Estimation of pigment distribution in optical micrograph by using spectral transmittance

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Abstract

Relative amounts of pigments on an over head projector (OHP) sheet printed by laser printer can be estimated by using spectral transmittance at each pixel of optical micrograph, and the multi–layered pigments also can be separated to each component. The transmittance spectra were estimated from multiband image using the Wiener estimation method. In the experiment, relative amount of pigments printed by one or two kinds of color pigments were estimated, and overlapped pigment by magenta and yellow was separated into each pigment. The result was accurate as compared with separation based on RGB values.

Introduction

Color of a printed image is usually predicted by Neugeabuer model, however this model is not accurate because it assumes that each ink layer has the same thickness, printed dots are randomly distributed, and the optical dot gain is not considered by the model. In real printed image, the thickness of ink is not equal at each point of image. We can observe from the optical micrograph that the optical density is not the uniform in the dot. In addition, many printing techniques do not use random dot halftoning algorithm. The other problem is that when we print four color inks, the spread of one ink on the another ink layer is different from printing on the bare papers because the upper ink layer is influenced by the unevenness on the lower ink layer.[1,2] The color prediction will be more accurate if we know two–dimensional spatial distribution of ink in each layer of the print.

From Neugeabuer model, the color is predicted by using density or colorimetric value, however when high image quality is concerned the spectral reflectance is usually considered. Broadband reflectance measurements are inappropriate for Neugeabuer model of a color printer because the real inks do not have constant reflectance.[3] Many researches have been studied to extend the Neugeabuer to spectral Neugeabuer model such as colorant estimation of printing,[4,5] compensation for optical interactions,[6] and art printing reproduction.[7–9]

In this paper, we propose the technique to estimate two–dimensional distribution of pigment for each printed color.

The relative amounts of pigments on each layer are estimated from total spectral transmittance that is obtained by multiband image. For this estimation, some measured transmittance spectra are used as reference spectra. The Wiener estimation method[10,11] is used for estimating the total spectral transmittance per pixel from multiband image. Multiple linear regression analysis is applied to extract relative amounts of pigment from the estimated transmittance spectra. Two–dimensional distributions of relative amounts of pigments printed by one or two kinds of color pigments are estimated, and the result is compared with that based on RGB values.

Model of Total Spectral Transmittance in Layered Pigments

In case of pigment on transparency, the negative logarithm of spectral transmittance is directly proportional to the concentration and to the thickness of the absorbing pigment based on Lambert–Beer's law. The relation is described at position (x,y) and wavelength λ as follows.

$$-\log T(x,y;\lambda)=\varepsilon(\lambda)c(x,y)d(x,y), \tag{1}$$

where $T(x,y;\lambda)$, $\varepsilon(\lambda)$, $c(x,y)$, and $d(x,y)$ denote the spectral transmittance, the characteristic function of the pigment, the concentration, and the thickness, respectively. The product $c(x,y)d(x,y)$ is proportional to amount of pigment at position (x,y).

Transmittance spectra of pigment are measured by using a microspectroscope at several random points in the dot. The measured transmittance spectra are averaged at each wavelength in order to reduce the error of measurement. We call average transmittance as reference transmittance. We assume that the position with reference transmittance in the image is a reference point (x_0,y_0) and that $c(x_0,y_0)d(x_0,y_0)$ is equal to 1. Equation (1) at reference point is given by

$$-\log T(x_0,y_0;\lambda) = \varepsilon(\lambda). \tag{2}$$

Relative amount of pigment to the reference point at arbitrary position (x,y) is expressed as $a(x,y)$. Using the relative amount $a(x,y)$, Eq.(1) becomes

$$-\log T(x,y;\lambda) = \varepsilon(\lambda)a(x,y). \tag{3}$$

Substituting Eq.(2) into Eq.(3), we can obtain following equation.

$$\log T(x,y;\lambda) = a(x,y)\log T(x_0,y_0;\lambda). \tag{4}$$

Figure 1 shows the schematic diagram of two–dimensional distribution of amount of pigment on transparency.

Equation (4) can be extended for the multi–layered pigment. When four kinds of color pigment, cyan, magenta, yellow, and black (C,M,Y,K) are layered at (x,y), the relationship between transmittance spectra and relative amounts is expressed as

$$\log T(\lambda) = a_c \log T_c(\lambda) + a_m \log T_m(\lambda) + a_y \log T_y(\lambda) + a_k \log T_k(\lambda), \qquad (5)$$

where $T(\lambda)$ denotes total spectral transmittance at (x,y). $T_c(\lambda)$, $T_m(\lambda)$, $T_y(\lambda)$, and $T_k(\lambda)$ denote transmittance spectra at reference point (x_0,y_0) of C,M,Y,K, respectively. a_c, a_m, a_y, and a_k denote relative amounts at (x,y) of C,M,Y,K, respectively. The coordinate (x_0,y_0) and (x,y) in the equation are not written for simplicity. Estimation method of relative amounts of pigments is shown in the next section.

Linear Operation to Estimate Relative Amount of Pigment

Considering the measurement error with respect to sampling wavelengths, Eq. (5) becomes

$$\left.\begin{aligned}
\log T(\lambda_1) &= a_c \log T_c(\lambda_1) + a_m \log T_m(\lambda_1) + a_y \log T_y(\lambda_1) + a_k \log T_k(\lambda_1) + e_1 \\
\log T(\lambda_2) &= a_c \log T_c(\lambda_2) + a_m \log T_m(\lambda_2) + a_y \log T_y(\lambda_2) + a_k \log T_k(\lambda_2) + e_2 \\
&\vdots \\
\log T(\lambda_N) &= a_c \log T_c(\lambda_N) + a_m \log T_m(\lambda_N) + a_y \log T_y(\lambda_N) + a_k \log T_k(\lambda_N) + e_N
\end{aligned}\right\} \qquad (6)$$

where λ_i, i=1,2,…, N, is sampling wavelength, and e_i . i=1,2,…, N, denotes the error.

Relative amounts a_c, a_m, a_y, and a_k are determined in order to minimize a sum of square error at each wavelength. The sum is represented as G;

$$
\begin{aligned}
G &= \sum_{i=1}^{N} e_i^2 \\
&= \sum_{i=1}^{N} \left[\log T(\lambda_i) - a_c \log T_c(\lambda_i) - a_m \log T_m(\lambda_i) - a_y \log T_y(\lambda_i) - a_k \log T_k(\lambda_i) \right]^2 .
\end{aligned}
\tag{7}
$$

Taking partial derivative with respect to a_c, a_m, a_y, a_k and setting the result equal to zero yields the minimum of G. For example, the calculation with respect to a_c is shown as follows,

$$
\begin{aligned}
&2\sum_{i=1}^{N} \left[\log T(\lambda_i) - a_c \log T_c(\lambda_i) - a_m \log T_m(\lambda_i) - a_y \log T_y(\lambda_i) - a_k \log T_k(\lambda_i) \right] \log T_c(\lambda_i) = 0 \\
&a_c \sum_{i=1}^{N} \log T_c(\lambda_i)^2 + a_m \sum_{i=1}^{N} \log T_c(\lambda_i) \log T_m(\lambda_i) + a_y \sum_{i=1}^{N} \log T_c(\lambda_i) \log T_y(\lambda_i) \\
&\qquad + a_k \sum_{i=1}^{N} \log T_c(\lambda_i) \log T_k(\lambda_i) = \sum_{i=1}^{N} \log T(\lambda_i) \log T_c(\lambda_i)
\end{aligned}
\tag{8}
$$

Using vector–matrix notation, the equation with a_c, a_m, a_y, a_k can be expressed as

$$
\mathbf{X}\boldsymbol{a} = \mathbf{Y} \tag{9}
$$

where

$$\mathbf{X}=\begin{bmatrix} \sum_{i=1}^{N}\log T_c(\lambda_i)^2 & \sum_{i=1}^{N}\log T_c(\lambda_i)\log T_m(\lambda_i) & \sum_{i=1}^{N}\log T_c(\lambda_i)\log T_y(\lambda_i) & \sum_{i=1}^{N}\log T_c(\lambda_i)\log T_k(\lambda_i) \\ \sum_{i=1}^{N}\log T_c(\lambda_i)\log T_m(\lambda_i) & \sum_{i=1}^{N}\log T_m(\lambda_i)^2 & \sum_{i=1}^{N}\log T_m(\lambda_i)\log T_y(\lambda_i) & \sum_{i=1}^{N}\log T_m(\lambda_i)\log T_k(\lambda_i) \\ \sum_{i=1}^{N}\log T_c(\lambda_i)\log T_y(\lambda_i) & \sum_{i=1}^{N}\log T_m(\lambda_i)\log T_y(\lambda_i) & \sum_{i=1}^{N}\log T_y(\lambda_i)^2 & \sum_{i=1}^{N}\log T_y(\lambda_i)\log T_k(\lambda_i) \\ \sum_{i=1}^{N}\log T_c(\lambda_i)\log T_k(\lambda_i) & \sum_{i=1}^{N}\log T_m(\lambda_i)\log T_k(\lambda_i) & \sum_{i=1}^{N}\log T_y(\lambda_i)\log T_k(\lambda_i) & \sum_{i=1}^{N}\log T_k(\lambda_i)^2 \end{bmatrix} \tag{10}$$

$$\boldsymbol{a}=\begin{bmatrix} a_c \\ a_m \\ a_y \\ a_k \end{bmatrix}, \tag{11}$$

$$\mathbf{Y}=\begin{bmatrix} \sum_{i=1}^{N}\log T(\lambda_i)\log T_c(\lambda_i) \\ \sum_{i=1}^{N}\log T(\lambda_i)\log T_m(\lambda_i) \\ \sum_{i=1}^{N}\log T(\lambda_i)\log T_y(\lambda_i) \\ \sum_{i=1}^{N}\log T(\lambda_i)\log T_k(\lambda_i) \end{bmatrix} \tag{12}$$

Let define matrix **K** and vector $\boldsymbol{t}$ as follows,

$$\mathbf{K}=\begin{pmatrix} \log T_c(\lambda_1) & \log T_m(\lambda_1) & \log T_y(\lambda_1) & \log T_k(\lambda_1) \\ \log T_c(\lambda_2) & \log T_m(\lambda_2) & \log T_y(\lambda_2) & \log T_k(\lambda_2) \\ \vdots & \vdots & \vdots & \vdots \\ \log T_c(\lambda_n) & \log T_m(\lambda_n) & \log T_y(\lambda_n) & \log T_k(\lambda_n) \end{pmatrix}, \tag{13}$$

$$\boldsymbol{t}=\begin{pmatrix} \log T(\lambda_1) \\ \log T(\lambda_2) \\ \vdots \\ \log T(\lambda_n) \end{pmatrix} \tag{14}$$

Using matrix **K** and vector $\boldsymbol{t}$, **X** and **Y** are represented as follows,

$$\mathbf{X} = \mathbf{K}^T\mathbf{K} \tag{15}$$

$$\mathbf{Y} = \mathbf{K}^T\boldsymbol{t} \ , \tag{16}$$

where $\mathbf{K}^T$ represents a transposed matrix for $\mathbf{K}$. Substituting Eqs. (15) and (16) into Eq. (9), we can obtain following equation,

$$\mathbf{K}^T\mathbf{K}\boldsymbol{a} = \mathbf{K}^T\boldsymbol{t} \ . \tag{17}$$

If $\mathbf{K}^T\mathbf{K}$ is nonsingular matrix, relative amount $\boldsymbol{a}$ is given by the following equation,

$$\boldsymbol{a} = [\mathbf{K}^T\mathbf{K}]^{-1}\mathbf{K}^T\boldsymbol{t}. \tag{18}$$

Experiment

Color laser printer (Phaser 550J, Tektronix) and the C,M,Y,K pigment were used for microscopic sample. A single printed dot of each pigment on OHP sheet and overlapped pigment by magenta and yellow were cut in size about 1cm×1cm for microscope. The spectral transmittance at reference point of each pigment is shown in Fig. 2. Transmittance spectra of micrograph of the OHP sheets were estimated from multiband image by using Wiener estimation based method.[10]

The matrix $\mathbf{K}$ was calculated from Eq. (13), and vector $\boldsymbol{t}$ was calculated from Eq. (14) using the estimated transmittance spectra of micrograph. Therefore, relative amount vector $\boldsymbol{a}$ of pigment was estimated from Eq. (18) using the obtained $\mathbf{K}$ and $\boldsymbol{t}$.

Figures 3 and 4 show the results of the estimated relative amount of cyan and black pigment, respectively. Figures 3(a) and 4(a) show the images of pigment, which are reproduced as RGB value from spectral transmittance at each position. The size of pigment is 10～15 μm in diameter. Figures 3(b) and 4(b) represent two–dimensional distribution of amount of each pigment. In the Figs. 3(b) and 4(b), The points on the axis for relative amount and the axes for pixel correspond to the estimated relative amount of pigment and the position (x,y) on micrograph taken by multiband CCD camera, respectively.

Figure 5 shows the reproduced image with magenta and yellow pigment as example of mixed pigment image. In Fig. 5, the upper left and the down right area in the image are printed with only magenta and yellow pigment, respectively, and the center area is layered with magenta and yellow pigment. Figure 6 shows monochromatic pigment images divided by Eq. (18) based on transmittance spectra, and Figure 7 shows the divided monochromatic pigment images based on RGB values. There is a difference between the divided images based on transmittance spectra and those based on RGB values.

Conclusion and Discussion

A method to estimate relative amount of printed pigment in optical micrograph has been proposed. Lambert–Beer's law is applied to pigment printed on transparency. The relative amounts of pigments at all pixels were calculated from the estimated spectral transmittance at each pixel and the measured spectral transmittance at reference point by using linear operation. The method was applied to micrographs of transparencies with one or two kinds of pigments. Two–dimensional distributions of the estimated relative amounts were drawn. The overlapped pigments image was separated into two component images.

Our technique can separate each layer image from multi–layered image, therefore it can be used in the printer model because dot area and dot profile from multicolor printed image are obtained. This will give an accurate prediction of color reflectance by spectral Neugebauer model.

We also applied this estimation method for separating micrograph of mouse testicle cell which is stained with two kinds of pigment, namely Hematoxylin and Eosin, on trial. The property of each pigment could be obtained from separated image successfully.[11] This method may have a capability to apply to the other pigment transparencies such as biological and medical sample.

In this paper, the pigment printed on transparency was studied to gain the dynamic range of measurement. We would to apply this method for estimating the relative amount of pigment from four color printed image on paper.

References

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Figure captions

Fig. 1. Schematic diagram of two–dimensional distribution of relative amount of pigment on transparency.

Fig. 2. Spectral transmittance at reference point of each pigment; (a) cyan, (b) magenta, (c) yellow, (d) black.

Fig. 3. (a) Reproduced image of cyan pigment, (b) two–dimensional distribution of estimated relative amount of cyan pigment.

Fig. 4. (a) Reproduced image of black pigment, (b) two–dimensional distribution of estimated relative amount of black pigment.

Fig. 5. Reproduced image with magenta and yellow pigment. upper left area: printed only magenta pigment, down right area: printed only yellow pigment, center area: printed both pigments.

Fig. 6. Divided pigment images based on transmittance spectra, corresponding density to relative amount, (a) magenta, (b) yellow.

Fig. 7. Divided pigment images based on RGB values, corresponding density to relative amount, (a) magenta, (b) Y pigment.

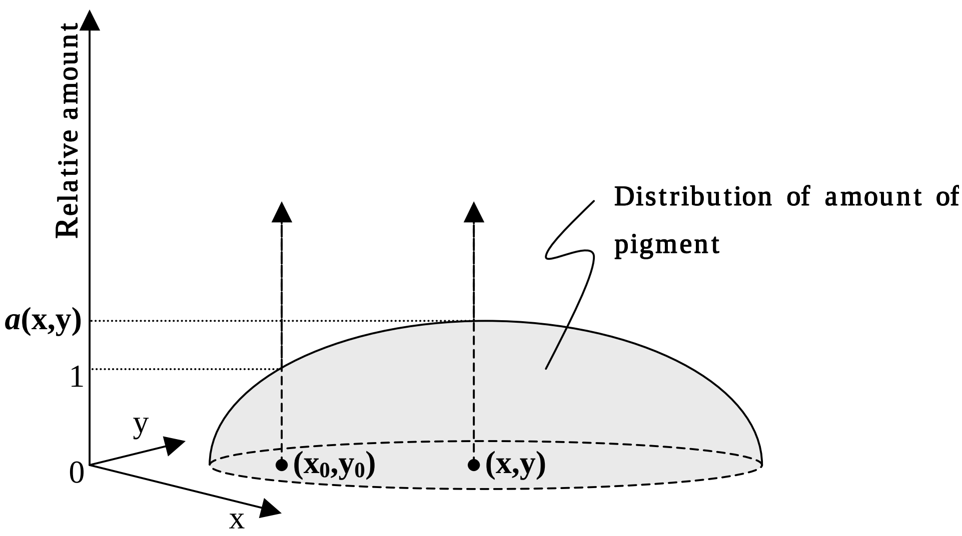
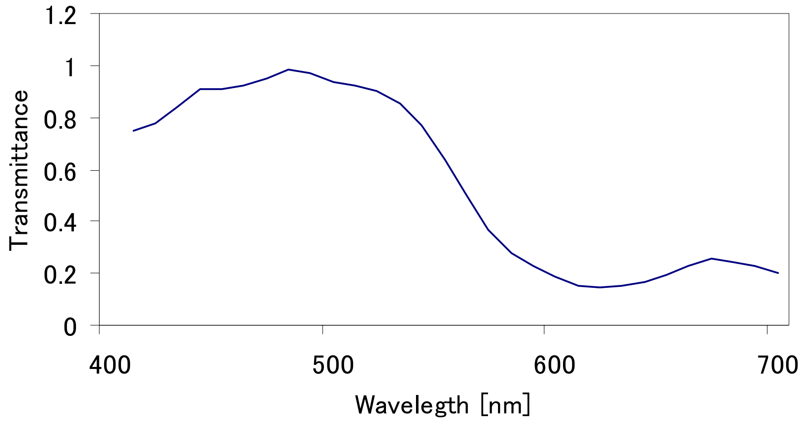
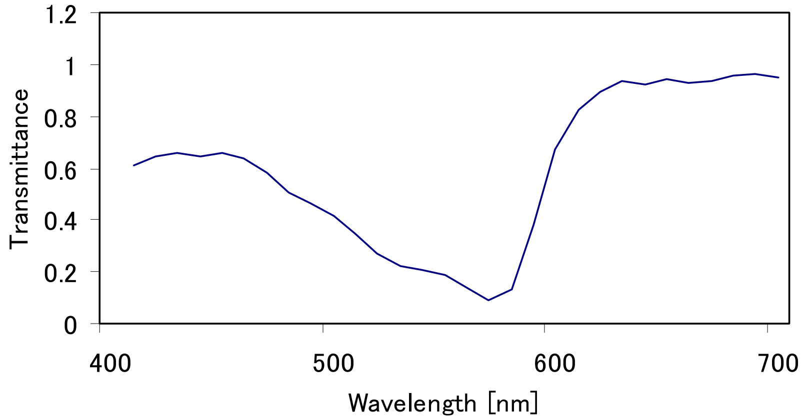

Relative amount
Distribution of amount of pigment
a(x,y)
1
0
y
x
(x_0,y_0)
(x,y)

Fig. 1.

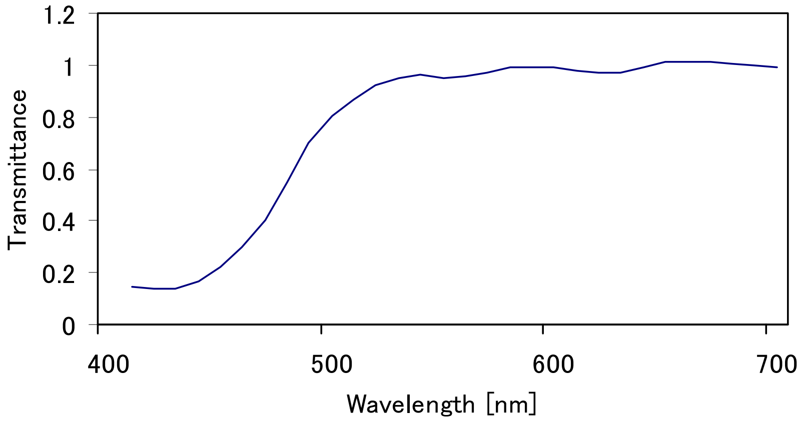

1.2
1
0.8
0.6
0.4
0.2
0
Transmittance
400
500
600
700
Wavelegth [nm]

(a)


1.2
1
0.8
0.6
0.4
0.2
0
Transmittance
400
500
600
700
Wavelength [nm]

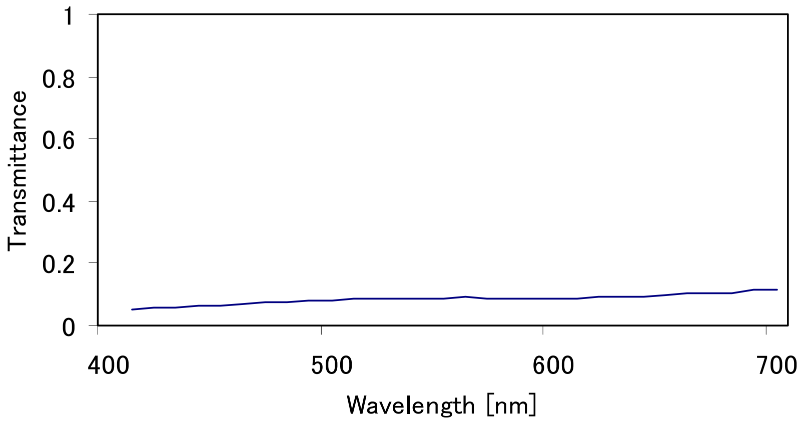
(b)

Fig. 2.



1.2
1
0.8
0.6
0.4
0.2
0
Transmittance
400
500
600
700
Wavelength [nm]

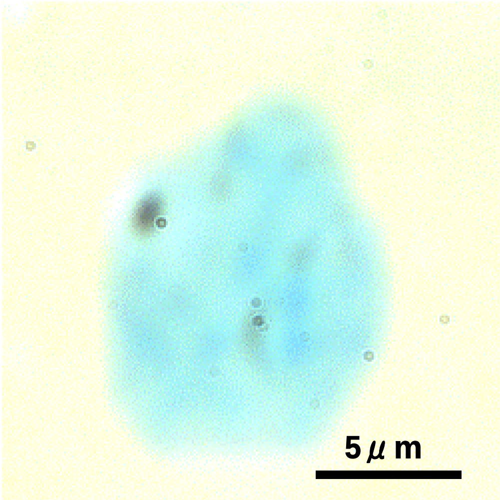
(c)



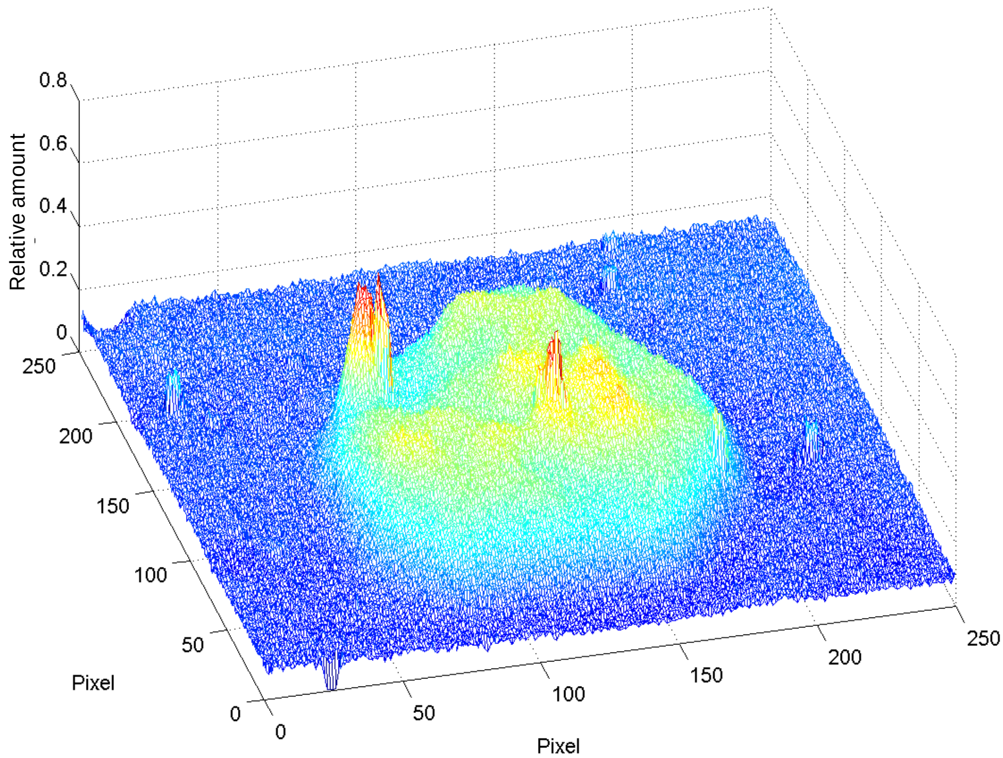
1
0.8
0.6
0.4
0.2
0
Transmittance
400
500
600
700
Wavelength [nm]

(d)

Fig. 2.

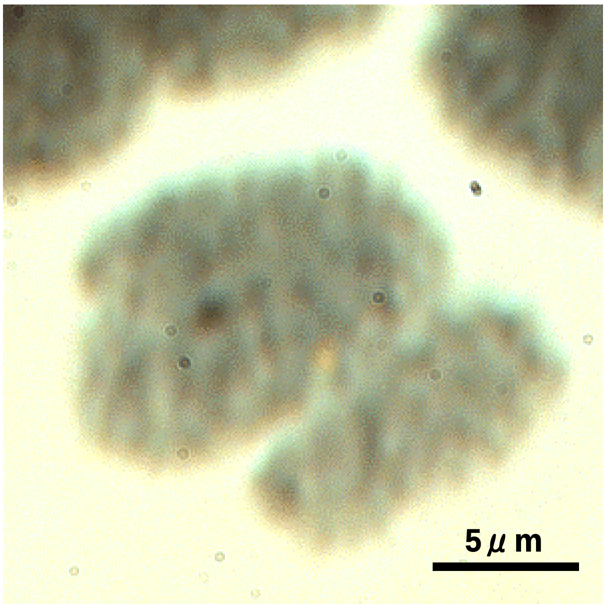

5 μm

(a)

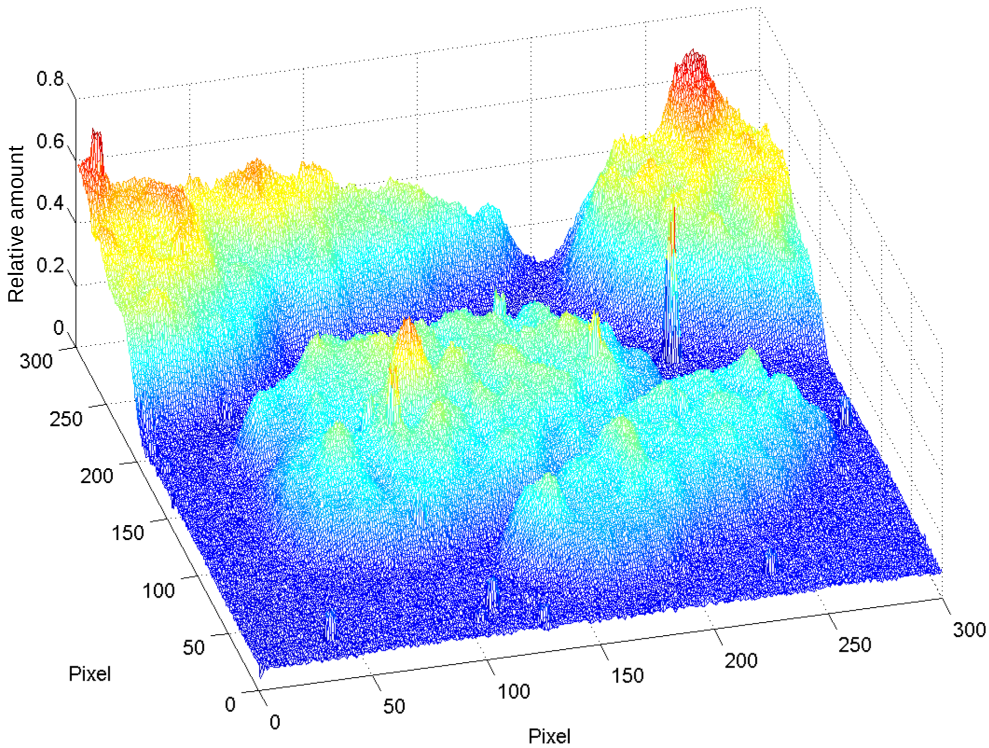

Relative amount
0.8
0.6
0.4
0.2
0
250
200
150
100
50
0
Pixel
0
50
100
150
200
250
Pixel

(b)

Fig. 3.


5 μ m

(a)


0.8
0.6
0.4
0.2
0
Relative amount
300
250
200
150
100
50
0
Pixel
0
50
100
150
200
250
300
Pixel

(b)

Fig. 4.

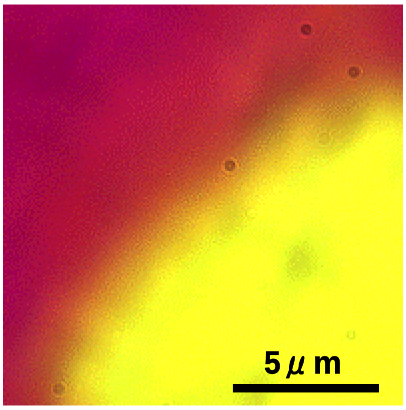
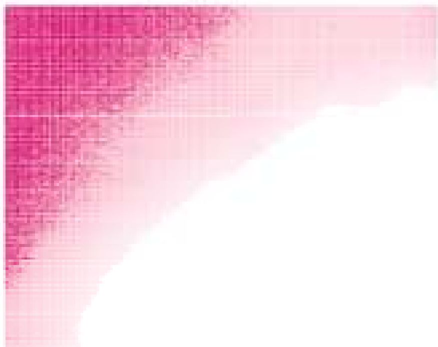


Fig. 5.



(a)

(b)

Fig. 6.

(a)

(b)

Fig. 7.