CWA2: Raman instruments twinning protocol

Contents

Foreword

This CEN Workshop Agreement has been developed in accordance with the CEN-CENELEC Guide 29 “CEN/CENELEC Workshop Agreements – A rapid prototyping to standardization” and with the relevant provisions of CEN/CENELEC Internal Regulations - Part 2. It was approved by a Workshop of representatives of interested parties on 2024-XX-XX, the constitution of which was supported by CEN following the public call for participation made on 2023-12-20. However, this CEN Workshop Agreement does not necessarily include all relevant stakeholders.

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Introduction

Raman spectroscopy is increasingly popular in different industries to characterize advanced materials at different stages, since it is a relatively easy technique to implement that offers a rapid characterization of the specific chemical fingerprint of each material. The growth in the use of this technique has led to the development in recent decades of new and different Raman instruments, ranging from portable to high resolution equipment [1][2]. As a consequence, there is a great interest in the standardization and harmonisation of Raman to increase interoperability in terms of comparability, reproducibility and reliability of the data obtained in different fields.

The first step to make data comparable is to have calibration protocols that allow obtaining valid and corrected Raman spectra in each Raman instrument. Nowadays, there are different studies and standards that indicate how to perform these calibrations, both in the x-axis of the Raman shift and in the y-axis of the relative intensity [3]. However, despite having the Raman instruments calibrated in the same way, equal Raman spectra that can be compared are not obtained, since these corrections do not allow obtaining the same Raman intensity counts and, therefore, do not fully harmonise the Raman spectra.

Despite the great interest in being able to compare Raman spectra in terms of Raman intensity, there are currently no harmonisation standards or studies with protocols to obtain equal Raman spectra. The main reason is that Raman intensity depends on a large number of factors and parameters of the Raman instrument, such as the optical components (i.e. objectives, gratings, mirrors…), the laser wavelength, or the quantum efficiency of the detector [4][5]. In addition to all these instrumental factors, the Raman intensity also depends on the Raman cross-section of the measured material. Calculating the contribution of all these factors to the total Raman intensity is not feasible due to the great difficulty in controlling each element that influences the Raman signal. For this reason, a new protocol has been developed that allows two different Raman instruments (previously calibrated) to be twinned, and from there to harmonise their Raman spectra in terms of Raman intensity. The protocol uses a reference sample that allows extracting quantitative information from Raman spectra.

The concept of Raman twinning in the protocol involves the calculation of an experimental constant that includes in a single value all the differences between all the experimental variables of two Raman instruments. To do this, it is necessary to have a homogeneous reference sample that always has a fixed Raman cross-section and to obtain the linearity of the Raman intensity with the laser power of each instrument.

This Raman twinning protocol will allow progress in the use of this technique since the same Raman spectra can be obtained despite having measured in different instruments, achieving solutions in the interoperability and harmonisation of the data between different Raman instruments, applications and industries.

1 Scope

This CEN Workshop Agreement (CWA) provides a procedure for twinning Raman instruments using a test sample to harmonise their Raman spectra in terms of intensity.

This twinning protocol allows to correlate different Raman instruments to obtain equal Raman spectra in terms of Raman intensity, improving comparability, reproducibility and reliability. It is intended to be applied by end-users of Raman spectroscopy instruments, Raman manufacturers or users of Raman data.

The twinning protocol is applicable to any kind of Raman instrument (non-confocal and confocal) within the boundaries described in Section 6.1. The protocol has been developed using Raman instruments using 532 nm and 785 laser sources. Prior to use, this protocol requires that the Raman instrument or the acquired test sample data has already undergone a full calibration on x and y axis. This twinning protocol has been tested after applying the calibration protocol described in CWA1. The protocol may have applications beyond the stated limits, such as other previous calibration protocols or systems using different excitation sources, but its effectiveness is not confirmed.

2 Normative references

CWA1: Raman instruments calibration and verification protocols

3 Terminology

Where used, this document follows the definitions outlines in ISO 18115:2022. For the purpose of this document, the following terms, definitions, symbols and abbreviations apply:

3.1 Terms and definitions

3.1.1

Calibrated instrument

Raman instrument that has followed a previous calibration protocol that allows obtaining Raman spectra corrected in both Raman shift and relative intensity.

3.1.2

Test material

Material that exhibits homogeneity and stability concerning specific properties and has been validated as suitable for its intended application within a measurement procedure.

3.1.3
Reference Raman instrument

Raman instrument that serves as a reference to twin the second Raman instrument (instrument to be twinned).

3.1.4
Instrument to be twinned

Raman instrument that wants to be paired with the reference Raman instrument to harmonise Raman spectra in intensity.

3.1.5
Laser power regression line

Correlation line between the Raman intensity of a Raman band versus the laser power with which the measurement was performed.

3.1.6
Correction factor

Experimental constant that corrects all the differences between the two Raman instruments twinned in terms of Raman intensity.

3.1.7
Twinned Raman instrument

Raman instrument for which a Correction Factor has been calculated to obtain corrected spectra with equal intensity as if they were acquired with the reference Raman instrument.

3.1.8
Reference Raman band

Band of a Raman spectrum that is used to obtain a power regression line in order to calculate the Correction Factor.

3.1.9
Laser power

Power in mW of the excitation laser beam.

3.1.10
Integration time

Amount of time used to obtain a Raman spectrum.

3.1.11
Raman cross-section

Measure of the probability of a molecule or material to scatter light at a specific wavelength during Raman spectroscopy. A higher cross-section indicates a greater probability of Raman scattering for a given molecule.

3.1.12

Baseline removal

Separation of the baseline from the Raman spectrum through a mathematical procedure to accentuate Raman peaks and minimize other effects.

3.1.13

Background

Apparent Raman signal caused by anything other than the substance for which the analysis is being made.

(edited version of ASTM E131 – 10)

3.1.14

Verification

Evaluation process to ensure that the model follows predetermined standards and criteria during its development

3.1.15

Validation

Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled.

[ISO 9000:2015]

3.1.16

Quality factor of harmonisation of Raman intensity

Parameter that allows comparing the degree of coincidence between two Raman spectra that have been previously harmonised after carrying out the calibration protocol and the Raman twinning protocol. This parameter allows measuring the quality of harmonisation between two Raman spectra.

3.1.17

Raman focus

The act of adjusting the focus on the sample to maximise the intensity of the Raman signal.

3.2 Symbols and abbreviated terms

a.u.c.: arbitrary units corrected.

A_{har}: area under the Raman spectrum of the twinned Raman instrument.

A_{ref}: area under the Raman spectrum of the reference Raman instrument.

CCD: Charge Coupled Device.

CF: Correction factor.

I_N: Normalised Raman intensity.

I_R: Calibrated raw Raman intensity.

LP_R: Laser power of the reference instrument.

LP_T: Laser power of the instrument to be twinned.

Q_{HI}: Quality factor for Harmonisation of Raman Intensity.

RI_R: Reference Raman instrument.

RI_T: Raman instrument to be twinned or that has already been twinned.

RRB: Reference Raman band.

S_{RIR}: Slope obtained from the RI_R power regression line.

S_{RIT}: Slope obtained from the RI_T power regression line.

t_R: Integration time used in the reference instrument.

t_T: Integration time used in the instrument to be twinned.

4 Overview and Significance

Two Raman instruments that are going to be twinned for Raman harmonisation in terms of Raman intensity shall be calibrated in the same way, using the same protocols and criteria in x and y axis. A suitable way to correct and calibrate Raman spectra would be with the CWA1.

This twinning protocol is mainly based on accounting for all the possible differences between two Raman instruments in a constant by setting a test sample that is used to evaluate the linearity between Raman intensity and laser power for each instrument in a comparable way. It is necessary to have a good test material to obtain a good Raman twinning that allows the harmonisation of Raman spectra (Section 5). Measurement of the test sample is required for obtaining the Raman spectra that will allow two different calibrated Raman instruments to be twinned following these steps:

- Test sample measurements.
- Pre-processing.
- Obtention of laser power regression lines.
- Verification.

Pre-processing is required for having Raman spectra taken with equivalent integration time and laser power conditions and with similar background. Obtention of laser power regression lines is required for calculating the correction factor (CF). Verification is required for verifying the twinning between the two instruments and controlling the degree of harmonisation similarity between two Raman spectra after the Raman twinning of two Raman instruments.

5 Requirements of the test sample

This twinning protocol needs a test sample with controlled and constant Raman cross-section so that it is not a variable affecting the Raman intensity. Therefore, prior to the twinning protocol, a test sample must be available with the following requirements:

- The test sample shall be of **high homogeneity**. It shall present a homogeneous composition to provide equal Raman signal throughout all parts of the test material within the experimental error of the Raman instrument. The degree of deviation of the Raman response of the sample when different areas are irradiated should be less than 3%. The Raman intensity provided by each Raman equipment shall not depend on sample and must be the result only of the different elements and components of each instrument in order to quantify its efficiency.
- The test sample shall be a **strong scattering**, resulting in a good Raman signal/noise ratio (see CWA1 for a reference of what a good SNR might be). It is necessary to have well-defined Raman spectra, since noise introduces a great error in quantification.
- The test sample shall have **stability over time**, and not be affected by temperature or humidity. Additionally, the test sample shall be chemically stable.
- The test sample shall have **thermal stability** to withstand laser power densities in the ranges generally used in Raman instruments without being altered or burned: when the test sample is measured at the maximum laser power of a instrument, it shall not show signs of burning, nor shall its Raman spectrum be altered with Raman shifts of one or more Raman bands.
- The test sample shall have low-roughness surface (i.e. S_a=0.75±0.1 µm) with an optical quality finish to provide **high reflectance**.
- The test sample should have a Raman cross-section and colour as similar as possible to the real material that will be measured in the twinned Raman instruments.

The selection of the test sample is a very delicate step. Annex A includes an example of a fully developed valid test sample for this Raman twinning protocol.

6 Steps of the twinning protocol

The general scheme in Figure 1 represents all essential steps for twinning two different calibrated Raman instruments. This requires that the Raman instrument or the acquired test sample data has already undergone a full calibration protocol on x and y axis (as proposed in CWA1) prior to use for Raman twinning.

To carry out the Raman twinning protocol, a test sample shall be used. This test sample shall be a homogeneous, stable and reproducible material, with a good Raman signal/noise ratio (section 5).

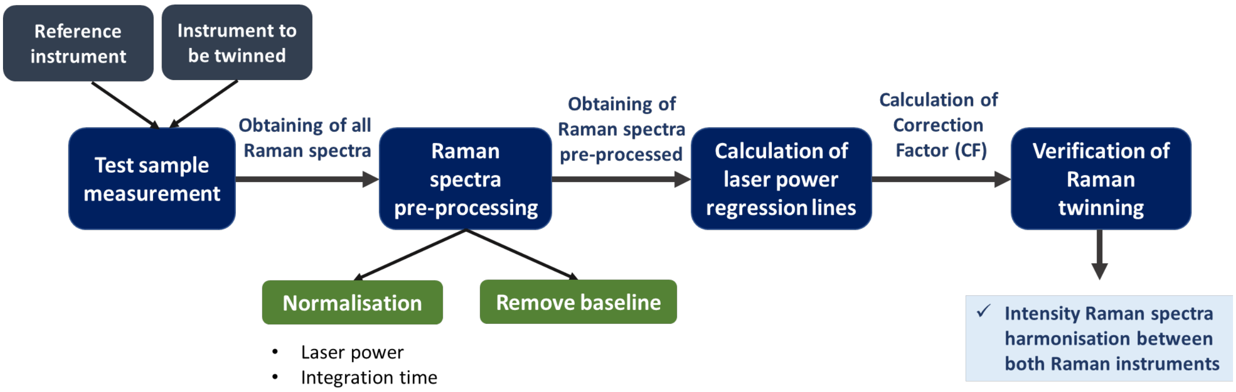

Figure 1: Scheme of the Raman twinning protocol.

6.1 Boundaries

Due to the breadth of Raman instrumentation, the Raman twinning protocol has the boundaries of use included in Table 1. This table is extracted and adapted from CWA1.

Table 1. Boundaries of use

Boundary	**Explanation**
Excitation wavelength	The protocol has been developed primarily with 532 nm and 785 nm Raman systems.
No polarisation	The effects of polarisation on the Raman peaks- has not been considered in this protocol.
No resonance	The effect of resonance on the Raman peaks has not been considered in this protocol.
Backscattered spontaneous Raman.	Only 180° backscattered Raman has been used to develop this protocol, and as such, techniques like transmission, SERS, etc. cannot be confirmed to use this method.
Stokes only, no THz/GHz Raman	Only Stokes Raman scatter is considered for this protocol, excluding GHz/THz Raman scatter. Anti-Stokes is not considered.

6.2 Test sample measurement

The Raman twinning protocol shall begin with the measurement of the test sample in both Raman instruments, the reference instrument (labelled as RI_R) and the instrument to be twinned (labelled as RI_T). The general scheme in Figure 2 represents all essential elements of the test sample measurement.

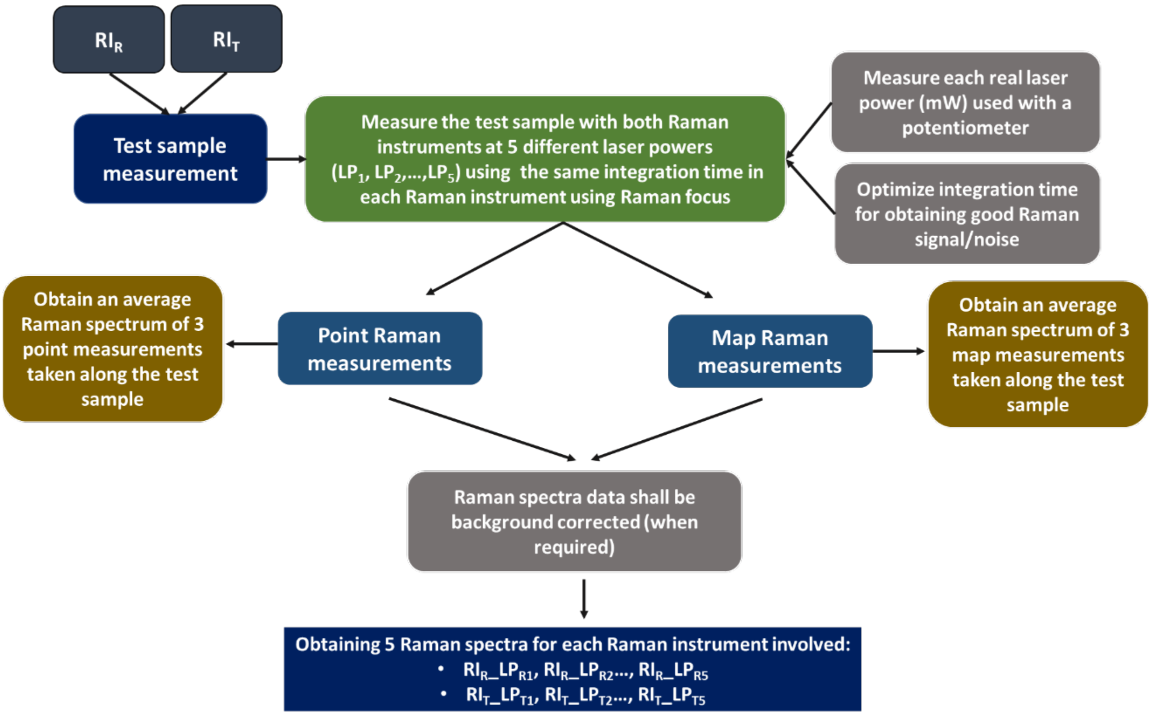

Figure 2: Scheme of the test sample measurement step.

The Raman spectra shall be taken using **Raman focus**, that is, focusing on the test sample where the Raman signal is maximized. This shall be checked before taking the final Raman spectra for the twinning protocol in both non-confocal or confocal Raman systems. The objective of measuring by performing Raman focus is to maximize the Raman signal of each measurement and sample, since the focal length will depend on the penetration depth, which depends not only on the laser wavelength, but also on properties such as the Raman cross-section or the actual colour of the sample.

The test sample shall be measured with at least **5 different laser powers** in each Raman instrument (LP_1, LP_2, LP_3, LP_4, $LP_{5…}$). The laser powers chosen shall be at different increments in the range of 5 to 100% of available laser power (i.e. 20, 40, 60, 80 and 100 %). Laser powers and integration times can be different in each Raman instrument. Average Raman spectra of the test sample shall be obtained with each of the laser powers selected for the reference instrument ($RI_R\_LP_{R1}$, $RI_R\_LP_{R2}$…, $RI_R\_LP_{R5}$) and for the instrument to be twinned ($RI_T\_LP_{T1}$, $RI_T\_LP_{T2}$…, $RI_T\_LP_{T5}$) to obtain a linear regression line for each instrument.

The real laser power (mW) used to measure each Raman spectrum in each Raman instrument shall be provided with maximum precision. For this, a calibrated power meter shall be used. The **laser power shall be measured several times** until confirming the certainty of the values and verify the stability of the laser power for each set value to calculate the average. The laser power shall be measured in the same way in the two Raman instruments to be twinned. Furthermore, ideally the same power meter should be used in both cases. The measurement error between the two power meters used shall be less than 5%. In Annex B there is more information on how to correctly measure laser power.

In each Raman instrument, a specific integration time shall be set. The **integration time shall be optimized** to obtain Raman spectra with a minimum level of Raman signal/noise ratio as provide as guideline on CWA1 (see CWA1 for a reference of what a good SNR might be) with all the used laser powers. Each Raman instrument can demand a different integration time, but it shall be the same for the 5 selected powers.

The way to measure the test sample shall be different depending on the type of Raman instrument. There are two possible options: Raman instruments with the capability to make only single-point Raman measurements and Raman instruments with mapping capability.

- **Single point:** In the case of Raman instruments with just the capability to make single point Raman measurements, to obtain good Raman spectra, at least 5 Raman single point measures shall be performed along the test sample with each laser power, in such a way that there is an average spectrum for each laser power used.
- **Mapping**: In the case of Raman instruments with the capability to make a Raman map, to obtain good Raman spectra, at least 3 Raman maps shall be performed along the test sample with each laser power, in such a way that there is an average Raman spectrum for each laser power used. The scan Raman measurements can correspond to a line or a surface (that is actually the sum of several scans' lines). The size and points per map will be optimized according to each Raman instrument. The length of the line or the area of the map should have the same size and resolution for in both reference and twinned instruments.
- **Note**: The Mapping shall be of a surface that represents correctly the sample distribution and represents a similar area of analysis of the illumination of the units to be twinned. e.g. reference unit has 1um laser spot (confocal) and other units' illumination spot is ~100um, the map shall be aprox 95-105um. The volume/deepth of sampling shall be considered too.

All the Raman spectra shall be **background corrected**. The background Raman spectrum **shall be removed** after the acquisition of the sample Raman spectrum.

Note: background correct refers to a spectrum in the same conditions of the sample, but with laser irradiation off, no variation in the adquisiton conditions shall be introduced.

Finally, the average Raman spectra of the test sample for the reference instrument ($RI_R\_LP_{R1}$, $RI_R\_LP_{R2}$..., $RI_R\_LP_{R5}$) and for the instrument to be twinned ($RI_T\_LP_{T1}$, $RI_T\_LP_{T2}$..., $RI_T\_LP_{T5}$) shall be obtained.

6.3 Raman spectra pre-processing

For Raman instruments twinning, the test sample Raman spectra shall be pre-processed for normalisation and baseline removal. A summary scheme is shown in Figure 3.

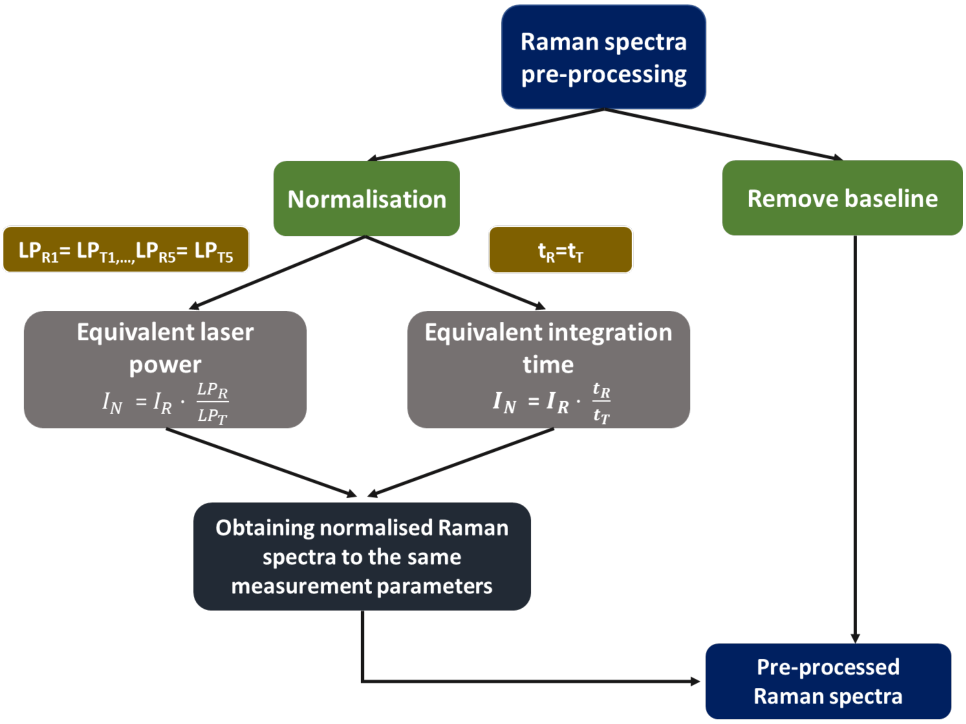

Figure 3: Scheme of the Raman spectra pre-processing steps.

Normalisation

The Raman spectra of the instrument to twin shall be normalised to the same measurement parameters of integration time and laser power used in the reference instrument, in order to have equivalent measurement conditions in all Raman spectra, as follows:

- When the selected laser powers for the measurements in the two Raman instruments have been different, the Raman intensity shall be normalised in the instrument to be twinned according to the laser powers used in the reference instrument, Equation 1 shall be applied.

$$I_N = I_R \cdot \frac{LP_R}{LP_T} \tag{1}$$

Where I_N is the normalised Raman intensity, I_R the calibrated raw Raman intensity, LP_R the laser power of the reference instrument and LP_T the laser power of the Raman instrument to be twinned.

- When selected integration time for the measurements in the two Raman instruments has been different, the Raman intensity shall be normalized in the instrument to be twinned according to the integration time used in the reference instrument. Equation 2 shall be applied.

$$I_N = I_R \cdot \frac{t_R}{t_T} \tag{2}$$

Where I_N is the Raman intensity normalised, I_R the calibrated Raman raw intensity, t_R the integration time used in the reference instrument and t_T the integration time used in the instrument to be twinned.

- When selected laser powers and integration times for the two Raman instruments have been different, Equation 1 shall be applied first, and then Equation 2.

After these operations, the RI_R and RI_T Raman spectra shall have equivalent laser power in each of the 5 Raman spectra and equivalent integration time.

Baseline removal

After normalisation, the baseline shall be removed from all the Raman spectra of both Raman instruments to obtain similar Raman spectra in terms of background. The baseline shall be removed with the same criterium and procedure in all cases. Annex C shows an example of how to remove the baseline.

6.4 Calculation of laser power regression lines

The twinning of Raman instruments is achieved by applying an algorithm that allows obtaining a CF. This experimental constant correlates the Raman intensities of the reference Raman instrument and the Raman instrument to be twinned. The CF accounts for the differences in efficiency between the Raman instruments, including all the variations that exist due to different parameters (such as spot size, power density, optical path, spectrometer, etc.). The CF is obtained from the ratio between the slopes of the laser power regression lines obtained by representing the intensity of a defined reference Raman band (RRB) in the post-processed Raman spectra versus the used laser power in both Raman instruments. This linearity will define the difference between the Raman instruments to be twinned. *Figure 4* shows a scheme with the fundamental elements of this step:

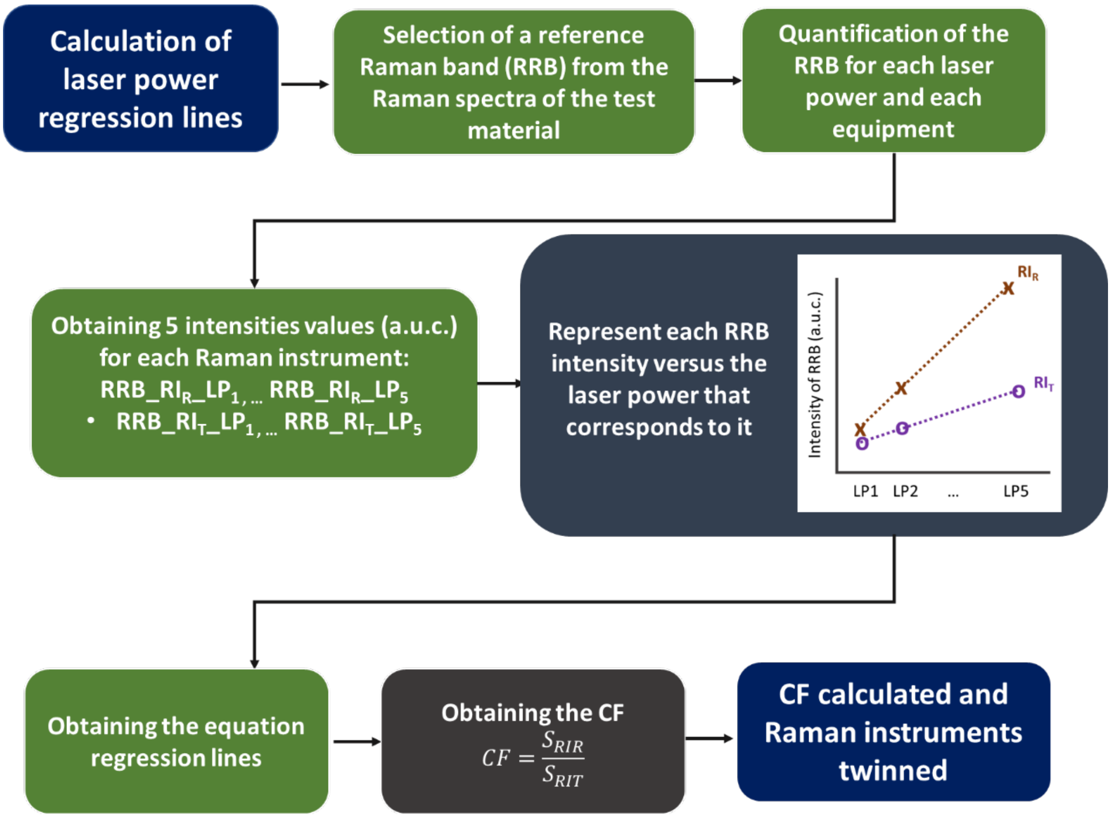

Figure 4: Scheme of the calculation of laser power regression lines step.

First, a reference Raman band (RRB) easily identifiable and quantifiable should be selected and its Raman intensity in the corrected Raman spectrum of the test sample shall be quantified for each laser power and each Raman instrument. For intensity quantification, the peak should be fitted with an adequate function, such as Lorenzian, Gaussian, Voigt or Pearson IV, depending on the specific case, to find the maximum of the signal, which will be the Raman intensity (in a.u.c) of the selected RRB.

Then, the laser power regression lines shall be obtained by representing each Raman intensity of the RRB vs. its laser power for both Raman instruments, and the linear regression equations of each instrument shall be obtained. The CF shall be calculated with Equation 3 as the ratio between the slopes (S) of the laser power regression lines for the reference Raman instrument and of the Raman instrument to be twinned.

$$CF = \frac{S_{RIR}}{S_{RIT}} \tag{3}$$

The calculated CF is the experimental result that correlates the variation in Raman intensity of both Raman instruments. With the CF the two Raman instruments are twinned and, therefore, their Raman spectra can be harmonised in terms of Raman intensity (Section 7).

6.5 Verification of Raman twinning

The verification of the effectiveness of the Raman twinning protocol shall be carried out in three different ways.

First of all, verification shall be done by multiplying the Raman intensities of the RRB_RI$_T$ by the CF and checking that the points overlap with the regression line of the RRB_RI$_R$ used to calculate the CF, indicating that the Raman intensities coincide throughout all the laser powers.

Besides, the verification shall be performed after multiplying the RI$_T$ Raman spectra of the test sample by the CF. It is recommended to be done for all laser powers. In this way, the Raman spectra of both Raman instruments should coincide in their Raman intensities.

In addition, the quality of the Raman twinning shall be evaluated by quantifying differences in Raman intensities. For this, a quality factor for the harmonisation of Raman intensity (Q_{HI}) shall be calculated with the Equation 4, considering the areas under the harmonised and the reference spectra as follows:

$$Q_{HI} = 1 - \frac{|A_{ref} - A_{har}|}{A_{ref}} \tag{4}$$

Where A_{ref} is the area under the reference Raman spectrum and A_{har} is the area under the twinned Raman spectrum considering the full spectral range. This equation shall be applied for each pair of laser powers and calculate their average. The higher the Q_{HI} value, the better the harmonisation between two Raman instruments, where Q_{HI}=1 means an ideal harmonisation and a Q_{HI} greater than 0.9, a very good harmonisation. It shall be done for all laser powers.

Furthermore, after having performed the verification with the data used to carry out the twinning protocol, it is recommended to carry out a validation with the same three procedures using Raman data obtained with 2 additional laser powers, different from those used in the twinning protocol.

Validation can also be carried out when the CF is applied to harmonize Raman spectra of a real sample if the real sample is also measured in the reference Raman instrument (see Section 7).

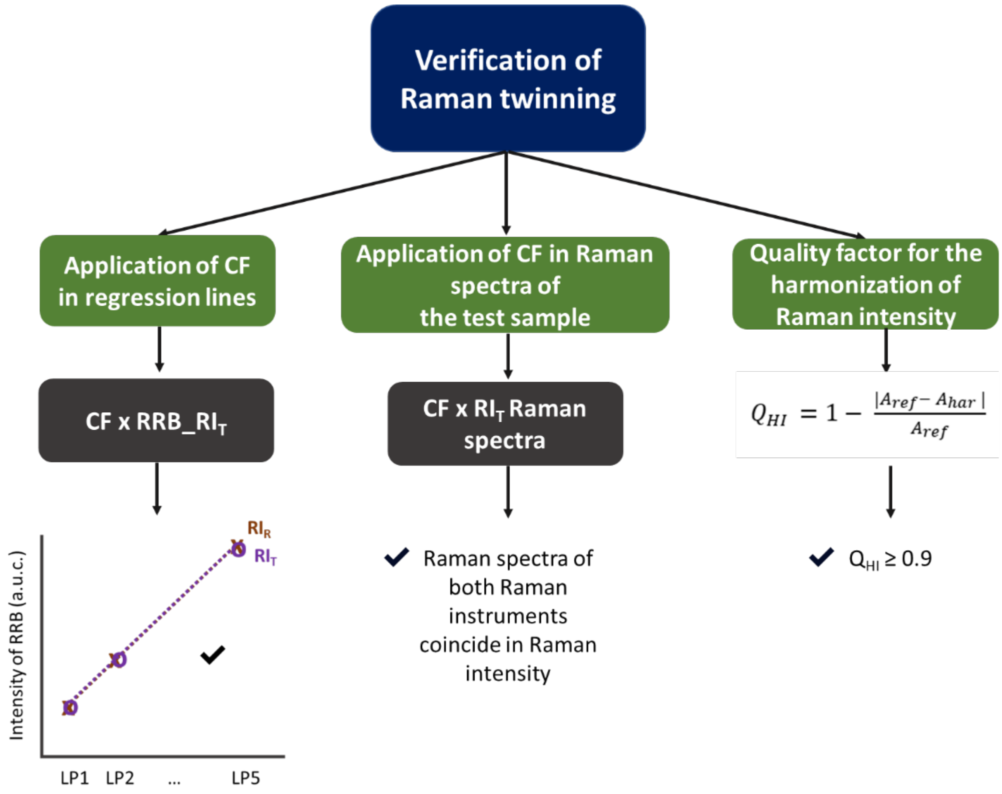

Figure 5: Scheme of the verification of Raman twinning.

7 Application of CF to harmonise Raman spectra

Once the CF is obtained, the two Raman instruments are twinned, and therefore, the Raman spectra of different real samples can be harmonised in terms of Raman intensity.

To obtain a harmonised Raman spectrum , the CF shall be multiplied by the RI_T Raman spectrum. The Raman spectrum shall be previously corrected and calibrated or taken on a calibrated equipment (in both axes). Furthermore, the Raman spectrum to be harmonised shall have equivalent measurement conditions in terms of laser power and integration time than those used to calculate the CF (the spectra can be taken under the same conditions of laser power and integration time or be normalised).

To check the quality of harmonization and validate the twinning protocol, Equation 4 can be applied if the sample is measured in both Raman instruments. An example of intensity harmonisation applying the CF is shown in Annex D.

Annex A (informative) Example of test sample

An example of a test sample for this Raman twinning protocol is a composite material of epoxy and anatase TiO_2 particles (0.5% by weight in this example). This test sample was developed to fulfil all the requirements of Section 5: it has a high homogeneity, strong scattering, stability over time, thermal stability and high reflectance.

TiO_2 has certain advantages for this application: it can be dispersed well in a polymer matrix to obtain a homogeneous material, it has a good Raman signal, and is stable, since it does not degrade in the presence of most chemical substances.

The manufacture of the test sample is carried out by homogenizing the TiO_2 particles in the epoxy matrix in three different steps. Initially, the epoxy resin and TiO_2 particles were mixed in a high-speed stirrer (2700 rpm, 2 min). Subsequently, the suspension was subjected to high shear (20000 rpm, 2 min), followed by final homogenization using a three-cylindrical roller. Then, the suspension was casted into a mould and subjected to the curing process recommended by the resin manufacturer. Then, the composite material was demolded and cut to thicknesses of approximately 2 mm. Finally, the surface is polished [8].

Figure 6a shows an example of Raman spectrum of this test material obtained by using a BWTek iRaman Lab instrument with a 20X objective, 785 nm laser, measured at 290 mW with 6 s integration time. Figure 6b shows the quantification of the Raman intensity of the main TiO_2 band, at 144 cm^{-1} (I_{144}), in 50 different points of the test sample. Raman intensities in all the measurements are very similar, having a deviation ≤2.8% across the test material, showing the high homogeneity of this test material.

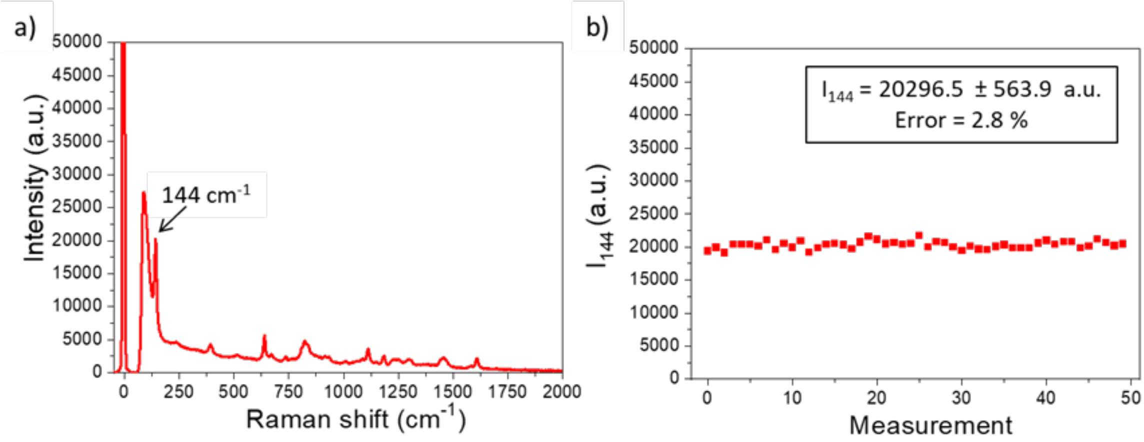

Figure 6: a) Average Raman spectrum of epoxy + 0.5% TiO_2 and b) maximum intensity of the TiO_2 band at 144 cm^{-1} (I_{144}) of each of the 50 measurement points measured at 290 mW and 6 s of integration time using a BWTek instrument with a 20X objective and a 785 nm excitation laser.

This twinning protocol has been tested with this test material, selecting as RRB its most intense Raman band at 144 cm^{-1}. However, for Raman instruments that with a cut-off at higher wavenumbers, it may also be possible to use any other TiO_2 Raman band, as for example, the 638 cm^{-1} Raman band, obtaining equivalent results.

Annex B (informative) Example of laser power measurement

To estimate the real value of the laser power, it shall be accurately measured with a calibrated power meter. The same model and type of power meter should be used in both Raman instruments to minimise errors.

There are different power meters with different types of sensors (photodiode, energy and thermal), which allow the precise measurement of the power of the lasers used in Raman spectroscopy.

In this example, laser power is measured with a power meter with a photodiode sensor. First of all, it is essential to select the wavelength of the laser that is going to be measured on the power meter. Next, the laser power meter photodiode sensor shall be placed where the sample would go. When the laser is turned on, it shall be ensured that the entire laser beam hits the sensor, as close as possible to the centre of the sensor. The value that shall be reported for each selected laser power shall be the average of at least 10 readings. This process shall be repeated for each optical path that is going to be twinned.

Annex C (informative) Example of baseline removal

Figure 7 contains an example of how to remove the baseline from a Raman spectrum. On the one hand, *Figure 7*a shows raw Raman spectra of a test sample (Annex A) taken at 5 different laser powers with both Raman instruments, where all Raman spectra present certain baseline. On the other hand, *Figure 7*b shows the Raman spectra with a flat baseline at 0 a.u.c. after its removal. The aim is to be able to quantify the intensity of just the Raman bands, without taking into account other contributions to the signal.

For baseline removal, different processing software or scripts can be used, or sometimes the Raman instrument control software itself presents this function. The user shall evaluate and decide in each case the best way to remove the baseline. In addition, the baseline shall be removed carefully to avoid creating spectral distortions. In this example, all the baselines have been removed with OriginPro 2022 calculation software, using the UserDefined baseline mode, the method 2nd derivate (zeroes) with 25 number of points and the spline interpolation method.

It should be noted that for this step to be valid all baselines shall be removed with the same criteria.

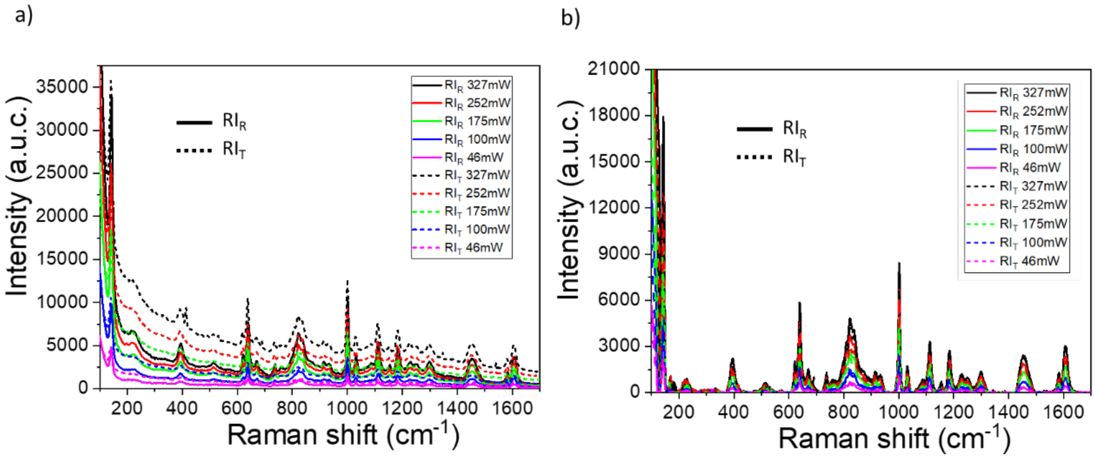

Figure 7: a) Raman spectra before removing the baseline and b) Raman spectra after removing the baseline.

Annex D (informative) Example of Raman intensity harmonisation

An example of Raman spectra harmonisation in terms of Raman intensity is shown in *Figure 8*. For this example, the Raman spectra of the test sample of Annex A has been used.

First, *Figure 8*a shows the Raman spectra obtained and calibrated (following the CWA1) in two different Raman instruments (RI$_R$ and RI$_T$) at 5 different laser powers after the pre-processing step of the Raman twinning protocol. *Figure 8*b shows the Raman spectra after harmonisation after applying the CF. These two Raman instruments were twinned following the protocol of this CWA2, where the CF obtained from the power regression lines that can be observed in *Figure 8*c, was 1.39. In this case, the main Raman band of the TiO_2 (144 cm^{-1}) has been used as RRB, but it can be used with any other Raman band since, as mentioned above, the method has also been validated with other TiO_2 Raman bands.

As it can be seen in *Figure 8*a, the Raman spectra between RI$_R$ and RI$_T$ do not coincide in Raman intensity. However, by multiplying the Raman spectra of RI$_T$ by the CF (1.39), it can be seen in *Figure 8*b how the Raman spectra coincide in each pair of laser power. This shows with an example how the Raman spectra of two previously twinned instruments can be harmonised in Raman intensity.

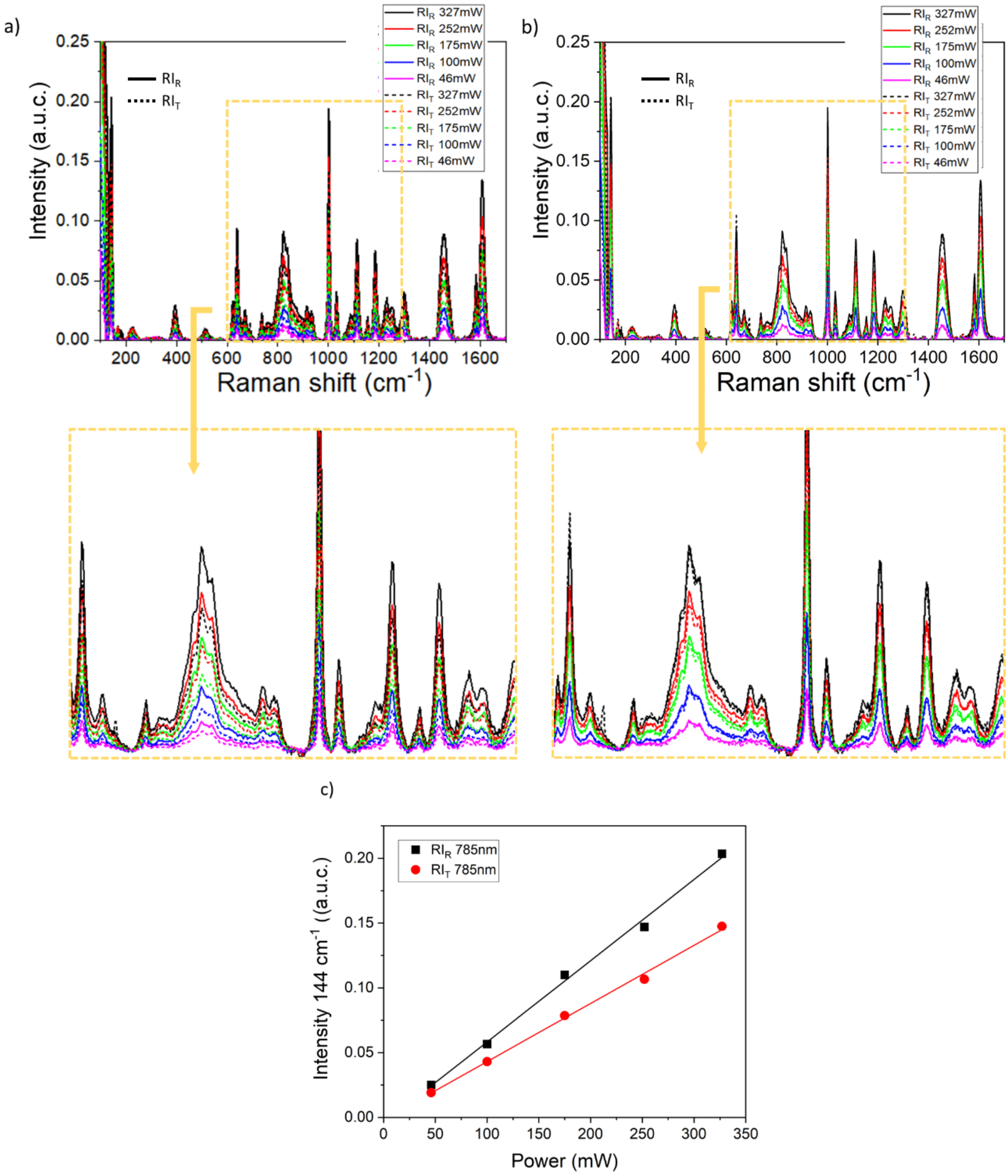

Figure 8: a) Raman spectra before applying the CF and b) after applying the CF and c) power regression lines obtained.

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