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 and the LEO/Oxford Systems. 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
- LEO 1450 Variable Pressure Scanning Electron Microscope
- Oxford Energy Dispersive X-ray System (SDD Detector)

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
- Manganese (Mn) / Rhodium (Rh) 5492 Standard
- Nitrogen gas, compressed (Purity Grade 5.0)

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 Load Perception software.

5.1.1.2 Vent chamber, place samples into specimen holder, and load holder into stage.

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 Turn on the power and saturate the beam.

5.1.2.4 Adjust the X-ray dead time on the copper standard to 10 % - 20 %.

5.1.2.5 Perform instrumental setup of each sample loaded into the sample stage previously. Include with these samples the instrument blank and control.

5.1.2.6 Enter sample description in Analysis, including Laboratory number and Forensic Scientist's name.

5.1.2.7 Select Perform EDS Check and verify EDS check after each analysis.

5.1.2.8 Set the stage point to the copper standard. Use the backscatter detector and adjust its operational threshold level.

5.1.2.9 When all settings are correct, select Save under Set-Up.

5.1.2.10 Start automated analysis.

5.1.3 Particle Relocation and Identification

5.1.3.1 Review results of automated analysis. Assess instrumentally identified particles and determine whether there are Characteristic Gunshot Residue Particles. Use this information to formulate conclusion on sample. Characteristic Gunshot Residue Particle(s) are spheroidal particle(s) that contain antimony, barium, and lead. Characteristic Gunshot Residue Particles may also contain the following elements: silicon, calcium, aluminum, copper, iron, sulfur, phosphorus, zinc, nickel, potassium, chlorine, and tin. Note additional particles present in the population, including particles that contain antimony, barium, and/or lead.

5.1.3.2 Relocate and create documentation of image and spectra of Characteristic Gunshot Residue Particle(s).

5.1.3.3 When analysis and relocation are complete, shut down the instrument and remove the samples.

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 ±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 ±5 %. If this value is not obtained ±5% maintenance shall be performed or a service engineer called. Once maintenance is performed and this criterion is met, the instrument may be returned to service.

5.1.4.5 Remove Copper Standard from stage, 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 LEO/Oxford SEM/EDX

5.2.1 LEO SEM Start-Up Procedure & Loading of Samples

5.2.1.1 Load the SmartSEM software.

5.2.1.2 Load Fantasia Software & open RemCOn32 software.

5.2.1.3 Using mounting tweezers, place samples in the holder and note position of each sample. Tighten the screws on the holder for each mounting position. Place the holder back on the stage, ensuring that the flat edge of the mount is against the flat area of the stage.

5.2.1.4 Turn on the Extra High Tension (EHT) and Filament.

5.2.1.5 Adjust saturation of the filament slighty below or at the second crossover.

5.2.1.6 Adjust the working parameters of the instrument as necessary by accessing Tools through the User Toolbar.

5.2.2 Setting up a GSR Analysis in Oxford

5.2.2.1 Load INCA Software. Select GSR Tab.

5.2.2.2 Identify the Project name and sample information for each adhesive lift in the holder.

5.2.2.3 Go to the Recipe tab. Create a database and select "NC Crime Lab GSR" recipe. ***NOTE* Do not "Lock" or "Embed database within the project file."**

5.2.2.4 Go to the Microscope tab. Set the stage point to the Mn standard. Increase the Mag to 766 and deadtime to 10 – 30%.

5.2.2.5 Go to the Quant Optimization tab. Choose Mn standard and acquire a spectrum.

5.2.2.6 Go to the Area Layout, select areas tab, and highlight the adhesive lift associated with each sample.

5.2.2.7 Go to the Feature Detection tab. Select Calibration and Mn/Rh standard. Acquire a spectrum and optimize the working parameters to between 145 and 255.

5.2.2.8 Run automated analysis.

5.2.3 Particle Relocation & Identification

5.2.3.1 Review results of automated analysis. Assess instrumentally identified particles and determine whether there are Characteristic Gunshot Residue Particles. Use this information to formulate conclusion on sample. Characteristic Gunshot Residue Particle(s) are spheroidal particle(s) that contain antimony, barium, and lead. Characteristic Gunshot Residue Particles may also contain the following elements: silicon, calcium, aluminum, copper, iron, sulfur, phosphorus, zinc, nickel, potassium, chlorine, and tin. Note additional particles present in the population, including particles that contain antimony, barium, and/or lead.

5.2.3.2 Relocate and create documentation of image and spectra of Characteristic Gunshot Residue Particle(s).

5.2.3.3 When analysis and relocation are complete, remove the samples.

5.2.4 Performance Check (Resolution vs. Process Time) – Performed monthly if in use.

5.2.4.1 User must exit INCA software to access the program for this check.

5.2.4.2 Click INCA Monitor icon.

5.2.4.3 Select Options, Monitor Options, and Engineer login. Enter the password.

5.2.4.4 In "Test Details" tab, Highlight Resolution vs. Process Time then click "add." Follow prompts until it asks for user to adjust counts on a standard.

5.2.4.5 Load a standard such as Manganese, then adjust count rate between 4000 and 8000 counts using SEM spot size. (Do not follow the on screen suggestion of 1000 counts; that specific criterion is only for detectors cooled by liquid nitrogen and is not the criterion for the SDD detector.)

5.2.4.6 Pass Criteria: The extrapolated strobe resolution at process time 5 or 6 shall be no greater than the detector resolution of 127 eV. If resolution requirement is not met, maintenance shall be performed or a service engineer called. Once maintenance is performed and this criterion is met, the instrument may be returned to service.

5.2.4.7 When test is complete, select REPORT icon and Print.

5.2.4.8 Load and analyze the Stainless steel 316 Standard. Print report. 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 this criterion is met, the instrument may be returned to service.

5.2.4.9 Fill out LEO/Oxford SEM-EDX Performance Check Log and place all printed reports in LEO/Oxford Notebook.

5.2.5 Shut Down Procedure

5.2.5.1 Turn off the filament & close the SEM operating window.

5.2.5.2 Close the SEM user software. Close the EMServer. Close the RemCon32 software.

5.2.5.3 Close all windows in INCA software.

5.2.6 Performance Verification for New Instrument Set-Up

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

5.2.6.2 Spectra shall be obtained from a Manganese/Rhodium 5492 Standard, Copper Standard and a Stainless Steel 316 Standard.

5.2.7 Standards & Controls - This instrument requires the use of Manganese/Rhodium 5492 standard for performance checks. In addition the Stainless Steel 316 Standard shall be used for performance verifications. These standards have no special storage requirements.

5.2.8 Maintenance - Routine maintenance shall be performed such as changing pump oil, checking liquid chiller status, and replacing a filament. Any maintenance performed shall be documented in the maintenance log for that particular instrument.

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

5.4 Calculations - N/A

5.5 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
09/05/2014	5	Updated header to Physical Evidence Section – Trace Unit, issuing authority to Physical Evidence Section Forensic Scientist Manager. 5.1.1.2, 5.1.1.3, 5.1.2.3, 5.1.2.5, 5.1.2.7, 5.1.2.8, 5.1.2.10, 5.1.3, 5.1.3.1,

		5.1.3.2, and 5.1.3.5 were updated to clarify the instructions for use. Added Appendix 1. Removed: 5.1.1.1, 5.1.1.4, 5.1.1.5, 5.1.1.6, 5.1.3.2, 5.1.3.4. Moved instructions to SEM Training.
07/01/2016	6	Added Instrumentation for GSR Analysis (Section 5.2). GSR samples can now be examined on the LEO/Oxford SEM/EDX.

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	Backscattered electron imaging mode
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.
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	