Brooke A. Weinger,[1,2] *M.S.; John A. Reffner,*[2] *Ph.D.; and Peter R. De Forest,*[1] *D. Crim*

A Novel Approach to the Examination of Soil Evidence: Mineral Identification Using Infrared Microprobe Analysis*

ABSTRACT: Identification of minerals using the infrared microprobe with a diamond internal reflection objective is a rapid and reliable method for forensic soil examinations. Ninety-six mineral varieties were analyzed, and 77 were differentiated by their attenuated total reflection (ATR) spectra. Mineral grains may be mounted in oil for conventional polarized light microscope characterization and their ATR spectrum obtained with little or no interference by the liquid. This infrared microprobe method can be used to identify silicates, phosphates, nitrates, carbonates, and other covalent minerals; however, ionic minerals, metal oxide and sulfide minerals, and minerals with refractive indexes greater than diamond do not produce identifiable spectra, but the lack of a spectrum or one with high absorbance values does provide useful information. This research demonstrates the overall utility that infrared microprobe analysis brings mineral identification in soil evidence.

KEYWORDS: forensic science, forensic geology, minerals, mineral identification, soils, soil analysis, infrared spectroscopy, infrared microprobe, light microscopy

The evidentiary value of soil and the minerals contained therein is well established. An early instance of soil examination being effectively used in a criminal investigation was in the late 19th century in Sir Arthur Conan Doyle's Sherlock Holmes stories (1). Although fictional, this provided many criminalists with ideas about the use and potential of this type of evidence. In 1893, Hans Gross, an Austrian magistrate, wrote in his Handbook for Examining Magistrates that "Dirt on shoes can often tell us more about where the wearer of those shoes had last been than toilsome inquiries." Although Gross did not reference any actual cases, this foretold what was to come in the field of forensic investigations (1).

The minerals in soil can provide a plethora of information in a civil or criminal investigation, including but not limited to associating a suspect with a location and provenance determinations (1–7). Despite the significant contribution geological evidence can have in the solving of a case, there is an underutilization of soil evidence in crime labs. This is especially the case for mineralogical identification using the polarized light microscope (PLM), which is the traditional tool for mineral analysis. Unfortunately, many criminalists are not adequately trained to do this type of work. As a result, there has been much research carried out to find complementary or alternative methods of mineral identification, with specific emphasis on instrumentation that can increase the accuracy and decrease the time associated with this analysis (3).

Forensic soil examination is believed to be very complicated because of the complexity of soil, but this diversity and complexity enables us to characterize, classify, and compare soil samples with high discriminating power. Prior studies reported on the ability of close proximity soils to be differentiated, and using a variety of techniques it was shown that soils can be differentiated at distances of 10 m and as close as 1 m (8,9).

Mineral identification is one of the most important aspects of soil discrimination because minerals are the major component of most soil varieties. Minerals are defined as "naturally occurring inorganic substance having relatively constant chemical composition and fairly definite physical properties" (10). Most soil samples contain between 3 to 5 mineral varieties. Although thousands of minerals exist in nature, only about 20 are common in soil specimens (1,11). However, it is not enough just to know the most prevalent minerals found in soil; it is an unusual mineral variety that often has the potential to be the most useful in a case.

There are numerous optical properties and morphological features that are used in the identification of minerals. These include: color, pleochroism, birefringence, relief (degree of shadowing), refractive index, extinction angle, sign of elongation, interference figures, shape, twinning, conchoidal fractures, cleavage, interfacial angles, crystal system, crystal habit, crystal structure, and chemical composition (1,11). Both stereomicroscopy and polarized lightmicroscopy were used for the determination of many of these optical and morphological properties of minerals; however, modern instrumentation has been used to supplement these traditional methods. These include X-ray diffraction (XRD) (3,12,13), scanning electron microscopy equipped with an energy dispersive X-ray spectrometer (3,12), transmission electron microscopy (3,12), X-ray fluorescence (3), Raman spectroscopy (14,15), cathodoluminescence (3,16,17), infrared spectroscopy (12,18), and infrared microspectroscopy (19). XRD is considered the gold standard for mineral identification but the problem with its widespread forensic use is the absence of an X-ray diffractometer in most forensic laboratories (3).

Infrared microprobe analysis unites light microscopy with infrared spectroscopy to create a powerful system capable of correlating microstructure with chemistry. The application of infrared

[1]Department of Science, John Jay College of Criminal Justice/CUNY, 445 West 59th Street, New York, NY 10019.
[2]Smiths Detection, 21 Commerce Drive, Danbury, CT 06810.

microprobe analysis for fiber and paint evidence analysis is well established and accepted in forensic science laboratories. With new advances, infrared microprobe analysis can be extended to a wide range of physical evidence from the identification of minerals and illicit drugs to the possible differentiation of glass evidence. Infrared microprobe analysis of minerals is made possible through the use of the diamond attenuated total reflection (D-ATR) microscope objective. The D-ATR microscope objective allows for the selective isolation of individual minerals for simultaneous collection of microscopical and infrared data, thus enabling the indisputable identification of minerals. Infrared microprobe analysis requires virtually no sample preparation and enables direct infrared spectroscopic analysis of unknown mineral grains. When coupled with a preliminary examination using traditional methods of polarized light microscopy, complete analysis of most unknown minerals can be performed quickly and easily. The purpose of this research was to complete an extensive evaluation of the use of the infrared microprobe for the identification of minerals in forensic soil samples.

Materials and Methods

Instrumentation

IlluminatIR[TM] II with D-ATR infrared microscope objective (Smiths Detection, Danbury, CT); Olympus BX-51 PLM (Olympus America Inc., Center Valley, PA); PixeLINK PL-A662 megapixel firewire camera (PixeLINK, Ottawa, ON, Canada); low-e MirrIR[TM] microscope slides (Kevley Technologies, Chesterland, OH).

Mineral Samples

Ninety-six different mineral varieties were analyzed in this study. The minerals standards were obtained from two Cargille No M-4 Comminuted Minerals Reference Sets (R.P. Cargille Laboratories, Inc., Cedar Grove, NJ), a set of minerals obtained from John P. Wehrenberg used in his Manual for Forensic Mineralogy (20), clay minerals used by the American Petroleum Institute and Columbia University to compile their "Analytical Data on Reference Clay Materials" (21), and minerals from Ward's Natural Science (Ward's Natural Science Establishment, LLC, Rochester, NY).

Seven unknown clay mineral fractions for the single blind study were provided by Dr. Christopher S. Palenik (Microtrace, LLC, Elgin, IL). Six of the samples were obtained from clay standards or mixtures of the standards that had been prepared for analysis by XRD. The samples were prepared from suspensions in water that were collected on a filter and air-dried. The samples on the filter paper were then mounted on glass slides. Sample 7 was collected from a soil sample, and was prepared in a similar way to the clay standards. However, the clay fraction was first separated from the rest of the soil by placing the soil sample in water and sonicating it. The suspension was centrifuged to pull all large particles to the bottom of the tube. The clay fraction of sample 7 which was collected by filtration represented any material that remained in suspension. The mineral composition of sample 7 was identified by XRD.

Test Procedure

Each sample was transferred to an infrared-reflective low-E glass microscope slide and observed using the PLM. A digital image of the sample was captured using either a glass microscope objective or the D-ATR objective. Infrared microprobe analysis with the D-ATR was performed on 10–20 mineral grains of the sample.
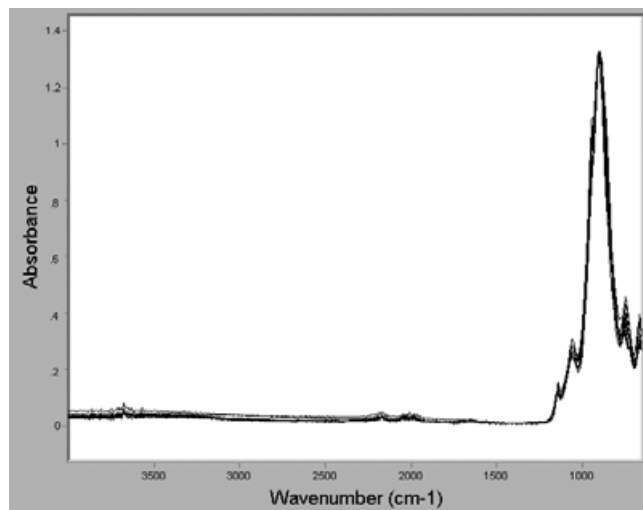

FIG. 1—*The repeatability of diamond attenuated total reflection analysis is shown by the 10 overlaid infrared spectra of 10 different grains from the same source of hornblende. The spectra were produced using Grams® AI software.*

When the mineral was brittle and shattered and scattered with pressure from the D-ATR objective thereby preventing infrared analysis, a drop of immersion oil ($n_D = 1.516$ @ 23°C) (Olympus Corporation, Tokyo, Japan) was added to the sample. Infrared microprobe analysis was performed on 10–20 mineral grains of the sample mounted in immersion oil in order to demonstrate repeatability of the method on a specific mineral and from a particular source, as shown with hornblende in Fig. 1.

Infrared Microprobe Analysis Method

Each infrared background and sample spectrum of a mineral grain was collected using an IlluminatIR I 1/2, a spectral resolution of 4 cm^{-1}, 64 co-added scans, and a 42-μm infrared beam diameter.

Spectral Processing

An unprocessed average spectrum was obtained for all samples using Grams® AI software (Thermo Galactic, Salem, NH) for an initial assessment of the spectra. Next, all individual spectra had the atmospheric carbon dioxide interference peak (2400–2300 cm^{-1}) deleted from the ATR spectrum. When the largest water vapor peak (in the range of 4000–3500 cm^{-1}) in the average spectrum had an absorbance value greater than 0.01, the water vapor spectrum was subtracted from each individual spectrum to reduce its interference. For those samples that were mounted in oil and from which the oil was still present in the spectra, the ATR spectrum of the oil was subtracted in order to reduce its contribution to the overall ATR spectrum. Next, all spectra were normalized to an area of 1 using OriginLab® software (OriginLab Corporation, Northampton, MA). The processed spectra from all the same mineral were averaged to obtain the mineral's average spectra. The process of normalization produced spectra with absorbance values that were effectively meaningless for comparison of absorbance strength between samples. The last step was to multiply the spectra by a factor to regenerate the absorbance values of the minerals originally measured. This factor was the average of the area under each individual spectrum's curve prior to normalization

(it was this factor that each spectrum was divided by to normalize the total area to one).

Mineral Library Database Analysis

After the mineral ATR library was compiled from the learning set, its validity was tested with a second source of the same mineral varieties that were used to make the library. The robustness was established by using a different instrument, an IlluminatIR II, to obtain three ATR spectra of each mineral variety. All minerals were analyzed using a spectral resolution of 4 cm^{-1}, 64 co-added scans, and a 42-μm infrared beam diameter. Each individual unprocessed ATR spectrum was then compared against the ATR spectra in the mineral library database.

Single Blind Study

A small sample of each of the samples was scraped off of the filter paper with a microspatula and mounted in immersion oil on an infrared-reflective low-E glass microscope slide. The PLM was used to screen the samples to determine the number of different minerals present in each sample. After observation with the PLM, D-ATR spectral analysis was carried out with both 42 and 21 μm infrared beam diameters. The number of spectra collected for each unknown varied based on the visual observations.

Results and Discussion

When using the D-ATR objective, the contact between the diamond and the sample must be significant in order to obtain quality ATR spectra. Because of the irregularity in the surface and the hardness of many minerals, the best contact comes after the sample is shattered by the increased pressure. However, in a dry mount preparation of a hard and brittle mineral, such as that of quartz in Fig. 2, the mineral grain shatters and scatters which prevents sufficient contact area and subsequent infrared analysis.

The solution to this problem is to mount the grain in an immersion oil. The mounting of a sample in oil has three major advantages: the highest quality image is obtained because of the reduction in visible light scattering; optical properties, such as refractive index, can be measured; and the viscosity of the oil prevents the scattering of the shattered fragments thus permitting infrared analysis. Microscopists and spectroscopists are concerned with the effect of the oil on the resulting spectra as these oils are organic liquids with some infrared absorption. However, as contact pressure is increased, the mineral's peaks increase and the peaks due to the oil decrease, as seen in Fig. 3. This is also true for softer solids, such as clays if the oil immersion technique is used. The high pressure contact between the diamond and the sample squeezes the oil from between the two, thus the resulting infrared spectrum is of the mineral without a significant contribution from the oil.

The forensic analysis of soil seeks to identify the minerals present, and as such the hardness of a questioned mineral is unknown at the onset of the infrared investigation. To prevent the loss of the sample by scattering during breakage, the authors recommend that all unknown mineral analysis by D-ATR analysis be performed on an oil-mounted sample. The broken fragments can be preserved for further analysis, but a potential problem of D-ATR infrared analysis is the necessary shattering of the mineral grains which prohibit subsequent microscopical examinations of the shattered grains.

Analysis with the D-ATR objective proved to be successful at identifying the majority of mineral varieties studied. Eighty-five of the ninety-six different mineral spectra were compiled into a mineral library database. Eleven minerals were excluded based on limitations explained directly below. The mineral library database was tested with different sources of the same mineral varieties and showed the infrared microprobe to be a promising tool for mineral identification in soil analysis.

There are three categories of minerals that cannot be specifically identified with infrared microprobe analysis, and include (i) halides, (ii) metal oxides and sulfides, and (iii) minerals with refractive indexes greater than or equal to that of the diamond. The first category, halides, includes the minerals halite (NaCl), fluorite (CaF$_2$), and cryolite (Na$_3$AlF$_6$), which were optically transparent in the infrared spectral region. The spectra of these minerals appear to be composed of atmospheric water vapor only. The second group includes most minerals composed of metal oxides and sulfides, and includes magnetite (FeFe$_2$O$_4$), hematite (Fe$_2$O$_3$), pyrite (FeS$_2$), ilmenite (Fe^{2+}TiO$_3$), rutile (TiO$_2$), and cassiterite (SnO$_2$). These produce distorted spectra with extraordinarily high absorbance values (greater than 3) or spectra which cannot be used for a positive identification because of a lack of constant results. Two metal oxides that do have unique and reproducible spectra are corundum (Al$_2$O$_3$) and zincite (ZnO). Lastly, for total internal reflection of the infrared beam to occur inside the diamond ATR objective, the refractive index of the mineral must be less than that of diamond ($n = 2.4$). Therefore, minerals such as goethite [Fe^{3+}O(OH)] and sphalerite [(Zn,Fe)S] (with refractive indices of 2.26–2.515 and 2.37–2.43, respectively) did not produce reproducible infrared spectra. Even though a definitive identification cannot be made for
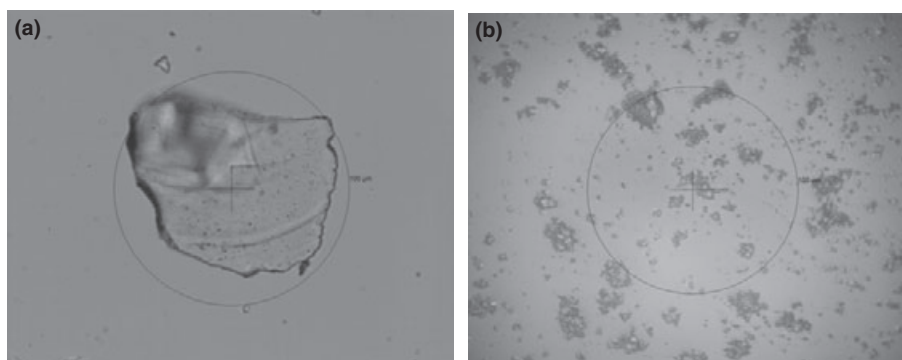

FIG. 2—(a) Photomicrograph of a dry-mounted quartz grain at 40 times magnification, prior to analysis, and (b) a photomicrograph of the same dry-mounted quartz grain at 36 times magnification after being shattered and scattered by the diamond attenuated total reflection objective. The length of the circle measures 100 μm.
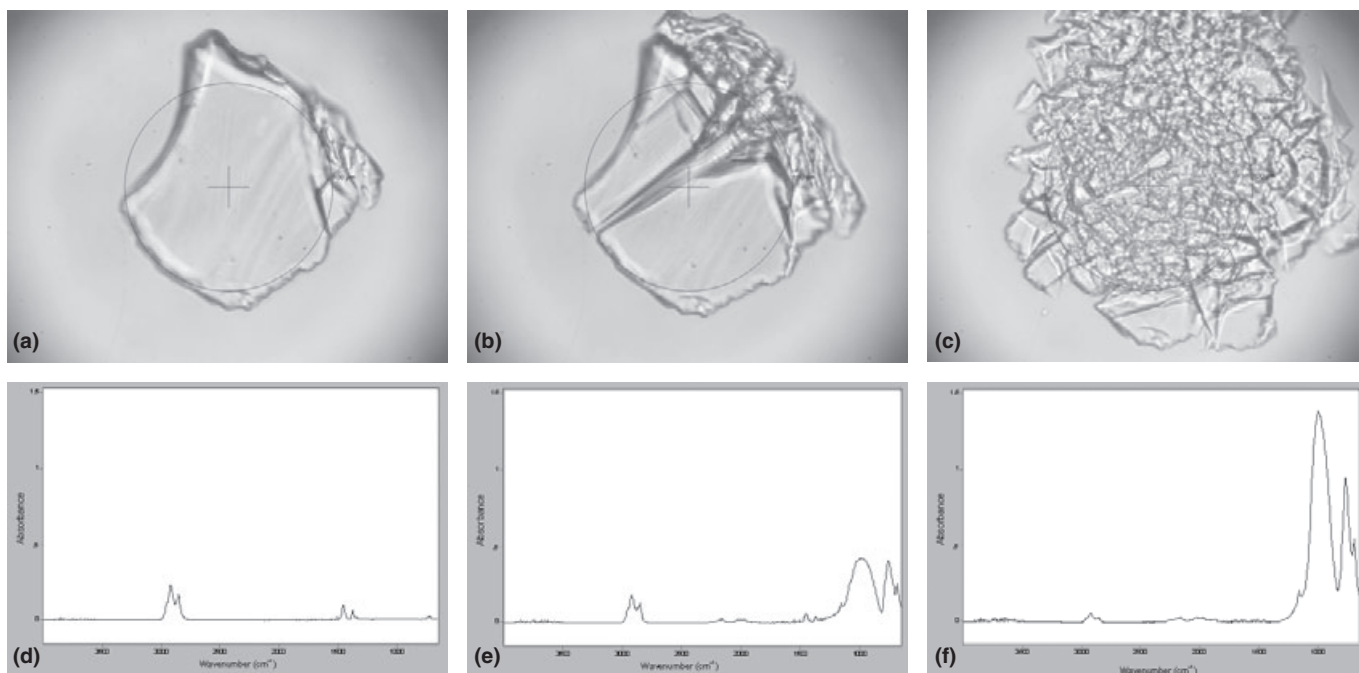
FIG. 3—*Photomicrographs of a quartz grain in mineral oil (n_D = 1.516 @ 23°C) at 36 times magnification using the diamond attenuated total reflection objective, varied by increasing the contact pressure: (a) after initial contact, (b) after significant contact, and (c) after being crushed but not scattered. The diameter of the circle measures 100 μm. Below each photomicrograph are their resulting infrared spectra (d–f). The spectra were produced using Grams® AI software.*

these minerals based on the generated spectra, the lack of a spectrum or one with exceedingly large absorbance values can provide valuable investigative information on the identity of the mineral in question.

There are a myriad of examples of the success of the D-ATR analysis of minerals. Aragonite and calcite, two polymorphs (minerals with identical chemical formulas that exist in two or more structures) of calcium carbonate ($CaCO_3$), were readily differentiated by their infrared spectra (Fig. 4). Although chemically identical to each other, these have different crystal lattices. The effects of these differences were easily observed within their infrared spectra. Two silicon

dioxide minerals, quartz (SiO_2) and opal ($SiO_2 \cdot nH_2O$), were also unmistakably distinguished by their infrared spectra (Fig. 4). D-ATR analysis was capable of making the distinction between albite ($NaAlSi_3O_8$) and labradorite [$(Ca,Na)(Si,Al)_4O_8$], two common plagioclase feldspars (Fig. 5). Garnet is a group of minerals that includes the minerals pyralspite (almandine) [$Fe^{2+}_3Al_2(SiO_4)_3$] and ugrandite (andradite) [$Ca_3Fe^{2+}_2(SiO_4)_3$]. These two varieties of garnet were studied, and they were plainly distinguished by their infrared spectra (Fig. 5). The clay minerals such as kaolinite [$Al_2Si_2O_5(OH)_4$] and montmorillonite [$(Na,Ca)_{0.3}(Al,Mg)_2Si_4O_{10}(OH)_2 \cdot nH_2O$] (Fig. 6) were also easily differentiated by their infrared spectra.
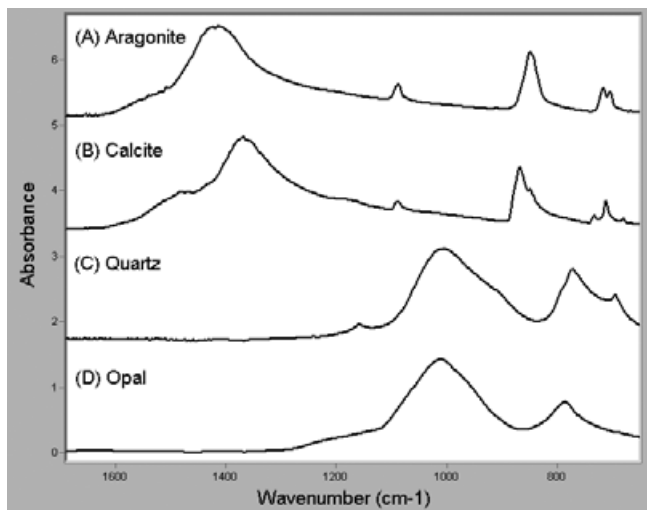

FIG. 4—*Infrared spectra of (a) aragonite, (b) calcite, (c) quartz, and (d) opal collected using the diamond attenuated total reflection infrared microprobe. The spectra were produced using Grams® AI software.*
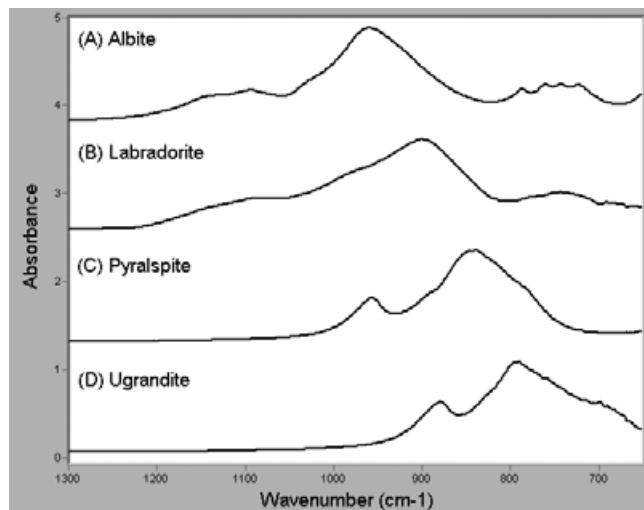

FIG. 5—*Infrared spectra of (a) albite, (b) labradorite, (c) pyralspite, and (d) ugrandite collected using the diamond attenuated total reflection infrared microprobe. The spectra were produced using Grams® AI software.*
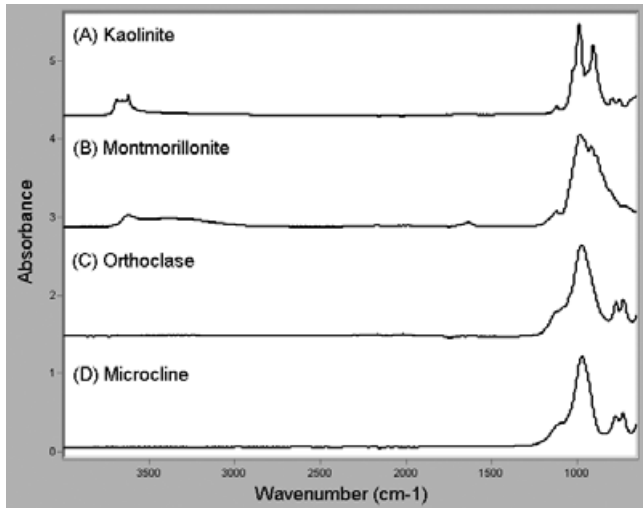
FIG. 6—*Infrared spectra of (a) kaolinite, (b) montmorillonite, (c) ortho-clase, and (d) microcline collected using the diamond attenuated total reflection infrared microprobe. The spectra were produced using Grams® AI software.*

This work on minerals showed some mineral varieties that had overlapping or indistinguishable spectra. This emphasizes the fact that the infrared spectroscopy of minerals is not an infallible stand alone method for mineral identification. However, it is very powerful as a complementary technique to polarized light microscopy and is much simpler and less costlier than XRD. The IlluminatIR infrared microprobe enabled the PLM observations to be paired with infrared analysis, thus making microscopy a much more powerful tool for mineral analysis.

Orthoclase and microcline, which are both composed of $KAlSi_3O_8$, proved to have indistinguishable infrared spectra. Both of these minerals have the same hardness and density and have very similar optical properties (refractive index, birefringence, etc…). One difference was their crystal structure: microcline is triclinic and orthoclase is monoclinic. Despite the difference in structure, their spectra (Fig. 6) were too similar to be discriminated. Their differentiation would be a trivial problem for XRD.

Chert is a fine grain, microcrystalline quartz (SiO_2). Chert and quartz are not distinguishable by XRD, and as expected, were not differentiated by their infrared spectra. However, visually they are readily differentiated from each other, thus supporting the need to use the PLM to observe the optical properties of the sample prior to infrared analysis.

Other minerals with indistinguishable spectra included epidote [$Ca_2(Fe^{3+},Al)_3(SiO_4)_3(OH)$] and serpentine [$Mg_6Si_4O_{10}(OH)_8$] as well as the intermediate plagioclase feldspars andesine (Al_2SiO_5) and labradorite [$(Ca,Na)(Si,Al)_4O_8$].

Within a given mineral variety, there proved to be some spectral variations. These did not prevent their identifications with the mineral library database, but is a factor to consider when doing infrared microprobe analysis. There are two reasonable explanations for spectral variations within a given mineral variety. The first is that natural minerals only have a "relatively constant chemical composition" (10). If there are differences in chemical composition, albeit small ones, then this will affect peak heights and can shift peak locations. Second, because the analysis is completed on single mineral grains, orientation effects may cause spectral differences (19). However, because the mineral grains are crushed prior to D-ATR analysis, the orientation effects are potentially minimized due to the

generation of random orientations. A study of orientation effects on the infrared spectra of mineral grains collected by D-ATR could be accomplished by polarizing the incident infrared radiation, but this was outside the scope of this study.

One potential limitation of D-ATR infrared analysis is that it is a surface technique in which the evanescent wave from the infrared beam is only capable of penetrating the sample to a depth of 0.5 to 4 μm. Thus, it is possible that the coating or weathering of soil mineral grains could be problematic. However, by crushing the mineral grains, the interior of each sample comes into contact with the D-ATR objective thus enabling the simultaneous analysis of the inside and outside of each mineral grain. A potential area for further study is the effects of coatings or weathering of mineral grains on their D-ATR infrared spectra.

The mineral database library was tested with a single blind study performed on six clay standards or mixtures of standards, and one true soil sample. This study was intended for mineral identification and not a soil identification which would also include a quantitative grain count. The first sample was correctly identified as illite. Sample 2 was determined to be saponite dispersed in a matrix of micro-cline or orthoclase and tremolite–actinolite. XRD did not identify the nonclay phases and correctly confirmed the major component to be saponite. The samples were transported together in a slide box, and some of sample 2 had visibly crumbled off, thus it is thought that there was contamination of saponite in some of the subsequent samples. Sample 3 was identified as kaolinite and illite dispersed in a matrix of quartz or chert, saponite, microcline or orthoclase, steatite, one mineral with a refractive index greater than or equal to that of diamond, and two minerals not in the library. The two major components of kaolinite and illite were confirmed by XRD but the nonclay phases were not characterized. Sample 4 was identified as illite and chlorite dispersed in a matrix of saponite, epidote or serpentine, opal, dolomite, and two unidentified minerals. Unknown #4 was confirmed by XRD to be chlorite and illite with minor dolomite (est. <5%). Sample 5 was identified as kaolinite, chlorite, and hectorite dispersed in a matrix of epidote or serpentine, dolomite, and one unidentified mineral. XRD confirmed this to be kaolonite and chlorite with plagioclase feldspar and possibly dolomite. Sample 6 was identified as kaolinite, illite, and chlorite dispersed in a matrix of epidote or serpentine and one unidentified mineral. XRD confirmed sample 6 to be kaolonite, illite, and chlorite with plagioclase feldspar and minor quartz. Sample 7, the true soil sample, was the most difficult to analyze, with many of the samples being unidentifiable or contaminated with filter paper. To avoid the contamination with paper, the use of a gold or metal filter would be advantageous for this type of analysis. Plasma ash could also be used to remove the paper (by burning the cellulose in a vacuum oven with low pressure oxygen plasma) without affecting the mineral grains. Alternative methods for isolating the mineral fractions from soil samples for infrared microprobe analysis should be investigated. Sample 7 was identified as illite dispersed in a matrix of calcite, quartz, zoisite, and 14 unidentified samples. XRD confirmed sample 7 to be chlorite, illite, kaolinite, calcite, with the minor presence of quartz and muscovite. This single blind study demonstrated the ability of D-ATR infrared analysis to identify both the major and minor minerals contained in a mixture.

Conclusion

This study shows that infrared microprobe analysis is rapid and reliable, thus making it an ideal additional tool for use in forensic soil examinations. The brittleness of a mineral and its scattering upon crushing cannot be anticipated so the optimal method of

preparation of an unknown mineral for D-ATR analysis is by mounting the sample in an immersion oil. The advantage of D-ATR analysis is that the sample preparation is minimal and requires no additional preparation beyond that for traditional polarized light microscopy. The time required for infrared microprobe analysis, once the sample is prepared, is roughly a minute for each mineral grain. The work presented here offers the soil examiner an alternate method to confirm the light microscopical identification of a mineral sample by using infrared microprobe analysis.

The disadvantage encountered was the inability to easily identify certain mineral types such as halide minerals, metal oxide and sulfide minerals, and minerals with refractive indices greater than that of the diamond. In addition, there were several minerals with infrared spectra that were indistinguishable. Although a definite identification of a mineral by its infrared spectrum alone is not always achievable, the data can be used to narrow down the mineral possibilities. When paired with polarized light microscopy (to observe optical and morphological properties), a definitive identification is almost always possible.

Infrared microprobe analysis offers an efficient means to aid in the identification of minerals and is a complementary technique to polarized light microscopy. The IlluminatIR enables a sample of interest to be analyzed with polarized light microscopy and sequentially analyzed with infrared spectroscopy. It has been shown that infrared microprobe analysis enhances and confirms the identity of mineral samples, and is a useful tool in the characterization of soil evidence. This ability to integrate polarized light microscopy with infrared microprobe analysis for the identification of minerals is unprecedented.

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Additional information and reprint requests:
Brooke A. Weinger, M.A., M.S.
Department of Forensic Science
John Jay College of Criminal Justice/CUNY
445 West 59th Street
New York, NY 10019
E-mail: brooke.weinger@jjay.cuny.edu