THE VAN LEEUWENHOEK SPECIMENS

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[*Plates* 1 *to* 6]

Introduction

In May 1674 the pioneer Dutch microscopist Antony van Leeuwenhoek prepared a series of sections of plant and animal material. They were cut by hand, using an open razor, and were carefully enclosed in small paper packets or envelopes. These in turn he put into a larger envelope which was fixed to a sheet of notepaper. On 1 June 1674 van Leeuwenhoek wrote a letter to the Royal Society's then Secretary, Henry Oldenburg, and the final page of this epistle was the sheet that bore the hand-made paper envelopes in which these historic specimens were secreted.

The material was preserved in the Society's archive collections with the other letters that form the four great files of correspondence in the strong-room at Carlton House Terrace. It has remained there apparently undisturbed ever since, repeatedly ignored by many scholars who have studied van Leeuwenhoek's letters. Earlier this year, during a systematic examination of his extant works, I had the good fortune to discover these important examples of van Leeuwenhoek's dexterity. They are in excellent condition, comparable with the best hand-cut sections that would be prepared today, and provide a uniquely interesting and revealing insight into the state of microtechnique in the seventeenth century. Additional inferences can be drawn on related topics, including van Leeuwenhoek's sources of inspiration, most notably his possible introduction to the field of microscopy through Robert Hooke's influential *Micrographia* of which editions were published in 1665 and 1667.

To the historian these specimens comprise a unique example of 'scientific archaeology', and it is invaluable that they have been allowed to remain undisturbed over the 307 years that have elapsed since they were prepared. This has made it possible to submit them to detailed optical and scanning electron micrography. However, in each case the majority of the sections have been left undisturbed in the hope that techniques as yet undiscovered may be utilized in the future to extract further information from these artefacts from the dawn of microscopy.

Van Leeuwenhoek's microscopy

Few pioneers in microbiology have had a career exceptional in so many ways as van Leeuwenhoek. He was an autodidact, a monoglot speaker of the Early Modern Dutch that was spoken in the united provinces of Holland in the seventeenth century, yet he received international acclaim and acknowledgement in his own lifetime. Without any affiliations to a university, and with no school of student disciples to follow him, he revolutionized the orthodox understanding of the minutiae of life through systematic observations which he carried out as an amateur. He was visited by noblemen from many countries, and made countless important and unprecedented observations on subjects that ranged from plant crystalline deposits to the spermatozoa in many animal species and included the first observations of bacteria and the cell nucleus, all undertaken with the aid of small hand-made single lenses mounted in metal plates. The metals he refined himself, and he was able to extract and refine gold and silver from ore in his home workshop.

Controversy persists over his work, however. He has been dismissed as a dilettante (1), his work as a botanical histologist denigrated (2) and his claims to have observed bacteria are still disputed (3). Sometimes his efforts have been dubbed as painfully superficial and amateurish (4) and it has been said—without justification—that he produced chaotic results unless under the direction of others (5). His recognition as a visionary amateur during his lifetime may have been unusual, but he is equally noteworthy in being widely forgotten for a prolonged period after his death; indeed at the first international meeting held to commemorate his life's work, which was organized in his home town of Delft in 1875, there was no British representative. Van Leeuwenhoek's British biographer, Clifford Dobell, reports that the invitation sent to the Royal Society at the time was not even acknowledged (6). Yet even this meeting had its illogicality: the organizer was the microbiologist Christian Gottfried Ehrenberg and his declared intention was to celebrate the bicentenary of van Leeuwenhoek's discovery of the protozoa 'in 1675'. The discovery was made a year earlier than this, however, and was reported in *Philosophical Transactions* in September 1674 (7).

The first major biography of Antony van Leeuwenhoek was published in 1875 (8) and it was followed by a monumental, if highly partisan, study which was published in commemoration of the three hundredth anniversary of his birth (9). But van Leeuwenhoek was also the subject of the opening chapter of a lighter and more popular volume which appeared five years before Dobell's definitive work. Paul de Kruif did much in this account to rekindle interest in a pioneer who had been needlessly neglected (10), and placed on record for the first time many of van Leeuwenhoek's greatest achievements. More recently, the lecturer in the History of Biology at Leiden, who was appointed Editor-in-Chief of the *Collected Letters of Antony van Leeuwenhoek* in 1942, A. Schierbeek, has published a two-volume biographical study in Dutch, which gave rise to a shorter version in English published in 1959 (11, 12). Maria Rooseboom, former Director of the National Museum of the History of Science at Leiden, contributed an important biographical sketch of van Leeuwenhoek to this review (13).

Of these books the comprehensive and admirably painstaking biography by Dobell (9) is the master-work. In terms of detail and broad-based approach it is impossible to fault, and it portrays to a remarkable degree the social milieu in which van Leeuwenhoek worked as well as analysing in detail so many aspects of his life and work, including such details as the seals he left behind, portraits in which he appeared, or might have appeared, and the anecdotes that lie behind the fate of the landmarks associated with his life. This book shows how slight has been the interest in preserving the sites of special interest, and it is easy to imagine the sense of frustration Dobell must have experienced when he located the house in which van Leeuwenhoek was born, commissioned photographs of it, only to find that they were reproduced before his own publication was ready (14) and that the building itself was demolished shortly afterwards (15). He refers to the only monument to van Leeuwenhoek that was at that time visible on the streets of Delft, a bronze plaque on the railings at the corner of Oude Delft–Boterbrug (= Old Delft–Butter-bridge), and bemoans the fact that it hangs at a corner which has no associations with any of the houses, or even streets, in which van Leeuwenhoek lived. The plaque was taken from the painting by Johannes Verkolje made in 1686, and it states that the discoverer of microbes 'lived and worked in this town' [*Stede*]. However, this was not the inscription which Dobell saw after the plaque was first erected in 1909 for, though he does not quote the words, he objects to its inaccuracy: 'Leeuwenhoek never lived on the spot where his modern bronze effigy with its false inscription now hangs. His real residence was in a neighbouring street—the *Hippolytusbuurt*—and has long since vanished without trace' (16). I have examined the plaque closely, and it is clear that the last word has in fact been altered. Doubtless the plaque, when it was first designed by J. C. Schultz, originally proclaimed that van Leeuwenhoek lived and worked 'in this street', which would explain Dobell's indignation. The fact that it now reads 'in this town', and that the last word has been subsequently brazed into position, is a concession to our growing insight into the details of van Leeuwenhoek's life.

There are aspects of Dobell's biography which cast an undignified light on his analysis. For example, he is quick to condemn in a most virulent fashion any detractor from van Leeuwenhoek's attainments. Thus von Uffenbach is dismissed as a 'complacent German diarist' making 'fatuous comments' and Dobell takes him to task for '[spelling] his name "Leuwenhoeck" throughout—a mistake I have taken the liberty of correcting'. Yet variations of the spelling exist elsewhere: van Leeuwenhoek used variants himself, and acquired the 'van' as late as 1685, apparently as an affectation. Dobell notes nineteen different ways in which van Leeuwenhoek's name was spelt in the columns of *Philosophical Transactions* (18), and does not allow that to disturb him as much (one of the possibilities he notes was exactly that used by Uffenbach). Similarly, Dobell takes Uffenbach to task for his use of Latin expressions, which he dismisses as pedantry. Yet in the time that account was written, 1710, it was perfectly acceptable to employ such phrases as '*in spiritu vini*'. Dobell resorts to such elegant expressions as *lapsus calami*, *sub voce*, *in litt.*, in his own writing and it can be argued (19) that this undignified use of what appear to be double standards detracts in some respects from the flavour of objectivity that such a magnificently comprehensive tome should embody.

A much more general area of neglect, however, covers van Leeuwenhoek's activities as a microtomist—a section-cutter who prepared material by hand in order to render it thinner, more translucent, and easier to observe in fine detail. The reason for this neglect lies in the great (and understandable) emphasis that has been placed on van Leeuwenhoek's pioneering discoveries as a microbiologist. The titles of the various biographical studies illustrate this preoccupation, and are replete with phrases such as 'little animals' (9), 'microbe hunters' (10), 'discoverer of protozoa' (*Ontdekker der Infusorien*) (8), and so forth. The standard accounts tend to begin with the letter sent from Delft to Henry Oldenburg and dated 7 September 1674 (20). In it, van Leeuwenhoek describes his journey to Berkelse Mere, two hours from his town. He collected some of the water in a glass phial and then wrote in rapture of the tiny green threads, the darting protozoa and rotifers, the slow-moving ciliates and the multifarious unicellular algae he could observe next day through his microscope:

> I found . . . some green streaks, spirally wound serpent-wise and orderly arranged, after the manner of the copper or tin coils which distillers use to cool their liquors as they distil over. The whole circumference of each of these streaks was about the thickness of a hair of one's head. Other particles had but the beginning of the foresaid streak; but all consisted of very small green globules joined together: and there were very many small green globules as well. Among these there were, besides, very many little

animalcules whereof some were roundish, while others, a bit bigger, consisted of an oval. On these last I saw two little legs near the head, and two little fins at the hindmost end of the body. Others were somewhat longer than an oval, and these were very slow a-moving, and few in number. These animalcules had divers colours, some being whitish and transparent; others with green and very glittering little scales; others again were green in the middle and before and behind white; others were yet ashen grey. And the motion of most of these animalcules in the water was so swift, and so various, upwards, downwards, and round about, that 'twas wonderful to see. (21)

In these words the foundations of microbiology were laid. It is tempting to see the organisms that van Leeuwenhoek describes so vividly as rotifers, ciliates and motile algae, with *Euglena* and *Spirogyra* clearly recognizable. But apart from this essentially vague exercise in diagnostic taxonomy the more important message of van Leeuwenhoek's words lies in the sense of wonder and excitement which the sight engendered in his mind. Freshwater microscopy became a major pastime for the Victorian microscopists a century ago, and in an era in which the optical microscope is being somewhat neglected in our schools and colleges it is arguable that the introduction of this simple yet entrancing demonstration would do much to restore a sense of perspective in the teaching of biology today.

Though this demonstration of the teeming freshwater populations was epoch-making, van Leeuwenhoek next refers to observations on aquatic micro-organisms in a letter written fifteen months later (22) but states only that 'some were incredibly small, less even than the animalcules which others have discovered in water, and which have been called by the name of Water-flea, or Water-louse'. One month after this, in January 1676, he wrote again to Oldenburg (23) and promises: 'Upon these [living creatures discovered by me in water] I have made divers notes, concerning their colour, figure, the parts whereof their body is composed, their motion, and the sudden bursting of their whole body; of which notes I keep a copy by me, which I shall send you at the earliest opportunity.'

The notes became the bulk of van Leeuwenhoek's most famous letter to the Royal Society. Dated 9 October 1676 (24) it covers eighteen pages of notepaper and sets the scene on which all the subsequent development of observational microbiology was based. The letter, though apparently corrected by van Leeuwenhoek, is not in his handwriting and it seems to represent a fair copy made from his daily diary. The narrative nature of the description may be sampled from his paragraphs on the observation of micro-organisms in an infusion of pepper-water:

> The 4th and 5th of May [1676] I examined it again, but perceived no living creatures. The 6th *ditto* I discovered very many exceedingly small animalcules. Their body seemed, to my eye, twice as long as broad. Their motion was very slow, and oft-times roundabout.
> The 7th *ditto*, I saw the last mentioned animalcules in still greater numbers.
> On the 10th *ditto*, I added more snow-water to the pepper, because the water was again so diminished that the pepper-corns began to dry out.
> The 13th and 14th *ditto*, the animalcules as before.
> The 18th of May, the water was again so dried away, that I added snow-water to it once more. (25)

The conclusion that van Leeuwenhoek observed bacteria in his infusions has been well documented by Dobell. He cites van Leeuwenhoek's description of a micro-organism '3 or 4 times as long as broad, though their whole thickness was not, in my judgement, much thicker than one of the hairs wherewith the body of a louse is beset' and, using the description together with the dimensions of the reference object van Leeuwenhoek uses (a hair on the body of a louse being approximately 3μm in thickness) he confidently identifies the organism as *Bodo caudatus* (Duj.) Stein, which is a common organism in suspensions of organic matter. The careful descriptions of the organism and its behaviour indeed makes the identification safe. Van Leeuwenhoek moves from this description to organisms that were, by comparison, 'incredibly small; nay, so small in my sight, that I judged that even if 100 of these very wee animals lay stretched out against one another, they could not reach to the length of a grain of coarse sand'. This apparently vague measurement can be quantified with some accuracy (26) at $\frac{1}{30}$ inch, in which case the organisms described are *less than* 8μm in length. He goes on to describe 'very tiny eels; but now I could not see their bending, when they moved, so very plainly as I had seen it before . . . my method for seeking the very smallest animalcules, and the little eels, I do not impart to others; nor yet that for seeing very many animalcules all at once; but I keep that for myself alone'.

This vagueness, to the modern mind, contains the seeds of doubt which have encouraged so many of van Leeuwenhoek's detractors. Suppose his estimate of proportion had been wrong; what if he had over- or under-estimated the dimensions of his sand-grain; did his manifest secrecy over the technique he claimed to use for observations on the smallest micro-organisms conceal an element of hyperbole or even charlatanism? To the student of van

Leeuwenhoek's writings, such questions seem abhorrent. His writings reveal a transparently self-critical honesty and any notion of deliberate fraud or exaggeration seems absurd. The doubts have been reinforced by our ready acceptance of the compound microscope, and the concomitant belief that a single lens cannot produce adequate resolution (3). Much has been written on the magnification and numerical aperture of van Leeuwenhoek's lenses (26) and it has been accepted that in theory he could have resolved bacteria, but these data have not convinced everyone that the hypothesis was ever translated into reality. Yet the capacity of a single lens to magnify has long been greatly underestimated. One can readily show that even a crudely melted bead-lens can produce relatively clear images with the minimum of sophistication (27) and the simple (i.e. single-lensed) microscopes of the eighteenth century were in many ways capable of producing results comparable with a basic compound microscope of today (28). It is possible to observe bacteria with such an instrument. Using the most powerful of the microscopes made by van Leeuwenhoek that is still known to exist (29) I have on several occasions resolved stained preparations of bacteria including *Sarcina lutea*, an unusually robust organism, and *Bacillus subtilis*. However, it is an error to draw extrapolative conclusions based on observations of specimens which were unavailable at the time, and a stained preparation on a modern microscope slide is not comparable with the fresh and unstained material that van Leeuwenhoek must have used. In addition, the microscope cited is most unlikely to be the most powerful that van Leeuwenhoek ever made. One recent examination of this microscope (which is illustrated in Figure 2) suggests the magnification is $\times 266$ with a resolution of 1.3 μm (29), whilst it has been argued that his 'lost' lenses may have included examples that were capable of magnifications of $\times$ 400 or even $\times 500$ (30), perhaps with correspondingly improved resolution. Work in progress as this paper goes to press suggests that the Utrecht lens may be capable of better resolution even than this, as I have resolved fungal hyphae with it and they appear to be 1 μm in diameter or perhaps even less. The only means we have of demonstrating the resolution of bacteria is to utilize single lenses and to apply them to material that is free from the modern artefact of microscopical preparation.

Accordingly I investigated unstained, fresh preparations of bacteria of the kind van Leeuwenhoek would have used. One series of experiments gave comparable micrographs of buccal mucosa smears seen through a modern high-resolution instrument and the single lens of a Wilson screw-barrel microscope (31). This comparison demonstrated that larger bacteria could be resolved satisfactorily with this mass-production lens. More recently I have undertaken some studies of living bacteria with the aid of a lens ground from the mineral spinel by Horace Dall, an amateur lens-grinder who lives and works in Luton, Bedfordshire. I have shown the magnification of this lens at a standard primary image distance of 250mm to be ×395, so it may represent van Leeuwenhoek's better lenses. With this it is possible to obtain clear images of living pond bacteria of the genus *Spirillum* (Figure 3) in confirmation of the claim advanced in van Leeuwenhoek's celebrated letter of 9 October 1676 (p. 41 *supra*). The capacity of single lenses has clearly been greatly underestimated, then, and the many major discoveries which were made using simple microscopes are due for reassessment (32). It is, however, important that due understanding is given to the preparation of material for experiments of this kind. Misunderstandings of the kind of specimens available are rife (54) and attempts to reconstruct the appearance of images generated by early microscopes err when they are based on observing sophisticated preparations through primitive instruments for which such material would not have been available (33). I have argued elsewhere, in surveying the development of microtechnique (34) that our limited understanding of this facet of microscopy is long overdue for attention. It is almost unthinkable, for example, that today one would examine a whole insect in the round, as it were, held in forceps on a microscope stage. But spring-loaded stage forceps were in use from the early years of the microscope (35) until the turn of the present century, and the view of nature they provided was closer to reality than the artificially cleared, flattened and mounted microscope preparations of the present day (36).

Equally important is the patient and conscientious use of the microscope, understanding its individual characteristics and the various means available of enhancing contrast and maximizing image distinctness. Antony van Leeuwenhoek noted the striations that are visible on voluntary muscle, for example (37), and using a later simple microscope by Banks I have obtained micrographs of this kind of material in which the striations remain unresolved, though with diligence and attention to the substage mirror (which affects the angle at which light strikes the specimen) and the distance of the light-source (which has marked effects on the capacity of the lens to resolve fine detail) they can subsequently be visualized with clarity. Not only is it important to take into account the limitations of specimen preparation and the technical limits of the microscope concerned, therefore, but the practical use of the instrument has the greatest relevance to whether a given structure could actually be observed by the microscopist or not. There is a tendency to place too much emphasis on the measurable parameters of a given lens (e.g. magnification, radius of curvature of the lens face, calculated numerical aperture, etc.) and not enough on the practical considerations, including the essential question: 'What did the specimen truly look like?' When we bear in mind that two lenses of similar theoretical performance can provide images of widely differing quality, the timeliness of this principle should need no emphasis.

However, it is difficult to recreate van Leeuwenhoek's experiments in detail as so little was recorded by him of his exact working methods. We have seen (p. 42, *supra*) that he was deliberately secretive about his techniques. Revealing as his observations of micro-organisms are, they say little of his abilities in the area of specimen preparation, for these most interesting objects were examined in the living state, unaltered and without any special preparation. According to van Leeuwenhoek (38) they were examined in fine glass tubes, or in some cases—since he describes how they would change on drying (39)—even on slivers of glass or mica. In either event the specimen was held with a little glue or wax on to the point of a spike which he mounted adjacent to the plates holding the lens between them (see figure 2). Such a procedure hardly amounts to microtechnique in any degree. We have seen that most of the interest in van Leeuwenhoek's observations centres on his investigations of micro-organisms, and the biographies tend to commence their examination of his work with the letter of 9 October 1676, *q.v.*, paying scant attention to what went before (40). For this reason I commenced a search through the letters that are stored in the Society's collections in the hope of uncovering evidence of his work on inanimate specimens and the techniques he might have used in preparing them for examination. It was through this investigation that I discovered a series of sections sent to London by van Leeuwenhoek in 1674. They have since been examined in detail.

The specimens

The first communication of van Leeuwenhoek's work to the Royal Society came about through Reinier de Graaf (41) who on 28 April 1673 wrote to Oldenburg that 'a certain most ingenious person here, named *Leewenhoeck*, has devised microscopes which far surpass those which we have hitherto seen. . . . The enclosed letter from him, wherein he describes certain things which he has observed more accurately than previous authors, will afford you a sample of his work'. The enclosed descriptions were of fungus sporangia, the sting, mouth-parts and eye of a bee, with some notes on the head-louse, and were published in *Philosophical Transactions* (42). They were well received, and van Leeuwenhoek began his work in earnest. Fourteen months later, on 1 June 1674, he sent his fourth communication to the Society. It extends over six folio pages and is written in his own hand. The subjects he discusses include the microscopy of blood, the structure of bone, teeth, liver and brain, and the growth of the epidermis. The final leaf of the letter is blank, however, but on turning it over I noticed a small envelope pasted to the last page. Cautiously I opened it, anticipating that it contained some artefact; inside were four smaller paper packets, and three of these proved to contain finely cut sections that van Leeuwenhoek had prepared with his own hand for the interest of the Royal Society. Doubtless he obtained these specimens in the days or weeks before sending them to London, in which case they date from May 1674. Such specimens, from the earliest days of microtechnique, give an exceptional opportunity for insight into van Leeuwenhoek's abilities which his recorded observations alone cannot provide.

Even to the naked eye the specimens were in part recognizable. Specimen # 1 was of cork from *Quercus suber*, prepared as finely translucent sections ranging in length from approximately 1.5mm to some 7mm. Even a cursory examination showed that they were of histological thinness, and compared favourably with the best hand-cut sections that might be obtained today. Specimen # 2 was clearly elder-pith from *Sambucus nigra*, this time in the form of rounded sections cut from young stems perhaps 15mm in external diameter. Packet # 3 was empty, but the labelling suggests it contained material from a writing-quill. Specimen # 4 was not immediately identifiable: it had the appearance of a sectioned aquatic stem with a thickened outer wall and a spongy medullary cavity. However, microscopy proved that this initial impression was erroneous. The sections were of optic nerve, the 'lacunae' being derived from the loss of material through degeneration over the centuries. The closing passages of van Leeuwenhoek's letter (quoted here from the translation (43)) provide the provenance:

> Which kind of progress of growing I apprehend may in some manner be seen in the pith of *Wood*, in *Cork*, in the pith of the Elder, as also in the White of a *Quill*, of which three last I have sent you and your curious Friends some small particles, cut off with a sharp Razor, thinking it well worth their observation. Only I would here advertise, that when any of these particles is applied to the pin of a Microscope, the instrument may be held within doors and in the shade, yet held to the free Air, as if with a Telescope, you would look upon the Stars in the Firmament.

What an abundance of information may be derived from so short a passage. We have an indication of the method van Leeuwenhoek used to cut his sections, an outline of the way they were held in position on the stage bracket of his microscope (29), and advice on the use of a restricted source of illumination which shows that he was aware of the benefits of limiting the diameter of the source in an attempt to control the aperture of the illuminant cone. Using these single-lensed microscopes against a light source of too great an angular size causes severe image degradation (for example, if the observer stands close to a larger window, or uses the instrument in the open air). But a dark room with a window some distance away, as van Leeuwenhoek seems to be suggesting, provides a restricted cone of illumination and this fits well with the optical limitations of the lens.

The most memorable impression that these delicate specimens left in the mind was of their pristine appearance: the elder pith sections are near-white and glistening, the cork material a pale pink colour as if taken from a high-quality cork of today. There is no sign of a foxed, discoloured or faded appearance. Similarly, the sections of nerve are clean and new-looking. The botanical material is desiccated but is free of visible attack by mites or damage by fungi. A unique feature is that the specimens had over the centuries been pressed together so that both sets of plant material were compacted into a single mass which careful dissection was able to unravel. The Society's Archivist, Mr Leslie Townsend, was kind enough to allow me to remove portions of the specimens for microscopy, but in the interests of preserving these historic sections for the benefit of study in future centuries, when sophisticated techniques of analysis may enable more detailed information to be derived, the majority of the sections were left undisturbed. One-third of them were removed and in each case one suitable specimen was selected for scanning electron microscopy.

The continuing search through the van Leeuwenhoek letters led to the discovery of five further packets of material. They are not as interesting in many ways: four of them contain portions of entire specimens, so they do not reveal any degree of microtechnique, and the dissected contents of the remaining specimen show a growth of fungus material which reduces its interest. The first two packets were found attached to a letter dated 2 April 1686. The first of these (designated here specimen # 5) contains a number of fragments of plant material, some of them thick transverse sections >200μm in thickness, which are identified as 'a cotton seed cut into 24 round slices' (44). The second of these packets, specimen # 6, contains larger portions of plant material and this is '9 seeds of the cotton tree, from which the membranes have been removed, and the leaves separated' (45). An initial examination showed that these specimens had been affected by an overgrowth of micro-organisms to some degree, doubtless because (as sections of fresh plant material) they were moist when packed away and in this manner acted as a suitable substrate for fungus growth.

Three further packets were discovered pasted to the final page of a letter dated 17 October 1687. They contain samples of thin papery materials, specimen # 7 being a blackened fragment apparently of paper, specimen # 8 an algal mat, and specimen # 9 described as: 'Paper which in 1686, 14 or 15 March in Courland, was said to have fallen from the sky'. The relevance of these specimens is revealed in the text:

> The same Gentleman told me, amongst other things, that there had fallen from the sky, on a field in Courland, on the 14th or 15th of March 1686, a piece of burned paper which was quite three sheets large, of which, so he said, he had a piece that he had observed through the microscope; but that he was unable to form an opinion of it. And as I made it clear that I would like to see this alleged paper, the said Gentleman sent me a piece (by letter), of which I hereby enclose about half.
>
> I had not had this supposed paper in my house for half an hour before I had (with the aid of the Microscope) formed such a clear idea of it, that I fancied it to be a plant which had come forth from the water. And moreover I took it for sure that, if it were true that it had fallen out of the Sky onto the Field, then this substance must first have been driven up into the air (by a Cloud which we call a Whirlwind); But I much rather believe that, owing to heavy rain, or melting snow, (if the country is a mountainous one) the water from a Morass or from Ditches, has flooded some piece of land, and that the water had left this green plant, from which the so-called paper is made, behind on a greensward or a field with young corn, with the result that the Sun and wind caused the plant to become dry and stiff, so that it took on to some extent the look of burned paper. (46)

I have quoted these words because, as well as accounting for the nature of the three specimens van Leeuwenhoek had enclosed, they convey the assurance and informed competence of the man in a situation where his microscope could give him evidence that could help to solve such a mystery and, what is more, it was evidence that could not be obtained in any other manner. As an example of problem-solving this is a worthy case to cite, and the methodical approach can be echoed in present-day investigations.

Specimens # 7, # 8, # 9 have been unaltered, and they do not therefore reveal anything of van Leeuwenhoek's technique. Specimen # 6 has been partly dissected, but is otherwise unhelpful in this respect; and # 5 (although described as 24 sections) represents such thick portions that 'slices', the word

FIGURE 1. The portrait of Antony van Leeuwenhoek which comprises the frontispiece of P. J. Haaxman's biography subtitled 'De Ontdekker der Infusorien' and was published in Leiden in 1875. This engraving is based on the 1686 portrait in oils by Johannes Verkolje (1650–1693). On the table in front of a globe lies the diploma conferring Fellowship of the Royal Society.

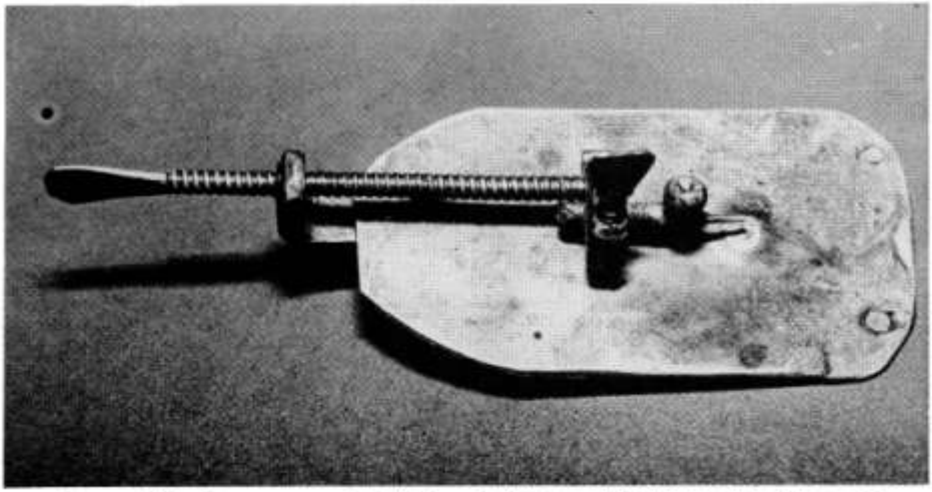

FIGURE 2. This microscope now in the University Museum in Utrecht is typical of those made by van Leeuwenhoek. The lens has a focal length of 0.94mm and a magnification of ×266 with a resolution of 1.3μm (29), and is held between two brass plates 46mm × 25mm in size.

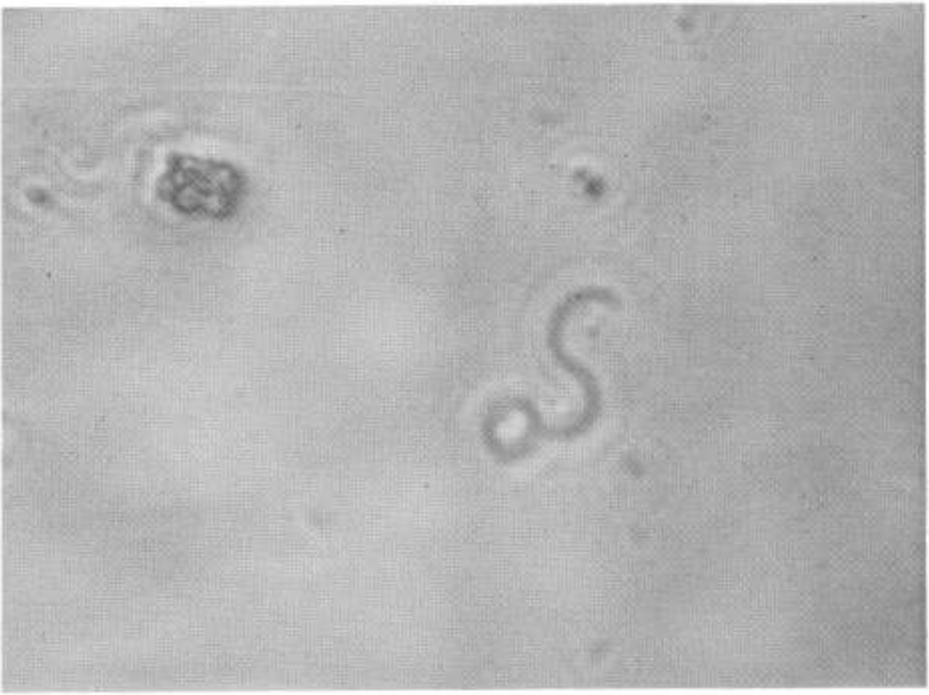

FIGURE 3. A study of pond bacteria of the genus *Spirillum* resolved by the author using a single lens of van Leeuwenhoek type. In this case the simple microscope contained a lens ground from the mineral spinel by H. Dall of Luton, Bedfordshire with a magnification of ×395. This seems to be typical of those high-power lenses van Leeuwenhoek made, estimates (30) suggest he may have produced lenses magnifying up to ×500.

van Leeuwenhoek employs (*schijfjens* in the original) is particularly apt. The fresh optic nerve is notoriously difficult to cut transversely, and in this case too (specimen # 4) the transverse sections are not of histological dimensions. Though van Leeuwenhoek was not to know this, the nerve trunk lacks cell bodies, and as the micrographs show his thinnest section gives an excellent insight into the organization of the supporting tissue around the nerve fibres. It is fair to say that these specimens (# 4–9 inclusive) are what might have been anticipated as typical of seventeenth-century microtechnique. Specimens # 1 and # 2, however, are in a different category altogether. They reveal a controlled and skilful ability to handle material, and portions of the sections are considerably less than single-cell thickness. Interestingly enough, the sections have a ridged appearance and this might be construed as indicating that an irregular cutting edge was employed. This is not the case. What van Leeuwenhoek contrived was a method of cutting which involved taking thin sections from the face of the specimen, whilst allowing the blade to rise gradually through the material. When he perceived that the specimen was being cut too thinly, and was breaking up, he allowed the direction of cut to alter slightly, taking a somewhat deeper 'bite'. In this manner he was able to obtain sections for microscopy in which extraordinarily thin portions were present in a matrix of somewhat thicker material. The thicker portions also acted as a support for the finest regions of these sections. Even the most solid-looking of the cork sections is less than 100μm at the thick edge, and as the micrographs show for large areas the thickness is <20μm and in parts around 5μm, with the finest tapering regions of the periphery being thinner even than that.

The plates show how the specimens that were prepared and stored in the moist condition have overgrown to a marked extent with organisms of spoliage. The preparations of cork, elder and nerve were essentially dry and they have retained their original appearance to a surprising degree. Occasional spores were demonstrated by scanning micrography and in some cases they had germinated. The specimens most affected were # 1, the cork sections. The smaller cell size encourages the retention of moisture and at the same time produces a more efficient *quasi*-filtering action than the sections of elder pith. Occasional traces of growth were visible, as the plates demonstrate, but in the material examined by the scanning electron microscope (SEM) there was no region in which the growth was significant. Fungus colonization of the optic nerve section in specimen # 4 was virtually absent. However, the nerve fibres themselves which make up the bundles known as fasciculi are now missing. The thin layers of perineurium which separate each bundle of fibres are well preserved, and give an open 'lacunate' structure rather like cell walls. Doubtless it was this parallel which took van Leeuwenhoek's attention. The outer sheath (which is thicker than is the case with most nerves, as it derives from both the dura and arachnoid membranes of the brain) is prominent and its cut surface shows the clean and even sectioning that was used. Overall, specimens #1, #2 and #4 are excellent examples of hand section-cutting at its finest, and the well-preserved condition of the material is testimony to van Leeuwenhoek's fortunate choice of container for his material. The simple expedient of wrapping the specimens in paper and storing them in an envelope has bequeathed to us and our descendants a unique selection of pioneering microscopical material in a state of preservation that it would be difficult to emulate through any other technique.

Sources of inspiration

Where did van Leeuwenhoek gain his inspiration for this monumental body of work? Does this investigation of some of his early specimens throw any light on his sources of understanding? I have proposed (47) that he may have been given some initial impetus by Robert Hooke's *Micrographia: or some Physiological Descriptions of Minute Bodies made by Magnifying Glasses. With Observations and Inquiries thereupon*, which as we have seen (p. 37) was the first major book devoted to the microscope. There are several reasons which encourage one to look closely at this likelihood.

a) Van Leeuwenhoek must have been professionally interested in the structure of cloth since after his birth on 24 October 1632 and his education in Warmond (north of Leiden) and Benthuizen (north-east of Delft) he found himself at the age of 16 in Amsterdam, apprenticed to a linen-draper. At that time hand lenses were used to 'count' cloth, i.e. to assess the density of the

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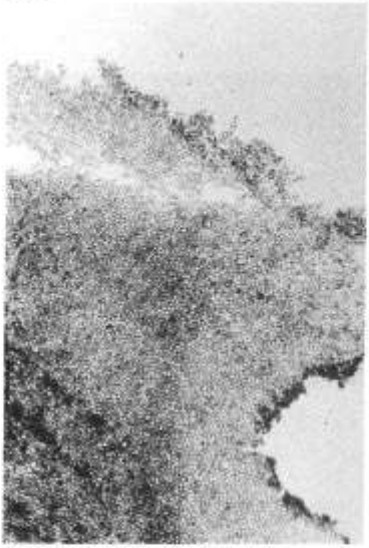
FIGURE 4. Specimen # 1: hand-cut sections of cork, with the packet in which they were found. The sections were neatly wrapped in white note-paper, each piece approximately the size of a cigarette paper. Approx. ×¾.

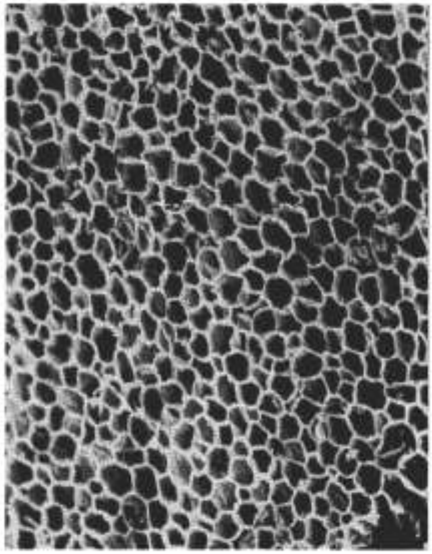
FIGURE 5. A single cork section viewed through a simple microscope made by Banks of London around 1820, showing the cell walls. ×40.

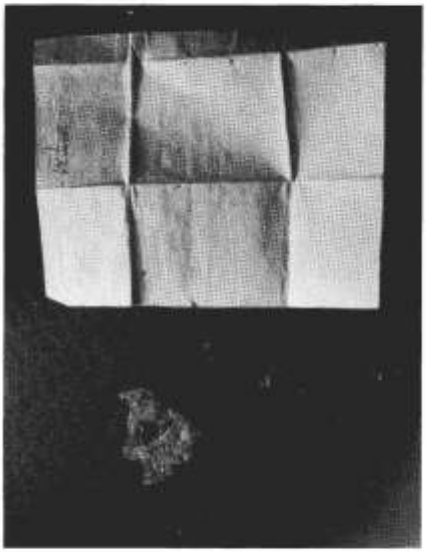
FIGURE 6. Scanning electronmicrograph of a single section from the packet in Figure 4. Note the ridged appearance caused by the cutting technique described on p. 49. ×50.

FIGURE 7. The extremely regular nature of van Leeuwenhoek's hand-cut sections shows clearly in this S.e.m. study of cork magnified ×250.

Plate 3







FIGURES 4 to 7. For description see opposite.

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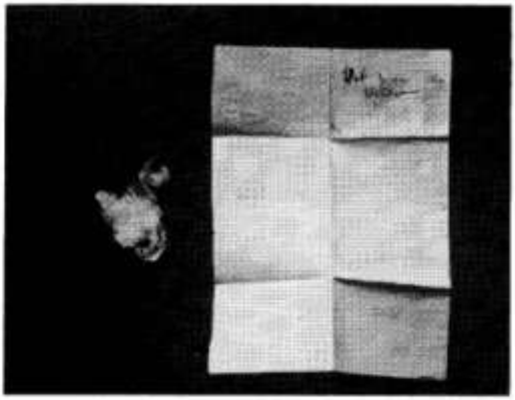

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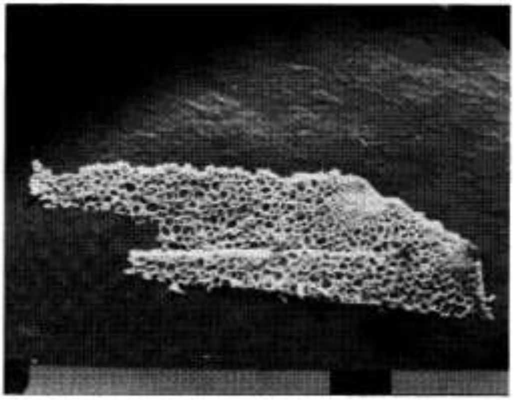

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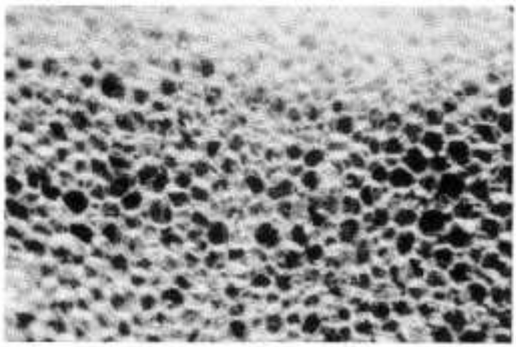

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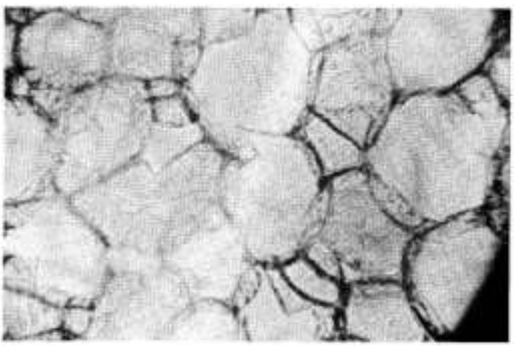

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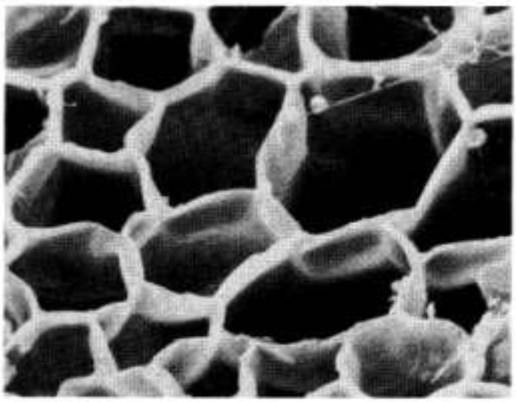

FIGURES 8 to 13. For description see opposite.

warp and weft, and the young van Leeuwenhoek would have known about their use.

b) Robert Hooke's opus was published first in 1665 and then, because it sold so well, a reprinted edition appeared in 1667. At the time it was a fashionable talking-point: Samuel Pepys wrote that he 'took home Hook's (*sic*) book of microscopy, a most excellent piece, and of which I am very proud' (48). In the late 1660s, it was a well-known volume and its message was widely understood.

c) In his letter of 7 September 1674 he states that he had been to England 'about six years ago', i.e. around 1668. His journey via Harwich took him along the Thames through Rochester and Gravesend to London. He writes that he was set thinking by the whiteness of the cliffs he saw and adds that he 'also tried to penetrate the parts of the chalk' (in the original *de deelen van het krijt te penetreren*) which Dobell takes to mean that he was using a microscope to examine the rocky samples he collected. In my view it is important to note that van Leeuwenhoek does not here say that he was able to 'penetrate' the structure; only that he tried to. The next sentence continues: '*At last* I observed that chalk consists of very small transparent particles; and these transparent particles lying one upon the other is, *methinks now*, the reason why chalk is white', (author's italics). Thus the uncertainty about the nature of the rock when he first saw it is complemented by references in a later period of time to his resolution of the matter. It is sensible therefore to suggest that at the time he was travelling to London his curiosity could not be satisfied, whereas at a *later* time he was able to investigate the structure of chalk further.

d) Among the specimens depicted in Hooke's book are some diagrams

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FIGURE 8. Sections of elder pith comprising Specimen # 2, shown with their paper packet annotated by van Leeuwenhoek.

FIGURE 9. A portion of Specimen # 2 viewed with a low-power simple microscope of van Leeuwenhoek type. ×8.

FIGURE 10. This single fragment of elder pith approximately 3mm in length shows the ridged appearance of van Leeuwenhoek's 'stepped' cutting technique. S.e.m. ×15.

FIGURE 11. A low-power study of elder pith, cut by van Leeuwenhoek, using a simple microscope. ×40.

FIGURE 12. Section of Specimen # 2 viewed with a high-power single lens of van Leeuwenhoek type, showing cell-wall detail. ×250.

FIGURE 13. This scanning electronmicrograph reveals strands of fungal hypae and occasion spores. They are too infrequent to have produced significant change in the material in over three centuries. ×500.

showing cloth samples under the microscope. It is inevitable that anyone who read the book would be attracted to depictions of subjects with which they were themselves concerned, hence it is clear that the studies of fabric would have been of noteworthy interest to the weavers and drapers of London.

Since van Leeuwenhoek was in the drapery business, it seems probable he would have been introduced to *Micrographia*. He was in London very shortly after the 2nd edition of that book was published, perhaps in the same year, and his inquiring mind would have made him an avid investigator of Hooke's findings. We could only conclude that he did not know of the book if we accept that he did not visit acquaintances in the drapery business or, if he did, they did not mention the fascinating coverage of their special subject of interest in one of London's most popular books of the time.

It is interesting that van Leeuwenhoek's earliest letters concern topics which are described by Hooke, indeed his first epistle of all (49) specifically takes Hooke to task, without naming him, for his published descriptions of fungal sporangia. Van Leeuwenhoek writes as follows: 'The mold . . . has been seen by some represented to be shott out in the form of the stalks of vegetables . . . but I do observe such Mold to shoot up first with a straight transparent stalk, and is follow'd by another globul, driving out the first either side-ways, or at the top . . . and this knob indeed consists of nothing else than of many small roundish knobs, which being multiplied, the big knob begins to burst asunder.'. Van Leeuwenhoek often resorts to oblique, anonymous references to people with whom he intends to disagree, and it is not unusual to see this device employed here. For example, the description on p. 41 is a reference to names coined by Jan Swammerdam (whose *watervlooy* is what we would know as the water-flea *Daphnia pulex*). But the note of chastisement for Robert Hooke becomes clear when we look at what he wrote about the sporangial structures of molds. Hooke has surprised many people by observing sporangia,

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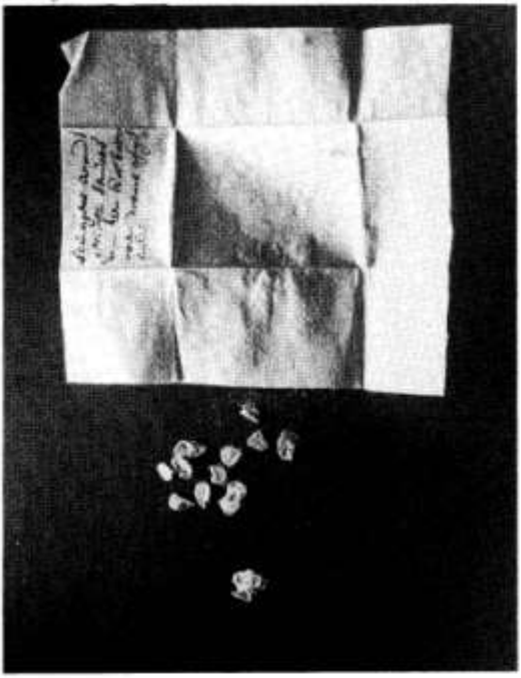
FIGURE 14. Specimen # 3 appears to have been material from a writing-quill. It was missing from its packet. However, it would have provided little information on van Leeuwenhoek's microtechnique as the material would not have been in transverse sections (see text).

FIGURE 15. The packet for Specimen # 4 contained approximately 18 sections of optic nerve from a cow.

FIGURE 16. A single section (visible, with its distinctive shape, on far left in Figure 15) viewed under a low-power optical microscope. ×10.

FIGURE 17. Portion of section in Figure 16 under the scanning electron microscope. Note even surface of nerve sheath and perineurium forming lacunate structure of nerve cord itself. ×100.

15



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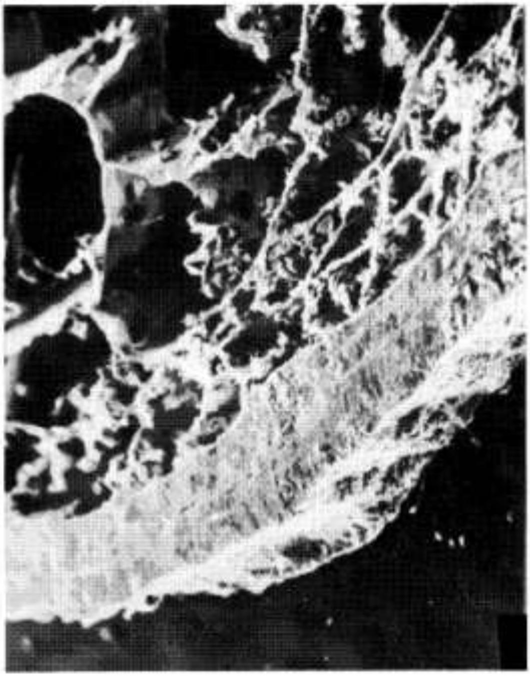

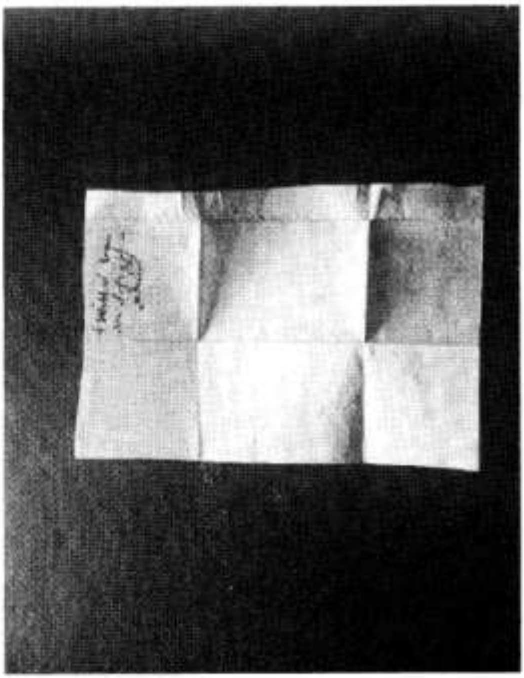

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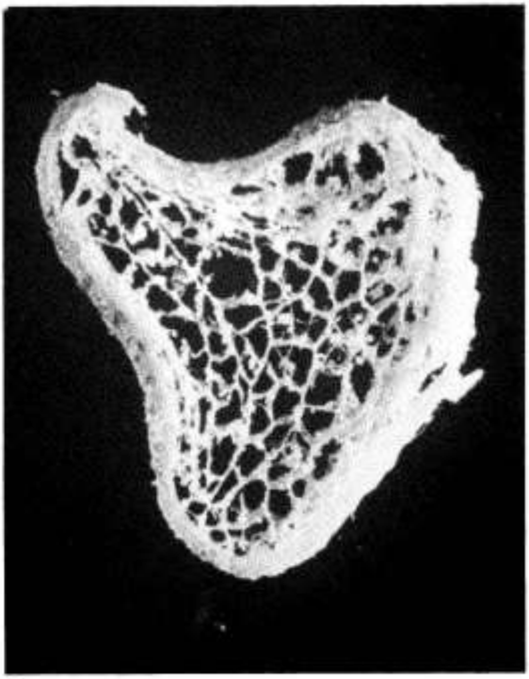

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FIGURES 14 to 17. For description see opposite.

Plate 6

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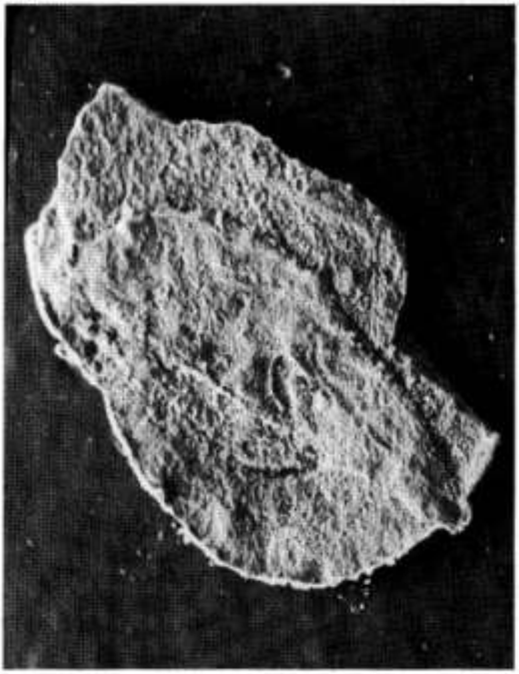

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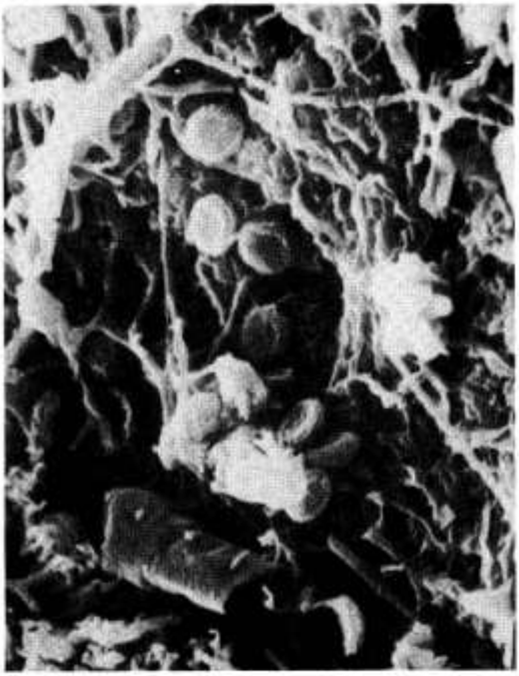

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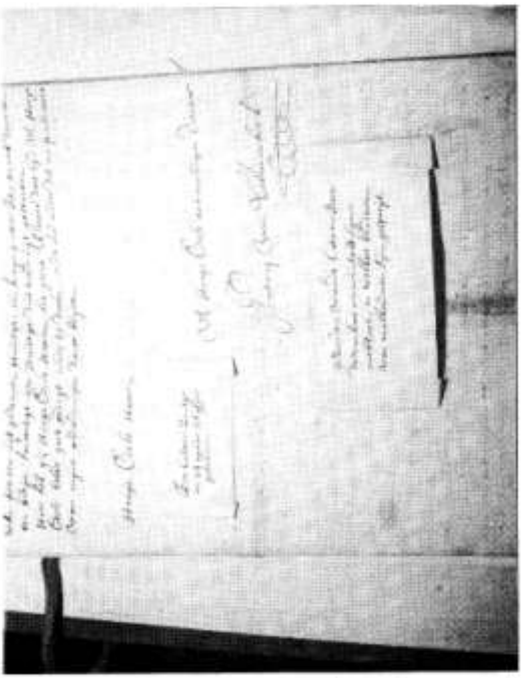

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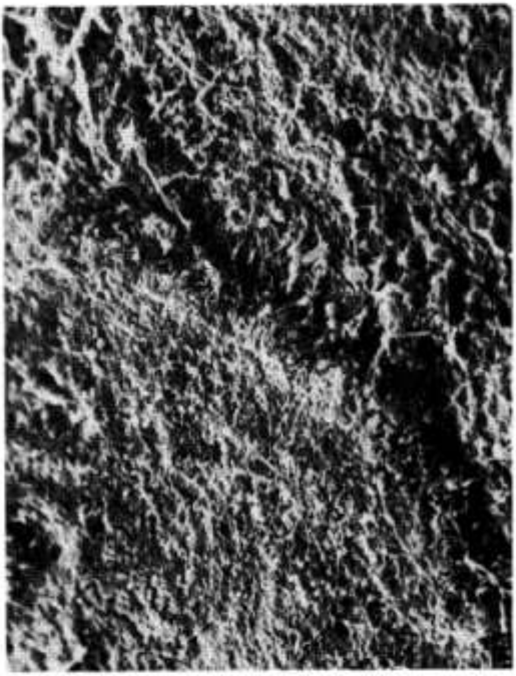

FIGURES 18 TO 21. For description see opposite.

and figuring them in the dehisced state, yet without observing any spores. It is this which van Leeuwenhoek finds equally surprising. Hooke's description (50) runs thus:

> A very pretty shap'd Vegetative body, which, from almost the same part . . . shot out multitudes of small long cylindrical and transparent stalks, not exactly streight, but a little bended with the weight of a round and white knob that grew on the top of each of them. What these heads contain'd I could not perceive [but I consider] that Mould require[s] no seminal property, but may be produc'd at any time from any kind of *putrifying* Animal or Vegetable substance . . .

There is not only a correlation of subject-matter too close for mere coincidence to be at work, but terminological quirks, such as the use of the term 'shot', which make the relationship unambiguous. It becomes clear from van Leeuwenhoek's writings that his instincts were deeply opposed to the notion of spontaneous generation, and this may well be the reason for his immediate rebuttal of Hooke's proposition. Van Leeuwenhoek's biographers make much of his inability to read English, but this is no argument. His second wife Cornelia Swalmius (married: 25 January 1671) was by all accounts a 'bluestocking', and in any event a knowledge of English is not necessary for him to have been captivated by Hooke's illustrated volume. The topics raised in *Micrographia* would have been discussed in van Leeuwenhoek's language, of course, and this reference to part of the book's contents dating from the dawn of his contact with the Royal Society shows that he was well able to know what it contained.

The specimens described in this article provide some further evidence in

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FIGURE 18. These two packets, containing Specimens # 5, # 6, were mentioned as 'drawn rectangles' when the letters were translated from microfilm. They contain a cotton seed cut into 24 sections, and dissected cotyledons respectively.

FIGURE 19. This section, largely complete, shows the cotton seed embryonic structures to be heavily overgrown with hyphae and considerably spoiled. ×25.

FIGURE 20. An enlargement of the central portion of Specimen # 6, sectioned cotton seed, shows severely degraded cellular structure due to fungus growth during storage. ×180.

FIGURE 21. High-power scanning electronmicrograph of the cotton seed section shows fungus spores and portion of arthropod exoskeleton, which appears to indicate spoilage by mites during storage. Only the moist specimens have been subject to damage of this sort, the fine sections that comprise Specimens # 1 & # 2 (cork and elder pith) were of dry material and are in pristine condition. ×1750.

favour of this thesis, for cork is one of the major topics discussed at length by Hooke. It is fitting that this type of material should have been bequeathed to us by van Leeuwenhoek, for it was in his description of the structure of cork that Hooke gave us the immortal coinage of the term 'cell' (a word whose origins are incorrectly described by the *Oxford English Dictionary* (51)). In describing the honeycomb structure of cork sections, Hooke wrote (52): 'These pores, or cells, were not very deep, but consisted of a great many little Boxes . . .' and in this fashion he proposed a term which is at the mainstay of modern biology. But if we read on we find, just two pages later, the following almost haunting words:

> Nor is this kind of Texture peculiar to Cork onely; for upon examination with my *Microscope*, I have found that the pith of an Elder, or almost any other Tree . . . have much the same kind of *Schematisme*, as I have lately shewn that of Cork . . . The pith also that fills that part of the stalk of a feather that is above the Quil, has much such a kind of texture.

Cork, elder pith and the shaft of a quill feather occur here, not merely in the same section of prose, but in the same order in which van Leeuwenhoek in turn listed them when he came to send his own specimens to the Royal Society. Not only do the marvellous sections illustrate how well-deserved is van Leeuwenhoek's reputation as the father of microscopy, but they show him a master of microtechnique and, at the same time, reflect some honour on Robert Hooke. Surely he can be seen as an important instigator, albeit an unwitting one, of van Leeuwenhoek's lifetime devotion to the microscope.

Postscript

It remains for us to account for the presence of these specimens in their original condition, secure in the Society's archives. The words in which van Leeuwenhoek stated that he was sending them to London have been extensively reprinted in the various editions and translations of his letters (53) and as we have seen, p. 46 *supra*, the fact is entirely unmistakable. Not only was the presence of the sectioned material recorded thus (it was mentioned in Dobell, for example) but the two packets of cotton seeds were recorded as 'drawn rectangles' in the translated letters published in Holland (54): 'In the manuscript two rectangles have been drawn at the bottom of the letter. One of these contains the words: "a cotton seed cut into 24 round slices" and the other "9 seeds of the cotton tree from which the membranes have been removed, and the leaves separated".'

The confusion doubtless arose because most of the translation work was done from microfilm, in which the packets would indeed look like 'drawn rectangles', although it seems surprising that the possibility that these were captioned envelopes was so easily overlooked in the preparation of a definitive work. The oversight is compounded by a footnote elsewhere in the letters (2 April 1686) where van Leeuwenhoek refers to the cotton seeds (*q.v.*): '8 or 9 of these seeds, from which the young cotton tree takes its origin, I send you herewith'. A footnote to this points out tersely: 'This slide is no longer in the library (*sic*) of the Royal Society.'. Three points deserve to be made:

First, it is surprising that this question is not raised elsewhere in connexion with preparations that are mentioned in the letters but which are apparently missing. The closing section of the letter dated 1 June 1674, for example, is positive in its assertion that specimens of cork, elder pith and quill are enclosed, yet the notes to the published translation have nothing to say on the matter.

Second, the error is compounded by the mention of 'this microscope slide'. No slide would have been expected to be found from such an early date, and even the bone or ivory slider which long predated the standard microscope slide (34) was yet to be invented.

Third, it is intriguing to observe that the same translation which notes that van Leeuwenhoek's promised gift of '8 or 9 of these [cotton] seeds' is now missing, goes on to describe the rectángles visible at the end of the letter and notes that one of them is labelled: '9 seeds of the cotton tree . . .', without further comment or investigation.

Technical Notes

Magnifications are stated in standard terms, i.e. the ratio of actual object size to image dimensions at a lens-to-image distance of 250mm. The micrographs utilizing single-lens magnifiers (e.g. the study of *Spirillum* in Figure 3) were taken on FP4 film with a Gillett & Sibert microscope adapted by the author for the purpose, with a lens-holder in place of the conventional objective nosepiece. Section thickness has been measured by three means: (a) lateral microscopy utilizing an eyepiece micrometer separately calibrated from a standard stage graticule; (b) by direct measurement from scanning electron and optical micrographs; (c) using a screw-gauge micrometer measuring slide/coverslip combinations with and without the specimen in position. Techniques were selected as appropriate for the specimen concerned. The optical micrographs were taken using the Olympus TTL automatic exposure system attached to a Leitz Dialux microscope through Phaco NP1 objectives. Scanning electron microscopy was carried out with the advice and technical assistance of Mrs C. Winters, using a Cambridge Stereoscan 600 operating at 15 to 25 kV. Selected portions of the material were gold coated in an Emscope sputterer after mounting with double-sided tape.

Acknowledgements

I am indebted to the comments and the encouragement of many friends and colleagues in several countries during the work which led up to this investigation. In particular I remain grateful to the following, who were generous with their assistance and their kind hospitality during my visits to the facilities in their charge:
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Notes

(1) Becking, L. M., A. van Leeuwenhoek, Immortal Dilettante, *Sci. Monthly*, New York, **18**: 547 (1924).

(2) Sachs, J., *History of Botany*, London and Munich (1875).

(3) Jones, R. V., [personal communication] has reported a recent examination question in which university students were instructed to account for the reasons why it is impossible to see bacteria with a simple microscope (1981).

(4) Sachs, J., *vide* ref: (2) p. 264 [quoted by Dobell, C., ref: (6) *infra*] (1875).

(5) Becking, L. M., *vide* ref: (1) (1924).

Notes (1)–(5) demonstrate the prevalence of inadequately researched conclusions on van Leeuwenhoek's work. Publications from primary sources include Dobell's study in Note (6) and van Cittert, P. H., 'The van Leeuwenhoek Microscope' in possession of the University of Utrecht, etc., *Proc., K. Ned. Akad. Wet.*, **35**: 1062–1063 (1932); op. cit., **36**: 194–196 (1933); op. cit., **37**: 290–293 (1934). Secondary sources which deal largely with later periods include Bracegirdle, B., *A History of Microtechnique*, Cornell University Press, N.Y., 1978 (notable Chapter 2) and Turner,

G. L'E., (in) *Essays on the History of the Microscope*, Senecio, Oxford (1980). The current findings in the present paper may be read in conjunction with the wide-ranging discourse of Bradbury, S. (in) *Evolution of the Microscope* (1967) and *The Microscope Past and Present* (1968), both published by Pergamon Press, Oxford.

(6) Dobell, C., *Antony van Leeuwenhoek and his "Little Animals"*, John Bale, Sons & Danielsson, London, pp. 340–341 (1932).

(7) van Leeuwenhoek, A., *Phil. Trans*: **9**, 179–182 (1674).

(8) Haaxman, P. J., *Antony van Leeuwenhoek, de Ontdekker der Infusorien*, Leiden (1875).

(9) Dobell, C., *vide* ref: (6) *supra* (1932).

(10) de Kruif, P., Leeuwenhoek: first of the Microbe Hunters [in] *Microbe Hunters*, pp. 9–31, Jonathan Cape, London and New York 1927, reissued (1930).

(11) Schierbeek, A., *Antoni van Leeuwenhoek, Leven en Werken*, (2 vols) Lochem (1950–1951).

(12) —— *Measuring the Invisible World: the Life and Works of Antony van Leeuwenhoek FRS*, Abelard-Schuman, New York (1959).

(13) Rooseboom, M., Leeuwenhoek's Life in the Republic of the United Netherlands, op. cit., pp. 13–42 (1959).

(14) Dobell, C., [in] ref: (6) *supra*, plate II (facing p. 20) (1932).

(15) —— op. cit., p. 341 (1932).

(16) —— op. cit., p. 339 *et seq.*, and see Plate 1 in the present publication.

(17) von Uffenbach, Z. C., *Merkwürdige Reisen durch Niedersachsen Holland und Engelland*, (3 vols) Ulm-Memmingen-Frankfurt-Leipzig, III: pp. 349–360 [ed: J. G. Schelhorn] (1753–1754).

(18) Dobell, C., [in] ref: (6) *supra*, p. 303 (1932).

(19) Ford, Brian J., [in] *The Revealing Lens, Mankind and the Microscope*, p. 65 *et seq.*, Harrap, London (1973). van Cittert, P. H., On the use of Glass Globules as Microscope-lenses, *Proc. K. Ned. Akad. Wet.*, **57** (B): 103–111 (1954).

(20) van Leeuwenhoek, A., MS in Royal Society Archives, 1835. L.1.7, extract in *Phil. Trans.*, **9** (108): 178–182 (1674).

(21) —— [in] Dobell, C., ref (6), which reproduces a reduced facsimile of the original MS. in Plate XVII, facing p. 110.

(22) Op. cit., p. 111.

(23) van Leeuwenhoek, A., MS in Royal Society Archives, 1851. L.1.22, extract in *Phil. Trans.*, **12** (133): 821–831 (1677). Note that there is a discrepancy over the dating of the letters, since the Gregorian Calendar was adopted in some Provinces of Holland in 1582 (the Province in which Delft was situated being amongst them) whilst in some other Provinces—including Utrecht and much of Northern Holland—the change was not instituted until 1700. In England the Old-style Julian Calendar was retained until an Act of Parliament of 1752, when 2 September was followed by 14 September. At the time of the letters referred to in this paper, the discrepancy was 10 days. Van Leeuwenhoek's dates, being already in New-style, agree with modern conventions.

(24) From the translation in Dobell, C., ref (6) *supra*, p. 134.

(25) Note: snow-water, sometimes kept in store for several years, was a regular source of pure water for drinking; van Leeuwenhoek used it as his source of *aq. dest.*

(26) [in] Schierbeek, A., ref (12) *supra*, Leeuwenhoek's Micrometry, pp. 55–57. Van Leeuwenhoek's 'counter' may be found in his letter of 4th October 1677. Also Dobell, C., *q.v.*, p. 313 *et seq.* (1932).

(27) Ford, Brian J., Recreating the Pioneer Microscopist's View, *New Scientist*, 51 (763): 324–325 (1971).

(28) —— A Recreation of the Microscopic View of Nature Two-and-Half Centuries Ago, *Brit. J. Photogr.*, **118** (5793) 682–685 (1971).

(29) For a recent description [see] van Zuylen, J., The Microscopes of Antoni van Leeuwenhoek, *J. Microsc.*, 121 (3) 309–328 (1981).

(30) Schierbeck, A., ref: (12) *supra*, p. 52 ¶ 3 (1959).

(31) Ford, Brian J., [in] *The Optical Microscope Manual: Past and Present Uses and Techniques*, David & Charles, England; Crane, Russak, New York; Reed International, Australia; p. 86 figs. 2 & 3, (1973).

(32) —— [paper] *Robert Brown FRS and the Discovery of the Nucleus*, subject of a Special General Meeting of the Linnean Society of London at Burlington House, Piccadilly, 5 November 1981.

(33) Chew, V. K., [in] *Physics for Princes*, HMSO, # 8, 'Object seen through the "Prince of Wales" microscope', shows cleared and mounted adult flea in a form unavailable at the time the microscope was made, 1755 (1968).

(34) Ford, Brian J. [in] ref: (31) *supra*, p. 156–160 incl. Fig. 9, 'Evolution of the microscope preparation', p. 159.

(35) Stage forceps (dubbed 'tongs' here) are referred to in the instruction sheet supplied about 1730 with Culpepper's 'Double Reflecting Microscope' [reproduced in] Disney, A. N., Hill, C. F., & Baker, W. E. W., *Origin and Development of the Microscope*, Royal Microscopical Society, plate 4, facing p. 165 (1928).

(36) Ford, Brian J. Microscopic views from the past (in press), *Brit. J. Photogr.*, (1981).

(37) van Leeuwenhoek's diagram and a translation of his observations appear, for example, in Schierbeek, A., [ref. (12)] plate 9 fig. 4 (p. 112), pp. 123–125.

(38) —— Reproduced in Dobell, C., *q.v.*, from letter dated 29 June 1708 [*vide: Phil. Trans.* **17** (318) 210–214 (1708)]: 'I brought a thin glass tube . . . over the bottom of the porcelain cup, where most of the particles that had come off the tongue lay all of a heap'.

(39) —— [in] 2nd Observation on Pepper-water, 23rd May 1676, ref: (23) *supra*, and elsewhere.

(40) Refer, for example, to refs (6) to (16) for some examples.

(41) Reinier de Graaf (1641–1673) studied medicine at Leiden before practising in Delft. His name is commemorated in the Graafian follicle of the ovary. At the age of 32 he died, in the same year that he had succeeded in introducing van Leeuwenhoek to the Royal Society.

(42) *Phil. Trans.* **8** (94) 6037, (1673).

(43) The *Collected Letters* are published by Swets & Zeitlinger Ltd, Amsterdam. Compilation of later volumes is still in progress.

(44) Translation from ref (43), shown in Plate 6.

(45) Op. cit., see also Figure 18.

(46) Op. cit., letter of 17 October 1687, p. 87 *et seq.*

(47) [in] ref: (19) pp. 52–53.
(48) Pepys, S., *Diary* entry for 20 January 1665.
(49) van Leeuwenhoek, A., [in] *Collected Letters*, ref (43) *supra* I:31.
(50) Hooke, R., *Micrographia, or Some Physiological Descriptions, etc.*, Martyn & Allestry, printers for the Royal Society, London, 1665 (reprinted by Dover Publications, New York (1961) pp. 125–127).
(51) In this source the term 'cell' is said to be: The ultimate element in organic structures, a minute portion of protoplasm, enclosed usually in a membranous investment.

The history of this sense appears to begin with Grew, who observed and described the cells of plants . . .

1672–3 GREW *Anat. Plants* (1682) 64 . . . an infinite Mass of little Cells or Bladders.

[*Oxford English Dictionary*, p. 213]

Hooke's coinage of the term in 1665 is described in ref: (52) *infra*.
(52) Hooke, R., [in] ref: (50) p. 113 ¶ 3 (1665).
(53) a: Copy of this letter dated 1 June 1674 was sent to Constantine Huygens and has been independently published (Vandevelde & van Seters, *infra*).

b: *Phil. Trans.* **9** (106) 121–128 (1674).

c: *Recueil d'expériences et observations sur le combat qui procède du mélange des corps, etc., de la traduction de M. Mesmin*, Paris (1679).

d: *Genees en heelk proeven en aanmerkingen* [H. van Someren], Amsterdam, pp. 278–287 (1775).

e: Vandevelde, A. J. J., en van Seters, W. H., *Verslagen en mededeelingen der Koninklijke Vlaamsche Academie*, pp. 174–180 (1925).

Extracts of the letter, without including any reference to the gift of specimens, have also been published in:

Journal des Sçavans, Amsterdam (1679)

Collection Académique, Dijon et Auxerre (1755)

Abhandl. z. Naturgesch. Physik und Ökonomie, Leipzig (1780).
(54) Letter of 2 April 1686 ref: (43) I, 43, note 49.