Basic TRF measurements with the Varioskan™ spectral scanning multimode reader

This technical note describes how to perform the basic time-resolved fluorescence measurements with Thermo Electron's Varioskan spectral scanning multimode reader and SkanIt software. The procedures of endpoint, scanning and decay time measurements are described using europium as a pattern fluorochrome.

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Introduction

Time-resolved fluorescence (TRF) has been utilized for more than 2 decades; the first commercial application, DELFIA, was launched in 1983.
Lanthanides are the most common group of the fluorochromes used. They have been chosen because of several features suitable especially for the time-resolved method; like long excited state lifetime and large Stoke's shift. In assays the lanthanides are used as chelate labels.

The advantages of TRF are especially wide dynamic range and very low background.
Time-resolved method is most useful in cellular and homogeneous assays. The diverse properties of the lanthanides also enable the usage of multiple labels in one assay.
Time-resolved fluorescence measurements utilize the difference between the lifetimes of the specific and background signals i.e. because the fluorescence lifetime of the lanthanides is long, the measurement can be started only after the non-specific background fluorescence has decayed. (Fig. 1)
In a TR-fluorometer the sample is pulsed very fast (e.g. 100 times per second) and the sample is measured in the period between these flashes after a specified delay.

The following measurement types of Varioskan are described below:

- **TRF measurement**
- **TRF scanning**
- **TRF decay**

The SkanIt software has default calculations for both decay and spectral reduction.

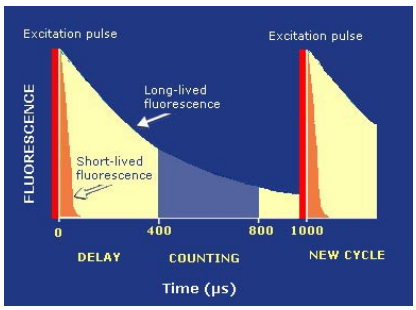

Figure 1. Basic principle of time-resolved fluorescence measurement. The excitation is made by very short light pulses and the emission is detected after a certain delay. The integration (counting) time in the figure is 400-800 µs.

Materials And Methods

TRF measurement (standard curve)
In Varioskan the excitation is made by a xenon flash lamp and detection with a photomultiplier tube. The wavelength selection is made by two sequentical monochromators for both excitation and emission sides of the system.

For the standard curve a dilution series between 10^3 and 10^9 amol/ml of Europium standard (Aldrich Chemical Europium standard solution, No 20,712-8) was made to the Enhancement solution (PerkinElmer, No 1244-105) and 80 µl of each dilution was pipetted to white 384 microtiter plate (Thermo Electron, No 8225) in 8 replicates. Enhancement solution was also used as a blank.
All the measurement parameters are described on the table below.

TRF Scanning

The chelate used in the assay has an effect on the lanthanide fluorescence spectra. By creating the spectra the optimal excitation and emission wavelengths can be chosen for each assay.
Of the spectra, the excitation spectrum describes the dependence of emission intensity at a single wavelength upon the excitation wavelength and the emission spectrum the wavelength distribution of the emission measured with a constant excitation wavelength.
In this application both excitation and emission spectra were run from a sample containing $5*10^5$ amol/well (50 µl) on the plate described above.

TRF decay

Fluorescence lifetime (τ) is the average interval between absorption and emission. The fluorescence intensity decays according to function:

$$I(t) = \alpha e^{-t/\tau}$$

This means that τ is the time where approximately 67% of the signal has decayed.

In SkanIt software the decay measurement is made in short steps and following parameters are used:

- Integration time: the length of the measurement window i.e. the time the emitted signal is collected for each measurement cycle.
- Start time: the time from the flash to the middle point of the first measurement.
- Stop time: the time from the flash to the middle point of the last measurement.
- Step : the time difference between two sequential middle points of the measurement

As a result of the SkanIt decay calculation in addition to the τ value also α and the recommended integration time for the assay is given.
For this study the decay curve was made from a sample containing $5*10^6$ amol/well on the plate described above.

Measurement parameters for the measurements were:

Parameter	Standard curve	Excitation spectra	Emission spectra	Decay curve
Delay time (µs)	200	200	200	-
Integration time (µs)	1000	1000	1000	100
Measurement time (ms)	2600	100	100	100
Excitation wavelength (nm)	340	-	340	340
Emission wavelength (nm)	615	615	-	615
Scanning start wavelength (nm)	-	270	400	-
Scanning end wavelength (nm)	-	500	700	-
Step size (nm)	-	1	1	-
Start time (µs)	-	-	-	50
Stop time (µs)	-	-	-	2350
Step (µs)	-	-	-	10

Results

TRF Measurement

The following standard curve (Fig.2) was achieved with the samples described above. The curve fit and display parameters can be specified for each run and ready and user made calculations may be added.

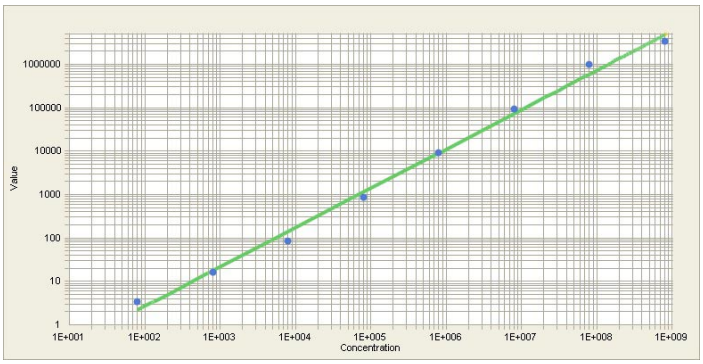

Figure 2. Europium standard curve. The calculated correlation coefficient of the curve is 0.9945

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Excitation and emission spectral scanning

The curves on Figures 3 and 4 were gained. The spectral calculation gives the maximum peaks to 338 and 616 nm. The curves and the maximum values correlate very well to the literature values (340 and 615 nm).

Decay measurement

The decay curve on Figure 5 was gained.

The calculated values reported were: τ = 725 µs, α = 19857, recommended integration time = 911 µs and R^2= 0.9998.
The τ value correlates very well on the literature value for Europium, which is 730 µs.

Conclusions

Varioskan offers a way to perform the measurements needed for both screening and research measurements and all the results of the measurements were as expected.
The spectral scanning and decay calculations are very advantageous features for assay development; all the important assay parameters can be defined. With the SkanIt software the visual examination of the results is very simple.

References

Principles of Fluorescence Spectroscopy, Lakowicz, J.R. Plenum Publishers, New York (1999)

Soini, E. and Hemmilä, I. Fluoroimmunoassay: Present status and key problems. Clin Chem. 25: 353-361 (1979)

Soini, E. and Kojola, H. Time-resolved fluorometer for lanthanide chelates: a new generation of non-isotopic immunoassays. Clin. Chem. 29: 65-68 (1983)

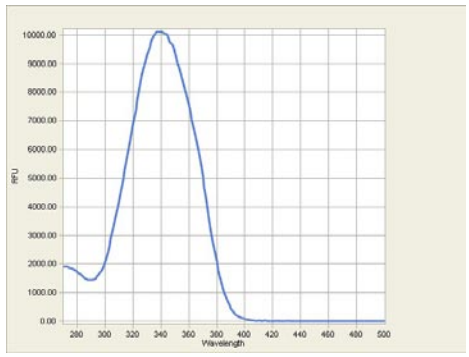

Figure 3. Europium excitation curve

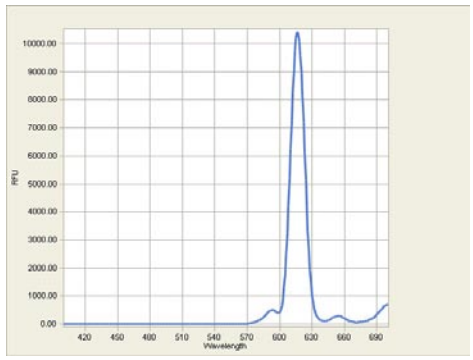

Figure 4. Europium emission curve

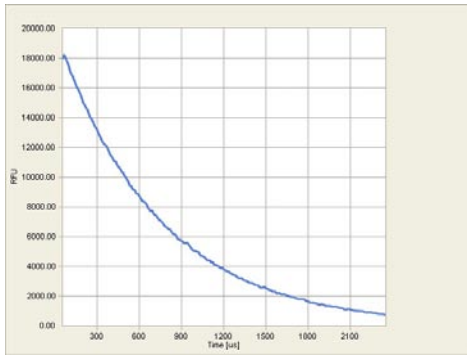

Figure 5. Europium decay curve.

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