GUIDELINE FOR THE FORENSIC EXAMINATION OF PAINT BY RAMAN SPECTROSCOPY

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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.

TABLE OF CONTENTS

1. AIMS

This guideline provides information and recommendations on the analysis of coating materials by Raman spectroscopy.

2. SCOPE

This guideline is intended to provide understanding of the possibilities, advantages, limitations and the proper use of Raman spectroscopy. It is designed to be used in conjunction to the Best Practice Manual for the Forensic Examination of Paint EPG-BPM-001 [1].

3. TERMS AND DEFINITIONS

For the purposes of this guideline, the relevant terms and definitions are given in ENFSI documents, in ILAC G19 [2], in ISO/IEC 9000 [3], ISO/IEC 17020 [4] and ISO/IEC 17025 [5] standards, and in ASTM E1610-18 [6] are applied. Specific technical terms used in this guideline include:

Term	Definition
Anti-stokes lines	Scattered light with higher energy (lower wavelength) than the exciting laser light
Confocal	Optical imaging technique used to increase micrograph contrast by using a pinhole to eliminate out-of-focus light
Dispersive instrument	Separating different wavelengths by using a grating-based dispersive unit (monochromator)
Edge filter	Band-pass filter that passes frequencies within a certain range and rejects frequencies outside this range. The edge filter rejects both the excitation laser line and the anti-Stokes lines.
Fluorescence	Radiation emitted from the excited sample that overlays the weak Raman signals
Laser	Device for emitting monochromatic light through a stimulated emission process
NIR	Near infrared radiation (wavelength interval of 700 to 1400 nm)
FT-Raman	Separating different wavelengths by measuring a time dependent interferogram and transforming it by Fourier Transformation into a frequency function (spectrum)
Notch filter	Band-stop filter with a narrow stop band rejecting the excitation laser line and passing both the Stokes and anti-Stokes lines
Polarizability	Relative tendency of a charge distribution (electron cloud) of an atom or molecule to shift under the influence of external stimulation
Raman scattering	Inelastic scattering of photons from a source
Raman shift	Difference between the excitation wavelength and the wavelengths of the scattered light
Raman spectroscopy	Spectroscopic method to compare and identify substances by measuring the shift of scattered light after monochromatic excitation
Rayleigh scattering	Elastic scattering of the excitation light by a sample
SERS	Surface enhanced Raman scattering is a surface sensitive technique that results in the enhancement of the Raman scattering by molecules adsorbed on a metal or metal colloid substrate, the enhancement factor can be as much as 10^{14} – 10^{15}.
SERRS	Surface enhanced resonance Raman scattering is a combination of the SERS technique with a laser excitation near the absorption maximum of the analytes
Stokes lines	Scattered light with lower energy (longer wavelengths) than the exciting laser light

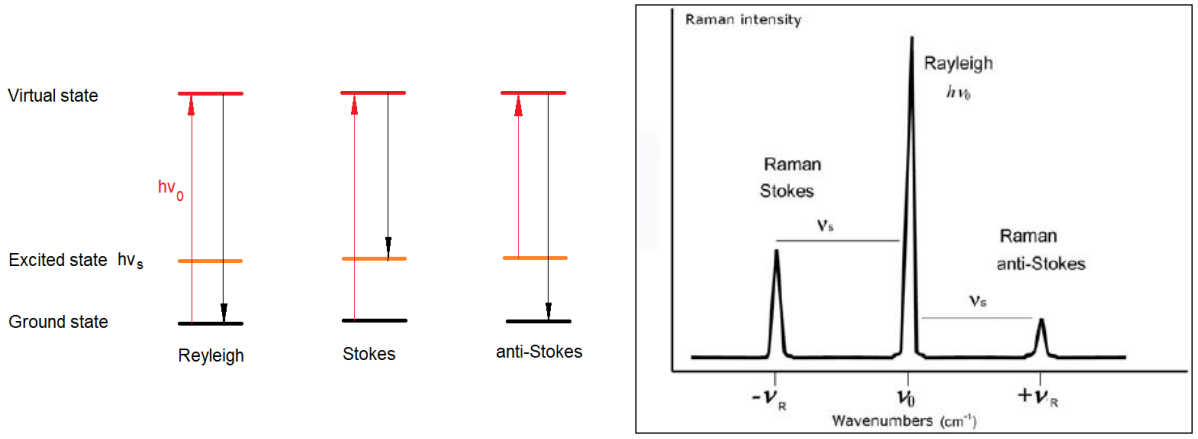
4. TECHNICAL BACKGROUND

4.1 Theoretical background

Infrared and Raman spectroscopy both measure vibrational transitions in molecules, but these methods are based on different selection rules. To be active in infrared the dipole moment of the molecule must change. For a transition to be Raman active there must be a change in polarizability of the molecule. Thus, Raman and Infrared spectroscopy are complementary.

Raman spectroscopy is based on the scattering of light by matter. When monochromatic light of energy hv_0 encounters a sample, there is a high probability that it will be elastically scattered i.e. with the same frequency (Rayleigh scattering). About 1 in 10^{10} incident photons however undergoes inelastic scattering i.e. with an energy that differs from hv_0 by an amount equal to a vibrational transition hv_s:

- Stokes Raman scattering occurs when a molecule absorbs an incident photon but relaxes to a higher vibrational state instead of the ground state;
- Anti-stokes Raman scattering occurs when a molecule in a vibrational excited state absorbs an incident photon and relaxes to the ground state instead of the initial excited state.



Stokes and anti-Stokes peaks are symmetrically positioned about the Rayleigh peak but the former is more intense. As the effects are weak and fluorescence can overwhelm the peaks, Raman spectra are usually recorded in the Stokes region only. Monochromatic excitation is performed using laser sources.

Raman spectra consist of scattered intensity plotted against Raman shift from the incident energy, usually expressed in wavenumbers (cm^{-1}).

4.2 Instrumentation

Raman spectroscopic measurement of paint particles requires the use of a spectrometer coupled with a microscope.

4.2.1 Raman spectrometer

Two types of instruments are available:

- Dispersive spectrometers separate the scattered wavelengths using a grating and measure them on a thermoelectrically cooled CCD detector. Resolution of these instruments depends on the grating used (number of lines per mm).
- FT-Raman spectrometers use an interferometer to modulate the beam similar to FTIR instruments. High sensitivity, liquid nitrogen cooled Ge detectors are used.

The choice of the laser wavelength is of prime importance for Raman spectroscopy. Measuring paint samples requires that several (at least three advised) different wavelength lasers should be available in order to avoid fluorescence and maximize detection capabilities (especially in case of mixed samples). Laser sources in the blue-green and red regions of the visible range and a NIR source are complementary and enable measuring most samples.

blue	457 nm	diode-pumped solid-state laser
blue	473 nm	diode-pumped solid-state laser
blue	488 nm	Ar ion (outdated)
blue	488 nm	optically-pumped diode laser
green	514 nm	Ar ion (outdated)
green	514 nm	diode-pumped solid-state laser
green	532 nm	solid-state laser
red	633 nm	He-Ne laser
red	638 nm	diode laser
NIR	785 nm	diode laser
NIR	1064 nm	Nd:YAG

Short wavelengths are usually detected with higher efficiency and less noise but are likely to excite more fluorescence. FT-Raman spectrometers use a 1064 nm laser.

Lasers should be tuned on and allowed to reach stability prior to operational runs (according to manufacturers' recommendations, approx. 20 min).

4.2.2 Microscope

Any stable reflected light microscope can be adapted to the spectrometer. A range of infinity corrected objectives are used ranging from 10x to 100x. The spot size of the laser beam on the sample and the depth of focus will decrease as the magnification increases (e.g. 50x objective, 514 nm laser gives a spot size of 6,5 µm and depth of focus of 10 µm). They will increase with the laser wavelength.

Confocal microscopes have an additional aperture (confocal hole) that prevents out-of-focus rays to reach the detector. Confocal mode will enable the reduction of the depth of focus to less than 2 µm (514 nm laser, 100x objective), but also reduces the Raman signal. This mode can be used to minimize fluorescence or to reduce interference from surrounding materials. Changing the laser wavelength is generally more efficient in minimising fluorescence.

4.2.3 Rejection filter

Rejection filters are inserted in the optical path after the sample and transmit the Raman signal to the detector while rejecting the intense Rayleigh line. They are matched to one

specific laser wavelength and have to be changed when changing the laser excitation wavelength.

Holographic notch filters can be used but have a limited lifetime.

Oxide coated edge filters reject both the Rayleigh line and the anti-Stokes scattering. As only Stokes scattering is used for measuring paint samples, edge filters are preferred.

4.3 Use in paint analysis

Many pigments and fillers used in paint formulations do not produce unambiguous information in the FTIR spectra due to unfavourable selection rules and the domination by the binders response. As a complementary technique to FTIR, Raman spectroscopy usually provides clear pigment and filler signals as opposed to weak response for the binders. It thus provides the means to compare these components and identify them using reference databases.

Different paints with the same pigment/filler composition can be indistinguishable using Raman spectroscopy.

The use of a microscope coupled with a Raman spectrometer has enabled the analysis of compact paint particles as well as smears. It is possible to analyse very small samples (0,01 mm or less) quickly, reproducibly and with a high degree of sensitivity. The diameter of the spot size is usually a few microns.

The method is practically non-destructive and provides additional or at least confirmatory information to FTIR analyses.

4.4 Limitations

Using a Raman microscope and the most commonly used objective 50x, the laser spot size at the sample surface is 2 µm in diameter (lateral resolution) and the penetration depth is approximately 2.5 µm (confocal resolution). The following limitations are given:

Amount : Minimum spot size 4 μm^2

Detection limit : Approx. 10 pg (2 µm ø x 2 µm H, density 1.5 g/cm^3)

Organic coloured pigments measured with specific laser wavelength may show resonance enhancement, yielding an improved detection limit.

A drawback of the method is the possible localised thermal degradation of the paint if the laser source power is not carefully managed.

A major limitation is the occurrence of fluorescence emission that can partially or totally mask the detection of the Raman signal. The use of multiple laser sources helps in overcoming this limitation.

5. SAMPLE HANDLING AND PREPARATION

5.1 Sample cleaning

Any impurity on the sample surface has to be documented, removed and, if forensically significant, analysed.

Depending on the sample, impurities can be removed either by scraping with a scalpel, or washing with a tissue soaked with water or a non-aggressive solvent like ethanol or methanol. Other organic solvents must be avoided because of possible chemical reactions with the sample or other alterations (dissolution, swelling).

5.2 Preparation techniques

Sample preparation techniques should be the same for all samples being compared.

Raman spectra are measured in reflectance. The samples are deposited either directly on a microscope slide, on a microscope slide covered with aluminium foil, or better still, on a slide of aluminium. The metal provides advantages due to its heat dissipation properties.

Smears of paint on other surfaces (e.g. metals, plastics, textiles, paint chips) can be measured directly on these surfaces. In this case a comparison measurement of the pure surface is necessary to eliminate signals of the latter.

In case of multilayer samples thin sections may eventually be prepared to provide selective analysis per layer. The thin sections should be placed on an appropriate sample holder or again on an aluminium (foiled) slide.

5.3 Special techniques: SERS, SERRS

SERS and SERRS enhance the Raman signal and quench fluorescence.

Several procedures exist to prepare SERRS solution, e.g. citrate-reduced silver colloid in aqueous solution of poly (L-lysine).

The techniques require good contact between the reagent and the sample, which is often difficult to obtain because pigments are trapped in the binder and thus are not in direct contact with the reagent spotted on the paint surface.

These special techniques are not yet applicable in routine analysis of paint.

6 MEASUREMENT CONDITIONS AND PARAMETERS

6.1 Calibration and validation

Instrument calibration and validation records shall be maintained in order to check the instrument performance and long-term stability.

6.1.1 Frequency calibration

Raman shift values are more prone to error than in FTIR systems. They have to be calibrated because small changes in the true wavelength of the laser sources can have a significant impact on the Raman shift accuracy.

Frequency calibration with absolute frequency standards is usually done by the manufacturer. A Ne, Ar or Hg lamp is placed near the sample position and the atomic emission lines are used as well as the lines from the laser itself (must correspond to zero Raman shift).

The ASTM committee collected a set of 8 Raman shift standards [6] with a standard deviation of less than 1 cm^{-1}. These standards can be used independently of the laser frequency, provided it is constant.

Among these standards, polystyrene is particularly suitable and is already used for FTIR calibration. It shows Raman shifts between 620 and 2940 cm^{-1} that are determined using 514 and 1064 nm lasers.

Another Raman shift standard is silicon with a sharp single band at 520 cm^{-1}.

One of these standards should be used for accuracy check when starting a session of measurements. Accuracy checks should concern laser lines and gratings that will be used for measurements.

6.1.2 Performance monitoring

The instrument response has to be controlled by checking the magnitude of the Raman signal under the same analytical conditions. This must be done for each laser source available with the instrument. The intensity of the silicon band at 520 cm^{-1} thus permits the detection of instrument changes (e.g. misalignment of the laser beam, output power decrease of the laser source).

In addition, a secondary standard similar to the sample of interest may be chosen, e.g. a known pigment from the laboratory collection or polystyrene. In this way positions and relative intensities of different peaks can be controlled in the spectral range of the sample interest.

6.1.3 Validation

Raman shift standards can be instrumental for validation and provide measurands in the form of the position and relative intensities of the peaks. The chosen peaks must cover a wide range of Raman shift.

In addition, other standards similar to the sample of interest should be chosen, e.g. a known pigment from the laboratory collection. Positions and relative intensities of different peaks must be controlled.

6.2 Analysis conditions

Analytical conditions have to be optimized according to instrument and sample type. Therefore only generic advice can be formulated.

6.2.1 Wavelength range

A range of 2000 – 200 cm^{-1} will be sufficient for measuring most pigments. Certain blue and green pigments also display spectral characteristics up to 4000 cm^{-1}.

6.2.2 Microscope objective

The choice of the objective will influence the size and depth of the area measured. Different objectives should be tested on the samples. Generally, 50x or 100x objectives will provide the best results.

6.2.3 Laser power

The laser power should not saturate the detector nor burn the sample. It is recommended to start with a low power and increase it depending on the result obtained. A high laser power may modify the molecular structure and thus the Raman spectra of heat sensitive samples (i.e. graphitization bands may appear).

6.2.4 Analysis time, number of accumulations, number of measurements

These parameters should be chosen so that an acceptable S/N ratio is obtained. As a start, the time of analysis can be short (i.e. 10 s), no accumulation, 1 measurement. If the S/N ratio is good, there is no need to adjust the parameters. Normally the same parameters are used for both the control and the questioned samples. Sometimes the intensity of the background fluorescence may require the use of different parameters for the control and the questioned samples, or even within the same sample.

Acquiring at least two accumulations may be useful to detect or automatically remove artefacts (if available in the software), such as ‘cosmic rays’ or ‘spikes’ in the spectrum.

6.2.5 Grating

Some instruments allow the grating being changed (from 300 to 1800 lines per mm). The choice of the grating depends on the laser wavelength. 300 lines per mm gratings offer a low resolution but fast measurements, and allow an overview of the Raman response over the spectral range. The higher number of lines per mm offers a high resolution but longer measuring times.

Normally the low resolution grating would be used to determine the region of interest where further acquisition should be done using a higher resolution grating.

6.2.6 Sample focus

The quality of the spectra depends on the correct focus on the sample. In confocal mode, it will allow to reduce interference from other surrounding materials. Tiny details in the sample could thus be investigated using a higher magnification and confocal mode.

6.2.7 Fluorescence

Fluorescence is an important issue in Raman spectroscopy. It can partially or even totally mask the Raman signal. Using another excitation wavelength (laser) can sometimes improve the Raman response. Fluorescence problems can therefore be more easily avoided when possessing multiple laser sources.

Another method is photo-bleaching, where the sample is irradiated by the laser for a long period of time (e.g. 15 min – 1 hr) in order to reduce or suppress fluorescence. Since Raman

spectroscopy is already a time-consuming technique, the use of photo-bleaching may be limited in routine work. SERRS will also quench fluorescence and enhance the Raman signal.

6.3 Spectra redundancy and documentation

Paint samples are notoriously inhomogeneous. Analysis should be performed on a sufficient number of measuring spots to account for compositional variations.

Spectra shall be stored in their raw form and unambiguously labelled, manipulated spectra shall be stored with appropriate annotations. The raw file format of the instrument manufacturer usually saves instrumental conditions and spectral corrections. It is recommended to store the raw data in this format.

7 INTERPRETATION OF RESULTS

7.1 Spectral comparison

In a first step the homogeneity of the spectra of the questioned and the control sample shall be assessed. Big variations have to be included in the interpretation of the results.

For comparison purposes, baseline corrected spectra are superimposed to check for similarities and differences. If some spectra are especially noisy, it may be difficult to observe them in all detail. Smoothing is possible but must be used with care as it may affect the presence of minor bands.

Given spectra of good quality, the patterns of major and minor peaks, their relative positions and intensities, of the control sample are compared to the recovered paint spectra. If the spectra are comparable then the samples are said to be indistinguishable. If peaks differ substantially in position or peaks are missing in one of the compared samples, then the samples do not match.

The Raman analysis is very local, especially when using higher magnification to focus on tiny details, and paint samples are known to be inhomogeneous. In addition the Raman signal is rather proportional than quantitative and resonance effects may significantly increase the signal of the pigment compared to other components such as fillers. The relative intensities may then vary when detecting multiple components in the same spectrum. The Raman spectra from different measuring spots may consequently slightly differ and it is sometimes desirable to compare several Raman spectra together to obtain an overview of the peaks present or absent from a sample.

7.2 Spectral interpretation using Raman libraries

Raman spectra contain information from the paint binder, its pigments and fillers. The dominant bands originate from pigments and fillers. When a mixture is present, one of the constituents is often dominant and can hide contributions of the others.

The number of pigments is limited to a few hundred. The ENFSI European Paint and Glass Expert Working Group (EPG) has created a Raman database using 458, 514, 633 and 785 nm lasers. This database contains almost all pigments available on the market. Each pigment has a unique Raman spectrum and its identification when used alone is relatively straightforward. When a mixture of pigments with a Raman contribution is present,

identification becomes more complicated and using several laser sources may be helpful to detect more than one pigment.

7.3 Use of KnowItAll software

Raman spectra of pigments and fillers can be compared to the EPG Raman library of pigments by using the KnowItAll software. Basic recommendations for its optimal use are:

7.3.1. Data correction

Data correction steps such as normalization and baseline correction may be done temporarily before the library search. It is preferable to use the same conditions for all spectra to be compared. A strong baseline correction is recommended before searching in libraries.

7.3.2. Search algorithm

Routines for spectra, peak and property search are available and can be used separately or in combination. A non-derivative search algorithm (Euclidian distance or Correlation) without prompting the automated baseline correction is sufficient.

8. REFERENCES

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9. AMENDMENTS TO PREVIOUS VERSION

Not applicable.

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