THE MICROSCOPE
Vol. 71:2, pp. 84–95, 2024
https://doi.org/10.59082/KQVK8045

CRITICAL FOCUS

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What Could the First Microscopists See?

We all remember key dates. Top of the list? It's July 4, 1776, when the Declaration of Independence was signed so the U.S. became a nation in its own right and set the time for a huge annual party of fun, feasting, and fireworks. Here's another for you: September 7, 1674. It's the day science met the microbe. On that date, Antony van Leeuwenhoek, the draper of Delft, penned a letter to London. He told how he'd recently returned from a trip by boat across a nearby lake and had collected a small glass bottle of the greenish growths that he saw in the water. Leeuwenhoek had been experimenting with home-made single-lensed microscopes and had taken a close look at his lakewater samples. What he saw revolutionized the world of science, for he was astonished to see a myriad tiny microbes swimming about. In that instant, our modern era of bioscience was born. Yet (to quote cell biologist Nick Lane in a recent video for the Royal Society) scientists still do not know what Leeuwenhoek saw. After reading this article, they will.

Much of what we are told about Leeuwenhoek is wrong. He is often described as the person who invented the microscope, though there were many investigators who had used lenses to magnify before his time. In the Netherlands we find Hans Lippershey, born in Germany in 1570, who emigrated to Middelburg where he set up business as a maker of eyeglasses. He took out a patent for a telescope in 1608, narrowly beating Jacob Metius of Alkmaar who applied for his own patent just three weeks later. But I have a copy of a book by an English military engineer, Thomas Digges, published in 1571, which reveals that telescopes were in use earlier than the reference works claim. Just read his words:

"Ye may by application of Glasses in due proportion cause any peculaire house, or roume thereof dilate and shew it selfe in as ample forme as the whole town first appeared, so that ye shall discerne any trifle, or reade any letter lying there open, especially if the sunne beames may come unto it, as plainly as if you were corporally present."

That describes a telescope some forty years earlier. A compound microscope is a telescope used backwards, as Galileo Galilei knew. He adapted his telescope for viewing minute objects, calling it an occhiolino or 'little eye', which he used for studies of insect anatomy. In 1625, Giovanni Faber coined the term 'microscope' based on Galileo's investigations. Websites and articles around the world say there is a microscope of that period in the collections of the Zeeuws Museum in Middelburg – but this curious artifact isn't a microscope. Scholars think it is, but it is actually a telescope viewing tube dated around 1680. Among the earliest to manufacture magnifiers specifically for microscopy were Hans Janssen and his son Zacharias of Middelburg, Netherlands, who are said to have invented a compound microscope around 1595. If so, microscopes were born almost 40 years before Leeuwenhoek. No, he was not their inventor.

Neither was Leeuwenhoek the first to envisage microbes. There had been ideas of sub-visible life forms existing long before they were discovered. Jainism, one

of the religions of the Indian subcontinent, taught of clusters of invisible entities they called nigodas some 2,500 years ago. In ancient Rome over 2,000 years ago, Marcus Terentius Varro – a scholar and agriculturist – speculated on invisible creatures that could be inhaled to cause disease, and in 1520 the Italian philosopher Girolamo Fracastoro coined the term 'seminaria' to denote sub-visible infectious agents that could spread epidemics. Leeuwenhoek first discovered bacteria in 1683 and has been criticized for not associating them with infectious disease, but I side with Leeuwenhoek. He saw the microbe world as teeming with minute life forms that were of scholarly interest. We know that only a small proportion of microbes cause disease, and Leeuwenhoek was right to regard the bulk of them as harmless fellow-travelers and objects of intense curiosity.

Leeuwenhoek was far from being the first to record the world beneath the microscope. There are close-up images of small creatures (like the honeybee *Apis mellifera*) among the relics of Ancient Egypt dating back 5,000 years. This was the symbol of Lower Egypt, and a honeybee I have scrutinized, carved in the chapel of Senusret I at Karnak some 4,000 years ago, is magnified some 25× and is the oldest magnified image in the world that embodies fine detail. It's incorrect, of course (there were no lenses available, other than those of the human eye) but deserves its place in the history of science. A Chinese Scholar, Han Yin, wrote that "Blossoms of snow, which are called ying, are always 6 pointed" 2,150 years ago, but the first magnified view of a snowflake that I have unearthed dates from Olaus Magnus, a Swedish historian, in 1555. (see "The Hidden Secrets of Snowflakes," *The Microscope* Vol. 62:4, pp. 171–181, 2014).

The Italian physician Marcello Malpighi used a microscope to delineate fine structure and there are minuscule features he recorded in the kidney and within insects that still bear his name today. Malpighi discovered fine blood capillaries in 1661 and, later, he made detailed studies of nerves. And of course, Robert Hooke in London published his great work *Micrographia* in 1665, and I have shown that it was Hooke's book that was to inform Leeuwenhoek and trigger the Dutchman's continuing devotion to microscopy. In 1668, the Italian experimenter Francesco Redi published some views of insects using a microscope, while the Dutch investigator Jan Swammerdam published detailed studies of microscopical structures in insects ranging from bees to lice, and also of the frog, in 1669. Christian Huygens carried out his own investigations into microscopes around 1670, and in 1677 he met Nicholas Hartsoeker, a prominent Dutch mathematician, who showed him how images could be greatly improved if ground lenses, rather than melted beads of glass, were used as magnifiers. None of these people studied microbial life as Leeuwenhoek had so painstakingly done, and when Nehemiah Grew published his well-illustrated book *The Anatomy of Plants Begun* in 1682, his images were stylized, cells being given a regular geometrical alignment that they do not have in nature, whereas the sketches from Leeuwenhoek's letters bear a closer resemblance to modern-day photomicrographs.

Everyone who knows of the man agrees that Leeuwenhoek was the 'father of microbiology'. I have said so myself. It is convenient shorthand – though it is only part of the truth. In fifty years of devotion to microscopical study, he documented a vast range of living organisms, ranging from chunky little rotifers to bacteria. There is no doubt that Leeuwenhoek was the first microbiologist. But, to be the 'father of' a discipline, you must have followers, and Leeuwenhoek did not. As I have also written in the past, when he passed, microbiology died with him. Robert Hooke and others replicated some of Leeuwenhoek's demonstrations of microbial life, but nobody took up the cause and advanced it.

The next microscopical investigator of note was the German naturalist Abraham Trembley, who published a great work on his experiments with *Hydra* in 1744. Unlike Leeuwenhoek, Trembley carried out extensive experiments, studying the eversion of the organism, its recovery from resection, and a host of similar demonstrations. *Hydra* had first been illustrated in Leeuwenhoek's momentous letter of 1702, and many have assumed that Trembley's work extended Leeuwenhoek's original discovery. It didn't: when Trembley first observed *Hydra* he believed that he was the first person to describe it. His work was independent, and owed nothing whatever to Leeuwenhoek's original research.

There were few others. John Needham, the first Roman Catholic to be elected a fellow of the Royal Society, claimed to observe 'microscopic eels' in samples from diseased wheat in 1745. The leading French naturalist of his generation, Georges-Louis le Clerck, Compte de Buffon, compiled a vast encyclopedia on all forms of life, published between 1749-1804, and observed microscopic life. He pioneered evolutionary theory, though did little to advance the study of the microbe. The Italian priest Lazzaro Spallanzani investigated spontaneous generation of bacteria, and published his refutation of the idea in 1765, though did

The ancient Egyptian temple at Karnak is replete with carved hieroglyphs and even enlarged portrayals of insects. These remarkable images were engraved nearly 4,000 years ago, and are the oldest known portrayals of magnified organisms.

This detailed relief of the bee *Apis mellifera* from the White Chapel of Senusret I at Karnak attempts to embody anatomical detail. Although the details are incorrect, they remind us of the inquisitiveness about tiny structures in ancient times.

nothing to increase his (or our) understanding of what they were.

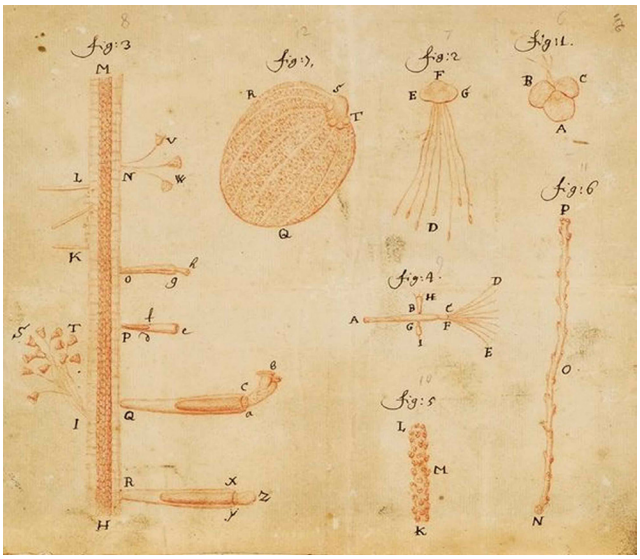
Protozoa and microscopic algae were largely ignored by academics after Leeuwenhoek's time until Christian Ehrenberg, a German biologist at the University of Berlin, turned his attention to them in 1830. Everything from *Euglena* to *Paramecium* came beneath his lens and when he died he bequeathed to the university over 40,000 microscope preparations, a truly astonishing total. Few showed interest in bacteria, until Ferdinand Cohn began classifying them in 1870. Cohn, a child prodigy who could read at aged 2 and gained his Ph.D. when only 19, recognized the four major morphological types (spheres and rods, threads and spirals) and – as Ehrenberg had done for protists – Cohn named so many of the types we know today. Leeuwenhoek was largely forgotten, until the biography by P. J. Haaxman of 1875. His great book was never translated into English, and I owe great gratitude to two eminent current-day microscopists, Peter Zoon and Debbie Stokes, who prepared for me meticulous translations of the full text. Haaxman's book meant that the name of Leeuwenhoek was recorded in the annals of formal scholarship. It is a great work.

Erroneous claims continue to be made about Leeuwenhoek to this day. It is said that he discovered blood cells in 1674, but in 1656 Pierre Borel, physician to King Louis XIV of France, had used his microscope to describe a type of 'worm' in human blood. Two years' later in Germany, a Jesuit priest Athanasius Kircher became the first person to record microscopical organisms when he claimed to have found 'worms' in blood from victims of the plague. Blood cells were first recorded by Malpighi in 1666. In his treatise, *De polypo cordis*, he initially imagined them to be globules of fat, though his description of rounded erythrocytes is unmistakable. When Leeuwenhoek first wrote of blood cells and illustrated them, he clearly showed them and even recognized the nucleated erythrocytes of fish. But how could he do this, with just a single lens?

To envisage what Leeuwenhoek had seen, I used a session with the single-lens Utrecht microscope attributed to him to capture micrographs of my own blood. This instrument is in the collection of the Museum of Science at Utrecht University, Netherlands, and magnifies some 266× so it represents the best kind of simple microscope available to the earliest pioneers. The micrograph shows the remarkably flat field of view created by this blown lens, and the erythrocytes are instantly recognizable. Note too, a leukocyte in the top right of this photomicrograph where we can even glimpse the lobed nucleus. This image has been plagiarized over the years, and features in several reference books.

Capturing an image of this sort is technically demanding; this demonstration has not been successfully repeated, though Brian Bracegirdle used the same microscope to image a blood smear and published results that remind us of the challenge. In his photomicrograph, no erythrocytes can be resolved. Leeuwenhoek was not only a pioneer, but also an extraordinarily gifted experimenter. To see what he saw (and faithfully recorded) required diligence, precision, and unerring objectivity. Leeuwenhoek deserves our highest respect for his groundbreaking work.

By the time Leeuwenhoek arrived on the scientific scene, microscopy was already an accepted branch of investigation. What singles him out is his meticulous



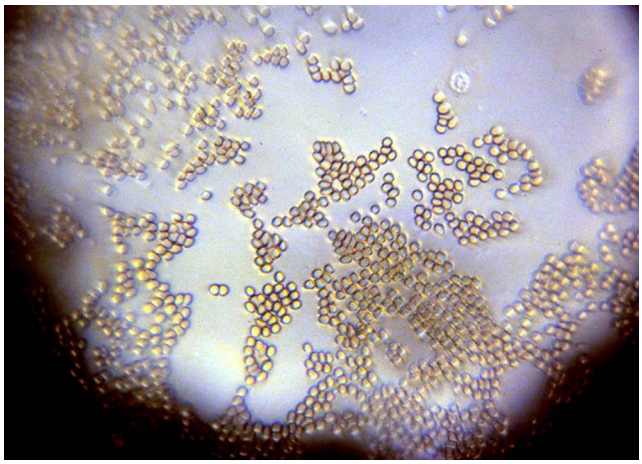
Lemna, the duckweed, is shown in this drawing from a Leeuwenhoek letter dated December 25, 1702. It shows *Vorticella* (fig 3 S), diatoms (fig 3 K) and sessile rotifers (fig 3 R) with a budding *Hydra* (fig 4). These sketches are as good as modern students might prepare today.

Clifford Dobell was uniquely gifted, and was the only microbiologist who could successfully culture enteric amebae. He lived and worked in London, and his widow Monica told me that he spent much of his spare time researching and documenting Leeuwenhoek's life.

microscopy, coupled with his clear and unambiguous descriptions of what he discovered. Yes, while the works of the great names in science were widely disseminated – Galileo and Newton, Hooke and Malpighi – Leeuwenhoek was largely eclipsed. The only comprehensive information on Leeuwenhoek for many decades was a biography by P.J. Haaxman, a distant descendant of Leeuwenhoek, which was published at Leiden in 1875. It has only ever been available in Dutch. Science could easily have forgotten about Leeuwenhoek's momentous revelation, had it not been for Clifford Dobell's biography. He was a brilliant British protozoologist who set out to expand Haaxman's work and publish a fuller, scholarly account of Leeuwenhoek's life and work. The result was a large, 435-page opus, *Antony van Leeuwenhoek and his Little Animals*, published in 1932 to celebrate the tercentenary of his birth. I knew several scientists who were colleagues of Dobell's; one of them, Lord Perry of Walton, described Dobell as a "crusty old bugger, very brilliant mind, but so difficult to get on with." Dobell died in 1949 though I came to know his widow, Monica, who used to invite me to the family home and reminisce. I have long owned a copy of Dobell's original biography, which had been originally purchased by R.S. Creed, an eminent medical man and a gifted lecturer who wrote a classic of neurology, *Reflex Activity of the Spinal Cord*. Turning the pages of Dobell's masterful book was so very enlightening, and I loved reading those pages. I still do.

I was invited by the Royal Society to write an article commemorating the centenary of Dobell's birth in 1886, and his widow Monica was delighted to tell me about his life. "His name was never Clifford," she told me. "He is really called Cecil, but he hated the name so told everyone to call him Clifford instead." Monica Dobell was dynamic company, an amusing conversationalist, and passed on to me copies of her husband's books on parasitic protozoa. "I want them to be in good hands," she explained as she signed them. When my article was published, she had a greater surprise for me (see: "The Leeuwenhoekiana of Clifford Dobell, *Notes and Records of Royal Society*," Vol. 41:1, pp. 95-105, 1986). She had shown me the author's original copy of the book on Leeuwenhoek, and now she told me to accept it as a personal memento. It was an unforgettable moment; that book is by me as I write.

Inside the front cover, Dobell has written the details of the publication. "Published (London) 14 Sept 1932 (1,000 copies printed). Issued in Holland by Swets and Zeitlinger (20 Oct 1932) Amsterdam (250 copies). (10 Oct 1932) in the USA by Harcourt, Brace & Co., N.Y. (750 copies)". It gets better – Dobell has marked a number of comments, sometimes pasting in clippings of important supplementary information. He has marked corrections too. One featured illustration is captioned as being 'The Shore at Scheveningen' but Dobell writes that Dr. van Seters has shown that it is not of Scheveningen at all, but shows Egmond aan Zee, some 50 miles north. Tucked inside the back cover of the book is van Seters' obituary of Dobell. It is an affectionate tribute.



Red blood cells, erythrocytes, predominate in this photomicrograph that I took through the single-lensed microscope in Utrecht, attributed to Leeuwenhoek. Once the slight color balance was corrected with Photoshop, the minor extent of chromatic aberration can be observed.

P. J. Haaxman was related to Leeuwenhoek, whose niece Maria Jans de Molijn had married Cornelis Haaxman in 1674. P.J. Haaxman was an apothecary of Rotterdam and in 1875 he published *Antony van Leeuwenhoek: De Ontdekker der Infusorien*, the first biography.

The book became the standard work on Leeuwenhoek and demand continued, so Dover Publications reissued it in 1960. When my book *The Leeuwenhoek Legacy* was published in 1991, I wrote that I saw it as a supplement to Dobell's great work. Since becoming interested in Leeuwenhoek in 1906, Dobell had taken pains to learn Early Modern Dutch so that he could translate many of Leeuwenhoek's letters in full, and the engraved version of the illustrations for the 1702 letter is one he decided to include. It was the first time a wider public had sight of what Leeuwenhoek had seen. Dobell was also first to translate several of Leeuwenhoek's most important letters.

Leeuwenhoek's correspondence remains in the same bound volumes where I have studied them too. Translating them all by the Dutch began in 1931 – and they are still translating them to this day. The first volume appeared in 1939, and the latest is volume 20, which covers the letters written up to 1716. It is taking twice as long to translate the research as it took Leeuwenhoek to do it. They're published in large scholarly volumes and costly. Most are out of print anyway, and those that can be found online cost around $300.

Most exciting of all, are Leeuwenhoek's vivid descriptions of microscopical life. He wrote how he first saw filaments of what we now know to be algae; and then, swimming in and out as if exploring, he observed minute animals with what seemed to be legs, and with two little fins at the rear. The two posterior projections give us the identification that we need. They were rotifers, organisms comprised of about 1,000 cells. Leeuwenhoek also saw occasional elongated, slower-moving organisms; these would have been single-celled ciliates, like *Paramecium*. We can match his written descriptions to what we now recognize, but how did those pioneering drawings represent reality? Many people have set out to reproduce the microscopical demonstrations of the first microscopists, though it has repeatedly proved to be more demanding than expected, and nobody has managed to reconcile the surviving drawings with what we can ourselves observe. In recent years, I have returned to those early decades of microscopy, and Photoshop allows us to align discrete images so that modern micrographs of microbes can be precisely positioned to recreate what the pioneers saw for themselves.

On the day in 1674 when science met the microbe, Leeuwenhoek immediately realized that these swimming entities were tiny animals and he wrote of them as dierkens. 'Dier' is the Dutch for animal, and 'kens' is the diminutive so, in an attempt to translate the word meaningfully (it has no exact counterpart in English) Henry Oldenburg, secretary of the Royal Society and the person to whom the letter was sent, decided to call them 'animalcules'. It is a fine and scholarly choice – though it is a word rarely used then, and never used now. In my view, it's better to simply translate them as microbes. Although the day microbes were first recorded is a momentous date for science, you won't find a single soul who knows it. Well, I do (though I just looked it up to check).

Why do I say that date important? It is because Leeuwenhoek's discovery was one of the greatest ever in the history of the world. Sure, the excavation of dinosaurs gave us incredible stories, but the ancients had found their gigantic bones thousands of years earlier, which is where the legend of dragons was born. There was nothing new about gigantic reptiles – reptiles were old hat. Scientists love to claim that Charles Darwin's

theory of evolution was the greatest of them all, though as I have shown, the theory had been recognized far earlier, and survival of the fittest is only one of a long list of factors governing biological evolution (see "Darwin, the Microscopist who Didn't Discover Evolution," *The Microscope,* Vol. 59:3, pp. 129-137, 2011). Electricity? We reviewed some of the great names in my last column, and harnessing its power was a tremendous step forward – but the ancients knew about static, and lighting was nothing new; so its existence wasn't a discovery. Galileo and his telescope showed us the rings around Saturn and the moons of Jupiter, but the planets had been studied for a thousand years and even pigeons know there are stars. Our understanding of the universe does nothing to change the way we live our lives; it was the microscope that did that.

Discovering a universe of tiny organisms, that nobody knew were there, was to revolutionize science. Our entire modern civilization is founded on an understanding of the microscopical world, yet we do not hear much of it in school, we see very little on TV, and hardly anybody out in the street would know a microbe. Over fifty years ago, I wrote a leading article for Nature in which I lamented the lack of public awareness of the microbe world (see "Microscopic Blind Spots," *Nature* Vol. 258, p. 469, 1975. https://www.nature.com/articles/258469a0) and I have encouraged broadcasters to embrace this untapped well of knowledge with little success. Indeed, it is 35 years since I last made a TV program in which I showed what wonders viewers might see through an everyday microscope at home (see tellymonitor. (2010, Sep 2). *Carol Vorderman Interviews Brian J. Ford* [Video file]. Retrieved from https://www.youtube.com/watch?v=LhGeApsKjas).

So, what did Leeuwenhoek really see? And how does his view stack up with modern science? To address this novel program of investigation we can abandon our customary preoccupations with magnification, numerical aperture, refractive index, and resolution. I have explained elsewhere how the earliest microscopists had no such preoccupations, and nor should we (see: "Did Physics Matter to the Pioneers of Microscopy?" *Advances in Imaging and Electron Physics* Vol. 158, pp. 27-87, New York: Academic Press, 2009). All that matters is how a specimen appears. Leeuwenhoek and his fellow pioneers were not looking at familiar subjects with conveniently conventional names. These were unique individuals observing living organisms and strange structures that nobody in the world had ever seen before. Everything was unprecedented, and it was all dazzlingly unfamiliar. None of them was concerned with convention, for all that mattered was seeing something. Anything. It mattered not what system of lighting was used, so long as you could see your specimen. How you embodied its characteristics in a record of what you saw was perplexing.

Leeuwenhoek wrote wonderfully discursive and flowery descriptions, often so good that we can tell what he was seeing from his written account. He also bequeathed visual records. We have our first evidence of his ventures into the microbial world in a letter that he wrote on December 25, 1702. While everyone else in Delft was celebrating Christmas, Leeuwenhoek was busily composing a letter to the Royal Society, describing what he'd observed. When I looked at the original letter, preserved to this day in London, I was captivated by turning the page and viewing the drawing he had attached to the final folio. It was captured in the red crayon (since the pencil was yet to be invented) and shows a specimen of the pondweed *Lemna minor*. Exquisite portrayals of microbes are seen, attached to the rootlets and delicately sketched by the limner whom Leeuwenhoek brought in to make his drawings. In the faint outlines, we see precisely which organisms he was studying.

For over fifty years, I have been exploring ways of reprising these earliest experiments and my journey of discovery has been widely published. As early as 1971, I wrote of my first stumbling steps (see: "Recreating the Pioneer Microscopists' View," *New Scientist*, Vol. 51:763, pp. 324-325, August 5, 1971, https://www.brianjford.com/a71-NSc_avl.pdf). When the Royal Society published some of my later experiments, *New Scientist* prominently featured them and they can be viewed online (see: "Leeuwenhoek's animalcules just as he saw them 340 years ago," https://www.newscientist.com/article/dn27563).

Just one question remained – how did Leeuwenhoek's view compare with real life? I resolved to compile a series of correlated images to reveal how accurate was the drawing, how meticulous were the engraved copies that were published in the Society's journal, how closely they compare with the same specimens viewed through a single-lens microscope (similar to what Leeuwenhoek used) and – most revealing of all – how the modern microscopist would view the same specimen. Many of my modern images were captured with the light microscope, others with scanning electron microscopes at Cardiff University and at the JEOL laboratories in London. Since microbes are given to change their orientation to suit the conditions of the time, it's not possible to find organisms that lie in precisely the same position as those drawn in the seventeenth century, though Photoshop can allow us to alter

the position of cells in a micrograph so that they more closely reprise the original representation. Not only has this never been done before, but nobody has even attempted this complex procedure.

As a result, we can match the pioneers' observations with observable reality for the first time in the history of science. The results are uniquely revealing, and the audience at the Inter/Micro 2024 conference in Chicago greeted the first disclosures with enthusiasm. The largest organism on the *Lemna* root in Leeuwenhoek's letter is a rotifer. These intriguing little creatures are eutelic – that is to say, every member of a given species has the same number of cells in the adult body, and their organs also are comprised of specific numbers of cells. Very few organisms exhibit this property, though small nematodes (like the popular experimental organism *Caenorhabditis elegans*) are eutelic, as are tardigrades and the Gastrotrichs. Leeuwenhoek supervised the sketches, so we can be sure that they represent what he observed.

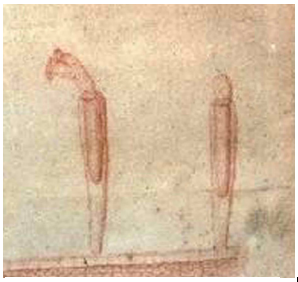
So now I set out on my unprecedented project. I would seek specimens that closely matched those figured by the earliest microscopists and would use Photoshop to adjust them carefully into positions that matched the original portrayal. This offers two benefits: first, we can directly compare the ancient and modern images, and secondly, it becomes possible to create a movie in which we can successively dissolve from one image to the next, seamlessly sliding through the centuries and watching how our present-day knowledge emerged from the studies of the past. We can derive a unique insight into the birth of microscopy, and can at last answer the essential question that had long bedeviled the history of science: just what did the microscope pioneers observe? And how accurate were their representations of the microscopical realities we know today?

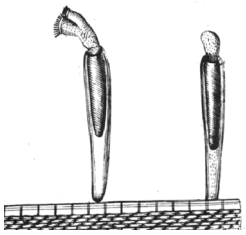
We will begin with the largest of the microscopical organisms in the diagram from Leeuwenhoek's letter of 1702. The microbe that Leeuwenhoek had portrayed is clearly a sessile bdelloid rotifer though we would need detailed microscopy at high magnification to make a precise identification. I have several as permanent preparations, though my Canadian colleague Robert Berdan has captured a video of a living representative of the group. We can adjust the precise positioning of the individual rotifers with Photoshop, in order to match the modern image with its predecessor, and the results reveal how Leeuwenhoek's meticulous observations are well matched to their contemporary counterpart. For decades I have compared the pioneering microscopists' studies with present-day images, obtained with single lenses, or with modern microscopes, sometimes using the scanning electron microscope, and often in comparison with the engravings that appeared in journals or alternative versions published by later investigators. Now I would ensure that the positioning of specimens made by disparate means were a match. The closely correlated images would offer an unprecedented insight into the accuracy of the earliest microscopical investigations.

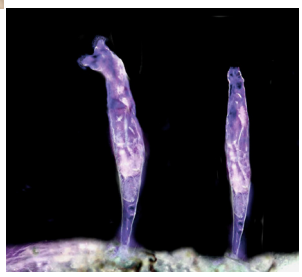
The drawing sent to London by Leeuwenhoek was engraved for publication by the Royal Society (see: *Philosophical Transactions* Vol. 23, pp. 1304–1311, 1702) and the engraving makes an interesting comparison. Every detail was meticulously transcribed. The faint sketch in red crayon has subtleties of line that only an observer would convey, while the engraving was made with a steel needle on a polished copper plate, producing a crisp, sharp image. These engravers copied the originals with meticulous care, each proportion being flawlessly captured. I can only assume that a simple *camera lucida* was always used. The printed picture was always a mirror image of the original, since the engraved plate, once inked, was inverted for printing and the image was thus transposed. So, to illustrate the comparison between original and printed page, I have horizontally flipped the printed pictures so that they match the originals.

There was lettering on the original drawing, which was included on the engraved plate. These letters indicated details enumerated in the text but they are not relevant when we are comparing the pictures with reality and so they have been removed. We don't want unnecessary clutter when interpreting a microscopical image. Set side by side, we can immediately see how the original fading drawing has been faithfully interpreted by the engraver. The printing process not only made the Leeuwenhoek images available to countless thousands of scholars, but conferred immortality upon them.

But how do these images relate to the appearance of living specimens? Berdan's videomicrograph was of a sessile bdelloid rotifer, possibly *Otostephanos*, attached to the cuticle of a larval midge fly *Chaoborus*. I have provisionally identified the example shown in the drawing that Leeuwenhoek had made as the flosculariidan rotifer *Limnias ceratophylli* (see: "The Rotifera of Antony van Leeuwenhoek," *Microscopy* Vol. 34:5, pp. 362-373, https://www.brianjford.com/a-avl-rotifers.pdf) though not being a specialist on rotifers, I may be wrong. In any event, the video shows a rotifer that is reminiscent of the organism in the drawing from 1702, and it is sufficiently similar to make an informed







Top: Leeuwenhoek sent images of sessile rotifers with his letter to London of 1702, and we might identify these as *Otostephanos*. Each can extend to some 250µm, and they spend time exploring their surroundings and maintaining a vortex from which they select suitable morsels of food. **Bottom left**: The images were published in *Philosophical Transactions of the Royal Society* in 1703, and attracted much interest among the fellows. Engravers were meticulous, and we can speculate that they used a *camera lucida* to ensure the proportions were accurately maintained. **Bottom right**: But how did the image compare with reality? Canadian microscopist Robert Berdan has captured vivid images of these organisms, and I utilised Photoshop to reposition them to match those observed by Leeuwenhoek. The published drawing did justice to reality.

comparison. A single frame can be selected from the video and two rotifers, comparable to those observed by Leeuwenhoek, can be identified. Photoshop allows us to orientate the organisms and rescale them so that they match the original images, and at last we can compare the eighteenth-century image with living rotifers as a present-day microscopist would view them. For centuries, scholars have speculated on how the earliest recorded images compare with the organisms they were intended to portray, and at last we can gain an insight into how faithfully Leeuwenhoek interpreted reality. The man was a master. Yet to the public he does not exist.

Tucked away on the edge of the page is a diminutive sketch of a clutch of curious creatures suspended on stalks. Every biologist will identify them as *Vorticella*, ciliates that graze on bacteria and microscopic algae and among the commonest of pond organisms. The drawing is delicate and perfectly portrays the proportions of these microbes. The engraver for the Royal Society captured the essence of the drawing with precision. The outline of the bell-shaped cells is carefully conveyed in the original drawing, and the printed version does justice to the original. There is no mistaking that this is *Vorticella*. Other sources have often said that it might be *Carchesium*, but this is a commonly made mistake. The contractile stems of *Carchesium* always branch; those of *Vorticella* are single strands connecting cell to substrate. What Leeuwenhoek studied was *Vorticella*.

Can we see this single-celled organism with a single lens? It is a microbe that is well matched to the simple microscope. I first took photomicrographs of this ciliate with a single-lensed (i.e. simple) microscope over forty years ago. The instrument that I used had an exquisite lens magnifying 395× ground from spinel ($MgAl_2O_4$) by the late Horace Dall of Luton, England, and set in a small brass mount. Spinel has a refractive index around 1.718 - 1.726, roughly the same as lead glass, and higher than soda-lime glass at 1.51 - 1.52. It has an Abbe number around 75, compared to soda-lime glass around 59. Regular glass is reckoned to show moderate dispersion, whereas spinel is rated as low; thus, there is reduced chromatic aberration. The image we obtain shows a simple microscope at the pinnacle of perfection and generates images that exceed the quality that Leeuwenhoek could have achieved. So it can show us the best that an optimized simple microscope can do, but cannot be taken to recreate what Leeuwenhoek would have known.

My research during the past twenty years has centered on single lenses ground for me from regular soda-lime glass by my colleague Esmond Reid of Cambridge. I have captured numerous images of living microorganisms, botanical and zoological histological preparations, aquatic life – including algae and pond microbes – and living human cells, from erythrocytes to spermatozoa. From time to time I have encountered attempts by others to emulate this research, and have thus acquired a unique library of images showing how to successfully recreate the experiments of the pioneers of microscopy (along with some remarkable failed attempts by an assortment of contemporary investigators).

Photomicrographs I have managed to take with single lenses substantiate the ease with which *Vorticella* could be studied in the seventeenth and eighteenth centuries. We can observe the beauty of the cell, the beating ring of cilia encircling the peristome, and the delicate tracery of the spirally contractile stalks that securely anchor each cell. The oral groove can easily be studied, with the food vacuoles and other cytoplasmic inclusions clearly visible. And here we have evidence of the kind of microscope that Leeuwenhoek must have used for these pioneering observations. This is not because of what we can now observe, but because of what Leeuwenhoek could not. Look again at the picture: although there are cell inclusions visible, there is no indication of the ring of cilia, and only a hint of the

oral groove. This drawing was not made with a higher-powered lens, but only with a low-powered instrument. Vacuoles were shown in the drawing. They vary in size but most measure some 4 μm in diameter. The stalks are typically 2 μm in thickness. The cilia are far smaller; measuring <1 μm and are not resolved. Leeuwenhoek could resolve each end of the ciliary crown, which he wrote of as 'horns', but could not perceive the cilia themselves, only the 'bustle' they created in the water.

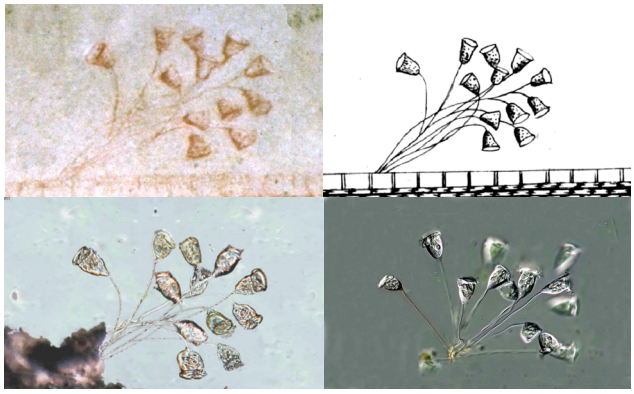
A magnification around 50× is sufficient to observe the behavior of *Vorticella*, and Leeuwenhoek describes this beautifully. In a letter dated June 28, 1713, he mentions his studies from the previous August, when he was once again studying *Vorticella*. His description is delicious:

"When we observe the microbes that are fixed by a long tail to something or other, like many that we have discovered on the little roots of duckweed, we see that they don't merely go round in a circle with the extreme part of their body (whereby they make, in proportion to the littleness of their body, a big bustle in the water); but the creatures can also pull their tails together, and that very quick too, so that when they stick their tails out again, they displace the water round about them and, being thus come into different water, they can get fresh food out of it."

He described the spiral stalk of *Vorticella* in vernacular terms that are utterly unambiguous:

"Their whole body lept back towards the globul of the tayl, which then rolled together Serpent-like, and after the manner of Copper or Iron wire that, having been wound about a stick, and unwound again, retains those windings and turnings …"

It's a vivid description that captures the essence of the organism, though says nothing of what goes on inside the cell. Leeuwenhoek is using a low-power lens. Several of his surviving microscopes are capable only of modest magnifications. Of those attributed to him in the collections of the Boerhaave Museum of Leiden, Netherlands, two magnify a mere 50×, and one only 35×. These are powerful enough to show *Vorticella* with the degree of detail visible in the drawing, but they lack the resolution necessary to reveal the finer details. Thus, we can surmise what kind of microscope might have been used in completing the drawing of December 25, 1702. In terms of magnification, it was nothing special. Bearing in mind that Leeuwenhoek had been

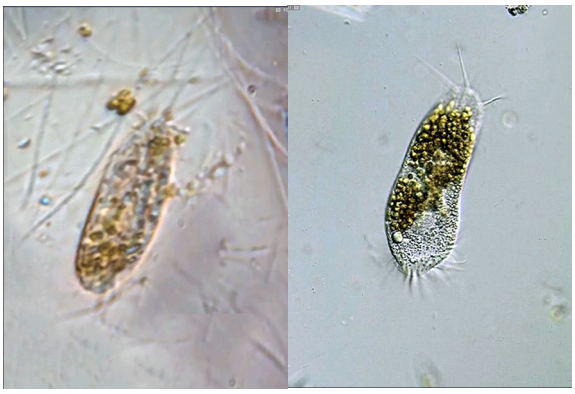


For the first time, we can compare the original studies with single-lens images. **Top left**: Leeuwenhoek's studies of *Vorticella* were sketched by his limner in the margin of the page (almost as if an afterthought) yet it is an unmistakable image, immediately recognizable by seasoned microscopists. This remarkable image underpinned the discovery of the microbial world. **Top right**: The engraved image reproduced in *Philosophical Transactions* in 1703 fully did justice to Leeuwenhoek's discovery. Each of the bell-shaped ciliated cells is meticulously positioned, and the delicate tracery of the living colony is embodied with consummate skill. **Bottom left**: It takes time and diligence to capture discrete images of the kind Leeuwenhoek might have seen, but these are individual *Vorticella* cells observed through a replica, single-lensed microscope. **Bottom right**: Phase-contrast microscopy provides what the modern-day microscopist expects to observe. Comparing this modern image with the appearance of these organisms with a single-lens microscope, and then with the original sketches, is uniquely revealing.

practicing microscopy – and making microscopes – for almost thirty years by that time, it is a surprising revelation that the microscope he had available at that time magnified so little.

I first set the original drawing alongside the engraved version as published, and we can appreciate the diligence and professionalism of the engraver. It is a meticulous copy. Taking single lens micrographs (using soda-lime lenses) and carefully setting them out in positions that approximate to the original drawing provides confirmation of what those simple microscopes could have provided. Finally, a living colony of *Vorticella* photographed using phase-contrast microscopy gives us the present-day view. These compilations consume time and patience, but the resulting comparisons are surely worth it. We can at last visualize what Leeuwenhoek was aspiring to achieve and can easily reconcile his ground-breaking endeavor with what we can ourselves observe today. It is a major step forward for historians of science.

The difficulty in reprising these observations becomes clear when we look at some of the attempts others have made. Some years ago I was invited to submit a proposal to the BBC for some programs about the living cell, and featuring the work of Leeuwenhoek. The



Left: Using a similar replica microscope, the appearance of *Stylonychia*, grazing through pond water, can be satisfactorily observed with a single lens, though the procedure is complex and time-consuming. Leeuwenhoek described this grazing behavior of such ciliates. **Right**: We can compare the view through a replica microscope with this study, kindly sent to me by Jason Oyadomari of Michigan. The cirri, produced from the fusion of cilia, show prominently in this micrograph, though they are barely perceived with the single lens..

The technical complexity of these experiments can be gained from this frame from an experiment broadcast by the BBC. In spite of the six-figure program budget, it proved impossible to discern a ciliate like *Stylonychia* with a replica Leeuwenhoek microscope by Hans Loncke.

suggestion came from Mark Thompson, who was Director-General of the BBC. He was to become President of the *New York Times* and has since been knighted, so is now Sir Mark. Mark thought it was a great idea, and passed it over to a senior television producer named George Entwistle – who promptly went off and made the programs, without another word to me. Entwistle produced a short series of programs, one of which was devoted to reprising the research of Leeuwenhoek. They chose a presenter named Adam Rutherford, who chided the stuffy old chaps in the early years of the Royal Society, heedless of the fact that – at the time – the members were as young as the youthful presenter. The program used a replica Leeuwenhoek microscope made by Hans Loncke in the Netherlands and set it up with a drop of lake-water in an attempt to capture micrographs of aquatic ciliates. Rutherford was duly excited at what was revealed: "That's astonishing … there is a tiny, tiny bug in there which is scooting around." The video showed some strands of chlorophyte algae and the 'bug' was visible only as an indistinct, blurred object that moved around in a corner. "I suppose it's a protozoa (*sic*)," purred Rutherford. It could have been anything, a rotifer perhaps, even the nauplius larva of a water flea, though I'd have thought it was *Stylonychia*. Being so indistinct, it was impossible to be certain and I smiled as I watched, knowing that Leeuwenhoek would have been content to know that the vast BBC budget could not provide images as good as those he'd obtained in the 1600s.

I kept screen grabs from the television program. They showed no more than blurred images, and it was impossible to tell what was what. So I set about capturing micrographs of living *Stylonychia* observed through one of the single lenses made by Reid. The best of them, magnifying some 200×, provided vibrant videos of the living protozoa and I demonstrated the comparison at a lecture that I was invited to present at the Royal Society in 2010. *Stylonychia* had also been photographed by Jason Oyadomari of Finlandia University, and he emailed me a copy. Set alongside the micrograph from the simple microscope (and the image from the BBC) we can clearly see the degree of detail that those early microscopes could create, and also see how unassailably difficult the task was found to be by others less experienced.

All the standard sources insist that, until chromatic aberration was overcome, microscopical images were inferior and degraded. The Whipple Museum for the History of Science of Cambridge University, England, published a view of the flea *Pulex* taken with an eighteen-century microscope, showing chromatic aberration, as an example of the inferior view that the early microscopists were obliged to endure. Browse to the site for the London Science Museum and you will find that they claim: 'Many researchers refused to use the early microscopes because they could not trust what they were seeing. Aberrations and impurities in the lenses caused distortions, which led to errors in observations.' The National Museum of Scotland posts the 'flaws of early microscopes and scientific practice'. Perhaps the worst of all is the BBC, with an authoritative page on their h2g2 site that speaks of the Janssens 'fooling around' with lenses, Galileo as a 'science freak' with his 'gizmos', Hooke a 'wierdo' while Leeuwenhoek invented a 'self-made two-lens microscope'. The proliferation of such inaccurate and misleading accounts emphasizes the need for the history of science to face facts. Few branches of scholarship are as riddled with fiction and trivialization.

Simply looking at these correlated images tells us so much about the origins of microscopy. They also

correct the widespread misrepresentation of Robert Hooke's pioneering investigations. His microscopical research was published in a folio-sized book, *Micrographia*, in 1665. On April 13, 1663, Hooke demonstrated the microscopical structure of cork to the fellows of the recently formed Royal Society. Plate XI of *Micrographia* records his demonstration of phellem from the cork oak, *Quercus suber*, revealing that it was comprised of minute, rectangular box-like structures that he named 'cells'. We use the term to this day, though to describe something entirely different; to us, the single cell is a unit of life, with its nucleus and organelles nestling within cytoplasm. What Hooke saw was the opposite – he was observing the dead, empty thickened walls where cells had once been. There was no life left; the cells he recorded were open spaces where life had long since disappeared.

In any event, a week earlier he had already demonstrated living cells – but didn't realize what he'd discovered. On the afternoon of April 8, Hooke had demonstrated to the Royal Society a specimen of the wall screw moss, *Funaria hygrometrica*, under his microscope. The image he obtained is beautifully engraved as Plate XXI of his magnificent book and I explained in an earlier article how it had revealed tissues comprised of living cells for the first time (see: "The Incredible, Invisible World of Robert Hooke," *The Microscope* Vol. 63:1, pp. 23-34, 2015).

Most sources dismiss the sections of cork that he prepared as 'bits' or crude 'slices' of cork, and the early microscopists are said to merely 'tear' their sections apart. It has been felt that the engraving Hooke published was an idealized version of what he could have observed at the time and there seemed to be no way to disillusion scholars of this erroneous belief until I unearthed Leeuwenhoek's original specimens at the Royal Society in 1981 (see: "The Story of the Leeuwenhoek Specimens," *The Microscope* Vol 59:1, pp. 11-19, 2011). Leeuwenhoek had repeated Hooke's demonstrations, and his cork sections were among those that I discovered. At last we had evidence of what the avid microscopist could achieve in the 1600s. Leeuwenhoek's sections were fine. They were far from the 'bits' of tissue 'torn' from a cork.

So I had cork sections contemporaneous with Robert Hooke and indeed, prepared to mirror his own demonstrations. Under low power microscopy, it was clear that they were of supremely high quality and comparable with what present-day microscopists would aspire to produce. How did the pioneer microscopists view them? I had taken some pictures of cork sections through a microscope of the kind used by Robert Hooke at the Science Museum in London over 50 years ago, and they had proved to be a puzzle, for you could not resolve the detail recorded in Hooke's engraving by looking through this early compound microscope. It proved to me that Hooke could not have used his microscope to make his observations. His grand instrument, made by Christopher Cock in London, was a status symbol and easy to use; but for fine detail he must have resorted to a simple microscope. Indeed, he described how to grind simple lenses and mount them in metal plates, and this was the technique that Leeuwenhoek copied (see: "The Incredible, Invisible World of Robert Hooke," *The Microscope* Vol. 63:1, pp. 23-34, 2015).

To find out exactly what these newly-discovered seventeenth-century cork sections looked like, I took micrographs with the Cambridge Stereoscan scanning electron microscope in the Department of Zoology at Cardiff University, where I was Research Associate, and also with the priceless single-lens Leeuwenhoek microscope at the University Museum in Utrecht. These I supplemented with higher-power video micrographs taken with the lenses made for me by Reid in Cambridge. And so I had the correlated images I needed, and could demonstrate that the engravings that Hooke had published were not refined and idealized. He had portrayed exactly what he observed. Another erroneous claim was laid to rest.

Leeuwenhoek passed away on August 26, 1723, still working with his microscopes. I presented a lecture to commemorate the date aboard the Seabourn cruise ship Sojourn (see tellymonitor. (2023, Sep 21). *Celebrating Leeuwenhoek* [Video file]. Retrieved from https://www.youtube.com/watch?v=SRfxSD6gmPg). It was the only lecture in the world to mark the date. For someone so influential in anticipating modern science it continues to astonish me that so little is generally known about the man, and so many erroneous claims continue to be made about his work.

This article has examined numerous claims about Antony van Leeuwenhoek and the pioneer microscopists. So many of the myths surrounding Leeuwenhoek's work can at last be dispelled. Until my research, we never knew how the first observations were carried out, how precise might be the early methods of specimen preparation, whether the first lenses could reveal the details claimed, how the earliest specimens appeared through the pioneers' microscopes, or how accurate was their portrayal. For me, it has been a marvelous journey of exploration and discovery over many decades, and it resolves the outstanding questions that have long perplexed and confused the historians of sci-

Robert Hooke included this exquisite microscopical study of a leaf of the nettle *Urtica dioica* in his book *Micrographia* of 1665. The engraving, shown here with captions removed, was based on his demonstrations to the Royal Society in the summer of 1663.

Painstaking choice of a specimen, and the judicious use of Photoshop to carefully align the stinging hairs, provides me with a comparable image of how the nettle leaf appears to a modern microscopist. This has never been achieved before.

ence. Just one question remained: what would Robert Hooke make of today's microscopy?

So to conclude, I selected one of Hooke's most vivid illustrations from one of my own copies of the original *Micrographia* – his carefully engraved study of the stinging nettle *Urtica dioica*. It's a familiar specimen. His eye-catching illustration has a three-dimensional quality, almost like a scanning electron micrograph. But what did the specimen look like? How much artifice was involved in transposing Hooke's vision to the printed page? Had he exaggerated details, altered proportions, used artistic license to clarify specific features? How did his engraving, based on observations made during the summer of 1663, compare with what a present-day microscopist would observe?

There is only one way to find out. I collected numerous samples of the fresh nettle leaves and found one in which many of the features mirrored those of the specimen that Hooke had studied. The needle-like stinging hairs were carefully adjusted in appropriate positions on the micrograph using Photoshop. The result is an astonishing comparison that bridges over 360 years of microscopical science. Everyone can now, for the first time in the history of science, compare the view bequeathed to us by Hooke, with what he would have observed in the microscopy laboratory of today. It is a vivid and unforgettable demonstration that, together with the other examples I present, dispels forever the notion of clumsy amateurs, crudely squinting through distorting lenses and sketching what they observed with little need for accuracy.

There are many misrepresentations in print from those early days, sure, published by opportunist plagiarists who lacked the skill and ability to make microscopical observations themselves. Seventeenth-century writers often reproduced the work of earlier pioneers – notably Robert Hooke – and claimed it as their own. Hooke himself even plagiarized Thomas Bartholin (see: "The Cheat and the Microscope - Plagiarism over the Centuries," *The Microscope* Vol. 58:1, pp. 21-32, 2010). In recent years a number of large and prestigious organizations have attempted crude recreations of my experiments, always with inferior results, while claiming their efforts as being done 'for the first time'. Since all my publications are freely available online it's hard to know why they would risk their reputations in the eyes of future scholars. Yet for some reason, microscopy is one area where so many individuals have found it beyond their capacity to obtain the results they desire, so they misappropriate someone else's and pretend it's theirs.

Back in the 1600s, the pioneers were remarkable investigators; diligent, dexterous, and devoted. Their discoveries were epoch-making, and their abilities outshine much of what we find in the laboratories of today. Meanwhile, their work endures - and publications in the annals of science are ultimately all that matters. It's a lesson that some still have to learn. ■