Application Note

PicoQuant

Performing high speed lifetime measurements of proteins using a 280 nm picosecond laser

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Introduction

When performing fluorescence measurements, a spectroscopist or microscopist needs to keep many factors in mind in order to obtain the best results. Choosing the right light source for exciting a specific sample is one of them. A large variety of sources is currently available, ranging from continuously emitting classic lamps to pulsed Light Emitting Diodes (LEDs) and all kinds of laser sources. These differ not only in the wavelengths at which they emit light but also on, e.g., spectral width, temporal pulse length (in the case of pulsed sources), light intensity, coherence, or polarization.

The fluorescence (or more generally photoluminescence) lifetime is an intrinsic characteristic for each fluorescent or phosphorescent species and can thus be used to characterize a sample. However, the lifetime can be affected by the chemical composition of its environment. Additional processes such as Förster Resonance Energy Transfer (FRET), quenching, charge transfer, solvation dynamics, or molecular rotation do also have an effect on a species decay kinetics. Changes in the lifetime are therefore a powerful tool to gain information about the local chemical environment of a photoluminescent species. Another advantage of lifetime information is that it is independent of sample concentration.

A common way to obtain photoluminescence lifetime data is Time-Correlated Single Photon Counting (TCSPC).[1] This method requires the use of a pulsed light source and, in a very simplified way, one measures repeatedly the time difference between sample excitation via a light pulse and the arrival of an emitted photon at the detector. The recorded time delays are then used to generate a histogram of photon arrival times, from which the lifetime can be extracted by mathematical means (i.e. fitting).

In the field of biology, life and materials science, the wavelength range between 280 and 300 nm is of particular interest for many microscopy and spectroscopy applications. Many important organic components such as aromatic amino acids like tryptophane or thyrosine, can be excited at these wavelengths. The fluorescence of these amino acids is very sensitive to changes in a protein's secondary or tertiary structure. For example, depending on hydrophobicity, the centre of a tryptophane emission band

Figure 1: The FluoTime 300 time-resolved spectrometer (bottom) can be equipped with a broad range of excitation sources, such as the VisUV-280 (top left) picosecond pulsed laser or the PLS-280 (top right) pulsed LED.

can shift by several tens of nanometers in addition to changes in its lifetime and quantum yield. Therefore, spectroscopic studies of tryptophane can provide insights into conformational changes even in proteins containing multiple of these amino acid residues.

Up to now the above mentioned spectral range was only accessible through either an expensive combination of amplified Ti:Sapphire laser with an Optical Parametric Oscillator (OPO), power inefficient pulsed LED or frequency doubled super-continuum lasers (which generate optical output levels in the µW range at MHz repetition rate).

PicoQuant recently added a new laser module to their VisUV laser platform range: the VisUV-280,[2] which generates picosecond laser pulses at 280 nm with variable repetition rates up to 80 MHz and an average optical output of more than 1 mW at 80 MHz. These characteristics make the VisUV-280 an excellent choice for many applications in life science applications, including fluorescence spectroscopy and time-resolved confocal microscopy.

To demonstrate this point, we performed steady state and time-resolved fluorescence measurements with Human Serum Albumin (HSA), an important protein for many pharmaceutical and biomedical applications, using a FluoTime 300[3] spectrometer equipped with a 280 nm pulsed LED (PLS-280)[4] or our new picosecond pulsed UV laser (VisUV-280).

The FluoTime 300 – a modular, high performance spectrometer

The FluoTime 300 is a versatile high performance fluorescence spectrometer featuring full automation and modular design. The system can accommodate a wide range of pulsed and continuous wave (CW) excitation sources such as picosecond diode lasers, LEDs as well as fiber amplified lasers or Xenon lamps.

With broad selection of detectors, TCSPC and Multichannel Scaling (MCS) electronics as well as accessories, the FluoTime 300 is an excellent choice for carrying out many types of spectroscopic applications such as steady state, time-resolved, anisotropy or phosphorescence measurements, covering time ranges from a few picosecond to several seconds.

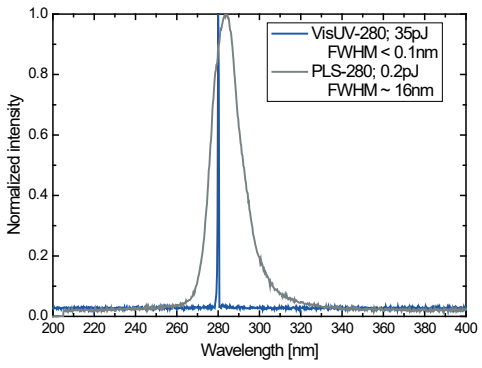

Figure 2: Optical spectrum of the pulsed LED PLS-280 (grey) and VisUV-280 (blue)

Comparing light sources: picosecond pulsed diode laser vs. pulsed LED

The VisUV-280 laser is a versatile and flexible platform based on a Master Oscillator Fiber Amplifier (MOFA) concept with frequency conversion. The master oscillator generates infrared picosecond pulses at 1064 nm with variable repetition rates up to 80 MHz using proven gain-switching techniques. The freely triggerable distributed feedback (DFB) laser diode generates sub-100 ps seed pulses that pass through a two-stage fiber amplifier.

The amplified pulses are fed into a commercial polarization maintaining Raman fiber, resulting in light pulses at 1120 or even 1180 nm. The Raman-shifted pulses are separated from residual fundamental light and then frequency doubled twice in a single pass cascade consisting of non-linear crystals with optimized focal conditions and spectral filtering.

In comparison to a pulsed LED (PLS-280), the optical characteristics of the VisUV-280 pulses are much better suited for spectroscopic applications. Not only is the spectral bandwidth is much narrower (< 0.1 nm vs. ~16 nm, see Fig. 2), but the temporal

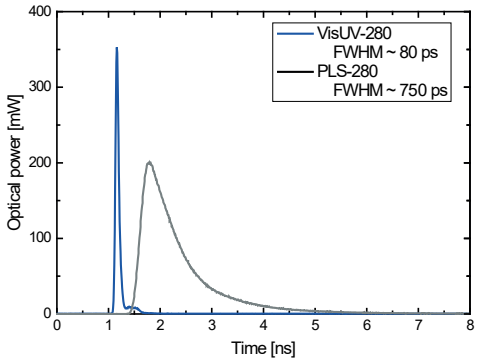

Figure 3: Temporal pulse trace of the pulsed LED PLS-280 (grey) and VisUV-280 (blue)

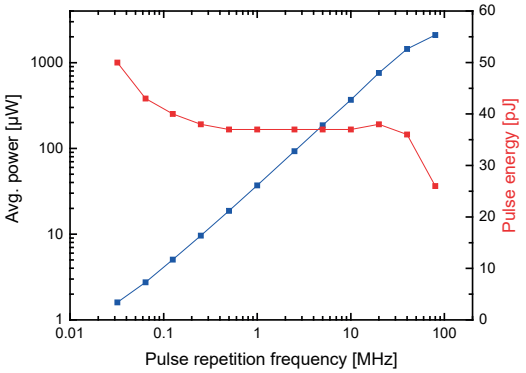

Figure 4: Optical output power (blue) and pulse energy (red) of the VisUV-280 as a function of repetition rate

width is much short in the case of the laser pulses (80 vs. 750 ps, full width at half maximum, see Fig. 3). This will be a decisive factor for time-resolved measurements as will be soon shown.

Furthermore, the picosecond pulsed laser source can achieve much higher light intensities than a conventional LED and has a well defined, nearly circular gaussian beam profile, with $M^2 < 1.1$ (for vertical direction) and $M^2 < 1.5$ (for horizontal direction).

Performance differences in real-world measurements

In this section, we will demonstrate the advantages of using a picosecond pulsed laser as excitation source for steady state and time-resolved fluorescence measurements with Human Serum Albumin (HSA) as a real-world sample. HSA is an important protein for many pharmaceutical and biomedical applications and can be well excited at 280 nm.

The following experiments were carried with a FluoTime 300 equipped with a double monochromator in the emission beam path. Incoming photons were detected via a PMA Hybrid 07 detection module connected to a TimeHarp 260 Pico. A high dynamic range clean-up filter was used to remove fundamental light background from the UV signal. The double monochromator was operated in substractive mode for time-resolved measurements (for improved time resolution) and in additive mode for recording steady state spectra (yielding higher spectral resolution).

Acquiring time-resolved data

Figure 5 shows the decay measured with a PLS-280 LED excitation source with a temporal width of approximately 800 ps (full width at half maximum). As a consequence of this pulse width, lifetimes of less than 250 ps cannot be determined as accurately as necessary for such samples. An additional issue was that acquiring this decay curve took about 12 minutes due to the LEDs low light intensity output. Low excitation power means that the sample will emit few fluorescence photons per time interval, leading to low count rates at the detector. As a consequence, collecting enough fluorescence events for good photon statistics will take more time than with a more powerful excitation source.

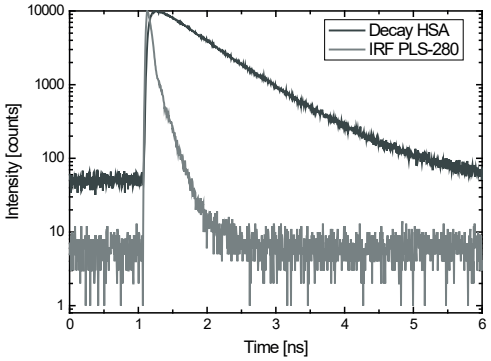

Figure 5: Decay (black) of HSA in water and Instrument Response Function (IRF, grey) obtained using the PLS-280 LED as excitation source

The decay curve shown in Figure 6 was measured with the same sample under the same experimental conditions, maintaining a repetition rate of 10 MHz, but using the VisUV-280 picosecond pulsed laser as excitation source. With this setup, the lasers excitation pulses have a temporal width of around 140 ps (full width at half maximum). Thanks to this narrower pulse width, shorter lifetimes can be determined more accurately that with the PLS-280 LED.

Also, the laser source produces a much higher optical output power, meaning that a higher fluorescence photon count rate was achieved. Thus, the measurement took only 20 seconds to complete compared to the previous 12 minutes.

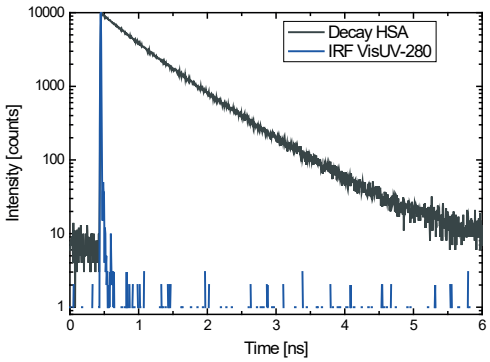

Figure 6: Decay (black) of HSA in water and Instrument Response Function (IRF, blue) obtained using the PLS-280 LED as excitation source

As expected, a tri-exponential model could be well fitted to the experimentally acquired decay data. The resulting lifetimes are given in Table 1. The values obtained with both excitation sources compare quite well to published data on HSA.[5] However, determining the shortest lifetime component (T_3) is more accurate when using the VisUV-280 as excitation source (0.33 vs. 0.19 ns).

Recording steady state spectra

The difference in optical output intensity between the LED and picosecond pulsed laser sources plays also an important role when recording steady state spectra. Under the same measurement conditions (i.e. an integration time of 1 s and a band pass of 2 nm), the steady state emission peak of HSA at 360 nm is 35 times more higher when using the VisUV-280 as excitation source (see Fig. 7). In order to get comparable result with the PLS-280 LED source, one would have to increase the integration time by a factor of 35 (e.g., to 45 s).

Another advantage of the VisUV-280 picosecond pulsed laser is that the excitation power can be varied

Table 1: Lifetimes obtained from fitting the decay data acquired with a pulesd PLS-280 LED or VisUV-280 laser.

Multi-exponential fit parameters	PLS-280 [ns]	VisUV-280 [ns]
T_1	8.33 ± 0.45	7.73 ± 0.09
T_2	5.37 ± 0.31	4.47 ± 0.16
T_3	0.33 ± 0.03	0.19 ± 0.03

without changing the temporal width of the pulses. This allows selecting the best excitation conditions (high count rates for short measurement times, outstanding temporal resolution) for a sample while reducing the risks of photobleaching by keeping the excitation power as low as possible.

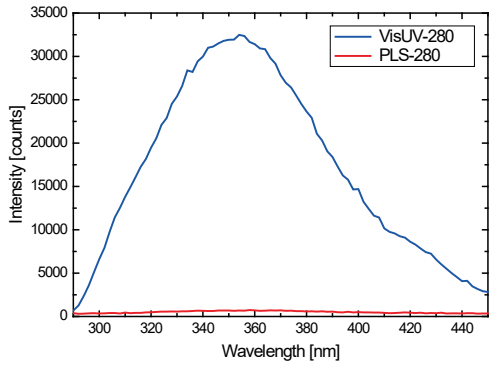

Figure 6: Decay (black) of HSA in water and Instrument Response Function (IRF, blue) obtained using the PLS-280 LED as excitation source

Conclusion

As the measurements reported here clearly show, using the VisUV-280 picosecond pulsed laser as excitation source instead of the PLS-280 LED allows for much shorter acquisition times for steady state and time-resolved measurements. The overall acquisition time for the full decay was about 35 times faster with the laser (from 12 min to 20 s). Furthermore, the narrower temporal pulse width of the VisUV-280 allows determining short lifetimes with higher accuracy. By combining these advantages, the VisUV-280 picosecond pulsed laser enables faster measurements with higher accuracy and less risk of photobleaching than a conventional UV LED such as the PLS-280.

Acknowledgment

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References and Further Reading

[1] M. Wahl, Technical Note on Time-Correlated Single Photon Counting, PicoQuant (2014)

[2] Website containing information regarding the VisUV high-power laser platform: https://www.picoquant.com/products/category/high-power-and-uv-lasers

[3] Website containing information regarding the FluoTime 300 time-resolved luminescence spectrometer: https://www.picoquant.com/products/category/fluorescence-spectrometers/fluotime-300-high-performance-fluorescence-lifetime-spectrometer

[4] Website containing information regarding the PLS Series sub-nanosecond pulsed LEDs: https://www.picoquant.com/products/category/picosecond-pulsed-sources/pls-series-sub-nanosecond-pulsed-leds

[5] M. Amiri, K. Jankeje, J. R. Albani, Journal of Pharmaceutical and Biomedical Analysis, 2010, vol. 51, 1097

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