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Standard Guide for Using Scanning Electron ~~Microscopy/X-Ray Spectrometry~~ Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDS) in Forensic ~~Paint~~Polymer 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)~~ covers recommended techniques and procedures intended for use by forensic ~~paint examiners. This guide is intended to supplement information presented in Guide~~ laboratory personnel that perform SEM/EDS analyses on polymer samples.~~E1610~~.

1.2 ~~The methods used by each examiner or laboratory or both depend upon sample size, sample suitability, and laboratory equipment.~~This guide describes various techniques and procedures used in the SEM/EDS analysis of polymers that include sample handling and preparation, instrument operating conditions, and spectral data collection, evaluation and interpretation.

~~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.~~

1.3 ~~This guide does not cover the~~ The theoretical aspects of many of the topics ~~presented.~~presented can be found in texts such as Scanning Electron Microscopy and X-ray Microanalysis (**1**).[2]

1.4 This guide is intended to be applied within the scope of a broader analytical scheme (for example, Guides E1610, E3260) for the forensic analysis of a polymer sample. An SEM/EDS analysis can provide additional information regarding the potential relationships between the sources of polymeric materials.

1.5 This guide ~~cannot replace knowledge, skill, or ability acquired through appropriate education, training,~~ is intended for use by competent forensic science practitioners with the requisite formal education, discipline-specific training (see Practices E2917, E3233, and E3234~~experience and should be used in conjunction with sound professional judgment.~~), and demonstrated proficiency to perform forensic casework.

1.6 The values stated in SI units are to be regarded as standard. ~~No other~~ Other units of measurement are included in this ~~standard.~~standard where applicable as a result of common usage (for example, keV).

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

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

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E620 Practice for Reporting Opinions of Scientific or Technical Experts
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
E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs
E2937 Guide for Using Infrared Spectroscopy in Forensic Paint Examinations
E3085 Guide for Fourier Transform Infrared Spectroscopy in Forensic Tape Examinations
E3233 Practice for Forensic Tape Analysis Training Program
E3234 Practice for Forensic Paint Analysis Training Program
E3260 Guide for Forensic Examination and Comparison of Pressure Sensitive Tapes

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),*~~ *aperture, 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.~~a beam-restricting orifice in an electron optical column; the orifice diameter influences the beam current and depth of focus.

~~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,*~~ *electron (BE) imaging, n*—~~primary beam electrons that are scattered from the sample after undergoing few inelastic interactions.~~a technique that uses high energy electrons that originate from the primary electron beam of the SEM and are elastically reflected by the specimen to create an image of the sample. The probability of backscattering is proportional to atomic number.

~~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.~~

3.1.3 *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.~~

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

3.1.4.1 *Discussion*—

This effect ~~may~~can interfere with image formation and X-ray analysis because of beam deflection. It can usually be eliminated by the application of a conductive ~~coating.~~coating or by the use of a low vacuum system.

[3] 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.

3.1.7 *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.

3.1.5 *electron probe microanalyzer (EPA, EPMA, EMMA),* *dead time, n*—electron beam instrument designed for quantitative X-ray analysis (electron probe microanalysis).the time (expressed as a percentage of real time) during which the energy dispersive X-ray spectrometer is not able to process X-rays.

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.

3.1.6 *energy dispersive X-ray spectrometryspectroscopy (EDS, EDXA, EDX), n*—EDX spectrometry is complementary to wavelength dispersive spectrometry (WDS).X-ray spectroscopy based on the simultaneous measurement of the energies of X-rays emitted by a sample.

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

3.1.7.1 *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 Si K-α fluorescence X-ray exits the detector crystal. It occurs at the principal peak energy minus the energy of the Si K-alphaK-α fluorescence X-ray (1.74 KeV).keV). The escape peak intensity is about 1 to 2 % of the parent peak.

3.1.8 *extraneous material,exclusionary difference, n*—material originating from a source other than the specimen (synonyms: contaminant and foreign material).a difference in a feature or property between compared items that is substantial enough to determine that they did not originate from the same source.

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.

3.1.9 *live time, n*—the time inover which the EDS energy dispersive X-ray spectroscopy electronics are available to accept and process incoming X-rays. Live time is often expressed as a percentage of real time.

3.1.15.1 *Discussion*—

Live time is often expressed as a percentage of real time.

3.1.10 *microtomy, n*—sample preparation methodapproach 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 (nonscanning) electron beam.

3.1.11 *pulse processor time constant, time, 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.the time designated to record a response by the detector.

3.1.11.1 *Discussion—*
A higher value (longer time) results in a more accurate determination of the detector amplifier pulse height (better spectral resolution). A lower value results in a higher count rate but with reduced spectral resolution.

3.1.12 *raster, n*—rectangularthe pattern scanned by the electron beam on a sample.sample; the raster dimensions change inversely with magnification.

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

3.1.13 *representative sample, sample (representative sample), n*—a representative portion of the specimen selected and prepared for analysis that is believedexpected 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.

3.1.14 *scanning electron microscopy (SEM), n*—a type of electron microscope in which a focused electron beam is scanned in a raster on a solid sample surface.surface; the term can also include the analytical technique of energy dispersive X-ray spectroscopy.

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.

3.1.15 *secondary electrons (SE), electron (SE) imaging, n*—imaging using 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, butvolume, with only those near the surface have enoughhaving sufficient 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.

3.1.16 *spectral artifacts, n*—spectral peaks other than characteristic peaks, produced during the EDS detection process.energy dispersive X-ray spectroscopy detection process; examples include escape peaks and sum peaks.

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

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

3.1.18 *sum peak, n*—peak occurring at the sum of the energy of two individual peaks.a peak resulting from the simultaneous detection of two photons; this is manifested as a peak at the combined energy of line(s) for the specific element(s) involved.

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.

3.1.19 *system peaks (stray radiation), n*—peaks that may can occur in the X-ray spectrum resulting from as a result of interaction of the electron beam or fluorescent radiation with components of the SEM itself scanning electron microscope 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.

3.1.20 *variable pressure scanning electron microscopy (LV, CP, VP, ESEM), mode, n*—type of SEM that is designed mode that allows some SEMs 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. varying chamber pressures.

3.1.20.1 *Discussion*—

The need for application of a conductive coating is minimized when using variable pressure mode; however, EDS can 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.~~

4.1 This guide is intended to advise and assist ~~laboratory analysts~~the analyst 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.~~preparation of polymer samples (for example, paint and tape) for SEM/EDS, the collection of data by SEM/EDS, and the interpretation of images and data resulting from these analyses.

4.2 When polymers are constructed as layered materials, SEM/EDS analysis is conducted on each polymeric layer individually. This analysis can be hindered by a non-discernable layer structure (for example, smear, irregular segregation within the layer system).

4.3 SEM-EDS data can be useful in:

4.3.1 *Layer Elucidation*—SEM images provide insight into the layer structure of a sample.

4.3.2 *Texture Elucidation*—SEM images and elemental maps provide insight into the texture (for example, surface topography, distribution of inclusions).

4.3.3 *Element Identification*—Determination of the elements detected in a sample layer.

4.3.4 *Relative Elemental Abundance Determination*—An EDS spectrum permits the relative abundance of elements in samples to be compared.

4.4 ~~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~~ In the context of a forensic polymer comparison, the evaluation of SEM/EDS results are intended to provide insight into the following forensic tasks:~~(1).~~

4.4.1 Comparison of structure, texture, and elemental data.

4.4.2 Support for results from other instruments (for example, the presence of calcium, oxygen, and carbon in the EDS spectrum obtained from discrete particles indicates the presence of calcium carbonate as observed in an infrared spectrum). Refer to Guides E2937 and E3085 for further details.

4.4.3 Significance of results given the presence of certain elements, layer structures, or textures.

~~**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.~~

5. Sample Preparation

5.1 ~~Samples shall first be examined with a stereomicroscope noting size, structure, overall homogeneity, and any material adhering to the sample.~~*Sample Handling:*

5.1.1 Practice E1492, the relevant portions of Guides E1610 and E3260, and the SWGMAT Trace Evidence Quality Assurance Guidelines and Trace Evidence Recovery Guidelines are followed for the collection, handling, and tracking of samples and specimens.

5.1.2 *Cleanliness*—Keep the work area and tools used for the preparation of samples free of all materials that could be transferred to the sample. Protect samples prepared for SEM/EDS analysis to minimize possible contributions from the laboratory environment.

5.1.3 *Labeling*—Label samples adequately throughout the examination process to ensure that identity and orientation (when relevant) are maintained. This is particularly important when comparison samples are mounted in the same preparation.

5.1.4 *Preservation*—When possible, maintain a portion of the evidence in its original, unaltered condition to ensure that adequate sample remains for potential future analyses. In the event that a limited sample size predicates the use of an entire sample, retain the prepared samples as evidence.

5.2 ~~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.~~*Initial Examination:*

5.2.1 Record the following minimum information in notes or images for the specific polymer sample being analyzed by SEM/EDS. This information is supplemented through an initial examination by stereomicroscopy. If this information has already been recorded as part of the broader analytical scheme for a sample, this information need not be duplicated:

5.2.1.1 Record if the submitted sample is a known or questioned item.

5.2.1.2 Record if the sample is suspected (or known) to be a particular type of material (for example, paint or tape).

5.2.1.3 Describe the type of sample (for example, chip, smear, tape fragment).

5.2.1.4 Determine if the polymer sample is a multilayered product. If so, determine and record the layer structure of the sample. Layer structure determined by means of stereomicroscopy could need to be refined after sample preparation and imaging at higher magnification (by light or electron microscopy).

5.2.1.5 Note features that could impact the SEM/EDS analysis (for example, surface imperfections or contaminants, inclusions within the layer).

~~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~~

5.3 ~~*Recognition and Removal of Extraneous Materials:*~~ *Visual Inspection for Debris*—A stereomicroscope is used to evaluate a sample for the presence of extraneous materials (for example, blood, adhesive from a tape lift, contributions from an underlying substrate). the presence of debris and the approach(es) used to mitigate the impact on sample visualization and elemental analysis results, such as the following:

5.3.1 *Physical Removal*—~~It is not unusual for extraneous materials to be present on~~ Under a stereomicroscope, larger particles and some residues are gently scraped or manually picked from 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.~~sample with intent to minimize disruption to the underlying sample surface.

5.3.2 *Washing*—~~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.~~A sample can be washed in a solvent (for example, water, alcohol) with sonication to assist as needed. Prior to washing, a small sample fragment can be subjected to the selected solvent to ensure that the sample is not soluble.

5.3.3 *Sectioning*—~~To immobilize extraneous materials, the technique of embedding described in~~The removal of cross-sections permits the ~~6.11.1.4~~ is effective. ~~Subsequent processing of the sample may then proceed without direct concern for the~~exposure of internal surfaces which are not subject to the presence of extraneous materials.

5.3.4 *Avoidance*—~~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.~~In some cases, microanalytical procedures provide imaging capabilities and an analytical volume that permit extraneous debris to be physically avoided during analysis.

~~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).~~

5.4 ~~*Demonstration of Internal Structure:*~~ *Layer Preparation*—Samples for comparison are prepared under the same conditions whenever possible. The preparation approach, details required to reproduce the preparation process, and differences between sample preparations are recorded. Samples are prepared in a manner that permits the resolution and analysis of individual layers. The following preparations represent a selection of the approaches available. Smears represent an exception and are treated in section 5.4.4.

5.4.1 *Hand-Cut Thin-Section Preparation:*

5.4.1.1 With the aid of magnification (for example, stereomicroscope), thin peels (thin slices through an individual layer) or cross-sections (thin sections which include all layers in a sequence of multilayered polymer samples) can be cut freehand using a scalpel or razor.

5.4.1.2 *Caution*—Polymers can contain layers that are too thin to isolate using the thin peel approach.

5.4.1.3 These approaches (thin peels and cross-sections) produce thin sections of varying thickness. Use caution when interpreting and comparing data collected from samples cut freehand as layer thickness differences complicate interpretation.

5.4.2 ~~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.~~*Stair Step Preparation:*

5.4.2.1 ~~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.~~For multilayered polymer samples, freehand thin peels are sequentially removed from the sample using a scalpel or razor with the aid of a stereomicroscope to produce a "stair step" sample in which a planar surface of each layer within a sample is exposed.

~~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.~~

5.4.2.2 ~~*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.~~Due to differences in the vertical height of each stair step, position the sample in the SEM such that the exposed "steps" are oriented with a direct line-of-sight to the EDS detector.

~~*(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.~~

5.4.2.3 *Caution*—Polymers can contain layers that are too thin to isolate or expose using the stair step approach.

5.4.2.4 This approach exposes layers of varying thickness. Use caution when interpreting and comparing data collected from samples prepared by this approach as both layer thickness differences and contributions from lower layers are possible.

5.4.3 *Embedded and Microtome-cut Preparations:*

5.4.3.1 Consider and establish the broader analysis scheme and sample preservation requirements prior to embedding a sample as recovery of embedded samples is impossible or difficult.

5.4.3.2 Samples can be embedded in a resin (for example, acrylic, epoxy) to produce a sample that is supported for microtomy. To select an appropriate resin, weigh factors that include: resin composition (to minimize contributions to analysis results); viscosity; curing time; wetting (to ensure that the polymer is firmly encased in the resin); and curing process (impact of heat and UV light on the sample). Record the type of resin used.

5.4.3.3 The position of samples within a mold is recorded to ensure that each sample is unambiguously identifiable.

5.4.3.4 To prepare a block for sectioning, ensure that samples are flat, as opposed to tilted, in the mold.

5.4.3.5 The sample block and respective sample layers are oriented relative to the blade in a manner to minimize potential smearing caused by the passage of the blade through the sample.

5.4.3.6 The embedded sample block (containing one or more samples) is cut on a microtome to produce thin sections.

5.4.3.7 *Caution*—Polymers can contain layers that are too thin to isolate during analysis due to excited volume impingement into neighboring layers.

5.4.3.8 Microtome-cut sections from the resin block or the sample within the resin block are analyzed. These thin sections can also be used for other instrumental techniques (for example, infrared spectroscopy). Refer to Guide E2937 for further details.

5.4.3.9 The resin of an embedded sample can contribute to the resulting SEM/EDS sample analysis. If not previously characterized, determine the elemental composition of the resin by SEM/EDS.

5.4.3.10 The sample block is retained as evidence following analysis.

5.4.4 *Smears:*

5.4.4.1 Examine smears by stereomicroscopy or polarized light microscopy for evidence of individual layer remnants. To the extent possible, individual layer remnants are recorded and analyzed.

5.4.4.2 When present on a substrate, smears are analyzed in situ (that is, on the substrate) or after isolating them from the substrate.

5.4.4.3 Analyze the substrate underlying the smear to account for the presence of elements originating from the substrate in the smeared sample. For example, include analysis of the underlying paint when a paint smear is observed on, and potentially commingled with, another paint system. At a minimum, include the top layer and disrupted layers in the substrate analysis.

~~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.~~

5.5 ~~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.~~*Mounting a Sample:*

5.5.1 Sample substrates (for example, carbon planchet, aluminum stub) provide physical support for samples to be examined in the SEM and a means by which to ground the sample and reduce charging. Polished beryllium, diamond, or pyrolytic graphite substrates are alternatives worth considering when working with small samples (for example, smears) where it is necessary to recover a particle. These alternatives are highly polished surfaces, exhibit a low background, and are reusable.

5.5.2 While a variety of sample substrate compositions are available, double-sided conductive carbon adhesive tabs, tape, or sheets are attached to the substrate and provide an adhesive surface for mounting and securing forensic polymer samples.

5.5.3 Samples are mounted on the substrate such that they are flat and adhered to the substrate.

5.5.4 To maximize conductivity of the sample to the substrate and thereby reduce or eliminate sample charging, a line of conductive carbon or silver can be applied between the sample surface and substrate or a conductive layer of carbon (that is, carbon coating) can be deposited. Charging can also be reduced or eliminated through the use of variable pressure mode in an SEM.

5.5.5 Record the type of substrate along with sample preparation, including any coating applied, as well as the position of samples on the substrate. A digital or hand-drawn map can be used to depict the identity and location of each specimen placed on an SEM stub. An indexing mark on the stub can also be included to assist in sample/location orientation during SEM examination.

5.5.6 A spectrum of the sample substrate can also be collected and retained.

~~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 cEDSr, 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~~