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TECHNICAL NOTE

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Application of Differential Interference Contrast Microscopy to the Examination of Paints

The value of paint flakes as evidence has been described throughout the forensic science literature. Experimental procedures and techniques as well as testing sequences have been reported in some detail by May and Porter [*1*], Crown [*2*], and Gothard [*3*]. Modern instrumental methods such as neutron activation analysis [*2,4*], X-ray fluorescence [*5*], atomic emission spectroscopy [*2,6*], pyrolysis gas chromatography [*3,7*], and spectrophotometry [*1,2,8*] have been applied to effect a greater degree of discrimination. The use of solubility testing, color change with acids and bases, and thin-layer chromatography has been advocated for some time [*6,9*]. The most prosaic of tests, microscopic comparison of color, layer sequence, and surface texture, has recently received attention by Gothard [*3*] and Fouweather et al [*10*].

The study of color, layer sequence, and surface texture is acknowledged as the initial procedure to be employed. This paper reports on the application of differential interference contrast and incident polarized light techniques to the observation and comparison of colors, layer sequences, and textures. Theoretical discussion of differential interference contrast microscopy has been published by Allen et al [*11*] and Lang [*12*]; therefore, it will be only briefly described here.

Differential interference contrast (DIC) microscopy, an amplitude contrast technique, is a refinement of phase contrast, the invention of Dutch physicist Zernicke [*13*], and equipment is available for both reflection and transmission microscopy. The principle has been applied by Michelson, Jamin-Lebedeff, and Normarski [*12*]. The instrument used for this study applies the DIC effect after the method of Normarski and is equipped for reflection microscopy, allowing observation of opaque and transparent specimens.

An explanation of the DIC effect is facilitated by consideration of the optical elements in the light path from the illuminating lamp to the eyepieces (Fig. 1). Light rays from the lamp *L* pass through a polarizer *P* and are reflected down the microscope tube by a beam splitter *B*. These rays pass through a modified Wollaston prism *W* and travel on to the specimen *S* through an objective lens *O*. Rays reflected vertically from the specimen return through the objective lens and Wollaston prism. These rays then pass through the beam splitter and an analyzer *A* before reaching the eyepieces.

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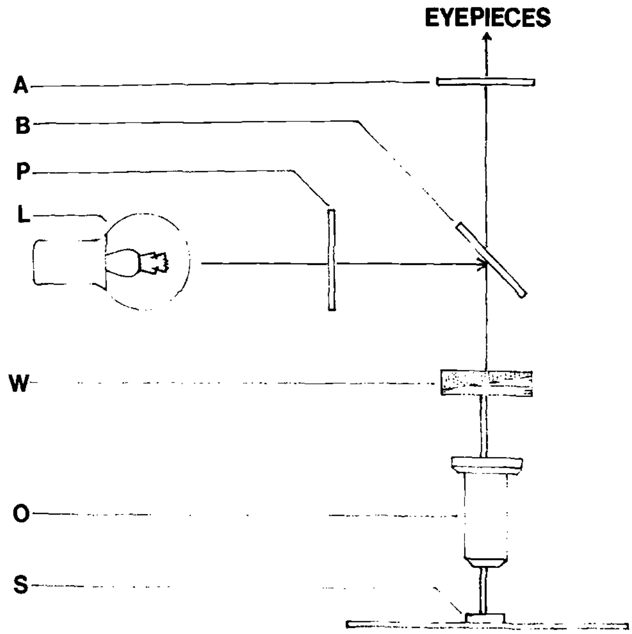

FIG. 1—*Optical components in the light path for differential interference contrast microscopy by incident illumination:* (A) *analyzer,* (B) *beam splitter,* (P) *polarizer,* (L) *illuminating lamp,* (W) *modified Wollaston prism,* (O) *objective lens, and* (S) *specimen under observation.*

The polarizer and analyzer are fixed at extinction with the Wollaston prism positioned at a 45-deg angle to the vibration direction of each. It is this prism which produces the DIC effect. The prism is made of some birefringent material, usually quartz or calcite crystal. Two wedge-shaped crystals are cemented together with their crystal axes at right angles [*12*]. A ray passing through the prism from the polarizer is split into two part-rays that are vertically (perpendicularly) polarized to each other. The ordinary ray continues its propagation in a straight line, but the extraordinary ray is laterally displaced [*14*]. This displacement has maximum values of about 0.5 mm (10× objective), 0.25 mm (20× objective), and 0.1 mm (40× objective). These part-rays reach the specimen at two different points, from which they are reflected up through the objective lens to the Wollaston prism. The part-rays are recombined by passing through the prism. Upon passing through the analyzer the combined part-rays are caused to vibrate in the same direction.

Given a smooth specimen surface the two part-rays traverse the same distance. Therefore, there is no phase displacement between the recombined part-rays. These rays will not interfere after passing through the analyzer and no contrast is generated.

A textured surface being examined has in effect plateaus, slopes, and valleys. In this situation the part-rays may traverse different distances and will experience some phase displacement. Upon passing through the analyzer they are brought into a common vibration direction and interfere. Interference is seen as intensity or color differences. Thus, it becomes evident that interference contrast by incident illumination is dependent on differences in the height of the specimen surface and that the intensity or color differences result from surface texture.

When the Wollaston prism is centered in the light path so that the thickness of each crystal wedge is equal, the zero order position, intensity differences are seen. By moving the prism left or right one sees the contrast as first order interference colors: blue, yellow, and red. The magnitude of displacement from the zero order position determines the predominant color seen. The direction of movement from the zero order position determines whether the specimen appears to be obliquely illuminated from the left or the right. Quite naturally, under these conditions a colored object may not be perceived in its true color.

Incident polarized light (IPL) is accomplished by simply removing the Wollaston prism from its slot. Although the use of polarized light to eliminate specular reflections is nothing new, it is particularly convenient with this equipment. Also, it is simple to perform examinations at shorter working distances (20× or 40× objectives), which are difficult with oblique illumination. This simplicity has proven itself to be of value when multilayered paint flakes are examined on edge.

Experimental Procedure

An American Optical Company Model 10-TG-DICV was used for all observations. The range of available magnifications was ×100 to ×800. Paint flakes prepared in the laboratory and flakes from actual cases were examined. Specimens were simply placed on microscope slides for observations of surface textures. Flakes selected for layer examination were set on edge in a drop of melted Parawax® on a microscope slide. When the wax had solidified the upper edge of the embedded flake was exposed and smoothed by scraping it with a fresh scalpel blade while the results were observed through a stereoscopic microscope. After this preparation the slide was then examined for layer colors and sequence.

Discussion

The prepared and evidence flakes displayed vivid colors in cross section with IPL as compared to ordinary incident light or oblique illumination. As might be anticipated, specular reflections were, for the most part, eliminated. The observed image was crisp. The inclusion of metallic particles and pigment grains was noted in some layers. Such inclusions could not be seen with ordinary incident light or oblique illumination. Because of the color changes possible with the Wollaston prism DIC is not recommended for layer or color studies.

The DIC technique did produce an excellent reproduction of the surface texture. The specimens were rendered in a bas-relief similar to that obtained with the scanning electron microscope, though less pronounced. The most useful and pronounced contrast was observed with the Wollaston prism only slightly displaced from the zero order position. The flakes were observed in colors because at or near the zero order position contrast is expressed as shadowing and intensity differences. Further displacement of the Wollaston prism, producing first order interference colors, did not provide additional information concerning the surface texture. Observations in these colored orders appeared to have some potential uses with other types of physical evidence [*15*].

The following examples are offered to provide the reader with some feeling for the potential of this technique.

Example 1

A paint specimen was prepared by applying aerosol spray paint to a length of 38- by 6-mm (1½- by ¼-in.) pine wood. The paint was allowed to dry for several hours. While the paint was still relatively soft the prepared surface was pressed against the teeth of a bench vise jaw. That test area was studied by incident and oblique lighting techniques. The presence of surface irregularities could be detected only with substantial effort. With DIC the impressions of the teeth in the paint were quite evident. An eyepiece micrometer allowed measurements of individual impression dimensions and spacing.

Example 2

Three specimens of burnt-orange paint were submitted for comparison. Item A was from a known source. Items B and C were questioned exhibits. Neither Item B nor Item C could be differentiated from Item A on the basis of color, gloss, or other layers. Microscopic examination with a stereoscopic binocular microscope showed no observable differences in the surface appearance. The DIC observation of Items A and C showed the surface to be smooth and almost free of imperfections at ×100 magnification. Item B showed a wavy surface different from Items A and C. Other examinations (infrared spectroscopy) confirmed the dissimilarity of Item B.

Example 3

In a recent case, paint flakes found in an automobile trunk were to be compared with flakes removed from a disassembled bicycle recovered with the remains of a murder victim. Several flakes from known and questioned sources were prepared in Parawax as mentioned above. Other flakes were simply placed on microscope slides for viewing the top and bottom layer surfaces. Specimens from each source were found to have six layers of different colors. The colors, sequence, thicknesses and, where present, the pigment grains were found to be in agreement.

Study of the outer, pale-green layer by DIC disclosed two significant imperfections present on flakes from both sources. One was the presence of a large number of randomly placed

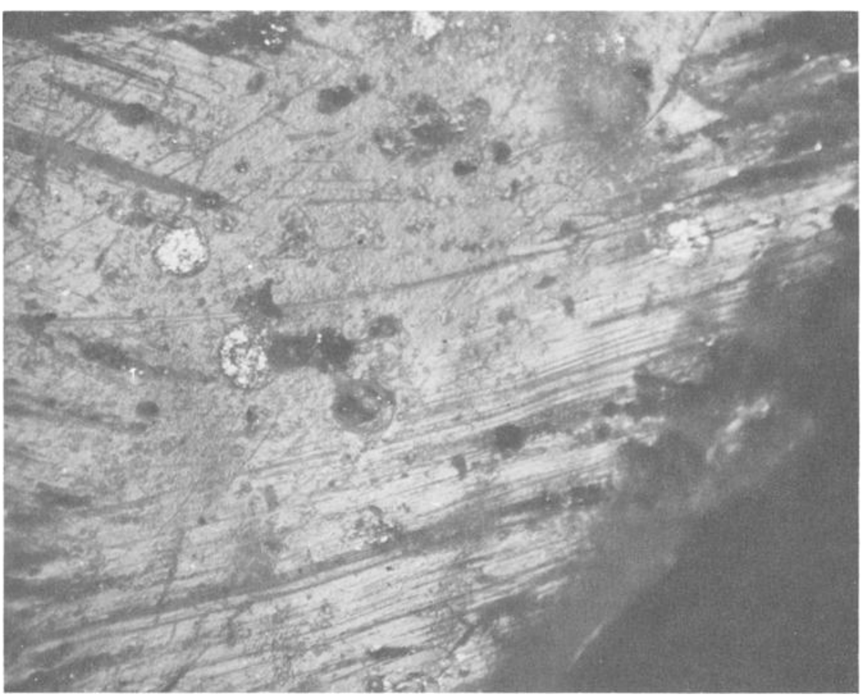

FIG. 2—*The surface of a paint flake cited in Example 3 as seen with differential interference contrast microscopy with the Wollaston prism slightly displaced from the zero order position.*

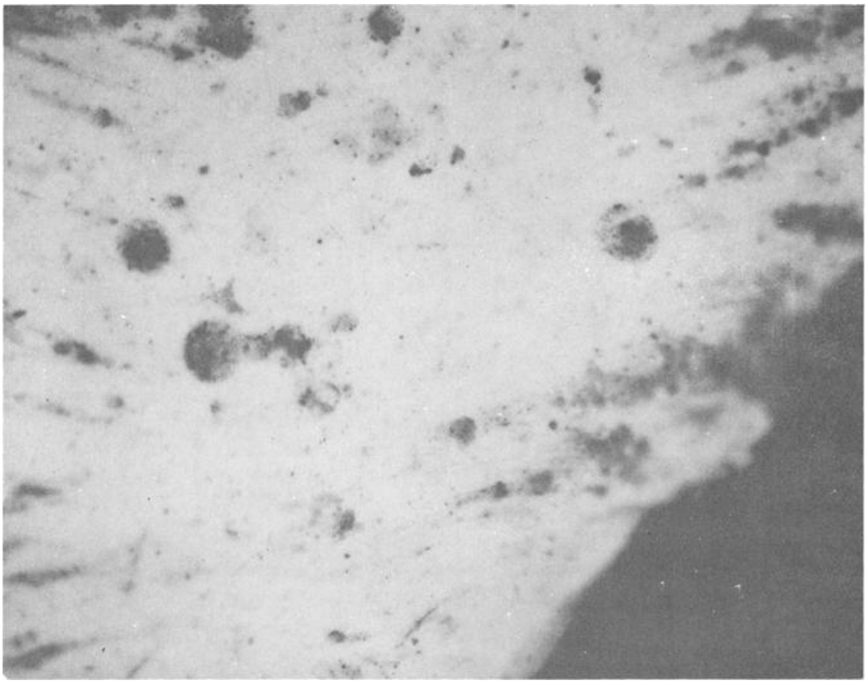

FIG. 3—*The paint flake from Fig. 2 with incident polarized light only.*

hemispherical depressions in the surface. These were similar to what automobile painters have called "fish-eye." Fish-eye results from a rejection of the fluid paint by spots of oil, grease, wax, or silicones on the surface being painted. The second imperfection was the presence of round, silver-colored spots of metallic paint. These were found to be essentially flush with the surface as though dissolved into the pale green paint (Fig. 2). Also, in a few places they were observed to have flowed into the fish-eye depressions. These two imperfections could not be clearly seen by oblique, incident, or incident polarized lighting (Fig. 3).

Subsequent gross examination of the bicycle frame showed that the silver paint spots were localized on the lower left rear part of the frame. The fish-eye depressions in the pale green paint were also most evident about this area. Coincidentally, this was the attachment site for the rear wheel, a potential source of oil from the rear axle, coaster brake assembly, and the drive chain. Furthermore, there was a substantial area of chipped paint where the coaster brake lever had been pried from the frame.

Although the number of paint layers present was not large the surface examination by DIC provided additional identifying characteristics establishing the origin of the flakes found in the automobile trunk.

Summary

Incident light differential interference contrast and incident polarized light techniques were applied to the comparative examination of paint flakes. The appearance of specimens so observed was superior to that obtained with oblique illumination or ordinary incident illumination. Differential interference contrast microscopy by incident illumination allowed the extraction of additional information concerning similarities and dissimilarities of the surface texture of paint flakes examined.

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