

Proposed Practices for Validating the Performance of Instruments Used for Automated Inorganic Gunshot Residue Analysis

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Abstract

The reliability of forensic inorganic gunshot residue (iGSR) analysis depends strongly on the performance of the scanning electron microscope with energy dispersive X-ray spectrometer (SEM/EDS) instrument and the software that automates the data acquisition. Without a record of the baseline performance of the instrument, it is often hard to know when performance has deteriorated. This paper provides a set of tests which can be performed when the instrument is commissioned, or when significant modifications are made, to establish the baseline performance and a smaller set of tests that can be performed regularly to document the current performance of the instrument. Together, these represent a powerful way to establish, document and evaluate the performance of the instrumentation used for iGSR analysis. Furthermore, most of these tests are suitable for characterizing SEM/EDS instruments used for other forensic or non-forensic uses.

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1 Introduction

Inorganic gunshot residue (a.k.a. firearm discharge residue) (iGSR) analysis is a forensic microscopy technique for discovering and characterizing the distinctive particles produced by the discharge of a firearm that often come to rest on proximate surfaces. Typically, the surface is the skin, hair, clothing or effects of an individual suspected to be near to a discharge. The technique involves sampling the surface and then examining the sample in a computer-automated scanning electron microscope with energy dispersive X-ray spectrometer (SEM/EDS). While iGSR-related particles can be discovered by manual investigation of the sample, typically computer automated methods are used to discern the often-sparse microscopic particles[1, 2].

An SEM/EDS is a complex piece of instrumentation with many components which must work in harmony to produce reliable results. There are multiple different ways and levels on which to verify the performance of the system. On one extreme, there are *comprehensive procedures* which evaluate the performance of the entire instrument and methods in operational scenarios. These are excellent for demonstrating that the system, in its entirety, is working correctly. However, the outputs of these methods are often not specific enough to be useful for diagnosing the root-cause of sub-optimal performance. On another level, there are *narrow-focused procedures* which characterize the performance of individual sub-systems. These tests are more useful for diagnosing the specific hardware problems that lead to sub-optimal performance. In between these two extremes, there are tests that evaluate the interaction between the sub-systems and can identify a whole different class of problems.

Comprehensive and narrow-focused test methods both have their place in a testing regime. Comprehensive test can be run frequently to efficiently demonstrate that the system overall is performing acceptably. The narrow-focused procedures are helpful for diagnosing a failure.

While no set of tests can fully characterize and diagnose all potential sources of problems, we have attempted to identify a realistic sub-set that can be implemented to characterize an instrument. It is necessary to establish baseline performance characteristics of the hardware sub-systems. So, as so often happens, there is a question about the current performance of the instrument, there will be a baseline against which the current performance can be compared. While performing all these tests may initially be time consuming, it is likely that they will save time and effort in the long run when it becomes necessary to diagnose a problem or to demonstrate that an instrument's performance has deteriorated.

These tests have been evaluated in the author's labs and represent many years of experience as both users and, in two cases, vendors or ex-vendors of scanning electron microscope (SEM) and energy-dispersive X-ray spectrometer (EDS) equipment including dedicated iGSR instruments.

Automated iGSR analysis is fundamentally dependent upon instrumentation to discover and classify the particles "characteristic of" or "consistent with" primer residue from the discharge of a firearm[3]. Samples are collected from individuals or surfaces using a sticky carbon tape on an SEM compatible stub to lift the particles. The instrumentation is involved in two phases – the *discovery phase* and the *confirmation phase*[3].

Discovery (detection & identification) During the discovery phase, candidate gunshot residue particles are identified and classified. The discovery phase must 1) discern a high fraction of the GSR-related particles present on a sample; 2) effectively discriminate the majority of non-iGSR-related particles while not overlooking true iGSR-related particles; and 3) efficiently search large sample areas.

Confirmation During the confirmation phase, candidate particles identified in the discovery phase are re-examined using the SEM/EDS in a manually controlled mode. New images and spectra

are acquired and examined to determine whether the particle has elemental constituents and morphology that suggest that it is a primer particle from the discharge of a firearm.

The discovery phase is important for ensuring that iGSR is efficiently discerned when present. The confirmation phase is critical for ensuring that only particles that are truly associated with iGSR are reported as such. The instrumentation performance is critical in both phases. The SEM/EDS systems currently performing iGSR analysis have a broad range of performance characteristics. The point of this document is not to exclude less high performance, but none-the-less useful, instruments from use. Rather, it is to document the instrument's best performance so that it can be maintained at that level. Any instrument performing iGSR analysis must meet the minimum performance specifications in the ASTM E1588-17 standard[3].

1.1 Validation and Quality Control

iGSR analysis is performed with a SEM/EDS. A modern SEM/EDS is a complex instrument with many different sub-systems each of which must be performing correctly to produce reliable results. *Validation* and *Quality Control* protocols are the mechanism by which an instrument and the way in which it used is evaluated to determine whether it is suitable for purpose. The difference between these two types of protocols are the specificity and the frequency of the tests.

Validation Validation is a process of demonstrating that the instrument and methods meet the required performance capabilities. Validation can be sub-divided into two types:

Instrument validation Measurement-based tests which are performed infrequently (see below) to document that various different components or sub-systems of the instrument are operating with the performance required for the intended use.

Method validation Method validation is defined by ASTM E2857-11[4] as

“Confirmation, by the provision of objective evidence and examination, that a method meets performance requirements and is suitable for its intended use.”

Instrument validation tests are performed “infrequently” such as when the instrument is brought into service, when a sub-system is replaced or when a problem is suspected. They document that various components or sub-systems of the instrument are operating at close to optimal performance for iGSR analysis.

Quality Control Quality control is defined in ASTM E882-10(2016)^{e1} item 6.1[5] as

“Quality control of analytical methods provides the information needed to ensure that procedures, equipment, and personnel are performing at the levels of precision and accuracy required by the intended use of the data.”

Quality control is performed on a regular basis to provide confidence that the instrument has been performing adequately since the last quality control check. Often a sub-set of the quality control tests are performed each day that the instrument is used and the remainder on a periodic (weekly/monthly) basis. The periodicity of the quality control checks is determined by documented laboratory procedures and can be thought of as the period over which the laboratory would be willing to discard previously collected data if the test were to fail. Quality control checks are as much about the quality of data from the recent past as about future data.

Validation and quality control are both critical aspects of a laboratories standard operating procedures. Most critically, this information can be used to demonstrate, when challenged, that during the time-period in which data was collected that the instrument was performing adequately. By bracketing a time-period with quality control tests, it is possible to provide strong evidence that during this time-period the instrument was performing correctly. The results from the tests allow the laboratory to both define how they expect their instrument to perform and then to demonstrate that in any given period time that it was performing at that level. With a well implemented validation and quality control program there should never be a question of whether the system is performing correctly. The validation tests exist to define correct performance and the quality control tests serve to flag when something is potentially wrong with the instrument. The goal is to waste as little time as possible collecting poor quality data and to provide high confidence that if poor quality data is collected due to instrumentation failure, the failure will be caught as soon as possible.

The tests in this document are intended for use by all laboratories that produce forensic iGSR data for use in a judicial environment. Most of these tests are suitable for use in other laboratories involved in SEM/EDS analysis.

This document addresses the performance of the instrumentation but does not address sample collection, sample preparation, data acquisition, data analysis or reporting.

The tests outlined in this document should be performed by the personnel primarily responsible for analyzing samples and verifying the performance of the instrument.

1.2 Document Overview and Use

This document consists of three parts. The first part (see Section 4) is a series of tests that isolate specific performance characteristics of the instrument and document their performance. The second part (see Section 5) is two checks that can be performed on a daily basis to quickly measure and document important instrument performance characteristics. The third part (see Section 6) is high-level tests to demonstrate that all the components work together harmoniously.

Most of the tests discussed in this document involve quantitative metrics of instrument performance. We have avoided placing firm limits on acceptable instrument performance because there is a broad range of instruments performing iGSR analyses with differing capabilities. While there are no doubt performance characteristics that would preclude the use of an instrument for iGSR analysis, it is the responsibility of the laboratory to document the instrument's performance and provide evidence that the performance is adequate for the intended use. There are minimum instrument requirements in ASTM E1588-17 [3]. At a minimum, all instrumentation used for iGSR analysis must meet these requirements.

Whenever possible, validation test data should be acquired using the iGSR automation software rather than through the SEM vendor's control software (when they differ). Often the iGSR automation software uses different scan control and data digitization hardware than the SEM vendor software. This is particularly true for the acquisition of image data but is likely also true for X-ray and stage data.

For clarity, a glossary containing definitions for many of the SEM/EDS specific terms and abbreviations used in this document is provided in Supplemental Material B. If additional background material is required a standard SEM/EDS textbook like Goldstein *et al* should be consulted[6].

2 Apparatus

The basic instrumentation for automated iGSR analysis include:

Scanning Electron Microscope (SEM) A microscope which produces images by rastering an energetic electron beam over the surface of a sample. The scanning electron microscope must have performance meeting the requirements in ASTM E1588-17 [3]. (The full system includes the hardware and software necessary to control the microscope and accessory detectors.)

Energy Dispersive X-ray Spectrometer (EDS) An accessory to the SEM for measuring an EDS spectrum generated by the energetic electron beam interacting with the sample. The performance of the energy-dispersive X-ray spectrometer must meet the performance requirements in ASTM E1588-17. EDS detectors typically consist of an integrated hardware and software system which may also take partial control of the SEM. Modern EDS are based on silicon drift detector (SDD) technology while many older ones are based on lithium-drifted silicon detector (Si(Li)) technology. Both types can provide adequate performance for iGSR.

Backscattered Electron Detector An imaging detector integrated into the SEM that is sensitive to energetic backscattered electrons.

Automation Software Software for automating the discovery and analysis of candidate iGSR particles. Automation software may be integrated into the SEM or EDS software, or may be a separate software package that takes control of the SEM and EDS.

In addition to the basic instrumentation, other samples and pieces of equipment are required to perform the tests in this document.

- A picoammeter and Faraday cup are a critical for evaluating the performance of both the SEM and EDS. It is not possible to adequately document instrument performance without knowledge of the probe current.
- A sample with flat, polished copper
- A sample with flat, polished aluminum
- A sample with flat, polished manganese
- A sample with flat, polished nickel
- A particle sample with many high atomic number particles of a few μm in diameter.
- A Planotec (PLANO GmbH, Germany¹) or equivalent GSR test sample
- A flat, polished sample with a sharp C/Cu interface or a Planotec.
- A certified reference material for calibrating SEM magnification meeting the requirements in ASTM E766 – 14^{e1}.

None of these tests involve hazards that are substantively different from those typical of routine operation of an SEM/EDS.

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3 Nominal Analysis Conditions

Unless otherwise specified, all tests should be performed at the instrumental settings used to perform iGSR. It may be necessary to first perform a couple of the test procedures as noted below to establish suitable values for these settings.

The *nominal analysis conditions* are:

Beam Energy The optimal beam energy for iGSR is determined by a desire to be able to use the energetic lead L-lines to distinguish lead from sulfur and molybdenum. 25 keV is common but values in the range 20 keV to 30 keV are acceptable. Each iGSR laboratory should select a single beam energy and use this setting for all analyses.

Probe Current A carefully selected, consistent probe current suited to both imaging and the acquisition of X-ray signals. (See Subsection 4.7.)

Working Distance A consistent working distance optimized for automated search and X-ray acquisition. (See Subsection 4.5.)

Image Settings Select the backscattered electron detector (BSED) and set the brightness and contrasts settings suitable for both bulk carbon and bulk copper (or comparable materials). Carbon should image close to black (≈ 16 out of 256 in an 8-bit image) and copper should image close to white (≈ 240 out of 256 in an 8-bit image). Be sure that if your backscattered electron detector is retractable that it is inserted to the measurement position. Also check that if the hole through the center of the backscattered electron detector is visible at low magnifications that it is roughly centered ($\pm 5\%$) on the image area.

Scan Speed The scan speed influences the signal-to-noise ratio in images collected on an SEM. With faster scan speeds, images can be collected more quickly but the pixel-to-pixel noise can hinder particle search and measure algorithms. Typically, a compromise scan speed is selected that balances speed and image processing. Some systems change scan speed dynamically to optimize the search and measurement procedures. (See Subsection 4.11.)

EDS Detector The energy-dispersive X-ray spectrometer should be configured with the pulse process time, offset (eV), gain (eV/channels), and channel count that is used to collect spectra for automated analysis. A moderate pulse process time is usually optimal. The offset, gain and channel count should be configured to span at least the range from 0 keV to E_0 where E_0 is the optimal beam energy. (See Subsection 4.7 and 4.8) A good choice is 10 eV/channel and 4096 channels as this setting captures the full range of energies from 0 keV to 25 keV whereas 5 eV/channel and 4096 channels does not. Functionally, the difference in quantitative performance is negligible between 5 eV/channel and 10 eV/channel. It is more important to always have access to the Duane-Hunt limit (see Figure 1.)

When a new instrument is commissioned, you will need to determine the nominal analysis conditions for your instrument and your analysis goals. Selecting these settings in the following order is efficient because it accounts for the interplay between the choices.

1. Select the optimal Beam energy. Select a beam energy with adequate overvoltage to excite the lead L-lines.
2. Select the optimal EDS throughput settings to balance throughput and resolution.

<i>Procedure</i>	<i>Section</i>	<i>Imaging</i>	<i>X-ray</i>	<i>Automation</i>
Beam Energy	4.1	✓	✓	✓
Probe Current	4.2	✓	✓	✓
Energy Scale Correction	4.3		✓	✓
Elevation angle	4.4		✓	✓
Optimal working distance	4.5		✓	✓
Detector alignment	4.6		✓	✓
Coincident Event	4.7		✓	✓
Detector Resolution	4.8		✓	✓
Count Rate Linearity	4.9		✓	✓
Instrumental Peaks	4.10		✓	✓
Response Time	4.11	✓		✓
Z-Contrast	4.12	✓		✓
Dimensional Calibration	4.13	✓		✓
Image Rotation Compensation	4.14	✓		✓
Stage-Field Registration	4.15			✓
Image Orthogonality	4.16	✓		✓
Stage Orientation	4.17	✓		✓
Stage Position - Short term	4.18			✓
Stage Position - Long term	4.19			✓

Table 1: A summary of the validation methods. Many of these tests are useful for instrument validation in forensic and other applications of SEMs and EDS. The columns *Imaging*, *X-ray* and *Automation* identify which tests are also relevant for imaging-only applications, EDS applications and automated particle analysis applications.

3. Select the optimal working distance - Using the protocol in section 4.5 to determine the working distance that optimizes the EDS count rate.
4. Select the optimal probe current - Optimize the probe current by considering the pulse pile-up (the coincidence artifact). Select a probe current that produces an acceptable sized coincidence artifact. See section 4.7 for more details. Also check that the selected probe current is capable of sufficient image resolution for the range or particle sizes that you expect to measure.
5. Backscattered electron detector settings - Using a sample containing two materials like carbon and copper, determine the optimal brightness and contrast settings for the backscattered electron detector.
6. Select the optimal scan speed - Select the fastest scan speed that produces sufficiently high signal-to-noise in the backscattered electron signal to allow reliable thresholding of particles of interest. See section 4.12.

There is a natural interplay between many of the parameters and there is no single universal set of optimal parameters for all instruments and case-loads. However, it is usually preferable to establish for each instrument a set of nominal analysis conditions use these conditions for all routine analyses. The nominal analysis conditions should be documented as part of the standard operating procedure (SOP) and operators should be trained in these choices.

4 Low-level Instrument Performance Metrics

The procedures in the following sections have been designed to isolate and evaluate various performance characteristics necessary for optimal automated iGSR analysis. Whenever possible, the data must be acquired through the iGSR automation software rather than through the SEM vendor's software. Often the iGSR automation software uses different scan generation and signal digitization (BSED and EDS) hardware than the SEM software. It is critical to evaluate the instrumentation and software that is used during the automated analysis which is not necessarily the same instrumentation and software used during routine imaging. Often the iGSR automation software is provided by the same vendor as the EDS software and is controlled by the same scan generation and signal digitization hardware. Often, it is possible to collect individual images using either the EDS vendor's software or the SEM vendor's software. When possible default to using the iGSR automation software. This ensures that the correct hardware and software is being tested. When this software isn't capable, use the same vendor's EDS software and only when neither of these pieces of software are capable, use the SEM vendor's software.

4.1 SEM: Beam Energy

4.1.1 Motivation

The image-forming and X-ray-producing electrons in an SEM are formed into a beam by an accelerating potential at a user specified voltage between the electron gun and the sample (specified in kilovolts (kV)) or equivalently at a user specified kinetic energy of the beam electrons as they strike the sample (specified in kiloelectron volts (keV)). The accelerating potential, the charge of an electron, and the bias of the sample relative to ground determine the beam energy. Usually, the sample is held at ground potential and the beam energy is equal to the $E_0 = V_0 q_e$ where V_0 is the electron gun potential relative to ground and $q_e = 1 e^-$ is the charge on a single electron. However, some instruments allow the sample to be voltage biased or, more frequently, the sample may accumulate charge (sample charging) and the resulting accelerating potential is related to the beam energy by $E_0 = (V_0 - V_S) q_e$ where V_S is the voltage on the sample surface relative to ground. The correct and stable beam energy is critical for ensuring imaging performance and reproducible X-ray production.

4.1.2 Method

The Duane-Hunt limit is a mechanism for measuring the beam energy from an EDS spectrum[7]. The method will involve collecting an EDS spectrum from bulk copper and examining the channel data to determine the incident beam energy.

1. Mount a copper sample in the SEM.
 - (a) Ensure that the sample is mounted at a working distance suited to collect an EDS spectrum.
 - (b) Ensure that the sample is mounted normal to the electron beam.
2. Use the SEM controls to select the nominal beam energy used to perform iGSR analysis.
3. Using either point-mode or area-raster of an area of less than $25 \mu\text{m}^2$, collect a EDS spectrum containing at least 100 000 total X-ray counts (1 000 000 is preferable).

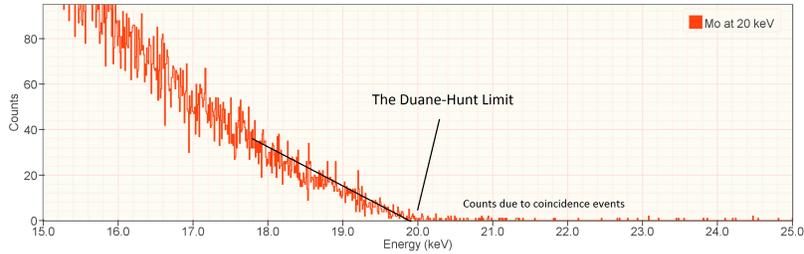


Figure 1: The Duane-Hunt limit is the energy at which the continuum (Bremsstrahlung) X-ray flux goes to zero intensity. It is equal to the incident energy of the electron beam producing the X-rays. Counts due to pulse pile-up may occur above the Duane-Hunt limit. The Duane-Hunt limit can be distinguished from the pulse pile-up signal by a flattening of the slope above the x-intercept representing the true Duane-Hunt limit.

4. Examine the spectrum in the range of channels a few keV above and below the nominal beam energy. Determine the Duane-Hunt limit.
 - (a) The Duane-Hunt limit is the energy at which the continuum X-ray signal decreases to be effectively zero as shown in Figure 1. Since the generated signal inevitably approaches zero at the Duane-Hunt limit, precise determination requires high count spectra.
 - (b) Be careful not to mistake pulse pile-up for true continuum X-ray signal. Pulse pile-up is spurious counts in an EDS spectrum produced when multiple X-rays enter the detector electronics faster than the electronics can separate them. Pulse-pileup can occur above the Duane-Hunt limit but usually can be distinguished by the slope of a line fit to a range of adjacent spectrum channels. Figures 1 and 2 clearly show pulse pile-up counts above 25 keV.
 - (c) As Figure 1 shows, the distribution of continuum intensity approaches the Duane-Hunt limit with a slight concave down curvature. If the sample is charging while the spectrum is being collected, the concave down curvature will be enhanced. If you suspect that the sample is charging while the spectrum is being collected look for evidence of changing brightness in the secondary electron signal. The secondary electrons represent very low energy electrons for which the production is very sensitive to local surface charging.
5. Compare the measured Duane-Hunt limit to the nominal beam energy. On most instruments, the Duane-Hunt limit should fall within 1% of the nominal beam energy.

4.1.3 Diagnosis

The measured Duane-Hunt limit may differ from the nominal beam energy because:

1. The electrons may not be striking the sample at the nominal beam energy.
 - (a) The sample might be charging (sample charging). See Figure 2.
 - (b) The path from the copper sample to the stage may not be conductive.
 - (c) The path from the stage to ground may not be conductive. Consider a broken or disconnected lead from the stage to the chamber, a missing picoammeter, or a missing BNC (or equivalent wiring interconnect) terminator.
 - (d) The accelerating voltage may not be as configured due to hardware failure.

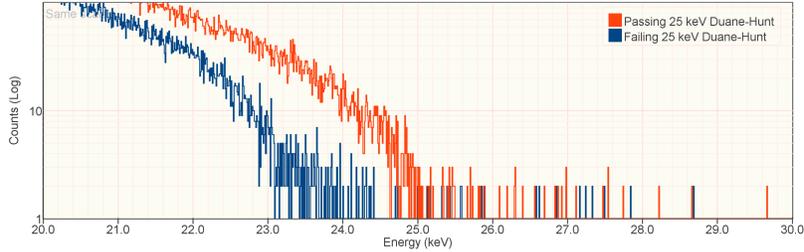


Figure 2: An example of spectra showing the Duane-Hunt limit for a nominal beam energy of 25 keV on a logarithmic vertical scale. The red spectrum shows the desired Duane-Hunt limit and behavior with the signal dropping off precipitously at 25 keV. The blue spectrum shows a Duane-Hunt limit from a charging sample in which the position of the limit is made ambiguous by a varying surface charge but clearly much below 25 keV.

- (e) The beam energy on some tungsten-filament SEMs is always a few hundred volts less than the set voltage due to a bias on the Wehnelt, a component of the electron gun assembly. A Duane-Hunt limit that is consistently low by a few hundred volts is not a problem so long as the over-voltage, U , on the lead L-lines is sufficient that they remain visible ($U > 1.5 \approx 20 \text{ keV}/13.0 \text{ keV}$).
- 2. The EDS spectrum display may not be calibrated correctly (see Section 4.3.) Ensure that the copper K and L-family peaks are located at the correct energies. It may also be worthwhile to check that higher energy K-lines like the Mo K or the Pd K are also in the correct positions as some detectors have subtle (but mostly benign) non-linearity in the energy scale.

4.2 SEM: Probe Current

4.2.1 Motivation

The probe current is a measure of the flux of electrons striking the sample measured in microamps (μA), nanoamps (nA) or picoamps (pA.) 1 nA is $6.25 \times 10^9 \text{ e}^-/\text{s}$ or $6250 \text{ e}^-/\mu\text{s}$ in the image-forming electron beam. It is critical that the probe current is suitable for the task and remains sufficiently stable over the full duration of the data acquisition. Stability is important for two reasons: 1) The backscattered electron signal is linearly proportional to the probe current; 2) The X-ray signal is linearly proportional to the probe current. Automated analysis is usually more sensitive to the first of these as small drifts in current can dramatically change which particles meet the fixed BSED intensity thresholds and are therefore discovered. A change in probe current can cause the system to miss iGSR particles or to needlessly analyze particles of lower mean atomic number.

4.2.2 Method

The probe current is measured with a Faraday cup and a picoammeter. See Figure 3 for an example measurement.

1. Mount the Faraday cup on the sample stage.
2. Perform the vendor-specified electron gun alignment procedure.
3. Connect the picoammeter to the ground-path connector, a wire that serves to conduct electrons that accumulate on the stage to instrument ground.

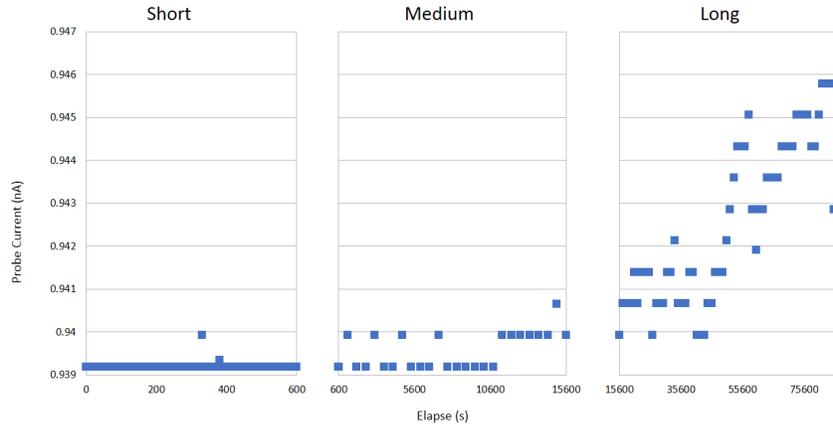


Figure 3: An example of a test of probe current stability on a modern Schottky field emission SEM. This represents an instrument with excellent short and long term stability. Tungsten filament, Lanthanum Hexaboride (LaB_6) and cold field emission microscopes are likely to show somewhat worse stability.

4. With the electron beam off (or blank the beam), measure the quiescent signal. The quiescent signal should be essentially zero. Record this number and subtract it from the beam-on measured values.
5. Turn on the electron beam, configure the probe current as described in the nominal analysis conditions and give the SEM (primarily optics and electron gun) time to stabilize (instrument dependent).
6. Do not modify the instrument settings while collecting probe current data.
7. Measure the probe current once every 10 s for 5 min (short-term).
8. Measure the probe current once every 10 min for 4 h (medium-term).
9. Measure the probe current once every 1 h for 24 h (long-term). If measuring every hour for 24 h is not feasible extend the measurement into the next work day for a total of 24 h intervals.
10. Plot the data. Calculate the minimum, maximum, average and RMS values for each data set.
11. Determine whether the measured short-term and long-term stability are adequate for the intended purpose.
 - (a) Often the probe current will drift progressively up or down over longer time periods. Either direction of drift introduces its own problems. Too much current can cause too many pixels to register as candidate particles (false positives). Too little current can cause too few pixels to register as candidate particles (false negatives).
 - (b) Each laboratory should set its own criteria for short-term and long-term stability but should record these criteria in their quality documentation.

4.3 EDS: Energy Scale Correction

4.3.1 Motivation

It is critically important that the X-ray detector and electronics assign characteristic X-rays to the correct energy bin in the EDS spectrum histogram. If the energy scale is incorrect, characteristic X-rays will be assigned to the wrong elements and blunders will occur. Often copper is used to calibrate the energy scale. The energy of the copper $K\alpha_1$ (IUPAC: K-L₃) transition has been tied to the SI through the work of Deslattes and Henins[8] and copper is readily available. While traceability to the SI is less direct, other elements or combinations of elements can also be used including Mn, Al with Cu or Mo. The iGSR automation software vendor may have a preference, in which case follow the vendor's suggestion.

Historically, the scale correction was performed using two potentiometers which controlled the gain and offset of the signal from the X-ray sensor going into the electronics that measured and stored the resulting voltage signal. By adjusting these two potentiometers it was possible to adjust the separation between the low and high energy peaks and to align the peaks at the correct energy windows.

Modern EDS systems have eliminated the physical potentiometers and replaced the offset potentiometer with an automatic mechanism for stabilizing the "zero offset". Also they have largely replaced the need to manually correct the gain with software algorithms that will automatically correct the gain when provided with spectra from an appropriate sample. On some older instruments, it may still be necessary to physically adjust potentiometers.

4.3.2 Method

1. Before checking the energy scale, ensure that the detector is configured according to the nominal analysis conditions. The detector should always be configured to a consistent number of eV/channel and a consistent resolution/throughput/time-constant setting.
2. Each vendor provides instructions and a method for ensuring that their detector's energy scale can be maintained. Follow the vendor's instructions.
3. Since verifying the energy scale is quick-and-easy and the consequences of an error are significant both in terms of spurious results and wasted effort, the energy scale should be verified frequently. Many operators perform this check each time they sit down at the instrument before they set up a new analysis.
4. Each time the scale is checked, you should archive an EDS spectrum (Cu or other consistent material) that can be used to demonstrate the performance of the EDS detector on the day in question. The National Institute of Standards and Technology (NIST) DTSA-II² (a pseudo-acronym) software package can be used to create quality control charts to track the performance of the detector from the archived spectra. Details are available at the download site and from this document (https://www.cst1.nist.gov/div837/837.02/epq/dtsa2/DTSA2_QC.pdf).
5. The QC spectrum should also be manually examined for indications of spurious signals, deterioration of the detector or other abnormalities. If you choose to use it, the NIST DTSA-II

²DTSA-II is available for free from <https://goo.gl/MI1Ku>

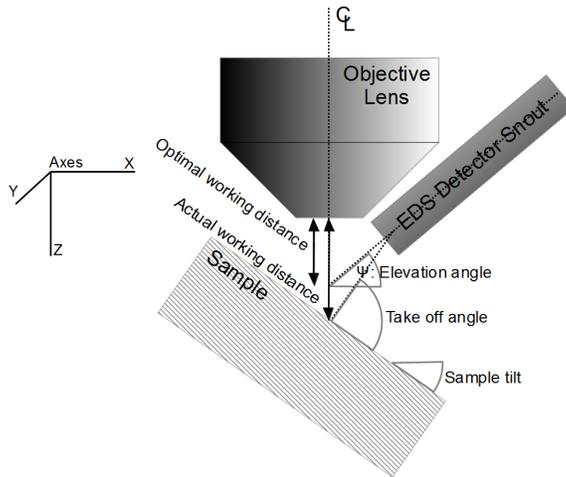


Figure 4: The relationship between the optic axis, EDS detector and sample positions as expressed in the instrument parameters, elevation angle and take-off angle.

software has many integrated quality checks that will catch many different kinds of performance degradation through the use of control charts that track detector resolution, scale and efficiency.

4.4 EDS: Elevation Angle

4.4.1 Motivation

Accurate knowledge of the EDS elevation angle is critical for accurate quantification and interpretation of spectra. While iGSR analysis is more qualitative than quantitative, the software needs to know the elevation angle to perform standards-based or standardless quantitative analysis.

4.4.2 Method

1. Measure the angle of the EDS detector snout relative to a plane perpendicular to the electron beam axis to an accuracy of $\pm 1^\circ$. See Figure 4 which shows how the elevation angle is defined relative to the optic axis (labeled Q_L) and the EDS detector snout axis.
 - (a) A level and protractor may be used.
 - (b) A protractor and plumb-line may be used.
 - (c) A digital inclinometer (like those found in many smart-phones) may be used.
 - (d) If the your SEM's stage tilt is perpendicular to the axis from the EDS detector to the sample, you can use the stage tilt to measure the elevation angle.
 - i. Select a large, flat sample. (Cu or Ni is suitable.)
 - ii. Start normal to the beam. Record the tilt (nominally zero.)
 - iii. While acquiring a spectrum, tilt the stage away from the detector until the input count rate goes effectively to zero as the sample occludes the X-rays generated in the sample from the detector.

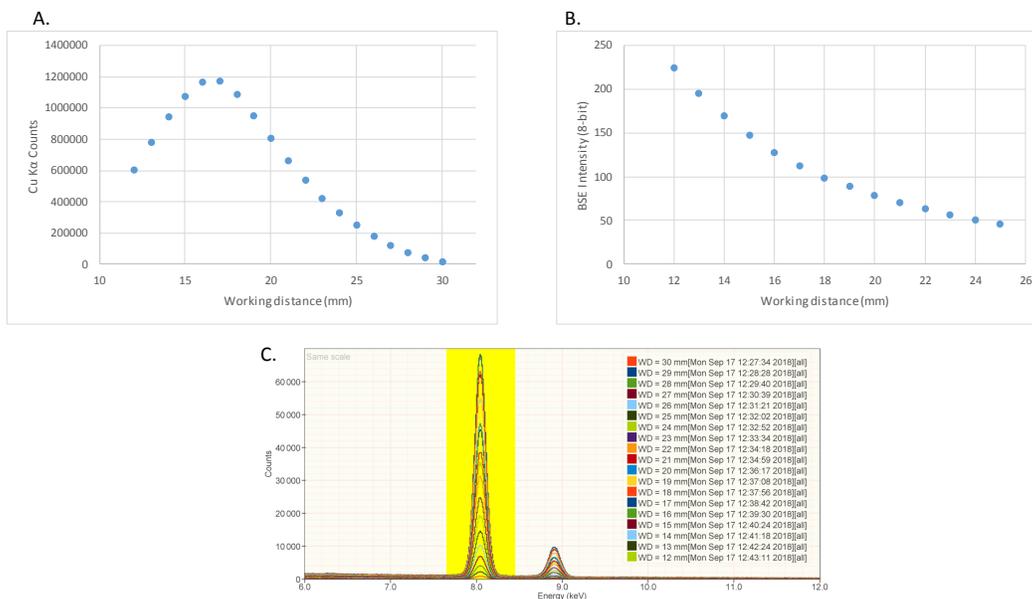


Figure 5: An example of determining the optimal working distance. A) shows a plot of the integrated counts in the yellow highlighted region in C). The integrated counts peak near 17mm working distance. This is also the region in which the count rate is least sensitive to errors in working distance. B) shows the variation in gray-scale intensity for the backscatter signal on the same instrument. While it is true that backscatter signal can often be enhanced by reducing the working distance, the sharp drop off in X-ray performance rarely makes the enhanced backscatter performance worthwhile.

- iv. Record the minimum angle at which the signal goes to zero and subtract the tilt measured at normal incidence.
2. Ensure that the measured elevation angle is consistent with the value recorded in the EDS software. Many vendors use the phrase “take-off angle” to refer to the elevation angle. This document distinguishes the elevation angle, which is a fixed property of the detector’s position in the instrument, from the take-off angle which involves the elevation angle but also takes into account the orientation of the stage when a spectrum is collected.
3. The elevation angle is measured relative to the center of the active area of the detector. For large detectors and detectors mounted close to the sample, the actual elevation of any point on surface of the detector can deviate significantly from the center value. Since the deviation is balanced in the sense that points above center balance points below, and since the deviation is the same for both standard and unknown spectra, this deviation is generally not considered to be a substantial concern in practice.

4.5 SEM & EDS: Optimal Working Distance

4.5.1 Motivation

The working distance is the distance from the the objective lens to the point at which the focused electron beam converges to its narrowest waist. The convergence point is usually determined by bringing a sample into focus at high magnification by adjusting the objective lens strength or by

adjusting the stage Z-axis until the image is in focus. Only then, when the image is in focus, can the working distance be calculated from the electron optics settings (primarily the objective lens and beam energy.) The working distance is distinct from the stage Z position as the working distance is a property of the electron beam.

Laboratories should establish and maintain a single, consistent working distance for each instrument at which all iGSR analyses will be performed. The optimal working distance should produce adequate imaging, and BSED and EDS detector performance. Shorter working distances tend to optimize image resolution. BSED performance tends to be poor at very small and large working distance and there is usually a range of working distances which produce near optimal performance. Since EDS acquisition often represents a dominant fraction of the total iGSR analysis time and since EDS detector performance can vary substantially with working distance but BSED performance varies slowly with working distance, a good way to select a operational working distance is to select the value that optimizes the EDS detector performance. This distance may be modified slightly to improve EDS detector alignment (see Section 4.6), and BSED signal or depth-of-focus performance while maintaining adequate X-ray throughput. Once determined for an instrument, a single, consistent working distance should be documented in laboratory procedures and maintained.

There is an inevitable trade-off between optimizing the BSED signal, the depth-of-field and the EDS signal. Each laboratory must make this decision for each instrument. Optimizing the BSED signal, typically by decreasing the working distance, will allow for faster search. However, the optimal BSED signal is usually at a working distance which is too short for optimal EDS performance and may not center the EDS detector active view field on image field (see Section 4.6). Longer working distances also typically produce larger depths-of-field and less sensitivity to rough or tilted sample surfaces.

4.5.2 Method

Use a copper sample to determine the working distance that produces the maximum X-ray flux.

1. Ensure that the EDS detector is configured for the pulse process time defined in the nominal analysis conditions.
2. Configure the instrument at the iGSR analysis beam energy.
3. Mount the copper sample on the stage with the surface perpendicular to the electron beam axis.
4. Select a probe current that produces a moderate dead time on the EDS detector ($\approx 5\%$ to 10% dead-time).
5. For each working distance in a set of 0.2 mm steps that encompasses the anticipated optimal working distance:
 - (a) Move the stage to the next working distance and refocus.
 - (b) Collect a EDS spectrum from the Cu sample.
 - (c) Integrate the number of counts in over a range of channels covering the Cu $K\alpha$ (IUPAC: K-L_{2,3}) peak. If your vendors software does not provide this functionality, you can export your spectra in ISO 22029:2012 EMSA/MAS spectrum file format to NIST DTSA-II for processing. It is not necessary to correct the integral for the continuum. Tabulate the working distance and the integrated counts.

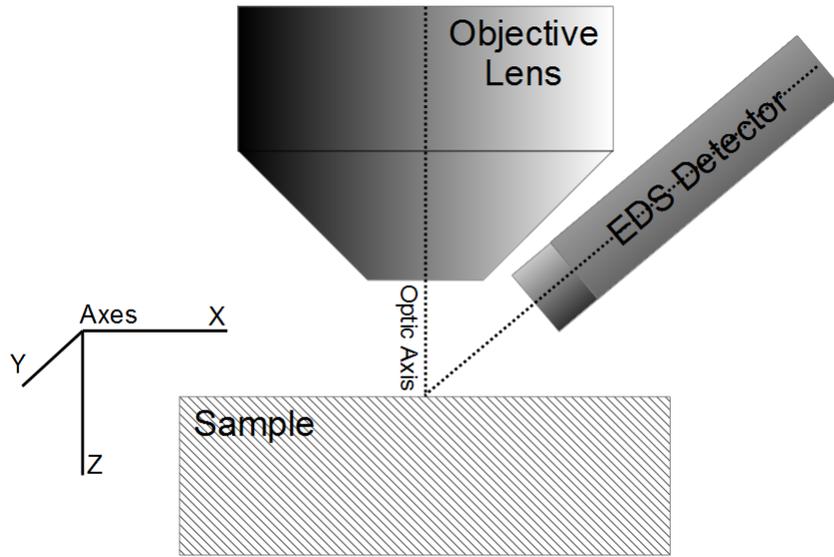


Figure 6: The snout of the EDS detector should be oriented such that the center of the detector's field is aligned with the optic axis and intersects the optic axis at the surface of the sample.

6. Plot the integrated counts against the working distance.
7. Identify the working distance that produces the maximum integrate counts.
8. If the plot does not show the count rates decreasing on both sides of the peak, repeat the procedure with a wider range of working distance.

Record the optimal working distance in a location specified in your laboratories iGSR analysis procedure. This value should be used consistently for all iGSR analyses performed on this instrument.

4.6 EDS: Detector Alignment

4.6.1 Motivation

EDS detectors are designed with a large acceptance angle but they only perform optimally when oriented correctly with respect to the intersection of the sample and the electron beam axis (See Figure 6). The orientation is usually fixed by a flange designed by the EDS vendor to mate their detector with another vendor's SEM. The flange design depends on the position, orientation and design of the SEM's vacuum port and the desired take-off angle, sample-to-detector distance and azimuthal angle (rotation about the optic axis). If the flange design is flawed, the EDS detector snout is damaged or the electron collimator is mounted incorrectly, the detector may not be oriented correctly with respect to the optimal working distance or the optic axis. The orientation can be further complicated by an insertion slide mechanism which should be designed to translate the detector snout along its central axis. This test should be performed at the insertion position used to collect iGSR data.

Almost all work in an SEM is performed at a field-of-view much less than 4 mm by 4 mm so the roll-off towards the edges is generally not significant so long as the highest intensity region is

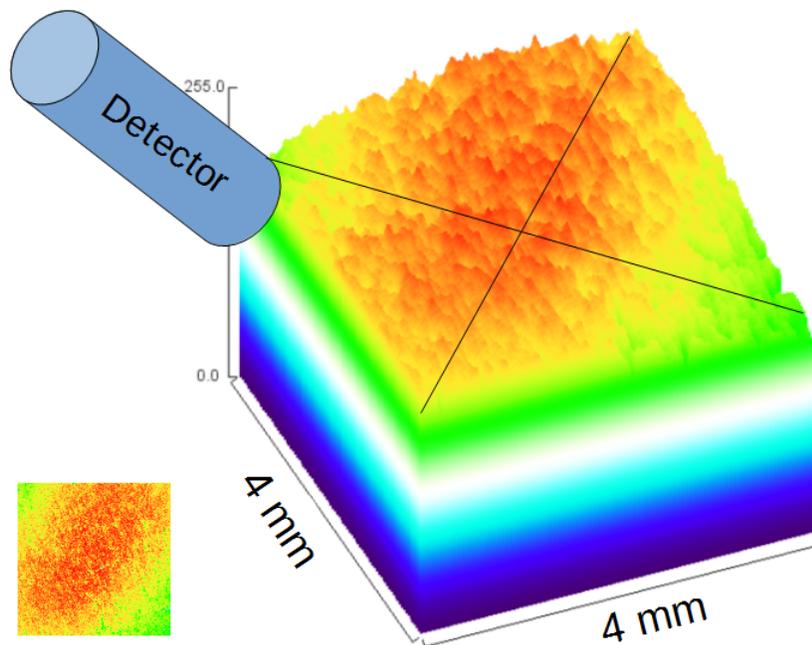


Figure 7: An image showing the variation in X-ray flux as a function of position. The vertical axis represents the relative number of X-rays measured. The image shows that the band of highest flux is oriented along the axis of the detector and is centered at the center of the image field. The X-ray flux is seen to drop off perpendicular to the detector snout. The red area represents the area over which the X-ray signal is relatively independent of beam position. With a misaligned detector, the highest flux area will not be centered on the image area. (The crossed lines show the center of the sample area.)

located in the center of the field-of-view. Typically, a field-of-view of a fraction of a millimeter is used to perform automated particle analysis. Additional care is required on instruments that use beam deflection to define electronic fields to ensure that, even when the beam is maximally deflected, the EDS detector remains geometrically efficient.

4.6.2 Materials and Apparatus

Sample A block of flat polished copper of dimensions greater than 4 mm by 4 mm mounted perpendicular to the incident electron beam.

4.6.3 Method

The orientation of the EDS detector can be evaluated by collecting and processing an X-ray spectrum image encompassing a large sample area. The raw count data should be peaked near the center of the image and roll off towards the edges of the image area.

1. Configure the instrument to the nominal analysis conditions. Ensure that the sample is mounted at the optimal working distance.
2. Select an image area of 4 mm by 4 mm (or the maximum field-of-view that can be achieved by your instrument at the optimal working distance)

3. Collect an X-ray spectrum image of dimensions at least 64 pixels by 64 pixels and an average X-ray count per pixel of at least 1000 events. (Calculate the average number of counts per pixel by dividing the output count rate by the dwell time per pixel.)
4. Create an image representing the raw intensities in a range of energies encompassing the Cu $K\alpha$ (IUPAC: K-L_{2,3}) and Cu $K\beta$ (IUPAC: K-M_{2,3,4,5}, N_{2,4,5}) characteristic peaks. This task may be performed many different ways. It is important to plot the raw intensities because the vendors often normalize the intensities in elemental maps which masks exactly the information we are trying to visualize. See Supplemental Material D for details on one method of converting the raw X-ray data into a intensity map. Figure 7 was generated using this method.
5. Verify that the region of highest flux is centered on the beam axis (center of the image) as shown in Figure 7.

If it is not possible to collect this data as a spectrum image and to process the spectrum image to extract raw counts, it is may be possible to collect equivalent data as a series of point spectra.

1. At the optimal working distance, image a 4 mm \times 4 mm field-of-view (or the maximum field-of-view that can be achieved) on the Cu sample.
2. Define a grid of points (at least an 8 \times 8 grid) covering the full image area.
3. Place the beam (by electronic deflection) in point-mode at each of the points on the grid and collect an EDS spectrum.
4. Process each spectrum to extract the intensity in a range of energies encompassing the Cu $K\alpha$ (IUPAC: K-L_{2,3}) and Cu $K\beta$ (IUPAC: K-M_{2,3,4,5}, N_{2,4,5}) characteristic peaks.
5. Plot the intensities and verify that the highest flux region is centered on the image area.

4.6.4 Remediation

If the detector is not aligned sufficiently well, you may choose to vary the optimal working distance slightly to bring it into better alignment. If this is not possible or requires a large compromise (10 %) in X-ray throughput, it may be necessary to contact the instrument vendor's service department. The resolution may involve redesigning the detector flange to correct for design errors or may involve repositioning other items in the SEM chamber. Usually, the SEM vendor's service engineer has better access to the information, tools and parts to perform these tasks than the EDS vendor but you may have to involve both.

4.7 EDS: Coincident Event Rejection

4.7.1 Motivation

A critical limit factor for EDS detector throughput is pulse pile-up rejection[9]. Pulse pile-up occurs when two X-rays enter the detector essentially simultaneously and the two X-rays are recorded as a single event with the sum of the individual events energies (See Figure 8). Higher order (pulse triplets, pulse quadruplets etc) are also possible but less likely and of lower intensity. Pulse pile-up produces spurious peaks that can appear to be characteristic X-rays from other elements and can make interpreting X-ray spectra more difficult.

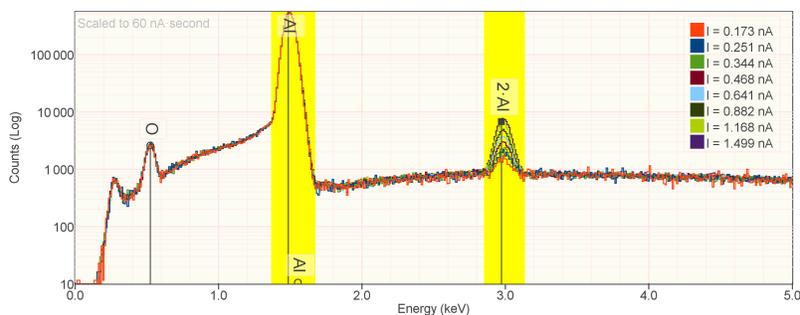


Figure 8: Spectra collected from bulk Al over a range of different probe currents from 0.173 nA to 1.499 nA. As the probe current increases, the intensity of the peak at twice the energy of Al K lines is seen to increase in intensity. This peak results from two Al K X-rays entering the detector simultaneously and being measured as though they were a single X-ray. It is not possible to eliminate coincident X-rays but the rate of coincidences can be reduced to an acceptable level.

4.7.2 Materials and Apparatus

Sample A sample consisting of flat polished aluminum mounted perpendicular to the incident electron beam.

Faraday cup A Faraday cup to measure the probe current.

Picoammeter A picoammeter attached to the sample current measurement port.

4.7.3 Method

1. Configure the instrument in the nominal analysis conditions.
2. Ensure that the EDS detector is configured using the energy axis scale (eV/channel) and pulse process time used for automated analysis and manual review. If a variety of settings are used for these phases, evaluate the detector at all settings.
3. Disable (if available) any pulse pileup correction provided by the EDS vendor's software.
4. Measure the probe current using the Faraday cup and the picoammeter.
5. Collect EDS spectrum from the aluminum block with at least 250 000 total counts in the range 100 eV to the nominal beam energy.
6. Compute the background corrected peak integral for the Al K peak - $I(\text{Al K})$.
7. Compute the background corrected peak integral for the region encompassing an equivalent with centered on twice the energy of the Al $K\alpha$ (IUPAC: K-L_{2,3}) X-ray $I(\text{Al K} + \text{Al K})$.
8. Compute the ratio $I(\text{Al K} + \text{Al K})/I(\text{Al K})$. The ratio should be less than 0.01 (1%). If the ratio exceeds the threshold, then the probe current should be reduced to reduce the incoming X-ray flux. If the ratio is significantly less than 0.01 then you may decide to increase the probe current.

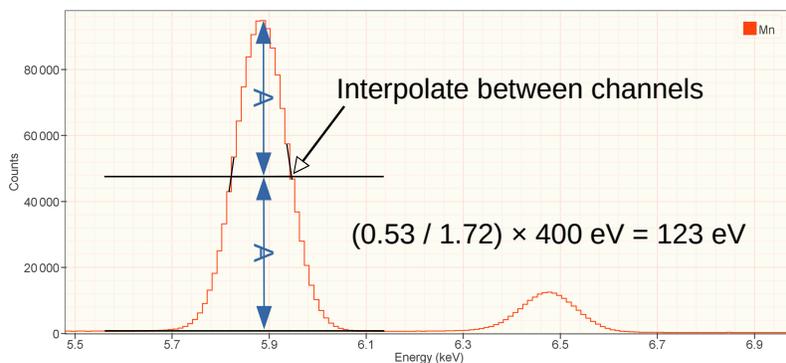


Figure 9: The FWHM of a characteristic X-ray peak may be estimated using a graphical approach.

Record the probe current that produces sufficiently low pulse pile-up in your SOP for this instrument. All automated and manual analyses are commonly performed at approximately this value ($\pm 10\%$). You may wish to examine spectra from iGSR particles at various probe currents to appreciate how pulse pile-up influences the perception of minor and trace elements. If your system enables pulse pileup correction while performing automated particle analysis, you may wish to leave the pulse pileup correction algorithm engaged while collecting automated data. In this case, you may be able to increase the probe current by a factor of two or more. Use caution though. Pulse pileup correction algorithms can cause spectrum artifacts (like holes and ripples) and require high-count spectra to work optimally.

4.8 EDS: Detector Resolution

4.8.1 Motivation

The ASTM E1588-17 standard specifies a minimum resolution for a detector used to perform iGSR analysis[3]. While almost any modern detector is capable of this resolution at best resolution setting (longest pulse processor process time), many older Si(Li) detectors may fail to meet this specification at realistic analysis settings. The validation test should be performed under the same settings that will be used for automated iGSR analysis and manual review.

4.8.2 Materials and Apparatus

Sample A bulk, flat polished specimen of pure manganese (Mn).

4.8.3 Method

The full method is presented in Annex A of ISO 15632 [10]. A summary is stated here.

1. Configure the instrument according to the nominal analysis conditions.
2. Collect an EDS spectrum from the flat, polished surface of a manganese specimen. Ensure that the characteristic peak intensities in the Mn $K\alpha$ (IUPAC: K-L_{2,3}) is at least 10 000 counts.
3. Compute the FWHM (FWHM) graphically by drawing a horizontal line half way between the continuum level and the top of the Mn $K\alpha$ (IUPAC: K-L_{2,3}) peak as shown in Figure 9. On each side of the peak draw interpolation lines between the center of the channels above

and below the half-max line. Measure the full-width distance between the intersection of the interpolation lines with the half-maximum lines. Measure the distance between energy calibration marks on the energy axis. Compute the ratio of the full-width distance and the energy calibration distance to estimate the width of the full-width line in eV.

4. Repeat at least five times and compute the mean.
5. The EDS vendor software may provide a means for extracting the detector resolution or it is possible to use the “Calibration Alien” tool in NIST DTSA-II to extract the detector resolution through a non-linear fitting algorithm although the results may differ by a fraction of an eV from the ISO 15632 value.

It is possible to use elements other than Mn to measure the detector resolution. Common alternatives include elements between Mn ($Z=25$) and Cu ($Z=29$). The resolution at Mn $K\alpha$ (IUPAC: K-L_{2,3}) can be calculated from the resolution, $FWHM_{ref}$, at the measured energy, E_{ref} , as

$$FWMH = \sqrt{2.5(E - E_{ref}) + FWHM_{ref}^2} \quad (1)$$

where E is the energy of the Mn $K\alpha$ (IUPAC: K-L_{2,3}) line (5.899 keV)[11].

4.9 EDS: Count Rate Linearity

4.9.1 Motivation

It is critical that the number of recorded X-rays per unit live-time is linearly proportional to the probe current. This basic assumption can fail if the detector electronics calculates the live-time incorrectly or if the picoammeter measures the probe current incorrectly. It also suggests when the detector is being over-driven and pulse pile-up is inhibiting linearity or a pulse pileup correction algorithm is not implemented correctly. Furthermore, tests like these can identify if a picoammeter is not linear over a range of currents.

4.9.2 Materials and Apparatus

Sample A block with a flat, polished specimen of copper.

Faraday cup A Faraday cup to measure the probe current

Picoammeter A picoammeter attached to the sample current measurement port.

4.9.3 Method

1. Configure the instrument in the nominal analysis conditions. Ensure that the detector is configured for the eV/channel and pulse process time used for automated analysis and manual review. If different settings are used for these phases, evaluate the detector at both settings.
2. Vary the probe current over a range from approximately 10 % to 200 % of the nominal probe current.
3. Collect a series of spectra each with at least 250 000 total counts.
4. Sum the number of X-ray events in a region of energies encompassing the Cu $K\alpha$ (IUPAC: K-L_{2,3}) and $K\beta$ (IUPAC: K-M_{2,3,4,5}, N_{2,4,5}) lines.

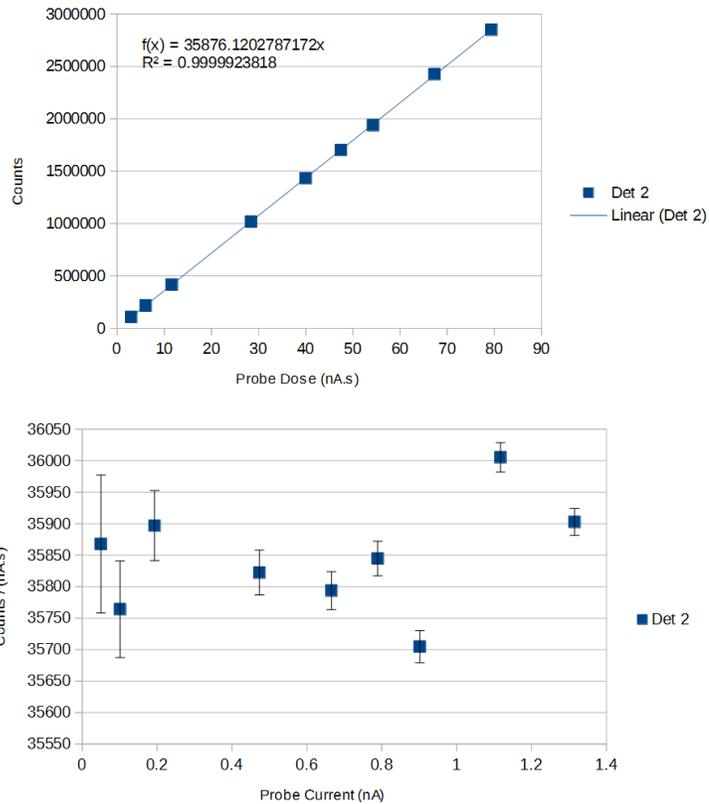


Figure 10: Two different presentations of the same linearity data. The top plot shows integrated counts as the probe dose (probe current times live time) is increased up to 80 nA·s by adjusting the probe current. This demonstrates the linearity but is not a sensitive way to show deviation from linearity. The bottom plot shows the integrated count divided by the dose. This ratio should remain constant.

5. Plot the counts/(nAs) vs. probe current. The line should be horizontal to within 1%. Figure 10 shows an example of the data plotted two different ways.

4.10 EDS: Instrumental Peaks

4.10.1 Motivation

Instrumental peaks are persistent trace-level characteristic peaks which are present in spectra but do not represent elements truly present in the sample[12]. These peaks often result from backscattered electrons generating X-rays in materials within the instrument chamber. The backscattered electrons strike the pole-piece, stage, detectors, sample or other items within the chamber generating X-rays which are sometimes detected. Backscattered electrons can be a particular problem with particles because enhanced scatter from the side of the particle can strike the interior of the chamber. Careful design of the chamber and detector can minimize instrumental peaks. Sometimes removing unnecessary accessories can help. Instrumental peaks can also be due to secondary fluorescence.

4.10.2 Materials and Apparatus

Sample A sample with high atomic number ($Z50$) particles of a few microns in diameter. The particles should not contain elements that are likely to be present within the sample chamber (like Al, Si, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo etc.)

Alternative A rough conductive sample of moderate to high atomic number that is not present within the sample chamber. Gold or platinum are a good choice.

4.10.3 Method

Collect a high count (1 000 000 counts) EDS spectrum from the material and examine the spectrum for trace-level evidence of peaks from other elements. Because of absorption, high energy peaks may be present in the absence of the usual lower energy peaks. When looking for trace peaks be careful to label all potential coincidence peaks so that they won't be mistaken for instrumental peaks.

Any instrumental peaks should be documented. If the elements represented by instrument peaks are relevant to iGSR, the presence of these peaks should be addressed and a strategy for addressing the peaks should be documented.

4.11 BSED: Response Time

4.11.1 Overview

The probe current and the scan speed are two parameters that influence the signal-to-noise ratio of the BSED. High signal-to-noise ratios are desirable to facilitate the identification of high mean-atomic-number particles by BSED signal. The signal-to-noise ratio is largely determined by the number of electrons that interact with the sample during the measurement interval. From this perspective, increasing the probe current by a factor-of-two is equivalent to decreasing the scan speed by a factor-of-two. Ideally, we would like a fast scan speed to maximize instrument throughput.

Since the probe current is typically limited by the EDS detector's performance, the scan speed must be adjusted to produce adequate sensitivity to small particles while optimizing throughput. The next two procedures will evaluate two detector characteristics that can limit scan speed.

4.11.2 Motivation

The efficient performance of the backscattered electron detector is critical for fast, reliable discovery of iGSR particles. Detector response time is important. The detector must respond fast enough to track sample changes as the beam is scanned over the surface of the sample. Poor response time limits sensitivity to small particles at faster scan speeds.

4.11.3 Materials and Apparatus

Sample - Option 1 A block containing a sharp interface between Cu and C

Sample - Option 2 The 100 μm feature in the lower-left corner of the Planotec to the left of the “SPS-TM-A” label. It may be necessary to change the brightness and contrasts to work with this higher mean-Z sample.

4.11.4 Method

1. Configure the instrument in the nominal analysis conditions.
2. Configure the scan speed, field-of-view and image dimensions to the settings used by the iGSR automation software while searching for candidate particles.
3. Be sure to perform this test with the iGSR automation software as the SEM vendor’s digitization hardware may perform very differently from the iGSR vendor’s digitization hardware.
4. Orient the interface vertically (along a column) in the center of the image with the C to the left and Cu to the right.
5. Collect a 256 pixel line scan of 100 μm length centered on the interface using the iGSR automation software.
6. Using the line-scan and line-scan tools to measure the width of the 10 % to 90 % rise interval in pixels over the interface.
7. The width of the rise/fall interval should be documented. Typically a value of 2 pixels or less is sufficient although larger value may be acceptable if the iGSR automation software provides compensation.
8. If the width of the rise/fall interval is too large, it may be necessary to reduce the scan speed.

4.12 BSED: Z Contrast

4.12.1 Motivation

The efficient performance of the BSED is critical for fast, reliable discovery of iGSR particles. The detector signal-to-noise ratio is critical. If the detector signal is too noisy, it is difficult to discern iGSR particles (particularly small particles) using the backscatter signal.

4.12.2 Materials and Apparatus

Sample A block containing clean, bulk, polished samples of Cu and Ni (at least 100 μm \times 100 μm each).

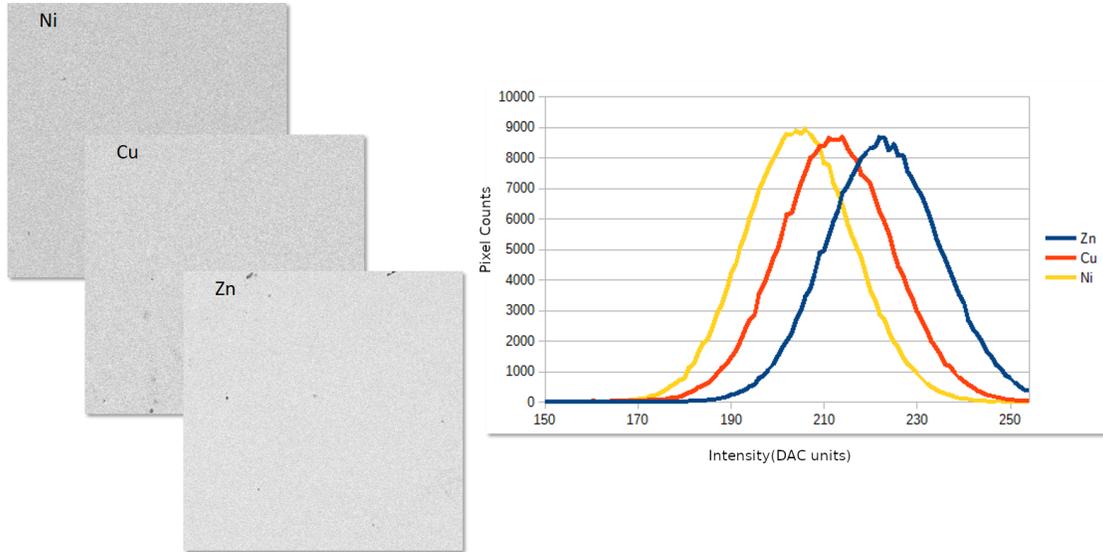


Figure 11: Backscatter electron images collected at 1 nA and 1 μ s/pixel from Ni, Cu and Zn and a plot of the associated histograms. The mean and standard deviation of the intensities were: for Ni, 204.7 ± 11.9 , for Cu, 212.3 ± 12.0 , and Zn, 222.6 ± 12.5 .

4.12.3 Method

1. Ensure that the sample appears clean and not oxidized using the secondary electron detector (SED). If there is surface contamination, clean the surface with a suitable detergent followed by ethanol. An ultrasonic cleaner can be useful.
2. Configure the instrument in the nominal analysis conditions.
3. Configure the scan speed and field-of-view to the settings used by the iGSR automation software while searching for candidate particles.
4. Set the brightness and contrast according to your iGSR protocol.
5. Collect an image with at least 10 000 pixels of an approximately 100 μ m field-of-view on the Cu sample
6. Collect an image with at least 10 000 pixels of an approximately 100 μ m field-of-view on the Ni sample
7. Calculate the mean and standard deviation pixel intensity for each image - $I_{Cu} \pm \sigma_{Cu}$ and $I_{Ni} \pm \sigma_{Ni}$.
8. Verify that $I_{Cu} - I_{Ni} > k\sqrt{\sigma_{Cu}^2 + \sigma_{Ni}^2}$ where k is a laboratory specified constant in the range between 0.1 and 1.0. The value of k should be selected to provide a level of BSED performance that reliably discerns both large and small (1 μ m) high-Z particles at the nominal probe current and scan speed.

4.12.4 Mediation

If the signal-to-noise ratio is not sufficiently good to reliably identify small candidate particles, the scan speed should be decreased to increase the time spent measuring each pixel.

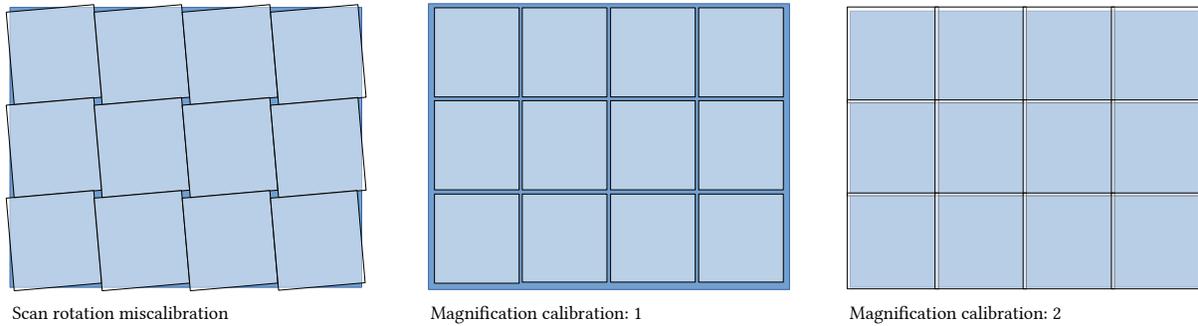


Figure 12: When the stage is used to tile multiple images to cover a sample, different types of image mis-calibration lead to either missed areas (darker blue) or multiply counted areas (lighter). A mis-calibration between the image rotations and the stage axes leads to some points around the image corners being missed. If the actual field-of-view is less than the calibrated value, some region between tiles will be missed. If the actual field-of-view is more than the calibrated value, some regions between tiles will be multiply counted. All of these images assume that the intent was to position adjacent images so they exactly abutted.

4.13 SEM: Dimensional Calibration

4.13.1 Motivation

Magnification is only of secondary importance in the identification of iGSR particles (See [3]). Primarily, iGSR particles are identified by their elemental constituents as evidenced in an EDS spectrum. However, ASTM E1588-17 does include particle size as a parameter worthy of consideration when interpreting EDS spectra.

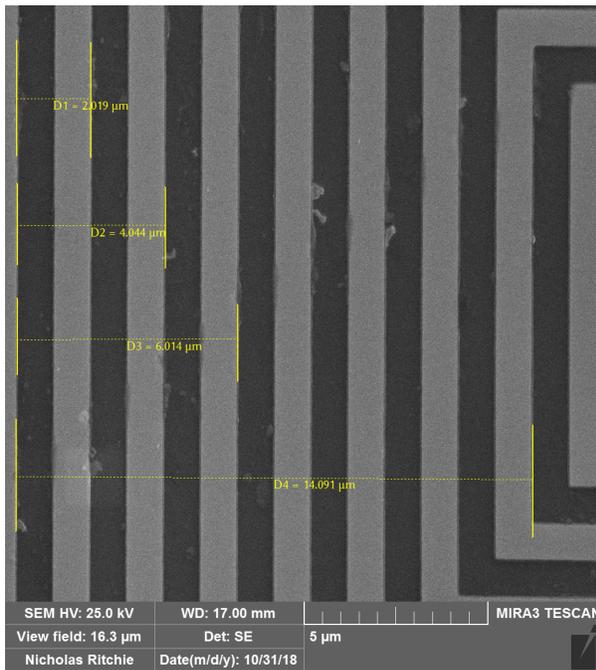
While it is common to speak of magnification, it is much better to think of the task as setting the relationship between the true and measured dimensions of a feature in an image. The dimensions of the feature are usually extracted from the image data in terms of a pixel representing one edge of the feature and a second pixel representing the other edge of the feature. The calibration is thus the multiplicative constant that translates the length in pixels into the length in SI-units (typically μm or nm). SEM are typically calibrated such that the multiplicative constant is the same in the horizontal and vertical image dimensions (“square pixels”). However, the scan mechanism in an SEM is complex (involving scan coils that don’t typically align with the image dimensions) and this characteristic can not be guaranteed.

The calibration isn’t a single number but a complex relationship between the scan generator, the scan coils, the electron optics, the beam energy and the working distance. This relationship is likely to work best in the regime in which the calibration is performed. If possible calibrate the magnification to perform best at the conditions (beam energy, field-of-view, image dimensions and working distance) used during iGSR analysis.

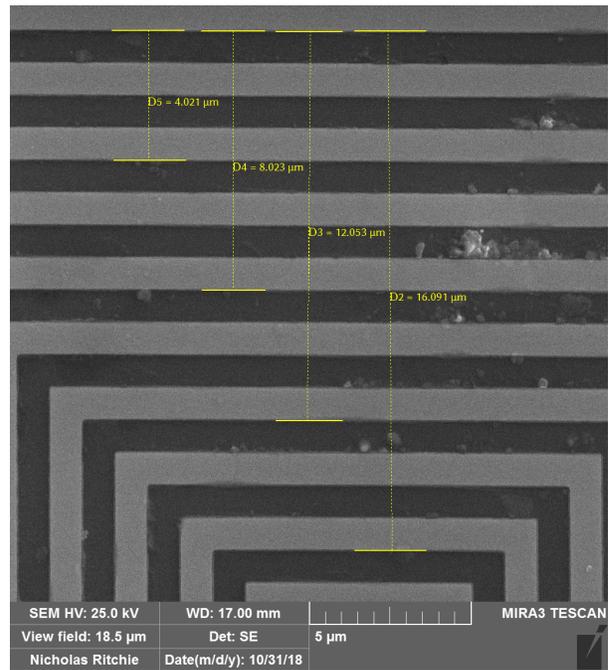
ASTM E766 – 14^{e1} provides a protocol for calibrating an SEM[13]. The ASTM E766 – 14^{e1} practice states in its scope that:

The relationship between true magnification and indicated magnification is a complicated function of operating conditions. Therefore, this practice must be applied to each set of standard operating conditions.

By limiting the variation in standard operating conditions as has been suggested throughout



(a) Horizontal



(b) Vertical

Figure 13: Measuring the calibration using a Geller MRS-4 length standard in the a) horizontal and b) vertical directions. Note that the nominal analysis conditions for this instrument are 25 keV and 17.0 mm working distance as evident in the marker bar.

this document, it is possible to limit the number of conditions under which the magnification should be calibrated. At a minimum, the magnification should be calibrated as per ASTM E766 – 14^{ε1} at the nominal analysis conditions. The calibration should be validated at both nominal magnification used for iGSR analysis as well as 10 × and 100 × the nominal magnification to verify that particle images will also be calibrated correctly. The emphasis in the validation process should not be on performing a magnification calibration but on verifying that the calibration is within tolerance.

4.13.2 Materials and Apparatus

Standard A certified reference material suitable for calibrating SEM magnification as specified in ASTM E766 – 14^{ε1}.

4.13.3 Method

1. Configure the instrument in the nominal analysis conditions.
2. Follow the protocol in ASTM E766 – 14^{ε1}. See Figure 13.
3. Measure the pitch of a feature of known physical dimension at the nominal magnification for iGSR analysis. Measure the pitch in both the horizontal and vertical directions. (The pitch is the distance from an edge to the corresponding edge on another feature.)
4. Measure the pitch of a feature of known physical dimension at one-hundred times the nominal magnification for iGSR analysis. Measure the pitch in both the horizontal and vertical directions as shown in Figure 13.
5. Verify that both magnification calibrations are accurate to within a fractional criterion defined within your laboratory quality documents. It should generally be possible to calibrate the image dimensions to within 5 % and 1 % may be achievable on high performance instruments.
6. If the magnification calibration falls outside your laboratories limits, you or your instrument service provider should follow the instrument manufacturer’s procedure to bring the instrument into compliance.

4.14 SEM: Image Rotation Compensation

4.14.1 Motivation

The objective lens’ magnetic field rotates the effective orientation of the scan coil raster. As a result, the image in an uncompensated system appears to rotate as the objective lens strength changes (as happens during focus). Modern instruments compensate for image rotation in a transparent manner through a calibrated procedure. As the objective lens strength changes, the beam scan orientation is changed dynamically to give the perception of a steady, un-rotating image.

However, image rotation compensation is an instrument calibration. Usually, the image rotation is calibrated such that at 0°, the faster changing scan direction (usually displayed horizontally in the image) is aligned with the X-axis. Mis-calibrations of the image rotation lead to a disparity between the raster directions and the stage motion directions. The disparity leads to a mismatch between adjacent image tiles that can lead to some regions being analyzed twice and others not analyzed.

4.14.2 Materials and Apparatus

Sample Almost any non-charging sample with a structured feature that is large enough to be imaged at the nominal iGSR analysis magnification is adequate.

4.14.3 Method

The strategy is to find a feature, move the stage until the feature is on the left side of the image, collect an image, move only the stage X-axis until the feature is on the right side of the image, and collect a second image. Compare the positions of the feature in the first and second image. The angle of misalignment equals $\theta = \arctan(\Delta y/\Delta x)$. Ideally, Δy will be small compared to Δx .

1. Configure the instrument in the nominal analysis conditions.
2. Select the magnification at which iGSR analyses are performed.
3. Set the image rotation to zero degrees.
4. Find a feature with a sub-feature which can be distinguished at the single pixel level. Move this feature to the left side of the image. Collect an image with at least 512 pixels \times 512 pixels in which the sub-feature can be easily distinguished.
5. Holding the Y-axis motion fixed, using only the X-axis motion move the feature to the right side of the image. Collect a second image with at least 512 pixels \times 512 pixels in which the sub-feature can be easily distinguished.
6. Identify the pixel coordinates of the feature in the first image (x_0, y_0) and in the second image (x_1, y_2) .
7. Compute the misalignment angle $\theta = \arctan\left(\frac{y_1 - y_0}{x_1 - x_0}\right)$. If the misalignment exceeds 0.5° , your or your service provider should follow the instrument manufacturer's procedure to bring the instrument into compliance.

4.15 SEM: Stage Field Registration

4.15.1 Motivation

Ideally, the automated image analysis process will cover the full area of the sample once and only once (see Figure 12.) Typically, the sample area is too large to analyze without using the stage to move the sample under the electron beam. Thus the stage must move the distance equivalent to the field-of-view of the image area. However, if the stage distance calibration is not equivalent to the image calibration, the stage translation may not exactly match the image field-of-view. If the stage moves too far, there will be regions between the image fields that are not analyzed. If the stage does not move far enough, there will be regions in the image fields that are multiply analyzed.

Perfect registration between stage and image cannot be maintained. However, it is possible to decide whether it is preferable to miss some regions and risk missing particles or to multiply scan some regions and risk double counting particles. Both strategies are acceptable. Leaving un-scanned regions between images will miss a small fraction of the particles present but will complete the sample analysis quicker. Multiply scanning some regions will risk double counting and the analysis will run slower but particles won't be missed. However, it is critical that the double counted particles are identified in manual review and not reported as though they were distinct particles.

4.15.2 Materials and Apparatus

Sample 1 A sample with an easily distinguished feature with dimensions of about 1% of the particle search field-of-view.

Sample 2 A sample with a known number and location of distinct features like the Planotec sample or a lab-constructed and characterized sample.

4.15.3 Method

The first test involves placing a feature from Sample 1 half-off one side (left or top) of the image. Move the stage by a distance equal to the image field-of-view to move the feature to the other side (right or bottom) of the image. This test should be performed at the field-of-view used for the particle search process. Ideally, the feature will move half-off the other side of the image. However, if the feature remains on the image, the stage is not moving far enough. If the feature leaves the image, the stage is moving too far.

1. Configure the instrument in the nominal analysis conditions.
2. Select the magnification at which iGSR analyses are performed. The magnification has been previously calibrated in Section 4.13.
3. This protocol assumes that the left, top image coordinates represent smaller X and Y stage coordinates than the right, bottom image coordinates. The protocol may be readily adapted to accommodate different image/stage mappings.
4. Identify a distinct, easily identifiable feature of dimension a few percent of the current field-of-view.
5. Increase the image field-of-view by 10%.
6. Mark the centroid of the particle on the image screen using an erasable marker or alternative means. Record the stage coordinates.
7. Move the stage along the X-axis by a distance equal to the nominal analysis field-of-view for automated analysis.
8. Locate the feature which should now be on the right-side of the image. Record the new stage coordinates.
9. Using the linear dimension measurement tool to measure distance between the initial (left hand) location of particle and the current (right hand) location of the particle.
10. The distance should be within 5% of the distance moved by the stage as determined from the coordinates measure in previous steps.
11. Repeat the procedure for the Y-axis using the top and bottom of the image.

The second test involves repeatedly performing automated analyses on Sample 2. Between each analysis, the analysis area must be reconfigured so that the stage field locations vary from analysis to analysis. Perform careful analysis on the resulting particle data to determine whether particles are being missed or double counted.

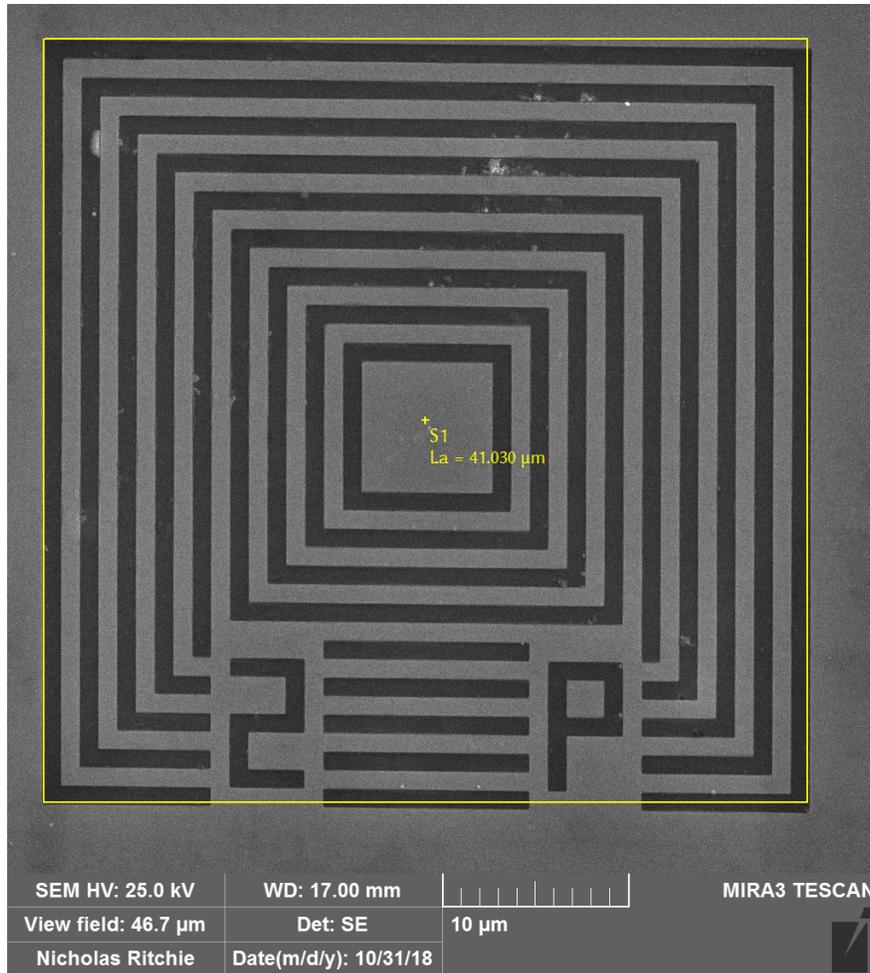


Figure 14: This image collected from a Geller MRS-4 standard shows that the scan axes on this instrument are not perfectly orthogonal. The overlaid yellow square matches the vertical scan direction but the horizontal scan direction is slightly off from the ideal 90°.

4.15.4 Mitigation

Mitigation is vendor dependent. The iGSR automation software vendor may provide calibration routines or guard bands that allow you to fine tune the motion of the stage under automation. Use the clues garnered from the first test to estimate the changes necessary to produce the desired registration.

4.16 SEM: Image Orthogonality

4.16.1 Motivation

The beam raster that produces an SEM image is create by two pairs of scan coils nominally mounted orthogonal to each other. If the scan coils are not perfectly orthogonal, then the image can appear distorted. This is complicated by whole-image rotations caused by the helical path of electrons in the objective lens' magnetic field.

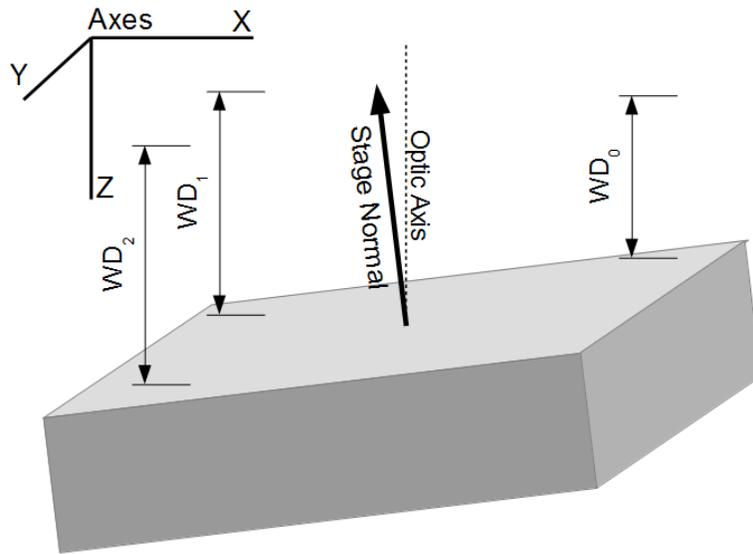


Figure 15: Ideally, the x-y motion of the stage should be orthogonal to the optic axis and a normal perpendicular to the surface of the stage should be parallel to the optic axis. However, if the stage or sample is tilted relative to the optimal alignment, the working distance (distance from the objective lens to the image point) will change as the stage is moved in the X and Y directions.

4.16.2 Materials and Apparatus

Standard A sample with known orthogonal features. A square chip will do.

4.16.3 Method

1. Configure the instrument in the nominal analysis conditions.
2. Collect an image in which one edge of the orthogonal feature is aligned with one edge of the image and the orthogonal edge of the feature is approximately aligned with another edge.
3. Compare the angle on the image with the known angle of the feature using image analysis tools such as those in ImageJ[14]. See Figure 14.
4. Rotate the image using the “image rotations” knob in 15° increments and repeat over the range 0° to 360°. The axes should remain orthogonal to better than a degree.

4.16.4 Mitigation

Although the positions of the scan coils can not be modified, image orthogonality problems can often be addressed by the SEM instrument vendor’s service department via a software-based fix.

4.17 SEM: Stage Orientation

4.17.1 Motivation

For optimal automated particle analysis performance, the surface of the sample should be maintained at a constant working distance. Maintaining a constant working distance requires a combination of different criteria. First, the stage must be oriented correctly. The optic axis defines the direction along which the working distance is measured (see Figure 15). The x-y motion of the stage should be perpendicular to the optic axis to ensure that the working distance does not change as the stage translates. Second, the sample should be flat and mounted parallel to the stage motion and perpendicular to the electron beam.

4.17.2 Materials and Apparatus

Sample A flat, conductive surface like the surface of the stage or a silicon wafer.

4.17.3 Method

Set and maintain the working distance at the nominal analysis conditions using the instrument focus control. At each of three (or more) positions dispersed around the full X and Y extent of the stage translation, using z stage motion only to bring the instrument into focus at a high magnification (approx. 20 μm field-of-view). Record the stage coordinates at each stage point - $\mathbf{P}_0 = (x_0, y_0, z_0)$, $\mathbf{P}_1 = (x_1, y_1, z_1)$, and $\mathbf{P}_2 = (x_2, y_2, z_2)$. Ideally, $z_0 \approx z_1 \approx z_2$. Any difference will be reflected in a tilt in the stage. To compute the tilt, compute the surface normal, a vector perpendicular to the surface of the stage, is

$$\mathbf{N} = \frac{(\mathbf{P}_1 - \mathbf{P}_0) \times (\mathbf{P}_2 - \mathbf{P}_0)}{|(\mathbf{P}_1 - \mathbf{P}_0) \times (\mathbf{P}_2 - \mathbf{P}_0)|} = (x_N, y_N, z_N).$$

The inclination angle equals $\theta = \arccos(\|z_N\|)$ and the orientation of the misalignment is given by $\phi = \arctan(y_n/x_n)$ where

$$z_N = \frac{x_0(y_1 - y_2) + x_1(y_2 - y_0) + x_2(y_0 - y_1)}{\sqrt{|x_1y_0 - x_2y_0 - x_0y_1 + x_2y_1 + x_0y_2 - x_1y_2|^2 + |x_1z_0 - x_2z_0 - x_0z_1 + x_2z_1 + x_0z_2 - x_1z_2|^2 + |y_1z_0 - y_2z_0 - y_0z_1 + y_2z_1 + y_0z_2 - y_1z_2|^2}}$$

and

$$\frac{y_n}{x_n} = \frac{x_0(z_2 - z_1) + x_1(z_0 - z_2) + x_2(z_1 - z_0)}{y_0(z_1 - z_2) + y_1(z_2 - z_0) + y_2(z_0 - z_1)}.$$

4.17.4 Example

While maintaining the image in focus at a working distance (focal length) of 17.0 mm, these three points were measured by moving the stage while not changing the objective lens current:

$$\mathbf{P}_0 = (-1.464, 18.600, 20.974)$$

$$\mathbf{P}_1 = (16.987, -8.267, 20.994)$$

$$\mathbf{P}_2 = (-20.430, -6.414, 20.871).$$

For which,

$$\mathbf{N} = (0.003372, 0.001560, -0.999993)$$

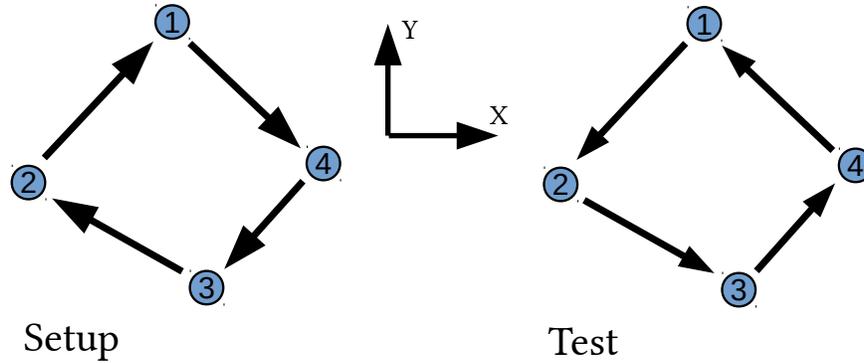


Figure 16: An arrangement of stage coordinates arranged in a rough cross shape allows for tests of approaching a coordinate from both directions on both axes. The coordinates are visited in opposite order between the setup and the test.

which suggest a moderate stage inclination of $\theta = 0.22^\circ$ at an orientation of $\phi = 24.8^\circ$. The stage inclination should be much less than a degree.

4.18 SEM: Stage Position Reproducibility - Short Term

4.18.1 Motivation

Accurate and consistent stage motion is critical for robust, reliable automated analysis. When an area is tiled, it is critical that the stage move consistently and reliably from one tile to the next. If the stage moves slightly less one step and slightly more the next, it leads to an inconsistency in the tiling. Furthermore, if the stage does not return to close to the same position each time, relocating particles (as is required to confirm iGSR particles) can be a challenge. Stage reproducibility can be divided into a less challenging short term reproducibility test and a more challenging long term reproducibility test. As a baseline, we'd like to be able to move between a handful of points and return to within a few micrometers regardless of the order in which the points are selected. A particular challenge is stage backlash. Poorly designed stages may come to rest in different positions depending upon whether a coordinate is approached from the above or below. There is often slop in the stage gears which results in a slight difference in resting position depending upon the direction in which the coordinate is approached. Some stage systems compensate for this by always approaching coordinates from the same direction. There are many other reasons why a stage may not return reproducibly to the same resting positions.

4.18.2 Materials and Apparatus

Sample A flat sample with distinct, recognizable features on the $1\ \mu\text{m}$ scale across a large fraction ($\gtrsim 80\%$) of the total stage travel area. A sample covered with distinctive particles is a good choice.

4.18.3 Method

The test involves setting up four stage coordinates associated with distinct features, collecting an image at each coordinate, and then revisiting the same four stage coordinates and collecting new images. A feature on the setup images is located on the relocation image and the offset (in μm) is

recorded. The test is repeated three times for coordinate separation typical of movements between points on a sample and three times for coordinate separation typical of movements across the full extent of stage motion.

1. Mount the sample centered on the extent of stage translation in the chamber. Ensure that the sample is flat and mounted perpendicular to the optics axis. Given the size of the sample, slight variation in working distance across the sample should be expected and can be tolerated.
2. Image the center of the sample at the *optimal working distance*.
3. Select a set of four stage coordinates that represent motions of approximately the distance from one side of a typical sample to the other. The coordinates should be arranged and labeled in the approximate positions shown in Figure 16.
 - (a) Initialize the stage by moving it to coordinate 4.
 - (b) Move the stage to each stage coordinate in order of label (1 to 2 to 3 to 4).
 - (c) Locate a distinctive feature and move the stage to localize the feature in the center of the image area. This can be accomplished by zooming in to a small field-of-view ($\approx 10\ \mu\text{m}$) and centering the particle in the field. Record the precise stage coordinates as reported by the instrument software.
 - (d) Collect and record an image of the particle with a field-of-view of approximately $100\ \mu\text{m}$.
 - (e) Once all four coordinates have been identified and images collected, reverse the order of traversal. Visit the points in the order 3 to 2 to 1 to 4.
 - (f) Enter the stage coordinates recorded in the setup traversal in the stage movement control and command the stage to that coordinate. Do not use the joystick to return to the stage coordinate. *Do not tweak the stage position* to return the feature to the center of the image.
 - (g) At each relocated stage coordinate, collect an image.
 - (h) Compare the image collected during the setup process with the equivalent image collected during the relocation process. Measure the offset in the image in each stage translation direction (X & Y) between the location of the feature in the setup image and in the relocated image. Tabulate these values.
4. Repeat these tests at least three times for stage coordinates separated by typical sample sized moves in different areas within the full stage travel.
5. Repeat these tests at least three times for stage coordinates separated by distances close to the full extent of stage travel.
6. Determine the typical and maximum offsets in the X and Y translation distances. Knowing this information helps analysts to understand how far afield they must search when attempting to relocate particles in the best-case scenario.

Note: It may be possible to use either the SEM software or the iGSR automation software to command the stage to move. It is preferable to use the automation software when possible because even when the SEM software implements and uses backlash correction, the automation software may or may not.

<i>Coordinate</i>	<i>Setup</i>	<i>Relocation</i>	<i>Tests</i>
1	4 → 1	2 → 1	-X vs +X
2	1 → 2	3 → 2	-Y vs +Y
3	2 → 3	4 → 3	-X vs +X
4	3 → 4	1 → 4	-Y vs +Y

Table 2: If the points are arranged according to Figure 16 then each point tests stage movement in predominantly along one stage axis as summarized in this table.

4.19 SEM: Stage Position Reproducibility - Longer Term

The short term stage reproducibility test evaluates under the best conditions how well the stage is capable of returning to the same point on the sample. For automated analysis, there may be many hours and hundreds of stage movements between when the particle is located and when it is relocated. Relocation under these conditions is much more challenging and fraught with potential pitfalls. There are numerous reasons why the relocation error may be larger like material expansion due to thermal processes and missed counts on the drive encoder. A more rigorous test involves running multiple automated analyses sequentially and relocating a fraction of the particles discovered in each analysis.

4.19.1 Materials and Apparatus

Samples Particulate samples similar in character to those routinely analyzed. The samples need not contain iGSR particles but should contain roughly the same number of particles as a routine case sample. The quantity of samples should be the number analyzed in a routine batch.

4.19.2 Procedure

1. Configure the system for the nominal analysis conditions.
2. Mount each particle sample in the instrument and configure the iGSR automation software for a routine iGSR analysis of each sample. The standard analysis termination criteria should be used.
3. Perform automated analysis on all the samples.
4. Select at least five particles from each sample at random and relocate these particles. Tabulate the stage coordinates at which the automated software reported the particle and the stage coordinates at which the particle was relocated. Repeat for each sample for a minimum of twenty particles total.
5. Record and plot the displacement between the stage coordinate where the particle was discovered and coordinate where it was relocated. This is a measure of how far afield it is necessary to search to relocate particles. As the displacement increases, it becomes increasingly difficult and time consuming to relocate particles and more likely that particles will be lost.

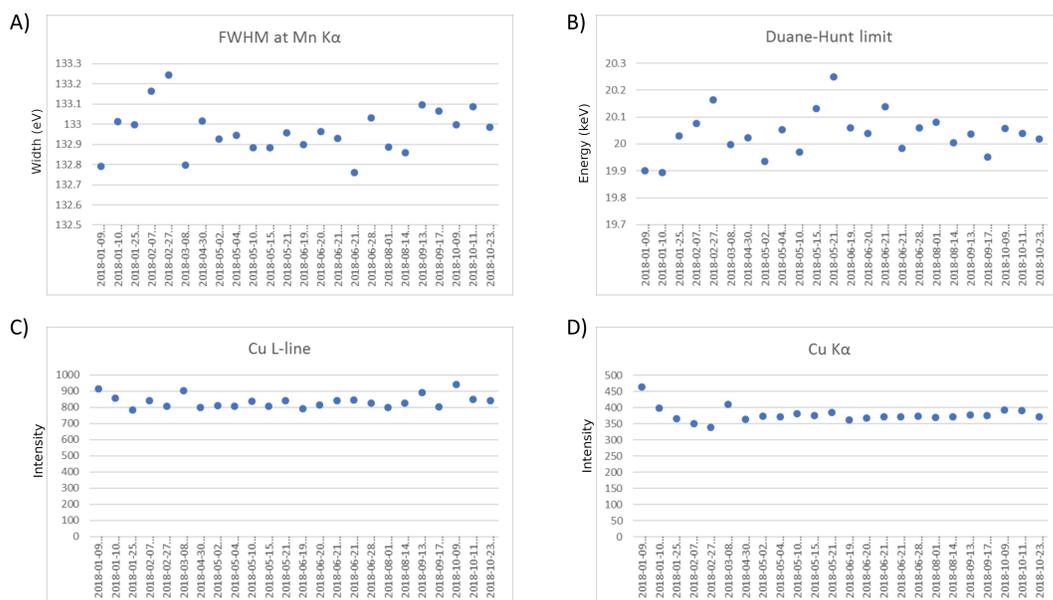


Figure 17: An example of tracking QC data collected from a Cu sample at 20 keV. A) The FWHM plot shows that the resolution of the detector is configured correctly and stable. B) The Duane-Hunt limit ensures that the beam energy is correct and that the sample is not charging. C) and D) The Cu K α (IUPAC: K-L_{2,3}) and L line data track detector efficiency, working distance and probe current.

5 Quality Control Checks

Quality control checks should be performed regularly and the results charted to catch deviations from optimal instrument performance quickly to prevent poor quality data from being collected.

5.1 EDS Detector Quality Control

5.1.0.1 Motivation

A single EDS spectrum measured from a simple controlled sample like Cu metal is capable of providing a large quantity of sensitive performance data. While the data might not provide specific information required to diagnose a problem, the data can be sufficient to flag when some part of the system is mis-performing. Collecting a single Cu EDS spectrum (and the probe current) can be done in a minute or two.

5.1.1 Materials and Apparatus

Sample A polished copper sample.

Faraday cup A Faraday cup to collect the probe current.

Picoammeter A picoammeter to measure the probe current.

5.1.2 Method

1. Configure the instrument in the nominal analysis conditions.
2. Measure the probe current.
3. Collect a EDS spectrum from the copper sample at the nominal working distance.
4. Extract the integrated K-line and L-line intensities, the Duane-Hunt limit, the total intensity from 0.1 keV to the Duane-Hunt limit and the FWHM at Cu K α (IUPAC: K-L_{2,3}) from the measured Cu spectrum. Use the measured probe current and live-time to normalize the measured X-ray intensities before plotting. Fit the position of the Cu K and L lines to extract the offset and gain calibration.
5. Plot and track these values using a control chart.
6. If one or more numbers are outside of laboratory established tolerances, identify the cause of the deviation and correct it.

5.1.3 Diagnosis

This simple test, while often non-specific as to the failure mode, is sensitive to many different instrument mis-configuration, mis-calibration or failure modes.

- The total intensity measurement is sensitive to changes in detector alignment, detector mis-configuration, detector failures, pulse processor failures, accelerating potential mis-configuration or failure, sample charging, Faraday cup or picoammeter mis-calibration or failures, and changes in working distance.
- The ratio of Cu K-lines to Cu L-lines is sensitive to changes in detector efficiency, detector obstruction and changes in take-off angle.
- The position of the Cu K-lines relative to the Cu L-lines is sensitive to the gain and offset calibration of the detector/electronics package.
- The Duane-Hunt limit is sensitive to changes in accelerating potential and to sample charging as discussed in Section 4.1.

5.2 Imaging Detector Validation

If we track the imaging detector performance under consistent conditions on a consistent sample, we can be confident that we will notice changes in the performance of the SEM components involved in forming an image.

5.2.1 Materials

- A sample consisting of a copper region and a carbon region. A small “glassy carbon” substrate half covered with copper tape works well.

5.2.2 Method

1. Configure the instrument in the nominal analysis conditions. The probe current, working distance and scan speed are particularly important.
2. Measure and adjust the probe current to the nominal value.
3. Set the brightness and contrasts on the BSED to consistent pre-established values.
4. Collect an image from the copper region at a 100 μm field-of-view and an image from the carbon region at a 100 μm field-of-view.
5. Extract histograms of the intensity data from the carbon and copper images.
6. Calculate the mean and FWHM from the intensity histograms.
7. Create a control chart to track the mean and FWHM.

6 GSR: Batch-based Validation

6.1 Quality Control Requirements in ASTM E1588-17

ASTM E1588-17 requires the laboratories to implement various quality control mechanisms for automated iGSR analysis[3]. None of the suggestions in this document should be taken to contradict or supercede these requirements. The quality control requirements set forth in ASTM E1588-17 include:

E1588-17 8.6.1.1 Establish and document a protocol.

E1588-17 8.6.1.2 Monitor EDS calibration and SEM beam current.

E1588-17 8.6.1.3 Regularly analyze a reference material with particles of known size and composition.

E1588-17 8.6.1.4 Incorporate environmental control samples into the analytical protocol.

6.2 Method Validation

6.2.1 Motivation

The validation tests up to this point have evaluated the various components to determine whether individually they are suitable for performing iGSR analysis. Quality control tests demonstrate that the system (hardware and software) is currently operating correctly. Whereas validation tests should be performed occasionally (only when the hardware changes), quality control tests should be performed on a regular basis to demonstrate that the instrument continues to perform correctly.

6.3 GSR Method Validation

6.3.1 Motivation

Method validation pulls together all the parts to determine whether the instrument, iGSR automation software and measurement protocol combination is currently capable of discovering, measuring and classifying characteristic iGSR particles, consistent gunshot residue particles, and/or associated iGSR particles under a realistic evaluation scenario[15, 16].

Method validation should not be a part of routine casework. It should be performed regularly (monthly, quarterly or certainly no less frequently than once-a-year). It should also be performed whenever there is potential for a significant change in the instrumentation such as after a preventive maintenance operation or other service-related operations.

Method validation should be performed on a pre-characterized sample that closely mimics routine casework. One possibility is to carefully characterize a laboratory constructed sample using a more careful and exhaustive search protocol. All iGSR-related particles on the sample above a threshold size should be identified, characterized and mapped. Alternatively, an engineered sample like the Planotec sample which consists of a glassy carbon substrate with deposited particles of known size, composition and placement. While the Planotec sample has a different substrate and lacks a diversity of other particle types, its advantages are a pre-established distribution of known particle sizes with a manufacturer provided map.

Two classes of errors can be characterized as *false positive* and *false negative*. A false positive occurs when a particle is either 1) detected and mis-characterized as iGSR; or 2) a true iGSR particle is counted more than once. A false negative occurs when an iGSR particle is either 1) not discovered in the search process; or 2) discovered but mis-characterized as not being iGSR by elemental analysis. Both laboratory constructed and engineered samples can be used to measure the false positive and false negative rates. However, since Planotec-like samples don't typically have many other non-iGSR particles, they are not good for identifying the first class of false positives (other particle types mis-identified as iGSR). This type of failure is less critical in practice because of the requirement that all iGSR particles must be manually confirmed before being reported.

The goal of this test is to ensure that the instrumentation, software and protocol are all working in harmony to identify and characterize iGSR particles. When a failure is discovered, it may be necessary to perform one of the earlier validation procedures to identify the specific cause of the failure. Alternatively, it may be as simple as being more careful in setting up the analysis. Regardless, this test should be performed by the casework analyst to ensure that their role in the process is also part of the evaluation.

6.3.2 Materials and Apparatus

Sample A simulated iGSR sample with an established number and position of particles similar to characteristic iGSR particles at known locations with a range of sizes from 0.5 μm to 2.0 μm (Planotec or equivalent). The sample should be sufficiently similar to a routine casework sample that an ASTM E1588-17-compliant analysis protocol can be used[3].

Alternative Sample Each laboratory can construct their own test sample as described in the introduction. As the characterization of this sample is critical, it is beneficial to ask another laboratory to verify your ground-truth results[15, 16].

The primary evaluated quantity is the number of characteristic iGSR particles (PbBaSb) with diameter greater than 1.0 μm . The measurement procedure should be demonstrated to be repeatable, unbiased and capable of detecting at least 90% of particles greater than 1.0 μm . There should not be substantial bias introduced by under- or over-counting of particles or by mis-classification of particles.

6.3.3 Method

1. Configure the instrument in the nominal analysis conditions.

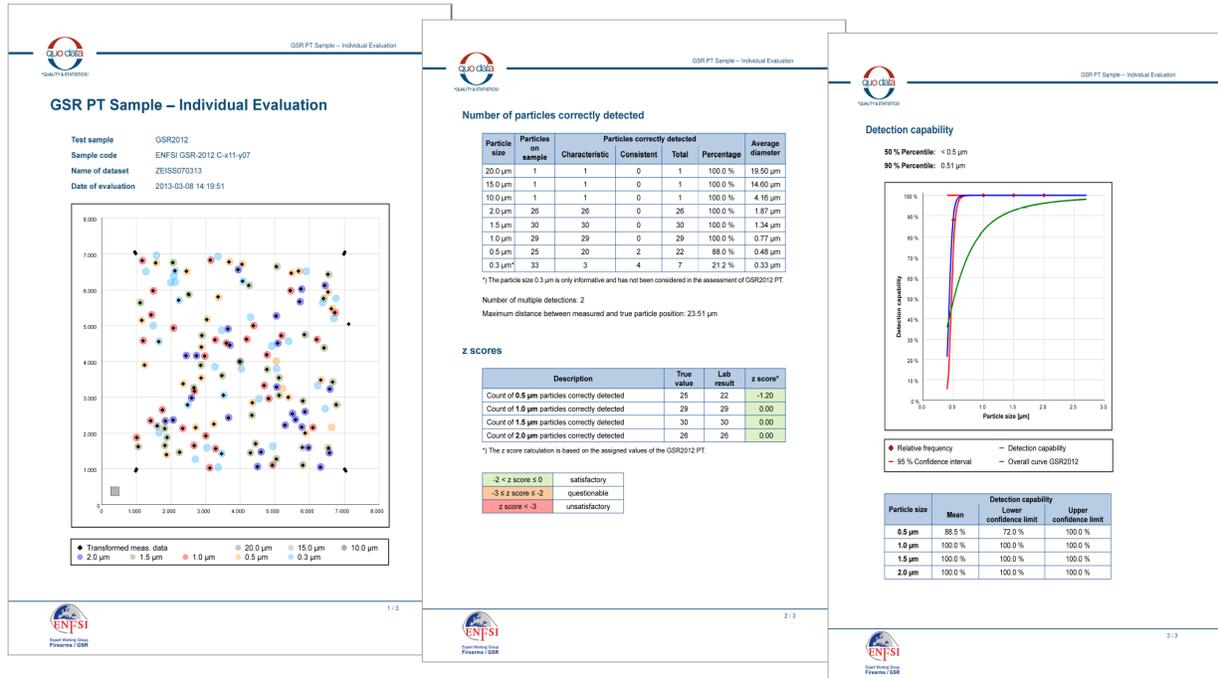


Figure 18: QuoData reports including the m_{90} statistic. Larger versions of these pages are presented in Supplemental Material C

2. Configure the iGSR automation software as though it were routine case work and compliant with ASTM E1588-17.
3. Perform an analysis of the sample.
4. Compare the results of the analysis with the pre-established known population and positions of the candidate iGSR particles.
5. Track the results with respect to efficiency of detection of particles within various size ranges.
6. Ensure that the following items are validated:
 - (a) Relative frequency of detected PbBaSb particles per particle size class
 - (b) Particles are automatically assigned to an appropriate actionable class
 - (c) Maximum distance between measured and true particle position
 - (d) Number of multiple detections
 - (e) Detection Capability m_{90} : The particle diameter, of which at least 90% of the existing/present particles are detected (as defined in the QuoData reports)
7. Plot and track the results with respect to efficiency of detection of particles within the various size ranges using a control chart.

The QuoData (Berlin, Germany) in collaboration with ENSFI has implemented an Internet-based web-tool to assist with the statistical evaluation of data collected from the Planotec simulated GSR sample (Plano GmbH, Wetzlar, Germany). This web tool combines knowledge of the particle

locations on the Planotec sample with sophisticated registration tools to generate reports like those shown in Figure 18. In addition, the web-tool offers an elaborated statistical evaluation for the minimal particle diameter, of which at least 90 % of the characteristic-like particles located on the Planotec sample are detected and correctly identified. The results of the evaluation are provided in a summary sheet (see Figure 18). A detailed description on the deployment of the evaluation tool is offered at: <https://gsr-ie.quodata.de/Quickguide.pdf>.

7 Conclusion

While it is a lot of work to document the performance of a new or modified SEM/EDS system, the effort usually pays off when there are questions about whether the instrument continues to perform at a high level. Quick tests performed daily will ensure that the major sources of instrument performance problems are identified quickly before they can compromise the data. Finally, method validation tests demonstrate that the entire instrument / software / protocol system is performing as a whole. Together, these tests form an important tool to ensure the integrity of iGSR results presented within the justice system.

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Supplemental Material A Standard Documents

This document attempts to be consistent with established relevant standards-body documents including:

A.1 ASTM Standards

ASTM International standards (<https://www.astm.org/>).

ASTM E766 – 14^{e1} Standard Practice for Calibrating the Magnification of a Scanning Electron Microscope[13]

ASTM E1588-17 Standard Guide for Gunshot Residue Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry[3]

ASTM E1508-12a Standard Guide for Quantitative Analysis by Energy-Dispersive Spectroscopy[17]

ASTM E2857-11 Standard Guide for Validating Analytical Methods[4]

A.2 ISO Standards

International Organization for Standardization (ISO) standards (<https://www.iso.org>).

ISO 15632 Microbeam analysis — Selected instrumental performance parameters for the specification and checking of energy dispersive X-ray spectrometers for use in electron probe microanalysis[10]

ISO 22309 Microbeam analysis — Quantitative analysis using energy-dispersive spectrometry (EDS) for elements with an atomic number of 11 (Na) or above[18]

ISO/TS 24597:2011 Microbeam analysis - Scanning electron microscopy – Methods of evaluating image sharpness[19]

ISO 16700:2016 Microbeam analysis - Scanning electron microscopy – Guidelines for calibrating image magnification[20]

ISO 22029:2012 Microbeam analysis – EMSA/MAS standard file format for spectral-data exchange[21]

A.3 ENFSI Documents

European Network of Forensic Science Institutes (ENFSI) documents (<http://enfsi.eu/>).

QCC-VAL-002 Guidelines for the Single Laboratory Validation of Instrumental and Human Based Methods in Forensic Science[22]

A.3.1 Other

International Union of Pure and Applied Chemistry (IUPAC) (<https://iupac.org/>).

IUPAC Gold Book Nomenclature, Symbols, Units and Their Usage in Spectrochemical Analysis - VIII Nomenclature System for X-ray Spectroscopy[23]

Supplemental Material B Glossary

accelerating potential The voltage between the SEM's electron gun and ground potential that serves to accelerate the beam electrons. The accelerating potential is usually measured in kilovolts (kV).

associated iGSR particle A particle type that is often seen in samples with characteristic iGSR particles but are also commonly seen in routine environmental samples. See GSR-related particle and ASTM E1588-17.

backlash correction A mechanism to improve the reproducibility of stage positioning. Many mechanical stages implement a mechanism to compensate for subtle positioning differences which occur when the stage axis approaches a set point from one direction rather than the other. Most often this involves making the final approach to the coordinate from the same direction each time. Typically, this involves overshooting the position when approaching from one direction and reversing the trajectory to perform the final approach.

backscattered electron detector The backscattered electron detector (BSED) is an imaging detector sensitive to the energetic backscattered electrons which scatter off the nuclei of the atoms in the sample. The backscattered electron detector signal is sensitive to differences in atomic number and less sensitive to sample topography. High atomic number elements produce more signal than low atomic number elements. The backscatter images tend to appear flatter than secondary images.

backscattered electron A backscattered electron is an energetic electron which scatters from the sample as the result of interaction with the incident electron beam. The energy of most backscattered electrons is greater than 50 % of the incident beam energy. Materials with a high mean atomic number produce more backscattered electrons than materials with a low mean atomic number.

beam energy The energy of the electrons in the probe beam when they strike the sample. This energy is typically determined by the accelerating potential unless the sample is (intentionally or unintentionally) biased relative to ground. The beam energy is usually measured in kiloelectronvolts (keV) and associated with the variable E_0 .

brightness and contrast Control the digitized image quality by changing the performance characteristics of the imaging detector (often SED or BSED). The brightness is essentially an offset that changes the levels of both the light and dark regions of the image. The contrast is a gain (or multiplier) that increases the images ability to reveal differences between low and high signal regions. The optimal brightness and contrast settings will make full use of the dynamic range of the imaging detector's digitization circuit.

BSED backscattered electron detector

candidate particle A feature that has been identified by the SEM/EDS as potentially of interest. A candidate particle may be identified by a combination of image analysis and EDS spectrum analysis.

certified reference material A reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an

accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence (see ISO Guide 31:2015).

characteristic iGSR particle Characteristic inorganic gunshot residue (a.k.a. firearm discharge residue) particles are “particles that have compositions rarely found in particles from any other source” other than discharge of a firearm. (From ASTM E1588-17.) The classic characteristic iGSR particle contains the three elements lead, barium and antimony (PbBaSb). (See GSR-related particle and ASTM E1588-17.)

characteristic X-ray X-rays of distinct energies produced by the relaxation of ionized atoms. Characteristic X-rays form element specific peaks in an EDS spectrum with the energy position of the peak defined by the shell structure of the element producing the X-ray. All elements with atomic numbers with $Z \geq 3$ (Li) produce characteristic X-rays. (See also <https://doi.org/10.1351/goldbook.C00985>)

coincidence artifact The coincidence artifact signal are events in an EDS spectrum that result from two or more X-rays entering the detector simultaneously. The deposited energies from the simultaneous X-rays can not be distinguished and the resulting signal is an event at recorded at the sum of the energies. The most intense characteristic peaks usually produce the most distinct coincidence signal at twice the energy of the characteristic peak. However, both characteristic and continuum X-rays can produce coincidence peaks which may even occur at energies above the incident electron beam energy.

cold field emission Cold field-emission is a technique for emission of electrons from an emitter through use of a needle-shaped source and a high applied electric field. Cold field-emission sources can show high brightness and very low energy dispersion and are well suited to the highest resolution electron microscopes but emission stability is poor. They are not well suited to analyses requiring stability for more than a fraction of an hour.

consistent gunshot residue particle Consistent inorganic gunshot residue (a.k.a. firearm discharge residue) particles are “particles that have compositions that are also found in particles from a number of relatively common, non-firearm sources. Particles within this group are produced through the operation of a variety of processes, equipment, or devices and can be found in the environment with varying levels of frequency.” (See GSR-related particle and ASTM E1588-17.)

continuum The continuum (or Bremsstrahlung) are X-rays generated when incident electrons are decelerated through interaction with material. Continuum X-rays can have any energy from zero up to the kinetic energy of the incident electrons. They form a slowly varying background which decreases in generated intensity with increasing X-ray energy. In contrast, characteristic X-rays form peaks at element specific energies. (See also <https://doi.org/10.1351/goldbook.B00735>)

degaussed Degaussing is the process of returning the objective lens to a state of predictable performance. The objective lens is predominantly an electromagnet and the lens strength is a linear function of the current in the wire coils. However, through time, the lens will build up a slight residual permanent magnetic field. This permanent field leads to a property called “hysteresis” in which the lens strength varies subtly from a reproducible linear function of current. Degaussing is a method of ramping the objective lens current to reset the residual

permanent field to a consistent value. Degaussing should be performed rarely particularly when configuring automated analyses as all focal conditions recorded before the degauss will no longer be valid after the degauss.

Duane-Hunt limit The Duane-Hunt limit is the highest energy at which continuum X-rays are observed in a measured spectrum. Due to conservation of energy, the deceleration of an energetic electron cannot produce a continuum X-ray with more than its kinetic energy. In fact, the intensity of the continuum decreases with X-ray energy until it goes to zero at the kinetic energy of the incident electrons (see beam energy). The energy at which the continuum goes to zero is therefore a measure of the incident electron energy and is diagnostic of problems with the electron source, accelerating potential, sample conductivity and the beam energy.

dwelt time per pixel The dwell time per pixel is the amount of clock time (“Real Time”) that the electron beam spends at each pixel while collecting image or spectrum image data. Typically, dwell times are approximately 1 μs /pixel to 100 μs /pixel for secondary electron and backscattered electron images and approximately 1 ms/pixel to 100 ms/pixel for spectrum images.

EDS energy-dispersive X-ray spectrometer

EDS spectrum An EDS data item consisting of a histogram of measured X-ray counts binned by X-ray energy.

electron optics The electro-magnetic lenses that manipulate and shape the final electron probe. The electron optics ensure that the beam electrons produced at the electron gun have the intended diameter and probe current at the sample surface. The electron optics are typically divided into a condenser lens which is responsible for controlling the final probe current and minimal achievable probe diameter and an objective lens which is responsible for ensuring that the smallest diameter of the beam is located at the sample surface. This is equivalent to saying that the objective lens is responsible for ensuring that the probe is fine as possible to resolve the smallest features possible (the image is “in focus.”)

electron gun Also called the *emitter*, the electron gun is the source of the electrons in the analytical beam. Common electron gun types include the tungsten filament gun, the Lanthanum Hexaboride (LaB_6) or CeB_6 gun, the Schottky field emission or the cold field emission gun.

electronic fields A mechanism to minimize the number of time-consuming stage movements by subdividing the image area into a grid of sub-images which are analyzed sequentially.

elevation angle The elevation angle is the angle between a plane perpendicular to the beam axis and the orientation axis of the EDS detector snout. The elevation angle and the sample position and orientation determine the take-off angle.

ENSFI ENSFI is the European Network of Forensic Science Institutes. It was founded to improve the exchange of information in the field of forensic science and to improve the quality of forensic science delivery in Europe. Besides the general work in the fields of quality and competence management, research and development and education and training, ENSFI has 17 Expert Working Groups in specialized forensic disciplines.

Faraday cup A Faraday cup is a sample designed to measure the full probe current. Faraday cups are essentially holes in which the electron beam can be fully captured. The hole ensures that

secondary and backscattered electrons are not lost to the probe current measuring process. (See also <https://doi.org/10.1351/goldbook.F02326>)

field-of-view The field-of-view is a measure of the sample area visible in an image. The horizontal field-of-view is the width of the full image area and the vertical field-of-view is the height of the full image area. If no direction is specified usually the horizontal direction is assumed. The field-of-view is typically measured in nanometers (nm), micrometers (μm), or millimeters (mm).

full-width half-maximum The full-width half-maximum (FWHM) is a measure of the resolution of a detector at a specific energy. Usually, the Mn K α (IUPAC: K-L_{2,3}) transition is used or the resolution at the measured energy is transformed to the equivalent of Mn K α (IUPAC: K-L_{2,3}). The full-width half-maximum is defined as the width of the peak measured at the low and high energies at half the intensity between the background (continuum) level and the peak intensity.

GSR-related particle One of various different types of particles that provide evidence that the sample is positively associated with the discharge of a firearm. GSR-related particles can be further subdivided according to the strength of the evidence. The strongest evidence is provided by characteristic iGSR particles which are commonly found in firearm discharge residue but are rarely found from other sources. Consistent gunshot residue particles provide less strong evidence as they are often found in firearm discharge residue but are also produced by other sources. Associated iGSR particles are commonly found associated with characteristic iGSR particles and consistent gunshot residue particles but, without a positive association, Associated iGSR particles are common enough that alone they do not provide much supportive evidence for the discharge of a firearm. (See ASTM E1588-17.)

guard bands A guard band is a region around the border of a stage field which is outside the imaged area. Guard bands are used to ensure that particles are not double counted.

iGSR inorganic gunshot residue (a.k.a. firearm discharge residue)

iGSR automation software Gun-shot residue automation software is a computer program that takes control of a scanning electron microscope with energy dispersive X-ray spectrometer to perform the search-and-measurement process for candidate characteristic iGSR particles and consistent gunshot residue particles. The automation software takes control of the SEM to collect images as necessary to discern iGSR particles. The images are processed to extract morphological metrics from the particles. The beam and EDS detector are automated to acquire X-ray spectra of the particles. The images and spectra are processed to assign candidate particles to classes based on elemental make-up. The GSR automation software is likely to be part of a more comprehensive package of EDS and SEM imaging tools from the same vendor. The automation software is likely to use the same data acquisition hardware as the more comprehensive toolset which may be different from the SEM vendor's data acquisition software. When performing validations tests for GSR attempt to perform the test first in the GSR automation software. When that isn't possible use the same vendor's EDS software and, finally if none of the other software is capable, use the SEM vendors software.

image rotation The image rotation is an adjustable parameter on most modern SEM instruments. The image rotation feature changes the direction in which the electron beam rasters over the

sample. The image rotation can be used to rotate the image of a feature by a full 360°. In modern SEMs, the image rotation is typically calibrated such that zero rotation corresponds to scanning image rows which are parallel to the x-axis stage motion. Older SEMs may require manual rotation compensation to ensure that the image remains aligned as the strength of the objective lens magnetic field is varied.

image dimension The dimensions of a digitized image in terms of the number of pixels in the horizontal and vertical dimensions.

Lanthanum Hexaboride (LaB₆) Lanthanum hexaboride crystal cathodes are sometimes used instead of tungsten-hairpin filaments for thermionic emission of electrons due to the relatively low work function (around 2.5 eV). The cathode lifetime and imaging performance tend to be better than tungsten filament but not as good as Schottky field emission or cold field emission. The current stability is usually better than a tungsten filament cathode. Sometime CeB₆ is used in place of LaB₆.

lithium-drifted silicon detector (Si(Li)) An older style of energy dispersive X-ray spectrometer that uses a large area capacitor-like cathode and anode structure to collect the electrons generate when an X-ray is absorbed by the detector. The large capacitance of these detectors limits throughput.

live-time The live-time is the time during which the X-ray detector is ready to measure X-ray events. The live-time is less than the *real-time*, the clock time, because the detector's electronics cannot measure new events while they are processing previous events. The number of measured X-ray events should increase linearly with live-time. Live-time is related to probe dose, the product of the live-time and the probe current.

magnification Magnification is defined as the ratio of the size of the image of the object to the size of the object. A display device (film, page, monitor, etc.) and the dimensions of the display device must be well defined to permit interpretation of the scale. Transferring an image from one device to another is likely to change the magnification. This ambiguity makes magnification a poor metric for digital images. Better choices to indicate scale are scale bars, field-of-view measures and pixel scale metrics.

method validation Tests performed to determine whether the combination of the instrumentation and SOPs are adequate for the intended purpose.

objective lens The objective lens is the final magnetic lens before the electron beam interacts with the sample. The objective lens can be adjusted to change the point at which the beam comes into focus.

optimal working distance The optimal working distance is the working distance at which the X-ray detector produces the maximum signal for a given probe current. Analyses should be performed at the optimal working distance because small variations in working distances around this value produce little variation in measured X-ray flux.

output count rate The number of X-ray events that are recorded in the spectrum per unit clock time (“real-time”). Output count rate (OCR) is often reported as “stores per second.” This should not be confused with the *input count rate* (ICR) which is the number of X-rays entering the detector each unit clock time.

over-voltage The over-voltage is a measure of how much the kinetic energy in an electron beam exceeds the threshold to ionize a specific shell in a specific element. The over-voltage is a dimensionless quantity equal to the ratio of the beam energy over the ionization edge energy. As a typical rule-of-thumb, it is suggested that an over-voltage of 2 or more is considered ideal to generate enough X-rays for quantification and an over-voltage of 1.5 is the practical minimum. The over-voltage, U , is equal to $U = E_0/E_s$ where E_0 is the beam energy and E_s is the edge energy, the minimum energy required to ionize the s -shell where s is typically one of the K, L_{III} or M_V shells.

particle size Particle size is a measure of the dimensions of a particle. Each vendor provides various different particle size metrics that characterize different attributes of the particles morphology. Most of these metrics are proprietary and while some can be compared crudely from vendor to vendor, they should be viewed as quantitative with large error bars. Some common particle size metrics include *average diameter*, *equivalent circular diameter*, *maximum diameter*, *minimum diameter*, *feret diameter* and *bounding box diameter*. The most robust of these metrics is probably the equivalent circular diameter because it uses all the pixels associated with the candidate particle. It is worth remembering that the SEM image projects a three-dimensional particle onto a two-dimensional plane discarding one-dimension of information. There are biases inherent in how the particle orients itself (for example, fibers tend not to orient on end) that limit our ability to make volumetric predictions from the particle size data.

picoammeter A current meter designed to measure currents in the picoamp (pA) or nanoamp (nA) ranges.

probe dose The probe dose is a measure of the number of electrons striking the sample while the X-ray detector is ready to measure the resulting X-ray. The probe dose equals the product of the live-time and the probe current. The measured number of X-ray events should be proportional to the probe dose (all else remaining constant).

probe current The probe current (or *specimen current*) is a measure of the number of electrons in the electron beam that strike the sample. It should not be confused with the emission current which is the number of electrons emitted from the gun. The probe current is usually measured in picoamps (pA) or nanoamps (nA) using a Faraday cup and a picoammeter.

pulse processor The pulse processor is an electronics package designed to convert the pulse of electrons generated when an X-ray strikes the active EDS detector element into a measure of the X-rays energy. Modern pulse processors convert the analog ramp signal into a digital (binary) signal early in the conversion process and then use this digital signal to detect pulse pile-up, to measure the X-ray amplitude and to determine live-time.

pulse pileup correction Pulse pileup correction is a software means to address the problem of pulse pile-up by processing the final spectrum to estimate the contribution of pulse pile-up at each X-ray energy and replace these events in their original location. It is a process which is based on a careful understanding of the pulse processor performance characteristics and a probabilistic algorithm which estimates for each energy how likely it is that a measured event represents two simultaneous X-rays rather than one X-ray.

pulse pile-up Pulse pile-up (or *sum peak*) is the detector artifact that occurs when two or more X-rays enter the detector almost simultaneously. If two X-rays enter the detector separated

by too little time, the signal from the X-rays merges and the resulting measured energy is the sum of the individual X-ray energies. Pulse pile-up can be limited by reducing the flux of X-rays entering the detector. This is usually done by reducing the probe current.

pulse process time The time dedicated by the EDS detector pulse processor to measuring the energy of each X-ray event. The pulse process time determines the throughput and resolution of an EDS detector. Longer pulse process times produce lower throughput but higher resolution. Shorter pulse process times produce higher throughput but lower resolution (broader characteristic peaks). Usually, the best trade-off of throughput vs resolution for iGSR analysis is at moderate pulse process time settings. Pulse process time is also called the throughput setting.

real-time The real-time is the measure of EDS acquisition time that is equal to the standard *clock-on-the-wall* time. Real-time is in contrast to live-time in which only the time during which the detector is not processing X-ray events is accumulated.

sample charging When the electron beam interacts with a sample, the electrons in the beam will form a current to ground if there is a conductive path. (Ground can be thought of as a large sink for electrons which defines what we locally mean by 0 V.) However, if there is no conductive path to ground, the electrons may accumulate on the sample leading to an excess of negative charge near the surface of the sample. The accumulated charges may lead to artifacts in image formation and X-ray production. With some combinations of sample and beam energy (typically below 3 keV), it is possible for positive charging to occur in which the interaction of the electron probe causes more electrons to exit the sample (as secondary electron and backscattered electron) than arrive in the probe.

scan generator The scan generator is the electronics package responsible for generating the currents in the scan coils that deflect the electron beam. The scan generator is typically controlled by a digital-to-analog converter that translates image pixel coordinates into scan coil currents leading to predictable amounts of beam deflection on the surface of the sample.

scan speed The scan speed is the speed at which the electron probe is rastered across the sample. Typically, scan speed is measured as the dwell time per pixel and is typically in the range 0.1 μs to 10 μs . Increasing scan speed (decreasing the pixel dwell) makes images more noisy.

Schottky field emission Schottky field-emission is a technique for enhancing thermionic emission of electrons from an emitter through use of a needle-shaped source and an applied electric field. Schottky field-emission is used to produce stable, high brightness and low chromatic dispersion electron guns.

secondary electron detector The secondary electron detector (SED) detects low-energy electrons that emerge from the sample as the result of interactions with the incident electron beam (secondary electron). Secondary electron detectors tend to produce a signal which accentuates topography in a way that looks similar to a visible light image illuminated from the position of the detector.

secondary fluorescence The incident electron beam generates X-rays which in turn may generate subsequent, less energetic, X-rays called secondary fluorescence. Secondary fluorescence may be generated in the sample or in the region around the sample.

secondary electron A secondary electron is a low energy (less than 50 eV) electron that is emitted as a result of the interaction of the electron beam with the sample. Emission of secondary electrons is very sensitive to the properties of the surface including surface layers and topography. Secondary electrons are also sensitive to the electronic band structure of the sample. Because of the complexity of the physics, there are few predictive models of secondary electron production.

SED secondary electron detector

SEM scanning electron microscope

SEM/EDS scanning electron microscope with energy dispersive X-ray spectrometer

silicon drift detector (SDD) A modern style of energy dispersive X-ray spectrometer that uses shaped fields to collect the electrons generated when an X-ray is absorbed by the detector to a tiny anode. The throughput and resolution of SDD detectors exceeds that of the older Si(Li) detectors.

SOP standard operating procedure

spectrum image A spectrum image is the X-ray spectrum equivalent of a backscattered electron detector or secondary electron detector image. Each pixel in the image is represented by an X-ray spectrum and not just a single intensity value. A spectrum image can be processed to produce elemental images (*X-ray elemental maps*) that represent the amount of an element at each pixel in the image. Spectrum images are a type of hyper-spectral image.

take-off angle The take-off angle is the angle from which detected X-rays emerge from the sample. The take-off angle is a function of the elevation angle and the position and orientation of the sample.

tungsten filament A tungsten filament cathode is a common and inexpensive source of thermionic emission of electrons for scanning electron microscopes. The image resolution achievable with a tungsten filament cathode can be an order-of-magnitude worse than that of a field-emission microscope. None-the-less tungsten filament cathodes are more-than-adequate for the most routine electron microscopy tasks and are particularly well suited to the current and imaging performance required for X-ray microanalysis.

working distance The working distance is the physical distance between the pivot point of the imaging scan coils (in the objective lens) and the sample surface. The working distance on modern instruments can be *estimated* by focusing the beam on the sample and reading the calculated working distance as reported by the SEM control software. The accuracy of this number can be variable depending upon many factors but it can be reproducible if the objective lens is degaussed before a careful final focus.

X-ray A quantized unit of electro-magnetic radiation (a photon) more energetic than optical photons but less energetic than gamma ray photons (approximately 50 eV to 100 keV). X-rays are typically produced via the relaxation of an ionized atom or the deceleration or deflection of an energetic electron.

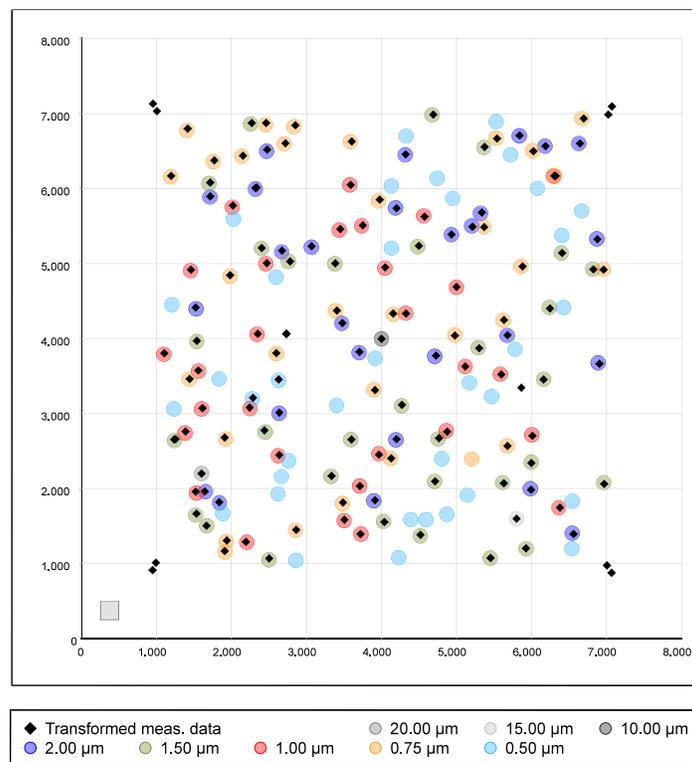
Supplemental Material C QUODATA Method Validation Report



GSR PT Sample – Individual Evaluation

GSR PT Sample – Individual Evaluation

Test sample GSR2013
Sample code ENFSI GSR-2013 A
Name of dataset Validation - run1
Date of evaluation 2015-10-16 11:05:13



1 / 3

Figure 19: ENFSI / QUODATA Method Validation Test Report page 1. A particle map from a single run of the validation sample showing both discovered and missed particles.

Number of particles correctly detected

Particle size	Particles on sample	Particles correctly detected				Average diameter
		Characteristic	Consistent	Total	Percentage	
20.00 µm	1	1	0	1	100.0 %	19.53 µm
15.00 µm	1	1	0	1	100.0 %	14.69 µm
10.00 µm	1	1	0	1	100.0 %	9.77 µm
2.00 µm	27	27	0	27	100.0 %	1.81 µm
1.50 µm	31	31	0	31	100.0 %	1.27 µm
1.00 µm	29	29	0	29	100.0 %	0.84 µm
0.75 µm	32	28	3	31	96.9 %	0.63 µm
0.50 µm	36	1	1	2	5.6 %	0.37 µm

Number of multiple detections: 2

Maximum distance between measured and true particle position: 29.4 µm

Z scores

Description	True value	Lab result	z score*
Count of 0.50 µm particles correctly detected	36	2	-9.4
Count of 0.75 µm particles correctly detected	32	31	-0.3
Count of 1.00 µm particles correctly detected	29	29	0.0
Count of 1.50 µm particles correctly detected	31	31	0.0
Count of 2.00 µm particles correctly detected	27	27	0.0

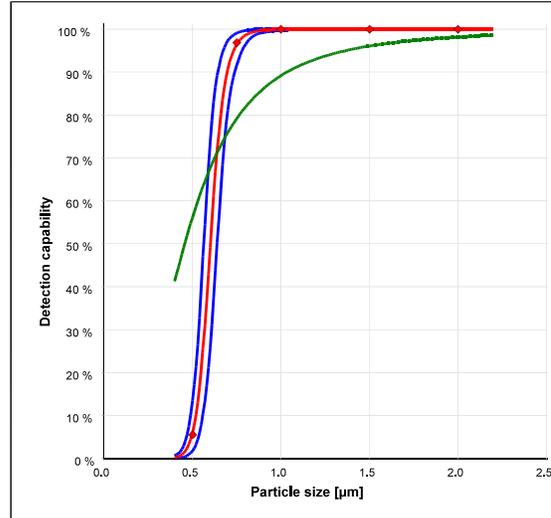
*) The z score calculation is based on the assigned values of the GSR2013 PT.

-2 < z score ≤ 0	satisfactory
-3 ≤ z score ≤ -2	questionable
z score < -3	unsatisfactory

Figure 20: ENSFI / QUODATA Method Validation Test Report page 2. Summary tables which report by size the number of particles discovered and missed and the z-score summarizing detection capability by particle diameter.

Detection capability

50 % Percentile: 0.60 μm
90 % Percentile: 0.70 μm



Particle size	Mean	Detection capability	
		Lower confidence limit	Upper confidence limit
0.50 μm	5.9 %	1.8 %	13.5 %
0.75 μm	96.5 %	91.7 %	99.1 %
1.00 μm	100.0 %	99.8 %	100.0 %
1.50 μm	100.0 %	100.0 %	100.0 %
2.00 μm	100.0 %	100.0 %	100.0 %

Figure 21: ENSFI / QUODATA Method Validation Test Report page 3. A plot showing detection capability as a function of particle size with confidence intervals.

Supplemental Material D Processing X-ray Spectrum Image Data using ImageJ

ImageJ[14] is a full-featured open-source software package for scientific image analysis. ImageJ is available bundled with many useful plug-ins through the “Fiji” (a self-referential acronym that stands for “Fiji is just ImageJ”) project at <https://fiji.sc/>[24]. ImageJ and Fiji require only the Java Runtime Environment (JRE) (Oracle Corporation, Redwood Shores, CA) which is available on many operating systems including the more recent Microsoft Windows, Apple OS X and Linux platforms. A recent implementation of the JRE is included with some of the FIJI installers.

Other scientific commercial and non-commercial imaging software is capable of performing similar tasks as ImageJ and can be used if they provide equivalent functionality.

1. Export the spectrum image data in a “block-of-bytes” or RPL/RAW format. Most EDS vendors permit exporting spectrum image data as a 3D binary matrix where each position in the matrix represents the number of X-ray events of a specific energy range recorded at that x-y coordinate.
2. Import the data block into ImageJ.
3. Rotate the data block until the block is visualized as an x-y image with the z-axis being the hidden dimension.
4. Identify a range of z-value associated with the Cu K-line family. This range of channels can be computed knowing the range of energies (7.75 keV to 9.15 keV) and the zero-offset (z) and gain (g) settings on the detector. The channel range is approximately $[(7750 - z)/g, (9150 - z)/g]$.
5. Use the stack tool to integrate the counts range of channels for each x-y coordinate.
6. Convert this into a gray-scale image.
7. Colorize the gray-scale image with the heat color map.
8. Display the heat color map in 3D projection.

Supplemental Material E Extracting image histogram data using ImageJ

1. Open a gray-scale image in ImageJ.
2. Select the → menu item.
3. Select the button to display the histogram in a separate window in table form.
4. Select the → menu item from the histogram table window to save the values as a Comma Separated Value (CSV) file.
5. Import the CSV file into a spreadsheet to view and plot.