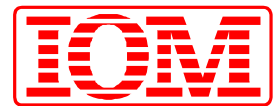


# fluorescence lifetime measurement in microplates at nano- and picomolar concentrations

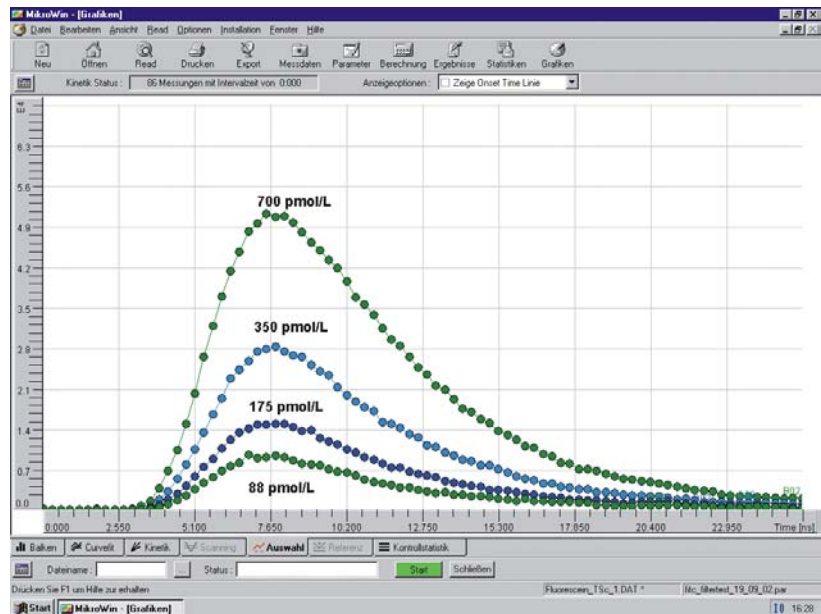


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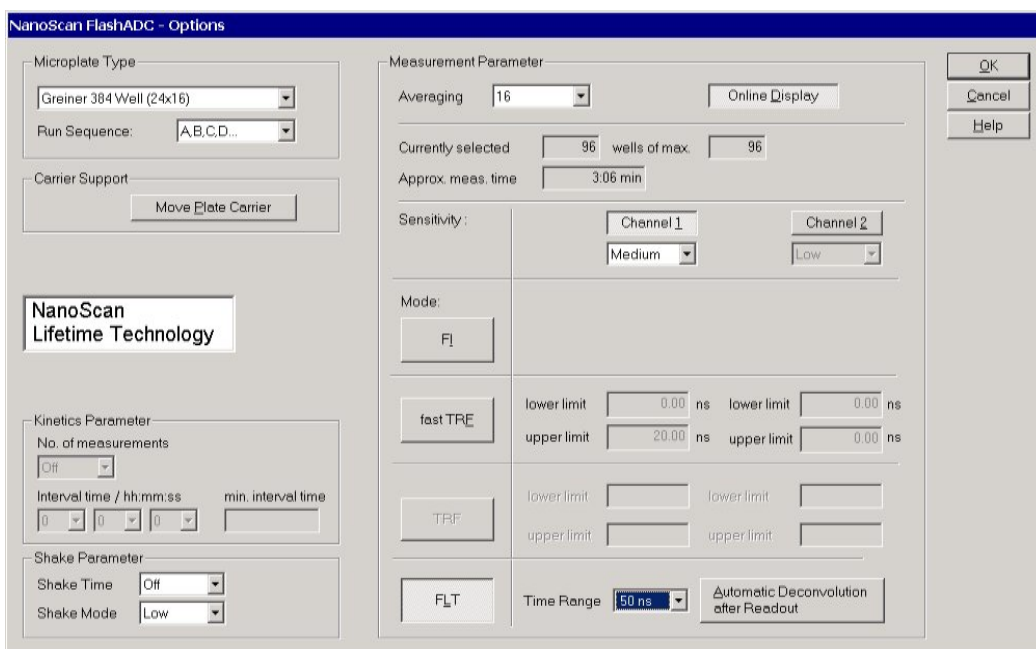
Application Note No. 7

Using the fluorescence reader **LF 401 NanoScan FLT** the customer is able to perform fluorescence lifetime measurements in common microplates at lowest concentration levels. The right diagram shows the example of four fluorescence decays of fluorescein in a black 96-well-plate (Nunc).

The **NanoScan** microplate readers use high-power pulsed lasers with low repetition-rates to excite the sample fluorescence. The fluorescence trace according to a single laser pulse is digitized. In order to improve signal-to-noise-ratio several laser pulse events are averaged. Typical pulse numbers to be averaged are 16 or 32

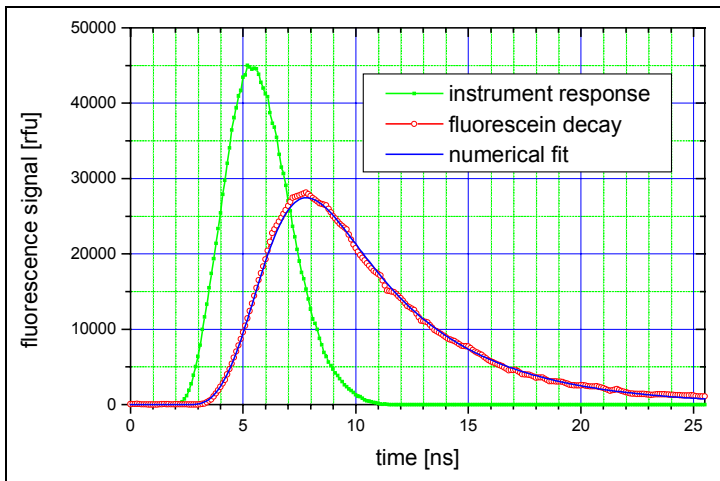


Instrument settings have been set according to the following window. The measurement was performed in a 384 well plate (Greiner).



The sample, fluoresceine disodium salt, was prepared to a stock solution of 140 nM in pH8 phosphate buffer. The sample volume in each cavity was set to 50  $\mu$ l. In the microplate a dilution series with a dilution factor of 2 was prepared..

The measurement was performed in the **FLT mode** in a time window of 50 ns.



After the measurement a numerical curve analysis of all decay curves has been performed. The curve analysis is integrated in the instrument software package and can perform an automatic or user-controlled curve fitting. This analysis recalculates the fluorescence lifetimes hidden in the decay curves.

<i>fluorescein concentration [nmol/L]</i>	<i>fluorescence lifetime [ns]</i>
34,8	4,63
17,4	4,64
8,7	4,65
4,3	4,63
2,2	4,59
1,1	4,51
0,5	4,55

The results indicated in the table above show a nearly constant value for the analysed fluorescence lifetime that is close to the literature value of 4,5 ns. The dynamic range of the FLT measurement for this experiment was more than 50 with a typical standard deviation of the lifetime analysis of 60 ps.

**Conclusion: Fluorescence lifetimes in the range of nanoseconds can easily be measured and analysed at nano- and picomolar concentrations using the LF 401 Nanoscan FLT.**