sample preparation to enable efficient dSTORM imaging. Considerations for multicolor imaging—such as the impact of dye choice on resolution—will also be addressed. Data analysis and visualization of multicolor 3D dSTORM will be introduced using the MATLAB-based Grafeo program and Abbelight NEO software. We will cover the fundamentals of spatial statistics, including Voronoi diagram-based filtering, clustering using Delaunay triangulation, Ripley's function, and the pair correlation function.

Workshop 4 / Ratiometric approaches using biosensor of intracellular pH in *Arabidopsis thaliana*

In this hands-on workshop, we will perform ratiometric measurements of intracellular pH in *Arabidopsis thaliana*. Participants will be introduced to the fundamental techniques for processing raw imaging data. We will also present and discuss simple scripts to automate routine calculations, enabling efficient and reproducible analysis of pH dynamics within plant cells.

Workshop 5 / Traditional confocal microscopy analysis combined with deconvolution on LEICA SP8 as a confocal microscopy control

We will mainly image microtubules on living or fixed samples, with different modalities and will apply deconvolution treatments to the images obtained as well as a set of images shared between the different workshops (wall, meiotic chromosomes and microtubules). In this way, we will be able to compare these data with the results of the different microscopy modalities seen during the week, and decide which one to use for which application.

Group 1	Group 2	Group 3
Angel CHAVEZ Martina ORVOSOVA Emma PASQUIER Isaac WOPEREIS	Rik FROELING Katarína HELDESOVÁ Marie ZILLIOX	Ignacy BONTER Katja FINK Jana KRUŽLICOVÁ

Group 4	Group 5	Group 6
Chen GAO Pavla NOVOTNÁ Konstantinos PANAGIOTOPOULOS	Valentina LEVAK Ira TRIVEDI Brian VUE	Rith Théa HAK João NEVES Vinaya RÖHRL

Program

Sunday June 29

2 PM – 6 PM: Welcome introduction
General presentation of the Summer School
Presentation of SPS and the SPS labs
Flash-talks of participants' research (2 to 3 Powerpoint slides, 5 min max.)

6:30 PM: Welcome cocktail

Monday June 30

9 AM – 10 AM: Session « Probes and multiplex imaging » (Building 19)

Arnaud Gautier (Sorbonne University, Paris, France)

“Illuminating and controlling cell function with chemogenetics” - Cells and organisms are complex machines driven by a set of dynamic biological events tightly orchestrated in space and time. The comprehensive molecular understanding of their inner workings requires acute molecular tools to observe and modulate the key triggers and cell signaling events. To study the molecular mechanisms that govern cells and organisms over different scales in time and space, we combine molecular chemistry with protein engineering and genetic tools to develop novel approaches for the quantitative imaging and acute modulation of individual small molecules, proteins, organelles or cells. Made of organic synthetic molecules coupled to genetic tags, these systems combine the advantage of synthetic molecules with the targeting selectivity of genetically encoded tags, challenging the paradigm of fully genetically encoded systems. During this talk, I will present how these systems can be used for imaging, sensing and controlling cell biochemistry with high spatial and temporal resolution.

1. Plamont et al. *Proc. Natl. Acad. Sci.* 113 (3), 497–502 (2016)
2. Tebo & Gautier. *Nature Communications* 10, 2822 (2019)
3. Li et al. *Angewandte Chemie International Edition* 59, 17917–17923 (2020)
4. Tebo et al. *Nature Chemical Biology* 17, 30–38 (2021)
5. Benaissa et al. *Nature Communications* 12, 6989 (2021)
6. Bottone et al. *Nature Methods* 20, 1553–1562 (2023)
7. El Haji et al. *Nature Communications* 16, 2594 (2025)

10 AM – 10:30 AM: Coffee break (Building 19)

10:30 AM – 12 PM: Session « Probes and multiplex imaging » (Building 19)

12 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM – 6 PM: Practical session A (Building 14)

Workshop 1 STED	Workshop 2 FRET-FLIM	Workshop 3 STORM	Workshop 4 Ratiometry	Workshop 5 Deconvolution	Workshop 6 FRET-FLIM analysis
Group 1	Group 6	Group 2	Group 3	Group 4	Group 5

6 PM – 9 PM: Poster session (Building 43) with snack food

Tuesday July 1

9 AM – 10 AM: Session « Nanoscopy » (Building 19)

Julien Gronnier (Technical University of Munich, Germany)

“Visualizing single molecule at work in living cells” - *Spatial partitioning is a fundamental property of biological that orchestrates cellular activities in space and time. Plasma membranes (PMs) are composed of billions of individual molecules arranged as an asymmetric lipid bilayer including integral and associated proteins. Over the past few decades, the study of fundamental aspects of development, reproduction, and interaction with microorganisms have revealed a common theme: the dynamic organization of the PM in various membrane domains¹. Here, I will present single-molecule localization microscopy (SMLM) approaches, in particular single-particle tracking photo-activated localization microscopy (spt-PALM)², and photochromic reversion³ to gain quantitative information into molecule motion and organization^{4,5}. Additionally, I will discuss the implementation of machine learning approaches to infer molecule functional state.*

1. Jaillais, Y., Bayer, E., Bergmann, D.C., Botella, M.A., Boulté, Y., Bozkurt, T.O., Caillaud, M.C., Germain, V., Grossmann, G., Heilmann, I., et al. (2024). Guidelines for naming and studying plasma membrane domains in plants. *Nature Plants* 2024 10:8 10, 1172-1183. 10.1038/s41477-024-01742-8.
2. Bayle, V., Fiche, J.B., Burny, C., Platre, M.P., Nollmann, M., Martinière, A., and Jaillais, Y. (2021). Single-particle tracking photoactivated localization microscopy of membrane proteins in living plant tissues. *Nature Protocols* 2021 16:3 16, 1600-1628. 10.1038/s41596-020-00471-4.
3. Arx, M.v., Xhelilaj, K., Schulz, P., Oven-Krockhaus, S.z., and Gronnier, J. (2024). Photochromic reversion enables long-term tracking of single molecules in living plants. *bioRxiv*, 2024.2004.2010.585335-582024.585304.585310.585335. 10.1101/2024.04.10.585335.
4. Levot, F., Hosy, E., Kechkar, A., Butler, C., Beghin, A., Choquet, D., and Sibarita, J.-B. (2015). SR-Tesseler: a method to segment and quantify localization-based super-resolution microscopy data. *Nature methods* 12, 1065-1071. 10.1038/nmeth.3579.
5. Wallis, T.P., Jiang, A., Young, K., Hou, H., Kudo, K., McCann, A.J., Durisic, N., Joensuu, M., Oelz, D., Nguyen, H., et al. (2023). Super-resolved trajectory-derived nanoclustering analysis using spatiotemporal indexing. *Nature Communications* 2023 14:1 14, 1-16. 10.1038/s41467-023-38866-y.

10 AM – 10:30 AM: Coffee break (Building 19)

10:30 AM – 11:30 AM: Session « Nanoscopy » (Building 19)

Eric Hosy (Interdisciplinary Institute for NeuroScience, Bordeaux, France)

“Super-resolution microscopy, from neuroscience to plant biology” - *Early 21st-century, single-molecule tracking techniques, such as quantum dots, revealed the dynamics of proteins at the cell surface. This was followed by the revolution of super-resolution imaging techniques—whether based on single-molecule localization or ensemble microscopy—emerging between 2006 and 2010. These advances provided unprecedented insight into both the diffusion properties and the nanoscopic organization of proteins. Through the presentation, we will explore how these techniques have conceptually transformed our understanding of neuronal and plant physiology with a range of examples.*

11:30 AM – 12 PM: Session « Nanoscopy » (Building 19)

Discussion

12 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM – 6 PM: Practical session B (Building 14)

Workshop 1 STED	Workshop 2 FRET-FLIM	Workshop 3 STORM	Workshop 4 Ratiometry	Workshop 5 Deconvolution	Workshop 6 FRET-FLIM analysis
Group 6	Group 5	Group 1	Group 2	Group 3	Group 4

Dinner not included in the Summer School

Wednesday July 2

9 AM – 10 AM: Session « FLIM & STORM: from theory to applications » (Building 19)

Joachim Goedhart (University of Amsterdam, The Netherlands)

“Engineering genetically encoded fluorescent probes for fluorescence lifetime imaging microscopy” - Fluorescence Lifetime Imaging Microscopy (FLIM) is a microscopy approach that measures the fluorescence lifetime of fluorophores. Since lifetimes are absolute measures, this imaging strategy delivers robust and quantitative information. This talk will give an overview of how we use FLIM, highlighting our efforts in (i) the engineering of fluorescent proteins with high quantum yield, (ii) the unmixing of fluorescent proteins based on lifetime, (iii) FRET imaging, and (iv) the development of single fluorescent protein biosensors with lifetime contrast.

10 AM – 10:30 AM: Coffee break (Building 19)

10:30 AM – 11:30 AM: Session « FLIM & STORM: from theory to applications » (Building 19)

Kalina Haas (Institute Jean-Pierre Bourgin for Plant Sciences, France)

“Fast, Focused, and Growing: New Tools to Uncover Plant Cell Expansion” - Plant growth is a multiscale process. At the subcellular level, it involves remodeling and expansion of the cell wall; however, the nature of these changes and their rapid regulation remain poorly understood. To advance our understanding of growth beyond long-term hormonal and genetic regulation, scale-appropriate methodologies are needed. In this presentation, I will discuss how super-resolution microscopy, rapid FLIM, and optogenetics can enhance our understanding of plant growth.

11:30 AM – 12 PM: Session « FLIM & STORM: from theory to applications » (Building 19)

Discussion

12 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM – 6 PM: Practical session C (Building 14)

Workshop 1 STED	Workshop 2 FRET-FLIM	Workshop 3 STORM	Workshop 4 Ratiometry	Workshop 5 Deconvolution	Workshop 6 FRET-FLIM analysis
Group 5	Group 4	Group 6	Group 1	Group 2	Group 3

7:30 PM – 9 PM: Dinner at the restaurant « Au chien qui fume », 72 rue de la Paroisse, 78000 Versailles

Thursday July 3

8 PM – 12 PM: Practical session D (Building 14)

Workshop 1 STED	Workshop 2 FRET-FLIM	Workshop 3 STORM	Workshop 4 Ratiometry	Workshop 5 Deconvolution	Workshop 6 FRET-FLIM analysis
Group 4	Group 3	Group 5	Group 6	Group 1	Group 2

12 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM – 6 PM: Practical session E (Building 14)

Workshop 1 STED	Workshop 2 FRET-FLIM	Workshop 3 STORM	Workshop 4 Ratiometry	Workshop 5 Deconvolution	Workshop 6 FRET-FLIM analysis
Group 3	Group 2	Group 4	Group 5	Group 6	Group 1

6 PM – 9 PM: Buffet dinner and Pétanque tournament (veranda of the INRAE cafeteria)

Friday July 4

Attention:
The rooms have to be vacated after breakfast (check-out). Make sure your things are packed and ready on time.

8 PM – 12 PM: Practical session F (Building 14)

Workshop 1 STED	Workshop 2 FRET-FLIM	Workshop 3 STORM	Workshop 4 Ratiometry	Workshop 5 Deconvolution	Workshop 6 FRET-FLIM analysis
Group 2	Group 1	Group 3	Group 4	Group 5	Group 6

12 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM – 6 PM: Restitution of the practical sessions and conclusion of the Summer School (Building 19)

6 PM: End of the Summer School

Abstracts of the participants

Mapping novel domains in the *Marchantia* meristem

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Plant development is characterized by cell proliferation and differentiation occurring within meristems. The gametophytic meristem of *Marchantia polymorpha*, a liverwort, differs in its cellular architecture and genetic regulation from shoot and root apical meristems of flowering plants. With approaches such as optical clearing of plant tissues we can now image all layers of the *Marchantia* meristem to reveal its structure and domains, while preserving nuclear and membrane localized fluorescent protein fusions. In this work we demonstrate that apical cells represent a sub-population of the meristem and represent a smaller domain compared to the YUCCA expressing cells - an auxin synthesis enzyme, previously used to mark meristems in *Marchantia*. Additionally, the stem cells are characterized by the expression of genes involved in the cytokinin biosynthesis pathway and a low auxin responsivity. We also show that PIN proteins are polarly localized inside the *Marchantia* meristem and might participate in meristem branching and maintaining the auxin concentration gradient around the meristem.

Loss of Glycosylated Sterols Alters Plasma Membrane Organization and Intercellular Communication in Arabidopsis

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Glycosylated sterols (GS), including steryl glycosides and acyl steryl glycosides, are sterol derivatives with a sugar moiety attached to the sterol core. Similar to free sterols, GS are proposed to localize within plasma membrane nanodomains, where they contribute to plant adaptation to biotic and abiotic stresses and play a key role in development. However, how these glycosylated lipids modulate membrane biophysical properties and their downstream effects on cellular processes remains poorly understood. To address this, we investigate how the absence of GS affects plasma membrane organization, protein-protein interactions, and intercellular communication. Using Arabidopsis GS-deficient mutants, we combine live-cell imaging to assess changes in membrane tension with proteomic and physiological assays to determine how these alterations impact protein interactions and overall plant physiology.

Our preliminary findings indicate that GS deficiency leads to the accumulation of the nanodomain marker REM1-3 and several plasmodesmata-associated proteins. These changes correlate with defects in cell-to-cell communication, suggesting that GS play a crucial role in maintaining membrane domain integrity and intercellular signaling.

Endophyte-Induced Immune Modulation in Potato: Real-Time Imaging of Calcium and Hormonal Signaling During Biotic Stress

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Plants rely on complex signaling networks to defend themselves against pathogens, with interactions between microbial communities playing a crucial role in shaping immune responses. Our study focuses on understanding how endophytic bacteria, such as *Bacillus subtilis* and *Paenibacillus sp.*, enhance potato immune response against pathogens like potato virus Y (PVY). Although the precise mechanisms remain unclear, these endophytes are known to modulate plant immunity, potentially by activating specific hormonal signaling pathways. By monitoring calcium fluxes and hormone-related signals (JA, ET, and SA) in plant cells, we aim to clarify the link between endophyte-induced immune effects and plant signal transduction mechanisms. Using transgenic potato plants expressing fluorescent sensors for stress-responsive hormones and calcium, we will assess immune responses in both leaves and roots under biotic stress conditions.

We will monitor these signals in real time in intact plant tissues, by employing live confocal microscopy at cellular level. We will adjust imaging protocols to enhance signal stability over extended periods, allowing us to track intracellular calcium dynamics and expression of hormone-dependent genes with high spatial and temporal precision.

This approach will help uncover spatial and temporal dynamics of calcium signaling and hormonal interactions with minimal disturbance of plant physiology. Ultimately, our findings will contribute to a deeper understanding of plant immune responses and provide new insights into the molecular basis of endophyte-mediated defense enhancement, with potential applications in sustainable crop protection strategies.

Mechanical Control of Plant Tissue Culture

Rik Froeling

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Growing tissues or entire plants from protoplasts or calli is a crucial step in plant breeding and cultivation. For many species, inducing the productive development of a pluripotent precursor forms a bottleneck in R&D workflows. In the animal field, emerging knowledge and technologies on the mechanical control of tissue development have ushered in a renaissance in mammalian tissue culture. Surprisingly, the mechanical dimension of tissue culture has been overlooked for plants, even though also plant morphogenesis is under mechanical control. This is largely due to a lack of tools to precisely apply and measure mechanical stresses in plant tissues. Yet, the ability to mechanically steer the ex-vivo culture of plant tissues holds the potential to improve commercial workflows and to form a basis for the fundamental understanding of how mechanics steer development. My research develops technologies and knowledge to gain mechanical control over plant tissue culture. We are making synthetic culture matrices, optimized to provide developing plant cells with a native-like mechanical context, and microfluidic systems for long-term and high-quality live-cell imaging. In tandem, we are engineering bespoke culture devices in which developing tissues can be subjected to mechanical stimuli during their growth. With this new technology, we will explore the optimal conditions to improve the efficiency of tissue generation from protoplasts, and study how mechanical stimuli alter cell physiology and developmental trajectories. Finally, we will explore the translatability of this technology to ex-vivo culture of crops and ornamental plants.

Revealing Ultrastructure of Polysaccharides' Network during Secondary Cell Wall Formation with 3D-dSTORM

Chen Gao

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Wood constitutes renewable biomass for sustainable energy and high-quality materials. Molecular understanding of wood formation would provide a new avenue for designing bio-inspired materials with remarkable strength and rigidity. A critical step in the wood forming process involves the cessation of primary wall synthesis and initiation of secondary cell wall synthesis. This process is poorly understood, mostly because the wood forming cells are located deep inside plant tissues and thus are difficult to observe. Here, we seek to elucidate the biomolecular and structural cell wall changes that take place during wood formation.

We will visualize wood-contributing carbohydrates dynamics during the transition from primary to secondary wall synthesis. The primary walls are typically composed of cellulose, hemicelluloses (e.g. xyloglucans and mannans) and pectins (homogalacturonan and rhamnogalacturonan I & II). The secondary walls are composed of cellulose, hemicelluloses (xylans) and the heterophenolic polymer lignin. We will activate secondary cell wall formation by transcriptional reprogramming of cells that are amenable for high resolution imaging to monitor wood formation in near-real time. Furthermore, we will use custom-designed super-resolution microscopy (3D-dSTORM) and cell wall probes to dissect cell wall changes. This study will provide a basis for future development of biotechnological applications of wood formation.

Key words: Wood formation, secondary cell wall, 3D-dSTORM, cell wall probes

Cell cycle and Cytoskeleton crosstalks for mitotic entry in plant cells

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In plants, morphogenesis relies on a complex coordination of cell division and elongation. Mitosis is regulated temporally by the cell cycle machinery, composed of cyclin/Cyclin Dependent Kinase (CDK) complexes, and spatially by the cytoskeleton, especially the microtubule one. Microtubules are highly dynamic and can assemble into highly ordered structures that serves as scaffold for cellular processes during the cell cycle: cortical microtubules in interphase, preprophase band at the G2/M transition, spindle in metaphase/anaphase and phragmoplast in telophase. In particular, the preprophase band or PPB is a ring-like structure of cortical microtubules specific to land plants that is a faithful predictor of the future division plane. Interestingly, key cell cycle players such as CYCA1s or CDKA;1 localize at the PPB, suggesting a dialog between the cell cycle and the cytoskeleton. The nature of this dialog is poorly described, and whether cell cycle players are regulating the PPB, or are gathered there to modify specific targets/regulators is unknown. My thesis aims at better characterizing this dialog, using genetic and microscopy approaches in live/fixed root meristems of *Arabidopsis thaliana*. Focusing first on CYCA1;1 and CDKA;1, we are looking at their subcellular (co-) localizations with the TON1/TRM/PP2A complex, known to regulate cortical microtubules organization. The biological significance of CYCA1;1/CDKA;1 presence at the PPB is assessed through functional analysis of knockout/hypomorphic mutants (microtubules organization, division positioning ...). Promising results showed that CDKA;1 is recruited at the PPB shortly after its initiation and low CDKA;1 kinase activity is associated with defects in PPB condensation and division plane positioning.

Key words: Mitosis – Microtubules – Cyclin/CDK – Preprophase Band – Microscopy

Lys10: Novel probe capturing pectin dynamics in plant cell walls

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The plant cell wall is a dynamic structure that undergoes constant remodelling to accommodate growth, differentiation, and responses to the environment. Pectic homogalacturonan (HG) is one of the key cell wall polysaccharides, which plays a crucial role in regulating cell wall mechanical properties and cell-to-cell adhesion. Despite its importance, tracking HG spatiotemporal dynamics *in vivo* remains challenging as traditional immunolabeling has been proven largely ineffective for such applications. To overcome these challenges, we developed and characterized a novel oligocationic peptide probe, a decamer of L-Lysine (Lys10). It is designed to associate with negatively charged regions of HG. Specificity analysis using a dot blot assay confirmed Lys10's binding to pectin isolates; the strength of the binding was relative to the level of methyl-esterification. Using fluorescence microscopy, we demonstrated that Lys10 efficiently penetrates live plant tissues and labels cell walls throughout *Arabidopsis* root, making it suitable for live-cell real-time imaging. This approach helped us to visualize HG's dynamic changes and distribution during root hair elongation and possible hot spots of pectin methylesterase activity. Furthermore, molecular docking revealed a high-affinity interaction between Lys10 and de-esterified homogalacturonan ($KD = 6,04 \times 10^{-9}$ M), comparable to antibody-based recognition. These findings demonstrate the potential of peptide-based probes for high-specificity cell wall imaging. Encouraged by Lys10's performance, we continue to develop additional probes targeting various polysaccharides, further expanding the tool repertoire available for studying cell wall remodelling during plant development.

Meiotic Dynamics and the Evolution of Neo-Sex Chromosomes in *Rumex hastatulus*: Insights into Chromosome Pairing and Synapsis

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Meiosis is a fundamental process in sexually reproducing organisms, ensuring proper gamete formation through chromosome pairing, synapsis, and recombination. While autosomes typically undergo complete pairing, heteromorphic sex chromosomes pair only within the pseudoautosomal region (PAR). Despite advances in meiosis research, the mechanisms governing PAR establishment during prophase I and its 3D structure during synapsis remain largely unexplored, particularly in young sex chromosome systems, such as those in plants. The plant species *Rumex hastatulus* exists in two cytotypes, XY and XYY, the latter arising ~0.2 MYA via an autosomal translocation onto ancestral sex chromosomes. This event created a neo-sex chromosome system with a reduced chromosome number and the formation of an X-Y-Y trivalent during prophase I. In this regard, this emerging model offers a unique opportunity to investigate how chromosomal rearrangements shape key meiotic processes, including synapsis and recombination. To explore this, we developed an oligonucleotide barcode system targeting the PAR, the translocated autosomal region, and the ancestral XY regions. Additionally, we optimized a robust immunolabeling approach to visualize synapsis between autosomes and sex chromosomes. Using high-resolution Structured Illumination Microscopy (SIM), we analyzed 3D meiotic cells, marking synaptonemal complex elements and localizing class I crossovers. Our study provides the first detailed insights into chromosome pairing and synapsis in this system, contributing to a broader understanding of sex chromosome evolution in plants. We aim to elucidate how recombination suppression and chromosomal rearrangements influence synaptic behavior and bivalent formation in neo-sex chromosomes.

Use of genetically encoded biosensors reveals that jasmonic acid and salicylic acid interact to determine spatial regulation of gene expression response in leaf to herbivore and mechanical wounding

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Understanding molecular background of plant defence response is key in the development of resilient plants. We studied spatiotemporal dynamics of response to herbivore and mechanical wounding using genetically encoded biosensors in potato plants. We generated two potato sensor lines, JAZ degradation sensor and multicystatin transcriptional reporter, both involved in jasmonic acid (JA)-signalling. Using confocal microscopy, we followed sensors' signal in vivo in intact leaves still attached to the plants potted in soil. Thus, we studied response to herbivore and mechanical wounding. In both types of wounding, biosensors revealed concentric locally restricted responses on the wounded leaf. Interestingly, JA-dependent gene expression was inhibited in the cells close to the wound, while JAZ was degraded confluent from the wound outwards. In search of the mechanisms of this response, we showed that salicylic acid (SA) is accumulating in the cells close to the wound using SA reporter. Additionally, we introduced multicystatin sensor in SA-depleted NahG plants, which showed uniform spread of multicystatin expression and thus confirmed the role of SA in attenuation of JA response close to the wound. Altogether, our results reveal JA-SA crosstalk is involved also in response to wounding. Now, we wish to upgrade our understanding of the molecular background of this phenomenon by employing roGFP sensor to follow redox state in the cytoplasm and plastids in cells in which we observe SA-JA crosstalk. Preferably, biosensors would be followed in the same plant, as we showed that we can separate overlapping fluorescent protein signals using LAS X Dye separation tool.

Impact of abiotic stress on organelles' motility

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Climate changes pose a huge threat to crop development and plants, as sessile organisms, need to cope with adverse environment to thrive. These conditions affect plant growth and survival to the extent that they must adapt to overcome these situations. To do it, plants can alter their developmental pattern, by modulating cellular processes, protein trafficking, and activating stress-responsive signals. Despite the physiology and oxidative responses of plants to stress are quite well documented, with the effect on the cytoskeleton organization has not been characterized yet. Alterations in cytoskeleton organization can have an impact in the organelle motility, and therefore on the correct distribution of vesicles and cargos to their destination. Thus, the objective of this work is to evaluate the organelle motility and cytoskeleton distribution under abiotic stress conditions, to assess changes in vesicle distribution. For this, several *Arabidopsis* lines expressing cytoskeleton markers along with specific organelle markers were grown in different abiotic conditions and the organelle motility was evaluated using Laser Scan Confocal Microscopy, with video time-lapses. Preliminary results on ER bodies showed that their movements are affected under specific abiotic stress conditions, and in some extreme situations the organelles stopped completely when compared to control situations. Further studies will involve the study of the impact of this lack of movement in cargo sorting and correct delivery of specific proteins.

The evolution of dosage compensatory mechanism in plants: a complete affair

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To balance gene expression between the biological sexes, several organisms have evolved a mechanism how to achieve a transcriptional balance between separate sexes, termed dosage compensation. While in some species DC affects almost the entire X chromosomes (e.g. *Drosophila*, *Caenorhabditis* and mammals), it appears that many organisms exhibit local or incomplete dosage compensation. Though rapid advances in the understanding of DC and the emergence of new exciting plant and animal models, the mechanisms by which incomplete DC evolves remain unexplored. In *Silene latifolia*, a dioecious plant with strongly heteromorphic XY chromosomes, it has been suggested that the maternal X chromosome is compensated similarly to X chromosome mammals. The similar phenomenon was suggested by cytological observations, however, clear link between early cytogenetic evidence and recent transcriptomic studies is missing. In this work, we present an efficient approach to identify parental X chromosome inheritance and the Click method adopted for sex chromosomes in *Silene*. Using advanced confocal microscopy, we examined replication timing and DNA methylation patterns between autosomes and sex chromosomes, on metaphase chromosomes and chromosomes in relaxed state. We found that replication status between paternal and maternal X chromosomes are substantially different, supported by distinct DNA methylation patterns, supporting previous findings of the paternal X chromosome genomic imprinting. We explore future approaches to test dosage compensation in the context of different ploidy levels and discuss the broader implications for the study of sex chromosome dosage compensation mechanisms in plants.

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Spatial regulation of plant phosphate uptake in the presence and absence of arbuscular mycorrhizal symbiosis in rice

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The sustainability of phosphorus fertilisation is a major challenge for agriculture in the upcoming decades. One of the proposed solutions to reduce the use of inorganic phosphate (Pi) fertilisers is the introduction of arbuscular mycorrhizal symbiosis (AMS) into agricultural use. This conserved and widespread symbiosis greatly improves Pi uptake and nutrition of the plant host, transporting Pi acquired by its hyphae from the soil into the root cells in exchange for carbon compounds. A crucial global crop, rice, can also benefit from this interaction, but current agricultural techniques, intense fertilisation in particular, prevent it from establishing in the field. To breed and engineer rice varieties that will benefit from the AM symbiosis in the field, we need detailed knowledge of how Pi uptake is regulated. Recent breakthroughs discover signalling components that coordinate direct (non-symbiotic) and symbiotic Pi uptake, but little is known about the spatial nature of Pi uptake across the root system. This is relevant as the rice root system is composed of distinct root types with different morphology, expression profiles and susceptibility to fungal colonisation. We are testing the hypothesis that direct and symbiotic uptake predominantly use different root types in rice and that these contributions change under varied fertilisation. The experiments include a genetic dissection of the uptake pathways and the use of fluorescent reporters for the transporters involved in both the direct and symbiotic Pi uptake to correlate their spatial expression. In combination with chemical imaging by Nano SIMS, the results will functionally map Pi uptake across rice root types and fertilisation conditions. This knowledge can be used to guide breeding and engineering of rice lines for more sustainable Pi use.

The HSP90 molecular chaperone as a potential regulator of gibberellin transport and signaling

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Gibberellins (GA) are essential phytohormones that regulate numerous developmental processes including germination, hypocotyl and root elongation, leaf expansion, pollen maturation, flower induction and plant adaptation to environmental stresses. In land plants, GA signaling is mediated via GIBBERELLIN-INSENSITIVE DWARF1 (GID1) cytoplasmic receptor. GA-bound GID1 receptor interacts with DELLA proteins which are negative regulators of development, promoting their degradation. The manipulation of GA biosynthesis and signaling is a widespread agricultural practice to optimize plant growth and yield. However, little information exists on how plants regulate GA transport. The most well-known gibberellin transporters belong to the nitrate and peptide transporter family (NPF).

The HSP90 molecular chaperone plays a key role in auxin signaling by stabilizing the cytoplasmic TIR1 receptor and in auxin transport by modulating the distribution of the PIN1 transporter. Furthermore, HSP90 is also involved in brassinosteroid (BR) signaling pathway, physically interacting with BRI1 and BAK1 receptors. Additionally, HSP90 plays a role in GA signaling pathway through its interaction with DELLA proteins, but its involvement in GA transport remains largely unexplored.

We aim to investigate the role of the HSP90 chaperone in gibberellin transport and signaling as well as the molecular mechanisms controlling its function. Towards this goal, we have shown that HSP90 physically interacts with key components of the GA signaling and transport pathways. Furthermore, we have investigated the impact of HSP90 pharmacological or genetic depletion on GID1 receptor stability and NPF3.1 transporter levels, providing new insights into the complex regulation of GA transport and signaling in plants.

Bioengineered condensates to interact and manipulate membrane-bound organelles in cells

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The dynamic interactions between the location and timing of biochemical processes within organelles is crucial for determining cell fate and function. Advanced biochemical, genetic, imaging, and “omics” approaches have revealed many important features of the biogenesis and functions of cellular organelles. In particular, recent studies suggest that communication between membrane organelles, through the establishment of membrane contact sites (MCS), could play crucial roles for their functions¹. However much less is known about putative contact sites between membrane-bound and membrane-less organelles (also known as biomolecular condensates)². One key limitation comes from the biochemical complexity of condensates that make them difficult to study and manipulate in a cell context. To further advance in that direction, we are developing a novel methodology allowing the controlled assembly/disassembly in cells of bioengineered membrane-less organelles and that recapitulate some biophysical features of liquid-like condensates³. Our engineered condensates are based on protein scaffolds designed to undergo phase separation in cells in a reversible manner^{3,4}. We demonstrated that it is possible to specifically target the assembly of engineered condensates on the surface of lipid droplets⁵ but also lysosomes, making them suitable for addressing fundamental questions about the interplay between condensates and lysosome interactions: How condensate nucleation and growth are modulated when interacting with the lysosomes? Conversely, how lysosome spatiotemporal dynamics is impacted when interacting with condensates? This method is versatile and could, in principle, be applied for studying other cellular organelles.

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Investigating the functions of ESV1 and LESV in starch metabolism

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Starch is a major part of our calorie intake and serves as a storage carbohydrate in plants. Despite its importance, its formation into compact, semi-crystalline starch granules remains unclear. Research in *Arabidopsis thaliana* has identified two non-enzymatic proteins, ESV1 (EARLY STARVATION 1) and its homolog LESV (LIKE EARLY STARVATION 1), which have recently been found to play a role in crystallizing starch granules. Since then, several additional hypotheses have been proposed to explain the functions of these two proteins – which of them is true remains to be discovered. Both proteins share a unique and highly conserved tryptophan-rich β -sheet domain. Yet, their mutant phenotypes differ: *esv1* mutants exhaust their starch reserves too rapidly, whereas *lesv* mutants seem to resemble wild-type plants, at least under normal growth conditions. However, like *esv1*, LESV-overexpressing plants are starved of starch. By taking several approaches involving *Arabidopsis*, yeast, and *in vitro* systems, we hope to analyze the proteins' structural domains and identify functional differences. We also hope to utilize super-resolution imaging techniques to reveal the proteins' localization on the starch granules, whether on the surface or embedded, which would offer key functional information. Understanding the functions of ESV1 and LESV will not only give us a better global understanding of starch metabolism but may also serve as a valuable resource for improving starch yields of important crops.

Analysis of the consequences of COP1/SPA-photoreceptor complex formation in Arabidopsis using FRET-FLIM

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Light is crucial for plant development, influencing processes from germination to flowering. Photoreceptors like cryptochromes and phytochromes allow plants to detect different light wavelengths. These light-activated photoreceptors interact with the CONSTITUTIVE PHOTOMORPHOGENIC (COP1)/SUPPRESSOR OF PHYTOCHROME A-105 (SPA) complex, inhibiting its role as a repressor of light signaling and initiating photomorphogenesis. In the darkness, uninhibited COP1/SPA functions as an E3 ubiquitin ligase, degrading positive regulators of photomorphogenesis and suppressing certain aspects of development. Light-induced COP1/SPA inhibition by photoreceptors occurs through various mechanisms. One such mechanism is the disruption of COP1-SPA interaction via SPA1's direct binding to red light photoreceptors, the phytochromes. Phytochromes (PhyA, PhyB) change conformation under red light and move to the nucleus, where they interact with SPA1, leading to COP1/SPA inhibition. The exact mechanism still remains unclear, which is the focus of my research. I am investigating which domains of SPA1 interact with phytochromes and contribute to COP1-SPA1 disruption. Yeast three-hybrid assays show that the SPA1 N-terminus is crucial for PhyA-mediated red-light-enhanced dissociation of COP1-SPA1. To further confirm these competitive interactions between SPA1 deletion constructs, COP1, and phytochromes, I aim to test them using in vivo methods such as Split-Luciferase and FRET-FLIM. My future research will explore whether phytochromes induce conformational changes in SPA1 that disrupt the COP1-SPA1 complex using intramolecular FRET-FLIM analysis.

How manipulating sphingolipids impair protein mobility and nanoscale organization in the plasma membrane

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Beyond serving as a structural barrier, plasma membrane (PM) senses environmental changes and orchestrates signal transduction, enabling adaptive responses. The PM is a complex asymmetrical proteo-lipidic structure which consists of more than 2,100 proteins for the *Arabidopsis thaliana* PM and 800 lipids comprising mainly phospholipids, sphingolipids, and sterols (Bahammou *et al.*, 2023). Nanoscale organisation and dynamics of the PM is crucial to achieve its role in viral context (Jolivet *et al.*, 2023). While PM proteins have been extensively studied, the role of PM lipids remains a mystery. GIPCs (GlycosylInositol PhosphoCeramides), the major lipids of PM, act as receptor for NLP (Necrosis and ethylene-inducing peptides), thus forming pore within PM (Lenarčič *et al.*, 2017). GIPCs, play also a role during salt stress by detecting the monocation ion Na^+ , thus opening the calcium channel (Jiang *et al.*, 2019). Sphingolipids-altered mutant have been poorly studied during biotic stresses. In our lab, one sphingolipid-altered mutant displays a decrease of 80% the levels of GIPCs and a reciprocal increase of IPC levels. This sphingolipid-altered mutant is less sensible to several pathogens compared to the WT. Using a ROS biosensor, it showed higher intracellular ROS near PM, while extracellular ROS remain the same as the WT, leading to the hypothesis that the sphingolipid-altered mutant PM is more permeable to H_2O_2 . Cell to cell communication, using the photoconvertible DRONPA-s, was not impaired in this mutant background. In the frame of my PhD work, I will study proteins mobility and nanoscale organisation in sphingolipids-altered mutants background and GIPCs organisation and dynamics within PM.

Integration of mechanical cues into the cell cycle progression by SOSEKIs

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Over the past few decades, the importance of mechanical forces in regulating plant development and growth is widely accepted. If mechanical forces are pivotal in regulating plant growth, it is essential for plants to translate these mechanical cues into biochemical signals. In animals, proteins have been identified that sense mechanical cues and convert them into biochemical responses, while the equivalent mechanosensing proteins have yet to be discovered in plants. Identifying such mechanosensors is critical, as it would reveal how mechanical forces influence plant development. We propose that the SOSEKI protein family may serve as key players in this process, acting as sensors that convert mechanical signals into biochemical ones. In 2019, they were first described as polar proteins, which localize to a specific corner of the cell. Recently, we discovered that cytoplasm localized SOSEKI is essential for proper cell cycle progression. The only circumstance when we observe translocation of SOSEKIs from the plasma membrane to the cytoplasm is when the plants are perceiving mechanical stress. This suggests that SOSEKI may integrate mechanical cues into biochemical signals through this translocation, linking mechanical stress to the cell cycle. By imaging SOSEKI protein behavior under mechanical stress in combination with molecular modeling, we aim to uncover the first molecular framework for mechanotransduction in plants. These findings may offer new insights into the molecular mechanisms that underlie plant growth and development in response to mechanical stimuli.

Imaging brown algae to get insights on the determinants of various 3D growth strategies

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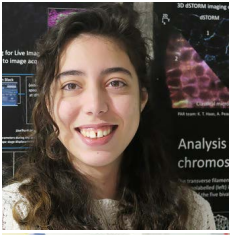
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Brown algae are photosynthetic multicellular organisms that have acquired complex multicellularity and have evolved independently of red and green algae (including land plants), animals, and fungi. Brown algal cells are surrounded by a semi-rigid cell wall, which prevents them from moving within the tissue, therefore their 3D growth relies on the location of the cell division plane. Our laboratory is investigating the 3D growth strategies of three brown algae species: *Sphacelaria rigidula*, *Fucus serratus* and *Saccharina latissima*, each employing a distinct 3D growth strategy at the onset of embryogenesis. *Sphacelaria* uses a strategy whereby the apical cell grows along a single axis and then sub-apical cells divide to grow in other dimensions. In contrast, *Fucus* initiates cell division without cell expansion, a process reminiscent of early embryo segmentation in metazoans.

I adapted *in vivo* time-lapse microscopy on confocal, two-photon and light-sheet and ad-hoc image analysis to these organisms. In particular, we developed the SBIS fluorescent membrane probe, which allows cell contours of growing embryos to be imaged in 3D for several days (Zilliox *et al.* 2025). These results will provide us with quantitative and qualitative data to build a comprehensive and dynamic map of the position and orientation of the cell division planes during algae's 3D growth, from which geometric cell division rules followed by these organisms will be inferred. Knowledge of these cell division rules, will enable a comparison of the mechanisms of construction of 3D tissues, such as embryos or meristems, between brown algae and plants.

Reference: M. Zilliox, M. Collot, B. Charrier: SBIS, a new orange fluorescent vital probe for the 4D imaging of brown algal cells. BioRxiv 2025 DOI: 10.1101/2025.02.02.636089.

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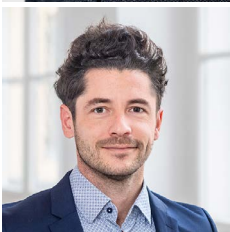
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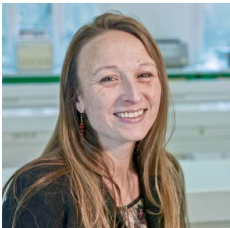
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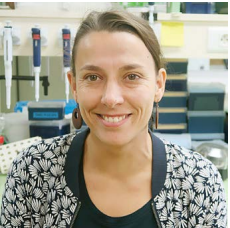
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