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, 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:*[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.

[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.

3.1.5 *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.

3.1.6 *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

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