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Imaging fingerprinting of excitation emission matrices

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ABSTRACT

The spectral fingerprinting of the excitation emission matrix (EEM) of fluorescent substances is demonstrated using polychromatic light sources and tri-chromatic image detectors. A model of the measured fingerprints explaining their features and classification performance, based on the polychromatic excitation of the indicators is proposed.

Substantial amount of spectral information is retained in the fingerprints as corroborated by multivariate analysis and experimental conditions that favor such situation are identified.

In average, for five different substances, the model shows a fitting goodness measured by the Pearson's *r* coefficient and the root mean square deviation of 0.8541 and 0.0247 respectively, while principal component classification patterns satisfactorily compare with the EEM spectroscopy classification and respectively explain 96% and 93% of the information in the fist two principal components.

The measurements can be performed using regular computer screens as illumination and web cameras as detectors, which constitute ubiquitous and affordable platforms compatible with distributed evaluations, in contrast to regular instrumentation for EEM measurements.

1. Introduction

As consumer electronic devices undergo a continuous improvement in performance and sophistication they become versatile platforms suitable with different purposes than those originally conceived for. Concurrently, their pervasive presence provides a distributed infrastructure compatible with chemical sensing purposes, as demonstrated by an increasing number of examples. Thus, flatbed scanners [1,2], DVD or CD drives [3] and computer screens in combination with web cameras (computer screen photo-assisted technique, CSPT [4,5]) have been demonstrated for diverse analytical and sensing uses.

CSPT in particular provides a ubiquitous platform supporting diverse optical experiments, including spectral fingerprinting [4,5], ellipsometry [6,7] and surface plasmon resonance [8]. In the case of spectral fingerprinting, computer screens are used as light sources displaying color sequences to illuminate chemically sensitive indicators imaged with a web camera [5].

The results of these measurements are distinctive optical fingerprints of those indicators and their response to target stimuli that enables to identify such stimuli [5]; however, the categorical description of CSPT fingerprints of fluorescent substances obtained in these conditions has been an elusive target. Recent progress on polychromatically excited fingerprints [9] has suggested the possibility to characterize the excitation emission matrix (EEM [10,11]) of fluorescent substances by CSPT. Excitation emission matrix spectroscopy [10–12] provides highly distinctive signatures of fluorescent substances and is a powerful method for the analysis of complex mixtures [10], which enables the identification of analytes, even in the presence of unknown interferences [12,13]. These characteristics make EEM spectroscopy attractive for chemical sensing, but unfortunately, the acquisition of EEM landscapes generally requires expensive and dedicated instrumentation not always compatible with distributed sensing purposes [12,14]. Thus, the potential to fingerprint EEMs with CSPT platforms becomes a key aspect to be investigated.

Here we contribute the mathematical model of CSPT fingerprints of fluorescent substances, and we demonstrate the link between such fingerprints and the EEM of the substances. The ability of CSPT to identify different fluorescent indicators is compared with the spectroscopy, revealing a substantial amount of the spectral information captured in these substance fingerprints, the capacity of the model to predict the classification patterns and the importance of the signal composition on the identification performance.

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2. Experimental

2.1. Materials

Five fluorescent substances are investigated in this work. Two of them are common fluorescent dyes (fluorescein and rhodamine B) and the others are fluorescent indicators used in chemical sensing [5]. Biladiene is a linear tetrapyrrole with two additional methyl groups at the 1,19 positions; GeTPC is the chlorogermanium complex of a triphenylcorrole and ZnTPPpol is a polymer, in which different ZnTPP units are linked by arylethynyl groups [15–17].

Solutions of fluorescent molecules, with concentration of 10 μM 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 in THF.

EEM measurements were carried out on a spectrofluorimeter (Hitachi F4500), operating in the [390, 700] nm detection range with excitations at 20 nm intervals in the same range. Samples were contained in 1 cm light path quartz cuvettes.

2.2. Instrumentation

The light source used in CSPT experiments was a Philips 170/s2 liquid crystal display (LCD) computer screen operating in normal conditions of intensity and contrast and at 1280 × 1024 pixel 32 bit color resolution at a standard refresh frequency of 60 Hz. During the spectral fingerprinting measurements the screen illuminated the samples with a 50 colors illuminating sequence at a rate of 1 color/s sorted according to the human perception of the visible spectrum. A Logitech Quickcam Pro 4000 with a 640 × 480 pixels charge coupled device detector recorded in synchronism with the illumination the image of the tested samples.

A sample holder was designed to provide measurements containing different contributions of transmitted and emitted light. CSPT is an imaging technique, and the holder provides such variety of signal composition distributed in the imaged area. In other assay formats such as microtiterplates [4], microstructures [18] and others [5,19] used in CSPT, a fixed transmission-emission proportion is captured. The present experimental arrangement aims at generalizing the possibilities found in those other practical formats.

The holder (Fig. 1a) provides symmetric illumination for the reference and sample cuvettes, which are simultaneously measured. In the back of the cuvettes partially reflecting sand blasted glass pieces coated with aluminium are used to increase the contribution of transmitted light in the lower 1 cm of the cuvette images, thus creating two regions with distinct signal compositions. A third water containing cuvette used as a beam splitter transversally guides the illumination. Fig. 1a shows a 3D disassembled experimental arrangement.

Fig. 1b shows the ray tracing of the experimental arrangement for three illuminating pixels at different positions on the screen. During the experiments the complete area is used as illumination and all pixels contribute simultaneously to the measurement. In blue is indicated the isotropic fluorescent emission. As indicated by the ray tracing and experimentally verified (Fig. 1c), the intensity of the illumination and consequently the emission decreases along the x coordinate.

2.3. Procedures

The result of the measurements was a collection of pictures, which were evaluated with software written in Matlab7R14. This software enables to select regions of interest (ROI) and to compute intensity signatures of these ROIs. In this work the ROI intensity fingerprints are the intensity for each illuminating color concatenated along the red, green and blue camera channel. Modeling and multivariate analysis were also performed with Matlab.

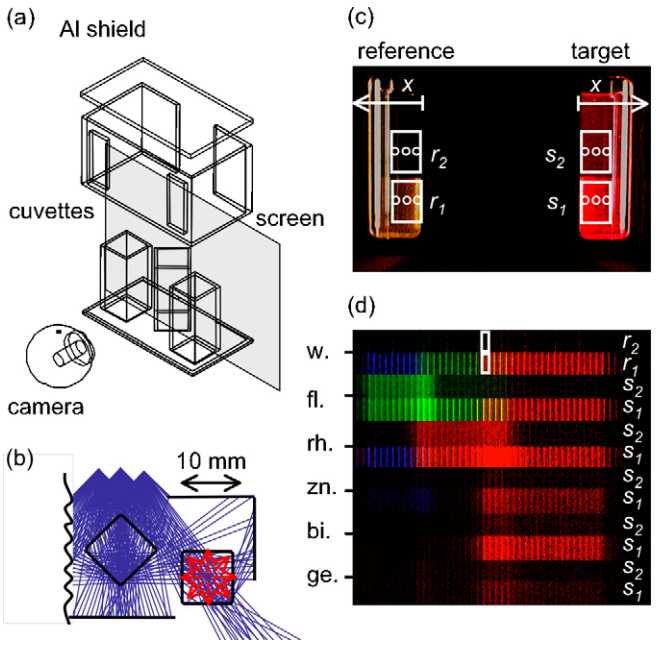

Fig. 1. (a) 3D scheme of the disassembled experimental arrangement. (b) Ray tracing of the setup, with the fluorescence represented in red. (c) One imaged frame, with samples under yellow illumination. The target substance is rhodamine B and the reference is distilled water. The coordinate x used in the text is indicated in white and the regions of interest (ROI) marked with white squared frames. (d) Collection of all ROIs for water and other 5 tested substances under 50 illuminating colors. fl = fluorescein, rh = rhodamine B, zn = ZnTPP, bi = biladiene and ge = GeTPC. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 1c shows one frame of the image acquisition sequence, in this case for a yellow illumination. White rectangular frames, in the upper and lower part of each cuvette, indicate two sets of ROIs used for processing. Spectroscopic measurements using a fiber optics spectrophotometer (Ocean Optics USB2000) are made at particular x positions and used to validate the modeling described below. r_2 and s_2 correspond to predominant fluorescent signals in the image composition, as can be inferred by the dark r_2 (water) and light s_2 (the sample is more intense than the reference), whereas r_1 and s_1 contain a larger proportion of transmitted light. The larger intensity of r_1 respect to r_2 is explained by diffuse reflection on the Al shield (not indicated in the ray tracing) back reflected toward the camera by the reflectors in r_1 and s_1.

3. Results and discussion

Fig. 1d collects the ROIs 1 and 2 for the reference cuvette and for each one of the 5 tested substances, providing an overview of the imaging experiments. r_2 darkness illustrate the satisfactory separation of excitation from fluorescence achieved by the setup. r_1 essentially shows the unaltered illuminating sequence (transmitted through water). In the case of fluorescein, s_2 show signals with maximum intensity for the corresponding absorption bands, while s_1 is a superposition of the fluorescence on the transmitted light.

Rhodamine shows same characteristics but emission and absorption occurs at longer wavelengths compared to fluorescein, and the emission peak appears for the green yellow illuminating conditions. In the case of fluorescein and rhodamine they have larger quantum yields than the other substances, and this is reflected by the relative intensity of the patterns.