"Out of stock"

Certified Reference Material

BAM-F012

Glass-based Multi-emitter Fluorescence Standard

as day-to-day intensity and wavelength standard for instrument performance validation (IPV) of fluorescence instruments

Certified Values

Normalized intensities of eight emission peaks ("emission intensity pattern") of a rectangular block of a multi-emitter (ME) glass BAM-F012. The intensities of the emission peaks were normalized by the sum of the signal maxima.

Table 1. Certified values of normalized emission intensities ("emission intensity pattern") at the specified peak position are extracts of measurements with an excitation wavelength (λ_{ex}) of 365 nm, spectral bandpasses of 4/8/4 nm of the excitation and 2/4/2 nm of the emission double monochromator and magic angle conditions (excitation polarizer set to 0° and emission polarizer set to 54.7°) at 25 °C. The values are the unweighted means of ten determinations at the reference spectrometer of BAM Federal Institute for Materials Research and Testing. The uncertainty is the expanded uncertainty with a coverage factor of $k = 2$ corresponding to a level of confidence of approximately 95%, as defined in the GUM (ISO/IEC Guide 98-3:2008).

Peak	Normalized intensity (I)	Expanded uncertainty $U(I)$
488 nm	0.0288	0.0023
542 nm	0.128	0.011
548 nm	0.1085	0.0081
591 nm	0.0909	0.0018
612 nm	0.307	0.013
620 nm	0.2070	0.0034
653 nm	0.0185	0.0024
701 nm	0.112	0.014

The intensity pattern was derived from the emission spectrum of the ME glass with typical cuvette dimensions (12.5 mm x 12.5 mm x 40 mm), measured with a fixed excitation wavelength λ_{exc} = 365 nm of a continuous excitation source and 1 nm wavelength intervals, and corrected for the instrument-specific spectral responsivity $s(\lambda)$ of the fluorometer's emission channel traceable to the SI unit of *spectral radiance*.[1-8] The certified values summarized in Table 1 are extracted from a spectrally corrected emission spectrum obtained at the measurement conditions given in heading of Table 1 and detailed in the section Measurement conditions and instructions for use. Deviations from these conditions may lead to deviations from the certified values and to increased uncertainties.

This certificate is valid for a period of 3 years starting with the dispatch of the reference material from BAM.

Date of dispatch:

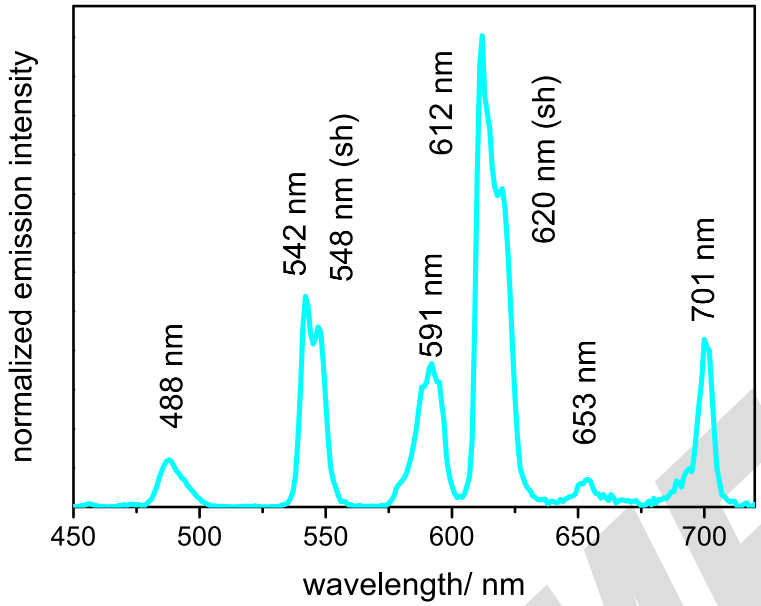

Figure 1. Normalized, spectrally corrected emission spectrum of eight bands and shoulders (sh) of BAM-F012; excitation was at 365 nm. The measurement conditions are detailed in Table 1.

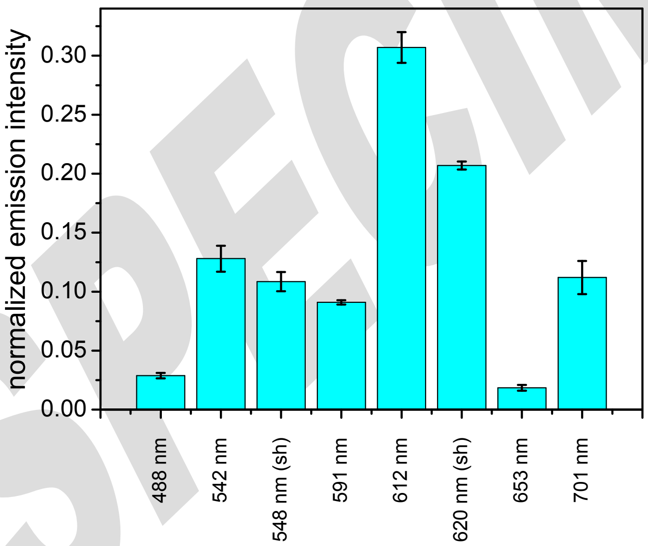

Figure 2. *Emission intensity pattern* of BAM-F012 (relative to the spectral radiance scale) with uncertainties using a coverage factor of k = 2 corresponding to a confidence interval of approximately 95 %.

Material Description

- The reference material consists of a rectangular block of a fluorescent multi-emitter (ME) glass with typical cuvette dimensions (12.5 mm x 12.5 mm x 40 mm) and four polished long faces. *) This CRM was prepared from a batch of a lanthanum phosphate glass melt doped with the luminescent rare earth (RE) metal ions Cerium(III), Terbium(III), and Europium(III).[9, 10]
- The fluorescence spectrum of the ME glass consists of several relatively sharp emission bands in the wavelength region between 450 nm and 720 nm as shown in Figure 1. Eight characteristic emission bands or shoulders (sh) at 488 nm ($^5D_4 \rightarrow {}^7F_{6;}$ Tb^{3+}), 542 nm ($^5D_4 \rightarrow {}^7F_{5;}$ Tb^{3+}), 548 nm (sh) ($^5D_4 \rightarrow {}^7F_{5;}$ Tb^{3+}), 591 nm ($^5D_4 \rightarrow {}^7F_{4;}$ Tb^{3+}/ $^5D_0 \rightarrow {}^7F_{1;}$ Eu^{3+}), 612 nm ($^5D_0 \rightarrow {}^7F_{2;}$ Eu^{3+}), 620 nm (sh) ($^5D_4 \rightarrow {}^7F_{3;}$ Tb^{3+}), 653 nm ($^5D_0 \rightarrow {}^7F_{3;}$ Eu^{3+}), and 701 nm ($^5D_0 \rightarrow {}^7F_{4;}$ Eu^{3+}) were evaluated.[11,9]
- The reference material is ready-to-use.
- The normalized emission intensities of eight characteristic bands used for certification of the emission intensity pattern were derived from an emission spectrum corrected for spectral responsivity of the emission channel of the BAM reference fluorometer, measured within the linear range of the previously calibrated emission detection system. The expanded uncertainties given in Table 1 include contributions from instrument-related uncertainties (i.e., uncertainties of the calibration of the wavelength accuracy and spectral responsivity of the BAM fluorometer,[7] measurement of fluorescence spectra of the ME glass) and material-related uncertainties (i.e., emission anisotropies, inhomogeneities of the emission spectra of different cuvette-shaped ME glasses obtained from the same glass melt, and from thermal stability studies of selected ME glasses) as determined by the propagation of uncertainties according to the Guide to the Expression of Uncertainty (GUM) and ISO Guide 35. Certification was performed according to ISO Guide 35 and according to the BAM Guidelines for the Production of BAM Reference Materials.[12]
- Standard Operation Procedure (SOP) for use of fluorescence standard BAM-F012 is included.

Recommended Use

- Reference material BAM-F012 is intended as standard for Instrument Performance Validation (IPV) of fluorescence measurement instruments with a continuous excitation source by regular evaluation of variations of the emission intensity pattern obtained, always using identical measurement conditions (i.e., identical excitation wavelength, spectral excitation and emission bandpass, polarizer settings, photomultiplier voltage, scan speed, integration time).
- The reference material may also be employed as wavelength standard for the validation of the wavelength accuracy of the emission channel, especially in the case of fluorescence instruments with low requirements on spectral resolution.
- BAM-F012 is also suitable to monitor temporal changes of the wavelength-dependent spectral responsivity of fluorescence instruments by comparison of the emission intensity pattern of the uncorrected spectra on a frequent, e.g., day-to-day or weekly basis using always identical instrument settings and providing a strict control of the excitation wavelength accuracy. A time dependence of the instrument-specific spectral responsivity can arise from aging of optical and opto-electronic instrument components in the emission channel. [1-8]
- BAM-F012 can be also employed to compare fluorescence instruments and for the determination of instrument-to-instrument variations, yet this requires use of identical measurement conditions and instrument settings and should only be done for instruments with continuous light sources. If not strictly identical, but very similar

---

*) Minor glass inhomogeneities (striae) due to differences in refractive index in the range of about ~10^{-6} obviously do not influence the standard-relevant optical properties.

measurement conditions are used, the assessment of the instrument-to-instrument intensity variations is only valid for the chosen set of parameters.

The parameters and requirements for use of BAM-F012 are described in the following clause.

Handling and instruction for use

Please follow the instructions provided in the SOP delivered with the material.

Prerequisites for the determination of emission intensity pattern using this reference material are the control of the wavelength accuracy of the excitation and emission channel(s) of the fluorescence instrument and operation of the detection system within its linear range. Spectral correction (i.e., consideration of the wavelength dependence of $s(\lambda)$ of the fluorescence instrument)[13] is mandatory to obtain an emission intensity pattern matching the certified values. Correction factors are often supplied with the spectrofluorometer software (e.g., as “correction file” or “correction curve”) by the instrument manufacturer. In this respect, the reference quantity (i.e., energy per unit time or photons per unit time) used for instrument calibration needs to be considered.[7] Only correction factors obtained relative to the spectral radiance scale can be expected to yield values very close to the BAM-certified emission intensity pattern. In the case of most of photon counting spectrofluorometers, the data and correction curves are usually provided in units of photons; here, the emission correction curve is commonly referenced to the spectral photon radiance scale. For comparison of the accordingly obtained emission intensity pattern with the BAM-certified data, each photon-based intensity value (photons per unit time) of the corrected emission spectrum has to be divided by the corresponding wavelength, followed by the normalization procedure described in the SOP attached. These calculations can be done in a spreadsheet program.
Mandatory in any case is the use of always identical measurement conditions for BAM-F012.
BAM-F012 should be placed in a standard cuvette holder. The ME glass BAM-F012 is optimized for a measurement geometry of 0°/90°, a spectral excitation bandpass of 4/8/4 nm, an emission bandpass of 2/4/2 nm, use of polarizers (e.g., magic angle conditions: excitation polarizer set to 0° and emission polarizer set to 54.7°), and ambient temperature of (25 ± 2) °C. Under these conditions, certification based on spectrally corrected emission spectra and calculations of the expanded uncertainties were performed.
The certified values of the emission intensity pattern are only valid for these conditions and the limits listed in Table 2.

Table 2. Recommended measurement conditions and limits.

Measurement condition	Region / limit
Excitation wavelength*	λ_{exc} = 365.0 nm ± 0.5 nm (continuous source)
Temperature T	(25 ± 2) °C
Spectral bandpass of the excitation monochromator of the instrument to be validated	4 nm (Certified values provided for spectral bandpasses 4/8/4 of the excitation double monochromator of the BAM fluorometer)**
Spectral bandpass of the emission monochromator of the instrument to be validated	2 nm (Certified values provided for spectral bandpasses 2/4/2 of the emission double monochromator of the BAM fluorometer)**
Polarizer settings (magic angle conditions)	Excitation polarizer set to 0° Emission polarizer set to 54.7°
Step width of the emission scan	(0.5; **1.0****) nm

* As BAM-F012 contains several RE ions, its emission spectrum and thus, its emission intensity pattern is dependent on excitation wavelength. Hence, care needs to be taken concerning the choice of the excitation wavelength and control of the wavelength accuracy of the excitation channel. If excitation wavelengths are used, which deviate from the recommended measurement conditions, the resulting emission profile of BAM-F012 will deviate from the certified emission pattern. Nevertheless, if always identical measurement conditions are used, BAM-F012 can be still utilized for instrument performance validation.

** parameters used for certification.

Data evaluation

Changes in the emission intensity pattern of BAM-F012 can principally arise from changes in the wavelength accuracy of the excitation and emission channels, and from changes in the spectral responsivity of the detection channel of the fluorescence instrument. Changes in the wavelength accuracy of the excitation channel and the spectral responsivity of the emission channel can result in changes of the intensity ratios (emission pattern), whereas changes in the wavelength accuracy of the emission channel result in changes of the spectral position of the emission bands. Changes in the spectral radiant flux reaching the sample affect only the intensities of all bands without changing the emission pattern.

Transport and Storage

The stability of the ME glass and its certified emission intensity pattern allows dispatch of the material at ambient temperature. After receipt, BAM-F012 should be stored at room temperature, wrapped in lens paper, and kept in a clean case in the dark. Exposure to direct sunlight should be avoided. The cuvette-shaped standard will remain stable for 3 years after dispatch provided that it is not exposed to extreme heat, humidity, chemicals, and mechanical stress.

Metrological Traceability

The certified relative emission intensity pattern of the glass-based fluorescence standard BAM-F012 is based on spectrally corrected emission spectra, measured with the calibrated BAM reference spectrofluorometer SLM 8100, which ensures metrological traceability of the emission intensity pattern to the spectral radiance scale.[1, 4] The performance of the instrument used for certification was assessed in an interlaboratory comparison of all National Metrological Institutes active in the field of fluorescence measurements (National Institute of Standards and Technology NIST, USA; National Research Council NRC, Canada; Physikalisch-Technische Bundesanstalt PTB, Germany; BAM Federal Institute for Materials Research and Testing, Germany).[14]

Legal Notice

Neither the BAM Federal Institute for Materials Research and Testing nor any person acting on their behalf make any warranty or representation, expressed or implied, that the use of any information, material, apparatus, method or process disclosed in this document may not infringe privately owned rights, or assume any liability with respect to the use of, or damages resulting from the use of any information, material, apparatus, method or process disclosed in this document.

Literature

1. Resch-Genger, U.; Pfeifer, D.; Monte, C.; Pilz, W.; Hoffmann, A.; Spieles, M.; Rurack, K.; Hollandt, J.; Taubert, D.; Schonenberger, B.; Nording, P., Traceability in fluorometry: Part II. Spectral fluorescence standards. *Journal of Fluorescence* **2005,** *15* (3), 315-336.
2. Pfeifer, D.; Hoffmann, K.; Hoffmann, A.; Monte, C.; Resch-Genger, U., The calibration kit spectral fluorescence standards - A simple and certified tool for the standardization of the spectral characteristics of fluorescence instruments. *Journal of Fluorescence* **2006,** *16* (4), 581-587.
3. Resch-Genger, U.; Hoffmann, K.; Nietfeldt, W.; Engel, A.; Neukammer, J.; Nitschke, R.; Ebert, B.; McDonald, R., How to Improve Quality Assurance in Fluorometry: Fluorescence Inherent Sources of Error and Suited Fluorescence Standards. *Journal of Fluorescence* **2005,** *15* (3), 337-362.
4. Hollandt, J.; Taubert, R. D.; Seidel, J.; Resch-Genger, U.; Gugg-Helminger, A.; Pfeifer, D.; Monte, C.; Pilz, W., Traceability in fluorometry - Part I: Physical standards. *Journal of Fluorescence* **2005,** *15* (3), 301-313.
5. Monte, C.; Resch-Genger, U.; Pfeifer, D.; Taubert, D. R.; Hollandt, J., Linking fluorescence measurements to radiometric units. *Metrologia* **2006,** *43* (2), S89-S93.
6. Hoffmann, K.; Monte, C.; Pfeifer, D.; Resch-Genger, U., Standards in Fluorescence Spectroscopy: Simple Tool for the Characterization of Fluorescence Instuments. *GIT Laboratory Journal* **2005,** *6*, 29-30.
7. Resch-Genger, U.; DeRose, P. C., Characterization of photoluminescence measuring systems (IUPAC Technical Report). *Pure and Applied Chemistry* **2012,** *84* (8), 1815-1835.
8. DeRose, P. C.; Resch-Genger, U., Recommendations for Fluorescence Instrument Qualification: The New ASTM Standard Guide. *Analytical Chemistry* **2010,** *82* (5), 2129-2133.
9. Hoffmann, K.; Resch-Genger, U.; Engel, A., Glass-based Reference Standards for Fluorescence Applications . *Luminescence* **2008,** *23*, 191-192.
10. Engel, A.; Ottermann, C.; Resch-Genger, U.; Hoffmann, K.; Schweizer, S.; Selling, J.; Spaeth, J. M.; Rupertus, V. In *Glass based fluorescence reference materials used for optical and biophotonic applications - art. no. 619110*, Conference on Biophotonics and New Therapy Frontiers, Strasbourg, FRANCE, Apr 03-05; Grzymala, R.; Haeberle, O., Eds. Strasbourg, FRANCE, 2006; pp 19110-19110.
11. Resch-Genger, U.; Hoffmann, K.; Hoffmann, A., Standardization of Fluorescence Measurements - Criteria for the Choice of Suitable Standards and Approaches to Fit-for-Purpose Calibration Tools. *Annals of the New York Academy of Sciences* **2008,** *1130*, 35-43.
12. BAM, Guidelines for the production of BAM Reference Materials, 2006. BAM, Ed. 2006.
13. Resch-Genger, U.; Pfeifer, D.; Hoffmann, K.; Flachenecker, G.; Hoffmann, A.; Monte, C., Linking fluorometry to radiometry with physical and chemical transfer standards: instrument characterization and traceable fluorescence measurements. In *Standardization and Quality*

Assurance in Fluorescence Measurements I: Techniques, Resch-Genger, U., Ed. Springer Verlag: Berlin-Heidelberg 2008; Vol. 5, pp 65-100.
14. Resch-Genger, U.; Bremser, W.; Pfeifer, D.; Spieles, M.; Hoffmann, A.; DeRose, P. C.; Zwinkels, J. C.; Gauthier, F.; Ebert, B.; Taubert, R. D.; Monte, C.; Voigt, J.; Hollandt, J.; Macdonald, R., State-of-the Art Comparability of Corrected Emission Spectra.1. Spectral Correction with Physical Transfer Standards and Spectral Fluorescence Standards by Expert Laboratories. *Analytical Chemistry* **2012,** *84* (9), 3889-3898.

Accepted as BAM-CRM on August 12, 2014

BAM Federal Institute for Materials Research and Testing

Prof. Dr. U. Panne
Head of BAM Department 1
“Analytical Chemistry;
Reference Materials”

Dr. U. Resch-Genger
Head of BAM Division 1.10
“Biophotonics”

This Reference Material is offered by: BAM

Catalog Key word: Glass-based Multi-emitter Fluorescence Standard

BAM Federal Institute for Materials Research and Testing
Richard-Willstätter-Str. 11, D-12489 Berlin, Germany

Phone: +49 30 8104 2061
Fax: +49 30 8104 1117

E-Mail: sales.crm@bam.de
Internet: www.webshop.bam.de