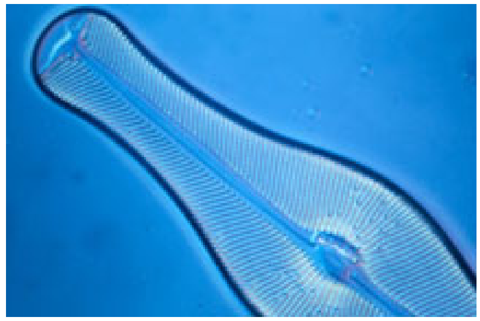
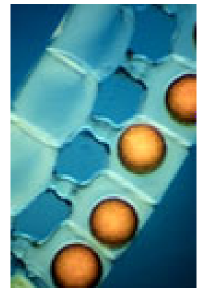




THE LIGHT MICROSCOPE

Written, produced and photographed by
Bruce Russell
Post-production by Eric Russell

A video program in BioMEDIA ASSOCIATES **Biology of--series.** Running time 20 minutes. Exploring the microworld can be enhanced using some easily applied techniques that are particularly useful for viewing living organisms.

(IMAGES IN THIS GUIDE ARE FROM THE VIDEO PROGRAM)

Simple microscopes

Hand held, single lens microscopes are useful tools for examining field collections. One type, called a DiscoveryScope, allows careful examination of aquatic samples in a small chamber, or in a custom plastic bag. These handy little scopes allow stable viewing of subjects in natural light and allow the investigator to study subjects over an extended period of time at dissecting microscope magnifications. Even a simple hand lens will reveal protozoans and other small organisms, when brightly lit against a dark background.

Compound microscopes

The typical classroom microscope extends the resolution of single lens microscopes. Much emphasis is placed on magnification, but itÕs important to remember that "magnification" simply refers to the degree of enlargement of the subject. Enlargement does not necessarily assure seeing more detail in the subject. Resolution of detail is determined by the interplay of three systems: the objective lens, the condenser lens and iris. This program illustrates how to manipulate these systems for the best possible viewing of living organisms and prepared slides.

The Limits of Light

Toss a pebble into a pool of water. Waves move out, four inches from crest to crest. A duck (12 inches at the water line) snoozing in the shallows rocks as the waves pass. Waves bounce from the duck and one could analyze these reflected and refracted wave patterns to assemble a crude picture of a duck. Nearby, a frog (two inches) is jounced, but fails to disturb the wave pattern sufficiently to create reflected or refracted waves. Other objects smaller than the wave length are passed over without perturbing the waves. The same is true of light waves. White light has an average wave length of around .6 micrometers. An extremely large virus, .3 micrometers in diameter, will not be seen. Two bacteria .3 micrometers apart will be seen as a single bacterium. This wave length limit explains why no further benefit can be gained by increasing the magnification of the microscope lens systems. To see smaller things, one must utilize the shorter wave lengths available from a beam of electrons, the principle of the electron microscope, but that's another story.

Function of the Condenser and Iris Diaphragm

Many student microscopes lack a focusing condenser. Instead they use a fixed condenser lens which is adequate for most observation. Focusing condensers must be focused so that the light source comes into sharp focus on the subject. With the condenser focused the objective lenses can achieve their designed resolution. The iris diaphragm is used to cut off light scattering into the objective lens. This "flair" lowers contrast and degrades the image. Lacking an adjustable iris, many student microscopes use a disc with holes of various diameters, sized to match the microscopeÕs objective lenses (smallest holes for the lowest magnification objectives).

Rotifer (*Keratella*) - iris open

Partially closing iris increases contrast and depth of field.

The iris has another function when viewing transparent subjects. The iris aperture can be reduced in order to increase contrast, making otherwise clear subjects stand out. Reducing the aperture in this way reduces resolution, but the improvement in contrast often provides much more information about the subject. For stained slides, where contrast is provided by the stain, the iris should just cut off flair light. To find the optimum aperture, remove the eyepiece and close the iris until it just appears around the edge of the field as you look down the tube. A quicker method, adequate for most observation, is to focus on the stained preparation with the iris wide open. Then, close the iris until the field just begins to darken. Obviously, a new iris setting is required for each objective lens used.

Oil immersion technique

Oil immersion objective lenses provide the highest resolution. By replacing the air gap between subject and lens with an oil having the same refractive index as glass, the some resolution degrading problems are eliminated. Ultimate resolution depends upon also making an oil connection between slide and condenser lens, a technique used with special equipment permitting the most critical microscope imaging. Most subjects observed in biology classes do not require viewing with oil immersion objectives. Stained bacteria, for example, can be distinguished using the 40X dry objective. The 100X oil immersion objective requires very careful manipulation so as to avoid jamming the slide. In preparation for using the oil immersion objective lens be sure something is in the center of the field of view using the 40X objective. Then the 40X is moved up and away, a small drop of immersion oil (fee of air bubbles) is added directly over the subject, the raised oil immersion lens is rotated into place and while looking from the side, the lens is lowered until it just makes contact with the oil. Now very slowly focus ever closer while looking for the first hazy image of the subject to appear.

TIP: For low contrast subjects, close the iris enough to create a high contrast image that will be easier to see as you focus the immersion lens ever closer to the coverglass. Once you have found the subject, open the iris all the way so that the subject is illuminated by the full condenser lens. The focused condenser provides the highest resolution, the whole purpose of going through this rather complex procedure.

Dark Field Viewing

Daphnia, Bright field

Daphnia, Dark field

Transmitted light (bright field viewing) is the normal microscope lighting technique, excellent for viewing stained slides. Dark field lighting provides an entirely different view of subjects and is particularly advantageous for viewing living organisms. The technique is most easily achieved by placing a black paper disc between the light source and the condenser. If your microscope has a filter holder, a clear plastic disc carrying the black "light stop" can be cut to fit the filter holder. When the condenser is focused and the iris opened all the way, beautiful dark field views are possible with the 4X and 10X objectives.

One of the most striking advantages of dark field viewing is that it provides a realistic rendering of color, something that is often lost I bright field viewing. Tip: substitute a disc of translucent colored plastic for the black paper disc to create colored background fields. Colored discs need be quite dense to achieve high contrast dark field lighting. The desired density is easily achieved by gluing several colored plastic discs together with clear contact cement.

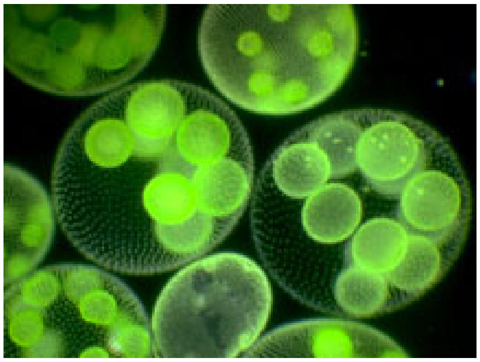
Polarized Lighting

Cyclops, crossed polarizers show muscle bands

Viewed between crossed polarizers many biological subjects will show surprising details not seen in other forms of lighting. Muscle fibers, skeletal elements, and crystals show up brightly when viewed between crossed polarizers. Try it on Paramecium and Daphnia.

Applying These Techniques

The last section of this program is a10 minute montage of living subjects viewed using the techniques described in the narrated portion of the program. They include: protozoans, rotifers, cnidarians, flatworms, the larval stages of marine invertebrates, microcrustaceans and tissue sections.



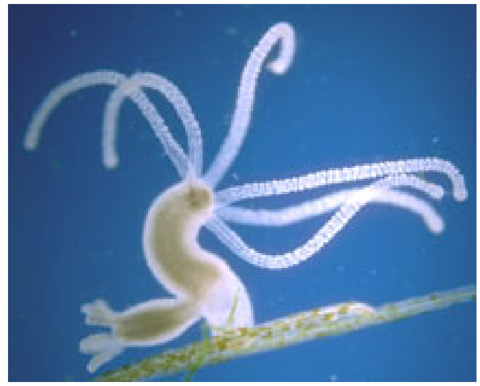
Volvox, dark field, 10X objective

Stentor, bright field, reduced aperture 4X objective

Dugesia (Planaria), dark field 4X objective

Bursaria, 10X objective

Hexarthra (rotifer), bright field, 40X objective



Hydra, blue stop, 4X objective

To learn more about microlife, and to practise using the microscope, collect water from local sources. Jars of pond water that contain some vegetation will often teem with populations of algae, protists and small animals.

To identify organisms found in natural collections we recommend the classic ***How to Know the Protozoa*** by T. L. Jahn, and ***Guide to Microlife*** by Rainis and Russell.

To further explore microlife see our galleries: The MicroNaturalists Notebook, The Ciliates, Life in a Drop of Pond, The Bacteria, and Babes in the Sea.