Estimation of transmittance spectra from multiband micrographs of fungi and its application to segmentation of conidia and hyphae

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Transmittance spectra of fungi were estimated from seven band optical micrographs. The optical microscope adjusted with single -chip CCD camera with seven band filters was used for image acquisition. The Wiener method was applied to estimate the transmittance spectra of five species which belong to one genus of fungi. The Wiener estimation operator was calculated using transmittance spectra of sixteen color transparencies and the corresponding camera responses. The estimated transmittance spectra were used for segmentation of conidia and hyphae in fungal optical micrographs, then the competitive learning in artificial neural network was applied to the segmentation.

Key words : spectral estimation, fungi, optical microscope, segmentation, competitive learning.

1. Introduction

Genebanks play a leading role in the preservation and documentation of genetic resources related to agriculture worldwide.[1] One of the most difficult issues in managing a genebank collection is accurate identification of conserved germplasm. The identification of fungi poses particular problems due to the lack of morphological characteristics to readily distinguish different species. Shape and color of fungi are important in correct identification of fungi. Since the numbers of accessions in a genebank are high in proportion to the number of experts who can identify them, it is necessary to develop a system which will enable fungi to be automatically identified.

Transmittance spectra have been used as a criterion for identification of objects, since spectroscopic distribution reflects physical and chemical features of materials.[2] On the other hand, the information of images with R, G and B channels is influenced by spectral characteristics of the imaging system and illuminant, as a consequence physical and chemical features are buried under these spectral characteristics.

To estimate transmittance spectra, a multiband imaging system using interference filters has been developed,[3,4] and an imaging system attached a spectral illuminant modulator using liquid crystal has been designed.[2] Transmittance spectra of micrographs were successfully estimated using the proposed imaging systems. However, in the system using interference filters, signal-to-noise ratio is low because of their narrow band characteristics and many band images are required for a wide range of wavelengths, in the later system, special equipment is required for filtering.

Recently, color correction techniques based on an estimation of reflectance spectra have been proposed [5-9] using broad band filters. The techniques were applied to various imaging systems such as art painting reproduction [10-12], human skin reproduction [13], internet shopping [14], and telemedicine [15]. The color spectra of natural samples have smooth shapes.[16,17] Thus high dimensional vectors of spectra are redundant to represent them, and the vectors will exist in limited vector subspace. This subspace can be represented by low dimensional basis. The low dimensional linear approximation method [5-9] or the Wiener method [11,18] are often used for the estimation of reflectance spectra from multiband images using several broad band filters. The signal-to-noise ratio is increased by the broad band characteristic of filters compared with interference filters.[19]

In this paper, the spatial distributions of transmittance spectra in optical micrographs are estimated from multiband images taken by CCD camera with broad band filters. Wiener's estimation method is used to estimate transmittance spectra of objects. In the next section, the image acquisition system and the Wiener estimation method are introduced. In section 3, we explain the process of estimating transmittance spectra from fungal micrographs. In section 4, segmentation of conidia and hyphae using an artificial neural network is explained, and segmentation based on transmittance spectra is compared with segmentation based on RGB images. In section 5, our conclusions are presented.

2. Image acquisition system

The imaging system to take multiband images in optical microscope is shown (Fig. 1). A digital camera (Kodak DCS420m, 1536x1024 pixels) is adjusted at the optical microscope (OLYMPUS BX50) with an objective (UPlanApo40×), a 40× magnification. The digital camera is controlled by a personal computer. During the experiment, seven filters with broad band were used to take multiband images, and each filter is inserted in turn between condenser lens and light source (Halogen lamp). The transmittance spectra of seven filters are shown (Fig. 2).

The principle of the image acquisition system and Wiener estimation method [11,18] are formulated as follows.

A. Formulation of Image Acquisition

The response $v_i(x,y)$ at position (x,y) of the digital camera with i-th color filter is expressed by

$$v_i(x,y) = \int_{410}^{700} t_i(\lambda)E(\lambda)S(\lambda)o(x,y;\lambda)\,d\lambda\ , \ i=1,\ldots,m, \qquad (1)$$

where λ is wavelength. $t_i(\lambda)$, $E(\lambda)$, $S(\lambda)$, and $o(x,y;\lambda)$ denote spectral transmittance of i-th filter, radiance of light source, total sensitivity of camera and spectral transmittance of object at position (x,y), respectively. We assumed that the number of bands m to take image is equal to seven and the noise in taking images can be ignored for the broad band filters.

The spectral characteristics of each element from 410 to 700 nm were sampled at 10 nm interval. Consequently, the number of elements l for discrete spectral data becomes 30. Using the vector-matrix notation, Eq. (1) can be expressed as,

$$\boldsymbol{v}=\mathbf{F}\boldsymbol{o}, \tag{2}$$

where $\boldsymbol{v}$ denotes a column vector with m elements representing the camera responses and $\boldsymbol{o}$ denotes a column vector with l elements representing the spectral transmittance of object. The coordinate (x,y) in $\boldsymbol{v}$ and $\boldsymbol{o}$ is eliminated for simplicity. These two vectors are related by a linear system matrix $\mathbf{F}$ with $m \times l$ components. The matrix $\mathbf{F}$ is expressed as

$$\mathbf{F}=\mathbf{TES}, \tag{3}$$

where

$$\mathbf{T}=[\boldsymbol{t}_1, \boldsymbol{t}_2, \ldots, \boldsymbol{t}_m]^t. \tag{4}$$

The vector $\boldsymbol{t}_i$, i = 1,2, …, m, denotes a column vector representing the transmittance of i-th filter and $[\cdot]^t$ represents transposition. The matrices $\mathbf{E}$ and $\mathbf{S}$ denote $l \times l$ diagonal matrices corresponding to the spectral radiance of the light source and the spectral sensitivity of the camera, respectively.

B. Wiener estimation

Estimated spectral transmittance $\boldsymbol{o}_{est}$ is given by the following linear operation of the Wiener method.

$$\boldsymbol{o}_{est}=\mathbf{G}\boldsymbol{v}. \tag{5}$$

The matrix **G** is determined to minimize the ensemble average of the square error ε between the original and estimated transmittance spectra,

$$\varepsilon=<(\boldsymbol{o}-\boldsymbol{o}_{est})^{\mathrm{t}}(\boldsymbol{o}-\boldsymbol{o}_{est})>. \tag{6}$$

Here <·> represents ensemble average. The matrix **G** is explicitly expressed by

$$\mathbf{G}=\mathbf{R}_{\mathrm{ov}}\mathbf{R}_{\mathrm{vv}}^{-1}, \tag{7}$$

where $\mathbf{R}_{\mathrm{ov}}$ and $\mathbf{R}_{\mathrm{vv}}$ denote correlation matrices defined as

$$\mathbf{R}_{\mathrm{ov}}=<\boldsymbol{o}\boldsymbol{v}^{\mathrm{t}}>,\ \mathbf{R}_{\mathrm{vv}}=<\boldsymbol{v}\boldsymbol{v}^{\mathrm{t}}>. \tag{8}$$

Equations (5) to (8) show that the Wiener estimation requires second order statistics with respect to original transmittance spectra and camera response. In this study, these correlation matrices were approximated by using the transmittance spectra of sixteen color transparencies and the corresponding camera responses.

3. Estimation of transmittance spectra from micrograph

Transmittance spectra of sixteen color transparencies were measured by a spectral photometer in advance, and the seven band images of the color transparencies were taken by the image acquisition system. The transmittance spectra of the sixteen color transparencies are shown (Fig. 3). The Wiener operator **G** in Eq. (7) was calculated using these measured transmittance spectra and camera responses. The estimated transmittance spectra by five to seven band filters for the sixteen color transparencies were compared with the original ones using normalized root mean square error (NRMSE) (Table 1). From the Table 1, we can see the efficiency of seven band images. Therefore, seven band images of fungi were taken by the proposed imaging system and the transmittance spectra of these images were estimated. Due to experimental limitations the number of band filters and color transparencies used are determined empirically.

In this study, we cultivated five species; *longibrachiatum*, *hamatum*, *harzianum*, *viride*, *polysporum* which belong to the genus *Trichoderma* [20,21] on four kinds of media; potato dextrose agar (PDA), special nutrient agar (SNA), corn meal dextrose agar (CMD), malt extract agar (ME). Transmittance spectra of fungi were calculated from camera responses in each pixel and the above estimation operator **G**. A schematic diagram of the above estimation process is shown (Fig. 4). In each pixel, spectral transmittance can be estimated from each camera response vector using the Wiener operator **G**. In this paper, we call this

2-dimensional distribution of transmittance spectra a transmittance spectra image.

Figures 5(a),(b) show examples of transmittance spectra extracted from conidia, hyphae, and medium of *T. longibrachiatum* on PDA and *T. hamatum* on CMD, respectively. The transmittance spectra of each constituent, that is conidia, hyphae, and medium, is different from each other, and there is a difference between transmittance spectra of the two species. Slices of transmittance spectra image of *T. longibrachiatum* at 450 nm and 600 nm are shown (Fig. 6).

4. Segmentation using artificial neural network

The information on the estimated transmittance spectra was used for the segmentation of fungal micrographs. Segmentation of conidia and hyphae is required to identify *Trichoderma*, because the criterion is defined by the shape and color of conidia and hyphae, respectively.[20,21] In this study, an artificial competitive learning neural network [22] was applied to the segmentation from fungal optical micrographs. As input vectors to the artificial neural network, color vectors of transmittance spectra and RGB values were considered in each pixel of the fungal image with 301 × 361 pixels. The architecture of the artificial neural network used in this experiment is shown (Fig. 7). When the dimensions of the input vector is n, the i-th neuron has n-dimensional weight vector $\boldsymbol{w}_i$. All weight vectors are initialized at the center of all color vectors in the image.

At every learning step, an n-dimensional input vector $\boldsymbol{p}$ is randomly extracted from all color vectors in the image, and the i-th neuron produces an internal value c_i according to the following equation.

$$c_i = -\| \boldsymbol{p} - \boldsymbol{w_i} \| + b_i , \tag{9}$$

where $\|\cdot\|$ is the operation of the Euclidean vector norm, and b_i is the bias in the i-th neuron.

The internal values compete in the layer, then the neuron which has the highest internal value in the layer is selected as a winner neuron. This neuron is given the value 1. All other neurons are given a value of 0.

The bias of the winner neuron is decreased and the weight vector is modified as follows.

$$\boldsymbol{w'} = \boldsymbol{w} + lr \times (\boldsymbol{p} - \boldsymbol{w_i}), \tag{10}$$

where $\boldsymbol{w'}$ is the modified weight vector, lr is the learning rate. The biases of the other neurons are increased, and the weight vectors are not modified in this learning method. The biases are modified so that all neurons have the similar opportunity to win.

An example of learning step is shown (Fig. 8). It is considered that the dimension of weight vector is two, the number of neurons is three, and weight vector $\boldsymbol{w_1}$ is the closest to the input vector $\boldsymbol{p}$. When the biases are the same in all neurons, the neuron with the closest weight vector to the input vector becomes the winner neuron.

The number of times the learning step is repeated is decided prior to an experiment. After the repeated learning steps are performed, final weight vectors, which are a cluster feature of some input vectors, are generated.

In this study, the number of neurons was set at three to separate it into three clusters, there were 150,000 learning steps, and learning rate was 0.1. The dimension of color vector was 21 for transmittance spectra sampled from 450 nm to 650 nm at 10 nm intervals and three for RGB values. The range of wavelength less than 450 nm and more than 650 nm is strongly influenced by noise, so this range was not used as a color vector.

At the end of the neuron learning steps, each color vector in the image is assigned to the neuron whose final weight vector is the closest to the color vector. Figures 9(a),(b) show segmentation images of *T. longibrachiatum* based on transmittance spectra and RGB values, respectively. In Fig. 9(a), black, gray, and white parts correspond to conidia, hyphae, and medium, respectively. In Fig. 9(b), black and white parts correspond to conidia and medium, respectively, however, the gray part include both hyphae and medium, namely, medium is segmented into hyphae. Segmentation based on transmittance spectra is more accurate than that based on RGB values.

5. Conclusions

A method to estimate transmittance spectra in optical micrograph has been proposed. The Wiener estimation operator was calculated from transmittance spectra of sixteen color transparencies and the camera responses, then transmittance spectra of all pixels in the micrograph were estimated from multiband images. The method was applied to analyze a fungal micrograph, and the estimated transmittance spectra were successfully used for segmentation of the micrograph.

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Figure captions

Fig. 1. Multiband imaging system for microscopy.

Fig. 2. Transmittance spectra of seven filters.

Fig. 3. Transmittance spectra of sixteen color transparencies.

Fig. 4. Schematic diagram for the estimation of transmittance spectra image.

Fig. 5. Examples of transmittance spectra extracted from conidia, hyphae, and medium; (a) *T. longibrachiatum* on PDA, (b) *T. hamatum* on CMD.

Fig. 6. Slices of transmittance spectra image at 450nm and 600nm.

Fig. 7. The architecture of the neural network used in the competitive learning method, where the highest internal value is c_2.

Fig. 8. Example of learning step with two dimensional weight vector $\boldsymbol{w}$, three neurons, and input vector $\boldsymbol{p}$. and $\boldsymbol{w}_1$ is the closest to input vector.

Fig. 9. (a) Segmentation image of *T. longibrachiatum* based on transmittance spectra. Black part: conidia, gray part: hyphae, white part: medium. (b) Segmentation image of *T. longibrachiatum* based on RGB value. Black part: conidia, gray part: hyphae and medium, white part: medium.

Table 1. Normalized root mean square error (NRMSE) between the original and estimated spectral transmittance of sixteen transparencies.

Number of band	5	6	7
NRMSE	0.0400	0.0369	0.0352