Advances in FORENSIC SCIENCE

Volume 2: INSTRUMENTAL ANALYSIS

Henry C. Lee / Robert E. Gaensslen

ADVANCES IN FORENSIC SCIENCES

Volume 2

Instrumental Analysis and Its Applications in Forensic Science

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PREFACE

The successful reception of volume 1 of this series by the forensic science community, and now the publication of volume 2, have been accompanied by a smooth transition from Biomedical Publications to PSG Publishing Co, Inc. *Advances in Forensic Sciences* is now firmly established as a source of timely information in the field. Future volumes will cover forensic serology, fingerprints, fire scene investigation and arson residue analysis, and crime scene reconstruction patterns.

The present volume is devoted to instrumental methods of analysis in forensic sciences. The chapters which follow include coverage of forensic microscopy, spectrometric and spectroscopic methods, chromatographic methods, and the use of small computers in laboratory administration and management, along with a chapter on the systematic application of instrumental methods in forensic analyses. It is recognized that it is not possible to include coverage of every instrumental method used in forensic science in a single volume of this size and scope. We believe, however, that this along with the other volumes in this series, provide current reviews of timely subjects within the forensic science field.

We take this opportunity to thank our contributors for their diligent efforts, the staff at PSG Publishing Co for making the transition to their company and the production of this volume smooth, and our wives for their continuing love and patience.

Henry C. Lee
R. E. Gaensslen

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Forensic Applications of Light Microscopy

Walter C. McCrone

If it is the criminalist's job to characterize, identify, and compare trace evidence, then no analytical tool suits his purpose better than the light microscope. A recent publication[1] attempts to detail the wide range of techniques, observations, and measurements possible on microscopic particles of trace evidence by polarized light microscopy (PLM). In this chapter the application of those PLM techniques to trace evidence is described and illustrated.

All of the early great criminalists relied heavily on the microscope. Hans Gross in his 1893 book on criminalistics included a chapter on microscopy in which he commented:

> Advanced though the construction of microscopes is today, and much as science can accomplish with this admirable artifact, the criminalist has as yet scarcely drawn upon the art of the microscopist. Studies of blood, determination of semen spots, and comparison of hairs is virtually all that the microscopist has to do for the criminalist. Other investigations occur only exceptionally, although there are innumerable cases in which the microscopist could provide vital information and perhaps clarify insoluble problems.[2]

and later in the same chapter: "Dirt on shoes can often tell us more about where the wearer of those shoes had last been than toilsome inquiries." George Popp became well known in the early 1900s as a result of his use of microscopy in solving crimes. One of his celebrated cases is well described in Thorwald's *Crime and Science*.[3] This same book covers many interesting cases solved, at least in part, by microscopy. Dr Victor Balthazard, Edmund Locard, Gaston Bayle, Albert Schneider, Arthur Kohler, and Edward Heinrich stand out as early forensic microscopists.

The development of forensic microscopy suffered a severe setback in the second half of this century with the development of a succession of more glamorous instruments such as infrared and mass spectrometers, x-ray and electron diffraction, electron and ion microprobes and, of course, the scanning and transmission electron microscopes.

An entire generation of chemists has now missed the opportunity to learn polarized light microscopy in college. An instrument invented in the seventeenth century, costing only a few thousand (or even a few hundred) dollars, and claiming no closer connection to the electronic age than an electric light source, was not expected to compete with the new generation of sophisticated electronic instruments. Still a few die-hard light microscopists (trained by Chamot and Mason, Norman Hartshorne, John Roberts, Ray Shelden, Mary Willard, Witt and Poe, etc) held on: Ray Allen, Maurice Anderson, Arthur Barron, Howard Bradley, Maria Brandstätter, Bill Bryant, George Cocks, John Delly, Ruth Giuffria, Don Grabar, Jim Hamill, Raymond Hasson, Andy Holik, Norman Hollies, Francis Jones, Joe Kohlbeck, John Krc, Lucy McCrone, Nydia Meyers, Scott Morrow, Skip Palenik, Dale Quackenbush, John Reffner, Ted Rochow, Mitch Siemenski, Dick Speight, Anna Teetsov, and George White represent a goodly proportion of them.

Partly as a result of the example of these light microscopists and the efforts of such criminalists as John Sullivan, the Law Enforcement Assistance Administration (LEAA) strongly supported a renaissance for PLM during the late 1970s. As a direct result of their sponsorship of intensive courses in forensic microscopy throughout the United States a (small) number of dedicated trace evidence examiners have, largely by continued self-study, begun to reinstate microscopy as an essential tool for trace evidence examination. These include Wayne Burrow, John Case, Hal Deadman, Mike Eyring, David Flohr, Mark Franey, Tom Hagney, Thom Hopen, John Kilbourn, Thom Kubic, Dave Metzger, Wayne Morehead, Larry Peterson, Nick Petraco, Ron Resua, Steve Shafer, John Simms, and Jim Small.

Although PLM is far from firmly reestablished and even farther from serving at its full potential, the tide seems to have turned. Increasingly, PLM is being used to examine trace evidence in the crime laboratory. Cornell has resumed teaching chemical microscopy and, to the surprise of most criminalists, including the recipient, a light microscopist received the 1984 Distinguished Service Award of the Criminalistics Section of the American Academy of Forensic Sciences.

There still remains a great unmet need for PLM in the forensic laboratory for the characterization of trace evidence. However, criminalists must first become aware of the role of PLM for such examinations. They must learn how to use PLM effectively and, finally, they must apply the methods to the study of known trace evidence particles. The proper use of the microscope has been described and the methods are covered in a variety of sources.[4–6] The logic of setting up a trace evidence section rather than a separate hair and fibers section (usually with serology), an explosives section, etc, follows from the fact that the same tools and techniques are used for all of the trace evidence categories. If you know how to measure refractive index, birefringence, and sign of elongation for ammonium nitrate, you also know how to make the same measurements on 1,3,5-trinitro-1,3,5-triazacyclohexane (RDX), nylon, Dacron, secobarbital, or copper phthalocyanine.

The ideal way to become a forensic microscopist is to start with the microscope — learn Köhler illumination and how to use the PLM to characterize fibers. Fibers are the easiest particles to master quickly because they usually have only two refractive indices (parallel and perpendicular to the length); glass fibers have only one index. Also, both indices are nearly always shown on every view since they usually lie flat. Later, one can add other trace evidence categories, starting with the inorganic explosives since there are relatively few of these. As time goes on, other categories can be added and one's skills sharpened.

A criminalist becomes a forensic microscopist as soon as, and only when, he thinks of the PLM first rather than infrared spectrometry (IR), x-ray diffraction (XRD), scanning electron microscopy (SEM), etc, when a new case crosses his desk. These other tools are, for most trace evidence, strictly supplementary and should be used only after first looking at the samples with the light microscope. Those who do look first with the PLM will find, with increasing experience, that the problem is quickly solved, usually with no need for additional study except as confirmatory tests.

The following discussion should illustrate the wealth of information that a trained forensic microscopist can expect to determine on even a single subnanogram particle. One must be able to isolate, manipulate, and mount such particles for study; techniques for this have been published in volume 1 of *The Particle Atlas*.[5] If a start is made on somewhat larger particles the ability to use the same techniques on smaller particles develops slowly but surely. Most of the characterization techniques, from refractive index measurement to microcrystal tests, can be carried out on subnanogram particles (about 2–10 μm in diameter). The various trace evidence categories include:

hairs, fibers, glass, paint, soils, drugs, explosives, gunshot residue, safe insulation, and miscellaneous. This listing was used in volume 5 of *The Particle Atlas* (pp 1379–1401),[7] but the treatment here is different and emphasizes those techniques one can use and measure on particles in each of these categories.

FIBERS

There are many thousands of fibrous substances in the world: cellulosic — plant-seed hairs, trichomes, grasses, stems, wood, etc; proteinaceous — animal hairs, silk, feathers, spider webs, etc; manmade organic fibers — polyamide, polyester, etc; miscellaneous — glass, carbon, metal, rubber; and mineral fibers such as chrysotile asbestos. More than 600 different manmade polymer fibers can be differentiated if one includes all polymers, copolymers, and mixed polymers (ie, each half of the crossection a different polymer) and crossectional configurations. In addition, there are many degrees of delustering and texturing as well as various surface treatments.

The forensic scientist should regard this wealth of fiber types as an opportunity. If one finds an unusual synthetic fiber as trace evidence, it can be a very valuable clue — far better than, say, cotton fibers, which are ubiquitous. The characterization of fibers is a matter of learning how to characterize any fiber morphologically and optically, then applying that ability to as many known fibers as possible. It is usually possible to determine the category almost at sight by its properties:

Glass — isotropic cylinders
Asbestos — very fine fibrils plus fiber bundles
Metal — opacity, luster
Rubber — elasticity
Trichome — short, tapered fibers
Fur — scales
Wood — pits
Manmade fibers — consistent crossectional diameter and same configuration along length; anisotropic

Of these categories, only the manmade fibers can be very long and have nearly constant crossectional shape and diameter. Figure 1-1 shows nylon fibers with a characteristic cylindrical crossection and smooth surface.

Natural fibers are much shorter and vary in diameter along their lengths. Cotton is shown in Figure 1-2 with twisted fibers unlike those from any other source. Because nearly all natural fibers are proteinaceous (eg, silk, hair, spider web) or cellulosic (eg, plant fibers), their optical properties are not very discriminatory. Instead, shape and

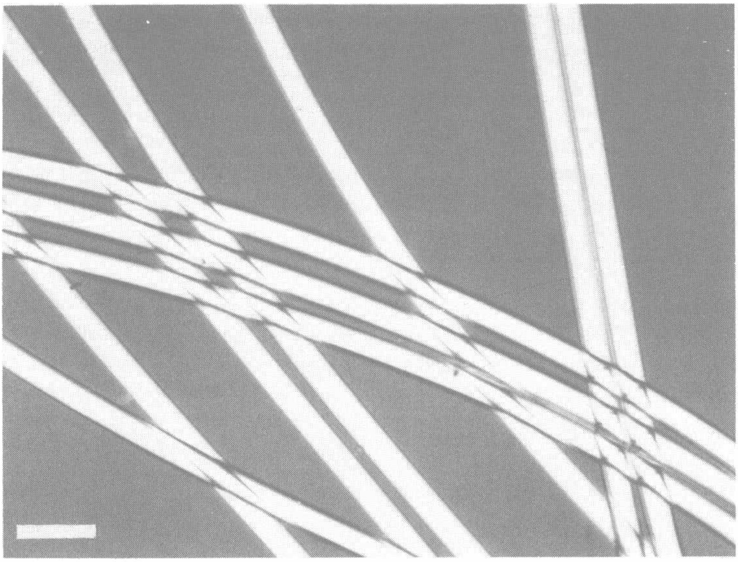

Figure 1-1 Typical fibers of nylon shown here with slightly uncrossed polars; bar = 100 μm.

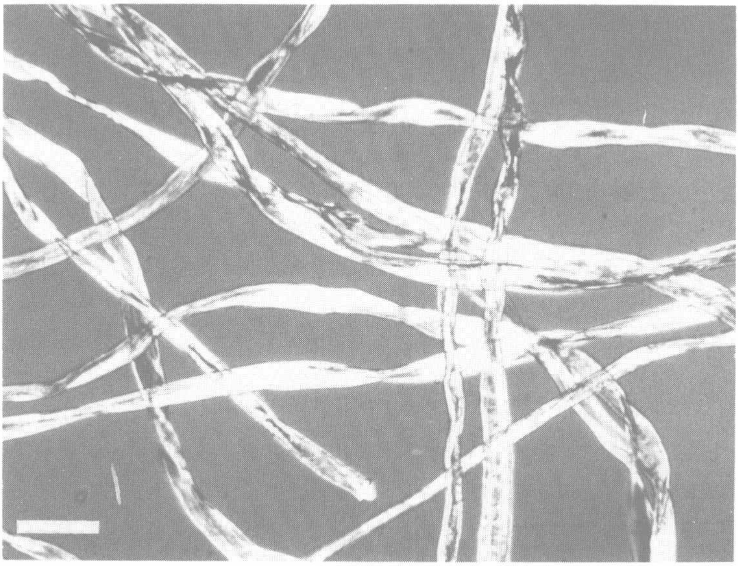

Figure 1-2 Cotton fibers showing the usual twists along the length, with slightly uncrossed polars; bar = 100 μm.

size variations and, in particular, changes along the length (eg, scales on animal hairs, pits on wood, twists for cotton, and nodes for bast fibers) are the most useful identifying characteristics.

A good summary of the microscopical characteristics (shape and size) of natural textile fibers appeared in (only) the first two editions of volume 1 of Chamot and Mason's *Handbook of Chemical Microscopy*.[8] Strelis and Kennedy[9] have also published a key to the identification of nonwoody plant fibers, most of them nontextile fibers (eg, coir, hemp, ramie, kenaf, sisal, kapok, esparto, sugar cane).

Recognition of wood fibers *per se* by noting the presence of pits on the ribbonlike fibers or of flat baggy cells is rapid; pits on ribbonlike fibers are generally softwood but multiple rows of pits on flat baggy cells dependably indicate hardwood. To proceed further to the identification of species of wood fibers requires careful study of the individual fibers and systematic keys such as those of Phillips.[10]

The identification of manmade fibers requires a different approach, usually involving composition-dependent optical properties. There are many different chemical compositions for manmade fibers and almost no longitudinal discontinuities such as the scales, nodes, and pits of natural fibers. This means we can identify manmade fibers by their composition-dependent properties. From a microscopist's point of view this means: measurement of optical properties (eg, refractive indices, birefringence, and sign of elongation), thermal properties (eg, glass transition temperatures, melting points, and eutectic melting points with *p*-nitrophenol), and spectroscopic measurements (Raman scattering, fluorescence, and absorption spectra), all of which are performed microscopically.

Useful refractive index data appear in volume 4 of *The Particle Atlas* (pp 872–976).[11] This includes the use of dispersion staining as a rapid means of identification. Wilkaan Fong[12] has also published a paper based on dispersion staining in a single liquid (n_D = 1.525) for identification of the most common manmade fibers.

The hotstage phase contrast microscope can also be used, although a comprehensive paper covering the data for a sufficient number of different fibers has yet to be written. Donald Grabar and Rita Haessley[13] made a beginning some years ago with their paper on the eutectic melting points of manmade fibers with *p*-nitrophenol (*p*-NP). Three melting points can be determined during a single heating cycle if at least two, even short, lengths of the fiber are visible in the field of view. One or more are placed in close contact with several small crystals of *p*-NP. On heating, the eutectic between the fiber and the *p*-NP will melt first, usually between 50°C and 100°C (Figure 1-3). On continued heating with slightly uncrossed polars the unmelted portions of the fiber will show characteristic polarization color changes,

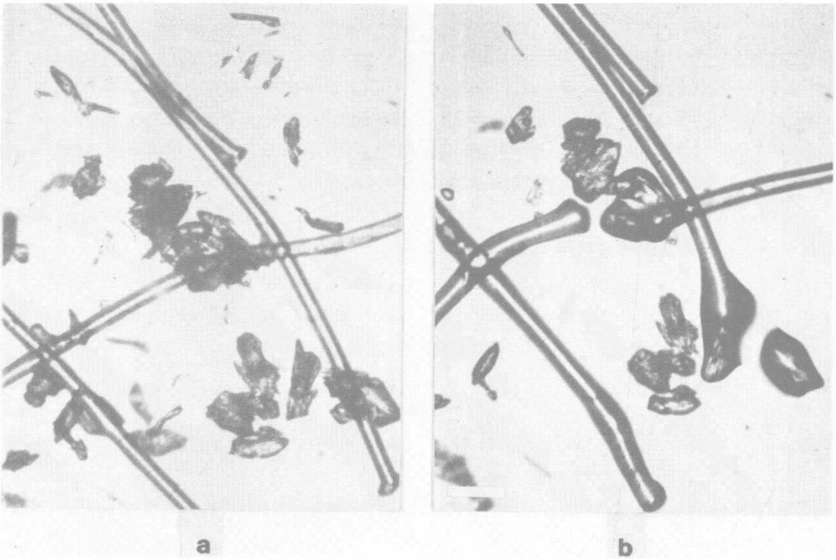

Figure 1-3 Nylon-6 fibers. a. Unmelted at room temperature. b. Partly melted by *p*-nitrophenol at 64°C in a hotstage; bar = 100 μm.

sometimes changing to zero retardation (glass transition temperature) before melting at a somewhat higher temperature.

Infrared absorption by Fourier transform infrared spectroscopy (FTIR) or Nanospec® is another way to characterize fibers. It is especially helpful for copolymers, mixed composition fibers (either coated or coextruded), and dyed fibers. The added information through use of polarized infrared absorption* should be considered for possible further characterization and discrimination of fiber and films.

Hairs, of course, are easily recognized as fibers and as hairs by the presence of scales. Occasionally, however, the scales may be worn completely away, for example, in rug wool. Then the medulla, if present, refractive indices, the response to a protein stain such as amido black, lack of good extinction between crossed polars, or the variation in diameter along the length (end taper or twisting of an oval crossection) may help. The best sources of information on the microscopy of hair are the reports of the Ad Hoc Committee on Forensic Hair Comparison[14] chaired by Barry Gaudette. The committee drew up definitions of the terms used by forensic hair microscopists and

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* Infrared light is not polarized with Polaroid filters but by reflection at the Brewster angle from a stack of thin silver halide sheets.

detailed a procedure for forensic hair comparison including an atlas of photomicrographs showing characteristic hair features. The final report covers training of hair microscopists, reporting data, and court testimony. The Federal Bureau of Investigation (FBI) has been very helpful in coordinating and supporting financially the meetings and symposia held to finalize these discussions.

The preliminary report of this committee lists the following categories of general characteristics of hair:

1. Color
    a. Hue
    b. Pigmentation
    c. Variation

2. Structure
    a. Form
    b. Diameter
    c. Crossectional shape
    d. Cortex
    e. Medullation
    f. Shaft aberrations

3. Cuticular traits
    a. Scales
    b. Thickness
    c. Margins
    d. Sequence
    e. Weathering

4. Acquired characteristics
    a. Proximal ends (roots)
    b. Distal ends (tips)

5. Length

Individual characteristics differ from general characteristics in that they occur infrequently. Individual characteristics include:

1. Artificial coloration
2. Abnormalities
3. Uncommon structural conditions
4. An unusual value for a particular general characteristic
5. Artifacts

The final report of this hair committee will undoubtedly supplant John Hicks's excellent booklet on hair examination[15] published by the FBI in a limited edition (available to crime laboratories).

GLASS

Of all the trace evidence categories, glass is the one most universally recognized as a legitimate subject for microscopical study. The refractive index, determined by noting the temperature of disappearance on heating of glass fragments in a standard silicone oil of known index and temperature coefficient of index, is a standard procedure in many forensic laboratories. Three views of glass chips are shown in Figure 1-4 at three different increasing temperatures. The above-best-focus Becke lines show the indices relative to the immersion liquid during heating in a hotstage microscope. This procedure will undoubtedly

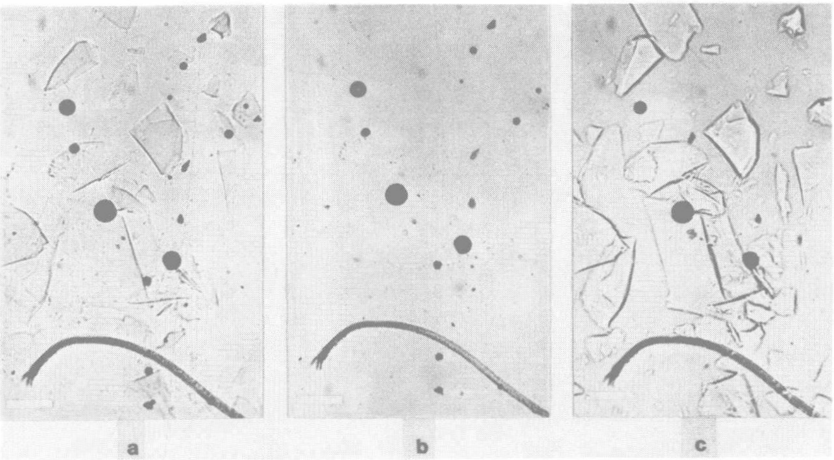

Figure 1-4 Glass chips during heating: a. n(liquid) > n (glass), b. n(liquid) = n(glass). c. n(liquid) < n(glass); bar = 100 μm.

become more widely used in the future with the publication by John Locke[16] of an automated procedure with software to give directly the refractive index of the glass. The commercial availability of the requisite stage, computer, and program will greatly encourage use of the technique. Elmer Miller[17] has suggested a hotstage microscopical procedure for the characterization of mineral wool.

Locke's new computerized procedure, titled GRIM (glass refractive index measurement), is a major advance. It will undoubtedly become the accepted procedure. It does, however, as usually applied, measure the refractive index at a single wavelength, usually n_D. A further step to measure the dispersion of refractive index is easy to do without modification of the GRIM program. The procedure used to determine n_D is made using an interference filter transmitting the 589-nm "band." If the same determination is made starting with the n_F filter and changing to the successively higher wavelength filters (n_D and n_C) during the heating cycle, three different match temperatures can be determined. These yield the three indices which can be used to further characterize the glass samples. Dispersion staining without a hotstage, yields dispersion of refractive index data directly but with some sacrifice in accuracy as normally used.[18]

There are glasses with the same n_D refractive index but with different n_F and n_C values. In any case, finding three indices in good agreement for both evidence and comparison sample can only be helpful in establishing probable identity of source. Table 1-1 shows

Table 1-1
Dispersion of Refractive Index

Glass	n_F	n_D	n_C	ν
Crown	1.5240	1.5172	1.5145	54.4
Flint	1.6391	1.6270	1.6221	36.9

dispersion of refractive index data for two different glasses. The dispersion number, ν, is calculated from the indices as:

$$\nu = \frac{n_D - 1}{n_F - n_C}$$

The shortness of this section on glass offers the possibility of a general discussion of trace elements as a potent tool for individualization not only of glass but of any other trace evidence particles. The microanalytical methods for trace element detection and quantitation have advanced rapidly during the twenty-plus years since Birks, Duncumb, Ely, and others developed the electron microprobe analyzer.

It is impossible to fully individualize hair or prove common origin of any substances because no one has had the time (and money) to survey the large number of background samples necessary before the uniqueness of such data can be evaluated. However, before neutron activation analysis (NAA) was discredited for the individualization of hair, a great deal of NAA data was accumulated. One can say that NAA would still be useful in proving absence of a common source for scalp hairs and would be strongly indicative for proof thereof. In other words, it almost worked.

Now, with very sensitive microprobes capable of plotting the concentrations of 40 or more trace elements along the length of a human hair at intervals corresponding to successive 15-minute growth periods, it would seem reasonable that trace analysis could individualize hair. This would be especially true given analyses for comparable growth periods on two hairs since the diurnal variation of each element can be plotted. This yields far more sophisticated data than average concentrations of trace elements over several centimeter-lengths of hair as measured by NAA. It seems quite possible, if not likely, that this degree of refinement beyond that of a method that "almost worked" would lead to success. Hopefully, this possibility can be investigated in the near future.

Trace elements can also be used to determine the mine-source of minerals. Trace elements have been used to help prove likely identity of origin for hair and for manmade fibers. In those cases, the ion

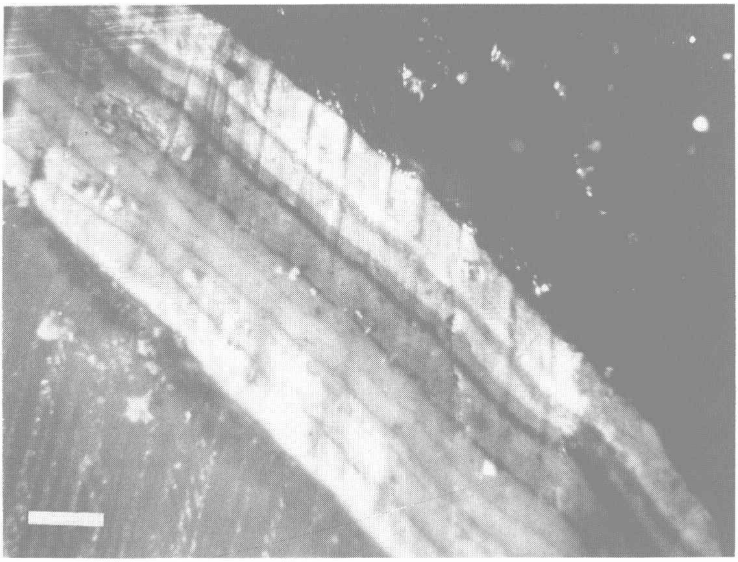

Figure 1-5 A razor-cut crossection of wall paint; bar = 200 μm.

microprobe, an even more sensitive trace microanalyzer, was used. This, then, is an area for more research but one held back today by the high capital cost of the instrumentation and the high cost of time for both operator and instrument.

PAINT

The examination of evidence and reference paint flakes begins as for glass, paper, a broken knife, tools, etc, that is, with a careful check for physical fit of the two fragments. If successful, the conclusion is absolute and further comparison is unnecessary (although it should be done if there is a chance the court might not agree on the certainty of the physical fit).

What can be done when, as in most cases, a physical fit of paint flakes is not possible? If both are in flake form, and especially if multilayered (Figure 1-5), each can be characterized as to:

1. Number of layers
2. Thickness of each layer (average and range)
3. Color
4. Surface characterization of substrate (composition and topography) based on replication by the base paint coat
5. Surface characterization of the outer paint surface