

OSAC PROPOSED STANDARD 2024-S-0012

Standard Practice for the Forensic Analysis of Geological Materials by Scanning Electron Microscopy and Energy Dispersive X-Ray Spectrometry

Trace Materials Subcommittee

Chemistry: Trace Evidence Scientific Area Committee (SAC)

Organization of Scientific Area Committees (OSAC) for Forensic Science



OSAC Proposed Standard

OSAC 2024-S-0012

Standard Practice for the Forensic Analysis of Geological Materials by Scanning Electron Microscopy and Energy Dispersive X-Ray Spectrometry

Prepared by
Trace Materials Subcommittee
Version: 2.0
January 2025

Disclaimer:

This OSAC Proposed Standard was written by the Trace Materials Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science following a process that includes an open comment period. This Proposed Standard will be submitted to a standard developing organization and is subject to change.

There may be references in an OSAC Proposed Standard to other publications under development by OSAC. The information in the Proposed Standard, and underlying concepts and methodologies, may be used by the forensic-science community before the completion of such companion publications.

Any identification of commercial equipment, instruments, or materials in the Proposed Standard is not a recommendation or endorsement by the U.S. Government and does not imply that the equipment, instruments, or materials are necessarily the best available for the purpose.

To be placed on the OSAC Registry, certain types of standards receive a Scientific and Technical Review (STR). The STR process is vital to OSAC's mission of generating and recognizing scientifically sound standards for producing and interpreting forensic science results. The STR shall provide critical and knowledgeable reviews of draft standards to ensure that the published methods that practitioners employ are scientifically valid, and the resulting claims are trustworthy.

The STR consists of an independent and diverse panel, which may include subject matter experts, human factors scientists, quality assurance personnel, and legal experts as applicable. The selected group is tasked with evaluating the proposed standard based on a defined list of scientific, administrative, and quality assurance based criteria.

For more information about this important process, please visit our website at: <https://www.nist.gov/organization-scientific-area-committees-forensic-science/scientific-technical-review-str-process>

Standard Practice for the Forensic Analysis of Geological Materials by Scanning Electron Microscopy and Energy Dispersive X-Ray Spectrometry

1. Scope

- 1.1 This practice covers recommended techniques and procedures for the use of Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM/EDS) for the forensic analysis of geological materials to include soils, rocks, sediments, and materials derived from them (for example, concrete).
- 1.2 The theoretical foundation of SEM/EDS is covered in numerous texts such as Scanning Electron Microscopy and X-ray Microanalysis (1). This document describes some options for specimen handling and preparation, instrument operating conditions, spectral and image data collection, evaluation of data quality, interpretation of EDS spectra for the identification of inorganic geological materials, documentation of morphology by SEM imaging, and criteria for sample comparison.
- 1.3 This standard is intended for use by competent forensic science practitioners with the requisite formal education, discipline-specific training (see Practice E2917) and demonstrated proficiency to perform forensic casework.
- 1.4 The values stated in SI units are to be regarded as standard. Other units are avoided, but photon energy is commonly reported in units of kiloelectronvolts (keV), data are collected as counts per second (cps), and compositional data based on EDS are reported in elemental weight percent (wt. %).
- 1.5 This standard is not intended for the characterization of building materials by SEM/EDS to assess engineering properties (C1723, C295, C856).
- 1.6 *This standard does not purport to address all the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*
- 1.7 *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:

- C1723 Guide for Examination of Hardened Concrete Using Scanning Electron Microscopy
- C295 Guide for Petrographic Examination of Aggregates for Concrete
- C856 Practice for Petrographic Examination of Hardened Concrete
- E620 Practice for Reporting Opinions of Scientific or Technical Experts
- E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory
- E1508 Guide for Quantitative Analysis by Energy Dispersive Spectrometry

E2917 Practice for Forensic Science Practitioner Training, Continuing Education, and Professional Development Programs

E1732 Terminology Relating to Forensic Science

E766 Practice for Calibrating the Magnification of a Scanning Electron Microscope

E3272 Guide for Collection of Soils and Other Geological Evidence for Criminal Forensic Applications

E3254 Practice for Use of Color in the Visual Examination and Forensic Comparison of Soil Samples

WK89493 Standard Guide for the Detection and Preservation of Forensic Trace Evidence (OSAC 2024-S-0012).

2.2 ISO Standards

ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories

ISO 22309:2011 Microbeam analysis — Quantitative analysis using energy-dispersive spectrometry (EDS) for elements with an atomic number of 11 (Na) or above

3. Terminology

3.1 *Definitions* – for additional terms commonly employed for general forensic examinations and SEM/EDS, see Terminology in **E1732** and **E1508** respectively.

3.1.1 *bulk analysis*, n - analysis of a material either by a large single area or by compiled results of multiple areas, typically intended to capture a representative property of the material.

3.1.2 *compositional domain*, n – compositionally distinct region of a particle.

3.1.3 *morphotype*, n - recognized subdivision of particles that is characterized by distinct morphological characteristics.

3.1.4 *morphometrics*, n - quantitative measurements of morphological features that can be used to characterize particles.

3.1.5 *overscan*, n – an EDS method in which the electron beam is rastered over a region of interest to obtain a bulk elemental analysis.

3.1.5.1 *Discussion*: The area of analysis should be as large as possible and is achieved by a single large area raster or the summed results from multiple smaller rastered areas.

3.1.6 *spot analysis*, n- an EDS mode in which a spectrum is collected when the electron beam is held static.

3.1.7 *standard*, n – a material with a known composition.

3.1.7.1 *Discussion*: The best standards have a simple flat geometry.

3.1.8 *variable pressure scanning electron microscope*, n - type of SEM that is designed to operate at higher chamber pressure than the conventional SEM.

3.1.8.1 *Discussion*: There are several alternative or closely related terms and abbreviations for variable pressure scanning electron microscope including: VP-SEM, ESEM or environmental SEM; low vacuum SEM; CP-SEM or controlled pressure SEM.

4. Significance and Use

- 4.1 This guide is intended to advise and assist analysts in the effective application of SEM/EDS to the analysis of geological materials. It is intended to be applicable to most modern SEM/EDS systems typically available in the forensic laboratory.
- 4.2 The goals of a forensic examination of geological evidence include identification of an unknown substance, comparison of two or more items for possible common origin, or estimation of provenance. SEM/EDS analysis is a component of an overall examination scheme and is not typically used on its own (7.1.1). The presence, absence, and relative abundance of mineral species or morphotypes determined by SEM/EDS in two samples can be used to evaluate whether there are exclusionary differences during a forensic comparison.
- 4.3 SEM/EDS can be used to determine the elemental composition of geologically-derived materials with high spatial resolution (sub-micrometer).
- 4.4 SEM/EDS is advantageous because it allows for the simultaneous imaging of individual particles to determine morphology and the measurement of the elemental composition of particles or discrete domains within particles.
- 4.5 The elemental compositions derived from SEM/EDS can be used to assign provisional mineral identifications. Provisional mineral identity can be determined by visual comparison of specimen EDS data to the following: reference EDS data published in mineralogy textbooks (2); empirically collected EDS data from known mineral standards; published mineral elemental composition data; or simulated EDS spectra (DTSA-II (3)).
- 4.6 Qualitative or semi-quantitative EDS data can provide additional information to test provisional mineral identifications made using other techniques (e.g., polarized light microscopy [PLM], Raman spectroscopy). In this document semi-quantitative EDS analysis consists of attributing peaks to elements and observing the peak height and area.
- 4.7 Imaging by SEM can provide additional information to test and provide support for provisional mineral identifications and morphometric evaluation.
- 4.8 *Limitations to SEM/EDS:*
 - 4.8.1 Detection limits depend on elements and matrices, and are typically around 0.1 weight % concentration for elements with atomic numbers above fluorine. For lighter elements, detection limits are typically around 1 weight % concentration. Alternative analytical techniques, such as inductively coupled plasma mass spectrometry or micro X-ray fluorescence, can provide lower detection limits.
 - 4.8.2 Some samples and SEM systems require covering the specimen with a conductive coating.
 - 4.8.3 Electron beam irradiation can irrevocably change some specimens (e.g., discoloration, loss of water, or migration of light elements).
 - 4.8.4 EDS only provides elemental information, which may limit its ability to identify certain minerals on its own.

4.8.5 EDS data obtained from a compositional domain (zone) of a particle might not be representative of the whole particle.

4.8.6 Procedures for quantitative methods using EDS are not covered in this guide; they are available elsewhere (ASTM [E1508](#) or reference [1](#)).

5. Specimen Preparation

5.1 The sample preparation methods selected depend upon the goals of the forensic examination, the type of material (consolidated versus unconsolidated, particle sizes, amount of material available), and any other methods of analysis to be applied before or after SEM/EDS. When differences arise between this standard and jurisdictional requirements, jurisdictional requirements can take precedence.

5.2 *General considerations*

5.2.1 Use specimen sample preparation practices that minimize the possibility of cross-contamination. Prepare questioned and known items on separate SEM specimen sample mounts (e.g., stubs) ([WK89493](#)).

Note 1 - The nature of the samples affects precautionary measures recommended to prevent cross contamination. Approaches for minimizing cross contamination include: preparing questioned and known samples in separate locations, storing prepared specimens samples within covered containers to minimize dust accumulation, and placing questioned and known items within the SEM/EDS instrument at separate times. Specimens of limited quantity and small particle size not embedded in mounting material require the greatest measures to mitigate potential cross contamination ([WK89493](#)).

5.2.2 Exposure of a sticky mount during sample preparation can provide a control specimen allowing tests for possible airborne contamination.

5.2.3 Document the location of the materials to be analyzed on the SEM mount when more than one specimen or subsample is placed on the same mount. Documentation methods can include sketches, photographs, captured video images, or index (fiducial) marks on the mount. Label samples with clear and unique codes/sample numbers.

5.2.4 Specimen preparation is commonly carried out with the aid of a stereomicroscope.

5.2.5 Once the SEM mounts are prepared, protect them from surface abrasion and atmospheric dust deposition with a protective lid.

5.2.6 Storing the prepared specimens in a vacuum chamber or desiccator reduces SEM chamber evacuation time.

5.2.7 Charging can be mitigated by applying a surface coating (e.g., carbon or metal) or adhering to the mount with a conductive paint (e.g., silver or carbon paint). Alternatively uncoated specimens can be analyzed using variable pressure mode ([3.1.8](#)) or using low accelerating voltage or beam current.

Note 2 - Variable pressure mode increases the width of the spot size and can reduce the EDS signal. The beam scatter could result in contributions to the EDS

spectrum from the mounting material or other material that is in proximity. The wider spot size could also worsen image quality and spatial resolution. The analyst must balance the benefits of retaining uncoated samples against the reduced data quality and reduced spatial resolution.

Note 3 - Lower beam current can reduce detection limits and lower accelerating voltage can affect the excitation of elements.

5.2.7.1 Specimen coating is commonly accomplished using a vacuum evaporator or sputter coater (1).

Note 4 - Metal (e.g., gold, palladium, platinum) coatings can interfere with X-ray peak assignments (e.g., determination of phosphorus and zirconium). If coating is required, carbon is preferred to metals when EDS analysis is performed.

5.2.8 If the intent of the analysis is the comparison of two or more samples, use the same specimen preparation technique for all samples to be compared.

5.3 *Unconsolidated Materials - Soils and Sediments*

5.3.1 Separation of unconsolidated materials

5.3.1.1 Particle separation can be beneficial prior to SEM/EDS analysis.

5.3.1.2 Processing of unconsolidated materials can include grain washing, particle size fractionation, density, and magnetic susceptibility separations, and hand picking of grains. Procedures for such separations related to forensic unconsolidated material examinations are detailed in published references (22,23). In most cases, some sample processing is required.

5.3.1.3 Grain size separation can be achieved with clean, laboratory-standard metal sieves or disposable plastic sieve cloth. Alternatively, grain size separation by Stokes' settling law can separate grain sizes.

5.3.1.4 Segregation of a sample component for SEM/EDS analysis, usually achieved by hand picking particles, is often used to determine the elemental composition of the component to confirm a provisional mineral identification. The morphology of individual grains should be considered when orienting them for optimal imaging and X-ray analysis (6.2.3.2).

5.3.1.5 When preparing subsamples of particulate material, use procedures that create representative subsamples.

5.3.1.6 Sample splitters, or cone and quartering (4) can be used to create representative subsamples, but both can be impractical for samples of limited quantity.

5.3.1.7 An alternative method of representative sub-sampling appropriate for small quantities of powder, is first to mix the particles, moistening (with an appropriate liquid such as water) to cause particle adhesion, and then selecting one or more sub-samples for analysis from this mixture.

Note 5 - Non-representative subsampling might introduce bias in the modal abundance of particles, affecting downstream analyses.

5.3.2 Preparing unpolished particles for SEM/EDS

5.3.2.1 Geological particles can be prepared as unpolished grains to: study their morphology; determine their qualitative elemental composition; or minimize alteration of samples of limited quantity.

5.3.2.2 The choice of mounting substrate is dependent on the analyses. Conductive carbon tabs are a common mounting substrate, but recovering particles from them can be challenging. Particles adhered to polished beryllium or carbon mounts with organic binders (e.g., collodion) or conductive paint are easily recovered.

Note 6 - Collodion (nitrocellulose) can be dissolved in acetone.

5.3.2.3 Mineral grains can be mounted either untreated or washed to remove adhering material (e.g., grain coatings, decomposition products, explosive residues) prior to analysis. When grains are treated to remove adhering material, it is recommended to retain this material for potential subsequent analysis. Grains can be either individually mounted or applied as a dispersion.

Note 7—Back sieving sand—or silt-sized grains (4) onto a sticky SEM stub allows grains to be well-spaced on the stub, avoiding the mixing of EDS spectra or blocking of EDS signal that can occur when grains are very closely spaced.

5.4 *Rocks and building materials (e.g., concrete, cinder blocks, bricks, pavers)*

5.4.1 SEM-EDS analysis can be used to examine the phases present and their petrographic textures.

5.4.2 The preparation of rocks and building materials for examination may include: direct examination with minimal sample preparation, polished thin-sections, or embedded and polished (5.5).

5.4.3 Direct examination allows for minimal sample preparation. The major elemental components of samples can be determined using this method of preparation. Elemental composition of surface minerals may not be representative of the original bulk source (e.g., due to chemical weathering).

5.4.4 More commonly, rocks and building materials are analyzed as polished sections (see section 5.5).

5.4.5 Aggregate within concrete can be extracted and grains within can be analyzed separately (24).

5.5 *Polished specimens*

5.5.1 Polished specimens can be prepared from bulk samples, lithic fragments, or individual grains to study grain morphology, internal texture, crystal zoning, weathering rinds, or inclusions.

5.5.2 Loose grains can be embedded in a mounting medium, typically epoxy, and polished.

5.5.3 EDS spectra collected from polished specimens are more reproducible than EDS spectra from unpolished specimens.

5.6 *Clay-sized fraction*

5.6.1 The clay-sized fraction is typically dispersed onto a substrate from a suspension. Dispersion onto a conductive substrate, or application of a conductive coating, will improve SEM imaging and EDS data quality. Due to particle size limitations the elemental analysis of clay-sized particles is commonly performed using the overscanning method (see 6.2.3.3 and 6.2.3.4).

5.7 *In situ analysis of particles on items of evidence*

5.7.1 When isolation of particles from their substrate risks sample loss or alteration, or when the nature of the contact between sample and substrate is of interest, *in situ* analysis can be conducted (e.g., minerals embedded in fabrics, or projectiles).

5.7.2 In some situations, it is necessary to excise portions of the substrate for placement in the SEM chamber.

5.7.3 Collect background EDS spectra of the questioned substrate to allow for the differentiation of the EDS signal of the adhering geological materials.

6. Instrumental Procedure

6.1 *SEM Imaging*

6.1.1 SEM quality assurance

6.1.1.1 Use laboratory-established protocols for instrument operation and maintenance.

6.1.2 Selection of Imaging Conditions

6.1.2.1 Select the analytical conditions for examination by SEM specific to case and specimen. The analyst can choose to conduct specific analysis types (6.1.4.2-6.1.4.5) to meet the goals of the examination.

6.1.2.2 Ensure electron gun and aperture alignment.

Note 8 - Significant changes in accelerating voltage could require adjustments to the aperture alignment.

6.1.2.3 Select an electron imaging detector suitable for observation of the features of interest.

Note 9 - Secondary electron images (SEI) are collected to capture topographic and surface features. Off-axis backscattered electron (BSE) detectors also provide topographic information.

Note 10 - The grayscale intensity value of BSE images is generally proportional to the specimen average atomic number and can be used for assessment of this compositional character of the specimen.

6.1.2.4 Adjust the image brightness and contrast conditions to best observe the specimen and features of interest.

6.1.2.5 Set the accelerating voltage to best observe the sample characteristics under investigation.

Note 11 - Lower voltages provide more surface sensitivity while higher voltages penetrate deeper into the sample.

6.1.2.6 Optimize the magnification, focus, and stigmatism to observe the feature of interest.

6.1.2.7 If you observe sample charging, use a mitigation method as described in section 5.2.7 and Note 2.

6.1.3 Image Acquisition

6.1.3.1 Collect sufficient images to record the features of interest within the specimen.

6.1.4 Assessing morphological characteristics

6.1.4.1 For morphometric assessment, confirm the instrument scale calibration (E766).

6.1.4.2 *Particle surface texture examination* - Examine and document the surface features of grains; these features are typically compared to reference atlases (5-8) or to known samples.

Note 12 - Individual particles, typically >250 µm in diameter, are selected for surface texture analysis. To improve imaging, these grain mounts are routinely coated with a conductive material (e.g., carbon or gold).

Note 13 - The range of characteristics useful for surface texture analysis is beyond the scope of this document and can be found in references (5) and (6); specific applications of quartz grain surface analysis for forensic purposes can be found in Morgan et al. (7).

6.1.4.3 *Particle shape examination* – Describe the morphology of particles based on: presence and nature of crystal faces, roundness (round to angular); and the relative grain dimensions (e.g., equant, elongated, bladed, platy (9)).

6.1.4.4 *Particle size and sorting* - Determine the particle dimensions and distribution of particle sizes (sorting) by SEM imaging.

Note 14 - Particle analysis can use a range of morphometrics and terminology which can be carried out by automated software.

Note 15 - If during sample preparation the sample was size-fractionated then any measurements or comparisons of particle size and sorting data should account for this preparation.

6.1.4.5 Document the morphology of microscopic particles of biological origin often found in soil (e.g., pollen, foraminifera, diatoms) by SEM imaging, to enable a level of taxonomic identification.

6.2 Elemental Analysis

6.2.1 EDS Instrument performance verification

6.2.1.1 Perform verification on a regular, documented schedule following instrument-specific requirements and after instrumental maintenance. This verification includes determination of energy calibration, energy

resolution, and visual assessment of the Bremsstrahlung background shape (10).

Note 16 - A reference material (often pure metals such as copper or aluminum) can be used to verify peak positions for both low-energy (~1 keV) and high energy (~8 keV) peaks.

6.2.2 *Selection of EDS conditions*

6.2.2.1 Use an accelerating voltage sufficient to generate X-rays of interest. This value is typically 1.5 to 3 times the energy of the X-ray line for an element of interest. An accelerating voltage of 15 - 25 kV is often sufficient for efficient X-ray generation. Lower voltages will reduce the analytical volume, increase light element sensitivity, and potential for specimen damage.

6.2.2.2 When L or M X-ray family lines are provisionally identified, increasing the accelerating voltage can confirm the presence of L or K X-ray family lines of that element.

6.2.2.3 Adjust the beam current, deadtime, and pulse processor time constant to optimize X-ray counts or spectral resolution.

Note 17 - Generally, deadtimes between 10-15 % (for a silicon-drift detector) provide high throughput while minimizing spectral artifacts (e.g., sum and escape peaks).

Note 18 - The pulse processor time constant influences collection speed and spectral resolution. Long time constants achieve greater spectral resolution but result in lower count rates.

6.2.2.4 The optimal working distance for the X-ray analysis is instrument dependent. Correctly adjusted, the working distance achieves a take-off angle that optimizes X-ray collection by the EDS detector.

6.2.2.5 Spectral acquisition times are specimen- and task-dependent. Elements present at low concentrations or light elements (e.g., boron, nitrogen, fluorine) typically require longer acquisition times, higher beam current, lower accelerating voltage, or a combination of these parameters to improve detection.

6.2.3 *Acquisition of elemental composition*

6.2.3.1 EDS spectra can be collected for a variety of purposes, such as: characterization of individual particles, detection of compositional domains within particles, or overscanning large areas to survey the elements present in a specimen.

Note 19 - Deflecting the beam from the center of the field of view can result in reduced X-ray counts. This effect is greater at low magnification (10).

6.2.3.2 Point analysis of a material is achieved by positioning a static beam to a spot of interest. For grains with significant topography, position the static beam on the top of the grain or on a side facing the detector to

prevent the specimen from blocking the emitted photon from reaching the detector (see 6.2.4.1).

6.2.3.3 Bulk analysis of a material is achieved by overscanning a selected region of interest. The degree of homogeneity of the material can be assessed by examining a backscattered electron image to select the size and location of the rastered area. BSE imaging will help to ensure analysis of single compositional domains. When analyzing a material at high accelerating voltages, signal contribution can come from phases/domains at depth.

6.2.3.4 Overscanning (see 3.1.6) large portions of a specimen when there are multiple phases/compositional domains present should not be used for quantitative analysis but can be used for detecting the elements present.

Note 20 - Overscanning is typically used for fine-grained (clay-sized) preparations. EDS data from the clay-sized fraction can be used together with other methods (e.g., X-ray diffraction, FT-IR, or staining) for identification of mineral phases present.

6.2.4 *Quality assessment of collected EDS spectra*

6.2.4.1 During and upon completion of the acquisition of an EDS spectrum, assess the data quality for: the shape, intensity, and continuity of the background; peaks; and spectral artifacts (e.g., sum peaks, escape peaks). Gaps in the background or sudden breaks in the continuum are an indication of a poor-quality spectrum resulting from specimen geometry (see 6.2.3.2). The low energy end of the continuum is particularly sensitive to problems. If the low energy continuum is suppressed, this often suggests occlusion of the X-ray signal. Reject spectra of poor quality and recollect.

6.2.4.2 Document artifact peak(s) or re-collect a spectrum under conditions that reduce or eliminate them. Unassigned or ambiguous peaks should be noted accordingly.

6.2.4.3 Once a quality spectrum is collected, X-ray peaks can be assigned to elements by comparison to published tables of elemental X-ray energies or with the assistance of software.

Note 21 - Modern instrument manufacturer software packages provide auto-identification of elements; if using auto-identification, confirm all peak assignments (11). Confirm the presence of multiple X-ray lines or check for the presence of higher energy X-ray family lines, when applicable. If only a single peak with low counts is assigned to an element, the element identification should be considered provisional. Provisional identifications can be confirmed by a collection of replicate spectra, those with a higher number of counts, or by complementary

techniques (e.g., X-ray fluorescence, or SEM or microprobe equipped with wavelength-dispersive X-ray spectrometers).

Note 22 - Use care when excluding an element whose peaks are occluded by another element. Performing a spectrum fit may be necessary to determine the presence of the element.

Note 23 - Due to the energy resolution limits of EDS detectors (approximately 130 eV), there is the potential for overlap of X-ray peaks from multiple elements. Examples of elements encountered in the analysis of geological materials that have overlapping peaks include Pb-S-Mo (molybdenite-MoS₂, galena-PbS), Ba-Ti (benitoite-BaTiSi₃O₉), and Y-P (xenotime-YPO₄). For additional examples of peak overlap, see Goldstein et al. (1). Peak interferences (like Pb-S-Mo) are best resolved by peak fitting as more than one element may be present.

Note 24 - Element detection using EDS depends on several factors including the matrix in which the element of interest resides, accelerating voltage, beam current, count times, or atomic number. At typical operating conditions (15 kV, ~1 nanoampere of current, and 30 seconds live time), elements (> fluorine) can be detected at ~0.1 weight percent.

6.2.4.4 The use of EDS spectral databases or software for the synthesis of spectra is recommended to confirm peak identification.

Note 25 - Element quantitation is beyond the scope of this document. For guidance on quantitation, see Goldstein et al. (1).

6.2.5 Automated EDS-based particle categorization or identification

6.2.5.1 The automated detection of particles and their elemental compositions can be used to categorize and count particles. The relative abundance of general groups defined by composition can be used in the forensic characterization of geological materials (12, 13).

6.2.5.2 Specialized software for automated provisional mineral identification by SEM/EDS has been shown to be useful in examinations of geological material (14-17).

6.2.5.3 Automated particle categorization or identification provides both quantitative modal abundance information as well as morphological assessment of mineral grains.

6.2.5.4 Procedures for automated mineral analysis are beyond the scope of this document.

7. Interpretation

7.1 Reports derived from the forensic analysis of geological materials typically address identification of material (7.2), restriction of the possible geographic source area (7.5 provenance), and the comparison of two or more materials to determine if they could share a common source (7.4). SEM/EDS analysis of any portion of a sample of geological evidence can be included in a report to aid in these three goals.

7.1.1 SEM/EDS analysis is usually a component of an overall forensic examination of geological evidence and is not typically used on its own. Use of additional orthogonal methods for forensic examinations is highly recommended. For example, SEM/EDS analysis is commonly conducted following color evaluation (E3254) and detailed PLM examination in the scheme of forensic soil comparisons.

7.2 *Material identification*

7.2.1 In forensic applications, mineral identifications based solely on qualitative EDS analysis are typically considered provisional. Some mineral varieties can be confidently identified by their EDS spectra alone (e.g., zircon, $ZrSiO_4$), but for other minerals EDS analysis alone is limited to provisional identification.

7.2.2 Provisional mineral identification based on qualitative EDS analysis is performed by visual inspection for the presence, absence, and relative peak area in a spectrum. The identification of a mineral group or species should be supported with the use of mineralogy reference materials, mineral database spectra, or synthesized EDS spectra (e.g., DTSA-II (3)).

Note 26 - EDS-based mineral identification and quantification are well established methods in widespread use in the Earth Sciences (2, 17-21). Validation of EDS-based mineral identification is instrument and specimen specific. Studies have shown strong agreement between X-ray diffraction (XRD) and EDS based mineral identification for natural (18) and synthetic (19) mineral mixtures.

Note 27 - Take caution in provisional identification of minerals within particular groups that display significant solid-solution (some examples include: members of the feldspar, garnet, pyroxene, amphibole, and tourmaline groups).

Note 28 - Some compounds occur as polymorphs (minerals having the same chemical composition but different crystal structures). The identification of a particular polymorph (e.g., SiO_2 , $CaCO_3$, Al_2SiO_5 , TiO_2 , and $KAlSi_3O_8$) is not possible by EDS alone. Differentiation of polymorphs can be achieved using morphology (see section 7.3), PLM, XRD, or Raman spectroscopy.

7.2.3 Element abundances calculated using standardless quantitation algorithms common in instrument software packages should be considered estimates and might not be accurate.

7.2.4 Mineral identifications can be confirmed with the use of an orthogonal instrumental technique (e.g., PLM, Raman spectroscopy, XRD, quantitative elemental analysis, or by distinctive morphology).

7.3 *Interpreting Morphology*

7.3.1 Morphological features including particle surface textures, grain coatings, grain shapes, particle size and sorting, the presence of crystal faces or cleavage, or the identification of microscopic biologically-derived particles (e.g., phytoliths, diatoms, foraminifera, and pollen) can be used in the interpretation of the geological history of a sample, as a basis of sample comparison (5,6,9), to

confirm a provisional mineral identification (7.2.4), or to aid in provenance interpretations (7.5).

7.4 *Sample comparisons*

7.4.1 The goal of forensic comparisons is to determine whether two samples have exclusionary differences indicating distinct sources. Alternatively, the lack of exclusionary differences supports the proposition that two or more samples could share a common origin.

7.4.2 The processes of transfer and persistence of particle assemblages can cause changes in relative proportions of particle types or sizes from the source. Knowledge of the history of the sample should influence the interpretation of similarities and differences. This can be partially mitigated by comparing similar fractions (size, or grain type).

7.4.3 Comparisons showing minimal differences - Two samples of geological material consisting of similar components (morphotypes, elemental types, minerals, ect.) in similar relative abundances could have been derived from a common source. If analysis by SEM/EDS supports the conclusion that two samples share similar components (morphological characteristics, provisional mineral identifications, and mineral elemental compositions) in similar relative abundances, then no exclusionary differences are detected.

7.4.3.1 EDS spectra of particles in two or more samples can be compared even if the identity of the particles is not known. For particles with similar specimen preparation and data acquisition, spectral overlay can be used for comparisons, where the presence or absence of peaks, peak shapes, and relative intensities are all considered in the evaluation as to whether exclusionary differences exist between compared samples.

7.4.3.2 The identification of less common features, including minerals, morphologies, mineral compositions, or bulk elemental profiles within both compared samples, increases the strength of association provided by the comparison. For example, soil mineral occurrence data can be used to substantiate the rarity of a given mineral, in general, or specifically within an area of interest.

Note 29 - Minerals that are rare, in general, might be common in the relevant areas of interest to the case. The local characteristics can be ascertained by using more expansive known exemplars, consulting published data, or contacting experts with localized knowledge (e.g., regional geological surveys, industry, academic literature, and persons with local expertise).

7.4.4 Comparisons showing differences - If SEM/EDS analysis indicates that there are significant differences with respect to relative abundance, variation, or the omission/addition of particle types (e.g., mineral types, grain morphologies, or chemical variants of the same mineral), or bulk elemental composition of the

fine fraction, the analyst shall evaluate possible explanations for these differences.

7.4.4.1 Comparisons showing explainable differences - Explainable differences can include: contamination or alteration of one of the samples due to the effects of fire, stomach acid, mixing or dissolution of soluble mineral phases (e.g., anhydrite/gypsum); task-relevant case information; transfer and persistence processes; sample size limitations; and the representativeness of the known exemplars with respect to time of collection or location. If there is no scientifically supported or logical explanation for the observed differences, then these would be considered exclusionary differences. Document the justification for the explainable differences.

7.4.4.2 Comparisons showing exclusionary differences - Samples with differences detected by SEM/EDS that are not explainable are considered exclusionary differences and indicate that these geological materials were derived from distinct sources.

7.5 Provenance:

7.5.1 The mineralogy, mineral elemental composition, or morphology determined by SEM/EDS can be used to interpret likely and unlikely sources of the material. The methods of this interpretation are beyond the scope of this document. See Pirrie et al. (14, 16) for a description and methods.

8. Documentation

8.1 Documentation of geological materials examinations by SEM/EDS should include:

8.1.1 Specimen preparation procedures (e.g., sieving, washing to remove grain coatings, density separation, conductive coating, embedding and polishing).

8.1.2 Instrumental conditions used: chamber vacuum conditions, working distance, magnification, beam current/ spot size, and accelerating voltage.

8.1.2.1 For imaging: detector, and a scale bar or field-of-view (FOV) dimension.

8.1.2.2 For EDS data: deadtime, time constant, and analytical collection interval (count time or defined total counts).

8.1.2.3 Relevant controls.

8.1.3 Information supporting the interpretations of mineral or material identification, provisional mineral or material identification, and references or databases used in identification (E620). Within representative spectra, annotate all peaks associated with elements that are being used for mineral identification.

8.2 For comparisons, document rationale for determining differences that are explainable or exclusionary.

8.3 Documentation should allow a second analyst to understand and evaluate all the work performed, and independently interpret the data.

8.4 Refer to E1492, E620, and ISO 17025 for further guidance.

9. Keywords

9.1 Energy dispersive X-ray spectrometry; EDS; scanning electron microscopy; SEM; particle analysis; mineral identification; geological material; elemental composition; soil.

10. References

- (1) Goldstein, J. I., Newbury, D. E., Michael, J. R., Ritchie, N. W. M., Scott, J. H. J., and Joy, D. C., *Scanning Electron Microscopy and X-ray Microanalysis*, 4th Edition, Springer, New York, NY, 2018, p. 550.
- (2) Nesse, W. D., and Baird, G., *Introduction to Mineralogy*, 4th Edition, Oxford University Press, p. 560.
- (3) DTSA II <https://www.cstl.nist.gov/div837/837.02/epq/dtsa2/>.
- (4) Gerlach, R. W., and Nocerino, J. M., "Guidance for Obtaining Representative Laboratory Analytical Subsamples from Particulate Laboratory Samples," EPA/600/R-03/027 200.
- (5) Mahaney, W. C., *Atlas of Sand Grain Surface Textures and Applications*, Oxford University Press, New York, NY, 2002, p. 237.
- (6) Krinsley, D. H., and Doornkamp, J. C., *Atlas of Quartz Sand Surface Textures*, Cambridge University Press., 1973, p. 102.
- (7) Morgan, R. M., Robertson, J., Lennard, C., Hubbard, K., and Bull, P. A., "Quartz grain surface textures of soils and sediments from Canberra, Australia: a forensic reconstruction tool," *Australian Journal of Forensic Sciences*, Vol 42, 2010, pp. 169-179.
- (8) Welton, J. E., *SEM Petrology Atlas*, American Association of Petroleum Geologists Methods in Exploration Series, Vol 4, 1984, p. 237.
- (9) Mange, M. A., and D. T. Wright, eds. *Heavy Minerals in Use*. Developments in Sedimentology Series; Elsevier: Amsterdam, The Netherlands, 2007; Vol 58, 1283p.
- (10) Ritchie, N. W., DeGaetano, D., Edwards, D., Niewoehner, L., Platek, F., and Wyatt, J. M., "Proposed practices for validating the performance of instruments used for automated inorganic gunshot residue analysis," *Forensic Chemistry*, Vol 20, 2020, 100252.
- (11) Newbury, D. E., "Mistakes encountered during automatic peak identification of minor and trace constituents in electron-excited energy dispersive X-ray microanalysis," *Scanning: The Journal of Scanning Microscopies*, Vol 31, No. 3, 2009, pp. 91-101.
- (12) Stoney, D. A., Neumann, C., and Stoney, P. L., "Discrimination and classification among common items of evidence using particle combination profiles," *Forensic Science International*, Vol 289, 2018, pp. 92-107.
<https://doi.org/10.1016/j.forsciint.2018.05.024>.
- (13) Stoney, D. A., Neumann, C., Mooney, K. E., Wyatt, J. M. and Stoney, P. L., "Exploitation of very small particles to enhance the probative value of carpet fibers," *Forensic Science International*, Vol 252, 2015, pp. 52-68.
<https://doi.org/10.1016/j.forsciint.2015.04.003>.
- (14) Pirrie, D., Rollinson, G. K., Power, M. R., and Webb, J., "Automated forensic soil mineral analysis; testing the potential of lithotyping," *Environmental and Criminal*

- Geoforensics* Geological Society, London, Special Publications, Vol 384, 2013, pp. 47-64.
- (15) McVicar, M. J. and Graves, W. J., "The Forensic Comparison of Soils by Automated Scanning Electron Microscopy," *Canadian Society of Forensic Science Journal*, Vol 30, No. 4, 1997, pp. 241-261, DOI: 10.1080/00085030.1997.10757104.
- (16) Pirrie, D., Crean, D. E., Pidduck, A. J., Nicholls, T. M., Awbery, R. P., and Shail, R. K., "Automated mineralogical profiling of soils as an indicator of local bedrock lithology: a tool for predictive forensic geolocation," *Forensic Soil Science and Geology*, Geological Society, London, Special Publications, 492, 2021, 261-280.
<https://doi.org/10.1144/SP492-2019-42>.
- (17) Schulz, B., Sandmann, D., and Gilbricht, S., "SEM-based automated mineralogy and its application in geo- and material sciences," *Minerals*, Vol 10, 1004, 2020, p. 24.
doi:10.3390/min10111004.
- (18) Han, S., Löhr, S. C., Abbott, A. N., Baldermann, A., Farkaš, J., McMahon, W., Milliken, K. L., Rafiei, M., Wheeler, C. and Owen, M., "Earth system science applications of next-generation SEM-EDS automated mineral mapping," *Frontiers in Earth Science*, Vol 10, 2022, doi: 10.3389/feart.2022.956912.
- (19) Dunkl I., von Eynatten H., Andò S., Lünsdorf K., Morton A., Alexander B., Aradi L., Augustsson C., Bahlburg H., Barbarano M., and Benedictus A., "Comparability of heavy mineral data—The first interlaboratory round robin test," *Earth-Science Reviews*, 2020, Vol 211, p. 27. <https://doi.org/10.1016/j.earscirev.2020.103210>.
- (20) Blannin R., Frenzel M., Tuşa L., Birtel S., Ivăşcanu P., Baker., and Gutzmer J., "Uncertainties in quantitative mineralogical studies using scanning electron microscope-based image analysis," *Minerals Engineering*, Vol 167, 2021, <https://doi.org/10.1016/j.mineng.2021.106836>.
- (21) Reed, S. J. B., *Electron Microprobe Analysis and Scanning Electron Microscopy in Geology*, 2nd Edition. Cambridge University Press, 2005, p. 206.
- (22) Ruffell, A., Pirrie, D., and Dawson, L., "Geological Evidence Analysis," Chapter 6 of A Guide to Forensic Geology, Donnelly, L., Pirrie, D., Harrison, M. A., Ruffell, A., and Dawson, L. eds., Geological Society, London, 2021, pp. 129-155, DOI.org/10.1144/GFG.6\.
- (23) Murray, R.C Evidence for the Earth second edition. Mountain Press, 2011 p. 200.
- (24) Ruffell A, and McKinley JM. "Concrete evidence: Analysis of aggregate and cement in a homicide investigation." *Forensic Science International: Reports*, Vol 9, 2024 100360. <https://doi.org/10.1016/j.fsir.2024.100360>.