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Chiral image contrast is demonstrated using enantiopure Eu(III) complexes that emit right or left-handed circularly polarised light of opposite sign, at selected wavelengths.

Photographic image contrast based on the relative intensity of emitted circularly polarised light is an unexplored phenomenon. Its observation requires a means of distinguishing left and right-handed circularly polarised light for which a pragmatic solution, in the absence of suitable broad spectrum chiral filters, is to use a quarter wave plate, linear polariser and an appropriate band-pass filter in the path of the observed light beam. Here, we describe the resolution of very bright, Eu(III) complexes by chiral HPLC, report the stability of the enantiopure complexes to racemisation and introduce a proof-of-concept study, revealing the ability to differentiate and detect objects labelled with an emissive Δ or Λ europium(III) complex using a time-gated camera, following near-UV flash excitation.

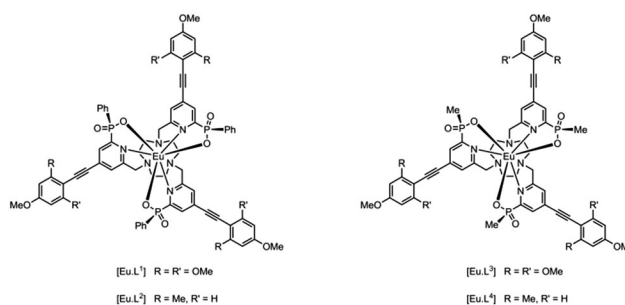
Circularly polarised luminescence (CPL) is the emission analogue of circular dichroism (CD) and intrinsically is a much more sensitive optical technique.^{1–3} Emission dissymmetry factors, g_{em} , (given as $g = 2(I_L - I_R)/(I_L + I_R)$) are typically very small for helical organic molecules but can be as high as 1.4 for lanthanide(III) complexes.⁴ Such behaviour contrasts with the size of g_{abs} values measured in CD that rarely exceed 10^{-4} . The ease of observation of circularly polarised photoluminescence from a chiral lanthanide(III) complex is a function of the brightness, B , of that complex at a given excitation wavelength, ($B_\lambda \sim \epsilon_\lambda \Phi_{em}$), and the nature of the lanthanide ion that determines the emission spectral form. Of particular importance, in this context, are the numerous series of water-soluble and highly emissive Eu(III) and Tb(III) complexes emitting in the visible region that are less prone to vibrational deactivation of the excited state and can be photosensitised from 337 to 405 nm.^{5–8}

Very bright, enantiopure europium(III) complexes allow time-gated chiral contrast imaging†

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Recently, a family of very bright europium(III) complexes has been introduced that are as bright as red fluorescent protein in aqueous media.^{9–13} The systems comprise a well-shielded nine-coordinate Eu(III) complex, cooperatively bound to the three ring nitrogen atoms of 1,4,7-triazacyclononane, three pyridyl nitrogens and three phosphinate oxygen groups. The aryl-alkynyl groups in the pyridine 4-position give rise to an internal charge transfer transition, that permits excitation in the range 340 to 375 nm, allowing efficient population of the europium 5D_0 excited state. Earlier crystallographic and solution NMR studies revealed that the complexes exist as a racemate, with an $RRR-\Delta-\delta\delta\delta$ or $SSS-\Lambda-\lambda\lambda\lambda$ configuration, specifying the chirality at phosphorus, around the helical axis (Δ is equivalent to P in this sense) and in the three ring NCCN chelates, respectively.^{9,12,14}

We have prepared the series of complexes, $[Eu\cdot L^{1-4}]$, in which either a P -phenyl or P -methyl group is present; the former series is the more lipophilic. The nature of the peripheral phenyl substituents has also been varied, allowing the excitation wavelength to be shifted closer to 355 or 365 nm, more appropriate for typical laser or LED excitation.



The complexes were prepared using previously published methods,^{11,14} and were purified by reverse-phase HPLC. The brightness of each complex in methanol solution at 355 nm falls in the range 14.5 to 33 $mM^{-1} cm^{-1}$ (Table 1 and Fig. 1). The broad absorption bands mean that at 365 nm (LED λ) the absorbance of the complexes of $[Eu\cdot L^1]$ and $[Eu\cdot L^3]$ was 92(± 2)% of that measured at the absorption maximum. Each of the complexes dissolves

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Table 1 Key photophysical properties of the europium(III) complexes (295 K, MeOH)

Complex	$\lambda_{\max}^a/\text{nm}$	Normalised absorbance at 365 nm/%	$\Phi_{\text{em}}/\%$	$\tau_{\text{Eu}}/\text{ms}$
[Eu-L ¹]	356	98	50	1.18
[Eu-L ²]	343	40	47	1.22
[Eu-L ³]	355	100	55	1.10
[Eu-L ⁴]	342	37	54	1.14

^a Extinction coefficients for these complexes are 65 000 (± 5000) $\text{M}^{-1} \text{cm}^{-1}$. Absorbance values are quoted at 365 nm as this is the LED excitation wavelength used in these experiments. Errors on quantum yield values are $\pm 15\%$ and on lifetimes, $\pm 8\%$.

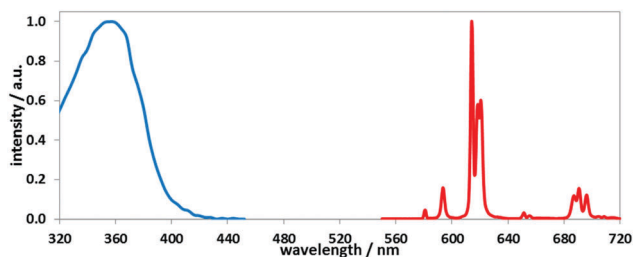


Fig. 1 Absorption and emission spectra for [Eu-L¹] (MeOH, 295 K), showing the fingerprint Eu emission profile arising from transitions from $^5\text{D}_0$ to the $^7\text{F}_n$ manifold ($n = 0-4$ shown here; see ESI† for all absorption and emission spectra).

readily in methanol solution and could be resolved using chiral HPLC, with columns based on chloro-substituted phenylcarbamate derivatives of cellulose or amylose (ChiralPak IC or ID; ESI†). The enantiomeric complexes eluted on the semi-preparative column with retention times differing by 8 to 18 minutes (295 K, MeOH, 4.4 mL min^{-1}).

The absolute configuration was assigned by comparison of their CPL spectra (Fig. 2), assessing the sign and sequence of observed transitions in these spectra, with those measured for

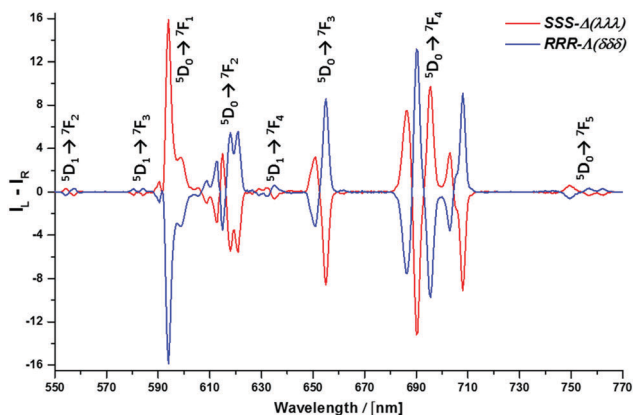
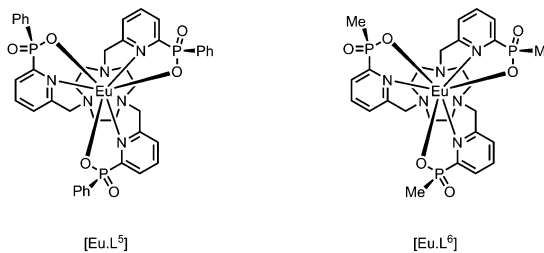


Fig. 2 Circularly polarised luminescence spectra for the enantiomers of [Eu-L²] (red Δ ; blue Λ) (5 μM complex, 4 min acquisition time, 3 averages, MeOH, 295 K); selected g_{em} values for Δ -[Eu-L²]: -0.19 (557 nm); $+0.15$ (599 nm); -0.19 (655 nm); -0.32 (708 nm); see ESI† for all g_{em} values, which are similar in size to recent reports.^{9,14} Similar total emission and CPL spectra were recorded for the samples on paper (Fig. 3 and Fig. S9, ESI†); for the sample immobilised on paper, the g value at 599 nm was 0.11.

the parent systems, *i.e.* [Eu-L⁵] and [Eu-L⁶], that lack the alkynyl-aryl moiety. Configurational assignment of these archetypal complexes has been established by X-ray crystallography and CD studies in earlier work.^{9,14}



The high signal intensity allowed the rapid measurement of the less commonly observed $^5\text{D}_0$ - $^7\text{F}_5$ CPL transitions (*ca.* 755 nm), as well as CPL associated with transitions from $^5\text{D}_1$ to $^7\text{F}_{2,3,4}$. No CPL signal was observed for the $^5\text{D}_1$ - $^7\text{F}_{0,1}$ transitions, and the $^5\text{D}_0$ to $^7\text{F}_6$ manifold was not observed.

The stability of the resolved complexes to thermally-activated racemisation was examined in methanol, monitoring the appearance of the enantiomeric europium complex by analytical HPLC. No evidence for racemisation had been observed in solution at room temperature, but at 60 °C the half-life for racemisation could be measured and was found to be 410 h for [Eu-L⁴]. For [Eu-L²], less than 0.5% of the other enantiomer could be discerned by chiral HPLC after 21 days under these conditions. These rates were independent of complex concentration, consistent with an intramolecular process, presumably involving stepwise dissociation of the Eu-oxygen bonds leading to epimerisation at each of the *P* centres, and a subsequent ring inversion of the triazacyclononane ring configuration ($\delta\delta\delta$ to $\lambda\lambda\lambda$). The half-life for racemisation of [Eu-L⁴] was considerably longer than that previously recorded for the parent complex (see ESI†).^{14a}

Time-gated photography has been achieved using an off-the-shelf DSLR camera (Nikon D5300) equipped with an i-TTL flash unit (Nikon SB910) paired with a wireless flash trigger and receiver (YN-622N). The complex [Eu-L¹], fluorescein and a co-spot of both compounds were applied as a solution in methanol to non-optimally brightened white paper and allowed to dry in air, creating a three-spot test paper (Scheme 1). A normal photograph under UV excitation shows, as expected, three spots of different colours (red from [Eu-L¹], green from fluorescein and yellow from the co-spot). Introduction of a band pass filter (595 ± 5 nm) to the camera, led to partial disappearance of the fluorescein spot, and all of the green-coloured emission, leaving three red spots. Total loss of the fluorescein spot was not achieved due to the long tail of the fluorescein emission. However, using distance-based time gating, placing the object 1.8 m from the camera (speed of light is 0.3 m ns^{-1}), the fluorescein spot was not observed, leaving just emission from [Eu-L¹].

Chiroptical contrast based imaging, *i.e.* the separation of left and right handed circularly polarised light emitted by the separate Λ - and Δ -[Eu-L²] enantiomers has been facilitated *via* modification of a time-resolved Zeiss Axiovert 200M epifluorescence microscope set-up.¹⁵ Enantiopure Λ - and Δ -[Eu-L²] were deposited as solutions in methanol onto white paper containing no



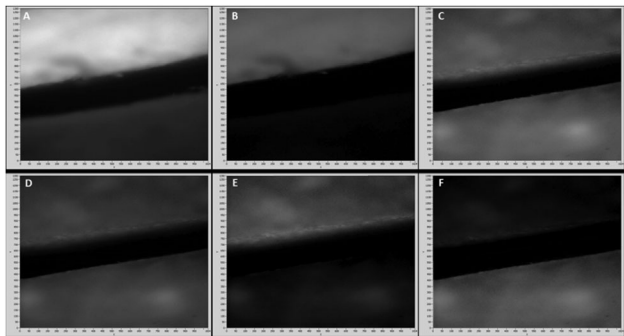


Fig. 3 Microscopy images following sample excitation using a 365 nm UV-LED ($t_{\text{acc.}} = 7.2$ ms per frame, 395 nm dichroic mirror, λ_{em} LP-420 nm) of fluorescein, A^- and Δ^- -europium(III) complex recorded on a custom-built microscope incorporating a chiroptical selector unit. (A) Ungated image of (top) A^- and Δ^- -[Eu-L³] (1:1) and (bottom) fluorescein, using 10 frame accumulation imaging sequence. (B) Time-resolved image of (top) A^- and Δ^- -[Eu-L³] (1:1) and (bottom) fluorescein ($t_d = 6$ μ s, λ_{em} LP-420 nm, 50 frame acc.). (C) Time-resolved image of (top) A^- -[Eu-L²] and (bottom) Δ^- -[Eu-L²] using ($t_d = 6$ μ s, λ_{em} LP 420 nm, 70 acc.). (D) Time-resolved image of (top) Δ^- -[Eu-L²] and (bottom) A^- -[Eu-L²] ($t_d = 6$ μ s, λ_{em} BP 595/10 nm, 150 frame acc.). (E) Time-resolved image of (top) A^- -[Eu-L²] and (bottom) Δ^- -[Eu-L²] using the RIGHT (horizontal polarisation) circularly polarised light chiroptical channel ($t_d = 6$ μ s, λ_{em} BP-589/20 nm, 370 frame acc.; contrast ratio $CR_{A:\Delta} = 3.23:1$) (F) Time-resolved image of (top) A^- -[Eu-L²] and (bottom) Δ^- -[Eu-L²] using the LEFT (vertical polarisation) circularly polarised light channel ($t_d = 6$ μ s, λ_{em} BP-589/20 nm, 370 frame accumulations; contrast ratio, $CR_{A:\Delta} = 1:3.64$).

In conclusion, using a very bright Eu complex to provide CPL with a predominant handedness of circular polarisation between 585 and 605 nm, we have demonstrated the concept of chiral image contrast in emission. Chiral liquid crystal displays (LCDs), especially nematic phase LCs, can act as linear polarisers or even controllable quarter or half wave plates, based on their orientation or rotation as a function of plane crystal surface, as used for 3D projection. Here, the situation is different; chiral circularly polarised emitters are used with a linear polariser: the incident angle of circularly polarised light is always 1/8th of the wavelength with respect to the axis of travel to the polariser at a point (rather than a slit, with linear polarised light). Rotating a linear polariser in the detection set-up, with circularly polarised emitted light does not make any contrast difference as a function of rotation angle.

In this example, circularly polarised light is translated to well-defined linearly polarised light using the birefringent

quarter-wave plate and the linear polariser. In such a setup, two linear polarisers separate the horizontal and vertical linearly polarised light generated. The H-LPL and V-LPL are a direct, wavelength-independent representation of the initially emitted left and right-handed CP light. This study therefore suggests a role for applications using the CPL emission of such Eu complexes in security tagging, among other possibilities.

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