

Supplemental Material to the article

“Experimental Verification of the Principle of Microscopic Reversibility in the Photoluminescence Decay Kinetics”

1. Derivation of Eq. (1) in the Wien approximation for a mixture of noninteracting phosphors that satisfy the Vavilov law and the Kennard–Stepanov relation. Let us consider a low absorbance solution containing a mixture of different phosphors that satisfy the Vavilov law and the Kennard–Stepanov relation. The time-resolved excitation-emission matrix of such a solution can be written as

$$F(\lambda_{\text{ex}}, \lambda_{\text{em}}, t) = K \sum_i c_i \varepsilon_i(\lambda_{\text{ex}}) \text{QY}_i \text{PL}_i(\lambda_{\text{em}}) \tau_i^{-1} e^{-t/\tau_i}, \quad (\text{S1})$$

where λ_{ex} is the excitation wavelength, λ_{em} is the emission wavelength, t is the time after the excitation pulse, K is a constant determined by the geometry of the experimental setup for luminescence measurements, i is an index running over all phosphors in the mixture, c_i is the concentration of the i th phosphor, $\varepsilon_i(\lambda_{\text{ex}})$ is the extinction coefficient of the i th phosphor as a function of the excitation wavelength λ_{ex} , QY_i is the fluorescence quantum yield of the i th phosphor, $\text{PL}_i(\lambda_{\text{em}})$ is the emission spectrum of the i th phosphor, normalized to unit area, τ_i is the fluorescence lifetime of the i th phosphor.

The emission spectrum of the i th phosphor is related to its absorption spectrum by the Kennard–Stepanov relation:

$$\text{PL}_i(\lambda_{\text{em}}) \propto \lambda_{\text{em}}^{-4} \exp\left(\frac{-hc}{\lambda_{\text{em}} k_{\text{B}} T}\right) \varepsilon_i(\lambda_{\text{em}}), \quad (\text{S2})$$

where k_{B} is the Boltzmann constant, T is the absolute temperature. Since we assume that the spectra $\text{PL}_i(\lambda_{\text{em}})$ are normalized to unit area, it follows from (S2) that

$$\text{PL}_i(\lambda_{\text{em}}) = \frac{\lambda_{\text{em}}^{-4} \exp\left(\frac{-hc}{\lambda_{\text{em}} k_{\text{B}} T}\right) \varepsilon_i(\lambda_{\text{em}})}{\int_0^{\infty} \lambda^{-4} \exp\left(\frac{-hc}{\lambda k_{\text{B}} T}\right) \varepsilon_i(\lambda) d\lambda}. \quad (\text{S3})$$

By substituting (S3) into (S1), we obtain

$$\lambda_{\text{em}}^4 \exp\left(\frac{hc}{\lambda_{\text{em}} k_{\text{B}} T}\right) F(\lambda_{\text{ex}}, \lambda_{\text{em}}, t) = K \sum_i \frac{c_i \text{QY}_i \tau_i^{-1} e^{-t/\tau_i} \varepsilon_i(\lambda_{\text{ex}}) \varepsilon_i(\lambda_{\text{em}})}{\int_0^{\infty} \lambda^{-4} \exp\left(\frac{-hc}{\lambda k_{\text{B}} T}\right) \varepsilon_i(\lambda) d\lambda}. \quad (\text{S4})$$

Since the right-hand side of Eq. (S4) is invariant under interchange of λ_{ex} and λ_{em} , the left-hand side of Eq. (S4) must also be invariant under interchange of λ_{ex} and λ_{em} , which proves Eq. (1) in the Wien approximation:

$$\lambda_{\text{em}}^4 \exp\left(\frac{hc}{\lambda_{\text{em}} k_{\text{B}} T}\right) F(\lambda_{\text{ex}}, \lambda_{\text{em}}, t) = \lambda_{\text{ex}}^4 \exp\left(\frac{hc}{\lambda_{\text{ex}} k_{\text{B}} T}\right) F(\lambda_{\text{em}}, \lambda_{\text{ex}}, t). \quad (\text{S5})$$

2. Derivation of Equation (1) in the Wien approximation for a cluster of luminescent particles that satisfy the Vavilov law and the Kennard–Stepanov relation, taking into account the Förster resonance energy transfer (FRET). Let us consider a cluster of N luminescent motionless particles in a medium with refractive index n at absolute temperature T . Let the sizes of the particles be small compared to the distances R_{ij} between them. Let the particles obey the Kennard–Stepanov relation and the Vavilov law. Let the i th particle be characterized by the fluorescence lifetime τ_i , by the fluorescence quantum yield QY_i , by the wavelength dependence of the absorption cross section $\sigma_i(\lambda_{\text{ex}})$, and by the emission spectrum $\text{PL}_i(\lambda_{\text{em}})$ normalized to unit area. These characteristics are assumed to be given for particles outside the cluster. Let the Förster resonance energy transfer occur between the particles in the cluster and the rate constants of the process be given by the Forster equation:

$$k_{ij} = \frac{9\kappa_{ij}^2 \text{QY}_i}{128\pi^5 n^4 \tau_i R_{ij}^6} \int_0^{\infty} \text{PL}_i(\lambda) \sigma_j(\lambda) \lambda^4 d\lambda, \quad (\text{S6})$$

where k_{ij} is the rate constant of transfer from the i th particle to the j th particle ($i \neq j$), κ_{ij} is the orientation factor, whose mean square is equal to $2/3$ for the case of free rotation. For $i = j$, we formally put $k_{ij} = 0$.

The emission spectrum in (S6) can be expressed through the absorption cross section using the Kennard–Stepanov relation:

$$\text{PL}_i(\lambda_{\text{em}}) = \frac{\lambda_{\text{em}}^{-4} \sigma_i(\lambda_{\text{em}}) \exp\left(\frac{-hc}{\lambda_{\text{em}} k_{\text{B}} T}\right)}{\int_0^{\infty} \lambda^{-4} \sigma_i(\lambda) \exp\left(\frac{-hc}{\lambda k_{\text{B}} T}\right) d\lambda}. \quad (\text{S7})$$

By substituting (S7) into (S6), we obtain

$$k_{ij} = a_i b_{ij}, \quad (\text{S8})$$

where

$$a_i = \frac{\text{QY}_i}{\tau_i \int_0^{\infty} \lambda^{-4} \sigma_i(\lambda) \exp\left(\frac{-hc}{\lambda k_{\text{B}} T}\right) d\lambda}, \quad (\text{S9})$$

$$b_{ij} = \frac{9\kappa_{ij}^2}{128\pi^5 n^4 R_{ij}^6} \int_0^{\infty} \sigma_i(\lambda) \sigma_j(\lambda) \exp\left(\frac{-hc}{\lambda k_{\text{B}} T}\right) d\lambda. \quad (\text{S10})$$

Here we have decomposed the coefficient k_{ij} into the factors a_i and b_{ij} : the former factor depends only on i , the latter factor is symmetric under interchange of i and j .

Let the probability for the i th particle in the cluster to be in the electronically excited state at time t after the excitation pulse be $p_i(t)$. Then, the migration of the excitation over the cluster is described by the following system of ordinary differential equations:

$$\frac{d}{dt} \mathbf{p}(t) = A \mathbf{p}(t), \quad (\text{S11})$$

where $\mathbf{p}(t)$ is the vector consisting of N functions $p_i(t)$, A is the $N \times N$ -matrix with elements

$$A_{ij} = -\delta_{ij} \left(\tau_i^{-1} + \sum_{r=1}^N k_{ir} \right) + k_{ji}, \quad (\text{S12})$$

where δ_{ij} is the Kronecker delta, that is, the number equal to zero for $i \neq j$ and equal to unity for $i = j$. The solution of the system (S11) can be expressed through the matrix exponential

$$\mathbf{p}(t) = e^{At} \mathbf{p}(0). \quad (\text{S13})$$

By substituting (S8) into (S12) and rearranging the result, we obtain:

$$A_{ij} = \left(-\delta_{ij} \left(\frac{1}{\tau_i a_i} + \sum_{r=1}^N b_{ir} \right) + b_{ji} \right) a_j. \quad (\text{S14})$$

It follows from (S14) that the matrix A can be expressed as a product:

$$A = SD, \quad (\text{S15})$$

where S is a symmetric matrix with elements

$$S_{ij} = -\delta_{ij} \left(\frac{1}{\tau_i a_i} + \sum_{r=1}^N b_{ir} \right) + b_{ji}, \quad (\text{S16})$$

while D is a diagonal matrix with elements

$$D_{ij} = \delta_{ij} a_j. \quad (\text{S17})$$

By taking into account the factorization (S15), the matrix exponential in (S13) can be expressed as

$$e^{At} = \sum_{p=0}^{\infty} \frac{A^p t^p}{p!} = \sum_{p=0}^{\infty} \frac{(SD)^p t^p}{p!} = D^{-1/2} \left(\sum_{p=0}^{\infty} \frac{(D^{1/2} S D^{1/2})^p t^p}{p!} \right) D^{1/2} = D^{-1/2} \exp(D^{1/2} S D^{1/2} t) D^{1/2}. \quad (\text{S18})$$

Equation (S18) gives us a representation the matrix exponential of the asymmetric matrix At in terms of the matrix exponential of the symmetric matrix $D^{1/2} S D^{1/2} t$. At the same time, it is known that the matrix exponential of a symmetric matrix is also a symmetric matrix.

Let us define the time-resolved excitation-emission matrix of the cluster $F(\lambda_{\text{ex}}, \lambda_{\text{em}}, t)$ as a response to an instantaneous excitation pulse carrying a unit photon flux per unit area J , with the light wavelength being λ_{ex} . In this case, the vector of probabilities for the particles in the cluster to be in the excited state immediately after such an excitation pulse becomes

$$\mathbf{p}(0) = J \vec{\sigma}(\lambda_{\text{ex}}), \quad (\text{S19})$$

where $\vec{\sigma}(\lambda_{\text{ex}})$ is the vector consisting of the quantities $\sigma_i(\lambda_{\text{ex}})$. The response, in our case, is the probability density of the distribution function of the emitted photons over the time of emission and the emission wavelength and is equal to

$$F(\lambda_{\text{ex}}, \lambda_{\text{em}}, t) = \sum_{i=1}^N p_i(t) \text{PL}_i(\lambda_{\text{em}}) \text{QY}_i \tau_i^{-1}. \quad (\text{S20})$$

By substituting (S7) into (S20), simplifying the result with the help of the definition (S9), and resorting to matrix representation by using (S17), we obtain:

$$F(\lambda_{\text{ex}}, \lambda_{\text{em}}, t) = \lambda_{\text{em}}^{-4} \exp\left(\frac{-hc}{\lambda_{\text{em}} k_B T}\right) (D \vec{\sigma}(\lambda_{\text{em}}))^T \mathbf{p}(t). \quad (\text{S21})$$

By substituting (S13), (S18), and (S19) into (S21) and rearranging, we obtain:

$$\lambda_{\text{em}}^4 \exp\left(\frac{hc}{\lambda_{\text{em}} k_B T}\right) F(\lambda_{\text{ex}}, \lambda_{\text{em}}, t) = J \vec{\sigma}(\lambda_{\text{em}})^T D^{1/2} \exp(D^{1/2} S D^{1/2} t) D^{1/2} \vec{\sigma}(\lambda_{\text{ex}}). \quad (\text{S22})$$

The product of matrices $D^{1/2} \exp(D^{1/2} S D^{1/2} t) D^{1/2}$ in the right-hand side of Eq. (S22) is a symmetric matrix. Transposing the right-hand side of Eq. (S22) interchanges the wavelengths λ_{em} and λ_{ex} . At the same time, the right-hand side of Eq. (S22) is just a number and therefore is invariant under the transpose. Hence, the right-hand side of Eq. (S22) is symmetric under interchange of the wavelengths λ_{em} and λ_{ex} . Consequently, the left-hand side of Eq. (S22) is also symmetric under interchange of the wavelengths λ_{em} and λ_{ex} :

$$\lambda_{\text{em}}^4 \exp\left(\frac{hc}{\lambda_{\text{em}} k_B T}\right) F(\lambda_{\text{ex}}, \lambda_{\text{em}}, t) = \lambda_{\text{ex}}^4 \exp\left(\frac{hc}{\lambda_{\text{ex}} k_B T}\right) F(\lambda_{\text{em}}, \lambda_{\text{ex}}, t). \quad (\text{S23})$$

3. Experimental data used for plotting Figure 4. Figures S1, S2, and S3 show the raw experimental data used for plotting Fig. 4, the fitting of these data, and an illustration of the correction for the noise, the photomultiplier dark count rate, and the instrument response function (IRF). The table S1 shows the experimental parameters used for measuring the fluorescence decay kinetics.

Table S1. Experimental conditions under which the fluorescence decay measurements have been performed

	Excitation (nm) / Detection (nm)			
	550/600	600/550	550/550	600/600
Time between pulses (ns)	400	400	100	100
Acquisition time (s)	442.3	4079.9	3600	3600
Bin width (ps)	8	8	4	4
Excitation attenuation	1	1	0.01	0.01
Detector attenuation	0.5	1	0.1	0.1
Signal rate (s^{-1})	43780	2050	67390	36470
Excitation bandpass (nm)	5	5	5	5
Detection bandpass (nm)	5	5	5	5

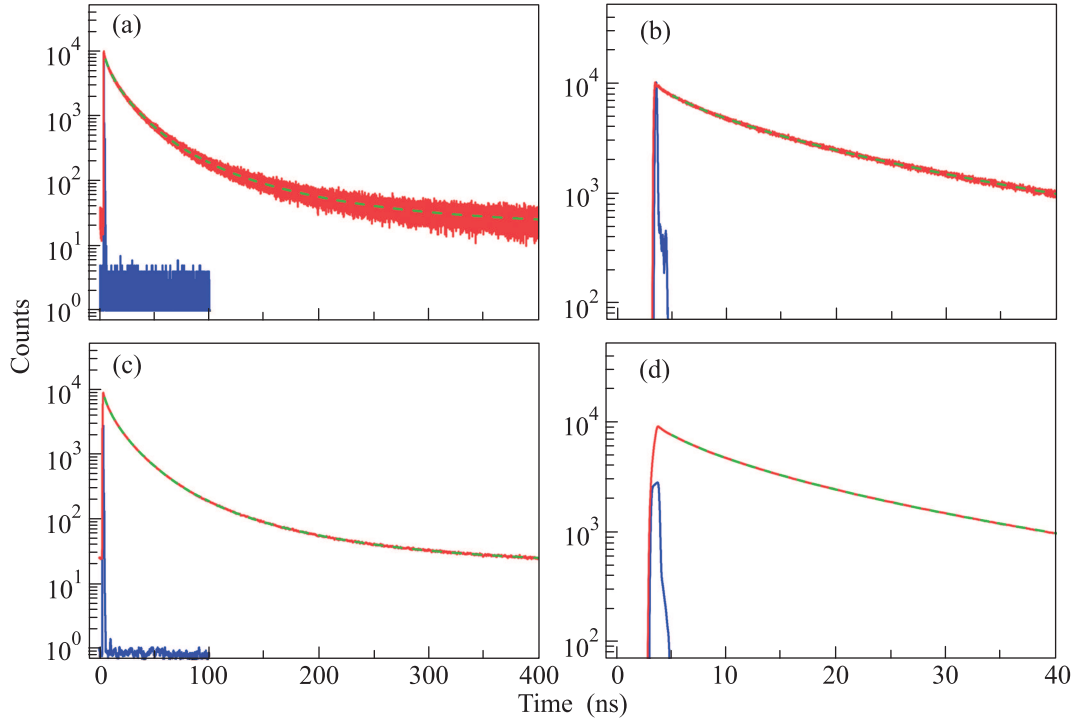


Fig. S1. Experimental fluorescence decay kinetics for excitation at 550 nm and detection at 600 nm (red line), the IRF measured using a scattering sample at 550 nm (blue line), and the fitting of the decay kinetics (green dashed line) (a). The magnified view of the same graphs (b). The same graphs, but the experimental decay curve and the IRF are smoothed using a rectangular window of 0.8 ns (c), (d)

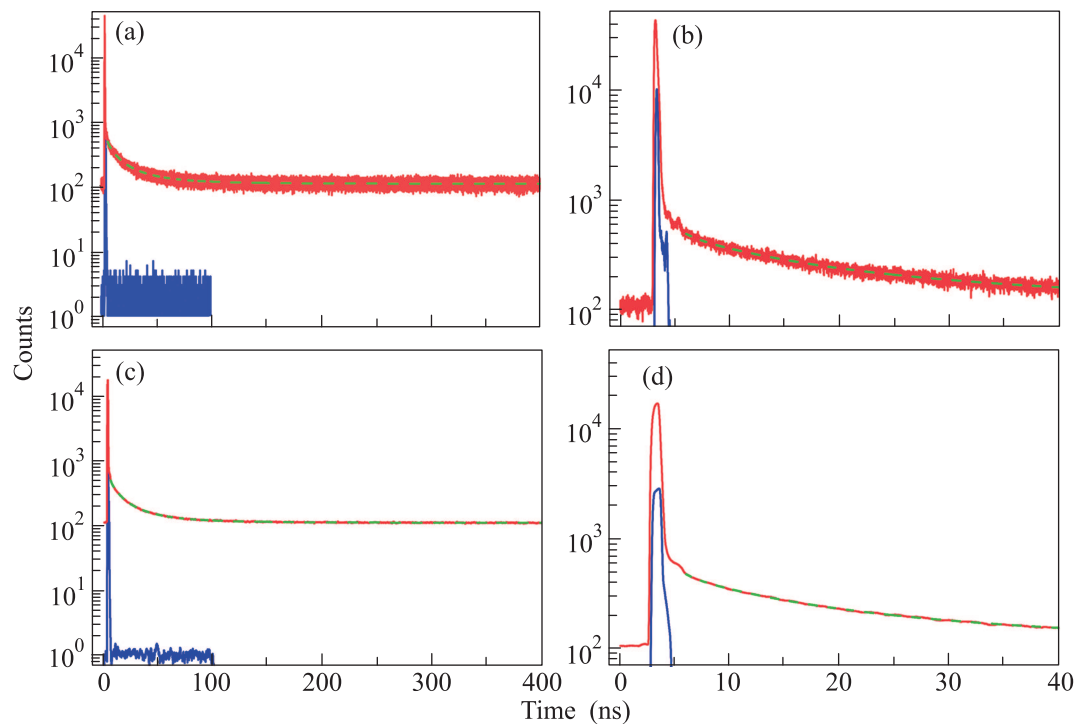


Fig. S2. The same as in Fig. S1, but with excitation at 600 nm and detection at 550 nm

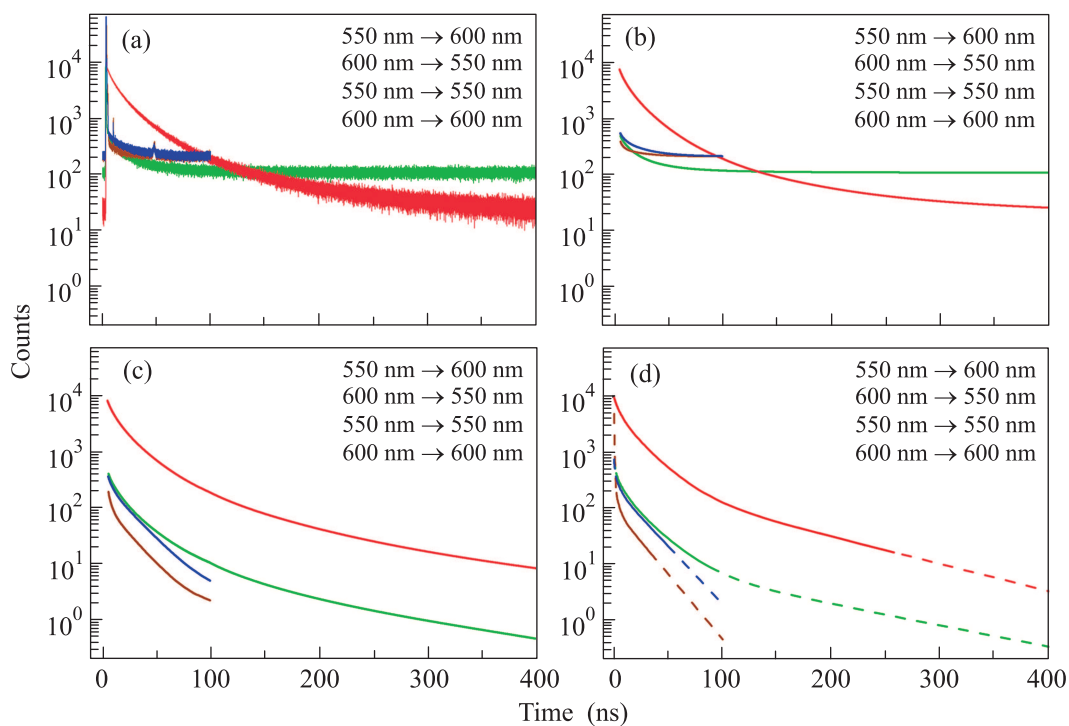


Fig. S3. Raw experimental fluorescence decay kinetics (a). Fitting of the experimental decays by the sum of the decreasing exponential functions with taking into account the dark count rate and the instrument response function (b). The same decays minus the dark count rate calculated under the assumption of no lifetimes larger than 200 ns (c). Hypothetical true fluorescence decay curves (d).

obtained as a result of the fitting (d); they differ from the curves in the previous panel in that the correction for the IRF is made; the portions of the curves that are statistically not reliable are plotted using dashed lines; the portions of curves were considered to be unreliable when the relative width of the confidence interval was larger than 10 %