Lessons from the history of light microscopy

Brad Amos

Historically, the late arrival of optical microscopy is something of a puzzle, although it may be a testament to the fact that the development of new technology is driven by the desire for scientific knowledge, and not the other way around. The explorations that began in the 17th century are by no means complete, but who is carrying them forward now?

According to conventional wisdom, the compound microscope was invented at the beginning of the seventeenth century and made possible the immediate discovery of cells. This is nonsense. The textbook progression, which invariably begins with the charming and ornate instrument of Hooke[1], is misleading. Hooke, who earned his living by staging impressive demonstrations for VIPs, naturally showed his 'great microscope', but admitted that he did not use it for critical observations. Leeuwenhoek, who followed Hooke, never used a compound microscope. Indeed, the great outpouring of scientific work in this period depended on single lenses. One of Leeuwenhoek's had a resolving power of 1.35 µm, which is quite enough for basic cytology[2]. Such lenses had been available from time immemorial — even the Vikings of the 11th and 12th centuries are now known to have fashioned quartz into optically ideal paraboloids for some unknown purpose[3].

The lesson is clear. It was not technology that caused the flowering of science, but vice versa. The astonishing and unprecedented change in human psychology that occurred in Europe during the Reformation needs to be remembered, celebrated and pondered, particularly by those who believe that they can curtail human curiosity and still have the benefits of technology. This includes not just the creationists but also students who refuse to dissect and the multitudes who would like to put a stop to all experiments in genetics or reproduction.

Who discovered cells and division?

The early masters of the single lens are often credited with the discovery of cells. However, they did not appreciate the significance of what they saw. Hooke, who coined the term, regarded the cell as a conduit for fluids through cork. Nehemiah Grew saw the polygonal-facetted form of plant cells in stems and roots, but suggested that they may actually be crystals, in which the form would indicate an acid or alkaline character. Trembley[4] described the division of the diatom *Synedra*, but had no idea that this was a universal process or that the bodies of larger organisms were composed of such units.

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The average cell biologist, if asked who discovered cell division, would probably give the credit to Schleiden and Schwann. This historical injustice has been exposed in a masterly book by Harris[5], who has studied the original sources. It emerges that Schleiden convinced himself that cells arise, nucleolus first, from a gummy substance in the intercellular space. The real credit belongs to one Dumortier, who studied filamentous algae or 'silkweeds', the group to which *Spirogyra* belongs. He accurately described a zone of multiplication just behind the tip where pre-existing cells developed a wall, which he showed was double by breaking the filament in two at the level of the new wall without leakage of the contents from either fragment. Dumortier's counterpart for animal tissues was Remak, who, like Dumortier, fully appreciated the general significance of his own observations of cell division. Dumortier is unknown because he fell victim to nationalist prejudice. Virchow summarized Remak's life's work (without giving due credit) and it is Virchow who is remembered, because of his ringing catchphrase "omnis cellula e cellula" — all cells from cells.

Discovering the right material

The modern concept of the cell was not developed until the end of the nineteenth century and required difficult and recondite study. Mitosis was observed by Nageli, Hofmeister and Strasburger[6] in the staminal hairs of *Tradescantia*, whereas van Beneden and later Boveri discovered the key facts of meiosis and fertilization in the eggs of a nematode, the latter taking advantage of the fact that *Ascaris megalocephala* var. *univalens* has only one paternal and one maternal chromosome[7]. It is interesting that these obscure and atypical animal models, or similar ones, are still in use in modern research[8,9] Should we conclude that all the useful exploration, at the level of the light microscope, was completed by the early

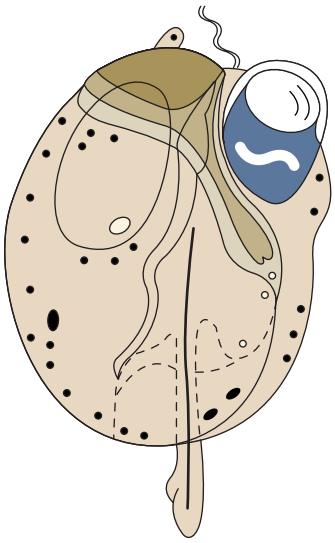

Figure 1 **Schematic diagram showing the eye-like structure in the marine planktonic dinoflagellate *Erythrodinium*. The eye (upper right, clad in dark pigment) appears to contain a receptor array, a lens and apparatus for focussing, but nothing is known of how the cell processes the information it receives[11,12].**

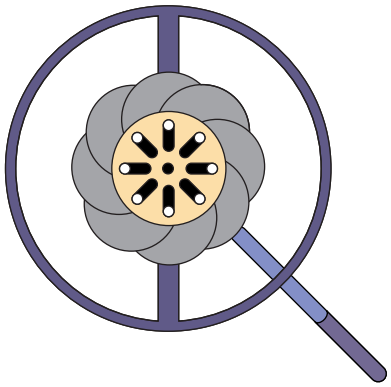

Figure 2 **Traviss' expanding stop (c. 1900, ref. 18). This apparatus was placed in the back focal plane of the condenser to obtain dark-field illumination. The petal-like structures with curved edges are thin opaque leaves of metal. The rod protruding from the centre to bottom right is a lever, by which the leaves are made to rotate and move out, thus collectively increasing the size of the central patch until light is just prevented from entering the objective directly. At this setting, the object is illuminated by the light that passes around the outside of the stop. This gives the brightest dark-field image of objects that diffract light. The device is, in effect, the inverse of an iris diaphragm.**

pioneers? The answer is no — important discoveries continue to be made, such as the revelation in 1978 of the existence of a new type of ciliate that aggregates into a fruiting body similar to that of the amoeba *Dictyostelium*[10]. Unfortunately, a grant proposal to seek the genuinely unknown at the light-microscope level, or even to search the old literature, would be unlikely to be funded.

But the old literature is well worth searching. For example, many modern researchers are struggling to reconcile the global influence of second messengers with the fact that neurones are capable of different types of integrative activity in different regions of the same cell. How would these researchers react if they knew that certain cells exist that contain an undeniable eye, complete with a lens, a curved retina-like receptor plate and circumferential structures that seem to constitute a focussing mechanism. These are marine dinoflagellates, in which investigation of the 'ocelloid' began in the 1900s (ref. 11; see Greuet[12] for a modern electron-microscopic study), but the physiology of which remains unknown.

A light-microscopic mystery

Historically, Lamarckian inheritance was dispatched by mendelian genetics and only briefly revived by Lysenko[13]. It has recently returned to centre stage with the discovery of prions. Sonneborn, however, found a clear example of Lamarckism in the inheritance of cortical pattern in the ciliate *Paramecium*[14]. In this organism, the cell surface is covered with rows of cilia that run from anterior to posterior, each row having a cytoskeletal fibre on one side, making it asymmetrical. Sonneborn found that when one ciliary row was experimentally inverted, the acquired trait was clonally inherited, and persisted even if the nuclear genes were replaced by repeated outbreeding. Grimes[15], working with different species, found that such traits persisted even through the process of encystment, in which the cilia disappear and the cytoskeleton is unrecognizably altered. In these situations the individual supramolecular assemblies (cilia and their associated fibres) are unaltered, but an abnormal global pattern, such as an inversion or duplication, is inherited. The lesson here is that some form of information transfer occurs from mother to daughter cell that can be explained neither by nuclear genes nor by prion-like transformation operating only at the molecular level.

Detecting sub-resolution objects

It was soon realized that objects smaller than the wavelength of light could be seen in the light microscope, provided that the contrast could be increased by viewing them like dust in a sunbeam, with oblique illumination and a dark background. Pijper[16] used South African sunlight to view bundles of bacterial flagella. The modern phase of dark-field research was started by Gibbons[17], who filmed the relative sliding of microtubule doublets in ciliary axonemes. Hotani[18] recorded the shape and transformations of bacterial flagella and individual microtubules. In his remarkable work, Hotani used low-magnification objectives of moderate numerical aperture, with a special dark-field condenser that was not readily available outside Japan. This has led to suggestions that the device invented by Traviss[19] (Fig. 2) should be brought back into use. Using dark field is tricky, as it is easily disturbed by the slightest contamination of the slide, coverslip or medium. Nevertheless, Higuchi (personal communication) has revived this method, using laser illumination to achieve a great improvement in the accuracy of determining the position of polystyrene beads in an optical trapping apparatus[20].

History of microscope development

Some inventions in microscopy have been directly stimulated by the needs of cell biologists, one example being the laser-scanning confocal microscope[21]. Often, however, a microscope has been developed first and applications have been sought. This has not always been successful. The multiple-beam interferometer microscope of Tolansky[22], although cheap and simple to make and capable of a vertical resolution of 0.1 nm, has found no application in cell biology because its lateral resolution is no greater than normal. Even sadder is the immense ingenuity invested in certain types of interferometer microscope[23] that measured the dry mass of a cell or of a limited area of a cell. This innovation proved to be of very limited interest to cell biologists. How do we avoid misapplied effort and make the best use of our physicists and engineers? The stock politician's answer is to encourage academic collaborations with industry, but anyone connected with the manufacturing industry knows that the problem is not a shortage of academics with ideas, but rather the manufacturer's difficulty in judging them. Perhaps grant-giving bodies should organize meetings to define the microscope requirements of biomedical research. The Society of Arts in Britain did this in the 1850s and the prize-winning design set the standard for an affordable but useful instrument.

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1. Hooke, R. *Micrographia* (Facsimile edition, Dover, New York, 1961).
2. Ford, B. J. *The Leeuwenhoek Legacy* (Biopress and Farrand, Bristol and London, 1991).
3. Schmidt, O., Wilms, K. H. & Lingelbach, B. *Optometry and Visual Science* **76,** 624–630 (1999).
4. Baker, J. R. *Abraham Trembley of Geneva* (Arnold, London, 1952).
5. Harris, H. *The Cells of the Body* (Cold Spring Harbor Laboratory Press, New York, 1995).
6. Strassburger, E. *Zellbindung und Zelltheilung* 3rd edn (Herman Dabis, Jena, 1880).
7. Boveri, T. Z. *Naturwissenschaften* **22,** 685–882 (1888).
8. Hepler,P. K. *J. Cell Biol.* **100,** 1362–1366 (1985).
9. Albertson, D. G., Rose, A. M. & Villeneuve, A. in *C. elegans II* Ch. 3, 47–78 (Cold Spring Harbor Laboratory Press, New York, 1997).
10. Olive, L. S. *Science* **202,** 530–532 (1978).
11. Kofoid, C. A. & Swezy, O. *The Free-Living Unarmored Dinoflagellata. Mem. Univ. California* **5.1** (1921).
12. Greuet, C. *Protistologica* **4,** 209–230 (1968).
13. Huxley, J. *Memories* 282–285 (Allen & Unwin, London 1972)
14. Beisson, J. & Sonneborn, T. M. *Proc. Natl Acad. Sci. USA* **53,** 275–282 (1965).
15. Aufderheide, K. J., Rotolo, T. C. & Grimes, G. W. *Eur. J. Protistol.* **35,** 81–91 (1999).
16. Pijper, A., Neser, M. L. & Abraham, G. *J. Gen. Microbiol.* **14,** 371–384 (1956).
17. Summers, K. E. & Gibbons, I. R. *Proc. Natl Acad. Sci. USA* **68,** 3092–3096 (1971).
18. Horio, T. & Hotani, H. *Nature* **321** 605–607 (1986).
19. Spitta, E. J. *Microscopy* (Murray, London, 1907).
20. Svoboda, K. & Block, S. M. *Annu. Rev. Biophys. Biomol. Struct.* **23,** 247–285 (1994).
21. White, J. G., Amos, W. B. & Fordham, M. J. *J. Cell Biol.* **105,** 41–48 (1987).
22. Tolansky, S. *Multiple-Beam Interference Microscopy of Metals* (Academic Press, London, 1970).
23. Hale, A. J. *The Interference Microscope in Biological Research.* (Livingstone, Edinburgh, 1958).