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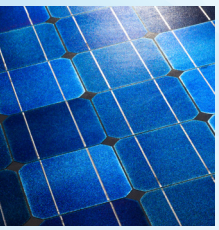
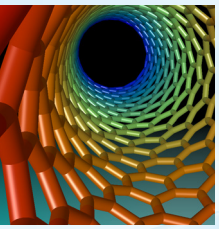
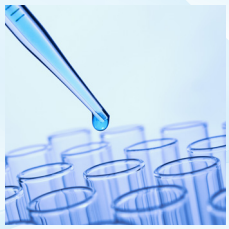
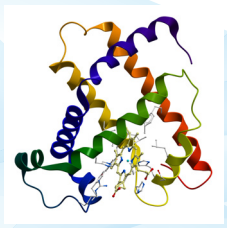


# Fluorolog-QM Enhancement with a Broadly-Tunable OPO Pulsed Laser

FL-TN-2020-04-23

ELEMENTAL ANALYSIS
FLUORESCENCE
GRATINGS & OEM SPECTROMETERS
OPTICAL COMPONENTS
CUSTOM SOLUTIONS
PARTICLE CHARACTERIZATION
RAMAN / AFM-RAMAN / TERS
SPECTROSCOPIC ELLIPSOMETRY
SPR IMAGING


## A Versatile Phosphorescence Solution for Multiple Application Areas

### Introduction

The Fluorolog-QM offers the most flexible photoluminescence (PL) platform on the market. Its hardware and software architecture allows easy addition of various light sources, detectors and third party components and devices, so the instrument can be configured for multiple experiments and diverse applications.

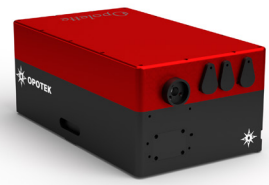

HORIBA Fluorolog-QM modular research spectrofluorometer

One of the strongest features of the Fluorolog-QM platform is its unique Single Shot Transient Digitizer (SSTD) mode allowing for rapid, real time acquisition of PL decays and time resolved spectra. It can be used with a variety of pulsed and modulated light sources, such as xenon flash lamps, HORIBA-IBH SpectraLEDs, modulated DPSS lasers, Q-switched lasers et al.

However, the ultimate versatility and performance of the SSTD mode can be realized with an addition of a pulsed OPO laser source. This technical note presents data obtained with an OPO-based Opolette laser (Opotek Inc., Carlsbad CA), fully integrated into the FLUOROLOG-QM platform and controlled by its FelixFL software. The Opolette is broadly and seamlessly tunable from 210 to 2400 nm, which covers the excitation wavelengths for most PL applications. Its pulse duration is 7 ns with the energy per pulse ranging from about 0.5 to 9 mJ, which corresponds to very impressive peak power ranging from 100 to 1800 kW. The Fluorolog-QM system with the Opolette option is capable of measuring PL decays and time-resolved (TR) excitation and emission spectra in the microsecond to milliseconds time domain.

Due to the inherently narrow linewidth (4 – 7 $cm^{-1}$) of the Opolette laser, the TR excitation spectrum is always measured with high resolution, which can benefit many applications in inorganic luminescence and materials science.

By adding an optional Tektronix oscilloscope, one can enhance the temporal resolution to less than 10 ns (detector dependent) for fluorescence lifetimes.
+ UV12 tunable laser was fiber coupled into the sample compartment for direct illumination of the sample.




Opotek LLC tunable Opolette laser model HE 355 LD + UV12

The Fluorolog-QM with the Opolette laser option can be used with a variety of detectors, such as UV-VIS and NIR photomultipliers (185-1700 nm), as well as with NIR photodiodes, such as InGaAs, InAs and InSb which can cover spectral ranges from 800 to 5500 nm.

This note illustrates the use of the Fluorolog-QM/Opolette system for materials science involving lanthanides in the UV-VIS-NIR region, singlet oxygen lifetime applications (NIR), room temperature phosphorescence of proteins

and studies of upconverting materials. We also illustrate the use of Tektronix oscilloscope as an additional option to enhance temporal resolution for shorter fluorescence lifetimes.

## Equipment Used

The Fluorolog-QM-75-21 shown below was equipped with a continuous 75 watt xenon lamp and an optional pulsed xenon lamp connected to a double excitation monochromator. It also featured a single emission monochromator with the standard cooled PMT housing and an optional NIR IGA detector. The Opotek HE 355 LD + UV12 tunable laser was fiber coupled into the sample compartment for direct illumination of the sample.

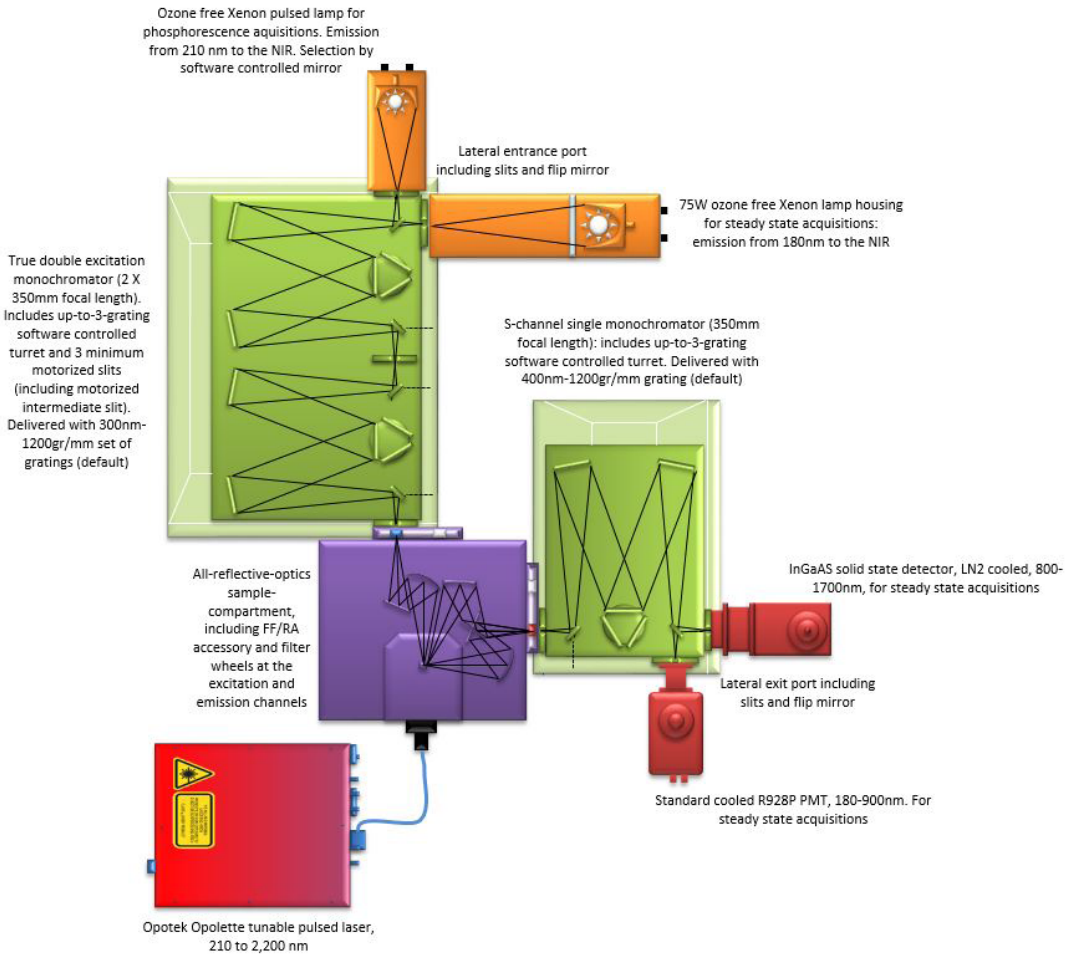
All data presented here was acquired with the OPO laser as the tunable excitation laser with the PL emission detected by the emission monochromator either with the cooled PMT housing, or the optional NIR IGA detector, as noted below.

With this configuration, laser illuminated, time-resolved emission and excitation spectra (TRES) are acquired directly with FelixFL software. The user simply specifies the time range, or window, for the delayed luminescence after the laser flash, and the corresponding spectrum is quickly acquired just like a normal scanning emission or excitation spectrum, except that it is time-resolved.

## How does the Opolette laser compare to other pulsed sources?

• *Microsecond Xenon flash lamp*
A Xe flash lamp is a broadband source, which requires a monochromator for wavelength and bandwidth selection. It is a very convenient and relatively inexpensive excitation source for phosphorescence and PL lifetimes in the µs-ms range. Like the Opolette, it can also be used for time-resolved excitation and emission spectral measurements. However, the energy per pulse of the Xe flash lamp is in a 1-20 µJ range (wavelength and bandwidth dependent), so the Opolette remains a preferable choice for weakly emitting samples, especially when measured with NIR photodiode detectors.

• *Supercontinuum lasers (such as Fianium Whitelase, NKT Extreme series)*
Unlike the Opolette, which outputs tunable monochromatic pulses, supercontinuum lasers emit broadband radiation and require a monochromator for wavelength selection. Their energy per pulse is typically in a µJ range (wavelength and bandwidth dependent), which is 2-3 orders of magnitude lower than that of the Opolette laser. These are mode-locked lasers and are not externally triggerable. While they are excellent choices for TCSPC fluorescence lifetimes in the ps-ns range, they are not suitable for phosphorescence and long PL lifetimes with the SSTD technique.



• *SpectraLEDs*
SpectraLEDs are unique long pulsed LEDs from HORIBA. These are quasi-monochromatic sources with a typical bandwidth of 10-30 nm. Their pulse duration and repetition rate are software controlled via TTL pulses and they can be easily applied to lifetimes in the us-ms range with SSTD. Their energy per pulse is within 20nJ – 4 mJ range (LED and pulse duration dependent), which is 3-4 orders of magnitude lower than that of the Opolette laser. They can be used with both the SSTD and TCSPC/MCS techniques with PMT detectors. Generally, they are not suitable sources for NIR photodiode detectors.

• *DPSS lasers with modulation option*
HORIBA offers a number of cw lasers with a modulation option (460, 520, 808 and 980nm; other wavelengths available on request) with power ranging from 200 mW to 2 W. These lasers can be modulated with software-controlled TTL pulses resulting in a pulse duration from about 10 µs to 100s of milliseconds. Depending on the laser and pulse duration selected, the energy per pulse can range from about 2 uJ to 500 mJ. These lasers are excellent choices for TRPL with SSTD and NIR photodiode detectors. They can be used with both the SSTD and TCSPC/MCS techniques with PMT detectors. Due to their high power, the 808nm and 980nm lasers are preferable choices for PL upconversion studies. The Opolette's advantage over DPSS lasers is its wavelength tunability, the ability to measure TRPL excitation spectra and the coverage of the UV-range down to 210 nm.

## Results

### 1. UV-VIS: Tb3+ and Eu3+ PL decays and TR spectra

The Fluorolog-QM FelixFL software controls the output wavelength of the Opolette laser so the system can scan the excitation wavelength over a user-specified spectral range while acquiring emission data. Therefore, in addition to a time-resolved (TR) emission spectrum the system can also acquire a TR excitation spectrum with high spectral resolution. Figure 1 shows both the TR excitation and emission spectra for $Tb^{3+}$ ions. This data was acquired with the cooled PMT housing and R928 photomultiplier tube (PMT), included with the base Fluorolog-QM system.

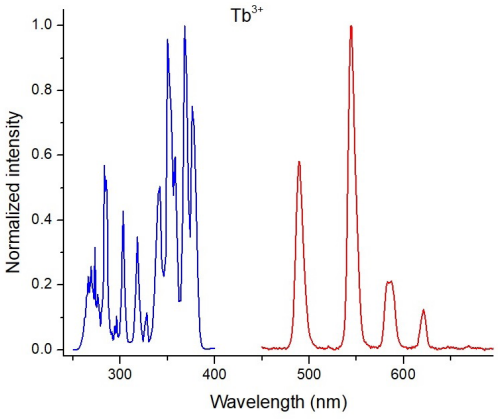



Figure 1: TR excitation and emission spectra of TbCl3 in aqueous solution measured with the R928 PMT operating in the analog mode. The integration time window was 850-3000µs.

The PL decay of the $Tb^{3+}$ sample is shown in Figure 2. The decay was averaged for 50 laser shots with the total collection time of only 2.5 s! The PL decay of the $Tb^{3+}$ sample is shown in Figure 2. The decay was averaged for 50 laser shots with the total collection time of only 2.5 s!

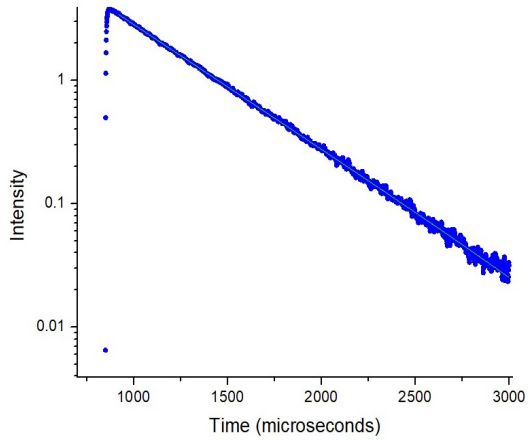



Figure 2: PL decay of TbCl3 collected at 350nm excitation and 545 nm emission with 50 laser shots at 20Hz (2.5 second acquisition time). The recovered lifetime is 430 µs.

Figures 3 and 4 show TR spectra and a decay of europium chloride excited by the OPO laser and detected with the R928 PMT.

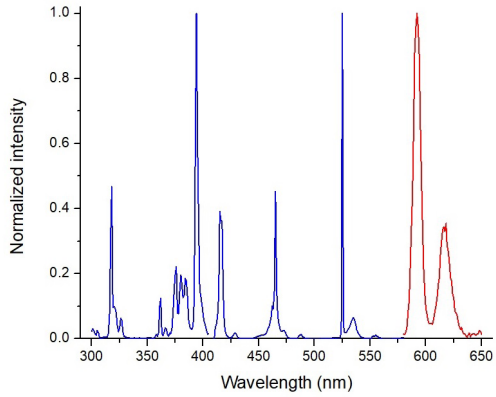



Figure 3: TR excitation and emission spectra of EuCl3 in aqueous solution measured with the R928 PMT operating in the analog mode. The integration time window was 900-1500 µs.

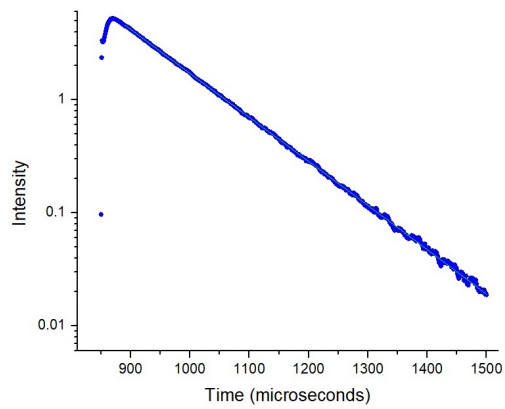



Figure 4: PL decay of EuCl3 collected at 465nm excitation and 592 nm emission with 100 laser shots at 20Hz (5 second acquisition time). The recovered lifetime is 112 µs.

## 2. NIR: $Nd^{3+}$ and $Ho^{3+}$ decays and TR spectra

For near infrared (NIR) detection, a liquid nitrogen cooled InGaAs (IGA) detector was added to a second exit port of the emission monochromator of the Fluorolog-QM. The FelixFL software then automatically switches the output light to this detector and acquires the signal from the IGA detector.

Figures 5 and 6 show TR spectra and a decay of $Nd^{3+}$-doped glass measured with a liquid nitrogen cooled InGaAs detector.

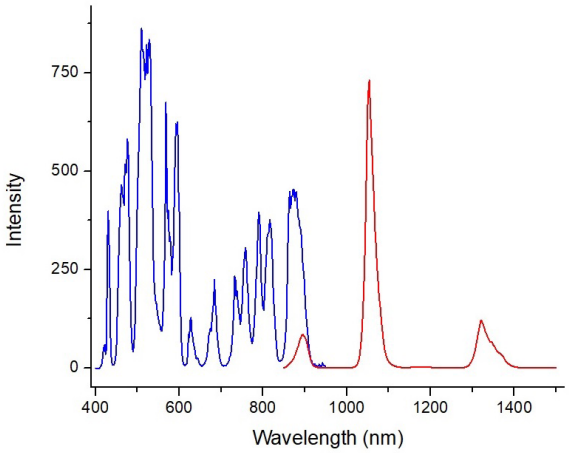



Figure 5: TR excitation and emission spectra of $Nd^{3+}$-doped glass measured with the LN-cooled InGaAs detector. The integration time window was 900-2000 µs.

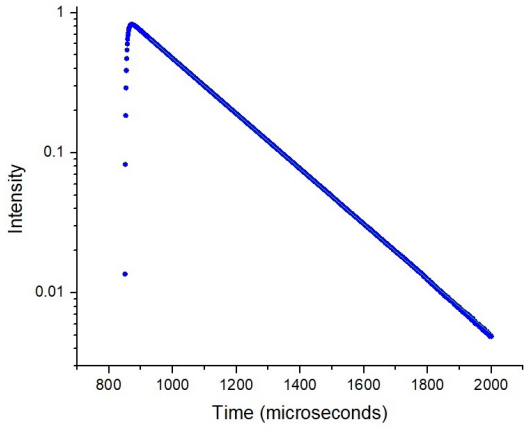



Figure 6: PL decay of $Nd^{3+}$-doped glass collected at 825nm excitation and 1053nm emission with 100 laser shots at 20Hz (5 second acquisition time). The recovered lifetime is 220 µs.lifetime is 220 µs.

A PL decay of $Ho^{3+}$-doped glass sample is shown in Figure 7. Since the decay exhibited two lifetimes, 14 and 85 us, the TR emission was measured at two different time windows in order to determine spectral contribution of each lifetime component (Fig. 8).

The TR emission spectra measured at different time delays/gates indicate that the short lifetime component is predominantly responsible for the emission bands at 1022 nm and 1372 nm, while the middle band at 1190 nm has contributions from both lifetime components.

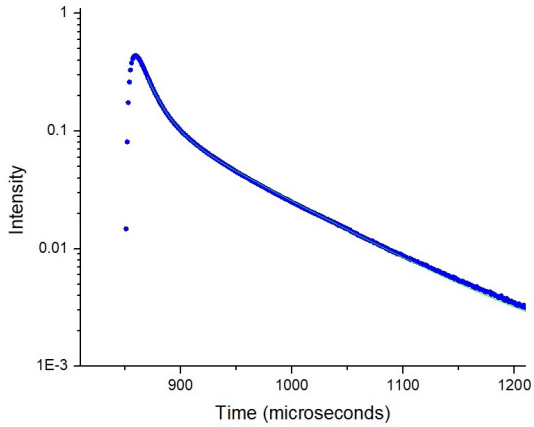



Figure 7: PL decay of Ho3+-doped glass collected at 450nm excitation and 1026nm emission with 100 laser shots at 20Hz. The decay required a double exponential fit with resulting lifetimes of 14 µs and 84.6 µs.

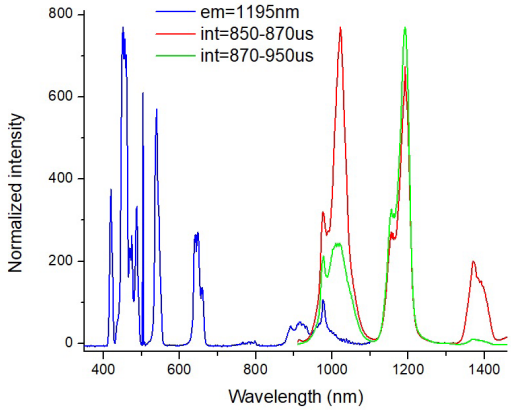



Figure 8: TR excitation and emission spectra of $Ho^{3+}$-doped glass measured with the LN-cooled InGaAs detector. The TR emission spectra were measured at two different time intervals after the excitation

## 3. NIR: Singlet oxygen phosphorescence

Determining singlet oxygen phosphorescence lifetimes is usually a difficult task due to its low quantum yield, especially in aqueous media. In our tests, using rose bengal as a photosensitizer, we were able to measure reliably the decays of singlet oxygen in methanol, acetonitrile and water and determine its lifetimes (Fig. 9).

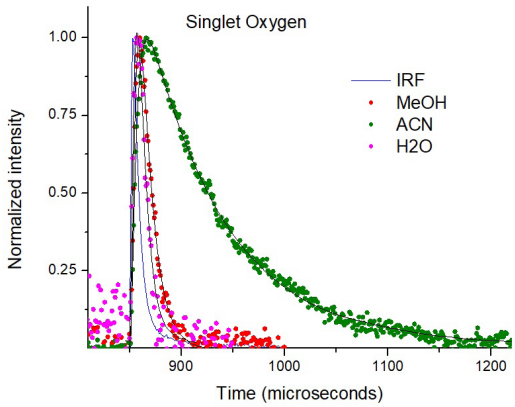



Figure 9: Singlet oxygen phosphorescence decays generated by the excitation of rose bengal at 557 nm and monitored at 1270 nm in methanol (8.2 µs), acetonitrile (75.3 µs) and water (4 µs) with the InGaAs detector.

## 4. Room Temperature Protein Phosphorescence

Tryptophan (Trp) residues in a protein, when buried inside the hydrophobic core, can often exhibit room temperature phosphorescence (RTP). The protein RTP lifetime and intensity are both very sensitive to any conformational changes, which expose the Trp to collisional quenching and oxygen molecules. The intensity of the RTP is usually very low and in addition the RTP spectrum is obscured by the overlapping tail of Trp fluorescence. The time resolved measurement allows for gating out the promptTrp fluorescence and revealing the hidden RTP.
Figure 10 shows the Fluorolog-QM equipped with the Opolette laser can readily measure the RTP spectrum of BSA with 280 nm excitation. The spectrum is obtained by integrating a part of the RTP decay after the sharp fluorescence peak (Fig. 11).

The RTP decay is double exponential with lifetimes of 2.5 ms and 7.6 ms, which likely reflect different conformational states of the BSA with different exposure of TRP to the solvent phase.

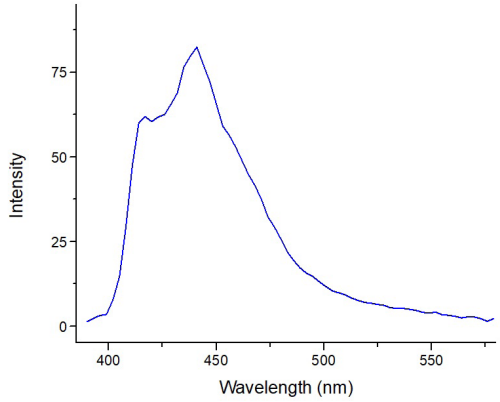



Figure 10: Time-resolved RTP of de-aerated BSA protein measured at 280 nm excitation. The spectrum is obtained by integrating the decay signal after the fluorescence peak. The integration time window was 1000-5000 µs.

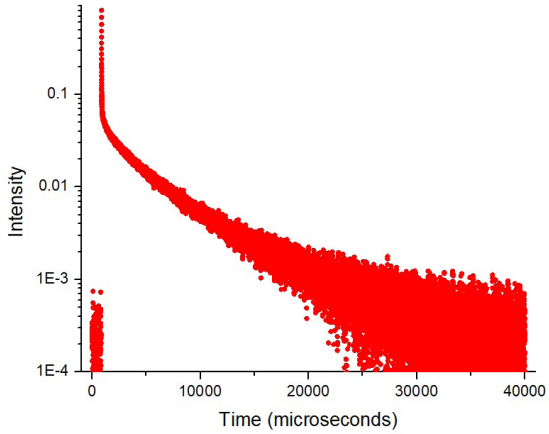



Figure 11: RTP decay of de-aerated BSA protein at 280 nm excitation and 445 nm emission. The lifetimes recovered from a 2-exp fit to the slow portion of the decay are 2.5 and 7.6 ms. 5.

## 5. Upconversion study

The Fluorolog-QM combined with the Opolette laser is an excellent choice for upconversion studies. The laser energy is high enough for sequential excitation of lanthanide materials to effect the upconversion process and the available broad spectral range makes it a versatile choice for different materials that require different excitation wavelengths.

Figure 12 presents the time-resolved upconversion spectrum of a powder material containing erbium ions and its upconversion decay curve is shown in Figure 13. The decay analysis reveals the existence of a rise time in addition to a decay time, which is consistent with the sequential two-photon excitation leading to a higher excited state.

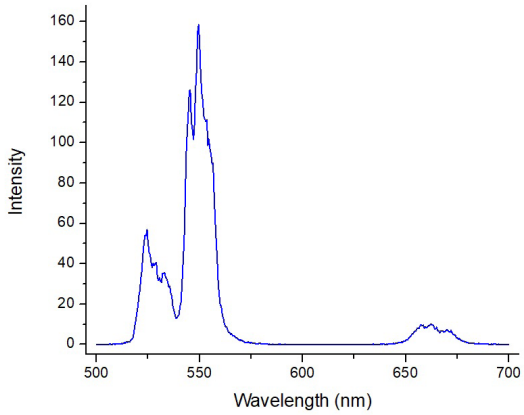



Figure 12: Time-resolved PL upconversion spectrum of a composite powder containing $Er^{3+}$ ions excited at 980 nm. The integration time window was 850-1500 µs.

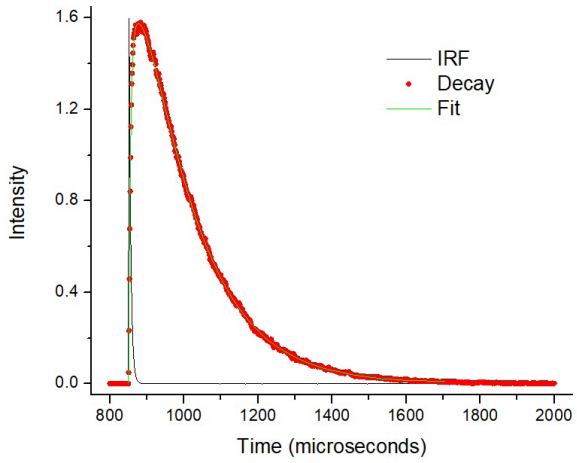



Figure 13: L upconversion decay of Er3+ powder with 980 nm excitation and 550 nm emission. The decay analysis resulted in a single lifetime of 145 µs and a risetime of 42 µs.

**6. Time resolution enhancement with digital oscilloscope** (under 10 ns to milliseconds from the UV to the NIR)

The temporal resolution of the standard Fluorolog-QM/ Opolette configuration is determined by the 1MHz transient digitizer built-in into the standard Fluorolog-QM electronic interface. This limits the lifetime range to ~ 1 us and higher. Since the OPO laser pulse duration is 7 ns, the temporal resolution of the entire system can be enhanced by adding an optional digital oscilloscope, which is also controlled by FelixFL software. With the combination of Fluorolog-QM, Opolette and faster digital oscilloscope, fluorescence lifetimes shorter than 10 ns can be measured with the SSTD detection technique and a photomultiplier detector. Below we present data acquired with the Tektronix DPO5000B 2 GHz oscilloscope, making this combination of system and components an extremely versatile PL platform across a wide wavelength range from the UV to NIR and a wide PL lifetime range from ~ 10 ns to milliseconds.

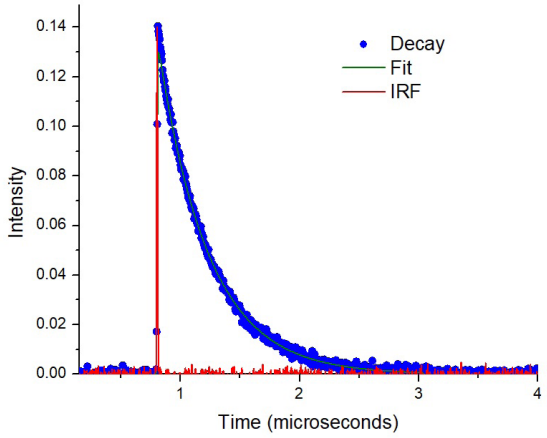



Figure 14: Fluorescence decay of deaerated pyrene in cyclohexane with OPO laser excitation in SSTD mode with Tektronix DPO5000B 2 GHz oscilloscope. Lifetime = 421 ns

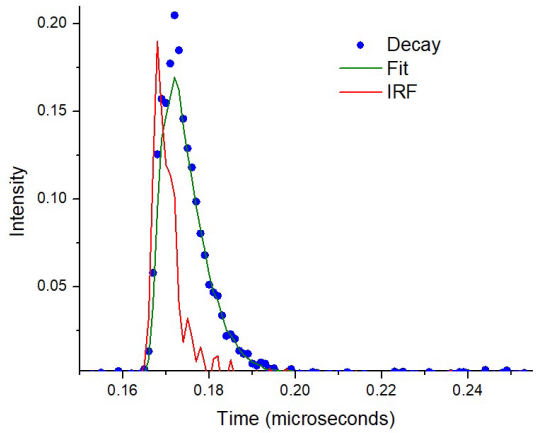



Figure 15: Fluorescence decay of perylene in propylene glycol with OPO laser excitation in SSTD mode with oscilloscope. Lifetime = 4.9 ns

Figures 14 and 15 illustrate the fluorescence lifetime measurements in the nanosecond time domain with an optional digital oscilloscope.

## Conclusion

The Fluorolog-QM equipped with the optional OPO-based Opolette laser provides the ultimate tool for time-resolved measurements in the microsecond to millisecond time domain for spectral ranges spanning from UV to NIR. The SSTD rapid acquisition mode and seamless software control of the OPO laser wavelengths make these measurements fast and easy. The versatility of the laser and the ability to utilize a broad range of detectors with the SSTD technique make this configuration suitable for diverse applications that include lanthanide material characterization and upconversion, conventional phosphorescence studies, room temperature protein phosphorescence, singlet oxygen detection and many others.

An addition of an optional digital oscilloscope can enhance the temporal resolution of this configuration to fluorescence lifetimes below 10 ns, making this combination an excellent system for comprehensive PL spectra and time-resolved research.