From the identification of 'Cells', to Schleiden & Schwann's Cell Theory, to Confocal Microscopy and GFP lighting up the Plant Cytoskeleton, to Super-Resolution Microscopy and Single Molecule Tracking: Here's...

A Short History of Plant Science Chapter 5:

A Short History of Plant Light Microscopy

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When the microscope was first introduced to scientists in the 17th century it started a revolution. Suddenly a whole new world, invisible to the naked eye, opened up to curious explorers. In response to this realization Nehemiah Grew, one of the early microscopists, noted in 1682 '*that Nothing hereof remains further to be known, is a Thought not well Calculated.*'[1]. And indeed, with ever increasing resolution, there really does not seem to be an end to what can be explored with a microscope.

The Beginnings: Plant Internal Structures and 'Cells' (1600-1835)

While simple lenses were being used as magnifying glasses for several centuries, the early **17th century** brought the invention of the compound microscope, and with it launched the scientific field of microscopy[2]. It is not clear who invented the first microscope, but it was most likely developed from early telescopes[2]. Galileo Galilei built his first telescope in the early 1600s and used it to chart the stars[2]. He subsequently published his treatise 'Sidereus nuncius' **(1610)** about his observations[2,3]. However, Galileo also observed that he can use his telescope to magnify objects if he moved the lenses further apart[2]. It is conceivable that this observation, made by others as well, has led to the development of the microscope[2]. One of the first documented microscope makers was Cornelius Drebbel, and Galileo built his first microscope based on a design by Drebbel in the **mid 1620s**[2]. This microscope was used by Federico Cesi and Francesco Stellut to observe a bee and a beetle, possibly the earliest documented use of a microscope[2]. Simple compound microscopes of the mid 17th century were basically hollow metal tubes containing a convex lens at each end – the objective lens to collect and focus the light coming from the object, and the eyepiece lens on the other end for additional magnification[2]. These earliest compound microscopes allowed for magnifications

of up to 25 times, but were quickly improved in the following years. Robert Hooke and Antonie van Leeuwenhoek were two pioneering microscopists in those years. Antonie van Leeuwenhoek, a drapery salesman, was simply interested in finding a tool to better examine the thread quality in the fabrics in his shop, which got him interested in lens making[4]. Eventually he was able to create tiny lenses, allowing for magnifications of up to 250 times[4]. Robert Hooke, a polymath, had already been interested in optics and light refraction when he came across the new compound microscopes[5]. He too started to experiment with custom-made instruments and self-made lenses to improve the quality of his microscopes[5]. Eventually, both started documenting their microscopic work. Robert Hooke used his microscope to document everything, from microbes to plants, to man-made objects[6]. This resulted in the publication of Robert Hooke's book '*Micrographia: or Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses. With Observations and Inquiries Thereupon*' by the Royal Society of London in **1665**[6]. '*Micrographia*' became a bestseller, with Samuel Pepys, British politician and famous diarist, confiding to his diary that '*Before I went to bed, I sat up till 2 o-clock in my chamber, reading of Mr. Hookes Microscopical Observations, the most ingenious book that I ever read in my life.*'[4]. Antonie van Leeuwenhoek also read this book, and started to publish his own observations in the form of letters to the Royal Society in the late **1670s**[4,7]. He focused mainly on insects and microorganisms, but did adventure a bit further as well. And so, in **1677**, he checked with the Royal Society of London if his latest work was publishable, writing *'If your Lordship should consider that these observations may disgust or scandalise the learned, I earnestly beg your Lordship to regard them as private and to publish or destroy them as your Lordship sees fit'*[8,9]. But the Society did consider van Leeuwenhoek's latest observations to be of scientific value, and so the first observation of sperm in human and animal ejaculate was published in **1678**[8,9]. From a plant microscopist's perspective, however, it is one figure that stands out among these earliest publications: In Robert Hooke's *Micrographia*, *Schem: XI, Fig: 1, A & B* shows a piece of cork (*click for Figure*)[6]. When examining this slice under his microscope, Hooke found that it had '*very little solid substance*', but was made up of little '*pores, or cells*'[6]. For Hooke, this observation demonstrated to him '*the true and intelligible reason of all the Phænomena of Cork*' - why it is so light relative to its size, why it floats on water, and why it is so springy when compressed[6]. But more important in retrospect is that this little sentence coined the word '*cell*' to describe cells[6].

The work of Robert Hooke and Antonie van Leeuwenhoek made them the "Fathers of Microscopy", and this new exciting field of research quickly got populated with other figures.

One of the next big publications for the plant sciences came in **1682** with Nehemiah Grew's '*The anatomy of plants - with an idea of a philosophical history of plants, and several other lectures, read before the Royal Society*'[1]. This came at a time when it was not even accepted that plants were made up of organs, or had any internal structures at all. The book opens with a dedication to King Charles II that beautifully describes how the invention of the microscope forever altered our perception of the world, or rather, how it opened up a completely new world, which previously remained hidden to the human eye:

'*Your majesty will here see, that there are those things within a Plant, little less admirable, than within an Animal. That a Plant, as well as an Animal, is composed off several organical parts; some thereof may be called its Bowels. That every Plant has Bowels of diverse kinds, containing diverse kinds of liquors. That even a Plant lives partly upon air; for the reception whereof it has those Parts which are answerable to Lungs. So that a Plant is, as it were, an Animal in Quires; as an Animal is a Plant, or rather several Plants bound up into one Volume.*

Again, that all the said Organs, Bowels, or other Parts, are as artificially made; and for their Place and Number, as punctually set together; as all the Mathematic Lines of a Flower or Face. That the Staple of the Stuff is so exquisitely fine, that no Silkworm is able to draw anything near so small a thread. So that one who walks about with the meanest Stick, holds a Piece of Natures Handicraft, which far surpasses the most elaborate Needle-Work in the World.

In sum your majesty will find, that we are come ashore into a new World, whereof we see no end.'[1].

In the following book Grew systematically describes the morphology and anatomy of several plants, covering seeds, leaves, stems, roots and flowers, always accompanied by beautiful illustrations of the entire organ, magnifications and cross-sections (*e.g., see plate XXXVI. B here*)[1]. At a time when it was not yet accepted that plants had any inner structures, let alone organs at all, his images showed that plants were indeed as complex as animals.

What the illustrations in these books also demonstrate, is that the authors not only had to be masters of microscopy, but also had to be great at sketching and drawing, to adequately document their observations. The days of cameras and detectors were still centuries away at that point. In the early 1800s, however, an invention by William Wollaston did bring some help. Thankfully for many microscopists coming after him, William Wollaston was, in his

own account, not good at drawing: '*Having* (...) *amused myself with attempts to sketch various interesting views without an adequate knowledge of the art of drawing, my mind was naturally employed in facilitating the means of transferring to paper the apparent relative positions of the objects before me.*'. This led him to develop a device called the camera lucida in **1807**[10]. The camera lucida is as simple as it is ingenious. A four-sided glass prism is placed in front of the eye piece of the microscope and above the piece of paper where the drawing is supposed to be made[10,11]. In the prism, two sides are arranged at a 135° angle to produce two reflections of the light coming from the microscope through total internal reflection, thereby producing a non-inverted or reversed image of the object under the microscope at the position of the eye[10,11]. Since the prism is above the piece of paper, the microscopist sees both, the reflected image from the object at the edge of the prism, and the drawing surface in front of him, and can sketch out the key points of the object onto the paper[10,11]. As the superimposed image and the paper will not be in the same focal plane, a lens is additionally placed between the prism and the paper, to bring both into the same focus[10,11]. The camera lucida, or similar devices such as Sömmering's mirror, were used well into the 20th century, and were instrumental in making the microscope the powerful tool it has become for scientists[11].

While Nehemiah Grew's observations made it clear that plants were indeed made up of several different structures, it was not yet clear how all these different structures are formed and connected, and how Hooke's cells fit in. Between **1800** and **1810**, the French botanist Charles-François Brisseau de Mirbel made his own microscopic observations of the anatomy of different plants[12]. These eventually led him to the understanding that green plants are made up of a single continuous membrane, which envelopes and interconnects the cells[12,13]. The individual cells, he argues, where made up from parenchyma, and grow from, between or inside of older cells[12–14]. This hypothesis got Brisseau de Mirbel a lot criticism from his contemporaries, who believed that cells were individual units, put together to form a tissue, and eventually this disagreement led him to further investigations to prove his point[14,15]. Going into this new work he declared that '*Thirty years have passed since I first published my opinions on several points. They were strongly attacked. Today now I want to submit them to my own review: I will try to be impartial.*' **(1835)**[14]. He decided to focus on a thorough investigation of one specific plant, rather than looking at several different ones for his re-examination, and chose the liverwort *Marchantia polymorpha*, instead of a plant with a stem, woody tissue, flowers and such, since '*it is the cellular tissue which I have chosen to investigate, and, consequently, a whole plant made of this tissue is more suitable than any other*'[14]. While Brisseau de Mirbel's view that the cellular tissue of plants is made from one

continuous membrane turned out to be wrong, and he acknowledged so, his work was still important in understanding where cells came from, as he was among the first to hypothesize that new cells arise somehow from older cells[15,16]. And furthermore, his description and illustrations of *M. polymorpha* contributed to the introduction of this liverwort as a model plant (*see Plate I here for one of his beautiful overviews*)[15].

Plant Cell Nuclei and Chromosomes, the Cell Theory and Cell Division (1830-1930)

The next big step in understanding plant life via microscopy came in the mid-19th century at the hands (and eyes) of Matthias Jacob Schleiden and Theodor Schwann[16]. Matthias Schleiden already believed that an organism was made up of a society of cells, and so he focused his work on discovering where these cells originated from. Brisseau de Mirbel's idea that they somehow arise from older cells was not accepted yet, and cells were sometimes suggested to just 'crystallize'[17]. Schleiden built his work in part on the finding of Robert Brown that all plant cells seem to have one nucleus **(1831)**[18]. So Schleiden came up with the idea that this body was the potential starting block of a new cell[19]. His first big discovery was that the nucleus contained another, smaller granule, the nucleolus[19]. Then, while monitoring the endosperm of palm seeds over time, he observed free-nuclear divisions of the endosperm (*see Fig. 1 a-e here*)[19]. Such divisions occur before the first zygotic division in the endosperm of the embryosac, resulting in 4 to 8 free nuclei before first cell walls are formed and the nuclei are separated into individual cells[20]. From these observations of an (as we now know) atypical cell-division event that only occurs in the endosperm, he logically, but incorrectly, concluded that all new cells are formed *de novo* around a free floating nucleolus[19]. According to his hypothesis, the nucleus is first formed around the nucleolus, which then starts to grow[19]. Once it has reached its full size, the cell emerges from the nucleus as a bubble and expands until it reaches its final size[19]. Then, the cell wall is laid down and the cell is fully established **(1838)**[19]. In 1837, while preparing his observations for publication, Schleiden met Theodor Schwann, his colleague at Johannes Peter Müller's Institute at the University of Berlin, for dinner[16]. On this occasion, '*Schleiden, this illustrious botanist pointed out to me the important role that the nucleus plays in the development of plant cells*', Schwann recalled later[16]. He had just recently observed cells with nuclei in the notochord (*chorda dorsalis*) of toads, and following his dinner with Schleiden he also observed the same in mammalian cartilage tissue[16,21]. Realizing these common principles between plants and animals, Schwann proposed a general cell theory in **1839**[16,21]. Based on his and Schleidens observations Schwann defined a cell as consisting of a nucleus (with nucleolus), and fluidic content contained within a

wall[21]. He further hypothesized that all organisms, be it a plant, animal or human, are made up of one or more cells, with the cell being the basic unit of structure and organization of an organisms[21]. Finally, he concurred with Schleiden that new cells are formed *de novo* around the nucleus, which therefore represented a common principle of development for all organic tissues[21]. This 'cell theory', while not completely correct, led Edmund Wilson to remark in **1896** that "*no other biological generalization, save only the theory of organic evolution, has brought so many apparently diverse phenomena under a common point of view or has accomplished more for the unification of knowledge*"[22]. It is therefore somewhat ironic that because the cell theory remained so compelling as a generalized model for how all organic tissues form and develop, it actually inhibited research into cell division for decades, due to its inclusion of the *de novo* cell formation aspect[17]. Still, the finally accepted fact that new cells are formed via division of existing cells was again based on the work of two plant microscopists: Hugo von Mohl and Carl Nägeli[23]. Von Mohl was an expert for microscopy and plant sample preparation. Among the many phenomena he observed and documented in the mid 19th century were the formation, opening and closing of stomata, and he also coined the term '*protoplasm*' to describe the content of a cell[23–26]. In regards to cell divisions, von Mohl actually observed and documented them in the algae *Cladophora glomerata* already in **1835** (see Fig. 3-5 *here*)[23]. Von Mohl's observation was later supported by Carl Nägeli, who observed cell division in pollen in **1842**[25,27]. While the working hypothesis of von Mohl and Nägeli were not accepted over the cell theory at the time, it did form the basis for subsequent studies confirming that new cells are indeed formed by cell division.

With the nucleus and nucleolus at the central focus of Schleiden's and Schwann's work, the further development of the microscope allowed researchers in the early 20th century to publish on the content of the nucleus: the plant chromosomes[28–30]. In **1907** *Arabidopsis* pioneer Friedrich Laibach completed his PhD by determining the number of chromosomes in different plant species, among them *Arabidopsis thaliana*[28]. *A. thaliana* was only featured in his complete thesis however, and was omitted from the publication, as it was not regarded as important enough at the time[31]. Laibach himself helped change this view in the years to come (See also 'A Short History of *Arabidopsis thaliana* (L.) Heynh. Columbia-0'[31]). Following this work, Emil Heitz analysed the chromosomes of liverworts in closer detail, thereby following in the footsteps of Marchantia pioneer Brisseau de Mirbel and *Arabidopsis* pioneer Laibach **(1928)**[29,30]. Finding density differences within the chromosomes during the telophase of mitosis, Heitz defined the terms '*euchromatin*' and '*heterochromatin*'[29,30]. Both, Laibach

and Heitz used the '*Abbe'scher Zeichenapparat*', to document their work. This was an improved version of the camera lucida, designed by Ernst Abbe for Zeiss microscopes[28,29].

Ernst Abbe may have pushed the boundaries for microscopists like hardly any other individual person[32]. In the **1860s** Ernst Abbe joined Carl Zeiss in his newly founded Zeiss Company as director of the research department, and later went on to become a co-owner of the company in the **1870s**[32]. During his time at Zeiss he studied the theory of optics and microscopy, and, based on his findings, started to develop and build much improved microscopes[32–34]. Some of his most important contributions to the field are the invention and implementation of apochromatic lenses into microscopes to focus light of different wavelengths to the same plane, the development of the first refractometer to determine the refractive indices of different samples and media, a definition of the numerical aperture for an objective lens, and a formula to define the resolution limit of a light microscope[33–36]. When the first ZEISS logo was issued in 1904 it featured the company's name inside a frame outlining Abbe's apochromatic doublet lens, highlighting the importance of this invention[37]. Another important Zeiss employee at that time was August Köhler. Köhler tackled another major problem of microscopy at the time, which was the uneven illumination of the field of view, which in addition often showed the illumination source (e.g. the light bulb filament) in the final image[38]. Köhler developed the Köhler-illumination technique, which utilizes a collector lens in front of the light source to defocus the light source from the sample plane, thereby removing it from the image **(1893)**[38]. Additionally, an adjustable field diaphragm is installed in front of the collector lens to get rid of any stray light[38]. Finally, a condenser lens focuses the light onto the sample, thereby ensuring a homogenous illumination of the entire field of view[38]. Thanks to the work of Abbe and Köhler, the general imaging conditions improved dramatically for microscopists at the end of the 19th century. And there was another development around the turn of the century that would radically change the way microscopists work; photomicrography. Photomicrography had been invented and patented already in **1850**, when Richard Hill Norris used it to image blood cells[39]. But two important developments really opened up the field of microscopy to photomicrography. The first was the aforementioned Köhler-illumination in 1893, since a homogenously illuminated field of view is a prerequisite to obtain a good photomicrograph. The second was the development of the Leitz Camera, or LeiCa in short, in the early 20th century[40]. The Leica 1 was released as a portable and easy to use camera in **1925**, and in combination with a microscope with Köhler-illumination finally enabled scientists to take photos of their observations, rather than having to draw them[40].

Plant Cell Organelles & the Cytoskeleton (1930-1980)

The work of Ernst Abbe and August Köhler advanced the common light microscope to a point where its potential was almost exhausted. At this point, new microscopy techniques were needed to increase the resolution and image quality further. The first such major improvement came in **1934** when Frits Zernike published the theoretical work that eventually resulted in phase-contrast microscopy (PCM) (**1938**)[41,42]. When light passes through a sample it is scattered, resulting in changed phases of the light waves compared to the non-scattered illumination light that did not pass through the sample[41,42]. These phase changes can be converted into differences in brightness to enhance the contrast in the final image[41,42]. In a phase contrast microscope this is achieved by filtering the non-scattered illumination light to decrease its amplitude, and by changing the phase of the non-scattered illumination light to match its phase with the phase of the scattered light, thereby creating constructive interference[41,42]. This technique was especially important for biologists at the time, as it increased the contrast, and hence the image quality, of non-labelled samples[41,42]. And since most samples were still unlabelled at the time, adding good contrast to the image meant a giant leap forward. Accordingly, Frits Zernike was awarded the Nobel Prize for Physics in **1953** for his invention[43,44]. One early publication utilizing PCM in the plant field came in **1955**, when Robert de Ropp analysed plant cells that he had cultured, trying to establish a proper plant cell culture[45]. While he failed to establish a true cell culture, as the cell protoplasts steadfastly refused to divide in the culture medium employed, the improved contrast in his images allowed him to not only see organelles in much closer detail, such as mitochondria and moving nuclei, he could even observe the streaming of the cytoplasm, and he also documented different stages of secondary cell wall formation (*see Fig. 7 here*)[45]. In the same year Helen Sorokin documented mitochondria, stomata, and plastids clustered around the nucleus in peeled lettuce epidermis cells (*see Fig. 2 here*), and also showed how Neutral Red and Janus Green B can be used to stain mitochondria. For the latter, she also demonstrated how the combination of PCM with vital stains can push the resolution even further[46].

Both de Ropp and Sorokin used PCM and photomicrographs to document their work, and can therefore be considered state-of-the-art microscopists. However, there are always talented people that push things a little further. Already ten years earlier, in **1946**, Henrik Lundegårdh published his work on root hair development in wheat[47]. For this, he designed and built a specialized experimental setup: First, he designed a little microfluidic chamber in which the

wheat seedling could grow in distilled water[47]. Through in- and outlets at each end of the chamber he was able to run different solutions through it, and along the root of the growing wheat plant[47]. This chamber was closed by a cover slip on top, and mounted onto a microscope[47]. To document the reaction of the root hairs to different solutions washed through the chamber, Lundegårdh had installed a film camera above the microscope with a clock work to automatically run 32 mm film through the camera, and an automatic electromagnetic shutter for the one second exposure time[47]. Using this setup, which preceded the modern microfluidic platform RootChip[48] by 65 years, he was able to, among other things, document that glucose accelerates hair growth, that a pH lower than 6 reduces growth, and that the addition of auxin or calcium can counteract this negative effect, at least at a pH of 5[47]. Though educational videos of growing roots or emerging lateral roots had been recorded since before the 1930s, this setup provided a whole new level of detail[47,49].

While plant microscopists were beginning to publish their work using PCM, Georges Nomarski already further developed this technique into differential interference contrast (DIC) microscopy **(1952-1955)**[50,51]. For DIC microscopy, two orthogonally polarized light rays are used, which both penetrate the sample slightly offset from each other, thereby experiencing slightly different phase retardations, depending on the refractive index and thickness of the sample at the point they pass through it[50,51]. Both rays are then re-combined but cannot fully reproduce the initial polarization of the illumination light due to the subtle differences in phase retardation experienced by both rays[50,51]. A polarization filter oriented perpendicular to the polarization of the illumination light is then used to reject the illumination light and transmit specifically such light rays that penetrated through optically inhomogeneous parts of the sample, leading to a substantial increase in edge contrast[50,51]. This effort led to the development of the ZEISS Nomarski System in **1965**. In **1966,** a prototype of this new DIC microscope found its way into Robert Allen's Department of Biology at Princeton University, and together with Andrew Bajer he created comparative images of *Haemanthus katheriniae* (cape tulip) cells undergoing mitosis using either PCM or DIC[52]. Having demonstrated the benefits of DIC microscopy for plant cells with this first paper, the pair immediately added a second publication containing a time-series of DIC images following a cell undergoing mitosis and cell plate formation (*see Fig. 2-7 here*)[53].

Helen Sorokin's use of Neutral Red and Janus Green B to stain mitochondria were the first examples shown here for another new trend in the middle of the twentieth century. While general stains have long been used, researchers now began to specifically develop and

synthesize new stains. One of the new vital stains identified at that time was 4′,6-diamidino-2-phenylindole (DAPI), originally developed as a drug against Trypanosomiasis in **1971**[54]. It unfortunately failed as a drug, but in **1975** it was shown that it could be used to label DNA in the nucleus of cultured human cells, and a year later, in **1976**, it was shown to also work in plant cells[55,56]. Another important DNA stain set was the series of Hoechst stainings **(1975/1976)**[57,58]. Later on, more dyes for specific structures and organelles were added, such as 3,3'-dihexyloxacarbocyanine iodide (DiOC6(3)) to mark the plant endoplasmic reticulum **(1986)**[59]. And during the 1980s, the field of plant biology underwent a major revolution due to several developments, namely: the adoption of *Arabidopsis thaliana* as a model organism for the plant field, the establishment of plant transformation, and the identification of the cauliflower mosaic virus 35S promoter (*see the Short History Chapters 1-3 for more on this plant science revolution*[31,60,61]). In addition, these developments also brought the first genetically encoded reporter for plant light microscopy[62]. This came in the form of the *Escherichia coli β-glucuronidase* (*GUS*) gene **(1987)**[62]. The enzyme encoded by the *GUS* gene converts a colourless substrate (mostly X-Gluc) into the blue diX-indigo[62]. Therefore, expression of *GUS* from a gene's specific promoter will visualize the expression pattern of the investigated gene *in planta*[62].

Moreover, another important 'staining' method was developed at the time; immunofluorescence microscopy **(1974)**[63]. In the early 1930s researchers were able to purify and label pneumococcus antibodies, despite not even knowing for sure if these antibodies were proteins or substances of a completely different nature[64]. This lead Albert Coons to test if he could use fluorescently labelled pneumococcus antibodies to actually locate antigens in tissue infected by pneumococcus[65]. By 1941, in the midst of World War II, Coons and his colleagues had managed to synthesize a fluorescein-antipneumococcal antibody, and were indeed able to stain pneumococcal antigens in the liver of an infected mouse[66]. Unfortunately, as mentioned by Coons concerning this breakthrough, '*I joined the Army in April, 1942, and the paper was written on a cross-country train. It was carefully re-written by Enders, who sent it off to the Journal of Immunology where it appeared in November, 1942. In the press of events, however, he forgot to send me a reprint, and I had no idea of its fate for many months. Finally, I subscribed to the Journal of Immunology. Six issues of it reached me at Brisbane in Australia on the day I boarded a ship to go North to New Guinea. In one of them I found our paper*'[67]. The photomicrograph, taken by Coons with a Leica 1 through a ZEISS fluorescence microscope, is the first immunostaining documented, and basically initiated the field of immunohistochemistry[67,68]. But in the early **1970s** Klaus Weber took the field a big step

further, by demonstrating that an organism will not just produce antibodies against actually infectious disease agents, but against almost every foreign protein injected into it[63]. The realization that antibodies can be raised against pretty much any protein, and then be used to label and visualize this protein in other cells, formed the basis of immunofluorescence microscopy **(1974)**[63]. In order to reach this breakthrough it came in handy that Weber had previously pioneered the technique of sodium dodecyl sulfate gel electrophoresis to separate and purify proteins based on their molecular weight[69]. In the early 1970s, this technique allowed Weber and his colleagues to obtain the pure antigens required to raise their antibodies[63]. The first antibody Weber and his team raised and used as fluorescent marker was an anti-actin antibody, and the fluorescent images of the actin network in chicken cells they obtained with it, served as the basis for the typical textbook view of the actin cytoskeleton used for the next decades[63]. Following this initial paper, the Weber lab added a string of publications lighting up the entire animal cytoskeleton with antibodies against actin, tubulin, myosin and several other proteins[63,70–72] (**1974-78**). He then helped the plant field by demonstrating that *Leucojum aestivum* (summer snowflake) endosperm microtubules can also be labelled with his anti-tubulin antibody, providing scientists with the first view of the plant microtubule network **(1977)**[73]. Lloyd et al. subsequently showed the labelling of microtubules in intact cells (*see Fig. 1 here*) **(1979)**[74]. The first images of the plant actin network were not obtained using antibodies, however. F-Actin was first shown in the green algae *Chara* in **1980** using nitrobenzoxadiazole-labeled phallacidin, while rhodamine-labelled phalloidin was used to label the actin in vascular plant cells in **1985**[75,76].

The addition of immunofluorescence microscopy to the scientific imaging toolbox represented a giant leap forward, and it set the path for the next major innovation. At this stage, another revolution was needed to move the field forward.

A Green Fluorescent Revolution and the Visualization of Proteins (1960-1999)

This revolution would eventually come with the concurrent development of the confocal laser scanning microscope (CLSM) and the GREEN FLUORESCENT PROTEIN (GFP) as a genetically-encoded fluorescent label. This, however, was a long process. First sketches of confocal beam paths using a pinhole can be found in papers from the **1940s** and early **1950s**, but the first prototype of a confocal microscope was invented, patented and built in **1955/56** by Marvin Minsky[77–79]. This is somewhat peculiar, as Minsky is not known as a spectroscopist, microscopist, or even biophysicist - he is a computer scientist, famous for being one of the pioneers of research into artificial intelligence (AI)[80]. And indeed, that is

what ultimately got in the way of him doing anything further with the confocal microscope prototype he had built[79]. In the early 1950s, his ideas on AI were not fully matured yet, so '*while those ideas were incubating I had to keep my hands busy and solving that problem of scattered light became my conscious obsession*'[79]. But since the field of AI got going around **1956**, Minsky abandoned his confocal at that point[79]. Thus, it was only in **1967** that the first images were taken on a confocal microscope, more precisely on a confocal microscope using a Nipkow spinning disc, named Tandem-Scanning Reflected-Light Microscope[81,82]. The Nipkow disc, perforated with several small pinholes, performed a dual-function, focusing the incandescent lamp illumination light beam to the layer of interest in the sample, and also filtering the emitted light to get rid of any scattering out of focus light (hence the 'tandem' in the name)[81,82]. Using this microscope, researchers imaged frog ganglions and noted that the axons were only visible when the Nipkow disc was inserted into the microscope, thereby demonstrating the ability of this technique to improve the resolution[81]. Since the image quality was not sufficiently good, however, they still needed to include a hand-drawn sketch in their paper, explaining what was apparently visible in the image[81]. This confocal was improved in **1969** with the construction of a scanning microscope featuring a helium–neon laser as light source, a moving objective lens, rather than having to move the sample, and an adjustable exit aperture to act as pinhole in front of a photomultiplier detector, instead of the Nipkow disc[83]. The developers, Davidovits and Egger, then went on to demonstrate its ability by imaging frog blood cells **(1971)**[84]. It is important to keep in mind, that these early CLSMs were still used to image unstained tissue. The following ten years brought several more refinements and additions, such as improvements in the depth of field by using confocal point scanning (the term 'confocal' is mentioned here for the first time)[85–87]. From **1983** onwards, computers could be used to control the microscope, and to store and process the images digitally[88,89]. And then, in **1985**, Brakenhoff et al. showed that they could perform optical sectioning of samples by using a computer-controlled mechanical stage that moved not just two-dimensionally, but also in the third dimension, allowing them to image several layers of the same sample in confocal mode, and computationally reconstruct the three-dimensional image afterwards[90]. They used this technique to show the three-dimensional arrangement of mithramycin(and therefore fluorescent)-labeled chromatin in mouse nuclei – demonstrating that the CLSM had finally arrived at a state where it could be used to answer a biological question **(1985)**[90,91]. When they tried to publish this groundbreaking work in *Nature*, their paper, which had a title focusing on the new microscopy technique, rather than the mouse, was immediately rejected on the grounds that *Nature* does not publish method papers. So the

authors changed the title to a less method-centric '*Three-dimensional chromatin distribution in neuroblastoma nuclei shown by confocal scanning laser microscopy*', and got the same paper published in *Nature*, since with this title it was clearly *Nature*-worthy[90,92]. At the time of this publication, a second paper showing a similar three-dimensional imaging approach on a CLSM was published by Carlsson et al. from Stockholm University[93]. But since their work was not published in *Nature*, it received less attention at the time[92]. It did however, result in the first commercially available CLSM, produced by the company Sarastro[92]. This happened in parallel with William Bradshaw Amos and John Graham White building their own CLSM, which they also intended to commercialize[92]. In **1987**, White and Amos were the first to develop a CLSM where the scanning was performed with the laser beam itself, instead of a moving stage, which significantly sped up the imaging[94]. So when they submitted their paper on the new CLSM to the Journal of Cell Biology, one of the editors immediately sent them a note, trying to purchase the microscope[92]. The big companies, such as ZEISS and Leica, were less enthusiastic, and so they eventually produced their CLSM with Bio-Rad, making the Bio-Rad MRC 500 the second commercially available CLSM next to the Sarastro CLSM 1000[92].

One of the first labs in the plant field to adopt the CLSM was the group of Elliott Meyerowitz, who were already instrumental in pioneering *Arabidopsis thaliana* as a general plant model (*see also* 'A Short History of *Arabidopsis thaliana* (L.) Heynh. Columbia-0'[31]). In the early **1990s**, Mark Running from the Meyerowitz lab developed CLSM to image *Arabidopsis* meristems, using propidium iodide as a marker for nuclei (*see Fig. 6 A here*)[95,96]. And plant microscopists were also quick to connect the CLSM with the new field of immunofluorescence microscopy. Using fluorescently-labelled tubulin, they were able to live-image the plant microtubule network in *Tradescantia* (spiderwort) on a CLSM **(1990)**[97]. For this, they injected fluorescein-labelled pig or sheep tubulin into plant cells, and then recorded how these building blocks were incorporated into the microtubules (*see Fig. 1 here*)[97]. Furthermore, they could image time-series of microtubule dynamics during mitosis and cytokinesis, and demonstrate the negative effect of the herbicide oryzalin on microtubule stability (*see Fig. 4 here*) **(1993)**[98]. Also in **1993**, Grabski et al. visualized the plant endoplasmic reticulum using DiOC6, and showed that it spans the entire plant cell as a net-like structure connected to the plasma membrane[99]. They then used the new CLSM to apply fluorescence recovery after photobleaching (FRAP) measurements in living plant cells, demonstrating that the membrane dye can actually move between cells, and that the cells' membrane systems therefore must be interconnected (*see Fig. 8 here*)[99].

The establishment of the CLSM, in combination with fluorescent markers, was another major advancement in the field of microscopy. But a second milestone had to be reached to utilize its full potential, namely the engineering of GFP as a genetically-encoded reporter and protein-tag. GFP was first observed in **1962**, when Osamu Shimomura and his colleagues isolated bioluminescent proteins from *Aequorea* jellyfish squeezates (the result of squeezing bioluminescent tissue of *Aequorea* through a handkerchief)[100]. They isolated aequorin, a photoprotein that emits blue light when calcium is added[100]. Interestingly, when stimulated in intact cells, the emitted light appeared green, rather than blue[100]. Shimomura and his colleagues eventually isolated the green fluorescent protein as well, and speculated that the blue luminescence of aequorin could excite the green protein *in vivo*, and that this energy transfer may explain the green luminescence observed in intact tissue[101]. This hypothesis was confirmed in **1974**, when the calcium-triggered energy transfer between purified aequorin and GFP was demonstrated *in vitro*[102]. The chromophore of GFP was then described by Shimomura in **1979** (with a slight correction published in **1989)**[103,104]. At the time, the focus was still quite heavily on the aequorin though, and in the early **1980s** Milton Cormier received a grant from Hoffman-La Roche to clone the *aequorin* gene[105]. The pharmaceutical company planned to use it as a bioluminescent marker for antibodies to use in diagnostics[105]. Cormier hired Douglas Prasher for this work[105]. For the project, Prasher and his colleagues regularly travelled to the island Puget Sound to go on fishing expeditions, catching fluorescent jellyfish to isolate proteins, DNA and mRNA from them[105]. Using reverse transcription of the isolated mRNA, Prasher constructed jellyfish cDNA libraries to eventually isolate the specific aequorin cDNA from there[105]. Since the protein structure of aequorin and GFP were already partially known, Prasher could create synthetic radio-labelled antisense DNA probes to screen for homologous sequences in his libraries[105]. Using this method, Prasher and his colleagues quickly progressed and were able to isolate and clone the aequorin cDNA (as well as four isotypes) in **1985**[106]. Aequorin is a holoprotein, meaning that it requires conjugation of a prosthetic chemical group to its apoprotein (apoaequorin) to become functional. In the case of aequorin, this is a luciferin, coelenterazine[106]. Once apoaequorin and coelenterazine have formed the functional aequorin, binding of two calcium ions triggers a conformational change and subsequent oxidation and excitation of the coelenterazine[106–108]. As the coelenterazine reverts from this excited state to its ground state, blue light is emitted[106–108]. Prasher and his team were able to demonstrate and describe this mode of action when they heterologeously expressed the aequorin cDNA in *E. coli* **(1985-89)**[106–108]. However, for Douglas Prasher, the *GFP* gene became much more interesting[105]. Aequorin was bioluminescent, meaning light is

emitted by the joint action of an enzyme (in this case apoaequorin) and a light-emitting molecule (coelenterazine), as well as a co-factor (calcium). GFP, however, seemed to be solitarily fluorescent – able to emit light simply as a result of being excited by light of higher energy. This independence of any co-factors made it a much more promising reporter in Prasher's mind[105]. Following his work identifying and cloning the *aequorin* gene of *Aequorea* in **1987**, Prasher received a tenure-track position at the Woods Hole Oceanographic Institution, where he started to work on cloning and expressing *GFP*, trying to demonstrate its usefulness as a fluorescent reporter[105]. However, not many shared his vision at the time[105]. In fact, even his colleagues, like William Ward and Osamu Shimomura, reportedly doubted that GFP would function as a stand-alone fluorophore[105]. And accordingly, it proved almost impossible for Prasher to acquire funding for this work[105]. On top of that, Prasher felt isolated and unsupported as a molecular biologist at an institution made up entirely of marine biologists and ecologists, who did not appreciate his work[105]. By the early **1990s** Prasher had grown so frustrated and depressed, that he decided to stop his tenure-track process at Woods Hole and began to look for a new job[105]. His paper describing the successful cloning of the *GFP* cDNA and gDNA was published in **1992** as his final work[109]. His last, passing-of-the-torch kind of act as an academic researcher was to mail out two envelopes containing the *GFP* gene, one to Martin Chalfie and one to Roger Tsien[105]. Both had read his paper and shared his vision of GFP as a fluorescent protein tag[105]. Some years later, in 2008, Chalfie and Tsien, together with Osamu Shimomura, were awarded the Nobel Prize in Chemistry for their work on '*the discovery and development of the green fluorescent protein, GFP*'[110–112]. At the time, Douglas Prasher was working as a courtesy van driver at a car dealership[105]. To acknowledge Prasher's contribution, Chalfie and Tsien made Prasher a co-author on their papers, and eventually invited himr and his wife to join them at the Nobel Prize award ceremony, all costs covered[105]. Once Chalfie and Tsien had received the *GFP* gene from Prasher in 1992, things went fast. Chalfie and his co-workers were quickly able to express the gene in *E. coli* and *Caenorhabditis elegans*, demonstrating that the protein could be produced, and is indeed fluorescent without any co-factors, in both pro- and eukaryotic cells[113]. For the imaging, the team used *'a variety of microscopes'*, as stated in their **1994** *Science* paper, which was simply because they actually did not own a fluorescence microscope, and therefore had Zeiss, Nikon and Olympus bring in demo microscopes, on which they performed their experiments[113]. Chalfie also passed the *GFP* gene on to his wife, Tulle Hazelrigg, who showed that it could be used in *Drosophila* in a publication in *Nature* that same year[114]. In his *Science* paper, Chalfie had already mentioned the suitability of *GFP* for expression in *Drosophila*, a personal

communication from Hazelrigg he was permitted to include in exchange for, (1) freshly prepared coffee, every Saturday at 8:30 am for two months, (2) preparation of a special French dinner, and (3) nightly emptying of the garbage for one month[112,113]. Expression in the model yeast *Saccharomyces cerevisiae* was demonstrated as well, anecdotally by the Tsien lab, and with first published images by Tim Stearns **(1994/1995)**[115,116]. But Roger Tsien was primarily interested in tinkering with the protein, and he quickly started publishing on new and improved variants of the fluorophore[111]. Single point mutations optimized its excitation properties by removing one of its two excitation peaks (395/475nm), and slightly shifting the remaining main peak to 488 nm **(1994/1995)**[115,117]. Furthermore, he and his team were able to create a 'cyan' variant (CFP) **(1994)**[115]. Further mutations resulted in improved brightness, and the creation of a second 'blue' fluorophore (BFP), which the team used to demonstrate its suitability for FRET-experiments (measuring energy transfer from BFP to GFP)[118]. One year later, Tsien and crystallographer James Remington and their teams had determined a crystal structure for GFP and evolved the 'yellow' YFP **(1996)**[119]. The only 'color' that could seemingly not be engineered with GFP was 'red'. But once the DsRed protein from *Discosoma* was described in **1999**, the Tsien lab quickly used it to produce several red fluorophores as well, such as the monomeric mRFP and the fruit collection (mCherry, tdTomato, etc.)[120–122]. An important triple-mutation not engineered by the Tsien lab was added to GFP in **1996** and significantly increased the brightness of the protein, resulting in the 'enhanced' EGFP[123]. Interestingly, in **2019**, the team of Nathan Shaner, a student of Roger Tsien, found that the crystal jelly *Aequorea victoria* had already naturally evolved pretty much all of the critical mutations that made the superior EGFP[124], but due to its very low expression level compared to the 'regular' GFP, this natural EGFP had so far been overlooked[124].

Thus, by 1995 GFP was successfully expressed and used in most model organisms. But foreshadowing what would become a common theme for plant microscopists trying to reproduce methods and techniques established in other organisms, things were a lot more complicated in plants. Expression of *GFP* in plant cells only seemed to work when a virus-system was used for expression of the gene, while stable transgenic *Arabidopsis* lines with strong emission could not be created **(1995)**[125,126]. It was later discovered that this was due to a cryptic intron, which was spliced out in plant cells and therefore removed part of the coding sequence from the *GFP* mRNA[127]. Only after codon usage optimization and removal of the splice site for the cryptic intron could plant scientists finally also employ GFP as a tag for their proteins **(1996/1997)**[128,129]. This optimized variant was first expressed in maize

protoplasts (*see Fig. 1 E here*), and then in stably transformed *Arabidopsis* lines (*see Fig. 4A-H here*)[128,129]. But as always, microscopists quickly turned to their favourite structure, the cytoskeleton, first showing microtubule dynamics using a new GFP-MBD (microtubule binding domain) reporter for live-imaging of different cell types (*see Fig. 5 A here*), and then showing a Golgi/ER/Actin co-staining (ERD2–GFP/rhodamine–phalloidin) to visualize the movement of Golgi vesicles along an ER/Actin network (*see Fig. 2 e-g here*) **(1998)**[130,131]. The latter is a great example for the capabilities of the new system, as movement of GFP-labelled proteins could now readily be tracked live over time[131].

With the advent of the CLSM and GFP a new era in microscopy began in the 1990s. The constant improvements with every new generation of CLSM resulted in superior images with higher resolution, and the possibility to finally label nearly every protein of choice genetically, by simply fusing the *GFP* gene to the respective coding sequence, allowed researchers to observe their proteins of interest in action *in vivo*. New and improved fluorescent proteins, still many of them based on GFP, are being continuously developed and released, showing that the potential of both, CLSM and GFP is not yet exhausted. The GFP family tree on FPbase.org is worth viewing as a very nice illustration of the wealth of fluorescent proteins derived from this single protein: click here[132]. But GFP also led the way toward the next big advance in microscopy, super-resolution, thanks to the '*on/off blinking and switching behaviour*' of GFP, as observed by Roger Tsien and William Moerner in **1997**[133].

Plasma Membrane Nanodomains and Single Molecule Tracking (2000-today)

Since the late 1980s, research on how to break the resolution limit as defined by Ernst Abbe intensified, and in the early **2000s** the first practical approaches were being devised and tested[134–136]. Among the first super-resolution imaging techniques successfully applied to resolve sub-diffraction limit structures in biological samples were stimulated emission depletion (STED), photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) **(2006)**[137–139]. The latter two of these techniques require a blinking behaviour of the fluorophores used for the imaging, as observed for GFP in 1997[133,135]. The density of fluorescent labels is limiting the resolution of individual proteins, as they will appear as one blur[135]. By getting them into a blinking state, only a portion of the proteins will be fluorescent at any given point, thereby allowing more precise localization of their individual positions, and better resolution of two or more proteins in close proximity[135]. In STED microscopy on the other hand, the transient reduction in label density is achieved by

'switching off' any fluorescent molecules in a circular area around the very centre of the focal spot with a circularly polarized high-energy depletion laser[135]. This reduces fluorescence to the central spot which can have a lateral resolution of way below 100 nm[135]. For the development of such techniques, Eric Betzig, Stefan Hell and William Moerner were awarded the 2014 Nobel Prize in Chemistry[140–142]. Another super-resolution technique is structured illumination microscopy (SIM), which uses structured light patterns generated by, e.g., reflecting off a grid, to scan the focal plane multiple times[135,143]. With every scan, the pattern is shifted laterally leading to a series of images with different interference patterns[135]. The different interference patterns recorded can then be computationally reconstructed into a super-resolution image[135]. Since SIM is less invasive than the aforementioned super-resolution techniques, and can be used with conventional fluorophores, it is more compatible with live-cell imaging[135]. Sadly, SIM-developer Mats Gustafsson passed away in 2011, thereby making him ineligible for the 2014 Nobel Prize for super-resolution microscopy[144]. Additionally, given the fact that SIM holds the potential for time-resolved live-cell super-resolution imaging, it is also conceivable that it will result in a Nobel Prize of its own in the future.

As always, adopting such complex new techniques to plants poses a big challenge, and thus there are only few publications so far reporting on super-resolution imaging of intact plant cells using these methods. This is in part because of the specialized microscopes required for these techniques. Super-resolution microscopes that allow for straight forward out-of-the-box super-resolution imaging are only now becoming more common, and the software to properly process such images is still highly complex and needs to be thoroughly understood[135,145]. However, PALM and STED have been successfully used in plants to image proteins in plasma membrane nanodomains, and to track the movement of individual proteins therein, while SIM was used to live-image the cytoskeleton (*see Fig. 2 a-d here*) **(2011-2019)**[146–149]. In the meantime, plant microscopists have taken advantage of the range of near super-resolution techniques, which can be performed on regular confocal microscopes with additional hardware components and better deconvolution software, such as total internal reflection fluorescence (TIRF) microscopy, the ZEISS AiryScan setup, or fluctuation-based super resolution microscopy techniques, such as super-resolution radial fluctuations (SRRF) imaging[150–154]. The AiryScan and single-molecule TIRF have also been successfully used in plants to study single proteins in plasma membrane nanodomains (*see Video 2 here*), while SRRF is used for less mobile structures like cell wall components (*see Fig. 2 E here*) **(2019-2021)**[150]. And, of course, these techniques have also been used on the cytoskeleton (*Fig. 1 A-*

F here)[149,151]. Beyond this, plant microscopists have achieved close to super-resolution images using spinning-disc confocal microscopes equipped with super-fast high-resolution cameras. Using such a microscope the group of Akihiko Nakano was able to simultaneously live-image the directed trafficking and sorting of several distinct proteins, labelled with different fluorophores, within the trans-Golgi network **(2021)**[155]. That same year, and again using such a spinning-disc confocal, the rearrangement of individual microtubules into thick, regularly spaced bundles, required for secondary cell wall pattern formation in single cells *in planta* was achieved (*compare the 2021 live-video S2 here to the 1955 phase-contrast image of the secondary cell wall in Fig. 7 here*)[45,156].

Overall, super-resolution-ready microscopes are now part of the product range of all the big microscope suppliers, such as ZEISS, Nikon, Leica or Andor, and some small manufacturers have also emerged specialized on specific super-resolution techniques. Among them, Nobel laureate Stefan Hell is one of the founders of Abberior Instruments, which focuses on the STED technique developed by Hell. One of their specialized STED microscopes has recently been employed to image the distinct localization of two chromosomal proteins in *Arabidopsis* at super-resolution (*see Fig. 1 A,B here*) **(2021)**[157]. Accordingly, it appears that the dawn of super-resolution imaging has now also arrived for plant microscopists.

Vertical-Stages, Light-Sheets and Open Science (2000-today)

At this stage, custom-built or customized microscopes are also becoming more common. For plant microscopists, this means that a problem unique to their field could now also be tackled: tilting the imaging stage into a vertical position. As plants grow along the gravitational vector – roots with, shoots against it – long-term live-imaging of developmental processes should ideally be performed with the plants positioned vertically. Use of a vertical-stage microscope was first reported in a **2009** paper studying the response of a root growing against a physical barrier[158]. Subsequently, it was used to study the interplay between gravity perception and hormone signalling in the root **(2017/18)**[159,160]. Today, several institutes have installed their own tilted microscopes, and more publications can be expected in the near future.

And, as the final microscopic method discussed here, the early 2000s also brought us the light sheet fluorescence microscope (LSFM)[161,162]. In a LSFM the excitation light is focused only along one axis to create a thin planar sheet of light, instead of a spot[162]. This planar sheet of light then illuminates a complete slice of a sample, which is imaged at once through an objective arranged at a 90 degree angle to the light sheet[162]. By moving the sheet through the

sample slice by slice along the Z axis, three-dimensional images can be quickly obtained[162]. The design and implementation of the first LSFM was published by Richard Zsigmondy in **1909**, and featured an illumination light path that converted polarized sunlight into a light sheet by simply channelling it through a thin slit[163]. Using this Ultramicroscope, as he called it, he was able to image gold particles in a colloidal gold solution, which could not be imaged with the standard microscopes at the time[163]. For this work he was award the Nobel Prize in chemistry in **1925**[164]. Following this breakthrough however, things got rather quiet around light sheet microscopy for nearly a century. A similar technique was published in **1993** as orthogonal-plane fluorescence optical sectioning, but like Zsigmondy's Ultramicroscope, it did not catch on[165]. Things only changed in **2004** when the lab of Ernst Stelzer published its selective plane illumination microscope (SPIM)[161]. Stelzer subsequently collaborated with plant microscopist Alexis Maizel to adapt the SPIM for studies with plants, using it first to create high-resolution three-dimensional time-series of growing roots and lateral roots (*see Video S1 here*) **(2011)**[166]. The SPIM was eventually commercialized by the EMBL-spin out company Luxendo, who's **2020** LSFM have also been used successfully in plants, as has the ZEISS Lightsheet Z.1, introduced by the company in **2012** [167,168].

The SPIM also stands as an example for the growing open science movement within the microscopy community[169]. In **2013**, Jan Huisken, first author of the 2004 SPIM paper from the Stelzer lab, teamed up with Pavel Tomancak to create the OpenSPIM platform (http://openspim.org/), making everything needed to custom-build one's own SPIM openly available to the community[169]. And, in similar fashion, this community-based thinking of the open science movement has benefitted microscopists in many other ways. Another prime example is the image-analysis software Fiji[170]. Based on the National Institutes of Health's ImageJ, Fiji is an open-source, customizable, all-in-one image analysis program, which nowadays is indispensable for microscopists from all fields (the paper has so far been cited over 25000 times, despite many authors neglecting to cite it in the methods section of their papers) **(2012)**[170,171]. ImageJ/Fiji also allows users to write and incorporate new tools and plug-ins, increasing its versatility even more, and the SRRF analysis open source toolkit mentioned before is an example of one such plug-in[172]. Furthermore, MorphoGraphX is an open-source 3D image processing/analysis program, which not only allows for three-dimensional image-reconstruction, but also cell segmentation and cell lineage tracing, and carries the additional advantage to plant microscopists that it was developed with plant scientists **(2015)**[173]. And since all the imaging data acquired must be managed, the Open Microscopy Environment (OME) was created by and for the community[174]. Finally, with the

ever-increasing selection of fluorescent proteins available to microscopists, Talley Lambert has recently created the community-editable FPbase database (https://www.fpbase.org), an invaluable resource of all information available for any fluorescent protein[132].

In the coming years, it can be expected that super-resolution microscopy will fully enter the plant field, as more groups specialize on the adoption and establishment of these techniques, and more companies produce custom-made microscopes that make it easier to apply them straight out-of-the-box. Plant optogenetics is another emerging research area with use and applicability of microscopy methods, which will become increasingly important in the coming years to engineer and control pathways in plants[175,176]. With an ever-growing open-science movement, improved data/image-analysis tools, programs and databases are constantly being developed and made publicly available, making every step from image acquisition to publication easier. Accordingly, we can expect many more beautiful (and informative) images of plants at an ever-increasing resolution in the years to come.

Further Reading:

- A Short History of Plant Science
  Chapter 1: A Short History of Arabidopsis thaliana (L.) Heynh. Columbia-0[31]
  Chapter 2: A Short History of the CaMV 35S Promoter[60]
  Chapter 3: A Short History of Plant Transformation[61]
  Chapter 4: A Short History of Vernalization[177]
- Nehemiah Grew - The anatomy of plants - with an idea of a philosophical history of plants, and several other lectures, read before the Royal Society[1]
- Howard Gest - The discovery of microorganisms by Robert Hooke and Antoni van Leeuwenhoek, Fellows of The Royal Society[4]
- Harald Volkmann - Ernst Abbe and his work[32]
- W. B. Amos & J. G. White - How the Confocal Laser Scanning Microscope entered Biological Research[92]
- Yudhijit Bhattacharjee - How Bad Luck & Bad Networking Cost Douglas Prasher a Nobel Prize[105]
- George Komis et al. - Super-resolution Microscopy in Plant Cell Imaging[178]
- Guido Grossmann et al. - Green light for quantitative live-cell imaging in plants[179]
- Nathanaël Prunet and Keith Duncan - Imaging flowers: a guide to current microscopy and tomography techniques to study flower development[180]

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