Effect of Filter Arrangement in the Estimation Accuracy of an Imaging Spectrometer

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ABSTRACT

We investigate the reason why increasing the number of basis spectra in a spectral imaging device does not always improve the estimation merit. A particular filter is not the cause of this observation but the components of the inverse of the transformation matrix which map the coefficient of the basis spectra to the color of the sample. We found out that the large magnitude of the components of the inverse of the transformation matrix results in error in the calculation of the coefficients. This error leads to a drop in the spectral estimation merit even when the number of basis spectra is increased. Therefore, it is not enough that the filter used in an imaging spectrometer is not a linear multiple of other filters and nonzero to any of the wavelengths in the range of interest. Filters must also be arranged in a sequence such that the inverse of the transformation matrix will have components with small magnitude.

INTRODUCTION

Microscopic spectral imaging techniques have been developed, but they require special optics to be attached to the microscope (Schrock et al., 1996; Youvan et al., 1997). Recently, simpler and more compact imaging spectrometers have been introduced (Saloma et al., 2004; Connah et al., 2001; Kasari et al., 1999; Andres et al., 2004) which use lesser number of images by employing dimension-reduction algorithms. Dimension-reduction algorithms such as singular-value decomposition (SVD) (Soriano et al., 2002) and principal component analysis (PCA) (Imai et al., 2000) calculate basis spectra so that the spectrum of a sample may be represented by a weighted superposition of the basis spectra.

Theoretically, as the number of images and basis spectra are increased, the accuracy of spectral estimation of the sample should also increase. However, this is not always the case. For example, Connah et al. (2001) report anomalous result in using six basis spectra. Kasari et al. (1999) observed that using four bases is better than using five or six, while Andres et al. (2004) found that the estimation error does not consistently fall with increasing number of bases. All of them were unable to explain why this deviation from the theoretical prediction happens.

Using our developed spectral imaging method, we have found an explanation why increasing the number of images does not always increase the amount of information in the spectrum of the sample. The result of this investigation is not applicable only to our developed method, but also to other spectral imaging techniques such as that of Connah, Kasari, Andres, and others which utilize filter and dimension-reduction algorithms.

METHODS

SVD rotates the component axes wherein the data are most widely spread. If we assume an existence of a spectral library (ensemble of spectra of different samples), the axes represent the basis spectra. The basis spectra are then arranged into decreasing eigenvalue; that is, the first basis spectrum has a greater eigenvalue than the second basis, and so on.

After obtaining the basis spectra $e_i(l)$, the estimated spectrum $C_{est}(l; x, y)$ of the reference spectrum $C(l; x, y)$ can be expressed in terms of the first few significant $e_i(l)$:

$$C_{est}(\lambda;x,y)=\sum_{n=1}^{N} a_n(x,y)e_n(\lambda), \tag{1}$$

where a_n is the coefficient of the nth $e(l)$. The summation is taken from $n = 1$ to $n = N$, where N is the number of $e_i(l)$ that is utilized for estimation. The basis spectra are already known after the application of a dimension-reduction algorithm. Thus, N can be easily adjusted.

Relating the estimated spectrum to the output of the camera, we obtain

$$Q=Ta, \tag{2}$$

where Q is an M-column vector containing the channel output of a color camera (pixel color) and T is $M\times N$ transformation matrix that maps the expansion coefficients in a to the image space colors Q (Soriano et al., 2002). The elements $\{T_{nm}\}$ of T is described by

$$T_{nm}=\int e_n(\lambda)S_m(\lambda)d\lambda, \tag{3}$$

where $e_n(l)$ is the nth basis spectra and $S_m(l)$ is the mth camera sensitivity given by

$$S_m(\lambda)=S_\eta(\lambda)F(\lambda), \tag{4}$$

where η may be 1 (red channel), 2 (green channel), or 3 (blue channel). $F(l)$ is the transmission of the lightly colored filter used.

Since $e_i(l)$ are immediately known after applying SVD and the sensitivity of the camera and the transmission of the filter used are either obtained from the manufacturer or measured independently, T_{nm} is just a constant for a set of basis spectra, camera sensitivity, and filter transmittance.

In spectral imaging, the image output channels $\{Q_m(x, y)\}$ and the spectral library $\{C_k(l)\}$are known and the immediate task is to determine the component values of a. After a is known, one uses Eq. (1), to solve for the corresponding spectral estimation $C_{est}(l; x, y)$ which describes the optical spectrum at the location (x, y) of the two-dimensional (2D) image of the fluorescing sample.

For a colored image, the values for the different $Q_m(x, y)$'s for every pixel location (x, y) of the two-dimensional image are taken from the red (R), green (G), and blue (B) channel outputs ($M = 3$) of the camera. The unknown coefficients $\{a_n(x, y)\}$ are determined via

$$a=T^{-1}(Q), \tag{5}$$

where T^{-1} is the inverse of T. The inversion matrix T^{-1} is defined only if T is a square matrix because the size of T is equal to $M\times N$, i.e., T^{-1} exists only if $N = M$.

To increase the number M of color channels, the sample is image-captured with a lightly colored transmission filter placed before the camera. With the insertion of a filter, the fluorescent sample is imaged under three more independent channels ($M = 6$), in addition to the original three (for R, G, and B) provided by the 3CCD camera in the absence of a filter (null filter). In this study, five filters are used—null, pale lavender, pale apricot, lime, and pale yellow green.

The accuracy of estimation was measured using fidelity f:

$$f=1-\frac{\langle C-C_{est}\rangle}{\langle C^2\rangle}, \tag{6}$$

where $\langle\cdot\rangle$ is the average value and C is the theoretical spectrum.

Fidelity describes the general similarity between theoretical and estimated spectrum. Perfect estimation occurs when $f = 1$.

RESULTS AND DISCUSSIONS

Increasing the number of basis spectra increases the cumulative eigenvalue. Thus, the information that can

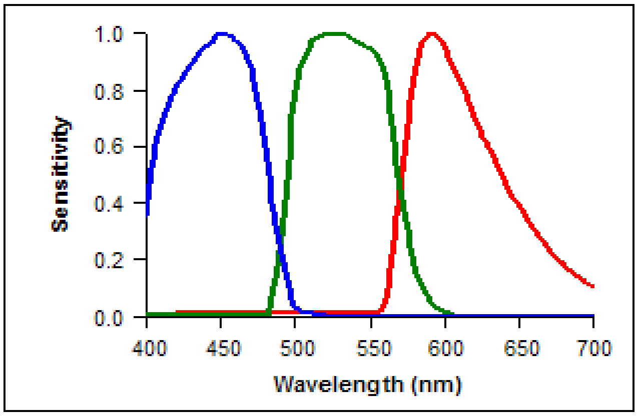

Fig. 1. Camera sensitivities of 3CCD camera with null filter.

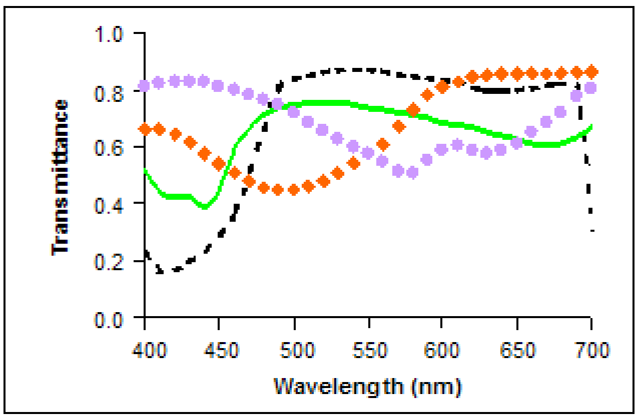

Fig. 2. Transmission of lightly colored filters: pale lavender (violet circle), pale apricot (orange diamond), lime (black dotted line), and pale yellow green (yellow green solid line).

estimate the spectrum of the sample are also increased. Figure 1 shows the camera sensitivities of the 3CCD camera used with a null filter. The transmission of the lightly colored filters is shown in Fig. 2.

Figures 3 and 4 illustrate the average fidelity of estimation of each spectrum in the spectral library (with 423 emission spectra of fluorescent dyes and microspheres) using the same set of filters but of a different sequence. For example, a null filter is used to generate the first three sensitivities of the camera and pale lavender for the next fourth to sixth sensitivities. As the number of basis spectra increases, fidelity approaches unity. Standard deviation also decreases both for the two cases. This means that with the increased number of bases spectral estimation becomes more accurate and more precise. However, a prominent drop in fidelity and a sudden increase in standard deviation are observed using 11 and 9 basis spectra for Figs. 3 and 4, respectively. This contradicts our prediction.

The 11th basis spectrum in Fig. 3 corresponds to the green channel of the camera with lime filter. If this filter causes the anomalous observation, we will expect that the effect must also be present to more set of basis spectra. However, this is not observed in using 12–15 bases.

It may be hypothesized that the anomalous effect of the lime filter cancels out as the number of basis spectra is further increased. Thus, the effect is present only in the 11th camera channel. If that is the case, if we rearrange the first three filters and make the last two filters fixed, we will expect to observe anomalous estimation merit again in using 11 basis spectra. However, the effect is seen in using nine basis spectra,

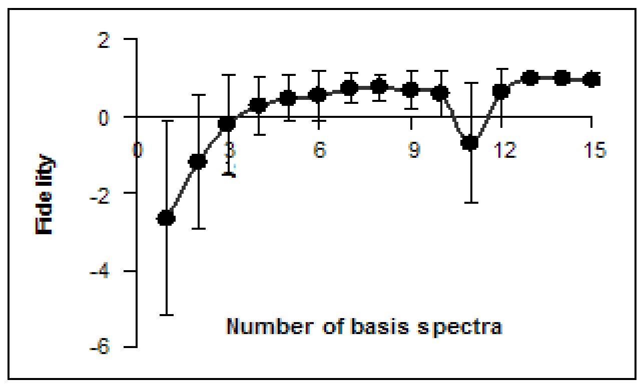

Fig. 3. Fidelity using filter arrangement of null–pale lavender–pale apricot–lime–pale yellow green.

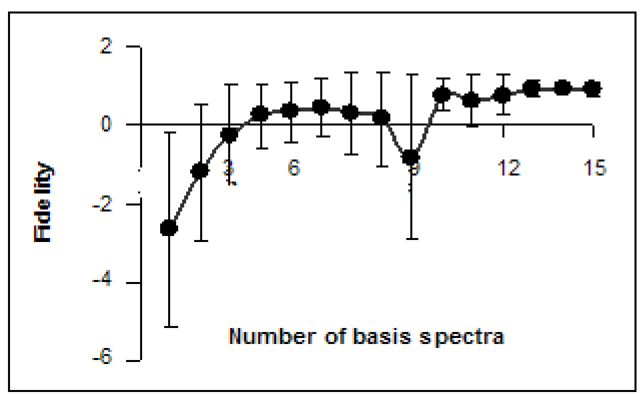

Fig. 4. Fidelity using filter arrangement of null–pale yellow green–pale apricot–lime–pale lavender.

as shown in Fig. 4. Pale apricot is the filter that corresponds to the ninth camera channel and the lime filter has no contribution yet since it is used in generating the 10th, 11th, and 12th camera channels. Therefore, the lime filter is not the cause of the deviation from the expected result.

From Eq. (1), spectral estimation is done using basis spectra and coefficients. For a given spectral library, the basis spectra are fixed values. Coefficients are calculated using the inverse of the transformation matrix and the pixel color as shown in Eq. (5). For a given sample, pixel color is fixed for a particular channel of the camera. Thus, the only freedom we have is choosing the filter to increase the number of camera channel. The sensitivity of the camera channel affects the calculation of the coefficient through the inverse of the transformation matrix.

Tables 1 and 2 show the components of the inverse of the transformation matrix of Figs. 3 and 4, respectively, using nine basis spectra. Comparing the two tables, Table 2 has components with larger magnitude, with a difference of one order indicated by the shaded cell, compared with Table 1. An anomalous result in using nine bases is observed only using the inverse of the transformation matrix of Table 2. This observation is also seen (not shown) in comparing the inverse of transformation matrices of Figs. 3 and 4 with 11 basis spectra.

Hence, the unexpected drop in fidelity as the number of basis spectra is increased is attributed to the large magnitude of the components of the inverse of the transformation matrix. This large magnitude leads to the instability of the equation resulting in a large calculation error of the coefficients.

Table 1. Components of the inverse of the transformation matrix using null–pale lavender–pale apricot–lime–pale yellow green filters.

2	7	2	-1	-6	-3	-2	-6	1
-5	-16	-6	2	14	9	5	14	-2
6	19	7	-3	-17	-10	-5	-16	2
-7	-22	-7	6	19	10	4	19	-2
0	1	4	-5	-1	-5	3	-2	0
7	20	9	6	-18	-12	-13	-18	1
-11	-43	-19	-4	38	29	16	38	-7
10	48	11	3	-42	-15	-14	-41	2
4	29	16	1	-27	-23	-6	-24	5

Table 2. Components of the inverse of the transformation matrixusing filters null–pale yellow green–pale apricot–lime–pale lavender filters.

-9	48	2	6	-59	-2	6	-8	-2
24	**-134**	-5	-15	**165**	5	-16	22	5
-31	**171**	6	20	**-211**	-6	21	-27	-6
65	**-378**	-14	-38	**465**	14	-47	60	14
-58	**369**	13	33	**-453**	-13	44	-60	-14
76	**-499**	-18	-39	**614**	18	-60	80	18
-47	**357**	15	22	**-440**	-14	38	-57	-16
32	**-292**	-8	-15	**358**	9	-27	47	8
6	**-106**	-5	-3	**129**	5	-5	19	5

In practice, camera output is taken from the average of the gray value level of several pixels depending on the signal-to-noise ratio (SNR) of the sample. Any noise or deviation in color values will propagate when multiplying with the inverse of the transformation matrix. Thus, an increase in error is observed.

On the other hand, using a transformation matrix with components of smaller magnitude gives a smaller error since standard deviation is less amplified. Thus, it becomes more robust to fluctuation, noise, and quantization errors of the digitizer.

For a limited set of filters an anomalous result may be avoided by choosing the best arrangement that will give small components of the inverse of the transformation matrix. Another way is by finding the optimum filter that will minimize the magnitude of each component for any number of basis spectra.

This result implies that the conditions that (1) the filter is not correlated with other filters used and that (2) in the wavelength range considered the filter transmittance must be nonzero (Soriano et al., 2002; Imai et al., 2000) are not enough considerations in choosing the filter for spectral imaging. We add another constraint; that is, that (3) the arrangement of the generated camera sensitivity from the filter should make the components of the inverse of the transformation matrix stable.

SUMMARY

Using our developed technique in spectral imaging, we illustrate why an increase in number of basis spectra is not always accompanied by an increase in the spectral estimation merit. It is shown that the cause of this is the large magnitude of the components of the inverse of the transformation matrix. We present that the cause is not a particular filter, but the arrangement of the filters.

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