

Fluorometer Lab Session

Abstract

In this experiment, we used a *PerkinElmer FL 8500* fluorometer to analyze the optical behavior of five distinct samples. The spectral range of the emission data collected by the fluorometer was between 380 and 780 nm. Each sample was measured to obtain its fluorescence characteristics, and from the data, individual Donaldson matrices were computed. These matrices, representing the fluorescence excitation-emission relationship, were subsequently applied to standard illuminants A and D65 to simulate their excitation conditions. The corresponding emission spectra were recorded and analyzed. Using the Donaldson matrices, we were also able to identify and distinguish the five samples based on their unique fluorescence profiles. This study demonstrates the utility of Donaldson matrices in characterizing and differentiating fluorescent materials under varying illumination conditions.

1 Introduction

In this lab, the fluorescence of five samples was analyzed using a fluorescence spectrometer. Fluorescence is an important material property that can be utilized in various fields such as earth science, aesthetics, security, and it is even harnessed by certain organisms (6).

A fluorescence spectrometer was used to capture the fluorescence emitted by the five samples. This data allowed for the determination of the composition of each sample's coating. Additionally, the same data enabled the determination of each sample's emission spectrum under various illumination conditions.

2 Theory

2.1 Fluorescence

It is well known that light energy is absorbed in specific units of energy called quanta. This amount of energy is dependent on the wavelength of the light being absorbed as seen in equation 1. Here, E is the energy (quanta), h is Planck's Constant, c is the speed of light, and λ is the wavelength of the light (2).

$$E = hv = \frac{hc}{\lambda} \quad (1)$$

All molecules have energy levels which the quanta can inhabit. The level with the least energy is called the ground state and any level above that is an excited state. The amount of energy that is introduced to the molecule determines which energy level its electron can be excited to. This quantity of energy is known as the photo-excitation energy (6). If a molecule absorbs energy and sends one of its electrons to an excited state, it must eventually come down. An electron that travels from a higher to a lower electron state will emit a photon, and

this phenomenon is called fluorescence. The photons associated with fluorescence will have a greater wavelength (and less energy) than the photon-excitation energy because energy will have been lost in the time between absorption and emission (2). Figure 1 is known as a Jablonski diagram and it allows us to visualize the process of electrons moving between energy levels.

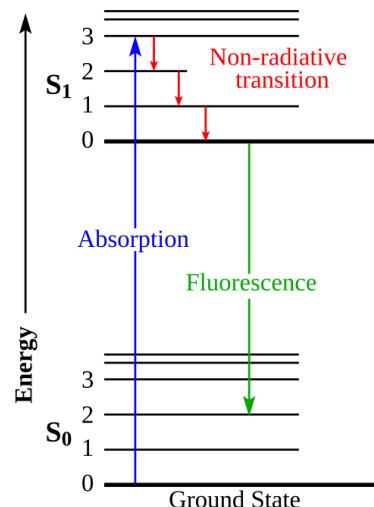


Figure 1: Jablonski Diagram (6)

The wavelength at which these photons are emitted is called the emission spectrum. Each band in the absorption spectrum will have a corresponding band in the emission spectrum (2). Samples which are illuminated by light with the same excitation spectrum will produce a different emission spectrum based on the molecular make-up of each material. In this way, emission spectra can be used to identify the basic components that a material is comprised of.

2.2 Fluorescence Spectrometer

It is possible to observe the emission spectrum of a sample using a fluorescence spectrometer. This device uses two monochromators - one for emission and one for excitation (figure 2). Within the fluorescence spectrometer, there is a light source which the excitation monochromator uses to illuminate the sample with specific wavelength bands throughout the specified range of wavelengths. Once the sample is illuminated, it presumably goes through fluorescence. This light is collected by the emission monochromator which is located at a 90° angle from the excitation monochromator. The emission monochromator also accepts only one specific wavelength band at a time. The light that is accepted will reach the photo-detector and then be recorded by the software (1).

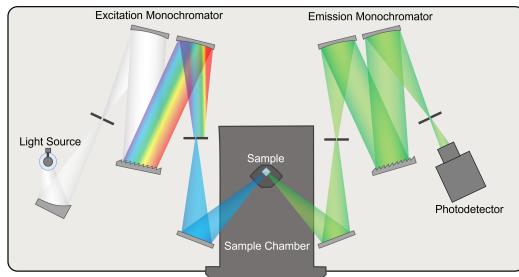
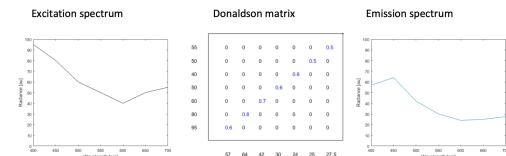


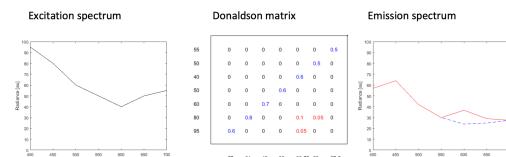
Figure 2: Inside of a fluorescence spectrometer (1)

2.3 Donaldson Matrix

The Donaldson matrix is a function of two wavelengths: the excitation wavelength and the emission wavelength of a certain material (5). The two wavelengths that make up the Donaldson matrix are the same wavelengths that are recorded from the excitation and emission monochromators. Therefore, the software associated with the fluorescence spectrometer will collect these data points and generate a Donaldson matrix to be used to analyze the samples. The Donaldson matrix can be used to generate a reflectance vs. wavelength plot. If the sample does not produce any fluorescence, the entries will be along the main diagonal of the matrix (figure 3(a)). However, if the sample does contain fluorescence, there will be additional non-zero entries below the main diagonal. This fluorescence will be obvious in the reflectance vs. wavelength plot because there will be bands visible at certain emission wavelengths which are due to the photon emission occurring at a lower energy (figure 3(b)).



(a) Donaldson matrix of a colored sample with no fluorescence



(b) Donaldson matrix of a colored sample with fluorescence

Figure 3: Excitation spectra, Donaldson matrix, and associated emission spectra of two colored samples (3).

3 Methodology

3.1 Material and Apparatus



(a) Samples under normal laboratory light



(b) Samples under UV light

Figure 4: Experimental Samples

Five samples were provided to the lab group with the information that all of the samples had been painted with either a fluorescent or non-fluorescent coating and two of the samples are coated with a mixture of two fluorescent coatings. The samples are shown in figure 4, both under normal laboratory lighting and under UV light.

The *PerkinElmer FL 8500* was the fluorescence spectrometer used to analyze the fluorescence of the aforementioned samples. The device is shown in figure 5. A 150 W Xenon Arc bulb is used within the device to provide the energy which excites the sample's electrons. This fluorometer is a highly accurate tool with precision up to 1.0 nm while measuring wavelengths up to 900 nm (4).

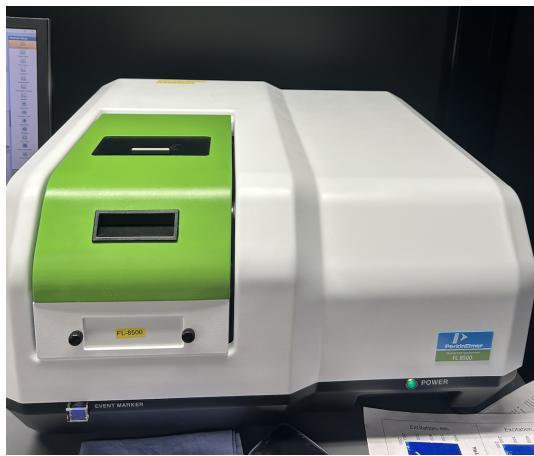


Figure 5: Fluorescence Spectrometer - *PerkinElmer FL 8500*

3.2 Procedures

This lab consisted of two tasks: deciphering which two of the five samples contain a mixture of two fluorescent paint colors, and calculating the emission spectra of the red colored sample (figure 6) when it is illuminated by CIE Standard Illuminant A and D65.

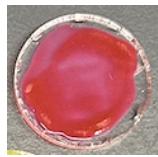


Figure 6: Red Sample

The associated software must be tuned with certain settings to ensure ample sensitivity without causing the data acquisition time to be too long. Specifically, the wavelength range and the number of scans must be ade-

quately set. The following settings were changed from the default to collect the spectral data of these samples:

- Method Setup - Spectral Scan
- Data Collection - Emission Scan
- Background Collection - Not Selected

Excitation

- Excitation Wavelength - 300 nm
- Excitation Slit Width - 10 nm
- Excitation Filter - Air

Emission

- Emission Start Wavelength - 380 nm
- Emission End Wavelength - 780 nm
- Emission Slit Width - 10 nm
- Scan Speed - 24000 nm/min

Acquisition

- Photomultiplier Voltage - 300
- Response Width - Auto
- Em. Corr - On
- Gain - x1

Scan Mode

- Scan Mode - 3D Scan
- Number of Scans - 41
- Excitation Increment - 10 nm

The settings under the **Accessory** tab were left as default. Additionally, it should be noted that the excitation and emission slit widths must be kept constant - in this case 10 nm. The scan mode should be set to 3D scan because this is the setting which corresponds to the creation of each sample's Donaldson matrix. These settings were then saved so they could be used for analysis.

Once the settings were tuned, one sample at a time was placed into the fluorescence spectrometer as seen in figure 7. The door must be completely shut before the data acquisition begins so that the sample is only illuminated by the wavelengths produced by the excitation monochromator. To begin collecting data, the '**Run**' button must be selected within the software. The data acquisition process took about 15-20 minutes for each sample. Following the process of cycling through each wavelength, the results were saved in the '**Previous Results**' page of the software then exported to the USB drive. The data collected from these trials were then used to complete both laboratory tasks.

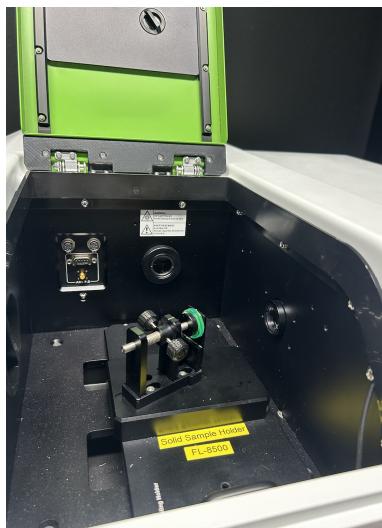


Figure 7: Sample inside of the fluorescence spectrometer

In order to complete the second task of this lab, the spectral information of both CIE Standard Illuminant A and D65 were provided.

4 Results

4.1 Donaldson matrices

A fluorescence spectrometer was used to analyze the five samples and obtain the corresponding Donaldson matrices - which are presented in the following subsections. The software saved the collected data as CSV files, which were processed and visualized using MATLAB to generate figures 8, 9, 10, 11, and 12.

4.1.1 Sample 1: Red

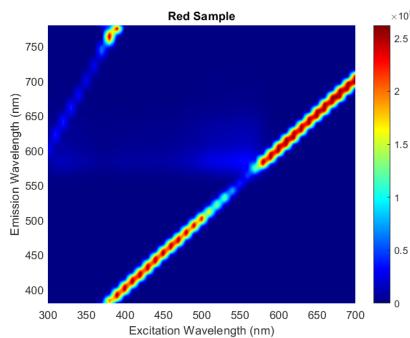


Figure 8: Donaldson matrix of Red Sample

All experimental samples exhibit fluorescence which is evident due to the horizontal band coming from the main di-

agonal in each Donaldson matrix. This band corresponds to the wavelength of light produced by emission when the sample is illuminated by the respective wavelengths. Figure 8 emits light from about 560 to 600 nm during fluorescence. These wavelengths correspond to orange light; therefore, this material emits orange light when its photons are released from the excited state.

4.1.2 Sample 2: Yellow

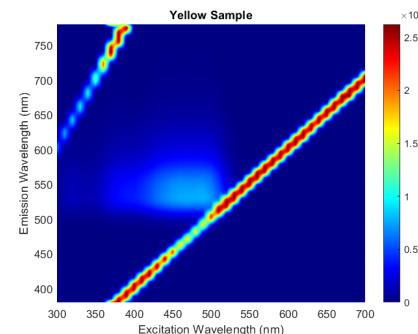


Figure 9: Donaldson matrix of Yellow Sample

Similarly, figure 9 depicts fluorescence over the wavelengths 520 to 560 nm. There are seemingly two peaks in this samples Donaldson matrix - one around 525 nm and another around 565 nm. For this reason, this sample conceivably consists of two different fluorescent paints. The range of the fluorescent emission wavelengths are primarily in the green range. Therefore, this sample will emit green light during fluorescence.

4.1.3 Sample 3: Green

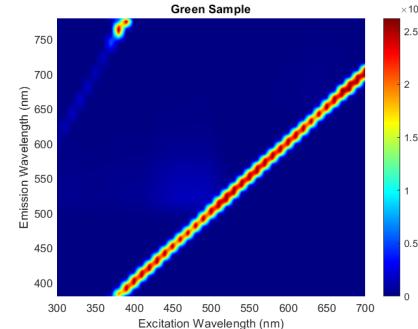


Figure 10: Donaldson matrix of Green Sample

The sample shown in figure 10 demonstrates much weaker fluorescence comparatively, but fluorescence still exists

between wavelengths of 500 and 550 nm. This sample which is visibly green under the laboratory lighting conditions will also emit green light during fluorescence.

4.1.4 Sample 4: Yellowish-Green

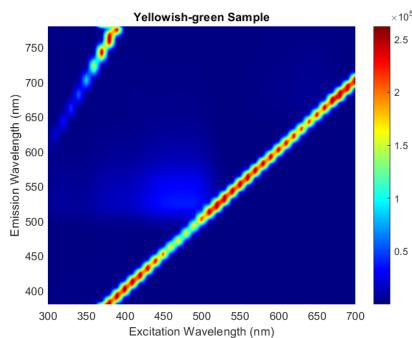


Figure 11: Donaldson matrix of Yellowish-Green Sample

Figure 11 is notably another one of the samples that is coated with a mix of two paint colors. The range of fluorescence of this sample is between 500 and 560 nm. The peak values of this sample's fluorescence occurs at a wavelength of about 520 nm and 550 nm. This range of fluorescent values suggests that paints containing the same pigments as the green and yellow samples are present.

4.1.5 Sample 5: Purplish-Blue

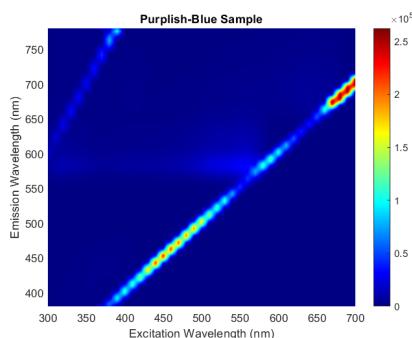


Figure 12: Donaldson matrix of Purplish-Blue Sample

Finally, the purplish-blue sample (as seen in figure 12) shows a band of fluorescence occurring around a wavelength of 575 nm. This sample emits light during fluorescence which is at the same wavelength as the light emitted by the red sample. This implies that the same paint that was used on the red sample was also used on this sample.

4.2 Emission Spectrum Analysis

Using the data recorded from the red sample, matrix multiplication with both the CIE A and CIE D65 standard illuminants (after inverting the array values to ensure accurate calculations) was performed to obtain the corresponding emission spectra for each illuminant.

4.2.1 Under illuminant A

The spectrum of standard illuminant CIE A under standard conditions prior to illuminating the red sample is shown in figure 13.

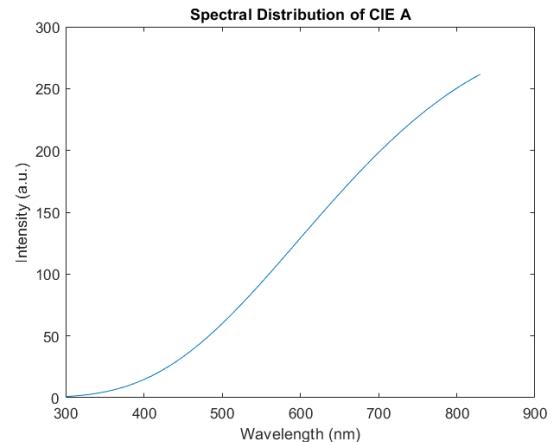


Figure 13: Spectral Distribution of CIE A

After illuminating the red sample, the resulting emission spectrum is shown in figure 14.

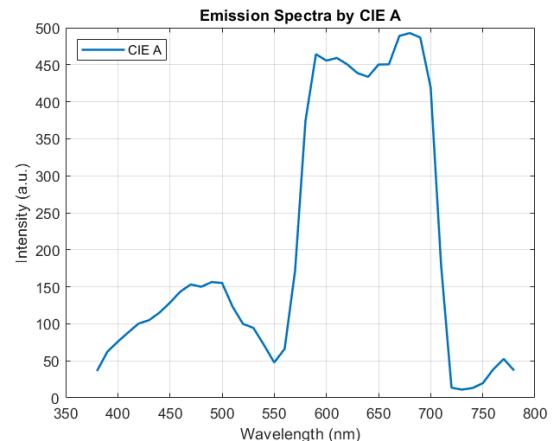


Figure 14: Emission Spectra by CIE A

4.2.2 Under illuminant D65

The spectrum of illuminant CIE D65 under standard conditions, prior to illuminating the red sample is shown in figure 15.

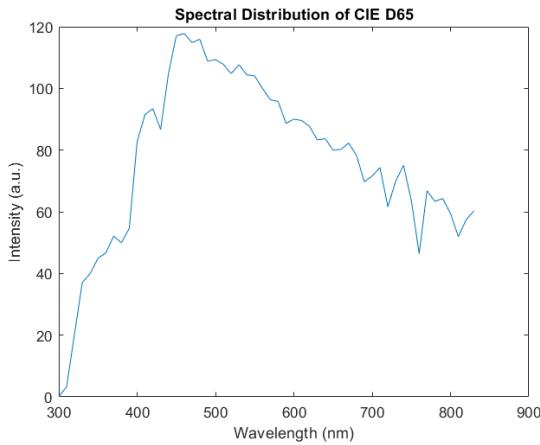


Figure 15: Spectral Distribution of CIE D65

After illuminating the red sample, the resulting emission spectrum is seen in figure 16.

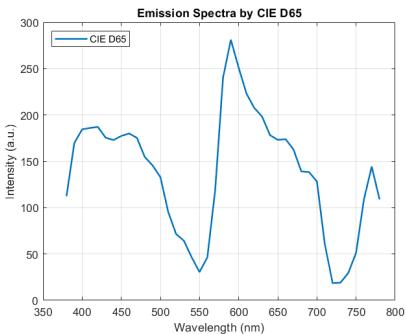


Figure 16: Emission Spectra by CIE D65

4.2.3 Comparison

Figure 17 displays both spectra prior to illumination, allowing for a clear comparison of their differences. Standard illuminant A has a positive relationship between intensity and wavelength while standard illuminant D65's intensity remains more constant across the entire wavelength spectrum.

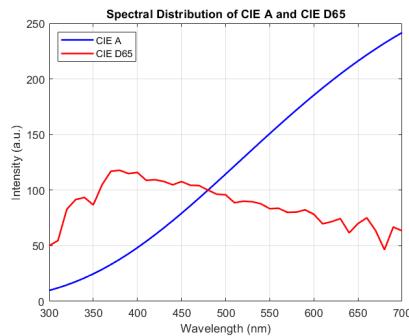


Figure 17: Spectral Distribution of CIE A and CIE D65

In figure 18, we observe that both spectra exhibit a similar emission pattern, though the intensity levels still differ.

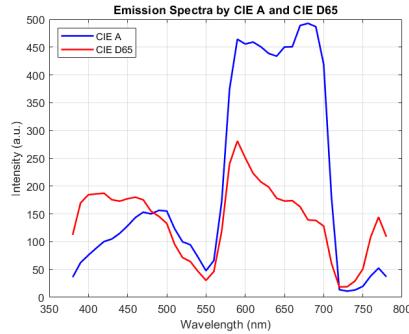


Figure 18: Emission Spectra by CIE A and CIE D65

5 Conclusion

This experiment successfully demonstrated the use of a *PerkinElmer FL 8500* fluorescence spectrometer to analyze and differentiate five fluorescent samples based on their excitation-emission characteristics. By capturing and analyzing the Donaldson matrices for each sample for a wavelength range of 380 to 780 nm, we were able to visualize their unique fluorescence profiles and identify the two samples that contained mixed coatings. By applying this analysis it was concluded that all five samples contained fluorescent pigmentation and the yellow and yellow-green samples both contained two different types of fluorescent paint.

Furthermore, by applying the Donaldson matrix of the red sample to standard illuminants CIE A and D65, we simulated and compared their respective emission spectra under these lighting conditions. Despite the similar spectral shapes under both illuminants, notable differences in intensity were observed, highlighting how illumination

conditions influence fluorescence behavior. Overall, this lab reinforced the value of Donaldson matrices in characterizing fluorescent materials and provided hands-on experience in processing and interpreting fluorescence spectral data.

References

- [1] Edinburgh Instruments. What is a fluorescence spectrometer?, 2024. Accessed: 2025-04-14. URL: <https://www.edinst.com/resource/what-is-a-fluorescence-spectrometer/>.
- [2] George G Guilbault. *Practical fluorescence*. CRC Press, 2020.
- [3] Hannu Laamanen. Fluorescence example. Class handout, University of Eastern Finland, 2025.
- [4] PerkinElmer. Fl 8500 fluorescence spectrophotometer. <https://www.perkinelmer.com/product/sys-fl-8500-analyzer-n4200031>, 2025. Accessed: 2025-04-29.
- [5] Shoji Tominaga, Keita Hirai, and Takahiko Horiuchi. Estimation of fluorescent donaldson matrices using a spectral imaging system. *Optics express*, 26(2):2132–2148, 2018.
- [6] Wikipedia contributors. Fluorescence. *Wikipedia*, The Free Encyclopedia, April 2025. Accessed: 2025-04-14. URL: <https://en.wikipedia.org/wiki/Fluorescence>.