

Determination of Lanthanide Fluorescence Lifetime and Spectral Properties with the Varioskan Spectral Scanning Fluorometer

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Abstract

Lanthanides are fluorochromes used in time-resolved fluorometry. An important characteristic of the lanthanide ions is their long fluorescent lifetime. It is defined as the time in which the initial fluorescence intensity of a fluorophore decays to 1/e. The lifetimes of the lanthanides are measured in microseconds.

When used as fluorochromes, the lanthanide ions are complexed with organic ligands. The structure of this chelate or cryptate has an effect on the fluorescent properties of the label. In this work the lifetime of some commercially used lanthanides were determined in different formats using a spectral scanning multimode reader. Additionally, excitation and emission spectra of the lanthanides were run to determine the optimal measurement parameters for each label in time-resolved measurements.

The results show that it is important to determine the parameters for the fluorochrome in the actual assay environment and to optimize the parameters of a time-resolved measurement with a spectral scanning instrument.

Introduction

The lifetime of the excited state is defined by the average time the molecule spends in the excited state prior to return to the ground state. Fluorescence emission is a random process and therefore the lifetime is an average value. This lifetime parameter t of the TRF label describes the lifetime of the fluorescence emission. Each lanthanide has its unique t value and it is the time it takes the signal to decrease to the value 1/e, meaning that after the time t from the excitation about 37% of the original I_0 signal remains. With lanthanides the measurement of the fluorescence decay and lifetime is easy to perform due to their microsecond scale lifetimes. Theoretical fluorescence emission decay rate can be calculated from the following formula:

$$I_t = ae^{-t/\tau}$$

Where: I_t = remaining fluorescence signal after the time t
 a = fluorescence signal at the time 0
 τ = fluorescence lifetime of the label used

It is important to determine the t value of the label or TR-FRET pair of the assay for assay optimization. This enables the determination of the optimal measurement delay and integration time for each assay. The possible disturbing factors in the assay can also be detected by following the fluorescence lifetimes of the fluorochromes. Many interfering factors have an effect on the fluorescence emission lifetime. Therefore if the t value is not altered from the literature information, there is no disturbing factors present in the assay.

When the fluorescence emission decay curve and the label's fluorescence lifetime t have been determined, the best possible measurement delay for each assay can be determined. Similarly, the optimal TRF integration time to be used to collect the fluorescence signal can be determined from the decay curve. In addition to the label's fluorescence decay curve and lifetime, a similar decay curve can be measured from the blank samples to determine the time after which any background fluorescence from the sample or stray light from the instrument's excitation light is not present in the measurement. By combining this background and label fluorescence emission information, it is possible to determine optimal measurement parameter settings for each application individually, both for simple TRF with one labels and for TR-FRET assays using two different labels.

Materials and Methods

Fluorescence spectra and emission decay curves were determined with three lanthanide labels, Europium, Terbium and Samarium, and with non-lanthanide Platinum label. The following commercial products were used in the measurements:

Fluorochrome stock solutions:

- Europium atomic absorption standard solution, Sigma, #27,712-8
- Terbium atomic absorption standard solution, Sigma, #305928
- Samarium ACP/DCP standard solution, Sigma, #35652-2
- FluoSpheres[®] platinum microspheres, Invitrogen Inc., #F-20886

Chelator solutions:

- DELFIAR[®] Enhancement solution, Perkin-Elmer Corp., #1244-105
- DELFIAR[®] Enhancer solution, Perkin-Elmer Corp., #C5000-100

TR-FRET pairs:

- Europium - APC complex, from LANCE[™] Tyrosine kinase kit, Perkin-Elmer Corp., #AD0121
- Terbium - Fluorescein complex, from Lanthascreen[™] TR-FRET Tyrosine kinase assay kit, Invitrogen Inc., #PV3149

First, excitation and emission spectra were measured from all labels. Europium and Samarium were measured as a recommended chelate complex using DELFIA Enhancement solution to dilute the lanthanide stock solutions. Terbium spectra was measured as recommended with DELFIA Enhancer solution and also directly from the stock solution. Platinum microspheres spectra were measured by diluting the microsphere suspension with distilled water. The effect of the chelate on the fluorescence spectra was also determined by measuring both excitation and emission spectra from the free Terbium ion without any chelates present using pure Terbium stock solution as a sample.

Fluorescence emission decay curves were then measured from the same samples. The following instrument settings were used in all decay measurements:

- Measurement time, 1000 ms (100 flashes)
- Delay start point, 50 μ s
- Delay stop time, 2000 μ s
- Delay step time, 10 μ s

All measurements were done with the Varioskan[®] spectral scanning time-resolved fluorometer connected to SkanIt[®] Software. After the measurement SkanIt Software generates automatically all decay curves and calculates the fluorescence lifetime and recommended integration time for each sample.

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Results

Fluorescence excitation and emission spectra of all labels used are shown in Figures 1. and 2. and collected information about the spectral peaks is shown in Table I. As seen, all excitation and emission peaks are mainly corresponding well with published reference information. There is a clear difference in the location of the excitation peaks of lanthanides when they are in their normal chelate form and when used as a TR-FRET donor. With Europium, excitation maximum is shifted from 340 to 329 nm and with Terbium the shift is from 315 to 335 nm. Emission spectra of these labels show clear changes in the intensities of the different peaks. When Europium is measured in normal chelate structure, 2. and 3. minor peaks are relatively small, but when Europium used as a LANCE donor is measured, these minor peaks are much bigger.

Fluorescence spectra of the Terbium ion in water and in DELFIA Enhancer are shown in Figure 3. Emission spectra of the free ion form and chelate form are clearly identical but excitation spectra show a very different behavior. In chelate form Terbium has only one excitation maximum at 315 nm when free Terbium has clearly double excitation peaks at 352 and 369 nm.

Fluorescence decay curves of all labels are shown in Figure 4. From these figures it is clearly seen that the fluorescence lifetime of the TRF label is different than the fluorescence lifetime of the TR-FRET complex where the same label is used as a donor molecule.

TRF Label	Excitation Peaks			Emission Peaks			Reference Data	
	Major Peak	2. Peak	3. Peak	Major Peak	2. Peak	3. Peak	Excitation peak	Emission peak
Europium (Eu) in Enhancement Solution	340 nm			616 nm	698 nm	592 nm	340 nm	615 nm
Europium donor from LANCE	329 nm			616 nm	700 nm	592 nm	340 nm	615 nm
Terbium (Tb) in Enhancer Solution	315 nm			545 nm	491 nm	584 nm	320 nm	545 nm
Terbium donor from Lanthascreen	335 nm			545 nm	490 nm	585 nm	320 nm	545 nm
Samarium (Sm) in Enhancement Solution	338 nm			644 nm	600 nm	564 nm	340 nm	642 nm
Platinum (Pt) microspheres	541 nm	397 nm	509 nm	650 nm	708 nm		390 nm	650 nm

Table I. Excitation and emission peaks of the different TRF labels

TRF Label	Fluorescence lifetime (t)	Recommended TRF integration time	Reference Data
Europium (Eu) in Enhancement Solution	709	890	730
Europium donor from LANCE	190	240	NA
Terbium (Tb) in Enhancer Solution	1045	1310	1050
Terbium donor from Lanthascreen	160	200	NA
Samarium (Sm) in Enhancement Solution	77	100	50
Platinum (Pt) microspheres	104	130	>100

Table II. Determined fluorescence lifetimes and calculated recommended TRF integration times of the TRF labels

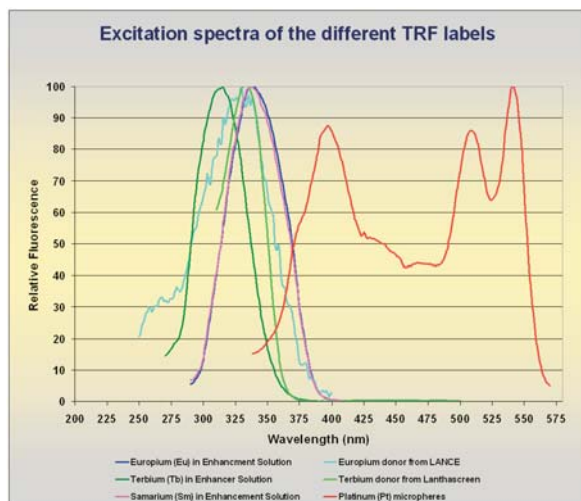


Figure 1. Fluorescence excitation spectra of the selected TRF labels.

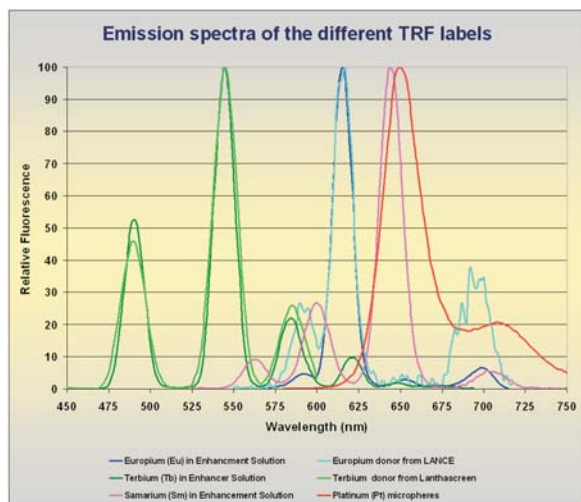


Figure 2. Fluorescence emission spectra of the selected TRF labels.

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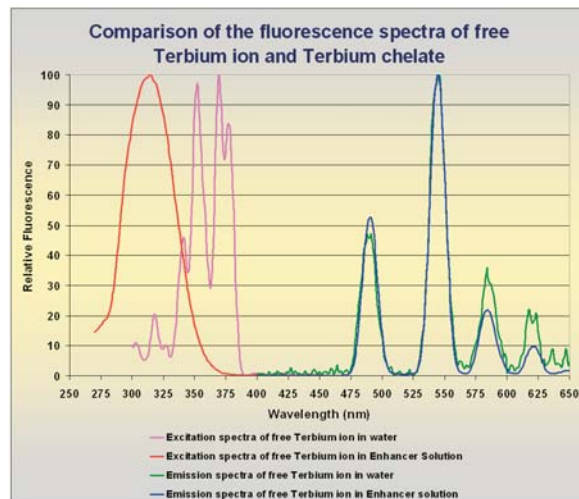


Figure 3. Fluorescence spectra of Terbium chelate and free Terbium ion.

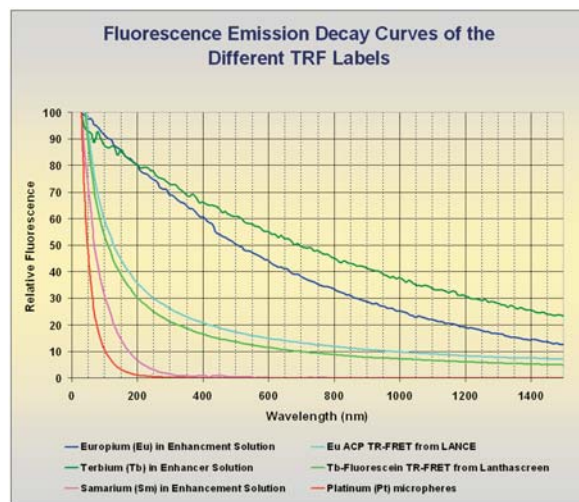


Figure 4. Decay curves of all TRF labels.

Conclusions

- Both fluorescence excitation and emission spectra of the TRF labels are effected by the chelate matrix used with the label.
- When the TRF label is used as a TR-FRET donor, the TR-FRET complex shows quite a different fluorescence lifetime than the label alone.
- There are no general parameters that would be optimal for the measurement of a TRF label. For optimal assay performance it is essential to determine optimal measurement parameters case by case, separately for each application.