



GUIDELINE for the forensic examination of paint by SEM/EDS

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2 GENERAL REMARK

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

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11 1. AIMS

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

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18 2. SCOPE

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

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23 3. DEFINITIONS AND TERMS

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

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

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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.

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

81

82

83 **5. SAMPLE HANDLING AND PREPARATION**

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

85

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

90

91 5.1 Preliminary examinations

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

99

100 5.2 Removal of extraneous materials

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

106

107 5.3 Exposing the internal structure for analysis

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

111

112 5.3.1 Hand cutting

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

118

119 5.3.2 Embedding for microtomy or polishing

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

121

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

127

- 128 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, 400nm - 580nm)
 - iii. Drill the hole (\varnothing 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

129
130 Follow the instructions given by the manufacturer and use moulds of 5 cm or more. It is
131 possible to embed more than one sample in each mould. The samples should be mounted in
132 such a way that they will not be mixed up (e.g. different shapes, documented positions).

133
134 As it is often difficult to mount small pieces of paint for embedding, it is recommended that
135 small pieces of paint are glued onto the tip of a needle, which is then fixed, before pouring the
136 embedding liquid over the paint on the tip of the needle. This work should be done under a
137 stereomicroscope.

138
139 **5.3.2.1 Microtomy**
140 The most commonly used microtome-knives for the cutting procedure have steel blades, hard
141 metal blades, glass blades, sapphire blades or even diamond blades. There are many factors
142 that affect the quality of the cutting. It is important to optimise the knife speed and angles (free
143 angle and inclination angle). The sample should not be too warm and the blade should be well
144 attached and not too short. For the sectioning of paint, a hardened steel knife of profile "C"
145 (wedge-shaped) and/or "D" (chisel-shaped) is preferred.

146
147 The recommended thin section thickness for use in transmission measurements is between 3
148 and 10 μm . This sample can also be used for qualitative SEM/EDS analysis.
149 The thin section thickness should be 20 μm or more in order to achieve the best results in
150 semi-quantitative SEM/EDS analysis.

151
152 **5.3.2.2 Grinding and polishing**
153 Grinding and polishing is recommended if semi-quantitative analysis is to be carried out.
154 A rotating wheel with speed of 300 revolutions per minute (rpm) or higher should be used.
155 Successively finer polishing materials are used in the process. It is recommended to start the
156 grind process with silicon carbide (SiC) paper, or other suitable abrasives, with a grit size of
157 approximately 300, followed by a grit size of 800 and finally a grit size of 1200. Rinse the
158 sample thoroughly with water in between the use of each type of paper.

159
160 **5.3.3 Direct mounting**
161 In situations where there is no need for the examination of layer structure (e.g. smearing), it is
162 possible to mount the paint sample directly onto a sample stub, which is commercially
163 prefabricated for each microscope manufacturer. The stub should be coated with conducting
164 glue, preferably a sticky tab of carbon.

165
166 In situations where prior analysis had been carried out using non-destructive techniques (e.g.
167 FT-IR using diamond cells), the paint sample can be recovered after analysis and mounted
168 onto a sample stub for SEM-EDS analysis.

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172 5.4 Coating

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

181

182 Using a low-vacuum SEM, coating or sputtering is not necessary, but it should be noted that
183 the low-vacuum leads to a beam broadening of the primary beam ("*skirt effect*"), which widens
184 the interaction volume of the beam even further. This effect depends on the primary beam
185 energy as well as the gas path length of the electrons and the chamber's gas pressure.
186 Furthermore, a continuous background signal and the characteristic X-ray lines of the chamber
187 gas are added due to electron-gas interaction and the signal-to-noise ratio is decreased.

188

189

190 **6. INSTRUMENTATION**

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192 6.1 Instrument calibration

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194 The energy calibration of the EDS system should be checked on a regular basis (preferably
195 weekly) or when there are concerns about its correct performance. The acquired reference
196 spectra must be inspected critically regarding peak shape and peak position to reveal
197 malfunctions. Modern spectrometer software allows an automatic two-point energy calibration
198 using a standard material of known composition, preferably a transition metal such as copper
199 providing high and low energy characteristic energies ($E(\text{Cu-L}\alpha) = 0.928 \text{ keV}$ and $E(\text{Cu-K}\alpha) =$
200 8.040 keV) or two different metals (e.g. aluminium, 1,486 keV and copper, 8,040 keV). The
201 calibration process should be performed as stated by the EDS system's manufacturer.

202

203 It is recommended to keep a permanent log of the calibration spectra and the determined
204 resolution (usually the FWHM of $\text{MnK}\alpha$).

205

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

208

209 6.2 Imaging

210

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

212

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

217

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

223 6.3 Elemental analysis

224

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

230

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

237 The beam current (or “*spot size*”) should be selected in a way leading to a dead time between
238 5 and 40 % for selected process time (preferably < 20 % to minimise coincidence peaks) with
239 respect to a reasonable acquisition time and to possible high-current sample damage. [5]

240 A spectral resolution of 10 eV/channel is recommended.

241

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

245

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

249

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

254

255

256 **7. LIMITATIONS**

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

263

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

266

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

270

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

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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 (MnK α)
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%

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8. REFERENCES

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9. AMENDMENTS AGAINST PREVIOUS VERSION

New document.

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