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A Subnanosecond Time-Resolved Fluorescence Lifetime Spectrometer Applying Laser Diodes

Stephan Landgraf* and **Günter Grampp**

Institute of Physical and Theoretical Chemistry, University of Technology, A-8010 Graz, Austria

Summary. Since the first application of laser diodes (LD) for the determination of fluorescence lifetimes in our group in 1995, great technical improvements have been made to increase the time resolution of the apparatus. Using the phase fluorometric method, measurements down to 100 ps are now possible. Values can be measured in steps of 10 ps with good reproducibility using a high-frequency signal generator and a GHz digital storage oscilloscope. No additional optical equipment is necessary for this new method. Following an easy calibration procedure, results are available within one minute without any adjustments. The components of the new apparatus including light source, sample chamber for standard fluorescence cuvettes, and detection unit are situated in a 12 × 13 × 9 cm setup. The apparatus was tested with different fluorophores, and the results correlate very well with known values in the range of 0.1 to 1000 ns.

Keywords. Laser diodes; Fluorescence lifetimes; LD-Modulation fluorometry; Dyes.

Introduction

Since the beginning of the 90s an increasing number of publications with the application of semiconductor light sources appeared. Three different techniques were used: single photon counting with short pulses [1, 2], phase-modulation fluorometry using a conventional modulation spectrometer [3–5], or a lock-in amplifier [6].

Using continuous wave laser diodes (LD) in the visible region, which are available from 690 to 630 nm (and, recently, down to 400 nm), a new compact fluorescence spectrometer was built [7], which replaces the former larger and slower setup on an optical rail [8]. The phase fluorometric method [9–11] was used for the determination of the fluorescence lifetimes. The fluorescence lifetime of liquid or solid samples can easily be calculated from the time delay between a reference signal and the signal of the fluorescence [12]. Neither further calibration nor adjustments of the apparatus with a stable frequency modulation are necessary. Compared to other applications of LDs for lifetime measurements, the apparatus has no external modulator [13], no external laser systems [14], and works completely independently from any other spectroscopic or optical tools, as for example a phase fluorometer [15].

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* Corresponding author

Results and Discussion

The calculation of the lifetime from the measured time delay follows Eq. (1).

$$\theta = \tan^{-1}(\omega\tau); \quad \tau = \frac{T \cdot \tan\left(\frac{\Delta t}{T} \cdot 2\pi\right)}{2\pi} \tag{1}$$

τ is the fluorescence lifetime, T is the period time of the modulation frequency, Δt is the measured time shift, and Θ is the phase angle ($\omega = 2\pi f$ and $f = 1/T$). For small phase angles of less than 10°, there is a simple approximation for Eq. (1): $\tau = \Delta t$. This equation describes only a monoexponential decay. More complex evaluations with more than one lifetime can also be made using phase and modulation measurements [10, 16]

Values can be measured in steps of 10 ps using relatively low frequencies between 0.1 and 300 MHz and close to 100% depth of modulation in the excitation light. The method was applied for three commercial laser dyes (nile blue, oxazine 1, oxazine 4) in several solvents and correlated very well with values published in the literature.

Different excitation wavelengths are available by simply changing the module of the light source, called "laser diode mounting" in Fig. 1. The sensitivity of the photomultiplier used as the detector in the experimental setup is very high; therefore, dye concentrations of less than 10^{-7} mol/dm^3 can be applied (only if the quantum field of fluorescence is equal to unity). Changes of the light intensity in the whole apparatus don't influence the results, because they are calculated only from the zero crossing points. Due to the low power consumption of the light source, this new method can be used for fluorescence sensor applications.

Experiments with oxazine dyes in various solvents correlate very well with known values [17–19] in the range of 0.4 to 4 ns (see Table 3). Typical for these dyes is a large solvent dependence of the fluorescence lifetime caused by the polarity of the solvent (Fig. 2). Figure 3 shows an example to elucidate the

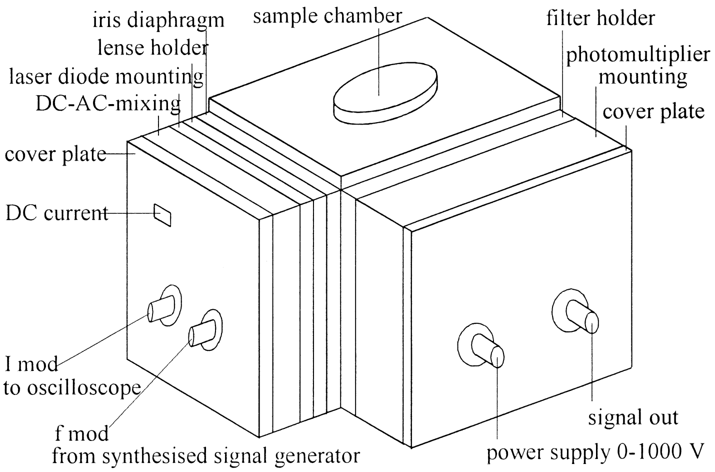

Fig. 1. Scheme of the modular laser diode apparatus

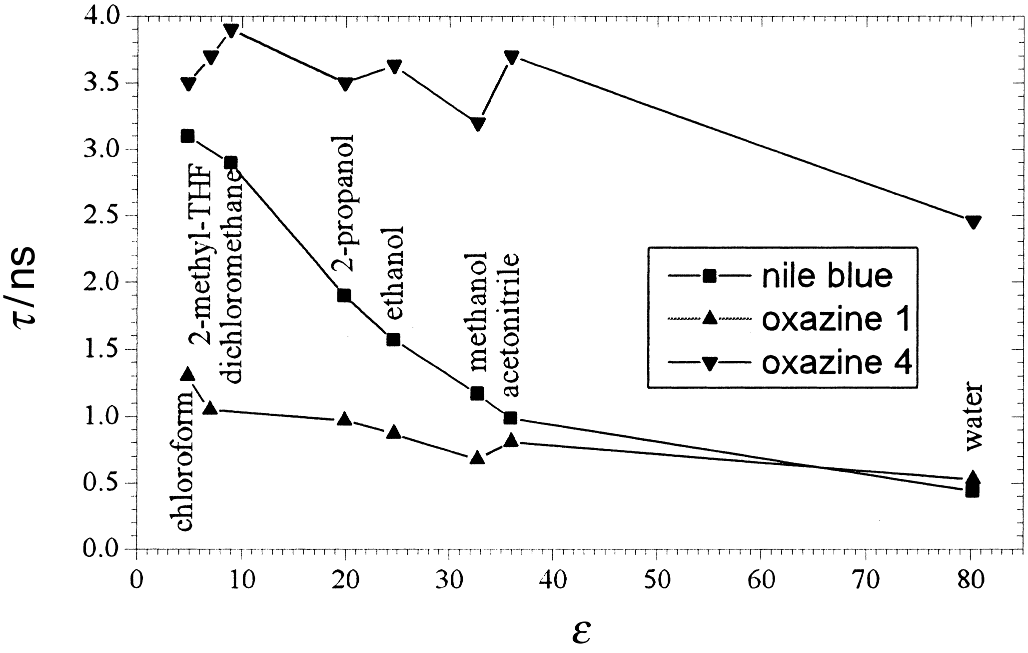

Fig. 2. Solvent polarity dependence of the fluorescence lifetime of the applied oxazine dyes

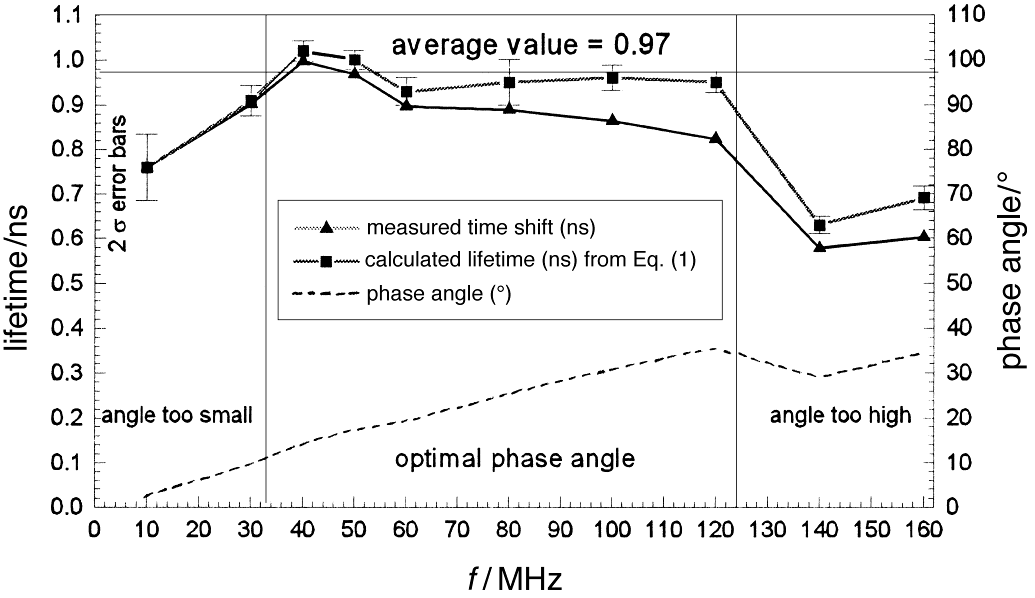

Fig. 3. Diagram of the measured and calculated values (oxazine 1, 10^{-5} *M*, 2-propanol) *vs.* the modulation frequency within a range of 10 to 160 MHz; the optimal conditions for the lifetime determination applying the new apparatus are shown

performance of the apparatus: oxazine 1 in 2-propanol. As indicated, best results for this lifetime are obtained at a phase angle between 10° and 40°. Below 10°, a small deviation of the zero crossing point determination results in a significant error for τ (Eq. (1).) Above 40°, the AC signal of the PM is significantly demodulated due to the fluorescence lifetime. The reproducibility is quite high within a large range of applied modulation frequencies. The two curves for the measured time shift and the calculated lifetime also show the applicability of Eq. (1). According to the fluorescence lifetime of the investigated system, the optimum modulation frequencies should result in a phase angle of about 10° to 40°. In this region the measurements are most precise and reproducible.

The wavelength range of inexpensive commercially available LDs is limited to the red region, but ultrafast light emitting diodes (LED) can also be installed. This increases the wavelength range to 370 nm with practically no interruption. Fluorescence lifetimes of some aromatic fluorophores have already been published using blue LEDs [20, 21]. Very short lifetimes of approximately 100 ps have been found for viologen-like laser dyes [22] applying the new ultraviolet LED (370 nm). Long lifetimes of the ruthenium-*tris*-bipyridinium complex have also been measured, noting excellent agreement with literature values: 890 ns in acetonitrile (840 ns [23]), 520 ns in dichloromethane (488 ns [24]), and 600 ns in water (630 ns [24]).

As the evaluation is not based on the light intensity, but exclusively on the time shift, the method is free of interference, like long term drifts in the light source or detector, as required in industrial and medical sensor applications. Additionally, the demodulation of the fluorescence signal can be measured, but in this case the advantage mentioned above is lost. Due to the modular arrangement of the apparatus it is very easy to add further components as for example a polarizer. Thus, a couple of effects can be analyzed using the present method. Altogether, the described experimental setup can be considered as an evaluation tool for lifetime determinations using any directly emitting semiconductor light source. According to recent technical improvements [25] it can be expected that laser diodes in the blue-green region will soon be available. These, however, could straightforwardly be intergrated in our apparatus; hence, the applicability of this method will increase rapidly [26].

Experimental

Some special aspects have to be mentioned regarding the design of a new apparatus for LD spectroscopy. A red LD typically works with a relatively low voltage of about 2.4 V and a current of 30 to 60 mA. So the total power consumption can reach more than 100 mW, which is quite high for the small size of the housing. Without a heat sink the temperature of the LD would increase rapidly, which could even damage the device. Table 1 shows typical values for the temperature dependence of some optical and electronic characteristics of LDs. Therefore, optimal working conditions at constant temperature (room temperature) and a mean optical output power of 60% of the maximum values should be maintained.

Table 1. Typical optical and electronic characteristics of laser diodes

Characteristics	Temperature dependence around room temperature
Threshold current	0.5–1 mA/K, exponential function
Centre wavelength	0.2–0.3 nm/K, linear function
Total lifetime	$\approx -10\%$/K, logarithmic function

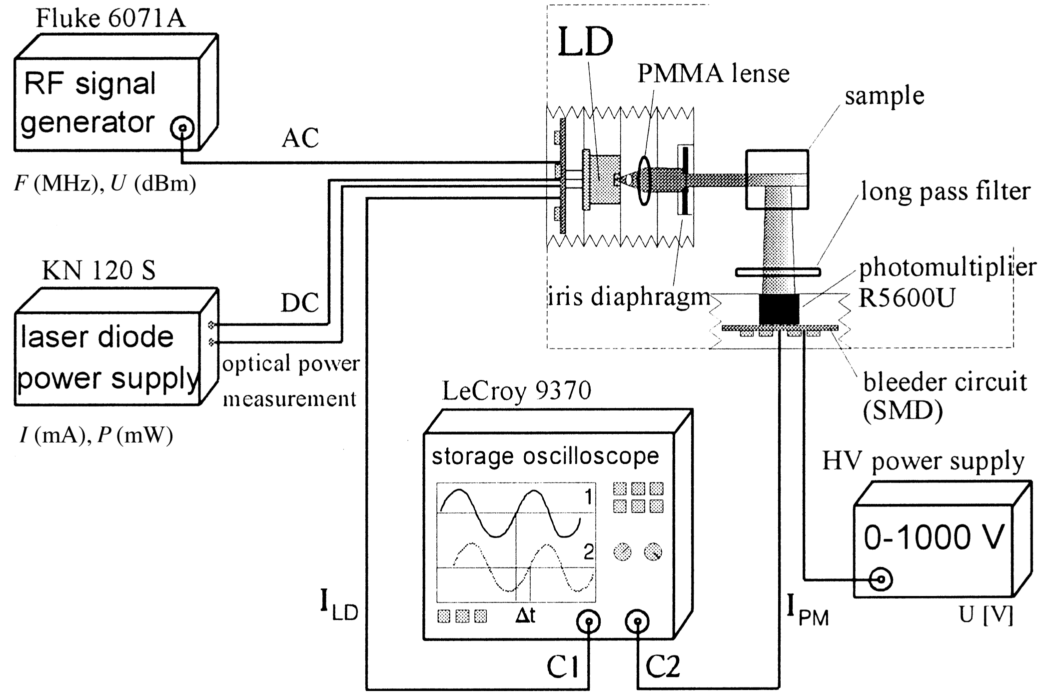

Fig. 4. Schematic description of the applied apparatus

On the other hand, since the apparatus works at relatively high frequencies, a closed shielding for the circuit boards has to be provided. Furthermore, variable wavelengths should be available without complicated replacing procedures. Thus, a modular technique was applied using square aluminum plate units with an edge length of 8 cm. The individual units were assembled forming a massive metal block. The sandwich structure works both as electromagnetic shielding and heat sink (see Fig. 1).

Only four external devices are necessary as shown in Fig. 4. The frequency generator is a Fluke 6071A synthesized RF signal generator with a 50 Ω output. The frequency can be varied from 100 kHz to 1 GHz with an output level of -139 to $+19$ dBm. For the modulation experiments, 0.1 to 300 MHz and -4 to 0 dBm are typically applied. The oscilloscope is a LeCroy 9370 digital storage oscilloscope with a DC to 1 GHz analog input range. Fastest single shot sampling rate is 1 Gsample/s. For the time shift detection, the RIS (random interleave sampling) mode is selected where a resolution of 10 points/ns is achieved. The zero crossing values are then calculated from the data points directly by an internal function of the oscilloscope ("delay"). The results are displayed in steps of 0.01 ns distance and are averaged over all zero crossing points. For each sample, various frequencies were applied (see Table 3). A monoexponential decay results in a frequency-independent value of τ according to Eq. (1). The final result is the averaged lifetime of all applied modulation frequencies. Depending on the investigated system, less expensive signal generators and digital storage oscilloscopes can also be applied. The LD power supply is a KN120S device (GFO, Germany). The LD current is regulated constantly with maximum current values of the LD and the integrated photodiode; this way a secure operation of the LD is possible. The final device is a photomultiplier power supply which can set an output voltage of 0 to -1000 V with 5 mA maximum current. The applied bleeder circuit for the dynode voltages of the photomultiplier consumes less than 1 mA. The internal devices of the new apparatus are described in more detail below.

Table 2. Characteristics of the applied laser diodes as described by the supplier (Hitachi)

Characteristics	LD 635 Type HL6312G	LD 670 Type HL 6722G
Centre wavelength	638.1 nm	674.8 nm
Threshold current	48.67 mA	34.41 mA
Maximum output power	5 mW	5 mW
Output power slope	0.396 mW/mA	0.461 mW/mA
Photodiode current	75 μA/mW	411 μA/mW
Vertical output angle	29.4°	30.4°
Horizontal output angle	7.7°	7.9°
Polarization at $P = 3$ mW	~300:1	~250:1

Table 3. Results of the fluorescence lifetime measurements (ns) and the applied frequencies (MHz); σ values are shown in parentheses, reference values in brackets[a]; ε is the static dielectric constant at $T = 298$ K

Solvent, ε	Dye	Nile blue	Oxazine 1	Oxazine 4
Pure water, 80.2	τ/ns	0.44(3) [0.42]	0.53 (4)	2.46(6)
	f/MHz	50–150	70–150	20–80
Acetonitrile, 35.9	τ/ns	0.99(7)	0.81(2)	3.6(2)
	f/MHz	40–120	40–120	10–30
Methanol, 32.7	τ/ns	1.17(6)	0.68(2)	3.2(1)
	f/MHz	30–100	50–140	10–30
Ethanol, 24.6	τ/ns	1.57(4) [1.9, 1.42]	0.87(6)	3.63(3)
	f/MHz	20–80	40–70	10–30
2-Propanol, 19.9	τ/ns	1.9(1)	0.97(4)	3.5(1)
	f/MHz	10–40	40–120	10-50
Dichloromethane, 8.93	τ/ns	2.90(5)	[b]	3.9(1)
	f/MHz	15–40		10–30
2-Methyl-*THF*, 6.97	τ/ns	[c]	1.05(5)	3.70(6) [3.6]
	f/MHz		40–100	10–30
Chloroform, 4.81	τ/ns	3.1(1)[3.36]	1.3(2)	3.5(2)
	f/MHz	15–40	20–80	10–30

[a] Refs. [17–19]; [b] the solution is not stable during the measurement; [c] the solubility of nile blue in 2-Me-*THF* is very low

Light source

The characteristics of the LDs used in the lifetime spectrometer are shown in Table 2. In Fig. 5 a typical *P-I*-curve of a laser diode (635 nm, 5 mW) is shown. Above a characteristic current, called threshold current I_{th}, the optical output power P varies linearly with I. Therefore, a sinusoidal modulation of the power supply leads to a sinusoidal modulation of the emitted light, which can reach approximately 100% modulation. The experimental slope (0.38 mW/mA), as shown in Fig. 5, was calculated from the displayed current of the power supply (KN120S) and the given characteristics of the integrated photodiode current.

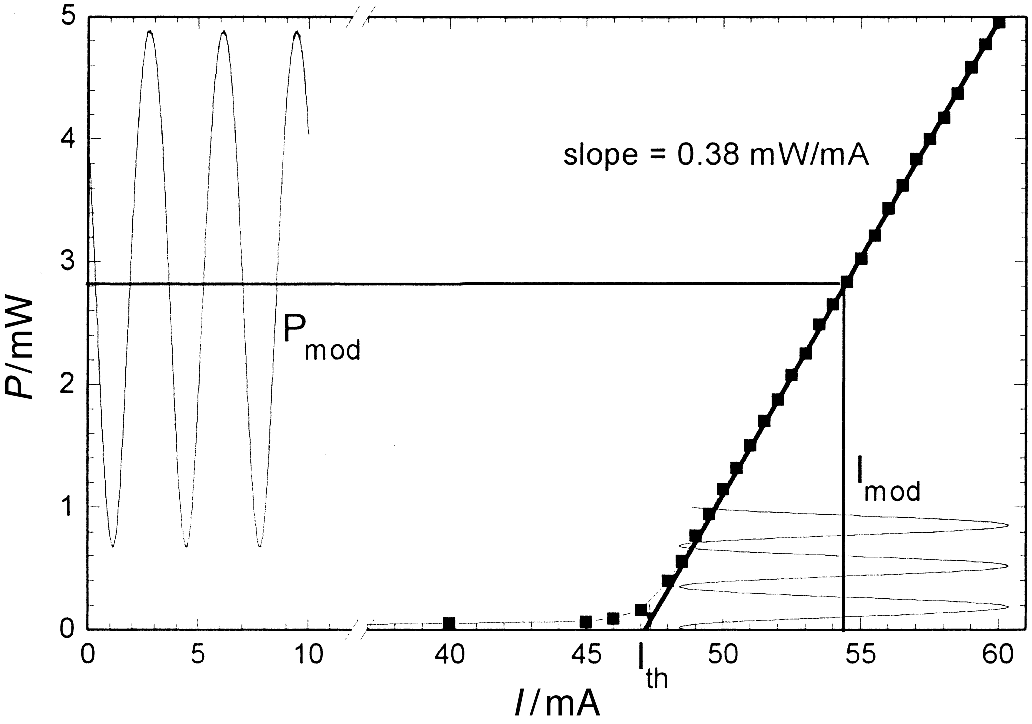

Fig. 5. Diagram of the optical output power P of a laser diode (635 nm) *vs.* the supply current I

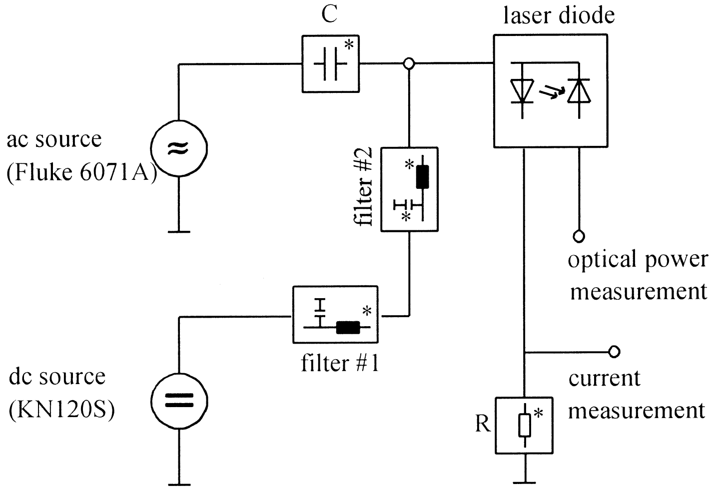

Fig. 6. Scheme of the electronic modulation of the laser diode; all devices marked with an asterisk are built in SMD technique; $C = 10\,\text{nF}$, $R = 10\Omega(\pm 1\%)$, filter #1 = 330 kHz, filter #2 = 3.3 MHz

The modulation is carried out by mixing the DC current of the LD power supply and the AC current of the frequency generator as shown in Fig. 6. The circuit board was designed completely in 50 Ω technique to avoid mutual effects and to maximize the frequency range. The cut-off frequency is about 670 MHz (−3 dB). Higher frequencies can be adjusted by increasing the RF signal level. At 880 MHz the amplitude is decreased to one tenth. In modulation measurements, this performance is not used completely because of the limiting properties of the photomultiplier (see *Light detection*). Due to the fast depolarization in the applied solvents [3, 27], which is more than ten times faster than the investigated fluorescence lifetimes, the setup was used without polarizer for the experiments presented in this paper. Additional polarizer modules are available if necessary, *e.g.* for measurements in highly viscous media.

Sample chamber

The heart of the apparatus is the sample chamber (Fig. 1). Two perpendicular holes in the cubic aluminum block allow 90° fluorescence experiments. In the center there is enough space for a 20 × 10 mm cuvette as well as for a standard 10 × 10 mm cuvette using an adapter. An additional hole in the cover plate is intended for inert gas cells with a bigger height. The optical reference signal is produced by replacing the sample in the chamber with a reflecting plate. For this calibration the long pass filter in the detection path has to be replaced by a neutral density filter (NG 4, Schott) to achieve approximately the same light intensity as used in the fluorescence measurement. The precise adjustment of the detector signal level is performed by the iris diaphragm (see Fig. 1).

Light detection

Common photomultipliers (PM), like the R928 (Hamamatsu), are not suitable for the experimental setup, because of their large size and low cut-off frequency. Even with special bleeder circuits, as described in the technical data sheets of the Hamamatsu company, the shortest pulses showed a length of 2 to 3 ns. The −3 dB frequency in modulation experiments was determined to be 83 MHz

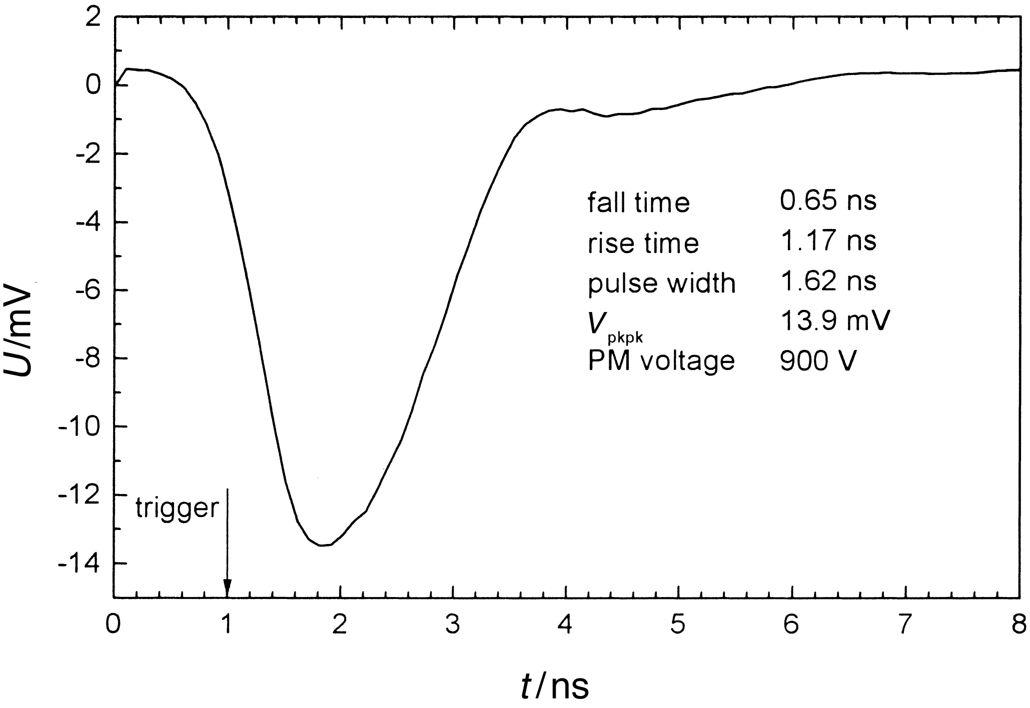

Fig. 7. Single photon response of the applied R5600 photomultiplier in the newly developed fast bleeder circuit board (measured with 1 GHz digital storage oscilloscope LeCroy9370, RIS mode, 2000 sweeps)

for the R928. This results in an AC signal demodulation at the PM, and the signal/noise ratio is decreased. Hence a faster PM, the R 5600U (Hamamatsu), was used, and a very small size bleeder circuit (5 × 5 cm) was developed. All resistors are built in the SMD (surface mounted device) technique, except of those that work as the necessary bridges. Hence, frequencies of more than 250 MHz can be adjusted for applications in the subnanosecond region. Besides this, the experimental setup is largely reduced using this fast PM type.

The performance of the whole detection unit can be characterized by the fall time of 650 ps that corresponds to the value of the data sheet [28]. The overshooting (Fig. 7) of less than 5% is extremely small compared to simple voltage divider circuits.

As already described, the recording of the PM output was achieved with a LeCroy 9370 oscilloscope. The zero crossing points are calculated using the integrated interpolation function. The delay times are displayed in steps of 10 ps.

Sample preparation

The investigated dyes nile blue, oxazine 1, and oxazine 4 were used as delivered (Lambda Physik, 99%). Water was distilled twice. Methanol, 2-propanol, dichloromethane, chloroform, and 2-methyltetrahydrofuran were dried over molecular sieve (3Å) and redistilled. Acetonitrile was used in far UV quality (Romil, 99.9%). Ethanol was used in Uvasol quality (Merck, 99.9%). All solutions had a dye concentration of $10^{-5}\,M$ and were saturated with dry nitrogen (AGA, 5.0 plus catalyst). For all experiments reported a 665 nm long-pass filter (Schott) was used; the *Rayleigh* scattering from the LD is not measurable.

Acknowledgements

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