Journal of Microscopy, Vol. 278, Issue 3 2020, pp. 110–113 doi: 10.1111/jmi.12903

Introduction: 11th International Botanical Microscopy meeting

M.D. FRICKER
University of Oxford, South Parks Road, Oxford, OX1 3RB, UK

The 11th quadrennial International Botanical Microscopy meeting took place in the new conference facility at Oxford Brookes University in April 2019, 24 years after Oxford Brookes last hosted the event. This was the latest in a long sequence of these specialist meetings that bring together some of the best plant microscopists from around the world to share cutting-edge developments in techniques, and their application to plant and fungal specimens. One of the great joys of the Botanical Microscopy meetings is that the topics covered are so diverse, both in terms of the biology and also the technology. In the space of four days talks ranged over the cytoskeleton and motors, the endomembrane system, mitochondria, autophagy, programmed cell death, chromatin organisation, plasmodesmata, lipid signalling, reactive oxygen species (ROS), reactive nitrogen species (RNS), calcium signalling, cell shape, biomechanics, plant–pathogen interactions and even vegetable vectors for human pathogens. The techniques ranged from confocal super-resolution, stochastic optical reconstruction microscopy (STORM), Förster resonance energy transfer (FRET), total internal reflection fluorescence microscopy (TIRF), Brillouin, correlative light and electron microscopy (CLEM), focussed ion-beam (FIB) SEM, serial-block face (SBF) SEM, second-harmonic imaging to whole-root imaging in transparent soil. Given this plethora of new approaches and biological systems, it is interesting to reflect on how much has changed between the two meetings held at Oxford Brookes nearly a quarter of a century apart.

The origin of the International Botanical Microscopy meetings is inextricably linked to developments in electron microscopy in the 1960–1970s (Gunning, 1996), and EM techniques dominated the early meetings. Ultra-rapid freezing techniques were introduced in the 1980s (Gilkey & Staehelin, 1986), but it was not until the development of high-pressure freezing (HPF) that major breakthroughs were achieved for thick plant specimens (Dahl & Staehelin, 1989; Kaeser *et al.*, 1989). By the fifth meeting in 1995 at Oxford Brookes, HPF was set to become the gold standard in sub-cellular preservation. However, very few HPF systems were available for plant scientists then (and now), which means the overall impact has been much more restricted than the technique deserves. For those groups with access, HPF and tomography has yielded beautiful and quantitative insights into plant cell structure (Seguí-Simarro *et al.*, 2004; Donohoe *et al.*, 2007; Wang *et al.*, 2019) and can also handle non-model systems (Gergely *et al.*, 2018; Steiner *et al.*, 2018). In the intervening decades, a number of additional approaches to quantitative three-dimensional (3-D) reconstruction at the EM-level have developed, including SBF SEM (Kittelmann *et al.*, 2016; Harwood *et al.*, 2020) and FIB SEM (Lütz-Meindl *et al.*, 2016). The visually stunning results achievable with these methods were show-cased in several presentations at the meeting and through a tour of the imaging facility at Oxford Brookes.

At the light microscopy level, in the mid-1990s confocal microscopy was just taking off with an explosion of new models and imaging modes, with increased numbers of excitation wavelengths, more efficient light budgets, and more detection channels. Nevertheless, confocal microscopes were still operating at the 'classical' diffraction limit of light microscopy. It was not until the early 21st century that super-resolution or fluorescence nanoscopy techniques were introduced (Sahl *et al.*, 2017). Although these require specialised equipment, it was impressive how many presentations included data acquired with these methods. It is also instructive to consider how closing the gap between classical LM and EM resolutions has revealed considerable additional cellular complexity, perhaps most obviously in presentations focussed on transient bilateral or trilateral associations between different organelles. Thus, the meeting covered numerous permutations of physical or signalling interactions between ER-organelles, ER–plasmamembrane–cell wall, or even ER–chloroplast–plasmamembrane. Many of these have been described previously at the EM level, but the LM techniques add dynamics, and much more facile identification of the components through fluorescent-protein tags rather than immunogold labelling. Furthermore, the advent of CLEM techniques offers the best of both worlds, with direct linkage between LM and EM levels.

In this respect, it is remarkable to note that fluorescent protein technology in plants was only just emerging in 1995, initially through virus (Baulcombe *et al.*, 1995; Heinlein *et al.*, 1995; Oparka *et al.*, 1995) or transient (Hu & Cheng, 1995; Niedz *et al.*, 1995; Sheen *et al.*, 1995) expression systems, but then adopted much more widely with the identification and removal of the cryptic intron site in green fluorescent protein (GFP, Haseloff & Amos, 1995; Haseloff *et al.*, 1997; Rouwendal *et al.*, 1997). Indeed, citations for GFP in plants increased from a handful of references in 1995 to several thousand by 1999. In parallel, GFP was absent from the fifth meeting at Brookes, but had appeared as an exciting new

Correspondence to: M.D. Fricker; e-mail: mark.fricker@plants.ox.ac.uk

© 2020 The Authors. *Journal of Microscopy* published by John Wiley & Sons Ltd on behalf of Royal Microscopical Society
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

technology by the sixth meeting in St Andrews. It is still remarkable that the agrobacterium-infiltration technique for transient expression of GFP-constructs in tobacco leaf epidermal cells only appeared in 2000 (Batoko *et al.*, 2000), but now forms the basis of so much research on the endomembrane system in plants. The importance of fluorescent-protein technology to live-cell imaging cannot be understated, and about this time, the Royal Microscopical Society (RMS) also recognised the pioneering work of Roger Tsien in this field with the award of an RMS Honorary Fellowship and The Pearse Prize in 2000.

It is also worth noting that the rise of GFP has biased subsequent research towards model systems that are readily transformable, whereas traditionally microscopy has not been constrained by species, and has embraced a broad range of systems that exhibit interesting biological behaviour. Notably, *Arabidopsis* was only just beginning to gain traction as a model system in the 1990s (Somerville & Koornneef, 2002), whereas now it dominates most plant cell and developmental biology, along with a limited number of test platforms such as *Nicotiana*, or more applied research in crop plants. In contrast, most microscopy techniques developed prior to the advent of transgenic tags are far more agnostic to the species, and historically there has been a rich history in the study of different systems which exemplify a particular facet of cell biology. Thus, I have some nostalgia for the systems that once defined their cognate field such as pollen tube growth in *Lilium*, mitosis in *Tradescantia* stamen hairs, signalling in stomatal guard cells of *Commelina* and *Vicia*, microfibril deposition in *Micrasterias*, cytoplasmic streaming in *Chara*, cap-patterning in *Acetabularia*, phloem physiology of Cucurbitaceae, or ultrastructural studies of *Dionea*.

At the meeting, the benefit of examination of non-typical model organisms was most elegantly apparent in the work presented on programmed cell death (PCD) in the lace plant *Aponogeton madagascariensis* by Fraser and colleagues (Fraser *et al.*, 2020). In this system, reproducible PCD takes place as part of normal leaf development to yield a highly fenestrated leaf, and it presents an ideal experimental system to test factors affecting induction, spatial limitation and execution of PCD. Being aquatic, it is also straightforward to apply pharmacological agents to probe the underlying signal transduction networks, in this case with a focus on calcium.

Continuing with this volume, the review by Renna & Brandizzi (2020) probes the subtle and complex behaviour of the plant trans-Golgi network, that is probably the most sophisticated sorting hub in any eukaryotic cell. They draw together information about the players, their locations and their likely roles that are emerging from fluorescent protein P-tagging, super-resolution live-cell imaging, mutants and pharmacology. What emerges is a much richer and deeper understanding of the intricate workings of this elaborate organelle that was originally identified as a partially coated reticulum (PCR) observed proliferating around dictyosome membrane profiles in EM sections of *Vigna* and *Chara* (Harris & Oparka, 1983; Pesacreta & Lucas, 1984).

The importance of 3-D imaging, reconstruction and quantitation to dissect the spatial organisation within plant cells is illustrated beautifully in the review by Elliott & Kirchhelle (2020) who take the canonical view of the *Arabidopsis* root (Dolan *et al.*, 1993) and start to map the fine-grained spatial localisation of proteins to cell faces, geometric edges and vertices within a radial co-ordinate system (inner-outer and apical-basal). These form biochemically distinct domains within the cell that presumably orchestrate highly localised variation in cell-wall composition and architecture, or incorporate sensors for mechanical stress or localised signals. Detailed description of the various exocytic and endocytic trafficking pathways linked to edge and face polarity are getting ever more refined. Nevertheless, knowledge of the cargos moved through each pathway and their downstream fate have proved much more recalcitrant to investigation.

Most notably, whilst techniques for protein labelling and visualisation with fluorescent tags are well advanced, there is still relative paucity in procedures to identify carbohydrate cargos, along with the structure, composition and organisation of the cell wall polymers. The paper by Bidhendi *et al.* (2020) reviews the range of possible approaches, including polysaccharide-specific antibodies and chemical fluorescent probes and describes a set of protocols for fluorescence labelling of cellulose and pectins in live and fixed material. Using a panel of chemical probes and antibodies, they document the heterogeneity in both composition, location and alignment of wall polymers in a range of different tissues. They also note that wall composition has a dynamic component that also benefits from live-cell imaging approaches.

Whilst it is assumed that the composition and architecture of the cell wall will affect its mechanical properties, actually quantifying these over the relevant length scales from polymer– interactions, through cells to tissues is much more challenging and requires a range of different approaches reviewed by Robinson & Durand-Smet (2020). Typically, rheological measures of these composite, heterogeneous and anisotropic polymers use atomic force microscopy (AFM) techniques *in situ* or in excised material. Measurements at the whole-cell and tissue level can be achieved by measuring responses to uniaxial stresses imposed by physical deformation to determine stress–strain relationships or tissue responses to imposed stress. Individual measurements tend to be limited in scope because of the experimental challenges, but often feed into mathematical models to extrapolate from the local to global scale, or to infer mechanical coefficients from tissue responses. Mechanical perturbation is not just a biophysical tool, but is also a normal part of the plant lifestyle, whether it be roots growing through soil, wind affecting aerial plant parts or through contact with other organisms. However, it is usually minimised in laboratory-grown material and tends not to feature in a typical portfolio of experimental treatments.

It is likely therefore that there will be considerable new insights emerging as biomechanical measurement and manipulation, and cell biology become more closely integrated.

It has become traditional at these meetings to include art as part of the programme. At previous meetings, we have been wowed by the beautiful imagery of artists, such as Rob Kessler, who incorporate botanical themes into their work. At this meeting, the converse occurred – Larry Griffing took time out from his normal research to provide a convincing excursion into the world of art history, specifically to track down possibly the only known portrait of Robert Hooke, the grandfather of our discipline (Griffing, 2020).

Whilst the science, and art, on display was spectacular, this year's meeting was also emotionally charged for many participants. The final session was dedicated to the memory of Ian Moore, who died in 2018. Poignantly it was Chris Hawes who wrote his obituary (Hawes, 2019) and who specifically wished this Special Edition to be dedicated to his memory. Although unbeknown at the time, this was also to be Chris Hawes's final meeting, as he died in July 2019 (Neumann, 2019). Shortly before writing this introduction, we also received the sad news that Nick Read, who was unable to attend the meeting through illness, died in March 2020. Nick was one of the pioneers of calcium measurements and live-cell imaging in plant and fungal cells, and co-organised earlier meetings in the Botanical Microscopy series. All were good colleagues and friends for more than 30 years and will be sadly missed.

References

Batoko, H., Zheng, H.Q., Hawes, C., & Moore, I. (2000). A rab1 GTPase is required for transport between the endoplasmic reticulum and Golgi apparatus and for normal Golgi movement in plants. *Plant Cell* **12**, 2201–2218.

Baulcombe, D.C., Chapman, S. & Santa Cruz, S. (1995) Jellyfish green fluorescent protein as a reporter for virus infections. *Plant J.* **7**, 1045–1053.

Bidhendi, A., Chebli, Y. & Geitmann, A. (2020) Fluorescence visualization of cellulose and pectin in the primary plant cell wall. *J. Microsc.* **278**, 164–181.

Dahl, R. & Staehelin, L.A. (1989) High-pressure freezing for the preservation of biological structure: theory and practice. *J. Electron Microsc. Tech.* **13**, 165–174.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K. & Scheres, B. (1993) Cellular-organization of the *Arabidopsis thaliana* root. *Development* **119**, 71–84.

Donohoe, B.S., Kang, B.H. & Staehelin, L.A. (2007) Identification and characterization of COPIa- and COPIb-type vesicle classes associated with plant and algal Golgi. *Proc. Natl. Acad. Sci.* **104**, 163–168.

Elliott, L. & Kirchhelle, C. (2020) The importance of being edgy: cell geometric edges as an emerging polar domain in plant cells. *J. Microsc.* **278**, 123–131.

Fraser, M., Dauphinee, A. & Gunawardena, A. (2020) Determining the effect of calcium on cell death rate and perforation formation during leaf development in the novel model system, the lace plant (Aponogeton madagascariensis). *J. Microsc.* **278**, 132–144.

Gergely, Z.R., Martinez, D.E., Donohoe, B.S., Mogelsvang, S., Herder, R. & Staehelin, L.A. (2018) 3D electron tomographic and biochemical analysis of ER, Golgi and trans Golgi network membrane systems in stimulated Venus flytrap (*Dionaea muscipula*) glandular cells. *J. Biol. Res (Thessalon.)* **25**, 1–16.

Gilkey, J.C. & Staehelin, L.A. (1986) Advances in ultrarapid freezing for the preservation of cellular ultrastructure. *J. Electron Microsc. Tech.* **3**, 177–210.

Griffing, L. (2020) The lost portrait of Robert Hooke? *J. Microsc.* **278**, 114–122.

Gunning, B.E.S. (1996) Introduction. *J. Microsc.* **181**, 95–98.

Harris, N. & Oparka, K. (1983) Connections between dictyosomes, ER and GERL in cotyledons of mung bean (*Vigna radiata* L.). *Protoplasma* **114**, 93–102.

Harwood, R., Goodman, E., Gudmundsdottir, M., Huynh, M., Musulin, Q., Song, M. & Barbour, M.M. (2020) Cell and chloroplast anatomical features are poorly estimated from 2D cross-sections. *New Phytol.* **225**, 2567–2578.

Haseloff, J. & Amos, B. (1995) GFP in plants. *Trends Genet.* **11**, 328–329.

Haseloff, J., Siemering, K.R., Prasher, D.C. & Hodge, S. (1997) Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic Arabidopsis plants brightly. *Proc. Natl. Acad. Sci. USA* **94**, 2122–2127.

Hawes, C. (2019) In memoriam – Ian Moore. *J. Cell Science* **132**, jcs229666.

Heinlein, M., Epel, B.L., Padgett, H.S. & Beachy, R.N. (1995) Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* **270**, 1983–1985.

Hu, W. & Cheng, C.L. (1995) Expression of Aequorea green fluorescent protein in plant cells. *FEBS Lett.* **369**, 331–334.

Kaeser, W., Koyro, H.W. & Moor, H. (1989) Cryofixation of plant tissues without pretreatment. *J. Microsc.* **154**, 279–288.

Kittelmann, M., Hawes, C. & Hughes, L. (2016) Serial block face scanning electron microscopy and the reconstruction of plant cell membrane systems. *J. Microsc.* **263**, 200–211.

Lütz-Meindl, U., Luckner, M., Andosch, A. & Wanner, G. (2016) Structural stress responses and degradation of dictyosomes in algae analysed by TEM and FIB-SEM tomography. *J. Microsc.* **263**, 129–141.

Neumann, U. (2019) In memoriam – Chris Hawes. *J. Microsc.* **276**, 3–12.

Niedz, R.P., Sussman, M.R. & Satterlee, J.S. (1995) Green fluorescent protein: an in vivo reporter of plant gene expression. *Plant Cell Rep.* **14**, 403–406.

Oparka, K.J., Roberts, A.G., Prior, D.A.M., Chapman, S., Baulcombe, D. & Santa Cruz, S. (1995) Imaging the green fluorescent protein in plants — viruses carry the torch. *Protoplasma* **189**, 133–141.

Pesacreta, T.C. & Lucas, W.J. (1984) Plasma membrane coat and a coated vesicle-associated reticulum of membranes: their structure and possible interrelationship in *Chara corallina*. *J. Cell Biol.* **98**, 1537–1545.

Renna, L. & Brandizzi, F. (2020) The mysterious life of the plant trans-Golgi network: advances and tools to understand it better. *J. Microsc.* **278**, 154–163.

Robinson, S. & Durand-Smet, P. (2020) Combining tensile testing and microscopy to address a diverse range of questions. *J. Microsc.* **278**, 145–153.

Rouwendal, G.J.A., Mendes, O., Wolbert, E.J.H. & Douwe de Boer, A. (1997) Enhanced expression in tobacco of the gene encoding green fluorescent protein by modification of its codon usage. *Plant Mol. Biol.* **33**, 989–999.

Sahl, S.J., Hell, S.W. & Jakobs, S. (2017) Fluorescence nanoscopy in cell biology. *Nat. Rev. Mol. Cell Biol.* **18**, 685.

Seguí-Simarro, J.M., Austin, J.R., White, E.A. & Staehelin, L.A. (2004) Electron tomographic analysis of somatic cell plate formation in meristematic cells of Arabidopsis preserved by high-pressure freezing. *Plant Cell* **16**, 836–856.

Sheen, J., Hwang, S., Niwa, Y., Kobayashi, H. & Galbraith, D.W. (1995) Green-fluorescent protein as a new vital marker in plant cells. *Plant J.* **8**, 777–784.

Somerville, C. & Koornneef, M. (2002) A fortunate choice: the history of Arabidopsis as a model plant. *Nat. Rev. Genet.* **3**, 883–889.

Steiner, P., Luckner, M., Kerschbaum, H., Wanner, G. & Lütz-Meindl, U. (2018) Ionic stress induces fusion of mitochondria to 3-D networks: an electron tomography study. *J. Struct. Biol.* **204**, 52–63.

Wang, P., Liang, Z. & Kang, B.H. (2019) Electron tomography of plant organelles and the outlook for correlative microscopic approaches. *New Phytol.* **223**, 1756–1761.

© 2020 The Authors. *Journal of Microscopy* published by John Wiley & Sons Ltd on behalf of Royal Microscopical Society, **278**, 110–113