Photonics Laboratory Report: Fluorescence Spectrophotometer

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Photonics for Security, Reliability, and Safety (PSRS)

LF00CX78 Photonics Laboratory

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Contents

1 Abstract

This study explores the fluorescence characteristics of five samples with varying fluorescent coatings using the PerkinElmer FL 8500 spectrometer. The primary aim is to identify samples with dual fluorescent paint coatings and determine specific paint combinations through Donaldson matrices. Additionally, the emission spectra of a selected sample are calculated under CIE standard illuminants A and D65. The results provide detailed excitation-emission matrices, aiding in the identification of dual paint combinations and offering insights into the emission behavior under different lighting conditions.

2 Theory

Fluorescence is a luminescence phenomenon where a substance emits lower energy light immediately after absorbing electromagnetic radiation with higher energy. In contrast with scattering and phosphorescence, fluorescence involves the rapid re-emission of light instantly after the material absorbs photons, while scattering involves the dispersal of light in various directions due to interaction with particles, without any delay in time. Phosphorescence, on the other hand, involves a delayed re-emission of light, continuing to glow for some time even after the excitation source has been removed due to forbidden energy state transitions [1].

2.1 Fluorescence Phenomena

The basics of fluorescence is that everything is quantized. This means that there will only be rotation, vibration, or electronic transitions if the energy of the incident light is just right to cause an electron from a lower rotation, vibration or electronic to a higher level, where the right energy means the right wavelength of incident light. Fluorescence deals with electronic transitions, which require higher energy compared to rotational and vibrational transitions.

Fluorescence involves the absorption of high-energy photons, often ultraviolet (UV) light, which excites electrons in the substance to higher energy states. Upon returning to their ground state, these electrons release energy in the visible spectrum, which is seen as fluorescence. Fluorescence of molecules are characterized by stokes shift, the difference between the wavelength of the incident light and the emitted light. [2].

2.2 Excitation and Emission

Excitation in fluorescence refers to the process where a molecule absorbs a photon of light, causing an electron to jump from a lower energy level (ground state, S0) to a higher energy level (excited state, S1 or S2). The energy of the absorbed photon must precisely match the energy difference between the ground state and the excited state. Once in the excited state, the molecule can undergo internal conversion, where it relaxes to the lowest vibrational level of the excited state without emitting light. The molecule then returns to the ground state by emitting a photon, a process known as fluorescence emission. The emitted photon has less energy, and therefore a longer wavelength, than the absorbed photon, due to energy loss during the internal conversion process. This difference in energy (and wavelength) between the absorbed and emitted photons is known as the Stokes shift. The Jablonski diagram effectively illustrates these excitation and emission processes by showing the pathways and transitions involved in fluorescence [2].

2.3 Jablonski Diagram

The Jablonski diagram is a visual representation that explains the processes of photon absorption, internal conversion, intersystem crossing, and emission in fluorescent materials [2]. When a molecule absorbs a photon, it is excited from the ground state (S0) to higher electronic states (S1 or S2). The molecule then undergoes internal conversion, relaxing to the lowest vibrational level of the first excited singlet state (S1). Some molecules may experience intersystem crossing to a triplet state (T1), which involves a spin change and typically has a longer lifetime. The return from T1 to S0 results in phosphorescence (delayed fluorescence), where the emitted photon has a longer wavelength and occurs over a longer timescale compared to fluorescence. Figure 1 shows a sample Jablonski diagram, illustrating all these concepts [3].

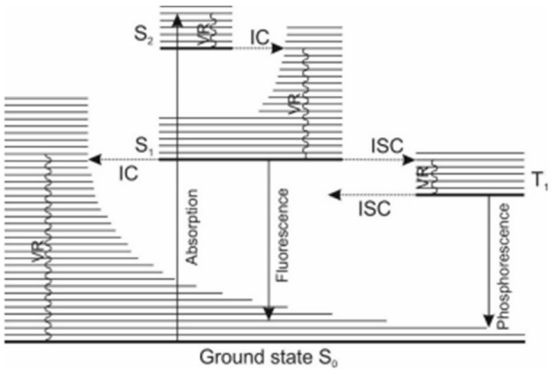


Figure 1: Jablonski diagram illustrating excitation, emission, fluorescence, and phosphorescence [2].

2.4 Donaldson Matrix

Donaldson matrices, also known as excitation-emission matrices (EEMs), measures fluorescence intensity as a function of both excitation and emission wavelengths. Each matrix is a 2D plot where the x-axis represents the *excitation wavelength*, and the y-axis represents the *emission wavelength*. The intensity at each point on the plot indicates the fluorescence response of the sample at that specific excitation-emission pair. It is used to analyze a sample's fluorescence characteristics by revealing its spectral features. Since these matrices are unique for different compounds, they are used as signatures for substances based on their distinct fluorescence patterns. In our experiment, Donaldson matrices help determine which samples are coated with single or dual fluorescent paints by comparing their fluorescence signatures.

The aim of this lab is to measure Donaldson matrices for five samples with different fluorescent coatings using the PerkinElmer FL 8500. The first objective is to identify which samples are coated with two paints and determine the specific paint pairs. The second objective is to calculate the emission spectrum for a sample under CIE standard illuminants A and D65.

2.5 Method

This section details the experimental design and how measurement was taken.

2.5.1 Experimental Setup and Sample Introduction

The experimental setup for this fluorescence measurement involved using the PerkinElmer FL 8500 fluorescence spectrometer (Fig. 2). This advanced spectrometer is equipped with various functions to precisely control excitation and emission settings.

Figure 2: Experimental setup: PerkinElmer FL 8500.

The excitation and emission wavelengths, along with other settings, were carefully selected with attention to the slit widths being kept consistent to ensure accurate measurements. The instrument was calibrated, and the control software is set to the presets recommended by our instructor and the manual for our experiment. Additionally, we set the photomultiplier voltage to 290 V.

2.5.2 Sample Introduction

The samples used in this experiment were specifically prepared to exhibit distinct fluorescent properties. Five samples in total were analyzed: three were coated with a single type of fluorescent paint, and two were coated with a combination of two different fluorescent paints. The samples were labeled and handled with care to avoid contamination and ensure accurate measurements. The samples were then securely mounted in the solid sample holder of the spectrometer, ensuring they were properly aligned to receive uniform excitation light and produce consistent emission data.

(a)

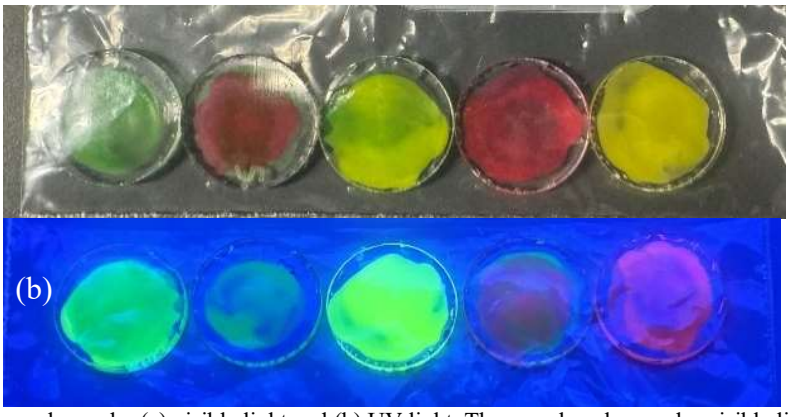


Figure 3: Experiment samples under (a) visible light and (b) UV light. The sample colors under visible light (from left to right) are sample 1: green, sample 2: green-red, sample 3: green-yellow, sample 4: red, and sample 5: yellow.

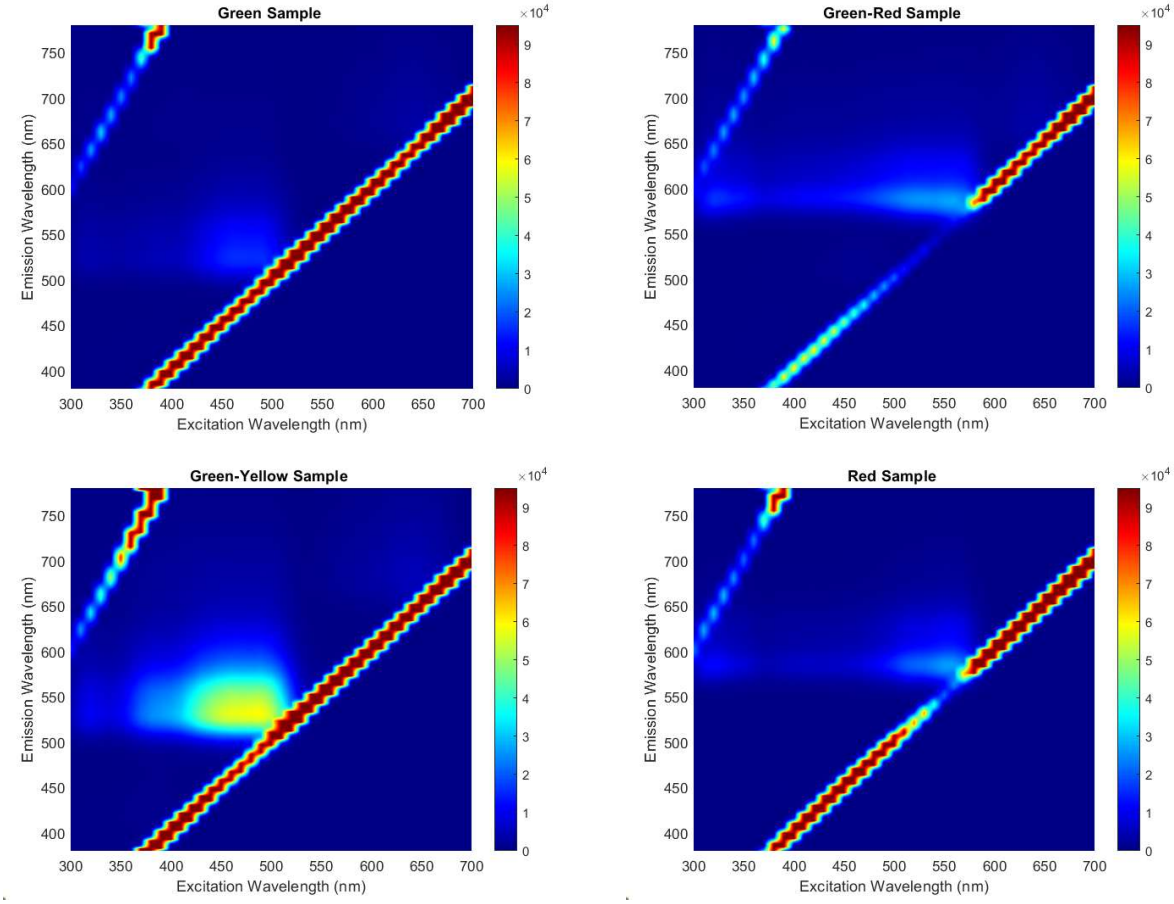
The samples were then excited, and the emitted fluorescence was captured for further analysis.

3 Results

The results of the fluorescence measurements using the PerkinElmer FL 8500 spectrometer are presented in this section. The primary objectives were to analyze the Donaldson matrices of the samples, identify the samples with dual paint coatings, determine the specific paint combinations, and calculate the emission spectra for a selected sample under CIE standard illuminants A and D65.

3.1 Donaldson Matrices Analysis

The fluorescence spectra of the five samples were successfully measured, and we obtained detailed Donaldson matrices for each. These matrices depict the relationship between excitation and emission wavelengths. Due to the relatively low photometric voltage (290 V), the fluorescence signal was quite weak. To improve visualization, we applied a threshold, capping all values above a certain limit. Initially, we experimented with different threshold values using a slider until we were satisfied with an optimal threshold of 95,000 for visualizing each sample's Donaldson matrices.



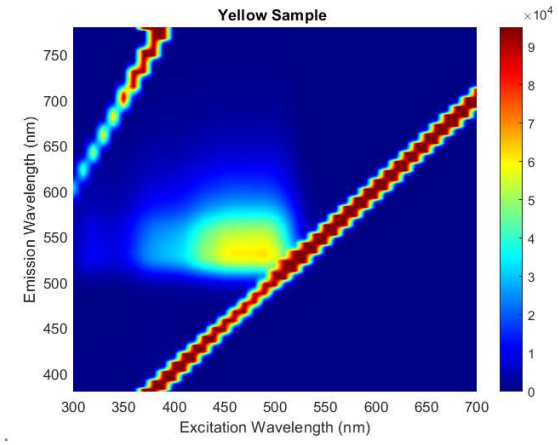


Figure 4: Excitation-emission matrix of the samples.

3.2 Identification of Dual Paint Combinations

By convention, fluorescence occurs when the absorbed UV light by the sample emits a photon that has a higher wavelength specifically in the visible range. When this happens, the 3D plot of the excitation emission spectrum shows a “spot" of emissions at these emitted wavelengths. And in principle, these wavelengths correspond color of the sample. From the plots it can be observed that the emitted wavelengths are around 540nm, 580nm, 610nm for samples 1, 5, and 4. These wavelengths correspond to green, yellow, and red colors respectively, hence these samples are green, yellow and red. Also, it can be observed that the emitted wavelengths for the 2nd and 3rd samples are within the Green-Red and Green-Yellow respectively, hence this infer that these samples 2 is a mixture of red and green paint while sample 3 is a mixture of green and yellow paints.

3.3 Emission Spectrum Calculation under CIE Standard Illuminants

For the second task, the green sample was selected to calculate the emission spectra under the CIE standard illuminants A and D65. Illuminant A represents a typical incandescent light source with a color temperature of approximately 2856 K, while illuminant D65 simulates average daylight with a color temperature of around 6500 K [4].

The aim of this task involved adjusting the raw fluorescence data to restore the emission spectrum from the excitation spectrum of the selected green sample.

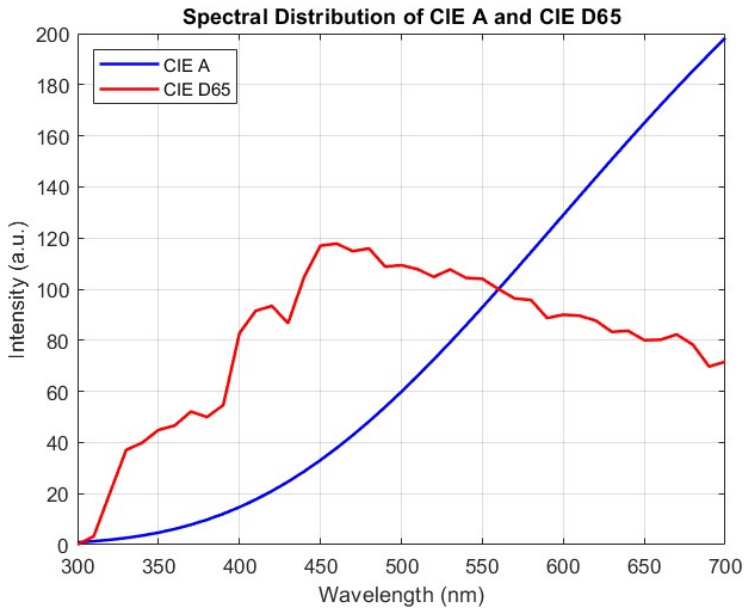


Figure 5: Excitation spectra by the CIE standard illuminants A and D65.

After extracting the wavelength range of 380 nm to 780 nm with 10 nm increments from the original matrix of the green sample, we obtained a modified Donaldson Matrix for the green sample. Then, we matrix-multiplied the spectral distributions of CIE A and D65, which are the excitation spectra, by the new Donaldson Matrix to obtain the emission spectra (Fig. 7).

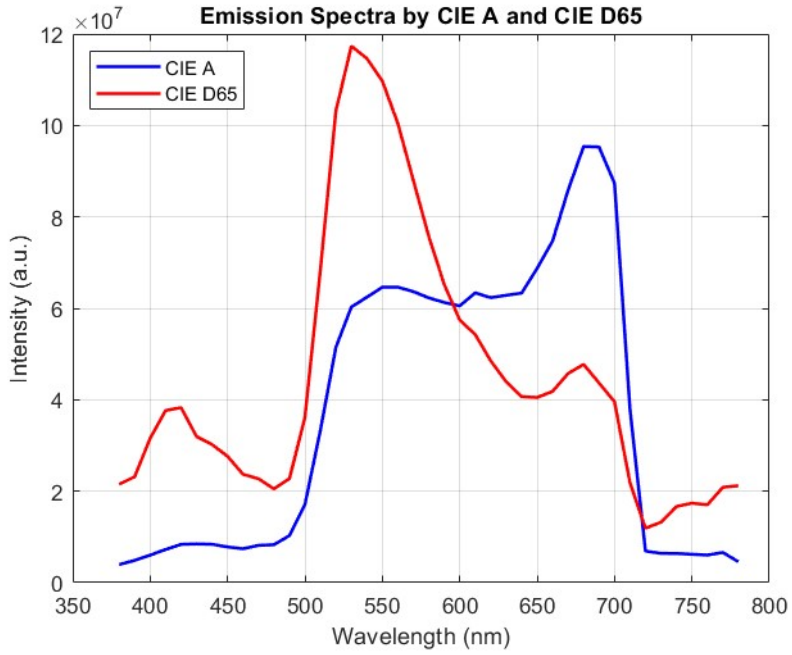


Figure 6: Emission spectra of the green sample by the CIE standard illuminants A and D65.

4 Conclusion

In conclusion, the study used the PerkinElmer FL 8500 spectrometer to analyze the fluorescence characteristics of five samples with different fluorescent coatings. The objectives were to identify samples with dual fluorescent paint coatings, determine specific paint combinations using Donaldson matrices, and calculate the emission spectra of a selected sample under CIE standard illuminants. To achieve this objective, the Donaldson matrices of five samples we reconstructed from their fluorescence spectra. The Results revealed that samples 1, 4, and 5 exhibited single fluorescent coatings with green, red, and yellow emissions, respectively, while samples 2 and 3 showed emissions indicating dual coatings: sample 2 with a green-red combination and sample 3 with a green-yellow combination. The emission spectra of the green sample (sample 1) were calculated under CIE standard illuminants A and D65 by adjusting the raw fluorescence data and using matrix multiplication with the spectral distributions of the standard illuminants to restore the emission spectra. This analysis revealed that the green sample's emission was more pronounced under illuminant D65, simulating daylight conditions. This lab helped us understand the working principle of fluorescence spectrometer, how it is used to measure the Donalson matrix and how these measurements can be used to recognize substances.

References

[1] P. M. V. Raja and A. R. Barron, "Photoluminescence, Phosphorescence, and Fluorescence Spectroscopy," Rice University, Aug 2022. [Online]. Available: https://chem.libretexts.org/@go/page/5588. [Accessed May 2024].

[2] J. Lakowicz, "Principles of Fluorescence Spectroscopy," in *Instrumentation for Fluorescence Spectroscopy*, Boston, MA: Springer, 2006, p. 27–61.

[3] R. Šípoš and J. Šima, "Jablonski Diagram Revisited," *Revista Cubana de Física,* vol. 37, pp. 125-130, 2020.

[4] CIE, "COLORIMETRY — PART 2: CIE STANDARD ILLUMINANTS," Inturnational Commission on Illumination, [Online]. Available: https://cie.co.at/publications/colorimetry-part-2-cie-standard-illuminants-0. [Accessed May 2024].

[5] S. Tominaga, K. Hirai and T. Horiuchi, "Estimation of bispectral Donaldson matrices of fluorescent objects by using two illuminant projections," *Journal of the Optical Society of America. A,* vol. 32, pp. 1068-1078, 2015.

Appendix

PHYRA47, "Photonics-Laboratory/4-fluoroscence-spectrometer/," GitHub, 2024. [Online]. Available: https://github.com/PHYRA47/Photonics-Laboratory/tree/main/4-fluoroscence-spectrometer. [Accessed: May 15, 2024].