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The mission of ASTEE is to encourage the exchange and dissemination of ideas and information within the field of trace evidence through improved contacts between persons and laboratories engaged in trace evidence analysis. The journal of the American Society of Trace Evidence Examiners is a peer reviewed journal dedicated to the analysis of trace evidence. All original articles published in JASTEE have been subject to double-blind peer review.

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JASTEE has also established a working arrangement with *The Microscope*, the journal established and edited by the McCrone Institute. Under this arrangement, articles published in *JASTEE* may be selected for publication in *The Microscope*, and vice versa.

INSIDE THIS ISSUE

Scott Ryland, B.S.[1]

Discrimination of Flat (Sheet) Glass Specimens Having Similar Refractive Indices Using Micro X-Ray Fluorescence Spectrometry

ABSTRACT: It has been reported that discrimination between sources of broken flat (sheet) glass can be substantially improved when trace element profiles are compared in addition to refractive index alone. This paper reports on the discrimination power afforded by micro X-ray fluorescence spectrometry on two sets of randomly encountered sources of broken flat glass having nominal refractive indices of 1.5184 to1.5185 (37 samples) and 1.5180 to 1.5182 (30 samples) at 20.0 degrees C. The results indicate discrimination powers on the order of 98.0 to 99.5 percent for both trial sets using either a conservative +/- 3 standard deviations criterion or a T-test criterion at 95 percent confidence level.

KEYWORDS: Forensic, glass comparisons, discrimination study, X-ray fluorescence spectrometry

Small fragments of broken flat window glass are occasionally encountered in forensic investigations involving homicide, home invasion, motor vehicle hit and run, and property crime. Typically, it is requested that fragments recovered at the scene or on the clothing of a suspect (questioned sample) be compared to the glass from a broken window of known origin (known sample) in an effort to determine if they are alike. One of the most common analytical techniques used for discrimination between samples is the precise determination of their refractive indices (1, 2). If the samples are found to be different it is concluded that the two could not have had a common origin. If they are found to be indistinguishable it is concluded that the questioned glass could have originated from the known broken window. In the author's experience, this characteristic alone is quite discriminating and often differentiates from ninety to ninety-eight percent of randomly selected samples of flat glass. However, it has been reported that discrimination between sources of broken flat (sheet) glass can be substantially improved when trace elements are compared in addition to refractive index alone (1, 2, 3, 4). One method for comparison of trace elements in small fragments of

[1] Florida Department of Law Enforcement, Orlando Regional Operations Center, 500 W. Robinson St., Orlando, FL, USA, 32801.

Table 1: Sample Set 1 (nD=1.5184-1.5185)

Sample Number	Year Entered	nD at 20° C.	Float	Tempered	End Use
W23	1980	1.5184	Yes	Yes	vehicle side window
W33	1980	1.5185	Yes	Yes	vehicle side window
W49	1982	1.5185	Yes	No	store window
W62	1984	1.5184	Yes	No	store window
W79	1986	1.5185	Yes	No	store plate glass
W83	1987	1.5184	Yes	Yes	vehicle side window
W95	1989	1.5185	Yes	Yes	vehicle side window
W103	1987	1.5184	Yes	Yes	vehicle side window
W107	1990	1.5185	Yes	Yes	vehicle side window
W129	1995	1.5185	No	Yes	sliding glass door
W132	1993	1.5185	Yes	No	display case
W142	1995	1.5185	Yes	No	laminated store window
W143	1995	1.5185	Yes	No	laminated store window
W152	1995	1.5185	Yes	No	bathroom window (outer)
W153	1995	1.5185	No	No	bathroom window (inner)
W165	1996	1.5184	Yes	Yes	store window
W174	1996	1.5184	Yes	Yes	vehicle side window
W193	1996	1.5185	Yes	Yes	vehicle rear window
W204	1997	1.5185	Yes	No	store window
W206	1997	1.5185	Yes	No	store window
W232	1997	1.5185	Yes	No	business window
W248	1998	1.5184	Yes	Yes	residence window
W255	1999	1.5185	Yes	Yes	business window
W259	1999	1.5184	Yes	Yes	business window
W266	2000	1.5185	Yes	No	business window
W285	2000	1.5185	Yes	Yes	business window
W319	2002	1.5185	Yes	No	laminated store window
W320	2002	1.5185	Yes	No	laminated store window
W323	2002	1.5185	Yes	Yes	vehicle side window
W340	2002	1.5185	Yes	Yes	vehicle side window
W350	2003	1.5184	Yes	Yes	vehicle side window
W351	2003	1.5184	Yes	Yes	vehicle rear window
W352	2002	1.5185	No	No	business window
W360	2002	1.5184	Yes	No	laminated store window
W368	2003	1.5184	Yes	No	residence window
W383	2004	1.5184	Yes	No	residence window
W399	2004	1.5184	No	Yes	business door

broken glass is micro X-ray fluorescence spectrometry (μ-XRF). It is not only sensitive to the major elements present in soda-lime-silicate glass but also to the minor and trace elements present, with detection limits on the order of tens of parts-per-million (ppm) for elements such as Ti, Mn, As, Rb, Sr, and Zr (5). Furthermore, its totally non-destructive nature permits it to be used at any point during the analytical scheme. Improved discrimination using μ-XRF has been previously reported in the literature (6, 7, 8), but often not with larger sets of samples known to have similar refractive indices.

This project evaluates the discrimination power of μ-XRF on two sets of randomly encountered sources of broken flat glass having nominal refractive indices of 1.5184 to 1.5185 (37 samples and designated Set 1) and 1.5180 to 1.5182 (30 samples and designated Set 2) at 20.0 degrees C. The samples were selected from a reference collection of sheet glass samples comprised of standards submitted in cases received from agencies throughout the state of Florida, USA, from 1979 to 2005 (Tables 1 and 2). Considering the sampling method, it is reasonable to assume that the samples in the reference collection are random and to some extent represent the population of broken flat glass in the state of Florida. Refractive index distributions in the glass collection of over five hundred samples indicate similar patterns between older architectural glass (received in casework from 1979 to 1997) and newer architectural glass (received in casework from 1998 to 2010). That is not the case for vehicle glass however. This observation is not at all surprising considering the difference in service life for architectural glass versus vehicle glass.

Individual panes of laminated architectural and vehicle windows were treated as separate samples since they are sometimes discriminated by either their refractive indices or elemental compositions.

Table 2: Sample Set 2 (nD=1.5180-1.5182)

Sample Number	Year Entered	nD at 20° C.	Float	Tempered	End Use
W2	1979	1.5181	No	No	unknown
W5	1979	1.5181	No	No	residence window
W37	1980	1.5181	Yes	No	unknown
W47	1982	1.5180	Yes	No	fire extinguisher case
W98	1989	1.5180	Yes	Yes	vehicle side window
W100	1989	1.5182	Yes	No	vehicle windshield (outer)
W106	1990	1.5181	Yes	Yes	store window
W144	1995	1.5181	Yes	Yes	vehicle side window (rear)
W145	1995	1.5181	Yes	Yes	vehicle side window (front)
W149	1995	1.5180	No	Yes	business window
W178	1996	1.5180	Yes	No	residence window
W189	1996	1.5182	Yes	Yes	business window
W210	1997	1.5180	Yes	Yes	business window
W213	1997	1.5181	Yes	Yes	vehicle side window
W219	1997	1.5180	No	Yes	store window
W222	1998	1.5182	Yes	No	store window
W224	1997	1.5180	Yes	Yes	vehicle side window
W234	1997	1.5180	Yes	Yes	business window
W235	1997	1.5180	Yes	No	business window
W241	1998	1.5182	Yes	Yes	business door
W245	1998	1.5181	Yes	Yes	vehicle sunroof
W252	1999	1.5182	Yes	No	residence window
W264	1998	1.5182	No	No	vehicle side view mirror
W272	2000	1.5180	Yes	Yes	vehicle side window
W282	2001	1.5182	Yes	Yes	vehicle side window
W293	2001	1.5181	Yes	No	business window
W295	2001	1.5181	Yes	Yes	business window
W346	2002	1.5182	Yes	Yes	vehicle side window
W393	2004	1.5181	Yes	No	business door
W394	2004	1.5181	Yes	No	business door

Samples for the two trial sets were chosen from high frequency refractive index populations without regard to age, heat strengthened type (tempered versus annealed), or end use application (architectural versus automotive). Obviously, once samples were selected based on their particular refractive index range the sample sets were no longer random. This is evidenced by the paucity of windshield glass in both sets. A majority of the samples are from architectural sources and there is a reasonably even distribution of tempered versus non-tempered glass in the trial sets. The refractive index values recorded for the reference collection are mean values reported to only four decimal places and corrected to 20.0 degrees C. Hence, they are nominal values and do not guarantee that some of the samples within each set could be discriminated by detailed side by side comparison using a sensitive method such as Foster + Freeman's GRIM[2].

METHODS OF ANALYSIS

The refractive indices of the first twenty-one of the thirty-seven samples of Set 1 were inter-compared using a Foster + Freeman GRIM in order to evaluate the discrimination power provided by pair-wise comparison of the nominally similar refractive indices using a sensitive method. This initial set was analyzed as part of a

[2] Foster + Freeman, Ltd., Vale Park, Evesham, Worcestershire, WR11 1TD, UK.

previous exercise designed to compare the discrimination power of micro X-ray fluorescence spectrometry, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), and laser induced breakdown spectrometry (LIBS) (7). Mean refractive indices, ranges, and standard deviations were determined for each of the twenty-one samples at one wavelength.

Following that, the full sets of thirty-seven samples and thirty samples having nominally similar refractive indices were analyzed by μ-XRF. They were inter-compared on a pair-wise basis using spectral overlay evaluation and comparison of selected element intensity ratios using a +/- 3 standard deviation criterion and a T-test criterion. Although the former intensity ratio criterion is what the author uses in routine casework, the latter was chosen to be more consistent with the criterion used in current publications evaluating LA-ICP-MS. Spectral overlay comparisons permitted detection of different elements and grossly differing element peak intensities (Figure 1). Peak intensity ratio comparisons permitted more precise comparisons of semi-quantitative characteristics and also served to minimize the effects of inherent take-off angle variations between replicate samples (Figures 2 and 3).

Figure 1: Samples W340 and W352 from Set 1 demonstrate clear differences in As and K content. The spectra are normalized to the most intense peak (Si Kα) and displayed with an expanded intensity scale to visualize trace components.

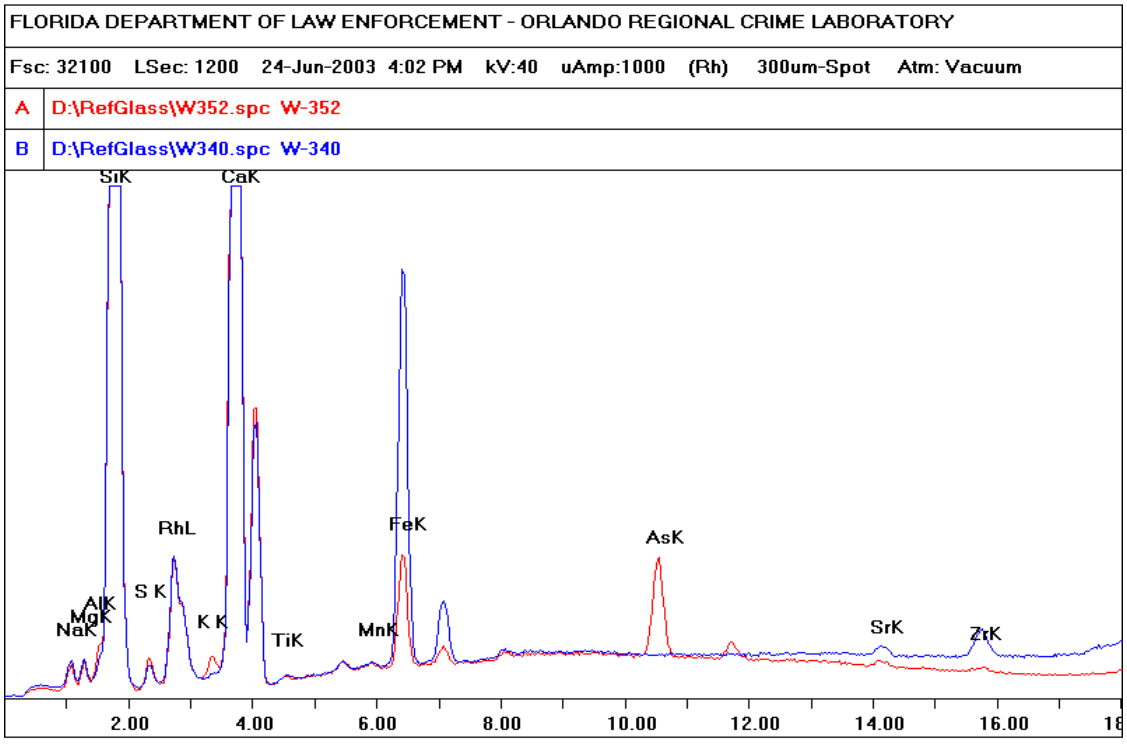


Figure 2: Samples W340 and W319 from Set 1 demonstrate semi-quantitative differences in their levels of Fe and Zr. The spectra are normalized to the most intense peak (Si Kα) with the lower spectra displayed at full scale intensity and the upper spectra displayed with an expanded scale to visualize trace components.

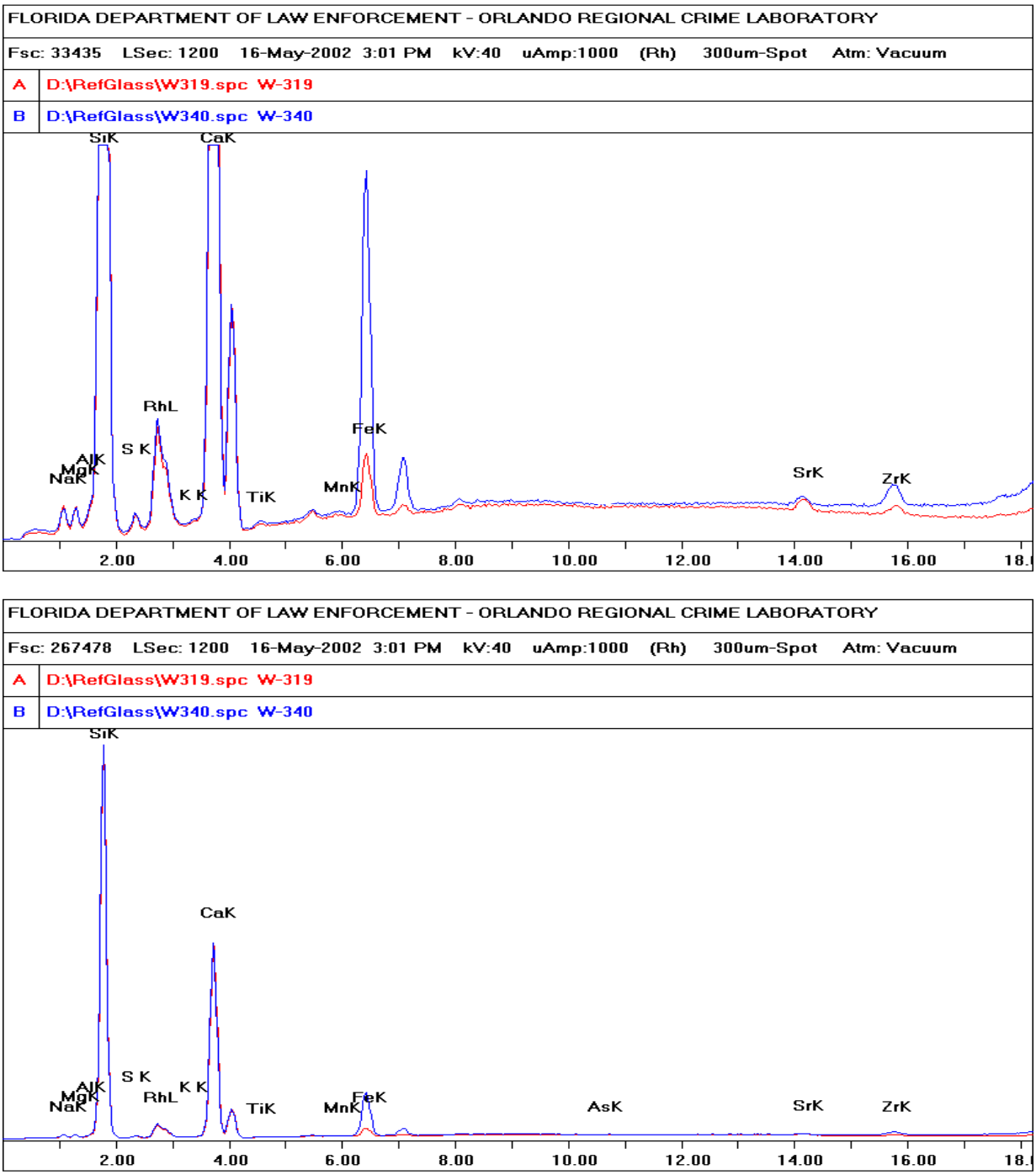
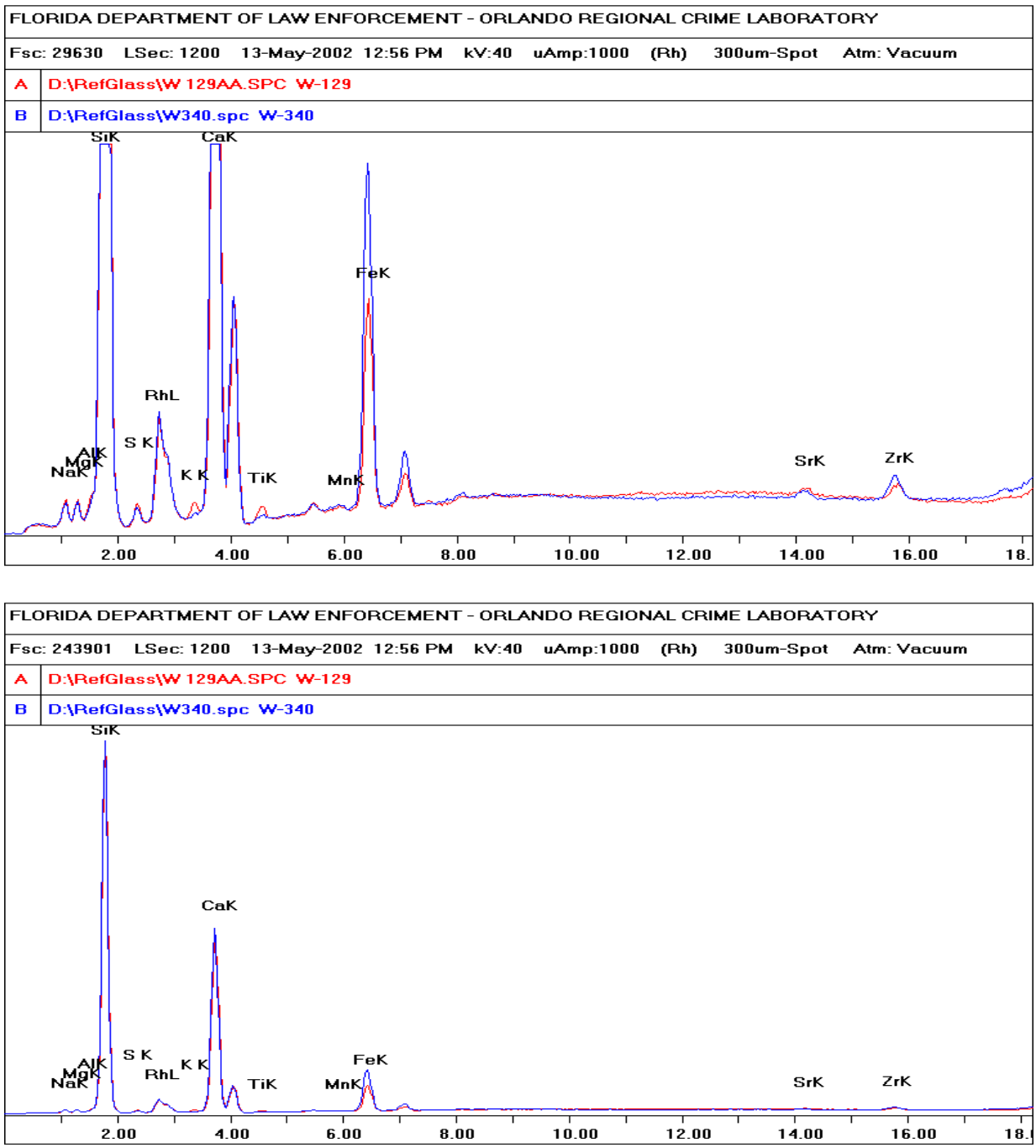


Figure 3: Samples W340 and W129 from Set 1 demonstrate semi-quantitative differences in their Ca/Fe, Ca/K and Fe/Ti peak intensity ratios. The slight apparent differences in the Sr/Zr ratios are indistinguishable using a +/- 3 standard deviation criterion for three replicates of each sample. The spectra are normalized to the most intense peak (Si Kα) with the lower spectra displayed at full scale intensity and the upper spectra displayed with an expanded scale to visualize trace components.



Glass Refractive Index Measurement System

Samples were cracked and five fragments were selected from each. Each of the five fragments was re-cracked and the refractive index was measured on three edges of each at 589 nanometers using a Foster + Freeman GRIM 2[2] system and the oil immersion technique. Locke B silicone oil[3] was used as the mounting medium and was calibrated using a series of Locke B glass reference standards[3]. The accuracy of the oil's refractive index calibration was subsequently checked using two external glass standards, NIST Standard Reference Material 1822[4] and the Locke B-5 glass standard[3]. The Locke B-5 standard was not used in creating the oil calibration curve. The mean of the fifteen measurements and their standard deviations were recorded for each. The means of each of the possible pairs were inter-compared using a variety of discrimination criteria, including +/- 2 .5 times the standard deviation, +/- 2.0 times the standard deviation, a fixed criterion of +/- 0.00010, range overlap, and the T-test with Welch modification (assuming unequal variance) using both 99 percent and 95 percent confidence levels. The latter two were calculated on Microsoft Excel[5] and are the most aggressive of the suite.

Micro X-ray Fluorescence Spectrometry

Newly cracked samples were washed in acetone and laid on the surface of a sheet of X-ray analysis film[6] stretched over an open aperture and held in place by a very small amount of contact adhesive[7]. Irregular shaped samples were positioned with the flattest bulk glass fractured surface (not original surfaces) facing upward using the contact adhesive as an aid in positioning. Three to five separate fragments were analyzed for each sample. In the first set of 37 samples three separate fragments were analyzed for most samples and five for some (those demonstrating elevated relative standard deviations for the Ca/Fe or Sr/Zr ratios) while in the second set of 30 samples five separate fragments were analyzed for each sample.

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[3] Locke Scientific Services Ltd., The Old Laundry Bridge Street, Southwick, Fareham, PO17 6DZ, UK.

[4] National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899, USA.

[5] Microsoft Corporation, One Microsoft Way, Redmond, WA 98052, USA.

[6] Kapton Polyimide X-ray film, 7.5 micron thickness, Chemplex Industries Inc., 2820 SW 42nd Ave. Palm City, FL 34990-5573, USA.

[7] Liquid Paper Dry Line transfer contact adhesive, Sanford, a Division of Newell Rubbermaid, 2707 Butterfield Road, Oak Brook, IL, 60523, USA.

Analyses were performed on an EDAX Eagle II micro X-ray fluorescence spectrometer[8] equipped with a Rhodium X-ray tube and 300 micron mono-capillary focusing optic under vacuum. The tube was run at a potential of 40kV with the current adjusted to provide a dead time of approximately 40 to 45 percent. The Beryllium windowed Si(Li) energy dispersive X-ray detector was operated at a 17 microsecond processing time constant collecting data for 1200 live seconds. One relatively flat area was targeted for analysis on each of the three to five replicates. Spectra were plotted at two attenuations for each analysis permitting visual comparison of both the high and low intensity peaks. The spectral data was also processed using EDAX software[8] for background subtraction, peak deconvolution, and peak intensity calculation. The peak intensity ratios and standard deviations for the replicates were recorded for Ca/Fe, Sr/Zr, Ca/Mg, Ca/K, Fe/Zr, Fe/Sr, and Fe/Ti and inter-compared in a pair-wise fashion using both a +/- 3 times the standard deviation criterion and a T-test with Welch modification (assumed unequal variance) at a 95 percent confidence level.

RESULTS AND DISCUSSION

A summary of the results of inter-comparisons of the refractive indices for the initial twenty-one samples of Sample Set 1 using a variety of discrimination criteria can be found in Table 3. While 93 percent of the 210 possible pairs are indistinguishable using the +/- 2.5 standard deviation criterion, only 37 percent of them were found to be indistinguishable using the aggressive T-test criterion at a 95 percent confidence level. It is indeed striking to see the wide spread of discrimination power provided by simply choosing different discrimination criterion. This speaks strongly for the need to develop a national consensus on the appropriate criterion. Much has been written on this topic over the years, with Bennett, et. al. (9) most recently cautioning on the use of parametric statistical methods given evidence that refractive index in a window is not normally distributed. They further demonstrate that acquisition of a truly representative standard is quite difficult, even if the analyst has the entire window at his/her disposal. Considering the more conservative +/- 2.5 standard deviation criterion (equivalent to a 97.5 percent confidence level), most of the samples chosen to have similar nominal refractive indices do indeed appear to be indistinguishable.

Pair-wise inter-comparison of the μ-XRF results for the 666 possible pairs in Set 1 using spectral overlay and +/- 3 standard deviation criteria discriminates all but three of the pairs (W142/W143, W319/W320, and W23/W323). Two of these three pairs are inside/outside panes of the same laminated windows. Application of the T-test as described above permits further discrimination of the W23/W323 pair. These two approaches yield discrimination powers of 99.5 percent or better on this set of

[8] EDAX Inc., part of AMETEK, Inc., Materials Analysis Division, 91 McKee Drive, Mahwah, New Jersey, 07430, USA.

randomly acquired flat glass samples having similar refractive indices. The W142 and W143 glass samples also served as controls in this study since they were found to be indistinguishable in the previously reported exercise using the first twenty-one samples of Set 1 (7).

Table 3: Discrimination power of various refractive index criteria for the first twenty-one samples in Set 1 using Foster + Freeman GRIM (210 possible pairs)

Criterion	Number Indistinguishable Pairs	Percent Indistinguishable
+/- 2.5 Standard Deviation	196	93
+/- 2.0 Standard Deviation	174	83
+/- 0.00010 Fixed Criterion	126	60
Range Overlap	176	84
T-test with Welch modification at 99% confidence level	120	57
T-test with Welch modification at 95% confidence level	78	37

Pair-wise inter-comparison of the μ-XRF results for the 435 possible pairs in Set 2 using spectral overlay and +/- 3 standard deviation criteria discriminates all but eight of the pairs (W234/W235, W393/W394, W234/W393, W234/W394, W235/W393, W235/W394, W252/W295, and W98/W213). Six of the pairs resulted from the four indistinguishable samples W234, W235, W393 and W394 that come from two separate laminated windows, where W234/W235 and W393/W394 are inside/outside panes of each window. Application of the T-test as described above permits further discrimination of the eight pairs leaving only the W234/W235 and W393/W394 pairs indistinguishable. This yields discrimination powers of approximately 98.0 to 99.5 percent respectively on this second set of randomly acquired flat glass samples having similar refractive indices.

As reported by Howden et. al. and Dudley et. al. (10, 11), X-ray fluorescence spectrometry analyses of small glass fragments are prone to reduced precision as a result of critical depth effects and variation in take-off angle effects. The samples analyzed in the previous two sets were infinite thickness with respect to X-ray penetration. That is, if the samples compared are thicker than approximately 1.5 millimeters, little to no critical depth effects are experienced (12). In order to evaluate the impact of sample thickness on discrimination power, small (on the order of 1 millimeter) thin samples of the initial set of twenty-one samples in Set 1 were also compared by μ-XRF for the previously reported study (7). Using the more conservative spectral overlay and +/- 3 standard deviation criteria, eleven of the 210 possible pairs

were found to be indistinguishable resulting in a discrimination power of approximately 95 percent.

CONCLUSION

This study corroborates the previously reported improved discrimination power afforded by trace elemental comparisons using μ-XRF in addition to refractive index comparisons. For each set of sheet glasses having quite similar refractive index ranges, 98.0 to 99.5 percent of the thicker sample pairs were discriminated by μ-XRF. This discrimination power is slightly reduced as samples become quite thin or their surfaces become markedly uneven. Hence, only 95 percent of smaller thin sample pairs from one subset were discriminated. The results demonstrate that despite these limitations, μ-XRF remains a very robust non-destructive technique in the discrimination of glass samples encountered in typical forensic casework.

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Particle Combination Analysis for Predictive Source Attribution: Tracing a Shipment of Contraband Ivory[4]

ABSTRACT

Dusts from within a shipment of contraband ivory were analyzed to help determine the original location where the ivory was packed. Key findings were the types of minerals, soil, and vegetation represented in the dust, as determined using a combination of light and electron microscopy, energy dispersive x-ray analysis, infrared microspectroscopy, palynology and non-human DNA analysis. Beginning with a possible origin within the continent of Africa, first-stage analysis of the recovered dusts was able to eliminate environments comprising approximately 91% of the area, including all areas of 36 countries. Of the remaining 12 countries, the analysis was able to eliminate 72% of their area, allowing the investigations to be focused within portions of these countries. Next steps were defined to further reduce the possible origins of the dust based on more detailed regional analyses.

INTRODUCTION

Fine dust particles, adhering to virtually any object and within virtually any product, are the result of a history of exposure. Routinely, such dusts contain a tremendous variety of particles, including those of mineral, botanical, zoological, microbial, and anthropogenic character. The large number of particles in these dusts, and their variety, provide an extremely rich source of potential information – translating into a powerful inferential tool – when the particles are appropriately analyzed and appropriately

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[3] Palynology Laboratory, Texas A&M University, College Station, TX.

[4] Portions presented at the INTERPOL, 6th International Conference on Environmental Crime, Lyons, France, October 13-17, 2008 and the Society for Wildlife Forensic Sciences, Inaugural Meeting, Ashland, Oregon, April 19-23, 2010. Work on case-related law enforcement and forensic laboratory analyses has been presented at these conferences and at the NIJ/FBI Trace Evidence Symposium, Clearwater Beach, Florida, August 2-7, 2009 by Ken Goddard, Director of the US National Fish and Wildlife Forensics Laboratory.

interpreted. When faced with alternative hypothetical geographical, environmental, and human activity exposures, the results of fine dust analysis allow the exclusion of many sources of exposure, and the appraisal of others with degrees of support for hypotheses ranging from highly unlikely to strongly compelling. The results can be extremely useful and allow predictive source attribution. That is, they contain an intelligible "signal" regarding provenance: where items or people originated, how they have been transported, and the people, conditions and environments to which they have been exposed.

This use of fine dusts, extracting signals allowing predictive source attribution, is distinguished from, and complementary to, those trace evidence tasks addressing either (1) comparison with specific possible sources or (2) classification among a set of pre-defined sources. These distinctions result in different limitations and they enable solving different problems.

Comparison with specific possible sources

The task of comparison with specific possible sources (individualization by direct comparison) has a frequent application in the comparison of trace evidence found on a suspect with that found at a particular (typically crime scene) location. Published case reports of this type are common.[1] The focus is on the presence and variability of specific particle types. Two questions are of primary importance for this task

- Are the differences that are seen between the two samples sufficient to exclude a common origin?
- To what extent do the corresponding particle types, and their estimated frequencies of joint occurrence, support a hypothesis of common origin?

The purpose of comparison with specific possible sources is to rule out a specific source or to include it with a high degree of specificity. The specificity obtainable depends on the individuality of the features that are analyzed, compared and found to be in correspondence between the two samples.

To compare with specific possible sources, one must have “known” comparative reference samples from a location and one must apply an analytical protocol that is sensitive to highly individual sample characteristics. The analytical results from the reference samples are compared to those from the “questioned” sample (usually associated with a suspect). Based on the comparison, there is either a conclusion that the questioned sample is *not* from the same source as the reference sample, or that it *could be* from the same source (along with an estimate of the specificity of the conclusion). This approach is very appropriate for certain types of problems, but predictive source attribution is distinctly different.

Classification among a set of pre-defined sources

The task of classification among a set of pre-defined sources has frequent application to the problem of assigning the origin of a specific type of product to one of a closed set of alternatives. In this type of analysis, samples are examined and compared with a library of alternative reference samples. This is the typical laboratory support for product liability cases and enforcement cases relating to importation of goods.[2] A typical question posed to the analyst is, “From which of these possible sources (manufacturers, countries) did the sample (asbestos, lead paint, oil spill, foodstuff or defective product) come from?

To conduct classification among a set of pre-defined sources, one must first compile “known” reference samples from each of the alternative sources in the closed set. A specific analytical protocol is then used that discriminates among the reference samples. This protocol must be based on stable, specific sample features sufficient for unambiguous discrimination. Incoming “questioned” samples are then analyzed using the same protocol and the results are compared with those from the reference set. Based on the analyses, a conclusion is made that the questioned sample is from one of the specific sources within the closed set, or that it comes from an alternative (unknown) origin. Again, this approach is very appropriate for certain types of problems, but predictive source attribution is distinctly different.

Predictive source attribution

In contrast to the comparisons with specific sources, or classification among alternative possible sources, the use of dusts for predictive source attribution works with a single sample and an open-ended set of possible sources. As noted above, the goal is to extract any intelligible "signal" that can reliably predict provenance: where items or people originated, how they have been transported, and the people, conditions and environments to which they have been exposed.

Examples of predictive source attribution from dusts are less frequent.[3] Predictive source attribution from dusts utilizes many different particle types – recognizing, analyzing and exploiting whatever particles happen to be present – and focuses on those particles providing relevant information to the source attribution questions of interest. There is a much richer signal, composed of many co-occurring particle types. With this rich, quantitative distribution of fine particles, hypotheses about the origin of the dusts can be tested, and the support for alternatives can be reliably estimated.

The exploitation of fine dusts for predictive source attribution requires a multidisciplinary approach – the goal is to recognize, identify and exploit information coming from any type of particle that is (1) present and (2) carries associated information that is useful in refining the hypothesis or solving the problem. Scientific

disciplines with consistent contributions are geology (minerals, soils), botany (pollen, plant DNA), entomology (insects, parasites), microbiology (fungi, protozoa, diatoms, foraminifera), chemistry (ingredients, drugs, explosives), industrial hygiene (work and pollution-related dusts), and forensic science (human activity traces).

We have been actively developing this capability for many years and applying it to major investigative problems including analysis and source attribution of dusts associated with counterfeits, clothing, weapons, laboratories and contraband items. Many of these investigations, at the national and international level, cannot be discussed in detail because of their ongoing or sensitive nature. The present case originated from Interpol, and approvals have been given for discussion and presentation.

The evidence resulted from a seizure of four locked steel boxes in Singapore where X-ray inspection of the boxes revealed them to be tightly packed with an estimated 1000 pounds of elephant tusks. Without opening, the locked steel boxes were tightly wrapped and shipped to the National Fish and Wildlife Forensics Laboratory in Ashland, Oregon, where we participated in the opening and sampling of evidence.

DESCRIPTION OF SAMPLES AND SAMPLE PROCESSING

A double-wrapped and tightly sealed metal shipping box was received and opened in the National Fish and Wildlife Forensics Laboratory biosafety area (Figure 1). Tusks were found tightly bundled in loosely woven fabric bags. Small particle samples (Figure 2) were collected from the fabric bags directly (falling onto clean parchment paper during bag examination), from the bottom of the shipping box, and by vacuuming of the bags using filter cassettes (see materials and methods).

Sub-samples for palynology (approximately 3 g) and non-human DNA analysis (approximately 250mg) were prepared from the vacuumed dusts by pooling small amounts from the individual cassettes. The debris recovered directly from the fabric bags and from the bottom of the box was processed for microscopical analysis and individual particle analysis. Large particles (greater than approximately 1mm) were first removed from both samples. Many of these larger particles were plant tissue, insect parts, tusk fragments and pieces of cardboard. Of the remaining, mostly fine debris, approximately one-quarter of each sample was taken by carding, resulting in a pooled sample of 1.9 grams. This sample is shown by stereomicroscopy in Figure 3. It is heterogeneous, with large quantities of mineral grains, together with smaller quantities of plant tissue and insect fragments.

Figure 1. Part of the initial opening and examination of the tusk shipment in the biosafety area of the National Fish and Wildlife Forensics Laboratory, Ashland, OR. Ken Goddard, Laboratory Director (Left) and David Stoney (Right). The tusks were tightly bundled in loosely woven fabric bags.

Figure 2. Debris recovered directly from fabric bags (top left), from the bottom of the box (top right) and by using vacuum cassettes (bottom).

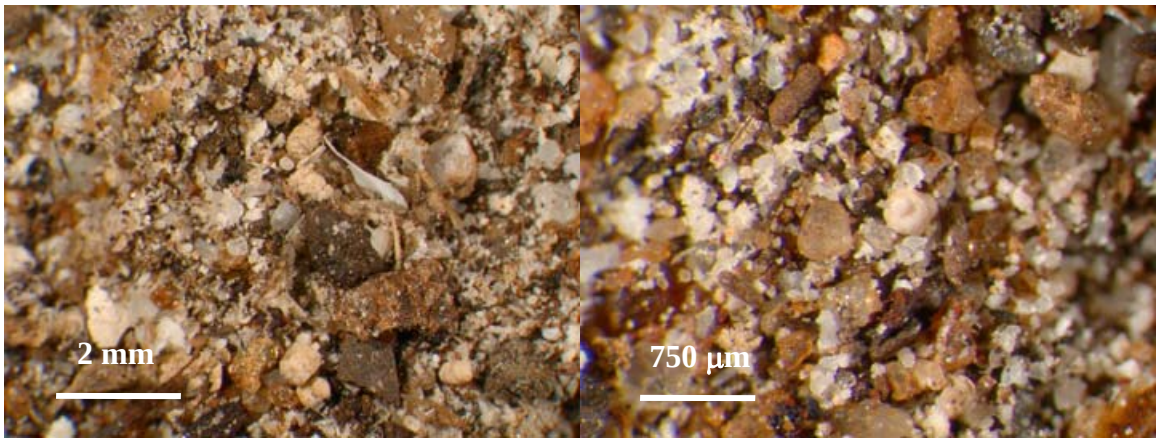


Figure 3. Overviews of the sample taken for microscopical analysis and individual particle analysis, following removal of larger particles, as shown at two different magnifications.

MATERIALS AND METHODS

Dust Recovery

Vaccum recovery of fine dusts was performed using a Staplex Model EC-1 Econometric™ Area Air Sampler, fitted with a length of Tygon® R-3603 Clear Laboratory Tubing (I.D 1/4in/6.4mm; wall thickness 1/16in/1.6mm; O.D. 3/8in/9.5mm) and a Pall® 37mm Air Monitoring Cassette (product number 4336, 3-piece unit with 0.8 µm GN-4 Metricel® membrane and support pad). The open-faced sampling configuration was used, sweeping the cassette over the fabric while in direct contact with the fabric surface.

Particle Microscopy

Stereoscopic microscopy and photomicrography were performed using a Leica MZ16 stereomicroscope fitted with a Nikon model 995 digital camera using a 1.0X Optem coupler. Compound light microscopy (transmitted, reflected and polarized) was performed using a Zeiss Axio Imager.A1m microscope fitted with a Zeiss MRc5 digital camera using a 1.0X Zeiss coupler.

Fractionation of the Sample by Settling Velocity

Particles were fractionated by settling velocity in distilled water. The sample was added to a 15ml centrifuge tube and approximately 10 to 12 ml of distilled water was added (sufficient to allow a settling distance of 10cm). The sample was sonicated briefly and stirred. After mixing, the sample was allowed to settle in the water for 30 seconds, after which the water and portion of soil still suspended were removed from the tube using a pipette. Clean water was added to the settled sample and the procedure was repeated until there was virtually no material suspended in the water after 30 seconds of settling. The clean, settled portion was designated as the "Sand" fraction. The remaining unsettled portion was mixed and allowed to settle for 60 minutes. The portion still

suspended was removed from the tube using a pipette and was labeled, designated as the “Clay” fraction. The settled portion was labeled, designated as the "Silt" fraction.

Fractionation of Larger Particles by Sieving

The particles from the "Sand" fraction were dry sieved into four size fractions using 38 mm diameter ASTM stainless steel wire mesh sieves (Endecotts Ltd., London) of sizes 1 mm, 500µm and 180µm mesh sizes. The resulting particle size ranges of the fractions were: > 1 mm, 500µm – 1 mm, 180 – 500µm, and <180µm.

Fractionation of the Particles by Density Separation

The finest sieved fraction from the settled "Sand" fraction (<180µm) was divided into heavy and light particles by a heavy liquid density separation. The particles were placed in a clean 15 mL centrifuge tube and approximately 6.0 mL of bromoform (density = 2.89) was added. The sample was mixed and centrifuged until the partitioning of particles (floating vs. sinking) was visually complete. The bottom of the tube (containing particles with densities greater than 2.89) was then immersed in liquid nitrogen and frozen. The top portion of the bromoform, containing the light particles, was transferred to a clean centrifuge tube. Acetone was added to both tubes to decrease the density of the bromoform until all particles sank. The samples were centrifuged and the liquid was removed with a pipette. The samples were washed with acetone until all bromoform was removed from the samples. The tubes were then left uncovered on a clean bench to evaporate the remaining acetone.

Mounting of Particles for Polarized Light Microscopy (PLM)

The particle fractions isolated by density separation were prepared for polarized light microscopy (PLM) by mounting in Cargille calibrated refractive index liquids (Cargille Laboratories, Cedar Grove, NJ), using refractive index of 1.540 ($n_{D,}$ 25°C, Cargille Series A) for the light particles and refractive index 1.660 ($n_{D,}$ 25°C, Cargille Series B) for the heavy particles.

Identification of Sand Fraction Particles

The particles present in the fractions were characterized optically and morphologically. Identifications of minerals were made using the PLM by comparison to known samples and reference data.[4, 5]

Quantitative Estimations and Quantitative Measurements of Particle Abundance

For quantitative estimates, each particle type (such as a particular mineral) was characterized as being a major, minor or trace component of the mixture, with major defined as any particle composing greater than 10% of the sample, minor as any particle composing 1–10% of the sample, and trace as composing less than 1% of the sample. These estimates were made on a visual basis, with ambiguities addressed by verbal

modifiers, such as "low major", or "high minor", as appropriate. For quantitative determinations, number percents were determined for particle types by point counting using the ribbon method.[6]

Silt and Clay Fraction Examination by PLM

The silt and clay fractions were each suspended in water and vortexed, after which a small drop of the suspension was examined under a cover slip by PLM.

Clay Analysis by X-Ray Diffraction

A sub-sample of the re-suspended clay fraction was taken for x-ray diffraction analysis. Four different assays were sequentially performed on the sample: one on the neat clay, then again after treatment with ethylene glycol, and then again after heat treatments at two different levels. The neat clay was prepared by spotting onto a glass slide and drying in air. After x-ray diffraction, the glass slide was placed overnight in a vacuum desiccator in contact with vapor saturated with ethylene glycol. After x-ray diffraction the glass slide was heated to 400 °C in a muffle furnace for one hour. After x-ray diffraction, the slide was heated to 550 °C in a muffle furnace for one hour, followed by the final x-ray diffraction. The x-ray diffraction was performed on a PANalytical X'Pert Pro diffractometer using copper radiation, with 40 mA current and 45kV voltage, and a step size 0.033° 2-theta, both using standard conditions (4° to 64° 2-theta, 160 sec) and using a slow scan (300 sec) over the clay region (4° to 34° 2-theta) with step size 0.033° 2-theta. The resulting diffraction patterns were then compared and analyzed using the X'Pert HighScore program utilizing the International Centre for Diffraction Data (ICDD) database.

Palynology of the Silt Fraction

A sub-sample of the re-suspended silt fraction was taken for palynology. The sample was extremely difficult to process for palynology due to clumping. It was initially processed with concentrated HF (to remove silicates), followed by rinsing in concentrated HCl (to remove the resulting fluorosilicates), and dispersal in glacial acetic acid. This was followed by acetolysis (one part sulfuric acid and nine parts acetic anhydride) to remove cellulosic materials, followed by a glacial acetic acid wash. To remove atypical clumping the sample was sieved carefully through a 40µm mesh NITEX screen with subsequent preparation of two samples, one from the material larger than 40µm in size (trapped on the screen surface) and the second from the materials that were smaller than 40µm in size (having passed through the screen). Subsequent preparation consisted of washing in ethanol, dilution to 1:1 ethanol and water, staining with saffarin, addition of glycerine and allowing the ethanol to evaporate overnight. Slides were prepared and examined from both fractions, combining the results. Strictly quantitative methods of pollen counting were not performed due to high quantities of

debris. Pollen types that were identified were assessed semi-quantitatively as follows, based on observing some 250 to 300 grains:

Abundant	=	dominant pollen types
Frequently Observed	=	> 10 pollen grains
Occasionally Observed	=	3-10 pollen grains
Rarely Observed	=	2-3 pollen grains
Single Grains	=	1 pollen grain

Non-Human DNA Analysis

DNA from botanical particles (present as fine dusts and fragments of botanical tissue) was isolated and purified from a total of approximately 0.1 g of the sample using the Qiagen DNeasy (Valencia, CA) extraction kit. 2.5 ng to 25 ng of DNA in three separate extracts was used in PCR along with primers targeting the nuclear-encoded Internal Transcribed Spacer II (ITS2) region.[7] Primer sequences used for the Internal Transcribed Spacer II region are ITS3 (forward: GCATCGATGAAGAACGCAGC) and ITS4 (reverse; TCCTCCGCTT ATTGATATGC). PCR product cloning and sequencing was performed using the Invitrogen Topo TA Cloning Kit for Sequencing following the manufacturer's guidelines. Twenty-five resulting colonies were selected for plasmid sequencing.

Nucleotide sequences were characterized using the National Institutes of Health's online GenBank, Basic Local Alignment Search Tool (BLAST), and its associated computational tools for distance-based tree drawing.

GIS Analysis

Spatial data processing, analysis, and cartographic visualization were conducted on a Dell Precision T3400 using tools within ArcGIS ArcView 9.2 software [8] with supplemental spatial data modeling performed using DIVA-GIS 5.2 software.[9] Base map data utilized in the generation of maps were obtained from ESRI.[10]

ANALYTICAL RESULTS

Judgment of Sample Suitability for Predictive Source Attribution

From preliminary results, we concluded that the quality of this sample was very well suited to this task and to the applied analytical methods. There were many particles, varied in type, suitable for addressing the questions of interest. These included geological, ecological, and human activity signals that were expected to contribute significantly to the result.

Based on our experience with similar samples, the particles recovered in this case, and the specific questions of interest, we expected to be able to: (1) limit the possible origin of this material to a narrowly defined region or regions within a single country, (2) define options for follow on actions that will further restrict the possible areas of origin, and (3) lead to sufficiently precise areas to allow for specific evaluations, exclusions or confirmation based on comparative reference information or samples.

The foundation for this assessment was:

- analysis of the ecological and geological references available for the regions of interest
- isolation and characterization of the amounts, variety and quality of particles contributing to ecological and geological signals: minerals, pollen and spores, non-human DNA and human activity related particles

Based on this overall sample assessment of suitability for predictive source attribution, a detailed first-stage analysis of the sample was conducted.

Masses of Separated Fractions

The masses of the separated fractions, and their percentages relative to the processed sample, are given in Table 1. The sample is composed of approximately 65% sand, 29% silt, and 6% clay. The sand is primarily fine sand (<180µm).

Table 1. Masses of Sample Fractions

Fraction	Size range (µm)	Mass (g)	Mass (%)
Bulk	all	1.91	100
Sand Fraction	> 1000	0.112	6
	500 – 1000	0.135	7
	180 – 500	0.367	19
	< 180	0.632	33
	Total	1.25	65
Silt Fraction	settles in 30 sec – 60 min	0.56[5]	29
Clay Fraction	does not settle in 60 min	0.11[5]	6

[5] The mass of the combined silt and clay fractions was taken as the bulk samples mass minus the combined mass of the sand fractions. The volumes of the silt and clay fractions after centrifuging each fraction were estimated as 0.7 mL (silt) and 0.2 mL (clay). Applying an experimentally determined correction factor of 0.7 to the clay volume (when used for mass estimates) results in 83% of the combined silt+clay mass (0.56 g) for silt, and 17% (0.11 g) for clay.

The masses of the separated heavy and light particles, from a sub-sample of the fine sand (<180µm), are given in Table 2.

Table 2. Masses of Heavy and Light Particles and Minerals

		Light (< 2.89 g/cc)		Heavy (> 2.89 g/cc)	
		Mass (g)	Mass (%)	Mass (g)	Mass (%)
Fine Sand < 180µm	All particle types	0.3312	66.44	0.1673	33.56
	Minerals (including Fe-oxides)[6]	0.1689	50.24	0.1673	49.76
	Minerals (excluding iron oxides)[7]	0.1510	76.78	0.0457	23.22

Abundant Particle Types in the Fine Sand Fraction (<180µm)

The two most abundant particle types present in the fine sand were aggregates of corn starch grains and iron oxides, each composing nearly 30% and together making up nearly 60% of the grains counted. It is uncertain whether the iron oxides are of geologic origin or of anthropogenic origin. Many of the mineral grains observed are iron-stained, indicating that at least a portion of the iron oxides are likely to be geologic in nature.[8]

Geological Particles in the Low Density Fine Sand Fraction

The minerals in the <180µm fraction were composed of nearly equal amounts of heavy and light minerals, with the light minerals (<2.89 g/cc) making up approximately 50% of the fraction. Overviews of the light mineral fraction by stereomicroscopy and PLM are shown in Figure 4. Point count percentages for low density (<2.89 g/cc) components of the fine size fraction are given in Table 3.

Quartz grains were the most abundant mineral present in the light mineral fraction, composing roughly 42% of the geologic material in the fraction. They are primarily well-

[6] Adjustments based on particle percentages determined by point counting. In the light fraction non-geological particles were 51% of those counted (primarily starch aggregates, and including minor portions of organic matter such as plant tissue and charred particles).

[7] Adjustments based on particle percentages determined by point counting. In the light fraction iron oxides accounted for 5.4% of the fraction. In the heavy fraction they accounted for 72.7% of the fraction.

[8] The iron oxides were included in the mineralogy assessment of the sample, although the possibility that they are anthropogenic in origin, and the implications that would have on the results, are considered in the discussion and conclusions.

rounded grains, although a small number of angular quartz grains are present as well. The quartz was often iron-stained. Altered, unidentifiable mineral grains were a major component of the light fraction, making up approximately 24% of the geologic material. Feldspar minerals were also a major component, together making up about 17% of the light minerals, with a 2:1 alkali feldspar to plagioclase feldspar ratio. The feldspar grains were somewhat weathered and many were iron stained. Iron oxides were a low major component of the light mineral fraction, composing 11% of the fraction. Biotite mica was a high minor component of the fraction (7%), and there were trace amounts of lithic fragments. Figures 5 and 6 illustrate the major and minor components of the light mineral fraction.

Table 3. Point Count Percentages for Geological Particles in the Lower Density (<2.89 g/cc) Fine Sand Fraction (<180µm)

Particle Type	Count	Percent
Quartz	150	41.7
Alterite	85	23.6
Alkali feldspar	38	10.6
Biotite mica	38	10.6
Iron oxides	26	7.2
Plagioclase feldspar	22	6.1
Lithics	1	0.3
Total	360	100.0

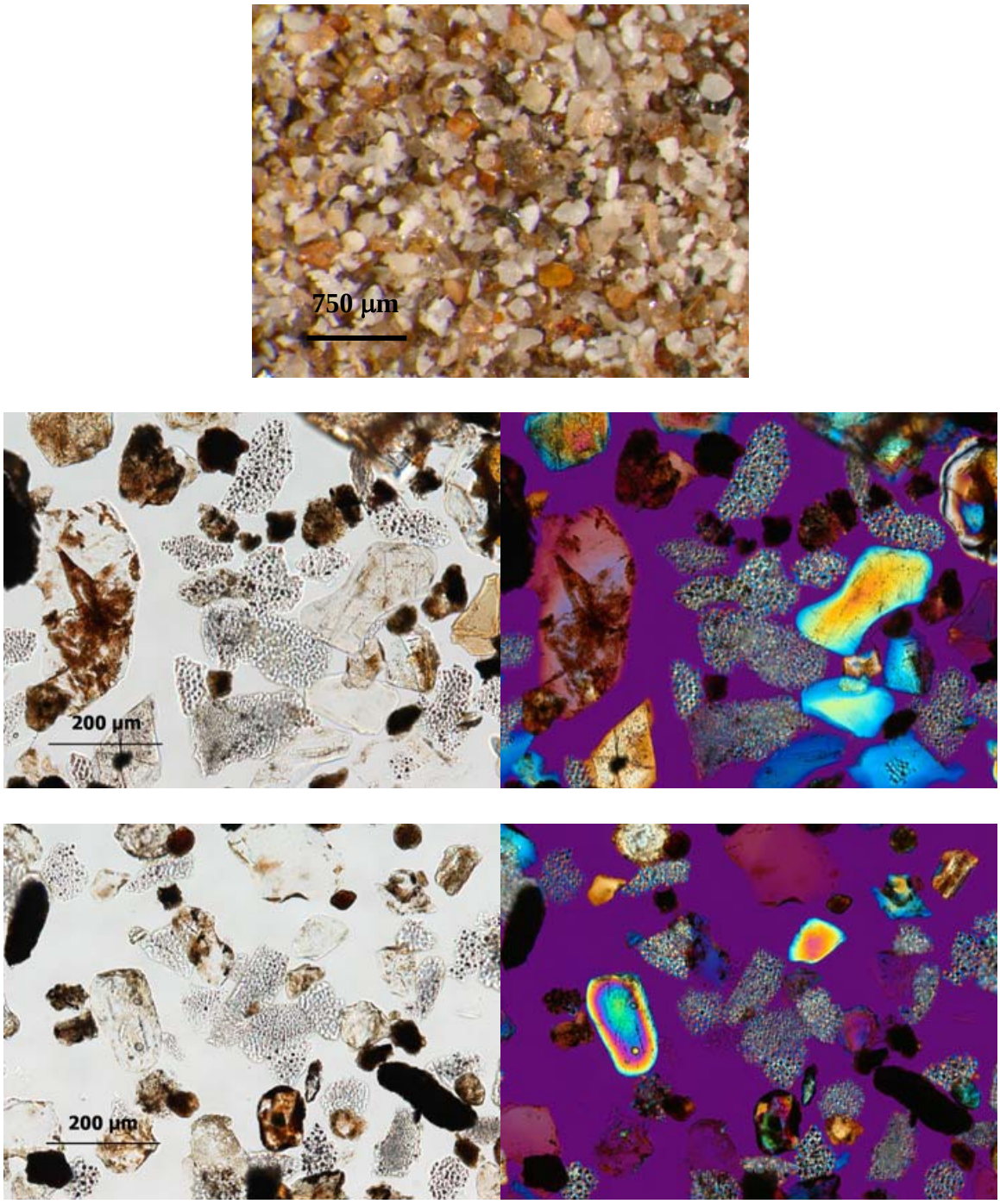


Figure 4. Top Row: Stereomicroscope overview of the light mineral fraction.
Middle and Bottom Rows: Overviews of the light mineral fraction shown in plane polarized light (left), and in crossed polars with a 530 nm compensator (right). Mounting medium is 1.540.

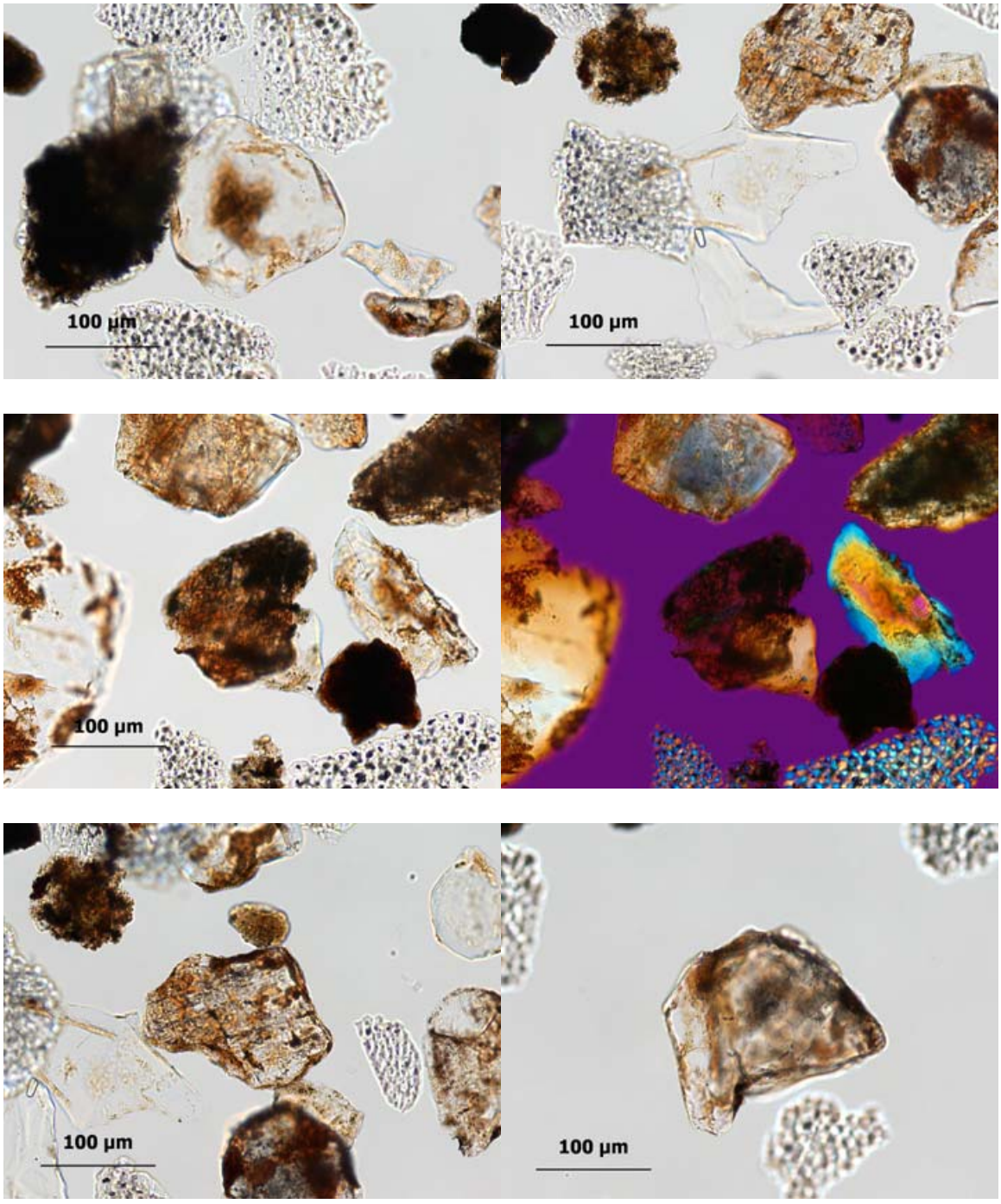


Figure 5. Top Row: A rounded quartz grain (left) and angular quartz grains (right). Second Row: Unidentified altered mineral grain in plane polarized light (left), and between crossed polars with a 530 nm compensator (right).
Bottom Row: Alkali feldspar (left) and plagioclase feldspar (right). Mounting medium is 1.540.

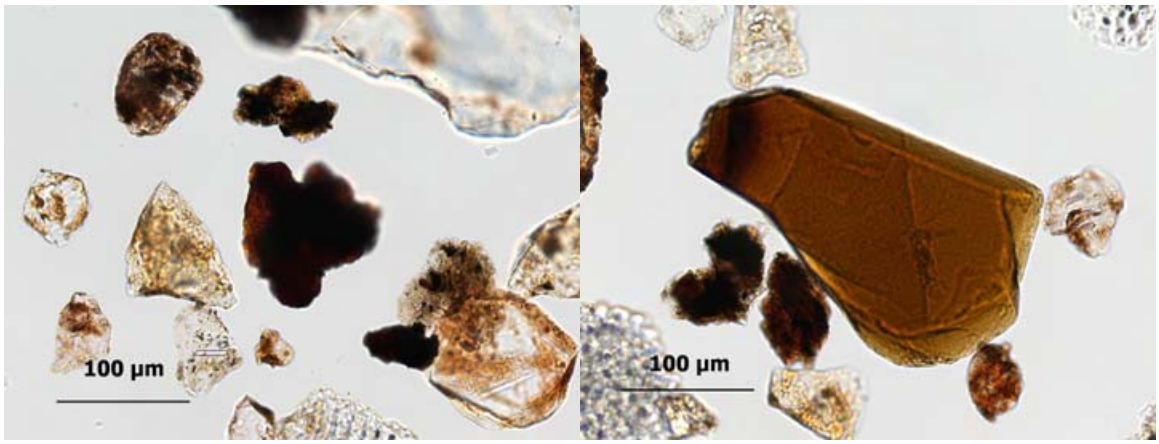


Figure 6. Iron oxide (left) and biotite mica (right). Mounting medium is 1.540.

Geological Particles in the High Density Fine Sand Fraction

The heavy minerals in the <180µm fraction comprised approximately 50% of the recovered mineral grains. An overview of the heavy mineral fraction by stereomicroscopy is shown in Figure 7 (top left). Point count percentages for high density (>2.89 g/cc) components of the fine size fraction are given in Table 4.

The heavy minerals were composed primarily of iron oxides, making up nearly 73% of the fraction. Hornblende grains were also a major component of the fraction, accounting for roughly 12% of the observed grains. Biotite mica (9%) was a high minor component. Opaque grains and muscovite mica were low minor components of the fraction. Trace components of the heavy mineral fraction were titanite, sillimanite, pyroxene, unidentifiable altered minerals, zircon, apatite, epidote, garnet, dolomite, rutile, glauconite, and an unidentified mineral. Tremolite was also observed during a survey scan following point counting. Figure 7 illustrates the major and high minor components of the heavy mineral fraction.

Particles in the Silt Fraction

There was a large amount of silt present in the recovered material (29%) and it was analyzed by PLM. The silt is dominated by corn starch grains. The minerals present consist primarily of quartz and feldspar, biotite mica, muscovite mica and iron oxides. There were smaller amounts of hornblende, opaque grains, and biological materials. Figure 8 shows an overview of the silt fraction and illustrates the primary particle components.

Table 4. Point Count Percentages for Geological Particles in the Higher Density (>2.89 g/cc) Fine Sand Fraction (<180µm)

Particle Type	Count	Percent
Iron oxides	812	72.7
Hornblende	129	11.5
Biotite mica	97	8.7
Opaques	29	2.6
Muscovite mica	15	1.3
Titanite	9	0.8
Sillimanite	5	0.4
Pyroxene	4	0.4
Alterite	3	0.3
Zircon	3	0.3
Apatite	3	0.3
Epidote	2	0.2
Garnet	2	0.2
Dolomite	1	0.1
Rutile	1	0.1
Glauconite	1	0.1
(Unidentified)	1	0.1
Total	1117	100.0

Clay Fraction by Polarized Light Microscopy

By PLM the clay fraction sample showed primarily fine clay minerals and micas (Figure 9, top row). There were also minor amounts of very fine, positively elongated particles with high birefringence (Figure 9, bottom row), along with trace amounts of iron oxides and opaque particles.

Clay Fraction by X-Ray Diffraction

The XRD spectra for the neat clay fraction, and those after treatments with ethylene glycol, heating to 400 °C and heating to 550 °C are shown in Figure 10.

The peaks in the spectra are listed in Table 5, and identified crystalline phases responsible for those peaks are listed in Table 6. The XRD results indicate that the sample is composed of major illite and minor kaolinite. The presence of kaolinite was confirmed due to its behavior during heat treatment.

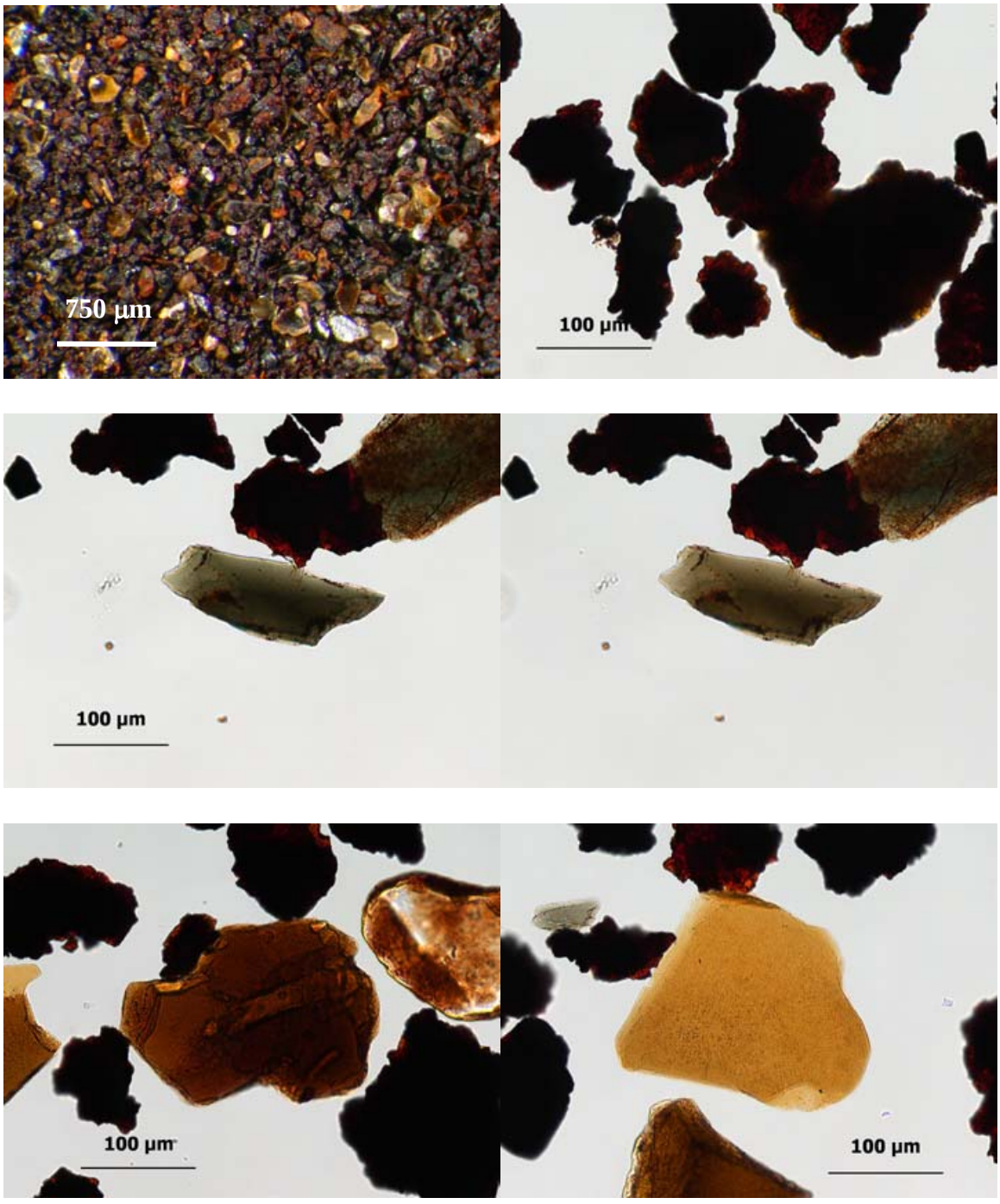


Figure 7. Top Row: Stereomicroscope overview of the heavy mineral fraction (left) and several iron oxide particles shown in plane polarized light (right). Second Row: Hornblende grain shown in plane polarized light with the polarizer oriented E-W (left) and N-S (right). Bottom Row: Biotite flakes. Mounting medium is 1.660.

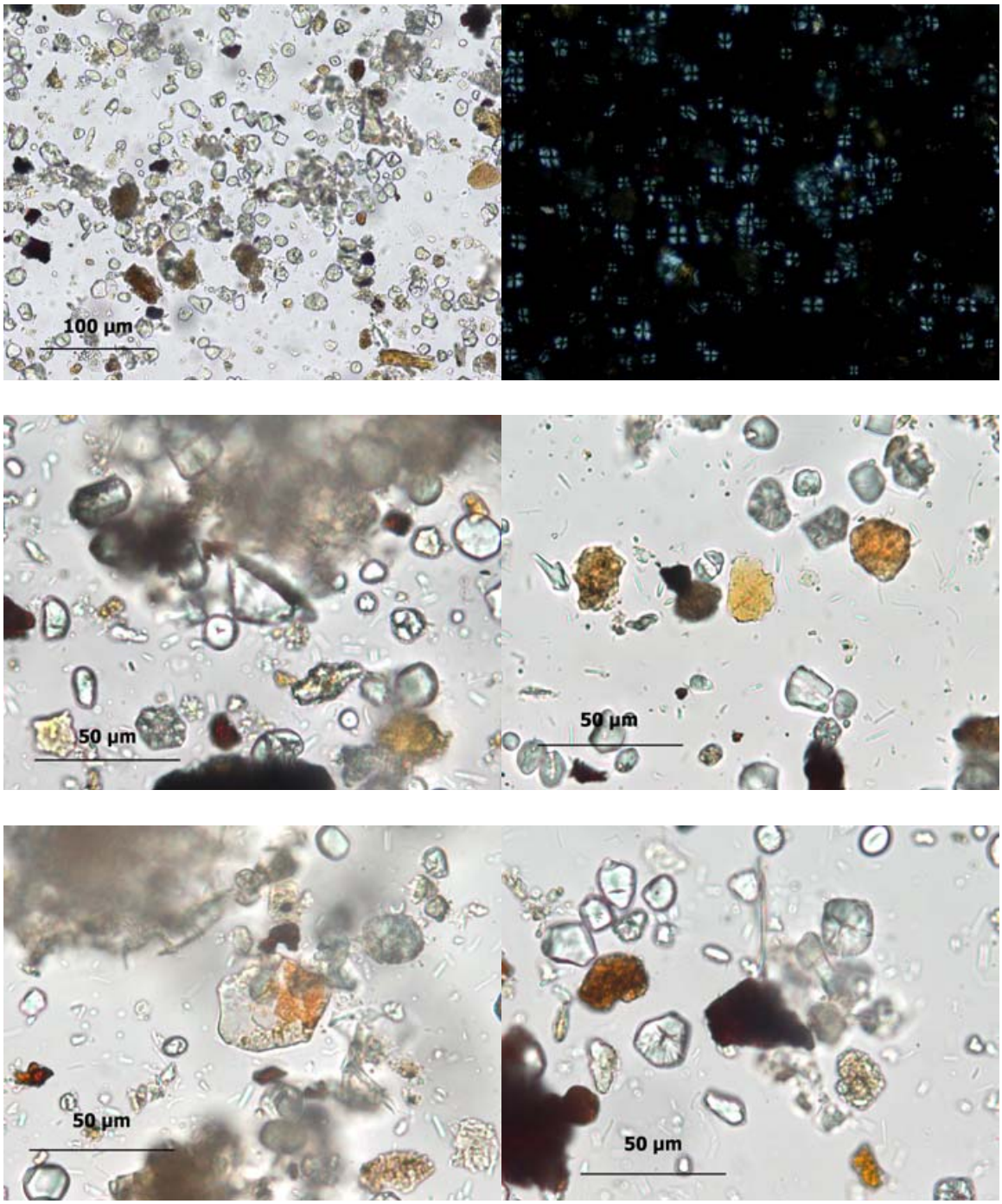


Figure 8. Top Row: Overview of the silt fraction shown in plane polarized light (left), and in between crossed polars (right). Middle Row: Quartz or feldspar grain (left) and biotite mica flake (right). Bottom Row: Muscovite mica flake (left) and iron oxide particle (right). Mounting medium is water.

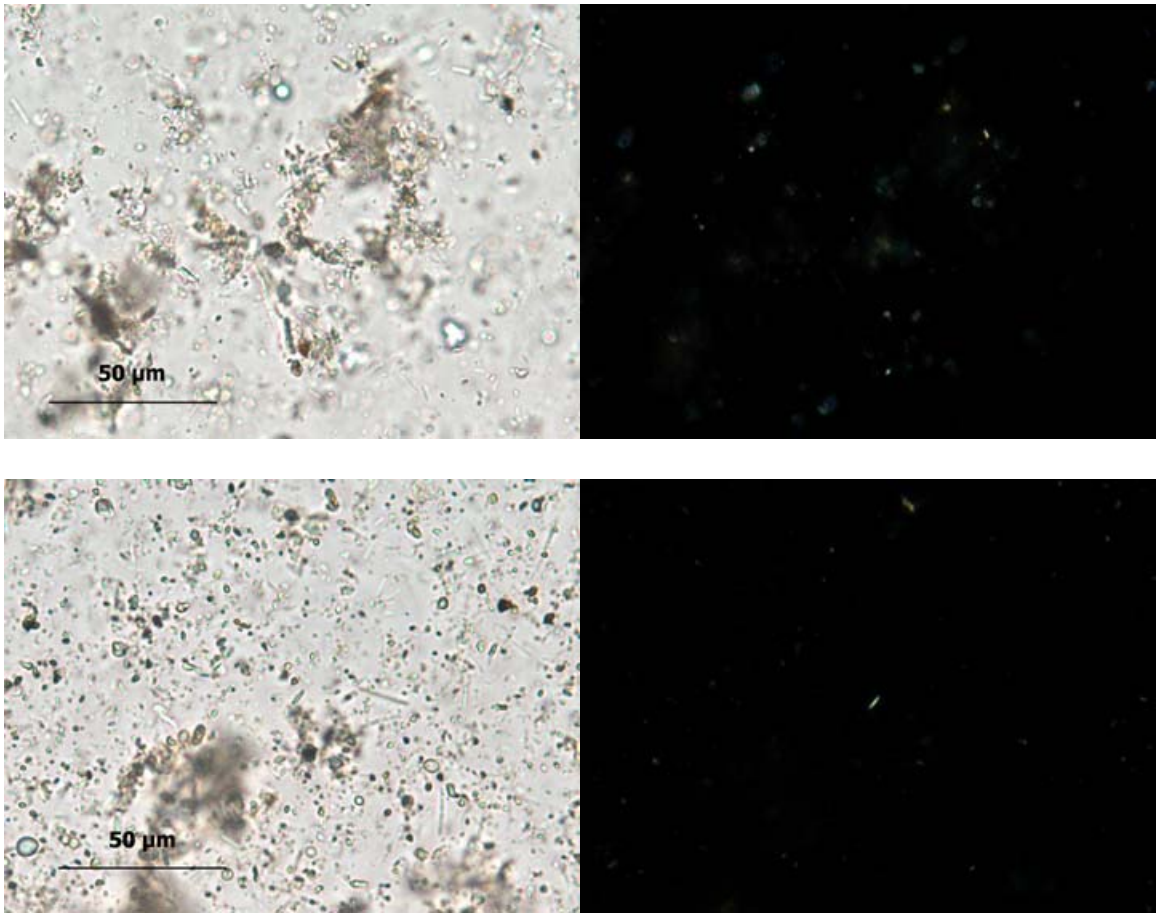


Figure 9. Top Row: Overview of the clay fraction shown in plane polarized light (top left) and in between crossed polars (top right). Bottom Row: Fine, highly birefringent particle with positive elongation shown in plane polarized light (bottom left) and in between crossed polars (bottom right). Mounting medium is water.

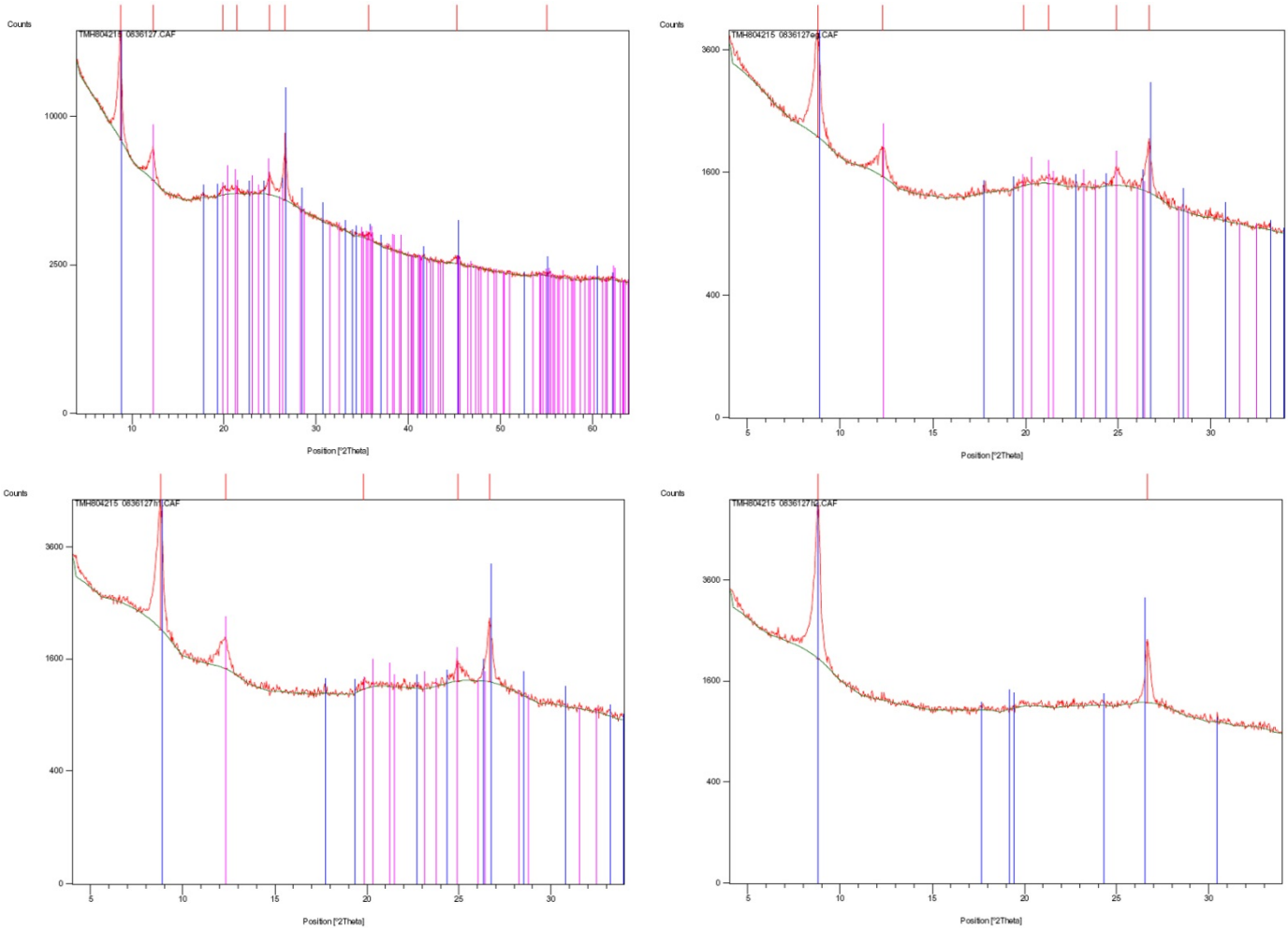


Figure 10. XRD spectra for of the clay fraction. Neat (top left), and after treatments with ethylene glycol (top right), heating to 400 °C (bottom left) and heating to 550 °C (bottom right).

Table 5. Peak Listings for Clay X-Ray Diffraction

Neat Clay

Pos. [°2Th.]	d-spacing [Å]	Area [cts*°2Th.]	Rel. Int. [%]	Matched by
8.8069	10.04093	1577.08	100.00	00-016-0344
12.3442	7.17046	247.97	23.59	01-080-0885
19.9082	4.45991	146.51	4.64	01-080-0885
21.4085	4.15064	297.85	4.05	01-080-0885
24.9229	3.57275	290.80	13.83	01-080-0885
26.6544	3.34446	476.00	45.27	00-016-0344; 01-080-0885
35.7555	2.51130	140.53	3.34	00-016-0344; 01-080-0885
45.3291	2.00069	156.97	2.49	00-016-0344; 01-080-0885
55.0171	1.66774	121.01	1.16	00-016-0344; 01-080-0885

Ethylene Glycol-Treated Clay

Pos. [°2Th.]	d-spacing [Å]	Area [cts*°2Th.]	Rel. Int. [%]	Matched by
8.8093	10.03829	482.71	100.00	00-016-0344
12.3027	7.19460	100.88	20.90	01-080-0885
19.9109	4.45932	35.06	2.42	01-080-0885
21.2458	4.18206	59.68	4.12	01-080-0885
24.9178	3.57347	59.04	12.23	01-080-0885
26.6589	3.34391	93.12	38.58	00-016-0344; 01-080-0885

400 °C Heat-Treated Clay

Pos. [°2Th.]	d-spacing [Å]	Area [cts*°2Th.]	Rel. Int. [%]	Matched by
8.8131	10.03397	497.67	100.00	00-016-0344
12.3354	7.17559	100.07	17.24	01-080-0885
19.8301	4.47731	36.64	3.68	01-080-0885
24.9272	3.57214	66.17	9.97	01-080-0885
26.6587	3.34393	182.56	36.68	00-016-0344

550 °C Heat-Treated Clay

Pos. [°2Th.]	d-spacing [Å]	Area [cts*°2Th.]	Rel. Int. [%]	Matched by
8.8150	10.03182	732.05	100.00	00-042-1437
26.6534	3.34458	200.65	27.41	00-042-1437

Table 6. Identified Crystalline Phases by X-Ray Diffraction

Neat Clay

Visible	Ref. Code	Score	Compound Name	Scale Factor	Chemical Formula
*blue	00-016-0344	47	fluorphlogopite	0.606	K Mg3 (Si3 Al) O10 F2
*pink	01-080-0885	36	Kaolinite 1\ITA\RG	0.189	Al2 (Si2 O5) (O H)4

Ethylene Glycol-Treated Clay

Visible	Ref. Code	Score	Compound Name	Scale Factor	Chemical Formula
*blue	00-016-0344	57	fluorphlogopite	0.606	K Mg3 (Si3 Al) O10 F2
*pink	01-080-0885	51	Kaolinite 1\ITA\RG	0.185	Al2 (Si2 O5) (O H)4

400 °C Heat-Treated Clay

Visible	Ref. Code	Score	Compound Name	Scale Factor	Chemical Formula
*blue	00-016-0344	55	fluorphlogopite	0.617	K Mg3 (Si3 Al) O10 F2
*pink	01-080-0885	42	Kaolinite 1\ITA\RG	0.164	Al2 (Si2 O5) (O H)4

550 °C Heat-Treated Clay

Visible	Ref. Code	Score	Compound Name	Scale Factor	Chemical Formula
*blue	00-042-1437	62	Phlogopite-1\ITM\RG, ferroan	0.889	K(Mg, Fe)3 (Al, Fe) Si3O10 (OH , F)2

Palynology

Pollen types and their incidence observed in the examination are given in Table 7.[9] In addition to those pollen listed in the table, there were many grains that were degraded beyond recognition and many unidentified pollen of a variety of types. The sample also contained many different taxa of fungi and many fragments of insect chitin.

Pollen types occurring abundantly, frequently or occasionally are illustrated in Figure 11. Some of the pollen types occurring more rarely in the sample are illustrated in Figure 12. Some of the many genera of fungal spores found in the sample are illustrated in Figure 13.

Dominant Pollen Types

Dominant pollen types in the sample were those of the African shrub *Adenodolichos paniculatus* (wáákén wuta, "fire bean") and the family POACEAE (grasses).

Adenodolichos paniculatu (wáákén wuta, "fire bean") is a shrub that grows best in direct sunlight. It is insect-pollinated with limited amounts of pollen. The pollen is ornate, large, and very fragile. It is not easily dispersed or carried by winds and degrades rapidly in soils.

In the POACEAE (grass family) there are hundreds of genera and thousands of species. The morphology is non-specific by light microscopy, with the principle difference among species being the pollen size. All species are wind pollinated.

Frequently Observed Pollen Types

Frequently observed pollen types on the sample were those of the species *Terminalia brownii* (hareri biiris), the genus *Rumex* (dock, sorrel), and the family COMBRETACEAE.

Terminalia brownii (hareri biiris) is a large tree that favors sandy soils and direct sunlight. The trees are commonly found in the scrublands and on the edges of savannas. It is insect-pollinated and the pollen are very small. The pollen are rarely dispersed and are only expected in soils close to the actual trees.

There are hundreds of species in the genus *Rumex* (dock, sorrel). All species need direct sunlight and they are most commonly found growing in grasslands and savanna regions. A large number of the species are wind-pollinated and disperse large quantities of pollen as far as several kilometers.

[9] Comprehensive pollen counts (200 to 300 pollen grains per sample) were not employed for this sample because of the large amounts of debris which obscured many of the grains.

The family COMBRETACEAE are flowering plants including over 600 species of trees and shrubs. *Terminalia brownii* is in this family, but there are additional taxa represented in the sample, difficult to differentiate and which remain unidentified.

Pollen Types Observed Occasionally

Pollen types observed occasionally in the sample were those of the species *Zenkerella egregia*, the genus *Brachystegi* (miombo trees), the tribe Helianthinae (sub-family of ASTERACEAE, high spine-type) and the combined group Cheno-Ams (family CHENOPODIACEAE and genus *Amaranthus*).

Zenkerella egregia is a lowland forest species of large, insect-pollinated trees. They are on the endangered species list. The pollen are only expected in soils close to the actual trees.

Brachystegi (miombo trees) are dominant and ecologically important trees occurring in large areas of open deciduous woodlands. They are insect-pollinated and the pollen are only expected in soils close to the actual trees.

The Helianthinae (ASTERACEAE , high spine type) are a large group of insect-pollinated plants, including many ornamental and weed types. They grow best in open sunlight, and are very common members of grasslands and savanna habitats.

Cheno-Ams are a very large group (more than 100 genera with more than 1000 species) whose pollen are indistinguishable. Many are common weeds. All are wind-pollinated annuals that grow easily in direct sun and grow quickly in disturbed habitats. They produce vast amounts of small pollen grains that can be carried easily on wind currents.

Rarely Observed Pollen Types

Pollen types observed rarely in the sample were those of the species *Diospyros mespiliformis* (jackal berry); the genera *Citrus* (orange, lemon, etc.), *Polygonum* (knotweed), and *Typha* (monocot type cattail); the tribe Lactuceae (sub-family of ASTERACEAE, dandelion type) and the families MELIACEAE (mahogany family), MYRTACEAE (myrtle family) and RHAMNACEAE (buckthorn).

Diospyros mespiliformis (jackal berry) is a large, insect-pollinated tree, common in savannas and preferring moist soils. They produce small amounts of pollen, which are only expected in soils close to the actual trees.

Citrus (orange, lemon, etc.) is the genus that includes the commercially important species of oranges, lemons, grapefruit and limes.

Polygonum (knotweed) is a large genus of rapidly growing, mostly herbaceous plants.

There are tens of species belonging to the genus *Typha* (cattail). All of these grow in wet environments. Cattails are wind-pollinated and produce large amounts of small pollen grains that are easily dispersed by the wind.

The Lactuceae (ASTERACEAE, dandelion type) are a large group of insect-pollinated plants commonly found in fields and gardens throughout the world. They require open areas and sunlight characteristic of savanna or grassland regions.

MELIACEAE (mahogany family) is a family of about 50 genera and 550 species, mostly tropical, and including (among African species) the economically important tree species of *Carapa procera* (crabwood), *Entandrophragma cylindricum* (zapele), Entandrophragma utile (utile, sipo), *Guarea cedrata* (Bossé), *Khaya ivorensis* (Ivory Coast mahogany) and *Khaya senegalensis* (Senegal mahogany).

MYRTACEAE (myrtle family) is a family with over 4500 species and 130 genera which include *Myrtus* (myrtle), *Eucalyptus* (gum), and *Psidium* (guava). There are 40+ species native or introduced in Africa, and 300+ cultivated species.

RHAMNACEAE (buckthorn) is a family primarily of trees and shrubs with over 850 species and 50 genera found worldwide, but mostly in subtropical and tropical regions.

Pollen Types Observed as Single Grains

Pollen types observed as single grains were those of the species *Gilbertiodendron deweveri* (limbali, abeum) and *Sida acuta* (common wireweed), as well as of the family CYPERACEAE (sedges).

Gilbertiodendron dewevrei (limbali, abeum) is a large African tree that grows on low, moist slopes of rivers and flooded basins. It is insect-pollinated and produces small amounts of pollen, which are expected only in soils close to the actual trees. It is commonly used for lumber, and is found in Cameroon, Central African Republic, Congo, Gabon, Ivory Coast, Liberia, Nigeria, Sierra Leone, and Zaire.

Sida acuta (common wireweed) is a shrub in the Mallow family with pantropical distribution that requires direct sunlight and grows aggressively in disturbed soils and in a wide range of environments. It is insect-pollinated and the pollen are large. The pollen are expected only in soils close to the actual trees.

Plants of the family CYPERACEAE (sedges) are most commonly found near water (marshes, bogs, edges of lakes). They are wind-pollinated, but produce low numbers of pollen which are dispersed close to the ground, so the pollen does not travel far. The pollen are fragile and degrade quickly in soils.

Table 7 Pollen Types Identified in the Sample[10]

Dominant Pollen Types (abundant)	Condition
Adenodolichos paniculatu (wáákén wuta, "fire bean")	Mix: Pristine and degraded
POACEAE (grass) with wide variety of taxa	Most degraded, few pristine
Frequently Observed Pollen Types (> 10 grains)	
Terminalia brownii (hareri biiris)	Pristine
Rumex (dock)	Most degraded, few pristine
COMBRETACEAE	Pristine
Pollen Types Observed Occasionally (3 to 10 grains)	
Zenkerella egregia	Pristine
Brachystegi (miombo trees)	Pristine
Helianthinae (ASTERACEAE, high spine type)	Pristine
Cheno-Ams	Pristine
Pollen Types Observed Rarely (2 or 3 grains)	
Diospyros mespiliformis (jackal berry)	Pristine
Citrus (orange, lemon, etc.)	Pristine
Polygonum (knotweed)	Pristine
Typha (cattail)	Pristine
Lactuceae (ASTERACEAE, dandelion type)	Pristine
MELIACEAE (mahogany family)	Pristine
MYRTACEAE (myrtle family)	Degraded
RHAMNACEAE (buckthorn)	Pristine
Pollen Types Observed as Single Grains	
Gilbertiodendron dewevrei (limbali, abeum)	Degraded
Sida acuta (common wireweed)	Pristine
CYPERACEAE (sedges)	Pristine

[10] Among 250 to 300 observed grains, Abundant = dominant pollen types, Frequently observed = > 10 grains, Occasionally observed = 3-10 grains, Rarely observed = 2-3 grains.

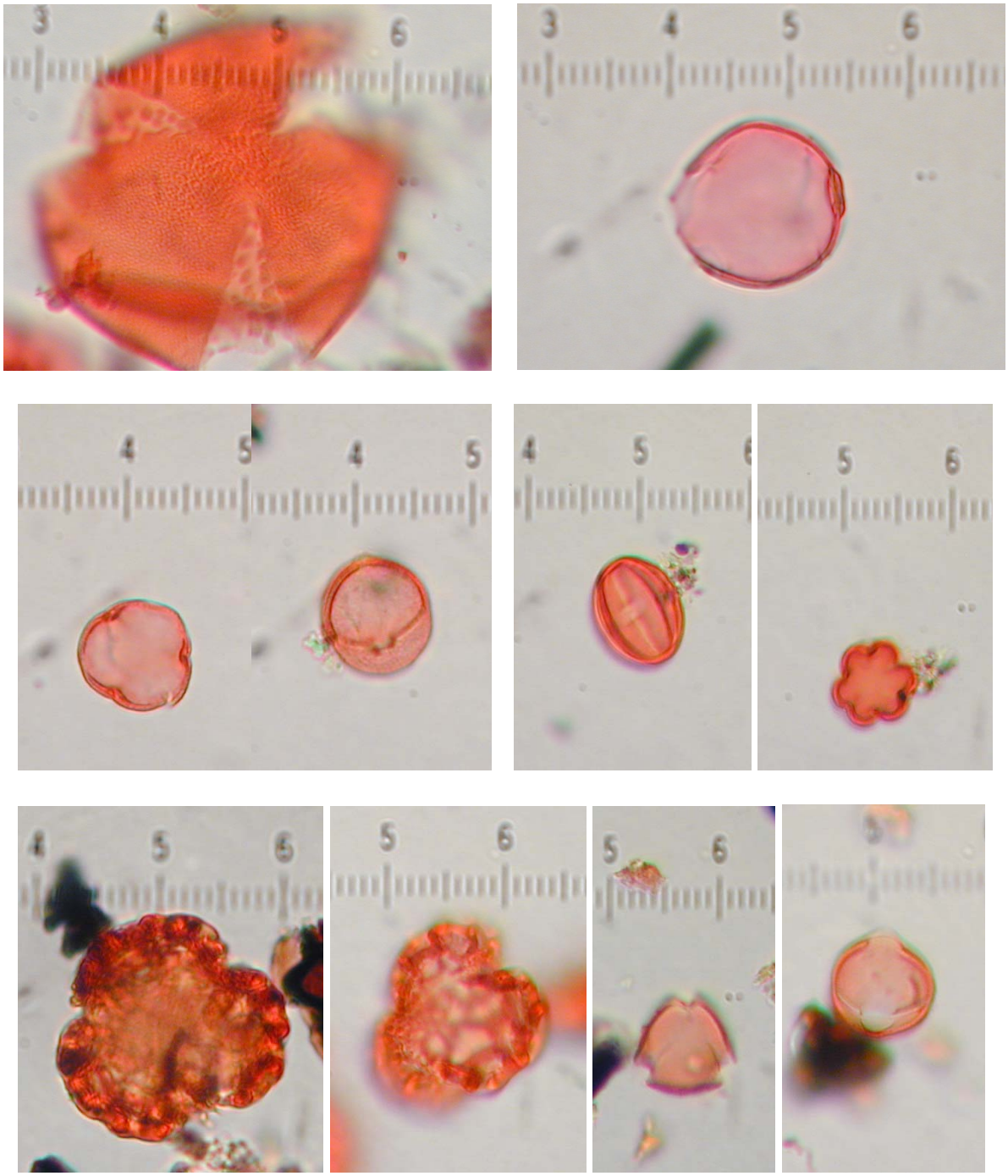


Figure 12. Some of the key pollen types in the sample. Top Row: Abundant, dominant pollen types: Adenodolichos paniculatu (left), and POACEAE (grass, right). Middle Row: Frequently observed pollen types: Rumex (two views, left) and Terminalia brownie (two views, right). Bottom Row: Occasionally observed pollen types: Brachystegi (two views, left) and Zenkerella egregia (two views, right). The distance between the smallest units on the scale is 2.5µm.

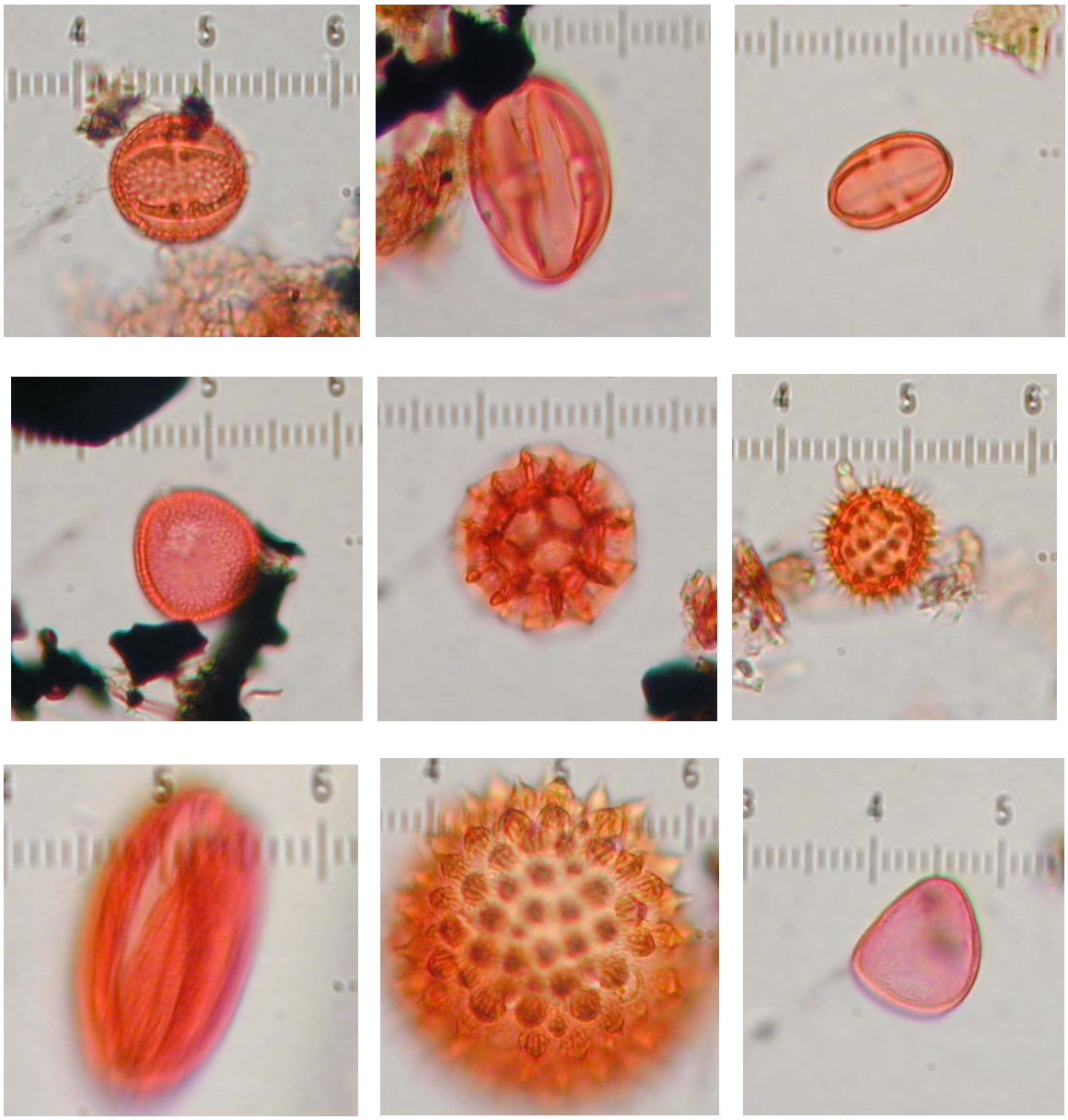


Figure 13. Some of the pollen types found in small numbers in the sample. Top Row: Citrus, Diospyros mespiliformis, and Polygonium. Middle Row: Typha, ASTERACEA (Lactuceae type), and ASTERACEA (Helianthinae type); Bottom Row: Gilbertiodendron dewevri, Sida acuta, CYPERACEAE. The distance between the smallest units on the scale is 2.5µm.

Figure 14. Some of the many genera of fungal spores found in the sample. The distance between the smallest units on the scale is 2.5µm.

Non-Human DNA

Table 8 shows the taxa identified by their DNA. Six plant taxa were identified along with three fungal taxa.

Botanical DNA

Colophospermum mopane (mopane tree) is a characteristic tree found in Africa that grows in hot, dry, low-lying areas.

Flueggea (bushweeds) is a genus of some 50+ species. The DNA recovered from this sample had over 50% of the data sequences of this type, which is most closely related to (but distinct from) available reference DNA of *Flueggea virosa* (Chinese waterberry) and *Flueggea leucopyrus.* Bushweeds are distributed in Eastern Hemisphere tropical zones.

Bauhinia (orchid tree) is a genus of 500+ species distributed in the tropics and warm temperate climates. Many species are grown as ornamentals.

The *Triticum* (wheat genus) plant DNA is identified to genus level and could be from domestic wheat or wild varieties. Similarly, the *Zea* (corn genus) plant DNA is also identified to genus level and could be from domestic corn or wild varieties.

The FABACEAE (legume family) is very large with 700+ genera and nearly 20,000 species. The plant DNA present in the sample is most closely related to (but distinct from) available reference DNA of the genus *Podalyria* and *Calpurnia.* Both are shrubs or small trees found in southern Africa.

Fungal DNA

Aspergillus penicilloides is a common aerobic, filamentous mold. *Eurotium amstelodami* is closely related to *Aspergillus* species, as its (distinctly separate) sexually reproductive stage. The *Malassezia* fungal DNA is identified to the genus level. It is a type of yeast, naturally found on the skin surfaces of many animals (including man).

Table 8. Taxa Identified by DNA Sequence Analysis and Molecular Systematics

Plants

Colophospermum mopane (mopane tree)
Flueggea (bushweed)
Bauhinia (orchid tree)
Triticum (wheat genus)
Zea (corn genus)
FABACEAE close to genus *Podalyria* or *Calpurnia*

Fungi

Aspergillus penicilloides
Eurotium amstelodami
Malassezia

ANALYTICAL SUMMARY AND INTERPRETATIONS

Overview

There was a strong signal of naturally occurring minerals, containing a wide variety that is well distributed by size into sand, silt and clay (enabling extensive analysis of each). There was also a very wide variety of pollen and spore types present, contributing to the signal through their individual identifications, relative quantities and condition, co-occurrences and correlations. Additional signals were identified using botanical DNA.

Geological Environment

The minerals in the sample are composed of both light and dark grains, with small quantities of plant matter and insect fragments. The grains are fairly well sorted, with most of the material being fine sand and silt, primarily in the < 180µm size range. Excluding starch and other non-geologic material, the fine sand is composed primarily of iron oxides, making up roughly 42% of the fine sand. Quartz was the next most abundant component, making up 21% of the sample and occurring primarily as rounded, iron-stained grains. Altered, unidentifiable grains were a low major component (12%); biotite (8%) and feldspar (8%) were high minor components. The feldspar was almost two-thirds alkali feldspar and one-third plagioclase, primarily occurring as iron-stained altered grains. Hornblende (6%) and opaque minerals (1%) were minor components of the sand, and there were trace amounts of plant opal, lithic fragments, muscovite mica, titanite, sillimanite, pyroxene, zircon, apatite, epidote, garnet, dolomite, rutile, glauconite, tourmaline, tremolite, and two unidentified minerals. The silt fraction contained generally similar minerals as the fine sand fraction. There was a significant amount of clay in the sample, dominated by illite along with minor kaolinite.

Excluding iron oxides (a major portion of which is likely anthropogenic), the fine sand is dominated by quartz grains (36%) and highly altered, unidentifiable minerals (20%). Feldspar minerals and biotite each make up 14% of the fine sand excluding iron oxides, and hornblende is 10% of this fraction. Opaque minerals and muscovite mica are low minor components, with trace amounts of plant opal, lithic fragments, titanite, sillimanite, pyroxene, zircon, apatite, epidote, garnet, dolomite, rutile, glauconite, tourmaline, tremolite, and two unidentified minerals.

The majority of the sediment has a composition consistent with an ironstone or iron-rich sandstone (if a major portion of the iron oxides are geologic in origin) or a feldspathic sandstone (if a major portion of the iron oxides are anthropogenic). There is likely an intermediate or mafic rock that contributed to the sediment, as indicated by the relatively large amounts of biotite and hornblende. The rounded nature of the quartz and weathered nature of the feldspar minerals indicates that this is likely from fairly mature sediment, supported by the significant amount of kaolinite clay. The virtual absence of carbonates (a single dolomite grain in the heavy fraction was the only carbonate observed) indicates that there is no limestone in the vicinity. The sample contained a relatively large amount of mineral grains significantly larger than 60µm, and there were no characteristic volcanic minerals or morphologies observed. This indicates that there are unlikely to be volcanic rocks contributing to the sediment.

Overall, the geological analysis indicates the minerals originated from ironstone or sandstone, or from a sedimentary deposit of aeolian or fluvial nature.

Ecological Environment

Based on the pollen, fungal spores and DNA there is strong support for a tropical region.

- Pollen from insect-pollinated plants are dominant. Most tropical plants are insect pollinated.
- A bimodal pollen size distribution was observed. This is typical for tropical regions, and atypical for temperate regions. In tropical regions (and in this sample), most of the pollen are either small (5 to 25µm) or large (50 to 100µm). In temperate regions, on the other hand, the vast majority of pollen types range in size between 25 and 45µm in diameter.
- Many fungi are present, in high diversity, along with many pollen grains that were degraded beyond recognition. This is typical for warm and humid habitats in tropical regions, where microbial activity and chemical oxidation are high.

The fungal spores are more resistant to oxidation, and much of the pollen become highly degraded.

- Botanical taxa identified by DNA are characteristic of tropical environments. This includes *Flueggea* (bushweeds), *Bauhinia* (orchid tree), *Podalyria/Calpurnia,* and *Colophospermum mopane* (mopane tree).

Within the tropical region, there is strong support for a dominant grassland or savanna habitat.

- The abundance and diversity of grasses indicate grasslands or savannas.
- All of the pollen types that were dominant, frequently observed, or occasionally observed come from plants that are typical of savanna and/or open brushland environments. For the most part, these plants are annuals, quick growing, and require open sunlight.

Near the specific site there is a dominant presence of the *Adenodolichos paniculatus* shrub (wáákén wuta, "fire bean") and *Flueggea* (bushweeds).

Within the dominant grassland environment, or closely nearby, there is strong support for scrubland and trees.

- The following trees and shrubs were present: *Terminalia brownii* (hareri biiris), *Brachystegi* (miombo), Colophospermum mopane (mopane) , *Bauhinia* (orchid tree) and *Podalyria* / *Calpurnia*.
- Pollen (occurring in small numbers) are present from an assortment of additional trees and shrubs:
    - *Diospyros mespiliformis* (jackal berry)
    - *Citrus* (orange, lemon, etc.)
    - *Polygonum* (knotweed)
    - MELIACEAE (mahogany family)
    - MYRTACEAE (myrtle family)
    - RHAMNACEAE (buckthorn family)
    - *Gilbertiodendron dewevrei* (limbali, abeum)

There is support for a nearby wetter area. Close by (within an easy wind-blown distance) are *Typha* (cattails), and CYPERACEAE (sedges).[11]

[11] *Although sedges are only indicated by a single pollen grain, this grain is in excellent condition and pollen of this type are (1) produced in low numbers, (2) dispersed close to the ground, and (3) quickly degraded in soil.*

GEOGRAPHICAL INFERENCES

A First Stage Source Attribution Estimate[12] was made based on four primary geographical inferences:

- an initial environmental estimate (based primarily on botanical findings)
- an initial soil estimate (based primarily on mineral findings)
- a taxonomic viability estimate for *Diospyros mespiliformis*
- a taxonomic occurrence estimate for *Colophospermum mopane*

A combined intersection of these estimates resulted in the overall First Stage Source Attribution Estimate.

An Initial Environmental Estimate

A review was conducted of ecoregion profiles [11,12] and their corresponding reports as described in *Terrestrial Ecoregions of Africa and Madagascar: A Conservation Assessment* [13] to assess their correspondence with the potential source environment as inferred from the initial analysis of the small particle traces from the sample. The geographical distribution of these reference data is mapped in Figure 15, using the WWF Terrestrial Ecoregions dataset.[14]

Based on the degree of correspondence with the initial fine particle analysis, twenty-six ecoregions were classified as Excluded and 15 were found to have Poor Fit correspondence (Table 9). This assessment was based primarily on botanical signals (pollen and plant DNA) and to a lesser extent on mineralogical signals (e.g. soil). The remaining 65 ecoregions within Africa contain at least one botanical or ecological feature that could contribute to the signals obtained from the sample. This initial environmental assessment was mapped using GIS by reclassifying the WWF Terrestrial Ecoregions dataset into three classes: Possible, Poor Fit, and Excluded as a potential source of the observed environmental signals. The result is shown Figure 16, where the dark gray shows Excluded regions, the lighter gray, Poor Fit regions, and the white showing possible remaining regions.

[12] *See discussion in the following section, "Next Steps."*

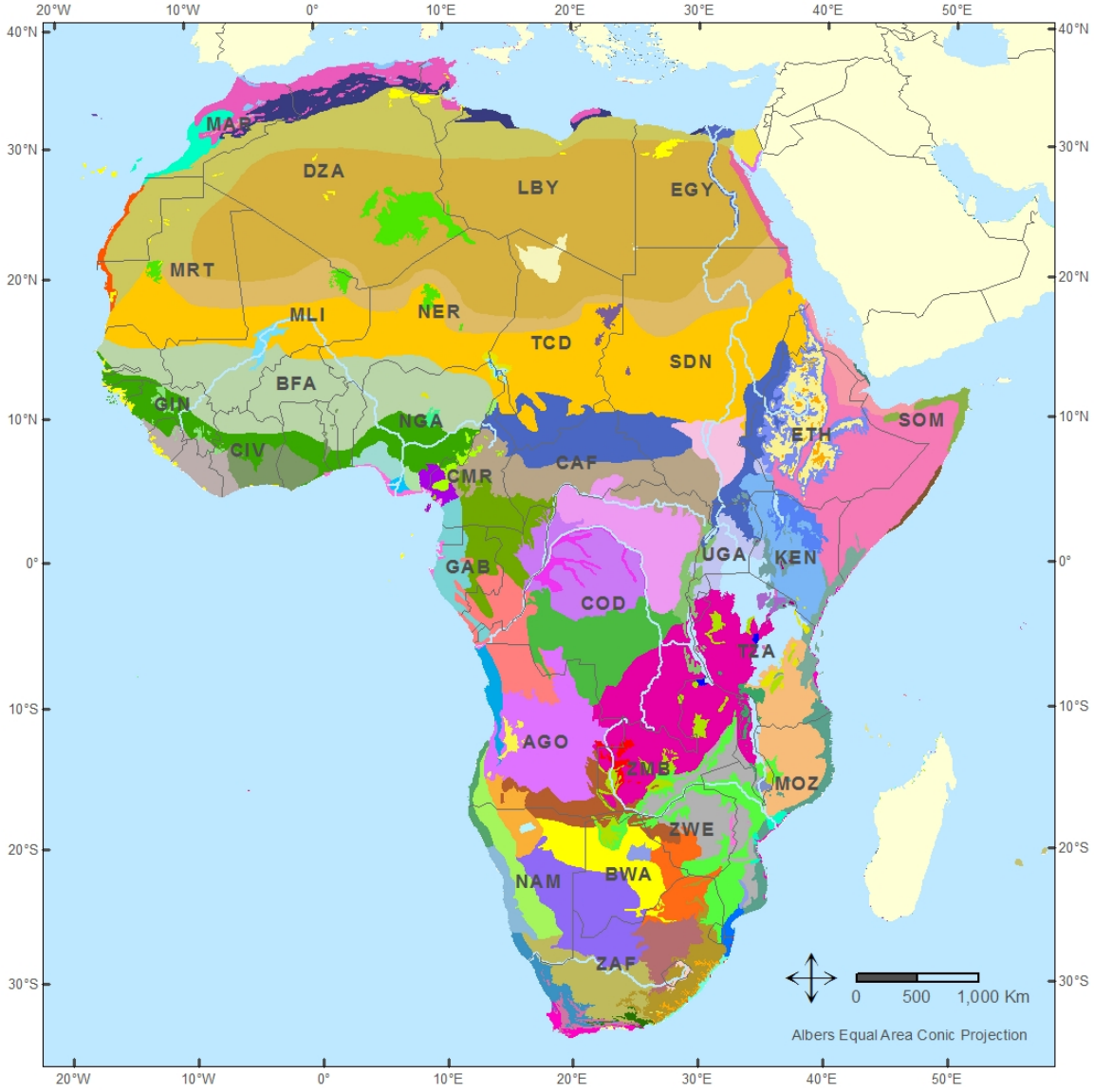


Figure 15. Map illustrating the WWF Terrestrial Ecoregions dataset within continental Africa. There are a total of 106 ecoregions within this area.

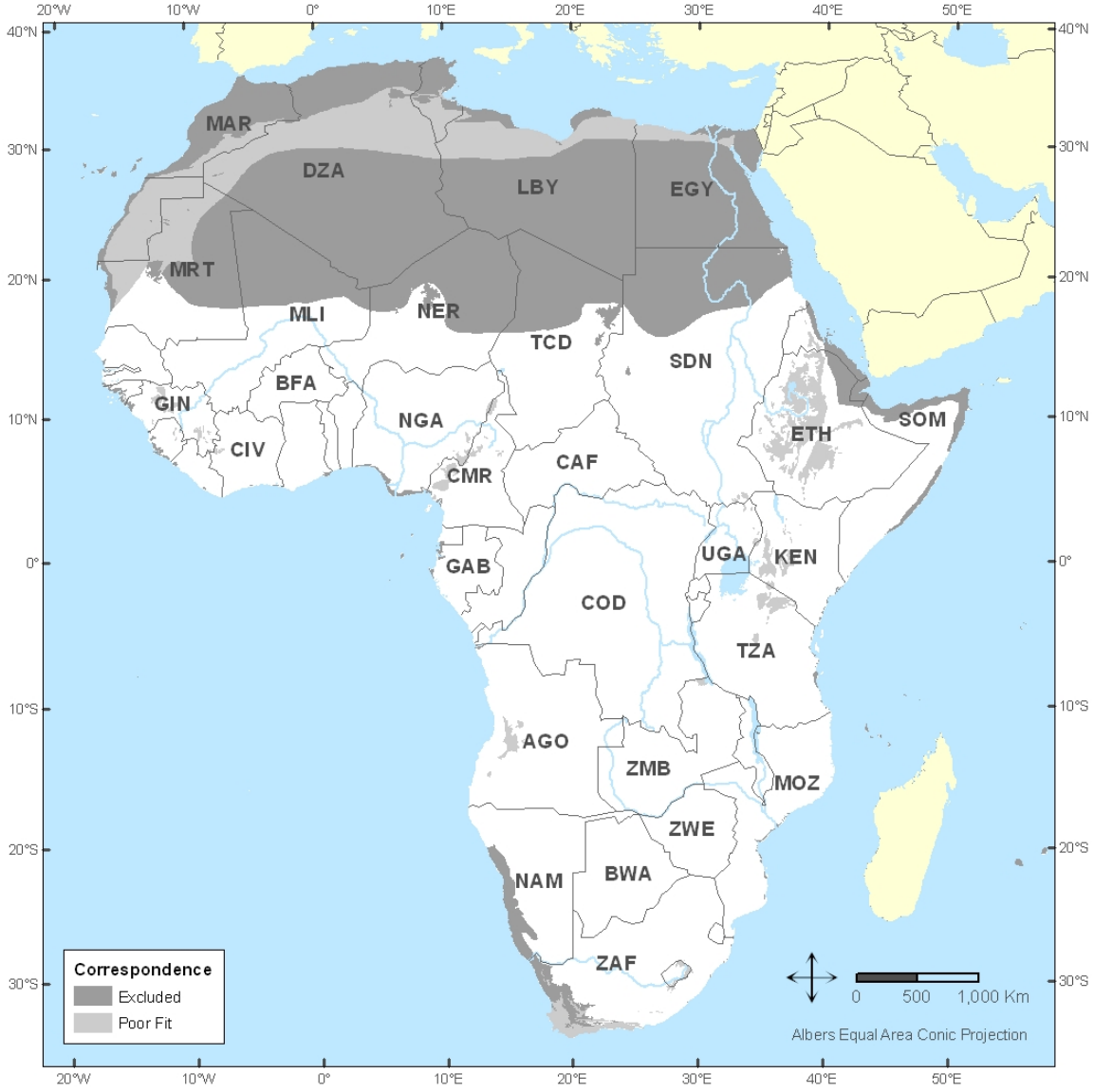


Figure 16. An initial environmental assessment for the African continent. Excluded areas are shown in dark gray. Highly unlikely Poor Fit areas are shown in light gray.

Table 9. Summary of Ecoregions Classified as Excluded or with Poor Fit Correspondence with Observed Environmental Signals

Eco-Code	Realm	Biome	Ecoregion	Class
AT0103	Afrotropic	Tropical and Subtropical Moist Broadleaf Forests	Cameroonian Highlands forests	Poor Fit
AT0108	Afrotropic	Tropical and Subtropical Moist Broadleaf Forests	East African montane forests	Poor Fit
AT0114	Afrotropic	Tropical and Subtropical Moist Broadleaf Forests	Guinean montane forests	Poor Fit
AT0115	Afrotropic	Tropical and Subtropical Moist Broadleaf Forests	Knysna-Amatole montane forests	Poor Fit
AT0121	Afrotropic	Tropical and Subtropical Moist Broadleaf Forests	Mount Cameroon and Bioko montane forests	Poor Fit
AT0708	Afrotropic	Tropical and Subtropical Grasslands, Savannas, and Shrublands	Itigi-Sumbu thicket	Poor Fit
AT0710	Afrotropic	Tropical and Subtropical Grasslands, Savannas, and Shrublands	Mandara Plateau mosaic	Poor Fit
AT0714	Afrotropic	Tropical and Subtropical Grasslands, Savannas, and Shrublands	Serengeti volcanic grasslands	Poor Fit
AT1001	Afrotropic	Montane Grasslands and Shrublands	Angolan montane forest-grassland mosaic	Poor Fit
AT1003	Afrotropic	Montane Grasslands and Shrublands	Drakensberg alti-montane grasslands and woodlands	Poor Fit
AT1007	Afrotropic	Montane Grasslands and Shrublands	Ethiopian montane grasslands and woodlands	Poor Fit
AT1008	Afrotropic	Montane Grasslands and Shrublands	Ethiopian montane moorlands	Poor Fit
AT1202	Afrotropic	Mediterranean Forests, Woodlands, and Shrublands	Lowland fynbos and renosterveld	Poor Fit
AT1203	Afrotropic	Mediterranean Forests, Woodlands, and Shrublands	Montane fynbos and renosterveld	Poor Fit

Eco-Code	Realm	Biome	Ecoregion	Class
AT1303	Afrotropic	Deserts and Xeric Shrublands	East Saharan montane xeric woodlands	Excluded
AT1304	Afrotropic	Deserts and Xeric Shrublands	Eritrean coastal desert	Excluded
AT1305	Afrotropic	Deserts and Xeric Shrublands	Ethiopian xeric grasslands and shrublands	Excluded
AT1307	Afrotropic	Deserts and Xeric Shrublands	Hobyo grasslands and shrublands	Excluded
AT1315	Afrotropic	Deserts and Xeric Shrublands	Namib desert	Excluded
AT1317	Palearctic	Deserts and Xeric Shrublands	Red Sea coastal desert	Excluded
AT1319	Afrotropic	Deserts and Xeric Shrublands	Somali montane xeric woodlands	Excluded
AT1322	Afrotropic	Deserts and Xeric Shrublands	Succulent Karoo	Excluded
AT1401	Afrotropic	Mangroves	Central African mangroves	Excluded
AT1402	Afrotropic	Mangroves	East African mangroves	Excluded
AT1403	Afrotropic	Mangroves	Guinean mangroves	Excluded
AT1405	Afrotropic	Mangroves	Southern Africa mangroves	Excluded
PA0513	Palearctic	Temperate Conifer Forests	Mediterranean conifer and mixed forests	Excluded
PA0904	Palearctic	Flooded Grasslands and Savannas	Nile Delta flooded savanna	Excluded
PA0905	Palearctic	Flooded Grasslands and Savannas	Saharan halophytics	Excluded
PA1010	Palearctic	Montane Grasslands and Shrublands	Mediterranean High Atlas juniper steppe	Excluded
PA1212	Palearctic	Mediterranean Forests, Woodlands, and Shrublands	Mediterranean acacia-argania dry woodlands and succulent thickets	Excluded
PA1213	Palearctic	Mediterranean Forests, Woodlands, and Shrublands	Mediterranean dry woodlands and steppe	Excluded
PA1214	Palearctic	Mediterranean Forests, Woodlands, and Shrublands	Mediterranean woodlands and forests	Excluded

Eco-Code	Realm	Biome	Ecoregion	Class
PA1303	Palearctic	Deserts and Xeric Shrublands	Arabian Desert and East Sahero-Arabian xeric shrublands	Excluded
PA1304	Palearctic	Deserts and Xeric Shrublands	Atlantic coastal desert	Excluded
PA1321	Palearctic	Deserts and Xeric Shrublands	North Saharan steppe and woodlands	Poor Fit
PA1325	Palearctic	Deserts and Xeric Shrublands	Red Sea Nubo-Sindian tropical desert and semi-desert	Excluded
PA1327	Palearctic	Deserts and Xeric Shrublands	Sahara desert	Excluded
PA1329	Palearctic	Deserts and Xeric Shrublands	South Saharan steppe and woodlands	Excluded
PA1331	Palearctic	Deserts and Xeric Shrublands	Tibesti-Jebel Uweinat montane xeric woodlands	Excluded
PA1332	Palearctic	Deserts and Xeric Shrublands	West Saharan montane xeric woodlands	Excluded

An Initial Soil Estimate

A review was conducted of dominant soil types as defined in the UN-FAO Digital Soil Map of the World[15] to assess their correspondence with the potential source location as inferred from the initial analysis of the small particle traces from the sample. The geographical distribution of these reference data within continental Africa is mapped in Figure 17.

Based on the degree of correspondence of soil descriptions with the initial fine particle analysis, 20 dominant soil types were excluded as possible source locations, and seven were classified as having poor fit as a potential source location (Table 10). The remaining 60 dominant soil type remained as possible source locations. This initial soil correspondence assessment was mapped using GIS by reclassifying the dominant soil types of the Digital Soil Map of the World dataset as Possible, Poor Fit, or Excluded potential source locations. The result is shown Figure 18, where the dark gray shows Excluded regions, the lighter gray, Poor Fit regions, and the white showing possible remaining regions.

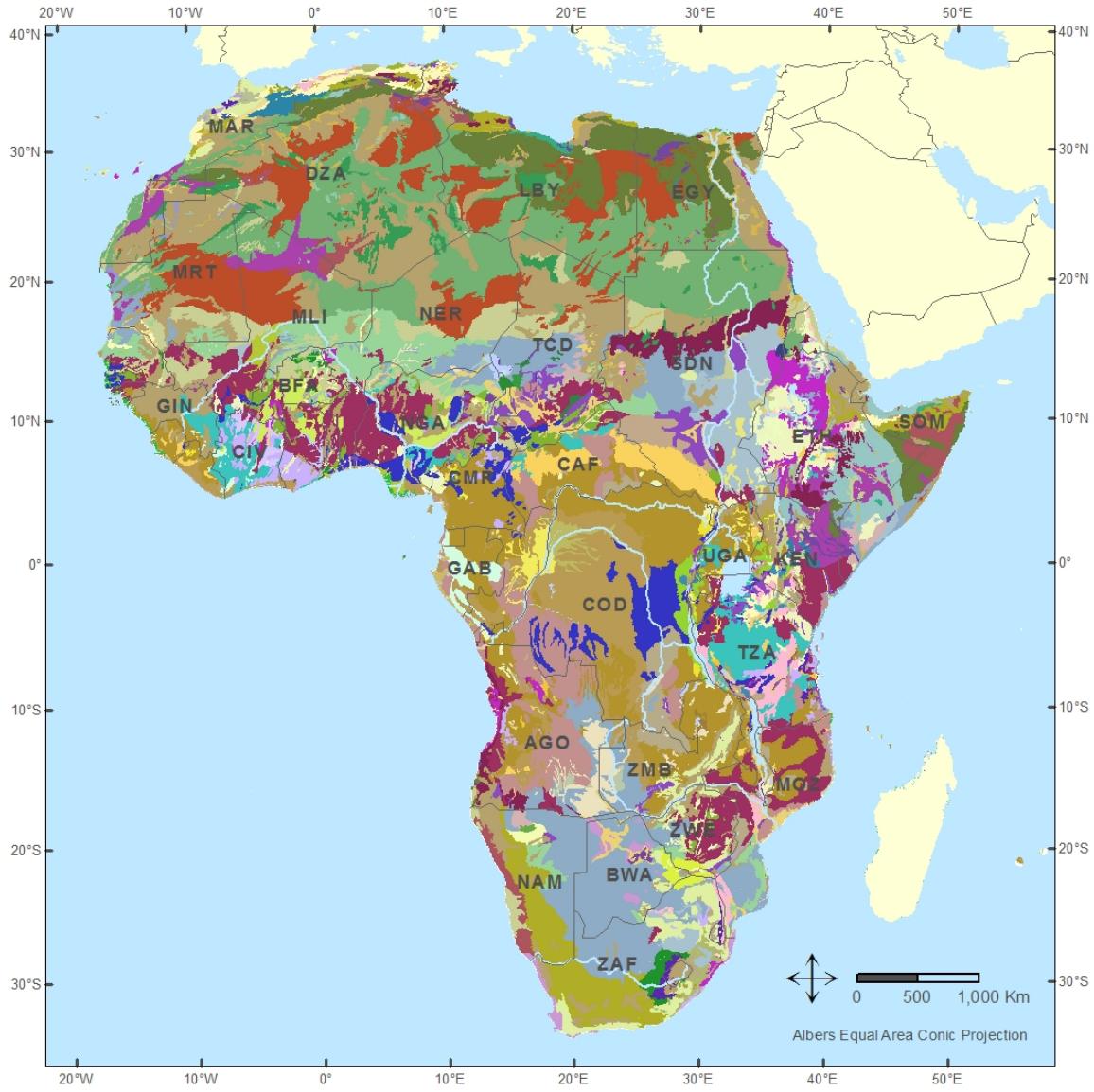


Figure 17. Map illustrating the UN-FAO Digital Soil Map of the World dataset within continental Africa. There are a total of 87 dominant soil types within this area.

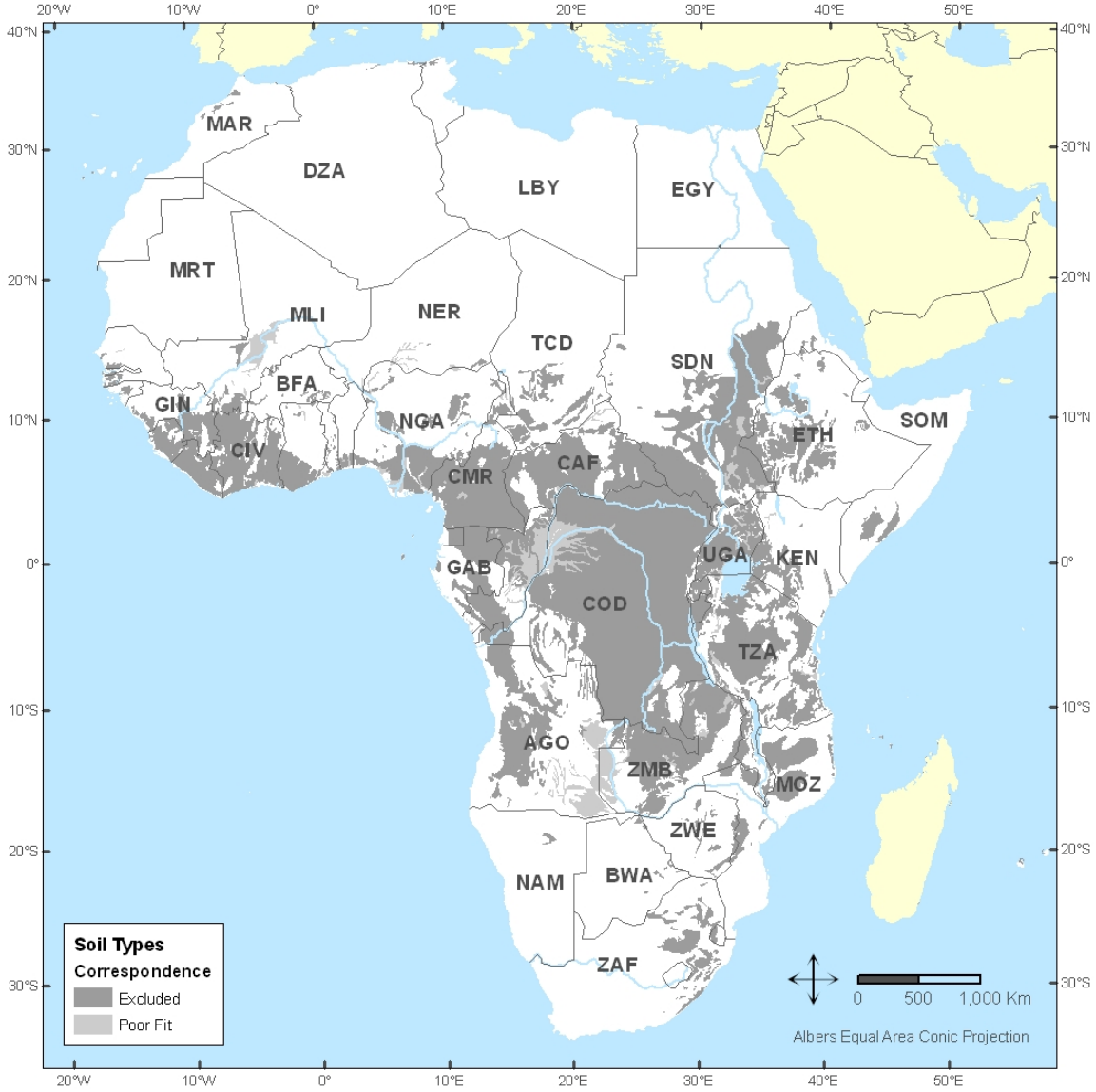


Figure 18. An initial soil assessment for the African continent. Excluded areas are shown in dark gray. Highly unlikely Poor Fit areas are shown in light gray.

Table 10. Summary of Soil Types Classified as having Poor Fit Correspondence with Geological Signals or Excluded as a Potential Source Location

Soil Type	Class
Af: Ferric Acrisols	Excluded
Ag: Gleyic Acrisols	Excluded
Ah: Humic Acrisols	Excluded
Ao: Orthic Acrisols	Excluded
Ap: Plinthic Acrisols	Excluded
Fa: Acric Ferralsols	Excluded
Fh: Humic Ferralsols	Excluded
Fo: Orthic Ferralsols	Excluded
Fp: Plinthic Ferralsols	Excluded
Fr: Rhodic Ferralsols	Excluded
Fx: Xanthic Ferralsols	Excluded
Gc: Calcaric Gleysols	Poor Fit
Gd: Dystric Gleysols	Poor Fit
Ge: Eutric Gleysols	Poor Fit
Gh: Humic Gleysols	Poor Fit
Gm: Mollic Gleysols	Poor Fit
Gp: Plinthic Gleysols	Poor Fit
Gx: Gelic Gleysols	Poor Fit
Nd: Dystric Nitosols	Excluded
Ne: Eutric Nitosols	Excluded
Nh: Humic Nitosols	Excluded
Th: Humic Andosols	Excluded
Tm: Mollic Andosols	Excluded
To: Ochric Andosols	Excluded
Tv: Vitric Andosols	Excluded
Vc: Chromic Vertisols	Excluded
Vp: Pellic Vertisols	Excluded

A Taxonomic Viability Estimate for Diospyros mespiliformis

Pollen present in the sample showed the proximity of the African ebony or jackal berry tree (*Diospyros mespiliformis*). This species is a medium to large sized tree that is widespread in tropical regions of Africa. It is most commonly found in savannas and savanna woodlands at elevations below 1,300 m, and is absent from the rain forests of the Guinea Congolian Region except locally along their northern fringes. A taxonomic viability estimate was made based on climate suitability using the EcoCrop modeling approach and DIVA-GIS software.[16] This climate suitability model uses long-term monthly temperature and precipitation data from the WorldClim database [17] to predict the adaptation of a specific plant over geographic areas based on the precipitation, temperature, and growing season thresholds. These climate suitability parameters for *Diospyros mespiliformis* were taken from the EcoCrop online database.[18]

The EcoCrop model generated six categories for the potential growth of *Diospyros mespiliformis*: Excellent, Very Suitable, Suitable, Marginal, Very Marginal and Unsuitable. Figure 19 shows the areas ranging from Excellent to Suitable as green, and those from Marginal to Very Marginal as yellow. The white areas are Unsuitable for the potential growth of *Diospyros mespiliformis*.

A Taxonomic Occurrence Estimate for Colophospermum mopane

The mopane tree, *Colophospermum mopane* was detected in the sample by DNA analysis. This species is a conspicuous and well-recognized one, which occurs as either a shrub or tree depending on local conditions. The range of the genus *Colophospermum* is described in a number of flora references [19-25]. It is endemic to the Zambezian floristic region of southern Africa, which encompasses approximately 3,770,000 sq km. There is a recorded distribution within parts of Angola, Botswana, Malawi, Mozambique, Namibia, South Africa, Zambia, and Zimbabwe. There are no significant records of this species being cultivated outside of its native distribution.

African ecoregion profiles [11-13] were reviewed for the recorded presence of *Colophospermum mopane* as a dominant, common, or noteworthy vegetation type. Ecoregions without a recorded presence of mopane were assessed to determine whether they could support this species in ecotone or transitional environments, such as in proximity to borders with ecoregions having a recorded presence of mopane. Nine ecoregions were found to have a recorded presence of mopane, and seven ecoregions were found to contain transitional areas and / or ecological features that could support the presence of mopane (Table 11). This taxonomic occurrence assessment was mapped using GIS (Figure 20) by reclassifying the WWF Terrestrial Ecoregions dataset [14] according to the recorded presence, possible presence, or no recorded presence of mopane in the respective ecoregions of Africa.

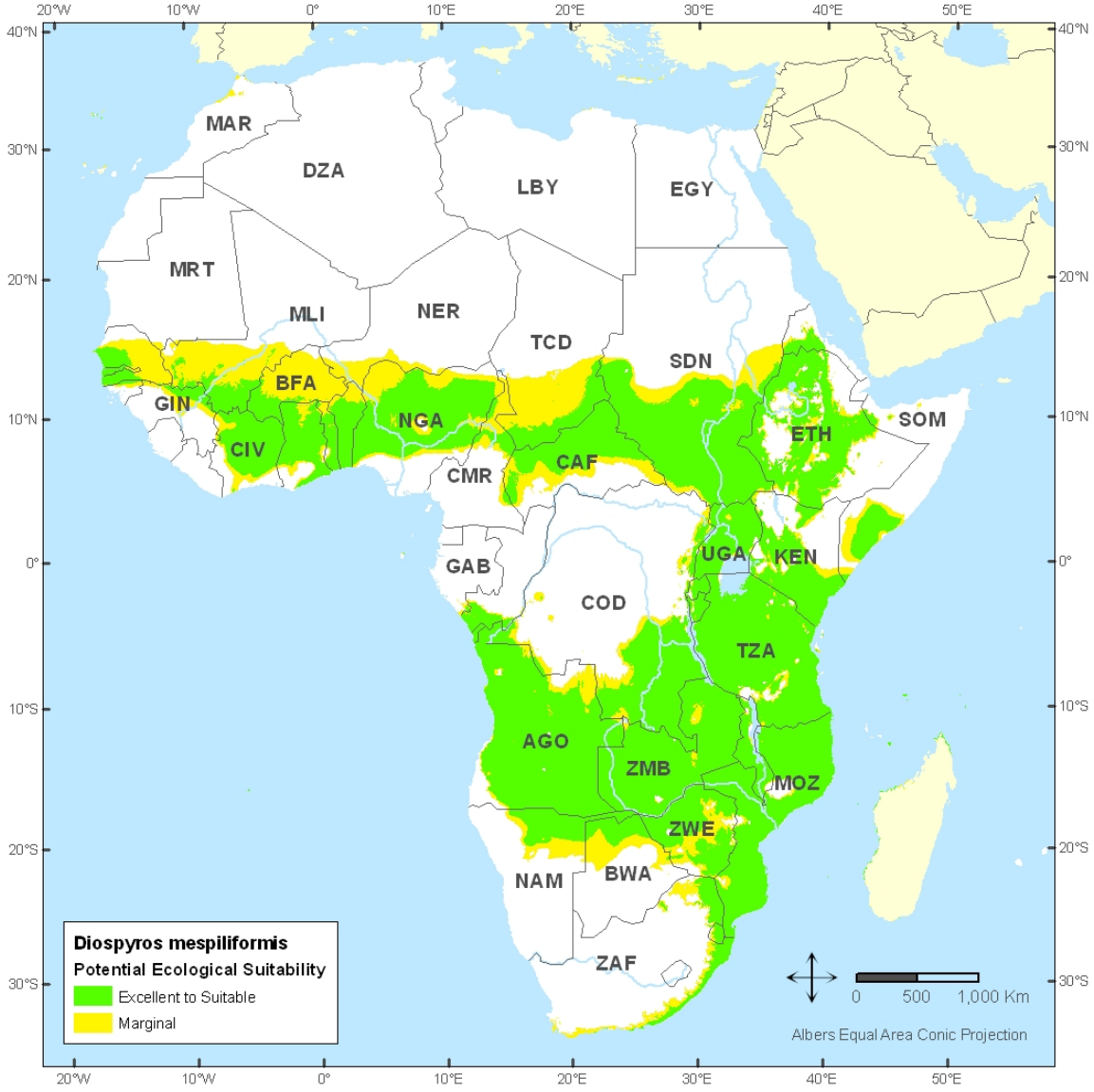


Figure 19. Taxonomic viability estimate for Diospyros mespiliformis *based on EcoCrop modeling. The areas in green are those with Excellent to Suitable ecology. Those in yellow are Marginal to Very Marginal. The white areas have Unsuitable ecology for the growth of this species.*

Table 11. Summary of ecoregions with the recorded or possible presence of mopane.

EcoCode	Ecoregion Name	Presence of *Mopane*
AT0203	Zambezian Cryptosepalum dry forests	Recorded
AT0701	Angolan Miombo woodlands	Recorded
AT0702	Angolan Mopane woodlands	Recorded
AT0704	Central Zambezian Miombo woodlands	Recorded
AT0706	Eastern Miombo woodlands	Possible
AT0709	Kalahari Acacia-Baikiaea woodlands	Recorded
AT0717	Southern Africa bushveld	Recorded
AT0719	Southern Miombo woodlands	Recorded
AT0725	Zambezian and Mopane woodlands	Recorded
AT0726	Zambezian Baikiaea woodlands	Possible
AT0902	Etosha Pan halophytics	Possible
AT0906	Zambezian coastal flooded savanna	Possible
AT0907	Zambezian flooded grasslands	Possible
AT1002	Angolan scarp savanna and woodlands	Possible
AT1310	Kaokoveld desert	Possible
AT1316	Namibian savanna woodlands	Recorded

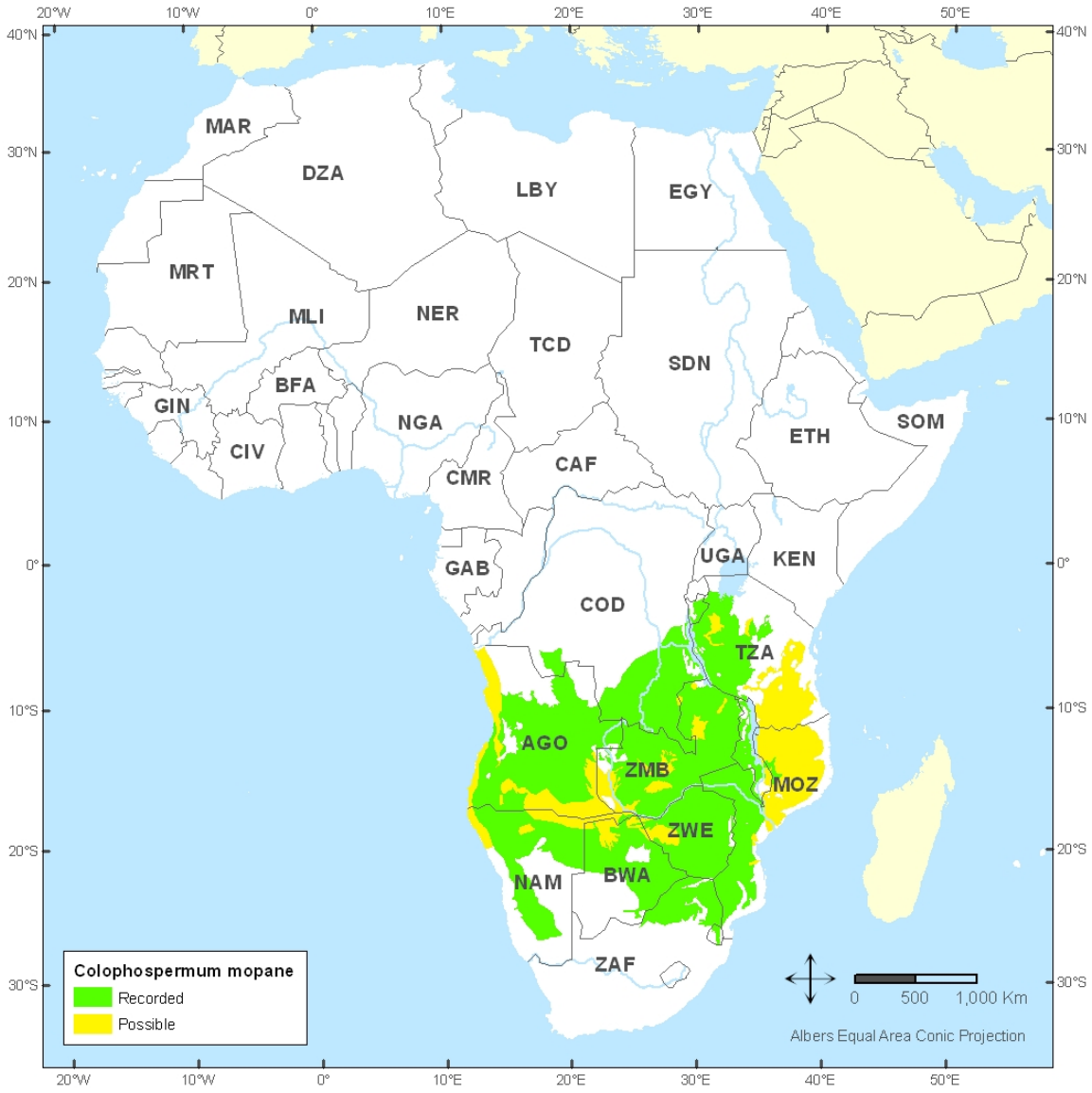


Figure 20. Taxonomic occurrence estimate based on ecoregions on the African continent with a recorded or possible presence of Colophospermum mopane. *White areas have no recorded presence of this species.*

Overall First Stage Source Attribution Estimate

A GIS intersection analysis was conducted using the four estimates: the initial environmental estimate, the initial soil estimate, the taxonomic viability estimate for *Diospyros mespiliformis* and the taxonomic occurrence estimate for *Colophospermum mopane*. Areas within continental Africa were eliminated by the following four criteria:

1. Those areas determined to be Excluded and Poor Fit from the initial environmental estimate (dark gray and light gray areas, Figure 16).
2. Those areas determined to be Excluded and Poor Fit from the initial soil estimate (dark gray and light gray areas, Figure 18).
3. Those areas determined to be Unsuitable for the taxonomic viability of *Diospyros mespiliformis* (white areas, Figure 19).
4. Those areas without the recorded presence of *Colophospermum mopane* (white areas, Figure 20)

The result is the area shown in Figure 21, which has 91.3% of the area in continental Africa eliminated.[13] Of the 48 continental countries, three quarters have been eliminated, leaving the 12 countries shown in Table 12.

Figure 22 shows a close-up of this region, and differentiates the area into Best Fit and Next Best Fit Areas. This differentiation highlights the areas where the modeled taxonomic viability of *Diospyros mespiliformis* is ranked as Excellent to Suitable (Best Fit; green areas in Figures 19 and 22) and those where the viability is ranked as Marginal to Very Marginal (Next Best Fit; yellow areas in Figures 19 and 22).

[13] In this GIS analysis, the total area of continental Africa is was calculated as 29,387,399 sq km, and the green region as 2,557,213 sq km.

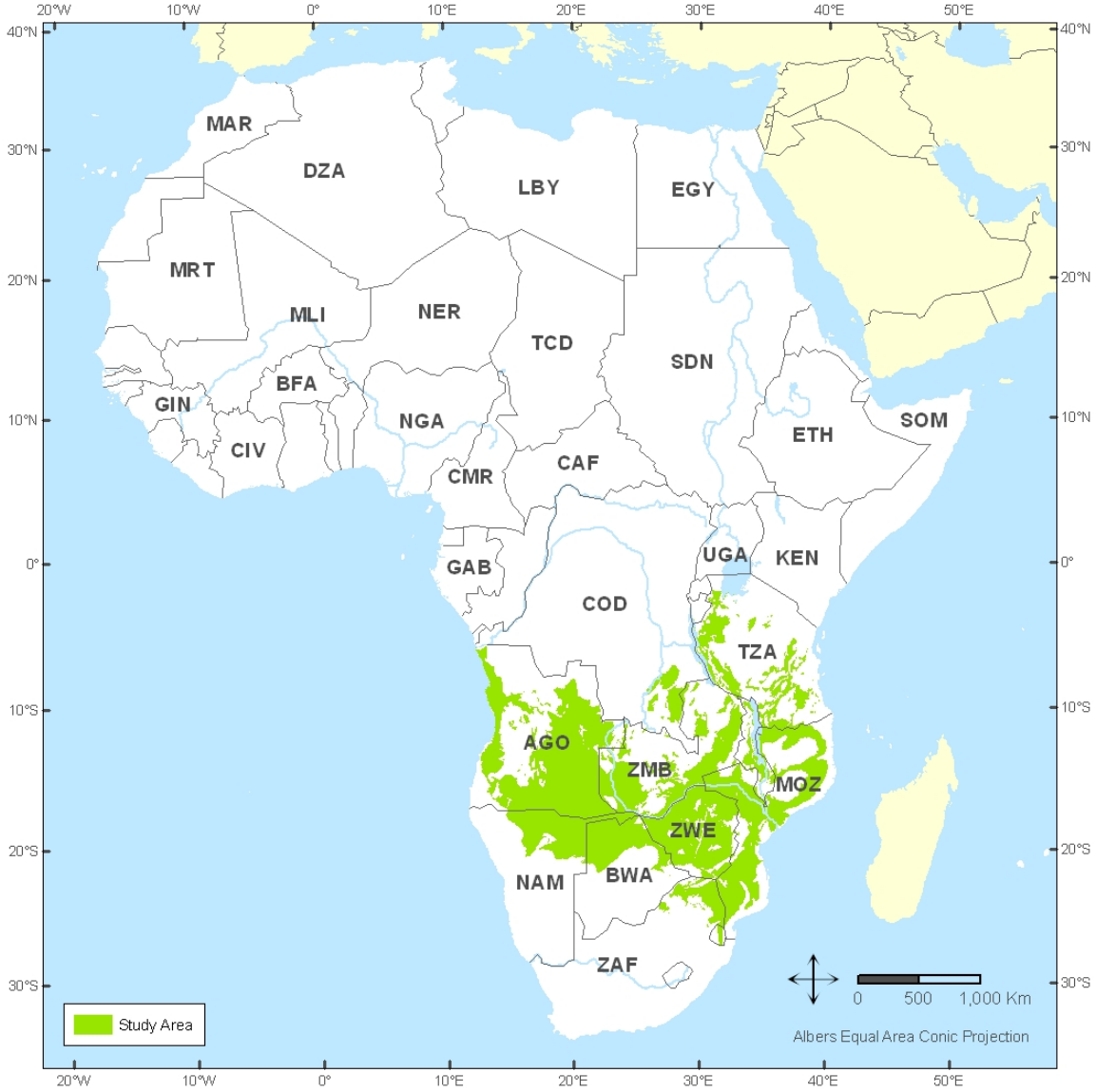


Figure 21. First stage source attribution estimate from the GIS intersection analysis combining the initial environmental estimate, the initial soil estimate, the taxonomic viability estimate for Diospyros mespiliformis *and the taxonomic occurrence estimate for* Colophospermum mopane.

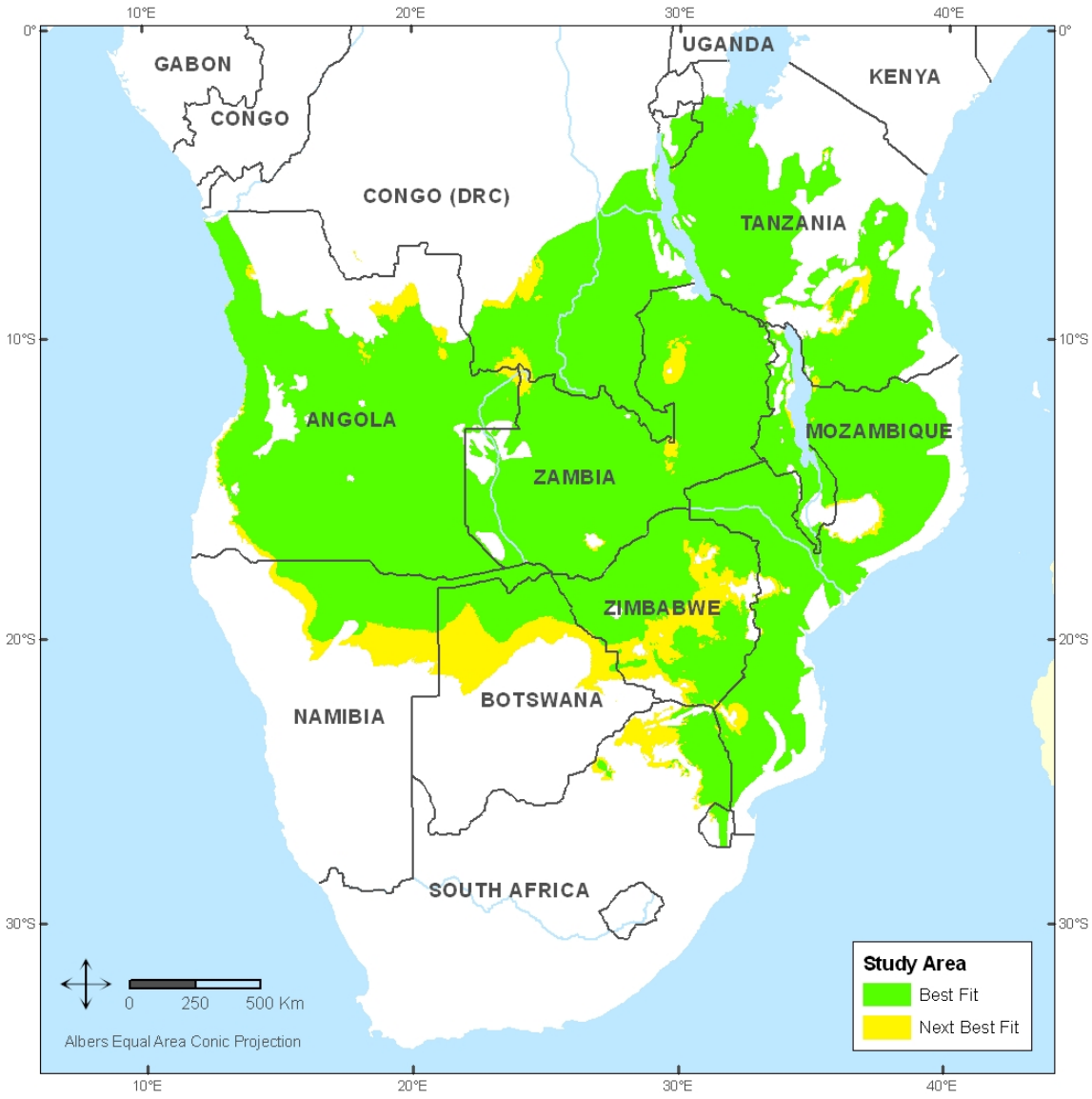


Figure 22. Close-up of the area shown in Figure 21, differentiated to show the Best Fit areas (where the taxonomic viability of Diospyros mespiliformis *is Excellent to Suitable) and Next Best Fit areas (where the taxonomic viability of* Diospyros mespiliformis *is Marginal to Very Marginal).*

Table 12. The Twelve Countries in Continental Africa Remaining in the First Stage Source Attribution Estimate, with Areas and Percentages of the Estimate (sq km)[14]

Country	Country Code	Total Area	Area in Estimate	Percent in Estimate	Percent of Estimate
Angola	AGO	1,252,935	698,893	55.8%	27.3%
Botswana	BWA	579,783	171,816	29.6%	6.7%
Burundi	BDI	27,098	50	0.2%	0.0%
Democratic Republic of the Congo	COD	2,336,471	64,722	2.8%	2.5%
Malawi	MWI	117,440	38,694	32.9%	1.5%
Mozambique	MOZ	793,980	436,166	54.9%	17.1%
Namibia	NAM	827,897	202,216	24.4%	7.9%
South Africa	ZAF	1,219,930	75,464	6.2%	3.0%
Swaziland	SWZ	16,823	5,692	33.8%	0.2%
Tanzania	TZA	942,536	206,308	21.9%	8.1%
Zambia	ZMB	753,941	321,909	42.7%	12.6%
Zimbabwe	ZWE	391,456	335,283	85.7%	13.1%
TOTAL AREA		**9,260,289**	**2,557,213**		

NEXT STEPS

As noted in the previous section, the result shown in Figures 21 and 22 is a First Stage Source Attribution Estimate, eliminating environments comprising approximately 91% of the area, including all areas of 36 countries. First Stage estimates enable source attribution refinements by reducing the area under investigation to one where Next Stage correspondence rankings can progress. These are based on a combination of more detailed reference information, more detailed sample characteristics, and integration with any independently obtained investigative information. In the specific case described here, the First Stage estimate, when combined with other investigative information, was sufficient to meet the case needs. One such combination is shown in Figure 23, where the areas shown in Figure 22 are reduced to those with the known and possible elephant ranges, as reported in the 2007 African Elephant Status Report.[26]

There are numerous options available for Next Stage estimates based on the sample data and variation within the First Stage estimate. For example, reference data covering the area shown in Figure 22 is shown in Figures 24 to 27 as differentiated by

[14] Total Area is that of the entire country, as calculated in this GIS analysis. Area in Estimate is the calculated area, within that country, that is in the First Stage Source Attribution Estimate. Percent in Estimate is the percentage of that country's area that is within the Estimate. Percent of Estimate is the percentage of the Estimate that is within that country.

ecosystems (ecoregions),[14] land cover,[27] soil types,[15] and geologic units.[28] From the reference data and the particles found in this sample, two of the most immediately apparent discriminating data types for the next stage are the minerals, which severely limit the possible geological units, and the close proximity of the *Zenkerella egregia* tree, whose geographical distribution is closely monitored due to its endangered species status.[29]

Based on our experience, we expect these Next Stage estimates to reduce the remaining regions on the African continent by at least another 90%, leaving less than 1%. This would take the case to a point where either another iteration of the process can be conducted (with further refinements) or where the Final Stage of source attribution is enabled.

Final Stage Source Attribution is where specific candidate sites can be indicated as hypotheses, where specific information can be provided to investigators to screen candidate sites, and where comparative testing, using traditional forensic trace evidence processes, can be employed as a test to reject or confirm specific sites.

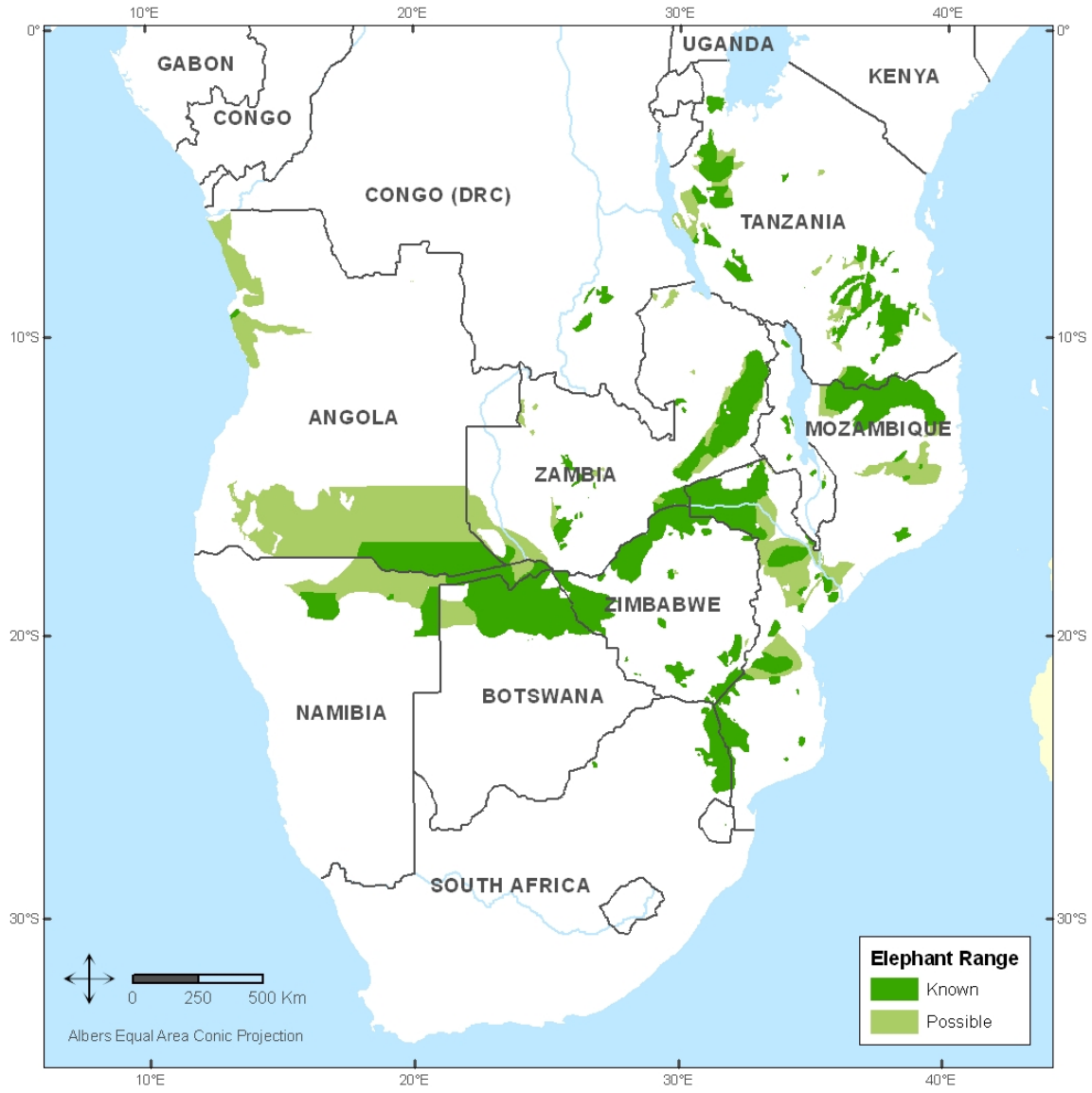
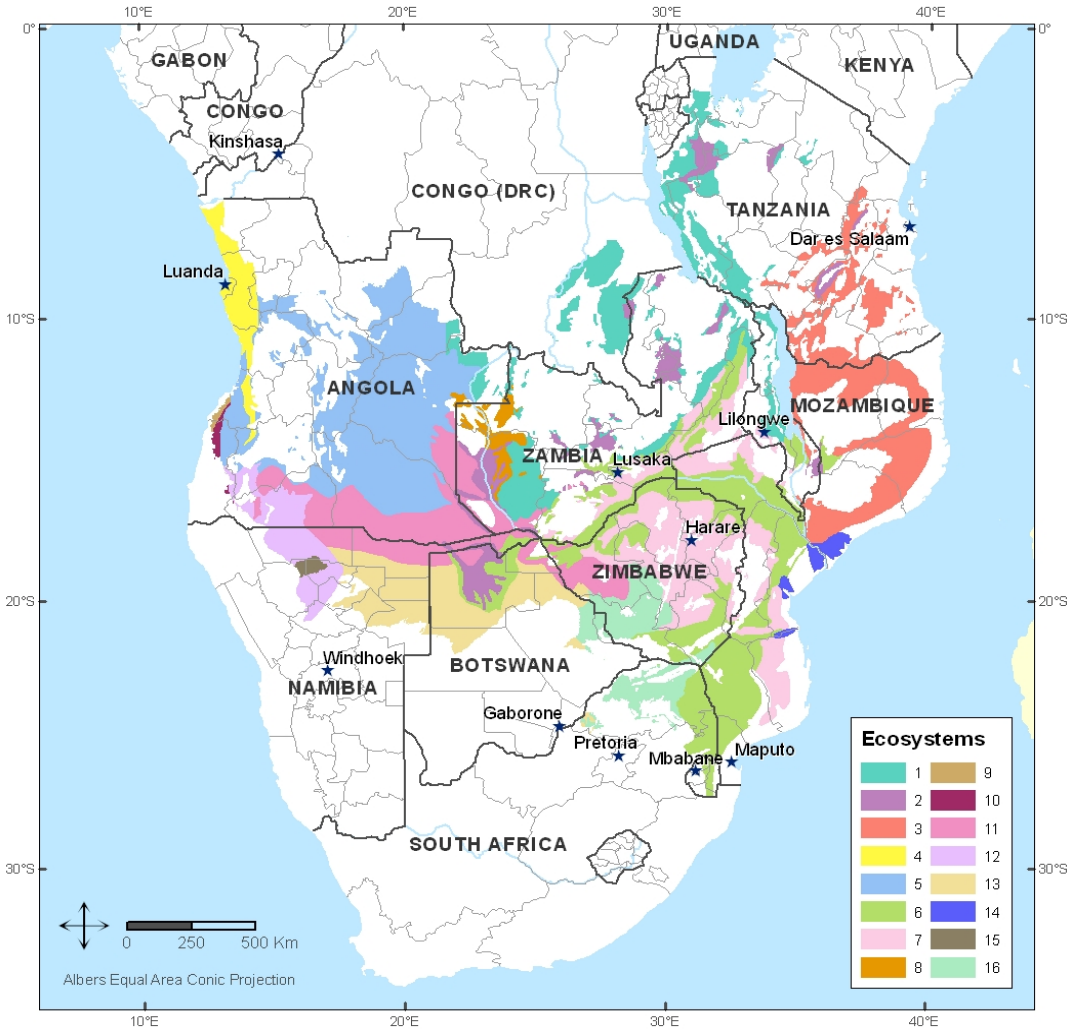


Figure 23. One of the combinations of the First Stage estimate with other investigative information: the areas within the estimate are combined with known and possible elephant ranges.



1	Central Zambezian Miombo woodlands	9	Kaokoveld desert
2	Zambezian flooded grasslands	10	Namibian savanna woodlands
3	Eastern Miombo woodlands	11	Zambezian Baikiaea woodlands
4	Angolan scarp savanna and woodlands	12	Angolan Mopane woodlands
5	Angolan Miombo woodlands	13	Kalahari Acacia-Baikiaea woodlands
6	Zambezian and Mopane woodlands	14	Zambezian coastal flooded savanna
7	Southern Miombo woodlands	15	Etosha Pan halophytics
8	Zambezian Cryptosepalum dry forests	16	Southern Africa bushveld

Figure 24. The First Stage estimate, shown differentiated by sixteen unique ecosystems (ecoregions).

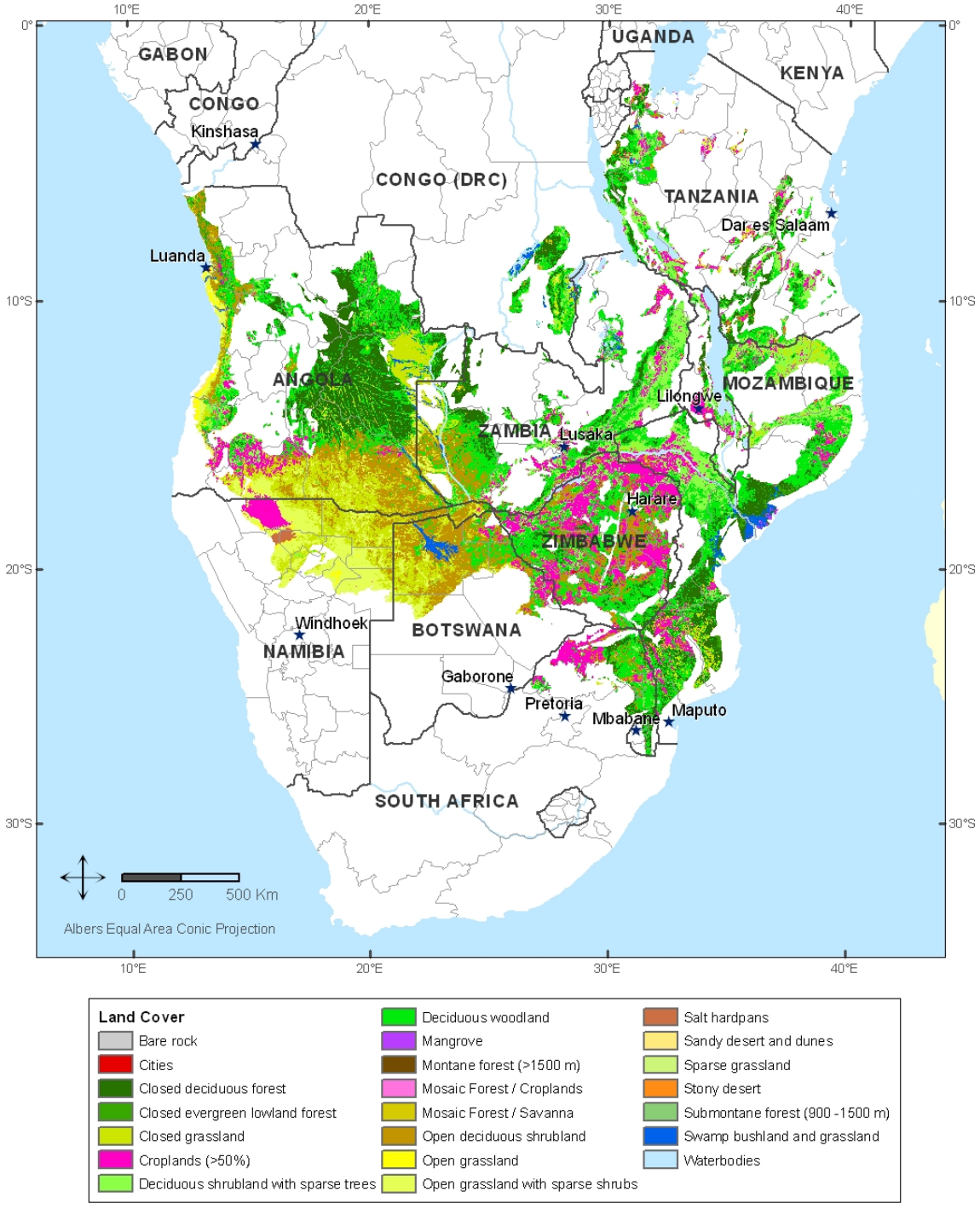


Figure 25. The First Stage estimate, shown differentiated by twenty-two land cover classes.

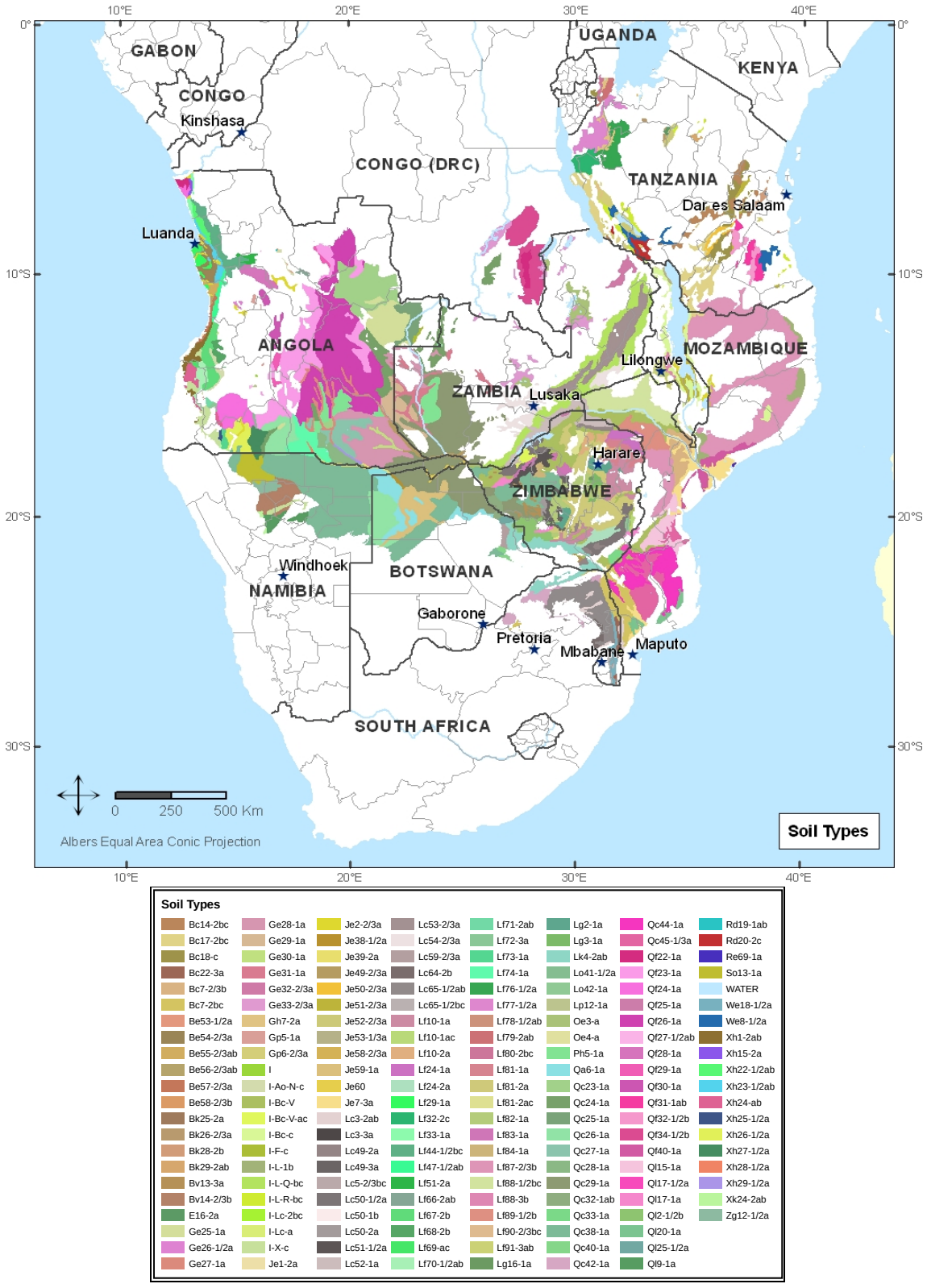


Figure 26. The First Stage estimate, shown differentiated by 172 soil types.

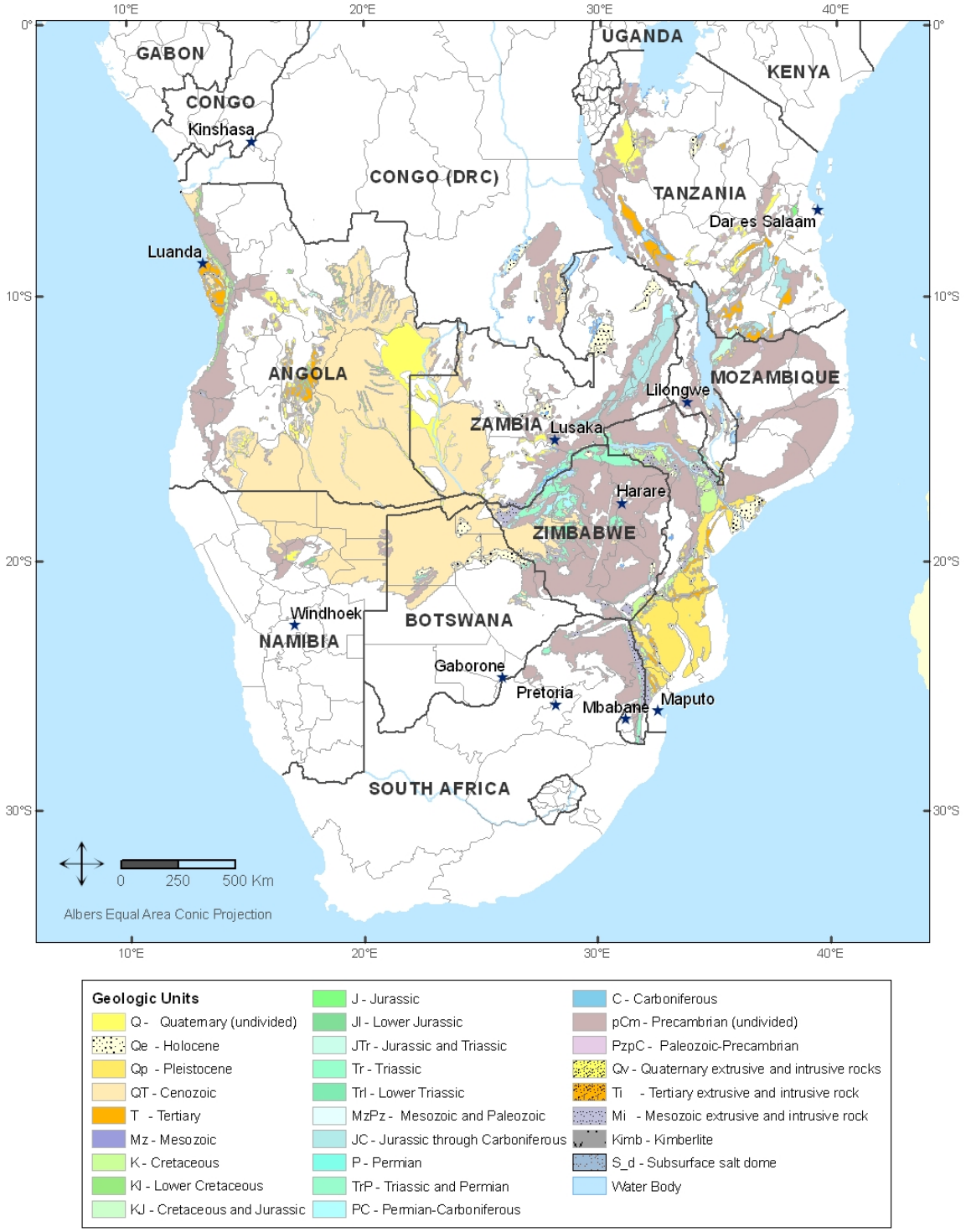


Figure 27. The First Stage estimate, shown differentiated by 27 geologic units.

DISCUSSION

The detailed analysis of dusts, when conducted with expertise appropriate for the sample, and combined with appropriate reference data, allows source attribution inferences that can resolve questions of investigative interest and allow the efficient geographical focus of investigative effort. Dusts are always present, but the specific content is highly variable, with portions that convey aspects of (for example) ecology, climate, botany, soil, geological environment, land use, geography and specific human activities. References providing the geographical distribution of these variables can be exploited using GIS analysis, and attribution hypotheses can be generated and tested for consistency with the entire dataset of particles in the sample.

In the present case, the First Stage source attribution estimate eliminated 91.3% of the geographical area under consideration, and three quarters of the countries. This, combined with other investigative information, met the international law enforcement needs of this case.

This work is an application of a new capability using Particle Combination Analysis (PCA). As this case illustrates, the PCA capability is easy to employ and does not interfere with investigation by traditional methods. For example, investigation of this case was also supported by latent print processing, x-ray examination, recovery of transfer evidence (bullet impacts, paint), elephant population genetics, entomological identifications and carbon dating.[30,31] PCA integrates with, supports and contributes to other investigation efforts, such as focusing ground investigative effort toward most likely regions or environments, and providing information on landmarks or nearby activities that can be used to screen candidate areas.

Notably for investigations involving organized crime, wildlife crimes and illegal trade, PCA enables “cross-linking” attribution among cases involving different types of evidence, endangered species and contraband. These can be linked through the analysis of combinations of particles occurring on or within the items. This is distinct from many (very useful) types of source attribution tools, such as those focused on the population genetics of a specific species,[31] the elemental and isotopic compositions of a specific foodstuff,[32] or linkages based on a library of results for the same type of contraband.[2]

Uniquely among methods for source attribution, PCA provides an iterative means to refine and test source attribution estimates. The First Stage Source Attribution Estimate, based on the combined analyses of one sub-set of particles, enables the Next Stage estimate, which can draw on a different sub-set of particles, a finer set of reference

data, or on different analytical methods used to analyze specific target particles. And, at the Final Stage of source attribution, the same samples can suggest candidate sites, guide the investigation on the ground, and test specific candidate sites.
What makes PCA work is that there are thousands of very small particles of almost unlimited type and character in the fine dusts that are present on virtually any item. The combinations are so complex that until recently there was no *practical* method to identify and interpret these combinations. The capability described here resulted from an ongoing, multi-million dollar post-9/11 federally-funded research program.

ACKNOWLDGEMENTS

The authors gratefully acknowledge the support of the National Fish & Wildlife Forensics Laboratory and INTERPOL in general, and Kenneth Goddard, Edgard Espinoza and Peter Younger in particular, for recognizing the potential of Particle Combination Analysis in support of predictive source attribution, and for providing the opportunity to work on this case. Often there is institutional resistance to the incorporation of new methodologies, such as PCA. The RJLee Group, Inc. performed the x-ray diffraction of clay, under the direction of David Exline.

Authors' Contributions. DAS developed the PCA approach, planned its application to this case, collected samples, supervised microscopical, DNA and GIS analyses, interpreted the PCA data, and wrote the manuscript. AMB performed microscopical analysis and related interpretations. VMB performed pollen analysis and related interpretations. EAC performed environmental modeling, GIS analysis and related interpretations. MTC performed DNA analysis and related interpretations. PLS developed the PCA approach, planned its application to this case, interpreted the PCA data and revised the manuscript. All authors read and approved the manuscript.

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Standard Guide for Using Infrared Spectroscopy in Forensic Paint Examinations

Scientific Working Group on Materials Analysis (SWGMAT)

Introduction

Paint samples received by forensic laboratories are usually in the form of small chips or smears. Infrared (IR) spectroscopy is one of the most commonly used tools available for the analysis of these types of samples and serves as a staple comparative technique in the assessment of whether or not a questioned sample could have come from a suspected object. IR spectroscopy provides molecular structure information on many of the organic and inorganic constituents contained within a single paint layer. This information can be used to classify both binders and pigments in coating materials. The classification information may then be utilized to identify probable types of paint such as architectural, automotive, or maintenance. Additionally, the use of automotive paint databases may allow the determination of information such as potential vehicle year, make and model. Databases may also aid in the interpretation of the significance (e.g. how limited is the group of potential donor sources) of a questioned paint.

1. Scope

1.1 This guide applies to the forensic IR analysis of paints and coatings and is intended to supplement information presented in the Forensic Paint Analysis and Comparison Guidelines also written by SWGMAT. This guideline will be limited to the discussion of Fourier Transform Infrared (FTIR) instruments and will provide information on FTIR instrument setup, performance assessment, sample preparation, analysis and data interpretation. It is intended to provide an understanding of the requirements, benefits, limitations and proper use of IR accessories and sampling methods available for use by forensic paint examiners. The following accessory techniques will be discussed: FTIR-microspectroscopy (transmission and reflectance), diamond cell and attenuated total reflectance. The particular methods employed by each examiner and/or laboratory will depend upon available equipment, examiner training, sample size, sample suitability, and purpose of examination. This guideline will not cover the theoretical aspects of many of the topics presented. These can be found in texts such as *An Infrared Spectroscopy Atlas for the Coatings Industry* (Federation of Societies for Coatings, 1991) and *Fourier Transform Infrared Spectrometry* (Griffiths and de Haseth, 1986).

1.2 This guide does not purport to address any safety concerns associated with this technology. It is the responsibility of the user to establish appropriate safety and health practices**.**

2. REFERENCED DOCUMENTS

2.1 *ASTM* Standards:
E1610-02 *Standard Guide for Forensic Paint Analysis and Comparison*
E1421-94 *Standard Practice for Describing and Measuring Performance of Fourier Transform Infrared (FT-IR) Spectrometers: Level Zero and Level One Tests*
E1492-05 *Standard Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Laboratory*

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3. Terminology

For definitions of terms used in this guide other than those listed here, see ASTM D 16 *Terminology Relating to Paint, Varnish, Lacquer, and Related Products* and ASTM E 131-93 *Terminology Relating to Molecular Spectroscopy*.

100% Line: This is calculated by ratioing two background spectra taken under identical conditions. The slope and noise of 100% lines are used to measure the performance of the instrument.

Absorbance (A): The logarithm to the base 10 of the reciprocal of transmittance T. $A = \log^{10} (1/T) = -\log^{10} T$.

Absorbance Spectrum: A representation of the infrared spectrum in which the ordinate is defined in absorbance units (A). Absorbance is linearly proportional to concentration and is therefore used in quantitative analysis.

Additive: (modifier) Any substance added in a small quantity to improve properties. Additives may include substances such as driers, corrosion inhibitors, catalysts, ultraviolet absorbers, and plasticizers

Attenuated Total Reflectance (ATR): A method of spectrophotometric analysis based on the reflection of energy at the interface of two media that have different refractive indices and are in intimate contact with each other.

Aperture: An opening in an optical system that controls the amount of light passing through a system.

Background: The signal produced by the entire analytical system apart from the sample.

Beam Condenser: A series of mirrors that focus the infrared beam in the sample compartment to permit the examination of smaller samples that would otherwise not be possible.

Beam Splitter: An optical component that partially reflects and partially transmits radiation from the source in such a manner as to direct part to a fixed mirror and the other part to a moving mirror.

Binder: A nonvolatile portion of the liquid vehicle of a coating, which serves to bind or cement the pigment particles together.

Coating: A generic term for paint, lacquer, enamel, or other liquid or liquefiable material that is converted to a solid, protective, or decorative film or a combination of these types of films after application.

Deuterated Triglycine Sulphate (DTGS)Detector: A thermal detector that operates at room temperature but lacks the sensitivity for use with microscope accessories.

Extraneous Material (contaminant, foreign material): Material originating from a source other than the specimen.

Interferogram: A plot of the detector output as a function of retardation.

Microtomy: A sample preparation method that sequentially passes a blade at a shallow depth through a sample, resulting in sections of selected thickness.

Mercury Cadmium Telluride (MCT) Detector: A quantum detector that utilizes a semi-conducting material and requires cooling with liquid nitrogen to be operated. This type of detector is commonly used in microscope accessories due to its sensitivity.

Paint: A pigmented coating.

Pigment: A finely ground, inorganic or organic, insoluble, and dispersed particle. Besides color, a pigment may provide many of the essential properties of paint such as opacity, hardness, durability, and corrosion resistance. The term pigment includes extenders.

Representative Sample: A portion of the specimen selected and prepared for analysis that exhibits all of the characteristics of the parent specimen.

Significant Difference: A difference between two samples that indicates that the two samples do not have a common origin.

Smear: A transfer of paint resulting from contact between two objects. These transfers may consist of co-mingled particles from two or more sources, fragments, or contributions from a single source.

Specimen: Material submitted for examination. Samples are removed from a specimen for analysis.

Transmittance (T): The ratio of the energy of the radiation transmitted by the sample to the background, usually expressed as a percentage.

Transmittance Spectrum: A representation of the infrared spectrum in which the ordinate is defined in %T. Transmittance is not linearly proportional to concentration.

Wavelength: The distance, measured along the line of propagation, between two points that are in phase on adjacent waves.

Wavenumber: The inverse of the wavelength; or the number of waves per unit length usually given in reciprocal centimeters (cm^{-1}).

4. Summary of Practice

4.1 The film forming portion of a paint or coating is the organic binder, also referred to as the resin. The binder forms a film that protects as well as displays the organic and inorganic pigments that make a coating both decorative and functional. Infrared spectroscopy is commonly employed for the analysis of paint binders, pigments and other additives that are present in detectable concentrations.

4.2 Paints and coatings absorb infrared radiation at characteristic frequencies that are a function of the coating's composition. These absorption frequencies are determined by vibrations of chemical bonds present in the various components.

4.3 The analysis of coatings using infrared spectroscopy can be carried out using either transmission or reflectance techniques. These measurements can be taken with a variety of equipment configurations and sampling accessories, the most common being the use of an infrared microscope. A variety of accessories can also be utilized in the system's main bench. However, one should be aware that the use of a non-microscope accessory typically requires a larger sample size than those that can be analyzed using a microscope.

4.4 For transmission FTIR, a thin-peel of each paint layer, or a thin cross-section of a paint sample is made either by hand with a sharp blade or using a microtome. It is then analyzed using either a microscope attachment or other suitable accessory, such as a diamond anvil cell. When thin samples suitable for transmission FTIR are not obtainable, reflectance techniques (ATR, reflection) may be employed using microscope objectives or bench accessories.

4.5 Basic Principles

4.5.1 Infrared spectroscopy (mid-range) is capable of utilizing a spectral range between 4000 and approximately 400 cm^{-1}. Extended range instruments are needed to take measurements down to approximately 200 cm^{-1}. The actual spectral cutoff will depend on the type of detector and optics used.

4.5.2 An FTIR spectrometer measures the intensity of reflected or transmitted radiation over a designated range of wavelengths. The spectrum of a sample is produced by ratioing the transmitted or reflected infrared spectrum to a background spectrum.

4.5.3 Transmission spectra may be plotted either in percent transmittance (%T) or in absorbance (A). Reflectance spectra may be plotted either in percent reflectance (%R) or in absorbance (A).

4.6 Instrumentation

4.6.1 An FTIR instrument consists of a source to produce infrared radiation, an interferometer, a detector and a data processing device. A micro-FTIR instrument also has a microscope equipped with a detector and infra-red compatible optics.

4.6.2 Most FTIR systems are equipped to collect data using the main bench in the range of 4000-400 cm^{-1}. Extended range systems are equipped with a beamsplitter and optics that allow transmission down to approximately 200 $cm^{-1.}$ Systems equipped with an FTIR microscope utilize a more sensitive detector type. Depending on the specific detector type, microscopic samples can be analyzed in the range of approximately 4000-450 cm^{-1}.

5. Significance of Use

5.1 FTIR spectroscopy may be employed for the classification of paint binder types and pigments as well as for the comparison of spectra from known and questioned samples. When utilized for comparison purposes, the goal of the forensic examiner is to determine whether any significant differences exist between the known and questioned samples.

5.2 This guide is designed to assist an examiner in the selection of appropriate sample preparation methods and instrumental parameters for the analysis, comparison or identification of paint binders and pigments.

5.3 It is not the intent of this guide to present comprehensive theories and methods of FTIR spectroscopy. It is necessary that the examiner have an understanding of FTIR and general concepts of specimen preparation prior to using this guide. This information is available from manufacturers' reference materials, training courses, and references such as: Forensic Applications of Infrared Spectroscopy (Suzuki 1993), Infrared Microspectroscopy of Forensic Paint Evidence (Ryland 1995), Use of Infrared Spectroscopy for the Characterization of Paint Fragments (Beveridge

2001), and An Infrared Spectroscopy Atlas for the Coatings Industry published by the Federation of Societies for Coatings Technology.

6. Sample Handling

6.1 The general collection, handling, and tracking of samples shall meet or exceed the requirements of ASTM 1492-92 as well as the relevant portions of the SWGMAT's Trace Evidence Quality Assurance Guidelines.

6.2 The work area and tools used for the preparation of samples must be free of all extraneous materials that could transfer to the sample.

6.3 A paint sample should first be examined with a stereomicroscope, noting its size, appearance, layer sequence, heterogeneity within any given layer, and presence of any material that could interfere with the analysis (for example traces of adhesive, surface abrasion transfers, or zinc phosphate conversion coating residue on the underside of the base primer). Some surface materials may be of interest and therefore may be worthy of analysis before removal.

6.4 Each layer of a multi-layered paint sample should be analyzed individually.

6.5 When analyzing difficult samples (e.g. smears, dirty samples, or heterogeneous samples) care must be taken when sampling the paint and in choosing appropriate analytical conditions. An attempt should be made to remove any extraneous material from the exhibit before sampling. It may be necessary to analyze a number of samples to ensure reproducibility and understand inter/intra-sample variation.

6.6 Extraneous material should be removed either by scraping with a suitable tool such as a scalpel or washing with water. If needed, alcohols or light aliphatic hydrocarbons can be useful for cleaning. However, it should be noted that the use of organic solvents for cleaning paint can alter the composition by extracting soluble components such as plasticizers or dissolve the paint binder. If solvents are used, known and unknown samples should be treated the same, making sure no residual solvent remains.

6.7 For the accurate comparison of paint samples, they should be prepared and analyzed in the same manner.

7. Analytical Techniques and Operating Conditions

7.1 Paints may be analyzed by transmission or reflectance utilizing the microscope accessory or the bench accessories. The technique chosen will depend on the physical nature of the paint, the quantity of sample, preparation and analysis time, available equipment, and access to reference libraries for that technique. The same technique should be used on both known and questioned samples. It may be necessary to use multiple preparation and/or analytical techniques in order to analyze all sample layers and characteristics.

7.2 The type of detector and beam splitter will dictate the spectral range of the FTIR spectrometer. Mid-range infrared instruments use alkali halide beam splitters that are made from either cesium iodide (CsI) or potassium bromide (KBr).

7.3 The most common infrared detector used on the main bench is a deuterated triglycine sulphate (DTGS) detector. The DTGS detector operates at room temperature. A spectrometer equipped with a DTGS detector and CsI optics has an approximate spectral range of 4000-200 cm^{-1}. With KBr optics and a DTGS detector, the spectral range of the spectrometer is approximately 4000-400 cm^{-1}.

7.4 The detector commonly used with the microscope accessory is a mercury-cadmium-telluride (MCT) detector. The MCT detector is approximately 40X more sensitive than the DTGS detector, but has a narrower spectral range with a lower limit of 700-450 $cm^{-1,}$, depending on the type.

7.5 Infrared data are collected from the sample and ratioed against a previously stored or newly acquired background. Ratioing the sample spectrum to the background enables removal of absorptions from the cell or support material (e.g. diamond absorptions) and/or from the atmosphere (carbon dioxide and water vapor). Absorptions from *the* atmosphere can be minimized by purging with dried and filtered air. The number of scans acquired for each specimen will vary depending on sample type and size.

7.6 Main Bench Transmission Techniques:

7.6.1 The most common bench transmission technique for the analysis of paint is the use of the diamond cell with a beam condenser.

7.6.1.1 Either prior to or after sample analysis, a background spectrum of the empty diamond cell is collected. The same background spectrum may be used for multiple samples or a new one may be collected for each sample.

7.6.1.2 A sample from a single paint layer is placed on the clean diamond cell and compressed between the windows to a desired thickness. Both high-pressure and low-pressure diamond cells can be used in conjunction with a beam condenser. Sample compression is normally done under a microscope to ensure uniform coverage. The cell is then placed in the sample holder in the main bench of the instrument. The instrument is allowed to equilibrate. This process will depend on the type of instrument and efficiency of the purge. A sample spectrum is then collected with the sample in place. Typically 16 to 256 scans are collected with a resolution of 4 cm^{-1}. These parameters may vary depending on the instrument and size and nature of the sample. The same sample parameters, including the number of scans, should be acquired for the background as for the sample.

7.6.1.3 Diamond absorbs infrared radiation in the 2300 to 1900 cm^{-1} region; therefore, sample absorptions in this region may be obscured if the diamond path length is too long.

7.7 Attenuated Total Reflectance (Main Bench)

7.7.1 A number of in-bench single reflection ATR accessories are available. The general principles of operation are the same for each accessory. The sample of interest is placed in direct contact with the internal reflecting crystal, such as diamond or KRS-5. Some accessories employ a viewing microscope to facilitate proper placement of the sample or area of interest.

7.7.2 In contrast to transmission methods, ATR methods require little or no sample preparation, although the pressure applied when using the ATR accessory may deform the sample.

7.7.3 Once in contact with the crystal, multiple scans are collected. The sample is removed and the crystal is cleaned. Background scans are collected with the sample removed, either before or after the sample scans. Typically 16 to 256 scans are collected at a resolution of 4 cm^{-1}. These parameters may vary depending on the instrument and size and nature of the sample.

7.8 FTIR Microscope Accessory

7.8.1 In forensic science, infrared microspectroscopy is the most commonly used method for acquiring the infrared spectrum of a paint sample. Spectra can be obtained from samples as small as 10-20 μm in diameter, using transmittance, reflectance and ATR methods. MCT detectors are commonly used with microscopes due to the higher sensitivity needed for small samples. They are available in configurations usually designated as narrow band and broad (wide) band with the lower

energy cut-off ranging from approximately 700 to 450cm^{-1}. There is a trade off between sensitivity and spectral range with these detectors. A detector with the spectral range of 4000 to 650cm^{-1} is typically used for paint examination since it offers the optimal balance between spectral range and sensitivity. These detectors must be cooled by liquid nitrogen before use. When using the lower sensitivity/broader spectral range detector, larger samples are required.

7.8.2 Transmission measurements:

7.8.2.1 Transmission methods generally require more extensive sample preparation. The sample must be thin enough not to over-absorb. For transmission data viewed in % transmittance, spectral peaks optimally should not fall below 10% T. For spectra displayed in absorbance, the maximum absorbance optimally should be 1.0 or less.

7.8.2.2 A prepared and mounted sample is placed on the microscope stage and focused. The condenser on some instruments may have to be adjusted to account for the thickness of a support window. The sample is observed with visible light and the area to be analyzed is centered in the field of view. The area of interest is isolated from the remainder of the field of view with one or two apertures.

7.8.2.3 The number of apertures will depend on the instrument configuration. The apertures control the area and location of the infrared beam striking the sample and the transmitted light reaching the detector.

7.8.2.4 Apertures also block unwanted radiation originating outside of the area of interest. If stray light is allowed to reach the detector, absorption intensity is reduced.

7.8.2.5 As a sample area of interest becomes smaller, or as the aperture(s) are reduced so that a portion of the sample can be isolated, diffraction effects rapidly increase. These effects can be experienced when using aperture sizes smaller than 25 µm x 25 µm.

7.8.2.6 In order to minimize the effects of sample heterogeneity, aperture areas greater than 2500 µm^2 (e.g. 50 µm x 50 µm or 25 µm x 100 µm) should be used when possible. Alternatively, multiple areas of the sample can be analyzed to determine the range of spectral characteristics.

7.8.2.7 Once the area of interest is isolated by adjusting the magnification and apertures of the microscope, the infrared spectrum of the sample is collected. Typically 16 to 256 scans are collected at a resolution of 4 cm^{-1} or better.

7.8.2.8 The background spectrum is collected from an unused area of the support window using the same aperture configuration as used for the sample.

7.8.2.9 If the sample size is limited, the resulting spectrum may be noisy. To increase the signal to noise ratio (S/N), the number of scans can be increased. It is important to collect spectra with good S/N to permit visualization of fine detail such as small sharp peaks or shoulders in the resultant spectrum.

7.8.3 Reflection measurements (microscope):

7.8.3.1 The FTIR microscope can also be used in the reflection mode. However, in most cases, transmittance methods are preferred for several reasons. Refractive index changes, and differences in infrared absorption coefficients for different wavelengths, give rise to distortions in reflectance spectra. Reflectance spectra are not absorption spectra and cannot be compared in detail to transmission spectra due to shifts in spectral peak wavelengths and variations in spectral peak intensities. Also, most of the reference data of coatings, binders, pigments and additives consist of transmission spectra. Furthermore, being surface analysis techniques, inconsistencies in the preparation of sample surfaces can present problems in detailed comparisons. Additionally, when

analyzing individual layers in cross section and using the requisite small apertures, signal-to-noise constraints are even greater than those encountered in transmission analyses.

7.8.3.2 If samples are compressed directly on a glass slide made of infrared light reflecting architectural glass (low e-glass), the microscope's reflection mode can be used to produce spectra mimicking double-pass transmission spectra. The technique is sometimes referred to as "transflection" or "reflection/absorption". Some wavelength maxima shifts may be observed in intense absorption bands.

7.8.3.2.1 For transflection, the thinned sample is placed on an infrared reflective surface, such as a glass slide made of infrared light reflecting architectural glass (low e-glass), or a gold mirror, and placed on the microscope stage. It is viewed using visible light and the area to be analyzed is centered in the field of view. The area of interest is isolated from the remainder of the field of view with an aperture and the infrared spectrum is collected. Typically, 16-256 scans are collected at a resolution of 4 cm^{-1} or better. The background spectrum is collected from an unused area of the reflective support using the same aperture configuration and number of scans as used for the sample.

7.8.4 ATR Objectives for infrared microscopes:

7.8.4.1 ATR microscope objectives may be fitted with a silicon, ZnSe, diamond, KRS-5, or germanium internal reflecting crystal offering a wide variety of penetration depths and crystal physical attributes. The sample is viewed using visible light and the area to be analyzed is centered in the field of view. The crystal is then placed in direct contact with the area of interest. Monitoring the single beam spectrum will provide an indication of whether there is sufficient contact between the sample and crystal. Typically 64-512 scans are collected at a resolution of 4 cm^{-1} or better and ratioed against an air background. The number of scans collected for each sample will vary depending on sample type and size.

8. Sample Preparation Methods and Sampling Accessories

8.1 The method chosen for sample preparation depends on the size, nature, and condition of the specimen, as well as the particular FTIR technique/accessory that will be employed for analysis. It may be necessary to use multiple preparation and/or analytical techniques in order to analyze all sample layers and characteristics.

8.2 Transmission techniques (Main bench):

8.2.1 Samples prepared for analysis by main bench transmission techniques must be thin enough to allow infrared radiation to pass through without being over-absorbed by the sample. For transmission data that are viewed in absorbance, the sample optimally should be thin enough to produce a maximum absorbance of 1 absorbance unit. For transmission data viewed in % transmittance, spectral peaks optimally should not fall below 10% T. This typically requires a sample thickness of approximately 5-10 µms.

8.2.2 The separation and analysis of individual layers is recommended in order to determine chemical composition and detailed spectral characteristics of each layer. This may be achieved by microtomy, or by hand using a sharp blade while observing the sample under a stereomicroscope.

8.2.3 Sample preparation techniques which may be employed for transmission analysis using the main bench include a thin peel stretched over an aperture, an alkali halide pellet (e.g. potassium bromide), or a diamond cell.

8.2.4 Thin peels of each layer can be placed on a glass microscope slide and compressed with the flat beveled surface of a scalpel blade, a roller bearing tool, or other equivalent technique, and then

placed directly over a small masking aperture for analysis. Given the small sample sizes encountered, a beam condenser is typically employed.

8.2.5 Potassium bromide (KBr) pellets are made by grinding a small sample of the individual paint layer with dry spectroscopic grade KBr using a mortar and pestle. The powder is then transferred to a die maker and a press is used to generate the pellet. As KBr is deliquescent, the pellets, KBr powder and die maker should be stored in an oven or desiccator. Given the small sample sizes encountered, one to three millimeter diameter pellets mounted in a beam condenser are typically employed.

8.2.6 The diamond anvil cell is a useful sampling technique when the greatest spectral range is required and for laboratories that do not have a microscope accessory for their infrared spectrometer. It is possible to obtain spectra down to approximately 200 cm^{-1} with cesium iodide (CsI) optics, or down to 400 cm^{-1} with KBr optics. Both high- and low- pressure diamond cells can be used in conjunction with a beam condenser. Pliable and powdered samples are amenable to low-pressure diamond cells, and harder paint samples may require the use of high-pressure diamond cells. Low-pressure diamond cells have the advantages of costing less, are compatible with an infrared microscope accessory and they do not obscure spectral information in the 2400 to 1800 cm^{-1} region because they contain thinner diamonds than high pressure cells.

8.2.7 Diamond cells permit relatively simple sample preparation. The cell consists of two diamond windows, a holder for each diamond and a means of compressing the sample to an appropriate thickness. There are a variety of designs available. The paint sample is simply placed on one of the diamond faces, the second diamond is positioned on top, and sufficient pressure is applied to form a film. This is normally done under a stereomicroscope to ensure uniform coverage of the diamond face.

8.2.8 For non-elastic paints, one diamond is typically removed prior to analysis. This leaves the thin compressed film adhering to one of the diamond faces and avoids diffraction fringes in the recorded spectrum resulting from the two parallel diamond faces.

8.2.9 The diamond cell sampling technique is essentially non-destructive because of diamond's inertness. The paint sample can be recovered uncontaminated from the diamond face using a scalpel blade or other suitable tool.

8.2.10 The chief drawback of this technique is that a larger sample is required than when a microscope accessory is used. This can be particularly significant when examining small paint chips that have multiple layers because extraction of sufficient paint from interior layers can represent a significant challenge. Furthermore, each sample must be placed in the cell and removed prior to mounting the next sample, unlike the microscope method where multiple samples may be placed on the support material and analyzed sequentially.

8.3 Internal Reflection Techniques (main bench)

8.3.1 Attenuated Total Reflectance (ATR) spectroscopy may be used to analyze exposed paint layers. Although ATR spectroscopy accessories are available in both multi-reflection (macro scale) and single reflection (micro scale), only single reflection accessories will be discussed because they are more suitable for forensic paint examination. In contrast to transmission methods, ATR methods require virtually no sample preparation when examining an already exposed surface. In some instances ATR methods may lend themselves to conducting the examination *in situ.* Since ATR is a surface technique it is necessary to remove any extraneous material from the area to be examined.

8.3.2 The bench ATR (single reflection) accessory utilizes an internal reflection crystal to condense the beam onto a spot-sized sampling area. The crystal is mounted horizontally in a purged box of the sample compartment. Mirrors within the accessory focus the beam onto the sample, and the infrared

light reflected by the sample is directed to the detector. The specimen is placed in contact with the center of the crystal, and an adjustable piston is used to apply sufficient pressure to ensure contact.

8.3.3 ATR techniques are subject to inter-sample variations resulting from variations in pressure on the sample surfaces and variations in surface contact areas. Being reflectance techniques, they are also prone to the same type of spectral distortions noted for reflection spectroscopy.

8.4 FTIR microscope accessory

8.4.1 The use of a microscope accessory is preferred for very small samples and has several advantages over bench techniques. Spectra can be obtained from flattened samples as small as 10-20 µm in diameter. The microscope attachment permits the sequential analysis of multiple samples placed on an appropriate support material. The method also affords the advantage of viewing the sample optically and choosing specific regions of interest in either heterogeneous specimens or those having varying thickness.

8.4.2 Although it is a popular IR accessory for paint analysis, it should be noted that the microscope attachment has some disadvantages. Unlike DTGS detectors, the MCT detectors used in microscope accessories require cooling with liquid nitrogen to minimize electronic noise. There is a trade off between sensitivity and spectral range with these detectors. Narrow band detectors that cut off in the 700 cm^{-1} range are more sensitive than the broad band detectors that cutoff in the 450 cm^{-1} range. Care must be taken with very small samples as the use of small measurement apertures can limit the energy from the longer wavelengths (smaller wavenumbers) from reaching the detector. Heterogeneity issues are also more pronounced when using very small apertures.

8.4.3 The chief drawback of this technique is its limited spectral range. Microscope optics and detectors have a cutoff of approximately 450 cm^{-1} as contrasted to the diamond anvil cell/beam condenser method with extended range optics affording a lower cutoff of approximately 200 cm^{-1}. The lower range can be advantageous in the classification and comparison of inorganic pigments.

8.4.4 Transmission measurements (microscope)

8.4.4.1 Transmission measurements are commonly used as they generate spectra with fewer artifacts. However, transmission methods generally entail more extensive sample preparation than reflection techniques. The paint sample must be thin enough to avoid over-absorption.

8.4.4.2 Analysis of individual layers is required in order to spectrally characterize each layer in a multi-layered paint sample. For transmission FTIR spectroscopy with a microscope accessory, individual layers of multi-layered paint samples can be analyzed either as thin peels of each layer or as individual layers in a thin cross section of the intact chip.

8.4.4.3 The paint layers may be separated by hand using a sharp blade while observing the sample under a stereomicroscope. Thin peels of each layer can be placed on a glass microscope slide and compressed with the flat beveled surface of a scalpel blade, a roller bearing tool, or other suitable technique. The sample obtained can then be placed either directly over a small masking aperture or on an appropriate salt plate for analysis. Sample thickness on the order of 5 µm is desired.

8.4.4.4 When selecting an appropriate infrared support material (e.g. salt plate), several factors should be taken into consideration including cost, availability, environmental sensitivity, transparency of the material to infrared radiation, and the durability of the material.

8.4.4.5 The low pressure diamond cell can also be used as a sample support medium under the FTIR microscope. The paint sample is simply placed on one of the diamond faces, the second diamond is positioned on top, and sufficient pressure is applied to form a film. This is normally done under a stereomicroscope to ensure uniform coverage. For non-elastic paints, one diamond is

typically removed prior to analysis. This leaves the thin compressed film adhering to one of the diamond faces and avoids diffraction fringes in the recorded spectrum resulting from the two parallel diamond faces. The sampling technique is essentially non-destructive because of diamond's inertness. The paint sample can be recovered uncontaminated from the diamond face using a scalpel blade or other appropriate tool.

8.4.4.6 The microscope attachment also affords FTIR analysis of multi-layered paint samples without physical separation of the layers. A thin cross section with intact layer structure can be cut by hand using a sharp blade or with a microtome (sample thickness on the order of 5 µm is typical). The cross sectioned sample is either flattened on a microscope slide and placed on a support material or flattened directly on the support material. If the sample is used for analysis by another technique (e.g. elemental analysis), contributions from the support material may be detected. Individual layers may then be analyzed after observing with visible light and centering the sample in the field of view, delineating areas of interest using the microscope's aperture controls. Care must be taken to avoid contributions from adjoining layers.

8.4.4.7 Cross section analysis has the advantage of viewing the sample optically and then selecting the specific region for specimen analysis, thus permitting rapid analyses of individual layers. It also permits analyses of specific regions of inhomogeneous materials and compositional mapping. However, it typically requires smaller target apertures which can result in diffraction effects, heterogeneity concerns, and signal to noise constraints.

8.4.5 Reflection measurements (microscope)

8.4.5.1 If samples are compressed directly on a glass slide made of infrared light reflecting architectural glass (low e-glass), the microscope's reflection mode can be used to produce spectra mimicking double-pass transmission spectra. The technique is sometimes referred to as "transflection" or "reflection/absorption". Some wavelength maxima shifts may be observed in intense absorption bands. Transflection samples need to be approximately half the thickness of an optimum transmission sample.

8.4.5.2 The FTIR microscope can also be used in the reflection mode, but in most cases, transmittance methods are preferred for several reasons. Refractive index changes, and differences in infrared absorption coefficients for different wavelengths, give rise to distortions in reflectance spectra. Reflectance spectra are not absorption spectra and cannot be compared in detail to transmission spectra due to shifts in spectral peak wavelengths and variations in spectral peak intensities. Also, most of the reference data of coatings, binders, pigments and additives consist of transmission spectra. Furthermore, being surface analysis techniques, inconsistencies in the preparation of sample surfaces can present problems in detailed comparisons. Additionally, when analyzing individual layers in cross section and using the requisite small apertures, signal-to-noise constraints are even greater than those encountered in transmission analyses.

8.4.6 Internal Reflection Techniques (ATR microscope objective)

8.4.6.1 ATR objectives are available for infrared microscope accessories. The technique requires little or no sample preparation and is non-destructive.

8.4.6.2 An ATR microscope objective may be used to analyze exposed paint layers. In some instances ATR methods may lend themselves to conducting the examination *in situ.* Since ATR is a surface technique it may be desirable to analyze any existing surface material of interest and then remove the surface material from the area and reanalyze the area.

8.4.6.3 It should be noted that the analysis of thin surface smears may result in contributions from substrate material or underlying layers. If the substrate material does not transmit infrared radiation, such as metal or glass, the ATR spectra will appear more like transmission spectra the thinner the

sample becomes. Hence, ATR spectra of thin samples (on the order of 1 to 2 microns) should not be directly compared to ATR spectra of thick specimens.

8.4.6.4 ATR is subject to inter-sample variations resulting from variations in pressure on the sample surface and variations in surface contact areas. Also, intra-sample variations may result from sample heterogeneity. Being a reflectance technique, it is somewhat prone to the same type of spectral distortions noted for reflection spectroscopy.

9. Performance Checks

9.1 The instrument must be checked to ensure it is operating properly. Results of the check must be documented. Before any performance checks, the instrument must be thermally stable. It is recommended that the system be left on, or in a stand-by mode, as continuous operation is better for performance, stability, and prolonging the lifetime of the IR-source. For detectors that require liquid nitrogen, the detector takes approximately twenty minutes to cool and stabilize.

9.2 Performance checks should be conducted at least once a month (or before use if used less frequently). This will depend upon how often the instrument is used or the laboratory's protocol. It is recommended that the built-in instrument test from the manufacturer be used for the performance checks, if available. Instrument performance tests should include evaluation of conditions such as wavenumber accuracy, signal-to-noise ratio, signal strength, and 100% line. Performance tests recommended by the manufacturer or those outlined in ASTM E 1421-94 may also be utilized. A performance check should also be used for FTIR accessories.

10. Classification, Comparison and Interpretation

10.1 Binder classification of commonly encountered coatings is based on the interpretation of characteristic infrared absorption bands. Similarly, some pigments and additives may be identified.

10.2 Classification of a coating may be achieved by evaluating the absorption bands present in the spectrum with respect to band position, band shape and band intensity. After evaluating the absorption bands, interpretation and classification of a spectrum may be accomplished through comparison of a sample spectrum to spectra of reference materials, use of flow charts and published data. Spectral libraries of known materials may also be used to characterize the binder, additives and/or pigments present in the paint.

10.2.1 There are many sources of flow charts and functional group frequency charts. Flow charts are easy to use and offer an examiner a place to start in classifying paints. However, classification should not be solely based on comparison to flow charts. The spectrum should be compared to representative reference spectra before a classification is made. It should be noted that new formulations may not be represented in existing paint classification systems.

10.3 Binder classification may be hindered by the presence of certain pigments. If the paint binder is soluble, a micro extraction may be performed to isolate the binder for analysis. If the paint is not soluble, then an alternative analytical technique, such as pyrolysis-gas chromatography, may be utilized to effect the classification of the binder.

10.4 Binder classification may be difficult in contaminated samples and smears. Contributions from substrate or other co-mingled materials must be considered. Particle picking may be utilized to obtain spectra which are suitable for interpretation. Spectral subtraction may also be utilized to assess the presence of co-mingled materials.

10.5 Classification of the binder type, pigments and/or additives may assist in determining the significance and end use of the coating. It should be noted that the classification of the binder may differ from the manufacturer's designation, due to changes in polymer chemistry or component

migration that occur during curing. Also, trade names given to the coating by the manufacturer may not reflect the actual chemistry of the paint. This conflict in designation results from the use of marketing trade names and historical designations that are technically inaccurate or incomplete.

10.6 Comparison of known and questioned samples may be conducted with both spectra displayed in transmittance and/or absorbance. It should be noted that certain information may be seen more readily in one format or the other.

10.6.1 There are a number of significant factors which should be considered when assessing whether or not spectra can be distinguished from one another: the presence or absence of absorption bands, their positions, shapes, and relative intensities.

10.6.1.1 Characteristic absorption bands present in one spectrum should be present in the comparison spectrum. The position of the absorption bands should have reasonable agreement with each other and is somewhat dependent on the shape of the absorption band. A rule of thumb is that the positions of corresponding peaks in two or more spectra be within ± 5 cm^{-1}. For sharp absorption peaks one should use tighter constraints. One should critically scrutinize the spectra being compared if corresponding peaks vary by more than 5 cm^{-1}. Replicate sample spectra may be necessary to determine reproducibility of absorption position.

10.6.1.2 The absorption bands should have comparable relative intensities and shapes for the spectra being compared. If there is variation between spectra it may be necessary to acquire additional spectra to determine if the variation is reproducible.

10.6.1.2.1 If there are notable differences among spectra of a single sample, the collection of additional spectra may be necessary to assess the range of variation.

10.6.1.2.2 If differences are noted between questioned and known samples, the collection of additional spectra may be necessary to demonstrate whether the differences are repeatable and therefore significant.

10.7 One of three conclusions can be reached after evaluating and comparing the known and questioned spectra.

10.7.1 Spectra are dissimilar if they contain one or more significant differences.

10.7.2 Spectra are indistinguishable if they contain no significant differences.

10.7.3 A spectral comparison is inconclusive if sample size or condition preclude a decision as to whether differences are significant.

11. Spectral Libraries

11.1 Several infrared spectral libraries and databases relating to paint are available. The Federation of Societies for Coatings Technology offers a library in both printed and digital formats. It is a compilation of the infrared spectra of the various chemical compounds commonly found in coatings. Other libraries relating to polymers, pigments, and additives found in coatings are marketed by various companies. The ability to search the library of spectra and compare them to the spectrum of a specific paint layer may assist in the identification of the chemical components in that layer.

12. Paint Data Query Database (PDQ)

12.1 For forensic purposes, one of the most comprehensive compilations of OEM (original equipment manufacture) automotive paint information is the Paint Data Query (PDQ) database. PDQ is used to

aid in the identification of make, model, and year of an unknown vehicle, to assess the relative significance of a paint system when conducting a paint comparison, and to stay current with automotive paint trends. PDQ consists of a text-based database and spectral libraries. It was created in the early 1970s and is maintained by the Royal Canadian Mounted Police Forensic Laboratory Services. This database is only available to law enforcement agencies through contractual agreement.

12.1.1 The PDQ text-based database consists of two parts; the source-based information and the paint layer information. The source-based information includes topics such as production plant, make, model and year for each paint sample in the database. The paint layer information includes the layer system (number, color, sequence, etc.) and the chemical composition of each layer.

12.1.2 The PDQ spectral libraries include the infrared spectra of individual paint layers.

12.1.3 To aid in the identification of make, model, and year of an unknown vehicle, the data acquired from an evidentiary paint system is coded according to PDQ format and is searched against the text-based portion of the database. The spectral library is also available for searches and comparisons to the data obtained from evidentiary samples to further narrow the range of possible sources.

12.1.4 The database may also serve to assist in assessing the relative significance of a correspondence between a questioned and known OEM paint system. For example, accessing the database will provide information as to how many other makes and models of vehicles in the database utilize the same paint system.

12.1.5 The database is also a source of information to keep the forensic paint examiner aware of current trends in OEM automotive coating systems. These include changes in technology such as paint compositions and paint layer structures.

12.2 PDQ is intended to be a source-based database, not a population-based database. In other words, PDQ does not contain information on finish systems of all vehicles in existence. This must be taken into consideration whenever the database is used.

13. Documentation

13.1 Case notes should include a copy of all of the instrumental data that was used to reach a conclusion. All hard copies should include a unique sample designation, the operator's name or initials, and the date of analysis.

13.2 Case notes should also include a description of the evidence analyzed by IR, the method of sample preparation, the analytical instrumentation used, and its operating parameters.

13.3 See SWGMAT's Trace Evidence Quality Assurance Guidelines for further requirements (http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/jan2000/swgmat.htm).

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Guideline for Forensic Examination of Pressure Sensitive Tapes

Scientific Working Group on Materials Analysis (SWGMAT)

1.0 Scope

This document is intended as an introductory guide for the forensic scientist in the examination and comparison of pressure sensitive tapes. Detailed analytical aspects of tape analysis will be addressed in separate documents. The methods and practices described have been peer-reviewed and are generally accepted within the forensic community.

2.0 Reference Documents

E1492-92 *Practice for Receiving, Documenting, Storing, and Retrieving in a Forensic Laboratory*

SWGMAT Trace Evidence Quality Assurance Guidelines [Online] (January 2000). Available: http://www.fbi.gov/hq/lab/fsc/backissu/jan2000/swgmat.htm

SWGMAT Trace Recovery Guidelines [Online] (October 1999). Available: http://www.fbi.gov/hq/lab/fsc/backissu/oct1999/index.htm

Pressure Sensitive Tape Council-14th edition Test Methods, Glossary of Terms

3.0 Terminology

Adhesive: A material that will hold two or more objects together solely by intimate surface contact.

Additives: Materials that are included in adhesive or backing formulations to increase overall volume, impart color, or provide other desired properties.

Backing: A thin flexible material to which adhesive is applied.

Backsizing: A layer applied to the top side of the backing. Its purpose is to coat and fill a porous surfaced backing with a material that is inert to the adhesive formulation to be used.

*Calendering:*The use of a multi-roll device to apply pressure sensitive adhesive at 100% solids to various backings by heat and pressure to produce adhesive tape.

Cellophane: Form of regenerated cellulose. A thin transparent film manufactured from wood pulp. Used as a backing material in tape products.

Celloulose acetate: A transparent film that is used for tape backings. A matte surface version is used for write-on tapes. It is more moisture-resistant than cellophane.

Creped: Paper that has small folds in it giving it high stretch and conformability. Used in masking tape (saturated paper tape).

Elastomer: A material that can be deformed, but when the forces are removed will return to its original form. Serves as the base material for PSAs.

Fill yarns: Fibers in the scrim fabric of reinforced tape that run crosswise, perpendicular to the warp direction. Also called weft yarns.

Flatback: Smooth paper backing sometimes used in masking tapes.

Migration: The movement over a period of time of an ingredient from one layer to another. This often occurs in PVC tapes where plasticizer in the PVC backing "migrates" into the adhesive.

Plasticizer: Material added to plastics to impart flexibility by creating spaces between the polymer chains and lowering the inter- and intra-chain attractive forces, allowing freer movement of the chains. Used in pressure sensitive backings (particularly PVC) as well as some adhesives to lower glass-transition temperatures and allow use at sub-ambient temperatures.

Pressure sensitive adhesive (PSA): PRESSURE SENSITIVE ADHESIVE (PSA) Consists of a polymeric base usually with appropriate plasticizers and tackifiers. It can form an adhesive bond with no physical or chemical change, and with no more than slight pressure.

Pressure sensitive tape (PST): PRESSURE SENSITIVE TAPE Consists of a flexible backing and PSA, which when applied to a surface, bonds immediately at room temperature with slight pressure. The bond can be broken (usually) without damage to the surface and without leaving a residue.

Prime coat: A coating of adhesive-like material between the tape adhesive and backing that serves as a bonding agent.

Scrim: A loosely-woven gauze-type cloth added to duct tape for reinforcement and strength.

Reinforcement: Cloth, scrim, glass filaments, or plastic filaments added to tape for stability and strength.

Release coat: A coating applied to the backing on the side opposite the adhesive that provides ease of unwind and prevents delamination or tearing.

Tack: Property of an adhesive achieved by the addition of a low molecular weight organic component that allows the elastomer to form a bond immediately with a surface under low pressure.

Tackifier: Material added to the adhesive base polymer to impart tack

Thickness: Distance from one surface of either a tape, backing, or adhesive to the other, usually expressed in mils or thousandths of an inch.

Warp yarns: Fibers in scrim fabric of reinforced tape that run lengthwise in the machine direction.

Weft yarns: See *Fill yarns*.

4.0 Summary of Guideline

4.1 The information contained in this guideline is intended to assist the examiner in characterizing and comparing evidentiary tape samples. The forensic examination of pressure sensitive tape encompasses the determination of physical construction and chemical composition of tape products. General information on product variability, construction, and composition is provided. This guideline provides an overview of techniques applied to the analysis of tape components.

4.2 Methods for the analysis of tape include examinations of physical characteristics, polarized light microscopy (PLM), Fourier transform infrared spectroscopy (FTIR), pyrolysis gas

chromatography (py-GC), scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS), X-ray fluorescence spectrometry (XRF), inductively coupled plasma (ICP) techniques, and X-ray powder diffraction (XRD). These different procedures provide complementary information and should be selected and employed in an order to obtain the most discriminating information consistent with the laboratory's capabilities. It is assumed that the forensic examiner has a basic familiarity with instrumental techniques used in the methods described.

4.3 Typically, a tape examination involves the comparison of samples to determine if they could share a common origin. The goal is to determine if any significant differences exist between the samples. The evaluation of tapes for class characteristics can associate known and questioned tapes to a group but not to a single, individual source. A physical end match of two tape ends provides individualizing characteristics that associate the two tapes to one another to the exclusion of all other tapes.

4.4 Questioned tape samples may be submitted with a request to identify possible product information, manufacturing, and retailing sources. Sourcing of a questioned tape can provide valuable investigative lead information. Physical characteristics and compositional data are useful for technical inquiries to tape manufacturing companies, comparisons with various brands of tape purchased at local commercial outlets, and for searching reference databases.

5.0 Significance and Use

5.1 The analysis and comparison of tape evidence in the forensic science laboratory can provide valuable information due to the variability of tape products. However, some classes of tape exhibit more variability than others. In general, the more complex the product (e.g., duct tape), the more variable it is. The common tape classes and their components are described further in section 6.0. Studies have shown differences between randomly selected rolls of tape, but because of the ever-changing tape markets, suppliers, and economics, it is not feasible to establish the statistical probability that a given sample would have the same physical and chemical characteristics as a randomly selected tape.

5.2 While tapes within a specific class may appear similar on a macroscopic level, differences may be found on closer analysis of the physical and chemical characteristics. Differences are readily observed in tapes manufactured in different plants.

5.3 Differences may also be found between batches of tape products within the same plant, due to changes in raw materials and processing that occur over time. Also, the many components that comprise a given tape product are subject to supply-and-demand fluctuations in the market. For example, a lower bid for some minor component may lead to its substitution from one batch to the next, resulting in compositional changes that can be detected in the forensic laboratory. While it is less likely to find differences in tape rolls produced by the same production line, the probability of finding differences between batches increases with time between batches.

5.4 It may be feasible to detect physical differences between rolls of tape produced in the same batch. For example, one batch of duct tape produced in a large sheet may be slit into nominal two-inch wide (~50.8 mm) individual rolls. There are numerous cutters spaced along the width of the sheet that can result in slightly different roll widths within the same batch. Differences in the warp yarn offset from the machine edge may also be found in rolls from the same batch.

5.5 Within-roll variability has been assessed using different analytical instruments. No significant within-roll variations have been reported.

5.6 In the comparison of tape samples, much information can be obtained from macroscopic and stereomicroscopical examinations. Exclusions at this stage preclude additional analysis. When

samples are found to be similar at this stage, the examiner should proceed with other examinations available and practical to adequately address the chemical, compositional, and physical properties of the tapes before rendering a conclusion. At that point if no significant differences are found, the tapes are consistent and could have come from the same source. Only in rare circumstances can a stronger statement be supported.

6.0 Tape Construction and Classes

6.1 Backings
The pressure sensitive tape backing, or film, provides a support material for the adhesive. There is a wide range of materials used for tape backings depending upon the commercial end use. These include, but are not limited to, polyethylene, polypropylene, polyvinylchloride, saturated paper, cellulose acetate, cloth, and polyester. Furthermore, fillers, colorants, plasticizers, release coats, primer coats, and preservatives may also be added to tape backings.

6.2 Adhesives
The formulation of pressure sensitive adhesives (PSA) consists of an elastomer to which tackifer resins and inorganic materials are added.

6.2.1 Elastomers
The following is a list of elastomers that are used in PSAs. PSAs may contain one elastomer or a blend of several different elastomers.

Natural rubber (polyisoprene)
Synthetic polyisoprene
Polybutadiene
Polyisobutylene
Styrene butadiene random copolymer
Styrene isoprene block copolymer (SIS)
Styrene butadiene block copolymer (SBS)
Styrene ethylene-butylene block copolymer
Ethyl or butyl acrylate
Silicones
Polychloroprene

Tackifying resins are blended with elastomers to lower the glass transition temperature, allowing freer movement of the polymer chains and thus giving PSAs their “sticky” adhesive property. The tackifying resin is typically a C-5 (5 carbon hydrocarbon component). Silicone and acrylic PSAs do not require a tackifier. More costly silicone-based adhesives may be found in adhesive formulations of tapes that are geared for high temperature or chemical resistance.

6.2.2 Additives
Inorganic materials are added to an adhesive formulation to either increase the overall volume or to impart color. Such materials include calcite, dolomite, iron oxide, kaolinite, talc, titanium dioxide (rutile or anatase), and zincite. In addition, zincite can also function as an “accelerator,” or cross-linker for a rubber-based adhesive. Other materials may be added to provide resistance to extremes in temperature and UV exposure.

6.3 Tape Classes

6.3.1 Polycoated cloth tape
Commonly referred to as duct tape, polycoated cloth tape consists of three basic components: the backing, the reinforcement fabric, and the PSA. These components in concert are what

determine a duct tape's appearance, strength, and end use. The final product will be designed for specific end usage, whether it is for general commodity use, construction, etc.

6.3.1.1 Duct tape backing
The backing, which is polyethylene, is available in various colors. Duct tapes that are silver or gray commonly contain a small amount of aluminum to impart the silver color. Other colored backings are achieved by adding colored pellets to the molten polyethylene. Inorganic materials may be added to the backing, such as talc, which improves water repellency and tear strength. The backing may consist of a single layer or multiple layers of polyethylene and can range in thickness from about 1.5 mils to 4 mils (1 mil = 0.0010 in). The backing may also exhibit characteristics imparted during the manufacturing process, such as calendering marks and striations. Additionally, lettering or designs may also be imparted on the surface or the underside of the polyethylene.

6.3.1.2 Duct tape adhesive
The PSA formulation for duct tapes consists of an elastomer to which tackifying resins and inorganic materials are added. The elastomer is typically natural rubber (polyisoprene) but could also be a mixture/blend of synthetic and/or natural elastomers. Other materials used as elastomers include styrene-butadiene copolymer and styrene-isoprene copolymer.

The tackifying resin is typically a C-5 (5 carbon hydrocarbon component) that is used to make the elastomer “sticky” or impart tack.

Inorganic materials are added to an adhesive formulation to either increase the overall volume or to impart color. In duct tape adhesives any of the following may be found: calcite, dolomite, kaolinite, talc, titanium dioxide and zincite.

6.3.1.3 Duct tape reinforcement fabric
The scrim is commonly constructed of cotton, polyester, or a blend of these two materials. Reprocessed cellulose may also be found. The scrim is generally manufactured as either plain weave or weft-insertion (having knit warp yarns and texturized fill yarns). Yarns in both the warp and fill directions can be twisted (spun), texturized, or filament. Variations of these can be seen.

6.3.1.4 Other components found in duct tape
Two additional layers that may be present within a duct tape product are a release coat and a primer coat.

6.3.2 Vinyl tape
A vinyl tape, also referred to as an electrical tape, finds use in applications that require heat resistance/retardance and insulating properties. The two main components are the backing and the PSA.

6.3.2.1 Vinyl tape backing
Polyvinyl chloride (PVC) is the most common material used to construct the backing. Plasticizers, typically phthalate or adipate compounds, are added to this material to impart flexibility to the PVC. Other plasticizers may include alkyl/aryl phosphate compounds and dialkyl tin compounds. Backings range in thicknesses of 4.5-7.5 mils and are commonly black in color, imparted by the addition of carbon black. However, a variety of colored backings are produced and available. In addition to plasticizers, inorganic materials such as lead stearate, lead carbonate, antimony oxide, kaolinite, calcite, and titanium dioxide may also be found.

6.3.2.2 Vinyl tape adhesive
The adhesive can be formulated in several ways, depending on the intended end use market, and can be either colorless or black, through the addition of carbon black. Commonly available vinyl tapes consist of acrylic-based PSA or highly cross-linked rubber-based PSA. The adhesive layer

may also exhibit plasticizers, either intentionally added by the manufacturer or as a result of migration from the backing layer.

6.3.2.3 Other components found in vinyl tapes
As with duct tape, two additional layers, a release coat and a primer layer, may be used in vinyl tapes.

6.3.3 Polypropylene packaging tape
Polypropylene packaging tape has been designed as a general-purpose tape used to seal packages. The two main components are the polypropylene backing and the adhesive.

6.3.3.1 Polypropylene packaging tape backing
Packaging tape backings are typically clear but also can be found in various shades of tan or brown. The polypropylene, which is in the isotactic form, can be subdivided into two distinct types based upon their tear resistant properties: monoaxially oriented polypropylene (MOPP) and biaxially oriented polypropylene (BOPP). A monoaxially oriented backing is formed into a thin film by stretching the polypropylene material as it is slowly cooled in one direction only (length-wise) prior to introducing it into the tape manufacturing process. A biaxially oriented backing is manufactured by stretching the film in two directions (length-wise and width-wise). There is a distinct end-use or consumer difference between a MOPP and a BOPP tape: a MOPP tape is marketed as a "hand-tearable" tape, and BOPP tapes require a cutting tool such as a dispenser. Total tape thicknesses are on the order of 1.5-2.0 mil. The thickness of the film alone typically varies from 0.9 to 1.0 mil but can range from 0.8 - 2.0 mil.

6.3.3.2 Polypropylene packaging tape adhesive
Packaging tape adhesives are typically clear but are available in shades of tan or brown. Generally, when the backing is colored, the adhesive will be clear and vice versa. While clear adhesives contain no inorganic material, the colored adhesive may contain inorganic material such as iron oxide and titanium dioxide. Adhesive formulations typically are isoprene-based, styrene-isoprene copolymer-based (SIS), or acrylic-based.

6.3.3.3 Other components found in polypropylene packaging tape
Two additional layers that may be present within a packaging tape product are a release coat and a primer coat.

6.3.4 Saturated paper tape
"Masking tape" consists of a paper backing, a saturant, and an adhesive. This type of tape is used as a masking material for paint applications and other general-purpose applications.

6.3.4.1 Saturated paper tape backing
The backing of a paper tape is either flatback or creped paper, which has been saturated with carboxylated butadiene styrene, acrylonitrile butadiene, or a similar material. The purpose of a saturant is to fill porous material and boost the strength of the backing. The paper alone typically exhibits weak internal and external strengths, and the saturant fills the voids between the paper fibers adding strength to the product and minimizing absorption of paint products.

6.3.4.2 Saturated paper tape adhesive
The adhesive for saturated paper tapes typically is an isoprene-based PSA or a styrene-butadiene block copolymer, either of which may contain inorganic filler. Acrylic-based adhesives have been used as well, but for outdoor or "clean release" formulations. These adhesives for saturated tapes are formulated with less tack since strong adhesion to a surface is less desirable in masking applications. As with most tapes, if the product is designed to endure exposure to high heat or chemical reagents, the formulation will be cross-linked to provide the needed strength.

6.3.4.3 Other components found within saturated paper tape
The backsize layer is applied to the side of the backing opposite of where the adhesive will be applied. The main purpose of this layer is to coat and fill the porous surface of the backing with a material that is inert to the adhesive formulation to be used. There are a variety of materials that can be used for this purpose, such as acrylic and polyvinyl acetate, and the material used will depend upon the adhesive formulation. In conjunction with the adhesive formulation, a primer coat may be present.

6.3.5 Other tapes
The previous sections have discussed the more common types of tapes encountered within forensic casework. There are numerous other types of tape that may also be found less frequently. These types include, but are not limited to, filament/strapping tape, cloth/ medical tape, and office tape.

6.3.5.1 Filament/strapping tape
Filament tapes are similar to packaging tapes in construction with the addition of reinforcement material. The backing for this type of tape is typically constructed of oriented polypropylene (low cost) or polyester (high cost). The reinforcement filaments can be glass, polyamide fibers, or polyester fibers running in the machine direction. Adhesives found on such tape products can be colored, dependent upon inorganic material content, or colorless. The elastomer can be either isoprene or styrene-isoprene block copolymer.

6.3.5.2 Cloth tape
Cloth tapes are most frequently used for medical and athletic purposes. Common cloth materials include natural and synthetic woven fabrics (e.g., cotton, polyester). Traditionally, adhesives were natural rubber-based, but in recent history have been largely replaced by acrylic copolymers and other synthetic elastomers.

6.3.5.3 Office tape
Office or stationery tape is comprised of a backing and a PSA. The most common tape backings include cellulose acetate, cellophane, and polypropylene and can range in appearance from clear glossy or matte to a translucent yellow. The PSA can be isoprene-based, acrylic-based, or styrene-isoprene copolymer-based. As mentioned in the previous tape discussions, a release coat and a primer layer may also be present.

7.0 Sample Handling

7.1 Due to concerns with the handling of tape as physical evidence, each laboratory must develop appropriate procedures concerning sample size, collection, packaging, preservation, and order of examinations.

7.2 Different forensic disciplines may be called upon to examine the same item of evidence. The order in which the examinations will be conducted needs to be resolved on a case-by-case basis. The order of examinations should be selected and conducted so as to preserve the most transient evidence and provide the greatest discrimination and most valuable information. Examiners must be aware or make the submitting agency aware of the effects that some disciplines' processing and examinations may have upon other specific examination requests. If another discipline is chosen before the tape examination, obtaining an unadulterated representative sample should be considered.

7.3 When the amount of a tape specimen present for comparison purposes is adequate in size—as deemed by the examiner—bulk or lot sampling is the sampling method of choice. Considerations involved with bulk sampling should include where the sample is taken, how much sample is taken, and if the sample is considered representative of the whole. The examiner must be able to explain how the samples were taken and why the sampling technique was used.

Nondestructive methods should be exhausted before subjecting the sample to any destructive tests.

7.4 Techniques to untangle tape specimens should be chosen with care to minimize alterations in the chemical or physical properties. Methods include mechanical separation using warm air, liquid nitrogen, or appropriate solvents.

7.5 The item of evidence should be preserved in a manner that does not interfere with future testing.

7.6 Tape samples submitted as evidence may be degraded by environmental exposure or subjected to physical damage. The strength of an association between a damaged piece of tape and a more pristine sample might be weakened depending upon the degree of damage. In some cases, the damaged tape may be unsuitable for comparison purposes.

8.0 Methods

This section provides an overview of suggested flow of analytical techniques to be utilized for the analysis of tape. The selection of methods is at the discretion of each examiner on a case-by-case basis and will vary depending upon sample size or condition, availability of laboratory instrumentation, and examiner training. Subsequent SWGMAT documents will address these methods in more detail specific to tape analysis.

8.1 Physical Characteristics
Macroscopic and stereomicroscopical observations (e.g., color, thickness, width, and reinforcement construction) provide initial and discriminating information for tape comparisons. Physical end matches can provide individualizing associations.

8.2 Polarized Light Microscopy
Characterization of inorganic materials and other tape additives are accomplished with the use of PLM. PLM is a useful adjunct to FTIR and elemental analysis. Optical properties of oriented polymers such as polypropylene (MOPP and BOPP) and polyester can also be determined. PLM is also used to evaluate and differentiate the reinforcement fibers of tapes (e.g., duct tape and strapping tape).

8.3 Fourier transform infrared spectroscopy
Organic and some inorganic constituents may be evaluated with the use of infrared spectroscopy. These components include the backing polymer, adhesive elastomer, plasticizers, additives, and reinforcement fibers. The use of a bench ATR (attenuated total reflectance) accessory is particularly useful for surface analysis of a larger area of the adhesive and backing.

8.4 Elemental techniques
Common analytical techniques that can be utilized for the characterization of the inorganic constituents of tapes include scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS), x-ray fluorescence spectroscopy (XRF), inductively coupled plasma (ICP) techniques and X-ray powder diffractometry (XRD). SEM/EDS, XRF and ICP provide elemental profiles of analyzed specimens while XRD provides crystalline structure information. Additionally, SEM has imaging capabilities to evaluate surface topography of tape backings.

8.5 Pyrolysis gas chromatography
Organic constituents may be further characterized by py-GC. This technique separates the formulation into it individual organic components. This is particularly useful when inorganic fillers in the tape obscure the FTIR interpretation. Py-GC can be coupled with mass spectrometry to obtain molecular information.

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Guideline for Assessing Physical Characteristics in Forensic Tape Examinations

Scientific Working Group on Materials Analysis (SWGMAT)

1.0 Scope

This document is part of a series of SWGMAT guidelines relating to the forensic analysis of tape and is intended to assist individuals and laboratories that conduct physical examinations and comparisons of pressure sensitive tapes. Its aim is to provide a description of the methods used to assess the physical characteristics of tape evidence.

2.0 Reference Documents

ASTM International Standards

D 1535 *Method for Specifying Color by Munsell System*
E308 *Test Method for Computing the Colors of Objects by using the CIE System*
E1459-92 (2005) *Standard Guide for Physical Evidence Labeling and Related Documentation*
E1492-05 *Standard Practice for Receiving, Documenting, Storing, and Retrieving in a Forensic Laboratory*

SWGMAT Trace Evidence Quality Assurance Guidelines [Online] (January 2000). Available: http://www.fbi.gov/hq/lab/fsc/backissu/jan2000/swgmat.htm.

SWGMAT Trace Recovery Guidelines [Online] (October 1999). Available: http://www.fbi.gov/hq/lab/fsc/backissu/oct1999/index.htm

SWGMAT Forensic Fiber Examination Guideline [Online] (April 1999) Available: http://www.fbi.gov/hq/lab/fsc/backissu/april1999/index.htm

SWGMAT Guideline for the Forensic Examination of Pressure-Sensitive Tapes [Online] (October 2008) Available: http://www.fbi.gov/hq/lab/fsc/backissu/Oct2008/index.htm

3.0 Terminology

Adhesive: A material that will hold two or more objects together solely by intimate surface contact.

Backing: A thin flexible material to which the adhesive is applied.

*Calendering:*The use of a multi-roll device to apply pressure sensitive adhesive at 100% solids to various backings by heat and pressure to produce adhesive tape.

CIE: International Commission on Illumination

Duct tape: Fabric-reinforced tape used for air duct installation or for general utility applications

Electrical tape: PVC-backed tape with specific dielectric properties designed for electrical applications

Fill yarn: Yarns in the scrim fabric of reinforced tape that run crosswise, perpendicular to the warp direction. Also called weft yarns

Filament tape: A fiber-reinforced tape in which the reinforcing fibers are only in the warp direction. Also referred to as strapping tape

Long-wave UV illumination: In the wavelength range from 400 nm – 315 nm with peak wavelength energy at 366 nm

*Machine direction:*The direction of the tape that runs the length of the tape.

Masking tape: Paper-backed tape having a creped, usually beige or buff-colored backing

Nominal width: The design width of the tape, usually in terms of round numbers. Measured width can vary from nominal width due to stretching or weathering.

Packaging tape: a) Pressure-sensitive tape consisting of an oriented polymer with a brown or clear adhesive layer, or b) Paper-backed tape, which has a moistenable adhesive.

Physical end match: A one-of-a-kind fit between two pieces of torn or cut ends

Scrim: A loosely-woven gauze-type cloth added to duct tape for reinforcement and strength

Scrim: The dimensional count of the scrim, in terms of threads per inch, expressed as warp count by fill count

*Short-wave UV illumination:*In the wavelength range from 280 nm- 100 nm with the peak wavelength energy at 254 nm

Stereomicroscope: A microscope containing two separate optical systems, one for each eye, giving a stereoscopic view of a specimen

Strapping tape: See filament tape.

Texturized yarn: A crimped feature in reinforcement fibers designed to give bulk

Twist: The direction of twist in yarns is indicated by the capital letters S and Z. Yarn has an S-twist if when it is held vertically, the spirals around its central axis slope in the same direction as the middle portion of the letter S, and Z-twist if they slope in the same direction as the middle portion of the letter Z.

Warp yarns: Yarns in scrim fabric of reinforced tape that run lengthwise (in the machine direction)

*Weft yarns:*See fill yarns.

Yarn: For the purposes of this document, yarns refer to lengths of fiber reinforcement: twisted staple fibers or filament fibers.

4.0 Summary of Guide

Tape specimens can be examined to determine a common source or possible manufacturer. This guide covers the visual and stereomicroscopic examinations of color, thickness, reinforcement, and backing and adhesive features. Structural details, such as design, construction, and composition, can provide information that may assist the analyst in reaching a conclusion.

A goal of a tape comparison is to assess the significance of any observed differences. If no significant physical differences are found between samples, instrumental analyses are warranted.

5.0 Significance and Use

Physical characterization of tape specimens is the initial step of a comprehensive forensic pressure sensitive tape analysis. The construction, composition, and color of tapes vary and, therefore, are useful characteristics for forensic examinations. Visual characteristics and physical measurements are the quickest, most discriminating and least invasive examinations.

6.0 Sample Handling

6.1 An effort should be made not to alter the condition of a questioned specimen before the preliminary examination. In some circumstances, it may be desirable to obtain a sample cutting from the tape before a sample is analyzed for latent fingerprints. Necessary precautions should be taken to eliminate loss or contamination of other types of evidence (e.g., latent prints, DNA, and other trace evidence).

6.2 Samples for testing should not be cut from the ends of the tape if there is a possibility of a physical end match between specimens. A sample should be obtained from an area that does not interfere with the existing end(s), and the location should be marked.

6.3 If tape is received in a tangled condition an attempt should be made to separate it manually with a careful peel. More aggressive techniques such as gentle heat, liquid nitrogen, freezing, or solvents can be used if necessary. However, these techniques could affect the outcome of subsequent analyses and should, therefore, be applied only to the extent necessary.

6.4 All procedures must be conducted in such a manner to ensure that no cross-contamination occurs. The item must be photographed or described prior to conducting any analyses in order to provide documentation of original condition. Transient evidence (e.g., hair, fiber, paint) should be preserved and documented.

6.5 Tape may not be in its original state due to weathering, stretching, chemicals, etc. These changes may limit the information obtained from the analyses. Tape does not always allow for the full range of examinations, the examinations and analyses that are performed should be reflected in the analyst's notes.

7.0 Analysis

Written descriptions, sketches, photography, or other imaging methods must be used to document each sample's characteristics.

Preliminary examination of tape construction should include its general appearance macroscopically and under a stereomicroscope, including any adhering matter.

For all pressure sensitive tapes, document and record any physical damage (e.g., worn, cut, torn, frayed). The following general macroscopic characteristics should be observed and documented:

- General condition
- Wad, flat pieces, or fragments
- Dimensions (e.g., nominal width and length)
- Number of pieces
- Colors

- Severed ends for possible physical matches

7.1 Physical end match
When conducting comparison examinations between two or more tape specimens, the free ends should be carefully examined for possible physical end matches. A physical end match is defined as free ends of separate pieces of tapes that physically fit together demonstrating that the two pieces were once one continuous piece. Even though this type of association is the most compelling type of association, the analyst may elect to continue with a complete analytical analysis of these specimens depending upon the quality of the end match.

7.1.1 General guidelines for examination of tape evidence for possible physical end matches:

- Observe the tear or cut pattern from the backing and adhesive side of both specimens to determine if a physical association is plausible. For finer detail, a stereomicroscope should be used to examine the ends.

- If the backing is distorted or folded over and adhered to the adhesive layer, gently straighten it out to restore the torn/cut edge. This may be accomplished with the careful use of forceps, gentle heat, or mild solvent.

- Depending on the type of tape, manufacturing marks, creping on a paper backing, printing or any other continuous surface features may be present across fractured edges and would provide additional points of comparison.

- Determine if there are individualizing characteristics (e.g., a flaw or mark) that extends across the fracture. This would be an accidental or anomalous mark that initiates on one piece and terminates across the fracture edge on the other.

- If the tape has a fabric reinforcement layer, solvent (e.g., hexane, chloroform, or xylene) may be used to remove a sufficient amount of adhesive to expose the fabric and ensure alignment of the yarns that have broken across the torn ends.

- Any physical associations must be documented with descriptive notes. Physical associations between specimens that link a suspect to a crime scene or to a victim should be imaged. The imaging method should be dimensionally accurate and include a measuring scale.

- It is strongly recommended that any/all associations between a question specimen and a known specimen be confirmed by another qualified analyst.

7.2 Physical Features
Tape examinations involve a process of documenting all of the physical characteristics exhibited.

The following characteristics should be documented when applicable:

- Color of adhesive and backing
- Surface texture
- Width measurement
- Overall thickness
- Backing thickness

Each of these characteristics can have a number of sub-elements, all of which can be characterized to complete the examination. Physical characteristics of a tape may change after removal from the original roll. The analyst must decide what is within an acceptable tolerance. Any measuring devices used should be properly checked with applicable quality assurance and control procedures.

7.2.1 Backing
The type of backing must be recorded (e.g., paper, polymer film). The backing should be examined both macroscopically and by using a stereomicroscope for color, texture and appearance under multiple illumination sources. For comparative examinations, a side-by-side color comparison of two or more backings is appropriate; otherwise, the Munsell or CIE color systems may be utilized.

7.2.1.1 Markings on the Backing
Under the stereomicroscope the tape should be examined for features such as calendering marks, striations, dimples, and inclusions. The shapes and type of markings should be documented.

7.2.1.2.1 Multiple Layer Backings
Multiple layers may be present in tape backing and should be examined to determine if multiple layers are present. There are a number of ways to cross-section tapes (e.g. hand sectioning, microtome). The multiple layers should be characterized and then analyzed with appropriate analytical instrumentation.

7.2.2 Adhesive
The adhesive should be examined both macroscopically and by utilizing a stereomicroscope for color and appearance under multiple illumination sources. For comparative examinations, a side-by-side color comparison of two or more adhesives is appropriate; otherwise, the Munsell or CIE color systems may be utilized.

7.2.3 Reinforcement
If reinforcement is present in a tape, it should be characterized.

7.2.3.1 Duct Tape Reinforcement
The three main features to examine in duct tape reinforcement are weave, yarn description and scrim count.

Assess the weave of the scrim fabric under the stereomicroscope. This may require separating the adhesive from the scrim. The most frequently encountered weave patterns are weft-insertion and plain weave. Weft-insertion has chain-stitch warp yarns with texturized filaments in the fill direction. A plain weave has a one over/one under pattern; the warp and fill directions can be a combination of any of the following types of yarns:

- twisted yarns (Z- or S-twist)
- filament fibers bound by another filament fiber
- texturized filament fibers
- straight filament fibers

The fluorescence of the threads should be examined using short- and/or long- wavelength illumination.

The number of filaments per bundle in the fill and warp directions may be counted.

The scrim count is the warp count per inch and the fill count per inch and should be recorded.

7.2.3.2 Strapping (Filament) Tape Reinforcement

The fibers in filament tape most often consist of synthetic or glass fibers. The fibers are only in the warp direction. The number of bundles across the width of the tape may be counted, and the fluorescence of the threads should be examined using short- and/or long- wavelength illumination.

8.0 Report Documentation

The goal is to produce documentation that will be meaningful to a reviewer in the absence of the recording analyst. The resulting notes must be sufficient to document the conclusions reached in the analyst's report. All pertinent data, including any documentation of physical end matches, should be placed into or referenced within the case file. For comparative tape examinations, if differences are observed in physical characteristics, no further testing is necessary, and a report can be issued. If no significant differences are observed, instrumental examinations should be performed before a report is issued. In sourcing cases, instrumental examinations may be necessary before a report is issued.

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Guideline for Using Light Microscopy in Forensic Examinations of Tape Components

Scientific Working Group for Materials Analysis (SWGMAT)
Scope | Referenced Documents | Terminology | Summary of Guidelines | Significance and Use | Sample Handling | Analysis | Report Documentation | References

1.0 Scope

This document is part of a series of guidelines, prepared by the Scientific Working Group for Materials Analysis (SWGMAT), relating to the forensic analysis of tape and is intended to assist individuals and laboratories that conduct microscopic examinations and comparisons of pressure-sensitive tapes. These methods emphasize the examination and comparison of duct tape (a fabric reinforced tape) and clear polypropylene packing tape (a non-reinforced tape). However, the methods are general and may also be used for other tape types.

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3.0 TERMINOLOGY

Biaxially oriented polypropylene (BOPP): An oriented polypropylene film in which the polymer has been stretched in both the machine direction and cross direction during the manufacturing process. Tapes with such films cannot be torn by hand.

Birefringence: The numerical difference in principle refractive indices for a substance.
Cross direction: The direction of the tape that runs across the width of the tape.

Delusterant: An agent used to alter the light reflected from a fiber causing a dulling effect.

Dispersion staining: A procedure involving central or annular stops in the objective back focal plane to induce colored images of transparent particles mounted in liquids with indices matching the particle at a wavelength in the visible range.

Extinction angle: As it applies to tape, the angle between the machine edge of a clear oriented polymer tape film under crossed polars and the point of extinction (appears dark under crossed polars).

Fill yarns: Yarns in the scrim fabric of reinforced tape that run crosswise, perpendicular to the warp direction. Also called weft yarns.

Machine direction: The direction of the tape that runs the length of the tape.
Monoaxially oriented polypropylene (MOPP): An oriented polypropylene film in which the polymer has been stretched in only one direction during the manufacturing process. Tapes with such films can be torn by hand.

Mounting medium: A liquid, polymer, or resin used to mount specimens for microscopical examinations.

Refractive index: The ratio of the velocity of light in a vacuum to the velocity in some medium.

Retardation: The actual distance of one of the doubly refracted rays behind the other as they emerge from an anisotropic substance. The amount of retardation is dependent on the difference between the two refractive indices and the thickness of the material.

Sign of elongation: A reference to the orientation of the refractive indices in an anisotropic substance as it relates to the elongated direction of a substance. If the slow wave (higher refractive index) is in the elongated direction it has a positive sign of elongation. If the fast wave is in the elongated direction it has a negative sign of elongation.

Warp yarns: Yarns in scrim fabric of reinforced tape that run lengthwise (in the machine direction).

Weft yarns: See fill yarns

4.0 Summary of Guidelines

The following layers of PSA tape will be examined:

- Clear and semi-opaque film backings are examined for optical properties and additives.
- Adhesives are examined for filler components.
- Fibers in fabric-reinforced tape are examined for construction and fiber classification.

Some microscopical examinations of tape involve separation of the layers. After mounting the samples on microscope slides, tapes are compared to determine if they have similar optical properties and morphological features.

5.0 Significance and Use

There is variability in tape films, adhesives, and fibers that can be readily noted with transmitted and polarized light. Some tapes may exhibit microscopic variability that cannot be readily detected in other instrumental or macroscopic examinations. Microscopical examinations of the tape components offer a simple way to assess the similarities and differences between tape products. If differences can be seen by this technique, further tests are not necessary.

6.0 Sample Handling

Other initial examinations, such as physical match, macroscopical/ stereomicroscopical examinations, and the collection of trace evidence such as hair and fibers adhering to the tape,

should be completed before proceeding with sample mounting for microscopical examinations. Refer to section 6 of the Physical Characteristics Guideline protocol.

If a questioned tape is submitted in a tangled condition, refer to section 6 of the Physical Characteristics Guideline protocol. Care must be taken with any heat methods to avoid stretching the tape's polymer backing.

7.0 ANALYSIS

Tape samples should be examined first under a stereomicroscope. Areas of the tapes that appear in their original state (i.e., not stretched out of shape and having a clean adhesive area) should be selected for analysis. Fingerprint powders or chemicals should be gently but thoroughly cleaned from the film backing.

7.1 Fabric-Reinforced Tape (Duct tape, Gaffer's tape, Strapping tape)

All three layers of fabric-reinforced tape may be mounted separately for microscopy. The adhesive and reinforcement fibers will have more discriminating microscopic features than the film backing. These methods describe the full examination; however, it is at the discretion of the examiner to choose those methods that are most suitable to the given case. Refer to the SWGMAT *Forensic Fiber Examination Guidelines*, "Microscopy of Textile Fibers" section 7.2 for mounting media recommendations.

7.1.1 Sample Preparation- Areas of the tape free of contamination are selected for analysis. This is best done under a stereomicroscope. Ends should be avoided when cutting samples. The tape is initialed at the site of the cut. The film **backing** is separated from the adhesive and fabric. A suitable solvent (e.g., hexane) may be used if mechanical separation is not feasible. The clean film is mounted in the appropriate medium and cover-slipped.

Microscopical examinations of the **adhesive** are useful only in opaque adhesives. The adhesive is separated from backing by pinching with tweezers and cutting with a scalpel and then transferred to a microscope slide. Care should be taken not to include fibers in this sample. Xylene or a similar solvent may be added to the adhesive sample on the slide to disperse the adhesive's rubber base. After drying, the sample is mounted in a suitable mounting medium and cover-slipped. Most minerals of PSAs can be evaluated in mounting media having refractive indices of 1.66 and 1.55.

Fibers from the scrim fabric can be gently pulled and clipped from the adhesive for mounting. If necessary, the fibers can be rinsed of any adhering adhesive using hexane or other suitable solvent. The warp and fill yarns may be cotton/polyester blends. Therefore, the whole bundle should be loosely mounted on a microscope slide in a mounting medium. Warp and fill yarn fibers are mounted separately.

7.1.2 Microscopical Examination of the Mounted Film Backings

Tape backings with some transparency may be cleaned of adhesive and mounted in a mounting medium. In duct tapes the gray color of polyethylene film backing is due to the presence of aluminum powder. Viewing mounted duct tape films under transmitted light on a comparison microscope may offer some comparative information about the density, size, and dispersion of the aluminum particles in tapes. Note that duct tape backings may be multilayered. A cross section of the duct tape backing should be examined for physical characteristics and chemical composition. In clear and matte backings from strapping tapes and office tapes, additives are looked for and noted in plane polarized and cross polarized light. In addition, clear backings may be suitable for the methods described in section 7.2.

7.1.3 Microscopical Examination of the Mounted Adhesives
The inorganic fillers of PSAs may be examined under transmitted plane and crossed polarized light. Mounting media with refractive indices of 1.66 and 1.55 are suitable for most mineral types that may be found in PSAs. The morphological and optical features of the different inorganic fillers can be noted. These particles are mixed with the elastomer and tackifying resin and include, but are not limited to, kaolinite, calcite, dolomite, rutile, zincite, or talc. Dispersion of adhesive samples first in xylene, as described in section 7.1.1, allows for a better assessment of these fillers. The identity of these minerals may be surmised from their optical properties along with the IR spectra and elemental composition.

7.1.4 Microscopical Examination of the Fiber Reinforcement
Refer to the SWGMAT *Forensic Fiber Examination Guidelines*, "Microscopy of Textile Fibers," for methods of determining the optical properties of the reinforcement fibers of the tape. Using these microscopic methods the following observations should be made separately for the warp and fill fibers:

Fiber class - usually cotton or polyester.
Diameter - of each class of fibers.
Delusterant - either absent, light, medium, or heavy.
Shape - may be round, polygonal, tri-lobal, etc.
Blending - cotton may be blended with polyester.

7.2 Non-Reinforced Tape - Examinations of Oriented Films (Clear Polypropylene Packing Tape)

The methods described in this section are recommended for clear packing tapes; however, they are applicable to other non-reinforced tapes with clear backings. Transparent 1/2" office tapes and some strapping/filament tape may have oriented polymer backings.

The variability in the polymer films in clear packing tape is imparted during the manufacturing process. Controlled heating, cooling and stretching produce films with both amorphous and crystalline areas. Biaxially oriented polypropylene (BOPP) is stretched in two directions with crystalline bundles lining up along the two stretched directions. Monoaxially oriented polypropylene (MOPP) is stretched in one direction. The differences in these two types of packing tapes can be distinguished with polarized light microscopy. Within each of these subclasses of packing tapes, variances may also be noted in the extinction direction with respect to the machine direction of the tape. Differences in interference colors will reveal differences in tape film thickness.

The polypropylene film of packing tapes behaves as an optically biaxial crystal. There are two perpendicular refractive indices in the plane of the film. One runs roughly in the cross direction and the other in the machine direction. The third refractive index runs normal to the plane of the film.

7.2.1 Sample Preparation - Select about an inch of tape that has both machine edges and appears to be in its original state (i.e., has not been damaged by heat, stretching, or contamination). Stick this piece directly onto a clean microscope slide, adhesive side down. An arrow noted on the mounted sample can help keep track of which direction is the machine direction.

There is no need to separate the adhesive from the film for the microscopic examination of clear packing tape. Brown packing tapes with clear film backings and colored adhesive must have the adhesive removed. The cleaned film may be mounted in an appropriate medium for microscopic examinations.

7.2.2 Determination of Polypropylene Film Orientation –
The following polarized light observations presume that the microscope is optimally aligned and illuminated. Refer to the SWGMAT *Forensic Fiber Examination Guidelines*, "Microscopy of Textile Fibers" section 7.3 for further details concerning the determination of optical properties referred to in the following discussions.

The surface of the clear packing tape sample is brought into focus in transmitted light at about a 100X magnification. The polars are crossed, and the extinction position is found. The stage is rotated just off extinction, and patterns are observed in the film. These patterns may be sharpened by refocusing and closing down the aperture diaphragm. A pattern of "X"s is seen in biaxially oriented tapes (BOPP) and shows the bi-directional stretching in the production process. The pattern seen in monoaxially oriented tapes (MOPP) shows the one direction of stretching. Its pattern may be hazy and show more than one interference color that streak in the one direction of the stretch.

The angles of the crosshatches in the BOPP tape pattern described above may vary from one tape film to another but will be consistent throughout a roll of tape. These angles can be determined with an appropriate eyepiece reticule.

7.2.3 Determination of the Extinction Angle Relative to the Machine Direction
The machine direction of the tape relative to the extinction direction may vary from 0 to 15 degrees between different tapes.

The surface of the tape is brought in focus in transmitted light. One of the machine edges of the tape is aligned with the vertical line of the eyepiece graticule. The stage position is noted in degrees. The polars are crossed and the stage is rotated until the tape film is at its nearest full extinction. The stage position is again noted. The difference in degrees is the extinction angle relative to the machine edge. Tape samples from the same roll will show similar extinction angles.

7.2.4 Determination of the Retardation - The thickness of the polymer film in clear packing tapes can vary within manufacturers. When the birefringence of the films of the different packing tapes is the same, the variance in the interference color of the films will be a function of the thickness. Slight differences in thickness will show noticeably different interference colors. These interference colors depend only on the tape film thickness, not the total tape thickness (film + adhesive). The clear adhesive layer is isotropic and does not contribute to the interference colors.

Using approximately 400X magnification, the surface of the tape is brought into focus and the polars are crossed. The interference color is noted with the stage rotated to maximum brightness (close to 45 degrees). The fast wave (lower refractive index) is found with one refractive index running roughly across the tape and the other running roughly lengthwise along the tape. The fast wave is aligned parallel to the slow wave of a quartz wedge or Berek compensator. The point of compensation is found and from this, the retardation can be calculated. The experience of the microscopist may dictate the easiest way to arrive at the retardation value.

The birefringence of the polypropylene tape film (BOPP) in the plane of the tape (the difference between the refractive indices of the machine and cross directions of the tape) has been reported in the range of 0.014 - 0.016. (Rappe 1991)

7.2.5 Other Observations - Some clear tape films may have additives that may be visible in transmitted or polarized light and their presence is useful for comparison purposes between tapes. Some assessment of their optical properties should be noted: size, distribution, relative interference colors, etc.

Irregularities in the thickness of the tape film may be observed under crossed polars as multiple interference colors in any given field.

Some tape films may not totally extinguish, or they may show undulose extinction (i.e., areas of lightness and darkness).

7.3 Microscopy of other Tape Classes - Any tape class that has inorganic fillers in the adhesive or backing, reinforcement fibers, or clear or semi-opaque film backings may lend itself to examinations described in these guidelines.

8.0 REPORT DOCUMENTATION

The examiner's notes should contain all descriptions, diagrams, photographs, and calculations that reflect the mcroscopical observations. These observations are only a part of the overall analysis of the tape samples that includes other macroscopical and instrumental exams. For comparative tape examinations, if significant differences are observed in microscopical characteristics, no further testing is necessary, and a report can be issued. If no significant differences are observed, further instrumental examinations should be performed before a report is issued.

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Guideline for Using Fourier Transform Infrared Spectroscopy in Forensic Tape Examinations

Scientific Working Group on Materials Analysis (SWGMAT)

1.0 Scope

This document is part of a series of SWGMAT guidelines relating to the forensic analysis of tape. Infrared spectroscopy (IR) is a valuable method for the identification and comparison of pressure sensitive tape components. This document provides basic recommendations and information about FTIR-spectrometer components and accessories, with an emphasis on sampling techniques specific to tape components. The particular method(s) employed by each examiner and/or laboratory will depend upon available equipment, examiner training, sample suitability, and sample size. It is assumed that the examiner has a basic knowledge of the theory and proficiency in the use of infrared spectroscopy.

2.0 Reference Documents

ASTM International Standards

E1492-05 *Practice for Receiving, Documenting, Storing, and Retrieving in a Forensic Laboratory*

E131-98 *Terminology Relating to Molecular Spectroscopy*

1421-99 (2004) *Standard Practice for Describing and Measuring Performance of Fourier Transform Mid-Infrared (FTMIR) Spectrometers: Level Zero and Level One Tests*

E 573-01 (2007) *Standard Practices for Internal Reflection Spectroscopy*

SWGMAT Trace Evidence Quality Assurance Guidelines [Online] (January 2000). Available: http://www.fbi.gov/hq/lab/fsc/backissu/jan000/swgmat.htm.

SWGMAT Trace Recovery Guidelines [Online] (October 1999). Available: http://www.fbi.gov/hq/lab/fsc/backissu/oct1999/index.htm

SWGMAT Forensic Fiber Examination Guideline [Online] (April 1999) Available: http://www.fbi.gov/hq/lab/fsc/backissu/april1999/index.htm

SWGMAT Guideline for the Forensic Examination of Pressure-Sensitive Tapes [Online] (October 2008) Available: http://www.fbi.gov/hq/lab/fsc/backissu/Oct2008/index.htm

3.0 Terminology

Absorbance, A: the logarithm to the base 10 of the reciprocal of the transmittance, (*T*). $A = \log_{10}(1/T) = -\log_{10}T$.

Absorbance band: a region of the absorption spectrum in which the absorbance passes through a maximum.

Absorbance spectrum: a plot or other representation of a function of absorbance against any function of wavelength.

Absorptivity: an absorbance divided by the product of the sample pathlength (b) and the concentration of the absorbing substance (c). The units of b and c shall be specified.
a = *A*/bc.

Aperature: an opening in an optical system that controls the amount of light passing through a system.

Attenuated total reflectance (ATR): a method of spectrophotometric analysis based on the reflection of energy at the interface of two media which have different refractive indices and are in intimate contact with each other; also known as Internal Reflection Spectroscopy (IRS).

Background: absorption caused by anything other than the substance for which the analysis is being made.

Beam condenser: a series of mirrors that focus the infrared beam to a small area in the sample compartment to permit the examination of smaller samples than would otherwise be possible.

Beam splitter: an optical component that partially reflects and partially transmits radiation from the source in such a manner as to direct part to a fixed mirror and the other part to a moving mirror.

Deuterated triglycine sulphate (DTGS) detector: a thermal detector that operates at room temperature but lacks the sensitivity for use with microscope accessories.

Double-pass transmission spectra: spectra that results from incident radiation passing through the sample, reflecting off the substrate, and passing through the sample a second time.

Far-infrared: the infrared region of the electromagnetic spectrum with wavelength range from approximately 25 to 1000μm (wavenumber range 400 to 10 cm^{-1}).

Filler/extender: an inorganic material that is added to a tape to modify a physical property or reduce cost.

Fourier transform (FT): the mathematical process which is used to convert an amplitude-time-spectrum to an amplitude-frequency spectrum, or *vice versa*. In FTIR spectrometry, retardation is directly proportional to time; therefore, FT is commonly used to convert an amplitude-retardation spectrum to an amplitude-wavenumber spectrum, and *vice versa*.

Fourier transform infrared spectrometry (FTIR): a form of infrared spectrometry in which an interferogram is obtained; this interferogram is then subjected to a Fourier transform to obtain an amplitude-wavenumber (or wavelength) spectrum.

Infrared: the region of the electromagnetic spectrum with wavelength range from approximately 0.78 to 1000μm (wavenumber range 12,800 to 10 cm^{-1}).

Infrared spectroscopy (IR): the study and interpretation of spectra within the infrared region of the electromagnetic spectrum.

Interferogram: a plot of the detector output as a function of retardation.
Interferometer: a device used to split a beam of radiant energy into two paths, generate an optical path difference between the beams, and recombine them in order to produce repetitive interference maxima and minima as the optical retardation is varied.

*Internal reflection element:*a high refractive index material (e.g., ZnSe, Ge, or diamond) used as a substrate for the sample to internally reflect the IR electromagnetic radiation.

Internal reflection spectroscopy (IRS): see Attenuated Total Reflection (ATR)

Low E-Glass: glass that is coated with an IR reflective surface. Such glass is suitable for use as a sample support when performing IR reflection techniques.

Mercury Cadmium Telluride (MCT) detector: a quantum detector that utilizes a semi-conducting material and requires cooling with liquid nitrogen to operate. This type of detector is commonly used in microscope accessories due to its sensitivity.

Mid-infrared: the infrared region of the electromagnetic spectrum with wavelength range from approximately 2.5 to 25µm (wavenumber range 4000 to 400 cm^{-1}).

Retardation: optical path difference between two beams in an interferometer; also known as the "optical path difference" or "optical retardation".

Spectrometer: an instrument for the measuring of some function of spectral power, or other physical quantity, with respect to spectral position within a spectral range.

Spectroscopy: the study of the theory and interpretation of spectra generated by any phenomenon, such as electromagnetic waves or particles, ordered in accordance with the magnitude of a common physical property (wavelength, frequency, or mass).

Spectrum: an arrangement of the component parts of any phenomenon, such as electromagnetic waves or particles, ordered in accordance with the magnitude of a common physical property.

Transmittance, (T): ratio of the energy of the radiation transmitted by the sample to the background, usually expressed as a percentage.

Wavelength: the distance, measured along the line of propagation, between two points that are in phase on adjacent waves.

Wavenumber: the number of waves per unit length, in a vacuum, usually given in reciprocal centimeters cm^{-1}.

4.0 Summary of Guideline

This guideline covers the analysis of components used in tape backings and adhesives by infrared spectroscopy. It can be applied to a wide range of infrared spectrometers and accessory configurations.

For the infrared analysis of the fibers in the reinforcement component of tapes, one should refer to SWGMAT Chapter 6 of Forensic Fiber Examination Guidelines: Infrared Analysis of Textile Fibers.

5.0 Significance and Use

This guide is designed to assist an examiner in the selection of appropriate sample preparation methods for the analysis, comparison, and identification of pressure sensitive tape components. If no significant differences are noted in the physical measurements, then IR should be the next step in the analytical scheme.

Infrared spectroscopy can provides molecular information regarding major organic and inorganic components. For various reasons, components in lesser amounts are typically more difficult to identify unequivocally. Reasons for this include interference of the absorption bands of the major components with the less intense bands of minor constituents and sensitivity issues whereby the minor components are present at concentrations below the detection limits of the instrument.

Infrared spectroscopy can be used to obtain spectra for elucidation of the chemical composition of a tape and for comparison of two or more samples. When used for comparison of spectra, the goal is to determine whether any significant differences exist between the samples.

6.0 Sample Handling

6.1 The general collection, handling, and tracking of samples should meet or exceed the requirements of ASTM 1492-05 as well as the relevant portions of the SWGMAT's Trace Evidence Quality Assurance Guidelines and Trace Recovery Guidelines.

6.2 The work area and tools used for the preparation of samples must be free of all materials that could transfer to the sample.

6.3 When analyzing difficult samples (e.g., residue, dirty samples, or inhomogeneous samples), care must be taken when sampling the tape and in choosing appropriate analytical conditions. An attempt should be made to remove any extraneous material from the specimen before sampling. In order to ensure reproducibility and/or evaluate intra-sample variations, repeat analysis of any samples is recommended.

6.4 If the tape has been processed by the latent print unit and an unprocessed piece of tape has not been retained, a number of options are available to obtain a representative sampling for infrared analysis. Tape backing can be cleaned with an appropriate solvent. Alternatively, a clean portion can be obtained by manually removing the residue from the latent print processing by gentle scraping of the surface. A representative adhesive sample can be obtained by exposing and sampling the underlying portion.

6.5 Attenuated Total Reflection (ATR), also known as Internal Reflection Spectroscopy (IRS), is a rapid sampling method for the analysis of the tape backing and the adhesive as virtually no sample preparation is necessary. Single or multiple reflection elements can be used depending on the amount of clean area available for sampling. When only a small clean area is available, a single reflection element is desirable to avoid contamination.

6.6 Transmission microspectroscopy is possible by sampling a small portion of the tape component (backing, adhesive) and analyzing it as a thin film.

6.7 A diamond anvil cell may be used to analyze both the backing and the adhesive portions of tape. This may be used in the bench with a beam condenser or placed under the microscope accessory.

6.8 If the sample backing is opaque, pyrolysis/IR may be used. The condensed pyrolyzate cast as a film on an IR window is normally sufficient to identify the polymer by transmission. No inorganic information can be obtained from this technique. Caution should be exercised when comparing spectra obtained by this technique as the pyrolysis conditions are not optimally controlled.

6.9 Tackifiers and/or plasticizers may be extracted from the adhesive or backing using a mild solvent such as hexane or acetone. They are subsequently analyzed in transmission by casting a thin film.

6.10 Samples being compared should be prepared and analyzed in the same manner.

7.0 Analysis

A standard mid-IR range FTIR spectrometer is acceptable to conduct the necessary analyses. The detector cutoff should be no higher than 750 cm^{-1}. A mid-infrared FTIR spectrometer with an extended range to near 200 cm^{-1} is optimum, as it is advantageous for the classification and comparison of inorganic fillers and pigments.

7.1 Instrument Parameters
7.1.1 Performance and Calibration
It is essential that instrument performance and calibration be evaluated routinely, at least once a month (or before use if used less frequently).

7.1.2 The preferred performance evaluation method is in accordance with ASTM-1421-99 (2004), Sections 1-7, 9.5 and 9.5.1. In brief, this includes evaluation of the following:

System throughput;
Single-beam spectrum;
100% T line;
Polystyrene reference spectrum.

7.1.3 Sample and background scans should be run under the same instrument conditions.

7.1.4 A resolution of 4 cm^{-1} is optimum (one data point every 2 cm^{-1}). Higher resolution may be used. The additional data points, however, typically yield no further analytical information for polymeric samples.

7.2 Main bench

7.2.1 Transmission

7.2.1.1 Samples prepared for analysis by transmission techniques must be thin enough to allow infrared radiation to pass through without being over-absorbed by the sample. For transmission data that are viewed in absorbance, the sample should be thin enough to produce a maximum absorbance of 1 absorbance unit. For transmission data viewed in % Transmittance, optimally, spectral peaks should not fall below 10% T. This typically requires a sample thickness of approximately 5 – 10 micrometers.

7.2.1.2 Sample preparation techniques that may be employed for transmission analysis in the main bench include backing and/or adhesive pressed in a diamond cell, a thin backing sample stretched over an aperture, or adhesive deposited onto an alkali halide pellet (e.g., KBr, NaCL, or AgCl).

7.2.2 ATR

7.2.2.1 ATR methods may lend themselves to conducting the examination of the tape intact. Since ATR is a surface technique it is necessary to remove any extraneous material from the area to be examined. The bench ATR (single reflection) is useful for forensic casework size samples. These accessories utilize an internal reflection crystal to condense the beam onto a spot-sized sampling area.

7.2.2.2 ATR is also useful in the analysis of duct tape backings for layer structure determination. The adhesive is removed, and the backing is analyzed on both sides. The compositions are then compared.

7.3 FTIR Microscope accessory

7.3.1 The use of a microscope accessory is preferred for very small samples. Spectra can be obtained from samples as small as 10-20 micrometers in diameter after flattening.

7.3.2. There is a trade off between sensitivity and spectral range with the MCT detectors. The low energy cut off for most detectors is in the 700-450 cm^{-1} range. The smallest apertures particularly limit the energy from the longer wavelengths (smaller wavenumbers) reaching the detector due to diffraction. Heterogeneity issues are also more pronounced when using very small apertures.

7.3.3 The microscope attachment permits the analysis of multiple samples placed on an appropriate support material. The method affords the advantage of viewing the sample optically and choosing the most appropriate area for analysis.

7.3.4 Spectral measurements using an FTIR microscope can be obtained in transmission, reflection, or ATR mode.

7.3.4.1Transmission

7.3.4.1.1 Transmission measurements are commonly used because they generate spectra with fewer artifacts. However, transmission methods generally entail more sample preparation than reflection techniques. The tape sample must be rendered thin enough not to over-absorb. Samples can be placed directly over a small aperture for analysis or placed on an appropriate salt plate. This typically requires a sample thickness of approximately 3-5 micrometers.

7.3.4.1.2 A diamond cell can also be used as a sample support medium under the FTIR microscope. The adhesive can simply be smeared on one of the diamond faces. The tape backing sample is placed on one of the diamond faces, the second diamond is positioned on top, and sufficient pressure is applied to form a film. For nonelastic samples, one diamond is typically removed prior to analysis once the sample has been compressed. This leaves the thin compressed film adhering to one of the diamond faces.

7.3.4.2 Reflection

7.3.4.2.1 If samples are flattened directly on an infrared light reflecting surface (e.g., low e-glass or gold mirror), the reflection mode can be used to produce spectra mimicking double-pass transmission spectra. The technique is sometimes referred to as "transflection" or “reflection/absorption.” Samples need to be approximately half the thickness of an optimum transmission sample.

7.3.4.2.2 The FTIR microscope can also be used in the specular reflection mode; however, it is not useful for tape unless the surface of the sample is highly reflective.

7.3.4.3 ATR
ATR objectives are available for infrared microscopes. Consistent pressure should be applied to each sample to mitigate spectral variations. Intra-sample variations may result from sample heterogeneity; therefore, multiple samplings should be considered.

8.0 Spectral Interpretation

8.1 Spectral comparison
Comparisons of the tape component spectra can be accomplished by digital overlays with full scale expansion.

8.1.1 Comparison of samples may be conducted with both spectra displayed in transmittance and/or absorbance. Certain information may be seen more readily in one format or the other.

8.1.2 There are a number of significant factors that should be considered when comparing spectra including the presence or absence of absorption bands, and their position (wavenumber), shape and relative intensity. Additional sample replicates may be necessary to evaluate reproducibility of these spectral characteristics

8.1.2.1 The presence of additional absorption bands could be from true differences between the samples or from extraneous material adhering to the tape. If extraneous material is suspected as the source of the difference, the sample should be cleaned or additional samples prepared. If the sample cannot be cleaned or resampled, then spectral subtraction may be an option.

8.1.2.2 For spectra to be considered indistinguishable, the position of the absorption bands should have reasonable agreement with each other. A rule of thumb is that the positions of corresponding peaks in two or more spectra being compared should be within a few wavenumbers of each other, depending on whether the peak is sharp or broad. For sharp absorption peaks one may use tighter constraints and with broad peaks the variation may be slightly greater.

8.1.2.3 For spectra to be considered indistinguishable, the shape of the absorption bands should be consistent between comparison samples. The peak width and the symmetry of each peak should be evaluated. Sample thickness may affect the peak width and resolution.

8.1.2.4 For spectra to be considered indistinguishable, the relative intensities of respective absorption bands should be similar between comparison samples. The relative intensity may be affected by the heterogeneity of the sample.

8.1.3 Three possible conclusions can be reached after evaluating and comparing spectra: 1) the spectra are dissimilar, 2) the spectra are indistinguishable, or 3) inconclusive.

8.1.3.1 The spectra are dissimilar if there are one or more significant differences in the spectra. Significant differences are differences in which the spectral variation cannot be explained other than as differences between samples.

8.1.3.2 The spectra are indistinguishable when there are no significant differences in the spectra. Differences are not significant if the spectral variation can be explained as something other than differences between samples.

8.1.3.3 An inconclusive determination is one in which the significance of the differences cannot be completely assessed due to the constraints of sample size and/or condition.

8.2 Component Characterization
8.2.1 Tape is often comprised of a number of components that result in overlapping bands in the IR spectra; therefore, caution must be exercised while evaluating the data. Not all of the components of tape can be elucidated by IR due to overlapping bands and/or relative concentration.

8.2.2 Tools that can assist in the characterization of the spectra include, but are not limited to, spectral libraries, flow charts, and reference standards. It should be noted that most commercial spectral libraries consist of transmission (as opposed to reflection) spectra. It is desirable to use reference spectra that were obtained using the same sample preparation and collection technique.

8.2.3 The following components, if present, may be characterized by IR spectroscopy depending on the condition of the tape and on the concentration of the material.

- Backing
  - Polymer film
  - Plasticizers
  - Fillers/Extenders
  - Flame retardants
- Adhesive
  - Elastomer
  - Tackifiers
  - Fillers/Extenders
- Release coating
- Fiber reinforcement

9.0 Documentation

9.1 When making comparisons of tape samples, similarity or dissimilarity in the IR spectra should be noted.

9.2 For chemical identification of tape components, the positions of the absorption bands according to wavelength or wavenumber and their relative intensities must be compared to those of known reference spectra. It is desirable to confirm the identification by other methods such as polarized light microscopy (PLM), pyrolysis gas chromatography (Py-GC), scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS), X-ray fluorescence (XRF), and/or X-ray diffraction (XRD).

9.3. Case notes should include a copy of all of the instrumental data that was used to reach a conclusion. All hard copies should include a unique sample designation, the operator's name/initials, and the date of analysis.

9.4. Case notes should also include a description of the evidence analyzed by IR, the method of sample preparation, the analytical instrumentation used, and its operating parameters.

9.5 See SWGMAT's Trace Evidence Quality Assurance Guidelines for further requirements.

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Guideline for Using Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy in Forensic Tape Examinations

Scientific Working Group on Materials Analysis (SWGMAT)

1.0 Scope

This document is part of a series of SWGMAT guidelines relating to the forensic analysis of tape and is an outline of methods for scanning electron microscopy (SEM). This document is a version of SWGMAT's Standard Guide for Using Scanning Electron Microscopy/X-ray Spectrometry in Forensic Paint Examinations, with modifications regarding material-specific information. The methods employed by each examiner and/or laboratory depend on sample size, sample suitability, and laboratory equipment. The term scanning electron microscopy occasionally refers to the entire analytical system, including energy dispersive X-ray spectroscopy (EDS).

This guide is not intended to be an instruction book, nor will it apply in every situation. It is the responsibility of the analyst to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to the use of this technique.

2.0 Reference Documents

ASTM. *Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory*, ASTM E1492-05

Scientific Working Group on Materials Analysis. Guideline for the Forensic Examination of Pressure Sensitive Tapes, *Forensic Science Communications* [Online]. (October 2008). Available: www.fbi.gov/hq/lab/fsc/backissu/oct2008/standards/2008_10_standards02.htm

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Scientific Working Group on Materials Analysis. Trace evidence recovery guidelines, *Forensic Science Communications* [Online]. (October 1999). Available: www.fbi.gov/hq/lab/fsc/backissu/oct1999/trace.htm

3.0 Definitions

The terms defined relate specifically to SEM/EDS analysis as described in this document. General tape definitions can be found in the SWGMAT Guideline for the Forensic Examination of Pressure Sensitive Tapes.

Background X-rays (Bremsstrahlung, braking radiation, continuous spectrum): Nonspecific X-ray radiation with a continuous energy range from zero up to the beam voltage. Background radiation results from the deceleration of beam electrons in the atomic Coulombic field. A typical X-ray spectrum consists of both a continuous background and peaks from characteristic X-rays.

Backscattered electrons (BE): Primary beam electrons that are scattered from the sample after undergoing few inelastic interactions. The probability of backscattering is proportional to the atomic number.

Bulk analysis: A type of scanning electron microscopy analysis that determines the representative elemental composition of a material. The area of analysis is as large as possible and may be achieved by a single large area raster or the summed results from multiple smaller area rasters.

Characteristic X-rays: X-ray emission resulting from de-excitation of an atom following inner shell ionization. The energy of the X-rays is related to the atomic number of the atom, providing the basis for energy dispersive X-ray spectroscopy. A typical X-ray spectrum consists of both a continuous background and peaks from characteristic X-rays.

Charging: Negative charge accumulation on either a nonconductive sample or a sample that is not properly grounded. This effect may interfere with image formation and X-ray analysis because of beam deflection. It can usually be eliminated by the application of a conductive coating or by the use of a low vacuum system.

Concentration: For the purpose of this guide, the following ranges shall apply: Major: greater than 10 percent; Minor: 1 to 10 percent; Trace: less than 1 percent.

Energy dispersive X-ray spectroscopy (EDS, EDXA, EDX): X-ray spectroscopy based on the measurement of the energy of X-rays. Energy dispersive X-ray spectroscopy is a complementary spectroscopy to wavelength dispersive spectroscopy.

Escape peak: A peak resulting from incomplete deposition of the energy of an X-ray entering the energy dispersive X-ray spectrometer detector. This peak is produced when an incoming X-ray excites a silicon atom within the detector crystal, and the resulting Si K-α fluorescence X-ray exits the detector crystal. It occurs at the principal peak energy minus the energy of the Si K-α fluorescence X-ray (1.74KeV). The escape peak intensity is about 1-2 percent of the parent peak.

Extraneous material (contaminant, foreign material): Material originating from a source other than the specimen.

Final aperture: The last beam-restricting orifice in an electron optical column. The orifice diameter influences the beam current and depth of focus.

Interaction volume: The sample volume in which the electron beam loses most of its energy. It is generally thought of as the volume in which detectable X-rays are produced. The actual volume varies depending upon beam voltage, average atomic number, and density of the sample.

KLM reference lines: The energies associated with the transitions of the K, L and M shell electrons. Each element will have a different series of KLM reference lines.

Live time: The time over which the energy dispersive X-ray spectroscopy electronics are available to accept and process incoming X-rays. Live time is often expressed as a percentage of real time.

Particle analysis: An analytical method intended to determine the elemental composition of a single particle such as a pigment particle in an adhesive. Usually performed with a static (non-scanning) electron beam.

Pulse processor time constant: Operator-selected value for pulse-processing time. A higher value (longer time) results in a more accurate determination of the detector amplifier pulse height (better spectral resolution). A lower value results in a higher count rate but with reduced spectral resolution.

Raster: The rectangular pattern scanned by the electron beam on a sample. The raster dimensions change inversely with magnification.

Sample (representative sample): A representative portion of the specimen selected and prepared for analysis that is believed to exhibit all of the elemental characteristics of the parent specimen.

Scanning electron microscopy (SEM): A type of electron microscope in which a focused electron beam is scanned in a raster on a solid sample surface. The strength of resulting emissions of signals varies according to sample characteristics such as composition or topography. These signals directly modulate the intensity of the display cathode ray tube. The electron beam of the scanning electron microscope and the display cathode ray tube are scanned synchronously, resulting in a two-dimensional image of the sample. By popular usage, the term scanning electron microscopy may also include the analytical techniques energy dispersive X-ray spectroscopy and wavelength dispersive X-ray spectroscopy.

Secondary electrons (SE): Low-energy electrons produced from the interaction of beam electrons and conduction band electrons of atoms within the interaction volume. They are produced throughout the interaction volume, but only those near the surface have enough energy to escape. The secondary electron signal is typically used to form topographic images.

Specimen: Material submitted for examination. Samples are removed from a specimen for analysis.

Spectral artifacts: Spectral peaks other than characteristic peaks, produced during the energy dispersive X-ray spectroscopy detection process. Examples include escape peaks and sum peaks.

Spectral resolution: A measure of the ability to distinguish between adjacent peaks in an X-ray spectrum. It is usually determined by measuring peak width at half the maximum value of the peak height or full-width-half-maximum.

Sum peak: A peak resulting from the simultaneous detection of two photons. This is manifested as a peak at the combined energy of line(s) for the specific element(s) involved.

System dead time: The time during which the energy dispersive X-ray spectrometer is not able to process X-rays. Dead time is typically expressed as a percentage of real time.

System peaks (stray radiation): Peaks that may occur in the X-ray spectrum resulting from interaction of the electron beam or fluorescent radiation with components of the scanning electron microscope itself.

Take-off angle: Angle between the specimen surface and the detector axis.

Thick section: For the purposes of this guide, a sample that is two micrometers or thicker.

Thin section: For the purposes of this guide, a sample with a thickness of less than two micrometers.

4.0 Significance and Use

4.1. The scanning electron microscope is one component of the analytical scheme of the forensic analysis of tape and can be used to define the bulk elemental composition of individual tape components (backing and adhesive) and the elemental composition of individual particulate components within tapes, as well as the surface morphology.

4.2. The methods described in this guide may have some limitations. These may include the inability to detect elements in trace concentrations, the need for a conductive coating of the sample (with a high vacuum SEM), and the discoloration of materials by irradiation.

4.3. Although quantitative and semiquantitative methods are available for energy dispersive X-ray spectroscopy, they are not appropriate for most tape analyses because of the typical condition of the tape.

4.4. The information available from a heterogeneous specimen may diminish as its size is reduced and its condition degrades. The smaller a specimen, the less valuable it may become for an association. As sample size is reduced, it may no longer be representative of the original material. This may also be true of a degraded specimen.

4.5. This guide is intended to advise and assist laboratory analysts in the effective application of SEM to the analysis of tape evidence.

4.6. It is not the intention of this guide to present comprehensive methods of scanning electron microscopy. It is necessary that the analyst have an understanding of scanning electron microscope operation and general concepts of specimen preparation prior to using this guide.

5.0 Sample Handling

5.1. The general collection, handling, and tracking of samples shall meet or exceed the requirements of ASTM 1492-05 as well as the relevant portions of the SWGMAT's Trace Evidence Quality Assurance Guidelines and Trace Evidence Recovery Guidelines.

5.2. The work area and tools used for the preparation of samples should be free of all materials that could transfer to the sample.

5.3. When samples are prepared for scanning electron microscopy, a map identifying sample location on an SEM mount can be constructed to assist in locating the sample when performing the analysis.

6.0Sample Preparation

6.1. Samples should first be examined with a stereomicroscope, noting size, color, structure, and any extraneous material adhering to the sample.

6.2. The choice of a specific method for sample preparation depends on the size, nature, and condition of the specimen, as well as the analytical objective. It may be necessary to use multiple preparation methods in order to analyze all sample characteristics.

6.3. In developing a strategy for analysis, the following should be considered:

- determination of the presence of extraneous materials and a strategy for removal
- method of attachment to a scanning electron microscopy mount
- method(s) for producing a uniform geometry
- the need for a conductive coating on the prepared samples
- determination of the presence of surface features of analytical interest

6.3.1. If the analytical objective is to determine elemental composition, then any possible contribution from extraneous materials should be eliminated or accounted for.

6.3.2. For the accurate comparison of elemental composition and structure, samples should be prepared in the same manner.

6.4. Recognition and removal of extraneous materials

6.4.1 It is not unusual for extraneous materials to be present on the surface of a specimen submitted for analysis, particularly on an adhesive component. Because the scanning electron microscopy method is a surface analysis, the presence of even a small amount of this material can prevent an accurate determination and comparison of composition. Therefore, recognition and removal or abatement of this material should be performed.

6.4.2. Depending on sample size and type, extraneous material may be physically removed with a brush, probe, or fine blade. Debris can also be removed from the backing surface with methanol and a cotton swab or lifted off the backing with office tape. Care should be taken that the adhesive from the office tape does not adhere to the sample surface, which might interfere with any subsequent organic or inorganic analysis. If necessary, a fresh surface may be exposed by scraping or cutting with a fine scalpel blade.

6.4.3. When extraneous materials cannot be removed, these materials should be avoided during analysis

6.5. Methods of attaching samples to SEM mounts

6.5.1 All samples to be analyzed in the scanning electron microscope should be attached to an appropriate SEM mount. Because the presence of a carbon peak in the spectrum does not usually interfere with elemental comparisons, mounts constructed of carbon are preferred.

6.5.1.1. The adhesive should be removed from the backing to ensure no contribution from the backing is in the resulting spectrum. The tape's adhesive is smeared directly onto the surface of the mount.

6.5.1.2 A backing may be attached directly to a mount using the tape's own adhesive. Contribution of the adhesive in the resulting spectrum is typically not a concern during backing analysis. If the backing has been separated from the adhesive or if a cross-section of the backing has been prepared, the backing or cross-section can be attached to the mount with a mounting adhesive. This mounting adhesive may be applied as a liquid or as a double-sided tape. If the tape is known to be multi-layered, both the outer and the adhesive side may be analyzed.

6.5.2. The geometry of each sample, including flatness and take-off angle, should be similar. Often, a backing can be pressed flat with clean glass in order to remove irregularities.

6.5.3. Generally it is necessary to apply a conductive layer to the sample surface to eliminate charging. Carbon is preferred, because the presence of a carbon peak in the spectrum usually does not interfere with elemental comparisons. The use of a variable pressure instrument may also eliminate charging.

7.0 Analytical Procedures

7.1. Instrument calibration

7.1.1. Prior to beginning an analysis, verification of the operational condition of the scanning electron microscope should be established. This may include the presence of system peaks, accuracy of magnification, and determination of spectral energy calibration and resolution.

7.1.2. The presence of system peaks is generally determined upon installation of the scanning electron microscope or following a modification or addition of accessories.

7.1.3. For a determination of accuracy of magnification, a percentage of error of magnification should be calculated. A scanning electron microscope's indicated value of magnification (such as a measurement marker) is compared to a measurement of a certified standard (such as NIST SRM 484D). A calibration check of the primary image output device to the certified standard should be performed periodically and a record kept in a permanent log. Relationships of measurements on display monitors, as well as any other image capture applications to the primary image output device, should also be recorded. Magnification standards for scanning electron microscopes are commercially available, with errors of less than five percent generally achievable.

7.1.4. Energy calibration should be established frequently for the energy dispersive X-ray spectrometer, including zero offset and gain, and a record kept in a permanent log. Energy calibration may be determined directly by measuring the centroid energy of a low- and high-energy peak or determined automatically using software provided by the instrument manufacturer. If automated methods are used, measured spectral energies typically do not deviate more than 10eV from that of actual energies. Automatic methods for calibration are described in documentation from the manufacturer.

7.1.5. Spectral resolution for the energy dispersive X-ray spectrometer may be determined regularly and a record kept in a permanent log. This may be determined automatically or can be determined manually by measuring the width of the Mn K-α peak (or other suitable metal) at half the maximum peak height. Automatic methods for calibration as well as recommended performance limits are often available from the manufacturer.

7.1.6. EDS system performance can also be evaluated by comparison (overlay) of system checks to a suitable reference spectrum.

7.2. Structural imaging

7.2.1 SEM imaging of pressure-sensitive tape backings at moderate magnifications (75-250X) yields structural information complementary to that of traditional light microscopical methods. It can be used to image very small striations, craters, and surface features on the backings of polymer-based tapes, such as black electrical tape and duct tape. It can be used to view the paper fibers in masking tapes, as well as show the cross-sectional structure of each of these tapes.

7.2.2 . A backscattered electron image is useful for defining structures based on the average atomic number of the matrix. Structures containing elements with higher atomic numbers will generally appear brighter than those with lower atomic numbers. This is often useful for evaluating homogeneity and layer structure.

7.2.3. SEM micrographs should include a measuring scale or magnification scale or both. The micrograph should also display which signal (backscattered electron or secondary electron) was used to produce the image.

7.3. Selection of scanning electron microscopy/energy dispersive X-ray spectroscopy operating conditions

7.3.1. The following suggested operating conditions are meant as general guides for starting conditions. As the analyst determines specific analytical needs, actual operating conditions may vary.

7.3.1.1. A beam voltage of 20-30 KeV is an adequate compromise between the need for sufficient over-voltage necessary for efficient X-ray excitation and X-ray spatial resolution. Most of the X-ray lines produced may be displayed with an energy range of 0 to 20KeV. The pulse processor time constant should be set at a midrange value, which is a compromise between maximum count rate and maximum spectral resolution. The beam current should be adjusted to yield an X-ray detector dead time of approximately 30 percent. A live time of 100-200 seconds is usually sufficient to provide reasonable counting statistics for minor peaks.

7.3.1.2. Generally, changes in the suggested initial conditions are required under the following circumstances:

- Beam voltage is increased when higher energy line excitation is required.
- Beam voltage is decreased when greater spatial resolution is required.
- Pulse processor time constant is lengthened when greater spectral resolution is required.
- Pulse processor time constant is shortened when a greater count rate is required (e.g., for trace element analysis or construction of elemental distribution maps).
- Detector to sample distance can be reduced to increase X-ray collection efficiency.
- Spectral energy display scale is expanded when sufficient detail is not evident.
- Beam current is increased when the X-ray count rate is too low. Decreasing the condenser lens current and/or increasing the final aperture size may increase beam current.
- Beam current is decreased when the X-ray count rate is too high. Increasing the condenser lens current and/or decreasing the final aperture size may decrease beam current.

7.4. Bulk spectra collection

7.4.1. Once heterogeneity of the material is evaluated, a spectrum of the average (bulk) elemental composition of the sample is obtained. The raster should include as much area of the sample as possible. Analyzing a single large area or summing the spectra from several smaller areas may achieve this.

7.4.2. When comparing samples, all data and micrographs should be collected in the same manner with the same conditions.

7.5. Qualitative analysis

7.5.1. Once an X-ray spectrum is collected, a qualitative analysis is performed in order to determine the elements present. The process is straightforward for the peaks of elements present in major amounts and those not overlapping. Misidentifications or omissions of minor components are possible unless a systematic approach to elemental identification is used which includes consideration of X-ray line families, spectral artifacts, escape peaks, sum peaks, and overlaps.

7.5.2. Reference lines, or energies, may be obtained from several sources, including energy slide rules, published tables, and computer-generated KLM reference lines that may be superimposed on the spectrum. Additionally, manufacturers often provide an automatic element identification application. These aids often are used in complementary fashion.

7.5.3. Identification begins with high-energy peaks and major peaks. High-energy peaks are generally less likely to overlap than lower energy peaks. If a major peak is present, generally a complete family of peaks can also be identified. Each line within the family is labeled with elemental symbols. Spectral artifacts, including sum peaks and escape peaks associated with major peaks, should be evaluated and labeled.

7.5.4. As spectral interpretation alternates between the identification of major and minor peaks, the vertical (counts) scale should be adjusted to reveal required detail. In addition to the higher energy peaks, the presence of any lower energy families and their expected relative intensities should be noted. Individual asymmetric peaks and inconsistent peak ratios within a family may indicate a peak overlap. Superimposing and scaling KLM reference lines on the spectrum or referencing the actual spectrum of an elemental standard aids elemental identification. The analyst should be familiar with the characteristic pattern and relative intensities of peaks of various atomic numbers. The identification of major elements is usually straightforward.

7.5.5. Following the identification of major elements, lower intensity peaks and overlapped peaks are identified. The limited number of characteristic peaks present for minor elements can limit their identification.

7.5.6. The presence of an element can be considered unequivocal only when a distinctive, unique set of lines is produced or when a single peak occurs at an energy where it cannot be mistaken for another element or spectral artifact. Unequivocal identification may not be possible if an element is present in low concentration or if lines required for confirmation are overlapped with the lines of other elements.

7.5.7. If identification is unequivocal, each individual peak is labeled with the corresponding elemental symbols (and X-ray line if the software permits). If the identification is probable but not absolute, the peak label should so indicate (such as by parenthesizing the elemental symbols).

7.5.8. Spectra should be displayed on a scale that clearly demonstrates the peaks identified. In order to display peaks from elements with significant differences in concentration, the peaks from the elements in low concentration may be viewed by displaying the spectra separately on different display scales.

7.5.9. If an automatic identification application is used, the analyst should confirm the resulting element identifications.

7.5.10. There may be an overlap of peaks in the energy dispersive X-ray spectroscopy spectrum of materials containing several elements. Some commonly occurring overlaps encountered in energy dispersive X-ray spectroscopy are as follows: Ti K β/V K-α, V K-β/Cr K-α, Cr K-β/Mn K-α, Mn K-β/Fe K-α, Fe K-β/Co K-α, Pb M-α/S K- α/Mo L-α, Ba L-α/Ti K-α, K K-β/Ca K-α, Zn L-α/Na K-α, P K-α/Zr L-α, and Al K-α/Br L-α.

In order to resolve these overlaps, several methods may be employed.

- The live time count can be increased.
- The processing time of the pulse processor may be increased to improve spectral resolution.
- Mathematical spectral subtraction (deconvolution) methods supplied by the energy dispersive X-ray spectrometer manufacturer can be employed.
- An alternative method of elemental analysis or X-ray diffraction may be used.

7.6. Individual component analysis

7.6.1. Additional evaluation of composition may be achieved by the spot (nonrastered) analysis of specific particles within layers. Generally, these particles appear bright in the backscattered electron image. Such an analysis may improve the detection limit beyond that achievable by a bulk analysis, as well as serve to associate elements detected by a bulk analysis. For example, the bulk analysis of a tape adhesive may reveal the presence of Al, Si, Mg, and O. Specific particle analysis may associate the elements Si, Mg, and O as being present in one type of particle, and Al, Si, and O in a second type. These associated elemental compositions would then indicate these particles could be talc and kaolinite, respectively. Polarized light microscopy, infrared spectroscopy, or X-ray diffractometry can be used to confirm the presence of some of the compounds.

7.6.2. Because the beam interaction volume may be considerably larger than an individual particle, inclusion of other matrix components may be expected in the spectrum from an individual particle. Lower beam voltages may be used to confine more of the interaction volume to the particle. It should be noted, however, that the use of lower beam voltages may result in the loss of characteristic lines that may be found at higher energies.

7.7. Analysis of a primarily organic matrix

7.7.1. Analysis of a substance that is primarily organic (e.g., duct tape backing, clear electrical tape adhesive) may be useful. Within such a matrix, the interaction volume is significantly larger than that of a substance that is primarily inorganic. This is a result of a lower average atomic number of the matrix. In order to reduce the interaction volume, the beam voltage may be reduced; however, the voltage should be sufficient to produce X-rays from all lines of analytical interest. Charging may also be an issue with such samples. Therefore, precautions may be taken to prevent this from occurring (e.g., sample coating or operation at low vacuum).

7.7.2. Because an organic matrix may contain small amounts of some elements, the counting time should be extended.

7.8. Heterogeneity versus analytical area

7.8.1. In order to compare the average composition of structures, the spectrum used for comparison should come from an area of the structure sufficient to produce representative composition.

7.8.2. The representative nature of a spectrum can be determined by the critical comparison of spectra from adjacent areas. If no differences are evident, the sampled area is homogeneous at that magnification. A representative bulk analysis can be achieved by rastering the beam across as large an area as the sample permits.

7.9. Assessment of results

7.9.1. Generally, comparisons are facilitated by direct spectral comparison.

7.9.2. If spectral differences are not detected, it is likely that the materials are similar in elemental composition.

7.9.3. If spectral differences are detected, it is likely that the materials are not similar in composition; however, several alternative explanations are possible and should be evaluated. Only after the considerations in Sections 7.8.1 through 7.8.2 have been addressed can the analyst confirm that spectral differences are indicative of compositional differences.

7.9.3.1. Differences in background shape may result from dissimilar sample geometry. To correct, see Section 6.5.2.

7.9.3.2. Differences in the composition of major peaks may indicate that the spectra are not representative of the bulk composition of a heterogeneous sample. This could occur as a result of the analysis of a sample too small to be representative or the analysis of a raster area too small to be representative.

7.9.3.3. If there are no differences in major peak ratios, differences in minor/trace components may result from the presence of extraneous materials. If the sample was a fragment or unable to be cleaned, a small amount of foreign material may have been present during the analysis. Consequently, some of the minor elemental peaks in the spectrum may have been produced from elements in the extraneous material. To correct, see Section 6.4.

7.9.3.4. Differences in carbon intensity may result from a contribution of carbon from the mount if the sample is very small. Furthermore, the presence of carbon, oxygen, and nitrogen in the tape matrix limits the usefulness of these elements in direct spectral comparison; therefore, they are typically not evaluated.

7.10. Interpretation of scanning electron microscopy/energy dispersive X-ray spectroscopy data

7.10.1. A conclusion regarding similarity results from the comparison of images and elemental composition of individual layers. Spectra may be critically compared by overlaying them.

7.10.2. If a comparative analysis did not demonstrate significant differences, then no differences were indicated in structure and composition within the limits of the analytical capability of scanning electron microscopy/energy dispersive X-ray spectroscopy.

7.10.3. If ratio differences between peaks exist, it can be concluded that these differences may result from either actual differences in the bulk composition of the materials or from the analysis of a small sample (or area) whose chemistry is not representative of the bulk composition of a heterogeneous sample. The latter should only be concluded following an extensive investigation of the heterogeneity of the known samples and demonstration that the range of variation present in one sample encompasses that observed in the other sample.

7.10.4. If there are no differences in major peak ratios but there are differences in minor/trace peaks, it can be concluded that no differences in major elemental constituents are indicated, although some differences in the bulk composition are evident. For example, if the specimen was a fragment and unable to be adequately cleaned, a small amount of foreign material may have been present during the analysis. Consequently, some of the minor elemental peaks present in the spectrum may have been produced from elements in the foreign material and not from elements in the questioned material. Equally so, the observed differences may be due to actual

differences in the composition of the samples. Therefore, with respect to the elemental composition of these samples, an inconclusive result for this technique is indicated.

7.10.5. If, after taking into consideration Section 7.9.3, a comparative analysis demonstrates significant differences between samples regarding structure and composition, it then can be concluded that the samples are different.

8.0 Documentation

8.1. Case notes should include a copy of all of the instrumental data that was used to reach a conclusion. All hard copies should include a unique sample designation, the operator's name/initials, and the date of analysis.

8.2. Case notes should also include a description of the evidence analyzed by SEM, the method of sample preparation, the analytical instrumentation used, and its operating parameters. The case notes should also include a statement or data confirming system calibration, as detailed in Section 7.1.

8.3. See SWGMAT's Trace Evidence Quality Assurance Guidelines for further requirements.

9.0 References

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