# MICROSCOPY 101

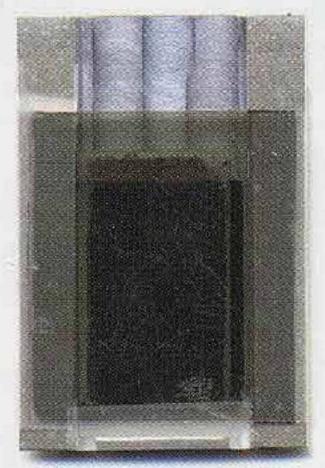
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## Petrographic Slides Projected in a 35mm Slide Projector

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In a second year geology class, students begin to explore the use of the petrographic microscope to look at the optical properties of rocks and minerals in thin section. The petrographic microscope is configured with a polarizing plate (polarizer) below the stage that transmits light in the N-S direction and an additional polarizing plate (analyzer) in the tube above the stage that passes light in the E-W direction. This configuration allows students to use various optical techniques for mineral identification taking advantage of the isotropic and anisotropic properties of the crystal and the way light is transmitted through it in thin section. As students



Slide holder showing slide in pocket behind polarized film

generate questions about their observations, the lab instructor is required to move from student to student looking through their microscope to describe the effects. In an effort to find a more efficient way to present this information to the class as a whole, we began to explore the various ways available for us to project the slides onto a large screen in real time.

The most obvious method was to use a high-resolution color video camera coupled to a microscope with the output

sent to a LCD projector. Cost was the major drawback of this technique and it limited us to a small field of view depending upon the microscope magnification used.

Another method we explored was to sandwich a thin section



slide between two pieces of polarizing film and place it in a microfiche reader. This method still had the limitation of a small field of view and the slide sandwich was much thicker than the microfiche was designed to handle. The resulting viewer image gave poor resolution and was not bright enough to be easily seen.

Looking at the design of a commercial petrographic slide holder (GeoScan Enabler from Meyer Instruments, Inc., Houston, Texas) we typically use for scanning glass slides in a 35mm slide scanner, I realized that a similar design might allow us to put the thin section sandwich into a 35mm slide projector. After a few tests, I was convinced this would work for our purposes.

Working with our machinist, I was able to rough out a holder that would allow us to put a petrographic slide between two pieces of polarizing film taped to both sides. With the projector slide tray removed, the holder can then be placed into the optical path of the projector in the same location that a normal 35mm slide would occupy. Using tape on the polarizing films allows them to be added or removed as needed to demonstrate various properties. The bright projected image displays a large portion of the slide and can be sized by placing the projector at different distances from the viewing screen.

### EMBEDDING MEDIA HEALTH HAZARDS AND MEDICAL DOCUMENTATION

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Questions have been raised from time to time on the MSA list server about potential health hazards associated with embedding media. Documentation of these health hazards in the biomedical and microscopy communities is extensive; however, many microscopists are not familiar with the occupational medicine literature. Articles directed to the microscopy community date back to the 1980's, but they appeared in publications which were not available to all university libraries or those using embedding techniques1-4. Hazards in the use of embedding media include exposure to carcinogens, irritants, allergens, and systemic toxicants. Although many of the chemicals used in dehydration, infiltration, and embedding are listed as potential carcinogens (these effects usually take years to appear), the most significant problems manifest themselves as a result of irritation or allergic reactions. These problems result either from low dose-long term (chronic) exposure, or short term-high dose exposure (accidental spills). This article will present documentation from the medical literature, as well as the author's personal experience gained over more than thirty years in electron microscopy research laboratories. A future article will address the use of personal protective equipment and laboratory safety involving embedding media.

Amine accelerators are strong irritants and probably represent the greatest hazard of all embeddding media components<sup>5</sup>. Commonly used accelerators such as benzyldimethylamine (BDMA), tris(dimethyl amino ethyl) phenol (DMP-30), and dimethyl aminoethanol (DMAE or S-1) are extremely volatile and should be used only in a properly functioning hood. DMAE has been documented as a cause of allergic asthma in industry6.

Although it was thought that low molecular weight compounds used in dehydration, as transitional solvents, and as embedding media were either irritants or possible carcinogens, there

also has been documentation of immunologic injury by these compounds7.8. Propylene oxide, the smallest epoxide and a commonly used transitional solvent, has been identified as the cause of corneal burns from its vapors9 and contact dermatitis in an electron microscopy technician10. The author is aware of electron microscopy technical staff that have developed systemic reactions to propylene oxide, even when using proper laboratory and industrial hygiene procedures. This problem was overcome by using alternatives to propylene oxide. Dimethyl formamide, used as a dehydrating agent and transitional solvent with acrylic resins, can be readily absorbed by the lungs as well as through the skin and is toxic to the liver. Prolonged or repeated skin contact with this compound may cause contact dermatitis9. Styrene, a transitional solvent and crosslinker in polyester resins, is an irritant of the eyes, mucous membranes, and a central nervous system depressant. Repeated contact also can lead to dermatitis9.

The medical literature lists a number of epoxy resin components associated with hypersensitivity reactions ranging from contact dermatitis to respiratory symptoms such as occupational asthma. Vinyl cyclohexene dioxide (VCD), the epoxy component of Spurr's low viscosity embedding medium", is extremely irritating. There are numerous anecdotal reports of electron microscopy personnel experiencing adverse systemic reactions (usually flulike symptoms), even when exercising appropriate precautions in handling and disposing of the VCD-containing medium. Antibodies to VCD were demonstrated in industrial plant workers who reported multiple complaints of ocular, nasal, skin, and chest symptoms12. 1,4-butanediol diglycidal ether, an epoxy diluent, was documented to cause contact dermatitis in industrial workers which resulted in the workers seeking other employment<sup>13</sup>. Adverse reactions to acid anhydride hardeners range from irritation of the skin and conjunctiva to respiratory hypersensitivity and asthma<sup>14, 15</sup>. Allergic contact dermatitis to dodecenyl succinic anhydride (DDSA), a common anhydride hardener in epoxy resins, has been reported in an electron microscopy technician16. In addition, asthma has been attributed to grinding epoxy resins cured with acid anhydrides17.

Acrylic resins such as LR white, LR Gold, Lowicryl, and methacrylate are used extensively in biological and materials applications and represent one of the most significant sources for occupational medical problems in the microscopy laboratory. Orthopedic surgeons demonstrated the necessity for wearing non-latex gloves when handling acrylics18. Latex gloves are penetrated rapidly by acrylics and exposure results in a painful contact dermatitis in susceptible individuals. The author is aware of microscopy personnel who could not even enter the laboratory when acrylic resins were being used for infiltration and embedding without developing contact dermatitis. Dental technicians have developed neurotoxic reactions and parasthesia of fingers from coming in contact with methyl methacrylate. The reaction experienced by the dental technicians was not the result of an irritant effect, but rather a destruction of membrane lipids and myelin sheaths in nerves by the strong solvent action of the acrylics. In addition, respiratory problems have been reported in dental technicians working with methyl methacrylates and cyanoacrylate glues<sup>19</sup>. A dental student who took no precautions to prevent inhalation of the dust created by grinding acrylic polymers developed alveolitis<sup>20</sup>. Transmission and analytical microscopic examination of polymer samples and lung biopsies were used in the diagnosis. Lowicryl induced contact dermatitis has been reported

in a number of electron microscopy personnel21. The same authors reviewed the toxicological properties and occupational hazards involved in using (meth)acrylate compounds in embedding media for electron microscopy<sup>22</sup>. More recently, bronchial symptoms were documented in workers exposed to methylmethacrylate<sup>23</sup>.

Although polyester resins (Vestopal W, Rigolac, Selectron, Rhodester) are used infrequently, there are hazards associated with media which contain polyester and styrene. Styrene, benzoyl peroxide and cobalt naphthenate can cause irritation and sensitization of the skin and mucous membranes9, 24. In addition, there are additional problems of skin de-fatting as a result of the strong solvent action of styrene.

From the discussion above and the reference literature, it is very clear that there are significant hazards associated with all aspects (unpolymerized and polymerized) and classes of embedding media. The key to avoiding problems is education and common sense approaches to laboratory safety.

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