

Introduction

Luminescent optical probes based on lanthanide chelates have attracted particular attention over the last decades in the biomedical field due to their versatile magnetic and photoluminescent properties. Lanthanides exhibit large Stokes shifts and therefore, reabsorption effects can be avoided. Their narrow emission bands allow for excellent spectral discrimination, they are resistant to photobleaching, and time-resolved detection can be facilitated thanks to their long photoluminescence lifetimes in the millisecond time regime. These characteristics make lanthanides useful for numerous applications. These include their broad employment in molecular imaging, such as in magnetic resonance imaging (MRI) as contrast agents.¹ Lanthanide nanoparticles have been widely used in high-sensitivity immunoassays^{2,3} as well as in the detection and imaging of fingerprints.⁴

In photoluminescence imaging, the downside of lanthanides is that they have weak absorption, limiting the emission's brightness. They are therefore usually excited through chelated ligands that act as absorption enhancers and transfer the excitation to the lanthanide. Diethylenetriaminepentaacetic acid (DTPA) is a common chelating ligand for metal ions. The covalent attachment of the sensitising chromophore carbostyryl 124 (cs124) to the DTPA to form DTPA-cs124 results in a chelating ligand with strong absorption in the UV to enhance lanthanide luminescence via energy transfer (Figure 1).

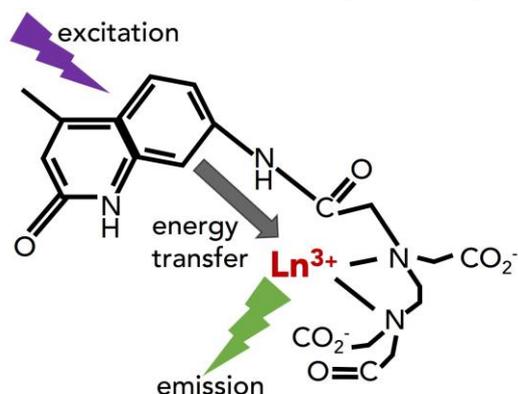


Figure 1 Schematic illustration of the energy transfer from the ligand to the lanthanide ion (Ln^{3+}).

In this application note, an Edinburgh Instruments FS5 Spectrofluorometer is used for a complete characterisation of the photoluminescence properties of Gd^{3+} , Eu^{3+} , and Tb^{3+} lanthanide complexes with DTPA-cs124.

Materials & Methods

Four samples were investigated: Gd^{3+} , Eu^{3+} , and Tb^{3+} complexes with DTPA-cs124 and the ligand DTPA-cs124 on its own with no metal ion. The samples were dissolved in deionised water to produce solutions of absorbance values between 0.1 and 0.15 at the excitation wavelengths to minimise inner filter effects. The spectral and quantum yield measurements were conducted using the main

excitation source of the FS5 Spectrofluorometer (Figure 2), a 150 W Xenon lamp. The samples were held in the SC-05 Cuvette Holder Module for the photoluminescence spectra and SC-30 Integrating Sphere for the quantum yield measurements. The FS5 was equipped with time-correlated single photon counting (TCSPC) and multichannel scaling (MCS) single photon counting electronics for measuring fluorescence and phosphorescence lifetimes, respectively. The TCSPC excitation source was a 340 nm picosecond pulsed LED (EPLD-340) and the MCS excitation source was the FS5's built-in microsecond Xenon flashlamp. The FS5 was equipped with a PMT-980 photodetector for photoluminescence detection.



Figure 2 Edinburgh Instruments FS5 Spectrofluorometer.

Absorption Spectra

Firstly, the absorption spectra of all samples were measured using the FS5's built-in transmission detector (Figure 3). Figure 3 shows that the absorption properties of the lanthanides reflect their ligand (DTPA-cs124) and not the lanthanides themselves.⁵

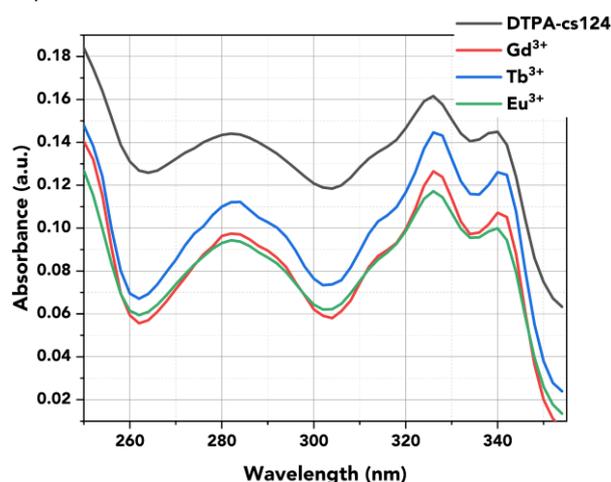


Figure 3 Absorption spectra of all samples.

Emission Spectra

Next, the emission spectra of all lanthanide complexes and their ligand were acquired. The Tb^{3+} and Eu^{3+} complexes were excited at 320 nm and 300 nm, respectively, and Figure 4 shows the emission spectra of the lanthanide complexes showing the energy transfer from the ligand to the lanthanide ion. The emission spectra of Tb^{3+} (Figure 4a) and Eu^{3+} (Figure 4b) complexes are characterised by narrow emission bands (between 480 nm and 720 nm) that were successfully resolved by

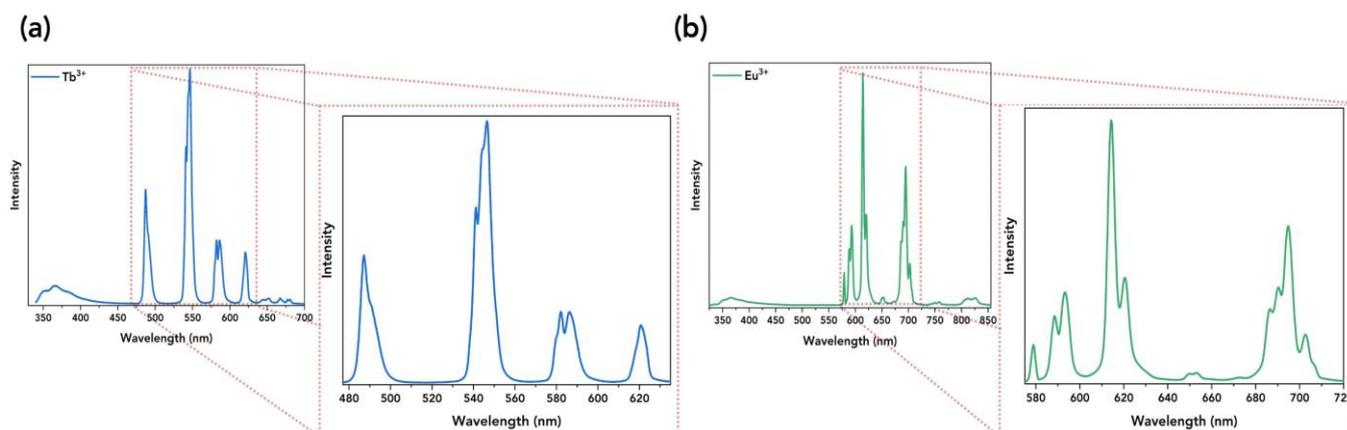


Figure 4 (a) Emission spectrum of the Tb^{3+} complex. Its narrow emission peaks are zoomed-in for better resolution. (b) Emission spectrum of the Eu^{3+} complex along with its well-resolved narrow emission bands.

setting low emission bandwidth on the emission monochromator ($\Delta\lambda_{em}=0.8$ nm), step size=0.2 nm and dwell time=0.2 s for Tb^{3+} complex and 1 s for Eu^{3+} complex. Their spectra also revealed a broader emission band within the 345 nm – 450 nm regime which is the direct DTPA-cs124 emission.

Figure 5 shows the emission of Gd^{3+} complex and DTPA-cs124. It can be seen that their emission spectra are similar showing that the energy transfer from the ligand to the metal ion is insufficient to excite Gd electrons, resulting in non-radiative decay.⁶

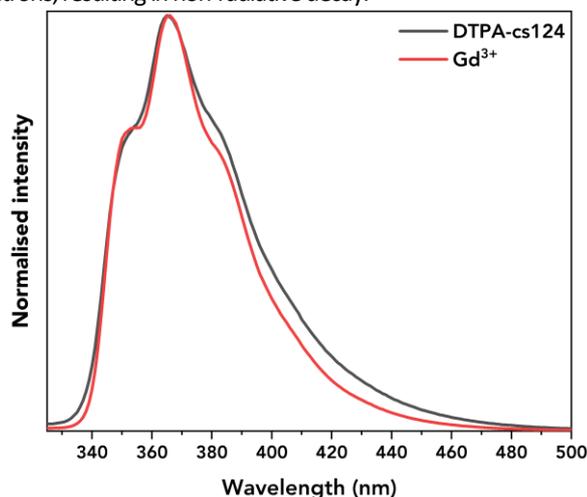


Figure 5 Normalised emission spectra of Gd^{3+} and DTPA-cs124.

TCSPC Measurements

The change in the lifetime of the direct DTPA-cs124 ligand fluorescence when chelated with the different lanthanides was then investigated using TCSPC. The monochromator's emission wavelength was set at the low emission bands of all samples (360 nm – 380 nm), where the fluorescence of DTPA-cs124 and the energy transfer from the ligand to the metal complexes occur.

The decays were fitted using the reconvolution fitting feature of the FSS's Fluoracle® software as a reconvolution of the instrument's

response (IR) of the detector and the exponential decay of the samples. Table 1 summarises the TCSPC measurements and Figures 6a to 6d show the graphs of the fast decays along with their fits and the IR pulse of each measurement. The excitation source was a picosecond pulsed LED (EPLD-340) with a central emission wavelength at $\lambda_{ex}=340.7$ nm and a 20 MHz repetition rate. The TCSPC data shows that the lifetime of DTPA-cs124 decreases from 512 ps to 443 ps when complexed with Gd^{3+} , 351 ps when complexed with Tb^{3+} , and 431 ps when complexed with Eu^{3+} . This demonstrates that the energy transfer between the ligand and the lanthanide ions occurs.

Table 1 Summary of the TCSPC measurements.

Sample	Lifetime (ps)	Std dev (ps)
DTPA-cs124	512	6.75
Gd^{3+}	443	2.23
Tb^{3+}	351	2.45
Eu^{3+}	431	17.3

MCS Measurements

The lifetime decays of the lanthanide ions after energy transfer was then measured using MCS mode. Figure 7 shows the slow decays of Tb^{3+} (in blue) and Eu^{3+} (in green). A microsecond flashlamp operating at 40 Hz was used as the excitation source.

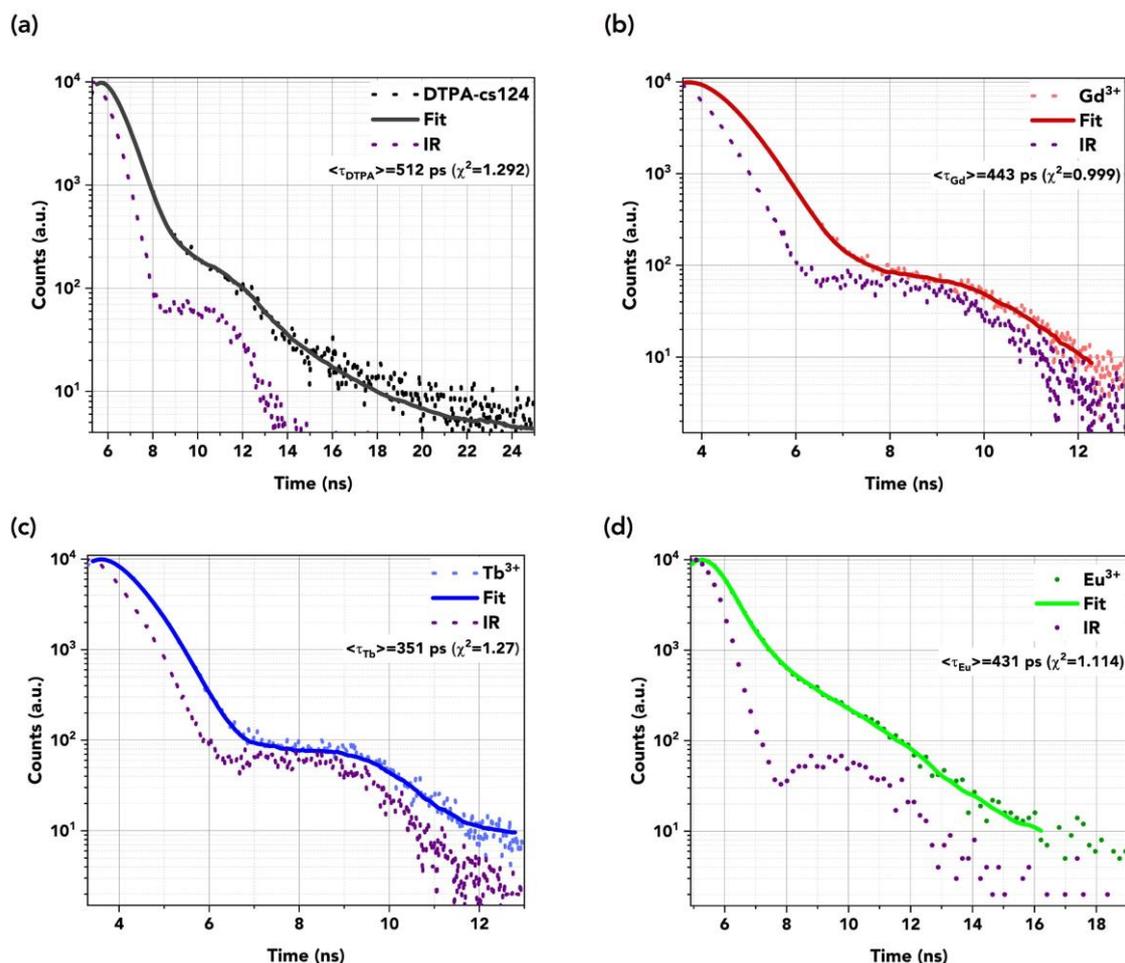


Figure 6 Lifetime decays in TCSPC mode of (a) DTPA (=512 ps) and (b) Gd^{3+} (=443 ps), (c) Tb^{3+} (=351 ps), and (d) Eu^{3+} (=431 ps). An EPLED-340 was used as the excitation source at a repetition rate of 20 MHz.

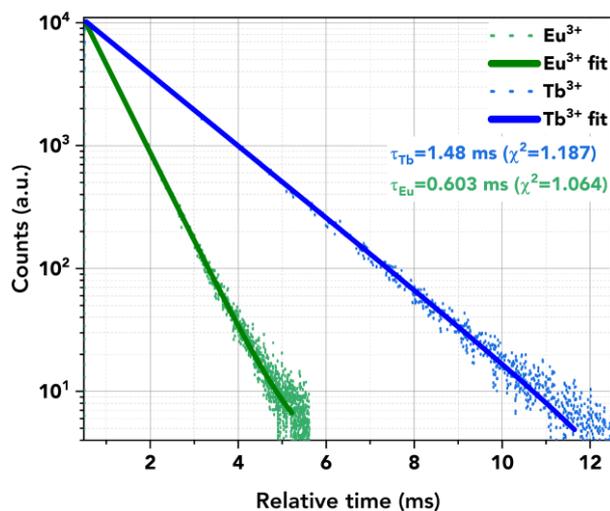


Figure 7 Lifetime decays of Eu^{3+} (green) and Tb^{3+} (blue), acquired in MCS mode using the microsecond Flashlamp at 40 Hz.

Quantum Yield Measurements

Lanthanide probes of high brightness are highly desirable, and the quantum yield is an important metric since it shows how efficiently a luminescent probe emits light. The quantum yield of all samples was determined using the FS5's integrating sphere (SC-30) and calculated through the built-in quantum yield wizard of Fluoracle[®]. The measurements comprised two steps: firstly, the emission and scattering of the lanthanide complexes and their ligand were acquired. Secondly, it was acquired the emission and scattering of the blank (deionised water). This process was repeated three times for each sample to check the consistency of the results and maintain reliability.

A representative quantum yield measurement of the Tb^{3+} complex can be seen in Figure 8 where its scattering and emission spectra are shown in blue along with the emission and scattering spectra of the blank (in violet). Both measurements (sample and blank) were performed in the FS5's integrating sphere.

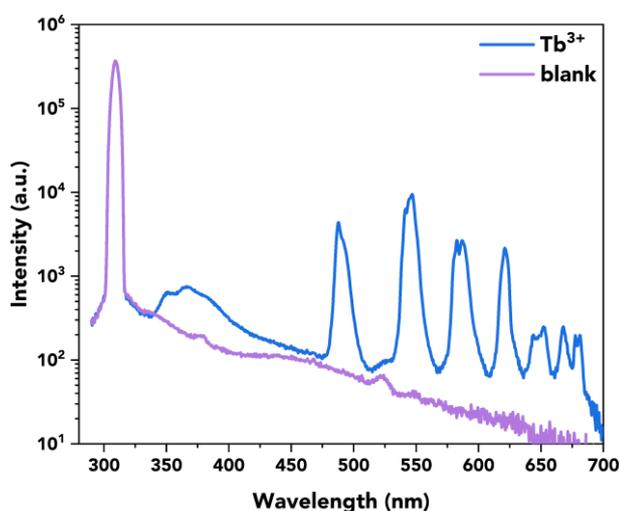


Figure 8 Example of a Tb^{3+} complex quantum yield measurement (=29.01%) by using the FS5's integrating sphere (SC-30). The measurement parameters were λ_{ex} =310 nm, $\Delta\lambda_{ex}$ =6 nm, $\Delta\lambda_{em}$ =0.6 nm, step size=0.5 nm, and dwell time=1s.

Table 2 summarises the quantum yield calculations (QY 1 to 3) along with the average quantum yield value of the samples and their standard deviation. The Tb^{3+} complex was found to have the best quantum yield at 28.35%.

Table 2 Summary of the quantum yield calculations of the lanthanide complexes and their ligand.

Sample	QY 1 (%)	QY 2 (%)	QY 3 (%)	Average (%)	Std dev (%)
DTPA-cs124	6.61	5.62	6.01	6.08	0.5
Gd^{3+}	8.35	7.45	7.5	7.76	0.5
Tb^{3+}	29.01	26.61	29.44	28.35	1.52
Eu^{3+}	5.92	5.41	5.05	5.46	0.44

Conclusion

An Edinburgh Instruments FS5 Spectrofluorometer was used for a complete study of the photoluminescence properties of lanthanides complexes (Gd^{3+} , Tb^{3+} , Eu^{3+}) along with their ligand (DTPA cs-124).

The FS5 Spectrofluorometer enables a complete characterisation of imaging probes, comprising absorption spectra, emission spectra, fluorescence and phosphorescence lifetimes, and quantum yield determination in one compact instrument.

Acknowledgements

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