Genuine or copy? Novel methods of authenticating new Leeuwenhoek microscopes.

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INTRODUCTION

Antony Leeuwenhoek (1632-1723) was a draper and civic official in Delft, Netherlands, who visited London in 1666. Clearly he was inspired by Robert Hooke's great work Micrographia (1665)[1] for he began his lengthy communication with the Royal Society of London by precisely reprising Hooke's observations and listing them in the same order as Hooke; I have previously shown that he also used Hooke's design for making his microscopes[2].

The observations published by Hooke were primarily of familiar subjects under low magnification – a louse, a flea, the sting of a bee, a mosquito larva, etc. – whereas Leeuwenhoek concentrated on truly microscopical objects including flagellates and rotifers, blood cells and, as his experience and abilities increased, ultimately bacteria. In consequence he became widely renowned as a pioneering investigative microscopist. In 1686, to reflect his growing importance, Leeuwenhoek resolved to adopt a change of name and was thereafter known as Antony van Leeuwenhoek. Other names by which he is known, including Antonie and Anton, are latter-day inventions by writers and were not current in Leeuwenhoek's lifetime. His work was comprehensively examined in a definitive biography published by Clifford Dobell[3].

In 1981 it emerged that Leeuwenhoek's original specimen packets still lay among his letters[4] and these gave us, for the first time, an indication of Leeuwenhoek's precise and meticulous microscopical techniques[5].

This revelation led to a review that brought together the artefacts that Leeuwenhoek had bequeathed to science, notably the legacy represented by his surviving microscopes[6]. There are also some mounted lenses associated with Leeuwenhoek's name, though it is his single-lens standard microscopes, based on the method of construction laid down by Hooke[2], for which he is widely renowned. It has long been accepted that there were nine of these although I now believe that doubts could be entertained about several examples. Of the total, one was recently auctioned and cost the new owner almost half a million dollars.

That microscope has since disappeared, and the owners remain obdurately determined to conceal its whereabouts[7].

FIGURE 1, (left-right)
1 Replica of the brass Leeuwenhoek microscope at Utrecht University Museum
2 Example of a generic replica kindly produced for the author by Mr. Chris Kirby
3 Boerhaave Museum replica of a brass Leeuwenhoek by Mr. Arie de Vink
4 Camacho/Pallas microscope discovered in mud deposits from a Delft canal
Scale bar = 10 mm

CURRENT REVELATIONS

The accepted number of nine standard Leeuwenhoek microscopes has recently increased from nine to twelve. The tenth was obtained by the Museum Boerhaave in Leiden under questionable circumstances and was announced twenty years after it was identified, in an article for an obscure, and now defunct, journal.[8] Its existence had not been published by other scholars.

In 2014 a silver microscope was revealed and we were asked to provide authentication. There was no reason to doubt its origins, though it lacked any

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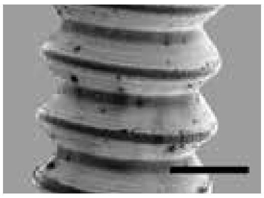

FIGURE 2 Variable-pressure SEM macrography with the Hitachi S-3400N microscope provides this image of the screw of the de Vink replica at 8 kV, initial magnification 10x. To some, the screw may appear acceptable to the naked eye, but the proportionality of the screw (Fig 18) indicates that it was cut with a post-industrial die

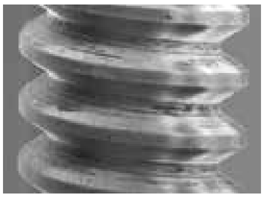

FIGURE 3 The long screw of the replica by Chris Kirby has squared crest and root, closer to that of a Leeuwenhoek microscope. This has been cut with a UNC die (fig 13) and Kirby selected a brass rod that has a diameter 0.33 mm smaller than that for which the die was manufactured. This exaggerates the flattened crest. Same scale as Fig 2

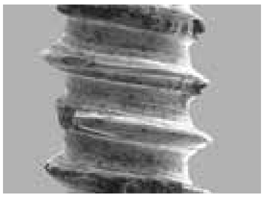

FIGURE 4 In Utrecht, the best of the microscopes attributed to Leeuwenhoek magnifies 266x and replicas of this remarkable instrument were made for some years by Mr. Hansen van Walle of Antwerp, who kindly donated one to the author. Under the SEM the cutting of the thread can be observed, and fragments of brass have been broken away by the industrial die.

FIGURE 5 The rolling of a thread by Leeuwenhoek would ideally produce a square contour as modelled by this modern example of a steel tube. Producing a thread by rolling involves apparatus that requires less precision than manufacturing a high-tensile tap and die of the industrial era. The threaded portion of a rod increases in diameter during the rolling process.

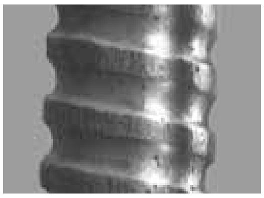

FIGURE 6 Excavated from mud dredged from a canal in Delft, this portion of the screw thread from the Camacho/ Pallas microscope is unlike any of the recent attempts to produce replica microscopes. Imaged with the Hitachi S-3400N at 40x and an accelerating voltage of 8 kV, this macrograph helps us to characterise Leeuwenhoek's instrument.

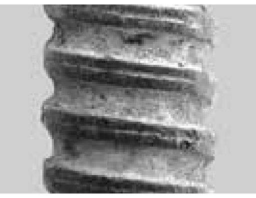

FIGURE 7 An unprotected portion of the same screw thread shows greater evidence of surface damage due to burial in mud for three centuries. Note the crest of the rolled thread, where characteristic grooves can be seen. This feature is referred to as a 'split crest' (Javier Fernandez Landeta *et al* [in] *Journal of Manufacturing Science & Engineering*, 137, June 2015).

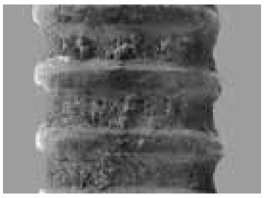

FIGURE 8 Present-day rolled threads are used for securing the spokes of bicycle wheels, and this well-used example provides a fitting comparison with the mud-encrusted thread in Fig 7. The characteristic squared profile is evident, with broad crest and root (Fig 12). Note also the deformation of the crest that is seen more clearly in the Leeuwenhoek microscope.

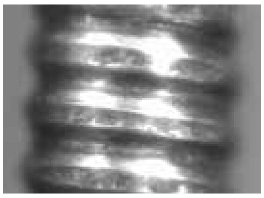

FIGURE 9 Close-up digital image of the recently-discovered silver microscope taken by the Museum Boerhaave, Leiden [13] may reveal a similar rolled structure of the thread. The lack of resolution of such images presents a problem and emphasises the value of macrography with a scanning electron microscope (SEM) as unambiguous sources of evidence.

FIGURE 10 The use of a loupe or hand lens can provide useful evidence of the thread structure. This is a selective enlargement from our studies of the Camacho/ Pallas microscope with a Zuiko Digital 35mm macro lens and Olympus E-500 camera which resolves sufficient detail to conclude that the thread was not produced by a post-industrial die.

FIGURE 11 Distorted industrial dies have been used to create non-standard screw threads, as in this example from the Boerhaave museum. Here the profile is not comparable with an industrial standard, though the parallel scoring reveals this thread was cut with a die, and not rolled. In a case of this sort, only the SEM can provide conclusive evidence.

Scale bar (for all these correlated screw thread images) = 1.00 mm

documentary provenance.[9] Finally, in December 2014 a twelfth Leeuwenhoek microscope emerged. In a story redolent of our times, this brass instrument was advertised for sale on the on-line auction site eBay for $99.99, though the auction was peremptorily cancelled when the instrument was privately purchased by a Spanish collector, Dr Tomás Camacho. The vendor was initially unwilling to part with the purchase, and insisted that it had been irretrievably mislaid, but eventually it was sent to its new owner in Spain[10].

Whenever these instruments have been reviewed, it is on the basis of a consensus that a given microscope is genuine. Authorities will assemble pointers towards authenticity, until there is a sense of genuineness. This traditional and widely-used approach results in an essentially subjective result, summed up by a recent paper that states: "a combination of characteristics must be considered, no single parameter will do."[11]

An assembly of supportive arguments and observations does not rely on falsification and thus falls into the trap of conventional epistemology. Popper sensibly drew attention to the fact that the practice of declaring an unfalsifiable theory to be scientifically true in this way is mere pseudoscience: "My proposal is based upon an asymmetry between verifiability and falsifiability; an asymmetry which results from the logical form of universal statements. For these are never derivable from singular statements, but can be contradicted by singular statements."[12] We must therefore move away from opinions of authenticity and seek single facts that would disprove genuineness.

In each of these two microscopes, there is a range of evidence to suggest that they were genuine. Having inspected microscopes of accepted provenance over several decades, one can become confident that the style and appearance of a recently discovered instrument can substantiate a claim that it might be genuine. The silver microscope discovered in 2014 was taken to the Boerhaave Museum where they supported my conclusion that it was probably authentic. In a report on the instrument sent to the author[13] it was concluded that:

> *Evaluating the authenticity of an historic artefact requires a holistic approach: functional and stylistic assessment, material analysis, historic awareness and even connoisseurship all put weight in the scale. In the present evaluation, the copy has been inspected by means of microphotography (sic), lens properties have been gathered, and material composition has been analysed with X-ray fluorescence. These data have been compared with those of a selection of original and replica instruments. In stylistically assessing the instrument, no features were found that give away a modern date of manufacture, or testify to working procedures differing from those of Leeuwenhoek.*

Some of these stated techniques were revealing; thus, the analysis of the silver alloy was compatible with that of known examples made by Leeuwenhoek, apart from evidence of elemental chromium (believed to be left by using contemporary silver polish to clean

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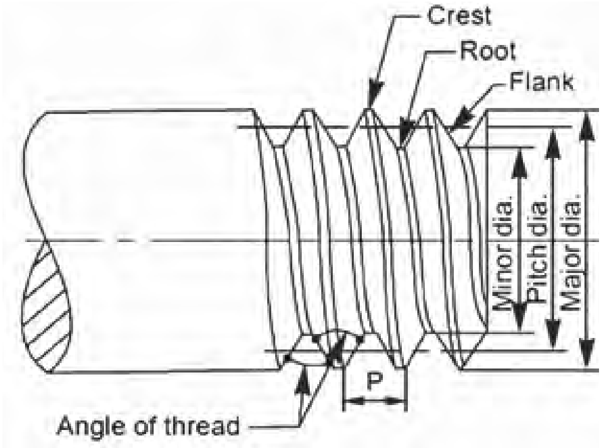

FIGURE 12 The nomenclature used in this paper is derived from the standard terms used by engineering in the production of a screw thread. Of importance in the present investigations are the crest and root of the thread, whereas the angle of the thread in the Leeuwenhoek microscope cannot be determined. The existence of a measurable angle typifies a more recent thread cut with a die.

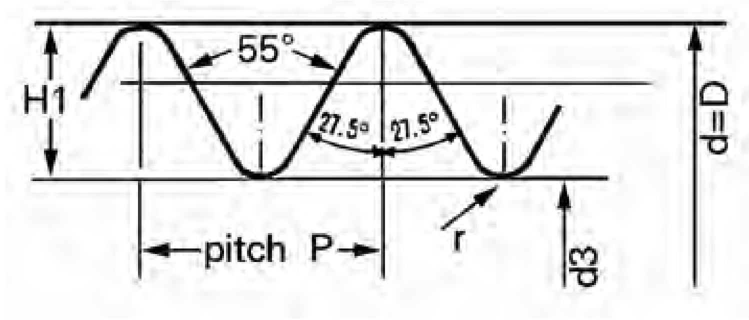

FIGURE 13 Devised by Sir Joseph Whitworth in 1841, this standardised screw has a thread angle of 55° and a depth of 0.640327p and a radius of 0.137329p, where p is the pitch. The need for mass production of weaponry during the Crimean War gave impetus to the adoption of the Whitworth standard, and it is still widely in plumbing fixtures and in some computer applications in the United States.

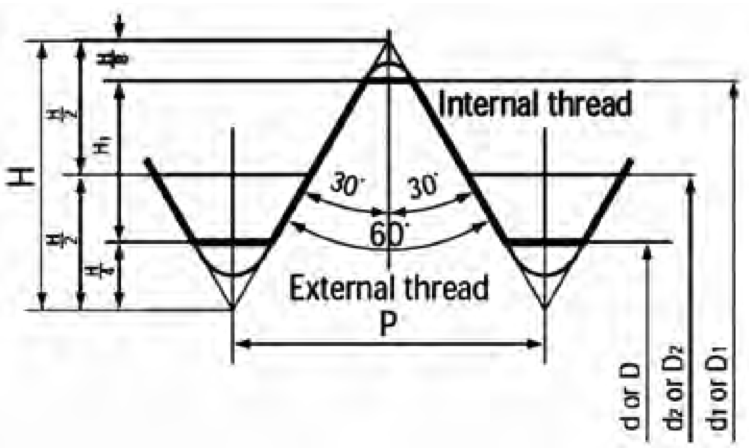

FIGURE 14 The United Coarse thread (UNC) is part of the Unified Thread Standard (UTS) adopted by the US in 1949. It features a thread angle that is increased to 60° while the crest and root have a flattened contour measuring one-eighth of the height of the thread. Even when rod of reduced diameter is used, so the crest profile appears more square, the SEM allows us correctly to characterise the thread.

the object). However, the convention of 'stylistic assessment' of a scientific instrument provides essentially a subjective conclusion. The reliance on such personal opinions is widely used in establishing authenticity through the world of art and antiques, and these microscopes served to emphasise that more objective and evidence-based criteria should more properly be used. A search for authenticity should be replaced by a quest for a single feature which proves that an example cannot be genuine.

In examining antique microscopes, several of the conventional examinations that are brought together can be questioned. Details of the alloy can be analysed by X-Ray Fluorescence (XRF) analysis as practised by the Boerhaave Museum, Leiden, or Energy-dispersive X-ray Spectroscopy (EDX) which my colleague JJ Rickard utilised at the Cavendish Laboratory, Cambridge[14]. Though revealing, these findings are not conclusive since forgeries of antique artefacts can easily be made by constructing components from contemporaneous alloys. Hallmarks are confidently cited, though they can be forged and it would be possible to construct a present-day replica using hallmarked silver from an earlier century. In any event, the hallmarks on the silver Leeuwenhoek microscopes date only from the nineteenth century. Many current conventions can be questioned when we seek an unambiguous answer to a recalcitrant problem.

It is the minutiae of construction that alone bear testimony to the origin of an artefact, though the images mentioned in the Boerhaave report as examples of 'microphotography'[13] proved to be unsatisfactory. They lack definition, display limited depth of focus, and are of limited value in interpreting the fine details (fig 9). By contrast, the depth of field and crisp resolution of a scanning electron microscope (SEM) were recognised as providing evidence of detail that would be inimitable by a more recent manufacturer. In my view, this could provide unequivocal evidence since details – such as the profile of a screw thread cut with an industrial die – would be characteristic. The new owner of the silver microscope, Mr. Bert Degenaar, was uninterested in this novel proposal, and Tiemens Cocquyt, the telescope specialist at the Boerhaave Museum – who these days also has responsibility for antique microscopes – informed us that their microscopes would not be available for examination under SEM due to the modernisation of the department in which they were displayed.[15]

However, the owners of the newly-discovered brass microscope, Dr Tomás Camacho and Dr Estrella Pallas, were convinced of the revelations that SEM analysis could provide, and their diminutive instrument was sent to Cambridge by secure courier without delay. Initial observations revealed many details of construction[16] and it was then possible to obtain images of the stage and specimen pin assembly[17] and to raise the possibility that other examples might yet be found.[18] The unprecedented SEM observations were filmed by a television crew for the national news[19].

The lens surface had been damaged after the microscope was completed – presumably by Leeuwenhoek – though it proved possible to attempt a reconstruction of the original profile so that the magnification of the lens could be retrospectively calculated[20]. This programme of detailed examination derives full benefit from the depth of field and high resolution offered by variable-pressure microscopy at the Cavendish suite in Cambridge. Rather than aiming at high magnification, lower magnifications (typically 8×) were obtained and this novel technique of SEM macrography has provided the novel insights that the project requires.

ESTABLISHING A PROTOCOL

It would be impracticable to subject each microscope to the complexities of comprehensive SEM examination. A rapid and repeatable short test should be the core component of a practical protocol. Even if not ultimately definitive as a test of authenticity, it should provide evidence of more recent manufacture or forgery. The production of screw threads by Leeuwenhoek at his home in Delft is an example of an inimitable procedure:

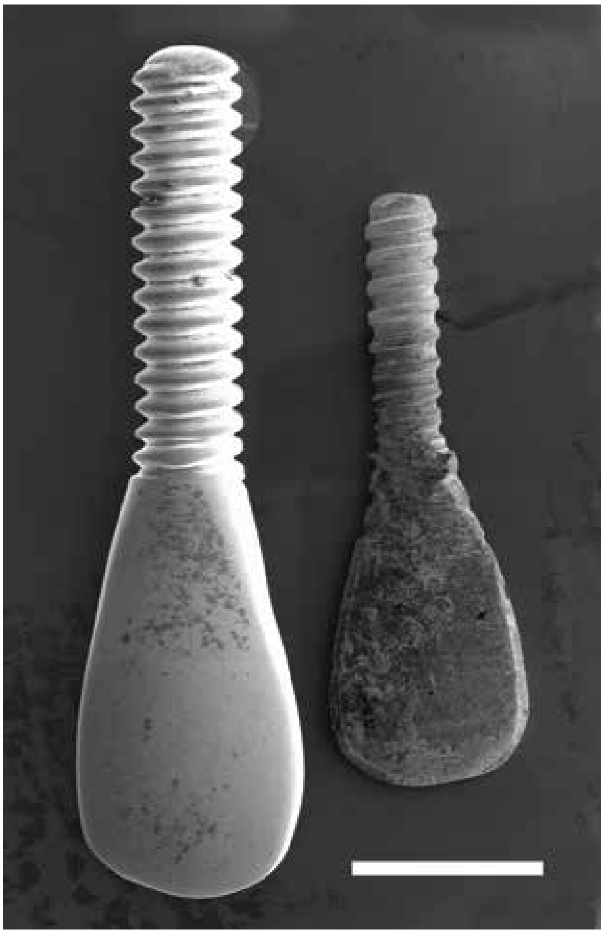

FIGURE 15 subjecting each Leeuwenhoek microscope to rigorous SEM examination would be unnecessary. Comparing the focussing screws provides the crucial evidence we seek. These two screws are from the Kirby replica (left) and the Camacho/Pallas microscope (right) at initial magnification 7x at 10 kV. The Kirby thread has clearly been cut, and has the characteristic UNC contour. Scale bar 5 mm

he had to devise methods of producing screws when modern manufacturing methods were not available. The manufacturer of replica microscopes currently marketed by the Boerhaave Museum states that they utilise a British Standard Whitworth 2.3 mm diameter screw thread to produce the screw threads and adds: "The Pitch is finer [than for a genuine Leeuwenhoek microscope] though, but tests have proven that only real experts will see the difference."[21]

What the tests might be is not stated, but there are many characteristics defined by the die produced to cut a Whitworth screw thread. This was the first standard screw in the world, devised in 1841 by Joseph Whitworth and intended to provide universality in an era when manufacturers each had their own procedures, which made mass production by a range of producers impractical.

Whitworth's standard specified a 55° thread angle and a thread depth of 0.640327p with radius 0.137329p, where p is defined as the pitch, the pitch increasing with diameter in defined steps. This system was subsequently adopted as the British Standard Whitworth and was crucial in the mass-production of weaponry for the Crimean War. Subsequently it became a global standard.[23]

Any microscope that embodies the details of a Whitworth thread cannot have been made by Leeuwenhoek: thus we have a single definitive criterion by which to detect non-genuineness. Leeuwenhoek's methods of screw production pre-date Whitworth's by over 160 years and have characteristic features that macrography with the SEM have revealed. Similarly, the long screw threads for the excellent replicas made by Mr. Chris Kirby are cut with a 6 x 32 UNC die, which he points out is the coarsest thread commonly available for that size.[22] He cuts his thread on 1/8 inch brass rod; it should be 0.013 inches (0.33 mm) greater in diameter but Kirby finds that this gives the thread a somewhat flatter crest than normal, giving it an appearance closer to that of a Leeuwenhoek original.

Neither of these is comparable to the details of a Leeuwenhoek screw when inspected with the SEM. Leeuwenhoek's thread profile is essentially square, rather than v-shaped, and the crest often bears an apical groove which is indicative of rolling, rather than cutting with a die. This method produces no swarf and the thread has a diameter greater than the blank rod from which it is made.[23] Because this is a cold-formed process, the hardness and surface finish are of higher quality and resistance to wear is minimised.[24]

It is proposed that we have recourse to SEM examination of the thread of the focussing screw of each purported Leeuwenhoek microscope. The focussing screw can easily be removed without compromising these valuable and historical instruments and the microscopic configuration will rapidly reveal whether a given microscope reveals standards that can only derive from industrial screw-cutting dies.

This can provide evidence, not of genuineness, but that a given microscope is a replica or fake. In one example (the brass microscope at the Deutsches Museum, Munich) the focussing screw is missing and in this instance the specimen pin could be imaged instead. Doubts have already been raised about the provenance of this instrument[6] and the screw thread appears to be no larger than the rod from which was formed – thus we have some *prima facie* evidence that it may not be a rolled thread. To an experienced eye, examination of these details even with a hand-lens may suffice to indicate that a given example is unlikely to be genuine.

CONCLUSIONS

It is the quest for signs of forgery on which we need to embark, not a concatenation of criteria that might subjectively support hoped-for authenticity. The procedures now developed are therefore based on identifying a single characteristic that demonstrates non-genuineness. The standard approach seeks to confirm the authentication of a given instrument with "a combination of characteristics"[11] or a "holistic approach" using several confirmatory tests[13] whereas this new protocol aims at identifying whether a microscope must be of more recent manufacture.

In some cases, high-quality close-up photography may be sufficient to demonstrate that a thread has been cut with a Whitworth or UNC die. In all cases, macrography with the SEM will substantiate which of the microscopes have clearly been faked: further procedures will be contingent upon what can be ascertained using the approach to SEM inspection already developed with the Camacho/Pallas microscope. The overriding question is to know which of the surviving Leeuwenhoek microscopes is a replica (or forgery): using this simple protocol, we can speedily determine which examples embody more recent manufacturing techniques and thus cannot be genuine. This innovative approach to a recalcitrant problem is proposed as a means of identifying which of the twelve accepted examples was not, after all, manufactured by Leeuwenhoek.

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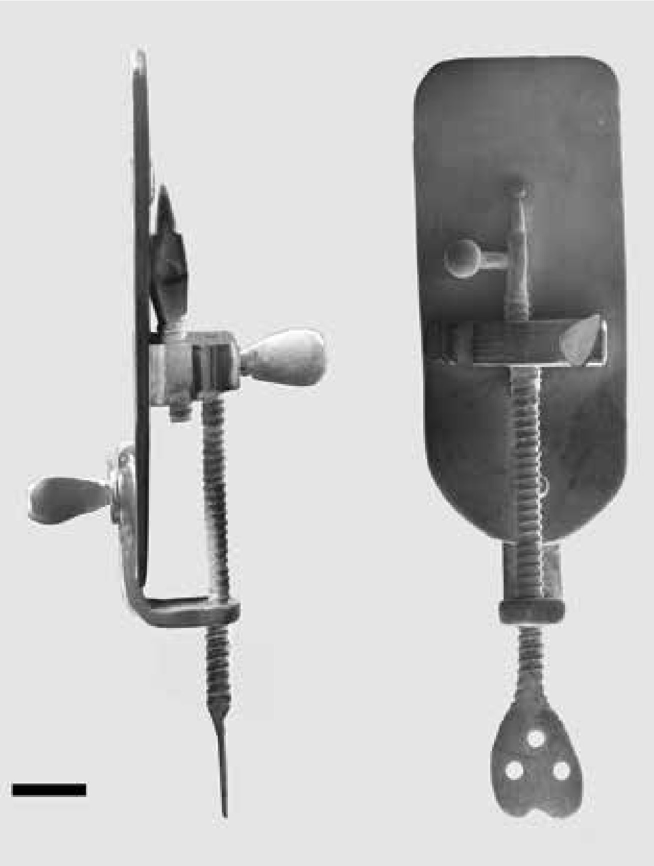

FIGURE 16, left, assembled from over 100 discrete macrographs, these composite SEM images allow us to review the detailed appearance of the microscope excavated from mud.[10] Under higher magnifications, the surface working can be inspected though it is the appearance of the focussing screw (centre) that may be used to demonstrate if an instrument is a replica. Scale bar = 5 mm

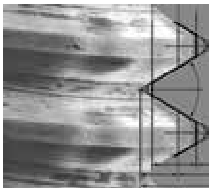

FIGURE 17 The procedure for thread cutting developed by Kirby for his replica microscopes provides a result that, to the naked eye, is reminiscent of an authentic rolled thread by Leeuwenhoek. However, we can superimpose the UNC thread profile directly onto the SEM macrograph, and this demonstrates unequivocally the precise nature of the die-cut thread

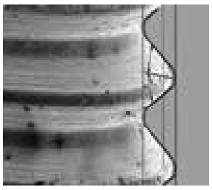

FIGURE 18 Replicas that were once made for the Boerhaave Museum had screw threads that were non-standard. However, with proportions adjusted to match the macrograph, compatibility with the Whitworth contour is unmistakeable and there is also a clear angle of the thread. This confirms post-industrial production, and such a distinction could be made with a hand lens

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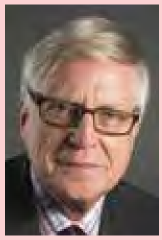
BIOGRAPHY



The author has researched this field for 50 years and in 1981 discovered the cache of original specimens sent by Leeuwenhoek to London in the seventeenth century. Professor Ford is a Fellow of Cardiff University, a former Fellow of the Open University, and is based at Gonville & Caius College, University of Cambridge.

ABSTRACT

Within the space of a single year, two previously unrecorded Leeuwenhoek microscopes have been presented to the author for authentication. Each had an intriguing provenance, yet the scientific investigations lacked objective criteria by which to confirm their origin. Using the high resolution of the scanning electron microscope at unprecedentedly low magnifications, previously undetectable details of manufacture were revealed. Several of the accepted Leeuwenhoek microscopes may prove to be forgeries, or replicas. Since Leeuwenhoek's microscopes are the oldest existing high-resolution instruments, of which a single example can cost $500,000, there is clearly a case for substantiating provenance and we may now have a rapid, repeatable and reliable source of objective reassurance. Current orthodoxies centre on a quest for features that together substantiate authenticity; it is here advanced that we identify a single criterion which proves that a microscope cannot be authentic. It is now proposed that macrography of a single component of each purported Leeuwenhoek microscope can provide a reliable intimation of non-genuineness.

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