

Estimation of Spectral Transmittance in Optical Microscopic Image and its Applications

Masaru TAKEYA*,**, Norimichi TSUMURA*, Hideaki HANEISHI*, and Yoichi MIYAKE*
**** Graduate School of Science and Technology, Chiba University Chiba, JAPAN***
***** National Institute of Agrobiological Resources Tsukuba, JAPAN***

Abstract

Spectral transmittance in optical microscopic image was estimated from multi-band image taken through five broad band filters based on Wiener estimation method. Matrix for Wiener estimation was calculated using the 16 spectral transmittances of color transparencies measured in advance by spectral photometer. The optical microscope adjusted for monochrome digital camera (Kodak DCS420m, 1536x1024 pixels) with five band filters was used for image acquisition. The fungi image taken by those imaging systems were analyzed by using the estimated spectral transmittances.

Introduction

The MAFF (Ministry of Agriculture, Forestry and Fisheries) genebank project has been done since 1985. In this project, genetic resources such as plant, microorganism, and animal have been collected and preserved. The rapid spread of improved varieties has resulted in the loss of genetic diversity and increased genetic uniformity. Industrialization and urbanization gave seriously damage the natural environment. One urgent objective of the MAFF genebank project is to keep a diversity of species before they disappear. On the management of the genebank, one of the most difficult operation is an identification of genetic resources, because the identification requires special knowledge and technique. Especially in identification of fungi, experts must collates shape or color of the fungi with literature or knowledge to identify them. There aren't enough experts to identify a lot of fungi for MAFF genebank project. Therefore we must develop the system to identify fungi speedily, easily, and exactly.

In this paper, spectral transmittance information is used as criterion of the identification, since the spectroscopic distribution reflects physical and chemical features of materials.[1] On the other hand, the information of RGB image is influenced by the imaging system such as light source, spectral sensitivity. The spatial distribution of spectral transmittance in optical microscopic image can be estimated from multi-band image. We apply Wiener estimation method to estimate spectral transmittance of the object from multi-band image.[2]

Image acquisition system

Figure 1 shows the imaging system to take multi-band image. Digital camera is adjusted at the optical microscope (OLYMPUS BX50) with an objective (UPlanApo40×), a magnifications of 40×. The digital camera is controlled by personal computer. In the experiment, five filters with spectral transmittance shown in Figure 2 were used to take multi-band image, and each filter is inserted in turn between condenser lens and light source (Halogen lump).

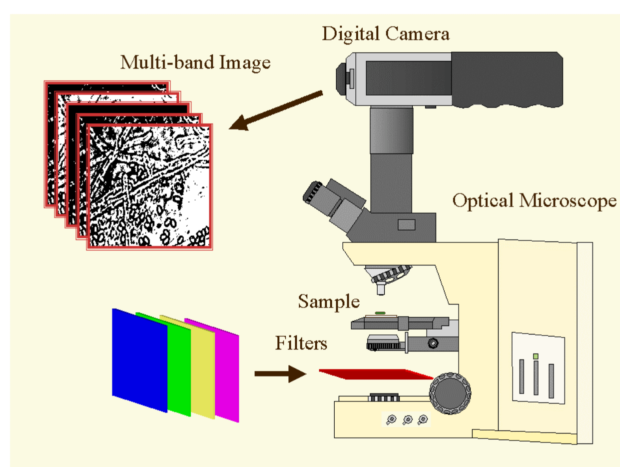


Figure 1:Imaging system to take multi-band image.

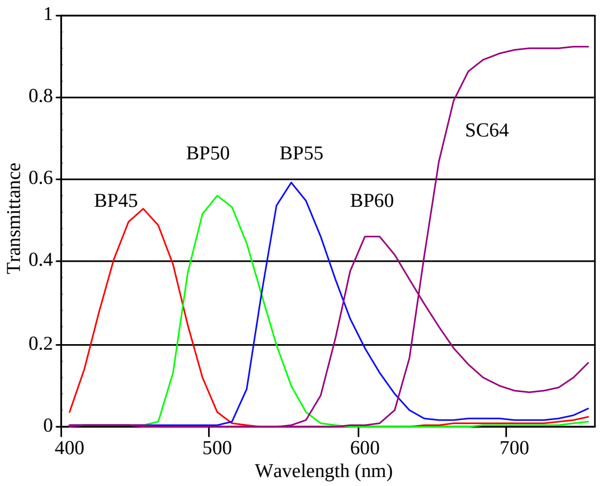


Figure 2:Spectral transmittance of five filters.

Formulation of Image Acquisition

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

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

where $t_i(\lambda)$, $E(\lambda)$, $S(\lambda)$, and $o(x,y;\lambda)$ are transmittance of i-th filter, radiance of light source, total sensitivity of camera and transmittance of color transparency, respectively. We assumed that m is equal to five and the noise is negligible for the wide band filters used to take image.

Spectral characteristic of each element from 410 to 700 nm was sampled in 10 nm interval. Consequently, the number of elements, l, for discrete spectral data becomes 30. Using the vector-matrix notation, Equation (1) can be expressed as,

$$\boldsymbol{v}=\mathbf{F}\boldsymbol{o} \qquad (2)$$

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

$$\mathbf{F}=\mathbf{TES} \qquad (3)$$

and

$$\mathbf{T}=[\boldsymbol{t}_1,\boldsymbol{t}_2,...,\boldsymbol{t}_m]^t \qquad (4)$$

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

Wiener estimation

A solution of Equation (2) by the Wiener method is given by the following linear operation,

$$\boldsymbol{o}_{est}=\mathbf{G}\boldsymbol{v} \qquad (5)$$

The matrix $\mathbf{G}$ is determined to minimize the ensemble average of the square error ε between the original and estimated spectral transmittance,

$$\varepsilon=\langle(\boldsymbol{o}-\boldsymbol{o}_{est})^t(\boldsymbol{o}-\boldsymbol{o}_{est})\rangle \qquad (6)$$

Here <> represents ensemble average. The matrix $\mathbf{G}$ is explicitly expressed by

$$\mathbf{G}=\mathbf{R}_{ov}\mathbf{R}_{vv}^{-1} \qquad (7)$$

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

$$\mathbf{R}_{ov}=\langle\boldsymbol{o}\boldsymbol{v}^t\rangle,\quad \mathbf{R}_{vv}=\langle\boldsymbol{v}\boldsymbol{v}^t\rangle \qquad (8)$$

Equations (5) to (8) show that the Wiener estimation requires the second order statistics with respect to original spectral transmittance and camera response. In this study, these correlation matrices were calculated approximately by using the transmittance spectra of 16 color transparencies and the corresponding camera outputs.

Estimation of spectral transmittance to microscopic image

Spectral transmittance of 16 color transparencies were measured by spectral photometer in advance, and the five band images of color transparency were taken by microscopic imaging system. Wiener operator $\mathbf{G}$ of Equation (8) was calculated using these measured spectral transmittance and camera response. As the result of Wiener estimation, average color difference between the original and estimated spectral transmittance in L*a*b* color space under the light source Halogen lump was 3.5 for samples of transparencies. Figure 3 shows color differences for 16 transparencies.

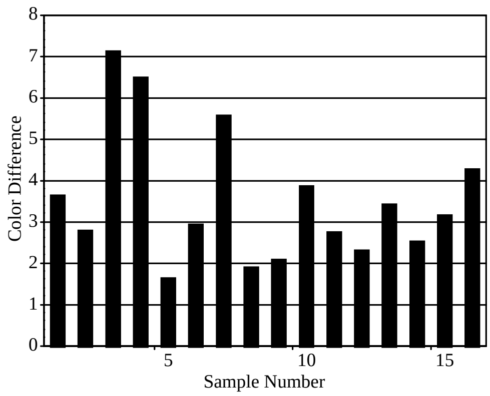


*Figure 3:Color difference in CIE L*a*b* space of 16 color transparencies.*

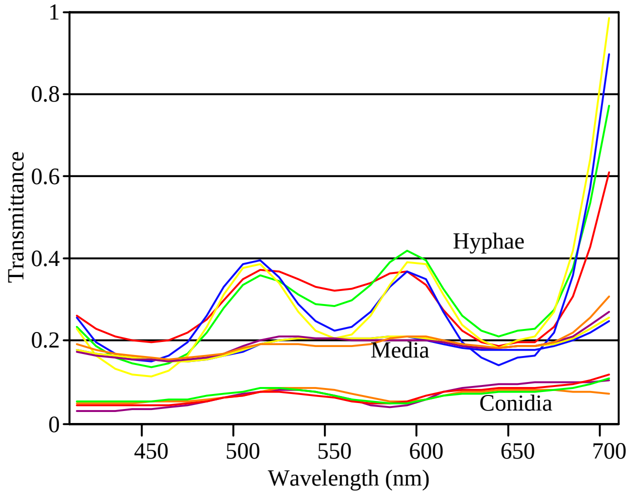
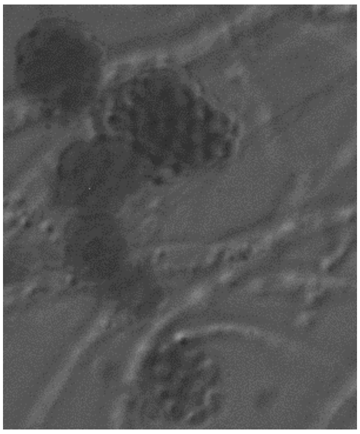
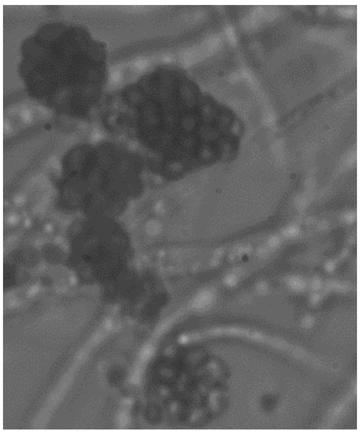


Figure 4:Estimated spectral transmittance.



450nm



600nm

Figure 5:Slices of spectral transmittance image at 450nm and 600nm.

Five band images of fungi were taken by those imaging systems. In this study, we cultivated five species (longibrachiatum, hamatum, harzianum, viride, polysporum) which belong to genus Trichoderma on four media (PDA, SNA, CMD, ME). Spectral transmittances of fungi were calculated from camera responses in each pixel and the above estimation matrix G.

Figure 4 shows examples of spectral transmittance extracted from conidia, hyphae, and media of *Trichoderma longibrachiatum* on PDA, respectively. It is clear that the spectral transmittance in each part is different from that of other parts. Figure 5 shows slices of spectral transmittance image at 450nm and 600nm.

Segmentation using Neural Network

Segmentation of conidia and hyphae is required in identifying *Trichoderma*, because the criterion is defined by the shape and color of conidia and hyphae, respectively.[3] In this study, competitive learning in neural network [4] was applied for the segmentation from spectral microscopic image.

Figure 6 shows the architecture of neural network used in this experiment. The *i*-th neuron has n-dimensional weight vector $\boldsymbol{w}_i$. All weight vectors are initialized to be on the center of the input vectors. At the every step of the learning, an n-dimensional input vector $\boldsymbol{p}$ is randomly extracted from the set of training vectors, and the *i*-th neuron produces the internal value c_i according to the following equation.

$$c_i = -\| \boldsymbol{p} - \boldsymbol{w}_i \| + b_i \tag{9}$$

where $\|.\|$ means the operation of Euclidean vector norm, b_i is the bias in the *i*-th neuron. The internal values are competed in the layer, then the neuron which has the highest internal value in the layer is selected as a "winner neuron". The "winner neuron" outputs the value 1. On the other hand, all neurons except the "winner neuron" output the value 0. The bias of the "winner neuron" is decreased and the weight vector is modified as follows.

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

where $\boldsymbol{w'}$ is the modified weight vector, lr is the learing rate. On the other hand, the biases of the other neurons are increased, and the weight vectors are not modified in this learning method. When the biases are same in all neurons, a neuron with the closest weight vector to the input vector becomes a "winner neuron". Figure 7 shows an example learning step where the 2-dimensional weight vector and 3 neurons with $\min(\| \boldsymbol{p} - \boldsymbol{w}_i \|) = \| \boldsymbol{p} - \boldsymbol{w}_1 \|$ are considered. The biases are modified so that all neurons have the similar oppotunity to win. These steps are repeated within the decided epoches. After the learning, all training vectors are assigned to the neuron whose weight vector is the closest to

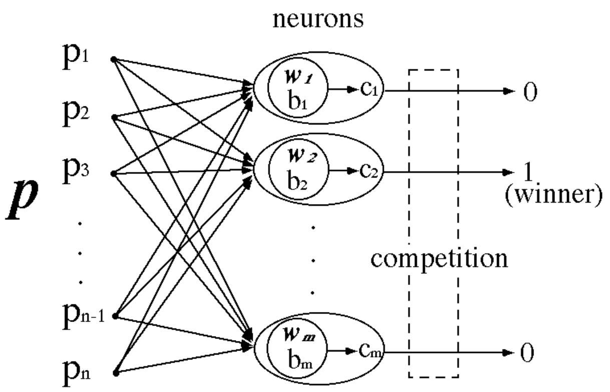


Figure 6:The architecture of the neural network used in the competitive learning method, in case of max(c_i)=c_2.

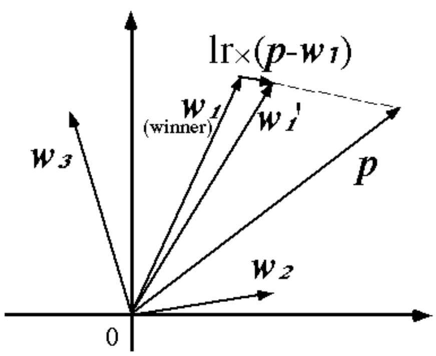


*Figure 7:An example of learning step where the 2-dimensional weight vector and 3 neurons with min(|| **p** - $\mathbf{w}_i$ ||)=|| **p** - $\mathbf{w}_1$ || are considered.*

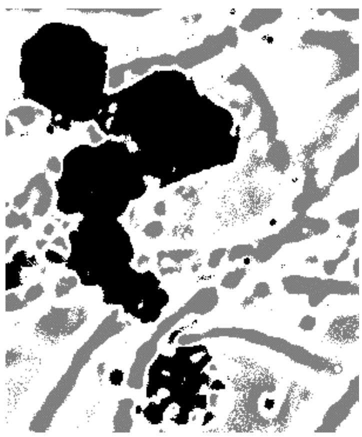


Figure 8:Segmentation image of T. longibrachiatum based on spectral transmittance. "black part: conidia", "gray part: hyphae","white part: media".

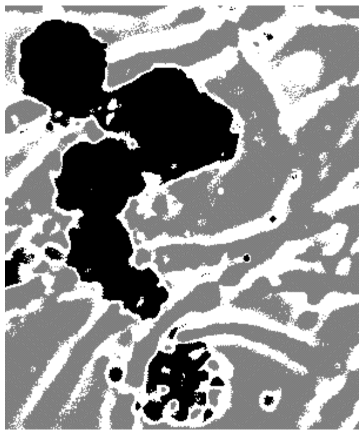


Figure 9:Segmentation image of T. longibrachiatum based on RGB value. "black part: conidia", "gray part: hyphae and media","white part: media".

it, then each neuron repsesents a cluster in vectors, respectively.

In the segmentation of image, the number of neuron was set at three to separate it into three clusters, learning epochs were 150,000 times, and learning rate was 0.1. The dimension of input vectors was 21 for spectral reflectance sampled from 450 nm to 650 nm in 10 nm interval and three for RGB values. Figures 8 and 9 show segmentation images of *Trichoderma longibrachiatum* based on spectral transmittance and RGB values, respectively. It is clear that segmentation based on spectral transmittance was better than segmentation based on RGB values.

Conclusion

A method to estimate spectral transmittance in optical microscopic image was proposed. Spectral transmittances of 16 color transparencies and camera response were measured and used to estimate the spectral transmittance of all pixels in the microscopic image. The method was applied to reproduce of fungi microscopic image, and the estimated spectral transmittances were successfully used for segmentation of microscopic image in order to identify fungi.

References

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