Standard Guide for
Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry[1]

1. Scope

1.1 This guide covers the analysis of gunshot residue (GSR) by scanning electron microscopy/energy-dispersive X-ray spectrometry (SEM/EDS) by manual and automated methods. The analysis may be performed manually, with the operator manipulating the microscope controls and the EDS system software, or in an automated fashion, where some amount of the analysis is controlled by pre-set software functions.

1.2 Since software and hardware formats vary among commercial systems, guidelines will be offered in the most general terms possible. For proper terminology and operation, consult the SEM/EDS system manuals for each system.

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

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

2. Summary of Practice

2.1 From the total population of particles collected, those that are detected by SEM to be within the limits of certain parameters (for example, atomic number, size, or shape) are analyzed by EDS (**1-3**).[2] Typically, particles composed of high mean atomic number elements are detected by their SEM backscattered electron signals and an EDS spectrum is obtained from each. The EDS spectrum is evaluated for constitu-

ent elements that may identify the particle as being consistent with or characteristic of GSR, or both.

3. Significance and Use

3.1 This document will be of use to forensic laboratory personnel who are involved in the analysis of GSR samples by SEM/EDS (**4**).

3.2 SEM/EDS analysis of GSR is a non-destructive method that provides (**5, 6**) both morphological information and the elemental profiles of individual particles.

3.3 Particle analysis contrasts with bulk sample methods, such as atomic absorption spectrophotometry (AAS) (**7**), neutron activation analysis (NAA) (**8**), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS), where the sampled material is dissolved or extracted prior to the determination of total element concentrations, thereby sacrificing morphological information and individual particle identification.

3.4 X-ray fluorescence spectrometry (XRF) is a technique that has been used to map the placement and distribution of GSR particles surrounding bullet holes in order to establish shooting distances (**9**). Unlike the solution-based bulk methods of analysis, XRF is non-destructive; however, XRF still does not provide morphological information and is incapable of individual GSR particle identification.

4. Sample Preparation

4.1 Once the evidence seal is broken, care should be taken so that no object touches the surface of the adhesive SEM/EDS sample collection stub and that the stub is not left uncovered any longer than is reasonable for transfer, mounting, or labeling.

4.2 Label the sample collection stub in such a manner that it is distinguishable from other sample collection stubs without compromising the sample; for example, label the bottom or side of the stub.

4.3 If a non-conductive adhesive was used in the sample collection stub, the sample will need to be coated to increase its electrical conductivity, unless an environmental SEM or variable-pressure/low-vacuum SEM is used for the analysis. Carbon is a common choice of coating material, since it will not interfere with X-ray lines of interest. For high-vacuum SEM, coat the sample sufficiently to eliminate charging of the sample.

5. Sample Area

5.1 Sample collection stubs for SEMs typically come in one of two diameters: 12.7 mm or 25.4 mm, which yield surface areas of 126.7 mm² and 506.7 mm² respectively.

5.2 Manual analysis of the total surface area of the stub is prohibitively time-consuming. Because the particles are collected onto an adhesive surface in a random manner and the particles do not tend to cluster, it is reasonable to analyze a portion of the stub surface by employing an appropriate sampling and analytical protocol (6, 10).

5.3 Automated SEM/EDS analysis can enable data collection from nearly the entire surface area of the sample collection stub. Due to the disparity between the shape of the sample collection stub (round) and the SEM field of view search area (square or rectangular), analysis of 100 % of the sample collection area may not be possible in some systems.

5.3.1 Analysis of the maximum allowable surface area of the sample is recommended, however, many automated systems can be programmed to terminate the analysis of a stub or series of stubs once a pre-established number of particles with specified classification(s) have been detected. The decision as to how many particles satisfy the requirements of a particular case is a matter for the analyst to decide but should be subject to guidelines set out in the laboratory's standard operating procedures.

6. Instrument Requirements and Operation

6.1 *General*:

6.1.1 Most commercial-grade SEM/EDS systems should be adequate for GSR analysis.

6.1.2 Automated data collection of GSR involves some portion of the data collection being controlled by pre-set software functions. The extent to which the SEM and EDS systems communicate and are integrated varies according to the manufacturers involved and the capabilities of the hardware/software architecture.

6.2 *Scanning Electron Microscope (SEM)*:

6.2.1 The SEM, operating in the backscattered electron imaging mode, must be capable of detecting particles down to at least 0.5 μm in diameter.

6.2.2 The SEM must be capable of an accelerating voltage of at least 20 kV.

6.2.3 Automated SEM/EDS systems include: communication and control between the SEM and EDS system, and a motorized stage with automated stage control. The system should have the ability to recall stage locations of particles for verification and software for particle recognition.

6.3 *Energy Dispersive Spectrometry (EDS)*:

6.3.1 The detector's resolution should be better (less) than 150 eV, measured as the full width at half the maximum height of the Mn Ka peak.

6.3.2 At a minimum, the EDS spectrum should be acquired at 20 eV per channel.

6.3.3 Display of the EDS output must encompass the X-ray lines of analytical utility, with a minimum range of 0–15 keV.

6.3.4 Automated systems will also include software capable of acquiring X-ray spectra for a specified collection time or total X-ray counts.

6.3.5 It is desirable that the spectrum obtained from the analysis of each particle of interest be stored. At a minimum, an automated system must be capable of storing all of the particle location coordinates.

6.4 *Sample Placement*:

6.4.1 Record the positions of the stubs (sample and standard/reference stubs) on the SEM stage when the samples are inserted.

6.4.2 If it is anticipated or required that additional analyses will be needed, it is desirable that the stub can be returned to the same orientation as before its removal. This may consist of marking the side of each stub and aligning it with marks on the microscope stage or by having stubs that fit into the stage in only one position (for example, stubs with a pin that is a half-circle in cross section).

6.5 *Detection and Calibration*:

6.5.1 Particles of GSR are detected by their backscattered electron signal intensity. The absolute signal intensity that a particle produces is related to the electron beam current, mean atomic number, and size of the particle (for particle sizes on the order of the beam diameter). Particles whose mean atomic numbers are high will appear brighter than those of lower atomic number composition. As the beam current increases, the amount of signal each particle produces also increases (11).

6.5.2 The brightness and contrast settings (low and high thresholds) of the backscattered electron detector system determine the limits of detection and discrimination of particles whose mean atomic number exceed the minimum threshold setting but fall below the maximum threshold setting. Threshold settings for the backscattered electron signal should be done with a suitable reference sample of known origin (often supplied by the EDS manufacturer) or pure element standards at the same parameters that will be used for the sample analysis. This calibration sample should, if possible, be in the microscope chamber at the same time as the samples to be analyzed.

6.5.3 The backscattered electron detector's brightness and contrast should be set to include the high atomic number particles of interest and exclude low atomic number particles that are not of interest. Typically, high contrast and low brightness settings provide an adequate range between threshold limits for ease of detection. If the beam current is changed or drifts, the brightness and contrast threshold limits, which were based on the previous beam current, may no longer be compatible with the new conditions and should be readjusted. The beam current may be measured with a Faraday cup, a