The Use of a Combined Polarizing Light/Epi-fluorescence Microscope for Examination and Analysis of Painted and Coated Objects and Samples

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Introduction

CONSERVATORS AND SCIENTISTS USE compound light microscopes to examine and analyze samples from painted and coated objects and surfaces. Two principal types of microscopes are commonly used: polarizing light microscopes and fluorescence microscopes. Polarizing light microscopes are used to identify fibers, wood, inorganic pigments, and many other particle samples. Epi-fluorescence microscopes are used to examine the visible fluorescence of samples and fluorescent stains. Either microscope may be used to examine surface color, reflectance, and texture using reflected visible light provided by an external light source.

Presented at the annual meeting were practical advantages of using a combined polarizing light/ epi-fluorescence microscope for examination and analysis of samples and object surfaces. The color photomicrographs used to illustrate the presentation cannot be reproduced in this postprints article. Instead, this article will briefly describe the use of polarizing light microscopes and epi-fluorescence microscopes, and the principal advantages of using a combined microscope for examination of samples and objects.

Polarizing Light Microscopes

Polarizing light microscopes permit one to measure the optical properties of transparent samples (e.g., thin-sections, pigments, and fibers) using transmitted polarized light. Polarizing filters inserted above and below the sample restrict the vibration direction of transmitted light to a single direction, e.g., N-S or E-W. When one filter is inserted into the light path, the light is said to be

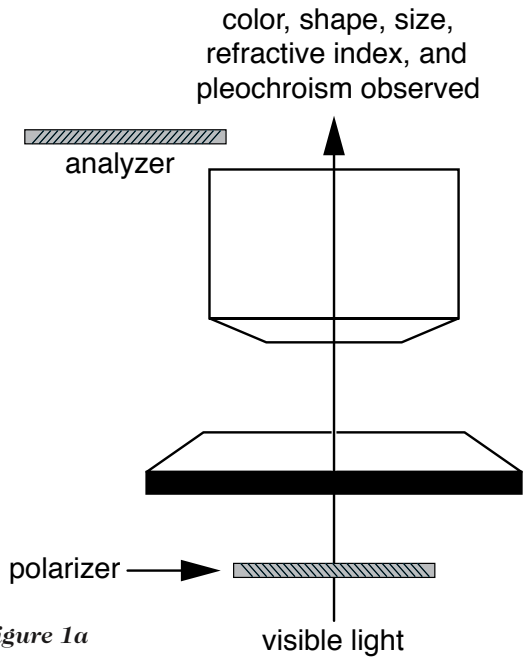

Figure 1a

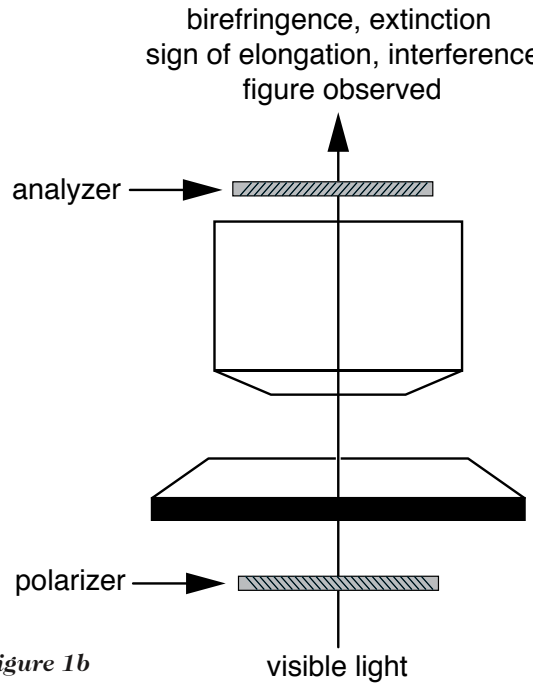

Figure 1b

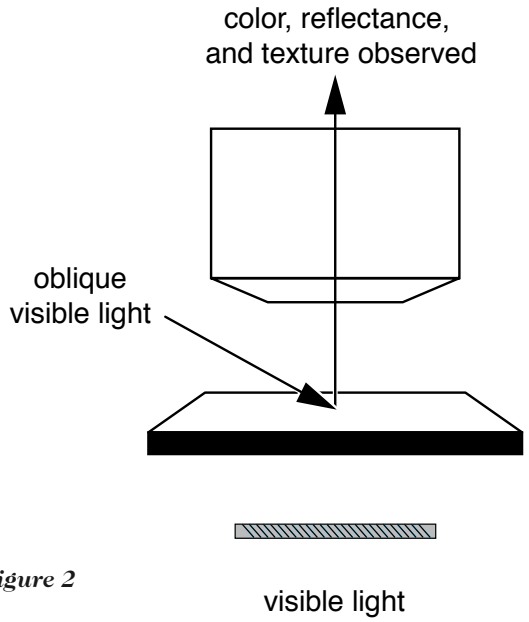

Figure 2

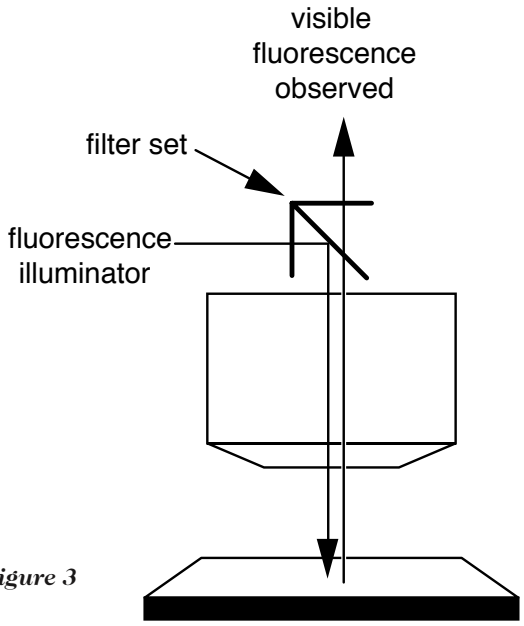

Figure 3

"plane-polarized" *(fig. 1a)* Plane-polarized light is used to observe color, opacity, and pleochroism, and to measure refractive index. When a second filter is inserted into the light path, perpendicular to the first, the light is said to be "cross-polarized." *(fig. 1b)* When viewed with cross-polarized light, specimens that are able to refract light from one vibration direction to the other appear light against a black field. These specimens are termed "anisotropic." Cross-polarized light is used to measure birefringence, and to observe extinction, sign of elongation, optic sign, and interference figures. Using an external visible light source one may observe surface color, reflectance, and texture. *(fig. 2)* Observations and measurements made using transmitted polarized light and reflected visible light form the basis of polarized light microscopy (PLM), a technique used to identify wood, and many fibers, inorganic pigments, and a diverse array of other particles.

Epi-fluorescence Microscopes

Epi-fluorescence microscopes permit one to oserve the visible fluorescence of opaque and transparent samples. Fluorescence is a phenomenon that occurs when light is absorbed by a material and is re-emitted at a longer wavelength. In an epi-fluorescence microscope, the wavelength of light reaching the sample (excitation), and of light viewed through the eyepieces (fluorescence) is regulated by different filter sets, which are constructed of an excitation filter, a dichroic mirror, and an emission filter *(fig. 3)*.

For example, near ultraviolet light might be used to excite blue fluorescence in a sample. Many coatings, binders, fibers, and some pigments and dyes are fluorescent, but very few materials exhibit unique visible fluorescence. Fluorescence microscopy is used to differentiate layers and particles that appear similar in visible light, and to study the distribution and composition of materials using fluorescent staining techniques and microspectrofluorometry. The energy associated with ultraviolet light may cause some organic pigments and dyes to fade, and may induce sample-plane temperatures in excess of 120 degrees Fahrenheit; therefore one must exercise caution when examining objects and samples using a fluorescence microscope. Using an external visible light source one may observe fluorescence in the context of surface color, reflectance, and texture.

A Combined Microscope

Polarizing light microscopes and fluorescence microscopes are often purchased and used separately. Combined microscopes may be purchased new, and older microscopes may often be refitted for individual and simultaneous illumination using polarized and fluorescence illumination. Among the advantages of a combined microscope are lower cost, more efficient and less time-consuming examination and analysis, and extended visual context.

For example, a single microscope may be used for polarized light microscopy and fluorescence

microscopy, allowing one to examine the visible light, color, reflectance, fluorescence, opacity of particles and fibers, and layers in cross-section and thin-section samples. These observations are used in characterizing layer structure and particle samples. Combined illumination is useful for viewing the homogeneity of samples prior to analysis using Fourier transform infrared microspectroscopy (FT-IR microscopy) and scanning electron microscopy (SEM), and for determining the layer-origin of detached particles from bulk cross-section and thin-section samples.

Using combined illumination, one may observe the opacity and particle content of transparent and opaque layers in bulk cross-section samples while also viewing surface color and fluorescence. Similar observations may be made along the edges of unmounted layered samples. These observations can help one to discern the presence of pigments and grime layers among transparent coating layers, and to observe the birefringence of particles in opaque paint layers. Using combined illumination, one may readily distinguish flakes of binder and coating in dispersed paint samples being examined using polarized light microscopy. One may also discern the presence of fluorescent pigments such as madder, zinc white, and toluidine red, and may discern the presence of fluorescent dyes used to top-off or enhance the color of inorganic pigments.

About the Author

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