

The search for authenticity – leeuwenhoek microscopes under the SEM

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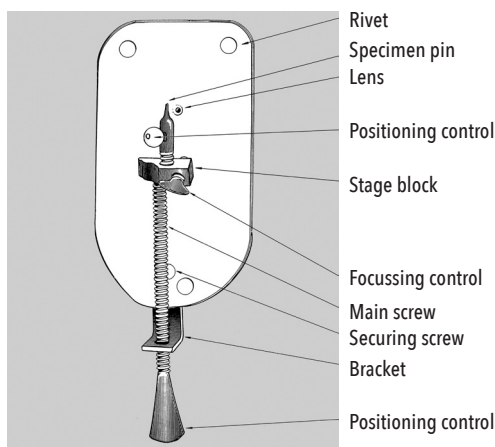
INTRODUCTION

It is popular to celebrate Antony van Leeuwenhoek as the 'founder of microbiology' (and sometimes, less accurately, as the 'father of the microscope'). Yet, when Leeuwenhoek died in 1723, microscopy died with him and he was largely forgotten. The only biographical book that had been published was written by P J Haaxman in 1875 [1], and it caught the attention of the British parasitologist Clifford Dobell, who decided to expand and update it. Dobell's widow Monica told me that he paid towards its production as a magnificent 435-page biography entitled Antony van Leeuwenhoek and his 'little animals'. It was published in 1932 [2], to match the tercentenary of Leeuwenhoek's birth. According to Dobell's notes, 1,000 copies were published by John Bale, Sons & Danielsson for a British audience, with 750 more for the United States published by Harcourt, Brace & Co., and a further 250 for the Netherlands by Swets & Zeitlinger. The title and author's name were printed in red for the title page, the same style that Robert Hooke had used for his momentous book *Micrographia* in 1665. Dobell dismissed Haaxman's pioneering volume as a 'booklet' (hardly fair; even though smaller than Dobell's monumental tome it still ran to a respectable 140 pages).

Neither writer alluded to there being still-unknown Leeuwenhoek microscopes (Figure 1). There were nine accepted as being genuine, and the rest (we had all assumed) had been dismissed and abandoned in the eighteenth century [3]. Following the publicity given to my research, four others have since emerged. Two of them – one made from brass, the other of silver – have now been scrutinised and imaged with the scanning electron microscope (SEM). For the first time we can see how they were made, and can identify features that authenticate them as genuine.

Until now, the identification of a Leeuwenhoek instrument has always been based on the subjective interpretations of the Dutch authorities. This is unsafe, indeed several of those attributed to Leeuwenhoek are likely not made by him at all. My new protocol provides an objective means of authentication.

In December 2014 I was invited to visit Christie's auction house in London by James Hyslop, their Head of Science and Natural History, who



believed they might be holding a Leeuwenhoek microscope. Using a loupe I could study the way the screw thread had been made and could see the placement of the rivets which held the plates together. In time you get to recognise individual handiwork much as you can identify a signature, and over the decades I had handled all the supposed Leeuwenhoek microscopes. I was confident that all the features of this diminutive, little instrument pointed to it being genuine.

My advice to Christie's was that I should study this microscope at length in the SEM, since that alone could provide the conclusive evidence of precisely how it had been manufactured. The vendor was not concerned with such refinements, and nor was the purchaser, who was an antique dealer keen to own it, though not to have it analysed in further detail. The microscope was also said to be genuine by the Boerhaave Museum in Leiden, who owned several examples with which it could be compared.

To everyone's astonishment, three months later another supposed (?) Leeuwenhoek microscope turned up [4]. The new owner had a scholarly interest in microscopes and was fully in accord with my wish to examine and analyse the instrument with the SEM at the Cavendish Laboratory, Cambridge University. It was possible to see how the two metallic plates had been fashioned, how their rivets had been disposed, the details of the stage block, and the method used to create the screw threads with which the specimen was positioned, and which had been made by hand in an era when taps and dies did not exist.

This microscope was also inspected

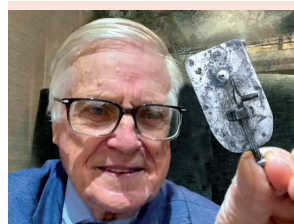
at the Boerhaave Museum in Leiden, where it was found to be a near-twin of a Leeuwenhoek microscope of known provenance in their collection. The Dutch were perturbed by the amounts of zinc in the alloy, arguing that zinc production was not known as a metal until 1746. Small amounts of selenium were also detected by their hand-held X-ray fluorescence analyser (XRF). Their conclusion was that the levels of zinc cast doubt on the authenticity of the brass, while the presence of selenium must indicate that it had been artificially aged with a patination fluid containing selenium dioxide. The Dutch authorities concluded that this was certainly a replica microscope.

In December 2023, a further example was found during a house clearance in East Anglia. This time Mr Hyslop was kind enough to bring it from Christie's to the Cavendish Laboratory, so that I could have a day to scrutinise it in detail. The SEM images that I obtained provided much information on the means of manufacture. A confidential report was also prepared by the Boerhaave Museum. They also concluded that the diminutive instrument seemed to be a genuine Leeuwenhoek microscope.

SEM ANALYSIS

It proved necessary to capture over 100 frames for a single microscope to be comprehensively imaged in the Hitachi S-3400N SEM. Piecing these together to form an image of the entire instrument was not a simple stitching procedure, since successive frames had disparate contrast and varying brightness and, where focus had been subtly adjusted, the magnification of an image frame

FIGURE 1: An idealised view of a Leeuwenhoek microscope, to illustrate its component parts. The single biconvex lens, often no more than a millimetre or two in diameter, could reveal plant anatomy and magnify protozoa with ease. The best (magnifying over 250x) could resolve spermatozoa and living bacteria.



BIOGRAPHY

Brian J Ford has associations with the universities of Cambridge, Kent, the Open University (of which he is a former fellow), Leicester (where he is a former visiting professor) and Cardiff (where he is a fellow). Ford has identified three newly discovered Leeuwenhoek microscopes and has now evolved a protocol for authenticating them. The author of some 35 books (many on the microscope) and 250 publications on Leeuwenhoek, he is also renowned as a lecturer, TV broadcaster, and research biologist. His interests range from intelligence in the living cell to the microbiology of foodstuffs.

ABSTRACT

Antony van Leeuwenhoek pioneered microbiology in the 1600s using small, single-lensed microscopes. Nine were said to exist, though there is documented provenance for very few; most arrived on the scene many years after Leeuwenhoek's death in 1723, and authentication has always been based on the 'feel' of the microscopes (to use their word) by the Dutch. It is likely that several of those accepted as genuine are actually fake. Following the publicity given to the author's research, four more have emerged. Two of these microscopes have been subjected to scanning electron microscopy, which alone can reveal details of manufacture.

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was correspondingly altered. The creation of a single image was done manually, with great care taken to avoid proportional disparity across the entire instrument. Each took two or three days to prepare, and the resulting images are over 1.5 m in length.

The impression and disposition of the matching plates were carefully aligned by Leeuwenhoek, and they were secured together by rivets, four at the corners in the case of the silver microscope, and six for its brass counterpart, with two further rivets at the mid-point along the long axis. The finishing of the rivets was meticulously carried out; their heads were virtually flush with the surrounding surface, though the countersunk hole that perforated the body plate was sometimes slightly greater in diameter than the rivet head itself.

The stage block of these microscopes is essentially rectangular, though the left-hand protrusion is characteristically contoured in a stepped configuration. Inserted into this block is a forged specimen pin. Each microscope originally supported its specimen (or the glass tube in which the specimen was constrained) by attaching it with a dab of gum to a pin protruding from the stage block. The authentic microscopes showed that the pin was forged, and the rounded faceting from this method of manufacture was clearly visible under the SEM (Figure 3), though was not detectable by the naked eye. The base of each specimen pin is threaded, the threaded portion of the brass microscope being significantly greater in diameter than the cylindrical base of the specimen pin.

The screw threads were of particular interest. They are mostly of consistent pitch, though of varying contour. Typical configuration showed a rounded, rectangular thread though the crest was often more substantial in some parts than in others. In both the brass and silver microscope, there was evidence of a characteristic valley at the crest of each turn in the thread. Others have not observed this aspect or have commented on it; but this feature is confined to hand-made threads that are not prepared by turning with a

die, as modern threads are made. This appearance is characteristic of threads that are rolled. Here we have a process where a former displaces metal to produce the thread. The crests frequently deform, leaving an unmistakable grooved appearance. Because no metal is removed in the rolling process, but merely displaced, the diameter of the thread crests is greater than that of the original rod onto which the thread is rolled. A conventional thread is cut, so the crests of the thread are acute and never show the characteristic grooved configuration. In a thread cut with a die, the diameter of the thread is always less than that of the original rod.

Finally, I carried out a detailed SEM inspection of a Leiden replica microscope made by their former technician Mr Arie Vink (Figure 2). These copies are remarkably faithful to the originals, and to the unaided eye it is very difficult to distinguish genuine from imitation. I was immediately able to note the absence of crest grooves in the threads and noted that the threaded portions were of narrower dimensions than the original rod, both of which intimate that the threads have been cut, rather than rolled (Figure 4). However, as I had predicted, it was the SEM which finally proved the point. The clear indications of post-industrial processing were abundantly evident. Were these signs detectable on the brass microscope on which the Dutch had cast doubts, then these findings – together with the disparity of the elemental analysis – would unequivocally indicate that the microscope was a forgery.

RESULTS VISUAL SCRUTINY

Examination of a subject of microscopical interest is ordinarily carried out with a specimen on a slide, and the first step is customarily taught as focusing under the low power objective. This is a mistake. Fully to acquaint one's self with a specimen, another step should precede microscopical scrutiny – how does a specimen look to the unaided eye? Inspecting the slide without a

FIGURE 3: Near right. TOP: The SEM image reveals how the specimen pin of the Vink replica had been produced. The parallel scouring marks left by the cutting tool show that it was turned on a lathe. The profile is neatly conical. CENTRE: An unmistakably faceted shape is seen in the newly discovered brass microscope. This specimen pin has clearly been forged, rather than turned. This manual processing has conferred an angular outline to the pin. BOTTOM: Softer facets can be discerned when the specimen pin of the silver microscope is subjected to examination under the SEM. The malleable nature of silver means that there are no sharply defined shoulders.

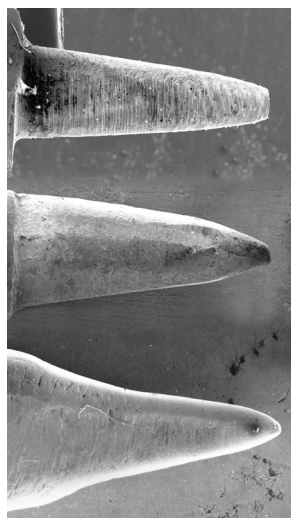


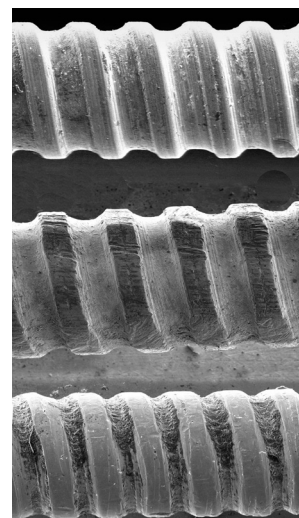
FIGURE 4: Far right. TOP: This view of the main screw from the replica reveals that Vink used an oversized die to cut the thread, thus conferring an antique appearance. The tooling marks are seen to be running in the direction of turn. CENTRE: The marks on the crest of the threads from the newly-found brass microscope run lengthwise, at right angles to those seen under the SEM in the replica. This thread was created by rolling, not by cutting with a die. BOTTOM: Although the metals have disparate qualities, the screw thread from the silver microscope matches the appearance of its brass counterpart (above). The only markings on the crest run lengthways; this thread was rolled.

microscope should always come first. The disposition of key features, or the most propitious area of a slide to examine, perhaps an obvious departure from the appearance of a routine specimen, an intrusive object that would not be anticipated, or the relative positions of key features of a section ... so much can be evinced by examining a slide without a microscope. So it is with a small object, like a Leeuwenhoek microscope.

Lying the newly discovered brass instrument on the bench alongside one of the replicas made by Vink in Leiden showed how similar they were. At first glance it would be easy to conclude that the brass microscope was a replica. However, it was clearly not a copy; the lower portion of the plates in the original microscope at Leiden was straight; the new brass microscope was rounded. This was the kind of disparity that exists between two instruments made by the same hand, but with variations from one microscope to the next. A forger, or the maker of a known replica, would ensure that the copy was exactly the same as the original. It was immediately less likely that the newly discovered microscope was a replica. Then there was the question of the screw; even without a lens I could detect the characteristic grooving of the thread crests that is inimitable in a hand-made rolled thread. This made it even less likely that the instrument was a copy. Finally, the question of mass. Leeuwenhoek produced fine sheets of brass. His microscopes are always lighter than replicas tend to be. Of the replica Leeuwenhoek microscopes that I have in my collections, they weigh 8.40 g, 9.44 g, and 14.96 g. The Vink replica weighs 14.69 g. But the new candidate weighed only 6.24 g, making it even more likely that it was genuine.

ELEMENTAL ANALYSIS

Although zinc and selenium were detected by the Dutch, these are not necessarily reliable findings. The microscope had been retrieved from landfill mud that had been dredged from a Delft canal [4] so any metallic components on the surface could have originated from the substrate in which the microscope lay. The



hand-held XRF device used by the Dutch is primarily intended for use on artefacts like antique oil paintings, and it is likely only to indicate elements on the surface. The data we obtained at the Cavendish laboratory was obtained with energy-dispersive X-ray (EDX) analysis as part of the SEM instrumentation which provides deeper penetration, and this gives us more accurate results of the components of the alloy.

In fact our EDX data were close to what the Dutch had claimed. As an alloy of copper and zinc, brass has been known for millennia. Brass with higher levels of zinc is more malleable, and it is said to have been available as ingots since the sixteenth century. Our EDX results showed 57.7% copper and 27.8% zinc, brass with 28% zinc has been reported from ancient times. The copper in Leeuwenhoek's brass came from the Falun mines, 230 km north-west of Stockholm. There is always selenium in the extracts from the Falun mine, indeed, when selenium was first discovered by the noted Swedish chemist Jöns Jacob Berzelius in 1817, it was in the same Swedish seams as those from which Leeuwenhoek was to derive his copper. It could be argued that the presence of selenium would substantiate the genuineness of Falun copper at the time, though the proportions of contaminants might not be reliably consistent.

In conclusion, the presence of these contaminating elements can show that the copper component of Leeuwenhoek's brass likely originated from the Falun mine. The presence of selenium is not only expected, but – since this element was first identified in that source – it could be argued that it serves to substantiate the microscope's authenticity.

SCANNING ELECTRON MICROSCOPY

This provides the definitive evidence and is the resource to which the Dutch authorities lacked access. First, the edges of the replicas are all cleanly cut; those of a Leeuwenhoek microscope exhibit a rounded profile under the SEM (Figure 5). This provides an immediate indicator of genuineness. Secondly, the specimen pin of a microscope hand-forged by Leeuwenhoek always displays

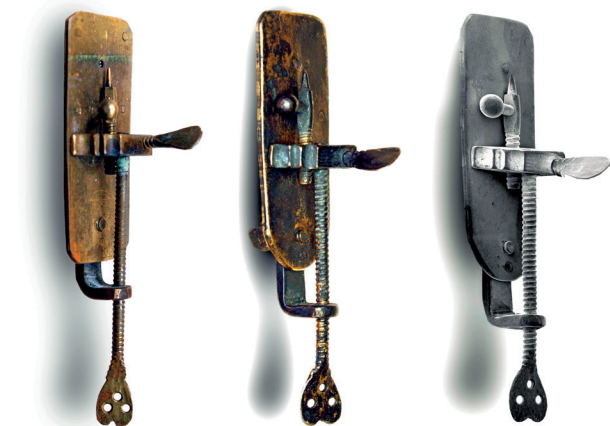


FIGURE 2: LEFT: In Leiden, Arie Vink was still making replica Leeuwenhoek microscopes during his eighties. This example is faithfully copied from an authenticated brass microscope in the Boerhaave museum collection. CENTRE: On close inspection, this previously unknown brass microscope looks virtually identical to the replica, and it would be tempting to assume it was a copy. Only the rounded lower profile of the plates is different. RIGHT: This newly-found brass instrument was examined in the scanning electron microscope and the image (a composite 1.5 m long, comprising about 100 separate frames) reveals diagnostic details to confirm authenticity.

a characteristic faceted appearance. The pins of replicas are turned on a lathe, and the SEM shows the tooling marks that run around the tapering metal (Figure 6). This is unequivocal evidence of authenticity. Thirdly, the thread under the SEM is unmistakably hand-made. Rather than being cut with a die, and the minute surface marks being oriented along the direction of the cutting tool, the Leeuwenhoek threads are rolled. Along many areas of the threaded rods, the grooved crest can be discerned that can only occur with a rolled thread. Any surface marks are oriented along the length of the rod, showing how it was manufactured, and these are at right-angles to the marks left by a thread that has been cut with a die. This is definitive and proves that this newly discovered brass microscope is certainly no replica.

The most widespread application of thread rolling in today's world is at the end of bicycle spokes. Were those threads cut, then the slightly narrower diameter of the thread (due to metal being cut away) would confer a weakness on the spoke. Since rolling displaces metal, rather than removing it, there is no loss of inherent strength. Present-day thread rolling is done with a rotating former, and so is consistent throughout its length. Leeuwenhoek was rolling threads in his home workshop, so the depth and contour of the thread varies from time to time. These findings substantiated that the brass microscope was certainly no modern replica.

We still need to demonstrate that it was made by Leeuwenhoek, and not by some other craftsman. The discovery of the silver microscope in East Anglia provides a comparison. This one has the appearance of an authentic Leeuwenhoek instrument and was accepted as genuine by the Dutch. The objective criteria on which I now insist were provided by examination with the SEM. This immediately provided evidence that was, in each case, entirely consistent with the results from the brass microscope.

The edges of each plate were rounded, not cut from a plate with shears (as is the case with replicas) (Figure 5). The evidence of forging of the specimen pin was comparable to that of the brass microscope, and the thread was shown to be exactly like that of the brass instrument, and entirely different from the cut thread of a replica. The Boerhaave museum staff had said there was a figure 4 stamped into the silver, but this was incorrect. The character is clearly engraved with a steel needle; only the SEM can provide such evidence. Curiously the Dutch authorities insisted that the silver microscope was also contaminated with selenium, which they insisted was due to patination of the microscope. This was entirely mistaken: first, we would find any such agent most abundant in the crevices marking the surface of the silver, but there is no selenium in any of the deepest recesses of the microscope. A minute trace of selenium was detected in one discrete area of

the microscope, but traces of carbon, oxygen, silicon, chloride, iron, copper and tantalum were also evident. Sampling the detritus from the deepest recesses of the engraved or hallmarked regions of the silver evinced no sign of the selenium which the Dutch had claimed, though traces were detected of the elements listed above. The notion that this instrument was once patinated with selenium is unsustainable.

Secondly, selenium cannot be used to patinate silver, and no one would expect to attempt it. Silver will acquire patination readily enough, though if unscrupulous individuals wish to patinate it artificially, they would utilise liver of sulphur. It contains no selenium. The composition is a mixture of potassium sulphide and potassium polysulfide with potassium thiosulfate, and is used to confer an artificially aged appearance to silver and copper. Selenium dioxide is employed to patinate only brass, never silver. The fact that this element was not found in any of the deep recesses of the silver microscope using EDX disproves the notion that selenium patination was ever used on this instrument.

CONCLUSIONS

We have considered the SEM examination of two microscopes by Leeuwenhoek, one made from brass, the other of silver (Figure 2). In each case, the microscopes share identical features that are characteristic of manufacture by hand, including rounded edges of the metal plates, a faceted appearance of the specimen pin, and the clear conclusion that the threaded rods were rolled, and not cut with a die. In both microscopes, the depth of the rolled screw threads was occluded with detritus accumulated over some 350 years. Indeed, the lens of the silver microscope was heavily encrusted with debris and would be unusable without careful cleaning. Every other feature (including the contours of the stage blocks) was identical in both microscopes, even though each had been made from disparate metals. A single individual constructed them both.

The analysis of the brass showed that it did contain selenium, an element first identified in the same Swedish mine from which Leeuwenhoek's supplies would have originated, and thus confirmed as authentic of its time. The brass microscope, which the Dutch had sought to identify without the benefits of the protocol here described, was believed by them to be a near-twin of a Leeuwenhoek microscope with accepted provenance, and thus looked authentic. Clearly, it was not a copy since the rounded contour of the body plate was not identical to the purported original (Figure 5). So many features point to its being genuine, even without recourse to the SEM. However, the fine structure that was to emerge proves that this is no latter-day replica, but an antique hand-made instrument.

Meanwhile, the findings from my examination of the silver microscope – already accepted as a Leeuwenhoek original – are identical. During his

FIGURE 5:
TOP: The corner of the Arie Vink replica shows how the brass plates were cut with shears, creating a rectangular cross-section with sharp edges. The two plates do not precisely coincide, and finishing scratches are visible. BOTTOM: Under the SEM, the brass instrument under higher magnification shows how Leeuwenhoek finished his microscopes. The fit is closer, with detritus sealing the gap. Most notable is the rounded profile of the two plates.

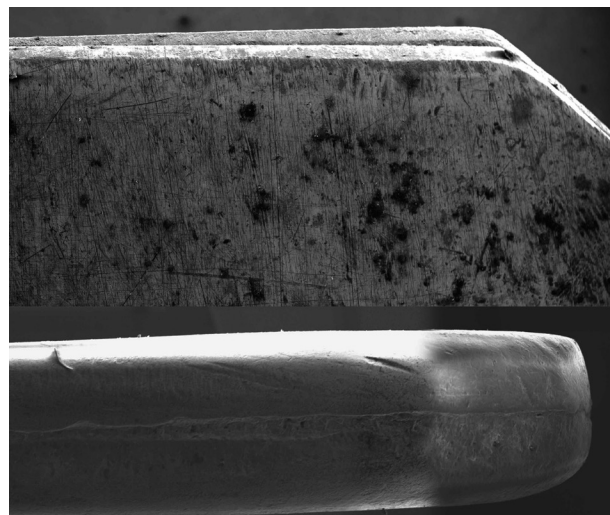
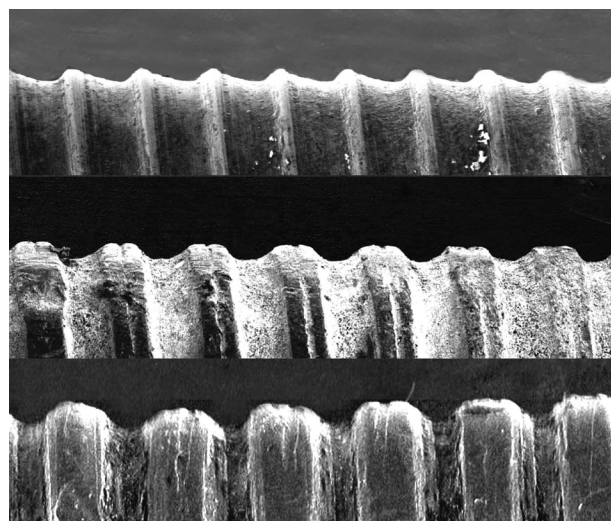


FIGURE 6:
TOP: Circular tooling marks are characteristic of the Vink replica and unequivocally prove that the thread was turned. By modifying an oversized die, an unusual profile is created, seeking to emulate an antique artefact. CENTRE: The hallmark of a rolled thread is that grooves sometimes appear on the crest of the thread, and this portion of the main screw of the brass microscope provides a definitive example. Metal has been displaced, not removed. BOTTOM: Comparable crest grooves are also discernible in the thread from the silver microscope. This provides a final indication of authenticity, and disproves the idea that either of these two microscopes could be a replica.



lifetime, Leeuwenhoek manufactured hundreds of his little microscopes, often leaving a specimen permanently attached, so that he could return to it subsequently for further study. At his death, 247 complete microscopes were left. And of what were they made? In a large and impressive book which the Royal Society produced to celebrate their tercentenary, they described Leeuwenhoek microscopes as "little wooden paddles" [5] though this is an idiosyncratic description for the Society to use; of course, none was ever made of wood. The metals Leeuwenhoek chose were gold, silver, or brass. Few were ever made of gold, and the intrinsic value of the metal would make it irrational to assume that any might still survive. Silver Leeuwenhoek microscopes have long been known, and we could assume that he used this metal earlier in his career, since silver is so much easier to work. Most seem to have been of brass, and this is the metal used to construct the majority of those that survive.

Any protocol for authenticating Leeuwenhoek microscopes must therefore apply to those made both of silver and brass. The observations I have made on both of the recently discovered microscopes present identical features, which can now be linked with provenance. Since publicity was given to my research, four previously unknown

Leeuwenhoek microscopes have been claimed. If others emerge, claiming their authenticity could rely for the first time on objective criteria. The era of subjective assessment is over, and the sense that an instrument "feels right" (the term often used by the Dutch) can no longer validate a Leeuwenhoek microscope on its own [6].

Of greater interest is the fact that these diagnostic features, now they have been identified, can be used to investigate the authenticity of microscopes currently accepted as genuine, but whose provenance is uncertain. Several of those claimed to be by Leeuwenhoek are likely to be fakes. Using the factors elicited above will allow us to prove which is authentic. The number of authentic Leeuwenhoek microscopes currently stands at 13; but the true total is certainly fewer than that. At last this new protocol will allow us to be assured of the difference. It could well be adapted to the study of other antique artefacts, such as scientific instruments, clocks and watches. A new era of authentication using the SEM is at hand.

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