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Polychromatic Fingerprinting of Excitation Emission Matrices

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Excitation emission matrix (EEM[1–3]) spectroscopy provide highly distinctive signatures of fluorescent substances and is a powerful method for the analysis of complex mixtures.[1,4] The multi-way characteristics of EEM data enable the mathematical identification of analytes, even in the presence of unknown interferences, which is known as the second-order advantage.[3,5] These characteristics make EEM spectroscopy attractive for chemical sensing; however, unfortunately, the acquisition of EEM landscapes generally requires expensive and dedicated instrumentation not always compatible with sensing purposes.[3–6]

In contrast to traditional instrumentation approaches, regular consumer optoelectronic devices co-opted as measuring platforms are an affordable and highly distributed alternative that is being increasingly explored. The rationale behind this approach is that successful sensing applications can be as pervasive as the devices supporting them. Thus, scanners,[7,8] DVD or CD drives[9] and computer screens in combination with web cameras (computer screen photo-assisted technique, CSPT[10–12]) have been demonstrated for diverse analytical and sensing uses. CSPT in particular utilises polychromatic sources with tuneable spectral radiances provided by ubiquitous computer screens and mobile phones. With this method, complex and distinctive signatures of fluorescent substances can be obtained and used for chemical sensing;[11] however, to crystallise the analytical potential of this method the missing categorical interpretation of the fingerprints and its theoretical description must be provided.

Here, we elucidate these key issues through the spectroscopic investigation of the polychromatic fingerprints of fluorescent substances and we propose a concurrent theoretical description explaining the complete range of polychromatic signatures of diverse substances as the collection of features from the EEMs.

Figure 1a shows the scheme of the experimental arrangement. Light from a computer screen illuminates a cuvette contained in a dark box with two transversally mounted fibre optics connected to a spectrophotometer. When the spectra are acquired from output E a significant part of the collected signal is due to fluorescence. The mirror (Al-coated glass) on the right wall of the cuvette is used to increase the fluorescent signal. From output T a transmission spectrum is obtained. Figure 1b depicts the measured spectral radiances of 50 polychromatic illuminating colours displayed by the screen, which in this case, as in CSPT experiments, constitute the light source.

Emission and transmission spectra of tested substances, for each particular illuminating colour i, are given by Equations (1) and (2) in which I and I_o are intensities measured through the dissolved target substance and the pure solvent used to dissolve it, respectively.

$$E_i(\lambda) = \left.\frac{I_i(\lambda)}{I_{0i}(\lambda)}\right|_{Eout} \quad (1)$$

$$T_i(\lambda) = \left.\frac{I_i(\lambda)}{I_{0i}(\lambda)}\right|_{Tout} \quad (2)$$

Imaging systems such as CSPT capture a weighed sum of these spectra,[11] and we choose that representation here to keep our discussion compatible with the interpretation of other such methods. Thus, we combine these spectra in a weighed (with positive weights $n_1+n_2=1$) total transmittance [Eq. (3)]

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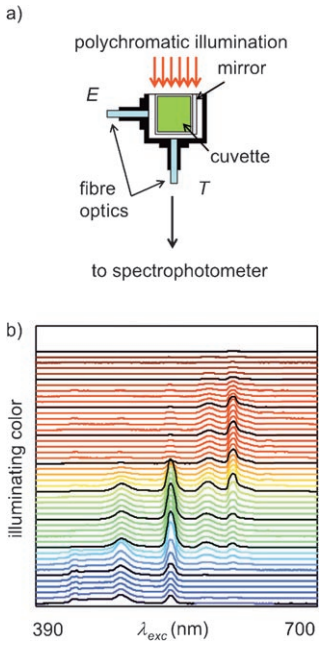

Figure 1. a) Cross-section scheme of the experimental arrangement. b) Collection of measured spectral radiances of illuminating polychromatic colours displayed on the screen. The colours of the lines correspond to the perceived colours of these radiances. Black lines are an aid to the eye highlighting changes in the radiance for different illuminating colours.

$$F_i(\lambda) = n_1 T_i(\lambda) + n_2 E_i(\lambda) \tag{3}$$

In CSPT experiments n_1 depends on the concentration and the molar absorptivity of the substances, and on the path length of the cuvette, whereas n_2 depends on the geometry of the setup and quantum yield of the substances. In this work their values are chosen to display a similar proportion of absorption and emission features, which is also a demanding scenario for the subsequent modelling.

In contrast to regular spectrophotometers that use wide band light sources, computer screens provide polychromatic spectral radiances as result of the combination of three partially overlapping bands, corresponding to the radiances of the screen primaries $R(\lambda)$, $G(\lambda)$, $B(\lambda)$. Thus, for any given colour i defined by the triplet of weights (r_i, g_i, b_i) the exciting spectral radiance is given by Equation (4)[12–14] in which γ is the correction for the nonlinearity of the intensity with respect to the displayed colour value.[14]

$$c_i(\lambda) = (r_i R(\lambda) + g_i G(\lambda) + b_i B(\lambda))^{\gamma} \tag{4}$$

The absorbance of a substance ($A(\lambda)$), as conventionally defined by monochromatic spectroscopy, is independent of the illumination, since it is obtained for a unique broadband illumination. Here, depending of the illuminating colours, some bands are highly modulated and the resulting absorbance ($\alpha_i(\lambda)$) does depend on the illumination according to Equation (5) in which $\tilde{c}_i(\lambda)$ is the normalised $c_i(\lambda)$. The transmittance associated to α can be calculated as usual from Equation (6).

$$\alpha_i(\lambda) = \tilde{c}_i(\lambda) A(\lambda) \tag{5}$$

$$\tau_i(\lambda) = 10^{-\alpha_i(\lambda)} \tag{6}$$

In addition to the transmitted light, each polychromatic excitation $c_i(\lambda)$ produces a collection of emissions (for each single excitation line stimulated by $c_i(\lambda)$) that we account as $\varepsilon_i(\lambda_{em})$ and is calculated from the EEM (for which $\rho(\lambda,\lambda_{em})$ is the normalised EEM landscape) of the tested substance obtained with a regular fluorescence spectrophotometer [Eq. (7)].

$$\varepsilon_i(\lambda_{em}) = \int_{\lambda} \bar{c}_i(\lambda)\rho(\lambda, \lambda_{em})\mathrm{d}\lambda \tag{7}$$

Finally, the total transmittance $\psi_i(\lambda)$ [Eq. (8) adopts the same form as Equation (3).

$$\psi_i(\lambda) = \eta_1 \tau_i(\lambda) + \eta_2 \varepsilon_i(\lambda) \tag{8}$$

In Equation (8) $\eta_1 + \eta_2 = 1$ are the free variables used to fit the model with the measured $F_i(\lambda)$. Thus, according to Equations (5)–(8) illuminations with varied spectral compositions (e.g., a sequence of illuminating colours) highlight different features of the EEM, which become embedded in the total transmittance.

Figure 2a illustrates the tested molecules and Figure 2b collects the contour plots of the excitation emission spectra measured with regular monochromatic excitation (fluorescence spectrometer). Two of these substances are common fluorescent dyes (fluorescein and rhodamine B) and the others are fluorescent indicators used in chemical sensing. Biladiene is a linear tetrapyrrole with two additional methyl groups at the 1,19-positions, GeTPC (TPC = triphenylcorrolate) is the chlorogermanium complex of a triphenylcorrole and ZnTPPpol (TPP = 5,10,15,20-tetraphenylporphinate) is a polymer in which different ZnTPP units are linked by arylethynyl groups.[15–17]

Figure 2c shows the measured spectra ($F_i(\lambda)$) of each of these substances for 50 polychromatic illuminations. The red line in the first panel of Figure 2c indicates $F(\lambda) = 1$; hence, values larger than 1 correspond to predominant fluorescence, because that means that the detector receives more light than through the pure solvent used as reference. As can be seen, absorption features in the polychromatic spectra are well aligned with the absorption peaks of the excitation–emission spectra. For clarity, only ten $F_i(\lambda)$ are highlighted in Figure 2 with their respective illuminating colours. Different illuminating colours specifically highlight characteristic features of the EEM. For instance, in the case of fluorescein, for blue illumination the whole absorption band can be obtained, while toward cyan illumination the absorption peak narrows, becoming a more efficient excitation just limited to the maximum absorption peak, showing a larger proportion of fluorescent signal. Illuminating colours in the red region are not able to excite fluorescence and this can be seen in the spectra for red light. The details of these tran-

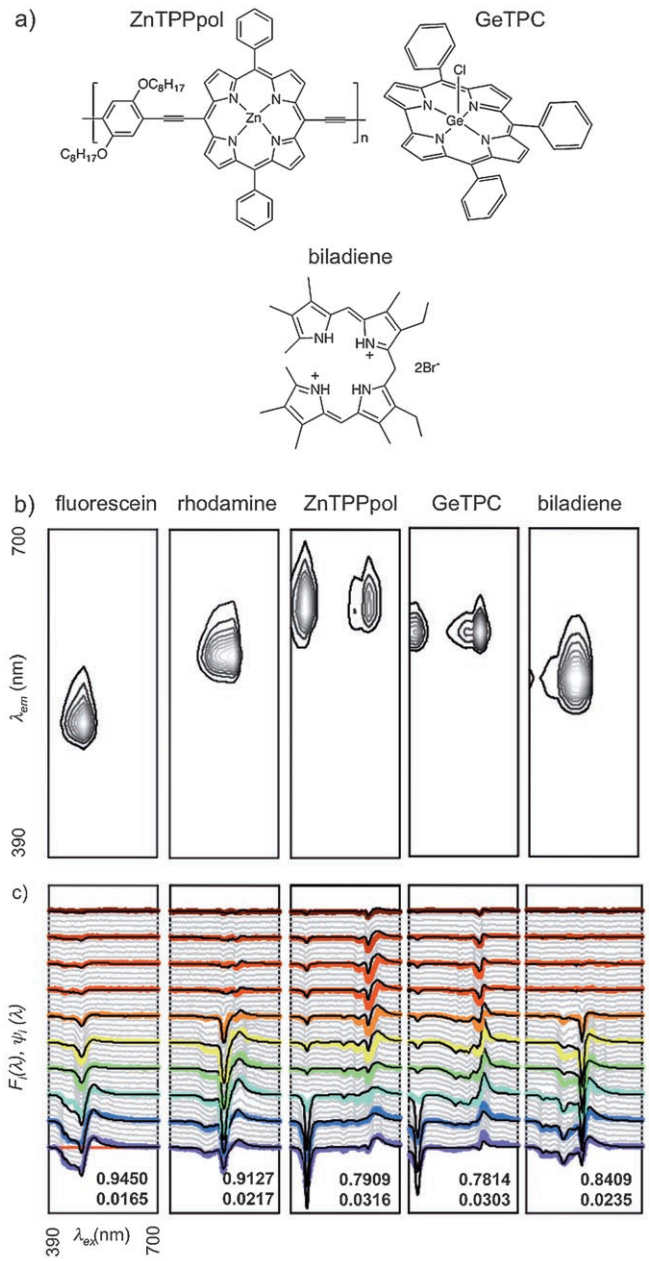

Figure 2. a) Molecular structure of the tested substances. b) Contour plots of excitation–emission spectra of fluorescein, rhodamine, Zn–porphyrin polymer, Ge–corrole complex and biladiene measured with a fluorescence spectrometer. c) Measured total transmittances for 50 different illuminating colours (colour and grey lines) for the substances given in a). The illuminating colours are indicated only on 10 transmittances. Solid black lines show the calculated total transmittances. The red line in the first panel is an aid to eye highlighting $F(\lambda)=1$, larger values correspond to fluorescence. r and rmsd fitting values are indicated for each substance.

sitions together with the shape of the spectra constitute a fingerprint of the EEM that become captured in $F_i(\lambda)$.

The complexity of the fingerprints in Figure 2c underscores the challenge to provide a unified model able to reproduce all nuances and to generalise to different substances by using a minimum number of free parameters. Modelled results are displayed as black curves in Figure 2c for the ten experimental results highlighted in colour. With only two free parameters (η_1 and η_2) the model properly reproduces the location of absorption and emission features as well as their specific shape and behaviour along the illuminating sequence, corroborating the fingerprinting of the EEM described by Equations (7) and (8) for all tested substances.

Standard measures of fitting goodness[18,19] were calculated for the collection of ten coloured spectra of each substance in Figure 2c. Pearson's r coefficient[18,19] evaluates the fitting to trends in the data and the root mean square deviation (rmsd) assesses the deviations from exact values.

A value of $r=1$ indicates a perfect fit and in our case an average $r=0.8541$ value indicates the ability of the model to generalise to different substances. For the evaluated concentrations of porphyrins, the spectra show absorption of the Q-bands larger than expected from the EEM, which is reflected in a disproportion of the Soret band with respect to the Q-band in the model (Figure 2c), producing lower r values for these substances although still satisfactory given the complexity of the fingerprints, while the average rmsd = 0.0247 is well within the close fit category (<0.06[20]).

Summarising, this work demonstrated the ability of a ubiquitous polychromatic excitation to support the fingerprinting of the EEM of fluorescent indicators. This possibility relies on the capacity of computer screens to deliver spectrally controlled illumination to systematically highlight different regions of the EEM. The goodness of the fitting denotes a correct description of the detection mechanism and underscores the substantial amount of the spectral information retained in the fingerprints.

Beyond the sensing possibilities of the present setup, this work elucidates the spectroscopy essence of ubiquitous sensing methods such as CSPT, for which a web camera used as an imaging detector replaces the spectrophotometer, enabling the simultaneous classification of arrays of diverse substances.[10,11,21]

Experimental Section

Solutions of fluorescent molecules, with concentration of 2 μM for the absorption and 10 μM for the emission measurements were prepared. Spectroscopic-grade THF (Fluka) and distilled water passed through Milli-Q purification system were used as solvents. Fluorescein and rhodamine were diluted in water, whereas the other substances were dissolved in THF. Absorption spectra were measured with a Shimadzu UV-1601PC, spectrophotometer. EEM measurements were carried out on a spectrofluorimeter (Hitachi F4500), operating in the 390–700 nm detection range, with excitations at 20 nm intervals within the same range. Samples were contained in 1 cm light path quartz cuvettes. Polychromatic excitation measurements were performed by using an LCD screen (Philips 170 s2, 1280×1024 pixels resolution at a 60 Hz refresh frequency) operating at normal conditions of intensity and contrast. In the case of the Ge–porphyrin complex, a CRT screen (CTX 1565 GM) was used, since the blue band in these screens is better aligned with the Soret band of the substance.

The screen illuminated the cuvettes, which were contained in the setup shown in Figure 1a. Optical fibres were connected to an Ocean Optics USB2000 spectrometer. During the measurements, the screen displayed a

sequence of 50 colours and for every colour the transmission and emission spectrum of both the reference (solvent) and the sample were recorded. Numeric processing and modelling was performed with software written in Matlab 7 code.

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