GUIDELINE FOR THE FORENSIC EXAMINATION OF PAINT BY SEM/EDS			
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GENERAL REMARK

This guideline assumes prior knowledge in the forensic discipline. It is based on consensus among the relevant forensic experts and reflects the accepted practices at the time of writing. The requirements of the judicial systems are addressed in general terms only.

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1. AIMS

This guideline provides information and recommendations on the analysis of paint and coating materials by Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS). It is designed to be used in conjunction to the Best Practice Manual for the Forensic Examination of Paint (EPG-BPM-001) [1].

2. SCOPE

This guideline is intended to provide understanding of the possibilities, advantages, limitations and the proper use of SEM/EDS for the forensic examination of paint samples.

3. TERMS AND DEFINITIONS

For the purposes of this guideline, the relevant terms and definitions given in ENFSI documents, the ILAC G19 Modules in Forensic Science Process [2], and in standards like ISO 9000 [3] and ISO 17025 [4] apply.

Specific technical terms used in this guideline are defined in ASTM E2809-22 [5].

4. BACKGROUND

4.1 Use of SEM/EDS for paint analysis

SEM/EDS can be used for the comparison of paint samples, mainly the inorganic components such as additives, pigments, aluminium flakes, mica, etc. By using an energy dispersive detector (EDS), components down to ~ 500 ppm (qualitative analysis) can be detected. Although databases exist for X-ray spectra collected at forensic laboratories [6], there are currently no X-ray spectral databases for the identification of cars and car manufacturers from paint samples [7].

SEM/EDS is a valuable tool for the comparison of paint samples. The electron microscope can be used for imaging samples and the EDS is used for obtaining information on the elemental composition of samples. SEM/EDS has the advantage of being able to detect a wide range of elements simultaneously in a short period of time and can be applied to small sample sizes. Due to the inhomogeneous nature of paint samples, care must be taken to ensure that spectra obtained at high magnifications are representative of the bulk composition. Besides providing information on bulk elemental composition, this technique can zoom in to smaller regions to characterise individual particles.

SEM/EDS is typically used for qualitative analysis and does not require much sample preparation. In rare occasions where semi-quantitative analysis is to be carried out, more sample preparation steps will be required, and this process can be time-consuming.

4.2 Types of signals used

The different types of signals that are widely used in analysis of paint are introduced in this section. Refer to [8]-[10] for introductory text on SEM/EDS.

4.2.1 Secondary electron (SE) images

Images formed by secondary electrons give excellent topographic views of a specimen but no chemical information. Topographic information for paint samples is seldom of interest for forensic interpretation.

4.2.2 Back scattered electron (BSE) images

The strength of the information gathered from the back scattered electrons is that they reflect compositional properties of the sample. BSE images provide an overview of the distribution of different elements with $Z > 6$ (carbon) in the paint matrix and provide qualitative compositional information.

4.2.3 Energy dispersive X-ray Spectroscopy (EDS)

By interaction of primary electrons with the paint sample characteristic X-rays are formed. These can be detected by an EDS system and provide insight into the atomic composition at micrometer scale. Electron microscopes are equipped with a lithium doped silicon (SiLi) or a Silicon Drift Detector (SDD), and are able to provide qualitative composition results within short times.

In certain circumstances semi-quantitative results can be obtained.

In low-Z matrices such as paint samples, the interaction volume from which X-rays arise can be as large as several micrometers laterally and vertically, resulting in the obtained spectrum being derived from the spot of interest and its surroundings.

5. SAMPLE HANDLING AND PREPARATION

In this section, some practical aspects of sample preparation and analysis will be outlined.

Generally, for qualitative analysis, the samples should be flat and several images and spectra should be taken to sufficiently capture the variations in elemental composition due to sample inhomogeneity. For semi-quantitative analysis, additional procedures, such as embedding and polishing the samples in order to obtain flat and smooth surfaces, will be required.

5.1 Preliminary examinations

The SEM/EDS method should be considered as a non-destructive method in the sense that samples can be re-measured using the same technique. However, it may be problematic to analyse it using other techniques due to the strong adhesion of the sample to its holder or when grinding and polishing was done for a semi-quantitative analysis and the sample was thereby physically altered. This has to be considered in the decision-making process as depicted in the Best Practice Manual for the Forensic Examination of Paint [1].

5.2 Removal of extraneous materials

Dirt, salt or any other non-significant material must be removed from the surface of the paint by scraping with a scalpel or washing with a soaked piece of tissue paper or a dipped cotton bud with water, methanol or ethanol. Aggressive solvents that may affect the paint samples should not be used.

5.3 Exposing the internal structure for analysis

If the sample consists of multiple layers, it is recommended to determine the elemental composition of each layer individually.

5.3.1 Hand cutting

The separation of individual layers (single layer peels) can often be advantageous. Sometimes, the individual layers of multi-layered paint chips are exposed by obliquely cutting the chip with a scalpel under a microscope. The sample is then transferred to an electrical conducting double-sided adhesive tape on a SEM stub ensuring that the sample is small and can be easily recognized.

5.3.2 Embedding for microtomy or polishing

A sample can be embedded in a suitable material for subsequent microtomy or polishing.

For the embedding procedure, it is recommended to use Technovit® 2000 LC, which is a liquid photocuring resin based on mono and bifunctional methacrylates. Other embedding materials such as two-component epoxy and acrylic resins may be used as well. Electrically conducting embedding materials are heat and pressure hardened and are not recommended for this procedure.

The following embedding procedure for using Technovit® 2000 LC is recommended:

i. Cast the Technovit® 2000 LC into the microtube (MIC 19, milian);
ii. 30 minutes curing under visible blue light source (Dulux S Osram 9W/71, 400 nm – 580 nm);
iii. Drill the hole (Ø 1mm, depth 1mm) in the Technovit® 2000 LC;
iv. Place the specimen into the hole using tweezers;
v. Overcast the specimen with Technovit® 2000 LC;
vi. Final polymerisation is always 30 minutes.

Follow the instructions given by the manufacturer and use moulds of 5 cm or more. It is possible to embed more than one sample in each mould. The samples should be mounted in such a way that they will not be mixed up (e.g. different shapes, documented positions).

As it is often difficult to mount small pieces of paint for embedding, it is recommended that small pieces of paint are glued onto the tip of a needle, which is then fixed, before pouring the embedding liquid over the paint on the tip of the needle. This work should be done under a stereomicroscope.

5.3.2.1 Microtomy

The most commonly used microtome-knives for the cutting procedure have steel blades, hard metal blades, glass blades, sapphire blades or even diamond blades. There are many factors that affect the quality of the cutting. It is important to optimise the knife speed and angles (free angle and inclination angle). The sample should not be too warm and the blade should be well attached and not too short. For the sectioning of paint, a hardened steel knife of profile “C” (wedge-shaped) and/or “D” (chisel-shaped) is preferred.

The recommended thin section thickness for use in transmission measurements is between 3 and 10 µm. This sample can also be used for qualitative SEM/EDS analysis.

The thin section thickness should be 20 µm or more in order to achieve the best results in semi-quantitative SEM/EDS analysis.

5.3.2.2 Grinding and polishing

Grinding and polishing is recommended if semi-quantitative analysis is to be carried out.

A rotating wheel with speed of 300 revolutions per minute (rpm) or higher should be used. Successively finer polishing materials are used in the process. It is recommended to start the grind process with silicon carbide (SiC) paper, or other suitable abrasives, with a grit size of approximately 300, followed by a grit size of 800 and finally a grit size of 1200. Rinse the sample thoroughly with water in between the use of each type of paper.

5.3.3 Direct mounting

In situations where there is no need for the examination of layer structure (e.g. smearing), it is possible to mount the paint sample directly onto a sample stub, which is commercially prefabricated for each microscope manufacturer. The stub should be coated with conducting glue, preferably a sticky tab of carbon.

In situations where prior analysis had been carried out using non-destructive techniques (e.g. FT-IR using diamond cells), the paint sample can be recovered after analysis and mounted onto a sample stub for SEM-EDS analysis.

5.4 Coating

When a high-vacuum SEM is used coating of the non-conductive paint sample is mandatory. For an EDS analysis metal sputtering (e.g. Au, Pt or Au/Pd) is less favourable then carbon coating due to the interference of the metals with the X-ray lines of the sample. A carbon film thickness between 5 nm and 10 nm is usually sufficient to prevent sample charging artefacts. Nevertheless, the *Duane-Hunt* limit (cut-off energy of the *bremsstrahlung* continuum) should be checked for each high vacuum EDS measurement. If there is a significant deviation from the primary beam energy the coating has to be checked.

Using a low-vacuum SEM, coating or sputtering is not necessary, but it should be noted that the low-vacuum leads to a beam broadening of the primary beam (“skirt effect”), which widens the interaction volume of the beam even further. This effect depends on the primary beam energy as well as the gas path length of the electrons and the chamber’s gas pressure.

Furthermore, a continuous background signal and the characteristic X-ray lines of the chamber gas are added due to electron-gas interaction and the signal-to-noise ratio is decreased.

6. INSTRUMENTATION

6.1 Instrument calibration

The energy calibration of the EDS system should be checked on a regular basis (preferably weekly) or when there are concerns about its correct performance. The acquired reference spectra must be inspected critically regarding peak shape and peak position to reveal malfunctions. Modern spectrometer software allows an automatic two-point energy calibration using a standard material of known composition, preferably a transition metal such as copper providing high and low energy characteristic energies (E(Cu-Lα) = 0.928 keV and E(Cu-Kα) = 8.040 keV) or two different metals (e.g. aluminium, 1,486 keV and copper, 8,040 keV). The calibration process should be performed as stated by the EDS system’s manufacturer.

It is recommended to keep a permanent log of the calibration spectra and the determined resolution (usually the FWHM of MnKα).

As the EDS detector is sensitive to temperature fluctuations, the room temperature should be held constant in the range specified by the manufacturer.

6.2 Imaging

It is imperative that paint samples are compared using identical conditions.

Depending on the type of experiment (imaging or chemical X-ray analysis), the set of conditions/parameters should be optimised. In this way, one parameter set is obtained for imaging, giving optimal images, and another set for X-ray analysis giving optimal results for qualitative and semi-quantitative measurements.

The image can be generated using either secondary electrons or backscattered electrons. For high-resolution SE imaging, the primary energy should be as low as possible (< 2 kV), whereas BSE imaging requires a higher beam energy (> 5 kV). It should be noted for thin layers, that higher beam energies lead to a larger BSE escape volume, which may exceed the dimensions of a single layer.

6.3 Elemental analysis

The parameters chosen for analysis are dependent on the instrument, such as the type of detector used and the type of electron emission source used. For example, a SDD detector with a field emission gun (FEG) will produce higher count rates compared to a SiLi detector with a tungsten filament source. With higher count rates, the instrument will require shorter counting times to produce good spectra.

As a starting point for the primary beam energy 20 to 25 kV should be used to ensure the detection of all elements in the sample. For detection of light or heavy elements an accelerating voltage of 5-15 kV or 20-25 kV (or higher) can be beneficial. The bremsstrahlung background diminishes when reducing the accelerating voltage. This can be useful to detect trace elements if the reduced voltage is at least twice the energy of the targeted X-ray line of a potential trace element.

The beam current (or "*spot size*") should be selected in a way leading to a dead time between 5 and 40 % for selected process time (preferably < 20 % to minimise coincidence peaks) with respect to a reasonable acquisition time and to possible high-current sample damage [5]. A spectral resolution of 10 eV/channel is recommended.

The counting time must be long enough to ensure a satisfying signal-to-noise ratio for the desired application (qualitative or quantitative measurements) on each single peak with a recommended minimum of 250,000 accumulated counts in the acquired spectral range.

For stage-tilt and working distance etc., consult the instruction manual. EDS measurements should be performed at or as close as possible to the manufacturer specific *analytical working distance* of the microscope.

When analysing multi-layered paint samples, it should be ensured that elements from layers next to the layer of interest do not interfere with the analysis. The analytical window should be defined at a safe distance from the various boundaries and the large EDS interaction volume must be considered.

7. LIMITATIONS

Care should be taken to avoid systematic and random errors or incomplete datasets in the analysis due to factors such as the presence of contaminants, inhomogeneities in the sample, instrumental conditions, spectral resolution and misinterpretation of data. If an automatic element identification algorithm is used for qualitative analysis, its results must be inspected critically. These algorithms are prone to misidentification especially with minor or trace elements and severe peak-overlap (Ba/Ti, Al/Br, S/Mo etc.).

When using a low vacuum SEM, the spread of the electron beam ("skirt effect") should also be considered as a possible influence on the analytical results (cf. section 5.4).

Due to the usually inhomogeneous distribution of inorganic materials in paint and the large escape volume of characteristic X-rays in paint a qualitative interpretation of the acquired spectra is encouraged and must be performed with caution.

Some other limitations in the SEM/EDS method are summarised in Table 1.

Table 1

Parameter	**Limits/ Limitations**
Elements detectable	Z>4, using atmospheric thin window (ATW)
	Z>3, using windowless detector
Minimum probe size	5 nm
Spatial resolution	>100 nm
Spectrometer resolution	130 – 150 eV (MnKα)
Escape peak	X-rays with energies >1.74 eV generate specific X-rays in the silicon of the detector
Relative accuracy	1 – 10%
	For trace elements (< 1 wt%) up to > 50%

8. REFERENCES

[1] EPG-BPM-001, Best Practice Manual for the Forensic Examination of Paint, Issue 2, 2022.

[2] ILAC G19:06/2022, Modules in a Forensic Science Process.

[3] ISO/IEC 9000:2015, Quality management systems – Fundamentals and vocabulary.

[4] ISO/IEC 17025:2017, General requirements for the competence of testing and calibration laboratories.

[5] ASTM E2809-22, Standard Guide for using Scanning Electron Microscopy/ Energy Dispersive X-ray Spectroscopy in Forensic Polymer Examinations.

[6] Ward D.C. 2000. Use of X-ray Spectral Databases in Forensic Science, Forensic Sci. Commun., Vol. 2, No. 3.

[7] Sjåstad K.E. 2000. Oral presentation of the results from the SEM/EDS questionnaire for the EPG, Krakow.

[8] Goldstein J.I., Newbury D.E., Michael R.R., Ritchie N.W.M., Scott J.H.J., Joy D.C. 2018. Scanning Electron Microscopy and X-Ray Microanalysis, 4th Edition, Springer-Verlag, New York.

[9] Scott V.D., Love G., Reed S.J.B. 1995. Quantitative Electron-probe Microanalysis, EllisHorwood Series in Physics and its Applications, 2nd Edition, EllisHorwood, New York.

[10] Newbury D.E., Ritchie N.W.M. 2013. Is Scanning Electron Microscopy/Energy Dispersive X-ray Spectrometry (SEM/EDS) Quantitative? Scanning, Vol. 35, 141–168.

9. AMENDMENTS TO PREVIOUS VERSION

Not applicable.

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