Lanthanide Complexes as Luminescent Labels

by

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Introduction

- *Lanthanide ions have some characteristics that make them very useful as photoluminescent labels.*
- *The most commonly used ions are trivalent **EUROPIUM** and **TERBIUM** in the form of **COMPLEXES** with organic ligands. Complexes of **SAMARIUM** and **DYSPROSIUM** are also used for special purposes.*
- *Several other lanthanides luminesce in inorganic host crystals and are used in inorganic phosphors but most do not emit efficiently (at least in the visible spectral range) in solution or as organic complexes*

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Lanthanide Complexes as Luminescent Labels

*The absorption and emission of lanthanide ions originates from **f-f transitions** which are 'forbidden' by spectral selection rules...in practice this means that both absorption and emission are very weak. In addition the presence of **WATER** in the coordination sphere of the ions quenches luminescence.*

***These problems can be overcome by complexing the ion with a suitably designed organic ligand**.*

This serves three purposes:

- *The ligand enhances the quantum yield of emission*
- *The ligand can absorb light efficiently and transfer absorbed energy on to the complexed lanthanide ion*
- *The ligand provides a means to add a reactive functional group to link the lanthanide complex to a target molecule*

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Why Use a Lanthanide for labelling?

- *The commonly used lanthanides (europium and terbium) have **sharply structured emission spectra** which helps in discriminating the emission from weakly structured background*
- *More importantly, **the lifetime of the luminescence is relatively long** (up to more than a millisecond, depending on the complex).*
- *This means that lifetime-gated imaging can be used to eliminate interference from the prompt fluorescence of unwanted impurities etc.*
- *In marked contrast to most other labels with long-lived luminescence, **lanthanide emission is not very sensitive to quenching by oxygen**:-this is a consequence of the shielding of the f-orbitals in the ions.*

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What Limits Sensitivity of Luminescence Detection?

Background signals nearly always set a limit to sensitivity in luminescence detection of a specific label

'Background' might be, for example:-

- ***Fluorescence from impurities***
- ***Scattered exciting light leaking through filters***
- ***Raman-scattered light***

All these signals can be eliminated if a label with a long emissive lifetime is used…time-gated detection can see the long-lived emission selectively

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'Real World' Example of Time-Gated Detection

A microarray of oligonucleotides on a Nylon substrate was imaged using a europium cryptate-based binding reagent

The cryptate requires short wavelength UV light to excite its luminescence…unfortunately this also excites impurities in the Nylon giving an extremely high background that obscures the europium emission (left hand image).

The time-gated image (right) shows that the prompt emission from the Nylon can be effectively suppressed allowing the cryptate emission to be detected selectively

Microarray on Nylon probed with Eu-Cryptate

Image without Time Gating

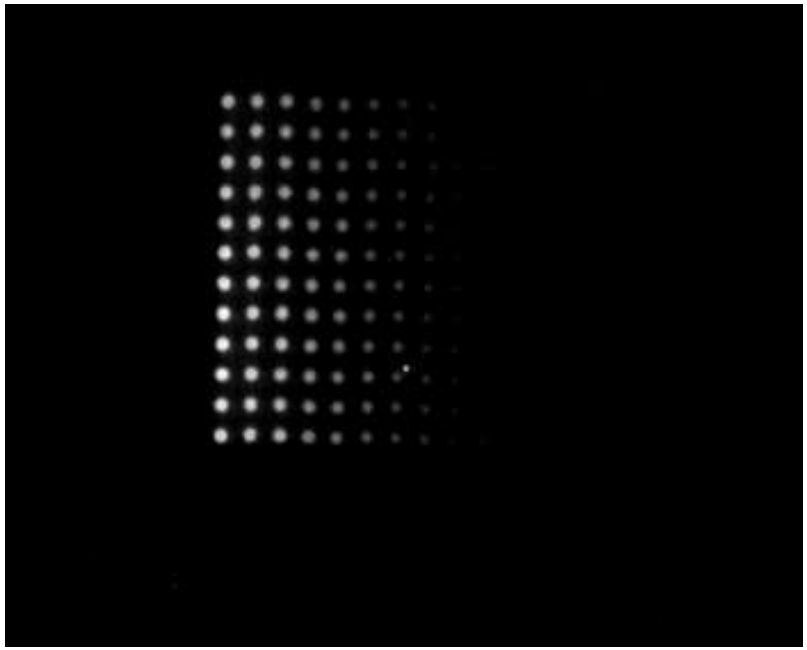

Image with Time Gating

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Prospects for Multiplexing with Lanthanide Labels

Europium and terbium-based labels can be easily discriminated spectrally.. The main emission of terbium complexes is in the green while europium emits primarily orange light. We can 'multiplex' labels on the basis of spectral properties and decay times jointly. This is a very important advantage of the new technology.

Labels emitting 'prompt' fluorescence can be combined with labels emitting long-lived emission

For example:-
red/orange emitter…rhodamine (short lifetime) and europium (long lifetime)

blue/green emitter…fluorescein (short lifetime) and terbium (long lifetime)

Applications of Imagex Technology To Lanthanide Detection

***Spectral** (wavelengths of excitation and emission) and **Temporal** (time-resolved) detection can potentially be used to detect a wide range of labels simultaneously*

This is one of the most important features of the Imagex technology..it offers a low-cost route to 'deep' multiplexed detection using labels having lifetimes ranging from nanoseconds to milliseconds and beyond in conjunction with spectral imaging where this is needed