Funded by
the European Union

AGILE Doctoral Training Event #2

Fundamentals of Microscopy Imaging for Plant Sciences

Self-study assignment: The Plant Imaging Challenge

As part of the training, we would like each of you to explore imaging modalities **other than the ones you use in your own project**, and to share what you learn with the group in a short online presentation. The aim is for all of us to get an overview of the different physical principles behind modern imaging, and to think about what each could bring to plant sciences, including nuclear imaging.

1. Choose your papers

- Pick **two papers** (or three, if you wish) from the list below. Each paper represents one imaging modality, and the list covers a range of principles: label-free, tomographic and deep-tissue, fluorescence with optical sectioning, and super-resolution.
- **Pick modalities you do not use yourself** in your own research.
- **Your papers must come from different categories** (the four sections of the paper list), so that you contrast different principles. For example, two light-sheet papers, or a review together with a paper on a technique it covers, are not possible.
- **Each combination of papers can be chosen only once.** If you pick three papers, none of your pairs should match another student's selection.
- **Each modality can be chosen by at most three students.** It will then be contrasted with a different modality each time.
- Selections are first come, first served.

2. Register your choice

Enter your name and your papers in the shared selection table (link will follow), by **November 25th**. The table checks the rules automatically: a red flag means your combination or a modality is already taken, and you should choose again. The *Papers* tab shows how many places are left for each modality. Verena and I will monitor the selections and confirm them.

AGILE

Funded by
the European Union

3. Prepare a 5-minute presentation

Your talk lasts **5 minutes**, whether you picked two papers or three; 4–6 slides are enough. Please stick to the time, so that every talk gets its share of questions. Build it in three parts:

1. **Modality in a nutshell** (one slide per modality). Explain the physical principle: what signal is measured, and how the image is formed. Then give the key figures, always in the same order so that modalities can be compared: lateral and axial resolution · maximum imaging depth · speed (time per image or per volume) · live imaging possible? · label needed? · sample preparation · main limitation (e.g. phototoxicity) · availability and approximate cost.
2. **Applications in plant sciences, including nuclear imaging:** real examples from the literature, or applications you imagine. Contrast your modalities: what can one do that the other cannot?
3. **The plant imaging challenge** (closing slide). **Your mission**: choose one of the five challenges below and explain which of your modalities you would use, and why, or why neither would work. You can also combine yours with a modality presented by someone else.
    - **Chromatin in motion.** Follow the movement of a tagged gene locus and changes in chromatin compaction in the nuclei of a living root meristem over several hours, without damaging the tissue.
    - **The nanoscale nucleus.** Resolve how heterochromatin (chromocenters), nuclear pores and lamina-like proteins (CRWNs) are arranged at the nuclear periphery of Arabidopsis cells, at a scale of tens to hundreds of nanometres.
    - **Deep inside the flower.** Image every nucleus of a developing ovule or anther in 3D, including the innermost cells such as the spore mother cells, and follow how their nuclei change during development.
    - **No labels allowed.** Measure how nuclear stiffness, chromatin density or dry mass change when a cell differentiates or responds to stress, for example in guard cells or root hairs, without any fluorescent label.
    - **From seedling to nucleus.** Monitor a whole seedling responding to a stimulus such as light, drought or a pathogen over several days, and zoom in on the nuclei of the cells that respond.

Before your talk: fill in your ID cards

Enter the key figures for each of your modalities in the **ID cards** tab of the selection table (numbers only, in the units shown). The **Map** tab then places every modality automatically on a chart of resolution against imaging depth. We will show this comparison map of all the modalities built together by the end of the presentations.

4. Presentation sessions

The presentations will take place online on **January 7th from 9:00 AM to 12:00 PM**. Each presentation will be followed by a 2-minute Q&A session with the instructors and fellow students. To ensure everyone participates, a random generator will assign one student to ask a question after each presentation. We will send you the presentation schedule and the meeting link once the order is finalized.

Verena and I will supervise the sessions. The other supervisors in the network are warmly invited to join, so you may get questions from people who know these techniques well, or from people hearing about them for the first time.

We look forward to your presentations!

Célia and Verena

Funded by
the European Union

5- Paper list

Table to fill in your choice and the ID cards https://shorturl.at/IZWy6

	Modality	Reference
Label-free imaging		
P01	Quantitative phase imaging	Mir M et al. (2011). Optical measurement of cycle-dependent cell growth. *PNAS*. doi:10.1073/pnas.1100506108
P02	Holotomography (digital holography)	Sandoz PA, Tremblay C, van der Goot FG, Frechin M (2019). Image-based analysis of living mammalian cells using label-free 3D refractive index maps reveals new organelle dynamics and dry mass flux. *PLoS Biology*. doi:10.1371/journal.pbio.3000553
P03	Raman imaging (CARS and SRS), review	Cheng J-X, Xie XS (2015). Vibrational spectroscopic imaging of living systems: an emerging platform for biology and medicine. *Science*. doi:10.1126/science.aaa8870
P04	Stimulated Raman scattering (SRS)	Freudiger CW et al. (2008). Label-free biomedical imaging with high sensitivity by stimulated Raman scattering microscopy. *Science*. doi:10.1126/science.1165758
P05	Brillouin microscopy	Elsayad K et al. (2016). Mapping the subcellular mechanical properties of live cells in tissues with fluorescence emission–Brillouin imaging. *Science Signaling*. doi:10.1126/scisignal.aaf6326. This study was done in Arabidopsis.
Tomography and mesoscale or deep-tissue imaging		
P06	Optical coherence tomography	Fujimoto JG (2003). Optical coherence tomography for ultrahigh resolution in vivo imaging. *Nature Biotechnology*. doi:10.1038/nbt892
P07	Optical projection tomography	Sharpe J et al. (2002). Optical projection tomography as a tool for 3D microscopy and gene expression studies. *Science*. doi:10.1126/science.1068206
P08	Photoacoustic tomography	Wang LV, Hu S (2012). Photoacoustic tomography: in vivo imaging from organelles to organs. *Science*. doi:10.1126/science.1216210
P09	Soft X-ray tomography	Larabell CA, Le Gros MA (2004). X-ray tomography generates 3-D reconstructions of the yeast, *Saccharomyces cerevisiae*, at 60-nm resolution. *Molecular Biology of the Cell*. doi:10.1091/mbc.e03-07-0527
P10	Cryo-electron tomography	Mahamid J et al. (2016). Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science*. doi:10.1126/science.aad8857
Fluorescence: optical sectioning and speed		
P11	TIRF	Mattheyses AL, Simon SM, Rappoport JZ (2010). Imaging with total internal reflection fluorescence microscopy for the cell biologist. *Journal of Cell Science*. doi:10.1242/jcs.056218
P12	Confocal and optical sectioning	Conchello J-A, Lichtman JW (2005). Optical sectioning microscopy. *Nature Methods*. doi:10.1038/nmeth815
P13	Two-photon	Svoboda K, Yasuda R (2006). Principles of two-photon excitation microscopy and its applications to neuroscience. *Neuron*. doi:10.1016/j.neuron.2006.05.019
P14	Light sheet (SPIM)	Huisken J et al. (2004). Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science*. doi:10.1126/science.1100035
P15	Scanned light sheet (DSLM)	Keller PJ et al. (2008). Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. *Science*. doi:10.1126/science.1162493

	Modality	Reference
P16	Lattice light sheet	Chen B-C et al. (2014). Lattice light-sheet microscopy: imaging molecules to embryos at high spatiotemporal resolution. *Science*. doi:10.1126/science.1257998
P17	Light-field and fast volumetric imaging	Weisenburger S, Vaziri A (2018). A guide to emerging technologies for large-scale and whole-brain optical imaging of neuronal activity. *Annual Review of Neuroscience*. doi:10.1146/annurev-neuro-072116-031458
Fluorescence: beyond the diffraction limit		
P18	Super-resolution overview	Schermelleh L et al. (2019). Super-resolution microscopy demystified. *Nature Cell Biology*. doi:10.1038/s41556-018-0251-8
P19	STED	Klar TA et al. (2000). Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission. *PNAS*. doi:10.1073/pnas.97.15.8206
P20	3D-SIM	Schermelleh L et al. (2008). Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy. *Science*. doi:10.1126/science.1156947
P21	PALM	Betzig E et al. (2006). Imaging intracellular fluorescent proteins at nanometer resolution. *Science*. doi:10.1126/science.1127344
P22	MINFLUX	Balzarotti F et al. (2017). Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. *Science*. doi:10.1126/science.aak9913
P23	Expansion microscopy	Chen F, Tillberg PW, Boyden ES (2015). Expansion microscopy. *Science*. doi:10.1126/science.1260088