APPLIED SCIENCES AND ENGINEERING

Neutron tomography of Van Leeuwenhoek's microscopes

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The technique of neutron tomography has, after 350 years, enabled a first look inside the iconic single-lens microscopes of Antoni van Leeuwenhoek. Van Leeuwenhoek's 17th-century discovery of "animalcules" marks the birth of microbiology. His skillfully self-produced microscope lenses remained unsurpassed for over 150 years. Neutron tomography now enabled us to reveal the lens types Van Leeuwenhoek used. We argue that Van Leeuwenhoek's instruments incorporate some innovations that testify to an awareness of concurrent developments. In particular, our analysis shows that for making his best-performing microscopes, Van Leeuwenhoek deployed a lens-making procedure popularized in 1678 by Robert Hooke. This is notable, as Hooke always wanted to find the secret of Van Leeuwenhoek's lenses, but never managed to do so. Therefore, Van Leeuwenhoek was far from the isolated scholar he is often claimed to be; rather, his secrecy about his lenses was motivated by an attempt to conceal his indebtedness to Hooke.

INTRODUCTION

Van Leeuwenhoek (1632–1723) was the founding father of microbiology. He made his discoveries using specialized, self-made, single-lens microscopes (*1*, *2*), such as shown in Fig. 1. Yet, he was very secretive about the most essential aspect of his microscopes: the lenses. The quality of his lenses was not surpassed until the introduction of achromatic microscope lenses in the 1830s (*3*, *4*). The discoveries he made in microbiology brought about a strong interest from his contemporaries in how he was able to produce lenses that enabled him to reveal the microworld.

So far, three lens-production methods have been put forward as having been deployed by Van Leeuwenhoek. Information about these methods has been derived from some scarce references given by Van Leeuwenhoek during his life, combined with earlier studies of his remaining microscopes (*4*). A first method is the classical method of grinding and polishing lenses to the required shape in a grinding mold (Fig. 2A). Van Leeuwenhoek referred this method to his contemporaries and praised the thin lenses it yields, and it is generally believed that many of his microscopes, including most of the preserved ones, held or hold such a lens. Second is the production of ball-shaped lenses after the method of Johannes Hudde, which involves flameworking: melting and collecting glass on a needle tip (Fig. 2B) (*5*). Van Leeuwenhoek wrote rather negatively about the glass contaminations this method produced and admitted that he only practiced it for a short time at the beginning of his career. No such lenses have been identified in his preserved microscopes. A third procedure is suggested by Van Leeuwenhoek's own testimony that "after ten years of trying, he had developed a glass blowing method with which nonspherical lenses could be produced" (*6*). This puzzling remark has given rise to much speculation, and the mysterious lenses referred to by Van Leeuwenhoek have tentatively been linked to the outstanding performance of his microscopes. In particular, for the Van Leeuwenhoek microscope with the highest magnification that is preserved, a 266-powered instrument with a measured resolving power of 1.35 μm, it has been suggested that Van Leeuwenhoek made the lens using a glass-blowing technique (*4*). Van Zuylen found that this lens, in contrast to the other preserved ones, shows such a smooth surface finish that it must have been made using a flameworking rather than a lens-grinding technique. The air bubbles present in this lens reinforce this attribution. Van Zuylen proposed that by blowing air, Van Leeuwenhoek shaped a glass tube into a bulb (Fig. 2C). The glob or "knot" at the end of this bulb, once broken off, has optical characteristics similar

Fig. 1. The two original Van Leeuwenhoek microscopes that were studied with neutron tomography. The lens sits mounted between the brass plates, at the position of the specimen pin. (**A**) A medium-powered (×118) instrument (Rijksmuseum Boerhaave, Leiden, inventory number V7017). Note that there is a redundant drill hole in the upper left corner of the instrument, not to be confused with its lens aperture, which is directly behind the pin. This microscope is numbered #1 by Van Zuylen. Photo credit: Tom Haartsen Fotografie, Ouderkerk aan de Amstel. (**B**) The instrument with the highest magnification among the preserved ones (×266) (Utrecht University Museum, inventory number UM-1). This microscope is numbered #3 by Van Zuylen. Photo credit: Utrecht University Museum.

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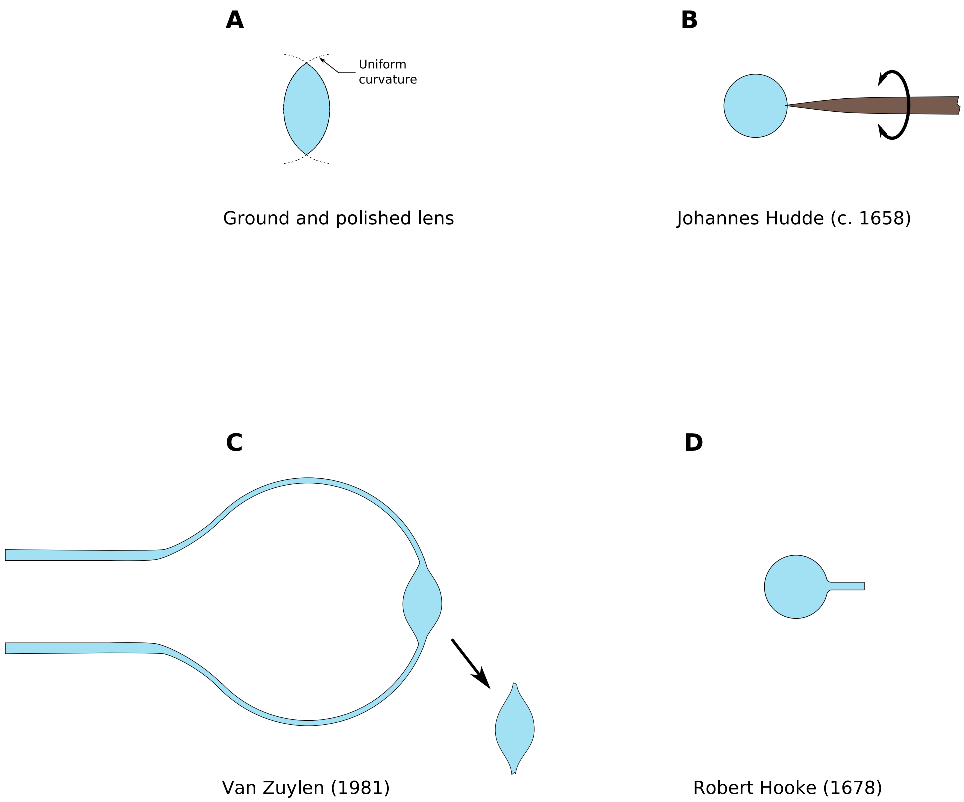

Fig. 2. Lens-making procedures possibly adopted by Van Leeuwenhoek. (**A**) Lens ground and polished in a grinding dish. (**B**) Method after Johannes Hudde, involving melting glass on a metal needle. (**C**) Hypothesis by Van Zuylen for the Utrecht Leeuwenhoek lens, making use of glass blowing. (**D**) Method after Robert Hooke, melting the tip of a glass filament into a ball-shaped ending.

to the lens in the Utrecht Leeuwenhoek microscope. These characteristics are as follows: a smooth, "fire-polished" surface, a very high magnification, and an impressive resolving power. Van Zuylen further attributes these outstanding optical qualities to the aspherical surfaces (i.e., lens surfaces with a carefully controlled, nonuniform curvature) that this method would give to lenses, a shape that he also tentatively detects on the surfaces of the original lens. Van Zuylen's hypothesis is important because he advances the preserved Utrecht microscope as proof that Van Leeuwenhoek did make use of "secret" lens-making techniques. Furthermore, his analysis implies that Van Leeuwenhoek was unique in deliberately, and successfully, mastering the benefits of aspherical lenses, thereby obtaining advantages in optical performance over any competing instrument at the close of the 17th century.

Deducing lens characteristics from Van Leeuwenhoek's preserved microscopes remains a challenging task. Often, the aperture (Fig. 1, the tiny hole behind the specimen pins), which ranges in size from 0.5 to 1.0 mm, leaves not more than a few percent of the lens visible, making the rest of the glass surface inaccessible for research. Hitherto, in earlier research, curvatures and focal properties of the lenses have been measured directly through this small available aperture, while properties of the lens surface hidden behind the metal plates, as well as lens thickness, needed to be estimated by indirect methods (*4*). This has made it additionally difficult to make accurate predictions about the lenses' shape and, thus, about the production methods of the lenses with which Van Leeuwenhoek made microbiological history.

METHODS

This limitation was overcome by imaging two of Van Leeuwenhoek's original microscopes, shown in Fig. 1, with neutron tomography. One of these microscopes is the 266-powered Utrecht instrument, for which Van Zuylen has proposed a glass-blowing production method. By applying computed tomography using neutron radiation, it has proven to be possible to visualize the entire shape of the lens, noninvasively, as it is held in between the brass plates. In this case, neutron tomography has clear advantages over x-ray imaging. Whereas the metal apertures would hinder x-ray radiation to detect the relatively low electron density of the glass compared to that of the metal (brass), the neutrons interact with the nuclei of atoms (rather than their electrons), yielding a more favorable contrast between the metal and glass.

RESULTS

The neutron tomography measurements were performed at the FISH beam line of the research reactor at the TU Delft, The Netherlands (*7*). The data collection took 15 hours, using a 100-μm scintillation

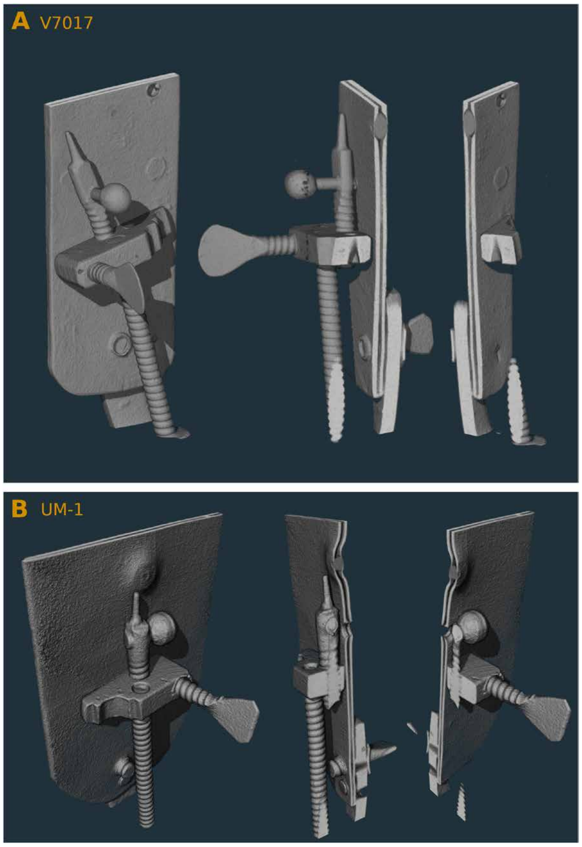

Fig. 3. Reconstructed tomograms and cross-cuts of the two investigated Van Leeuwenhoek microscopes, as shown in Fig. 1. For both microscopes, the lenses become visible in the cross-cuts. (**A**) Medium-powered instrument (V7017). This instrument has its specimen pin turned aside for unobstructed analysis of the lens. (**B**) High-powered instrument (UM-1).

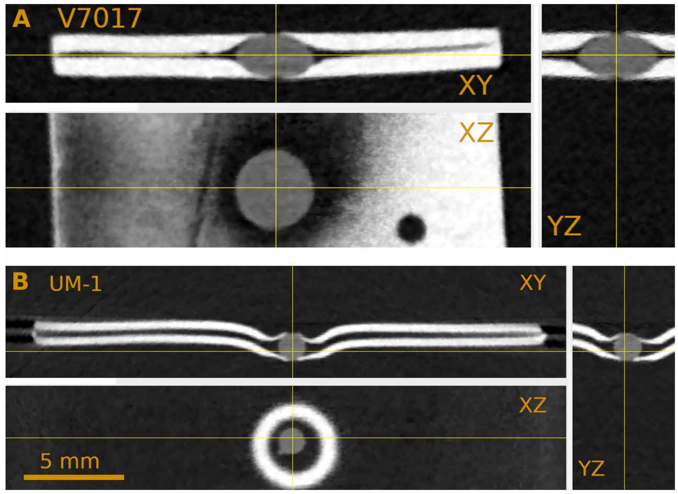

Fig. 4. Orthogonal cross sections of computed tomography of the Van Leeuwenhoek microscopes from Leiden and Utrecht, as shown in Fig. 1. (**A**) The cross sections of the lentil-shaped lens of the medium-powered microscope (V7017). (**B**) The circular cross section of the high-powered microscope (UM-1). The XZ projection shows that this ball-shaped lens has a tiny glass stem connected to it.

screen, which renders a spatial resolution of approximately 150 μm. After filter corrections for dead camera pixels on the individual images, standard filtered back projection was used to reconstruct the three-dimensional computer model shown in Fig. 3.

Physical features of the lenses

When the data are viewed as orthogonal cross sections, the tomogram of the medium-powered microscope (Fig. 4A) clearly shows a "lentil-shaped" profile for the lens, with a 2.7-mm diameter of the rim and a thickness of 1.5 mm. In particular, it shows a relatively thin glass lens, bounded by a rim, with a front and back face having a uniform radius of curvature over the full lens surface. This radius of curvature matches the curvature measurement of the 1.91/1.96-mm radius that was derived earlier over the limited, clear aperture (*4*). The pointed rim is indicative of an abrasive production method, as a glass-blowing or flameworking method would prevent the formation of sharp edges (*5*). Within the resolution of the experimental data, there is also no sign of any particular roughness of the rim of this lens, which could have otherwise pointed to a production method where the lens is broken off, unpolished, from a larger piece of glass (*4*). The shape revealed by the tomogram, in other words, corresponds exactly with what one would expect for a ground and polished lens.

The cross sections also inform us how Van Leeuwenhoek assembled this microscope. The XY and YZ cross sections show how the brass plates form an accurate envelope holding the lens, most likely achieved by indenting the interior sides of the plates. The exterior faces of the brass lens plates indeed show corresponding filing marks around the apertures. The XZ cross section shows the circular perimeter of the lens, and the inner side of one of the brass plates, with an inclined clamping mark on the left and a through-hole. It must be noted how closely the recess in the brass plates matches the shape of the glass lens, yielding a brass edge around the lens' aperture of only a few tenths of a millimeter thickness. This design choice would have guaranteed Van Leeuwenhoek ample room to position and to manipulate the specimen in front of the lens' surface. The care with which the lens is fitted between the brass plates sheds new light on Van Leeuwenhoek's working methods. Traditionally, the Delft scholar was viewed as a dedicated tinkerer, who carefully hand-crafted one microscope after the other. Given the sheer number of instruments he produced during his life (more than 500), recently this view has shifted to allow the possibility of some form of series production (*8*). The tomography data obtained here show that fitting the lens in the microscope remained, nonetheless, a delicate and custom procedure. Judging from the preserved copies, each microscope that Van Leeuwenhoek produced held a lens with a distinct curvature and magnification. The tight fitting of the lens that the tomogram shows then suggests that the brass plates were specially adapted to hold this specific lens.

The XY cross section for the high-powered instrument, UM-1 (Fig. 4B), reinforces the suggestion that closeness of the specimen to the lens was a prime consideration when Van Leeuwenhoek constructed his microscopes. Where the lens sits, the brass plates are recessed such that the front surface of the lens lies out of the plane of the brass plates, sticking out toward the specimen pin (downward direction in Fig. 4B). Also, as is the case with the medium-powered instrument, the brass rim that holds the lens is very thin, bringing the lens very close to the surface. Of further note is that in this high-powered instrument, Van Leeuwenhoek used differently sized lens apertures at the object side (0.55 mm) and at the eye side (0.70 mm) of the lens. This results in the lens being stopped down to a numerical aperture (NA) of 0.37, primarily by virtue of the eye-side aperture. Van Leeuwenhoek's deployment of differently sized lens apertures is in line with, and may reflect a familiarity with, studies and rules of thumb for single-lens microscope apertures that were being developed in the circles of Christiaan Huygens around 1678 (*5*, *9*).

The biggest surprise, however, comes from the lens itself. The tomogram for this high-powered lens reveals a substantially different picture compared to the medium-powered one. In particular, the high-powered lens has an obvious circular cross section in all directions and one can discern in the XZ cross section of Fig. 4B a tiny glass thread (a stem) that is connected to it, which sits with the lens in between the brass plates. Within the limits of measurement accuracy, the glass globule, apart from the stem, shows a constant diameter of 1.3 mm in all directions. This discovery stands in sharp contrast with how this lens was previously taken to be thinner along the optical axis than the lens is wide—i.e., to be more lentil-shaped (*3*, *4*, *10*).

The association of thin lenses with Van Leeuwenhoek's microscopes goes back a long time. Already in the 18th century, Uffenbach reported (*6*) that, during his 1710 visit, Van Leeuwenhoek claimed never to use globular lenses and argued that such lenses would also not fit inside his rather thin construct of the two brass plates. In 1954, Van Cittert, physicist and curator of the Utrecht microscope, wondered why Van Leeuwenhoek did not consider the use of globular lenses, especially after he demonstrated how a self-fashioned globular lens of 1.1 mm diameter would yield a performance comparable to the Utrecht instrument. Van Cittert remained convinced that the original lenses were nonglobular, and wrote "the chromatic as well as spherical aberration of the lens of Van Leeuwenhoek being greater than that of an equally strong globule, it remains a mystery why Van Leeuwenhoek preferred ground lenses" (*10*). A few years later, Walther (*11*) confirmed, on mathematical grounds, that spherical aberrations indeed would have been smaller if Van Leeuwenhoek would have used globular lenses, provided that they were used in combination with carefully controlled lens apertures.

Our encounter of a ball-shaped lens also substantially affects its presumed production methods. In particular, it contradicts the lens-production method advanced by Van Zuylen. The nonspherical shape of the lens in Van Zuylen's method and the subsequent rough rim that would follow upon the breaking out of this lens from the glass body, as shown in Fig. 2C, are clearly absent in the high-powered lens. Instead, the globular shape, in addition to the tiny stem that sticks out of the lens, indicates that Van Leeuwenhoek used a much simpler and, at that time, much more widely circulating production method. Van Zuylen's misattribution probably stems from a wrong interpretation of the term "glass blowing". To a 17th-century practitioner, the "blowing" in "glass blowing" would refer to the feeding of oxygen with the mouth to a flame to obtain a sufficient temperature for melting glass, rather than blowing air into a glass tube to control its resulting shape (*5*).

The lens shape revealed by neutron tomography completely fits the description for an "exceeding easie to make" [sic] microscope lens that Robert Hooke published in 1678 (*12*). Hooke described how the end of a thin glass thread can be turned into a ball-shaped ending by melting it in a flame (Fig. 2D). The remaining stem, sticking out of the globule, then serves as a handle for the lens or can be used for mounting the lens between two plates. The 1678 description was a variation on a method Hooke already described in his *Micrographia* (1665), the book that predated Van Leeuwenhoek's microscopic career and, most likely, even served as an inspiration for Van Leeuwenhoek to start microscopizing in the first place (*1*, *8*). Details about this lens-production method, therefore, certainly circulated in print before Van Leeuwenhoek started his career. Hooke's earlier description differed in that in 1665, concerned about the blurring caused by multiple refractive surfaces, he suggested the stem to be ground off, leading to a plano-convex lens (*13*). Hooke's 1678 revision of the lens recipe, instead, acknowledged that it might equally well be used as an equiconvex, globular lens, with the intact stem serving as a handle, exactly as neutron tomography now revealed to be the case for the high-powered instrument. Hooke's lens-making procedures are in some sense adaptations of similar globular lens recipes, making use of glass melting that gained popularity in the second half of the 17th century (*2*, *4*, *5*, *8*). Yet, through his publication of the lens recipe, particularly in the hugely successful *Micrographia*, by the 1670s, the association of this specific lens-production method with Hooke would have been unmistakable.

As mentioned before, at some point in his career, Van Leeuwenhoek testified to having deployed globule lenses using a method developed around 1658 by Amsterdam scholar Johannes Hudde, which, making use of a needle, yields lenses without a protruding stem. He claimed that he used the Hudde lenses only briefly, abhorring them because of their contaminations. Yet, Hooke's procedure, not making use of a needle to collect the glass, poses virtually no risk of introducing contaminations. Van Leeuwenhoek always remained silent about any of Hooke's lens-making methods. A lens made after these descriptions by Hooke had never been associated with Van Leeuwenhoek before, and it thus seems that here, we have proof of a third, be it unexpected, lens-making procedure that can positively be associated with Van Leeuwenhoek's microscopes.

DISCUSSION

Competition with Hooke

The encounter of a globular lens after Hooke in Van Leeuwenhoek's microscopes is ironic. Ever since Van Leeuwenhoek started communicating his discoveries to the Royal Society in 1673, Hooke, who was then the Society's secretary, was unsuccessful in obtaining details about Van Leeuwenhoek's observation methods. Such details were deemed important because of the controversial nature of Van Leeuwenhoek's findings, and because transparency and reproducibility were important values to the Society (*14*). Underlying the controversy, too, was a contrast of characters (*15*). While Hooke had high esteem for an openness about research procedures, Van Leeuwenhoek remained secretive about his methods. In 1678, Hooke complained that "The manner how the said Mr. Leeuwenhoeck doth make these discoveries, he doth as yet not think fit to impart, for reasons best known to himself" (*12*). Lacking a methodology for the reproduction of Leeuwenhoek's observations, in his (printed) correspondence, Hooke suggested his own techniques instead. It was in this particular context—as a remedy for Van Leeuwenhoek's secrecy—that Hooke's 1678 recipe for his "easy-to-make" microscope lens saw wider circulation. Even in 1685, when Hooke sent the later Society Fellow Thomas Molyneux to Delft to find out details about the lenses, Van Leeuwenhoek kept the production details to himself. We may now assume that Van Leeuwenhoek's silence was a deliberate choice. The answer to the secret may have been known to Hooke much better than he ever imagined. Van Leeuwenhoek adopted the very lens-making procedure by Hooke soon after he published it, and brought it to a great success, but never told anyone about it.

Available microscopes: Limitations and opportunities

Van Leeuwenhoek's microscopes enabled the foundation of microbiology, yet due to their maker's secrecy, the lens-making procedures involved have to be deduced from very scarce sources and references. Of the hundreds of microscopes produced by Van Leeuwenhoek, only a handful has been preserved. Two of these have been submitted to neutron tomography in this research. As a result, a discussion of Van Leeuwenhoek's lens-making procedures can never be exhaustive; no single procedure can ever be ruled out completely. For instance, a reference in his correspondence informs us that in 1674, Van Leeuwenhoek used the egg of a codfish as a lens, positioned in front of his microscope, to observe a small, reversed image of his surroundings (*16*). Exceptional as this experiment may be, it reminds us of the originality and unorthodoxy of Van Leeuwenhoek's working procedures and warns us that surprises should not be ruled out.

Given the limited amount of evidence we have at our disposal, what current research can do, nonetheless, is positively identify lens-making procedures that are attested in Van Leeuwenhoek's preserved microscopes. Subsequently, the identified procedures can be matched with the known optical performance of the lenses. This allows us to judge the extent to which any unattested, custom lens-making method would have given Van Leeuwenhoek an actual observational benefit. In other words, it teaches us whether the head start that Van Leeuwenhoek had over his competitors should necessarily be attributed to secret lens-making procedures. Last, identifying attested lens-making procedures lets us compare these with concurrent techniques and allows us to position Van Leeuwenhoek's practice in the scholarly social networks of the 17th century.

Implications: Van Leeuwenhoek's microscopes

Hitherto, details about Van Leeuwenhoek's lenses' shape and optical characteristics hidden behind the metal retaining plates had remained unavailable. It was commonly agreed that many preserved microscopes held a ground lens, while the high-powered Utrecht lens was considered to be an exception. In our research, neutron tomography allowed us to peek inside the microscopes, determining the lenses' characteristics, and identifying their production methods. The two microscopes that were imaged are well-suited choices for investigating Van Leeuwenhoek's lens-making practice. One of them, the medium-powered instrument, is one of only three preserved microscopes (of which one misses the lens) having a provenance that can be fully traced back to Van Leeuwenhoek's ownership, through his family estate (*4*, *8*). The other one, the high-powered Utrecht microscope, has a provenance going back to the 1840s and has been put forward as a central instrument in the discussion about Van Leeuwenhoek's secret lens-making procedures.

Neutron tomography of these microscopes, then, has offered visually conclusive proof that Antoni van Leeuwenhoek did not limit himself to only a single lens type for making his pioneering discoveries but adopted distinct lens-making procedures that circulated at that time and integrated these into his microscopes. The neutron tomography results confirm that Van Leeuwenhoek ground and polished many of his lenses and carefully mounted these between crudely finished, yet custom-made brass plates, each specifically adapted to the lens it was intended to hold. The tomograms also confirm that the high-powered microscope preserved in Utrecht does not contain a ground lens but a flameworked one. Unexpectedly, its shape completely fits the "exceedingly easy" lens-making procedure that Robert Hooke published in 1678. This is ironic, as Hooke always wanted to find out the secret of Van Leeuwenhoek's lenses but never managed to do so. The encounter of this type of lens contradicts Van Zuylen's assertion that the Utrecht microscope testifies to a custom glass-blowing technique developed personally by Van Leeuwenhoek for the lenses of his highest-powered microscopes and, consequently, that Van Leeuwenhoek managed to take advantage of the optical benefits of the aspherical surfaces that such a secret method would create. Yet, with Van Zuylen, and with Zuidervaart and Anderson, our find does confirm that flameworking played a more prominent role in Van Leeuwenhoek's lens production than is often assumed. On a larger scale, it underlines how glass melting and flameworking techniques brought quality microscopic observations within the reach of a far broader audience in the second half of the 17th century.

Taking into account the discovered lens types and the revised production methods revealed through neutron imaging—how can we now explain the superiority of Van Leeuwenhoek's microscopes and, in particular, the superiority of his high-powered instruments? First of all, the encounter of a "Hooke-type" lens in the Utrecht microscope implies that Van Leeuwenhoek did not need to resort to secret lens-making procedures to achieve superior performance. Rather, Van Leeuwenhoek deployed several lens-making procedures. As we have shown, one of these was Hooke's globular lens recipe, which—as geometrical analyses show—was inherently promising in terms of performance and resolving power, as long as the globule was manufactured with care and the aperture was tuned for an optimal performance of the lens. Our data reveal that, although his microscopes were relatively "crude" in finish, Van Leeuwenhoek did indeed pay specific attention to such considerations. For the medium-powered microscope, he carefully matched the lens plate recess to the lens. For the high-powered microscope, in which lens configuration is even more critical, Van Leeuwenhoek adopted Hooke's promising globular lens design, and incorporated this into his microscopes with specific attention for the object distance and a carefully controlled lens aperture. The different dimensions he gave to the object-side versus the eye-side aperture further attest to this care. Essentially, through these considerations, he pushed the design to the best of its capabilities.

In particular, Walther derived that, for any single-lens "Leeuwenhoek-type" microscope under normal viewing conditions, spherical aberration is likely the prime harmful effect that needs to be addressed, but a good balance between magnification and resolving power can be achieved—preferably with globular lenses—by choosing the lenses' working aperture carefully, and proportional to the magnifying power. This optimal balance persists up to magnifications of about ×270 (*11*). Limited as the data from his preserved microscopes may be, the preserved instruments do show that Van Leeuwenhoek came a long way in approaching these ideal conditions. For the Utrecht instrument for instance, we observed that Van Leeuwenhoek gave his globule a working aperture (NA) of 0.37. In the diffraction limited case, such a system may theoretically be able to resolve details down to 1.0 μm in size. Comparing this to the actual resolving power of the microscope, one does not end up disappointed. Van Leeuwenhoek's skills were clearly of a sufficient level to maintain high standards when making and implementing such high-powered lenses. The fact that, even after 300 years, Van Leeuwenhoek's partially damaged (*3*, *4*) lens still resolves 1.35 μm shows how well he was able to deploy and improve Hooke's recipe up to the best of its theoretical capabilities.

Consequently, if Van Leeuwenhoek's observational head start was less due to secret lens-making techniques, it was craftsmanship and careful aperture control that made the difference. It is notable that Van Leeuwenhoek's much-heralded 266-powered microscope, unsurpassed in optical performance until the 1830s, turns out to have been produced by a relatively easy and straightforward method, one that was accessible to many. However, notwithstanding his personal skills, Van Leeuwenhoek was definitely well aware of the production methods reported by his contemporaries, and he experimented and implemented their methods as well. Through the successive production of his hundreds of microscopes, Van Leeuwenhoek seems to have fully mastered these grinding and flameworking techniques, to have combined them with appropriate apertures, and to have brought them to perfection, resulting in the superiority of his iconic microscopes in which all the attention and efforts were guided toward their one essential component: the lens.

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Acknowledgments: We are grateful to the director and curators of the Utrecht University Museum for making their Van Leeuwenhoek microscope available for research. **Funding:** This work was financially supported by The Netherlands Organization for Scientific Research/Netherlands Institute for Conservation, Art and Science (NWO/NICAS) grant no. 628.007.032. **Author contributions:** All authors contributed equally to this article. **Competing interests:** The authors declare that they have no competing interests. **Data availability:** All data needed to evaluate the conclusions in the paper are present in the paper.

Submitted 15 October 2020
Accepted 29 March 2021
Published 14 May 2021
10.1126/sciadv.abf2402

Citation: T. Cocquyt, Z. Zhou, J. Plomp, L. van Eijck, Neutron tomography of Van Leeuwenhoek's microscopes. *Sci. Adv.* **7**, eabf2402 (2021).

Science Advances

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Sci. Adv., 7 (20), eabf2402. • DOI: 10.1126/sciadv.abf2402

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