Fluorescence Spectral Imaging

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Laboratory Practice Report

May 2018

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S. Alam, K. Oyemakinwa, A. Leinonen *Fluorescence Spectral Imaging*, 15 pages
University of Eastern Finland
Master's Degree Programme in Photonics

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Abstract

Fluorescent materials were used to study their response under the illumination of Ultraviolet (UV) light. However, direct exposure of UV light source can be very dangerous for human health if suficient protection is not taken. Three different samples namely fluorescent colored paper, fluorescent highlighter and make-up materials were measured for this purpose, and correction was done for camera sensitivity as well as non-uniform illumination.

The measured fluorescent colors corresponded to the peaks of their respective color spectra. The peak of the light source was found to be much lower than the peaks for fluorescent materials. Some errors were caused by low sensitivity of the camera in the UV region. This affected the corrections made to the data and created noise in the spectra but the effect of these errors were not significant.

Contents

Chapter I

Introduction

Emission of light by hot bodies could either be incandescent or luminescent. Incandescent emission is due to the high temperature of the body, all other forms are luminescent [1]. Luminescence occurs as a result of energy loss and the continuous luminescence can be achieved if there is a supply of energy which could either be internal or external depending on the radiation. It is desirable that the supply of energy be external and it can be achieved in different ways.

However, when the energy supply is due to the absorption of energy from either ultraviolet, visible or infrared light, the phenomenon is called photoluminescence which is divided into fluorescence and phosphorescence. The property of absorption of light at a certain wavelength and emission at a longer wavelength by atoms or molecules in a short interval is called fluorescence. [2] This short interval of time is called the fluorescence time on the contrary, in case of phosphorescence a longer excited state period is observed.

In general, the fluorescent process is characterized by three major stages: excitation of an atom or molecule caused by the absorption of photon which occurs in femtoseconds, rotational or vibrational relaxation of the excited state electron to its lowest energy state in picoseconds, and emission of a longer wavelength photon and the return of the atom or molecule to its ground state in nanoseconds [2]. The shift to a longer wavelength in the emission spectra was discovered by Sir George G. Stoke, hence the term Stokes shift, which will be discussed later in this study. Fluorescence is an interesting field of study still under investigation and a useful tool in genetics and cell biology.

In this study three samples were chosen and measured using a hyperspectral

camera with powerful ultraviolet light source. Different ways to correct the uneven irradiance of the light field and the shape of the measured spectral power distribution of the emitted light will be determined. Chapter 2 provides detailed information on fluorescence phenomenon, Donaldson matrix, and safety precautions in working under high powered UV light. In Chapter 3 the measurements, development of data correction will be presented, and the results will be discussed. Finally, in Chapter 5 the conclusions will be given.

Chapter II

Theory

In this Chapter fluorescence phenomenon will be presented. Also, the construction of the Donaldson matrix and the protection against UV radiation will be discussed.

2.1 Fluorescence

Fluorescence is a kind of Photoluminescence which is caused by the absorption of photon and resultant excitation of the specific materials in a certain wavelength region, namely ultraviolet or visible region. After some energy loss the material again goes to ground state by emission of electromagnetic radiation in the longer wavelength region. [1, 3]

Fluorescence phenomenon is described by the Jablonski diagram that is presented in Fig. 2.1. It is basically based on the excitation of electron from the ground state to higher energy state. The energy states are represented by S_0, S_1, S_2 while the vibrational energy levels are denoted by $1, 2, 3$ and 4. When absorption occurs, a transition of electrons from the lowest vibrational energy level of the ground state to the 1st vibrational energy level of state S_2 happens. After that, by loosing some energy the molecule goes to the lowest vibrational energy level of intermediate state S_1. This process may take 10^{-14} s to 10^{-11} s and is known as vibrational relaxation. This step can be followed by two different steps. If the transition occurs from S_1 to S_0 with the emission of photon then the process is called fluorescence. Another process known as phosphorescence or delayed fluorescence may take place if the molecule in the intermediate state S_1 goes to the lowest excited triplet state T_1 by transferring the energy with a neighboring molecule and it is called inter system crossing. If the excitation energy is greater than the emission energy, which means

the wavelength of the emitted energy is greater than the wavelength of the excitation energy, the difference between two maxima is then called Stokes shift. However, if the emitted energy wavelength is less than the excitation energy wavelength then the difference between two maxima is called anti Stokes-shift.

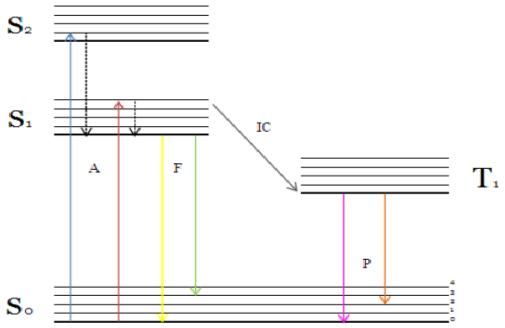


Figure 2.1: Jablonski diagram. [3]

Reflectance is a measure of appearance of object color. In case of fluorescent material total reflectance is the sum of reflectance and fluorescence and can be written as

$$R_T(\mu, \lambda) = R_R(\lambda) + R_L(\mu, \lambda), \tag{2.1}$$

where $R_R(\lambda)$ is reflectance and $R_L(\mu, \lambda)$ is fluorescence, μ is excitation wavelength, and λ is emission wavelength. [3]

2.2 Donaldson Matrix

Donaldson matrix $D(\lambda_{em}, \lambda_{ex})$ is a function of two different wavelengths: excitation wavelength λ_{ex} and emission wavelength λ_{em}. This is also called Bispectral radiance factor. Donaldson matrix is the sum of two factors: reflected radiance factor $D_R(\lambda_{em}, \lambda_{ex})$ and luminescent radiance factor $D_L(\lambda_{em}, \lambda_{ex})$. It is represented as

follows:

$$
\begin{aligned}
D &= D_R + D_L \\
&= \begin{bmatrix} 0 & \cdots & 0 & S_1 & 0 & \cdots & 0 \\ 0 & & 0 & 0 & S_2 & \ddots & \vdots \\ \vdots & & \vdots & \vdots & \ddots & \ddots & 0 \\ 0 & \cdots & 0 & 0 & \cdots & 0 & S_N \end{bmatrix} \\
&\quad + \begin{bmatrix} \alpha_1\beta_1 & \cdots & \alpha_1\beta_{M-N} & 0 & 0 & \cdots & 0 \\ \alpha_2\beta_1 & & \alpha_2\beta_{M-N} & \alpha_2\beta_{M-N+1} & 0 & \ddots & \vdots \\ \vdots & & \vdots & \vdots & \ddots & \ddots & 0 \\ \alpha_N\beta_1 & \cdots & \alpha_N\beta_{M-N} & \alpha_N\beta_{M-N+1} & \cdots & \alpha_N\beta_{M-1} & 0 \end{bmatrix} \\
&= \begin{bmatrix} \alpha_1\beta_1 & \cdots & \alpha_1\beta_{M-N} & S_1 & 0 & \cdots & 0 \\ \alpha_2\beta_1 & & \alpha_2\beta_{M-N} & \alpha_2\beta_{M-N+1} & S_2 & \ddots & \vdots \\ \vdots & & \vdots & \vdots & \ddots & \ddots & 0 \\ \alpha_N\beta_1 & \cdots & \alpha_N\beta_{M-N} & \alpha_N\beta_{M-N+1} & \cdots & \alpha_N\beta_{M-1} & S_N \end{bmatrix}
\end{aligned}
\tag{2.2}
$$

From the matrix representation of Eq. (2.2) it becomes clear that the reflected radiance factor $D_R(\lambda_{em}, \lambda_{ex})$ is diagonal and when $\lambda_{em}= \lambda_{ex}$ it has values. The reason is that incident light has the same wavelength as the reflected monochromatic light. However, as we described earlier, in Stokes shift the emission wavelength is greater than excitation wavelength in case of luminescent energy. So, luminescent radiance factor $D_L(\lambda_{em}, \lambda_{ex})$ has value when $\lambda_{em} > \lambda_{ex}$ is satisfied. $D_L(\lambda_{em}, \lambda_{ex})$ is represented as the product of emission $\alpha(\lambda_{em})$ and excitation $\beta(\lambda_{em})$ spectra: $D_L(\lambda_{em}, \lambda_{ex}) = \alpha(\lambda_{em})\beta(\lambda_{em})$. $S_i(i = 1, 2, ..., N)$ represent the discrete reflectance spectra. [4]

2.3 Ultraviolet radiation

Ultraviolet (UV) radiation is a part of the electromagnetic spectrum defined to be the wavelength range of $100 - 400$ nm. UV is divided into three different ranges: UV-C at $100 - 280$ nm, UV-B at $280 - 315$ nm, and UV-A at $315 - 400$ nm. Most of the UV light reaching earth is UV-A, since all of the UV-C and $90 - 95\%$ of UV-B are absorbed by the atmosphere. [5]

UV radiation can be harmful especially to skin and eyes. This is due to the short wavelength of the radiation damaging the cells on the first layers of the skin and penetratingdeeper inside. Penetration causes damage to the cells deeper inside the body by genetic mutation in the DNA. This damage causes sunburn and possibly even skin cancer. Although sunburn sounds like the damage is caused by the heat radiation which is incorrect since UV radiation only damages the cells it interacts with. Similarly UV radiation incident on the eye will damage the Cornea and other cells inside the eye. Exposure of UV radiation may cause irritation, light sensitivity, and tearing in eyes, which can be felt within 30 minutes. Long exposure to high amounts of UV radiation may cause permanent retinal damage. [5]

A good way to protect against UV radiation is to wear black clothes and a protective mask on the face. Clothing should be thick and dark to absorb UV radiation in order to prevent damage on skin. Also, gaps between clothing such as gloves and pants, should be avoided. If a high power UV light source is used a protective shield between user and light source should be used at all times. It should also be made sure that there are no surfaces which reflect light in UV region or extra personnel in the vicinity of the source. By taking these precautions most of the harmful effects of UV radiation can be avoided completely. [5]

Chapter III

Measurements and calculations

In this Chapter, the procedures and precautions taken to acquire the data from samples and a detailed explanation of how corrections for the UV light and sensitivity of camera will be discussed.

3.1 Measurement procedure

The experiment was conducted using a Line Scanning Spectral Imaging System. It includes a scanner 1×1.5 m and a visible V10 camera with a spectral range of $400 - 1000$ nm which scan samples in the visible region. Ultraviolet radiation was used to measure the spectral information of three different fluorescent samples. The UV light source was an LED with a 365 nm wavelength. Samples used were a drawing on white paper made with fluorescent highlighter, pieces of different color fluorescent papers, and two make-up boxes. The system was calibrated to measure the spectral power distribution in uniform illumination under halogen light. The used UV light source was placed at a fixed position. The light was inclined at an angle close to the movable board where measurements would be taken. This was achieved by using a clamp setup. Samples were positioned so that it was close to the UV light source when measurements were taken.

As discussed earlier, before measurements UV protection was taken for personnel who worked with the device. For this purpose, thick, black clothing and specialized UV protecting visors were used. Focusing of the camera and software controlling the device had been properly set before the measurements were taken. Each sample was scanned separately. Due to low intensity of the UV light source the exposure time was increased considerably to get sufficient data from the samples. The experiment

was carried out in a dark room to avoid samples being exposed to other light sources.

3.2 Data correction

As a result of the measurement set-up described previously, it was seen that there was non-uniformity in the spectral power distribution of the samples. Hence, a need for corrections to be made on the data to get a uniform spectral power distribution was realized.

The spectral power distribution of the white reference and the samples were stored in a spectral cube containing three dimensional pixel information (x, y, λ). This information contained the transmission sensitivity of the camera under halogen light. Corrections were done by dividing the measured halogen spectra by the real halogen spectra. The real spectra for halogen was calculated from Planck's radiation law for a temperature of $3250K$. The transmission sensitivity of camera can be represented as

$$TP = \frac{A}{B}. \tag{3.1}$$

Where TP is the transmission sensitivity of the camera, A is the measured data and B is the halogen spectra calculated by the Planck's radiation law. The calculated spectra is presented in Fig. 3.1.

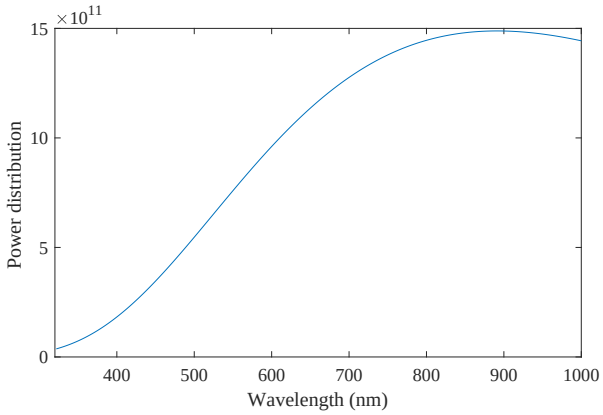


Figure 3.1: The spectra of halogen light calculated for a temperature of 3250K.

The correction factor D was calculated by taking the reciprocal of the normalized

camera sensitivity TP_{norm} over the spectral range.

$$D = \frac{1}{TP_{norm}}. \tag{3.2}$$

Spectral power distribution of the UV light located in a spectral cube had also been obtained for the samples measured. Corrections for this were also done by dividing each pixel by the maximum value in the spectra as described by the equation below:

$$V_{norm} = \frac{V_i}{V_{max}}. \tag{3.3}$$

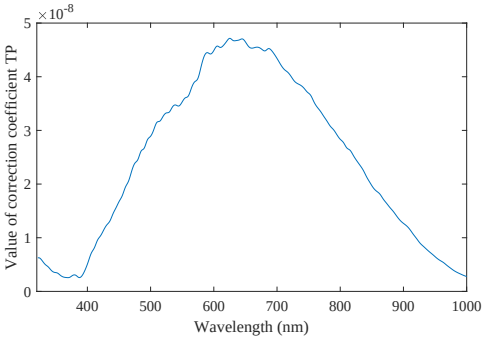
Where V_i specifies each pixel ranging from the first pixel to the nth pixel and V_{max} is the pixel with the highest value in the spectra. The correction factor was also calculated by taking the reciprocal of the normalized V over the spectral range. This can be represented as

$$C = \frac{1}{V_{norm}}. \tag{3.4}$$

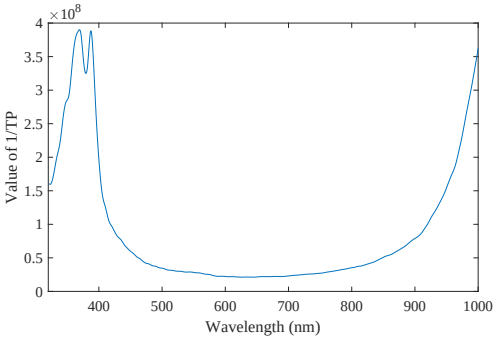
From corrections calculated for the samples, the overall correction factor k was then calculated by taking the product of C and D: $k = C \times D$. The actual spectra of the measured samples are then calculated by multiplying the correction factor k with the measured samples.

Figs. 3.2(a) and 3.2(b) show the correction for the cameras sensitivity. One can clearly see from these figures that the sensitivity is poor in the UV and IR region. Wihtout applying corrections the light source would be poorly visible on the spectrum.

The correction for non-uniform UV light is presented in Fig. 3.3. The light source is located near the pixel 0 which is located at the top of the figure. It can be clearly seen that the peak of the UV light is located at 365 nm, as expected. The region which has higher value is the region to be corrected since the reciprocal value was taken, as stated in Eq. (3.4).



(a) The sensitivity of the camera TP.



(b) Reciprocal of the sensitivity TP.

Figure 3.2: Correction for the sensitivity of the camera.

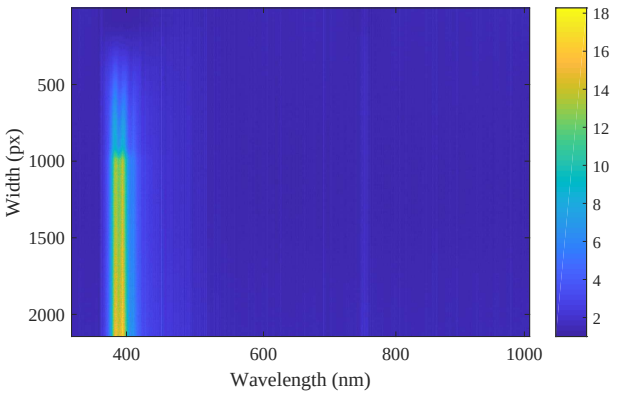
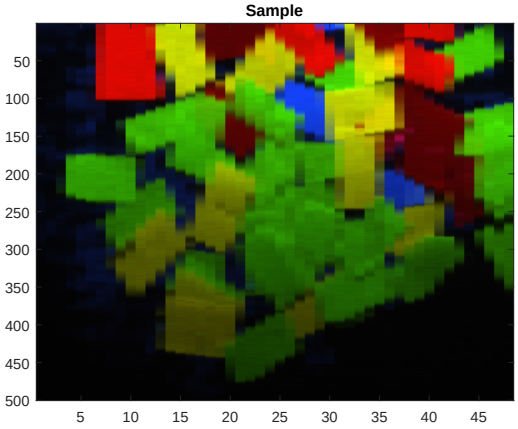


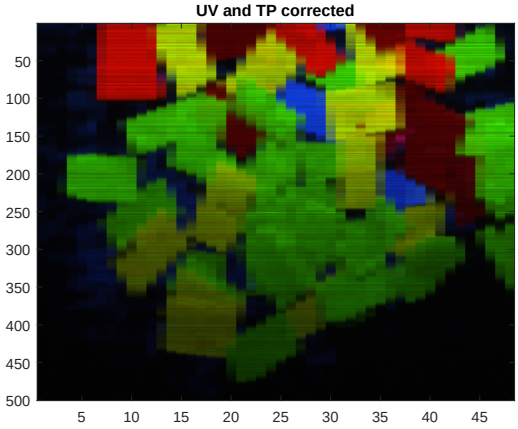
Figure 3.3: The correction coefficient C as a function of width and the wavelength.

3.3 Results

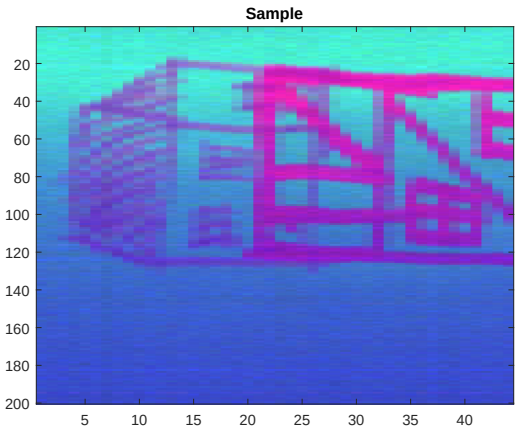
The corrections worked as expected but some errors can be spotted from Figs. 3.4(a) to 3.4(d) and in Figs. 3.5(a) and 3.5(b). These errors are mostly seen as horizontal lines in the corrected images but their overall effect is small. Some noise can also be seen in these images, especially in Fig. 3.5(d). This noise may be caused by the combination of low intensity of the light and the poor sensitivity of sensors in the UV region.



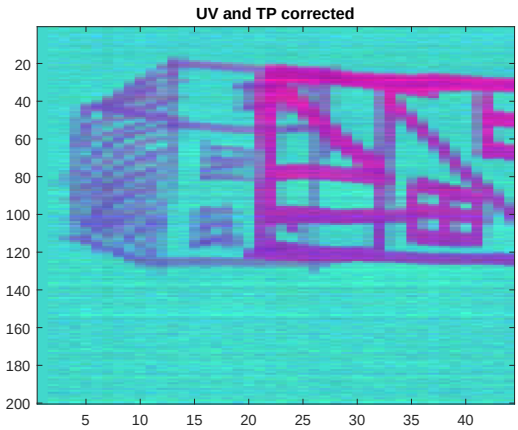
(a) RGB presentation of measured fluorescent papers.



(b) RGB presentation of corrected fluorescent papers.

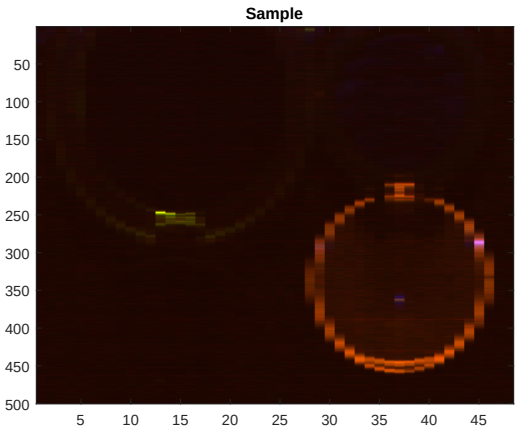


(c) RGB presentation of measured highlighter.

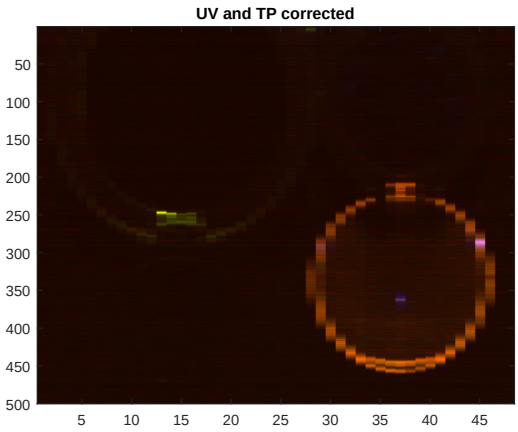


(d) RGB presentation of corrected highlighter.

Figure 3.4: Fluorescent paper and highlighter samples used in the measurement. These pictures are RGB presentations of the fluorescence measurements.



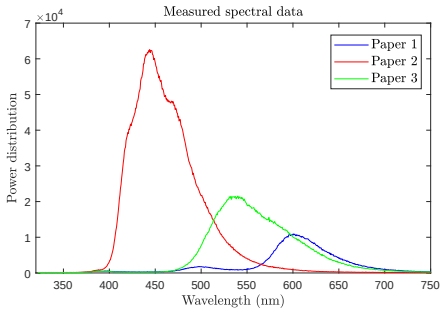
(a) RGB presentation of measured makeup samples.



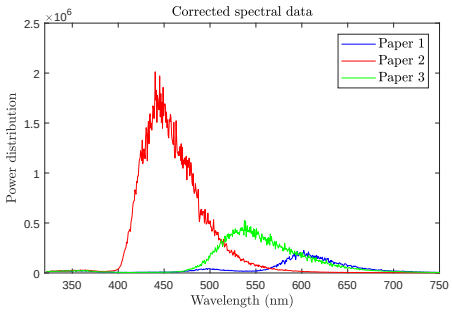
(b) RGB presentation of corrected makeup samples.

Figure 3.5: Makeup samples used in the measurement. These pictures are RGB presentations of the fluorescence measurements.

The spectra of the fluorescent papers of Figs. 3.5(a) and 3.5(b) are presented in Figs. 3.6(a) and 3.6(b). The peak of the light source can not be seen in the measured Fig.3.6(a) but a tiny peak can be spotted in the corrected Fig. 3.6(b). These peaks caused by the fluorescence seem to correspond to their respective colors nicely. Some noise can be seen in the corrected data which was most likely caused by the correction and low sensitivity of the camera in UV region.



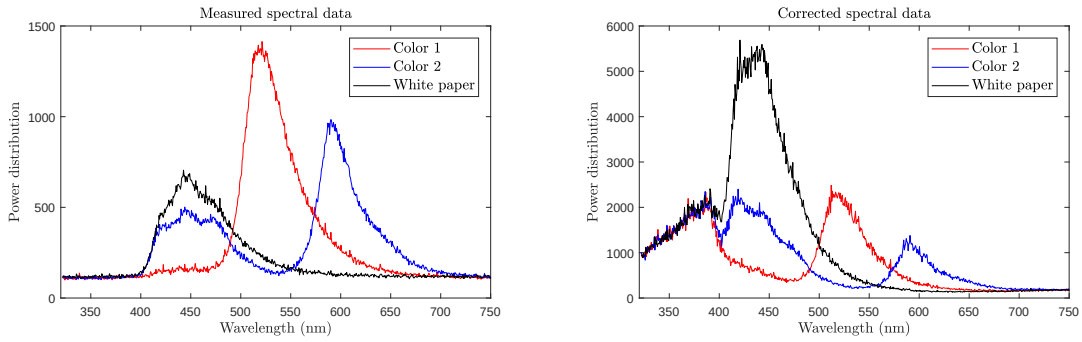
(a) Spectra of the measured data.



(b) Spectra of the corrected data.

Figure 3.6: Spectra of fluorescent papers presented in Figs. 3.4(a) and 3.4(b). Paper 1 corresponds to the blue color, paper 2 to the red color, and paper 3 to the green color.

In the case of the highlighter seen in Figs. 3.4(c) and 3.4(d). The fluorescence peaks of spectra shown in Figs. 3.7(a) and 3.7(b) seem to correspond nicely with their respective colors. By comparing these two figures the spectral data shows the effect of the sensitivity of the camera nicely. By correcting the sensitivity the peak of the light source becomes visible but the intensity is much lower than the fluorescent peaks. Intrestingly, the fluorescent peak of the white paper gains much power after corrections but the other peaks do not gain as much. Again the effect of the noise is visible in the corrected Fig. 3.7(b), especially in the UV region where the sensitivity of the camera is low.

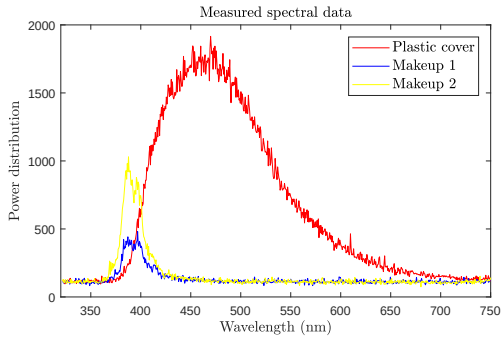


(a) Spectra of the measured data.

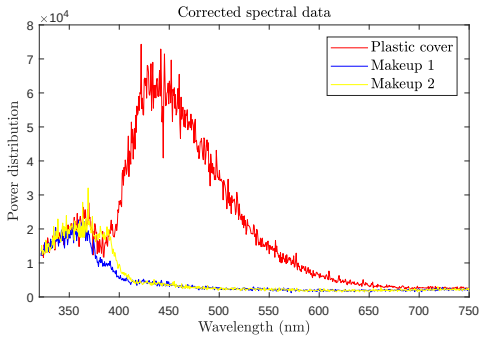
(b) Spectra of the corrected data.

Figure 3.7: Spectra of highlighter presented in Figs. 3.4(c) and 3.4(d). Color 1 presents the purple color and color 2 presents the dark purple from Figs. 3.4(c) and 3.4(d).

The makeup measurements shown in Figs. 3.5(a) and 3.5(b) also have noise in the spectrum after corrections. The spectras are presented in Figs. 3.8(a) and 3.8(b). Once again the peak of the light source can be seen after correcting the data. When choosing the measurement samples the makeup was thought to be fluorescent but the spectra confirms that the material was not fluorescent, although the plastic covers were found to be fluorescent.



(a) Spectra of the measured data.



(b) Spectra of the corrected data.

Figure 3.8: Spectra of makeup and the plastic cover presented in Figs.3.5(a) and 3.5(b).

Chapter IV

Conclusions

The concept of fluorescence was studied in this practice. Three samples namely fluorescent colored paper, fluorescent highlighter, and make-up materials were studied under UV light. Suffient protection was taken for working under UV. The used UV light source illumination was not uniform for the samples, therefore correction was done for this purpose. Also, sensitivity of camera was corrected to get the overall correction by dividing the measured spectra with the actual spectra calculated from the Planck's law.

Different peaks were observed for different colors as expected and it was found that the peak of the light source was much lower than the fluorescent colors. The appearance of horizontal lines in the corrected pictures was due to the correction and some errors in calculations. The noise in the pictures is due to combination of low intensity of light and sensors poor sensitivity in the UV region. It was found that fluorescent paper was the most fluorescent sample. Measured make-up materials were not fluorescent, which was unexpected, but the plastic covers of the boxes were found to be fluorescent.

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