Designation: E2809 – 13

Standard Guide for
Using Scanning Electron Microscopy/X-Ray Spectrometry in Forensic Paint Examinations[1]

This standard is issued under the fixed designation E2809; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide is an outline of methods for scanning electron microscopy (SEM) intended for use by forensic paint examiners. This guide is intended to supplement information presented in Guide E1610.

1.2 The methods used by each examiner or laboratory or both depend upon sample size, sample suitability, and laboratory equipment.

1.3 The term "scanning electron microscopy" occasionally refers to the entire analytical system including energy dispersive X-ray spectrometry (EDS) or wavelength dispersive X-ray spectrometry (WDS) or both.

1.4 This guide does not cover the theoretical aspects of many of the topics presented.

1.5 This guide cannot replace knowledge, skill, or ability acquired through appropriate education, training, and experience and should be used in conjunction with sound professional judgment.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E766 Practice for Calibrating the Magnification of a Scanning Electron Microscope
E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory
E1508 Guide for Quantitative Analysis by Energy-Dispersive Spectroscopy
E1610 Guide for Forensic Paint Analysis and Comparison
E1732 Terminology Relating to Forensic Science

3. Terminology

3.1 *Definitions*—For additional terms commonly employed for general forensic examinations, see Terminology E1732.

3.1.1 *background X-rays (Bremsstrahlung, braking radiation, continuous spectrum), n*—nonspecific X-ray radiation with a continuous energy range from zero up to the beam voltage in which background radiation results from the deceleration of beam electrons in the atomic Coulombic field.

3.1.1.1 *Discussion*—A typical X-ray spectrum consists of both a continuous background and peaks from characteristic X-rays.

3.1.2 *backscattered electrons, n*—primary beam electrons that are scattered from the sample after undergoing few inelastic interactions.

3.1.2.1 *Discussion*—The probability of backscattering is proportional to the atomic number.

3.1.3 *bulk analysis, n*—type of scanning electron microscopy (SEM) analysis that determines the average elemental composition of a material in which 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.

3.1.4 *cathodoluminescence, n*—emission of photons in the ultraviolet (UV), visible (Vis), and infrared (IR) regions of the electromagnetic spectrum as a result of electron beam interaction with certain materials.

3.1.5 *characteristic X-rays, n*—X-ray emission resulting from de-excitation of an atom following inner shell ionization in which the energy of the X-rays is related to the atomic number of the atom, providing the basis for energy dispersive X-ray spectrometry (EDS).

3.1.5.1 *Discussion*—A typical X-ray spectrum consists of both a continuous background and peaks from characteristic X-rays.

---

[1] This guide is under the jurisdiction of ASTM Committee E30 on Forensic Sciences and are the direct responsibility of Subcommittee E30.01 on Criminalistics.

Current edition approved Feb. 15, 2013. Published April 2013. DOI: 10.1520/E2809-13.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3.1.6 *charging, n*—negative charge accumulation on either a nonconductive sample or a sample that is not properly grounded.

3.1.6.1 *Discussion*—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.

3.1.7 *detector fluorescence peak (dead-layer peak, silicon internal fluorescence peak), n*—peak resulting from the emission of characteristic X-rays in a thin layer of inactive crystal area in the front of an EDS detector.

3.1.7.1 *Discussion*—The peak is characteristic of the type of detector, such as silicon for a lithium-drifted silicon detector. In a silicon detector, this peak may appear at 0.2 % apparent concentration.

3.1.8 *electron probe microanalyzer (EPA, EPMA, EMMA), n*—electron beam instrument designed for quantitative X-ray analysis (electron probe microanalysis).

3.1.8.1 *Discussion*—It is related to SEM but with multiple wavelength spectrometers and is designed to work at reproducible and stable beam currents and specimen-beam-X-ray detector geometries. Electron probe microanalysis is the determination of elemental concentration by X-ray emission from the microvolume of material in which a static electron beam interacts.

3.1.9 *embedding, n*—procedure for casting a sample in a block of material that polymerizes, or otherwise hardens, to permit handling during further preparation.

3.1.10 *energy dispersive X-ray spectrometry (EDS, EDXA, EDX), n*—EDX spectrometry is complementary to wavelength dispersive spectrometry (WDS).

3.1.11 *escape peak, n*—peak resulting from incomplete deposition of the energy of an X-ray entering the EDS detector.

3.1.11.1 *Discussion*—This peak is produced when an incoming X-ray excites a silicon atom within the detector crystal and the resulting silicon (Si) K-alpha fluorescence X-ray exits the detector crystal. It occurs at the principal peak energy minus the energy of the Si K-alpha fluorescence X-ray (1.74 KeV). The escape peak intensity is about 1 to 2 % of the parent peak.

3.1.12 *extraneous material, n*—material originating from a source other than the specimen (synonyms: contaminant and foreign material).

3.1.13 *final aperture, n*—last beam-restricting orifice in an electron optical column.

3.1.13.1 *Discussion*—The orifice diameter influences the beam current and depth of focus.

3.1.14 *interaction volume, n*—sample volume in which the electron beam loses most of its energy.

3.1.14.1 *Discussion*—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.

3.1.15 *live time, n*—time in which the EDS electronics are available to accept and process incoming X-rays.

3.1.15.1 *Discussion*—Live time is often expressed as a percentage of real time.

3.1.16 *microtomy, n*—sample preparation method that sequentially passes a blade at a shallow depth through a sample resulting in sections of selected thickness as well as a flat block.

3.1.16.1 *Discussion*—Each may be used for the determination of sample characteristics.

3.1.17 *particle analysis, n*—analytical method intended to determine the elemental composition of a single particle such as a pigment particle in a paint layer.

3.1.17.1 *Discussion*—Usually performed with a static (non-scanning) electron beam.

3.1.18 *pulse processor time constant, n*—operator-selected value for pulse-processing time in which a higher value (longer time) results in a more accurate determination of the detector amplifier pulse height (better spectral resolution) and a lower value results in a higher count rate but with reduced spectral resolution.

3.1.19 *raster, n*—rectangular pattern scanned by the electron beam on a sample.

3.1.19.1 *Discussion*—The raster dimensions change inversely with magnification.

3.1.20 *representative sample, n*—representative portion of the specimen selected and prepared for analysis that is believed to exhibit all of the elemental characteristics of the parent specimen.

3.1.21 *sample polishing, n*—sample preparation method using progressively finer abrasives to achieve a flat, smooth sample surface.

3.1.21.1 *Discussion*—Generally, this is required for quantitative analysis.

3.1.22 *scanning electron microscopy (SEM), n*—type of electron microscope in which a focused electron beam is scanned in a raster on a solid sample surface.

3.1.22.1 *Discussion*—The strength of resulting emissions of signals varies according to sample characteristics such as composition or topography. As the electron beam of the SEM scans the surface of a sample, a signal is continuously registered by the imaging system which produces a two-dimensional image of the sample on the display monitor. By popular usage, the term SEM may also include the analytical techniques EDS and WDS.

3.1.23 *secondary electrons (SE), n*—low-energy electrons produced from the interaction of beam electrons and conduction band electrons of atoms within the interaction volume that are produced throughout the interaction volume, but only those near the surface have enough energy to escape.

3.1.23.1 *Discussion*—The secondary electron signal is typically used to form topographic images.

3.1.24 *smear, n*—transfer of paint resulting from contact between two objects and consisting of comingled particles, fragments, and possible pieces of one or both surfaces.

3.1.25 *specimen, n*—material submitted for examination.

3.1.25.1 *Discussion*—Samples are removed from a specimen for analysis.

3.1.26 *spectral artifacts, n*—spectral peaks other than characteristic peaks produced during the EDS detection process.

3.1.26.1 *Discussion*—Examples are escape peaks and sum peaks.

3.1.27 *spectral resolution, n*—measure of the ability to distinguish between adjacent peaks in an X-ray spectrum and it is usually determined by measuring peak width at half the maximum value of the peak height or full-width half-maximum.

3.1.28 *sum peak, n*—peak occurring at the sum of the energy of two individual peaks.

3.1.29 *dead time, n*—time during which the EDS is not able to process X-rays.

3.1.29.1 *Discussion*—Dead time is typically expressed as a percentage of real time during which the detector is not collecting X-ray data.

3.1.30 *system peaks (stray radiation), n*—peaks that may occur in the X-ray spectrum resulting from interaction of the electron beam or fluorescent radiation with components of the SEM itself

3.1.31 *takeoff angle, n*—angle between the specimen surface and the detector axis.

3.1.32 *transmission electron microscopy (TEM), n*—type of electron microscopy in which an image of a sample prepared as a thin section is formed by the interaction of the beam passing through the sample.

3.1.33 *variable pressure scanning electron microscopy (LV, CP, VP, ESEM), n*—type of SEM that is designed to operate at higher chamber pressure than the conventional in which the need for application of a conductive coating is minimized when using a variable pressure SEM; however, EDS may be complicated because of the electron beam spread experienced at higher operating pressures.

3.1.34 *wavelength dispersive spectroscopy (WDS, WDXA), n*—X-ray spectroscopy that separates and identifies X-rays based on their differences in wavelength.

3.1.34.1 *Discussion*—WDS is a complementary spectroscopy to EDS.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *concentration, n*—for the purpose of this guide, the following ranges shall apply: major: greater than 10 %; minor: 1 to 10 %; and trace: less than 1 %.

3.2.2 *sample size, n*—for the purposes of this guide, the following terms are used to describe sample size with the actual size demarcation between each being somewhat arbitrary.

3.2.2.1 *fragment, n—only within this guide*, sample or specimen smaller than approximately 0.2 mm.

(*1*) *Discussion*—If the material from which the fragment originated was layered, then the fragment may also show a layered structure with light microscopy inspection and SEM analysis. A fragment is frequently not of sufficient size to permit multiple tests.

3.2.2.2 *particle, n—only within this guide*, sample or specimen whose greatest dimension is less than approximately 50 µm.

(*1*) *Discussion*—Material of this size generally has none of the overall structural characteristics that can be associated with the material from which the particle originated. A particle is generally not of sufficient size to permit multiple tests.

3.2.2.3 *piece, n*—sample or specimen larger than approximately 0.2 mm.

(*1*) *Discussion*—If the material from which the piece originated was layered, then the piece may show a layered structure. A sample of this size is sufficient to perform all of the suggested cross-sectional preparation and analytical methods.

3.2.3 *thick section, n*—for the purpose of this guide, a sample that is 2 µm or thicker.

3.2.4 *thin section, n*—for the purpose of this guide, a sample with a thickness of less than 2 µm.

4. Significance and Use

4.1 The SEM can be used to define and compare the layer structure of multilayered samples, the structure of individual layers, the bulk elemental composition of individual layers, and the elemental composition of individual particulate components within paints and coatings.

4.2 The test methods described in this guide may have some limitations. They include the inability to detect elements in trace concentrations, the need for a conductive coating of the sample, the inability to remove a sample from most embedding materials after analysis, and the discoloration of materials by irradiation.

4.3 Although quantitative and semiquantitative methods are available for EDS (see Guide E1508), they are not appropriate for most paint analyses because of the typical heterogeneity of paint. Application of quantitative methods is further complicated by an inability to predict what compounds may be present (see 7.12.1).

4.4 The information available from a specimen may diminish as its size is reduced and its condition degrades. The smaller a specimen is, the less valuable it becomes for association with a known because it may contain fewer characteristics of the original material. As specimen size is reduced, it may no longer be representative of the original material. This may also be true of a degraded sample.

4.5 This guide is intended to advise and assist laboratory analysts in the effective application of scanning electron microscopy to the analysis of paint evidence. It is intended to be applicable to most modern scanning electron microscopes typically used in the forensic laboratory.

4.6 It is not the intention of this guide to present comprehensive methods of SEM. It is necessary that the analyst have an understanding of SEM operation and general concepts of specimen preparation before using this guide. This information is available from manufacturers' reference materials, training courses, and references such as Scanning Electron Microscopy and X-ray Microanalysis: A Text for Biologists, Materials Scientists, and Geologists (**1**).[3]

5. Sample Handling

5.1 The general collection, handling, and tracking of samples shall meet or exceed the requirements of Practice E1492 as well as the relevant portions of the "Trace Evidence Quality Assurance Guidelines" (**2**) and "Trace Evidence Recovery Guidelines" (**3**).

5.2 The work area and tools used for the preparation of samples shall be free of all materials that could transfer to the sample. Samples prepared for SEM analysis shall be maintained in a protective container such as a petri dish or box.

5.3 When samples are prepared for SEM, construct a map identifying sample location. This may be in the form of a sketch, a photomicrograph, or a captured video image and shall include an index mark on the mount.

6. Sample Preparation

6.1 Samples shall first be examined with a stereomicroscope noting size, structure, overall homogeneity, and any 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 request. It may be necessary to use multiple preparation methods to analyze all sample characteristics.

6.3 In developing a strategy for analysis, consider:

6.3.1 Determination of the presence of extraneous materials and a strategy for removal;

6.3.2 Method of attachment to an SEM mount;

6.3.3 Method(s) for exposing internal structure if the specimen is inhomogeneous;

6.3.4 Method(s) for producing a uniform geometry;

6.3.5 Necessity of applying a conductive coating to the prepared samples; and

6.3.6 Determination of the presence of surface features of analytical interest.

6.4 If an analytical goal is to determine elemental composition, then any possible contribution from extraneous materials shall be eliminated.

6.5 If an analytical goal is to determine structure, then the internal structure shall be exposed using an appropriate method.

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

6.7 Although embedding with subsequent polishing or microtomy may be considered labor-intensive, these methods permit precise, reproducible sample preparation.

6.8 If sufficient sample size permits, mounting flat, intact specimens may allow visualization and analysis of surface features

6.9 *Recognition and Removal of Extraneous Materials:*

6.9.1 It is not unusual for extraneous materials to be present on the surface of a specimen submitted for analysis. Because the SEM 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, a strategy for the recognition and removal or visualization and abatement of this material shall be used.

6.9.2 Depending on sample size and type, extraneous material may be physically removed with a brush, probe, or fine blade. Debris can also be lifted off the sample with tape. Samples that are too small to be effectively taped can be rolled on a thin adhesive layer. Care shall be taken that the adhesive 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.

6.9.3 To immobilize extraneous materials, the technique of embedding described in 6.11.1.4 is effective. Subsequent processing of the sample may then proceed without direct concern for the extraneous materials.

6.9.4 When extraneous materials cannot be removed and the sample is not embedded, note their location during light microscopy or backscatter electron SEM or both observations. During analysis, avoid areas with extraneous material. Note that some surface extraneous materials may not be visible by light microscopy alone.

6.10 *Methods of Attaching a Sample to a SEM Mount:*

6.10.1 All samples to be analyzed in the SEM shall be attached to some form of an SEM mount. These mounts are usually made of aluminum, carbon, beryllium, or brass. Because the presence of a carbon peak in the spectrum does not usually interfere with elemental comparisons, mounts constructed of carbon are preferred. Carbon mounts are available either as spectroscopically pure or pyrolytic. Pyrolytic carbon offers the advantage of a hard, flat, glasslike surface that results in a featureless background when imaged. Samples may be attached directly to a SEM mount, with the prior application of an adhesive layer. Ideally, the adhesive shall be organic with minimal inorganic content and soluble in a solvent that evaporates rapidly. The adhesive may be applied to the mount dropwise by a micropipette or spread into a thin film by drawing out the drop with a coverslip. The thickness of adhesive may be adjusted by regulating the size of the drop (**4**).

6.10.2 Electrically conductive carbon paints are commercially available and may be used for directly attaching samples onto the surface of an SEM mount. The paints typically consist of micronized carbon suspended in an organic solvent. A small streak of carbon paint can be placed on the mount using a fine tipped brush while viewing under a stereomicroscope at low magnification. The sample may then be touched to the surface of the paint just before it goes to dryness causing it to adhere to the surface of the mount with an electrically conductive attachment.

6.10.3 Various carbon-conductive adhesives and double-sided tapes are commercially available and may be used. Their elemental compositional purity shall be characterized before use (**5**).

---

[3] The boldface numbers in parentheses refer to a list of references at the end of this standard.

6.11 *Demonstration of Internal Structure:*

6.11.1 For characterization, the sample shall be prepared so that the internal structure is exposed. A variety of methods are presented in 6.11.1.1-6.11.1.4. If the specimen is too small to carve manually, pieces and fragments may be prepared in cross section by freehand cutting, polishing, or microtomy of the embedded sample.

6.11.1.1 The sample may be cut and attached on edge to an SEM mount or shaved after attaching to an SEM mount. This method is suitable only for large samples. This method can be performed rapidly; however, layers can separate, extraneous materials can be dragged onto the surface to be analyzed, and the geometry between samples may not be consistent.

6.11.1.2 Some samples may be slowly carved, exposing each individual layer. This may be done by holding the sample in place, either with forceps or in some other manner, then peeling the layers away with a clean, sharp scalpel blade or diamond knife. The cutting tool shall be held at a very low angle to produce thin peels and avoid excessive pressure on the sample. Alternatively, a focused ion beam (FIB) may be used. Thin peels of the individual layers may then be harvested and mounted by one of the techniques described in 6.10. This method does require substantial sample manipulation but provides the advantages of reproducible flat sample geometry, no potential for the interaction volume to extend into neighboring layers, and the availability of large analytical surface areas. Sample size should be relatively large, and preparation by this method does not provide an opportunity to image a cross section of the specimen. Furthermore, detection of minor elemental constituents requires longer analytical acquisition times owing to the reduced analytical volume afforded by the thin peel.

6.11.1.3 Some samples may be stair-stepped by cutting a layered structure on intralayer planes and peeling to expose underlying layers for analysis. Although this method can expose a large area of each layer for X-ray analysis and potentially avoid spectral variations caused by inhomogeneity, the interaction volume may extend into an underlying layer. Sample size should be relatively large, and preparation by this method does not provide an opportunity to image a cross section of the specimen.

6.11.1.4 *Embedding*—Before microtomy or polishing, a sample is embedded to provide support. The sample is placed in a mold with an identifying label, and the mold is filled with embedding material that is allowed to polymerize or harden. Several mold types are available, such as a silicone flat holder, capsules, slotted stub, and ring mounts. Embedment and subsequent exposure of the specimen's cross section offers the advantages of abating extraneous materials, providing precise control and manipulation of samples smaller than 0.2 mm, and processing of several samples simultaneously. Disadvantages are the possibility of selective removal of soft or soluble layers, trapping of polishing materials, and extension of the beam interaction volume into the adjacent layers when thin layers are encountered.

(1) Microtomy of Embedded Samples—Microtomes are generally of two types: histomicrotome and ultramicrotome, either of which may be used for the preparation of paints. A glass knife is usually used in an ultramicrotome, although diamond or tungsten carbide knives may be used for hard materials. A steel or tungsten carbide knife is used in a histomicrotome. In addition to producing a flat sample block for subsequent SEM analysis, sections may be cut for light microscopy, ultraviolet-visible (UV-Vis) microspectrophotometry, and infrared (IR) microspectroscopy. Multiple samples may be embedded in the same mold for microtomy. Their relative positions shall be indexed such that their cross sections may be identified in the sample block. This may be accomplished by mounting a taggant fragment in the mold and noting its position relative to the questioned and known samples before microtomy. Microtomy produces a sample block that is flat across the entire face. Slight variations in takeoff angle may exist between samples if embedded in separate molds. Mounting samples for comparison in the same mold minimizes these variations. In doing so, however, care shall be taken to assure that the paint fragments lie parallel to one another so that the beam/sample geometry between samples does not vary.

(2) Polishing of Embedded Samples—Polishing is a process by which the embedded sample is exposed to a series of successively finer abrasives. Individual paint samples may be embedded in a single block or embedded individually and mounted in a holder containing multiple sample slots. Individual embedding permits individual sample height adjustment, whereas if several samples are mounted simultaneously, only one final polishing plane is possible. Various types and combinations of polishing materials are available and suitable. Diamond abrasives, however, are recommended for the final polish step because they do not leave particle residues that may be mistaken for paint components. When paints are simultaneously polished, the analyst is assured that each has been prepared in the same manner. Each is equally flat, scratch-free, and in the same plane. However, edge rounding may occur between areas of differing hardness.

6.12 *Uniform Geometry:*

6.12.1 If samples are to be compared, the takeoff angle of each specimen shall be similar. Only then are spectral differences indicative of differences in the chemistry of the samples.

6.12.2 Similar geometry can be achieved if the samples are microtomed or polished simultaneously.

6.12.3 If microtomy is selected as a preparation method and multiple blocks are used, each block shall be microtomed at a similar angle.

6.13 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.

7. Procedure

7.1 *Instrument Calibration:*

7.1.1 Before beginning an analysis, verification of the operational condition of the SEM shall be established. This includes presence of system peaks, accuracy of magnification, and determination of spectral energy calibration and resolution. A method for spectral sensitivity calibration should also be performed. That is, that the detector performance over the energy range used for analysis has a consistent and useful sensitivity as seen by measuring characteristic X-ray intensities from a standard material at a variety of energies encompassing the range desired.

7.1.2 The presence of system peaks is generally determined upon installation of the SEM or following a modification or addition of accessories. Goldstein (**1**) describes a procedure.

7.1.3 For a determination of accuracy of magnification, a percentage of error of magnification shall be calculated. An SEM'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 shall 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, shall also be recorded. Magnification standards for SEMs are commercially available, with errors of less than 5 % generally achievable. Additional information on magnification calibration can be found in Practice E766.

7.1.4 Energy calibration shall be established frequently for the eEDSr, 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 exceed 10 eV from that of actual energies. Automatic methods for calibration are described in documentation from the manufacturer.

7.1.5 Spectral resolution for the EDS shall 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 manganese (Mn) K-alpha peak at half the maximum peak height. Automatic methods for calibration as well as recommended performance limits are often available from the manufacturer.

7.2 *Structural Imaging:*

7.2.1 Light microscopy is useful for defining layers and structures based on color characteristics. The end-on view can be either an edge-mounted, microtomed, or embedded sample observed with reflected light. When the samples have been prepared by a method producing a flat surface, such as polishing or microtomy, the entire field of view is in focus at high magnification. Mounted on a glass slide, the thick section is observed with transmitted light. Light microscopy demonstrates layer structure as well as some structural detail within each layer.

7.2.2 A backscatter electron image is useful for defining layers and structures based on the average atomic number of the matrix. For comparison purposes, a magnification similar to that of the light microscopy image is suggested. Higher magnification images may be useful for demonstration of structural details.

7.2.3 A cathodoluminescence image can also be used to provide structural information and discrimination and is complementary to the light microscope and backscatter electron images (**6**).

7.2.4 SEM micrographs shall include a measuring scale or magnification scale or both. The micrograph shall also display which detector was used to produce the image (BE detector or SE detector).

7.3 *Selection of SEM/EDS 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 requirements may vary.

7.3.1.1 A beam voltage of 20 to 25 KeV is an adequate compromise between the need for sufficient overvoltage 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 20 KeV. The pulse processor time constant shall be set at a value which provides maximum count rate and maximum spectral resolution. The beam current shall be adjusted to yield an X-ray detector dead time between 5 and 40 % depending upon your detection system. A live time of 100 to 200 s is usually sufficient to provide reasonable counting statistics for minor peaks. This is instrument and sample dependent.

7.3.1.2 The beam/sample/X-ray detector geometry shall be optimized for X-ray collection efficiency, particularly when attempting to analyze nonflat samples.

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

(1) Beam voltage is increased when higher energy line excitation is required.

(2) Beam voltage is decreased when greater spatial resolution is required.

(3) Pulse processor time constant is lengthened when greater spectral resolution is required.

(4) Pulse processor time constant is shortened when a greater count rate is required (for example, for trace element analysis or construction of elemental distribution maps).

(5) Detector-to-sample distance can be reduced to increase X-ray collection efficiency.

(6) Spectral energy display scale is expanded when sufficient detail is not evident.

(7) Beam current is increased when the X-ray count rate is too low. Decreasing the condenser lens current or increasing the final aperture size or both may increase beam current.

(8) Beam current is decreased when the X-ray count rate is too high. Increasing the condenser lens current or decreasing the final aperture size or both may decrease beam current.

7.4 *Bulk Spectra Collection:*

7.4.1 Once the structure of the material is defined, the average or bulk elemental composition of each layer is determined. The raster shall include as much area of a layer as possible. This may be achieved by analyzing a single large area or summing the spectra from several smaller areas.

7.4.2 It may be useful in some comparisons to use a combination of mapping and pixel by pixel spectral interpretation allowing both average layer analysis and individual component analysis.

7.4.3 Because the X-ray analytical volume may be larger than the raster and X-ray fluorescence may be significant, the analyst shall consider the possibility of the contribution of X-rays produced in adjacent layers.

7.5 *Qualitative Analysis:*

7.5.1 Once an X-ray spectrum is collected, a qualitative analysis is performed 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, however, unless a systematic approach to elemental identification is used that 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 spectral line markers 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, shall be evaluated and labeled.

7.5.4 As spectral interpretation alternates between the identification of major and minor peaks, the vertical (counts) scale shall 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 shall be noted. Individual asymmetric peaks and inconsistent peak ratios within a family may indicate a peak overlap. Elemental identification is aided by superimposing and scaling K, L, and M reference lines on the spectrum or referencing the actual spectrum of an elemental standard. The analyst shall become familiar with the characteristic pattern and relative intensities of peaks of various atomic numbers. The identification of major components is usually straightforward.

7.5.5 Following the identification of major components, lower intensity peaks and overlapped peaks are identified. The number of characteristic peaks present in a spectrum often limits minor element 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 in which 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 an 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 unequivocal, the peak label shall indicate so (such as by parenthesizing the elemental symbols).

7.5.8 Spectra shall be displayed on a scale that clearly demonstrates the peaks identified. To display peaks from elements with significant differences in concentration, the (small) 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 shall confirm peak identification.

7.5.10 Although the natural X-ray line width is approximately 2eV, EDS resolution is approximately 140 eV. As a result, there may be an overlap of peaks in the EDS spectrum of materials containing several elements. Some commonly occurring overlaps encountered in EDS are as follows: titanium (Ti) K-beta/vanadium (V) K-alpha, V K-beta/chromium (Cr) K-alpha, Cr K-beta/Mn K-alpha, Mn K-beta/iron (Fe) K-alpha, Fe K-beta/cobalt (Co) K-alpha, lead (Pb) M-alpha/sulfer (S) K-alpha/molybdenum (Mo) L-alpha, barium (Ba) L-alpha/Ti K-alpha, potassium (K) K-beta/calcium (Ca) K-alpha, zinc (Zn) L-alpha/sodium (Na) K-alpha, phosphorus (P) K-alpha/zirconium (Zr) L-alpha, and aluminum (Al) K-alpha/bromine (Br) L-alpha.

7.5.11 To resolve these overlaps, several methods may be used:

(1) The processing time of the pulse processor may be increased to improve spectral resolution.

(2) Mathematical spectral subtraction (deconvolution) methods supplied by the EDS manufacturer can be used.

(3) A WDS scan can be performed over the questioned energy range.

(4) An alternative method of elemental analysis or X-ray diffraction may be used.

7.5.12 If peaks remain that are not identified by the above scheme, a comprehensive tabulation of X-ray line energies may be consulted.

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 electrons 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 paint layer may reveal the presence of Al, Si, Mg, Ba, S, and oxygen (O). Specific particle analysis may associate the elements Si, Mg, and O as being present in one type of particle; Ba, S, and O present in a second type; and Al, Si, and O in a third type. These associated elemental compositions would then indicate these particles to be consistent with talc, barium sulfate, and clay, respectively. Polarized light microscopy, IR spectroscopy, or X-ray diffractometry can be used to confirm the presence of some of the compounds.

7.6.1.1 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.

7.6.2 As an alternative to using the spot analysis mode, individual particles may be teased out of the sample using a fine tungsten needle and individually mounted for discreet analysis.

7.7 *WDS Analysis:*

7.7.1 WDS may be used in conjunction with EDS to provide greater sensitivity (×10) and resolution (5 versus 140 eV) of characteristic peaks (**7**). Stand-alone spectrometers with multiple diffracting crystals can be interfaced to many SEMs. Some manufacturers provide software to integrate the WDS functions with EDS in such a way that qualitative scans may be performed from the EDS screen as a graphic-user interface.

7.7.2 WDS generally requires flat samples for analysis. Higher beam currents and higher magnifications than are frequently used for EDS are required to provide a sufficient count rate. However, the thermal energy produced from this higher beam current has the potential for damaging the sample.

7.8 *Comparison of a Small Particle to a Bulk Sample*—Any individual particle or fragment from an inhomogeneous paint may not be compositionally representative of the bulk and, therefore, would not be expected to produce spectra similar to the bulk material. To compare the compositional characteristics of a particle with a bulk sample, the spectrum of the particle shall be compared to the nonrepresentative spectra obtained from multiple, randomly selected areas having a similar analytical volume to that of the particle.

7.9 *Analysis of a Smear*—A smear is composed of comingled particles, fragments, and possible pieces of one or both original contact surfaces. Initially, a light microscopical search is made for the largest fragments present. Particles that are approximately 50 µm can often be selected individually and attached to a mount for analysis. It is also possible to lift a collection of deposited particles with a sticky lift, attach the lift to a SEM mount, and analyze the particles directly.

7.10 *Analysis of a Primarily Organic Layer:*

7.10.1 Analysis of a layer that is primarily organic, such as a clear coat or varnish, may be useful for comparison. Within such a layer, the interaction volume is significantly larger than that of a layer containing pigments. This is a result of a lower average atomic number of the matrix. To reduce the interaction volume, the beam voltage may be reduced; however, the voltage shall be sufficient to produce X-rays from all lines of analytical utility.

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

7.11 *Inhomogeneity Versus Analytical Area:*

7.11.1 To compare the average composition of structures, the spectrum used for comparison shall come from an area of the structure sufficient to produce an average composition.

7.11.2 The representative nature of a spectrum can be determined by the critical comparison of spectra from adjacent areas. If no differences are evident, the structure 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.

7.11.3 A representative bulk analysis is most difficult to achieve when analyzing a thin, inhomogeneous layer in a cross-sectioned specimen. In these types of samples, a representative spectrum may not be achieved until the individual spectra from 10 or even 20 areas have been summed.

7.12 *Assessment of Results During Analysis:*

7.12.1 Generally, comparisons are facilitated by direct spectral comparison.

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

7.12.3 If spectral differences are detected, it is likely that the materials are not similar in composition; however, several alternative explanations are possible and must be evaluated. Only after the considerations in 7.12.3.1-7.12.3.5 have been addressed can the analyst confirm that spectral differences are indicative of compositional differences.

7.12.3.1 Differences in background shape may result from dissimilar sample geometry. To correct, see 6.12.

7.12.3.2 Differences in the composition of major peaks may indicate that the spectra are not representative of the bulk composition of an inhomogeneous specimen. 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. To correct, see 7.8.

7.12.3.3 Compositional differences may result from unequal contributions from elements in adjacent layers. To correct, see 7.4.

7.12.3.4 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 a smear and 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 6.9.

7.12.3.5 Differences in carbon intensity may result from a contribution of carbon from the mount.

7.13 *Interpretation of SEM/EDS Data:*

7.13.1 A conclusion regarding similarity results from the comparison of images and elemental composition of individual layers. Collect all data and micrographs in the same manner over as short a time as reasonable. Spectra may be critically compared by overlaying them.

7.13.2 Compositional comparisons may be made by methods such as calculating the ratios of the integrated peak intensities of a spectrum to that of a reference peak and comparing the values to those of the comparison sample. If a peak ratio method of comparison is used, the ratios shall be displayed in tabular form.

7.13.3 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 SEM/EDS.

7.13.4 If a comparative analysis demonstrates significant differences between samples regarding structure and composition, then it can be concluded that the samples are different.

7.13.5 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 an inhomogeneous specimen. The latter shall only be concluded following an extensive investigation of the heterogeneity of the known specimens and demonstration that the range of variation present in the known sample encompasses that observed in the questioned sample.

7.13.6 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 sample was a fragment or smear 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

8. Documentation

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

8.2 Case notes shall 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 shall also include a statement or data confirming system calibration, as detailed in 7.1.

8.3 See "Trace Evidence Quality Assurance Guidelines" (**2**) for further requirements.

9. Keywords

9.1 dispersive X-ray spectrometry; EDX; EDS; embedding; forensic paint examination; scanning electron microscopy; SEM

REFERENCES

(1) Goldstein, J. I., Newbury, D. E., Echlin, P., Joy, D. C., Romig, A. D., et al., *Scanning Electron Microscopy and X-ray Microanalysis: A Text for Biologists, Materials Scientists, and Geologists*, 2nd edition, Plenum Press, New York, 1992.

(2) Scientific Working Group on Materials Analysis, "Trace Evidence Quality Assurance Guidelines," *Forensic Science Communications [Online]*, January 2000, available from: http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/jan2000/index.htm/swgmat.htm.

(3) Scientific Working Group on Materials Analysis, "Trace Evidence Recovery Guidelines," *Forensic Science Communications [Online]*, October 1999, available from: http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/oct1999/index.htm/trace.htm.

(4) Ward, D. C., "A Small Sample Mounting Technique for Scanning Electron Microscopy and X-Ray Analysis," *Forensic Science Communications [Online]*, July 1999, available from: http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july1999/index.htm/ward.htm.

(5) Wrobel, H. A., Millar, J. J., and Kijek, M., "Comparison of Properties of Adhesive Tapes, Tabs, and Liquids Used for the Collection of Gunshot Residue and Other Trace Materials for SEM Analysis," *Journal of Forensic Sciences*, Vol 43, No. 1, 1998, pp. 178–181.

(6) Stoecklein, W., and Goebel, R., "Application of Cathodoluminescence in Paint Analyses," *Scanning Electron Microscopy*, Vol 6, No. 3, 1992, pp. 669–678.

(7) Bearden, J. A., "X-Ray Wavelengths," *Reviews of Modern Physics*, Vol 39, No. 1, 1967, p. 78.