Application Note

PicoQuant

Measuring steady-state and time-resolved photoluminescence from a positionable, micrometer-sized observation volume with the FluoMic Microscope

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Introduction

Over the years, luminescence spectroscopy has established itself as one of the fundamental methods for analyzing the photophysical properties of a variety of samples, ranging from simple organic molecules, complexes, luminescent proteins to semiconductors. The commonly used steady-state methods (i.e. excitation and emission spectroscopy) provide valuable insights into the photophysics of a sample. However, such results give only a partial view of the sample's behavior after photoexcitation. A further piece of the puzzle is often revealed by performing time-resolved luminescence spectroscopy.

Studying luminescence lifetime data of a material is a very powerful analytical tool for spectroscopists and microscopists alike, as it provides insights into the excited state dynamics of molecules, complexes, nanoparticles, or semiconductors. The fluorescence or phosphorescence lifetime is an intrinsic characteristic of luminescent species. It indicates how long the species under consideration will remain in an electronically excited state before returning to the ground state. Each emitting species has a characteristic luminescence lifetime that can be influenced by its environment.

A series of spectroscopy and microscopy methods based on luminescence lifetime have been developed and allow obtaining information that would be otherwise not accessible through steady-state experiments. For example, fluorescence lifetime imaging (FLIM) is a very well established imaging method in life science where the lifetime information is combined with spatial localization in the sample, allowing investigating biochemical or physical processes.[1] This combination of data can help detecting changes in the local environment such as pH, temperature, or ion concentration, identify molecular interactions or conformation changes via Förster Resonance Energy Transfer (FRET). Time-resolved methods are commonly used not only in biological studies, but also a powerful tool in materials science for the characterization of key parameters like e.g. charge carrier dynamics and mobility in semiconductors.

Combining the spectral and time information of a sample's luminescence signals often provide a good understanding of the dynamic processes occurring in it. This understanding can be further expanded by including spatial information. Getting steady-state and time-resolved spectroscopic data form multiple, well defined points of the sample can help in inferring structural-to-photophysical relationships.

The aim of this White Paper is to highlight that the combination of FluoTime 300 and FluoMic Microscope is a powerful tool which brings your research to a new level by giving you photophysical information (i.e. spectral, temporal, and spatial) of your sample. The abilities of this combination will be demonstrated with a series of examples reflecting a broad range of applications.

The FluoTime 300, a high-end spectrometer for photoluminescence studies

The FluoTime 300 "EasyTau" is a modular, high performance photoluminescence spectrometer for steady-state and time-resolved measurements with full automation.[2] Thanks to its design and broad range of accessories, the spectrometer can be

adapted to specific sample and measurement needs in a simple and cost effective way.

As the FluoTime 300 supports data acquisition by means of either Time-Correlated Single Photon Counting (TCSPC)[3] or Multichannel Scaling (MCS) techniques, luminescence decays with lifetimes ranging from a few picosecond to several seconds can be investigated. Multiple detector options and a broad range of easily exchangeable light sources (including picosecond pulsed and continuous wave lasers, pulsed LEDs, and Xenon lamps) are available, which provide access to a broad spectral range from UV up to NIR.

The spectrometer can be equipped with double monochromators in both excitation and emission pathways. Due to their very high stray light rejection (signal-to-noise ratio of 29.000:1 (RMS) using standard water Raman test), the FluoTime 300 can be used to also study samples with very strong scattering contributions with extreme sensitivity and temporal resolution. The operation of the emission double monochromator can be switched from additive to subtractive mode via the spectrometer's graphical user interface. Additive mode is ideally suited for applications requiring high spectral resolutions and can reach values as high as 0.15 nm. Temporal resolution can be significantly increased in subtractive mode, which allows studying very short luminescence lifetimes. In combination with appropriate excitation sources, TCSPC electronics and detectors, an Instrument Response Function (IRF) below 60 ps can be achieved.

All instrument operations are controlled from the intuitive and easy-to-use "EasyTau2" system software. Specifically designed application wizards guide the user through the necessary optimization steps for performing typical measurement tasks. A customized measurement mode with full instrument control is available for those who are familiar with the techniques. More sophisticated application tasks, like, for example, alternating between time-resolved decays and steady-state spectra at different temperatures over night or automation of routine processes can be easily performed through scripted data acquisition using the integrated scripting language.

All time-resolved data can by analyzed with the integrated analysis and fitting module of the EasyTau2 software, which features global decay analysis and iterative re-convolution (up to fourth order) with non-linear error minimization through an easy-to-use graphical user interface. This feature set makes the FluoTime 300 the ideal analysis platform for studying many types of photoluminescent samples in materials science not only for experts but also for novice users.

The FluoMic Microscope: getting information from well defined points of a sample

In many cases, characterizing materials such as semiconductors or solar cells requires measuring their photophysical properties at various points with spatial resolution. However, scanning over a large sample area is often not necessary, as single point measurements at various sites are sufficient.

The FluoMic Microscope (see Fig. 1) provides a fast, easy, and reliable way to bring the power of the FluoTime 300 spectrometer to a sample with a micrometer sized, positionable observation volume. On the spectrometer side, installation of the FluoMic requires only sliding the specialized mounting unit into the sample chamber.

Thank to its pre-aligned optical fibers, both pulsed and CW light sources of the FluoTime 300 can be used to excite a sample located in an external device. The FluoMic includes a special microscope fiber coupler unit that can be attached to various microscopes (such as, e.g., the Olympus BX43).

Figure 1: The FluoMic Microscope consists of a specialized sample mounting unit (A), a series of pre-aligned optical fibers (B), a fiber in-coupler (C) and out-coupler (D) unit that can be attached to various microscopes (here an Olympus BX43 is shown).

Emitted light is collected from a small area (down to 2 µm) and brought back to the detection arm of the FluoTime 300 via a detection fiber. Thus the outstanding flexibility in both spectral (from UV to NIR) and time (from ps to ms) range of the FluoTime 300 can be extended to samples located outside the spectrometer and then measured with spatial resolution. Furthermore, the FluoMic provides access to the broad palette of wizard-guided or customized steady-state and time-resolved applications supported by EasyTau 2.

Application examples

Recording steady-state and time-resolved spectra of fluorescence standards

To check the performance of the FluoMic , excitation and emission spectra measured with the SFG and SFO polymer reference blocks from Starna Scientific (UK). Steady-state emission and excitation spectra were recorded with a FluoTime 300 equipped with a FluoMic Microscope, a Xe arc lamp as excitation source, a 20x objective and a 50/50 beam splitter. For comparison, these spectra were measured under the same conditions, but using the conventional sample holder of the FluoTime 300.

All recorded spectra are shown in Figure 2, with data from SFG displayed in green and SFO in orange. Lines indicate excitation and emission spectra (solid and dashed, respectively) obtained using the FluoMic Microscope. Data obtained using the conventional sample holder is represented by circles: filled for excitation and empty for emission. Both set of spectra are identical and in good agreement with the curves reported by Starna.

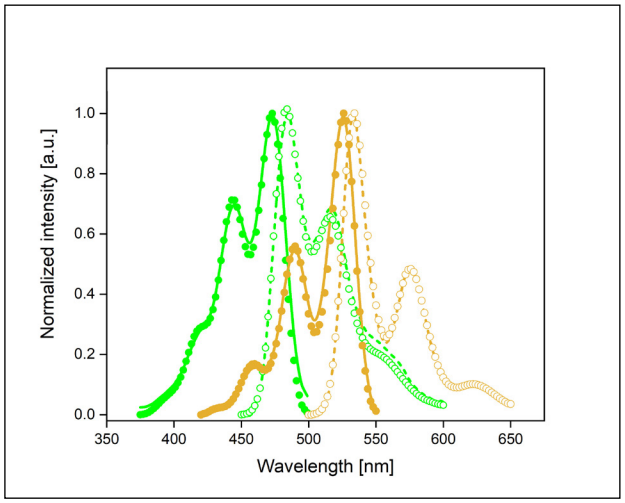

Figure 2: Excitation and emission spectra of the fluorescence polymer reference block SFG (green) and SFO (orange). Spectra obtained using the FluoMic Microscope are shown as lines (solid = excitation, dashed = emission) and those recorded using a conventional sample holder as circles (filled = excitation, empty = emission).

Time-resolved emission spectra (TRES) and fluorescence decays were acquired from both samples using picosecond pulsed lasers from PicoQuant with excitation wavelengths of 438 nm (LDH-P-C-440) or 510 nm (LDH-P-C-510B) for SFG and SFO, respectively. Again, one set of measurements was performed using the FluoMic Microscope (with a 20x objective and a 50/50 beamsplitter) and another with the conventional sample holder.

A single exponential model can be well fitted to each recorded decay. For both samples, the resulting lifetimes are nearly identical regardless of whether the FluoMic Microscope is used or not. The values for SFG (3.8 ns) and SFO (3.5 ns) match well the lifetimes reported by Starna. The TRES curves for both samples are shown in Figure 3 with data from SFG in green and SFO in orange. For both samples, the spectra are nearly identical regardless of of whether the FluoMic Microscope was used (lines) or not (circles).

All of these measurements nicely show that the FluoMic allows bringing the full power and versatility of the FluoTime 300 to samples outside the spectrometer. Thanks to the add-on, the spectrometer's high accuracy and precision in both spectral and time dimensions can be applied to any point of a sample.

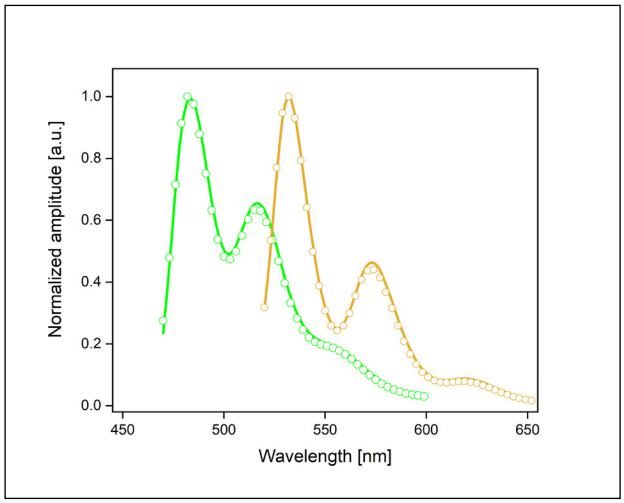

Figure 3: TRES recorded from the fluorescence polymer blocks SFG (τ_{fl} = 3.8 ns) and SFO (τ_{fl} = 3.5 ns)

Using FluoMic to record TRES of gummy bears

Gummy bears are a popular type of gelatin-based candies from Germany, which were created by Hans Riegel, Sr. in 1922. Combing in all sizes and shapes, they are not only a popular treat (see Fig. 4 A) but are also nicely luminescent.

The luminescence properties a yellow and a red gummy bear were investigated with the help of the new FluoMic Microscope. Steady-state emission spectra of both were recorded upon excitation with a 440 nm pulsed diode laser (Fig. 4 B). The two candies exhibit a similar emission band with a maximum at about 520 nm, with the red one having a broader tail towards the red spectral range. This is not surprising as their main ingredients are gelatin, sugars, starch, and will be differing only in flavoring and food coloring.

Recording TRES from the same candies yields more information (Fig. 4 C and D): In both cases, a tri-exponential model fits the data very well. The two longer-lived components (blue and red curves) have a similar spectral shape with maxima at around 510 nm. The shortest-lived component (probably stemming from the colorant) shows additional peaks at 585 and 610 nm for the red gummy bear.

Recording luminescence spectra from larger and irregularly shaped samples becomes very easy and fast using FluoMic. Plus, the spectroscopist get to enjoy the gummy bears afterwards.

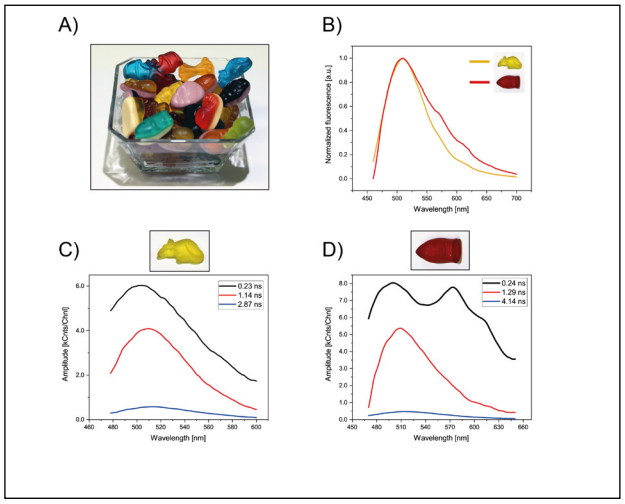

Figure 4: Gummy bears (A) and their spectral properties: steady-sate spectra of a yellow and red gummy bear recorded upon excitation at 440 nm (B), TRES of the yellow gummy bear (C) and of the red one (D)

Studying the photoluminescence of a solar cell

A FluoTime 300 equipped with the FluoMic Microscope is very well suited for studying the photophysics of semi-conductors or solar cell. In this example, steady state and time-resolved spectra from a Copper Indium Germanium diSelenide (CIGS)-based solar cell have been measured. The sample was kindly provided by Dr. G. Brammertz, IMEC, Leuven (Belgium).

The cell is built up as a stack starting with a Mo back contact, followed by a 1.5 µm thick CIGS layer, a 50 nm CdSe buffer layer, a 150 nm iZnO layer and topped off with a 300 nm thick ITO contact. A silver grid is layered over the top contact (see Fig. 5). Steady-state emission spectra and photoluminescence decays were recorded from two spots: one close to a silver grid line and the other roughly in the middle between two lines.

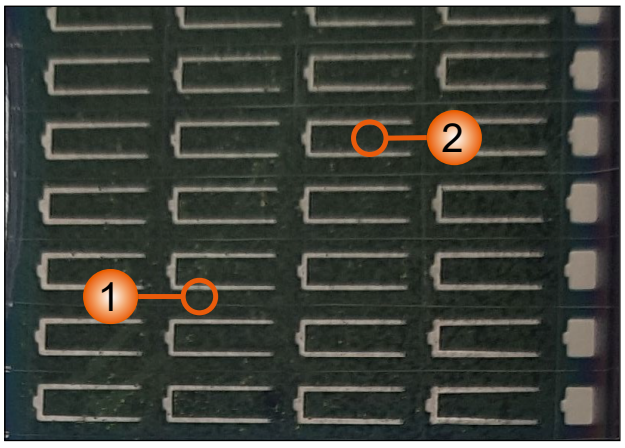

Figure 5: Photograph of the CIGS solar cell used in this example (top view) with two measurement points indicated as 1 and 2. Sample courtesy of Dr. G. Brammertz, IMEC, Leuven (Belgium)

From this sample, both steady-state and time-resolved data was recorded using a FluoTime 300 spectrometer equipped with the FluoMic Microscope. In all experiments, a picosecond pulsed laser module (VisUV-560) emitting at 560 nm was used as excitation source.

The FluoMic Microscope was outfitted with a 10/90 beam splitter (excitation/detection) and either a 20x or 40x objective. A change in objective leads to a size difference in both excitation and detection spots: the 20x objective provides an excitation spot size of ca. 60 µm and a detection area of about 10 µm, while these values change to ca. 30 µm (excitation) and 5 µm (detection) for the 40x objective. This spot size variation is useful for checking whether the photoluminescence depends on the excitation power density.

The emission spectra recorded at both spots are identical: a broad, featureless band with a maximum at 1250 nm. These steady-state spectra are also not influence by a change in objective. However, the luminescence decays recorded at 1250 nm are significantly affected by the objective change, as can be seen in Figure 6. The difference is even more pronounced at position two, where not only the average lifetime is quite shorter, but also the shape of the decay curve changes massively.

This example highlights that being able to acquire data from well defined excitation/detection area

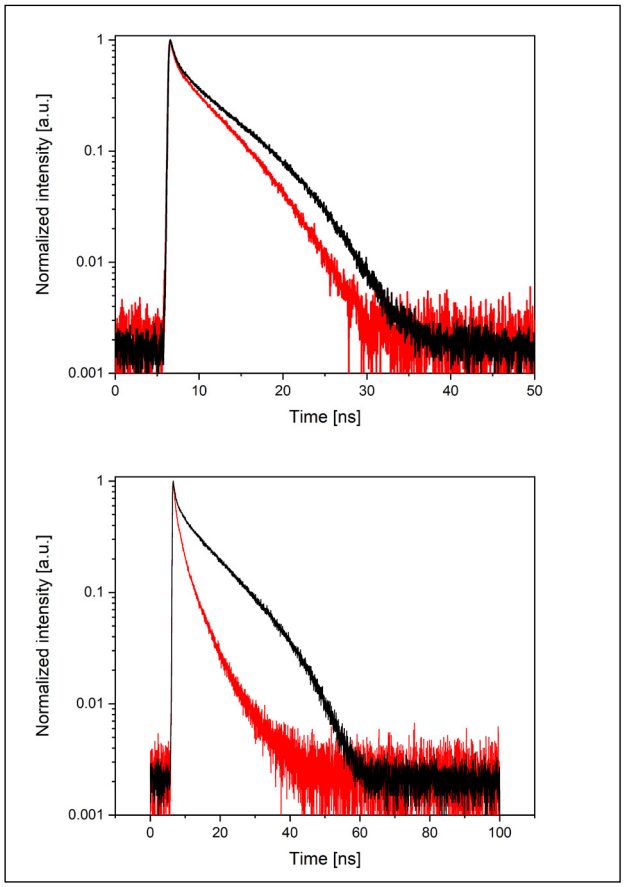

Figure 6: Photoluminescence decays acquired from spot 1 (top) and 2 (bottom). The red curves were obtained using a 20x objective and the black ones with a 40x objective.

can provide deeper insights into the relationships between structure and photophysical behavior. This information is not readily available when investigating such a sample with a conventional spectrometer as the luminescence signal is averaged over a lager area (typically 1 mm^2 or more).

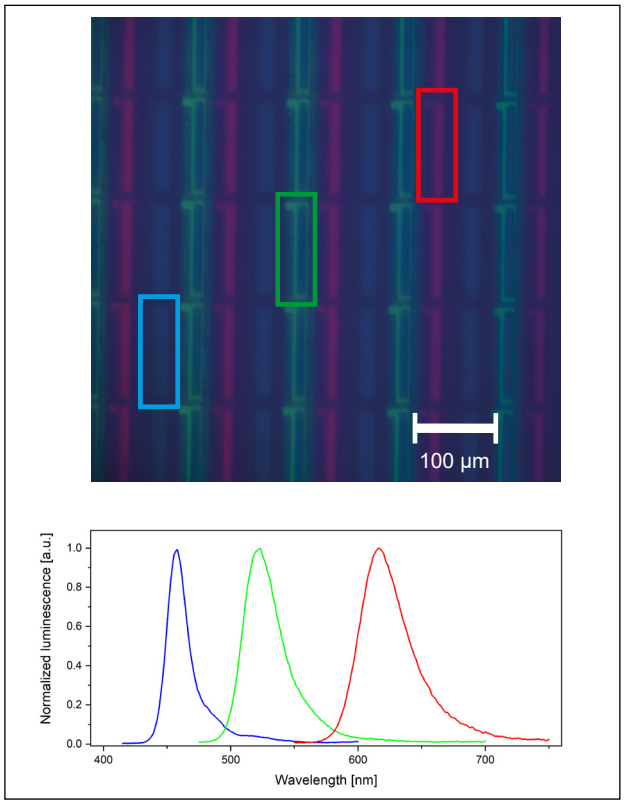

Figure 7: Top: microscopy image of a smartphone screen captured through a 20x objective. The blue, green and red pixels selected for the measurements are indicated by boxes in the corresponding colors.
Bottom: steady-sate emission spectra recorded from the marked pixels.

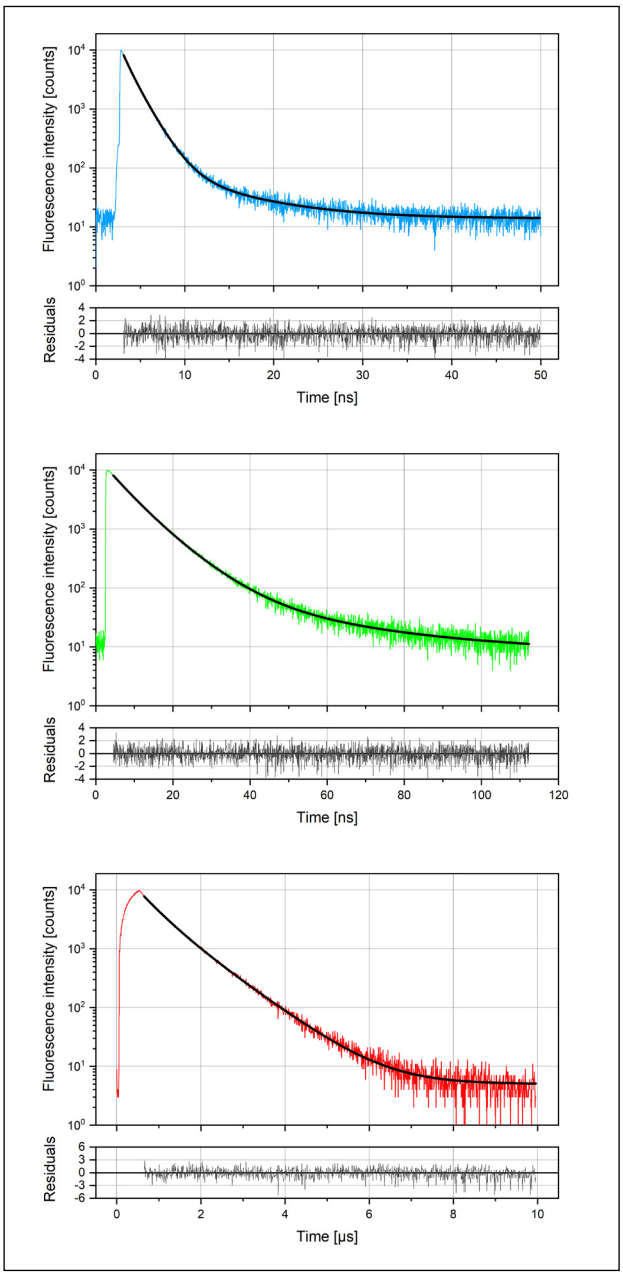

Figure 8: Luminescence decays recorded from the selected pixels as well as fitted single exponential functions. From top to bottom: blue, green, and red pixel.

Investigating pixels on a LED display

The stage of Olympus BX43 upright microscope can accommodate even rather large or unconventionally shaped objects. As an example, we placed a regular smartphone on the stage to acquire steady-state as well as time-resolved data from individual pixels of its screen.

The microscope stage was used to move a white part of the screen into the 20x objective's focal point (see Fig. 7, top). The detection spot was then moved to either a blue, green, or red pixel where the corresponding emission (Fig. 7, bottom) and luminescence decay curves (Fig. 8) were acquired. The sample was excited at 440 nm using a pulsed diode laser from PicoQuant. Luminescence decay of blue pixels was acquired at 460 nm, green pixels at 522 nm, and red pixels at 620 nm, respectively. It should be mentioned that the laser was used in burst mode (100 kHz effective repetition rate, 500 ns burst length) when luminescence kinetics of red pixels was collected.

Conclusions

The FluoMic Microscope gives you the opportunity to quickly and easily carry out time-resolved as well as steady-state luminescence measurements on wide range of solid objects outside of the spectrometer. It extends the power and capabilities of the FluoTime 300 with the ability to gather data from a well defined observation spot that can be freely placed. This additional spatial information enables you to get a more detailed picture of the processes and dynamics

occurring in your sample by letting you know what happens where.

Further reading

[1] S. Trautmann, V. Buschmann, S. Orthaus, F. Koberling, U. Ortmann, R. Erdmann, Application Note on Fluorescence Lifetime Imaging (FLIM) in Confocal Microscopy Applications, PicoQuant (2012)

[2] https://www.picoquant.com/products/category/fluorescence-spectrometers/fluotime-300-high-performance-fluorescence-lifetime-spectrometer

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

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