Supporting Information

Determination of the fluorescence quantum yield of quantum dots: suitable procedures and achievable uncertainties

Markus Grabolle [1], Monika Spieles [1], Vladimir Lesnyak[2], Nikolai Gaponik[2], Alexander Eychmüller[2], Ute Resch-Genger*[1]*

[1)] Federal Institute for Materials Research and Testing (BAM), Richard-Willstaetter-Str. 11, 12489 Berlin, Germany, Email: ute.resch@bam.de

[2)] Physical Chemistry, TU Dresden, Bergstr. 66b, 01062 Dresden, Germany, Email: alexander.eychmueller@chemie.tu-dresden.de

1. Terminology

Measured fluorescence spectra, $I_m(\lambda_{ex},\lambda_{em})$, contain sample- and instrument-specific contributions. Removal of background signals such as scattering and fluorescence from the solvent and dark counts at the detector is obtained by subtraction of a *background spectrum*, $I_b(\lambda_{ex},\lambda_{em})$, that was recorded under identical measurement conditions for a cuvette containing only the solvent used for the sample. This procedure yields spectrally *uncorrected spectra*, $I_u(\lambda_{ex},\lambda_{em}) = I_m(\lambda_{ex},\lambda_{em}) - I_b(\lambda_{ex},\lambda_{em})$. Spectra that are additionally corrected for the spectral characteristics of the respective fluorescence instrument are termed *corrected spectra*,

$I_c(\lambda_{ex},\lambda_{em})$. *Corrected emission* and *excitation spectra* are obtained from $I_u(\lambda_{ex},\lambda_{em})$ by application of experimentally determined *emission* or *excitation correction curves*. These curves represent the (wavelength- and polarization-dependent) relative spectral responsivity $s(\lambda_{em})$ of the emission channel and the (wavelength- and polarization-dependent) relative spectral irradiance of the excitation channel at the sample position, respectively. $I_c(\lambda_{ex},\lambda_{em})$ are not corrected for sample-related effects such as so-called inner filter effects (attenuation of the exciting light beam, reabsorption of fluorescence light), fluorescence quenching by oxygen, and effects caused by different refractive indexes of the solvents used.[1,2,3] For the symbols used here, the subscripts *ex* and *em* denote *excitation* and *emission*, λ indicates that the quantity refers to a spectral bandwith of 1 nm and *p* that the quantity gives a number of photons (photonic units) instead of an energy value (energy units). The term intensity (*I*), that represents the recorded fluorescence signal, is used as description of radiant flux or radiant power.

For each luminescence technique, the measured fluorescence signal or photocurrent per unit bandwidths (excitation: $\Delta\lambda_{ex}$ and emission: $\Delta\lambda_{em}$) $I_m(\lambda_{ex},\lambda_{em})$ is determined by both instrument- and analyte-specific quantities, see equation 1.[4,5,6,7] Instrument-specific quantities include the spectral irradiance E_λ at the wavelength λ_{ex} reaching the sample, i.e., $E_\lambda(\lambda_{ex})$, and the spectral responsivity $s(\lambda_{em})$ of the emission or detection channel per unit spectral bandwidth of emission. E_λ is controlled by the spectral radiance L_λ of the excitation light source and the transmittance T of optical components like lenses, mirrors, filters, monochromator gratings, beam splitters, and polarizers in the excitation channel. a_x is the cross-sectional area of the sample in the detection region irradiated by the excitation beam. $s(\lambda_{em})$ is determined by the transmittance or reflectance of the optical components in the

emission channel and the spectral responsivity of the detector. Accordingly, both quantities (i.e. $s(\lambda_{em})$ and $E_\lambda(\lambda_{ex})$) strongly depend on wavelength.

$$I_m(\lambda_{ex},\lambda_{em})\,\Delta\lambda_{ex}\Delta\lambda_{em} = f(\lambda_{ex}) \times F_\lambda(\lambda_{ex},\lambda_{em}) \times E_\lambda(\lambda_{ex}) \times s(\lambda_{em}) \times K \times a_x \Delta\lambda_{ex}\Delta\lambda_{em}$$

(eq. 1S)

Analyte-specific quantities, that control measured fluorescence signals from the material side, are the analyte's absorption factor $f(\lambda_{ex})$, formerly known as absorptance, at the excitation wavelength (λ_{ex}), and its spectral fluorescence yield $F_\lambda(\lambda_{ex},\lambda_{em})$.[6,7] $F_\lambda(\lambda_{ex},\lambda_{em})$ reveals the spectral shape of the fluorescence spectrum of the analyte. f is nonlinearly linked to absorbance and thus, to the concentration by the Beer-Lambert law, see equation 2S (A equals the absorbance (at the excitation wavelength λ_{ex}), ε the molar (decadic) absorption coefficient (at the excitation wavelength λ_{ex}), c the analyte concentration, l the optical pathlength) and T the transmittance (at the excitation wavelength λ_{ex}). F_λ is linked to the integral quantum yield of photoluminescence Φ_f, see equation 3S, that represents the number of emitted photons N_{em} per number of absorbed photons N_{abs}, see equation 4S,[6,7] and *Technical Note on the Determination of the Photoluminescence Quantum Yield*.[8,9] K is a factor which takes into account the geometry of the instrument, including the overlap between the excitation and emission volumes within the sample.[4,5,6]

$$f(\lambda_{ex}) = 1 - 10^{-A(\lambda_{ex})} = 1 - 10^{-\varepsilon(\lambda_{ex})\,c\,l} = 1 - T(\lambda_{ex}) \qquad \text{eq. 2S}$$

$$\Phi_f = \int_0^\infty F_\lambda(\lambda_{exc},\lambda_{em})\,\mathrm{d}\lambda_{em} \qquad \text{eq. 3S}$$

$$\Phi_f = N_{em}(\lambda_{ex}) / N_{abs}(\lambda_{ex}) \qquad \text{eq. 4S}$$

Table 1S Symbols used

Symbol	Name	Unit
$I_m(\lambda_{ex},\lambda_{em})$	fluorescence signal	A nm^{-2}
$E_\lambda(\lambda_{ex})$	spectral irradiance	W m^{-2} nm^{-1}
$E_{p,\lambda}(\lambda_{ex})$	spectral photon irradiance	m^{-2} nm^{-1} s^{-1}
$s(\lambda_{em})$	spectral responsivity	A W^{-1} nm^{-1}
$\Delta\lambda$	spectral bandwidth	nm
$L_\lambda(\lambda_{ex})$	spectral radiance	W m^{-2} sr^{-1} nm^{-1}
$L_{p,\lambda}(\lambda_{ex})$	spectral photon radiance	m^{-2} sr^{-1} nm^{-1} s^{-1}
$F_\lambda(\lambda_{ex},\lambda_{em})$	spectral fluorescence yield	nm^{-1}
Φ_f	fluorescence quantum yield	no units
K	optical geometry factor	no units
ε	molar absorption coefficient	L cm^{-1} mol^{-1}
l	optical pathlength	cm
a_x	irradiated area of sample	m^2
c	concentration	mol L^{-1}
c_0	velocity of light in vacuo	m s^{-1}
T	transmittance	no units
A	absorbance	no units

2. Characterization of Spectrofluorometers

Range of Linearity of the Emission Detection System. Typical methods for the determination of the linear range of a detection system include: i.) the variation of the spectral radiance of a lamp by means of attenuators, such as optical filters with known and homogeneous transmission characteristics or polarizers (via polarizer settings),[5,6] ii.) exploiting the quadratic distance dependence of diffuse illumination using a lamp, a non-fluorescent white standard and a setup for the variation of the distance between both standards as used by us,[6] iii.) the double aperture method,[10] iv.) the variation of the light intensity via

chromophore concentration,[6,11] and v.) the measurement of ratios of signals.[5,6] Methods i.), ii.) and iii.), that require expensive and sophisticated optical components and set ups, are only recommended for expert laboratories. The reliability of method iv.), that is recommended in ASTM E 578-83[11] in conjunction with quinine sulfate dihydrate and is recommendable for non-expert users, depends on the chosen chromophore(s). A suitable dye should display well-separated unstructured absorption and emission bands to minimize inner filter effects and should not be prone to quenching and aggregation or dimerization. Also, use of very dilute dye solutions, e.g., with absorbances preferably below 0.05 or at least below 0.1 for 1 cm-cells at the excitation wavelength is recommended, as only then a linear dependence of fluorescence intensity on dye concentration can be anticipated.[6] In addition, it is recommended to use the concentration dependence of both the fluorescence intensity and the shape of the normalized uncorrected emission spectrum for determination of the range of linearity of the detection system. Method v.) previously published by us,[6] is robust, simple, and not very susceptible to additional measurement uncertainties. Signal ratioing can be achieved e.g. either using different settings of an emission polarizer, an attenuator like a filter in the emission channel or different emission wavelengths. One approach pursued by us is the controlled modulation of the spectral radiance of the instrument's excitation light source or a second lamp with attenuators in front of the light source, a white standard at the sample position and an emission polarizer. Then, the ratios of the light fluxes of the lamp scattered from the white standard at the sample position towards the detection system are determined for two different emission polarizer settings as a function of lamp intensity. Deviations from a constant value exceeding the (previously determined) uncertainty of fluorescence measurements reveal the upper limit of the linearity of the emission detection system. For this method, the attenuation factor should be on the order of 2 to 5. Also, this methods requires knowledge of the uncertainty of positioning the used optical components, here the polarizers. A straightforward alternative of method v.) for the broad majority of fluorescence users is the

use of dilute dye solutions (absorbances at the excitation wavelength preferably at maximum 0.05 or at least below 0.1) and the variation of the light intensity reaching the detector via dye concentration.

Relative Spectral Responsivity ($s(\lambda_{em})$). Generally, the (relative) spectral responsivity of the emission channel of a fluorescence instrument, see equation 1, can be obtained with a source-based standard like a lamp or chromophore-based emission standards that preferably emits a broad, unstructured spectrum in the spectral region under consideration, typically the UV/vis, vis/NIR or UV/vis/NIR spectral region.[4,5,6,7] The wavelength-dependent spectral radiance or corrected emission spectrum of these standards must be known and should be preferably certified with a stated uncertainty. The instrument´s (relative) spectral responsivity $s(\lambda_{em})$ equals the quotient of the measured (uncorrected) emission spectrum and the certified spectral radiance or corrected emission spectrum of the standard. A very stringent requirement on the reliability of the determination of $s(\lambda_{em})$ is its measurement under application-relevant measurement conditions including e.g. emission slit width, detector voltage and mode, filters, attenuators, and polarizers in the emission channel, integration or scanning or averaging time, measurement geometry.

Typical methods for the determination of $s(\lambda_{em})$ include i.) use of a calibrated physical source-based transfer standard such as a tungsten ribbon lamp or an integrating sphere-type radiator as performed by us, ii.) use of the previously characterized excitation channel (with a calibrated detector) as calibrated light source in a synchronous scan of the excitation and emission channel with a calibrated white (diffuse reflector) standard at the sample position, or iii.) use of chromophore-based spectral fluorescence or so-called emission standards. All of these approaches are in principle traceable to a radiometric scale. Method i.) that requires sophisticated attenuation procedures due to the high spectral radiances of typical source-based transfer standard which decrease the spectral radiance to a level that is within the linear range

of the fluorometer's detection system, is recommended only for expert laboratories. This is similarly the case for method ii.) that relies on the use of a white standard, the wavelength dependence of the reflectance or spectral radiance factor of which was determined (and certified) for the employed measurement geometry, and on the reliability of the synchronization of the emission and excitation monochromators. Due to its comparatively little error-proneness and the meanwhile commercial availability of suitable sets of emission standard, method iii.) is the recommended method for the UV/vis region for the broad community of fluorescence users. For the determination of $s(\lambda_{em})$ in the NIR region, i.e., for wavelength above 770 nm, at present, method i.) is the only option.[12,13,14]

Due to radiometric conventions and the calibration of the transfer standards used, the emission correction curves–and thus also corrected emission spectra–are typically obtained traceable to the spectral radiance ($L_\lambda(\lambda)$) scale.[5,6]

Relative Spectral Irradiance at the Sample Position ($E_\lambda(\lambda_{ex})$). Instrument-independent excitation spectra and the comparison of (integral) emission intensities measured at different excitation wavelengths require knowledge and consideration of the spectral irradiance at the sample position. For the determination of the relative spectral shape of $E_\lambda(\lambda_{ex})$, that is sufficient in the majority of cases, the wavelength and polarization dependence of the excitation light flux reaching the sample (in relative units) needs to be obtained.[6] Here, it is typically assumed that the illuminated volume does not change in between instrument characterization and measurement of fluorescent samples to be corrected. For the few cases where the absolute values of $E_\lambda(\lambda_{ex})$ are desired such as the direct comparison of fluorescence intensities generated by different instruments or the determination of absolute fluorescence quantum yields, additional knowledge of the illuminated volume of the spectral responsivity transfer standard (and the sample) is mandatory.

The following procedures have been described for the determination of $E_\lambda(\lambda_{ex})$[5,6,7]: i.) use of a calibrated spectral responsivity transfer standard such as a calibrated detector, typically a silicon photodiode (simple or integrating sphere-type, trap detector) placed at sample position, ii.) application of the previously characterized emission channel, as "calibrated detector" in a synchronous scan of the excitation and emission channel with a white standard at sample position,[4] iii.) use of chromophore-based so-called excitation standards with known corrected excitation spectra, iv.) use of a pyroelectric detector, v.) use of a quantum counter, vi) use of an actinometer, and vii.) the comparison of the absorption and excitation spectrum of a chromophore.

The most common and most reliable method for the measurement of $E_\lambda(\lambda_{ex})$ or the excitation light flux reaching the sample (in relative units) is method i.). The determination of corrected excitation spectra employing this method has been described in detail by us in ref. 6. As typically flux-calibrated detectors (calibrated spectral responsivity, radiometric units) are used, this method yields corrected excitation spectra that are traceable to the *spectral responsivity scale* thereby not considering the photonic nature of the excitation light. Method iii.) critically relies on suitable sets of excitation standards and dilute dye solutions. Drawbacks of method iii.) are the lack of certified excitation standards and generally, the limited reliability of literature data. Not advisable are the application of methods iv.)-vii.). Also the most simple method vii.) can lead to a comparatively high calibration uncertainty if the dye photophysics are not very well known. For example, the fluorescence quantum yield of the dye may depend on excitation wavelength for an excitation involving two different electronic transitions.

For the comparison of (integral) fluorescence intensities obtained at different excitation wavelengths (measurement of Φ_f at two different λ_{ex}) and for the comparison of corrected excitation spectra with measured absorption spectra, both the wavelength- and polarization

dependence of the excitation channel (equaling the wavelength- and polarization dependence of the excitation light reaching the sample or $E_\lambda(\lambda_{ex})$) and the photonic nature of the excitation light need to be taken into account. Accordingly, the corresponding quantities obtained need to be divided by the respective photon energies or multiplied by the wavelength λ_{ex} thereby establishing the spectral photon irradiance $E_{p,\lambda}$ as reference quantity ($E_{p,\lambda}(\lambda_{ex}) = E_\lambda(\lambda_{ex}) \times \lambda_{ex}/(hc_0)$).

3. Supporting Measurements with Quantum Yield Standards

Excitation wavelength independence of the fluorescence quantum yield of coumarin 153. The excitation wavelength independence of Φ_f of the quantum yield standard coumarin 153 was confirmed by its matching absorption spectrum (the wavelength dependence of ($f(\lambda_{ex})$) and corrected excitation spectrum (in photonic units), see Figure 1S.

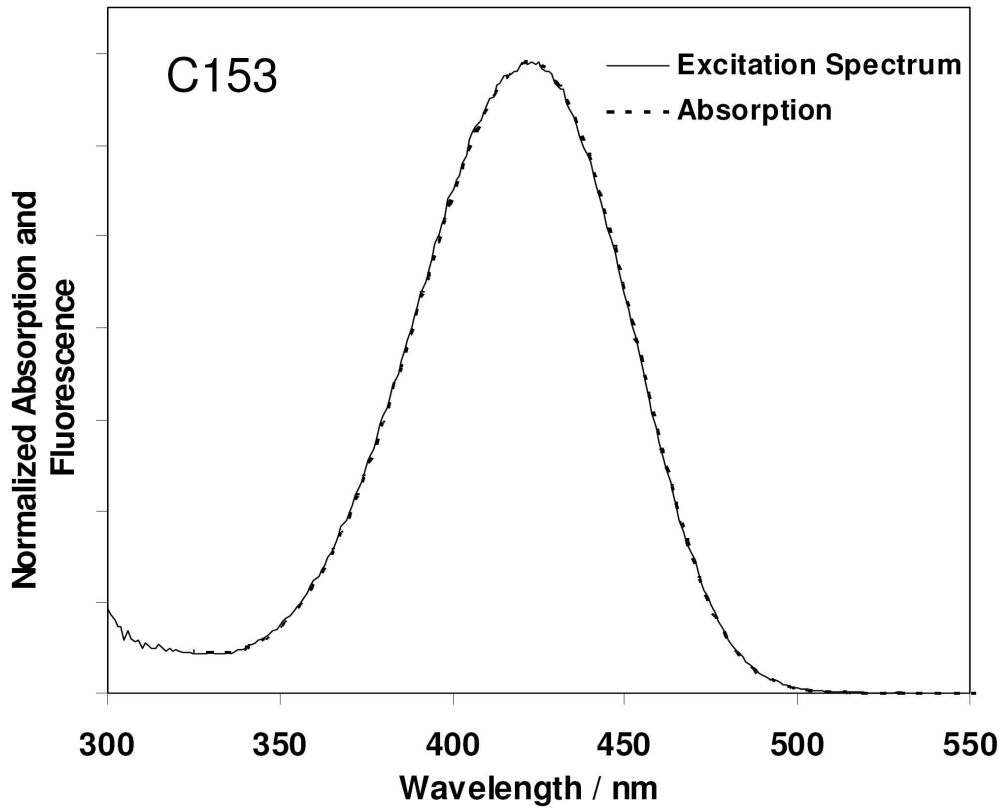

Figure 1S Comparison of the absorption spectrum (the wavelength dependence of ($f(\lambda_{ex})$) and the corrected excitation spectrum of a dilute solution of coumarin 153 in ethanol measured at emission wavelengths of 540 nm. The photonic nature of the exciting light was considered upon division of the corrected excitation spectrum by the energy of the exciting photons. Matching of the spectra confirms the excitation wavelength independence of Φ_f for this dye.

Concentration dependence of the absorption spectra of fluorescein 27 and rhodamine 6G. The concentration independence of the normalized absorption spectra of fluorescein 27 (Figure 2S, left) and rhodamine 6G (Figure 2S, right) indicates that within the concentration range used, there are no signs for dye aggregation. The normalized absorption spectra of both standards were obtained for absorbances of 0.025 (measured with 50 mm-cells) and 2.5 (measured with 1 mm-cells) at the main absorption band and the given absorbances refer to the maximum of the main absorption band and an optical pathlength of 10 mm. This observation provides the basis for our assumption of concentration-independent fluorescence quantum yields of these dyes.

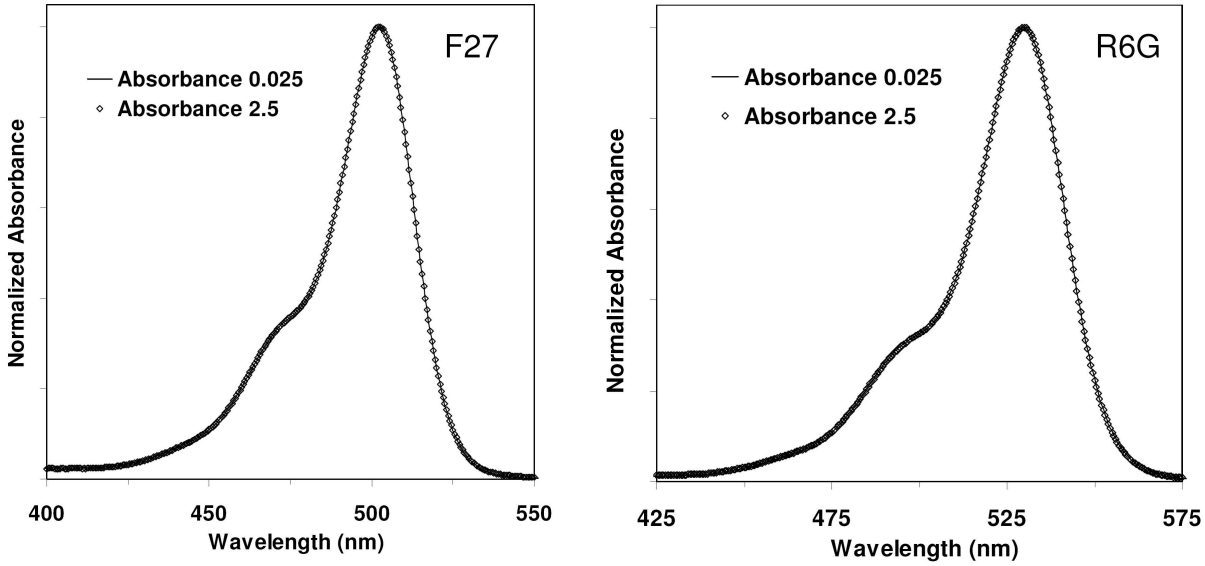

Figure 2S. Normalized absorption spectra of fluorescein 27 (left) and rhodamine 6G (right). The absorbances of the dilute dye solutions were measured with 50 mm-cells and the absorbances of the concentrated solutions with 1 mm-cells, respectively. The given absorbances refer to the maximum of the main absorption band and an optical pathlength of 10 mm.

Concentration dependence of additional CdTe QD samples

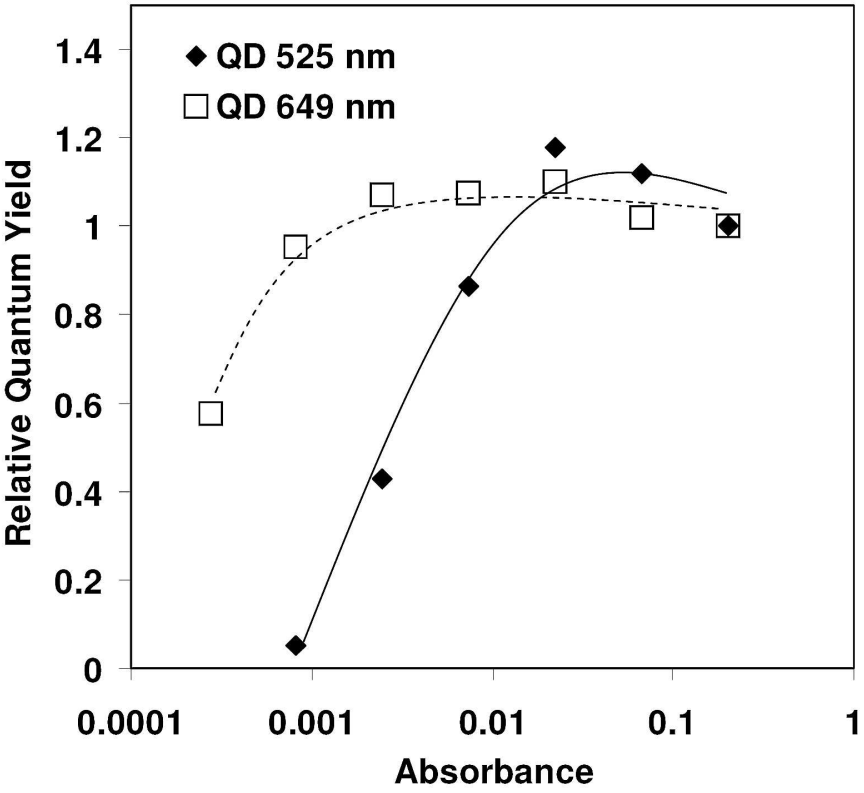

Figure 3S. Dependence of the relative fluorescence quantum yield on the particle concentration for a short wavelength (525 nm) and a long wavelength (649 nm) emitting CdTe QD in water. The surface ligand was TGA. For better comparison, the quantum yield values are normalized at the absorbance 0.2. The absorbances refer to the first excitonic maximum. The quantum yields, obtained for the highest concentration (absorbance 0.2) were 0.20 for the 525 nm and 0.57 for the 649 nm

emitting particles (fluorescence standard: coumarin 153 in ethanol and cresyl violet in methanol (QY = 0.54[15])

References

1. Verhoeven, J. W., Glossary of terms used in photochemistry, *Pure Appl. Chem.* **1996**, *68*(12), 2223-2286.
2. Melhuish, W. H., Nomenclature, symbols, units and their usage in spectrochemical analysis VI: Molecular luminescence spectroscopy, *Pure Appl. Chem.* **1984**, *56*(2), 231-245.
3. Mielenz, K. D., *Appl. Opt*. **1978**, *17*(18), 2876-2877.
4. DeRose, P. C.; Early, E. A.; Kramer, G. W., *Rev. Sci. Instrum*. **2007**, *78*, 033107.
5. 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,* vol. 5*,* U. Resch-Genger, ed., *Springer Series on Fluorescence*, O. S. Wolfbeis (series editor), Springer-Verlag Berlin Heidelberg 2008, pp. 65-100.
6. Resch-Genger, U.; Pfeifer, D.; Monte, C.; Pilz, W.; Hoffmann, A.; Spieles, M.; Rurack, K.; Taubert, D. R.; Schönenberger, B.; Nording, P., *J. Fluoresc.* **2005**, *15*, 315-336.
7. Resch-Genger, U.; DeRose, P. C., Characterization of photoluminescence measuring systems, (IUPAC Technical Note 2008), submitted to the Fluorescence Task Force Group, IUPAC.

8. Rurack, K.; Resch-Genger, U., Determination of the Photoluminescence Quantum Yield of Dilute Dye Solutions (IUPAC Technical Note), submitted to the Fluorescence Task Force Group, IUPAC.

9. Rurack, K., Fluorescence quantum yields: methods of determination and standards. In: *Standardization and Quality Assurance in Fluorescence Measurements I: Techniques,* vol. 5*,* U. Resch-Genger, ed., *Springer Series on Fluorescence*, O. S. Wolfbeis, ed., Springer-Verlag Berlin Heidelberg 2008, pp. 101-145.

10. a.) Mielenz, K. D., Eckerle, K. L., *Appl. Opt*. **1972**, *11*, 2294. b.) Zwinkels J. C., Gignac D. S., *Appl. Opt*. **1991**, *30*, 1678.

11. ASTM E 578 -01 (2001, original version 1983), Linearity of fluorescence measuring system. In: Annual book of ASTM standards, vol 03.06.

12. a.) Monte, C.; Hoffmann, K.; Pfeifer, D.; Hoffmann, A.; Resch-Genger, U., *J. Fluoresc*. 2006, *16*, 581. b.) Federal Institute for Materials Research and Testing (BAM) (2006) Certificates of analysis, Certified Reference Material BAM-F001, BAM-F002, BAM-F003, BAM-F004, and BAM-F001. Spectral fluorescence standard for the determination of the relative spectral responsivity of fluorescence instruments within its emission range. Certification of emission spectra in 1 nm-intervals. The corresponding product numbers from Sigma-Aldrich for the ready made standards are 97003-1KT-F for the Calibration Kit and 72594, 23923, 96158, 74245, and 94053 for BAM-F001, BAM-F002, BAM-F003, BAM-F004, and BAM-F005, respectively. c.) U. Resch-Genger and D. Pfeifer, Certification report, Calibration Kit Spectral Fluorescence Standards BAM-F001 – BAM-F005, BAM (2006).

13. DeRose, P. C.; Wang, L.; Gaigalas, A. K.; Kramer, G. W.; Resch-Genger, U.; Panne, U., 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: Techniques, Resch-Genger, U. (ed.), Springer Series on Fluorescence, Vol. 5, Wolfbeis, O. S. (series editor), Springer-Verlag Berlin Heidelberg 2008, pp. 33-64.

14. Gardecki, J. A.; Maroncelli, M., *Appl. Spectr.* **1998***, 52*, 1179-1189.

15. Demas, J. N., Measurement of Photoluminescence, in *Optical Radiation Measurements*, Vol. 3, Mielenz, K. D., Ed.; Academic Press: New York, 1982; Vol. 3, pp 195-248.