National Institute of Standards & Technology

Certificate

Standard Reference Material® 2940a

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Orange Emission

Lot 1

This Standard Reference Material (SRM) is intended for use for the evaluation and calibration of the relative spectral responsivity of steady-state fluorescence spectrometers with a continuous excitation source and for determining the day-to-day or instrument-to-instrument intensity variations of a single or similar fluorescence instrument(s), respectively. This SRM is certified for the relative, corrected emission spectrum, E, in relative energy units from emission wavelengths λ_{EM} = 500nm to 800 nm at 1 nm wavelength intervals at a fixed excitation wavelength (λ_{EX}) of 412 nm. The SRM should be positioned with the excitation beam normal to and centered on one polished face and with the emission being collected from the center of an adjacent polished face at 90° with respect to the excitation beam. The long-frosted side should face away from the detection system. Each SRM has its own serial number etched into the top face, which should face up when in use. The frosted face may be used with a front-face or epifluorescence geometry, or the polished faces may be used with geometries different from that prescribed above; however, the certified values become reference values in these cases. This SRM consists of a single cuvette-shaped piece of solid glass.

Certified Values: NIST certified values are values for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST [1,2]. The certified values for this material are listed in Table 1. The values were certified at 25.0 °C ± 0.5 °C with an excitation bandwidth ($\Delta\lambda_{EX}$) of 3.0 nm and an emission bandwidth ($\Delta\lambda_{EM}$) of 3.0 nm. The certified values for E and corresponding total uncertainties at the 95 % confidence level, U_{95}, at each emission wavelength are given in Table 1. Metrological traceability of E is to the NIST spectral radiance scale, as expressed in relative energy units. Metrological traceability of wavelength is to the SI unit of meters.

Reference Values: NIST Reference values are non-certified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision and may not include all sources of uncertainty [1,2].

Expiration of Certification: The certification of **SRM 2940a** is valid, within the measurement uncertainty specified, until **01 September 2028**, provided the SRM is handled and stored in accordance with the instructions given here (see "Instructions for Use"). The certification is nullified if the SRM is 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 or register online) will facilitate notification.

Overall direction and coordination of the technical measurements required for certification of this SRM were performed by P.C. DeRose of the NIST Biosystems and Biomaterials Division.

Production and certification of this SRM were performed by P.C. DeRose, and J.R. Anderson and A.V. Kirchhoff of the NIST Fabrication Technology Division.

Statistical consultation was provided by J. Lu of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

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Certificate Issue Date: 10 October 2018

Steven J. Choquette, Director
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Table 1. Certified Relative Corrected Emission Spectrum of SRM 2940a Lot 1 at λ_{EX} = 412 nm

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
500	0.0337	0.0049	559	0.5369	0.0313	618	0.9981	0.0368	677	0.6225	0.0267
501	0.0362	0.0052	560	0.5486	0.0315	619	0.9978	0.0368	678	0.6143	0.0264
502	0.0396	0.0056	561	0.5596	0.0316	620	0.9965	0.0367	679	0.6056	0.0262
503	0.0429	0.0060	562	0.5740	0.0319	621	0.9935	0.0367	680	0.5965	0.0259
504	0.0466	0.0065	563	0.5857	0.0321	622	0.9909	0.0366	681	0.5872	0.0256
505	0.0502	0.0069	564	0.5992	0.0323	623	0.9858	0.0365	682	0.5793	0.0253
506	0.0543	0.0073	565	0.6121	0.0325	624	0.9846	0.0365	683	0.5727	0.0251
507	0.0584	0.0078	566	0.6257	0.0328	625	0.9820	0.0364	684	0.5645	0.0248
508	0.0629	0.0083	567	0.6392	0.0330	626	0.9775	0.0363	685	0.5543	0.0245
509	0.0676	0.0088	568	0.6511	0.0331	627	0.9737	0.0362	686	0.5484	0.0243
510	0.0725	0.0093	569	0.6636	0.0333	628	0.9710	0.0362	687	0.5425	0.0241
511	0.0778	0.0098	570	0.6757	0.0334	629	0.9702	0.0362	688	0.5335	0.0238
512	0.0833	0.0103	571	0.6886	0.0336	630	0.9644	0.0361	689	0.5264	0.0236
513	0.0886	0.0108	572	0.7017	0.0338	631	0.9590	0.0359	690	0.5175	0.0233
514	0.0943	0.0114	573	0.7132	0.0339	632	0.9546	0.0358	691	0.5089	0.0230
515	0.1003	0.0119	574	0.7264	0.0341	633	0.9522	0.0358	692	0.5029	0.0228
516	0.1065	0.0125	575	0.7402	0.0343	634	0.9455	0.0357	693	0.4931	0.0225
517	0.1131	0.0130	576	0.7503	0.0343	635	0.9387	0.0355	694	0.4859	0.0222
518	0.1196	0.0136	577	0.7613	0.0344	636	0.9327	0.0353	695	0.4775	0.0219
519	0.1262	0.0141	578	0.7737	0.0346	637	0.9294	0.0353	696	0.4714	0.0217
520	0.1339	0.0147	579	0.7837	0.0346	638	0.9237	0.0352	697	0.4641	0.0215
521	0.1417	0.0154	580	0.7934	0.0347	639	0.9167	0.0350	698	0.4569	0.0213
522	0.1491	0.0159	581	0.8051	0.0348	640	0.9096	0.0348	699	0.4475	0.0209
523	0.1563	0.0164	582	0.8138	0.0348	641	0.9048	0.0347	700	0.4417	0.0207
524	0.1647	0.0170	583	0.8226	0.0349	642	0.8984	0.0345	701	0.4343	0.0205
525	0.1723	0.0175	584	0.8315	0.0349	643	0.8934	0.0344	702	0.4283	0.0203
526	0.1805	0.0181	585	0.8410	0.0350	644	0.8867	0.0343	703	0.4222	0.0201
527	0.1887	0.0186	586	0.8515	0.0351	645	0.8829	0.0342	704	0.4137	0.0198
528	0.1979	0.0192	587	0.8593	0.0351	646	0.8750	0.0340	705	0.4067	0.0195
529	0.2062	0.0197	588	0.8692	0.0352	647	0.8690	0.0338	706	0.3984	0.0192
530	0.2156	0.0203	589	0.8802	0.0354	648	0.8614	0.0336	707	0.3927	0.0190
531	0.2235	0.0207	590	0.8872	0.0354	649	0.8555	0.0335	708	0.3857	0.0188
532	0.2328	0.0212	591	0.8940	0.0355	650	0.8488	0.0333	709	0.3805	0.0186
533	0.2426	0.0217	592	0.9023	0.0355	651	0.8439	0.0332	710	0.3751	0.0184
534	0.2527	0.0222	593	0.9103	0.0356	652	0.8345	0.0330	711	0.3678	0.0182
535	0.2610	0.0226	594	0.9195	0.0358	653	0.8279	0.0328	712	0.3606	0.0179
536	0.2711	0.0231	595	0.9253	0.0358	654	0.8198	0.0326	713	0.3536	0.0176
537	0.2815	0.0236	596	0.9311	0.0358	655	0.8126	0.0324	714	0.3468	0.0174
538	0.2916	0.0240	597	0.9382	0.0359	656	0.8059	0.0322	715	0.3401	0.0171
539	0.3018	0.0245	598	0.9425	0.0359	657	0.7978	0.0320	716	0.3348	0.0170
540	0.3119	0.0249	599	0.9487	0.0360	658	0.7882	0.0317	717	0.3287	0.0167
541	0.3226	0.0253	600	0.9552	0.0361	659	0.7816	0.0315	718	0.3237	0.0166
542	0.3334	0.0257	601	0.9595	0.0362	660	0.7715	0.0312	719	0.3165	0.0163
543	0.3438	0.0261	602	0.9654	0.0362	661	0.7645	0.0310	720	0.3104	0.0161
544	0.3550	0.0265	603	0.9678	0.0362	662	0.7561	0.0308	721	0.3047	0.0159
545	0.3659	0.0268	604	0.9719	0.0363	663	0.7483	0.0306	722	0.2985	0.0156
546	0.3767	0.0272	605	0.9773	0.0364	664	0.7378	0.0303	723	0.2922	0.0154
547	0.3888	0.0276	606	0.9790	0.0364	665	0.7289	0.0300	724	0.2874	0.0152
548	0.3997	0.0279	607	0.9842	0.0365	666	0.7214	0.0298	725	0.2823	0.0150
549	0.4114	0.0282	608	0.9883	0.0366	667	0.7122	0.0295	726	0.2755	0.0148
550	0.4240	0.0286	609	0.9914	0.0366	668	0.7028	0.0292	727	0.2695	0.0145
551	0.4356	0.0289	610	0.9924	0.0366	669	0.6956	0.0290	728	0.2663	0.0144
552	0.4480	0.0292	611	0.9923	0.0366	670	0.6835	0.0286	729	0.2606	0.0142
553	0.4603	0.0296	612	0.9951	0.0367	671	0.6773	0.0284	730	0.2550	0.0140
554	0.4737	0.0299	613	0.9968	0.0367	672	0.6676	0.0281	731	0.2493	0.0138
555	0.4858	0.0302	614	0.9955	0.0367	673	0.6595	0.0279	732	0.2430	0.0135
556	0.4978	0.0305	615	0.9983	0.0367	674	0.6509	0.0276	733	0.2392	0.0134
557	0.5102	0.0307	616	1.0000	0.0368	675	0.6406	0.0273	734	0.2357	0.0133
558	0.5239	0.0310	617	0.9977	0.0367	676	0.6330	0.0270	735	0.2298	0.0131

Table 1 Continued

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
736	0.2247	0.0128	753	0.1617	0.0104	770	0.1153	0.0086	787	0.0817	0.0073
737	0.2221	0.0127	754	0.1593	0.0103	771	0.1135	0.0085	788	0.0796	0.0071
738	0.2172	0.0126	755	0.1566	0.0102	772	0.1107	0.0084	789	0.0778	0.0071
739	0.2129	0.0124	756	0.1534	0.0101	773	0.1087	0.0083	790	0.0771	0.0069
740	0.2092	0.0123	757	0.1493	0.0100	774	0.1074	0.0083	791	0.0770	0.0070
741	0.2031	0.0120	758	0.1471	0.0099	775	0.1042	0.0082	792	0.0745	0.0069
742	0.1990	0.0119	759	0.1455	0.0098	776	0.1021	0.0080	793	0.0719	0.0068
743	0.1955	0.0117	760	0.1419	0.0097	777	0.0999	0.0080	794	0.0696	0.0067
744	0.1930	0.0116	761	0.1377	0.0095	778	0.0978	0.0079	795	0.0680	0.0066
745	0.1897	0.0115	762	0.1358	0.0094	779	0.0973	0.0078	796	0.0670	0.0065
746	0.1869	0.0114	763	0.1328	0.0093	780	0.0956	0.0078	797	0.0661	0.0065
747	0.1827	0.0113	764	0.1301	0.0092	781	0.0930	0.0077	798	0.0650	0.0065
748	0.1781	0.0111	765	0.1284	0.0091	782	0.0906	0.0076	799	0.0642	0.0064
749	0.1740	0.0109	766	0.1258	0.0090	783	0.0889	0.0075	800	0.0643	0.0064
750	0.1710	0.0108	767	0.1236	0.0089	784	0.0857	0.0074			
751	0.1679	0.0107	768	0.1205	0.0088	785	0.0837	0.0072			
752	0.1634	0.0105	769	0.1165	0.0087	786	0.0841	0.0073			

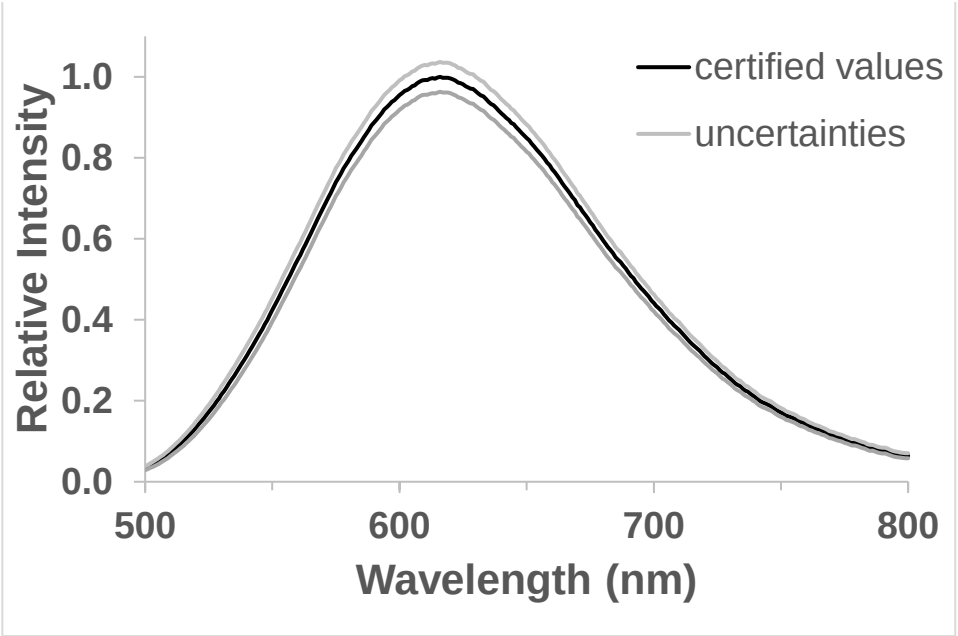

Figure 1. Spectrum for SRM 2940a Lot 1.

Information Values: A NIST information value is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess adequately the uncertainty associated with the value or only a limited number of analyses were performed [1,2]. A NIST information value is provided for information purposes only. Information values for the relative temperature coefficient of the E value at 620 nm and the fluorescence anisotropy (r) at 620 nm of SRM 2940a are listed in Table 2. Information values cannot be used to establish metrological traceability.

Table 2: Temperature Coefficient of the E Value at 620 nm and the Fluorescence Anisotropy at 620 nm of SRM 2940a

Relative Temperature Coefficient for E at 620 nm:	–0.21 % $°C^{-1}$ (range: 11 °C to 39 °C)
Fluorescence Anisotropy at 620 nm:	0.021

Physical Description: SRM 2940a is a manganese-doped (0.11 % MnO_2 by weight) borate matrix glass. Each unit of this SRM is a rectangular solid block with standard cuvette dimensions 12.5 mm × 12.5 mm × 45.0 mm, with three of the four long faces optically polished and one long face, the top face and the bottom face ground to a frosted finish using a 400-grit polish. The serial number of each unit is etched on the top face.

Photostability: After irradiating the SRM with a white light source with a nominal intensity of 13 mW cm^{-2} nm^{-1} from 400 nm to 700 nm for more than 17 hours, no change in the absolute intensity or shape of the emission spectrum was observed within an uncertainty of ± 0.4 % (k = 2) at the peak maximum. This amount of irradiation corresponds to about 242 hours of irradiation with our fluorometer's excitation beam under the conditions used for certification.

Certification Measurements: The emission monochromator was calibrated for wavelength using a Hg line of a pen lamp at 404.66 nm. The excitation monochromator was calibrated for wavelength by setting the excitation monochromator at 412.0 nm and then scanning the emission monochromator over the same wavelength position. A diffuse reflector was placed at the sample at a 45° angle relative to the incident beam to reflect the light from the excitation beam into the detection system. A calibrated light source was used to determine the relative responsivity of the detection system as a function of wavelength with the aid of a calibrated reflector at the sample position to reflect the light from the calibrated source into the detection system [3]. The spectrum of each SRM was then collected from an emission wavelength of 500 nm to 800 nm at 1 nm increments and a fixed excitation wavelength of 412 nm. The excitation and emission bandwidths were set to 3 nm, and the relative excitation intensity was collected simultaneously with the fluorescence intensity, enabling the measured SRM spectrum to be corrected for variations in excitation intensity. The resulting SRM spectrum was then corrected for the responsivity of the detection system and a small emission wavelength bias. The certified spectrum shown in Figure 1 is an average of the corrected spectra for all SRM units in this batch, which has also been normalized to one at 620 nm. The certified quantity E is the relative amount of fluorescence emitted from the sample, expressed in relative energy units. The absolute peak intensity was also found to vary by less than 2 % for all units in this batch.

Assignment of Uncertainties: Standard uncertainty components equivalent to the estimated standard deviation were assigned for sample inhomogeneity, sample variation within the batch, and measurement uncertainties. These values were then combined with systematic uncertainties due to wavelength accuracy, bandwidth accuracy, temperature accuracy, spatial uncertainty of the excitation beam's position on the sample (causing secondary inner filter effect uncertainties), variation of F and G polarization ratios [4] among instruments, and uncertainty in the spectral shape correction (due to uncertainty in the radiance and reflectance values of the calibrated light source and reflector), using the root-sum-of-squares method. An expansion factor of k = 2 was applied so that the expanded uncertainties given in this certificate express an interval (E ± U_{95}) within which the true value is expected to fall with a level of confidence of approximately 95 % for a normal distribution [2].

Handling and Storage: This SRM should be handled only while wearing a pair of clean, powder-free plastic (nitrile recommended) or cloth disposable gloves. The SRM should be grasped with two fingers in an area away from where the excitation beam will be incident on or where the fluorescence will be collected from the SRM. The supplied case should always be used to store the SRM after it has been wrapped in a clean piece of lens paper. The SRM should be stored in a desiccator or other low humidity environment around room temperature (15.0 °C to 35.0 °C). It should not be exposed to direct sunlight and should be kept in the dark whenever possible. The faces of the SRM can be washed with absolute ethanol and gently dried with lens paper, if necessary.

INSTRUCTIONS FOR USE

WARNING TO USERS: This standard and its certified values cannot be used for spectral correction of fluorescence spectrometers with pulsed light sources. The certified values cannot be applied to instruments using pulsed light sources.

For Correction of Detection System Responsivity: Place the SRM at the sample position of the steady-state fluorescence spectrometer using a standard cuvette holder, with the long-frosted side facing away from the detection system. The excitation beam should be horizontally centered on the entrance and exit faces of the SRM. Measurements should be taken with the SRM at a temperature of 25.0 °C ± 0.5 °C. Set the excitation and emission bandwidths as close to 3 nm as possible and set the excitation wavelength to 412 nm. Scan the emission monochromator from 500 nm to 800 nm using a 1 nm increment. Collect the detection system signal and, if possible, the simultaneous excitation intensity at each point. Correct the measured fluorescence signal for the excitation intensity, if possible, by dividing the former by the latter. Normalize this spectrum by dividing the intensity values at all wavelengths by the intensity value at 620 nm. Divide each certified value by its corresponding normalized, measured value (preferably excitation intensity corrected) to obtain a correction factor for the detection system responsivity at each emission wavelength. For user convenience, a list of the certified values and uncertainties in

ASCII format and a Microsoft EXCEL-based program to produce a similar list with a user-specified λ_{EM} range and step size can be downloaded from the data file link at https://www-s.nist.gov/srmors/view_detail.cfm?srm=2940a.

For Day-to-Day Intensity Standard: Excite the SRM at a wavelength between 400 nm and 550 nm, preferably at 412 nm, and measure the fluorescence intensity, preferably at the peak maximum, and the excitation intensity, if possible. Day-to-day intensity variations can be determined by periodically measuring the fluorescence intensity (preferably excitation intensity corrected) under the same experimental conditions and comparing the intensity values over time.

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/sites/default/files/documents/srm/SP260-136.PDF (accessed Oct 2018).

[2] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Oct 2018); 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 https://www.nist.gov/pml/nist-technical-note-1297 (accessed Oct 2018).

[3] DeRose, P.C.; Early, E.A.; Kramer, G.W.; *Qualification of a Fluorescence Spectrometer for Measuring True Fluorescence Spectra*; Rev. Sci. Instrum., Vol. 78 (2007).

[4] Mielenz, K.D.; *Measurement of Photoluminescence*; Mielenz, K.D. Ed., Optical Radiation Measurements, Vol. 3, Academic Press: New York, NY pp. 58−76 (1982).

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) 948-3730; e-mail srminfo@nist.gov; or via the Internet https://www.nist.gov/srm.