OPTICAL FILTER DESIGN OF FLUORESCENCE IMAGING SYSTEM USING LINEAR DISCRIMINANT ANALYSIS

Taemin Kim
Korea Advanced Institute of Science & Technology
335 Gwahak-ro, Yuseong-gu
Daejeon 305-701, Korea
tmkim@kaist.ac.kr

Byoung-Kwan Cho
Chungnam National University
79 Daehangno, Yuseong-gu
Daejeon 305-764, Korea
chobk@cnu.ac.kr

ABSTRACT

Based on a fluorescence imaging model, the optical filters, such as excitation and emission filters, are determined by linear discriminant analysis. The discriminability is a quadratic Rayleigh quotient of one filter if the other filter is assumed to be constant. The optical filters are optimized alternatingly and iteratively by solving their generalized eigenvalue problems. The proposed method is validated to design a fluorescence imaging system for poultry fecal inspection.

Index Terms — Fluorescent Imaging, Excitation and Emission Filters, Linear Discriminant Analysis

1. INTRODUCTION

Many applications of fluorescence imaging have been reported in biological and chemical sciences. The emerging fluorescence techniques are able to detect trace amount of fluorescent substances. It reveals only objects of interest in an otherwise dark background, while permitting selective tagging. The ability to label multiple distinct targets in a single biological sample greatly enhances the power of fluorescence imaging. The only way to capture a hyperspectral image is to take multiple images different optical filters and combine them electronically to yield a single, composite image [1]. Most fluorescence instruments, including microscopes, use optical filters to control the spectra of the excitation and emission light. It is possible to make optical filters with multiple pass-bands so the more than one fluorophore is excited, or the emission from more than one fluorophore observed, with a single filter [2].

Hyperspectral imaging techniques have been utilized in many scientific disciplines, from microscopic studies to airborne remote-sensing applications. A hyperspectral image is a three-dimensional data product containing two-dimensional information measured at a sequence of individual wavelengths across a sufficiently broad spectral range. The resultant spectra can be used, in principle, to characterize and identify any given material. Hyperspectral imaging and optical systems are prevailed in biological inspection. A laboratory-based hyperspectral imaging system mounting a pushbroom method was developed for food quality and safety [4]. Hyperspectral imaging systems were developed for detection fecal and ingesta contaminations on poultry carcasses [5].

Many researchers choose to focus on a few wavelengths for their application [6]. The ratio image of the 565-nm to the 517-nm wavelengths was able to identify fecal and ingesta contaminates [5]. Key wavelengths of visible and near infrared lights were chosen by extensive experiments for poultry fecal inspection [7]. Several pre-processing methods are investigated to enhance the accuracy of the (565/517) band ratio image [8]. Principal component analysis is employed to detect poultry skin tumors in hyperspectral fluorescence imaging [9][10].

An efficient method to design optical filters, such as excitation and emission filters, is proposed for a multispectral fluorescence imaging system. The optical filters are optimally determined by linear discriminant analysis (LDA) based on a fluorescence imaging model [14]. The discriminability is a quadratic Rayleigh quotient of one filter if the other filter is assumed to be constant. The optical filters are optimized alternatingly and iteratively by calculating the first generalized eigenvector, which has positive elements and maximizes the discriminability by the Perron-Frobenius theorem. The proposed method is validated to design a fluorescence imaging system for poultry fecal inspection.

2. MULTISPECTRAL IMAGING MODEL

The multispectral imaging model is proposed in [14] and summarized here. The multispectral imaging system for spectrofluorimetry consists of a light source, excitation and emission filters, and a camera (Figure 1a). Without loss of generality, the white light source is assumed to emit all wavelengths evenly and the camera has the uniform sensitivity for all wavelengths. A hyperspectral response of...
a specimen is represented by a two-dimensional function of emission and excitation wavelengths (Figure 1b). Suppose that a specimen has a hyperspectral response \( h(u,v) \) with random noise \( n(u,v) \). Simply, \( n(u,v) \) is assumed to be Gaussian:

\[
n(u,v) \sim N(0, \sigma^2(u,v)),
\]

where \( \sigma^2(u,v) \) is variance at location \((u,v)\).

In multispectral imaging system, the hyperspectral response is condensed into a smaller dimensional quantity by convolution. For simplicity, a single dimensional intensity will be derived by convolution of hyperspectral response and optical filters. The response is:

\[
r(v) = \int s(u)h(u,v)v(u,v)du + n(u,v),
\]

where \( r_s(v) = \int s(u)h(u,v)du \) and \( r_n(v) = \int s(u)n(u,v)du \) be signal and noise responses, respectively. The mean and variance of \( r(v) \) are calculated by:

\[
\bar{r}(v) = E[r(v)] = r_s(v) + r_n(v),
\]

\[
\sigma^2 = \text{Var}[r(v)] = \int \text{Var}[r_s(v)]du + \int \text{Var}[r_n(v)]du.
\]

The intensity \( g \) through a filter \( f(v) \) is:

\[
g = \int f(v)r(v)dv = g_s + g_n,
\]

where \( g_s = \int f(v)r_s(v)dv \) and \( g_n = \int f(v)r_n(v)dv \) are signal and noise of intensity. The sample mean and variance of the intensity are obtained by:

\[
\bar{g} = E[g] = g_s + g_n,
\]

\[
\sigma^2 = \text{Var}[g] = \int s(u)h(u,v)f(v)du dv,
\]

\[
= \int \int s^2(u)\sigma^2(u,v)f^2(v)du dv,
\]

The excitation and emission filters are chosen to maximize the discriminant power of multiple specimens. Suppose we have \( c \) specimens to be classified. The mean and variance of the intensity of the \( k \)th specimen are:

\[
\bar{g}_k = E[g | o_k] = \int s(u)h_k(u,v)f(v)du dv,
\]

\[
\sigma^2_k = \text{Var}[g | o_k] = \int \int s^2(u)\sigma^2_k(u,v)f^2(v)du dv,
\]

where \( h_k \) and \( \sigma^2_k \) are mean and variance of the \( k \)th spectrofluorimetric response. The total mean of intensity is:

\[
\bar{g} = \sum_{k=1}^c p_k \bar{g}_k,
\]

where \( p_k \) is prior probability of the \( k \)th specimen. The discriminability is defined by

\[
J(f,s) = \frac{s^2_k(f,s)}{s^2_k(f,s) + \sigma^2_k(f,s)},
\]

where \( s^2_k(f,s) \) and \( \sigma^2_k(f,s) \) are within and between variances, respectively [3]:

\[
s^2_k(f,s) = \sum_{i=1}^c p_i \sigma^2_i,
\]

\[
\sigma^2_k(f,s) = \sum_{i=1}^c p_i (\bar{g}_i - \bar{g})^2.
\]

The discriminability varies with form of \( f \) and \( s \) but not by scalar product. Therefore, their function space is restricted to positive unit functions:

\[
(f, s) = \arg \max_{f, s \in B(\mathbb{R}^+)} J(f,s),
\]

where \( B(\mathbb{R}^+) \) is a collection of all positive unit functions.

3. ALTERNATING SCHEME

The optimal excitation and emission filters are obtained numerically. Suppose that excitation and emission filters are sampled by \( m \) and \( n \) wavelengths, respectively. Let \( s \) the excitation vector and \( f \) be the emission vector. The hyperspectral response is written by:

\[
r = (H + N)s,
\]

where \( H \) and \( N \) be \( n \) by \( m \) matrices of the spectral response and random noise, respectively. \( N = \{n_y\} \) is assumed to be a Gaussian random noise matrix such that
\[ n_y \sim N(0, \sigma_y^2). \]  
Then equation (4), (5) and (6) become 
\[ g = f' H_s + f' N_s, \]
\[ \overline{g} = f' H_s, \]
\[ \sigma^2 = \sum_{i,j} (f_i \sigma_i s_j)^2. \]  

For multiple specimens, within and between scatters are derived in quadratic forms of respective excitation and emission vectors. Let \( H_k \) and \( N_k \) be the hyperspectral response and noise of the \( k \)th specimen. The within scatter is:
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4. EXAMPLARY RESULTS

Spectrofluorimetric responses of poultry feces (intestines, duodenum, small intestine, cecum, rectum) and organic materials (blood, skin, and flesh) were collected for poultry fecal inspection. Experimental setup was the same with [14], but spectrofluorimetric data were collected in Korea. The digestive tracts were eviscerated from chicken carcasses and their feces and organic materials were gathered. Spectrofluorimetric responses of specimens were measured by a spectrophotometer (Fluorolog III, Horiba Industries, Edison, N.J., USA). The light source was excited from 350nm to 610nm by 5nm steps and the emissions were measured from 370nm to 750nm by 2nm steps. Figure 2 plots representative spectrofluorimetric responses of poultry skin and feces.

The proposed method provides optimal excitation and emission filters, quantitatively, which is consistent with qualitative studies of Cho and Kim (2007). Excitation and emission filters were discretized by the same resolution, initialized with constant functions, and optimized alternatingly. Figure 3 shows the optimal relative attenuations of excitation and emission filters. The proposed method provides pictorial shapes over the range, while the...
previous research presented selective bandwidths experimentally. An excitation filter composed of UV-A (about 360nm) and blue light (about 460nm) and a band-pass filter with 710-750nm bandwidth were most appropriate.

The optimal optical filters are efficiently obtained by solving the first eigenvector of generalized eigenvalue problems. MATLAB software was used to calculate discriminability from spectral data and to obtain the optimal excitation and emission filters. It takes more than a couple of hours on a computer (Intel® Core™ 2 Duo Quad CPU Q6600 @ 2.40GHz) if the nonlinear optimization is used in an alternating way. This slow solution is because of nonlinear constraints and singularity that exists between scatter matrices. The new proposed method obtains the solutions in several iterations and just a few seconds.

5. CONCLUSION

The optimal excitation and emission filters of multispectral imaging system were designed by linear discriminant analysis (LDA). A mathematical model for hyperspectral imaging system was proposed and its system parameters, such as excitation and emission filters, were determined to maximize the discriminability for substances of interest. Optical filters are optimized alternatingly and iteratively by solving generalized eigenvalue problems. It is shown by the Perron-Frobenius theorem that the first generalized eigenvector have positive elements and maximize the discriminability. The optimal excitation and emission filters of a multispectral imaging system for poultry fecal inspection were consistent with those which experts provided in previous research. Future studies should guide practical implementation of optical filters.

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6. REFERENCES