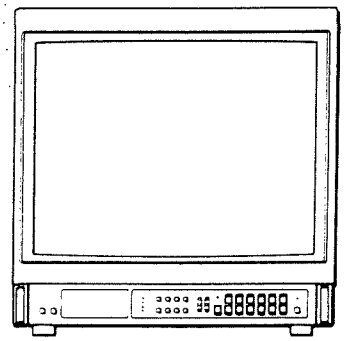
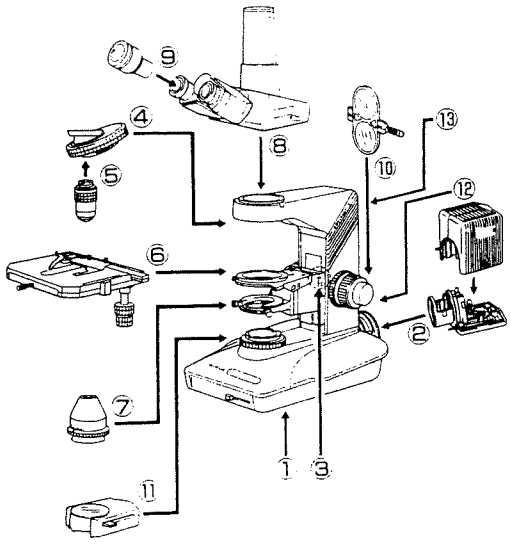
FINAL REPORT:

VIDEO IMAGE TRANSFER THROUGH A MICROSCOPE





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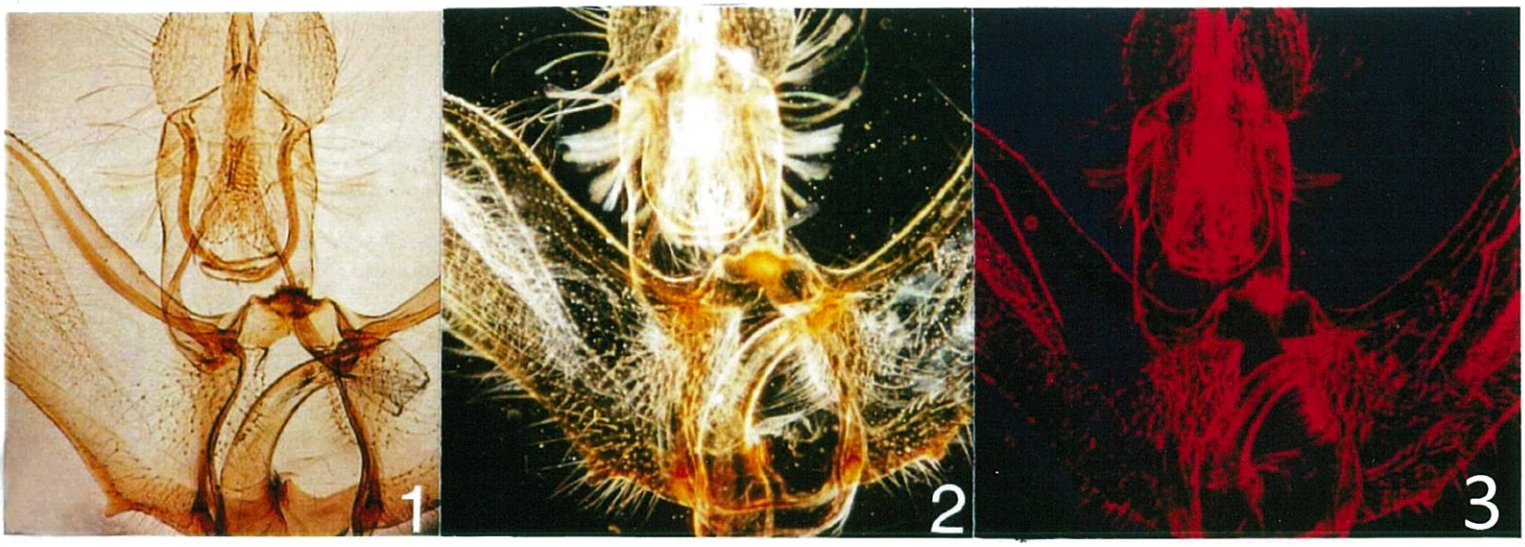
Abstract: This report reviews the various illumination systems available on a light microscope normally encountered at APHIS ports. For the pilot study, the advantages of selecting Nikon microscopes are discussed. Other video systems are presented for evaluation by the BATS staff. A vendor directory of video imaging technology, recently published by The Microscope Society of America, is avaiable upon request.

MODES OF ILLUMINATION: DARK FIELD AND RHEINBERG ILLUMINATION

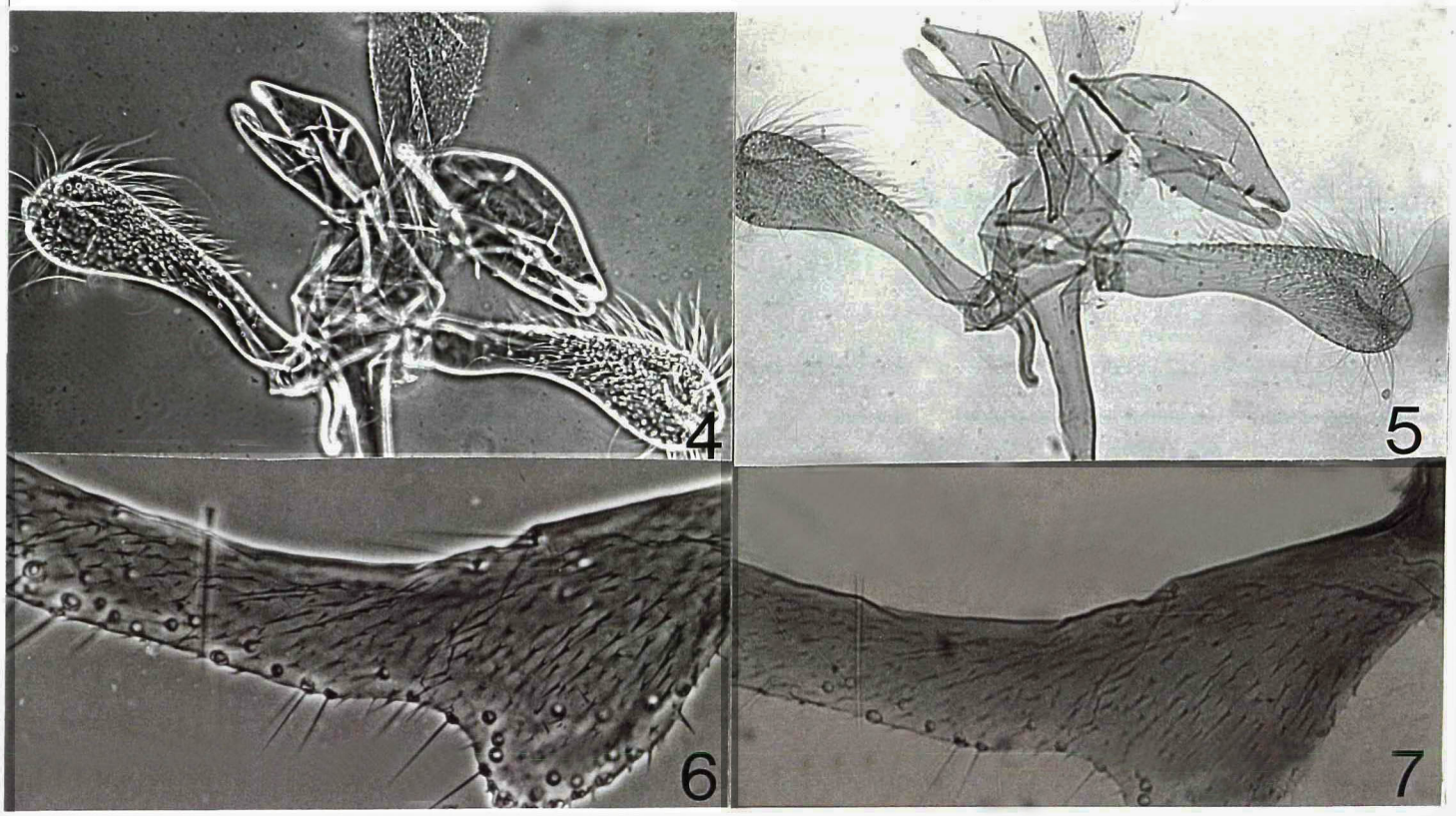
Most microscope work is carried out in the brightfield mode (moth genitalia, fig. 1), which is characterized by a solid cone of light passing through the specimen. One alternative method of enhancing this image is called darkfield illumination, where an opaque disc is placed within or below the condenser so that the image is viewed in a hollow cone of light (fig. 2). Note how the pores and setae stand out more clearly. At low powers (up to 40x), a darkfield stop is cheap and easy to add in a phase contrast condenser. For higher powers, a special condenser must be purchased. Sometimes a darkfield image may be obtained by using a phase condenser with a brightfield objective, instead of the normal phase contrast objective, so that the phase ring modifies the cone of light to approach the same condition seen with a darkfield stop.

There is no reason why a darkfield stop must be opaque, although it is almost always sold that way. In theory, the stop and the outer ring may be of contrasting colors. This is the basis for Rheinberg illumination (fig. 3), where a red inner stop was used with a purple outer ring. This optically stains the specimen and background any color desired. Rheinberg filters are no longer sold and most microscopists make their own using plastic Wratten filters available from Kodak through a camera store. Note that the color intensity is greater on more sclerotized subjects.



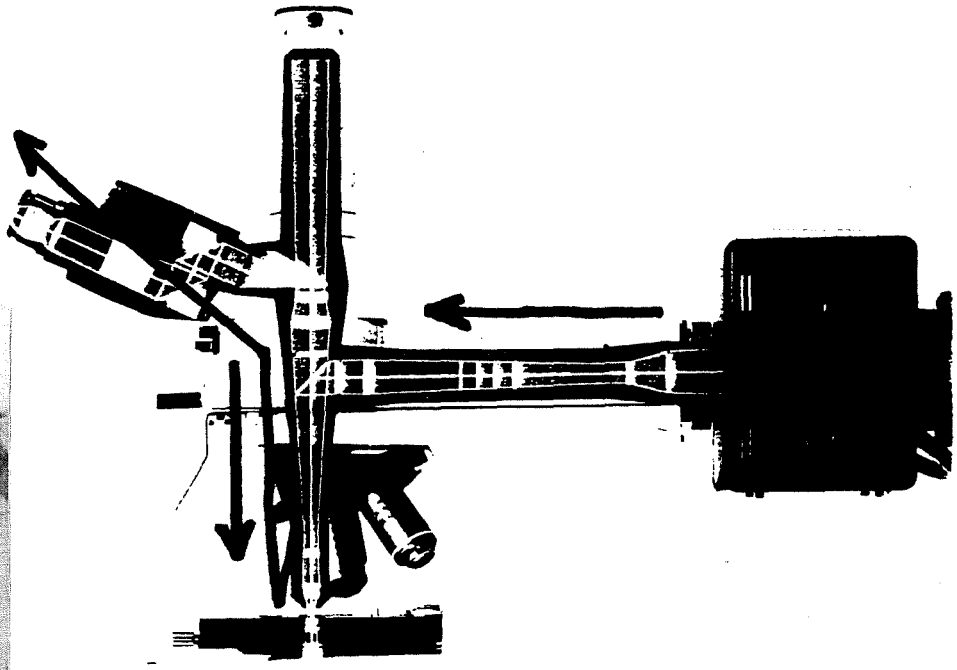
MODES OF ILLUMINATION: PHASE CONTRAST

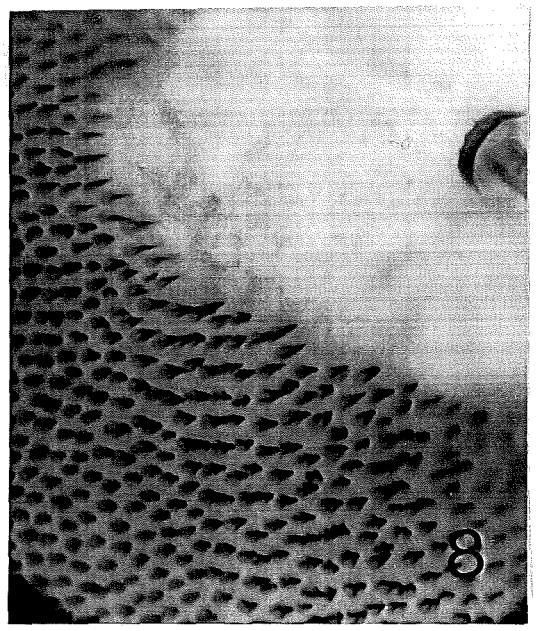
Most large ports have at least one phase contrast microscope. Phase contrast is obtained by aligning a ring in the objective with a ring of exactly the same size in the condenser. This mode works best on thin subjects, but care must be taken to keep the phase rings perfectly aligned with a special eyepiece called a phase telescope. There are two types of phase contrast, depending on the ring in the objective. In bright medium phase contrast (moth genitalia, fig. 4), the subject stands out brillant white against a darker backround. For comparison, the same subject is shown in brightfield illumination (fig. 5). The opposite effect is seen in dark medium phase contrast where the subject is dark against a lighter backround (moth genitalia, fig. 6). Compare how prominent the spines are compared to the brightfield image of the same object in fig. 7. The biggest disadvantage of phase contrast is the halo which often appears around the subject. When the object is thick, or structurally complex, it is often difficult to discern how one part is oriented with respect to another. Phase contrast is of moderate cost. Be sure to ask your salesperson what types of phase contrast are available because the intensity of the subject and the background may vary.



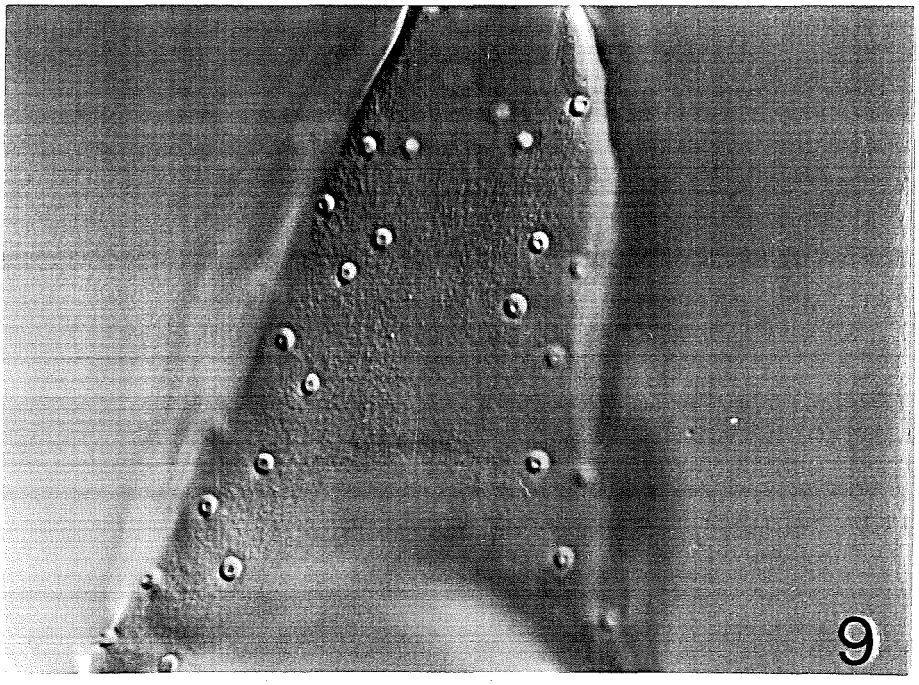
MODES OF ILLUMINATION: REFLECTED LIGHT

Most entomologists never deal in objects that are completely opaque. However, this is not the case for geologists who study rocks, botanists who study leaf texture, or the electronics industry that examines micro-circuit boards for flaws. In these disciplines, reflected light microscopes are common. The reflected light microscope bounces light downward outside the objective and then collects this light upward through the objective (see diagram). In this way, the compound scope functions as a "super-stereoscope" with the ability to see subjects at 400x without the need to make slides. I find this mode critical for examination of cuticular texture (tortricid larva, fig. 8), egg micropyles, and early instar Lepidoptera. Any of the techniques available for transmitted light (phase contrast, darkfield, etc.) will also be an option on reflected light microscopes, however, it may be necessary to change nosepieces on some models. Be sure to investigate long working distance objectives if the loss of resolution is not critical.





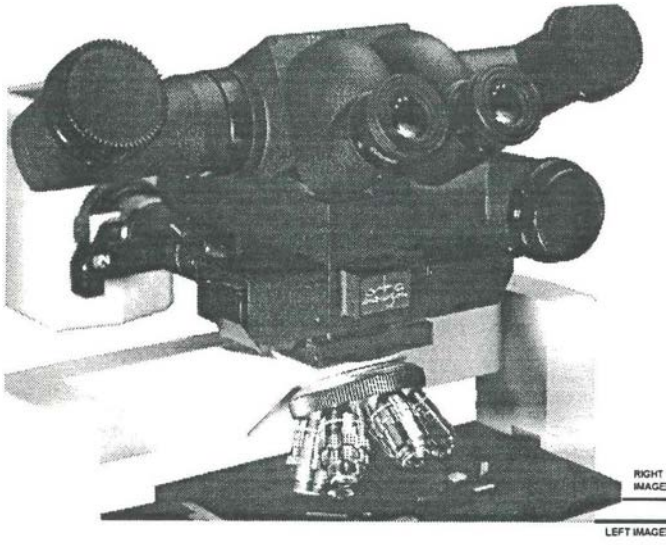
There are two main contrast enhancement systems which produce a nearly identical image using different, theoretically complex, methods. Nomarski differential interference microscopy (abbreviated DIC) has two prisms which separate and then recombine the image under polarized light. In Hoffman Modulation, the objective and condenser each contain a slit which must be aligned, also under polarized light. Details of images seen under DIC and Hoffman Modulation appear in relief as if you were viewing the object obliquely. Pores, pits, depressions, and ridges all stand out strongly without any halo common to phase contrast systems. An example of pores from moth genitalia in fig. 9 clearly shows the advantages of DIC. Unfortunately, DIC can be expensive and difficult to adjust. Hoffman Modulation, and perhaps a newer technique from Zeiss called Varel, are cheaper but slightly less flexible in that only modified objectives can be utilized. Larger ports with high volume should investigate these techniques.



MODES OF ILLUMINATION: **THREE** DIMENSIONAL IMAGING

Microscope images normally appear flat with hardly any depth of field. A new kind of microscope developed by the Edge company (see below) rectifies this problem by using four independent lights to give a true 3-D image. The cost of $30,000 is well beyond the means of most APHIS stations, but it is of possible utility to the Professional Development Center as a teaching aid, or perhaps to our more research oriented staff in the Methods Development Section.

A cheaper method called stereo-polarization can mimic the 3-D effect of an Edge microscope, but at a reduced cost of only $50! It is based on the "Mercer effect". Polarized light is sent up each eyepiece with the vibration plane at right angles to each other. It is the same principle as cheap 3-D glasses that view blue and red images separately. Stereo-polarization does not work with optics that are not strain free, but it is worth the time to learn this technique if you view insect whole mounts.



FROM EDGE'S TRUE-VIEW 3D MICROSCOPE HEAD ....

...TO THE CLASSROOM, CONFERENCE ROOM, AND AUDITORIUM

- \+ View 3D directly through the microscope eyepieces
- \+ View 3D directly on the monitor
- \+ Create 3D photographs and video recordings
- \+ Teach and conduct peer-group discussions working directly from the microscope

For additional information call Edge at (310) 396-9333 or Microstereopsis at (301) 738-8165.