

Analytical Chemistry**Fluorescence Quantum Yields of a Series of Red and Near Infrared Dyes Emitting at 600–1000 nm**

Knut Rurack* and Monika Spieles

Div. I.5, Federal Institute for Materials Research and Testing (BAM), Richard-Willstätter-Straße 11,
D-12489 Berlin

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I. Basic equations for fluorescence quantum yield determination

The intensity of the fluorescence depends on the ability of a fluorophore to convert a number of absorbed photons, $N_{\text{abs}}(\lambda_{\text{ex}})$, into emitted photons, $N_{\text{em}}(\lambda_{\text{ex}})$, in a particular environment, i.e., the fluorescence quantum yield Φ_f , eq. S1.

$$\Phi_f = N_{\text{em}}(\lambda_{\text{ex}}) / N_{\text{abs}}(\lambda_{\text{ex}}) \quad (\text{S1})$$

Fluorescence quantum yield and emission spectrum are directly related by eq. S2, in which $F_{p,\lambda}(\lambda_{\text{ex}}, \lambda_{\text{em}})$ is the spectral fluorescence photon yield.

$$\Phi_f = \int_{\lambda_{\text{em}}} F_{p,\lambda}(\lambda_{\text{ex}}, \lambda_{\text{em}}) d\lambda_{\text{em}} \quad (\text{S2})$$

The latter is connected to the actually measured emission spectrum $I_m(\lambda_{\text{ex}}, \lambda_{\text{em}})$ by eqs. S3 and S4.

$$P_{p,\lambda}(\lambda_{\text{ex}}, \lambda_{\text{em}}) = \varepsilon(\lambda_{\text{ex}}) c F_{p,\lambda}(\lambda_{\text{em}}) \int_{\Delta\lambda} \int E_{\text{ex},\lambda}(\lambda_{\text{ex}}, \vec{r}) P(\vec{r}) dV d\lambda_{\text{ex}} \quad (\text{S3})$$

$$I_m(\lambda_{\text{ex}}, \lambda_{\text{em}}) = \int_{\Delta\lambda} s(\lambda_{\text{em}}) P_{\lambda}(\lambda_{\text{ex}}, \lambda_{\text{em}}) d\lambda_{\text{em}} \quad (\text{S4})$$

Here, $P_{\lambda}(\lambda_{\text{ex}}, \lambda_{\text{em}})$ is the spectral radiant power, obtained from $I_m(\lambda_{\text{ex}}, \lambda_{\text{em}})$ by taking into account the spectral responsivity of the emission channel, $s(\lambda_{\text{em}})$, and the spectral bandwidth $\Delta\lambda$.^{S1,S2} $P_{p,\lambda}$ finally is additionally determined by the sample-specific quantities $\varepsilon(\lambda_{\text{ex}})$, concentration, c , spectral irradiance at position \vec{r} in the sample, $E_{\text{ex},\lambda}(\lambda_{\text{ex}}, \vec{r})$, and the detection probability of a photon emitted at position \vec{r} , $P(\vec{r})$. Moreover, $F(\lambda_{\text{em}})$ used in eq. 1 of the paper is equivalent to the emission-related part, i.e., $P_{p,\lambda}(\lambda_{\text{em}})$.

II. Relative uncertainties

(Because of the multiplicative and quotient forms of the respective equations and because correlations between the quantities are assumed to be negligible, summation of the squares of the relative uncertainties was performed.^{S3})

II.1 Absorption coefficient (ε)

The absorption coefficient at the longest wavelength absorption maximum is determined according to $\varepsilon = \frac{E}{cd}$ with E = absorbance (see II.1.3), c = concentration (see II.1.1 and II.1.2) and d = optical path length (see II.1.2).

II.1.1 Preparation of 3 different stock solutions:

- a) Weighing of ca. 0.5 mg sample for individual stock solution (balance Satorius supermicro Type S4: ± 0.0001 mg); $u_{rel}^w = 0.02$ %
- b) Dissolving in 10 mL solvent (dispenser ± 0.05 mL); $u_{rel}^s = 0.5$ %

II.1.2 Preparation of measurement solution in 50 mm optical path length quartz cell:

- a) Addition of 1 mL stock solution (Eppendorf Reference pipette ± 0.01 mL) to 14 mL solvent (dispenser ± 0.05 mL); $u_{rel}^p = 1$ %, $u_{rel}^d = 0.5$ %
- b) Cell length (± 0.02 mm); $u_{rel}^l = 0.04$ %

II.1.3 Performing 2 repetitive measurements of the 3 stock solutions:

- a) Absorbances at $\lambda_{max} \sim 0.15$ (photometric accuracy of spectrometer ± 0.0003); $u_{rel}^a = 0.2$ %
- b) Repeat accuracy of measurement ($n = 25$: ± 0.00008); $u_{rel}^i = 0.05$ %

II.1.4 Experimental standard deviation for replicate measurements: $u_{rel}^r \leq 2.2$ %

II.1.5 Relative uncertainty of ε : $u_{rel}^{\varepsilon^2} = u_{rel}^w^2 + u_{rel}^s^2 + u_{rel}^p^2 + u_{rel}^d^2 + u_{rel}^l^2 + u_{rel}^a^2 + u_{rel}^i^2 + u_{rel}^r^2$

$$u_{rel}^{\varepsilon} = 2.53 \text{ %}$$

II.1.6 Bias of fluorescence: A possible negative bias due to fluorescence from the sample reaching the detector has to be considered. It can be approximated from the ratio of the solid angle detected to 4π . The instrument used is equipped with f/8 optics so that the contribution is technically below -1 %.

II.2 Relative fluorescence quantum yield (Φ_f)

The relative fluorescence quantum yield is determined according to $\Phi_f^i = \Phi_f^s \frac{f_s(\lambda_{ex})}{f_i(\lambda_{ex})} \frac{\int_{\lambda_{em}} P_{p,\lambda}^i(\lambda_{em}) n_i^2}{\int_{\lambda_{em}} P_{p,\lambda}^s(\lambda_{em}) n_s^2}$ with

Φ_f = fluorescence quantum yield (see II.2.4), f_x = absorption factor at λ_{ex} ($f_x(\lambda_{ex}) = 1 - 10^{-A_x(\lambda_{ex})}$) (see II.2.1),

$F^x(\lambda_{em})$ = fluorescence intensity F^x at λ_{em} (see II.2.3) and n = refractive index (see II.2.3); super- and subscripts i and s refer to sample and standard.

II.2.1 Absorption measurements: According to II.1.3 for absorbances at $\lambda_{ex} \sim 0.06$; $u_{rel}^A = 0.55$ %

II.2.2 Transfer of 3 mL of the absorption solution into a 10 mm optical path length quartz cell: since no dilution step is involved, only contribution from cell length (± 0.01 mm); $u_{rel}^L = 0.1$ %

II.2.3 Fluorescence measurements:

a) Relative uncertainty of the emission spectrum, containing all contributions from repeat measurements and traceable calibration,^{S4} across the respective wavelength range for the UV/vis (for dyes QS, C-102, C-153, DCM, Rh-101) and the vis/NIR (for all other dyes) channels: $u_{rel}^{em} \leq 3$ % (UV/vis) and ≤ 6 % (vis/NIR)

b) For the integrated fluorescence intensities, points of intersection were chosen at $3 \times$ noise. The integral ex-

pressions in the equations in II.2.5 account for the maximum possible error;
$$\frac{\int_{\lambda_{em}} F^i + u_{rel}^{em} - \int_{\lambda_{em}} F^i}{\int_{\lambda_{em}} F^i} \leq 0.06$$
 %

c) Relative uncertainty of the excitation spectrum, containing all contributions from repeat measurements and traceable calibration, across the UV/vis wavelength range (for determination of Φ_f of Rh-101 against QS according to path A): $u_{rel}^{ex} \leq 1.3$ %

d) Relative uncertainty of refractive index with $n_D \sim 1.3$ (accuracy of refractometer ± 0.0001); $u_{rel}^n = 0.008$ %

e) Experimental standard deviation for replicate measurements, u_{rel}^r :

dyes (range)	QS, C-102, C-153, DCM, Rh-101 (UV/vis)	CV, Ox-170, Ox-1, HEDITCP, HPDITCP, HITCI (vis/NIR)	IR-140, IR-125 (vis/NIR)	ONITCP (vis/NIR)	Cc, ODNITCP (vis/NIR)
u_{rel}^r [%]	≤ 0.4	≤ 1.47	≤ 2.15	5.12	≤ 8.25

II.2.4 Fluorescence quantum yield of standard (quinine sulfate): $u_{rel}^{\Phi_s} = 5$ % (according to NIST)^{S5}

II.2.5 Relative uncertainty of Φ_f^i according to

a) Path A:

$$u_{rel}^{\Phi_i^2} = 2u_{rel}^A{}^2 + 2u_{rel}^L{}^2 + 2u_{rel}^{ex}{}^2 + \left(\frac{\int_{\lambda_{em}} F^i + u_{rel}^{em} - \int_{\lambda_{em}} F^i}{\int_{\lambda_{em}} F^i} \right)^2 + \left(\frac{\int_{\lambda_{em}} F^s + u_{rel}^{em} - \int_{\lambda_{em}} F^s}{\int_{\lambda_{em}} F^s} \right)^2 + 2(u_{rel}^n)^2 + u_{rel}^r{}^2 + u_{rel}^{\Phi_s}{}^2; \quad u_{rel}^{\Phi} = 5.40 \%$$

b) Paths B and C:

$$u_{rel}^{\Phi_i^2} = 2u_{rel}^A{}^2 + 2u_{rel}^L{}^2 + \left(\frac{\int_{\lambda_{em}} F^i + u_{rel}^{em} - \int_{\lambda_{em}} F^i}{\int_{\lambda_{em}} F^i} \right)^2 + \left(\frac{\int_{\lambda_{em}} F^s + u_{rel}^{em} - \int_{\lambda_{em}} F^s}{\int_{\lambda_{em}} F^s} \right)^2 + 2(u_{rel}^n)^2 + u_{rel}^r{}^2 + u_{rel}^{\Phi_s}{}^2$$

1. Chain link $u_{rel}^{\Phi} = 5.08 \%$

2. Chain link $u_{rel}^{\Phi} = 5.16 \%$ (u_{rel}^n is not effective because same solvent used from 2. chain link on)

etc.

II.2.6 Propagation of relative uncertainty in UV/vis transfer chains (best currently achievable values in blue):

a) UV/vis dyes vs. QS^{SRM}, different excitation wavelengths (Path A):

chain link	1.	2.	3.	4.	5.
dye	QS ^{SRM}	C-102	C-153	DCM	Rh-101
$u_{rel} [\%]$	5	5.40	5.40	5.40	5.40

b) Rh-101 and commercially available QS vs. QS^{SRM} direct, single excitation wavelength (Path B):

chain link	1.	2.	3.
dye	QS ^{SRM}	Rh-101	QS
$u_{rel} [\%]$	5	5.08	5.08

c) UV/vis range (Path C):

chain link	1.	2.	3.	4.	5.
dye	QS ^{SRM}	C-102	C-153	DCM	Rh-101
$u_{rel} [\%]$	5	5.08	5.16	5.23	5.31

II.2.7 Propagation of relative uncertainty in vis/NIR transfer chains (best currently achievable values in blue):

a) For Cresyl Violet, Oxazine 170 and Oxazine 1:

chain link	1.	2.	3.	4.
dye	Rh-101	CV	Ox-170	Ox-1
$u_{rel} [\%]$	5.08	5.35	5.60	5.85

b) For HEDITCP:

chain link	1.	2.
dye	Ox-170	HEDITCP
$u_{rel} [\%]$	5.60	5.85

c) For HPDITCP, HITCI and Cryptocyanine:

chain link	1.	2.	3.	4.
dye	CV	HPDITCP	HITCI	Cc
$u_{rel} [\%]$	5.35	5.60	5.85	10.14

d) For IR-140, ONITCP and ODNITCP:

chain link	1.	2.	3.	4.
dye	HITCI	IR-140	ONITCP	ODNITCP
$u_{rel} [\%]$	5.85	6.28	8.14	11.62

e) For IR-125:

chain link	1.	2.
dye	HITCI	IR-125
$u_{rel} [\%]$	5.85	6.28

II.2.8 Propagation of relative uncertainty in transfer chains with $u_{rel}^I = 1.5 \%$ assuming lower $u_{rel}^{\Phi_s}$ for primary chain link:

chain link	1.	2.	3.	4.	5.	6.	7.	8.
$u_{rel} [\%]$	4	4.35	4.67	4.96	5.25	5.51	5.77	6.01
$u_{rel} [\%]$	3	3.45	3.84	4.20	4.53	4.84	5.13	5.40
$u_{rel} [\%]$	2	2.62	3.12	3.56	3.94	4.29	4.61	4.92
$u_{rel} [\%]$	1	1.97	2.60	3.11	3.54	3.93	4.28	4.60

II.2.9 Relative uncertainty of $\Phi_f^{Rh-101}(T)$

a) Path B in II.2.5 is basically effective, except

- I. That the fluorescence excitation spectra are evaluated instead of the absorption spectra: u_{rel}^{ex} is effective instead of u_{rel}^A ;
- II. That additional uncertainties are introduced by the use of tabulated data of $n(T)$ and $\rho(T)$:^{S6} $u_{rel}^n = 0.008 \%$, $u_{rel}^\rho = 0.01 \%$ and
- III. By the measurement of T itself: $u_{rel}^T \leq 0.01 \%$.
- IV. Experimental standard deviation for replicate measurements: $u_{rel}^r = 6.0 \%$

$$u_{rel}^{\Phi_i^2} = 2u_{rel}^{ex^2} + 2u_{rel}^{L^2} + \left(\frac{\int_{\lambda_{em}} F^i + u_{rel}^{em} - \int_{\lambda_{em}} F^i}{\int_{\lambda_{em}} F^i} \right)^2 + \left(\frac{\int_{\lambda_{em}} F^s + u_{rel}^{em} - \int_{\lambda_{em}} F^s}{\int_{\lambda_{em}} F^s} \right)^2 + 2(u_{rel}^n)^2 + 2u_{rel}^\rho + 2u_{rel}^T + u_{rel}^{r^2} + u_{rel}^{\Phi_s^2}; \quad u_{rel}^\Phi = 7.71 \%$$

III Photostability of the dyes

The photostability of the dyes was assessed with a customized setup employing a 150 W Xenon lamp that irradiates a sealed 10 mm optical path length quartz cell at a chosen wavelength (see Table S1; upper limit 650 nm) with a slit width of 15 nm and a spot size of 1.5 cm² at sample position. 2500 µL dye solution, adjusted to an absorbance of ca. 0.11 at the absorption maximum, were exposed for 17 h to excitation intensities (measured with a Si-diode calibrated by the PTB) as listed in the table. The degradation of the dye, i.e., the decrease in concentration was calculated from the data of two measurements according to the Beer-Lambert law (relative uncertainty of measurement ca. 3 %).

Table S1. Photostability data of the dyes investigated.

dye	λ_{irr}	P_{irr}	bleaching
	nm	mW cm ⁻²	%
Rh-101	560	1.56	2.1
CV	560	1.53	3.1
Ox-170	600	1.73	5.3
Ox-1	600	1.72	0.2
Cc	650	0.39	2.3
HITCI	650	0.40	0.5
IR-125	650	0.42	4.5
IR-140	650	0.43	0.3
HEDITCP	600	1.72	11.2
HPDITCP	600	1.74	3.0
ONITCP	650	0.40	26.0
ODNITCP	650	0.40	11.3
QS ^{SRM}	350	0.48	1.2
QS (Fluka)	350	0.48	0.9
C-102	390	2.59	55.6
C-153	420	2.63	8.2
DCM	470	2.98	3.7

Despite the fact that the apparatus employed did not allow for irradiation at wavelengths > 650 nm so that Cc, HITCI, IR-125, IR-140, ONITCP and ODNITCP could only be irradiated in the vibronic shoulder of the longest wavelength absorption band, the table reveals that most of the dyes are reasonably stable (≤ 5 % degradation)

even under prolonged irradiation. All these dyes do not show any modification of the absorption before and after irradiation. Moreover, whereas ONITCP and ODNITCP show only a reduced yet spectrally unchanged (down to 400 nm) absorption spectrum after irradiation, Ox-170, HEDITCP, C-153 and especially C-102 show clear signs of photoproduct formation (see Figure S1). However, as stated in the Experimental Section of the paper, measurements and comparison of the absorption spectra before and after measurement of the fluorescence spectrum did not show any signs of photobleaching within experimental error. All the dyes used are thus virtually stable for at least ca. 30 min under the excitation conditions of the SLM fluorometer employed. In the long run, a substitute for C-102 should be searched for.

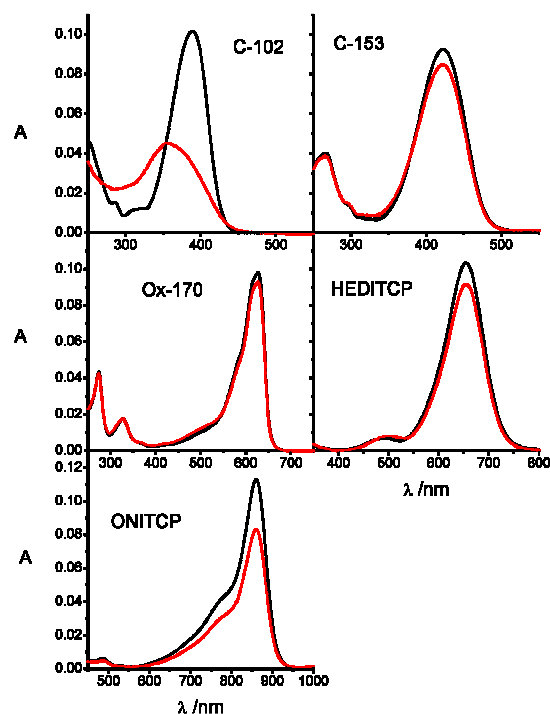


Figure S1. Absorption spectra of C-102, C-153, Ox-170 and HEDITCP before (black) and after (red) 17 h irradiation as described in the text. Whereas C-153, Ox-170 and HEDITCP show slight spectral changes, C-102 shows clear signs of a photochemical reaction. ONITCP, which only degrades with no traces of vis/NIR photoproduct formation is included for comparison.

IV References

- S1 Hollandt, J.; Taubert, R. D.; Seidel, J.; Resch-Genger, U.; Gugg-Helminger, A.; Pfeifer, D.; Monte, C.; Pilz, W. *J. Fluoresc.* **2005**, *15*, 301-313.
- S2 Because Φ_f is the ratio of emitted to absorbed photons (cf. eq. S1), the spectral radiant power that reaches the emission channel from the illuminated volume is preferably expressed as photon quantity with $P_{p,\lambda} = P_\lambda \lambda/hc$.
- S3 Evaluation of measurement data—Guide to the expression of uncertainty in measurement, Joint Committee for Guides in Metrology JCGM, Paris, first edition 2008, corrected version 2010.
- S4 Monte, C.; Resch-Genger, U.; Pfeifer, D. R.; Taubert, D. R.; Hollandt, J. *Metrologia* **2006**, *43*, S89-S93.
- S5 Velapoldi, R. A.; Mielenz, K. D. *NBS Spec. Publ. 260-64*, National Bureau of Standards: Washington, DC **1980**.
- S6 Landolt–Börnstein compendium, at <http://www.springermaterials.com/navigation/> (accessed Dec 1, 2010).