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Low cost polarized light microscopy

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This paper describes a simple and inexpensive adaptor for the conversion of essentially any transmitted light microscope into a polarized light microscope. Although the focus is on the specific application of asbestos identification, microscopes adapted in this manner have general utility for identification of various crystalline and noncrystalline materials. The conversion allows one to study extinction characteristics, birefringence, pleochroism, sign of elongation, isotropy/anisotropy, as well as dispersion staining colors. Key components of the adaptor are a polarizer, an analyzer, a retardation plate, a stage rotation device, and central and annular stops. Construction details are included and allow fabrication of the adaptor at very low cost with minimal machining, using readily available materials. Instructions for its use are also provided and serve to complement a working knowledge of identification techniques. Polarized light microscopy has great utility for a number of industrial hygiene applications. Hopefully the adaptor described herein will serve to broaden its accessibility. **Jones, W. G.; Thorne, P. S.; Clere, J. L.**:Low cost polarized light microscopy. *Appl. Ind. Hyg.* *4*:191-195; 1986.

Introduction

Polarized light microscopy (PLM) has for many years been a basic tool for use in identification of crystalline materials. In the field of industrial hygiene (and general environmental health), this technique has found specific utility in identification of asbestos in bulk samples. It is, in fact, one of the methods recommended by the Environmental Protection Agency for determination of asbestos in building materials.[1]

PLM has certain advantages over other identification techniques, and, in the hands of a well-trained microscopist, nearly all components of bulk insulation samples can be quickly and accurately identified. Further, the various amphiboles can be differentiated[2] and percentages of sample components can be estimated.

PLM is generally taken to be an inexpensive technique; however, this is in relation to very expensive alternatives (SEM, TEM and X-ray diffraction). Polarized light microscopes are actually rather expensive. They range in cost from approximately $1500 for a monocular unit to several thousand dollars for binocular instruments.

In order to identify asbestos, as well as other particles, using polarized light microscopy, characterization of the following properties is required:[1]

- Morphology
- Color and Pleochroism
- Refractive Indices
- Birefringence
- Extinction Characteristics
- Sign of Elongation
- Dispersion Staining Colors

Determining these properties with a polarized light microscope generally involves viewing the particles under plane polarized light as well as under crossed polars. The latter condition involves viewing the specimen between two polarizing films with the transmission axes of the polaroids aligned perpendicular to each other. Polarized light microscopes are therefore equipped with polarizing film placed between the specimen and the light source (referred to as the polarizer) and between the specimen and the ocular (the analyzer). At least one of these must be able to be rotated through an angle of at least 90 degrees.

Determination of extinction characteristics and sign of elongation are made under crossed polars, and both require rotation of the particle with respect to the polarizing film. Thus, polarized light microscopes come also equipped with rotating stages. Sign of elongation determination requires insertion of a retardation plate between the crossed polars, so these microscopes also have a slot through the body tube to accommodate this plate.

Dispersion staining requires insertion of a central or annular stop in the back focal plane of a low power objective and use of plane polarized light. This is a rather specialized technique, and although dispersion staining objectives are commercially available,[3] they are typically not an integral part of a PLM.

Polarizing film is commercially available at reasonable cost,[3] and one can usually find a satisfactory location for insertion of this film both above and below the specimen on standard microscopes. Further, it is typically not a problem to contrive a means to allow rotation of one of the films. It should be mentioned that the difficulty of doing this is typically inversely associated with cost of the microscope. Part of the

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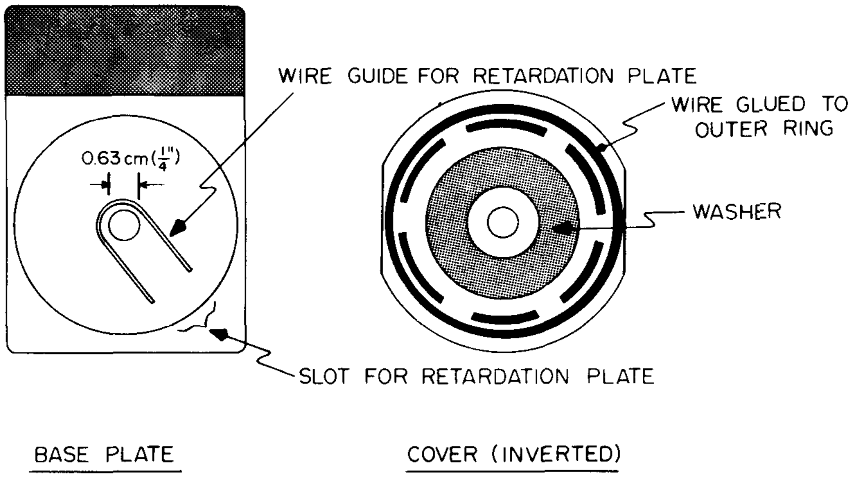

Figure 1—PLM adaptor (construction notes).

expense of high cost microscopes is in the ability to add on various items (including PLM accessories) at a later date. With these instruments, there are usually various filter holders available which make conversion quite simple, and if the converted microscope has a rotating stage most of the above-mentioned tests can be performed. Unfortunately, however, rotatable stages are rare on non-PLM microscopes.

These ideas are not particularly new; people have been making such modifications on their own (to varying degrees) for years. The purpose of this study however was to develop a convenient adaptor for quick conversion of relatively low-cost *fixed-stage* biological type microscopes (the type that are available at all universities, secondary schools, and most labs) into essentially full-featured polarized light microscopes for use in particle identification. The balance of this paper deals with the building and use of such a device.

Construction details

The initial design scheme was to develop a low cost device, fabricated from easily available materials, which would mount on the stage of the microscope and provide means for specimen rotation as well as a housing for one fixed polarizer and means for insertion and removal of a retardation plate. A Gelman Sciences, Inc. Analyslide® Ann Arbor, MI (product #7231) seemed to offer a reasonable starting point. This is a slim plastic container which is commonly used in laboratories to store and examine filters. The Analyslide measures approximately 4 × 8 cm, (2 × 3"), and is about 0.6 cm thick. It is actually designed to fit conveniently into a microscope mechanical stage.

The initial machining step was to drill a 0.64 cm (¼ in.) hole through the center of both the base plate and cover of the Analyslide (Figure 1). The next step was to modify the cover to allow it to rotate freely on the base plate. This was required since the initial fit was much too tight to allow smooth rotation. This was accomplished by inverting the cap and wedging a 15 cm (6 in.) length of wire (we used 20 AWG Nylon Jacketed) between the outer and inner rings as shown in Figure 1. Thus, when the cover was placed on top of the base plate it was elevated enough to allow free rotation. The rotation was also aided by the smooth surface of the wire which presented little friction. Initially, 0.16 cm diameter solder was used for this, but later wire (with its hard sheath) was found to provide better movement. It is important that the wire lie flat around the entire cover and that it be pushed to the outside edge prior to gluing. Super glue (Super Glue Corp., Hollis, NY) worked fine for this and all other gluing operations involved in building the PLM adaptor. Next a washer was glued to the underside of the cap as shown in Figure 1. This added mass helped smooth out movement. Finally a handle was glued to the cap, in this case, the inexpensive handle portion of a binder clip. Obviously there are many other possibilities for this design feature.

A retardation plate slider was then fabricated out of sheet metal (approximately 5 × 1.2 cm) according to the specs given in Figure 2. In order to provide a guide for insertion of the retardation plate, solder was first formed around the retardation plate and then glued to the base plate (Figure 1). Next, it was necessary to provide a slot through which the plate could be inserted into the guide. This step was accomplished by cutting a slot, flush with the bottom of the base plate, into the rim of the plate using a hack saw blade. This slot and associated retardation plate guide can be located in whatever position is most convenient. The dimensions of the slider can also be altered to suit individual needs.

The next task was to find a low-cost material for the retardation film. Insertion of this film between crossed polars enables one to determine sign of elongation, which has particular utility for resolving crocidolite from other asbestos forms. It has been reported that cellophane and transparent tape are birefringent materials and can act as half-wave retardation plates for light of various colors as a function of the material thickness.[4] After experimenting with various materials, it was found that 24 layers of transparent tape (Scotch® 600, 3M Co., P.C./Part No. 021200-07457 St. Paul, MN) was adequate, and this was used in the first few adaptors. Later, however, it was found that just *two layers* of a commercial book tape (Scotch® 845 Book Tape P.C./Part No. 021200-07383) had acceptable optical properties, and this was used for subsequent conversions. It should be noted that in the PLM adaptor, the retardation plate is inserted into the light path between the light source and the specimen in a horizontal position; therefore, optical clarity was not a limiting factor.

The polarizing film for the adaptor was purchased from McCrone accesso-

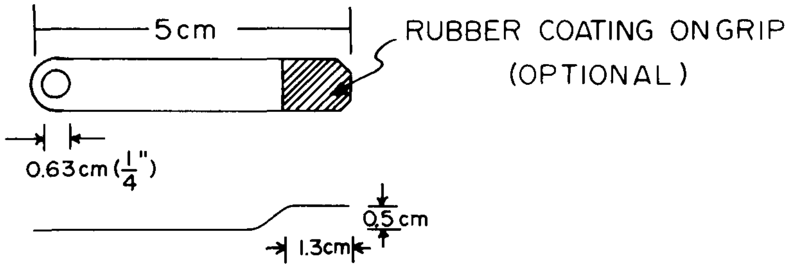

Figure 2—Retardation plate specs.

ries and Components[3] and is available in 5 × 5, 15 × 15, and 30 × 30 cm sheets. Circular portions of polarizing film are also available in "kit" forms for specific microscopes. Furthermore, at least one manufacturer (AO Reichert™) will supply them at no cost to their customers. The fixed polarizer which is mounted in the base plate can be conveniently cut with a 0.64 cm (¼ in.) paper punch. The retardation material can be likewise cut. The polarizing film is thinner than the base plate. This is important since it provides for a gap both above and below the film after it is pressed into the hole, thus it is protected from scratches from the microscope stage or the retardation plate. Neither the fixed polarizer nor the retardation material should be glued in place until certain adjustments to their position are made.

The adaptor unit can be refined a bit at this point. A large drill bit or counter sink can be used to bevel (from the top) the hole drilled in the cover. Also one can spray paint the entire unit flat black and add rubber grip material to the end of the rotation handle and retardation plate. Stage clips can also be fabricated from thin metal and glued to the cover. This is not a requirement, however, unless the microscope is used in a tilted position. Figure 3 is a diagram of the completed adaptor.

It should be noted that the refractive index oils that are used for particle identification are corrosive to the plastic and paint of the rotating stage of the PLM adaptor. A smooth surface is important so that the slides can be moved freely on the stage when scanning a preparation. The wide tape that was used for the retardation material is not affected by the oils and can serve as a suitable protective covering. Aluminum tape (also available in wide rolls) is equally effective.

Central and annular stops for dispersion staining were fabricated by cutting out circular portions of black electrician's tape and mounting them on 18 mm circular glass cover slips (Figure 4). The exact size of the stops will be a function of specific microscope conditions, so one can start with 0.64 cm (¼ in.) and

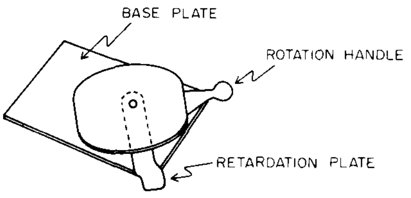

Figure 3—Schematic diagram of PLM adaptor.

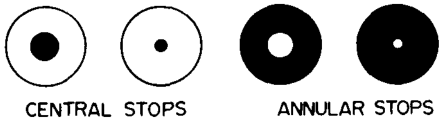

Figure 4—Dispersion staining stops.

0.32 cm (⅛ in.) stops since they can be easily cut with punches. If these do not work, one must resort to different sizes. These stops were placed in the back focal plane of a low power objective. This was accomplished by simply removing the objective and attaching the cover slip to the objective by means of a temporary adhesive. Handy Tak (Super Glue Corp., Hollis, NY 11423) is a reusable adhesive putty-like substance that was quite adequate for this application. For a more permanent bond, Barge cement (Div. Natl. Starch and Chemical Corp., Towaco, NJ) was used.

Others have reported on methods for observing dispersion staining colors using phase contrast microscopes.[5] The stops required for this are part of the phase illumination system and thus need not be constructed. Therefore, if you are converting a microscope that has phase illumination, this may be a more convenient approach.

Use of the PLM adaptor

The PLM adaptor is designed to fit conveniently into a mechanical stage device and can therefore be moved around the stage just as a slide would be. A mechanical stage is not a requirement, however, and often it is more convenient to remove the mechanical stage altogether when converting such a scope.

The first step in using the PLM adaptor is to center stage rotation. This is accomplished by bringing a specimen into focus and, while rotating it, observing where the center of rotation lies. The center is then brought into the center of the cross hairs within the ocular by moving the PLM adaptor on the stage. Often the center of rotation is initially outside the field of view altogether, but with practice one can become proficient at estimating its location and moving the adaptor accordingly. The rotation is rechecked and the procedure repeated until center of rotation is in the center of the field. It is true that a mechanical stage makes the centering procedure a bit easier; however, once it is centered, it is very easy to knock it out of alignment, particularly if the mechanical stage movement is not stiff. For these scopes, a better method is to first remove the mechanical stage device and then apply the temporary adhesive putty to the four corners of the bottom of the base plate. The adaptor is then pressed onto the stage. Light coming through the 0.64 cm hole ensures that the initial position is somewhat close to center. The adhesive holds the adaptor firmly but does allow the movement required to center rotation. After centering is accomplished, the adhesive holds the device firmly in place for subsequent use. Registration marks can also be made on the stage at this point to aid in realignment.

Up to this point nothing about the analyzer (polarizing film placed above the specimen) has been mentioned. Since the polarizer is fixed within the adaptor base plate, this film must also be rotatable. There are several possible solutions.

One approach is to cut a circular portion of film and mount it in the ocular. This is obviously more appropriate for a monocular scope, but could also be used in one (or possibly both) oculars of a binocular scope. The ocular can be easily rotated by hand for viewing under both crossed and plane polarized light.

Another approach is to mount the analyzer film in the back focal plane of the objective. This is done by cutting a circular portion of film (approx. 1.5 cm diameter), removing the objective, and attaching the film by means of the temporary adhesive to the back focal plane (top of objective). The objective is reinserted into the turret but left loose so that it may now be rotated through 90 degrees. The rotation can be made directly by moving the objective by hand; however, a simple spring loaded plastic clothes pin, clipped to the objective, serves as an ideal rotation handle. Obviously the movement of the objective requires readjustment of focus. This has not proved to be a problem, however, since microscopists instinctively have their hand on fine focus as they view specimens.

The next step is proper alignment of the fixed polarizer. This is important in order to accurately determine extinction characteristics. The fixed polarizer should be inserted so that its vibration direction is oriented east-west. The polarization direction can be checked as previously described by looking through it at a source of polarized light of known direction.[6] Reflected light from a nonmetallic shiny surface when viewed at an angle will be polarized in an E-W direction and serves as a suitable reference. Fine adjustment of the alignment can be achieved by viewing under crossed polars an anisotropic straight fiber (such as hair) with known parallel extinction. The polarizer should be adjusted so that extinction of the fiber occurs parallel to the ocular cross hairs. If the polarizing film fits loosely in the adaptor, a small amount of putty can be used to hold it in place while still allowing small adjustments. After alignment, a mark can be made on the edge of the film to indicate vibration direction. If the polarizer is already fixed in the adaptor, adjustment can be made by rotating the entire adaptor on the microscope stage.

The final item is to permanently attach the retardation film (3M tape) to the retardation plate. This is done by placing the tape in general position in the retardation plate and rotating it between crossed polars until the deepest red color is obtained. A fiber of known sign of elongation can be viewed at this point to ensure that a fiber with positive sign of elongation is blue in the upper right to lower left position and yellow in the upper left to lower right position. If not, this can be corrected by rotating the tape to the next position where the red background occurs. The tape can then be glued permanently in the retardation plate.

Discussion

The authors have converted a wide variety of microscopes and found them all to be quite adequate for the identification of asbestos in bulk insulation samples. Biological scopes of reasonable quality are readily available and can be purchased second hand for as little as $100. These are often binocular and usually have a 10X objective which is suitable for identification work. Higher power objectives can also be fitted with the polarization film and used; however, the play that is inherent in the PLM adaptor movement becomes somewhat annoying at high magnification. When looking for a scope for PLM conversion, a good illumination system is an important factor. The scope should have a strong light source and, ideally, the ability to set up Köhler illumination. Since the working distance is changed when using the adaptor, adjustment will be required to re-establish Köhler illumination.

The PLM adaptor can be made quite easily, and the cost is indeed low. Also, the mechanics of the tests used for identification can be learned fairly quickly. That is not to say, however, that someone can build one and immediately be proficient at identification. Proper training is critical. A course in PLM, such as those offered by McCrone Research Institute (Chicago, IL) or in some university geology departments, is strongly recommended for beginners. Several fundamental texts in optical mineralogy are also referenced.[7-11] This is just the beginning, however, and proficiency only comes with hours of studying known samples and building of a "personal file" of quickly identified particles. Morphology is a very important component of particle identification, particularly with fibers.

Researchers at the Pittsburgh Graduate School of Public Health have exploited a special utility for the PLM adaptor to take advantage of the stock of biological microscopes available at the university. Ten such scopes were modified for identification duty and a lab in asbestos identification where each student has his or her own polarized light microscope is now offered. The cost of the adaptors is so nominal that motivated students can be given the adaptors to enable them to continue to improve their skills. This has worked out quite well for the department, and other industrial hygiene departments may likewise benefit. The cost of supplying students with conventional polarized light microscopes would otherwise be prohibitive.

Another advantage of the PLM adaptor which is a direct function of its low cost is the ability to take an adapted scope out into the field to make identifications at the work site. Many field situations would not be compatible with high cost conventional polarized light microscopes. Often in these instances where identification of bulk samples is required, there is some construction work *in progress.* Having the ability to immediately determine the presence or absence of asbestos can result in rapid and appropriate response to control exposure when results are positive and can control anxiety when results are negative. Contrast this with a typical situation where a sample is collected, sent to a lab, and in a few days or perhaps weeks the results are received (long after a decision should have been made).

If there is no hood available, identification of asbestos samples at the job site does carry some risk of exposure. However, since identification requires the transfer of very small amounts of materials directly from a sample container to refractive index liquid, the potential for exposure is less than it is for bulk sample *collection* where larger amounts of material must be extracted from sometimes awkward and occupied locations. Both tasks, however, should be done in a fashion that minimizes the release of fibers into the air. A recent publication describes practical means for doing so.[12]

It should be noted that polarized light microscopy has uses beyond the identification of asbestos. In the field of industrial hygiene, examples include the identification of both non-fibrous (silica, calcite, olivine) and fibrous (glass fiber, mineral wool, cellulose) materials.

Objectives used for polarized light microscopy are typically rated as "strain free." These are manufactured carefully to avoid causing strains in the glass which could give the glass birefringent properties under crossed polarization. No problems have been found in using standard 10X objectives in any of our converted microscopes. We suggest, though, that objectives for PLM be first examined between crossed polars for evidence of strain.

There is certainly nothing sacred about this specific design, and changes can be made to suit individual needs. Should anyone have improvements or modifications they can suggest or problems adapting a particular microscope, the authors would appreciate being informed. One modification currently being tested is a field identification instrument constructed from a small flashlight (as a light source) in combination with a magnification device fashioned from the objective and ocular of a standard microscope. The main advantage here is in terms of portability in that it can be easily carried in a briefcase (or even a pocket). Another advantage is that anyone owning a microscope already has the major components required to fabricate this device. Although this unit has not been fully evaluated, it may have potential as a field screening instrument.

In another variation on this theme, a Zeiss IM-35 inverted microscope has been converted for polarized light work. As mentioned earlier, with high quality microscopes it is easy to find locations for the polarizer, analyzer, and retardation material, and the Zeiss was no exception. This scope did not have a rotating stage however. This problem was solved via a modification of the basic PLM adaptor design. For this application, rather than drilling a 0.64 cm (¼ in.) hole, a 2.54 cm (1 in.) hole was drilled through both the base plate and cover. This hole was then large enough for the objective to come up from the bottom through the adaptor. The slide which sits on the cover could then be rotated above the objective. Obviously this design does not allow insertion of a compensator within the rotation device, but again, with a scope like this, there is no problem finding a suitable location for this or for the polarizing film. The cost to convert this scope conventionally would be literally thousands of dollars. Our cost was a few dollars in materials and approximately one hour of quite enjoyable and gratifying labor.

Recommendations

This paper has described a simple, effective, and inexpensive method for converting essentially any biological microscope into a polarized light microscope with the features required for many identification tasks encountered in the field of industrial hygiene. We recommend its use particularly for the applications of training and field screening/identification.

A final recommendation or message we wish to convey, however, is simply that we should not allow ourselves to be intimidated or infatuated by technology, but rather seek to exploit our individual abilities in the course of finding practical solutions to occupational health problems with materials at hand.

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