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In memoriam – Chris Hawes

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'I've just loved plants all my life. [. . .] when I was at school, we picked up microscopes for the first time - and that was it! Once you've used a microscope you will never want to use any other instrument'. Chris Hawes, Nature Live Talk 'Leaves and lasers' at the Natural History Museum on 15 October 2015 (https://www.youtube.com/watch?v=eAFZbIdHZ7c).

I am deeply saddened to report that Christopher Robert Hawes (born 11 June 1953), Professor of Plant Cell Biology and former Research Lead at Oxford Brookes University (Figure 1), passed away after a long-term illness in the evening of Thursday, 4 July 2019. Although not unexpected, his sudden death came as a shock to his colleagues and friends in Science all over the world generating avalanches of emails on the weekend when the news was known to a wider circle of friends and collaborators. Within less than a year, with Ian Moore, Associate Professor in Plant Sciences at the University of Oxford passing away on 31 August 2018, we have lost another outstanding plant cell biologist and English gentleman far too early, taking with him an immense knowledge of microscopy and plant cell biology that will leave a gaping hole in the scientific community. It was Chris himself who wrote a beautiful obituary for Ian in the Journal of Cell Science (2019), which must have been an extremely difficult task given that he was also suffering from the same type of cancer at the time. Of course, Chris was not only a brilliant scientist but a loving husband, brother, uncle and great uncle and a dear friend to many within and outside Science. To me, he was first an excellent mentor during my postdoc in his lab between 2001 and 2003 (setting up the high-pressure freezing unit at Oxford Brookes), then someone always willing to share and support my passion for microscopy and in addition, fortunately, a dear friend in later years. Chris will be deeply missed by everyone who was privileged to know him and our thoughts are with Kay, his family and close friends (including Lexie, the latest of Chris's beloved Newfoundland dogs) at this sad time.

Internationally renowned, inspirational and fun to be around

Chris was an internationally acknowledged researcher and a highly respected colleague at Oxford Brookes University which he joined in 1989 as Senior Lecturer, becoming then Reader, and ultimately attaining the highest grade of professorship awarded by the University. As head of an internationally renowned research group studying the organisation and function of the plant endomembrane system, notably aspects relating to ER and Golgi body biogenesis and interaction, Chris made unique contributions to the development of cell biology research at Oxford Brookes University. These contributions would never have been possible but for his deep biological knowledge coupled to an incredible ability to raise funds for his research and for state-of-the-art equipment from various funding agencies (overall approx. 5 million GBP during his career). This combination was crucial for the development of the Bioimaging Unit that Chris initiated in the Department of Biological and Medical Sciences and which received a steady flow of visitors who benefited not only from access to cutting-edge equipment but also from Chris's inestimable advice and expertise.

He was not only passionate about research (and University politics or politics in general) but also about passing on his enthusiasm and skills to others. He was head of the Oxford Brookes Biology Doctoral Training Programme and will be remembered for his commitment and kind support by the many generations of students he taught and tutored. He supervised to completion more than 20 postgraduate research degrees and the work of almost as many postdoctoral researchers. Professor Linda King, pro vice-chancellor for Research and Global Partnerships at Oxford Brookes University, comments: *'Those of us privileged to work with him knew him both as a tireless and dynamic scientist, but also as a great collaborator and often as a personal friend; someone with a big heart for others, who built his research on relationships as much as on experiments and equipment'*.

Chris also played a leading role in the Royal Microscopical Society since becoming a member in 1980. During his involvement with the Society over four decades, he occupied a number of senior positions. He joined the Electron Microscopy Committee in 1988, becoming its honorary secretary in 1992, and Chairman in 1993. He served as RMS vice president in 2002 before becoming president 2 years later. In 2015, he was honoured with the highly prestigious honorary fellowship of the RMS, a distinction that meant a lot to him. His 9-year term as executive honorary secretary came to an end only the day before he died, and it was typical of Chris's dedication that he remained fully active in the RMS throughout his battle with

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Fig. 1. Chris Hawes at the 9th International Botanical Microscopy Meeting in 2011 at Wageningen University.

cancer, and he even planned to attend the Annual General Meeting on 3 July 2019 during the Microscience Microscopy Congress in Manchester. He served as a scientific editor of the *Journal of Microscopy* from 2000 to 2018 (in addition to roles in numerous other prestigious journals), and organised the RMS Immunocytochemistry course held annually at Oxford Brookes University from 1991 to 2003, as well as the RMS Introduction to EM course from 1985 to 2010.

Chris's lasting accomplishments as a plant microscopist spanning more than 40 years made use of a wide range of microscopy techniques (Figure 2), and he often pioneered the application of emerging new techniques and technology to plant specimens, concurring with his friend Peter Hepler that *'new discoveries in cell structure and function are closely intertwined with new developments in instrumentation, and specimen preparation, together with exploitation of model organisms'* (Hepler, 2016). His outstanding scientific quality is expressed in more than 160 high-impact scientific papers and about 50 review articles with currently more than 13 000 citations and a Google Scholar h-index of 64. In addition, he authored 19 book chapters and coedited 4 books: 'Endocytosis, exocytosis and vesicle traffic in plant cells' (Chris Hawes, Julian Coleman & David Evans, Cambridge University Press, 1991), 'Electron Microscopy of Plant Cells' with John Lloyd Hall (Academic Press, 1991), 'Plant Cell Biology: A Practical Approach' with Béatrice Satiat-Jeunemaitre (Oxford University Press, 2001) and 'The Plant Endoplasmic Reticulum. Methods and Protocols' with Verena Kriechbaumer (Humana Press, 2018) which all represent seminal works in the field, providing key resources for plant scientists.

'Mister 1 000 000 Volt': high-voltage EM and thick sections to study fungal and plant ultrastructure

Chris started his scientific career at the University of Bristol in 1971, obtaining a PhD in 1977 supervised by Dr. Alan Beckett on various aspects of conidium structure and germination of the fungal plant pathogen *Ceratocystis adiposa*, causal agent of oak wilt, using both light and electron microscopy – a combination he continued to use whenever possible in future research projects. Following two 1-year positions, first as postdoctoral research assistant to Professor David Smith at the University of Bristol, then as senior electron microscope technician at Bristol Polytechnic, Chris moved to the University of Oxford in April 1979. For the next 4 years, Chris worked as postdoctoral research assistant to Professor Frederick Robert Whatley in the Department of Plant Sciences and Professor Sir Peter Hirsch in the Department of Metallurgy and Science Materials, where he used high-voltage electron microscopy (at 1 million volt) to study biological materials with a focus on the spatial organisation of the plant cell cytoplasm, in particular the endomembrane system (Hawes, 1981). Chris always easily connected to people and in March 1983, he founded the Oxford EM Users' Group, together with David Ferguson (EM unit at the John Radcliffe Hospital Oxford) and Barry Martin, EM technician in the group of David Shotton, Department of Zoology at Oxford University. The monthly meetings soon included members of Reading University (the group was formally renamed to the Oxford and Reading EM Users' Group in 1987), and the popularity of the group resulted in joint annual meetings with the Cambridge EM Users' Group in later years. It was also in the early 1980s that Chris's life-long research interest and love for plant ER–Golgi relationships first emerged. Thereafter, he would exploit many new advances in specimen preparation and imaging techniques to delve ever more deeply into plant cells, always with a sharp eye on plant endomembranes and their interrelationships. For instance, the combination of the so-called ZIO impregnation technique, used in the 1960s to study synaptic vesicles and later adapted to plant material by Nick Harris (1978), together with thick sections and beam tilt stereoscopy for high-voltage electron microscopy, provided new insights into ER organisation during mitosis and started to reveal ER/Golgi connections (Hawes *et al.*, 1981; Juniper *et al.*, 1982). PEG embedding for TEM (Hawes & Horne, 1985 – his first publication in the *Journal of Microscopy*), imaging

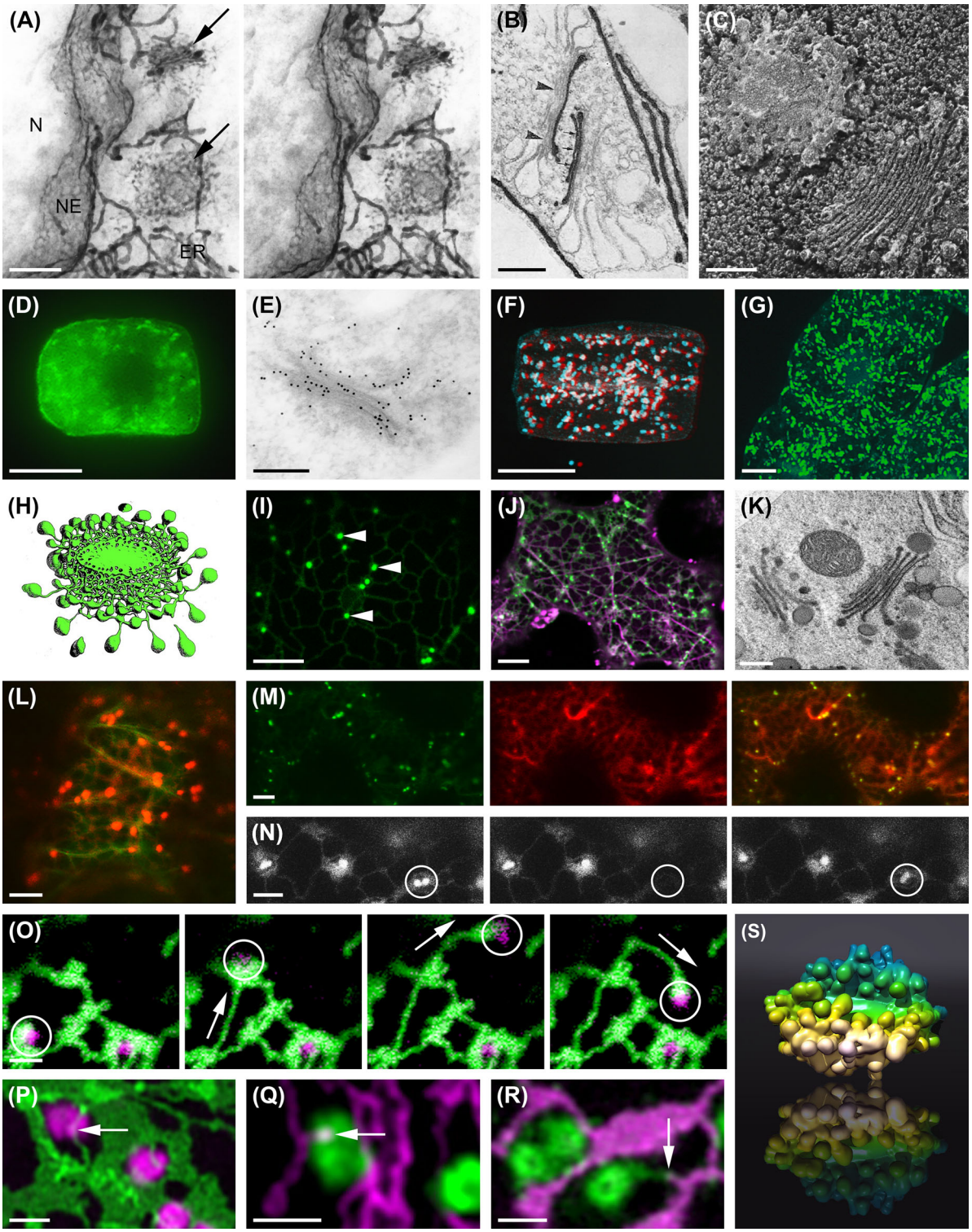

Fig. 2. The glorious and gorgeous Golgi in the course of time – snapshots by Chris Hawes and collaborators. (A–C) Golgi electron micrographs from the 1980s. (A) HVEM stereo pair of a 1 μm thick section through a ZIO-impregnated maize root cap imaged at 1 MV showing two Golgi stacks (arrows) in the vicinity of the nucleus (N)/nuclear envelope (NE). (B) Selective ZIO staining of Golgi stacks in an ultrathin section of a maize root cap cell viewed with conventional TEM at 80 kV: impregnated cisternae at the *cis*-side (small arrows) *versus* non-impregnated cisternae at the *trans*-side (arrowheads). (C) Adjacent Golgi stacks in ultrarapid-frozen, deep-etched suspension-cultured carrot cells in parallel and cross fracture (upper left and bottom right image corner respectively). (D–G) Golgi stacks uncovered by JIM84 antibodies in maize root tip cells (D–F) and suspension-cultured BY2 cells (G). Visualisation by epifluorescence microscopy (D), immunogold-TEM (E) and early 3D reconstructions of confocal sections to be viewed with red/green anaglyph glasses (F)

of extracted whole, critical-point dried cells at 1 million volt (Hawes, 1985) or rapid freeze/deep etching of plant material (Hawes and Martin, 1986) were alternative attempts to visualise membranes and organelle ultrastructure. It is noteworthy that decades later, Chris would enthusiastically dig out his 35-year-old ZIO-impregnated maize root blocks, relying on their strong contrast, to put them straight into the SEM chamber and demonstrate the value of ZIO impregnation for serial block face SEM.

Fluorescent probes to study coated vesicles and endocytosis in plants

During the early years at Oxford University, Chris developed his independence as a researcher and in 1983 became one of the first cohort of highly prestigious Royal Society University research fellows. Together with his co-fellow and long-term colleague David Evans, specialist in biochemistry, Julian Coleman and Louise Cole, he made the exciting discovery that brain clathrin antibodies could recognise coated vesicles in carrot suspension culture cells (Cole *et al.*, 1987), a project that involved a lot of time spent on plant cell culture as David remembers: *'We were a small team making real progress; and where Chris's skills in imaging meant we could visualise not only coated vesicles, but even clathrin triskelions, measuring the lengths of heavy and light chains for comparison with mammalian studies'*. The project led Chris and his colleagues at Oxford University to explore the emerging field of endocytosis in plant cells, adding live cell imaging techniques to his repertoire. Searching for the right marker to visualise the dynamics of endocytic processes at the light microscopy level was the foundation of a long-term collaboration with Karl Oparka (Cole *et al.*, 1990, 1991; Oparka & Hawes, 1992). While at Oxford University, Chris also teamed up with French CNRS researcher Béatrice Satiat-Jeunemaitre who arrived as a visiting member for a duration of 6 months at the end of 1988, working on exocytosis and cell wall morphogenesis. In the end, 6 months turned into 7 years and into another fruitful collaboration that would prove significant during Chris's next move 'up the hill'.

A new Bioimaging Unit and two powerful new tools: an antibody and a drug to dissect the plant Golgi apparatus

In September 1989, Chris moved to Oxford Polytechnic, now Oxford Brookes University, as Senior Lecturer, being charged with setting up the first EM suite. On appointment, Chris immediately convinced Oxford Polytechnic that they were buying the wrong electron microscope and should instead purchase a 120 kV machine, like the one he had just persuaded Oxford University to buy for his laboratory there! In addition, he equipped the lab with a high-end fluorescence microscope, fore-runner of the cutting-edge confocal laser scanning microscopes so important to Chris's later research. He was allowed to recruit Louise Cole, expert on plant electron microscopy as PhD student and Barry Martin, who would work with Chris for more than 20 years until his retirement in 2011. A new Bioimaging Unit at Oxford Brookes University was emerging.

In the early years at Oxford Brookes University, while Chris was heavily involved in building up his teaching, Béatrice's experience as a plant cell biologist using immunocytochemistry both at the light as well as at the electron microscopy level helped immensely in the coordination of daily research. Together, Chris and Béatrice deciphered key aspects of Golgi function and biogenesis with the help of two powerful new tools: JIM84, an antibody that specifically labelled Golgi stacks (Horsley *et al.*, 1993; later shown to identify Lewis a epitopes – Fitchette *et al.*, 1999), and the fungal drug Brefeldin A, which triggers the redistribution of Golgi membranes into the ER and *de novo* formation of Golgi upon washing it out. This new toolbox yielded a series of highly cited publications (Satiat-Jeunemaitre & Hawes, 1992a,b). Béatrice recalls: *'There was a pile of Falcon tubes full of antibodies behind a door, made at the John Innes Institute by Chris's PhD student David Horsley. They were supposed to be made against clathrin, but proved to be*

or maximum-projected (G). (H) Chris's favourite logo, depicting Golgi membrane connections and dynamics. (I, J) Illuminating Golgi stacks (arrowheads) and the polygonal network of cortical ER by virus-mediated expression of ERD2-GFP in *Nicotiana clevelandii* leaves, unravelling the colocalisation of the ER/Golgi system with actin cables (labelled by rhodamine-phalloidin, magenta in J). (K) TEM micrograph of Golgi stacks in high-pressure frozen and freeze-substituted tobacco root cap cells. (L–N) Elucidating Golgi dynamics in tobacco leaf epidermal cells *via* Agrobacterium-mediated transformation. (L) ST-YFP-labelled Golgi stacks (red) align on talin-GFP-tagged actin cables (green). (M) Golgi stacks and ER export site markers (labelled by ERD2-GFP and Sar1-YFP respectively) form a mobile secretory unit. (N) Fluorescence recovery of ERD2-GFP after latrunculin treatment and photobleaching of two adjacent Golgi stacks (circle). (O) Shaping the ER by optical tweezers: ER tubule formation (arrows) induced by movement of a trapped Golgi body (circle) pulled across the cytoplasm. (P–R) Revealing membrane connections between the Golgi and the ER (arrows) by high-resolution Airyscan imaging of various fluorescent fusion proteins (P: GFP-HDEL & ST-mRFP; Q: ST-GFP & mRFP-HDEL; R: mRFP-HDEL & Sec24-GFP). (S) 3D Golgi body from an *Arabidopsis thaliana* root cell stained with the ZIO technique. Data were produced using SBF-SEM followed by region of interest segmentation and thresholding based on staining intensity. Scale bars: 500 nm (A), 250 nm (B, C, E, K), 10 μm (D, F, G, I, J, M), 5 μm (L, N), 2 μm (O), 1 μm (P, Q, R).
Micrographs in (A) and (B) adopted from Hawes (1981) and in (C) from Hawes & Satiat-Jeunemaitre (1996) and reproduced with permission from Elsevier. Micrograph in (E) adopted from Satiat-Jeunemaitre *et al.* (1996) and in (O) from Sparkes *et al.* (2009a) and reproduced with permission from John Wiley & Sons. Unpublished micrographs courtesy of Béatrice Satiat-Jeunemaitre (D, F, G), Petra Boevink (I, J), Federica Brandizzi (L, M, N), Verena Kriechbaumer (P, Q, R), and Louise Hughes (S).

useless as clathrin markers on Western blots. As I was struggling at the time to find a right plasma membrane marker, Chris handed me a sample marked "JIM84", saying "B, you may try this one, not sure what you may find". So I "tried", on my systems, at the light and EM level. Probably our first attempt at correlative light and electron microscopy! And here it was, immunogold staining revealing clouds of gold particles around exocytic vesicles and Golgi stacks, immunofluorescence permitting for the first time to visualise the entire Golgi apparatus of one plant cell at the light microscopical level. At the same time, there was this young girl Jennifer Lippincott-Schwartz, who was using a funny drug to alter the organisation of mammalian Golgi, Brefeldin A. The way to explore some key features of plant Golgi biology was open! It has been a wonderful exciting time, source of many collaborations and experiments to come'.

Beauty comes from within: live cell imaging of glowing Golgi and ER

A new era of microscopy certainly began when, in combination with the enormous progress in instrumentation (CLSM), GFP was successfully introduced into plant cells and delivered to the secretory pathway by adding targeting and retention signals to the fluorescent protein (Hawes *et al.*, 2001; Brandizzi *et al.*, 2002a). This period of Chris's research is intimately linked with the work of Karl Oparka and collaborators at the Scottish Crop Research Institute, now James Hutton Institute, in Dundee. With the help of viral vectors, GFP was successfully targeted to the ER and to Golgi stacks, ground-breaking work that was described in the highly cited paper by Boevink *et al.* (1996). Witnessing the moment when static images of fixed material moved on to images of living cells and when fluorescence tagging allowed dynamics and interactions of organelles and proteins to be observed live must have been a career-altering experience for any cell biologist and microscopist! Petra Boevink, a postdoctoral researcher in Karl's lab at the time, remembers Chris's excitement: *'The ER was indeed beautiful [. . .] and it was fantastic to see its mobility (though on the old, somewhat slower confocal that made it hard to capture!). I think visualising the Golgi, and particularly the Golgi and ER together [. . .] were what really hit the spot with Chris. Observing the close association I think sparked a whole new hypothesis for him and a fruitful line of research'*.

This was also a scientific topic that could captivate a wider public, especially because it dispelled the wrong yet wide-spread notion of plants being inert and immobile – and hence boring. It was highly entertaining when Chris, in the Oxford Brookes University's Christmas lecture in 2001 described the new technology to a broad audience using images from the 1964 Goldfinger movie to explain the use of laser power in confocal microscopy (the famous scene when Auric Goldfinger *alias* Gerd Fröbe wants to slice up James Bond *alias* Sean Connery with a laser beam) and an image of *The Incredible Hulk* to "provide proof" that long before plants and mice, human beings had already been transformed and made green by GFP! When talking about spectral variants of GFP (basically green and red at the time) in one of his last slides he jokingly put the slogan of a big telecommunication company 'The future is orange'. This yielded the expected laughter, but also anticipated the much wider palette of new fluorescent and fluorogenic labels with emission ranging from blue to far-red that are now available for live-cell imaging.

The following years saw an explosion of plant cell biology papers based on the new GFP-technology and Chris and his collaborators pushed forward our knowledge of the interrelated dynamics of the plant endomembrane system and the plant cytoskeleton. The most outstanding is surely the famous '*Stacks on tracks*' paper (Boevink *et al.*, 1998), with currently more than 800 citations (Google Scholar) and one of the papers that Chris was most proud of. It showed that Golgi stacks moved rapidly over the cortical ER network in *Nicotiana clevelandii* leaf epidermal cells and that the precise architecture of the ER network was matched by an underlying actin network. Moreover, it demonstrated that Golgi movement was dependent on actin cables, work that built upon previous complementary studies using actin-depolymerising drugs and JIM84 immunofluorescence labelling of the Golgi apparatus (Satiat-Jeunemaitre *et al.*, 1996).

The molecular era of the new millennium: studying the regulatory machinery of membrane trafficking

At the beginning of the new millennium, the confocal microscopes in Chris's group were innovatively employed to decipher many aspects of the plant secretory pathway. Chris's microscopy work during those years was fundamentally supported by molecular approaches identifying regulatory proteins like small Rab GTPases involved in the plant trafficking machinery (Andreeva *et al.*, 1997, 1998) and was shaped by a close collaboration with the group of Ian Moore at Oxford University. Although at first, when Ian was appointed to the Department of Plant Sciences, Chris was '*reticent about another plant secretory pathway researcher coming to Oxford just one km down the hill*', it turned out to be a '*wonderful on-off collaboration that lasted for 23 years*' (Hawes, 2019). One outcome of Chris and Ian putting their heads together was the method by which tobacco leaf epidermal cells were transformed in order to transiently express fluorescent protein fusions or other constructs of interest. The idea emerged after witnessing the infiltration of water into pea leaves during a visit to Karl Oparka's lab at the SCRI in Dundee in the mid-1990s. Quickly, the method by which a diluted *Agrobacterium* suspension was delivered to the intercellular mesophyll spaces of tobacco leaves became daily routine for everyone in both Oxford labs up and down Headington Hill imaging fluorescently labelled proteins by confocal microscopy (Batoko *et al.*, 2000) and a standard

procedure used internationally in the following years (Sparkes *et al.*, 2006). In addition to the close collaboration with Ian's lab, Chris also teamed up with Jürgen Denecke, Leeds University, to corroborate microscopy results with more easily quantifiable biochemical data based on a functional assay of α-amylase secretion and the transient synthesis of heterologous proteins in tobacco protoplasts (Leborgne-Castel *et al.*, 1999) that could be used to measure protein transport through the plant endomembrane system. Federica Brandizzi, long-term collaborator of Chris, with a total of 27 shared publications, personifies that era and depth of collaboration: a postdoc first in Ian's and then in Chris's group, she made numerous trips to Leeds visiting Jürgen Denecke's lab. Chris was always open to new microscopy advances. Federica remembers Chris taking his group to the Royal Society in London where a confocal microscope was equipped with selective photobleaching capabilities for florescence recovery after photobleaching (FRAP) analyses. '*He was so excited about the microscope and its potential, like a little boy in a candy shop! He asked the microscope personnel to help photobleach a Golgi stack in a sample that we had brought from the lab. To Chris's and everybody else's surprise, the fluorescence in the Golgi stack recovered. Chris was over the moon and exclaimed: "You see, Fede, it recovers, it recovers!". I do not know if it was more exciting to see his excitement or seeing the Golgi recover*!' recalls Federica. Her outstanding and highly cited work with Chris using FRAP technology immensely contributed to our knowledge on the energy requirements of protein transport, the tight interrelationship between endomembranes and the cytoskeleton, and the formation and dynamics of ER-export sites (Brandizzi *et al.*, 2002b; Saint-Jore *et al.*, 2002; DaSilva *et al.*, 2004).

LN_2 fog and big bangs – high-pressure freezing at Oxford Brookes University

Confocal laser scanning microscopy studies in Chris's lab on dynamics of the plant endomembrane system were often complemented by structural and immunogold transmission electron microscopy – superresolution at its best as Chris would say. In 2001, Chris received a BBSRC grant that enabled him to purchase a high-pressure freezer, becoming the first UK lab to apply this technology to plant specimens. One of Chris's outstanding qualities was his deep knowledge of both light and electron microscopy and his very clever way of applying them in tandem whenever possible (Osterrieder *et al.*, 2009; Wang *et al.*, 2011). With the facility being open to all BBSRC grant holders, new UK collaborations were initiated. One of the first customers to use this facility was Lorenzo Frigerio (Warwick University) – at the time working on the transport of ricin through the Golgi to storage vacuoles in the *Ricinus communis* endosperm (Jolliffe *et al.*, 2004). Chris and Lorenzo would successfully continue their collaboration with no fewer than 16 joint publications, the latest as recently as 2018.

Pushing the limits of confocal laser scanning microscopy: optical tweezers, FRET-FLIM and superresolution microscopy

Although the green GFP-triggered revolution remains unmatched in its relevance to progress in the field of plant cell biology, many cutting-edge developments were added to the portfolio of confocal laser scanning microscopy techniques and technologies in following years. Whenever possible, Chris would make sure that these became available to him and his lab members to further advance his knowledge of the ER, the Golgi apparatus and the tight relationships between these compartments - either on site at Oxford Brookes University or through UK and European collaborations.

In addition to the use of photoactivatable GFP (Runions *et al.*, 2006) and the application of new analytical tools to monitor ER network remodelling under various conditions (Sparkes *et al.*, 2009b), it was the so-called optical tweezers used to micromanipulate Golgi stacks and ER strands (Sparkes *et al.*, 2009a; Hawes *et al.*, 2010) that helped to address the question of whether the Golgi and ER are physically attached to one another. A question difficult to address by standard confocal microscopy techniques – which made it the perfect research question for Chris! His description of the first optical tweezer experiments with Imogen Sparkes, postdoctoral researcher in his lab at the time (2003–2009) and close colleague with 23 joint publications, Tijs Ketelaar and Norbert de Ruijter, collaborators at Wageningen University, overflowed with infectious enthusiasm and the experiments yielded amazing movies that Chris never got tired of showing. Tijs Ketelaar remembers: *'Collecting data was hard work, first we tried to trap Golgi bodies in untreated cotyledons, but the cytoplasmic streaming was more than the tweezers could handle. After we depleted all latrunculin stocks in the whole building, we were able to successfully trap Golgi bodies and move them around. [. . .] the screams of joy and excitement could be heard from a long distance'.* Chris clearly enjoyed weaving new ER networks by grabbing Golgi stacks and moving them around the cell. Imogen Sparkes recalls the unique experience: *'After completing the objectives it then of course descended into whether we could write our names in the trailing ER! We never managed to succeed. [. . .] these experiments with Chris are one of my best scientific experiences. We were able to try something really wacky and novel driven by our curiosity, just how science should be'.* The research on remodelling ER membranes was further fuelled by work on reticulons in close collaboration with Lorenzo Frigerio (Tolley *et al.*, 2008; Sparkes *et al.*, 2010) and much advanced in recent years by Verena Kriechbaumer (Kriechbaumer *et al.*, 2015, 2018), postdoctoral researcher in Chris's lab for a few months in 2007 and again from 2012 until 2017, then becoming a close colleague of Chris and co-lead of the Plant Endomembrane Group at Oxford Brookes University.

Before his acquisition in 2015 of two confocal systems fitted with a new type of detector providing improved signal-to-noise ratio and superresolution, Chris made use of the unique Central Laser Facility of the Rutherford Appleton Laboratory at

the Harwell Campus in Oxfordshire. During two consecutive programmes (2009–2016 and 2016–2020) funded by the Science and Technology Facilities Council that granted access to the FRET-FLIM, single-molecule imaging and optical tweezer systems, numerous visits enabled Chris and his team to image details of the endomembrane system at previously unprecedented resolution, depth and speed. Verena Kriechbaumer recalls the experiments: *'Harwell weeks always mean tons of preparation work and long hours in cold, dark rooms but also so much excitement, especially when Chris would drop in ready to be bombarded with all the fantastic results and to glue his nose to the screen with us. His expectation was no less than a paper per week!'*

In addition to working on the ER membrane remodelling mentioned above, FRET and FLIM applications were pivotal in assessing protein interactions between Golgi tethering factors and small GTPases (Osterrieder *et al.*, 2009), in studying the distribution of Golgi-resident enzymes in a time-resolved manner (Schoberer *et al.*, 2013), in characterising protein complexes at ER–plasma membrane contact sites in collaboration with Patrick Huessy from Durham University (Wang *et al.*, 2014), as well as in elucidating the role of ER–Golgi-tethering proteins (Osterrieder *et al.*, 2017).

Much of the methodological knowledge that Chris gained, particularly during the last decade, on the structure and remodelling of the ER network was further advanced by his final PhD student Charlotte Pain in collaboration with Mark Fricker, culminating in the last paper that Chris lived to see published describing integrated tools to quantitatively analyse the plant ER architecture and dynamics (Pain *et al.*, 2019).

Big machines – big toys: 3D-EM

Apart from his unlimited curiosity and enthusiasm for anything related to (plant) cell biology, Chris was known for his almost childlike joy to drive any type of microscope (Figure 3). Summoning Chris to the microscope for help was a risky business as, once the control knobs were handed over to him, it could be challenging to get into the pilot's seat again. Therefore, it goes without saying that he was very proud of the Oxford Brookes Bioimaging Unit that he created over the last 30 years and that must have felt like a vast microscopy theme park to him. In 2013, Chris obtained a BBSRC equipment grant worth 750 000 GBP for a serial sectioning FEGSEM and received significant support from RMC Boeckeler to update the preparative equipment of the Bioimaging Unit which, in 2015, became a member of the Carl Zeiss labs@location programme, a collaborative partnership on biological imaging. As always, Chris very early recognised the potential of automated 3D EM to open up a new era of electron microscopy, as witnessed by the Special Issue of the *Journal of Microscopy* 'Three-dimensional SEM – the future of cell imaging' (2015) that he coedited with Eric Hummel, product manager at Carl Zeiss AG (Oberkochen, Germany) and former postdoctoral researcher in Chris's lab from 2006 until 2009, and he quickly applied it to his own research (Kittelmann *et al.*, 2016). The impression that 3D-SEM made on Chris was probably comparable to that of seeing the GFP-illuminated ER network live and in 'living colour' for the first time. In his Nature Live Talk 'Leaves and lasers' at the Natural History Museum on 15 October 2015 to mark the 350th anniversary of Robert Hooke's *Micrographia*, Chris comments – with his typical good humour – on the

Fig. 3. Playing with microscopes – Chris Hawes in front of the TEM in the newly built Sinclair Annexe Building at Oxford Brookes University in July 2016. Picture courtesy of Louise Hughes.

benefits of automated microscopy: *'It's great now doing microscopy. You set it up and you can go home and have a beer. Actually, you can have 24 hours' worth of beers and come back and collect your data'*. In real life, Chris would not immediately take advantage of the time liberated for other tasks or activities by automated imaging processes. Louise Hughes, bioimaging researcher and microscopist who ran the Oxford Brookes Bioimaging Unit from 2011 until 2018, describes the excitement of the early days of 3D-SEM: *'During the first automated run we were doing, he was sitting in front of the machine, commenting on every single image that appeared on the screen one after the other'*.

Bringing people together

Teaming up with Chris, either as a postdoc or a more senior collaborator was a win–win situation. Chris' dedication to plant microscopy was evidenced not only by his quantifiable output of scientific publications but also by his animation of the international community of plant cell biologists, promoting the exchange and discussion of new and challenging scientific ideas and concepts in friendly rivalry. This is well illustrated by his role in organising the RMS International Botanical Microscopy Meetings, which he resuscitated in the early 1990s and which were subsequently held every 4 years. His positive, encouraging, enthusiastic and unpretentious character set the tone of these meetings, which felt more like a gathering of friends and family than is usual for a scientific conference. Despite being already seriously affected by his illness, it is due to his immense will-power, mental strength and absolute determination that he was able to coorganise and host the 11th International Botanical Microscopy Meeting at Oxford Brookes University in April 2019, attending every single talk. Even more remarkably, he gave a presentation on the ER–Golgi interface summarising many puzzling observations made over many years which 'had fallen together' and challenging current views on the topic. Chris was very touched by the fact that so many people made it to the meeting. The only thing he regretted was that David Robinson, his scientific sparring partner of long duration, with whom he and Federica Brandizzi could have endless discussions over the existence of COPII vesicles and the exact nature of ER export sites (see also the very special paper by Robinson *et al.*, 2015), was not able to attend the meeting. I am certain that Chris's presentation entitled 'The ER–Golgi interface – revealed at last?' will remain in the memories of all those who had the privilege to be there.

Concluding remarks

In Chris, we have lost a fantastic person and an excellent scientist – he will be sorely missed! The global community of plant cell biologists that Chris helped to create will continue as one of his greatest legacies and I am convinced that his science will inspire generations of plant cell biologists to come. Among many fond and funny memories of Chris, I will always remember his answer to students (and colleagues) when confronted with their cell biology-related questions: '*Don't ask why – ask how!*'

To honour and commemorate Chris's work, a symposium will be held at Corpus Christi College and Oxford Brookes University on 8 November 2019 with a series of invited lectures by renowned international speakers on scientific topics that fascinated Chris over his long, but prematurely curtailed career.

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