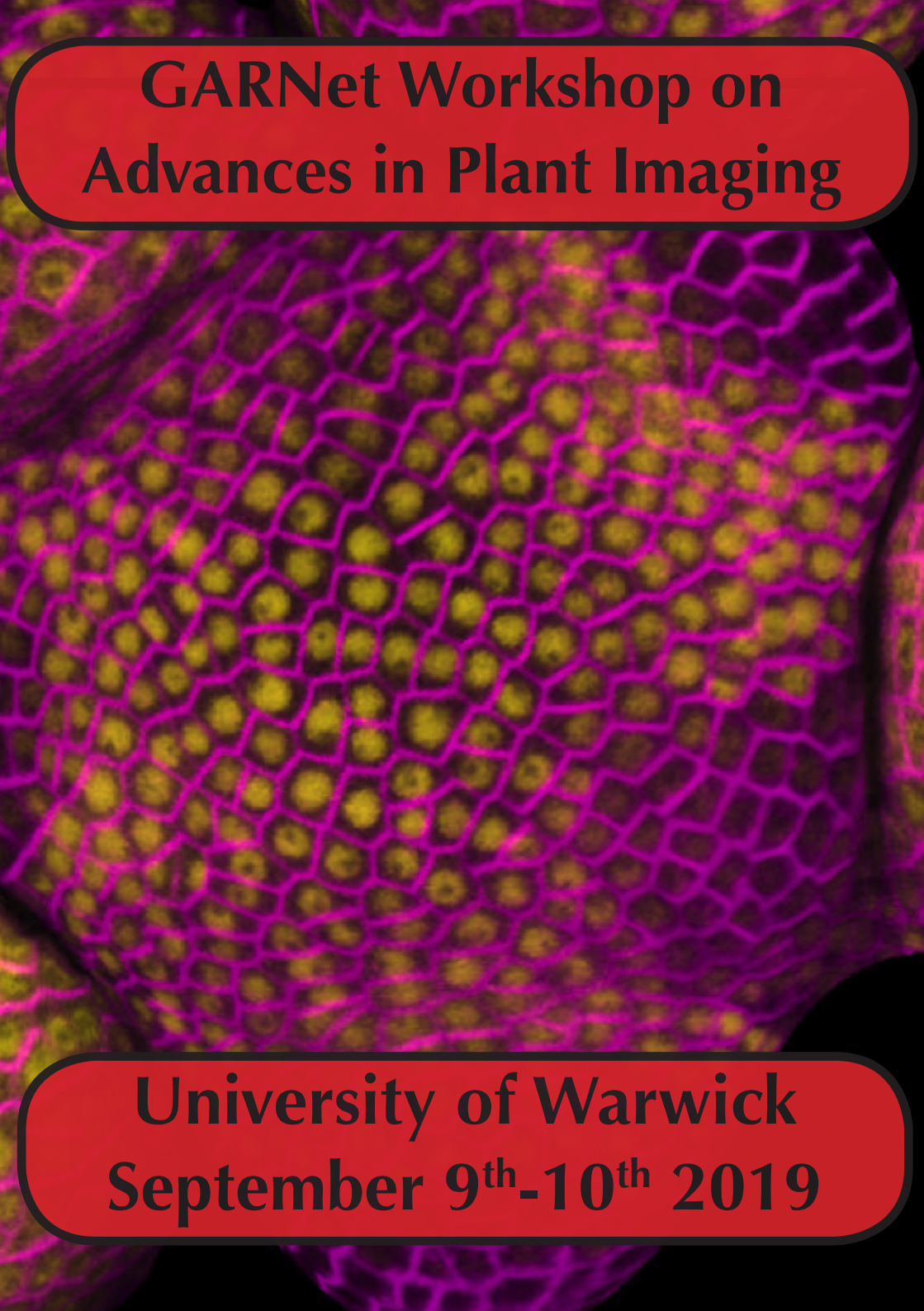
GARNet Workshop on Advances in Plant Imaging



University of Warwick
September 9th-10th 2019

GARNet Imaging

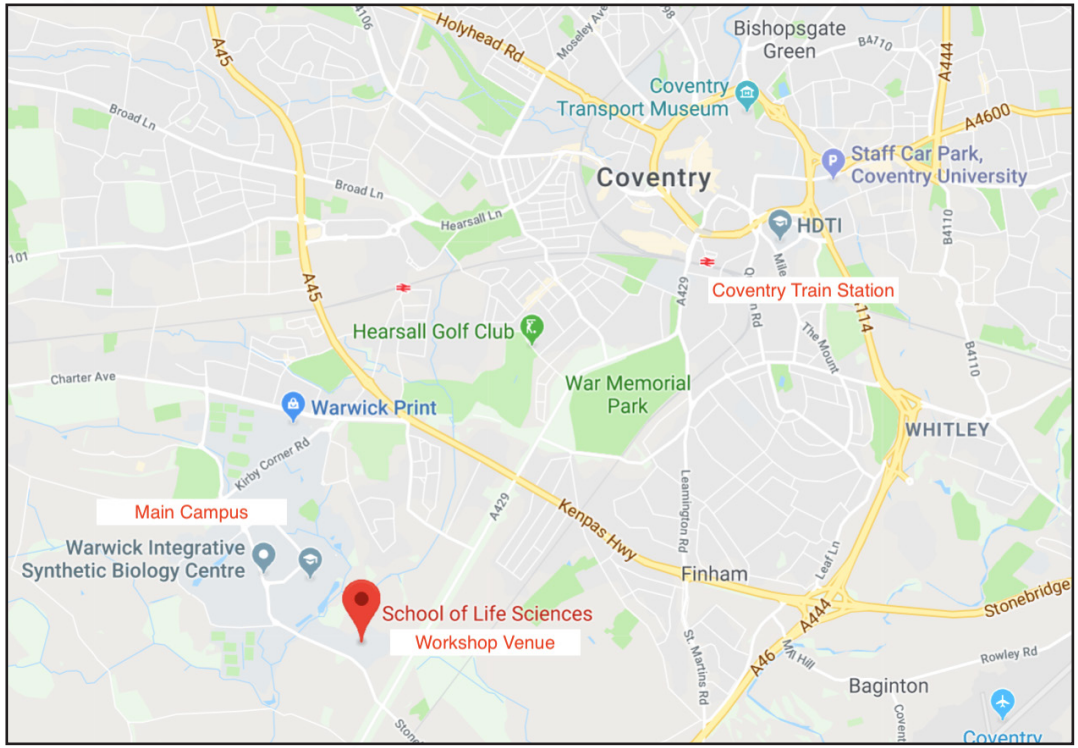
Meeting Information

For all questions about the workshop please contact:

Geraint Parry, GARNet Coordinator
> geraint@garnetcommunity.org.uk
> 07411967414

The meeting will take place at:

School of Life Sciences
University of Warwick Gibbet Hill Campus
Gibbet Hill Road
Coventry
CV4 7AL



Travel to the University of Warwick

The closest railway station to University of Warwick is Coventry.

From Coventry station please take the National Express 12X or the National Express 11 bus and alight at the Gibbet Hill Campus stop.

Other general travel information can be found on the University of Warwick website: https://warwick.ac.uk/about/visiting/directions/

The Gibbet Hill campus is a pleasant 15-20 minute walk from Main Campus through Tocil Wood.

If you need to drive to the meeting then please park on main campus. There is a frequent shuttle bus that runs between main campus and Gibbet Hill campus. Information about the shuttle bus can be found at the link below and the map is on page 6.

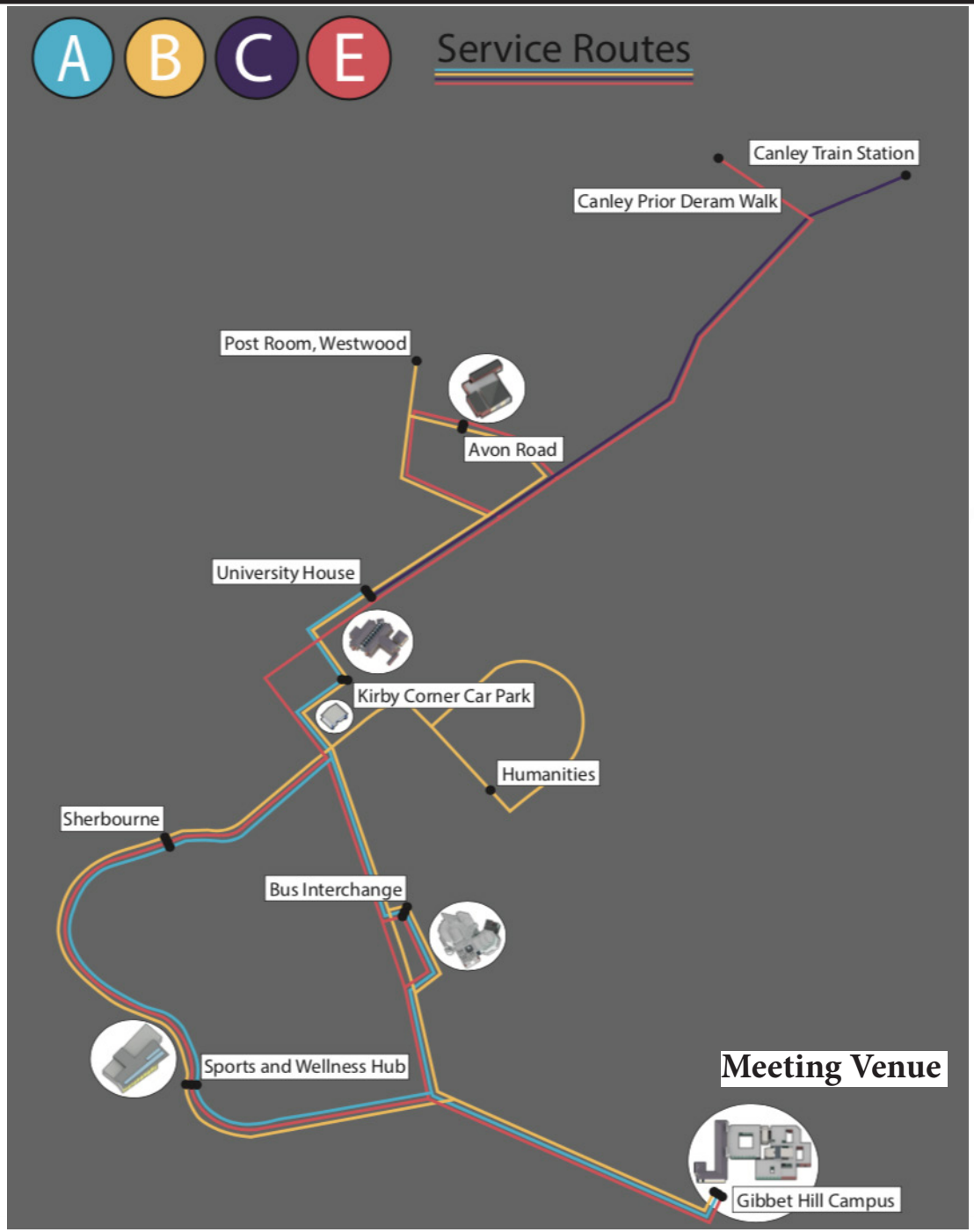
https://warwick.ac.uk/services/estates/transport/buses/shuttlebus/

Parking permits for Gibbet Hill Campus are in very short supply but if you have a medical or other important reason for requiring a pass then please contact Geraint Parry.

The School of Life Sciences at Gibbet Hill

Registration, catering and the poster sessions will take place in the Atrium area.

The talks all take place in the GLT1 lecture theatre



GARNet Imaging

A Date for the Diary!

The 32nd International Conference on Arabidopsis Research (ICAR2021) will hosted by GARNet and take place in Belfast.

Please put June 21st-25th 2021 into your diaries!

32ND INTERNATIONAL CONFERENCE ON ARABIDOPSIS RESEARCH

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Meeting Schedule

Monday September 9th 2019

2:00-3.15pm: *Registration with Coffee and Tea*

3.15-3.30pm: **Lorenzo Frigerio**
Welcome to the University of Warwick

Murray Grant
Introduction to Horizon Scanning session:
Delegates are encouraged to use post-it notes to indicate what they think will be important for the future of plant imaging

High Resolution Microscopy for Plant Cell Biology
(20minute talks with 10 minutes for questions)

3.30-4.00pm: **Joe McKenna (Oxford Brookes Bioimaging)**
Connecting the dots: Sub-diffraction limited imaging of plasma-membrane nanodomains

4.00-4.30pm: **Martin Goldberg (University of Durham)**
The structure of the nuclear pore complex and other intra-cellular molecular complexes in plant cells.

Talks selected from Abstracts

4.30-4.45pm: **Nataliia Gnyliukh (Institute of Science and Technology Austria)**
High Spatial and Temporal Resolution Imaging of Single Events of Clathrin Mediated Endocytosis in Live Plants

4.45-5.00pm: **Chris Morgan (John Innes Centre)**
Untangling the adaptive evolution of polyploid meiosis using super-resolution microscopy

5.00-5.45pm: **Flash talks: Two minutes, Two Slides.**
List of speakers on page 18

6pm-later: *Evening Entertainment: BBQ and socialising*

Tuesday September 10th 2019

Imaging across scales

9.00-9.30am: **Anis Meschichi (Swedish University of Agricultural sciences)**
Single Molecule RNA FISH: quantitative applications for gene expression analysis

9.30-10.00am: **Angharad Jones (Cardiff University)**
Tracking cells in complex tissues

10.00-10.30am: **Giovanni Sena (Imperial College)**
Light-sheet microscopy for plant roots

10.30-11.00am: **Emily Morris (University of Nottingham)**
Using X-ray CT scanning to understand how root branching is regulated by water availability

11.00am: *Tea Break*

Imaging with Novel Genetically encoded Reporters

11.30-12.00pm: **Phil Mullineaux (University of Essex)**
Introducing Redox biosensors

12.00-12.30pm: **Myriam Charpentier (John Innes Centre)**
Calcium Imaging in Plants

12.30-1.00pm: **Annalisa Rizza (The Sainsbury lab, Cambridge University)**
The Makings of a Gradient: Spatiotemporal distribution of Gibberellin in Arabidopsis roots

1.00-1.30pm: **George Littlejohn (University of Plymouth)**
Seeing the light: achieving signal and contrast generation in plants with minimal physiological impact and in multiple modes of microscopy

1.30pm: Lunch

2.15-3.15pm: Poster Session to provide plenty of time for discussion and advice.

3.15-4.30pm Discussion Session on Horizon Scanning in Plant Imaging

4.30pm Meeting end

GARNet Imaging

Meeting Sponsors

Thanks to these people for supporting the workshop.

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GARNet Imaging

September 9th 3.30pm

Connecting the dots: Sub-diffraction limited imaging of plasma-membrane nanodomains

Joe McKenna

Oxford Brookes University

The plant plasma-membrane (PM) contains a plethora of proteins which are vital for perceiving the environment, resource acquisition and transport of hormones which define plant development. The PM has been classically described and understood by the fluid mosaic model to be a diffuse mix of proteins moving through the membrane. However, in order for signalling or enzymatic complexes to function quickly and efficiently there must be some structure to the PM, ensuring components are in the correct place when required.

A number of groups have demonstrated that when imaged with sub-diffraction limited techniques, protein heterogeneity and distinct nanodomains can be observed within the PM. However, how do these nanodomains form and how are their size and dynamics regulated?

In order to answer this we used a protein involved in hormone transport (PIN3) and pathogen perception (FLS2) and characterised their dynamics and nanodomain size using both TIRF-single particle and Airyscan sub-diffraction limited imaging. Both proteins have differently sized nanodomains within the PM. Upon depolymerisation of the actin or microtubule cytoskeleton we observe differential effects on PIN3 and FLS2 nanodomains.

However, perturbation of the cell wall by either inhibiting cellulose biosynthesis, pectin methylation or enzymatic degradation causes increases in both proteins nanodomain dynamics and size. Therefore, we propose a model in which the cell wall and in part the cytoskeleton contribute to the formation and functionality of nanodomains.

GARNet Imaging

September 9th 4.00pm

The structure of the nuclear pore complex and other intra-cellular molecular complexes in plant cells.

Martin Goldberg

University of Durham

We are interested in the structure and structural interactions of intra-cellular molecular complexes. The nuclear pore complex (NPC) is a massive membrane associated protein structure that controls the movement of soluble macromolecules and membrane bound proteins to and from the nucleus. It is a selective barrier that allows free, size dependent, diffusion of small molecules, but restricts the passage of larger molecules unless complexed with a transport carrier. Although the scaffold of the NPC structure, which has been studied by cryo-electron microscopy combined with X-ray crystallography, cross-linking and other studies, is understood to some extent at the molecular, even atomic, level, the nature of the selective barrier is still to be determined.

Furthermore, although the NPC is anchored to the nuclear lamina inside the nucleus, in metazoan, and possibly to the cytoskeleton, these connections are poorly understood. Although all eukaryotes have structurally similar NPCs, containing similar proteins, there are differences that are yet to be understood.

We have developed surface imaging methods using scanning electron microscopy (SEM) to determine the structural organization of plant and other NPCs and their intra-nuclear and –cytoplasmic interactions. Although plants do not have obviously equivalent proteins to metazoan intermediate filaments, we do find evidence for a structure similar to the vertebrate nuclear lamina which anchors the NPCs in the nuclear envelope (NE). We are also interested in using these and other methods to determine how the cytoskeleton associates with the NE in plants.

GARNet Imaging

September 9th 4.30pm talk selected from abstracts P6

High Spatial and Temporal Resolution Imaging of Single Events of Clathrin Mediated Endocytosis in Live Plants

Nataliia Gnyliukh[1], Alexander Johnson[1] and Jirí Friml[1]

1- Institute of Science and Technology Austria, Am Campus 1, Klosterneuburg, 3400 Austria

Clathrin-mediated endocytosis (CME) is essential in plants as it has roles in growth, development, signaling pathways and many more essential processes. While the importance of CME is unquestionable, the precise details of the functioning and composition of the CME machinery remain unknown. A major reason for this is the lack of reliable methods allowing direct imaging of cell surface and single endocytic events.

To overcome this and increase our knowledge about CME we developed the use of Total Internal Reflection Fluorescence Microscopy (TIRF-M) to look directly at the plasma membrane; a major site of CME. Combining this with automated, unbiased high throughput analysis, we can examine the spatial temporal dynamics of single endocytic events by using the departure of the clathrin as a marker for the completed CME event. We validated this approach using dual channel imaging of the endocytosis adaptor protein TPLATE, and found that it is recruited in tandem with clathrin, as expected. We, therefore, provide a useful tool to the community, which can be used for in depth study of potential proteins, involved in CME.

GARNet Imaging

September 9th 4.45pm talk selected from abstracts P11

Untangling the adaptive evolution of polyploid meiosis using super-resolution microscopy

Chris Morgan[1], Martin White[2], Chris Franklin[3], Nancy Kleckner[2], Kirsten Bomblies[4]

1- Department of Cell and Developmental Biology, John Innes Centre, Norwich, UK.
2- Department of Molecular and Cellular Biology, Harvard University, Cambridge, USA.
3- School of Biosciences, University of Birmingham, Edgbaston, Birmingham, UK.
4- Institute of Molecular Plant Biology, Department of Biology, ETH Zürich, Zürich, Switzerland.

Autopolyploid species possess multiple (>2) homologous copies of each chromosome and are widespread within the plant kingdom. During autopolyploid meiosis the formation of crossovers (COs) that simultaneously link >2 homologous chromosomes leads to the formation of multivalent chromosomal associations at metaphase I that can cause homologue mis-segregation and reduced fertility. Multivalents are common in newly-formed autopolyploids, but in established autopolyploid species they are in almost all cases very rare, suggesting that solutions can evolve to prevent them.

To understand how meiosis can evolve to overcome these challenges we have used single-cell immunocytological analysis coupled with super-resolution (3D-SIM & dSTORM) microscopy to dissect chromosomal pairing patterns and CO localisation in spread prophase I cells from evolved autotetraploid, diploid and synthetic-tetraploid (double-diploid) lines of the model plant species *Arabidopsis arenosa*. We find that differences in CO frequency and chromosome organisation and pairing behaviour are observed between these three lines and that these changes promote stable bivalent formation during metaphase I in the established autotetraploids.

September 9th 5.00pm: List of Short Talks to introduce posters

P1: Hee-Kyung Ahn (Sainsbury lab, Norwich, UK)
Re-localization of paired immune receptor NLRs upon immune activation. Abstract page 36

P2: Joanna Chustecki (University of Birmingham, UK)
The social network of mitochondria and their dynamics in plant cells: Page 37

P4: Trupti Gaikwad (University of Plymouth, UK)
Fluorescence imaging as a technique to monitor plant health and improve cropping in vertical farming systems: Page 39

P5: Anna Gardner (University of Birmingham, UK)
Leaf physiology and morphology under elevated CO2 in a mature oak woodland.: Page 40

P7: Ciaran Griffin (University of Plymouth, UK)
The use of image analysis in rice blast disease: Page 41

P8: Hanna Hõrak (University of Tartu, Estonia)
Chlorophyll fluorescence imaging reveals systemic autoimmune damage in young leaves of npr1-1 during local bacterial infection in mature leaves: Page 42

P9: Léa Jacquier (University of Geneva, Switzerland)
Symplastic connections in differentiated Arabidopsis roots: Page 43

P10: Jen McGaley (University of Cambridge, UK)
Spatiotemporal dynamics of nutrient exchange at the arbuscule: Page 44

P12: Luca Piccinini (Sant 'Anna School of Advanced Studies Pisa, Italy)
Development of sensors for molecules involved in flooding response in plants: Page 45

P13: Jim Rowe (Sainsbury lab, Cambridge University, UK)
ABACUS2- ABA Concentration and Uptake Sensor 2 allows dynamic quantification of ABA in vivo.: Page 46

P14: Jennifer Sloan (University of Sheffield, UK)

GARNet Imaging

September 9th 5.00pm: List of Short Talks to introduce posters

An integrated 3D model of rice photosynthesis, eLeaf: Page 47

P15: Moumita Srivastava (University of Durham, UK)
SUMO Mediated regulation of BR signalling pathways: Page 48

P16: Bijun Tang (Sainsbury lab, Cambridge University, UK)
Growth reprogramming and GA repatterning of Arabidopsis during stress response: Page 49

P17: Ludi Wang (Aberystwyth University, UK)
'Poppidopsis': An in vitro Live-cell Imaging System in Arabidopsis Pollen Tubes Provides Important Insights into Self-incompatibility-induced Programmed Cell Death (SI-PCD) in Papaver: Page 50

P18: Emily Breeze (Univesity of Warwick, UK)
Targetting the Arms Factory: The endoplasmic reticulum is a key battlefield in the plant defence response.
Page 52

GARNet Imaging

September 10th 9.00am

Single Molecule RNA FISH: quantitative applications for gene expression analysis

Anis Meschichi[1], Stefanie Rosa[1]

1- Swedish University of Agricultural Sciences, Uppsala – Sweden

Single cell measurements of gene expression have started to reveal surprising variability otherwise hidden in bulk measurements. Moreover, relative changes in gene transcription determined by qRT-PCR lack potentially important information relating to sub-cellular localization of RNA molecules.

In my talk I will present an in situ hybridization method capable of detecting individual mRNA molecules and thus allowing for accurate quantification and localization of mRNAs within fixed samples. This method is based on multiple single-labeled oligonucleotide probes generating diffraction-limited signals that can be detected by fluorescence microscopy. I will present some aspects of the protocol and sample preparation and discuss some applications of this technique from our own research.

Reference:

Duncan S., Olsson T., Hartley M., Dean C. & Rosa S. A method for detecting single mRNA molecules in Arabidopsis thaliana. Plant Methods 12, 13 (2016)

GARNet Imaging

September 10th 9.30am

Tracking cells in complex tissues

Angharad Jones, Jim Murray

School of Biosciences, Cardiff University, Cardiff, CF10 3AX

Size is a fundamental cellular property that must be tightly regulated; if a cell is too large, molecules cannot diffuse across it efficiently and it may lack structural integrity, but conversely if too small, the formation of essential intracellular structures may be restricted.

In multicellular organisms, cell size can also affect the overall size and structure of the tissue with consequences for function and development; tissues composed of large cells could be less structurally robust whereas building tissues from small cells may require more nutrient resource in additional cell walls and nuclei per unit area. Whereas cell size has been studied in unicellular organisms using synchronised culture techniques, cell size control in multicellular organisms needs to be studied within the developmental context of the tissue.

In order to do this, I am using a combination of confocal time-lapse imaging and lineage analysis to track the growth and division of individual cells. This data can be combined with computational approaches to produce mathematical models of cell size control that will allow us to predict how the size and structure of tissues will change under different developmental and environmental conditions.

GARNet Imaging

September 10th 10.00am

Light-sheet Microscopy for Plant Roots

Giovanni Sena

Imperial College London

Live imaging is a necessary tool to characterize the dynamics in developmental processes. While the details of the phenomenon under investigation will dictate the optimal magnification, spatial resolution, frequency of observations (temporal resolution), and duration of the observation, innovative technical solutions are required to keep the sample alive, in the field of view and in focus during long imaging sessions. Fluorescence microscopy offers many advantages, especially when internal tissues are to be imaged. If the sample is relatively transparent, a common solution is optical sectioning, usually achieved through one of the existing realizations of confocal microscopy. Unfortunately, such methods rely on scanning the sample with a laser beam focused on a single point, resulting in high energy levels absorbed locally by the tissue.

Therefore, frequent and prolonged time-lapse imaging through confocal microscopy can easily lead to severe damage and possibly death of the specimen. Light-sheet fluorescence microscopy (LSFM) offers unique advantages for studying the dynamics of developmental processes at high spatial and temporal resolutions, over long periods. The laser beam is focused into a thin “sheet” of light to illuminate at once only a small section of the sample at any given time, resulting in significantly less energy being absorbed by the tissue and allowing prolonged and frequent observations.

I will describe a simple imaging setup based on light-sheet microscopy, specifically developed in my lab to track and image Arabidopsis root tips at high spatial and temporal resolutions for many days. With hardware composed by off-the-shelf optical elements and live-imaging software developed in MATLAB, the system can be recreated at low costs in any lab.

We applied the method to a quantitative analysis of mitotic dynamics during normal growth and de novo regeneration of the Arabidopsis root meristem. I will describe the raw data and a basic suite of analysis, showing interesting dynamics.

GARNet Imaging

September 10th 10.30am

Using X-ray CT scanning to understand how root branching is regulated by water availability

Emily Morris

University of Nottingham

Root system architecture is shaped by the soil it grows in. As soil is an extremely complex environment the root receives many signals simultaneously that influence how the root grows. For instance, water is spread heterogeneously in the soil profile. Due to differing sized pores and structures such as cracks and bio-pores, the root will experience localised water deficit even when water is present in the soil. In order to understand how root development is shaped by environmental signals, such as water availability, it is necessary to study root development in soil. However, this is challenging due to the opaque nature of soil.

In the Hounsfield Facility at the University of Nottingham, X-ray Computed Tomography (CT) has enabled us to visualise 'the hidden half' of plant biology. X-ray CT can image root systems and soil structure simultaneously, in a non-destructive manner. Using this technique we can observe how water availability is a key regulator of root branching. Branching mainly occurs on the side of the root in contact with water, termed hydropatterning, and branching is repressed when the root grows through a localised water deficit, termed xerobranching. Interestingly, not all types of lateral roots respond to water deficit with the same response.

Laser ablation tomography (LAT) was used to get a closer look at what changes occur in the cellular anatomy of the root during localised water deficit. The data collected highlighted that branching is repressed at an early stage of initiation in water deficit. Therefore, by using X-ray CT scanning and LAT we have observed that the availability of water in the soil significantly alters root system architecture.

GARNet Imaging

September 10th 11.30am

Introducing Redox biosensors

Phil Mullineaux

School of Life Sciences, University of Essex, Wivenhoe Park, Colchester, CO4 3SQ, UK.

There is considerable evidence that reactive oxygen species, in particular hydrogen peroxide (H_2O_2) but also organic hydroperoxides (ROOH) such as those derived from the oxidation of phospholipid fatty acids by singlet oxygen, are engaged in a multitude of cellular signalling functions. These ROS are critical in determining the response of plant cells to changes in their environment and during many stages of organ development.

Until recently, studies have been severely hampered by a lack of information on the temporal, quantitative and spatial dynamics of these ROS. However recently, genetically-encoded fluorescent protein biosensors that report H2O2 are appearing from several laboratories that hold great potential for overcoming these limitations. We are increasingly using these biosensors, plus ones that report the redox state of glutathione, to understand the spatial and temporal components of ROS-mediated chloroplast-to-nucleus (retrograde) signalling.

I will describe our experiences of developing and using two types of peroxide sensor - the HyPer series and the redox-relay roGFP2-based probes. We have used both types to good effect and in specific experimental situations, but also become aware of the limitations of each type of biosensor. I will describe these and also unpublished data which show we are making progress on the use of pairs of probes that can be used to delineate H2O2 and organic peroxide dynamics in vivo.

GARNet Imaging

September 10th 12.00pm

Calcium Imaging in Plants

Myriam Charpentier

John Innes Centre, Cell and Developmental Biology, Colney Lane, NR47UH, Norwich, UK.

Spatiotemporal changes in cellular calcium (Ca2 +) concentrations are essential for signal transduction in a wide range of plant cellular processes. Understanding how such a versatile secondary messenger encodes specific cellular response requires precise knowledge of the dynamics of its release with both spatial and temporal resolution. Within the same cell, diverse stimuli can induce specific calcium signals varying in amplitude, duration, and frequency, in the cytoplasm or organelles.

To gain insight into the specific calcium signals dynamics, calcium imaging techniques based on chemical indicators and genetically encoded calcium indicators have been developed. Here, using root legume as a model system, I will present how genetically encoded calcium indicators can dissect the spatial and temporal dynamic of microbial-elicitors-induced calcium release.

GARNet Imaging

September 10th 12.30pm

The Makings of a Gradient: Spatiotemporal distribution of Gibberellin in Arabidopsis roots

Annalisa Rizza[1], Leah Band[2], Markus Owen[2] and Alexander M. Jones[1]

1- The Sainsbury Laboratory, Cambridge University, United Kingdom
2- Centre for Plant Integrative Biology, University of Nottingham, United Kingdom

Gibberellin (GA) promotes cell elongation and influences many aspects of plant development from seed germination to flowering. GA dynamics at the whole plant or organ level are the result of GA levels in myriad cells and tissues that are determined by a series of enzymes and transporters. This complexity calls for studying GA regulation at high spatial and temporal resolution.

To visualize and quantify GA, we are using GPS1 (Gibberellin Perception Sensor 1), the first FRET biosensor which allows for high-resolution GA measurement in vivo. By measuring the emission ratio of nuclear localised GPS1, we mapped and monitored endogenous and exogenous GA distribution in different tissues. In Arabidopsis root tips we observed a gradient of endogenous and exogenous GA with low levels in the cell division zone grading to high levels in the cell elongation zone.

A central question was how this gradient of a mobile small molecule is created and maintained. Thus, we perturbed the activities of GA enzymes and transporters to investigate the consequences on GA patterning. We now show that patterning of GA biosynthesis and cell permeability are both crucial for shaping GA gradients in roots. Although it is clear that GA gradient regulates root growth, we now aim to determine the precise quantitative relationship between GA patterns and plant growth patterning. Technical aspects of imaging nlsGPS1 biosensors and growing roots in RootChip microfluidic devices will also be discussed.

GARNet Imaging

September 10th 13.00pm

Seeing the light: achieving signal and contrast generation in plants with minimal physiological impact and in multiple modes of microscopy

George R. Littlejohn

School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK

All biological imaging requires the generation and detection of signals which may be differentiated from background. Achieving sufficient signal and contrast to yield meaningful information in an environment as optically and chemically complex as plant tissue has some distinct challenges, but many of the apparent difficulties which present themselves also offer solutions and techniques for imaging which work particularly well in plants.

Here we will discuss distinct opportunities in plant cell biology and consider the use and limitations of several modes of advanced plant imaging. We will consider the need for live cell imaging and physiological biosensors in understanding plant cell biological processes and discuss how making appropriate choices, and where necessary compromises, of imaging modality and conditions is required by microscopists to answer biological questions with minimal physiological impact.

Poster P1

Re-localization of paired immune receptor NLRs upon immune activation

Hee-Kyung Ahn, Jonathan D G Jones

The Sainsbury Laboratory, University of East Anglia, Norwich, NR4 7UH, United Kingdom

Plants are surrounded by pathogenic microbes and have developed immune signalling pathways to evade pathogen challenges. Intracellular immune receptors of the nucleotide-binding domain, leucine-rich repeat (NLR) superfamily recognize effectors secreted into host plant cells and induce immune signalling. RRS1-R and RPS4 are unique paired NLRs that function together as a complex for the recognition of effector AvrRps4, which is secreted by bacterial pathogen *Pseudomonas syringae pv. pisi*.

Estradiol-inducible system was deployed to induce expression of effector proteins in planta, which effectively mimicks the native system of type III secretion by bacteria. This enabled the observation of early changes in the RPS4-RRS1 complex upon effector secretion. N-terminal truncated Venus protein was fused to the N-terminus of RRS1-R (nVenus-RRS1-R), and C-terminal truncated CFP protein was fused to the N-terminus of RPS4 (cCFP-RPS4) with estradiol-inducible effector cassettes expressing either AvrRps4 for mutant effector AvrRps4E187A for bifluorescence complementation (BiFC) analysis. RRS1-R and RPS4 interact in the nucleoplasm before estradiol induction.

Upon induction of AvrRps4 for several hours, interaction of RRS1-R and RPS4 was seen at nuclear puncta at the nucleolar periphery. However, upon induction of mutant control AvrRps4E187A, no change in localization of RRS1-R and RPS4 interaction was seen. Furthermore, RRS1-R was tagged with GFP (RRS1-R-GFP), and RPS4 was tagged with YFP (YFP-RPS4) to observe localization changes of each protein upon effector induction. RRS1-R-GFP localization did not alter from nucleoplasmic localization with or without estradiol, but punctate patterns within the nucleolus were additionally observed after effector induction with estradiol.

On the other hand, YFP-RPS4 localization shifted from the nucleoplasm to nucleolar rings upon induction of AvrRps4. YFP-RPS4 localization remained nucleoplasmic upon induction of AvrRps4E187A. These results suggest that changes in the localization of immune receptors may be involved in immune activation by plants.

GARNet Imaging

Poster P2

The social network of mitochondria and their dynamics in plant cells

Joanna Chustecki

University of Birmingham

The amount of energy plant cells have access to is vital for their growth. Mitochondria are very dynamic and move around the cell delivering energy to where it is needed most. There is little understood about their motion, and how it is controlled. We aim to understand how and why mitochondria are so dynamic within plant cells, how this links to the exchange of genetic material and the bioenergetics of the cell. It is important to understand why the plant invests so much energy in keeping these mitochondria moving, and whether we can perturb this system to make the plant more efficient- key for securing food sources in the future.

Using a light-scanning confocal microscope, we can collect video data of fluorescent mitochondria. We image single cells of the hypocotyl of young Arabidopsis thaliana seedlings, which gives us a pseudo-2D system of organellar motion. Extracting coordinates over time, we build up social network structure of mitochondrial interactions, and characterise this movement using various summary statistics.

We are also modelling this motion, to conclude the best parameters to simulate wild type motion and the behavior in mutant lines. These currently include friendly, a mutant affecting correct distribution of mitochondria, and msh1, a DNA mismatch repair protein, affecting the mitochondrial genome and plastid development. Imaging and modelling dynamics in these mutants will help further our understanding of the role organellar motion plays in plant cells.

Using these novel approaches to organellar motion we aim to further understand these mitochondria, their interactions with other organelles such as the chloroplast, and why the plant cell needs to invest energy to keep them moving.

Poster P3

flg22-induced root growth-immunity trade-offs can be overcome by de-repression of cell cycle arrest

Ruth Eichmann[1], Jessica Finch[1], and Patrick Schäfer[1,2]

1- School of Life Sciences, The University of Warwick, Coventry, CV4 7AL, United Kingdom.

2- Warwick Integrative Synthetic Biology Centre, The University of Warwick, Coventry, CV4 7AL, United Kingdom.

Plants use plasma membrane receptors to detect conserved molecules of potentially harmful microbes, and thereupon activate pattern triggered immunity (PTI) to protect plants against pathogen invasion. However, PTI activation appears to have some trade-offs and is well known to inhibit plant growth and yield. These growth-immunity trade-offs may impair efforts to breed for plants with high resistance and full growth and yield levels.

In Arabidopsis, flg22 (active flagellin epitope) detection by the receptor FLAGELLIN-SENSING 2 has been key to uncovering PTI regulation and we scrutinised the effects of flg22 on root growth inhibition. We found that flg22 inhibits cell division as a key growth determinant in the root meristem but does not affect stem cell niche function. Moreover, flg22-induced PTI affects the G2-mitosis pathway and specific stimulation of this pathway by over-expressing CYCLIN-DEPENDENT PROTEIN KINASE B1;1 resulted in regular growth upon full PTI activation. Our findings demonstrate the feasibility of uncoupling negative growth-immunity trade-offs. Considering the conservation of cell cycle regulation, it may inform breeding strategies for crops with high resistance traits without impairing growth.

GARNet Imaging

Poster P4

Fluorescence imaging as a technique to monitor plant health and improve cropping in vertical farming systems

Trupti P. Gaikwad*, Naofel Aljafer*, Angela Harrop and George R. Littlejohn.

University of Plymouth

Vertical farming systems offer local, controllable and sustainable food production. We are developing image acquisition and analysis tools and techniques to optimise food production using plant health monitoring systems deployed in vertical farms. Specifically, we are using measurement of photosynthesis parameters to diagnose nutritional deficiency, physiological state and early disease (pre)symptoms. We are working within a suite of related projects which are developing vertical farming platforms, imaging and robotics to automate the growth, health monitoring, disease control and harvesting of crops.

Plant stress results in changes in photosynthesis efficiency. Imaging techniques: such as Chlorophyll Fluorescence (CF) Imaging and Normalised Difference Vegetative Index (NDVI), are very valuable tools could provide spatial and temporal information about these processes as chloroplasts play a vital role in integrating multiple environmental stimuli including both biotic and abiotic factors. The aim of the current study is to analyse and optimize the various changes in measured parameters associated with plant nutritional deficiency and disease. We are also using the same approach to assess plant health with regard to harvesting.

We aim to provide a tool which food producers or robotic applications may use to diagnose diseased individuals and act by applying chemicals or removing infected or unhealthy plants before disease can spread throughout the crop or time and space is devoted to low yielding individuals. In order to achieve this, we are also developing image analysis methods to diagnose plant health and inform decisions which can be acted upon by the grower or robots.

Poster P5

Leaf physiology and morphology under elevated CO2 in a mature oak woodland.

Anna Gardner[1], J.Pritchard[1], A.R.Mackenzie[2]

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This study investigates the effect of elevated carbon dioxide (eCO2) on oak leaf physiology and morphology in a mature temperate woodland. Rising CO2 is expected to stimulate photosynthesis and reduce stomatal conductance, but limited studies have been conducted on mature temperate forests. This experiment has been conducted at the Birmingham Institute of Forest Research Free Air Carbon Enrichment Experiment (BIFoR-FACE). BIFoR-FACE is set in a mature oak (*Quercus robur. L*) woodland manipulated to simulate future atmospheric conditions (+150ppm) to experimental plots. This study used a paired plot design (n=3) of elevated CO2 plots (eCO2) (550ppm) and ambient control plots (aCO2) (400ppm).

In situ instantaneous leaf gas exchange measurements were taken in the upper oak canopy (June to October, 2018 and 2019) using a Li-6800 portable photosynthesis system. Preliminary results suggest a) increased photosynthetic rates (A) b) decreased stomatal conductance (gsw) and c) increased intrinsic water-use efficiency (iWUE) under eCO2 treatment. The strength of the treatment effect was influenced by both seasonal and diurnal fluctuations of environmental variables such as; light intensity, air temperature and water availability. Our next steps will be to use microscopy as a tool to study leaf morphology, in particular stomatal density, to better understand the leaf physiological response to eCO2. We would also like to scale up, from leaf to canopy, to better understand carbon balances in forests. This study has provided important data required in terrestrial biome modelling (TBM's), to be used in the accurate calculation of global carbon budgets.

GARNet Imaging

Poster P7

The use of image analysis in rice blast disease

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Accurate, reliable, and fast quantification of infection severity in plants can play an important role in curtailing the spread of disease in crops. Many current analysis techniques rely on visually checking samples, using a numbered and subjective severity scale which often requires expertise to determine. The use of automated image analysis in infection severity quantification can allow for high throughput image processing, gleaning detailed and unbiased information in a very short time span.

The disease quantification programme only requires a camera, blank background, and calibration square, and so can analyse images captured in the field and lab, alike. Image processing can also be used for transforming complex growth patterns into simple graphs, or for quick characterisation and counting of several regions of interest (ROIs). Here, we present a range of automated image processing techniques using ImageJ for rice blast disease quantification, spore recognition, and fungal growth analysis.

GARNet Imaging

Poster P8

Chlorophyll fluorescence imaging reveals systemic autoimmune damage in young leaves of npr1-1 during local bacterial infection in mature leaves

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Diseases caused by bacterial pathogens severely reduce crop yields. Improvement of crop disease resistance requires a deep understanding of the physiology and molecular mechanisms underlying plant-pathogen interactions. Many important components of the plant immune system have been identified using the Arabidopsis thaliana - *Pseudomonas syringae* (Pst) model system; among them the protein NPR1 (NONEXPRESSER OF PR GENES 1) that acts as a central positive transcriptional regulator of salicylic acid (SA) responses that mediate plant immune response to Pst infection.

Chlorophyll fluorescence imaging can be applied to detect disease symptoms in plants before visual damage occurs. We used whole-plant chlorophyll fluorescence imaging of Fv/Fm in Arabidopsis to assess Pst-induced damage (manifested in reduction of Fv/Fm) in wild-type and npr1-1 mutants, which express a dysfunctional version of NPR1. Surface inoculation with Pst resulted in clear reduction of Fv/Fm in young leaves in the centre of Arabidopsis rosette of *npr1-1*, but not wild-type plants. Infiltration inoculation of mature leaves with Pst resulted in similar reduction of Fv/Fm in young leaves, indicating a systemic response. As SA is involved in systemic signalling, we tested whether infiltration of SA into mature leaves affects Fv/Fm in systemic young leaves. Fv/Fm in systemic young leaves was reduced in SA-infiltrated npr1-1 plants.

In the SA-biosynthesis deficient *npr1-1sid2-2* double mutants, Pst infection did not cause a reduction in Fv/Fm in systemic young leaves, indicating a requirement for SA biosynthesis for the systemic damage. In line with this, inoculation with the coronatine-deficient Pst strain, which cannot suppress SA response through jasmonic acid pathway, caused a more severe reduction in Fv/Fm in the young leaves of *npr1-1*. We propose that in addition to a positive role in SA signalling, NPR1 functions as a negative (auto)regulator of SA-mediated immune responses, thus protecting young leaves from autoimmune damage triggered by excess SA levels.

Poster P9

Symplastic connections in differentiated Arabidopsis roots

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The tight regulation of water and nutrients uptake from soil is essential for plants to grow and to adapt to the environmental resources. In vascular plants, there are three major pathways for nutrient uptake: apoplastic, symplastic and coupled trans-cellular. Besides nutrients, the symplastic pathway also supports the movement of varied molecules such as phytohormones, transcription factors and viral pathogens through plasmodesmata (PD). PD are membrane-lined channels that connect neighbouring cells and provide a cytoplasmic continuum called symplast. Due to the importance of cell-to-cell communication, movements through PD needs to be tightly regulated. At the moment, the only known mechanism for PD regulation is callose deposition. This deposition influences the communication between the cells by modulating the PD aperture. Additionally, nutrient uptake is known to be affected by endodermal nutrient barrier (Casparian strip and suberin lamella).

This nutrient barrier is a specialized structure in endodermal cells that is made of lignin and suberin, a feature of differentiated endodermis. Since, this barrier is known to have an impact on the apoplastic pathway, we want to study how it might be affecting symplastic pathway. Our first goal is to reconstruct the symplastic connections to understand the directionality of this movement if any. For this purpose, we will use high-resolution 3D confocal microscopy to monitor the movement of GFP, which is known to move freely through PD, expressed under root cell-type specific promoters. Photoactivable fluorophores will be also tracked to follow their movement across close cell types. Finally, electron microscopy will help us to visualize the PD structure. Preliminary results show a differential diffusion in between the different cell types. Our second goal is to study PDs regulation (callose deposition, size of the pore, cytoplasmic sleeve) under abiotic stresses. This work will provide a better understanding of cell-to-cell communication in a context of differentiated roots.

Poster P10

Spatiotemporal dynamics of nutrient exchange at the arbuscule

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Arbuscular mycorrhizal symbiosis is an intimate, mutually beneficially relationship between Glomeromycotina fungi and almost all land plant species. Central to the symbiosis is nutrient exchange: mineral nutrients (e.g. phosphate and ammonium) are transported from fungus-to-plant, while organic carbon (e.g. lipid) is transported from plant-to-fungus. This bi-directional nutrient transport takes place at a specialised fungal structure called the arbuscule, hosted within plant root cortical cells and surrounded by a plant-derived peri-arbuscular membrane (PAM).

To visualise the spatiotemporal dynamics of nutrient exchange at this interface between symbiotic partners, the PAM-localised transporter proteins responsible for each direction of nutrient transport have been tagged via fluorescent fusion protein constructs. Confocal and multiphoton microscopy will be combined with time-lapse techniques to image transporter protein localisations throughout arbuscule development, maturity and collapse. This will illuminate the dynamics – and give insights into the regulation – of reciprocal nutrient exchange during arbuscular mycorrhizal symbiosis.

Poster P12

Development of sensors for molecules involved in flooding response in plants

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Climate changes cause a continuous increase in flooding events that can affect the whole plant or some parts of it. These events are harmful for crops, considering that the most part of them are not able to survive a prolonged submersion. Indeed, most crops are particularly sensitive to the oxygen deficiency caused by flooding, although some evolved strategies intended to induce tolerance or allow avoidance of this stress condition.

Oxygen, or lack thereof, is likely not the only gaseous molecule involved in the regulation of flooding response. The aim of my project is to expand the toolbox of genetically encoded sensors for reporting the abundance of fermentation metabolites such as pyruvate, acetaldehyde, ethanol and lactate with a focus on the SAM (shoot apical meristem).

In the frame of this project, I am generating Forster Energy Transfer (FRET) and transcriptional reporters. I will exploit "Laconic" (San Martìn *et al*. 2013) and "Pyronic" (San Martìn *et al*. 2014), two FRET sensors based on bacterial transcription factors, developed and tested in mammalian cells, in order to quantify intracellular lactate and pyruvate, respectively. I will also exploit the alc gene-expression system from the filamentous fungus *Aspergillus nidulans* to detect ethanol and acetaldehyde. The output of the sensors will be measured by different methods, comprehensive of confocal microscopy imaging, gus staining and FRET measurements.

GARNet Imaging

Poster P13

ABACUS2- ABA Concentration and Uptake Sensor 2 allows dynamic quantification of ABA in vivo.

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Abscisic acid (ABA) coordinates environmental cues with endogenous signalling to regulate plant development dynamically, optimising growth to the situation. Quantifying ABA dynamics in real time under different conditions is therefore critical to understanding how plants adapt growth to their environment (Jones 2016). By connecting the ABA receptor (PYL1) and a truncated co-receptor (ABI1) to a Förster Resonance Energy Transfer (FRET) pair, we previously developed a novel ABA biosensor, called ABACUS1 (Jones et al. 2014).

This biosensor had a large positive FRET ratio change in response to ABA, but had too low affinity to report unstressed ABA levels. ABACUS1 also had an ABA hypersensitivity phenotype, due to overexpression of PYL1. To develop ABACUS2 we introduced several mutations to the PYL1 ABA binding pocket, increasing affinity. Linker truncations have improved ratio change even further. A mutation has also been introduced to weaken interaction with endogenous signalling and reduce unwanted phenotypes. ABACUS2-150n and ABACUS2-800n detect ABA in planta at high spatiotemporal resolution showing differential accumulation in different tissue types. Combining ABACUS with inducible ABA biosynthesis, catabolism and deconjugation lines will allow us to probe how ABA accumulation and depletion are regulated in real time to control development and stress responses.

Poster P14

An integrated 3D model of rice photosynthesis, eLeaf

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Photosynthesis depends on flux of gas into the leaf to the plastid-containing cells. Therefore, the size, shape and spatial distribution of cells, and the air channel network connecting them to the outside world, will influence physiological performance.

We are quantifying the cellular architecture of IR64 rice leaves grown at ambient and elevated CO2 levels, using a combination of imaging techniques including confocal and TEM, coupling this with measurements of physiological performance. This information is fed into a 3D computational model of rice leaf photosynthesis, allowing the testing of hypotheses of leaf structure/function.

GARNet Imaging

Poster P15

SUMO Mediated regulation of BR signalling pathways

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Brassinosteroids (BRs) are essential steroid phytohormone that regulate growth, development and stress response in plants (Depuydt and Hardtke, 2011). During BR signalling, the interaction between BRASSINOSTEROID INSENSITIVE2 (BIN2) and Brassinazole Resistant1 (BZR1) is crucial to regulate its transduction to the downstream gene expression (Kim *et. al.*, 2014). BZR1 acts as a direct physical convergence point for signalling pathways and the underlying mechanism of its fine-tuning is still not well understood.

Posttranslational modification of proteins by Small Ubiquitin-like MOdifier (SUMO) is emerging as an important mechanism to transduce environmental signals into cellular signalling, reflected by recent studies (Orosa et. al., 2018). In this study, we have identified the SUMO protease Ubiquitin-like-specific protease1a (ULP1a) mediates the deSUMOylation of BZR1 to repress the BR signalling. SUMOylation of BZR1 promotes the nucleocytoplasmic transport of BZR1 by inhibiting its interaction with BIN2 kinase in cytoplasm. In addition, under abiotic stress, ULP1a gets accumulated in cytoplasm and its accumulation leads to the deSUMOylation of BZR1. In contrast, BR treatment promotes the degradation of ULP1a allowing SUMOylated BZR1 to accumulate and promote growth.

This study uncovers a regulatory mechanism of BR signalling pathway to shape plant growth and development.

Poster P16

Growth reprogramming and GA repatterning of Arabidopsis during stress response

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The nuclear-localised Gibberellin Perception Sensor 1 (nlsGPS1), a Förster Resonance Energy Transfer (FRET) based biosensor, was previously used in transgenic Arabidopsis plants to obtain a spatiotemporal understanding of gibberellin (GA) levels in vivo. A positive correlation between GA levels and cell length was reported for certain tissues and a gradient of GA was shown in roots and etiolated hypocotyls of Arabidopsis under optimal growth conditions (Rizza *et al.* 2017). In the present study, we observed that the external application of GA does not cause increased length in roots. Our hypothesis is that in Arabidopsis grown in favorable conditions, GA signalling for growth is largely saturated.

Since nlsGPS1 is not rapidly reversible, a reversible sensor is required to investigate the decline of GA. A next-generation of nlsGPS1-CE was generated to be more rapidly reversible and to reduce interference with endogenous GA signaling (i.e. increase orthogonality). Reduction of GA in vivo can be detected with this sensor. We continue to attempt the engineering of a next-generation of GA sensor based on nlsGPS1-CE sensor with higher affinity, more rapid reversibility, and better ratio-change.

With the next-generation of the GA sensor, we will be able to investigate the repatterning of GA in a spatiotemporal manner when Arabidopsis experiences stress conditions that limit growth. By characterising cell growth patterning under these conditions, we can gain knowledge on the relationship between GA repatterning and the growth reprogramming in Arabidopsis during stress.

Overall, understanding how GA affects plants in different stress conditions will provide more insight on how to improve crop yield and maintain the biodiversity in ecosystems where plants are under various stresses, such as nutrient limitation, alkaline soils, drought, etc. Moreover, the next-generation of nlsGPS1 biosensor will allow us to further understand the mechanisms of gibberellin regulation and signalling.

References:
1. A. Rizza, A. Walia, V. Lanquar, W. Frommer, and A. Jones. In vivo gibberellin gradients visualized in rapidly elongating tissues, 2017, 3: 803-813, Nature Plants.
2. E. Colebrook, S. Thomas, A. Phillips, and P. Hedden. The role of gibberellin signalling in plant responses to abiotic stress, 2014, 217: 67-75, Journal of Experimental Biology.

GARNet Imaging

Poster P17

'Poppidopsis': An *in vitro* Live-cell Imaging System in Arabidopsis Pollen Tubes Provides Important Insights into Self-incompatibility-induced Programmed Cell Death (SI-PCD) in Papaver

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Self-incompatibility (SI) is a genetically controlled mechanism to prevent inbreeding in higher plants. During pollination, the gametophytic SI system in poppy (*Papaver rhoeas*) utilises cell-cell recognition to reject incompatible pollen, which is mediated by the interaction of two S-determinants: a stigma-expressed secreted protein (PrsS) and a pollen-expressed plasma-membrane localised protein (PrpS). Cognate PrpS-PrsS interaction triggers a signalling network causing rapid growth arrest and eventually programmed cell death (PCD) in incompatible pollen tubes. The actin cytoskeleton is a key target of this signalling network and undergoes rapid depolymerisation followed by the formation of F-actin foci. Once formed, these structures are stable and increase in size over time. However, current knowledge of these dramatic changes to the actin cytoskeleton consists of still images of fixed cells from a few time points after SI-induction.

The transgenic Arabidopsis 'SI' system, which exhibits all the hallmark features of poppy SI, provides the opportunity to perform live-cell imaging of the dynamic alterations of the actin cytoskeleton in incompatible pollen tubes. Here, we analyse the SI induced F-actin remodelling in pollen tubes utilising Arabidopsis 'SI' lines expressing a fluorescence marker, Lifeact-mRuby that labels the F-actin cytoskeleton. This approach enables us to characterise the spatial and temporal dynamics of SI-induced F-actin remodelling. The combination of Lifeact-mRuby marker lines with other fluorescence markers, such the [Ca2+]cyt indicator YC3.6 and the [pH]cyt indicator pHGFP, provides important insights into how actin integrates with other regulatory components in mediating SI-PCD.

GARNet Imaging

Poster P18

Targetting the Arms Factory: The endoplasmic reticulum is a key battlefield in the plant defence response.

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As the gateway to the cell's secretory pathway, the endoplasmic reticulum (ER) provides the critical environment for lipid biosynthesis and protein production, folding and quality control. The ER is a highly dynamic interconnected network of tubules and cisternae (sheets) that extends throughout the cytoplasm and across cellular boundaries, associating with other organelles and the plasma membrane. Hence, the ER is central to the maintenance of cellular homeostasis. During pathogen infection the demand for de novo protein and lipid biosynthesis increases significantly, necessitating rapid, but highly regulated, ER expansion and remodelling as part of a successful defence response ('defensive strategies'). The ER is, therefore, critical to the perception and regulation of adaptive host responses to biotic stress, and, as such, is also a prime target for manipulation by the pathogen as part of its virulence strategy orchestrated through the secretion of small effector molecules ('offensive tactics').

Here, we present a detailed characterisation of the active remodeling of the ER architecture during both PTI and ETI (pathogen-/ effector- triggered immunity) as part of the plant's response to infection with the bacteria Pseudomonas syringae pv tomato DC3000. In addition, we have identified several ER-localised effectors from a variety of oomycete pathogen species including Phytophthora infestans, Plasmopara halstedii and Hyaloperonospora arabidopsidis, one of which is putatively targeted to ER-chloroplast contact sites. Such effectors may therefore represent a common strategy by the pathogen to detrimentally manipulate ER morphology, activity and/or inter-organellar communication in order to cause disease.

Poster P19

Effect of temperature environmental stress on oilseed rape reproduction

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Agriculture is facing the crucial challenge of adapting crop productivity to changes in the climate; environmental stress during flowering has a direct and negative impact on yield. This is due to pollen and ovule development being highly sensitive to environmental stress, with reduced male and female fertility, and therefore ultimate seed set. Over 70 million tons of oilseed rape are being produced worldwide yearly and therefore understanding environmental sensitivity within Brassica is pivotal for optimising crop performance under changing climates. The BnaDFFS panel of approximately 100 diverse Brassica lines grown world-wide, was used to compare the effects of both cold and heat stress on different genotypes – with a focus on pollen viability, pollen germination, ovule development and final seed yield.

This analysis has resulted in 9,000 microscope images, 3,000 photos and over 100,000 individual data points. High-throughput computer image analysis is therefore essential to be able to extract all the data to compare the lines. Computer learning and computer segmentation has been used for image analysis, along with reutilisation of image J programs for a novel purpose. GWAS analysis will then be used to provide candidate genes for future breeding programs and further study to to combat environmental stress losses by targeting robust fertility under changing environments.

The knowledge gained will help to address the delivery of more robust oilseed rape lines to genetically mitigate the potentially deleterious effects of future climate scenarios.

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GARNet Imaging

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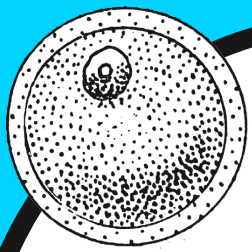

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