



Don't ask why - ask how!

In memoriam of Chris Hawes Hon FRMS

Corpus Christi College, Oxford, UK
8 November 2019

Organisers: Federica Brandizzi, Verena Kriechbaumer, Ulla Neumann, Béatrice Satiat-Jeunemaitre, Allison Winton, Jessica Cole & Lucy Ridler

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Friday 8th November 2019

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Béatrice Satiat-Jeunemaitre & Allison Winton

Symposium schedule:

9:00-9:30	Arrival /registration Corpus Christi College, Oxford University		
9:30-10:00	David Evans	Welcome	
10:00-10:50	<i>Béatrice Siatat- Jeunemaitre</i> “From clathrin cages to Golgi coats”	Karl Oparka “Words for a friend” Petra Boevink “Conversion of a molecular biologist” Clare Steele-King “A PhD with Chris Hawes- Life on the Diamond Knife”	Mark Fricker “A tale of trivia”
10:50-11:40	<i>Federica Brandizzi</i> "The green revolution in plant imaging"	Jürgen Denecke "Spending time at the microscope: better than a detective story"	Nadine Paris “How fluorescent proteins shed light on plant cell proton homeostasis”
11:40-12:30	Flash presentations		
	Lunch Break		
13:30-14:20	<i>Imogen Sparkes</i> “Stacks on and off tracks: pushing the boundaries in light microscopy”	Lorenzo Frigerio “Webs and dustbins”	Chris Stubbs “Ultrafast femtosecond lasers for time domain imaging in plants”
14:20-15:10	<i>Ulla Neumann</i> "Through thick and thin – 40 years of plant EM”	Louise Hughes "Stack it up: serial section and serial-block face SEM”	Eric Hummel "Trend Setting Microscopy finding new ways in plant imaging"
15:10-15:40	Coffee/tea break		
15:40-16:30	<i>Verena Kriechbaumer</i> “Veni, Vidi, Imaging: Measuring up to the plant endomembrane system”	Patrick Moreau “A journey in the secretory pathway: from lipids to SNAREs”	Patrick Hussey “Building an ER-PM Contact site”
16:30-17:00	<i>Allison Winton, Susan Anderson & Pete Nellist</i>	Chris & the RMS	
17:00	Ending Corpus Christi day		
18:00-18:30	Brookes Bioimaging Tours at Oxford Brookes University		
18:30-20:30	Dinner at Oxford Brookes University (Taylors Yard, John Henry Brookes Building)		
21h onwards	Gathering in Oxford Pub		

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In memoriam – Chris Hawes

ULLA NEUMANN

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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=eAFZbldHZ7c>).

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

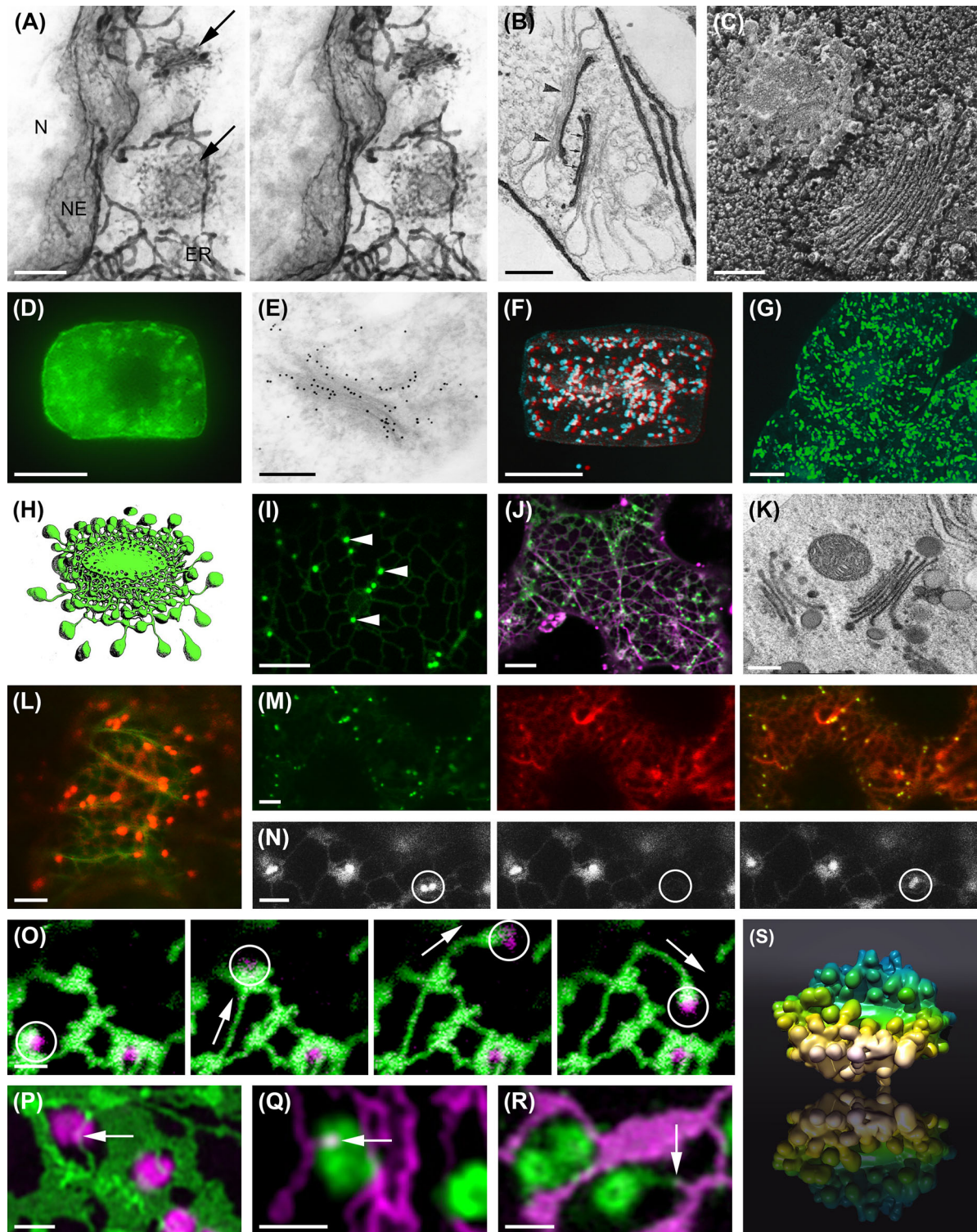


Fig. 2. The glorious and gorgeous Golgi in the course of time – snapshots by Chris Hawes and collaborators. (A–C) Golgi electron micrographs from the 1980s. (A) HVEM stereo pair of a 1 µm thick section through a ZIO-impregnated maize root cap imaged at 1 MV showing two Golgi stacks (arrows) in the vicinity 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 clelandii* 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 μ m (D, F, G, I, J, M), 5 μ m (L, N), 2 μ m (O), 1 μ m (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 fluorescence 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).

LN₂ 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 Kriebaumer 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 Huebner 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.

Acknowledgements

Many people have helped in writing this obituary by sharing fond memories of Chris and by contributing images: I particularly would like to thank Béatrice Satiat-Jeunemaitre, Petra Boevink, Federica Brandizzi, Imogen Sparkes, Tijs Ketelaar, Verena Kriechbaumer and Louise Hughes as well as David Evans, Barry Martin and Linda King for their invaluable contributions. I would also like to express my gratitude to Richard O'Connell for two rounds of in-depth proofreading.

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Big dogs, fluorescent proteins and toy microscopes

Karl Oparka

I first met Chris Hawes in 1980 when I was a postdoc at Durham University. At the time Chris was at Oxford University where he was using the million-volt electron microscope to produce thick-section images of plant cells. In Durham, along with Nick Harris, I was using the zinc iodide-osmium (ZIO) technique to highlight the ER and Golgi systems in plant cells. Chris and I were invited to give back-to-back seminars at an SEB meeting in Durham on the cortical ER system in plants. Before our talks, Chris said he had to let his dog out for a stroll. I accompanied him to the car and genuinely thought he had a cow in the back of his small car. That was my first introduction to Newfoundlands, of which there was a succession over the next forty years.

Chris and I hit it off immediately and we became the best of friends both inside and outside of science. Our careers moved closely in parallel and we made frequent visits to each other's labs (Chris subsequently moved to Oxford Brookes and I moved to the Scottish Crop Research Institute, SCRI, in 1983). Whenever I visited Chris and Kay, I always made sure to pack several pairs of trousers to accommodate the saliva of the latest Newfoundland. Our working relationship was built on complete trust and our confidence in the ability of the other to deliver the necessary science. This was cemented by our lasting passion for microscopy in all its forms (the term 'imaging' hadn't been coined then). To put things in a historical context, imaging at the time was seen as the poor relative to the rapidly expanding area of molecular biology and wasn't seen as at all 'trendy'. This did not put us off in any way and we continued to push the boundaries of plant imaging wherever and whenever we could, mostly using TEM or basic fluorescence microscopy. We became quite the double act and began to explore the use of newly developed fluorescent probes for cell biology. We had a brief foray into the field of plant endocytosis (at the time not thought to be possible due to the high turgor of plant cells). We continued to hone our imaging skills and were poised to exploit the advent of confocal imaging when it arrived. During this period, our groups published many papers together.

In 1996, my group was working on plasmodesmata (PD) and I was developing and increasing interest in the exploitation of PD during the cell-to-cell movement of viruses. At this time, along with Simon Santa Cruz and Sean Chapman, we developed the first viral vectors capable of expressing the newly discovered GFP. This was highly significant because the transgenic expression of GFP *in planta* was hindered by a cryptic splice site that took a few years to overcome. This crucial time window gave us a unique opportunity to exploit viral expression of GFP for all sorts of purposes. I recall clearly, as did Chris, a phone call we had in which I suggested to him that we target GFP to the ER using the potato virus X (PVX) expression system. Chris was, of course, very keen and so began a revolution in plant-based GFP imaging. It seems ludicrous now, but we applied to the Leverhulme Trust for a grant to target GFP to the ER using the HDEL retention signal. One of the reviewers was pessimistic, recording for posterity that this venture was too technically challenging! We got the grant and during a lecture tour of Australia I bumped into Petra Boevink who was finishing off her PhD in Canberra. She had a strong background in plant virology and was keen to move into cell biology. Petra moved to my lab and within a short space of time had targeted GFP-HDEL to the ER, producing the first images of the dynamic nature of the cortical ER system in plants. Although commonplace now, these images were groundbreaking at the time, and revealed the enormous potential for targeting GFP to a plethora of plant organelles for non-invasive imaging. Shortly after this, we targeted GFP to the Golgi using the sialyl transferase (ST) sequence from rat. I remember, distinctly, sitting in the confocal room at SCRI along with Chris and Petra looking at the Golgi whizzing around the cortex of plant cells. We stared at the screen in total disbelief for ages before Chris said 'we need rhodamine phalloidin'. When we stained the actin network we saw the intimate relationship between the Golgi bodies and the underlying actin network. Chris shouted at the screen, 'Stacks on Tracks'! Our publication of this seminal paper (Boevink et al., 1998), and the images it contained, remained a subject of pride for Chris and I throughout our careers. The rest, as they say, is history. However, it is nice to remember the origins of the work that led to so many other future publications on Golgi/ER dynamics in plant cells.

Of course time moved on, and we continued to pursue our personal research interests. However, we never stopped collaborating on topics of mutual interest. When I moved to Edinburgh we went on to produce the first super-resolution images of PD (Fitzgibbon et al., 2010) and more recently my group (Knox et al., 2015) in collaboration with Chris's group (Kriechbaumer et al., 2015) examined the role of reticulons in primary PD formation.

Our fascination with imaging and in pushing microscopes to their limits never faltered and our enthusiasm never waned. Countless students from both our labs will recall that we behaved like young children whenever we were placed in front of a microscope, often side-lining the poor operator while we feverishly collected images that were placed into temporary files with original-sounding titles such as 'Good 1' (there were a lot of images in this file). When I started lecturing at Edinburgh University, Chris kindly sent me copies of all his lectures to plagiarise as I saw fit. It is testament to the passage of time that all the undergraduate students we taught were mesmerised and motivated by the images that Chris and I collected over our careers.

I will miss my good friend and colleague very much.

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Endocytosis, Exocytosis, Golgi Biology : 10 years of close collaboration (1989-2000), 30 Years of friendship

Satiat-Jeunemaitre Béatrice (CNRS) and Hawes Christopher (University of Oxford & Oxford Brookes University)

Objectives

Fully enjoy Research in plant cell biology.

Strategy

Friendship, mutual liking and regard on plant cell biology. Channel one's energy & enthusiasm to have fun in Science

MATERIAL & METHODS



Material: Any plant roots, epicotyls, hypocotyls, (no arabidopsis!)

Methods : Light and transmission electron microscopy, immunolocalisation, pharmacological approaches

AND...a mad team for serious business



Louise,
Julian,
David
Larry,
Barry,
Clare,
Kim,
Ann, ...



RESULTS :

Hundreds of TEM negatives; thousand of multiwell slides and TEM grids, countless observations in darkness with microscope for only companion, or in darkrooms for micrograph printing and plates making; thousands of exchanges around liters of tea (*with Milk for me please*) or *coffee (more water please)*; a very few arguments on how to handle research and life, but endless enthusiastic discussions with diagrams and schemes; always animated cross-fertilization of ideas on how a Golgi may work and how a vesicle may reach its target at the right time at the right place; 15 co-authored papers, 7 book chapters, 1 co-edited book; wonderful collaborators all over the world; 10 years of CH/BSJ close collaboration, 30 years of friendship.

JIM84, a first multiscale marker for Golgi; BFA compartments and plant Golgi Biology; map for trafficking pathways in plant cells ; Golgi intracellular organisation & actin dependency

The Brookes Cell Biology floor



ACKNOWLEDGEMENTS

Thank you Chris to have been there

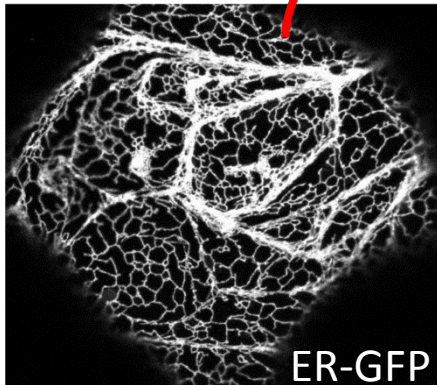


The conversion of a molecular biologist

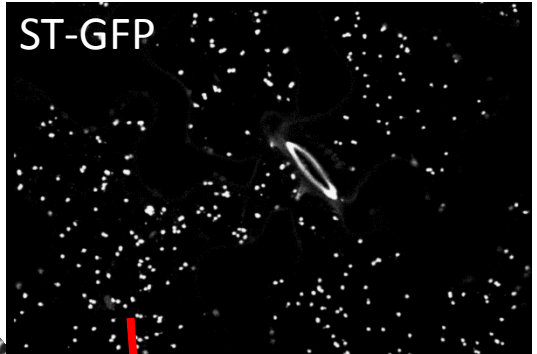
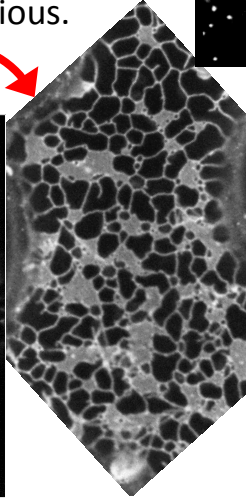
Petra Boevink, The James Hutton Institute, Dundee

I arrived in the UK a molecular biologist and was converted to cell biology by the beauty of the plant endomembrane system, encouraged by Chris and Karl. Using the power and speed of viral expression we first lit up the ER, swiftly followed by the Golgi, then both together. Chris' excitement at seeing the Golgi stacks moving along the ER was joyous and infectious.

Of course we had to play with Chris' favourite inhibitor, Brefeldin A.

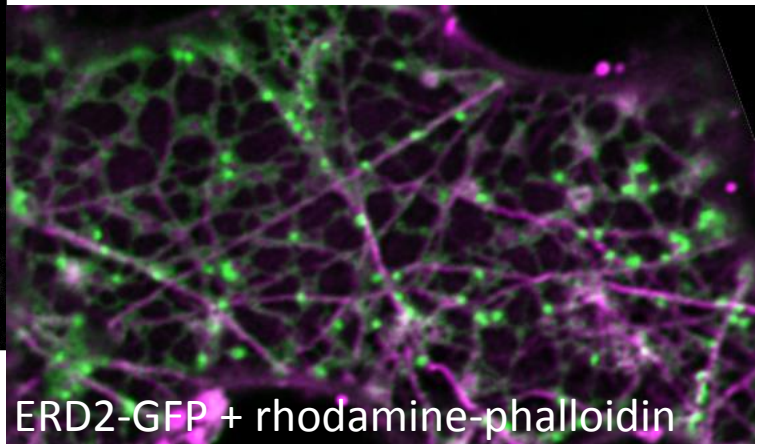
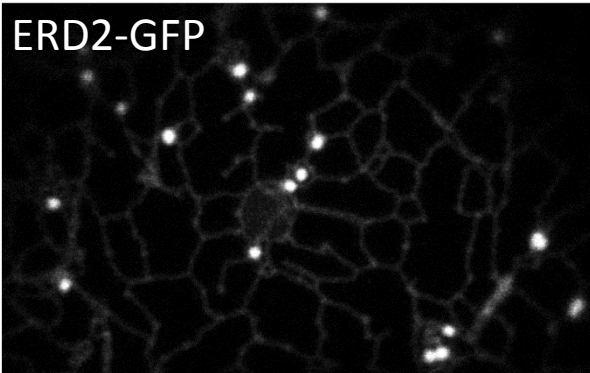


+ BFA

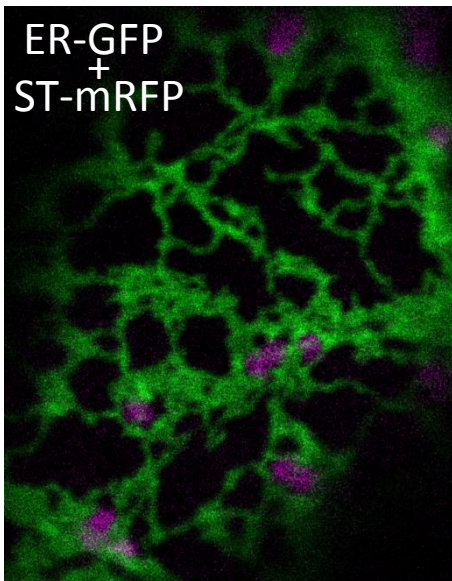


Stacks on Tracks! Chris used to email me regularly with updates on the number of citations for this paper; 'a beer when we get to 100', 'champagne at 500'.

ERD2-GFP

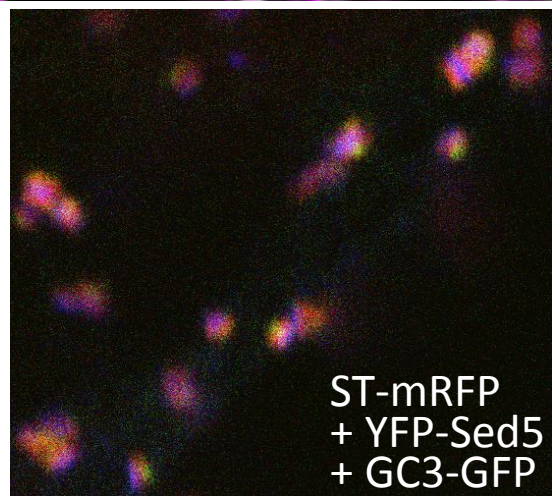


ER-GFP
+
ST-mRFP



Independent labelling of Golgi and ER allows greater specificity (and great movies!)

Triple-labelling of Golgi! Maita finds the plant golgins



The Oxford Brookes minibus links endomembranes to red traffic lights



What might look like a stereo pair image at first glance is in reality proof of my alleged offence of contravening a red traffic light on 4 September 2001, at exactly 14h20, on the A40 near Thornhill Park & Ride at East Oxford. Had the images been taken from the front of the car, you would see myself driving the vehicle with Chris (and an Oxford Brookes driving instructor) sitting next to me!

At the time, I was post-doc in Chris's lab and it was the period of intense collaboration with Ian Moore's group from Plant Sciences, University of Oxford. In September 2001, we were all to attend the 4th ENPR meeting at Heidelberg and Chris had the excellent idea that we should drive down together in the Oxford Brookes minibus. However, in addition to Chris, we needed a second driver. Having a driving licence and – as a German - being used to drive on (the right side of) German highways made me the ideal candidate for the job. For insurance reasons, I had to take a kind of dummy driving test before being allowed to drive the minibus. So one lunch time, Chris, the driving instructor and myself set out for a little joyride. All went really smoothly until we approached the red traffic light at the above mentioned junction and the traffic light turned red. I hesitated for a fraction of a second and decided I would better accelerate instead of having to do a full break application. However, I had overestimated the accelerating capacity of the minibus and didn't quite make it, passing the traffic light precisely 1.4 seconds after the signal changed to red (as stated in the police report).

Anyway, we finally made it to Heidelberg and back – safe and sound. Another memory I have of that trip down to Heidelberg is seeing Ian in the rear mirror of the minibus while driving. His usual self, he had worked frantically for the best part of the previous night on the presentation of and with Hazel Betts, his PhD student at the time. As a result, Ian was desperate to get some sleep before we arrived at the meeting. In order to achieve that, he had pulled his navy-coloured jacket over his head and was bouncing from side to side while sleeping in the back of the car. So I could see him moving in and out of the field of view offered by the rear mirror which looked very funny indeed.

So these two police camera images bring back a lot of memories. And while weeks after the meeting and the incident of the red traffic light, I was cursing when the bill for my offence amounting to about 65 GBP finally arrived, Chris was fascinated by the incredible image quality of the police camera...what else!?!

By Ulla Neumann, MPI for Plant Breeding Research, Cologne, Germany

Not a matter of taste but of security: appropriate outfit for high pressure freezing



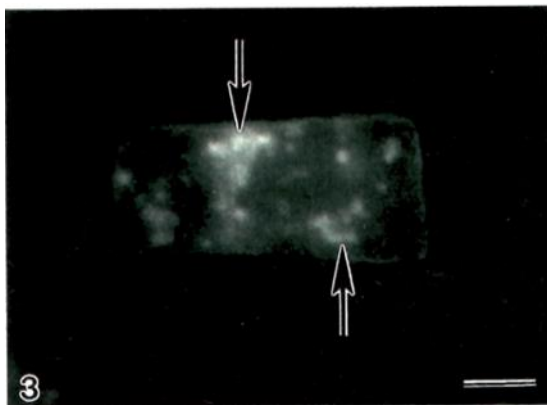
In 2001, Chris received a grant from the BBSRC entitled „High pressure freezing to capture endoplasmic reticulum and Golgi dynamics“. With a background in plant light and electron microscopy, I was offered a three-year postdoctoral position to work alongside Barry Martin to help setting up and running the high pressure freezing unit at Oxford Brookes University. As the unit was also open to other BBSRC grant holders, a small promotional announcement was to be published in „Business“, the quarterly magazine of the BBSRC at the time. Apart from information on what the unit had to offer and how it could be used, the announcement was supposed to feature a picture of the two people running the unit – Barry and myself.

So one day, Sheona Bellis, photographer at the Department of Biological and Medical Sciences, took a series of pictures which we proudly presented to Chris (upper left image). To our great surprise, he was rather annoyed by the lack of safety equipment (no lab coat, no gloves, no goggles!!!) and immediately sent us back to re-take a publishable image. So we did! And we made sure to wear every piece of protective clothing available in the lab (see right image)!!!

Of course, this wasn't the image that was published in „Business“ in the end - we had to go for the boring one with us looking very well-behaved and Barry exceptionally well-combed (lower left image). But the funny picture remained on the wall of the high pressure freezing lab at Oxford Brookes University for many years and I frequently used it for presentations on sample preparation for electron microscopy. In fact, in 2011, after giving a talk at the High Pressure Freezing Meeting in London, I was approached by Andres Käch, now head of the facility at the Centre for Microscopy and Image Analysis at the University of Zürich, telling me that he knew me – not from my scientific publications but from the photo in Chris's lab!

By Ulla Neumann, MPI for Plant Breeding Research, Cologne, Germany

When I was 21 and approaching the end of my undergraduate degree, I was lucky enough to end up with a choice of three PhD projects, all with funding, but it was the project with Chris that I really wanted. Chris had me interested in vesicle trafficking from the first few minutes I met him and, insignificant as it may seem, he was the only one that offered to pay my interview travel expenses! As a cash-strapped student that really stuck in my mind. I was to discover, of course, that this was typical of Chris's approach to the people in his lab: the science was important, challenging and exciting, but Chris saw the whole person, and paid attention to make sure they were thriving. Chris's enthusiasm for all things plant and microscopical was utterly infectious. He was never pompous or self-important, just brimming with excitement and ideas to share.



I was the first of Chris's PhD students to work on the Golgi Apparatus. This was before Chris had a confocal microscope and much of my time was spent preparing individual maize root cells for immunolabeling with JIM 84 using a cellulase/pectinase cocktail and a blunt glass rod to make root squashes. The success of a whole week's work could hinge on the batch number of the Cellulase Onozuka! In my third year, the dream of our own confocal started to take shape. I remember Chris telling me that if I could find a way to secure funding for it, he'd get my PhD awarded on the spot! (Just for the record - I didn't, and my PhD was secured by sheer hard graft, just like everyone else's.) When Chris finally had the cash, I went with him to a few of the manufacturers to see the confocal microscopes in action. I remember one visit where the demonstrator confidently set up the software to prepare a Z stack of about 20-30 optical sections. It was going to take some considerable time to process it, so we all went

for lunch, but when we returned, excited to see this cutting edge technique, we found that the whole system had crashed; needless to say, we didn't buy that one!



Chris was a stickler for making sure things were done properly; he couldn't abide shoddy work in the lab, particularly microscopy, and would tut and frown at substandard micrographs in a publication. This fitted perfectly with my perfectionist streak, particularly when it came to preparing material for the TEM. Chris told me that I could have a diamond knife to use on the ultramicrotome only once I could line up a pre-trimmed block and cut a perfect 70-80nm gold section with the first cut using a glass knife. Only after I had practised, achieved it and demonstrated my new skills did he confess he was joking. I got the knife though and he had the good grace to look impressed! It's a sectioning challenge I still set myself today!



My time in Chris' lab taught me what science is really all about and has given me some of my happiest memories. It was such a brilliant combination of friendships, science, challenge, excitement - and Champagne on a Friday afternoon!

Clare Steele-King
University of York

Early days – remembering Chris

By Louise Cole

South Parks Road, Oxford. He was athletic, unstoppable. His research questions unmistakable:

Do plants have clathrin-coated vesicles? Do plant cells even endocytose?

The sound of *Daucus carota* suspension-cultured cells oscillating in flasks filled the lab. Discarded coconut shells, drained of liquid for media, littering and dirtying - the bench.

Jude and I knitted sweaters over lunch – always laughing, joking - and talking about microscopy. We were all part of a plant cell community - with Chris at the core.

Scientific discussions often continued over morning and afternoon tea. Win stood behind the enormous metal tea kettle ready to quench our thirst and chat. Chris always bought mini cheddars; he was like that.

Birthdays were celebrated with sugary jam doughnuts and lab suppers were almost always spicy curry nights - at Chris and Kay's cottage - with Biffa stealing the limelight.

We all followed Chris – as a new professor - up Headington Hill to the *real* university in Oxford! This is where Chris shone brightly. His group grew into a phenomenon and people came from far and wide to work on the plant Golgi apparatus and endoplasmic reticulum.

I can still feel the weight of his hands on my shoulders, pressing down, as he was excited to hear about the results of the weekend's experiments. Yes, we all worked long hours to produce data to make Chris proud, and smile at our scientific achievements.

That smile, bordered by *that* moustache - that would disappear once a year - when Chris went on vacation.

That smile is imprinted in all our minds...

an incredible indelible mark...

like his impact on plant science...that will never be forgotten.

Many happy memories of Chris ——

I first met Chris in the 1980's when he was on a Royal Society Fellowship at the Botany School, University of Oxford. This was home to F.A.L. (Lionel) Clowes and Barrie Juniper, authors of a landmark textbook "Plant Cells" (1968).

Chris was using heavy metal staining (osmium zinc iodide) to reveal the ER in plant cells, and making innovations in specimen preparation methods and microscopy, projects that continued for the rest of his life at Oxford Brookes University.

Immediately he made a strong impression on me as a very cheerful person who obviously thought a lot about his work and would readily and openly discuss practical problems and possible solutions in studying plant cell biology.

His subsequent work, and that of his students, colleagues and collaborators on the endomembrane system, vesicle trafficking, exocytosis and endocytosis is an outstanding achievement that deserves the greatest praise and recognition by all his peers. The accumulation of numerous research papers, reviews and books over the years attests to a brilliant mind working hard and productively. [Brian Gunning said "Very saddened by your news. I had enormous respect for all of his work."]

Chris was a very much appreciated and loved contributor to many conferences. Early days at SEB meetings are fondly remembered for his excellent contributions and good humour during 'social' events. His promotion and organisation of International Botanical Microscopy meetings were invaluable for both young and older attendees. Sincere thanks will flow from around the world for these events.

On a personal level, 'Chris 'n Kay' and the dog were always very welcoming and hospitable to myself and family. We greatly enjoyed visits to the 'Didcot towers' neighbourhoods and enjoyed their return visits to Ireland, where they had various adventures at the hands of the weather (their tent blew away in a storm on one occasion).

So, Chris will be greatly missed by everyone who knew him, but we will draw comfort from the privilege of that contact and the cheerful smile that never faded (——and the socks!).

Martin Steer
Ireland



Memories from Poland:
Magda Krzeslowska, Anna Kaspruwicz - Maluski
Slawek Samardakiewicz, Przemyslaw Wojtaszek
Faculty of Biology, Adam Mickiewicz University in Poznan



In 2005 in Poznan (Poland) the 2nd Conference of Polish Society of Experimental Plant Biology took place at the Adam Mickiewicz University. At the invitation of the organizers, a lecture entitled "Fluorescent proteins and live cell imaging in plants" was then given by Chris Hawes. It was a great event for us because we knew Chris as an outstanding specialist in the field of confocal microscopy. To this day, we remember the unusual images presented by Chris showing the dynamics of organelle movements in plant cells.

During the meeting we met with his extraordinary kindness. When it turned out that one of the lecturers canceled the lecture, Chris spontaneously proposed the lecture "Introduction to fluorescent proteins".

Chris presented these issues at the highest level, very interestingly, with specific swaddling. It shows how much he lived what he did and how willingly he shared it with others.



Slawek - I met Chris again a few years later (2013) on the occasion of a scientific conference in Lijiang (China). To my surprise, he perfectly remembered his stay in Poznan. At that time, I also remembered one scene that testified about how he loved life, how he liked to have fun. During the concert of the folklor group, it was proposed that the conference participants join the band's play. Chris was the first to pick up the call, and he joyfully followed the local musicians

Magda - I met Chris regularly at the Botanical Microscopy Meetings since 2007. I've always been impressed by Chris's lectures, his personality and sense of humor, amazing presentations of the results as songs. I included these songs in my cell biology lectures and the students were always delighted with them. I think that they may forget many facts from the plant cell life but they will always remember that ER and GA "are in love" and connected with each other ☺

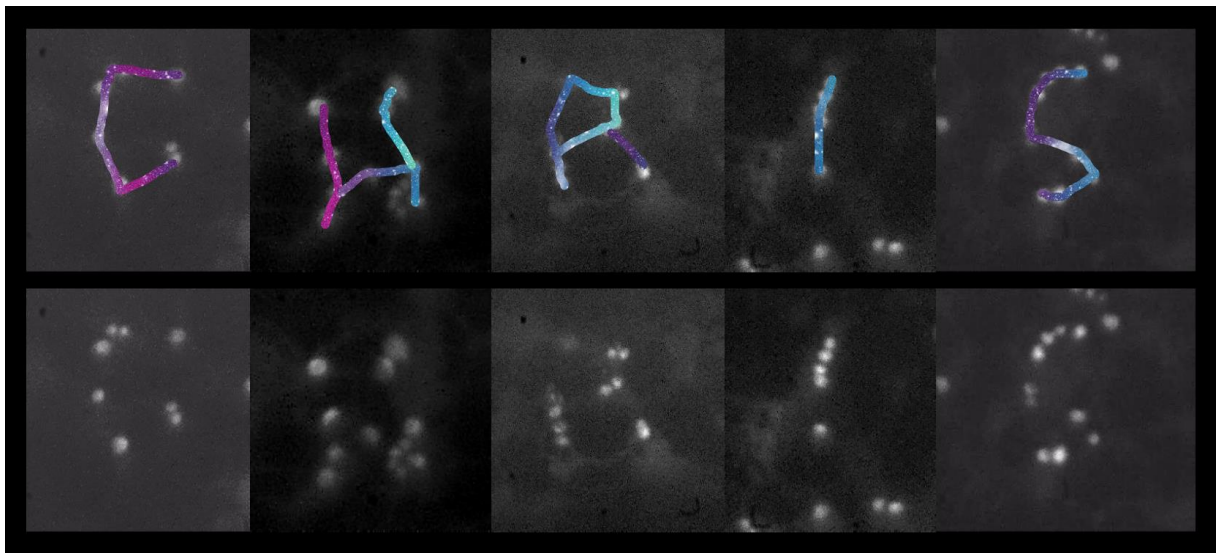


Chris' Golgi constellation

Imogen Sparkes, Bristol University

Chris was an amazing, enthusiastic, supportive scientist with a drop of mischief thrown in for good measure! An example of mischief and science came from the optical tweezer experiments we carried out at Wageningen University. The aim was to trap Golgi and determine if the movement of the Golgi in turn dragged the ER, testing the hypothesis of a physical association between the two organelles. To our amazement it worked! Mischief then took the form of trying to write our names using the trailing ER. We never quite managed in the short amount of time we had left after all the experiments.

Chris, mate, the image below is for you – it might not be your name in the ER, but it is spelt using your favourite organelle Golgi! I will miss you very much, thank you for everything. Chris, before you ask, your name was spelt during lunch / dinner breaks so no experiments were 'harmed'!



Images taken of *N. tabacum* leaf epidermal cell expressing StGFP. Samples were treated with latrunculin b to depolymerise actin and stop organelle movement. Optical tweezers were used to trap and move Golgi into position to generate letters.

Dogs, Coconuts and Onion Sandwiches

Background

It is a fact that dogs smell and that big dogs smell more than little dogs. However, this is a fact often not recognised by your average dog owner

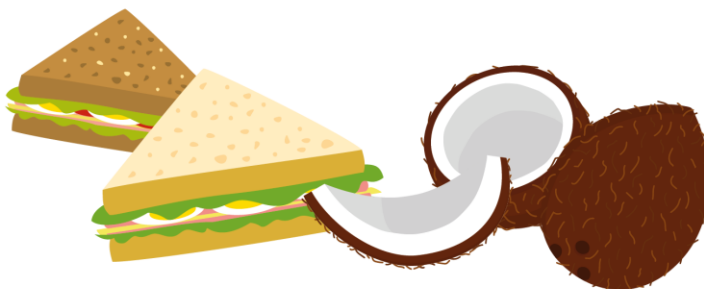
Coconuts were a big part of life in the early days in Oxford. Coconut milk was an essential ingredient in carrot tissue culture medium and carrot cells were forever growing in the lab. Every 4 to 6 weeks a posse would head off down to the covered market and return with a sack of coconuts to milk.

On this particular occasion the coconut run was down to Louise (Cole) and Chris. It was pouring with rain so I cadged a lift into town to get lunch. Lou and I regretted our decision as soon as the car door opened. There was a very pleased to see us, very wet Biffa in the boot of the car and those of you in the 'dog smell know' will be aware that the increase in smell of a wet Newfoundland is logarithmic over normal big dog smell! Louise rode shotgun, while I was in the back with the over enthusiastic dog leaning over my shoulder. The smell was horrific! Louise and I had the windows open, gagging for air while the rain poured in on us.

A small rest bite as we raced into the market for coconuts and lunch and then back for more torture by dog odour.....

We arrived back at Plant Sciences not a moment too soon, gasping for breath and practically falling out the car in our haste to escape the smell. Chris actually noticed our mild discomfort at this point.

**'Sorry about the smell.
Onion sandwiches today!'**



Judith Sheldon (Jude)

SOLVED: The mystery of the John Innes Sherry

Going to conferences

with Chris was always fun: The Good beer Guide route to get there; Getting to meet EVERYBODY as Chris knew EVERYBODY; the acceptance, purely by association; Chris's ability to mend any slide projector; and of course the registration.

'Chris Hawessss **H**Aw'



The Botanical Microscopy meeting at the John Innes Institute and UEA in the early 1980s was a classic. It pre-dated me working with Chris, but I was there with him and my bestie (and Chris's first post-doc) Jane Stephenson. Everybody that was anybody was there and much was learned, mostly not in the lectures and including cell biology sign language.

The conference dinner was a messy affair. Friendships were made, hearts were broken, lots of people got food poisoning and we never got to know where the staples went. As the evening wound up Chris got word from Clive Lloyd and Keith Roberts not to take the wine (it was on sale or return) but to help ourselves to the Sherry. Several of us duly obliged and we wandered (staggered) back across campus to continue frivolities back at our accommodation (another story!). Half way back one of our party dropped one of the sherry bottles he was juggling. It made a very loud bang as it smashed. Thinking on my feet (just) I grabbed his second bottle, before it received a similar fate, and put it in the only place I knew it would be safe, Chris's jacket pocket. Even in those early days I knew he would not waste alcohol.....

I didn't discover the fate of the sherry for many years. Fast forward 12 to 15 years, through gaining my PhD, a post-doc with Chris in Oxford, two children and a career break, and following Chris up the hill where I discovered he was now a grown up. I was telling an anecdote from the conference in a lab meeting about a visitor we were expecting, but got the 'don't you dare tell' look from Chris so seamlessly changed to the sherry tale.....

As I finished Chris piped up 'I always wondered where that bottle of John Innes Sherry had come from. We found it at the back of the drinks cabinet when we moved last year.'



Judith Sheldon (Jude)

Thank you for showing us the light, Chris!

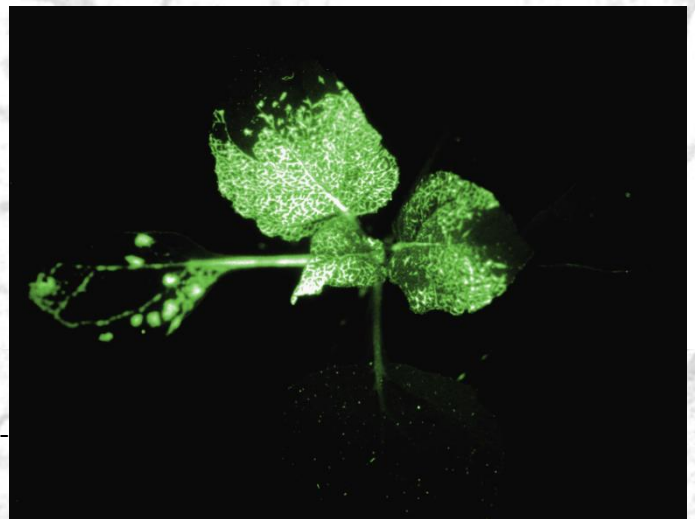
Federica Brandizzi

Chris Hawes was a pioneer in plant cell biology and a wonderful mentor. He was shamelessly adventurous in exploring new microscopy frontiers and have fun in the process.

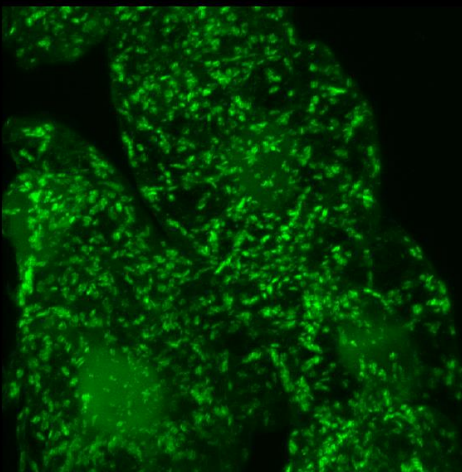


Along with Ian, Chris developed transient tobacco transformation (above panel), which is now widely adopted by labs around the globe to express genes and monitor their function in live tissues. This approach superseded earlier, more difficult, and economically more costly approaches, such as virus-mediated transformation (left panel).

I joined Chris' lab after a brief postdoc with Ian Moore at the University of Oxford. At that time, GFP was just surfacing on the plant cell biology arena. Chris had the intuition to tag GFP to the endomembranes to see them come to life at the microscope.



Golgi Apparatus of Tobacco Cells



I was fortunate to join the lab of an enthusiastic scientist that provided me with the freedom to explore the field. This gave me terrific opportunities to work with incredible people, like Nadine Paris, Jurgen Denecke, Jennifer Lippincott-Schwartz, Erik Snapp, Patrick Moreau, just to name a few.

With these collaborations, we moved the fields to new frontiers by implementing quantitative imaging based on multi-spectral imaging, photobleaching.

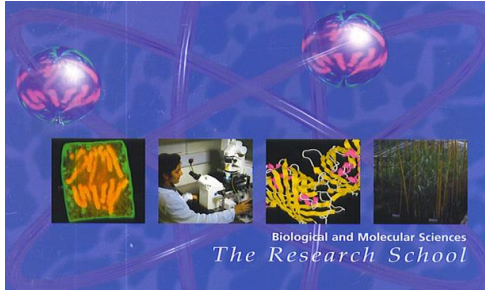
There is no doubt that a strength of Chris was to surround himself by bright and enthusiastic students and post-docs. He generated a safe atmosphere in which wonderful, life-lasting friendship bloomed. Ulla, Claude (she took the beautiful BY-2 cell image above), Jorunn, Amanda, Einat, Arunika, Sasha and so many others that I was so privileged to meet during my postdoc.



Thank you for everything, Chris!

Chris, your passion for plant cell biology is contagious!

Arunika Gunawardena, Professor
Biology Department, Dalhousie University, Canada
<http://pcdlab.biology.dal.ca>



I remember my first day at the confocal microscope (1997). I was a bit scared to use that expensive piece of equipment, but not only did you reduce my fear, you also inspired me to love confocal microscopy (I became popular; I was on the cover of the research school magazine). I fell in love with the confocal and since then I have been using it! Thank you for everything.

Characterisation of programmed cell death during aerenchyma formation induced by ethylene or hypoxia in roots of maize (*Zea mays* L.)

Arunika H. L. A. N. Gunawardena^{1,*}, Deborah M. Pearce¹, Michael B. Jackson², Chris R. Hawes¹, David E. Evans¹

¹Research School of Biological and Molecular Sciences, Oxford Brookes University, Gypsy Lane Campus, Headington, Oxford, OX3 0BP, UK

²IACR-Long Ashton Research Station, Department of Agricultural Sciences, University of Bristol, Long Ashton, Bristol, BS41 9AF, UK

Received: 12 April 2000 / Accepted: 18 June 2000



Thank you for being such an inspirational mentor!



In 2019, I attended the 11th International Botanical Meeting (after 20 years from my first meeting) in Oxford. It was a very happy get-together!

In 1999, when I was a graduate student, you took us to St Andrews, Scotland to attend the 6th International botanical microscopy meeting.



I still remember how you enjoyed the Sri Lankan rice and curries I made for our lab potlucks (salmon curry was your favorite!)

Chris, your passion for plant cell biology is contagious and I think all those who have worked with you or been mentored by you would agree! The world of plant cell biology research will not be the same without you, but we will try our best to carry on your legacy. We miss you Chris and your beautiful smile will stay with us forever! We all love you!

Memories from and inspiration by Chris Hawes



My first confocal microscope (1998)



Freeze drying samples



Always happy to talk about Golgi in plants



Group trip to the RMS meeting in St. Andrews

Phrases by Chris that stayed on with me:

"Imaging science reflects the art of nature"

"A good scientist should be able to give a lab meeting using only a marker and a white board"

"A good presentation will amaze your audience! Here are some rules..."



Physiological and Molecular Plant Pathology
Volume 65, Issue 3, September 2004, Pages 129-135

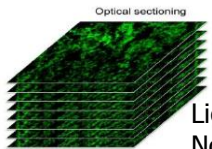


An immunocytochemical and ultra-structural study of the extracellular matrix produced by germinating spores of *Stagonospora nodorum* on natural and artificial surfaces

E. Zelinger ^{1,*}, C.R. Hawes ¹, R.B. S.J. Gurr ², F.M. Dewey ^{1,2}

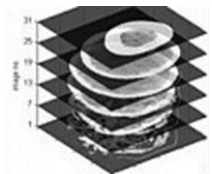
My first paper

The love of microscopy as a research tool has led me to run a microscopy unit pursuing correlative imaging.



Optical sectioning

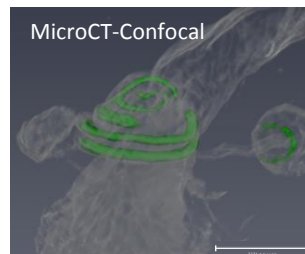
Light beam
Non-destructive



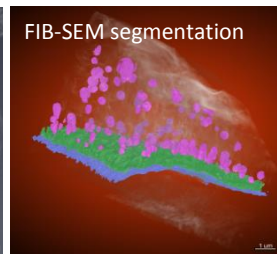
X-ray beam
Non-destructive



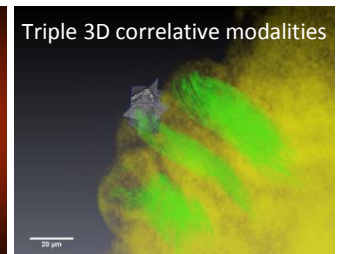
Ion beam
destructive



MicroCT-Confocal



FIB-SEM segmentation



Triple 3D correlative modalities

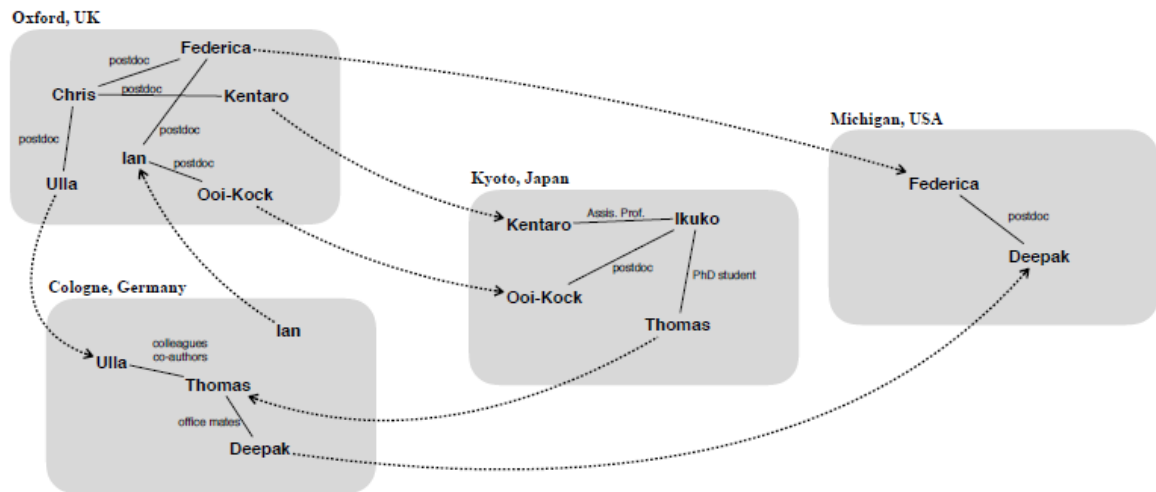
In a project focused on the *Drosophila* female sperm storage organs, we used triple correlative approach. We show spatial distribution of fluorescent markers in localized areas of the seminal receptacle, making visible the cellular differences between vesicle formation in the seminal receptacle before and after mating. We also examined the lumen lining of the distal seminal receptacle before and after mating and noted a close interaction between sperm and the cellular secretory layer lining the lumen, suggesting a possible interaction.

Cross-encounter at the three-way (or even more) junctions

Ryohei Thomas Nakano,

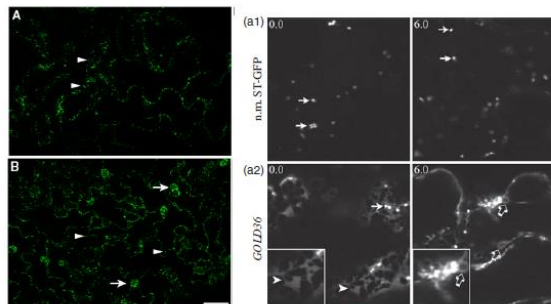
Max Planck Institute for Plant Breeding Research, Cologne, Germany

It was a strange coincidence that I first came across with Chris, when I was starting my PhD in the Ikuko Hara-Nishimura's lab. I was trying to identify the responsible genes for my *endoplasmic reticulum morphology (ermo)* mutants. I had already spent more than a year during my master, simply sequencing any possible candidate genes within the genomic region that I obtained from the map-based cloning. Then Kentaro Tamura, who was an assistant professor in the lab at that time, just walked to me with mixed feelings and told me that one of the mutants he isolated in the Chris' lab, which had been taken over by Federica Brandizzi, that exhibited very similar phenotypes with my *ermo2* mutant possessed a causative mutation somehow close to my genomic region. I sequenced the gene, *SEC24a*, and that was it. Truth to be told, we first suspected that there might have been contamination of seeds, because the two mutants, G92 and *ermo2*, isolated from two independent forward genetic screenings had the same base pair substitution at the same position that caused the same missense amino acid mutation in the same *SEC24a* gene. But indeed it was really a surprising coincidence between the two labs, somehow enrolling in the same scientist, Kentaro Tamura. We managed to carefully coordinate with Federica and, fortunately, it ended up with back-to-back publications. Some years later, I was again about to identify the causative mutation for *ermo3*, but then a paper was published from Natasha Raikhel's group, reporting the identification of the same gene with the same mutant phenotype, which made me utterly disappointed at the middle of my PhD. Then Kentaro again contacted me to tell me that it happened again - another mutant from the work by Chris and Federica ended up with the same gene. This time it was a bit tricky to organize a joint manuscript and I needed to somehow twist my story to get my *ermo3* work published after two publications. It was the moment I learnt the fun and the tough sides of forward genetics at the same time... This was how I encountered Chris in the first place, not in person but indirectly via online, as a collaborator and/or as a competitor. At the end of my PhD, I shifted my work from canonical ER structures to the ER bodies, and then, of course, I bumped into Chris' work again. I finally came to meet Chris in person when he visited the MPIPZ in 2015, where I started my postdoc project with Paul Schulze-Lefert. I took lunch together with Chris and Ulla Neumann, chatting only about the ER and it was a memorable time for me. When I moved to Germany in 2013, I thought I completely switched my subject and there would not be any connections to what I did in Kyoto. However, it was not true, not surprisingly at all, as it is indeed a small world and a lot of people that I have had opportunities to work with are actually somehow connected to Oxford (see below). Thus, though I have not been directly connected to Oxford, I feel like I'm a part of the community and I already look forward to another encounter with Oxford-related people, like at the ER three-way junctions where major important cellular phenomena take place.



Chris Hawes Lab @ Oxford

Mutant screening for aberrant Golgi morphology/trafficking using **ST-GFP**
... by Federica Brandizzi and Kentaro Tamura



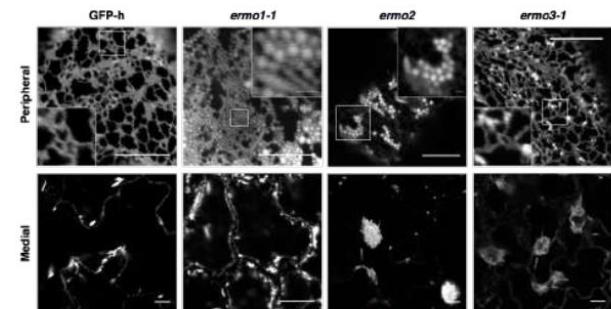
"G92" from Hawes Lab:
SEC24a At3g07100, G → A substitution, causing *R692K* missense mutation

"ermo2" from Hara-Nishimura Lab:
SEC24a At3g07100, G → A substitution, causing *R692K* missense mutation

Faso *et al.*, 2009; Nakano *et al.*, 2009 [back-to-back in THE PLANT CELL]

Ikuko Hara-Nishimura Lab @ Kyoto

Mutant screening for aberrant ER morphology using **SP-GFP-HDEL**
... by Ryo Matsushima and myself



"GOLD36" from Hawes Lab:
MVP1 At5g39500, C → T substitution, causing P80L missense mutation

"ermo3" from Hara-Nishimura Lab:
MVP1 At5g39500, G → A substitution, causing abnormal splicing

MVP1 was originally isolated by **N. V. Raikhel** from the mutant screening for aberrant vacuolar morphology/trafficking

Agee *et al.*, 2009; Marti and Stefano *et al.*, 2010; Nakano *et al.*, 2012

Endocytosis and Asafoetida

By Dr Kim Twinn (née Crooks)

In 1989, when I was an undergraduate at Oxford Polytechnic, I attended a talk on fungi by a 'new kid on the block', Senior Lecturer, Dr Chris Hawes. It was a superb talk which clearly showed Chris's knowledge and passion for his subject. Unlike many lectures of that time, Chris's talk came with a whole carousel of 35mm slides containing fabulous images of interesting fungi. I was completely enthralled and wanted to learn more about the research he had eloquently described; as soon as I could, I switched my degree to Cell Biology.

I adored my new subject and worked hard to impress Chris. I desperately wanted to use the cutting-edge microscopes that he talked so fervently about. My efforts paid off when, after graduating, Chris offered me work in his department which led to a full-time PhD with Chris and Dr Julian Coleman as my joint supervisors.



A birthday party for Chris in our office at Brookes University

My research focused on endocytosis in carrot cells; a challenging task, but I loved every minute of it (especially the hours I spent on the light and electron microscopes). Working with Chris meant that I was part of a dynamic team who helped me every step of the way: Louse Cole was my mentor; Clare Steele was my rock; Béatrice Satiat-Jeunemaitre was my 'fount of all knowledge' and Barry Martin, Anne Kearns and Jan Evins were the most helpful technicians anyone could wish for.

We worked hard and played hard; if Béatrice was about on a Friday afternoon, we often drank champagne in the office (which was also our party venue for every occasion)!

One part of my PhD studies I remember well was a visit to The University of Rouen/CNRS to work with Loic Faye and his team. Chris flew out to see how I was getting on and insisted we talk over a delicious 'plateau de fruit de mer' in a local restaurant. The next day, Chris flew back and I went down with a bad case of food poisoning! Fortunately, the visit was well worth the effort and helped me move forward in my research.

Having Chris as a PhD supervisor was an amazing experience; not only did Chris teach me everything there was to know about plant cells, microscopy and endocytosis, he also enlightened me on how to make authentic curries. This included several trips to Cowley Road in Oxford to show me what spices to buy and which grocers to buy them from. I had never heard of asafoetida before (Chris called it essence of poppadum) and, from the smell, I wasn't convinced that it deserved a place in any meal - but I was wrong. Nowadays, I always have a jar of asafoetida in my cupboard for making curries and, every time I use it, I think of Chris and smile :)



Chris and PhD colleagues at my wedding in 1997

FISHING IN THE CANADIAN SHIELD

Larry Fowke,

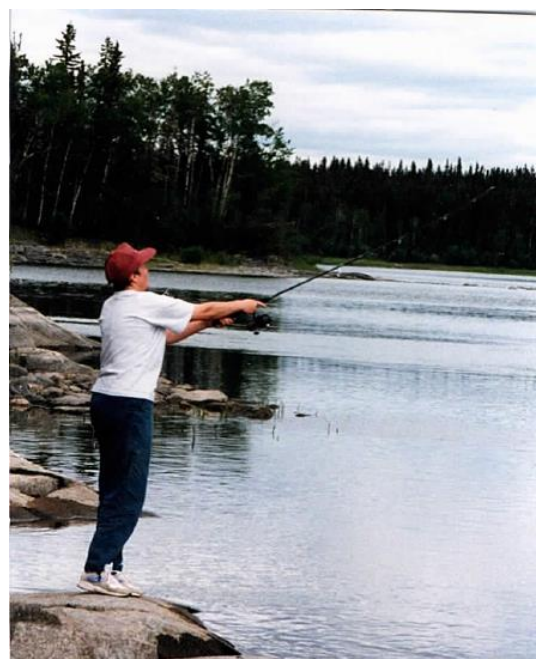
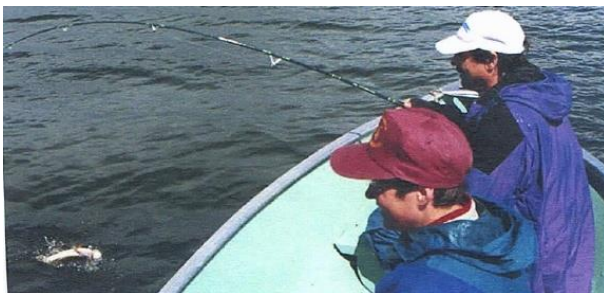
Biology, U of S, Saskatoon SK, Canada



In 1991 I had the good fortune to spend 5 months of my sabbatical leave in Chris Hawes' lab working on a mutual project. Chris and Kaye made us feel very welcome. The work in the lab was both interesting and productive. It was nice working in a well equipped lab with an enthusiastic and talented scientist. We were very impressed with Oxford.

In 1995 Chris and Kay came to Canada for Chris to attend a conference. Following the conference we drove about 5 hours north of Saskatoon well into the Canadian Shield, a heavily forested, rocky area populated by hundreds of lakes. Our destination was the tiny village of Missinipe located on the Churchill river, home to many fish. We set up camp and made plans for the next days fishing. Out in the boat we were very busy. Chris and Kay had their lines out as we trolled a short distance from the shore. It wasn't long before the fish started biting. It got so busy that I spent most of my time unhooking their fish and releasing them. They even both had fish on their lines at the same time. Most of the fish were small pike (1-2 lbs) with an occasional pickerel. We ate fresh fish for supper every night. It was a blast.

Chris will be missed by his colleagues and friends. He left a legacy of first rate science.



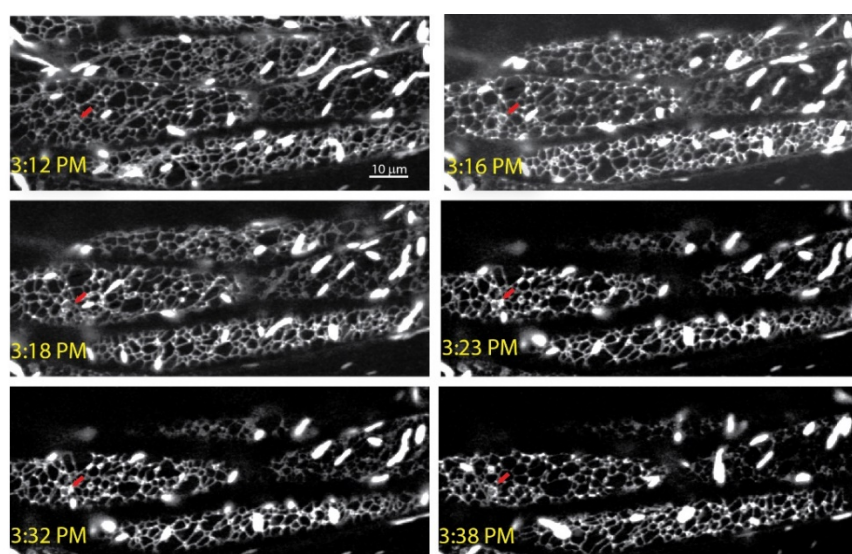
What I learned from the never-ending confocal session.

Lawrence Griffing, Texas A&M University, College Station, TX

When I took my sabbatical with Chris Hawes in 2007, I was just finishing a couple projects that used video of grizzly bears taken 24/7 during the summer months in Alaska. There were no grizzly bears in Chris' lab, so instead of taking exhaustive video sequences of grizzly bears, I took exhaustive video sequences of the endoplasmic reticulum in transgenic Arabidopsis (and tobacco) expressing GFP-HDEL. The hypothesis was "ER naturally cycles through multiple forms, without changing anchor sites (now called contact sites)". However, the real question being tested was— how long can Griffing monopolize the confocal?

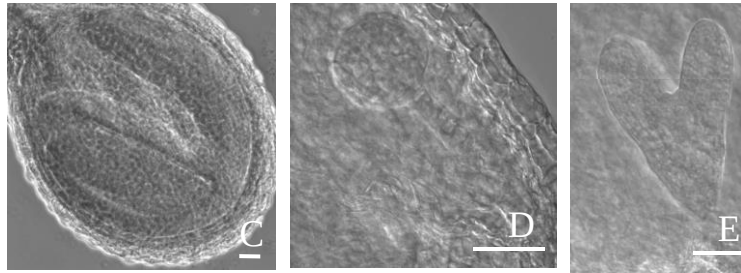
Most confocal sessions started around 10AM. After the obligatory several cups of prime British Gold Nescafe, sometimes courtesy of Chris, who had gigantic jars of the stuff in his office, I would mount my seedlings and ride off into the dark world of the wonderful Zeiss LSM, across the hall from Chris' office. I could always tell how busy Chris was by whether or not he left his keys in his office door. Sometimes I would be "on the scope" when he left at night and then when he arrived the next morning. Peeking in, he would, with that serious chortle of his, ask "Have you been here all night, Larry?" Never all night, but during the weekends, I would sometimes stay for 16 hours. My time was limited only by the bus schedule from Oxford Brookes to my rooms in The Chestnuts, a bed and breakfast in Cumnor operated by the ancient, inimitable, Mrs. Jones and her husband, a former engineering don, now fading like the gigantic trees in the front of the property.

What I learned was, yes, I was most welcome to monopolize the confocal for extended periods. Chris was extremely generous with his time and his facilities, traits he taught his many students as an exemplary role model. I also learned that, as shown in the below unpublished figure, that ER of Arabidopsis hypocotyls can undergo dramatic changes in form over periods of a half an hour. This is presumably due to evaporation of water and increasing pressure of the coverslip (its reversible if you just add water) but maintain during that time a variety of contact sites including those with the chloroplast-plasma membrane (red arrow). During my sabbatical, I developed the image processing techniques to identify contact sites and the persistent and motile cisternae and tubules, which I was able to apply to the work of Imogen Sparkes and John Runions in a Plant Cell paper. These long-term observations set the stage for on-going analyses of known contact site proteins and understanding their role in contact site maintenance and the function of contact sites.



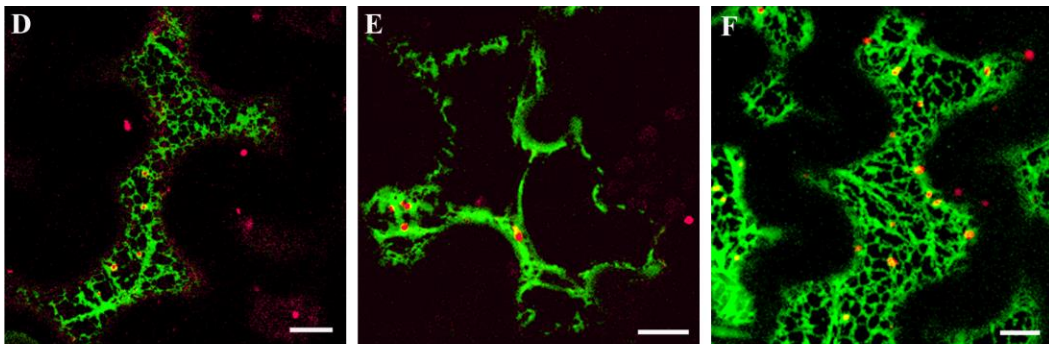
An *Arabidopsis pex10* Null Mutant Is Embryo Lethal, Implicating Peroxisomes in an Essential Role during Plant Embryogenesis¹

Imogen A. Sparkes, Federica Brandizzi, Stephen P. Slocombe, Mahmoud El-Shami², Chris Hawes, and Alison Baker*



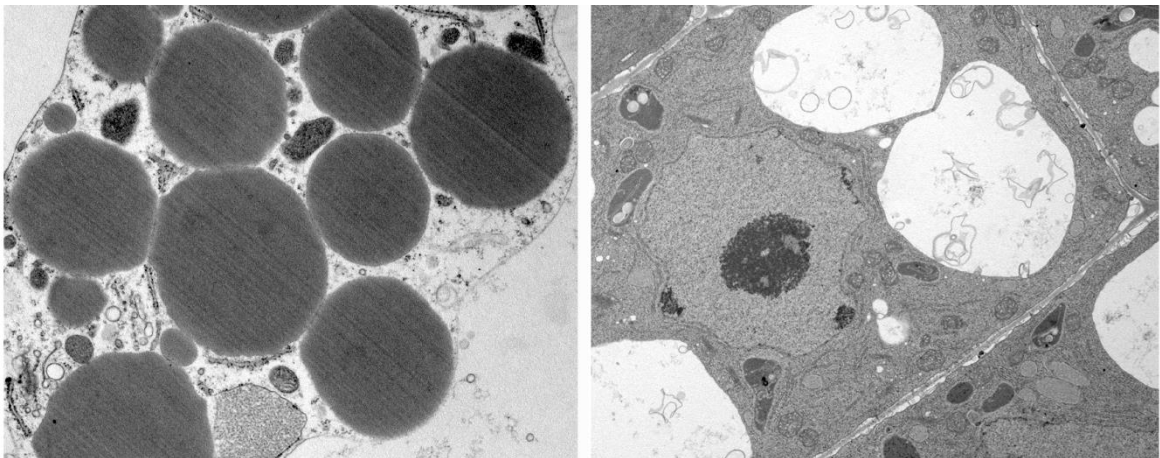
AtPEX2 and *AtPEX10* Are Targeted to Peroxisomes Independently of Known Endoplasmic Reticulum Trafficking Routes^{1[w]}

Imogen Averil Sparkes*, Chris Hawes, and Alison Baker



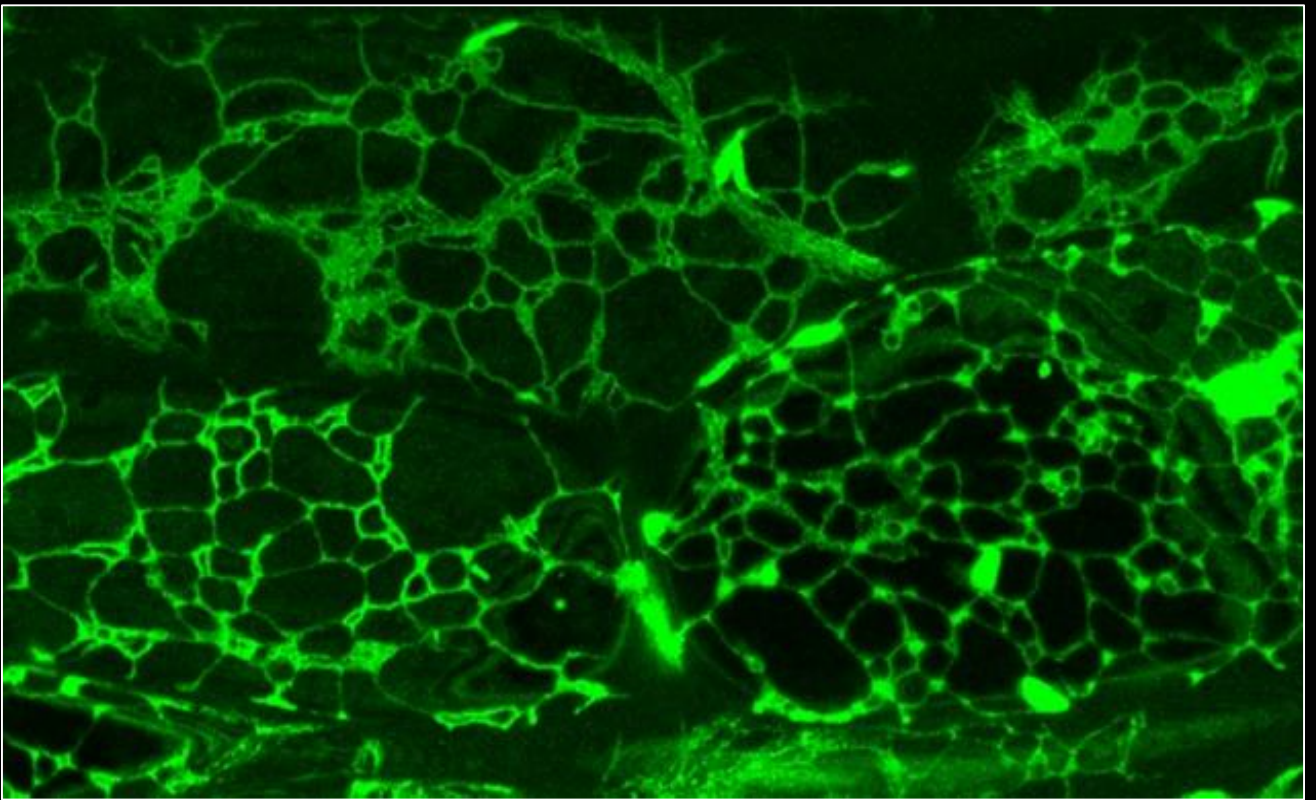
An inhibitor of oil body mobilization in *Arabidopsis*

Laura-Anne Brown¹, Tony R. Larson², Ian A. Graham², Chris Hawes³, Rupesh Paudyal¹, Stuart L. Warriner⁴ and Alison Baker¹



Fond memories of a wonderful scientist and person

„The amazing cortical ER network
in *Arabidopsis* hypocotyl cells...
Such observations will always be
linked to your memory, Chris!“



© David Blum, diploma student at University Vienna

A handwritten signature in white ink, reading "Ingeborg", with a long, sweeping horizontal stroke underneath.

ao. Univ.-Prof. Mag. Dr. Ingeborg Lang

Department Ecogenomics and Systemy Biology
University of Vienna
Althanstraße 14 (UZA I)
A-1090 Wien, Austria
ingeborg.lang@univie.ac.at



universität
wien

**Don't ask me why,
I just love it**



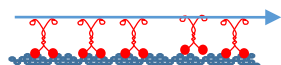
**Endoplasmic reticulum of the exocrine pancreas,
high pressure frozen and freeze substituted to Epon, Paul Verkade**

Walking along plant cell biology trails

With Chris Hawes and myosin tails

Einat Sadot, Volcani Center, Israel

2002 Wageningen



2005 A visit to Israel



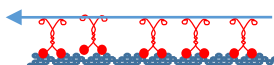
The Microscopy Day - on Tuesday, 5 April 2005

Cohen Auditorium, The Volcani Center, ARO, Bet-Dagan.

Programme

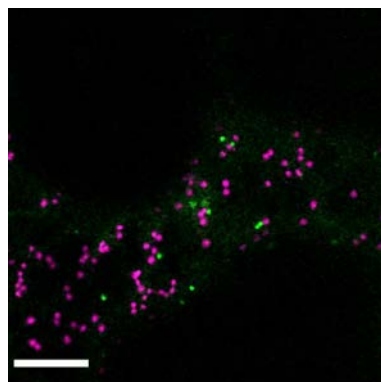
0930-0935 Welcome address by E.R. Pridmore - ARO Director.
0935-0945 Plant structure analysis: at the beginning of the 21st century. - Emory Joel (ARO).
0945-0950 Live cell imaging - confocal microscopy and fluorescent proteins to study organelle dynamics and protein transport. - Chris Hawes (Oxford Brookes University).
0950-1015 Confocal microscopy at the ARO - cell biology studies of agricultural crops. - Einat Sadot (ARO).
1015-1030 Discussion
1030-1100 Coffee Break
1100-1145 High-Pressure Freezing for EM preparation - immunocytochemistry and tomography to study secretory pathway organelles. - Chris Hawes (Oxford Brookes University).
1145-1245 Quantitative microscopy mark easy. - Ben Hammett (Tel-Aviv University).
1245-1245 Discussion
1245-1400 Lunch Break
1400-1445 Laser Microdissection as a tool to obtain cell-type specific proteins, metabolites and transcript profiles. - Julia Kuhl (Max Planck Institute for Molecular Plant Physiology).
1445-1455 Discussion
1455-1515 Whole genome studies and the power of microscopy: agricultural research applications using *A. thaliana* GeneChips. - Arnon Greenberg (Eisenberg Tree).
1515-1530 Discussion and Circulating remarks.

2009 A joint paper in Plant Physiology



A Comparative Study of the Involvement of 17 Arabidopsis Myosin Family Members on the Motility of Golgi and Other Organelles^{1[W][OA]}

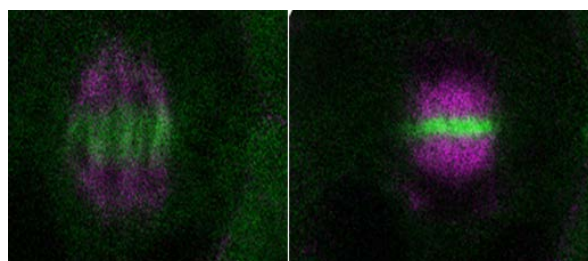
Dror Avisar, Mohamad Abu-Abied, Eduard Belasov, Einat Sadot*, Chris Hawes, and Imogen A. Sparkes



2018 the JXB editor accepting our paper

Myosin XI-K is involved in root organogenesis, polar auxin transport, and cell division

Mohamad Abu-Abied^{1,*}, Eduard Belasov^{1,*}, Sapir Hagay¹, Valera Peremyslov², Valerian Dolja² and Einat Sadot^{1,†}



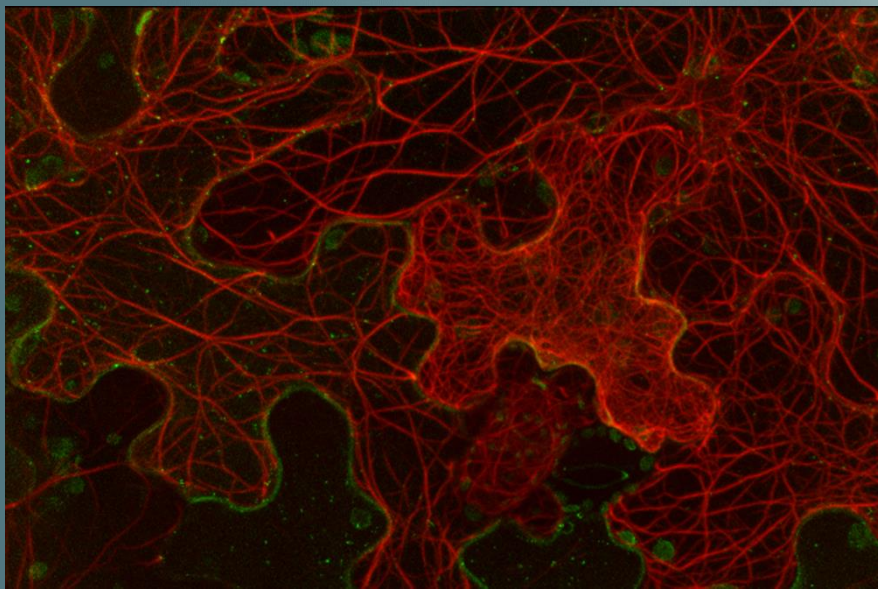
2019 RMS



שלום חבר

Two things clearly stand out when I reflect on my meetings with Chris. Firstly, he has been great cell biologist, creating a special scientific spirit and stimulating discussions, which I really miss now. Secondly, he always encouraged to go to unknown territories and exploring new technologies, in particular new microscopy approaches. I would like to thank Chris for welcoming me to the cell biologist community and being such a great colleague.

Silke Robatzek



How incredibly lucky I was to work with the Plant Cell Group at Oxford Brookes

Pengwei Wang

Key Laboratory of Horticultural Plant Biology, Huazhong Agricultural University, Wuhan, China

• KMS period, Oxford (2006-2010)



I became a member of the Hawes' lab in 2006, the aim of the project was to study unidentified Golgi matrix proteins. However, none of them localized to the Golgi, so I became an ER person in the end....

(The name of KMS, Killing Me Slowly, comes from Eric Hummel, who may had enough of microtome sectioning over that period)

• RTNs and VAP27 (2006-2010)

Putting the Squeeze on Plasmodesmata: A Role for Reticulons in Primary Plasmodesmata Formation¹

Kirsten Knox², Pengwei Wang^{2,3}, Verena Kriebbaum, Jens Tilsner, Lorenzo Frigerio, Imogen Sparkes, Chris Hawes, and Karl Oparka*

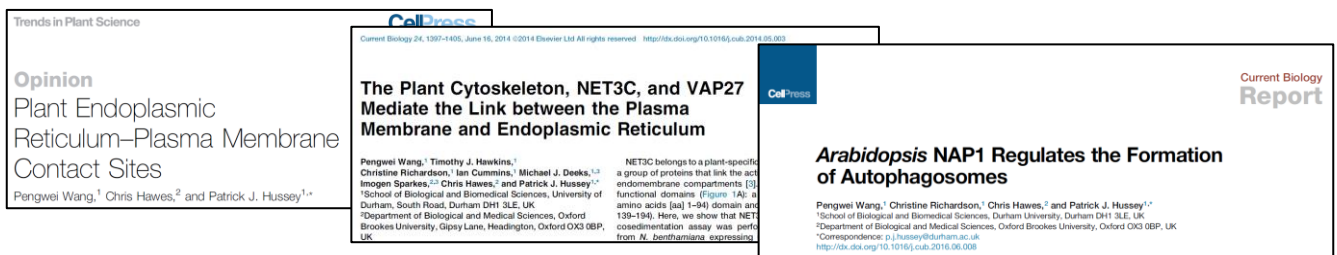
Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh EH9 3BF, United Kingdom (K.K., K.O.); Plant Cell Biology, Oxford Brookes University, Oxford OX3 0BP, United Kingdom (P.W., V.K., C.H.); Biomedical Sciences Research Complex, University of St. Andrews, St. Andrews KY16 9ST, United Kingdom (J.T.); Life Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom (L.F.); and Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, United Kingdom (I.S.)

ORCID IDs: 0000-0002-5487-1763 (K.K.); 0000-0002-0362-878X (P.W.); 0000-0003-3782-5834 (V.K.); 0000-0003-3873-0650 (J.T.); 0000-0003-4100-6022 (L.F.); 0000-0003-4856-7690 (C.H.).

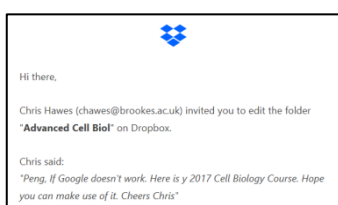


After the PhD, I stayed for a short post-doc with Chris, and was trapped in the ER network further. I started to study the reticulon and VAP27 proteins, benefiting from this work, I have produced a number of related publications.

• Collaborating with Chris (2011-present)



I moved to Patrick Hussey's Lab in 2011, and started a productive and enjoyable collaboration between two labs.



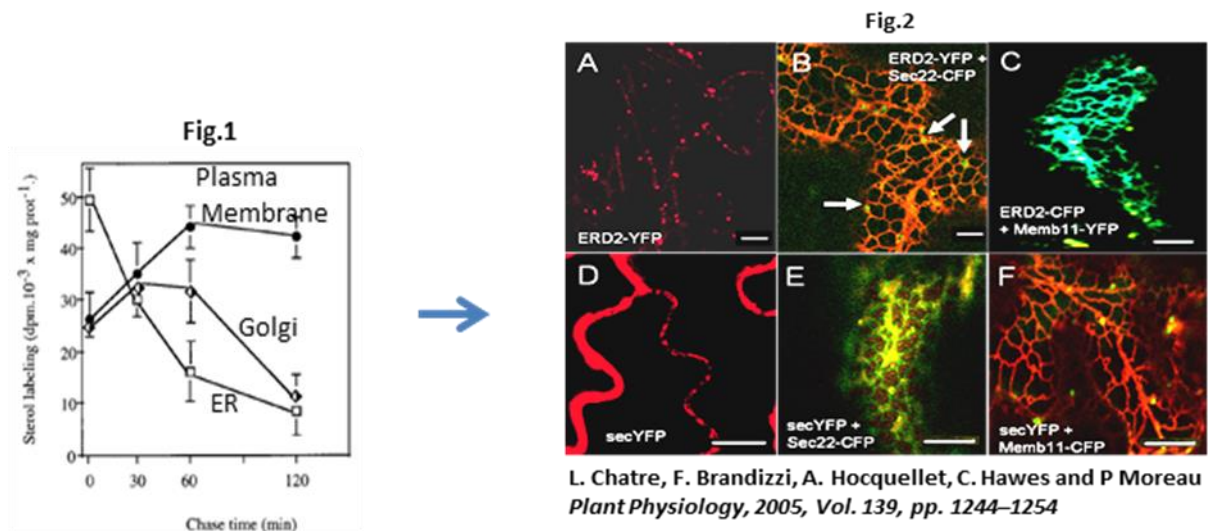
I wrote to Chris regularly to share some excitements of new data, it was a good feeling to know that he was there to help. May 2019, Chris sent me all his lecture presentation for the Cell biology course, that is the last email I received from him, but our work never ends...

A journey in the Secretory Pathway: From Lipids to SNAREs

Patrick Moreau

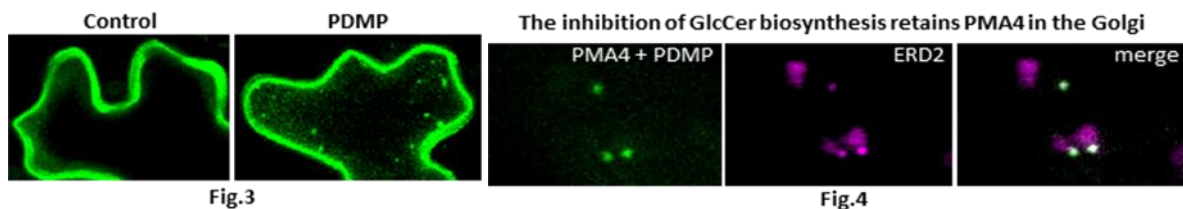
UMR 5200 CNRS - Université de Bordeaux, INRA Bordeaux Aquitaine, Villenave d'Ornon, France

For more than 15 years (1986-2002), **labeling and membrane fractionation** was used to investigate membrane traffic, as shown for example in Fig.1 for sterol trafficking between ER and plasma membrane. Then, thanks to Béatrice Satiat-Jeunemaitre, **meeting Chris Hawes was a great starting point** to use his new approaches (**expression of fluorescent XFP-tagged proteins in vivo**) to study protein trafficking in my team. We were the recipients of a **Franco-British Research Partnership Program Alliance in 2002** to collaborate on the study of SNAREs at the ER-Golgi interface. Laurent Chatre, my PhD student, started to work with Federica Brandizzi, postdoctoral fellow in Chris Hawes lab at that time. As shown in Fig.2, effect of the overexpression of Sec22 and Memb11 on ERD2 dynamics and secretion of the secYFP cargo was studied. A redistribution of ERD2 from Golgi to ER (Fig.2B-C) and a secYFP block in the ER (Fig.2E-F) were observed.

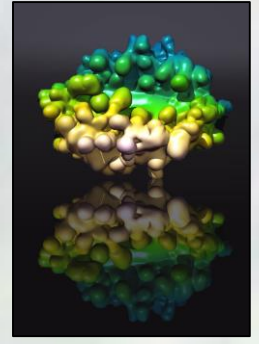
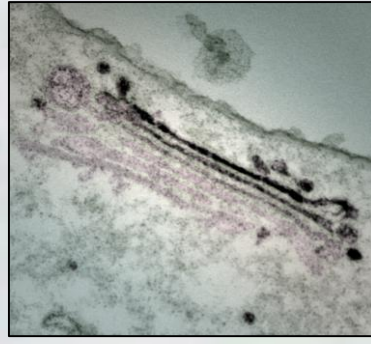
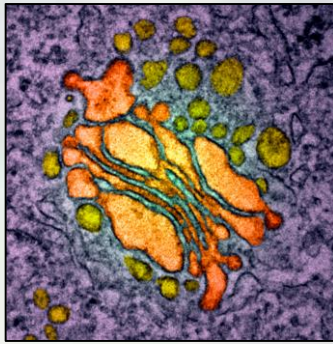
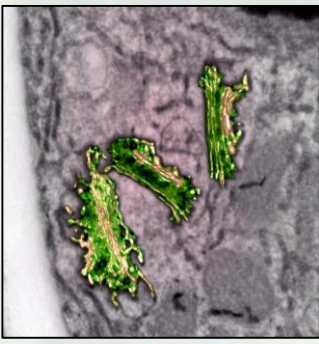


(Moreau et al. *Plant Physiology*, 1998)

Then, after this collaboration with Chris, my team started to **combine these in vivo fluorescent approaches with biochemical approaches (inhibitors of lipid biosynthesis)** to investigate the role of lipids in protein trafficking. As an example, Fig.3 shows a partial block of the transport of the ATPase PMA4 to the plasma membrane with appearance of intracellular dots after inhibition of Glucosylceramide synthesis by PDMP. These dots were identified as Golgi bodies (Fig.4), (Melser et al, *Traffic* 2010).



Today, we have succeeded several studies deciphering the links between lipid metabolism and the functioning of the plant secretory pathway. Thanks to the **pioneering work of Chris Hawes**, we were able to establish new ways of investigating the plant secretory pathway. The story will continue with the collaboration we still have with his team (with Verena Kriechbaumer), with in mind a great memory and our gratitude.



“Of course, you know these cells are really plants, don’t you? They have [insert a plant cell structure], which makes them plants...”

Chris Hawes, insisting that everything was a plant if you looked hard enough

In 2011 I joined Oxford Brookes university as the Bioimaging Unit Manager and was lucky enough to work closely with Chris for just over 7 years. Chris was an inspirational scientist. He renewed my faith in science, was a great mentor and became a good friend. His innovative approach to microscopy was both exciting and refreshing and we shared a love for all things related to electron microscopy. Chris and I spent many hours together on the microscopes, especially if there were Golgi bodies involved! In fact, it was difficult to get him away, even when we had automated 3D imaging techniques that meant we could leave the microscope to collect data by itself. We did numerous outreach events together and I will always remember driving into London with him, debating the validity of different types of music for several hours. But I will mostly remember Chris for being a great person. He cared about the people around him. He helped me through several very tough times when my mother became ill. He came to my wedding on a hill in mid Wales and happily camped in the field despite the weather. He shared a love of Triumph motorbikes with my husband. He paid attention and encouraged me in all aspects of my life, not just my work with him. Chris was an amazingly supportive line manager, an enthusiastic colleague and a refreshing scientist. He is missed. Louise Hughes

Golgi bodies in Venus’ fly trap gland cells captured using electron tomography (top left), from an animal cell and from a maize root cell using TEM (top middle left and right, respectively) and rendered in 3D (top right). Chris on the TEM in 2016 after moving into the new facility (above). Chris posing on Paul’s motorbike (2018, bottom left), making pasta filling at a ZEISS labs at location partner event (2018, bottom middle) and when the new 3D SEM arrived in 2014 (bottom right).



Chris and his inspiration for plant glycobiology at BOKU Vienna

Jennifer Schoberer and Richard Strasser

Department of Applied Genetics and Cell Biology, University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria, jennifer.schoberer@boku.ac.at, richard.strasser@boku.ac.at

When we first meet Chris at one of the Pharma-Planta meetings in 2004 we were immediately fascinated by his enthusiasm about the secretory pathway, in particular his enthusiasm about the Golgi apparatus. At that time, we had already been working on different Golgi-resident glycosyltransferases and glycosidases involved in N-glycan processing and had established their stepwise trimming reactions as well as a basic idea of their Golgi-localization across the stack. Chris instantly liked our work and suggested to collaborate to more precisely determine the sub-Golgi location of Golgi-resident proteins and use the enzymes with defined *cis*-to-*trans* sub-Golgi location as tools to study the overall Golgi organization as well as Golgi biogenesis.

An early highlight of our common studies was work on Golgi stack disassembly and reformation. Blockage of secretion resulted in a retrograde absorption of Golgi-resident glycosyltransferases and glycosidases into the endoplasmic reticulum (ER) starting with the *trans*-Golgi enzymes, followed by a sequential redistribution of medial- and *cis*-Golgi enzymes (Fig. 1A) (Schoberer et al., 2010). These findings indicate the presence of a distinct retrograde pathway that remains to be uncovered. With a Programme Access Grant, Chris opened the doors for us to use the two photon FRET/FLIM microscopes at the Harwell-Campus, Didcot. Using fluorescence lifetime imaging of *cis*-, medial- and *trans*-Golgi enzymes it was possible to determine the interaction of different N-glycan processing enzymes and establish the formation of homomeric and heteromeric complexes in the Golgi (Fig. 1B) (Schoberer et al., 2013). In our recently published paper (Schoberer et al., 2019), we identified an amino acid signal motif within the cytoplasmic tail of the ER- α -mannosidase MNS3 that acts as a specific Golgi retention signal and thus prevents the interference with ER-associated degradation of misfolded glycoproteins (Fig. 1C).

As an outstanding cell biologist, Chris taught us (specifically Jenny) the secrets of live cell imaging of fluorescent proteins with confocal microscopy. Moreover, as collaboration partner in many successful funding applications, Chris significantly contributed to the progress of our scientific careers. We will deeply miss Chris, but we are committed to share his excitement for science and will continue our joint work to solve some of the unanswered questions in plant cell biology (Fig. 1D-E). Let it be tubules!

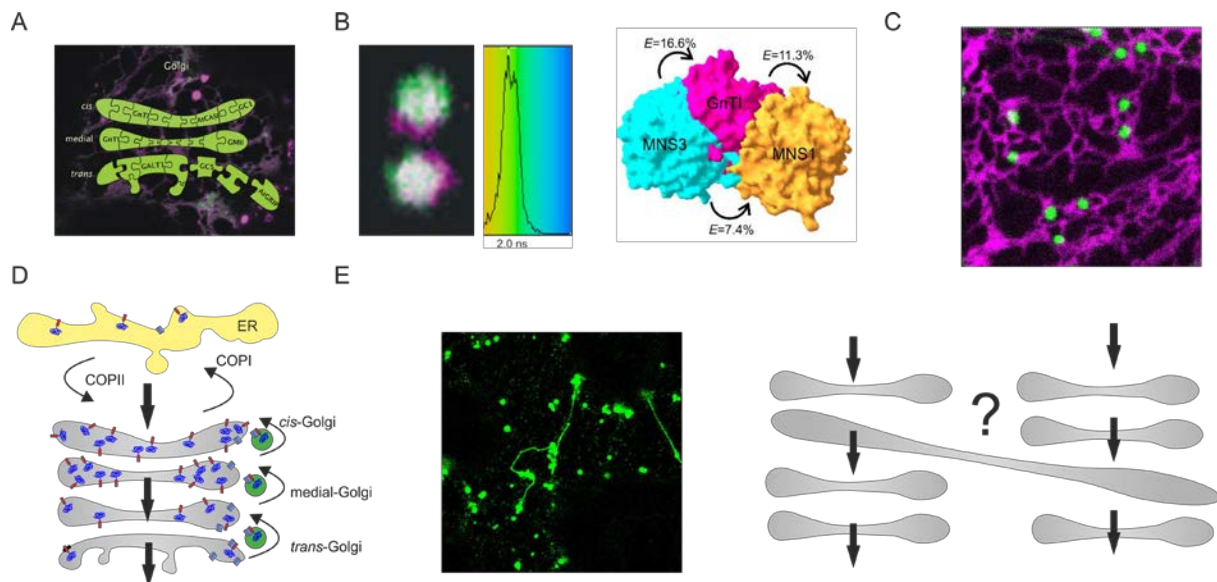


Figure 1. (A) The Golgi disassembly starts at the *trans*-face of the Golgi. (B) FRET-FLIM was key to determine the *in planta* interaction of N-glycan processing enzymes with differential intra-Golgi locations. (C) MNS3 exclusively resides in the Golgi apparatus at steady-state. (D) Proposed Golgi transport model based on vesicular transport carriers. (E) Frequently observed tubular connections between individual Golgi stacks and a simplified illustration of a potential role for exchange of cargo or Golgi resident proteins and lipid components.

- Schoberer J, Runions J, Steinkellner H, Strasser R, Hawes C, Osterrieder A (2010) Sequential depletion and acquisition of proteins during Golgi stack disassembly and reformation. *Traffic*. 11: 1429-1444. doi: 10.1111/j.1600-0854.2010.01106.x.
- Schoberer J, Liebminger E, Botchway SW, Strasser R, Hawes C. (2013) Time-resolved fluorescence imaging reveals differential interactions of N-glycan processing enzymes across the Golgi stack in planta. *Plant Physiol*. 161: 1737-1754. doi: 10.1104/pp.112.210757.
- Schoberer J, König J, Veit C, Vavra U, Liebminger E, Botchway SW, Altmann F, Kriechbaumer V, Hawes C, Strasser R. (2019) A signal motif retains Arabidopsis ER- α -mannosidase I in the cis-Golgi and prevents enhanced glycoprotein ERAD. *Nat Commun*. 10: 3701. doi: 10.1038/s41467-019-11686-9.

Tribute to Chris Hawes' contribution to the Gatsby Plant Science Summer School

A lot of cell biologists have contributed to the Gatsby summer schools and continue to do so brilliantly; but Chris was always my “go to” person if I wanted to bounce any ideas around as to who to involve and what to include on the topic of plant cell biology.

After the success of the first summer school in 2005 and our consideration as to who to invite for a plenary talk in 2006, Chris was an obvious choice to excite students about plant cells. Here is his photo and abstract for his talk in 2006 at Bretton Hall.



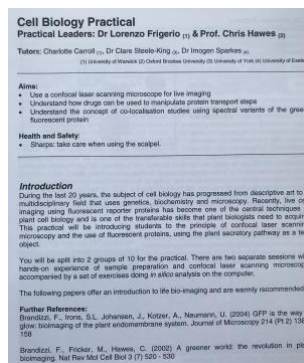
Lighting up the Cell – Fluorescent Proteins reveal Endomembrane Dynamics Professor Chris Hawes, Oxford Brookes University

Time: Thursday 13th July, 9.00 – 9.45am

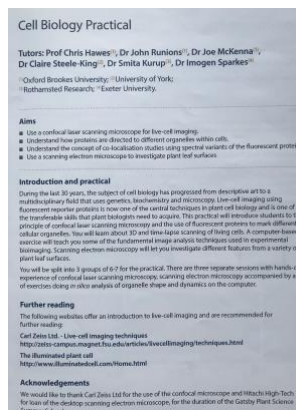
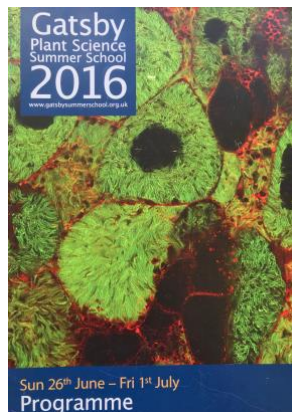
Abstract:

The plant cell can be considered as a factory which is manufacturing a myriad of different products with a wide range of uses such as growth, defence and storage. One important production line in this factory is the secretory pathway comprising the endoplasmic reticulum (ER), Golgi apparatus and associated organelles. This system is responsible for the synthesis and transport of a wide range of proteins and complex polysaccharides that are either destined for the vacuoles or the cell surface. In order to be able to efficiently exploit this pathway for biotechnological uses, such as improving the quantity and quality of seed proteins or the production of useful compounds including biopharmaceuticals, a full understanding of the mechanisms regulating production rates and transport through the pathway is necessary. In this lecture I will describe how the application of recent technology based around the use of a jellyfish Green Fluorescent Protein (GFP) has revolutionised our ability to investigate the plant secretory pathway in living cells. By tagging GFP or coloured variants, to organelles, regulatory molecules and secretory product (cargo) the structure and dynamics of the ER and Golgi can be studied in real time, as can the transport of secretory product through the pathway.

Looking back through summer school programmes, I next find photos of Chris in 2012, though he will have contributed in the intervening years too. Everyone will recognise I'm sure how he shared the excitement of what he saw under the microscope to the students and how they hung on to his every word.



I next found him in the 2016 programme.



Again, captivating another group of students and this time with a desk top scanning EM. It was Chris' idea to introduce the desktop scanner to the practical and it was an instant hit with students, tutors, visitors, in fact anyone on site who wanted to know what we were all so excited about. I took the picture on the right to show the image on Chris' laptop, but I think the grin on Chris' face is the best part of this photo, as he was clearly delighted with the image.

Chris was always a pleasure to spend time with; he was interested in everything and everyone. On behalf of the teams at the University of Leeds who ran the summer schools from 2005-2013 and for the Gatsby Plant Science Education Programme team at the University of Cambridge who have run the school since 2014, we'd like to thank Chris for his significant contribution to the school's success. We will miss him a great deal.

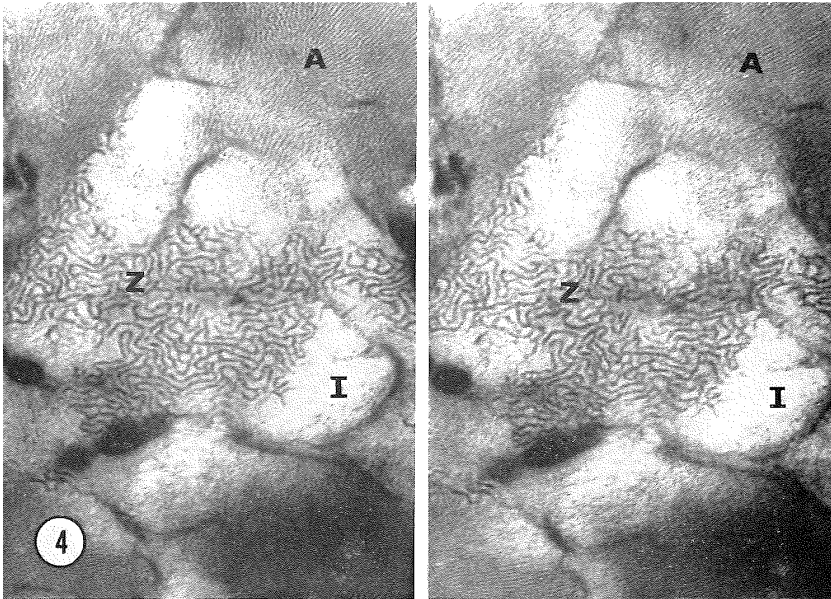
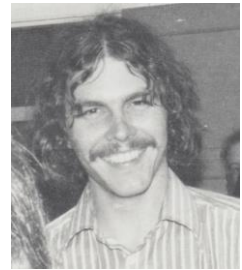
Celia

Dr Celia Knight MBE was the founder and academic coordinator of the Gatsby Plants summer school, later called the Gatsby Plant Science Summer School, from 2005 – 2017 and is now the academic advisor for the school.

Chris Hawes: outlaw to in-law

Jim Hardie, Imperial College and Royal Entomological Society.

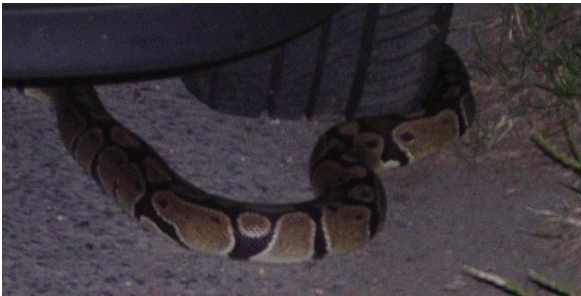
I was first introduced to Chris by my sister, Kay, at a party in Birmingham in 1977, and it soon became apparent that we had some common interests – motorcycles, beer and electron microscopy – as well as my sister.



Microscopist - Chris and I were both post-docs and a while later we used Chris's expertise on the high-voltage electron microscope (HVEM) at Oxford University to clarify something arising from my PhD studies. I had worked on the ultrastructure of insect muscle and was interested in the perforated Z discs in supercontracting muscles. In some body-wall muscles, such as the blow fly maggot, the Z disc was rigidly aligned and standard thin-sectioned material readily showed the reticular structure of the Z material. By comparison, in crane fly larvae, thin sections indicated that the Z material was more fragmented and possibly comprised discrete Z bodies. Using thick 1

µm sections and 3D representations from the HVEM, Chris and I could show that the Z material of crane fly muscle also had a reticular arrangement but the perforations were larger and the Z disc less tightly aligned within the muscle fibre (stereopair image: x1700, tilt angle 12°; **Hardie & Hawes**, 1982, The three-dimensional structure of the Z disc in insect supercontracting muscles. *Tissue & Cell* 14, 309-317).

Herpetologist - One evening in 2002 we spotted a 2 m python lying behind my car outside Chris's house. Chris collected some makeshift equipment and we set about a rescue. The snake had other ideas, slithered under the car



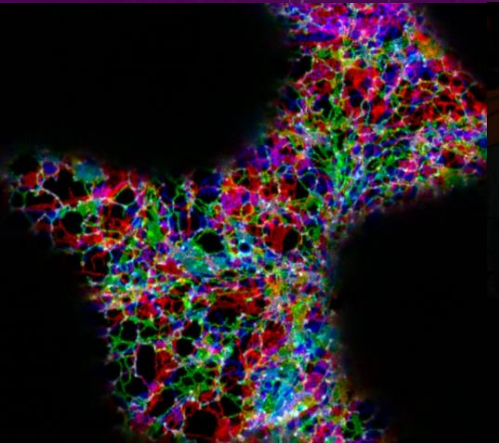
and wrapped itself around the exhaust pipe. Chris's attempts to talk it down failed so the engine was started and when the exhaust became uncomfortably warm the snake decided to move on. Chris grabbed it and popped it into a handy pillow case. No one nearby admitted to owning the snake so a local pet shop rehoused it.



Motorcyclist – Chris rode motorcycles when we first met and his favourite was a Triumph – a 1980 T140D 750cc Bonneville Special. Bought new and not replaced until 33 years later, by a 2013 Bonneville.

Outlaw to in-law? Chris finally became my official brother-in-law when he and Kay married in 2018. Until then he was merely my brother-out-law. He was also, of course, a very good friend.

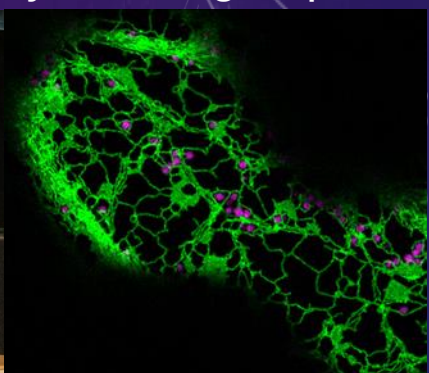




We say goodbye to a wonderful colleague,
teacher, mentor and friend...

Thank you for everything
and all the memories

With love,
Verena & your lab group





Pardon Me for Not Getting Up

*Oh dear, if you're reading this right now,
I must have given up the ghost.*

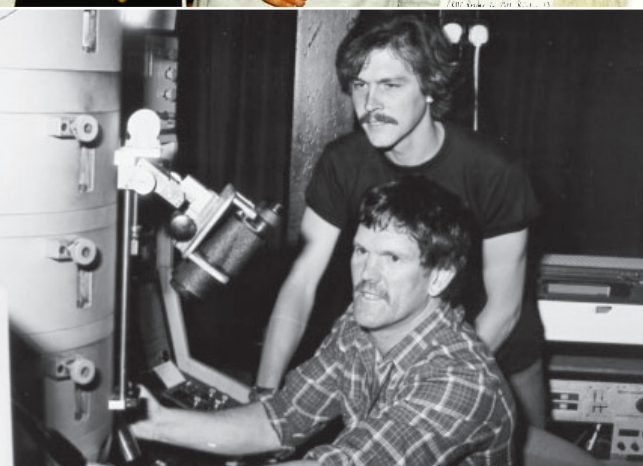
*I hope you can forgive me for being
Such a stiff and unwelcoming host.*

*Just talk amongst yourself my friends,
And share a toast or two.*

*For I am sure you will remember well
How I loved to drink with you.*

*Don't worry about mourning me,
I was never easy to offend.*

*Feel free to share a story at my expense
And we'll have a good laugh at the end.*



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