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An American National Standard

Standard Guide for Using Scanning Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (SEM/EDS) in Forensic Polymer Examinations[1]

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1. Scope

1.1 This guide covers recommended techniques and procedures intended for use by forensic laboratory personnel that perform SEM/EDS analyses on polymer samples.

1.2 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 theoretical aspects of many of the topics 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 is intended for use by competent forensic science practitioners with the requisite formal education, discipline-specific training (see Practices E2917, E3233, and E3234), and demonstrated proficiency to perform forensic casework.

1.6 The values stated in SI units are to be regarded as standard. Other units of measurement are included in this 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.*

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

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

[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.

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

[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.

3.1.2 *backscattered electron (BE) imaging, n*—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.

3.1.3 *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.

3.1.4 *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 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 or by the use of a low vacuum system.

3.1.5 *dead time, n*—the time (expressed as a percentage of real time) during which the energy dispersive X-ray spectrometer is not able to process X-rays.

3.1.6 *energy dispersive X-ray spectroscopy (EDS, EDXA, EDX), n*—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 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 Si K-α fluorescence X-ray exits the detector crystal. It occurs at the principal peak energy minus the energy of the Si K-α fluorescence X-ray (1.74 keV). The escape peak intensity is about 1 to 2 % of the parent peak.

3.1.8 *exclusionary difference, n*—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.

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

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

3.1.11 *pulse processor time, n*—operator-selected value for 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*—the pattern scanned by the electron beam on a sample; the raster dimensions change inversely with magnification.

3.1.13 *sample (representative sample), n*—a representative portion of the specimen selected and prepared for analysis that is expected to exhibit all of the elemental characteristics of the parent specimen.

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; the term can also include the analytical technique of energy dispersive X-ray spectroscopy.

3.1.15 *secondary 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, with only those near the surface having sufficient energy to escape.

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

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

3.1.18 *sum peak, n*—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.

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

3.1.20 *variable pressure mode, n*—mode that allows some SEMs to operate at 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.

4. Significance and Use

4.1 This guide is intended to advise and assist the analyst in the 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 In the context of a forensic polymer comparison, the evaluation of SEM/EDS results are intended to provide insight into the following forensic tasks:

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 Preparation

5.1 *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 *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).

5.3 *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*—Under a stereomicroscope, larger particles and some residues are gently scraped or manually picked from the surface of a sample with intent to minimize disruption to the underlying sample surface.

5.3.2 *Washing*—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*—The removal of cross-sections permits the exposure of internal surfaces which are not subject to the presence of extraneous materials.

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

5.4 *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 *Stair Step Preparation:*

5.4.2.1 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.

5.4.2.2 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.

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.

5.5 *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.

6. Instrumental Operating Conditions

6.1 The following are recommended operating parameters that can be altered to optimize conditions for various analytical needs. As the analyst determines specific analytical needs, actual operating conditions can vary.

6.1.1 Samples being compared are analyzed under the same operating conditions.

6.1.1.1 *SEM Magnification (if images are collected through the SEM software) and EDS Software Magnification (if images are collected through the EDS software)*—Magnification standards for SEMs are commercially available. A magnification tolerance of less than 5% is generally achievable. Refer to Practice E766 for additional information on magnification calibration.

6.1.1.2 *EDS Resolution*—In some software packages, this process can be automated. Alternatively, the resolution can be manually checked by measuring the full width of the manganese (Mn) Kα peak at half of the maximum peak height.

6.1.1.3 *EDS Energy Calibration*—Check energy calibration at each time constant used. In some software packages, this process can be automated. For manual measurement, the peak centroid energy of a low- and high-energy peak are measured and checked against reference values.

6.1.2 Record the values for each parameter, evaluate them against established tolerances, and compare them to historical values obtained from the same SEM/EDS system.

6.1.3 Recalibrate the EDS detector if the measured parameters fall outside of the laboratory defined tolerance. Consult

the appropriate operation manual for system specific calibration instructions. Do not use the instrument unless each parameter of the performance check falls within its defined tolerance.

6.2 *Instrument Setup:*

6.2.1 Refer to the appropriate operating manual for instrument specific setup instructions.

6.2.2 Record conditions that are necessary to reproduce or review the data. Many of these conditions are automatically captured as metadata within images or spectral data files.

6.2.3 Variation of the operating values is permitted.

6.2.4 Use the same operating conditions when samples are being compared.

6.2.5 *SEM Setup:*

6.2.5.1 *Vacuum Mode*—It is recommended that samples be analyzed under high vacuum conditions. If variable pressure is used, beam spread can result in contributions from outside the pre-defined raster area such as adjacent layers or the sample substrate. Beam spread increases with higher chamber pressure and reduces spatial resolution for acquired images. In variable pressure mode, record the pressure at which an analysis is conducted.

6.2.5.2 *Beam Saturation*—For systems with a filament, attain a stable beam current prior to operation.

6.2.5.3 *Voltage*—For EDS analysis of polymers, an electron accelerating voltage should be chosen to sufficiently excite and allow for the detection and confirmation of the presence of high atomic number elements. Caution should be exercised when using higher beam voltages, as they can damage material with less inorganic content (for example, colorless electrical tape adhesive, automotive clearcoat, duct tape backing). Lower accelerating voltages can be used to excite X-ray transitions from lower elemental electron shells, but the analyst will need to be aware of possible peak overlaps such at Ti K-line and Ba L-line.

6.2.5.4 *Beam Current*—The beam current selected when conducting these analyses are typically in the picoamps to nanoamp range; however, the absolute value is not generally measured or specified in SEM/EDS analyses. It is important to achieve a stable beam current (as per instrument instructions). This is typically achieved by letting the beam stabilize at a specific setting for a period of time (established empirically or by manufacturer recommendation). Therefore, no guidance on the absolute scale of this value is provided in this document; however, the beam current is generally set to provide a balance between image resolution and X-ray output. Adjustments to the beam current can be made by varying a setting that may be described as beam current, spot size, or probe current (varies by manufacturer).

6.2.5.5 *Working Distance*—The optimal working distance for collecting EDS X-ray spectra is fixed by the SEM/EDS geometry. Consult the installation specifications to determine the optimal working distance for a given SEM/EDS configuration. Conduct analyses at this optimal working distance unless precluded by specific sample constraints (for example, sample size). Note deviations from the optimal working distance.

6.2.5.6 *Condenser Aperture*—Select an appropriate condenser aperture size for imaging and EDS analysis. Check the aperture centering at the start of each analysis session. The SEM operating manual provides instrument-specific centering instructions.

6.2.5.7 *Focus and Stigmation*—Ensure that the sample is in focus and that the stigmators have been adjusted prior to imaging and analysis.

6.2.5.8 *Magnification*—Magnification can be adjusted to permit imaging and EDS analysis of a sample at different scales. For instance, lower magnifications can be helpful to record the overall sample or a particular layer, while higher magnification can be used to study a specific inclusion.

6.2.6 *EDS Setup:*

6.2.6.1 *Deadtime*—Set the deadtime through adjustment of SEM beam current and EDS time constant to a desired value (refer to EDS instrument specific operating instructions for guidance). Higher dead times are more likely to produce sum peak artifacts in EDS spectra.

6.2.6.2 *Pulse Processor Time*—Set the pulse processor time (for example, time constant) to provide a balance between resolution and total analysis time. Use automatically selected time constants with caution due to the possibility that samples being compared could be analyzed under different time constants.

6.2.6.3 *Live Time*—Set a live time that provides sufficient signal-to-noise to meet the analytical needs should be selected. For example, with paint samples, 100 s live count time can provide sufficient signal for a heavily filled paint layer versus 200 s for a clearcoat with minimal inorganic contribution.

6.3 *Imaging:*

6.3.1 Different detectors exist for imaging:

6.3.1.1 *Secondary Electron Imaging,* which generally provides topographic information about a sample, is typically used in polymer analyses to examine original surfaces (such as surface deposits or surface textures).

6.3.1.2 *Backscatter Electron Imaging,* which provides image contrast that is generally proportional to the average atomic number, is typically used in polymer analyses to examine flat/prepared surfaces to determine the number of layers present within a multi-layer sample or to visualize the size and distribution of inclusions (for example, fillers). Components that exhibit cathodoluminescence display contrast that is disproportionally high BSE contrast relative to its atomic number.

6.3.2 Position a sample or layer of interest in the field of view and center it under the electron beam. Adjust the magnification and move the sample to select the area to be imaged. Typically, the sample focus and beam stigmation adjustments are made while viewing the sample at a magnification greater than that to be used for analysis or imaging. Select the appropriate scan speed and acquire a secondary electron or backscatter electron image of the sample.

6.3.3 Evaluate the images for charging effects. If charging is detected, mitigate it by using one of the following strategies:

6.3.3.1 Moving to a different area of the sample.

6.3.3.2 Reducing the accelerating voltage. Changes to accelerating voltage will impact the energy range over which elements will be excited, the excitation efficiency of those