Andrew M. Bowen[1]

Identification of General Unknowns

ABSTRACT: Trace evidence examiners are sometimes faced with the task of identifying small samples of unknown materials. Microscopical examination is recommended as the first step in this process, enabling the analyst to characterize, classify, and often identify the unknown substance. When results are ambiguous, the observations made under the microscope are used to guide subsequent analyses with other instruments. Techniques commonly employed in follow-up analysis include Fourier transform infrared micro-spectroscopy, scanning electron microscopy with energy dispersive spectroscopy, Raman micro-spectroscopy and X-ray diffraction. Microchemical analyses are extremely useful for clearing up remaining ambiguities. The best results are obtained when multiple analyses are performed on the same particle or subsamples derived from a single particle that has been crushed or cut into fragments. Once identified, known standards should be obtained for comparison to ensure that the identification is accurate. A number of casework examples are provided along with guidance on the selection of appropriate analytical techniques.

KEYWORDS: general unknowns, stereomicroscopy, polarized light microscopy, Fourier transform infrared micro-spectroscopy, scanning electron microscopy, energy dispersive spectroscopy, Raman micro-spectroscopy, X-ray diffraction, microchemistry

Introduction

Small amounts of unknown materials are often present on items of evidence submitted for trace evidence analysis. Occasionally their significance needs to be determined to aid an investigation. It is typically the responsibility of the trace evidence examiner to identify these unknown substances and determine whether they are relevant to the investigation at hand.

Materials observed on evidence items during visual inspection are usually fairly large (≥ 100 um), as smaller particles are difficult to see without the aid of lenses. However, significantly smaller particles of unknown substances (as small as several micrometers) can be observed during microscopical examination of tape lifts or debris collected by vacuuming.

[1]Stoney Forensics, Inc.,14101 Willard Road, Suite G, Chantilly, VA 20151, www.stoneyforensic.com

Received 14 June 2010; and in revised form 18 June 2010; accepted 18 June 2010

As a general rule, larger particles are more significant in a sample due to their limited transport distances. Particles smaller than 10 um can remain suspended in the air and travel large distances from their source (1). They may therefore be unrelated to any activity or environment to which the evidence was directly exposed. Particles larger than 100 um, meanwhile, tend to settle quickly to the surface once strong winds subside, and are therefore unlikely to travel long distances (1). Their presence on evidence usually indicates that the evidence item was recently exposed to their source. Despite their potential transport distances, small particles sometimes need to be identified as well. This is true when they are present in great abundance in a sample or when they are unusual in nature, especially if they are observed in both a questioned sample and a known sample.

Stereomicroscopy

Larger particles are generally easier to work with than smaller particles as they can often be physically divided into smaller subsamples, each of which can be analyzed using a different instrument. The first step in the identification of every material, however, should be careful examination by stereomicroscopy. Forceps or a tungsten needle can be used to turn the particle over such that its entire exterior surface is examined and representative orientations documented photographically. If at all possible, the stereomicroscope should be equipped with transmitted light, oblique reflected light, and coaxial illumination and the substance observed under all available lighting conditions. Two examples of large particles observed on evidence items, turned over to reveal different features on different sides, are illustrated in Figures 1-2.

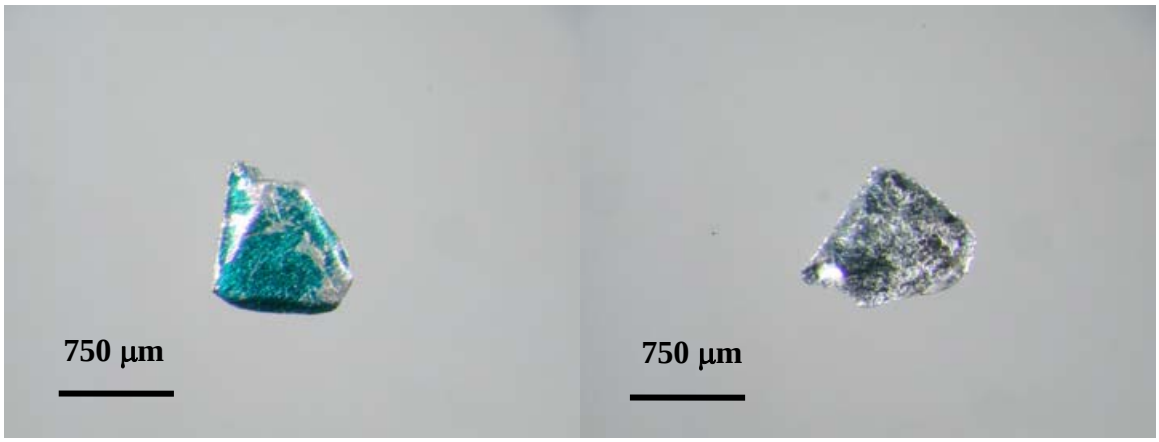

Figure 1. A metallic flake with green coating shown by stereomicroscopy on both sides.

It is not uncommon for the stereomicroscopic examination to reveal the general class of the material (i.e., botanical matter, man-made polymer, insect part, metal fragment). Even if stereomicroscopy does not enable the substance to be placed into a definite group, the process of characterizing its properties begins at this stage. Important properties observed by stereomicroscopy include a substance's opacity/transparency,

color, size and shape, surface texture, and luster in reflected light. Additional properties can be determined by manipulation of the unknown. Gently pushing on the substance with a tungsten needle or probe can reveal information about its hardness, elasticity and tackiness. If a substance appears metallic, its magnetic properties may be elucidated by bringing a magnet near it and observing its behavior. Care should be taken to ensure that the particle does not stick to the magnet. Placing a piece of clear plastic over the magnet prior to bringing it close to the unknown substance is a useful safeguard. That way if the particle sticks to the magnet, the plastic can be placed back down on the microscope stage (with the particle underneath it) and the magnet carefully pulled away from the sample, leaving the particle in its original location. Figures 3-5 illustrate examples of particles readily classified by stereomicroscopy.

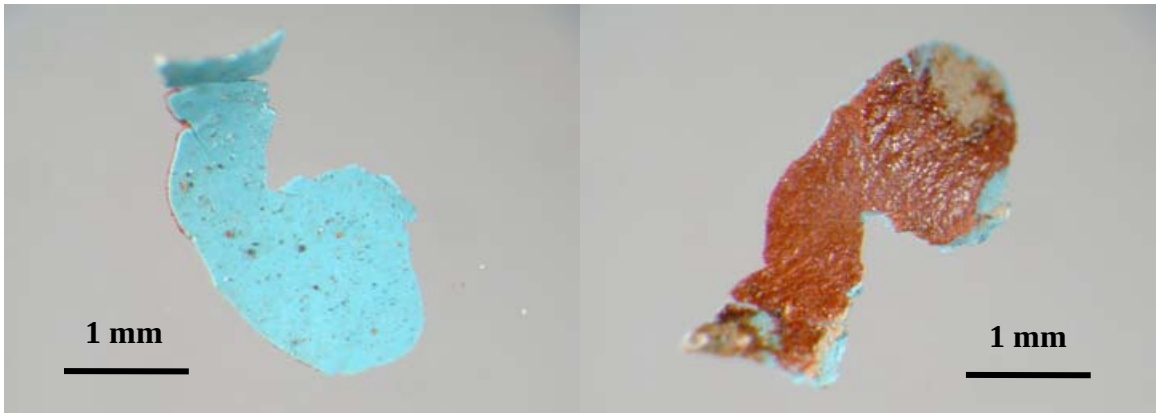

Figure 2. A multi-layer coating flake shown by stereomicroscopy on both sides.

Figure 3. Particles of botanical matter from evidence items shown by stereomicroscopy.

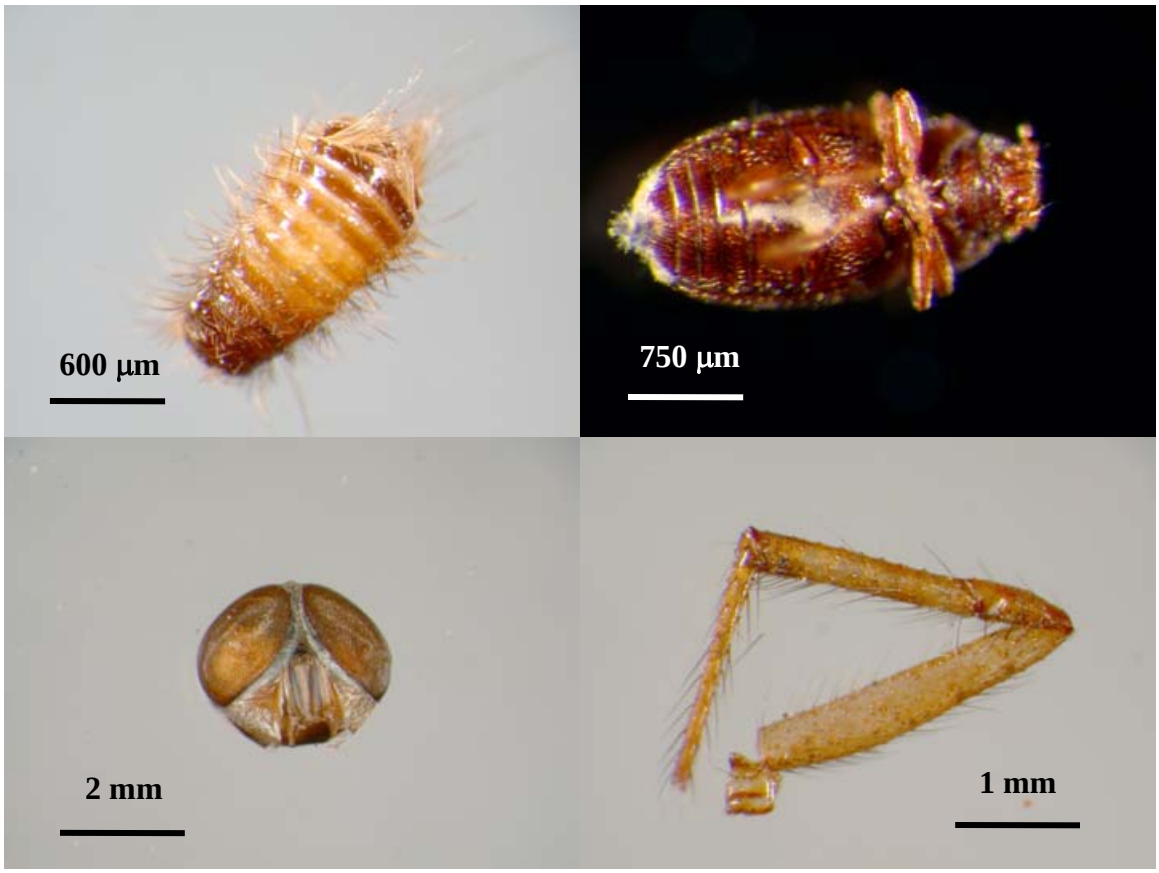

Figure 4. Insects and insect parts from evidence items shown by stereomicroscopy.

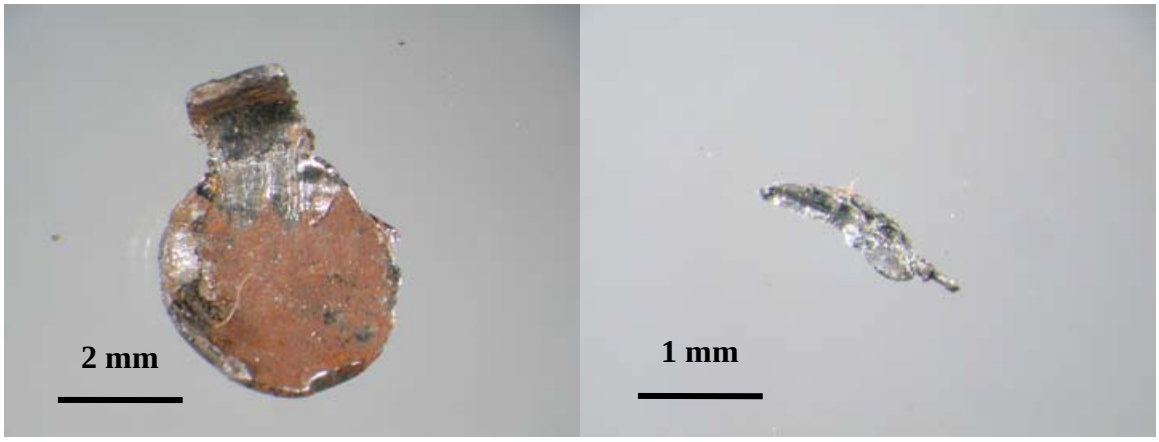

Figure 5. Metal fragments from evidence items shown by stereomicroscopy.

Often a general classification of a substance by stereomicroscopy is sufficient for identification purposes. A leaf fragment may not need to be identified any further if the type of plant from which it originated is not believed to be relevant to the investigation. Whenever identification is concluded at such an early stage, a note should be made describing analyses that could be conducted to provide additional information. For example, a report indicating the presence of leaf fragments should include a note that

DNA analysis could be used to identify the plant family, genus or species at a future date. If the leaf fragment in question had been found on a victim, and similar leaf fragments were later observed on items submitted from a suspect, additional analysis may then be warranted. Knowing the identity of the leaf fragments might assist in determining whether they came from a common or unusual plant, and may help investigators decide whether their presence on both victim and suspect can be adequately explained by the suspect's version of events. Knowledge of the geographic distribution of a particular plant may be helpful for investigative purposes.

Where more precise identification is warranted, the general appearance of the substance under the stereomicroscope should drive the subsequent analyses. If the material is biological in origin, an appropriate expert (in botany, entomology, etc.) should be consulted. In most other cases, additional analyses are performed by the trace evidence examiner. Typically it is best to begin with a single, fairly large particle of the unknown substance and crush or cut it into smaller portions for subsequent analyses. This ensures that data obtained from different instruments all relates to the same substance, as opposed to different components in a mixture. This process is performed under a stereomicroscope with a piece of clean paper placed underneath the sample to serve as a high-contrast background. Black paper should be used as a background when working with a light-colored particle; white paper should be used when working with a dark-colored particle. If the particle is believed to be a polymer or is elastomeric, it can be transferred to a plastic slide and a small portion excised using a clean Teflon-coated razor blade. If the substance is hard or appears to be crystalline, it can be placed between two clean glass microscope slides and crushed by pressing the slides together. During this process, the hardness and brittleness of the substance can be observed. Some materials (such as metals) are too hard to be crushed by glass slides. Small fragments of metals or other hard materials can be scraped off a large particle using a diamond scribe; alternatively the entire particle can be analyzed without fractionation. Smaller particles must be analyzed in their entirety.

Polarized Light Microscopy (PLM)

As a general rule, the next step in the identification of unknowns (after stereomicroscopy) is analysis by PLM. If the material was cut with a razor, the small excised portion should be transferred to a clean glass slide with forceps or a tungsten needle. If the material was crushed between two glass slides, the slides should be separated and the slide retaining the smallest amount of the substance should be used for PLM analysis (typically the top slide). A coverslip can be placed directly on top of the crushed material and a mounting medium introduced at the edge of the coverslip. The two mounting media commonly used for identification of general unknowns are Cargille liquids with refractive indices of 1.540 and 1.660. In general, dark-colored substances are first mounted in a 1.660 liquid while light-colored substances are initially mounted

in a 1.540 liquid (with exceptions made at the analyst's discretion). Often an additional preparation is required in a different mounting medium based on observations made in the first liquid. Temporary (liquid) mounting media are used so that particles can be recovered for additional testing if necessary.

Most substances can be readily classified by PLM, including those that escaped classification under a stereomicroscope. The presence of cellular structure is indicative of botanical matter (Figures 6-7); the presence of starch suggests foodstuffs (Figures 8-9); insect parts are often light brown and are highly organized (Figure 10); and anisotropy with sharp extinction is typical of crystalline substances. After careful determination of optical properties most crystalline substances can be identified microscopically with reference to determinative tables (2, 3). With experience many common minerals (Figures 11-12) and chemicals (Figures 13-14) can be identified on sight, and subsequent analytical techniques can be selected as appropriate to confirm the microscopical identification.

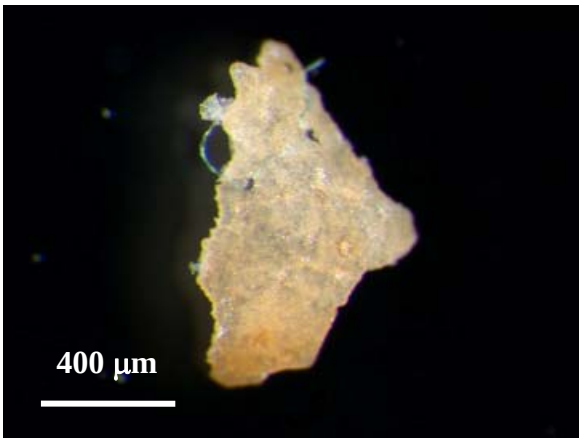

Figure 6. A tan particle from an evidence item shown by stereomicroscopy. The nature of the particle was unclear by stereomicroscopy alone.

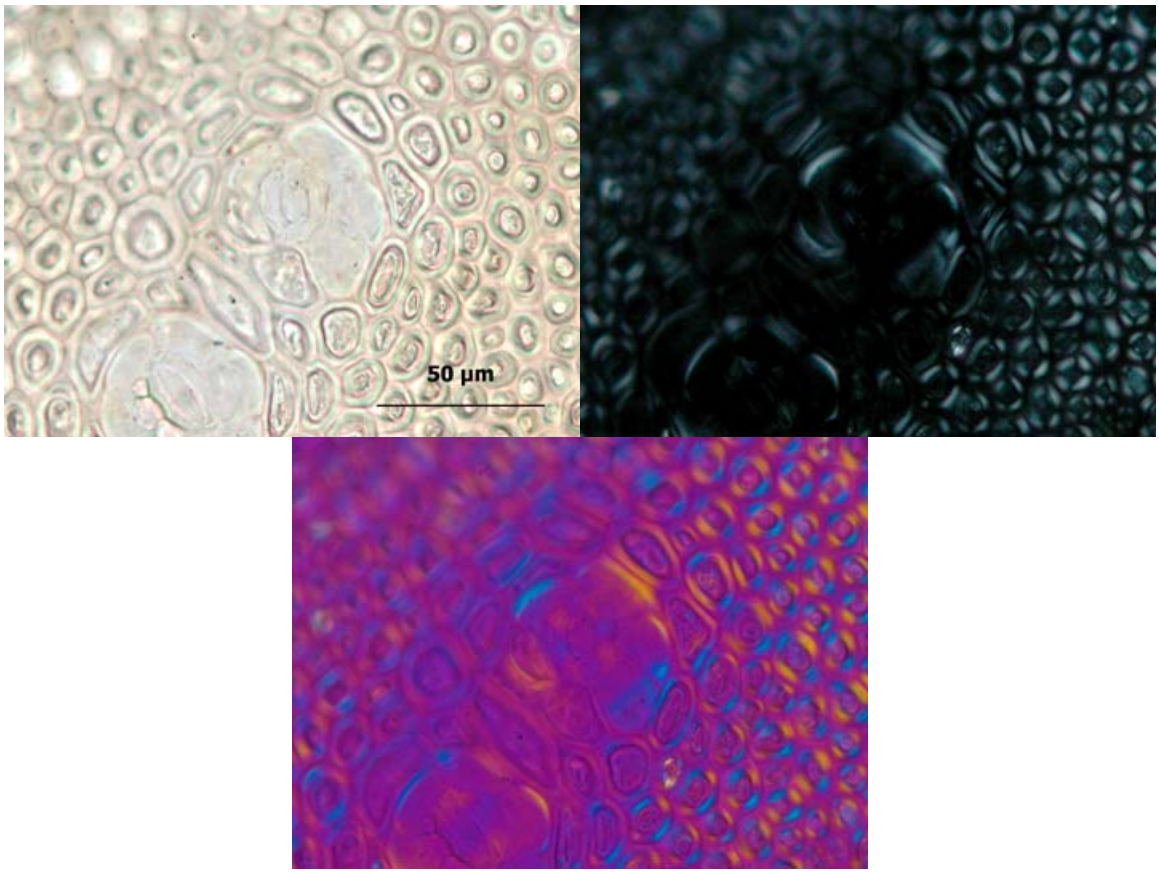

Figure 7. A portion of the particle from Figure 6 shown in plane polarized light (top left), in between crossed polars (top right), and again in crossed polars with a 530 nm compensator (bottom). The cellular structure indicates that the material is botanical, and the presence of stomata suggest it is a leaf fragment. Mounting medium is 1.660.

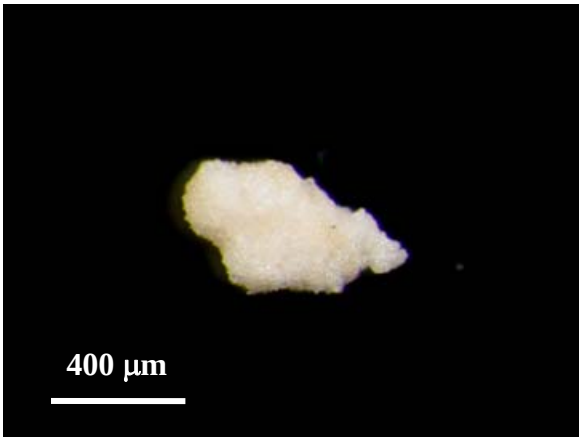

Figure 8. An off-white particle from an evidence item shown by stereomicroscopy. The nature of the particle was unclear by stereomicroscopy alone.

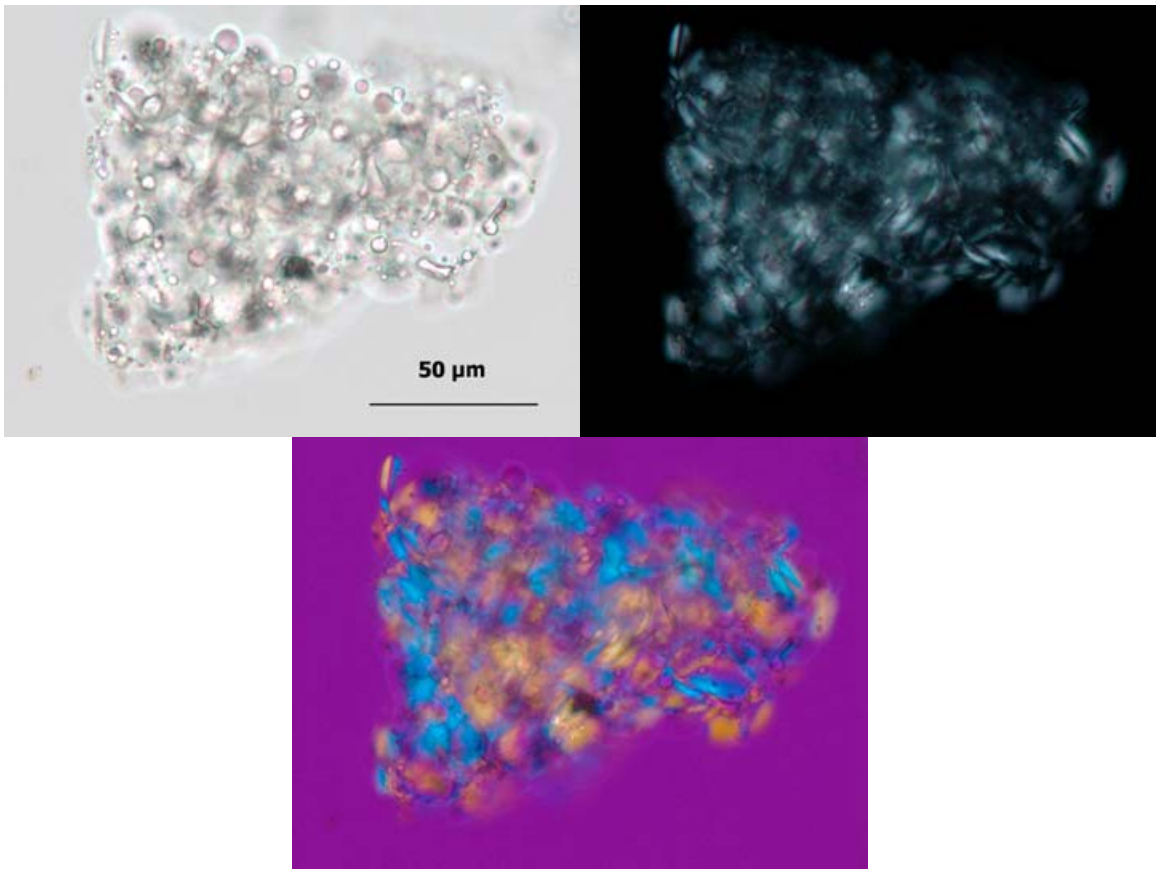

Figure 9. A portion of the particle from Figure 8 shown in plane polarized light (top left), in between crossed polars (top right), and again in crossed polars with a 530 nm compensator (bottom). The material consists of an aggregate of starch grains with adhering liquid droplets, suggestive of a foodstuff. Mounting medium is 1.540.

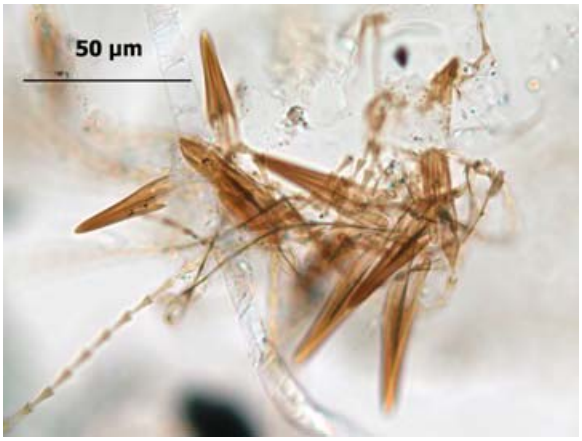

Figure 10. Insect parts shown in plane polarized light. Mounting medium is 1.540.

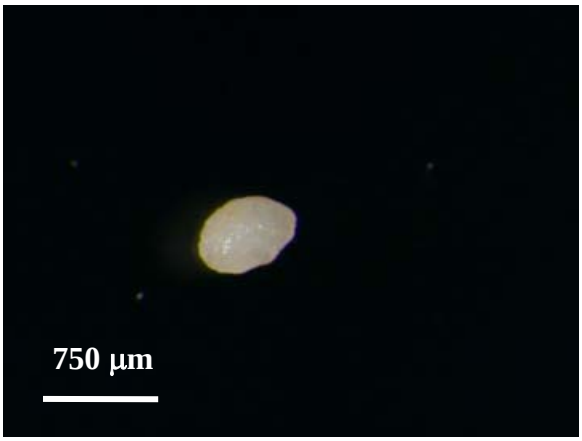

Figure 11. An off-white particle from an evidence item shown by stereomicroscopy. The nature of the particle was unclear by stereomicroscopy alone.

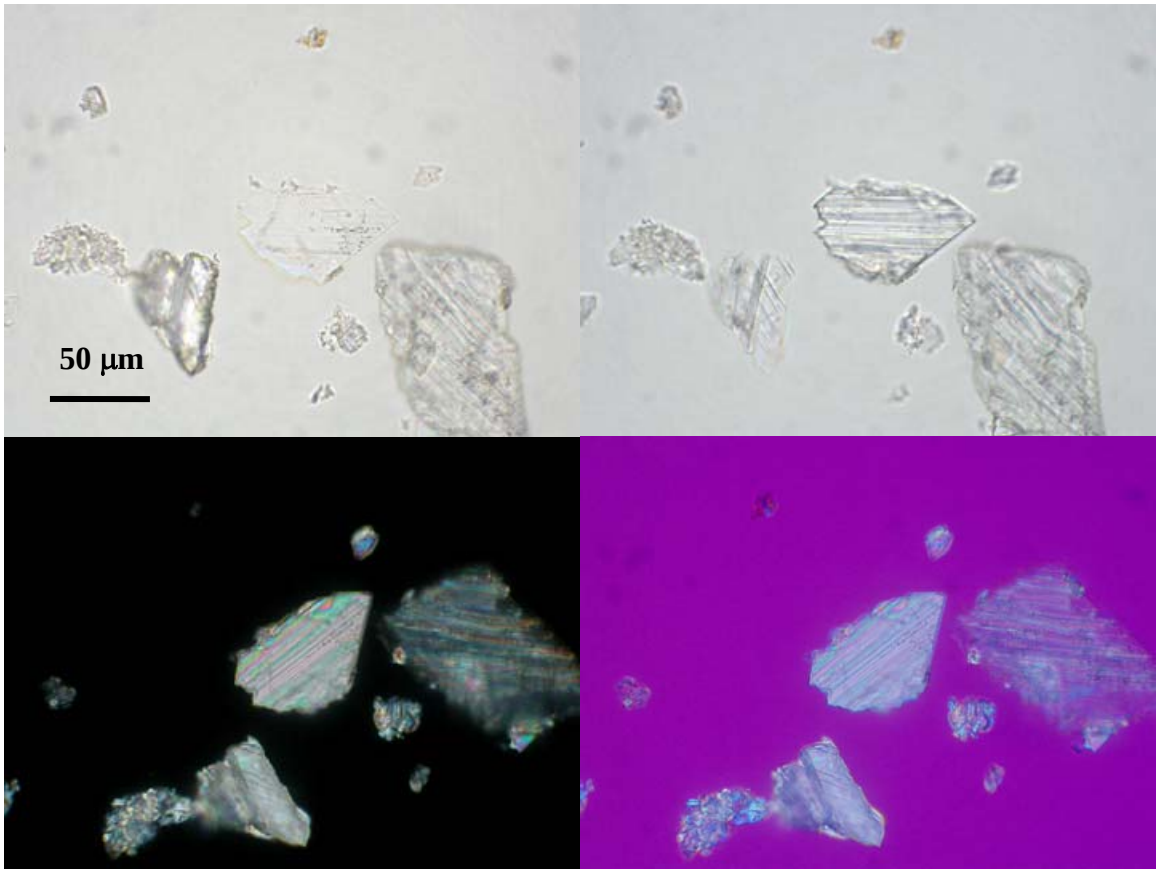

Figure 12. Crushed fragments of the particle from Figure 11 shown in plane polarized light with the polarizer oriented E-W (top left), with the polarizer oriented N-S (top right), in between crossed polars (bottom left), and again in crossed polars with a 530 nm compensator (bottom right). The morphology and optical properties are characteristic of calcite, a commonly occurring mineral. Mounting medium is 1.660.

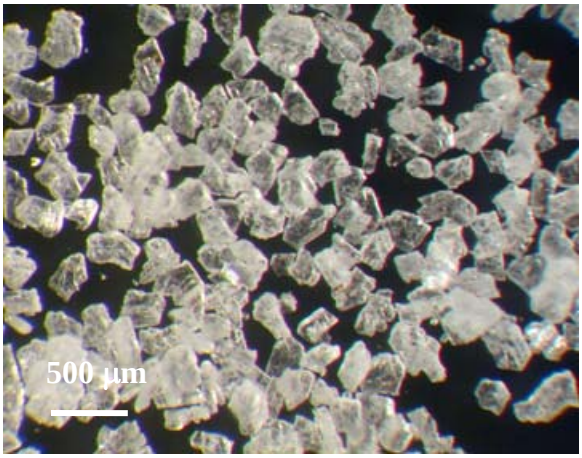

Figure 13. A white powder submitted for analysis shown by stereomicroscopy. The identity of the material was unclear by stereomicroscopy alone.

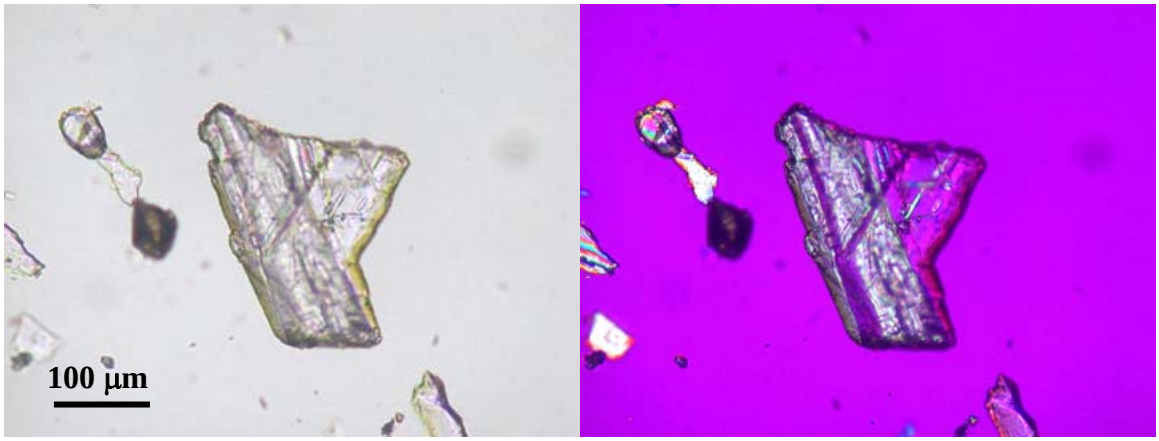

Figure 14. A particle of the white powder from Figure 11 shown in plane polarized light (left) and again in between crossed polars with a 530 nm compensator (right). The morphology and optical properties are characteristic of sodium bicarbonate (baking soda). Mounting medium is 1.660.

Substances that cannot be identified by PLM can still benefit tremendously from microscopical analysis. A trained microscopist can easily determine if a sample is homogeneous or a mixture, and if the latter, how many components are present. This information is critically important for interpreting subsequent instrumental data. If a substance were seen by PLM to consist of a polymer with inorganic filler, results from subsequent instrumental analyses could be interpreted in that context. The crystallinity of a substance can be inferred on the basis of the optical behavior of a substance, providing guidance on the possible contribution of XRD analysis. If two or more separate, similar particles are observed on an evidence item, PLM analysis can confirm that the two are in fact the same compound. In addition, when a substance cannot be identified by PLM analysis, a tremendous number of substances can be ruled out as possible candidates by an experienced microscopist.

Fourier Transform Infrared Micro-Spectroscopy (FTIR)

In many cases where PLM is unable to unambiguously identify a substance, FTIR analysis aids significantly in identifying the material. Fourier transform infrared spectroscopy is a vibrational spectroscopy technique that provides information on the covalent bonds present in a substance. It is particularly well suited for identification of polymers and organic compounds, although it can be useful for inorganic analysis as well. In general materials that are fairly soft, tacky or elastomeric are good candidates for FTIR analysis since many organic substances have these properties. A small portion of the unknown particle is excised using a clean razor, or a small amount of powder from a crushed particle isolated. This material is transferred to a salt plate and pressed into a thin film for FTIR analysis. For identification of complete unknowns database searches are helpful. In the absence of a database match, spectral interpretation can indicate whether a substance is organic or inorganic and can provide insights into the chemical class of the compound (i.e., hydrocarbon, amide, carbonate). In some cases this is sufficient for identification purposes when combined with the properties determined by PLM and stereomicroscopy. With experience the identity of complete unknowns can be determined by spectral interpretation alone.

Many unknown substances turn out to be mixtures, and PLM analysis is critical in understanding the FTIR spectra generated from these materials. Polymer fragments are commonly encountered as evidence and often contain inorganic fillers. Calcite is a common filler that is easily recognizable by PLM (Figures 15-16). Other common fillers include titanium dioxide (with broad absorption between 400 and 700 cm^{-1}) and kaolinite clay (with characteristic sharp absorptions in the water region as well as the Si-O region). When calcite is present in a polymer, FTIR spectra of the material will include a strong broad absorption near 1400 cm^{-1} and a moderate sharp absorption around 870 cm^{-1}, along with a few weaker absorptions. The peaks not attributable to the calcite are due to the polymer itself, which can be identified using selected region searches or by subtracting a standard calcite spectrum from the mixture spectrum (Figure 17). Another example encountered in casework was a circuit board fragment in the form of a fiberglass-reinforced polymer (Figures 18-20).

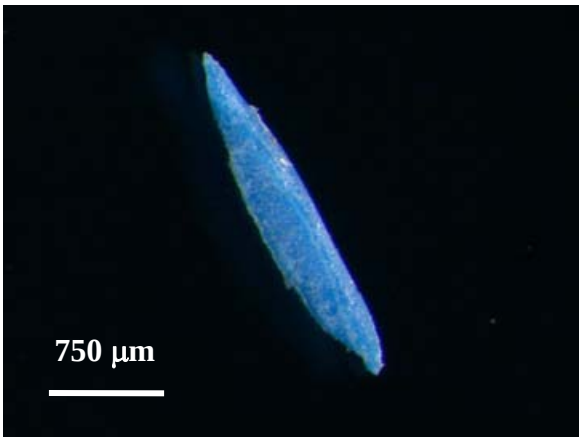

Figure 15. An elongated blue polymer particle from an evidence item shown by stereomicroscopy.

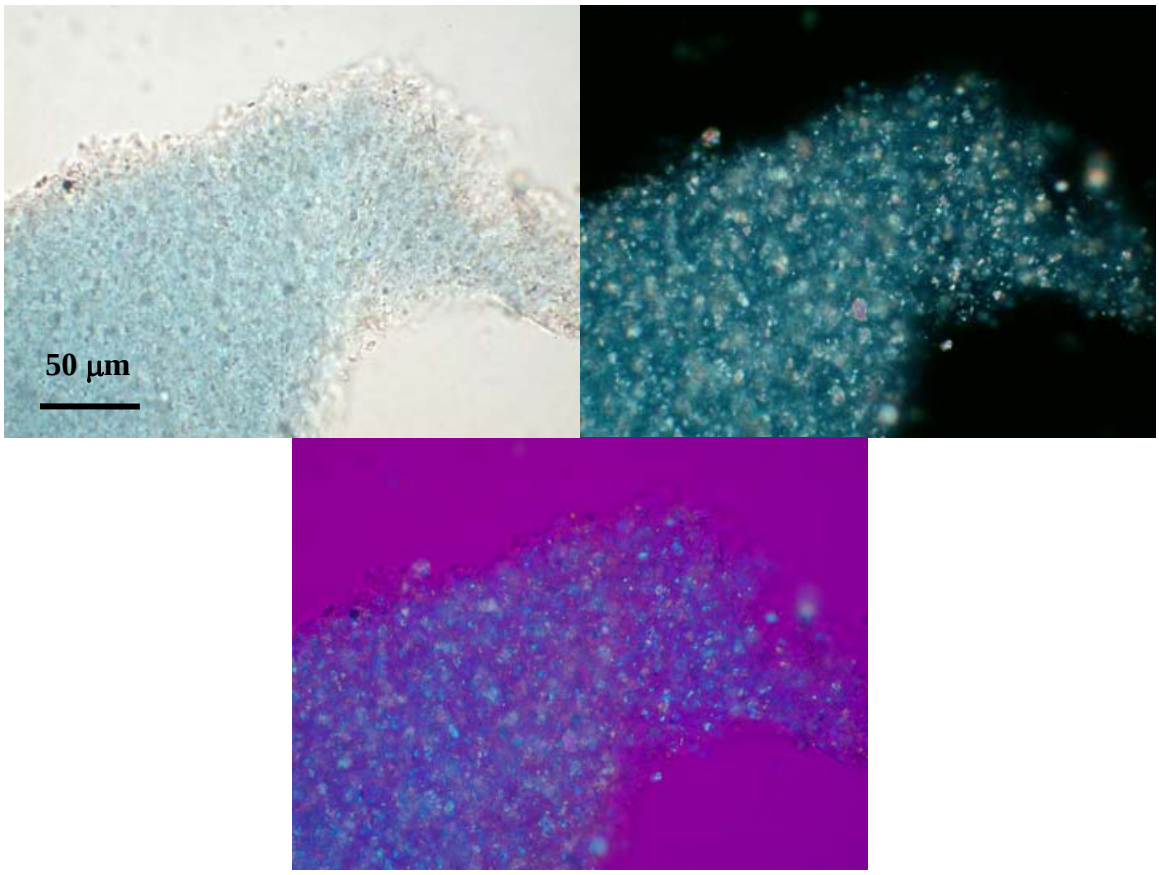

Figure 16. A thin slice of the blue particle from Figure 15 shown in plane polarized light (top left), in between crossed polars (top right), and again in crossed polars with a 530 nm compensator (bottom). The small, highly birefringent particles are calcite, present in an isotropic polymer matrix. Mounting medium is 1.660.

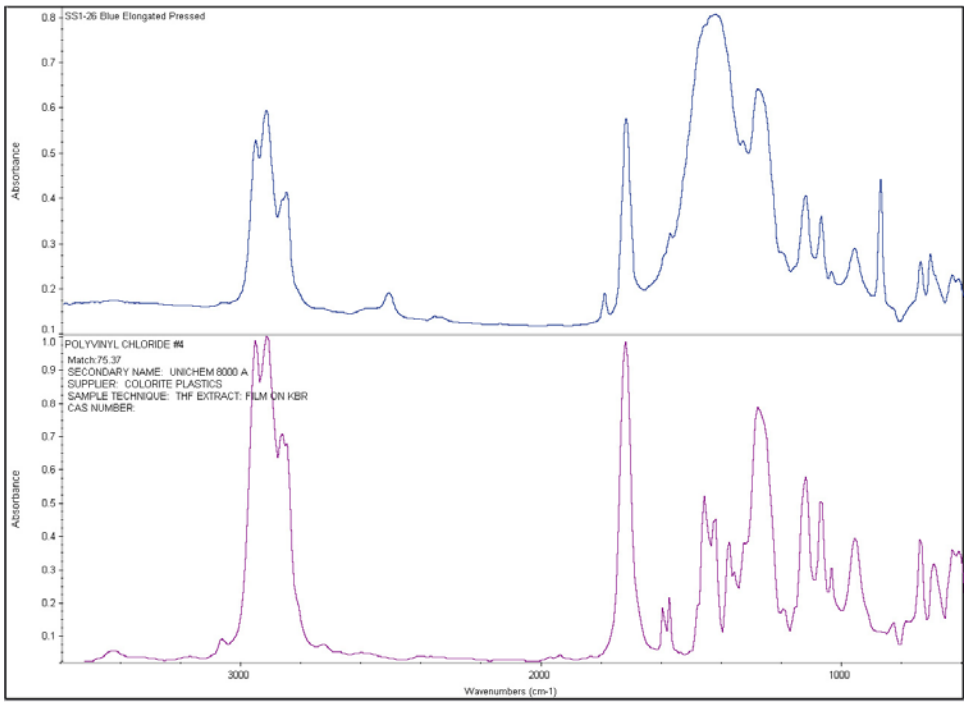

Figure 17. The FTIR spectrum of a fragment of the elongated blue particle from Figure 15 (top) and a library spectrum of PVC (bottom) for comparison. The differences are primarily attributable to the calcium carbonate present.

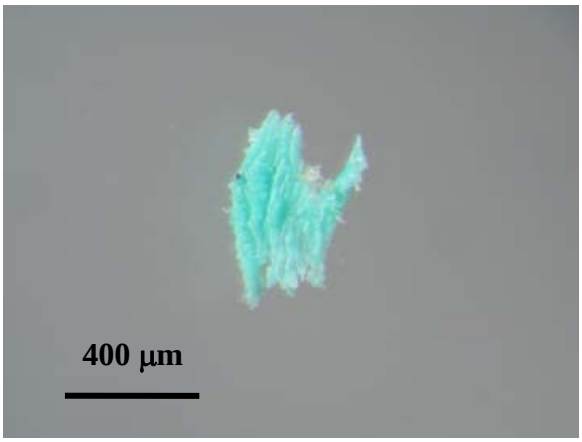

Figure 18. A green particle from an evidence item shown by stereomicroscopy.

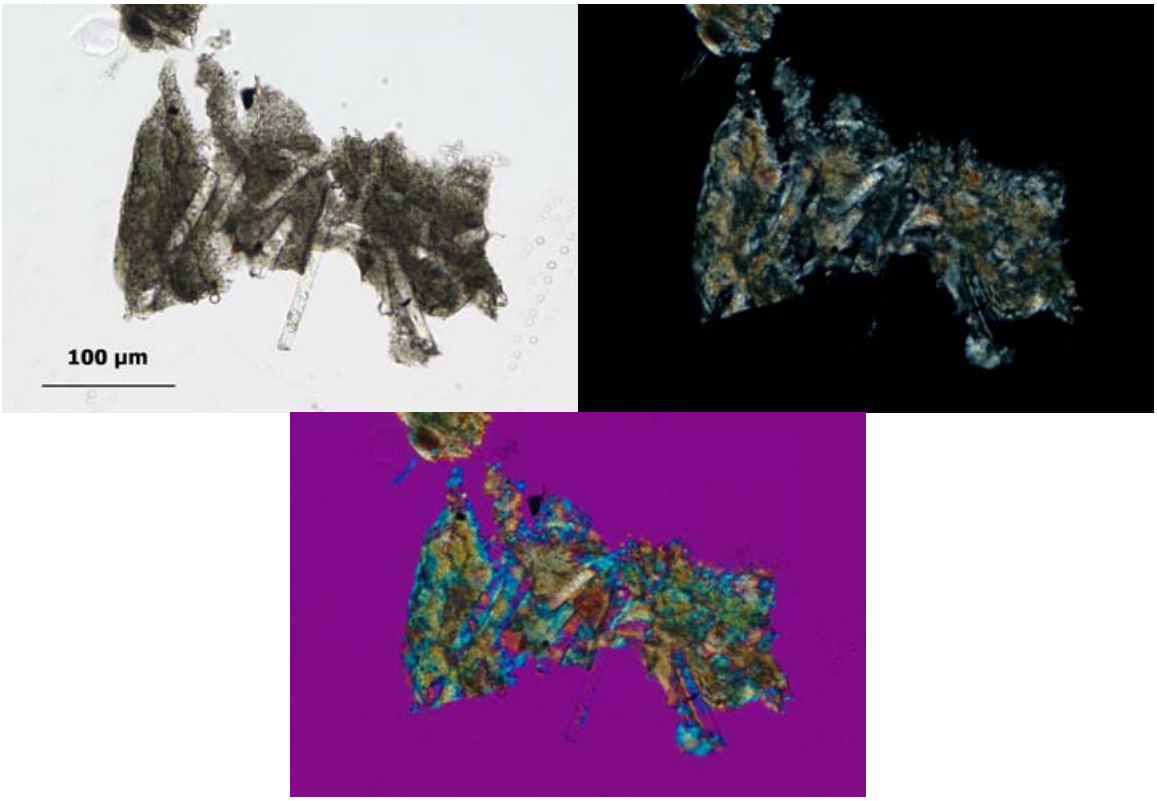

Figure 19. A portion of the green particle from Figure 18 shown in plane polarized light (top left), in between crossed polars (top right), and again in crossed polars with a 530 nm compensator (bottom). Mounting medium is 1.660.

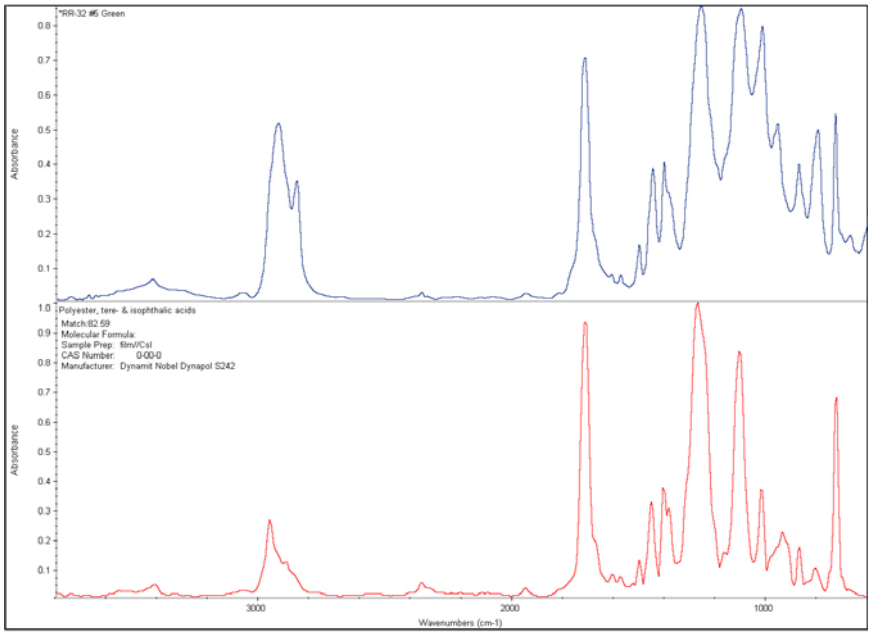

Figure 20. The FTIR spectrum of the green particle from Figure 18 (top) and a library spectrum of polyester (bottom) for comparison. The broad absorption envelope near 1000 cm^{-1} in the unknown material is likely due to the fiberglass Si-O bonds.

Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS)

Another instrument that is widely available in crime laboratories and extremely useful for the identification of general unknowns is the SEM, especially when equipped with an EDS detector. The EDS accessory provides semi-quantitative data on the elemental composition of the substance. Typically, SEM-EDS analysis is indicated for materials that are opaque, materials that are hard, materials that are dark in color and materials that do not produce useful IR spectra (or those with IR spectra lacking C-H stretches). Commonly encountered opaque substances include metals (Figures 21-22) and combustion products (Figures 23-24) while common hard materials include abrasives (Figures 25-26) and minerals (Figures 27-28). A small, golden sphere in a dust sample was determined to have come from a lighter based on SEM-EDS data (Figures 29-30). Purely ionic substances do not produce IR spectra and SEM-EDS data can often provide insights into their identity (Figures 31-33).

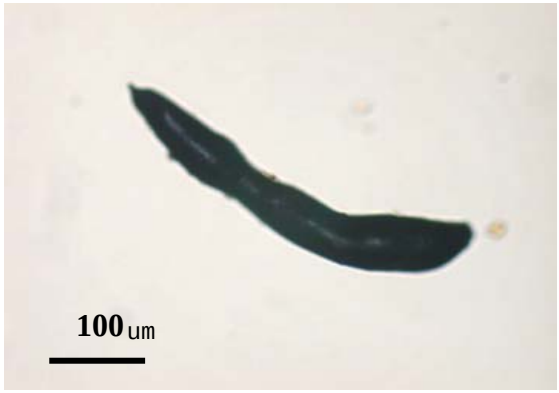

Figure 21. An opaque particle shown in plane polarized light. Mounting medium is 1.660.

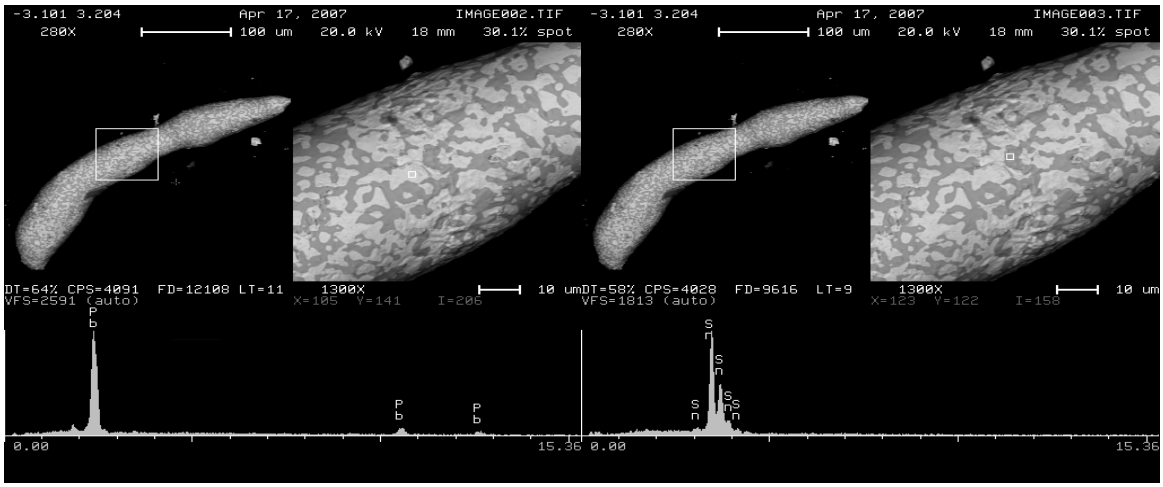

Figure 22. Two SEM images of the opaque particle from Figure 21, areas selected for EDS analysis and the EDS spectra of the selected areas (bottom). The light gray areas on the particle are lead, while the dark gray areas are tin, indicating the metal particle is solder.

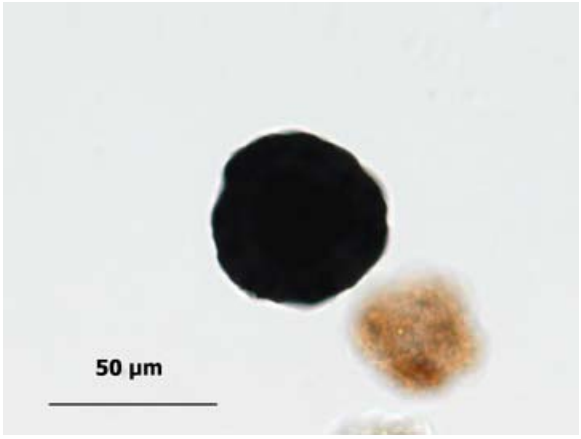

Figure 23. An opaque grain shown in plane polarized light. Mounting medium is 1.540.

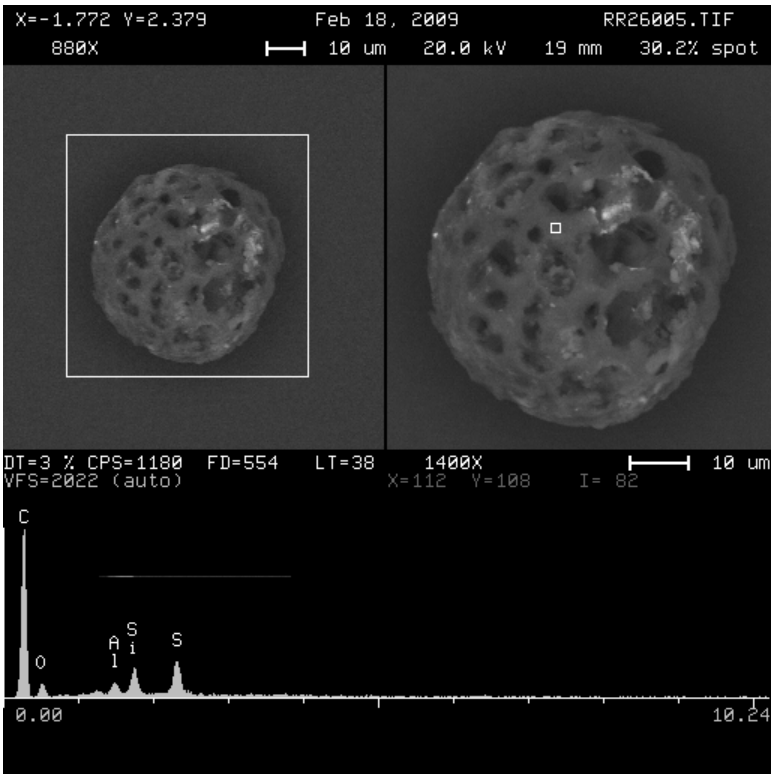

Figure 24. An SEM image of the opaque particle from Figure 23 (top left), the area selected for EDS analysis (top right), and the EDS spectrum of the selected area (bottom). The material is carbon-rich, and is likely coal or a similar combustion product.

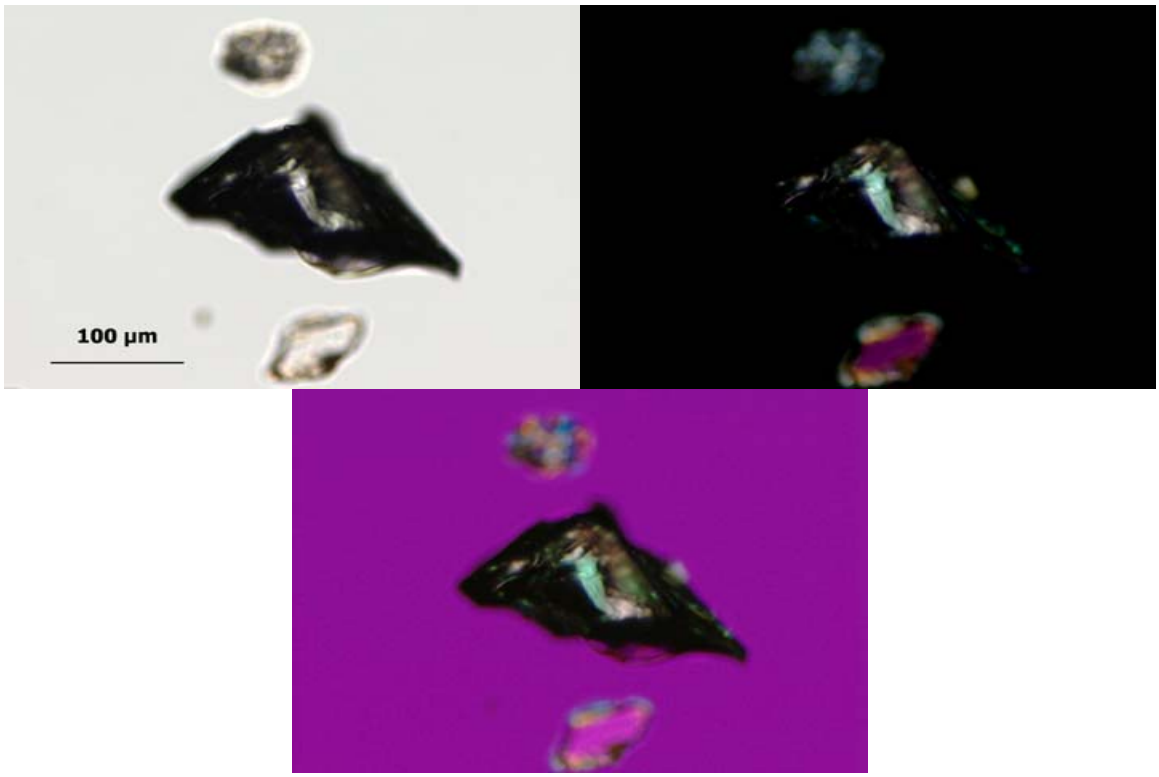

Figure 25. A carborundum particle shown in transmitted plane polarized light (top left), in between crossed polars (top right), and again in crossed polars with a 530 nm compensator (bottom). Mounting medium is 1.660.

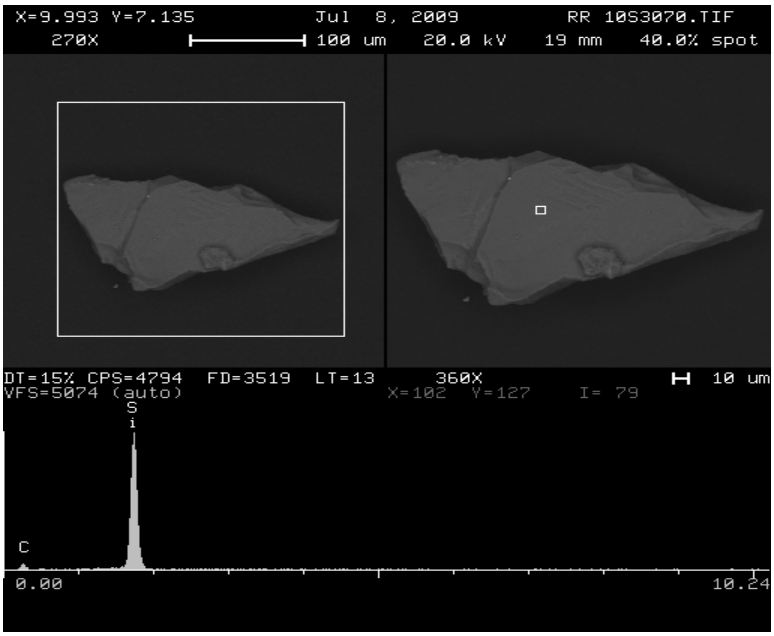

Figure 26. An SEM image of the carborundum particle from Figure 25 (top left), the area selected for EDS analysis (top right), and the EDS spectrum of the selected area (bottom). The EDS spectrum is consistent with carborundum, but would be ambiguous without the PLM data.

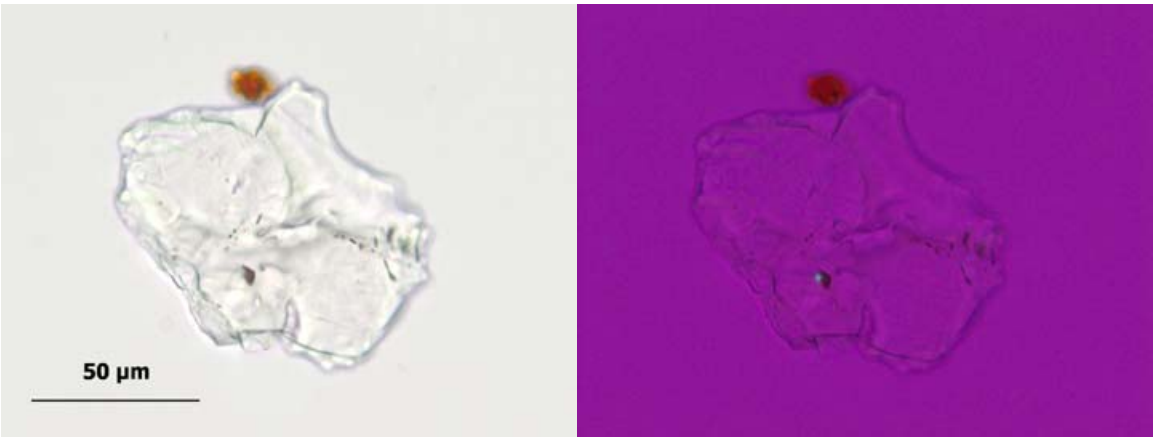

Figure 27. A garnet grain shown in plane polarized light (left) and again in between crossed polars with a 530 nm compensator (right). Mounting medium is 1.660.

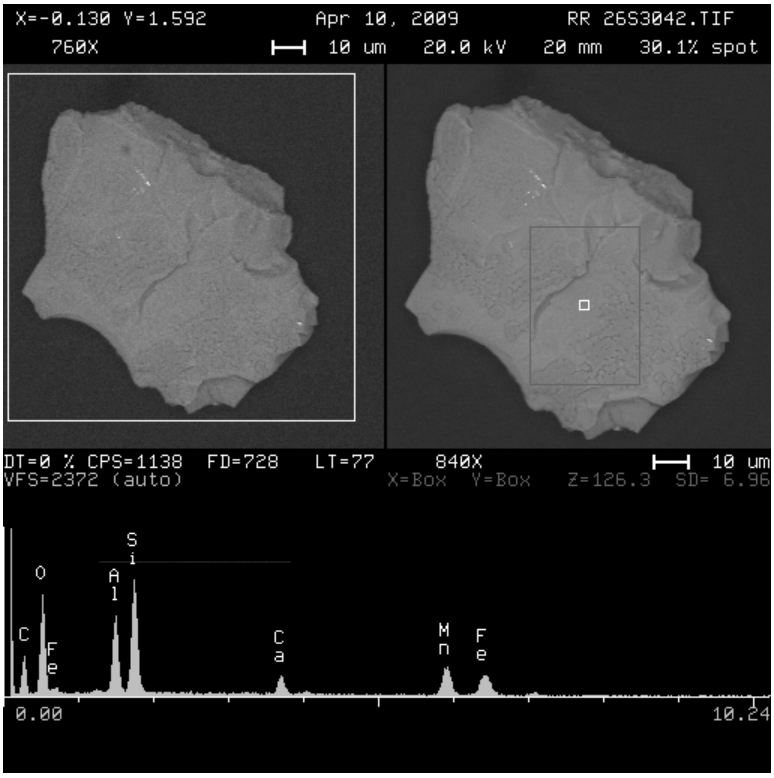

Figure 28. An SEM image of the garnet grain from Figure 27 (top left), the area selected for EDS analysis (top right), and the EDS spectrum of the selected area (bottom).

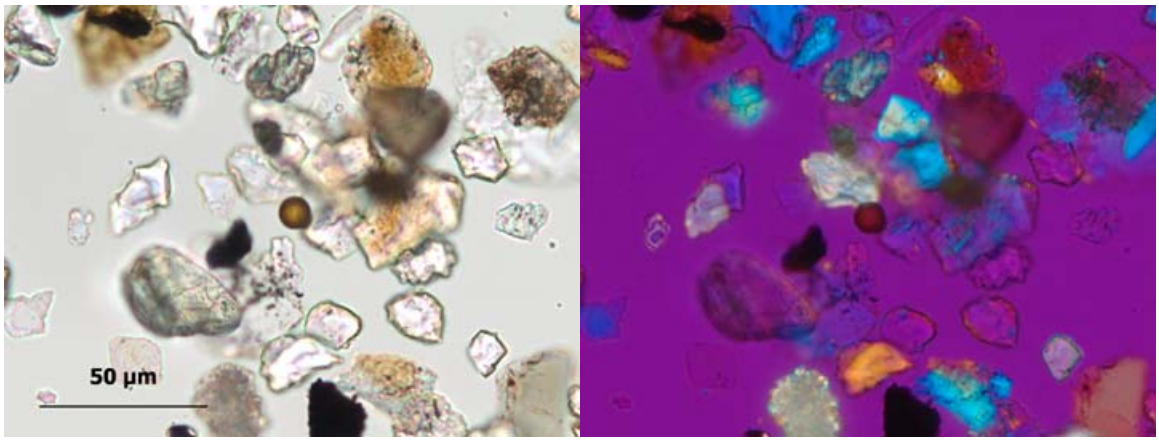

Figure 29. A golden sphere shown in plane polarized light (left) and again in between crossed polars with a 530 nm compensator (right). The morphology suggests formation at high temperature. Mounting medium is 1.660.

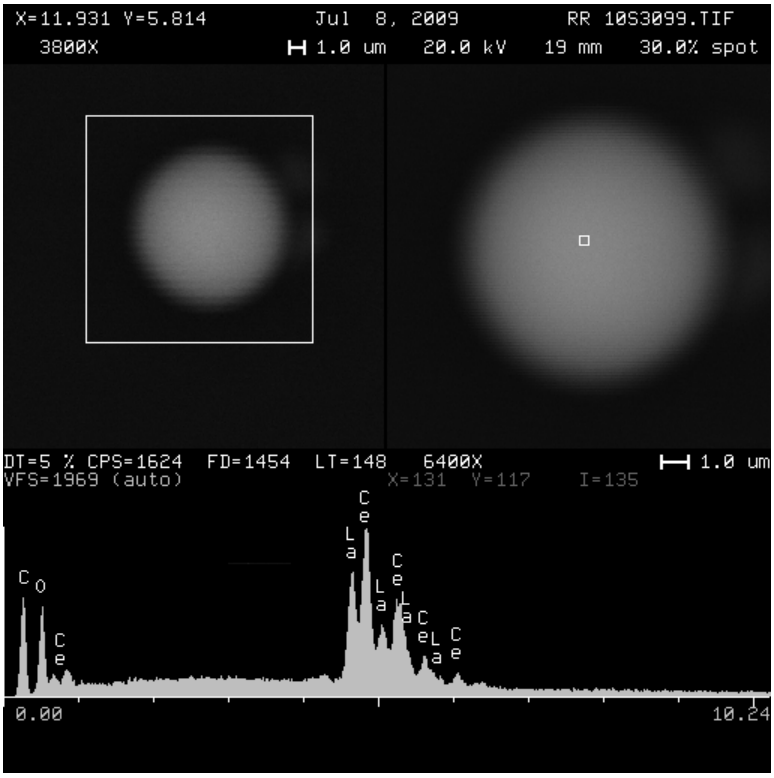

Figure 30. An SEM image of the sphere from Figure 29 (top left), the area selected for EDS analysis (top right), and the EDS spectrum of the selected area (bottom). The EDS spectrum is consistent with mischmetal, used as lighter flint.

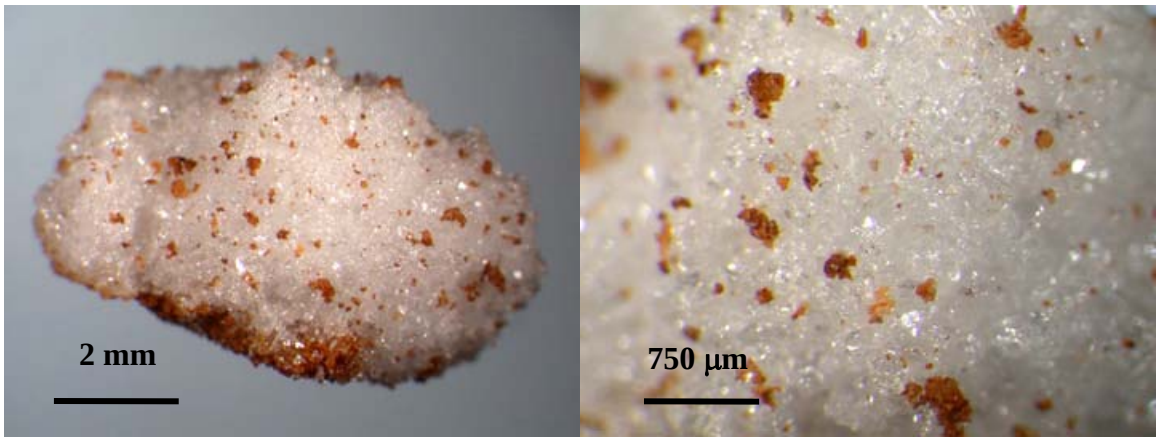

Figure 31. Clump of unknown white material submitted for analysis shown by stereomicroscopy at two different magnifications.

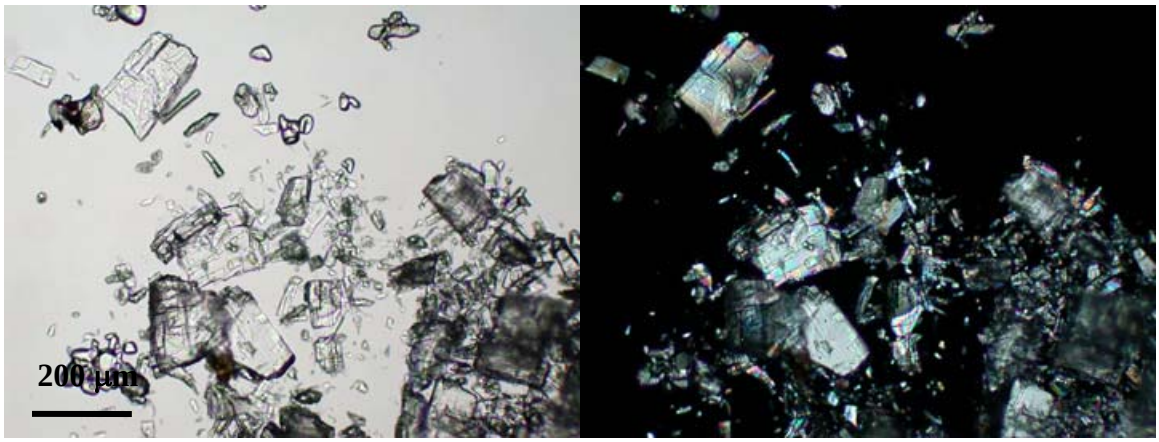

Figure 32. Some crushed fragments of the white substance from Figure 31 shown in plane polarized light (left) and again in between crossed polars (right). Mounting medium is 1.660.

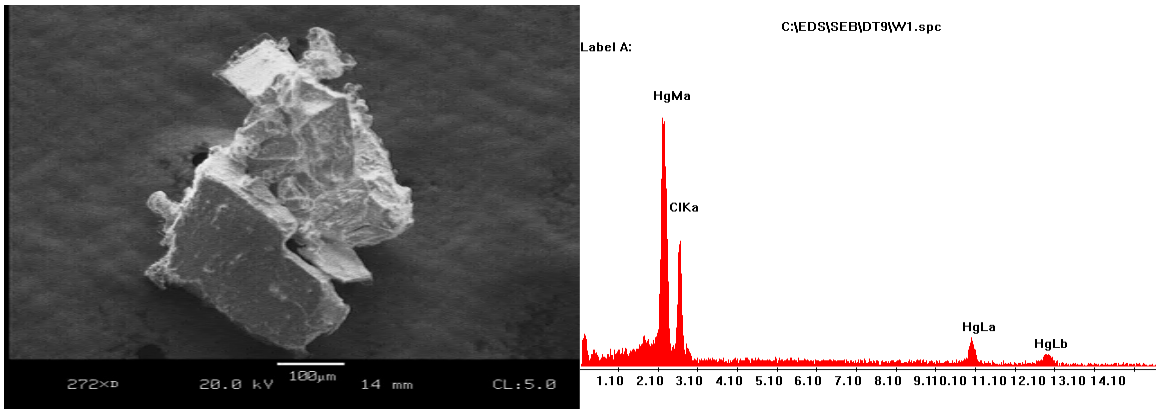

Figure 33. An SEM image of crushed fragments of the white substance from Figure 31 (left), and its corresponding EDS spectrum (right). The material was identified as mercuric chloride on the basis of its EDS spectrum and optical properties.

While EDS data is very useful for describing a substance, it is almost never sufficient for definitive identification of a material. Even in the simple example shown in Figures 31-33, the EDS data could not distinguish between mercurous chloride (HgCl) and mercuric chloride ($HgCl_2$), and optical properties determined by PLM were required to make this distinction.

It is sometimes necessary for all three of the above techniques (PLM, FTIR and SEM-EDS) to be used together for identification purposes (Figures 34-36). When a single small particle is all that is available, it should be analyzed by PLM first, followed by SEM-EDS on a temporary mount (tacked down on a Be stud using collodian), and finally recovered and pressed flat for FTIR analysis. For larger particles, separate subsamples can be used for each analysis.

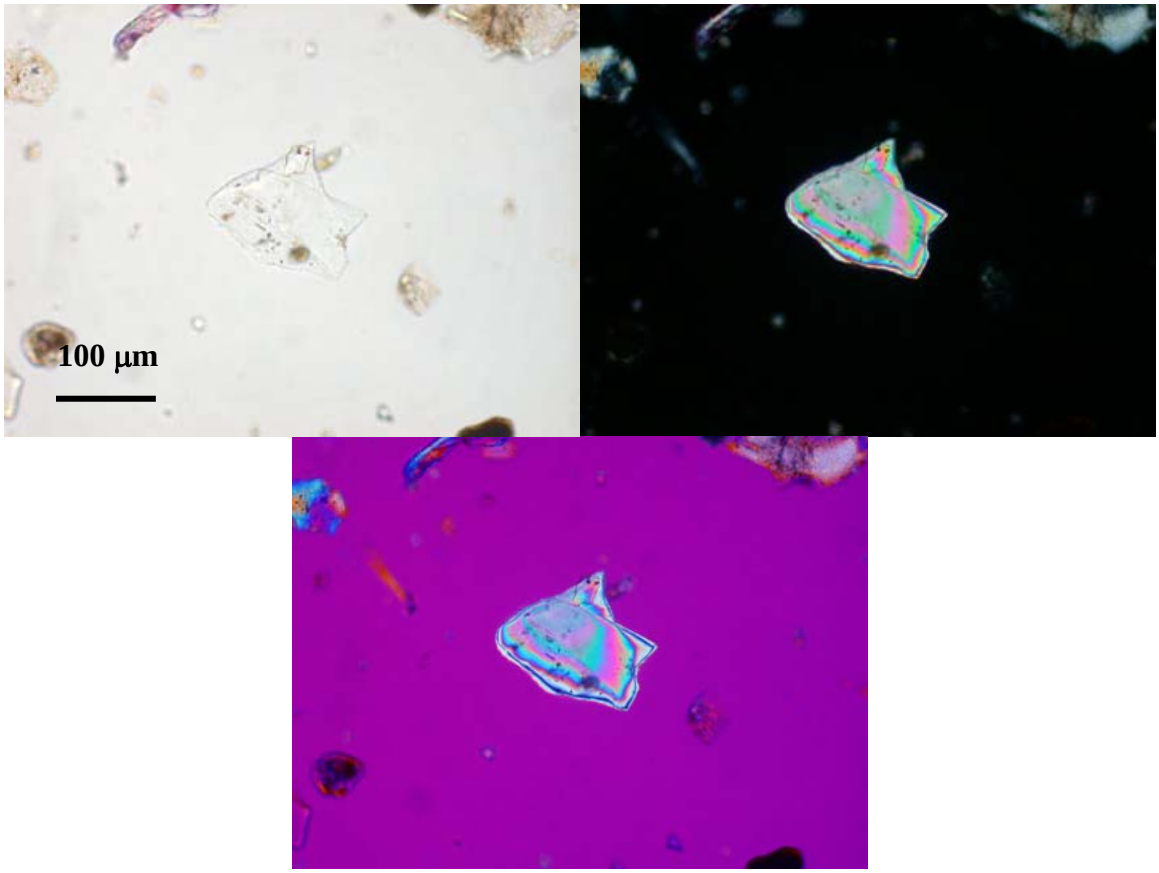

Figure 34. A grain of monosodium glutamate from a dust sample shown in plane polarized light (top left), in between crossed polars (top right), and again in crossed polars with a 530 nm compensator (bottom). Mounting medium is 1.540.

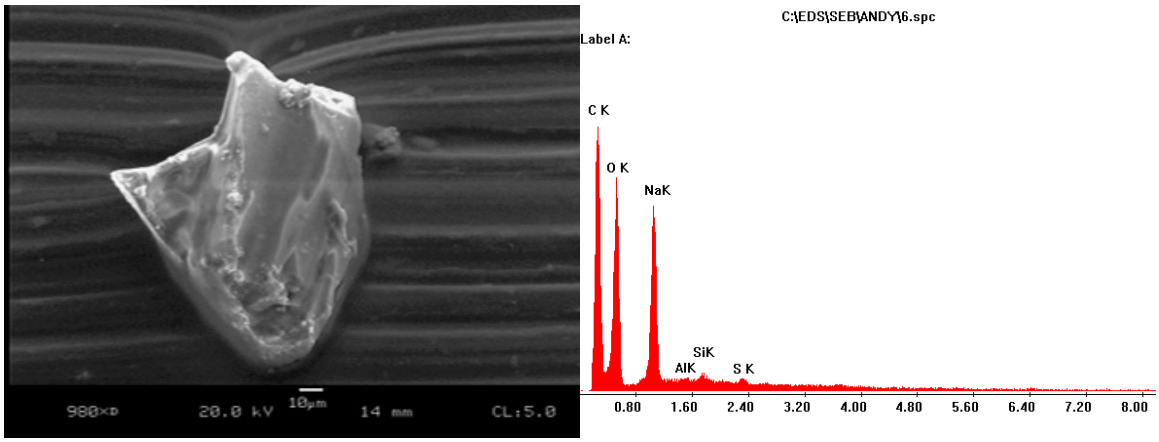

Figure 35. An SEM image of the particle from Figure 34 (left), and its corresponding EDS spectrum (right). The EDS data indicated that it is an organic sodium salt.

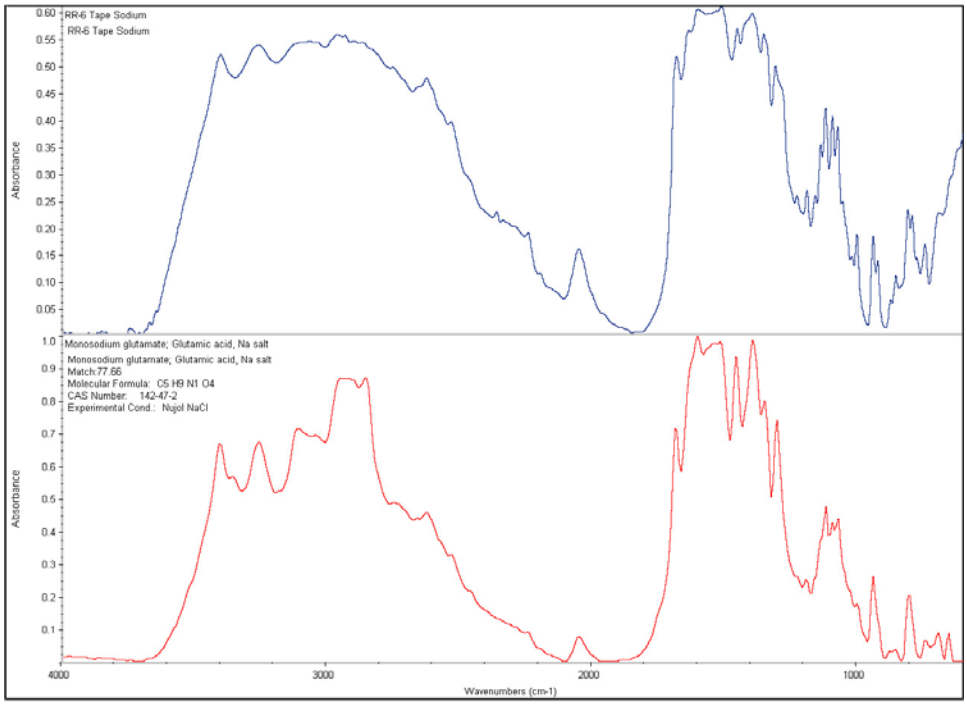

Figure 36. The FTIR spectrum of the particle from Figure 34 (top) and a library spectrum of monosodium glutamate (MSG) (bottom) for comparison. The differences are attributable to Nujol used in the library sample preparation but not with the unknown.

Microchemistry

Microchemistry, typically in the form of microcrystal tests or spot tests, provides qualitative information on chemical species present in a compound (4). This data can be critical for making a definitive identification. In many cases the oxidation state of an element detected by EDS is unclear, and microchemistry is particularly well suited to determining this. For example, if a compound cannot be identified on the basis of its optical properties or FTIR spectrum, and is shown to contain Na, S, and O in its EDS

spectrum, the likely hypothesis would be an inorganic sodium salt. However, the oxidation state of the sulfur, the presence or absence of water of hydration, and possibly other important pieces of information would remain unknown. Microchemical tests are specific for polyatomic ions (i.e., sulfate, sulfite, thiosulfate) and the presence or absence of these ions can be determined by an appropriate test. In addition, the oxidation state of cations can often be determined by microchemical tests. Once the specific ions and their oxidation states have been determined, optical properties determined by PLM can be used to distinguish different hydrates and polymorphs.

Raman Micro-Spectroscopy

Another vibrational spectroscopy method exists that is complementary to FTIR. This method is Raman spectroscopy, and with the aid of a microscope very small particles can be analyzed (sometimes smaller than 1 um). Raman can be used to distinguish between different polymorphs and allotropes (Figures 37-39) as well as identifying both organic and inorganic compounds. Raman can be applied to particles on SEM stubs without remounting the particles. The primary disadvantages of Raman are the fluorescence exhibited by many substances along with the limited number of libraries available for database searching.

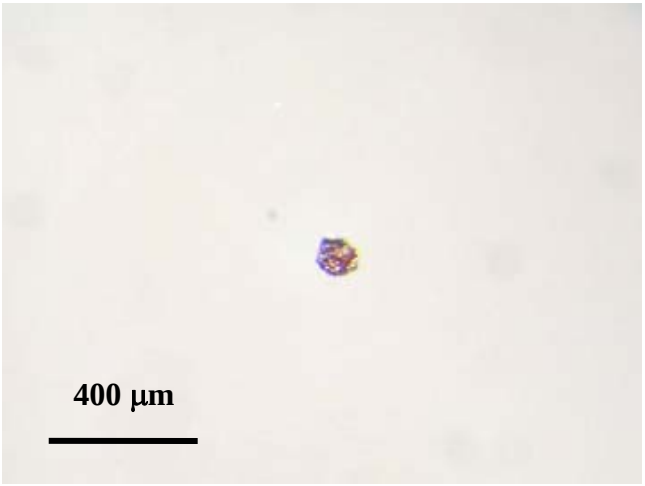

Figure 37. A red, opaque particle found on an evidence item shown by stereomicroscopy.

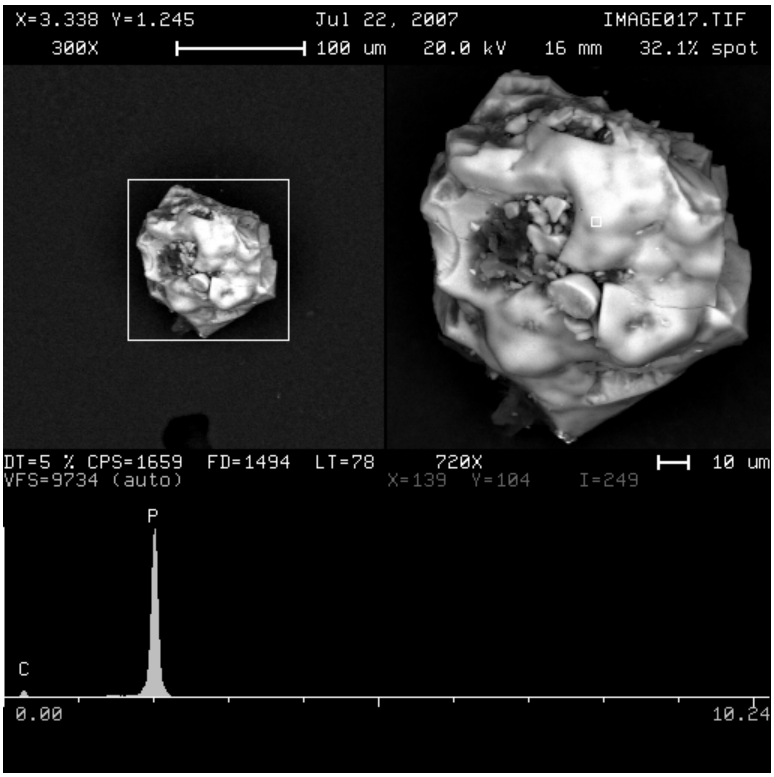

Figure 38. An SEM image of the red particle from Figure 37 (top left), the area selected for EDS analysis (top right), and the EDS spectrum of the selected area (bottom). The EDS spectrum is consistent with elemental phosphorus.

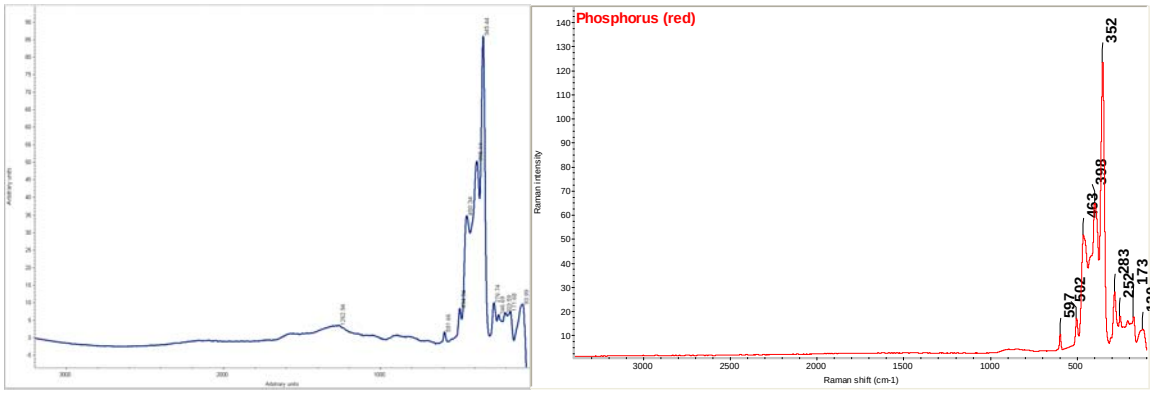

Figure 39. The Raman spectrum of the particle from Figure 37 (left) and a library spectrum of red phosphorus (right) for comparison. Raman distinguished red phosphorus from white phosphorus.

X-Ray Diffraction (XRD)

While it is inaccessible to many crime labs, XRD is extremely useful for identifying crystalline substances. Different hydrates and polymorphs can be distinguished, as can materials containing ions with different oxidations states. Provided a substance is crystalline and its diffraction pattern is in the available database, it can usually be identified by XRD. Powder XRD analysis, the most common type, requires fairly large

sample sizes and may be inappropriate for many trace evidence samples. However, single crystal instruments exist and would be a powerful tool for any laboratory that has to identify small particles of unknown crystalline substances. One example where XRD was crucial for identification purposes involved a detergent submitted for analysis. Present in the detergent was an unknown sodium phosphate phase. The identity of the phase was determined to be sodium tripolyphosphate hexahydrate by XRD analysis after ambiguous PLM, SEM-EDS and FTIR results (Figures 40-43, Table 1).

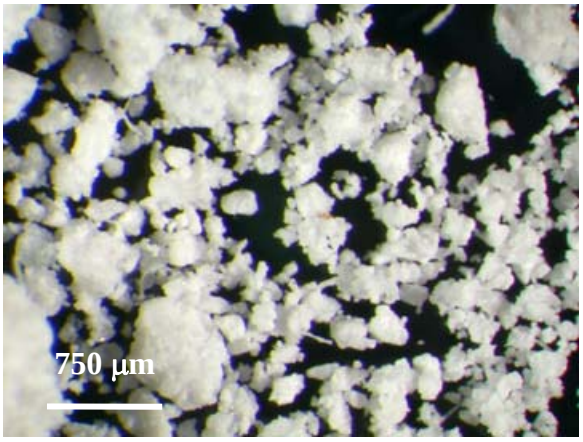

Figure 40. A white powder submitted for analysis shown by stereomicroscopy.

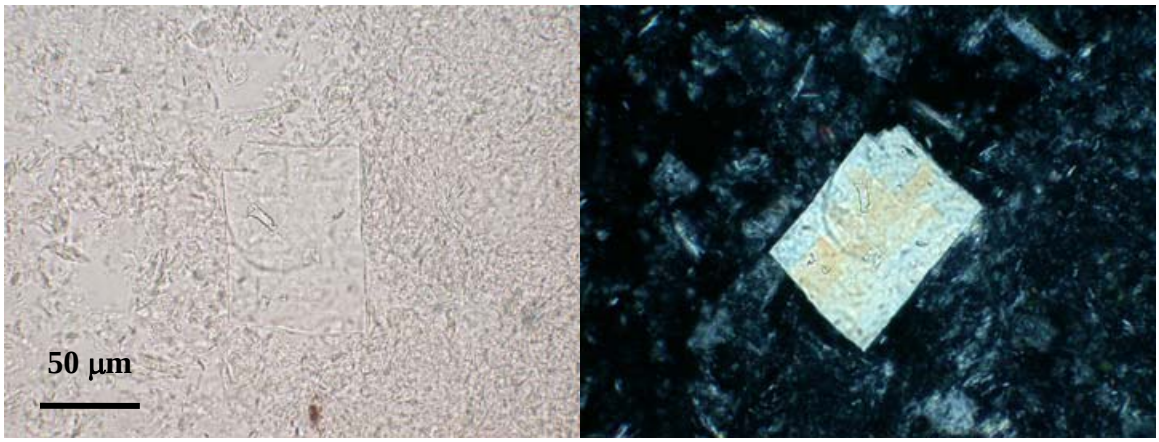

Figure 41. A portion of the white powder from Figure 40 shown in plane polarized light (left) and again in between crossed polars (right). The rectangular plate is a sodium phosphate phase that was consistent optically with two separate compounds. Mounting medium is 1.470.

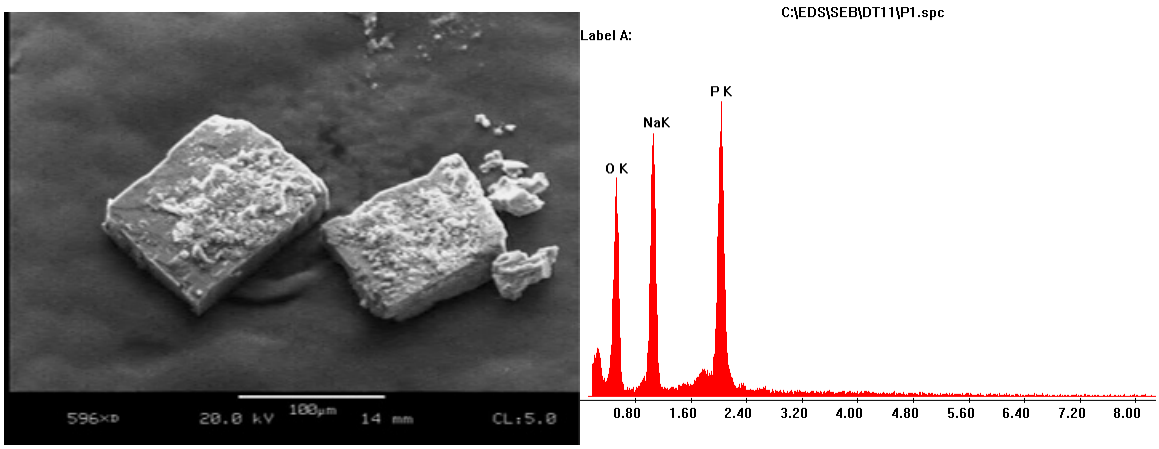

Figure 42. An SEM image of two rectangular plates from the white powder in Figure 40 (left), and the corresponding EDS spectrum of the plate on the left (right). The material is an unknown sodium phosphate.

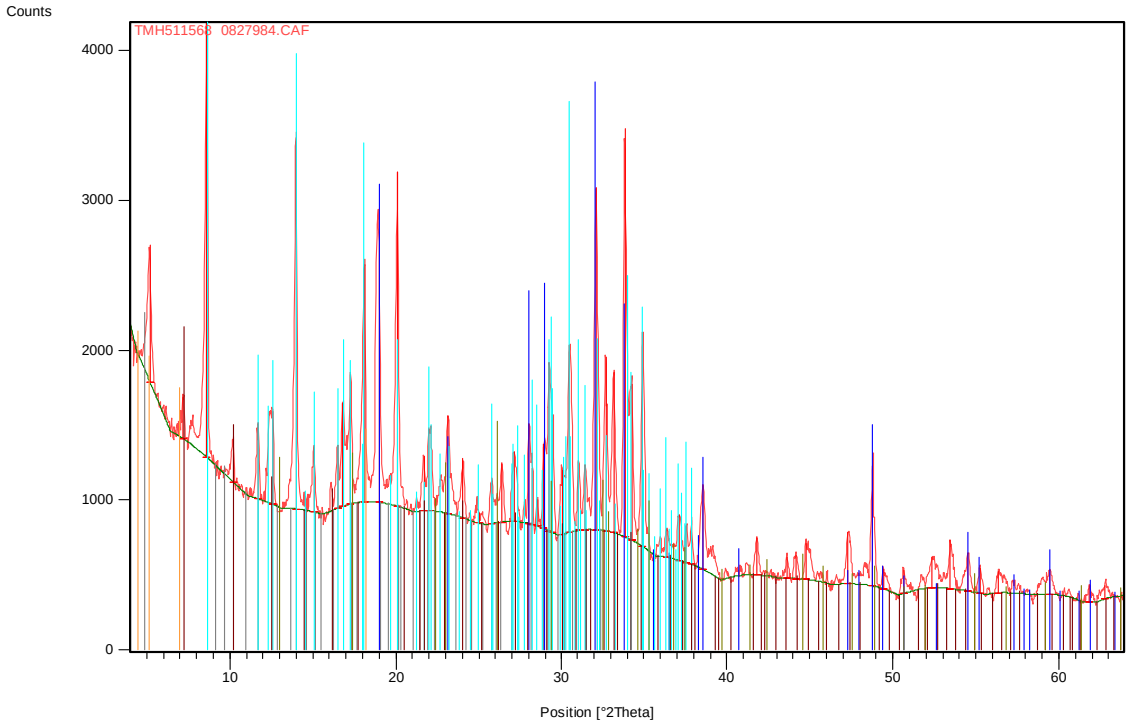

Figure 43. Powder XRD spectrum of the white powder from Figure 40.

Table 1. Identified Crystalline Phases in the XRD spectrum in Figure 43.

Ref. Code	Chemical Formula	Compound Name	Displacement [°2Th.]	Scale Factor	RIR	SemiQuant [%]
00-037-1465	Na2 S O4	Thenardite, syn	-0.034	0.673	0.000	-
00-010-0186	Na5 P3 O10 ▪ 6 H2 O	Sodium Phosphate Hydrate	-0.027	0.720	0.000	-
01-077-0379	Na Si Al O4	Sodium Aluminum Silicate	0.043	0.171	4.980	-
00-004-0013	C18 H29 Na O3 S	Dodecylbenzene sodium sulfonate	0.397	0.367	0.000	-
00-003-0394	Na P O3	Sodium Phosphate	0.009	0.151	0.000	-

Conclusions

It can be daunting to attempt the identification of a completely unknown substance, particularly when a very limited sample size is available. However, examination by stereomicroscopy is often sufficient to classify a substance, and at a minimum enables an analyst to characterize it in a non-destructive manner. Subsequent analysis typically begins with PLM, and microscopical results then direct additional analyses by FTIR micro-spectroscopy or SEM-EDS. In some instances Raman micro-spectroscopy or XRD analysis are required as well. The best results are obtained when all of the different techniques are applied to the same particle or fragments of a single particle that has been divided into subsamples. This ensures that all of the data collected on different instruments corresponds to the same material, as opposed to different components in a mixture.

Once a substance has been identified, a reference standard of the material should be obtained and analyzed using all of the techniques employed for comparative purposes. This process enables absolute confidence to be attached to the identification. While this type of work can pose a considerable challenge, it is one of the most rewarding types of problems faced by trace evidence examiners.

References

1) Guthrie, G.D., Jr. and Brooke, M.T., Eds. (1993) Reviews in Mineralogy Volume 28: Health Effects of Mineral Dusts. Mineralogical Society of America.
2) Winchell, A.N., and Winchell, H. (1989) The Microscopical Characters of Artificial Inorganic Solid Substances: *Optical Properties of Artificial Minerals*. 3rd Ed. McCrone Research Institute, Chicago, IL.
3) Winchell, A.N. (1987) The Optical Properties of Organic Compounds. 2nd Ed. McCrone Research Institute, Chicago, IL.
4) Chamot, EM, and Mason, CW. Handbook of Chemical Microscopy. Volume II: Chemical Methods and Inorganic Qualitative Analysis. 2nd Ed. McCrone Research Institute, Chicago, IL, 1989.