National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2243

Relative Intensity Correction Standard for Raman Spectroscopy: 488 nm and 514.5 nm Excitation

This Standard Reference Material (SRM) is a certified spectroscopic standard for the correction of the relative intensity of Raman spectra obtained with instruments employing either 488 nm or 514.5 nm laser excitation. SRM 2243 consists of an optical glass that emits a broadband luminescence spectrum when excited with either of these two laser wavelengths. The relative spectral intensity of the glass luminescence, for each excitation wavelength, has been determined through the use of a white-light, uniform-source, integrating sphere that has been calibrated for its irradiance at NIST. The shape of the luminescence spectrum of this glass is described by a polynomial expression that relates the relative spectral intensity to the wavenumber (cm^{-1}) expressed as the Raman shift from the excitation laser wavelength. This polynomial, together with a measurement of the luminescence spectrum of the standard, can be used to determine the spectral intensity-response correction that is unique to each Raman system. The resulting instrument-intensity-response correction may then be used to obtain Raman spectra that are instrument independent.

This SRM is the third in a series of SRM's (2241, 2242, 2243) that provide relative intensity correction for Raman spectrometers employing lasers commonly used for Raman spectroscopy. This SRM is intended for use in measurements over the range of 20 °C to 25 °C.

Certification: The polynomials describing the relative luminescence spectrum of SRM 2243 as a function of excitation wavelength are given in Tables 1 and 2.

Expiration of Certification: The certification of **SRM 2243** is valid until **01 January 2014**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Use"). The certification is nullified if the SRM is physically damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Production and certification of this SRM were performed by S.J. Choquette, W.S. Hurst, and D.H. Blackburn of the NIST Chemical Science and Technology Laboratory.

The SRM units were cut and polished by J. Fuller of the NIST Fabrication Technology Division.

Statistical consultation was provided by S.D. Leigh and N.A. Heckert of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

Laurie E. Locascio, Chief
Biochemical Science Division

Robert L. Watters, Jr., Chief
Measurement Services Division

Gaithersburg, MD 20899
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See Certificate Revision History on Last Page

Table 1. Coefficients of the Certified Polynomial[a] and of the Relative ± 2σ Confidence Curves[b] Describing the Luminescence Spectrum of SRM 2243 for 488 nm excitation. (Valid for Temperatures of 20 °C to 25 °C)

Polynomial Coefficient	Certified Value Polynomial Coefficient[c] 20 °C to 25 °C	Polynomial Coefficient[c] of the Relative[d] ± 2σ Confidence Curves[b]	
		+ 2σ CC	– 2σ CC
A_0	6.914 6 E–03	0.049 033 7	–0.049 033 7
A_1	0	–1.084 59 E–04	1.084 59 E–04
A_2	6.066 55 E–08	1.202 87 E–07	–1.202 87 E–07
A_3	–6.115 22 E–11	–7.498 51 E–11	7.498 51 E–11
A_4	5.754 29 E–14	2.650 81 E–14	–2.650 81 E–14
A_5	–1.569 01 E–17	–4.646 92 E–18	4.646 92 E–18
A_6	1.284 18 E–21	3.096 85 E–22	–3.096 85 E–22

Table 2. Coefficients of the Certified Polynomial[a] and of the Relative ± 2σ Confidence Curves[b] Describing the Luminescence Spectrum of SRM 2243 for 514.5 nm excitation. (Valid for Temperatures of 20 °C to 25 °C)

Polynomial Coefficient	Certified Value Polynomial Coefficient[c] 20 °C to 25 °C	Polynomial Coefficient[c] of the Relative[d] ± 2σ Confidence Curves[b]	
		+ 2σ CC	– 2σ CC
A_0	–0.024 4612	0.028 485 8	–0.028 485 8
A_1	3.176 90 E–04	–3.178 86 E–05	3.178 86 E–05
A_2	–4.847 06 E–07	1.081 68 E–08	–1.081 68 E–08
A_3	4.900 77 E–10	–1.499 80 E–12	1.499 80 E–12
A_4	–1.703 40 E–13	1.934 33 E–15	–1.934 33 E–15
A_5	2.385 45 E–17	–7.062 38 E–19	7.062 38 E–19
A_6	–1.169 21 E–21	6.877 58 E–23	–6.877 58 E–23

[a] A NIST certified value represents data, reported in a SRM certificate, for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated or accounted for by NIST [1].

[b] The confidence curves were calculated point-by-point using the uncertainty analysis of [2].

[c] Where $I_{SRM}(\Delta\upsilon = A_0 + A_1 \times (\Delta\upsilon)^1 + A_2 \times (\Delta\upsilon)^2 + A_3 \times (\Delta\upsilon)^3 + A_4 \times (\Delta\upsilon)^4 + A_5 \times (\Delta\upsilon)^5 + A_6 \times (\Delta\upsilon)^6$; for Δυ = **200 cm^{-1} to 4800 cm^{-1} and is in units of photons sec^{-1} cm^{-2} $(cm^{-1})^{-1}$**

[d] Relative ± 2σ Confidence Curve(Δυ) calculated as ± 2σ Confidence Curve(Δυ)/I_{SRM}(Δυ).

Certified Values: A NIST certified value [1] represents a value derived from data reported in an SRM certificate for which NIST has the highest confidence in its accuracy to the extent that all known or suspected sources of bias have been fully investigated or accounted for by NIST. The certified values of the coefficients of the sixth-order polynomial describing the shape of the luminescence spectrum of SRM 2243, excited at 488 nm (20 492.35 cm^{-1}), are listed in Table 1. The spectrum and its associated expanded uncertainty [3] (relative $\pm$ 2σ confidence curves (CC)) are shown in Figures 1 and 2. The dependent variable of this polynomial expression is the relative spectral intensity of the luminescence. The independent variable of this polynomial is the wavenumber expressed in units of Raman shift (cm^{-1}) from the laser excitation wavelength of 20 492.35 cm^{-1}. This polynomial is certified to describe the luminescent response of the SRM when it is measured between the temperatures of 20 °C to 25 °C. This polynomial certifies the shape of the luminescence spectrum between 200 cm^{-1} and 4800 cm^{-1} Raman shift for excitation of this SRM with a 488 nm laser.

The certified values of the coefficients of the sixth-order polynomial describing the shape of the luminescence spectrum of SRM 2243, excited at 514.5 nm (19 435.18 cm^{-1}), are listed in Table 2. The spectrum and its associated expanded uncertainty [3] (relative $\pm$ 2σ confidence curves (CC)) are shown in Figures 3 and 4. The dependent variable of this polynomial expression is the relative spectral intensity of the luminescence. The independent variable of this polynomial is the wavenumber expressed in units of Raman shift (cm^{-1}) from the laser excitation wavelength of 19 435.18 cm^{-1}. This polynomial is certified to describe the luminescent response of the SRM when it is measured between the temperatures of 20 °C to 25 °C. This polynomial certifies the shape of the luminescence spectrum between 200 cm^{-1} and 4800 cm^{-1} Raman shift for excitation of this SRM with a 514.5 nm laser.

Certification Uncertainty: The coefficients of the polynomials that express the expanded uncertainty (the relative $\pm$ 2σ confidence curves) of the certified polynomial of $I_{SRM}(\Delta\upsilon)$ are listed in Tables 1 and 2. The relative $\pm$ 2σ Confidence Curves($\Delta\upsilon$) are calculated as $\pm$ 2σ Confidence Curve($\Delta\upsilon$) / $I_{SRM}(\Delta\upsilon)$. To calculate the upper and lower 95 % confidence curves for the luminescence spectrum of SRM 2243, use the formula:

$$(95\%\ CC) = I_{SRM}(\Delta\upsilon) + (\pm \text{relative } 2\sigma\ CC\ (\Delta\upsilon) \times I_{SRM}(\Delta\upsilon))$$

The relative $\pm$ 2σ confidence curves, as calculated from the coefficients of Tables 1 and 2, are shown in Figures 2 and 4. The calculated upper and lower 95 % CC are shown in Figures 1 and 3.

The confidence curves were calculated by applying the point-by-point uncertainty analysis of [2] to the fitted polynomial. This method was used to evaluate the uncertainties of the shape of the luminescence spectrum of SRM 2243 based on measurements using three different instruments. Components of the uncertainty include the uncertainty in the white-light, uniform-source, integrating sphere irradiance calibration and various systematic errors from the operation of the Raman instruments used in the measurements. Careful measurements of the glass have shown it to be spatially homogeneous in spectral luminescence. No significant changes in the shape of the luminescence spectrum occur over the range of laser power densities commonly used in Raman instruments.

Physical Description: SRM 2243 is a manganese-doped (0.15 wt % MnO_2) borate matrix glass. Each unit of this SRM consists of a glass slide that is approximately 10.7 mm in width × 30.4 mm in length × 2.0 mm in thickness, with one surface optically polished and the opposite surface ground to a frosted finish using a 400 grit polish. The frosted surface of the slide is characterized by a surface average roughness (root-mean-square) in the range of 1.30 µm to 1.49 µm, as determined by stylus profilometry [4]. The slide is held in a 12.5 mm square cuvette-style optical mount. This mount is designed for the typical 12.5 mm sampling accessories widely used in chemical spectroscopy, i.e., absorbance, fluorescence, etc. This mount can easily be placed on its side for use on a microscope stage. The mount holds the glass slide, frosted side up, in place with a clip. The glass slide extends approximately 0.3 mm above the sides of the mount to allow its use with close focus objectives.

Measurement Conditions: The certification measurements of the luminescence spectrum of SRM 2243 were made using three Raman spectrometers. One was a commercial Raman microscopy system, while the other two used commercial 0.5 m focal length spectrometers designed for array detectors. Each had its own dedicated, line-tuneable argon ion laser. All Raman systems were operated in a 180° backscattering geometry. The collection optics ranged from short focus objective lenses (microscope system) to an achromatic 200 mm focal length collection lens. The x-axis of each spectrometer was calibrated for wavelength using a neon emission pen lamp. The y-axis (relative spectral intensity) of each system was calibrated with a white-light, uniform-source, integrating sphere that had been calibrated for irradiance at NIST. The spectral resolution of the systems ranged from approximately 3 cm^{-1} to 6 cm^{-1} as measured by the full-width-at-half-maximum of the neon emission lines. All certification data were acquired at nominal room temperature (21°C $\pm$ 1°C).

INSTRUCTIONS FOR USE

SRM 2243 is used to provide Raman spectra corrected for relative intensity. This requires a measurement of its luminescence spectrum on the Raman instrument and then a mathematical treatment of both this observed luminescence spectrum and the observed Raman spectrum of the sample.

For proper use of this procedure, attention must be paid to the following experimental conditions. Due to polarization effects that are present in Raman instrumentation, a polarization scrambler should be employed in the Raman light-collection optics, most preferably in a region of collimated light. Raman spectral bands that exhibit various degrees of polarization will not be properly intensity-corrected without the use of a scrambler. To acquire the luminescence spectrum of SRM 2243, the surface of the glass should be placed at the same position from which the Raman spectrum of the sample is collected. It is important that the laser excitation be incident only on the frosted surface of the glass. The shape of the spectral luminescence will have some sensitivity to the placement of the glass surface relative to the collection optics of the spectrometer, which is minimized by scattering from the frosted surface. Measurement conditions should be arranged to furnish a spectrum of optimum signal-to-noise ratio. The luminescence spectrum should be acquired over the same Raman range as that of the sample.

The relative intensity of the measured Raman spectrum of the sample can be corrected for the instrument-specific response by a computational procedure that uses a correction curve. This curve is generated using the certified polynomial and the measured luminescence spectrum of the SRM glass. For the spectral range of certification, $\Delta\upsilon = 200\ \text{cm}^{-1}$ to $4800\ \text{cm}^{-1}$, compute the elements of the certified relative spectral intensity of SRM 2243, $I_{SRM}(\Delta\upsilon)$, according to equation 1:

$$I_{SRM}(\Delta\upsilon) = A_0 + A_1 \ (\Delta\upsilon)^1 + A_2 \times (\Delta\upsilon)^2 + A_3 \times (\Delta\upsilon)^3 + A_4 \times (\Delta\upsilon)^4 + A_5 \times (\Delta\upsilon)^5 + A_6 \times (\Delta\upsilon)^6 \qquad (1)$$

where $(\Delta\upsilon)$ is the wavenumber in units of Raman shift (cm^{-1}) and the A_n's are the coefficients listed in Tables 1 or 2. The elements of $I_{SRM}(\Delta\upsilon)$ are obtained by evaluating equation 1 at the same data point spacing used for the acquisition of the luminescence spectrum of the SRM and of the Raman spectrum of the sample. $I_{SRM}(\Delta\upsilon)$ has been normalized to unity and is a relative unit expressed in terms of photons $\text{sec}^{-1}\text{cm}^{-2}(\text{cm}^{-1})^{-1}$, or alternatively:

$$\frac{\text{Photons}}{\text{sec} \times \text{cm}^{-1} \times \text{cm}^2}$$

The data sets that are the measured glass luminescence spectrum, S_{SRM}, and the measured Raman spectrum of the sample, S_{MEAS}, must have the units of Raman shift (cm^{-1}). The elements of the correction curve $I_{CORR}(\Delta\upsilon)$, defined by equation 2, are obtained from $I_{SRM}(\Delta\upsilon)$ and the elements of the glass luminescence spectrum, $S_{SRM}(\Delta\upsilon)$ according to equation 2:

$$I_{CORR}(\Delta\upsilon) = I_{SRM}(\Delta\upsilon) / S_{SRM}(\Delta\upsilon). \qquad (2)$$

The elements of the intensity-corrected Raman spectrum, $S_{CORR}(\Delta\upsilon)$, are derived by multiplication of the elements of the measured Raman spectrum of the sample, $S_{MEAS}(\Delta\upsilon)$, by the elements of the correction curve according to equation 3:

$$S_{CORR}(\Delta\upsilon) = S_{MEAS}(\Delta\upsilon) \times I_{CORR}(\Delta\upsilon) \qquad (3)$$

In measurements with a dispersive spectrometer, the x-axis is directly related to the wavelength (nm) of the measured spectrum. The transformation from the wavelength scale to the Raman shift scale requires a multiplicative factor of λ^2 be applied to the relative intensity. The certified relative spectral intensity polynomial, $I_{SRM}(\Delta\upsilon)$, includes this λ^2 factor. As a result of equations 1 through 3, the elements $S_{MEAS}(\Delta\upsilon)$ and $S_{SRM}(\Delta\upsilon)$ may either include this λ^2 factor or not, so long as it is consistently applied, since it will cancel out if it is included. A detailed description of this procedure for the intensity correction of Raman spectra can be found in references 5 and 6.

The polynomial expression, equation 1, is certified for use **between 200 cm^{-1} and 4800 cm^{-1}**. This is the spectral range common to the three instruments used for the certification. The polynomial fits are intended as simple numerical descriptors of the spectral response observed over the wavenumber range studied. They are **NOT** intended for use in extrapolation; in fact, such use is strongly discouraged. Nor are these fits intended as physically meaningful models. Additional studies have measured the luminescence of SRM 2243 from –1000 cm^{-1} and 7000 cm^{-1} Raman shift from the respective laser excitation wavelength. The relative intensity corrected spectra of SRM 2243 for the extended range is shown in Figure 5. This figure is provided for information purposes only.
The polynomial coefficients listed in Tables 1 or 2 can not be used to extrapolate the certification to this extended range without incurring significant error. Extrapolation of the polynomial outside the certification limits of 200 cm^{-1} and 4800 cm^{-1} is not a supported use of this SRM.

If additional information regarding the extended luminescence of this SRM is desired, please contact NIST [7]. Certified values of the polynomial coefficients given in Tables 1 or 2 are for use at temperatures between 20 °C to 25 °C.

Use of this SRM at temperatures other than the certification temperature is not currently supported. However, the temperature response of this SRM has been studied between the ranges of 5 °C and 60 °C. Unlike previous SRM's in this series, the white light corrected and normalized luminescence spectra of SRM 2243 does not demonstrate a temperature dependence.

This SRM is not intended for use as a standard for the determination of absolute spectral irradiance or radiance.

Handling and Storage: To maintain the certified properties of SRM 2243, the glass slide should be handled only in its mount. When not in use, the SRM should be stored in the container provided or in one providing comparable mechanical protection.

Luminescence Spectrum on the Wavelength Scale: The equation describing the luminescence spectrum of the glass SRM is given in equation 1, where ($\Delta\upsilon$) is the wavenumber in units of Raman shift (cm^{-1}). For correction of spectra where the x-axis is in wavelength with units of nanometers, the same polynomial coefficients can be used to calculate $I_{SRM}(\lambda)$ through the following coordinate transformation:

$$I_{SRM}(\lambda) = [10^7/\lambda^2] \times [A_0 + A_1 \times Z^1 + A_2 \times Z^2 + A_3 \times Z^3 + A_4 \times Z^4 + A_5 \times Z^5 + A_6 \times Z^6] \quad (6)$$

where:

$$Z = 10^7 \times [(1.0/\lambda_L) - (1.0/\lambda)] \quad (7)$$

and λ_L is the wavelength of the laser in nm and λ is the wavelength in nanometers. The factor of 10^7 in the first term of equation 6 is needed only if it is desired to preserve the numerical value of spectral areas computed relative to the two x-axis coordinate systems.

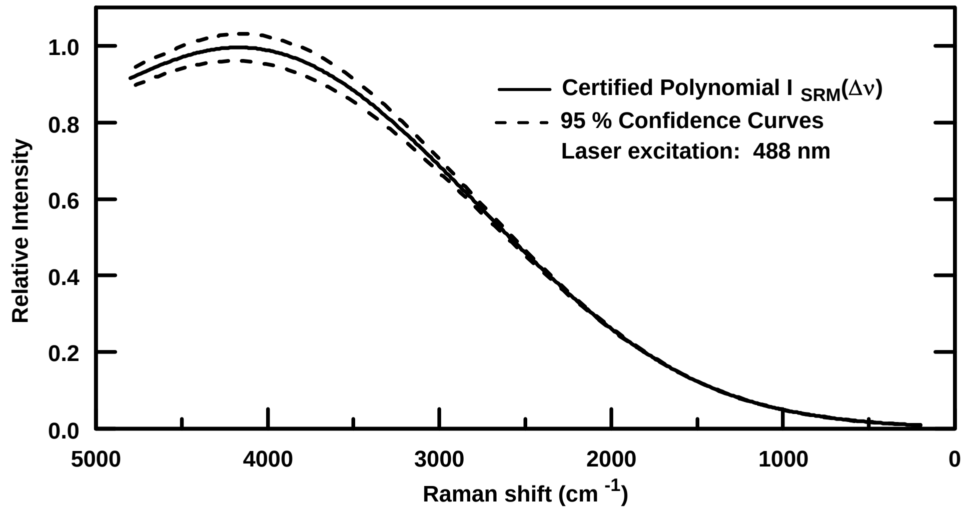

Figure 1. Sixth-order certified polynomial for SRM 2243 when excited at 488 nm. The x-axis is expressed in Raman shift (cm^{-1}) relative to 488 nm (20 492.35 cm^{-1}). The y-axis is on a relative scale and normalized to unity with the dimensions of photons $s^{-1}cm^{-2}(cm^{-1})^{-1}$. Dashed lines indicate 95 % confidence curves.

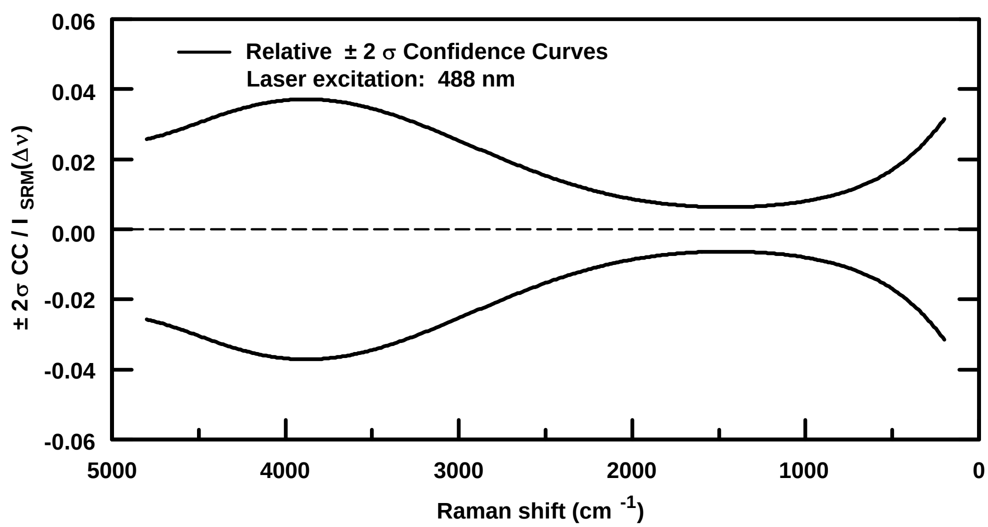

Figure 2. Relative ± 2σ confidence curves describing the luminescence spectrum of SRM 2243. The two solid black curves represent for SRM 2243 at room temperature, respectively, the quotient ± 2σ CC/ $I_{SRM}(\Delta\upsilon)$. The certified polynomial is valid over the temperature range of 20 °C to 25 °C. The x-axis is Raman shift (relative to 488 nm, 20 492.35 cm^{-1} laser excitation).

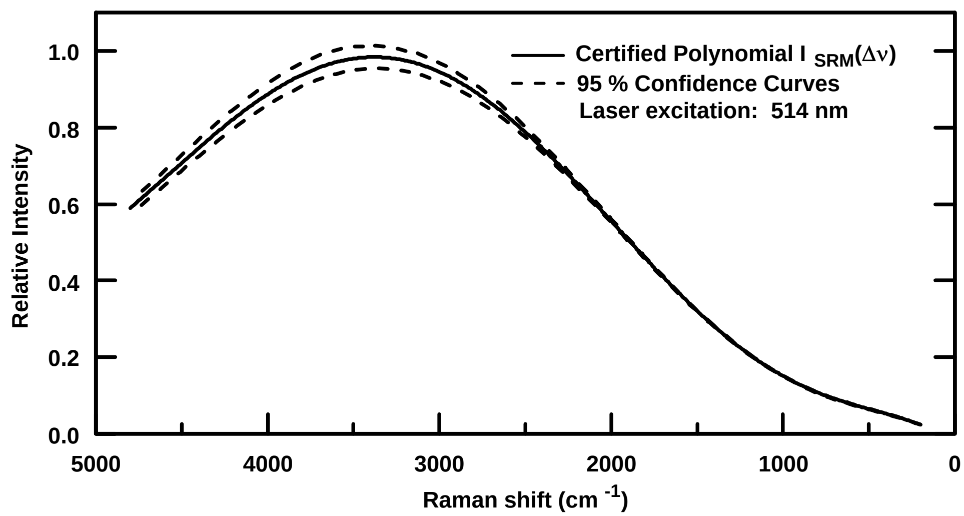

Figure 3: Sixth-order certified polynomial for SRM 2243. The x-axis is expressed in Raman shift (cm^{-1}) relative to 514.5 nm (19 435.18 cm^{-1}). The y-axis is on a relative scale and normalized to unity with the dimensions of photons $s^{-1}cm^{-2}(cm^{-1})^{-1}$. Dashed lines indicate 95 % confidence curves.

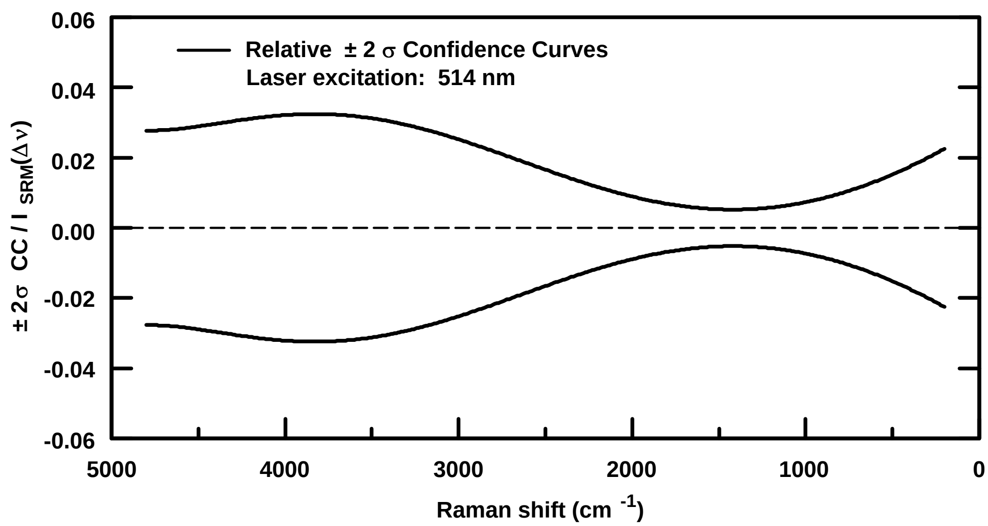

Figure 4. Relative ± 2σ confidence curves describing the luminescence spectrum of SRM 2243. The two solid black curves represent for SRM 2243 at room temperature, respectively, the quotient ± 2σ CC/ $I_{SRM}(\Delta\upsilon)$. The certified polynomial is valid over the temperature range of 20 °C to 25 °C. The x-axis is Raman shift (relative to 514.5 nm, 19 435.18 cm^{-1} laser excitation).

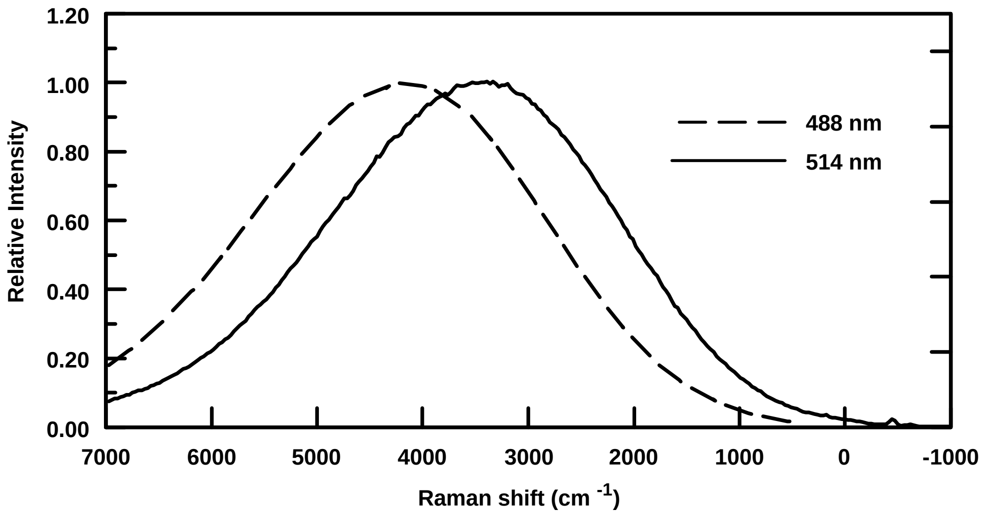

Figure 5. Extended Range Scan of SRM 2243 when excited by 488 nm or 514.5 nm laser irradiation. The relative intensity corrected spectra are provided for information purposes only.

REFERENCES

[1] May, W.; Parris, R.; Beck, C.M.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurement*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at http://www.cstl.nist.gov/nist839/NIST_special_publications.htm.

[2] Levenson, M. S.; Banks, D. L.; Eberhardt, K.R.; Liu, H.K.; Vangel, M.G.; Yen, J.H.; Gill, L.M.; Guthrie, W.F.; Zhang, N.F.; *An Approach to Combining Results From Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105 Number 4, pp. 571-579 (2000).

[3] ISO; *Guide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed.; International Organization for Standardization: Geneva, Switzerland (1993); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://physics.nist.gov/Pubs/.

[4] J.F. Wong; T.V. Vorburger, *Stylus Profiling at High Resolution and Low Force*; Appl. Opt. 30, 42 (1991).

[5] Ray, K.G.; McCreery, R.L.; *Simplified Calibration of Instrument Response Function for Raman Spectrometers based on Luminescent Intensity Standards*; Appl. Spectrosc., Vol. 51(1), pp. 108-116 (1997).

[6] Frost, K.J.; McCreery, R.L.; *Calibration of Raman Spectrometer Response Function with Luminescence Standards: An Update*; Appl. Spectrosc., Vol. 52, Number 12, pp. 1614-1618 (1998).

[7] Additional information regarding this SRM and its extended luminescence spectrum may be obtained by contacting Steven Choquette at Steven.Choquette@nist.gov, 301-975-3096, NIST, Gaithersburg, MD 20899-8312.

Certificate Revision History: 11 June 2009 (This revision extends the expiration date with editorial changes); 02 July 2004 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.