Supporting Information

Narrow-band emitting solid fluorescence reference standard with certified intensity pattern

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Normalization procedure.

To compare the results of emission measurements of the glass samples, a standardized procedure was selected out of 10 possible normalization alternatives. The relative standard deviation of the individual intensity maxima is most crucial for the evaluation of the fluorescence intensity pattern. From six independent data series of fluorescence spectra, the relative standard deviations were determined. These results are shown in the column graph in Figure S-1.
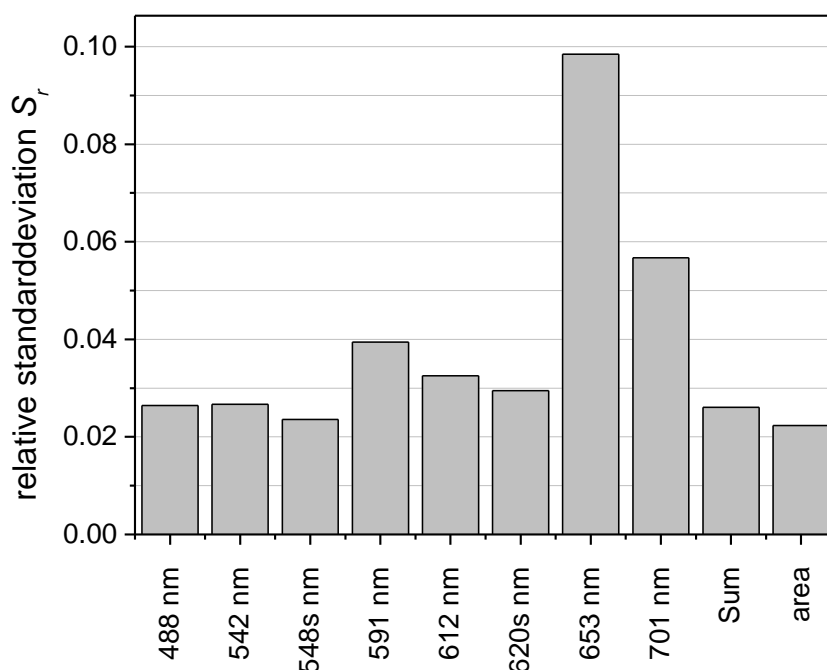

Figure S-1. Relative standard deviations S_r of normalized spectra based on 10 different normalization procedures. The fluorescence intensity of 8 individual emission peaks or shoulders, respectively, the integrated emission (area under the emission profile), and the sum of the intensities of eight selected peak maxima were evaluated.

Striking is the large uncertainty of the peak at 653 nm. Its very weak intensity is probably strongly influenced by spectral noise. Concerning the normalization of the emission spectra, the three favorite options are a normalization procedure using the peak intensity at 548(sh) nm, the sum of the peak intensities, and the area under the emission curve. A peak shoulder, e.g. the peak at 548 nm is not really suitable for a reliable normalization because its intensity strongly depends on the spectral resolution of the fluorescence measuring device. The normalization factor with the lowest uncertainty is shown to be the peak area. This evaluation, however, is prone to

uncertainties in regard to baseline correction and peak integration and renders an easily reproducible normalization difficult. The normalization procedure based on the sum of the individual peak intensities at previously defined wavelengths turned out to be highly reproducible and reveals a sufficiently small uncertainty.

Homogeneity study. The band positions as well as the band intensity of the eight band maxima were examined. Statistical analysis of the determination of the band positions from the set of eight intensity maxima was performed using one-factorial analysis of variance (ANOVA). Here, a possible spread of the results of analysis of the four faces of the samples was compared with those between the individual glass cuvettes. No significant inhomogeneity of the peak position of the samples were detected, all eight peak positions are highly homogeneous over the batch. In addition to the peak positions also the normalized peak emission intensities of the eight band maxima were tested for their homogeneity. The largest relative standard deviations s_r was observed for the band at 653 nm with 4.1% and at 701 nm with 2.8% (see Fig. S-2) possibly due to an unfavorable signal-to-noise ratio in the long wavelength region.
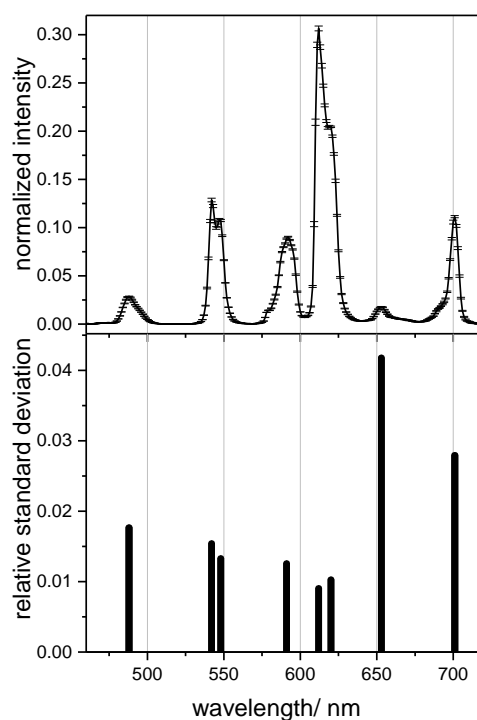

Figure S-2. Normalized fluorescence emission (top), and relative standard deviation S_r of the peak intensities of the selected fluorescence bands of the ME-glass materials (bottom) resulting from 48 measurements on 12 selected samples.

Statistical evaluation of the normalized peak intensities using one-factorial analysis of variance (ANOVA) only revealed a significant between-unit difference for the double peak 542/548 nm but are still homogeneous at a 1% significance level.

Thus, the material can be considered sufficiently homogeneous according to the requirements of ISO Guide 35. Furthermore, all homogeneity contributions to the measurement uncertainty are calculated duly and are taken into account in the uncertainty budget of the certified intensity values.
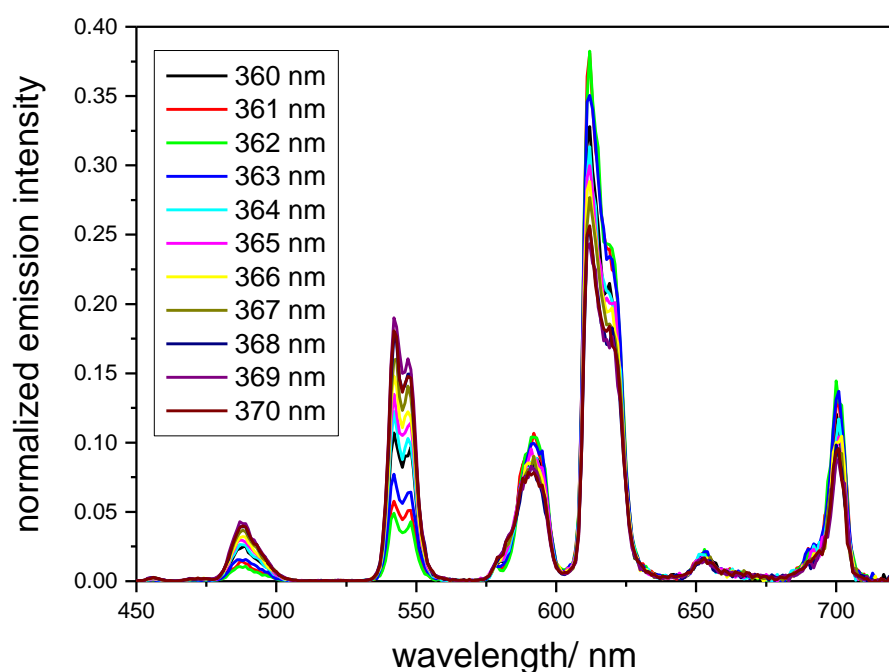
Excitation wavelength dependence.

Figure S-3. Emission spectra of a multi-emitter (ME)-glass recorded upon excitation with varied wavelength in the range of 360-370 nm with 1 nm increments. All other parameters correspond to the standard measurement conditions.
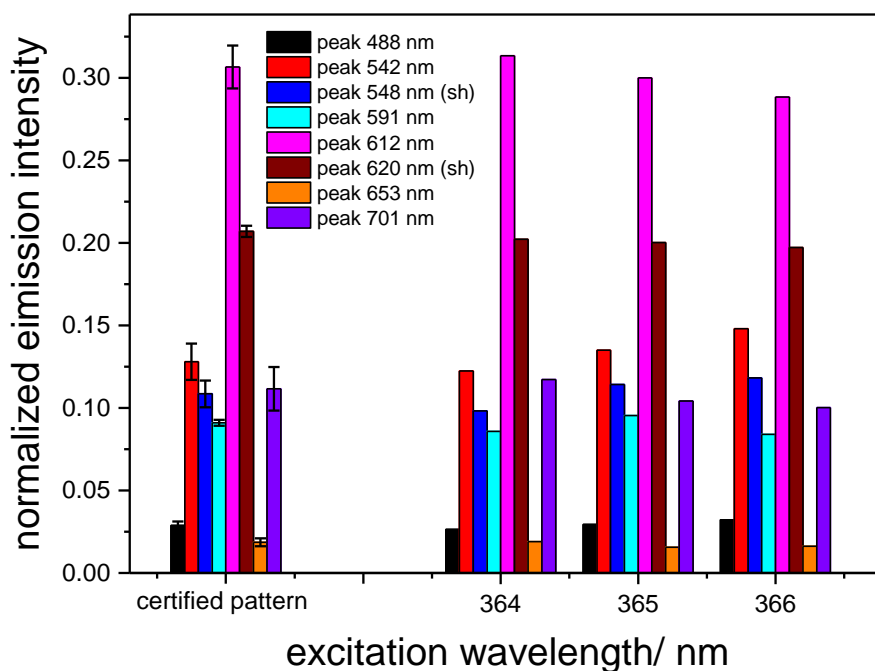
Figure S-4. Observed changes in the emission pattern of BAM-F012 resulting from slight deviations (± 1 nm) of recommended excitation wavelength 365 nm.

Uncertainty contributions due to variations in excitation wavelengths u_{exc} are summarized in Table S-1. The peaks at 612 nm, 542 nm, and 548 nm show the highest uncertainty contributions, whereas the relatively broad peaks at 591 nm and 653 nm contribute to a lesser extent. These values were calculated from the first derivative at the recommended excitation wavelength of 365 nm and the previously determined uncertainty estimates for excitation wavelength for influence of both of instrument wavelength setting (nm), and spectral width (nm). [1]

Table S-1 Estimation of uncertainty of the certified normalized emissions due to variation in excitation wavelength.

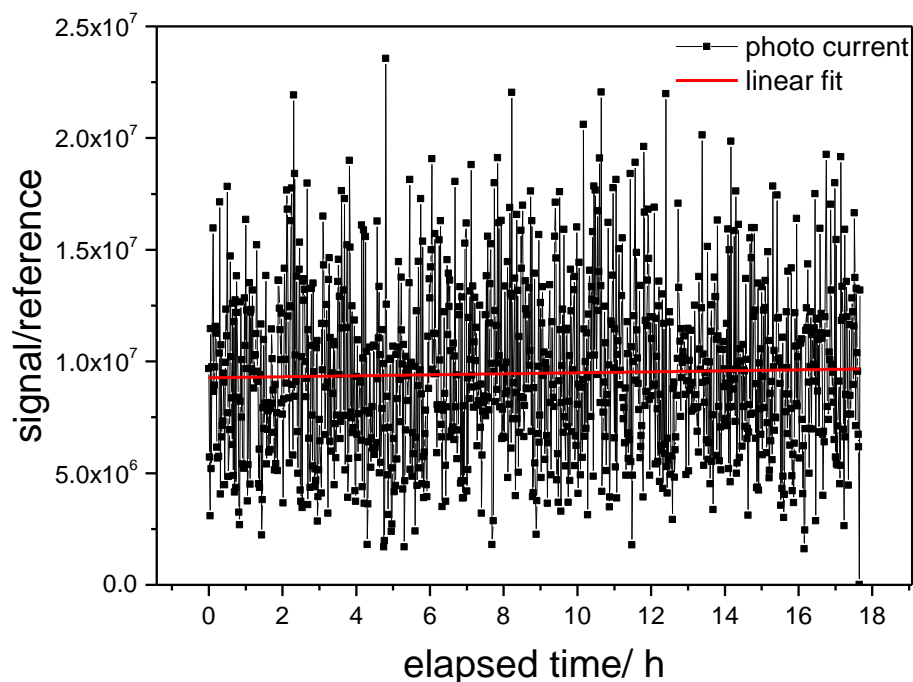|	488 nm	542 nm	548 nm	591 nm	612 nm	620 nm	653 nm	701 nm
slope	0.0029	0.01289	0.00100	-0.0009	0.0125	0.0025	0.0014	0.0085
$u_{contrib}$	0.0011	0.0050	0.0039	0.0003	0.0049	0.0010	0.0005	0.0033

Photostability

Figure S-5. Photostability study (excitation wavelength 365 nm). The photo-current recorded is proportional to the optical transmission of the glass sample.

Fluorescence anisotropy.

Table S-2 Normalized emission intensities of eight selected bands recorded with two different polarizer settings. Excitation polarizer was always set to 0°, and emission polarizer set to either 0° (Pol=0°;0°) or 90° (Pol=0°;90°), respectively and the emission anisotropy r calculated from Eq. 1.

Peak/ nm	relative emission intensity		Anisotropy r
	Pol=0°;0°	Pol=0°;90°	
488	0.0339±0.00183	0.03289±0.00128	0.01011
542	0.14915±0.00563	0.14964±0.00457	-0.0011
548	0.12564±0.00531	0.12231±0.00269	0.00898
591	0.09444±0.00635	0.09638±0.00306	-0.00678
612	0.33586±0.02089	0.32861±0.01112	0.00729
620	0.22856±0.01174	0.22258±0.00789	0.00888
653	0.02215±0.00703	0.01896±0.00116	0.05307
701	0.12637±0.02798	0.12113±0.00409	0.01421

The relative uncertainty contribution u_{aniso} resulting for measurements of the nearly isotropic emitter under different polarization conditions account to between 0.1% and 6% depending on the peak under study. The peaks at 488 nm, 548 nm, and 591 nm are insensitive to polarization effects.
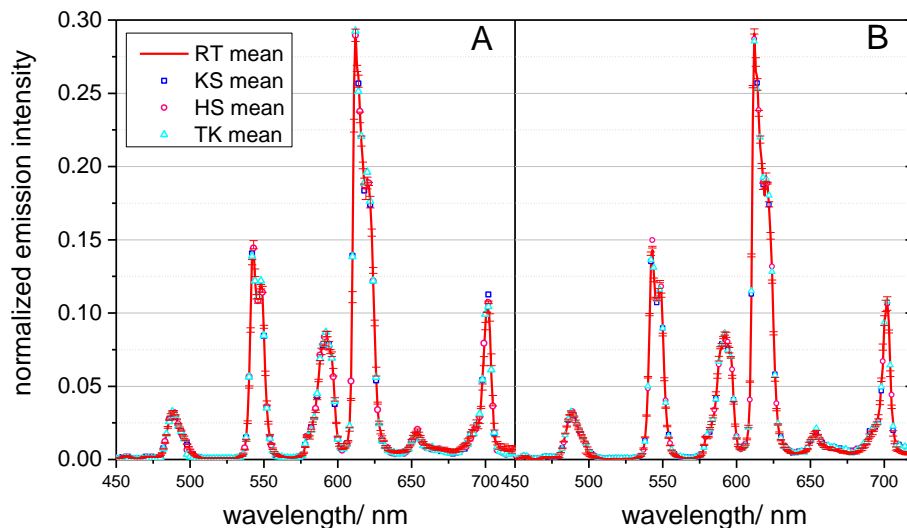
Long-term stability

Figure S-6. Normalized emission profiles of 12 samples before the start of the long-term study (A) and after 21 weeks (B) at various storage temperatures, with associated standard deviations.

To assess the thermal long-term stability, relative standard deviations s_r of the band intensities were examined. The 12 samples kept at various storing conditions were measured at different predefined maturity dates of the campaign (Figure S-7). Largest deviation was found for the band centered at 653 nm, most likely because of its low signal-to-noise-ratio. Therefore, this band was not used in the assessment of a trend. From the calculated relative standard deviations no clear trend could be determined.
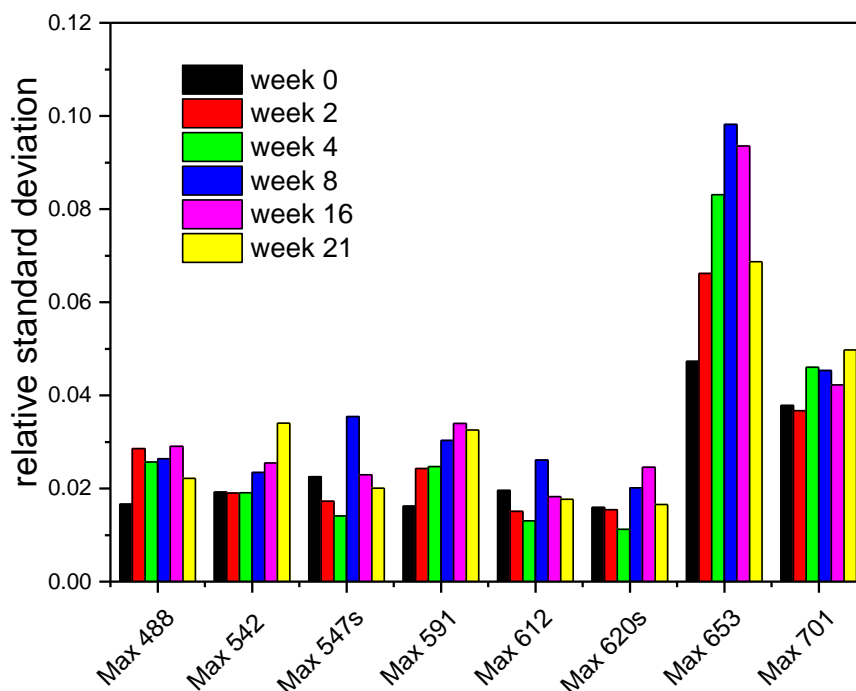
Figure S-7. Comparison of relative standard deviations of the band intensities of 12 samples stored at various storing conditions and measured at different dates of the campaign (0 weeks (black), 2 weeks (red), 4 weeks (green), 8 weeks (blue), 16 weeks (pink), and 21 weeks (yellow).

The long-term stability of the glass material also follows from Fig. S-8, were no significant change in I_{SC}/I_{RT} over time for the storage conditions (TK, KS, and HS) has been observed. Here, the results of measurements with samples kept at room temperature were defined as reference value, as the glass material was expected to be extremely stable at this storage condition (RT).
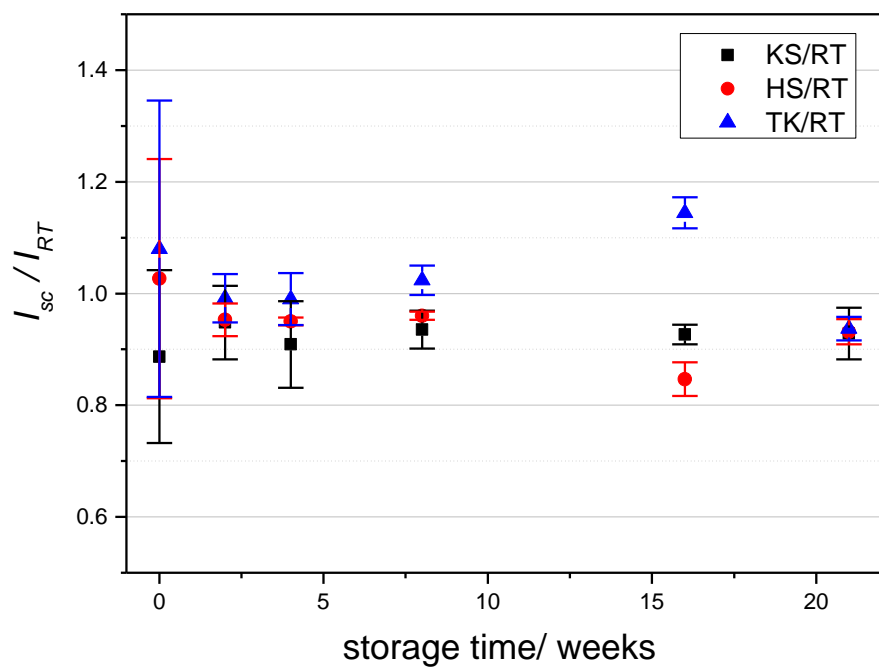
Figure S-8. Data obtained from samples stored at different storage conditions I_{SC} compared to the data measured on samples stored at RT, defined as reference values I_{RT}.

For the production of the fluorescent glass material different rare earth dopants were used. To uncover possibly different thermal effects on the band intensities of the rare earths ions three selected peaks were studied in detail. These emission bands were chosen to originate either exclusively from the dopant Tb^{3+} (488 nm) or Eu^{3+} (701 nm), or include contributions of both of the emitters (612 nm).
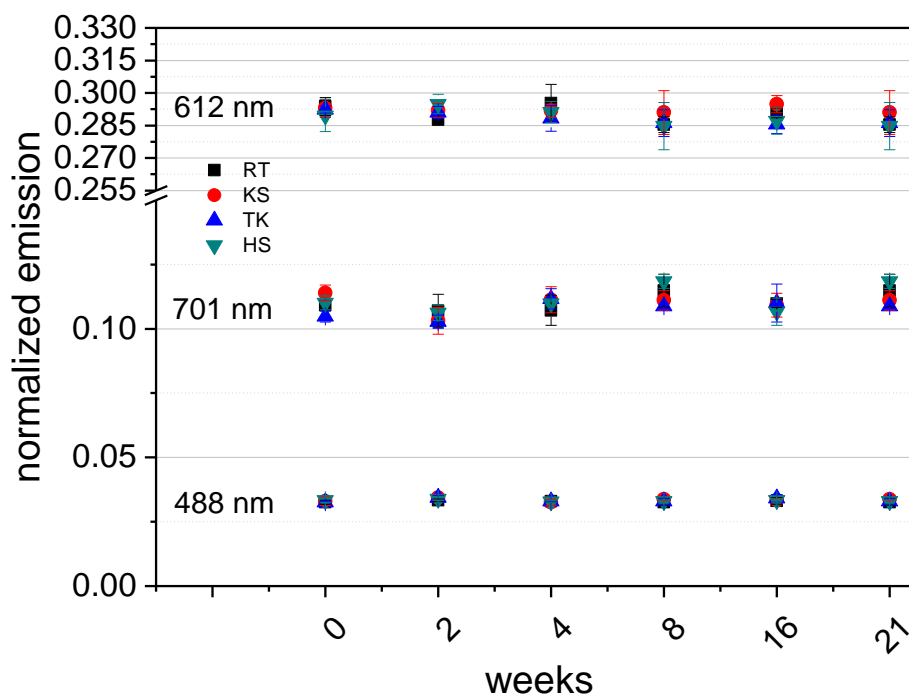

Figure S-9. Normalized fluorescence intensities of the ME-Slides for three selected bands at 488 nm related to Tb^{3+}, the mixed band between 612 nm and 625 nm and the peak at 701 nm, related to Eu^{3+} at different storage conditions TK: Freezer -19 ° C, KS: refrigerator 8 ° C, RT : Room temperature 25 ° C, HS: 70 ° C heating chamber and different storage times.

Generally, the three band intensities at 488 nm, 612 nm, and 701 nm reveal no obvious trend, in which the peak at 488 nm showed the smallest deviations in the intensities, possibly attributed to the comparatively broad band shape.

Long-term stability of the glass standard has been assessed by repeated measurements of 12 selected samples stored at the recommended storage temperature (room temperature) initially, and after 17 and 32 months of storage. Replicate measurements have been consolidated to sample means.
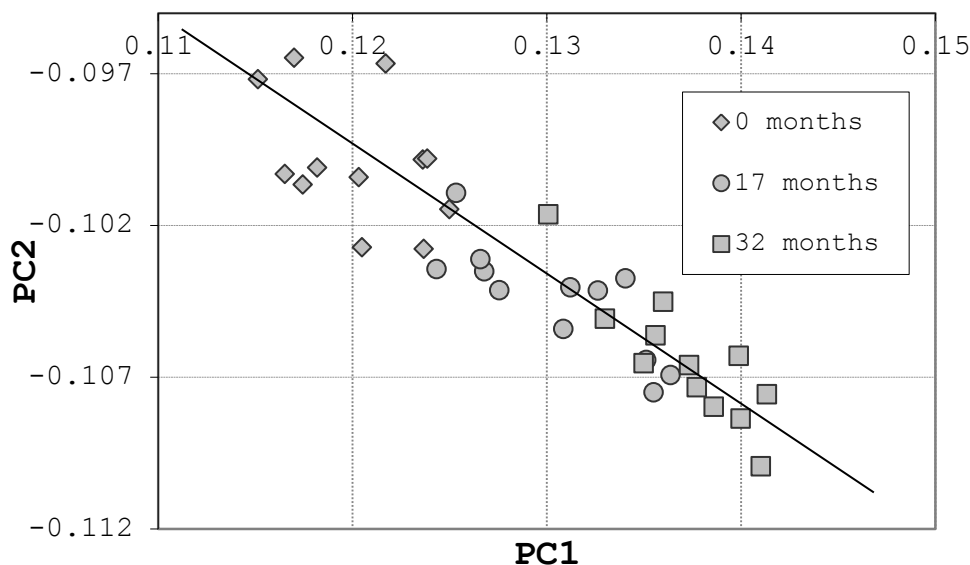
Figure S-10. PCA of the stability. Plotted are the two first principal components. A clear time dependence of the spectral pattern is obvious.

The data set at the initial point of the stability was subjected to a principal component analysis, and loadings have been determined for the 8 input variables (peaks in the spectrum). These loadings have been used to determine the scores (two principal components) for the instances in time 0, 17, and 32 months. Figure S-10 displays the results. From the graph, a change of the spectral pattern over time is obvious. The first two principal components explain roughly 60 % of the overall variance. In order to obtain estimates for the shelf life of the samples, the data sets were investigated peak-by-peak using either one-factorial ANOVA with time as a factor or regression analysis for the determination of the degradation coefficients. Relative changes of peak intensities over a period of 72 months are displayed in Figure S-11.
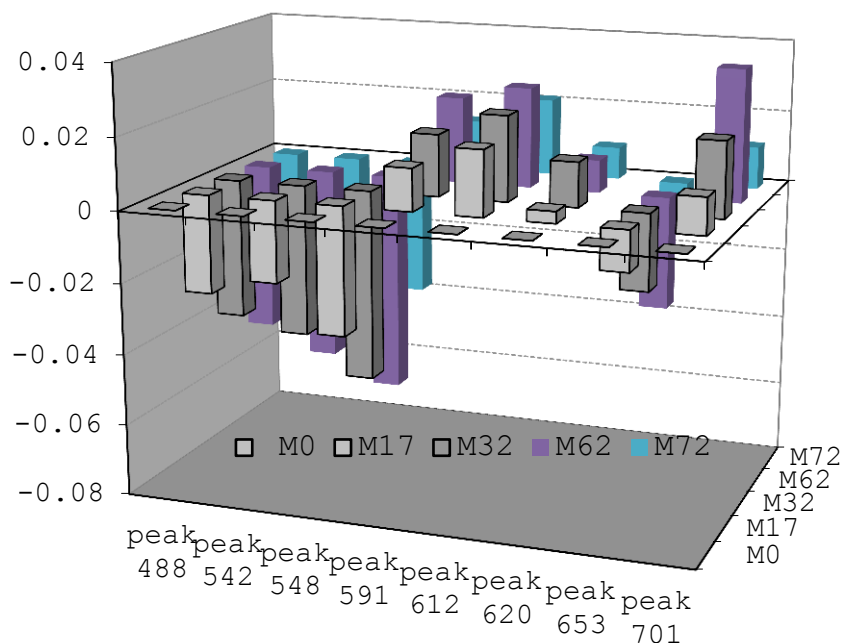
Figure S-11. Relative changes in the spectral emission pattern over time (0 – 72 months).

All ANOVA analyses revealed a significant change over time for all peaks, supported by a MANOVA over all peaks at once. The slopes of the regression lines over the changes in time were used to estimate shelf lives for the different peaks. Shelf lives are calculated in a way that they constitute the maximum time elapsed at which the degradation of the measurand will cause the value fall beyond (below or above) the upper or lower confidence limit of the certified value.

Table S-3 Degradation rates and shelf life times for the individual peaks

Peak/ nm	Time dependency	Shelf life/ month
488	−0.000576	137
542	−0.000672	123
548	−0.0006955	107
591	0.000224	88
612	0.000343	122
620	8.334E−05	199
653	0.000228	568
701	0.000358	336

From long-term studies of the material over almost 72 month, a shelf life time of the fluorescence standard of 7 years has been estimated. An additional allowance u_{lts} in the uncertainty budget had to be made only for peak position 591 nm, which degrades slightly faster than other peaks.

References

(1) 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. *Anal. Chem.* **2012**, *84*, 3889-3898.