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History of Science. — *The "*VAN LEEUWENHOEK *Microscope" in possession of the University of Utrecht.* II. By Dr. P. H. VAN CITTERT. (Communicated by Prof. L. S. ORNSTEIN.)

(Communicated at the meeting of February 25, 1933.)

In a previous paper the magnification of the VAN LEEUWENHOEK microscope, which is in possession of the University of Utrecht, has been stated to be about 270 diameters in accordance with the measurements of HARTING and contrary to the description of MAYALL [1]). Further measurements have been made on the properties of the lense. The lense is apparently a ground lense, as it is biconvex with radii of curvature of about 0.75 mm. and a thickness of about 1.1 mm. The objectdistance is about 0.5 mm., the numerical aperture about 0.4 and the theoretical resolvingpower 0.7 μ. The lense is diafragmed on both sides: on the objectside by a diafragm of 0.5 mm. diameter, on the other side by a diafragm of 0.8 mm. The objectside of the lense is badly scratched, which is not to be wondered at, as this side of the lense is totally unprotected. As a consequence of these scratches a part of the image is spoiled, and of course the other parts of the image are also affected. Therefore the image is not so good as it undoubtedly has been in VAN LEEUWENHOEK's time [2]).

[1]) As far as is known in Utrecht, there has never been more than one VAN LEEUWENHOEK microscope in possession of the University of Utrecht. The microscope was originally a possession of the Physical Institute, but was borrowed by HARTING, who was the director of the Zoological Institute. After HARTING's death the microscope remained in care of the Zoological Institute untill recently: it was returned only some years ago to the care of the Physical Institute as a consequence of the foundation of the "Utrechtsch Universiteitsmuseum". The microscope described in 1886 by MAYALL must therefore be identical with the specimen described by HARTING in 1850. When my attention was drawn through DOBELL's book to the description of the microscope given by MAYALL as an inferior instrument with a magnification of only 40 diameters, I was urged to compare the instrument with the descriptions and I found that HARTING's account was in accordance with the facts. I cannot imagine how it was possible for MAYALL to give a description which is so totally in disaccordance with the instrument. Perhaps DOBELL hits the nail on the head when he says: "MAYALL apparently never saw the microscope which he described" (DOBELL, pg. 327)."

[2]) I have had made a glassglobe of about 1.1 mm. diameter, and used this as a lense with diafragm of about 0.5 mm. The resolving power and the magnification were of the same order as that of the VAN LEEUWENHOEK lense. All investigations, described hereafter, have been done with both lenses. It is astonishing, what can be seen through such a simple globe of glass.

To determine the resolvingpower of the microscope, a microphotograph was made of an object-micrometer divided in $^1/_{100}$ mm. On this photograph the diffraction pattern of the different lines was measured and a resolving-power of about 1 μ was calculated. This is in fair accordance with the microphotograph of an old "diffraction plate of NOBERT" reproduced in fig. 1 (magnification 200 diameters). The distances of the lines in the different rows are: 2.66 μ, 2.38 μ, 2.22 μ, and 2.02 μ. There is no doubt that the limit of the resolving power has not been attained in this photograph. (The spots on the microphotograph are not due to faults in the lense, but are real spots on the object).

The observation of stained bacteria was possible down to 1—2 μ. Of course with bacteria of this order of magnitude no details in form could be seen. Observations were made for example on: Bacterium coli ($\pm 3\,\mu$), Staphylococcus pyogenes citreus (1—2 μ), Sarcina flava (3—4 μ, the quadrilateral symmetrie was easily observable) and the bacteria of the teeth. With unstained living bacteria the limit was higher. It was however possible to observe the Bacillus mesentericus ($\pm$ 5 μ), but this, I think, was the limit. Smaller objects could however be observed if they were moving very rapidly: they are then detected by their motion, and not by their form. All these investigations have been done by the aid of transmitted light with an aperture of about 1 : 4. Further it was quite easy to observe the cilia of the paramaecium and of some rotifers, and the spirilla in canalwater.

Observations have also been made with capillary tubes partly filled with aquarium- or canalwater in the manner described by VAN LEEUWENHOEK (comp. DOBELL, page 211: "I took a little of this water and put it in a glass tube, whose diameter was about a fifteenth of an inch and which was filled for about an inch of its length with the water"). Protozoa could be observed very clearly, but their fast swimming made it very difficult to see details. Moreover the different depths at which they swam, made observation very difficult. But in the neighbourhood of the meniscus observation was quite easy. Near *a* (fig. 2) the protozoa are caught by the

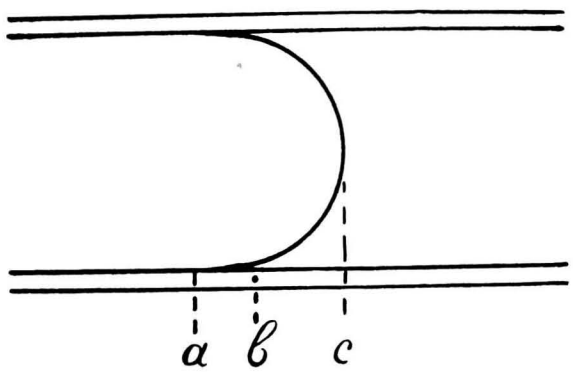

Fig. 2

capillary forces and can therefore be observed at leisure. Sometimes hundreds of them can be found there together, partly lying still, partly trying to escape from the trap, that has caught them, and, what is very important, they are all lying in one plane, so that it is quite easy to focuss on them. When VAN LEEUWENHOEK says: "My method for seeing very many animalcules all at once, I do not impart to others, but I keep that for myself alone" (DOBELL, pg. 144), it is quite possible that he was referring to observation in the meniscus. The animalcules often remain so quiet, that it is possible to photograph living ones with an exposure of several seconds. Such a photograph is reproduced in fig. 3 (magnification 200 diameters). The photograph has been taken purposely, when there were only a few protozoa in the meniscus, since, if there are too many, the mass is never at rest, and it is impossible to photograph them. Between *a* and *b* the protozoa can be observed swimming freely (of course not to be seen on the photograph on account of the long time of exposure). In the tube itself the protozoa can only be photographed by putting some gelatine in the water (fig. 4). This photograph has been made not with the original VAN LEEUWENHOEK lense, as is the case with the other photographs but with the glassglobe which we made ourselves. The magnification is 240 diameters, the object was canalwater. A number of another kind of protozoa (cyclidia?) and some spirilla are to be seen. Between *b* and *c* however there is automatically a dark ground illumination, caused by the refraction in the meniscus, and here it is indeed possible to observe the protozoa in dark ground illumination. It was impossible to see the organs of movement of these protozoa, but when it happened that one was at rest in the dark region, it was possible to focus on it. Then the bright outline of the animal caused by diffraction and a bright spot in the middle, caused by refraction, were to be seen. Immediately it began to move, however, a bright fan could be seen extending from it. In fig. 5 a photograph of this dark ground region is reproduced (it is of course overexposed on the liquid side and underexposed in the dark region itself).

DOBELL has suggested that VAN LEEUWENHOEK must have used dark ground illumination. Of course, it is impossible for me to say if this was actually the case, but, if VAN LEEUWENHOEK did observe his animalcules in the meniscus, he must have observed that the appearance of such an animal totally altered when it passed the dark ground region, and therefore, he had in any case the opportunity to hit upon the idea. On the other hand, experiments made with oblique illumination and with illumination along the length of the tube gave very unsatisfactory results. The observation of objects illuminated in this manner was very difficult and owing to the one sided illumination it was often impossible to recognise the object, when seeing the dark ground image.

I am very much indebted to Prof. Dr. W. C. DE GRAAFF and Dr. H. VELTHORST for their help with the microbiological part of this investigation.

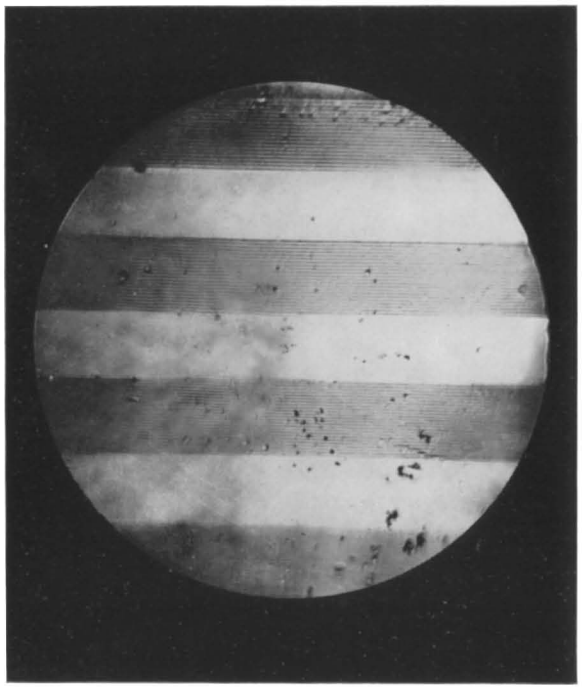

Fig. 1

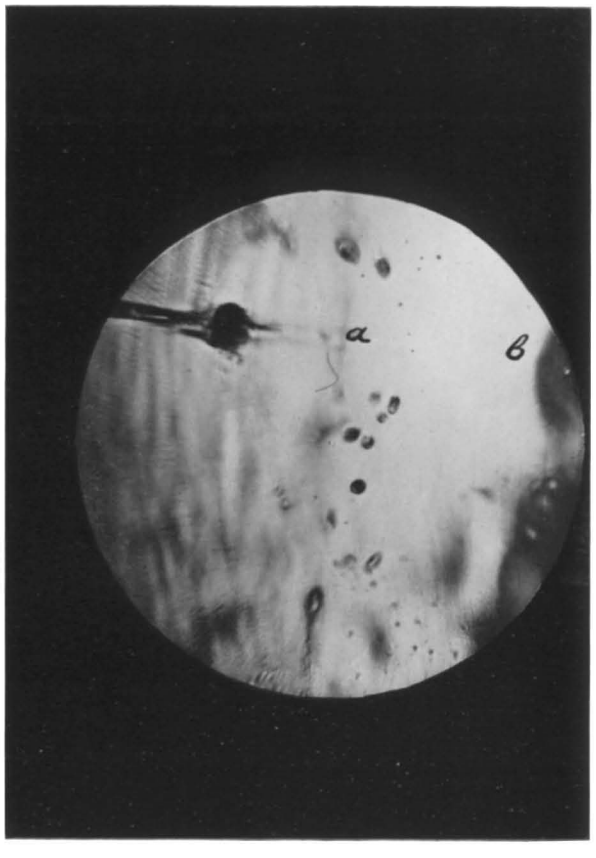

Fig. 3

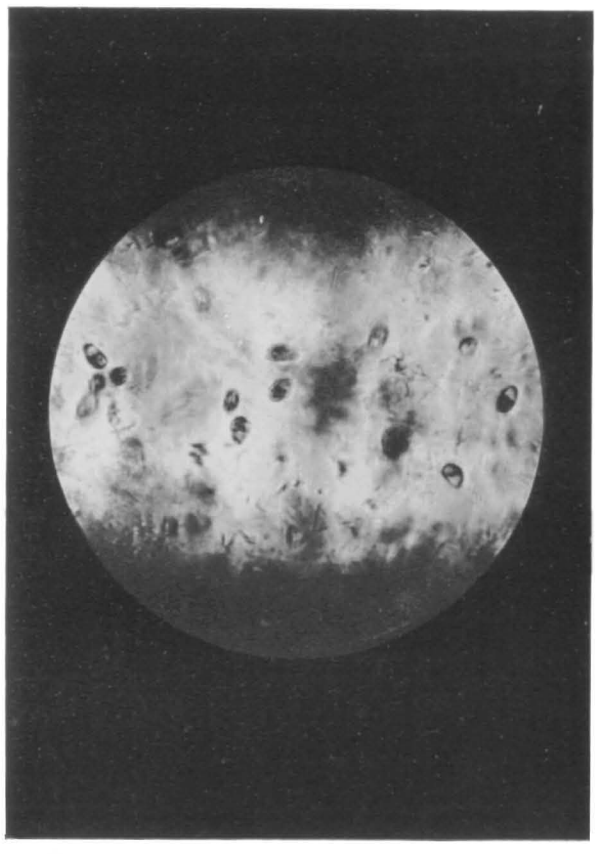

Fig. 4

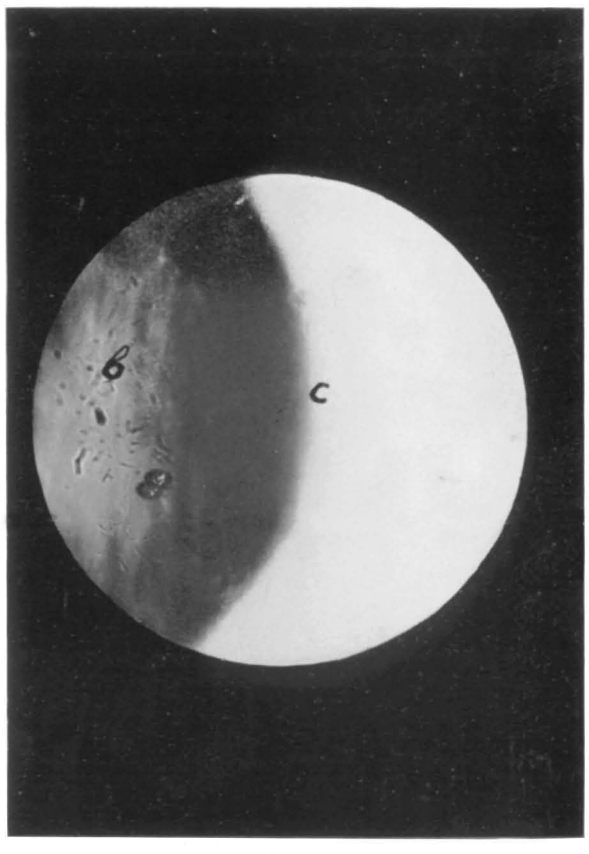

Fig. 5