National Institute of Standards & Technology

Certificate

Standard Reference Material 1931

Fluorescence Emission Standards for the Visible Region

This Standard Reference Material (SRM) is intended for use in the evaluation and calibration of the relative spectral response of fluorescence spectrometers. It consists of four fluorescence standards and a "blank" specimen mounted in anodized aluminum cuvette-sized holders. The fluorescence standards are certified for the relative corrected emission spectrum, E(λ) in energy/wavelength units. The certified values of the blue, green, yellow and orange emission spectra at 2 nm wavelength intervals are given in Tables I, II, III and IV, respectively. The fluorescence emission standards are:

Fluorescence Emission Standard	Excitation Wavelength, nm*	Emission Wavelength Range, nm*
B (Blue)	340	400 - 550
G (Green)	280	490 - 600
Y (Yellow)	410	490 - 740
O (Orange)	235	530 - 740

*Excitation and emission monochromator bandpass set at 5.3 nm, temperature was 25°C

The fluorescence emission standards are composed of inorganic phosphors (10% by weight) and polytetrafluoroethylene (PTFE) which were pressed into wafers and sintered. The phosphors selected emit in the blue, green, yellow or orange wavelength regions (Figure 1). The PTFE wafer is mounted in an anodized aluminum cuvette-sized holder. The fluorescence excitation beam angle of incidence is 60 degrees from the normal to plane of the sintered wafer. SRM 1931 L and R are oriented for left and right-handed spectrofluorimeters, respectively, as defined by viewing the fluorescent sample along the excitation beam. If the first emission optical component is located to the right of the sample then this orientation is designated R and if it is to the left then it is designated L. The anodized holder is engraved with the first letter of the color of the emission (B, G, Y or O) over the excitation beam aperture. A PTFE blank in a non-engraved holder is also included in the set for diagnostic purposes.

The technical measurements leading to certification of spectral emission were performed by A. Thompson and K.L. Eckerle of the Radiometric Physics Division.

The overall direction and coordination of this project was provided by K.D. Mielenz and J.J. Hsia, NIST Radiometric Physics Division.

The technical and support aspects involved in the issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by R.L. McKenzie.

Gaithersburg, MD 20899
August 15, 1989

Stanley D. Rasberry, Chief
Office of Standard Reference Materials

(Over)

CALIBRATION MEASUREMENTS: Calibration measurements were done with s emission polarization (perpendicular to the plane of incidence at the monochromator grating) to minimize polarization errors. Ten spectra were measured for each phosphor standard in the master set, except for the yellow phosphor for which only nine spectra were measured. In an individual emission spectrum, the fluorescence value at each wavelength was the mean net signal from 100 measurements of the fluorescence signal and ten dark measurements. These values have been corrected for the following instrument parameters: spectral responsivity of the detection system, photomultiplier tube nonlinearity, and monochromator wavelength error. The corrected values are used to calculate the relative emission spectrum, $E(\lambda)$, which is presented in Tables I, II, III, and IV. The total standard error , $TSE[E(\lambda)]$, (3 sigma) of $E(\lambda)$ is given in Tables I, II, III, and IV. These error limits include quadrature sums of random and systematic uncertainties for: instrument parameters, the measurement of the emission spectra, and the measurement of the calibration values for the spectral responsivity. In addition to the master set calibration, commercial spectrofluorimeter measurements were carried out on 18 sets out of 50 sets to estimate the uniformity of the SRM 1931 batch preparation. Variation in the standards emission spectra due to: UV instability, long-term stability, sample nonuniformity, sample to sample variation, temperature, excitation wavelength and excitation and emission polarization were estimated and their values will be given in an NIST 260-series Special Publication (currently in preparation). Values for the emission spectra in photon, wavelength and wavenumber units will also be presented in this 260-series Special Publication.

STORAGE AND USE OF SRM 1931: SRM 1931 should be stored in the aluminum container supplied in a location with stable temperature and humidity. The PTFE wafer will readily adsorb tobacco smoke and other organic aerosols. To avoid the adsorption of fluorescent contaminants SRM 1931 should not be exposed to these environments. The left or right-handed orientation of the wafer should not be changed by the user, if reorientation is necessary the standards should be returned to the NIST Radiometric Physics Division (534). The measured corrected emission spectra of SRM 1931 is certified at a bandpass of 5.3 nm. A procedure to correct these spectra for bandpass error is used in the 260-series Special Publication (in preparation) to calculate the emission spectra at zero bandpass.

The measurement of SRM 1931 is a front surface fluorescence measurement. Therefore the proper alignment of the excitation and emission optical paths is essential, otherwise the emission monochromator may view only the fringe of the emission spot or a non-emitting portion of the wafer. The cuvette holders of commercial spectrofluorimeters have a degree of positional uncertainty in the placement of the cuvette. This allows one to place the standard in the holder and to adjust the position of the cuvette in the excitation beam until the fluorescence signal is maximized. Typically this is done with the emission monochromator near the fluorescence maximum. It is also necessary to filter out the excitation light from the emission monochromator. A simple UV glass filter is sufficient for this purpose and prevents first and second order scattered excitation radiation from distorting the emission spectra. This filter must then remain in the instrument for all subsequent fluorescence measurements for which the calibrations generated using these standards are to be used.

The calibration of the yellow master standard required over 80 hours of measurement time. After this long measurement time the standard was observed to have darkened in the region of the excitation spot accompanied by a diminution of the fluorescence signal intensity and a slight change in the shape of the emission spectrum for wavelengths less than 570 nm. Therefore as a conservative precaution, the certified values for the yellow fluorescence standard relative emission spectra encompass the wavelength range from 570 to 740 nm with the values less than 570 nm included for information only. For high accuracy calibrations it is recommended to use the certified values of the green and the orange standards to cover the excluded wavelength region of the yellow fluorescence standard. In typical calibrations the average measurement time will be on the order of several minutes and the small changes observed in the emission spectrum of the yellow master would entail little problems in accuracy. A more detailed treatment of the errors involved will be found in the 260-series Special Publication. For optimum accuracy in the calibration of a spectrofluorimeter, it is recommended to use the standards at wavelengths where the relative emission is greater than 0.10 whenever possible.

Table I. The relative corrected emission spectrum, E(λ), of the blue phosphor, the total standard error in E(λ), TSE[E(λ)] (3 sigma), these error limits include random and systematic uncertainties added in quadrature.

λ, nm	E(λ)	TSE[E(λ)]	λ, nm	E(λ)	TSE[E(λ)]
400	0.04878	0.00123	476	0.67794	0.00506
402	0.05966	0.00127	478	0.64256	0.00477
404	0.07666	0.00146	480	0.60298	0.00446
406	0.09738	0.00092	482	0.56859	0.00455
408	0.12213	0.00092	484	0.53476	0.00486
410	0.15203	0.00098	486	0.49894	0.00408
412	0.18512	0.00112	488	0.46922	0.00440
414	0.22375	0.00122	490	0.43649	0.00373
416	0.26715	0.00194	492	0.40798	0.00370
418	0.31380	0.00125	494	0.38092	0.00403
420	0.36546	0.00143	496	0.35374	0.00372
422	0.41954	0.00226	498	0.32907	0.00324
424	0.47674	0.00202	500	0.30583	0.00330
426	0.53385	0.00206	502	0.28345	0.00332
428	0.59184	0.00144	504	0.26244	0.00235
430	0.65132	0.00149	506	0.24259	0.00229
432	0.70840	0.00170	508	0.22577	0.00215
434	0.76155	0.00164	510	0.20872	0.00209
436	0.81095	0.00212	512	0.19354	0.00206
438	0.85735	0.00261	514	0.17914	0.00189
440	0.89676	0.00169	516	0.16503	0.00173
442	0.93083	0.00266	518	0.15332	0.00174
444	0.95797	0.00248	520	0.14130	0.00155
446	0.97772	0.00250	522	0.13065	0.00141
448	0.99213	0.00318	524	0.12070	0.00133
450	0.99757	0.00215	526	0.11112	0.00115
452	1.00000	0.00242	528	0.10303	0.00108
454	0.99535	0.00378	530	0.09498	0.00102
456	0.98189	0.00356	532	0.08794	0.00098
458	0.96785	0.00471	534	0.08111	0.00093
460	0.94216	0.00347	536	0.07475	0.00084
462	0.91896	0.00475	538	0.06920	0.00082
464	0.88925	0.00378	540	0.06366	0.00069
466	0.85539	0.00433	542	0.05892	0.00069
468	0.82415	0.00435	544	0.05433	0.00064
470	0.78743	0.00421	546	0.04996	0.00055
472	0.75231	0.00490	548	0.04629	0.00054
474	0.71694	0.00511	550	0.04254	0.00051

Table II. The relative corrected emission spectrum, E(λ), of the green phosphor, the total standard error in E(λ), TSE[E(λ)] (3 sigma), these error limits include random and systematic uncertainties added in quadrature.

λ, nm	E(λ)	TSE[E(λ)]	λ, nm	E(λ)	TSE[E(λ)]
490	0.07105	0.00066	546	0.52942	0.00568
492	0.09572	0.00090	548	0.48144	0.00542
494	0.12615	0.00136	550	0.43342	0.00509
496	0.16349	0.00181	552	0.39022	0.00488
498	0.20628	0.00205	554	0.35026	0.00432
500	0.25826	0.00252	556	0.31230	0.00379
502	0.31545	0.00309	558	0.28026	0.00328
504	0.37757	0.00354	560	0.24862	0.00285
506	0.44751	0.00440	562	0.22153	0.00251
508	0.52091	0.00606	564	0.19692	0.00212
510	0.60521	0.00607	566	0.17425	0.00175
512	0.68943	0.00731	568	0.15547	0.00169
514	0.77264	0.00824	570	0.13734	0.00145
516	0.84898	0.00861	572	0.12177	0.00128
518	0.91209	0.01006	574	0.10801	0.00117
520	0.95862	0.01030	576	0.09537	0.00101
522	0.98784	0.01041	578	0.08408	0.00089
524	1.00000	0.01050	580	0.07485	0.00080
526	0.99402	0.01001	582	0.06643	0.00070
528	0.97492	0.00966	584	0.05896	0.00061
530	0.94302	0.00977	586	0.05208	0.00057
532	0.90401	0.00992	588	0.04663	0.00046
534	0.85611	0.00934	590	0.04122	0.00045
536	0.80295	0.00863	592	0.03666	0.00039
538	0.75121	0.00847	594	0.03284	0.00041
540	0.69238	0.00731	596	0.02912	0.00034
542	0.63746	0.00718	598	0.02603	0.00033
544	0.58267	0.00675	600	0.02330	0.00034

Table III. The relative corrected emission spectrum, E(λ), of the yellow phosphor, the total standard error in E(λ), TSE[E(λ)] (3 sigma), these error limits include random and systematic uncertainties added in quadrature. The values in italics (wavenlengths 490 to 568 nm) are not certified, and are presented for purposes of Information Only.

λ, nm	E(λ)	TSE[E(λ)]	λ, nm	E(λ)	TSE[E(λ)]	λ, nm	E(λ)	TSE[E(λ)]
490	*0.05577*	*0.00300*	574	0.99115	0.01089	658	0.19842	0.00291
492	*0.06341*	*0.00276*	576	0.98291	0.01057	660	0.18755	0.00286
494	*0.07398*	*0.00295*	578	0.97375	0.01031	662	0.17766	0.00277
496	*0.08653*	*0.00325*	580	0.96069	0.01005	664	0.16806	0.00238
498	*0.10042*	*0.00341*	582	0.94821	0.00978	666	0.15854	0.00241
500	*0.11699*	*0.00366*	584	0.93378	0.00998	668	0.15042	0.00224
502	*0.13508*	*0.00401*	586	0.91577	0.00980	670	0.14205	0.00216
504	*0.15411*	*0.00422*	588	0.89850	0.00948	672	0.13436	0.00210
506	*0.17543*	*0.00452*	590	0.87739	0.00935	674	0.12702	0.00190
508	*0.19787*	*0.00452*	592	0.85788	0.00869	676	0.12001	0.00175
510	*0.22300*	*0.00461*	594	0.83751	0.00906	678	0.11394	0.00176
512	*0.24977*	*0.00503*	596	0.81368	0.00899	680	0.10745	0.00144
514	*0.27833*	*0.00536*	598	0.79210	0.00839	682	0.10166	0.00145
516	*0.30845*	*0.00528*	600	0.76586	0.00814	684	0.09645	0.00138
518	*0.34007*	*0.00592*	602	0.74265	0.00801	686	0.09115	0.00131
520	*0.37325*	*0.00582*	604	0.72051	0.00795	688	0.08665	0.00129
522	*0.40713*	*0.00599*	606	0.69391	0.00763	690	0.08218	0.00122
524	*0.44221*	*0.00616*	608	0.67077	0.00776	692	0.07789	0.00111
526	*0.47887*	*0.00619*	610	0.64501	0.00762	694	0.07417	0.00105
528	*0.51535*	*0.00644*	612	0.62066	0.00741	696	0.07037	0.00105
530	*0.55208*	*0.00695*	614	0.59752	0.00714	698	0.06704	0.00104
532	*0.58959*	*0.00758*	616	0.57128	0.00685	700	0.06385	0.00094
534	*0.62623*	*0.00774*	618	0.54897	0.00678	702	0.06064	0.00102
536	*0.66228*	*0.00777*	620	0.52502	0.00675	704	0.05762	0.00082
538	*0.69798*	*0.00842*	622	0.50147	0.00639	706	0.05502	0.00075
540	*0.73159*	*0.00823*	624	0.47867	0.00595	708	0.05264	0.00090
542	*0.76552*	*0.00909*	626	0.45644	0.00563	710	0.05011	0.00067
544	*0.79626*	*0.00936*	628	0.43651	0.00550	712	0.04795	0.00074
546	*0.82586*	*0.00920*	630	0.41521	0.00561	714	0.04574	0.00065
548	*0.85557*	*0.00984*	632	0.39552	0.00496	716	0.04369	0.00077
550	*0.88183*	*0.01038*	634	0.37593	0.00475	718	0.04205	0.00064
552	*0.90526*	*0.01129*	636	0.35701	0.00465	720	0.04028	0.00057
554	*0.92653*	*0.01133*	638	0.34006	0.00442	722	0.03854	0.00057
556	*0.94435*	*0.01132*	640	0.32249	0.00424	724	0.03698	0.00062
558	*0.96078*	*0.01106*	642	0.30667	0.00426	726	0.03504	0.00063
560	*0.97309*	*0.01119*	644	0.29031	0.00399	728	0.03372	0.00055
562	*0.98352*	*0.01114*	646	0.27481	0.00381	730	0.03265	0.00049
564	*0.99064*	*0.01060*	648	0.26146	0.00354	732	0.03122	0.00058
566	*0.99277*	*0.00961*	650	0.24707	0.00354	734	0.03003	0.00059
568	*1.00000*	*0.01066*	652	0.23405	0.00330	736	0.02896	0.00049
570	0.99841	0.01034	654	0.22145	0.00319	738	0.02809	0.00062
572	0.99498	0.01055	656	0.20956	0.00317	740	0.02681	0.00053

Table IV. The relative corrected emission spectrum, E(λ), of the orange phosphor, the total standard error in E(λ), TSE[E(λ)] (3 sigma), these error limits include random and systematic uncertainties added in quadrature.

λ, nm	E(λ)	TSE[E(λ)]	λ, nm	E(λ)	TSE[E(λ)]	λ, nm	E(λ)	TSE[E(λ)]
530	0.05955	0.00078	600	0.91150	0.00822	672	0.37641	0.00547
532	0.07052	0.00084	602	0.93258	0.00891	674	0.35497	0.00484
534	0.08308	0.00099	604	0.95190	0.00964	676	0.33378	0.00456
536	0.09717	0.00117	606	0.96739	0.00940	678	0.31525	0.00393
538	0.11198	0.00134	608	0.98043	0.01011	680	0.29606	0.00349
540	0.12838	0.00141	610	0.98893	0.01014	682	0.27753	0.00332
542	0.14553	0.00169	612	0.99655	0.01053	684	0.26075	0.00319
544	0.16366	0.00201	614	1.00000	0.01075	686	0.24390	0.00288
546	0.18253	0.00204	616	0.99764	0.01039	688	0.22961	0.00291
548	0.20208	0.00234	618	0.99496	0.01087	690	0.21502	0.00289
550	0.22286	0.00266	620	0.98860	0.01108	692	0.20139	0.00262
552	0.24360	0.00315	622	0.97871	0.01075	694	0.18875	0.00244
554	0.26466	0.00335	624	0.96646	0.01063	696	0.17650	0.00221
556	0.28691	0.00359	626	0.95130	0.01006	698	0.16517	0.00216
558	0.30904	0.00375	628	0.93603	0.01012	700	0.15420	0.00207
560	0.33159	0.00399	630	0.91689	0.01026	702	0.14413	0.00195
562	0.35557	0.00419	632	0.89531	0.00979	704	0.13512	0.00200
564	0.37951	0.00412	634	0.87181	0.00955	706	0.12585	0.00183
566	0.40434	0.00394	636	0.84712	0.00944	708	0.11756	0.00157
568	0.43125	0.00474	638	0.82353	0.00956	710	0.10966	0.00152
570	0.45850	0.00486	640	0.79665	0.00917	712	0.10238	0.00148
572	0.48691	0.00510	642	0.77064	0.00942	714	0.09560	0.00152
574	0.51608	0.00547	644	0.74192	0.00904	716	0.08861	0.00146
576	0.54674	0.00563	646	0.71389	0.00871	718	0.08284	0.00114
578	0.57758	0.00587	648	0.68536	0.00826	720	0.07723	0.00115
580	0.61010	0.00608	650	0.65873	0.00851	722	0.07140	0.00105
582	0.64235	0.00631	652	0.62988	0.00797	724	0.06721	0.00091
584	0.67579	0.00672	654	0.60332	0.00786	726	0.06202	0.00105
586	0.70836	0.00667	656	0.57459	0.00766	728	0.05810	0.00106
588	0.73973	0.00674	658	0.54767	0.00723	730	0.05434	0.00120
590	0.77222	0.00705	660	0.52094	0.00693	732	0.05009	0.00132
592	0.80306	0.00741	662	0.49549	0.00683	734	0.04674	0.00152
594	0.83334	0.00813	664	0.46983	0.00622	736	0.04361	0.00116
596	0.86123	0.00844	666	0.44395	0.00593	738	0.04029	0.00096
598	0.88814	0.00846	668	0.42125	0.00584	740	0.03806	0.00168
			670	0.39798	0.00561			

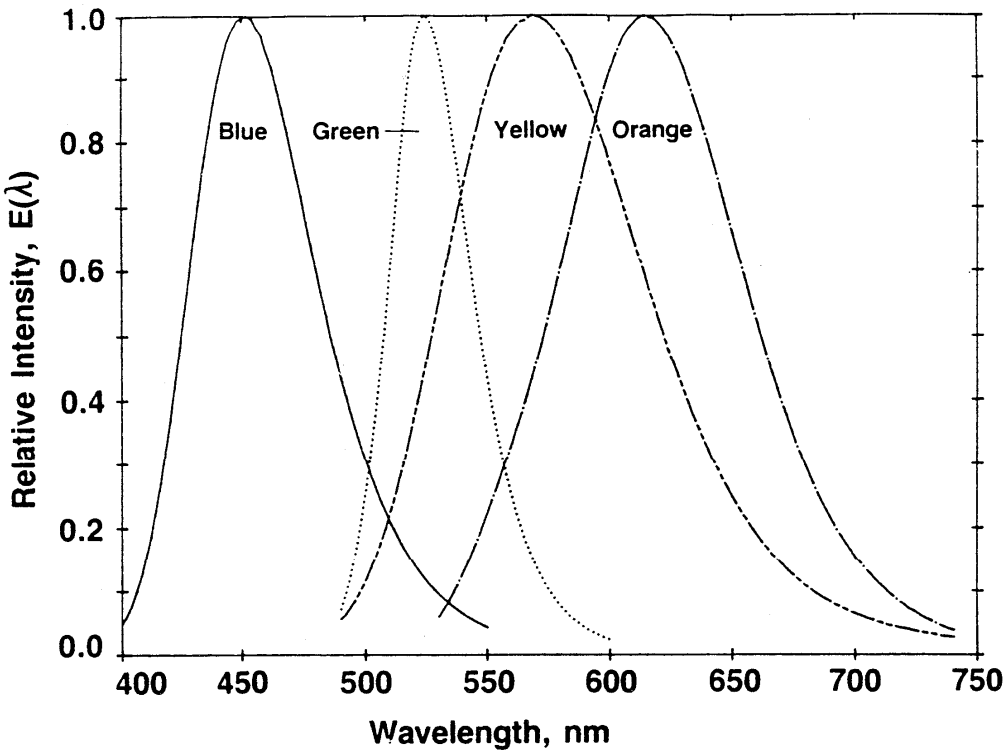

Figure 1. Relative Corrected Emission Spectra of SRM 1931