Chapter 13

Trace Evidence in the Real Crime Laboratory

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1. Introduction to Trace Evidence

Trace evidence includes all the small bits and pieces of material that can be used to assist with the investigation of crimes and accidents. Trace evidence originates from the accumulation of material fragments left behind at the scenes of crimes or accidents that serve as reminders of someone or something that was present there during the crime or accident. The traces are often microscopic in size and, therefore, are not noticed right away, particularly by the perpetrators of crime. Fortunately for the police, the criminal is usually too busy to realize that his hairs and clothing fibers were left behind or that he took away fragments of glass on his clothing.

1.1. The Locard Exchange Principle

Material traces are left behind when someone touches something or when things rub together because, whenever any two objects come in contact, they transfer material from one to the other. The more violent the contact, the more likely the transfer will occur. Edmond Locard described these transfers in the early part of the 20th century. Dr. Locard was director of the crime laboratory in Lyon, France, between about 1910 and 1940. Since the earliest crime laboratories, forensic scientists have explained these transfers as a result of the Locard Exchange Principle. Locard explained that the microscopic debris that covers our clothing and bodies actually are silent witnesses of all our contacts.

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Thereafter, forensic scientists all over the world soon recognized that the principle applies to all criminals because wherever they step, whatever they touch, or whatever they leave behind, traces will serve as evidence against them. Not only their fingerprints or their DNA, but their hair, the lint from their clothes, the paint they scratch, the glass they break, and the dirt on their shoes will be there as evidence of the encounter.

1.2. Collecting Trace Evidence

Locard recommended that traces that can be seen with a magnifier should be collected directly using needles and tweezers and placed into folded paper packets. Many crime laboratories also collect trace evidence by shaking or scraping garments over clean paper on the laboratory bench. In the middle of the 20th Century, Dr. Max Frei-Sulzer, of the Zurich Police Department Crime Laboratory, first recommended collecting trace evidence completely invisible to the naked eye by pressing transparent tape to the surface where microtraces were suspected to be present, lifting the traces and placing the tape on a transparent plastic card. Today, tape is routinely used in the crime laboratory to collect hairs and fibers.

Gun shot residue (GSR) particles are also collected using tape, but of a different kind. GSR analysis using scanning electron microscopy (SEM) depends on finding traces of small particles left as a residue on the shooter after a gun is fired. The particles are collected from hands and clothing using double-sided tape placed on a metal disk for the SEM. Just as with fibers, areas on a suspect's clothing, such as a pocket where a gun was placed after it was fired, can be targeted with the tape lift.

1.3. Associative Evidence

Linking a suspect to the crime scene or linking a victim to a suspect is the essence of solving crimes. The term associative evidence was defined by James Osterburg, along with the New York City crime laboratory, and signifies that some connection or association has been established between crime scene and criminal. Fingerprints are used to associate a person with a place or object, such as a bank vault or a gun. Blood on a suspect's clothing is used to associate the victim with the suspect. Likewise, traces of hairs, fibers, paint, glass, and soil are used in the same way to prove that a person came in contact with an object, a place, or another person.

For example, the trace evidence left behind as a result of a hit and run accident, where a pedestrian is struck and injured, is a good example of associative evidence. As the car strikes the pedestrian, blood, hair, and clothing fibers are transferred from the victim to the car; and at the same time, paint and headlight glass are transferred from the car to the victim's clothing. Car parts, such as pieces of headlights, turn-signal lenses, and grill, are left behind after

the car leaves the scene. When the suspect car is found, with all this evidence it is usually a relatively simple matter to associate the victim with the car, the car with the victim, and the car with the scene of the accident. In higher speed crashes where the driver and passenger bounce around inside the car, the driver is associated with the car by saliva and makeup on the airbag if it deployed, by trouser fibers on the dash, or by shirt fibers on the seatbelts. The passenger's blood and hair may be caught in the broken windshield.

1.4. Types of Trace Evidence

Different groups of associative evidence are usually dealt with by different crime laboratory specialists. For example, impressions, such as fingerprints and footprints and those on bullets and cartridge casings or broken objects, are examined by the identification specialist and firearms examiner, respectively. Genetic markers from blood, semen, and saliva are identified in the DNA laboratory. Although the types of materials that might be used as trace evidence are nearly unlimited, hairs, fibers, paint, glass, and soil are the most common types analyzed in the microscopy laboratory.

Hairs are useful as trace evidence because they originate directly from the body. They are associated with the victim or suspect by comparing the questioned hairs found in the trace evidence with known samples collected later from animals or persons suspected of leaving the hairs. They are either associated by comparison of microscopic features or by analysis of DNA in the hairs.

Fibers, paint, and glass differ from hairs in many ways and are therefore analyzed in a different way. These microtraces are all minute portions of manufactured products. Fibers are manufactured in large quantities and distributed throughout the world to be made into the textile products (clothing, carpet, upholstery, etc.) we use in our everyday lives. Likewise, paint and glass are made in large batches and then used in cars and homes. They are identified and compared through microscopic materials analysis. All are characterized by their size, shape, and construction (morphology), by their optical and physical properties (color, refractive index, birefringence, density), and by their chemical composition. But seldom can any of these analyses determine that a particular microscopic portion originated from the suspected source (sweater, carpet, window sill, car, window) to the exclusion of identical materials that may be used elsewhere in the same product. Unlike fingerprints, footprints, and broken fragments, which can be matched to a single individual or object, fibers, paint, and glass cannot. Nevertheless, in many cases, these manufactured traces assist immensely with the investigation and determining their likely source by comparison often helps solve crimes of all types.

Many natural samples (soil, pollen, leaves), originating from the ecological environment, are also useful as associative evidence. These materials are either compared directly with known samples from the site where the questioned

samples likely originated; or, in some cases, the identification of these natural samples provides leads to the location of the crime scene. A model case was described recently by Schierenbeck *(1)*. A botanist was given some leaves collected from the clothing of a missing child, apparently left behind by the murderer. After determining the plant species and predicting where the species would be found, the botanist and the detective visited the site where the clothing was found. They drove further up the mountain and visited several sites where the species of interest were found. At the fifth site, as the detective took global positioning system readings, the botanist climbed down a hill to examine the leaf litter. Just as she noticed that the leaves matched in every detail to the leaves from the clothing, she noted the strong odor of decay and a blanket near a log. The missing child's body was then discovered.

2. Forensic Microscopy

Traces are usually very small and, therefore, must be analyzed microscopically. As many questions can be answered quickly and inexpensively with a microscope, it is the beginning point in nearly all trace evidence cases. The basic tools for forensic microscopy are a stereomicroscope, a polarizing light microscope, and a transmitted light comparison microscope. Where necessary to complete a comparison, light microscopy is used to prepare samples for infrared or electron microscopy.

2.1. Stereomicroscopy

The stereomicroscope, also known as a dissecting microscope, is the starting point of virtually every analysis. The main difference between the stereomicroscope and the more common compound microscope is that the compound microscope views the sample from a single direction, whereas the stereomicroscope views the object from two slightly different angles, just like our normal vision. There is also plenty of room under the microscope lens to handle evidence while observing its appearance and preparing samples. The stereoscopic microscope, however, views objects mainly by means of reflected light and its power, typically ranging from 5 to 50× magnification, is much less than the compound microscope. Nevertheless, the lower power has advantages because what you see with the naked eye compared to what you see through the stereoscopic microscope are similar, making it easy to use.

2.2. Polarized Light Microscopy

Polarized light microscopes are built on the higher power (1000× magnification possible) compound microscopes by adding two rotating polarizing

filters in the light path, one before the specimen (polarizer) and one after the specimen (analyzer), in order to control the planes of vibration of the light used to view the sample. By manipulating the polarizing microscope and using appropriate attachments, a number of fundamental optical characteristics used to identify and compare trace evidence can be measured including refractive index and birefringence. Polarized light microscopy (PLM) is the principal means to identify fibers and soil minerals, for example. Polaroid®, 35 mm, digital, or video cameras can be added to the microscope to take pictures of the trace evidence. Nearly every trace evidence sample finds its way to the polarizing microscope.

2.3. Fluorescence Microscopy

Through the use of special filters and lights on a compound light microscope, the fluorescence of materials may be observed, allowing microtraces that are similar in normal or polarized light to be distinguished simply by their fluorescent colors, in the same way that a "black light" is used to create the unusual colors of some Halloween costumes. Dyed fibers are compared in this way.

2.4. Comparison Microscopy

The final step in most comparisons is to use two microscopes side-by-side, connected with an optical bridge, to view the questioned and known samples at the same time. The comparison microscope contains an arrangement of prisms whereby half the field of view from each microscope is viewed simultaneously in the eyepieces, with the left half of the image from the left microscope and the right half from the other. Two matching polarizing microscopes or two matching fluorescence microscopes are ideal for the comparison of hairs, fibers, paint layers, or any evidence requiring detailed comparison of microscopic features.

2.5. Infrared Microscopy

Infrared spectroscopy (IR) is the study of a substance's interaction with infrared light and is frequently used in crime laboratories to identify drugs, plastics, paint, fibers, and explosives. Fourier transform infrared spectrometers (FTIRs) are more sensitive and can be used in combination with a microscope to identify the small microtraces found in trace evidence.

2.6. Electron Microscopy

Electron microscopes use electrons instead of light to view trace evidence. For example, in the SEM, samples are bombarded with a focused beam

of electrons instead of light, which produces more electrons and X-rays. These backscattered and secondary electrons produce high resolution images at magnifications 100 times greater than a light microscope with extreme depth of field making them appear almost three-dimensional.

X-rays generated by the electron beam striking the sample surface are captured with an energy dispersive spectrometer (EDS) and are used to determine the chemical elements in the sample. The EDS gives a quantitative measurement for virtually all the elements on the periodic table from carbon through uranium. Modern instruments can analyze thousands of particles automatically, such as particles collected from GSRs. (*See* ref. 2 for additional information on electron microscopy.)

2.7. Duct Tape

To illustrate how all of these microscopes might be used together, consider the case where ordinary duct tape found on a bomb package might be compared with a partial roll of tape in the suspect's workshop. First, the tape from the package is inspected for fingerprints and hairs, fibers, or other microtraces that might be adhering to the adhesive. The tape readily picks up traces from the bomb-maker's environment. Second, if the tape from the bomb and workshop look similar in size and color, the cut or torn ends are inspected to determine whether an irregular torn end from the package can be matched with an end from the roll, like a jig-saw puzzle. When possible matches are found, they are confirmed using a stereomicroscope and proven with photography. If a fracture match does not prove that the tapes are from the same roll, a materials analysis is next. Duct tape is comprised of an adhesive layer usually containing a filler (such as calcium carbonate), a cloth reinforcing material, and a polymer backing with a colored coating. Each of these materials is compared, in turn, and each must coincide before it can be concluded that the tape on the package could have originated from the roll from the workshop.

Each sample is compared visually and with a stereomicroscope for color, texture, structure and constituent parts. The adhesive is identified by infrared microspectroscopy (FTIR) and the filler is analyzed by PLM and SEM-EDS. The cloth weave is studied with a stereomicroscope. The reinforcing fibers are identified by PLM and FTIR, and any coatings on the fibers are analyzed by FTIR and SEM-EDS. The backing is identified by FTIR and melting point. The colored coating is compared with alternate light sources and microspectrophotometry (MSP) and its composition is determined by PLM, FTIR, and SEM-EDS. After the tape comparison is complete, it would be time for the crime laboratory to begin comparing the hairs, fibers, paint, glass, soil, and other microtraces trapped in the adhesive with samples from the suspect and workshop.

3. HAIRS

Human hairs are the most often used microscopic trace evidence. Hairs are readily shed and unwittingly left behind; they are relatively easy to discover; and they are not easily destroyed. The violence of homicidal and sexual assaults tends to result in the transfer of hairs from one person to another, to the crime scene, and to weapons. One of the earliest hair cases was the murder of Fanny Sébastiani, the Duchesse of Praslin, in her bedroom at the Hôtel Sébastiani, 55 rue du Faubourg, St. Honoré, Paris, on August 18, 1847. The Duke could not explain how his loaded pistol, found under the body, had bits of the victim's skin and hair adhering to the handle indicating that the Duchesse had been violently struck with it during the struggle. He died in prison.

Human head and pubic hairs are most often used as trace evidence, but animal hairs from domesticated animals (pets and farm animals) and from textiles (furs) are found occasionally. For example, clothing brought to a dry-cleaners by a robber and subsequently left on the counter when the robber fled was covered with dog hairs which were later compared with the suspect's pet. In cases of sexual assault, pubic hairs are sometimes transferred between victim and assailant, which is why the victim's pubic hair is combed during the medical examination. Consequently, hairs are often found and need to be compared with possible sources. The only disadvantage of hairs is that their origin cannot always be matched with sufficient certainty to the suspect.

3.1. Hair Comparison

The principal means for determining the source of a hair is by microscopic comparison between the questioned hairs and hairs collected from individuals who are possible sources. For human hairs, these characteristics can be broadly grouped into color, structure, and treatment. The structure of hair can be likened to an ordinary lead pencil consisting of an outer skin called the cuticle (like the paint), a shaft (like the wood), and an inner medulla (like the graphite). As with pencils, color is probably the most useful characteristic for distinguishing hairs from different sources. In addition, the tip ends may be freshly cut, split, or singed. The shape of the root (eraser end) may indicate whether the hair had been pulled. Bleached or dyed hair can usually be identified by a distinct demarcation line seen when the hairs grows out revealing the untreated "roots."

If all the features of two hairs are alike after comparisons using a stereomicroscope, polarizing microscope, and comparison microscope, then a conclusion that the hairs are similar in all respects (a match) is justified and the samples could have originated from the same individual. Conversely, if two samples of hairs are not alike, then it is logical to conclude that they did not

originate from the same individual. The crime laboratory can often state that two hairs originated from different sources, but when two hairs appear similar they can only say, without DNA analysis, that they *could* have originated from the same source.

3.2. DNA Analysis

Following a microscopic comparison and an assessment of the root characteristics, hairs are selected for DNA analysis. Genomic DNA is found in the nucleated cells of the root tissue attached to the hair when pulled from the skin and, if sufficient DNA is recovered, the origin of the hair can be determined to a virtual certainty, in the same way the donor of blood and semen can be determined. In addition, the entire hair shaft contains mitochondrial DNA (mtDNA) that can be amplified and typed using PCR technologies. Although mtDNA is not as useful as genomic DNA for identifying the donor because the mtDNA is always the same as the person's mother, it is very stable, provides exclusions, and enhances the value of any microscopic associations. In summation, the combination of microscopic analysis and DNA analysis of hair can provide extraordinary trace evidence often associating victims with assailants.

4. FIBERS

Textile fibers are a good illustration of the Locard Exchange Principle. Whenever two people come together during an assault, there is often a cross transfer of fibers where the victim's clothing fibers transfer to the assailant's clothing and vice versa. Likewise, if someone rolls around on a carpet, carpet fibers will transfer to the person's clothing, as best illustrated by the case of the Atlanta Child Murders where many of the victims' bodies contained carpet fibers from Wayne Williams' bedroom and cars.

Fabrics and carpets are made from fine individual fibers formed into yarns or tufts used to construct the textile. There are many different natural and man-made fibers, many different ways to produce them, many different colors, and many more ways to make them into textiles. Therefore, each fiber and finished product is made to exacting specifications depending on the designed end use. The microscopist in the crime laboratory is tasked to first identify the fiber and then compare them with possible sources.

The fibers are recovered and compared in the crime laboratory using a variety of microscopic techniques that will identify, characterize, and compare the material composition of the fibers. If, after microscopic and chemical comparison, the questioned and known fibers cannot be distinguished, a match is

proclaimed and the forensic scientist can conclude that the fibers could have a common origin. The match is not certain because there are thousands of pounds of fibers made the same and hundreds of garments or carpets made from the same fibers. The value of the fiber evidence depends on the circumstances of the case and on the likelihood that similar fibers will be found in the victim's or assailant's environment, that is, how common the fibers are. Fibers smashed on the car hood that hit a pedestrian are good evidence the car was involved, even if the fibers are a common cotton/polyester blend. Some judgment regarding the commonness can be made simply by looking around, such as in a shopping center, and observing how many people are wearing the same item of clothing.

4.1. Fiber Comparison

Once in the laboratory, fibers are first examined with a stereomicroscope. If the sample contains fragments of fabric, the construction (weave, knit) is also compared and the possibility of physically refitting the fabric with its source is considered. PLM is used to identify the generic class of individual fiber fragments, i.e., whether the fiber is cotton, wool, silk, rayon, polyester, nylon, acrylic, glass, etc., by considering the fiber size and shape and measuring two fundamental optical characteristics, refractive index, and birefringence. The fibers are contrasted further by comparing their fluorescence, their melting point, chemical composition, and dyes.

4.1.1. Refractive Index

PLM is used to determine refractive index, the primary means to identify the different types of fibers. The same methods are used for glass comparisons and for the identification of soil minerals. The refractive index of a transparent material (whether ice, glass, fiber, or gem) is equal to the ratio of the speed of light in a vacuum to the speed of light passing through the material. The difference in the speed causes the light to bend as it passes into and out of the material, just as when looking at a swizzle stick in a martini. In addition, all transparent fibers other than fiberglass display two refractive indices, one for light polarized parallel to the long axis of the fiber and one for light polarized perpendicular to the fiber.

The relative refractive index is determined by observing whether the fiber is higher or lower in refractive index than the liquid it is in, using the Becke line test. By changing the immersion liquid of which the refractive index is known (refractive index liquid), the absolute value can be determined when the object disappears in the right liquid. At that point, the liquid and the fiber have the same refractive index.

4.1.1.1. Becke Line

The edge of a small transparent object immersed in a liquid, such as a fiber or glass fragment immersed in a refractive index liquid, acts as a lens deviating the beam of light coming up through the microscope either toward or away from the object. As the focus is changed by moving the microscope stage up and down, a bright band of light, known as the Becke line, can be seen moving back and forth across the edge. On increasing the distance between objective lens and stage (lowering the stage), the Becke line moves toward the medium with the higher refractive index; conversely, on lowering the objective lens (raising the stage), the Becke line moves toward the medium with the lower refractive index. In this way, the microscopist always knows, with an easy turn of the focusing knob, whether the fiber has a refractive index higher or lower than the liquid, the refractive index of which should always be known. This helps to rapidly identify polyester fibers. For example, if the liquid has a refractive index of 1.66, only polyester fibers will have a refractive index greater than the liquid in one orientation with respect to the polarizer and lesser in the other orientation.

4.1.2. Birefringence

Most fibers have two refractive indices: one for each orientation of the polarizer. The birefringence is defined as the difference between the refractive index parallel and the index perpendicular to the plane of the polarized light. Absolute birefringence is determined by measuring both refractive indices by the Becke line test. The interference colors of the fiber as seen with a polarizing microscope are dependent on the fiber thickness and birefringence based on the formula: Retardation (nm)/Birefringence = 1000× Thickness (mm). The retardation causing the interference colors can be estimated by comparing the colors with a Michel-Lévy chart. The birefringence is then calculated from the formula or estimated from the chart. For a birefringent fiber, the sign of elongation is negative when the parallel index is less than the perpendicular index. Only acrylic fibers have negative signs of elongation.

4.1.3. Fluorescence

Fiber fluorescence usually results from the dyes—there are thousands of dyes. Even fibers similar in color may fluoresce differently when compared using various combinations of filters and a comparison microscope.

4.1.4. Infrared Spectroscopy

FTIR microscopy assists with the identification of man-made fibers because each of the different types has different chemical compositions. Polyester

is made from polyethyleneterphthalate, acrylic from polyacrylonitrile, rayon from regenerated cellulose, and nylon is a polyamide. Even within the broad generic classes of fibers, differences may be detected in their composition owing to variations in co-polymers. For example, there are at least nine varieties of acrylic fibers, each with different mixtures of co-polymers that can only be identified by FTIR.

4.1.5. Thermomicroscopy

Melting point can be used to distinguish between different types of nylon or olefin carpet fibers. Nylon 6 melts at about 213°C while nylon 6,6 melts at about 250°C; polyethylene olefin melts at 135°C while polypropylene melts at 170°C.

4.1.6. Comparison Microscopy

If all of the characteristics are still the same, the next step is to examine the fibers with a comparison microscope. Individual fibers are compared side by side; features, such as crimp, color, pigmentation, thickness, luster, and cross-sectional shape, are noted. This side-by-side and point-by-point examination is the best technique to discriminate between fibers from different sources.

4.1.7. Microspectrophotometry

MSP is the most useful way to distinguish fibers that are visually the same, but, in fact, differ only when seen in different light. As different wavelengths of light are passed through the fiber, MSP results in a graph of the color (absorbance vs wavelength of light) and the shape of the graph defines the color. Fibers from the same source will produce the same graphs.

4.1.8. Thin Layer Chromatography

The final step in most fiber comparisons is to extract the dyes from the fibers and compare the constituent dyes using thin layer chromatography. Extracts of the fiber dyes are spotted near the bottom of an absorbent plate, the bottom end of the plate is dipped in a suitable developing solvent, and, as the eluting solvent rises up, the absorbent it rises past the spots of dye, like when toilet paper is dipped in water. The individual dyes from the fiber are moved upwards at differing rates, separating the dyes into colored spots. Extracts from the questioned fibers and the known fibers are run side-by-side and the different dyes are compared with respect to color and position on the plate. Fibers from the same source will contain the same individual dyes. If, after all of these analyses, the questioned and known fibers are indistinguishable, it is likely they originated from the same source.

5. PAINT

In general, the purpose of the analysis and comparison of paint trace evidence is to determine whether two or more samples of paint are from the same source. Paint on a pry-bar used to break open a homeowner's window is a typical example. Traces of paint are recovered from the pry-bar and samples are collected from the window for comparison. The comparison is accomplished through microscopic and chemical analyses. If two samples cannot be distinguished, after completing all appropriate analyses, then a conclusion that they could have originated from the same source is possible.

In other cases, it is important to try to determine the source of the paint without the benefit of a comparison sample. For example, in order to search for a vehicle involved in a hit and run accident, the color, layer structure, and chemical composition of paint collected from the accident site are compared with databases of paint used on all the recently produced cars and trucks. Then the laboratory usually can tell the investigator the color, make, and model of the car they are looking for.

5.1. Collecting Paint

Loose paint chips on cars and window frames can be picked from the surface with tweezers. Intact paint films must be scraped with a strong sharp knife into folded paper packets. The entire paint layer, all the way down to the wood or metal, must be collected. In that way, all the layers will be present for analysis. If clothing is received, the clothing is placed on clean paper for inspection. First, the clothing is searched visually for evidence of a paint transfer. Next, debris is scraped with a clean spatula onto the paper and the debris is searched with a stereomicroscope for paint chips. Chips are examined with a stereomicroscope; the questioned and known samples are compared with respect to colors and layers. Samples are then isolated for PLM, FTIR, or SEM-EDS.

5.2. Paint Comparison

Paint is a protective film applied to a wide variety of products. After evaporation of the solvent vehicle used to spray or spread the coating, the dried paint layers consist of a polymeric binder film containing pigments and fillers (extender pigments). The binder provides a protective coat designed to meet the needs of its intended use. Therefore, the composition of the binder is specifically designed and will differ markedly between paints produced for different uses, such as the differences between exterior and interior house paints. The pigments are added to provide opacity and also to color the film. The number of possible

colors is practically innumerable, as seen in a hardware store paint department. The color is caused by a nearly unlimited number of pigments, from the common titanium dioxide white to pearlescent-coated micas used on new cars. Therefore, the forensic paint comparison involves a detailed analysis of color, binder, and pigments for the usual purpose of determining whether two paints are similar.

Most surfaces are painted with multiple coats. For example, most cars are painted with a primer layer, a base coat, a pigmented layer, and a clear coat on the top. The colors, thicknesses, and sequence of the layers are compared. Paint on houses and a myriad of other structures is often applied repeatedly producing a random accumulation of many different layers of different paints of different colors. The number and sequence of the layers, their thickness, and their color, is often sufficient to prove that two multilayered chips of paint originated from the same source. The match is confirmed by aligning the layers under a comparison microscope.

Fracture matches of larger chips are always possible, but usually the paint chips are studied microscopically and analyzed using PLM, SEM-EDS, and FTIR. The comparison begins using a stereomicroscope with accessories for photography and continues using a polarizing microscope, fluorescence microscope and comparison microscope. SEM-EDS assists in identifying and comparing the pigments in each layer of the paint. FTIR is used to identify the binder. Finally, pyrolysis gas chromatography (PGC), a destructive technique that burns the paint and separates the components by gas chromatography, is used to identify the binder constituents and compare the questioned and known paints.

5.3. Quality Control

Complete written and photographic records are required for all trace evidence cases, but paint can be used to illustrate the types of information that are maintained with each case. For example, in addition to taking detailed written notes, during the initial inspection and subsequent analysis and comparison, the paint chips might be photographed. SEM-EDS, FTIR, and PGC results are recorded and maintained in the case file. All correspondence, chain of custody records, complete notes describing samples, testing, results of testing, and conclusions are kept in the project file. Additionally, all equipment used in the testing of samples is calibrated to insure proper performance. Most crime laboratories have approved written procedures that detail the proper calibration, maintenance, and operation of the instruments. Instruments not operating properly cannot be used.

6. GLASS

Glass is found in our kitchens and the restaurants we frequent, in our cars, in the stores where we shop, and in the windows of our houses and offices. The various types of glass are:

1. Flat glass used for windows, doors, display cases, and mirrors.
2. Container glass used for bottles and jars.
3. Tableware glass.
4. Optical glass used for eyewear.
5. Decorative glass, such as stained glass.
6. Specialty glass used for headlamps and cookware.

Fortunately for the forensic investigator, glass broken by impact can produce hundreds of small particles that can be used as trace evidence. Glass might be recovered at the scene of a hit and run accident or on the clothing of a burglar. In any of these cases, the first possibility is that pieces of glass can be physically matched back to the window or bottle from which they were broken.

6.1. Fracture Matching

To prove that two or more pieces definitely originated from the same object, they must be fracture matched. Fractured fragments are broken or torn objects split apart by force into separate pieces, e.g., a broken tool, paint chips, torn paper, or vehicle headlight lens. A fracture match is the re-assembly of two or more separated fragments that proves that the pieces were once one in the same.

Fracture matching in the crime laboratory, like all other analyses of associative evidence, always involves a comparison to determine whether the questioned evidence could have originated from the suspected source. Accidental (individual) characteristics in the fracture, such as imperfections or irregularities produced accidentally during manufacture, growth, or use, or those caused by abuse, corrosion, or damage when broken, are required to distinguish it from all others. As with all trace evidence, an absolute match cannot be made in the absence of individual characteristics. Although an arrangement in shape and printed design are sufficient to allow the putting together of a jigsaw puzzle, more seems to be required in the crime laboratory. Jigsaw puzzles are mass produced, so without the accidental markings caused by the tearing of the pieces, pieces of other puzzles might also match.

In the crime laboratory, the first of four criteria for a fracture match is that the objects must have been separated by either fracturing or tearing, not purposely cut

like a puzzle. Second, the separated pieces must be realigned so that the object seems to fit together again. Third, the separated pieces must fit together like a lock and key in one or more of the following ways:

1. Along an irregular zig-zag-like edge.
2. Verified by surface markings like the puzzle picture.
3. Verified by a three-dimensional fit, so that all surfaces fit around the object including the upturned broken edges.

Finally, of course, the markings or broken edges must possess individual characteristics, such as growth rings in wood, machine marks on a tool, or the random marks on the edge of broken glass caused by the break. The match should be tested by moving the two fractured pieces back and forth, observing that they do not fit together in more than one way. There will be only one way in which they actually align in every detail. If they cannot be so aligned, the two pieces were never one and the same.

6.2. Collecting Glass

When looking for small fragments in clothing, the cuffs and pockets are turned out to free any glass that may be caught there. The item can then be tugged, shaken, or brushed over clean paper on the laboratory bench and the paper searched with a stereomicroscope for glass fragments. Shoe soles, weapons, and tools are examined directly with a stereomicroscope, looking for any damaged areas where glass might be embedded. Any cuts or holes in the soles of footwear are probed for glass.

A sharp conchoidal (shell-like) broken edge and transparency distinguishes freshly broken glass from plastic and sand grains. Glass is further distinguished from fragments of plastic and minerals by PLM. Glass is not birefringent like most minerals. Color can usually distinguish between sources of glass and the surface of some glass should fluoresce under short-wave ultraviolet light. Surface features formed during manufacture and a curved surface can help distinguish window glass from container, decorative, and eye glass. Finally, if both the inside and outside surfaces are still part of the glass fragment, the thickness can distinguish between different window glasses.

6.3. Comparison of Glass

If a physical match is not possible, physical and chemical properties of the glass are compared in order to determine whether two pieces could have originated from the same batch. There are two fundamental physical properties used by crime laboratories to compare glass. The first is density.

6.3.1. Density

The density of glass is measured by placing a glass fragment into a liquid, so it floats without sinking or rising to the surface. If the glass remains suspended, the glass and liquid have the same density. The density of the liquid is then either measured directly or the same liquid is used with another fragment of glass to determine whether they are of the same density. Density is very sensitive to small changes in composition. If two samples of glass differ in density, they could not have originated from the same source.

6.3.2. Refractive Index

The second fundamental physical property of glass is refractive index, which also depends on the chemical composition of the glass as well as its manufacturing history. The crime laboratory either uses a manual method or automated image analysis to microscopically measure the refractive index. Both methods are based on a double variation technique, developed by Richard Conrad Emmons at the University of Wisconsin circa 1928, and promoted to the forensic scientists during the second half of the 20th century by Walter C. McCrone. By the proper manipulation of both the temperature and wavelength of light, it is possible to obtain not only the refractive index of the glass, but also how the index varies with the wavelength of light used, called the dispersion curve of the glass. A hot stage is used on the microscope to control the temperature because the refractive index liquids used for these tests depend on the temperature. Color filters are used to control the wavelength of light. In both the manual and automated procedures, the match point of the refractive index is determined by observing the Becke line, like when measuring the refractive indices of fibers, and noting the point where the glass particle disappears (no Becke line), which is the point where the refractive index of the glass matches the refractive index of the liquid. With automated image analysis, the camera and computer detect the point where the glass disappears, records the temperature of the stage, and, thereby, the refractive index of the liquid and the glass. If two samples of glass can be differentiated by their refractive index, they could not have originated from the same source.

6.3.3. Elemental Analysis

The concentrations of most elements in glass are intentionally controlled by the manufacturers in order to make a particular glass product. However, all glass products display variations in the concentration of these elements and contain small concentrations of uncontrolled trace elements. Therefore, element concentrations may be used to differentiate among glasses made by different

manufacturers and from different production lines. The methods for determining the elements in the glass include: SEM-EDS, X-ray fluorescence spectrometry (XRF), and inductively coupled plasma-atomic emission and mass spectrometry (ICP). ICP burns the sample up; therefore, all nondestructive examinations must be completed prior to any ICP.

SEM-EDS measurements are nondestructive, applicable to very small samples, and the most readily-available means for elemental analysis in most crime laboratories. The questioned and known glass samples are compared by measuring concentrations of the elements sodium (Na), aluminum (Al), magnesium (Mg), calcium (Ca), and potassium (K). The disadvantage is that SEM-EDS is not as sensitive for some elements as XRF and ICP. XRF requires a larger piece of glass than SEM-EDS, but the element detection for most elements are generally better for XRF than for SEM-EDS. Although destructive, ICP techniques are even more sensitive and measure lower concentrations of more elements, such as Al, barium (Ba), Ca, iron (Fe), Mg, manganese (Mn), Na, titanuium (Ti), strontium (Sr), and zirconium (Zr), providing more points of comparison and improving discrimination between glass sources.

Like all trace evidence comparisons, each of the observations and measured values must be consistent between questioned and known glass in order to prove that glass came from the same source. If any of the findings suggest that the glass differs, then the conclusion must be that the questioned glass sample cannot be associated with the suspected source.

7. Soil

Mud and clods of dirt are sometimes found on vehicles, shovels, or suspects' shoes. Although soil differs greatly from other types of trace evidence, it serves the same purpose. The usual question is whether the soil came from the crime scene.

7.1. Collecting Soil

Although collecting the questioned sample from shoes, for example, is relatively straightforward, there is nothing else in criminalistics where the choice of a control sample is more difficult than with soil. The difficulty stems from the fact that soil is a dynamic accumulation of particles constantly changing—and usually changing over very short distances. Soil sometimes varies within inches across the landscape and within inches down into the ground. Primary known samples are chosen from the spot where it is suggested the questioned sample originated, such as the location of a shoe, tire print,

or grave. Usually several known samples are required to be sure they adequately represent the site where the questioned soil could have originated. Alibi samples are collected from spots where the suspect says the soil is from, that is, the places used as an excuse for the mud on their shoes or their shovel. Alibi samples are of paramount importance. When the suspect's excuses are disproved, the soil evidence is strengthened.

7.2. Soil Comparison

The approach to a forensic soil comparison depends on the background and interests of the examiner. Some examiners have special skills in soil science or mineralogy, others have special skills in palynology (study of pollens and spores), whereas others might pay more attention to the building materials in the soil. Regardless of the emphasis, all approaches compare the microscopic constituents in the questioned soil sample with the microscopic constituents in samples collected from possible sources of the soil. In the end, irrespective of the approach, the conclusion whether the soil could have originated from a suspected source will be based on similarities between the questioned soil and one or more known samples.

The most common approach for comparing soil is to consider its:

- Color.
- Relative amounts of gravel, sand, silt, and clay.
- Mineral composition including the types of rock fragments, heavy minerals, and clay.
- Biological materials like pollen.
- The presence of man-made materials, such as shingle sand, mining and industrial contaminants, and, possibly in unusual circumstances, chemical traces of pesticides.

Color is the most useful of all the soil features for comparison.

More specifically, the color of each sample is noted and then the soil is passed through a set of soil sieves in order to determine the proportion of sand and silt. The color of the sand and silt is compared again. The coarse and medium sands are washed to remove coatings. Heavy minerals (those with specific gravity greater than the more common quartz and feldspars) are separated and the colors are compared again. The types of soil minerals are then identified using PLM and the number of each type counted. At any point where the samples can be distinguished, the process can end or more samples can be collected. When the samples remain indistinguishable throughout the whole process, the crime laboratory can conclude that the soil could have originated from the suspected source.

8. CONCLUSION

Trace evidence encompasses virtually anything that can be touched, broken, pulverized, transferred, shed, and left behind during a crime if a piece of it, no matter how small, can be found during the investigation. Trace evidence can originate from people and their clothing, from the products they make and use, and from the ecological environment. Therefore, it is impossible to describe every type of trace evidence. Furthermore, trace evidence can be used to investigate virtually every imaginable crime, from murder to the theft of Christmas trees. In the latter case, if the trees are taken from the ground and moved to another location, the soil in the ball can be compared with the holes left at the nursery. After recognizing the possibilities, the detective investigating the case must continue the investigation, following all the leads, so the appropriate samples can be obtained and compared in the crime laboratory. The value of trace evidence depends on what can be learned from its analysis and how it fits into the investigation. If the right evidence is brought to the crime laboratory, the crime laboratory has all the tools necessary to associate the trace evidence to the right culprits.

GLOSSARY

Absorbance:	The ratio of light (visible, ultraviolet or infrared) absorbed by a substance relative to the corresponding absorption of a blackbody or reference; when measured over a range of wavelengths, the resulting spectrum of absorbance is used to characterize materials.
Accidental or individual characteristics:	Characteristics of materials caused by unique growth patterns or by wear and tear, used to individualize evidence such as fingerprints or footwear impressions.
Alternate light source:	Usually hand-held crime scene investigation equipment used to produce visible and invisible light at various wavelengths to enhance or visualize potential items of trace evidence, such as body fluids, fingerprints, and clothing fibers.
Birefringence:	The numerical difference between the maximum and minimum refractive indices of anisotropic substances (minerals and fibers, for example); birefringence may also be determined with the aid of compensators, or estimated through use of the Michel-Lévy Interference Color Chart.
Comparison microscope:	Two matching compound microscopes joined together with an optical bridge that allows specimens on both microscopes to be viewed simultaneously, thereby

allowing a direct side-by-side comparison between the two specimens.

Compensator: A birefringent section of optical quartz, gypsum, mica, or similar material that is positioned between the polarizer and analyzer in a polarizing microscope; the plate can sometimes be tilted and/or rotated to measure retardation.

Conchoidal: The fracturing properties of certain kinds of stone, glass and ceramic; for example, in flint and glass, a fractured surface will exhibit roughly circular ridges radiating outwards from the point of impact, shaped like the exterior surface of a conch shell.

Condenser lens: A lens mounted below the compound microscope stage whose purpose is to focus or condense the light onto the specimen; condenser lenses are not used on low-power stereomicroscopes.

Cuticle: The outermost layer of the shaft of a hair, comprised of overlapping scales.

Density: Density is the mass of a substance per unit volume, expressed, for example, as grams (g) per cubic centimeter (cc), pounds per square inch, etc.; specific gravity is the ratio of the density of a substance to the density of some standard substance, such as pure water with a density of 1 g/cc, usually taken as the standard.

Depth of field: The distance between the nearest and farthest points that appear in acceptably sharp focus in the plane of the specimen; depth of focus is the distance between the nearest and farthest points that appear in acceptably sharp focus in the plane of the image; both vary with lens aperture, focal length, and lens-to-object distance.

Dispersion: In optics, dispersion is a phenomenon that causes the separation of a light wave into spectral components with different wavelengths (colors of the rainbow); in microscopy it describes the variation of optical properties owing to the use of different wavelengths of light.

Energy Dispersive Spectrometer: An instrument, usually attached to a scanning electron microscope, that collects X-rays produced by interaction of the electron beam of the microscope with the material analyzed and which have energies characteristic of the elements in the specimen; the abundance (concentration) of the elements can also be

determined as a percentage of the total amount of sample analyzed.

Eyepiece: Also called an ocular, the lens system closest to your eye when looking through a microscope; a small tube that contains the lenses needed to bring a microscope's focus to a final image in the eye; the magnifying power of the microscope is the magnification of the eyepiece times the magnification of the objective times any tube factor which may be present; a binocular or stereo microscope has two matching eyepieces, a monocular microscope has one eyepiece.

Fourier transform infrared spectrometry: Infrared spectrophotometers record the interaction of IR radiation with substances which allows identification of the sample's functional groups in the chemical makeup; when the effects of all the different functional groups are taken together, the result is a unique molecular "fingerprint" that can be used to confirm the identity of a sample. A Fourier transform is a mathematical operation used to translate a complex curve into its component curves. In a Fourier transform infrared spectrometer, the complex curve is an interferogram, or the sum of the constructive and destructive interferences generated by overlapping light waves, and the component curves are the infrared spectrum. The standard infrared spectrum is calculated from the Fourier-transformed interferogram, giving a spectrum in percent transmittance (%T) vs. light frequency (cm-1).

Gas chromatography: GC is a type of chromatography in which the sample, usually dissolved in a solvent, is vaporized and carried by an inert gas through a column packed with a sorbent to any of several types of detectors; each component of the sample, separated from the others by passage through the column, produces a separate peak in the detector. Pyrolysis gas chromatography is where the sample is made volatile by burning rather than by dissolving in a solvent.

Gunshot residue: Gunshot residue (GSR) testing is used in the investigation of suspected use of firearms; the residues usually include gun powder particles and components from the primer, the bullet, the cartridge case and the firearm itself; some of the primer particles (containing lead, antimony and barium) in the residue are unique to GSR.

Heavy mineral:	Heavy minerals (e.g. rutile, zircon) have a specific gravity greater than 2.89; heavy mineral grains are often found as a minor but important component in soil; they are separated from the lighter minerals (quartz, feldspars) by flotation in a heavy liquid such as bromoform (tribromomethane) which has a density of 2.8899 g/cc; that is, the heavy minerals sink in the bromoform while the light minerals float.
Hot stage:	A platform that sits on a microscope stage under the objective lens and which holds the specimen slide and controls the temperature of the slide, refractive index liquid and sample; the sample can then be observed while the temperature of the specimen is raised and lowered in a controlled manner over a temperature range between room temperature usually to about a maximum of 300 degrees Celsius.
Image analysis:	Image analysis is the extraction from digital images of useful information such as sizes and shapes by means of computer processing techniques; image analysis tasks can be as simple as reading bar-coded tags or as sophisticated as identifying a person by his face.
Immersion liquid:	A liquid of known refractive index used to microscopically measure and compare the refractive index of small particles.
Inductively coupled plasma atomic emission:	A method for measurement of the concentration of elements in a substance; in ICP the plasma is generated from radio frequency magnetic fields in a torch resulting in extremely high temperatures; samples are injected into the center of the plasma where the sample molecules undergo instantaneous vaporization, dissociation, ionization and excitation. Atomic Emission Spectrometry is based on the principle that during reversion to the ground state an excited atom or ion releases absorbed energy as light (photons) with wavelengths that are characteristic of an element and intensities that can be measured to determine the concentration of the element.
Interference color:	In microscopy, these are the Newtonian series of colors seen when observing samples with more than one refractive index (anisotropic samples) between fully-crossed polarizers in non-extinction

	orientations; interference colors are illustrated in Michel-Lévy Interference Color Charts.
Luster:	Brightness or reflectivity of fibers, yarns, carpets, or fabrics; synthetic fibers are produced in various luster classifications including bright, semi-bright, semi-dull, and dull; bright fibers usually are clear (have no white pigment) whereas the duller designations have small amounts of white pigments (delusterants) such as titanium dioxide.
Magnification:	The magnification is simply the number of times the image of an object is enlarged when viewed through the microscope; the total magnification is the product of the eyepiece magnification, the objective magnification, and the variable tube factor if there is one.
Mass spectrometry:	Instrumental method for identifying the chemical constitution of a substance by separating gaseous ions according to their differing mass and charge, often combined with other analytical techniques, such as gas chromatography (GC), liquid chromatography (LC) and inductively coupled plasma (ICP) mass spectrometry (MS).
Medulla:	The central canal found in many human and animal hairs, usually visible with a microscope as a black line because the canal is filled with air.
Michel-Lévy birefringence chart:	The Michel-Lévy Interference Color Chart, also known as the Michel-Lévy Table of Birefringence, graphically relates the thickness, retardation (optical path difference), and birefringence (numerical difference between the principal refractive indices) for transparent anisotropic substances viewed between the crossed polars of a polarizing microscope.
Microspectrophotometry:	A technique for measuring the spectral absorption of light, usually in nanometers, by comparing the difference between the absorption of the sample and a reference sample.
Objective:	The lens in a microscope closest to the specimen that first gathers light from the specimen and forms the first magnified image.
Ocular:	The eyepiece of a microscope which serves to further magnify the image formed by the objec-

	tive lens and focuses the image for viewing or photography.
Palynology:	The branch of science dealing with microscopic, decay-resistant remains of certain plants and animals, particularly pollen and spores, both living and fossil.
Particle:	A minute portion of matter; a very small piece or part of something bigger.
Polarizing microscope:	A compound microscope employing polarized light to show changes in internal structure and composition of material not discernible with ordinary light.
Polymer:	Any of numerous natural and synthetic compounds, usually of high molecular weight, consisting of up to millions of repeated linked units where each unit is a relatively light and simple molecule, usually formed into a plastic.
Refractive index:	The ratio of the velocity of light in a vacuum to the velocity of light in a medium (solid, liquid, gas); expressed as n, the refractive index varies with wavelength and temperature.
Retardation:	The number of wavelengths by which the polarized rays passing through a substance between crossed polars of the polarizing microscope fall behind each other; the amount of retardation increases with thickness and the birefringence of the crystal and is expressed in interference colors.
Scanning electron microscope:	An electron microscope with a fine beam of electrons that systematically sweeps or scans (moves point-to-point) over the surface of a specimen, producing highly magnified, detailed images of the surface with excellent depth of field; most scanning electron microscopes are equipped with accessories for doing elemental analysis as well.
Sign of elongation:	A term used to describe the location of the high and low refractive indices in an elongated, anisotropic substance; by convention, a specimen is described as positive (+) when the higher refractive index ("slow direction") is lengthwise ("length slow"), and negative (-) when the lower refractive index ("fast direction") is lengthwise ("length fast").

Thin layer chromatography: A procedure for separating compounds by spotting them on a glass or plastic plate coated with a thin layer of silica or alumina gel; a solvent is allowed to move up the plate by capillary action to separate components of the sample into visible spots; TLC is used for identifying and comparing materials which are highly colored or which fluoresce under ultraviolet light, such as fiber dyes.

Trace element: An element making up only a very small portion of the substance, often in parts per million.

Trace evidence: Trace evidence might be anything that can be described as small bits of solid material used in a forensic investigation, such as particles of dust creating a footwear impression, torn scraps of paper from a threatening note, gunshot residue particles, clothing fibers, paint chips, pubic hairs, or diatoms; trace evidence associates people with people, people with places, objects with people, objects with places and objects with objects; trace evidence, thereby, provides evidence to help understand the behavior of parties to accidents and crimes.

Wavelength: The distance between two successive points of an electromagnetic waveform, such as light and X-rays, usually measured in nanometers (nm).

X-rays: X-rays are a type of electromagnetic radiation between ultraviolet light and gamma rays in wavelength, frequency, and energy; X-rays have short wavelengths (and high frequency) as compared to visible light and therefore can pass through most solid objects.

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