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Robertson, Lesley A.

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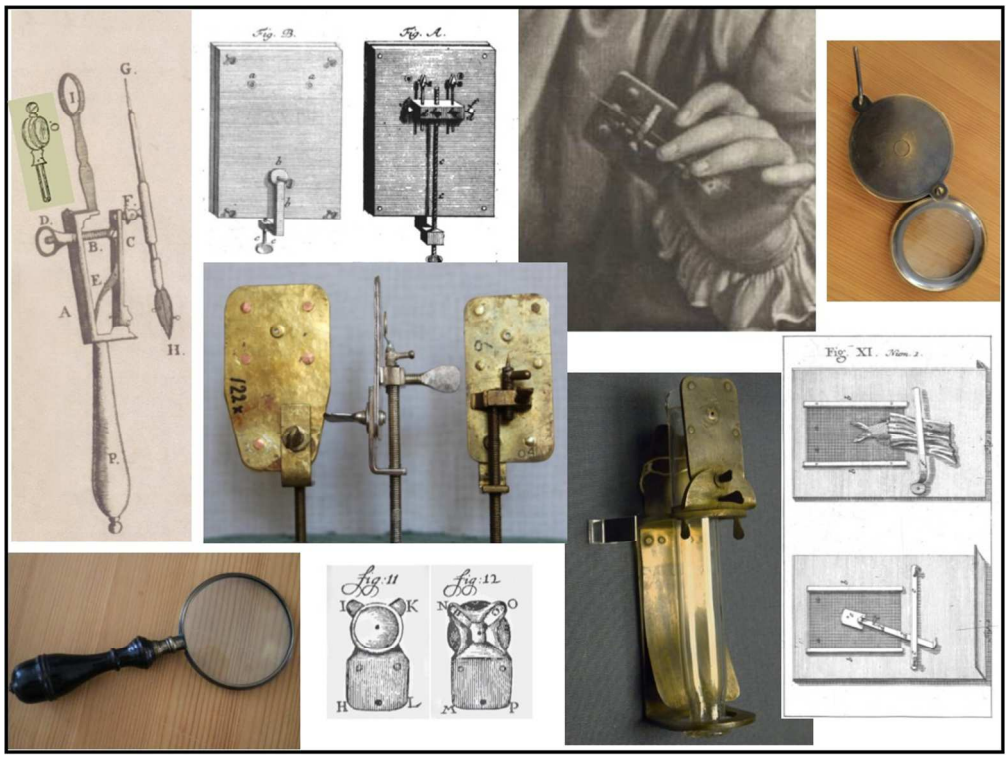

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Running title: Lighting Van Leeuwenhoek's samples.

Lesley A. Robertson

Department of Biotechnology and Delft Science Centre,

Delft University of Technology,

Mijnbouwstraat 120,

2628RX, Delft,

The Netherlands

l.a.robertson@tudelft.nl

tel +31 15 2782421

Keywords: single lens microscopes, Van Leeuwenhoek, opaque samples

Single sentence summary: The problems associated with viewing opaque samples using Van Leeuwenhoek microscopes are demonstrated, possible solutions are reviewed, and the possible identity of his "particular method of observing" is discussed.

Abstract

Possible techniques for lighting opaque samples while using Van Leeuwenhoek microscopes have been tested, and the results are presented in relation to published material. The design of the microscope causes the sample to be in shadow with any form of top-lighting. It is therefore suggested that Van Leeuwenhoek's hinted "particular method of observing" might refer to a different style of microscope as shown in the frontispiece of the sale catalogue for his microscopes, and available at that time for purchase from sellers of optical equipment.

Introduction

Despite the many descriptions of his experimental methods scattered throughout his letters, Antoni van Leeuwenhoek has a reputation for being secretive about his experimental methods. This might partly be because the versions of his letters published by the Royal Society (Phil Trans) were generally only edited highlights. If one reads letters by other scientists published in the same volumes, their methods are also rarely described in detail. To find more detailed descriptions of some of his work, the reader must either seek out his privately-published collections, most of which can be downloaded as pdf files from the Internet Archive and other sites (generally in Dutch or Latin), or consult the volumes of Collected Letters, volumes 1-15 of which can be downloaded as pdf files (DNBL). Of course, perhaps to avoid plagiarism, he did not describe everything.

Since Van Leeuwenhoek's time, people have speculated about how he lit his samples. Anyone who has used one (or a copy) will know that they are very effective with transparent samples. Van Leeuwenhoek commented that they are best used with diffuse light from the sky, a lamp or a candle, but never the sun because of distortion (Van Leeuwenhoek, 1694, 1699). He also mentioned using a curved mirror or a magnifying glass to improve the lighting, and this is probably how he achieved dark field views (Robertson, 2015a; Robertson et al, 2016). However, despite the fact that many of the samples in his huge body of research were opaque, his microscopes are not very effective with them. Opaque samples only appear as silhouettes with transmitted lighting, and the level of detail shown in the drawings that accompanied his letters cannot be seen without top (or reflected) lighting (see, for example, the drawing of a parasitic wasp, *Aphidius* sp., published by Van Leeuwenhoek in 1702a).

"Living history", where experts use historical equipment such as agricultural implements or adopt domestic lifestyles from the past rather than theorizing, can provide a great deal of useful information and eliminate (or even confirm) theories. Historical

microbiology is a limited form of this where historically important experiments are repeated with original equipment or accurate copies to test how such experiments could have been done in the days before detailed method descriptions in publications (Robertson, 2015a). It seems reasonable to expect that repeating selected experiments from Van Leeuwenhoek's work would also shed light on his lighting techniques.

Materials and methods.

The following magnifiers were used:

A facsimile Van Leeuwenhoek microscope (Loncke, 2006a, b) with a magnification of about 65x.

A late 19th century Carl Zeiss Jena "jug handled" compound microscope (Anon, 1906) as a control.

A 20th century botanist's hand-lens with a magnification of 30x. This hand-lens was chosen to represent the 17th century originals because, like the microscopes sold in Van Leeuwenhoek's time for viewing opaque samples, its lens is mounted in a simple metal ring, rather than in a metal plate.

As Van Leeuwenhoek frequently remarked, artificial lighting is necessary for work in the evenings or bad weather. Photography also imposes small compromises in experimental design. It has previously been shown (Robertson, 2015A) that similar results are obtained with different light sources, including a candle. For continuity with previous experiments and ease of photography, all magnifiers were lit using an LED light and a thin diffusing screen (to give even lighting for the sensor in the camera), unless otherwise specified.

Photography was done using a Canon EOS M10 digital camera body fitted with a Bresser microscope adaptor which had been modified by lining the stainless steel inner tub with black adhesive material to eliminate internal reflection. To simulate the focusing controls of the two types of microscope, samples for the hand-lens were mounted on a glass rod

attached to a macro focusing slide. All photographs are the result of focus stacking between 4 and 15 images to correct for the limited depth of field imposed by the camera.

To allow the moth's wing to be moved between the microscopes and the hand-lens, it was attached to a glass coverslip using a drop of colourless adhesive.

Sample selection was governed by the availability of Van Leeuwenhoek drawings for comparison as well as the ability to mount the samples in all three magnifiers. Moth and butterfly wings were convenient as representatives of the many samples Van Leeuwenhoek examined, especially because they are transparent at their edges and opaque over the rest of the surface. Similar results were obtained with both, so only the results with moth wings are presented.

Results

It is not possible to top-light samples using a classical Van Leeuwenhoek microscope (Fig. 1A) because the metal lens mount obstructs the light path, casting a shadow. Several researchers (Baker, 1739; Harting, 1859; Clay & Court, 1932) have suggested that Van Leeuwenhoek lit opaque samples from the side, using a lens or curved mirror. However, while the use of a lens or mirror to focus light on the sample works well for transparent samples and to achieve dark field microscopy (Robertson et al, 2016), it does not work from the top or the side. Depending on the strength of the lens in use, the distance between it and the sample pin when the sample is focused varies from 8mm -1mm with magnifications between 68x -303x, respectively (Fig. 1A, centre microscope). Light from the side reached the sample perpendicular to the light path between the sample and the observer's eye, and gave little or no improvement in visible detail.

It has also been suggested that Van Leeuwenhoek made silver microscopes so that they could be polished and reflect light onto the upper surface of the sample, or else used a thin mirror between the microscope and the sample (Baker, 1739; Anderson, 2017). This was tested by covering the facsimile microscope (leaving a small hole for the lens) with a flat

piece of aluminium foil with the highly reflective side facing the sample. Again, this gave little or no improvement. The lens mount is flat and therefore does not focus reflected light onto the sample. With the lens and sample necessarily so close together, there is no room to curve the lens mount in the manner of the cup-shaped reflector that first appeared early in the 18th century (Baker, 1739, 1742), but eventually became known as a Lieberkühn.

Fig. 2A shows the backlit wing of a large yellow underwing moth, as photographed with the facsimile Van Leeuwenhoek microscope. Van Leeuwenhoek's drawing of scales from a silkworm moth is inset. At the upper edge of the wing, where the light only had to travel through a single layer, the appearance of the scales in the two images is similar. With thicker layers of scales, little or no detail can be seen. Figs 2B and C show the same wing under the Zeiss microscope with top and back lighting, respectively. B resembles A, but only C shows all of the scales. As Fig. 3A shows, Van Leeuwenhoek was also able to see the scales covering a butterfly wing (Van Leeuwenhoek, 1678). When the hand-lens was used, results similar to those in Fig 2B were obtained by backlighting the wing. However, it was also possible to position the light at the front, beside the eye or camera (Figs 3B and C), allowing the surface of the wing and the scales, to be lit. The distance between the hand-lens and the sample was approximately 20mm which permitted a lighting angle greater than 90° because the narrow metal band mount for the lens did not interrupt the light path and did not cast a shadow.

Discussion

Antoni van Leeuwenhoek is generally remembered for his discovery of microorganisms (Van Leeuwenhoek, 1676), for which the level of magnification delivered by his traditional microscopes (Fig. 1A) was certainly necessary. Aqueous samples are generally transparent, and it is even possible to use dark field lighting (Robertson et al, 2016). However, his research covered a wealth of subjects, often with samples that were not transparent, did not require such strong magnification, or both. It is clear from his own words

(below) that he viewed his microscopes as tools for a particular job rather than objects in their own right:

"I have said heretofore how I composed my instruments, which some people would have made far finer and more accurate. …. I have so far trained myself that I have for many years made the tools that I needed for several matters. And that is why what I required for my use was only made a bit roughly by myself." (Van Leeuwenhoek, 1689).

Robert Hooke mentioned the impossibility of lighting samples with a single lens microscope in one of his Cutlerian Lectures (Hooke, 1679):

"The only inconvenience in these kinds of Microscopes, is, that the object is necessarily brought so near the glass, that none but such as are transparent, and to be viewed by a through light are capable of examination by them"* (* "glass" in this context in the 17th century meant "lens").

Van Leeuwenhoek must have found a way around the problem. His approach to the apparatus he called an *aalkijker* for viewing blood circulation in eels and various fish shows that he was willing to adapt his tools as necessary (1689, 1695). The original version of the *aalkijker* used the same lens holder as his microscopes (Fig. 4A). However, in his second paper on the subject, he described how, in order to be able to shine more light on his sample, he removed most of the metal on the lens holder (Fig 4B). To protect his eye from the light, it was then necessary to add an eye cup. Some writers (e.g. Baker, 1739; Priestly, 1772; Harting, 1850) described this cup as a reflector surrounding the lens to direct light onto the sample, the arrangement which eventually became known as a "Lieberkuhn". They even suggested that Van Leeuwenhoek should be credited with its invention. However, as pointed out above, the very small distance between the sample and the lens on a Van Leeuwenhoek microscope does not allow a curved reflector to be used. There is no room. If one considers Van Leeuwenhoek's own description (his *figs 11 and 12* are shown in Fig 4B), it is clear that those authors were looking at the apparatus from the wrong side (Van Leeuwenhoek, 1689):

"…screwed on to this instrument is the brass plate into which, again, a magnifying glass has been riveted, as is shown here in fig: 11, HIKL., over which magnifying glass I have soldered a small cup, in order that the eye might the better see the objects, for I had filed away the brass around the magnifying glass as much as it could bear, to bring as much light as was practicable on to the objects that one might wish to see…. on fig: 12. MNOP, where one can see the same instrument from the other side".

The cup is there to protect the viewer's eye from the light aimed at the sample. The credit for inventing the "Lieberkühn" belongs to Descartes (1637).

Van Leeuwenhoek was obviously aware of the advantages of being able to light his sample from all angles. If he adapted his *aalkijkers* to do this, why would he not also adapt his microscopes for opaque samples? He could possibly have used the reduced lens holders shown in Fig 4B, but when he needed a weaker lens, is it possible that he was using a different style of microscope? This might explain the inclusion of something that strongly resembles a microscope commonly sold at that time for viewing opaque samples (Fig. 5A) in the frontispiece of the sale catalogue for his microscopes after his daughter's death (Fig. 6H; Rees, 1747).

Most of Van Leeuwenhoek's microscopes have vanished, and the assumption that they were all alike is common despite the fact that he clearly adapted his tools as required. He twice altered his original *aalkijker* (the 3rd time to make viewing easier for visitors by holding the fish against a piece of glass) but versions 2 and 3 (Figs 4B and C) have not survived except as written descriptions or illustrations (Van Leeuwenhoek, 1695, 1708; Von Uffenbach, 1754). The catalogue for the sale of his microscopes (Rees, 1747) mentions microscopes with two and three lenses, something which some authors have believed to indicate that he was also using compound microscopes (e.g. Harting, 1850) even though pictures of such microscopes made at the time clearly show two or three lenses side by side (Fig. 1B and C, Verkolje, 1686; Rees, 1747; Von Uffenbach, 1754). Those microscopes have also not survived, neither have those mentioned in the catalogue as being able to hold two

samples, those with a hole to mount a capillary tube in the sample block (Fig. 1C) or those made from gold.

Van Leeuwenhoek frequently hinted (Dobell, 1932) that he had another “particular method of observing” which he did not allow guests to use, but which allowed him to see more. The “burning glasses” (e.g. Fig 5B, C) mentioned in the inventory of his house after his daughter’s death (Geesteranis, 1745) would not have been strong enough for a lot of his work, although they would have permitted top lighting. We can never be certain, but it is attractive to speculate that he was talking about top lighting samples using the type of microscope shown in Figs. 5A and 6H. Top lighting reveals a lot of additional detail (Figs 2C and 3), but it would have been more complicated to set up than his traditional microscopes, and he was famously impatient with visitors and the time they cost him. In addition, would he have wanted his visitors to report that for some of his work he was using microscopes of a type readily available from opticians, even if he improved the lenses?

There was clearly more variation in Van Leeuwenhoek’s magnifying toolkit than can be seen from the few surviving microscopes. All of the equipment shown in Fig. 6 can be identified in his letters or the reports of his visitors (Robertson et al, 2015b) except the magnifier, H, which resembles that shown in Fig. 5A.

The digital abstract for this paper shows Van Leeuwenhoek’s magnifying toolkit as it might have been.

Acknowledgement

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CONFLICT OF INTEREST

None

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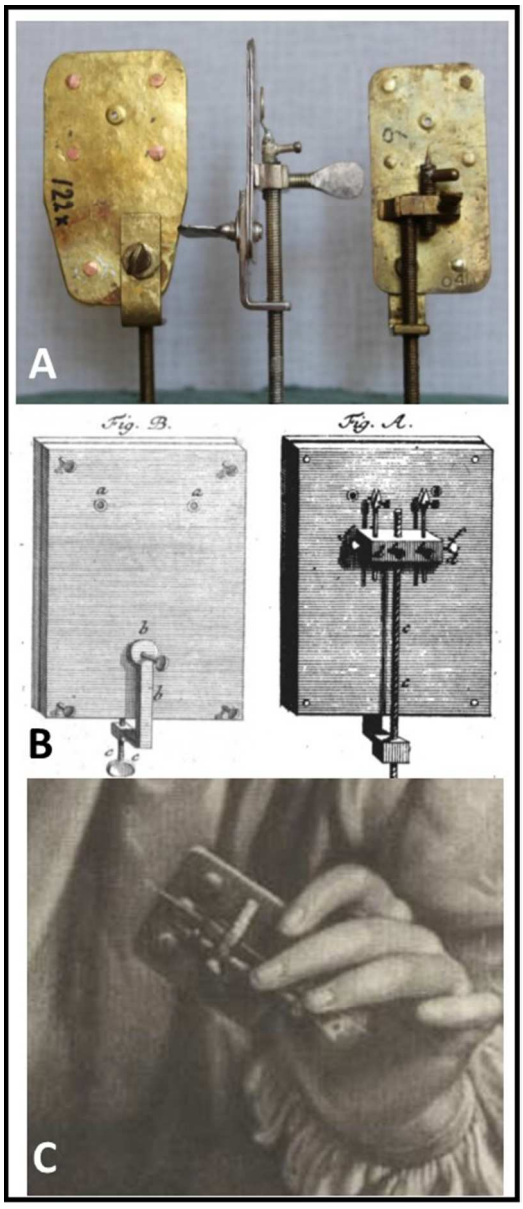

Figure 1: Three versions of Van Leeuwenhoek's microscope. A: Facsimiles of the well-known form. B: Version shown by Von Uffenbach (1754) with 2 lenses and 2 sample holders. C: Version shown by Verkolje (1686) with 3 lenses, 1 sample pin and a holder for a capillary tube.

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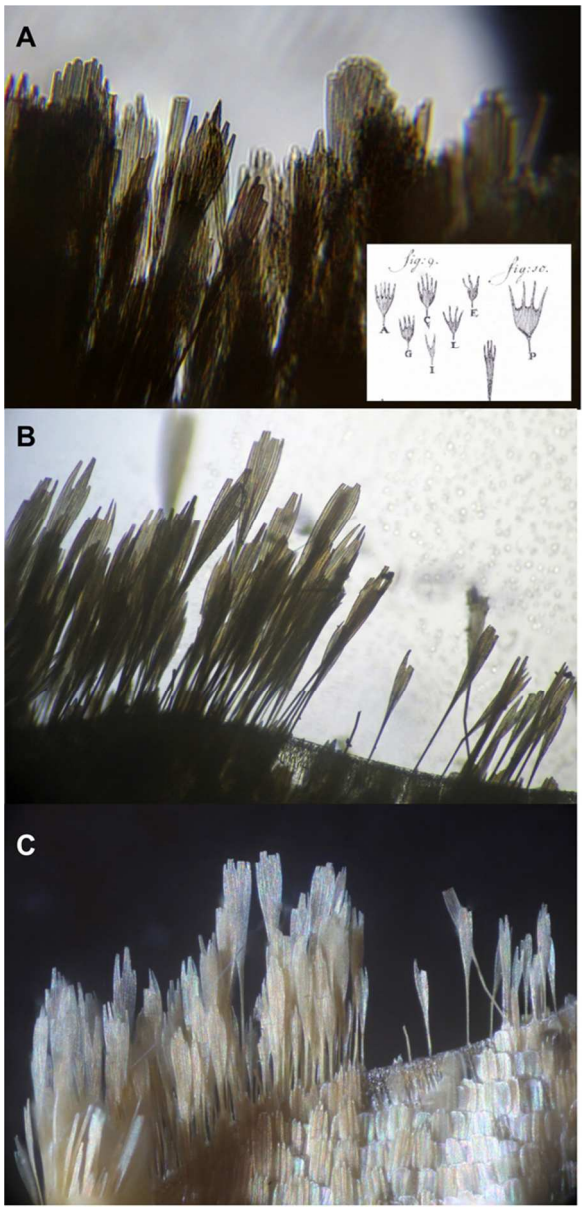

Figure 2. A: The edge of the hindwing of a large yellow underwing moth as photographed through the facsimile Van Leeuwenhoek microscope, inset: Van Leeuwenhoek's drawings of the scales from the wing of a silkworm moth (Van Leeuwenhoek, 1702b). B and C: the same sample photographed through the Zeiss microscope under back and top-lighting, respectively.

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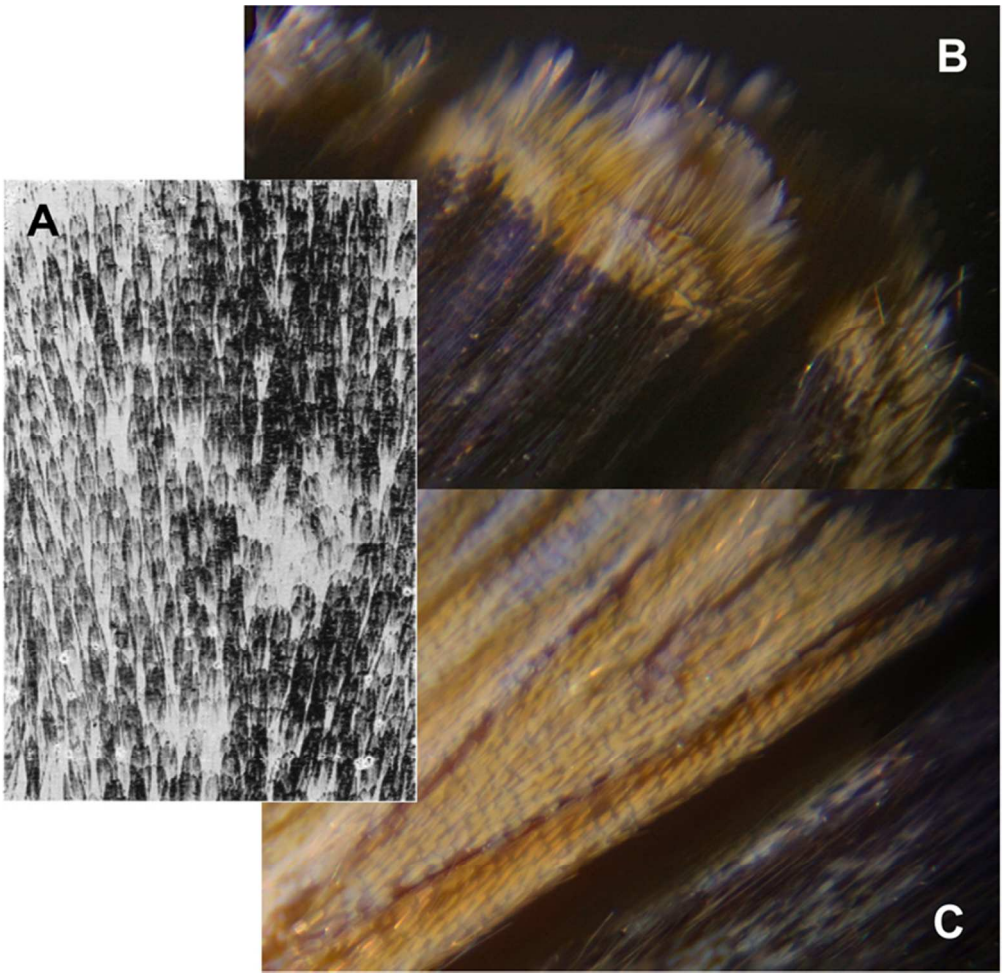

Figure 3. A: The scales seen by Van Leeuwenhoek on the surface of a butterfly's wing (Van Leeuwenhoek, 1678). B and C: The same moth's wing as in Fig. 2, photographed through the modern hand lens with the light positioned beside the camera to give top lighting.

140x135mm (150 x 150 DPI)

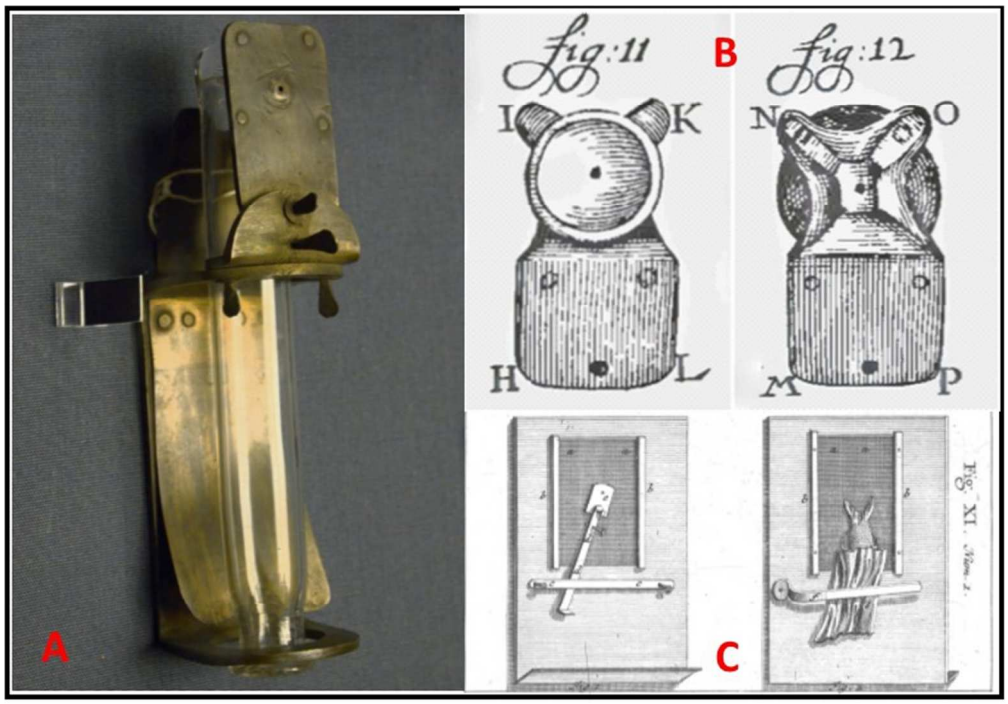

Figure 4. A: Facsimile of Van Leeuwenhoek's original aalkijker with the same lens holding plate as used in his microscope (Fig. 1A). B: The reduced lens holder with the eye cup attached to Van Leeuwenhoek's first modification of his aalkijker. This lens could be attached to the aalkjiker frame in place of the familiar rectangular flat plate shown in Fig. 4A, "fig 11" shows the observer's side, and "fig 12", the sample side. C: The 3rd version of the "aalkijker" with the glass tube for holding a fish or eel replaced by a flat glass plate, a clamp and a piece of wet fabric (see also Fig 6B).

140x98mm (150 x 150 DPI)

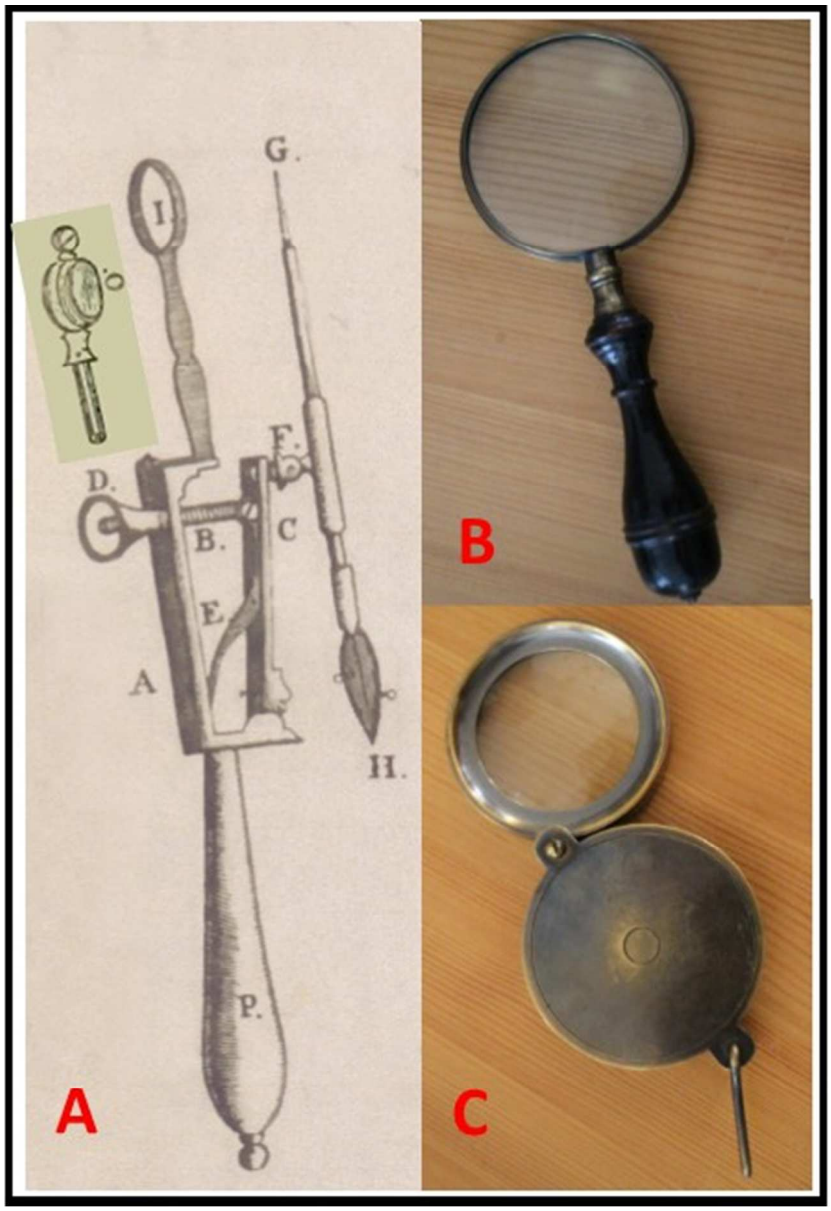

Figure 5: A: Low powered microscope for opaque samples (Baker,1739) which strongly resembles the instrument marked B in Fig 6. It could be used with larger lenses (O) mounted in a metallic ring (I), or fitted with a smaller, stronger lens surrounded by a reflector (often called a "Lieberkuhn") which could also be attached to the microscope at (I). B and C: Facsimiles of "burning glasses" in use in the 17th and 18th centuries, as sold by Museum Boerhaave in Leiden.

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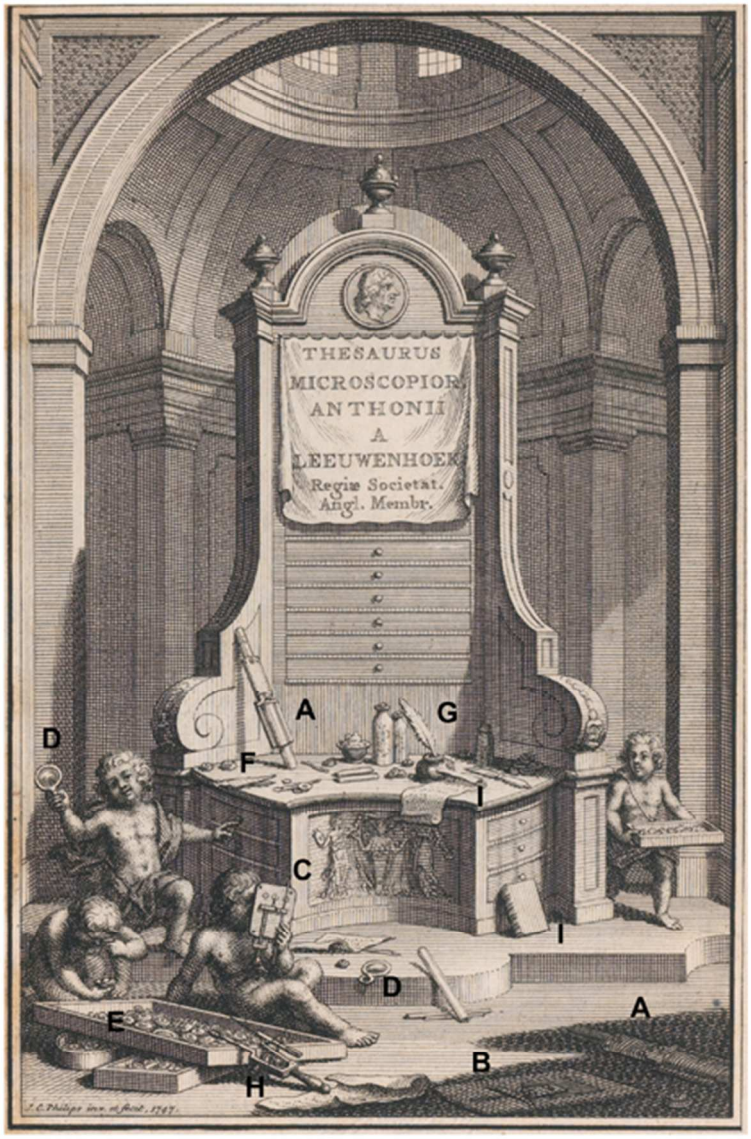

Figure 6. The frontispiece of the catalogue for the sale of Van Leeuwenhoek's microscopes after the death of his daughter, Maria (Rees, 1747, Robertson 2015b). (A) original aalkijker; (B) second modification of the aalkijker; (C) microscope with three lenses side by side, a sample pin and a capillary sample tube; (D) magnifying glass; (E) loose lenses, tweezers; (G) quill pen and ink; (H) microscope for opaque samples; (I) bound book.

106x149mm (125 x 125 DPI)