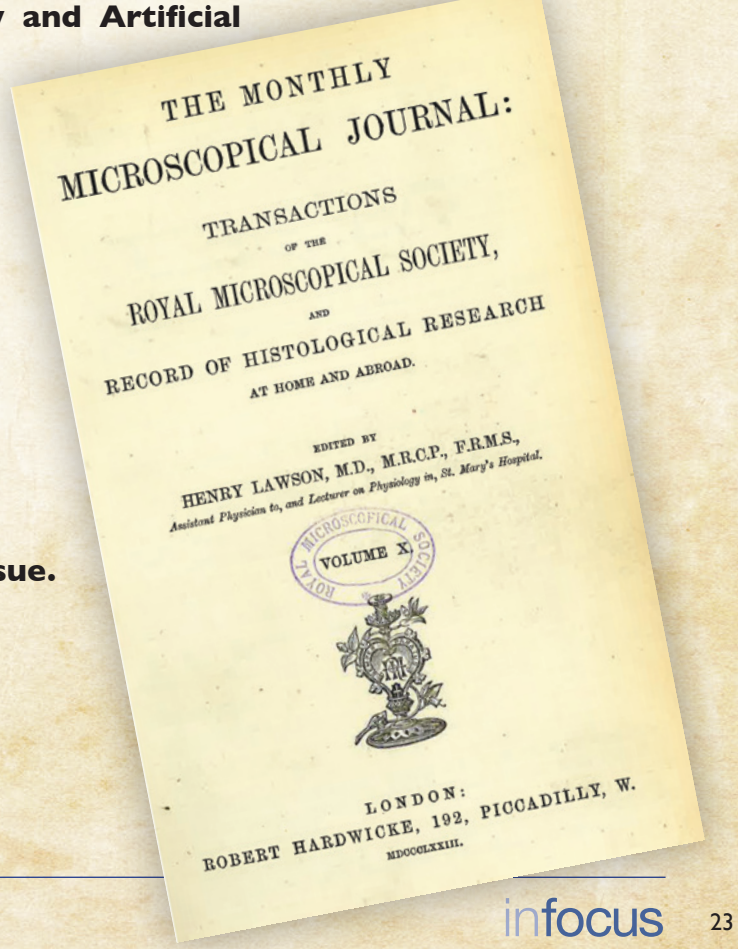
In commendation of Ye microscope

Of all the Inventions none there is
Surpasses
The noble Florentine's Dioptrick Glasses
For what a better, fitter guift Could bee In
this World's Aged Luciosity
To Help our Blindness so as to devize
A paire of new Artificial eyes
By whose augmenting power we now see more
Than all the world Has ever doun Before

These sentiments are as appropriate today as they were when Henry Power, author of the first book on microscopy written in English, wrote them in 1664. 'Let me see', 'I'll look into that' are everyday expressions but they take on special and particular meaning in microscopy. It is now 175 years since the Microscopical Society of London (which became the Royal Microscopical Society in 1866) published its journal – just two years after it was founded. Since then the journal has chartered all the major developments in the art of microscopy and has published many important papers striving to develop a series of 'new and Artificial eyes'.

In order to mark this anniversary the editors of the journal have you chosen a selection of papers to be republished in this special anniversary issue.


THE MONTHLY
MICROSCOPICAL JOURNAL:
TRANSACTIONS
OF THE
ROYAL MICROSCOPICAL SOCIETY,
AND
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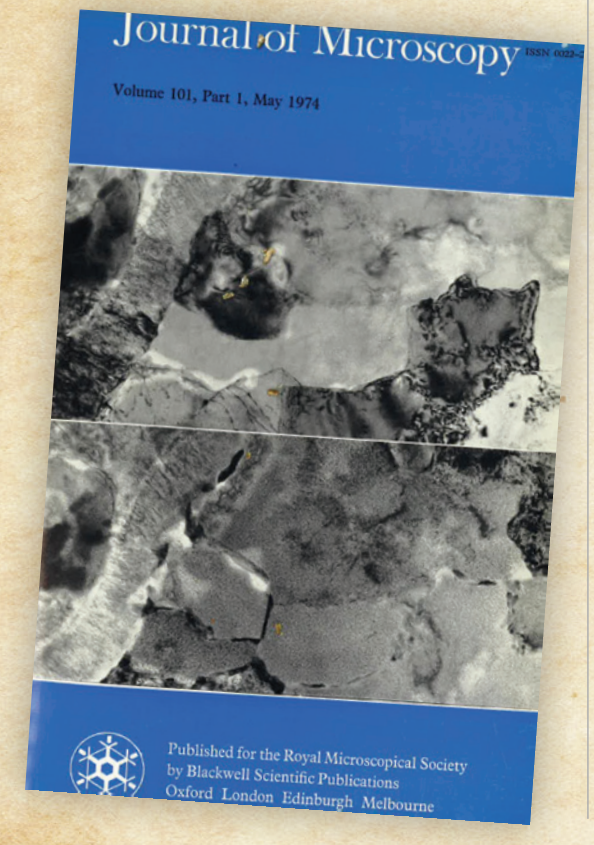
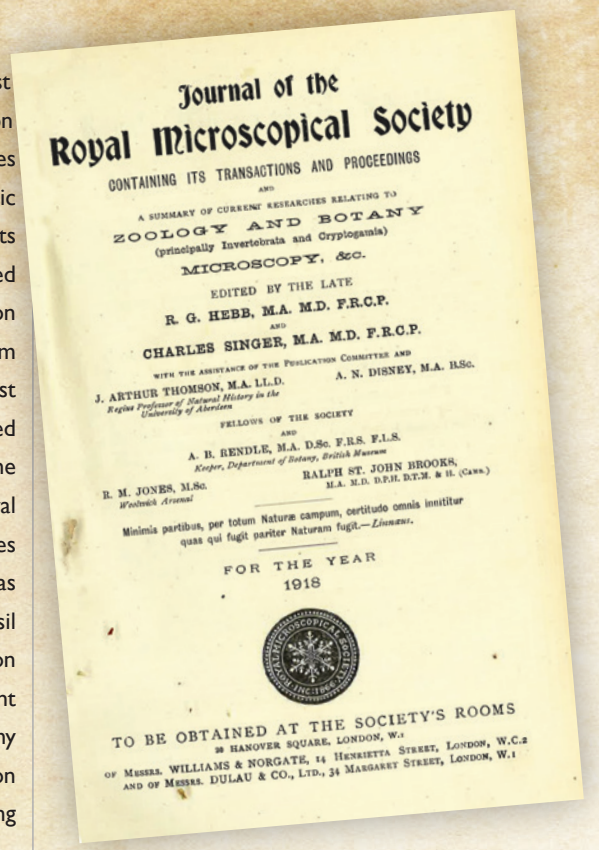
AT HOME AND ABROAD.
EDITED BY
HENRY LAWSON, M.D., M.R.C.P., F.R.M.S.,
Assistant Physician to, and Lecturer on Physiology in, St. Mary's Hospital.
ROYAL MICROSCOPICAL SOCIETY
VOLUME X.
LONDON:
ROBERT HARDWICKE, 192, PICCADILLY, W.
MDCCCLXXIII.

Sir Richard Owen (1804 – 1892) became the first president of the Microscopical Society of London when it was founded in 1839 and edited many issues of its journal – then known as the Microscopic Journal. Owen was a giant among naturalists even when many giants of that species roamed the Earth. He laid the foundations of the London Museum of Natural History and coined the term dinosaur (terrible lizard). Owen was an anatomist and palaeontologist who must have already earned an enviable reputation as early as 1836 when he became the Hunterian professor in the Royal College of Surgeons. For it was then that Charles Lyell suggested to Charles Darwin that Owen was the man to classify and catalogue Darwin's fossil collection brought back from South America on HMS Beagle. Owen continued to make important contributions to zoology and comparative anatomy for the rest of his life, including his seminal work on invertebrates, fish, reptiles and birds - both living and extinct.




Journal of the
Royal Microscopical Society
CONTAINING ITS TRANSACTIONS AND PROCEEDINGS
AND
A SUMMARY OF CURRENT RESEARCHES RELATING TO
ZOOLOGY AND BOTANY
(principally Invertebrata and Cryptogamia)
MICROSCOPY, &c.
EDITED BY THE LATE
R. G. HEBB, M.A. M.D. F.R.C.P.
AND
CHARLES SINGER, M.A. M.D. F.R.C.P.
WITH THE ASSISTANCE OF THE PUBLICATION COMMITTEE AND
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Woolwich Arsenal
Minimis partibus, per totum Naturæ campum, certitudo omnis inuitur
quas qui fugit pariter Naturam fugit.—*Linnæus.*
FOR THE YEAR
1918
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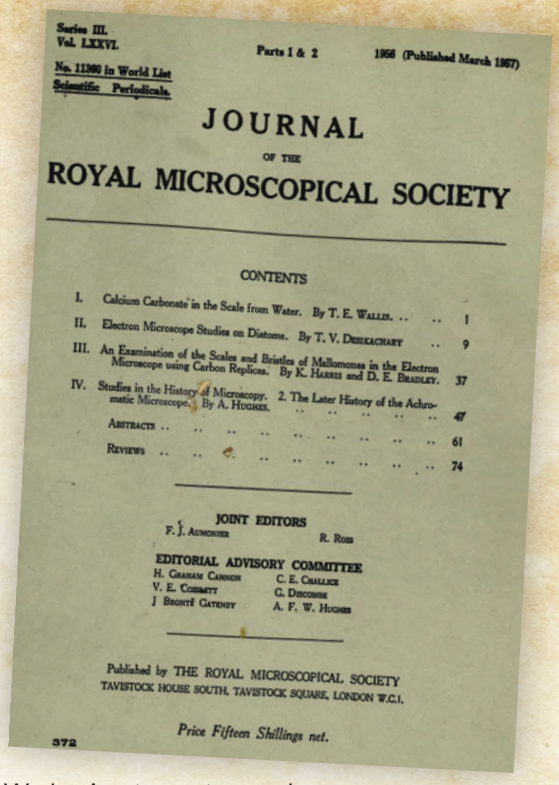
Two of the earliest papers in The Microscopic Journal describe Owen's discovery of a new genus of fossil fish: *On the structure of the teeth of Dendrodus strigatus and Dendrodus compressus* [Owen 1841] and *On the structure of fossil teeth from the central or corn-stone division of the old red sand-stone, indicative of a new genus of fishes, or fish-like Batrachia, for which is proposed the name of Dendrodus* [Owen 1841]. The teeth of these extinct animals, which he classified in the Labyrinthodontia, had a remarkably complex structure which could only be revealed by microscopy of sections. Unfortunately, like those dinosaurs in the movies, Owen eventually clashed with the biggest of the giants, T. H. Huxley and Charles Darwin, and lost. This, together with accusations of plagiarism, tarnished his once glittering reputation and he died a bitter and grumpy old man.

Francis Herbert Wenham (1824 – 1908) began his career as a marine engineer and specialist in propellers working on Brunel's huge ship, the SS Great Britain. He later coined the word aeroplane and had such a reputation in aircraft design that Wilbur Wright referred to him as one of the ablest and most useful men who ever laboured in the cause of human flight. As if this weren't enough, Wenham was a noted microscopist and cell biologist. One of his articles in the Transactions of The Microscopical Society was a serious contribution to the cell theory which was then being formulated and was to become one of the cornerstones of biology, along with the theory of evolution and the laws of genetics. But most microscopists will know him for his contributions to the design of microscopes, especially to binocular microscopy.

In the same way that stereophonic broadcasting significantly enriched listening to music in the twentieth century binocular microscopes may well have had a similar impact on the microscopists of the mid-19th century and they proved so successful that all serious research microscopes today have two eyepieces. Although forerunners of the modern binocular dissecting microscope, consisting of two microscopes with optical axes converging on the specimen, may have been common before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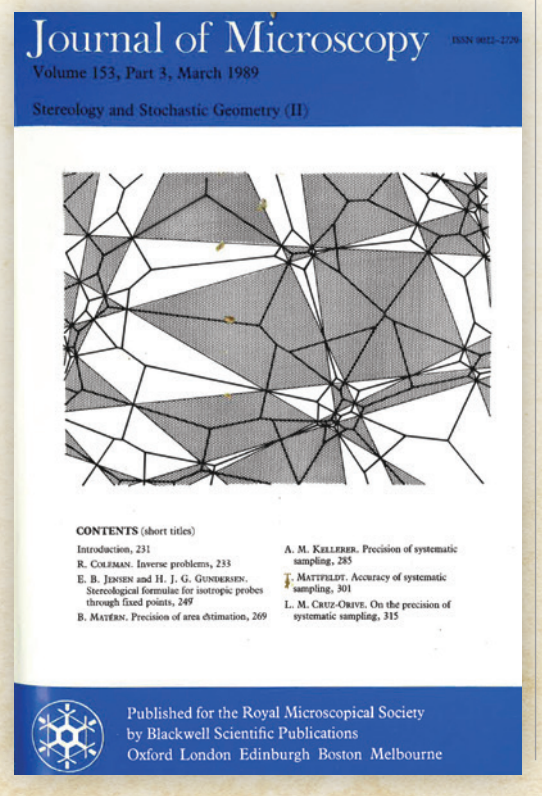
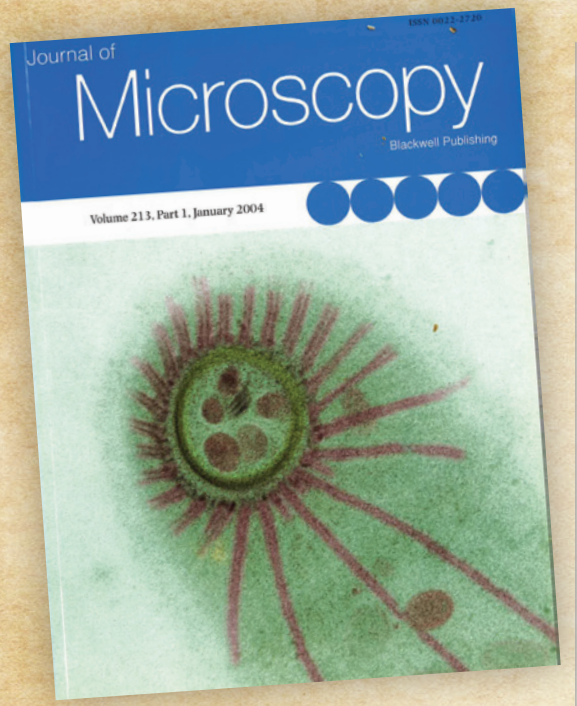
Wenham's innovations, these were not capable of high resolving power because of the difficulty of positioning two high-aperture objectives close enough to the specimen. Wenham's aim was to produce a binocular microscope with a single objective of the highest resolving power then available.

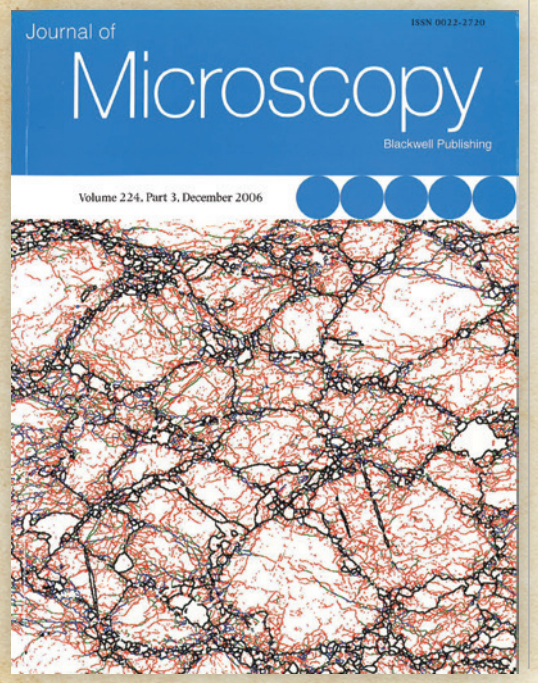


His first efforts were specifically designed to maintain or even enhance the stereoscopic effect. These were symmetrical prism systems placed in the optical path behind the objective so that the imaging beams diverged to two separate eyepieces in a 'Y' configuration. Because of the inversion of the microscope image, the stereoscopic effect was also reversed so that more distant parts of the specimen appeared closer. Further innovations removed this defect, by crossing the two optical paths, but this was at the expense of a more sophisticated prism system that proved very difficult to manufacture. Finally Wenham abandoned the idea of enhancing the stereoscopic effect, which was not of much importance anyway when the specimen was thin, and used a simple beam-splitting cube and a single



right-angled prism to separate and diverge the two imaging beams. This became the famous Wenham binocular microscope which is still familiar to microscope collectors today with its asymmetrically disposed eyepiece tubes – ugly but much coveted.

Further developments of the prism system soon followed, mainly in the Zeiss works in Germany,

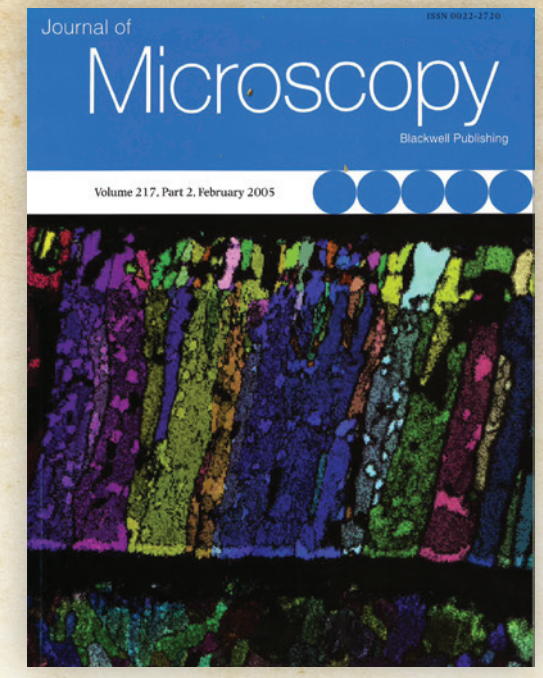


leading to the binocular head on a modern research microscope. Interestingly, although Zeiss and others still toyed with the idea of enhancing the stereoscopic effect, using two holes in a substage diaphragm and 'D'-shaped diaphragms in the eyepieces, it became clear that the main advantages of high-power binocular microscopy lay in allowing an unstrained and natural vision - as Wenham had eventually realised. Most of Wenham's innovations in binocular microscopy were published over several years in forerunners of the Journal of Microscopy.

Sir George Stokes, who had named and explained the phenomenon of fluorescence in 1852, was Lucasian Professor of Mathematics at the University of Cambridge when he read a paper to the Royal Microscopical Society *"On the Question of a Theoretical Limit to the Apertures of Microscopic Objectives"*, which was later published in the Journal of the Royal Microscopical Society, Vol 1, Issue 3, pages 139–143 (1878). This paper criticized computations by Professor R. Keith of a new microscope objective. In this era of microscopy, design of new optical lenses was as contentious as the design of electron optical lenses today!

It is probably impossible to overstate the importance of the contribution which Ernst Abbe (1840 – 1905) played in the understanding and development of microscopy and there is no need to list his contributions. He was elected to an Honorary Fellowship of the Royal Microscopical Society in 1878. He read a number of papers to the Society including one in 1881 *"On the Estimation of Aperture in the Microscope"*. In this paper he explains the importance of numerical aperture to 'afford a definition of aperture for the practical comparison of objectives, which should exhibit the true relation of aperture to the actual performance of the microscope, a relation which is entirely concealed by the angular expression'.

The electron microscope, of course, had not been invented when the Journal of Microscopy was first published but it is a happy coincidence to find a

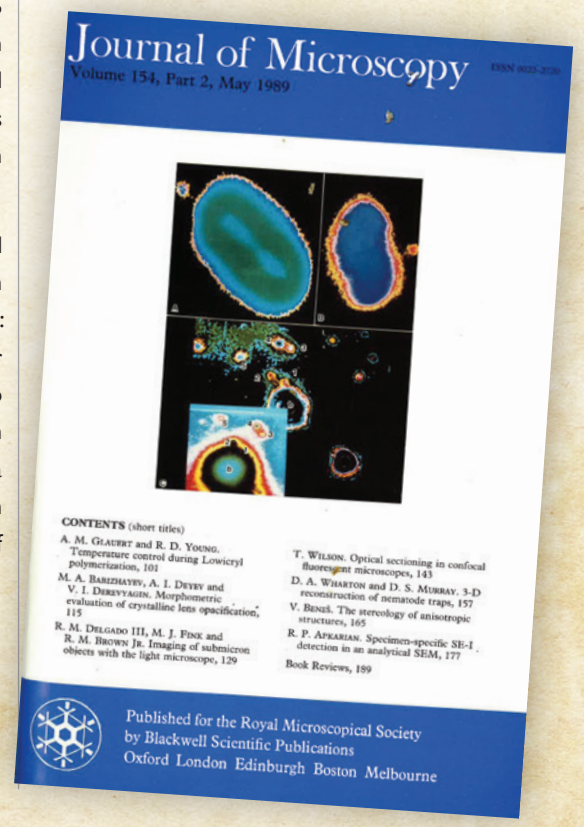


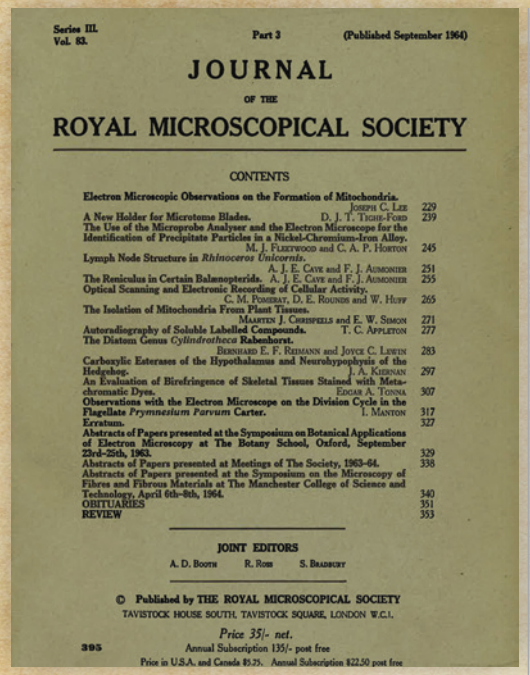
paper on transmission electron microscopy (TEM) that is 50 years old, and written by the co-inventor of the electron microscope, Ernst Ruska, who was honoured with the award of a Nobel Prize in 1986 for his contribution. The paper was published in 1965, and follows a lecture presented to the Royal Microscopical Society by Ruska in 1964. Ruska was awarded an Honorary Fellowship by the RMS in 1963.

The subject of the paper is one that has obsessed many electron microscopists since the invention of the instrument, and continues to do so today: improving the resolution of the microscope. For decades it was a source of deep frustration to those working in high-resolution TEM that although the typical deBroglie wavelength of electrons in a TEM is ~2 pm, TEM instruments struggle to reach a resolution of 0.1 nm (1 Å), almost 3 orders of magnitude worse.

In the paper, Ruska systematically works through many of the factors that had been identified as limiting the resolution of the microscope. It is striking that the factors that identified in this paper, only 30 years after the invention of the electron microscope, are still very much in the mind of those trying to reach the highest resolutions 50 years later.

The paper starts with some examples of current best performance. An image showing 0.2 nm lattice fringes from a thin film of gold is shown. Getting beyond 0.1 nm took a further 40 years after this paper was published, and has been hard won. It then goes on to address the most important limitation of electrons lenses – their large inherent spherical and chromatic aberration. Ruska describes the importance of the symmetric condenser-objective lens, proposed by Glaser in 1941, with the sample immersed in the magnetic field close to its maximum strength. This arrangement gives a very short focal length, together with lower spherical and chromatic aberration, and continues to be the standard design for the primary TEM imaging lens today. Ruska describes experimental field measurements within the bore of a condenser-objective lens made in his laboratory, and from these estimates of the lens

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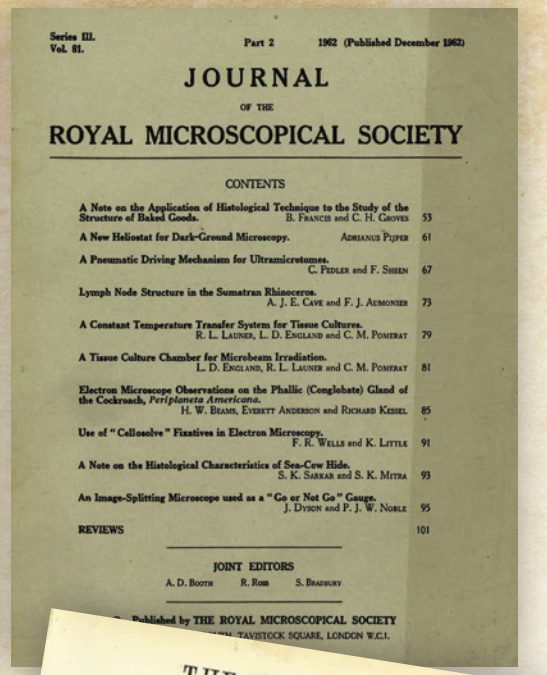
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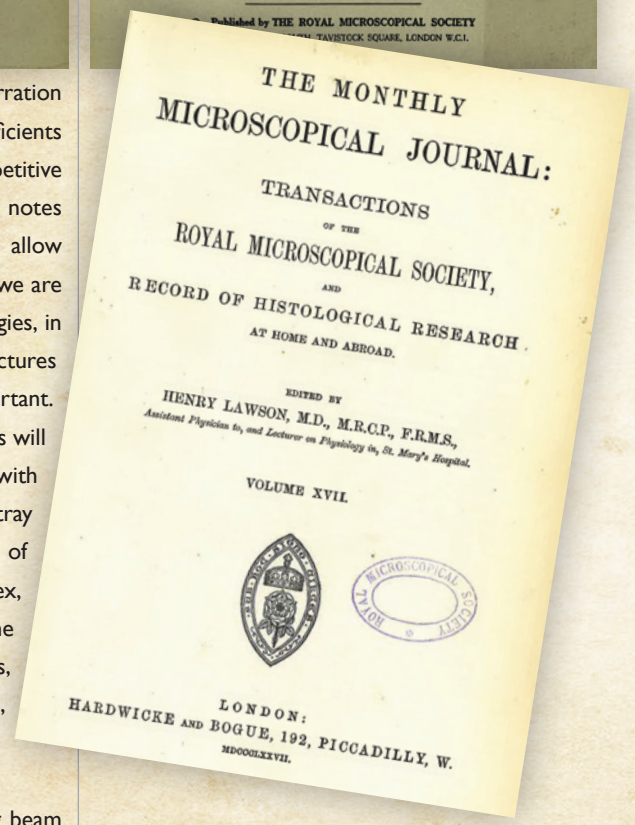

THE MONTHLY MICROSCOPICAL JOURNAL:

TRANSACTIONS OF THE ROYAL MICROSCOPICAL SOCIETY, AND RECORD OF HISTOLOGICAL RESEARCH AT HOME AND ABROAD.

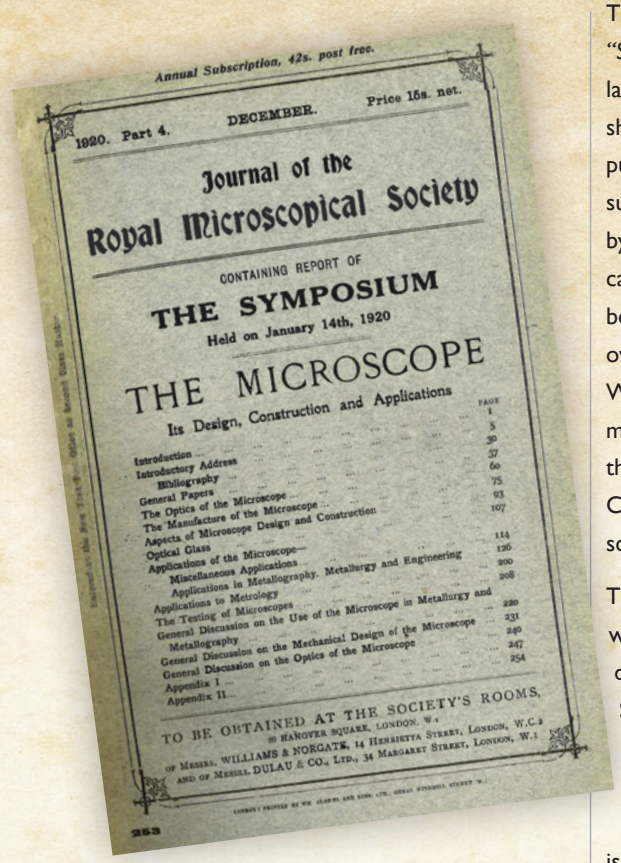
EDITED BY HENRY LAWSON, M.D., M.R.C.P., F.R.M.S., Assistant Physician to, and Lecturer on Physiology in, St. Mary's Hospital.

VOLUME XVII.

LONDON: HARDWICKE AND BOGUE, 192, PICCADILLY, W. MDCCCLXXVII.

parameters are made. With spherical aberration coefficients of less than 1 mm and small coefficients of chromatic aberration, the lens is competitive with any available today. Interestingly, Ruska notes that reductions in lens aberrations will allow lower beam energies to be used, and today we are seeing increased interest in low beam energies, in particular for the study of carbon nanostructures where knock-on radiation damage is important. Ruska is concerned that lower beam energies will increase heating and charging effects, along with making the microscope more susceptible to stray fields. Today we know that the dependence of sample damage on beam energy is complex, and depends on the damage mechanism. The increased sensitivity at low beam energies, however, remains a concern. In a similar vein, the paper goes on to discuss the impact of "errors", by which it refers to limitations on imaging due to spread in the illuminating beam energy and ripples in the lens power supply. Today, the spread in beam energies is still regarded as an important limiting factor, and the use of both cold field-emission and monochromators is found to be beneficial, particularly at lower beam energies.

The paper then goes on to consider a number of practical issues associated with achieving high resolution in the TEM. These include use of a cold trap to improve the vacuum conditions in the vicinity of the sample and the use of sample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1920. Part 4. DECEMBER. Price 15s. net.

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Held on January 14th, 1920

THE MICROSCOPE

Its Design, Construction and Applications

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cooling to reduce damage. Again, both of these are now very much standard practice in modern TEM instruments. The possibility of the use of zone-plates plates for increased phase contrast is mentioned as a potential significant advance, and again we are currently seeing significant activity in the area of zone plates

The paper concludes by predicting that future advances leading to improved resolution would be rather incremental. Although the principles of aberration correction for electron lenses had been published in 1947 by Scherzer, their potential for resolution improvement was ignored by Ruska in the present paper. Their successful implementation and commercial availability took another four decades or so after the publication of this paper, but we can now know that they did create a step change in performance with a resolutions of around 50 pm being achieved.

The short invited review, from Albert Crewe, "*Scanning Transmission Electron Microscopy*", is a landmark paper being the first time that micrographs showing individual, isolated atoms had ever been published in the Journal of Microscopy. The initial such observation was published a few years earlier by Crewe in the journal *Science* [1]. Albert Crewe came from a particle physics background, and had been Director of the Particle Accelerator and later overall Director at Argonne National Laboratory. While at Argonne, Crewe became interested in microscopy, stimulated by the biology programme there. In 1967 he moved to the University of Chicago to focus on his project to develop the scanning transmission electron microscope (STEM).

The key development, as highlighted in the paper, was the field-emission electron gun (FEG). The development of this gun unlocked the potential of STEM, and the STEM is now firmly established as a key instrument for imaging and spectroscopy at atomic spatial resolution. The FEG is one of the brightest sources of radiation known to man and is about 10,000 times greater than 3rd generation synchrotrons, such as the Diamond Light Source in the UK. The FEG is now commonplace across both conventional TEM and STEM instruments.

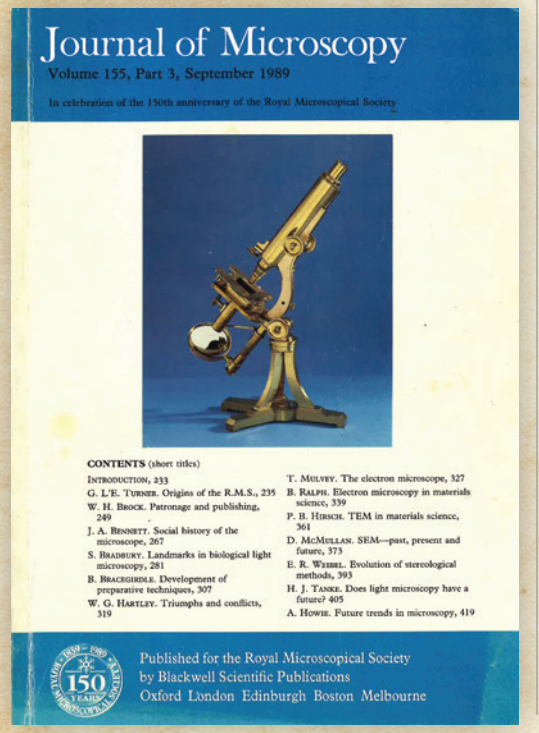
The short review concludes with a discussion of the detectors that can be used with STEM and the electron scattering processes that lead to image contrast. The annular dark-field (ADF) detector is highlighted, and it is the atomic-number contrast seen in images from this detector that allowed the single atoms to be seen.

The Journal of Microscopy has published what can be considered to be some of the seminal papers in the field of biological cryo-microscopy over the second half of the last century. Here we have chosen two papers for re-publication and we refer to others that highlight the development of cryosectioning or CEMOVIS (cryo-EM of vitreous sections) from its early days in the 1980s (Dubochet 1982, 2011), through to the use of cryo-EM tomography to

enable higher resolution data to be extracted from the sections.

Back in 1983 one of the pioneers of cryo-EM, Jacques Dubochet, published a paper in the Journal entitled "Electron microscopy of frozen hydrated sections of vitreous ice and vitrified biological samples". This was one of a trio of ground breaking cryo-microscopy papers, the others covering EM of frozen water and frozen biological suspensions (Dubochet et al. 1982, Lepault et al. 1983). In these papers Dubochet demonstrated the importance of vitrification of water for cryo-EM and demonstrated the sectioning of vitreous ice, rat liver and catalase crystals. Vitrification was found not only to be necessary for good ultrastructure through lack of ice crystal damage but also for obtaining high quality sections, as crystalline ice proved to be extremely difficult to cut. From work such as this cryo-ultramicrotomy for low temperature EM was born, which is now a routine, although still skill-demanding, procedure for CEMOVIS.

We also mention the two papers from Martin Müller's laboratory published in 1991 and 1992

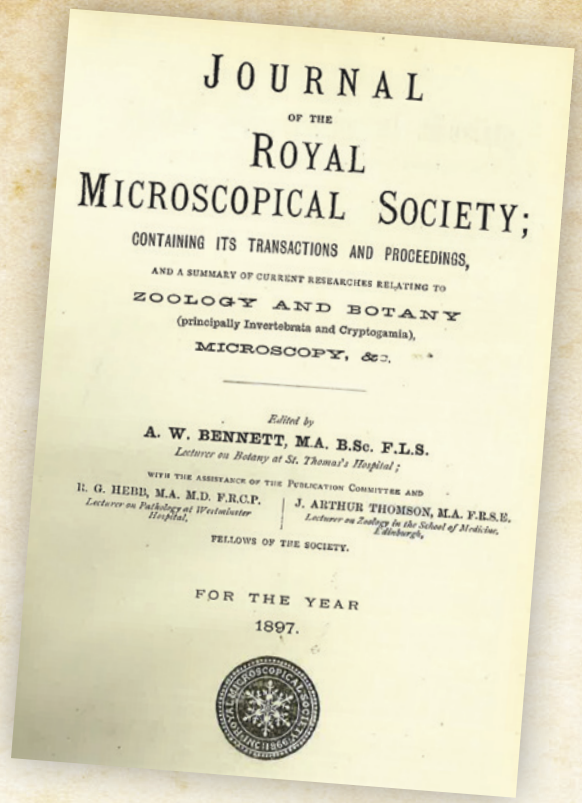


where Martin Michel first published micrographs from high-pressure frozen, unfixed apple leaf tissue (Michel et al. 1991, 1992). Although the second paper concentrated on the advantages of using diamond knives for cryo-sectioning, they both showed, as far as I am aware, the first good quality micrographs of unfixed, sectioned plant material. These remarkable micrographs revealed two forms of vacuoles in leaf palisade cells, near perfect nuclear envelopes with nuclear pores, mitochondria, endoplasmic reticulum, Golgi stacks and microtubules and of course beautiful chloroplasts with thylakoids and granal stacks. This work perfectly validated the ultrastructure of plant material as observed in conventionally chemically fixed and resin embedded material and showed a way to structurally analyse plant tissue in a more native state.

Finally in 2008 the Journal published one of the first reports on the 3D structure of the Golgi apparatus in high pressure frozen Chinese hamster ovary cells using -EM tomography on 200 nm thick cryo-sections (Bouchet-Marquis et al. 2008). In addition to the avoidance of fixation artefacts this methods has the advantage of extremely high resolution in 1.6 nm virtual sections extracted from the tomograms. The authors could reveal two forms of COPI vesicles budding from Golgi cisternal and intra cisternal connections when Golgi were induced to secrete large cargo molecules such as pro-collagen I. Cross and longitudinal sections of microtubules clearly showed the 13 protofilaments that make of this cytoskeletal element.

Although cryo-electron microscopy requires special skills and equipment and can be much more challenging than conventional electron microscopy, the two chosen publications clearly show the advantages of preserving the native ultrastructure of the biological specimen and imaging the actual molecules in the tissue.

The Journal of Microscopy is also the Journal of the International Society for Stereology and we republish two important papers here. The first is by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FELLOWS OF THE SOCIETY.
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1897.

Roger Miles, who was President of the International Society for Stereology, 1984-87, and professor in Canberra where he published a series of ground-breaking papers which laid down the theoretical foundation for stereology, and facilitated the practical implication of stereology in microscopy, medicine, biology, mineralogy, metallography and many other fields. His paper, republished here, *"A comprehensive set of stereological formulae for embedded aggregates of not-necessarily-convex particles."* is a major break-through because it shows theoretically for the first time that stereological estimators do not only work for convex particles but for arbitrarily shaped particles, as long as the observer can recognize which profiles belong to which particles in a section plane.

DC Sterio is the *nom de plume* of a famous applied stereologist, who worked for his entire career at Aarhus University, Denmark. Aarhus University has since the 1970's had one of the strongest environments in both theoretical stereology as well as applied stereology in biomedicine. Here we republish *"The unbiased estimation of number and sizes of arbitrary particles using the dissector"*. This paper describes the disector which is a method for number-weighted sampling of arbitrarily shaped particles using sections, a problem that had puzzled scientists for over a century. The famous sphere size problem described by Wicksell in 1925 can also be solved by the disector. The original used two physical sections, but soon it was presented in an optical version, and together with the ingenious fractionator principle, it has been the gold standard for cell number estimation ever since. The impact of the disector in applied biomedicine has been enormous with several thousand citations and it started the revolution of "design-based" stereology.

In the same way that the Journal charted advances in optical microscopy in its early years it has continued to do so throughout its history. The last 30 or 40 years, in particular, have witnessed many important advances which have been chronicled in the Journal. The journey has taken us through the development of the confocal microscope. Here we cite an early paper of Fred Brakenhoff where he muses about a number of optical systems to provide enhanced lateral resolution which was the initial driver before the importance of the instrument's optical sectioning ability was fully appreciated. The desire to combine enhanced resolution with optical sectioning lead to a number of new microscope geometries based on interference [Gustafsson, 1999] and structured illumination. The latter technique being particularly attractive for optical sectioning as well as improving the lateral resolution [Gustafsson, 2000]. In addition to these 'optical' approaches chemistry continues to play its part in microscopy by using, for example, actively controlled single molecules to enhance resolution [Moerner, 2012]. W E Moerner was awarded the Nobel Prize for chemistry in 2014.

Tony Wilson
General Editor – Journal of Microscopy

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