Special Microscopy Using a Standard Student Microscope

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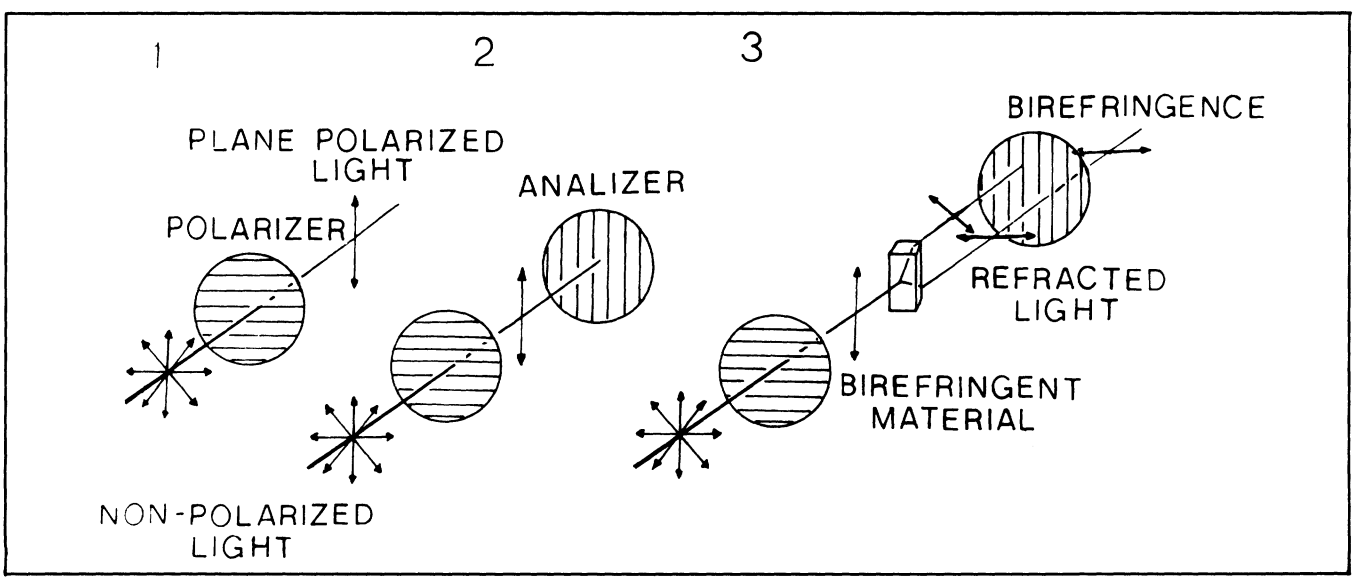
The student microscope is a basic tool for teaching biology. With a few simple and inexpensive modifications, it can provide even more useful information (and inspiration) to the student. In this article I explain the procedures used in my General Botany course to obtain polarization, fluorescence, and dark field images using a standard student microscope.

Polarization Microscopy

Transparent crystalline materials possess the property of optical birefringence. This means that light passing through such material will be bent (refracted) into two planes different from the light originally striking the material. Optical birefringence is of great diagnostic value in studying plant anatomy because certain structures such as cell walls, starch, and crystals are birefringent. If the incoming light is passed through a polarizing filter (the polarizer), only the light wave exactly perpendicular to the plane of the polarizing material will pass through (fig. 1). If a second polarizing filter (the analyser) is oriented perpendicular to the polarizer, it will also absorb the plane polarized light (fig. 2). At the point of precise perpendicular orientation, maximum darkness (extinction) is achieved. If birefringent material is placed between the polarizer and analyser, the plane polarized light striking the material will be refracted into planes which may pass through the analyser, thus forming a bright image against a dark background (fig. 3).

The principle of the polarizing microscope can easily be demonstrated using two sheets of polarizing film and a piece of cellophane. Hold two polarizing filters up to a window, one in front of the other. Rotate one polarizer until the transmitted light is completely extinguished. Without changing the relative positions of the polarizers, slip the piece of cellophane between the filters. Cellophane is birefringent, thus light will pass through the crossed polarizers where the cellophane is located but the rest of the field will remain dark.

To convert a student microscope into a polarizing microscope, place a polarizer between the specimen and the light source and set an analyser on top of the ocular lens. Many microscopes have a built-in filter holder below the iris diaphragm, which will hold the



FIGURES 1-3. Polarization microscopy. Fig. 1. Incoming non-polarized light, passing through a polarizing filter, the polarizer, is changed to plane polarized light. Fig. 2. Light passes through polarizer as in fig. 1. A second polarizing filter, the analyser, is rotated 90° from the polarizer. As a result, no light passes through the analyser. Fig. 3. Light passes through a polarizer as in figs. 1 and 2, however, birefringent material is placed in the path of the plane polarized light. The plane polarized light is refracted and any light not in the original plane will pass through the analyser and appear as birefringence.

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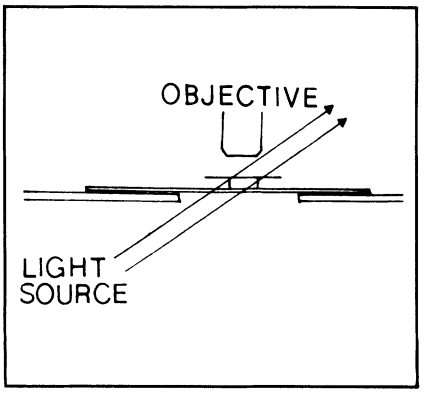

FIGURE 4. Dark-field illumination without a condenser. Light source is positioned to the side of the specimen so that no light enters the objective lens directly (arrows). Only light reflected or refracted by the specimen will enter the objective.

polarizer. The polarizer may be taped to the bottom of the stage if your microscope has no filter holder. The analyser is rotated until extinction is achieved. Any birefringent materials on the slide will appear bright against a dark background.

Secondary walls of xylem, sclereids, and most phloem fibers, as well as starch grains and crystals, may easily be identified using polarized light. This technique works equally well on hand sections of fresh material or stained prepared slides—the latter are often spectacular in appearance. Some suggested laboratory exercises using a polarization microscope are given in Rock (1981).

Fluorescence Microscopy

Many chemical substances absorb light of one wavelength and reemit light with a longer wavelength. This phenomenon is called fluorescence. In fluorescence microscopy the specimen is illuminated (excited) with ultraviolet light (UV). Any transmitted UV is blocked with a barrier filter between the specimen and the observer so that only fluorescent light is seen. *Protective goggles should be worn because UV light is harmful to the eyes!* This safety precaution should be followed, regardless of the UV source, to protect against possible stray UV light from the set-up.

To convert a standard student microscope into a fluorescent microscope, simply change the light source and provide a barrier filter. The least expensive and safest fluorescence source is a "black light." This lamp may either be placed directly under the stage or in front of a microscope with a mirror. Two microscopes can share one lamp but stray UV radiation should be avoided by covering unused portions of the lamp with aluminum foil. A medium yellow Wrattan photographic filter can serve as a barrier filter. The fluorescence obtained from this setup is weak but will be adequate in a completely darkened room.

Many substances occurring in plants fluoresce when illuminated with UV light. These include cutin, suberin, lignin, chlorophyll, and many phenolic compounds. Thus, fluorescence microscopy can be used to identify specific structures. In addition, tissue may be stained with fluorescent dyes to increase visibility. For example, staining tissue with aniline blue may be used to localize callose with a fluorescence microscope. Cellulose, carboxylated polysaccharides, and callose are fluorescent under UV when treated with optical brighteners such as Calcofluor White or dilute solutions of commercial laundry detergents which contain optical brighteners.

Dark-Field Microscopy

The purpose of dark-field microscopy is to render minute objects or details of larger objects plainer or actually visible by increasing the contrast of the object against the background. In dark-field microscopy, as the name implies, the field of view is dark while the objects are light. This is because the incoming light is either partially blocked or directed in such a way that no light passes directly into the microscope. Instead, the object bends the light into the microscope, thus appearing to produce its own

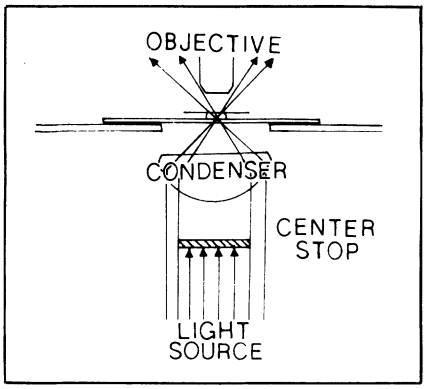

FIGURE 5. Dark-field illumination with a condenser lens. An opaque filter, the center stop, is placed in the light path below the condenser so that no light will pass directly into the objective (arrows). Only light reflected or refracted by the specimen will enter the objective lens.

light in an otherwise dark background.

It is important that the whole field is not covered by the object. If there are no intervening empty spaces, the whole field will appear bright. Thus, ordinary microscope sections are *not* suitable subjects for dark-field work. Dark-field is most useful for such material as blood, saliva, bacterial cultures, starch grains, pond water, etc. in which small objects are distributed throughout a liquid.

There are several methods of obtaining dark-field effects. The method used depends on the type of microscope available.

1) *Student microscope without condenser or diaphragm.* With this type of microscope, light must be directed through the object from below the stage at a very oblique angle (fig. 4). The purpose is to have the light so oblique that none will go directly into the microscope. As a result, the only light that will pass into the objective lens is that which is reflected or refracted by the object.

2) *Student microscope with an annular diaphragm.* This is the simplest type of microscope with which to obtain dark-field illumination. Rotate the diaphragm ring until the field darkens.

3) *Student microscope with a condenser and iris diaphragm.* With this type of microscope a special central

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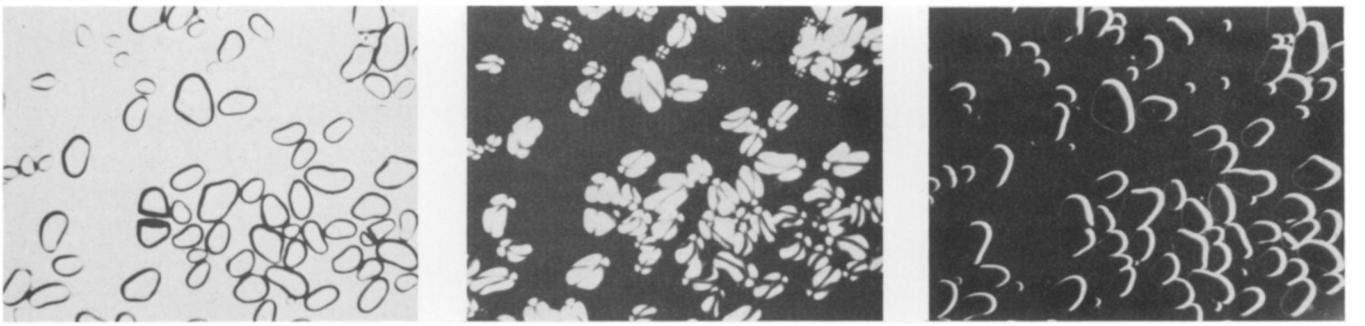

FIGURES 6-8. Starch grains seen with various types of light microscopy. All photomicrographs were made of the same preparation using a standard student microscope and techniques outlined in this paper. Fig. 6. Image with standard bright-field illumination. Fig. 7. Image using polarization microscopy. Fig. 8. Image using dark-field microscopy.

stop must be prepared. The size of the stop must be such that it excludes all light rays from passing directly into the condenser and allows those to pass which are of a greater aperture than that of the objective in use (fig. 5). The correct size can be determined as follows:

a) Focus on a specimen with the objective to be used;
b) Open the iris diaphragm wide;
c) Remove the eyepiece lens and observe the brightly lit back lens of the objective;
d) Close the iris until its edges are clearly seen;
e) Slowly open the iris until it just fits the field. This indicates the size of the required central stop;
f) Turn the microscope on its side and measure the size of the iris opening;
g) Make a central stop, the size of the iris opening or slightly larger, out of opaque material (e.g., india ink on a glass slide);
h) Insert the slide in a holder centered below the condenser or tape it to the bottom of the condenser;
i) Observe an appropriate object. If the field looks gray instead of black it is because the central stop is too small, the stop is not centered, or the object is not appropriate—too large or too numerous so that the entire field is lightened.

Note: Different powered objectives have different apertures and will therefore require different sized center stops. In general, the higher the magnification, the greater the aperture and the larger the required center stop. However, a large center stop for a high power objective will work equally well for lower magnifications. Because more light is needed for higher powers than lower powers, a brilliant light source is necessary when using high power with dark-field. A good description of the theory and application of dark field microscopy is found in Gage (1925).

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Sources for Materials

Polarizing Material (Fisher Scientific (1600 W. Glenlake Ave., Itasca, IL 60143) Linear polarizing film, Cat. #1B-789A). This is a 6×6 inch sheet from which filters of the appropriate size can be cut with a scissors.

U.V. Light. A "black light" may be purchased at many light shops.

U.V. Protective Goggles (Fisher Scientific UV-Absorbing Goggles, Cat. #11-405-1B).

U.V. Barrier Filter (Wratten, Medium Yellow #8 Photographic filter)—may be purchased at many photography stores.

References

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ROCK, B.N. 1981. Three-dimensional plant anatomy via hand sectioning differential staining. *In* Glase, J.C. (ed.) *Tested studies for laboratory teaching: Proceedings of the Second Workshop/Conference of the Association for Biology Laboratory Education (ABLE).* Dubuque, Iowa: Kendall/Hunt Publishing.

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