Simulated Abridged Spectral Fluorescence Imaging

Mahnaz Mohammadi and Roy S. Berns

Munsell Color Science laboratory, Chester F. Carlson Center for Imaging Science, Rochester Institute of Technology, Rochester, New York, USA

Abstract

The true spectral reflectance of a series of fluorescent paints was reconstructed using an abridged method proposed by Allen and adopted for imaging using a multi-channel digital camera. The reconstructions were simulated using a "virtual" camera. The performance of the system was highly dependent upon the two sharp short–wavelength cutoff filters known as fluorescence-weakening and -killing filters. The purpose of these filters was reducing and removing the amount of luminescence emerging from a fluorescent sample, respectively. The performance of the theory to reconstruct the true reflectance in a spectral region where both reflectance and luminescence occur simultaneously was dependent upon an independent-wavelength constant. The results show that the Allen method is most effective for samples that highly fluoresce.

I. Introduction

The measured spectral radiance of a fluorescent sample using a spectrophotometer with polychromatic illumination and monochromatic detection is a curve that consists of reflected and luminescent components,

$$\beta_T(\lambda) = \beta_S(\lambda) + \beta_L(\lambda) \tag{1}$$

where $\beta_T(\lambda)$, $\beta_S(\lambda)$, and $\beta_L(\lambda)$ are the total, reflectance, and luminescence radiance factors, respectively. Since the luminescence radiance factor of a fluorescent sample is illuminant dependent, the total radiance factor is also dependent upon the illuminant.

In order to accurately measure the reflected and luminescence components, a bispectrometer is used, having independently controlled monochromatic illumination and detection [1]. An abridged method to separate the two components is also a technique proposed in the literature [2, 3, 4]. Allen [2] introduced an abridged method using just two sharp short-wavelength cutoff filters to calculate the true reflectance (reflected radiance factor). The idea behind his theory is reducing the luminescence in the overlap region, in which both excitation and emission occurs at the same or shorter wavelengths, the anti Stokes shift [5], and excluding all the excitation wavelengths to measure the true reflectance in wavelengths larger than the emission range. These two sharp short-wavelength cutoff filters are called fluorescence-weakening and fluorescence-killing filters.

The Allen idea was employed in spectral imaging of a series of fluorescent paints. The samples were the Golden Acrylics Fluorescent paints containing blue, green, magenta, orange, red, and yellow colorants. A set of fluorescence-weakening and -killing filters was simulated using a cubic-spline function for each fluorescent paint based on Allen theory. The optimized filters were used in a simulated abridged spectral fluorescence imaging system to estimate the true spectra reflectance. The calibration target was the GretagMacbeth ColorChecker Rendition Chart (CC). A SVD-based pseudo inverse technique was used to derive a transformation matrix to transform simulated digital counts to true spectral reflectance. The spectral accuracy of the method was analyzed by root mean square error (RMS%) between the estimated and the measured true reflectance by a Labsphere 450 bispectrometer.

II. Theory of the Method

Calculation Method

In the calculation method proposed by Allen [1], the total radiance factor under three different illuminants should be measured. The first illuminant can be any desired one but preferably a daylight source. The second should be the same source with a sharp short-wavelength cutoff filter in front of it and the third should also be the same source but using a sharp short-wavelength cutoff filter with longer cutoff wavelengths. These filters are called fluorescence-weakening and fluorescence-killing filters, respectively.

To derive a formula to calculate the true spectral reflectance, Eq. 1 is rearranged as the following for the total radiance factor under illuminant no. 1,

$$\beta_{T1}(\lambda) = R_S(\lambda) + \frac{F(\lambda)}{E_1(\lambda)} \tag{2}$$

where $F(\lambda)$ is the true emission, which is illuminant independent, $E_1(\lambda)$ is the spectral power distribution of illuminant no. 1, and $R_S(\lambda)$ is the true reflectance. The corresponding formula for illuminant no. 2 is

$$\beta_{T2}(\lambda) = R_S(\lambda) + \frac{KF(\lambda)}{E_2(\lambda)} = R_S(\lambda) + \frac{KF(\lambda)}{E_1(\lambda).\tau(\lambda)} \tag{3}$$

Here, K is an independent-wavelength constant, which reduces the true emission, $F(\lambda)$, using illuminant no. 2 and $\tau(\lambda)$ is the transmittance of the fluorescence-weakening filter. Solving for $R_S(\lambda)$ by eliminating $F(\lambda)/E_1(\lambda)$ between Eq. (2) and (3) gives,

$$R_S(\lambda) = \frac{[\beta_{T2}(\lambda)\cdot\tau(\lambda) - \beta_{T1}(\lambda)\cdot K]}{[\tau(\lambda) - K]} \tag{4}$$

The constant K is derived from Eq. (4) as,

$$K = \frac{\tau(\lambda_k)[\beta_{T2}(\lambda_k) - R_S(\lambda_k)]}{[\beta_{T1}(\lambda_k) - R_S(\lambda_k)]} \tag{5}$$

where λ_k is a wavelength, in which the true reflectance can be discerned using the fluorescence-killing filter at this wavelength. A possible λ_k for the Golden Acrylic Orange is shown in Figure 2, schematically.

Based on Allen's theory, the spectrum is divided into three regions: part I, part II, and part III. These regions are shown in Figure 1. In part I, where no emission occurs, the detected radiance with no filter corresponds to the spectral true reflectance. In part III, the measured radiance using the fluorescence-killing filter in the excitation path is the true reflectance. The reason is that the fluorescence-killing filter excludes all the excitation wavelengths and there would be no energy reaching the sample that might excite luminescence. The true reflectance can be calculated in part II using Eq. (4) with having K solved in Eq. (5).

Simulated Abridged Fluorescence Imaging

The idea of the Allen method was employed in fluorescence imaging. Simulated abridged fluorescence imaging was based on a high performance Roper Scientific, Inc. Photometric Quantix 6303E that uses a Kodak blue enhanced KAF6303E CCD, a set of six glass colored filters optimized for the best colorimetric and spectral performance [6], and a set of fluorescence-weakening and -killing filters for each fluorescent paint. The spectral sensitivity of the camera was measured previously [6,7]. The calibration target was the GretagMacbeth ColorChecker Rendition Chart (CC) and the fluorescent samples were the Golden Acrylics Fluorescent paints. Illuminant CIE D65 was used in all computations. The digital counts for all pixels of the calibration target with known reflectance factor was computed as,

$$D_i = \sum_{\lambda=380}^{750} R(\lambda) \cdot \phi(\lambda) \cdot S(\lambda)_i + \eta$$
$$\phi(\lambda) = E(\lambda) \cdot \tau(\lambda) \tag{6}$$

where λ is wavelength, D_i is the digital count of the i*th* channel, $R(\lambda)$ is spectral reflectance factor of a pixel, and ϕ is the spectral power distribution of the desired illuminant. Having an excitation filter with transmittance of $\tau(\lambda)$ in front of the illuminant, CIE D65, results in a new illuminant, $\phi(\lambda)$. The S_λ, is the spectral sensitivity of the camera for the i*th* channel combined with the transmittance of the colored filters, an IR cut-off filter, and any desired excitation and emission filters. A noise coefficient, η, was generated consisting of 50 values with normal distribution and zero mean value and standard deviation of 2.5% of the digital count of each patch of the calibration target. Based on a singular value decomposition technique [7], a transformation matrix, **T**, converting digital counts to reflectance values was derived for each part of the spectrum defined as above. It means that for part I, with no emission, no short-wavelength cutoff filter should be in the excitation path and a sharp long- wavelength cutoff filter should be used as an emission filter in front of the camera for excluding all the emissions. The simulated digital counts would be related to the true spectral reflectance in part I. In part III, the fluorescence-killing filter was used in this excitation path to remove all the excitation wavelengths reaching the sample. All the simulated signals by this configuration would correspond to the true spectral reflectance in part III. Using the fluorescence-weakening filter in front of the illuminant would reduce the amount of emission but not exclude all. The true spectral reflectance in the overlap region can be calculated using this excitation filter. Therefore, three transformation matrices for the three parts of the spectrum were derived.

The same excitation and emission filters described above were used to simulate the digital counts for the fluorescent samples. Since employing the fluorescence-weakening and -killing filters in the excitation path means changing the illuminant, the spectral luminescence would vary under these illuminants. Therefore, Eq. (6) is not valid for the fluorescent samples. In this case, the luminescence under illuminants nos. 2 and 3 should be calculated. The inner summation in Eq. (7) is the emerged total radiance from a fluorescent sample under a new illuminant.

$$D_i = \sum_{380}^{750} \left(\sum_{300}^{750} \left(D_T(\mu,\lambda) \cdot \phi(\mu) \right) \right) \cdot S(\lambda)_i + \eta \tag{7}$$

where, $D_T(\mu,\lambda)$ is the Donaldson total radiance factor matrix [2], measured with a bispectrometer (the Labsphere 450), μ and λ are the excitation and emission wavelengths. The other terms are the same as before.

Digital counts for each part of the spectrum for the fluorescent samples were calculated using Eq. (7). By having the transformation matrix and digital counts of the fluorescent sample for each part of the spectrum, one can estimate the true reflectance of the sample according to Eq. (8),

$$\hat{\mathbf{R}} = \mathbf{T} \cdot \mathbf{D} \tag{8}$$

where, $\hat{\mathbf{R}}$ is the estimated true reflectance matrix-vector, **D** is the digital count matrix, and **T** is the transformation matrix.

The constant K was calculated from Eq. (5) based on the estimated total radiance under non-filtered and filtered illuminants at λ_k. Once K is known, the true reflectance in the overlap region is calculated using Eq. (4). In order to select λ_k, the true reflectance can be calculated at each wavelength longer than the emission peak and the wavelength with the best spectral performance would be a proper wavelength. Usually a very long wavelength should not be chosen because the total radiance with and without any excitation filter becomes too similar and the precision of the calculation would be reduced. The same reason makes Allen's method difficult to employ for samples with low luminescence.

III. Results and Discussion

In order to develop a spectral fluorescence imaging system based on Allen's theory, it was assumed that a virtual spectroradiometer was, at first, the device detector. For each fluorescent paints, a set of fluorescence-weakening and -killing filters was simulated using the cubic-spline function. The true spectral reflectance of the fluorescent paints was estimated using Allen's method. The optimized excitation filters for the Golden Acrylic Fluorescent Orange along with the excitation and emission spectra of the sample are shown in Figure 1. As can be seen, the fluorescence-killing filter excludes almost all the excitation wavelengths, which might excite luminescence in the visible region. The fluorescence-weakening filter reduces the emission by removing some excitation wavelengths. The cross region between the cyan and the green spectra in Figure 1 is the overlap region. Since the true reflectance and emission cannot be separated completely in this region, Allen's method would be a proper technique to be employed for this purpose. The calculated total radiance factor of the Golden Acrylic Orange with and without the excitation filters is plotted in Figure 2. As it was expected, the fluorescence-weakening filter reduced the amount of fluorescence but the fluorescence-killing filter excluded all the excitation wavelengths and the calculated total radiance was close to the true reflectance at wavelengths longer than the emission peak.

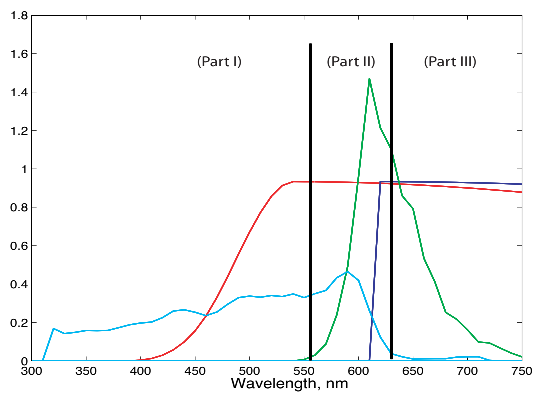

Figure 1. Emission (green line) and excitation (cyan line) spectra of Golden Acrylic Orange along with the optimized excitation filters; the red line is transmittance of the fluorescence-weakening filter, and the blue line is transmittance of fluorescence-killing filter.

Figure 3 shows the spectral performance of the Golden Acrylic Orange using the described algorithm. The accuracy of the method to calculate the true reflectance in the overlap region can be seen in Figure 3. The noise in measuring the total radiance factor using the Labsphere 450 makes a slight difference between the total radiance factor and true reflectance at the short wavelengths.

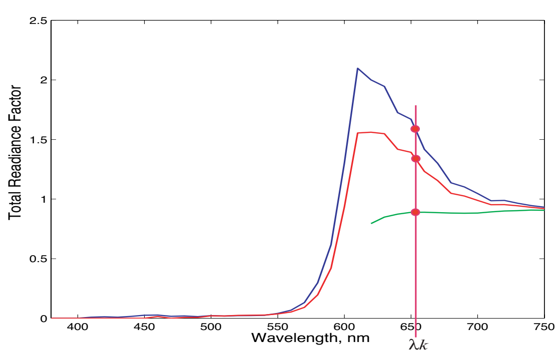

Figure 2. Total radiance factor of Golden Acrylic Orange with and without excitation filters; the blue line is without the filter, the red line is with the fluorescence-weakening filter, and the green line is with the fluorescence-killing filter.

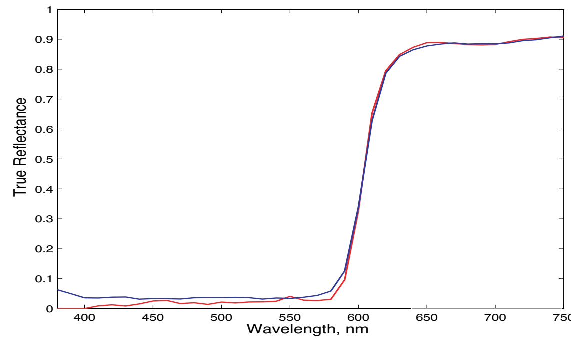

Figure 3. True reflectance of Golden Acrylic Orange; the blue line is measured with the Labsphere 450 bispectrometer and the red line is reconstructed using Allen's method.

The same performance was seen for the other fluorescent paints. In the case of the fluorescent blue paint, which has slight luminescence and a small overlap region in the short wavelengths (Figures 4 and 5), Allen's theory was not a very effective method to calculate the true reflectance. The reason is that the total radiance factors for non-filtered and filtered illuminants are not significantly different. Hence, the K value would be close to the throughput of the fluorescence-weakening filter at λ_k. On the other hand, since the fluorescent blue paint does not fluoresce highly as shown in Figure 4, the fluorescence-weakening filter cannot be very effective in reducing the luminescence. This fact can be seen in Figure 5. Two different fluorescence-weakening filters with the same fluorescence-killing filter were selected to reconstruct the true reflectance. The fluorescence-weakening filter shown in the first row of column (a) could cut off the very short wavelengths and most of the excitation wavelengths could excite the luminescence but the one shown in column (b) could cut more excitation wavelengths. These filters were selected as the extreme possible fluorescence-weakening filters. The RMS% spectral error between the reconstructed true reflectance using these two different filters and the measured one are 2.42% and 1.36%, respectively. The spectral performance using the other filters between these two selected filters are within this range. This result demonstrates that the fluorescence-weakening filter does not significantly affect the spectral performance for low luminescence samples such as the fluorescent blue paint.

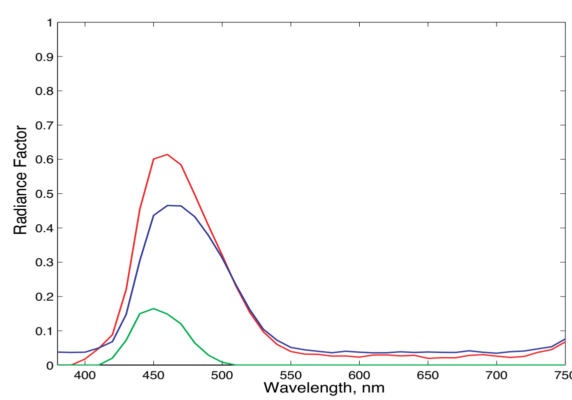

Figure 4. Radiance factor of Golden Acrylic Blue; the red, blue, and green lines are total, reflectance, and luminescence radiance factors, respectively.

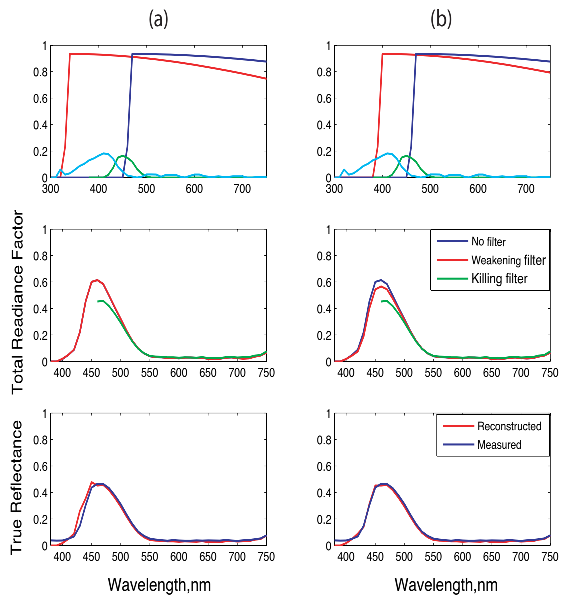

Figure 5. Emission (green line) and excitation (cyan line) spectra of Golden Acrylic Blue along with the different excitation filters; the red and blue lines are transmittance of fluorescence-weakening and -killing filters, respectively, in the first row. In the second row, the total radiance factor with and without excitation filters; the blue line is without filter, the red line is with the fluorescence-weakening filter, and the green line is with the fluorescence-killing filter. The third row is the true reflectance.

The selected fluorescence-weakening and -killing filters for each of the paints were used in the fluorescence imaging system. For the region where no emission occurred, no filter was employed in the excitation path and a sharp long-cutoff filter was used in the emission path to exclude all the radiance above this region. Each of the excitation filters was used in the path between the illuminant, CIE D65, and the fluorescent sample. The total radiance in the pass band of each filter was simulated separately. A transformation matrix for each described region was derived using Eq. (6) for the GretagMacbeth Color Checker Rendition chart as a calibration target. The digital counts corresponding to the total radiance factor were simulated for all the fluorescent paints based on Eq. (7). The true reflectance in each region was estimated using the derived transformation matrix and the simulated digital counts by Eq. (8). The *K* value based on the estimated total radiance using the fluorescence-weakening and-killing filters and also with no filter illumination was calculated for each of the paints. The true reflectance in the overlap region was calculated using the *K* value. The estimated total radiance at wavelengths longer than the cutoff wavelength of the fluorescence-killing filter would correspond to the true reflectance in this region and the estimated total radiance corresponding to the region less than the shortest wavelength of the emission curve would also be the true spectral reflectance. The goodness of the theory to estimate the true reflectance for the Golden Acrylic Orange paint is shown in Figure 6. Errors in the very long wavelengths might be attributed to a lack of accuracy of the transformation matrix at long wavelengths. The same trend has been seen in estimating the true reflectance of the other paints.

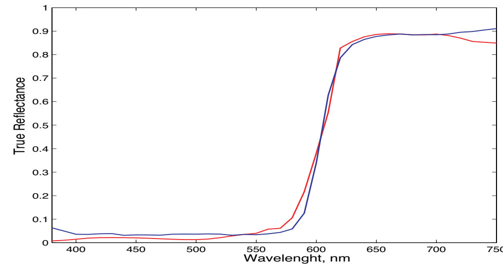

Figure 6. True reflectance of Golden Acrylic Orange; the blue line is measured with the Labsphere 450 bispectrometer and the red line is reconstructed using abridged fluorescence imaging.

IV. Conclusions

The abridged theory proposed by Allen [1] to separate the reflectance and luminescence component of the total radiance emerging from a fluorescent sample was studied and implemented, via simulation, for abridged spectral fluorescence imaging. The performance of the technique was very dependent upon the selection of the fluorescence-weakening and -killing filters. Also the performance was highly dependent upon the wavelength-independent constant to calculate the true reflectance in the overlap region. The theory was very effective for the samples with a large amount of luminescence.

Optimizing a set of filters to employ for all the six fluorescent paints or some groups of them is suggested as a future research. The study of the noise effect in fluorescence imaging is also recommended. Finally, these methods require testing experimentally.

References

[1] R. Donaldson, Spectrophotometry of fluorescent pigments, *Brit. J. Appl. Phys.* **5**, 210-214 (1954).

[2] E. Allen, Separation of the spectral radiance factor curve of fluorescent substances into reflected and fluoresced components, *Applied Optics* **12**, 289-293 (1973).

[3] D. Eitle and E. Ganz, Eine Methode zur Bestimmung von Normfarbwertrn für fluorezierende proben, *Textilveredlung* **3**, 389-392 (1968).

[4] F. T. Simon, The two-mode method for measurement and formulation with fluorescent colorants, *J. Color Appearance* **1**(4), 5-11 (1972).

[5] F. W. Billmeyer, Jr., Colorimetry of fluorescent specimens: A state-of-art report, *NBS-GCR* 79-185, 1979.

[6] S. Quan, Evaluation and Optimal design of Spectral Sensitivities for Digital Color Imaging, PhD thesis, Rochester Institute of Technology, 2002.

[7] See www.Art-si.org for the related papers.

Acknowledgments

This research was supported by the Andrew W. Mellon Foundation, the National Gallery of Art, Washington, DC, the Museum of Modern Art, New York, and the Institute of Museum Library Services, as part of the art spectral imaging project.

Biography

Mahnaz Mohammadi received her B.S. degree in Textile engineering from AmirKabir University at Tehran in 1993 and a M.S. in Polymer Engineering with an emphasis in color science from AmirKabir University in 1996. She is currently a Ph.D. student in Imaging Science at the Munsell Color Science Laboratory of Rochester Institute of Technology.