# Living Images from the Birth of Microscopy

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## Introduction

Microscopes are probably used in more branches of scientific research than any comparable instrument, and the light microscope has become an instantly recognizable icon. Curiously, this single instrument is widely misrepresented; the story of its development has for decades been misleading, and the general understanding of microscopy is weak. Worse, the media have done a great disservice to the instruments in general—they are poorly portrayed, wrongly interpreted, and both print and broadcast media have failed to do justice to the all-important role that the microscope has played in the advancement of our understanding.

Ask yourself: when did you last see a worthwhile presentation of microscopic images on television or in a magazine? The chances are it was a considerable time ago. Similarly, when did you see a successful film of what the pioneering microscopists could see with their diminutive instruments? This is easier to answer: never. There have been occasional attempts to recreate the images from the dawn of microscopy, but none has been successful. The standard works always emphasized the crude nature of early microscopes and the distorted and chromatic images that their inferior lenses could generate, and this has been perpetuated by recent television documentaries.

Yet our research has radically reformed this accepted view. Even the minute, single lenses used by the pioneering microscopists can create images of startling clarity. As this research began, a number of micrographs were produced to show how good the results could be, and some of these have been honored with awards in Britain and America [1]. More recently, videomicrographs have been successfully obtained, and these provide, for the first time, a living view of how the first microscopists viewed their specimens. The results belie the misleading accounts and demonstrate that the pioneer microscopists were observing nature with a clarity that is surprisingly close to what we might expect in today's laboratory.

This might seem to be an extreme statement, but consider: in so many fields of endeavor, the present-day version is much better than the first version, e.g., aircraft, automobiles, dwellings, computers, etc. Current devices make the earliest examples seem greatly inferior. Surprisingly, this is not the case with the conventional light microscope. Optical constraints impose a resolution limit of approximately 0.3 µm, and it has been shown that the single-lens microscope hand-made by Antony van Leeuwenhoek and preserved at the University Museum for the History of Science at Utrecht, Netherlands, could resolve objects as fine as 0.7 µm. The resolution of this early microscope was therefore within a factor of 3 of the best that a conventional light microscope could theoretically achieve. This may be unique in the history of science [2].

## Materials and Methods

Obtaining video images through these minute lenses is demanding, but not technically complex. The lens supplied on an SVGA webcam was removed and replaced with a customized bracket into which a small lens can be inserted. We have used original eighteenth- and nineteenth-century lenses from botanical and aquatic microscopes, as well as modern-day replica lenses. The best of these were plano-convex soda-glass lenses approximately 1 mm in diameter that were ground and polished by my colleague Es Reid of Cambridge University. The assembly is fitted onto a purpose-built bracket that provides an extension from the mechanical stage of an Olympus BH laboratory microscope. Use of the mechanical stage controls provides for movement of a specimen in the *x*-*y* directions; whereas, the coarse- and fine-focusing controls allow us to adjust for optimum image clarity. Illumination is provided by a single light-emitting diode mounted along the optical axis of the assembly and some 7–15 cm (about 3–6 inches) beneath the specimen. Actual early lenses ranged in magnification from 25 to 600 and allowed various experiments that recreated the observations recorded in the literature by pioneering microscopists.

## Recreations of Early Observations

In our previous experiments, still micrographs were taken with the original microscope made by the pioneer of high-power microscopy, Antony van Leeuwenhoek of Delft, Netherlands, around 1690 and preserved at Utrecht. These modern photos taken through the original microscope showed that Leeuwenhoek's own lenses could generate an image of astonishing quality. This was the microscope used by Bracegirdle in 1981 to image a blood smear. The results were disappointing, and no cells could be seen in the resulting image (Figure 1a). However, using a mount that fitted the diminutive microscope to a modern Olympus OM2n film camera allowed us to demonstrate how clear the image could be: a fresh blood smear could be observed in which, not only were the predominant erythrocytes clearly visible, but even the lobed nucleus of granulocyte could be resolved (Figure 1b). Thus, the resulting image taken with Leeuwenhoek's original seventeenth-century microscope compares favorably with a present-day micrograph.

In recent years, other attempts have been made to recreate observations made by pioneers from earlier centuries. The BBC program entitled *Cell* took the No. 2 lens from the microscope owned by Robert Brown in the 1820s and used it to reprise Brown's observation of the

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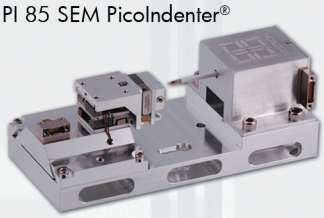

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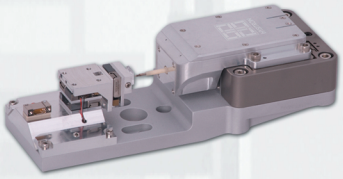

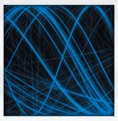

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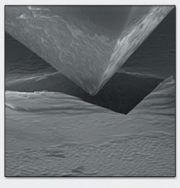
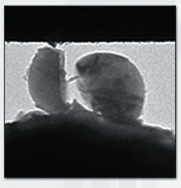
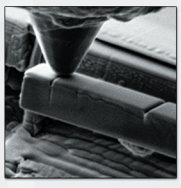
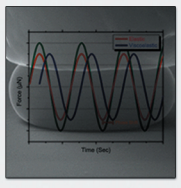
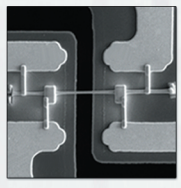
|  |  |  |  |  |  |  |  |

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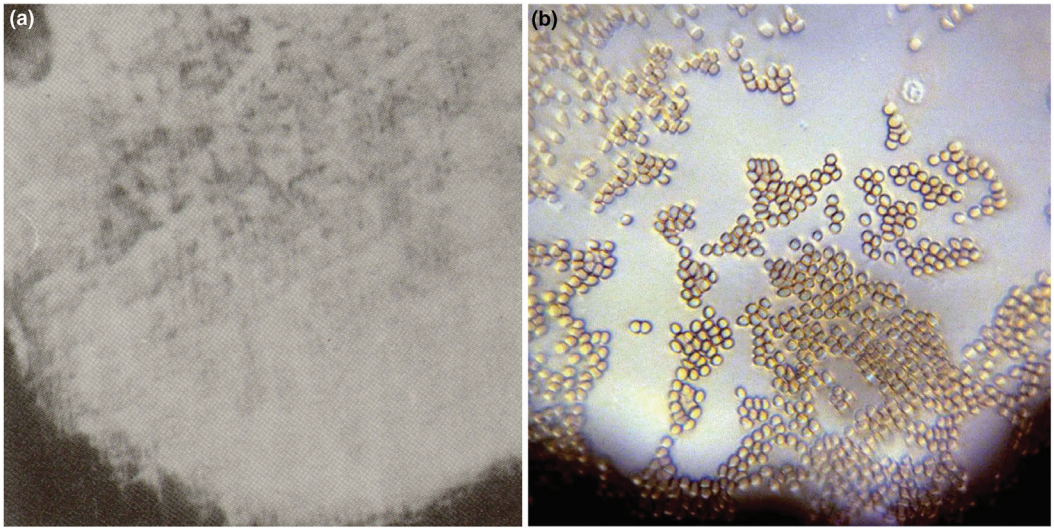


**Figure 1:** (a) Bracegirdle's image of blood cells through Leeuwenhoek's original microscope at the University of Utrecht [3]. Nothing can be discerned in this image, though the aperture of the handmade microscope can be seen at the periphery. (b) Image of human blood cells from the same microscope under optimized conditions shows the image clarity that could be obtained. Erythrocytes are clearly resolved, and the lobed nucleus of a granulocyte (top right) can be observed. This lens was made by Leeuwenhoek blowing a large bubble of glass and extracting the terminal pellet, as described in the text.

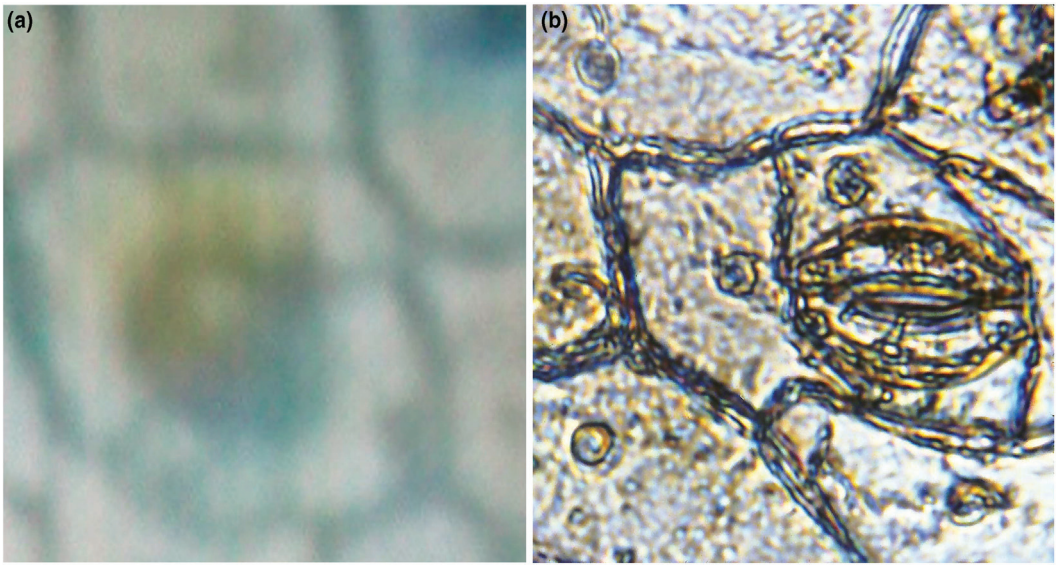


**Figure 2:** Robert Brown named the cell nucleus during studies of orchid tissue. (a) In a reprise of Brown's epoch-making observations, orchid epidermis was imaged for the BBC program *Cell* using the #2 lens from Robert Brown's microscope preserved at the Linnean Society of London. The results are disappointing, and nothing of cytological significance was resolved. (b) Specimens of orchid epidermis examined successfully with the same microscope. Our experiments imaged peels of the same orchid tissue through this #2 lens. After careful adjustment, the results clearly reveal the cell nuclei. Stomata are also resolved (right), and some fine cytological details are apparent within the epidermal cells.

cell nucleus in tissues taken from orchid leaves. Brown was a meticulous observer, and he used his unsophisticated single-lens microscope (made by Bancks of London) in some extraordinary research. For example, he identified the fertilization of the naked ovule in the Gymnospermae, an observation that present-day microscopists would find difficult or impossible to emulate. The BBC's technical teams undertook to repeat Brown's naming of the cell nucleus, but the results were disappointing (Figure 2a). Little could be seen in their images. However, using still photography and, in the recent research, digital video recording, we have shown that considerable cytological detail could be perceived (Figure 2b). Not only did we use the same microscope for our research, but even the identical No. 2 lens.

One crucial paradox remains unchallenged, and it featured in another BBC program, *The Age of Revolution*. In it, Robert Hooke's great work from 1665 titled *Micrographia* was examined [3]. The best-known illustration from that monumental work showed the flea *Pulex* engraved on a large folding plate (Figure 3). It features impressive detail and immense clarity of line, appearing not unlike a modern-day scanning electron micrograph. The program presented this to the viewer, followed by a video sequence of a flea as seen through Hooke's compound microscope (made for him in London by Christopher Cock) (Figure 4a). The recreation of his results was interesting—but, none of the fine detail that Hooke painstakingly recorded could have been resolved by his compound microscope.

Close scrutiny of *Micrographia* provides the answer. Hooke is well known for using his large and expensive compound microscope for everyday observations, but he had a trick up his sleeve. For high-magnification observations he resorted to a small and undistinguished single-lens instrument. He describes how to make them and how the lenses were ground. I have elsewhere shown [2] that it was this very design that Leeuwenhoek took from *Micrographia* when he began to manufacture microscopes of his own. Hooke's single-lens magnifiers are revealed by a few words in the preface

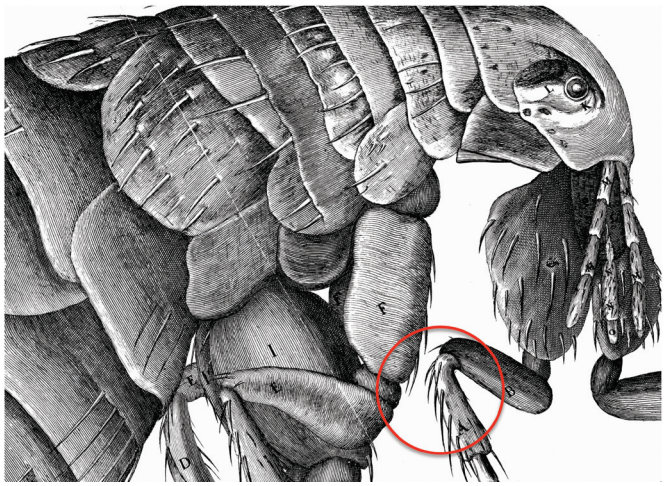

**Figure 3:** Detail from Robert Hooke's impressive engraving of a flea showing fine detail that cannot have been observed with his compound microscope. This poses a profound paradox, for he was clearly not guided by guesswork. Thus, Hooke must have employed a single-lens microscope with a ground plano-convex lens for his high-magnification studies. The circle indicates the region for the image in Figure 4b.

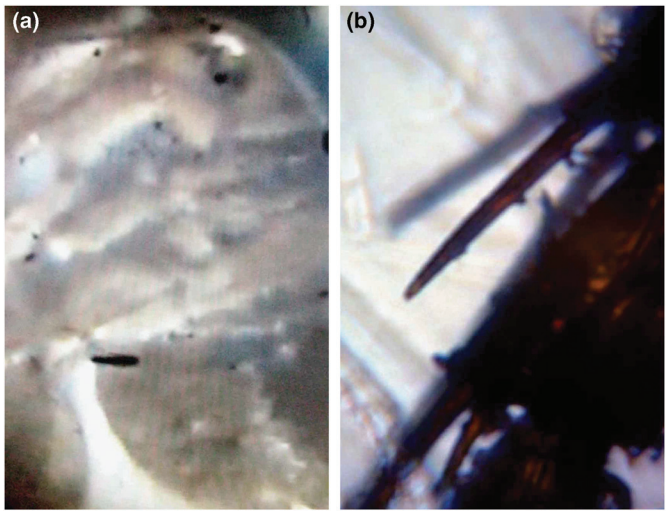


**Figure 4:** (a) In a television documentary, *The Age of Revolution*, this image of the flea *Pulex* was presented to show what Robert Hooke had observed. This video sequence was taken by the BBC through a Hooke microscope made in London by Christopher Cock around 1665. The results are disappointing because of technical problems and the limitations of a primitive compound microscope. (b) This frame from our videomicrograph of a flea was taken using a single-lens microscope of the kind used in the seventeenth century. The lens, magnifying 200×, is here focused on a single chitinous hair on the flea's forelimb (see Figure 3). The resolution is impressive, and the single lens provides evidence of each feature included by Hooke in his engraved image.

of Hooke's masterwork. He found them inconvenient: "These," he wrote, "are very troublesome to be used … but make some objects more distinct [4]." As the present research has shown, these small lenses offer a short working distance, which results in far higher magnification and breathtaking resolution for so diminutive an instrument. The absence of other optical components in the light path minimizes the aberration levels inherent in Hooke's compound microscope.

One widespread misstatement is that these single lenses were beads of glass. Most of the traditional accounts say so, but Hooke's description shows that this was not the case. His technique begins by melting glass, certainly, but the resulting droplet is then ground with jeweler's abrasive to provide a plano-convex lens used with the plane face next to the specimen. Leeuwenhoek made his lenses in exactly this way, and he also wrote of occasions when he tried "blowing" lenses, which seems impossible [5]; however, my late colleague Dr. Jan van Zuylen of Zeist in the Netherlands solved the puzzle. If you seal the end of a glass tube in the flame and then blow hard, a large fragile bubble is the result. At the termination of this balloon-like structure the glass is thicker and this small pellet, asymmetrical in contour, can be separated from the surrounding thin shards of glass and used as a lens. The resulting field of view is flat across the center, and this unusual kind of magnifier is found in the Utrecht microscope. We have attempted to create models of the process but found it exceedingly difficult to do successfully. Truly, Leeuwenhoek was a superb craftsman and a great innovator, far from the "dilettante" as he has often been described.

## Capturing the Recreation of Experiments Using Video

Using video, it has now been possible to recreate a range of these original experiments. Fittingly, the first results of this research were revealed in a presentation in London on October 29, 2010, at the Royal Society, where Robert Hooke was the demonstrator in 1665 and which elected Antony van Leeuwenhoek a member on February 26, 1680. A full range of the results will be presented for the first time in a plenary lecture of Microscopy & Microanalysis, the annual meeting of the Microscopy Society of America, on August 4, 2014.

Textbooks still describe the early microscopes as inferior, their images being chromatic and of poor quality; television reconstructions have recently perpetrated the myth. We can now put these ill-informed accounts to rest. The skill and diligence of the pioneer microscopists deserves our respect and highest admiration.

## Discussion

Diligent attention to detail allows one to optimize the results obtained with minute single lenses. The differences between the popular previous images and those one actually can obtain are marked: an unsuccessful attempt to capture images of blood cells using the Leeuwenhoek microscope in Utrecht can be compared with a micrograph in which the cells are vividly resolved (Figure 1). Similarly, it was found that Robert Brown's observations of the cell nucleus had been poorly represented on television, whereas the same lens (and identical specimen material) is capable of resolving cytological details in orchid epidermis (Figure 2). Most interesting were observations of the flea *Pulex* through Hooke's compound microscope, for the detail he included in his engraving is incapable of resolution with this instrument. Studies of the same specimen with a single-lens microscope,

by contrast, allows us to examine single hairs in detail (Figure 4b).

This research allows us to conclude that the conventional portrayals of pioneering microscopy are grossly misleading. Devotion to detail can produce fine images from the same lenses, which demonstrates to us the degree of skill and intelligence that was brought to bear on nature by the microscopists who founded our discipline.

## Conclusion

Far from offering poorly resolved and indistinct views of the microscopic realm, the original handmade early microscopes provided a clarity that is comparable with what we would expect today. Our video reprise of the crucial experiments that laid the groundwork for our modern understanding of the microscopical realm allows us all to appreciate the results of their skill and to marvel at the meticulous investigation that set in train today's era of microscopical biology.

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