A dozen favourite books related to microscopy

This article is a slightly abridged version of that which I was asked to write for the *Quekett Journal of Microscopy* (see *QJM* vol.41 part 3: 219-225, Summer 2010).

I suppose most of us amateur microscopists have accumulated books about microscopy, natural sciences and imaging in general. My microscopy library takes up about 25 feet of shelving full of books (which is modest by some standards) and, yes, it is very difficult to make a selection of just a dozen books. I find that a chapter or a part of a single book has its merits, and I value the way a particular subject is approached by one author over another. So, like most of us, I dip into my books.

Unusually, one book both pleases and frustrates me in the same chapter! This is the second chapter on basic light microscopy in *Essential Cell Biology* [1]. The chapter is written by some very eminent scientists formerly from the Advanced Light Microscopy Facility at the European Molecular Biology Laboratory in Heidelberg. I like their approach to teaching the principles of (fluorescence) light microscopy. They introduce the subject not from a physical-mechanical viewpoint, but rather from that of the sample, for this is what the researcher is most concerned about. To quote: 'Biological scientists will usually raise questions such as where the cell/protein localises, where and when it moves, whether it co-localises or interacts with something, rather than asking what he or she could do with a certain type of lens'

This chapter has protocols of a practical nature, which I also like because these provide a useful resource to indicate to students. However – and I feel strongly about this, for it is not the only microscopy book to do so wrongly [2] – the protocol describing Köhler illumination is incomplete, dealing only with the adjustment of the illuminated field diaphragm (IFD), and does not mention the aperture diaphragm in the condenser at all. I suspect that either the author was recalling how to adjust an epi-fluorescence microscope (where some feel that the illuminating aperture iris, or condenser diaphragm, can remain wide open, and where in some fluorescence microscopes this diaphragm is not available), or it is an omission.

Because of my role as a microscopy facility manager and the need to teach others, my final choice of a dozen books will reflect my involvement in, and enjoyment of, teaching light microscopy.

My first choice must be ***An Introduction to the Optical Microscope*** by Savile Bradbury [3]. This was the first in the series of Microscopy Handbooks published by the Royal Microscopical Society in 1984. It is only 85 pages, published in monochrome (I also have bigger, more colourful texts) but for me this little book is an absolute gem, imbued with the character and personality of the author like no other.

Over 20 years ago I was an under-employed, restless, junior technician who had been given the task of buying a research-grade fluorescence microscope for the place where I then worked. I arranged demonstrations, and spoke to company representatives and very quickly found out how desperately little I knew about either end of a microscope.

At that time Baird & Tatlock operated a franchise arrangement for Zeiss West (Oberkochen), and I enquired of the rep. "what is a diatom?" He looked at me with pity and bemusement and said "You really don't know *anything* about microscopy, do you?" as if to say 'why have you of all people been given this purchase exercise?' The stress on 'anything' was embarrassing, and I had to admit the plainly obvious - that indeed I did not know anything about microscopes. I have never forgotten his advice by way of a reply. "What you must do" he said firmly and cheerfully "is read the newly-published RMS handbook on the light microscope by Savile Bradbury".

I was grateful for the advice, intrigued by the author's unique Christian name, and promptly went out and bought my copy. A few years later I was fortunate to be employed as a research technician at the department of Human

Anatomy, Oxford (now DPAG: Physiology, Anatomy & Genetics) where Savile was a senior member of staff. He very kindly took me under his wing, encouraged and taught me, and very much helped me in my career.

Savile was an accomplished and prolific author, clear and lucid, and very widely-read with a most comprehensive library of his own. It would be interesting to know what his choice of a dozen books would have been. I dare say one of them would have been *Principles of Biological Microtechnique* [4] written by John Baker, his D-Phil supervisor at Brasenose, for whom Savile had enormous regard and respect and to whom he referred in his Presidential address to the Quekett Microscopical Club [5] in 1993.

Contrast Techniques in Light Microscopy [6], handbook No. 34 in the RMS Handbook series by Savile Bradbury and Peter Evennett, has to be my second choice. My everyday copy of Bradbury & Evennett is dog-eared from constant use, like any good bible. I refer to it frequently, and recommend it to all who will listen. I have three copies: two were given to me by the authors, and are priceless for their inscriptions, because Savile and Peter taught me pretty much all I know regarding light and electron microscopy. This is a favourite book for me not merely because of its clarity and completeness of instruction, but because I can see these two friends of mine in my mind's eye, teaching students, as I read their work. I have been very fortunate to have been involved with them in teaching on the Royal Microscopical Society's summer school in light microscopy, and have not only witnessed their skill at first-hand, but have benefited also from their superb teaching. The third well-used copy has 'working copy' in large red letters on the inside front cover, so I know which one to lend out and which to refer to and teach from. You will not find a better explanation of phase contrast or interference contrast anywhere, and I have been recommending this book to all my students, be they Professors, PhDs or lesser-mortals, ever since its publication.

Occasionally a book will come along that you treasure as a well-loved tool. Ron Oldfield's ***Light Microscopy: an illustrated guide*** [7] is one such. I turn to it frequently to clear up a point, and I like the familiarity of knowing my way around the book. Again it is dog-eared (at A4 size I wish it were in hardback and more rigid), and I would happily have more than one copy to work from, but this book appeared quickly to go out of print, which is a great shame, for it is a very good book indeed, and copies are not easy to find. In my view, *Light Microscopy: an illustrated guide* is good because it was developed out of the material from a teaching course that Ron organised. I particularly value the practical exercises that are included at the end of each chapter.

These days I find myself using two more recently-published books more frequently. The first of these is Spector & Goldman's **Basic Methods in Light Microscopy** [8], and Sluder & Wolf's *Digital Microscopy* [9]. I really value the chapter in Spector & Goldman by John Murray on fluorescence optical sectioning, which is the best that I have seen on the subject, and it is probably the hardest choice that I have to make for this selection in distinguishing between these two books. However, I think that recently I have pulled Sluder & Wolf down from the bookshelf more often. Therefore, my fourth choice must be ***Digital Microscopy*** 3rd edition, Methods in Cell Biology, volume 81. This text arose from two earlier editions edited by the same editors, and has remained popular for over 10 years. There are good chapters on image formation, microscope alignment, fluorescence microscopy and most aspects of cameras and digital imaging, but the chapter that I find most useful in a professional capacity is that on live-cell fluorescence imaging by Jennifer Waters.

Another book which is never far out of reach, and which I have either bought or recommended for every light microscopy facility that I have worked in, is the first edition of Douglas Murphy's ***Fundamentals of Light Microscopy and Electronic Imaging*** [10]. A hallmark of the success of this book by Murphy is the fact that it is carried by major scientific supply houses in their general catalogues. I especially like the treatment of Abbe's theory for image formation in the microscope in Chapter 5, and the explanation of spatial resolution in Chapter 6 neatly illustrated (Figure 6-1) with a beautiful illustration of *Pleurosigma angulatum*.

Sixth on the list must be Needham's ***The Practical Use of the Microscope*** [11] for in this book you will find a description of most of the microscopical gear available post-war. Arguably, the golden hey-day of the light microscope was the first two-thirds of the 20th century. Where now can you get the esoteric accessories that are no longer made, yet are so useful: the apertometer, Abbe test plate, Lieberkühn illuminator, Traviss expanding dark-field stop or Nelson's cassegrain dark-ground illuminator? With my interest in test diatoms, I particularly like Figures 49 (page 127) and 53 (page 144) comparing the resolution of the frustule of *Amphipleura pellucida* into striae and puncta by, respectively: oblique illumination, near UV and electron microscopy.

Arthur Barron's ***Using the Microscope*** [12] is seventh on my list. I have the third, and last, edition of 1965, which I particularly like for its clear explanation on how to set up a microscope with a mirror and external light source. It also has a very clear, and readable, section on the polarising microscope which I refer to often.

Bradbury's ***The Evolution of the Microscope*** [13] is my eighth choice. This book is a very clear and readable history of the light microscope from the earliest times, and I am fortunate to have the author's own copy. In a similar vein Gilbert Hartley's ***The Light Microscope: its use and development*** [14] also describes the history and development of the light microscope, but in a different style to Bradbury. I am fond of it for its sheer readability and enjoyment. It is difficult to pick any one passage, but I particularly like reading Hartley's thesis regarding the discovery of the microscope following the invention of the telescope, and am amused by the illustration in Figure 59 on page 131 showing the trinocular microscope for three people in use! I am fortunate to have two copies, so keep one at home and the other at work.

My tenth choice is Carpenter & Dallinger's 8th edition of **The Microscope and its revelations** [15], a favourite of several serious amateur microscopists. I had been looking for this exalted book for quite a while when an example bound as two volumes came up for sale for £30. At the time I had a young family to feed, and spare cash for microscopy was not in abundance. I enquired, put the 'phone down, and took a few minutes too long to rationalise my decision (i.e. what can I, in all honesty, plausibly say to my wife); when I 'phoned back it had gone. I very much regretted this, and so leaped at the chance of a single-bound copy when this presented itself. The book is beautifully bound and illustrated, and my copy naturally falls open at Plate XII, an illustration of *Arachnoidiscus japonicus*, which I absolutely love. I have photographed this, and have a print of it at work by my desk.

Aglow in the Dark, my eleventh choice [16], is the smallest book in my selection, measuring only 17 x 14.7 x 1.8 cm (6.7 x 5.8 x 0.7 inches). It describes how bioluminescence has been put use in the service of mankind, and is a wonderfully entertaining, yet informative, read about how genetically-engineered fluorescent proteins have revolutionised light microscopy within the last decade. You can find this gem quite cheaply on-line in the second-hand book market for about £10; in my view, money well-spent by any criterion. Here is a quotation from pages 141-142 to whet your appetite:

'Heim, Tsien and other scientists swapped virtually every amino acid in the 238 amino acids of the jellyfish protein. To check for fluorescence, Heim used an old spectrofluorimeter, referred to as the Green Monster. The operation was low-tech. Heim looked through different colored Kodak filters that he taped to his lab goggles while changing the color light by hand on the old Green Monster. "At Berkeley they were ready to throw it away, but as an old pack rat or magpie, I refused to let it go." Tsien says of the spectrofluorometer.'

The three men instrumental in the discovery and application of Green Fluorescent Protein (GFP) and its derivatives – Shimomura, Chalfie and Tsien - jointly shared the Nobel Prize for Chemistry in October 2008 [17, 18], and very rightly so. Unfortunately, a Nobel prize cannot be shared between four people: circumstances conspired to keep Douglas Prasher from sharing this prize for which his work was a crucial foundation – as the three recipients acknowledged.

My last choice is a very recent (for 2010) publication. It is Randy Wayne's ***Light and Video Microscopy***, [19]. There is a decided historical slant to the tuition, which I find grows on me, and I particularly like the exercises and clear diagrams. Given that this book is also one that has arisen from a formal taught course in microscopy, I'm intrigued by the discourse on the 'Microscopist's Model of the Photon' in the Appendix. If I were cast away on a desert island, getting to the bottom of this book's Appendix would keep going for quite a while!

I shall argue for a 'baker's dozen' in my choice because naturally I want you to have value! My thirteenth choice is not a book (so I can honestly claim to have selected only 12 books), but a journal. I am tempted to select the Royal Microscopical Society's 150th anniversary issue (vol. 155 part 3, September 1989) of the *Journal of Microscopy* with its excellent articles by eminent Fellows on landmarks in light microscopy; the social history of the microscope; development of preparative techniques and triumphs and conflicts. Instead I have selected *Nature Methods*, an influential journal series, with an impact factor of 25, in which I very often find useful and stimulating articles describing the cutting-edge and future development of microscopical and imaging science.

Besides books, I use papers and articles on a frequent basis in my professional life as a microscopist. I have already mentioned *Nature Methods*. The editors selected super-resolution fluorescence microscopy for their Method of the Year 2008. The commentaries and research articles in this issue of *Nature Methods* [20] give an exciting overview of the future of biological light microscopy. I find the primer in this issue by Daniel Evanko [21] most helpful, as well as those by Patterson[22], Lippincott-Schwartz [23], Fernandez-Suarez [24] and Davis [25] most helpful.

To turn full circle, and return to exploring the limits of resolution in the 19th century, one of my favourite historical papers is that by Turner and Bradbury [26]. Their study settled once and for all the argument surrounding Friedrich Nobert's final twenty-band test plate, the last of his famed 19th century series of diamond-ruled resolution tests [27]. Nobert ruled his finest 20th band at an intended line separation of 0.113 μm in 1873, just before the theoretical work of Abbe on resolving power and image formation was published in English in 1875. We know now that these finest bands could never have been resolved with the light microscope. When the 19th band of the nineteen-band test plate (intended spacing 0.225 μm) was resolved by Dr JJ Woodward in 1869, Nobert promptly ruled another.

The finest ever band resolved by the light microscope, the 11th band of a line separation of 0.21 μm, was seen and demonstrated circa 1884 by that doyen of 19th century microscopy, Edward Milles Nelson [28]. Controversy raged for about 100 years as to whether Nobert had actually ruled the unresolvable finest bands. Some, including Webb, whose own diamond ruling work did not match that of Nobert, thought that the diamond would have ploughed a series of furrows so fine that they would have naturally collapsed into a featureless trench from the mechanical stress of ruling the glass substrate which he described as

'aerial polarized black lines of light as to embarrass the minds of some gentlemen, and driven them to resort to a declaration of "spectral lines" without giving the slightest hint of their source'.

Indeed Webb, in July 1873, decided to henceforth refer to Nobert's finest-ruled lines as 'incisions', saying: 'I now advisedly adopt the word incision, for the word line applies no more to these diamond cuttings than it does to the Suez Canal'.

Others, including Woodward, suspected the veracity of the ruled lines, not merely from familiarity with the appearance of the test plates and working with them, but also from calculating Fraunhofer's equations for the spectral colour reflected by the ruled lines which, as Woodward later pointed out, is 'altogether independent of our ability to resolve the lines with the microscope'. We see the same phenomenon of spectral colour today reflected from the surface of a CD burnt with data.

Turner & Bradbury showed unequivocally (with a carbon-coated plastic replica or 'peel' of the surface of the test plate) that the 18th, 19th and 20th bands were ruled at an average line spacing of 0.13 μm, 0.12 μm and 0.11 μm

respectively [Figure 11 in ref. 26]. Any modern museum curator who read their methodology would have fifty fits, and this important work would never be carried out today in the same fashion … disassembly, dissolving the ruled coverslip free of the original cement, glueing it onto a modern slide with Farrant's medium and taking a formvar replica! I have the original plate for Figure 4 of this paper given to me by one of the authors, inscribed on the reverse, framed and hung at home.

I have pushed my luck by claiming a baker's dozen in my choice, and thus simply cannot comment on any other favourites. In a former life I was a histologist as well as a microscopist; I am particularly fond of Bracegirdle's *A History of Microtechnique: the evolution of the microtome and the development of tissue preparation* [29] not least because I like to know the history behind a subject and have enjoyed using a modern-day Cambridge rocking microtome. I have also found Clayden's *Practical Section Cutting and Staining* [30] to be invaluable whilst training. There are other practical texts on histology, but these two resonate with me and are the histological choices I would make were I allowed a greater number of books in my selection.

This has been a very enjoyable exercise and I encourage other Quekett Club members to peruse their bookshelves, however large or modest, and write up their own list of a dozen favourite books. It has been fun to read what others have selected and discover the reasons for their choice. It has also been refreshing to look over my own choice, to remind myself why certain books bring so much pleasure, and to make this selection for you.

Jeremy Sanderson

My shortlist of a dozen favourite books in microscopy

1. Bradbury, S (1984) ***An Introduction to the Optical Microscope***
2. Bradbury, S & Evennett, PJ (1996) ***Contrast Techniques in Light Microscopy***
3. Oldfield, R (1994) ***Light Microscopy: an illustrated guide***
4. Sluder, G & Wolf, DE (2007) ***Digital Microscopy*** 3rd Edn.
5. Murphy, DB (2001) ***Fundamentals of Light Microscopy and Electronic Imaging*** 1st Edn
6. Needham, GH (1958) ***The Practical Use of the Microscope***
7. Barron, ALE (1965) ***Using the Microscope*** 3rd Edn.
8. Bradbury, S (1967) ***The Evolution of the Microscope***
9. Hartley, G (1993) **The Light Microscope: its use and development**
10. Carpenter, WB & Dallinger, WH (1901) ***The Microscope and its revelations***
11. Pieribone, V & Gruber, DF (2005) ***Aglow in the dark: the revolutionary science of biofluorescence***
12. Wayne, R (2009) ***Light and Video Microscopy***

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