CWA1: Raman instruments calibration and verification protocols

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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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The following organizations and individuals developed and approved this CEN Workshop Agreement:

-	Consejo Superior de Investigaciones Científicas (CSIC)	Raquel Portela Miguel Bañares José Francisco Fernández María Fernández Alberto Moure Nicolás Coca
-	Elodiz ltd	Enrique Lozano – WS Chair James Thomson – CWA leader Muhammad Faizan
-	Encapsulae	Julián Reinosa
-	Fraunhofer LBF	Dirk Lellinger
-	Goyalab	Thomas Kuntzel Ludovic Guilbert
-	Horiba France SAS	Hajar Elazri Philippe de Bettignies
-	Ideaconsult ltd	Nina Jeliazkova Iliev Luchesar Slava Tsoneva Nikolay Kochev Evgeni Marinov
-	Iran Nano Innovation Council (INIC)	Hassan Pouy Pouy
-	Laboratoire de Chimie Théorique, Sorbonne Université	Mónica Calatayud
-	Lightnovo ApS	Yaroslav Aulin, PhD;

		Yurii Pilhun
		Oleksii Ilchenko
-	CEN/TC 352 Nanotechnologies	Emeric Frejafon
-	National Institute of Advanced Industrial Science and Techonology of Japan (Osaka)	James Taylor
-	National Institute of Standards and Technology (NIST)	Angela Hight Walker
-	National Metrology Institute of Japan (NMIJ)	Nobuyasu ITOH
-	National Physical Laboratory of UK	Dimitrios Tsikritsis
		Natalie Belsey
		Charles Clifford
-	National Technical University of Athens	Charitidis Costas
		Afroditi Ntziouni
-	Osaka University	Katsumasa Fujita
		Yasuaki Kumamoto
		Kazuli Bando
-	Real Casa de la Moneda (FNMT)	Vicente García
		Esther Gómez
		José Lorenzo
		Andrés Ruiz
-	Spanish Association for Standardization (UNE)	Iván Moya – WS Secretary
-	Temas Solutions GmbH (TEMASOL)	Devendra Joshi
		Blanca Suárez
-	Topsøe A/S	Søren Birk Rasmussen
-	Unichim	Massimiliano Rocchia
-	Wasatch Photonics	Dieter Bingemann

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Introduction

Although Raman spectroscopic data intrinsically depends on how the Raman signal is obtained - instrument and acquisition configuration, sample and sampling environment- and (pre)treated/processed to generate and analyse the Raman spectra, there is consensus neither within academia nor among standardisation bodies or manufacturers on the best way to calibrate Raman instruments and harmonise Raman data. The need for the user to calibrate, verify and maintain the Raman instrument is a daily challenge and a source of deviations. An example of the existing divergence in the procedures is the relative intensity correction, fundamental to compare spectra acquired with different instruments or excitation wavelengths. A limited number of manufactures offer intensity correction procedures, using external or internal references, which can be luminescent glasses certified by metrology institutes (wavelength specific) or a calibrated irradiance source (valid for multiple excitation wavelengths), as indicated in ASTM E2911. The calibration frequency and protocol depend on the type of Raman instrument, target application/user, or availability of references. Differences created by the optical path, reference material, operator, or algorithms in the resulting calibration are unclear. Operations relative to the y-axis calibration can be affected by several factors, such as the use of polarisers, sampling geometry, focal point volume, depth of analysis, dispersion, detector-related factors (integration time, saturation, gain, etc.), fluorescence or luminescence effects, electronic noise, environmental light contamination, cosmic radiation, etc.. Operations relative to the x-axis calibration are more frequent and better understood and covered in a good number of available standard protocols and methods (sometimes diverse). These operations include:

- Spectrometer wavelength/wavenumber calibration to correlate pixels with an equivalent wavelength/wavenumber (in absolute nm or cm^{-1})
- Raman shift calculation (in relative cm^{-1}), defined by the laser line
- Spectral dispersion over the observation window, which drives the valid spectral range - and consequently pixel resolution
- Spectral resolution
- Pixel adjustments, related to, e.g., the elimination of hot pixels and data point interpolation

The existing standard reference materials and guides for calibration and validation in Raman spectroscopy have proven to be valuable for many years, but they are incomplete. For example, how spectral resolution might affect calibration is not indicated, Raman shift values provided in ASTM E1840 were obtained with single-mode laser lines only, mainly with FT-Raman systems -a

technique currently in decline-, and no corrections were introduced for polarizability or resonance effects, or the now extensively used multimode lasers. Moreover, they lack interconnection, which is critical for easy and extended applicability and to avoid divergencies, and there is no clear indication to run a complete system calibration or to harmonise data in the standardisation landscape.

This has created a need for a more comprehensive protocol which not only calibrates the x and y axes, but verifies the resolution variation across the entire spectral range. This document introduces one such protocol, which aims to facilitate inter-instrument comparison of data.

1   Scope

This CEN Workshop Agreement (CWA) provides a data harmonisation protocol for Raman instruments that allows:

- − Calibration adjustment of data and instruments already calibrated by the manufacturer;
- − Calibration of instruments without any previous calibration;
- − Verification of the calibration for instruments that were calibrated with this protocol in the past;

The protocol consists of the following calibration and verification stages: x-axis positions, x-axis resolution and y-axis relative intensity correction. In addition to the use of the full protocol to harmonize an instrument, independent sections of the protocol can be used to verify/calibrate certain qualities (x-axis calibration, resolution, or y-axis calibration) of the instrument.

The protocol is applicable to any kind of Raman instrument within the boundaries described in chapter 5. Primary use of this protocol is for fixed grating dispersive spectrometer systems. In scanning spectrometer systems, the exact settings of the scanning must be considered as part of the optical path.

The protocol may have application beyond the boundaries stated, such as systems using 514.5 nm or 633 nm excitation sources, but it has been developed using only 532 nm and 785 nm instruments, and as such its effectiveness with other excitation wavelengths is unconfirmed.

NOTE: Term 'calibration' can have legal meaning in a metrology environment, this is not the focus of this CEN Workshop Agreement

2   Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the cited edition applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

- ASTM E1840 Standard Guide for Spectrometer Calibration and Validation of the Raman shift axis.[1]
- ASTM E2911 Standard Guide for Relative Intensity Correction of Raman Spectrometers.[2]
- ATSM E2529 Guide for testing the resolution of a Raman spectrometer.[3]

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

Counts corrected - Units for the y-axis of the Raman spectrum after an intensity correction algorithm has been used.

3.1.2

Laser zero position - The wavelength of the laser excitation, which becomes 0 Raman shift (cm^{-1}) on the Raman shift x-axis.

3.1.3

Optical path - The list of components (e.g. collection lens, slit, grating) that the Raman scatter passes through, from the sample, to reach the detector.

3.1.4

Pixel resolution - FWHM of neon emission on either the pixel, wavelength, wavenumber, or Raman shift x-axis.

3.1.5

Pixel resolution curve - A function fit of the FWHM vs the position of the neon peaks in the spectral range, in either the pixel, wavelength, wavenumber, or Raman shift x-axis.

3.1.6

Primary Raman data - Raman data as obtained from the software, and thus has already some level of mathematical treatment to create a full Raman spectrum (e.g. pixel binding, stitching data segments, spectrometer offset correction or applying corrections that contaminate the spectrum such as hot pixels). This data will be already displayed in cm^{-1} or Δcm^{-1}.

3.1.7

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

3.1.8

Raw Raman data - A set of (unprocessed) data that is given directly as output from the detector. (Based on an except from CWA 17815:2021)[4]

3.1.9

Spectral distribution - The width of wavelengths/wavenumbers the pixel collects. Taken as the halfway point between Pix(n-1) and Pix(n) - the halfway point between Pix(n+1) and Pix(n), for Pix(n)

3.1.10

Spectral resolution - The minimum distance between two peaks to be separated unambiguously, based on the FWHM of calcite on the Raman shift x-axis.

184 3.1.11
185 **Spectral resolution curve** - An adjustment of the pixel resolution curve based upon the spectral
186 resolution(s).

187 ## 3.2 Symbols and abbreviated terms

c.c.	Counts corrected
ASTM	American Society for Testing and Materials
CRM	Certified reference material
FWHM	Full width half maximum
NIST	National Institute of Standards and Technology
SpeD:SRes	Ratio of spectral distribution to spectral resolution
SRM	Standard reference material, a registered trademark (™) belonging to NIST
SNR:	Signal-to-noise ratio

188 # 4 Overview and Significance

189 This calibration and verification protocol focuses on three parameters of the Raman
190 instrument:

191 – x-axis positions

192 – x-axis resolution

193 – y-axis relative intensity correction

194 X-axis positioning is required to transform the raw Raman data from the detector pixels into a
195 spectrum, with signal intensity as a function of wavelength or Raman shift, in a accurate,
196 reproducible manner, allowing for noting peak shifts or for successful identification -primary
197 Raman data-. X-axis resolution assessment is required for adjusting and comparing data
198 between instruments of varying resolution. Y-axis relative intensity correction is required to
199 evaluate relative intensities, especially for comparing visible laser instruments to NIR laser
200 instruments, as their detectors have wildly different quantum efficiency profiles, though they
201 differ even in systems of the same laser wavelength.
202 The objectives that motivate the development of the harmonization protocol are:

203 1. Create more accurate x-axis calibrations.

204 Current x-axis calibrations are defined by the manufacturer. This can lead to variability in the
205 precision, and therefore peaks from two spectrometers may not align within accepted
206 tolerance if the instrument variance exceeds the expected variation in the peak position.
207
208 2. Facilitate correction of each instrument based on their resolution across the whole range.

209 Resolution of instruments is often taken as a flat figure, normally evaluated at one point in the
210 spectrum. However, it is known that the resolution of an instrument does in fact vary across
211 the detector. If this resolution behaviour across the entire detector can be defined, then a

212   function that adjusts the spectrum can be used to better compare two instruments with
213   different resolution curves.

214

215   3.   Correct the intensity for quantum efficiency-based intensity discrepancies.

216   The instrument response depends on many factors such as the sample properties (geometry,
217   size, density, cross-section, matrix), sampling parameters (time, accumulations, temperature),
218   excitation wavelength and power density, spectrometer, grating, detector, optics, focus,
219   refractive indexes, polarization of irradiated or scattered light, or type of filters. A known issue
220   when comparing Raman spectra originating from systems with a different excitation
221   wavelength, is that the detector responds differently to different wavelengths of light. This is in
222   part due to the transmission curves of the optics, favouring some wavelengths over others.
223   Primarily the difference in intensities seen at different wavelengths is due to the quantum
224   efficiency of the sensor.

5   Requirements of the calibration and verification protocol

The next considerations shall be met in order to assure the harmonization protocol provides the desired results. Any instrument beyond the scope of these boundaries can attempt the full protocol to qualify the behaviour of the instrument, but the resulting calibration has not been confirmed to be within the accepted tolerance of calibration and verification.

5.1   Boundaries

Due to the breadth of Raman instrumentation, the calibration and verification protocol has the boundaries of use included in Table 1.

Table 1. Boundaries of use

Boundary	Explanation
Excitation wavelength	The protocol has been developed primarily with 532 nm and 785 nm Raman systems.
Not designed for full calibration of scanning systems	The protocol can be used to calibrate one mode of a scanning system. However, due to the movement of certain components within the optical path, separate experimental days will require independent calibration.
No polarisation	The effects of polarisation on the Raman peaks have not been considered in this protocol.
No resonance	The effect of resonance on the Raman peaks has not been considered in this protocol.
No discrimination between confocal vs non-confocal	The effect of confocality on the Raman peaks has not been considered in this protocol.
Pixel resolution <0.8 nm	Defined resolution useable with the protocol, based on the FWHM of the Ne lines. (Note: Pixel resolution above that threshold can use the protocols, but the results have not been evaluated)
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 scattering is considered for this protocol, excluding GHz/THz Raman scattering. Anti-Stokes is not considered.
Valid only for one optical path	The calibration spectra must be collected with the same optical path and laser. Changes to either require their own calibration. x-axis calibration can be shared by similar optical paths, but requires specificverification. Y-axis calibration must be done for each optical path.
Post-acquisition calibration of data	The protocol is designed for post-acquisition calibration of data. It can be used: *to calibrate new instruments by manufacturers. *to create a virtual calibration, with existing instruments. It does not add a new calibration to the instrument.

Full protocol is required for calibration of an instrument	The individual steps and sections must ALL be followed to consider the system calibrated. The protocol is broken down into branches for ease of understanding. Individual branches are useful for verification of parameters.

234

5.2   Reference materials

The required qualities of the materials used in the protocol are defined in Table 2. Sample-specific guidance on how to measure is included in Table 4 (Section 5.3.2) and Annex A.

Table 2. Samples and their required quality

Material	Quality
Neon	Must not contain non-neon lines which interfere with the analysis of the neon lines. The relative intensity of the peaks should be a close match for literature values[5,6], e.g. 585 nm peak should be the strongest peak. Variation in exact intensity is expected as spectrum will not be intensity calibrated.
Silicon	Only one silicon sample is required The surface can be smooth or rough . The dopant of the silicon can be undoped (N) or doped with boron (B) or phosphorus (P). The orientation of the silicon crystal can have (100) or (111) crystal orientation if undoped or (100) crystal orientation for any of the mentioned dopants.
Calcite	The spectrum must not have strong fluorescence. The fluorescent maximum should not be higher than 20% of the $1085 cm^{-1}$ peaks raw maximum. Calcite should be cut, as this reduces the likelihood of fluorescence being observed, compared to forced cleaving.
Polystyrene	The surface must be polished. Must be a solid extruded, clear, with minimal additives. The thickness of the sample should be 5 mm or larger, with a sampling area of minimum 2 mm diameter or larger. Molecular weight of the polystyrene is recommended to be approximately 185000 g/mol. Significantly lower values (e.g. 35000 g/mol) cause thermal instability, and significantly higher values (e.g. 400000 g/mol) have deviations from the anticipated peak positions.
NIST SRMs	Specific dependent on wavelength of excitation (only 532 nm and 785 nm covered).[7,8]
ELODIZ LED	Specific dependent on wavelength of excitation (only 532 nm and 785 nm covered).
White light source	Traceable source will preferably function over the entire spectral range

5.3 Data quality

5.3.1 Data quality

The reference spectra need to be checked for quality according to the information included in Table 3.

Table 3. Data quality considerations

Issue	Explanation
Saturation	The peaks being analysed and the areas in close proximity must not be saturated.
SNR	Signal to noise ratio of peaks to be analysed must be at least >8, and ideally >100. SNR is calculated using (S-B)/N, where S is the peakmaximum, B is the peak base level, and N is the standard deviation of the noise from a flat region of the spectrum without any peaks.
Spikes	Spikes must be removed from the spectra using a spike removal algorithm, as they can distort peak fitting and other processing steps.
Pedestalling	The spectrum should not have pedestalling, which is where the entire spectrum is above a y-axis value. The y-axis offset should be set to zero in the spectra.
Environmental contamination	Background subtraction must be applied to each spectrum to remove effects from the environment, such as light emissions, etc. The background must be collected using the same acquisition parameters (i.e., exposure time, number of averages/accumulations) with the sample and probe in the same positions as during the spectrum acquisition, with the laser off. No shutters or covers should be used that prevent the environmental light from reaching the detector during background acquisition.
Raw-data files	The data acquired should not have any pre-processing (smoothing, baseline correction, etc.) except those necessary to generate a readable spectrum file (such as binding or stitching).

5.3.2 Sample specific guidance

The particular guidance to be considered for the samples is defined in Table 4.

Table 4. Sample specific considerations

Reference material	Specific consideration
Neon	Make sure the emission port for the neon is in the focal plane. It may be required to use aditional optics for delivery of the emission to the optical path, or reduction of intensity to avoid saturation. These accessories must not interfere with the resulting spectrum. Adjust the acquisition time to ensure the peaks are not saturated. In uncooled detectors, a second spectrum may be required to see some weaker peaks. This will require a longer exposure time than the first, and will result in some of the originally visible peaks becoming saturated. The widest range of peaks should be usable.

Silicon	Due to polarization phenomena, rotate the sample in the plane orthogonal to the laser to maximise signal.
	Different silicon types have different tolerances – see Appendix 1
	Rougher surface is a stronger scatter when exposed to 785 nm laser excitement.
	Maximise signal using Raman focus.
	WARNING: NIR lasers may easily heat the sample, creating a broad peak near 2500 cm^{-1}. This broad emission may need to be saturated (using increased acquisition time) to allow for better visibility of the silicon peak at 520.45 cm^{-1}. Limit sample exposure to laser radiation before taking a spectrum.
Calcite	Maximise signal using Raman focus
	Due to polarization phenomena, rotate sample in the plane orthogonal to the laser to further maximise signal.
Polystyrene	Maximise signal using Raman focus.
	WARNING: Caution with highly focused collection optics, since high power density can cause deformation and damage the sample. Limit the laser power to prevent damage.
NIST SRMs	Use as indicated by the NIST and ASTM E2911.
ELODIZ LED	Warm up LED for 60 seconds to achieve stability.
	Maximize the intensity at the centre of the irradiation, focus on the surface of the glass.
White light source	Use as indicated by the manufacturer

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5.3.3 Metadata

Some information is required to accompany the spectra and the resulting calibration.

Mandatory metadata include:

- Make and model of the instrument
- Serial number of the instrument
- Instrument type
- Optical path details
    - Laser wavelength
    - Focusing optics
        - Numerical aperture
    - Grating
    - Slit size
    - Pinhole, if present
- Acquisition parameters
    - Exposure time
    - Number of averages/accumulations
    - Laser power in mW at sample positon

Optional metadata include:

- Number of data points
- Cut off filter wavelength/Raman shift
- Temperature in the lab
- Laser spot size
- Any stitching or binding processing used to create the full spectrum

6   X-axis calibration and verification protocol

6.1   Prerequisites and purposes

Prerequisits for the x-axis calibration protocol are:

- A.   Neon spectrum[1]
- B.   Silicon spectrum[2]
- C.   Calcite spectrum[3]
- D.   Polystyrene spectrum[4]
- E.   Laser wavelength integer

Detailed information on these prerequisites can be found in Annex A. Sample-specific guidance on how to measure the samples is included in Table 4 and sample requirements in Table 2.

A.   Neon spectrum

The neon peak positions are used to create a wavelength x-axis. These need to be correlated with NIST absolute peak position values. The neon peak widths are used to find the pixel resolution.

B.   Silicon spectrum

The silicon spectrum is used to calculate the Raman shift x-axis based on the silicon peak position in the wavelength x-axis.

C.   Calcite spectrum

The calcite spectrum is used to to verify the calibration steps and to make a final adjustment to the Raman shift x-axis (with the polystryene peaks), as well as to assess the Raman spectral resolution.

D.   Polystyrene spectrum

The polystyrene spectrum is used to verify the calibration steps and make a final adjustment to the Raman shift x-axis (with the calcite peaks).

E.   Laser wavelength integer

The laser wavelength integer is required to turn the Raman shift x-axis that already exists on an instrument to an approximate wavelength x-axis.

---

[1] NIST tabulated neon values – found in ANNEX A, Table 5

[2] Tabulated silicon values – found in ANNEX A, Table 6

[3] Tabulated calcite values – found in ANNEX A, Table 7

[4] ASTM tabulated polystyrene values – found in ANNEX A, Table 8

307 Algorithm/techniques required to be used are:

    a) Peak base approximation / baseline
    b) Peak finding / peak candidate generation
    c) Peak fitting using
        o Gaussian peak shape
        o Pearson IV peak shape
        o Voigt peak shape
    d) Polyharmonic spline

316   a) Peak base approximation / baseline

317 Peak base approximation is required to give the peak fitting method a grounding point for the
318 peak to be fit upon.

320   b) Peak finding / peak candidate generation

321 Peak candidates are the rough parameters of the peak used as a first approximation of the
322 peak. The included factors are position (of the highest pixel), height (amplitude of highest pixel)
323 and FWHM (geometrically calculated).

325   c) Peak fitting

326 Peak shapes used are Gaussian, Voigt and Pearson IV. The peak shapes are used to find the
327 precise position and the FWHM of the peak observed. Shape selected is based upon the
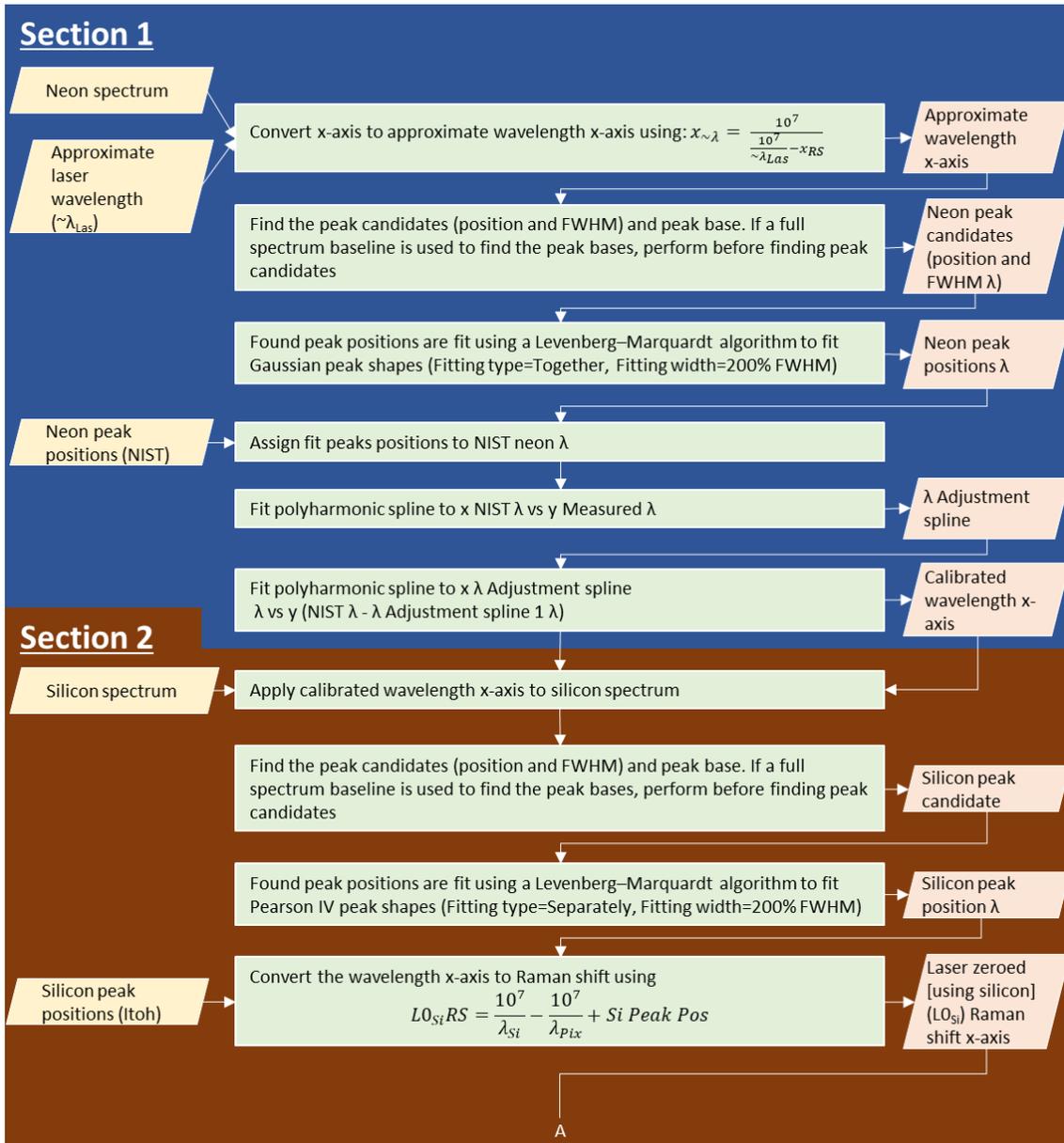328 associated error of the fit and the accuracy and precision of the results.

330   d) Polyharmonic spline

331 Polyharmonic spline is a function used to interpolate and extrapolate the rest of the x-axis from
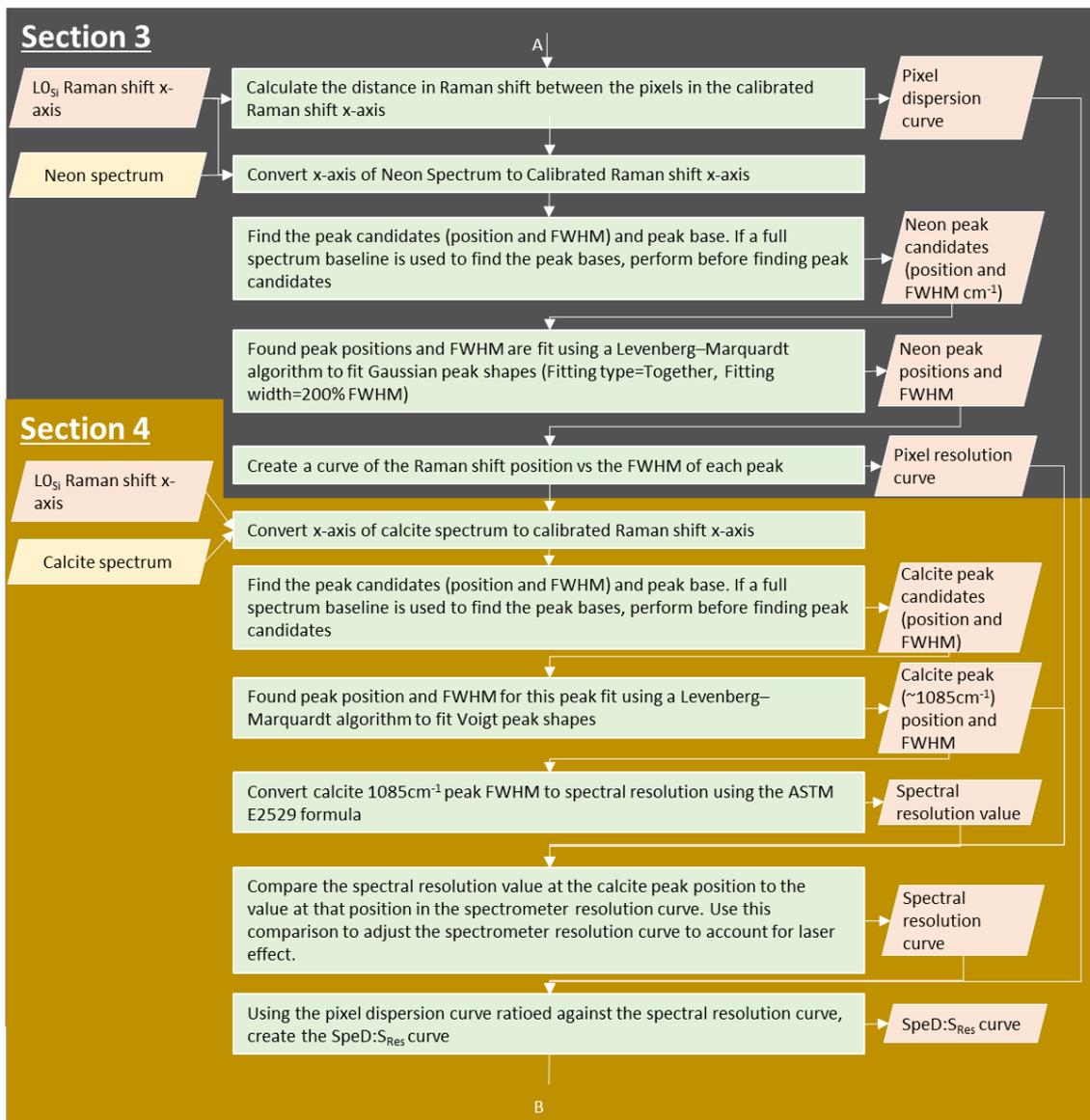332 the ideal/absolute values and the observed values.

333

6.2   Position and resolution calibration and verification protocol

The calibration and verification protocol for X-axis positioning and resolution is described in Figure 1, Figure 2 and Figure 3, where:

- Green shaded box = instruction
- Yellow shaded box = acquired before protocol
- Orange shaded box = acquired during protocol
- Blue shaded box = needs testing / confirmation

Section 1

Neon spectrum

Approximate laser wavelength ($\sim\lambda_{Las}$)

Convert x-axis to approximate wavelength x-axis using: $x_{\sim\lambda} = \dfrac{10^7}{\frac{10^7}{\sim\lambda_{Las}} - x_{RS}}$

Approximate wavelength x-axis

Find the peak candidates (position and FWHM) and peak base. If a full spectrum baseline is used to find the peak bases, perform before finding peak candidates

Neon peak candidates (position and FWHM λ)

Found peak positions are fit using a Levenberg–Marquardt algorithm to fit Gaussian peak shapes (Fitting type=Together, Fitting width=200% FWHM)

Neon peak positions λ

Neon peak positions (NIST)

Assign fit peaks positions to NIST neon λ

Fit polyharmonic spline to x NIST λ vs y Measured λ

λ Adjustment spline

Fit polyharmonic spline to x λ Adjustment spline λ vs y (NIST λ - λ Adjustment spline 1 λ)

Calibrated wavelength x-axis

Section 2

Silicon spectrum

Apply calibrated wavelength x-axis to silicon spectrum

Find the peak candidates (position and FWHM) and peak base. If a full spectrum baseline is used to find the peak bases, perform before finding peak candidates

Silicon peak candidate

Found peak positions are fit using a Levenberg–Marquardt algorithm to fit Pearson IV peak shapes (Fitting type=Separately, Fitting width=200% FWHM)

Silicon peak position λ

Silicon peak positions (Itoh)

Convert the wavelength x-axis to Raman shift using

$$LO_{Si}RS = \frac{10^7}{\lambda_{Si}} - \frac{10^7}{\lambda_{Pix}} + Si\ Peak\ Pos$$

Laser zeroed [using silicon] (LO_{Si}) Raman shift x-axis

A

Figure 1. Section 1 & 2 of the x-axis position and resolution calibration and verification protocol.

Section 3

LO$_{Si}$ Raman shift x-axis	Calculate the distance in Raman shift between the pixels in the calibrated Raman shift x-axis → Pixel dispersion curve
Neon spectrum	Convert x-axis of Neon Spectrum to Calibrated Raman shift x-axis
	Find the peak candidates (position and FWHM) and peak base. If a full spectrum baseline is used to find the peak bases, perform before finding peak candidates → Neon peak candidates (position and FWHM cm^{-1})
	Found peak positions and FWHM are fit using a Levenberg–Marquardt algorithm to fit Gaussian peak shapes (Fitting type=Together, Fitting width=200% FWHM) → Neon peak positions and FWHM

Section 4

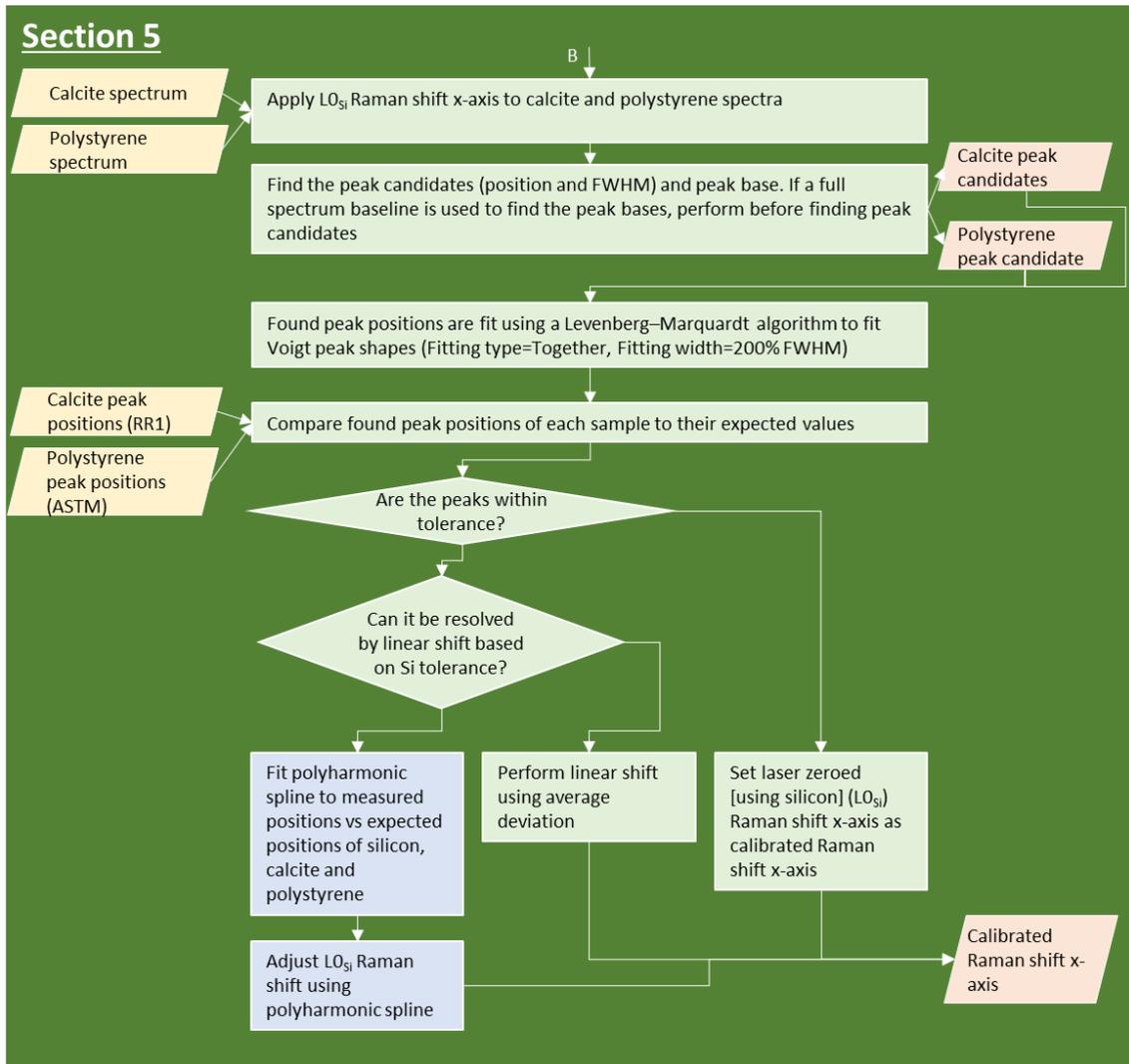|	
LO$_{Si}$ Raman shift x-axis	Create a curve of the Raman shift position vs the FWHM of each peak → Pixel resolution curve
Calcite spectrum	Convert x-axis of calcite spectrum to calibrated Raman shift x-axis
	Find the peak candidates (position and FWHM) and peak base. If a full spectrum baseline is used to find the peak bases, perform before finding peak candidates → Calcite peak candidates (position and FWHM)
	Found peak position and FWHM for this peak fit using a Levenberg–Marquardt algorithm to fit Voigt peak shapes → Calcite peak (~1085cm^{-1}) position and FWHM
	Convert calcite 1085cm^{-1} peak FWHM to spectral resolution using the ASTM E2529 formula → Spectral resolution value
	Compare the spectral resolution value at the calcite peak position to the value at that position in the spectrometer resolution curve. Use this comparison to adjust the spectrometer resolution curve to account for laser effect. → Spectral resolution curve
	Using the pixel dispersion curve ratioed against the spectral resolution curve, create the SpeD:S$_{Res}$ curve → SpeD:S$_{Res}$ curve

343

344   *Figure 2. Section 3 & 4 of the x-axis position and resolution calibration and verification protocol.*

Figure 3. Section 5 of the x-axis position and resolution calibration and verification protocol.

6.2.1   Section 1– Wavelength x-axis

Use a neon spectrum and an approximate wavelength x-axis to match the neon peaks to their NIST assignments, and then calibrate the x-axis to a wavelength x-axis (Figure 1).

6.2.2   Section 2– Laser zeroed Raman-shift x-axis

Apply the wavelength x-axis to the silicon spectrum. The silicon peak is then used to find the laser zero position and convert the wavelength x-axis to the Raman-shift x-axis (Figure 1).

6.2.3   Section 3– Pixel dispersion & Spectrometer resolution curves

Use the laser zeroed Raman-shift x-axis to find the pixel dispersion across the detector. The laser zeroed Raman-shift x-axis is then applied to the neon spectrum to create a spectrometer resolution curve from the FWHM of the neon peaks (Figure 2).

6.2.4   Section 4– Spectral resolution & SpeD:SRes curve

Applies the laser zeroed Raman-shift x-axis to the calcite spectrum to find the Raman spectral resolution, which in turn is used to adjust the pixel resolution curve to make a Raman spectral resolution curve & SpeD:SRes curve (Figure 2).

6.2.5   Section 5– Calibrated Raman-shift x-axis

Apply the laser zeroed Raman-shift x-axis to the calcite and polystyrene spectra using the peak position information to make a final adjustment to the laser zeroed Raman-shift x-axis to create the calibrated Raman-shift x-axis (Figure 3).

7   Y-axis (relative intensity) correction protocol

Relative intensity correction shall be performed after the calibration of x axis, as the conversion formula requires the use of the x-axis values.

Y-axis correction involves the measurement of a certified reference material that can be:

- NIST SRM fluorescent glass sample
- Traceable LED light source (e.g. ELODIZ Raman intesity correction LEDs)
- Traceable white light source (e.g. Tungsten lamp)

The expected curve will accompany the sample, usually in the form of a formula, and must be compared to the measured curve. The ratio between the two is used to apply a multiplier to each pixel's intensity value.

Y-axis after calibration should be in a.u.c. units to indicate that a correction algorithm has been applied to the data.

8   Calibration files

Calibration files contain the necessary information needed to apply the calibration to an instrument (by a manufacturer) or to data (post-acquisition, by an end user).

For x-axis calibration, the file contains:

- Metadata as listed in section 5.3.3
- Date of the calibration
- Calibration curve, consisting of points of uncalibrated shift values as independent variable and calibrated shift values as dependent variable
- The silicon peak position in wavelength/wavenumber
- The calibrated laser wavelength in nm (optional)

The intended purpose of the calibration curve points is to generate a spline function with these points, and then to retrieve the calibrated Raman shift values. Thus, the calibration curve may or may not have as many points as the original spectra. However, the spline generated by the curve points must match the original calibration data with sufficient accuracy. The first and the last point should correspond with the minimum and maximum shift value of the spectral range, respectively.

For y-axis calibration, the file contains:

- Reference to x-axis calibration used
- Metadata as listed in section 5.3.3
- Date of the calibration

400	- Calibration curve, consisting of points of calibrated shift values as independent variable
401	and intensity factors as dependent variable.
402	
403	The intended purpose of the calibration curve points is to generate a spline function with
404	these points, and then to retrieve the intensity factors as function values of this spline. Thus,
405	the calibration curve may or may not have as many points as the original spectra. However,
406	the spline generated by the curve points must match the original calibration data with
407	sufficient accuracy. The first and the last point should correspond with the minimum and
408	maximum shift value of the spectral range, respectively.
409	

Annex A (Normative) Requirements for x-axis calibration and verification protocol

The following spectra must be acquired for x-axis calibration, before the beginning of the calibration protocol, in the same analysis session. Neon and polystyrene may require collection of a second over-exposed spectrum to enhance low intensity peaks within the spectrum.

- Neon spectrum
  - o Neon over exposed spectrum
- Silicon spectrum
- Calcite spectrum
- Polystyrene spectrum
  - o Polystyrene over exposed spectrum

Laser wavelength integer is the wavelength of the laser in the Raman instrument, as indicated by the supplier. It is infrequent that the manufacturer provides an accurate laser wavelength value but the unit will be sold with a laser wavelength such as 532 nm, or 785 nm, which is what is referred to as the laser wavelength integer.

Table 5 Neon peak positions (NIST)[9]

Peak #	Peak position in wavelength / nm	Uncertainty / nm	Peak #	Peak position in wavelength / nm	Uncertainty / nm
1	533.07775	0.00004	38	705.91079	0.00004
2	540.05616	0.00004	39	717.39380	0.00004
3	556.27662	0.00004	40	724.51665	0.00004
4	565.66588	0.00004	41	748.88712	0.00004
5	571.92248	0.00004	42	753.57739	0.00004
6	574.82985	0.00004	43	754.40439	0.00004
7	576.44188	0.00004	44	794.31805	0.00004
8	580.44496	0.00004	45	808.24576	0.00004
9	582.01558	0.00004	46	811.85495	0.00004
10	585.24878	0.00005	47	813.64061	0.00004
11	587.28275	0.00004	48	830.03248	0.00004
12	588.18950	0.00005	49	836.57464	0.00004
13	590.24623	0.00004	50	837.76070	0.00010
14	594.48340	0.00005	51	846.33569	0.00004
15	596.54710	0.00004	52	849.53591	0.00004
16	598.79074	0.00004	53	854.46952	0.00004
17	602.99968	0.00005	54	857.13535	0.00004
18	607.43376	0.00005	55	859.12583	0.00004
19	609.61630	0.00005	56	863.46472	0.00004
20	612.84498	0.00004	57	870.41122	0.00010
21	614.30627	0.00005	58	877.16575	0.00010
22	616.35937	0.00005	59	878.37539	0.00004
23	618.21460	0.00004	60	885.38669	0.00004

24	621.72812	0.00005
25	626.64952	0.00005
26	630.47893	0.00005
27	633.44276	0.00005
28	638.29914	0.00005
29	640.22480	0.00010
30	650.65277	0.00005
31	653.28824	0.00005
32	659.89528	0.00005
33	667.82766	0.00005
34	671.70430	0.00005
35	692.94672	0.00004
36	702.40500	0.00004
37	703.24128	0.00004

61	891.95007	0.00010
62	898.85564	0.00010
63	914.86720	0.00010
64	920.17588	0.00010
65	927.55191	0.00010
66	930.08532	0.00010
67	932.65072	0.00010
68	937.33079	0.00010
69	942.53797	0.00010
70	945.92110	0.00010
71	948.66825	0.00010
72	953.41640	0.00010
73	954.74052	0.00010
74	966.54200	0.00005

426

Table 6. Silicon peak positions

Dopant	Orientation	Peak position in Raman Shift / cm^{-1}	Standard deviation / cm^{-1}	Source
None	100	520.45	0.28[‡]	Itoh et al[10]
None	100	520.66*	0.53	RR1 Silicon study
None	111	520.47*	0.46	RR1 Silicon study
Boron	100	520.40*	0.34	RR1 Silicon study
Phosphorus	100	520.27*	0.41	RR1 Silicon study
Phosphorus	100	520.45*	0.47	RR1 Silicon study
All	All	520.45*	0.46	RR1 Silicon study

* average of all spectra collected in Round Robin 1 (RR1) Si study performed by CHARISMA H2020 project (GA 95921, see Annex B), normalised to 520.45 cm^{-1} (Raman shift) value provided by Itoh et al[10]

[‡] expanded uncertainty (coverage factor k = 2)

Table 7. Calcite peaks positions

Peak position in Raman Shift* / cm^{-1}	Standard deviation / cm^{-1}	Source
155.21	1.37	RR1 Calcite study
281.26	1.08	RR1 Calcite study
711.95	0.71	RR1 Calcite study
1085.91	0.56	RR1 Calcite study
1435.22	0.67	RR1 Calcite study
1748.91	0.7	RR1 Calcite study

* Spectra collected in Round Robin 1 (RR1) study performed by CHARISMA H2020 project (GA 95921, see Annex B), were calibrated using neon and silicon

Table 8. Polystyrene peak positions from ASTM E1840

Peak position in Raman Shift / cm^{-1}	Standard deviation / cm^{-1}	Relative intensity	Source

620.9	0.69	16	ASTM E1840
795.8	0.78	10	ASTM E1840
1001.4	0.54	100	ASTM E1840
1031.8	0.43	27	ASTM E1840
1155.3	0.56	13	ASTM E1840
1450.5	0.56	8	ASTM E1840
1583.1	0.86	12	ASTM E1840
1602.3	0.73	28	ASTM E1840
2852.4	0.89	9	ASTM E1840
2904.5	1.22	13	ASTM E1840
3054.3	1.36	32	ASTM E1840

437

Annex B (informative) Harmonization protocol development process

439 This protocol is the culmination of several tasks and studies performed by the H2020
440 CHARSIMA (Grant Agreement No. 95921)

441 Round robin 1.1

442   This round robin utilised the materials defined earlier in the CHARISMA project. Each
443   sample was analysed using every optical path of each instrument available within the
444   consortium. This collection of data is the cornerstone of the protocol development as
445   these are the training development. The data collected here would primarily be used to
446   harmonise the x-axis for both position and resolution purposes.

447   The instruments within the consortium include various configurations (i.e., handheld,
448   portable, and scanning instruments) as well as different laser wavelengths, though the
449   only wavelengths that appear in more than 1 instrument were 532 nm and 785 nm.

450 Silicon study

451   This study utilised several silicon materials. It was noticed by a consortium member
452   that the Raman peak for silicon could noticeably change by moving the sample, as well
453   as by day to day, which adds on to the well-known effect of sample rotation in an axis
454   perpendicular to the beam. Thus, a study was devised to analyse the silicon samples in
455   different positions and orientations over several days, to assess this variability. With
456   laser power, humidity and temperature recorded to check for any dependence in the
457   ranges analysed.

458 Polystyrene study

459   This study utilised the polystyrene defined earlier in the CHARISMA project, as well as
460   other recommended standard polystyrene source and polystyrene samples with
461   divergent characteristics (e.g., high molecular weight). These were studied to see the
462   effect of the different polystyrene characteristics on the Raman spectrum.

463 Peak fitting and protocol development task force

464   To facilitate the development of the algorithms and fine tuning of the data processing
465   used for the protocol, as task force was assembled of members from WP3, 4 and 5.
466   This task force has experimented with different methods of peak fitting and x-axis
467   calibration.

468   Round robin 1.2 (Relative intensity correction)

469   The second part of the internal consortium round robin utilised the material defined
470   earlier in the CHARISMA project and is still under development. These data are used to
471   harmonise the relative intensity response of the instrument.

472   Note on existing standards

473   ASTM E2911 was created in tandem with the NIST SRMs, and outlines the method of
474   relative intensity correction in more detail. In late 2022, this standard was temporarily
475   removed without explanation. As such, CHARISMA have developed a relative intensity
476   correction protocol, which is performed with ELODIZ LED light sources

477

Annex C (informative) Software (Altaxo, Oranchada)

479   Two software have been developed as part of the H2020 project CHARISMA that can be used
480   for calibration purposes. These are described in this annex.

C.1 Oranchada

482   Orange, an open source data mining and machine learning tool, primarily focuses on workflow
483   design for data analysis and modelling.[11,12] Orange provides a visual interface for designing and
484   executing data analysis workflows. The primary purpose of workflow design is to create a
485   structured sequence of data processing and analysis steps. Orange provides a user-friendly
486   visual interface where users can create and modify workflows using drag-and-drop
487   components called widgets. These widgets represent data processing and analysis tasks. Users
488   can design workflows by connecting widgets in a modular fashion. Each widget performs a
489   specific function, such as data loading, data pre-processing, visualization, and modelling. The
490   visual canvas allows for flexibility in structuring the workflow. Workflows can include data
491   transformation steps, where users apply various operations to manipulate and pre-process
492   data. This might involve filtering, scaling, imputation, and feature selection. Users can include
493   widgets for data visualization, allowing them to gain insights from the data and assess the
494   results of their analyses. Orange provides widgets for machine learning and predictive
495   modelling, enabling users to build and evaluate models for classification, regression, clustering,
496   and more. Data flows through the workflow from one widget to another, and users can observe
497   how data changes at each step, making it a powerful tool for interactive data analysis. Like
498   dedicated workflow systems, Orange supports iterative design and experimentation. Users can
499   modify their workflows, re-run analyses, and refine their models based on the results. Orange
500   offer some scripting and programming capabilities, which allow for customization and the
501   integration of Python code within the workflow and / or developing custom widgets or set of
502   widgets, known as add-ons.

503   Oranchada add-on guide is available at Zenodo.[13]

504   Figure 4 shows the Orange main screen with Oranchada Pro widgets on the left : Add Baseline,
505   Add Noise, Subtract, Find Peaks, Fit Peaks, Generate Spectra, HDR Merger, HHT Sharpening,
506   Load File, Load Test Spectra, Merger, Moving minimum, Normalize, RS to WL, Recover Spikes,
507   Resample NUDFT, Select, Set X axis, WL to RS, Xaxis fine calibration and Load File Names.[14]

508
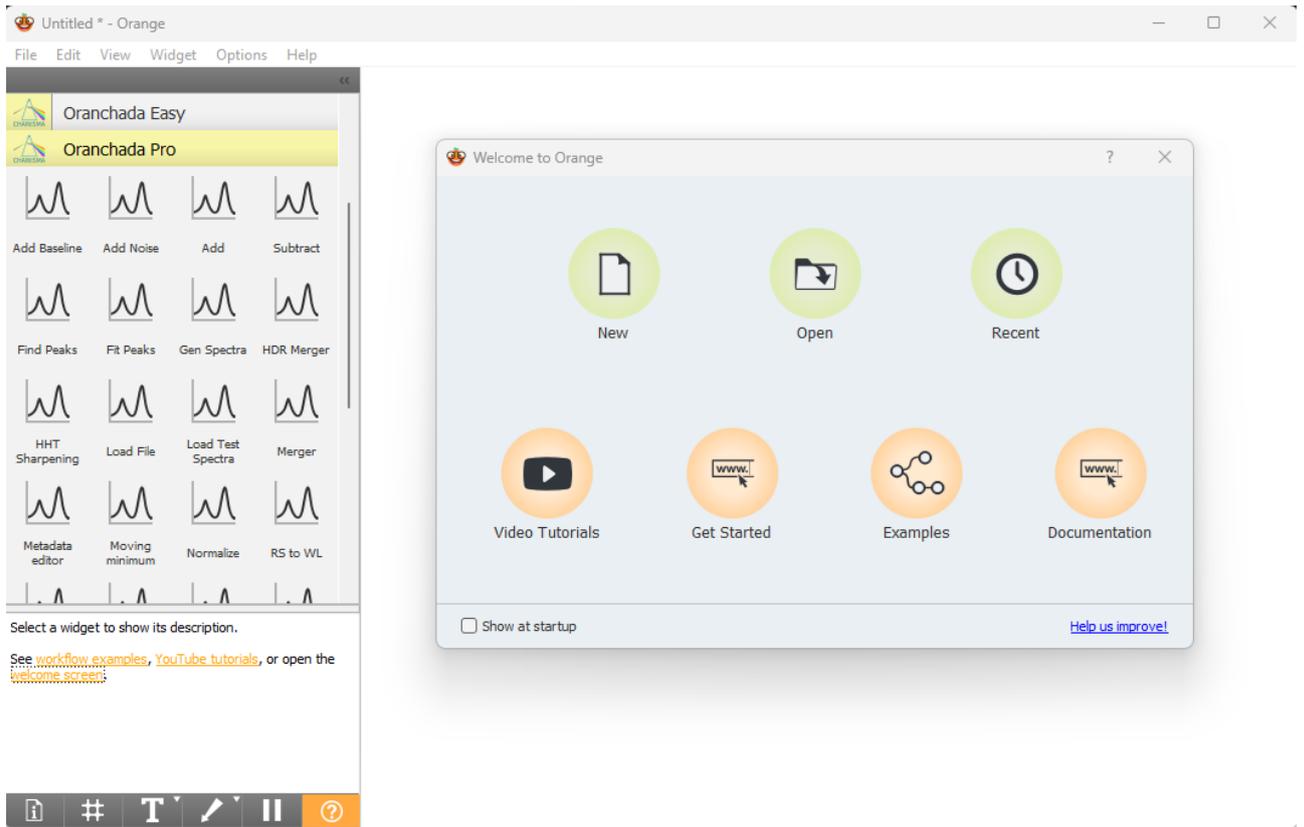
Figure 4. Main screen of Orange data mining with Oranchada add-on. "Welcome to Orange" shows some important basic functions create a new project, open an existing one, or choose from the ones we recently worked on. There are also links to helpful information on how to work with Orange.

Oranchada widgets for spectra processing have a common structure, described at Figure 5.

513
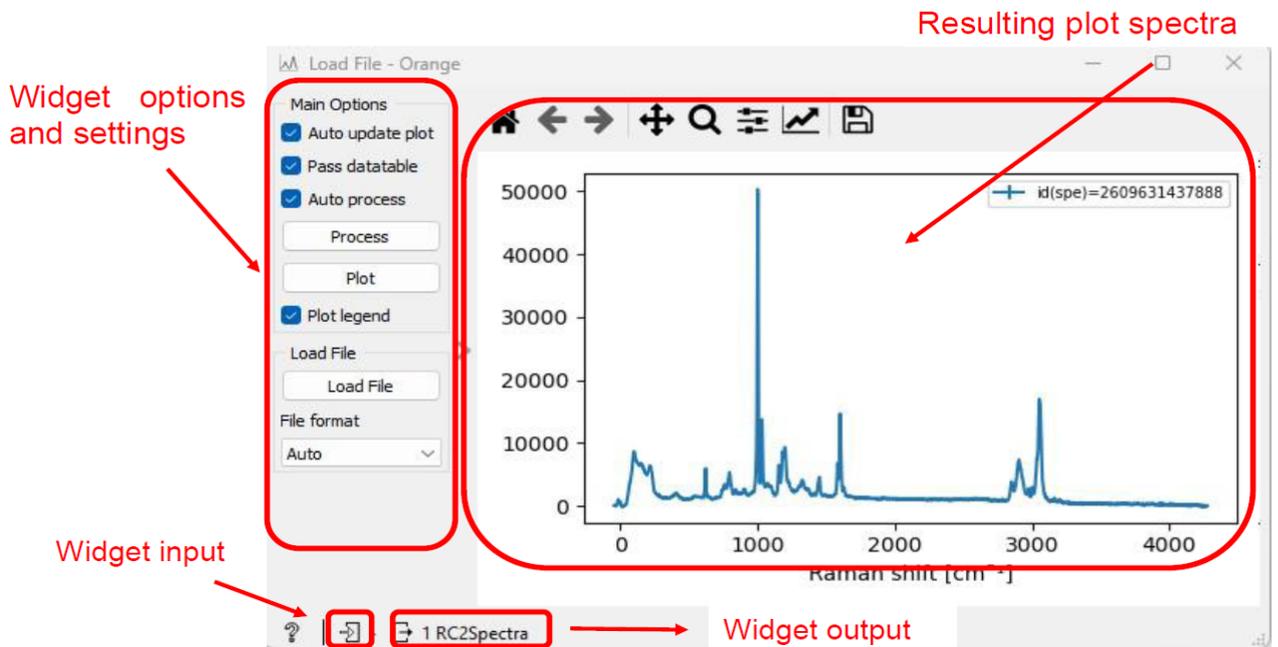
Figure 5. Basic structure for Oranchada Spectra processing widgets

Widgets have input and output channels. For example, the "HHT Sharpening" widget Input channels are "RC2Spectra" and respectively Outputs channels are "RC2Spectra" and "Data"

27

517   (Figure 6 left). When connecting a widget to another widget with multiple channels, as in the

518   example workflow (Subtract widget at Figure 6 right), a popup window asking users for

519   information which channels to connect will appear.

520
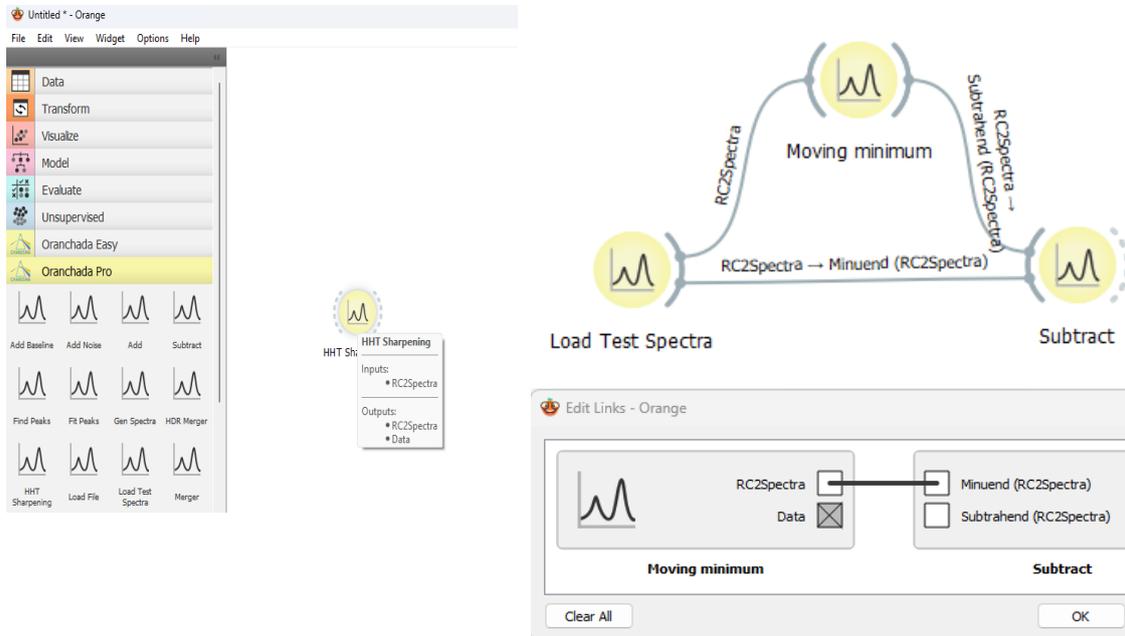
521   *Figure 6. Widgets have input and output channels.*

522

C.1.1 Spectral import

524   Spectral import is done by using the load file widget (Figure 7). Various spectral file formats are

525   supported. After a spectrum is loaded, it can be visualized by clicking the Plot button.
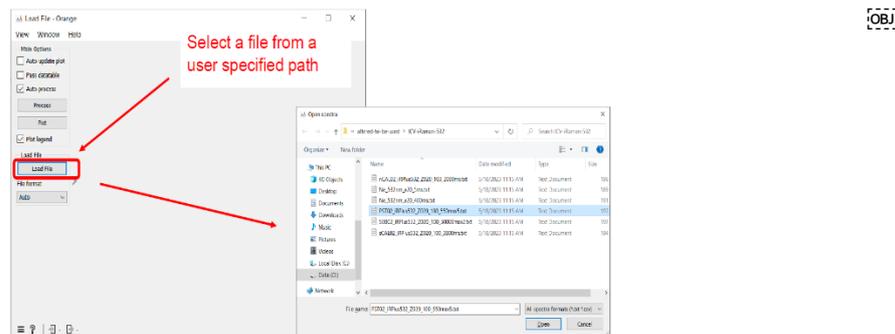
526   *Figure 7. Load file widget allows selecting, loading and visualize one or more spectra in supported formats.*

C.1.2 Calibration

528   Orange workflow implementing the calibration protocol is available as .ows file at

529   https://github.com/h2020charisma/oranchada/tree/main/examples .

530   The pending Oranchada release will feature a single widget for calibration.

531   The calibration can also be done by Python code using ramanchada2 library .

C.1.3 Applying calibration to acquired data

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28

C.2 Altaxo

538   Altaxo is an open source, general purpose graphical data analysis and plotting program
539   developed since 2002 by one of the consortium members.[15,16] It is a project-oriented program.
540   This means that everything is stored in one project file, which is a .zip file containing the project
541   items. There are different types of project items. The most relevant are tables, in which the data
542   are stored, 2D and 3D graphs for the visualization of the data, and notes using the Markdown
543   language syntax. The project items can be organized in a hierarchical way, i.e. they can be
544   organized in folders and subfolders. In this way, even very large projects (in the GB range) can be
545   realized without losing overview.

546   There are special menu points for treating repeating task: project items and entire folders can
547   be moved and copied. Data can be mass imported. Project items can be mass exported in various
548   formats, including to Excel format for tables, Word format for Notes, and various image formats
549   for 2D and 3D graphs. By utilizing data sources for each table, tables remember where the data
550   has come from. In this way, data analyses can be repeated by executing the data source again.

551   If the built-in menus are not appropriate, tasks can also be scripted. There is a built-in script
552   editor for C# with syntax highlighting and code completion This makes scripting really easy.
553   Altaxo has a huge mathematical and signal processing library. Vector and matrix operations are
554   accelerated by the Intel Math Kernel library.

555   The main window of Altaxo is shown in Figure 8. The structure of the program windows is flexible,
556   i.e. both document windows and toolbar windows can be docked to every side, stacked, and
557   even moved outside of the main window.

558   Installation of Altaxo is easy: use the graphical installation with a .msi installation file. In fact, no
559   installation at all is necessary, because Altaxo runs on all computers with Windows 7, Windows
560   10 and Windows 11 out of the box, simply by expanding a provided .zip file into a folder on the
561   computer or even a USB memory stick. Details on how to install Altaxo can be found on the
562   homepage [13].

563
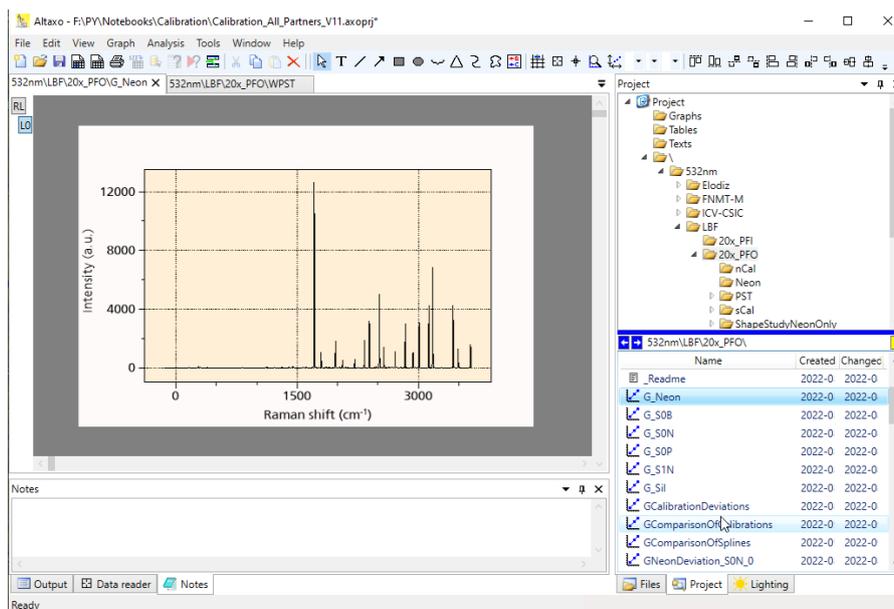
Figure 8 Main window of Altaxo. On the left side, the document area shows the currently visible documents. On the
*right side, the project explorer shows the content of the project.*

564
565

For the CHARISMA project, some features were added to Altaxo, which are described in the
following sections.

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567

C.2.1 Spectral import

568

Spectral data can be imported into Altaxo in various formats:

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- ASCII format. This is the most flexible option. Altaxo is able to recognize the structure of most of the ASCII files, including different separation strategies (separation by tabs, spaces, columns with fixed width etc.) and different cultures (numbers with dot or comma as decimal separator). There is a menu point to import multiple ASCII files in one table, which is useful when having multiple spectra belonging together, for instance a time series of spectra.

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577
578
579
- .SPC files. This is a binary file format, developed by Thermo Galactic, for containing the spectral data to be processed in GRAMS (a spectral software). .SPC files can contain multiple spectra. Currently, most of the spectrometers are able to export spectra using this format.

580
581
- JCamp-DX files. It is a text-based file format for storing spectroscopic data, originally for infrared spectroscopy.

582
- Renishaw Wire .wdf files. This is a binary, vendor specific file format from Renishaw.

583
584
- Import using an import script. Arbitrary other file formats can be imported by writing an import script in C#.

585

In Figure 9, the import menu points of Altaxo are shown.
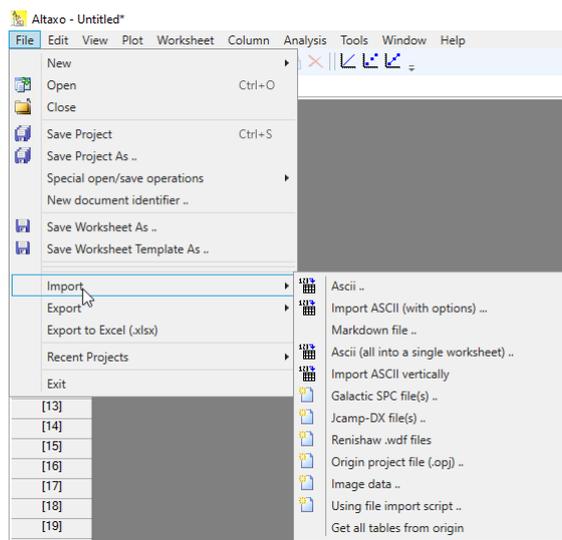
587 *Figure 9 Import menu of Altaxo*

C.2.2 Calibration

589 Altaxo contains fully automated routines for performing x-axis calibration as well as y-axis
590 calibration of a Raman instrument. In order to perform an x-axis calibration, a spectrum of
591 neon (obtained by using a neon lamp) and a Raman spectrum of a silicon sample are
592 necessary.[17] After both spectra are imported into Altaxo, the user first chooses the neon
593 spectrum, then uses the menu point Analysis->Spectroscopy->Raman->Neon calibration. The
594 neon calibration dialog opens (Figure 10). Almost all options available can be left to their
595 default value. Only the approximate wavelength of the laser must be entered.

596
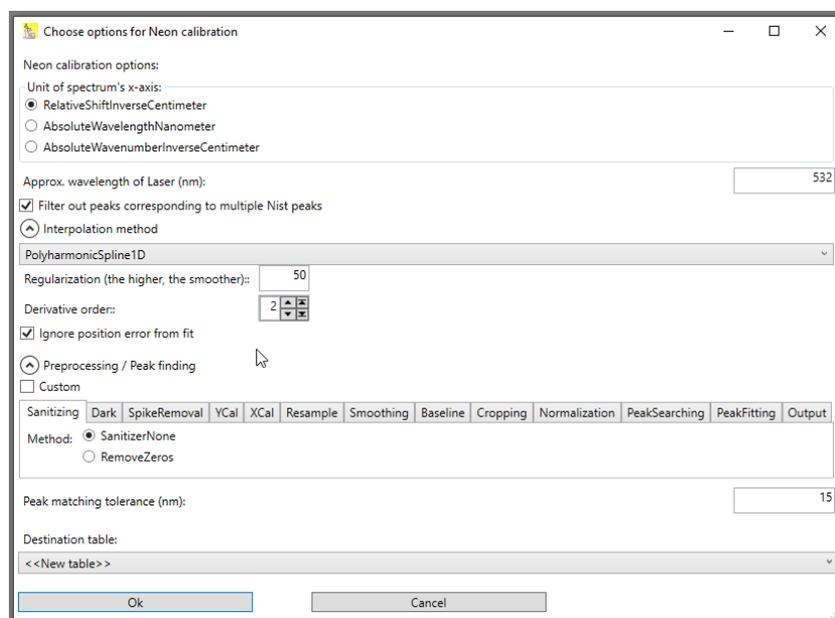
598 *Figure 10 Neon calibration dialog of Altaxo.*

599 After confirming the dialog with OK, a new calibration table is created, in which the results of
600 the neon calibration are stored. The results already allow to assign each point of the spectrum,
601 which usually has shift as its x-value, an absolute wavelength value. But the calibration is not
602 yet complete, because the exact laser wavelength is still unknown. In order to get this, the user

603 chooses the silicon spectrum, and by using the menu Analysis->Spectroscopy->Raman->Silicon
604 calibration opens the silicon calibration dialog (Figure 11). Again, the option can be left to their
605 defaults. If the temperature of the measurement (of the silicon wafer) is known, it can be
606 entered in the dialog box. The destination table is set to the same table as where the neon
607 calibration is already stored.
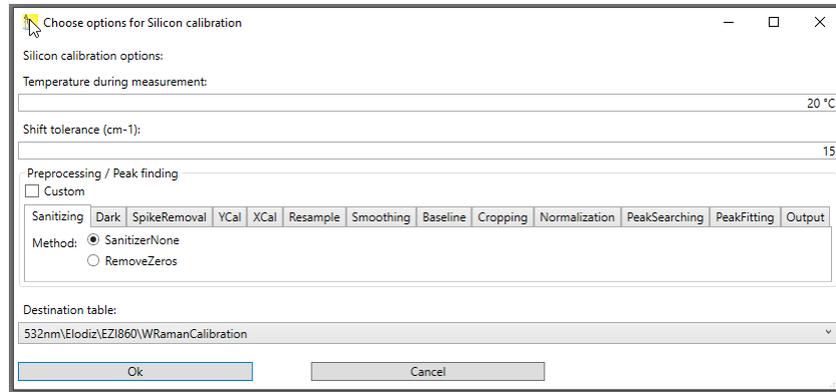
608

609 *Figure 11 Silicon calibration dialog of Altaxo.*

610 By confirming with OK, the silicon calibration is executed, and the calibration table is amended
611 with the results of the silicon calibration. The calibration table now contains all information in
612 order to map the uncalibrated shift values of the experimental spectra to calibrated shift
613 values. Additionally, it contains the calibrated laser wavelength.

614 The y-calibration is done with a spectrum of a calibrated LED or the Raman spectrum of a NIST
615 calibration standard. After the spectrum is imported, the intensity calibration dialog (Figure 12)
616 can be opened by using Analysis->Spectroscopy->Intensity calibration.
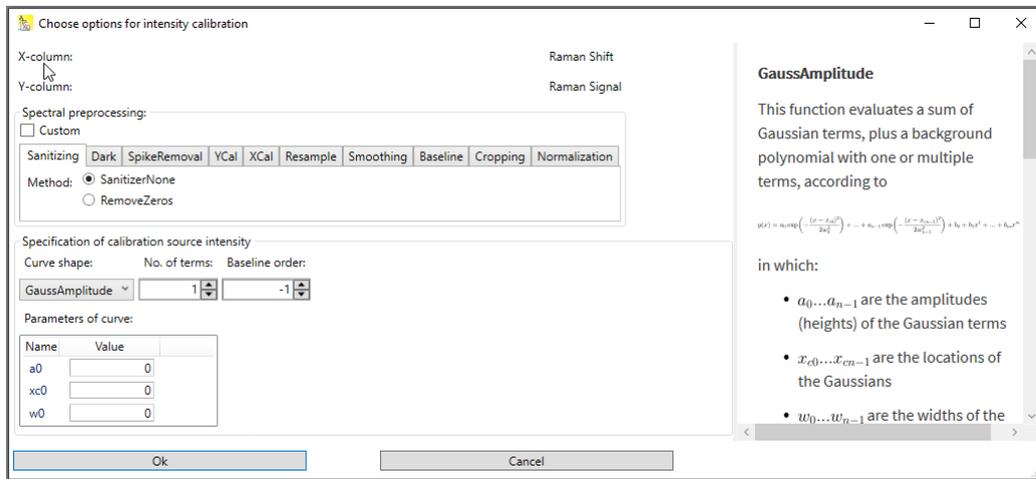
617

618 *Figure 12 Intensity calibration dialog of Altaxo.*

619 In the upper part of the dialog, the pre-processing options can be chosen. For intensity
620 calibration to be performed, it is important to have a spectrum for which the dark spectrum is
621 already subtracted. If this is not yet the case, it can be done here by choosing the appropriate
622 pre-processing option. In the lower part of the dialog, the calibrated curve shape of the LED or
623 NIST standard can be entered. In the case above, a Gaussian shape is chosen, for which the
624 parameters amplitude, position, and sigma can be entered. Other shapes, more peak terms, and
625 parameters for a polynomial baseline can be entered as well. After confirming the dialog with

626   OK, a new calibration table will be created, which contains factors by which the y-values of the
627   spectrum will be multiplied with in order to obtain an intensity calibrated spectrum.

C.2.3 Applying calibration to acquired data

629   If a calibration was successfully performed, it can be applied to other uncalibrated spectra, in
630   order to get a calibrated spectrum. This can be achieved by first import the spectrum or the
631   spectra for which the calibration should be applied into a worksheet. Then a preprocessing is
632   applied to those uncalibrated spectra. Select the y-columns of all spectra for which the
633   calibration should be applied, then choose from the menu
634   Analysis->Spectroscopy->Preprocess. In the spectral preprocessing dialog, go to the tab "XCal",
635   choose "XCalibrationByDataSource", and then choose in "Tables containing calibrations" the
636   table which contains the x-calibration that should be applied to the spectrum or spectra (Figure
637   13).

638   Likewise, in order to apply the y-calibration, go to the tab "YCal", choose
639   "YCalibrationByDataSource", and then choose in "Tables containing calibrations" the table
640   which contains the y-calibration that should be applied to the spectrum or spectra (Figure 14).

641   In the other tabs of the preprocessing dialog, choose the additional options that you want to
642   apply to the spectrum or spectra. Since the spectral preprocessing dialog is also used for peak
643   analysis, you can also apply a x- and y-calibration before making a peak analysis of spectra. You
644   can find it using the menu Analysis->Spectroscopy->Peak finding and fitting.
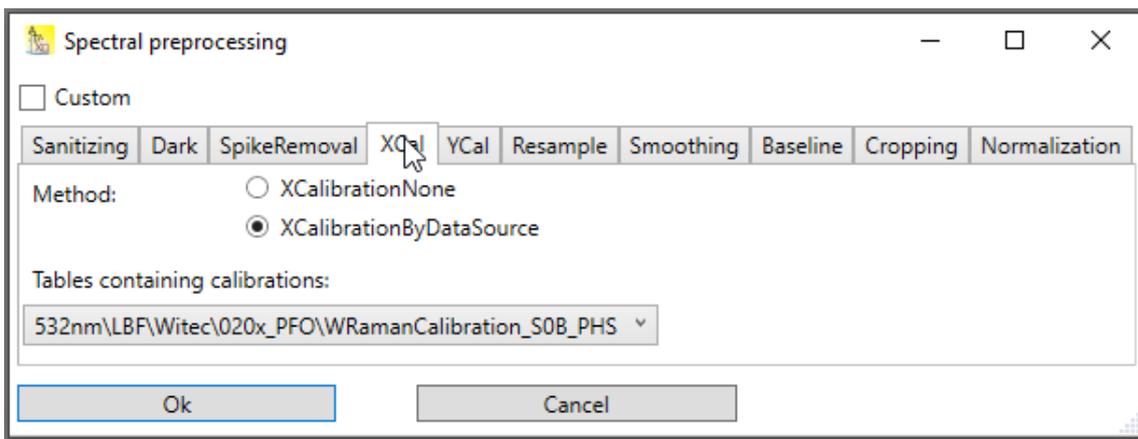
645

646   *Figure 13 Spectral preprocessing dialog showing the "XCal" tab.*

647

648

649 *Figure 14 Spectral preprocessing dialog showing the "YCal" tab.*
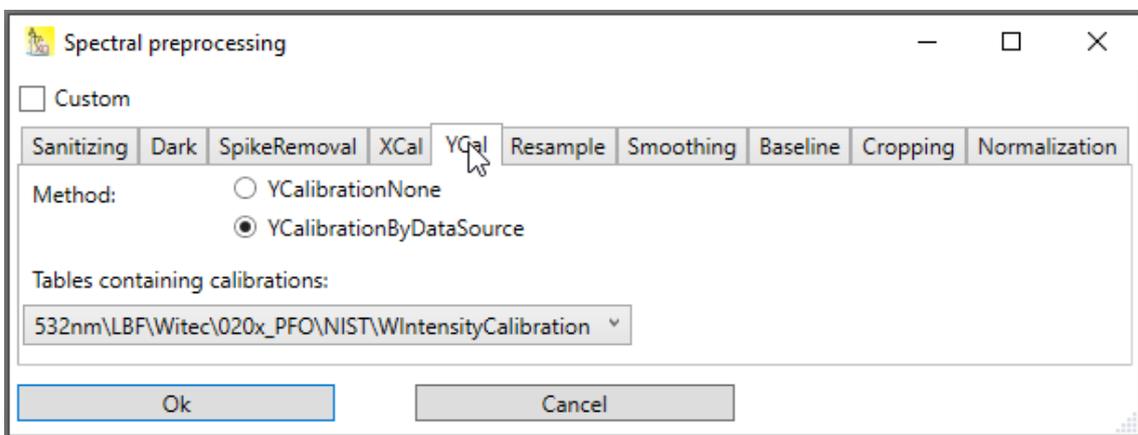
Annex D (informative) Correlation with theoretical methods

651 Peak position and relative peak intensity can be obtained by theoretical calculations and used to
652 validate Raman spectra. Calculations can be performed by widely used ab initio techniques such
653 as density functional theory (DFT) without the input of experimental data, with robust
654 implementation in materials science codes (VASP, CRYSTAL etc). The following issues should be
655 considered:

656 - Experiment-free Raman spectra do not depend on meaurement conditions (hardware,
657 software, cosmic rays, operator). Their accuracy relies on: i) the physico-chemical laws included
658 in the theoretical protocol followed: electronic interactions such as exchange, correlation or
659 dispersion, laser and temperature corrections, computational algorithms used to compute
660 frequency (harmonic approximation) or polarizability (DFPT, finite differences, AIMD). Typically
661 fluorescence and resonance are not included in regular codes, nor overtones. ii) the structural
662 model as a representation of the real sample. Well-characterized crystalline materials such as
663 calcite can be properly represented by its correponsing lattice parameters and atoms Wyckoff
664 positions, whereas polymers need a proper sampling of molecular models. Impurities or
665 mixtures need to be included in the structural model to assess their impact in the computed
666 spectrum. The computed Raman spectra should be carefully analyzed before comparison with
667 measurements.

668 -The computational protocol should be adjusted to provide the closest results to the
669 observations. This is particularly important for calibration purposes. Note that the computational
670 protocol is very sensitive to the nature of the material: simple ionic crystals and polymers will
671 not necessarily need the same computational protocol.

672 - CHARISMA protocols for computed Raman spectra (2 MODA sheets available, for static and
673 dynamic approaches) were developped and tested. Besides the peak position and intensity,
674 which defines the spectra shape, static approaches can be used to assign vibrations to each peak,
675 providing valuable information on the nature of the signal by identifying vibrations (stretching,
676 bending, collective). This may be used for calibration purposes to assess the robustness of a
677 peak. For instance, several polystyrene models (finite, inifite, syntactic, atactic, helicoidal) show
678 very robust peaks for vibrations associated with phenyl C-H stretching, whereas other peaks
679 appear within a larger range of Raman shift because of their backbone nature (they are thus
680 sensitive to the polymer structure and chain end). A document summarizing the main results of
681 computed polystyrene Raman spectra can be found here https://hal.science/hal-04344053.

682

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684

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