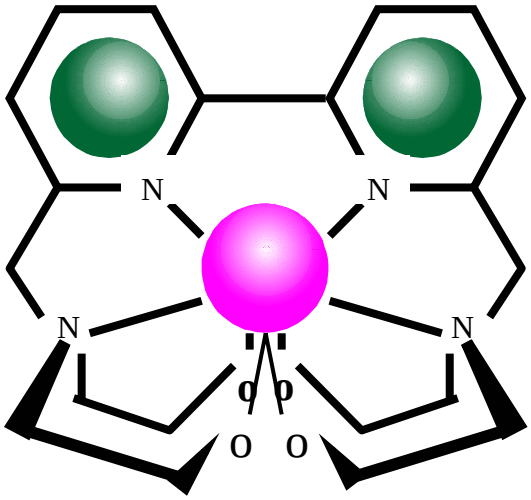
TRF：Time-Resolved Fluoremetry

時 差 式 螢 光

TRF 是利用鑭系元素的螯合物(Lanthanid Chelates)標示在欲分析的分子如 DNA、Protein 上。其螯合物的結構簡圖如下：

Binding arm：
Isothiocyanate (ITC)
Iodoacetamido (IA)
Amino
Dichlorotriazine (DTA)



Chelate：
N1 (DTTA)
DTPA
W2014
W1024
W8044
W14016 …...

Sc														
Y														
La	**Ce**	**Pr**	**Nd**	**Pm**	**Sm**	**Eu**	**Gd**	**Tb**	**Dy**	**Ho**	**Er**	**Tm**	**Yb**	**Lu**
Ac	**Th**	**Pa**	**U**	**Np**	**Pu**	**Am**	**Cm**	**Bk**	**Cf**	**Es**	**Fm**	**Md**	**No**	**Lr**

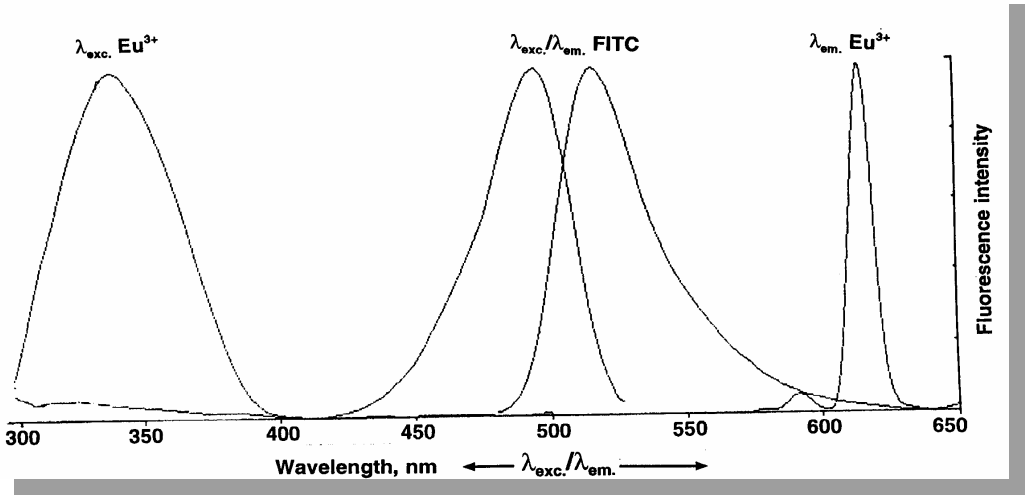
與一般的有機螢光的比較表如下：

	Eu-chelate	Fluorescein
Excitation (nm)	340	490
Emission max. (nm)	613	515
Strokes' shift (nm)	273	25
Decay time (ns)	730,000	3
Emission width	Narrow	Broad
Background interference	Low	High
Self quenching	No	Strong
# of repeated measurements	High	Low
Applicability of multi-label	Good	Limited

TRF 的特點：

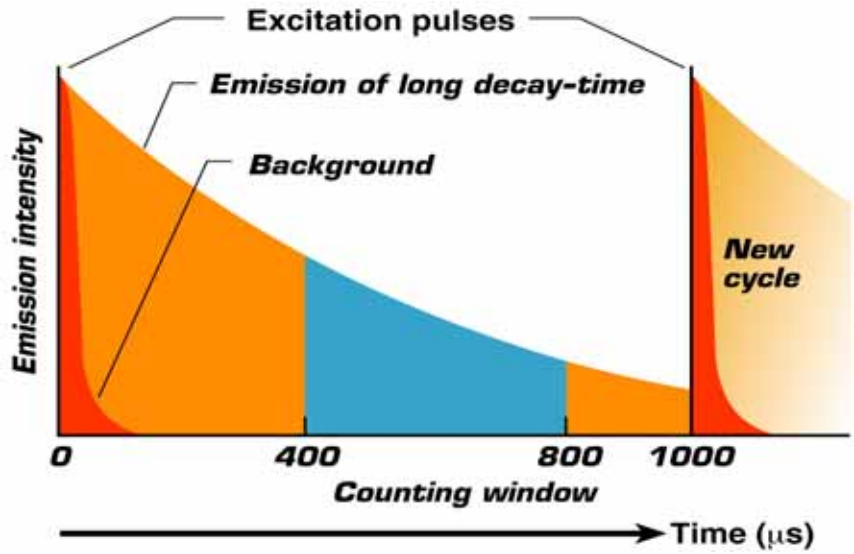
A. Large Strokes Shift：

激發與發射光譜不會相互干擾，沒有自發的淬滅(Internal quenching)現象，因此得到最多的可用訊號，靈敏度增加。



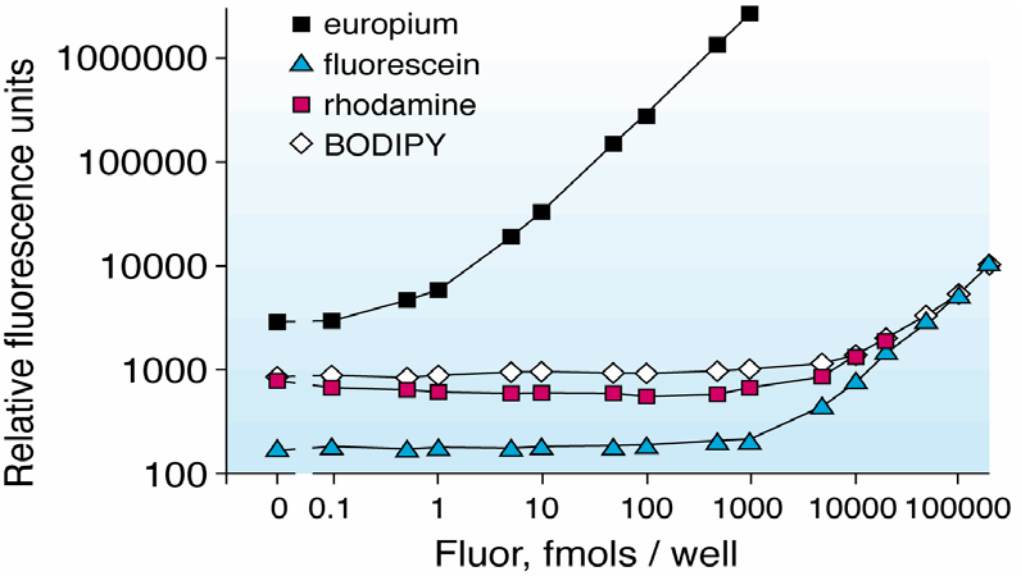
B. Long Emission Time：

Eu-chelate 的發射光(Emission)能持續數百個微秒(microsecond)；一般的螢光只能維持數個奈秒(nanosecond)。因此可利用適當的機器偵測自激發光(Excitation)後的 400-800 微秒的螢光訊號，藉此排除背景的螢光雜訊。



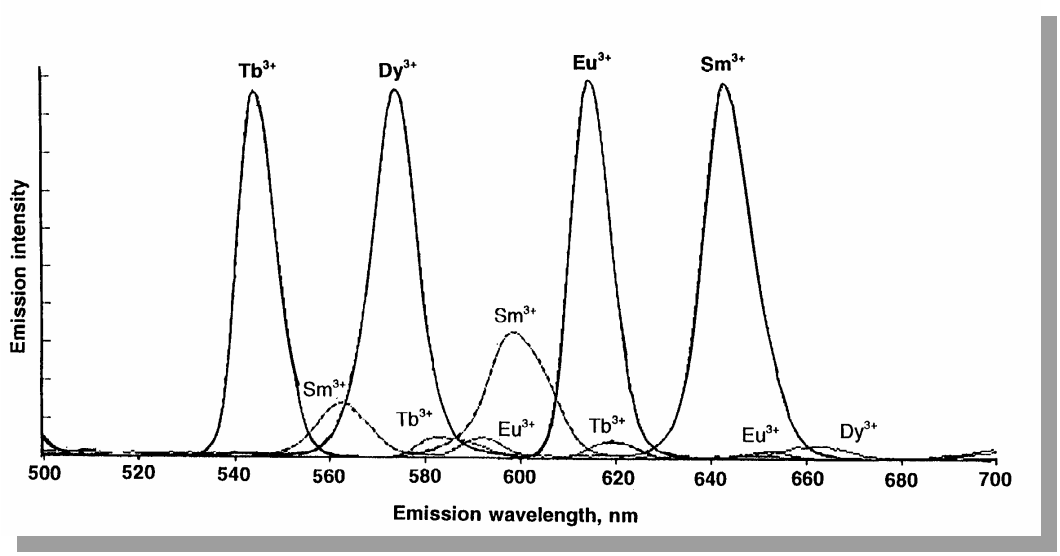
C. High Sensitivity：

因為背景降低且沒有自發的淬滅(Internal quenching)現象，相對地提高 S/N 比值，所以 TRF 的靈敏度比一般的螢光來得高。



D. Multiple Labels：

鑭系元素中：Tb、Dy、Eu、Sm 的螢光圖譜如下圖所示，這四個的發射光波長差異非常明顯，適用於同時多種標定的實驗。



TRF 的應用：

PerkinElmer 公司在 TRF 領域已有超過 20 年的經驗，其螯合物的技術領先全球。並有多種產品上市，其中又以新生兒檢驗試劑的使用率最高。本公司提供的 TRF 相關產品，依照其部份分析原理的差異又分為三類：

DELFIA：dissociation enhanced lanthanide FIA

LANCE：time-resolved fluorescent resonance energy transfer assay

TruPoint：time-resolved fluorescence quench assay

相關產品資訊請參閱伯森公司網站 www.blossombio.com.tw 或請洽本公司各區域業務代表。

TRF 主要的應用原理是將鑭系元素的螯合物(Lanthanid Chelates)標示在欲分析的分子如 DNA、Protein 上，藉以進行分子間結合、酵素活性....等實驗，再以 TRF 讀儀偵測其訊號。TRF 相關應用的文獻眾多，在此僅列出部分文獻供作參考：

1. Immunoassays

- Madersbacher S, Shu-Chen T, Schwarz S et al. "Time-resolved immunofluorometry and other frequently used immunoassay types for follicle-stimulating hormone compared by using identical monoclonal antibodies" *Clin Chem* 1993; 39: 1435-9.

2. Kinase assays

- Gaarde WA, etc., "Development of a Nonradioactive, Time-Resolved Fluorescence Assay for the Measurement of Jun N-terminal Kinase Activity *J Biomol Screen* 1997 Aug **2:4** 213-23

3. Receptor-ligand binding assays

- Inglese J, etc**.,** "Chemokine receptor-ligand interactions measured using time-resolved fluorescence" *Biochemistry* 1998 Feb 24 **37:8** 2372-7
- Moore KJ, etc., "A Homogenous 384-Well High Throughput Screening for Novel Tumor Necrosis Factor Receptor: Ligand Interactions Using Time Resolved Energy Transfer" *J Biomol Screen* 1999 Aug **4:4** 205-14
- Makishima M, etc., "Identification of a nuclear receptor for bile acids" *Science* 1999 May 21 284:5418 1362-5

4. GPCR function assays: ex. cAMP, GTP binding

- Valenzano, K.J., Miller, W., Kravitz J. N., Samama, P., Fitzpatrick, D., and Seeley, K. "Development of a fluorescent ligand-binding assay usinf the AcroWell filter plate" *J Biomol Screen* 2000, **5**:455-461

5. DNA hybridization

- Sjoroos M, etc., "Triple-label hybridization assay for type-1 diabetes-related HLA alleles" *Biotechniques* 1995 May 18:5 870-7

6. Cytokine assay

- Stenroos K, etc., "Homogeneous time-resolved IL-2-IL-2R alpha assay using fluorescence resonance energy transfer" *Cytokine* 1998 Jul 10:7 495-9

7. Biodistribution

- Neville ME, etc., "A comparison of biodistribution of liposomal and soluble IL-2 by a new method based on time-resolved fluorometry of europium" *Cytokine* 2000 Nov 12:11 1702-11

8. Molecular interaction

- Hamy F, etc., "Merged Screening for Human Immunodeficiency Virus Tat and Rev Inhibitors" *J Biomol Screen* 2001 Jun **6:3** 179-87

9. Enzyme assays

- Boisclair MD, etc., "Development of a ubiquitin transfer assay for high throughput screening by fluorescence resonance energy transfer" *J Biomol Screen* 2000 Oct 5:5 319-28