

Characterization of Standard Reference Material 2943, Cu-Ion-Doped Glass, Spectral Correction Standard for Blue Fluorescence

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Abstract: Standard Reference Material (SRM) 2943 is a cuvette-shaped, Cu-ion-doped glass, recommended for use for relative spectral correction of emission and day-to-day performance verification of steady-state fluorescence spectrometers. Properties of this standard that influence its effective use or contribute to the uncertainty in its certified emission spectrum were explored here. These properties include its photostability, absorbance, dissolution rate in water, anisotropy and temperature coefficient of fluorescence intensity. The expanded uncertainties in the certified spectrum are about 5 % around the peak maximum at 446 nm, using an excitation wavelength of 330 nm. SRM 2943 can replace SRM 936a quinine sulfate dihydrate, which is no longer sold by NIST, for many applications, as it covers the same spectral range. SRM 2943 is significantly more photostable than organic dyes, but unlike the other fluorescent glass SRMs in this series, it does photodegrade gradually under lamp-based excitation.

Keywords: fluorescence; instrument qualification; Cu glass; spectral correction; SRM; standards

Introduction

The increasing use of quantitative fluorescence-based assays in clinical, biotechnological, pharmaceutical and other health-related areas has fueled demand for fluorescence standards. [1] A variety of certified reference materials [2,3,4,5,6,7] and related standardization documents [8,9,10,11,12,13] have recently become available in response to this demand. Standard Reference Material (SRM) 2943 is the fourth fluorescence SRM in a series of recently released NIST standards certified as spectral correction standards for emission. Many of its fluorescence properties have been characterized and are described here to understand better the uncertainties and limitations of its use as a standard. Similar characterizations of SRMs 2940,[14] 2941 [15] and 2942 [16] have been reported previously. SRM 2943 can be used in combination with SRMs 2940-2942 to calibrate fluorescence instruments throughout the near UV and visible regions from 320 nm to 780 nm. These SRMs can also be used as day-to-day intensity standards for performance verification, due to their resistance to photodegradation.

SRM 2943 is a ready-to-use, cuvette-shaped, Cu-ion-doped, solid glass standard whose certified values can be used to correct fluorescence emission spectra for distortions in the measured spectral shape, i.e., relative intensity correction, due to the changing responsivity with wavelength of the detection system of a steady-state fluorescence

spectrometer. SRM 2943 can be used in combination with SRMs 2940, 2941 and 2942 to calibrate fluorescence instruments through the near UV and visible regions from 320 nm to 780 nm.

The certified values of SRM 2943 are to be used as follows to obtain correction factors as a function of emission wavelength. The SRM is excited at a fixed wavelength of 330 nm while the emission is collected from 350 nm to 640 nm, preferably using the instrument parameters given in the certificate. [2] Due to larger signal to noise levels near the peak maximum, the emission range from $\lambda_{EM} = 380$ nm to 560 nm is recommended as optimal for most instruments and applications. The measured spectrum is then normalized to a peak intensity of one at the peak maximum, nominally 446 nm, i.e., divide all measured intensity values by the corresponding value at the peak maximum. Each certified value is then divided by its corresponding normalized, measured value to obtain correction factors. The measured emission spectrum of an unknown sample that falls in the effective emission range of the SRM can then be corrected by multiplying its measured intensities by the correction factors at the corresponding emission wavelengths. Even though the correction factors must be determined using the SRM at a 330 nm excitation wavelength, they may be applied to the spectral correction of emission independently of the excitation wavelength of a sample.

Due to glass fabrication limitations, SRM 2943 was produced from four separate glass melts. Although the same “recipe” was used for each melt, the optical properties of each are statistically discernable. Accordingly, SRM 2943 was produced and certified in four batches, labeled Series A, B, C and D, and will be released in succession. All batches display very similar, but not identical, behavior. The results shown here are representative of all four batches, except where noted, so the suffix A, B, C or D will only be used when a difference between batches was observed.

In the past, NIST had certified SRM 936a, quinine sulfate dihydrate, [17,18] as a spectral correction standard covering the same emission range as SRM 2943. Since SRM 936a is no longer available from NIST, this new SRM can be used in its place. Although, it should be noted that SRM 2943 is to be excited at a different wavelength than SRM 936a, 330 nm versus 347.5 nm, respectively. SRM 2943 is also significantly more photostable and does not require any sample preparation before use.

Experimental

A more detailed experimental description of many of these procedures has already been reported. [15] All uncertainties given here are expanded using $k = 2$, i.e., 2σ uncertainties, unless specified otherwise.

Samples: The glass was melted at 1300 °C in a high purity alumina crucible, using a base glass composition with mass fractions of $P_2O_5 = 71$ % ($Ca(H_2PO_4)_2H_2O$ and $NH_4 H_2PO_4$ used), $CaO = 26$ % ($Ca(H_2PO_4)_2H_2O$ used), $Al_2O_3 = 3.0$ % (Al_2O_3 used), and a dopant mass fraction of $Cu_2O = 0.014$ %. Reducing conditions were maintained during melting by flowing a 95 % N_2 , 5 % H_2 gas mixture into the crucible. The addition of copper to

most glasses made in an oxygen-rich environment results in a blue color attributed to Cu^{2+} . However, if oxygen is excluded and sufficiently strong reducing conditions are employed during the melt, a colorless copper-containing glass may be formed, as demonstrated here, which fluoresces blue light strongly. [19,20,21,22] This fluorescence is emitted by Cu^+ in the glass. It is very difficult to produce a copper-containing glass that is completely free of Cu^{2+} , which accounts for a very slight blue tint in some of the SRM 2943 samples.

The glass was cut into cuvette-shaped pieces (12.5 mm x 12.5 mm x 45.0 mm) with three long sides polished, to be used with a 90° transmitting, detection geometry, and one long side frosted, to be used with a front-face detection geometry [23] for minimizing excitation beam penetration and as a surface on which to focus for microscope applications. One glass batch had a final composition with mass fractions of $\text{P}_2\text{O}_5 = 77 \% \pm 15 \%$, $\text{CaO} = 17 \% \pm 3 \%$, $\text{Al}_2\text{O}_3 = 6 \% \pm 1 \%$, $\text{Cu}_2\text{O} = 0.012\% \pm 0.002 \%$ and other trace oxides = $0.19 \% \pm 0.04 \%$, determined using X-ray fluorescence. Similar results were found for the other three batches.

Fluorescence Measurements: All steady-state fluorescence spectra were taken on a SPEX Fluorolog 3 [24] (Jobin Yvon, Edison, NJ) spectrofluorometer using a continuous 450 W Xe lamp excitation source, except where noted. A small fraction of the excitation beam was reflected, using a fused silica window, to a “reference” photodiode just before the sample to monitor the relative excitation intensity as a function of time and wavelength. The wavelength accuracy achieved over the entire wavelength range of the instrument was ± 0.2 nm for both emission and excitation, determined using atomic lamps. The relative radiometric accuracy as a function of wavelength of the reference (excitation) and signal (emission) detection systems was corrected using a calibrated detector and a calibrated light source, respectively, traceable to the NIST realization of the International System of Units (SI). [25,26,27,28,29] All fluorescence measurements were taken at 25°C using a 90° transmitting geometry with the excitation beam incident on and normal to one of the polished glass surfaces. The excitation wavelength was 330 nm, and the typical scanning range for emission spectra was from 350 nm to 640 nm, using excitation and emission bandwidths of 3 nm. The ratio of signal to reference intensities is given as the “fluorescence intensity” in what follows to correct for signal intensity fluctuations due to changes in the excitation intensity with time, and all emission spectra are corrected for the responsivity of the detection system. A more detailed description of the qualification of the fluorescence spectrometer, related uncertainties and experimental conditions for certification and the determination of spectral correction factors is given elsewhere. [30]

A fluorescence spectrometer with pulsed excitation (Varian Eclipse) was used with 5 nm and 2.5 nm bandwidths for excitation and emission, respectively, pulse duration = 2 μs , PMT voltage = 600 V to 800 V, PMT gate = 40 μs with no delay time between the excitation pulse and the gate. Correction factors for relative spectral correction were determined for this instrument using Federal Institute for Materials Research and Testing - Germany (BAM) certified reference materials (CRMs) [6], so corrected spectra could be compared between instruments using pulsed and continuous excitation. CRMs were used here to save the time needed to set up physical transfer standards, such as a

calibrated light source. This emphasizes the easy-of-use of NIST SRMs and other CRMs, which can be measured in the same way as typical samples.

Polarizers: Glan Thompson polarizers were used just after the excitation monochromator and just before the emission monochromator to measure the fluorescence intensities I_{VV} , I_{VH} , I_{HV} and I_{HH} , which were then used to determine fluorescence anisotropy (r), where the first and second subscripts indicate the polarization setting of the excitation and emission polarizers, respectively, using V to indicate vertical or 0° polarization and H to indicate horizontal or 90° polarization. These measurements were taken at a fixed emission wavelength, corresponding to the peak maximum for SRM 2943. F and G values [31] were determined as described previously. [15,30]

Photostability Testing Methods: The fluorescence intensity of SRM 2943 was measured periodically after several hours of continuous irradiation. These measurements were taken on the Fluorolog 3, after the sample was removed from the irradiation chamber and its temperature was allowed to equilibrate in the sample compartment of the fluorometer. The irradiation chamber used a 150 W Xe arc lamp with a Hoya U-340 bandpass filter between the lamp and chamber to pass wavelengths between 280 nm and 380 nm.

A fiber optic with a 400 μm diameter aperture attached to an Ocean Optics S2000 spectrometer with an 8 nm bandwidth was used to measure the irradiance of the light incident on the samples as a function of wavelength. The relative spectral responsivity of the spectrometer was calibrated using a calibrated tungsten halogen lamp. The excitation irradiation incident on the samples, when they were excited in our fluorometer at an excitation bandwidth and wavelength of 3 nm and 330 nm, respectively, was measured using both a calibrated Si detector and the fiber optic spectrometer. The comparison of the two measurements was used to calibrate the absolute responsivity of the fiber optic spectrometer.

Lifetime: Fluorescence lifetimes were measured on an ISS K2 fluorometer with a K2LF accessory. A monochromator was used to set the excitation wavelength at 330 nm. An NG-11 glass filter was placed before the emission PMT to block UV scattered light when measuring SRM 2943. A Ludox suspension (excitation light scatterer) was used as a lifetime reference with a lifetime of 0.0 ns. No emission wavelength selector was used with the lifetime reference. The K2 was scanned over 10 frequencies in the range from 0.1 kHz to 200 kHz.

Results and discussion

Corrected Fluorescence Spectra and Uncertainties

The corrected emission spectrum is a single broad peak with a nominal maximum at 446 nm and a full-width at half the maximum intensity (FWHM) of 96 nm. This spectral shape is consistent with Cu-ion-doped glasses reported previously, although the positions and broadness of peaks have been found to change with the base glass composition. [32,33] Homogeneity of the glass was measured on a centimeter scale by collecting the

spectrum for each SRM 2943 sample in both a normal and a raised (0.5 cm) position and comparing them. Both spectra were found to be statistically identical for all samples, implying that they are spatially homogeneous.

The total uncertainty in the relative fluorescence intensity was calculated for each certified intensity value in the fluorescence spectrum by adding in quadrature the 1σ uncertainties due to 1) spatial uncertainty of the excitation beam's position on the sample (causing secondary inner filter effect uncertainties), 2) variation of F and G polarization ratios between instruments, 3) temperature uncertainty, 4) excitation and emission wavelength and bandwidth uncertainty, 5) uncertainty in the spectral shape correction (due to uncertainty in the radiance and reflectance values of the calibrated light source and reflector) and 6) standard deviation of the certification data. The total 1σ uncertainties were then multiplied by an expansion factor $k = 2$ to obtain the total expanded uncertainties (U_{95}). The spectrum of SRM 2943 and the associated uncertainties in the certified values are shown in Fig. 1 and reported in the certificate. [5] The values for U_{95} are about 5 % near the peak maximum and 8% at the wings. The sides or wings of the peak refer to the regions of the spectrum to either side of the peak maximum where the intensities are 10 % to 20 % of the peak maximum.

The excitation spectrum of SRM 2943, spectrally corrected for excitation intensity, has a peak maximum at 288 nm and a FWHM of 37 nm (see Fig. 2). Absorbance of the excitation beam by the glass matrix becomes significant at wavelengths less than 300 nm, causing large inner filter effects. For this reason, we chose an excitation wavelength of 330 nm.

The certified values will only yield effective spectral correction factors when the SRM is excited at 330 nm, because the shape of the emission spectrum is excitation wavelength dependent. A 1.0 nm shift of the excitation wavelength in either direction causes the resulting emission spectrum to deviate from the certified values by less than 3 % in the optimal region from 380 nm to 560 nm. Deviations due to a 1.0 nm change in the excitation bandwidth are less than 2 % in the optimal region, and those due to a 1.0 nm change in the emission bandwidth are insignificant, being less than 0.3 % across the entire certified emission spectrum.

When excited at wavelengths shorter than 280 nm, the SRM photodegrades rapidly. For instance, we observed a photodegradation rate of 0.9 % per hour when excited at 260 nm with an irradiance of 0.5 mW cm^{-2} . Therefore, the SRM should not be exposed to less than 280 nm wavelength light and is not recommended for use as a day-to-day performance standard at these short excitation wavelengths.

Corrosion Study

The weight of a Cu-ion-doped glass immersed in deionized water was measured over a period of 30 days. The rate of dissolution was $7.2 \text{ ng}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$, which is equal to a log dissolution rate of $-8.1 \text{ g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$. SRMs 2940 and 2941, both displayed a log dissolution rate of $-6.6 \text{ g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$. [14,15] These two SRMs are composed of a borate glass matrix, in contrast to SRM 2943, which uses a phosphate glass matrix. Our data

suggest that the dissolution rate is dependent on the composition of the glass matrix and not on the dopant. Window glass is reported to have a log dissolution rate in the range of $-8.0 \text{ g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ to $-8.5 \text{ g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$. [34]

Absorbance and Inner Filter Effects

The borate glass matrices of previous SRMs caused them to strongly absorb wavelengths less than 375 nm, with transmittance (T), dropping to 40 % by 350 nm and to 1% or less below 300 nm. To make an SRM that would transmit excitation and emission below 350 nm, we needed to use a phosphate matrix for SRM 2943, which has a value of T= 98 % at 330 nm (see Fig. 3). The transmittance of the glass continues to decrease with decreasing wavelength, e.g., T = 10 % at 280 nm and T = 0.3 % at 260 nm. Absorbance was measured with a Lambda 900 (PerkinElmer) spectrophotometer using a 1 nm increment and a 3 nm bandwidth.

Inner filter effects (IFE) are due to absorption by the sample of either the excitation beam before it reaches the detection region at the center of the sample, known as the primary IFE, or the emission before it leaves the sample, known as the secondary IFE. Both cause the measured fluorescence intensity (F) to decrease, the extent of which can be easily calculated using the measured absorbances $A(\lambda_{\text{EX}})$ and $A(\lambda_{\text{EM}})$ of the sample at the excitation and emission wavelengths, respectively. [35, 36] Samples with $A(\lambda_{\text{EX}})$ and $A(\lambda_{\text{EM}})$ values less than 0.04 (T = 91 %), corresponding to intensity changes of less than 5 %, are generally considered to be small enough to ignore, as is the case here.

SRM 2943 has a primary IFE at its excitation wavelength of 2 %, but all IFEs will be observed with the same magnitude whenever the SRMs are measured under the same conditions, so they should not matter when the conditions specified on the SRM certificate are followed. On the other hand, the positions of the excitation beam and detection path on the sample can change over time or between instruments, resulting in a corresponding change in IFE values. Ideally, the detection region should always be at the center of the cuvette, where the excitation beam and emission detection path overlap. In reality, this position can change due to misalignment of the excitation source, optics and sample over time or due to differences in optical alignment between samples. A 1 mm change in the position of the excitation beam or detection path would cause a 1.1 % change in the measured fluorescence intensity at the peak maximum. These absolute intensity differences due to IFEs can affect the SRM when being used for day-to-day intensity verification of instrument performance.

When these SRMs are being used with their certified values for relative spectral correction, only changes in relative intensity versus λ_{EM} are significant. This means that the primary IFE, which is independent of λ_{EM} , will not affect SRM performance. Only changes in the secondary IFE with λ_{EM} can affect the spectral correction when the position of the detection region changes. The percent error in the measured relative emission spectrum due to IFEs was calculated with the same 1 mm change in position. As might be expected, the relative IFE errors are even smaller than the corresponding absolute errors, given above, with those for SRM 2943 being less than 0.2 %.

Photostability

Possibly, the most important characteristic of a solid, robust fluorescent reference material that is meant to be used repetitively in the field is photostability. The SRM was irradiated with UV light (> 280 nm) from a 150 W Xe lamp to test the glass under conditions close to those expected under normal use. Irradiation of SRM 2943 for 25 h showed no changes in spectral shape, but did show a gradual decrease in the fluorescence intensity at a rate of $0.06 \% \pm 0.03 \%$. The irradiance incident on the SRM was measured to be about 2.1 mW cm^{-2} at 330 nm, using a spectral bandpass of 8 nm, with a comparable irradiance throughout the region between 280 nm and 380 nm. The exposure time in the irradiation chamber (25 h) corresponds to about 180 h (7.5 days) of continuous excitation in our fluorometer. This correspondence was calculated by considering the intensity in the irradiation chamber at other excitation wavelengths where sample absorption produces fluorescence. The intensity values of the excitation fluorescence spectrum at each wavelength were used to weight the corresponding excitation intensity values in the irradiation chamber. Ultraviolet (UV) light shorter than 280 nm from the Xe lamp was blocked, using a filter, to prevent UV solarization of the glass, which is known to change the absorption of metal-ion-containing glasses. [37, 38] SRM 2943 should not be employed as a performance verification standard at excitation wavelengths below 280 nm due to this effect.

Anisotropy and Polarization Effects

Samples with non-zero values for fluorescence anisotropy (r) will show different fluorescence intensities and spectral shapes on different instruments, since each fluorometer has its own polarization ratios or factors, where $I_{V,EX} / I_{H,EX}$, referred to as the F factor, is the ratio of the vertically and horizontally polarized components of the excitation intensity and R_V / R_H , referred to as the G factor, is the ratio of the responsivities of the detection system to vertically and horizontally polarized light. The values of these polarization factors are dependent on the unique components of individual instruments, such as gratings, other optics, lamps and detectors.

The r value for SRM 2943 was measured to be 0.046 ± 0.002 at its fluorescence peak maximum. The anisotropy of SRM 2943 did change with emission wavelength (λ_{EM}), as shown in Fig. 5. Y error bars representing 1σ standard deviations for the average r values and a trendline are also given in the figure. With excitation and emission polarizers in place, the intensity of detected fluorescence from the Cu glass became too weak at emission wavelengths less than 380 nm and greater than 530 nm to measure accurate r values. Therefore, r values were extrapolated using a third order polynomial fit and a linear fit of the measured values at wavelengths below 380 nm and above 530 nm, respectively.

The F factor at 330 nm is 0.8. The range of G factors for our instrument is from 2.7 to 0.3 over the emission wavelength range of the SRM. These F and G values are typical for monochromator-based systems. [39] We estimated $\pm 25 \%$ to be a typically expected instrument-to-instrument difference between the F and G values of our instrument and those of other users for conventional fluorometers designed to cover the emission region

from about 350 nm to 750 nm with greatest sensitivity. With this assumption, differences in the absolute intensity at the peak maximum and in the relative intensities across the emission spectrum that can be expected due to variations in F and G values between instruments were calculated. [40] The absolute intensity difference at the peak maximum was calculated to be 0.9 % for SRM 2943. The differences in the relative intensity across the emission spectrum normalized to one at the peak maximum were calculated to be less than 1 % in the peak region and as much as 4% at the blue wing of the spectrum, see Fig. 6.

Temperature Dependence

The fluorescence peak intensity as a function of temperature was measured between 10 °C and 40 °C (see Fig. 7). The slope of the linear least-squares fitted straight line to the plotted points was taken to be the temperature coefficient. This value corresponds to 0.41 % / °C \pm 0.02 % / °C for SRM 2943 at 25 °C. It should be noted that the fluorescence intensity of this glass increases with increasing temperature, whereas the fluorescence intensity of the other three glasses in this series (SRMs 2940-2942) decreases with increasing temperature as is typical for fluorescent materials, in general. This uncommon behavior suggests complex excited state dynamics occurs in this glass, requiring further investigation to be understood.

By graphing the percent difference between the spectra, normalized to one at the peak max, at other temperatures with that at 25 °C, the temperature dependence of the spectral shape as a function of emission wavelength was determined. To average out noise and get a smooth percent difference curve, the percent difference plots at 40 °C and 10 °C were both fitted to polynomials using a least squares fit. SRM 2943 was certified at 25 °C with an uncertainty of \pm 0.5 °C. The temperature dependence of the percent difference was found to be linear with changes in temperature. In addition, the fit at 40 °C shows a slightly larger percent difference than that at 10 °C, with both temperatures deviating from the certification temperature by 15 °C (see Fig. 8). Therefore, the percent difference fit at 40 °C, as the larger of the two, was used to calculate the uncertainty in the certified values corresponding to the uncertainty in temperature, by taking the percent difference values as a function of wavelength and dividing each by 30 (15 °C / 0.5 °C = 30). Spectral differences due to a \pm 0.5 °C change in temperature were found to be insignificant, less than 0.2 %, for SRM 2943 across its emission wavelength range.

Fluorescence Lifetimes and Pulsed Excitation

The time decay of fluorescence for SRM 2943 was found to be single exponential. The fitted lifetime for SRM 2943 was $\tau = 37 \mu\text{s} \pm 3 \mu\text{s}$ with $\chi^2 = 16$. This lifetime is within 30 % of those of Cu-doped glasses reported in the literature. [41]

The corrected emission spectrum for SRM 2943 was also determined on an instrument with pulsed excitation and compared to the certified spectrum taken on the Fluorolog 3. The fluorescence spectra look very similar using either pulsed or continuous excitation (see Fig. 9) with the relative intensity values from the pulsed instrument differing by 10% or less throughout the spectrum from the certified values. They differ by less than 2 % in

the peak region. These differences are within the combined uncertainties of the certified values and the uncertainties related with the pulsed instrument measurements. These results imply that the fluorescence emitted within 40 μ s of the excitation pulse has the same spectral profile as the longer, time-averaged fluorescence. The 40 μ s PMT gate duration was chosen as a typical value for conventional pulsed fluorometers, suggesting that SRM 2943 can also be used as a spectral correction standard for instruments with pulsed excitation.

Conclusion

SRM 2943, a Cu-ion-doped glass in the shape of a standard cuvette, has been certified as a relative spectral correction standard for fluorescence emission from 350 nm to 640 nm. The expanded uncertainties in the certified values are about 5 % near the peak maximum at 446 nm. Errors in the measured emission spectrum due to variations in the polarization ratios (F and G factors), experienced over time or between instruments, were found to be as much as 4 % across the entire wavelength range, but less than 1 % in the peak region. This assumes a 25 % difference between the F and G values of our instrument and those of other conventional fluorometers. Corresponding errors due to inner filter effects were found to be insignificant. This assumes a 1 mm displacement of the detection region from the center of the cuvette for IFEs. The fluorescence anisotropy and temperature coefficient of fluorescence intensity for the SRM were measured to be 0.046 ± 0.002 and $0.41 \% / ^\circ\text{C} \pm 0.02 \% / ^\circ\text{C}$, respectively, at the peak maximum at 25 $^\circ\text{C}$. A positive temperature coefficient is atypical for fluorescent materials. SRM 2943 can be used in the place of SRM 936a for many applications, as it covers the same spectral range. SRM 2943 is also significantly more photostable than SRM 936a, although, gradual photodegradation was observed under common lamp-based excitation conditions.

Acknowledgements

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Figure captions

Fig. 1 : The certified fluorescence spectrum of SRM 2943 with intensity in relative power units and the corresponding uncertainty envelope obtained by adding and subtracting the total expanded uncertainty (U95) to the certified values. The relative percent uncertainty is labeled in the peak and wing regions of the spectrum. The certified spectrum is normalized to one at the peak maximum at 446 nm.

Fig. 2: The fluorescence excitation spectrum of SRM 2943 with emission collected at 446 nm. The spectrum is normalized to one at the peak maximum at 288 nm.

Fig. 3: Transmittance spectrum of SRM 2943 with phosphate glass matrix.

Fig. 4: The fluorescence intensity as a function of irradiation time, being irradiated with a nominal irradiance of 2.1 mW cm^{-2} from 280 nm to 380 nm at a spectral bandpass of 8 nm. The error bars for fluorescence intensity represent 1σ standard deviations. The uncertainty in wavelength is smaller than the point size used.

Fig. 5: The dependence of the fluorescence anisotropy (r) of SRM 2943 on emission wavelength. The uncertainty in wavelength is smaller than the point size used. The error bars for anisotropy represent 1σ standard deviations.

Fig. 6: Percent error in the measured fluorescence emission spectrum of SRM 2943 due to a $\pm 25\%$ change in the polarization ratios (F and G factors) of a fluorescence spectrometer from those of the instrument used to certify the standard.

Fig. 7: The temperature dependence of the fluorescence intensity of SRM 2943 at the peak maximum. The error bars represent 1σ standard deviations.

Fig. 8: The percent difference in the fluorescence spectrum of SRM 2943 caused by a $\pm 15^\circ\text{C}$ change in temperature from 25°C is shown along with the positive and negative values of the fitted trendline at 40°C .

Fig. 9: Spectrally corrected fluorescence spectra of SRM 2943 taken on instruments with pulsed and continuous excitation.

Figure 1

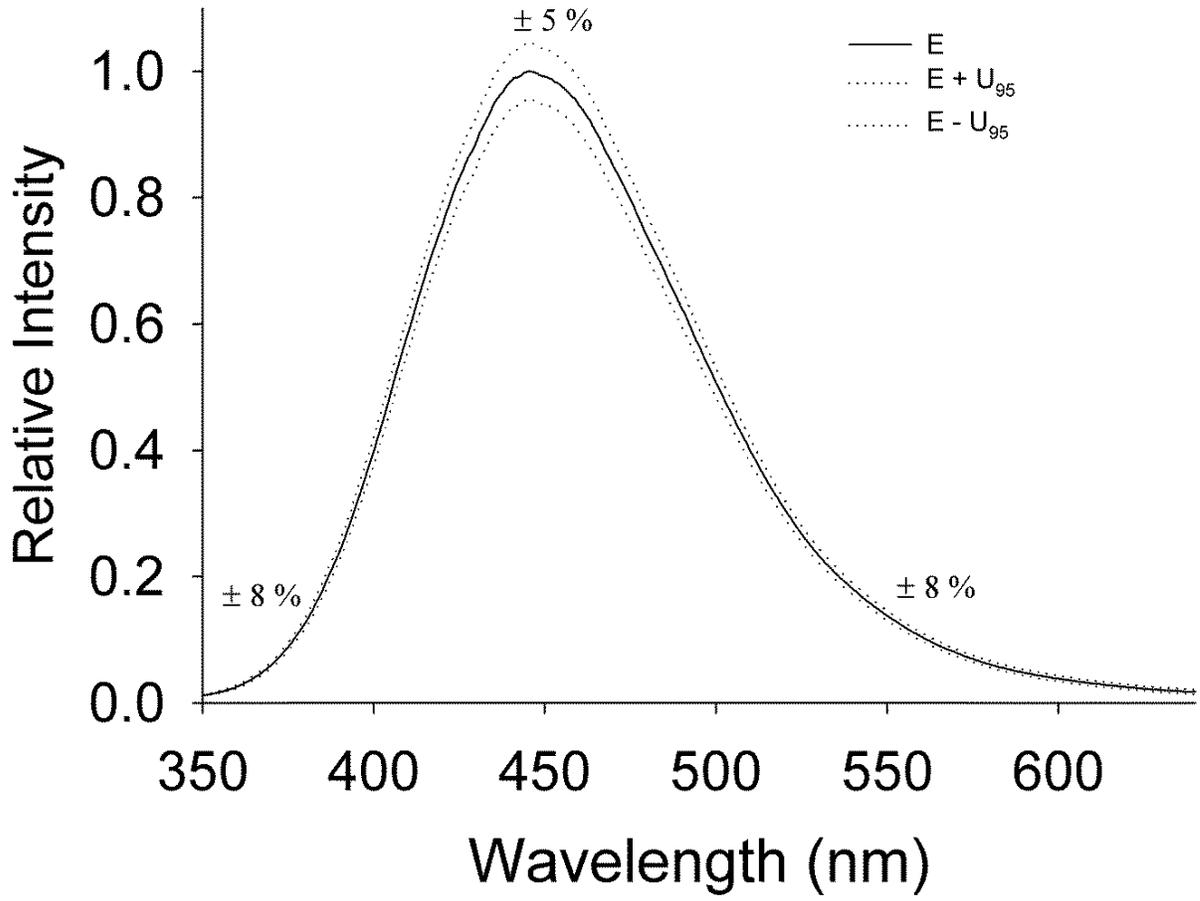


Figure 2

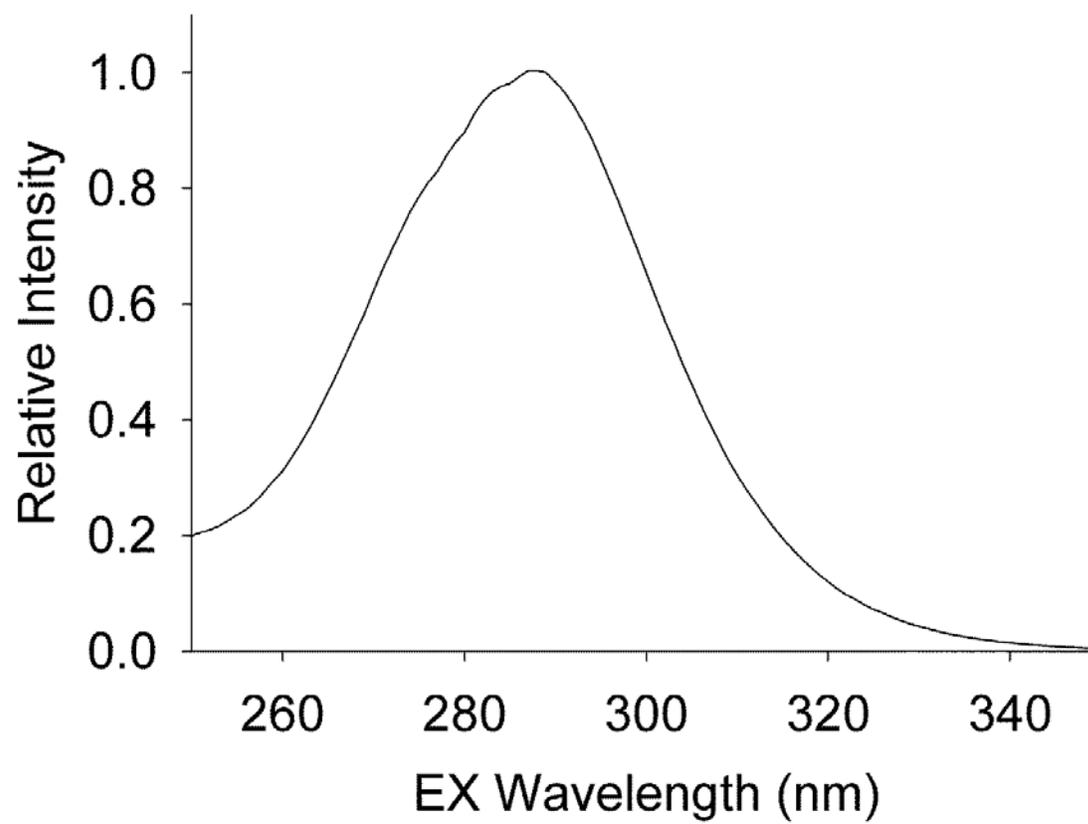


Figure 3

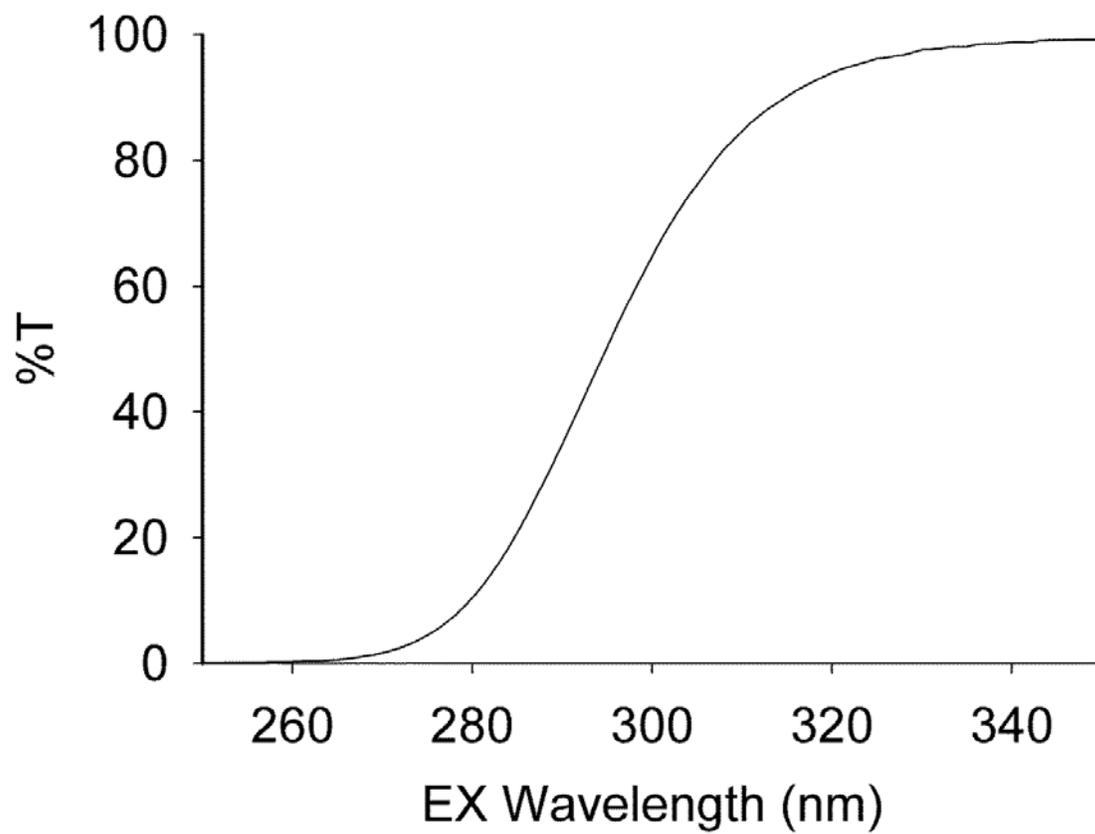


Figure 4

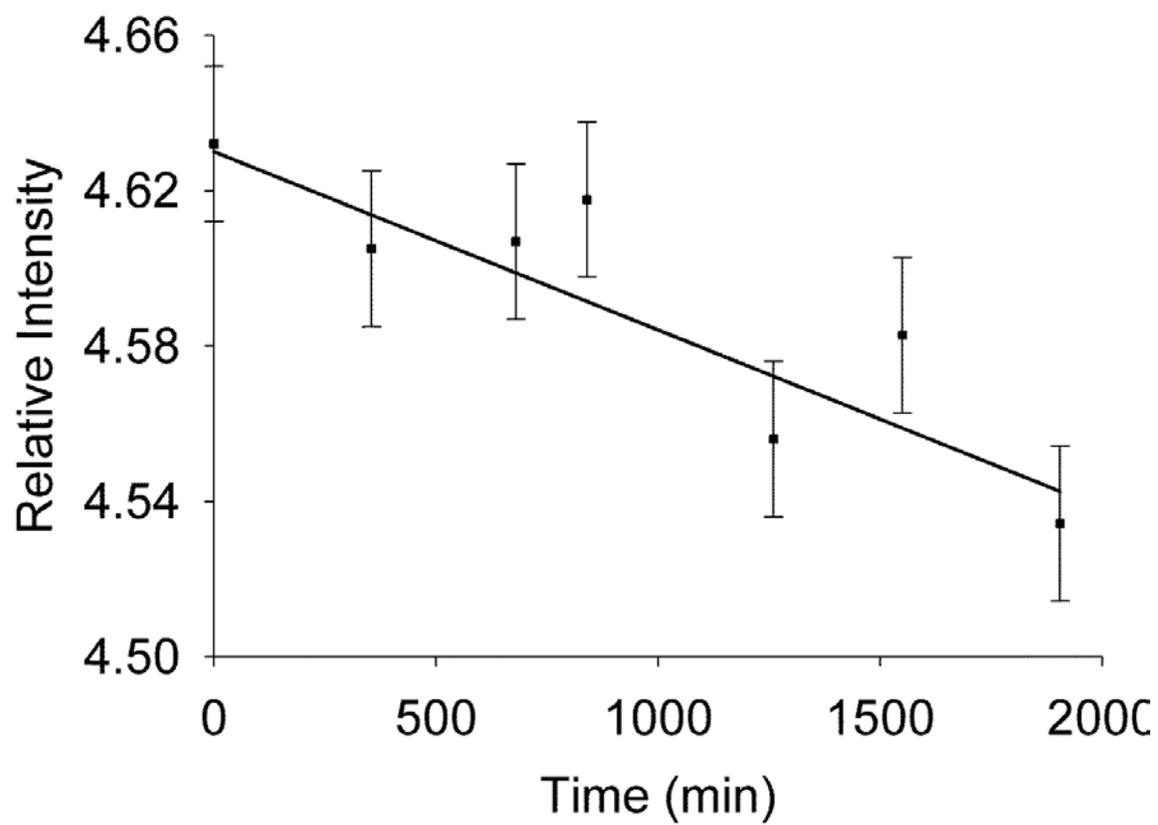


Figure 5

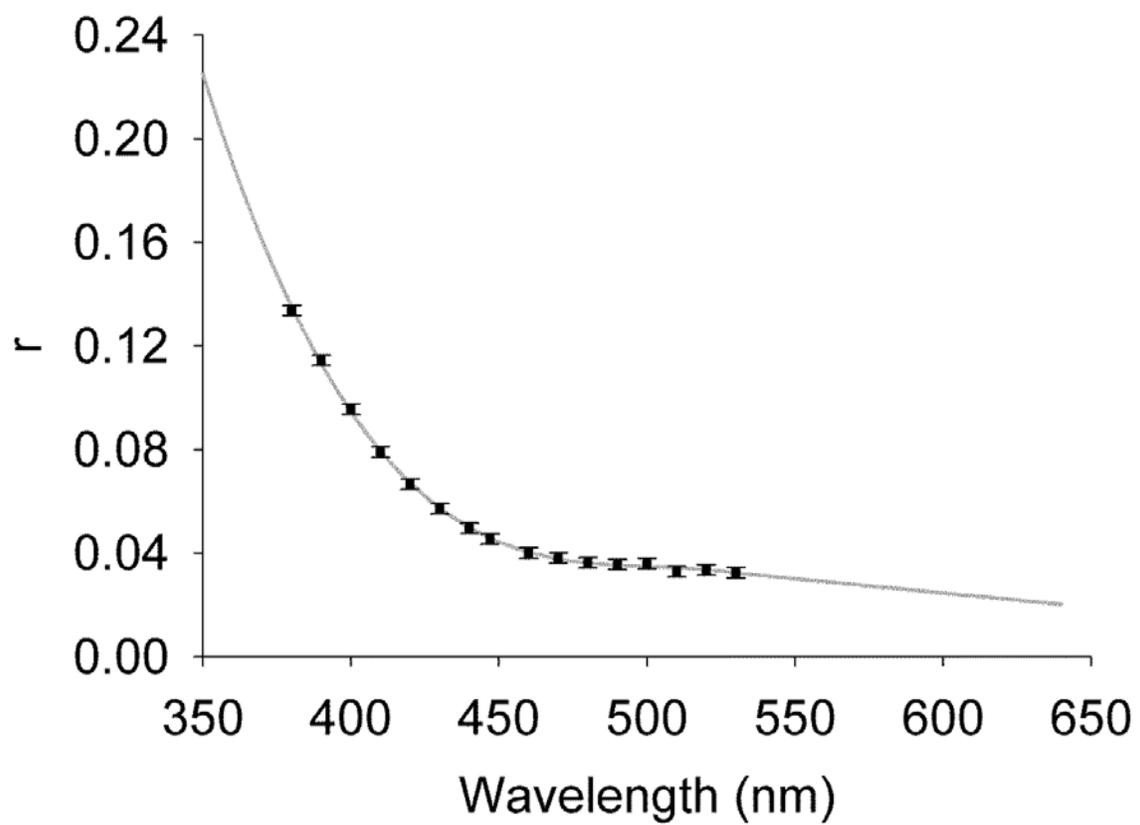


Figure 6

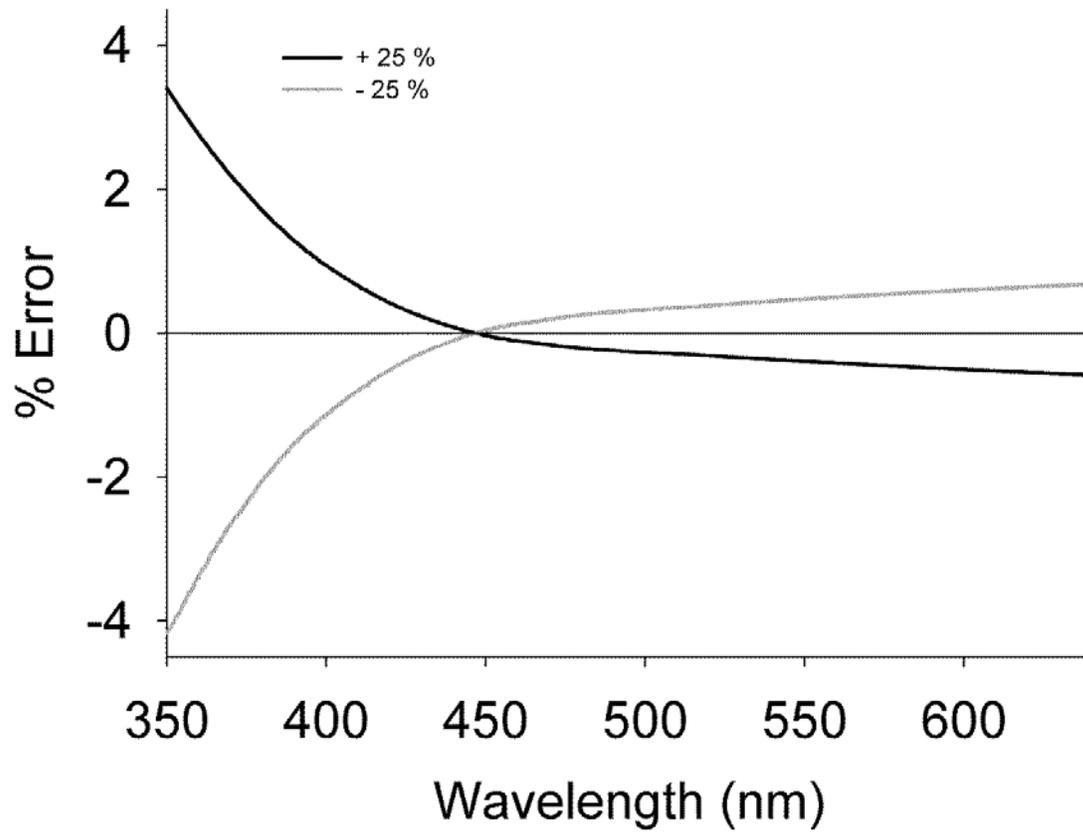


Figure 7

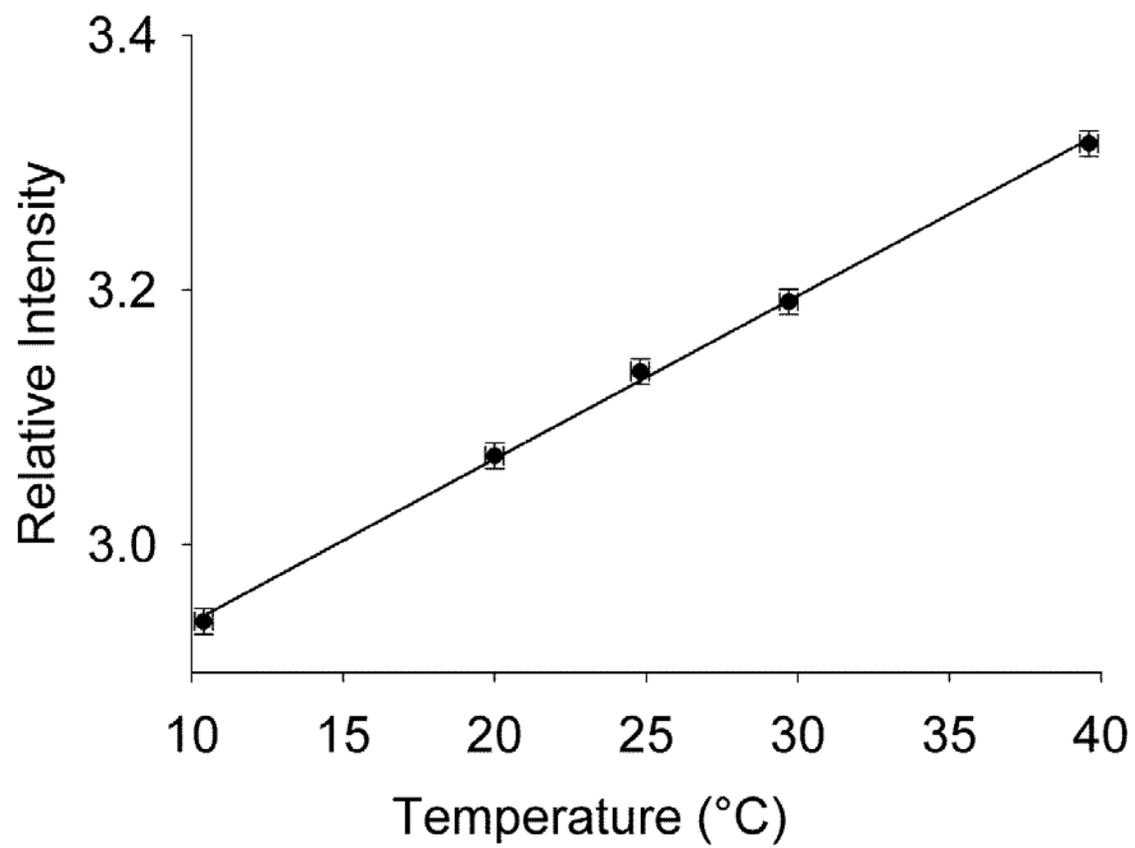


Figure 8

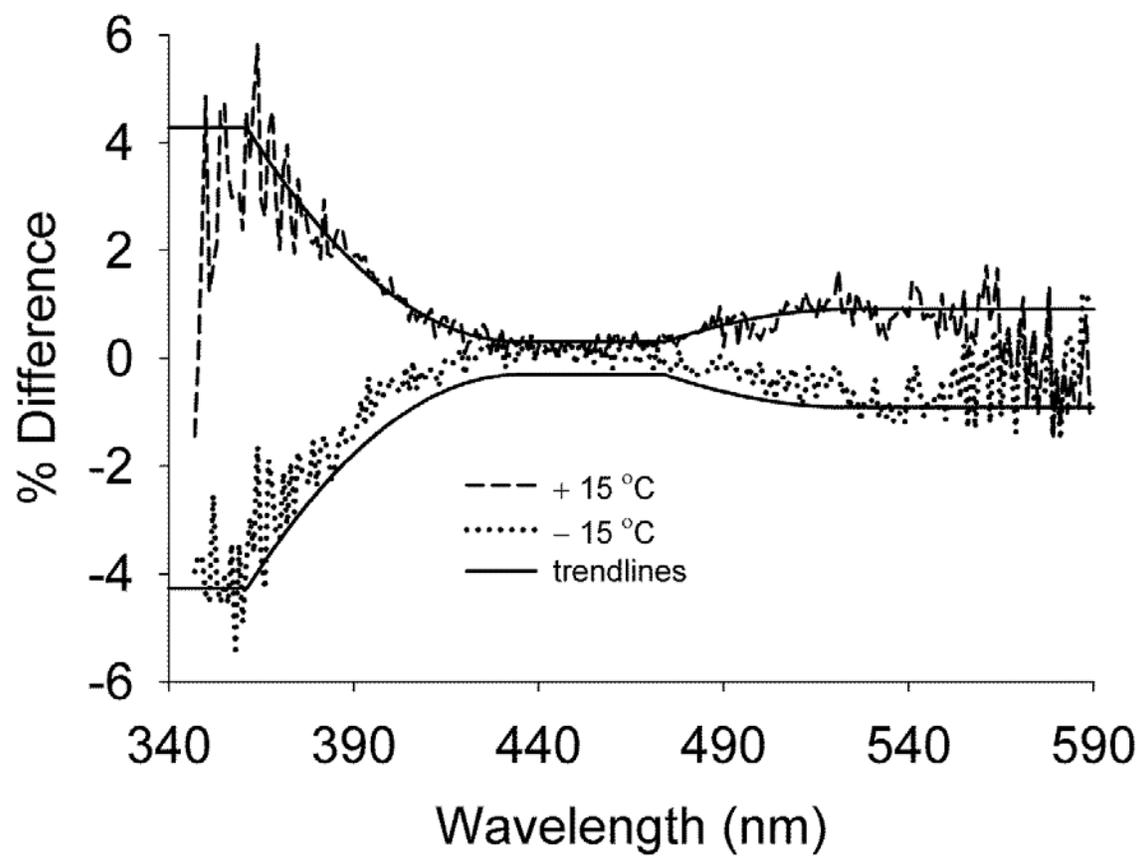
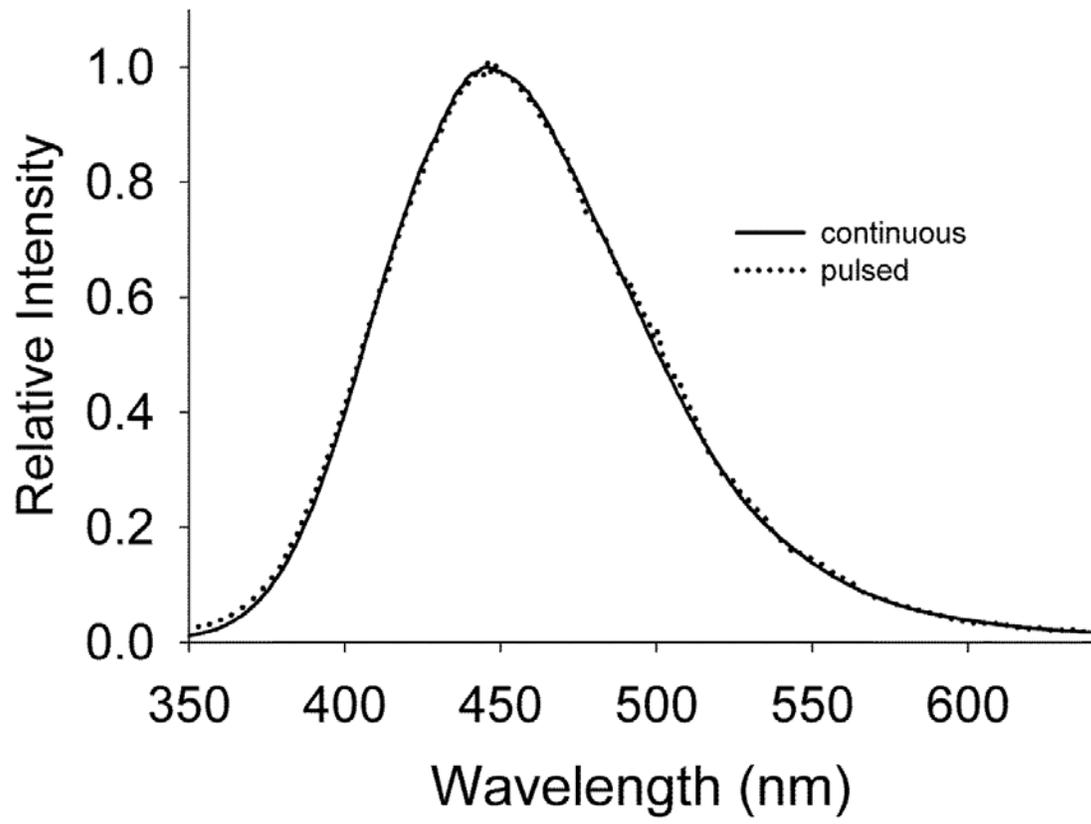


Figure 9



¹ P.C. DeRose, U. Resch-Genger, L. Wang, A.K. Gaigalas, G.W. Kramer, U. Panne, “Need for and Metrological Approaches towards Standardization of Fluorescence Measurements from the View of National Metrology Institutes,” in *Standardization and Quality Assurance in Fluorescence Measurements I*, Vol. 5 of Springer Series on Fluorescence (Springer-Verlag GmbH, Berlin Heidelberg, 2008).

² Certificate of analysis, Standard Reference Material 2940, Relative Intensity Correction Standard for Fluorescence Spectroscopy: Orange Emission. National Institute of Standards and Technology, 2007. (https://www-s.nist.gov/srmors/view_detail.cfm?srm=2940)

³ Certificate of analysis, Standard Reference Material 2941, Relative Intensity Correction Standard for Fluorescence Spectroscopy: Green Emission. National Institute of Standards and Technology, 2007. (https://www-s.nist.gov/srmors/view_detail.cfm?srm=2941)

⁴ Certificate of analysis, Standard Reference Material 2942, Relative Intensity Correction Standard for Fluorescence Spectroscopy: Ultraviolet Emission. National Institute of Standards and Technology, 2009. (https://www-s.nist.gov/srmors/view_detail.cfm?srm=2942)

⁵ Certificate of analysis, Standard Reference Material 2943, Relative Intensity Correction Standard for Fluorescence Spectroscopy: Blue Emission. National Institute of Standards and Technology, 2009. (https://www-s.nist.gov/srmors/view_detail.cfm?srm=2943)

⁶ Certificate of analysis, Certified Reference Materials BAM-F001-BAM-F005, Calibration Kit, Spectral Fluorescence Standards; Federal Institute for Materials Research and Testing: Berlin, 2006.

⁷ Certificate of analysis, Standard Reference Material 1932, Fluorescein Solution. National Institute of Standards and Technology, 2003. (https://www-s.nist.gov/srmors/view_detail.cfm?srm=1932)

⁸ ASTM E 2719, “Standard Guide for Fluorescence-Instrument Calibration and Validation”, in *Annual book of ASTM standards* (ASTM International: West Conshohocken, PA, 2010) Vol 03.06.

⁹ P.C. DeRose, U. Resch-Genger, *Anal.Chem.* 82 (2010) 2129.

¹⁰ U. Resch-Genger, P.C. DeRose, *Pure Appl. Chem.* in press.

¹¹ IUPAC Project no. 2004 021 1 300.

¹² P.C. DeRose, Recommendations and Guidelines for Standardization of Fluorescence Spectroscopy, NISTIR 7457 (National Institute of Standards and Technology: Gaithersburg, MD, 2007)

¹³ G.E. Marti, R.F. Vogt, A.K. Gaigalas, C.S. Hixson, R.A. Hoffman, R. Lenkei, L.E. Magruder, N.B. Purvis, A. Schwartz, H.M. Shapiro, A. Waggoner *Fluorescence Calibration and Quantitative Measurements of Fluorescence Intensity, Approved Guideline* (NCCLS, I/LA24-A, 2004) Vol. 24 No. 26.

¹⁴ P.C. DeRose, M.V. Smith, K.D. Mielenz, D.H. Blackburn, G.W. Kramer, *J. Lumin.* 129 (2009) 349.

¹⁵ P.C. DeRose, M.V. Smith, K.D. Mielenz, D.H. Blackburn, G.W. Kramer, *J. Lumin.* 128 (2008) 257.

¹⁶ P.C. DeRose, M.V. Smith, K.D. Mielenz, J.R. Anderson, G.W. Kramer, *J. Lumin.* 131 (2011) 1294.

¹⁷ Certificate of analysis, Standard Reference Material 936, Quinine Sulfate Dihydrate. National Institute of Standards and Technology, 1979.

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- ¹⁸ Certificate of analysis, Standard Reference Material 936a, Quinine Sulfate Dihydrate. National Institute of Standards and Technology, 1994.
- ¹⁹ A.R. Rodriguez, C.W. Parmelee, A.E. Badger, *J. Amer. Ceram. Soc.*, 26 (1943) 137.
- ²⁰ G.O. Karapetyan, *Bulletin of the Academy of Sciences of the USSR – Physical Series*, 25 (1961) 535.
- ²¹ K. Wilke, *Z. Phys. Chem.*, 219 (1962) 153.
- ²² D.S. Shionoya, E. Nakazawa, *Appl. Phys. Lett.*, 6 (1965) 118.
- ²³ P.C. DeRose, *NIST Interagency Report 7457*, National Institute of Standards and Technology (2007).
- ²⁴ Certain commercial equipment, instruments, or materials are identified in this paper to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.
- ²⁵ J. H. Walker, R. D. Saunders, J. K. Jackson, D. A. McSparron, *Natl. Bur. Stand. (U.S.) Spec. Publ. 250-20* (U.S. GPO, Washington, D.C., 1987).
- ²⁶ J. H. Walker, R. D. Saunders, A. T. Hattenburg, *Natl. Bur. Stand. (U.S.) Spec. Publ. 250-1* (U.S. GPO, Washington, D.C., 1987).
- ²⁷ V. R. Weidner, J. J. Hsia, *Natl. Bur. Stand. (U.S.) Spec. Publ. 250-6* (U.S. GPO, Washington, D.C., 1987).
- ²⁸ T. C. Larason, S. S. Bruce, C. L. Cromer, *J. Res. Natl. Inst. Stand. Technol.*, 101 (1996) 133.
- ²⁹ T. C. Larason, S. S. Bruce, A. C. Parr, *Natl. Bur. Stand. (U.S.) Spec. Publ. 250-41* (U.S. GPO, Washington, D.C., 1998).
- ³⁰ P.C. DeRose, E.A. Early, G.W. Kramer, *Rev. Sci. Instru.* 78 (2007) 033107.
- ³¹ K. D. Mielenz, "Photoluminescence Spectrometry", in *Measurement of Photoluminescence, Optical Radiation Measurements*, K. D. Mielenz, Ed. (Academic Press, New York, 1982) Vol. 3, pp. 58-76.
- ³² M. Elisa, B. Sava, A. Diaconu, D. Ursu, R. Patrascu, *J. Non-Cryst. Solids* 355 (2009) 1877.
- ³³ K. Annapurna, A. Kumar, R.N. Dwivedi, N. Sooraj Hussain, S. Buddhudu, *Mater. Lett.* 45 (2000) 23.
- ³⁴ B.C. Bunker, G.W. Arnold, J.A. Wilder, *J. Non-Cryst. Solids* 64 (1984) 291.
- ³⁵ M.C. Yappert, J.D. Ingle, Jr., *Appl. Spec.* 43 (1989) 759.
- ³⁶ C.A. Parker, W.J. Barnes, *Analyst* 82 (1957) 606.
- ³⁷ W.A. Weyl, *Coloured Glasses* (Society of Glass Technology, Sheffield, UK, 1999) pp. 497-521.
- ³⁸ B. Hylan, B. Turner, *Fusion* 54(2) (2007) 19.
- ³⁹ R.A. Velapoldi, K.D. Mielenz *NBS Spec Pub 260-64* (U.S. Government Printing Office, Washington, DC, 1980).
- ⁴⁰ E.D. Cehelnik, K.D. Mielenz, R.A. Velapoldi, *J. Res. Nat. Bur. Stand.* 79A (1975) 1.

⁴¹ Turner, W.H. (1973) "Photoluminescence of Color Filter Glasses," *Appl.Optics*, **12**, 480.