SEEING IS BELIEVING

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Since the first century A.D., mankind has found ways to see better[1]. During the next twelve centuries, convex lenses were made from clear minerals for eyeglasses, in order to overcome far-sightedness. Then, in the 1300's, clear, artificial glass became available for the same purpose. By the sixteenth century, concave lenses were made for the near-sighted. It was not until the 17th century that a combination of lenses led to the sciences of astronomy and microscopy[1].

One of the earliest important microscopists was the Englishman Robert Hooke (1635-1703). He owned a compound microscope with an objective lens and an eyepiece, much like Galileo's telescope. But Hooke thought that to see more and more detail was to have more and more magnification; so he experimented with a third (field) lens. He looked at green "tarnished" water to see "whether this was like moss - yet so ill and imperfect are our microscopes that I could not certainly discriminate any"[1]. However, the resolving power of Hooke's microscope was sufficient to reveal the structure of cork in units which he pictured artistically as "cells", because he was reminded of monks' cells[2].

Curiously, in his book, Hooke described how to make a simple (single lens) microscope by means of a very small glass bead: draw a thread of glass, run it into a bead in a flame, and then snap off the apex. Grind that region flat with jeweler's abrasives. Fit the tiny lens in a flat piece of metal. Impale the specimen, or hold it with wax (or place a drop of a liquid specimen) on a spike hand-made into a screw for focusing the lens[1,2].

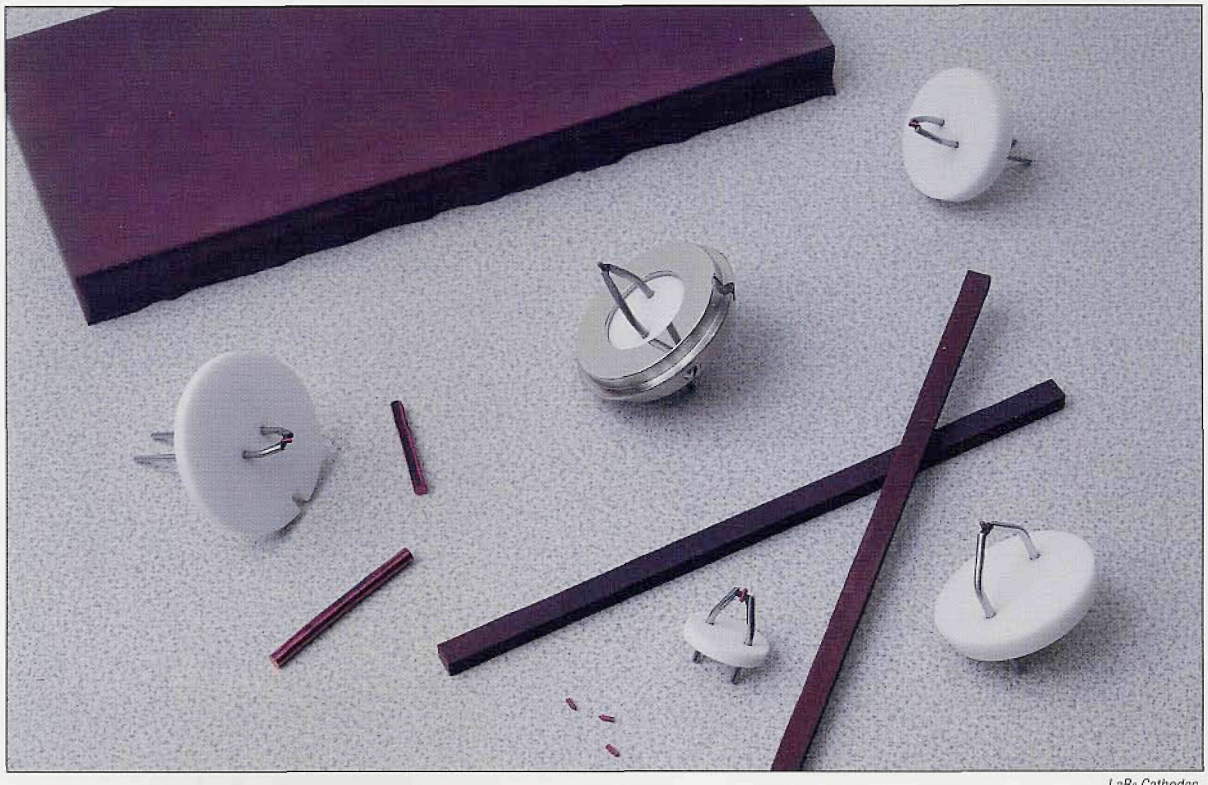
Anthony van Leeuwenhoek (1632-1723), a Dutch draper, was forty years old before he began making simple microscopes according to Hooke's directions. Of the more than 400 that he made, only nine remain. More importantly, he reported seeing a wide variety of microorganisms, including bacteria, which he termed "animalcules". For two and a half centuries, however, a number of scientists have doubted Leeuwenhoek's resolutions. Nevertheless, recent work, especially that of Brian J. Ford and his colleagues, who have tested Leeuwenhoek's surviving microscopes and modern versions of it, have completely substantiated Leewenhoek's results[1].

How did Leewenhoek's method differ from Hooke's? Hooke was dealing with the "far field" just as his contemporary, Galileo, did with his telescope. Both men, though, were unaware of the optical aberrations in their instruments. During the next two and a half centuries these aberrations were understood and corrected. Moreover, the limitations of visible light were learned, and the advantages of ultraviolet and infrared "light" were understood and employed. Knowing the overall limitations of light led to the use of microscopy by means of electrons, x-rays, or acoustics[1].

Back in the 17th century, how and why did Leewenhoek see "animalcules" and "beasties"? With his kind of microscopes the object was placed very close to the lens, as was the eye. In this way, the kinds and extents of aberrations were negligible. Professor Michael Isaacson uses the simple analogy of an operating water hose. If the observer stands in the "far field" of spraying water, there is little information obtainable about the size and shape of the nozzle. But if one stands close to the nozzle, a great deal of information is obtainable. In theoretical physics, "near" and "far" fields are on opposite sides of a lens, but the water-hose example is sufficient to explain Leeuwenhoek's advantage over Hooke.

The conception of near-field optics by E.H. Synge in 1926 has been "rediscovered" by Professor M. Isaacson[3]. There has been an "explosion of new interest in the development of near-field optics[4]. "By the next century, nearfield imaging should be available at close to nm spacial resolution using visible light"[3].

1. T.G. Rochow and P.A. Tucker, *Introduction to Microscopy by Means of Light, Electrons, X-rays, or Acoustics, 2nd ed.* (New York: Plenum, 1994)
2. R. Hooke, Micrographia (Royal Society of England, London, 1665. reprinted by Dover: New York, 1961).
3. M. Isaacson, "Three Hundred Years after Hooke and Van Leeuwenhoek; Optical Microscopy in the Twenty-First Century," Microscopy Today (January/February, 1995), 22.
4. M. Isaacson, ed., *Proc. of the 2nd International Conference on Near Field Optics*, Raleigh, NC (1994, in press).



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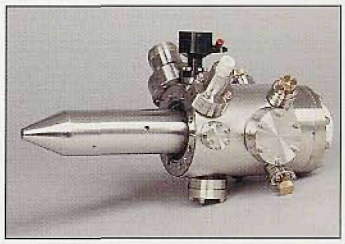

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Collecting Material For Specimen Preparation

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Most workers wishing to prepare material for microscopy will study a limited range of organisms, and already be familiar with raising, culturing or collecting the species in question because of their research interests or adopted field of study. For those new to microscopy who have not yet defined a field of interest, it is suggested that they read a practical introductory text such as that of Gravé (1991).

The diversity and abundance of animal, plant and microbial life available for collection means that gathering material can be a relatively simple task. Nevertheless, a methodical approach ensures that specimens are less likely to suffer damage and full details of their natural habitat are known, which will place any serious study into scientific context. Concomitant with the proper collection of material is an understanding of taxonomy. Readers wishing to know more about this subject are advised to consult Jeffrey (1989) and Margulis and Schwartz (1988). For our purposes, we can regard specimens as aquatic, static terrestrial (in general, plants) and mobile terrestrial forms. These notes are confined to remarks on collecting microbial, herbaceous or invertebrate life from the wild. Subculturing and propagating research material, or raising chordate populations, requires special facilities and is beyond the scope of this text.

A variety of microbes can be cultured using s simple hay infusion. A handful of chopped grass can be added to tap water that has previously been allowed to stand for a day or so to remove the chlorine, and after a few days bacteria will accumulate. The culture can be further enriched by the addition of horse manure. Likewise, animal pellets and soil samples can be collected and dissected into water or buffer to provide material for investigation.

Botanical specimens can be collected into polythene bags, or kept pressed between two lightweight boards lined with paper. In humid climates collection in alcohol vapour is preferred to prevent decay. Alternatively, specimens can be dissected and immersion-fixed in the field. Likewise, fungi can usually be dissected into small cubes for fixation in the field. Spore samples can be taken as imprints from the fruiting body by placing the hymenial surface directly onto the slide and fixed by air drying. Further details for collecting botanical specimens can be found in Forman and Bridson (1992).

Many insects live and feed on plants; they can be beaten or shaken into an umbrella or net, or else picked or sucked off with an aspirator. Insects are best killed using a bottle containing a swab soaked in ethyl acetate, or cyanide, or by immersion into 70% alcohol (which also fixes the specimen). Some insects are phototropic and can be caught using a light trap, while others respond to chemical repellents or attractants. Those insects which inhabit woodland floor detritus can be sifted using Tüllgren or Berlèse funnels. Further details are given in Borror *et al.* (1989), in addition to the guides published by the Natural History Museum for collectors of insects and other invertebrates.

Aquatic invertebrate species can be collected directly in glass vials or screw-top jars, or dredgings from plankton nets taken to provide species trapped in the algal weed. Benthic animals can be dislodged by stirring the water and overturning stones upstream of the net. Empty the contents of the net into a white dish, or translucent container with a white sheet or paper background. The animals will at once crawl out from the detritus, and can be identified and selected. Sorting is much easier if living forms are sorted; when dead they resemble the dredgings and, lacking movement, are much harder to discriminate. Many invertebrates will survive transport amongst damp weed kept in an air-tight tin better than they will in overcrowded bottles of water. If bottles are used, they should be cleaned with only a small amount of detergent and rinsed several times with tap water. Just prior to use, rinse out the bottle with pond water before sampling. When filling bottles, they should be left two thirds empty to provide a sufficiently high surface area to volume ratio between the water and air.

Whatever the species collected, a hard-backed notebook should be used to