

Technical Procedure for Scanning Electron Microscope/ Energy Dispersive X-Ray System (SEM/EDX) for GSR Casework

- 1.0 Purpose** – This technical procedure shall be followed for the operation of the Scanning Electron Microscope/Energy Dispersive X-Ray System (SEM/EDX). This procedure shall be used for GSR casework.
- 2.0 Scope** – This procedure applies to the ASPEX System. These instruments are used for high resolution and magnification imaging with enhanced depth of field for trace evidence and non-destructive elemental analysis of gunshot residue particles, paint, metals, powders, and other trace particulate material.
- 3.0 Definitions** – N/A
- 4.0 Equipment, Materials, and Reagents**
- 4.1 Equipment**
- ASPEX Variable Pressure Scanning Electron Microscope/Energy Dispersive X-Ray System
- 4.2 Materials**
- Mounting tweezers for SEM lifts
 - Kimwipes
 - Nitrile gloves
 - Adhesive lifts with carbon-backed tape for blank standard
 - Copper (Cu) Standard
 - Stainless Steel 316 Standard
 - PLANO GSR Standard
- 4.3 Reagents** – N/A
- 5.0 Procedure**
- 5.1 ASPEX Variable Pressure SEM/EDX**
- 5.1.1 Start-Up Procedure & Loading of Samples**
- 5.1.1.1** Turn instrument ON. Enter user name and password when prompted. The ASPEX engine will start automatically.
- 5.1.1.2** After ASPEX engine loads, double click “Perception” icon.
- 5.1.1.3** Move black lever on the stage from the downward position towards the upward position. This will begin the process of venting the chamber. The black lever will automatically move to the full upward position once the chamber is at atmospheric pressure.

- 5.1.1.4 Pull out the stage from the microscope column and load the sample(s) into the specimen holder using mounting tweezers. Insert specimen holder into stage and tighten the thumb screw on the right side of the stage.
- 5.1.1.5 Gently slide stage back inside the microscope column. Push black lever downward to begin the vacuum process. Choose "Variable Pressure" checkbox in the Perception software. Default variable pressure setting is 0.1 Torr. Click Power Button (turns bright green when power is ON and SEM/EDX is at proper vacuum).
- 5.1.1.6 Set stage point to copper standard. Saturate the beam and align filament at 20 kV by adjusting the filament drive up to around 68 % or until an image appears. Turn the filament drive up slowly until the image is at maximum brightness. Select "Source Image Mode" from the drop down menu to view the emission image. Adjust filament drive until the emission image is slightly under saturation. Select "Line Scan" option from the drop down menu. Adjust the X and Y axis controls to adjust the line scan to its maximum height. Close the "Line Scan" mode.

5.1.2 Setting up a GSR Analysis in ASPEX

- 5.1.2.1 All GSR method parameters shall be found in APPENDIX 1.
- 5.1.2.2 Identify the sample name and information for each adhesive lift in the specimen holder.
- 5.1.2.3 Set the three stage positions for each adhesive lift and store these points along with the correct working distance.
- 5.1.2.4 Adjust the X-ray dead time on the copper standard to 10 % - 20 %.
- 5.1.2.5 Click on GSR tab, then Set-Up. Select New and choose 13 Pin Sampler.
- 5.1.2.6 Enter sample description in Analysis, including Laboratory number and Forensic Scientist's name.
- 5.1.2.7 Select Quality Check then click on "Perform EDS Check" Box, select "Point 1" box, and verify EDS check every "1" analysis.
- 5.1.2.8 Set the stage point to the copper standard. Make sure both the background and the copper standard are visible within the screen. Select Analysis and Thresholds to adjust brightness and contrast so a threshold setting between 16 and just above 224 is achieved.
- 5.1.2.9 When all settings are correct, select Save under Set-Up.
- 5.1.2.10 Select "Go" button to start automated analysis.

5.1.3 Collection and Storage of Data GSR on ASPEX

- 5.1.3.1 Review results of automated analysis.
- 5.1.3.2 Select Report icon. Report icon provides a list of particles found on the adhesive lifts.
- 5.1.3.3 Select a particle of interest and click Relocate.
- 5.1.3.4 Perform an elemental analysis on a particle and, if the particle contains barium, antimony and lead and is spheroidal in morphology, select Save.
- 5.1.3.5 Click “Report” button to generate a particle report for all confirmed gunshot residue particles.
- 5.1.4 **Performance Check** – Performed monthly if in use.
 - 5.1.4.1 Load a Copper standard; saturate the beam and align the filament at 20 kV. Obtain an image of the standard and focus on it.
 - 5.1.4.2 Select Control, Right Mouse click and click on Calibrate from the drop-down menu.
 - 5.1.4.3 Collect X-Ray spectra of the Copper Standard. The zero offset and gain of the pulse processor are being extracted while the spectrum is being collected. When the pulse processor is calibrated, zero and gain will be displayed in Green. Record the date, value of the Gain (10.000 $\pm$ 5 %), and initials on the Aspex SEM/EDX Performance Check Log.
 - 5.1.4.4 Pass Criteria: The value of the Gain shall be 10.000 $\pm$ 5 %. If this value is not obtained $\pm$ 5% maintenance shall be performed or a service engineer called. Once maintenance is performed and this criteria is met, the instrument may be returned to service.
 - 5.1.4.5 Remove Copper Standard from stage and load the Stainless Steel 316 Standard.
 - 5.1.4.6 Saturate the beam and align the filament at 20 kV. Obtain an image of the standard and focus on it. Identify the peaks and save the spectra.
 - 5.1.4.7 Print out the spectra and place in the Aspex Performance Check Manual.
 - 5.1.4.8 Pass Criteria: The following peaks should be present: Fe, Ni, Si, Mo, Cr, and Mn. If these peaks are not present maintenance shall be performed or a service engineer called. Once maintenance is performed and the criteria met, the instrument may be returned to service.
- 5.1.5 **Shut Down Procedure**
 - 5.1.5.1 The SEM vacuum always remains on.

5.1.5.2 Ensure that the X-Ray system is not actively collecting spectra.

5.1.5.3 Turn filament drive to 0 % and close Perception software.

5.1.5.4 Select Start icon then Shut Down.

5.1.6 Performance Verification for New Instrument Set-Up

5.1.6.1 A new SEM with EDX detector shall be installed by a certified engineer according to the manufacturer's guidelines.

5.1.6.2 Spectra shall be obtained from a Copper standard and a Stainless Steel 316 standard.

5.1.6.3 An analysis shall be performed on a PLANO GSR Standard and then compared to the known amount of GSR particle's on that standard.

5.1.6.4 If the spectra are acceptable and all major peaks identified, the instrument is approved for use in casework.

5.1.7 **Standards and Controls** – This instrument requires the use of a Copper Standard for calibration performance check. In addition the Stainless Steel 316 and PLANO GSR standards are used for performance verifications. These standards do not require special storage requirements.

5.1.8 **Maintenance** – Routine maintenance shall be performed such as changing pump oil, changing out a filament, etc. Any maintenance performed shall be documented in the Maintenance Log for that particular instrument.

5.2 **Sampling and Sample Selection** – No sampling is performed. When sample selection occurs, it shall be based on the Forensic Scientist's training and experience

5.3 **Calculations** - N/A

5.4 **Uncertainty of measurement** - N/A

6.0 **Limitations** – N/A

7.0 Safety

7.1 The greatest safety concern is radiation from the X-ray tube. The x-ray system is monitored for leaks on a regular basis.

7.2 There is a high voltage/current safety concern which can cause electrocution. Avoid contact with any live circuitry components. Potentially lethal voltages exist with the high voltage x-ray supply.

8.0 **References**

ASTM Standard E 1588 – 95 (2001), “Standard Guide for Gunshot Residue Analysis by Scanning Electron Microscopy/ Energy—Dispersive Spectroscopy.” ASTM International, West Conshohocken, PA, 2001, www.astm.org.

Andrasko, J. “Detection of Gunshot Residue on Hands by Scanning Electron Microscopy.” *Journal of Forensic Sciences* 22.2 (1977): 279-287.

DeGaetano, Douglas, et al. “A Comparison of Three Techniques Developed for Sampling and Analysis of Gunshot Residue by Scanning Electron Microscopy/Energy Dispersive X-Ray Analysis (SEM/EDX).” *Journal of Forensic Sciences* 37.1 (1992): 281-300.

Nesbitt, R.S., et al. “Detection of Gunshot Residue by Use of the Scanning Electron Microscope.” *Journal of Forensic Sciences* 21.3 (1976): 595-610.

Wolten, G.M., et al. “Final Report on Particle Analysis for Gunshot Residue Detection.” The Aerospace Corporation, ATR-77 (7915)-3, 1977.

9.0 Records

- ASPEX SEM/EDX Performance Check Log
- Maintenance Log
- Results For Instrumental Analysis Of Evidence For Gunshot Residue

10.0 Attachments

- APPENDIX 1: Guidelines for ASPEX GSR Acquisition parameters.

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original ISO Document
02/01/2013	2	Request for Instrumental Analysis of Evidence for Gunshot Residue was added as a record. Revised title for Attachment 1 and measure dwell time.
09/30/2013	3	5.1.4 - Removed from performance check that it shall be performed by first person to use the instrument
10/18/2013	4	Added issuing authority to header

APPENDIX 1: Guidelines for ASPEX GSR Acquisition parameters

The default parameters for GSR are saved as gsr.atp template file.

Parameter	Setting	Description
Detector	BSED	Silicon Drift X-ray Detector
Video	Manual: Default	
Background stabilize	Off	Enabling background stabilize will acquire and analyze the stage field before analyzing the stage field. Enabling this option increases the analysis duration
Magnification	Field size is 178 µm	Each electronic field analyzed will measure 178*178 µm. The magnification is depended upon the size of the display area. Displaying the same field size on a larger monitor is equivalent to an increase in the magnification.
Focus	Use stage coordinates	
Number of electronic fields	5x5	
Search Thresholds	128-255	
Search dwell time	4 µs	Decreasing the search dwell time will decrease the analysis duration on the expense of possibly missing particles low in contrast.
Search grid dimensions	512x512	The search grid dimensions together with the electronic field size determine the beam spacing. The default beam spacing is 0.35 µm.
Measure Thresholds	64-255	
Measure Dwell time	16 µs	Increased dwell time results in more accurate morphology.
Randomize field order	Off	
Max. number of stage fields	1000	
Maximum number of particles	4000	
Maximum analysis duration	600	
Maximum number of particles per e-field	100	
Guard band	0	Electronic fields are abutting
Save stage field images	Off	
Morphology: Size criteria	0.3-89	Minimum and maximum size criteria based upon selected field size and search grid dimensions.
EDS: Collect compositional Data	On	
Nominal duration	1	Collect a 1 second spectrum to classify

		particle.
Maximum duration	2	Collect a 2 second spectrum when classification rule calls for MaxEDS.
Minimum counts	300	
Target counts	2500	
Elemental threshold	1	
EDS result	Normalized Counts	
EDS vector file	Gsrchem	
Classification rule file	Gsrclass	
Raster style	Chord Raster Mode	