The Clarity of Images from Early Single-Lens Microscopes Captured on Video

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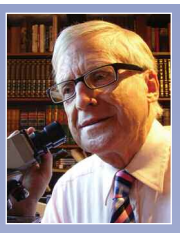
BIOGRAPHY



Brian Ford is a microscopist and author of books, articles and papers on the microscope and its development. Prof Ford was a NESTA Fellow. His research work ranges from haemostasis to microbiology, and he is an authority on Antony van Leeuwenhoek. Of the author's 30 books, many have been on microscopy and the history of cell biology.

ABSTRACT

Microscopes of seventeenth century design are the starting-point for an investigation into the pioneers of microscopy. The techniques for using early microscopes can be exacting and diligence is required to derive the best results. Using modern digital techniques it has proved possible to recreate the observations originally made as far back as the 17th century. Accounts in standard books, and now in television documentaries, create an impression that the images obtained with early microscopes are invariably of low quality, exhibiting chromatic aberration and poor focus. Although the early microscopes are demanding instruments to use, this programme of research has shown that it is possible to obtain videomicrographs of remarkably high quality. Published images both from print and television are here compared with the best results that early microscopes can reveal.

KEYWORDS

microscopy, microscope, imaging, chromatic, aberration, micrograph, television, Leeuwenhoek, history of science, Robert Brown

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INTRODUCTION

The first microscopes created images that are far superior than is currently claimed. We often hear that early microscopists were handicapped by their inferior instruments, their lenses generating images that were distorted and fringed by rainbow-hued fringes. We are all reminded of these assertions and entire television programmes have been founded on this principle. The same view abounds in standard books on microscopy, and even in university examinations, where a question asked medical students to explain why it was impossible for Leeuwenhoek to have observed what he claimed. The reality is very different. Our research has shown that a single lensed instrument of the 1600s can come close to the resolution of a present-day compound microscope – the remarkable conclusion is that most of the fundamental discoveries made with light microscopes could have been made with a Leeuwenhoek lens.

The claim that early microscopes produce inferior images is copied from source to source, but is not backed by scientific evidence. Here is an example: the description of Charles Darwin's microscope by James Smith (1846) by the Whipple Museum for the History of Science at the University of Cambridge states: "The 1/8-inch object glass was an impressive lens, even by modern standards. It allowed Darwin to achieve a maximum magnification of over 1300 times. The resolution and image quality at this magnification would have been poor, however." This begins as a paean of praise, but ends in speculative critique. But note that the writer concludes that the image "would have been poor" – this is a surmise, not a finding. The Whipple Museum has a fine collection of early microscopes, but modern museums lack expertise in their use. Thus, a published image of a flea taken with an early Cuff microscope at the Whipple (Figure 1) is indistinct and poorly lit; but must be admitted that this is the result of limited expertise, rather than a drawback with the instrument. This photomicrograph is mistakenly identified as a microphotograph, which is a fundamental misuse of the term. Microphotographs are tiny photos of portraits, scenes, secret documents or eroticism that were popular during the Victorian era; by contrast, a photographic image taken through a microscope is a photomicrograph. You would hope that museum staff handling microscopes would remember such things.

Yet even the museums closest to the subject often fail to handle it well. The Boerhaave Museum in Leiden has a unique collection of microscopes made by the pioneering microscopist Antony Leeuwenhoek. To mark a specialist exhibition on Leeuwenhoek, they summarised their expertise in a catalogue devoted to his work. One of their illustrations was of an elder pith section viewed through an original lens made by Leeuwenhoek some three centuries earlier (Figure 2a). Little could be seen; the indistinct images of the cell walls are little better than an observer can obtain with the unaided eye (elder pith contains cells large enough to be seen without a microscope).

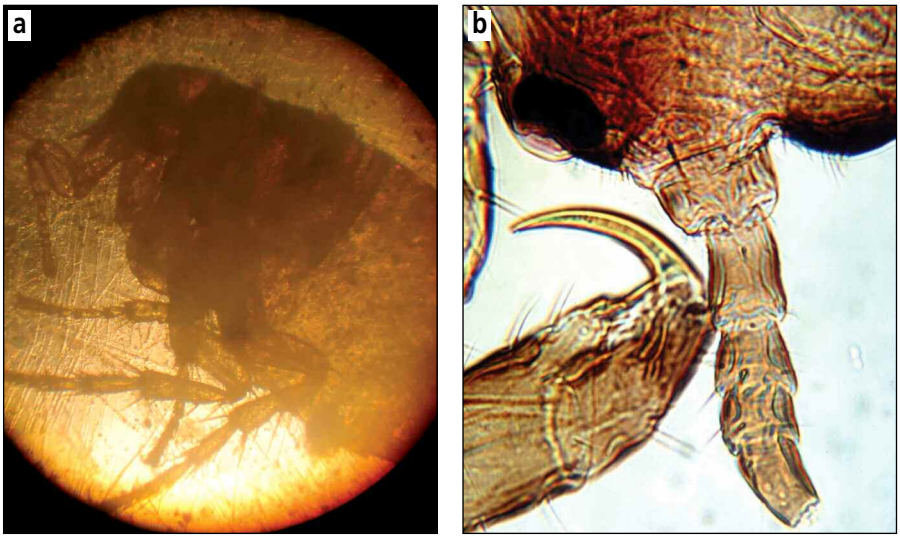

Figure 1:
(a) Image of a flea taken on an 18th-century Cuff microscope, published by the Whipple Museum, Cambridge. The substage lighting is poorly adjusted and little detail is visible. Original magnification 30x. (b) Antenna and claw of the louse Pediculus imaged by the author with a replica simple microscope made by the late Horace Dall. Much finer details are resolved. 275x.

The problem lay, not with the microscope, but with the techniques that were employed to put it through its paces. I had also undertaken the same observation, with a team at the Utrecht University Museum to assist me comprising Dr Robert Frederik, Jaap Stolp, Pieter Smiesing and Karel Snethlage. The Museum Director, the late Professor Peter-Hans Kylstra, generously allowed me full access to the Leeuwenhoek microscope in his care and this allowed me to take micrographs of Leeuwenhoek's hand-cut sections through his own microscope. The results were breathtaking; the images were comparable with those you would obtain from a modern light microscope and it was possible to discern fine fibres and hyphae down to 0.7 µm in thickness (Figure 2b).

Reflect for a moment on the significance of this discovery – we are already within a factor of 4 of the limits of light microscopy (200 nm). The catalogue was entitled Antony van Leeuwenhoek in the original Dutch edition and when the exhibition transferred to England the translated edition was given a new title, 'Beads of Glass'. This is doubly unfortunate, for it helped perpetuate a widespread myth about Leeuwenhoek, namely that he worked with bead lenses. This is not the case; all his lenses were biconvex or planoconvex. The micrograph was a serious underestimation of that great man's technical abilities, for as we can see Leeuwenhoek's microscopes were capable of revealing images many orders of magnitude better than that.

Television programmes have recently promulgated the same view. The first professional microscopist was Robert Hooke, who in 1663 was given the responsibility of providing regular microscopical demonstrations to the members of the recently formed Royal Society. His microscope was included in a recent Channel Four television programme from London. In so many ways this was an exciting and memorable documentary, though not when Richard Dawkins attempted to recreate Hooke's observations. The image obtained was of such poor quality that one could see little that was not visible to the naked eye (Figure 3a). In fact, Hooke certainly used a single lensed microscope to make his detailed observations, for he describes the technique clearly in his great book Micrographia of 1665 (Figure 3b). Using a microscope of this sort we can see the great improvement in image quality (Figure 3c): the details of the specimens portrayed in Micrographia are simply not available to an observer using a compound microscope of the kind Hooke possessed.

In a series entitled The Cell, the BBC attempted to recreate the view of nature that Leeuwenhoek would have enjoyed. The programme was high on exuberance and enthusiasm, but the attempts did not succeed. Their first demonstration was of aquatic microorganisms which they set out to film with a replica microscope made by a Dutch enthusiast named Hans Loncke. The microscope was expertly filmed, but the view through the lens was faint and blurred (Figure 4a). The commentator presumed that it might be a protozoon we could see 'scooting about' though it could have been any other kind of organisms visible to the taken eye (a rotifer, for instance). Although the script emphasised that this was a view which compared favourably with that obtained through a modern microscope, the truth is that the results were too poor to enable any serious observations to be made. The experiment was not well conceived; the BBC resorted to the use of darkground microscopy in order to maximise contrast (there are suggestions that pioneers, including Hooke and Leeuwenhoek, may have done this but no certain source to substantiate the idea). Regretfully, it did not work.

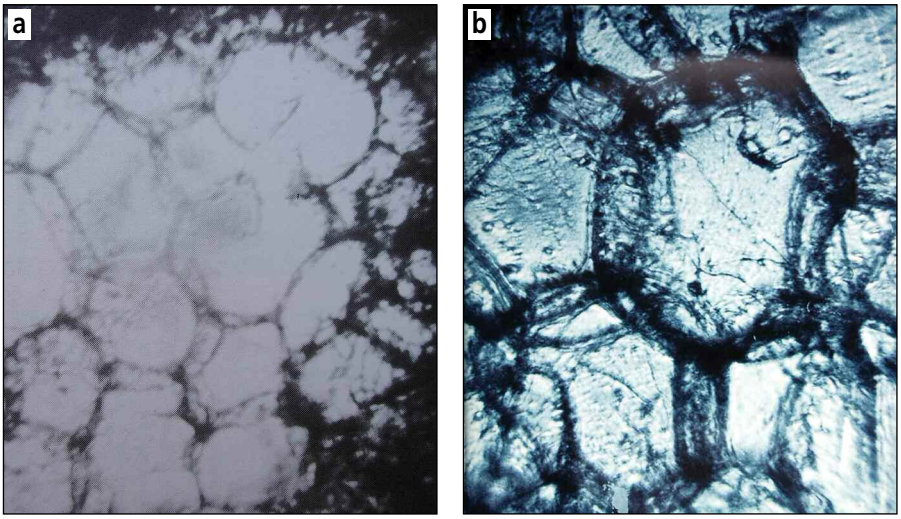

Figure 2:
(a) In their exhibition catalogue entitled Beads of Glass, the Boerhaave Museum published this micrograph of cells of the elder tree Sambucus taken with an original Leeuwenhoek microscope. 100x. (b) The author, with the original 17th century Leeuwenhoek microscope in Utrecht, obtained this image of Sambucus cells in which fine pits in the cell walls are visible. 150x.

Having taken clear pictures through an original Leeuwenhoek microscope, I was keen to attempt to repeat the BBC's experiments, using a replica microscope and using video rather than still photography. To my relief, the resulting videomicrographs did show vividly how Leeuwenhoek viewed his specimens. Using a relatively crude Victorian lens it was possible to see filamentous algae and ciliated protozoa far more clearly than the documentary had claimed (Figure 4b). Using a planoconvex lens of higher magnification we could clearly resolve the organelles and intracellular particles of ciliates. A still frame of Stylonychia provides a revealing comparison. The image could almost have come from a present-day microscope. Even a single lens can provide a reasonably comparable image.

In another documentary, this time on Victorian microscopy, the presenter Michael Mosley set out to show how important was the introduction of histological stains. To prove the point, he was provided with a view of living cells *in vitro* and unstained, and he had to describe the view as looking like 'translucent mush' (Figure 5a). The 'mush' resulted from poor focusing, inadequate use of the substage, and flooding the specimen with too much illumination – in short, deficient science. Curiously, I had made precisely the same demonstration with Carol Vorderman for a BBC science programme entitled 'Take Nobody's Word for It' and had demonstrated what all microscopists know: with judicious adjustment, the cell could be induced to reveal itself in surprising detail, even without stains (Figure 5b).

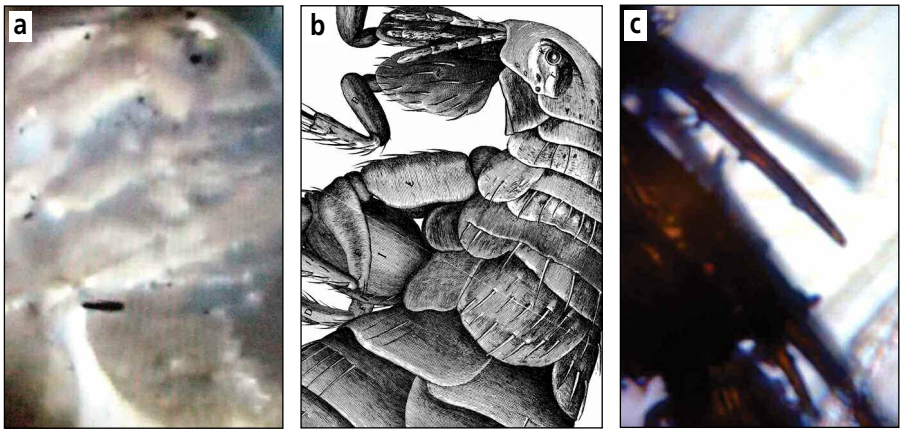

Figure 3:
(a) Pulex, the flea, imaged through Robert Hooke's microscope of the 1660s by Richard Dawkins for the Channel Four series Genius of Britain. 50x.
(b) Hooke's published engraving shows details far finer than are visible in (a). He used a single-lens microscope for detailed study. 50x.
(c) Frame from a video by the author through a single lensed microscope successfully resolves the fine hairs on the flea's appendage. 325x.

In their series on 'The Cell' the BBC also set out to recreate the view that Robert Brown had obtained with his single lens microscopes of the 1820s. These demonstrations were also unsuccessful; even the best sequences could not manage to resolve the cell walls and the nuclei which Brown noted (he coined the word 'nucleus' for this feature in 1828). A comparison between the two images is highly significant (Figure 6 a,b): here we have images using the original lens, not a replica. In each case we have identical specimens, identical methods of mounting, and the same lens. The lens was capable of providing images that are far superior images to those presented to the television audience.

How was it done? To obtain video sequences through these primitive lens systems, a routine video camera was stripped down and the lenses removed. An adaptor was constructed so that the single lens effectively became the new camera lens. The image struck through the lens is captured directly by the sensor, without there being any intervening camera lens that can interfere with the formation of that image. A mechanical stage mechanism is then modified to allow one to move the specimen at will through the field of view of the new single-lensed video microscope, and a fine-adjustment focusing assembly is adapted to permit one carefully to focus the array on the specimen at will. This is time-consuming and delicate work, but the results are so very worthwhile. It is only through scientific productions in television that anyone has previously attempted to obtain video sequences to show what the pioneers observed. For all the visual success of the bulk of those programmes, the microscope reconstructions are always disappointing. The public, as a result, are given a profoundly misleading account.

Yet, with a little diligence and ingenuity, it has proved possible to record what the pioneers truly observed – and the results are remarkable. This has been a worthwhile research programme and the audiences who have seen these videos have found them so very exciting to watch. The abilities of those pioneer microscopists were so much greater than has been recognised, and it is hard to envisage any other area of television film-making where such misleading findings could be perpetrated. Nobody could imagine a programme which implied that the Wright Brothers never flew or which reported that the Great Depression never occurred – we know the facts, for the facts have been presented to us all on television. For some inexplicable reason, mistakes and technical deficiency have crept through the fact-checking process when the microscope is brought into a TV documentary.

No longer is microscopy properly taught, and the consequences are regrettable. We have enjoyed doing the work – and these unprecedented results have at last become a fitting tribute and, in some ways, a vindication of the first microscopists who bequeathed to us the modern era of cell biology.

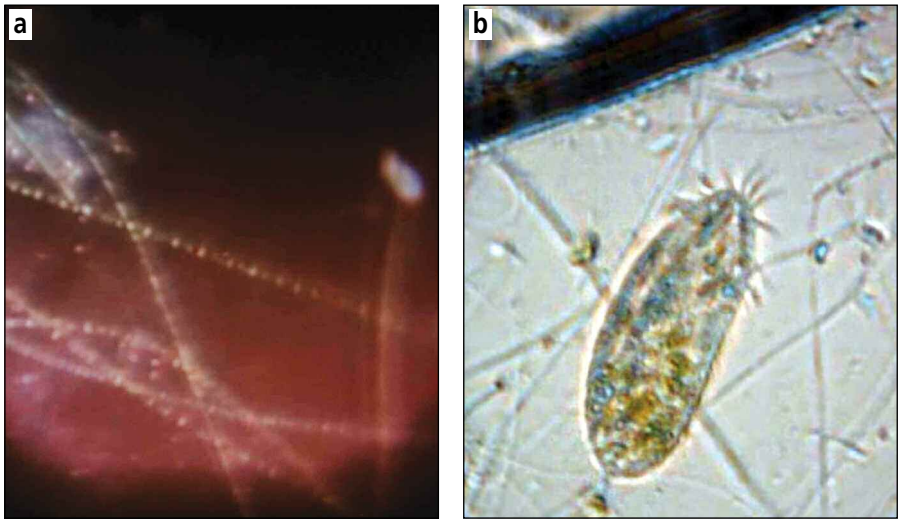

Figure 4:
(a) A sample of pond water was imaged through a replica Leeuwenhoek microscope by BBC4 in their series The Cell. The blurred results are little improvement on naked-eye observations. 15x. (b) A single-lensed replica microscope was later used by the author to observe Stylonychia and an algal filament. This is the view Leeuwenhoek would have enjoyed. 275x.

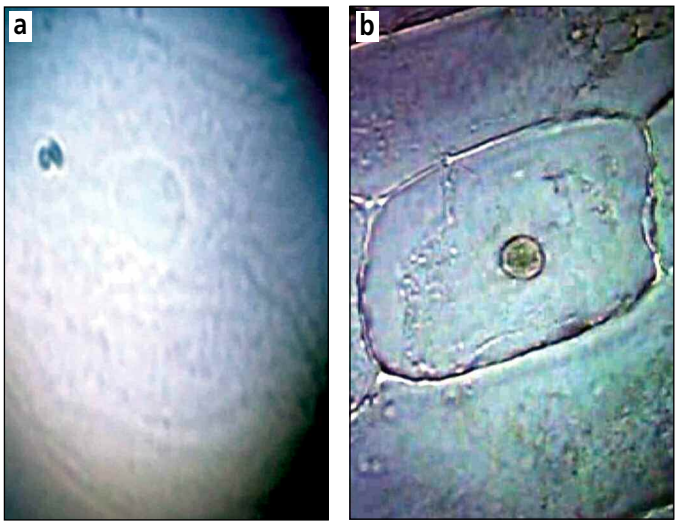

Figure 5:
(a) A plant cell imaged by the BBC for their recent series Story of Science. The presenter said that, until staining was developed, this was the best view available. The cell, he added, looked like 'mush'. 125x.
(b) In an earlier BBC science programme, the author made the identical demonstration with care being taken to adjust condenser focus and substage aperture. Even without stains, cytological detail is visible. 125x.

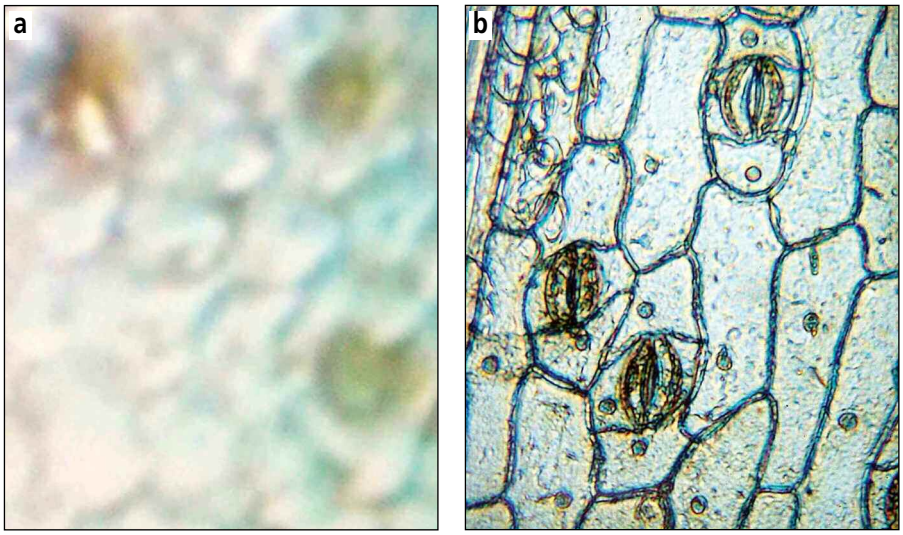

Figure 6:
(a) The BBC demonstrated the appearance of orchid epidermis through the No. 2 lens of Robert Brown's microscope of the 1820s. No cell structure is resolved. 75x. (b) Here the author examined an identical specimen through the same lens. With care in focusing and substage adjustment, much fine cytological structure is discernible. 75x.

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