

# HTS-FLT Measurement of Ruthenium Complexes

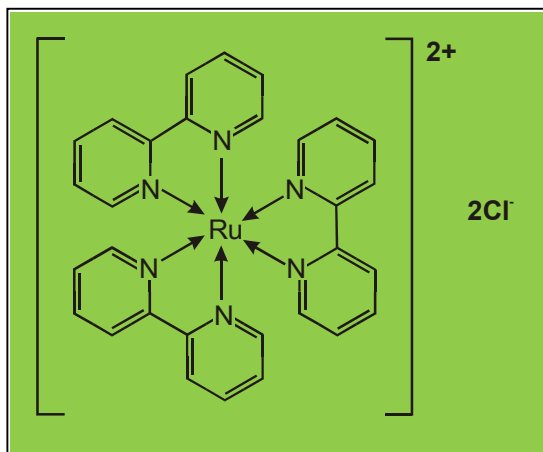


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Application Note No. 8

With the fluorescence microplate readers **LF 401 NanoScan** customers can measure the fluorescence lifetime of fluorescence labels not only in the nanosecond but also in the microsecond range at lowest concentration levels.

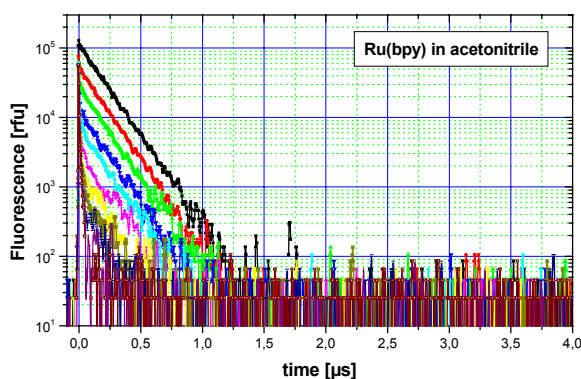
To demonstrate this experiments with a Ruthenium complex have been performed since  $\text{Ru}^{2+}$ -complexes like other transition metal complexes ( $\text{Cr}^{3+}$ ,  $\text{Os}^{3+}$ ,  $\text{Ir}^{3+}$ , from Hemmilä: "Applications of Fluorescence in Immunoassays", John Wiley & Sons, 1991) have lifetimes ranging from 0,1 to 10  $\mu\text{s}$ . Additionally, their fluorescence is characterised by a large stokes shift.



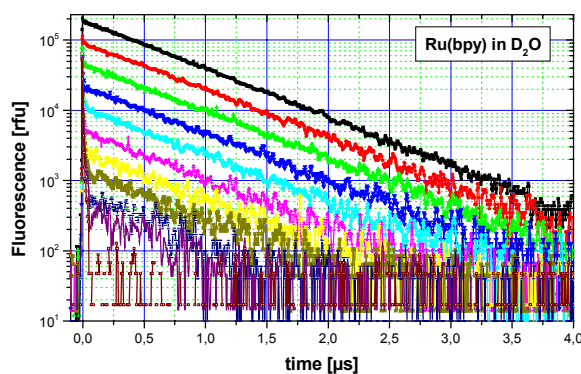
We used Tris-(2,2'-bipyridyl)-Ruthenium(II)-Chloride ( $\text{Ru}(\text{bpy})$ , Aldrich) which is known to have different decay times depending on the solvent. (Klimant: Thesis – "Development of optical oxygen sensors on the basis of luminescent transition metal complexes", University Graz, 1993).

## Measurement:

Measurements with the **LF 401 NanoScan FLT** have been performed in the solvents acetonitrile and  $\text{D}_2\text{O}$  and mixtures of both in a black 384-well microplate (Nunc). The sample volume was 50  $\mu\text{L}$ . Excitation wavelength was set to  $456 \pm 5$  nm. The fluorescence was collected at  $630 \pm 25$  nm. The detection time range was set to 4  $\mu\text{s}$ .



Graph 1: fluorescence decay curves of  $\text{Ru}(\text{bpy})$  in acetonitril



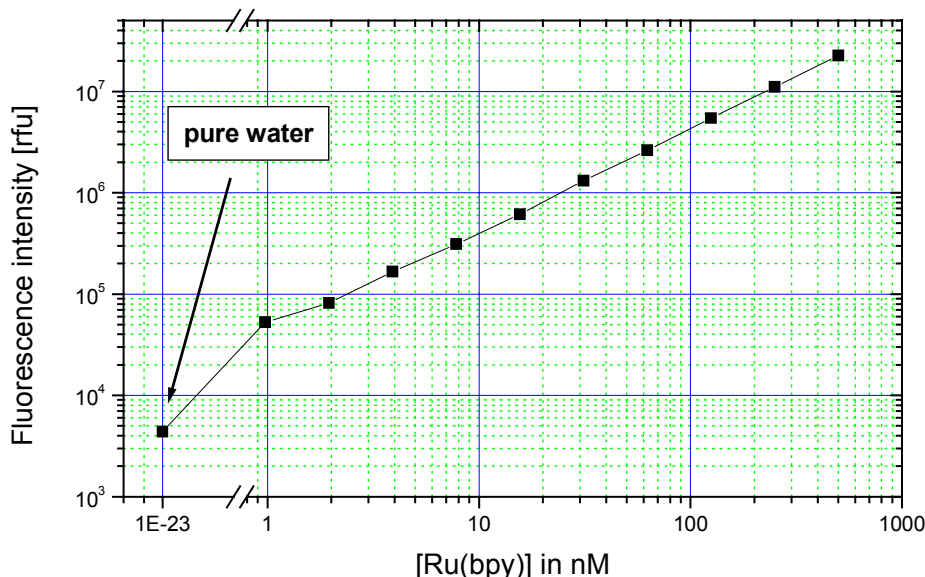
Graph 2: fluorescence decay curves of  $\text{Ru}(\text{bpy})$  in  $\text{D}_2\text{O}$

Dilution series of the  $\text{Ru}^{2+}$ -complex in the different solvents have been prepared starting with 500 nM at the highest concentration and a 1:2 stepwise dilution. The 10<sup>th</sup> sample had the lowest concentration of 0,976 nM. The graphs 1 and 2 show as an example the measured decay curves in pure Acetonitrile and  $\text{D}_2\text{O}$ .

Each decay curve represents the average of 64 laser shots.

**Results:**

The integral of the decay curves is identical to the steady state fluorescence intensity. Integration revealed a detection limit of this Ru-complex in D<sub>2</sub>O well below 1 nM and a linear dynamic range of the instrument of more than 3 decades for one setting of the instrument sensitivity..



Graph 3: Integrated fluorescence of Ru(bpy) in D<sub>2</sub>O

The numerical curve analysis which is integrated in the software package reveals automatically the lifetime from the decay curves. The next table contains the results of the curve analysis in dependence on the dye concentration and in dependence on the solvent.

<b>Concentration</b>	<b>acetonitril</b>	<b>50/50</b>	<b>60/40</b>	<b>80/20</b>	<b>D<sub>2</sub>O</b>
	lifetime	lifetime	lifetime	lifetime	lifetime
[nM]	[µs]	[µs]	[µs]	[µs]	[µs]
500	0,170	0,294	0,353	0,503	0,641
250	0,166	0,295	0,355	0,500	0,664
125	0,164	0,288	0,361	0,507	0,657
62,5	0,171	0,283	0,360	0,503	0,657
31,25	0,169	0,289	0,364	0,510	0,655
15,625	0,182	0,271	0,359	0,489	0,643
7,8125	0,153	0,291	0,374	0,490	0,649
<b>Mean value</b>	<b>0,168</b>	<b>0,287</b>	<b>0,361</b>	<b>0,500</b>	<b>0,652</b>

Table 1: resolved fluorescence lifetimes of Ru(bpy) in acetonitrile and D<sub>2</sub>O and mixtures of both

The calculated lifetime values remain constant over a concentration range of nearly 100. In the case of pure acetonitrile at the lowest concentration values, the error for the lifetime estimation increases. The reason is the noise increase on the decay curve due to a lower signal intensity.

Therefore, the accurate estimation of the fluorescence lifetime of a certain fluorescence label is possible down to concentrations of the level of app. a factor of 10 above the detection limit. Below this borderline a possible influence of background fluorescence may disturb the re-calculation resulting in wrong lifetime values.

**Conclusion:**

**The measurement of luminescence lifetimes of fluorescent compounds in the lower microsecond range is possible with the LF 401 NanoScan FLT. The accurate estimation of lifetimes in this time range can be done within a concentration range of 100. For intensity measurements the linear dynamic range of the instrument covers at least three decades in each of the three available sensitivity settings.**