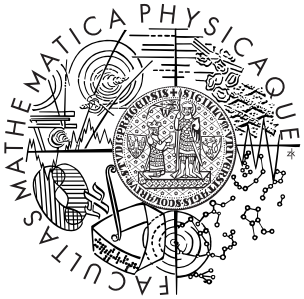
Univerzita Karlova v Praze
Matematicko-fyzikální fakulta

BAKALÁŘSKÁ PRÁCE



Jakub Zahumenský

Fázová fluorimetrie nízkomolekulárních látek

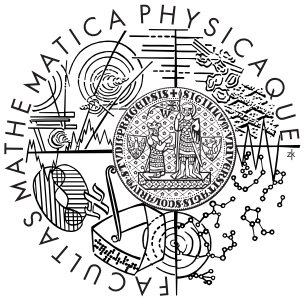
Fyzikální ústav Univerzity Karlovy - oddělení biofyziky

Vedoucí bakalářské práce: doc. RNDr. Petr Heřman, CSc.
Studijní program: Fyzika, obecná fyzika

2009

Charles University in Prague
Faculty of Mathematics and Physics

BACHELOR THESIS



Jakub Zahumenský

Frequency domain fluorescence spectroscopy of low molecular weight substances

Institute of Physics of the Charles University - Biophysics Division

Thesis supervisor: doc. RNDr. Petr Heřman, CSc.
Study program: Physics, General physics

2009

I would like to thank my supervisor, doc. RNDr. Petr Heřman, CSc. for his competent guidance of my bachelor thesis, for his helpful advice and useful comments.

I declare that I have written my bachelor thesis independently and exclusively using the cited sources. I agree with lending of the work and its publishing.

In Prague, 25^{th} May 2009 Jakub Zahumenský

Contents

Název práce: Fázová fluorimetrie nízkomolekulárních látek
Author: Jakub Zahumenský
Katedra (ústav): Fyikální ústav Univerzity Karlovy - oddělení biofyziky
Vedoucí bakalářské práce: doc. RNDr. Petr Heřman, CSc.
e-mail vedoucího: Petr.Herman@mff.cuni.cz

Abstrakt: V předložené práci studujeme fluorescenční vlastnosti 23 laserových barviv, jejichž emisní maxima leží ve viditelné a blízke UV oblasti. Z těchto barviv vybírame sadu referenčních standardů vhodných pro časově rozlišené fluorimetrické měření ve frekvenční doméně. Získané výsledky, které zahrnují absorpční a emisní spektra, doby života a křivky dohasínání studovaných fluoroforů, uvádíme v práci spolu s jejich základními chemickými vlastnostmi ve formě katalogu. Obsahem katalogu je taktéž sada referencí získaná z literatury. Měrením pomocí časově korelovaného čítaní jednotlivých fotonů jsme zjistili, že 15 ze studovaných barviv je vhodných jako reference pro časově rozlišená měření ve frekvenční doméně.

Klíčová slova: doba života, frekvenční a časová doména, dohasínání fluorescence

Title: Frequency domain fluorescence spectroscopy of low molecular weight substances
Author: Jakub Zahumenský
Department: Institute of Physics of the Charles University - Biophysics Division
Supervisor: doc. RNDr. Petr Heřman, CSc.
Supervisor's e-mail address: Petr.Herman@mff.cuni.cz

Abstract: In the present work we study fluorescence properties of 23 laser dyes, whose emission maxima lie in the visible and in the near UV part of the spectrum. From these dyes, we select a set of reference standards suitable for time-resolved fluorimetric measurements in frequency domain. The acquired results, that include absorption and emission spectra, lifetimes and decay curves of the studied fluorophores, are stated in the work in the form of a catalog along with their basic chemical properties. The catalog includes a set of reference compounds from the literature, as well. Utilizing the time-correlated single-photon counting, we found out that 15 of the investigated

dyes are suitable as references for time-resolved measurements in frequency domain.

Keywords: lifetime, frequency- and time-domain, decay of fluorescence

Chapter 1

Introduction

1.1 Principle and properties of fluorescence

1.1.1 Jablonski diagram

The phenomenon of fluorescence is best explained with the help of Jablonski diagram (figure 1.1). The singlet ground, first excited and second excited states are depicted by S_0, S_1 and S_2, respectively, the first excited triplet state is depicted by T_1 and various vibrational levels are depicted by 0, 1, 2; 0 having the lowest energy. Transition of electrons between various energy states are depicted as vertical lines/arrows.

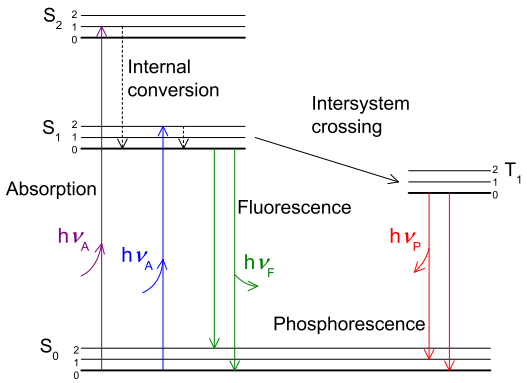


Figure 1.1: Jablonski diagram

Upon excitation of an atom, it accepts a certain quantum of energy in a form of a photon. This is called *absorption*. The atom can be excited

to various vibrational energy levels of S_1 state (most commonly), S_2, or sometimes even higher states. The excited atom usually immediately relaxes to the lowest vibrational level of S_1 (singlet state). This process is called *internal conversion*. The atom can also relax to the T_1 state (triplet state) due to spin-orbital interaction that changes the spin of the electron. This is called *intersystem crossing*. Both of these processes are non-radiative and are accompanied by a loss of energy of the electron. Internal conversion generally occurs in 10^{-12} s. The loss of energy results in longer wavelength of the emitted photon in comparison to the absorbed one. This phenomenon is called the *Stokes's shift* and can be directly observed in the absorption and emission spectra (see any absorption/emission spectrum in chapter 5).

The radiative transition from S_1 or T_1 states to any of the vibrational levels of S_0 is generally called *luminescence*. Substances that are luminescent are called *luminophores*. Depending on the excited state, we distinguish between *fluorescence* (transition from S_1 to S_0) and *phosphorescence* (transition from T_1 to S_0). For fluorescence, lifetimes (see section 1.1.2) near 10 ns or shorter are typical, whilst for phosphorescence, the lifetimes range generally from milliseconds to seconds. The reason for this large difference in lifetimes is that the transition from T_1 to S_0 is forbidden due to quantum mechanics (a spin conversion is essential) while the transmission from S_1 is allowed. In our work, we focused on the phenomenon of fluorescence.

1.1.2 Fluorescence lifetimes and quantum yields

Fluorescence lifetime and quantum yield are ones of the most important characteristics of a fluorophore. Their meaning is best described by the simplified Jablonski diagram (figure 1.2). Here, the processes leading to the relaxed S_1 state are not explicitly depicted. Instead, this diagram concentrates on the transitions from S_1 to S_0, particularly on the emissive rate Γ of the fluorophore and its rate of non-radiative decay to the ground state k_{nr}.

$Lifetime$ of a luminophore is the mean time, during which an electron occupies an excited state prior to its radiative transition to any of the vibrational energy levels of the ground state. During this time, the fluorophore can undergo many interactions with its environment, for example *quenching*, which is a non-radiative deexcitation of the fluorophore. As mentioned above, lifetimes of fluorescence are typically in nanosecond range.

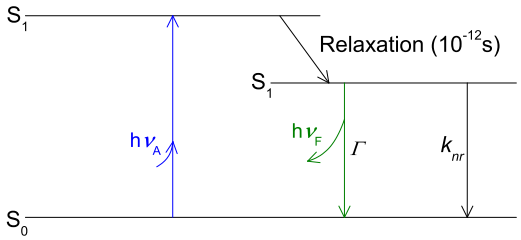


Figure 1.2: Simplified Jablonski diagram

Let us assume a very short light pulse that excites a fluorophore to the first excited state resulting in an initial population n_0. Using the emissive and the non-radiative decay rate, we can describe the depopulating of the excited state by a differential equation

$$\frac{\mathrm{d}n(t)}{\mathrm{d}n} = (\Gamma + k_{nr})n(t), \tag{1.1}$$

where $n(t)$ is the population of the excited state in the time t following the excitation. Solving this equation leads to an exponential decay of the excited state population: $n(t) = n_0 e^{-t/\tau}$. The same principle applies to intensity leading to a single exponential decay law:

$$I(t) = I_0 e^{-t/\tau}, \tag{1.2}$$

where the lifetime τ is the reciprocal of the total decay rate: $\tau = (\Gamma + k_{nr})^{-1}$. It is important to note that this is only true for fluorophores with mono-exponential decay, which is not always the case.

Quantum yield is defined as the ratio of the photons emitted to the number of absorbed photons. The number of absorbed photons per second is given by the sum of the emissive rate and the rate of non-radiative decay. The number of emitted photons per second is equal to Γ. Hence, the fluorescence quantum yield Q can be noted as

$$Q = \frac{\Gamma}{\Gamma + k_{nr}}. \tag{1.3}$$

Fluorophores with greater quantum yields display brighter emission.

1.2 Lifetime measurements

Lifetime measurements belong to so-called time-resolved methods. There are two among many that are dominant: the time-domain and frequency-domain method.

In *time-domain* or *pulse measurements (TD)* a short pulse of light of an appropriate wavelength is used to excite the fluorophore. Following the excitation, the time dependent intensity is measured and the decay time is calculated from the plot of $\ln I(t)$ or from the time that it takes the intensity to drop to $1/e$ of the intensity at t=0. More common is to fit the experimental data to assumed decay model (usually mono- or multi-exponential). The intensity decays are always measured through a polarizer. In order to avoid the effects of rotational diffusion on the intensity decay, the "magic angle" condition is used (see [1] pg. 43).

On the contrary, by *frequency-domain* or *phase-modulation method (FD)* we use an intensity-modulated light instead of a short pulse for excitation of the investigated sample. The modulated light is typically sine-shaped. To get reliable results from the measurements, the reciprocal of the modulation frequency ω has to be comparable with the decay time. When this method of excitation is used, the sample is forced to emit light with the same modulation frequency, but of less modulation. Furthermore, due to the intermolecular processes that the excited electron undergoes prior to the actual fluorescence, the emission is delayed in time relative to the excitation (see figure 1.3). This time delay can be measured as a phase angle shift between excitation and emission. By measuring of this *phase angle* (often also called *phase shift*) ϕ we can estimate the decay time τ of the sample using the equation

$$\tau_\phi = \omega^{-1} \tan\phi, \tag{1.4}$$

where ω is the modulation frequency in radians.s^{-1}. The polarizer in the magic angle position should be used by FD measurements as well.

The sample excited at the peak of the excitation waveform continues to emit when the excitation is at its minimum, which is the reason why the emitted light has a lesser intensity modulation than the excitation. This effect is called *demodulation* and its extent is dependent on the decay time of the sample and the modulation frequency. Thus, knowing the value of the frequency, we can estimate the decay time through measuring of demodulation m. The meaning of the demodulation can be described with the help

of figure 1.3, where one can see parameters a and b that mean peak-to-peak high and average intensity of the incident light, respectively. The parameters A and B have the same meaning for the emitted light. The ratios a/b and A/B are called the modulation of the incident and emitted light, respectively. The demodulation parameter m is then calculated as a ratio of the modulation of the emitted light relative to the modulation of the incident light: $m = (B/A)/(b/a)$. The decay time can be estimated by measuring of the demodulation parameter following a calculation according to the formula

$$\tau_m = \frac{1}{\omega}\sqrt{\frac{1}{m^2} - 1}. \tag{1.5}$$

In the case of a mono-exponential decay the phase (τ_ϕ) and demodulation (τ_m) lifetimes have the same value equal to the actual lifetime τ of the fluorophore. If the decay is more complex, then equations (1.4) and (1.5) yield apparent lifetimes that represent the weighted average of the decay components. (For further information see [1], pg. 99).

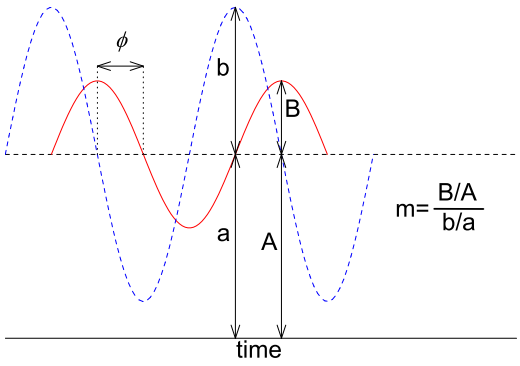


Figure 1.3: Demodulation: the ratios b/a and B/A represent the modulation of the excitation and emission respectively

In the past, using the FD instrumentation, one was able to determine the mean lifetime, but was not able to resolve any complex decay law. This is no longer true and one can use FD for determining of any type of decay function with reliable results. In the measurements of biological molecules, mono-exponential decay is rather an exception. It is therefore essential to understand other types of decay, as well. The simplest complex decay is multi-exponential decay, which can be explained as a linear combination of mono-exponential decays:

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i}, \tag{1.6}$$

where α_i and τ_i are amplitudes and lifetimes of individual decay components.

It can be useful to know, how specific components of the decay contribute to the overall steady-state intensity of the emission. To calculate the contribution of a certain component, one can use the equation

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j}. \tag{1.7}$$

This is only true, if the used multi-exponential model describes the actual decay. It is important to note that one can fit a multi-exponential model to almost any experimental data, so one has to be very careful, whether the obtained values actually have the physical meaning one wishes for.

When using the multi-exponential model, it is often useful to determine the mean lifetime $\bar{\tau}$. To calculate this value, we use the equation

$$\bar{\tau} = \langle \tau \rangle = \frac{\sum_i \alpha_i \tau_i^2}{\sum_i \alpha_i \tau_i} = \sum_i f_i \tau_i. \tag{1.8}$$

It is clear from this equation that for a mono-exponential decay, $\bar{\tau}$ has the meaning of actual lifetime.

In FD measurements, the phase angle and demodulation are measured over a wide range of frequencies. These data are called the *frequency response* of the sample. The characteristic features of the frequency response of a sample are illustrated in figure 1.4 for a single- and double-exponential decay.

If the decay is mono-exponential, we can take any of the measured values of m and ϕ and calculate the actual lifetime using equations (1.5) and/or (1.4). If the decay is more complex, it is essential to measure the modulation and phase shift over the largest possible interval of modulation frequencies. To estimate the decay times one takes the interval of frequencies, where the phase angle is frequency dependent and the modulation still measurable. It is the interval close to the intersection of the two experimental curves (in figure 1.4 (right) it is approximately the interval between 10 and 100 MHz).

In actual FD measurements one should perform a comparison of the phase shift and demodulation of the sample's emission relative to scattered light. Due to spectral dependence of photomultiplier tubes (PMTs), it is

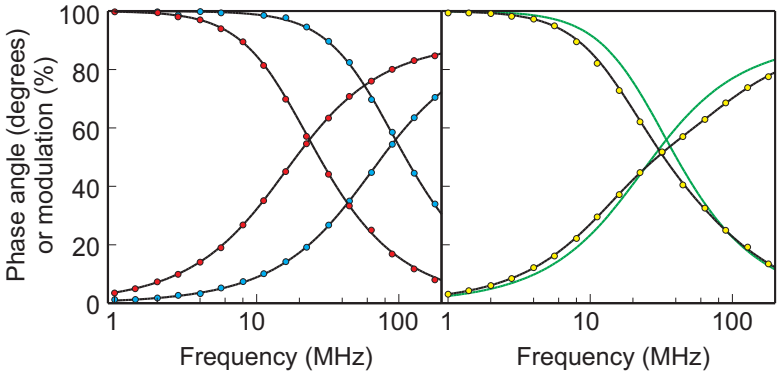


Figure 1.4: Simulated FD data for two independent fluorophores with lifetimes $\tau_1 = 10$ ns (red) and $\tau_2 = 2.5$ ns (blue) fitted with the singe-exponential model (left) and a single fluorophore with two components with lifetimes $\tau_1 = 10$ ns and $\tau_2 = 2.5$ ns (right). The upper lines show fitted demodulation and the lower lines show fitted phase shift dependence on the change of modulation frequency. For illustration purpose, there is also a single-exponential fit in the right picture (green).

advantageous to do this comparison on the same wavelength and use well-characterized reference compounds for this purpose. Mono-exponential decay and accurate knowledge of the fluorescent lifetime of the reference is an essential requirement for valid FD measurements. For further information on the method see [1], pg. 168.

1.3 Goals of the thesis

Time-resolved fluorescence spectroscopy is a very significant method when studying structure and dynamics of macromolecules and biological systems. For accurate phase measurements, use of an appropriate reference fluorophore is essential and strict requirements on its photostability, fluorescence lifetime and spectral characteristics are needed to be fulfilled. The main goal of our work was to create a well-characterized set of fluorescence standards for time-resolved frequency-domain measurements. Emission maxima of these compounds lie in the visible and near UV part of the spectrum. The created set of references will be used in the future for measurements of important organic molecules.

Chapter 2

Material and methods

We selected twenty-three fluorophores (laser dyes) (table 2.1), by which we expected short lifetimes and single-exponential decays. Then we prepared dilute solutions of the selected dyes in spectroscopic methanol. We preferred methanol as solvent to water, even though methanol is a volatile liquid. The reason for this is simple. Because stock solutions of the compounds are usually used, we had to consider that over a long period of time, microorganisms could start to grow in water, even in distilled water of high purity. Furthermore, the solubility of the dyes in methanol is better than in water. Therefore, we decided to use methanol and store the solutions in a freezer at $-20°C$.

To obtain the absorption spectra we used *Cary 50 UV-Vis spectrophotometer*[1] that uses a Xenon lamp as an excitation source. It is important to use solutions of a proper concentration when acquiring the absorption spectra. Too dilute samples give unsatisfactory signal-to-noise ratio and too dense solutions cause deformation of the peaks due to a non-linear response of the instrument.

After having measured absorption spectra of the samples, we measured their emission spectra using a *Fluoromax-2* spectrometer[2]. In these measurements, we excited the samples with a light beam from a Xenon lamp at the maximum of the sample's absorption. Once again, it was necessary to prepare samples with appropriate concentration to avoid an inner filter effect ([1] pg. 55).

[1] Varian, Inc.

[2] ISA Jobin Yvon-Spex Instruments SA, Inc.

The actual lifetime measurements were performed with a pulse dye laser and the time-domain method using time-correlated single-photon counting ([1], pg. 103). The reason for this was that some samples exhibited very short lifetimes and for lifetimes near 100 ps we would need a modulation frequencies near 1 GHz, which was beyond the capability of our equipment. We therefore had to use the time-domain method.

Dye	Supplier	Molecular formula	MW
Bis-MSB	-	$C_{24}H_{22}$	310,44
Butyl PBD	λChrome	$C_{24}H_{22}N_2O$	354,45
Coumarin 440	Exciton	$C_{10}H_9NO_2$	175,15
Coumarin 450	Exciton	$C_{13}H_{15}NO_2$	217,00
Coumarin 460	Exciton	$C_{14}H_{17}NO_2$	231,30
Coumarin 480	Exciton	$C_{16}H_{17}NO_2$	255,32
Coumarin 515	λChrome	$C_{21}H_{21}N_3O_2$	347,42
Coumarin 535	Exciton	$C_{20}H_{19}N_3O_2$	333,39
Coumarin 540	Exciton	$C_{20}H_{18}N_2O_2S$	350,44
Cresyl Violet 670	Exciton	$C_{16}H_{11}N_3O.HClO_4$	361,74
DCM	Exciton	$C_{19}H_{17}N_3O$	303,37
DOTC Iodide	-	$C_{25}H_{25}N_2O_2.I$	512,39
LDS 698	λChrome	$C_{19}H_{23}N_2O_4Cl$	378,85
LDS 722	Exciton	$C_{19}H_{23}N_2.ClO_4$	378,86
LDS 820	Exciton	$C_{23}H_{25}N_2S.ClO_4$	460,98
PPD	-	$C_{14}H_{10}N_2O$	222,24
Rhodamine 560 Chloride	Exciton	$C_{20}H_{14}N_2O_3.HCl$	366,80
Rhodamine 610 Chloride	Exciton	$C_{28}H_{31}N_2O_3.Cl$	479,02
Rhodamine 640 Perchlorate	Exciton	$C_{32}H_{31}N_2O_3.ClO_4$	591,05
Rhodamine 800	λChrome	$C_{26}H_{26}N_3O_5Cl$	495,52
Stilbene 1	Exciton	$C_{26}H_{18}O_6S_2K_2$	568,74
Stilbene 420	Exciton	$C_{28}H_{20}O_6S_2.2Na$	562,56
Uranin	λChrome	$C_{20}H_{10}O_5.2Na$	412,30

Table 2.1: Selected dyes for lifetime measurements; MW=molecular weight

The measurements were carried out with an apparatus that consisted of two lasers, an actively mode-locked argon ion pulse laser - *Spectra Physics, model 171* and a dye laser - *Spectra Physics, model 375*, used for excitation and a detector, PMT - *Hamamatsu, R3809U-50*, oriented 90° relative to the excitation beam. The pulse laser produces short, 5 ps wide, light pulses at

the frequency of 4 MHz. The wavelength of this pulses is 514,5 nm. This light beam is then shifted to 630 nm in the second laser that uses Rhodamine 6G (Rhodamine 590) laser dye as a medium. The beam is then doubled in a BBO crystal to create a beam with a 315 nm wavelength[3]. As the instrumentation does not allow tuning over a broader interval of wavelengths, we excited all of the samples with this beam of pulsed light. To eliminate scattered light and influence of the excitation beam, we used a total of three long-pass filters to cut off wavelengths shorter than the emission maximum of the investigated dye, leaving the longer wavelengths and the emission peak untouched. We used filters with cut-off wavelengths of 350, 405 and 550 nm. In addition, we put a monochromator between the filter and the PMT to select the emission maximum. To change the intensity of the excitation light, we used neutral density filters.

Prior to and after measuring of the actual decay of the samples, we acquired the so-called *instrument response function (IRF)*, which is the response of the instrument to the excitation pulse. To get the IRF, one carries out a lifetime measurement with a scattering sample, typically Ludox. The function depends on the shape of the excitation pulse and how the pulse is detected. It is not a δ-function and its shape has to be therefore taken into account when analyzing the experimental data. The reason for acquiring the IRF prior to the actual measurement and after it as well is to be able to detect any drifts of the instrumentation, which would lead into a difference between the two measured functions. If the difference was too big, one would need to check the instrumentation for failures and perform the lifetime measurements of the samples again. When there is a slight, but not a big difference between the two IRFs, one can use the first one for the analysis of the first half of the experimental data and the second one for another half. This leads to more accurate results.

To analyze the decays, we used a computer software that uses the least-square analysis to fit the obtained data to a single- or multi-exponential decay model. Through this procedure we were able to determine the decay functions of the selected fluorophores and hence the lifetimes of the substances.

To illustrate the use of the created set of lifetime standards, we performed one FD measurement. For this purpose we used *ISS K2 multifrequency phase*

[3]The reason for this experimental set-up is that the apparatus is generally used for protein studies that have to be excited with wavelengths from the UV part of the spectrum.

fluorometer with a LED and a monochromator set at 521 nm as the excitation source. To cut off unwanted emission, we used a 570 nm long-pass filter placed in front of the PMT. The measurement itself was performed on Rhodamine 640 with Rhodamine 610 as reference using fifteen modulation frequencies from 1 to 150 MHz, where we measured both, demodulation and phase shift. The lifetime of the investigated sample was calculated via the least-square analysis used to fit the experimental data with a suitable function.

Chapter 3

Results and discussion

3.1 Absorption and emission spectra

We measured absorption and emission spectra of twenty-three laser dye solutions in spectroscopic methanol. Absorption and emission maxima are listed in table 3.1.

The gained absorption spectra are in good correspondence with the spectra listed in [2], although these spectra were measured for solutions of the dyes in ethanol. For full absorption and emission spectra of the investigated dyes see chapter 5.

3.2 Lifetime measurements

To obtain the lifetime of the dyes, we performed time-resolved TD measurements using time-correlated single-photon counting. We used the least-square analysis to calculate the decay curves from the acquired data. In case of multi-exponential decays, we used (1.8) to calculate the mean lifetimes and (1.7) to calculate the fractional intensities of the individual components of the decay. The (mean) lifetimes are listed in table 3.1 that is sorted according to emission maxima of the dyes. For full information on the dyes, including the measured decay curves, along with the fit of the data, see chapter 5.

The distribution of weighed residua can seem not exactly random in some cases, which can be caused by small impurities in the prepared samples.

The lifetime of Rhodamine 610 (Rhodamine B) in methanol obtained from our measurement is $\tau = 2,47$ ns, which is in good correspondence with the value stated in [3] (see also table 5.2), according to which the lifetime is $\tau = 2,5 \pm 0,1$ ns.

Dye	λ_{max}^{abs}[nm]	λ_{max}^{em}[nm]	τ[ns]
Butyl PBD	304,5	363,5	1,03
PPD	280,5	342	1,33
Stilbene 1	342,5	408	0,888*
Bis-MSB	347,5	416	1,64*
Stilbene 420	349,5	422	1,09*
Coumarin 440	352	439	3,80*
Coumarin 450	366	443	3,92
Coumarin 460	374	449	2,06
Coumarin 480	390,5	467	4,78
Coumarin 515	413,5	478	1,73
Coumarin 535	438,5	489	1,98*
Coumarin 540	459	502	2,45
Uranin	491,5	516	4,39*
Rhodamine 560 Chloride	502	520	4,09
Rhodamine 610 Chloride	546	568	2,47
Rhodamine 640 Perchlorate	568,5	586	4,54
DCM	465	614	1,37*
Cresyl Violet 670	596	618	3,28
LDS 698	483	663	0,210
LDS 722	496	686	0,344
Rhodamine 800	678,5	698	1,76
DOTC Iodide	683,5	704	1,25*
LDS 820	578	790	0,375

Table 3.1: Measured lifetimes of the selected laser dyes; an asterisk marks compounds with multi-exponential decay and the value is the mean lifetime. The table is sorted according to the emission maxima of the dyes.

To validate our results, we measured the fluorescence decay of Rhodamine 640 using time-resolved FD with Rhodamine 610 as the reference. We obtained a good fit with lifetime of Rhodamine 640 $\tau = 4,47$ ns, which is in good correspondence with the value obtained from the TD measurement. For plot of the FD data see figure 3.1.

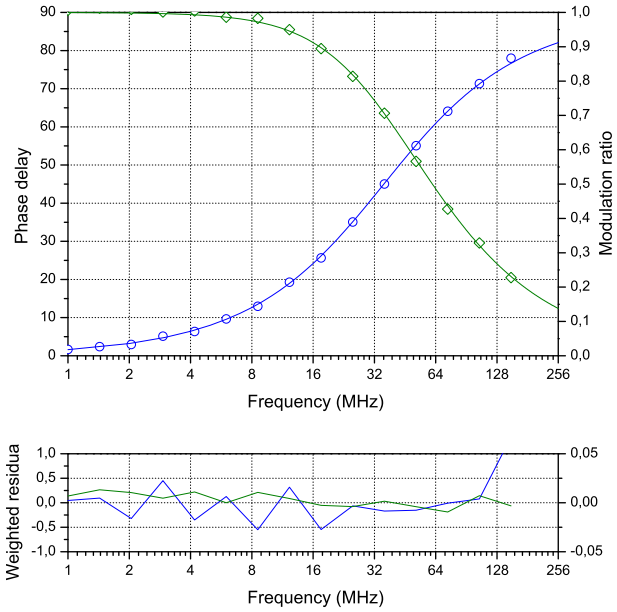


Figure 3.1: Frequency-domain data for Rhodamine 640 using Rhodamine 610 as the reference compound. The green points and lines suit the data acquired for modulation, the blue lines and points suit the data acquired for phase angle shift.

The sum of the least-squares $\chi^2 = 1,72$ is in this case higher than in TD measurements, which is caused by different noise characteristics of the obtained data.

Chapter 4

Conclusion

We have measured a total of twenty-three laser dyes, whose emission maxima range from the UV across the whole visible spectrum. We acquired their absorption and emission spectra and calculated their decay functions $F(t)$. In the case of multi-exponential decays, we also calculated the contribution of the decay components to the overall emission intensity of the samples using (1.7) and the mean lifetimes using (1.8). Fifteen of the investigated samples displayed mono-exponential decay; seven showed double-exponential decay and one showed a tripple-exponential decay (DOTC Iodide). Samples with mono-exponential decay are good lifetime references for FD measurements. All of our measurements of the selected laser dyes were carried out with their dilute solvents in methanol at a room temperature near 25°C.

The lifetimes range from 210 ps for LDS 698 to 4,78 ns for Coumarin 480. All dyes with their absorption and emission maxima and lifetimes are listed in table 3.1. For further information on the dyes, such as full absorption and emission spectra, chemical formulas, appearance etc., see chapter 5, where they are listed alphabetically.

Chapter 5

Appendix

In this chapter, we list all measured data for all studied laser dyes in the form of a catalog. For transparency, each emission/absorption spectrum contains the calculated lifetime of the dye. An asterisk next to the lifetime suggests that the dye displays a multi-exponential decay, consistently with table 3.1.

At the end of the appendix, there is also a table of additional lifetime standards from the literature. This table is sorted alphabetically, as well.

5.1 Bis-MSB

$C_{24}H_{22}$; 1,4-bis[2-(2-methylphenyl)ethenyl]-benzene

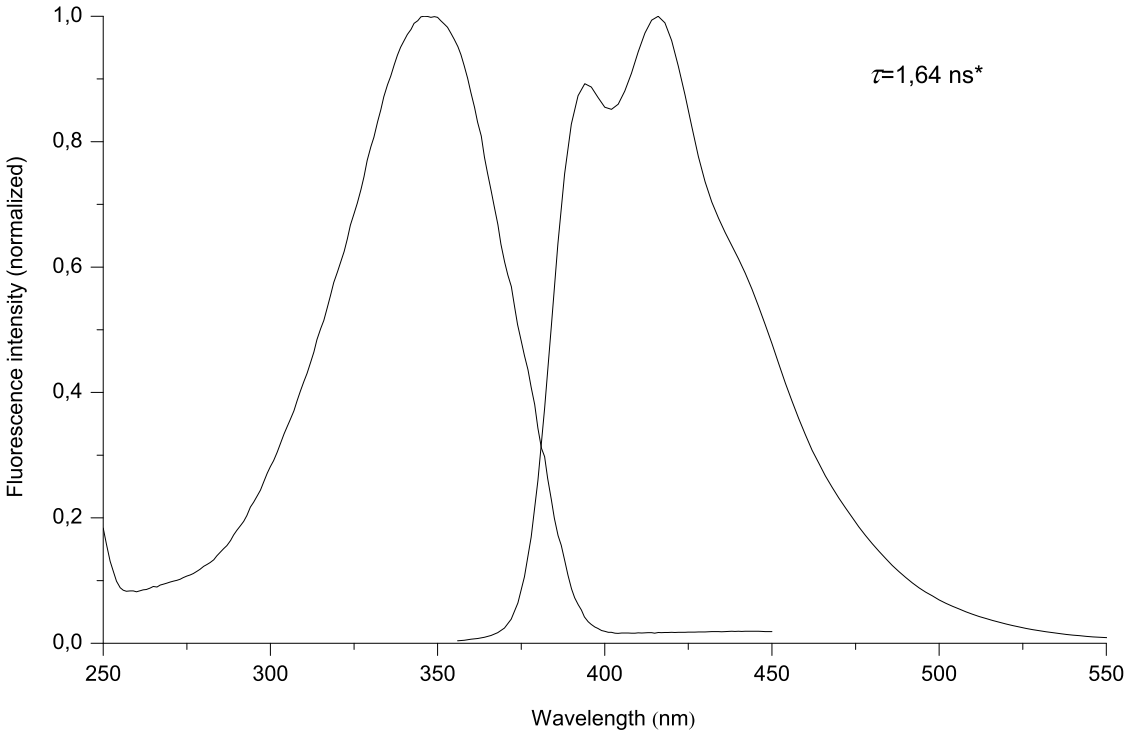


Figure 5.1: Emmision and absorption spectrum

Molecular weight: 310,44
Quantum yield: 0,88 in methanol; 0,89 in ethanol; 0,98 in cyclohexane [4]
Appearance: greenish yellow, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 347,5$ nm
Emission maximum: $\lambda_{max}^{em} = 416$ nm
Decay function: $F(t) = 0,73e^{-t/1,45} + 0,27e^{-t/2,00}$
Intensity ratio of the components: 0,66:0,34
Mean lifetime: $\bar{\tau} = 1,64$ ns
Reduced $\chi^2 = 1,025$

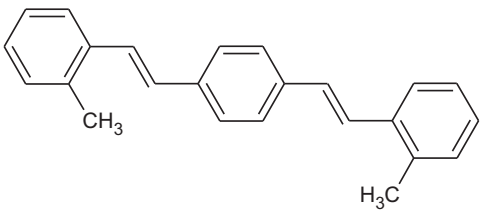


Figure 5.2: Chemical formula

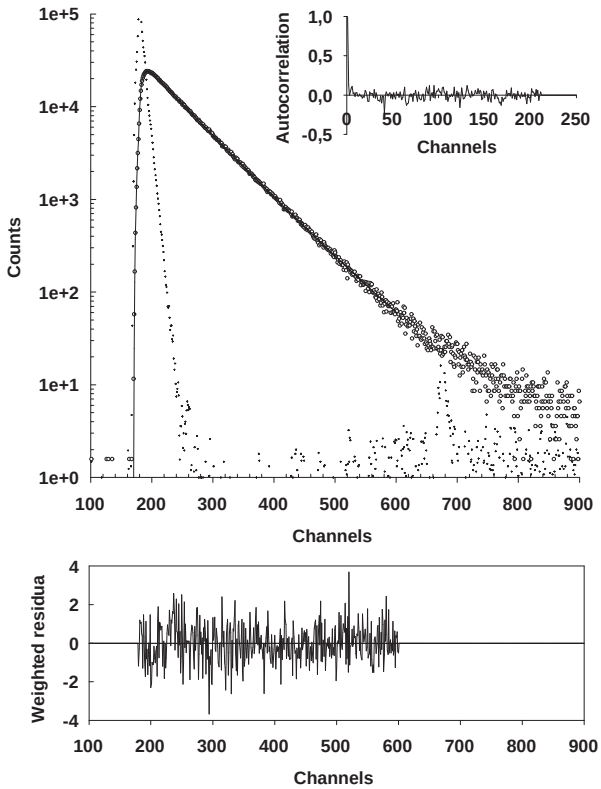


Figure 5.3: Fluorescence decay

5.2 Butyl-PBD (BPBD-365)

$C_{24}H_{22}N_2O$; 2-(4-biphenylyl)-5-(4-t-butylphenyl)-1,3,4-oxadiazol

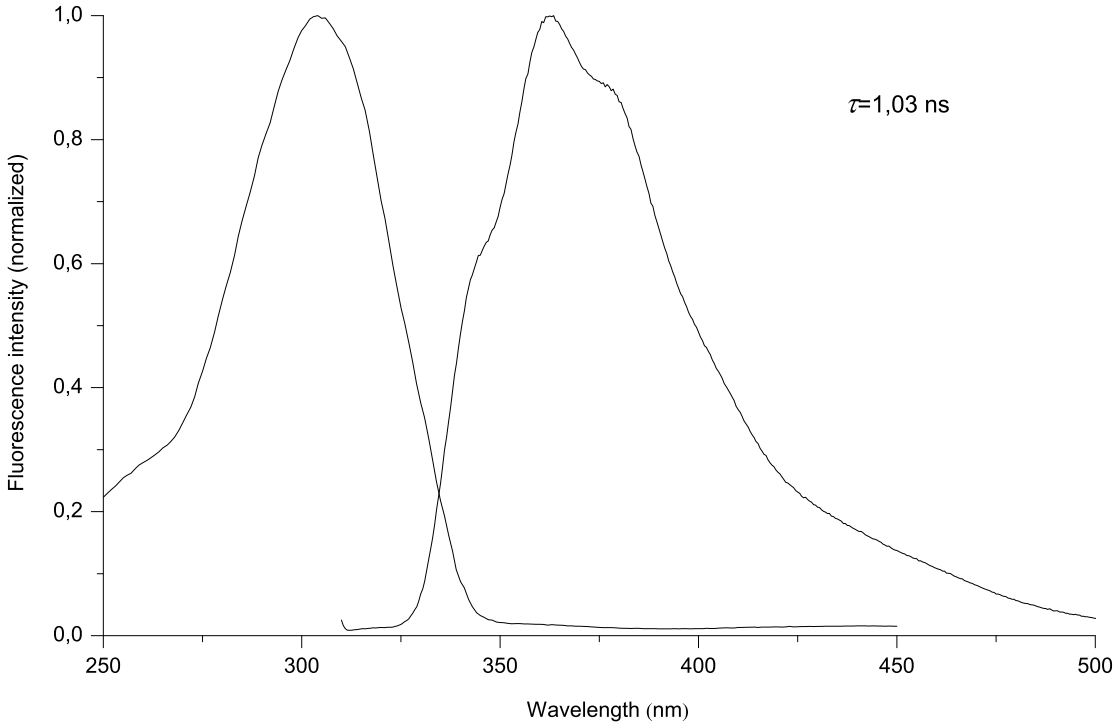


Figure 5.4: Emmision and absorption spectrum

Supplier: LambdaChrome
Molecular weight: 354,45
Appearance: white, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 304,5$ nm
Emission maximum: $\lambda_{max}^{em} = 363,5$ nm
Decay function: $F(t) = e^{-t/1,03}$
Mean lifetime: $\bar{\tau} = 1,03$ ns
Reduced $\chi^2 = 0,990$

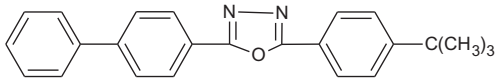


Figure 5.5: Chemical formula

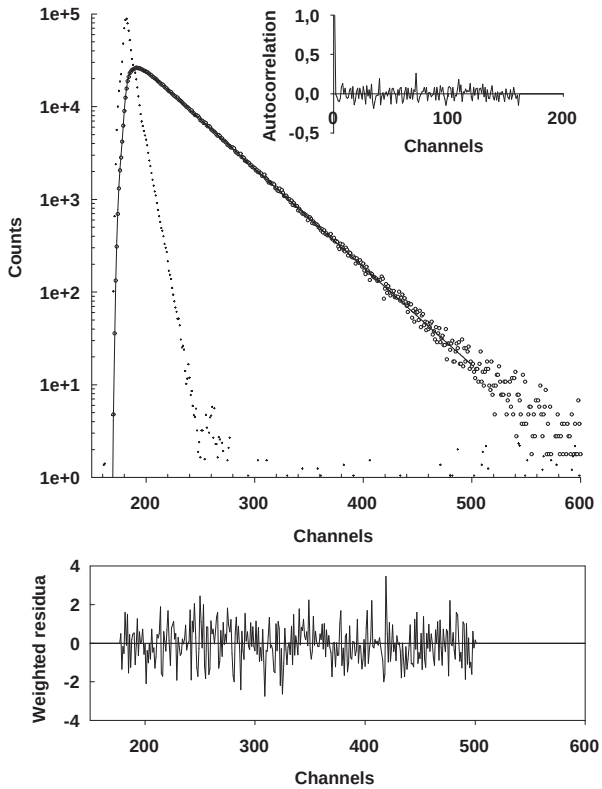


Figure 5.6: Fluorescence decay

5.3 Coumarin 440 (Coumarin 120)

$C_{10}H_9NO_2$; 7-amino-4-methyl-2H-1-benzopyran-2-one

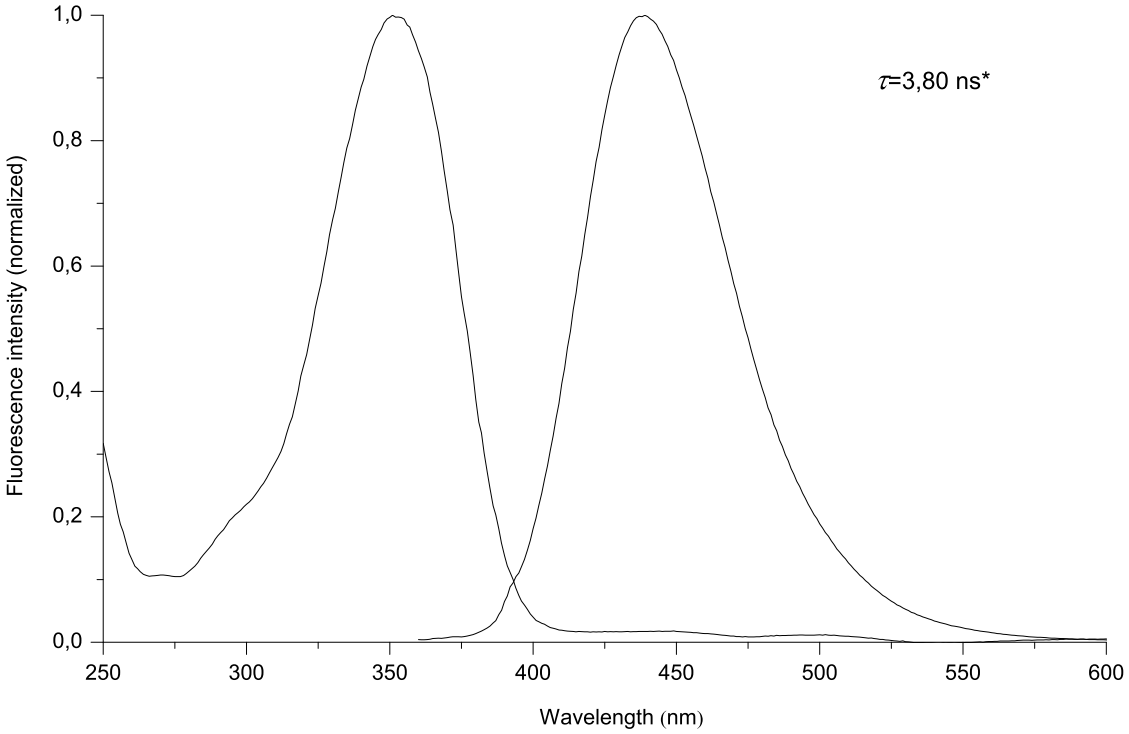


Figure 5.7: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 175,15
Appearance: light yellow, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 352$ nm
Emission maximum: $\lambda_{max}^{em} = 439$ nm
Decay function: $F(t) = 0,25e^{-t/0,986} + 0,75e^{-t/4,02}$
Intensity ratio of the components: 0,07:0,93
Mean lifetime: $\bar{\tau} = 3,80$ ns
Reduced $\chi^2 = 1,033$

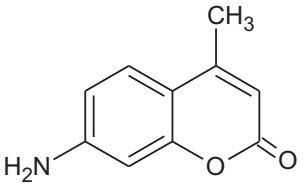


Figure 5.8: Chemical formula

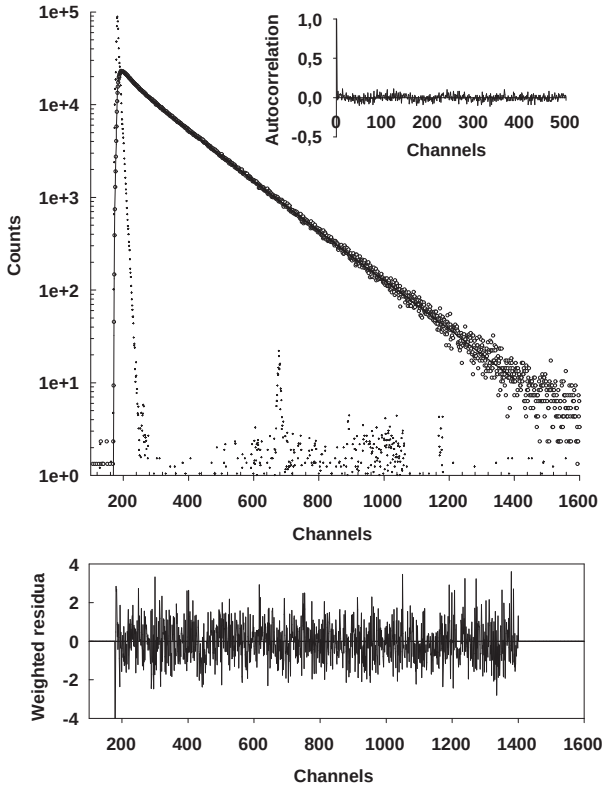


Figure 5.9: Fluorescence decay

5.4 Coumarin 450 (Coumarin 2)

$C_{13}H_{15}NO_2$; 7-(ethylamino)-4,6-dimethyl-2H,-1-benzopyran-2-one

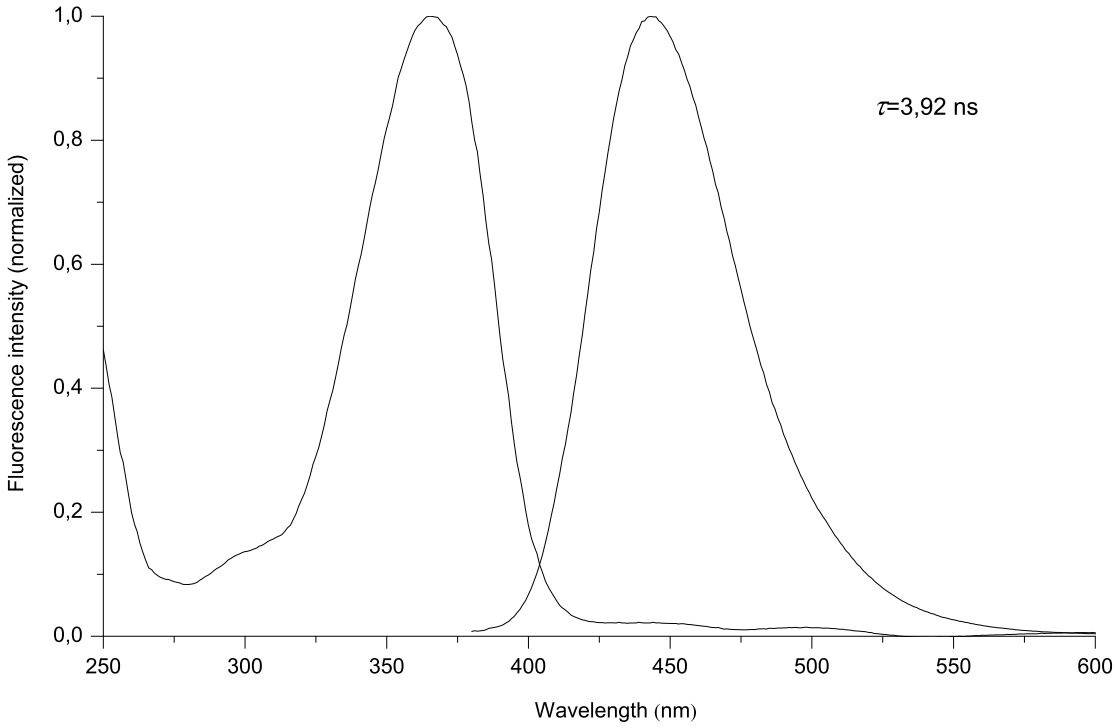


Figure 5.10: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 217,00
Appearance: pale yellow, crystalline powder
Absorption maximum: $\lambda_{max}^{abs} = 366$ nm
Emission maximum: $\lambda_{max}^{em} = 443$ nm
Decay function: $F(t) = e^{-t/3,92}$
Mean lifetime: $\bar{\tau} = 3,92$ ns
Reduced $\chi^2 = 0,983$

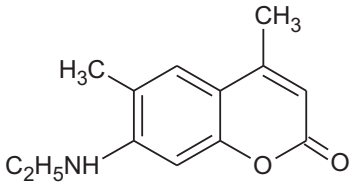


Figure 5.11: Chemical formula

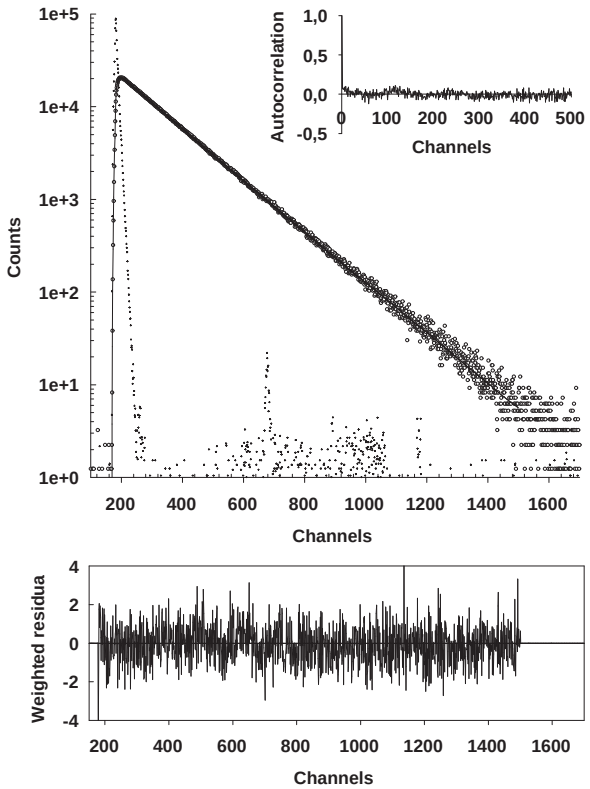


Figure 5.12: Fluorescence decay

5.5 Coumarin 460 (Coumarin 47; Coumarin 1)

$C_{14}H_{17}NO_2$; 7-(diethylamino)-4-methyl-2H-1-benzopyran-2-one

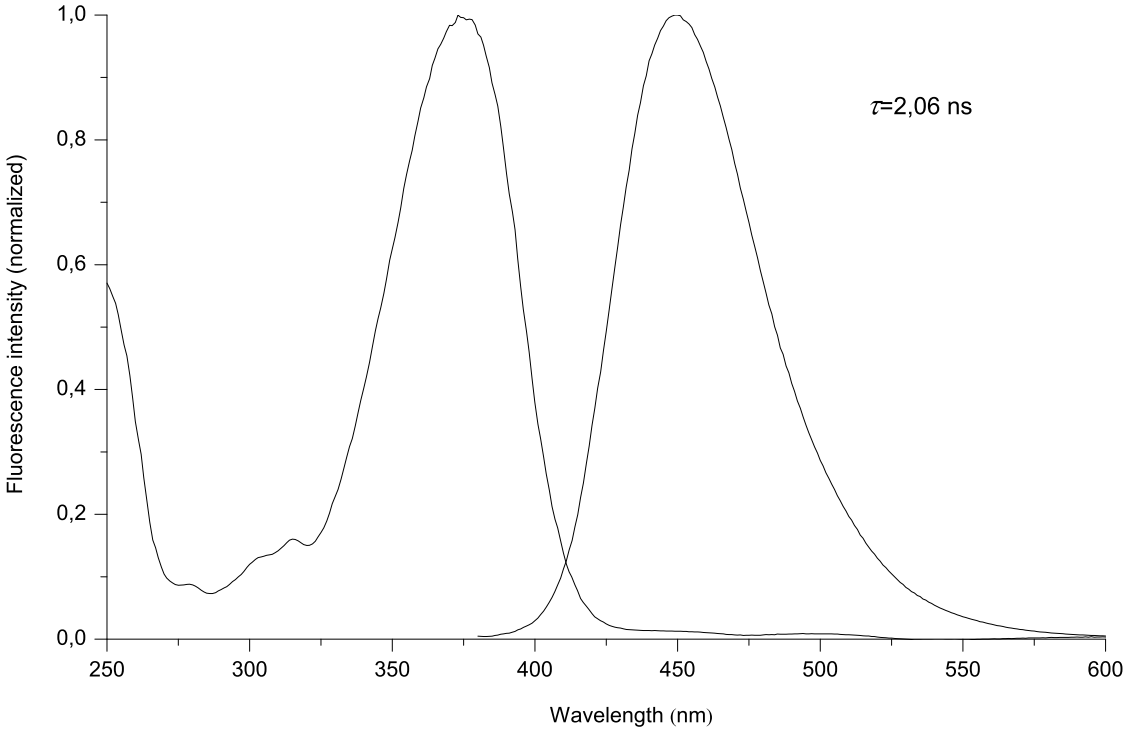


Figure 5.13: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 231,30
Quantum yield: 0,70 in ethanol [5]
Appearance: white, crystalline powder
Absorption maximum: $\lambda_{max}^{abs} = 374$ nm
Emission maximum: $\lambda_{max}^{em} = 449$ nm
Decay function: $F(t) = e^{-t/2,06}$
Mean lifetime: $\bar{\tau} = 2,06$ ns
Reduced $\chi^2 = 1,183$

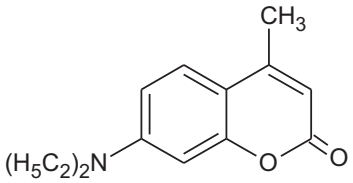


Figure 5.14: Chemical formula

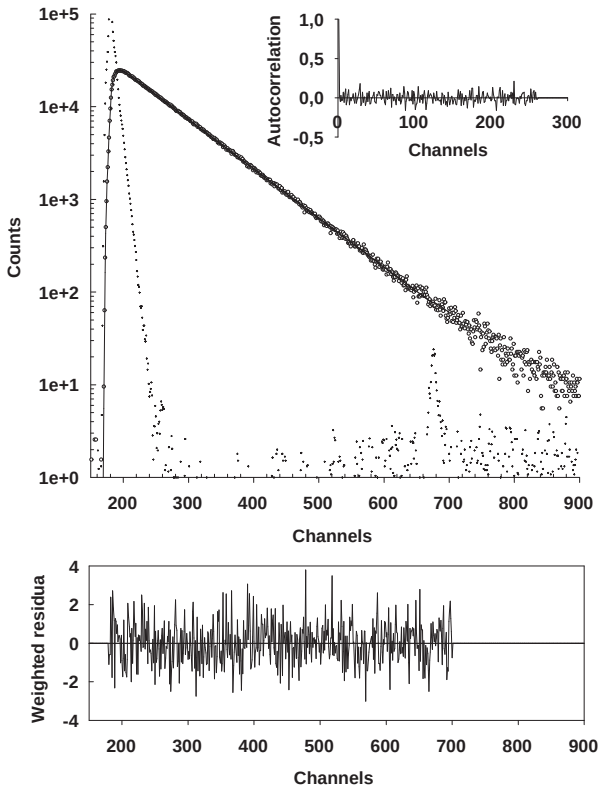


Figure 5.15: Fluorescence decay

5.6 Coumarin 480 (Coumarin 102)

$C_{16}H_{17}NO_2$; 2,3,6,7-tetrahydro-9-methyl-1H,5H,11H-[1]benzopyrano-[6,7,8-ij]quinolizin-11-one

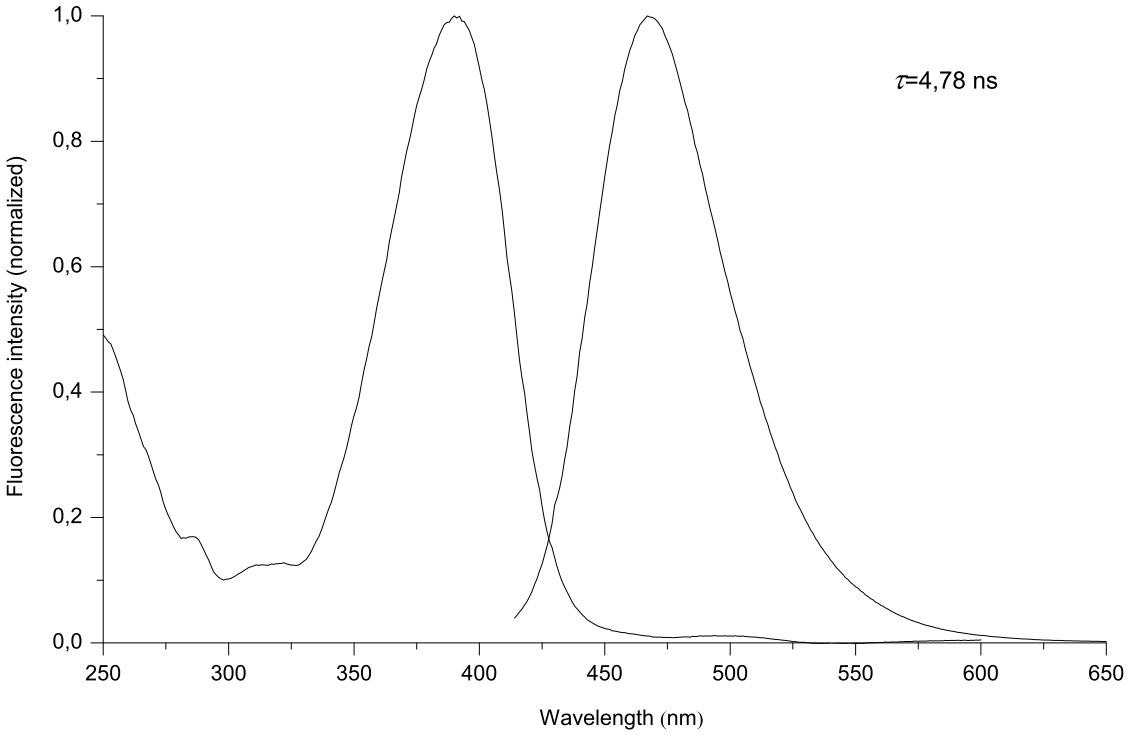


Figure 5.16: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 255,32
Appearance: pale yellow, crystalline powder
Absorption maximum: $\lambda_{max}^{abs} = 390,5$ nm
Emission maximum: $\lambda_{max}^{em} = 467$ nm
Decay function: $F(t) = e^{-t/4,78}$
Mean lifetime: $\bar{\tau} = 4,78$ ns
Reduced $\chi^2 = 1,010$

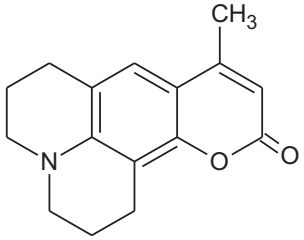


Figure 5.17: Chemical formula

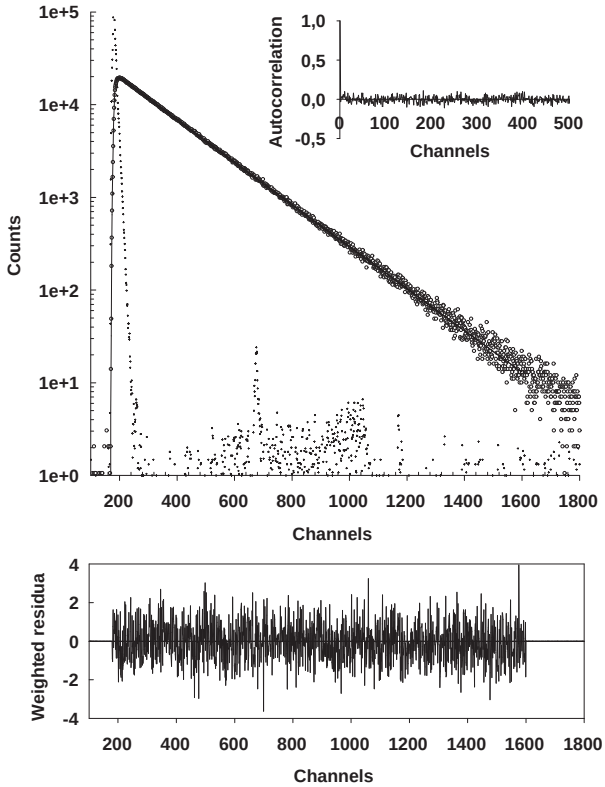


Figure 5.18: Fluorescence decay

5.7 Coumarin 515 (Coumarin 30)

$C_{21}H_{21}N_3O_2$; 7-(diethylamino)-3-(1-methyl-1H-benzimidazol-2-yl)-2H-1-benzopyran-2-one

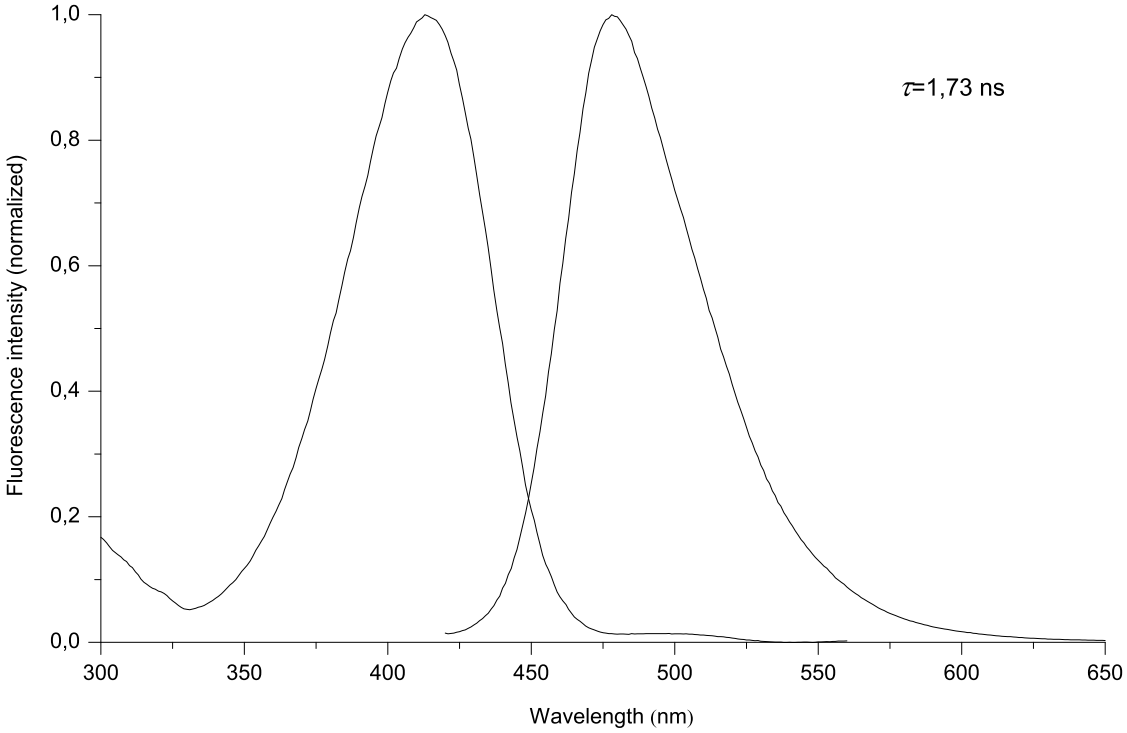


Figure 5.19: Emmision and absorption spectrum

Supplier: LambdaChrome
Molecular weight: 347,42
Appearance: yellow, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 413,5$ nm
Emission maximum: $\lambda_{max}^{em} = 478$ nm
Decay function: $F(t) = e^{-t/1,73}$
Mean lifetime: $\bar{\tau} = 1,73$ ns
Reduced $\chi^2 = 1,155$

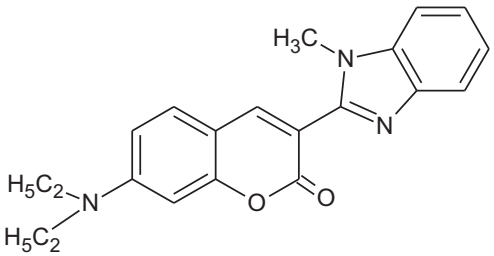


Figure 5.20: Chemical formula

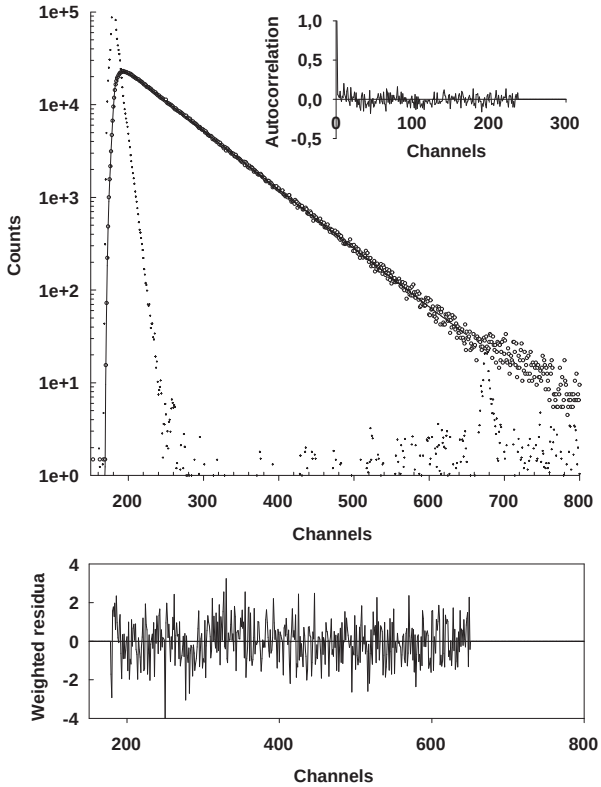


Figure 5.21: Fluorescence decay

5.8 Coumarin 535 (Coumarin 7)

$C_{20}H_{19}N_3O_2$; 3-(1H-benzimidazol-2-yl)-7-(diethylamino)-2H-1-benzopyran-2-one

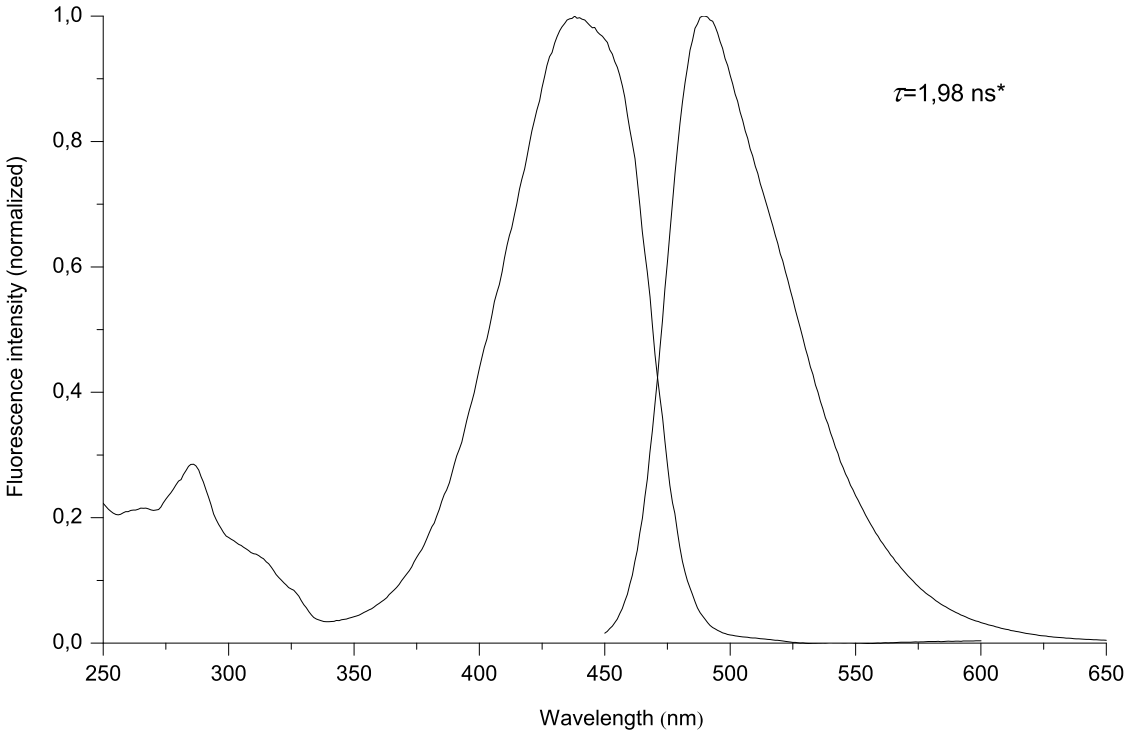


Figure 5.22: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 333,39
Appearance: yellow-orange, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 438,5$ nm
Emission maximum: $\lambda_{max}^{em} = 489$ nm
Decay function: $F(t) = 0,89e^{-t/1,78} + 0,11e^{-t/3,00}$
Intensity ratio of the components: 0,83:0,17
Mean lifetime: $\bar{\tau} = 1,98$ ns
Reduced $\chi^2 = 1,065$

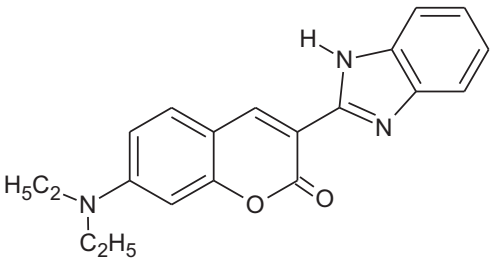


Figure 5.23: Chemical formula

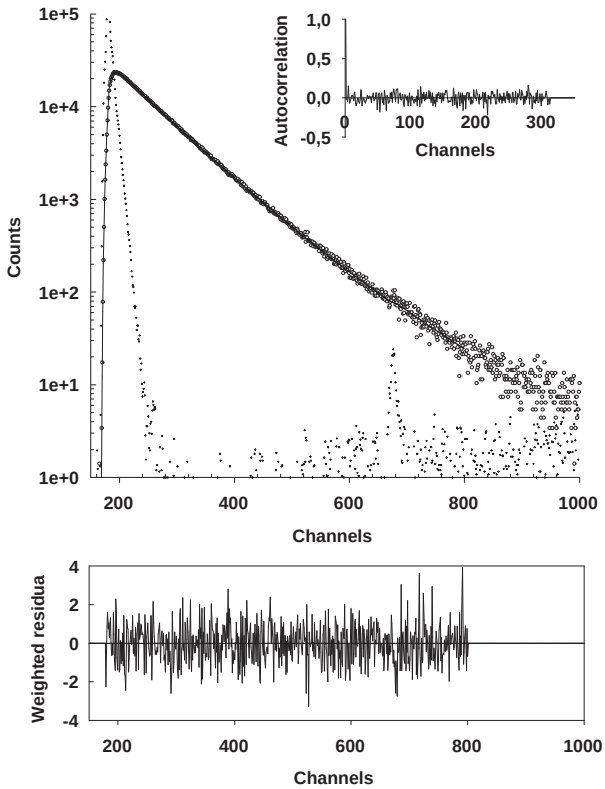


Figure 5.24: Fluorescence decay

5.9 Coumarin 540 (Coumarin 6)

$C_{20}H_{18}N_2O_2S$; 3-(2-benzothiazolyl)-7-(diethylamino)-2H-1-benzopyran-2-one

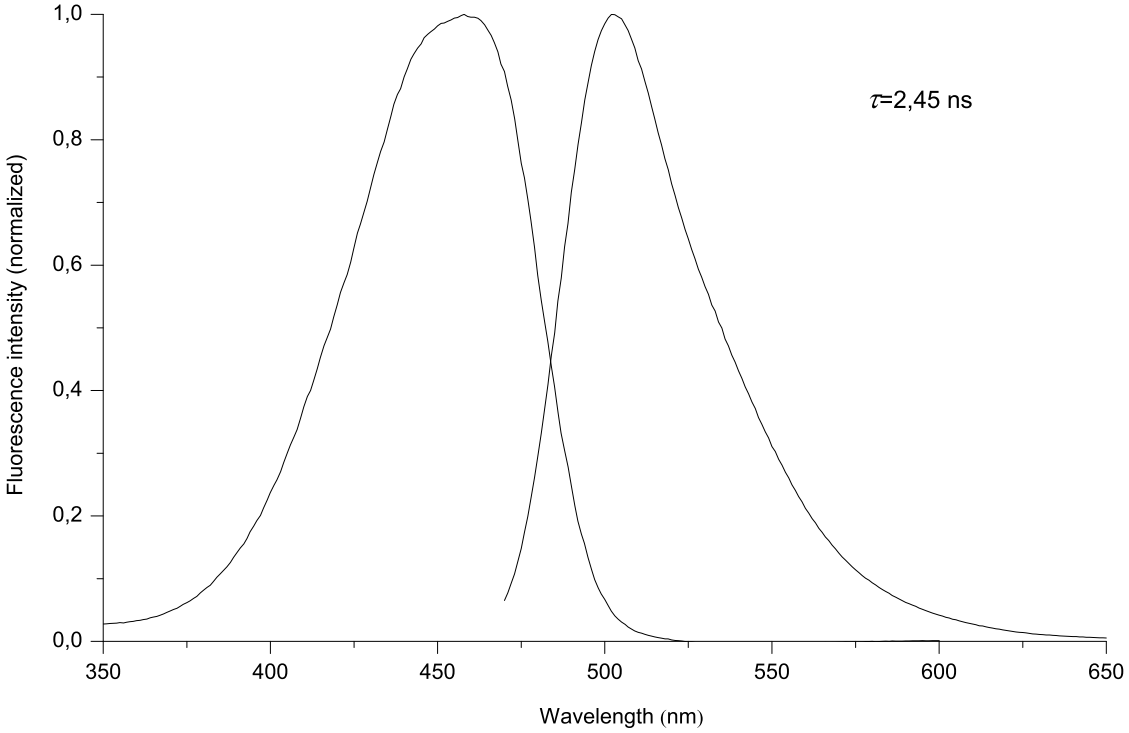


Figure 5.25: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 350,44
Quantum yield: 0,85 in ethanol [6]
Appearance: orange, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 459$ nm
Emission maximum: $\lambda_{max}^{em} = 502$ nm
Decay function: $F(t) = e^{-t/2,45}$
Mean lifetime: $\bar{\tau} = 2,45$ ns
Reduced $\chi^2 = 1,056$

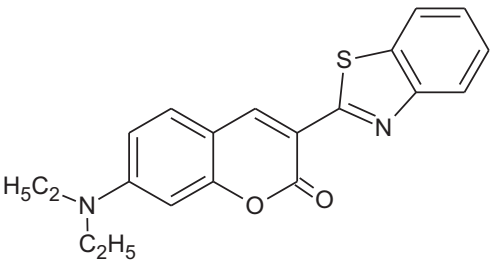


Figure 5.26: Chemical formula

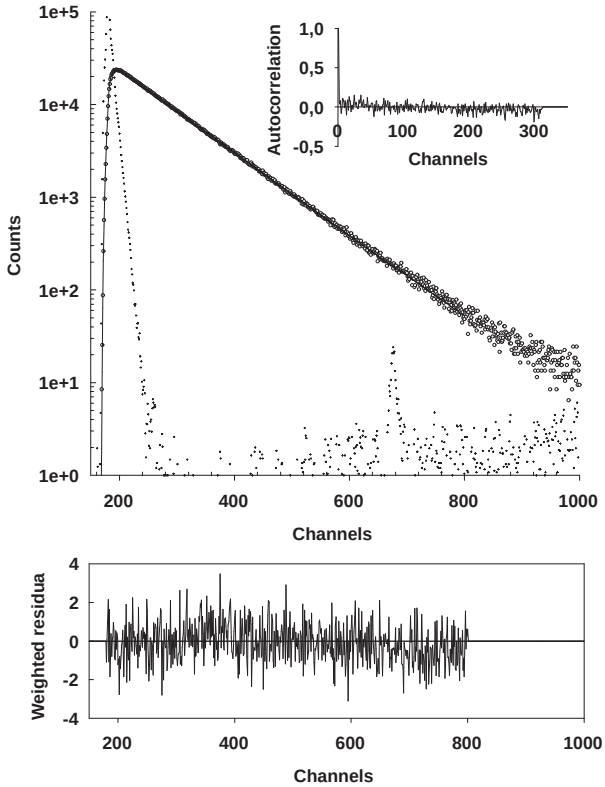


Figure 5.27: Fluorescence decay

5.10 Cresyl Violet 670 (Cresyl Violet)

$C_{16}H_{11}N_3O.HClO_4$; 5-imino-5H-benzo[a]phenoxazin-9-amine monoperchlorate

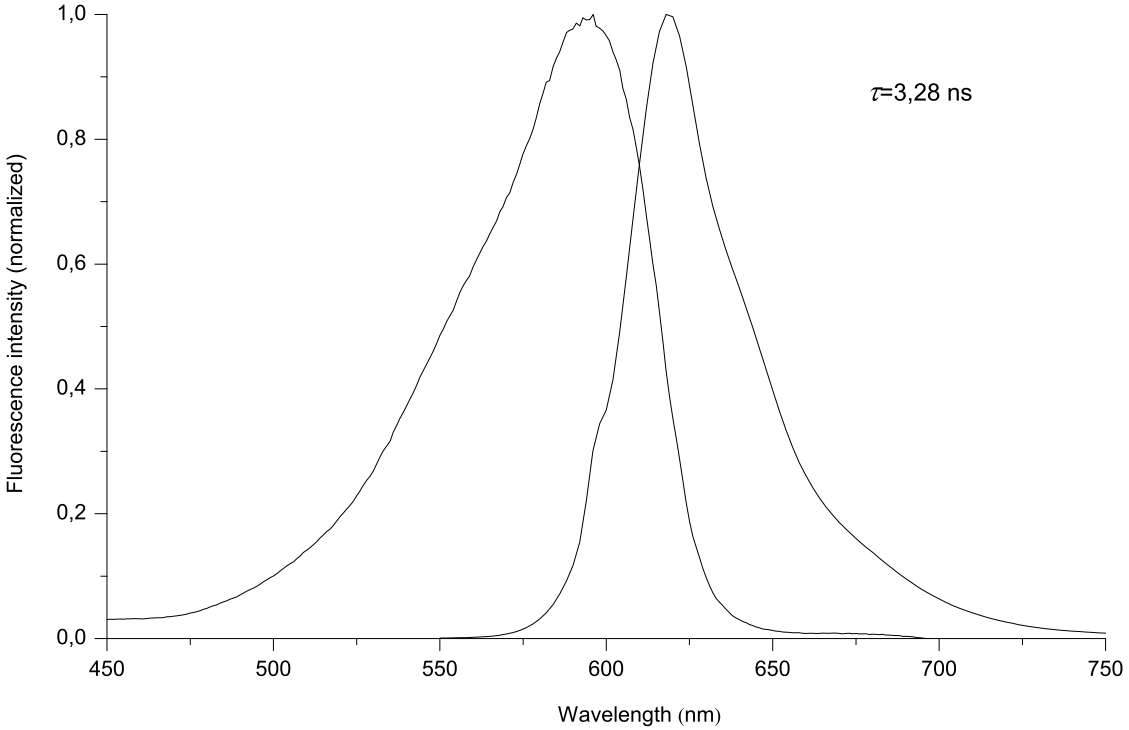


Figure 5.28: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 361,74
Quantum yield: 0,55 in methanol [3]; 0,54 in methanol [7]; 0,59 in ethanol [6]
Appearance: green, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 596$ nm
Emission maximum: $\lambda_{max}^{em} = 618$ nm
Decay function: $F(t) = e^{-t/3,28}$
Mean lifetime: $\bar{\tau} = 3,28$ ns
Reduced $\chi^2 = 1,038$

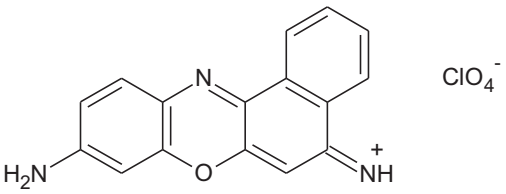


Figure 5.29: Chemical formula

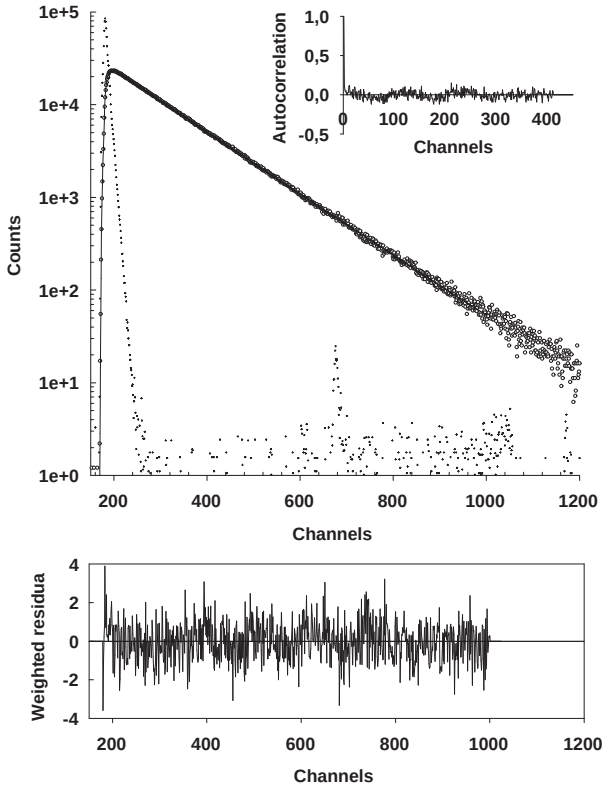


Figure 5.30: Fluorescence decay

5.11 DCM

$C_{19}H_{17}N_3O$; [2-[2-[4-(dimethylamino)phenyl]ethenyl]-6-methyl-4H- pyran-4-ylidene]-propanedinitrile

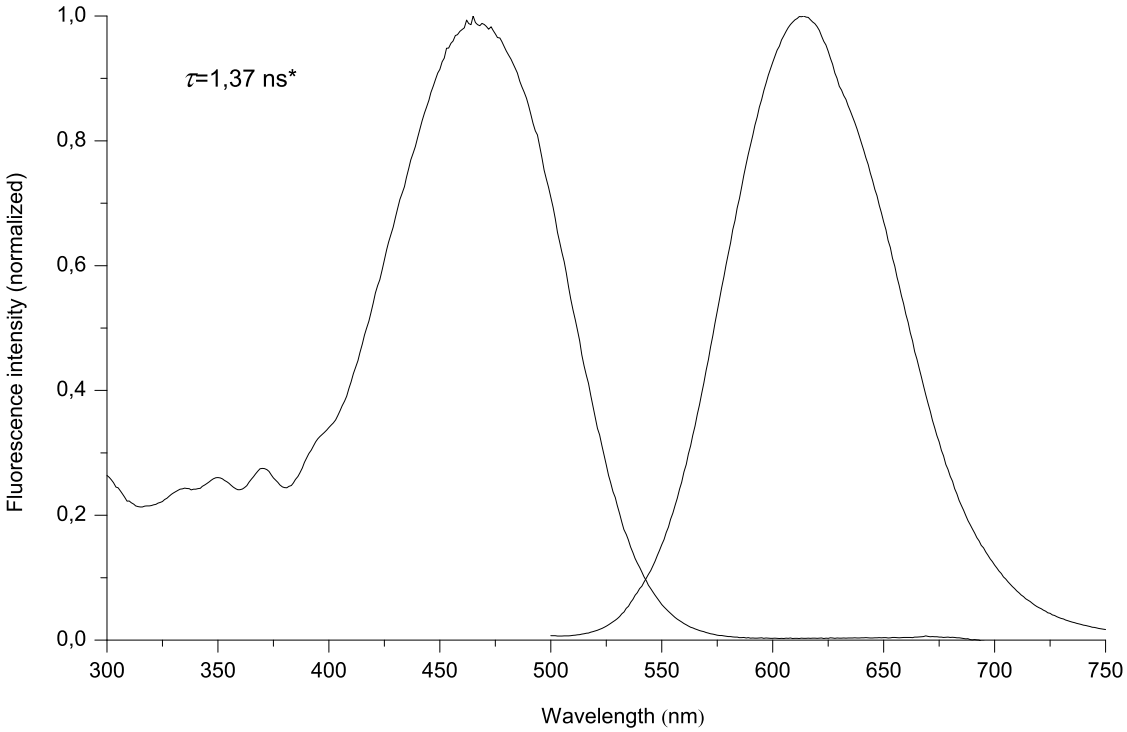


Figure 5.31: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 303,37
Appearance: red or orange-red, crystalline solid or powder
Absorption maximum: $\lambda_{max}^{abs} = 465$ nm
Emission maximum: $\lambda_{max}^{em} = 614$ nm
Decay function: $F(t) = 0,12e^{-t/0,624} + 0,88e^{-t/1,41}$
Intensity ratio of the components: 0,06:0,94
Mean lifetime: $\bar{\tau} = 1,37$ ns
Reduced $\chi^2 = 0,935$

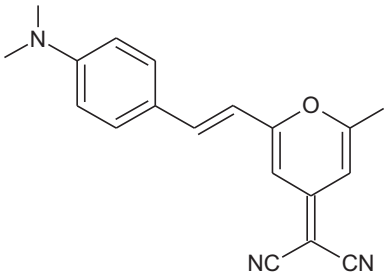


Figure 5.32: Chemical formula

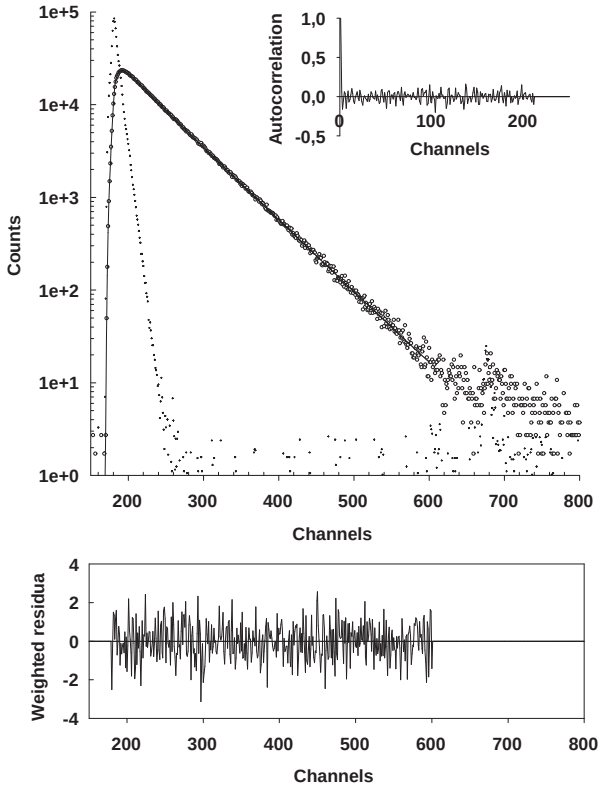


Figure 5.33: Fluorescence decay

5.12 DOTC Iodide

$C_{25}H_{25}N_2O_2.I$; 3-ethyl-2-[7-(3-ethyl-2(3H)-benzoxazolylidene)-1,3,5- heptatrienyl]-benzoxazolium iodide

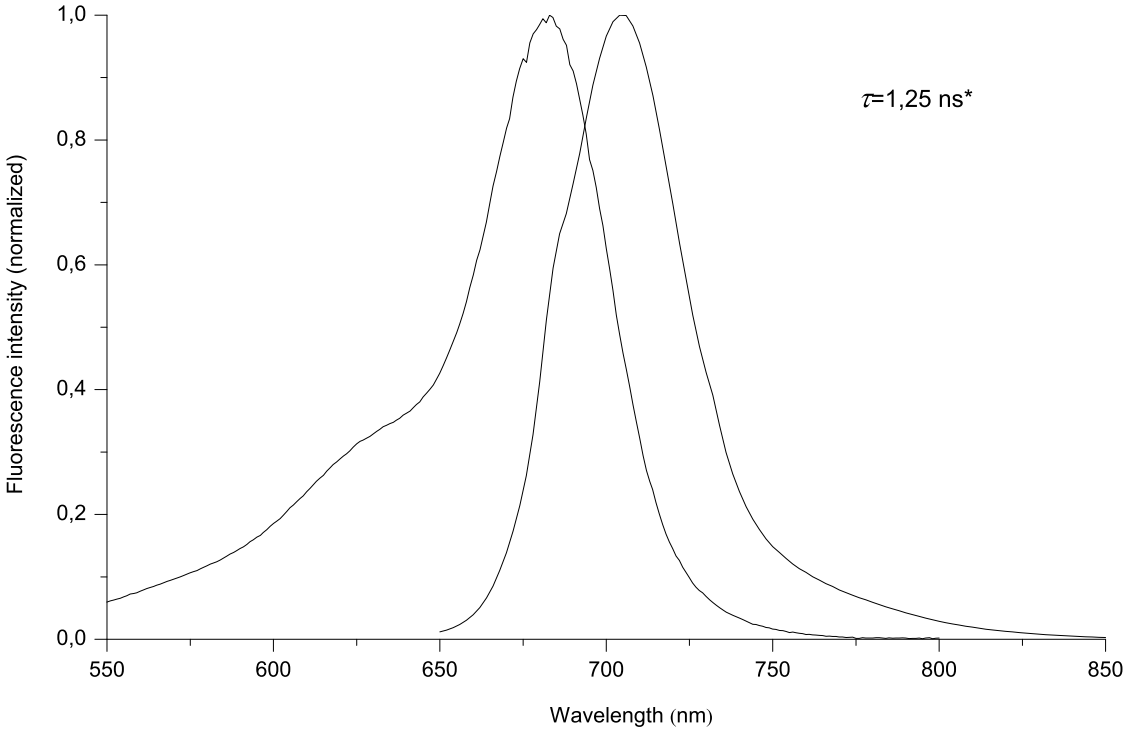


Figure 5.34: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 512,39
Quantum yield: 0,44 in ethanol [6]
Appearance: steely blue-gray, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 683,5$ nm
Emission maximum: $\lambda_{max}^{em} = 704$ nm
Decay function: $F(t) = 0,40e^{-t/0,220} + 0,32e^{-t/1,03} + 0,28e^{-t/1,62}$
Intensity ratio of the components: 0,10:0,38:0,52
Mean lifetime: $\bar{\tau} = 1,25$ ns
Reduced $\chi^2 = 1,109$

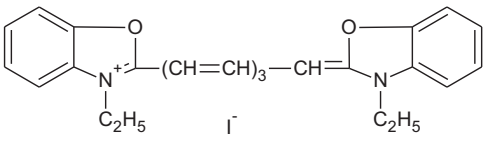


Figure 5.35: Chemical formula

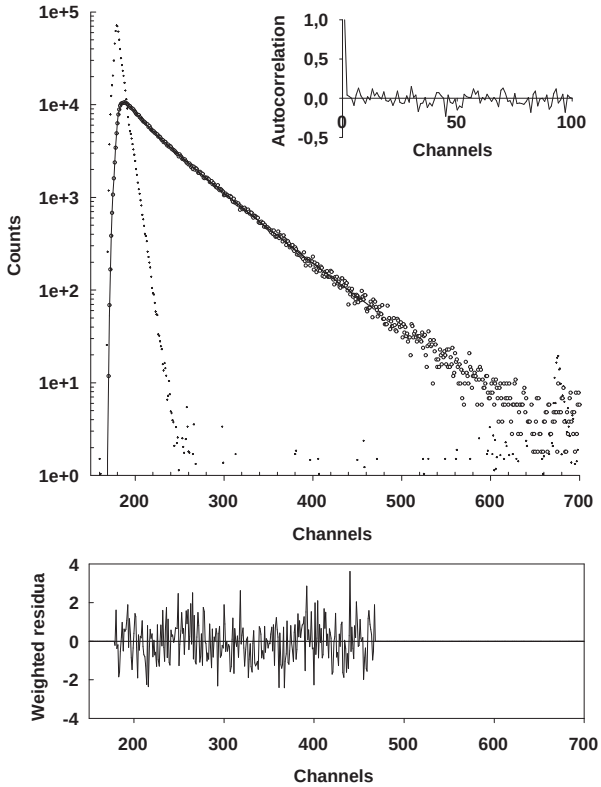


Figure 5.36: Fluorescence decay

5.13 LDS 698 (Pyridine 1)

$C_{19}H_{23}N_2.ClO_4$; 1-ethyl-2-(4-(p-dimethylaminophenyl)-1,3-butadienyl)-pyridinium perchlorate

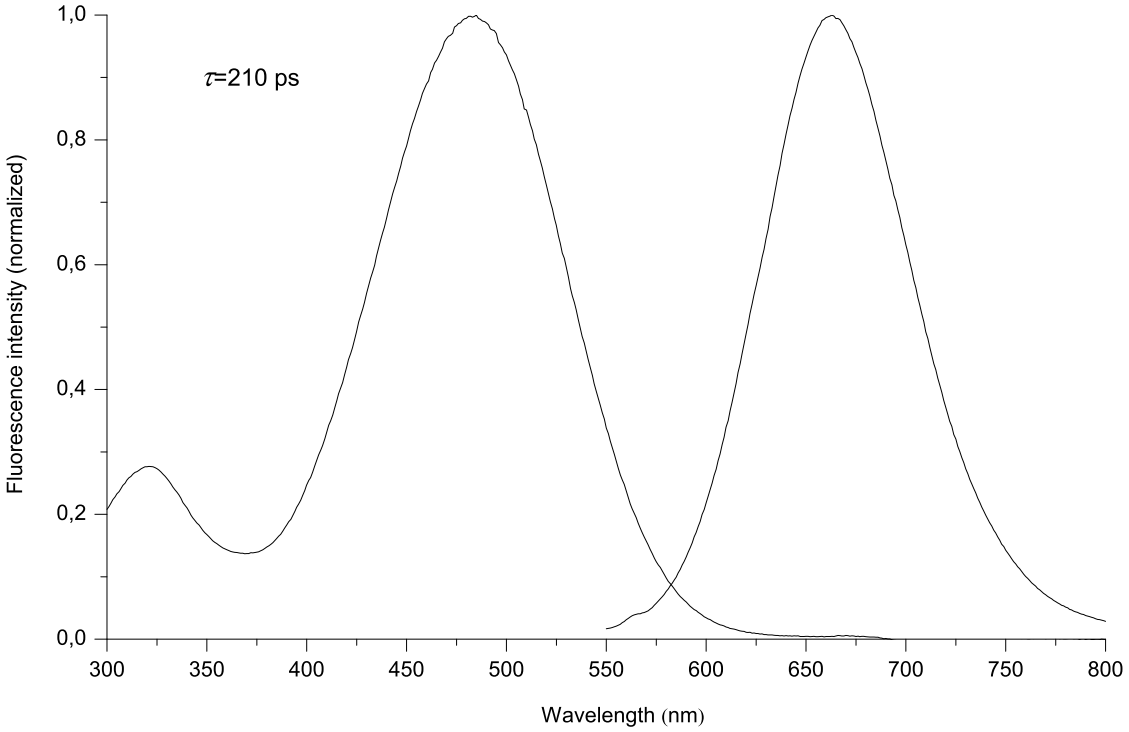


Figure 5.37: Emmision and absorption spectrum

Supplier: LambdaChrome
Molecular weight: 378,85
Appearance: red, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 483$ nm
Emission maximum: $\lambda_{max}^{em} = 663$ nm
Decay function: $F(t) = e^{-t/0,210}$
Mean lifetime: $\bar{\tau} = 210$ ps
Reduced $\chi^2 = 1,289$

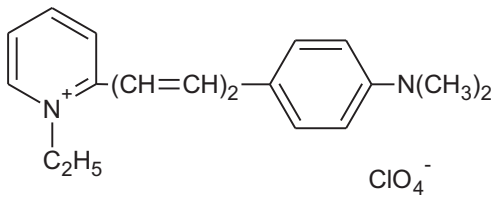


Figure 5.38: Chemical formula

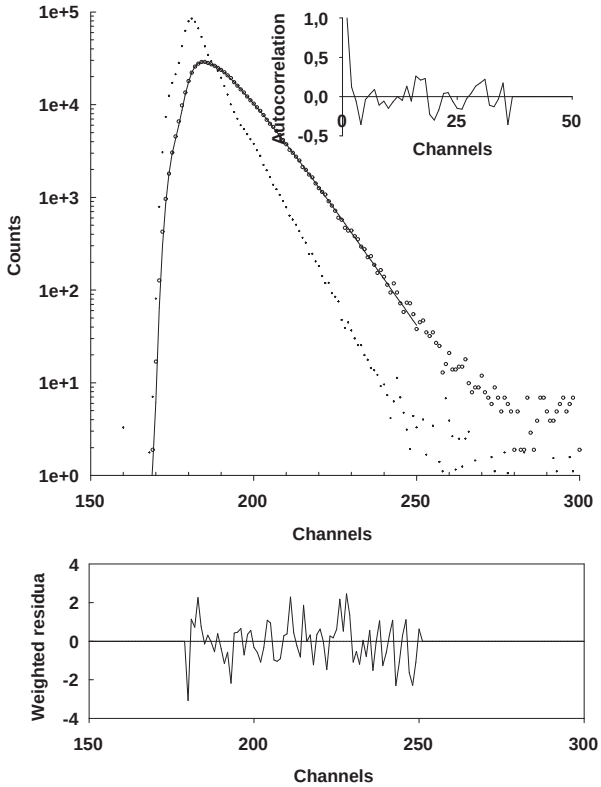


Figure 5.39: Fluorescence decay

5.14 LDS 722 (Pyridine 2)

$C_{19}H_{23}N_2.ClO_4$; 4-[4-[4-(dimethylamino)phenyl]-1,3-butadienyl]-1-ethyl-pyridinium perchlorate

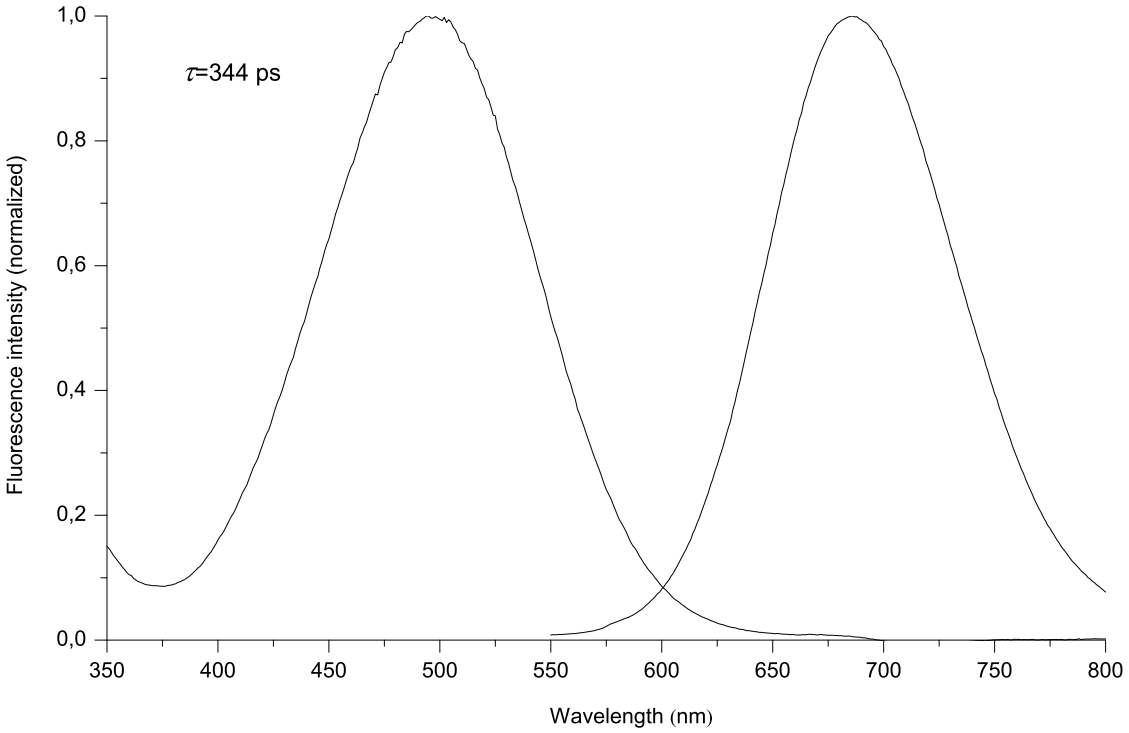


Figure 5.40: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 378,86
Appearance: purple, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 496$ nm
Emission maximum: $\lambda_{max}^{em} = 686$ nm
Decay function: $F(t) = e^{-t/0,344}$
Mean lifetime: $\bar{\tau} = 344$ ps
Reduced $\chi^2 = 1,104$

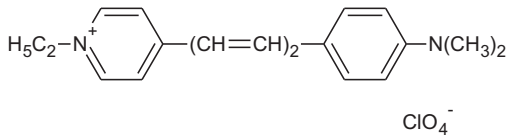


Figure 5.41: Chemical formula

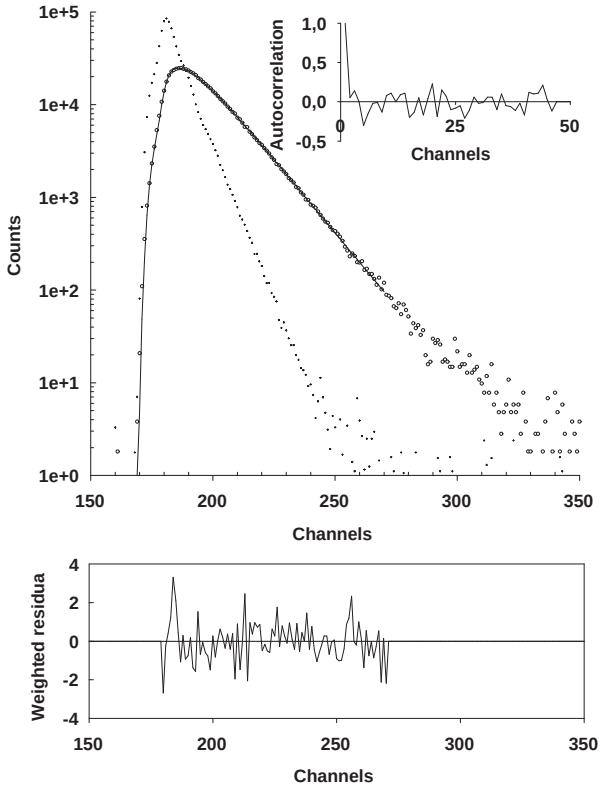


Figure 5.42: Fluorescence decay

5.15 LDS 820 (Styryl 9)

$C_{23}H_{25}N_2S.ClO_4$; 2-[6-[4-(dimethylamino)phenyl]-1,3,5-hexatrienyl]-3-ethyl-benzothiazolium perchlorate

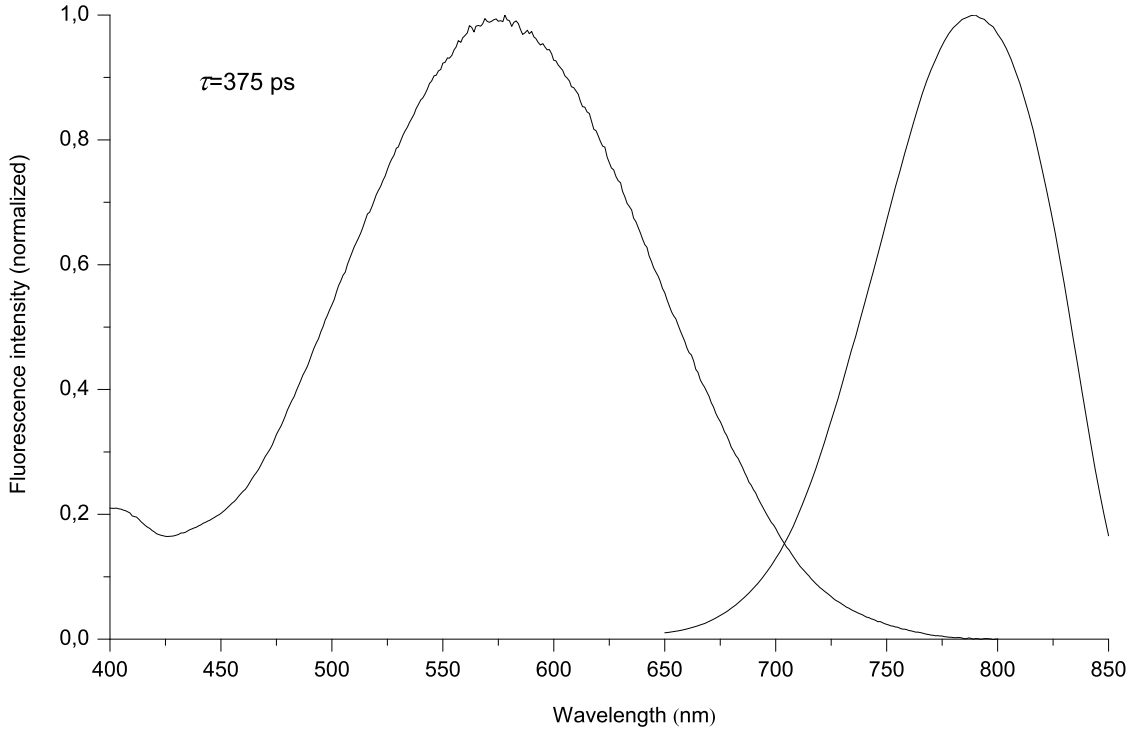


Figure 5.43: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 460,98
Appearance: dark green, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 578$ nm
Emission maximum: $\lambda_{max}^{em} = 790$ nm
Decay function: $F(t) = e^{-t/0,375}$
Mean lifetime: $\bar{\tau} = 375$ ps
Reduced $\chi^2 = 2,587$

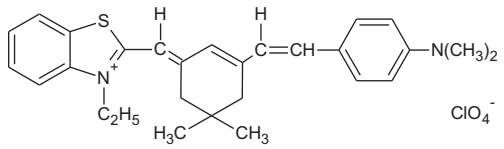


Figure 5.44: Chemical formula

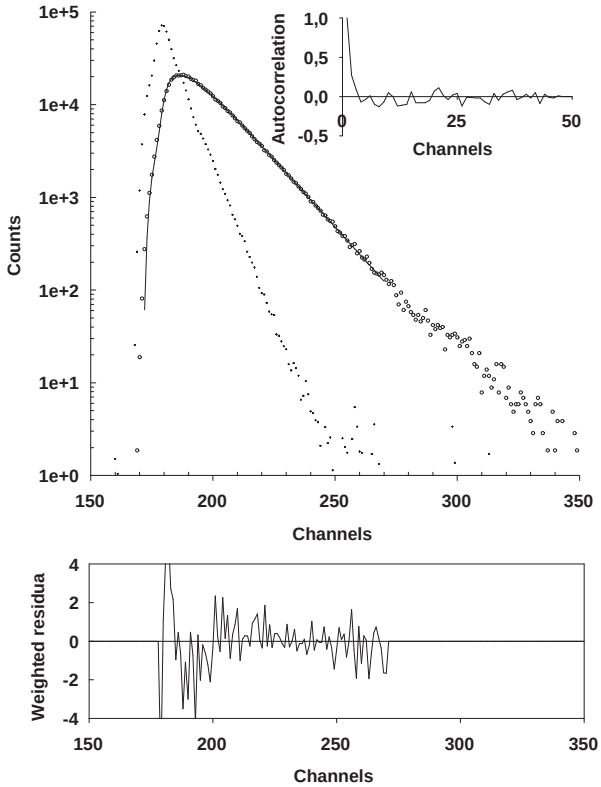


Figure 5.45: Fluorescence decay

5.16 PPD

$C_{14}H_{10}N_2O$; 2,5-Diphenyl-1,3,4-oxadiazole

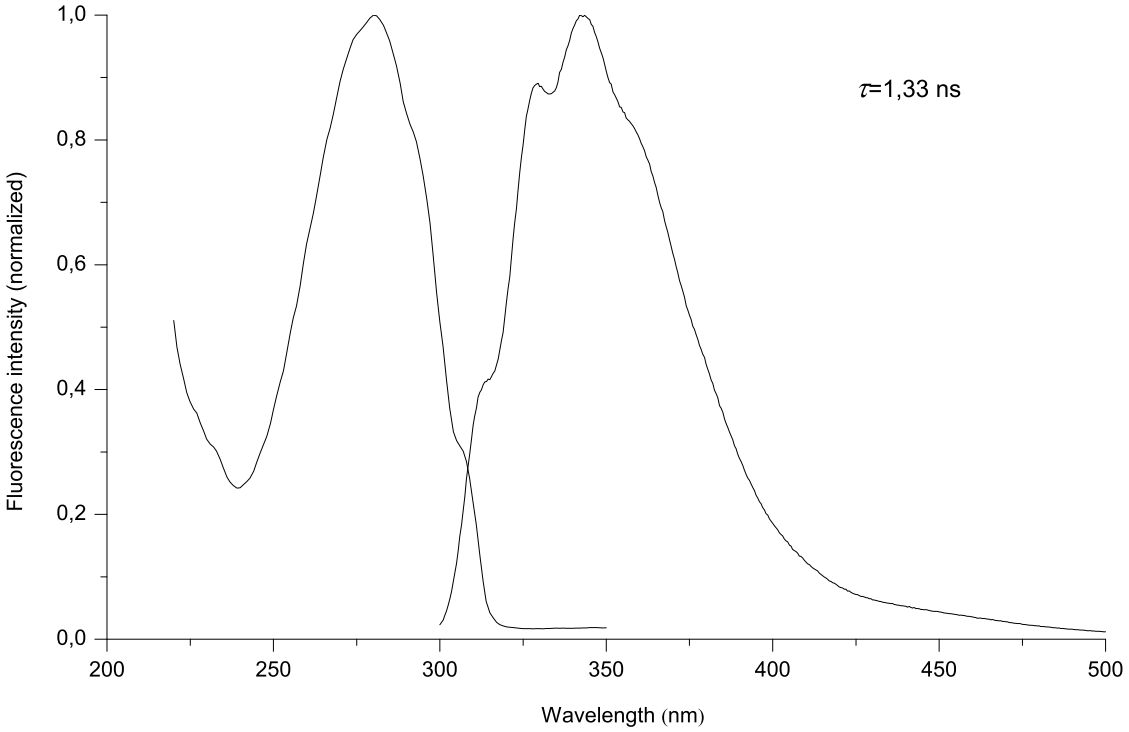


Figure 5.46: Emmision and absorption spectrum

Molecular weight: 222,24
Quantum yield: 0,80 in cyclohexane [3]
Appearance: pale blue, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 280,5$ nm
Emission maximum: $\lambda_{max}^{em} = 342$ nm
Decay function: $F(t) = e^{-t/1,33}$
Mean lifetime: $\bar{\tau} = 1,33$ ns
Reduced $\chi^2 = 0,900$

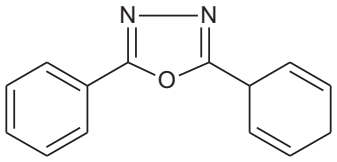


Figure 5.47: Chemical formula

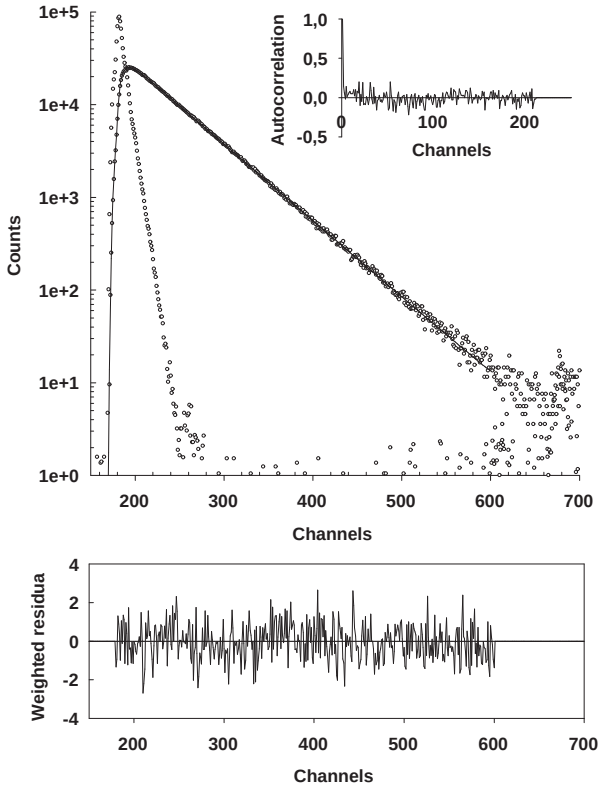


Figure 5.48: Fluorescence decay

5.17 Rhodamine 560 Chloride (Rhodamine 110)

$C_{20}H_{14}N_2O_3$.HCl; 2-(6-amino-3-imino-3H-xanthen-9-yl)-benzoic acid chloride

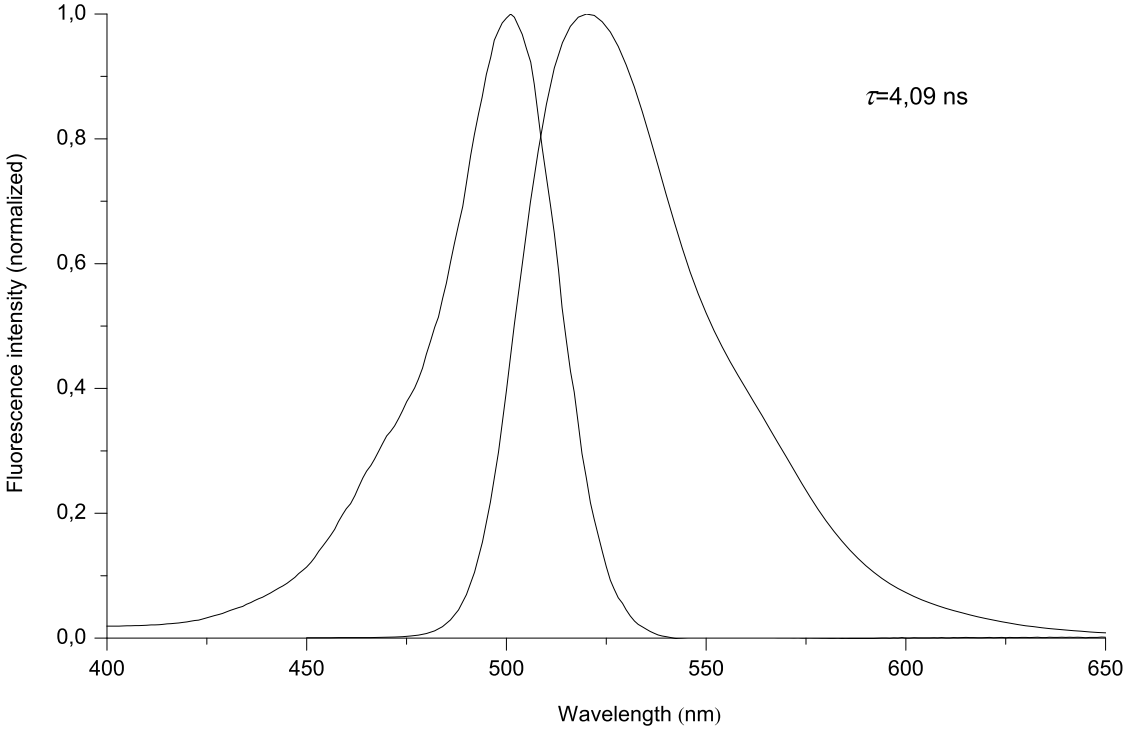


Figure 5.49: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 366,80
Appearance: red, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 502$ nm
Emission maximum: $\lambda_{max}^{em} = 520$ nm
Decay function: $F(t) = e^{-t/4,09}$
Mean lifetime: $\bar{\tau} = 4,09$ ns
Reduced $\chi^2 = 0,910$

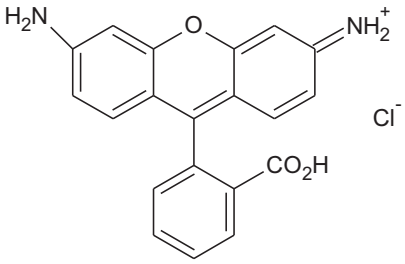


Figure 5.50 Chemical formula

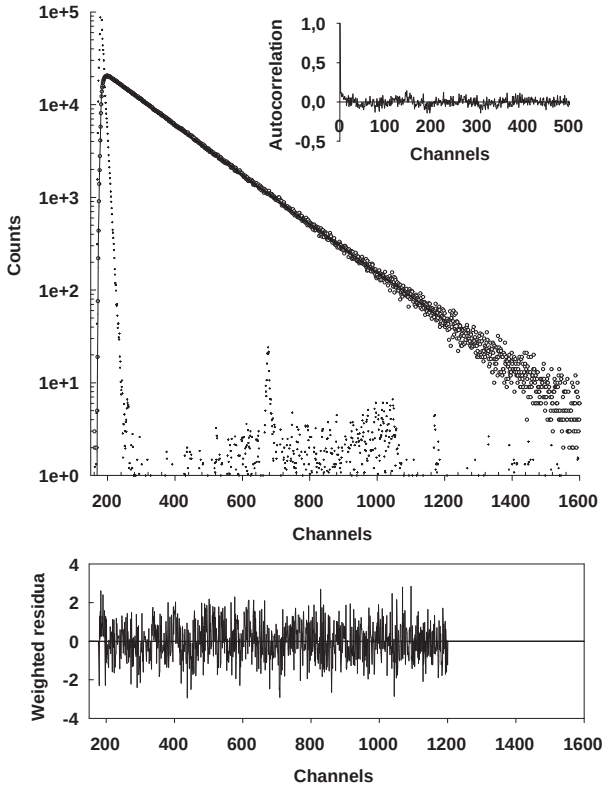


Figure 5.51: Fluorescence decay

5.18 Rhodamine 610 Chloride (Rhodamine B)

$C_{28}H_{31}N_2O_3.Cl$; N-[9-(2-carboxyphenyl)-6-(diethylamino)-3H-xanthen-3-ylidine]-N-ethylethanaminium chloride

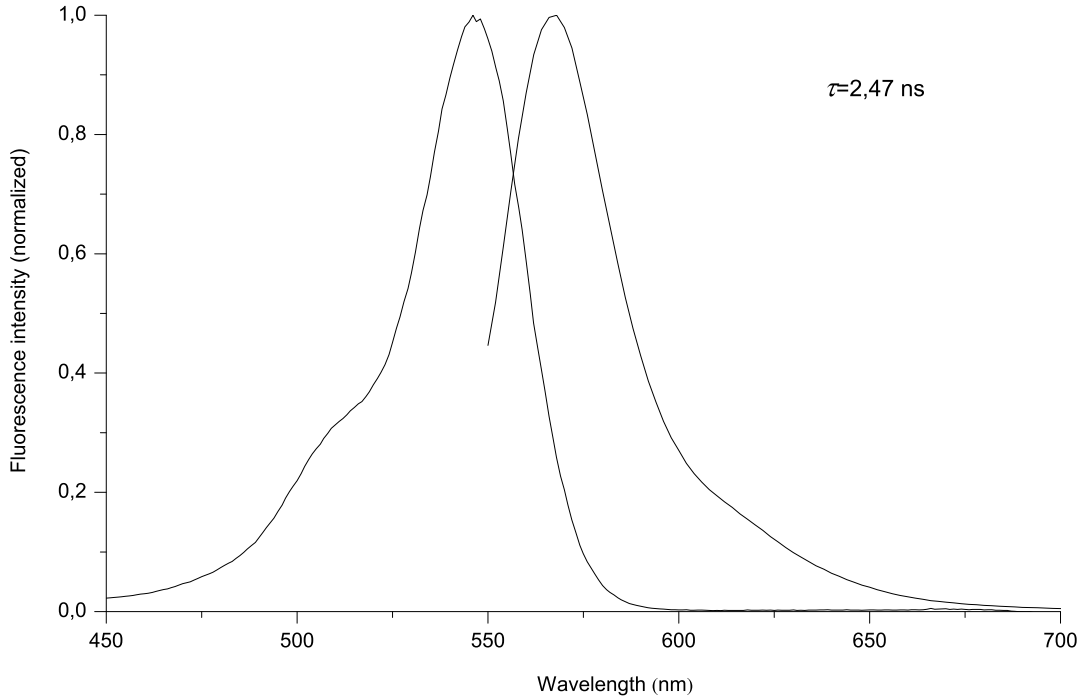


Figure 5.52: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 479,02
Quantum yield: 0,66±0,01 in methanol; 0,41±0,01 in water [3]; 0,31 in water [8]
Appearance: green, crystalline solid or reddish-violet, powder
Absorption maximum: $\lambda_{max}^{abs} = 546$ nm
Emission maximum: $\lambda_{max}^{em} = 568$ nm
Decay function: $F(t) = e^{-t/2,47}$
Mean lifetime: $\bar{\tau} = 2,47$ ns
Reduced $\chi^2 = 0,967$

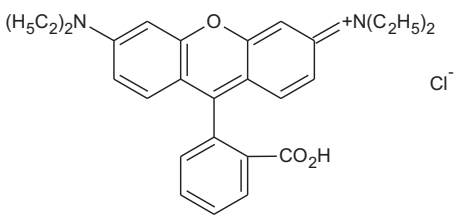


Figure 5.53: Chemical formula

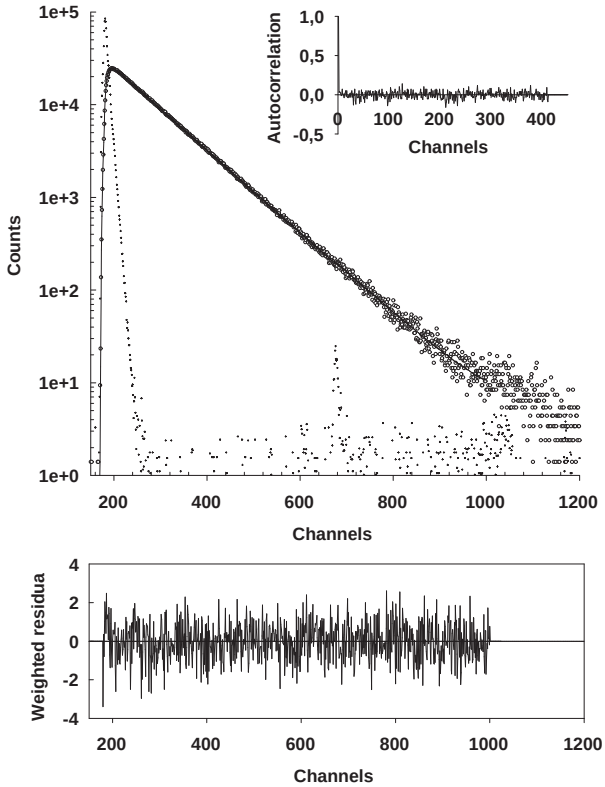


Figure 5.54: Fluorescence decay

5.19 Rhodamine 640 Perchlorate (Rhodamine 101)

$C_{32}H_{31}N_2O_3.ClO_4$; 9-(2-carboxyphenyl)-2,3,6,7,12,13,16,17-octahydro-1H,5H,11H,15H,-xantheno [2,3,4-ij:5,6,7-i'j']-diquinolizin-4-ium perchlorate

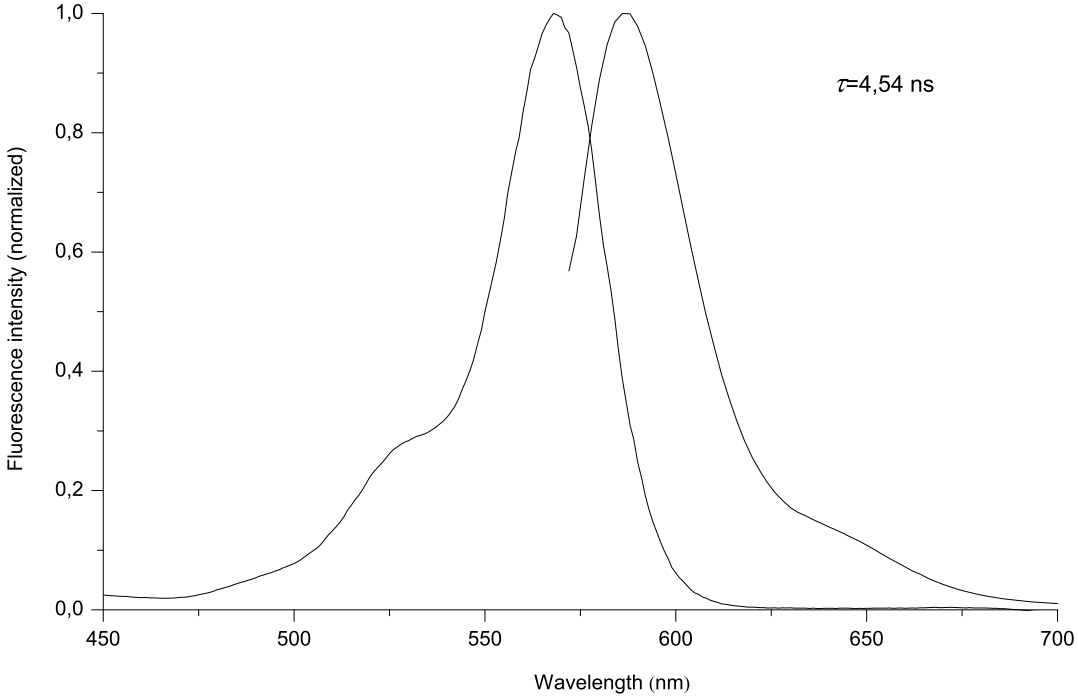


Figure 5.55: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 591,05
Quantum yield: 1,0 in ethanol; 1,0 in ethanol + 0,01% HCl [9]
Appearance: dark green with bronze sheen, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 568,5$ nm
Emission maximum: $\lambda_{max}^{em} = 586$ nm
Decay function: $F(t) = e^{-t/4,54}$
Mean lifetime: $\bar{\tau} = 4,54$ ns
Reduced $\chi^2 = 1,018$

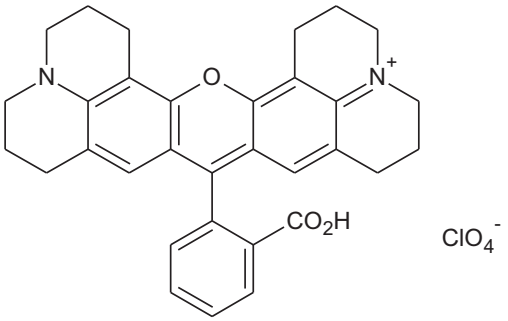


Figure 5.56: Chemical formula

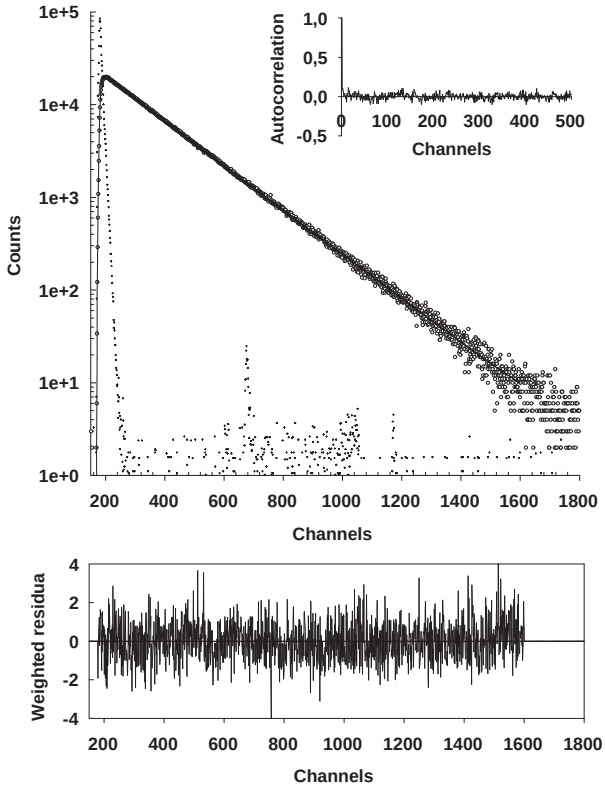


Figure 5.57: Fluorescence decay

5.20 Rhodamine 800

$C_{26}H_{26}N_3O_5Cl$; 8-Cyano-2,3,5,6,11,12,14,15-octahydro-1H,4H,10H,13H-diquinolizino [9,9a,1-bc:9',9a',1-hi]xanthylium Perchlorate

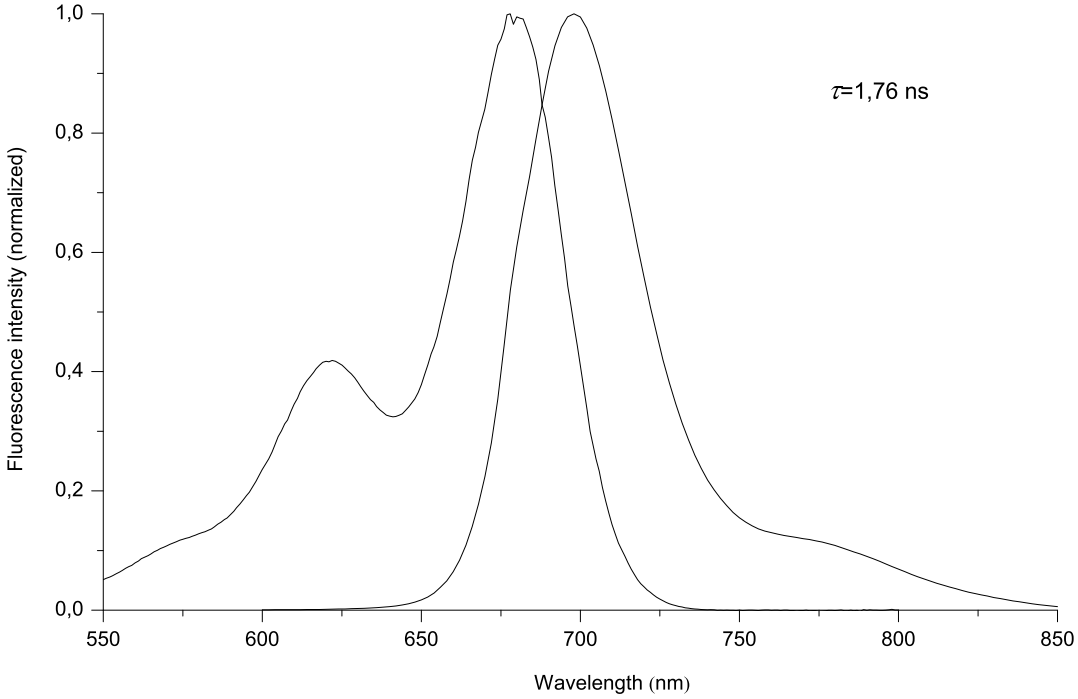


Figure 5.58: Emmision and absorption spectrum

Supplier: LambdaChrome
Molecular weight: 495,52
Appearance: green, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 678,5$ nm
Emission maximum: $\lambda_{max}^{em} = 698$ nm
Decay function: $F(t) = e^{-t/1,76}$
Mean lifetime: $\bar{\tau} = 1,76$ ns
Reduced $\chi^2 = 1,234$

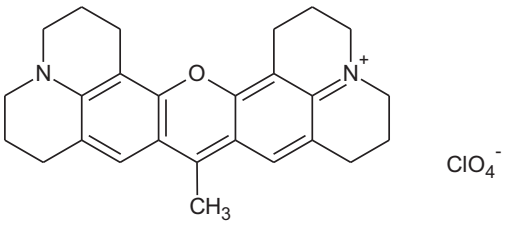


Figure 5.59: Chemical formula

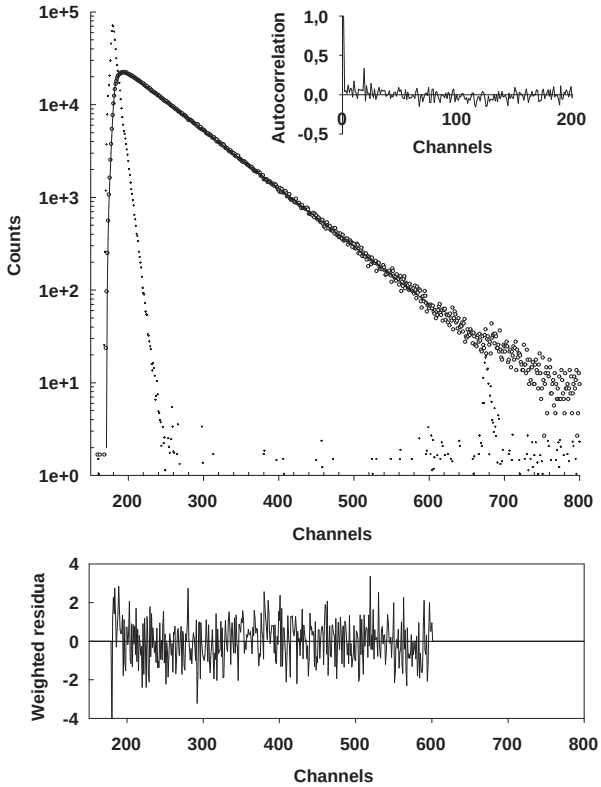


Figure 5.60: Fluorescence decay

5.21 Stilbene 1

$C_{26}H_{18}O_6S_2K_2$; [1,1'-Biphenyl]-4-sulfonic acid,4',4"-1,2-ethene-diylbis-, dipotassium salt

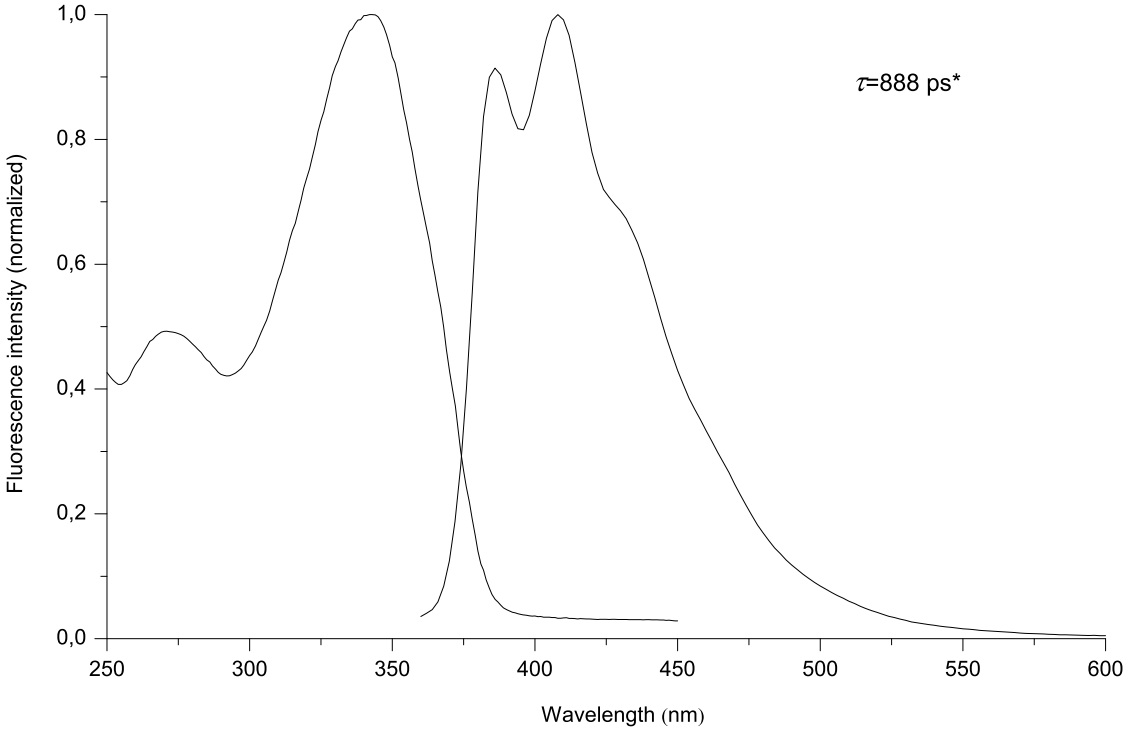


Figure 5.61: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 568,74
Appearance: slightly yellow, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 342,5$ nm
Emission maximum: $\lambda_{max}^{em} = 408$ nm
Decay function: $F(t) = 0,79e^{-t/0,831} + 0,21e^{-t/1,06}$
Intensity ratio of the components: 0,75:0,25
Mean lifetime: $\bar{\tau} = 888$ ps
Reduced $\chi^2 = 1,116$

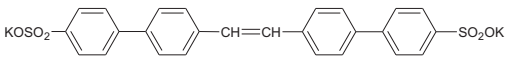


Figure 5.62: Chemical formula

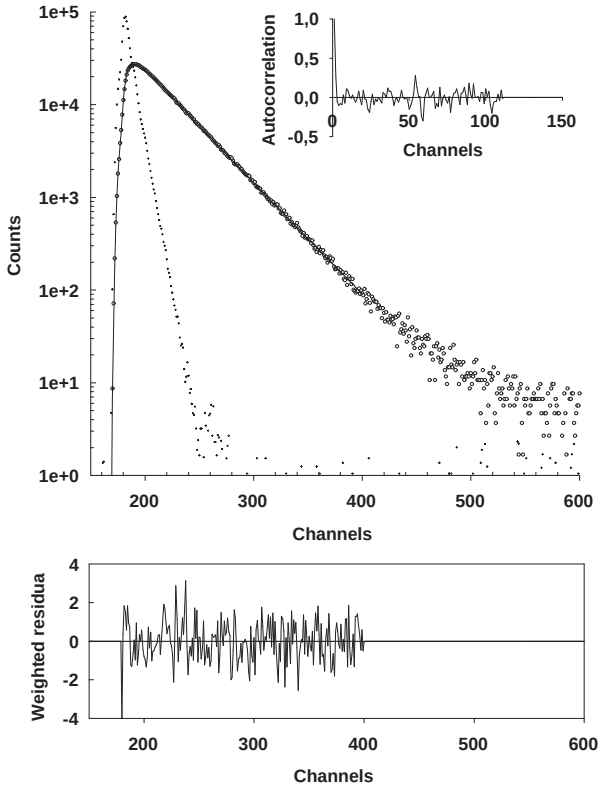


Figure 5.63: Fluorescence decay

5.22 Stilbene 420 (Stilbene 3)

$C_{28}H_{20}O_6S_2.2Na$; 2,2"-([1,1'-biphenyl]-4.4'-diyldi-2,1-ethenediyl)bis-benzenesulfonic acid disodium salt

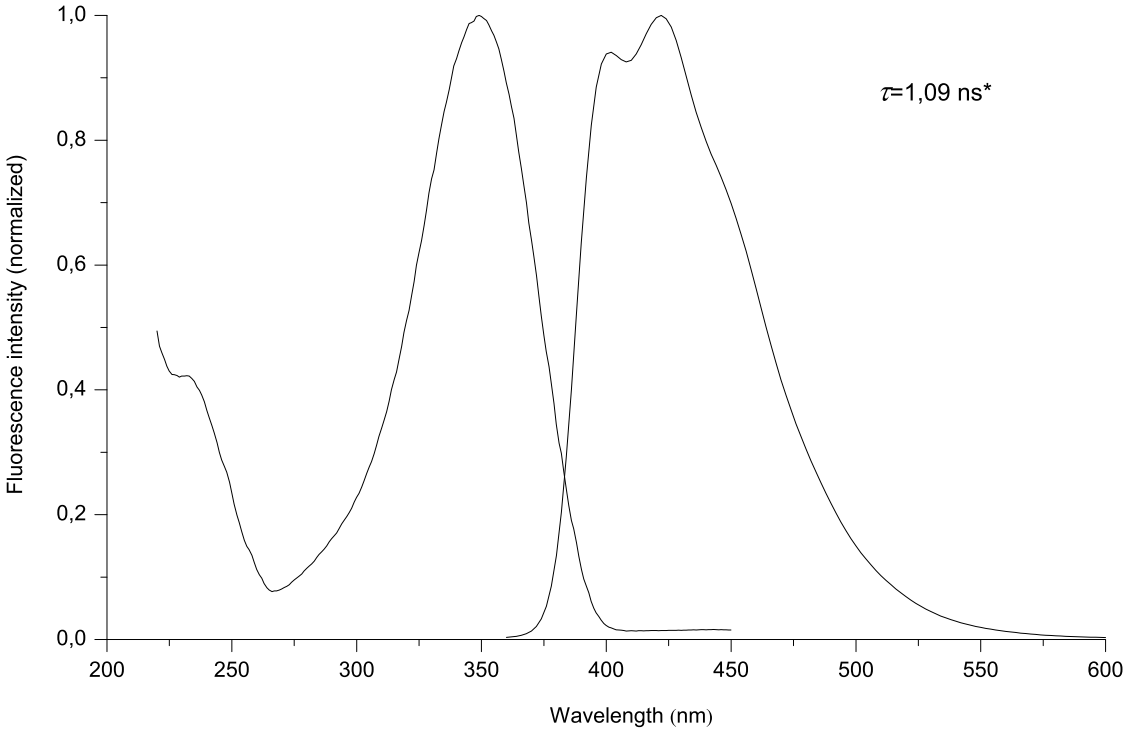


Figure 5.64: Emmision and absorption spectrum

Supplier: Exciton
Molecular weight: 562,56
Appearance: yellow, powder
Absorption maximum: $\lambda_{max}^{abs} = 349,5$ nm
Emission maximum: $\lambda_{max}^{em} = 422$ nm
Decay function: $F(t) = 0,99e^{-t/0,976} + 0,01e^{-t/4,41}$
Intensity ratio of the components: 0,97:0,03
Mean lifetime: $\bar{\tau} = 1,09$ ns
Reduced $\chi^2 = 1,046$

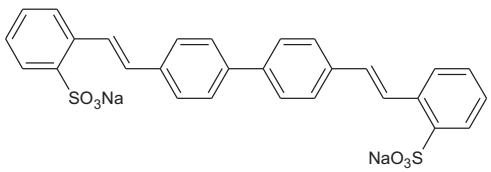


Figure 5.65: Chemical formula

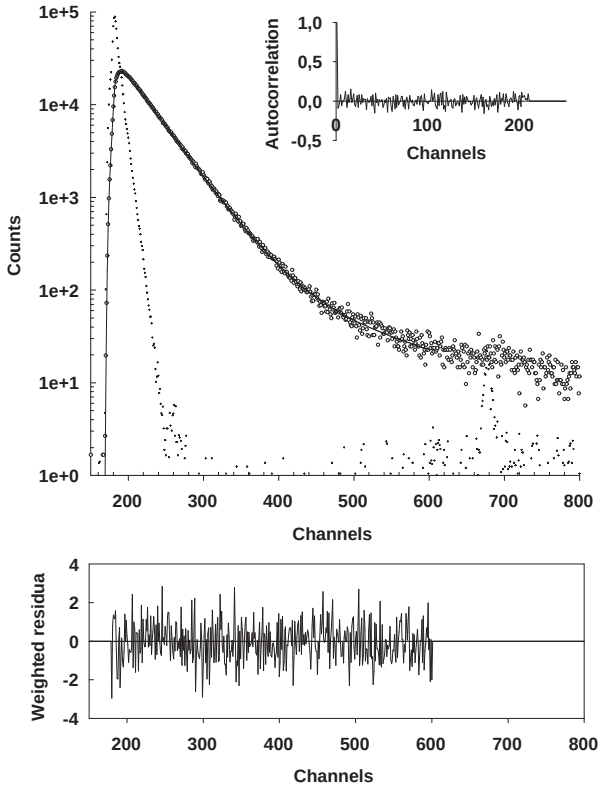


Figure 5.66: Fluorescence decay

5.23 Uranin (Disodium Fluorescein)

$C_{20}H_{10}O_5.2Na$; 3',6'-dihydroxy-spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, disodium salt

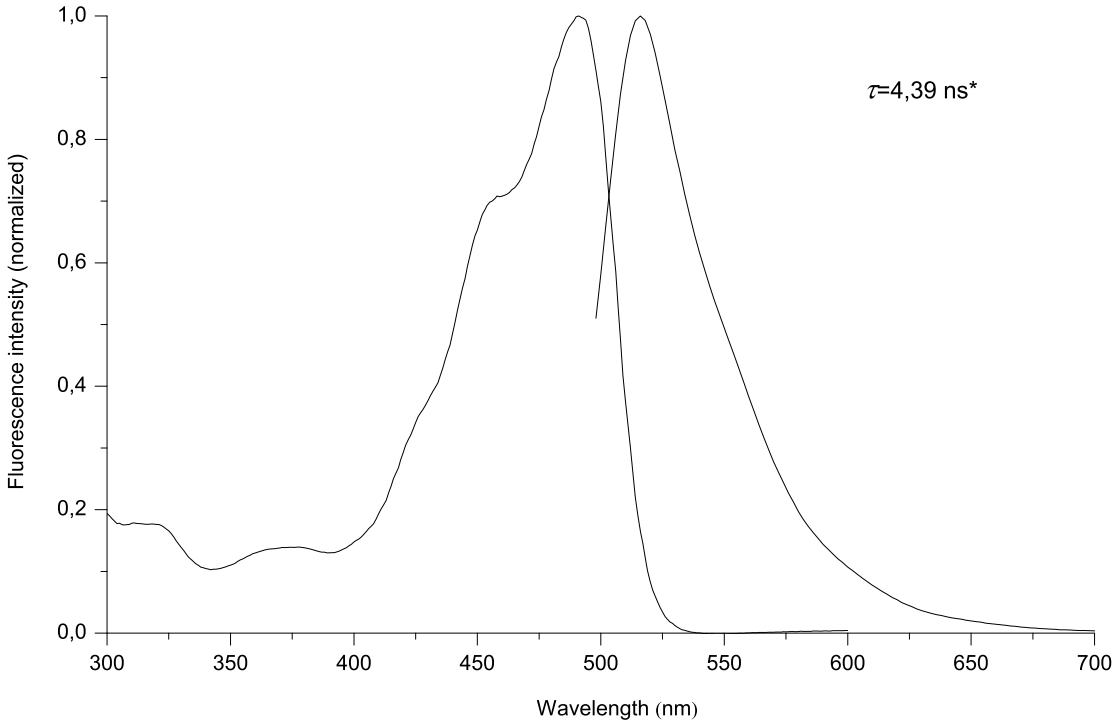


Figure 5.67: Emmision and absorption spectrum

Supplier: LambdaChrome
Molecular weight: 412,30
Appearance: red, crystalline solid
Absorption maximum: $\lambda_{max}^{abs} = 491,5$ nm
Emission maximum: $\lambda_{max}^{em} = 516$ nm
Decay function: $F(t) = 0,06e^{-t/2,54} + 0,94e^{-t/4,46}$
Intensity ratio of the components: 0,03:0,97
Mean lifetime: $\bar{\tau} = 4,39$ ns
Reduced $\chi^2 = 0,966$

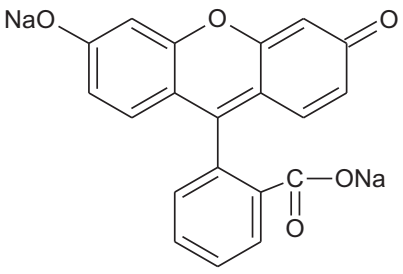


Figure 5.68: Chemical formula

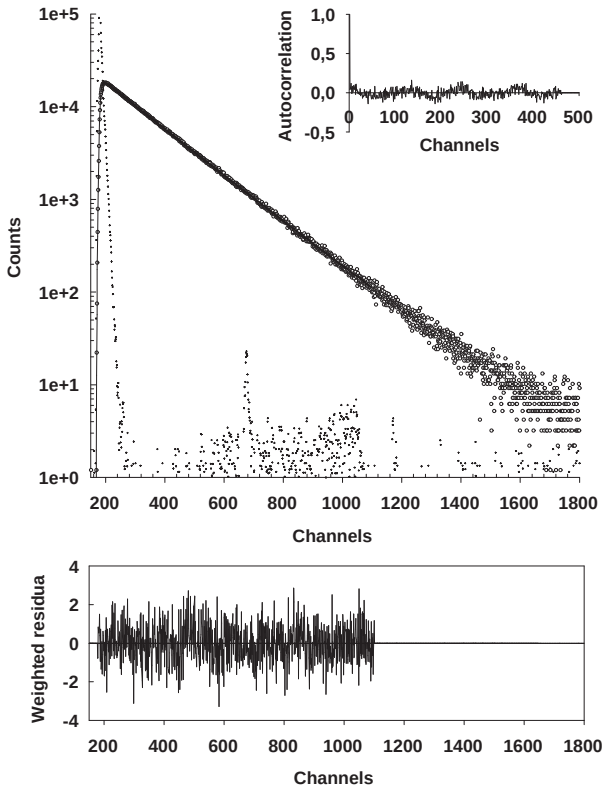


Figure 5.69: Fluorescence decay

5.24 Other fluorophores

Compound	Solvent	T[°C]	λ^{ex}[nm]	λ^{em}[nm]	τ[ns]	Ref.
1,2-dimethylindole	hexane (D)	20	-	330	5,71	[10]
1-cyanonaphtalene	hexane (D)	20	-	345	18,23	[10]
1-methylindole	cyclohexane (D)	20	-	330	6,24	[10]
2-aminopurine	water	-	290	380	11,34	[11]
2-methylindole	cyclohexane (D)	20	-	330	4,36	[10]
3-methylindole	hexane (D)	20	-	330	8,17	[10]
9-cyanoanthracene	cyclohexane	20	295-360	400-450	12,7±0,7	[3]
9-cyanoanthracene	ethanol	-	-	440	14,76	[12]
9-cyanoanthracene	ethanol	25	-	-	11,85±0,03	[13]
9-cyanoanthracene	methanol	20	295-360	400-480	16±1	[3]
Anthracene	cyclohexane	20	295-360	375-442	5,3±0,1	[3]
Anthracene	cyclohexane (D)	20	-	405	5,23	[10]
Anthracene	cyclohexane (U)	20	-	405	4,1	[10]
Anthracene	ethanol	-	-	380	5,47	[12]
Anthracene	ethanol	25	-	-	4,21±0,02	[13]
Anthracene	methanol	20	295-360	375-442	5,1±0,3	[3]
Anthranilic Acid	water	-	290	400	8,9	[11]
Coumarin 153	methanol	20	295-442	495-550	4,3±0,2	[3]
DPA	cyclohexane	20	295-360	400-475	7,5±0,4	[3]
DPA	methanol	20	295-360	400-475	8,7±0,5	[3]
Erythrosin B	water	20	488-568	550-580	0,089±0,003	[3]
Erythrosin B	methanol	20	488-568	550-590	0,47±0,02	[3]
Fluorescein, dianion	NaOH/water	-	400	490-520	4,1±0,1	[8]
Indole	water	-	290	360	4,49	[11]
L-Tyrosine	water	-	285	300	3,27	[11]
NADH	0.1 M PB 7.4	20	330-370	400-600	0,4	[14]
NATA	0.1 M PB 7.0	20	275	310-400	3	[14]
NATA	water	20	295-309	330-410	3,1±0,1	[3]
N-methylcarbazole	cyclohexane	20	290-325	350-400	14,1±0,9	[3]
POPOP	aq ethanol	25	-	-	0,87±0,01	[13]
POPOP	cyclohexane	20	295-360	380-450	1,12±0,04	[3]
POPOP	cyclohexane	25	-	-	1,14±0,01	[13]
POPOP	ethanol	-	-	370-540	1,35±0,2	[15]
POPOP	ethanol	-	-	400	1,38	[12]
POPOP	ethanol	25	-	-	1,32±0,01	[13]
POPOP	ethanol abs.	-	280-390	370-540	1,35	[11]
Dimethyl-POPOP	ethanol	-	300-400	390-560	1,45	[11]
Dimethyl-POPOP	ethanol	-	-	390-560	1,45±0,2	[15]
PPD	ethanol	-	-	310-440	1,2±0,2	[15]
PPD	ethanol	-	240-340	310-440	1,2	[11]

Table 5.1: Nanosecond lifetime standards; NATA = N-Acetyl-L-tryptophanamide, POPOP = 1,4-bis(5-phenyloxazole-2-yl)benzene, PPD = 1.5-diphenyl-1,3,4-oxadiazole, (D)=degassed, (U)= undegassed, PB = phosphate buffer

Compound	Solvent	T[°C]	λ^{ex}[nm]	λ^{em}[nm]	τ[ns]	Ref.
PPO	cyclohexane	20	290-325	360-450	1,36±0,05	[3]
PPO	cyclohexane (D)	20	-	440	1,42	[10]
PPO	cyclohexane (U)	20	-	440	1,28	[10]
PPO	ethanol	-	-	330-480	1,4±0,2	[15]
PPO	ethanol	-	280-350	330-480	1,4	[11]
PPO	ethanol	-	-	400	1,6	[12]
PPO	methanol	20	295-330	340-400	1,65±0,05	[3]
p-Terphentyl	cyclohexane	20	290-315	330-390	0,98±0,03	[3]
p-Terphentyl	methanol	20	284-315	330-380	1,17±0,08	[3]
p-Terphenyl	ethanol	-	280-320	310-412	1,05	[11]
p-Terphenyl	ethanol	-	-	310–412	1,05±0,2	[15]
Rhodamine B	methanol	20	295, 488-568	550-630	2,5±0,1	[3]
Rhodamine B	water	20	488-575	560-630	1,74±0,02	[3]
Rhodamine B	water	-	400	583	1,7	[8]
Rubene	methanol	20	300, 488, 514	550-610	9,9±0,3	[3]
SPA	water	20	300-330	466-520	31,2±0,4	[3]

Table 5.2: Nanosecond lifetime standards; PPO = 2.5-diphenyl-oxazole, (D)= degassed, (U)= undegassed

Compound	Solvent	T[°C]	λ^{ex}[nm]	λ^{em}[nm]	QY	τ[ps]	Ref.
DBS	cyclohexane	25	280-385	375-475	0,11	176	[11][16]
DBS	cyclohexane	37	-	-	-	133	[16]
DBS	toluene	35	-	-	0,12	168	[16]
DBS	toluene	5	-	-	-	248	[16]
DCS	cyclohexane	25	280-420	300-500	0,06	66	[11][16]
DCS	cyclohexane	37	-	-	-	57	[16]
DCS	toluene	25	-	-	0,06	116	[16]
DCS	toluene	5	-	-	-	186	[16]
DFS	cyclohexane	25	280-375	375-450	-	328	[11]
DFS	cyclohexane	37	-	-	-	252	[16]
DFS	toluene	25	-	-	0,16	305	[16]
DFS	toluene	5	-	-	-	433	[16]
DMS	cyclohexane	25	280-375	375-475	0,59	880	[11][16]
DMS	cyclohexane	37	-	-	-	771	[16]
DMS	DMF	25	-	-	0,27	572	[16]
DMS	ethylacetate	25	-	-	0,15	429	[16]
DMS	toluene	25	-	-	0,32	740	[16]
DMS	toluene	5	-	-	-	921	[16]
Phenylalanine	H_2O at neutral pH	-	260	282	0,02	6,8	[17]
Rose Bengal	methanol	25	556	572	-	519	[11]
Tyrosine	H_2O at neutral pH	-	275	304	0,14	3,6	[17]

Table 5.3: Picosecond lifetime standards; DBS = 4-dimethylamino-4-bromostilbene, DCS = 4-dimethylamino-4-cyanostilbene, DFS = 4-dimethylamino-4-fluorostilbene, DMS = 4-dimethylamino-4-methoxystilbene

Bibliography

[1] Lakowicz J. R.: *Principles of Fluorescence Spectroscopy, 3rd Ed.*, Springer Science+Business Media, LLC, New York, 2006.

[2] Brackmann U.: *Lamdachrome® Laser Dyes, 3rd Ed.*, Lambda Physik AG, Goettingen, Germany, 2000.

[3] Boens N., Qin W., Basarić N., Hofkens J., Ameloot M.: *Fluorescence Lifetime Standards for Time and Frequency Domain Fluorescence Spectroscopy*, J. Anal. Chem. **79** (2007) 2137-2149.

[4] Sujatha J., Mishra A. K.: *Bis-MSB, a Laser Dye as a Polarization Probe for Microheterogeneous Media*, Journal of Photochemistry and Photobiology A: Chemistry **101** (1996) 245-250.

[5] *Protocol For Fluorescence Quantum Yield Determination Employed By Nanoco Technologies Ltd.*, Nanoco Technologies Limited, Manchester, 2008.

[6] Kodak: *Optical products catalog*, The Laboratory and Research Products of Eastman Kodak Company, USA [1]

[7] Magde D., Brannon J. H., Cremers T. L., Olmsted J.: *Absolute Luminescence Yield Of Cresyl Violet. A Standard For The Red*, J. Phys. Chem. **83** (1979) 696–699.

[8] Magde D., Rojas G. E., Seybold P.: *Solvent Dependence of the Fluorescence Lifetimes of Xanthene Dyes*, Photochem. Photobiol. **70** (1999) 737.

[1] available at bruckner.chem.uconn.edu/PDFfiles/Kodak-Optical_Products.pdf

[9] Karstens T., Kobe K.: *Rhodamine B and Rhodamine 101 as Reference Substances for Fluorescence Quantum Yield Measurements*, J. Phys. Chem. **84** (1980) 1871–1872.

[10] Lampert R. A., Chewter L. A., Phillips D., O'Connor D. V., Roberts A. J., Meech S. R.: *Standards for Nanosecond Fluorescence Decay Time Measurements*, Anal. Chem. **55** (1983) 68–73.

[11] Lakowicz J. R.: *Principles of Fluorescence Spectroscopy, 2nd Ed.*, Kluwer Academic/Plenum Publishers, New York, London, Moscow, Dordrecht, 1999.

[12] Zuker M., Szabo A. G., Bramall L., Krajcarski D. T., Selinger B.: *Delta Function Convolution Method (DFCM) for Fluorescence Decay Experiments*, Rev. Sci. Instrum. **56(1)** (1985) 14–22.

[13] Kolber Z. S., Barkley M. D.: *Comparison of Approaches to the Instrumental Response Function in Fluorescence Decay Measurements*, Anal. Biochem. **152** (1986) 6–21.

[14] Lakowicz J. R.: *Principles of Fluorescence Spectroscopy, 1st Ed.*, Plenum Press, New York, London, 1983.

[15] Lakowicz J. R., Cherek H., Balter A.: *Correction of Timing Errors in Photomuliplier Tubes Used in Phase-Modulation Fluorometry*, J. Biochem. Biophys. Methods **5** (1981) 31-146.

[16] Lakowicz J. R., Gryczynski I., Laczko G., Gloyna D.: *Picosecond Fluorescence Lifetime Standards for Frequency- and Time-Domain Fluorescence*, J. Fluoresc. **1** (1991) 87–93.

[17] Demchenko A. P.: *Ultraviolet spectroscopy of proteins*, Springer-Verlag, New York, 1981.