Application Note

PicoQuant

# Microvolume Fluorescence Spectroscopy Measurements with the FluoTime Spectrometer

**Eugeny Ermilov, Maria Loidolt-Krüger, Bita Rezania**

PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany, info@picoquant.com

## Advanced analysis of low-volume nucleic acid and protein samples

In scientific research as well as in diagnostics, efficiency, sensitivity, and cost-effectiveness are important. An ever-increasing demand for more accurate results, coupled with the need to conserve precious patient samples and minimize reagent use and waste, is challenging laboratories across disciplines, including cell culture and microbiology.

Highly sensitive instruments which can precisely detect low concentrations of analytes in small sample volumes help to address these challenges. In this application note, we demonstrate microvolume fluorescence spectroscopy and lifetime measurements with the FluoTime 250 Lifetime Fluorometer, which can be applied to a range of tasks, including nucleic acid analysis, protein assays, enzyme assays with fluorogenic substrates, fluorescent ion indicators, metabolite quantification, and immunoassays. It should be mentioned that the same experiments can be carried out with the FluoTime 300 as well.

Our approach emphasizes minimal sample consumption, ensuring that valuable specimens such as cell cultures, microbiological cultures, and patient samples are utilized efficiently. Moreover, our approach is designed to optimize reagent usage without compromising performance, thereby reducing overall costs associated with laboratory analyses. Furthermore, our strategy prioritizes waste reduction, contributing to sustainable laboratory practices while maintaining the quality of experimental data.

## Fluorescence spectroscopy methods for advanced analytical applications

Among other analytical techniques, steady-state and time-resolved fluorescence spectroscopy can be used for identification, quantification, and purity evaluation of substances.

Steady-state emission spectroscopy is characterized by its simplicity and wide applicability. By analyzing the emission spectrum, researchers can identify substances based on their unique fluorescence signatures. This technique finds extensive use in the identification and detection of various compounds, including fluorophore-labeled biomolecules, environmental pollutants, and pharmaceuticals.

Furthermore, emission spectroscopy serves as a reliable tool for evaluating the purity of substances or quality of materials. Discrepancies in fluorescence spectra can indicate the presence of contaminants or structural alterations in the sample.

Time-resolved fluorescence spectroscopy represents a sophisticated extension of steady-state emission techniques. By measuring the decay of fluorescence emission over time following pulsed excitation, researchers can extract kinetic information about the sample.

The fluorescence lifetime decay is characteristic for each fluorophore and its environment and can thus be used for identification, complementing spectral information.

Moreover, this methodology is particularly valuable in applications involving environmentally sensitive fluorophores, where changes in the local environment, such as pH, temperature, concentrations of various ions, or viscosity, modulate the fluorescence lifetime. By exploiting the sensitivity of fluorescence lifetimes to environmental changes, researchers can design sensors capable of quantitatively measuring ion concentrations in biological and environmental samples. This capability opens avenues for real-time monitoring with temporal resolution.

The FluoTime Lifetime Fluorometer series (FluoTime 250 and FluoTime 300) makes these techniques readily available, as it is designed for ease of use, acquisition speed, and simple maintenance.


*FluoTime 250*

*FluoTime 300*

## Exemplary results and discussion

For proof of principle experiments, we used the common fluorophore Fluorescein dissolved in Ethanol. Two solutions with a constant volume of 60 µl and 4000-fold different concentration of 100 nM and 25 pM Fluorescein were prepared, placed in a small cuvette in the fluorometer, and measured. The same type of cuvette is also available for 28 µl instead of 60 µl volume. Using the smaller cuvette yields the same results, since the optical path for 28 µl and 60 µl cuvettes is the same (1.5 mm).

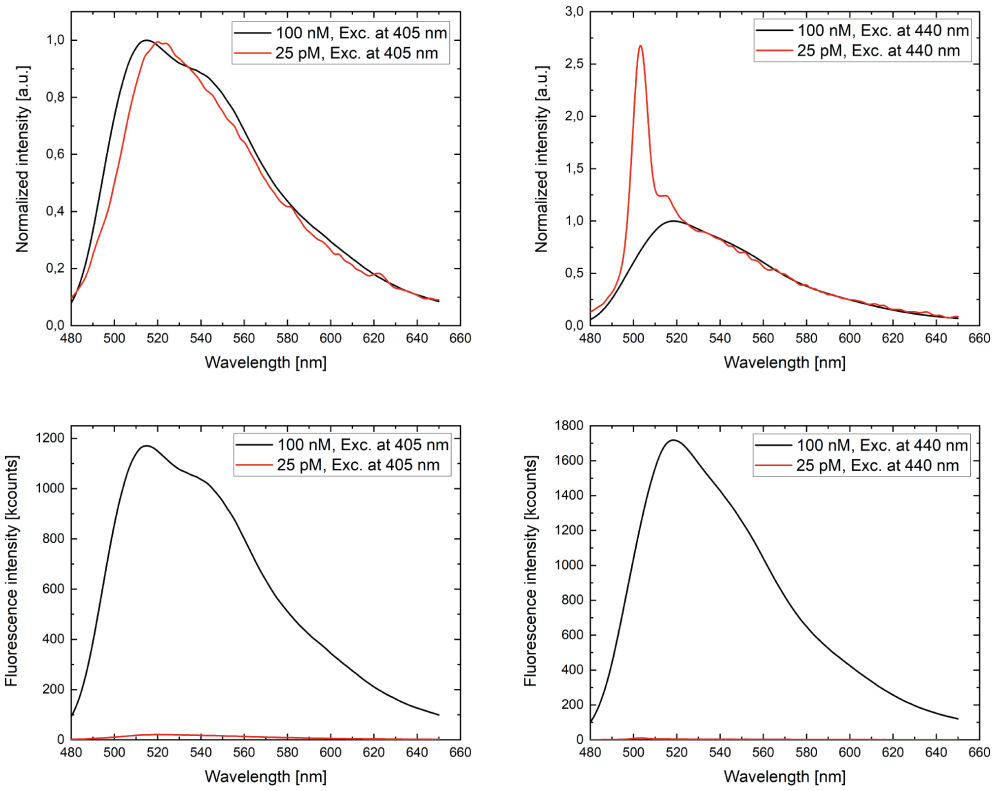


*Figure 1: Normalized (top) and original (bottom) fluorescence emission spectra of Fluorescein in EtOH with a concentration of 100 nM (black) or 25 pM (red) acquired after excitation with either 405 nm (left) or 440 nm (right).*

The acquired fluorescence emission spectra of Fluorescein in EtOH are shown in Fig. 1. The original spectra (bottom) clearly show the large decrease in fluorescence signal collected from the 4000-fold diluted 25 pM sample compared to the 100 nM sample. Yet the shape of the emission spectra is very similar upon excitation with 405 nm, as one can see after normalization (top left). This demonstrates the great sensitivity of the FluoTime 250.

After excitation with 440 nm a large Raman peak at about 500 nm is clearly visible in the emission spectrum of the 25 pM sample (top right). This highlights the practical need to select the proper excitation wavelength to minimize the spectral overlap of the fluorescence and the Raman signals. Fortunately, the FluoTime 250 offers this flexibility in configuration.

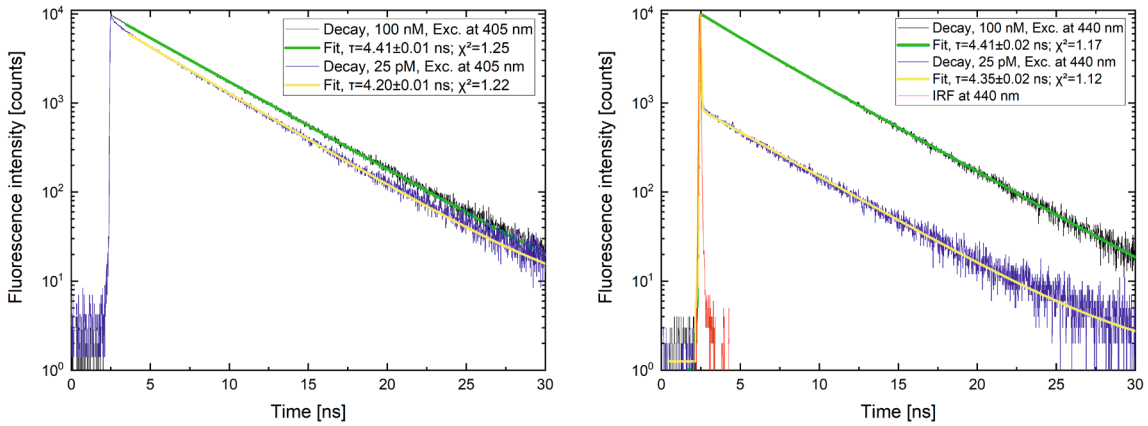


*Figure 2: Fluorescence lifetime decays of Fluorescein with a concentration of 100 nM (black) or 25 pM (blue) acquired at 516 nm after excitation with either 405 nm (left) or 440 nm (right). Fluorescence lifetimes obtained from tail fit (left) and reconvolution fit (right) are indicated in the legends.*

As pulsed excitation lasers and time-resolved detection are used, we can obtain fluorescence lifetimes as well. Fig. 2 shows the fluorescence decays of Fluorescein at both concentrations, acquired after excitation with either 405 nm (left) or 440 nm (right) and detected at 516 nm. One can perform a tail fit or a reconvolution fit of the decay to obtain the fluorescence lifetime, which is approximately 4.3 ns for Fluorescein in EtOH. The measured lifetimes agree very well for all conditions, highlighting the robustness and reliability of this method even at low signal levels.
In the fluorescence lifetime decay of 25 pM Fluorescein after 440 nm excitation, the Raman signal is visible as well, as a sharp decrease at the very beginning of the decay curve. By performing a reconvolution fit, this effect could be very easy taken into account in the analysis of experimental data as a “scattered light”.

## Conclusion

The FluoTime spectrometer series is versatile and extremely sensitive, and can thus be used for reliable determination of fluorescence emission spectra and fluorescence lifetimes, even at very low concentrations in small sample volumes. The FluoTime spectrometers can measure the lifetime precisely and reliably, enabling a robust read-out of lifetime-based reporter fluorophores. Their sensitivity enables you to preserve precious samples and minimize reagent use and waste.

PicoQuant GmbH
Rudower Chaussee 29 (IGZ)
12489 Berlin
Germany

Phone +49-(0)30-1208820-0
Fax +49-(0)30-1208820-90
Email info@picoquant.com
Web www.picoquant.com